Supplementary Material

Table S1. *Interpretation of ionic populations in AT/heparin mass spectrum based on the limited charge reduction data*.

	Compositions				
	Heparin	**AT/heparin complex**			
detected m/z range[1]	1000-4200	4300-5100	5000-6500	6000-7500	>7000
theoretical m/z range[2]	ND	3500-6100	4600-7000	5800-8200	>6800
number of AT	0	1	2	3	4 and more
mass of heparin	< 42 kDa	< 25 kDa	< 36 kDa	< 49 kDa	ND
major charge states	+3, +4, +5, +6, +7, +8, +9, +10	+13, +14, +15, +16	+20, +21, +22, +23, +24	+26, +27, +28, +29, +30	+32, +33, +34

[1]Detected m/z range for each composition is defined as the first and the last m/z isolation window where the species with the same stoichiometry could be resolved.

[2]Theoretical m/z range is typically larger than the detected m/z range for AT heparin complexes. It includes those weak ionic signals that may contribute to the entire shape of mass spectrum but cannot be detected during limited charge reduction analysis due to the suppressed electron transfer efficiency in presence of other abundant ionic population with adjacent stoichiometry. As we summarize in this table, the major charge states for AT/heparin complex are basically independent to the mass of involved heparin, thus the theoretical m/z range for each composition could be estimated using the equations as below:

$$m/z_{(\min|theoretical)} = 1 + \frac{Mass_{(\min|detected)}}{Charge_{(\max|detected)}}$$

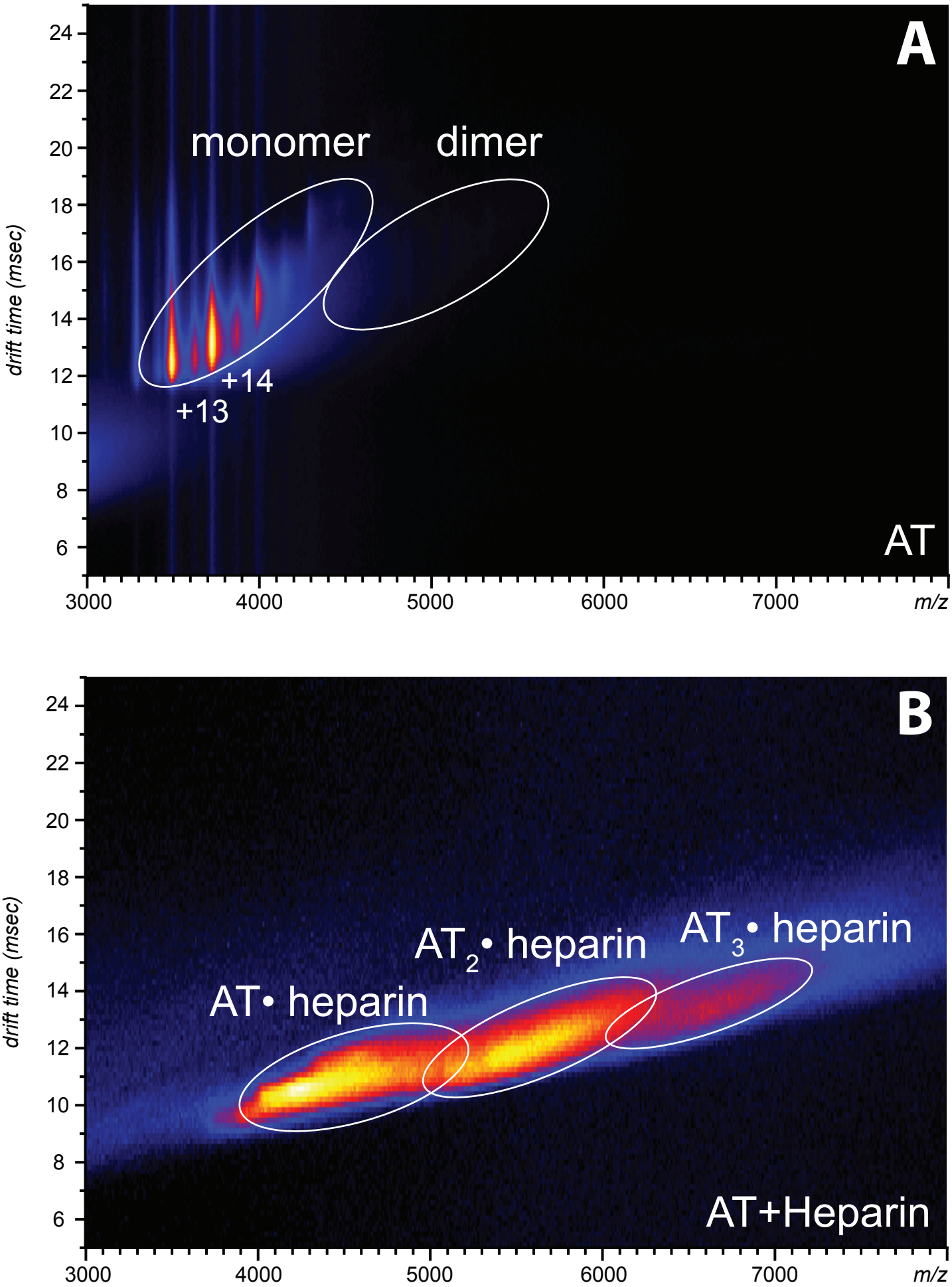
$$m/z_{(\max|theoretical)} = 1 + \frac{Mass_{(\max|detected)}}{Charge_{(\min|detected)}}$$

For unbound heparin, the charge depends on mass of heparin and hence the theoretical m/z range cannot be estimated based on current limited charge reduction data.

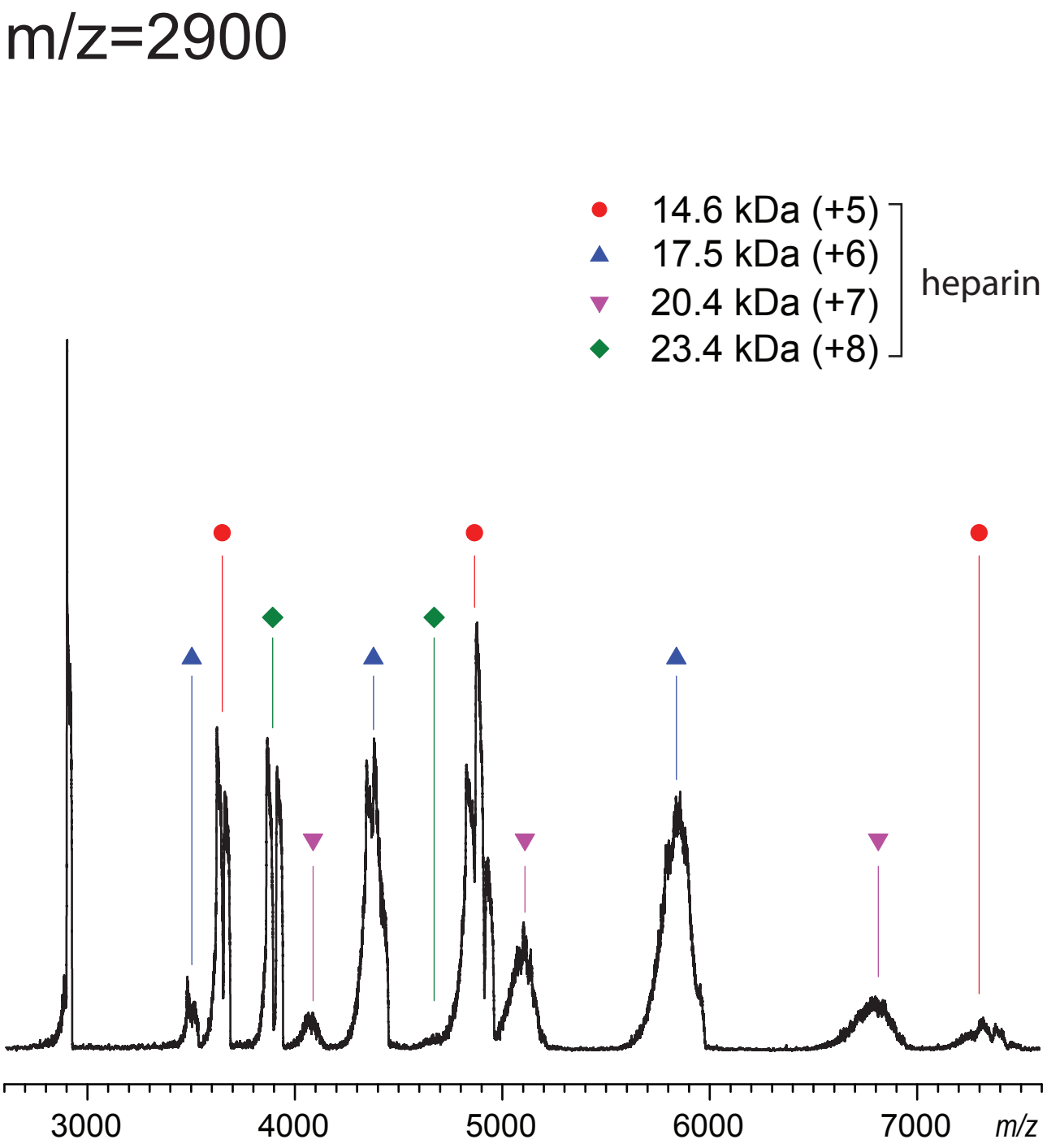
Supplementary Figure Legends

Figure S1. Ion mobility mass spectra (2-D diagram) of 4 µM of AT in 30 mM ammonium acetate (pH 6.0) acquired in the absence of (panel A) and the presence of 0.03 mg/mL of intact heparin (panel B). Distinct ionic populations are circled and their compositions in panel B are labeled upon limited charge reduction analysis.

