

## Supplementary Information for:

### Conformational Dynamics of $\alpha$ -Synuclein: Insights from Mass Spectrometry

#### Authors

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#### SI Methods

##### TEM

All Transmission Electron Microscopy (TEM) experiments were conducted on a Philips CM120 (Philips, Eindhoven, The Netherlands).

Prior to analysis TEM grids were prepared as follows, 4 $\mu$ L of sample was spotted onto 200m mesh formvar and carbon coated copper grids (TAAB, UK). Grids were incubated at room temperature for five minutes then dried by peripheral application of filter paper. Grids were rinsed with DI water then dried by peripheral application of filter paper. The samples were then stained by application of 4 $\mu$ L uranyl acetate (1% working solution) and incubated at room temperature for 35 seconds. Excess uranyl acetate was removed by peripheral application of filter paper and grids were allowed to dry for a minimum of 5 minutes prior to storage and 12 hours prior to analysis. All manipulation was conducted with high precision, anti-magnetic stainless steel fine tweezers (TAAB, UK).

## Supplementary information captions

### Figure S1

Spectra A and C from Figure 1 main text superimposed. Following pH modification the distribution of species intensity is altered. There is an obvious shift to the exhibition of lower charge states, indicated by the example monomer charge states labelled. The pH 3.5 spectrum also has a higher baseline between  $m/z$  2500 and 5000, indicative of aggregation.

### Figure S2

TEM images of  $\alpha$ -synuclein prior to (A) and following 96 under MS compatible *in vitro* aggregation conducive conditions (1mg/mL, 50mM ammonium acetate, 37°C and agitation at 200rpm). The scale bar represents 10nm. Note the absence of fibrils at Day 0 (A) and the presence of fibrils following 96 hours (Day 4, B).

### Figure S3

The effect of aggregation on observable  $\alpha$ -synuclein species demonstrated by nESI-MS. Chosen monomeric and dimeric species are highlighted.

### Figure S4

Comparison of nESI-XL-MS data of  $\alpha$ -synuclein. **A.**  $\alpha$ -synuclein following crosslinking reaction conducted at pH 4 **B.**  $\alpha$ -synuclein following crosslinking reaction conducted at pH 8. Note the reduction in the width of the charge state distribution and the lack of resolution of low order oligomeric species.

### Figure S5

Comparison of the position of monomeric and dimeric charge states selected for ECD- FT-ICR MS analysis within the charge state distribution of  $\alpha$ -synuclein. Dashed lines highlight charge states not analysed at pH 3.5.

### Figure S6

Plot of theoretical CCS values versus calculated radius of gyration obtained from MD simulations on solvated  $[\text{Insulin}+3\text{H}]^{3+}$  monomer. This training set is used to provide a correlation between  $R_g$  and CCS values.

### **Figure S7**

A. An annotated MS Spectrum of the  $\alpha$ -synuclein monomer,  $[\text{aSyn}+6\text{H}]^{6+}$  (pH 6.8) following ECD fragmentation. The lack of fragmentation is clearly visible and is likely the results of the low charge nature of the isolated species.

B. An annotated MS Spectrum of the  $\alpha$ -synuclein monomer,  $[\text{aSyn}+7\text{H}]^{7+}$  (pH 6.8) following ECD fragmentation. There is limited resolution of C and Z fragments, likely the result of the low charge nature of the isolated species.

### **Figure S8**

An annotated MS Spectrum of the  $\alpha$ -synuclein monomer,  $[\text{aSyn}+8\text{H}]^{8+}$  (pH 6.8) following ECD fragmentation. In line with accepted theory, the number of resolved fragments increases with charge state. In concurrence with other charge states and at both pH's analysed, the fragmentation is limited to the N terminus, fragments are resolved up to Y46 (C46/3+). The inset zoom regions highlight fragment ions resolved.

### **Figure S9**

An annotated MS Spectrum of the  $\alpha$ -synuclein monomer,  $[\text{aSyn}+9\text{H}]^{9+}$  (pH 3.5) following ECD fragmentation. In comparison with lower charged isolated species, greater numbers of fragments are resolved. The fragmentation is limited to the N terminus of the protein, fragments are resolved up to T75 (C75/6+). The inset zoom regions highlight fragment ions resolved.

### **Figure S10**

An annotated MS Spectrum of the  $\alpha$ -synuclein monomer,  $[\text{aSyn}+10\text{H}]^{10+}$  (pH 6.8) following ECD fragmentation. The most highly charged monomeric species fragmented, the number of fragments resolved is greater than other less charged species. The fragmentation is limited to the N terminus of the protein, fragments are resolved up to E83 (C83/5+), with the exception of the C139 fragment observed. This is likely to be the result of the greater coulombic repulsion suffered by higher charge state monomers leading to the disruption of the inferred structure. The inset zoom regions highlight fragment ions resolved.

### **Figure S11**

An annotated MS Spectrum of the  $\alpha$ -synuclein dimer,  $[(aSyn)_2+13H]^{13+}$  (pH 3.5) following ECD fragmentation. The limited fragmentation observed for the lowest charged dimer species analysed is highlighted. The inset zoom regions highlight fragment ions resolved.

### **Figure S12**

An annotated MS Spectrum of the  $\alpha$ -synuclein dimer,  $[(aSyn)_2+15H]^{15+}$  (pH 6.8) following ECD fragmentation. In line with accepted theory and in concurrence with monomeric  $\alpha$ -synuclein data, the number of resolved fragments increases with charge state. The inset zoom regions highlight fragment ions resolved.

