Characterization of Oligodeoxynucleotides by Electron Detachment Dissociation Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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Electron detachment dissociation (EDD), recently introduced by Zubarev and co-workers for the dissociation of multiply charged biomolecular anions via a radical ion intermediate, has been shown to be analogous to electron capture dissociation (ECD) in several respects, including more random peptide fragmentation and retention of labile posttranslational modifications. We have previously demonstrated unique fragmentation behavior in ECD compared to vibrational excitation for oligodeoxynucleotide cations. However, that approach is limited by the poor sensitivity for oligonucleotide ionization in positive ion mode. Here, we show implementation of EDD on a commercial Fourier transform ion cyclotron resonance mass spectrometer utilizing two different configurations: a heated filament electron source and an indirectly heated hollow dispenser cathode electron source. The dispenser cathode configuration provides higher EDD efficiency and additional fragmentation channels for hexamer oligodeoxynucleotides. As in ECD, even-electron d/w ion series dominate the spectra, but we also detect numerous a/z (both even-electron and radical species), (a/z – B), c/x, (c/x – B), and (d/w – B) ions with minimal nucleobase loss from the precursor ions. In contrast to previous high-energy collision-activated dissociation (CAD) and ion trap CAD of radical oligonucleotide anions, we only observe minimum sugar cross-ring cleavage, possibly due to the short time scale of EDD, which limits secondary fragmentation. Thus, EDD provides fragmentation similar to ECD for oligodeoxynucleotides but at enhanced sensitivity. Finally, we show that noncovalent bonding in a DNA duplex can be preserved following EDD, illustrating another analogy with ECD. We believe the latter finding implies EDD has promise for characterization of nucleic acid structure and folding.

Tandem mass spectrometry (MS/MS) is a well-established technique for oligonucleotide sequencing, including characterization of genetic markers, such as short tandem repeats and single-nucleotide polymorphisms. Most MS/MS strategies, such as collision-activated dissociation (CAD) and infrared multiphoton dissociation (IRMPD), involve the dissociation of even-electron anions. In those cases, neutral base (B) loss is a major fragmentation pathway. However, sequence-specific backbone cleavage, mainly in terms of (a – B) and w ions, is also observed, in which a ions contain the 5′ end of the oligonucleotide and w ions contain the 3′ end. Similar behavior has been reported for modified oligonucleotides. A major disadvantage of CAD and IRMPD, i.e., vibrational excitation, is secondary fragmentation, such as water and additional base loss, which complicates spectral interpretation and reduces sensitivity. Enhanced sensitivity has been reported through incorporation of a 7-deazapurine analogue, eliminating extensive depurination. Additional and complementary information can be obtained through fragmentation of radical anions, as demonstrated by McLuckey et al. through ion–ion reactions and by Hvelplund and co-workers through high-energy collisions with noble gas atoms. Particularly, reduced nucleobase loss from the precursor ions is seen.

Electron capture dissociation (ECD) also involves radical ion chemistry and provides unique fragmentation patterns for peptides and proteins, peptide nucleic acids, polymers, and...
lantibiotics. In all cases, additional and complementary structural information is obtained compared to MS/MS of even-electron ions. In particular, extremely “soft” fragmentation is achieved for peptides and proteins: backbone bonds can be cleaved without losing labile posttranslational modifications, allowing their localization. Also, backbone covalent bonds can be ruptured without breaking noncovalent interactions of a protein’s higher order structure. The latter feature has been exploited in the investigation of protein gas-phase folding and unfolding. We have found that ECD dissociation channels differ from other MS/MS techniques for oligonucleotides as well. However, with our first implementation, the fragmentation was nucleobase specific and rather limited. Recent results with an improved electron injection system and the ability to mass-selectively accumulate precursor ions (improving sensitivity) demonstrate more extensive fragmentation, resulting in complete sequencing of small (5–7-mer) oligonucleotides.

