Supporting Information

Extending native top-down electron capture dissociation to MDa immunoglobulin complexes provides useful sequence tags covering their critical variable complementary determining regions

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Cross-correlation analysis: Spectral comparison of native top-down ECD spectra involved an in-house implementation of the cross-correlation approach pioneered for mass spectra by Yates and coworkers.[1,2] Cross-correlation provides a metric for the pairwise comparison of spectra. The approach is ideally suited for cases where fragment isotope envelopes expand well beyond the first isotopic peak, and this peak's intensity is diminished relative to the base peak in the envelope. For charge-deconvoluted spectra, we report the normalized cross-correlation values for a 1000 *m/z* mass window slid over the spectra in steps of *10 m/z*.

Table S1. Typical instrument transmission and ECD cell parameters for IgM and hexameric IgG1 RGY

Instrument					
S-Lens RF Level	200	IST	-100 (V)	Skimmer Volt.	15.0 (V)
Inj. Flat. Offset	3.0 (V)	Bent. Flat. DC	2.0 (V)	Gate Lens Volt.	2.13 (V)
ECD cell (V)					
L1	2.12	L2	-24.2	LM3	7.0
L4	6.7	FB	1.5	LM5	7.0
L6	-26.2	L7	1.5		
Isolation windows (m/z)	IgM	14100 w 2500	Hex IgG1 RGY	12266 w 2000	
	mono	[5500-7300]	mono	5964 w 50	

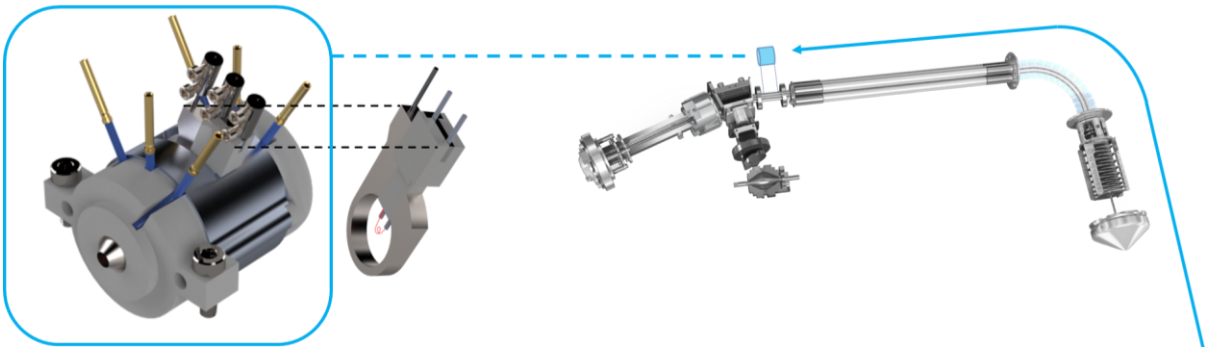


Figure S1. Schematic of the ECD cell implementation

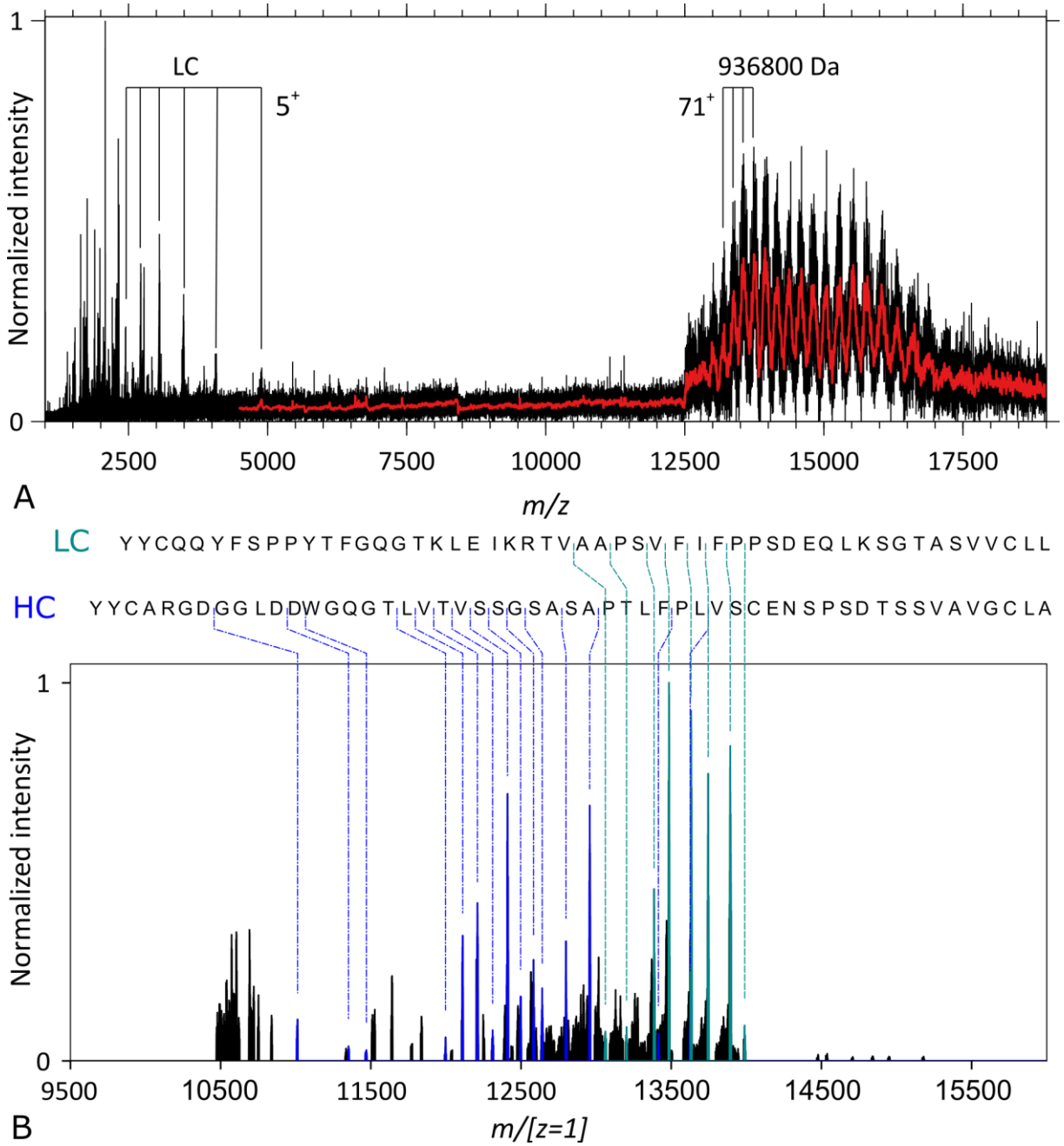


Figure S2. Native top-down CID MS2 spectra of the aWTA IgM assembly. A) Raw CID spectra with the precursor ion signal overlapping with high *m/z* product ions in the upper *m/z* range while in the region below *m/z* 5000 primarily isotopically resolved intact LCs and some backbone fragments are detected. The isolation window is centred on *m/z* 14100 with a width of 2500. B) Charge deconvoluted mass spectrum with LC and HC *b*-ion sequence tags highlighted in dark cyan and blue, respectively.