## **Supplementary information figures**

### **Figure S1**

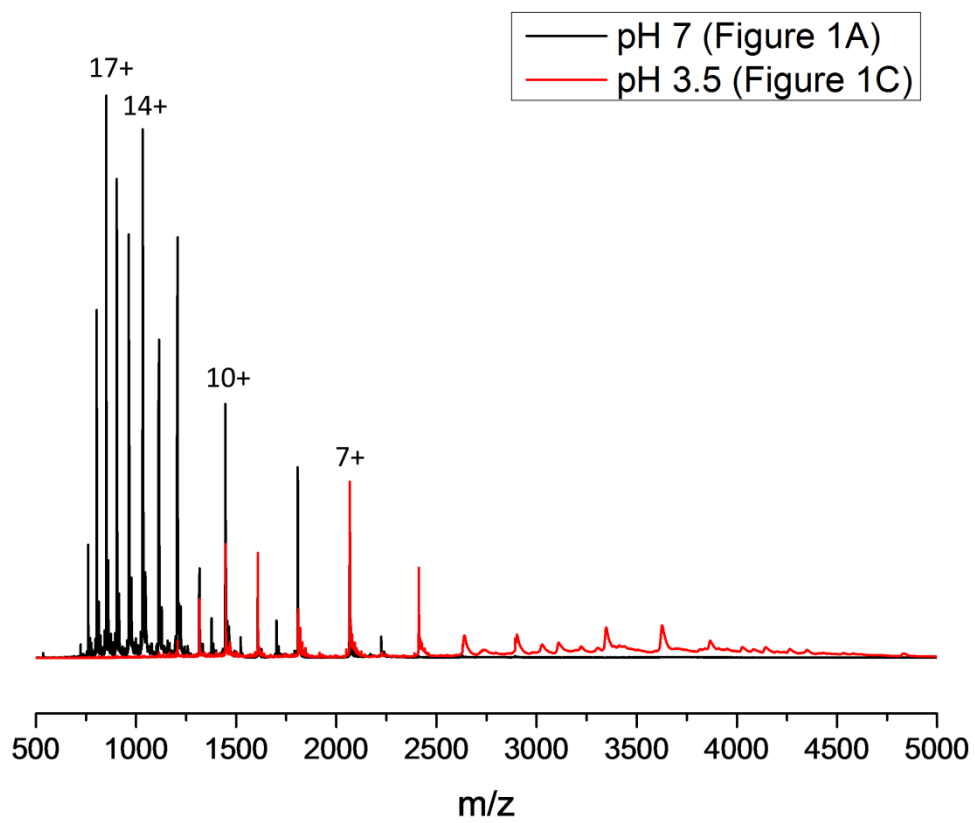
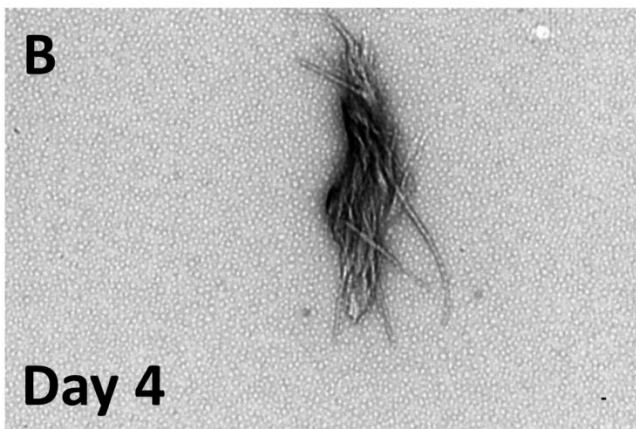
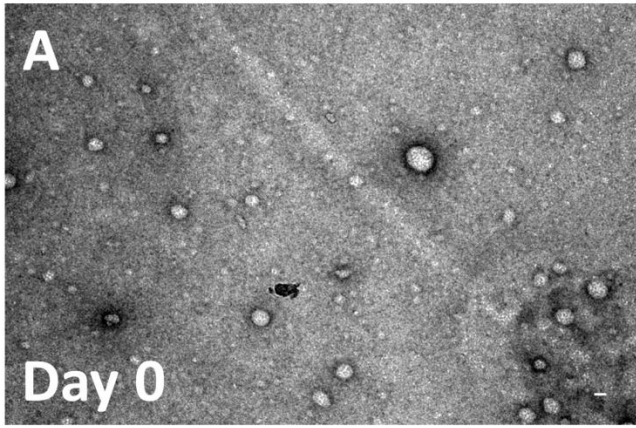


