Direct determination of molecular weight distribution of calf-thymus DNAs and study of their fragmentation under ultrasonic and low-energy infrared irradiations. A charge detection mass spectrometry investigation

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Direct determination of molecular weight distribution of calf-thymus DNAs and study of their fragmentation under ultrasonic and low-energy infrared irradiations. A charge detection mass spectrometry investigation

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Rationale: Calf-thymus (CT-DNA) is widely used as a binding agent. The commercial samples are known to be "highly polymerized DNA" samples. CT-DNA is known to be fragile in particular upon ultrasonic wave irradiation. Degradation products could have dramatic consequences on its bio-sensing activity, and an accurate determination of the molecular weight distribution and stability of commercial samples is highly demanded.

Methods: We investigated the sensitivity of charge detection mass spectrometry (CDMS), a single-molecule MS method, both with single-pass and ion trap CDMS ("Benner" trap) modes to the determination of the composition and stability (under multiphoton IR irradiation) of calf-thymus DNAs. We also investigated the changes in molecular weight distributions in the course of sonication by irradiating ultrasonic waves to CT-DNA.

Results: We report, for the first time, the direct molecular weight (MW) distribution of DNA sodium salt from calf-thymus revealing two populations at high (~10 MDa) and low (~3 MDa) molecular weights. We evidence a transition between the high-MW to the low-MW distribution, confirming that the low-MW distribution results from degradation of CT-DNA. Finally, we report also IRMPD experiments carried out on trapped single-stranded linear DNAs from calf-thymus allowing extraction of their activation energy for unimolecular dissociation.

Conclusions: We show that single-pass CDMS is a direct, efficient and accurate MS-based approach to determine the composition of calf-thymus DNAs. Furthermore, ion trap CDMS allows us to evaluate the stability (both under multiphoton IR irradiation and in the course of sonication by irradiating ultrasonic wave) of calf-thymus DNAs.

1 | INTRODUCTION

Calf-thymus (CT-DNA) has been commercially available for a long time. CT-DNA is very popular because it mostly resembles the mammalian DNA structure and is easy to extract from the thymus gland of calf. Thus, it has been widely used as a DNA sample, such as testing anti-dsDNA antibody activity,[1] nuclease activity,[2] DNA binding anticancer agents[3] and DNA binding agents[4] that modulate DNA structure and function. CT-DNA is also used in physicochemical studies of DNA behavior in solution, in particular conformation and rheological properties.[5] Recently, the interaction of silver (Ag) clusters with CT-DNA has been used as an efficient template method to prepare luminescent Ag clusters.[6]

The commercial samples are known to be "highly polymerized DNA" samples which contain both double- and single-stranded forms. However, double-stranded DNA is supposed to be the predominant

form. Information about their molecular weight distribution is not available from the suppliers and only an estimation of the molecular weight is reported to be between 10 and 15 million daltons (MDa). In the literature, the average molecular weight (MW) of CT-DNA samples ranges from 6 MDa[7] to more than 8 MDa.[8,9] Quantitative information on the molecular weight distribution of CT-DNA is, to the best of our knowledge, missing completely. Recently, a plausible CT-DNA molecular weight distribution was estimated from size-exclusion chromatography (SEC) with dual low-angle light scattering/refractometric detection at a sufficiently low flow rate.[9] Indeed, SEC separation is substantially disturbed by diverse flow-retardation effects; however, the nonbiased molecular weight distribution can be obtained using a sufficiently low mobile phase flow rate. A broad distribution was observed and extended over three orders of magnitude in molecular weight (from 10^5 to 10^8 Da).[9] This high polydispersity was attributed mainly to be the result of mechanical and shear degradation of CT-DNA during its isolation. Indeed, CT-DNA is known to be fragile in particular upon ultrasonic wave irradiation.[8,10] Degradation products could have dramatic consequences on the bio-sensing activity of the double-stranded CT-DNAs, and an accurate determination of the composition of commercial samples is highly required.

