Time-of-flight mass spectrometry of DNA for rapid sequence determination

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Peter Williams (Principal Investigator)

Neal Woodbury (co-Principal Investigator)

Department of Chemistry and Biochemistry

Arizona State University

Tempe, AZ 85287-1604

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Fig. 3. SIMION ion optical simulation of ion source optics. The focussing action of the Wehnelt electrode at the sample potential allows focussing of a divergent beam originating on the optic axis (e.g. in UV ablation) or of a forward-peaked, low-divergence beam originating from a wide sample area, by moving the sample along the ion-optic axis..

III.a DNA test samples

We synthesized a set of single-stranded DNA samples of different lengths ranging from 8 to 60 bases to evaluate our technique. These segments¹ contain mixtures of the four DNA bases, and the sequences were specified to preclude either self-annealing or intersegment annealing. To simulate short Sanger mixtures, we constructed 6-component test mixtures. For ice film experiments, the lengths were chosen so that no two segments differed in base number by a factor of 2. This allows unambiguous detection of possible artifacts such as dimer formation and doubly-charged ion formation. For UV experiments a negligible tendency to dimerize could be demonstrated in ancillary experiments and so the segment mass ratio criterion was relaxed. The compositions of the synthetic DNA fragments are listed in Table 1.

¹ Note: we use the term "segment" for these short DNA strands in order to reserve the term "fragment" for DNA that may be fragmented as a result of the ablation process.

Fig. 4. Mass spectrum of equimolar DNA test mixture: 8, 14, 20, 26, 32, 60 nucleotides, ablated from frozen aqueous solution film. The sequences of the segments are listed in Table 1. The spectrum is an average of 9 laser shots. Amount of sample loaded was ~ 20 pmol/DNA segment. Amount sampled to obtain this spectrum was ~ 20 fmol/DNA segment. Laser power 1.8 mJ into $\sim 100 \times 200 \mu\text{m}$ spot; wavelength 589 nm. The data are unprocessed.

III.b Ablation/ionization of DNA from ice films

Figure 4 shows the best multi-shot mass spectrum obtained to date with a mixed base DNA test mixture containing equimolar amounts of segment lengths 8, 14, 20, 26, 32, 60 nucleotides. This positive ion mass spectrum demonstrates most of the features required for successful sequence ladder readout:

Resolution: All six segments from the 8-mer to the 60-mer bases yielded clean, well-

peaks, with no sign of adducts, clusters or multiply-charged ions. An intense pulse of ions at low mass is always seen, arising from the ablation of the copper oxide substrate; this is well separated in time from the DNA molecular ion peaks and has no adverse influence on the high mass region of the spectrum. Most significantly, the peaks lack the extended tails which characterize the "hot" DNA peaks obtained using UV chromophore matrices (see section III.c), and there are no detectable fragment peaks, nor a continuous fragment background. *Such a clean mass spectrum is essential for accurate sequence ordering.* The resolving power (full-width, measured at 10% peak height) is ~50 at the highest mass (~ 18,500 Da) (see Fig. 6, where DNA mass spectra from ice and from a UV chromophore matrix are compared). For mixture analysis, this full-width resolving power is the required criterion. *The performance in Fig. 1 is considerably better than any reported time-of-flight data for DNA at this mass.* A resolving power only ~5 times better would allow real sequencing problems to be addressed (read lengths ~ 250-300 bases). However, due to the difficulty of obtaining reproducible mass spectra (see below), we have not yet devoted much attention to improving the resolving power; given that the resolution does not appear to be limited by peak broadening arising from the ionization process, significant improvement appears possible.

Sensitivity: The peak intensities for singly-charged ions differ by no more than a factor of 2 over the mass range, for an equimolar mixture. Uniform sensitivity over a wide mass range is an essential attribute for error-free mixture analysis. The data in Fig. 4 comprise a 9-shot average in a small region of a frozen film where adjacent shots all gave good spectra (the sample is moved for each shot because the entire film in the irradiation area ~100 x 200 μm is removed on each shot). The total amount of sample loaded was ~ 20 pmol of each DNA segment in a film smeared over ~ 2 cm^2 ; however, the amount sampled to obtain the mass spectrum was much smaller, ~ 20 fmol. Each individual shot consumed only ~ 2 fmol/component. Clearly, the sensitivity exists to attack Sanger mixtures, if reproducibility could be improved.

