Supplementary data for:

Ion Mobility Separation coupled with MS detects two structural states of Alzheimer's disease Aβ1-40 peptide oligomers.

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Fig. S1. ESI-MS spectrum of Aβ1-40 peptide at 100 μM concentration in 10 mM ammonium acetate at pH 7.4 is shown in red. The spectrum in the main panel contains strong signals at 1082.80 and 866.44 *m/z*, corresponding to +4 and +5 charged monomeric species (denoted MON^{4+} and MON^{5+}). However, the spectra also contain signals that cannot be attributed to monomeric species but correspond to signals expected for oligomeric non-covalently stabilized species. The *m/z* values along with their assignments to the oligomeric species are shown in Supplementary Table 1. For example, the signal at *m/z* 1856.61 corresponds exactly to the *m/z* expected for the +7 charge state of the trimeric species, and the observed spacing and distribution of a well separated isotopic envelope confirms the unique assignment of this signal to the +7 trimeric (TRI^{7+}) form. The signal at *m/z* 2164.62 can be attributed to both a monomer with +2 charges and a dimer with +4 charges. Closer inspection of the isotopic envelope in the region from *m/z* 2164-2169 (Fig. S1 inset D), shows the presence of two groups of signals; the stronger - spaced by 0.5 Da whilst the weaker is spaced by 0.25 Da, thus revealing the presence of both the monomeric and dimeric species. In principle the signal at 2406.48, deprived of well resolved isotopic envelope, can be attributed either to a pentamer with +9 charges or a decamer with +18 charges, etc. However, the presence of a strong signal from the decameric +18 species would require the presence of the decameric +17 species which is not the case and we can therefore assign *m/z* 2406.48 to the pentamer +9, although its isotopic envelope is, in this case, not well resolved. Other signals are similarly assigned to oligomeric species in an unequivocal fashion. The presence of oligomers as large as hexadecamers (i.e. containing sixteen Aβ peptide units) of molecular mass 69,278 Da can be detected. The signal amplitude decreases with the mass of oligomer, however there is no correlation between the signal amplitude

and species concentration, as the sensitivity of a mass spectrometer fitted with an electrospray ion source depends upon several factors, including sequence composition, molecular mass, and other competing chemical species that maybe present within the sample. **The insets A, B, C, D** (W-mode spectrum) show the isotopic envelopes corresponding to peaks of MON^{4+}, TRI^{7+}, TET^{9+} and MON^{2+}/DIM^{4+}, respectively. Note the shape and spacing in peak of 2164-2169 *m/z* (inset D), which represents a mixture of doubly charged monomer (spacing 0.5 *m/z*) and +4 charged dimer (spacing 0.25 *m/z*) signals. The insets **E, F, G** (V-mode) present the enlarged areas corresponding to a 2300–4000 *m/z* value range, where the signals of higher oligomeric forms are observed. **Inset E** presents the enlarged (54x) region of the spectrum from 2300 to 2950 *m/z* where peaks corresponding to HEP^{13+} to DIM^{3+} are observed. **Inset F** presents the enlarged (327x) region of the spectrum from 2950 to 3600 *m/z* where the peaks correspond to UDC^{16+} to TTD^{17+} species are observed. **Inset G** presents the enlarged (1011x) region of the spectrum from 3580 to 4000 *m/z* where peaks corresponding to PEN^{6+} to DDC^{13+} are detected.

Fig. S2. IMS spectrum signals corresponding to PEN^{7+} and PEN^{8+} species of Aβ1-40 oligomers. Drift time distribution is shown in left panels and the corresponding isotopic envelope is shown in right panels. Whereas for PEN^{7+} only one form, assigned to compact species is present in a drift time distribution spectrum, at higher charge of +8 a more extended form is prevalent (peak denoted “extended”) and the compact form is less populated.

Fig. S3. Fragment of the IMS spectrum of Aβ1-40 (shown in full in Fig. 1), centered at *m/z* 1733 and drift time of 10 ms. It shows two isotopic envelopes of different drift times (one at 8.1 ms and the other at 10.6 ms) but of the same isotopic envelope characteristic to dimeric species charged +5. This indicates the presence

of two coexisting dimeric forms – one of more compact structure and the second more extended.

