

Mapping a Conformational Epitope of Hemagglutinin A Using Native MS and Ultraviolet Photodissociation

M. Rachel Mehaffey¹, Jiwon Lee^{2,3}, Jiwon Jung⁴, Michael B. Lanzillotti¹, Edwin E. Escobar¹, Keith R. Morgenstern¹, George Georgiou^{3,4,5,6,7}, and Jennifer S. Brodbelt^{1*}

¹Department of Chemistry, ³Department of Chemical Engineering, ⁴Department of Biomedical Engineering, ⁵Institute for Cellular and Molecular Biology, ⁶Center for Systems and Synthetic Biology, and ⁷Department of Molecular Biosciences, University of Texas at Austin, Austin, TX 78712

²Thayer School of Engineering, Dartmouth College, Hanover, NH 03755

Corresponding author:

Jennifer S. Brodbelt
University of Texas at Austin
Department of Chemistry
Austin, TX 78712-0165
jbrodbelt@cm.utexas.edu
Phone: (512)-471-0028

Supporting Information:

Experimental. Detailed descriptions of the protein expression and purification, and on-line size-exclusion chromatography experiment.

Figure S1. Expressed protein sequences of the antibody and antigen.

Figure S2. Crystal structure of HA1 bound to the Fab fragment of an IgG antibody.

Figure S3. MS/MS spectra and sequence coverage maps of the antigen.

Figure S4. ESI-MS and MS/MS spectra of the antibody.

Figure S5. Sequence coverage maps of the antibody.

Figure S6. ESI-MS for optimization of the in-source trapping voltage.

Figure S7. ESI-MS and MS/MS spectra of the antibody-antigen complex.

Figure S8. Sequence coverage maps for each component of the antibody-antigen complex.

Figure S9. Comparison of sequence coverage of the antigen before and after complexation with the antibody.

Figure S10. Sequence ion type distributions for the four distinct regions of the antigen.

Figure S11. Number of sequence ions produced adjacent to each residue.

Figure S12. Distributions of ion types produced adjacent to each residue.

Table S1. Identified fragment ions from MS/MS spectra of the antigen.

Table S2. Identified fragment ions from MS/MS spectra of the antibody.

Table S3. Identified fragment ions from MS/MS spectra of the antibody-antigen complex.

EXPERIMENTAL

Recombinant Protein Expression & Purification

Genes for the antibody and antigen involved in the study were purchased as gBlocks (Integrated DNA Technologies) and cloned into the pcDNA3.4 vector (Invitrogen). For monoclonal antibody expression, heavy and light chain plasmids for D1 H1-17/H3-14 IgG were transfected into Expi293 cells (Invitrogen) at a 1:3 ratio. After incubating for 5 days at 37 °C with 8% CO₂, the supernatant containing secreted antibodies was collected by centrifugation at 500×g for 15 min at room temperature. Supernatant was passed over a column with 1 mL Protein A agarose resin (Thermo Scientific) three times to ensure efficient capture. After washing the column with 20 cv of PBS, antibodies were eluted with 3 mL of 100 mM glycine-HCl, pH 2.7 and immediately neutralized with 1 mL of 1 M Tris-HCl, pH 8.0. Antibodies were buffer-exchanged into PBS utilizing Amicon Ultra-30 centrifugal spin columns (Millipore). Similarly, for the HA1 antigen expression, after incubating for 5 days at 37 °C with 8% CO₂, the supernatant containing secreted protein was collected by centrifugation at 500×g for 15 min at room temperature. Supernatant was passed over 2 mL Ni-NTA agarose (QIAGEN) affinity column in gravity mode. Flow-through sample was collected and passed through the column three times. The column was washed with 15 cv of 10 mM imidazole, 150 nM NaCl, 100 mM Tris-HCl, pH 7.5 prior to eluting with 5 cv of 250 mM imidazole, 150 nM NaCl, 100 mM Tris-HCl, pH 7.5. Eluates were then buffer-exchanged into PBS using Amicon Ultra-30 centrifugal spin columns (Millipore).

Size-Exclusion Chromatography

The on-line size-exclusion chromatography experiments were performed using a Dionex LC system (Thermo Scientific) interfaced to the modified Q Exactive UHMR mass spectrometer. Separation was carried out on a 2.1 mm × 150 mm Waters ACQUITY UPLC Protein BEH SEC column with a 200 Å pore size and 1.7 µm particle size (Milford, MA). An isocratic mobile phase comprised of 20 mM ammonium acetate (pH 6.8) and flowing at 100 µL min⁻¹ was used to elute a 5 µL injection of the 1:2 antibody:antigen sample (1 µg/µL) after deglycosylation. An applied voltage of 4 kV on the HESI source interfacing the LC to the MS was used to ionize the effluent. An in-source trapping value of -150 V allowed efficient desolvation of the antibody-antigen complex. ESI-MS were collected at a resolving power of 12500 at *m/z* 200.

Figure S1. Expressed sequences of the (A) D1 H1-17/H3-14 IgG monoclonal antibody and (B) HA1 domain (residues 57-267) of the corresponding HA protomer from H1N1 A/California/04/2009 used for all experiments. Cysteine residues are shown in yellow font, and expected disulfide linkages are represented as dotted lines. Calculated average masses for each sequence account for disulfide bonds. A GOF glycan is expected to exist at N306 on the heavy chain of the antibody (highlighted in blue).

(A) D1 H1-17/H3-14 IgG monoclonal antibody (145,650 Da)

Heavy chain (49,713 Da)

N	QVQLQESGPGLVKPSESLSLT	C SVSGGSVSSNLHYWSWIRQLPGKGLEWI	50
51	GYISYTGSTKYNPSLNGRVTLSIDASKNQFSLELSSVTAADTAVYY	C ARD	100
101	FFEKLIAADDLNNAFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAA		150
151	LG C LVKDYFPEPVTLWNNSGALTSGVHTFPALQSSGLYSLSSVVTPSS		200
201	SLGTQTY C NVNHKPSNTEVDKKVEPK C DKTHT C PPC PAPELLGGPSVF		250
251	LFPPKPKDTLMISRTP E VT C VVVDVSHEDEPEVKFNWYVDGVEVHNAKTKP		300
301	REEQY N STYRVVSVLTVLHQDWLNGKEY C KVSNKALPAIEKTISKAKG		350
351	QPREPQVYTLPPSRDELTKNQVSLT C LVKGFYPSDIAVEWESNGQPENNY		400
401	KTPPPVLDSDGSFFLYKLTVDKSRWQQGNVF C SVMHEALHNHYTQKSL		450
451	LSPGK	C	

Light chain (23,112 Da)

N	DIRVTQSPSSLSASVGDRV T IT C RASQSISRSLNWYQQRPGKAPKFLIYA	50
51	ASNLQSGVPSRFSGGGSGTDFTLT I SSLQPEDFATYY C QETYSRTFGQGT	100
101	KADIKRTAAAPS F IFPPSDEQLKSGTASVV C LLNNFYPREAKVQWKVDN	150
151	ALQSGNSQESVTEQDSKDSTYSLSSTL C SKADYEKHKVY A C E VTHQGLS	200
201	SPVTKSFRNR G C	

(B) HA1 domain (residues 57-267) from H1N1 A/California/04/2009 (25,402 Da)

N	APLHLGK C NAGWILGNPE C ESLSTASSWSYIVETPSSDNGT C YPGDFID	50
51	YEELREQLSSVSSFERFE I FPKTSSWPNHDSNKGVTAAC C PHAGAKSFYKN	100
101	LIWLVKKGNSYPKLSKSYINDKGKEVLVLWGIHHPSTSADQQSLYQNADT	150
151	YVFVGSSRYSKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGN	200
201	LVVPRYAFAMERNAGSGHHHHHHHH	C

Figure S2. Crystal structure with a 180° view of the HA1 domain of an HA protomer (H3N2 A/Texas/50/2012) bound to the antigen binding fragment (Fab) region of the S5V2-29 IgG monoclonal antibody (PDB ID: 6E4X) with subunits labelled. The two epitope regions are highlighted (red, green) along the HA1 subunit (gray) with the corresponding epitope sequences from the HA strain expressed for this study shown (numbering is based on the HA protomer sequence; these residues correspond to T35-R55 and F163-Y193 in the expressed HA1 sequence shown in **Figure S1B**). The heavy chain (HC) and light chain (LC) of the antibody are colored separately (turquoise and orange, respectively).

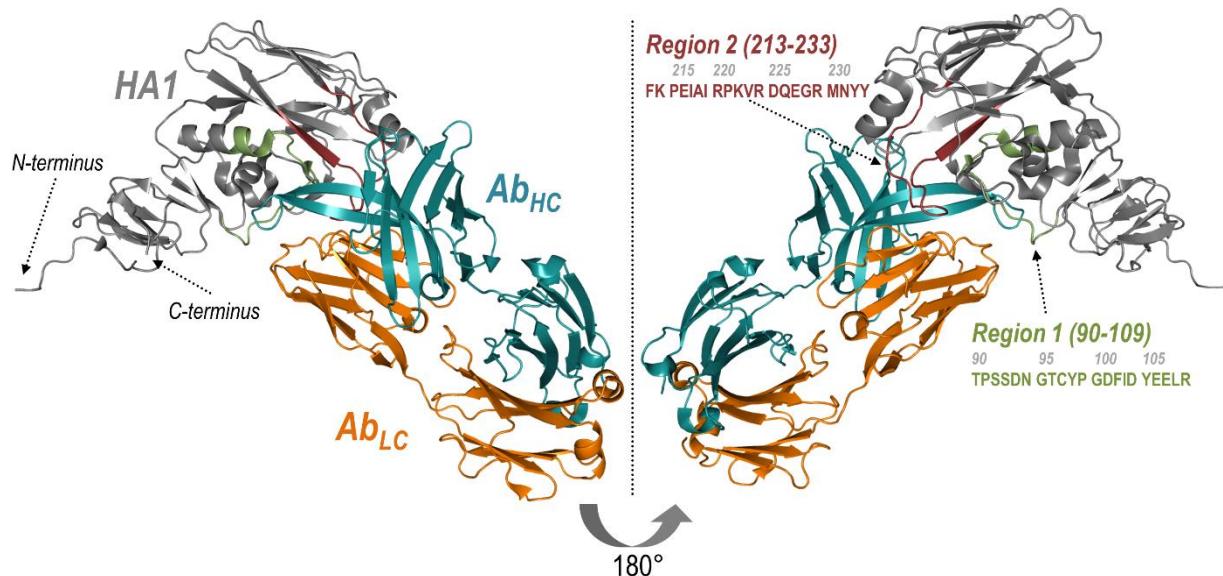


Figure S3. (A) MS/MS spectra resulting from activation of the 10+ charge state of HA1 with HCD (left) and UVPD (right). Several abundant fragment ions are identified and labelled. (B) Corresponding deconvoluted MS/MS spectra in which the precursor is denoted with a filled circle. Monoisotopic masses identified as assignable fragment ions originating from backbone cleavages of HA1 are used to create the sequence coverage maps in (C). Searches accounted for disulfide bonds at all four cysteine residues (highlighted in gray). Lists of all matched fragment ions are given in **Table S1**.

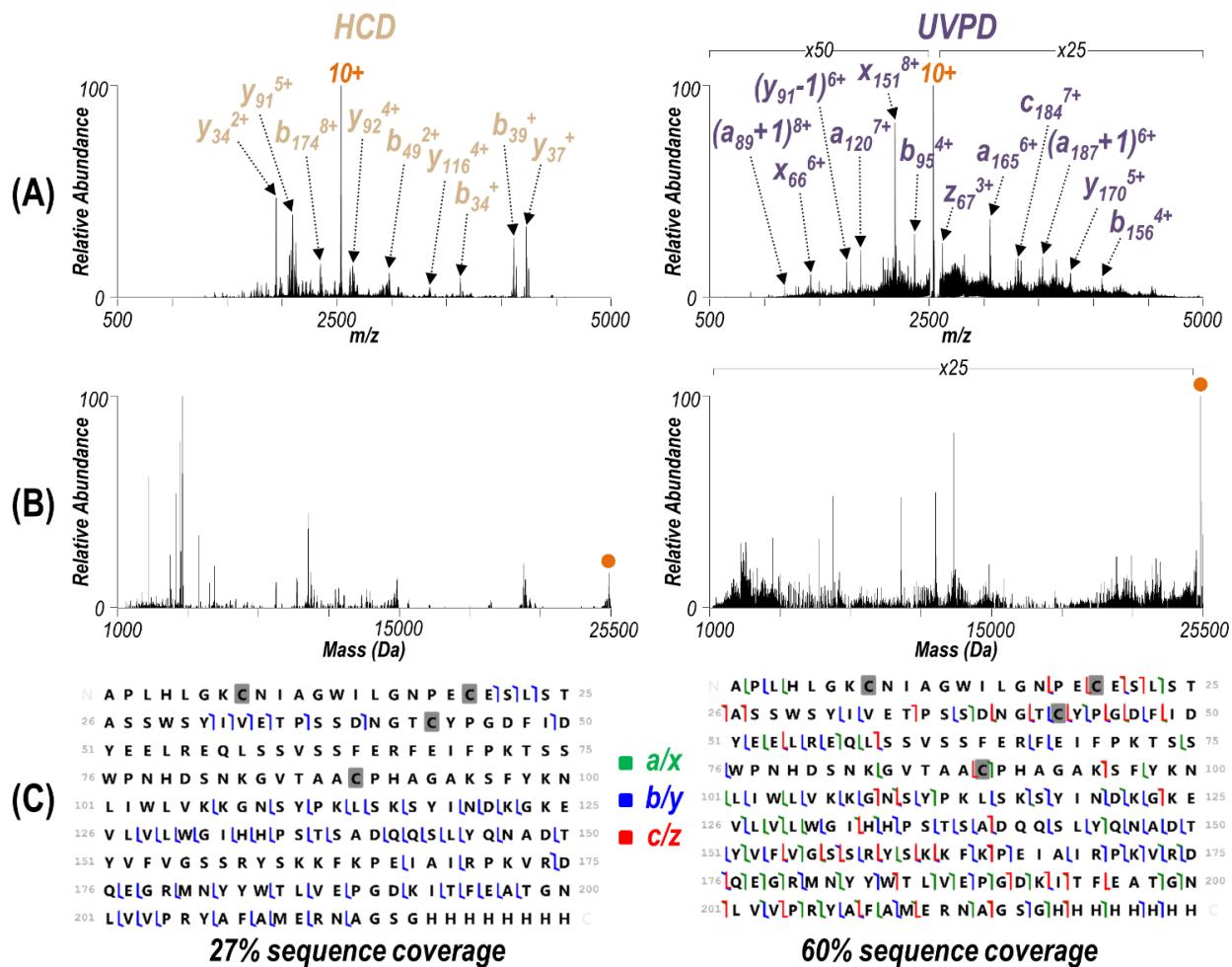


Figure S4. (A) ESI-MS of the D1 H1-17/H3-14 antibody in 20 mM ammonium acetate after reaction with PNGase F. Observed charge states are labelled and glycoforms are denoted with turquoise colored circles. Even with the short reaction time, the most abundant species corresponds to removal of the G0F glycan from both heavy chains. The 25+ charge state of the antibody without glycans was selectively isolated and activated with (B) HCD and (C) UVPD. Several abundant fragment ions resulting from HCD (tan) and UVPD (purple) are labelled corresponding to the sequences of the light chain (light color) or heavy chain (dark color).