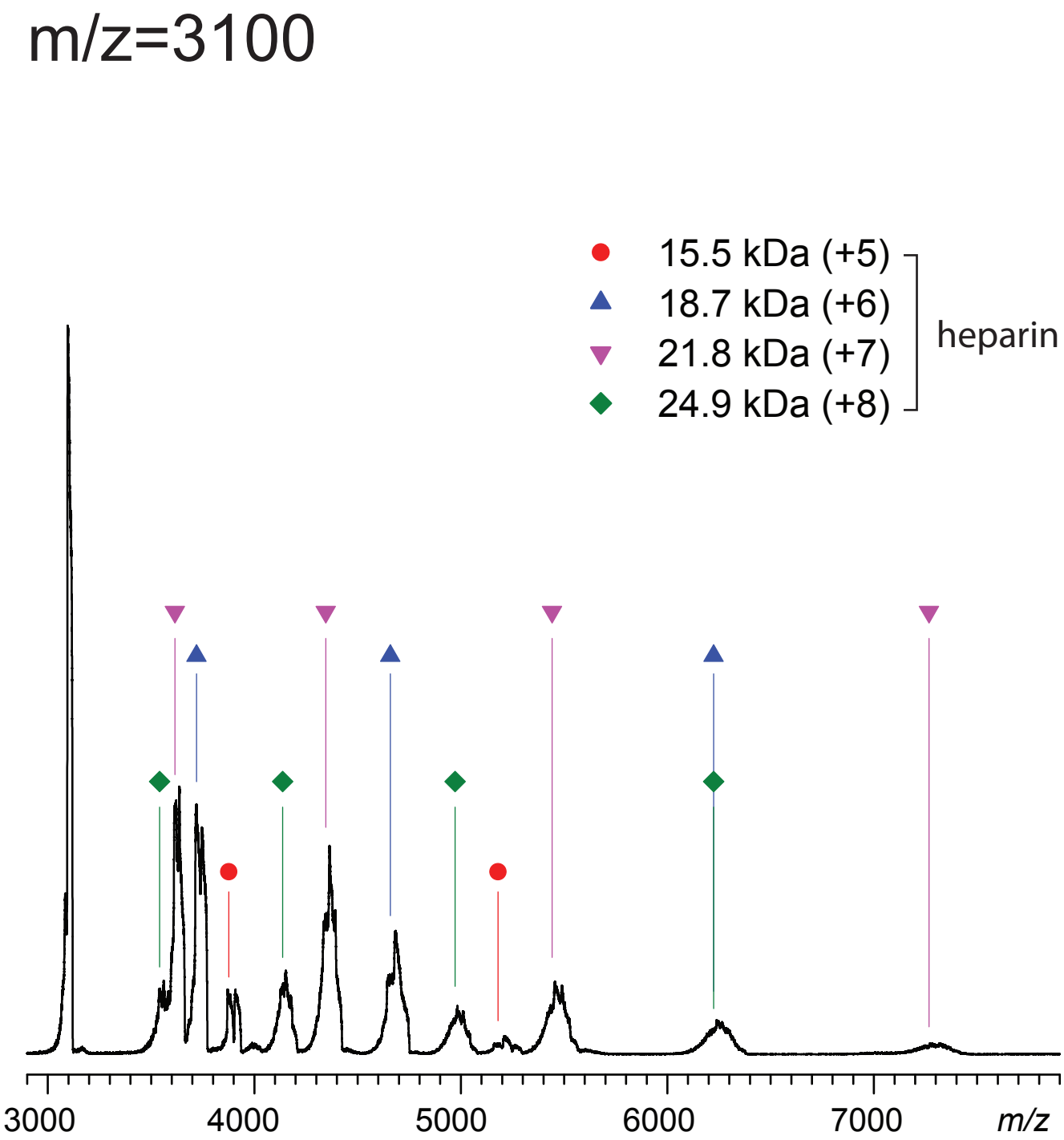
Figure S2. A complete set of the limited charge reduction data acquired by moving selection window onto different regions of interest. Interpretations of mass/charge/composition for each spectrum are labeled in different colors and symbols. Wide selection window (LM resolution = 3) was placed at m/z 4500, 4800, 5200, 6000, 6200, 6400, 7000 and 7200; narrow selection window (LM resolution = 4) was placed at m/z 2900, 3100, 3200, 3300, 3500, 4200, 4300, 4400, 4500, 4600, 5000, 5800, 5900, 6000.



