Fourier Transform Ion Cyclotron Resonance Mass Resolution and Dynamic Range Limits Calculated by Computer Modeling of Ion Cloud Motion

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(Abstract)

Particle-in-Cell (PIC) ion trajectory calculations provide the most realistic simulation of FT-ICR experiments by efficient and accurate calculation of the forces acting on each ion in an ensemble (cloud), including Coulomb interactions (space charge), the electric field of the ICR trap electrodes, image charges on the trap electrodes, the magnetic field, and collisions with neutral gas molecules. It has been shown recently that ion cloud collective behavior is required to generate an FT-ICR signal and that two main phenomena influence mass resolution and dynamic range. The first is formation of an ellipsoidal ion cloud (termed "condensation") at a critical ion number (density), which facilitates signal generation in an FT-ICR cell of arbitrary geometry because the condensed cloud behaves as a quasi-ion. The second phenomenon is peak coalescence. Ion resonances that are closely spaced in m/z coalesce into one resonance if the ion number (density) exceeds a threshold that depends on magnetic field strength, ion cyclotron radius, ion masses and mass difference, and ion initial spatial distribution. These two phenomena decrease dynamic range by rapid cloud dephasing at small ion density and by cloud coalescence at high ion density. Here, we use PIC simulations to quantitate the dependence of coalescence on each critical parameter. Transitions between independent and coalesced motion were observed in a series of the experiments that systematically varied ion number, magnetic field strength, ion radius, ion m/z, ion m/z difference, and ion initial spatial distribution (the present simulations begin from elliptically-shaped ion clouds with constant ion density distribution). Our simulations show that mass resolution is constant at a given magnetic field strength with increasing ion number until a critical value (N) is reached. N dependence on magnetic field strength, cyclotron radius, ion mass, and difference between ion masses was determined for two ion ensembles of different m/z, equal abundance, and equal cyclotron radius. We find that N and dynamic range depend quadratically on magnetic field strength in the range 1-21 Tesla. Dependences on cyclotron radius and Δm/z are linear. N depends on m/z as $(m/z)^{-2}$. Empirical expressions for mass resolution as a function of each of the experimental parameters are presented.

Keywords: FT-ICR, FTMS, mass resolving power, coalescence

Running Title: Simulated FT-ICR Mass Resolution and Dynamic Range

Introduction

In FT-ICR mass spectrometry, the measured signal results from induced charge on the FT-ICR trap detection electrodes caused by synchronous motion of ions in a static magnetic field. Ion motion synchronization is produced by radiofrequency-sweep excitation of ion cyclotron motion and results in a separate, synchronous ion cloud for each m/z. It is well known that during excitation and detection, ions undergo complex three-dimensional motion. For an ideal hyperbolic Penning trap, the motion in the plane perpendicular to the magnetic field is a combination of cyclotron and magnetron motions. Magnetron motion is induced by the radial component of the trap electric field and the axial magnetic field and is manifested as a modulation of the center of ion cyclotron rotation at a frequency typically ten thousand times smaller than the cyclotron frequency. In a hyperbolic trap and spatially homogeneous magnetic field, ions synchronized during cyclotron excitation continue to be synchronized indefinitely in the absence of collisions with neutrals and ion-ion interactions due to complete decoupling of cyclotron, magnetron and axial oscillations. In an actual ICR trap, the three modes of motion are coupled and the cyclotron and magnetron frequencies depend on the axial oscillation amplitude, resulting in ion cloud dephasing and "comet" structure formation. Ion cloud dephasing results from accumulated phase difference in the measured (cyclotron minus magnetron) motion for ions with different z-oscillation amplitude in an ion cloud; which can be evaluated for any ion trap by calculating the radial electric field dependence on axial z-coordinate. After some time, the phase difference across the original cloud becomes 2π, corresponding to the head of the comet reaching the tail and resulting in complete loss of signal from the cloud. FT-ICR MS resolving power is proportional to the duration of synchronous motion and is therefore limited by the time needed for dephasing to take place, which depends on ion trap geometry and magnetic field homogeneity [1]. Higher magnetic field strength results in smaller difference in measured cyclotron frequencies of ions in each cloud and smaller rate of phase difference accumulation.

