Low-energy (1-500 eV) ion-biomolecule interactions in the condensed phase

By

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Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.
In the middle of difficulty, lies opportunity.

Albert Einstein
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### Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>DD</td>
<td>Dipolar Dissociation</td>
</tr>
<tr>
<td>DEA</td>
<td>Dissociative Electron Attachment</td>
</tr>
<tr>
<td>DSB</td>
<td>Double Strand Break</td>
</tr>
<tr>
<td>GCR</td>
<td>Galactic cosmic Rays</td>
</tr>
<tr>
<td>HZE</td>
<td>High energy (E) and high charge (Z) particles</td>
</tr>
<tr>
<td>ICRP</td>
<td>International Commission on Radiation Protection</td>
</tr>
<tr>
<td>ISD</td>
<td>Ion Stimulated Desorption</td>
</tr>
<tr>
<td>ISS</td>
<td>International Space Station</td>
</tr>
<tr>
<td>LEO</td>
<td>Low Earth Orbit</td>
</tr>
<tr>
<td>LET</td>
<td>Linear Energy Transfer</td>
</tr>
<tr>
<td>LNT</td>
<td>Linear non-threshold model</td>
</tr>
<tr>
<td>NCRP</td>
<td>National Council on Radiation Protection</td>
</tr>
<tr>
<td>OER</td>
<td>Oxygen Enhancement Ratio</td>
</tr>
<tr>
<td>QMS</td>
<td>Quadrupole Mass Spectrometer</td>
</tr>
<tr>
<td>RBE</td>
<td>Relative Biological Effectiveness</td>
</tr>
<tr>
<td>SE</td>
<td>Secondary Electrons</td>
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<tr>
<td>SOBP</td>
<td>Spread Out Bragg Peak</td>
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<tr>
<td>SSB</td>
<td>Single Strand Break</td>
</tr>
<tr>
<td>UHV</td>
<td>Ultra High Vacuum</td>
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<td>WR</td>
<td>Weighting Factors</td>
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Abstract

An understanding of all nascent events leading to radiolytic DNA damage is required to achieve a complete description of ionizing radiation effects on living cells. These early, sub-picosecond events, involve the production of numerous low-energy (E < 20 eV) secondary electrons, neutral radicals, and secondary ions. Secondary ions, created either by the primary radiation, or by secondary electrons via resonant processes, are produced all along the primary radiation track with energies from a few eV up to several hundreds eV depending on their formation mechanism. Although much recent attention has focused on DNA damage initiated by secondary electrons, the subsequent reactive damage induced by the equally reactive non-thermal ionic species remain virtually unexplored.

This work focuses on the interaction dynamics of low energy (1-500 eV) ions (secondary ions or primary ions at track-end energies) with films of DNA components (thymine, deuterated thymine, uracil, bromouracil), or other biomaterials, to understand the basic mechanisms of low energy ion-induced DNA damage. A novel ultra-high vacuum ion beam system, adapted to the study of organic solids, has been specially designed and constructed to conduct these studies. The unique design of the apparatus allows irradiation of biomolecular films with highly focused, mass and energy selected atomic and molecular ion beams in the 1-500 eV energy range.

We show that atomic and molecular, singly or doubly charged primary cation impact (e.g. $D_n^+$, n=1-3, $He^+$, $N_2^+$, $Ar^+$, $Ar^{++}$), at ion energies between 10-500 eV in 1-2 nm thick films of thymine completely fragment the molecules, even at energies as low as 15 eV (near 0.5 eV/amu for $Ar^+$), and result in the formation of over 30 different secondary cation and anion fragments with energies below 5 eV. Many of these fragments react in the films before desorption, and result in formation of new products. We also show that fragment anions with
energies below 5 eV, produced by secondary electrons, can lead to further physico-chemical damage in organic films. Our experiments also reveal strong reactive scattering channels for some projectiles, e.g. HeH\(^+\) formation and desorption for thymine films irradiated with 10-300 eV He\(^+\) ion beams.

Our present work brings, for the first time ever, clear evidence that hyperthermal ion-interactions with DNA bases lead to severe damage, even at incident ion energies as low as 10-15 eV. The results of our preliminary studies, presented here, already challenge traditional models stipulating that heavy ion irradiation causes DNA damage via similar pathways as conventional ionizing radiation, by showing that primary and secondary ions incident on organic media induce significantly greater damage that reaches beyond single ionization, or free radical production. This has important implications for example in radiation therapy where the significant contribution of secondary ions and primary ions at track end energies cannot be forgotten anymore and left out of dose calculations.
Résumé

La compréhension des événements ultra-rapides menant au dommage radiolytique de l’ADN est nécessaire à une connaissance approfondie des effets de la radiation ionisante sur les cellules vivantes. Ces événements, intervenant dans le domaine de la picoseconde, impliquent la production massive d’électrons secondaires de basse énergie (<20eV), de radicaux et d’ions secondaires. Ces ions secondaires, créés soit par la radiation primaire elle-même, ou par les électrons secondaires via des processus résonants, sont produits tout au long de la trace de la radiation primaire avec des énergies allant de quelques eV à plusieurs centaines d’eV, selon leur mécanisme de formation. Si beaucoup d’attention a été consacrée ces dernières années aux dommages à l’ADN initiés par les électrons secondaires, le dommage réactif subséquent induit par les espèces ioniques, non thermiques et tout aussi réactives, demeure en revanche inexploré.

Ce travail cible la dynamique d’interaction d’ions (secondaires, ou primaires en fin de trace) de faible énergie (1-500 eV) avec des films de constituants de l’ADN (thymine, thymine deutéée, uracile, bromouracile) ou autres biomolécules, afin d’appréhender les mécanismes fondamentaux des dommages induits à l’ADN par les ions de basse énergie. Un nouveau système expérimental adapté à l’étude des solides organiques a été spécialement conçu et développé à cet effet. La conception unique de l’appareil permet l’irradiation de films moléculaires avec des faisceaux d’ions atomiques ou moléculaires, hautement focalisés et caractérisés à la fois en masse et en énergie sur la gamme d’énergie comprise entre 1 et 500 eV.

Nous montrons que l’impact de cations atomiques, moléculaires, simplement ou doublement chargés (dont D\textsubscript{n}+, n=1-3, He\textsuperscript{+}, N\textsuperscript{+}, N\textsubscript{2}\textsuperscript{+}, Ar\textsuperscript{+}, Ar\textsuperscript{++}), d’énergie entre 10 et 500 eV sur des films de thymine de 1 à 2 nm d’épaisseur, fragmente complètement les molécules,
même à des énergies incidentes aussi faibles que 15 eV (près de 0.5 eV/amu pour Ar⁺), et résulte en la formation de plus de 30 fragments positifs et négatifs secondaires différents, dotés d’énergies inférieures à 5 eV. Beaucoup de ces fragments réagissent dans le film lui-même avant de désorber, formant ainsi de nouveaux produits. Nous montrons également que les fragments négatifs produits par les électrons secondaires, possédant une énergie typiquement inférieure à 5 eV, ont la capacité d’induire de surcroît un dommage physico-chimique dans les films organiques. Nos expériences révèlent aussi l’existence de diffusions réactives privilégiées pour certains projectiles, notamment avec la formation et la désorption d’ions HeH⁺ durant l’irradiation de films de thymine par des ions He⁺ entre 10 et 300 eV.

Les résultats de notre étude témoignent pour la toute première fois de l’interaction d’ions hyperthermiques avec des bases d’ADN en démontrant clairement qu’elle conduit à des dommages sévères, même à des énergies incidentes aussi faibles que 10-15 eV. Les résultats de nos études préliminaires exposés ici, en montrant que les ions secondaires et primaires sont responsables de dommages conséquents allant au-delà de simples ionisations ou de la production de radicaux libres, contredisent les modèles traditionnels stipulant que le dommage à l’ADN induit par les ions lourds emprunte les mêmes voies que la radiation conventionnelle. Ceci a des répercussions majeures notamment en radiothérapie où la contribution avérée des ions secondaires, ou primaires en fin de trace, ne peut plus être ignorée et non comptabilisée dans les calculs de distribution de dose.
CHAPTER 1 - INTRODUCTION

1.1 Temporal and spatial energy distribution of ionizing radiation

The timescale for the interaction of ionizing radiation with matter is commonly divided into three categories (stages) and are governed by physics, chemistry, and biology; this is shown in fig. 1. Events occurring in the 0-10^{-12} \text{s} time interval are referred to as ultrafast events and are themselves divided into three stages (Sanche, 1989a; Sanche 1993).

![Temporal distribution of ionizing radiation induced events.](image)

Events taking place within attoseconds following the propagation of the incident radiation in the medium constitute the primary stage. The primary stage is characterized by ionizations and excitations leading to the formation of cations, anions, excited molecules, neutral fragments, and a large number a secondary electrons (SE) (~ 4.10^4 SE formed per deposited MeV, with an energy distribution mainly below 100 eV). The numerous SE react with their environment during the secondary stage to produce reactive cations, anions and radicals with kinetic energy below 10 eV via dissociative electron attachment or additional ionizations and excitations. Mainly in the case of high incident energy photons and electrons, it is shown that most of the biomolecular damage is not due to the primary radiation itself but results from
the interaction of the abundant secondary particles produced along the radiation track. Because they are non thermal, the reactive cations, anions, neutral radical fragments, and excited neutrals produced in large amounts via primary energy depositions as well as secondary events can rapidly react within picoseconds with the surrounding environment to cause further damage in what is called the reactive stage.

The energy distribution of radiation over space is not uniform. Ionizing particles passing through absorbing material lose energy in a large series of discontinuous steps. Depending on the amount of energy deposited, secondary species formed via primary and secondary processes are present in spurs, blobs or along the δ-ray tracks formed by some of the most energetic secondary electrons. Energy deposition for low LET radiation is typically illustrated in the left panel of fig.2 with the track of a fast proton in silicon. Although classified as heavy charged particles, high energy protons present essentially similar track characteristics as low LET fast electrons. Energy distributions of fast electrons in water forms spurs containing on average 100 eV and typically spaced out 100 nm apart (von Sonntag, 1987).

![Fig. 2 Spatial track distribution with examples of simulations for 1000 events in a 6-μm silicon tube. The simulation results including all secondary particles for 100 MeV protons are depicted on the left and for 100 MeV α particles on the right. Most significant are the large number of δ electrons surrounding the track. The greater ionization of the α particles is obvious in the density of these secondary particles (Kobayashi et al., 2004).](image)
Electrons tracks are less dense than the tracks of heavy charged particles, and the spurs are more widely spaced with more frequent delta rays terminating with blobs. Heavy charged particle tracks are characterized by a higher ionization density as illustrated by the 100 MeV $\alpha$ particle shown in the right panel of fig. 2. Spurs come nearer to one another forming a very small central core of ionization caused by the particle itself, surrounded by a much wider area of ionization caused by the secondary particles ejected from the central core. Thus, most of the volume of the track is secondary radiation and a living cell in the path of the track would probably be affected mostly by secondary ions and electrons.

Because of their high capacity of interaction within very short times (less than a picosecond), the physicochemical context at later times where the diffusion-limited chemistry begins, will essentially be a consequence of these secondary species interactions. Secondary reactive species, not only by their formation, but also by their subsequent reaction, lead to substantial physical and chemical modifications of the medium and basically determine the starting input for all the subsequent radiation chemistry and corresponding biological endpoints. The physical and chemical damage produced by such secondary ions is therefore of great radiobiological relevance.

1.2 The physics of heavy charged particles

As outlined above, knowledge of basic physics of radiation interaction and energy transfer is fundamental to the understanding of the late biological effects of radiation on living tissues.

The basic mechanism for the slowing down of moving charged particles is coulombic interactions between the particles and the medium. This energy loss mechanism is common to all charged particles. Heavy charged particles lose energy primarily through collisions
with atomic electrons of the medium while traversing matter. Direct collisions with electrons and coulombic interactions with orbital electrons lead to ionizations, while coulombic interactions with valence electrons leave the atom in an excited state. In addition to collisions with atomic electrons, incident heavy charged particles can also interact with the target nuclei. Nuclear reactions leading to target or projectile fragmentation occur at very high incident particle velocity whereas elastic nuclear collisions, relevant for our energy range, become the dominant energy loss process at low velocities. In contrast to electronic projectiles which experience major radiative energy losses via bremsstrahlung, the deflection resulting from collisions between heavy charged particles and atomic electrons is completely negligible. However, because of the frequent small angle nuclear scattering interactions, the path of a heavy charged particle in matter deviates from a perfect straight line. This multiple Coulomb nuclear scattering causes notably a pencil beam of charged particles to spread into a diverging beam as it penetrates a target. The widening of the beam as a function of depth is called beam straggling and has a great impact in radiotherapy with heavy charged particles since it can significantly lower the dose that can be concentrated in a deep-seated tumor. Beam straggling for electron beams is more pronounced due to the large momentum transferred to them, and has also a significantly larger range due to the substantially smaller mass of electrons compared to heavy charged particles.

The energy transferred by a heavy charged particle in a single electronic collision is only a small fraction of its energy. The maximum energy that can be transferred to an electron in a single collision can be evaluated classically with the assumptions that the particle moves rapidly compared to the electron and that the energy transferred is large compared to the binding energy of the atomic electron. Under these conditions, the atomic
electron can be considered to be initially free and at rest, as depicted in fig. 3 and the maximum energy transfer is given by:

$$Q_{\text{max}} = \frac{1}{2} M V_1^2 - \frac{1}{2} M V_2^2 = \frac{4 m M}{(M + m)^2} E$$  \hspace{1cm} (1)

where $E = MV_1^2 / 2$ is the initial kinetic energy of the heavy particle.

**Fig. 3 Classical representation for a collision of a particle of mass $M$ and speed $V$ with an electron of mass $m$, initially free and at rest ($v_1 = 0$). The two entities move with speeds $V_2$ and $v_2$ along the initial line of travel of the incident particle.**

For an electronic collision, the maximum energy transferred by the heavy particle to the atomic electron $Q_{\text{max}}$ is effectively very small since $m \ll M$. Expression (1) shows for example that a 10 MeV proton can lose only up to 0.22% of its energy in a single electronic collision. The situation where the two colliding entities have the same mass ($m = M$) gives $Q_{\text{max}} = E$ showing that the incident particle can transfer all of its energy in a head-on (or billiard-ball type) collision. Eq. (1) is accurate for classical particles and does not hold at extremely high energies where the exact relativistic expression for the maximum energy transfer must be used.
1.3 Linear energy transfer of heavy charged particles

As mentioned in the previous section, heavy charged particles interact with the traversed medium via their electric field, primarily with the molecular electrons along their path. The interaction taking place between a charged particle P and a molecule M can be visualized by the classical picture given in fig. 4.

Fig. 4 Schematic representation of the interaction between a charged particle and a molecule. (a) The particle P passes the molecule M at a certain distance. (b) The magnitude of the transversal electric field at the site of the molecule as a function of distance. (c) The same field as a function of time. (from Dertinger and Jung, 1970).

The electric field felt by the molecule, plotted as a function of the distance between the charged particle and the molecule in fig. 4b, shows that the Coulombic forces acting on the molecule are maximum at low impact parameter when the distance between the two entities is minimum. The magnitude of the electric field at the site of the molecule is independent of the velocity of the charged particle. The same field plotted as a function of time for both slow and fast charged particles in fig. 4c shows that slow charged particles interact with the molecule over a longer period of time, thereby giving it a larger momentum. The visualization of fig. 4 highlights three important aspects of energy transfer by charged particles: 1- the energy transferred by the moving charged particle decreases with its
velocity; 2- the energy transferred by the charged particle increases with its electric charge; 3- the energy transferred is independent of the mass of the charged particle.

The amount of energy transferred to the medium by the heavy charged particle is of fundamental importance in radiation physics, dosimetry and radiation biology. The average energy loss of the heavy charged particle per unit length of its track in the medium, designated by $-dE/dx$, is called the stopping power $S(E)$ of the medium for the particle, and is also referred to as the linear energy transfer (LET) of the particle, usually expressed as keV/$\mu$m in water. The LET, given here in SI units (Meesungnoen and Jay-Gerin, 2005), is quantitatively given by the Bethe-Bloch equation for heavy charged particles (Bethe, 1930; Bloch, 1933a; Bloch, 1933b):

$$S(E) = -\frac{dE}{dx} = \left( \frac{1}{4\pi\varepsilon_0} \right)^2 \frac{4\pi e^2 (ze)^2}{mn} n Z \left[ \ln \frac{2mV^2}{I} - \ln \left( 1 - \beta^2 \right) - \beta^2 \right]$$

where $V$ and $ze$ are respectively the velocity and charge of the heavy charged particle, $m$ and $e$ the rest mass and charge of the electron, $n$ the number of electrons in the medium per m$^3$, $Z$ the target atomic number, $I$ the mean ionization potential of the medium, and $\beta = V/c$. The three important aspects mentioned above are echoed in the Bethe-Bloch equation, of which $V$ and $ze$, the two only parameters characterizing the incident heavy charged particle, constitute the dominant part. The $1/V^2 \approx 1/E$ dependence yields an increase in energy loss with decreasing particle energy and the $ze$ dependence with increasing particle charge. The fact that the charge of the projectile enters in eq. (2) with a power of two is of importance: an $\alpha$-particle which has twice the charge of a proton has a four-fold LET compared with that of a proton. Like velocity and charge for the incident particle, the electronic density $n$ is the only important parameter to characterize the medium. At high particle energies that substantially exceed the binding energies of all the electrons, all of the latter have to be considered. At
lower particle energies, only the outer-shell electrons can contribute. Mean excitation energies \( I \), calculated or measured in experiments can be estimated for an element with atomic number \( Z \) by the following approximate empirical formulas (Turner, 1986):

\[
I \approx 19.0 \text{ eV}; Z = 1 \text{ (hydrogen)} \\
I \approx (11.2 + 11.7 Z) \text{ eV}; 2 \leq Z \leq 13 \\
I \approx (52.8 + 8.71 Z) \text{ eV}; Z > 13
\]

Estimated values were given in the ICRU report No. 37 (ICRU, 1984) and can also be found at http://physics.nist.gov/PhysRefData/XrayMassCoef/tab1.html.

1.4 Linear energy transfer over distance: the Bragg peak

At low particle velocities, eq. (2) diverges and becomes therefore synonymous with an infinitely large energy transfer. This results from the assumption that the charge \( ze \) of the particle remains constant over all the path of the particle. This assumption is not valid because in the low energy region, a positively charged particle captures and loses electrons as it traverses the medium, thus reducing its net charge and stopping power. The process of electron capture becomes important when the speed \( V \) of the heavy particle is less than or comparable to the speed that an electron needs to orbit about an atomic nucleus. According to Bohr's theory, the orbital speed of an electron in the ground state about a nucleus of charge \( ze \) is \( ze^2/h \). Thus a condition for electron capture by the projectile and loss from the target is \( ze^2/hV \geq 1 \). The electron capture probability increases as the particle slows down, and in turn becomes less strongly interacting. The Bethe-Bloch equation has thus to be modified and to reflect processes occurring at low energies; Barkas (Barkas, 1963) suggested the following expression \( z^* \) for the charge of the particle:
\[ z^* = z \left(1 - \exp \left( \frac{-125 \beta}{z^{2/3}} \right) \right) \] (3)

At low speeds (\( \beta = V/c \to 0 \)) \( z^* \) tends to 0 so that the whole expression in (2) also tends to 0. At the same time, since \(-dE/dx\) also falls off as \(1/V^2\) at higher energies, the energy loss must pass through a maximum at low particle energies. This maximum of energy loss is known as the Bragg peak, and is seen in Fig. 5 in which the LET of a charged particle is shown as a function of its distance of penetration in the medium.

For most of the charged particle track, the rate of energy loss increases roughly as \(1/E\) as predicted by eq. (2). However, near the end of the track, the charge is reduced by electron pickup and the curve falls off. The linear rate of energy loss is at its maximum as the particle velocity approaches 0 and falls off rapidly to 0 once past the Bragg peak.

![Fig. 5 Rate of energy loss of a 62 MeV proton beam in water (from http://www.lns.infn.it/catanaweb/latest.htm).](image)

However, when the energy transfer by charged particles is greatly reduced past the Bragg peak through interactions with target electrons, projectile energy transfer by nuclear
collisions becomes the dominant process at low particle velocities. The cross section for elastic nuclear collisions increases as the particle energy decreases, even below the Bragg peak. As mentioned earlier, the electric field of a charged particle interacts with the electric field of a nucleus. In the same manner as a sufficiently high energy transfer can knock out an atomic electron (and thus ionize the atom), a sufficiently high imparted momentum can remove an atom from its molecule. As nuclear collisions involve much larger masses than ionizations, the incident particle must be moving sufficiently slowly and must past very close to the nucleus. This is the reason why the probability of nuclear collisions is smaller by about three orders of magnitude than that of interactions with electrons at high particle energies.

1.5 Radiation-induced DNA damage

Absorption of energy from radiation (X- or γ-rays, charged or uncharged particles) in biological material produces excitations or ionizations by direct action (direct effect) of the incident radiation, or late chemical indirect action (indirect effect). Indirect effects involve the interaction of radiation with other atoms or molecules (e.g. water) in the cell to produce free radicals that are able to diffuse far enough to reach and damage the critical targets in the cell. Direct effects are generally the more important process for high LET radiation in terms of the quality of the lesions, because (a) the high ionization density produced leads to a high probability of interacting directly with the critical targets, and (b) in comparison to the direct effects, the indirect effects are less complex in terms of the severity of the lesions. Low LET radiation in contrast interacts mainly by indirect processes: about two thirds of the biological damage induced by X-rays are caused by indirect effects (Hall, 2000).

Current experimental evidence leaves little room for doubt that deoxyribonucleic acid (DNA) is the principal cellular target of lethal radiation effects (reproductive death of the cell
which loses its ability to divide and produce progeny cells. The structure of a strand of DNA is illustrated in fig. 6. Alternating sugar (deoxyribose) and phosphate groups form the backbone which holds the bases, attached on the sugar molecules. Two strands held together by hydrogen bonds between the bases form the DNA molecule. Bases on opposite strands must be complementary, pyrimidine bases thymine and cytosine pairing with purine bases adenine and guanine, respectively.

![DNA Structure](image)

**Fig. 6 The structure of a single strand of DNA (Hall, 2000)**

In a living cell, radiation damage to DNA is due to the combination of direct and indirect effects. The majority of damage produced by indirect effects (especially low LET radiation) is due to the radiolysis of water in the vicinity of the DNA molecule, leading to free radical induced-damages. These free radicals damage the DNA sugars and bases, producing mainly single-strand breaks (SSB), base loss, and a large number of modified DNA bases.

A much smaller number of double-strand breaks (DSB) are produced by direct ionization of DNA. In spite of the fact that the frequency of DSB is much lower than that of other types of lesions, DSB are believed to be of primary importance. In mammalian cells,
DSB are considered to be the primary lesion involved in cellular lethality (Elkind, 1985), mutagenesis, and, maybe more significantly in terms of cancer risk, the lesion that is more difficult for cells to accurately repair.

DSB can also be the ultimate consequence of multiple single lesions produced in close proximity. Ward (Ward, 1981; Ward, 1988; Ward, 1994) has been the first to highlight the importance of clustered damage, showing that lethal events such as DSB (or SSB accompanied by other lesions ultimately leading to DSB), occur in regions of high radical damage density and consequently, most probably in the spurs (von Sonntag, 1987).

---

**Fig. 7** Schematic representation of tracks of sparsely and densely ionizing radiations compared with relevant biological targets (chromatin fiber, nucleosomes and DNA double helix) (Belli et al., 2002).

Clustered damage thus appears to be the signature of high radiation doses. Tracks of alpha particles and electrons were examined for clusters of ionizations of differing complexity in spherical volumes of DNA size targets. The data show that the clusters for
alpha particles show higher frequency for clusters with larger numbers of ionizations and indicate that the frequency of more complex clusters increases with higher LET (Nikjoo and Charlton, 1995). The LET could thus determine both the frequency and the complexity of damage. The LET as given by eq. (2) is an average value indicative of the incident particle energy loss over its entire path in the medium. Low-LET radiations such as high-energy electrons and photons produce sparse and spatially well-separated ionization events. As shown in fig. 7, high-LET radiations such as carbon ions and $\alpha$ particles are characterized in contrast by a higher number of ionizations produced with a high density along the track. High LET radiation seems to typically produce concentrated damage identified by Ward as “locally multiply damaged sites”. This clustered damage often shows a high concentration of DSB and is believed to be lethal for the cells.

1.6 RBE of heavy charged particles

The temporal and/or spatial differences between high and low LET tracks make the early physicochemical damage from high LET radiation qualitatively different from low LET radiation damage, and not repairable in the same way. The enhancement on biological effects produced by densely ionizing radiation (neutrons, heavy charged particles) with respect to sparsely ionizing radiation (high energy electrons, photons, for which primary events are well separated in space, as seen in fig. 7) is represented by the introduction of the concept of relative biological effectiveness (RBE). The definition of RBE is illustrated for cell survival curves on fig. 8. The RBE of a given radiation ($r$) is defined as the ratio between the absorbed dose of a referenced radiation (sparsely ionizing 220 keV X-rays (ICRU, 1986)) and that of the test radiation required to produce the same well-defined biological effect on a certain biological system.
Fig. 8 Definition of RBE, illustrated for cell survival curves. RBE is defined as the ratio of X-rays dose to particle dose to produce a same effect in the same biological system (Kraft, 2000a).

For doses up to a few Gray, the reference X-rays curve can be approximated by a linear quadratic expression in dose $D$ where $S$ is the surviving fraction: $S = S_0 \exp\{\alpha D + \beta D^2\}$. For particle radiation of increasing LET, $\beta$ becomes smaller so that the dose relationship becomes linear (Kraft, 2000a). The reference X-rays curve shown in fig. 8 being linear quadratic, means that RBE is dose-dependent: RBE for ions is larger for the same low doses of X-rays. Another immediate observation that can be made from fig. 8 is that high-LET radiations are more effective than electrons and photons in inactivating the hit cells by a factor that can be as large as 3 at the 10% survival level but even lower than 1 at extremely high LET values as illustrated in fig. 9.

Fig. 9 illustrates the LET dependence of the RBE, and shows why LET near 100 keV/μm have the greatest RBE for biological effects, often related to the occurrence of DSB as mentioned in section 5. The low LET radiation is depicted as producing sparse ionizations too well separated to cause unrepairable and lethal damage to the cell. Consequently, the number of cells killed for a total number of ionizations events being small, low LET
radiations have a small RBE in contrast to an idealized radiation of optimum LET, for which the average distance between ionizing events and between cellular critical sites would match exactly, and would be of 2 nm, the diameter of the DNA double helix.

![Graph showing RBE vs LET with examples of X-ray, 100 keV/µm, and 200 keV/µm](image)

**Fig. 9** LET dependence of RBE. The general trend is that RBE is maximal for LET around 100 keV/µm and decreases for very large energy depositions (Hall, 2000).

This density of ionizations corresponds to an LET of about 100 keV/µm (or 100 eV/nm) and to a radiation possessing therefore the greatest RBE. As the LET is further increased, so is the ionization density thereby making the radiation less efficient at cell killing than lower LET radiation because of the overkill effect (the number of ionizations largely exceeds the number of damaged cells and so a large part of the delivered energy is wasted on already dead cells).

RBE is a complex quantity that depends not only on the LET of the radiation but has been also shown to strongly depend on the atomic number and the energy of the incident charged particle as shown in fig. 10. This strong observed dependence suggests that RBE rather depends on local ionization density, than on the total energy transferred to the critical targets. Because of the different radiosensitivities of different types of tissues, RBE also depends on both the biological system and the biological endpoint considered. Tissues that have a reduced repair capacity are radiosensitive, and do not exhibit an enhanced RBE when
irradiated with high LET radiation, because the dose threshold for the biological endpoint is already reached.

![Graph showing RBE dependence on atomic number. A same critical ionization density is realized for heavy ions at higher energy for larger LET values while at lower energy and smaller LET values for lighter ions (Kraft, 1999).]

**Fig. 10** RBE dependence on atomic number. A same critical ionization density is realized for heavy ions at higher energy for larger LET values while at lower energy and smaller LET values for lighter ions (Kraft, 1999).

Tissues having a high repair capacity are in contrast radioresistant and show a highly increased RBE when irradiated with high LET radiation. This is one major benefit of radiation therapy with heavy ions: slowly growing tumors, usually very radioresistant to photon irradiation, generally show the largest effect in RBE when exposed to carbon beams, while for radiosensitive and fast proliferating tumors the gain in RBE is smaller.

![Graph showing comparison of the microscopic structure of carbon tracks at different energies with a simplified depiction of a DNA molecule. Ionization and consequently the damage to the DNA is low at high particle energies but increases significantly at lower particle energies yielding clustered damage that is difficult to repair. (Kraft, 2000b).]

**Fig. 11** Comparison of the microscopic structure of carbon tracks at different energies with a simplified depiction of a DNA molecule. Ionization and consequently the damage to the DNA is low at high particle energies but increases significantly at lower particle energies yielding clustered damage that is difficult to repair. (Kraft, 2000b).
This observation can be related again to the local ionization densities reached along the track, which can be in turn directly correlated with local densities of DNA damage, as illustrated in fig. 11 which shows a comparison of the microscopic structure of carbon tracks at different energies. It has been shown that when the LET is larger than 10-20 keV/μm the dense ionizations produced along the particle track induce one or more DSB when it crosses the cell’s DNA. It has also been shown that the ratio of DSB to SSB increases with LET (Kiefer, 1985), which corroborates the previous discussion about clustered damage. The increase of RBE with LET can be correlated to the occurrence of clustered damage, typical of high radiation doses and that may be particularly difficult to repair.

1.7 Heavy charged particles in radiation therapy

Radiation therapy can be performed by using external radiation sources (external beam therapy – charged particles exposure by accelerator beams), or by using internal radiation sources (brachytherapy – long-lived radioactive sources in close vicinity of the tumor). Both approaches require careful treatment planning since radiation therapy is technically difficult and potentially dangerous. Radiation therapy with heavy charged particles is also called hadrontherapy because when passing through an absorber, singly charged ions loose all electrons (Bohr, 1948), and become hadrons (i.e. atomic nuclei). For instance, projectiles such as carbon are fully stripped off their electrons to become high LET C⁺⁶ hadrons at energies necessary for therapy.

The major potential of heavy ions in therapy is both the Bragg peak of their depth-dose profile, and their corresponding modulated RBE, which allows one to deliver effective depth doses to deeply-seated localized tumors while sparing surrounding healthy normal body tissues. This is difficult to achieve with the high energy photons and electrons used in
conventional radiation therapy because of their poor depth-dose distribution. This is illustrated in fig. 12 which shows the percentage of absorbed dose (in water) for 20 MeV electrons and 8 MV photon beams, together with 200 MeV protons. It can be seen that

![Graph showing depth dose curves in water for different radiation types.](image)

**Fig. 12** Depth dose curves in water for electrons (20 MeV), photons (from a Cobalt source and an 8 MeV linear accelerator), neutrons, and 200 MeV protons. With protons the highest dose is released near the end of the range in tissue giving rise to the Bragg peak (from Amaldi, 1999).

electron beams are characterized by a maximum range in the tissue (depending on the initial energy of the beam), beyond which lies a low intensity tail due to bremsstrahlung photons, while photon beams are characterized by an exponential profile of absorption after a maximum reached at 2 cm for beams of 8 MeV maximum energy. Dose-depth distributions for 10 MeV electrons and photons, measured with a water phantom at a medical accelerator are presented in appendix 1.

The depth of the Bragg peak depends on the initial energy of the ions and its width depends on the energy spread of the beam. As illustrated in fig. 13, the Bragg peak is often spread for therapeutic applications so that the region to be treated is covered in depth as well as in width by interposition of an absorbing material of variable thickness in the beam path (Chu et al.,
1993), or modulation of the beam energy during the irradiation, to superimpose many narrow Bragg peaks and obtain a spread-out Bragg peak.

![Graph showing Bragg peak superposition](image_url)

*Fig. 13 Schematic drawing of the superposition of many Bragg peaks to produce a flat Spread Out Bragg Peak (SOBP) (from Amaldi, 2004).*

The dose which expresses the local deposition of energy at the macroscopic level is not the only critical parameter to consider. Tumor tissue irradiation incorporates two other parameters: 1-the modalities of energy transfer to the tissues expressed at the microscopic level by the LET; and 2- the oxygen contact of the irradiated tissues. Tumor tissues tend to be scarcely vascularized and their content in oxygen is subsequently generally low (especially for tissues found at the center of a slowly growing tumor). In this case, the relevant quantity is the ratio between the doses required to produce a given effect in the absence and presence of oxygen, which is termed oxygen enhancement ratio (OER). Another equally important property of heavy charged particles is that in extended Bragg peaks, the magnitude of the radiobiological oxygen effect is significantly reduced (Blakely et al., 1978). The oxygen effect is reduced more effectively with particles of higher atomic numbers, which would suggest using the heaviest available projectile. This is however tempered by the fact that energetic heavier nuclei are more subject to fragmentation which leads to the reduction of depth-dose effectiveness.
CHAPTER 1 – Introduction

Fractionation of dose plays an important role in terms of the potentiation of the effects of heavy charged particle radiation injury which has been demonstrated in tissues (Goldstein et al., 1981) and in carcinogenic studies (Burns and Albert, 1979). Neon and heavier particles applied in split doses produced an enhanced cell killing effect (Ngo, 1982). Studies showing that most cells in a population of V79 cells exposed to heavy ions are arrested in the G2 stage just preceding cell division (Lucke-Huhle et al., 1979; Blakely et al., 1980) indicated that heavy ion RBE is greater for division delay than for cell killing, an additional property strengthening the justification for using heavy ions in therapeutic applications.

The increased and modulated RBE of heavy charged particles is what motivates their use in radiation therapy. The high ionization densities achieved near the Bragg peak produce many DSB and clustered damage when crossing the DNA of a cell nucleus. The effects on the cells are thus qualitatively different from those produced by sparsely ionizing radiation which interact mainly indirectly with DNA producing mostly repairable SSB and DSB. Due to the much larger proportion of direct effects, heavy charged particles have a greater RBE for most biological endpoints, such as cellular damage. First clinical studies were undertaken at the San Francisco medical center and the Lawrence Berkeley Laboratory in California with protons in 1954, helium in 1957, and with heavy ions, mostly neon, in 1975. The treatment of deep-seated tumors entered a new phase of development in the years 1993-1994 (Amaldi, 1994) with the wide use of proton beams; however, in the late nineties it became evident that the optimum therapeutic beam is found around $Z=6$, which defined the carbon ion, at present the second most frequently used charged particle.

Two major arguments in the use of carbon ions are: (a) For carbon ions, the high LET region is restricted to the end of the particle range, while repairable DNA damage dominates
for all the range before the Bragg peak, due to a low ionization density. For neon ions (for which \( Z^2 = 100 \)) the LET is already too high in the first layers of traversed tissue, and can produce dangerous retarded effects in addition to damaging the healthy tissue in front of the tumor. This can be seen in fig.14 showing the RBE of different ions as a function of the penetration depth. For protons, values summarized in table 1 show that the RBE at the Bragg peak is too low, while helium ions reach LET values approaching 20keV/\( \mu \)m only in the last mm of their path.

(b) Another reason is the small beam straggling. Heavy charged particles generally have small beam scattering; thus, because heavier carbon ions experience less multiple scattering than protons, carbon ions allow a sharper lateral energy deposition. However, nuclear fragmentation increases with atomic number and at the energies used in radiation therapy, projectile fragmentation becomes a concern. This is important, because lower charge and lower mass ions produced by fragmentation at high projectile energy usually have a longer range and therefore reach beyond the Bragg peak of the parent ions, thereby producing a tail that hinders the precision of tumor irradiation.
Fig. 15 NSRL computer display of Bragg curve behind polyethylene absorber for a 0.75 GeV/u iron beam (from http://srhp.jsc.nasa.gov/newsletter/Volume4-1/Index.cfm).

The crucial post-Bragg peak ionization zone is illustrated in fig. 15, showing the dose deposition of a 0.75 GeV/u iron beam behind polyethylene absorber. As seen in table 1, by considering the dose behind the tumor, carbon ions fragment into smaller ions much less than heavier nuclei such as iron. Because of the increased RBE, carbon ion treatment exceeds the possibilities of protons in hadrontherapy and may represent an optimum of biological efficiency in tumor therapy also on the molecular level of DNA damage and repair along a therapeutic beam (Heilmann et al., 1996).

Appendix 2 lists all the centers which have treated patients with hadrons and gives the statistics of the treated patients up to 2004. The number of locations shows the potential impact of hadrontherapy in the forthcoming decades.
Table 1. Properties of ions for a fixed range corresponding to 200 MeV protons (Brahme et al., 2001):

<table>
<thead>
<tr>
<th>Charged particle</th>
<th>( E/A ) (MeV/( \text{amu} ))</th>
<th>( p ) (MeV/( \text{c} ))</th>
<th>( p ) at inject. (MeV/( \text{c} ))</th>
<th>LET (( \text{keV}/\text{mm} )) at various residual ranges in water (MeV/( \text{c} ))</th>
<th>Dose at ( R_p ) after 10 cm SODP</th>
</tr>
</thead>
<tbody>
<tr>
<td>( ^1 \text{H}^+ )</td>
<td>56.0</td>
<td>56.0</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>( ^1 \text{H}^+ )</td>
<td>209.0</td>
<td>645</td>
<td>115</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>( ^2 \text{D}^+ )</td>
<td>136.0</td>
<td>1045</td>
<td>160</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>( ^3 \text{T}^+ )</td>
<td>108.0</td>
<td>1385</td>
<td>345</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>( ^4 \text{He}^+ )</td>
<td>238.5</td>
<td>2125</td>
<td>345</td>
<td>1.6</td>
<td>2.0</td>
</tr>
<tr>
<td>( ^4 \text{He}^+ )</td>
<td>212.0</td>
<td>2580</td>
<td>457</td>
<td>1.8</td>
<td>2.2</td>
</tr>
<tr>
<td>( ^4 \text{Li}^+ )</td>
<td>234.1</td>
<td>4905</td>
<td>380</td>
<td>3.7</td>
<td>4.6</td>
</tr>
<tr>
<td>( ^4 \text{Be}^4 )</td>
<td>283.7</td>
<td>7050</td>
<td>1055</td>
<td>5.9</td>
<td>7.2</td>
</tr>
<tr>
<td>( ^6 \text{B}^6 )</td>
<td>323.5</td>
<td>9550</td>
<td>1260</td>
<td>8.5</td>
<td>10.2</td>
</tr>
<tr>
<td>( ^6 \text{C}^6 )</td>
<td>391.0</td>
<td>11250</td>
<td>1370</td>
<td>11.1</td>
<td>13.4</td>
</tr>
<tr>
<td>( ^8 \text{N}^8 )</td>
<td>430.5</td>
<td>13910</td>
<td>1600</td>
<td>14.5</td>
<td>17.3</td>
</tr>
<tr>
<td>( ^8 \text{O}^8 )</td>
<td>468.6</td>
<td>16710</td>
<td>1830</td>
<td>18.2</td>
<td>21.6</td>
</tr>
<tr>
<td>( ^8 \text{Ne}^{10} )</td>
<td>540.8</td>
<td>22847</td>
<td>2297</td>
<td>26.8</td>
<td>31.5</td>
</tr>
</tbody>
</table>

The energies of column 2 correspond to a range of 26.2 cm in water. The other columns give the momenta at the end and the beginning of the acceleration cycle, the LET values at different residual ranges and the dose at the practical range for a 10 cm SOBP.