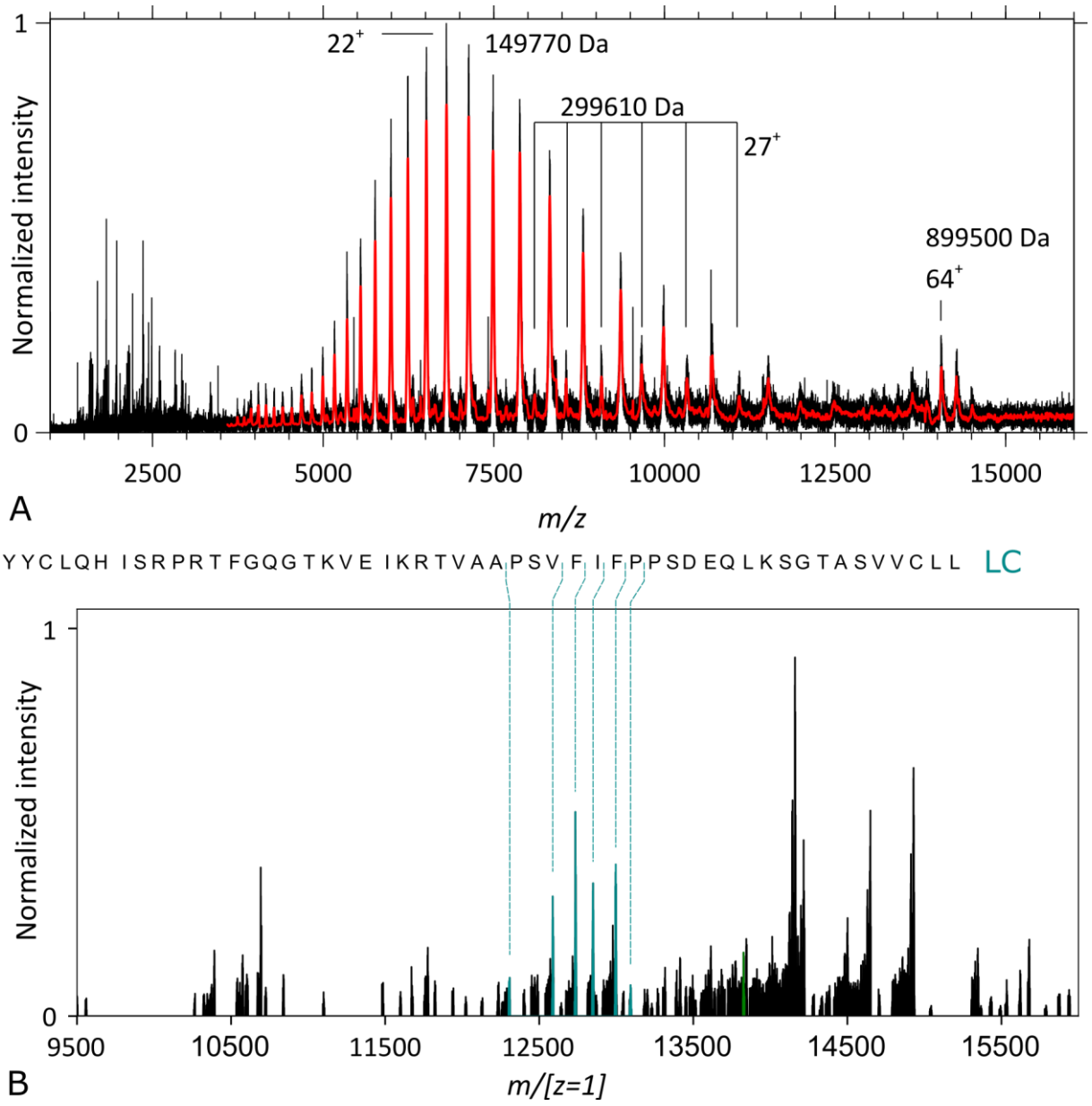


Figure S3. Native top-down CID MS2 spectra of the aCD52 hexameric IgG1 assembly. A) Raw CID spectra with the precursor ion signal at high *m/z*, intact IgG1 monomers in the 3500≤*m/z*≤12500 range, and backbone fragments below *m/z* 3500. The isolation window extends from *m/z* 12000 to *m/z* 15000. B) Charge deconvoluted mass spectrum with a LC *b*-ion sequence tag highlighted in dark cyan.

