Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary information

Electron capture dissociation and drift tube ion mobility-mass spectrometry coupled with site directed mutations provide insights into the conformational diversity of a metamorphic protein

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Experimental Details

DT IM-MS

All analyses were performed using 100 µM protein prepared in 20 mM ammonium acetate, pH 6.8. Three different day repeats were performed and the CCS obtained averaged. Similar tuning conditions were employed for each sample; capillary voltage 1.6 kV, cone voltage 56 - 60V, source temperature 80 ºC and pusher period 90-130 µs. The temperature and pressure of helium in the drift cell were approximately 28 ºC and 4.0 Torr respectively. Measurements were made at eight different drift voltages from 60 to 15 V.

Theoretical CCS

Before deriving the CCS values, all three NMR structures (PDB identifiers 1J9O, 2JP1 and 2HDM for Ltn10, Ltn40 and monomeric CC3 mutant, respectively) plus Ltn10 1-72 (PDB 1J9O with ID tail removed), were minimised *in vacuo* using the sander engine of either Amber11 or 12 (Ltn1-72) [1] and implementing Amber99SB-ILDN [2]. In the minimisation process first 5000 steps of steepest descend algorithm and then as many steps of conjugated gradients as sufficient to obtain a gradient convergence criterion (drms) equal to 0.005 kcal/mol•Å were excuted. For non-bonded interactions an infinite radial cut-off (999 Å) was used.

S1. WT 1-72

DT 1M-MS- experimentally determined CCS.

Species	**Experimental CCS / Å²**
$[M_{1\text{-}72}+4H]^{4+}$	856 ± 4
$[M_{1\text{-}72}+5H]^{5+}$	916 ± 8
$[M_{1\text{-}72}+6H]^{6+}$	950 ± 9
$[M_{1\text{-}72}+7H]^{7+}$ *	1037
$[M_{1\text{-}72}+7H]^{7+}$	1229 ± 12
$[M_{1\text{-}72}+8H]^{8+}$	1416 ± 8

Table S1: The average experimental CCS for all observed species of monomeric WT 1-72, errors are calculated from three different day repeats. * represents species observed in all repeats but only resolvable in one.

ECD Fragmentation- MS spectra obtained on FT ICR MS instrumentation

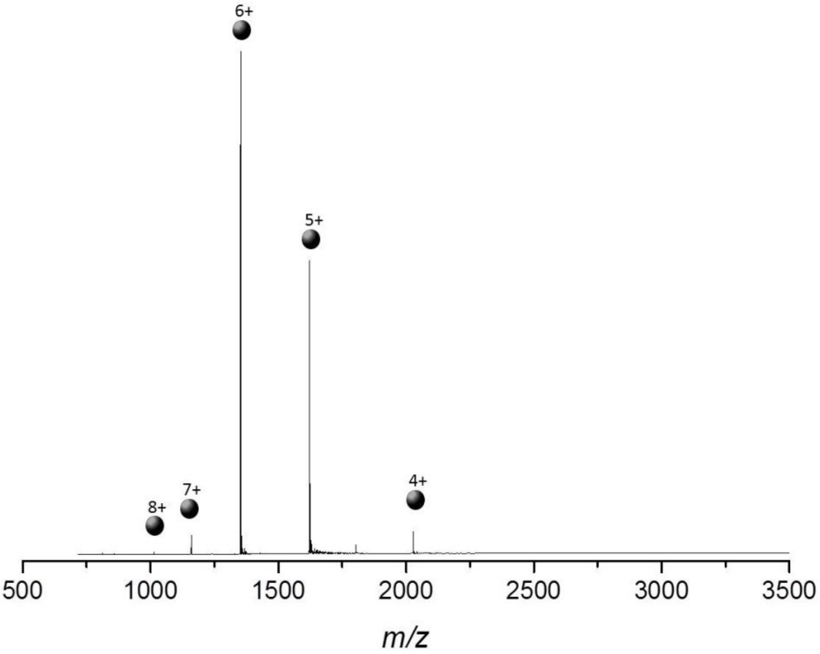

Figure S1: Mass spectra obtained on a 12T Apex Qe FTICR for WT 1-72 Ltn. Acquired using nano-electrospray ionisation of 5 μM Ltn in 100 mM ammonium acetate