Conventional mass spectrometry (MS) is a powerful tool for DNA mass analysis.[11] However, conventional MS measures the mass-to-charge ratios (m/z) of an ensemble of ions and usually requires homogeneous samples, or limited heterogeneity, to bridge the gap in the megadalton (MDa) mass range.[12] In contrast, charge detection mass spectrometry (CDMS) is a single-molecule MS method.[13] In CDMS, an ion is passed through a metal tube. CDMS allows the simultaneous measurement of the charge (z) from the image charge on the tube and of m/z from the time-of-flight (TOF) through the tube (and the ion's kinetic energy) of each single ion composing the sample leading to the direct determination of each corresponding mass.[14-17] Benner and colleagues demonstrated the potential of CDMS for the analysis of megadalton DNAs (between 2.8 and 31 MDa), both with a single-pass CDMS experiment,[18-20] and with ion trap CDMS ("Benner" trap) (see Figure 1).[21]

More recently, Antoine and coworkers have published a series of articles demonstrating the use of CDMS to study the photofragmentation of single-stranded DNA macromolecules.[22-26] By coupling a CO_2 laser to a "Benner" trap, infrared multiphoton dissociation (IRMPD) was performed on megadalton-size DNA ions. For double- and single-stranded DNAs, the experiment revealed several fragmentation pathways having distinct signatures which cannot be addressed by investigations associated with average statistical reaction rates.

In this paper, we report, for the first time, the direct molecular weight distribution of DNA sodium salt from calf-thymus (CT-DNA). We also investigate, by CDMS, the changes in molecular weight distributions in the course of sonication by irradiating ultrasonic wave to CT-DNA and observe a transition between the high-MW to the low-MW distribution. Moreover, we show IRMPD experiments on trapped single-stranded linear DNAs from calf thymus (~15 MDa), allowing extraction of their activation energy for unimolecular dissociation.

2 | EXPERIMENTAL

Experiments were performed on a custom-built spectrometer with an electrospray ionization (ESI) source to generate the highly charged macro ion beam and a vacuum interface. The ions are guided up the terminal vacuum stage chamber which contains two identical charge detection devices (CDD) to work in a single-pass CDMS[27] and ion trap CDMS modes.[24] The first CDD consists of a conductive tube collinear to the ion beam and connected to a field-effect transistor (JFET). The picked up signal is amplified by a low-noise charge-sensitive preamplifier and then shaped and differentiated by a home-built amplifier. The signal is recorded, in single-pass CDMS mode (see Figure 1), with a waveform digitizer card. The data are transferred to a desk-top computer where they are analyzed with a custom-written user program. Calibration in charge was performed using a test capacitor that allowed a known amount of charge to be pulsed onto the pick-up tube. The second CDD is surrounded by ion mirrors. Thus the second CDD is used as