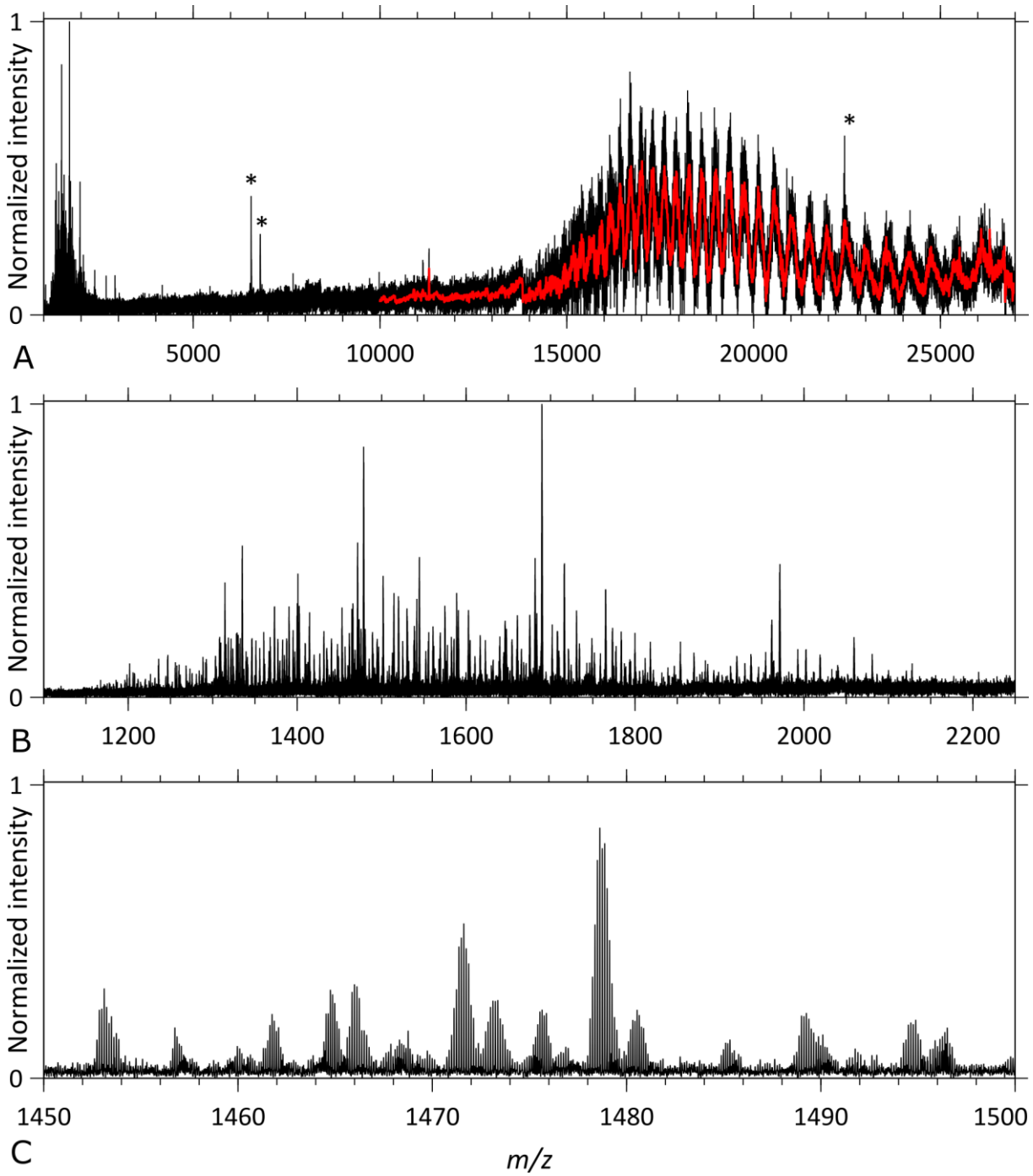


Figure S4. Native top-down ECD MS2 spectra of the aWTA IgM assembly. All these ECD MS2 spectra were taken with the "Analyzer CE-Inject (V) UHMR" at its default value of 3200. A) displaying the precursor ion signal at high *m/z*, and B) backbone fragment ions below *m/z* 3000. C) Illustration of some of the isotopically resolved fragment ion signals.

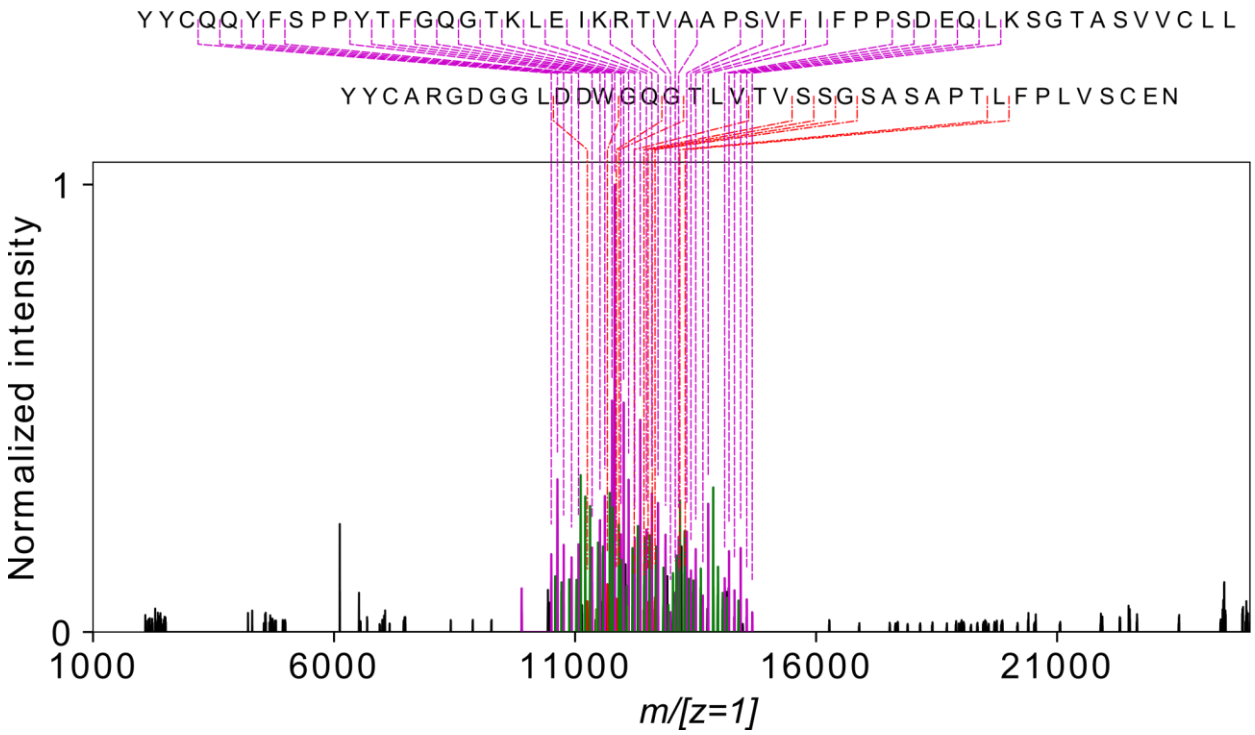


Figure S5. Charge deconvoluted native top-down ECD MS2 spectra of the aWTA IgM assembly.