Figure S2



**Figure S3**

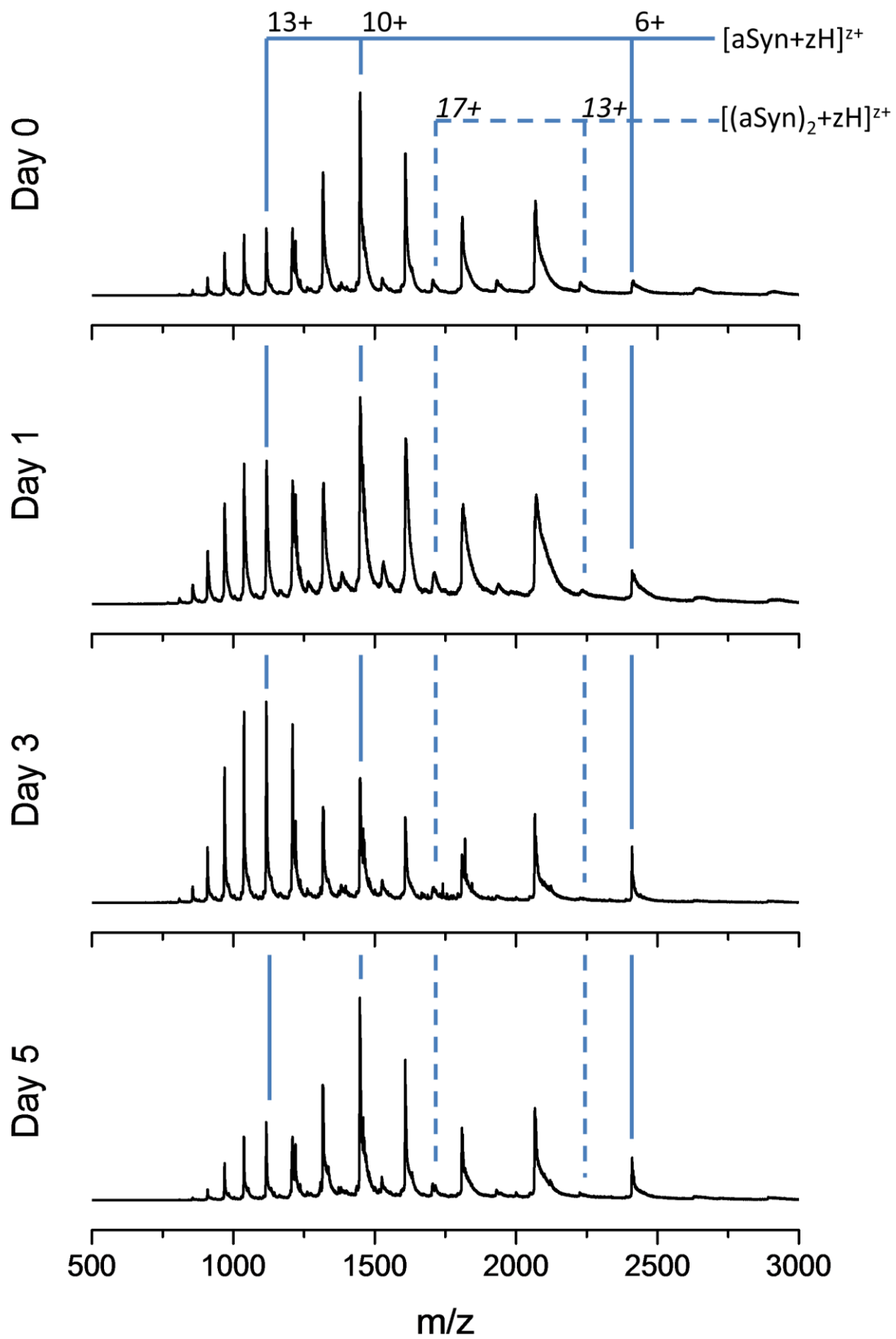
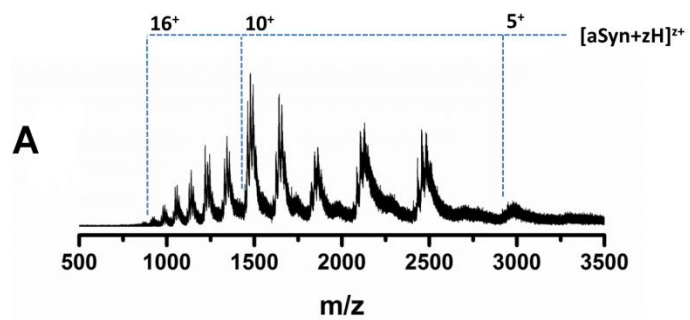
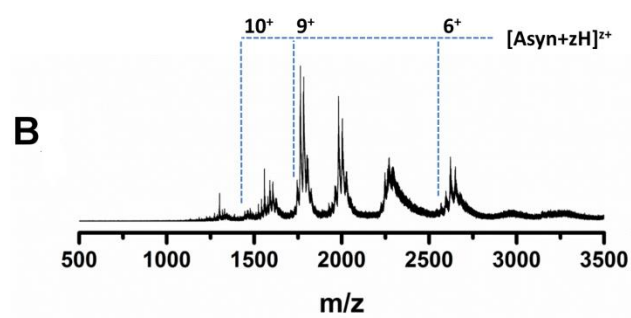


Figure S4



pH 4  
4 mod.



pH 8  
10 mod.