Zhao, et al., **Figure S1**

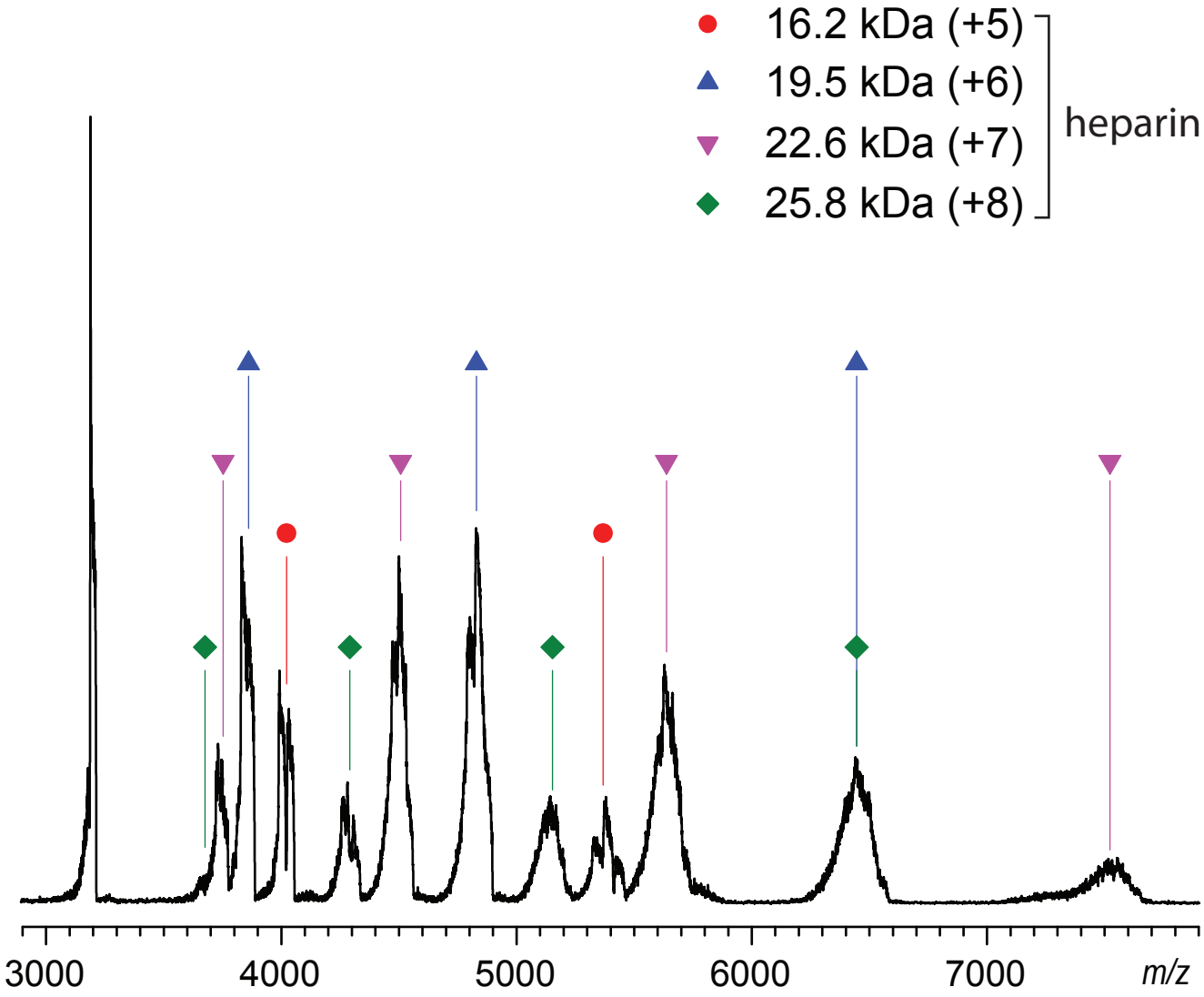


Zhao, et al., **Figure S2**



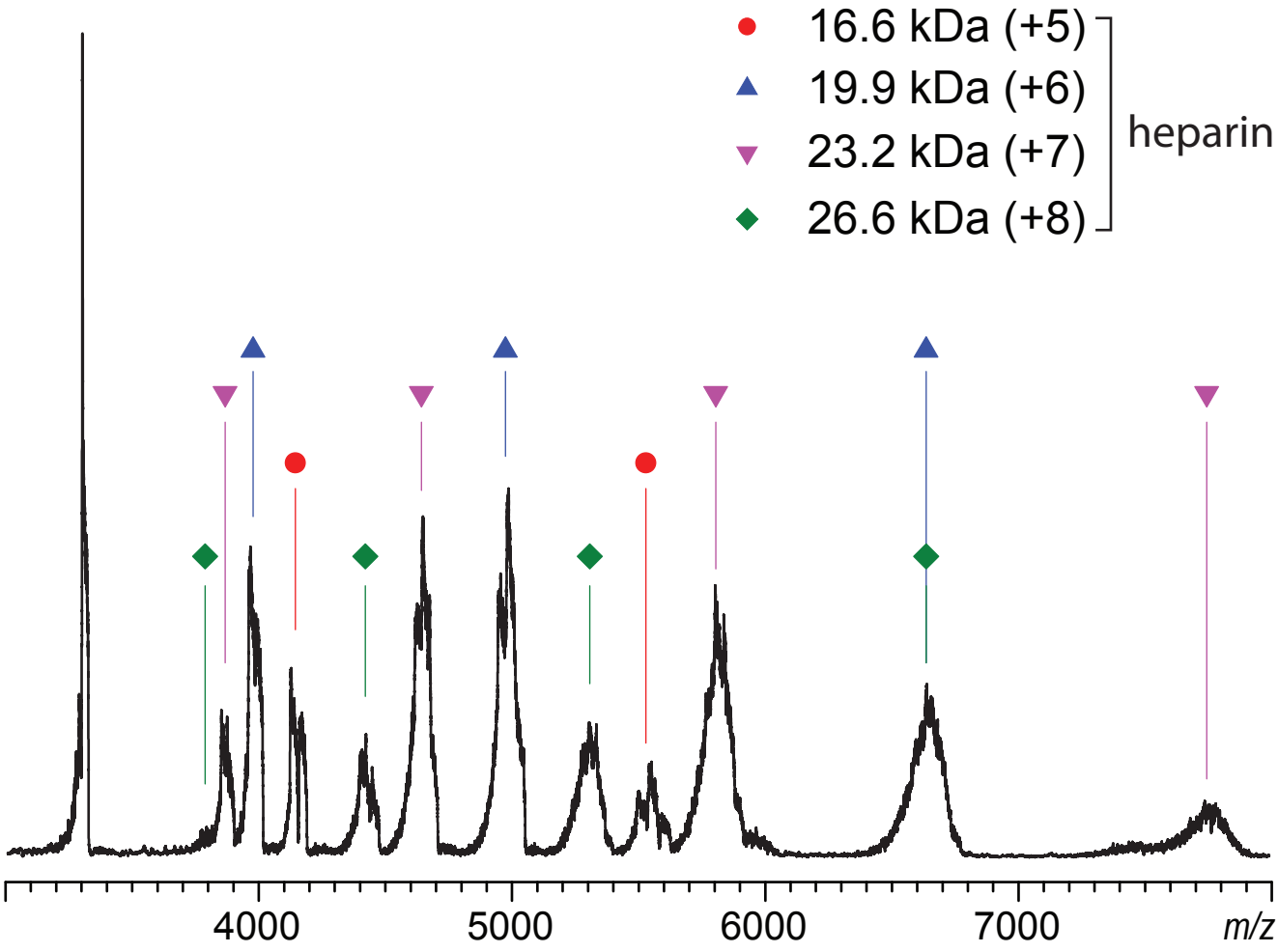
Zhao, et al., **Figure S2**

m/z=3200



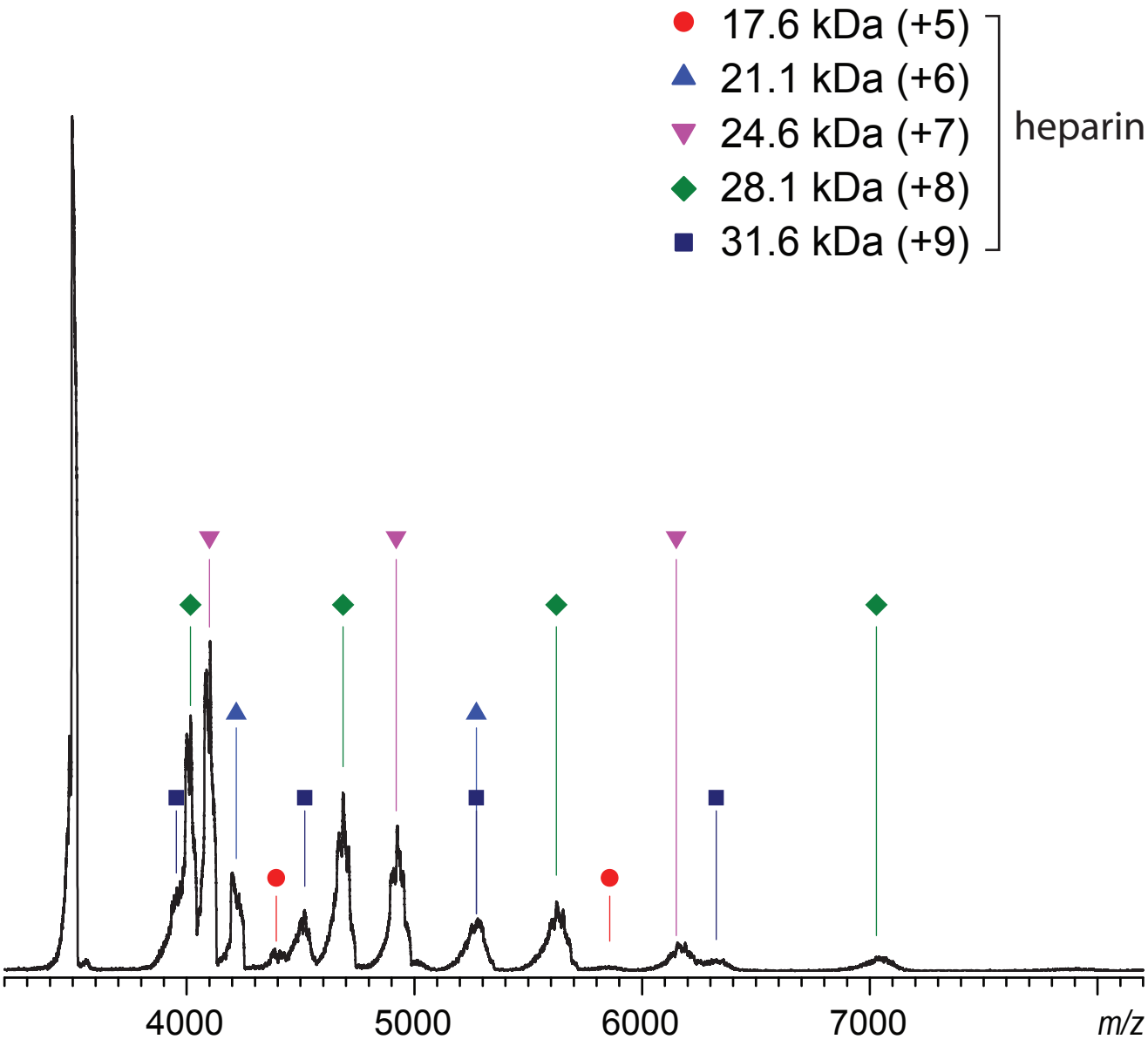
Zhao, et al., **Figure S2**

m/z=3300

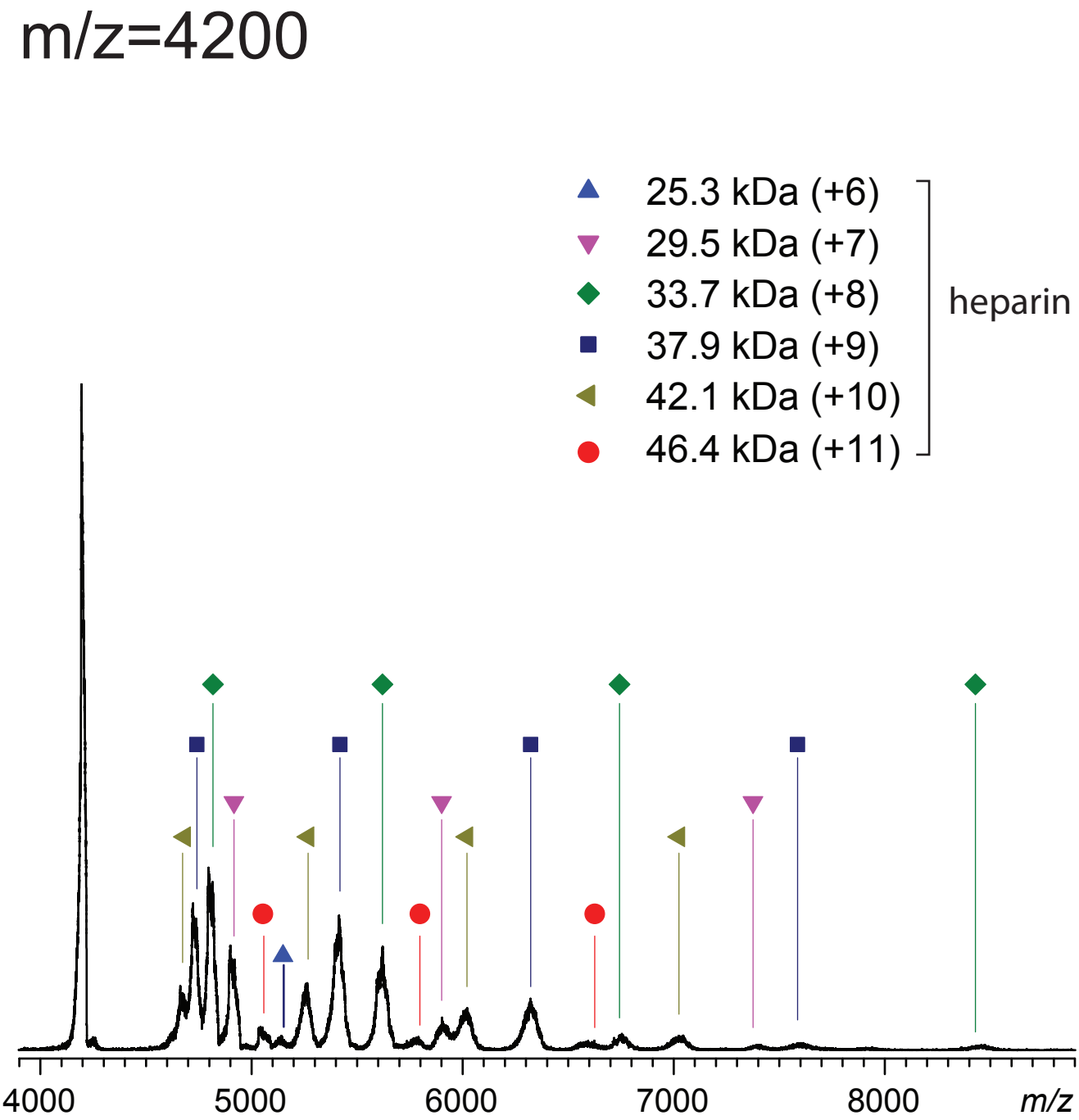


Zhao, et al., **Figure S2**