1.8 Heavy charged particles: major space radiation hazard

Health effects from space radiation are a concern of growing importance since the International Space Station (ISS) and Mars mission programs require increasingly longer stays in space for astronauts. The space radiation field in low Earth orbit (LEO) and in interplanetary space is detailed in appendix 3.

The energetic electrons (< 6 MeV) and protons (< 250 MeV) trapped by the geomagnetic field in the Van Allen belts predominate at LEO while the main contribution of dose during a space mission outside the geomagnetic field's protection originates from galactic cosmic rays (GCR) and solar particle events (SPE) which pose both the greatest threat to unprotected crew in interplanetary orbits. Protons provide the greatest contribution to the dose equivalent produced by the high fluxes of charged particles encountered during
the energetic (up to several GeV) solar flares and coronal mass ejections characterizing SPE. SPE constitute the main limiting factor to extravehicular activities because although rare, their prediction is very difficult.

GCR are charged particles that originate from sources beyond our solar system with energies ranging from several tens of MeV up to $10^{12}$ MeV as shown in fig 16. GCR primary composition

Fig. 16 Energy spectra of the more abundant GCR nuclei as measured near Earth. Below a few GeV/nucleon, these spectra are strongly influenced by the solar modulation as shown in the left part of the curves. The different curves for the same species (hydrogen, helium, C+O, iron) at those energies represent measurements at various levels of general solar activity, the lowest intensity being observed at the highest activity level (Meyer et al., 1974).
consists in 2% leptons (electrons and positrons) and 98% hadrons which include 85% energetic protons, 14% helium particles, and about 1% high-energy (E) and high-charge (Z) ions called HZE particles. HZE particle spectra are characterized by a relative abundance of iron ions (Z = 56) and ranges from Li to U, although ions heavier than Ni are rare. The relative greater abundance of even atomic number GCR particles is shown in fig. 17 together with a measure of their important ionizing power. Although the less abundant, HZE particles are high-LET and highly penetrating (due to their high energy) radiations, two characteristics that make them, together with intense and sudden solar flares (National Research Council, 1996), the most threatening component of space radiation. There is a major concern about the potential RBE for these HZE particles to produce genetic damage.

![Graph showing relative abundance of GCR nuclei]

*Fig. 17* Relative abundance of the even numbered GCR nuclei (solid bars) in comparison to their abundances weighted by the square of the charge of the particle to give a measure of the “ionizing power” \( R = J \cdot Z^2 \) (Wefel, 1978).

(Wu *et al.*, 1999; Wu *et al.*, 1997), genomic instability (Morgan and Murmane, 1995; Ponnaiya *et al.*, 1997), and cancer (National Research Council, 1996). In addition to their
high RBE for radiobiological damage, the high energy of these particles raises another concern: because cross sections for nuclear fragmentation are still significant at these extremely high energies, a large number of secondary particles are produced as the primary particles pass through the spacecraft and the astronaut bodies. The number of secondary particles can exceed the number of primary particles after only a cm or less of traversed material. The nuclear interactions between primary charged particles and target nuclei lead either to target fragmentation or projectile fragmentation. Collisions between high energy charged primary particles and heavy target nuclei lead typically to target fragmentation, producing knockout protons, neutrons and α-particles as well as recoil heavy nuclei. Reactions between HZE particles and target nuclei lead to projectile fragmentation, producing high energy neutrons and protons in addition to larger projectile fragments that retain much of the kinetic energy of the primary HZE particles (Cucinotta et al., 1996). In general, target fragments are slow and thus highly ionizing, whereas projectile fragments are fast and highly penetrating with a forward distribution. These secondaries, produced either by target or projectile fragmentation, some of them, such as low-energy nuclear recoils with LETs even exceeding those of primary HZE particles, can again interact with the material along their path and cause further damage passing through the body of an astronaut.

Traditional radiation protection is usually provided through increased shielding. The shielding for the space shuttle for example varies from 2 to about 30 g.cm⁻² of Al depending on equipment type and repartition. Fig. 18 presents the energy-range relationship for protons in Al or skin and shows that a 5 g.cm⁻² Al thickness is able to stop all protons at energies below 65 MeV. This thickness is thus efficient for the trapped particles of the Van Allen belts at average ISS altitudes (~400 km) where protons with energies lower than 10 MeV dominate, but proves unsuitable for incident particles of higher energy.
Fig. 18 Energy-range relationship for protons in Aluminium or skin. The range is expressed in g.cm$^2$. Grey horizontal lines represent typical thickness of the Al spacecraft walls and of the human skin. Ranges were calculated by SRIM2000 (Durante, 2002).

Doubling the presently used wall thickness would decrease the dose by a factor of 1.2 but would increase both weight and cost by more than 30-50 %. Furthermore, increasing the shield thickness in an attempt to stop the highly penetrating energetic radiation is at best inefficient, if even worse, because of the above-mentioned nuclear interactions of the GCR with the shield produce large numbers of secondary particles of generally lower energy but sometimes higher quality factors than the primary particles.

The nature of the shield has thus a dramatic effect on the radiation field composition and hence on the biological response (via the RBE of the secondaries). This is illustrated in fig. 19 showing the attenuation of dose equivalent and cell transformation frequency in a one-year exposure at solar minimum (hence at maximum GCR contribution) behind several shield materials.
Fig. 19 Attenuation of dose equivalent $H(x)$ and cell transformation frequency $T(x)$ in one-year exposure at the solar minimum behind several shield materials. GCR transport calculated by HZETRN. (a) Equivalent dose, calculation based on ICRP-60 (ICRP, 1991); (b) neoplastic cell transformation, calculation based on track-structure Katz's model (Waligorski et al. 1987). (from Wilson et al., 2001).

It can be seen that both thin and thick high $Z$ material shields provide at best no protection, lead being identified as the worst shield material due to the amplified low LET fragment production. Low $Z$ shields provide, in contrast, a good protection which can be understood by considering the total nuclear cross section $\sigma_p$ projected by the nuclei in a unit mass ($\text{cm}^2/\text{g}$) of shield material with atomic mass number $A_T$ approximated by the following expression (Schimmerling et al., 1986):

$$\sigma_p \approx N_0 \pi r_0^2 \left( \sqrt{A_p} + \sqrt{A_T} \right)^2 / A_T$$

(4)

Here $N_0$ is Avogadro’s number, $r_0$ the effective nucleon radius and $A_p$ the atomic mass number of the projectile. Eq. (4) indicates a strong preference for interaction between massive projectiles and light targets and shows that the cross section decreases with increasing the target atomic number. Fig. 20 shows the energy-range relationship for iron ions in light and heavy materials and confirms, as it can also be seen in fig. 19, that liquid
hydrogen is much more efficient than lead in stopping heavy ions (Wilson et al., 2001). Liquid hydrogen being a low-temperature (explosive) liquid, polyethylene appears so far as a good compromise (Miller et al., 2003; Abdo et al., 2003; Simonsen et al., 2000). However, further materials development is required since the optimum material for space radiation shielding has not been identified and awaits further understanding of the biological responses to specific ion types.

![Energy-range relationship for iron ions in liquid hydrogen, lucite, aluminium, and lead.](image)

*Fig. 20 Energy-range relationship for iron ions in liquid hydrogen \((\rho = 0.0715 \text{ g.cm}^{-3})\), lucite \((\rho = 1.2 \text{ g.cm}^{-3})\), aluminium \((\rho = 2.7 \text{ g.cm}^{-3})\) or lead \((\rho = 11.34 \text{ g.cm}^{-3})\). Ranges were calculated by SRIM2000 (Ziegler and Biersack, 1999). (from Durante, 2002).*

1.9 Heavy charged particle exposure: risk assessment

The fundamental problem in radiation protection is assessing the level of risk, and specifically determining the cancer types and the probability of their induction as a result of exposure to mixed fields of charged particle radiation, problem common to both astronauts and cancer patients treated with heavy ions. For the latter the risk of secondary cancer after surviving the primary cancer treatment at a relatively young age is becoming a future concern, given the increasing cancer rates among young people. A realistic estimate of the
uncertainty is of great importance since too large an estimate of risk would result in excessive costs (e.g. increase shielding mass for maximum protection deemed needed raises mission costs), or inability to control the tumour, while an estimate too small would result in excessive risk for the astronaut or cancer patient.

Fig. 21 Maximum contributions of main biological and physical factors to space radiation risk assessment (adapted from NASA, 1998).

What is needed in addition to the biological information is adequate knowledge of the physical characteristics that can cause the risk to vary, as shown in fig. 21. The uncertainties in physical characteristics concern knowledge of the radiation field composition and energy spectra, knowledge of physical transport of particles through materials and tissues, and knowledge of the amount of material surrounding each tissue site. In the absence of direct data, current understanding of the risk for humans of contracting cancer following exposure to radiation is founded on data from Japanese atomic bomb survivors. However, because the atomic bomb survivors were exposed to high doses of mainly low LET radiations (principally γ-rays), extrapolating these data to the low dose rate exposure to high LET protons, heavy ions and secondary radiation encountered in space increases the uncertainty in
estimating the level of risk. This extrapolation is embodied in the linear non-threshold (LNT) model which postulates that any dose, no matter how small, is potentially harmful (NCRP, 1993).

![Diagram of induced cancer risk vs. mean number of particle traversals per nucleus]

**Fig. 22** Schematic of the possible relative contributions of the direct and bystander effects to the total cancer risk produced by radiation exposure on a Mars mission (Brenner and Elliston, 2001).

This model is however challenged in particular by the adaptive response model which postulates that certain doses of low dose radiation may be beneficial (Olivieri *et al.*, 1984; Azzam *et al.*, 1994; Mitchel *et al.*, 1999), and the bystander effect model which postulates that low dose radiation may be even more damaging than what is predicted by the LNT model (Nagasawa and Little, 1999; Sawant *et al.*, 2001; Azzam *et al.*, 2001; Azzam *et al.*, 2004; Azzam and Little, 2004), as shown above in fig. 22. Since there exist no epidemiological studies of humans exposed to the kinds of radiations encountered in space, estimates of risks for biological effects induced by high LET radiation can in principle be empirically based on the risk estimates for exposure to low LET radiation, but multiplied by weighting factors (WR) that express the effectiveness of an absorbed dose of such radiation in terms of equivalent doses. Physical and radiobiological studies of the spatial and temporal distributions of radiation energy deposition are however needed to validate or invalidate this
predominantly empirical approach. These data are mainly obtained by the Monte Carlo method, with an accuracy limited however by the knowledge of input cross sections which are in great demand and necessitate experimental investigations. WR values, as well as derivation of the related quality factors (Kellerer, 2004) are based on many experimental RBE values for stochastic effects, and are selected by advisory groups such as the ICRP (International Commission on Radiation Protection). Uncertainties related to quality factors are among the largest, which is critical since uncertainties in these quality factors translate directly to uncertainties in risk. These values are highly dependent on an improved understanding of RBE as a function of particle type and energy transferred for tumor induction over a range of LETs. Evaluation of RBE of these mixed fields in any point within the patient’s body is particularly important in hadrontherapy where RBEs enter directly in the treatment planning.

1.10 Focus on ion-biomolecule interactions – the present project

As discussed above, the formation and the subsequent reactions of secondary ions are responsible for physical and chemical changes in the medium that determine the initial conditions for all the further radiation chemistry and its eventual biological endpoints. Understanding the basic mechanisms of interaction of the energetic secondary ions produced by the primary (heavy ion) radiation in the nascent stages, as well as the mechanisms by which these same ions are produced by an initial attack to, e.g., a DNA component and cause the final genotoxic damage, is therefore of crucial importance. DNA degradation induced by SE (Boudaïffa et al., 2000; Huels et al., 1998; Antic et al., 1999; Antic et al., 2000; Herve du Penhoat et al., 2001; Abdoul-Carime et al., 2001; Sanche, 2002; Martin et al., 2004) and free thermal radicals (von Sonntag, 1987; Huttermann et al., 1992; Zaider et al., 1994: Jones et
al., 1994; La Vere et al., 1996; Paul et al., 2000; Cadet et al., 2003; Cadet et al., 2004; Stinefelt et al., 2005) has received considerable attention in the past decades, yet the (primary or secondary) ion-induced radiobiological damage remains virtually unexplored particularly on a molecular level of DNA. While some studies have focused only recently on ion energies of several hundreds of keV or in the low MeV range (Lacombe et al., 2004; Coupier et al., 2002; de Vries et al., 2003a; de Vries et al., 2003b; Schlathölter et al. 2004), a dramatic lack of knowledge exists in the low energy range below 1000 eV. This energy range is of crucial importance since it concerns damage produced by (1) primary ions between the Bragg peak and the high LET track ends with energies usually of ~100-500 eV, (2) ballistic secondary ions with energies from several eV up to several hundreds of eV, such as recoil ions produced by primary ion impact (Schlathölter et al., 2004), (3) secondary ions with kinetic energy from hyper-thermal up to several tens eV produced by ion irradiation as well as any other ionizing radiation (secondary ions produced by electrons (primary or secondary), X-ray and γ-ray irradiation via valence excitation have energies typically below 5 eV; some ions formed via Auger-decay of specific core excitations can have energy up to tens of eV (Eberhardt et al., 1987; LeBrun et al., 1993)), and (4) primary or secondary ions anywhere along the track during energy depositions of several hundreds of eV.

The different types of ions produced during irradiation of biological media by heavy ions, electrons, X-rays, or γ-rays, are in different charge states (positive or negative, single or multiple) with very different potential and kinetic energies, as well as chemical reactivity. The physico-chemical and kinetic damage produced by such secondary ions will therefore be of great radiobiological relevance. In dense media, energetic (hyperthermal to several 100 eV) secondary ions will scatter inelastically over short distances (several nm), and may induce complex DNA damage clusters that can not be easily repaired by the cell. For similar
reasons, DNA damage at the primary ion track-ends, i.e. at macroscopic distances beyond the Bragg peak, is also of great consequence in hadrontherapy, since it may involve damage to distal regions, (beyond the tumor volume) which likely contain important healthy organs. Since at the track-ends the remaining primary ion energy is also deposited on nanometer scales, similar DNA damage clusters as those induced by the secondary ions may result. While the possibility of such unreparable and lethal DNA lesions is of great biological importance, the propensity of either low energy secondary ions, or primary ion track-ends, to cause such damage is as of yet unknown, and is thus neglected from radiation track calculations, or risk estimates. Thus, an understanding of the basic mechanisms of ion interaction with the biomedia is crucial, since only a detailed knowledge of the early damage events will allow us to link absorption of ionizing radiation and biological damage, thereby leading to the development of more efficient methods to control radiation effects.

These issues are of essential importance not only in an applied radiation therapy context, but also in current efforts to protect astronauts from harmful effects of space radiation during missions. In radiation therapy, low energy secondary ions generated along the primary radiation track can be noxious to healthy tissues (leading to secondary cancer after treatment), while secondary ions produced by primary ion fragmentation, and which extend beyond the Bragg peak of primary ions to form a tail, can result in harmful effects to critical tissues in the near environment of the target. For space radiation protection, the scarcity of data for late effects from primary protons and heavy ions in model systems, as well as from charged fragments produced by fragmentation, is a major barrier in providing estimates and even induce delay in NASA Mars mission programs (current dose projections for a Mars mission (Durante, 2002) exceed the career limits recommended by NCRP (NCRP, 2000)), while current uncertainties pose severe limits on plans for extended stays on the ISS.
Because no human epidemiological data are available for heavy ions, experimental studies are vital to risk assessment both for radiation therapy and space radiation protection.

Although the very damage produced by low energy ions is certain, no data however exists to document either the action of secondary ions formed with energies from near 0 eV up to several hundreds of eV via different processes all along the primary radiation track, or what occurs at primary ion track end energies, in the tail of the Bragg peak shown in fig. 15. The lack of data on this crucial very low energy range raises numerous questions: Just how harmful are low energy ions to DNA, or its components? How do their interactions lead to biologically relevant damage (e.g. base damage, strand breaks, clustered damage)? Low energy ions can cause damage by single ionization or bond cleavage, but is that the extent of the damage? Do low energy ions have other interaction mechanisms besides ionization to induce the damage? Do they induce mainly single ionizations, and simple bond dissociations? What are the transient species produced from DNA or its components at secondary ion and primary ion track-end energies, and what are the fragmentation energy thresholds? What is the effect of kinetic scattering and potential scattering for positive ions, multiple scattering, and binary collisions, as well as physico-chemical reactions in the relevant sub-keV ion energy range?

To find answers to some of these questions, we selected two different experimental approaches, both suited to investigate ion-biomolecule interactions in the very low 0.5-500 eV energy range:

The first method uses a low energy electron beam to initiate ion reactions in situ in O$_2$-benzene/toluene condensed films, by low energy O$^-$ ions formed via DEA to O$_2$ molecules. Benzene and toluene are used as model systems for DNA subunits because their aromatic ring reproduces some of the basic structures of DNA pyrimidine bases. Here, ion
formation via low energy electron resonant processes (DEA) reproduces the actual conditions of the secondary and reactive events (fig. 1) following primary energy deposition in matter, by yielding anions in close proximity to reactants. The disadvantage is a restriction of both ion energy (broad energy distributions up to 5 eV for O\(^-\) ions formed by DEA to O\(_2\) (Huels \textit{et al.}, 1995a; Huels \textit{et al.}, 1995b; Bass \textit{et al.}, 1998) and ion type, and is therefore a major limitation of this experimental method.

The second experimental approach is to design and construct a UHV ion beam system to investigate damage to DNA components by 0.5-500 eV ions. This approach is experimentally much more complex since a stable, highly focused, mass and energy selected ion beam is required over the entire ion energy range, and commercial instruments of this type are not available, due to the great technical difficulties of producing well characterized ion beam devices below a few 100 eV. Consequently, the ion beam apparatus constructed here is one of only a handful of such devices in the world. However, one of the many advantages of this approach is a better control over the experimental parameters by significantly increasing the range of available ion masses (e.g. H\(^+\) to Ar\(^+\) or Xe\(^+\)), ion type (atomic and molecular), charge state (anions and cations, and multiply charged ions, e.g. Ar\(^{++}\), or Xe\(^{n+}\)), as well as ion energy range and selectivity accessible compared to the \textit{in situ} method. The two experimental approaches used here are presented in detail in the following chapter 2. Chapters 3 to 7 are dedicated to the very first experimental results ever obtained on ion interactions with DNA components in the sub-keV ion energy range, namely films of thymine (chapters 4 to 6), uracil (chapter 7), and bromouracil (chapter 7), irradiated with a variety of incident ion beams. Finally, the experimental results are summarized and further discussed in chapter 8, along with goals for future work using the low energy ion beam system.
CHAPTER 2 – EXPERIMENTAL METHODS

2.1 Experimental investigation of ion-biomolecule interactions

While multiple experimental setups exist for the gas phase, investigation of ion-molecule interactions in the condensed phase are more complex, especially at the very low particle energies, which are our present concern. Three different experimental methods are currently available in our department to study ion interactions with solid films at very low energy.

The first method, available in the group of Leon Sanche, consists of irradiating films with a low energy (0–20 eV) electron beam to create low energy ions in situ via DEA, and monitor the products of their subsequent interactions with the surrounding film molecules. More details regarding this method are given in section 2. The second method, available in our group (not used in the present work), involves forming cations by electron impact on a pure gas and accelerate them (kT − 100 eV) towards an organic film spin-coated on the inner side of a cylinder. After irradiation, reaction products are analyzed by HPLC, or mass spectrometry. The third available technique, developed in our group as part of this work and used in the present studies, is presented in detail in the last section of this chapter. It consists of irradiation of biomolecule films with ion beams of various ionic species by ion stimulated desorption of positive and negative ions. In contrast to the first method detailed in the next section, ions in this method are generated outside the film in an ion source and used to bombard the films at energies up to 500 eV. The many advantages of this last method are mainly to provide a great flexibility on the type (species, mass and charge state) of ions produced, as well as on their incident energy which can be tuned from 0.5 up to 500 eV to cover the entire energy range relevant to our studies.
2.2 Electron stimulated desorption experiment

The experiments are performed in a UHV chamber which reaches a base pressure of approximately $5 \times 10^{-10}$ torr. The UHV chamber is enveloped by two layers of $\mu$-metal shielding to reduce residual magnetic fields in the experimental volume.

![Diagram of ESD apparatus]

**Fig. 0 Schematic view of the ESD apparatus**

A schematic view of the electron stimulated desorption (ESD) apparatus is given in fig. 0. Electrons emitted by the filament enter a custom designed hemispherical electron monochromator of about 2.5 cm central radius which produces a monoenergetic electron beam with an estimated resolution of 80 meV full width at half-maximum. The electron beam impinges onto the target film at an angle of 70° with respect to the surface normal with a measured intensity of about 1.0 nA for incident energies between 1 and 20 eV. Target films are prepared by condensation of a gas or vapor onto the surface of a 0.00075 cm thick polycrystalline Pt foil held at a temperature of 20K. Target film thicknesses are determined
by a volumetric dosing procedure (Sanche, 1979) with an accuracy of about 30% and an overall reproducibility of 0.2 monolayers (ML) (Bass et al., 1998).

The impinging monoenergetic electron beam can induce formation of anions via DEA and DD in the films. DEA fragments can possess kinetic energies up to about 5 eV (Huels et al., 1995b) and several different reactive scattering pathways with surrounding film molecules are available prior to desorption. Depending on their energy, the resulting charged products can either desorb or remain at the film surface or in its bulk. It has been found that heavier fragments which receive less energy than lighter fragments in the energy repartition resulting from energy and momentum conservation, may often remain trapped in the film (Sanche and Deschêne, 1988; Bass and Sanche, 1991). Only fragments possessing sufficient kinetic energy to overcome the charge induced polarization energy $E_p$ of the solid, estimated to be around 1 eV for hydrocarbon films (Sanche et al., 1989b) are able to escape from the film and emerge into the vacuum (Sanche et al., 1995). Reactive scattering by DEA fragment ions in the condensed phase has been first observed in the case of O$_2$ coadsorbed with alkanes where a considerable fraction of O$^-$ ions, directly resulting from DEA to O$_2$, reacts with surrounding hydrocarbon molecules to form OH$^-$ ions of sufficient energy to desorb and be detected (Sanche and Parenteau, 1987; Sanche and Parenteau, 1990).

A fraction of the fragments emerging into vacuum enters an rf quadrupole mass spectrometer (QMS), positioned at approximately 20° from the surface normal and is detected by pulse counting electronics. An ion lens system containing a set of retardation grids precedes the QMS with an angle of acceptance of about 25°. The QMS may be operated in the ion yield mode to give a particular ion yield as a function of electron incident energy, or in the ion energy mode where the anion signal intensity is obtained as a function of the retardation voltage applied on the grids. Further details about the apparatus can be
found in several publications (for a complete description, see Huels et al., 1994; Huels et al., 1995b).

2.3 1 - 500 eV ion stimulated desorption experiment

We report here on the construction of an UHV ion beam system, developed as part of this project and dedicated to the investigation of ion-biomolecule interactions in the condensed phase in the 1-500 eV ion energy range.

This work is the subject of an article entitled “A Novel Ion Beam System for Irradiation of Biomolecular Solids with 1 – 500 eV Singly and Doubly Charged Atomic and Molecular Ions” by Marjorie Imhoff, Zongwu Deng, Mahmoud Rajabian, Frank Brüning, and Michael A. Huels. This article is currently in preparation for submission to Review of Scientific Instruments.

This apparatus has been used for all the experimental results presented here, except for the results presented in chapter 3.
A Novel Ion Beam System for Irradiation of Biomolecular Solids with 1 – 500 eV Singly and Doubly Charged Atomic and Molecular Ions

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Abstract

Dedicated to condensed phase ion-biomolecule interaction studies aimed to better understand ion-induced DNA damage, we have developed an ultra-high-vacuum ion beam apparatus which unique design allows irradiation of solid biomolecular targets with a highly focused, mass and energy selected ion beam in the 0.5-500 eV energy range. Various ion beams with intensities in the 5 – 300 nA range, with about 1 eV FWHM, are routinely obtained. A high resolution mass spectrometer monitors desorbing (positive and negative) species during primary ion impact, or after bombardment by thermal desorption mass spectrometry. Films of DNA components are produced in vacuo by evaporation below 100°C and condensation on a clean Pt substrate at ambient temperature, whereby the molecular evaporation rate is calibrated (ng/cm²) by a quartz crystal micro-balance. Here, we present details of the ion beam transport optics used to produce well characterized ultra low energy beams (1-200 eV), projectile mass selection, and film preparation. We also present first measurements obtained with this apparatus of low energy (10 – 500 eV) He⁺, N⁺, N₂⁺, Ar⁺, and Ar⁺⁺ impact on Al surfaces and thymine films; for the latter we observe both anion and cation formation and desorption via endocyclic and exocyclic fragmentation of thymine, even for projectile incident energies below 20 eV.

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I. Introduction

It is now established that many of the genotoxic effects of ionizing radiation (X- or γ-rays,) on living cells are not caused by the primary radiation itself but by the abundant secondary species such as non-thermal low-energy ions, radicals, excited neutrals and ballistic secondary electrons generated along its track within atto- to femtoseconds\(^1\). Produced in copious amounts\(^2\) (5.10\(^4\)/deposited MeV) with an energy distribution lying essentially below 70 eV and a most probable energy below 10 eV\(^3,4\), low energy secondary electrons further react with molecules within irradiated biological media to produce in turn radicals and secondary reactive ionic species (E ≤ 10 eV) by dissociative electron attachment (DEA) and dipolar dissociation (DD)\(^5\). While most secondary ions produced by electrons (primary or secondary), as well as X- and γ-rays, via valence or core excitations have energies typically from thermal up to tens of eV\(^6,7\), secondary ions produced anywhere along an ion track can have energies ranging from several eV up to several hundred eV\(^8\) depending on their formation mechanism (see chapter 5 for ion formation dynamics). These non-thermal ionic and radical species can rapidly react on nm scales and induce further genotoxic damage.

Many studies have focused on DNA damage initiated by low energy secondary electrons\(^9,10,11,12,13,14\), showing recently that secondary electrons can cause single- and double-strand breaks (SSB and DSB) in supercoiled DNA at very low energies\(^15,16,17\), even below 3 eV\(^18\). However, The subsequent damage produced by the equally reactive non-thermal ionic species remains virtually unexplored on a molecular level. If recent studies have focused on ion energies in the hundreds of keV or low MeV range\(^19,20,21,22\), no data exists in the low energy range below 1000 eV. This energy range is of especially critical relevance for generalized heavy ion radiation therapy where we end up with ~ 100-500 eV slow particles at and beyond the Bragg peak maximum where the RBE reaches its highest value. It is therefore
crucial to determine the action of these either primary or secondary low energy reactive ionic species within vital cellular components, particularly DNA, to explain the mechanisms leading to radiobiological damage, making thereby an attempt to provide a first real link between nanoscopic dose and biological effect.

In this context, we report in this paper on the design, construction and characterization of a mass-resolved low-energy ion beam system, with emphasis on ion scattering experiments in the 1-500 eV energy range. Several experimental techniques to study low energy ion-molecule interactions exist for the gas phase\textsuperscript{23,24} and the condensed phase\textsuperscript{25,26,27,28,29,30,31} but very few of them have been adapted to fit the constraints and challenges generated by reactive scattering experiments both at hyperthermal energies and on organic films, in a fairly simple way and at limited costs. We use here Ion Stimulated Desorption (ISD) of ions and neutrals to extract the fundamental information about ion irradiation effects in the target films by recording the desorbing ionic and neutral species during the sample bombardment. The ISD technique at very low energies requires a well focused beam, resolved in both mass and energy.

The main requirements on our system are: (1) a low-energy ion beam that is mass selected and has a narrow energy spread, (2) efficient differential pumping to keep the source gas out of the collision chamber, (3) a UHV collision chamber with facilities for cleaning (sputtering and annealing) the sample, and (4) \textit{in situ} analytical techniques for beam and target surface characterisation, as well as (5) a film deposition method adapted to biomolecules. The system needs to be operated under UHV conditions to help to increase the particle mean free path as well as to maintain a low surface contamination rate to allow sample deposition times of typically a few min. The electrode elements in charge of carrying the beam from the source up to the target have to be designed to faithfully produce the
electric fields necessary to deflect the charged particles (see the Beam Collimation sections). Due to the radiobiological importance of both positive and negative ions, the source should be able to produce a wide variety of atomic and polyatomic ions of both positive and negative charge with adjustable ion energies.

II. Ion beam system

1. Overview

The configuration of the low-energy ion beam system is summarized in the schematic diagram shown in fig. 1. Five sections make up the apparatus: the ion source chamber, the MMA entrance chamber, the MMA exit chamber, the main chamber and the load-lock chamber. Three apertures of a few mm diameter create the four stages of differential pumping used to reduce the pressure from $10^{-5}$ - $10^{-6}$ Torr in the ion source, to a working pressure of $10^{-9}$ Torr in the main chamber. The three differential pumping apertures are identified in fig. 1 and consist in aperture A1 located after the source, and the exit aperture of the magnetic mass analyzer, and aperture A2 located before the main chamber.

A 1200 l/s oil (Santovac 5) diffusion pump (Varian) with a liquid nitrogen trap, backed up by a roughing pump is used for the ion source chamber. The ion source chamber is isolated by a pneumatic gate valve which closes in case of power outage and stays closed when the power returns while the diffusion pump and the roughing pump both stay off. This pumping facility was chosen due to the requirement of removing excess gas from the ion source.

The magnetic mass analyzer (MMA) entrance and exit chambers are pumped by turbomolecular pumps of 250 l/s and 65 l/s respectively. A 440 l/s ionic pump is used for the UHV main chamber. The load-lock chamber is evacuated with a 65 l/s turbo pump to a
Fig 1. Schematic view of the UHV ion beam system. The four differential pumping apertures are A1 (0.10 in. diameter), MMA exit aperture (0.075 in. each), and A2 (0.075 in. for pressures ranging from near 10^-5 to 10^-9 Torr in the ion source chamber to 10^-7 Torr in the main chamber during experiments with the gate valve open.
pressure of about $10^{-9}$ torr, pressure roughly the same as in the main chamber, which keeps negligible the contribution of background residual species. All turbomolecular pumps are hydrocarbon free pumping stations. The gate valve isolation located between the MMA exit chamber and the main chamber allows these pumps to be used independently of each other in addition to allow venting of the system for maintenance without air exposing the main chamber. The vacuum in the target chamber is further improved by proper baking at 100-120°C prior the experiments for all chambers excepting the ion source.

2. Ion Source

The ion source built here uses electron-impact ionization and is water-cooled to ensure temperature dissipation. The ion source is cylindrically symmetric and consists of: (1) an anode, formed by a stainless steel chamber closed by the extraction aperture plate through which the ions can be extracted; (2) a custom-made filament in the form of a spiral wound tungsten wire, kept about 1 mm distant from the anode extraction plate; (3) two isolated stainless steel rods holding the filament in the chamber. The high pumping speed of the diffusion pump is used to create a large pressure difference between the inside and the outside of the source. While the pressure outside the source is of about $10^{-5}$-$10^{-6}$ Torr during the experiments, the pressure inside the source is in the 0.1 – 1 torr range. The gas inlet system is connected to the ion source chamber through a variable leak valve. After introduction of an appropriate parent gas in the source, a discharge produced by applying a potential difference between the heated filament and the anode accelerates the electrons and leads them to an energy range suitable for ionization. Ions produced by electron impact near the source aperture are extracted by a draw out (DO) lens that follows the ion aperture plate. The filament bias relative to the source generally starts at -130 V. The filament current is around 12 A while the arc (discharge) current is generally around 400 mA, with a gas
pressure of typically $5 \times 10^{-6}$ torr in the source chamber. The source can produce both positive and negative ion.

3. Beam Collimation from source to MMA entrance aperture

Beam collimation is achieved by means of focusing ion lenses and deflectors. Ions formed in the source are first extracted by the DO and collimated into a beam up to the MMA entrance aperture before mass-selection in the MMA’s magnetic sector. The mass-selected ion beam is then collimated from the exit of the MMA up to the target where it is focused onto the target about 2 cm away from the end of the last lens of the beam line in the main chamber. Charged particle beams behaving like photon beams plus Coulombic interactions, the charged particle analogue of Snell’s law $\sqrt{E_1} \sin \alpha_1 = \sqrt{E_2} \sin \alpha_2$ is the property exploited in charged particle optics to make lenses by shaping the equipotential surfaces. The particle velocity that varies continuously across an electrostatic lens plays the equivalent role of the refraction index in light optics. All electronic elements constituting the beam line have a cylindrical symmetry with a central inner diameter $D$ of 0.5 in. and are made out of non-magnetic stainless steel which has the advantage of being bakeable, strong and easy to machine. The spacing between the cylinders is the object of a special care and its dimension must be carefully chosen$^{32}$. Our lens system is designed with a gap $g = 0.1$ D maintained by $\text{Al}_2\text{O}_3$ insulating spacers which also ensure electrical isolation between all the electronic components of the beam line. Since the presence of insulating elements can create a perturbation and undesirably affect the lens focal properties, all electrodes have been designed with overlap, so that external electric fields do not penetrate the gap between the electrodes (as it can be seen later in fig. 5a). The ion energy with respect to ground is defined by the difference of potentials applied to the ion source and the target which is at ground. All
ion optics are floated (biased) on the source potential. This is essential to maintain a well focused ion beam down to 1 eV, while maintaining high primary beam currents.

In addition of DO and A1, the beam line extending from the source to the entrance of the MMA is composed of 8 cylindrical lenses, two of them being X,Y deflectors. Each of the two deflectors is composed of 4 identical electrostatic pieces insulated from each other and electrically connected as two dipole doublets, with independently controlled voltages. The two deflectors act as stigmatic lenses capable of having different magnifications in two normal planes, and reshape the beam to keep it circular and focused before A1 and the MMA entrance apertures, respectively. Transmission through the beam line is independent of the final KE since all voltages are referenced to the potential of the ion source. Beam transmission through the lenses before the MMA is around 20%, relatively low value compared to that of the beam line elements extending from the MMA exit up to the target which is almost 100%.

4. Mass Selection

Ion beams leaving the source need to be mass-selected before impinging on the target surface. This is achieved with a 90° magnetic sector by using the characteristic relation:

\[ R = \frac{144 \sqrt{mE}}{qB} \]

which gives the radius of curvature R (cm) of ions of energy E (eV), mass m (amu), and charge q, in a magnetic field B (gauss). Deflection of ions in a perpendicular magnetic field being proportional to the particle momentum per unit charge, the action of varying B by varying the current through the magnet will separate all particles entering the magnetic field with the same energy according to their mass. For singly charged ions, \( R = 20.16 \text{ cm} \), and 1 T = \( 10^4 \) Gauss, we have \( B = 7.142 \sqrt{mE} \), where \( m \) is in (amu), \( E \) in (eV) and \( B \) in (Gauss).
The MMA mass tube (wave guide) is a UHV stainless steel tube with a rectangular cross section of 2.0" x 1.0" and a central turn radius of 8.0". Ions linearly drift over a distance of 16.54" between the 90° magnetic sector and both the entrance and exit apertures of the mass tube. The magnetic sector of the mass tube uses a variable magnetic field up to 2000 Gauss for mass selection generated by a power supply which has been extended up to 30 A to refine the MMA resolution of ions with high pass energies in the MMA.

The theoretical mass resolution of a magnet following Barber's rule (entrance and exit slits of the mass tube are aligned with the center of curvature of the magnet) is:

$$\frac{\Delta m}{m} = \frac{w}{R(1 - \cos \theta) + l \sin \theta}$$

where $w$ is the dimension of the entrance aperture, $\theta$ is the angle subtended by the magnetic sector, $R$ corresponds to the radius of curvature, and $l$ corresponds to the drift length. Here the location of the entrance and exit slits of the mass tube are not aligned with the center of curvature of the magnet, which means that the magnet does not follow Barber's rule. In the case of our 8" radius magnet, if the location of the entrance and exit slits were to follow Barber's rule, the previous expression would yield a $\Delta m/m$ of 0.004 (0.007) for $w = 0.075\text{in}$ (0.125\text{in}), $\theta = \pi/2$, and $l$ corresponding to 7.92\text{in} in a first approximation. The theoretical mass resolution limit of the magnet used here, based on transport calculations used to design the pole faces (see below) is 0.002 at 200 amu.
Fig. 2a Design of the electromagnet iron pole faces. The function of the two arc-shaped grooves in the pole is to tailor the shape of the magnetic field. Dimensions are inches [mm]. The dashed lines indicate the iron return plates, the dotted lines represent the coil windings.

In the present MMA, the double focusing (horizontal and vertical focusing) magnetic field is produced between the two arc-shaped pole faces of a custom made electromagnet (modeled by Dr. N. White at Diamond Semiconductor Group LLC\textsuperscript{33}, using POISSON magnetic transport simulation software), of which a schematic view is given in fig. 2a. It consists of an outer (9.84") and inner (5.91") curvature radius of the magnet pole, which has a central turn radius of 8.0". The entrance and exit surfaces of the poles are curved and non-normal to the beam axis along which the ion beam enters into the magnetic sector field, each having curvature radius of 5.15". This shape at the pole entrance and exit, as given by the transport calculations, is based on experience with similar magnets, tested and modeled in great detail\textsuperscript{34}. The function of this shape is to provide vertical magnetic focusing via the fringe fields at the curved entrance and exit of the pole face, in addition to the horizontal focusing already present. Vertical focusing of the ion beam is important here to prevent beam
intensity loss due to beam divergence inside the 2” x 1” cross section of the mass tube wave guide, and maximize beam transmission throughout the MMA.

Fig. 2b Schematic side view of the magnet. The electromagnet is composed of two identical pole faces, with a central gap of 1.18” and a total height of 7.12”. Central radius is 8.0”. Dimensions are indicated in in. (mm).

In addition to the arc shaped pole entrance and exit, the two pole faces of the magnet have two arc-shaped grooves parallel to the beam centerline, as indicated in fig. 2a,b. The function of these two grooves is to tailor the shape of the magnetic field between the pole faces in the horizontal plane, such that the field fall-off outward from the centerline is minimized to null second-order aberrations, which could otherwise kill the resolving power.
The pole faces must be flat and parallel to 0.1% of the gap (± 0.03 mm) to avoid blurring the focus.

Fig. 2b presents a schematic side view, showing a winding space of 20x40 mm cut out on each pole to accommodate 600 Amp-turns per coil (1200 total for both coils). The ribbon conductor used here for the coils minimizes the total number of windings per coil, as well as the needed power to yield the required 600 Amp-turns per coil. It is a 38mm wide and 0.178mm thick Cu copper ribbon wire manufactured by HM Wire International Inc\textsuperscript{35}. It is insulated with Kaladex \textsuperscript{®} 2000, a 25μ thick polyethylene-naphtalate film, and is characterized by a minimum breakdown voltage of 7.5 kV at 180°C. The benefits of this material, in addition to its geometric characteristics, are low moisture absorption, and high solvent resistance. Fig 2b features also a side view of the concentric grooves seen in fig. 2a.

Fringe fields at the beam entrance and exit in this pole face configuration compress the ion beam in the vertical direction as it passes through the sector gap which helps to improve beam transmission in both vertical and horizontal planes. Fig. 3 presents the horizontal (top curve) and vertical (bottom curve) MMA beam transmission modeled with the transport simulations (POISSON). The horizontal main axis in the figure corresponds to the beam centerline, and is representative of the beam path throughout the MMA (first drift length, magnet section, second drift length). On the two vertical axis, the top curve above the horizontal axis represents the MMA focussing in the horizontal plane, and the bottom curve below the horizontal axis represents the MMA focussing in the vertical plane. Units in the figure are given in cm. The two dashed lines in the figure correspond to the horizontal width (5.08 cm) and vertical height (2.54 cm) of the mass tube wave guide. The beam dimensions between the entrance (object) and the exit (image) of the mass tube go from 0 to 0.5 mm full width in the horizontal plane, and from 4.6 mm to 9.7 mm in the vertical plane.
Dist from 4 to X-waist = .09 m.; to Y-waist = .23 m.  
Radius of X-waist = .5 mm.; radius of Y-waist = 6.9 mm.  
Divergence at X-waist = 40.2 mrad.; at Y-waist = 28.8 mrad.  
At end of 4 X = .5 mm.; Y = 9.7 mm.  
\[ \Delta P/P \] = .00 %, \hspace{1cm} \Delta L = 33.6 mm.  
X-resolving power = 414.18; Y-resolving power = .00  
Elapsed time =****** microsec.