Figure S5



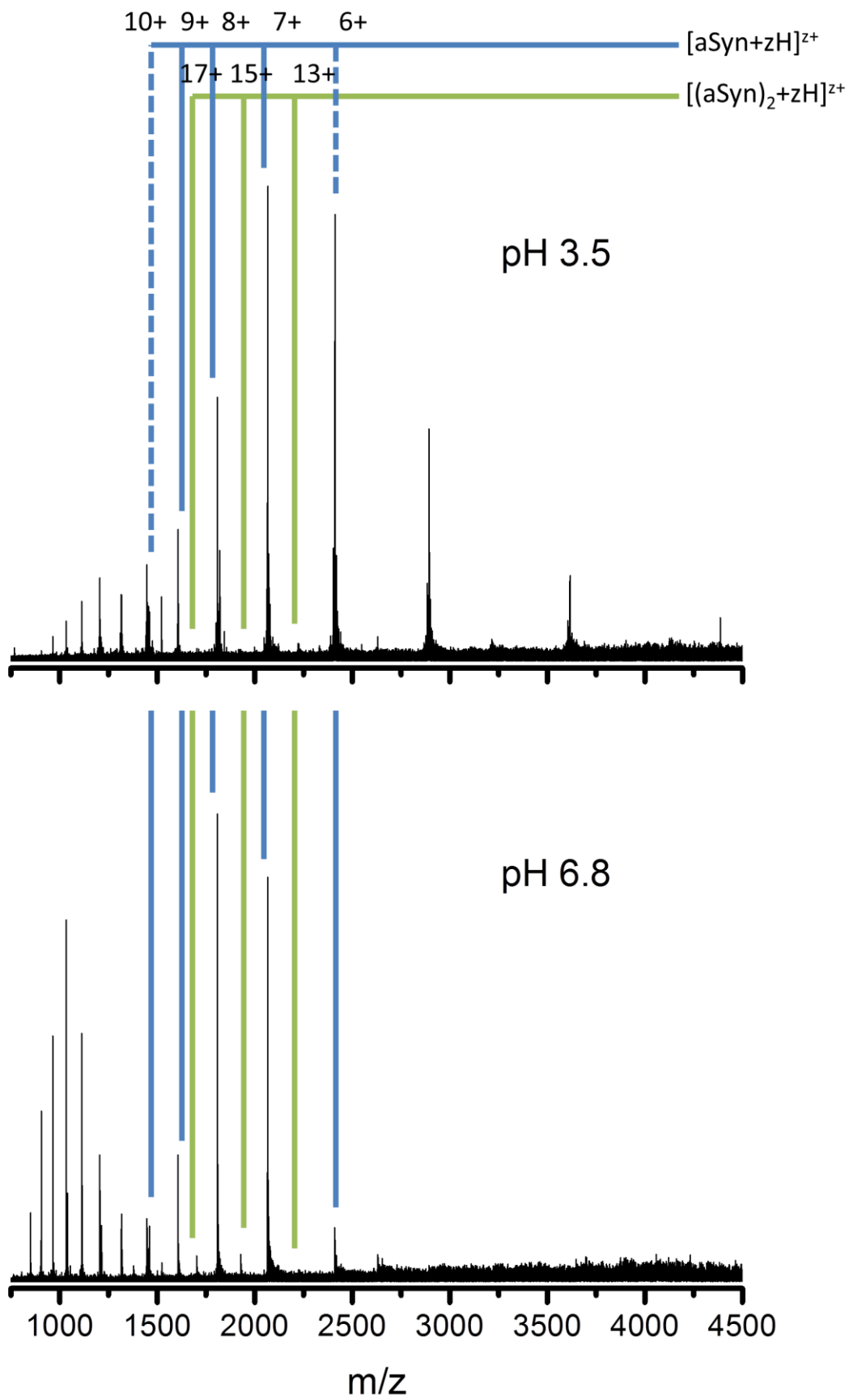


Figure S6

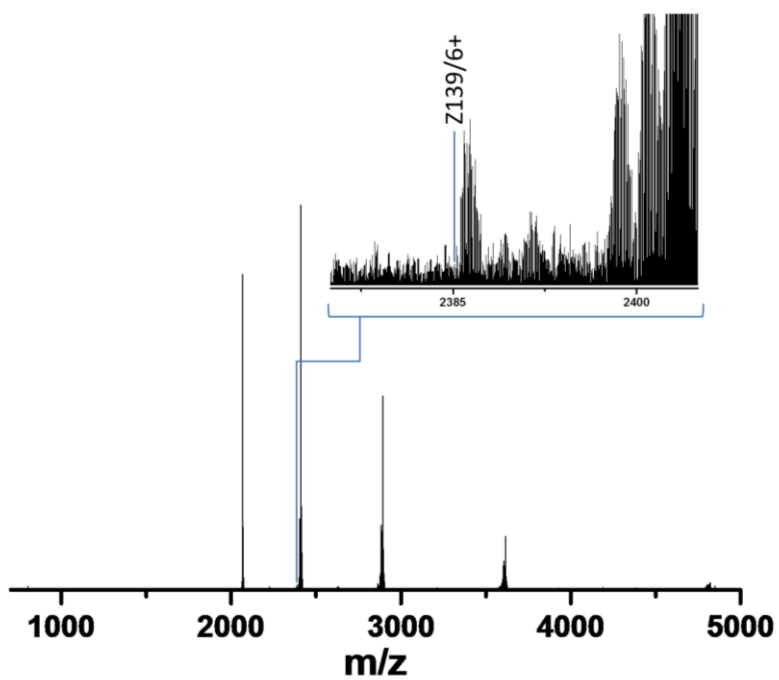
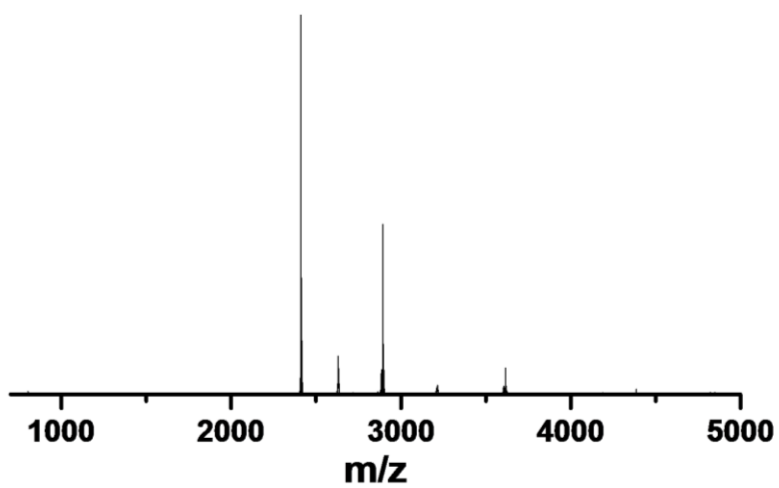