The described phenomena are observed by particle-in-cell computer modeling [2]. For a cubic cell and small ion number, comet structures form, leading to disappearance of the ICR signal after about 1 second at 7 T (extending prior simulations at 1 T [1]) corresponding to mass resolution in accordance with the expression for FT-ICR resolution as a function of observation period (see **Figures 1 and 2**). Moreover, this phenomenon takes place at only small number of ions in the cell. As for the prior 1 T simulations, above a critical ion number, ions of the same m/z move synchronously independent of their z-amplitude. In this work we have determined the dependence of minimum number, N, of ions in the cloud for onset of coalescence on magnetic field strength (B), cyclotron radius (R), ion mass (m), and difference between ion masses ($m_2 - m_1$), for two ion ensembles of different m/z and equal abundance, excited to the same

nominal cyclotron radius. Empirical expressions for N as a function of each of the above listed experimental parameters are presented.

Methods

The present supercomputer simulations begin from previously developed computer code based on a Particle-in-Cell algorithm for calculation of electric forces acting on individual ion in an ion cloud from other ions in the cloud, from the field of trap electrodes, and from image charges on these electrodes [1]. Briefly, the ion density distribution is determined at every node of the mesh by dividing the total charge extrapolated to that particular node by the ions contained in the volume of each elementary cube of the mesh. By use of a direct fast Fourier transform (FFT) Poisson solver with the trap boundary conditions, corresponding to DC and RF potentials on the trap electrodes, the charge density on each grid points is converted into potentials at those points and electric field is determined from the spatial derivatives of the potentials. The electric field at an individual ion position is calculated by interpolating the electric fields from the nearest grid points based on the same weighting algorithm as for charge projection to each grid point. The mathematical description of the whole procedure for solving the Poisson equation by the FFT method is described in [2]. The particle positions and velocities for the next time-step are calculated with the Boris integrator [2,8,9]. We used two grid resolutions: 32x32x32 and 64x64x64. For computational simplicity, we chose either a 1x1x1 or 2x2x2 inch cubic cell. The number of time steps for one cyclotron period was 50 or 100 depending on the magnetic field strength. The number of stored snapshots per simulation was determined from the snapshot repetition rate, namely, after every 13,560 steps. For the simulation results presented in Fig. 10, snapshots were recorded for every 10 steps. The number of ions ranged from 10,000 -1,000,000. For some simulations, ions with 10 charges were used. All simulations are based on spatially homogeneous static magnetic field, with coherent cyclotron motion induced by single-frequency resonant dipolar excitation. RF amplitude, V_{p-p} = 10 V, was used for excitation. Excitation duration, T_{exc}, to a final radius, R = 6 mm (for all plots except Figure 5) was determined from R = A V_{p-p} T_{exc} /B [7], and A = 300 (cm V^{-1} s^{-1}) (determined empirically). For coalescence simulations, m/z and mass differences, $(m_2 - m_1)/z$ were: m/z = 100 Th, 100.3, z = 1; m/z = 500, 500.3 Th, z = 1; m/z = 499.95, 500.05 Th, z = 1; m/z = 999.95, 1000.05, 1000.15 Th, z = 1; (cyt C m/z $\approx$ 512, z = 23). Simulations were performed with either the Moscow State Regatta supercomputer or a Florida State University Dell station. The progression from independent motion to collective motion modes of ion clouds was observed from a series of simulations with different number of trapped ions and other parameters fixed.

Results and Discussion

Coalescence. When two ion clouds with small m/z difference coherently rotate in their cyclotron orbits, collective motion of the two clouds occurs due to Coulomb interaction [3], ultimately resulting in phase locking when the mutual E x B drift velocity exceeds the ion cyclotron velocity difference between the clouds. The measured mass spectrum then contains only one m/z averaged peak rather than two peaks at their respective mass-to-charge ratios.

Here, a systematically varied set of simulations enables a quantitative description of coalescence development as a function of the number of ions (N) in the interacting clouds, magnetic field strength (B), cyclotron radius (R), mass (m) and mass difference. Modeling was performed for ions trapped in 25.4 and 50.8 mm cubic cells. Simulations for the 25.4 mm cell (ion cloud initial radius at Z = 0 is ~2 mm (for an ellipsoidal ion cloud), post-excitation ion cyclotron orbital radius, R = 6 mm (i.e., ~50% of the cubic cell inscribed radius), reveal the following relationships (**Figures 3 and 4**):

$$N = 200{,}000 \, B^2 \quad (m = 100.15 \text{ Da}, z = 1, m_2 - m_1 = 0.3) \tag{1}$$

$$N = 10{,}000 \, B^2 \quad (m = 500.15 \text{ Da}, z = 1, m_2 - m_1 = 0.3) \tag{2}$$

$$N = 3{,}000 \, B^2 \quad (m = 500 \text{ Da}, z = 1, m_2 - m_1 = 0.1) \tag{3}$$

Here and elsewhere, $m = (m_1 + m_2)/2$.