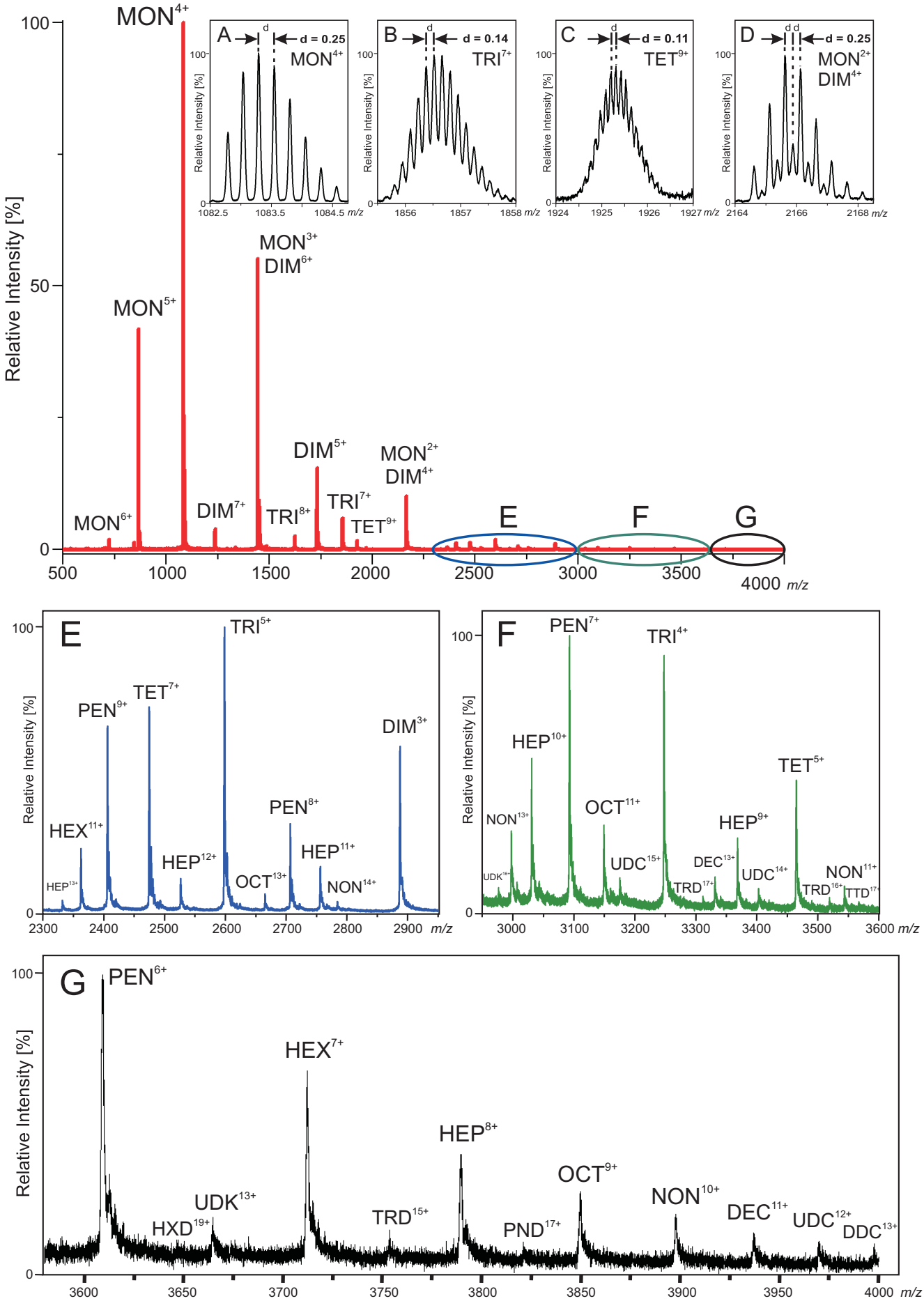
Fig. S4. IMS spectrum of Aβ1-40 after dilution by 10x **(A)** and 100x **(B)** to the final concentration of 20 μM and 2 μM, respectively. Oligomeric signals are retained in the spectra after dilution, at 20 μM nonameric species and at 2 μM pentameric species are detectable. Higher order oligomers may not be visible due to insufficient spectrometer sensitivity.

Fig. S5. Experimental values of drift time of the strongest signals corresponding to smallest oligomers (dimers to hexamers) bearing charges from n+1 to n+3 (n denotes the number of monomeric units in oligomer), presented as a function of inversed ion charge. The lines show the result of a two parameter fit of equation (1) to data points collected for each oligomer. The value of exponent *X* from Equation (1) obtained from each fit is shown. All values of *X* are very similar indicating a good calibration of its value in the presented experimental set.

