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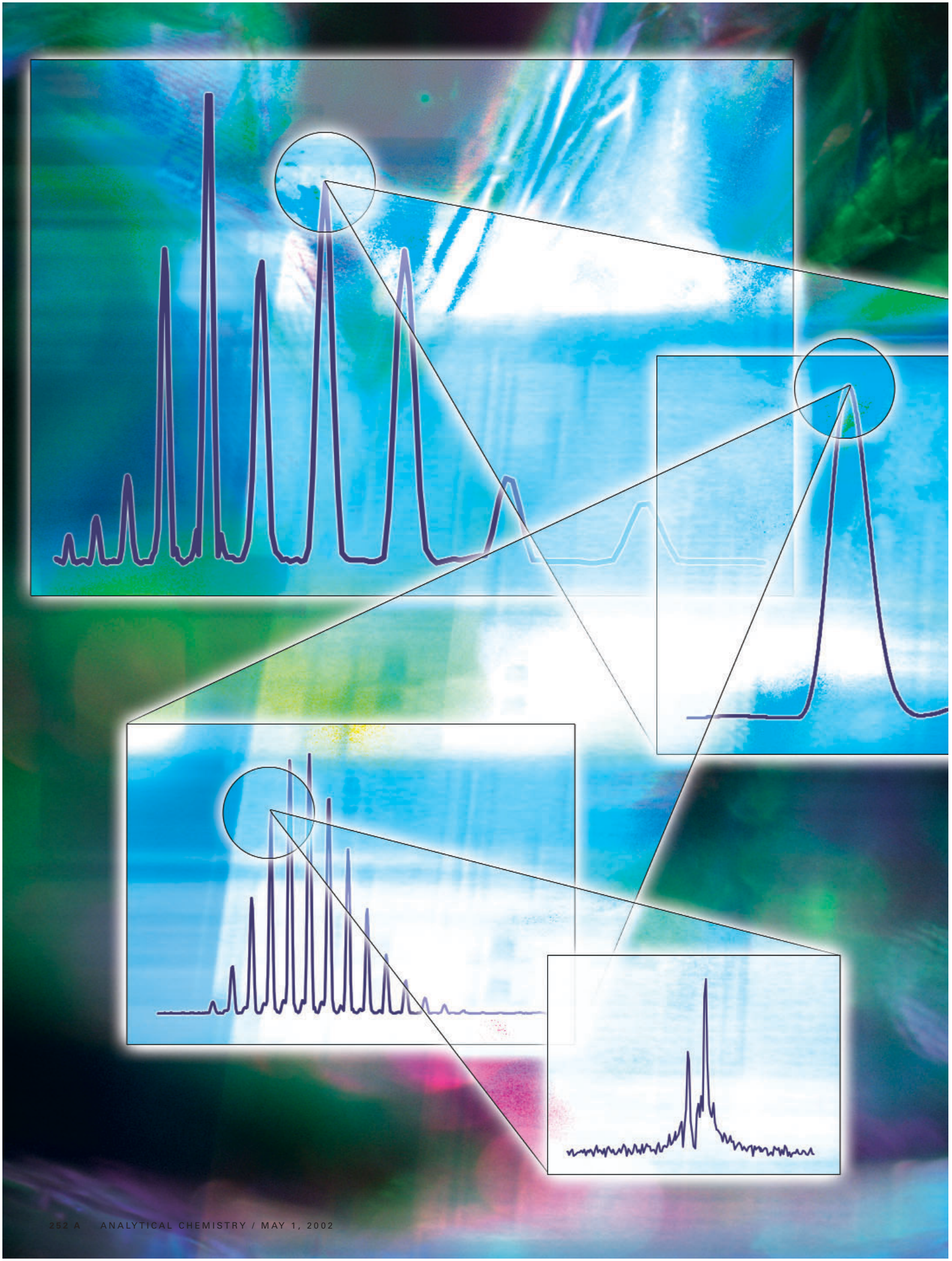

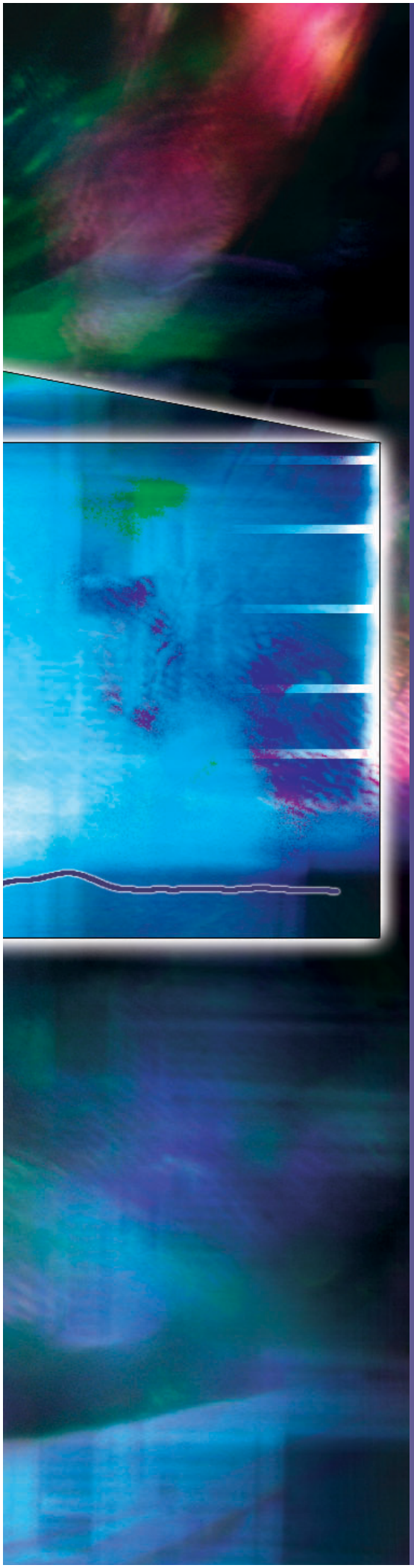

Scaling MS Plateaus with High-Resolution FT-ICRMS

A guide to increasing resolution and higher masses by MS.