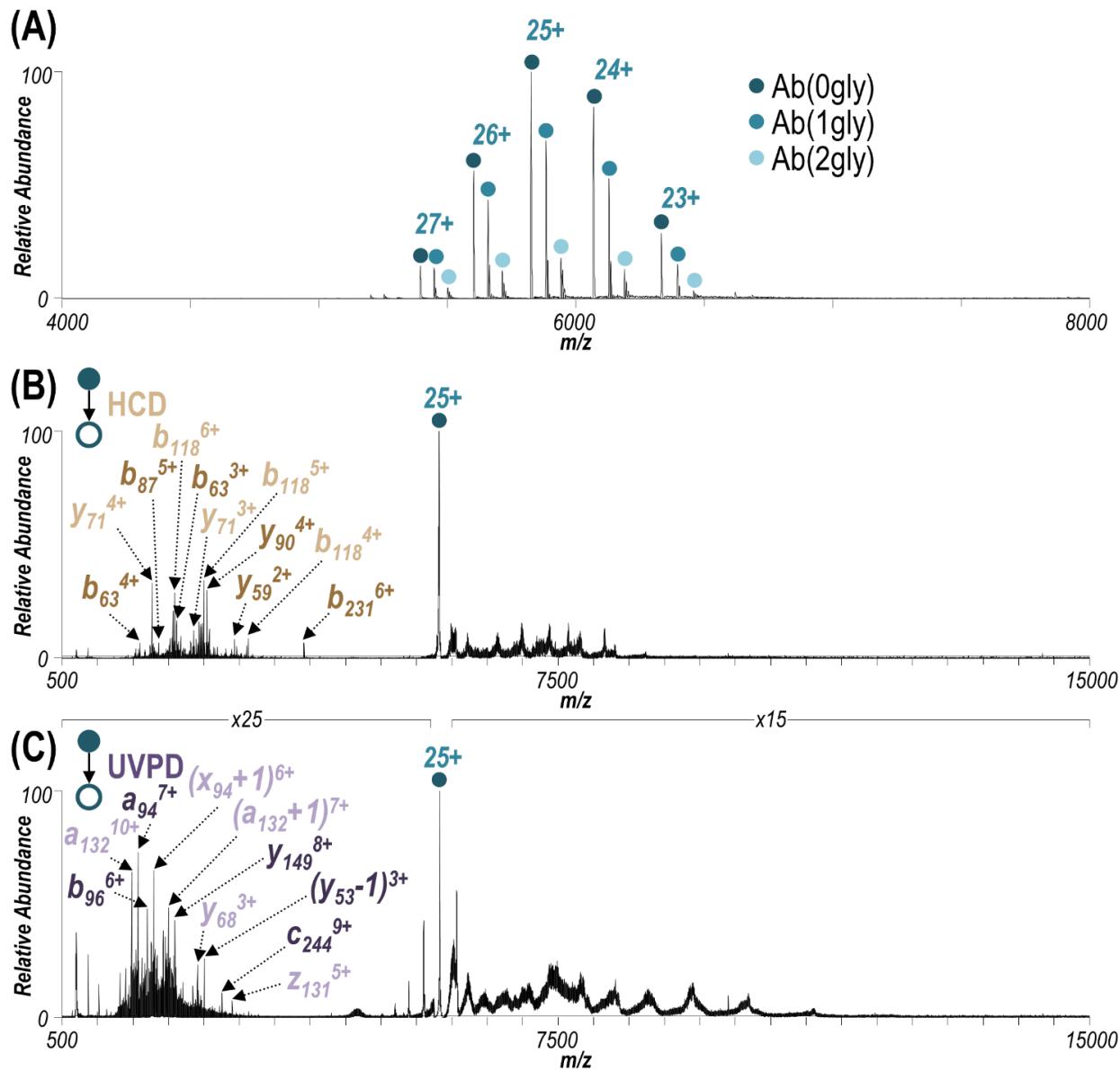


Figure S5. (A) Spectra resulting from deconvolution of the HCD and UVPD MS/MS spectra of the D1 H1-17/H3-14 antibody shown in **Figure S4**. Monoisotopic masses identified as assignable fragment ions originating from backbone cleavages of the (B) light chain and (C) heavy chain of the antibody are used to create the sequence coverage maps. The eight disulfide bonds that stabilize the antibody structure (highlighted in gray) were assumed to be intact for fragment ion identification. **Table S2** gives a list of all matched fragment ions.

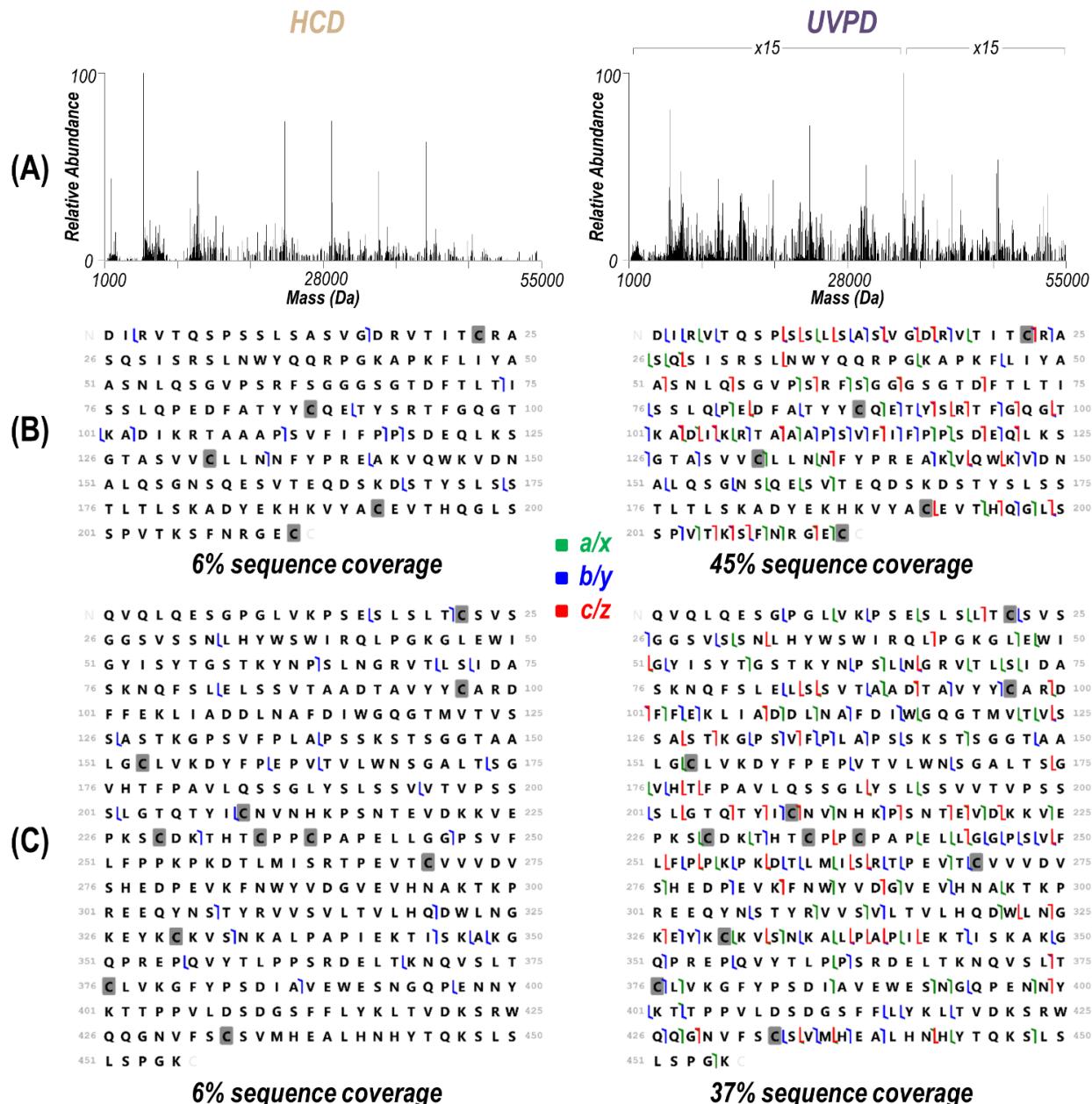


Figure S6. ESI-MS of the antibody-antigen complex collected using various in-source trapping energies (-10 V to -250 V) to determine the ideal value for analysis. Up to three species are observed including HA1 monomer (orange), 1:2 antibody:antigen complex (turquoise), and 1:1 antibody:antigen complex (green). At low IST values (top), the complex is poorly desolvated resulting in many adducts, while at high IST values (bottom) significant disruption of the complex and ejection of one HA monomer occurs.

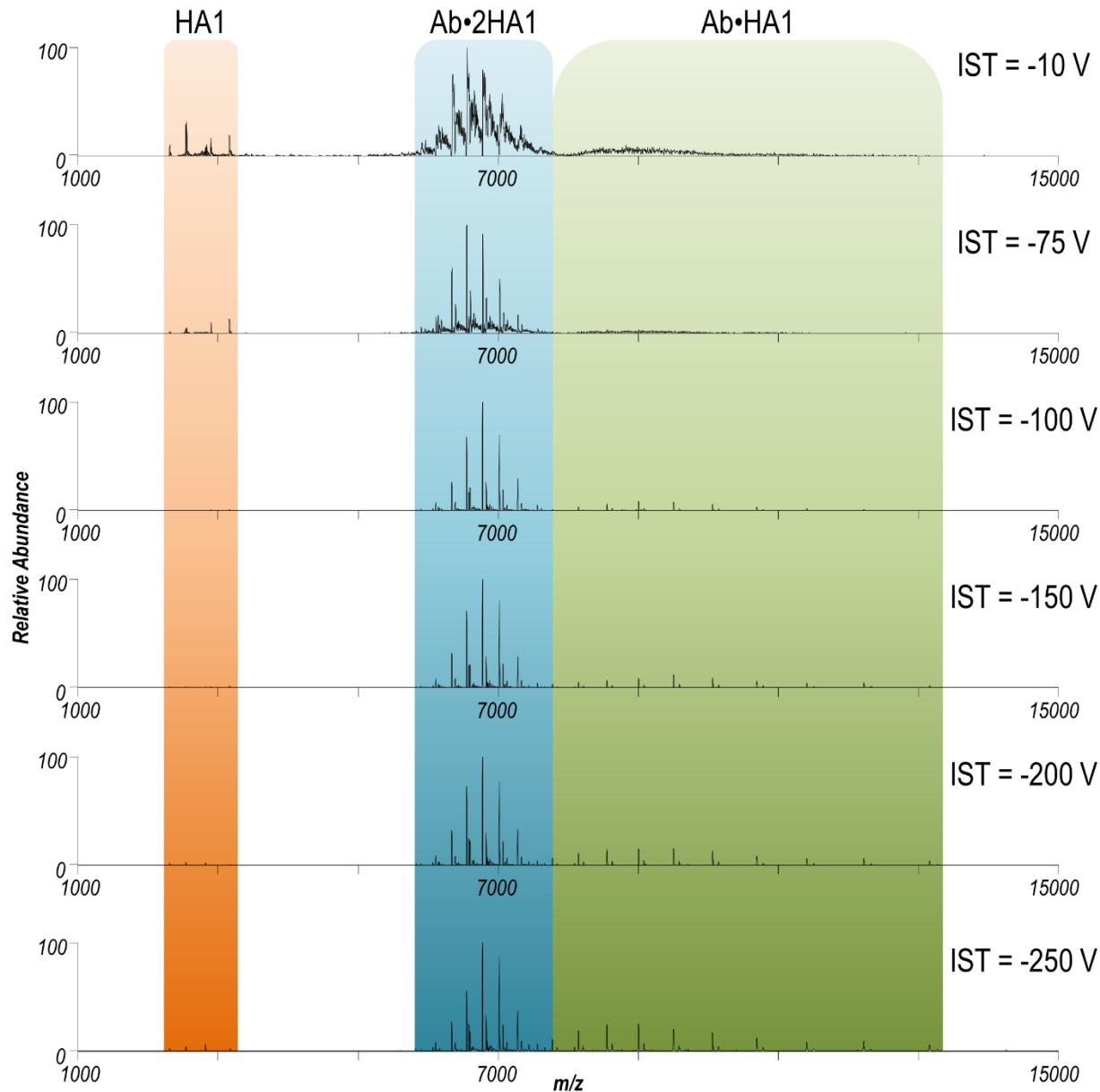


Figure S7. (A) ESI mass spectrum of a solution containing 1:2 antibody:antigen using an in-source trapping energy of -100 V. Charge states of the two observed species are labelled: Ab•2HA1 (turquoise) and Ab•HA1 (green). The 29+ charge state of the antibody-antigen complex was selectively isolated and activated with (B) HCD or (C) UVPD to yield the MS/MS spectra. Observed charge states of intact subunits of HA1 (orange) or Ab•HA1 (green) are identified, suggesting antigen ejection was a more favored fragmentation pathway for HCD compared to UVPD.