Supplementary Table 1. Calculated and measured mass-to-charge (*m/z*) values of the monomer and different oligomeric forms of the Aβ1-40 peptide identified in the ESI MS spectrum. Whenever a monoisotopic signal could be identified, monoisotopic masses are compared (denoted mon.); otherwise, average masses are compared (denoted av.). In column four the deviation is given (in parts per million) for the calculated vs. observed *m/z* values. In column five the value of charge/monomer for each form is presented.

Supplementary Table 1

Name	Calculated *m/z*	Experimental *m/z*	Δ (ppm)	Charge / monomer
Monomer^{7+}	619.17 (mon.)	619.17	0.00	7.00
Monomer^{6+}	722.20 (mon.)	722.20	0.00	6.00
Monomer^{5+}	866.44 (mon.)	866.44	0.00	5.00
Monomer^{4+}	1082.79 (mon.)	1082.80	9.24	4.00
Monomer^{3+}	1443.39 (mon.)	1443.41	13.86	3.00
Monomer^{2+}	2164.58 (mon.)	2164.62	18.48	2.00
Dimer^{7+}	1237.34 (mon.)	1237.35	8.08	3.50
Dimer^{5+}	1731.87 (mon.)	1731.90	17.32	2.50
Dimer^{3+}	2887.58 (av.)	2887.56	-6.93	1.50
Trimer^{10+}	1299.96 (av.)	1299.93	-23.08	3.33
Trimer^{8+}	1624.70 (av.)	1624.66	-24.62	2.67
Trimer^{7+}	1856.66 (av.)	1856.61	-26.93	2.33
Trimer^{5+}	2598.92 (av.)	2598.89	-11.54	1.67
Trimer^{4+}	3248.40 (av.)	3248.39	-3.08	1.33
Tetramer^{9+}	1925.39 (av.)	1925.38	-5.19	2.25
Tetramer^{7+}	2475.21 (av.)	2475.19	-8.08	1.75
Tetramer^{5+}	3464.89 (av.)	3464.89	0.00	1.25
Pentamer^{11+}	1969.12 (av.)	1969.12	0.00	2.20
Pentamer^{9+}	2406.48 (av.)	2406.48	0.00	1.80
Pentamer^{8+}	2707.17 (av.)	2707.16	-3.69	1.60
Pentamer^{7+}	3093.76 (av.)	3093.76	0.00	1.40
Pentamer^{6+}	3609.22 (av.)	3609.22	0.00	1.20
Hexamer^{13+}	1999.40 (av.)	1999.46	30.01	2.17
Hexamer^{11+}	2362.75 (av.)	2362.74	-4.23	1.83
Hexamer^{7+}	3712.31 (av.)	3712.32	2.69	1.17
Heptamer^{13+}	2332.47 (av.)	2332.48	4.29	1.86
Heptamer^{12+}	2526.76 (av.)	2526.76	0.00	1.71
Heptamer^{11+}	2756.37 (av.)	2756.39	7.26	1.57
Heptamer^{10+}	3031.91 (av.)	3031.90	-3.30	1.43
Heptamer^{9+}	3368.67 (av.)	3368.69	5.94	1.29
Heptamer^{8+}	3789.63(av.)	3789.68	13.19	1.14
Octamer^{13+}	2665.54 (av.)	2665.54	0.00	1.63
Octamer^{11+}	3149.99 (av.)	3150.02	9.52	1.38
Octamer^{9+}	3849.77 (av.)	3849.81	10.39	1.13
Nonamer^{14+}	2784.49 (av.)	2784.53	14.37	1.56
Nonamer^{13+}	2998.60 (av.)	2998.63	10.00	1.44
Nonamer^{11+}	3543.62 (av.)	3543.65	8.47	1.22
Nonamer^{10+}	3897.88 (av.)	3897.96	20.52	1.11
Decamer^{13+}	3331.67 (av.)	3331.73	18.01	1.30
Decamer^{11+}	3937.24 (av.)	3937.28	10.16	1.10
Undecamer^{16+}	2977.78 (av.)	2977.88	33.58	1.45
Undecamer^{15+}	3176.24 (av.)	3176.35	34.63	1.36
Undecamer^{14+}	3403.04 (av.)	3403.11	20.57	1.27
Undecamer^{13+}	3664.73 (av.)	3664.82	24.56	1.18
Undecamer^{12+}	3970.04 (av.)	3970.18	35.26	1.09
Dodecamer^{17+}	3057.38 (av.)	3057.45	22.89	1.42
Dodecamer^{13+}	3997.78 (av.)	3997.91	32.52	1.08
Tridecamer^{17+}	3312.07 (av.)	3312.18	33.21	1.31
Tridecamer^{16+}	3519.02 (av.)	3519.15	36.94	1.23
Tridecamer^{15+}	3753.55 (av.)	3753.71	42.62	1.15
Tetradecamer^{17+}	3566.77 (av.)	3566.94	47.66	1.21
Pentadecamer^{17+}	3821.47 (av.)	3821.66	49.72	1.13
Hexadecamer^{19+}	3647.20 (av.)	3647.28	21.93	1.19