The main caveat for analyzing oligonucleotides by ECD is the requirement for positively charged precursor ions. Negative ion mode results in higher sensitivity due to the sugar-phosphate backbone, which undergoes facile deprotonation. Electron detachment dissociation (EDD), recently introduced by Zubarev and co-workers, involves higher energy (≥10 eV) electrons than ECD and has been shown to provide fragmentation similar to ECD but for negatively charged peptide ions. For peptide diamions, electron detachment is followed mainly by N–C₉ and C₉–C bond cleavage. As for ECD, more extensive backbone fragmentation can be obtained with EDD compared to techniques based on vibrational excitation, resulting in enhanced peptide sequence coverage. Further similarity between these two ion-electron reaction techniques is evident from the retention of a labile sulfate group, rendering EDD a potential tool for localizing posttranslational modifications in acidic peptides. Here, we show that two different EDD configurations result in fragmentation patterns similar to ECD for hexamer oligonucleotides, allowing complete sequencing at enhanced sensitivity. In addition, we demonstrate preliminary results indicating that EDD is as “soft” as ECD in that noncovalent bonding is preserved. That characteristic opens up an array of opportunities for exploring nucleic acid structure in the gas phase.

**EXPERIMENTAL SECTION**

**Sample Preparation.** All oligodeoxynucleotides were purchased from TriLink BioTechnologies, Inc. (San Diego, CA) as their crude sodium salts. Stock solutions of 0.1 mM in 0.1% formic acid (FA) (Acros Organics/Fisher, Fair Lawn, NJ) were prepared for desalting by C₁₈ ZipTips (Millipore, Billerica, MA). Oligonucleotides were washed with water containing 0.1% FA five times and eluted in 2 × 5 μL of 1:1 (v/v) acetonitrile/water (Fisher) with 0.1% FA and diluted 5–10-fold prior to electrospray ionization. The electrospray solvent consisted of 1:1 (v/v) 2-propanol/water (Fisher) with 10 mM ammonium acetate (Fisher).

**Fourier Transform Ion Cyclotron Resonance Mass Spectrometry.** All experiments were performed with an actively shielded 7.0-T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer with a quadrupole front-end (APEX-Q, Bruker Daltonics, Billerica, MA). The oligonucleotide solutions were infused via an external Apollo electrospray ion source at a flow rate of 80 μL/h with the assistance of N₂ nebulizing gas. The off-axis sprayer was grounded, and the inlet capillary was set to 4 kV for generation of oligonucleotide anions. N₂ drying gas (212–287 °C) was applied to assist desolvation of ESI droplets. Ions were accumulated in the first hexapole for 0.1 s, transferred through the mass-selective quadrupole (7–10 Da isolation window), and mass-selectively accumulated in the second hexapole for 1–4 s. Ions were transferred through high-voltage ion optics and captured by gas-assisted dynamic (hollow cathode and IRMPD experiments) or static (filament experiments) trapping with argon as the collision gas in an Infinity ICR cell. The experimental sequence up to the ICR cell fill was looped 2–4 times to achieve a precursor ion signal-to-noise ratio of 200–400. For static trapping, the potential was −1.2 V, and for dynamic trapping, the initial potential was −2.5 V followed by a 4-s pumping delay. Prior to excitation and detection (for dynamic trapping), the trapping voltages were lowered to −0.8 V. Thus, the observed mass accuracies were higher for the hollow cathode and IRMPD experiments. All mass spectra were acquired with XMASS (version 6.1, Bruker Daltonics) in broadband mode from m/z 200 to 2000 with 256 or 512k data points and summed over 10–30 scans. Data processing was performed with the MIDAS analysis software: A Hanning window function was applied, and the data set was zero filled once prior to fast Fourier transformation followed by magnitude calculation. A peak list was generated and exported to Microsoft Excel for internal frequency-to-mass calibration with a two-term calibration equation (the calibration could not be performed with MIDAS due to compatibility issues with the Bruker data files). The calculated masses of the [M + 2H]²⁺

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precursor ions and the charge-reduced \([M - 2\text{H}]^+\) species were used for calibration. Only assignments better than 20 ppm were included. The reason behind that relatively high tolerance is the deterioration of the mass accuracy as a result of the simultaneous storage of anions and electrons, which drastically deviates from ideal space charge conditions. Above \(m/z\) 700, product ions were only assigned if a clear \(^{13}\text{C}\) isotopic peak was present. For lower mass ions, a combination of mass accuracy and ion abundance was used.