The history of spectroscopy is the history of resolution, and spectroscopic resolution has traditionally progressed in quantized steps. For example, the UV–vis absorption or emission spectrum of a hydrogen atom was first coarsely resolved into different series of transition frequencies—Lyman (n = 1 to n = 2, 3, 4, . . .), Balmer (n = 2 to n = 3, 4, 5, . . .), Paschen (n = 3 to n = 4, 5, 6, . . .), Brackett (n = 4 to n = 5, 6, 7, . . .), etc., in which n is the principal quantum number that indexes the various electronic energy states. As instrumental resolution improves, no additional peaks are seen until the more closely spaced Δj = 0,1 transitions are suddenly resolved (e.g., s- to p-orbital transitions). Further improvement in resolution reveals no more peaks until (in a strong electric field and with circularly polarized radiation) Δm = ±1 transitions appear. For molecules in a magnetic field, even finer detail emerges from nuclear spin transitions.

Mass spectrometers, including magnetic sectors, two- and three-dimensional quadrupoles, time-of-flight (TOF), and FT-ion cyclotron resonance (ICR), actually measure m/z, which is inherently quantized because charge occurs only in integer multiples of the elementary (e.g., electron) charge, and mass is quantized according to molecules, functional groups, elements, isotopes, and elemental compositions. Consequently, mass spectral resolution

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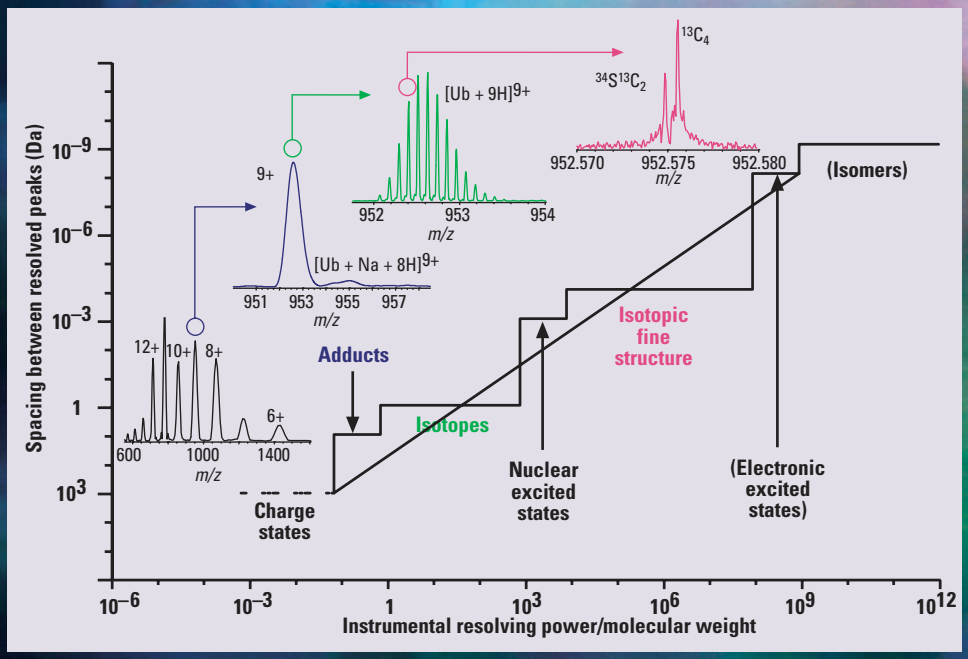


FIGURE 1. Mass spectral resolution versus the instrumental resolving power per unit of molecular weight, with experimental ESI FT-ICR mass spectra (insets) for the protein bovine ubiquitin with a monoisotopic mass of 8559.62 Da.

As instrumental resolving power improves, ions of different charge (but the same mass) are resolved first, followed by ions differing in nominal closest-integer mass; ions of the same chemical formula but different isotopic composition; ions of the same nominal mass but different elemental composition; and ultimately, ions of different internal energy or isomers with different heats of formation. Parentheses indicate splittings that have not yet been observed experimentally.

progresses along a series of stair steps (Figure 1). With continuously increasing instrumental mass resolving power, whole new families of peaks suddenly separate upon reaching each of several m/z plateaus. In Figure 1, the vertical axis is mass spectral resolution, scaled in Daltons. The horizontal axis, $1/\Delta m_{50\%}$ (in which $\Delta m_{50\%}$ is the mass spectral peak full width at half maximum peak height), may be thought of as the ratio of instrumental mass resolving power, $m/\Delta m_{50\%}$, to the ion mass m. The horizontal axis thus provides a mass-independent measure of resolving power.

In this article, we consider the new molecular information that becomes accessible as instrumental mass resolving power "climbs each step" in Figure 1 by increasing the resolving power of the mass spectrometer and/or by analyzing species of lower molecular weight. Because FT-ICRMS offers the highest available mass resolving power, mass resolution, and mass accuracy (*1–3*), it is convenient to illustrate each of the various mass spectral plateaus with FT-ICRMS examples. (FT-ICRMS resolving power may easily be degraded to match that of lower-resolution mass analyzers, simply by truncating the time-domain ICR data [*4*].) Examples range from drug screening to resolution and analysis of thousands of components in complex petroleum or biological samples. We also discuss the important distinctions between mass resolving power, mass resolution, mass precision, and mass accuracy. High mass resolving power is necessary not only to reach a higher mass at each plateau, but also to make sure that mass measurement precision translates into high mass accuracy for identifying unknowns.

Different charge states of ions of the same mass

An ionized atom or molecule may be characterized by its m/z. One of the most important ways of generating charged molecules is electrospray ionization (ESI) in which one or more protons are added or removed from an initially neutral molecule (*5, 6*). For example, potentially all of the arginine and lysine residues in a protein may protonate. Because there is typically ~1 such residue per kilo-Dalton of protein mass, a protein of 50 kDa could have a large number of different positive charge states (30+, 31+, 32+, . . . 50+). Therefore, the first plateau for molecular MS is to resolve different charge states, z, of ions of the same mass (Figure 2, bottom).

Obviously, if multiple charge states are present and resolved, and if they are not obscured by the presence of other species in the sample, then it is possible to match the ratios of their different m/z values to the best-fit ratios to determine all the charge states for ions of a given mass. Once the charge states are known (and a much better way of determining them is available at higher mass resolution), then the spectrum can be deconvolved from an m/z spectrum to a zero-charge mass spectrum (Figure 3) (*7*). For purposes of illustration, the ICR time-domain data in Figure 3 have been truncated to the first 15 ms, to reduce the mass resolving power to the level of a quadrupole or linear (nonreflectron) TOF mass analyzer.