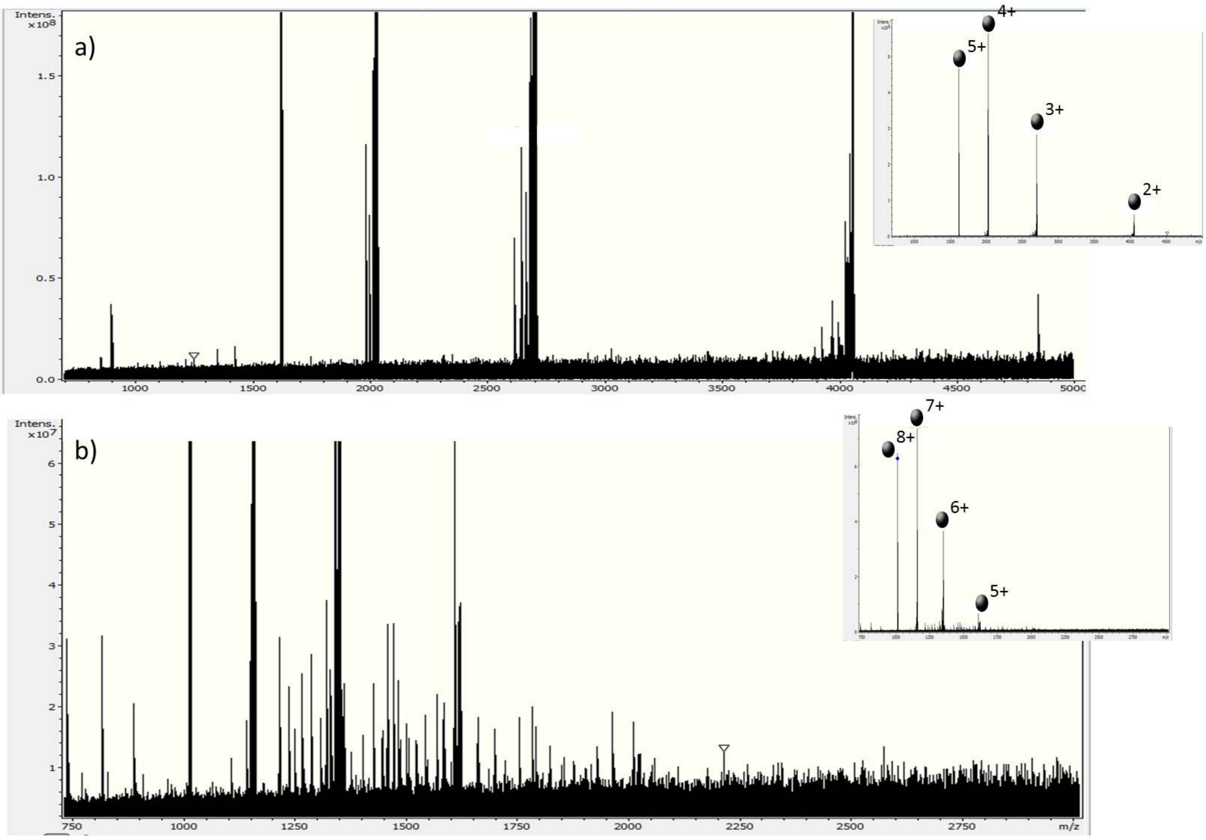

Figure S2: Mass spectra obtained on a 12T Apex Qe FT-ICR for WT1-72 Ltn after ECD fragmentation zoomed in to show low level fragmentation for A) $[M_{WT1\text{-}72}+5H]^{5+}$ and B) $[M_{WT1\text{-}72}+8H]^{8+}$. Inserts in both cases show full range spectra, high intensity species are due to charge reduction with no fragmentation

S2. CC3 Ltn

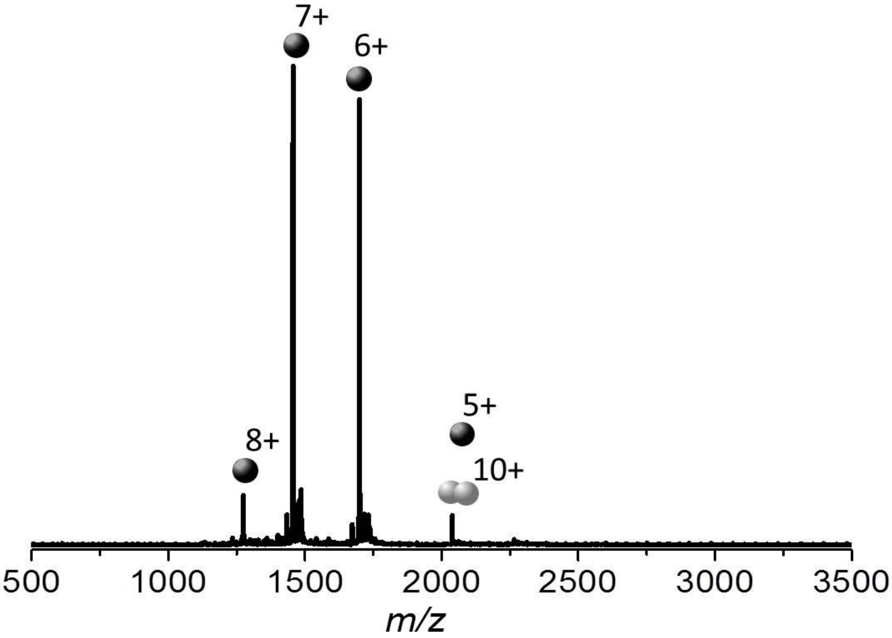

Figure S3: Mass spectra obtained on the DT IM-MS instrument for 100 μM WT 1-72 in 20 mM ammonium acetate

DT 1M-MS- experimentally determined CCS.