**Electron Detachment Dissociation.** Our first EDD configuration consisted of a directly heated rhenium filament (3.3-A heating current), placed \(-100\) mm behind the ICR cell. The filament was positively biased (11 V), except for during the EDD event when the bias voltage was pulsed to \(-21\) to \(-23\) V for 0.2–12 s. A recently installed improved EDD configuration utilizes an indirectly heated hollow dispenser cathode,\(^{39}\) which replaced the directly heated filament. The inner and outer diameters of the cathode are 3.5 and 7.6 mm, respectively, and the distance from the cathode to the cell is 88 mm. A heating current of 1.8 A was applied to a heater element located behind the cathode. During EDD, the cathode voltage was pulsed from 11 V to \(-16\)–\(-17\) V for 1 s. A lens electrode (6-mm inner diameter) located immediately in front of the cathode was kept at the same voltage as the (pulsed) EDD cathode bias to focus the electron beam.

**Infrared Multiphoton Dissociation.** IRMPD was performed with a vertically mounted 25-W, 10.6-\(\mu\)m, \(\text{CO}_2\) laser (Synrad, Mukilteo, WA). The laser beam is deflected by two mirrors for alignment through the hollow dispenser cathode to the center of the ICR cell. The beam enters the vacuum system through a BaF\(_2\) window. Photon irradiation was performed for 150 ms at 40% laser power.

**RESULTS AND DISCUSSION**

**Filament Electron Detachment Dissociation of \(dA_6\) Anions.** An example of oligonucleotide EDD from our first directly heated filament configuration is shown in Figure 1. Doubly deprotonated \(dA_6\) was dissociated at a filament bias voltage of \(-21\) V with an optimum irradiation time of 200 ms. Mainly even-electron d/w-type ions are observed (these ions cannot be distinguished based on mass alone due to the symmetry of \(dA_6\)). In fact, the entire series is present in the spectrum, allowing complete sequencing. In addition, one a/z-type radical ion (labeled a\(_z^+\)) and one (c/x – B) ion are seen (McLuckey nomenclature\(^{39}\)). The presented spectrum is much more information-rich than our previous filament ECD data for \(dA_6\), which only resulted in charge reduction to the [M + 2H]\(^+\) radical species and very minor adenine base loss.\(^{30}\) However, no base loss from charge-reduced \(dA_6\) is observed in EDD. Thus, for \(dA_6\), EDD has the added advantage (besides negative mode operation) of providing significantly more sequence information than ECD under similar conditions. In addition to the expected singly charged product ions discussed above, two doubly charged products are observed: d\(_{52}^+\)/w\(_2^+\) and [M – B – 2H]\(^+\). Such ions were also detected in recent ECD experiments and were suggested to be formed from a zwitterionic precursor ion structure.\(^{31}\) In EDD, an alternative explanation could be direct dissociative electronic or vibrational excitation (electron-induced dissociation, EID\(^{39}\)) as an accompanying pathway. However, further experiments are needed to fully elucidate the origin of these ions. The two rather abundant doubly charged peaks observed at \(m/z\) 964 and 989, respectively, are not present in rf-only mode and are hypothesized to result from ion–molecule reactions in the quadrupole.

Comparison of our EDD data to previous radical oligonucleotide anion dissociation experiments reveals similarities as well as differences. Electron transfer from \(\text{CCl}_3^+\) followed by CAD of [dA\(_3\) – 2H]\(^-\) in an ion trap resulted mainly in d/w ions, as in EDD.\(^{10}\) In addition, one a/z ion was observed although as an even-electron species rather than the radical a/z ion observed in EDD. Transfer of a hydrogen atom should be facilitated at the longer activation period during ion trap CAD compared to EDD. In stark contrast to EDD, the ion trap experiments resulted in sugar cross-ring cleavage, which may again be explained by the longer activation period, allowing for secondary fragmentation processes. Similar cross-ring cleavage products were also observed in 100-keV collisions of [dA\(_3\) – 2H]\(^+\) with He gas, which results in abundant charge-reduced radical species from electron loss.\(^{11}\) In the latter experiments, similar to EDD and ion trap CAD of odd-


electron ions, d/w ions dominate and a/z ions are observed, in contrast to low-energy CAD of even-electron ions. In addition, high-energy CAD experiments yield (a/z – B) ions, similar to EDD. However, in contrast to EDD and ion trap CAD of odd-electron ions but similar to ECD, base loss from the charge-reduced species is observed. High-energy collisions also result in a doubly charged [M – B – 2H]+ ion that can be formed as explained above for EDD.