A
B
C
D
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d = 0.14
d = 0.11
d = 0.25
MON4+
TRI7+
TET9+
MON2+
DIM4+
Relative Intensity [%]
m/z
MON4+
MON5+
MON3+
DIM6+
DIM5+
MON2+
DIM4+
DIM7+
TRI8+
TRI7+
TET9+
MON6+
E
F
G
Relative Intensity [%]
m/z
TRI5+
TET7+
PEN9+
DIM3+
HEX11+
PEN8+
HEP12+
HEP11+
OCT13+
NON14+
HEP13+
PEN7+
TRI4+
HEP10+
TET5+
NON13+
OCT11+
HEP9+
UDC15+
DEC13+
UDC14+
NON11+
TRD17+
TRD16+
TTD17+
UDK15+
G
PEN6+
HEX7+
HEP8+
OCT9+
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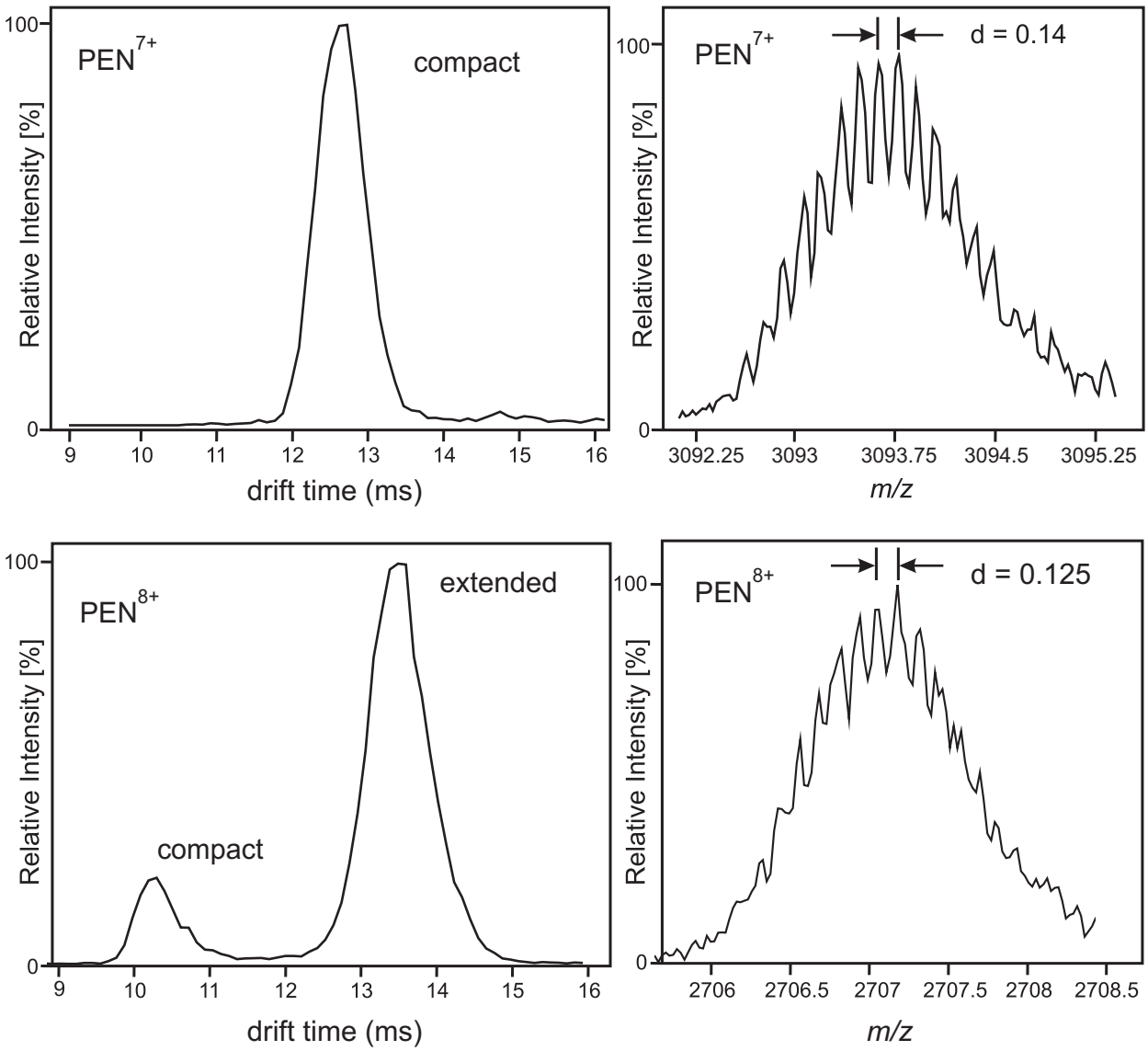
UDK13+
HXD19+
TRD15+
PND17+