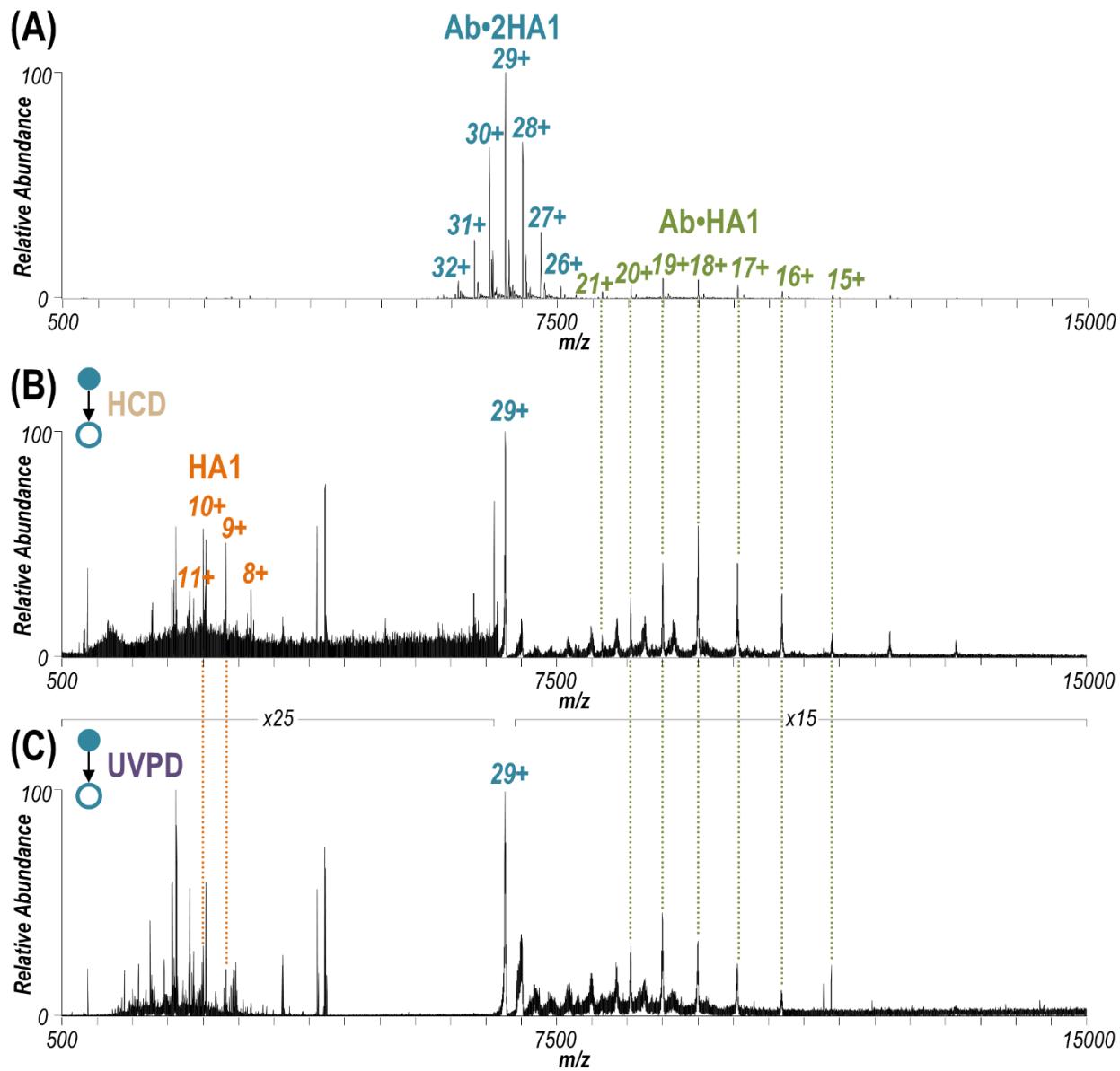


Figure S8. (A) Deconvoluted HCD (left) and UVPD (right) mass spectra corresponding to the MS/MS spectra of the antibody-antigen complex in **Figure S7**. Fragment ions originating from backbone cleavages were searched against the sequences of (B) HA1, (C) antibody light chain, and (D) antibody heavy chain to create the sequence coverage maps. All cysteine residues (highlighted in gray) were assumed to be disulfide bound. None of the observed fragment ions matched expected backbone cleavages for more than one of the three sequences. Sequence coverages are reported for each protein in the complex. A list of all identified fragment ions is given in **Table S3**.

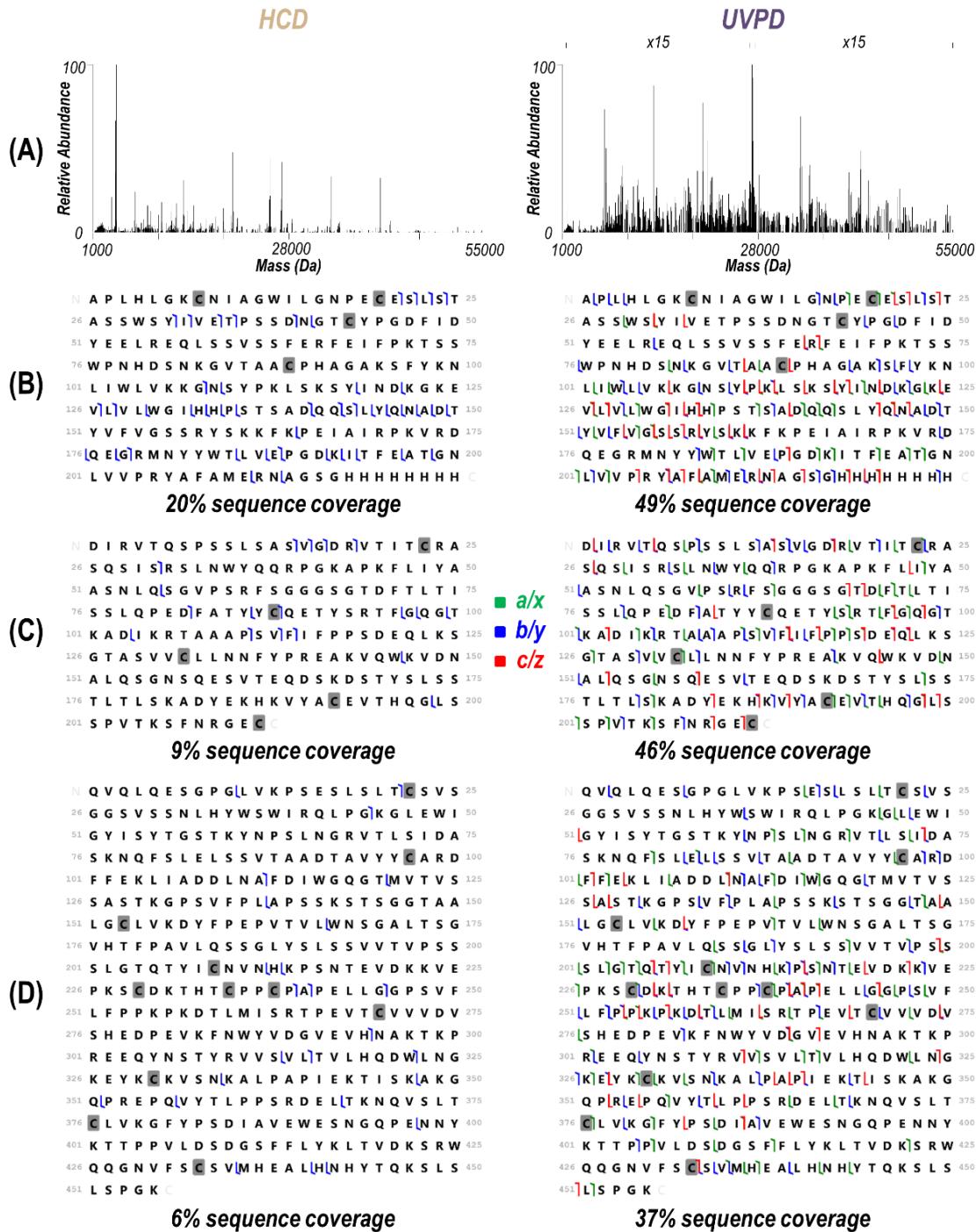


Figure S9. Comparison of the sequence coverage of HA1 afforded by HCD (left) and UVPD (right) for activation of (A) unbound HA1 and the (B) 1:2 antibody:antigen complex. Corresponding MS/MS spectra are shown in **Figure S3** for unbound HA1 (10+) and **Figure S7** for the complex (29+). Sequence coverages are reported below each map with the complex yielding lower sequence coverage than the unbound hemagglutinin A for both HCD and UVPD. The two epitope regions are highlighted: epitope region 1 (green) and epitope region 2 (red).

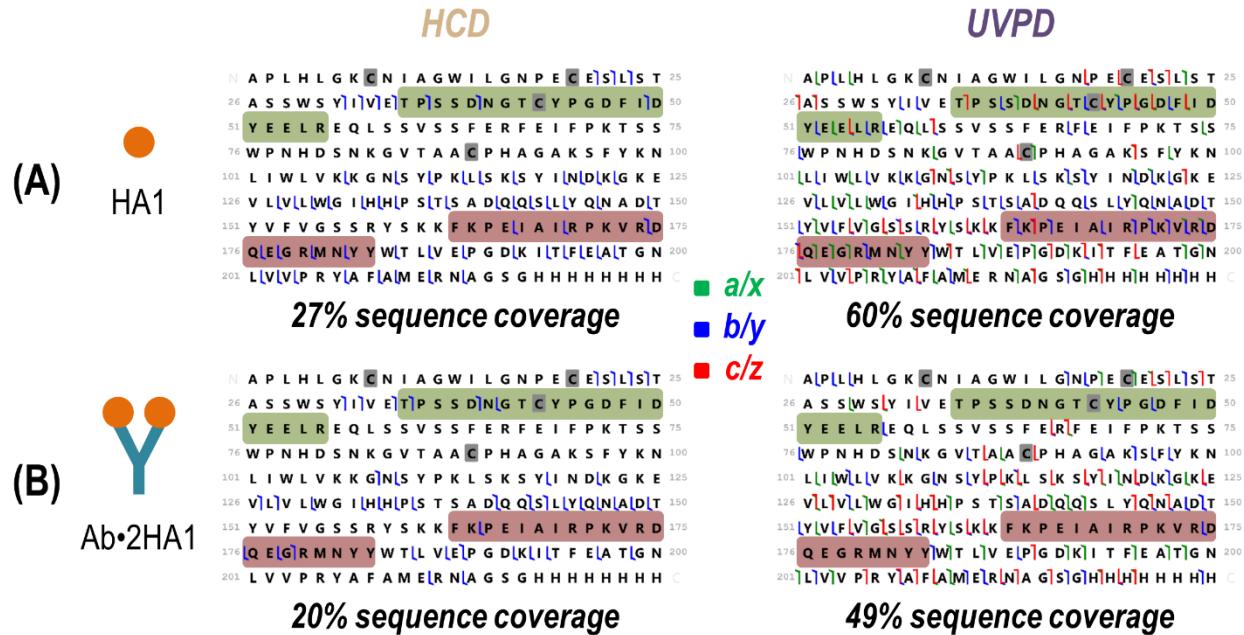


Figure S10. Bar graphs of the distribution of fragment ion types across several different regions of HA1 for (A) HCD and (B) UVPD showing pairs of stacked bars representing unbound HA1 (left bar) and the Ab•2HA1 complex (right bar), corresponding to the sequence coverages shown in **Figure 3** for the entire protein, epitope region 1, epitope region 2 and the rest of the protein. Residues 35-55 and 163-183 of the expressed HA1 sequence encompass the two expected epitope regions (or residues 90-109 and 213-233 in the HA protomer sequence). The rest of the protein represents the entire sequence excluding those two regions.

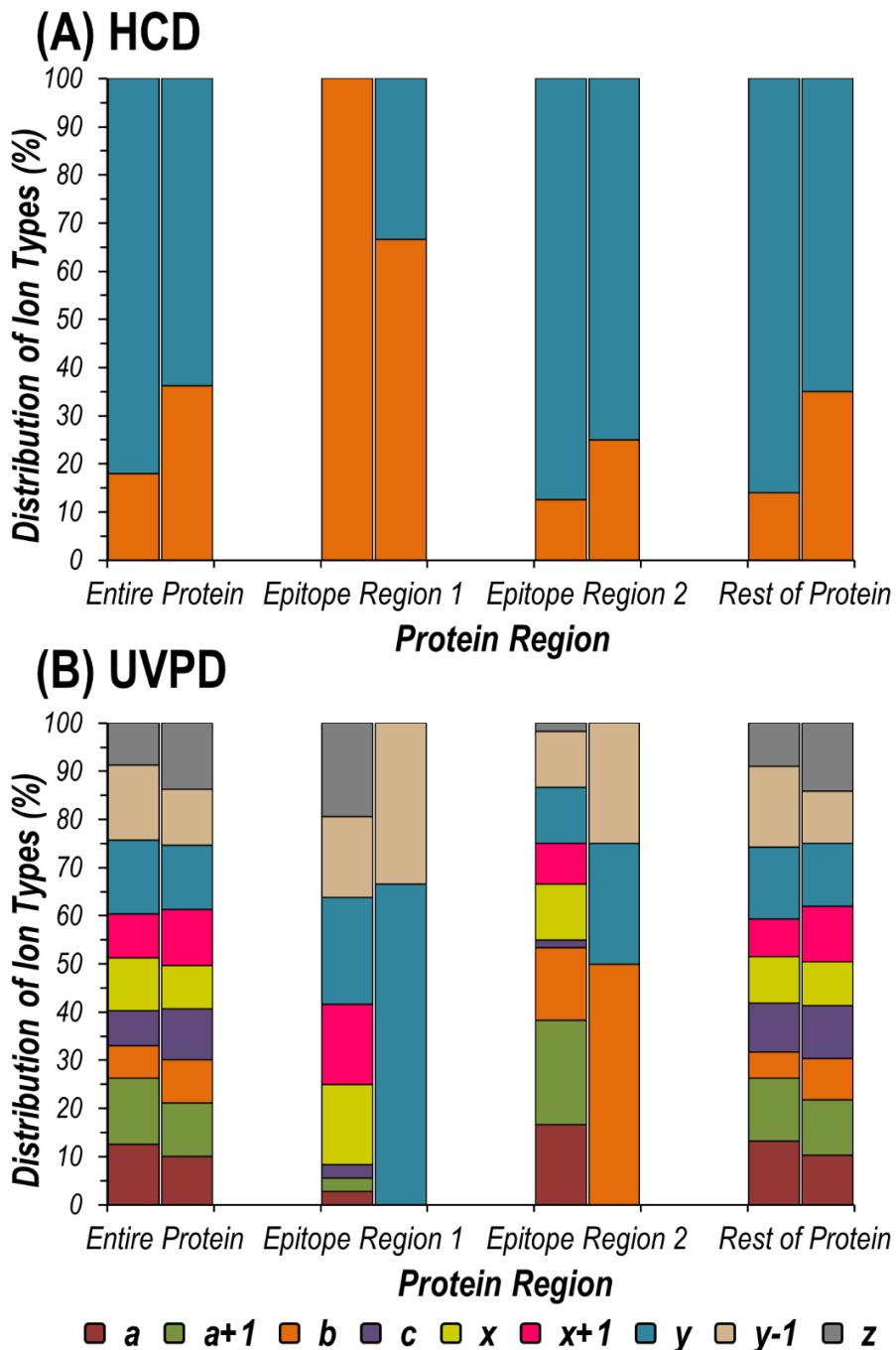
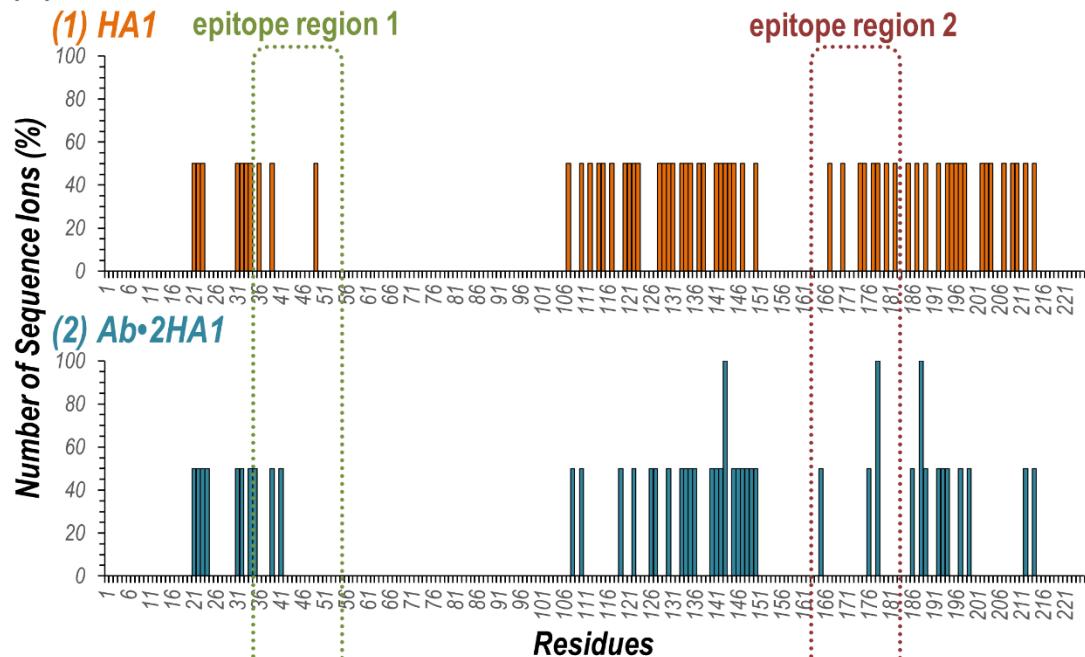


Figure S11. Plots of the number of HA1 sequence ions (originating from backbone cleavages along the protein sequence) as a percentage of the total number possible per residue resulting from activation of (1) unbound HA1 (10+) (orange) or (2) antibody-antigen Ab•2HA1 complex (29+) (turquoise) using (A) HCD and (B) UVPD. The two epitope regions (residues 90-109 and 213-233 in the HA protomer sequence, or 35-55 and 163-183 in the expressed sequence) are outlined with dashed lines. Subtraction of the corresponding values yielded the difference plots in **Figure 4**.