Small peaks are visible at times corresponding to 4 and 7-nucleotide DNA; initially we thought these were doubly-charged ions of the 8-mer and 14-mer, although it was puzzling that doubly-charged ions of the higher mass segments were not seen. Now, after discussion with the synthesizer personnel, we believe these peaks reflect impurities in the synthetic mixture arising from the DNA synthesizer; this then indicates a little of the capability of the technique to detect

low-level components.

Reproducibility: The reproducibility of ice film data has been poor. Only on about one sample in four could a region be found from which molecular ion signals were detected, and it was typically necessary to search a substantial area of each sample film to find such regions. Often, areas adjacent to a region which had yielded a single shot spectrum gave no molecular ion peaks, so that finding a region from which several successive shots could be averaged was much more difficult. The best data were obtained when the laser impinged on or near an edge of the frozen film.

III.c DNA ablation from UV chromophore matrices

We have also searched for materials which might act as ablation/ionization drivers for DNA under UV laser irradiation at 355 nm (frequency tripled Nd-YAG). These studies have had several purposes: (a) to compare the UV chromophore and ice film technologies to gain some fundamental understanding of the two processes, (b) to verify the high mass performance of our mass spectrometer using massive protein molecular ions, (c) to search for viable UV chromophore matrices for DNA which would allow robust analytical systems with inexpensive UV lasers. There appear to be two key criteria: (i) the chromophore material should have a high extinction coefficient at 355 nm, and (ii) the material should maximize incorporation of DNA into the chromophore crystals. UV matrices which work for proteins, such as α -cyano 4-hydroxycinnamic acid, are only moderately hydrophilic, dissolve poorly in water, and appear to incorporate DNA strands very poorly into the crystal when dried. We have evaluated anthranilic acid (2-amino benzoic acid).

Fig. 5 shows the negative ion mass spectrum of a DNA test mixture (segment sizes were 8, 10, 14, 20, 26, 32 bases) ablated from an anthranilic acid matrix at 355 nm. This mass spectrum is typical of spectra obtained for mixed-base DNA samples using most UV matrices. (Note that a number of authors have obtained very good results with UV laser ablation of polythymidilic acids, but more comprehensive studies have shown that mass spectra of homopolymers of the other bases, and heteropolymers, are far more difficult to obtain.) Although, depending on the matrix and preparation method, it may still be necessary to search the sample for a good ion-emitting region, with care, spectra such as that in Fig. 5 can be obtained from each sample loaded.

Fig. 5. Negative ion mass spectrum of equimolar 6-component mixture (8, 10, 14, 20, 26, 32 nucleotides) ablated from a solid anthranilic acid matrix. Laser wavelength 355 nm; pulse energy $\sim 0.1 \text{ J/cm}^2$; 50 shot average.

Three features of Fig. 5 are noteworthy:

- a) the peaks ride on a fairly intense background signal. This is not an effect of saturation of the detector by low-mass signals -- the mass spectrum was obtained at low power and the signal at low mass is not intense. Instead, this background must arise from *fragmentation* of the ablated DNA traversing the acceleration gap.
- b) the resolved DNA peaks are quite broad, show tails to lower mass, and for the 8-, 10- and 14-mers fragment peaks begin to be resolved at the low mass side.
- c) peak intensity decreases abruptly beyond the 10-mer. Ice film data (Fig. 4) show that this drop in intensity is not a detector problem, and must be related instead to extensive fragmentation

of the higher-mass DNA segments. These observations suggest that UV laser ablation is a far "hotter" process than ice film ablation (see section IV), and that sufficient energy is coupled into the DNA molecules in the former approach to rapidly disintegrate larger molecules during transit across the acceleration gap.

IV. Comparison of ice and UV chromophore data

It is quite instructive to compare the performance of the ice and anthranilic acid matrices for DNA mixtures. Figure 6 shows an expanded view of the region between ~ 26 and $42 \mu\text{s}$ in the

Fig. 6. Expanded comparison of mass spectra from ice film (upper) and anthranilic acid (lower) in the time region $\sim 26 - 42 \mu\text{s}$. Peaks due to the 8-mer and 14-mer are visible in the ice spectrum. An additional 10-mer component was present in the anthranilic acid matrix and gives a peak $\sim 33.5 \mu\text{s}$. The negative ion peaks from the anthranilic acid matrix appear at longer times due to deceleration rather than acceleration at the electron multiplier.