Fig. S1.


100
PEN7+
compact
Relative Intensity [%]
0
9
10
11
12
13
14
15
16
drift time (ms)
100
PEN7+
d = 0.14
Relative Intensity [%]
0
3092.25
3093
3093.75
3094.5
3095.25
m/z
100
PEN8+
extended
compact
Relative Intensity [%]
0
9
10
11
12
13
14
15
16
drift time (ms)
100
PEN8+
d = 0.125
Relative Intensity [%]
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2706
2706.5
2707
2707.5
2708
2708.5
m/z

Fig. S2.

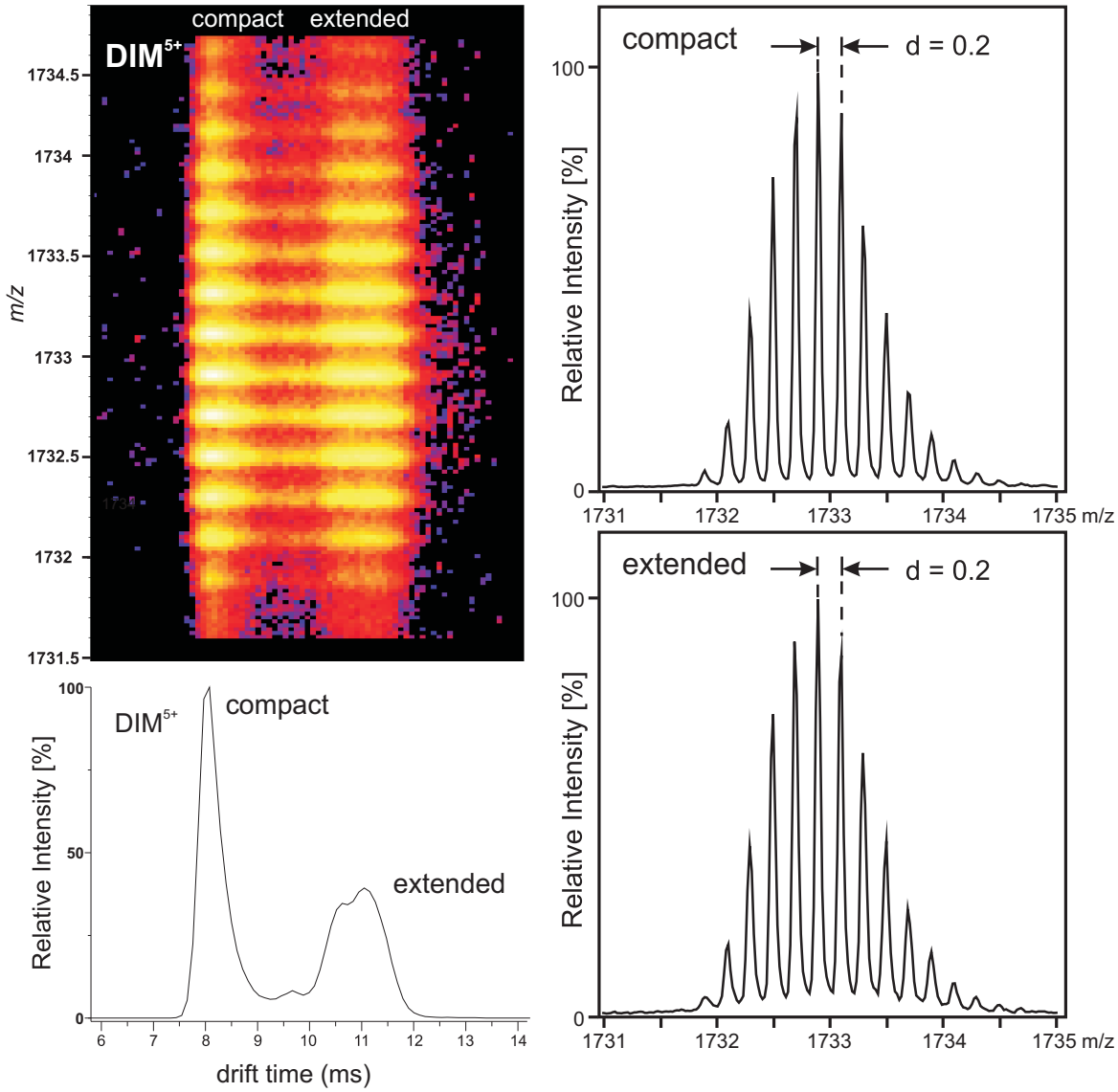
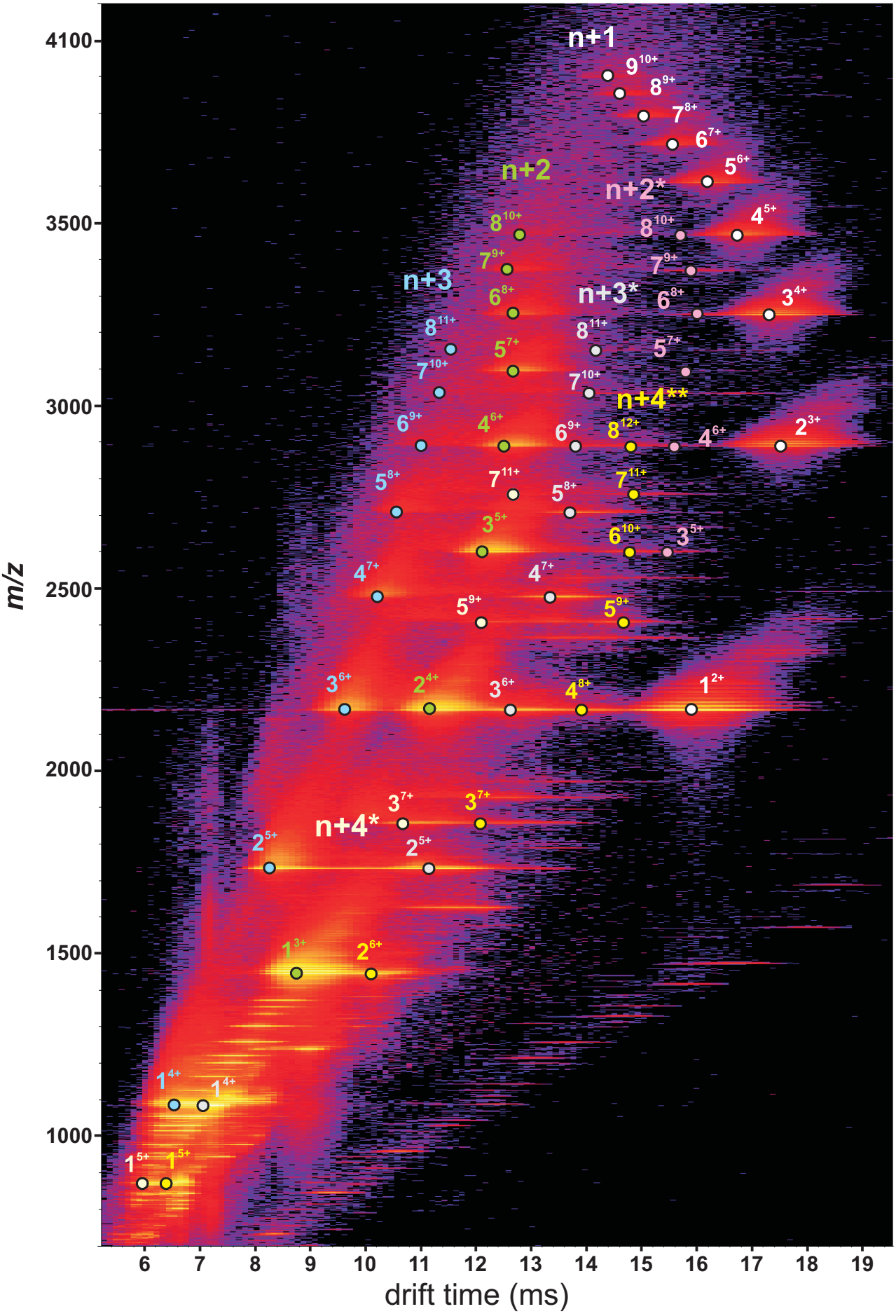