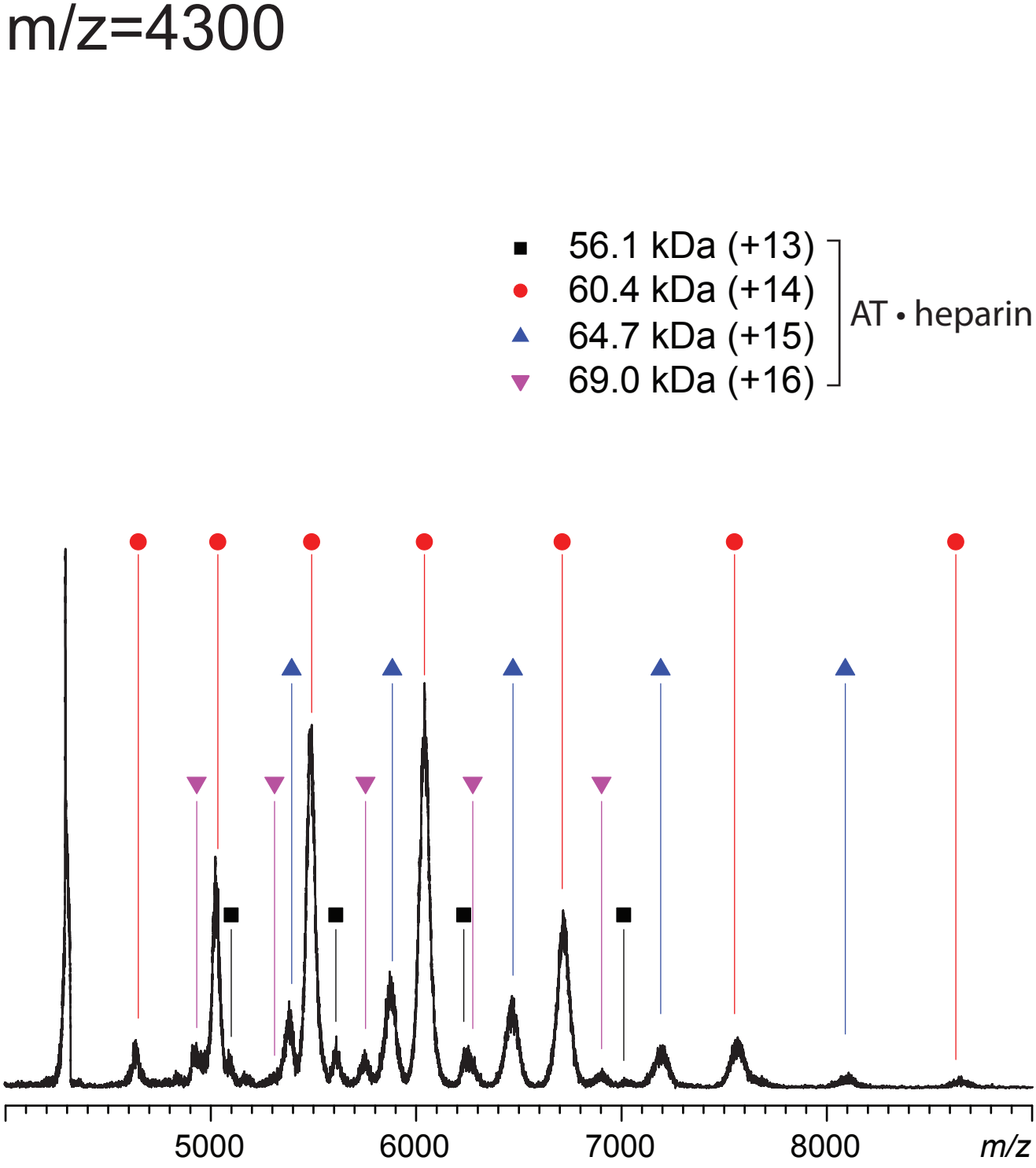
m/z=3500



Zhao, et al., **Figure S2**

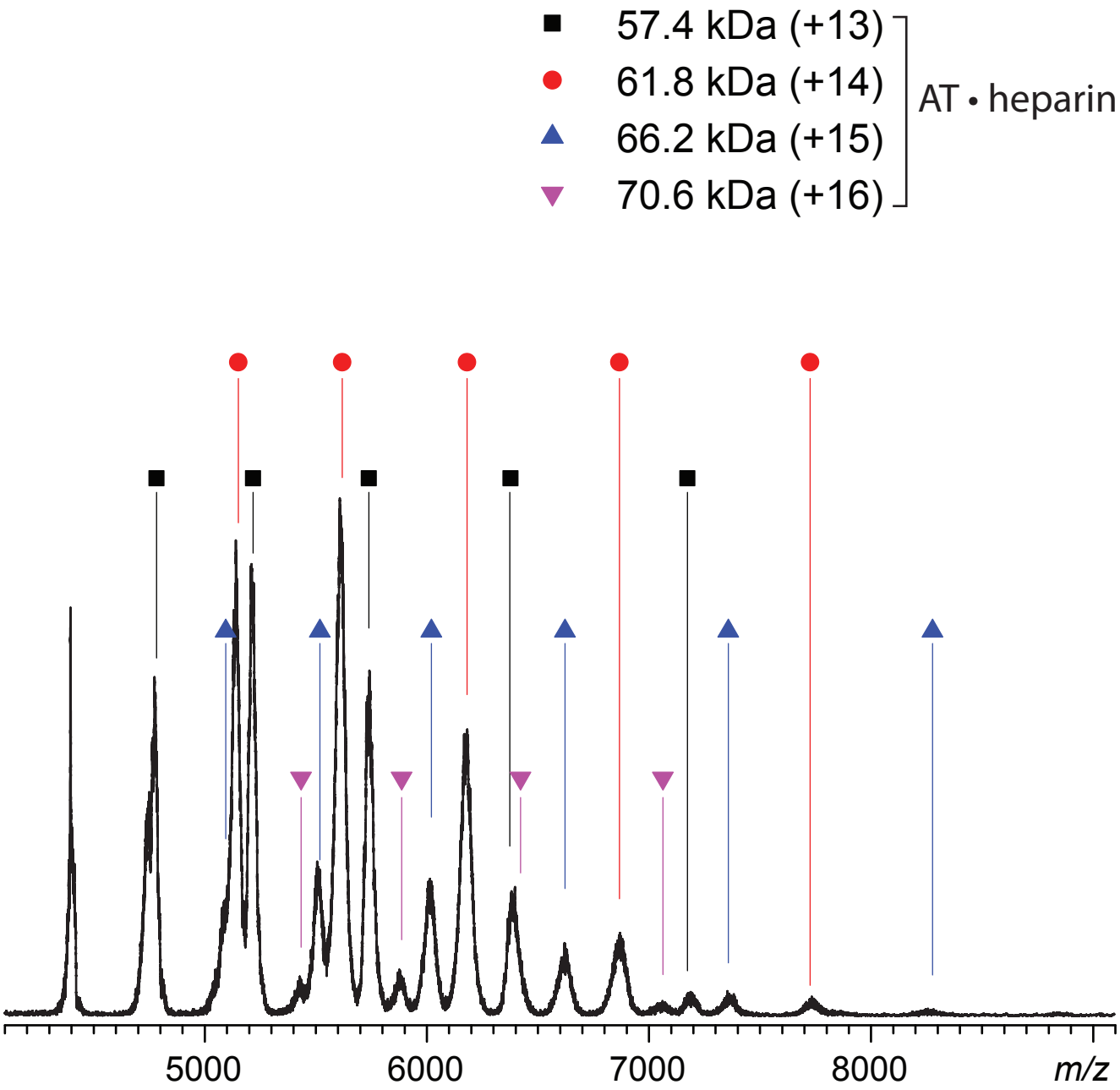


Zhao, et al., **Figure S2**



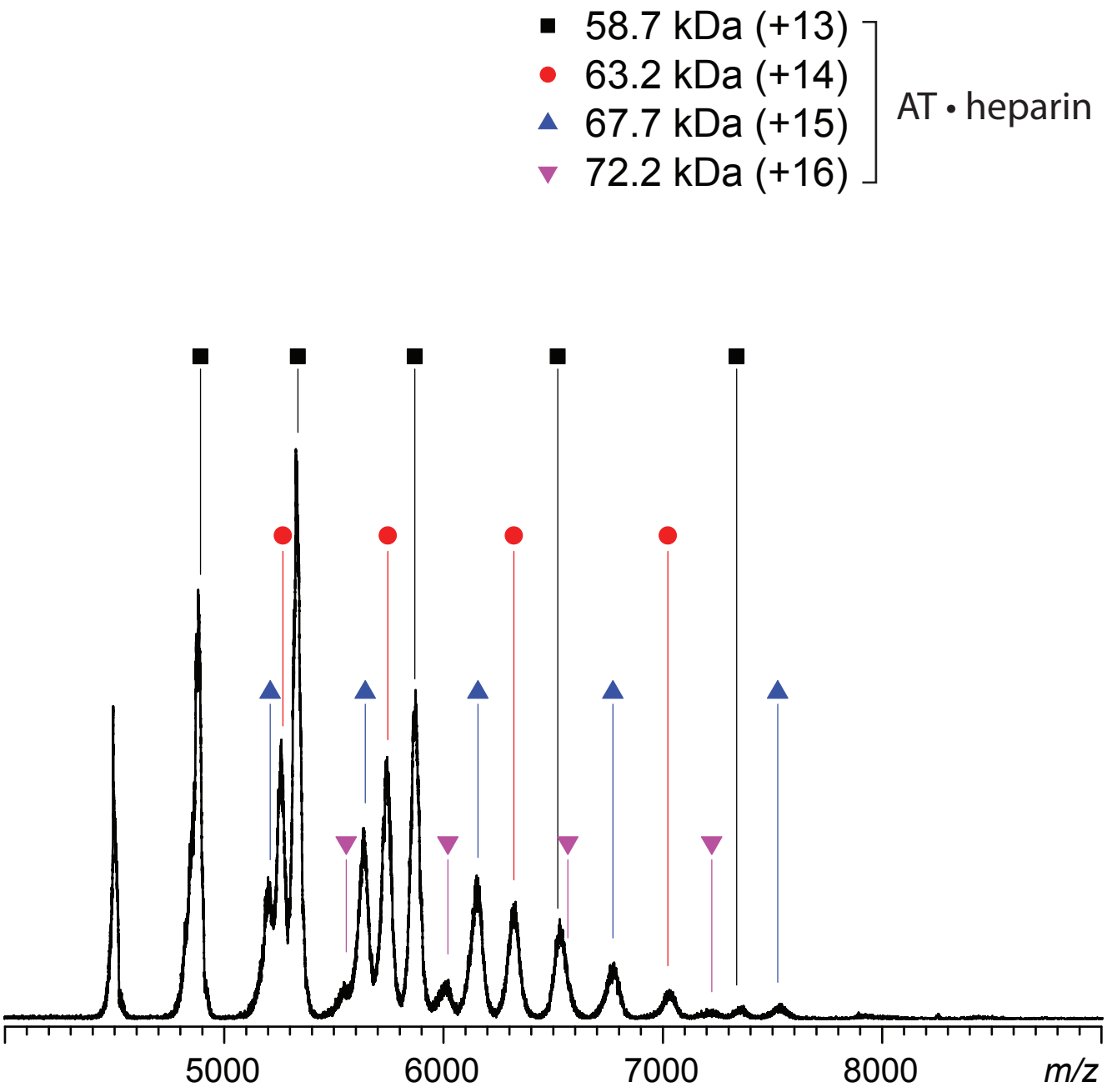
Zhao, et al., **Figure S2**

m/z=4400



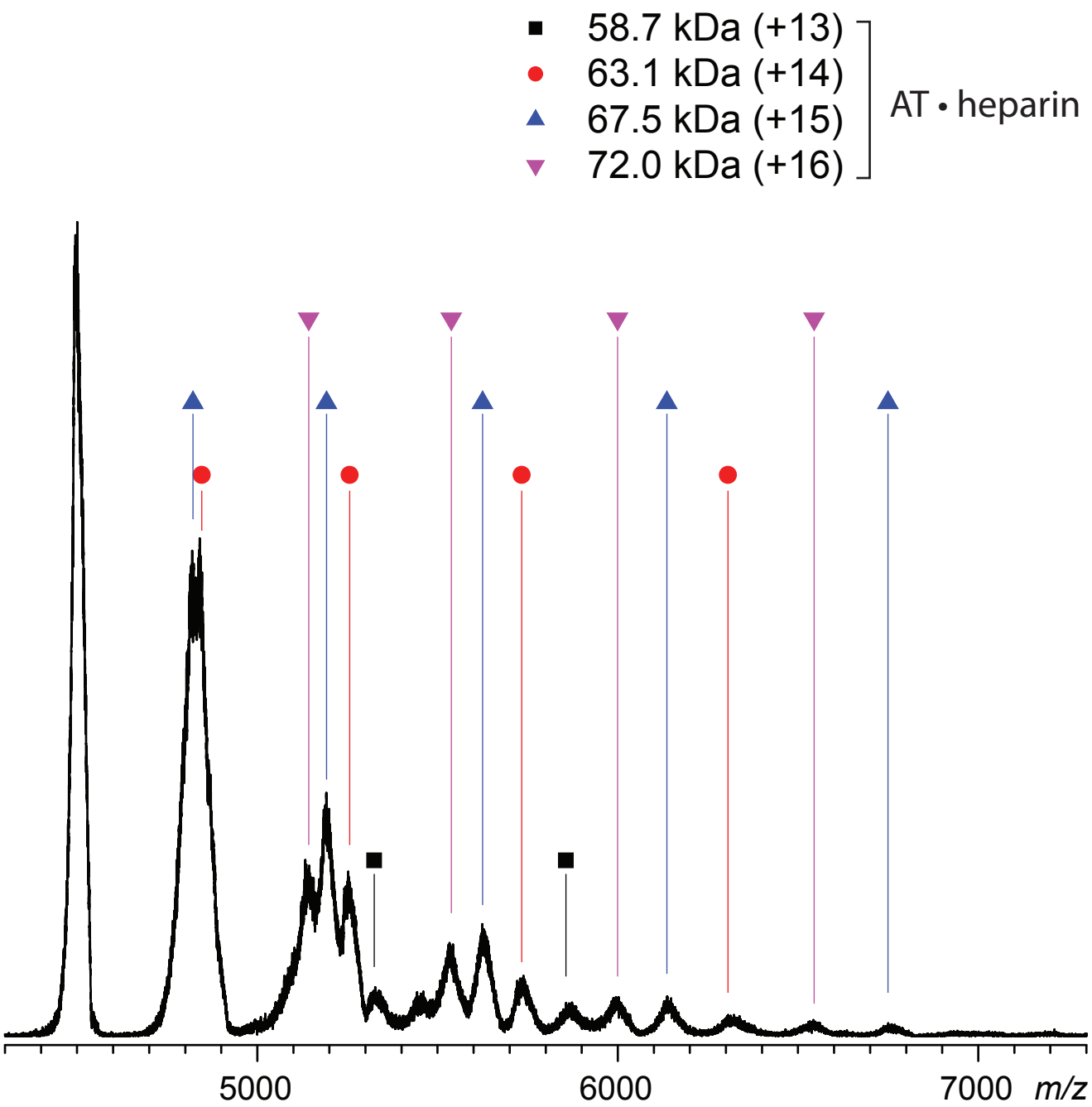
Zhao, et al., **Figure S2**

m/z=4500 (narrow window)