(A) HCD



(B) UVPD

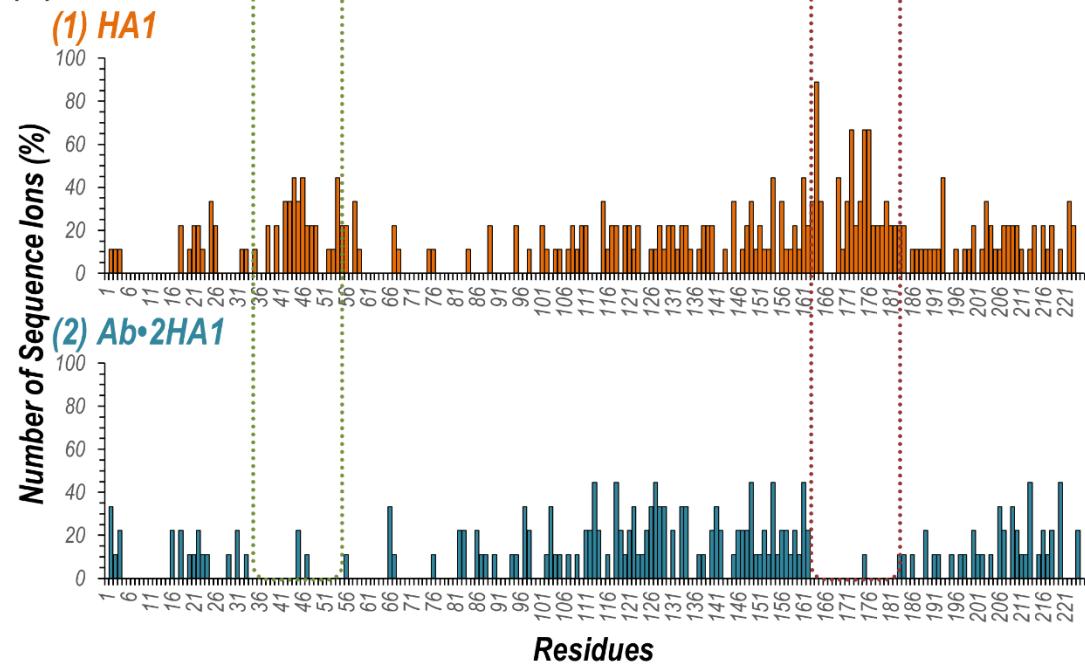
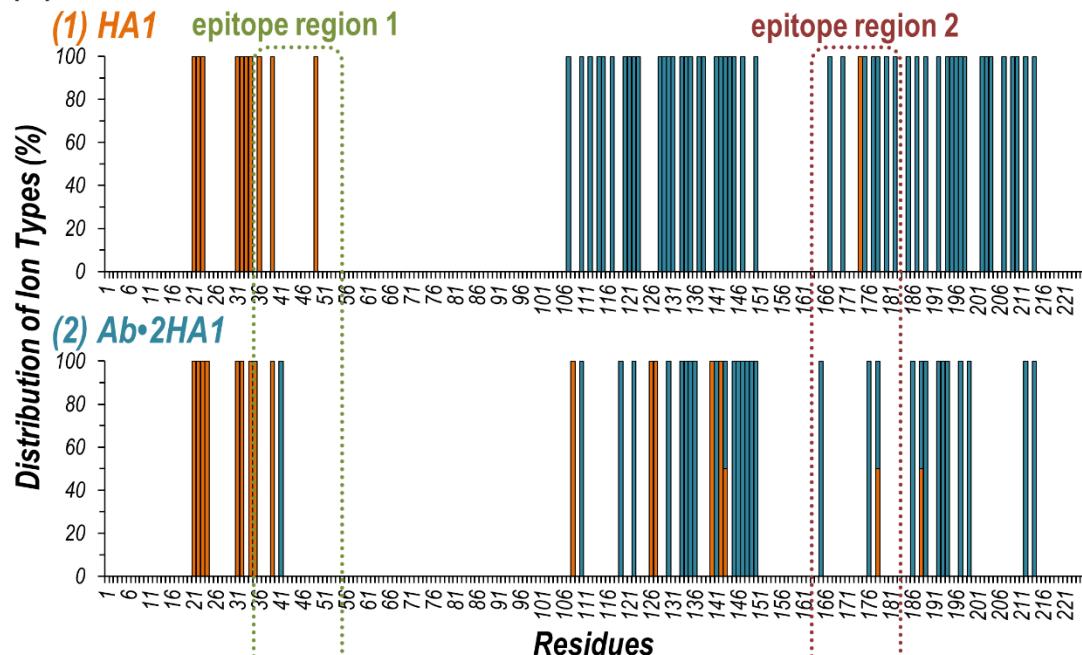


Figure S12. Distribution of ion types corresponding to the plots in **Figure S11** of the number of sequence ions originating from backbone cleavages at each residue for activation with (A) HCD and (B) UVPD of (1) unbound HA1 and (2) antibody-antigen complex. The dashed lines highlight the two epitope regions.

(A) HCD



(B) UVPD

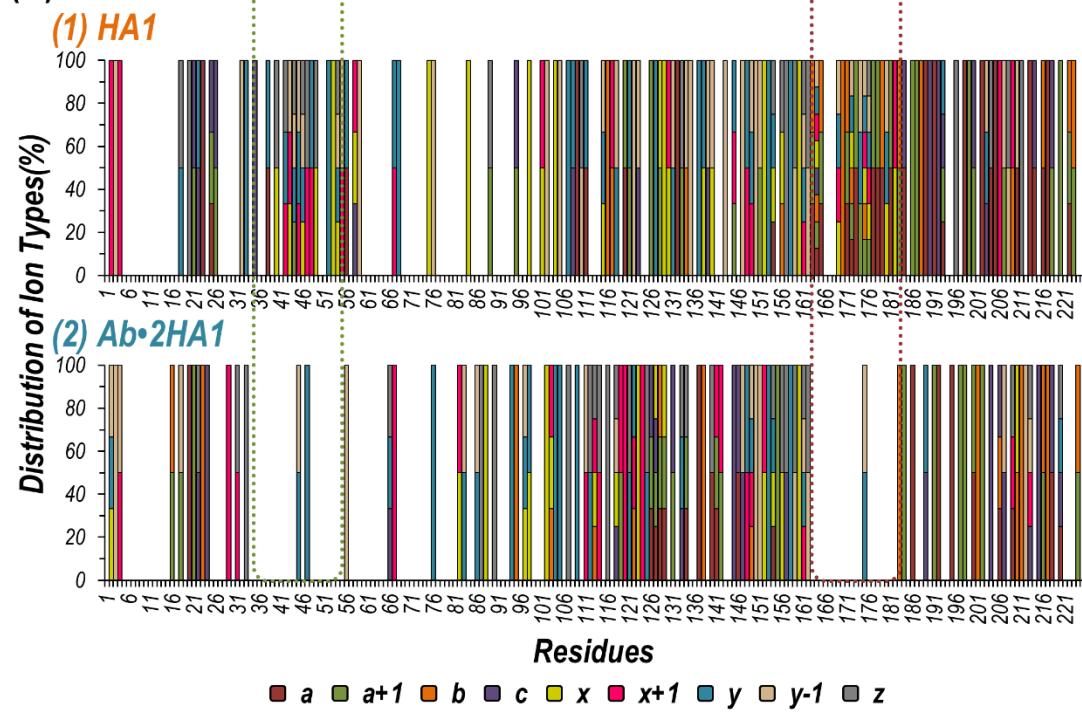


Table S1. Fragment ions identified as backbone cleavages produced upon activation of the 10+ charge state of unbound HA1 using (A) HCD and (B) UVPD. Identifications were made within ± 10 ppm mass error.

(A) *HCD of unbound HA1*

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
b21	2214.071	2214.088	7.5
b22	2301.103	2301.120	7.3
b23	2414.208	2414.187	-8.7
b31	3283.543	3283.573	9.0
b32	3396.657	3396.627	-8.8
b33	3495.721	3495.695	-7.4
b34	3624.738	3624.770	8.8
b36	3822.839	3822.864	6.7
b39	4111.963	4111.930	-8.2
b49	5178.360	5178.319	-7.8
b174	19437.666	19437.771	5.4
y12	1386.594	1386.608	9.8
y14	1656.738	1656.753	9.4
y16	1916.832	1916.821	-5.9
y17	1987.875	1987.858	-8.4
y19	2205.964	2205.981	7.9
y22	2622.181	2622.194	5.0
y23	2721.249	2721.276	9.8
y24	2820.345	2820.318	-9.7
y28	3205.514	3205.538	7.7
y29	3276.551	3276.580	8.8
y30	3405.594	3405.619	7.4
y31	3552.688	3552.662	-7.2
y32	3653.710	3653.740	8.2
y34	3894.889	3894.921	8.2
y37	4163.990	4164.023	8.1
y39	4392.141	4392.101	-9.2
y41	4606.260	4606.233	-6.0
y44	5118.466	5118.438	-5.3
y46	5363.522	5363.528	1.1
y48	5576.644	5576.681	6.5

y49	5705.687	5705.729	7.4
y51	5948.773	5948.815	7.2
y56	6585.191	6585.238	7.1
y59	6882.429	6882.396	-4.8
y76	8886.443	8886.508	7.3
y79	9186.550	9186.619	7.5
y81	9477.672	9477.734	6.6
y82	9590.798	9590.756	-4.4
y83	9677.788	9677.865	8.0
y84	9805.847	9805.921	7.6
y85	9933.995	9933.905	-9.0
y88	10207.001	10207.088	8.5
y89	10308.049	10308.137	8.6
y91	10492.134	10492.230	9.0
y92	10629.193	10629.248	5.2
y93	10766.334	10766.252	-7.6
y95	10936.437	10936.357	-7.4
y96	11122.436	11122.428	-0.7
y97	11235.520	11235.596	6.8
y98	11334.589	11334.661	6.3
y103	11860.900	11860.943	3.6
y104	11988.995	11989.088	7.8
y105	12104.073	12104.022	-4.2
y106	12218.065	12218.112	3.9
y109	12581.245	12581.330	6.8
y111	12796.372	12796.476	8.1
y112	12909.456	12909.564	8.4
y114	13134.603	13134.560	-3.3
y116	13384.766	13384.699	-5.0
y119	13683.944	13683.858	-6.3

(B) UVPD of unbound HA1

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
(a+1)21	2187.084	2187.076	-3.9
c21	2231.098	2231.110	5.7
c22	2318.130	2318.139	4.1

a23	2386.193	2386.206	5.6
(a+1)25	2575.280	2575.270	-4.0
a25	2574.272	2574.270	-0.9
c25	2619.293	2619.297	1.3
c26	2690.331	2690.340	3.4
(a+1)26	2646.317	2646.328	4.1
c35	3742.812	3742.815	0.8
a38	3968.908	3968.896	-2.8
(a+1)44	4622.119	4622.111	-1.7
a56	6084.768	6084.770	0.3
c58	6370.932	6370.909	-3.6
(a+1)89	9722.494	9722.521	2.8
c95	10327.810	10327.853	4.1
(a+1)95	10283.796	10283.842	4.4
c108	11904.723	11904.793	5.9
a109	11973.744	11973.812	5.7
a111	12223.840	12223.903	5.1
b116	12805.194	12805.189	-0.4
b117	12892.226	12892.277	4.0
(a+1)120	13255.429	13255.405	-1.8
a120	13254.421	13254.421	0.0
(a+1)121	13370.456	13370.535	6.0
c123	13599.586	13599.586	0.0
(a+1)126	13911.778	13911.817	2.8
(a+1)128	14123.931	14123.984	3.7
a132	14592.192	14592.190	-0.1
(a+1)133	14730.258	14730.210	-3.3
b134	14894.304	14894.390	5.8
c139	15354.532	15354.469	-4.2
(a+1)145	16044.842	16044.844	0.1
(a+1)151	16737.119	16737.213	5.6
a154	17081.316	17081.408	5.3
b156	17253.365	17253.405	2.3
(a+1)163	18122.865	18122.863	-0.1
b163	18149.852	18149.852	0.0