At this point, we digress to note that a couple of different methods have been proposed to reduce the charge of ES ions, preferably to a single charge, which renders the final mass spectrum simpler to interpret visually by eliminating all but the lowest charge state(s) species (i.e., reduction in chemical noise) (*8, 9*). However, FT-ICR mass resolving power varies inversely with ion m/z for isotopically resolved mass spectral peaks from different charge states of the same molecule (Figure 2, top). Thus, provided that individual isotopic peaks can be resolved, as in FT-ICRMS for ions of up to 112 kDa (*10*), charge reduction is never desirable because it actually lowers mass resolving power by a factor proportional to z (*11*).

Molecules of different nominal mass

If more than one chemical species is present in a mixture, then the next mass resolution plateau is the separation of ions of different nominal (nearest-integer) mass. For example, if a peptide can be broken at each of a series of backbone amide linkages, then each successive mass difference represents the mass of one amino acid (minus the mass of H_2O). At unit mass resolution, it is thus possible to discriminate 18 (out of 20 commonly occurring natural amino acids) different residue masses. Leucine and isoleucine have identical elemental composition and thus virtually identical mass, whereas lysine and glutamine have the same nominal mass but dif-

fer by 36 mDa, which represents, respectively, a CH_4 group versus an O atom. Of course, such analysis depends on starting from an isolated analyte—that is why low-resolution mass analyzers are typically coupled with prior off-line separation methods, such as polyacrylamide gel electrophoresis, or on-line techniques, such as GC or LC, to isolate individual compounds for mass analysis.

Another increasingly important application of MS is for screening of candidate (combinatorial) drugs or agents that bind to a particular biological receptor. Empirically, only the most strongly bound noncovalent adducts ($\leq 10^{-6}$ M dissociation constant in solution) survive ESI to remain adducted in the gas phase (*12, 13*). Thus, if different adduct complexes can be resolved from each other (*14*), then the most strongly bound ligands can be identified. In this way, the most strongly bound ligands from a library of hundreds of candidates can be identified from a single mass spectrum (*12, 13*). By screening multiple targets against ligand mixtures, a screening speed of 40,000–140,000 interactions per day can routinely be achieved in a real-world application (*15–17*).

Isotopic distribution of heavy atoms

The ability to measure mass to the nearest integer number of Daltons gave rise to several special applications. The heavy isotopes of elements found in organic and biological molecules are low in relative abundance per atom: 1.1% for ^{13}C, 0.015% for ^{2}H, 0.37% for ^{15}N, 0.20% for ^{18}O, and 4.2% for ^{34}S. The mass spectrum of ions containing just a single carbon will thus consist primarily of ^{12}C ions. However, for an ion with 70 carbons, the relative abundance of the $^{13}C_1{}^{12}C_{69}$ elemental composition becomes ~70 × 0.011, or ~77% that for $^{12}C_{70}$. A small protein such as ubiquitin contains hundreds of carbons, so that the relative abundance of species containing one or more ^{13}C (and/or ^{15}N, etc.) becomes higher than for the monoisotopic species in which all carbons are ^{12}C, all hydrogens are ^{1}H, all nitrogens are ^{14}N, all oxygens are ^{16}O, all sulfurs are ^{32}S, etc. (Figure 2, top).

The isotopic complexity of large molecules creates at least three major problems. First, the same total number of ions is distributed over many different masses, so that S/N (and thus the precision with which mass can be measured) is reduced. Second, even if the signals from species with different numbers of heavy atoms can be resolved to 1 ppm, determination of the correct ion mass to the nearest Dalton depends on accurate measurement of ion relative abundances (*18*). Third, the wide isotopic distribution makes it more difficult to identify adducts (or deuterium substitution for hydrogen in experiments designed to reveal the surface accessibility of a biomacromolecule [*19*]). Those difficulties subside if the molecule can be doubly depleted in ^{13}C and ^{15}N (as by expressing a protein from a minimal medium consisting of ^{13}C-depleted glucose and ^{15}N-depleted ammonium sulfate [*20*]), as shown for the Cdc42 protein in Figure 4. The monoisotopic peak now rises to prominence, thereby eliminating ambiguity in the molecular weight and allowing for direct identification of intact, undigested proteins from a proteome by LC/MS (*21*).

As noted earlier, ESI of high-mass (>1 kDa) polar molecules typically generates multiply charged ions. Those same ions also exhibit a distribution of several heavy atoms. Thus, because mass analyzers measure *m/z*, the interval between successive spectral peaks—for example, ions whose masses differ by one ^{13}C or ^{15}N atom—must be $1/z$. In other words, provided that mass can be resolved to within 1 Da, the charge state of an ion may be determined from the peak spacing in the *m/z* spectrum—a dividend of nature's isotopic complexity (*22*).

Figure 5 offers a spectacular example, in which an ESI FT-ICR *m/z* spectrum of a GluC digest (i.e., enzymatic cleavage at the carboxyl end of glutamic acid residues) of a 191-kDa protein exhibits several thousand resolved peaks (*23*). Automated peak-pattern recognition, in which the charge state of each peptide is determined from the spacing in its isotopic distribution, identifies more than 580 peptides, including the sequence location of the desired target, namely, a single chemically modified amino acid residue