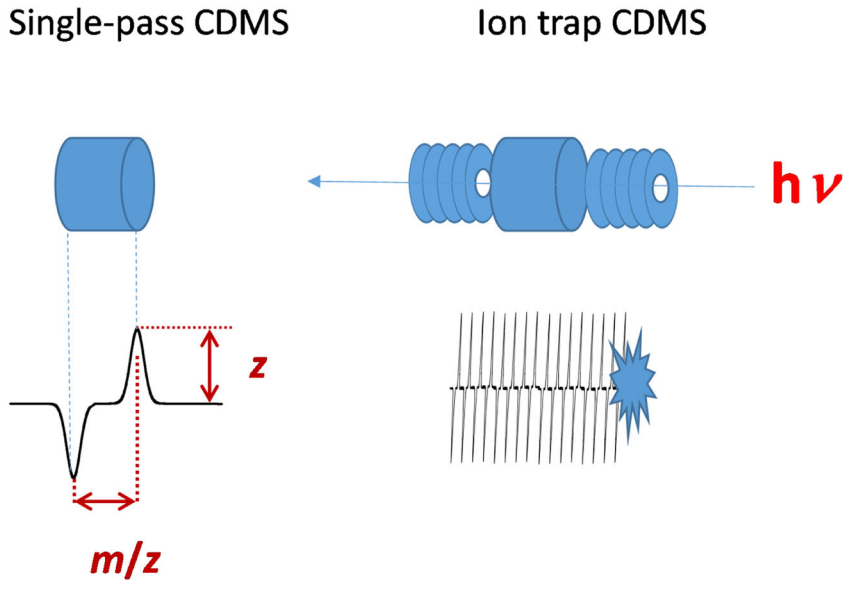

FIGURE 1 Schematics principles of charge detection mass spectrometry. (left) single pass CDMS; the charge z of single ions is determined by the amplitude measured by image charge through the tube and the m/z value is determined from the TOF through the tube (and the ion's kinetic energy). The detector tube can be embedded in an electrostatic ion trap (right), so a single ion's charge can be measured hundreds or thousands of times as the ion oscillates back and forth through the tube. This version is called ion trap CDMS. A CO_2 laser can be injected in the axis of the ion trap, and single trapped ions can be irradiated by low energy (hν~0.1 eV) IR photons. Fragmentation information can be extracted based on the shape of the induced charge waveform at the end of ion trapping (represented by a flash bang icon)

a gated electrostatic ion trap ("Benner" trap, see Figure 1) by the use of a delay generator and a discriminator. During the trapping time a continuous wave CO_2 laser is used in a synchronized manner to irradiate the trapped ion through a ZnSe window. Laser power dependent experiments are performed by changing the duty cycle of the laser.

Double-stranded (dsDNA, Sigma, D 3664, ~10–15 MDa) and single-stranded (ssDNA, Sigma, D 8899, ~15 MDa) DNAs from calf-thymus were bought commercially. A standard electrospray sample solution was made by diluting the CT-DNA to 0.0125 mg/mL in 1:1 water/acetonitrile. The positive mode of ionization was chosen for ESI. The spray conditions in negative ion mode using the standard pressurized electrospray inlet were much more difficult to reproduce and maintain than those in positive ion mode. Note that negative ion CD mass spectra of MDa DNA ions were reported by Benner et al[19] and the mass values obtained were similar in both ion modes. The sonication of DNA solutions was performed with an Elmasonic S series sonicator (37 kHz, the highest power 300 W). A tube containing the CT-DNA solution (5 mL) was immersed into the bath solution about 1 cm below the level, and the solution was irradiated for 0 to 25 min at a fixed irradiation intensity, at room temperature.

3 | RESULTS AND DISCUSSION

3.1 | Double-stranded DNAs from calf thymus

The two-dimensional (2D) graph (charge vs. mass) shown in Figure 2 reports mass measurements on around 10,000 single DNA ions; *m* (the molar mass) of each macro ion is obtained from a combination of both *z* (the charge) and *m/z* values. A broad distribution extends over two orders of magnitude in *m* (from 0.5 MDa to higher than 50 MDa) explaining the high polydispersity index reported in earlier works.[9] The charge distribution is also very broad ranging from ~300 e to larger than 2000 e. Interestingly, two populations could be seen on the CDMS 2D graph (Figure 2). They could be further distinguished by their time-of-flight (data not shown). The main population, the "high" mass population, had a mean mass of ~10 MDa. In addition, a "low" mass population (around 2.4 MDa) is clearly observed. The differences in the charge *vs* molar mass slopes corresponding to the two populations (Figure 2) indicate that the "low" mass population (slope ~1.4 10^{-4} *z/m*) is highly charged; much more than the "high" mass population (slope ~3.8 10^{-5} *z/m*). This might be correlated to the difference in surface/volume ratios in such biopolymers. This behaviour was already observed for CDMS analysis of entire amyloid fibers,[28] and also DNA samples.[25] These two charge distributions could reflect that ions are present in solution in two different conformations with different charging ability (an extended conformation more prone to be charged than a more folded one), each leading to different charge distributions as already mentioned in previous studies by Benner and co-workers on megadalton-DNA electrospray ions using CDMS.[19]

It was suspected that the high polydispersity for CT-DNA was mainly a result of mechanical and shear degradation of CT-DNA during its isolation. To better explore this hypothesis, we conducted sonication experiments by irradiating ultrasonic wave to CT-DNAs for 0 to 25 min. Interestingly, the two populations seen on the CDMS 2D graph are not changed by sonication (data not shown). However, the relative content of "low" and "high" mass populations is strongly dependent on the sonication time. Figure 3 shows the change in molecular weight distribution of the double-stranded CT-DNA, in the course of sonication time (0, 12, and 25 min). Clearly the "high" mass population is depleted to the benefit of the "low" mass population when the sonication time is increased. This confirms that the low-MW distribution observed in commercial samples results from degradation of CT-DNA.