In Eqs. 1-3 and those that follow, multiply-charged ions may be represented by *m/z* (in Th) rather than *m (in Da)*.

We recently developed an expression relating the minimum number of ions required to induce coalescence as a function of magnetic field B, mass m, mass difference $(m_2 - m_1)$ and ion cyclotron radius *R* for ion clouds of arbitrary form:

$$N = \frac{2}{\nu} \frac{RB^2 (m_2 - m_1)/m}{km} \tag{4}$$

in which k is the Coulomb force constant, $k = 1/4\pi\varepsilon_0 = 8.9 \; 10^9 \text{ N m/C}^2$, and ν is a coefficient derived from the form of cloud interaction potential. [4]

The cloud shape for our simulations is usually ellipsoidal. For a 25.4 mm cubic cell, the ellipse has semi-axes a and b = 4a, and the numerically calculated interaction potential is $\nu = 0.274/a^2$, so that

$$N = 7.3 \frac{a^2 RB^2 (m_2 - m_1)/m}{km} \tag{5}$$

which may be rewritten as

$$N = 4.87 \ 10^8 \ a^2 \ R \ B^2 \ (m_2\text{-}m_1)/m \tag{6}$$

in which R is in mm, $((m_1 + m_2)/2z)$ and $((m/z)_2 - (m/z_1))$ are in Th, B is in tesla, and N is the total number of ions in both clouds (each interacting cloud has the same ion number). ÷

From this formula we obtain (assuming $a \approx 2$ mm and $R \approx 6$ mm)

$$N \ (100.15/0.3) = 283{,}400 \ B^2 \tag{7}$$
$$N \ (500.15/0.3) = \ \ 12{,}320 \ B^2 \tag{8}$$
$$N \ (500/0.1) = \ \ \ \ \ 4{,}100 \ B^2 \tag{9}$$

which agrees well with the simulation results.

From the simulation for a 50.8 mm cubic cell (**Figure 5**):

$$N = 800{,}000 \ R[cm] \text{ for ions of m/z 499.95 and 500.05 at } B = 7 \ T \tag{10}$$

$$\text{Radius of cloud taken from simulation is } a = 2.2 \text{ mm.} \tag{11}$$

For the 50.8 mm cell, the ion cloud spreads more axially than radially. For an ellipsoidal cloud with semi-axes a and b = 8a in a 50.8 mm cell and by use of Equation 4 (as for 1:1:4 ellipsoids in the 25.4 mm cell) we obtain ν and an expression for N:

$$N = 13.6 \frac{a^2 RB^2 (m_2 - m_1)/m}{km} \tag{12a}$$

or $N = 84{,}235 \ R \ [mm]$ \hfill (12b)

Thus, the analytic description [4] is in good agreement with our numerical simulations. Analytic estimation shows that the coalescence condition depends not only on B, R, m, and $(m_2\text{-}m_1)$, but also on the

shape and size of the interacting clouds, which depend on the cell geometry and the number of ions in the trap and may distort the dependence on B, R, m, and *(m₂-m₁)*.

Coalescence Threshold and Frequency Shifts. Close inspection of the simulations used to generate the data in Figures 3 and 4 reveals complex and unstable progression from independent cloud rotation to coalescence (Figures 6-9). **Figure 6** (B = 7 T, $m/z \approx$ 500 Th, $(m_2-m_1)/z$ = 0.1 Th, detection period = 0.5 s) and **Figure 7** (B = 1 T, $m/z \approx$ 100 Th, $(m_2-m_1)/z$ = 0.3 Th, detection period = 0.5 s), displayed frequencies of two ion clouds of different m/z as a function of the total number of ions, 2*N. At small N, two distinct mass spectral peaks of different frequencies are evident, whereas at large N, the two resonances coalesce into a single peak at a frequency close to the average cyclotron frequencies of both clouds. For intermediate N values, a third peak much broader than the other two appears. The broadening corresponds to instability of cloud coherence (see Figures 8 and 9. In **Figure 8** (B = 7 T, $m/z \approx$ 500 Th, $(m_2-m_1)/z$ = 0.1 Th) two independent clouds become coupled during the detection period, starting ~0.03 s after excitation.. In **Figure 9** (B = 1 T, $m/z \approx$ 100 Th, (m_2-m_1) = 0.3 Th), two initially coupled clouds become independent. After excitation ion clouds.have similar radius and phase. If the total ion population of a cloud is close to the limit, 2*N, coalescence is unstable. Thus, if mutual Coulomb interaction results is loss of some ions, an initially bound joint ion cloud separates into independent ion clouds of different m/z, as seen in Figure 9 starting at ~0.15 s after excitation.