Hollow Cathode Electron Detachment Dissociation of dA₆ Anions. A recently installed improved EDD configuration consists of an indirectly heated hollow dispenser cathode, which has replaced the directly heated filament used above. The major advantage of a dispenser cathode over a filament is the high electron generation efficiency and narrow energy distribution. Figure 2 shows an EDD spectrum of dA₆, obtained by applying a –17-V bias voltage to the hollow dispenser cathode for 1 s. Product ion assignments are given in Table 1. The spectrum shows much more extensive fragmentation compared to the filament EDD above, and the relative fragment ion abundances are higher. As for the filament, the complete d/w ion series dominates the spectrum. One additional radical a/z ion and two additional (c/x – B) ions are observed. In addition to those species, four (a/z – B) ions are detected. With the filament, one possible (a/z – B) ion was seen but with a rather high (25 ppm) mass error, rendering the assignment uncertain. Other ions not present in the filament spectrum include four c/x products and three (d/w – B) products. Doubly charged d₅⁺/w₂⁺ and [M – B – 2H]²⁻ ions are detected at higher relative abundance than in filament EDD. Thus, it is more likely those species result from an EID-type or electronic excitation process rather than being formed from a zwitterion precursor because the electrospray and ion-transfer conditions were identical in the two cases.

The hollow cathode EDD spectrum is very similar to our previous ECD data obtained with a solid dispenser cathode electron source. EDD results in three more c/x-type ions and one more (d/w – B) ion compared to ECD. In addition, the detected a/z ions are radicals in EDD and even-electron species in ECD. However, because the ECD experiments were performed on a different instrument with different cell pressure and ion number, it is difficult to make a direct comparison. Neither ion–ion reactions in an ion trap nor high-energy collisions with He resulted in c/x, (c/x – B), or (d/w – B) ions, illustrating unique behavior in EDD. Thus, use of a dispenser cathode provides additional fragmentation pathways and higher efficiency EDD.

Hollow Cathode Electron Detachment Dissociation of dC₆ Anions. Inspired by the improved EDD of dA₆ obtained with the hollow dispenser cathode, we performed similar experiments for the remaining set of hexamer homodeoxyoligonucleotides.
overall appearance of the dC\textsubscript{6} EDD spectrum is similar to the one obtained for dA\textsubscript{6} with the dispenser cathode (see Supporting Information for the mass spectrum and ion assignments). Again, the entire d/w ion series is present as well as one complementary radical a/z ion and several (a/z − B), (c/x − B), and (d/w − B) ions. The most abundant product ion corresponds to doubly deprotonated d/w\textsubscript{5}, and a high-magnitude [M − B − 2H]\textsuperscript{2−} ion is also observed. Because cytosine has a higher proton affinity than adenine,\textsuperscript{14} polydC is more likely to have a zwitterion structure than polydA, correlating with our observations. However, the discussion for dA\textsubscript{6} above suggested more probable direct dissociative vibrational/electronic excitation as the source of doubly charged products. We believe electronic excitation is more probable than vibrational excitation because IRMPD (i.e., vibrational excitation) of cytidine-containing oligonucleotides displays facile cytosine loss from the charge-reduced species, which does not occur in EDD. The dC\textsubscript{6} EDD spectrum also contains one (d/w + H\textsubscript{2}O) ion. Similar species have been observed in ECD of polydC and were suggested to be formed via a rearrangement involving the terminal hydroxyl groups.\textsuperscript{30}

Liu et al. investigated a cytidine-rich oligonucleotide, d(GC-CCC), in their high-energy CAD experiments.\textsuperscript{12} As for dA\textsubscript{6}, d/w ions dominate the spectrum, in correlation with our EDD data. They also detected three even-electron a/z ions and two (a/z − B) ions. However, in contrast to EDD, they observed sugar cross-ring cleavage and neutral cytosine loss from the charge-reduced species, which can be explained by accompanying vibrational excitation.