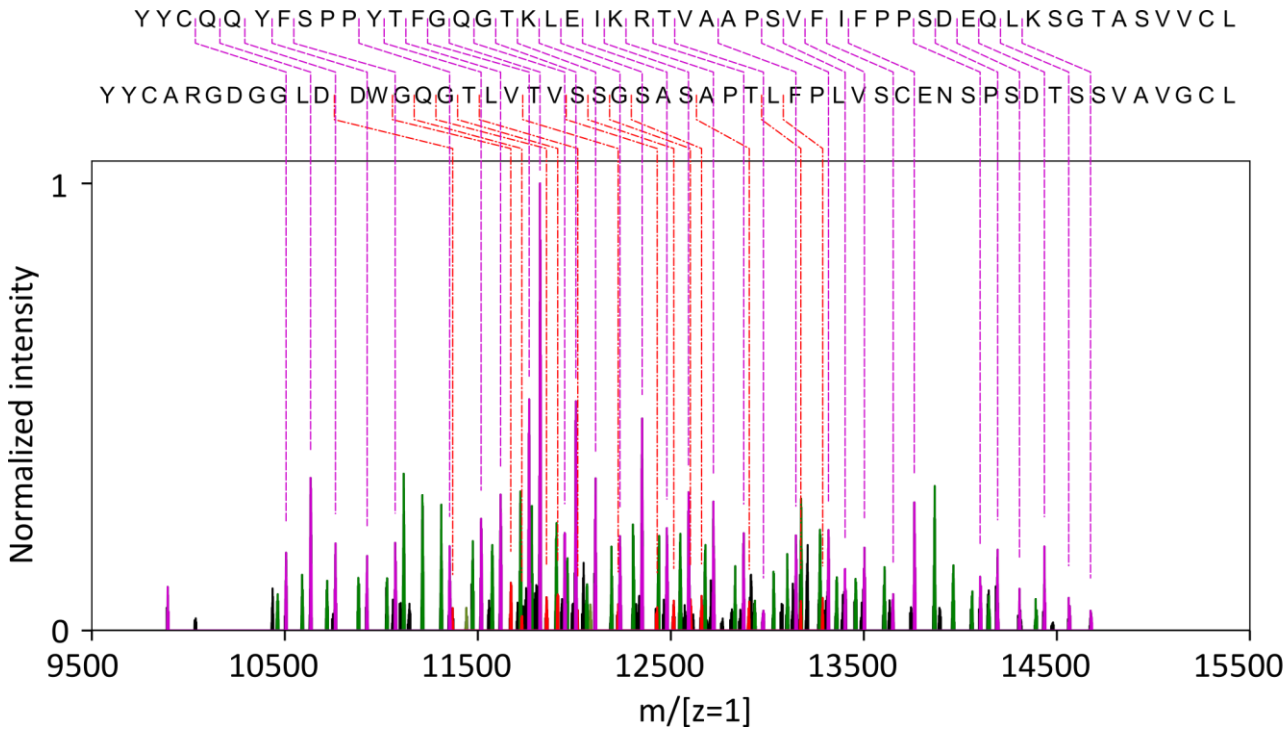


Figure S6. Native top-down ECD MS2 spectra of the aWTA IgM assembly with the Analyzer CE-Inject (V) UHMR 3700.

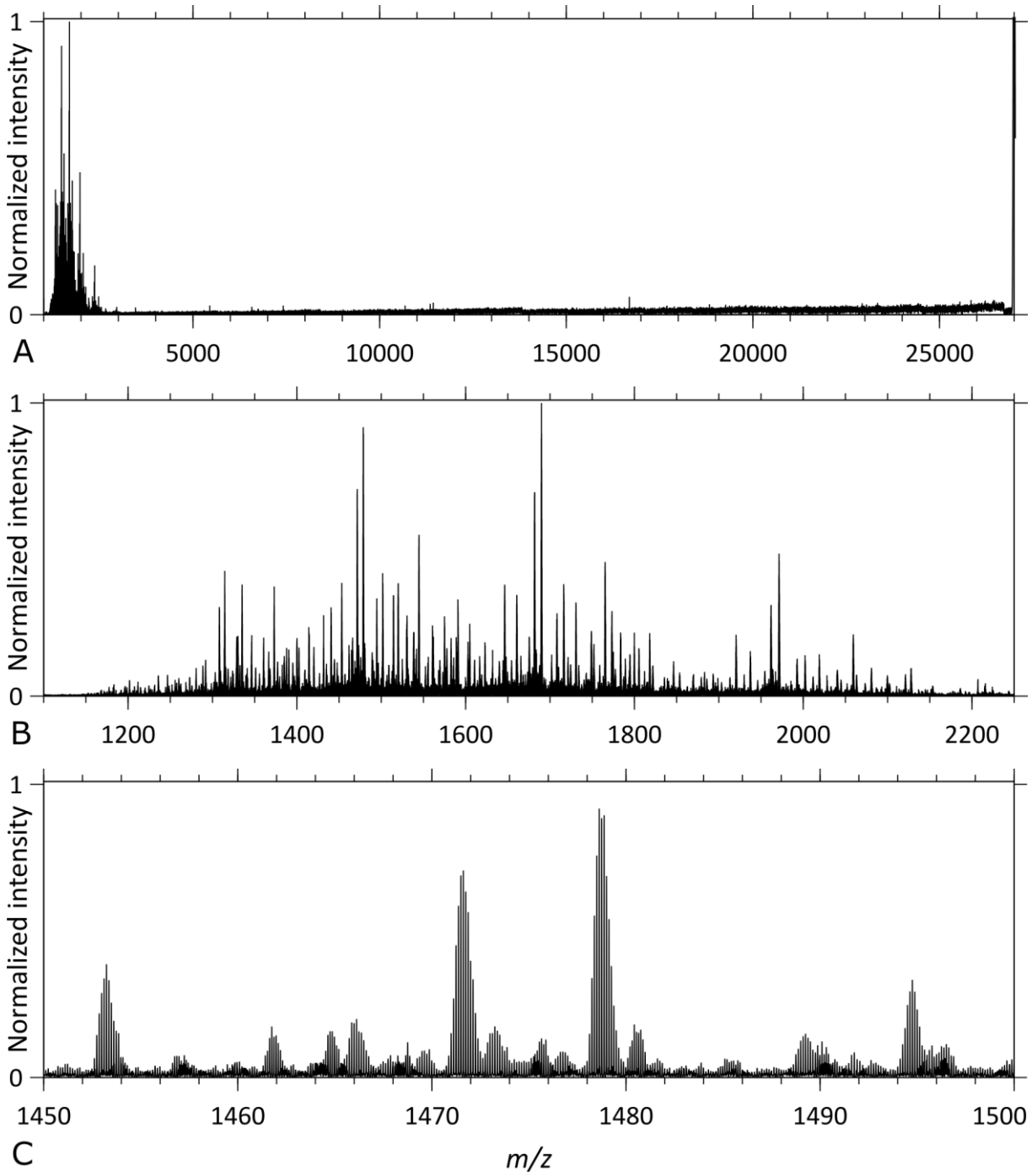


Figure S7. Native top-down ECD MS2 spectra of the aWTA IgM assembly. All these ECD MS2 spectra of the aWTA IgM assembly were taken with the "Analyzer CE-Inject (V) UHMR" at a value of 3700, A) displaying the precursor ion signal at high *m/z*, and B) backbone fragment ions below *m/z* 3000. C) Illustration of some of the isotopically resolved fragment ion signals.