a163	18121.857	18121.862	0.3
c164	18294.973	18294.956	-0.9
a164	18249.952	18249.949	-0.1
b164	18277.947	18277.949	0.1
(a+1)164	18250.960	18250.964	0.2
a165	18347.004	18346.990	-0.8
(a+1)165	18348.012	18348.011	-0.1
b165	18374.999	18375.010	0.6
b170	18957.348	18957.351	0.1
(a+1)171	19027.414	19027.408	-0.3
b171	19054.401	19054.399	-0.1
a171	19026.406	19026.407	0.0
a172	19154.501	19154.494	-0.4
(a+1)172	19155.509	19155.508	0.0
b172	19182.496	19182.496	0.0
(a+1)173	19254.577	19254.577	0.0
a173	19253.570	19253.574	0.2
(a+1)174	19410.678	19410.680	0.1
b175	19552.692	19552.691	-0.1
(a+1)175	19525.705	19525.708	0.1
(a+1)176	19653.764	19653.758	-0.3
a177	19781.799	19781.801	0.1
(a+1)177	19782.807	19782.821	0.7
(a+1)178	19839.828	19839.829	0.1
a178	19838.820	19838.825	0.2
a179	19994.921	19994.921	0.0
b179	20022.916	20022.919	0.1
(a+1)181	20241.013	20241.010	-0.1
a181	20240.005	20240.010	0.3
(a+1)183	20567.139	20567.141	0.1
b183	20594.126	20594.132	0.3
c184	20797.232	20797.308	3.7
a184	20752.211	20752.291	3.9
(a+1)186	20967.350	20967.461	5.3
(a+1)187	21066.419	21066.433	0.7

b188	21222.448	21222.572	5.8
a189	21291.506	21291.616	5.1
c190	21393.549	21393.663	5.4
a191	21463.555	21463.672	5.5
c192	21636.671	21636.783	5.2
a193	21704.734	21704.821	4.0
c193	21749.755	21749.857	4.7
(a+1)193	21705.741	21705.871	6.0
a198	22253.977	22254.038	2.8
(a+1)199	22312.006	22312.094	3.9
c200	22470.063	22470.130	3.0
(a+1)200	22426.049	22426.161	5.0
a202	22637.194	22637.288	4.1
c203	22781.284	22781.393	4.8
a204	22833.315	22833.365	2.2
a205	22989.416	22989.533	5.1
(a+1)207	23224.524	23224.652	5.5
b209	23469.617	23469.574	-1.8
a210	23572.663	23572.659	-0.2
a213	23971.849	23971.925	3.2
a214	24042.886	24042.973	3.6
c214	24087.908	24087.995	3.6
b216	24214.935	24214.795	-5.8
a216	24186.940	24187.037	4.0
a217	24243.961	24244.083	5.0
c218	24426.041	24426.169	5.2
(a+1)218	24382.028	24382.068	1.6
(a+1)220	24656.146	24656.276	5.3
b222	24957.251	24957.243	-0.3
(a+1)222	24930.264	24930.259	-0.2
a222	24929.256	24929.363	4.3
(a+1)223	25067.323	25067.440	4.7
b223	25094.310	25094.451	5.6
z15	1769.762	1769.754	-4.5
(y-1)16	1915.813	1915.816	1.2

(x+1)17	2014.845	2014.857	5.8
(y-1)18	2133.919	2133.916	-1.1
z18	2118.908	2118.909	0.5
(y-1)19	2204.956	2204.950	-2.7
(x+1)20	2396.014	2396.026	4.8
z22	2606.162	2606.166	1.4
y23	2721.249	2721.236	-5.0
(y-1)23	2720.241	2720.244	1.1
z30	3389.575	3389.557	-5.1
y33	3766.794	3766.815	5.7
(x+1)44	5145.426	5145.420	-1.2
x44	5144.418	5144.427	1.8
(y-1)46	5362.514	5362.512	-0.4
y46	5363.522	5363.524	0.4
x46	5389.501	5389.504	0.5
x50	5859.725	5859.725	0.0
(x+1)50	5860.733	5860.734	0.2
(y-1)50	5832.738	5832.741	0.5
y50	5833.746	5833.778	5.6
z50	5817.727	5817.760	5.7
(y-1)51	5947.765	5947.761	-0.6
x51	5974.752	5974.750	-0.3
y51	5948.773	5948.774	0.2
(x+1)51	5975.760	5975.765	0.9
y52	6104.874	6104.872	-0.3
(y-1)52	6103.866	6103.876	1.7
(y-1)54	6331.029	6331.031	0.3
x54	6358.016	6358.024	1.2
y54	6332.037	6332.049	1.9
(x+1)57	6725.262	6725.261	-0.2
y57	6698.275	6698.274	-0.1
x57	6724.254	6724.255	0.1
(y-1)57	6697.267	6697.271	0.6
(x+1)62	7263.574	7263.573	-0.1
(y-1)62	7235.579	7235.581	0.3

y62	7236.586	7236.591	0.6
x62	7262.566	7262.574	1.1
z64	7495.731	7495.755	3.2
(y-1)64	7510.742	7510.769	3.5
(x+1)65	7666.832	7666.799	-4.3
z65	7623.826	7623.835	1.2
(y-1)65	7638.837	7638.863	3.4
y65	7639.845	7639.877	4.3
x66	7752.856	7752.878	2.8
(y-1)67	7888.932	7888.966	4.3
z67	7873.921	7873.966	5.7
y68	8046.041	8046.087	5.6
z69	8117.055	8117.019	-4.3
z70	8204.087	8204.088	0.2
x70	8246.085	8246.087	0.3
z72	8360.176	8360.154	-2.7
y72	8376.195	8376.163	-3.9
x72	8402.174	8402.172	-0.3
y73	8523.264	8523.224	-4.6
x74	8648.311	8648.328	1.9
(y-1)75	8784.388	8784.397	1.1
(y-1)76	8885.435	8885.486	5.7
(y-1)77	9000.462	9000.478	1.8
(x+1)77	9028.457	9028.495	4.2
y77	9001.470	9001.511	4.6
y78	9072.507	9072.520	1.4
(x+1)78	9099.494	9099.534	4.3
(y-1)79	9185.542	9185.594	5.7
y81	9477.672	9477.690	1.9
(x+1)81	9504.659	9504.705	4.9
(y-1)83	9676.780	9676.805	2.5
x86	10074.911	10074.943	3.1
(y-1)86	10047.924	10047.963	3.9
(y-1)87	10118.961	10118.994	3.2
y88	10207.001	10207.013	1.2

x88	10232.981	10233.029	4.8
y89	10308.049	10308.048	-0.1
(y-1)91	10491.126	10491.158	3.1
(y-1)92	10628.185	10628.248	6.0
z93	10750.233	10750.294	5.7
y95	10936.357	10936.311	-4.2
(y-1)95	10935.349	10935.399	4.5
(x+1)96	11149.423	11149.423	0.0
x96	11148.416	11148.433	1.6
x97	11261.500	11261.511	1.0
x98	11360.568	11360.624	4.9
y99	11447.673	11447.698	2.2
(y-1)103	11859.892	11859.887	-0.4
(y-1)104	11987.987	11988.012	2.0
y105	12104.022	12104.068	3.8
y108	12494.213	12494.249	2.9
(y-1)108	12493.205	12493.278	5.8
(x+1)109	12608.232	12608.197	-2.7
y111	12796.372	12796.297	-5.9
x111	12822.351	12822.317	-2.6
(y-1)111	12795.364	12795.377	1.0
y115	13297.667	13297.726	4.5
z116	13368.680	13368.755	5.6
(y-1)116	13383.691	13383.771	6.0
y118	13555.763	13555.726	-2.7
y119	13683.858	13683.906	3.5
(y-1)121	13910.014	13910.092	5.6
x122	14050.085	14050.138	3.8
(y-1)124	14322.261	14322.311	3.5
(x+1)125	14463.340	14463.257	-5.7
x125	14462.332	14462.271	-4.2
x128	14867.533	14867.556	1.5
z137	15722.939	15722.975	2.3
x142	16164.149	16164.213	3.9
(y-1)150	17115.593	17115.679	5.0

x151	17229.612	17229.524	-5.1
y158	18006.055	18005.972	-4.6
y159	18153.124	18153.048	-4.1
(x+1)159	18180.111	18180.042	-3.8
(y-1)167	19031.525	19031.578	2.8
x168	19171.596	19171.549	-2.4
(x+1)168	19172.604	19172.589	-0.8
y170	19402.718	19402.825	5.5
(x+1)171	19585.806	19585.845	2.0
(y-1)171	19557.811	19557.865	2.8
z172	19655.884	19655.910	1.3
(y-1)172	19670.895	19670.945	2.5
y172	19671.903	19671.970	3.4
x172	19697.882	19697.957	3.8
x173	19826.925	19827.010	4.3
y174	19929.988	19929.970	-0.9
x177	20347.142	20347.164	1.1
z177	20305.144	20305.191	2.3
y178	20468.231	20468.237	0.3
(x+1)178	20495.218	20495.325	5.2
z179	20567.239	20567.141	-4.8
(x+1)179	20610.245	20610.316	3.4
y180	20640.279	20640.319	1.9
x180	20666.258	20666.332	3.6
(y-1)180	20639.271	20639.365	4.6
z180	20624.260	20624.372	5.4
(x+1)181	20764.319	20764.266	-2.5
y181	20737.332	20737.391	2.9
(y-1)181	20736.324	20736.440	5.6
z182	20884.376	20884.385	0.4
y182	20900.395	20900.424	1.4
(y-1)182	20899.387	20899.460	3.5
x183	21028.376	21028.373	-0.2
(x+1)183	21029.384	21029.466	3.9
(y-1)183	21001.389	21001.495	5.0

y184	21103.444	21103.448	0.2
(x+1)184	21130.431	21130.544	5.4
z184	21087.425	21087.548	5.8
x186	21300.488	21300.374	-5.4
z186	21258.490	21258.563	3.4
y188	21476.568	21476.576	0.4
y193	21989.811	21989.816	0.2
(y-1)194	22101.887	22101.871	-0.7
y204	23172.367	23172.276	-3.9
z206	23387.392	23387.497	4.5
y208	23629.506	23629.596	3.8
z208	23613.487	23613.599	4.7
(x+1)222	25132.251	25132.256	0.2
(y-1)223	25217.340	25217.348	0.3
(x+1)224	25342.388	25342.417	1.1

Table S2. List of fragment ions resulting from (A) HCD and (B) UVPD of the 25+ charge state of the D1 H1-17/H3-14 antibody identified as sequence ions corresponding to the (1) light and (2) heavy chains. A ±10 ppm mass error was used for identification.

(A) *HCD of antibody*

(1) Light chain

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
b16	1584.811	1584.809	-0.8
b74	7842.980	7842.993	1.6
b102	11014.417	11014.400	-1.6
b111	11937.936	11937.903	-2.7
b117	12628.310	12628.384	5.9
b118	12725.363	12725.288	-5.9
b135	14469.222	14469.214	-0.5
y38	4211.042	4211.075	8.1
y44	4849.333	4849.375	8.7
y71	7821.744	7821.774	3.9
y112	12282.051	12282.024	-2.2
y122	13380.561	13380.517	-3.3
y210	22869.226	22869.440	9.4

(2) Heavy chain

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
b21	2178.153	2178.148	-2.5
b63	6836.410	6836.360	-7.2
b231	24486.171	24485.951	-9.0
b246	25957.830	25958.020	7.3
b307	32965.312	32965.185	-3.8
b320	34461.163	34461.171	0.3
b333	36010.910	36010.809	-2.8
b345	37286.665	37286.484	-4.9
b387	41928.079	41928.448	8.8
y59	6727.278	6727.220	-8.5
y87	9816.782	9816.870	9.0
y100	11316.544	11316.546	0.2
y107	12108.968	12108.936	-2.6
y108	12180.005	12180.002	-0.3

y247	27773.853	27773.957	3.8
y254	28550.260	28550.119	-4.9
y261	29207.593	29207.796	6.9
y282	31324.672	31324.719	1.5
y292	32367.217	32367.405	5.8
y295	32692.381	32692.314	-2.0
y317	34858.435	34858.518	2.4
y329	36014.064	36014.103	1.1
y373	40814.313	40813.938	-9.2
y383	41917.874	41917.751	-2.9
y385	42117.990	42117.694	-7.0
y423	46540.260	46540.399	3.0
y439	48004.924	48004.812	-2.3

(B) UVPD of antibody

(1) Light chain

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
b13	1341.689	1341.678	-8.0
b14	1428.721	1428.721	0.3
b16	1584.811	1584.803	-4.6
(a+1)17	1672.851	1672.843	-4.7
c17	1716.864	1716.860	-2.2
b18	1855.939	1855.930	-4.7
c23	2389.214	2389.197	-7.3
b23	2372.188	2372.193	2.0
b24	2528.289	2528.271	-7.0
(a+1)27	2787.430	2787.427	-1.0
c51	5606.940	5606.898	-7.5
c55	6049.158	6049.186	4.6
(a+1)59	6345.319	6345.338	2.9
c60	6476.365	6476.377	1.9
a62	6734.513	6734.580	9.9
a63	6821.545	6821.487	-8.5
c65	6980.609	6980.561	-6.9
a65	6935.588	6935.606	2.6

c70	7397.759	7397.780	2.9
(a+1)80	8441.337	8441.333	-0.4
a89	9559.738	9559.816	8.1
b90	9716.776	9716.828	5.4
c92	9997.913	9997.983	7.0
b92	9980.887	9980.971	8.4
c94	10241.046	10240.976	-6.9
b96	10472.136	10472.208	6.9
c97	10546.184	10546.229	4.3
b100	10815.285	10815.283	-0.2
(a+1)100	10788.298	10788.352	5.0
(a+1)102	10987.430	10987.469	3.5
a102	10986.422	10986.475	4.8
c102	11031.443	11031.498	5.0
b102	11014.417	11014.481	5.8
a103	11101.449	11101.439	-0.9
c104	11259.554	11259.609	4.9
b104	11242.528	11242.585	5.0
b106	11526.724	11526.794	6.1
c108	11715.835	11715.870	2.9
b108	11698.809	11698.856	4.0
(a+1)108	11671.822	11671.884	5.3
a108	11670.814	11670.877	5.4
a109	11741.851	11741.890	3.3
a110	11812.888	11812.940	4.4
(a+1)110	11813.896	11813.949	4.5
b110	11840.883	11840.937	4.5
b111	11937.936	11937.986	4.2
b112	12024.968	12025.021	4.4
(a+1)112	11997.981	11998.054	6.1
(a+1)113	12097.049	12096.968	-6.7
a113	12096.042	12096.054	1.0
b113	12124.036	12124.103	5.5
a114	12243.110	12243.110	0.0
c114	12288.131	12288.164	2.7

