Formation of gas phase macromolecular targets by laser desorption from surfaces

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Abstract. To study biomolecular fragmentation induced by low energy ions, electrons, photons and radicals requires the production of a characterised gas phase target. Previous studies have used thermally evaporated DNA or RNA bases however this technique cannot be applied to a wide range of biomolecules because of fragmentation or decomposition. The technique described here involves the use of IR laser desorption for the production of the target in conjunction with time of flight mass spectrometry for analysis of molecular fragmentation. Characterisation of the targets will be carried out by fluorescent dye tagging and laser induced fluorescence imaging of the target spatial density profiles.

1. Introduction
A revolution in tumour therapy has come from the exploitation of the pronounced Bragg peak inherent in the passage of high energy ions (typically a few 100 MeV u⁻¹) through tissue. The energy deposition profiles of these ions are such that their destructive power can be focussed on the tumour with minimal damage to surrounding tissue. Consequently, there are improved clinical outcomes with higher clear-up rates and lower side-effects reported [1]. When the heavy ions reach the depths corresponding to the Bragg peak their kinetic energy has reduced to between several 100 keV u⁻¹ and zero. At the Bragg peak kinetic energies, the linear energy transfer to the tissue is highest resulting in maximum damage. In addition to direct destruction of DNA due to the primary particle, secondary species such as radicals and low energy electrons [2] and ions [3] also cause considerable damage. Secondary, singly ionised species can be formed through fragmentation events while multiply charged secondary ions are likely to be produced through Auger de-excitation following core ionisation by the primary particle [4]. Recently [5] we have investigated the interaction of singly and doubly charged, low energy, carbon ions with solid phase plasmid DNA and observed single, double and multiple double strand breaks. It was also observed that, for the same kinetic energy (2 keV), doubly charged ions induced more strand breaks than singly charged ions, indicating that the release of the potential energy of the ion was also important in the interaction.
The next phase of these ion irradiation studies is the investigation of these effects at a single molecule level in the gas phase where it is possible to use spectroscopic and mass spectrometric techniques. For example, time of flight analysis of fragment ions produced as a result of the interaction of the ions with the biomolecule will enable detailed information to be obtained on the susceptibility to breakage of specific molecular bonds. Information on absolute cross sections and severity of damage to the target may also be gathered. Such studies require the preparation of well defined target systems. However, the production of isolated gas phase biomolecules is not trivial since, as molecular mass increases, so does the likelihood of thermal decomposition. Thermal evaporation has been used to provide targets of DNA building blocks such as nucleobases for ion irradiation studies [6].

To produce a well defined gaseous target, two critical challenges have to be met, (i) the minimal loss of molecules to fragmentation processes in the production stage and (ii) the production of sufficient and known target densities for interaction studies to be performed. One approach is to use a well established laser desorption technique, such as MALDI [7], developed for mass spectrometry of large molecules. This laser (predominantly UV) desorption process can yield substantially more neutral molecules (10^4 - 10^5 more molecules than ions) [8,9] and is therefore a very promising possibility for the production of a neutral gas phase target. However, the use of a matrix results in a target with both matrix and analyte components making it difficult to separate matrix interaction effects from those with the analyte. Previous work [10], used a fluorescence imaging technique to record the plume of matrix and analyte molecules produced by laser desorption from a surface, showed that lighter mass matrix molecules disperse in a cosine distribution with respect to the normal to the surface. The analyte molecules (protein) were desorbed in a forward directed plume with a small radial extent resulting in a dense localised target of the heavy molecule. Since plume velocities of the order of 10^5 cm s^{-1} were measured in this work, it should be possible, after 10 – 20 µs has elapsed, to achieve a target that is predominantly analyte. Alternatively, a matrix free desorption technique [11] using infrared laser radiation may be employed in which a surface such as graphite or silicon absorbs the laser energy leading to desorption of the molecules.