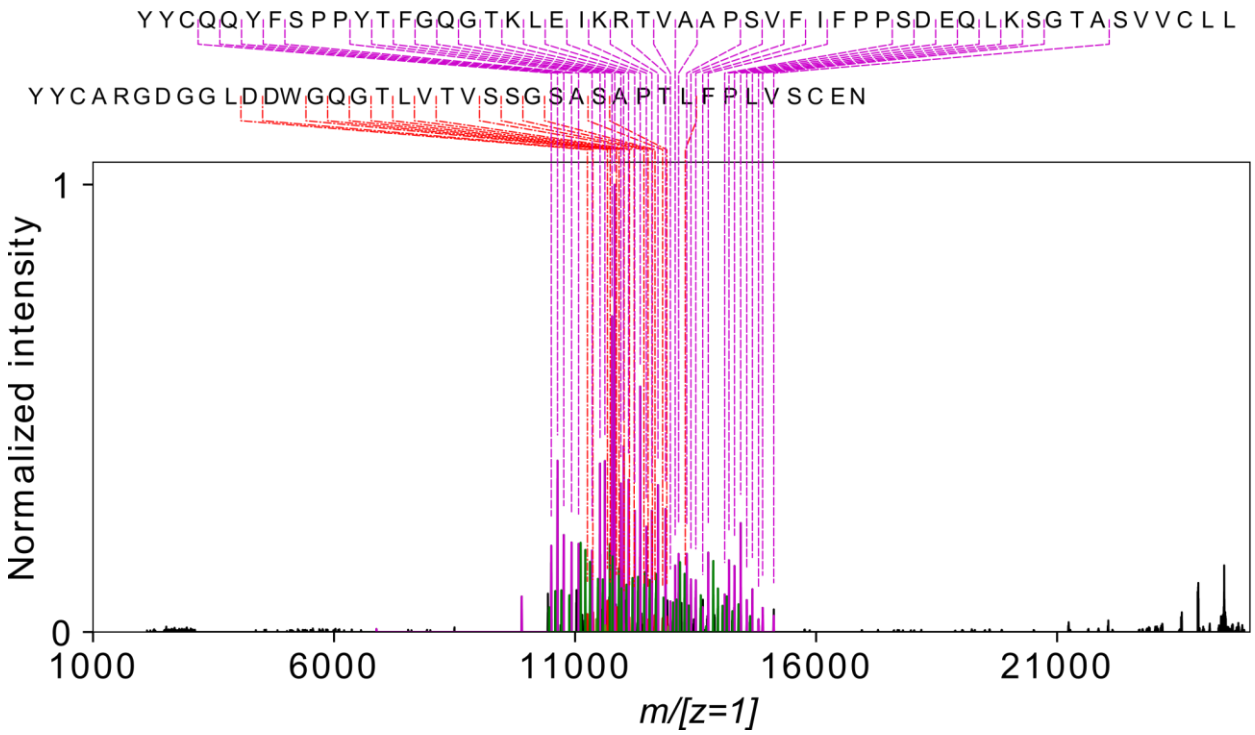


Figure S8. Charge deconvoluted native top-down ECD MS2 spectra of the aWTA IgM assembly.

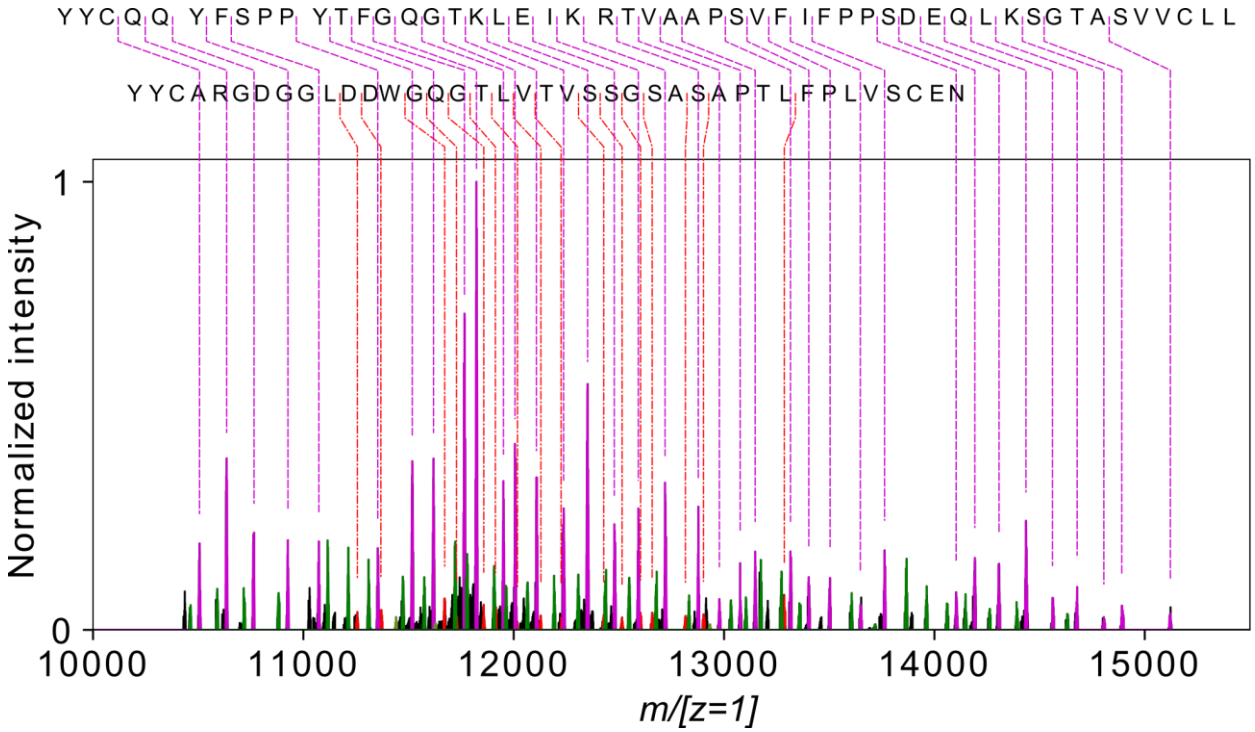


Figure S9. Charge deconvoluted native top-down ECD MS2 spectra of the aWTA IgM assembly. Identical to corresponding figure part in main text.