*Fig. 3* Horizontal (upper curve) and vertical (lower curve) beam focusing of the MMA. Ion drift length before and after the magnetic sector is 16.54" (42.01 cm). All units are in cm.

The slight enlargement of the beam at the exit of the mass tube, well within the limits of its rectangular cross section represented by the two horizontal dotted lines in the figure, show the effective double focusing of the MMA. The position of the focus is slightly adjusted by moving the magnet radially with respect to the beam axes.

The mass spectrum of various beams obtained from a D₂ source gas discharge, with the MMA set at a pass energy of 600 eV, is shown in fig. 4a and demonstrates the MMA sensitivity for low masses of 1-10 amu. D⁺, HD⁺, D₂⁺, HD₂⁺ and D₃⁺ ions corresponding respectively to masses 2, 3, 4, 5, 6 amu can be easily separated. The MMA mass resolution with a N₂O source gas, with the MMA pass energy set at 450eV is shown in fig. 4b. The mass lines corresponding to N₂⁺, NO⁺ and O₂⁺ can be easily separated. The experimental
mass resolution is determined by the gauss (G) width at 10% peak intensity (i.e. 10% valley to peak ratio) vs peak separation of two adjacent ion masses, which corresponds for example to 9 G vs 52 G for $D^+$ and $HD^+$ ($\Delta m \approx 0.17$ amu, $\Delta m/m \approx 0.085$ for $m=2$ amu), and 8 G vs 28 G for $N_2^+$ and $NO^+$ respectively ($\Delta m \approx 0.28$ amu, $\Delta m/m \approx 0.01$ for $m = 28$ amu).

![Magnetic Mass Spectrometer (MMA) Mass Resolution](image)

**Fig. 4 MMA mass resolution** (a) Mass resolution of the MMA shown in ion current as a function of magnetic field for a $D_2$ source. The pass energy is of 600 eV; (b) Mass resolution of the MMA shown in ion current as a function of magnetic field for a $N_2O$ source. The pass energy is 450 eV.

These measurements indicate a better mass resolution for low masses. The decreased mass resolution at higher masses is due to the 15 A limitation imposed by the magnet power supply which does not allow to reach magnetic field intensities high enough to transmit the beam at higher pass energies, which is needed to reach the high mass resolution obtained for low masses. This problem has now been solved by modifying the magnet power supply to extend its maximum to 30 A. The MMA tube is electrically isolated from the rest of the vacuum system so that it can be floated at a desired potential with respect to the ion source.
Accidental contact with high electrostatic potential was prevented by covering the electrically isolated MMA tube with a Plexiglas shield, equipped with cooling fans.

5. Beam Collimation from MMA exit aperture to target

Mass-selected ion beams leaving the MMA are decelerated and refocused onto the target through the ion lens system shown in fig. 5a. Located at the beginning of the beam line sections extending before and after the gate valve, only slight voltage adjustments of the deflectors $D_1$ and $D_2$ are needed in order to guide the beam through $A_2$ and the exit aperture of $L_6$. Defocusing of the beam in the grounded drift space of the UHV gate valve is avoided by an electrically isolated movable lens specially designed to fit inside the modified gate valve, when the latter is open; to close the gate valve this lens is retracted into a UHV storage space welded onto the gate valve body.

The conical shape of $A_2$ shields the beam from the ground gap between the gate valve and its lens. The ionic lens system depicted in fig. 5a yields about 95-98% transmission of the mass-selected ion beam. This is based on measuring and comparing the intensities of the ion beam (a) as it exits the MMA, using $L_1$ as a collector, (b) inside the lens system using $D_1$, $L_2$ and $A_2$ tied together as a collector, and (c) as it impinges on the target or beam detector faraday cup. This high transmission rate is preserved over the whole 500–1 eV ion energy range. SIMION 6.0 was used to optimize the design of the beam line during the design of the ion beam optics. The 2D SIMION model generated for the post-MMA beam line elements shown in fig. 5a is presented in fig. 5b, which displays the simulated trajectories of 45 ions each of 1 amu with 200 eV initial KE. Thirteen electrodes are included in the simulation of the mass-selected ion beam line, including the exit aperture of the MMA mass tube and the gate valve lens. The position and shape of each electrode drawn within the SIMION array are identical in scale to the actual electrostatic lenses used experimentally represented in fig. 5a
and correspond to a lens inner diameter D of 0.5” and a spacing between consecutive lenses of 0.05” maintained by the ceramic insulating plates fitted between them. In the simulation the resolution of the 2D matrix was 1 grid unit / 0.1 mm in the real beam line. As shown in Fig. 5a, most lenses have “overlap”, such that external fields do not enter the gaps between the lenses.

**Fig 5** Beam collimation from MMA exit aperture to target (a) Design of the actual lens system corresponding to the simulation. The lens system focusing mass-selected ion beams from the MMA mass tube exit aperture up to the target achieves a near 100% ion transmission. Lenses inner diameter is 0.5”, the gap between the electrostatic elements is 0.05” and total length is 10.4”. L1 entrance aperture is 0.157” diameter, A2 exit aperture is 0.078” and L6 exit aperture is 0.19”. (b) 2D representation of the post-MMA beam line using SIMION 6.0. The figure shows simulated trajectories for 45 ions of 1 amu traversing the electrostatic lens section in the x-y plane in the absence of collision gas. Ions origins are placed on the x axis at the center of the mass tube exit aperture, at a distance of 0.06in from L1 entrance aperture with an initial trajectory angle of 45°. The potentials of the electrostatic elements are indicated in the table below the simulation.
6. Beam Characterization

To monitor the ion flux and test the characteristics of the ion beam such as its intensity, energy and spatial width, we have designed and assembled a 2 element Faraday Cup, mounted below the target on a translatable and rotatable manipulator. Its aperture designed to be in the sample plane, the Faraday cup is brought up at the target position to monitor the beam intensity and focus. After adjusting the ion beam current in the Faraday cup, the sample holder is simply lowered to bring the target into the beam. Beam intensities of 5-200 nA can be achieved, depending on the ion species. The beam width can be estimated by mechanically translating the Faraday cup vertically through the ion beam, until the edge of the Faraday cup passed through the beam edge. The current density being

![Graphs showing beam intensity as a function of retardation voltage for Ar⁺ and D₂⁺ ions at different energies.](image)

**Fig. 6** (a) Incident Ar⁺ ion beam current at 3, 11, 22 and 53 eV measured with target and (b) Incident D₂⁺ ion beam current at 1.5, 11, 21.5 and 52.5 eV, measured with a custom-made multigrid retardation analyser, as a function of an applied retardation voltage.
assumed to be uniform within the area of the Faraday cup, our measurements currently indicate a beam diameter of less than 4 mm full width for energies between 1 and 500 eV, for all ion beams used here. Beam energy spreading can be more accurately determined with a multigrid array placed in front of another two element Faraday cup, and allows beam energy width to be measured by retardation.

Fig. 6a shows the $\text{Ar}^+$ ion beam current incident on the target at 3, 11, 22 and 53 eV measured as a function of retardation voltage applied to the target. The ion currents are nearly unchanged until just a few volts below that needed to repel the ion beam, then they drop off to zero over a few volts. Taking $\text{d}I/\text{d}V$, the differential of the intensity $I$ measured over the voltage $V$ applied to the second lens, we measure the beam energy width (full width at half maximum, FWHM) of the incident ion beam. With the assumption that the measured FWHM is equal to the energy spread of the ion beam, we estimate the $\text{Ar}^+$ beam energy spread to range from 0.9 eV at 3 eV, to 2 eV at 53 eV. These large measured FWHM are indicative of defocusing and deflection effects inherent to using the target for retardation. However, use of the retardation grid Faraday cup with a $\text{D}_2^+$ beam, fig. 6b, shows that the energy FWHM is somewhat smaller, i.e. ranging from 0.8 eV at 1.5 eV, to 1.8 eV at 21.5 eV.

Experimental uncertainty has however to be accounted for in these values, which represent a convolution of real energy width contributions from various effects, with measurement uncertainty. While thermal effects within the hot discharge plasma can add up to 0.5 eV, field effects in the source/draw out region have also a greater contribution. The geometry of the equipotential field lines inside the source, i.e. between filament, the source extraction aperture plate, and the draw out, generate an electric field gradient, existing even within the 1mm thick extraction region of the source extraction aperture. This electric field gradient makes precise location of the ion extraction critical, since a few volts can easily be added
over extremely short distances, e.g. mm. This is particularly important since extracting ion beams with narrow kinetic energy distributions from the source will thus yield better mass and energy selected beams at the exit of the MMA. Another contribution are measurement uncertainty, most of which comes from contact potentials. While their contribution can be estimated around 0.4-0.5 eV, contact potentials are however of greater importance to absolute energy values.

![Graph showing Ar⁺ target current vs Ar⁺ beam energy](image)

**Fig. 7** Typical Ar⁺ beam current at the target as a function of beam energy. The total Ar⁺ current is constant from 500 to ~1 eV with a maximum fluctuation of ± 5%.

A typical current vs beam energy curve is shown in fig. 7 for an Ar⁺ ion beam. We see that the total beam current for the Ar⁺ ion beam can be kept constant in the whole ion energy range from 500 to ~1 eV with a fluctuation of less than ± 5% or better. The intensity peaks at 11 eV, which corresponds here to the energy at which the beam has been optimized (tuned). For the energy range shown in the figure, only the source voltage has been changed (with respect to ground, i.e. the target) without any readjustment of the beam transport potentials. For ions other than Ar⁺, the range over which the ion current can be maintained constant is reduced to 500-10 eV, the current dropping by at most 40% between 10 and 1 eV.
7. Film Preparation

Molecules subject to irradiation are condensed on a polycrystalline Pt foil at room temperature in the main chamber. The Pt foil is fitted on a 0.4 x 0.75 in. insulating sapphire inserted into an oxygen free copper holder. A Chromel - Alumel thermocouple spot welded to the edge of the Pt foil monitors target temperature, while the electrical connections used to flash heat the target by DC current is used to measure the incident ion current read on a electrometer (floated or grounded). The sample holder unit is further mounted on a fully rotating and vertically transferable manipulation rod allowing exact positioning of the target along the central vertical axis of the main chamber. For film preparation, thymine obtained from Aldrich (99%) and used without further purification is first loaded into a miniature molecular evaporator in the load-lock chamber and degassed by heating for several hours well below the evaporation onset at about 45°C. To prepare the films, the sample is raised 18 cm above the ion beam, and positioned opposite a gate valve that leads to a load lock chamber, containing a molecular evaporator.

The molecular evaporator, made out of oxygen free copper, is 1.15” long, 0.25” inner diameter, 1.0” wide body, and a 0.1” diameter nozzle aperture. It is designed to accommodate a 0.5” diameter heater element (1200°C UHV button heaters (#101137), HeatWave Labs, Inc.). Chromel - Alumel thermocouples measure body and nozzle temperatures during operation of the molecular evaporator, which is fixed at the end of a magnetic transfer rod. The magnetic rod transfers the molecular evaporator into the main chamber, placing the nozzle about 1 cm close to the Pt surface for molecule deposition, and can also rotate around its axis, allowing the molecular evaporator to be equipped with a rotary activated shutter. Pieces composing the transfer rod are graduated to measure the molecular evaporator translation and rotation.
Thymine films are prepared by evaporation at temperatures between 90 and 100°C, temperatures compatible with evaporation times of a few minutes, well below the decomposition temperature close to 320°C. XPS results show that such evaporated films of DNA bases consist of intact molecules\textsuperscript{36,37}, which is also verified by thin film chromatography\textsuperscript{37}. The deposition rate of thymine molecules on the Pt surface is monitored and calibrated in situ by a quartz crystal microbalance (QCMB) in the load-lock. The sensitive crystal of the QCMB is vertically transferred in front of the opened molecular evaporator, at the same distance as between the molecular evaporator and the clean Pt surface to reproduce actual film deposition conditions on the target during calibration of molecules evaporation. The continuous molecule condensation at the piezoelectric surface induces a change in its resonance frequency which allow precise determination of the mass load\textsuperscript{38}.

Once a required evaporation rate achieved and stabilized, the molecular evaporator is transferred in front of the target into the main chamber for vacuum condensation of the molecules onto the Pt substrate. Thymine film thickness is determined by the evaporation rate, characterized in ng/min/cm\textsuperscript{2} in relation to the ~0.5 cm\textsuperscript{2} active crystal area. Assuming a uniform condensation, these quantities are converted to nominal monolayers by using the known density of thymine. This assumption however does not reflect actual thymine condensation which proceeds by cluster growth until it reaches full surface coverage in the 2-3 nominal monolayer regime\textsuperscript{39}. Films of 50-200 ng/crystal area which provide here the best thickness range for the measurement of ionic fragment desorption correspond thus roughly to 1-4 nominal monolayers. Before sample deposition, the Pt surface is bombarded with 500 eV Ar\textsuperscript{+} ions for several hours at room temperature followed by flash heating at about 1000 °C for 3 to 4 seconds before target molecule condensation. The mass spectra are collected
immediately after the condensation of the sample in the UHV experiment chamber. Charging effects have been found negligible on films below 800 ng/cm² in our studies⁴⁰.

8. Detection system

Desorbing species from the solid targets are mass analyzed by means of a high resolution quadrupole mass spectrometer (QMS) either during ion irradiation, or after by SIMS or thermal desorption- mass spectrometry (not presented here). The QMS entrance lens is 2 cm away from the center of the sample surface and is mounted at 90° to the mass-selected ion beam axis in the main chamber. With respect to the surface normal, the incident ion beam axis is at 60° and the QMS central axis at 30°. This configuration offers the best detection of secondaries, with a higher sensitivity ensured by the large solid angle of acceptance of the entrance lens of the QMS. The QMS has been purchased from Hiden Analytical Ltd. Its RF power supply has been modified by the manufacturer from an IDP standard system with mass range from 0.4-300 amu to 0.4-200 amu to give a better sensitivity and mass resolution at lower masses such as H and D. The QMS currently measures desorbed positive and negative ions with in vacuo energies between 0 and 5 eV.

The QMS can be operated under two modes: in the ion mass mode, the incident ion energy is fixed and mass spectra of desorbing positive and negative ions are obtained. In the ion yield mode, desorption energy thresholds are obtained by measuring the intensity of several specific ionic fragments as a function of incident ion energy.

With two filaments installed as part of an integral ionizer between the entrance ion optics and the quadrupole mass filter, the QMS can also be used for neutrals detection by fragmentation and ionization of neutral gas phase molecules via 4-150 eV electron impact ionization (positive or negative). For the moment, only cations yields can be recorded during electron impact, i.e. when the QMS is used in the RGA neutral detection mode; installation of
a RGA anion option may soon allow neutral detection in the RGA mode via 4 – 20 eV
dissociative electron attachment (however, in the ion stimulated desorption (ISD) mode the
ionizer is turned off, and both desorbing anions and cations are detected by the QMS).

The target needs to be in a field-free region at ground potential for compatibility to
probing instruments characterizing the beam-target interactions. To help ensuring an electric
field free environment around the target, the last lenses forming the end of the beam line
shown in fig. 5a are behind a grounded shield which can alternately be connected to a
variable potential. For the same purpose, another shield with the same possibilities hides the
entrance lenses of the QMS. An electric field free environment around the target would
constitute an idealistic situation. In practice, the switch of polarity of the QMS extraction
lenses voltage during the scan of positive and negative desorbing species slightly affects the
beam intensity reaching the target.

III. First results

1. Ion impact on metal surfaces

A separate target constituted of a technical Aluminum foil has been used to adjust the
focused mass-selected ion beam on the target with respect to the QMS axis. The technical Al
foil was cleaned with methanol and minor hydrocarbon surface contamination was easily
removed by ion impact. After sputter cleaning the only detectable contaminant is Na.

Fig. 8 shows energy thresholds for desorption of Na$^+$ and Al$^+$ ions induced by He$^+$,
N$^+$, N$_2^+$ and Ar$^+$ ion irradiation from the Al foil surface. Except for He$^+$, the maximum
sputtered Na$^+$ yield occurs at higher ion incident energy than that of Al$^+$, and the energy
position of the maximum of both yields increases as a function of incident ion mass. For all
Fig. 8 Desorption energy threshold of Na$^+$ and Al$^+$ induced by He$^+$, N$^+$, N$_2^+$, and Ar$^+$ impact on sputter cleaned Al.

Projectiles used here, sodium exhibits a lower desorption energy threshold than aluminum, and a greater yield for incident ion energies up to 230 eV for He$^+$, 50 eV for N$^+$ and 120 eV for both N$_2^+$ and Ar$^+$ irradiation. This could be explained both by the lower ionization potential of sodium\cite{41} and by the lower binding energy of sodium compared to aluminum: the weakly bound sodium can diffuse in the crystal and can be more easily sputtered out the surface of the Al foil. This is especially true during He$^+$ irradiation where the weakly bound sodium can be easily removed from the surface by the small amount of energy transferred upon light projectile impact. The desorbing Na$^+$ yield decreases with irradiation time but the signal reconstructs itself if irradiation is stopped, suggesting Na diffusion from within the Al foil (data not shown here).

Desorption energy thresholds for aluminum are observed to decrease as the projectile mass increases, except during N$^+$ irradiation where sodium has its lowest desorption energy
threshold. The different functional behaviors observed near desorption threshold energies (i.e. concave for Al⁺, convex for Na⁺) may be indicative of different sputtering mechanisms.

All yields in fig. 8 are observed to drop beyond 200 eV ion incident energy on average, which may indicate a decrease of the sputtering/ionization cross section. However, Na⁺ and Al⁺ ions desorbing at higher projectile energy will desorb with higher kinetic energy, and may thus escape from the QMS detection window, which can detect ions with KE up to 5 eV only. The signal drop may also be due to the projectile energy itself. High energy light projectiles will penetrate into the bulk more deeply, while heavy projectiles with lower incident energy will be stopped near the surface. Al⁺ and Na⁺ yields induced by He⁺ irradiation of the Al foil, when compared to Ar⁺ irradiation, support the latter hypothesis. At same energy, He⁺ can go deeper into the bulk than the heavier Ar⁺ ion.

The tendency observed for Al⁺ and Na⁺ desorption, in particular during He⁺ impact, may lead to an interesting development of nondestructive SIMS performed at low energy. Here, with current intensities of several nA, sputtering of sodium is observed with high yield, accompanied with a relatively low, or no Al⁺ yield, for energies below 50 eV for N⁺ and N₂⁺. This can be extended to higher energies in the case of He⁺ irradiation, which is more sensitive to sodium than aluminum below ~200 eV. High sensitivity towards the contaminants without damage to the surface could make low energy SIMS a quite efficient but nondestructive technique.

2. Ion impact on organic films

A typical ISD mass spectra of positive and negative ions obtained with our apparatus with a thymine film of 100 ng irradiated with an Ar⁺ beam of 200 eV is displayed in fig. 9. Spectra showing simultaneous desorption of positive and negative fragments are the best characterization of the interaction processes occurring in the condensed sample film under
irradiation. The abundant positive and negative fragments are identified by their relative yield as a function of their m/q ratio as detected by the QMS during irradiation of the thymine film.

While chemical identification of the desorbing fragments is discussed elsewhere, it is achieved by uses of isotopically substituted thymine (e.g. deuterated thymine). The major cations are $\text{H}^+$, $\text{CH}_x^+$ $(i=[0, 3])$, $\text{HNCH}^+$, $\text{C}_3\text{H}_3^+$, $\text{OCNH}_2^+$, $\text{HNC}_2\text{H}_4^+(\pm\text{H})/\text{OC}_3\text{H}_3^+(\pm\text{H})$, $[\text{T-OCNH}]^+$, $[\text{T-O}]^+(\pm\text{H})$ and $[\text{T+H}]^+$, while the major negative ions are identified as $\text{H}^-$, $\text{O}^-$, $\text{CN}^-$, $\text{OCN}^-$ and $[\text{T-H}]^-$. Desorbing ionic species can be distinguished as originated from endocyclic or exocyclic bond dissociation of the thymine molecule. In this manner, $\text{HNCH}^+$, $\text{C}_3\text{H}_3^+$, $\text{OCNH}_2^+$, $\text{HNC}_2\text{H}_4^+(\pm\text{H})$, $[\text{T-OCNH}]^+$, $\text{CN}^-$, and $\text{OCN}^-$ probably originate from endocyclic thymine bond breaking while $\text{H}^+$, $\text{CH}_x^+$, $[\text{T-O}]^+(\pm\text{H})$, $\text{H}^-$, and $\text{O}^-$ can result from exocyclic bond dissociation of the thymine molecule.

![Fig. 9 Positive and negative ISD mass spectra from 200 eV Ar$^+$ irradiated 100ng thymine film. Ar$^+$ beam intensity is 18 nA](image_url)
It is interesting to note that the positive fragments mass spectrum of a thymine film irradiated by 200 eV Ar$^+$ ions is dominated by endocyclic fragments. The damage caused to the thymine molecule can then be identified by the endocyclic and exocyclic bond breaking and can be further investigated by looking at the energy dependence of the bond dissociation. Fig. 10 shows ISD energy thresholds of four typical positive fragments desorbing from 100 ng thymine films under Ar$^+$, Ar$^{++}$, and D$_3^+$ ion impact. Exocyclic bond dissociation being

Fig. 10 ISD energy thresholds of CH$_3^+$, HNCH$^+$, C$_2$H$_5^+$, and HNC$_3$H$_4^+$ under Ar$^+$, Ar$^{++}$, and D$_3^+$ ion impact on 100 ng thymine films.
typically characterized by subtraction of H or CH$_3$ fragments, CH$_3^+$ is representative of exocyclic bond dissociation while HNCH$^+$ and HNC$_3$H$_4^+$ are indicative of endocyclic bond breaking of the thymine molecule.

Except under D$_3^+$ ion impact, where CH$_3^+$ is the last to appear around 25-35 eV, HNCH$^+$ and HNC$_3$H$_4^+$ both appear at lower energies near 15-20 eV under Ar$^+$ and Ar$^{++}$ irradiation. This shows that endocyclic bond dissociation occurs at lower energies than exocyclic bond dissociation under heavy ion impact (Ar$^+$, Ar$^{++}$, and also N$_2^+$, not shown) as opposed to higher energies under light ion impact (D$_3^+$, similar to D$^+$ and D$_2^+$ not shown), indicating that heavy primary ions cause endocyclic damage more efficiently than light primary ions. The observed increase of the fragment desorption threshold energy as the incident ion mass decreases also tends to indicate that heavy ions cause more severe damage to DNA bases than light ions, observation that are confirmed elsewhere (see chapter 6).

IV. Summary

If much is known about DNA damage initiated by low-energy secondary electrons, much less is known about DNA damage induced by the secondary ionic species. To remedy this, we have developed an UHV apparatus designed to perform studies of low-energy reactive ion-surface interactions in the 0.5 – 500 eV energy range. The system consists of four differential pumping stages with ion optics that ensure beam uniformity, a low energy spread of 0.8–1.5 eV and high ion selectivity, achieved by a specially designed magnetic mass analyzer. Ions produced in the source yield beams that can reach intensities between 5–200 nA depending on ion species. Beam intensities are constant (± 5% at worst) over the 500–10 eV incident energy range for all ions with a good stability over the day. Experiments are performed on biomolecular films produced by in vacuo evaporation below 90°C and
condensed on a clean Pt substrate at ambient temperature, assisted by a quartz crystal micro-
balance monitoring with a 0.5 ng.cm⁻² precision, before being submitted to various ion
irradiations. First results of fragmentation induced by 200 eV Ar⁺ ion impact on 2-4 nominal
ML thymine films reveal abundant positive and negative desorbing fragments indicative of
severe damage to DNA bases caused by ion impact at low energies. Future developments
will consist in modifying the load-lock system to irradiate samples prepared outside the ion
beam system, thus extending the sample range to self-assembled ML, other DNA
components, or DNA. Extension of the projectile range to more reactive or negative ions to
investigate the radiobiological damage caused by these ions is also considered.

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CHAPTER 3 – Low Energy Electron and O’ Reactions in Films of O₂ Coadsorbed with Benzene or Toluene

3.1 Introduction

While reactive scattering by low energy ions (such as those produced by DEA) has been well explored in the gas phase (Massey, 1976; Champion and Doverspike, 1984), few such studies exist in the condensed phase (Sanche and Parenteau, 1990; Azria et al., 1990; Kimmel et al., 1994; Lacombe et al., 1997). Here we present measurements of reactive scattering of O⁻ ions formed by DEA to O₂ initiated by 0-20 eV electron impact on O₂-C₆H₆/C₆H₅CH₃ solids. This work is presented in the following article entitled “Low Energy Electron and O’ Reactions in Films of O₂ Coadsorbed with Benzene or Toluene”, by Marjorie Imhoff, Luc Parenteau, Leon Sanche, and Michael A. Huels. This article is in press in the journal Physical Chemistry Chemical Physics.
3.2 Low Energy Electron and O\textsuperscript{\scriptsize{2}} Reactions in Films of O\textsubscript{2} Coadsorbed with Benzene or Toluene

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Abstract

A detailed understanding of nascent reactive events leading to DNA damage is required to describe ionizing radiation effects on living cells. These early, sub-picosecond events involve mainly low energy (E < 20eV) secondary electrons (SE), and low energy (E < 5eV) secondary ion (and neutral) fragments; the latter are created either by the primary radiation, or by SE via resonant mechanisms, i.e. dissociative electron attachment (DEA). While recent work has shown that SE initiate DNA strand break formation via DEA, the subsequent damage induced by the DEA ion fragments in DNA, or its basic components is unknown. Here, we report 0 – 20 eV electron impact measurements of negative ion desorption from condensed films containing O2 and either Benzene (C6H6), or Toluene (C6H5CH3); these molecules represent the most fundamental structural analogs of pyrimidine bases. Our experiments show that all of the observed OH− yields are the result of reactive scattering of 1–5 eV O− fragments produced initially by DEA to O2. These O− reactions involve hydrogen abstraction from Benzene or Toluene, and result in the formation of Benzyl radicals, or Toluene radicals centered on either the ring or exocyclic methyl group. O− scatters over nm distances comparable to DNA dimensions, and reactions involve a transient anion collision complex. O− scattering and reactions are found to depend on both, the temperature of hydrocarbon film formation (morphology), and the order of overlayer adsorption, e.g. O2 on Benzene, or Benzene on O2. Our measurements support the notion that in irradiated DNA similar secondary-ion reactions can be initiated by the abundant secondary electrons, and may lead to clustered damage.
1. Introduction

Secondary electrons (SE) are produced abundantly \((5 \times 10^4 \text{/MeV})^{1,2}\) along radiation tracks with initial kinetic energies well below 20 eV\(^{3,4,5}\). Along the tracks they produce ions and radicals with a yields different from those produced by the primary particle, due to the fact that multiple scattering is no longer negligible at low electron energies. Molecular orbitals become highly perturbed by slow electrons, hence allowing exchange interactions and multiple electron scattering. These perturbations leads to localized phenomena called resonances, i.e. the formation of transient negative ions (TNI). Depending on its lifetime, the TNI has several accessible decay channels, one being dissociative electron attachment\(^{6,2}\) (DEA), which leads to the production of a stable anion and a neutral radical. SE energy losses have been shown to be often dominated by resonant process in condensed media. Anions and neutral radicals formed by DEA can have energies up to a few eV, and thus can react rapidly with the surrounding medium by forming new chemical species\(^7\). Hence, it is crucial to characterize subsequent ultra-fast reactive events which result from the interactions between the TNI and the surrounding matter.

Although much is known about initial radiation track formation, as well as biochemical damage, even at times as short as \(10^{-9} \sim 10^{-11} \text{ s}\), very little is known about the sequences of reactive ion-induced events that occur on picosecond timescales following the initial energy deposition, but before diffusion of slow reactive species begins (near \(10^{-12} \text{ s}\)). We know however that interactions between DEA fragments and adjacent DNA constituents are very likely to occur. This has been suggested by a recent study that reported single and double strand breaks in low energy electron irradiated DNA, that were attributed to DEA\(^8\), and implied the involvement of DEA fragment reactions. The fact that SE, and most likely
ionic and radical species are involved in nascent DNA damage underlines the importance of understanding the mechanisms of such events.

The present study concerns interactions between condensed phase molecules and radical ionic species resulting from DEA. In the present experiments we induce ion formation by DEA within a condensed film bombarded with a low energy electrons. This method has the advantage that the reactive DEA anion products are formed in proximity to their prospective targets. Here we use DEA to O₂ that has the advantage of being well characterized as function of incident electron energy⁹ and resulting O⁻ kinetic energies¹⁰. O⁻ is also a radical anion produce by DEA in DNA¹¹, and is therefore our first choice to study post-DEA ion reactions. Here O⁻ are formed in heterogeneous films of condensed benzene (C₆H₆) and toluene (C₆H₅CH₃) coadsorbed under or over several O₂ monolayers (ML). We analyze electron induced dissociative processes within the films by detecting desorbing O⁻ and H⁻ yields from pure O₂ and hydrocarbon films, and from heterogeneous films formed by coadsorption of the two. Reactive scattering is observed by detection of desorbing OH⁻ resulting from a hydrogen abstraction reaction initiated by O⁻ within the films. Finally, using partially deuterated toluene (C₆H₅CD₃), we investigate the site-specificity of ion-molecule interactions.

2 Experimental Method

The electron stimulated desorption (ESD) apparatus used for the experiments has been discussed in detail elsewhere¹²¹³⁶. All the components are placed in an ultra high vacuum (UHV) system held at 10⁻¹⁰ Torr. The UHV chamber is μ-metal shielded to eliminate stray magnetic fields. Films are grown by vapor deposition: the molecular vapors are transferred into the chamber by a gas doser, and consense on a Pt substrate maintained at
a temperature of 20 K by means of a closed-cycle cryostat. The thin polycrystalline Pt foil is electrically isolated, and is cleaned by resistive heating to 1000 K. The volumetric dosing procedure\textsuperscript{14} allows an estimation of the deposited film thickness with a precision of ± 30% and a reproducibility of ± 0.2 ML. The condensed films are irradiated by an incident 0-20 eV monochromatic electron beam produced by a high-resolution electron monochromator. The electron beam has an intensity of 4 nA, a diameter of about 2 mm and an estimated resolution of 80 meV full width at half maximum (FWHM). The electronic impact on the solid stimulates the formation of negative ions that are either able to desorb directly or react with surrounding molecules prior to desorption. They can also become trapped inside the film. Anions that are able to desorb (i.e. overcome the charge induced polarization potential in the film) are detected by a quadrupole mass spectrometer(QMS). The QMS can be operated in three modes: (1) record the mass spectrum of the desorbing fragments at a fixed incident electron energy, (2) record the desorption yield of a particular fragment as a function of the incident electron energy, and (3) measure the kinetic energy distribution of specific desorbing anions by applying a variable retarding voltage to two grids inside the QMS entrance optics \textsuperscript{10}. The resulting ion yield functions from (2,3) are repeatable to within 5% with an estimated experimental uncertainty of about 12%.

The electrically isolated Pt substrate also acts as an electron collector. Measuring the onset of the transmitted electron current through a film during irradiation allows us to calibrate the electron energy scale to within ± 0.3 eV with respect to the vacuum level (E_{vac} = 0 eV). Changes in the onset are also representative of the charging of the films by trapped or charges during the experiments\textsuperscript{15}. This allows us to verify that all data reported here was obtained from essentially charge free films. While O\textsubscript{2} diffusion in hydrocarbon films is generally slow, ESD anion yields may change slightly due to internal diffusion of the target
molecules through the films; thus each anion yield curve is obtained immediately after film deposition on the substrate. This also minimizes contamination of the surface by background gases.

The stated purity of O₂ is 99.998%. Benzene and toluene samples have a minimum purity of 99%. Both are liquid at room temperature and were purified by several freeze-pump-thaw cycles prior to deposition. Measurements of desorbing species and the calibration of film thicknesses, given in ML, are performed at 20 or 27 K. To investigate different hydrocarbon film morphologies, films were also condensed at higher temperatures, and then cooled to 20 – 27 K for the experiments with adsorbed O₂. Other hydrocarbon films were irradiated with electrons at the higher temperature at which they were condensed. The film temperature is monitored by a chromel-gold thermocouple connected to the cryostat at a distance of about 1 cm to the film surface. The temperature of the Pt substrate is estimated to be about 2 or 3 K higher than the thermocouple value.

3 Results and Discussions

3.1 DEA in homogeneous films: pure O₂ and toluene films

DEA to O₂ proceeds via the formation of a transient negative ion (TNI) O₂⁻* formed by the temporary capture of an incident electron, viz.

\[ \text{e}^- + O_2 \rightarrow O_2^{-*} \rightarrow O^- + O \quad (1) \]

Depending on its electron autodetachment lifetime (\( \tau_A \)) the O₂⁻* TNI can decay by several decay channels and has to fulfill several requirements to decay via DEA\(^{16} \): (i) \( \tau_A \) has to be at least of the order of a vibrational period of the molecule (10\(^{-13}\) – 10\(^{-14}\) s), to give the O₂⁻* time to dissociate before autodetachment can occur, (ii) the TNI has to be dissociative in the Franck-Condon region of the ground state neutral molecule, and (iii) one of the two possible
fragment has to possess a positive electronic affinity. Among the many TNI states that can be formed only a few of satisfy all the requirements to yield dissociating $O + O^+$.  

In the gas phase, only one transient state is involved in $O^-$ formation via DEA to ground state $O_2$ ($^3\Sigma_g^-$): the $O^-$ yield exhibits a single peak centered on about 6.7 eV that is attributed to the $^2\Pi_u$ transient state correlated to the $O^- (^3P) + O(^3P)$ dissociation limit. In the condensed phase, adjacent molecules can lead to the loss of the $O_2$ cylindrical symmetry$^{17}$. Thus, in the condensed phase high-density environment, gas phase forbidden $O_2$ transitions, e.g. ($^3\Sigma_g^-$) to ($^1\Sigma^+$) states, can then be observed$^{18}$. It has been shown that the $O^-$ yield signature seen in the $O^-$ yield from pure condensed $O_2$ films in Figure 1a essentially involves three distinct $O_2^{*-}$ symmetries: the gas phase $^2\Pi_u$, and the $^2\Sigma_g^+$ and $^3\Sigma_u^+$ states. This leads to $O^-$ formation peaks near 8 - 9 eV, and 13.5 eV as shown in Figure 1a. The $^2\Pi_u$ and $^3\Sigma_u^+(I)$ $O_2^{*-}$ transient states are both correlated to the $O^- (^3P) + O(^3P)$ dissociation limit whereas $^2\Sigma_g^+(I)$ evolves non adiabatically to the $O^- (^3P) + O(^1D)$ dissociation limit via a curve crossing with another $O_2^{*-}$ transient state of same symmetry ($^2\Sigma_g^+(II)$). The continuous increase of the $O^-$ ion yield above 17 eV is in part due to dipolar dissociation, a non-resonant ion pair formation mechanism: $e^- + O_2(^3\Sigma_g^-) \rightarrow (O_2)^* + e^- \rightarrow O^+ + O^- + e^-$. However, electron energy loss (EEL) to $O_2$, followed by DEA (to the low lying $O_2$ resonances) also occurs at E(e) above 11 eV, and has been shown to dominate the $O^-$ ESD yields at these energies$^{10a}$.

For the dissociation of a parent $O_2^{*-}$ into $O^- (^3P)$ and $O(^3P$ or $^1D$) in the condensed phase, the kinetic energy ($E_K$) of the ion after its desorption from the film is given by$^{12}$:

$$E_K (O^-)_{\text{film}} = \frac{1}{2} (E_o - E^* - E_d + E_A(O) + E_p) - E_p \quad (2)$$

where $\frac{1}{2} = (1-\beta)$, with $\beta = m(O)/m(O_2)$. $E_o$ is the incident electron energy and $E^*$ is the total internal energy of the neutral oxygen atom following dissociation. $E^* = 0$ eV for the first dissociation limit $O^- (^3P) + O(^3P)$ and 2 eV for the second dissociation limit $O^- (^3P) + O(^1D)$.  

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$E_d = 5.08 \text{ eV}$ is the $O_2$ bond energy, and \( E_{A(O)} \), the electron affinity of $O$ is $1.46 \text{ eV}$. The polarization energy $E_p$ of the surrounding $O_2$ molecules induced by the $O_2^{*}$ TNI, and is estimated at $0.8 \text{ eV}$; it also represents the polarization barrier that must be overcome by the $O^{-}$ fragment to escape the film. EEL of the incident electron prior to DEA and losses of kinetic energy by $O^{-}$ due to inelastic collision processes prior to desorption have been neglected. We also make the implicit assumption that the polarization energy of the film could be reduced to an average value $E_p$ for the whole film, both in its bulk and at the surface, independently of some possible morphology effects.

The DEA fragments which desorb possess kinetic energies $E_k > E_p$; for condensed phase $O_2$, and at $5 < E(e) < 16 \text{ eV}$, the \textit{in vacuo} kinetic energy distributions of $O^{-}$ from DEA to $O_2$ are generally characterized by\cite{10}: (1) a most probable kinetic energy, $E_k(\text{mp})$, that varies between 0.25 and 3.5 eV, and (2) a maximum possible kinetic energy, $E_k(\text{max})$, that varies between 1 and 5 eV. Thus, near the surface (prior to desorption or reactions) the $O^{-}$ kinetic energies are larger by an average $E_p$ ($\approx 0.8 \text{ eV}$ for the disordered $O_2$ films condensed here at 20 K). This is demonstrated in figure 1, which shows $O^{-}$ yield functions for different retarding voltages ($V_R$) applied to the QMS lens grids: as $V_R$ is increased so does the yield function signature change due to the fact that only desorbing $O^{-}$ with an \textit{in vacuo} energy above $V_R$ are able to enter the QMS. This has been shown to relate to the various $O_2^{*}$ states contributing to the DEA process\cite{10}. Further ESD measurements from $O_2$ adsorbed on thick CH$_4$ and C$_2$H$_6$ substrates\cite{19} show that the $O^{-} E_k$ distributions are not strongly affected by the presence of these hydrocarbons, other than the slightly higher average $E_p$ ($\approx 1 \text{ eV}$ for hydrocarbons)\cite{20}.

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**Fig. 1** O⁺ electron stimulated desorption (ESD) yields from 5 monolayer (ML) films of pure O₂ condensed at 20 K, as functions of incident electron energy. (a) ESD O⁺ yield without retarding voltage ($V_R$) applied to the Quadrupole Mass Spectrometer grids, and (b) ESD O⁺ yields for $V_R = -1$, -1.3, and -1.5 V.