2. Sensitivity Requirements

Matrix free desorption techniques have been the subject of a limited number of studies. As a result, the plumes produced have yet to be characterised. In order to assess the sensitivity of our experimental system, we have carried out some calculations based upon a matrix assisted desorption plume. Sample preparations typically use a matrix to analyte ratio of ~ 1000:1. Previous measurements [12] have demonstrated matrix concentrations of ~ 10^{16} cm^{-3} in a plume of 3-Hydroxypicolinic acid (3-HPA) molecules, at a distance of 3 mm from the target surface. Therefore, an analyte concentration of approximately 10^{13} cm^{-3} can be expected. A representative example is an analyte of Deoxy ribonuclease I (DNase I) protein labelled with two covalently bonded tetramethyl rhodamine (TMR) dye molecules produced in a plume 10 mm in length and 2 mm diameter with the above density. With a mean absorption cross section of σ ~ 2 x 10^{-16} cm^{2} for randomly oriented fluorophores and a quantum efficiency of 0.28, the fractional absorption from the probe beam will be 8 x 10^{-4} and (for a 200 µJ probe pulse at 532 nm – ~2.5 x 10^{14} photons) this will result in emission of ~ 6 x 10^{8} fluorescent photons emitted into 4π steradians. The present system has a collection solid angle of ~ 5 x 10^{-3} steradians so that, assuming no absorption or reflection losses in the optics, ~ 2 x 10^{8} photons would be incident on the ICCD photocathode per probe dye laser pulse. With a typical gain of 100 counts per photon incident on the photocathode each fluorescent image excited by the probe beam will result in ~ 2 x 10^6 counts/image. Each pixel on the CCD is 13.5 µm square, therefore, for the recorded image of 10 mm × 2 mm we can expect ~ 2000 counts per pixel (with a typical noise of 900 counts per pixel).
3. Experimental Approach

Figure 1 shows the experimental layout. An aqueous solution containing the molecules of interest is deposited and dried on a stainless steel, graphite or silicon substrate. The biomolecules of primary interest will be oligonucleotides. These short lengths of single stranded DNA provide a model system incorporating many of the features of DNA found in a biological system but remaining sufficiently simple to enable unambiguous mass spectrometric characterisation. These oligonucleotides will be tagged with appropriate dye molecules allowing fluorescent imaging. Two lasers are available for desorption of the deposited material; (i) a standard Nitrogen laser (337 nm; 150 µJ) or (ii) an optical parametric oscillator (OPO) pumped by a Nd:YAG laser. The nitrogen laser is used for calibration of the system using the sample composition and preparation technique described above (derived from [9]). The OPO system provides output in the UV (355 nm; 7 mJ) and in the IR (tunable between 2.6 and 3.1 µm; < 1 mJ) and will be the desorption laser used during the matrix free study.

Figure 1: The apparatus for the production and characterisation of matrix-free, gas phase, biomolecular targets

Following desorption, a second laser is used to excite the dye tags attached to the oligonucleotides. This dye laser is pumped by a Nd:YAG laser and provides tunable light output in the UV and visible. Additionally, the pump laser wavelength (532 nm) may be used if higher energy probing is required. This probe beam is focused to a planar sheet and passed through the desorbed plume yielding fluorescent emission and allowing imaging of the plume by an ICCD camera (Andor Technology ISTAR™).

Application of a variable time delay (typically 5-60 µs) to the probe beam provides a series of snapshots of the expanding plume. It will be possible to measure plume dimensions and densities from the fluorescence emission intensity. A linear time of flight mass spectrometer attached to the chamber allows direct monitoring of ions produced in the desorption process providing a diagnostic to enable optimization of the yield of intact oligonucleotide molecules.

Preliminary tests were carried out, with Rhodamine B dye and using the 532 nm output from the dye laser system, to determine the sensitivity of the imaging system. Solutions with concentrations in the range \(10^{12} - 10^{17} \text{ molecules cm}^{-3}\) were used with a sheet beam from the laser (0.5 × 20 mm²). This experiment demonstrated detection of fluorescence with number densities as low as \(10^{12} \text{ molecules cm}^{-3}\).

Imaging of Tetra Methyl Rhodamine (TMR) dye tagged DNase I Protein has very recently been carried out, successfully demonstrating functionality of the imaging system. The first gas phase
fluorescent images of this system can be seen in Figure 2. The images reveal the time evolution of the desorbed protein molecules. Each image is the result of one desorption laser shot using the 355nm output of the OPO system. The broad absorption of the TMR dye was pumped by a Nd:YAG laser (output at 532nm) yielding fluorescence at 580nm. The time at which the probe beam passed through the plume relative to the desorption laser is shown at the top of the image. A colour gradient scale is shown at the bottom of the figure ranging from black to white, white indicating the most intense region of the plume. An approximation of distance from the target surface is indicated on the right of the figure.

![Figure 2: Time evolving plume images of TMR tagged DNase I Protein molecules](image)

Initial calculations have been performed to determine the average number density of protein molecules for a 2mm wide region 4mm from the target surface. The change in number density as a function of plume expansion time is shown in Figure 3.

![Figure 3: Plume Density of DNase I Protein 4mm from the Target Surface as a function of plume expansion time.](image)
Preliminary data reveals that an average number density of ~ $10^{12}$ molecules/cm$^3$ is readily attainable and that this density remains over a sufficiently long time frame for interaction studies to be performed.

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