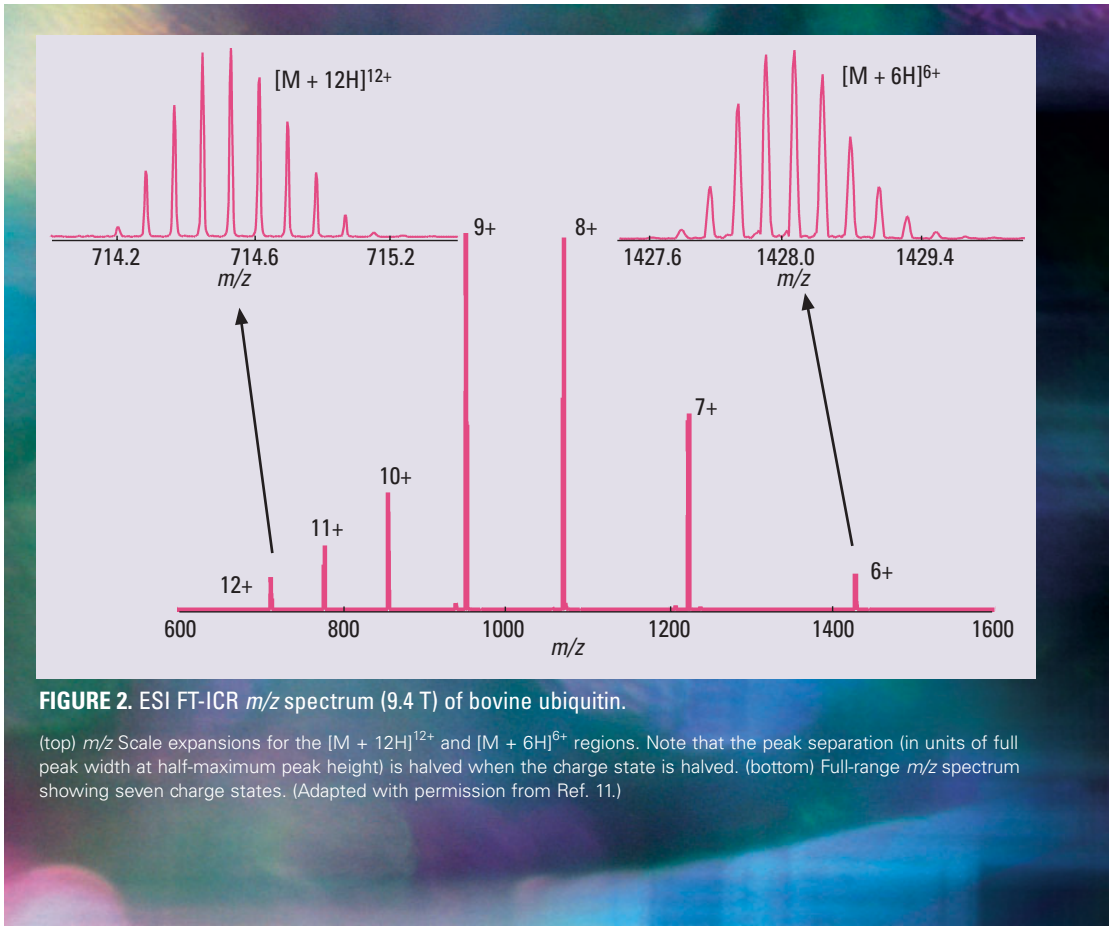


FIGURE 2. ESI FT-ICR *m/z* spectrum (9.4 T) of bovine ubiquitin.

(top) *m/z* Scale expansions for the $[M + 12H]^{12+}$ and $[M + 6H]^{6+}$ regions. Note that the peak separation (in units of full peak width at half-maximum peak height) is halved when the charge state is halved. (bottom) Full-range *m/z* spectrum showing seven charge states. (Adapted with permission from Ref. 11.)

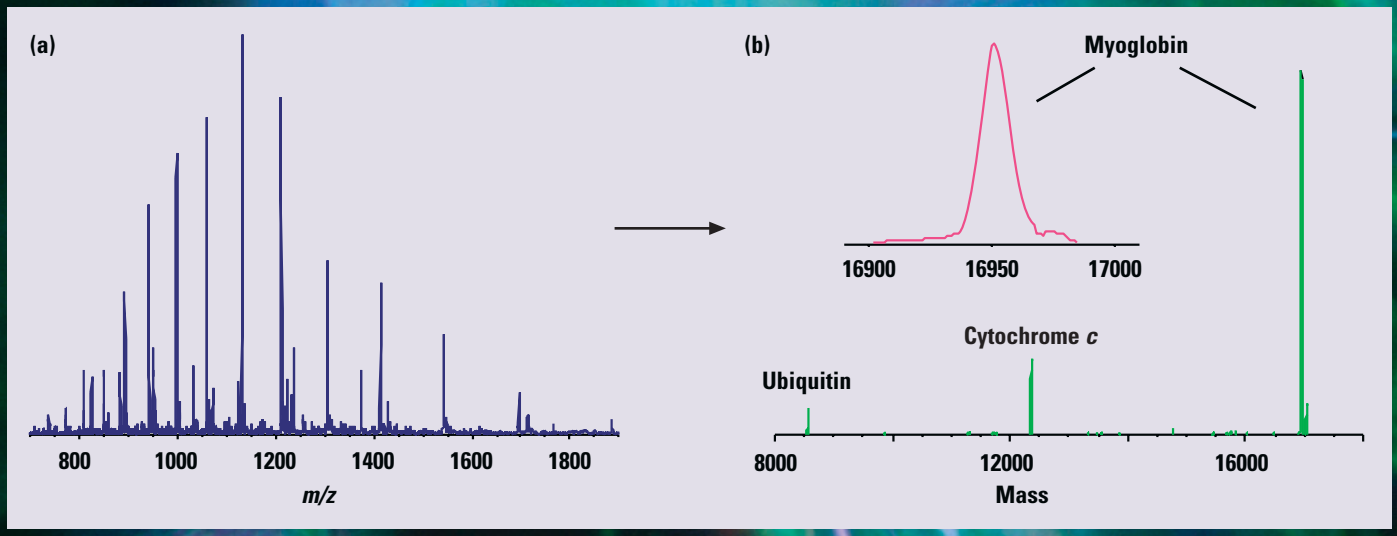


FIGURE 3. (a) Low-resolution ESI FT-ICR mass spectrum (16,000 data points and $m/\Delta m$ ~2000) is deconvolved into (b) a mixture of three proteins.

Note that n proton masses have been subtracted from each $(M + nH)^{n+}$ ion to yield the corresponding zero-charge mass in the deconvolved spectrum. (Adapted with permission from Ref. 7.)

(*24*). Note that enzymatic cleavage fragments up to ~30,000 Da are clearly resolved in this complex mixture. As another example, if mass can be measured to within 1 Da, then the oxidation state of a metal atom in a metalloprotein may be determined from its mass alone (*25–27*). In this way, multiple oxidation states of various gas-phase proteins have now been demonstrated (*28*).

Elemental compositions from fine structure

The full power of mass analysis emerges from resolution at the milli-Dalton level because each nuclide has a different mass defect (i.e., difference between the exact mass and the nominal mass): ^{12}C is 12.00000, ^{1}H is 1.007825, ^{16}O is 15.9949, etc. Thus, every different elemental composition, $C_cH_hO_oN_nS_s$ . . . ., has a different mass, so that the chemical formula of a molecule can be determined uniquely from a sufficiently accurate mass measurement. At the mass resolution and accuracy of FT-ICRMS, it is possible to resolve and identify each of up to several thousand elemental compositions in mixtures as complex as petroleum crude oil.