Species	**Experimental CCS / Å²**
$[M_{CC3}+5H]^{5+}$	1012 ± 2
$[M_{CC3}+6H]^{6+}$	1014 ± 3
$[M_{CC3}+7H]^{7+}$	1163 ± 4
$[M_{CC3}+8H]^{8+}$	1243 ± 5

Table S2: The average experimental CCS for all observed species of monomeric CC3, errors are calculated from the standard deviation of three different day repeats.

ECD Fragmentation buffered CC3- MS spectra obtained on FT ICR MS instrumentation

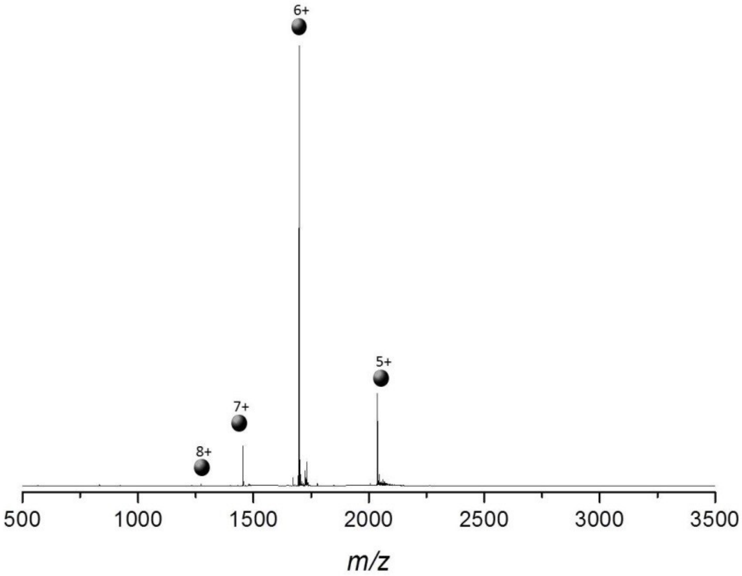

Figure S4: Mass spectra obtained on a 12T Apex Qe FTICR for CC3. Acquired using nano-electrospray ionisation of 30 μM CC3 in 100 mM ammonium acetate