3.2 | Single-stranded DNAs from calf thymus

Positive ion CD mass spectra of ssDNAs from calf-thymus samples are shown in Figure 4A. These plots represent the measurement of about 2000 individual trapped ions. Mass and charge were individually

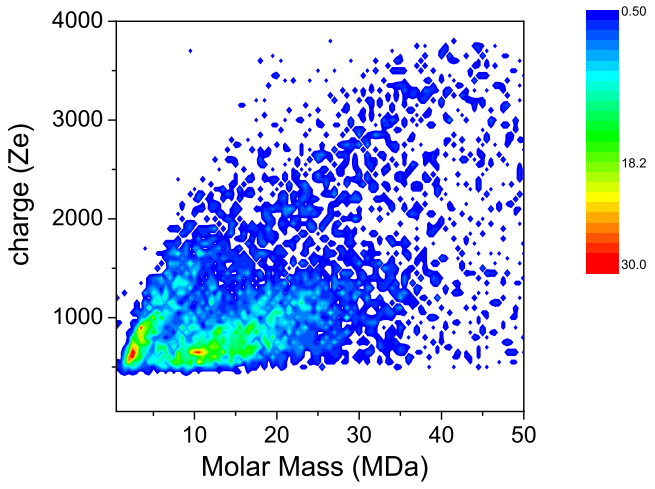

FIGURE 2 2D graph of CDMS measurements performed on a sample of ds-CT-DNAs

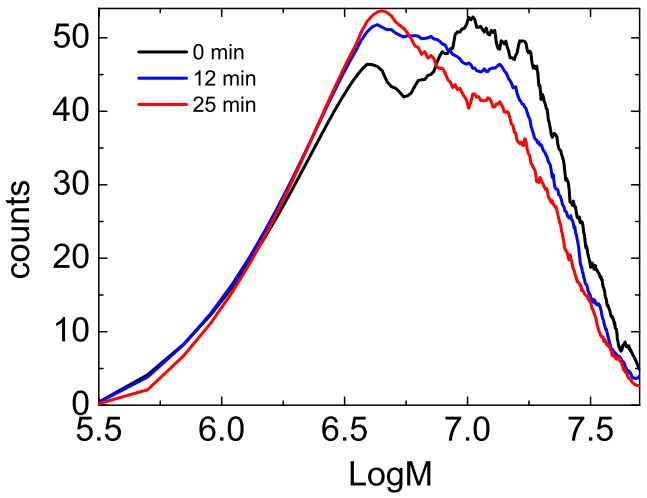

FIGURE 3 Measured molecular weight distributions in log scale (logM) of ds-CT-DNAs in the sonication process, determined by CDMS measurements. Irradiation time in min: 0, 12, and 25