(a+1)115	12357.202	12357.253	4.1
b115	12384.189	12384.277	7.1
b116	12531.257	12531.293	2.8
(a+1)117	12601.323	12601.355	2.5
a118	12697.368	12697.347	-1.6
(a+1)118	12698.376	12698.379	0.2
c120	12944.448	12944.510	4.8
b121	13056.464	13056.539	5.7
c122	13201.549	13201.565	1.2
a122	13156.528	13156.581	4.0
b122	13184.523	13184.626	7.8
b125	13512.734	13512.763	2.2
b128	13741.840	13741.861	1.5
(a+1)132	14102.023	14101.981	-3.0
c136	14600.291	14600.362	4.8
b142	15346.630	15346.622	-0.5
(a+1)143	15447.738	15447.778	2.6
b147	16016.026	16016.014	-0.7
b148	16115.094	16115.043	-3.2
(a+1)161	17417.687	17417.597	-5.2
a195	21224.470	21224.415	-2.6
c196	21406.550	21406.600	2.3
b197	21517.583	21517.699	5.4
(a+1)198	21547.617	21547.798	8.4
c199	21704.714	21704.722	0.3
a202	21930.810	21930.859	2.2
(a+1)202	21931.818	21931.913	4.3
b202	21958.805	21958.964	7.2
a203	22029.878	22029.912	1.5
c204	22175.947	22175.736	-9.5
c205	22304.042	22303.990	-2.3
b205	22287.016	22287.072	2.5
c206	22391.074	22391.212	6.1
a207	22493.122	22493.097	-1.1
a208	22607.164	22607.210	2.0

c210	22865.308	22865.430	5.3
(a+1)210	22821.295	22821.433	6.1
a210	22820.287	22820.493	9.0
(a+1)211	22950.337	22950.410	3.2
(y-1)6	722.281	722.287	9.0
z13	1393.630	1393.617	-9.0
x13	1435.628	1435.615	-8.7
y13	1409.648	1409.653	3.4
x17	1870.851	1870.847	-1.9
z20	2158.011	2158.011	0.0
(x+1)53	5865.750	5865.729	-3.6
(x+1)55	6122.851	6122.891	6.4
(y-1)57	6295.931	6295.875	-8.9
y57	6296.939	6296.972	5.3
(y-1)61	6681.127	6681.187	8.9
z66	7193.387	7193.336	-7.1
x68	7549.523	7549.548	3.4
y68	7523.543	7523.535	-1.1
z68	7507.525	7507.560	4.7
(x+1)69	7649.599	7649.571	-3.6
(x+1)77	8655.102	8655.129	3.1
x94	10397.953	10398.055	9.8
x107	11752.693	11752.783	7.7
(y-1)108	11853.801	11853.909	9.1
z109	11951.874	11951.893	1.6
x110	12108.899	12108.851	-4.0
(x+1)110	12109.906	12110.025	9.8
(y-1)113	12382.091	12382.077	-1.1
z113	12367.080	12367.159	6.4
y113	12383.099	12383.210	9.0
z119	13013.399	13013.290	-8.4
(y-1)121	13278.505	13278.588	6.2
(y-1)128	14165.830	14165.843	0.9
z131	14483.951	14484.008	3.9
(x+1)131	14526.957	14527.037	5.5

y133	14726.066	14725.991	-5.1
y137	15141.272	15141.205	-4.4
y166	18038.728	18038.792	3.5
(x+1)171	18637.063	18636.973	-4.9
z179	19623.536	19623.555	1.0
z185	20266.901	20266.721	-8.9
(x+1)186	20437.965	20437.909	-2.8
x187	20523.990	20524.168	8.7
(x+1)193	21169.316	21169.448	6.2
y195	21397.499	21397.490	-0.4
z196	21496.507	21496.317	-8.8
z198	21652.597	21652.465	-6.1
y198	21668.616	21668.795	8.3
y200	21826.685	21826.840	7.1
x200	21852.664	21852.846	8.3
z201	21897.698	21897.863	7.5
x202	22052.780	22052.998	9.9
z203	22097.814	22097.907	4.2
z204	22184.846	22184.890	2.0
(x+1)204	22227.852	22227.994	6.4
(y-1)204	22199.857	22200.027	7.7
y204	22200.865	22201.079	9.6
(y-1)208	22613.048	22613.141	4.1
(x+1)209	22740.111	22740.332	9.7
(y-1)210	22868.218	22868.197	-0.9
x210	22895.205	22895.428	9.8
y211	22982.310	22982.466	6.8

(2) Heavy chain

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
c20	2094.132	2094.141	4.2
b25	2553.287	2553.277	-3.9
c42	4541.288	4541.266	-4.9
(a+1)47	4949.550	4949.533	-3.5
a56	6061.060	6061.038	-3.6
a64	6895.447	6895.462	2.2

a89	9526.834	9526.798	-3.7
c91	9757.919	9757.881	-3.8
(a+1)91	9713.906	9713.893	-1.3
b93	9912.977	9912.929	-4.9
b96	10338.173	10338.150	-2.1
c99	10684.338	10684.352	1.3
b100	10782.339	10782.334	-0.5
c100	10799.365	10799.415	4.6
(a+1)101	10902.420	10902.443	2.1
b102	11076.476	11076.482	0.5
(a+1)103	11178.531	11178.514	-1.6
b103	11205.518	11205.534	1.4
a103	11177.524	11177.579	4.9
(a+1)107	11603.832	11603.815	-1.5
c107	11647.845	11647.902	4.9
(a+1)108	11718.859	11718.890	2.7
a108	11717.851	11717.892	3.5
a110	11945.962	11945.986	2.0
a112	12131.042	12131.058	1.4
b112	12159.037	12159.097	4.9
(a+1)115	12507.229	12507.262	2.6
b115	12534.216	12534.254	3.0
c129	13943.877	13943.897	1.4
b133	14296.052	14296.053	0.1
c134	14412.146	14412.130	-1.1
(a+1)135	14515.201	14515.131	-4.8
b135	14542.188	14542.143	-3.1
b136	14639.241	14639.210	-2.1
b138	14823.362	14823.401	2.6
(a+1)144	15383.667	15383.672	0.4
c205	21547.776	21547.798	1.0
c207	21811.887	21811.870	-0.8
b208	21907.944	21907.895	-2.3
(a+1)209	21982.959	21982.977	0.8
c209	22026.972	22027.034	2.8

a211	22195.062	22195.034	-1.3
b214	22602.254	22602.203	-2.3
c215	22716.333	22716.377	1.9
c218	23018.456	23018.437	-0.8
c219	23147.498	23147.526	1.2
(a+1)219	23103.485	23103.543	2.5
(a+1)220	23202.553	23202.458	-4.1
c221	23361.594	23361.549	-1.9
a224	23671.831	23671.922	3.9
(a+1)224	23672.839	23672.944	4.5
b232	24587.219	24587.181	-1.6
c244	25860.813	25860.783	-1.2
(a+1)268	28411.193	28411.133	-2.1
a276	29211.567	29211.637	2.4
b280	29717.743	29717.820	2.6
c283	30090.975	30090.887	-2.9
a283	30045.954	30045.877	-2.6
(a+1)286	30494.152	30494.058	-3.1
c289	30915.325	30915.191	-4.3
(a+1)290	30928.333	30928.402	2.3
(a+1)293	31255.512	31255.434	-2.5
(a+1)310	33358.537	33358.533	-0.1
(a+1)313	33643.705	33643.764	1.7
b314	33769.761	33769.664	-2.9
a321	34548.195	34548.283	2.6
c324	35006.422	35006.594	4.9
c326	35191.539	35191.510	-0.8
b327	35303.555	35303.677	3.5
b328	35466.618	35466.759	4.0
a333	35982.915	35982.929	0.4
b344	37173.581	37173.425	-4.2
(a+1)344	37146.594	37146.620	0.7
b351	37886.004	37886.000	-0.1
b362	39163.681	39163.726	1.2
b374	40534.396	40534.309	-2.1

c374	40551.423	40551.487	1.6
b376	40737.445	40737.393	-1.3
a377	40822.534	40822.420	-2.8
(a+1)386	41830.055	41830.121	1.6
(a+1)392	42531.357	42531.403	1.1
(a+1)393	42645.400	42645.217	-4.3
(a+1)398	43170.618	43170.821	4.7
c399	43328.674	43328.503	-4.0
a402	43675.859	43675.986	2.9
b426	46484.271	46484.239	-0.7
a427	46584.335	46584.255	-1.7
a428	46641.356	46641.192	-3.5
c428	46686.378	46686.563	4.0
b435	47404.665	47404.853	4.0
(a+1)437	47645.777	47645.679	-2.1
b437	47672.764	47672.768	0.1
b439	47872.844	47873.078	4.9
a442	48209.035	48209.206	3.5
(a+1)448	48954.398	48954.544	3.0
(a+1)454	49508.705	49508.635	-1.4
x12	1333.688	1333.681	-4.8
z13	1428.749	1428.750	0.9
z19	2130.073	2130.063	-4.8
(x+1)19	2173.079	2173.082	1.4
(y-1)20	2276.125	2276.130	2.5
z21	2360.182	2360.184	0.8
(x+1)21	2403.188	2403.199	4.5
(y-1)22	2462.225	2462.230	2.1
y37	4198.035	4198.016	-4.5
(y-1)40	4601.270	4601.289	4.3
y41	4715.361	4715.364	0.6
(y-1)53	5976.936	5976.936	0.0
y55	6207.086	6207.093	1.1
y61	6952.389	6952.418	4.2
x94	10641.148	10641.197	4.6

y100	11316.544	11316.541	-0.3
y106	11980.873	11980.896	1.9
(y-1)111	12507.209	12507.262	4.3
x111	12534.196	12534.254	4.6
z114	12850.383	12850.364	-1.4
x115	13005.465	13005.446	-1.5
(y-1)116	13075.531	13075.510	-1.6
y116	13076.538	13076.557	1.4
z116	13060.520	13060.543	1.8
(x+1)116	13103.525	13103.567	3.2
z117	13131.557	13131.539	-1.3
z118	13228.610	13228.620	0.8
y118	13244.628	13244.643	1.1
(x+1)119	13384.699	13384.712	0.9
y121	13556.844	13556.832	-0.9
(x+1)123	13784.906	13784.839	-4.9
z123	13741.901	13741.861	-2.9
x123	13783.899	13783.890	-0.7
x125	14011.062	14011.031	-2.2
z133	14903.510	14903.440	-4.7
y149	16803.518	16803.529	0.7
x159	18103.139	18103.101	-2.0
(y-1)162	18398.290	18398.358	3.7
y186	21142.570	21142.558	-0.5
(y-1)190	21567.773	21567.731	-2.0
(y-1)191	21668.821	21668.795	-1.2
z192	21809.911	21809.826	-3.9
(x+1)192	21852.917	21852.846	-3.2
(y-1)192	21824.922	21824.860	-2.8
z193	21896.943	21896.927	-0.7
x194	22052.025	22051.984	-1.9
(x+1)194	22053.033	22053.044	0.5
x196	22296.150	22296.107	-1.9
(y-1)196	22269.163	22269.121	-1.8
y197	22371.218	22371.192	-1.2

z198	22470.226	22470.220	-0.3
(x+1)198	22513.232	22513.316	3.7
(y-1)200	22710.385	22710.312	-3.2
x201	22865.467	22865.430	-1.6
(x+1)202	22963.528	22963.531	0.1
z202	22920.522	22920.538	0.7
(x+1)203	23060.580	23060.512	-3.0
y203	23033.593	23033.574	-0.8
z204	23164.643	23164.596	-2.0
x206	23466.793	23466.690	-4.4
(x+1)206	23467.801	23467.758	-1.9
z206	23424.795	23424.789	-0.3
(y-1)206	23439.806	23439.825	0.8
y207	23539.883	23539.868	-0.6
y208	23626.915	23626.981	2.8
(y-1)209	23722.959	23722.925	-1.5
y210	23780.989	23780.989	0.0
x211	23863.989	23864.060	3.0
(x+1)211	23864.997	23865.072	3.1
x212	23977.074	23977.138	2.7
x214	24219.200	24219.243	1.8
z219	24641.399	24641.358	-1.7
(x+1)224	25222.613	25222.665	2.1
x227	25566.729	25566.671	-2.3
z234	26321.211	26321.104	-4.1
z253	28421.157	28421.134	-0.8
(y-1)255	28636.284	28636.276	-0.3
(x+1)264	29507.713	29507.647	-2.2
z267	29827.886	29827.810	-2.6
x267	29869.884	29869.945	2.0
z277	30827.425	30827.429	0.1
z278	30928.473	30928.402	-2.3
y279	31081.550	31081.619	2.2
(x+1)280	31207.606	31207.596	-0.3
z281	31221.622	31221.659	1.2

(y-1)287	31752.887	31752.928	1.3
y293	32466.285	32466.418	4.1
x303	33682.820	33682.759	-1.8
(y-1)307	33968.013	33968.109	2.8
y315	34674.350	34674.201	-4.3
(y-1)320	35138.601	35138.451	-4.3
y320	35139.609	35139.566	-1.2
(y-1)324	35568.823	35568.808	-0.4
z328	35927.008	35926.923	-2.4
(y-1)331	36187.120	36186.993	-3.5
z331	36172.109	36172.221	3.1
x332	36313.175	36313.112	-1.8
(x+1)333	36415.231	36415.152	-2.2
(x+1)339	36988.489	36988.549	1.6
x340	37173.561	37173.425	-3.6
(y-1)340	37146.573	37146.620	1.3
x353	38631.301	38631.432	3.4
(x+1)356	39041.472	39041.593	3.1
x366	40152.948	40152.913	-0.9
(y-1)367	40196.998	40197.076	1.9
z370	40469.135	40469.248	2.8
(x+1)370	40512.141	40512.263	3.0
z371	40556.167	40556.337	4.2
(x+1)372	40712.257	40712.167	-2.2
y372	40685.270	40685.415	3.6
(y-1)372	40684.262	40684.448	4.6
(x+1)383	41944.861	41944.751	-2.6
x384	42030.885	42031.011	3.0
(x+1)386	42246.025	42245.925	-2.3
z389	42515.210	42515.065	-3.4
y389	42531.228	42531.403	4.1
y390	42645.271	42645.217	-1.3
y393	42942.440	42942.446	0.1
y404	44220.033	44219.909	-2.8
z405	44261.036	44261.231	4.4

(x+1)405	44304.042	44304.250	4.7
(x+1)407	44603.205	44603.086	-2.7
z423	46524.241	46524.112	-2.8
(x+1)425	46768.322	46768.359	0.8
y426	46828.367	46828.197	-3.6
(y-1)433	47400.635	47400.682	1.0
(x+1)436	47744.763	47744.977	4.5
(x+1)439	48031.911	48032.094	3.8
(y-1)442	48317.044	48316.887	-3.2
(x+1)444	48572.202	48572.146	-1.2
(y-1)447	48811.365	48811.543	3.6

Table S3. (A) HCD and (B) UVPD fragment ions produced by activation of the 29+ charge state of the 1:2 antibody:antigen complex. The sequences of (1) HA1, as well as the (2) light and (3) heavy chains of the D1 H1-17/H3-14 antibody were searched. The mass error for identification was ± 10 ppm.