Figure S7

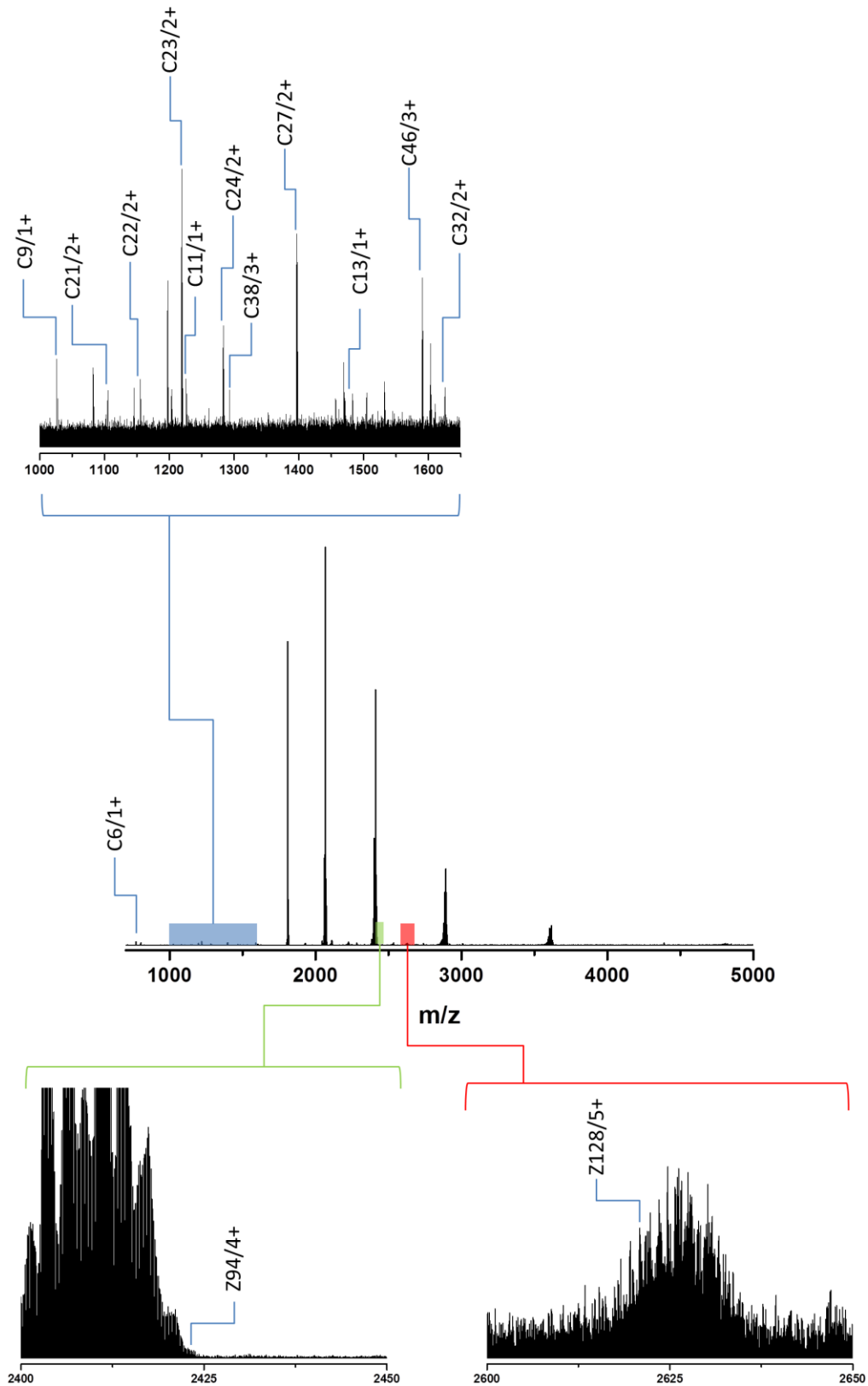


Figure S8

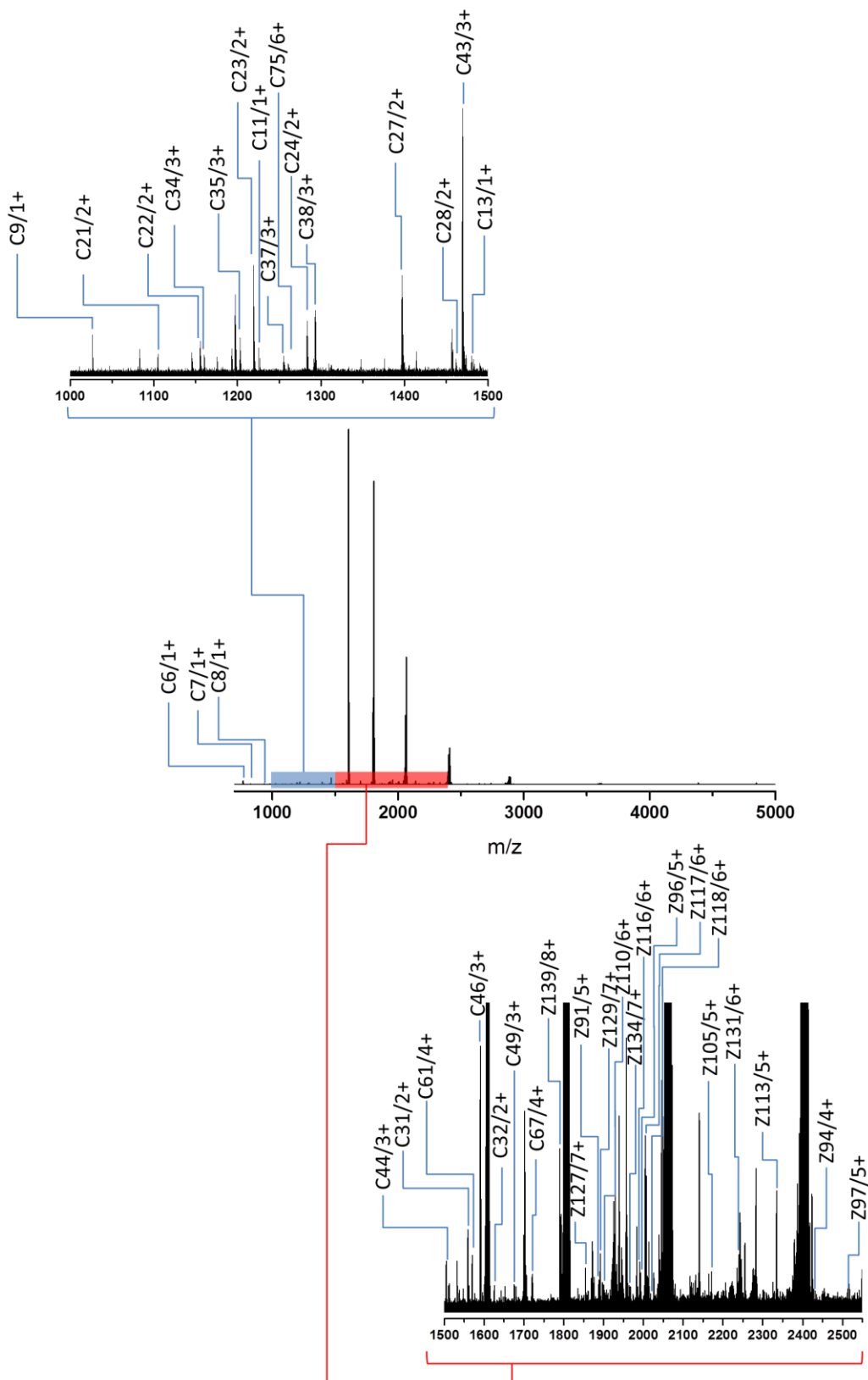


Figure S9

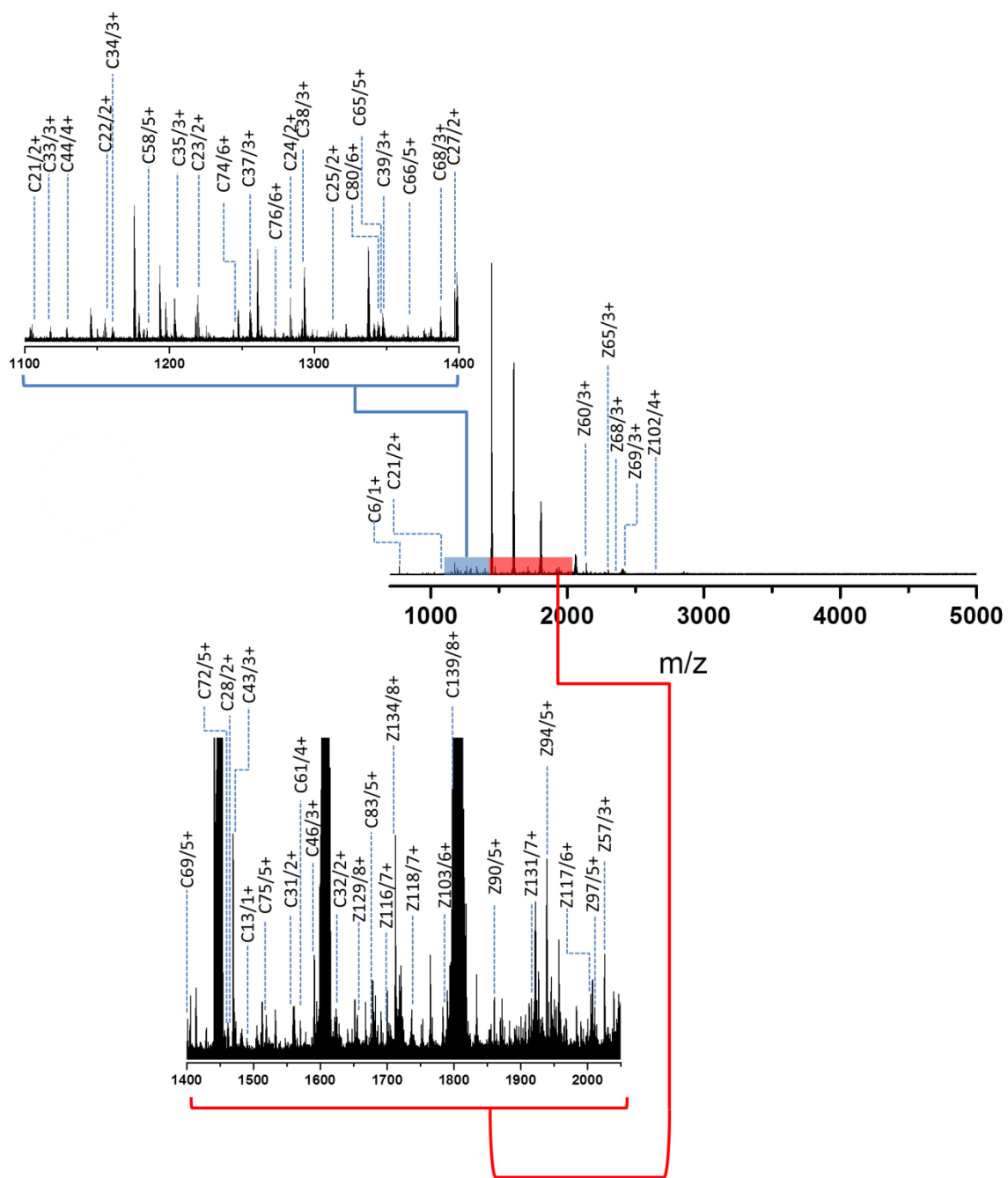


Figure S10

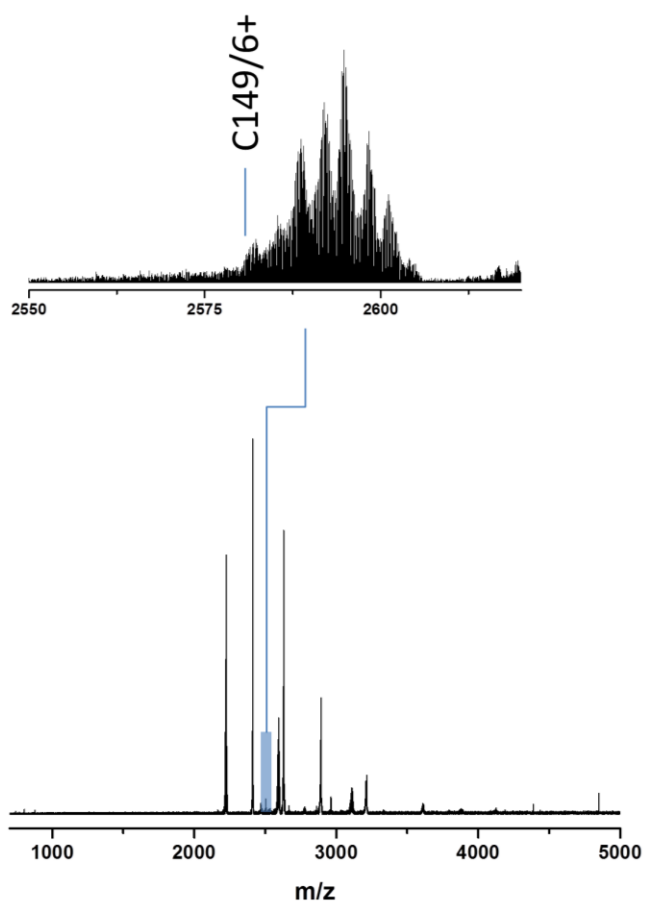