DEA to Toluene has not been previously reported, but is found to be similar to DEA in Benzene\(^{12, 21}\). Toluene is a benzene derivative formed by the addition of a methyl group on the aromatic ring structure. Comparing DEA anion yields obtained from pure films of benzene and toluene can help to estimate the influence of the methyl group on the resonant electron attachment process. H⁻ ESD yield measurements from benzene exhibit a single DEA peak centered at 8.2 eV with a threshold near 6.8-7 eV, resulting from the formation of a (C₆H₆)⁻⁺ TNI:

$$e^- + C_6H_6 \rightarrow (C_6H_6)^{*-} \rightarrow H^- + C_6H_5^*$$ \hspace{1cm} (4)

DEA to toluene (Figure 2) can lead to DEA not only via ring C-H bond cleavage but also via dissociation of exocyclic C-H bonds, part of the aliphatic methyl group, i.e:
\[ e^- + C_6H_5CH_3 \rightarrow (C_6H_5CH_3)^* \rightarrow H^- + \cdot C_6H_4CH_3 \]  \hspace{1cm} (5)

\[ \rightarrow H^- + C_6H_5CH_2^+ \]  \hspace{1cm} (6)

The \( H^- \) yields for toluene displayed on the top curve of figure 2 show a maximum centered on about 9 eV and, like benzene, an onset near 7 eV. For both toluene and benzene, all features below 15 eV are characteristic of DEA to the physisorbed molecules, and the steep rise of \( H^- \) yield with an energetic threshold near 14.5 eV is attributed to DD of ring or exocyclic C-H bonds, and multiple scattering prior to DEA. Anion yields for benzene and toluene show a strong dependence on the incident electron energy \( E_o \) and appear essentially similar in structure and intensity. For the deuterated molecules, experiments conducted on

**Fig. 2** ESD \( H^- \) yields from 8 ML thick toluene (\( C_6H_5CH_3 \)) films as functions of incident electron energy for different film condensation temperatures.
condensed C₆D₆ films¹² show a single D⁻ DEA peak centered at 8.74 eV, an increase of about 0.5 eV compared to the H⁻ peak from a C₆H₆ film but with an identical energy threshold near 7 eV. Two H⁻ contributions are found here in the total H⁻/D⁻ yields from experiments on partially deuterated toluene (C₆H₅CD₃ - not shown). The H⁻ signal arising from DEA to the ring structure of the partially deuterated toluene molecule gives a peak at about 8.7 eV while DEA to the methyl group resulted in a D⁻ resonance structure centered at about 9.3 eV. The superposition of these two resonances gives rise to the total H⁻ yield signature from C₆H₅CH₃ shown in Figure 2. A similar superposition of endocyclic and exocyclic H⁻ contribution has been observed in the ESD signature of partially deuterated aniline²², a benzene derivative formed by addition of an amino group to the benzene ring.

The other curves in Fig. 2 show H⁻ yields from several pure 8 ML toluene films, each deposited at different temperatures up to 142K. As the film deposition temperature increases the characteristic desorption signature is conserved, while the intensity varies. Similar changes were observed in the D⁻ signal versus incident electron energy collected at various temperatures between 27K and 140K from 20-bilayer porous amorphous ice films²³. The magnitude changes of the desorbed signal are related to the morphology of the film which depends on its deposition temperature. The temperature at which the films are deposited also controls the surface roughness, i.e. the porosity and the structural state of hydrocarbon films, which can considerably affect the outcome of ESD measurements.

The H⁻ yield temperature dependence is summarized in figure 3, which shows the integral H⁻ DEA signal (from 6 – 12 eV) versus the measured temperatures of the 8 ML toluene films in Fig. 2. The DEA peak signal increases with the film temperature until a plateau is reached between 90K and 125K. As the temperature increases beyond 125 K, the H⁻ intensity drops rapidly down to 150K. This behaviour in cyclic hydrocarbon films is
Fig. 3 Integrated ESD H\textsuperscript{-} yields for the 8 ML films of toluene in Fig. 2; here the H\textsuperscript{-} yields have been integrated across the 6 – 12 eV DEA resonances of toluene – see text.

related to the film morphology\textsuperscript{21}: here molecules condensed at 24K, i.e. well below their sublimation temperature, are randomly oriented with a amorphous-porous film structure, characterized by high surface area and low density due to numerous voids and pits. As the film deposition temperature is increased, the film gains in density, displaying an amorphous non-porous structure, before entering a crystalline phase characterized by ordered molecules and an absence of pores. Here, this is believed to occur above 90K. The observed H\textsuperscript{-} yield increase as the toluene temperature is raised from 24K up to 90K is likely related not to a change of morphology (amorphous) but to a change of porosity inside the film, before its transition to the crystalline phase occurs, such that the higher molecular density associated with the collapse of the pores leads to the observed increase of the signal. However, for a same condensed quantity of toluene, the incoming electron is likely to suffer a similar number of collisions irrespective of the fact that it scatters within a high or low-porosity film\textsuperscript{24}. The incoming electron can penetrate many ML of a film\textsuperscript{25} but anions, generated by electronic impact via DEA, have shorter reaction mean free paths\textsuperscript{36}. When the film is less
porous, developing a denser structure, more electron interactions occur at or near its surface, thus making ions less likely to scatter. The small DEA H⁻ yield decrease observed between 90 - 130K may be due to a molecular rearrangement at the film-vacuum interface that reduces the DEA cross section and/or the desorption probability, while the rapid H⁻ signal drop observed above 130K is related to toluene evaporation.

In addition to deposition temperature, film morphology has also been shown to strongly depend on the angle of the gas doser nozzle, relative to the surface normal. Films grown with a D₂O beam striking a Pt surface at an angle of 85° with respect to the surface normal will present a porosity twice that of films grown at a 45° angle. It was shown that pore formation may result from a columnar structure developed by the molecules of the film from the early stages of its formation. For small angles of incidence of the particles from the vapor phase molecules tend to accumulate where they initially hit the film, forming little peak structures. Those peaks generate small shadow areas, blind zones for following incident particles according to a “hit and stick” picture that leads to the formation of relatively small pores. The shadow areas grows with the angle of incidence. This shadowing effect, associated with a low molecule mobility at low condensation temperatures, then adds height to the peaks, and thus widening the gap between peak and valley structures which leads to formation of larger pores.

The condensation of benzene and toluene films grown in the present experiments at 20 - 27 K is close to beam diffusion as a tube carries the molecular vapor to the cryogenic surface at an angle of 70° with respect to the surface normal. In contrast, O₂ at 20-27K is close to sublimation temperature; on the polycrystalline Pt substrate used here this results in disordered non-porous O₂ films.
3.2 **DEA and O' reactions in heterogeneous films: O₂ on benzene substrates**

Hydrocarbon substrate morphology effects on DEA to coadsorbed O₂ are shown in Figure 4 which show O' yields obtained from heterogeneous films composed of various quantities of O₂ adsorbed on 5 ML benzene films condensed at a temperature of about 27K, and 80K, respectively. Benzene films condensed at 27K display an extremely porous structure whereas those condensed at 80K are expected to be crystalline. Fig. 4a shows the evolution of the O' yield with an increasing quantity of O₂ deposited on the amorphous-

Fig. 4 ESD O' yields as functions of incident electron energy for variable quantities of O₂ deposited at 27 K on (a) 5 ML thick benzene (C₆H₆) films condensed at 27 K, and (b) 5 ML thick benzene films condensed at 80 K and cooled down to 27 K. Shown in (c) is the OH yield obtained from 3.6 ML of O₂ deposited at 27 K on a 5 ML benzene film also condensed at 27 K.
porous benzene film; it can be seen that only for high O₂ coverage will the DEA O⁻ yield signature resemble that obtained with pure O₂ films in Fig. 1. Although in Fig. 4a the O⁻ yield at 20 eV is somewhat reduced relative to the intensities at the two peaks⁵¹, we find that the basic resonance signature of DEA to O₂ is essentially unchanged relative to a pure O₂ film (as are the KE distributions¹⁹ of O⁻ from DEA).

We also note in Fig. 4a that when the O₂ coverage is decreased down to 0.5 ML the DEA O⁻ yield maximum shifts from 8 eV to about 6.5 eV, suggesting a greater contribution from the gas phase ²Πₒ O₂⁻* state. This seems to indicate that for sub-ML O₂ quantities the molecules infiltrate into its pores, and thus have a very low surface density. This phenomena is not observed for sub-ML O₂ coverage on a crystalline benzene film as seen Figure 4b. Here, as the quantity of adsorbed O₂ increases, they start to accumulate at the film surface and quickly form an O₂ ML. Above 2 ML of O₂ on crystalline benzene the DEA O⁻ yield signature strongly resembles that from a pure multilayer O₂ film (Fig. 1) or that for 6.6 ML O₂ on the amorphous-porous C₆H₆ film (Fig 4a). The higher the amorphous-porous benzene film thickness, the higher is the amount of O₂ needed to fill the pores. By comparing O⁻ yields in Fig 4a and b we find that it takes about 2.5 times more O₂ molecules on the amorphous-porous benzene film to reproduce the O⁻ signal obtained with the crystalline film for an O₂ coverage of 2.6 ML. Thus, while O₂ will accumulate on a crystalline benzene substrate, when adsorbed on an amorphous-porous benzene film O₂ molecules migrate into the bulk, accumulate in the pores and hence reducing the O₂ density at the film-vacuum interface. Thus the O⁻ yield increases slower with O₂ coverage on amorphous-porous compared to crystalline benzene films. 

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The above is in agreement with the changes seen in the H$^-$ yields from amorphous-porous and crystalline films of benzene covered with O$_2$. This is in shown in Figure 5, where the integral (6-12 eV) DEA H$^-$ signal from 5 ML films of benzene is measured as a function of amount of O$_2$ condensed (at 27 K) on benzene substrates prepared at 27 and 80 K. For O$_2$ adsorbed on a crystalline (80K) benzene substrate it takes only 0.5 ML of O$_2$ to attenuate the H$^-$ signal to its 1/e intensity relative to a pure benzene film, while for an amorphous-porous (27K) benzene substrate it takes about 2.1 ML of coadsorbed O$_2$.

A comparison of the O$^-$ and H$^-$ yield changes from these films suggests a competition between O$^-$ and H$^-$ formation, since the increasing O$_2$ coverage of the hydrocarbon substrates leads to an increase of the O$^-$ yields (Fig. 4) and a decrease of the H$^-$ yield: infiltration and accumulation of a considerable fraction of the O$_2$ molecules into the porous allows the presence of both type of molecules at the film surface, thus still keeping some hydrocarbon molecules available for DEA. This is in fact similar to pre-mixed O$_2$/hydrocarbon films that are formed by adsorbing a mixture of molecules$^7$. As O$_2$ thickness
increases, and the pores fill up, the coverage of the film surface by \( O_2 \) molecules also increases and leads to the more gradual decrease of the \( H^+ \) yield, and a corresponding gradual increase of the \( O^- \) yield (Fig. 4a). Conversely, the \( H^+ \) yield obtained from increasing amounts of \( O_2 \) condensed on the crystalline hydrocarbon substrate decreases at a much faster rate, since the \( O_2 \) molecules immediately start to accumulate at the surface, and a smaller \( O_2 \) quantity is required to achieve a complete coverage of the crystalline hydrocarbon film. This is then associated with a much faster increase of the \( O^- \) signal (Fig. 4b).

\( O_2 \) coverage as well as the proximity to the underlying Pt substrate may account for the significantly lower \( O^- \) yield for low \( O_2 \) coverage of the amorphous-porous benzene films. As \( O_2 \) molecules infiltrate into the porous \( C_6H_6 \) film, \( O^- \) ions produced via DEA to \( O_2 \) inside the pores form closer to the Pt metal surface. Proximity with the Pt substrate has an effect on both the \( O^- \) ion and the \( O_2^- \) resonant state as it can be responsible for the quenching of the latter (electron transfer to the metal or polarization induced by hydrocarbon molecules which affects the resonance lifetime).\(^{32}\) \( O^- \) ions formed closer to the metal surface also experience a greater image force potential, which reduces their desorption probability. \( O^- \) formed close to the metal surface need more KE in order to desorb which may explain why the intensity of the 13 eV peak, yielding higher energy \( O^- \), is similar to that of the 6 to 8 eV peak for low \( O_2 \) coverage in Fig. 4a. In addition to the effect of the proximity of the metal surface, \( O^- \) ions formed within the porous benzene film can also suffer inelastic collisions within the film prior to desorption. Those collisions reduce the ion KE and hence the observed \( O^- \) yield by lowering the \( O^- \) desorption probability, and may also lead to reactions.

This is demonstrated in Fig 4c which shows \( OH^- \) yields from 3.6 ML \( O_2 \) deposited on an amorphous porous \( C_6H_6 \) film, as the one displayed in Fig. 4a. The \( OH^- \) results from reactive scattering\(^7\) within the benzene film via the following reaction pathway:
\[ O^- + C_6H_6 \rightarrow (C_6H_6O)^- \rightarrow OH^- + C_6H_5^* \] (7)

O\(^-\) ions produced by DEA to O\(_2\) react with adjacent C\(_6\)H\(_6\) molecules, forming a negative transient molecular complex that can decay via several pathways, including hydrogen abstraction leading to formation of OH\(^-\) ions. Similar O\(^-\) reactions have been observed in films of linear hydrocarbons\(^7\), aniline\(^{22}\) and tetrahydrofuran\(^{33}\). OH\(^-\) formation is attributed to a hydrogen abstraction via eq. 7, in part because: (1) the OH\(^-\) yield signature more closely resembles the yield of O\(^-\) ions desorbing from a pure O\(_2\) film with KE \(\geq 1.5\) eV (see Fig. 1b) than that of H\(^-\) ions resulting from DEA to the pure hydrocarbon film, (2) the retarded O\(^-\) yield (Fig.1b) and OH\(^-\) yield have similar energy thresholds near 5.5-6 eV, which is below the 6.8-7 eV H\(^-\) desorption onset from benzene (or toluene), and (3) H\(^-\) + O\(_2\) low energy scattering does not yield OH\(^-\) in the gas phase, where the only observed anion product is O\(_2\)\(^-\) \((^2\Pi_g)^{34}\). Although HO\(_2\) has a positive electron affinity (EA) of about 1 eV\(^{35}\), stable HO\(_2\)\(^-\) cannot be formed in gas phase H\(^-\) + O\(_2\) scattering, since the reaction exothermicity cannot be dissipated. Here in the condensed phase, HO\(_2\)\(^*\) may in principle still be formed (in some predissociative state), since the surrounding medium could absorb some of the exothermicity. However, in that case any OH\(^-\) formed by autodissociation of such HO\(_2\)\(^*\) would bear the signature of H\(^-\) formation via DEA (seen in Figure 2b - top curve), which is completely different than the actual OH\(^-\) signature observed here. Similarly, experimental conditions here rule out DEA to HO\(_2\) by subsequently arriving electrons as a source of OH\(^-\)\(^{33}\).

While the OH\(^-\) yields in Fig. 4c are low, they are extremely repeatable, and are a result of the experimental fact that for O\(_2\) adsorbed on porous films, most of the O\(_2\) resides within the pores, and only a small quantity is found at the surface. Also, most of the OH\(^-\) formed within the film’s pores must scatter out to desorb and be detected, which will lower
their survival probability in the film. For O\textsubscript{2} on crystalline benzene films (Fig. 4b), only very small amounts of OH\textsuperscript{-} are observed. This is due to the fact that in this case O\textsuperscript{-} must scatter into the crystalline hydrocarbon film, and backscatter as OH\textsuperscript{-} through the O\textsubscript{2} overlayer, which will drastically reduce its desorption probability.

This is demonstrated in Figure 6, which shows the OH\textsuperscript{-}/O\textsuperscript{-} yield ratios at an electron energy of 13 eV from increasing amounts of O\textsubscript{2} adsorbed at 27 K on 5 ML crystalline (prepared at 90K and cooled to 27K) and amorphous-porous (prepared at 27 K) benzene substrates.

![Graph showing the OH\textsuperscript{-}/O\textsuperscript{-} yield ratio at 13 eV incident electron energy for variable quantities of O\textsubscript{2} deposited at 27 K on 5 ML thick benzene films condensed either at 27 K, or at 90 K and cooled down to 27 K.](image)

**Fig. 6** OH\textsuperscript{-}/O\textsuperscript{-} yield ratio at 13 eV incident electron energy for variable quantities of O\textsubscript{2} deposited at 27 K on 5 ML thick benzene films condensed either at 27 K, or at 90 K and cooled down to 27 K.

We find that the OH\textsuperscript{-}/O\textsuperscript{-} ratio decreases for both hydrocarbon substrate morphologies as the O\textsubscript{2} coverage thickness increases. However a significantly more gradual diminution of the OH\textsuperscript{-} ion yield is observed when O\textsubscript{2} is adsorbed on the amorphous-porous benzene
substrate. Here, the OH\(^-\) yield is believed to be produced by both: (a) the O\(^-\) that originates within the film pores and, on a trajectory towards the vacuum, scatters from benzene in the bulk, or at the surface, and emerges as OH\(^-\), and (b) the small amounts of O\(^-\) are formed at the film surface and react with benzene prior to desorption as OH\(^-\). As the total film thickness and density increases (as the pores fill with O\(_2\)), it reduces the ion mean free paths and prevents them from desorbing. Because OH\(^-\) formed in the bulk of the porous film have a higher probability to suffer inelastic collisions prior to desorption as the film thickness and density increases, they have a lower desorption probability than OH\(^-\) formed at the surface. However, once the pores are filled and O\(_2\) accumulates at the surface, the OH\(^-\) has to also scatter through the increasing O\(_2\) overlayer, which may reduce their kinetic energies sufficiently to prevent desorption, even by elastic momentum transfer collisions with O\(_2\) (this will be discussed below).

For O\(_2\) on crystalline benzene substrates the OH\(^-\) yield is rapidly attenuated, and drops to almost zero for only 1 ML of O\(_2\). Here O\(^-\) formed at the O\(_2\)-benzene interface must first scatter into the benzene film, abstract a hydrogen, and scatter back (through large angles) as OH\(^-\) in order to desorb. Thus, from an experimental point of view, O\(_2\) on crystalline hydrocarbon substrates is the least favorable condition\(^{33}\) to observe low energy O\(^-\) reactions, such as OH\(^-\) formation.

Identical experiments have also been performed for O\(_2\) adsorbed on amorphous-porous and crystalline toluene (C\(_6\)H\(_5\)CH\(_3\)) substrates (not shown here for sake of brevity). The resulting OH\(^-\), O\(^-\), and H\(^+\) yields show very similar dependence on electron energy and hydrocarbon film morphology as the measurements shown above.
3.3 O reactions in heterogeneous films: hydrocarbons on O₂ substrates

Shown in Figure 7 are the O⁻, OH⁻ and OD⁻ yields obtained from 1.2 ML of partially deuterated toluene C₆H₅CD₃ condensed on 5 ML of O₂ at 24K. The OH⁻ yields display the same signature as those O⁻ that desorb with E_k > 1-1.5 eV from pure O₂ films (Fig. 1b), but with lower intensity. The combined contribution of the 2Πₓ, 3Σ⁺(I) and 2Σ⁺(I) transient states of O₂⁻ near 8 eV, and those of the 3Σ⁺(II) and 2Σ⁺(II) near 13 eV are clearly visible in both the OH⁻ and O⁻ yields from the heterogeneous film. We note that here, for small quantities of hydrocarbon adsorbed on 5 ML O₂, the OH⁻ yield signature is more clearly resolved. As will be discussed below, this is mainly attributed to a more favorable O⁻ and OH⁻ scattering and desorption geometry. Here, O⁻ will react with a hydrocarbon on its outgoing trajectory towards the vacuum, and large angle scattering of the OH⁻ is not required.

![Figure 7: O⁻, OH⁻, and OD⁻ ESD yields as functions of incident electron energy obtained from 1.2 ML of C₆H₅CD₃ deposited at 24 K on a 5 ML thick O₂ film.](image-url)
for desorption (e.g. as is the case for O\textsubscript{2} on crystalline hydrocarbon substrates, where backscattering of OH\textsuperscript{−} through overlayers will reduce its yield and also broaden, or smear out, its energy distribution needed for desorption).

In analogy to the present results for benzene, and other hydrocarbon substrates\textsuperscript{7}, here the OH\textsuperscript{−} and OD\textsuperscript{−} yield is attributed to post DEA reactions of O\textsuperscript{−} with toluene. Hydrogen abstraction from toluene occurs in the same way as for the benzene molecule with a slight distinction, however, in that it depends on the site where the hydrogen abstraction takes place, i.e. the methyl group or the ring structure, i.e.:

\[
\begin{align*}
\text{O}^- + \text{C}_6\text{H}_5\text{CD}_3 & \rightarrow (\text{C}_6\text{H}_5\text{CD}_3\text{O})^- \rightarrow \text{OH}^- + \text{CD}_3\text{C}_6\text{H}_4^+ \quad (8) \\
& \rightarrow \text{OD}^- + \text{C}_6\text{H}_5\text{CD}_2^+ \quad (9)
\end{align*}
\]

We note that this produces two distinctly different neutral (reactive) radical products. Despite the small energy difference between the methyl group CD (or CH) and the ring CH of the C\textsubscript{6}H\textsubscript{5}CD\textsubscript{3} molecule (discussed below), the overall OH\textsuperscript{−} and OD\textsuperscript{−} yields are essentially identical; this suggests that the probability for O\textsuperscript{−} to abstract a hydrogen from the ring or the methyl group of the C\textsubscript{6}H\textsubscript{5}CH\textsubscript{3} molecule is similar.

Using the same bond energy (D\textsubscript{b}) values\textsuperscript{36} for H-C\textsubscript{6}H\textsubscript{5} and H-C\textsubscript{6}H\textsubscript{4}CH\textsubscript{3} ring CH bonds (about 4.8-4.9 eV), the known electron affinities\textsuperscript{37} (EA) for O (1.46 eV) and OH (1.83eV), and an OH bond energy of 4.42 eV, we find that hydrogen abstraction from either the benzene or toluene ring is thermoneutral (or slightly endothermic by 0.11 eV), while hydrogen abstraction on the methyl group of the toluene molecule is exothermic by 0.96 eV, given a CH bond energy of 3.8 eV for the methyl group in toluene. This may in part explain why here, and in benzene, the OH\textsuperscript{−} desorption threshold is usually slightly higher than that for O\textsuperscript{−} desorption, and why in toluene the OD\textsuperscript{−} yield threshold is slightly lower (by about 0.9 eV) than the OH\textsuperscript{−} yield threshold (Fig. 7). The OH\textsuperscript{−} yield signatures from undeuterated
toluene (C₆H₅CH₃ – not shown) have a similar signature as those in Fig. 7, with a desorption threshold as that observed in Fig. 7 for OD⁻.

For the preceding and following discussions we note the following points: In general, OH⁻ (and OD⁻) formation and desorption will also be determined by (a) repartitioning of the total reaction energy (including the center of mass collision energy, E_cm) contained in the dissociating (OC₆H₆)⁺* or (OC₆H₅CH₃)⁻* collision complex, as well as (b) elastic (i.e. binary recoil) and inelastic collisions of the O⁻ and OH⁻ within the film, or at the surface, prior to reaction or desorption. Thus, only O⁻ with a certain minimum E_k may abstract a hydrogen and still have sufficient E_k to scatter (as OH⁻) in the film, and overcome the E_p of the solid. This is outlined below:

(a) Using a binary collision approximation⁸, the surface kinetic energy of the OH⁻ ion, E_k(OH⁻), formed by the dissociative decay of the transient [OM]⁺ * collision complex (with total mass M, here = 94 and 108 amu, respectively, for (OC₆H₆)⁺* or (OC₆H₅CH₃)⁻*), relates to the product ion mass, m_ion (17 amu) via

\[ E_k(OH^-) = \{1 - (m_{ion}/M)\} \times \{\Delta_R H + E_{cm} - E^*\} \]  

(10).

Here Δ_R H is the thermodynamic reaction enthalpy estimated above, which depends on the differences of EA and D_o of the reactants and products, E_cm = (m_T/M)E_k, where m_T is the mass of the target (78 and 92 amu, for benzene and toluene), E_k is the O⁻ projectile energy¹⁰ at the surface, or in the bulk, and E* the internal excitation energy of the products³⁹. The factor \{1 - (m_{ion}/M)\} = 0.82 and 0.84, for benzene and toluene, respectively. For an O⁻ E_k of 1.5 eV, hydrogen abstraction from the ring CH in either benzene or toluene via reaction 7 or 8, with Δ_R H = 0, will yield an E_k(OH⁻) of about 1 eV, while hydrogen abstraction from the methyl CH in toluene, reaction 9 with Δ_R H = 0.96 eV, will result in a E_k(OH⁻) of about 1.9 eV. In either cases we assume E* = 0; however it is likely that OH⁻ production via H-
abstraction from hydrocarbons will lead to repartitioning of some of the total reaction energy into internal ro-vibrational excitation \((E^* > 0)\) of both the \(\text{OH}^-\) and neutral molecular dissociation fragments; this will reduce the \(\text{OH}^-\) kinetic energy upon formation. Since the \(E_p\) of the various \(\text{O}_2/\text{hydrocarbon}\) films is estimated to be about \(0.8 - 1\ \text{eV}\)\(^{40}\), depending on the amount of \(\text{O}_2\), and film morphology, the above \(E_b(\text{OH}^-)\) values show that only \(\text{O}^-\) of a certain initial \(E_k > 1 - 1.5\ \text{eV}\) (i.e. those in Fig. 1b) can contribute to the \(\text{OH}^-\) yields observed here.

(b) As known from low energy ion transmission through condensed films\(^{26}\) and classical mechanics, even in single binary elastic recoil collisions\(^{38}\) prior to desorption, at the surface or in the film bulk, the \(\text{OH}^-\) (17 amu) will suffer energy losses in collisions with benzene (78 amu), toluene (92 amu), or \(\text{O}_2\) (32 amu): e.g. for projectile scattering angles of 90°, the final energy of the projectile is \(E_f = \{(m_T - m_p)/M\}E_i\), where \(E_i\) is the initial \(\text{OH}^-\) projectile energy, \(m_T\) and \(m_p\) the target and projectile mass \((m_T > m_p)\), and \(M\) the total mass of the \(\text{OH}^- + \text{molecule}\) collision system. Thus, an \(\text{OH}^-\) formed in the bulk, or at the surface, of an \(\text{O}_2\) dosed hydrocarbon film will loose about 36%, 31%, and 69% of its initial energy in a single recoil collision with a benzene, toluene, or \(\text{O}_2\), respectively. Consequently, most of the \(\text{OH}^-\) formed at very low electron energies will not desorb, even after only one recoil collision (more energy will be lost in inelastic collisions involving ro-vibrational excitations of the projectile and/or target). Thus, it is clear that different morphologies of the hydrocarbon substrate in part determine the intensities and signatures of the \(\text{OH}^-\) desorption yields observed, depending on if the \(\text{OH}^-\) is formed by (a) \(\text{O}^-\) scattering out of an amorphous-porous hydrocarbon film (e.g. Figs. 4a and c), (b) by \(\text{O}^-\) scattering into the crystalline hydrocarbon substrate (e.g. Figs. 4b and 6), and (c) by \(\text{O}^-\) scattering through the hydrocarbon overlayer (e.g. Fig. 7). In a sense, case (a) and (c) are related, and depend on what type of film is
formed in (c) by adsorption of the hydrocarbons on the multilayer O\textsubscript{2} substrate. This is discussed below.

**Fig. 8** (a) O\textsuperscript{−} and (b) OH ESD yields as functions of incident electron energy obtained from variable quantities of benzene deposited at 24 K on 5 ML thick O\textsubscript{2} films. Shown in (c) is the OH/O\textsuperscript{−} yield ratio from a film of 3.3 ML benzene condensed on 5 ML of O\textsubscript{2}.

Figure 8a shows the O\textsuperscript{−} yield obtained for increasing quantities of C\textsubscript{6}H\textsubscript{6} condensed on a 5 ML O\textsubscript{2} film at a temperature of 24K. It can be seen that the O\textsuperscript{−} yield retains its characteristic shape for various C\textsubscript{6}H\textsubscript{6} coverages. All O\textsubscript{2}\textsuperscript{*} resonant states contribute to the O\textsuperscript{−} signal that uniformly decreases as the C\textsubscript{6}H\textsubscript{6} overlayer thickness is increased.

While the slow initial attenuation of the O\textsuperscript{−} signal emerging from increasing C\textsubscript{6}H\textsubscript{6} coverage, up to 1.7 ML, suggests a large scattering mean free path through the overlayer, we
note that at low benzene coverage it may also be related to the existence of a mixture of the two molecules at the film surface. Although the O$_2$ substrate is non-porous, during adsorption the more massive C$_6$H$_6$ molecules (with room temperature kinetic energies) can penetrate the 24K O$_2$ film and thus preserve an effective O$_2$ density available for DEA at the film surface. However, here the creation of a mixed surface layer, does not result from the same mechanism as for O$_2$ adsorbed on a porous benzene substrate: for the latter the O$_2$ molecules diffuse into the pores of the amorphous-porous hydrocarbon substrate, whereas here the non-porous O$_2$ substrate can appear soft to the more massive hydrocarbon molecules that may become embedded into it. Here, the O$_2$ substrate being close to its sublimation temperature (20K), the O$_2$ molecules have a higher mobility than the more massive C$_6$H$_6$ molecules. Above 1.7 ML of benzene, this mixed film surface disappears under the increasing hydrocarbon layer, which quickly attenuates the O$^-$ yield as the C$_6$H$_6$ film thickness is increased.

In any case, O$^-$ ions resulting from DEA reactions to O$_2$ by the incident electrons that penetrate the C$_6$H$_6$ overlayers can produce OH$^-$ ions. The desorbing OH$^-$ ions yield obtained under similar conditions is displayed in Fig. 8b. All DEA O$_2$ * resonant state contributions seen in Fig. 8a yields are visible in the OH$^-$ yields, particularly the $^3\Sigma_u^+(II)$ and $^3\Sigma_u^+(II)$ states near 13 eV; this is because they yield higher kinetic energy O$^-$ that can more easily abstract a H from the C$_6$H$_6$ molecule, and produce OH$^-$ that can more easily overcome the $E_F$ of the film in order to desorb. While the O$^-$ yield displayed in Fig. 8a decreases, we see that the OH$^-$ yield at first increases with C$_6$H$_6$ coverage. The slow increase of the OH$^-$ yield may be related to the formation of a mixed zone gradually formed near the film surface as C$_6$H$_6$ molecules condense on the O$_2$ substrate. A mixture of C$_6$H$_6$ and O$_2$ molecules at the film surface at low C$_6$H$_6$ coverage would favor O$^-$ and OH$^-$ ion formation at the film surface,
which lowers their probability to suffer inelastic collisions prior to desorption. An increasing quantity of C₆H₆ deposited over O₂ provides more potential reactive sites for H abstraction, but a uniform decrease of the OH⁻ yield is observed at a C₆H₆ coverage above 1.7 ML up to 8 ML of benzene (not shown). As the C₆H₆ film thickness increases, C₆H₆ molecules progressively form a uniform hydrocarbon overlayer at the surface of the film, which reduces (a) the probability of the incoming electrons to reach the O₂ layers, and (b) the probability that either OH⁻ may scatter out, or that O⁻ reaches the surface to react.

We note that the OH⁻/O⁻ yield ratio depends not only on the amount of hydrocarbon deposited on the O₂ substrate but also on the electron energy. As shown in Fig. 8c, for 3.9 ML of C₆H₆ condensed on 5 ML of O₂, the OH⁻/O⁻ ratio is maximum for an incident electron energy near 11.5 eV; while at this energy the O⁻ yield is at a local minimum (i.e. where the O₂⁻ resonances have minimum overlap), it is in the energy range where the yield of O⁻ ions formed with about 3 eV of E_k is at a maximum. While H abstraction from benzene is at best thermoneutral, this suggests that as the electron energy increases to 12 eV, the O⁻ E_k increases to 3 eV, and so does the reaction probability to form sufficiently energetic OH⁻ that can scatter out of the film and desorb. However, for O⁻ E_k above 3 eV (formed by electrons above 12 eV), the collision time the O⁻ spends near a particular benzene decreases (20 to 10 fs/Å, for 3-5 eV O⁻), which will continuously decrease the probability for reactive scattering channels such as H abstraction.

Hydrogen abstraction by O⁻ leads to OH⁻ formation for both benzene and toluene overlayers on O₂ substrates, with similar dependence on hydrocarbon coverage. This is summarized in Figure 9, which shows the attenuation of the integrated O⁻ yields (from 5-16 eV), and the integrated OH⁻/O⁻ yield ratios (from 10-15 eV) for increasing quantities of C₆H₆ and C₆H₅CH₃ adsorbed at 24 K on a 5 ML film of pure O₂.
**Fig. 9** Integrated $O^-$ yields (from 5-16 eV), and the integrated $OH^+/O^-$ yield ratios (from 10-15 eV) for increasing quantities of $C_6H_6$ and $C_6H_5CH_3$ adsorbed at 24 K on a 5 ML film of pure $O_2$.

We find that for medium hydrocarbon coverage (1-3 ML) the $O^-$ signal attenuation is somewhat slower for an $O_2$ film covered by benzene than by toluene, which may be related to a lower $O^-$ reaction probability in the benzene overlayer, where H abstraction is at best thermoneutral, or slightly exothermic. However at higher hydrocarbon coverage $O^-$ attenuation tends to the same saturation point near about 5 ML for either benzene or toluene overlayers. Simultaneously the integral $OH^+/O^-$ yield ratio first increases at the same rate for both hydrocarbon overlayers, reaching a maximum near 1.5 ML, then gradually decreases for increasing hydrocarbon coverage. The integral $OH^+/O^-$ yield ratio for $O_2$ films covered with benzene is generally about 20 times smaller than for those covered with toluene, which is in part due to the greater exothermicity of H abstraction in toluene than in benzene. However in
both cases the optimum condition for $O^-$ collisions leading to formation and desorption of $OH^-$ is obtained for about 1.5 ML of hydrocarbon adsorbed on the O$_2$ substrates.

4 Summary and Conclusion

Our experiments conducted with heterogeneous films of cyclic hydrocarbon molecules, benzene, toluene and partially deuterated toluene, coadsorbed with O$_2$ provide evidence of reactive scattering within the films. 1 – 5 eV $O^-$ ions produced via DEA to O$_2$ react with hydrocarbon molecules to form new reactive ionic species and neutral hydrocarbon radicals. OH$^-$ ions are formed by hydrogen abstraction reactions induced by $O^-$ ions which are able to abstract a H on both the methyl group and the ring of the C$_6$H$_5$CH$_3$ molecule with similar efficiency. Since hydrogen abstraction can occur potentially occur on any site of the molecule, it will lead to the formation of different reactive hydrocarbon radicals. This observation has implications on our understanding of how secondary reactive anions can affect the DNA molecule. In DNA, similar hydrogen abstraction from thymine or cytosine may induce the formation of a radical pyrimidine base. This radical may either stabilize by reacting with adjacent H$_2$O molecules or surrounding proteins, leading to reactive damage, or oxidation by an oxygen atom that can come occupy the radical site. Oxidization of the base can prevent it to be correctly identified during replication, which may lead to gene mutations. The radical site may also be transferred to the DNA sugar backbone and induce a strand break.

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References


31 This is generally observed here, and is mainly due to different EEL effects in mixed films prior to DEA to O₂, and other extrinsic film effects on DEA and DD, discussed elsewhere.  


38 A gas-phase-like binary collision approximation, based on energy and momentum conservation, is used here, because (a) in the present dielectric physisorbed systems (cryogenic, van der Waals solids) the molecular binding energies (10 – 100 meV range) are much smaller than the average O⁻ projectile E_k(1-5 eV), and (b) typical periods for lattice vibrations (10⁻¹³ s) are larger than the average collision times (10⁻¹⁴ s) (see ref. 10a and 26 for detailed discussions). For projectile E_k significantly below 1 eV, this approximation is no longer valid; however for E_k < 0.8 eV ≈ E_p no ions desorb, and the present approximation is believed to be valid for the initial O⁻ + M collisions in the films.

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Here, for ion reactions in the condensed phase, effects of $E_p$ on the reaction/dissociation dynamics can be neglected, since all involved states, i.e. the initial ($O^- + M$), transient $(OM)^*$ and final ($OH^- + M^+$), are anionic states, that are equally affected by the $E_p$ of the surrounding medium. This is different from DEA (neutral to anion state transition).


This is suggested by semi-classical reactive collision principles, such as the well known Langevin reaction model, in which the cross section for slow reactions is $\sigma_L = k_L/v$, where $k_L$ is the Langevin reaction rate constant, and $v$ the relative collision velocity. The slower the collision velocity, i.e. the more time the reactants are in close proximity, the greater the reaction probability.
CHAPTER 4 – Chemical Identification of Ion Fragments Produced by Low Energy Ion Impact in Deuterated Thymine Films

4.1 Introduction

The very first results on condensed phase thymine fragmentation by 10-200 eV Ar\(^+\) ions are presented in this chapter, using the experimental methods detailed in section 2.3 of chapter 2. The work in this chapter is in press as an article in the *International Journal of Mass Spectrometry*, entitled “*Identification of Ion Fragments Produced from Thymine and Deuterated Thymine by Low Energy Ion Impact in Films and Electron Impact in the Gas Phase*”, by Marjorie Imhoff, Zongwu Deng, and Michael A. Huels.
4.2 Identification of Ion Fragments Produced from Thymine and Deuterated Thymine by Low Energy Ion Impact in Films and Electron Impact in the Gas Phase

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Abstract

The chemical composition of charged fragments desorbing from thymine-methyl-d$_{3}$-6-d (T$_{d}$) films during 10 – 200 eV Ar$^{+}$ ion irradiation is studied by mass spectrometry of positive and negative ions. The resulting mass spectra are compared to those obtained from similar films of thymine (T), and from 70 eV electron impact on both T and T$_{d}$ in the gas phase. Ion impact on T and T$_{d}$ films produces numerous positive and negative ion fragments via endocyclic and exocyclic bond cleavage, even at ion energies well below 60 eV. The major cations desorbing from T films are identified as HNCH$^{+}$, HN(CH)CCH$_{3}^{+}$, [T-OCN]$^{+}$, OCNH$_{2}^{+}$, C$_{x}$H$_{y}^{+}$ (x = 1-3, y = 0-4), and [T+H]$^{+}$. While ion impact on T and T$_{d}$ films produces a new fragment [T-O]$^{+}$, [T$_{d}$-O]$^{+}$, not seen in gas phase electron impact, the latter yields CO$^{+}$ fragments that are not observed during ion impact on the films. Anion desorption is dominated by H$^{-}$, O$^{-}$, CN$^{-}$, OCN$^{-}$, and [T-H]$^{-}$ formation, with lesser dissociation channels leading to desorption of C$_{2}$$^{-}$, C$_{2}$H$^{-}$, C$_{2}$CN$^{-}$, NC$_{3}$H$_{2}^{-}$, HNC$_{3}$H$_{5}^{-}$, OC$_{3}$H$_{5}^{-}$, C$_{2}$OCN$^{-}$, and C$_{3}$H$_{x}^{-}$ (x = 2, 3). Measurements of the primary ion energy dependence of the fragment desorption yields show that positive ion fragments appear at energies near 15 – 20 eV, while generally the endocyclic fragments appear at lower energies than the exocyclic (anion and cation) fragments. Our results show that even at very low ion energies thymine is sensitive to complete fragmentation, whereby the loss of the HNCH (or HNCD from T$_{d}$) fragment, i.e. ring cleavage involving the N1, C6 atoms, dominates; in cellular DNA this would correspond to complete loss of the base.

Keywords: DNA bases; Ion impact; Ion desorption; Condensed phase
1. Introduction

Ionizing radiation (protons, heavy ions, electrons, x- and γ-rays) treatment of biological media causes severe damage to DNA mainly by producing single and double strand breaks of DNA and complex clustered lesions [1]. This damage is associated with abundant secondary particles produced along the radiation tracks, which include low-energy ions, radicals, and ballistic secondary electrons [2,3]. Traditionally, DNA damage has been linked to thermal, or solvated, secondary electrons, and the formation of reactive radicals [4]. Hence, in the past decades, most experimental studies have focused on DNA damage by free radicals and solvated electrons.