(A) *HCD of 1:2 antibody:antigen complex*

(1) HA1

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
b21	2214.071	2214.072	0.2
b22	2301.103	2301.115	5.1
b23	2414.187	2414.190	1.1
b24	2501.219	2501.237	6.9
b31	3283.543	3283.537	-1.8
b32	3396.627	3396.628	0.3
b34	3624.738	3624.734	-1.0
b35	3725.786	3725.786	0.0
b39	4111.930	4111.931	0.4
b108	11887.696	11887.693	-0.3
b126	13938.765	13938.777	0.9
b127	14051.849	14051.781	-4.8
b140	15452.533	15452.439	-6.1
b142	15708.650	15708.582	-4.3
b143	15795.682	15795.696	0.9
b178	19866.815	19866.856	2.1
b188	21222.448	21222.367	-3.9
y12	1386.594	1386.595	1.1
y14	1656.738	1656.740	1.3
y27	3104.466	3104.476	3.3
y29	3276.551	3276.552	0.2
y32	3653.710	3653.711	0.3
y33	3766.794	3766.802	2.2
y34	3894.889	3894.892	0.9
y37	4163.990	4163.995	1.3
y38	4293.032	4293.040	1.8
y40	4505.185	4505.176	-2.0
y48	5576.644	5576.642	-0.4
y50	5833.746	5833.756	1.7

y61	7108.492	7108.462	-4.2
y76	8886.443	8886.460	1.9
y77	9001.470	9001.534	7.2
y78	9072.507	9072.518	1.2
y79	9186.550	9186.552	0.2
y80	9314.609	9314.608	0.0
y81	9477.672	9477.676	0.4
y83	9677.788	9677.787	-0.1
y85	9933.905	9933.902	-0.3
y90	10395.081	10395.090	0.8
y91	10492.134	10492.125	-0.8
y92	10629.193	10629.201	0.8
y93	10766.252	10766.250	-0.2
y96	11122.436	11122.439	0.2
y104	11988.995	11988.957	-3.2
y107	12331.149	12331.122	-2.2
y116	13384.699	13384.727	2.1
y185	21160.466	21160.412	-2.5

(2) Light chain of antibody

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
b14	1428.721	1428.724	2.0
b15	1527.789	1527.777	-8.2
b16	1584.811	1584.810	-0.3
b18	1855.939	1855.928	-6.0
b30	3101.565	3101.550	-4.8
b82	8712.393	8712.378	-1.8
b88	9459.674	9459.689	1.6
b111	11937.936	11937.935	0.0
b113	12124.036	12123.997	-3.3
b114	12271.105	12270.993	-9.1
y14	1522.732	1522.745	8.0
y66	7209.405	7209.364	-5.7
y109	11967.892	11967.953	5.1
y113	12383.099	12383.099	0.0
y115	12568.179	12568.197	1.4

y116	12625.201	12625.265	5.1
y126	13902.727	13902.739	0.9
y157	17065.205	17065.124	-4.7

(3) Heavy chain of antibody

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
b21	2178.153	2178.153	0.1
b44	4678.336	4678.363	5.7
b112	12159.037	12159.082	3.7
b239	25320.486	25320.474	-0.5
b240	25391.524	25391.534	0.4
b245	25900.809	25900.573	-9.1
b294	31419.558	31419.628	2.2
b315	33882.845	33882.920	2.2
b322	34762.269	34762.128	-4.1
y14	1558.810	1558.804	-4.4
y15	1695.869	1695.868	-0.5
y20	2277.132	2277.118	-6.3
y58	6598.235	6598.223	-1.9
y88	9917.830	9917.908	7.9
y99	11188.485	11188.478	-0.6
y104	11795.793	11795.696	-8.2
y108	12180.005	12180.088	6.8
y121	13556.844	13556.756	-6.5
y142	16011.105	16011.045	-3.7
y242	27207.638	27207.831	7.1
y243	27344.697	27344.933	8.6
y289	32054.017	32053.837	-5.6
y318	34929.472	34929.174	-8.5
y335	36618.353	36618.562	5.7
y445	48658.299	48658.102	-4.0

(B) UVPD of 1:2 antibody:antigen complex

(1) HA1

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
b16	1642.889	1642.883	-3.6
(a+1)16	1615.902	1615.911	5.6

(a+1)18	1826.998	1827.008	5.9
a20	2057.034	2057.036	1.2
(a+1)21	2187.084	2187.092	3.4
c22	2318.130	2318.140	4.3
b23	2414.187	2414.173	-5.9
c24	2518.246	2518.242	-1.6
c66	7250.341	7250.302	-5.3
b95	10310.784	10310.752	-3.1
b103	11362.333	11362.285	-4.2
b113	12476.982	12477.051	5.5
c118	13072.315	13072.259	-4.3
(a+1)119	13141.386	13141.481	7.2
b122	13525.538	13525.474	-4.7
c126	13955.792	13955.764	-2.0
(a+1)126	13911.778	13911.798	1.5
a126	13910.770	13910.798	2.0
(a+1)127	14024.862	14024.868	0.4
c127	14068.876	14068.885	0.7
a127	14023.854	14023.943	6.3
(a+1)128	14123.931	14123.947	1.2
a128	14122.923	14122.921	-0.2
b128	14150.918	14150.958	2.8
(a+1)129	14237.015	14237.061	3.3
a129	14236.007	14236.061	3.8
c131	14524.129	14524.127	-0.1
(a+1)131	14480.115	14480.177	4.3
c133	14774.272	14774.207	-4.4
(a+1)134	14867.317	14867.349	2.2
a134	14866.309	14866.254	-3.7
a137	15151.442	15151.349	-6.1
b138	15266.469	15266.460	-0.6
a140	15424.538	15424.583	2.9
(a+1)141	15553.604	15553.638	2.2
a141	15552.597	15552.638	2.7
(a+1)142	15681.663	15681.678	1.0

c145	16088.856	16088.844	-0.8
a146	16171.893	16171.914	1.3
c146	16216.914	16216.961	2.8
c147	16330.957	16330.915	-2.6
b149	16499.995	16500.019	1.5
a154	17081.316	17081.298	-1.1
(a+1)155	17139.346	17139.464	6.9
c157	17357.423	17357.413	-0.6
b183	20594.126	20594.073	-2.6
(a+1)184	20753.219	20753.357	6.7
a186	20966.342	20966.488	6.9
c189	21336.527	21336.549	1.0
(a+1)191	21464.562	21464.603	1.9
a192	21591.650	21591.748	4.6
a195	21952.850	21952.874	1.1
(a+1)197	22153.937	22154.098	7.3
(a+1)198	22254.985	22254.873	-5.0
(a+1)200	22426.049	22426.083	1.5
a200	22425.041	22425.221	8.0
b201	22566.120	22566.100	-0.9
(a+1)202	22638.202	22638.383	8.0
c204	22878.336	22878.506	7.4
c206	23197.501	23197.356	-6.2
b206	23180.474	23180.484	0.4
a206	23152.480	23152.599	5.2
c207	23268.538	23268.630	4.0
a209	23441.622	23441.644	0.9
b210	23600.658	23600.764	4.5
b211	23729.700	23729.567	-5.6
c213	24016.871	24017.019	6.2
c215	24144.929	24144.962	1.4
b216	24214.935	24214.867	-2.8
(a+1)216	24187.948	24188.050	4.2
b217	24271.956	24272.101	5.9
c218	24426.041	24425.861	-7.4

a218	24381.020	24380.940	-3.3
c220	24700.159	24700.103	-2.3
a220	24655.138	24655.085	-2.2
(a+1)224	25204.382	25204.474	3.6
b224	25231.369	25231.545	7.0
z6	824.345	824.346	1.1
y6	840.364	840.361	-3.8
z13	1484.618	1484.613	-3.3
(x+1)13	1527.624	1527.625	0.8
(y-1)13	1499.629	1499.633	2.8
(y-1)14	1655.730	1655.719	-6.5
x16	1942.800	1942.812	6.2
(x+1)17	2014.845	2014.855	4.7
z17	1971.839	1971.851	6.1
(y-1)19	2204.956	2204.949	-2.9
y37	4163.990	4164.020	7.3
(y-1)51	5947.765	5947.759	-1.0
(y-1)64	7510.742	7510.739	-0.4
z64	7495.731	7495.745	1.8
(y-1)65	7638.837	7638.843	0.8
(x+1)65	7666.832	7666.809	-3.0
z65	7623.826	7623.815	-1.4
y65	7639.845	7639.897	6.9
x66	7752.856	7752.908	6.6
(y-1)67	7888.932	7888.916	-2.0
z67	7873.921	7873.956	4.4
y68	8046.041	8046.088	5.8
z69	8117.055	8117.018	-4.5
x70	8246.085	8246.084	-0.1
z70	8204.087	8204.090	0.5
z72	8360.176	8360.153	-2.8
y72	8376.195	8376.163	-3.9
x72	8402.174	8402.171	-0.4
y73	8523.264	8523.224	-4.6
x74	8648.311	8648.329	2.0

(x+1)74	8649.319	8649.358	4.5
(y-1)75	8784.388	8784.398	1.2
(y-1)76	8885.435	8885.486	5.7
(y-1)77	9000.462	9000.477	1.7
(x+1)77	9028.457	9028.496	4.3
y77	9001.470	9001.512	4.7
y78	9072.507	9072.521	1.6
(x+1)78	9099.494	9099.533	4.2
(y-1)79	9185.542	9185.594	5.6
(x+1)84	9832.834	9832.811	-2.4
(x+1)85	9960.892	9960.940	4.8
z86	10032.913	10032.974	6.1
z92	10613.174	10613.172	-0.2
z93	10750.233	10750.253	1.9
y93	10766.252	10766.322	6.5
x97	11261.500	11261.440	-5.3
x99	11473.652	11473.635	-1.5
y101	11675.784	11675.751	-2.8
z101	11659.765	11659.775	0.8
(x+1)102	11830.866	11830.902	3.0
x103	11886.880	11886.924	3.8
y104	11988.995	11988.940	-4.6
(x+1)104	12015.982	12016.050	5.6
z105	12088.003	12087.962	-3.4
y105	12104.022	12104.059	3.0
(x+1)106	12245.052	12245.119	5.4
(x+1)107	12358.136	12358.165	2.3
(y-1)108	12493.205	12493.248	3.5
z108	12478.194	12478.256	5.0
x108	12520.192	12520.291	7.9
z110	12693.321	12693.330	0.7
(x+1)112	12936.443	12936.485	3.3
z112	12893.437	12893.526	6.9
x113	13063.530	13063.567	2.9
(x+1)113	13064.538	13064.600	4.8

z113	13021.532	13021.601	5.3
y114	13134.603	13134.589	-1.1
z114	13118.585	13118.581	-0.3
(y-1)115	13296.659	13296.667	0.6
(x+1)115	13324.654	13324.686	2.4
y117	13498.742	13498.846	7.7
z119	13667.839	13667.824	-1.1
y121	13911.021	13911.098	5.5
y122	14024.105	14024.043	-4.5
(x+1)123	14237.172	14237.061	-7.8
x123	14236.164	14236.061	-7.2
x124	14349.248	14349.292	3.0
x128	14867.533	14867.548	1.0
y128	14841.554	14841.591	2.5
(y-1)129	14987.615	14987.524	-6.1
x129	15014.602	15014.616	0.9
y129	14988.623	14988.638	1.0
y132	15274.787	15274.802	1.0
z136	15620.938	15620.975	2.3
x138	15835.975	15835.947	-1.7
z139	15865.014	15864.989	-1.6
y140	15982.080	15982.053	-1.7
(y-1)140	15981.072	15981.053	-1.2
(y-1)143	16265.257	16265.251	-0.4
y143	16266.265	16266.266	0.1
x144	16406.287	16406.260	-1.6
(x+1)144	16407.295	16407.283	-0.7
y150	17116.601	17116.594	-0.4
(x+1)159	18180.111	18180.101	-0.5
z160	18293.206	18293.093	-6.2
y160	18309.225	18309.211	-0.7
(y-1)170	19401.710	19401.732	1.1
(x+1)179	20610.245	20610.241	-0.2
y181	20737.332	20737.329	-0.1
(y-1)181	20736.324	20736.329	0.2

z193	21973.792	21973.841	2.2
z195	22249.940	22250.022	3.7
(x+1)195	22292.946	22293.080	6.1
(x+1)197	22566.057	22566.100	1.9
z204	23156.348	23156.447	4.3
(y-1)208	23628.498	23628.337	-6.8
(x+1)222	25132.251	25132.256	0.2
(y-1)222	25104.256	25104.419	6.5
(y-1)223	25217.340	25217.348	0.3
x224	25341.380	25341.466	3.4
y224	25315.401	25315.561	6.3
(y-1)224	25314.393	25314.593	7.9

(2) Light chain of antibody

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
b8	896.472	896.466	-6.6
b12	1270.652	1270.650	-1.6
b13	1341.689	1341.690	0.9
c13	1358.715	1358.728	9.8
a14	1400.726	1400.722	-2.9
b14	1428.721	1428.719	-1.6
(a+1)17	1672.851	1672.845	-3.1
a17	1671.843	1671.841	-1.1
b17	1699.838	1699.846	5.0
c17	1716.864	1716.873	5.2
b20	2056.055	2056.035	-9.5
(a+1)23	2345.201	2345.186	-6.5
b38	4177.085	4177.097	3.0
a48	5256.782	5256.794	2.4
(a+1)63	6822.553	6822.549	-0.5
c68	7181.684	7181.663	-3.0
c69	7282.732	7282.760	3.9
(a+1)71	7500.814	7500.764	-6.6
b78	8243.212	8243.161	-6.2
(a+1)81	8570.379	8570.410	3.6
b83	8859.462	8859.437	-2.8

a93	10039.924	10039.999	7.5
c96	10489.162	10489.194	3.1
(a+1)96	10445.149	10445.251	9.8
(a+1)97	10502.170	10502.228	5.5
c98	10674.242	10674.236	-0.6
(a+1)99	10687.250	10687.294	4.1
b99	10714.237	10714.293	5.2
a100	10787.290	10787.286	-0.4
c102	11031.443	11031.463	1.8
a104	11214.533	11214.602	6.1
b105	11370.623	11370.622	-0.1
(a+1)109	11742.859	11742.793	-5.6
b109	11769.846	11769.951	8.9
(a+1)111	11910.949	11910.880	-5.8
(a+1)112	11997.981	11998.040	5.0
a113	12096.042	12096.124	6.9
b113	12124.036	12124.133	7.9
c114	12288.131	12288.168	3.0
a114	12243.110	12243.223	9.3
c116	12548.284	12548.255	-2.3
(a+1)117	12601.323	12601.332	0.7
a118	12697.368	12697.422	4.3
(a+1)118	12698.376	12698.437	4.8
c119	12829.421	12829.425	0.3
a119	12784.400	12784.410	0.8
c121	13073.491	13073.387	-7.9
b121	13056.464	13056.437	-2.1
b122	13184.523	13184.537	1.1
c122	13201.549	13201.668	9.0
(a+1)126	13542.768	13542.759	-0.7
b129	13828.872	13828.886	1.0
a132	14101.016	14101.036	1.5
(a+1)132	14102.023	14102.118	6.7
b133	14242.095	14241.974	-8.5
b142	15346.630	15346.709	5.2

c152	16545.312	16545.256	-3.4
c158	17146.557	17146.598	2.4
a173	18771.268	18771.425	8.4
b179	19401.590	19401.732	7.3
(a+1)180	19461.635	19461.479	-8.0
c184	19982.871	19982.703	-8.4
c187	20377.068	20376.921	-7.2
b187	20360.041	20360.190	7.3
b188	20488.136	20488.077	-2.9
c189	20604.231	20604.174	-2.8
b190	20750.268	20750.469	9.7
(a+1)192	20896.319	20896.521	9.6
a193	21024.354	21024.261	-4.5
b194	21151.417	21151.496	3.7
b197	21517.583	21517.517	-3.1
c198	21591.630	21591.748	5.5
(a+1)198	21547.617	21547.823	9.6
c199	21704.714	21704.910	9.0
(a+1)200	21747.733	21747.873	6.4
a202	21930.810	21930.718	-4.2
b203	22057.873	22057.926	2.4
a205	22259.021	22259.089	3.0
b207	22521.116	22521.134	0.8
c209	22808.287	22808.243	-1.9
c211	22994.351	22994.510	6.9
x17	1870.851	1870.852	1.0
(x+1)18	1972.906	1972.903	-1.4
y51	5652.662	5652.715	9.4
(x+1)57	6323.926	6323.879	-7.4
(y-1)62	6752.164	6752.122	-6.3
(x+1)63	6894.202	6894.147	-8.0
z67	7379.466	7379.493	3.6
x70	7776.686	7776.741	7.1
(x+1)82	9196.383	9196.367	-1.7
z90	9896.795	9896.826	3.1