two spectra. This region contains peaks due to the 8-mer and 14-mer, and also a 10-mer (in the anthranilic acid spectrum, lower). The effects of fragmentation and other post-ablation chemistry in the UV-induced spectrum are clearly evident. The entire spectrum rides on a continuous sloping background, whereas for the ice data only detector electronic noise is visible in the baseline. There are tails extending to the low-mass sides of the UV-induced 8-mer and 10-mer peaks and at the 14-mer position little is visible but a step in the baseline and an extended fragment tail. The UV-induced peaks tail also towards higher mass, which may reflect formation of adducts of the matrix as is commonly seen in protein work. The UV peaks are broader than the ice peaks at the 50% height and much worse near the baseline -- so much so that it is impossible to establish a baseline resolution. This is important in sequence mixture analysis because lower intensity peaks adjacent to peaks such as those seen here could be lost in the tails. This comparison tends to underline our conclusion that ablation from UV-chromophore matrices produces vibrationally hot analyte molecules which fragment rapidly as they are accelerated away from the sample surface. In contrast, ablation from ice appears to produce cold, stable molecular ions. This is particularly significant when we begin work to improve the mass resolving power by using an ion reflector to compensate for initial energy spread in the ion pulse. Reflectors have proved of no utility in UV ablation of high mass proteins because the majority of the protein molecular ions decompose en route to the reflector. With vibrationally cold molecular ions resulting from ice ablation, the prospects for significantly improved mass resolving power with a reflector appear excellent.

Very recently, Becker *et al* reported that a new matrix material, 3-hydroxypicolinic acid, gave much improved mass spectra with mixed-base ss-DNA [3]. Fig. 7 shows a negative ion mass spectrum of our 8-mer - 60-mer mixture ablated from this new matrix. Indeed the results are strikingly improved. Sensitivity only begins to drop at around the 26-mer (in this mixture, the 8, 14, 20, 26 and 32 nucleotide components were present in equimolar quantities), and the 60-mer is clearly detectable (at 4 times the concentration of the 32-mer). However, resolution is still considerably worse than for the ice film data in Fig. 4.

The one major area in which UV ablation is considerably better than ice ablation is that of reproducibility. Although on UV chromophore targets it is often necessary to search the crystallized matrix deposit for good ion-emitting regions, such regions can usually be found on

Fig. 7. Negative ion mass spectrum of 6-component mixture ablated from 3-hydroxypicolinic acid matrix. Laser wavelength 355 nm. The 8, 14, 20, 26 and 32-mers were equimolar ($\sim 1 \mu\text{M}$); the 60-mer concentration was increased to $\sim 4 \mu\text{M}$. Approx. $5 \mu\text{L}$ of each component were loaded. The data have not been processed.

a properly-prepared deposit. In contrast, on ice film targets ion-emitting regions were much more difficult to locate. Despite significant improvements in our control of sample preparation, the ablation/ionization process has remained tantalizingly erratic. Good mass spectra are still only obtainable on small regions of the sample films, often after several hours of searching. There is clearly a variable which we can not yet control (as discussed in Section VIII, we believe that the problem lies in the ionization process). Below we discuss the steps so far taken to improve sample reproducibility.

V. Process control

From our preliminary work ion production appeared to be controlled by three key parameters [2,4]:

- a) ice films needed to be sufficiently thin (on the order of a few micrometers) to be completely vaporized by a shock wave driven by laser vaporization of a surface layer of the copper substrate.
- b) the state of the copper substrate surface appeared to be important, corroded surfaces giving less fragmentation in our collector experiments and somewhat more reproducible mass spectra, presumably due to better absorption properties and/or poorer heat conduction, both of which would lead to faster heating at the substrate/ice interface.
- c) ionization appeared to be more efficient and reproducible when the ablating laser was tuned to a sodium atom transition; arguably, this increased production of sodium ions which could attach to the ablated DNA molecules.

We have devoted a major part of our efforts towards understanding, controlling and optimizing these processes. At the same time we have continued to search for alternate routes to ablation and ionization which might offer better controllability and reproducibility and better potential compatibility with the high-throughput operation necessary for large-scale DNA sequencing.