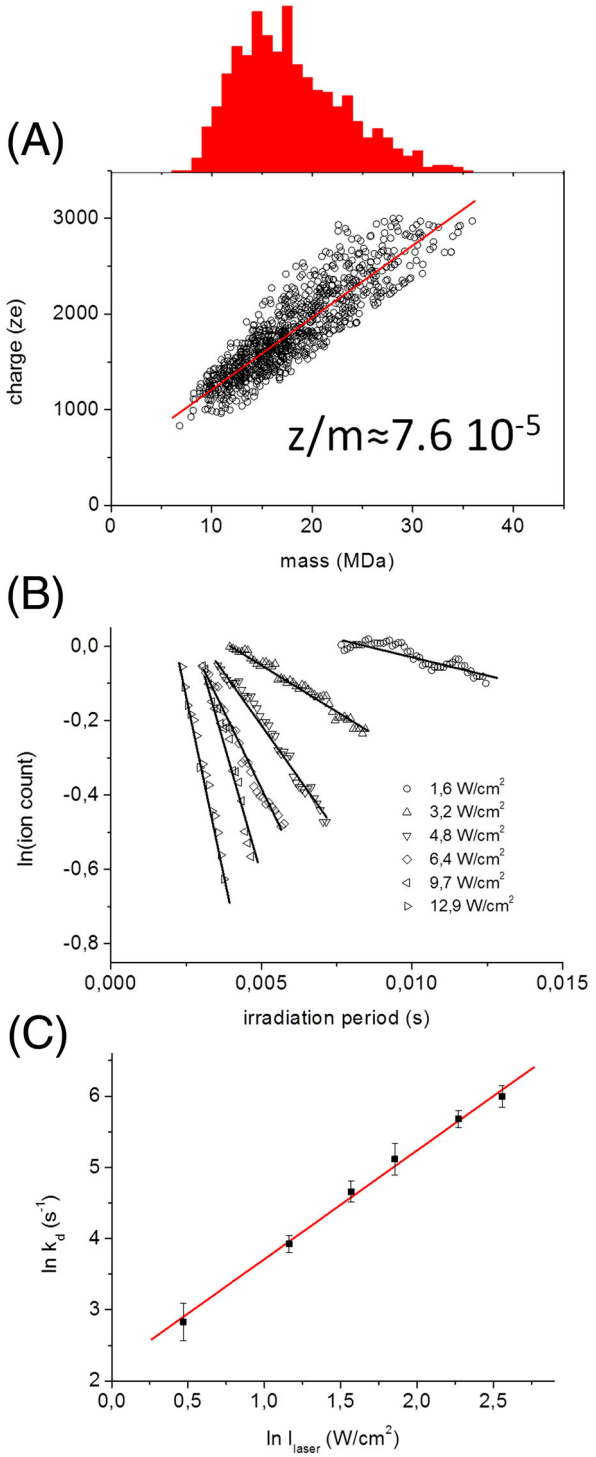

FIGURE 4 A, CDMS scatter plots of charge versus mass for ss-CT-DNA samples. Each point corresponds to an individual measurement. Projection of the scatter plots on the mass axis provides the corresponding mass histograms (bin size = 1 MDa). Solid red lines are representative of the charge distributions (i.e., z/m~7.6 10^e^{-5}). B, Logarithm of ion count versus time for the dissociation of ss-CT-DNAs under different laser power irradiation. The plots were constructed by analyzing ~100 wavelets of individual ions at each laser power in order to construct a frequency histogram of the distribution of ion counts. The line corresponds to a linear fit of the data. C, Respective plot of the logarithm of the first-order unimolecular dissociation rate constant, k_d, versus the logarithm of the laser intensity for ss-CT-DNA samples. The activation energy for dissociation, E_a, is obtained from the slope of the linear fit

extracted from each transient waveform.[25] A single broad mass distribution ranging from ~9 MDa to higher than 30 MDa is obtained. The charge distribution is also very broad ranging from ~1000 e to larger than 3000 e. The corresponding z/m slope is ~7.6 10^{-5}. This slope is about 2 times higher than the slope obtained for the "high" mass population of dsDNAs from calf-thymus samples, meaning that ssDNAs can hold more charges as compared to dsDNAs. This higher charge ability for ssDNAs may be due to a higher conformational flexibility allowing a better unfolding of DNA strands.

A single DNA macro ion selected in mass and charge can be trapped in our ion trap CDMS instrument during several tens of milliseconds which corresponds to several hundreds of oscillations (see Figure 1). Infrared multi-photon dissociation (IRMPD) experiments were carried out on single-stranded CT-DNA ions. At high CO_2 laser power, drastic changes are observed in the trapping duration. Indeed while the trapping time can exceed 30 ms without the CO_2 laser, it does not exceed 5–15 ms (see Figure 4B) and dissociation is usually very fast (less than a few ms). Furthermore, the induction period which corresponds to the averaged irradiation time needed to start the dissociation increases as the laser power decreases (see Figure 4B). From the linear fits of survival rate plots at each laser power we could obtain the corresponding dissociation constants (k_{diss}) of the studied DNA (see Figure 4C). From the slope of the linear evolution of k_{diss} as a function of laser intensity in a log–log plot, using the formalism described in our previous work,[23,29] we could extract the activation energy for unimolecular dissociation of ss-CT-DNA E_a = 0.3 eV.