For example, Figure 6a shows the resolution and identification of nine different elemental compositions within a mass window of just 0.25 Da. Such a spectrum provides a highly detailed fingerprint for recognition of patterns associated with properties of a mixture. Unlike a chromatogram, however, the mass spectrum provides considerable chemical detail about each component. First, the chemical formula identifies the number of heteroatoms, $O_oN_nS_s$, which is also known as the molecular "class". Second, because every ring or double bond reduces the number of hydrogens by two and because saturated hydrocarbons have the general formula C_cH_{2c+2}, the value of z (i.e., the compound "type") in the formula C_cH_{2c+z} reveals the number of rings plus double bonds for each molecular class. For example, $z = -12$ means that there are seven rings plus double bonds (*29*). Third, because every additional methylene group ($-CH_2$) does not change the class or type but does increase the mass by 14.01565 Da, a complete alkylation profile for all ions of a given class and type may be produced solely from accurate mass measurements (Figure 6b).

The upper mass limit for the unique determination of elemental composition from mass alone (at a mass accuracy of ~1 ppm) is ~300 Da. That limit may be extended if, in the case of crude oil, long series of homologous compositions are present, such as the alkylation patterns in Figure 6b, and the limit is extrapolated along the series from lower to higher mass (*30, 31*). Fortunately, however, as molecular mass increases, the nature of the problem can also change, because most large molecules are made from building blocks of limited elemental combinations, and knowing the construction rules can therefore identify the analyte.

For example, the primary goal with peptides is to determine amino acid composition and primary amino acid sequence rather than elemental composition. As another example, resolving the isotopic fine structure in a 16-kDa protein makes it possible to count the number of sulfur-containing amino acids on the basis of relative ion abundances rather than ion mass measurement (*32*). Ultrahigh-resolution MS makes possible the fingerprint identification of a complex substance in the presence of other contaminants, for example, the identification of arson accelerants (e.g., gasoline, kerosene, mineral spirits, turpatine), each of which is a mixture of hundreds of chemically distinct components, from the residue after a fire (*33*).

A particularly popular direction for complex mixture analysis is proteomics, in which one tries to identify a protein from the mass of one or more of its enzymatically cleaved fragments. For those applications, the main goal is to match the measured pep-

Defining the masses

Mass peak width ($\Delta m_{50\%}$)
Full width of mass spectral peak at half-maximum peak height

Mass resolving power ($m/\Delta m_{50\%}$)
A well-isolated single mass spectral peak

Mass resolution ($m_2 - m_1$ in Da, or $(m_2 - m_1)/m_1$ in ppm)
The smallest mass difference between equal magnitude peaks such that the valley between them is a specified fraction of either peak height

Mass precision
Root-mean-square deviation in a large number of repeated measurements

Mass accuracy
Difference between measured and actual mass

Mass defect
Difference between exact and nominal mass

tide masses with those expected from the analysis of the database of known proteins, for which mass measurement need not be as accurate as for determination of elemental composition. Moreover, if one is searching a particular subset of the protein database for, say, a 40,000-protein genome, then the number of possible amino acid compositions is very greatly reduced. In fact, such a database protein has been identified from as few as one to three of its trypsin-cleaved fragments at a mass accuracy of ~10 ppm (*34*). Commercial protein identification software is just beginning to incorporate accurate mass measurement capability.

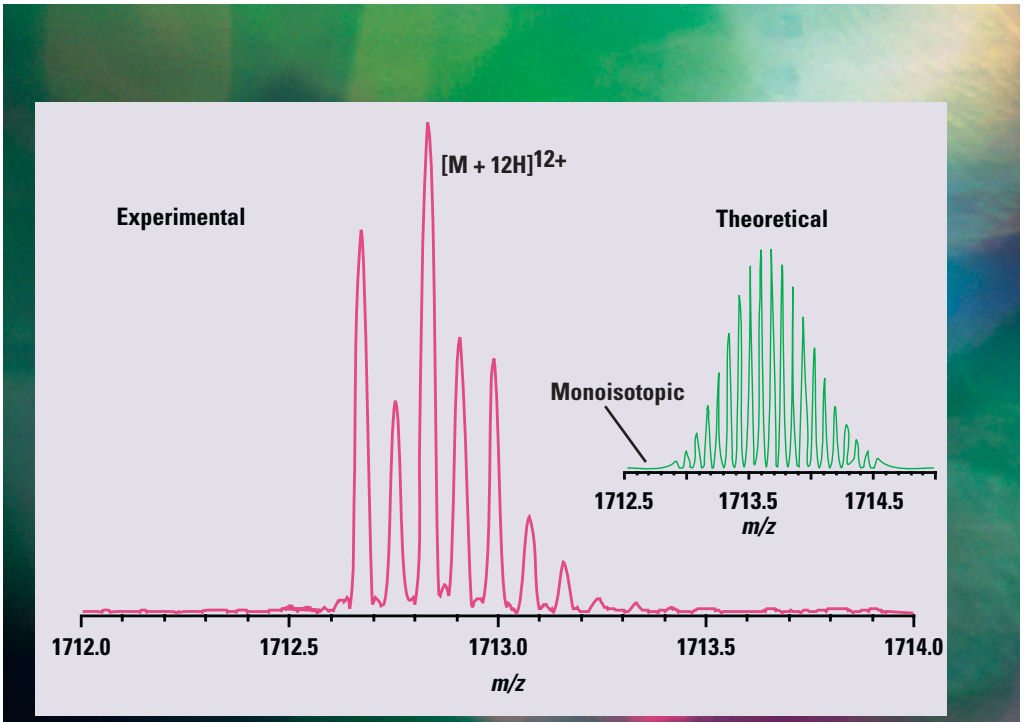


FIGURE 4. Experimental ESI/FT-ICR mass spectrum of a doubly depleted mutant Cdc42 protein versus the theoretical isotopic distribution for natural abundance Cdc42 mutant.

The His-tagged Q61L mutant protein has a monoisotopic mass of 20,540 Da ($C_{932}H_{1471}N_{231}O_{276}S_7$) whose relative abundance jumps from ~0.005% in the (right) natural abundance protein to ~80% in the (left) doubly depleted protein. (Adapted with permission from Ref. 44.)