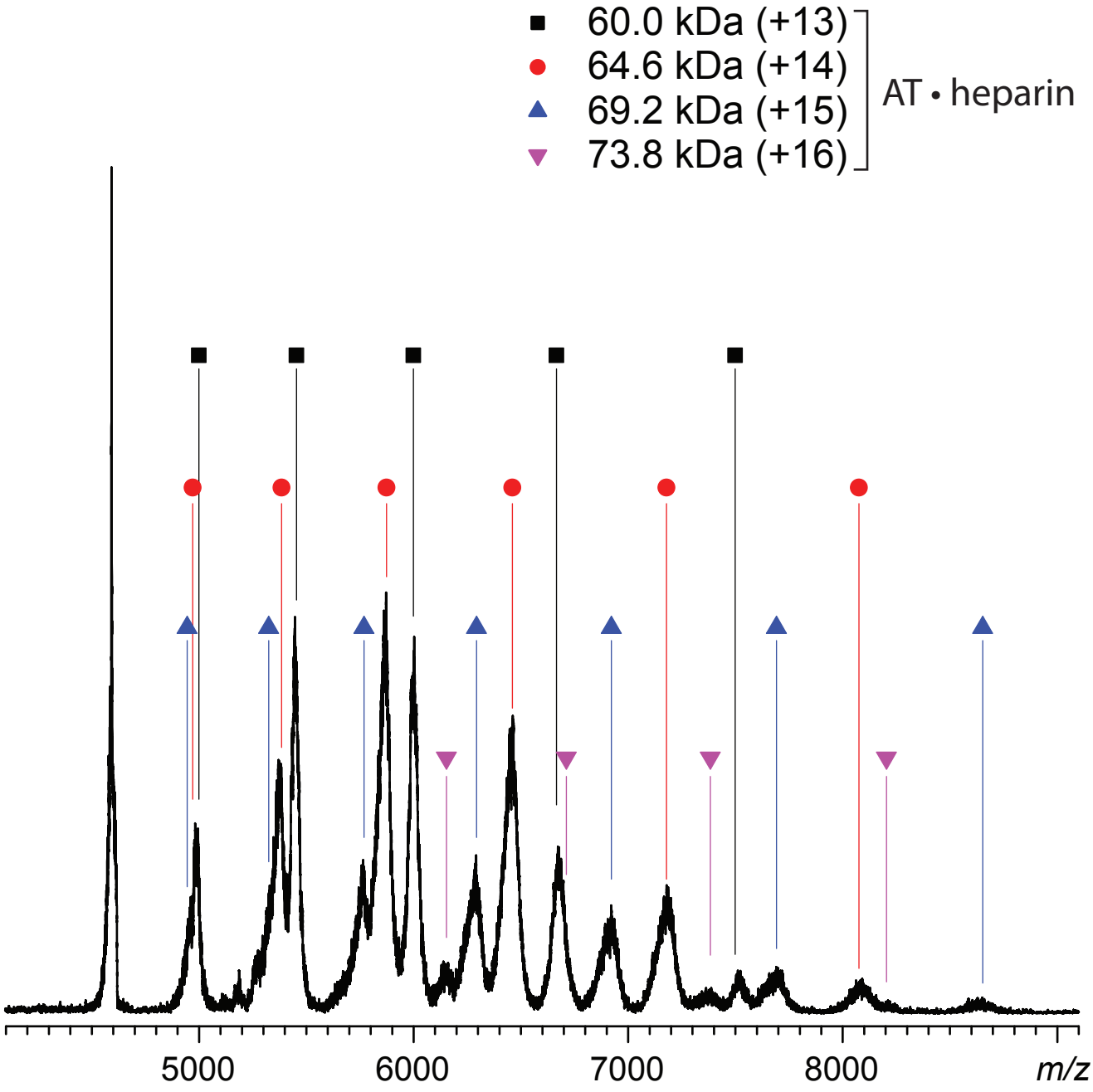
Zhao, et al., **Figure S2**

m/z=4500 (wide window)