However, most of the secondary electrons created along ionizing radiation tracks in solids and liquids have non-thermal initial energy distributions below 100 eV, with a most probable energy below 10 eV. Some secondary electrons such as Auger electrons may have energies up to several 100 eV, or a few keV for “delta rays”. Non-thermal secondary electrons can also produce secondary ions, by either ionization, excitation, or dissociative electron attachment (resonances). Recently, this has been shown to cause severe damage to DNA and its components at electron energies as low as 3 eV via resonant mechanism [5,6,7,8,9,10,11]. Simultaneously, hyperthermal (E < 100 eV) secondary ions are created along any radiation track as a result of dissociative ionizations. High energy electrons or photon tracks usually produce secondary ions with low kinetic energies (typically below 10 eV), but with different chemical reactivities. Some of the ions formed via Auger-decay of specific core excitations can have energies of several tens of electron volts. More important for the present studies, during heavy ion therapy [12,13] secondary atomic ions with energies of several hundred eV and multiple charge states can be produced [14]. Furthermore, while heavy ion therapy relies on the ion’s highly localized relative dose distribution (Bragg peak), the primary ion’s linear
energy transfer is highest at the ion track ends where the ionization density is highest, and where the primary ion energy is reduced below a few keV. Thus, the chemical and kinetic damage produced by either track-end primary ions, or hyperthermal secondary ions in subsequent scattering events, is of great biological relevance in heavy ion therapy, since it will occur over short distances (a few nm) and result in complex DNA damage clusters that can not be easily repaired by the cell.

In a recent preliminary study [15], we have found that ion impact on condensed phase thymine, at ion energies below 200 eV, results in significant film degradation via both kinetic and potential scattering (i.e. the primary ion’s potential energy). However, to understand the sequence of events leading to DNA damage during irradiation requires a detailed knowledge of the initial fragmentation pathways and products, and the possible reactivity of the initial fragments. Thus, the identification of the chemical composition of the fragments produced by ion (or electron) irradiation is a prerequisite for the further study of their reactivity.

Here, we report detailed mass spectroscopy measurements of the energetic (1-5 eV) anion and cation fragments produced by 10 – 200 eV Ar⁺ ion impact on condensed phase thymine-methyl-d₃-6-d (Td) and thymine (T), with molecular structures shown in Figure 1. The resulting ion stimulated desorption (ISD) mass spectra are compared to those produced in the same experimental set-up from gas phase T and Td by 70 eV electron impact, and the chemical identity of fragments produced by ISD is determined.

Our results show that the most abundant cation fragments produced by hyperthermal Ar⁺ impact are HNCH⁺, HN(CH)CCH₂⁺, C₃H₃⁺, OCNH₂⁺, [T-OCN]⁺, [T-O]⁺, where the first two are found to result from specific ring cleavage at, respectively, the C2-N1 and C6-C5, and C2-N1 and C5-C4. For [T+H]⁺ formation we find a strong 4 : 1 preference for abstraction of nitrogen bound hydrogen from adjacent T, relative to carbon bound hydrogen, opposed to the
stoichometric NH to CH ratio of $1 \div 2$. The most prevalent desorbing anions include $H^-$, $O^-$, $CN^-$, $OCN^-$, and $[T-H]^-$ formation; the latter involves deprotonation almost exclusively via NH bond cleavage, while $H^-$ formation occurs with stoichometric ratio from NH and CH sites. Lesser dissociation channels also lead to desorption of $OH^-$, $C_2^-$, $C_2H^-$, $C_2CN^-$, $NC_3H_2^-$, $HNC_3H_3^-$, $OC_3H_5^-$, $C_2OCN^-$, and $C_3H_x^-$ ($x = 2, 3$). Here, $OH^-$ (or $OD^-$ from $T_d$) formation likely involves subsequent reactive scattering of $O^-$ prior to desorption, leading to $H$ abstraction from NH and CH bonds. More importantly, both exocyclic and endocyclic fragmentation is observed for ion-beam impact well below 1 eV/amu, and results in the formation of ions that still have sufficient kinetic energy ($> 1$ eV) to overcome the charge induce polarization barrier at the film surface and desorb into the vacuum.

\[
\text{Thymine (T)} \quad \text{Thymine - methyl - d$_3$-6-d (T$_d$)}
\]

**Figure 1:** Molecular structures of thymine ($T$) and thymine-methyl-$d_3$-6-d ($T_d$).

2. Experimental Method

The experiments were carried out on an ion-beam apparatus developed in-house [15], and which will be discussed in detail elsewhere. Here we give only a brief description of the experimental method: a low energy ion beam system delivers a highly focused, mass- and energy-resolved positive or negative ion beam in the 1–500 eV energy range into a UHV ($10^{-9}$ torr) reaction chamber for sample film irradiation. A high resolution quadrupole mass
spectrometer (QMS) (Hiden Analytical Ltd.) is installed perpendicularly to the ion beam line to monitor desorbing positive and negative ions during ion impact. A large diameter lens is used at the mass spectrometer entrance to ensure a large solid angle of acceptance and thus a higher sensitivity. The QMS is optimized to detect ions of up to 5 eV kinetic energy.

In this work, we focus mainly on 10 - 200 eV Ar\(^+\) ion irradiation of 200 ng/cm\(^2\) thymine (T and T\(_a\)) films on Pt substrates, corresponding roughly to 4 nominal monolayers of thymine assuming no clustering of the molecules [8]. For negative ion desorption we use 20 ng/cm\(^2\) films. Films are prepared by in vacuo evaporation onto an atomically clean polycrystalline Pt substrate held on a manipulator at room temperature (22 - 24\(^\circ\)C), while the thymine condensation rate is calibrated to within 0.5 ng/cm\(^2\) by a quartz crystal microbalance. Prior to film deposition, the Pt substrate is cleaned by resistive heating to 800\(^\circ\)C, and/or 500 eV Ar\(^+\) sputtering. The evaporation temperature of \(\leq 100\)^\(\circ\)C is well below the thymine decomposition temperature of about 320\(^\circ\)C. X-ray photoelectron spectroscopy and thin film chromatography results show that the films consist of intact molecules [16,17]. The sample film is positioned in the center of the reaction chamber at 2 cm from both the exit of the ion beam line and the entrance of the mass spectrometer, with an incident beam angle of 30\(^\circ\) and QMS observation angle of 60\(^\circ\), both with respect to the sample surface, and subjected to ion irradiation. In the ion stimulated desorption (ISD) mode, positive and negative ions that desorb during primary ion impact at a fixed beam energy are recorded as a function of mass/charge ratio. The base pressure in the target chamber during experiments is \(\sim 2 \times 10^{-9}\) Torr.

With a co-axial, low profile ionizer (open grid on line of sight) installed between the entrance ion optics and the quadrupole mass filter, the QMS is also used in the residual gas analyzer (RGA) mode to fragment and ionize neutral gas phase molecules. The results of
electron impact on thymine in the gas phase, presented here, were taken in this mode with the electron impact energy set at 70 eV. The electron impact mass spectra were obtained after careful baking of the UHV system and degassing of the sample in the load-lock chamber to reduce the RGA background signal (particularly the H$_2^+$ and H$_2$O$^+$) to minimum. A relatively large amount of thymine is deposited on the Pt substrate, which is then placed in front of the entrance of the QMS. Subsequently, the thymine molecules are gently evaporated from the Pt into the QMS, by warming up the Pt substrate to 85 - 95 °C, and subjected to electron impact. For the moment, only cations yields can be recorded during electron impact, i.e. when the QMS is used in the RGA neutral detection mode; installation of a RGA anion option may soon allow neutral detection in the RGA mode via 4 – 20 eV dissociative electron attachment (however, in the ion stimulated desorption (ISD) mode the ionizer is turned off, and both desorbing anions and cations are detected by the QMS). A background spectrum, taken immediately prior to the sample evaporation, is subtracted from the thymine electron impact mass spectra presented here. The gas phase electron impact mass spectra obtained here can more easily be compared directly to our ISD mass spectra, since the QMS filter and detection system are the same for both measurements; thus, we can ignore differences in transmission and detection efficiencies between our QMS and mass spectrometers used in previous measurements of electron impact on thymine [18].

To facilitate chemical identification of the fragments, thymine (T) and partially deuterated thymine (thymine-methyl-d$_3$-6-d, T$_d$) were used in all of the present experiments. The stated purities are 99% for T, and 99% isotopic purity for T$_d$ (both from Sigma Aldrich); to remove impurities prior to evaporation, all compounds are gently degassed in the separate load lock chamber by heating for several hours near 40 °C, well below the evaporation onset for thymine (ca. 65 - 75 °C).
3. Results and Discussions

3.1 Positive ion formation

Both electron and ion impact on thymine produces abundant cation fragments. Figure 2 shows typical mass patterns of positive ion fragments produced by (a) 70 eV electron impact in the gas phase and (b) 200 eV Ar\(^+\) ion irradiation of a 200 ng/cm\(^2\) thymine film on Pt. The molecular parent cations are observed at 126 and 127 amu, respectively, for electron

![Mass spectrum of thymine fragments](image)

**Figure 2**: Global positive ion fragmentation pattern from thymine (T) produced by (a) 70 eV electron impact in the gas phase, and (b) 200 eV Ar\(^+\) ion irradiation of a 200 ng/cm\(^2\) thymine film on Pt substrate. The inset shows the parent molecular ions, and a fragment from the loss of an oxygen atom induced by Ar\(^+\) ion irradiation in (b). The chemical identification of the fragments is discussed in detail in the text. Both mass spectra have been normalized in intensity, such that the integral intensity of either mass spectrum from 0 – 200 amu is equal to one.

and ion impact. The appearance of the parent ion at 127 amu during ion irradiation of the film is due to protonation. Protonation is frequently observed during surface desorption, such as
secondary ion mass spectrometry, and is also observed in the production of daughter ions as discussed below.

During Ar\(^+\) ion irradiation of thymine films, three fragment peaks are observed at 109-111 amu, which can be assigned to the loss of an oxygen atom from thymine \([\text{T-O}]^+\) and its derivatives \([\text{T-O+H}]^+\). It is not clear whether this fragment involves breaking of endocyclic bonds. These peaks are not seen in the mass spectrum of electron impact, suggesting the absence of this specific oxygen loss fragmentation channel.

As shown in Figure 3, in the region of 1-15 amu we observe hydrocarbon fragments \(\text{CH}_x^+\) (x=0-3), and \(\text{H}^+\) fragments for Ar\(^+\) impact of films of T. These fragments are more clearly observable during irradiation by low mass cations, such as \(\text{He}^+\) or \(\text{N}^+\). He\(^+\) ion impact on thymine in particular also leads to enhanced \(\text{H}^+\) desorption via a specific reactive scattering channel, and will be discussed elsewhere.

Figure 3: Close up of cation mass spectra between 0 and 20 amu induced by (a) electron impact on gas phase thymine, and (b) 200 eV Ar\(^+\) impact on a 200 ng/cm\(^2\) thymine film on a Pt substrate. The relative ion intensity is in arbitrary units (see Fig. 2).
Below 20 amu, 200 eV Ar⁺ impact on T films yields mainly CH₃⁺ and some O⁺ fragments (the 15 amu peak from Ar⁺ irradiation of T films shifts to 18 amu for T_d films, the 16 amu peak does not shift), while 70eV electron impact on gas phase T also results in CH₄⁺ elimination. In the latter case the formation of H₂⁺, OH⁺ and H₂O⁺ is believed to result directly from gas phase electron-thymine interactions, since their yields are substantially larger than that observed in the subtracted background mass spectrum; however a small contribution from residual water in the UHV chamber can not be ruled out for the electron impact spectra.

In order to facilitate fragment identification, close ups of the mass spectra for the major fragments of thymine-methyl-d_5-6-d (T_d) produced by electron and ion impact are shown in Figures 4 and 5, respectively, along with those of thymine (T). We first discuss the

![Diagram]

**Figure 4:** Detailed positive ion fragment patterns produced by 70 eV electron impact on (a) thymine (T), and (b) thymine-methyl-d_5-6-d (T_d), both in the gas phase. The relative ion intensity is in arbitrary units (see Fig. 2).
assignments from high m/e fragments to low m/e fragments (from right to left of both figures).

In the gas phase electron impact spectra shown in Fig. 4, the fragment of thymine at 83 amu is found to shift to 87 amu in the case of T_d, indicating that it contains the four deuterium atoms. This fragment is assigned to the loss of an OCNH group from thymine. Further loss of a hydrogen gives a fragment at 82 amu, which shifts to 85 and 86 amu in the mass spectrum of thymine-methyl-d_3-6-d as a result of further loss of carbon-bound deuterium or nitrogen-bound hydrogen, respectively. The branching ratio between mass 85 and 86 amu in T_d is in reasonable agreement with the stoichiometric ratio of 4:1 for CD and NH sites.

In the ion impact mass spectra on T and T_d films in Fig. 5, most of the [T-OCNH]^+ (83 amu) or [T_d-OCNH]^+ (87 amu) fragments desorbs in the protonated form as [T-OCN]^+ or

![Image of mass spectra](image_url)

**Figure 5:** Detailed positive ion fragment patterns produced by 200 eV Ar^+ ion irradiation of 200 ng/cm^2 films of (a) thymine (T), and (b) thymine-methyl-d_3-6-d (T_d), both on a Pt substrate. The relative ion intensity is in arbitrary units (see Fig. 2).
\([T_d\text{-OCN}]^+\) at 84 or 88 amu, respectively. Loss of hydrogens from \([T\text{-OCNH}]^+\) yields fragments at 82 and 81 amu, while loss of H or D from \([T_d\text{-OCNH}]^+\) yields fragments at 86 and 85 amu, which is in agreement with the assignment of this fragment from the gas phase electron impact mass spectra of T and \(T_d\).

The fragment at 55 amu from electron impact (Fig. 4) is assigned to \(\text{HNC}_3\text{H}_4^+\) as it shifts to 59 amu in the case of \(T_d\). Further loss of a hydrogen (deuterium) atoms gives fragment(s) at 54 amu (for T) and 57, 58 amu (for \(T_d\)). A possible fragment \(\text{OC}_3\text{H}_5^+\) may also contribute to the peak at 55 amu, which should shift to 58 amu (\(\text{OC}_3\text{D}_5^+\)) in the case of \(T_d\). However, further loss of a deuterium from this 58 amu fragment should yield a fragment at 56 amu in the case of \(T_d\), the low yield of which suggests a negligible contribution of this dissociation channel.

During \(\text{Ar}^+\) ion irradiation of T and \(T_d\) films (Fig. 5), part of the \(\text{HNC}_3\text{H}_4^+\) (55 amu) and \(\text{HNC}_3\text{D}_4^+\) (59 amu) fragments appear in protonated form (56 and 60 amu). However, as seen in Fig. 5, the loss of a hydrogen from the 55 amu \(\text{HNC}_3\text{H}_4^+\), resulting in desorption of a 54 amu fragment, may involve hydrogen loss from either NH or CH groups, i.e. formation of \(\text{NC}_3\text{H}_4^+\) or \(\text{HNC}_3\text{H}_5^+\), since for \(T_d\) films both 58 and 57 amu fragments are observed, i.e. \(\text{NC}_3\text{D}_4^+\) and \(\text{HNC}_3\text{D}_5^+\). In either case, \(\text{HNC}_3\text{H}_4^+\) is the second most dominant cation fragment.

In the 36-48 amu region, both gas phase electron impact on T (Fig. 4) and \(\text{Ar}^+\) ion irradiation of T films (Fig. 5) produce a series of hydrocarbon fragments \(\text{C}_3\text{H}_x^+\) (\(x=1-4\)) at 37-40 amu, which shift to 38, 40, 42 and 44 amu in the case of \(T_d\). For \(\text{Ar}^+\) impact on T films (Fig. 5) these fragments are dominated by \(\text{C}_3\text{H}_5^+\). The fragment at 44 amu from T by both \(\text{Ar}^+\) and electron impact may be assigned to \(\text{OCNH}_2^+\), and may be the result of the O atom in the \(\text{OCNH}^+\) fragment abstracting a neighboring hydrogen from either the -CH$_3$ group or an N-H site prior to fragmentation, or desorption. Thus, this fragment can contribute to both the 44
amu (OCNH$_2^+$) and 45 amu (OCNHD$^+$) fragments in the case of T$_d$, which excludes residual CO$_2$ as a major contribution to the 44 amu peak in the thymine mass spectra. The 41 – 43 amu fragments are presently unassigned, and a minor contribution of NCN$^+$ to the 40 amu fragment can not be excluded.

Between 24 to 34 amu we find the dominant cation fragments produced by both electron and ion impact. However, the gas phase electron impact spectra become complicated in the case of T$_d$. Instead, we first discuss the condensed phase ion impact spectra in Fig. 5.

For ion impact on T films, the dominant fragment shifts from 28 amu in T, to 29 amu in T$_d$, indicating the inclusion of a deuterium atom in the fragment, and thus presence of a hydrogen atom in the case of T. As a consequence, this fragment is uniquely and exclusively assigned to HNCH$^+$ (HNCD$^+$) as the first dominant fragment, and not CO$^+$. The other minor fragments in this region are assignable to hydrocarbon fragments C$_2$H$_x^+$ (x=1-3) and possibly a minor contribution from HCN$^+$ (27 amu).

During gas phase electron impact (Fig. 4), spectrum of T is similar to that of ion impact, but those of T$_d$ are quite different. First of all, we can assign the hydrocarbon fragments C$_2$D$_x^+$ (x=1-3) at 26, 28 and 30 amu, and fragment HNCD$^+$ at 29 amu. Judging from the branching ratios of C$_2$H$_x^+$ and C$_2$D$_x^+$ fragments, the 28 amu peak has another major contribution from CO$^+$, which is absent in the case of ion irradiation. For gas phase electron impact on T, a possible contribution from background N$_2$ and/or CO to this 28 amu peak is excluded because (1) a background spectrum has been subtracted from the spectra in Fig 4, and (2) the 28 amu peak of the background spectrum exhibits no significant increase when warming up a clean Pt substrate following the same procedure used to take the electron impact spectra of gas phase thymine and thymine-methyl-d$_3$-6-d. Hence, in the gas phase electron impact spectrum of thymine (Fig. 4), the 28 amu peak contains contributions from
both HNCH\(^+\) and CO\(^+\), while in the condensed phase ion impact spectrum (Fig. 5), it consists of uniquely of the HNCH\(^+\) fragment. This suggest the absence of a specific CO\(^+\) fragmentation channel in the case of ion impact on thymine films.

![Figure 6: Detailed view of ion stimulated desorption (ISD) mass spectra for parent molecule \([M+H]^+\) formation by 200 eV Ar\(^+\) impact on 200 ng/cm\(^2\) films of (a) thymine (T), and (b) thymine-methyl-d\(_7\)-6-d (T\(_d\)), both on a Pt substrate. The relative ion intensity is in arbitrary units (see Fig. 2).](image)

Notably, we also find that protonation of certain fragment species desorbing under ion impact more often than not seems to involve preferential abstraction of a hydrogen from nitrogen sites from and adjacent T\(_d\), than from a carbon (e.g. for \([\text{HNC}_3\text{D}_4 + H]^+\), Fig. 5), while hydrogen elimination from a specific fragment may involve both NH and CH sites (e.g. \([\text{NC}_3\text{D}_4]^+, [\text{HNC}_3\text{D}_3]^+, \text{or } [\text{T}_d - \text{OCN} - \text{H}, \text{or } - \text{D}]^+ \text{ from } \text{T}_d, \text{Fig. 5})\). This is clearly observed in the desorption of the protonated parent molecule from films of T and T\(_d\) as shown in Figure 6. From integration of the mass peak signals we find that for \([T+H]^+\) formation and desorption there appears a strong 4:1 preference for abstraction of nitrogen bound hydrogens, relative to carbon bound hydrogens, opposed to the stoichiometric NH to CH ratio of 1:2. This strong preference for formation of \([\text{T}_d + \text{H}]^+\) versus \([\text{T}_d + \text{D}]^+\), even when accounting for small
quantities of natural $^{13}$C in T or $\text{T}_{d}$, e.g. [T$_{d}(^{13}$C)+H]$^+$ (132 amu), may be the result of the relatively weaker binding energy of NH compared to CH bonds in thymine.

### 3.2 Negative ion formation

Figure 7 shows the negative ion ISD mass pattern produced by 200 eV Ar$^+$ ion irradiation of a 20 ng/cm$^2$ thymine film on Pt substrate. Most of the anion fragments are more clearly observable at low coverage, which is related to their specific fragmentation dynamics [15]. The major fragments at 1, 16, 26, 42, and 125 amu are assigned to H$,^-$, O$,^-$, CN$,^-$, OCN$,^-$, and [T-H]$^-$. With the exception of H$,^-$, the known electron affinities (EA) [19] of these intense fragments are quite high, i.e. 1.46 eV (O), 3.86 eV (CN), 3.6 eV (OCN), compared to 0.75 eV for H$^-$; here the [T-H]$^-$ fragment involves deprotonation almost exclusively at the NH sites (to

![Figure 7](image-url)

**Figure 7**: Global negative ion fragment ISD pattern of thymine (T) produced by 200 eV Ar$^+$ ISD of a 20 ng/cm$^2$ thymine film on Pt substrate. The inset shows an enlargement of the region from 75 – 115 amu. The spectrum has been normalized in intensity, such that the integral intensity from 0 – 200 amu is equal to one. The chemical identification of the fragments is discussed in detail in the text.
be discussed below), and its EA has been estimated to be between 3-4 eV [20]. In any case, the strong intensity of these fragments suggests that their EAs contribute to their formation mechanism by low energy ion impact [15].

The minor anion fragments in Fig. 7 are assigned to CH\textsuperscript{−}, OH\textsuperscript{−}, C\textsubscript{2}H\textsuperscript{−}, C\textsubscript{2}CN\textsuperscript{−}, C\textsubscript{2}OCN\textsuperscript{−}, NC\textsubscript{3}H\textsubscript{2}\textsuperscript{−}, HNC\textsubscript{3}H\textsubscript{3}\textsuperscript{−}, OC\textsubscript{3}H\textsubscript{3}\textsuperscript{−}, and hydrocarbon fragments C\textsubscript{x}H\textsubscript{y}\textsuperscript{−}. While the fragments such as NC\textsubscript{3}H\textsubscript{2}\textsuperscript{−}, HNC\textsubscript{3}H\textsubscript{3}\textsuperscript{−}, OC\textsubscript{3}H\textsubscript{3}\textsuperscript{−} originate from direct fragmentation of thymine, there may be a contribution from carbon impurities in the Pt substrate to C\textsuperscript{−}. The small yield of mass 109 and 111 amu fragments in Fig. 7 is tentatively assigned to [T - OH]\textsuperscript{−} and [T - CH\textsubscript{3}]\textsuperscript{−}, respectively. While the latter assignment is consistent with a shift from 111 to 112 amu ([T\textsubscript{d}-CD\textsubscript{3}]\textsuperscript{−}) upon T\textsubscript{d} substitution (not shown), this is complicated by the fact that the 109 amu peak also shifts to 112 amu, suggesting [T\textsubscript{d} - OD]\textsuperscript{−} formation. The slightly larger peaks at 79, 81, and 82 amu are not yet assigned, but may in principle result from [T-OCNH\textsubscript{2} - nH]\textsuperscript{−} (n = 0, 1, 3) formation; however, the presence of trace amounts of 5-bromouracil contaminants in the sample might also yield 79 and 81 amu anions (\textsuperscript{79,81}Br\textsuperscript{−}).

Analogous to the cation fragments, Figure 8 allows identification of many of the anion species produced by Ar\textsuperscript{+} impact by comparing results from T and T\textsubscript{d} films from high to low mass fragments. For T films, the 66 amu fragment is assigned to C\textsubscript{2}OCN\textsuperscript{−}, which does not shift when T\textsubscript{d} is substituted for T. The small 68 amu satellite peak from T is tentatively assigned to [C\textsubscript{2}OCN+2H]\textsuperscript{−} since it shifts to 69 amu, viz. [C\textsubscript{2}OCN+H+D]\textsuperscript{−}. However, it may also be related to the 64/65 amu peaks from T which remains unassigned. Between 48 and 60 amu for T we find a unique fragment pattern with peaks at 50, 52, 54, and 55 amu, the first of which is assigned here to C\textsubscript{2}CN\textsuperscript{−}, since it does not shift upon T\textsubscript{d} substitution. The latter three are assigned to NC\textsubscript{3}H\textsubscript{2}\textsuperscript{−}, HNC\textsubscript{3}H\textsubscript{3}\textsuperscript{−}, and OC\textsubscript{3}H\textsubscript{3}\textsuperscript{−}; for T\textsubscript{d} substitution this three peak pattern shifts to 54 amu (NC\textsubscript{3}D\textsubscript{2}\textsuperscript{−}), 57 amu (HNC\textsubscript{3}D\textsubscript{3}\textsuperscript{−}), and 58 amu (OC\textsubscript{3}D\textsubscript{3}\textsuperscript{−}), whereby the lack of a
59 amu peak (HNC\_3D\_4) precludes contributions of a HNC\_3H\_4\^- fragment to the 55 amu peak from T.

**Figure 8:** Detailed negative ion fragment patterns produced by 200 eV Ar\(^+\) ion irradiation of 20 ng/cm\(^2\) films of (a) thymine (T), and (b) thymine-methyl-d\(_3\)-6-d (T\(_d\)), both on a Pt substrate. The relative ion intensity is in arbitrary units (see Fig. 7).

Except for OCN\(^-\), the sequence of fragments between 37 to 45 amu in Fig. 8 is more difficult to interpret, since more than one fragment may in principle contribute to the peaks at 38 and 40 amu in T films. Hence, some of the anion peaks in the 37 to 45 amu range in Fig. 8 are only tentatively assigned. However, we note that (a) the 39 amu peak in T is most likely C\(_3\)H\(_5\)^-, since C\(_3\)H\(_3\) has an EA of 2.7 eV, and it is almost completely absent in T\(_d\), where a C\(_3\)D\(_3\)^- fragment would contribute to the already very intense OCN^- peak at 42 amu, and (b) the 43 amu peak in T does not shift in T\(_d\), suggesting formation of OCNH^- involving either of its two NH positions in T; this seems reasonable since it involves cleavage of two bonds, rather than three if the hydrogen in OCNH^- were to originate from H abstraction at a carbon site.
Moreover, since the peak at 40 amu in T does not shift to 44 amu in T<sub>d</sub>, it is likely that it originates from the formation of CN<sub>2</sub><sup>-</sup> (EA of CN<sub>2</sub> is 1.8 eV) and not C<sub>3</sub>H<sub>4</sub><sup>-</sup>.

The peak assignments at 24 and 25 amu in T films to C<sub>2</sub><sup>-</sup> and C<sub>2</sub>H<sup>-</sup> in Fig. 7 are confirmed by the observation that for T<sub>d</sub> films the 24 amu peak is unchanged while the 25 amu peak is absent (not shown), since the C<sub>2</sub>D<sup>-</sup> fragment (26 amu) will contribute to the strong CN<sup>-</sup> peak. We note that both the C<sub>2</sub> and C<sub>2</sub>H have high EA's of 3.3 and 3 eV [19], respectively, which may contribute to their formation mechanism. Between 10 and 20 amu for T (Fig. 8) we find that the CH<sup>-</sup> peak at 13 amu in T shifts to 14 amu in T<sub>d</sub>, and thus suggests assignment to CD<sup>-</sup>. The 17 amu peak in T is assigned to OH<sup>-</sup>, since it shifts to 18 amu in T<sub>d</sub>; since this is also associated with a shift of CH<sub>3</sub><sup>-</sup> (15 amu) to CD<sub>2</sub><sup>-</sup> (18 amu), this assignment is more tentative. However, we find that upon T<sub>d</sub> substitution the OH<sup>-</sup> peak decreases in relative intensity, while the 18 amu peak increases more than can be accounted for by the small amount attributable to CD<sub>2</sub><sup>-</sup>. Thus, the 18 amu peak is mainly attributed to OD<sup>-</sup>. Since the thymine molecules here have no OH groups, this suggests that here some of the large amount of O<sup>-</sup> formed by ion impact in the film may react by H abstraction prior to desorption as OH<sup>-</sup> or OD<sup>-</sup>. This mechanism has been observed in electron irradiated mixed films of O<sub>2</sub> containing hydrocarbons [21]. Here, the observation that both OH<sup>-</sup> and OD<sup>-</sup> can be formed in T<sub>d</sub> films, suggests that H abstraction by desorbing O<sup>-</sup> occurs at both CH and NH sites.

Regarding the formation of the lowest and highest mass anions, i.e. H<sup>-</sup> and [T-H]<sup>-</sup>, we note that the site of H removal is not identical to both. This is shown in Figure 9, which shows that while H<sup>-</sup> and D<sup>-</sup> formation from T<sub>d</sub> occurs with 1:2 stoichiometric ratio, deprotonation of T (or T<sub>d</sub>) seems to clearly occur mainly at the NH sites, and almost always results in formation of [T-H]<sup>-</sup> (125 amu) and [T<sub>d</sub>-H]<sup>-</sup> (129 amu). This suggest that the formation mechanisms are likely different for H(D) than for [M-H]<sup>-</sup> formation [15].
Figure 9: Detailed view of ion stimulated desorption (ISD) mass spectra for parent molecule [M-H]$^-$ formation by 200 eV Ar$^+$ impact on 20 ng/cm$^2$ films of (a) thymine (T), and (b) thymine-methyl-$d_3$-$d_6$-$d_9$ ($T_d$), both on a Pt substrate. The inset in (b) shows the H$^+$ and D$^+$ yield from $T_d$. The relative ion intensities are in arbitrary units (see Fig. 7).

3.3 Fragment origins and desorption energy thresholds

Given the chemical identification of several ionic fragments, it is possible to further specify their site origins within the thymine molecule. According to the molecular structure of thymine, the major cation fragment HNCH$^+$ (HNC$D^+$) is a result of cleaving the N(1)-C(2) bond and the C(5)-C(6) bond, and the HNC$_3$H$_4^+$ (HNC$_3$D$_4^+$) fragment is a result of breaking the N(1)-C(2) bond and the C(4)-C(5) bond. Fragment C$_2$H$_5^+$ (C$_3$D$_3^+$) arises from dissociation of the N(1)-C(6) bond and the C(4)-C(5) bond, with further loss of a hydrogen atom. The fragments [T-OCNH]$^+$, OCNH$_2^+$, CN$^-$ and OCN$^-$ can have three possible site origins, which are not distinguishable on the basis of the present results. Fragments [T-O]$^-$, O$^-$, H$/^+$ and CH$_3^+$ are results of breaking specific exocyclic bonds of thymine.

Since the fragmentations involve either endocyclic or exocyclic bond cleavage, both the positive and negative ion fragments can be sorted into two groups. In this respect, the cation fragments HNCH$^+$, C$_3$H$_5^+$, OCNH$_2^+$, HNC$_3$H$_4^+$($\pm$H), [T-OCNH]$^+(\pm$H), and the anion
fragments CN⁻, OCN⁻ clearly indicate endocyclic bond cleavage, while the H⁺, CH₃⁺, [T-O]⁺(±H), H⁻ and O⁻ originate from exocyclic bond cleavage. Thus, one is able to identify ion impact damage to the endocyclic and the exocyclic sites in thymine, as well as the dependence of fragment desorption on incident ion projectile and energy.

Figure 10 shows the relative yields of cation fragment desorption as a function of incident energy during Ar⁺ ion irradiation of a 200 ng/cm² thymine film, from which the desorption energy thresholds of major fragments can be approximated. Most cation fragments appear at energies near 15-30 eV, namely, HNC₃H₄⁺ (15-18 eV), HNCH⁺, [T-OCNH]⁺(±H), [T-O]⁺(±H) (~20 eV), and C₃H₃⁺ (~30 eV). The CH₃⁺ and H⁺ fragments appear at higher energies of about 40 and 60 eV, respectively.

![Relative Ion Yield vs. Ar⁺ Kinetic Energy](image)

**Figure 10:** Relative yields of positive ion fragment desorption as functions of incident ion energy during Ar⁺ ion irradiation of a 200 ng/cm² thymine film on Pt substrate. The yield curves for the various ions have been normalized here in intensity at 120 eV to compare their energy dependence. Their relative abundance can be seen in Figure 2.
The anion fragments appear generally at higher energies with H at 40 eV, CN\(^-\) and OCN\(^-\) at 60 eV, and O at about 70 eV (not shown here). It is interesting to note that for Ar\(^+\) impact the endocyclic cation fragments HNC\(_3\)H\(_4\)+, HNCH\(^+\), [T-OCNH]\(^+\), etc. appear at lower energies than the exocyclic fragments CH\(_3\)+ and H\(^+\). The detailed fragmentation dynamics leading to the observed ion desorption is discussed elsewhere [15].

Most of the major fragments observed here by Ar\(^+\) ion irradiation of thymine are also seen during irradiation with other ions, including Ar\(^{++}\), He\(^+\), CO\(^+\), NO\(^+\), O\(^+\), N\(_2\)+, N\(^+\), D\(_x\)+ (x=1-3). While these measurements can not be discussed here for sake of brevity, we find it worthwhile to note the general trends: (1) Anion fragments appear usually at higher incident ion energies than the cation fragments. (2) High mass incident ions tend to fragment thymine molecules more efficiently than low mass ions, and also result in the desorption of larger fragment ions. For example, during 50-200 eV Ar\(^+\) irradiation, the cation fragment spectra are dominated by HNCH\(^+\), HNC\(_3\)H\(_4\)+, etc., while during 50-200 eV He\(^+\) irradiation, the H\(^+\) fragment, instead of HNCH\(^+\), dominates the cation fragment yields, which suggests an enhancement of CH or CD bond cleavage by lower mass ion projectiles. (3) The energetic sequence of damage to endocyclic and exocyclic bonds of thymine also depends on the ion projectiles. During heavy ion (Ar\(^+\), Ar\(^{++}\), N\(_2\)+, N\(^+\)) impact, endocyclic fragments appear at lower energy than exocyclic fragments, while during light ion (He\(^+\), D\(_x\)+ (x=1-3)) impact, exocyclic fragments appear at lower energies than endocyclic fragments. These studies continue and will be discussed in detail elsewhere.

4. Summary and Conclusions

Thymine and thymine-methyl-d\(_3\)-6-d are irradiated by low energy (10 – 200 eV) ions in the condensed phase, and by 70 eV electrons in the gas phase. The ionic fragmentation products are measured by a quadrupole mass spectrometer. Both electron and ion impact
produces abundant positive ion fragments. The chemical composition of the ionic fragments has been identified by comparing the mass spectra of thymine and thymine-methyl-d3-6-d. The major cation fragments produced by Ar⁺ impact on thymine films are identified as HNC₃H₂⁺, HN(CH)CCH₃⁺, C₃H₃⁺, OCNH₂⁺, [T-OCN]⁺, [T-O]⁺, and [T+H]⁺. While ion impact produces an additional fragment [T-O]⁺, gas phase electron impact also leads to a fragment CO⁺ that is not seen in condensed phase ion impact. The anion fragments produced by Ar⁺ impact on thymine films are dominated by H⁻, O⁻, CN⁻ and OCN⁻, with smaller amounts of NC₃H₂⁻, HNC₃H₃⁻, OC₃H₃⁻ and other hydrocarbon anions. Both positive and negative ion fragment desorption involves exocyclic and endocyclic bond cleavage, which extends down to about 15-18 eV for positive ion fragments.

In the radiobiological context of heavy primary ion interactions in biological media, our measurements suggest that: (a) even at primary ion track-ends, i.e. well beyond the Bragg peak, significant ion damage to cellular DNA may occur, and (b) secondary hyperthermal ions, produced anywhere along the primary ion track, are also likely to cause similar damage. In both cases, DNA damage even at the lowest ion energies, e.g. below 1 eV/amu, involves complex fragmentation channels (damage clusters) that reach far beyond simple molecular ionization.

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References


CHAPTER 5 – Fragmentation Dynamics of Condensed Phase Thymine by Low Energy (10–200 eV) Heavy Ion Impact

5.1 Introduction

To better understand some of the fundamental ion-biomolecule interactions that lead to radiolytic DNA damage, we have begun a series of studies focused on low-energy ion damage to films of DNA and its components. After the first step, chapter 4, which focused on the identification of the charged fragments desorbing from films of thymine and deuterated thymine irradiated by low energy 10–200 eV Ar$^+$ ions, we now address the fragmentation dynamics of thymine induced by low energy (10-200 eV) Ar$^+$ ion irradiation that leads to the desorption of ionic fragments.

Here, we present measurements of the desorption dynamics of ionic fragmentation products formed during ion-beam irradiation of condensed DNA bases, and the desorption energy thresholds of these products. The fragmentation and desorption dynamics of thymine induced by such low energy ions also is investigated as a function of thymine film thickness, as well as ion irradiation time. Abundant fragments both in positive and negative charge states are observed. Cation fragment desorption appears at much lower threshold energies (near 15 eV) than anion fragment desorption, where the latter depends strongly on the film thickness. It is proposed that anion fragment formation and desorption results from projectile impact induced excitation of either (1) a neutral thymine molecule, followed by fragmentation and charge exchange between the energetic neutral fragment and the substrate (or film) and/or (2) a deprotonated mono-anionic thymine molecule to a dissociative state, followed by unimolecular fragmentation of the excited thymine anion. The H$^-$ and O$^-$
fragment formation may have a further contribution from dipolar dissociation, e.g. formation of electronically excited neutral thymine, followed by dissociation into O⁻ + [T-O]⁺, due to their reduced sensitivity to the film thickness. Positive ion fragment desorption exhibits no significant dependence on film thickness before the emergence of surface charging, and originates from a kinetically-assisted charge transfer excitation (i.e. kinetic and potential scattering¹). The results suggest that the potential energy of the incident ion plays a significant role in lowering the threshold energy of kinetic fragmentation of thymine. Measurements of the time dependent film degradation yields for 100 eV Ar⁺ suggest a quantum efficiency for degradation of about six thymine molecules per incident ion.

While the present experiments are focused on chemically inert Ar⁺ ions, which induce molecular damage by virtue of their kinetic and potential energy (I.P. = 15.7 eV), it is important in two aspects. First, it marks the low energy limit of kinetic damage to DNA caused by ion impact. Second, both the identification and dynamics of the fragmentation allow us to distinguish such kinetic and potential scattering effects from physico-chemical damage induced by reactive cations such as N⁺, particularly at ion energies below 10 eV. Such experiments are currently underway.

The following results and discussions are part of an article in press in The Journal of Chemical Physics by Zongwu Deng, Marjorie Imhoff, and Michael A. Huels, with the title: “Fragmentation Dynamics of Condensed Phase Thymine by Low Energy (10–200 eV) Heavy Ion Impact”. The same experimental method presented in section 2.3, and also used to obtain the results of chapter 4 is again used here.
5.2 Fragmentation of Condensed Phase Thymine by Low Energy (8 – 200 eV) Ar$^+$ Ions

Figure 1 shows typical mass patterns of both positive and negative ion stimulated desorption (ISD) produced by 200 eV Ar$^+$ ion irradiation of 200 ng/cm$^2$ thymine (T) films (~4 nominal ML). The major cation fragments are assigned, from low m/e (mass/charge ratio) to high m/e, to H$^+$, CH$_x^+(x=0-3)$, HNCH$^+$, C$_3$H$_3^+$, OCNH$_2^+$, HNC$_3$H$_4^+(\pm H)$, [T-OCNH]$^+(\pm H)$, [T-O]$^+(\pm H)$, as well as the parent cation [T+H]$^+$. Major anion fragments include H$, O^-$, CN$^-$ and OCN$^-$, and OH$. The parent anion [T-H]$^-$ is more clearly observable at lower film thickness.