Ground and excited states

In keeping with Einstein's special theory of relativity, a difference in internal energy, ΔE, between a ground and excited state of a particle corresponds to a mass difference, Δm, according to

$$\Delta E = \Delta m c^2 \qquad (1)$$

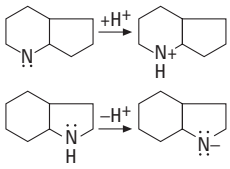
in which c is the speed of light in a vacuum. (Incidentally, contrary to popular opinion, the mass of a particle is a fundamental property that is independent of its speed. There is no such thing as "rest mass" or "relativistic mass"; rather, a particle may have "rest energy" or "relativistic energy" [*35*].) Unfortunately, an energy difference of ~1 eV corresponds to a mass difference of only ~1 nDa (*36*). Although several atomic masses have been measured by ICR-related methods to that level of precision (*37*), experimental efforts to detect the Δm (~10 nDa) between a ground and excited electronic state of an atomic ion have not yet succeeded. Of course, there is really no "chemical" reason to perform such a measurement given that spectroscopic transition frequencies can be measured much more accurately. Moreover, such measurements are unlikely to be feasible for molecular ions because the excited state must survive for >100 s so that its ICR frequency, and thus its mass, can be determined accurately.

However, the difference in mass between ground and excited (isomeric) nuclear states is much larger and has actually been resolved for atomic rubidium ions (*38*). In that case, the energy difference was ~100 keV, so that the corresponding Δm (~0.1 mDa) could be determined experimentally by an ICR-related method.

Isomers

Pushing the relativity argument to its extreme, it is, in principle, possible to distinguish between positional isomers based on their different heats of formation. For example, the heats of formation of dimethyl ether and ethanol differ by ~3 eV, corresponding to a Δm of ~3×10^{-9} Da. However, their resolution would require a mass resolving power >10^{10}, which is a factor of >10^3 higher than even FT-ICR currently offers. Still, it's fun to think that even differences in chemical bonding between isomers can, in principle, be distinguished by mass measurement alone.

In practice, there are other mass-related ways to distinguish between isomers. For example, the two molecules shown below



can, in principle, be distinguished by ESI because the more basic molecule (top) will protonate preferentially and thus appear in positive-ion ESI, whereas the more acidic molecule (bottom) will deprotonate preferentially and thus appear in negative-ion ESI (*30, 39*). Alternatively, various MS/MS experiments have been designed to distinguish optical isomers based on enantioselective gas-phase ion–molecule reactions (*40, 41*).

Mass resolving power, resolution, and accuracy

The present discussion has focused on quantum jumps in mass spectral resolution with continuously increasing instrumental mass resolving power. Those jumps explain why commercial mass analyzers are targeted to reach the highest-mass plateau available. In that respect, one way to think about FT-ICRMS is that it reaches each of the plateaus in Figure 1 at ≥100 times higher mass than do other mass analyzers.

However, even on top of a plateau, it is always desirable to have the highest possible resolving power to distinguish between different members of a mixture. To understand why, consider the following relationships. Mass resolving power, $m/\Delta m_{50\%}$, is defined for a well-isolated single mass spectral peak. Mass resolution, $\Delta m_{50\%}$, is equivalent to the smallest Δm between peaks of equal magnitude, such that the valley between them just disap-

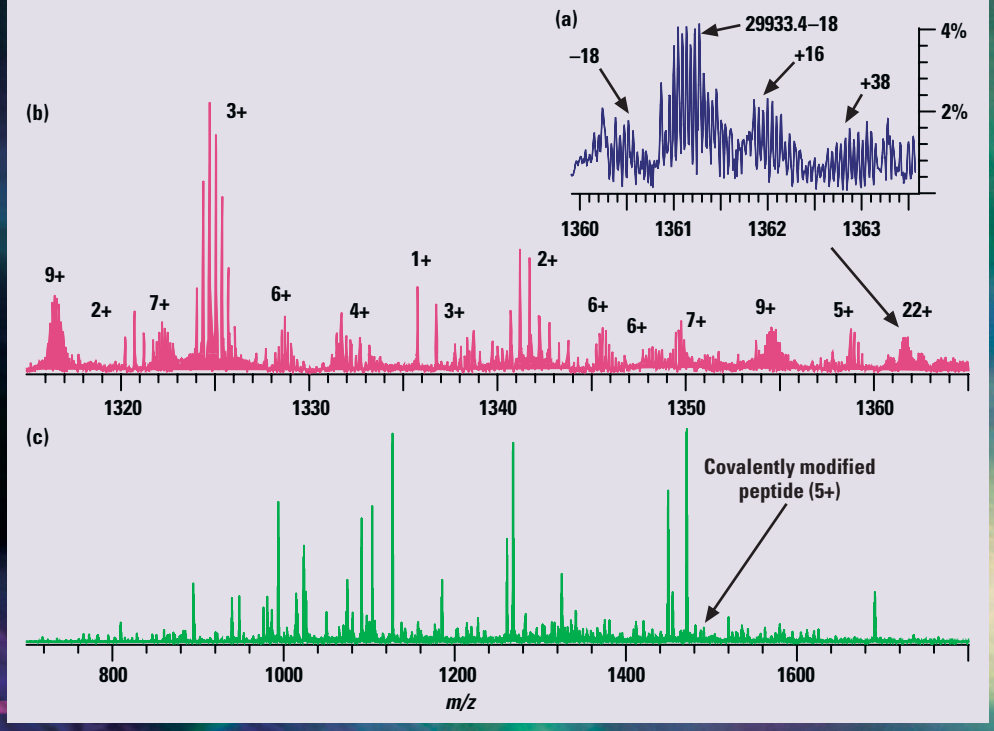


FIGURE 5. (a, b) Two *m/z* scale-expanded segments from (c) an ESI FT-ICR broadband mass spectrum of a GluC digest of a 191-kDa protein.

The charge state of each peptide in (a) and (b) was determined from the reciprocal of the spacing between adjacent peaks in each isotopic distribution, of which there were 824. From the single broadband spectral data in (c), 581 individual peptides could be identified, along with the site of a single amino acid chemical modification.

pears. Mass precision is the root-mean-square deviation in a large number of repeated measurements. Mass accuracy is the difference between the measured and actual masses. Although these figures of merit are clearly related to each other, some care is needed when applying them to a given analytical situation and in understanding commercial instrument performance claims.