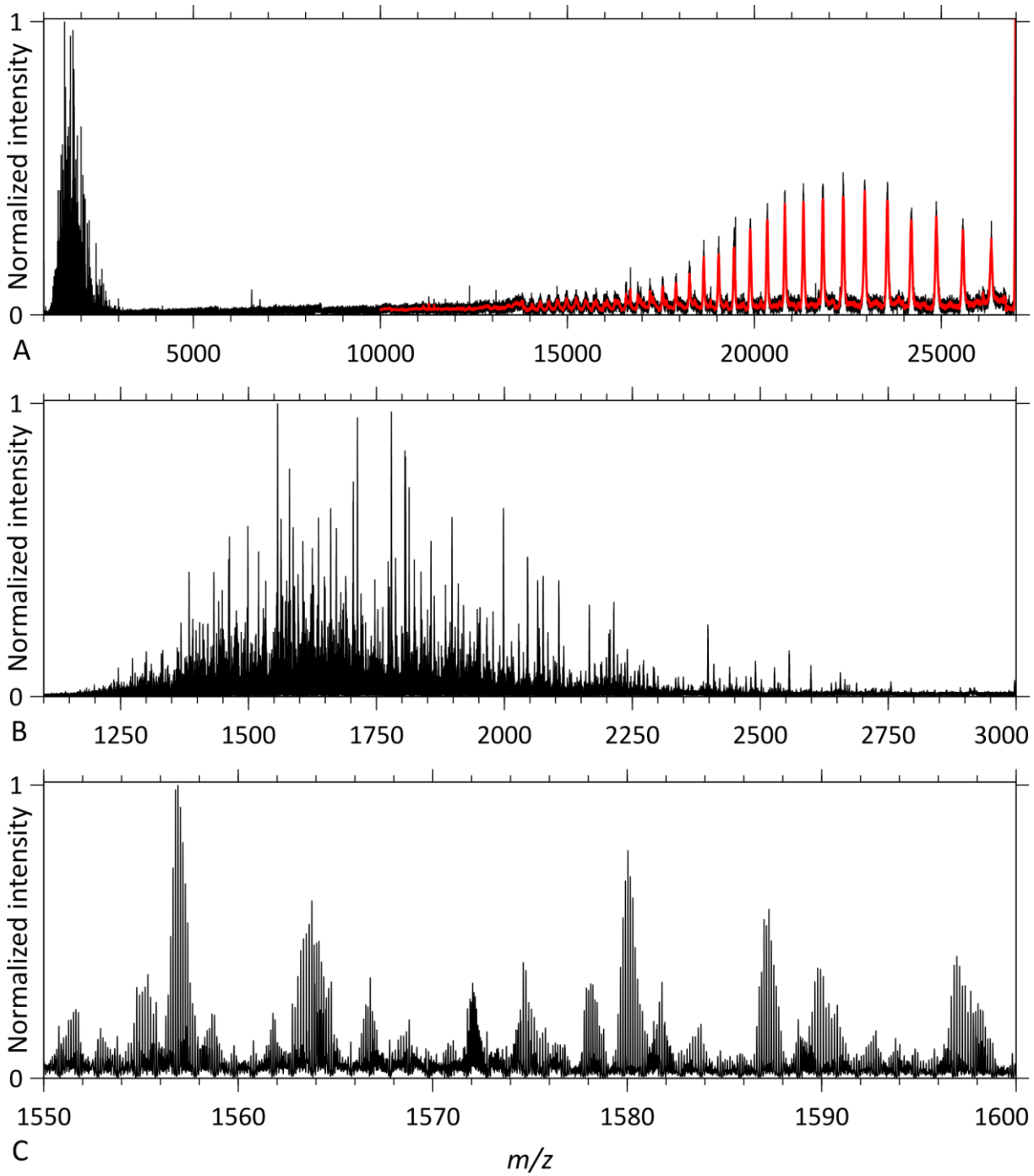


Figure S10. Native top-down ECD MS2 spectra of the hexameric aCD52 IgG1 assembly. All these spectra were taken with the "Analyzer CE-Inject (V) UHMR" at its default value of 3200. A) displaying the precursor ion signal at high *m/z*, and B) backbone fragment ions below *m/z* 3000. C) Illustration of some of the isotopically resolved fragment ion signals.

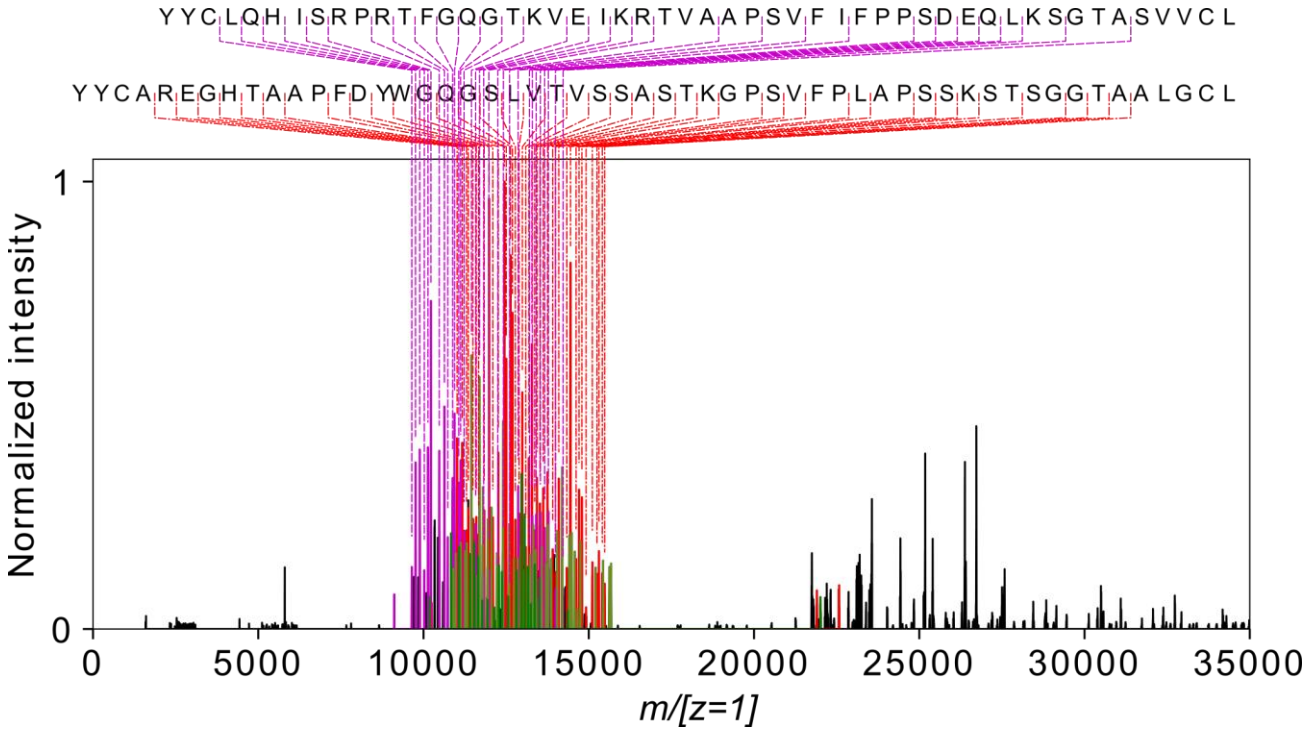


Figure S11. Charge deconvoluted native top-down ECD MS2 spectra of the hexameric aCD52 IgG1 assembly.