**Figure 1** Typical positive (top panel) and negative (bottom panel) ion stimulated desorption (ISD) mass spectra from a 200 ng/cm$^2$ thymine (T) film on Pt, produced by 200 eV Ar$^+$ ion irradiation. The chemical identification of fragments was achieved by comparing ISD mass spectra of thymine and isotopically-labeled thymine; see text. The insets show close-ups of the deprotonated and protonated thymine signal; the structure of thymine is also indicated in the lower panel. Both cation and anion mass spectra have been normalized in intensity, such that the integral intensity of either mass spectrum from 0 – 200 amu is equal to one.

Chemical identification of the fragments is achieved by comparing mass spectra from thymine and partially deuterated thymine\(^2\) (thymine \(\alpha\alpha\alpha\alpha\beta_d\)) during both ion irradiation in the condensed phase, and electron impact (70 eV) in the gas phase; for the latter measurements we use the low profile electron impact ionizer of the QMS to obtain electron impact cracking patterns of the unirradiated thermally (100 °C) desorbing films. Therefore, the ion fragments in Fig. 1, produced by ISD, can be sorted into two groups in terms of their formation by either endocyclic or exocyclic bond cleavage. In this respect, the cation fragments HNCH\(^+\), C\(_3\)H\(_3\)\(^+\), OCNH\(_2\)\(^+\), HNC\(_3\)H\(_4\)\(^+\)(±H), [T-OCNH]\(^+\)(±H), and the negative ion fragments CN\(^-\), OCN\(^-\) clearly indicate endocyclic bond cleavage, while the fragments H\(^+\), CH\(_3\)\(^+\), [T-O]\(^±\)(±H), H\(^-\) and O\(^-\) originate from exocyclic bond cleavage. Similarly, while the small anion yields of 12 and 13 amu fragments (C\(^-\) and CH\(^-\)) may result from either, endo- or exo-cyclic bond cleavage, e.g. at the C7 or C6 position, respectively, the 24 and 25 amu fragments (C\(_2\)\(^-\) and C\(_2\)H\(^-\)) also indicate ring fragmentation most likely at the C5 or C6 positions.

The desorption yields of the ionic fragments strongly depend on the incident ion energy. Figure 2 shows the measured relative ISD yields of some of the typical positive and negative ion fragments as a function of incident Ar\(^+\) ion energy, from which the fragment desorption energy thresholds are derived. Most cation fragments appear at energy thresholds near 15-30 eV, including HNCH\(^+\), HNC\(_3\)H\(_4\)\(^+\) (15 eV), [T-OCNH]\(^+\)(±H) (20 eV), C\(_3\)H\(_3\)\(^+\), [T-O]\(^+\)(±H) (25 eV), and H\(^+\), CH\(_3\)\(^+\) (30 eV), while the protonated thymine has a desorption threshold of ~20 eV. The anion fragments appear at higher energies than the cation fragments, with H\(^-\) near 25 eV, CN\(^-\) and OCN\(^-\) at 40 eV, and O\(^-\) at 50 eV. Therefore, it appears that during Ar\(^+\) ion irradiation, damage to the endocyclic bonds of thymine, as
Figure 2 Desorption energy thresholds of typical positive (top panel) and negative (bottom panel) ion fragments produced by \(\text{Ar}^+\) ion irradiation of thymine films. The relative fragment ion yields are normalized at 100 eV incident \(\text{Ar}^+\) ion energy for ease of comparison. The cation fragments are measured from 200 ng/cm\(^2\) films, and the anion fragments from 100 ng/cm\(^2\) films.

<table>
<thead>
<tr>
<th>Fragment</th>
<th>H(^+)</th>
<th>CH(_3)^+</th>
<th>HNCH(^+)</th>
<th>C(_3)H(_5)^+</th>
<th>HNC(_3)H(_4)^+</th>
<th>[T-OCNH(_n)](^+)</th>
<th>[T-O(^-)]</th>
<th>[T+H(^+)]</th>
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<tr>
<td></td>
<td>40*</td>
<td>40*</td>
<td>20</td>
<td>30</td>
<td>18</td>
<td>[T-OCNH(_n)](^+) (n=0-2)</td>
<td>20*</td>
<td>20*</td>
</tr>
<tr>
<td>H(^-)</td>
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<td></td>
</tr>
<tr>
<td>O(^-)</td>
<td>40</td>
<td>70</td>
<td>40</td>
<td>35*</td>
<td></td>
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</table>

Table 1: Measured desorption thresholds (in eV) for some of the positive and negative ion fragments observed by \(\text{Ar}^+\) impact on 200 ng/cm\(^2\) Thymine films. The experimental uncertainty in the threshold values is generally about ±2 eV, except for fragments that have low intensity near threshold (marked with an asterisk), where the estimated uncertainty is about ±5±2 eV.
indicated by the desorption of HNC3H3\(^{+}\)(±H), HNCH\(^{+}\) and [T-OCNH]\(^{+}\)(±H), etc. tends to occur at somewhat lower incident ion energies than exocyclic bond cleavage, leading to the removal of H or CH\(_3\) moieties. The desorption energy thresholds of the major positive and negative ion fragments are summarized in Table 1.

Another notable characteristic of the ISD yields is their film thickness (coverage) dependence. Figure 3 shows the evolution of the mass spectra of H\(^{-}\), O\(^{-}\), OH\(^{-}\), CN\(^{-}\) and OCN\(^{-}\) produced by 200 eV Ar\(^{+}\) ion irradiation of thymine films as a function of the film thickness (coverage) in the range of 20-800 ng/cm\(^2\).

![Figure 3 Mass spectra evolution of major negative ion fragment desorption as a function of thymine film thickness. A 200 ng/cm\(^2\) thymine film corresponds to ~4 nominal monolayers. Note that the desorption of CN\(^{-}\) and OCN\(^{-}\) fragments is suppressed by multi-layer films.](image)

The anion yields tend to decrease with increasing thymine film thickness. However, the desorption of CN\(^{-}\) and OCN\(^{-}\) fragments exhibit a more significant dependence on the film thickness than the H\(^{-}\) and O\(^{-}\) fragments, which is also demonstrated quantitatively in Figure 4. The desorption yields of OCN\(^{-}\) and CN\(^{-}\) fragment exhibit a sharp decrease in the film thickness range of 20 –200 ng/cm\(^2\) and are nearly suppressed from films above 200 ng/cm\(^2\), while the H\(^{-}\) and O\(^{-}\) desorption yields exhibit a more gradual decrease in the whole thickness range. It was estimated that a nominal monolayer of thymine consists of ~2.1-3.3\times10^{14}
molecules/cm² depending on the order or randomness of the molecular orientation if the molecules are evenly distributed on the surface. In this context, a 200 ng/cm² thymine film corresponds to about 4 nominal monolayers. However, in the sub-monolayer regime, it has been shown that the thymine molecule is adsorbed on metal surfaces with its molecular plane perpendicular to the substrate surface. In that case, a nominal monolayer of thymine may require more molecules. Other measurements have shown that clustered film growth of evaporated thymine reaches full metal coverage in the 2-3 nominal monolayer regime. Hence, the sharp decrease in CN⁻ and OCN⁻ desorption seems to occur prior to the full surface coverage of the Pt substrate by thymine molecules.

**Figure 4** Film thickness dependence of typical negative and positive ion fragment yields. For clarity, the relative yields are all normalized to their maxima. Note the strong dependence of anion fragments, typically CN⁻ and OCN⁻, on the film thickness. The two cation fragments show no significant change from film thickness between 20-400 ng/cm². The sharp decrease above 400 ng/cm² is due to the emergence of charging effect caused by the poor electric conductivity of thymine films on Pt substrate.

Several dynamic scenarios may account for the characteristics of the observed negative ion fragment desorption. The first involves kinetic fragmentation and subsequent charge exchange between the substrate and the energetic fragments. Impact of an incident heavy ion on thymine transfers part of its kinetic energy into the electronic and/or vibrational energy of the thymine molecule and promotes it to a manifold of dissociative excited states,
followed by subsequent decay and rupture of molecular bonds. The resultant energetic neutral fragments with positive electron affinity (EA) are then ionized via tunneling charge exchange from the substrate or film (e.g. via capture of an electron from the substrate), yielding a desorbing anion. This would require neutral fragments with high EA's (in addition to some kinetic energy) which here includes CN and OCN, both with EA's near 3.8 eV. A salient feature of this tunneling charge exchange mechanism for anion formation is an exponential dependence of the ionization probability on an energy barrier determined by the difference of the electron affinity of the departing particle and the surface work function of the substrate. The latter is highly sensitive to the surface adsorbates. While adsorption of low work function materials, such as alkalis, strongly enhances ion stimulated electron and anion desorption, which occurs prior to the full coverage of the substrate, adsorption of dielectric materials will raise the surface work function, and thus decrease anion desorption. This might be the case of thymine adsorption on Pt here, and explain the strong dependence of anion desorption on the film thickness. The above scenario is basically a kinetically assisted fragmentation mechanism.

A second possible anion fragment formation mechanism involves the adsorption state of thymine molecules on metal surfaces. Several studies have demonstrated that in the sub-monolayer regime, adsorption of thymine on metal surfaces leads to the deprotonation of the molecule at the nitrogen positions, and results in the adsorption of a mono-anionic thymine molecules on the surface. Here the negative charge is delocalized within the heterocyclic ring and the exocyclic groups, due to its resonance delocalized bond structure, with its electronic structure depending on its tautomeric forms. As a consequence, ion impact may transfer part of the projectile's kinetic energy into electronic excitation energy of the anionic ground state thymine molecule, and promote it to a dissociative state. Subsequent
unimolecular dissociation then leads to the formation and desorption of the negative ion fragments. Such a mechanism was proposed to account for electron and anion desorption from oxidized metal surfaces induced by photon and low energy ion impact.\textsuperscript{13,14,15} Since thymine deprotonation upon adsorption is a result of interactions with the metal substrate, it occurs only in the monolayer and sub-monolayer regime. Multi-layer films may impede the access of the incident Ar\textsuperscript{+} to the anionic thymine molecules on the Pt substrate, since the incident heavy ions will interact mainly at the film surface. This is also consistent with the observation of a more significant ISD parent anion [T-H\textsuperscript{−}] yield from the sub-monolayer films compared to multi-layer films. Hence, the anion desorption yields would also exhibit a strong dependence on thymine film thickness (coverage). This scenario is different from the previous in that the parent anion formation occurs upon thymine adsorption.

We note that the desorption yields of H\textsuperscript{−} and O\textsuperscript{−} are significantly less sensitive to the film thickness than the CN\textsuperscript{−} and OCN\textsuperscript{−} yields, and occur with significant intensity in multi-layer films. This suggests a possible contribution of another formation mechanism, most likely dipolar dissociation (DD), following the electronic excitation of a neutral thymine molecule by transfer of the incident ion's kinetic energy. Dipolar dissociation of the excited T\textsuperscript{*} along a C-H or C=O bond will lead to simultaneous formation of H\textsuperscript{−} + [T-H\textsuperscript{−}], or O\textsuperscript{−} + [T-O\textsuperscript{−}]. The measured O\textsuperscript{−} desorption threshold is near 50 eV, such that below that energy [T-O\textsuperscript{+}] formation will not involve DD (or DD will not create [T-O\textsuperscript{+}] with sufficient kinetic energy to escape the charge induced polarization barrier at the surface and desorb). While the very small yield of [T-H\textsuperscript{+}] precludes its desorption threshold to be established with certainty, it is estimated to be at least 20 eV above the H\textsuperscript{−} formation threshold which is at near 25 eV, and DD leading to H\textsuperscript{−} + [T-H\textsuperscript{+}] formation is not likely to occur below 45 eV incident Ar\textsuperscript{+} energy. Alternatively, hydrogen bonding between molecules may lead to partial negative charge on a
CO, NH, or CH moiety, resulting in H, or O\(^-\) formation upon ion impact excitation. Although OH\(^-\) formation may also occur in that case, the presence of enol forms in the film, or even reactive scattering of 1 – 5 eV secondary O\(^-\) in the film prior to desorption will also result in OH\(^-\) formation. The latter has been observed to occur in electron irradiated hydrocarbon films containing O\(_2\).\(^{16}\) In any case, all of the above anion formation mechanisms require a significant energy transfer to excite and dissociate the thymine molecule, in agreement with the higher energy thresholds of anion fragment desorption.

Since low energy electron interactions with thymine also lead to the formation of anion fragments via dissociative electron attachment,\(^{17}\) any secondary electrons produced by the incident ion impact within the film or substrate may in principle also contribute to the observed negative ion yields. Here, secondary electron emission is observed during ion impact on clean Pt substrate and thin thymine films, and appears as a background in the QMS negative ion mass spectrum with near-exponentially decreasing intensity. The secondary electron emission strongly depends on the primary ion type, the film thickness, and primary ion energy. Light ions (He\(^+\), D\(_x\)\(^+\)(x=1-3)) lead to much stronger electron emission from thin thymine films than heavy ions (Ar\(^+\), Ar\(^{++}\), N\(_2\)\(^+\) and N\(^+\)), since lighter projectiles are more likely to penetrate the dielectric films and reach the Pt substrate. Increased deposition of thymine molecules on Pt suppresses the electron emission as well as the negative ion desorption for both heavy and light ion impact. The Ar\(^+\) induced secondary electron emission energy thresholds from clean platinum (near 65 eV) are close to the negative ion desorption energy thresholds from thin thymine films, while for thick films secondary electrons are not observed at Ar\(^+\) energies below 200 eV. However, significantly less anions both in yield and species are observed during light ion impact in contrast to their stronger electron emission. Hence, secondary electron interactions are not believed to be a
major contribution to the anion desorption observed here. Moreover, this is also in agreement with the substantially different mass spectra of the present ISD anion yields, compared to those from low energy (40 eV) electron impact on thymine films, where $H^-$ is the most abundant fragment by almost three orders of magnitude, compared to the small or trace amounts of $O^-$, $CN^-$ and $OCN^-$ fragments.\(^3\),\(^18\)

The desorption of positive ion fragments occurs at much lower threshold energies than the negative ion fragments. It also exhibits a much weaker thickness (coverage) dependence than the anion fragments, as also shown in Figure 4. The positive ion fragment yields show no significant decrease from films between 20-400 ng/cm\(^2\), but decrease rapidly thereafter, probably due to the emergence of surface charging, caused by the incident ions coupled with the poor electric conductivity of thymine films. This is more clearly seen in Figure 5 which shows the mass spectral evolution of typical cation fragments $HNCH^+$, $C_3H_3^+$ and $HNC_3H_4^+$ as a function of film thickness (coverage) in the range of 20-800 ng/cm\(^2\). Desorption of $HNCH^+$, $C_3H_3^+$ and $HNC_3H_4^+$ from multi-layer films is obvious, suggesting that the positive fragment yields are much less sensitive to the film thickness.

\textbf{Figure 5} Mass spectra evolution of typical positive fragments as a function of the thymine film thickness. They appear not sensitive to the film thickness between 20-400 ng/cm\(^2\). The sharp decrease above 400 ng/cm\(^2\) is due to the emergence of positive surface charging caused by the poor electric conductivity of thymine.
Since Ar atoms are chemically inert, fragmentation of thymine by Ar$^+$ ions is caused by its kinetic and/or potential energy, i.e. charge state and ionization potential (I.P.). We first consider a purely kinetic fragmentation mechanism. The minimum energy ($E_{\text{min}}$) required for the production and desorption of a specific fragment ion is $E_{\text{min}} = D_o + E_i + K E_F$, where $D_o$ is energy required for breaking the molecular bonds to yield the fragment, $E_i$ the energy to ionize the fragment, and $KE_F$ the kinetic energy needed by the fragment ion to overcome the image charge induced desorption barrier at the metal, or the film surface.

For example, for the production of HNCH fragments, the total $D_o$ to cleave the C5-C6 and N1-C2 bonds is about 10.5 eV.$^{19}$ For thin thymine films on Pt, e.g. sub-monolayers, $E_i$ for HNCH is $\approx 1.4$ eV, and is determined by the difference of its ionization potential (I.P. $\approx 7$ eV,$^{20}$ and the surface work function of the metal substrate ($\approx 5.64$ eV$^{21}$).$^7$ In order to desorb, the ion fragment must have sufficient kinetic energy to escape the image charge potential ($E_{\text{icp}}$) at the metal surface, which is given by $E_{\text{icp}} \approx e^2/4(z+L_s),$ $^{22}$ where $e$ is the ion’s charge, $z$ its distance to the metal, and $L_s$ the Fermi-Debye “screening length” of about 0.5-1 Å. If the HNCH$^+$ is formed at about 1-2 Å from the metal surface $E_{\text{icp}} = 2-1$ eV. Thus, for HNCH$^+$ desorption from sub-monolayer films $E_{\text{min}} \approx 13-14$ eV. For thick multilayer thymine films $E_i = 7$ eV (I.P. of HNCH) at the surface, since the metal’s work function is now largely screened by the dielectric molecular layers, but now the fragment ion must overcome a charge induced polarization potential ($E_p$) at the surface of the thick dielectric thymine film; this $E_p$ depends in part on the polarizability of thymine, and molecular orientation of the adjacent molecules. For simple hydrocarbon films 4 – 5 monolayers thick, $E_p$ is estimated to be $\approx 1.1$ eV.$^{23,24}$ It is likely that an $E_p$ of similar strength is induced here in thymine films, given the similar film thickness. Thus, at the surface of thicker multilayer films the $E_{\text{min}}$ needed for desorption of HNCH$^+$ is about 18.6 eV.
In the present experiments the fragmentation of a thymine molecule likely involves only a single incident Ar\(^+\) ion, given the timescales of unimolecular dissociation (\(\sim 10^{14}\) second) compared to the ion arrival timescale of \(\sim 10^9\) second/ion, i.e. the incident ion current density (\(\sim 10^9\) ions/second). Using a classical binary collision approximation\(^{25,26}\), the maximum kinetic energy (in the laboratory frame) that can be transmitted to a target particle by momentum transfer from the low energy incident ion is \(4m_1m_2/(m_1+m_2)^2 \cdot E_{\text{lab}}\), where \(m_1\) and \(E_{\text{lab}}\) are the mass and the laboratory kinetic energy of the incident particle, respectively, and \(m_2\) is the mass of the specific target involved in the collision. Since the formation of HNCH\(^+\) likely involves a single binary collision between an incident Ar\(^+\) ion and a thymine molecule (or a C or N atom-center of thymine), this yields a maximum energy of \(\sim 0.7E_{\text{lab}}\) received by either target (i.e. whole molecule, or atom-center); thus for the observed desorption threshold for HNCH\(^+\) of 15 eV Ar\(^+\) ion energy (i.e. \(E_{\text{lab}}\)) the target receives about 11 eV. This is \(\sim 2\) and 7.6 eV below the \(E_{\text{min}}\) for submonolayer and multilayer thymine films, respectively. Furthermore, given the collision and desorption geometries in the present experiments, fragment desorption requires very large recoil angles and at least two collisions (that of the projectile with the target, and that of the fragment prior to desorption), which significantly reduces the energy accessible to the fragmentation and desorption processes. Hence, at the observed threshold of 15 eV, the energy received by the thymine target via purely kinetic scattering mechanisms is insufficient to produce the observed HNCH\(^+\) fragments.

Alternatively, the Ar\(^+\) ion may ionize and excite a thymine molecule to a dissociative state via charge exchange (potential energy transfer). However, since the maximum potential energy that can be transferred is equal to the I.P. of Ar (ca. 15.7 eV)\(^{21}\) \(\leq E_{\text{min}}\) for mono- or multilayer films (13 - 18.6 eV), most of the resultant fragments would remain in the film,
rather than desorb from the surface, due to the low kinetic energy provided by this purely potential scattering mechanism. Therefore, the potential energy of Ar$^+$ alone cannot lead to the ion-stimulated desorption observed here.

Therefore, near the desorption threshold, both the kinetic and potential energy of the Ar$^+$ ions are likely involved in the fragmentation and desorption processes, i.e. kinetic and potential scattering$^1$. It is expected that a thymine molecule is ionized and excited via charge exchange (potential energy transfer) with an incident Ar$^+$ ion, whereby the kinetic energy of the incident Ar$^+$ ion further contributes, e.g. via momentum transfer or inelastic scattering, to the molecular fragmentation; this provides the required kinetic energy for the fragments to overcome the charge induced image and/or polarization barrier to desorb from the film. Due to the poor electric conductivity of dielectric thymine films on the Pt substrate, the lifetime of the electronically excited cation state of thymine, as produced via charge exchange, is long enough to allow for the molecular dissociation to occur. In a sense, this is related to ion impact induced “electronic sputtering”, whereby electronic excitation energy is transferred to the target molecule either by inelastic projectile energy-loss, or by the electric field oscillations induced along the ion’s track in the film; particularly in dielectric/insulating molecular solids with large band gaps, these electronic excitations are greater than the inter-molecular binding energies, and are furthermore well localized. Thus the electronic excitations can induce nuclear motion and/or bond deformation, which may lead to dissociation.

Within this scenario, charge exchange with the metal substrate may not be a necessary step for the desorption of positive fragments, which accounts for its insensitivity to the film thickness. Meanwhile, ionization and excitation by potential energy transfer (charge exchange between the molecule and Ar$^+$) is energetically more efficient than momentum
transfer, which may account for the much lower desorption energy thresholds of positive ion fragments. In other words, the potential energy transfer of an incident positive ion plays a significant role in lowering the threshold energy of kinetic dissociation of thymine, and desorption of the fragments.

Figure 6 shows the irradiation time dependence for the desorption of [T+H]^+, [T-O]^+, as well as the ring fragments [T-OCN]^+ and HNC_3H_4^+ for 100 eV Ar^+ incident on a 200 ng/cm^2 (4 ML) film of thymine. The beam current was 18 nA (1.12x10^{11} ions/s), and the beam spot on the target film was about 4 ± 1 mm in diameter. We note that while the signal of [T+H]^+ and [T-O]^+ decays rapidly with irradiation time in an exponential fashion, the [T-OCN]^+ and HNC_3H_4^+ fragment yield decays much slower.

![Figure 6 Irradiation time dependence for select ion fragments desorbing from a 200 ng/cm^2 thymine film during 100 eV Ar^+ impact. The solid curve is an exponential fit to the [T+H]^+ data. The HNC_3H_4^+ has been multiplied by a factor of 0.5 for ease of comparison, and the inset shows the ion impact energy threshold for formation and desorption of [T+H]^+. The yield ratio N_i/N_f corresponds to integrated signal of a specific desorbing ion mass divided by the total integrated ion desorption yield of all masses from 0 – 200 amu.](image)

This is attributed to the fact that while formation of the latter fragments can proceed even when a thymine molecule has been damaged by a previous ion collision, e.g. HNC_3H_4^+ formation from [T-O] or [T-OCN] in the film is still possible by subsequent ion impact,
formation and desorption of \([T+H]^+\) and \([T-O]^+\) can only occur if the incident Ar\(^+\) collides with an intact thymine molecule. Therefore the irradiation time dependent signal decay of \([T+H]^+\) is a measure of the total film degradation (or surviving thymine) induced by fragmentation and/or desorption. From a fit to the initial low exposure (linear, low ion dose limit) region of the \([T+H]^+\) signal decay we find a \((N_t/N_i)\) degradation rate of 0.005/sec. From the known Ar\(^+\) beam density of 9x10\(^{11}\) ions/s/cm\(^2\) and the density of thymine (1.5g/cm\(^3\)) we can estimate a total degradation yield of about 5.5-6/ion. In other words the impact of one Ar\(^+\) ion with 100 eV will degrade about six thymine molecules in the film to the extent that subsequent ion impact can no longer yield desorption of \([T+H]^+\). The responsible mechanisms includes all degradation channels, namely: (a) channels that lead to desorption of the entire molecule (e.g.[T+H]\(^+\) desorption), (b) channels that lead to formation of fragment ions (and/or neutrals - not measured here) followed by fragment desorption, and (c) channels that lead fragmentation without desorption.

This is consistent with "knock-on" sputtering models in secondary ion mass spectroscopy, and suggests that the incident heavy ion will undergo multiple scattering events at the film surface or upon entering the film, leading to desorption of thymine and/or the initial fragments formed by the first and subsequent binary encounters. Furthermore here, at 100 eV Ar\(^+\) ion energy, many of the initial fragments will have trajectories into the film, or along its surface, and may carry significant kinetic energies; thus they are also likely to damage adjacent thymine molecules in the ensuing collision cascade.

For example, in a multilayer film, using the classical binary collision model\(^{25, 26}\) for target recoil angles near 90\(^o\) (e.g. into the film or along its surface), the kinetic energy transferred to a thymine in the first encounter is about 0.7\(E_{lab}\) (supra vide) of the incident 100 eV Ar\(^+\) energy, or 70 eV; if the Ar\(^+\) has been neutralized in the collision, the total energy
transferred to the thymine is about 86 eV. In either case, the Ar kinetic energy after the encounter is reduced to 30 eV, but still sufficient for endocyclic or exocyclic bond cleavage in one or several adjacent thymine molecules. If the 86 eV transmitted to the first thymine molecule results in the formation of HNCH\(^+\) (D\(_o\)+E\(_t\) \approx 17.5\) eV) the excess energy (\(\Delta E\)) is about 68.5 eV. From momentum conservation the kinetic energy of the HNCH\(^+\) fragment E\(_f\) = \{1- (m\(_f\)/M)\} \times (\Delta E - E\(^*\)), where m\(_f\) and M are the fragment and molecule masses (28 and 126 amu), respectively, and E\(^*\) the internal excitation energy of the products. For E\(^*\) = 0, the E\(_f\) will be near 53 eV for HNCH\(^+\), and 15.5 eV for the associated C\(_4\)H\(_4\)NO\(_2\) radical fragment, i.e. quite sufficient to damage several adjacent thymine molecules to such an extent that subsequent ion impact will no longer result in the desorption of [T+H]\(^+\). Alternatively, if the 86 eV transferred by the Ar\(^+\) in the initial binary collision does not result in fragmentation, but is redistributed into ionization and kinetic recoil energy of the entire molecule (I.P. of thymine \(\approx 9.5\) eV\(^{27}\)), the resulting T\(^+\) may posses a kinetic energy up to 76 eV; thus, scattering into the film or along its surface, it may also damage several adjacent molecules.

We note that the measured desorption threshold for [T+H]\(^+\) is near 17—20 eV (inset Figure 6); in a kinetically-assisted charge transfer excitation, proposed here, the energy transferred by the 20 eV Ar\(^+\) to the thymine target may be as large as \(0.7E_{lab}(Ar^+) + I.P.(Ar) \approx 30\) eV, while the total energy required to ionize thymine (\(\approx 9.5\) eV) and for the T\(^+\) to remove a hydrogen from an adjacent molecule (ca. 5-6 eV\(^{19}\)) is at most 16 eV. Even if one neglects the energy released by the H-T\(^+\) (or \(^7\)H-T) bond formation, this leaves sufficient \(\Delta E \approx 14\) eV to be redistributed into kinetic (or internal) energy of the desorbing [T+H]\(^+\).
5.3 Summary and Conclusions

To better understand the type of ion-biomolecule interactions that can lead to radiation damage of DNA by either secondary ions, or at primary ion track ends, we have performed a series of studies on the fragmentation of condensed phase thymine by low energy ions. In this work, the degradation of thymine films induced by 8 – 200 eV Ar⁺ irradiation is investigated by ion stimulated desorption (ISD) of positive and negative ions. The dependence of fragment ISD yields on incident ion energy, film thickness, and ion irradiation time is presented. Ar⁺ ion irradiation of thymine films produces a multitude of fragments that are sufficiently energetic to overcome the image charge/polarization barrier at the surface and desorb. Observed fragments include H⁺, CHₓ⁺(x=0-3), HNCH⁺, C₃H₄⁺, OCNH₂⁺, HNC₃H₄⁺(±H), [T-OCNH]⁺(±H), [T-O]⁺(±H), [T+H]⁺, H⁻, O⁻, CN⁻, OCN⁻, and many others. The positive ion fragments appear at much lower primary ion impact energies than the negative ion fragments.

The desorption of negative ion fragments exhibits a strong dependence on film thickness. It is proposed that at small film thickness the anion fragments result from a kinetic (projectile impact) excitation of either (1) a neutral thymine molecule, followed by charge exchange between the substrate and the fragment, or (2) a deprotonated mono-anionic thymine ion to a dissociative state, followed by unimolecular dissociation. The ISD yields of H⁻ and O⁻ may have another contribution from dipolar dissociation due to their reduced sensitivity on film thickness, compared to the ring fragments CN⁻ and OCN⁻. Conversely, the cation fragments exhibit a much weaker dependence on the film thickness before the emergence of surface charging, and are likely to originate from a kinetically-assisted charge transfer excitation, especially near their desorption thresholds. In terms of these results, it is concluded that the potential energy of an incident heavy cation plays a significant role in
lowering the threshold energy of kinetic fragmentation of thymine, and desorption of the products.

Much like traditional models of molecular excitation, single ionization, or free radical production induced by high energy photon or electron tracks in solid media, the fundamental ion-molecule interactions observed here will be generally observable in any molecular system (however somewhat modulated by the particular physical and chemical environment of the target). Thus, they are expected to occur in living cells as well, and a full understanding of the biological effects of highly ionizing heavy-particle radiation must incorporate a detailed knowledge of the secondary ion and radical reaction cascades induced along the primary ion tracks.

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References


18 In these experiments it is found that the very small yields of O\textsuperscript{−}, CN\textsuperscript{−}, and OCN\textsuperscript{−} can only be observed under specific conditions, namely: (1) thick thymine films above 10 ML, and (2) high electron beam currents up to 500 nA. The reason is that electron impact (even at 40 eV) in films of DNA bases produces anion fragments with insufficient kinetic energies to overcome the charge induced polarization barrier (ca. 1.1 eV) at the film surface. Thus, in order to desorb sufficient negative charge must accumulate in the thick films by the high incident electron beam currents, such that the anion fragments are expelled by the increasing adjacent charge density. However, due to the high electron beam currents it is not clear if the fragments are formed from intact, or already degraded bases. See ref. 3, and (b) H. Abdoul-Carine, P. Cloutier, and L. Sanche. Radiat. Res. 155, 625 (2001), and (c) M.-A. Hervé du Penhoat, M. A. Huels, P. Cloutier, J.-P. Jay-Gerin, and L. Sanche, J. Phys. Chem. B 108, 17251 (2004).


A gas-phase-like binary collision approximation, based on energy and momentum conservation, is used here, because (a) in the present dielectric physisorbed systems (condensed van der Waals solids) the molecular binding energies (meV range, up to 1 eV for H-bonding in the thymine films) are much smaller than the Ar⁺ projectile energies (8 - 200 eV), and (b) typical periods for lattice or phonon vibrations (10⁻¹² s)²⁵ are larger than the average collision times of about 10⁻¹⁴ s, i.e. the time for a 20 – 200 eV Ar⁺ to travel a distance of 1 Å in the film. Collision times, however, are similar to times for intra-molecular vibrations (≈ 10⁻¹⁴ s).

CHAPTER 6 – Low energy (1-500 eV) atomic and molecular ion damage to thymine and partially deuterated thymine films

This chapter focuses on the fragmentation of thymine and deuterated thymine (thymine-methyl-d$_3$-6-d) by 10-500 eV He$^+$, D$^+$, D$_2$$^+$, D$_3$$^+$, N$^+$, N$_2$$^+$, Ar$^{++}$ ions investigated by ISD mass spectrometry. Thymine and deuterated thymine are condensed on a polycrystalline Pt substrate and irradiated following the experimental method described in section 2.3 of chapter 2. The results in this chapter are compared to our previous results from Ar$^+$ irradiation of similar films, and are part of two articles currently in preparation for publication, entitled: “10-100 eV He$^+$ Ion Induced Damage to DNA Components in the Condensed Phase” by Zongwu Deng, Marjorie Imhoff, Ilko Bald, Eugen Illenberger, and Michael A. Huels, and “Low Energy (5-100 eV) Atomic and Molecular Ion Interactions with Condensed Phase Thymine”, by Zongwu Deng, Marjorie Imhoff, and Michael A. Huels. While the former article discusses hyperthermal He$^+$ damage to thymine, deuterated thymine, as well as D-ribose, 2-deoxy-D-ribose, and thymidine, here (in section 6.3) we focus only on the results on thymine and deuterated thymine, which are part of that paper.

6.1 Introduction

As detailed in chapter 1, low energy ion-biomolecule interactions can occur in all types of ionizing radiation contexts, and involve damage induced by primary or secondary ions. To date, their propensity to damage relevant targets in cells, such as DNA, or its components, remains greatly unexplored on a molecular level. This is particularly the case for (a) protons or primary heavy ions, and the abundant secondary ions produced along their
tracks with energies up to several hundreds of eV, in addition to their different charge states, as well as (b) the secondary ions produced with energies from thermal up to tens of eV in conventional radiation therapy, which can all interact in biomedia via different pathways and lead DNA damage via either kinetic, potential, as well as reactive scattering.

In the results presented in chapters 4 and 5, we have demonstrated that fragmentation of thymine by Ar⁺ ion impact can occur at energies as low as 15 eV, and leads to the desorption of abundant cations and anions, including H⁺ and H⁻, as well as over 30 different fragments related to endocyclic and exocyclic bond cleavage. Here we show that H⁺ desorption appears to be strongly enhanced during He⁺ ion impact, compared to much heavier projectiles such as Ar⁺, or N₂⁺. As DNA base pairs are formed by hydrogen bonds involving only N-bound H atoms of the bases, damage of the N-H bond by ionizing radiation is of particular biological importance. Indeed, H loss from DNA subunits is observed as a predominant event in both the gas and condensed phase during low energy electron impact as a result of dissociative electron attachment. A preferential scission of the N-H bonds of DNA bases was also observed in the gas phase leading to the formation of deprotonated base anions (Abdoul-Carime et al., 2004). In the following section (section 6.2) of this chapter, we first report on an observation of the ionic H desorption induced by low energy He⁺ ion irradiation of thymine films condensed on polycrystalline Pt substrate, with the emphasis on their chemical origin and the dynamics of He⁺ ion enhanced H⁺ desorption, as well as reactive scattering of the He⁺, leading to H-abstraction and HeH⁺ formation.

The last section (section 6.3) of this chapter focuses on the role played by the different incident ion characteristics such as mass, charge state, energy, and chemical reactivity. We extend the projectile range to Ar⁺⁺, N₂⁺, N⁺, D₃⁺, D₂⁺ and D⁺ ions to particularly investigate the following aspects: (a) the difference caused by the charge state of the ion projectiles
(singly or doubly charged positive ions, or molecular ions); (b) the role of the potential energy (I.P.) of positive ions; (c) the role of the kinetic energy carried by the projectiles; and (d) the role of chemical reactivity of individual ions.

6.2 10-100 eV He\(^+\) Ion Induced Damage to DNA Components in the Condensed Phase

6.2.1 Results and discussion

Figure 1 shows the typical mass patterns of both positive and negative ion fragments produced by 100 eV Ar\(^+\) and He\(^+\) ion irradiation of 200 ng/cm\(^2\) thymine films (~4 nominal monolayers).

![Diagram](image)

**Figure 1** Typical positive and negative (insets) fragment pattern of ion stimulated desorption from 200 ng/cm\(^2\) thymine (T) films on Pt substrate during 100 eV (a) Ar\(^+\) and (b) He\(^+\) ion irradiation. Note the strong enhancement of H\(^+\) desorption induced by He\(^+\) ion irradiation as well as the appearance of HeH\(^+\) ions.
The cation fragments are identified from low m/e (mass/charge ratio) to high m/e as 
\( H^+ \), \( CH_2^+(x=0-3) \), \( HNCH^+ \), \( C_2H_3^+ \), \( OCNH_2^+ \), \( HNC_2H_4^+(\pm H) \), \( [T-OCNH]^+(\pm H) \), \( [T-O]^+(\pm H) \), as well as \( [T+H]^+ \). Major negative ion fragments are \( H^- \), \( O^- \), \( CN^- \) and \( OCN^- \), etc. Chemical identification of the fragments was achieved by comparing mass spectra of thymine and thymine-methyl-d\(_3\)-6-d (T\(_d\)) during ion irradiation in the condensed phase, and electron impact (70 eV) in the gas phase (see chapter 4). The desorption dynamics of the major positive and negative ion fragments induced by \( \text{Ar}^+ \) ion irradiation has been discussed in chapter 5 on the basis of their dependence on the incident ion energy and the film thickness. We note that the in the anion mass spectrum for \( \text{He}^+ \) impact there is a small background signal, that decreases exponentially with mass. This background is due to secondary electrons entering the QMS, and is observed here only for lower mass atomic ion beams, which can more easily penetrate the organic films and create secondary electrons at the Pt substrate.

In figure 1 we immediately note the following: (1) the relative \( H^+ \) yield by \( \text{He}^+ \) impact is almost two orders of magnitude higher than for \( \text{Ar}^+ \) impact; (2) \( \text{He}^+ \) impact induces less endocyclic bond cleavage in both anion and cation yields, compared to \( \text{Ar}^+ \); (3) 100 eV \( \text{He}^+ \) impact leads to formation and desorption of \( \text{HeH}^+ \); and (4) \( \text{He}^+ \) impact at 100 eV (25 eV/amu) still induces desorption of \( [T+H]^+ \), alias at smaller yields compared to 100 eV (2.5 eV/amu) \( \text{Ar}^+ \) impact.

Desorption of the anion fragments has higher threshold energies than the cation fragments, and exhibits a significant dependence on film thickness. Figure 2a shows the yield dependence of \( \text{Ar}^+ \) stimulated \( H^- \), \( O^- \), \( CN^- \), \( OCN^- \), \( \text{HCNH}^- \) and \( \text{HNC}_2\text{H}_4^+ \) fragments on the film thickness (coverage). The anion fragment yields decrease rapidly with the increase in film thickness. It was proposed that the negative ion fragments result from kinetic fragmentation via several possible pathways: (1) kinetic excitation and fragmentation of a
neutral thymine molecule followed by ionization of the fragments via charge exchange with the substrate. The ionization probability strongly depends on the surface work function of the substrate, which is highly sensitive to surface adsorbate. Adsorption of dielectric materials such as thymine raises the surface work function and thus decreases anion desorption. (2) kinetic excitation of a deprotonated monoanionic thymine molecule to a dissociative state followed by unimolecular fragmentation. It is known that in the submonolayer regime, adsorption of thymine on metal substrates leads to the formation of deprotonated monoanionic thymine molecule as a result of the interaction with the substrate (McNutt et al., 2002; Li et al., 1999; Haiss et al., 1998).

![Graph](image)

**Figure 2** Thickness dependence of (a) Ar$^+$ stimulated H, O, CN, OCN, HNCH, HNC$_3$H$_4^+$ and (b) He$^+$ stimulated H, O, H$^+$ desorption yields. The yields are normalized to the maximum yield for clarity. Anion yields decrease rapidly with increase in film thickness, while the cation yields shows only a slight decrease between thickness of 20-400 ng/cm$^2$. Above 500 ng/cm$^2$, charging effects emerge, which cause the sharp decrease from 500 to 800 ng/cm$^2$. 

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Both of the above two scenarios explain the high desorption threshold energy and the strong film thickness dependence. (3) The H\(^+\) and O\(^-\) fragments may have another contribution from, e.g., dipolar dissociation following kinetic excitation of neutral thymine molecules due to their lesser sensitivity to the film thickness.