compact
extended
DIM5+
m/z
1734.5
1734
1733.5
1733
1732.5
1732
1731.5
DIM5+
compact
extended
Relative Intensity [%]
100
50
0
6
7
8
9
10
11
12
13
14
drift time (ms)
compact
d = 0.2
Relative Intensity [%]
100
0
1731
1732
1733
1734
1735 m/z
extended
d = 0.2
Relative Intensity [%]
100
0
1731
1732
1733
1734
1735 m/z

Fig. S3.


m/z
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3500
3000
2500
2000
1500
1000
6
7
8
9
10
11
12
13
14
15
16
17
18
19
drift time (ms)
n+1
n+2
n+2*
n+3
n+3*
n+4**
n+4*

Fig. S4 A.

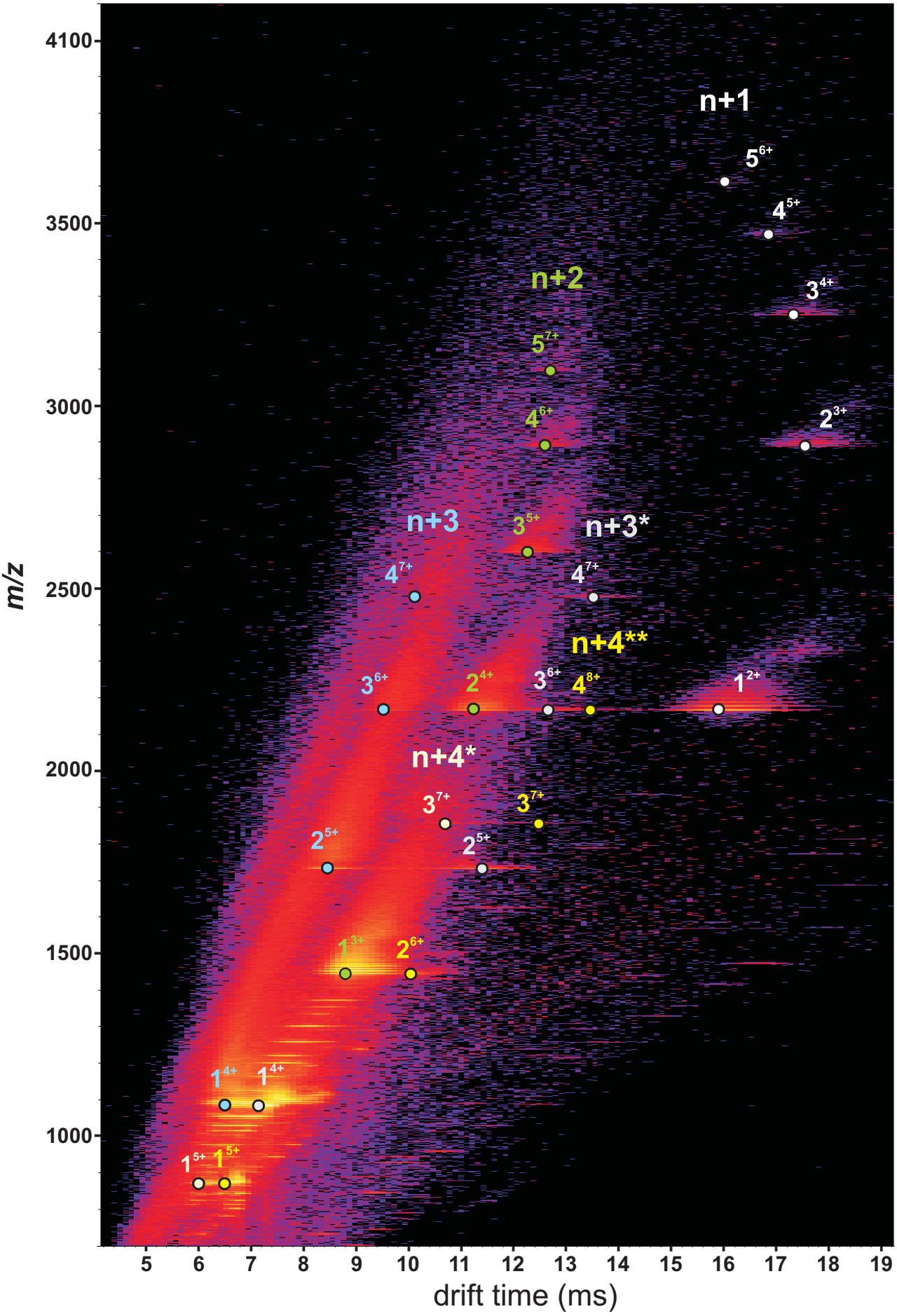
m/z
4100
3500
3000
2500
2000
1500
1000
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
drift time (ms)
n+1
5^6+
4^5+
n+2
3^4+
5^7+
4^6+
2^3+
n+3
3^5+
n+3*
4^7+
4^7+
n+4**
3^6+
2^4+
3^6+
4^8+
1^2+
n+4*
3^7+
3^7+
2^5+
2^5+
1^3+
2^6+
1^4+
1^4+
1^5+
1^5+