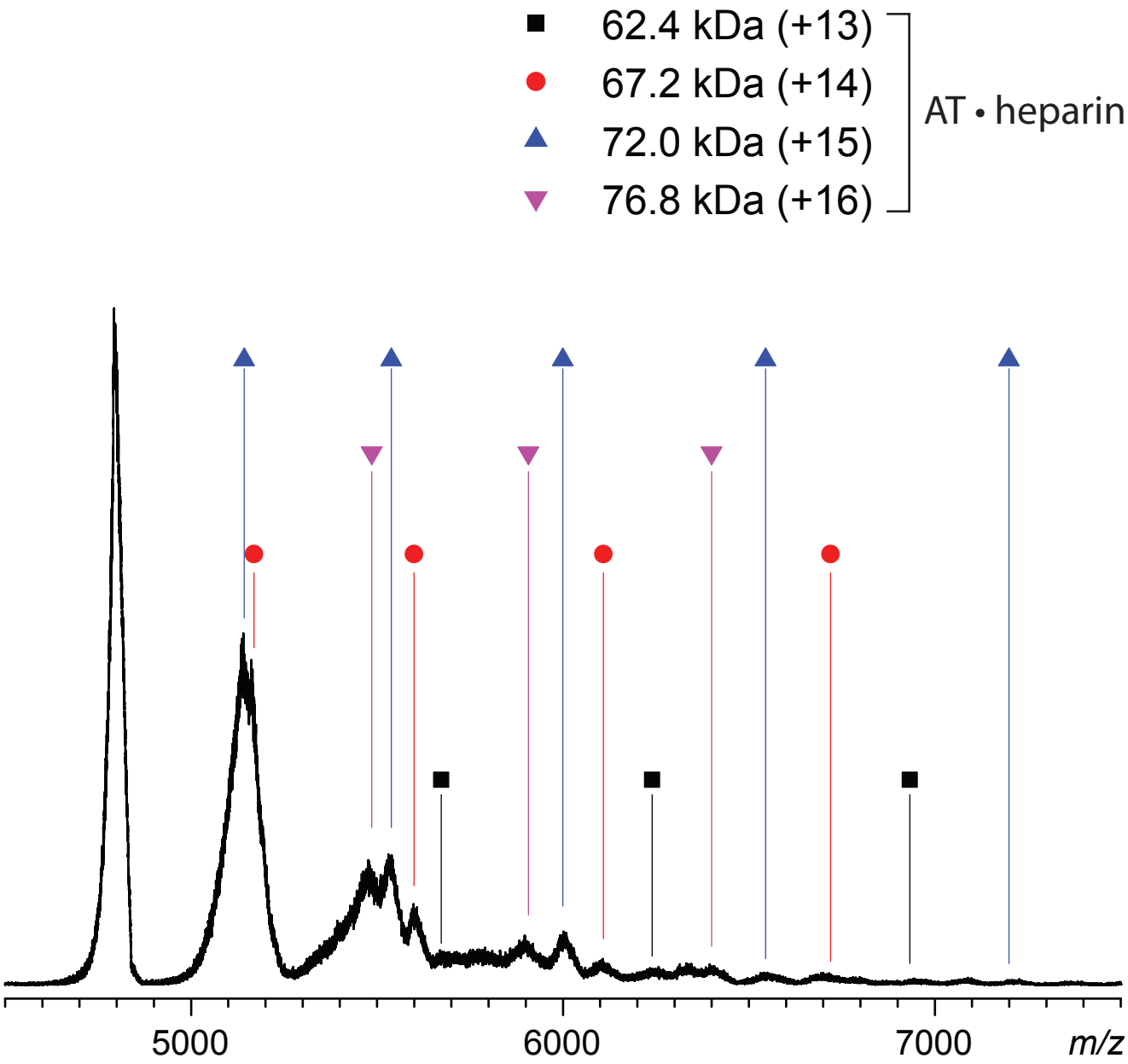
Zhao, et al., **Figure S2**

m/z=4600

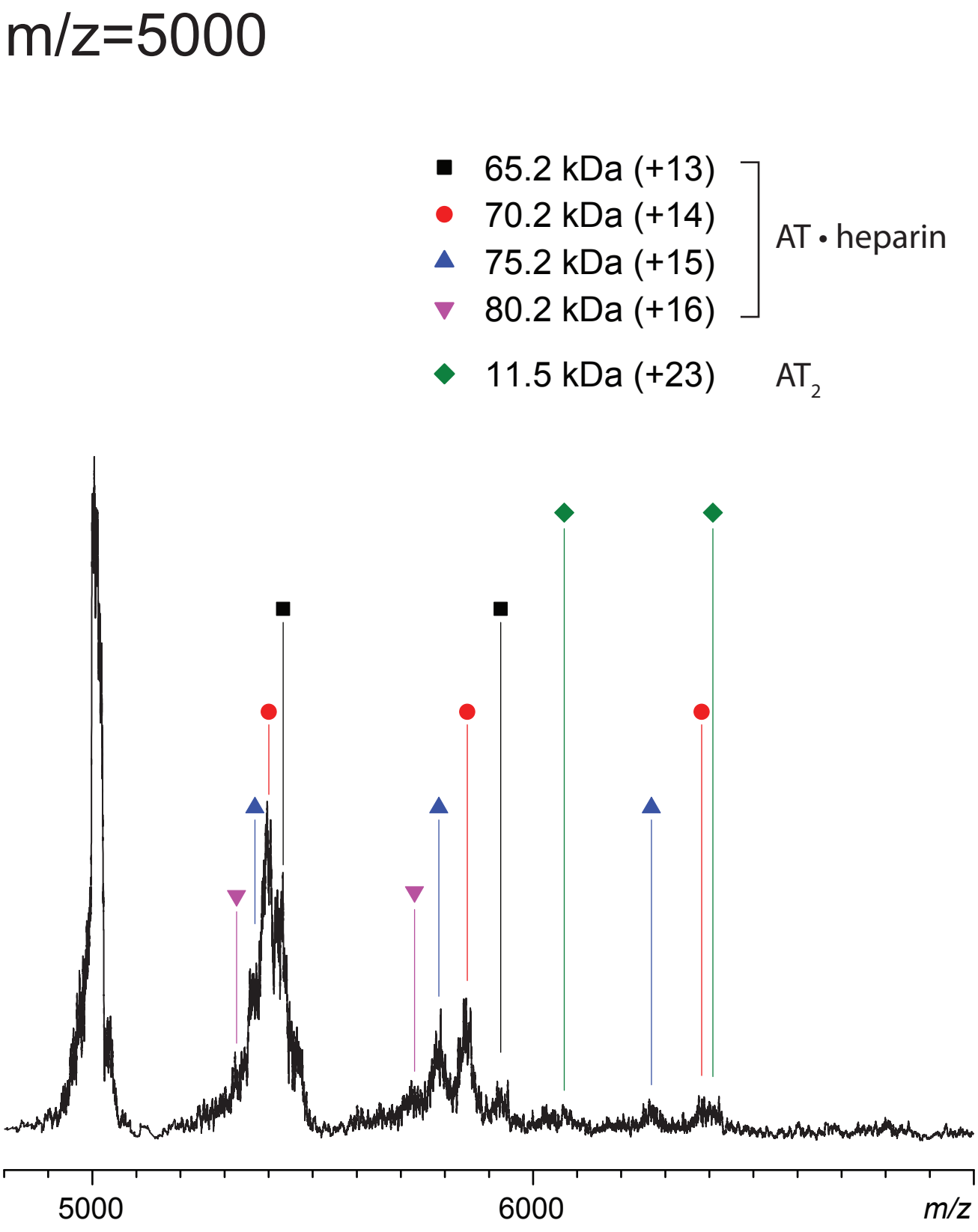


Zhao, et al., **Figure S2**

m/z=4800

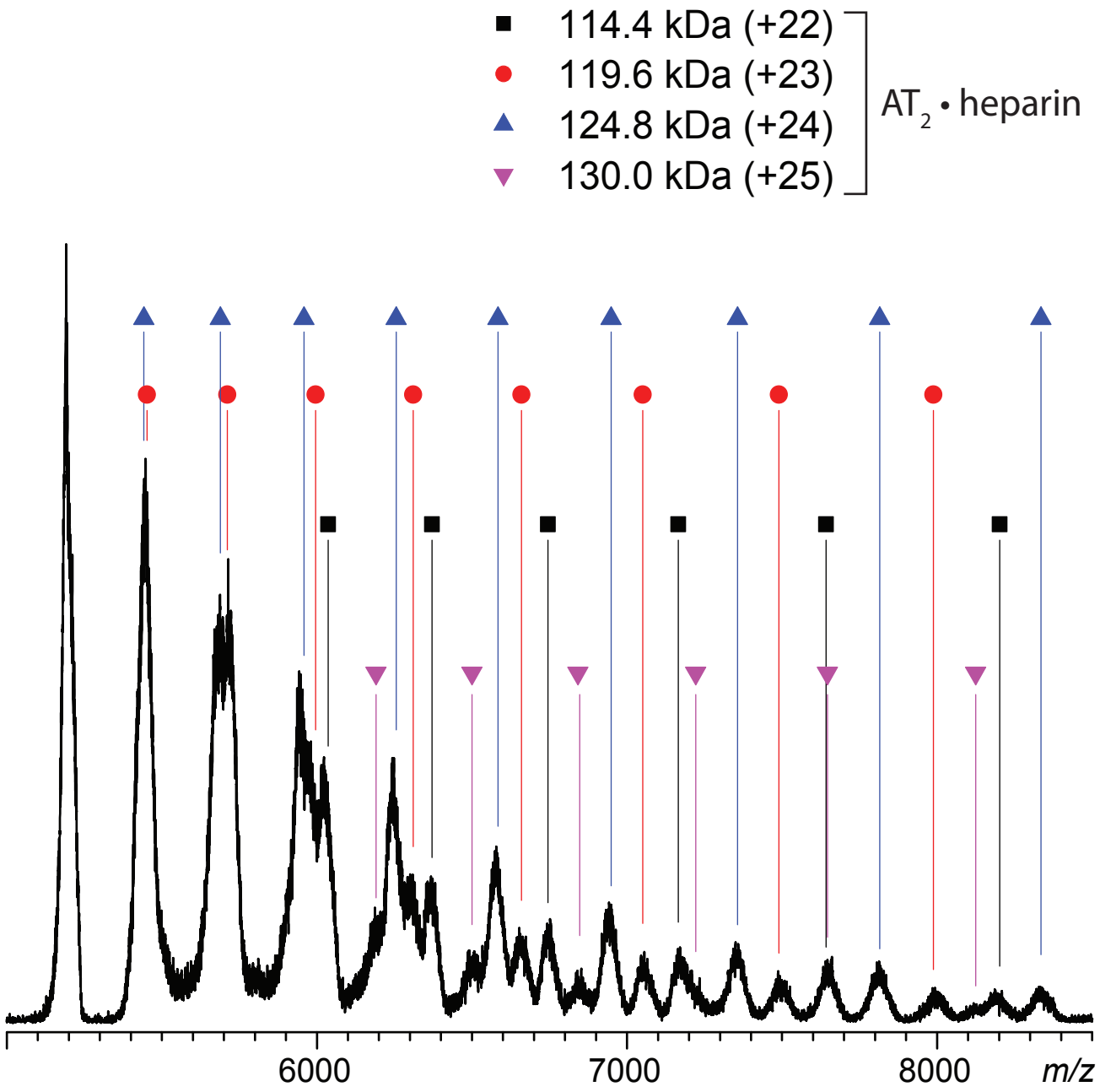


Zhao, et al., **Figure S2**

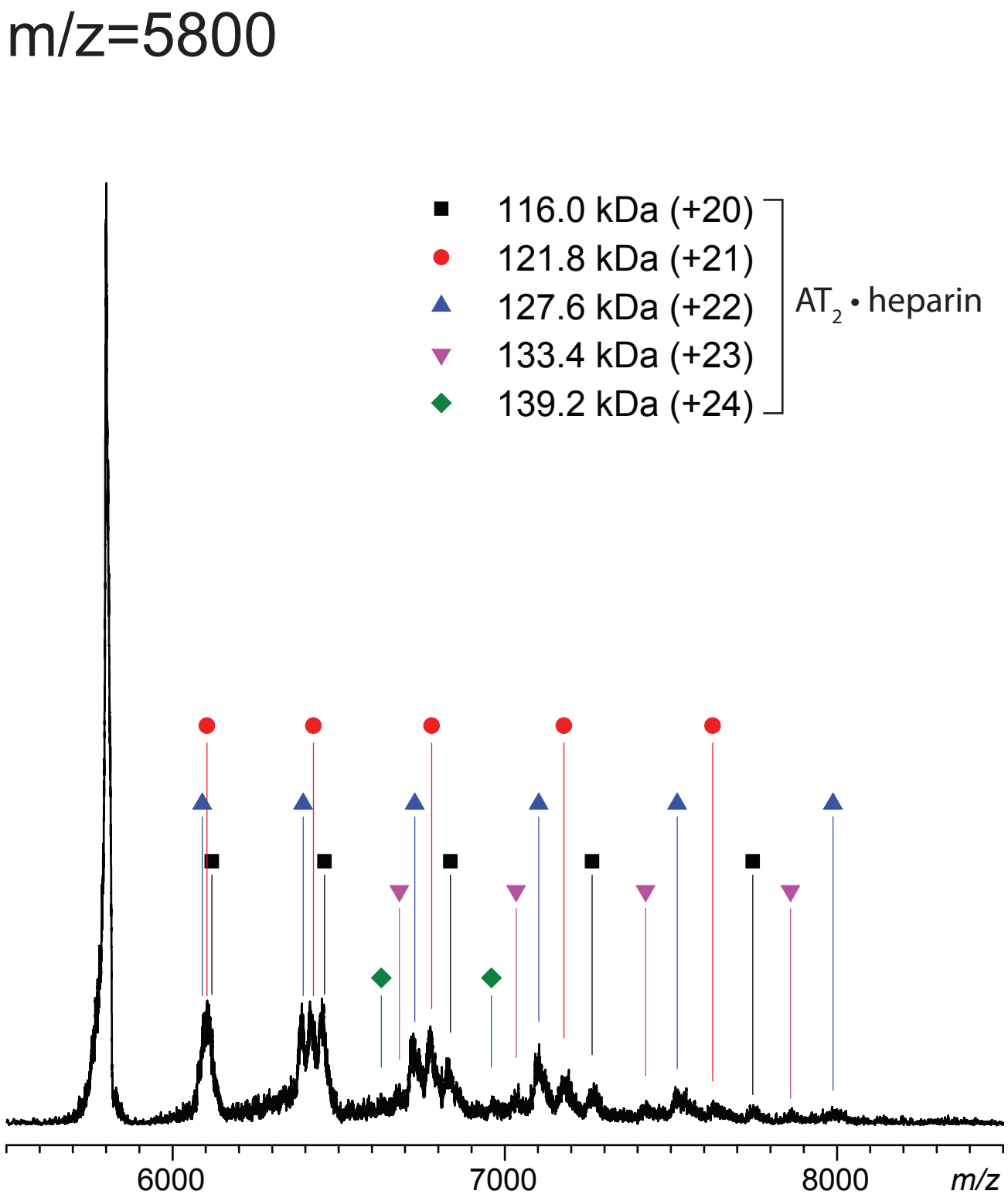


Zhao, et al., **Figure S2**

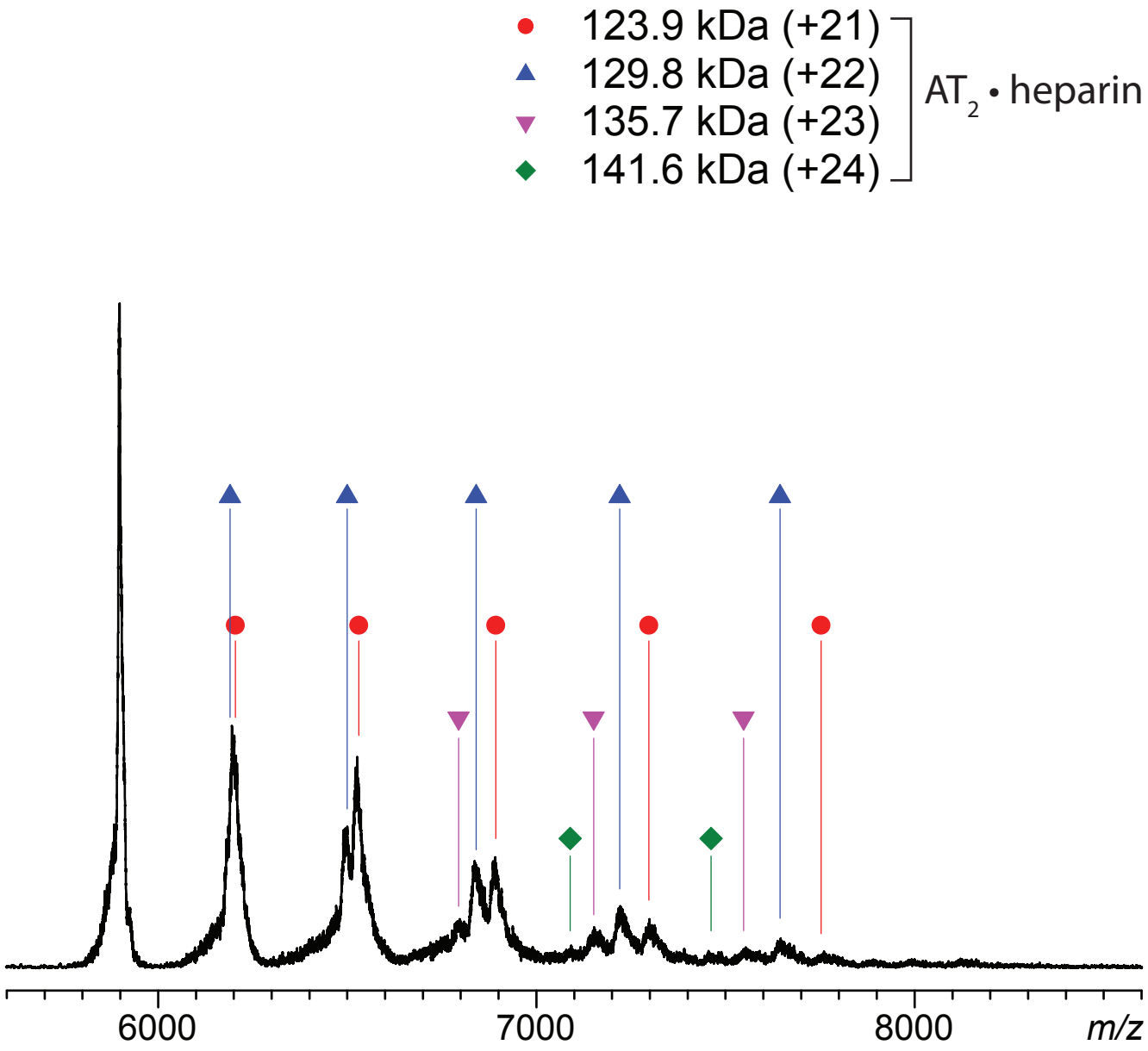