The positive fragment desorption involves both the kinetic and the potential energy of the incident ions. Thymine molecules are ionized and thus excited via charge exchange with the incident ions. The kinetic energy of the incident Ar\(^+\) ion contributes via momentum transfer to breaking the molecule into fragments and provides the required kinetic energy for the ionic fragments to overcome the induced polarization potential to desorb from the film. Hence, charge exchange with the substrate may not be a necessary step for the positive fragment desorption, in agreement with the lesser sensitivity of positive fragment yield to the film thickness (Fig. 2a). Meanwhile, ionization and excitation by potential energy transfer (charge exchange) is energetically more efficient than the momentum transfer, which account for the much lower desorption energy thresholds of the positive fragments. It is believed that these dynamics also hold for the major positive and negative fragment desorption during He\(^+\) ion irradiation of thymine films given the similar thickness dependence of the fragment desorption (Fig. 2b).

In the present work, we focus on Ar\(^+\) and He\(^+\) stimulated desorption of H\(^+\) and H\(^-\) fragments as seen in Fig. 1. Figure 3 shows the relative ion yield of H\(^+\) and H\(^-\) fragments as a function of incident energy during Ar\(^+\) and He\(^+\) ion irradiation of thymine. The measurements yield appearance energy thresholds near 40 - 50 eV for H\(^+\) and H\(^-\) desorption in both cases with a slightly lower energy for the H\(^-\) ions. In contrast to the general fact that the positive fragments appear at lower energies than the negative fragments, it may imply different channel(s) for the H\(^+\) desorption from other positive fragments.
Figure 3 Relative ion yield of H⁺ and H desorption as a function of incident ion energy during Ar⁺ and He⁺ ion irradiation of 200 ng/cm² thyme films.

We will try to address two questions in the following discussion: (1) the origin of the desorbed H⁺ ions. Chemically, there are two kind of hydrogen in thymine, i.e., carbon- or nitrogen-bound hydrogen, both of them may contribute to the H⁺ desorption. Because only the latter is involved in the hydrogen bond of DNA base pairs, it is interesting to investigate whether ion irradiation shows any preference for breaking the N-H and C-H bonds, as observed during electron irradiation (Hervé du Penhoat et al., 2001; Antic et al., 1999). (2) the dynamics that leads to the strong enhancement of H⁺ desorption during He⁺ irradiation. In contrast to the significant desorption of H⁺ ions during both Ar⁺ and He⁺ ion irradiation, the enhancement of H⁺ desorption suggests specific desorption channel(s).

For the first question, irradiation of thymine-methyl-d₃-6-d helps us to distinguish the chemical origin of H⁺ desorption. Figure 4 shows the negative ion mass spectra between m/e 0.4-4 of thymine-methyl-d₃-6-d as a function of (a) Ar⁺ and (b) He⁺ ion energy. It appears that ion irradiation of thymine-methyl-d₃-6-d leads to the desorption of both D⁺ and H⁺ ions, suggesting that H⁺ desorption is originated from both N- and C-bound hydrogen of thymine.
The branching ratio of $D_2/H_2^-$ in this energy range is $\sim 1.5 \pm 0.3$. In contrast to the stoichiometric $D/H$ ratio of 2.0 in thymine molecule, it suggests that the origin of $H^-\text{ ion desorption}$ shows a slight preference for N-bound hydrogen. The desorption dynamics is believed to be kinetic as discussed above.

**Figure 4** Anion mass spectra between m/e 0.4-4 for ion stimulated desorption of thymine-methyl-$d_3$-$d_6$ as a function of primary (a) $Ar^+$ and (b) $He^+$ ion energy, showing desorption of both $D^-$ and $H^-$ ions.

We note that at 60 eV, for $Ar^+$ impact the integral $H^-$ signal is stronger than the $D^-$ signal, while near 100 eV the $D^-$ signal begins to exceed the $H^-$ signal; this suggests that for $Ar^+$ impact near threshold there exists a preference for cleavage of N-H bonds, which is not observed for $He^+$ impact.

Hydrogen loss from C-H and N-H bonds also occurs for $He^+$ induced $H^+$ desorption. Figure 5 shows the positive mass spectra between m/e 0.4-8 of (a) thymine and (b) thymine-methyl-$d_3$-$d_6$ as a function of $He^+$ ion energy up to 120 eV. Both $D^+$ and $H^+$ ions are
observed with, however, a poor mass resolution. The branching ratio of \( \text{D}^+/\text{H}^+ \) near 80 – 100 eV is ~3.0, suggesting a slight preference for C-bound hydrogen at those energies.

![Cation mass spectra](image)

**Figure 5** Cation mass spectra between m/e 0-8 of ion stimulated desorption from (a) thymine and (b) thymine-methyl-d3-6-d as a function of He\(^+\) ion energy. Note the appearance of a peak at m/e 5 for thymine and a peak at m/e 6 for thymine-methyl-d3-6-d, which suggests the formation of HeH\(^+\) and HeD\(^+\) ions.

It is also noted that for He\(^+\) impact we observe (a) formation of HeH\(^+\) from thymine and HeD\(^+\) from thymine-methyl-d3-6-d that suggests H abstraction by the incident He\(^+\) preferentially at the carbon sites, and (b) backscattered He\(^+\) near 40 eV, not observed for Ar\(^+\) impact; since the QMS detects desorbing ions with energies below 5 eV, this suggests that unlike the Ar\(^+\), a 40 eV He\(^+\) may undergo significant energy loss events in the film and scatter out with a greatly reduced energy.

Figure 6 shows the relative ion yield of D\(^+\) and H\(^+\) desorption (obtained by deconvolution of the mass spectra) as a function of He\(^+\) ion energy, which increase
monotonically with He\(^+\) ion energy from 40-100 eV and yields a common appearance energy threshold near 40 eV for both D\(^+\) and H\(^+\) desorption.

![Graph showing relative ion yield of D\(^+\) and H\(^+\) desorption as a function of incident ion energy during He\(^+\) ion irradiation of a 200 ng/cm\(^2\) thymine-methyl-d\(_3\)-6-d film, and the HeH\(^+\) yield for He\(^+\) impact on a 200 ng/cm\(^2\) thymine film.](image)

**Figure 6** Relative ion yield of D\(^+\) and H\(^+\) desorption as a function of incident ion energy during He\(^+\) ion irradiation of a 200 ng/cm\(^2\) thymine-methyl-d\(_3\)-6-d film, and the HeH\(^+\) yield for He\(^+\) impact on a 200 ng/cm\(^2\) thymine film.

In addition to projectile induced direct bond rupture, there exists the possibility that the observed H\(^+\)/H\(^+\) desorption may also involve isolated hydrogen adsorbed on the Pt substrate as a result of, e.g., dissociative adsorption of thymine. Many organic molecules and also water are found to dissociate upon adsorption on metal surfaces. Subsequent photon or electron irradiation may lead to H desorption. For example, water adsorption on Pt and Ti surfaces dissociates into H, O and OH (Fisher and Gland, 1980; Fisher and Sexton, 1980; Stockbauer et al., 1982) and, under photon irradiation, results in H\(^+\) desorption, which occurs in the submonolayer regime and is suggested to be associated with the presence of OH on the surface (Stockbauer et al., 1982). Indeed, several studies have demonstrated that adsorption of thymine on metal surfaces leads to deprotonation from the N-H sites, which occurs in the submonolayer regime, and results in the adsorption of an anionic form of thymine on the metal.
surfaces (McNutt et al., 2002; Li et al., 1999; Haiss et al., 1998). Deprotonated adsorption of thymine may, on the other hand, results in adsorption of isolated hydrogen on the metal surfaces. In our case this may explain the appearance of Ar$^+$ stimulated [T-H]$^-$ desorption in thin films of T, but its absence from Ar$^+$ irradiated thick multilayer films (while the [T-H]$^-$ anion may also be indicative of collisional H loss, it is rather attributed to the adsorbate-substrate interaction in the present case). Furthermore, Ar$^+$ ion irradiation of sub-monolayer thymine-methyl-d$_3$-6-d yields exclusively [T$_d$-H] anions, in agreement with the assumption that deprotonation upon adsorption occurs only at the N-H sites, while the C-H bonds remain intact. In this respect, if the H$^{+/+}$ desorption involves isolated H precursors, it is expected to yield a branching ratio of D$^{+/+}$/H$^{+/+}$ lower than 2.0. In terms of our measured values, it appears that the H$^-$ and D$^-$ desorption may have some contribution from isolated hydrogen (D$^-$/H$^-$ $\approx$ 1.5), but the He$^+$ induced H$^-$ and D$^+$ desorption (D$^+$/H$^+$ $\approx$ 3) is believed to originate mainly from a direct ion-thymine interaction as a result of breaking of the N-H and C-H bonds.

We now turn to He$^+$ induced H$^+$ desorption. Many dynamics have been proposed to account for electron, photon or ion stimulated H$^+$ desorption from small molecules adsorbed on metals. In photon and electron stimulated desorption, H$^+$ desorption is discussed within the framework of Knotek and Feibelman, or Auger stimulated desorption mechanism. For example, photon or electron irradiation of OH adsorbed on metal surfaces may create an O 1s core hole if energetically permitted, and subsequent Auger decay of the core hole results in the formation of double or multiple holes in the valence band. If these valence holes are localized and not dissipated efficiently, the resultant Coulomb repulsion may break the chemical bonds and lead to H$^+$ desorption. Multi-charged ion bombardment of hydrogen-contaminated surfaces also leads to abundant secondary H$^+$ desorption, and is also ascribed to a possible Coulomb explosion mechanism (Della-Negra et al., 1988; Kakutani et al., 1995). In
a study on ultraviolet photon stimulated H⁺ desorption from dissociatively adsorbed water on Ti surface (Stockbauer et al., 1982), the photon energy threshold of H⁺ desorption was found to be ~25 eV, close to the O 2s energy level, with a second threshold close to the Ti 3p level, suggesting the involvement of O 2s and Ti 3p core hole excitations in the desorption dynamics. More particularly, Souda investigated He⁺ ion induced H⁺ desorption from H, O, and OH coadsorbed Si and Mg surfaces, and proposed that the formation of an O-2s core hole in the surface OH species by quasi-resonant charge exchange may initiate the subsequent desorption of H⁺ ions, since the He 1s hole is energetically accessible to the O 2s electrons (Souda, 2000). As a summary, the proposed H⁺ desorption dynamics all involve some sort of core level excitations energetically accessible to the primary particles.

Here, however, the finding that H⁺ desorption originates from either the nitrogen- or the carbon-bound hydrogen excludes the dynamics that involve core-hole formation. Because the C 2s and 2p electrons are all involved in chemical bonding, there is no core electron energetically accessible to the He⁺ ion in the case of He⁺ interaction with carbon-bound hydrogen. An alternate possibility is that impact of He⁺ ions on H atoms of thymine ejects the two valence electrons of the C-H or N-H bond via Auger process and ruptures the chemical bond, resulting in the desorption of the H⁺ fragment.

In addition to the H⁺+/- desorption, we also note in Fig. 5 the appearance of a small but discernible peak at m/e of 5, which shifts to m/e 6 in the case of thymine-methyl-d₃-6-d. The intensity of these peaks first increases with He⁺ ion energy from 20-100 eV and then decreases with further increase in He⁺ ion energy. These peaks clearly indicates the formation of HeH⁺ and HeD⁺ ions during He⁺ ion irradiation of thymine and thymine-methyl-d₃-6-d, which may occur during the collision between backscattered He⁺ ions and thymine molecules, more particularly, the hydrogen atoms of thymine molecules. By decreasing the energy below
60 eV, it is observed that He\(^+\) ions have a high probability to survive neutralization during impact with Pt and thymine films, this is not observed for Ar\(^+\) impact. As the QMS measures ions of 0-5 eV, the appearance of He\(^+\) ion in the 40 eV mass spectra of Fig. 5 also indicates that they can even survive multiple collision energy losses, some of which lead to HeH\(^+\) formation and desorption, with an apparent threshold near 15-20 eV (Fig. 6).

The HeH\(^+\) ion is a chemically stable molecule, the formation of which via He\(^+\) impact with H\(_2\) or He impact with H\(_2\)^+ in the gas phase has been intensively and extensively studied both experimentally and theoretically. These collision systems provide the simplest model reactions for the study of the dynamics and mechanisms of state-to-state chemical reactions (Jones et al., 1980; Dhuicq et al., 1996; Aguillon, 1998 (and references therein); Kumar et al., 1993; Pollard et al., 1991), among which He\(^+\) impact with H\(_2\) may provide some clues to account for our present results. He\(^+\) impact with H\(_2\) can proceed via several reaction channels, such as He\(^+\)+H\(_2\)->HeH\(^+\)+H, He\(^+\)+H\(_2\)->He+H\(^+\)+H, yielding ionic and neutral products H\(^+\), HeH\(^+\), H\(_2\)^+ , H, He, etc. and their associated products (Jones et al., 1980). These reactions are thought to proceed via the formation of an intermediate molecular ion (He-H\(_2\))^+ (Sidis, 1996). However, studies of He\(^+\) impact with CH\(_4\) yield no H\(_2\)^+ , H\(_3\)^+ and HeH\(^+\) ions, which is suggested to proceed via a direct charge exchange to produce highly excited CH\(_4\)^+ ions rather than via an intermediate complex ion (Adams and Smith, 1976). Here, the formation of HeH\(^+\) ions during He\(^+\) impact with thymine molecules suggests the existence of a reaction channel He\(^+\)+RH->HeH\(^+\)+R (1), probably via the formation of an intermediate molecular ion (He-H-R)^+, where RH is thymine. Similar to the He\(^+\) ion impact on H\(_2\), here the possible formation of an intermediate (He-H-R)^+ ion may result in another decay channel of the intermediate molecular ion, i.e. dissociation into He, H\(^+\) and R (2), which may in part explain the He\(^+\) ion enhanced H\(^+\) desorption observed here. Another possibility is that some of the HeH\(^+\) ions are
produced in excited states, which dissociate into He and H⁺ during desorption from the surface. However, the final results are the same as the reaction channel (2).

An isotopic effect was observed on the laboratory energy thresholds of H⁺ and D⁺ formation during He⁺ impact with H₂ and D₂, which, however, yields a common center of mass energy threshold for H⁺ and D⁺ formation (Jones et al., 1980), as well as HeH⁺ and HeD⁺ formation. Here, however, Figure 6 shows a common laboratory energy threshold for H⁺ and D⁺ desorption from He⁺ impact with thymine-methyl-d₃-6-d, i.e., no isotopic effect. A possible explanation for the absence of isotopic effect here is that the reduced mass of the collision system here (light on heavy) is dominated by the mass of the whole thymine molecule, even though the impact point has to be a hydrogen (deuterium) atom. In that sense it is noteworthy that classical collisions allow for maximum momentum transfer from the projectile to the target (atom) only for symmetric, or near symmetric, collisions where the mass of the projectile mass approaches that of the target.

In comparison with the stoichiometric ratio of D/H = 2 in thymine-methyl-d₃-6-d, here the measured D⁺/H⁺ branching ratio induced by He⁺ irradiation shows a slight preference for breaking the C-H bond. Such a preference is also observed during low energy electron impact on thymine films, where a low contribution from the N-H sites to the total desorption yield was observed (Hervé du Penhoat et al., 2001). A possible explanation is as follow. It is established that in the sub-monolayer regime, thymine molecules adsorb with their π plane perpendicular to a metal surface, with its carbon-bound hydrogen atoms sticking out of the surface (Furukawa et al., 2003; McNutt et al., 2002; Li et al., 1999; Haiss et al., 1998). Such a geometric structure allows a preference for access of carbon-bound hydrogen to the incident primary irradiation and the departure of the resultant products.
6.2.2 Summary and conclusions

Ion irradiation of thymine-methyl-d$_3$-6-d reveals that the H$^{+/-}$ desorption occurs as a result of breaking of both nitrogen- and carbon-bound hydrogen. The branch ratio of D$^+$, H$^-$ ions from C-H and N-H sites is slightly lower than the stoichiometric ratio in thymine, whereas that of the He$^+$ induced D$^+$, H$^+$ ions is slightly higher than the stoichiometric ratio. H$^-$ desorption is likely a result of kinetic fragmentation of neutral and/or deprotonated thymine molecules, but may also involve sputtering of isolated hydrogen adsorbed on the substrate. The enhanced He$^+$ induced H$^+$ desorption is likely a result of direct ion-thymine interaction via several possible dynamics, such as Auger stimulated desorption. It is also found that He$^+$ ions can abstract a hydrogen atom from the thymine molecule to form a HeH$^+$ ion, which occurs mainly on the carbon-bound hydrogens in thymine, and likely involves formation of an intermediate molecular ion (HeT)$^+$. Dissociation of either (HeT)$^+$ to He + (T-H) + H$^+$, or HeH$^+$ to He + H$^+$ during desorption, may also contribute to the enhanced H$^+$ desorption during He$^+$ impact.

6.3 Low Energy (5-100 eV) Atomic and Molecular Ion Interactions with Condensed Phase Thymine

6.3.1 Results and discussion

We report here on damage to films of thymine (T), and thymine-methyl-d$_3$-6-d (T$_d$) by Ar$^+$, Ar$^{++}$, He$^+$, N$_2^+$, N$^+$, and D$_x^+$ (x=1-3) ions by measuring ion stimulated desorption (ISD) of ionic fragmentation products.
6.3.1.1 Fragmentation products

Generally, ion impact on thymine films produces abundant ionic fragments. Figure 7 shows typical mass spectra of positively and negatively charged fragments (insets, for D⁺, N⁺ and N₂⁺ projectiles) desorbed from 200 ng/cm² thymine films during 200 eV (a) D⁺, (b) D₂⁺, (c) D₃⁺, (d) N⁺, (e) N₂⁺, and (f) 400 eV Ar⁺⁺ ion irradiation. Major positive fragments are identified from low m/e (mass/charge ratio) to high m/e as H⁺, CH₃⁺(x=0-3), HNCH⁺, C₃H₃⁺, OCNH₂⁺, HNC₃H₄⁺(±H), [T-OCNH]⁺(±H), [T-O]⁺(±H), as well as [T+H]⁺; major negative fragments are H⁻, O⁻, CN⁻ and OCN⁻, which is also the case for most of the other ion projectiles studied here.

The mass spectra of negative desorbing fragments for D₂⁺ and D₃⁺ irradiation are not shown because of the great similarity they bear with the one for D⁺. The negative mass spectrum obtained with Ar⁺⁺ is not shown either, due to its great resemblance with the negative mass spectrum acquired while Ar⁺ irradiation seen in the inset of figure 1a. The chemical identification of the fragments is achieved here by the method detailed in chapter 4. The chemical formulae of the fragments are given to express their original sites in thymine molecule. Both the positive and negative ion fragments can be sorted into two groups in terms of their formation by either endocyclic or exocyclic bond cleavage. In this respect, the positive ion fragments HNCH⁺, C₃H₃⁺, OCNH₂⁺, HNC₃H₄⁺(±H), [T-OCNH]⁺(±H), and the negative ion fragments CN⁻, OCN⁻ clearly indicate endocyclic bond cleavage (endocyclic fragments), while the fragments H⁺, CH₃⁺, [T-O]⁺(±H), H⁻ and O⁻ are likely formed by direct exocyclic bond cleavage (exocyclic fragments). Accordingly, we are able to distinguish the damage caused to the endocyclic and the exocyclic bonds of thymine molecules and the incident energy dependence of such fragmentations. Figure 8 shows the Ar⁺ ISD energy thresholds of the major positive and negative fragments obtained from 200 ng/cm² thymine.
Figure 7 Typical cation fragment patterns for ISD of 200 ng/cm² thymine (T) films on Pt substrate during 200 eV impact of (a) D⁺, (b) D₂⁺, (c) D₃⁺, (d) N⁺, (e) N₂⁺, and (f) 400 eV Ar⁺⁺. Insets show anion fragments for D⁺, N⁺ and N₂⁺ and enlargements of H⁺ and D⁺ desorption for D₂⁻ and D₃⁻ irradiation.
films (Shown here only for comparison; from Deng, et al., 2005a). Most positive ion fragments appear at energy thresholds between 18-30 eV, with H\(^+\) and CH\(_3\)\(^+\) at higher energies, near 40 eV. The negative ion fragments appear at higher energies, typically above 40 - 60 eV. It also appears that during Ar\(^+\) ion irradiation, damage of the endocyclic bonds of thymine, e.g. as indicated by the formation of HNCH\(^+\), HNC\(_3\)H\(_4\)(\pm H), [T-OCNH]\(^+\)(\pm H), etc. occurs at much lower incident energies than damage of exocyclic bonds leading to the formation of H\(^+\) or CH\(_3\)\(^+\), etc.

![Positive ion fragments](image)

**Figure 8** Desorption energy thresholds of positive and negative ion fragments induced by Ar\(^+\) ion impact of 200 ng/cm\(^2\) thymine films on Pt substrate. Each curve is an average of 3-7 experiments. The relative yield of HNCH\(^+\) is the sum of HNCH\(^+\) and [HNCH+H]\(^+\) as they represent the same fragmentation process. Such are also the cases of HNC\(_3\)H\(_4\)\(^+\), [T-OCNH]\(^+\) and [T-O]\(^+\) (Deng, et al. 2005a).
In these respects, in contrast to the fragment pattern similarity among different ion irradiation, the fragmentation efficiency, the branching ratios of the fragments and the energy thresholds of fragment desorption strongly depend on the ion projectiles. Generally, heavy ions tend to fragment thymine molecules more efficiently than light ions and also result in more high mass fragments. For example, during 50-200 eV Ar\(^+\) irradiation, the positive

**Figure 9** Desorption energy thresholds of typical fragments CH\(_3\)^+, HNCH^+, HNC\(_3\)H\(_4\)^+ and O\(^-\) from 200 ng/cm\(^2\) thymine films on Pt substrate induced by different ion projectiles.
fragments are dominated by endocyclic fragments, specifically, HNCH\(^+\), HNC\(_3\)H\(_4\)\(^+\), etc. (fig. 1a), while during 50-200 eV He\(^+\) irradiation, the H\(^+\) fragment instead of HNCH\(^+\) dominates the positive fragments (Fig. 1b), which suggests an enhancement of endocyclic bond cleavage by heavy ion projectiles. A similar case occurs to the negative ion fragments (insets in Fig. 7) where OCN\(^-\) and CN\(^-\) ions are absent of the negative spectra acquired while D\(^+\), D\(_2\)\(^+\) or D\(_3\)\(^+\) ion irradiation.

Figure 9 shows desorption energy thresholds of typical fragments CH\(_3\)\(^+\), HNCH\(^+\), HNC\(_3\)H\(_4\)\(^+\) and O\(^-\) from 200 ng/cm\(^2\) thymine films induced by different ion projectiles, which are also summarized along with other fragments in Table 1 at the end of the chapter. Data for D\(_3\)\(^+\) projectiles are omitted in figure 9 because of their great similarity with those for D\(_2\)\(^+\). Overall, the threshold energy of fragment desorption increases with decrease in incident ion mass. The lowest fragment desorption energy thresholds as described in eV/amu range from 0.4 (Ar\(^+\)) to 7.5 (He\(^+\)). It is also noteworthy that during heavy ion (Ar\(^+\), N\(_2\)\(^+\), N\(^+\), etc.) irradiation, the endocyclic fragments appear at lower energies than exocyclic fragments; while during light ion (D\(_3\)\(^+\), D\(_2\)\(^+\), D\(^+\)) irradiation, exocyclic fragments appear at lower energies than endocyclic fragments, which also suggests that heavy primary ions cause endocyclic damage more efficiently than light primary ions.

This is summarized in figure 10 which shows energy thresholds for desorption of (a) HNCH\(^+\) and (b) HNC\(_3\)H\(_4\)\(^+\) from 200ng/cm\(^2\) thymine films induced by different ion projectiles. If the HNCH\(^+\) and HNC\(_3\)H\(_4\)\(^+\) fragments both appear at an incident ion energy of \~15-20 eV during Ar\(^+\), N\(_2\)\(^+\), N\(^+\) and Ar\(^{++}\) irradiation, during He\(^+\) irradiation the HNCH\(^+\) and HNC\(_3\)H\(_4\)\(^+\) fragments appear at a much higher ion incident energy of \~40 eV. We also note the different shape of the ion energy dependent HNC\(_3\)H\(_4\)\(^+\) fragment yield for N\(_2\)\(^+\) and Ar\(^{++}\) in the region below 100 eV, compared to N\(^+\) or Ar\(^+\). Whether or not this relates to the higher ionization
potential of Ar$^{+}$, and the likelihood of N$_2^+$ fragmentation during a binary collision (yielding two energetic fragments interacting with the HNC$_3$H$_4$ moiety of thymine), is not known at the present.

![Graphs showing relative yield versus incident ion energy](image)

**Figure 10** Energy thresholds for desorption of (a) HNCH$^+$ and (b) HNC$_3$H$_4^+$ fragments from 200 ng cm$^{-2}$ thymine films induced by different ion projectiles.

### 6.3.1.2 Fragmentation dynamics

The desorption dynamics of the major positive and negative ion fragments induced by Ar$^+$ ion irradiation has been discussed in chapter 5 in terms of their dependence on the incident ion energy and the film thickness. Negative ion fragment desorption appears at much higher energy than the positive ion fragments, suggesting kinetic dynamics. It also exhibits a
strong dependence on the film thickness, i.e. decreases rapidly with increase in film thickness, suggesting a strong involvement of the substrate. Several fragmentation pathways have been proposed in terms of our understanding of ion-surface interactions and adsorption of thymine on metal surfaces: (1) The first possible scenario is kinetic fragmentation of a thymine molecule followed by ionization of the fragments via charge exchange with the substrate. The ionization probability strongly depends on the surface work function of the substrate, which is highly sensitive to surface adsorbate. Adsorption of dielectric materials such as thymine raises the surface work function and thus decreases anion desorption. (2) It is revealed that adsorption of thymine on metal substrate leads to the formation of deprotonated monoanionic thymine molecule as a result of the interaction with the substrate in the submonolayer regime (Furukawa et al., 2003; McNutt et al., 2002; Li et al., 1999; Haiss et al., 1998). Accordingly, another possible scenario is kinetic excitation of this deprotonated monoanionic thymine molecule into a dissociative state followed by unimolecular fragmentation. (3) Here, for all of the projectiles studied, the H$^+$ and O$^+$ fragments are less sensitive to the film thickness than the CN$^-$ and OCN$^-$ fragments, and may have another contribution from, e.g. dipolar dissociation following kinetic excitation of neutral thymine molecules.

The positive ion fragments appear at much lower energies than the negative ion fragments and are less sensitive to the film thickness. Energetic analysis suggests that both the kinetic and the potential energy of the incident cation ion are involved in the formation of positive fragments, such that an incident cation ionizes the thymine molecule via charge exchange (potential energy transfer) and promotes it to an excited state, while the kinetic energy contributes to breaking the molecule into fragments and providing the required kinetic energy for the fragments to overcome an induced polarization potential to desorb from the film. However, in comparison with: (a) the high energy kinetic sputtering regime, where
multiple atoms or molecules are set into motion by both primary and recoiled particle impact and (b) the potential sputtering regime, where impact of low energy but highly charged ions deposits a high density of potential energy at the impact spot, which results in multi-ionization, Coulombic explosion, fragmentation and desorption, here the picture of cation fragment desorption under the present conditions of singly charged low energy ion impact is less violent than in (a) or (b) (Aumayr and Winter, 2004). At the present low ion energies it likely occurs in such a way that in a single collision the combination of charge and momentum transfer “shakes up” and fragments the ionized target molecule, yielding ionic and associated neutral fragments, and does not require contributions from recoil particles as in (a) or multiple ionization as in (b). This picture is to some extent more closely related to a “slow” physico-chemical process.

It is believed that these dynamics also hold for the major positive and negative fragment desorption during other ion irradiation of thymine films given the similarity in both fragmentation pattern and thickness dependence of the fragment desorption. In this respect, the observed higher desorption threshold energies as well as the dominance of light fragments in the desorption mass spectra during light ion irradiation may result from the less kinetic energy (momentum) received by the fragment from the ion impact, which is required for the ionic fragments to overcome an induced polarization potential to desorb from the film. The H\(^{+}\) desorption dynamics will be further discussed in the following subsection.

6.3.1.3 Hydrogen loss

DNA base pairs are bound by hydrogen bond involving only N-bound H atoms of the bases. Damage of the N-H bond during ionizing radiation is therefore of particular biological importance since breaking of these specific N-H bonds may mean breaking of base pair.
Indeed, H loss from DNA subunits is observed as a predominant event in both gas phase and condensed phase during low energy electron impact as a result of dissociative electron attachment. A preferential scission of the N-H bonds of DNA bases was also observed in the gas phase leading to the formation of deprotonated base anions (Abdoul-Carime et al., 2004). Ion irradiation of condensed thymine on Pt substrate also results in H desorption as both H⁻ and H⁺ ions. The appearance energy thresholds of hydrogen desorption depend on the incident ion projectiles, as summarized in Table 2. The chemical origin is distinguished by irradiation of thymine-methyl-d₃-6-d films.

**Figure 11** Negative ion mass spectra in between m/e 0.4-4 of ion stimulated desorption of thymine-methyl-d₃-6-d as a function of primary (a) Ar⁺, (b) N₂⁺, (c) N⁺ and (d) He⁺ ion energy, showing the desorption of both D⁻ and H⁺ ions.

Figure 11 shows negative ion mass spectra between 0.4-4 amu of ion stimulated desorption of thymine-methyl-d₃-6-d films during Ar⁺, N₂⁺, N⁺ and He⁺ irradiation. Both D⁻
and H\(^-\) ions are observed, suggesting that H\(^-\) desorption is originated from both N- and C-bound hydrogen of thymine.

The branching ratio of D\(^7\)/H\(^-\) in this energy range is near 1.5±0.3, slightly lower than the stoichiometric D/H ratio of 2.0, and suggesting a slight preference for N-bound hydrogen. However, the branching ratio of D\(^7\)/H\(^-\) at low Ar\(^+\) energies (e.g. near 40-60 eV), is inverse to that of the other projectiles, which may suggest a different formation mechanism near threshold for the Ar projectiles. However, we can not exclude a contribution from isolate hydrogen adsorbed on Pt as a result of dissociative adsorption of thymine (Furukawa et al., 2003; McNutt et al., 2002; Li et al., 1999; Haiss et al., 1998). Ion stimulated H\(^+\) desorption as compared with other positive fragments is strongly enhanced by light mass ions with respect to the weak desorption during Ar\(^+\) irradiation, as seen in Fig. 1a, which is not the case for H\(^-\) or D\(^-\) desorption. While both D\(^+\) and H\(^+\) ions are observed during ion irradiation of thymine-methyl-d3-6-d films, the branch ratio of D\(^+\)/H\(^+\) exhibits dependence on primary ions, which is ~1.0 or less in the cases of Ar\(^+\), Ar\(^{++}\), N\(^2+\), and N\(^+\) irradiation, and ~3.0 in the case of He\(^+\) irradiation.

The H\(^+\) desorption dynamics can be described as a two-step event. Incident ion impact with a target hydrogen atom breaks the chemical bond via momentum transfer, while ionization proceeds via resonant charge exchange between the incident ion and the target hydrogen atom. As seen in Fig. 7, the H\(^+\) desorption yield strongly depends on the mass of the projectile, and may also relate to the similarity in ionization potential between the projectile and H. Light mass projectiles (e.g. D\(^+\), D\(^2+\), He\(^+\), etc.) yield stronger H\(^+\) yields than higher mass projectiles, possibly because their mass similarity makes them more likely to transfer most of their momentum to a hydrogen atom, which may also account for the lower appearance energy thresholds of H\(^+\) ions during D\(^3+\), D\(^2+\) and D\(^+\) ion irradiation (Table 2 at the
end of this chapter). The maximum kinetic energy (in the laboratory frame) that can be transmitted to a target particle by momentum transfer from the low energy incident ion is given by

\[ E = \frac{4m_1 m_2 (m_1 + m_2)^2}{m_1} E_{\text{lab}} \quad (1) \]

where \( m_1 \) and \( E_{\text{lab}} \) are the mass and the laboratory kinetic energy of the incident particle, respectively, and \( m_2 \) is the mass of the specific target involved in the collision. The equation suggests that more momentum will be transmitted to the target when its mass is close to that of the incident particle. A match in ionization potential may result in a higher ionization probability (Ionization potentials (eV): Ar, 15.76; N₂, 15.58; N, 14.53; D₂/H₂, 15.42; D/H, 13.6). A similar case is observed in Ne⁺ enhanced F⁺ desorption from LiF surface with respect to He⁺ and Ar⁺ ion irradiation (Ionization potentials (eV): Ne, 21.56; F, 17.42) (Rabalais, 1994). Hence, the relatively low H⁺ yield during Ar⁺ and N₂⁺ irradiation may be due to their mismatch in both mass and ionization potential with hydrogen. We also note in Fig. 7 that for Dₙ⁺ (n=1-3) projectiles, back scattered D⁺/D⁻ is observed, similar to He⁺ projectiles. Since low mass projectiles have a larger penetration depth than heavy projectiles, and the QMS is detects ions with energies below 5 eV, this observation shows that (a) Dₙ⁺ (n=1-3) projectiles with 200 eV can experience significant energy loss scattering in the film or at the Pt substrate, and desorb as D⁺ or D⁻ with energies below 5 eV, and (b) D₂⁺ and D₃⁺ will experience fragmentation and inelastic scattering to desorb as a low energy cations or anions.

In these respects, the ionization process(es) leading to H⁻ desorption is independent of the primary ions. Instead, it is probably determined by the difference between the surface work function and the electron affinity of the H atom, or the charge or polarization state of the H atom in the thymine molecule upon bond breaking. Hence, the observed H⁻ desorption may represent the bond-breaking capability of individual primary ions.
6.3.1.4 Reactive Scattering

It was shown previously (Deng et al. 2005b) that hyperthermal nitrogen cations can abstract a carbon atom, with 4 to 1 preference at the 5- C position at 10 eV, from both D-ribose, 5-13C D-ribose, and 2-deoxy-D-ribose molecules to form CN\(^-\) ions. Here during Ar\(^+\), N\(_2\)\(^+\) and N\(^+\) ion irradiation CN\(^-\) is also observed (Fig. 7). While CN\(^-\) desorption during Ar\(^+\) ion irradiation is a result of fragmentation of thymine molecules, carbon abstraction by nitrogen ions is still resolved by the measurements of its appearance energy thresholds. Figure 12 shows CN\(^-\) appearance energy thresholds during Ar\(^+\), N\(_2\)\(^+\) and N\(^+\) ion irradiation of (a) 200 ng/cm\(^2\) and (b) 40 ng/cm\(^2\) thymine films.

\[\text{Fig. 12 Desorption energy thresholds of CN}\(^-\)\) ions during Ar\(^+\), N\(_2\)\(^+\) and N\(^+\) ion irradiation of (a) 200 ng/cm\(^2\) and (b) 40 ng/cm\(^2\) thymine films. All ion yield are normalized at 60 eV for ease of comparison.}\]

While the relative desorption yield of CN\(^-\) ion exhibits significant dependence on the film thickness, its appearance energy thresholds do not. CN\(^-\) desorption appears at energies down to \(-10\) eV during N\(^+\) ion irradiation, and 30-35 eV during N\(_2\)\(^+\) and Ar\(^+\) ion irradiation, respectively. It is thus believed that carbon abstraction is the dominant CN\(^-\) formation channel.
during low energy (below 40 eV) N⁺ ion irradiation, while during higher energy irradiation both carbon abstraction and molecular fragmentation contribute, but are not distinguishable. During N₂⁺ ion irradiation additional energy is required to break the molecular nitrogen ion into atoms such that contribution from both channels appears concurrently. Contribution from carbon abstraction is manifested by the increase in relative intensity of CN⁻ ion with respect to other anions during N₂⁺ ion irradiation (insets in Figure 7 and 1).

In that sense we also note that the 15 amu yield during N⁺ and N₂⁺ is disproportionately high compared to that of other projectiles (compare Figure 7 and 1). While for non-nitrogen projectiles the mass 15 amu fragment is attributed to CH₃⁺, for N⁺ and N₂⁺ ion impact it is believed to contain a strong contribution from NH⁺. This is suggested by the fact that for both D-ribose, 1-D D-Ribose, and 2-deoxy-D-ribose molecules, NH⁺ via H abstraction by the incident N⁺ is observed with a maximum at 5 eV, and no threshold in incident ion energy (Deng et al. 2005b). However, for thymine this will need to be verified by isotopic 15-N substituted projectiles, or target molecules.

6.3.2 Summary and conclusions

In our condensed phase thymine fragmentation experiments, both positive and negative ion fragments are observed to originate from either endocyclic or exocyclic bond cleavage. Molecular fragmentation of thymine strongly depends on the ion projectile and incident ion energy. Kinetic, potential energy, or chemical dynamics are involved in the fragmentation processes. Fragment desorption occurs at energy as low as 18-40 eV depending on the type of the ion projectiles. Heavy primary ions cause more severe damage than light primary ions. The results suggest that ion damage to biomolecules may virtually occur in all types of ionizing radiation. In particular, during heavy particle therapy where secondary ions
with energy up to several hundreds eV can be created, ion damage is much more significant and needs to be carefully considered, in addition to the contribution of the secondary electrons. Tables 1 and 2 summarize the energy thresholds of several fragments.

**Table 1** Summarized energy thresholds (–eV) of major fragment desorption during different primary ion impact of thymine films on Pt substrate. The experimental uncertainty is generally about ±2 eV, except for low intensity fragments, the estimated uncertainty is about ±5/2 eV for those marked with an asterisk. The right-hand column shows the lowest desorption energy threshold (LDET) in eV/amu, where the values for $D_{1,3}^+$ come from the data in Table 2.

<table>
<thead>
<tr>
<th>Primary ions</th>
<th>O$^-$</th>
<th>CH$_3^+$</th>
<th>HNCH$^+$</th>
<th>C$_3$H$_3^+$</th>
<th>HNC$_3$H$_4^+$</th>
<th>[T-OCNH]$^+$</th>
<th>[T-O]$^+$</th>
<th>LDET eV/amu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ar$^+$</td>
<td>70</td>
<td>40</td>
<td>20</td>
<td>30</td>
<td>18</td>
<td>20</td>
<td>20</td>
<td>0.4</td>
</tr>
<tr>
<td>Ar$^{++}$</td>
<td>90</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20*</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>N$_2^+$</td>
<td>55</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>18</td>
<td></td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>N$^+$</td>
<td>55</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>20</td>
<td></td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>He$^+$</td>
<td>55</td>
<td>35*</td>
<td>40</td>
<td>40*</td>
<td>35*</td>
<td></td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>D$_3^+$</td>
<td>30*</td>
<td>30*</td>
<td>60*</td>
<td>60*</td>
<td></td>
<td></td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>D$_2^+$</td>
<td>30*</td>
<td>30*</td>
<td>40*</td>
<td>50*</td>
<td></td>
<td></td>
<td>&lt;2.5</td>
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</tr>
<tr>
<td>D$^+$</td>
<td>30*</td>
<td>50*</td>
<td>60*</td>
<td>70*</td>
<td></td>
<td></td>
<td>&lt;5</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** Appearance energy thresholds (–eV) of $H^+$ and $H$ desorption during different primary ion impact of thymine films on Pt substrate. The experimental uncertainty in the threshold values are the same as stated in Table 1.

<table>
<thead>
<tr>
<th>Fragments</th>
<th>Ar$^+$</th>
<th>Ar$^{++}$</th>
<th>N$_2^+$</th>
<th>N$^+$</th>
<th>He$^+$</th>
<th>D$_3^+$</th>
<th>D$_2^+$</th>
<th>D$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$^+$</td>
<td>40*</td>
<td>80*</td>
<td>35*</td>
<td>15-20</td>
<td>35</td>
<td>15*</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>H$^+$</td>
<td>40*</td>
<td>50*</td>
<td>35*</td>
<td>30</td>
<td>35</td>
<td>10*</td>
<td>20*</td>
<td>15*</td>
</tr>
</tbody>
</table>
CHAPTER 7 - 10-200 eV Ar$^+$ Ion Impact on Uracil and 5-Br-Uracil films

In this chapter we extend our studies to two other molecules, uracil and 5-Br-Uracil (BrU). Uracil and BrU have both the same fundamental chemical structure as thymine, and are therefore expected to provide additional information regarding the basic interaction mechanisms of the incident ions with the irradiated thymine molecules. Here we present the results of irradiation experiments of uracil and BrU films by Ar$^+$ ions with incident energies between 10 and 200 eV. These results are part of an article in preparation, by Marjorie Imhoff, Zongwu Deng, and Michael A. Huels.