At the coalescence threshold, we observe ion cyclotron frequency shifts due to changes in the number of charges in the ion clouds, a trend that persists until the abrupt onset of coalescence. Analysis of the cyclotron motion in the simulations used to construct Figure 7 (B = 1 T) reveals decreasing frequency, (ν as in Equation 4) of the 100 Th cloud (the faster moving mass), which is approximated by

$$\nu \text{ (Hz)} = -2.4 \ 10^{-4} \text{ N} + 152936 \tag{13}$$

Cloud dephasing for the slower (100.3 Th) orbiting cloud begins at lower ion number (150,000 ions), so we fitted only the first 6 data points:

$$\nu \text{ (Hz)} = -2.2 \ 10^{-7} \text{ N} + 153398 \tag{14}$$

Similarly, at 7 T for m/z 500 (Figure 6), we see a clear decrease in frequency of the faster moving ions of m/z 499.95 Th.

$$\nu \text{ (Hz)} = -1.3 \; 10^{-5} \, N + 214985 \tag{15}$$

Dephasing of the slower cloud (500.05 Th) begins with 725,000 ions in the two clouds, so we fit only the first 6 points:

$$\nu \text{ (Hz)} = -5 \; 10^{-7} \, N + 214940 \tag{16}$$

Remarkably, the frequency shift of the faster moving clouds is consistently ~1000 times greater than for the slower moving cloud. We can explain the increased frequency shift of smaller m/z ions by stronger coulomb interaction effect for them because they are outside of larger m/z cloud and experience the action of Coulomb forth from it..

An analytic–description of ion cyclotron frequency versus ion number can be taken from [5] We consider an ion trap with potential

$$\Phi_0(x, y, z) = V_{trap}(\gamma + \frac{a}{2\alpha^2}(2z^2 - x^2 - y^2)) \tag{17}$$

in which V_{trap} is the applied trapping voltage, a is the trap diameter, and γ and α are cell geometry constants. The resulting detected cyclotron frequency is

$$\omega = \frac{qB}{m} - \frac{2GtV}{B} \tag{18}$$

in which G is the trap geometry factor) Addition of the ion-ion interaction potential (we use the potential of a uniformly charged ellipsoid) [5] gives:

$$\omega = \frac{qB}{m} - \frac{2GtV}{B} - \frac{q\rho G_i}{\varepsilon_0 B} \tag{19}$$

in which Gi is the geometry factor for an ellipsoidal ion cloud, ρ is ion cloud density; and ε_0 is vacuum permittivity). Eq. 19 predicts a linear decrease in ion cyclotron frequency with increasing ion number (density). Thus, the observed linear frequency shifts are in good agreement with the theoretical description.

Ion cloud motion during coalescence and ion cloud shape. Visualization of the detailed interaction between coalescing ion clouds helps to understand the underlying physics and to build accurate and

predictive analytical models. **Figure 10** shows a series of ion cloud snapshots that represent three different m/z clouds (m/z 999.95 in green, 1000.05 in red, and 1000.15 in blue) in a 7 Tesla magnetic field and 50.8 mm cubic cell during one cyclotron period under complete coalescence conditions (Figure 10A, 102,000 ions per cloud) and partially coalesced conditions (Figure 10B, 34,000 ions per cloud). Note that the ions of different m/z in coalesced clouds do not mix (i.e., ions of each m/z maintain separate coherence inside the coalesced cloud). Further, note that in Figure 10A, each of the three coalesced clouds completes one revolution around the cloud axis during each cyclotron rotation, and that the three m/z clouds are positioned such that ions of higher m/z are always closer to the center of rotation. This redistribution takes place after cyclotron excitation that excites ions of all m/z to the same radius.

