Supplementary Information

Generating informative sequence tags from antigen-binding regions of heavily glycosylated IgA1 antibodies by native top-down electron capture dissociation

Jean-Francois Greisch[1,2,#], Maurits A. den Boer[1,2,#], Frank Beurskens[3], Janine Schuurman[3], Sem Tamara[1,2], Albert Bondt[1,2], Albert J.R. Heck[1,2,*]

[1] *Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute of Pharmaceutical Sciences, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands*

[2] *Netherlands Proteomics Center, Padualaan 8, 3584 CH Utrecht, The Netherlands*

[3] *Genmab, Utrecht, Uppsalalaan 15, 3584 CT Utrecht, The Netherlands*

[#] *equal contributing first authors*

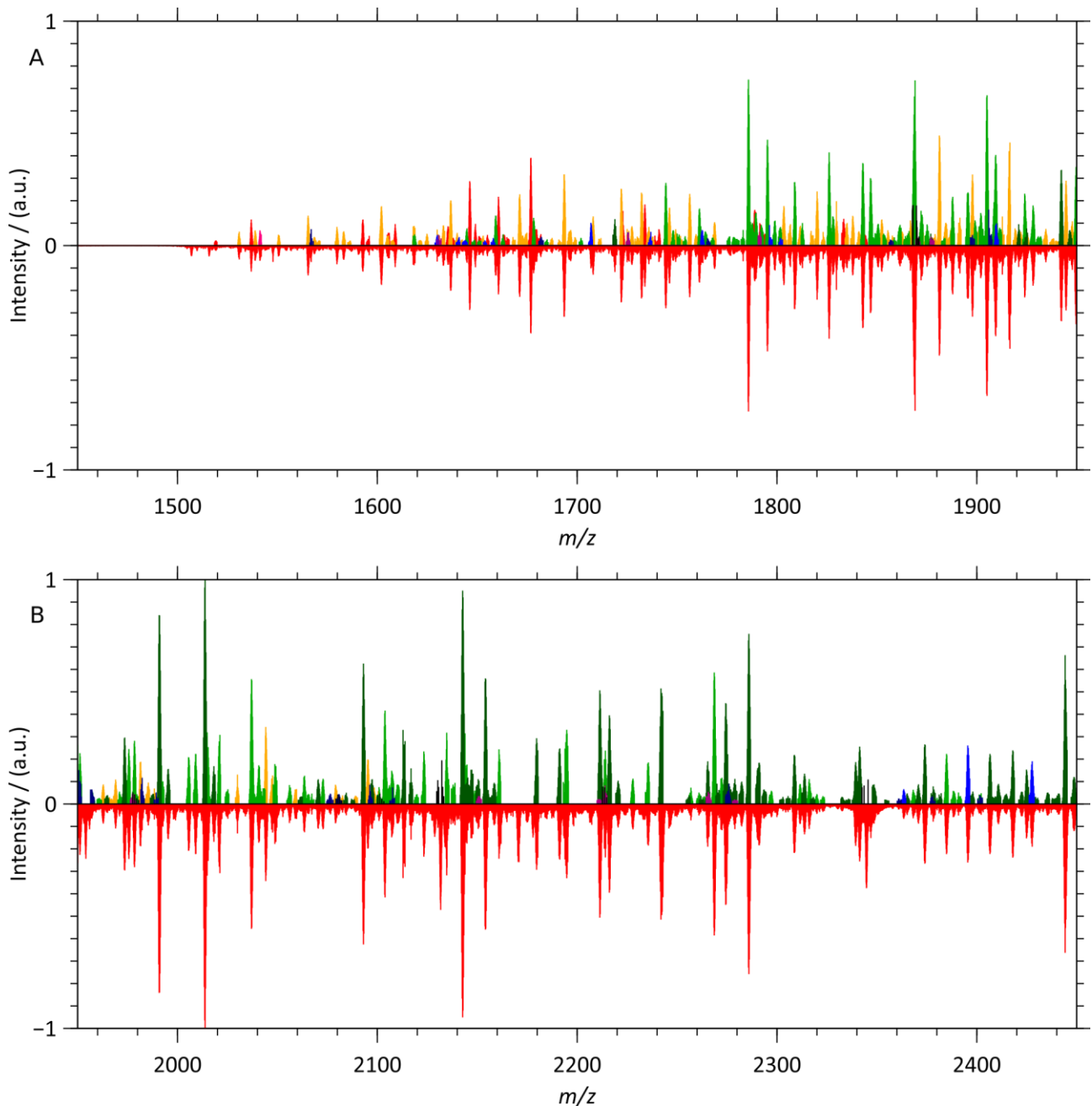[*] *to whom correspondence should be addressed, a.j.r.heck@uu.nl*