m/z=5200



Zhao, et al., **Figure S2**

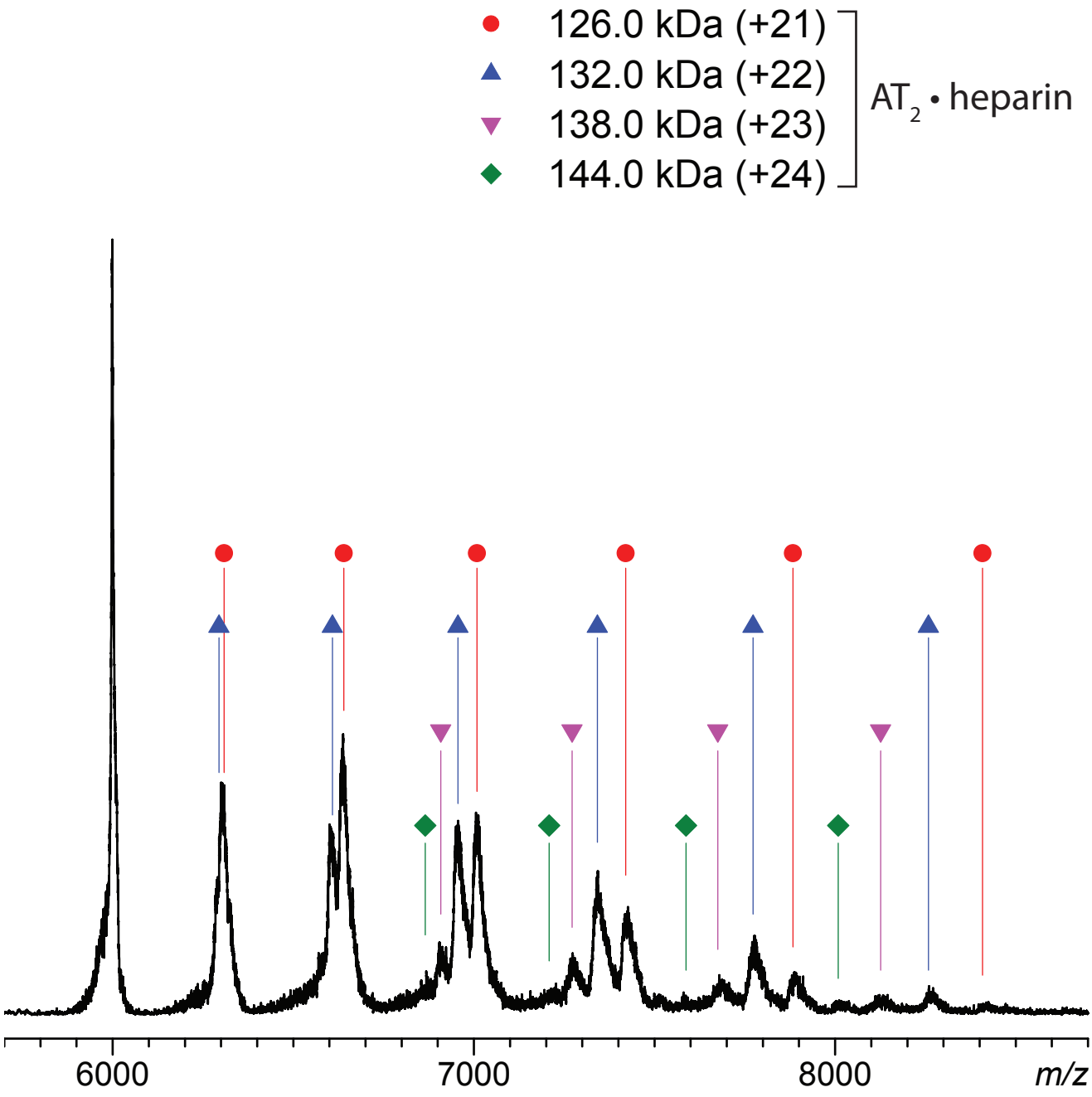


Zhao, et al., **Figure S2**



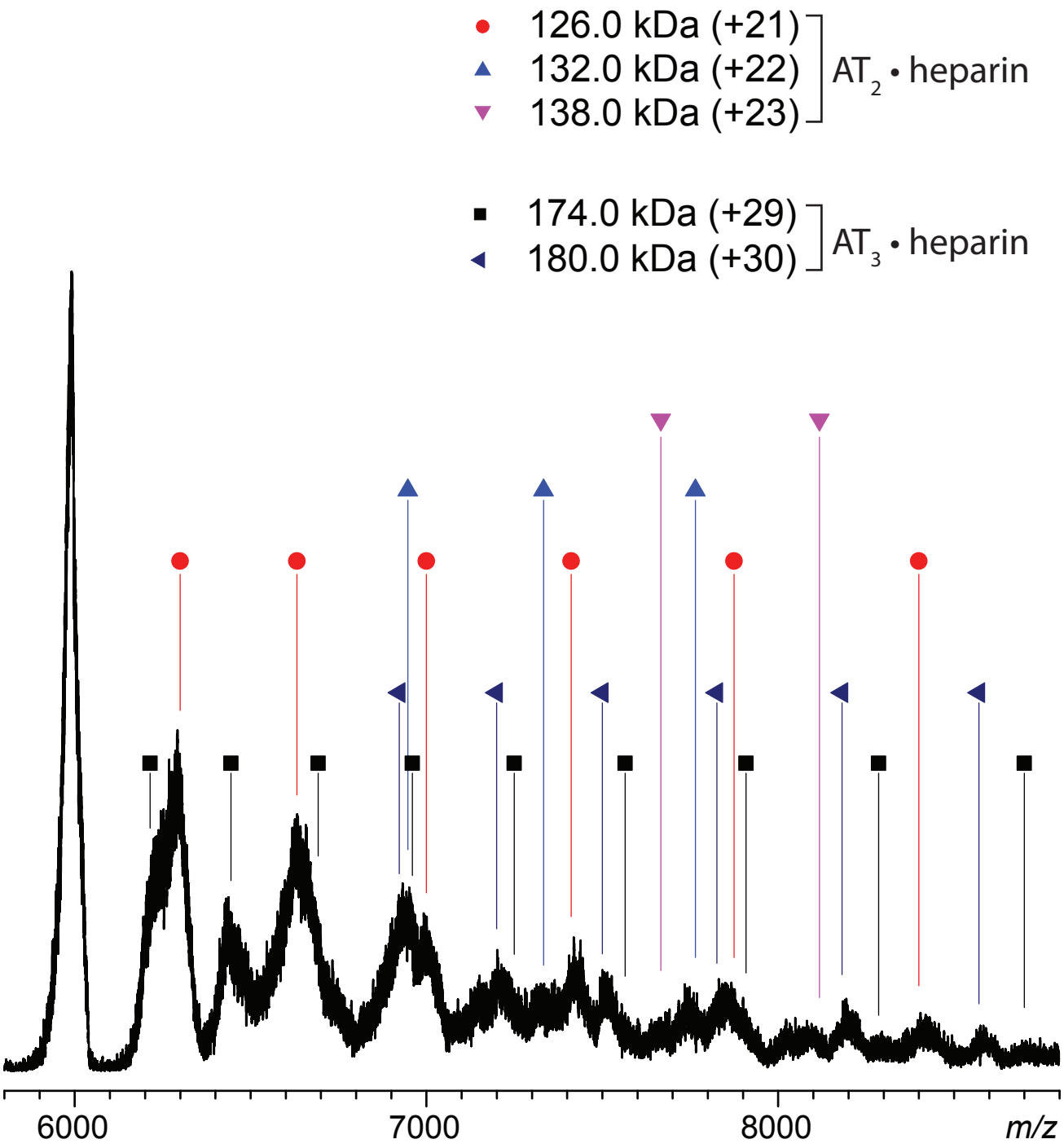
Zhao, et al., **Figure S2**

m/z=6000 (narrow window)

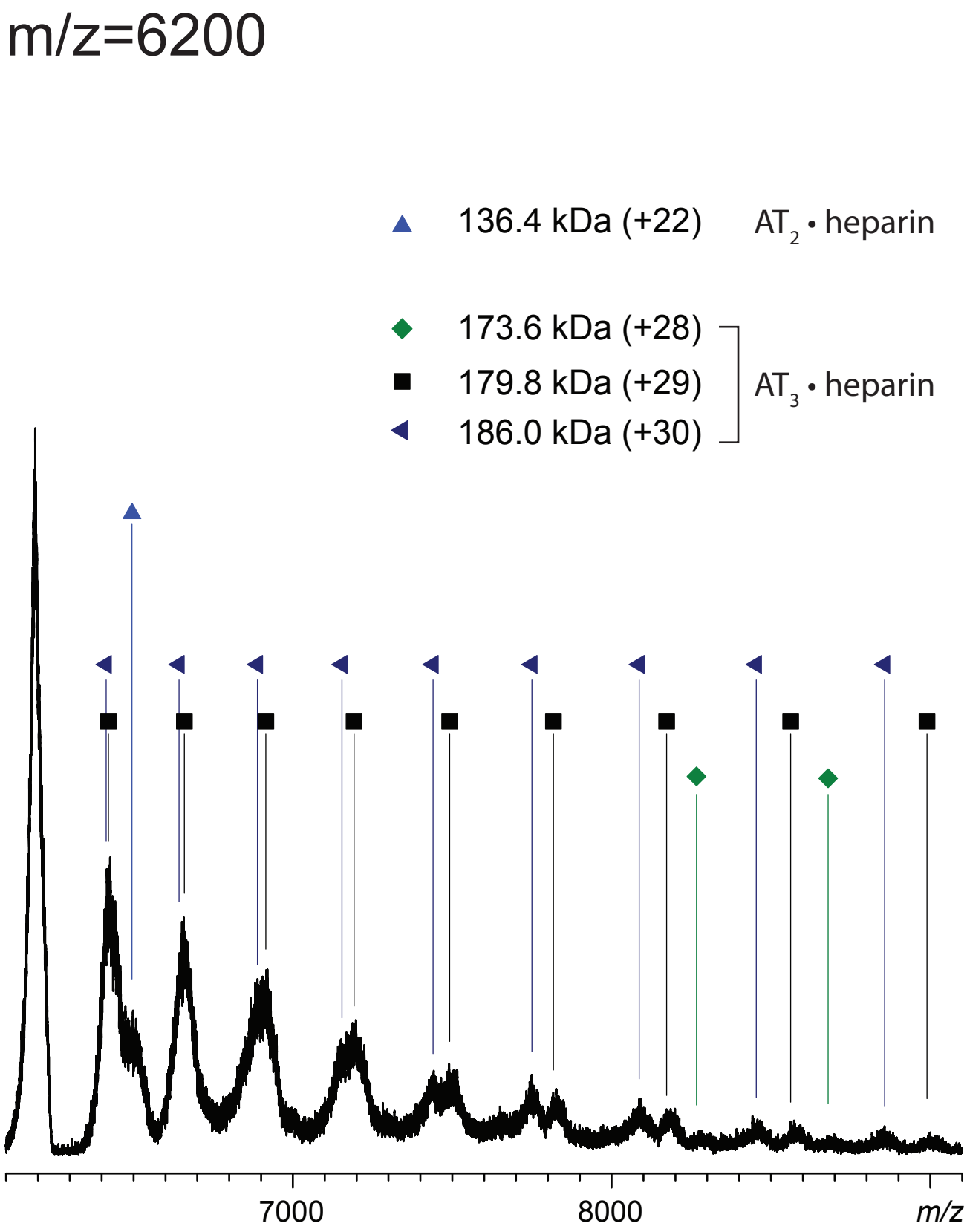


Zhao, et al., **Figure S2**

m/z=6000 (wide window)