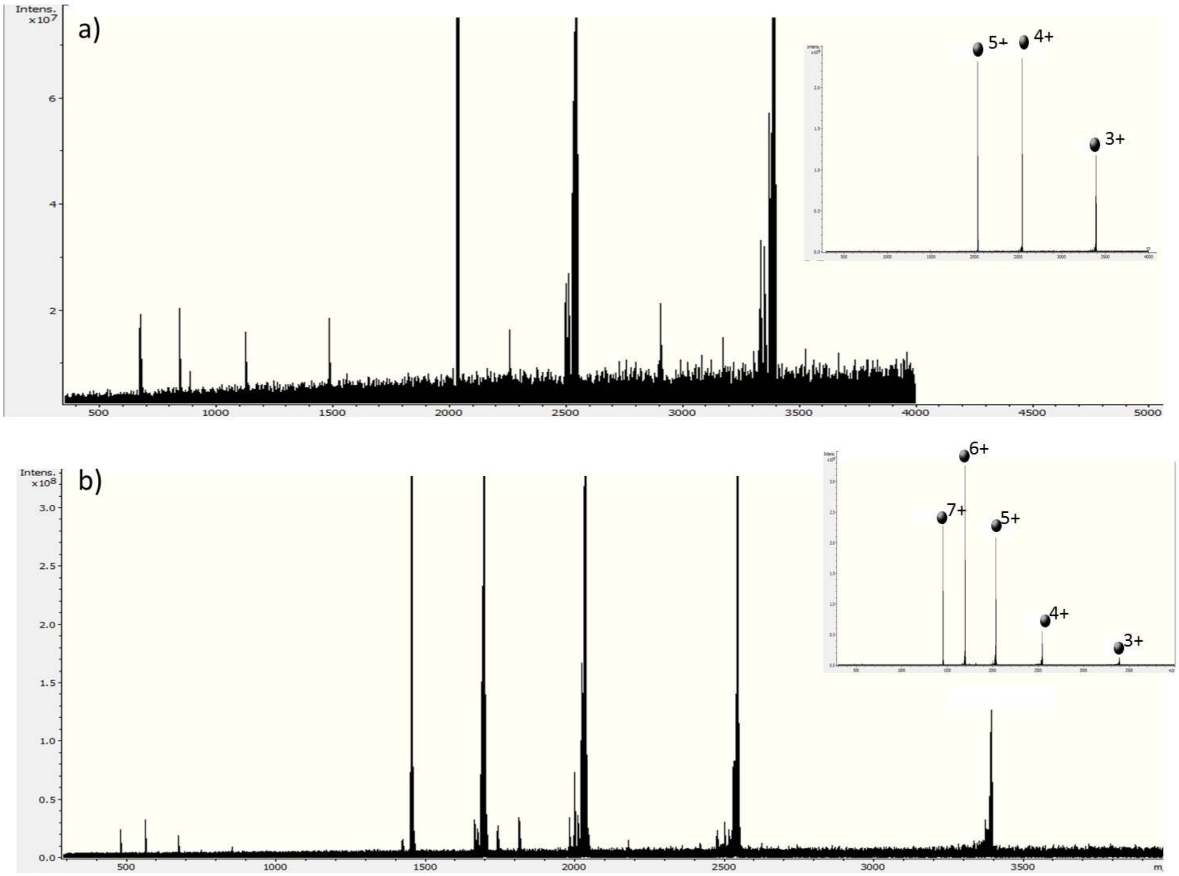

Figure S5: Mass spectra obtained on a 12T Apex Qe FT-ICR for CC3 Ltn after ECD fragmentation zoomed in to show low level fragmentation for A) $[M_{CC3}+5H]^{5+}$ and B) $[M_{CC3}+7H]^{7+}$. Inserts in both cases show full range spectra, high intensity species are due to charge reduction with no fragmentation

Super charged CC3

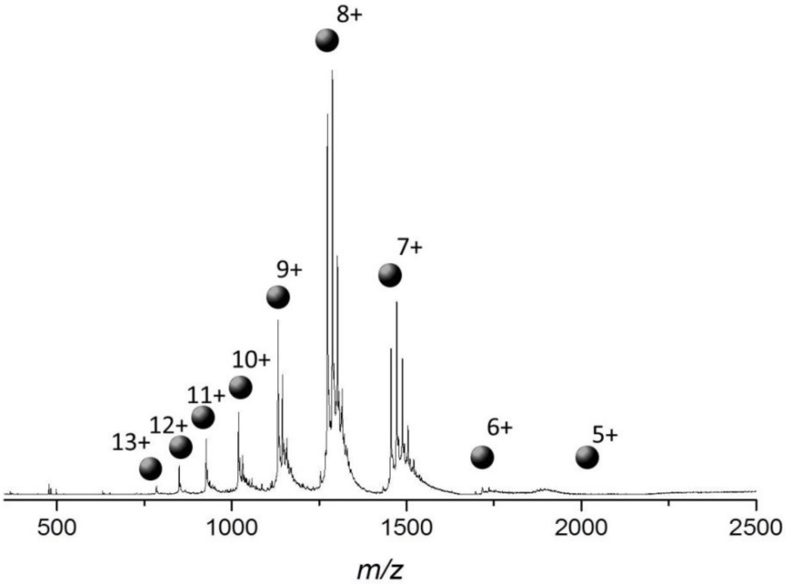

Figure S6: Mass spectra obtained on the DT IM-MS instrument for 100 µM WT 1-72 in 20 mM ammonium acetate + 1 % *m*-NBA

DT 1M-MS- experimentally determined CCS of supercharged CC3.

Species	**Experimental CCS / Å²**
$[M_{CC3}+5H]^{5+}$	1023 ± 8
$[M_{CC3}+6H]^{6+}$	1046 ± 15
$[M_{CC3}+7H]^{7+}$	1252 ± 30
$[M_{CC3}+8H]^{8+}$	1439 ± 25
$[M_{CC3}+9H]^{9+}$	1498 ± 10
$[M_{CC3}+10H]^{10+}$	1622 ± 17
$[M_{CC3} + 11H]^{11+}$	1697 ± 1
$[M_{CC3}+12H]^{12+}$	1757 ± 28
$[M_{CC3}+13H]^{13+}$	1824 ± 6

Table S3: The average experimental CCS for all observed species of monomeric CC3 obtained under supercharged conditions with 1% *m*-NBA, errors are calculated from the standard deviations of three different day repeats. * represents species observed in all repeats but only resolvable in one.

ECD Fragmentation- MS spectra obtained on FT ICR MS instrumentation

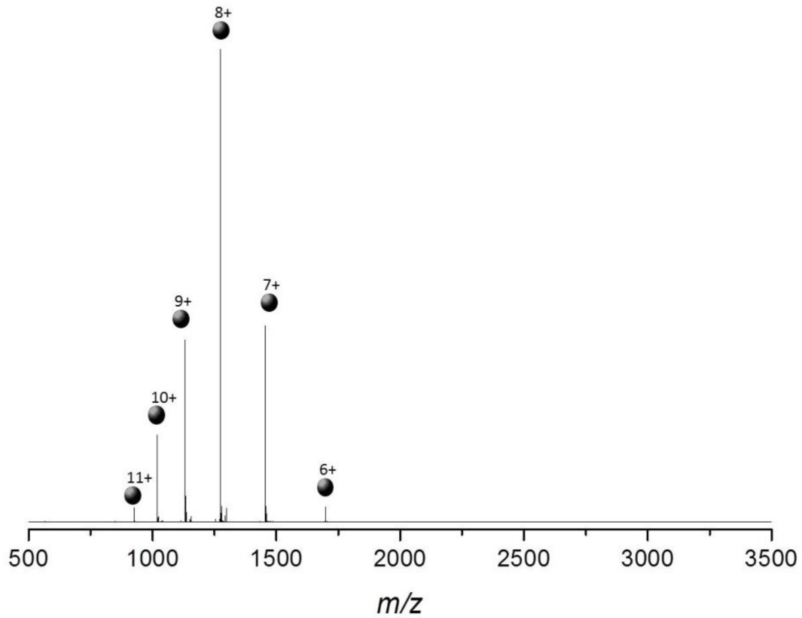

Figure S7: Mass spectra obtained on a 12T Apex Qe FTICR for CC3. Acquired using nano-electrospray ionisation of 30 μM CC3 in 100 mM ammonium acetate + 1% *m*-NBA.