Film thickness: We built an ancillary sublimator, with a small glass bell jar which allows good visual observation of the subliming film and can allow observation of interference fringes from thin films on polished substrates. With this it has become fairly routine to obtain films that appear to have sizeable areas of a suitable thickness and from which intact *neutral* DNA can be ablated routinely (see section VI). A film produced in this sublimator produced the mass spectrum shown in Fig. 4. This capability now is incorporated directly into a sample introduction lock on the instrument. We also built a simple spin-cast system, and have produced controllable spun films a few thousand angstroms thick.

Substrate surface: Copper substrates are corroded controllably by exposure for several minutes to acidified potassium permanganate solution. This produces a uniform black copper oxide surface which is both an excellent light absorber, and is also highly hydrophilic, so that aqueous samples can be spread uniformly over the surface.

Resonant ionization: This early observation led to our decision to purchase a dye laser

for ablation, and all ice film data reported here were obtained with the dye laser tuned to the 589 nm sodium D line transition. However, due to the continued lack of reproducibility of the process, it has not been possible to make a good comparison of on- and off-resonance conditions.

VI. Collector experiments: ablation mechanisms

By studying only the DNA mass spectra, it is impossible to isolate problems of ablation, ionization or detection, and it is difficult to investigate mechanisms of ablation. For this reason, we have continued our collector studies. By collecting ablated material, studying the angular distribution of the deposit and analysing the mass distribution by electrophoresis, the ablation process can be studied in isolation. Some of this work has been done with proteins rather than DNA because the electrophoresis is simpler (non-radioactive samples can be used and protein angular distributions can be visualized by staining). Both dry solid (2,4 dihydroxy-benzoic acid, DHB, a UV-absorbing matrix) and frozen (water matrix) samples have been studied.

For ice film ablation, the ablated vapor plume undergoes a fairly well-developed supersonic jet expansion. The angular distribution is characterized by a fit to a power cosine distribution, $\cos^n \theta$, where $n \sim 35-40$ (Fig. 8). This corresponds to Mach numbers $\sim 3-5$ in the

Fig. 8. Angular distribution of lysozyme ablated from thin frozen solution film on oxidized copper substrate. Wavelength 589 nm.

expansion from which it can be calculated that the initially ablated material cools by a factor of 3-4 in times on the order of a microsecond [4]. We believe that this cooling, together with the initially low matrix and sample temperatures arising from the indirect heating of the ice film, is responsible for the absence of fragmentation in the DNA mass spectrum of Fig. 4.

Observations that both matrix and analyte molecules ablated from UV chromophore matrices, have similar velocities have led to arguments that UV ablation also results in cooling. However, we find angular distributions of both analyte and matrix ablated from a UV chromophore (2,4 dihydroxybenzoic acid) which are only weakly forward-peaked ($n \sim 4-5$ in the power cosine fit as shown in Fig. 9). This signals at best a Mach 1 expansion which would result in only minor cooling; again this is consistent with the "hot" appearance of the mass spectra from UV chromophores (Figs. 5,6).

Fig. 9. Angular distribution of lysozyme ablated from solid 2,4 dihydroxybenzoic acid matrix (room temperature). Wavelength 355 nm.

Similar effects are seen in the fragment distributions determined by gel electrophoresis; ice samples produce little fragmentation of the protein lysozyme upon ablation, whereas the DHB matrix produces varying amounts of fragmentation depending on the power density. Consistent with our early results for DNA [1], the extent of fragmentation decreases as the power density is increased.

Ablation of non-radiolabelled DNA from ice films did not allow determination of an angular distribution; however we confirmed once again that intact DNA could be ablated from such films. A gel electrophoresis assay of the 8-mer - 60-mer 6-component mixture showed that all components were ablated substantially intact. Survival of intact DNA ablated from an ice film $\sim 1 \text{ cm}^2$ in area is additional confirmation that failure to observe ions in the mass spectrum is an ionization, not an ablation problem.

VII. Other studies

Given the difficulties of the visible laser ice ablation technique, we have investigated a number of alternative approaches.

a) IR laser ablation

A pulsed IR laser operating at $2.9 \mu\text{m}$ couples directly into the O-H stretch with a high extinction coefficient ($\epsilon \sim 10^3$), allowing direct ablation of water or ice films without an extrinsic chromophore. This would remove the sample thickness constraint in our approach since a thin layer of ice could be ablated from a thick frozen layer. Other groups have demonstrated that ions can be obtained with pulsed IR ablation, although ionization mechanisms are unclear. However, these groups used lasers with $\sim 200 \text{ ns}$ pulse lengths, which continue to heat the vapor plume for an extended time, and their mass spectra show evidence of this heating in fragmentation. We arranged to evaluate, with an option to purchase, an optical parametric oscillator (OPO) which produces a tunable, few nanosecond pulse when pumped by our YAG laser.