Very generally, for a well-isolated single spectral peak, precision, $m/\sigma(m)$, in which $\sigma(m)$ is the root-mean-square deviation for a large number of repeated measurements, is proportional to the product of the S/N and the square root of the number of data-points-per-peak width (*42*). Thus, a reflectron TOF mass analyzer, with a $m/\Delta m_{50\%}$ of only 10,000, can nevertheless potentially achieve a maximum mass precision and accuracy to ±1 ppm (or 1% of the peak width), provided that S/N and the number of data-points-per-peak width are sufficiently high (*43*). However, actual mass spectra typically exhibit much lower S/N, and mass accuracy can be further degraded to the point that it is much poorer than the mass precision if a given signal consists of two or more unresolved peaks. Thus, it is always best to have the highest possible mass resolution to ensure that mass accuracy approaches mass precision. That fact constitutes the main justification for ultrahigh-resolution FT-ICR mass analysis, because one never knows for certain in advance whether an impurity or contaminant or other analyte will have a mass very close to the analyte mass of interest.

Then and now

MS is changing the way in which chemical and biochemical analysis of complex mixtures is conducted. Traditionally, it was necessary to preseparate components because the selectivity of the final analysis had to be sacrificed to achieve acceptable sensitivity using methods such as refractive index, electrical conductivity, optical absorbance, electrochemistry, etc. However, MS now offers all three qualities: ultrahigh resolution with a peak capacity of >100,000 in a single mass spectrum, high sensitivity routinely in the femtomole range, and high information content per component (e.g., elemental composition and its associated class, type, and carbon distribution information; amino acid composition; metal ion oxidation state; etc.). The information is extracted in successive stages: charge state; functional groups; isotopic distributions; and ultimately, isotopic fine structure. The final message is that one should not be deterred by the visual complexity of an ultrahigh-resolution mass spectrum, because it is precisely that complexity that allows the resolution and identification of its components and the differentiation of complex mixtures.

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References

(1) Comisarow, M. B.; Marshall, A. G. *Chem. Phys. Lett.* **1974,** *25,* 282–283.
(2) Marshall, A. G.; et al. *Mass Spectrom. Rev.* **1998,** *17,* 1–35.

(3) Marshall, A. G. *Int. J. Mass Spectrom.* **2000,** *200,* 331–336.
(4) Marshall, A. G.; Verdun, F. R. *Fourier Transforms in NMR, Optical, and Mass Spectrometry: A User's Handbook;* Elsevier: Amsterdam, 1990.
(5) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989,** *246,* 64–71.
(6) Smith, R. D.; Loo, J. A.; Ogorzalek Loo, R. R.; Busman, M.; Udseth, H. R. *Mass Spectrom. Rev.* **1991,** *10,* 359–451.
(7) Zhang, Z.; Marshall, A. G. *J. Am. Soc. Mass Spectrom.* **1998,** *9,* 225–233.
(8) Stephenson, J. L. J.; McLuckey, S. A. *J. Mass Spectrom.* **1998,** *33,* 664–672.
(9) Scalf, J.; Westphall, M. S.; Smith, L. M. *Anal. Chem.* **2000,** *72,* 52–60.
(10) Kelleher, N. L.; Senko, M. W.; Siegel, M. M.; McLafferty, F. W. *J. Am. Soc. Mass Spectrom.* **1997,** *8,* 380–383.
(11) Marshall, A. G.; Hendrickson, C. L. *Rapid Commun. Mass Spectrom.* **2001,** *15,* 232–235.
(12) Cheng, X.; et al. *J. Am. Chem. Soc.* **1995,** *117,* 8859–8860.
(13) Wigger, M.; et al. Proceedings of the 46th American Society for Mass Spectrometry Annual Conference on Mass Spectrometry & Allied Topics, Orlando, FL, 1998; 1142.
(14) Freitas, M. A.; Hendrickson, C. L.; Marshall, A. G.; Rostom, A. A.; Robinson, C. V. *J. Am. Soc. Mass Spectrom.* **2000,** *11,* 1023–1026.
(15) Sannes-Lowery, K. A.; Drader, J. J.; Griffey, R. H.; Hofstadler, S. A. *Trends Anal. Chem.* **2000,** *9,* 481–491.
(16) Hofstadler, S. A.; et al. *Anal. Chem.* **1999,** *71,* 3436–3440.
(17) Griffey, R. H.; Hofstadler, S. A.; Sannes-Lowery, K. A.; Ecker, D. J.; Crooke, S. T. *Proc. Natl. Acad. Sci. USA* **1999,** *96,* 10,129–10,133.
(18) Senko, M. W.; Beu, S. C.; McLafferty, F. W. *J. Am. Soc. Mass Spectrom.* **1995,** *6,* 229–233.
(19) Zhang, Z.; Smith, D. L. *Protein Sci.* **1993,** *2,* 522–531.
(20) Marshall, A. G.; et al. *J. Am. Chem. Soc.* **1997,** *119,* 433–434.
(21) Li, W.; Hendrickson, C. L.; Emmett, M. R.; Marshall, A. G. *Anal. Chem.* **1999,** *71,* 4397–4402.
(22) Senko, M. W.; Beu, S. C.; McLafferty, F. W. *J. Amer. Soc. Mass Spectrom.* **1993,** *4,* 828–830.
(23) McLafferty, F. W.; Fridriksson, E. K.; Horn, D. M.; Lewis, M. A.; Zubarev, R. A. *Science* **1999,** *284,* 1289–1290.
(24) Horn, D. M.; Zubarev, R. A.; McLafferty, F. W. *J. Am. Soc. Mass Spectrom.* **2000,** *11,* 320–332.
(25) McLafferty, F. W.; Guan, Z.; Haupts, U.; Wood, T. D.; Kelleher, N. L. *J. Am. Chem. Soc.* **1998,** *120,* 4732–4740.
(26) Wu, Q.; Van Orden, S.; Cheng, X.; Bakhtiar, R.; Smith, R. D. *Anal. Chem.* **1995,** *67,* 2498–2509.
(27) He, F.; Hendrickson, C. L.; Marshall, A. G. *J. Am. Soc. Mass Spectrom.* **2000,** *11,* 120–126.
(28) Johnson, K. A.; Shira, B. A.; Anderson, J. L.; Amster, I. J. *Anal. Chem.* **2001,** *73,* 803–808.
(29) McLafferty, F. W.; Turecek, F. *Interpretation of Mass Spectra;* University Science Books: Sausalito, CA, 1993.
(30) Qian, K.; Rodgers, R. P.; Hendrickson, C. L.; Emmett, M. R.; Marshall, A. G. *Energy & Fuels* **2001,** *15,* 492–498.
(31) Hughey, C. A.; Hendrickson, C. L.; Rodgers, R. P.; Marshall, A. G.; Qian, K. *Anal. Chem.* **2001,** *73,* 4676–4681.
(32) Shi, S. D.-H.; Hendrickson, C. L.; Marshall, A. G. *Proc. Natl. Acad. Sci. U.S.A.* **1998,** *95,* 11,532–11,537.
(33) Rodgers, R. P.; Blumer, E. N.; Freitas, M. A.; Marshall, A. G. *J. Forensic Sci.* **2001,** *46,* 268–279.
(34) van der Rest, G.; He, F.; Emmett, M. R.; Marshall, A. G.; Gaskell, S. *J. Am. Soc. Mass Spectrom.* **2001,** *12,* 288–295.
(35) Okun, L. B. *Physics Today* **1989,** *42,* 31–36.
(36) Cohen, E. R.; Taylor, B. N. *Rev. Mod. Phys.* **1986,** *59,* 1121.
(37) DiFillip, F.; et al. *Physica Scripta* **1995,** *T59,* 144–154.
(38) Bollen, G.; et al. *Phys. Rev. C.* **1992,** *46,* R2140–R2143.
(39) Qian, K.; Robbins, W. K.; Hughey, C. A.; Cooper, H. J.; Rodgers, R. P.; Marshall, A. G. *Energy & Fuels* **2001,** *15,* 1505–1511.
(40) Grigorean, G.; Lebrilla, C. B. *Anal. Chem.* **2001,** *73,* 1684–1691.
(41) Tao, W. A.; Gozzo, F. C.; Cooks, R. G. *Anal. Chem.* **2001,** *73,* 1692–1698.
(42) Chen, L.; Cottrell, C. E.; Marshall, A. G. *Chemom. Intell. Lab. Sys.* **1986,** *1,* 51–58.
(43) Lee, H.-N.; Marshall, A. G. *Anal. Chem.* **2000,** *72,* 2256–2260.
(44) Marshall, A. G.; Emmett, M. R.; Freitas, M. A.; Hendrickson, C. L.; Zhang, Z. In *Mass Spectrometry in Biology and Medicine;* Burlingame, A. L., Carr, S. A., Baldwin, M. A., Eds.; Humana Press: Totowa, NJ, 2000; pp 31–52.