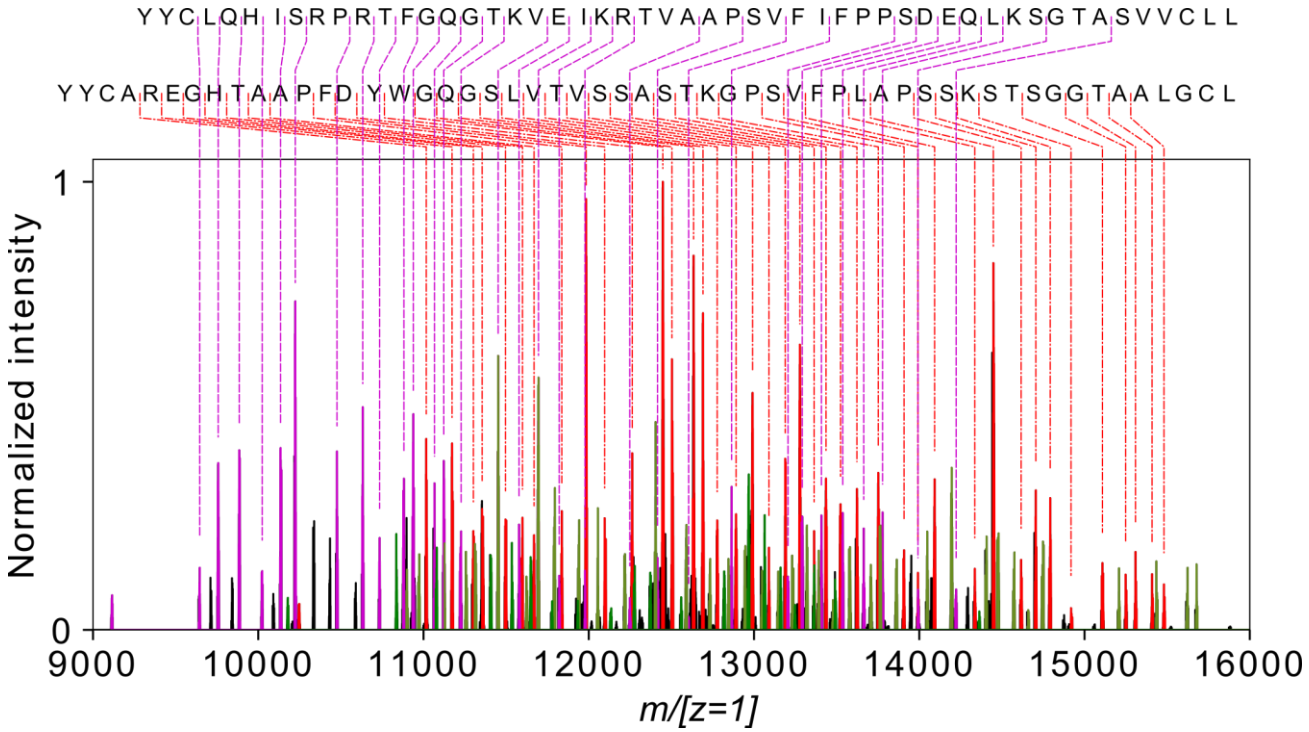


Figure S12. Charge deconvoluted native top-down ECD MS2 spectra of the hexameric aCD52 IgG1 assembly. Identical to corresponding figure part in main text.