Figure S1A-B. Comparison of the low *m/z* region of the native top-down ECD spectra of intact anti-CD20 IgA1 spectrum reconstructed from charge-deconvoluted spectra corresponding to fragment charge states ranging from 1⁺ to 13⁺ (top spectra), with the unprocessed fragment ion spectrum (bottom spectrum). **A)** [1450, 1950] *m/z* spectral range. **B)** [1950, 2450] *m/z* spectral range. See Figure 3 for the complete spectral range.
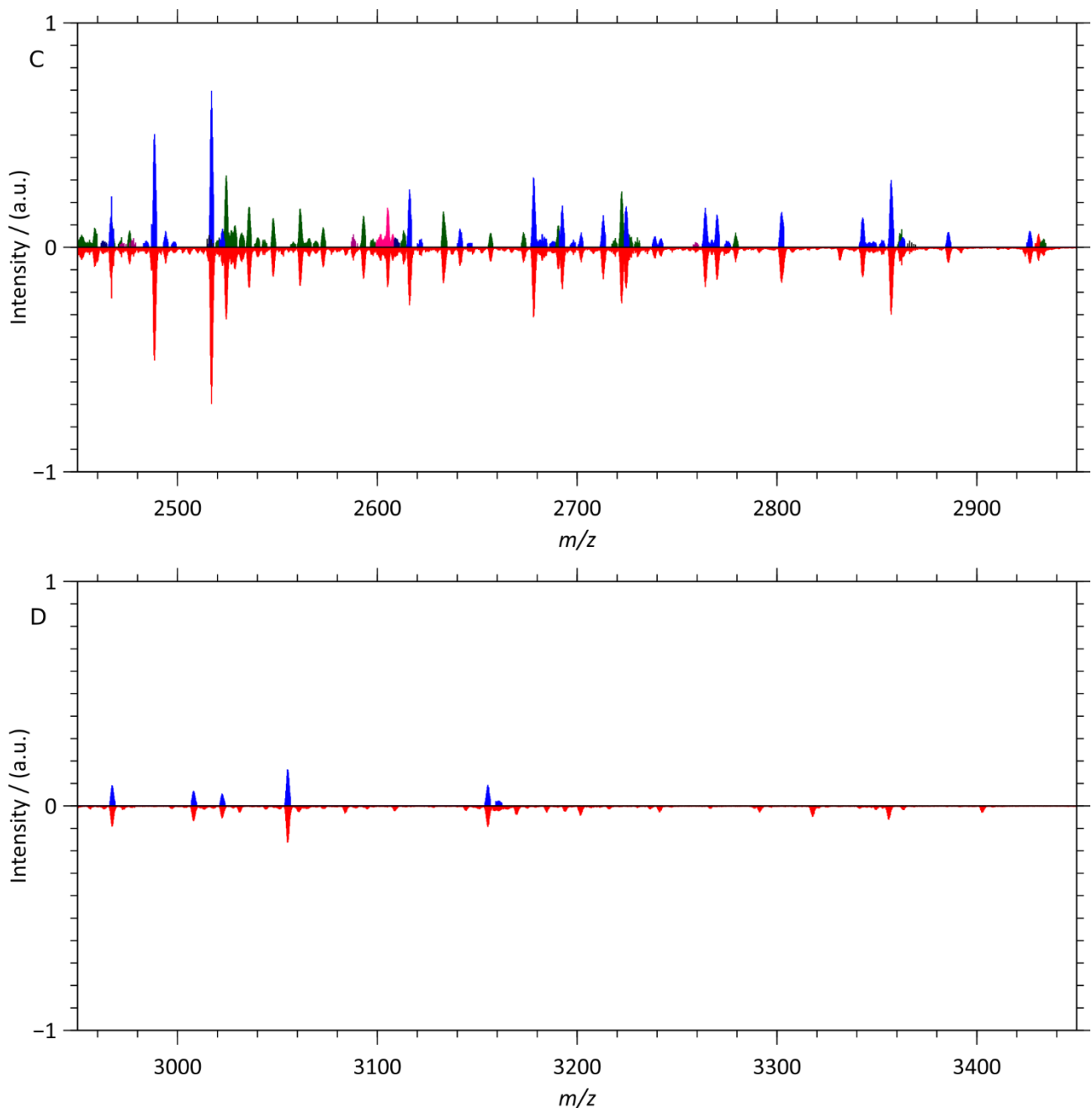