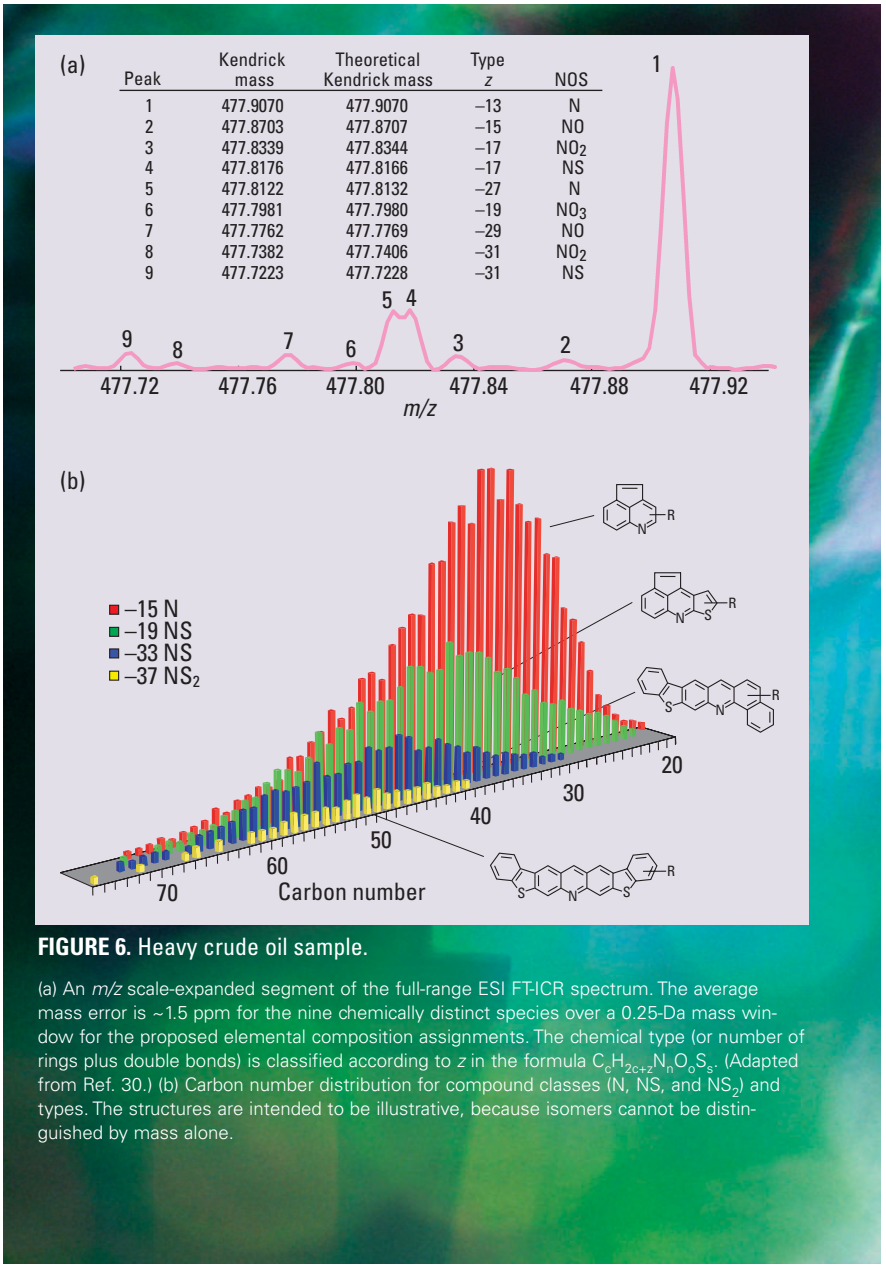

FIGURE 6. Heavy crude oil sample.

(a) An *m/z* scale-expanded segment of the full-range ESI FT-ICR spectrum. The average mass error is ~1.5 ppm for the nine chemically distinct species over a 0.25-Da mass window for the proposed elemental composition assignments. The chemical type (or number of rings plus double bonds) is classified according to *z* in the formula $C_cH_{2c+z}N_nO_oS_s$. (Adapted from Ref. 30.) (b) Carbon number distribution for compound classes (N, NS, and NS_2) and types. The structures are intended to be illustrative, because isomers cannot be distinguished by mass alone.