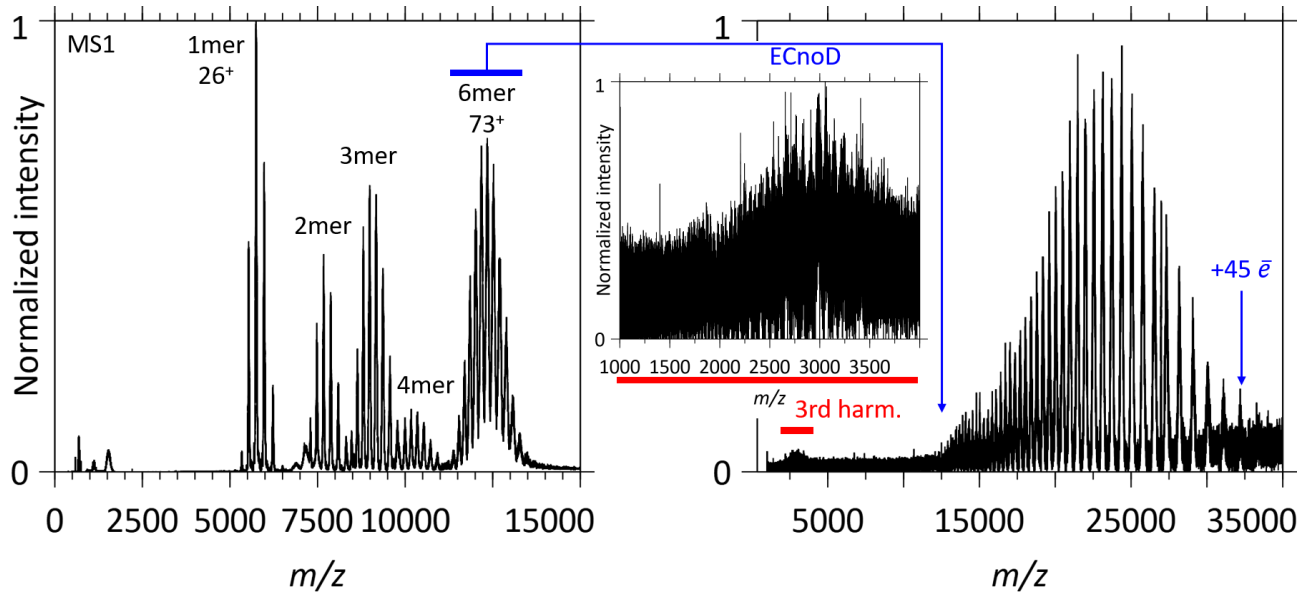
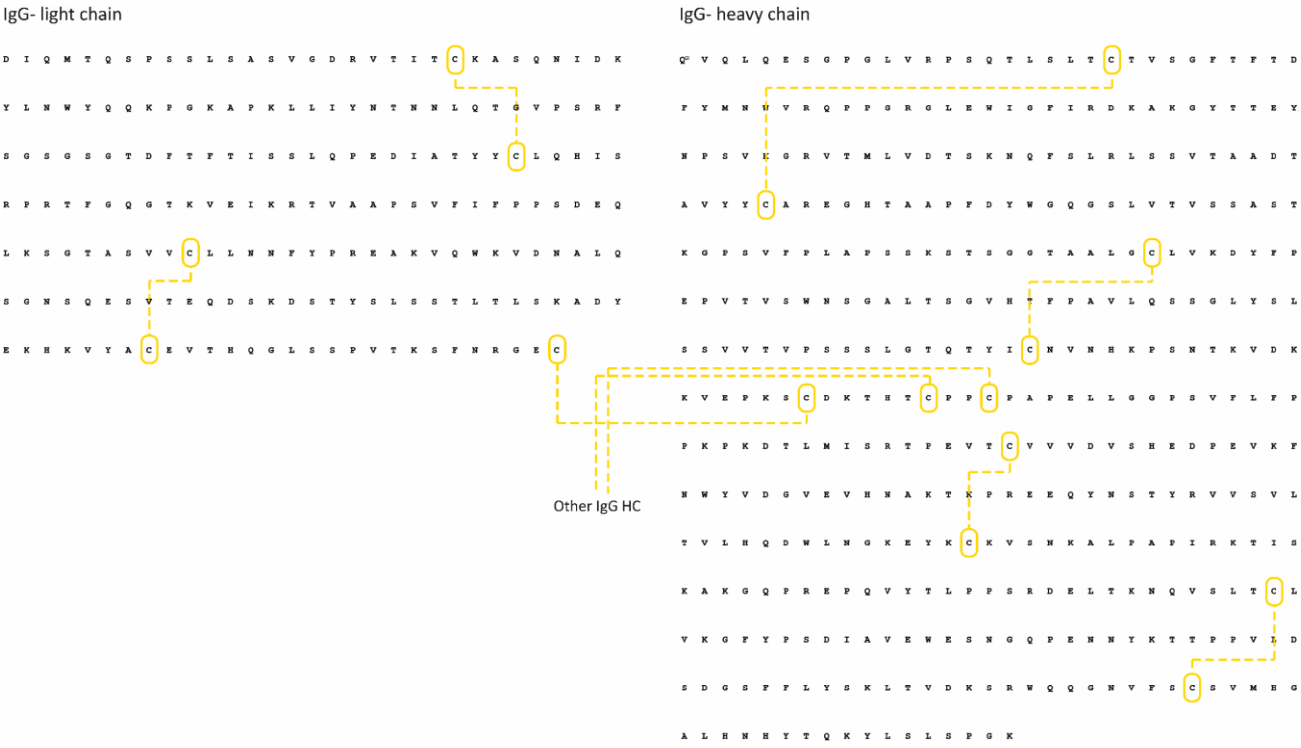
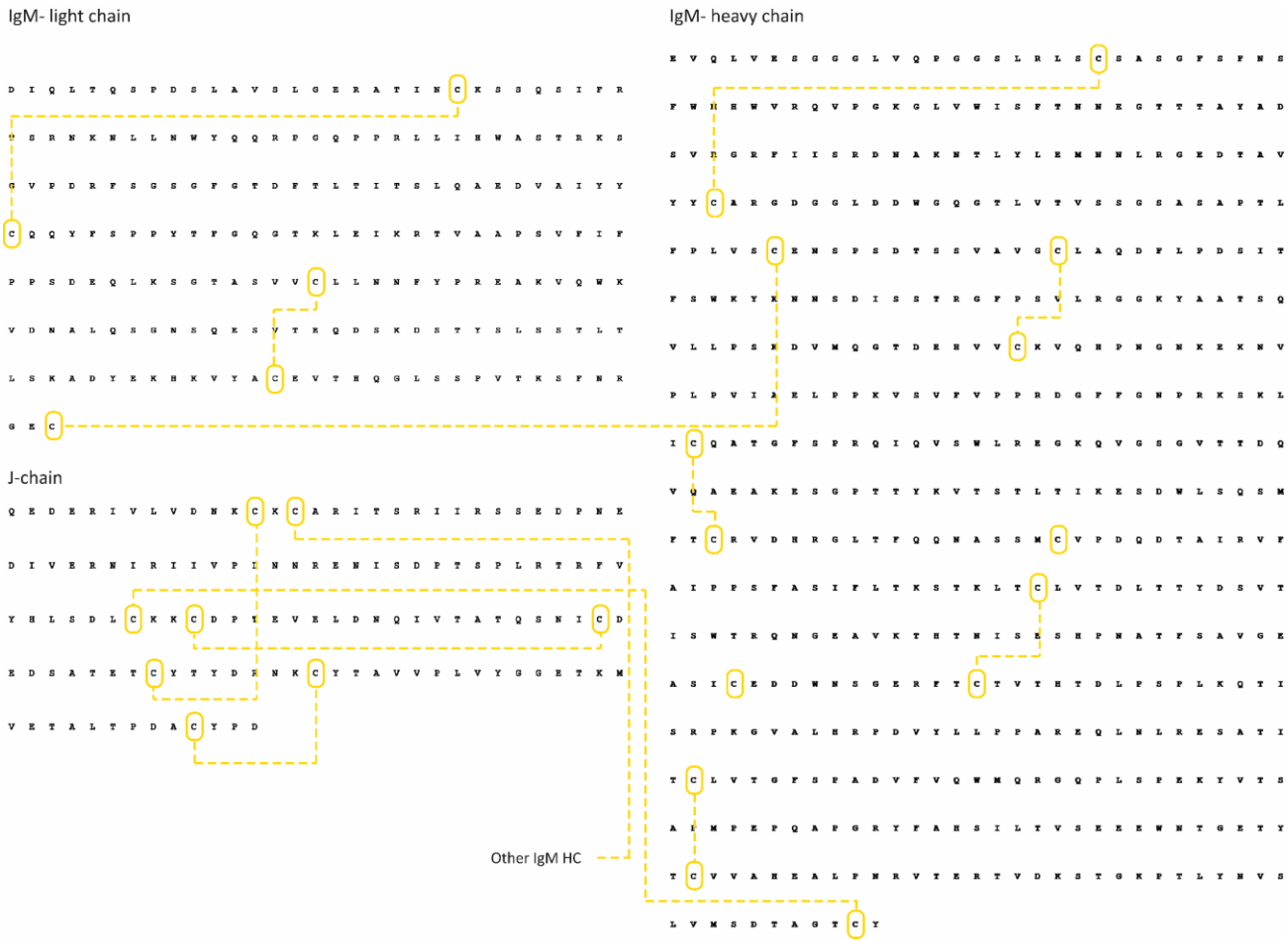


Figure S13. (left) MS1 of the hexameric aCD52 IgG1 solution. (Right) ECnoD spectrum of the hexameric aCD52 IgG1 assembly with the 3rd harmonic as inset. Up to 45 electrons are captured by the hexamer.

Figure S14. Disulfide connectivity maps within IgG and IgM





All raw data files can be accessed at: https://doi.org/10.6084/m9.figshare.16873249

REFRENCES

1. J. D. Venable, T. Xu, D. Cociorva, J. R. Yates, Cross-Correlation Algorithm for Calculation of Peptide Molecular Weight from Tandem Mass Spectra, *Anal. Chem.*, 2006, **78**, 1921–1929, doi:10.1021/ac051636h.

2. J.-F. Greisch, M. A. den Boer, F. Beurskens, J. Schuurman, S. Tamara, A. Bondt, A. J. R. Heck, Generating Informative Sequence Tags from Antigen-Binding Regions of Heavily Glycosylated IgA1 Antibodies by Native Top-Down Electron Capture Dissociation, *J. Am. Soc. Mass Spectrom.*, 2021, doi:10.1021/jasms.0c00461doi:10.1021/jasms.0c00461.