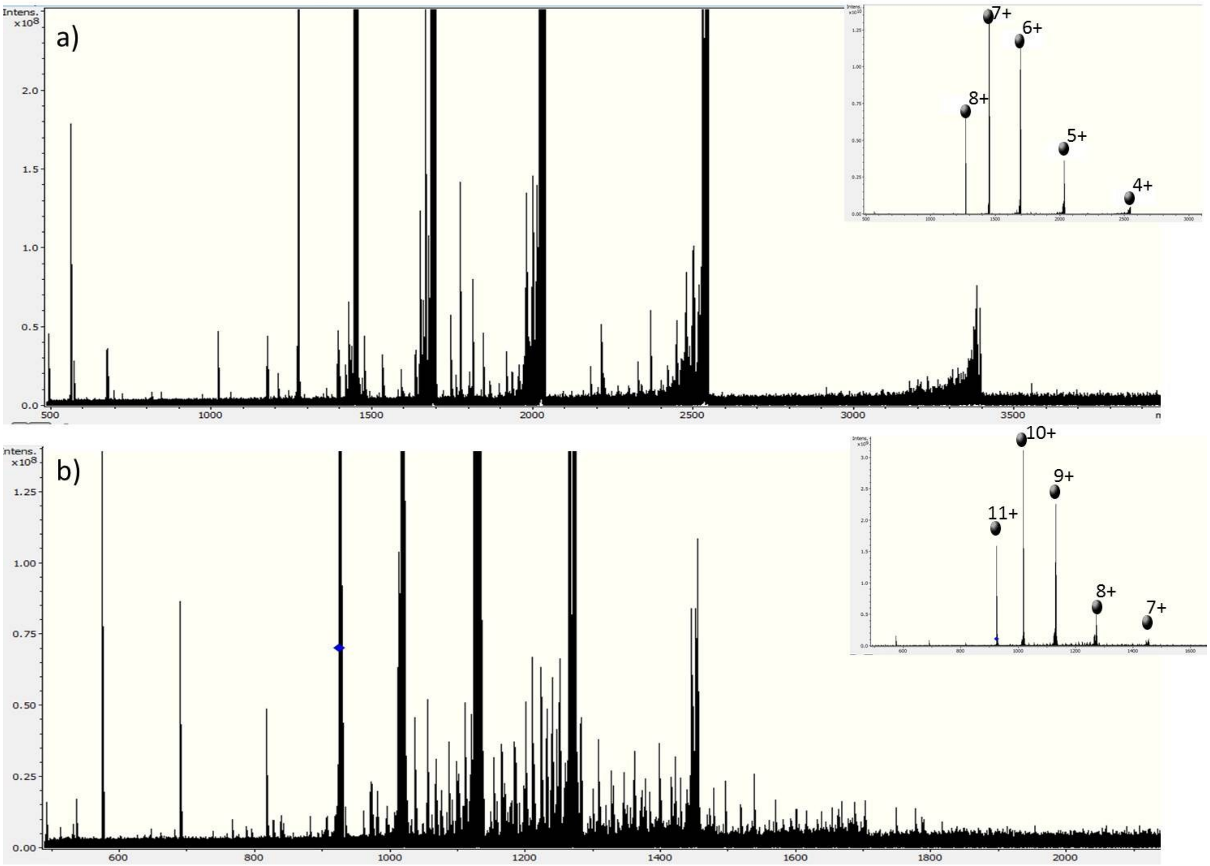

Figure S8: Mass spectra obtained on a 12T Apex Qe FT-ICR for supercharged CC3 Ltn after ECD fragmentation zoomed in to show low level fragmentation for A) $[M_{CC3}+8H]^{5+}$ and B) $[M_{CC3}+11H]^{11+}$. Inserts in both cases show full range spectra, with high intensity species are due to charge reduction with no fragmentation