Ion cloud shape is important because it influences the onset of coalescence [4]. **Figure 11** shows the progression of ion cloud shape as a function of ion number for ions of $m/z = 499.95$ and 500.05 Th at 7 T, in a 50.8 mm cubic cell, and 1 V trapping potential, at a post-excitation initial ICR orbital radius of 6 mm. The ion cloud shape corresponds to an elongated ellipsoid ($x^2/a^2 + y^2/b^2 + z^2/c^2 = 1$) that progresses from a = b < c, in which a ≈ 2.2 mm and c ≈ 10 mm for 100,000 ions to a ≈ 4 mm, b ≈ 6 mm, and c ≈ 20 mm for a coalesced cloud of 900,000 ions, which is near the cell capacity limit for 1 V trap potential. Note that all of our simulations proceed from an ellipsoidal initial ion distribution, and that no subsequent significant change of ion cloud shape was observed. A more accurate definition of initial ion cloud shape would require simulation of ion injection and capture.

Coalescence for an isotopic cluster of Cyt *c*. The analytical description presented above considers coalescence of two equal-abundance ion clouds. However there are typically many (up to 50,000) interacting clouds and the practical limitation of resolving power associated with coalescence may be different from the threshold predicted by the formulas derived from simulation of just two interacting clouds. In **Figure 12** we show simulated coalescence of the more realistic case of the 23+ charge state isotopic cluster of cytochrome *c*, with relative isotopic abundances calculated from Molecular Weight Calculator [10] and each isotope peak color-coded as in Figure 12b.

Note that the individual m/z clouds initially rotate at equal cyclotron radius, but as the number of ions increases, the radius of each cloud changes so that lower m/z (faster) clouds have larger radius than higher m/z (slower) clouds and eventually only one ion cyclotron resonance frequency is observed. This behavior is similar to that from Figure 10, in which three different m/z clouds coalesced. Figure 12a also shows that higher ion number in the isotopic cluster results in increased rate of ion cloud dephasing and noticeably impacts the calculated spectrum if the number of charges in the isotopic cluster exceeds 100,000.

Coalescence is first observed if the number of charges exceeds 350,000, in good agreement with the value predicted by Eq. 4 for m/z = 512, m/z difference of 0.043 Th, B = 7 T, and an initially ellipsoidal cloud (a = b = 4 mm and c = 4a).

Comparison of the calculated signal magnitude (Figure 12a, top) with snapshots of ion cloud position for a noncoalesced isotopic cluster (100,000 charges) shows that the maximum signal magnitude corresponds to constructive interference of the isotopes, which occurs periodically when the clouds tend to converge at one azimuthal angle [6]. Typical FT-ICR excitation results in equal ion cyclotron radius at all m/z [7], which requires each pair of different m/z clouds to pass through each other at their respective ICR difference frequency. For example, the monoisotopic cloud for a singly-charged peptide at m/z 1000 will pass through the $^{13}C_1$ cloud ~100 times during a 1 s detection period. Our modeling shows that ion clouds adopt an ellipsoidal structure with length determined by the number of ions (charges). If ion density inside a cloud approaches the Brillouin limit [3], the ion potential energy is independent of ion position in the cloud, so that a small ion cloud will spread throughout a larger cloud during their interaction and will continue to be axially dispersed after emerging from the larger cloud. Axial dispersion causes ion cyclotron motion dispersion and loss of ions after multiple passes, and is manifested as a loss of low abundance components of the simulated Cyt C isotopic cluster (Figure 12).

Conclusions

Dependence of coalescence on magnetic field is not easily measured with a single instrument because the magnetic field must be varied. The most practical recourse is to make realistic simulations of ion cloud behavior in magnetic fields of different intensity. The present simulations show that mass resolution stays constant at a given magnetic field strength with increasing ion number until a critical number N is reached, triggering the abrupt onset of coalescence. We have determined the dependence of N on magnetic field strength (B), cyclotron radius (R), ion mass (m), and difference between ion masses $(m_2 - m_1)$ for two ion ensembles of different m/z and equal abundance, excited to the same nominal cyclotron radius. We find that N depends approximately quadratically on magnetic field strength in the range 1-21 Tesla, as does dynamic range. Dependences on cyclotron radius and $(m_2 - m_1)$ are linear, and N depends on m as $1/m^2$. Empirical expressions for coalescence as a function of each of the above listed experimental parameters are presented. A recent analytical theory of ion cloud coalescence [4] agrees well with our simulation results.

Acknowledgments

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Figure Legends

Figure 1. Simulated x-y and y-z projections of ion clouds taken at 0.3 s after excitation (top) and time-domain ICR signals (bottom, 0.5 s of duration) for 5000 singly charged ions, m/z = 500 Th, at 7 T Left: Cubic cell. Right: Quadrupolar trapping potential with excitation and detection electric fields taken from the cubic cell.