Figure S1C-D. Comparison of the low *m/z* region of the native top-down ECD spectra of intact anti-CD20 IgA1 spectrum reconstructed from charge-deconvoluted spectra corresponding to fragment charge states ranging from 1$^+$ to 13$^+$ (top spectra), with the unprocessed fragment ion spectrum (bottom spectrum). **C)** [2450, 2950] *m/z* spectral range. **D)** [2950, 3450] *m/z* spectral range. See Figure 3 for the complete spectral range.
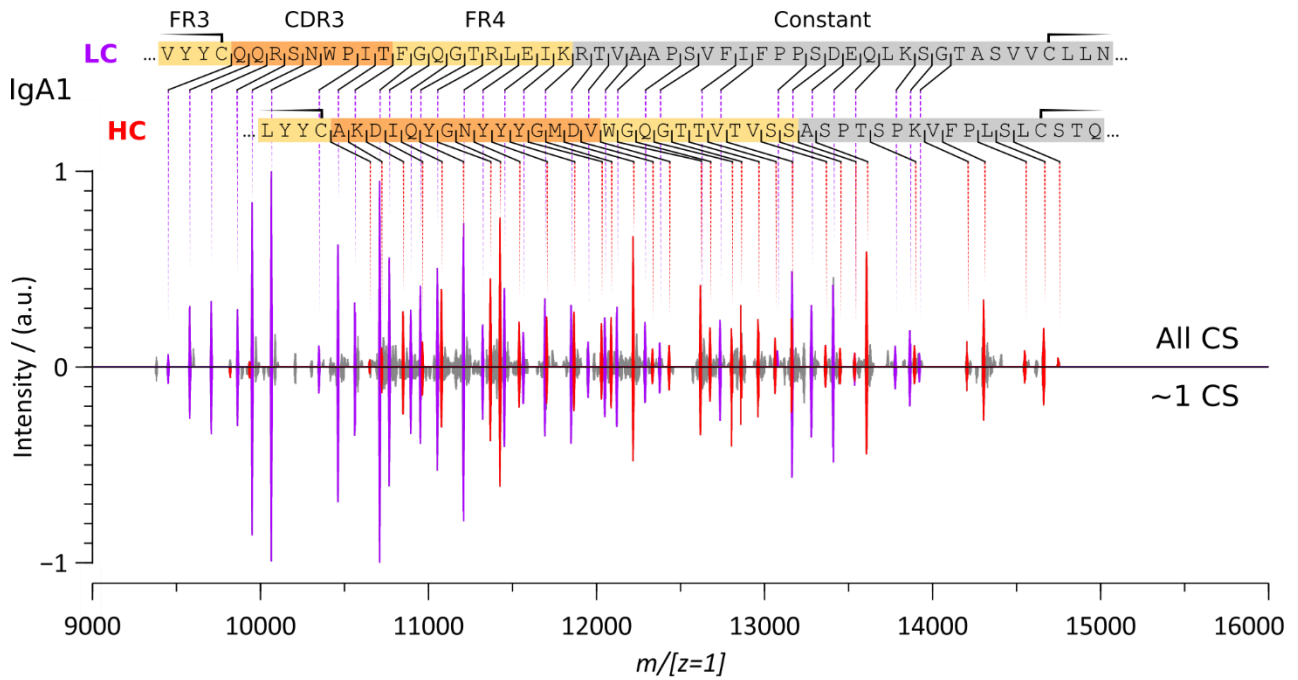
Figure S2. Charge-deconvoluted native top-down ECD spectra of intact anti-CD20 IgA1 selecting as precursor either all charge states (top) or a much narrower isolation window of 210 *m/z* targeting the charge state 25+ (bottom). LC *c*-ion fragments are annotated in purple and the HC *c*-ion fragments in red. Notably, the resulting ECD spectra look nearly identical.
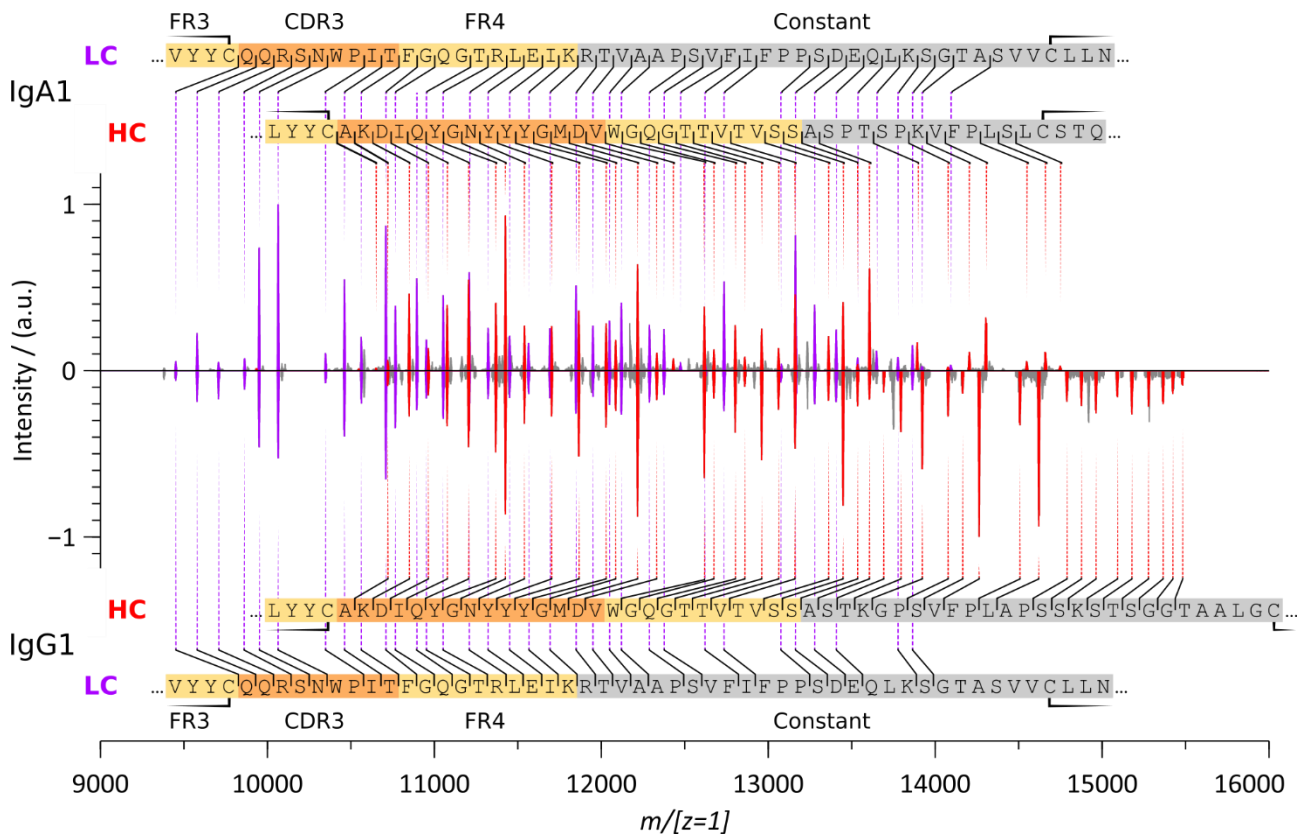