S3. W55D Ltn

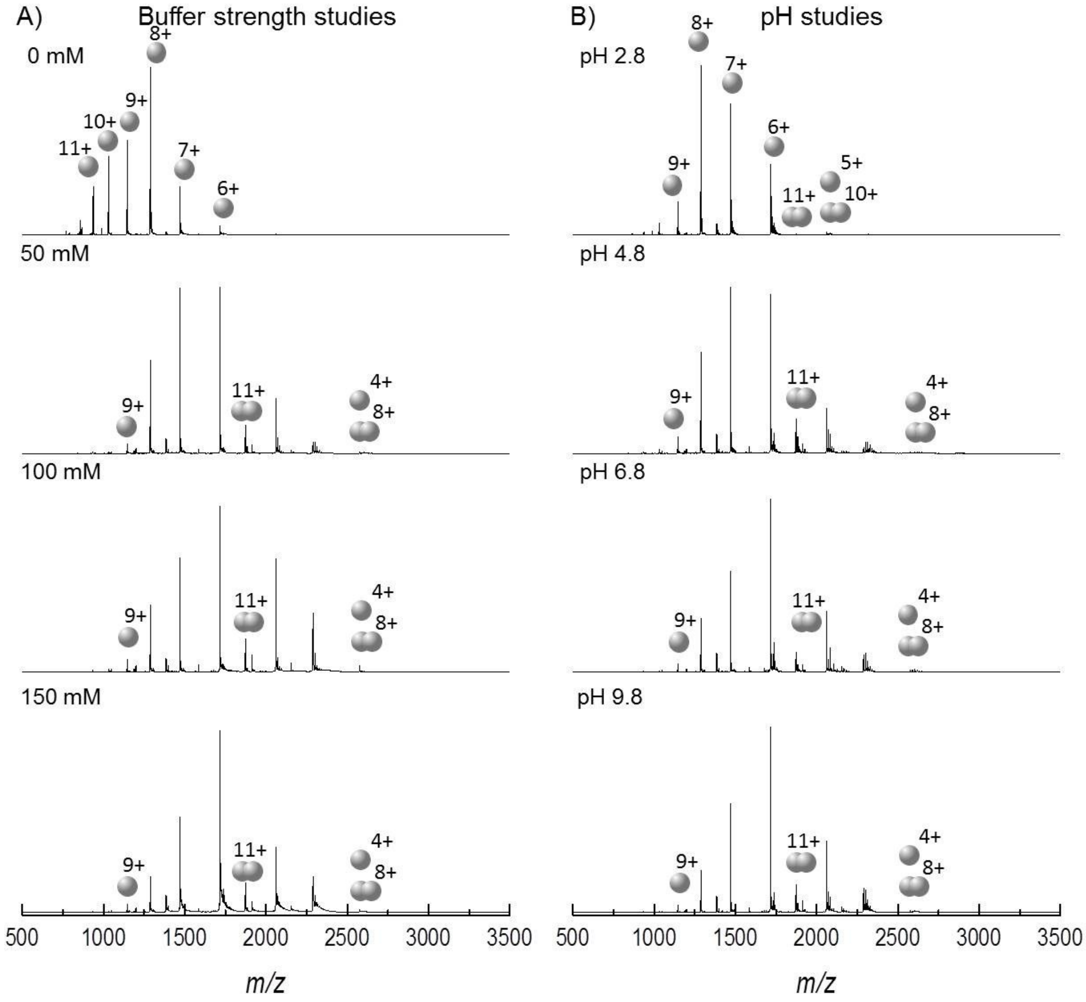

Figure S9: Mass spectra obtained on a waters Q-TOF ultima for 50 μM W55D A) effect of increasing buffer strength B) effect of changing pH in 20 mM ammonium acetate, through addition of formic acid or ammonium.

Species	**Experimental CCS / Å^2**
$[M_{W55D} + 4H]^{4+}$	778 ± 8
$[M_{W55D} + 5H]^{5+}$	839 ± 7
$[M_{W55D} + 6H]^{6+}$	1057 ± 12
$[M_{W55D} + 7H]^{7+}$	1150 ± 26
$[M_{W55D} + 8H]^{8+}$	1401 ± 6
$[M_{W55D} + 9H]^{9+}$	1407 ± 9
$[D_{W55D} + 8H]^{8+}$	1509 ± 6
$[D_{W55D} + 9H]^{9+}$	1641 ± 8
$[D_{W55D} + 9H]^{9+}$ *	1674
$[D_{W55D} + 10H]^{10+}$	1704 ± 6
$[D_{W55D} + 11H]^{11+}$	1829 ± 19
$[D_{W55D} + 11H]^{11+}$ *	2020
$[D_{W55D} + 12H]^{12+}$	2062 ± 8
$[D_{W55D} + 13H]^{13+}$	2103 ± 67
$[D_{W55D} + 13H]^{13+}$	2237 ± 97
$[D_{W55D} + 14H]^{14+}$	2246 ± 5
$[D_{W55D} + 15H]^{15+}$ *	2469
$[D_{W55D} + 16H]^{16+}$	2519 ± 12

Table S4: The average experimental CCS for all observed species of monomeric and dimeric W55D, errors are calculated from the standard deviations of three different day repeats. * represents species observed in all repeats but only resolvable in one