Under CO_2 laser irradiation, three specific pathways of dissociation were observed, based on the shape of the induced charge waveform as a function of time (see Figure 1 and Doussineau et al[25] for more details). "Sudden loss" type pathways display a sharp decrease in measured signal and are due to dissociation events where the fragment ion is lost from the trap. "Funnel" type pathways leads to a gradual loss of charge. A third type of pathway is called "staircase" and is characterized by a significant loss of charge followed by periods where the charge remains constant. At an irradiation power of 6.4 W/cm^2, the observation of "funnel" and "staircase" pathways is equally observed. This particular statistical repartition of pathways is similar to the one of non-hybridized dimers of single-stranded DNA determined in a previous study.[25] This may be attributed to the ability of these relatively unfolded DNAs to easily lose fragment upon irradiation.

4 | CONCLUSIONS

In this study, we show that single-pass charge detection mass spectrometry can be used as a direct, efficient and accurate MS-based approach to detect and measure the composition of commercial samples of calf-thymus DNAs. The mass distribution reveals two populations at high (~10 MDa) and low (~3 MDa) molecular weights. Furthermore, ion trap CDMS allows evaluation of the stability (both under multiphoton IR irradiation and in the course of sonication by irradiating ultrasonic wave) of calf-thymus DNAs. In particular, we evidence a transition, upon sonication between the high-MW to the

low-MW distribution, confirming that the low-MW distribution results from degradation of CT-DNA. Moreover, IRMPD experiments on the trapped single-stranded linear DNAs from calf-thymus (~15 MDa) disclose that ssCT-DNA follows unimolecular dissociation with an activation energy of 0.3 eV.