As expected, this laser showed ablation depths $\sim$ several μm , allowing multiple shots on the same area of a thick film. Protein mass spectra were obtained comparable to the results of other groups. However, spectral quality was no better than we obtained with UV chromophores, and acceptable DNA mass spectra were not obtained from ice or other solid IR chromophores such as succinic acid within the evaluation period. In particular, obtaining any high-mass ion

signals was an erratic process. Many shots failed to give acceptable spectra -- which turns out to be the experience, unfortunately unreported in the literature, of the other groups. We concluded that this approach was not sufficiently promising to divert our efforts into.

b. New sample preparation approaches

Our ice film mass spectra, and the angular distribution studies, are consistent with our belief that ablation of relatively thick (few μm) matrix films, achieved by minimal heating, can yield "cold", high quality mass spectra of DNA. However, there are a number of concerns with this approach. First, there are clear difficulties in incorporating freezing and sublimation requirements into any large scale sequencing application of mass spectrometry. Second, we are concerned that to get good molecular dispersion (solid solution) of DNA into any solid (including UV chromophores) is difficult. Both crystallization and freezing tend to exclude foreign material from the growing crystal lattice. In many ways, the ideal matrix for this approach is a room-temperature liquid that is vacuum compatible and also a good DNA solvent. Following this line of thought, we have discovered that solutions of tris-EDTA (TE) buffer, when sufficiently concentrated ($\sim 1\text{M}$ tris/ 0.1M EDTA), *do not evaporate to dryness in vacuum, but become viscous, highly-concentrated liquid films*. On polished metal substrates it is possible to produce such liquid films with thickness ranges measurable by interference fringes ranging from a few thousand angstroms to a few micrometers. At optimum TE concentration, the films retain approximately 80% water (by weight). Thin TE gel films can be cleanly ablated from a metal chromophore substrate by visible laser pulses of sufficient power. If suitable UV chromophores are incorporated into the solution, UV pulses ablate layers some tens of micrometers thick from the front surface. Such liquid, room temperature films *of a natural buffered solvent for DNA* may not only allow remarkably simple sample preparation but also avoid the concerns about solute segregation that arise when DNA solutions are frozen or when UV chromophore matrices are dried. To date we have obtained no DNA mass spectra from the thin TE films; again we think we are not controlling ionization in these samples. But we intend to continue to evaluate the ability of these films to produce mass spectra using either visible (thin film on metal chromophore substrate) or UV (thick film with UV chromophore) laser pulses.

VIII. Ionization discussion

It is abundantly clear from our collector studies that ablation of intact massive DNA

segments can be achieved routinely from ice films of appropriate thickness (few μm) and that we have no difficulty making such films (typically collector experiments ablate $\sim 1\text{ cm}^2$ film areas). It is equally clear that the vast majority of the film area yields *no* ionized DNA. However, from some regions ionization *does* occur. Thus, our focus has been to understand the reason for this and to develop an effective consistent means of ionization.

Our cooling calculations suggest that when cooling in the vapor expansion terminates, the molecules in the plume have temperatures $\sim 300\text{ K}$ or lower. At this temperature all molecules are in their ground electronic states and so ionization should indeed be unlikely. However, the mass spectrum always shows evidence of an intense flux of ions ablated from the substrate by the high power laser pulses used: why do these ions not attach to the DNA? Our belief is that the ions are usually physically separated from the ground-state DNA molecules by a thick vapor layer: typically as the sample film freezes the DNA concentrates near the outer surface of the frozen film so that immediately after ablation the substrate ion flux is separated from the DNA by several μm of vapor at much greater than atmospheric density. The water vapor plume must expand by a factor of $10^7 - 10^8$ in volume (i.e. to linear dimensions $\sim 1\text{ mm}$) before the mean free path for the gas phase ions becomes long enough for them to penetrate the plume and attach. However, the ions are accelerated by the high electric field in the sample region ($\sim 10^3\text{ V/mm}$), so that by the time the ions can contact the DNA they will have kinetic energies far too high for attachment to be possible. When we do see molecular ions, the laser typically is sampling at or near an edge of the ice film; it appears that at such a site, there may be some ions ablated from a substrate region not submerged by ice which reach the front of the ablated vapor plume before they have been appreciably accelerated by the field. Although it has been difficult to do controlled experiments to test this hypothesis there is other evidence supporting this view:

a) In our prototype mass spectrometer, which we abandoned because resolving power was inadequate, DN mass spectra appeared to be obtained more readily than in our present system. A key difference between the two may have been the absence of a large accelerating field in the prototype (operating with accelerating potentials of only $10 - 20\text{ V/mm}$) [2].