(x+1)96	10593.066	10593.133	6.3
y97	10713.148	10713.251	9.7
z98	10810.213	10810.255	3.9
(y-1)98	10825.224	10825.300	7.0
(y-1)101	11158.393	11158.448	4.9
(y-1)104	11397.520	11397.543	2.1
(x+1)105	11496.552	11496.467	-7.4
x107	11752.693	11752.783	7.7
(y-1)112	12281.044	12281.062	1.5
z116	12609.182	12609.242	4.8
(x+1)117	12799.256	12799.348	7.2
(x+1)119	13056.405	13056.437	2.5
(x+1)120	13143.437	13143.516	6.0
z128	14150.819	14150.958	9.8
x131	14525.949	14525.958	0.6
(x+1)134	14881.111	14881.175	4.3
x140	15494.467	15494.492	1.6
(x+1)141	15596.523	15596.422	-6.5
x142	15742.583	15742.507	-4.9
z143	15815.612	15815.543	-4.4
(y-1)151	16480.889	16480.987	6.0
y152	16637.998	16638.073	4.5
x154	16848.062	16848.033	-1.7
y162	17578.460	17578.626	9.4
z165	17909.625	17909.649	1.3
(x+1)176	19203.356	19203.484	6.7
(y-1)178	19524.504	19524.575	3.6
(x+1)180	19779.626	19779.797	8.7
(y-1)181	19838.663	19838.694	1.6
(x+1)184	20222.875	20222.713	-8.0
y186	20410.978	20410.833	-7.1
x186	20436.958	20436.913	-2.2
z186	20394.960	20395.067	5.3
y189	20725.149	20725.011	-6.6
(y-1)189	20724.141	20724.072	-3.3

x191	20954.177	20954.380	9.7
x194	21267.377	21267.512	6.3
y197	21569.547	21569.591	2.0
y198	21668.616	21668.730	5.3
(x+1)198	21695.603	21695.776	8.0
(x+1)204	22227.852	22228.041	8.5
x205	22323.897	22323.688	-9.4
z207	22496.990	22497.065	3.4
y207	22513.008	22513.183	7.7
y208	22614.056	22614.225	7.5
(y-1)210	22868.218	22868.413	8.5
y211	22982.310	22982.220	-3.9
z211	22966.291	22966.452	7.0

(3) Heavy chain of antibody

Identified Ion	Theoretical Mass	Observed Mass	Mass Error (ppm)
(a+1)16	1649.886	1649.882	-2.4
a16	1648.878	1648.884	3.4
b16	1676.873	1676.880	3.9
(a+1)63	6809.423	6809.405	-2.5
a65	7008.531	7008.522	-1.3
(a+1)68	7336.704	7336.689	-2.0
c73	7894.034	7894.018	-2.1
(a+1)80	8640.382	8640.362	-2.2
a83	8968.532	8968.536	0.4
b98	10511.211	10511.192	-1.8
(a+1)99	10640.325	10640.297	-2.6
b99	10667.312	10667.320	0.7
c101	10946.434	10946.399	-3.2
a102	11048.481	11048.515	3.1
(a+1)110	11946.970	11946.997	2.3
c110	11990.983	11991.017	2.9
b111	12088.000	12087.962	-3.1
a111	12060.005	12060.047	3.5
a113	12278.110	12278.129	1.5
(a+1)115	12507.229	12507.263	2.7

(a+1)116	12693.308	12693.330	1.7
b135	14542.188	14542.185	-0.2
(a+1)148	15685.789	15685.828	2.5
(a+1)163	17287.593	17287.608	0.9
b188	19837.892	19837.947	2.8
a193	20347.141	20347.124	-0.8
a197	20745.394	20745.428	1.7
c199	20974.500	20974.528	1.4
(a+1)202	21217.635	21217.593	-1.9
a203	21273.648	21273.730	3.9
a204	21374.696	21374.764	3.2
c205	21547.776	21547.823	2.2
c206	21648.823	21648.825	0.1
(a+1)207	21767.873	21767.892	0.8
a207	21766.865	21766.884	0.9
a209	21981.951	21981.888	-2.8
a210	22095.994	22095.909	-3.8
b210	22123.989	22123.959	-1.4
b211	22223.057	22223.065	0.3
b214	22602.254	22602.185	-3.0
b215	22699.307	22699.314	0.3
a216	22758.344	22758.286	-2.5
b217	22900.382	22900.357	-1.1
c222	23489.689	23489.679	-0.4
b223	23600.757	23600.764	0.3
(a+1)225	23801.881	23801.942	2.5
(a+1)229	24216.062	24216.014	-2.0
b237	25121.432	25121.435	0.1
(a+1)238	25196.447	25196.488	1.7
(a+1)239	25293.499	25293.460	-1.6
a239	25292.492	25292.511	0.8
b239	25320.486	25320.561	3.0
(a+1)240	25364.537	25364.574	1.5
a240	25363.529	25363.571	1.7
c240	25408.550	25408.626	3.0

(a+1)241	25461.589	25461.551	-1.5
c241	25505.603	25505.553	-2.0
a241	25460.581	25460.570	-0.5
c245	25917.835	25917.938	4.0
(a+1)252	26621.217	26621.191	-1.0
b252	26648.204	26648.188	-0.6
b253	26745.257	26745.184	-2.7
(a+1)254	26815.323	26815.283	-1.5
a254	26814.315	26814.349	1.3
c256	27084.484	27084.501	0.6
a259	27383.632	27383.608	-0.9
b266	28210.069	28210.039	-1.1
b282	29945.854	29945.817	-1.2
c289	30915.325	30915.328	0.1
b289	30898.298	30898.395	3.1
c291	31071.414	31071.357	-1.8
a301	32187.035	32186.949	-2.7
c311	33501.618	33501.550	-2.1
(a+1)312	33556.673	33556.618	-1.6
(a+1)315	33855.858	33855.859	0.0
(a+1)316	33956.906	33957.004	2.9
c324	35006.422	35006.336	-2.5
b325	35046.417	35046.291	-3.6
(a+1)325	35019.430	35019.491	1.7
(a+1)326	35147.525	35147.406	-3.4
b327	35303.555	35303.494	-1.7
a329	35566.718	35566.850	3.7
b334	36124.953	36124.987	0.9
b337	36437.169	36437.294	3.4
c340	36719.338	36719.330	-0.2
a356	38465.317	38465.390	1.9
a376	40709.450	40709.346	-2.6
a380	41106.719	41106.558	-3.9
c386	41874.068	41874.016	-1.2
a386	41829.047	41829.047	0.0

b387	41928.079	41927.928	-3.6
a387	41900.084	41900.104	0.5
b404	43901.954	43901.820	-3.1
(a+1)405	43972.020	43971.890	-3.0
(a+1)413	44792.380	44792.322	-1.3
(a+1)422	45900.013	45899.892	-2.6
c433	47235.591	47235.415	-3.7
a435	47376.670	47376.649	-0.4
(a+1)437	47645.777	47645.603	-3.7
c450	49198.528	49198.382	-3.0
a451	49266.591	49266.508	-1.7
(x+1)12	1334.696	1334.696	0.1
(x+1)14	1585.797	1585.791	-3.8
x16	1834.933	1834.926	-3.8
y16	1808.953	1808.951	-1.1
(x+1)19	2173.079	2173.081	1.1
y20	2277.132	2277.136	1.5
y21	2376.201	2376.199	-1.0
y22	2463.233	2463.237	1.7
(x+1)46	5295.566	5295.556	-1.9
x71	8078.846	8078.873	3.4
z73	8220.933	8220.914	-2.2
y77	8732.200	8732.209	1.1
(y-1)87	9815.774	9815.785	1.1
(x+1)88	9944.817	9944.794	-2.3
x91	10300.963	10300.938	-2.4
z94	10599.150	10599.191	3.8
z96	10809.287	10809.280	-0.7
y96	10825.306	10825.300	-0.5
(y-1)97	10925.346	10925.351	0.4
z101	11397.578	11397.543	-3.0
(y-1)102	11541.631	11541.617	-1.3
z103	11682.722	11682.762	3.5
z111	12492.198	12492.224	2.1
y112	12609.264	12609.242	-1.7

y115	12979.486	12979.528	3.3
z116	13060.520	13060.562	3.2
z117	13131.557	13131.591	2.6
y118	13244.628	13244.650	1.6
y121	13556.844	13556.895	3.7
x123	13783.899	13783.913	1.0
(x+1)125	14012.070	14012.104	2.4
z128	14362.224	14362.247	1.6
x133	14945.508	14945.547	2.6
(y-1)151	17079.616	17079.673	3.3
(y-1)154	17465.760	17465.797	2.1
x166	18809.478	18809.468	-0.5
x180	20555.247	20555.260	0.6
(y-1)181	20627.328	20627.283	-2.2
z181	20612.317	20612.324	0.3
(x+1)181	20655.323	20655.344	1.0
(x+1)183	20869.418	20869.443	1.2
(y-1)185	21039.560	21039.485	-3.6
y185	21040.568	21040.536	-1.5
z187	21227.598	21227.588	-0.5
(x+1)189	21498.715	21498.775	2.8
x191	21695.808	21695.776	-1.5
x193	21938.941	21938.944	0.1
z193	21896.943	21897.005	2.8
x195	22183.066	22183.082	0.7
(x+1)195	22184.073	22184.146	3.3
y196	22270.170	22270.126	-2.0
x196	22296.150	22296.159	0.4
(y-1)197	22370.210	22370.196	-0.6
z197	22355.199	22355.266	3.0
(x+1)198	22513.232	22513.183	-2.2
(y-1)198	22485.237	22485.284	2.1
y199	22614.340	22614.278	-2.7
(y-1)199	22613.332	22613.306	-1.2
y200	22711.393	22711.369	-1.0

z201	22823.469	22823.549	3.5
z202	22920.522	22920.529	0.3
x202	22962.520	22962.565	2.0
(y-1)203	23032.585	23032.588	0.1
(x+1)203	23060.580	23060.581	0.0
(y-1)205	23292.738	23292.742	0.2
x207	23565.862	23565.819	-1.8
y208	23626.915	23626.940	1.1
x209	23749.947	23750.030	3.5
(x+1)209	23750.954	23751.044	3.8
x210	23806.968	23807.013	1.9
y211	23838.010	23837.995	-0.6
y215	24290.274	24290.210	-2.6
y216	24361.311	24361.232	-3.3
z216	24345.292	24345.237	-2.3
x217	24484.343	24484.253	-3.7
x224	25221.605	25221.507	-3.9
z224	25179.607	25179.510	-3.9
(y-1)224	25194.618	25194.566	-2.1
y225	25323.721	25323.650	-2.8
z225	25307.702	25307.711	0.3
y226	25438.748	25438.726	-0.9
z236	26535.307	26535.277	-1.1
(x+1)237	26707.355	26707.298	-2.1
z240	26966.472	26966.398	-2.7
(y-1)240	26981.483	26981.429	-2.0
(x+1)242	27234.625	27234.552	-2.7
(x+1)248	27913.924	27914.035	4.0
y250	28151.048	28151.160	4.0
x251	28305.086	28305.075	-0.4
(x+1)255	28664.279	28664.248	-1.1
z256	28708.305	28708.212	-3.2
(x+1)256	28751.311	28751.408	3.4
(y-1)258	28907.401	28907.349	-1.8
y269	30014.010	30014.066	1.8

x271	30214.054	30213.934	-4.0
(x+1)289	32081.004	32080.882	-3.8
(y-1)298	33098.558	33098.564	0.2
z298	33083.547	33083.583	1.1
x300	33368.667	33368.788	3.6
(y-1)305	33825.939	33826.059	3.5
z306	33881.965	33881.918	-1.4
(x+1)307	33996.008	33996.044	1.1
y308	34070.068	34069.971	-2.8
(y-1)313	34458.215	34458.128	-2.5
x317	34884.414	34884.392	-0.6
(y-1)320	35138.601	35138.565	-1.0
(y-1)322	35384.738	35384.855	3.3
(y-1)326	35753.939	35753.870	-1.9
z328	35927.008	35926.954	-1.5
z329	35998.045	35998.041	-0.1
y336	36719.400	36719.330	-1.9
x343	37548.740	37548.624	-3.1
y343	37522.761	37522.757	-0.1
x349	38147.995	38148.145	3.9
z352	38460.260	38460.224	-0.9
x355	38925.438	38925.298	-3.6
(x+1)359	39370.612	39370.567	-1.1
x366	40152.948	40153.059	2.8
(y-1)368	40298.046	40298.094	1.2
(y-1)371	40571.178	40571.138	-1.0
y372	40685.270	40685.220	-1.2
(x+1)372	40712.257	40712.276	0.5
y373	40814.313	40814.179	-3.3
(x+1)373	40841.300	40841.172	-3.1
x382	41830.769	41830.604	-3.9
(y-1)382	41803.782	41803.808	0.6
(x+1)383	41944.861	41944.695	-4.0
(x+1)385	42144.977	42144.980	0.1
y385	42117.990	42118.144	3.7

(y-1)394	43055.475	43055.319	-3.6
z405	44261.036	44260.881	-3.5
y408	44705.260	44705.360	2.2
x409	44844.324	44844.375	1.2
(x+1)410	44902.353	44902.469	2.6
y419	45940.974	45940.921	-1.2
y432	47314.611	47314.511	-2.1
(x+1)435	47631.679	47631.849	3.6
y438	47917.892	47917.826	-1.4
x440	48159.946	48159.810	-2.8
(y-1)448	48868.387	48868.274	-2.3
(y-1)453	49453.662	49453.548	-2.3