ECD Fragmentation of W55D- MS spectra obtained on FT ICR MS instrumentation

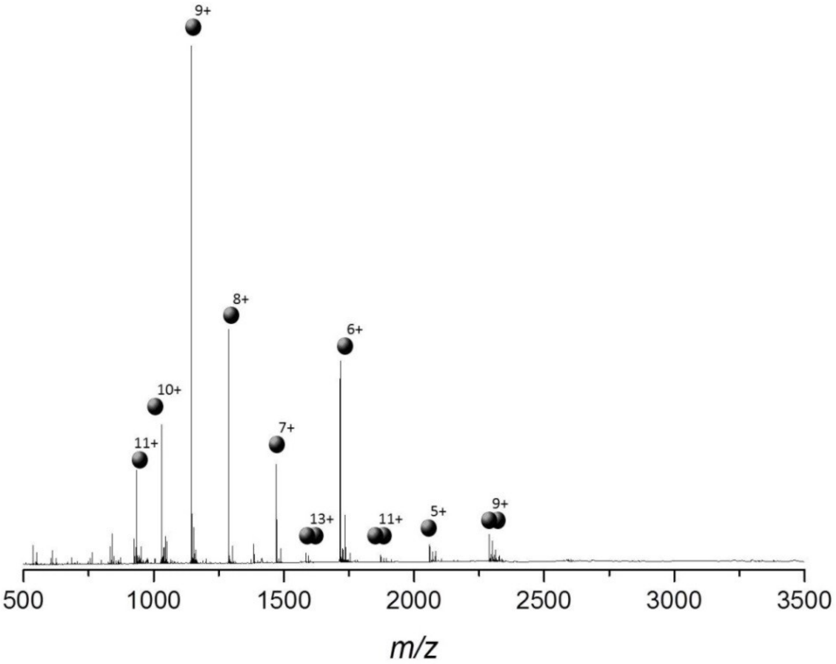

Figure S10: Mass spectra obtained on a 12T Apex Qe FTICR for W55D. Acquired using nano-electrospray ionisation of 30 µM W55D in 100 mM ammonium acetate.

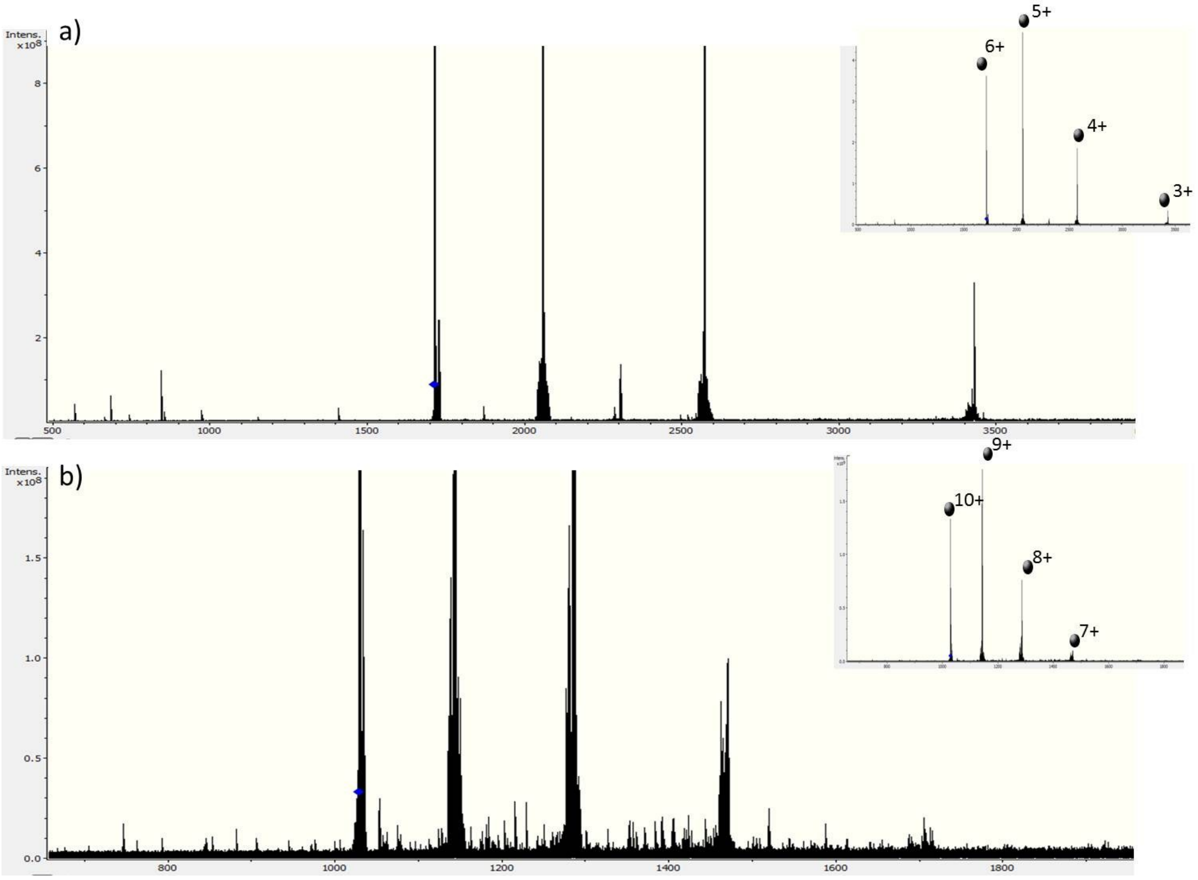

Figure S11: Mass spectra obtained on a 12T Apex Qe FT-ICR for monomeric W55D Ltn after ECD fragmentation of zoomed in to show low level fragmentation for A) $[M_{W55D}+6H]^{6+}$ and B) $[M_{W55D}+10H]^{10+}$. Inserts in both cases show full range spectra, high intensity species are due to charge reduction with no fragmentation