Zhao, et al., **Figure S2**



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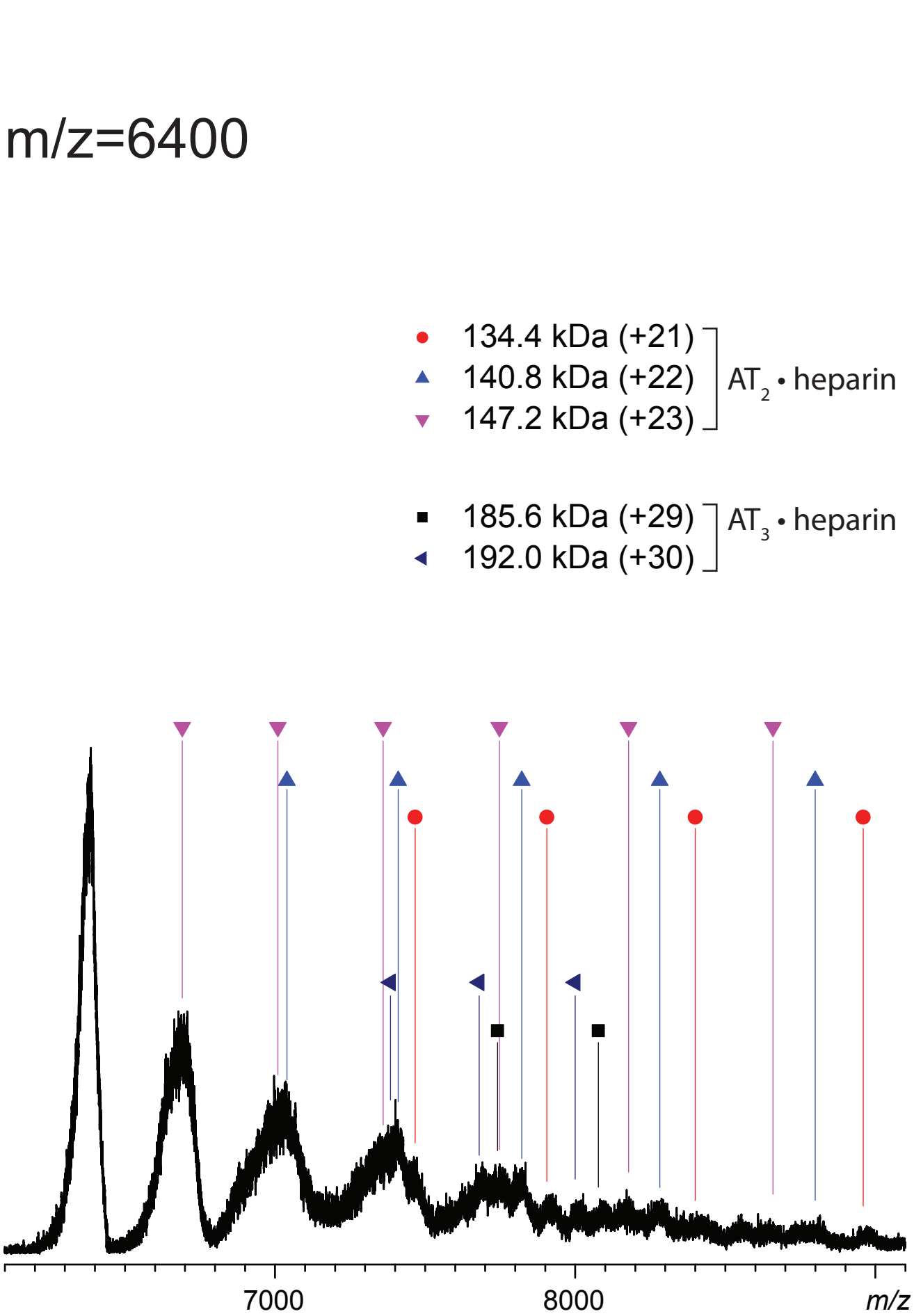

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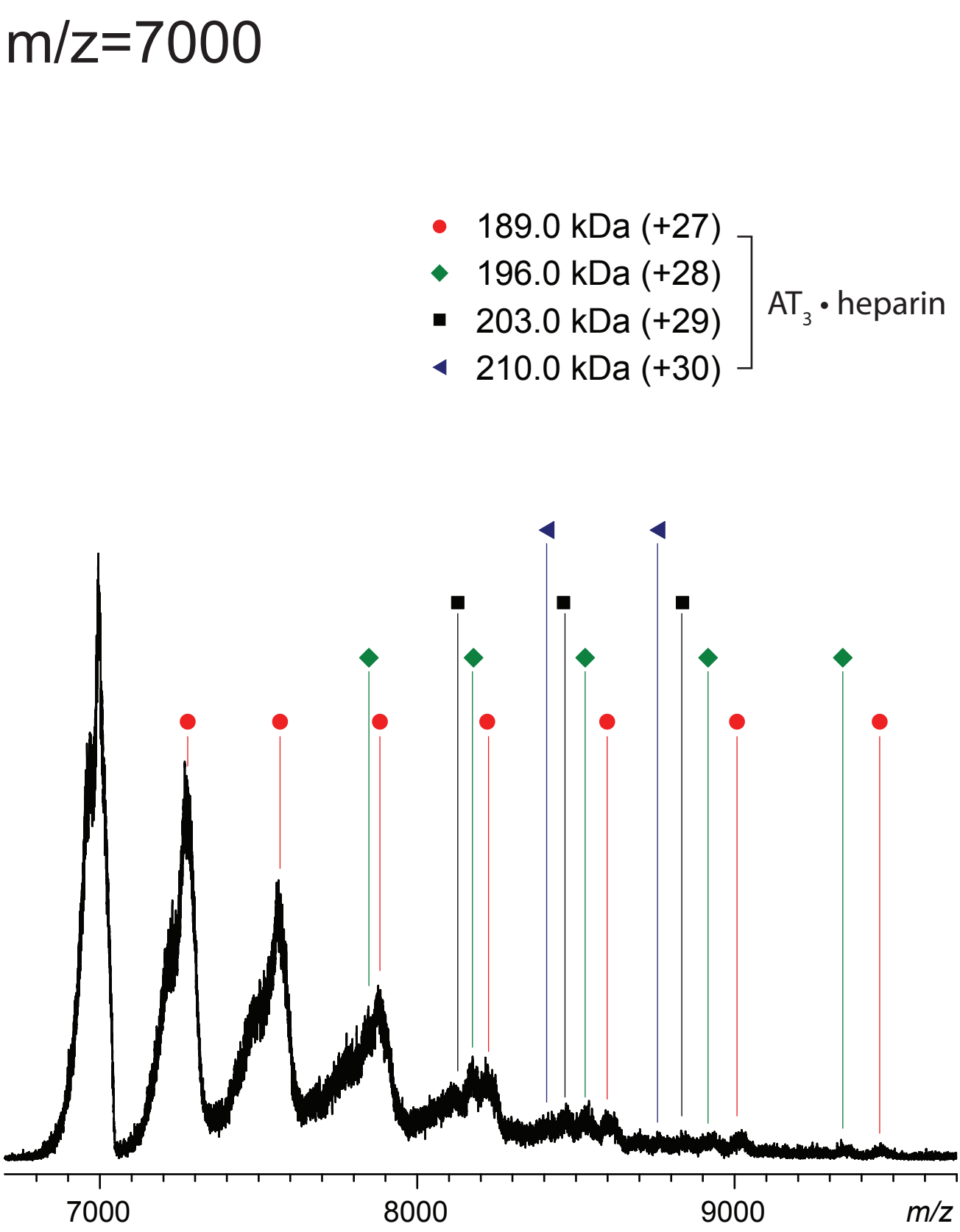

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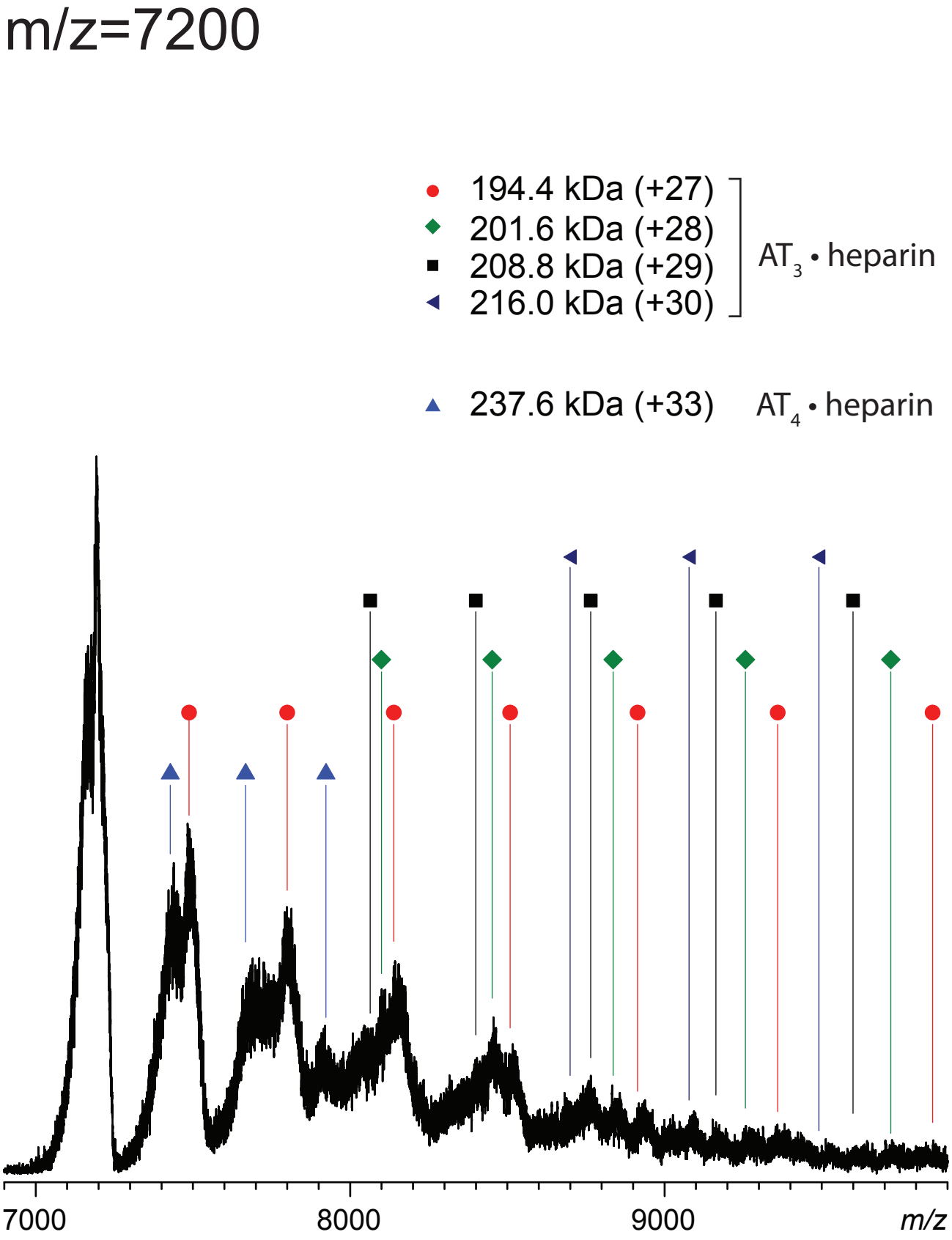

Zhao, et al., **Figure S2**