Figure S11

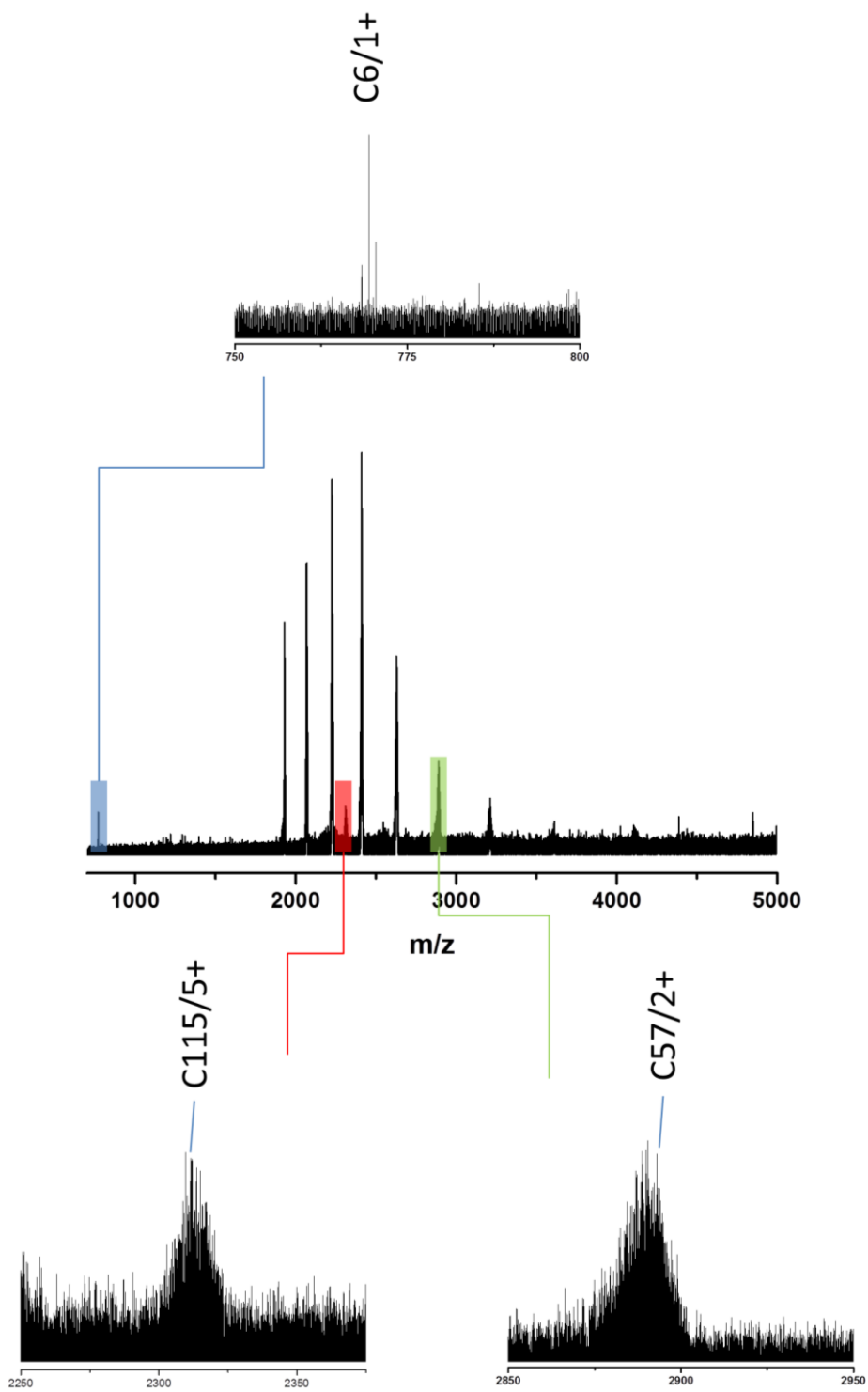


Figure S12

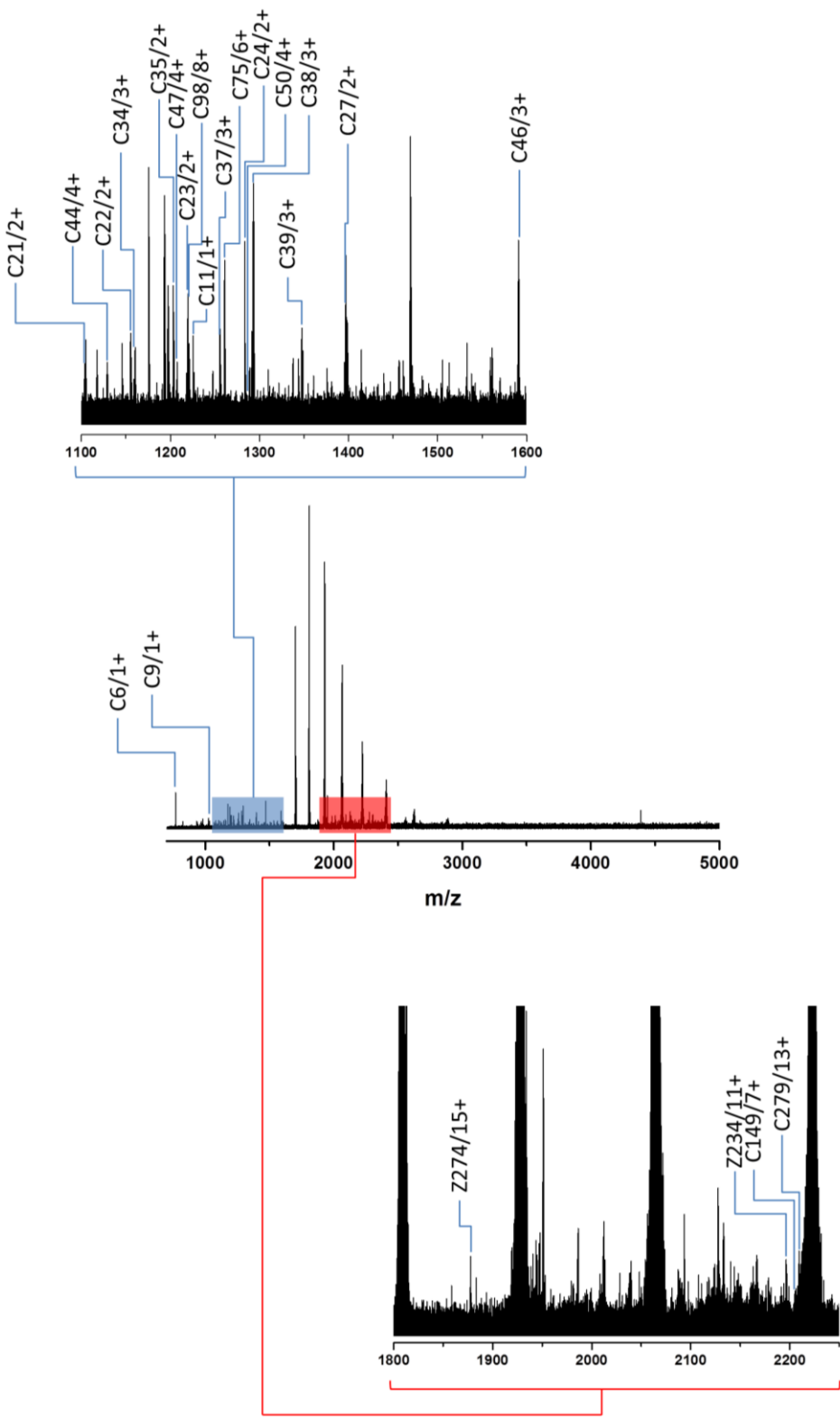


Figure S13



Figure S14

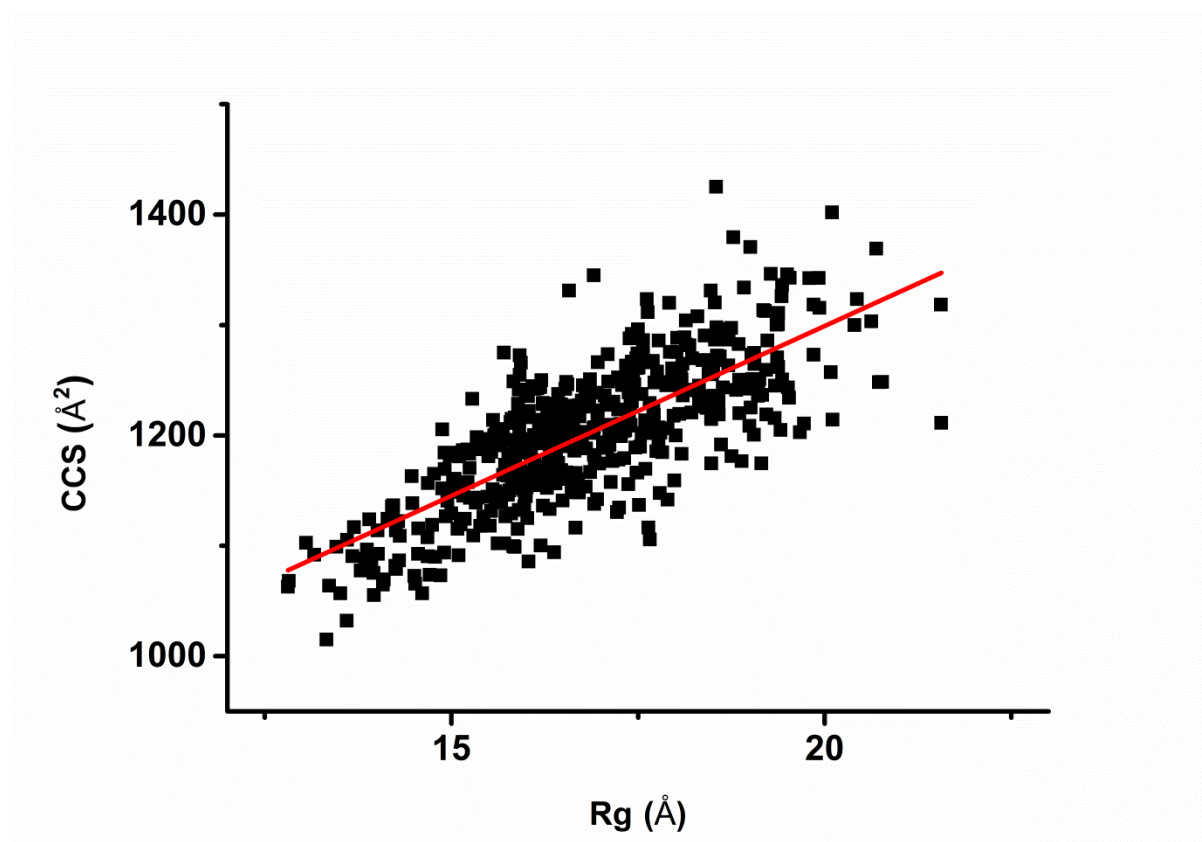


Table S1

Experimental Collision Cross Section (Å <sup>2</sup> )	Theoretical Radius of Gyration (Å)
1200	16.8`
1500	26.5
2350	54.1

Table S1

The collated calculated Radius of gyration values for the  $\alpha$ -synuclein conformational families identified following Crosslinking-IMMS, based on a training set ( Figure S14) of MD simulations of theoretical CCS values of solvated [Insulin+3H]<sup>3+</sup> versus calculated radius of gyration values.