Fig. S4 B.

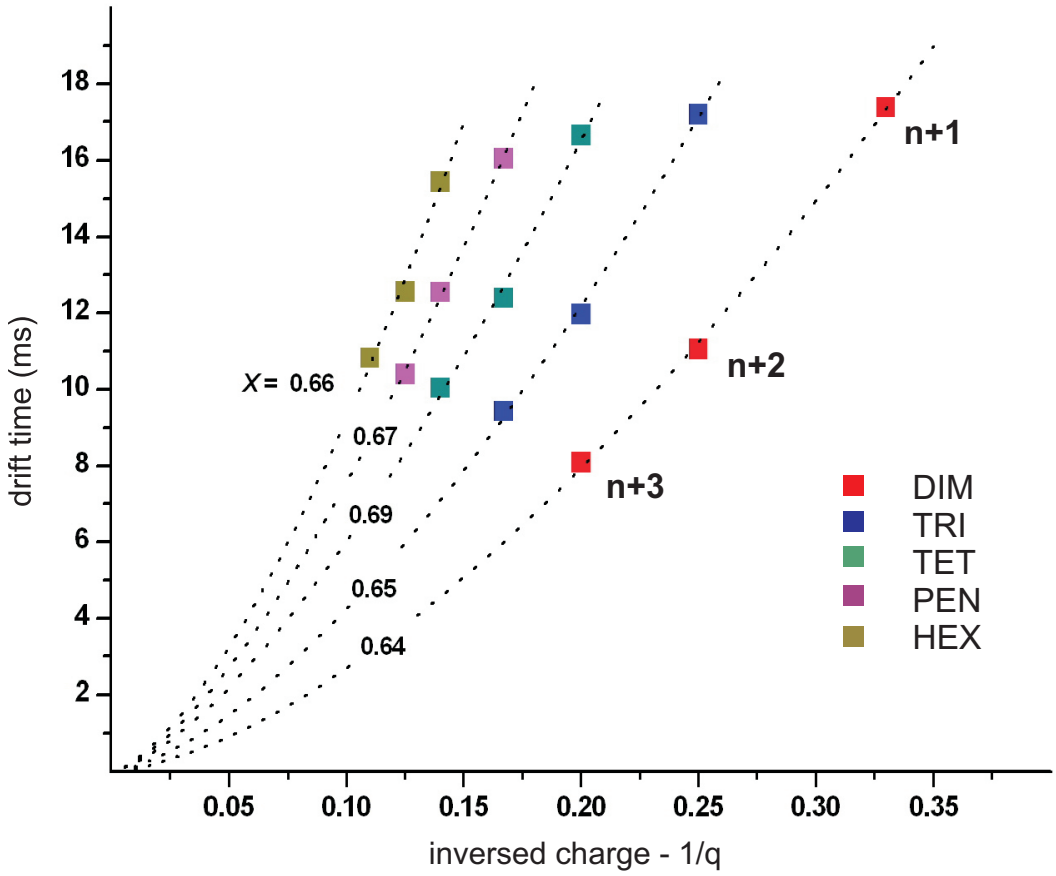
drift time (ms)
inversed charge - 1/q
X = 0.66
0.67
0.69
0.65
0.64
n+1
n+2
n+3
DIM
TRI
TET
PEN
HEX

Fig. S5.