b) Hunziker et al recently reported a method for ablating and ionizing fluorocarbon polymers on a metal substrate [5]. They ablate a thin layer of the polymer over a few mm^2 area with an excimer laser and ablate ions from the metal substrate with a subsequent intense pulse from a

focussed Nd-YAG laser. The metal ions attach to the polymer molecules giving a good mass spectrum of the polymer distribution. Again, two key differences from our technique: (i) the process takes place in low or zero field (a Fourier transform mass spectrometer is used), and (ii) the ablated polymer layer is thin (a few hundred angstroms) so that the substrate ions can penetrate through the vapor layer after it expands.

c) Hillenkamp et al [6] have recently shown that ablated protein molecules can be ionized by attachment of Na^+ ions ablated from a nearby surface by a second laser. Again the process occurs in zero field conditions.

If ablation at a sample edge produces ionized DNA, an obvious question is why the edge of a previous laser crater in the film does not serve as an ionization site for the next ablation pulse. There appear to be several factors mitigating against ion formation at laser-created edges. First, the power available in the tail of the beam is considerably lower than in the central focus. Second, the previous crater has been laser-cleaned, i.e. heated to such a high temperature that surface oxide is removed and with it all or most volatile material, particularly sodium contamination. Thus we may get little or no ion flux from such regions. This might also explain our observation that performance seems to improve at the Na resonant wavelength: resonant ablation has been shown to produce ions from metal surfaces at power levels where non-resonant ablation has no effect. Our random successes may be due to the edge of the laser spot occasionally impinging on ice film-free metal surface regions which produce rich ion fluxes -- e.g. sodium-rich pockets which are typically found on contaminated surfaces. This is most likely when the spot overlaps the edge of the ice film and strikes the original alkali-contaminated oxide surface.

There are several possible solutions to this ionization problem. They include:

- (a) reconfiguring the laser focus to produce *two* intense foci separated by $\sim 50\text{-}200\text{ }\mu\text{m}$ so that a trailing crater is irradiated by an intense laser pulse as an adjacent ice region is ablated
- (b) use of a second laser pulse (or a pulse split off from the ablating laser) to produce ions at a nearby surface (this would need to occur in a zero field environment with subsequent pulsed extraction),
- (c) incorporation of ionizable material near the surface of the frozen sample films, or in chemical combination with the DNA.

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Presentations

1. "Progress towards DNA sequence determination using laser ablation time-of-flight mass spectrometry", David M. Schieltz, Robert M. Thomas, Cong-Wen Luo, Chau-Wen Chou and Peter Williams, paper presented at 40th ASMS Conference on Mass Spectrometry and Allied Topics, Washington, D.C., June 1992.
2. "Laser ablation time-of-flight mass spectrometry for DNA sequencing", Peter Williams (Invited), *Desorption '92*, Bad Waldeck, Germany, September 1992.
3. "Time-of-flight mass spectrometry of DNA laser-ablated from frozen aqueous solutions: applications to the Human Genome Project", Peter Williams (Invited), *Advances in DNA Sequencing Technology*, SPIE Symposium, Los Angeles, January 21, 1993

² This work grew out of our use of fullerenes for mass spectrometer calibration and evaluation and the consequent availability of a capability for rapid evaluation of small quantities of fullerenes; < 5% of instrument time was made available to collaborators during gaps in DOE-supported work.

4. "Power-dependent calibration shifts in pulsed laser ablation time-of-flight mass spectrometry", Chau-Wen Chou and Peter Williams, 41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, June 1993.
5. "Collector studies of angular distributions and fragmentation of proteins and oligonucleotides ablated from ice and UV-absorbing matrices", Congwen Luo and Peter Williams, 41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, June 1993.
6. "Matrix-assisted ablation using nanosecond pulses of tunable mid-IR radiation", D.M. Schieltz, D. Dogruel and Peter Williams, 41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, June 1993.

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