Hollow Cathode Electron Detachment Dissociation of dG\textsubscript{6} Anions. The mass spectrum resulting from EDD of dG\textsubscript{6} is very similar to EDD of dA\textsubscript{6} and dC\textsubscript{6} in that all d/w ions are observed (see Supporting Information). In addition, four (a/z − B) and one (c/x − B) ion are detected. However, no a/z or c/x ions are seen, correlating with the labile nature of guanine. Depurination is a dominant fragmentation pathway in vibrational excitation of nucleic acids.\textsuperscript{9} Also, facile guanine loss is observed in ECD of both DNAs\textsuperscript{30,31} and PNAs.\textsuperscript{17} Base loss from charge-reduced dG\textsubscript{6} is seen in EDD, in contrast to dA\textsubscript{6} and dC\textsubscript{6}. Finally, one product ion is observed, corresponding to (d/w − B) with an additional loss of water. A combination of water and base loss was previously observed from the precursor ions in ECD of dG\textsubscript{6}.\textsuperscript{30,31}

**Hollow Cathode Electron Detachment Dissociation of dT\textsubscript{6} Anions.** Our current EDD approach allows analysis of thymidine-rich oligonucleotides, which was not possible with ECD due to the low proton affinity of thymine nucleobases. Although promoter and coding regions in DNA have been shown to have a high GC content, we believe it is important to establish the effect of each nucleotide individually before ECD/EDD can be widely applied to nucleic acid characterization. The hollow cathode EDD spectrum from dT\textsubscript{6} is shown in Figure 3 (for accurate masses of assigned product ions, see Supporting Information). As for the other homooligodeoxynucleotides, the entire d/w ion series is present, easily allowing complete sequencing. Three complementary a/z ions are also detected. For a\textsubscript{3}, a mixture of even- and odd-electron ions is observed; see the inset in Figure 3. Other detected ions include four c/x, four (a/z − B), and two doubly charged d/w ions. As polydT is unlikely to exist as a zwitterion species due to its low proton affinity, the latter ions are most likely formed through direct dissociative electronic or vibrational excitation. Finally, for dT\textsubscript{6}, a minor species corresponding to sugar cross-ring cleavage, resulting in a so-called Σ ion,\textsuperscript{11} is observed.

Gross, Hillenkamp, and co-workers have undertaken extensive characterization of T-rich oligonucleotides to elucidate their fragmentation mechanism in CAD.\textsuperscript{7,42–45} In matrix-assisted laser desorption/ionization postsource decay (PSD) spectra of singly deprotonated d(TGTT), d(TTGT), d(GTTT), d(TTGG), and d(TGTTCT), they observed several c, x, a, and Σ-type ions.\textsuperscript{42}

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in analogy with EDD. As PSD is induced by high-energy collisions, electronic excitation is plausible.

**Hollow Cathode Electron Detachment Dissociation of d(GCATGC).** We also performed hollow cathode EDD of d(GCATGC), which contains all four deoxynucleotides. Product ion assignments are listed in Table 2. For this species, it is possible to distinguish between d/w, a/z, etc., ions in some cases. For example, both d5 and w5 ions are present. In addition, a radical z5 ion but no a5 ion is detected. Thus, it is likely that the assigned d/w, a/z, etc., ions for the homooligonucleotides discussed above are mixtures of two different species. Two d-type ions were observed by Liu et al. in high-energy CAD of d(GCCCCCC). However, those d ions were of lower abundance than the detected w-type ions in the same mass range. Assuming an analogy with EDD, both the d5/w5 and d4/w4 species should contain contributions from the w ion series and complete sequencing is again possible. However, further experiments with terminally modified oligonucleotides are needed to establish the exact nature of those products. For d(GCATGC), a doubly charged w5 ion as well as doubly charged species corresponding to adenine, cytosine, and guanine base loss are observed.

### Table 2. Product Ions Observed Following Indirectly Heated Dispenser Cathode EDD of d(GCATGC)

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*Conditions: 1-s irradiation, −16-V bias, and 20 scans.