Figure 2. Simulated x-y projection of ion clouds taken at 0.05 s and 0.3 s after excitation (top and middle) and time-domain ICR signals (bottom, 0.5 s) for three different numbers of singly charged ions of m/z = 500 Th, at 7 T in a 50.8 mm cubic cell. Trapping potential 1 V.

Figure 3. Minimum number of ions at which FT-ICR peak coalescence takes place as a function of magnetic field strength, for equally abundant ions of m/z = 100 and 100.3 Th in a 25.4 mm cubic ICR cell. The smooth curve represents an analytical formula that approximately matches the simulated onsets. (In all simulations, coalescence appears to occur entirely during detection, but could possibly begin during excitation. We cannot distinguish between these possibilities, because the excitation period is too short for clouds to separate from each other.

Figure 4. Minimum number of ions at which FT-ICR peak coalescence takes place as a function of magnetic field strength, for equally abundant ions in a 25.4 mm cubic ICR cell. Left: ions of the same m/z difference $((m/z)_2 - (m/z_1)) = 0.3$ but different m/z (~100 and ~500 Th). Right: ions of the same m/z (~500 Th) but different m/z difference $((m/z)_2 - (m/z_1)) = 0.1$. Each smooth curve represents an analytical formula that approximately matches the simulated onsets.

Figure 5. Dependence of the number of charges in a 25.4 mm cubic FT-ICR cell for the onset of coalescence of two FT-ICR MS peaks (*m/z* = 499.95, 500.05 Th, equally abundant ions) on ion cyclotron radius (B = 7 T).

Figure 6. Top: Effective cyclotron frequencies of equally abundant ions of m/z = 499.94 and 500.05 Th as a function of the number of charges, N, at (25.4 mm cubic cell B=7 T. Bottom: Simulated FT-ICR mass

spectra for N = 100,000, 720,000, and 900,000. In Figures 6-10, lower m/z clouds are in blue; with higher m/z in red. (Gleb—true for Figure 10?)

Figure 7. Top: Effective cyclotron frequencies of equally abundant ions of m/z = 100 and 100.3 Th as a function of the number of charges, N, at B = 1 T, in a 25.4 mm cubic cell. Bottom: Simulated FT-ICR mass spectra for .N = 100,000, 325,000, and 500,000.

Figure 8. Top: x-y projections of ion clouds for two initially independently orbiting equally abundant ion clouds. Note the redistribution of ions and comet tail formation for ions of lower m/z. (B = 1 T, N = 325,000 charges, *m/z* = 100 and 100.3 Th, 25.4 mm cubic cell .) Bottom: Time-domain ion signal (left) and corresponding FT-ICR mass spectrum (right).

Figure 9. Top: x-y projections of ion clouds for two initially synchronously orbiting ion clouds of m/z = 499.95 and 500.05 Th. Bottom: Time-domain ion signal (left) and corresponding FT-ICR mass spectrum (right). (B = 7 T, 725,000 charges, 25.4 mm cubic cell.

Figure 10. Top: x-y projections of ion clouds of three different m/z values (999.95 in green, 1000.05 in red, and 1000.15 in blue) at 7 T , in a 50.8 mm cubic trap, and 1 V trapping voltage. (A) completely synchronized motion (~100,000 ions of each m/z). (B) two clouds synchronized and one independent (34,000 ions of each m/z).

Figure 11. Axial dimensions of equally abundant ion clouds (m/z = 499.95, 500.05) as a function of the number of charges. Total ion number: a) 100,000, b) 200,000, c) 300,000, d) 400,000, e) 500,000, f) 600,000, g) 700,000, h) 800,000, i) 900,000 charges (50 mm cubic cell with 1 V trapping voltage and 7 T magnetic field, 0.0147 s following excitation to an ion cyclotron radius of 6 mm.

Figure 12 x-y projections of ion clouds (left), and simulated time-domain ICR signals (top and right) and FT-ICR mass spectra (middle) for the Cyt *c* isotopic distribution (m/z ≈ 512, z = 23+) ion clouds, for different total ion numbers. Color-coding for members of the natural abundance isotopic distribution (individual peaks separated by m/z = 0.043) is shown at the bottom of the Figure. (B = 7 T ; 0.3 s detection; >100 steps per shortest cyclotron period; 0.0004 sec, 50 V_{p-p} excitation potential "chirp" excitation to yield post-excitation ICR orbital radius of 10 mm; 5 V trapping potential.