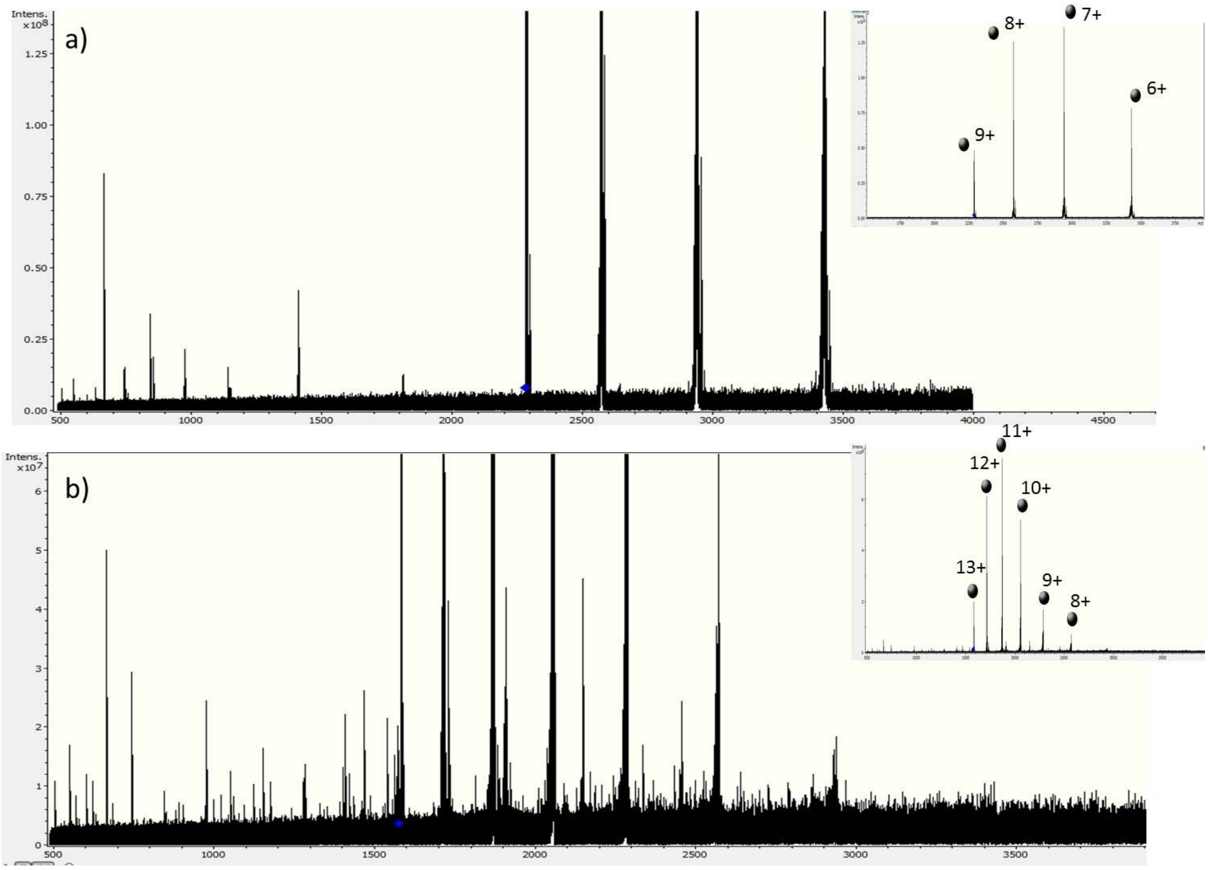

Figure S12: Mass spectra obtained on a 12T Apex Qe FT-ICR for dimeric W55D Ltn after ECD fragmentation zoomed in to show low level fragmentation for A) $[D_{W55D}+9H]^{9+}$ and B) $[D_{W55D}+13H]^{13+}$. Inserts in both cases show full range spectra, high intensity species are due to charge reduction with no fragmentation

References

(1) D.A. Case, T. A. D., T.E. Cheatham, III, C.L. Simmerling, J. Wang, R.E. Duke, R.; Luo, R. C. W., W. Zhang, K.M. Merz, B. Roberts, B. Wang, S. Hayik, A. Roitberg,; G. Seabra, I. K., K.F. Wong, F. Paesani, J. Vanicek, J. Liu, X. Wu, S.R. Brozell,; T. Steinbrecher, H. G., Q. Cai, X. Ye, J. Wang, M.-J. Hsieh, G. Cui, D.R. Roe, D.H.; Mathews, M. G. S., C. Sagui, V. Babin, T. Luchko, S. Gusarov, A. Kovalenko, and; Kollman, P. A. *AMBER 11*; University of California: San Francisco, 2010

(2) Lindorff-Larsen, K.; Piana, S.; Palmo, K.; Maragakis, P.; Klepeis, J. L.; Dror, R. O.; Shaw, D. E. *Proteins: Structure, Function, and Bioinformatics* **2010**, *78*, 1950.