**Figure 4.** EDD (16-eV electrons, 1-s irradiation, 10 scans, top) of a d(GCATGC) duplex with an indirectly heated hollow dispenser cathode electron source. Charge reduction is the major outcome although some dissociation into the constituent monomers is observed. In addition, one product corresponding to cleavage of the phosphodiester backbone and loss of a 5′-guanosine residue but with retention of the duplex is detected. In stark contrast, the major fragmentation pathway in IRMPD (10.6 μm, 150-ms irradiation at 10-W laser power, bottom) involves dissociation to the monomer constituents. The open circles (top and bottom) denote the calculated isotopic distributions for the charge-reduced duplex and the singly deprotonated monomer, respectively. Noise spikes are labeled with asterisks. These data illustrate the potential of EDD to characterize gas-phase nucleic acid structure.
We performed EDD of the triply deprotonated duplex (an odd-charge state was selected to avoid overlap with monomer signal) in the two different EDD configurations. Filament EDD resulted solely in charge reduction to form a radical species. The absence of any product ions is similar to ECD of large proteins and some posttranslationally modified peptides and can be explained through retention of the noncovalent interactions, preventing product ions from separating. Thus, EDD shows promise for being a useful tool in the characterization of nucleic acid gas-phase structure when combined with IRMPD or CAD. With the hollow cathode (Figure 4, top), charge reduction was also the main outcome (see inset). The deviation from the calculated isotopic distribution (open circles) can be attributed to the limited number of scans. Also, we have noticed additional distortion upon electron irradiation, due to the drastic change of space charge conditions in the ICR cell. In addition to the charge-reduced species, some dissociation of the duplex is observed to form a doubly deprotonated monomer. This behavior can be explained from the excessive heat radiated by the cathode, which can promote blackbody infrared radiative dissociation. However, one product ion, corresponding to cleavage of the phosphodiester backbone and loss of a 5′-guanosine residue from one of its constituent oligonucleotides but with retention of the duplex, is observed (1.4 ppm error). Detection of the latter (M + w2) ion clearly illustrates that EDD, similar to ECD, can disrupt covalent bonds without affecting weaker noncovalent interactions.

For comparison, we have included an IRMPD spectrum of the same triply deprotonated d(GCATGC) duplex (Figure 4, bottom). The two major products from vibrational excitation are the constituent monomers in their doubly and singly deprotonated forms. The inset shows the absence of doubly deprotonated duplex in the region of the singly deprotonated monomer. In addition to disruption of the duplex, the constituent monomers further dissociate into the expected (a − B) and w-type ions. Neutral guanine base loss is also observed. Interestingly, minor peaks corresponding to base loss from the intact duplex and to the same (M + w) ion as in EDD (but triply deprotonated instead of doubly deprotonated) are detected, illustrating the strength of the duplex. The latter data further support the formation of a specific duplex.

CONCLUSION

To our knowledge, this article demonstrates for the first time EDD experiments performed with a standard heated filament electron source. The achieved oligonucleotide data show much more extensive fragmentation than previous ECD experiments under similar conditions. Implementation of a hollow dispenser cathode electron source increases EDD efficiency and sensitivity. In particular, we demonstrate that EDD provides information-rich fragmentation patterns complementary to vibrational excitation for small oligonucleotides. For example, d-type ions and radical a- and z-type ions, absent from CAD and IRMPD spectra, are commonly observed as well as c/x and (c/x − B) ions. Complete oligonucleotide sequencing is easily achieved. The observed fragmentation is very similar to dispenser cathode ECD of the same species and, to some extent, to spectra obtained from other techniques involving dissociation of oligonucleotide radical ions. In contrast to previous high-energy CAD and ion trap CAD of oligonucleotide radicals, we only observe minor sugar cross-ring cleavage, possibly due to the shorter time scale of EDD, minimizing secondary fragmentation. In comparison to ECD, enhanced sensitivity is achieved through negative ion mode operation (on average a 10-fold improvement so far in terms of concentration sensitivity). The detection of doubly charged product ions from doubly charged precursors indicates that EDD conditions promote direct dissociative electronic excitation although other explanations for the formation of those ions are also discussed.

Intriguing results for an oligonucleotide duplex also demonstrate that EDD, similar to ECD, can cleave covalent bonds without disrupting labile noncovalent interactions, rendering the former technique amenable to probing the gas-phase structure of acidic biomolecules. Of particular interest is the application of this technology to characterize RNA structure and folding. Other future directions include characterization of nucleic acid-containing complexes as well as a further establishment of fundamental EDD behavior for larger nucleic acids.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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