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REFERENCES

1. Gilkeson GS, Pippen AMM, Pisetsky DS. Induction of cross-reactive anti-dsDNA antibodies in preautoimmune nzb/nzw mice by immunization with bacterial-DNA. *J Clin Invest*. 1995;95:1398-1402.
2. Maver ME, Greco AE. The nuclease activities of cathepsin preparations from calf spleen and thymus. *J Biol Chem*. 1949;181:861-870.
3. Rehman SU, Yaseen Z, Husain MA, Sarwar T, Ishqi HM, Tabish M. Interaction of 6-mercaptopurine with calf thymus DNA – Deciphering the binding mode and photoinduced DNA damage. *PLoS ONE*. 2014;9:e93913.
4. Yang H, Tang P, Tang B, et al. Studies of DNA-binding properties of lafutidine as adjuvant anticancer agent to calf thymus DNA using multi-spectroscopic approaches, NMR relaxation data, molecular docking and dynamical simulation. *Int J Biol Macromol*. 2017;99:79-87.
5. Bravo-Anaya L, Rinaudo M, Martínez F. Conformation and rheological properties of calf-thymus DNA in solution. *Polymers*. 2016;8:51-70.
6. Buceta D, Busto N, Barone G, et al. Ag2 and Ag3 clusters: Synthesis, characterization, and interaction with DNA. *Angew Chem Int Ed*. 2015;54:7612-7612.
7. Sundaresan N, Suresh CH, Thomas T, Thomas TJ, Pillai CKS. Liquid crystalline phase behavior of high molecular weight DNA: A comparative study of the influence of metal ions of different size, charge and binding mode. *Biomacromolecules*. 2008;9:1860-1869.
8. Tanigawa M, Suzuto M, Fukudome K, Yamaoka K. Changes in molecular weights and molecular weight distributions of differently stranded nucleic acids after sonication: Gel permeation chromatography/low angle laser light scattering evaluation and computer simulation. *Macromolecules*. 1996;29:7418-7425.
9. Porsch B, Laga R, Horský J, Koňák Č, Ulbrich K. Molecular weight and polydispersity of calf-thymus DNA: Static light-scattering and size-exclusion chromatography with dual detection. *Biomacromolecules*. 2009;10:3148-3150.
10. Fukudome K, Yamaoka K, Nishikori K, Tatehata H, Yamamoto O. Ultrasonic scission of deoxyribonucleic acid in aqueous solution II. Precipitational fractionation and molecular weights of sonicated samples. *Polymer J*. 1986;18:81-88.
11. Tost J, Gut IG. DNA analysis by mass spectrometry – past, present and future. *J Mass Spectrom*. 2006;41:981-995.
12. Snijder J, Rose RJ, Veesler D, Johnson JE, Heck AJR. Studying 18 MDa virus assemblies with native mass spectrometry. *Angew Chem Int Ed*. 2013;52:4020-4023.
13. Keifer DZ, Pierson EE, Jarrold MF. Charge detection mass spectrometry: weighing heavier things. *Analyst*. 2017;142:1654-1671.
14. Doussineau T, Bao CY, Antoine R, et al. Direct molar mass determination of self-assembled amphiphilic block copolymer nanoobjects using electrospray-charge detection mass spectrometry. *ACS Macro Lett*. 2012;1:414-417.
15. Doussineau T, Désert A, Lambert O, et al. Charge detection mass spectrometry for the characterization of mass and surface area of composite nanoparticles. *J Phys Chem C*. 2015;119:10844-10849.
16. Doussineau T, Mathevon C, Altamura L, et al. Mass determination of entire amyloid fibrils by using mass spectrometry. *Angew Chem In. Ed*. 2016;55:2340-2344.
17. Warren NJ, Mykhaylyk OO, Ryan AJ, et al. Testing the vesicular morphology to destruction: Birth and death of diblock copolymer vesicles prepared via polymerization-induced self-assembly. *J Am Chem Soc*. 2015;137:1929-1937.
18. Fuerstenau SD, Benner WH. Molecular weight determination of megadalton DNA electrospray ions using charge detection time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom*. 1995;9:1528-1538.
19. Schultz JC, Hack CA, Benner WH. Mass determination of megadalton-DNA electrospray ions using charge detection mass spectrometry. *J Am Soc Mass Spectrom*. 1998;9:305-313.
20. Schultz JC, Hack CA, Benner WH. Polymerase chain reaction products analyzed by charge detection mass spectrometry. *Rapid Commun Mass Spectrom*. 1999;13:15-20.
21. Benner WH. A gated electrostatic ion trap to repetitiously measure the charge and m/z of large electrospray ions. *Anal Chem*. 1997;69:4162-4168.
22. Antoine R, Doussineau T, Dugourd P, Calvo F. Multiphoton dissociation of macromolecular ions at the single-molecule level. *Phys Rev A*. 2013;87:013435.
23. Doussineau T, Antoine R, Santacreu M, Dugourd P. Pushing the limit of infrared multiphoton dissociation to megadalton-size DNA ions. *J Phys Chem Lett*. 2012;3:2141-2145.
24. Doussineau T, Bao CY, Clavier C, et al. Infrared multiphoton dissociation tandem charge detection-mass spectrometry of single megadalton electrosprayed ions. *Rev Sci Intrum*. 2011;82:084104.
25. Doussineau T, Paletto P, Dugourd P, Antoine R. Multiphoton dissociation of electrosprayed megadalton-sized DNA Ions in a charge-detection mass spectrometer. *J Am Soc Mass Spectrom*. 2015;26:7-13.
26. Halim MA, Clavier C, Dagany X, et al. Infrared laser dissociation of single megadalton polymer ions in a gated electrostatic ion trap: the added value of statistical analysis of individual events. *Phys Chem Chem Phys*. 2018;20:11959-11966.
27. Doussineau T, Kerleroux M, Dagany X, et al. Charging megadalton poly (ethylene oxide) s by electrospray ionization. A charge detection mass spectrometry study. *Rapid Commun Mass Spectrom*. 2011;25:617-623.
28. Pansieri J, Halim MA, Vendrely C, et al. Mass and charge distributions of amyloid fibers involved in neurodegenerative diseases: mapping heterogeneity and polymorphism. *Chem Sci*. 2018;9:2791-2796.
29. Paech K, Jockusch RA, Williams ER. Slow infrared laser dissociation of molecules in the rapid energy exchange limit. *J Phys Chem A*. 2002;106:9761-9766.

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