7.1 Introduction

The very first results, ever obtained, of 10 - 200 eV ion fragmentation of 1-2 nm thick thymine films were presented in chapters 4, 5 and 6, and showed that atomic and molecular ion impact has the potential to completely fragment the molecules, even at energies as low as 15 eV, resulting in the formation and desorption of over 30 different secondary cation and anion fragments. For Ar$^+$ ion impact at 100 eV, we found notably a quantum yield of thymine destruction of nearly 6 molecules/incident Ar$^+$, while for reactive nitrogen cations we found tantalizing evidence for atom abstraction reactions.

To deepen our understanding of the initial fragmentation pathways and products, as well as of the initial fragment’s possible reactivity, we discuss in this chapter ion damage to films of uracil and 5-Br-Uracil (BrU). BrU (uracil) has essentially the same structure as thymine except for a Br (H) atom which replaces the methyl group at the C5 position, as shown in fig. 1 below.
BrU is not normally found in DNA but it can be substituted for thymine and incorporated in DNA during replication. While BrU is known for its mutagenesis properties, i.e. incorporation of BrU into DNA induces a substitution-type mutation of A-T to G-C (Hu et al., 2004), its radiosensitization properties are however the most relevant for our present studies. When BrU is incorporated into cellular DNA in place of thymine, cells become much more sensitive to the lethal effects of both UV and X-rays (Hutchinson, 1973; Fujimoto et al., 1995; Cook and Greenberg, 1996; Chen et al., 2000; Watanabe and Nikjoo, 2002). This sensitization is due to the fact that BrU, and 5-halouracils (5-BrU and 5-IU) in general, are much more radiation sensitive than the normal bases, a fact which has led to clinical trials of BrU combined with X-ray therapy (Greenberg et al., 1994; Mitchell et al., 1986; Kinsella et al., 1984). Recent studies performed with low energy electron beams (Abdoul-Carime et al., 2000; Abdoul-Carime et al., 2001) have shown an induced dissociation of BrU into halogen Br\(^{-}\) ions and extremely reactive uracilyl radicals which are believed to be the precursors (Bhatia and Schuler, 1973) of DNA damage (McLaughlin et al., 1993).

In this chapter, we present results of uracil and 5-Br-Uracil (BrU) film degradation induced by 10-200 eV Ar\(^{+}\) ion impact by focusing on the chemical identification of the cation and anion fragments we observe.
7.2 Results and discussion

7.2.1 Cation fragment formation

Here we discuss the chemical identification of the fragments desorbing from uracil and BrU films under Ar⁺ impact by comparison with the data obtained for thymine in chapter 5. The positive and negative ion fragments desorbing from thymine films under Ar⁺ impact were identified in chapter 4 by comparing mass spectra obtained from thymine and deuterated thymine (thymine-methyl-d₃-6-d) irradiated by 200 eV Ar⁺ ion in the condensed phase, and by 70 eV electrons in the gas phase. We first discuss the chemical identification of the positive fragments of uracil and BrU films simultaneously by drawing a parallel with the structures seen for thymine in fig. 1 of chapter 5, starting from high m/e down to low m/e fragments.

Figure 2 shows typical ISD spectra of positive ion fragments induced by 200 eV Ar⁺ ion irradiation of 200 ng/cm² (a) uracil and (b) BrU films condensed on Pt. Assuming no clustering of the molecules and considering the same parameters applicable for thymine, this thickness should correspond roughly to 4 nominal monolayers (Herve du Penhoat et al., 2001). The molecular parent ions can be seen under their protonated form at m/e = 113 for [U+H]⁺ and m/e = (190, 192) for [BrU+H]⁺. Following the structures of the positive mass spectrum of thymine, peaks observed in the uracil spectra at m/e = (95,96,97) and (70,69,68) are respectively assigned to [U-O]⁺ (±H) and [U-OCNH]⁺ (±H), while peaks observed in the BrU spectra at m/e = (174, 176) and m/e = (146, 147, 148, 149, 150) are respectively assigned to [BrU-O]⁺ (±H) and [BrU-OCNH]⁺ (±H).

Peaks corresponding to m/e = 44 in both the uracil and BrU spectra are assigned to fragment OCNH₂⁺, as identified in the thymine spectra. Fragment HNC₃H₄⁺ observed in thymine spectra arising from N1-C2 and C4-C5 bond cleavage corresponds to uracil
fragment HNC$_2$H$_2^+$ at m/e = 41 and to BrU fragment HNC$_2$HBr$^+$ observed in its hydrogenated form HNC$_2$HBrH$^+$ at m/e = (120, 122). Due to the fragmentation channel present in thymine, the m/e = (120, 122) in BrU spectra is rather attributed to fragment HNC$_2$HBrH$^+$ than to fragment OC$_3$BrH$^+$.  

![Image of mass spectra](image)

**Fig. 2** Positive ion fragment patterns produced by 200 eV Ar$^+$ ion irradiation of 200 ng/cm$^2$ films of (a) uracil, and (b) bromouracil (BrU), both on a Pt substrate. Both mass spectra have been normalized in intensity, such that the integral intensity of either mass spectrum (from 0 – 120 amu for uracil in (a) and from 0 – 200 amu for BrU in (b)) is equal to one. The positive mass spectrum of BrU is completed up to 200 amu in the inset in (b).

Following the assignment of fragment HNC$_2$H$_2^+$ to m/e = 41, observed for both uracil and BrU positive mass spectra, the peak corresponding to m/e = 40 is assigned to fragment [HNC$_2$H$_2$ - H]$^+$ while signals at m/e = 42 and 43 can respectively arise from [HNC$_2$H$_2$ + H]$^+$ and [HNC$_2$H$_2$ + 2H]$^+$ fragment contributions for both uracil and BrU spectra. The signal possibly corresponding to OCNH$^+$ and OCN$^+$ at m/e = 43 and 42 in thymine is
small. However, since the relative contribution of fragments \([\text{U-OCN}]^+\) is three times more intense than \([\text{T-OCN}]^+\), signals at \(m/e = 43\) and \(42\) are possibly attributed also to fragments \(\text{OCNH}^+\) and \(\text{OCN}^+\) in both uracil and BrU spectra, in agreement with the attribution of signal at \(m/e = 44\) to fragment \(\text{OCNH}_2^+\).

In the same manner, thymine fragment \(\text{C}_3\text{H}_4^+\) resulting from N1-C6 and C4-C5 bond cleavage can result in uracil fragment \(\text{C}_2\text{H}_2^+\) at \(m/e = 26\) and BrU fragment \(\text{HC}_2\text{Br}^+\) at \(m/e = (104, 106)\). Fragment \(\text{HNCH}^+\) observed at \(m/e = 28\) is the most intense fragment observed for all irradiated films studied here. Peaks at \(m/e = 29\) could arise from a contribution of \(\text{COH}^+\) fragments. According to what is observed for thymine, peaks corresponding to \(m/e = 12-15\) are attributed to \(\text{CH}_i^+\) (\(i = 0 - 3\)), and \(m/e = 1\) to \(\text{H}^+\), for both uracil and BrU.

Fragments \(\text{CH}_i^+\) (\(i = 0 - 3\)) desorbing from thymine films were thought to involve the methyl group since the shifted peaks in the deuterated thymine positive mass spectra could be assigned to fragments \(\text{CD}_i^+\) (\(i = 0 - 3\)). However, here the presence of these peaks in both uracil and BrU spectra tend to rather involve hydrogen abstraction by ring carbon ions instead.

The major differences between uracil and BrU positive mass spectra first lie in certain fragment intensities. While the other major positive fragments have comparatively the same relative intensities, the relative intensity of fragments \(\text{OCNH}_2^+\) and \(\text{HNC}_2\text{H}_2^+\) are considerably bigger in BrU spectra, as well as the intensity of fragments \(\text{C}_2\text{H}_2^+\) and the signal at \(m/e = 29\) possibly attributed to \(\text{COH}^+\). If fragment \(\text{HNCH}^+\) remains the most intense positive fragment in both cases, \(\text{HNC}_2\text{H}_2^+\) and fragment \(\text{OCNH}^+ / [\text{HNC}_2\text{H}_2 + 2\text{H}]^+\) are significantly enhanced in the BrU positive ion mass spectra. The second difference observed between uracil and BrU spectra are unassigned structures. Although the two spectra both feature unidentified series of peaks for \(50 \leq m/e \leq 60\), the structure of these peak series
appears different. The peak at m/e = 52 could tentatively be assigned to OC$_3^+$ fragments, originating from the C4-C5-C6 part of the uracil and BrU molecule. Following this, the OC$_3^+$ fragment could pick up some hydrogens in scattering events prior to desorption to form OC$_3$H$,^+$, OC$_3$H$_2^+$, and OC$_3$H$_3^+$ fragments, contributing to peaks observed at m/e = 53, 54 and 55 respectively. Since no clear structures which would correspond to fragments OC$_3$HBr$^+$ and OC$_3$H$_2$Br$^+$ are seen at m/e = (132, 134) or (133, 135), fragments at m/e = 54 and 55 result from debrominated BrU molecules. Fragments OCNHC$^+$ originating from the C2-N1-C6 part of the ring structure could also contribute to the signal detected at m/e = 55, as well as m/e = 56 with fragments OCNHCH$^+$ formed by hydrogen abstraction. The signal at m/e = 56 could also receive a contribution from N3-C4-C5 HNCOCH$^+$ fragments which, here again, would leave apart bromine atoms in BrU molecules. It is interesting to note that the fragment corresponding to the peak observed at m/e = 57 is a dissociation channel specific to BrU since this signal is not detected either in thymine or uracil.

The BrU positive ion mass spectra present an additional unassigned series of peaks around m/e = 70. The peaks observed at m/e = 67–71 could however result from the loss of the fragments assigned to m/e = 40–44 to the debrominated BrU molecule.

7.2.2 Anion fragment formation

Negative ISD mass spectra obtained from 200 ng/cm$^2$ uracil and BrU films under 200 eV Ar$^+$ ions are presented in fig. 3a and 3b. The two anion fragment patterns are essentially alike except for the two peaks present in the BrU spectra at m/e = 79, 81. These two peaks correspond to BrU fragments $^{79}$Br$^-$ and $^{81}$Br$^-$, observed with a similar intensity matching the natural distribution of the two bromine isotopes (50.69 % for $^{79}$Br and 49.31 % for $^{81}$Br (de Bievre and Taylor, 1993)). The chemical identification of the other major negative ion
fragments desorbing from the uracil and BrU films is achieved by comparison with the known negative fragments desorbing from thymine films of similar thickness (see fig. 1 in chapter 5), taking into account the greater thymine film thicknesses (see chapter 5 for fragment desorption dynamics).

While a small deprotonated uracil signal \([\text{U-H}]^-\) is seen at \(m/e = 111\), the corresponding \([\text{BrU-H}]^-\) fragment yield is barely visible in the BrU spectra. For both

![Negative ion fragment patterns produced by 200 eV Ar\(^+\) ion irradiation of 200 ng/cm\(^2\) films of (a) uracil, and (b) bromouracil (BrU), both on a Pt substrate. Both mass spectra have been normalized in intensity, such that the integral intensity of either mass spectrum (from 0 – 120 amu for uracil in (a) and from 0 – 200 amu for BrU in (b)) is equal to one. The negative ion mass spectrum of BrU features no signal of significant intensity beyond 85 amu and is shown only up to 120 amu here for this reason.](image)

uracil and BrU negative ion mass spectra, the major peaks correspond to \(m/e = 42, 26, 16,\) and \(1\) and are, respectively, attributed to fragments \(\text{OCN}^-, \text{CN}^-, \text{O}^-,\) and \(\text{H}^-\). Peaks of smaller intensity observed at \(m/e = 41, 40, 39\) and \(38\) are assigned to fragments \(\text{OC}_2\text{H}^-, \text{HNC}_2\text{H}^-\),
NC$_2$H$^+$, and NC$_2^-$, in accord with the identification of the thymine anion fragments. In the same manner, small peaks corresponding to m/e = 25 and 24 may be due to fragments C$_2$H$^-$ and C$_2^-$ while fragments OH$^-$, CH$^+$, and C$^-$ may contribute to peaks observed at m/e = 17, 13, and 12. Fragments C$_2$H$^-$ and C$_2^-$ that that arise from C5 and C7 positions in thymine, would in that case give rise to uracil fragment CH$^-$, and BrU fragment CBr$^-$. The latter fragments are not observed, suggesting that C$_2$H$^-$ and C$_2^-$ fragments rather originate from the molecule ring structure. As for thymine, peaks at m/e = 50 and 66 can result from the contribution of fragments C$_2$CN$^-$ and C$_2$OCN$^-$.

We showed in chapter 4 that H$^-$ and D$^-$ formation occurs with 1:2 stoichiometric ratio in deuterated thymine. However, with 1/3 less H atoms in the uracil molecule, the relative fraction of H$^-$ observed from uracil films is 25% less than what would be expected from the difference in H content (compare to Fig 1, chapter 5, 200ng/cm$^2$ thymine film). This observation tends to indicate that H atoms from the methyl group located on the C5 position in thymine are preferred atoms for H$^-$ formation. In the case of BrU films, with 50% less H atoms in the molecule compared to thymine, the relative contribution of H$^-$ is approximately only 1/5 of the relative H$^-$ yield obtained from thymine, designating again the C5 position as a preferred site for H abstraction in thymine.

Comparing uracil and BrU negative ion mass spectra of fig. 3a and 3b, it can be seen that the relative contribution of both H$^-$ + Br$^-$ yields from BrU films is similar to the relative H$^-$ yield for uracil, for a same number of equivalent sites. While U has four H sites, BrU has three H and one Br site, and one would expect a 25% difference in H$^-$ yield between U and BrU. However, the H$^-$ yield from BrU corresponds to only half of that from uracil, despite the only 25% difference in H content. At the same time, the results of fig. 3 show that Br$^-$ alone contributes 2/3 of the H$^-$ + Br$^-$ yields taken together, which, when compared to uracil,
indicates a strong interaction preference with the C5 position in BrU. We note that this is associated with formation of a neutral Uracil-yl radical (Uyl'), that has been associated with enhanced formation of strand breaks, and lethal interstrand cross links, in single stranded regions of double stranded BrU sensitized DNA (Cecchini et al. 2004; Cecchini et al. 2005).

For BrU, this observation is likely related to the high electron affinity of Br atoms ($E_A(\text{Br})=3.36 \text{ eV}$ (Emsley, 1991)) which could partially explain the high Br$^-$ yield detected. Furthermore, the affinity level of a fragment is considerably lowered with respect to the vacuum level due to the image charge interaction with the surface, and broadened due to the energy-time uncertainty, both of which facilitate negative ion formation. The energy transferred upon impact from an incident Ar$^+$ ion can initiate a fragmentation at any site of the molecule, followed by a rearrangement of the electronic distribution in the molecule induced by the electronic affinity of the bromine atoms, subsequently leading to the cleavage of the C5-Br bond. Bond dissociations involving bromine atoms can occur according to three different general pathways: (a) we can have (Uyl − Br) → Uyl' + Br$, followed by electron pick up by Br$^-$ due to the high electronic affinity to give Uyl' + Br$^-; (b)$ dipolar dissociation can lead to (Uyl − Br) → Uyl$^+$ + Br$^-; and (c) (Uyl − Br) → Uyl' + Br$^+; based on the absence of Uyl$'$ signal in the BrU anion mass spectra (and only weak Br$^+$ yields), the latter channel is probably the least favored reaction of the three in BrU. In U, however, the small (U-H)' , i.e. Uyl$'$ yield, partially associated with the H$^+$ yield, suggest that this bond cleavage channel is accessible in uracil.

7.3 Desorption energy thresholds

Figure 4 shows the relative yields of cation (a, b) and anion (c, d) fragment desorption as functions of incident ion energy, measured during Ar$^+$ ion irradiation of
200ng/cm² uracil (a, c) and BrU (b, d) films. The data presented in fig. 4 are used to estimate the desorption energy thresholds of major positive and negative fragments from the uracil and BrU films. Most cations desorbing from uracil films (fig. 4a) appear at energies between 25 eV ([U+H]⁺, [U-O]⁺, [U-OCN]⁺, HNC₂H₂⁺, HNCH⁺) and 30 eV (CH₃⁺, H⁺). These desorption energy thresholds are slightly higher than those of major cation fragments desorbing from thymine films of same thickness, with the exception of CH₃⁺ and H⁺ fragments, which appear in thymine around 40 eV, but appear here at incident ion energies near 30 eV.

![Graphs showing relative ion yield vs. incident Ar⁺ energy for uracil and BrU.](image)

**Fig. 4** Relative yields of positive (in (a) for uracil and (b) for BrU) and negative ((c) for uracil and (d) for BrU) ion fragment desorption as functions of incident ion energy during Ar⁺ ion irradiation of 200ng/cm² uracil and BrU films on Pt.
The yields of major cations desorbing from BrU films presented in fig. 4b indicate even lower desorption energy thresholds for BrU. Most BrU positive fragments appear between 10 and 20 eV, which is slightly less than for thymine. This can be seen with \( \text{CH}_3^+ \) fragments, detected here near 20 eV instead of 40 eV for thymine. It is interesting to note that, as for thymine, the endocyclic fragments such as \([\text{U}-\text{OCN}]^+\), \(\text{HNC}_2\text{H}_2^+\), \(\text{HNCH}^+\) for uracil or \(\text{HNC}_2\text{HBrH}^+\) for BrU appear at lower incident ion energy than the exocyclic fragments \(\text{CH}_3^+\) and \(\text{H}^+\) under \(\text{Ar}^+\) irradiation.

The relative yields in fig. 4c indicate that most of the major anion fragments desorbing from uracil films appear around 70 eV, a higher value than for thymine for which \(\text{H}^-\) appears at 40 eV and \(\text{CN}^-\) and \(\text{OCN}^-\) at 35-40 eV. The opposite trend can be seen in fig. 4d, showing that the major negative ion fragments desorbing from BrU films mostly appear between 25 and 35 eV, much less than for both uracil and thymine. If BrU deprotonates upon adsorption (similar to thymine), the strong electron affinity of the Br group could render the \((\text{BrU-H})^-\) less prone to neutralization by the incoming projectile; in that case collisional excitation would yield greater numbers of surviving anion fragments, and lower the apparent desorption threshold. It is noteworthy, however, that in BrU the \(\text{H}^-\) threshold is lower than for \(\text{Br}^-\), despite the greater electron affinity of Br, and the smaller C-Br bond energy compared to C-H or N-H.

While the desorption energy thresholds of both cations and anions are greater than in thymine for uracil, and smaller for BrU, overall, anion fragments appear at higher incident ion energies than cation fragments in all cases, for uracil, BrU, and thymine.
7.4 Summary and conclusions

The positive and negative ISD mass spectra of uracil and BrU show the similar major fragmentation channels as those identified for thymine. The major dissociation channels found for thymine in chapter 4 are also found in both uracil and BrU mass spectra. The cation fragments produced by Ar$^+$ ion impact on uracil (BrU) films are dominated by \([\text{U}+\text{H}]^+\) ([BrU+H]$^+$), \([\text{U}-\text{O}]^+(\pm \text{H})\) ([BrU-O]$^+$ (±H)), \([\text{U}-\text{OCNH}]^+(\pm \text{H})\) ([BrU-OCNH]$^+$ (±H)), OCNH$_2^+$, HNC$_2$H$_2^+$, HNCH$^+$, and CH$_i^+$ (i = [0, 3]). The anion fragments are dominated by H$^-$, O$^-$, CN$^-$, OCN$^-$ and an additional contribution of $^{79,81}$Br$^-$ ions for BrU. [U-H]$^-$ and [BrU-H]$^-$ are more clearly observable at low Pt coverage because of their formation dynamics discussed in chapter 5. Some structures around 50 ≤ m/e ≤ 60 remain unassigned for both uracil and BrU, comparison with thymine ISD mass spectra not allowing for a clear identification.

As observed in the case of thymine during Ar$^+$ impact, anion fragments appear at lower incident ion energies than cation fragments. Both positive and negative ion fragment desorption involve exocyclic and endocyclic bond cleavage. Desorption energy thresholds for cations and anions are both higher for uracil, and significantly lower in the case of BrU, than those of thymine fragments. Desorption thresholds as low as 10 eV (HNC$_2$H$_2^+$ fragment from BrU) are likely to allow for significant ion damage to cellular DNA containing BrU, by fragmentation at even lower ion incident energies than previously found in thymine.

At the same time, the relatively large amounts of $^{79,81}$Br$^-$ ion fragments produced during 200 eV Ar$^+$ ion irradiation of BrU films can be synonymous of large amounts of non-thermal uracil-yl radicals, ready to interact with surrounding molecules. While BrU generates much interest because of the potential application of its radiosensitization properties in radiation therapy, its underlying mechanisms of action are not yet fully
understood. Recent studies performed with incident low energy electrons have shown a reduction of BrU molecules into molecular anions that subsequently dissociate into Br⁻ ions and reactive uracil-yl radicals. These studies point out that these uracil-yl radicals as possible precursors for biological damage by enhancing DNA strand breaks.
CHAPTER 8 – Summary, Conclusions, and Future Work

Radiation induced transformations at the molecular and cellular level are initiated by the interaction of fast primary particles (photons, electrons, ions) with cellular constituents, which triggers a series of primary, secondary, and reactive sub-picosecond events (Sanche, 1989a). Via primary ionizations or excitations, initial high-energy particles produce secondary electrons, cations, anions, excited molecules and neutral fragments, which contain essentially all of the energy of the primaries (La Verne and Pimblott, 1995). All these initial ballistic transients (electrons, ions, neutral fragments) thermalize within 10^{-12}s via multiple energy loss interactions with their environment.

The primary energy depositions by photons and electrons are now fairly well understood (Inokuti, 1971; Mott and Massey, 1965), and available data (LaVerne and Pimblott, 1995; Ahnesjö and Aspradakis, 1999; Goodhead and Nikjoo, 1989; Holley et al., 1990) serve to calculate energy absorption in biological tissue from different types of low LET radiation, e.g. X- and γ-rays, or electrons, via suitable Monte Carlo based codes, which also serve to link absorbed dose to relative biological effects. There exists, however, a large gap of knowledge between the primary events induced by energetic protons or heavy ions, which determine dose and the initial distribution of secondary reactive transients, and slower chemical or biological events, which describe the endpoints of such high LET radiation (DOE, 1998). We can determine quite precisely the energy deposited by an ion in a given volume of matter, but we do not have a precise understanding of the subsequent sequence of secondary ion (or electron) induced events because at this time there exists no nanoscopic,
molecular level picture for primary or secondary energetic ion damage to DNA, its
components, or other biomolecules.

As part of this work, we have completed the design and construction of a novel ion
beam apparatus, used here to investigate ion damage to films of thymine via the use of
energy (1-500 eV) and mass selected ion beams. Considering the technical difficulties of
obtaining well focused, mass and energy selected ion beams at energies below 5 eV, it is
worth mentioning that this apparatus is one of only a select few in the world with similar
capabilities. It is presently believed that heavy ion irradiation causes DNA damage via
similar pathways as conventional ionizing radiation (Brons et al. 2003; Krämer and Kraft,
1994), involving mainly simple ionization, single bond cleavage, and slow radical attack, but
modulated by the different structure and higher ionization density of the ion track. However,
it is our hypothesis that primary and secondary ions incident on organic media induce
significantly greater damage that reaches beyond traditional models of single ionization, or
free radical (OH) attack. This was confirmed in our recent experiments on bio-organic model
systems which showed that:

(a) atomic and molecular, singly or doubly charged primary cation impact (e.g. \( D_n^+ \),
n=1-3, \( \text{He}^+ \), \( \text{N}^+ \), \( \text{N}_2^+ \), \( \text{Ar}^+ \), \( \text{Ar}^{2+} \)), at ion energies between 10-500 eV in 1-2 nm thick films of
thymine completely fragments the molecules, even at energies as low as 15 eV (near 0.5
eV/amu for \( \text{Ar}^+ \)), resulting in the formation of over 30 different secondary cation and anion
fragments with energies below 5 eV; many of these fragments react in the films before
desorption, and result in formation of new products, e.g. \( \text{OH}^- \) desorption from thymine under
\( \text{Ar}^+ \) impact. For \( \text{Ar}^+ \) impact at 100 eV (2.5 eV/amu) we find notably a quantum yield of
thymine destruction of about 5.5-6 molecules/incident \( \text{Ar}^+ \).
(b) We also observe strong reactive scattering channels for some projectiles, e.g. HeH⁺ formation and desorption for thymine films irradiated with 10-300 eV He⁺ ion beams. For He⁺ impact we also observe strong enhancements in C-H and N-H bond cleavage, compared to Ar⁺ or Ar⁺⁺ at comparable energies; this enhancement seems to also extend downward in He⁺ energy, and can still be observed at He⁺ impact energies near 4 eV/amu. This suggests a potential scattering mechanism for base damage by He cations likely involving dissociative Auger processes by the 24.58 eV potential energy of the He⁺, allowing for ionizations of some of the molecular core orbitals of thymine. Furthermore, our experiments have shown that reactive scattering of nitrogen cations can also lead to carbon and hydrogen abstractions, viz. formation of CN⁻ and NH⁺.

(c) The notion that fragment anions with energies below 5 eV, produced by secondary electrons, can lead to further physico-chemical damage in organic films is confirmed by electron beam initiated ion reactions: reactions of 1-4 eV O⁻ via hydrogen abstraction occur in condensed benzene and toluene “base-like” model systems, where hydrogen abstraction from benzo vs. methyl groups occurs with similar probability.

First of all, the abundant positive and negative ion fragments produced by ion impact on thymine films suggest multiple fragmentation channels, indicating that ion impact can cause severe damage to DNA bases. In addition, the production of the detected ionic fragments is associated with the production of many radicals and neutrals, some of which can be anticipated from the desorbed ionic fragments. For example, formation of [T-O]⁺ fragments is associated with formation of O radicals, since O⁻ ions are formed at a much higher threshold energy than [T-O]⁺, while formation of CH_i (i=0-3) fragments via C-CH₃ bond cleavage is associated with the formation of uracil-yl radicals. These neutral fragments are believed to be also non thermal and to induce further damage in the biological medium.
For example, uracil-yl radicals produced via C-Br bond cleavage of 5-Br-uracil during irradiation (Abdoul-Carime et al., 2000) are believed to be reactive precursors (Bhatia and Schuler, 1973) to strong enhancement in cellular genotoxic damage (McLaughlin et al., 1993), when 5-Br-uracil is introduced in DNA as a radiosensitizing substitute for thymine. In the same manner, the benzo or toluene type radicals, formed by hydrogen abstraction reactions induced by O\textsuperscript{-} ions can (a) lead to reactive damage by reacting with surrounding molecules (H\textsubscript{2}O or proteins), (b) become oxidized, thereby preventing correct identification of the base during replication and possibly leading to gene mutations, or (c) be transferred to the DNA sugar backbone to induce a strand break. The further investigation of the chemical reactivity of these observed ionic fragments, to evaluate their potential in producing damage to DNA during the subsequent diffusion-limited reaction cascades, is therefore of a great biological interest.

Second, the measured energy thresholds of fragment desorption suggest that ion impact can cause kinetic damage to thymine at very low energies. Furthermore, in ISD experiments, in order to desorb from the surface, an ionic fragment needs additional kinetic energy to overcome the polarization barrier induced by the interaction between the ionic fragment and the metal substrate as well as the dielectric film, and is thus sensitive to both the distance of the ionic fragment from the Pt substrate, and the molecular polarizability of the solid (Hervé du Penhoat et al., 2001; Michaud and Sanche, 1990). This polarization charge induced desorption barrier is estimated to be \sim 1.1 \text{ eV} in simple hydrocarbon films of 4-5 ML (Rowntree et al., 1991). It is likely that a polarization potential of similar strength is induced here in thymine films, given the similar film thickness. As the kinetic energy of the fragments is believed to originate mainly from the primary ion impact via momentum transfer, and only part of the incident ion kinetic energy is transferred to the target molecule
(depending on the projectile ion type), the actual energy thresholds for thymine damage can in fact be much lower than the measured desorption thresholds. In other words, molecular damage in the film is likely to continue at energies significantly below 10-15 eV, yielding however ionic fragments with kinetic energies insufficient for desorption from the film.

As a consequence, it is believed that ion damage to biomolecules virtually occurs in all types of ionizing radiation. During conventional irradiation of DNA by electrons, X- and γ-rays, abundant secondary ions are produced via dissociative ionization or electron attachment processes. Dissociative ionization can result from either (a) valence excitation or (b) core excitation:

(a) Valence excitation refers to promotion of a valence electron to a higher unoccupied molecular orbital or the ionization continuum, resulting in an excited molecule. Subsequent decay may lead to the dissociation of the molecule, producing secondary ions as well as radicals/neutrals with energy typically below 5 eV. Fragmentation mechanism and patterns are similar to low energy (e.g. 70 eV) electron impact on gas phase molecules due to the fundamental similarity of the excitations. Dissociative electron attachment also produces very low energy secondary ions. These fragment ions, with energy much lower than the threshold energies as measured here, may not cause kinetic damage to DNA or its subunits, but may still cause physico-chemical damage depending on their individual chemical reactivity, as was recently observed in nitrogen cation irradiated films of DNA sugars, even at energies near 0.5 eV (Deng et al. 2005b).

(b) If core excitation occurs, it generates a core hole as a result of the promotion of a core electron to an unoccupied molecular orbital or Rydberg state or the ionization continuum. In light elements like C, N, and O, a core hole decays preferentially via Auger processes than by X-ray emission. Auger decay results in many different excited states
including single- or multi-charged states, some of which may be dissociative. Dissociative Auger decay following core excitation has been extensively studied in both gas phase and condensed phase (Eberhardt et al., 1983; Eberhardt et al., 1987; Romberg et al., 2000; Nagaoka et al., 2001). Usually, Auger decay produces a double-charged state. The two positive charges may locate in one atom in case of intra-atomic Auger decay or two adjacent atoms in case of inter-atomic Auger decay. These ionized molecules are highly unstable and fall apart following decay. In combination with Coulombic repulsion, this process usually produces fragment ions with energy higher than valence excitation, for example, up to 15 eV (Eberhardt et al., 1987; LeBrun et al., 1993). These processes may also occur during irradiation of DNA if the charge cannot be dissipated efficiently in the DNA medium. Multi-charged states can also be produced. In particular, a triple-charge state can be produced if one more valence electron is shaken off during Auger decay (Eberhardt et al., 1987; Tanaka et al., 2002), as well as by a double Auger decay process. Furthermore, if two core excitations are produced in one molecule such as a DNA subunit, Auger decay of the two core holes will place even more charge on the molecule. In these cases, the resultant Coulombic repulsion leads to more violent molecular dissociation and results in fragments with even higher kinetic energy. On the basis of the present results, given an energy above the threshold, the fragment ions produced via decay of specific core excitations are probably capable of producing secondary kinetic damage to DNA by impacting its neighboring DNA subunit, in addition to the chemical damage, which depends on the chemical reactivity of individual fragments.

During heavy charged particle radiation therapy, secondary ions with even higher energy can be produced. This is demonstrated by recent studies on high energy ion-biomolecule interactions (Schlathölter et al., 2004; de Vries et al., 2003ab; Coupier et al., 2002), where secondary ions such as O^{n+}, N^{n+} and C^{n+} (n=1-3) with energy up to 300 eV are
created during 0.5 MeV $^{129}$Xe$^{25+}$ impact with tymine molecules (Schlathölter et al., 2004). In combination with our present measurements, these results suggest that those secondary ions may play a significant role in DNA damage following heavy particle irradiation. While it is traditionally suggested that the severe damage produced by heavy ion irradiation is mainly due to the high density of ionization along the primary ion track (Krämer and Kraft, 1994), our results suggest an additional difference between heavy ion irradiation and conventional radiation: in addition to its unique dose distribution profile, the contribution of secondary ions produced along the primary ion track is extremely significant, and needs therefore to be carefully considered when evaluating DNA damage caused by heavy particle radiation therapy.

Our present work brings, for the first time ever, clear evidence that hyperthermal ion interactions with DNA bases lead to severe damage, even at incident ion energies as low as 10-15 eV. Our studies also indicate what type of energetic secondary ions are likely produced by primary heavy ion track-ends in DNA components, and furthermore confirm the hypothesis that the subsequent reactions of these fragments may lead to further damage and chemical transformation. It is our working hypothesis that, much like the traditional models of molecular excitation, single ionization, or free radical production, induced by high energy photon or electron tracks, the fundamental primary or secondary ion reaction pathways observed here for the first time are generally observed, or will be observable, in any molecular system (however somewhat modulated by the particular physical and chemical nature of the target). Thus, they are expected to occur in living cells as well, and the studies performed here illustrate ion damage to DNA components at a molecular level, and will provide a real indication about the link between physics and biology, that is, between nanoscopic damage and biological effect.
Armed with the remarkable results of our present studies, we now propose to complement our investigations on DNA with other molecules, including thymidine (recently completed, Z. Deng, and M. A. Huels, to be published), sugars (studies recently undertaken in our laboratory by Ilko Bald, visiting student from Berlin) and other nucleotides, as well as to extend our investigations to more complex molecules involving mono- or oligo-nucleotides, plasmid DNA, or proteins. Non-volatile molecules like oligonucleotides, plasmid DNA, and proteins are synonymous with non-vacuum prepared samples and require therefore a modification of the load-lock chamber, which has been completed. To further investigate low energy ion interaction mechanisms, the range of projectiles can be expanded to heavier ions like Xe\(^+\), Xe\(^++\), and Xe\(^+++\), or reactive ions such as H\(_2\)O\(^+\), O\(^+\), O\(^-\), OH\(^+\), OH\(^-\), CO\(^+\), and NO\(^+\), or reactive protonated ions including CH\(_3\)\(^+\), NH\(_4\)\(^+\), and H\(_3\)O\(^+\) ions (O\(^+\)\(^-\), OH\(^+\)\(^-\), H\(_2\)O\(^+\) and H\(_3\)O\(^+\) also being associated with water radiolysis). Particularly for the reactive type ion species, we will concentrate on the lowest ion beam energies in the 0.5 to 10 eV range, which corresponds to those secondary ion energies found along heavy ion, as well as traditional radiation tracks.

Furthermore, ion reactions in the films are currently measured by mass selected fragmentation and reaction yields of fragments which leave the surface during ion impact, via the QMS. A further improvement will be to measure reaction end products which remain behind in the solid after ion impact, either by thermal desorption mass spectrometry, or by traditional biochemical analysis methods, such as HPLC. In fact, energetic neutral fragments in similar, or possibly greater abundance, as the ion fragments observed here are believed to be produced in ion impact events. Among them, some neutral radicals can be very reactive and will play significant roles in radiation damage. However, we are currently focused on the ionic fragments for two reasons: First, in order for the neutral fragments to be detected by our
mass spectrometer, they are ionized by electron impact, which also induces further fragmentation and makes the identification of the initial desorbing neutral fragments more complicated; consequently, secondary neutral mass spectrometry is technically more sophisticated and costly, and requires, e.g., laser photo ionization of the neutrals. Second, to some extent, the desorption of abundant ionic fragments already reveals much information on chemical bond breaking, and more importantly, the events which may occur during ion irradiation and before fragment desorption, e.g. hydrogen abstraction reactions. Our ion desorption mass spectra do, however, already give some impression of what type of desorbing neutral fragments are associated with the desorbing ion fragments. In the very beginning of this type of new study, concerning ion interactions with DNA and its components, we thought it appropriate to first focus on the more easily measured effects, i.e. the ionic fragments, to obtain and interpret first results. The formation of neutral products that remain in the films is also important, and is currently being investigated on a second apparatus (L. Sellami and M. A. Huels, to be published), which allows hyperthermal Ar$^+$ ion irradiated thymidine films to be recovered and analyzed by standard biochemical methods (HPLC or LC-MS/MS). The first results are very promising, and show cleavage of the glycosidic bond by impinging Ar$^+$, even at energies down to 2 eV, as well as formation of possibly six new stable neutral degradation products. These experiments will however require further thorough analysis, and identification of the neutral products.

In any case, it is clear that the combination of such measurements during and after ion impact, with characterization of ion reaction pathways and final end products, will allow us to make a major contribution to our understanding of the fundamental DNA damage mechanisms induced by radiation tracks: The results of our preliminary studies, presented here, already suggest that nascent DNA damage mechanisms by ion tracks reach far beyond
conventional models of simple ionization, single bond cleavage, or slow radical damage. This challenges the traditional views that (a) DNA damage by heavy ion tracks involves similar nascent transients and damage pathways as those initiated by conventional (γ or X-ray) radiation tracks, and (b) the higher relative biological effectiveness of ion tracks is determined merely by the different structure and higher ionization density of the ion track compared to conventional radiation tracks.
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Appendix 1: Dose-depth distributions for 10 MeV electrons and photons

Dose-depth distributions of 10 MeV electron and photon beams, measured with a water phantom at a radiation therapy facility in France (MIR.Sys, private data).
## Appendix 2: World wide charged particle patient totals

– January 2005 –

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Source: J. Sisterson, Particles, 35, January 2005
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<tr>
<td><strong>Effect of spacecraft shielding</strong></td>
<td>\begin{itemize} \item Little attenuation through ionization \item Projectile and Target Fragmentation by heavy ions \item Target fragmentation by protons \end{itemize}</td>
<td>\begin{itemize} \item Electrons fully attenuated, some production of Bremsstrahlung \item Attenuation of low-energy protons \item Target fragmentation by higher-energy protons \end{itemize}</td>
<td>\begin{itemize} \item Low-energy protons attenuated \item Higher-energy protons and heavy ions can undergo nuclear interactions \end{itemize}</td>
</tr>
<tr>
<td><strong>Models/predictive capability</strong></td>
<td>\begin{itemize} \item Good agreement with measurements \item Badhwar/O'Neill \item CREME96 (Nynmik) \end{itemize}</td>
<td>\begin{itemize} \item Electrons: AE-8 (empirical model, old dataset, some refinements) \item Protons: AP-8 (empirical model, old dataset, some refinements) underpredicts at solar max overpredicts at solar min \end{itemize}</td>
<td>\begin{itemize} \item Little predictive capability \item NOAA Space Environment Center \item GOES satellites \item Neutron ground monitors \end{itemize}</td>
</tr>
<tr>
<td><strong>Relative contribution in LEO</strong></td>
<td>\begin{itemize} \item Dominates for high-inclination orbits \item ( \sim 50% ) of ISS dose \end{itemize}</td>
<td>\begin{itemize} \item Dominates for low-inclination, high-altitude orbits \item ( \sim 50% ) of ISS dose \end{itemize}</td>
<td>\begin{itemize} \item More severe in higher-inclination orbits \item Unknown contribution to ISS Dose \end{itemize}</td>
</tr>
<tr>
<td><strong>Relative contribution in free space</strong></td>
<td>Omnipresent</td>
<td>N/A</td>
<td>Presents potentially large risk, especially during solar maximum</td>
</tr>
</tbody>
</table>

In LEO, relative contributions from all sources are dependent on orbital parameters (orbital inclination, altitude), spacecraft orientation, solar cycle and temporal variations in the geomagnetic field in a highly complex way.