Figure S3. Charge-deconvoluted native top-down ECD spectra of anti-CD20 IgA1's (top) and IgG1's (bottom) Fab arms. LC *c*-ion fragments are annotated in purple and the HC *c*-ion fragments in red.
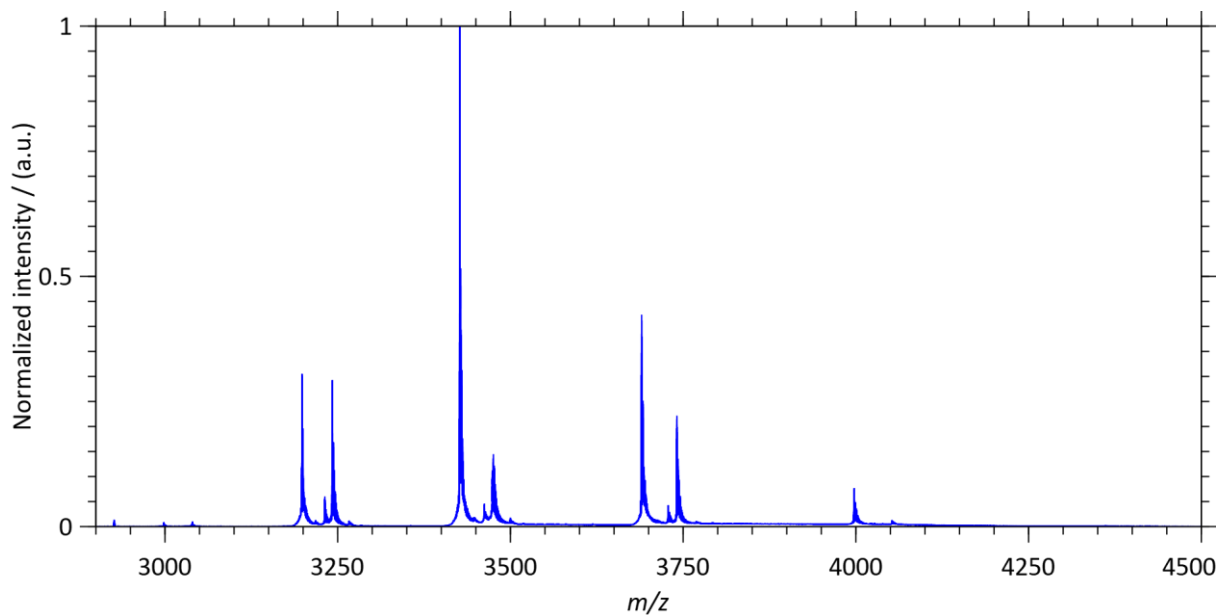
4

Figure S4. MS1 spectrum of the anti-CD20 IgA1 Fab. Two distinct Fab fragments are observed of 47 958 and 48 618 Da with the latter due to a missed cleavage of the OpeRATOR® enzyme, adding 295 (TPP) and 365 (HexNAc+Hex) to the mass of the main fragment.
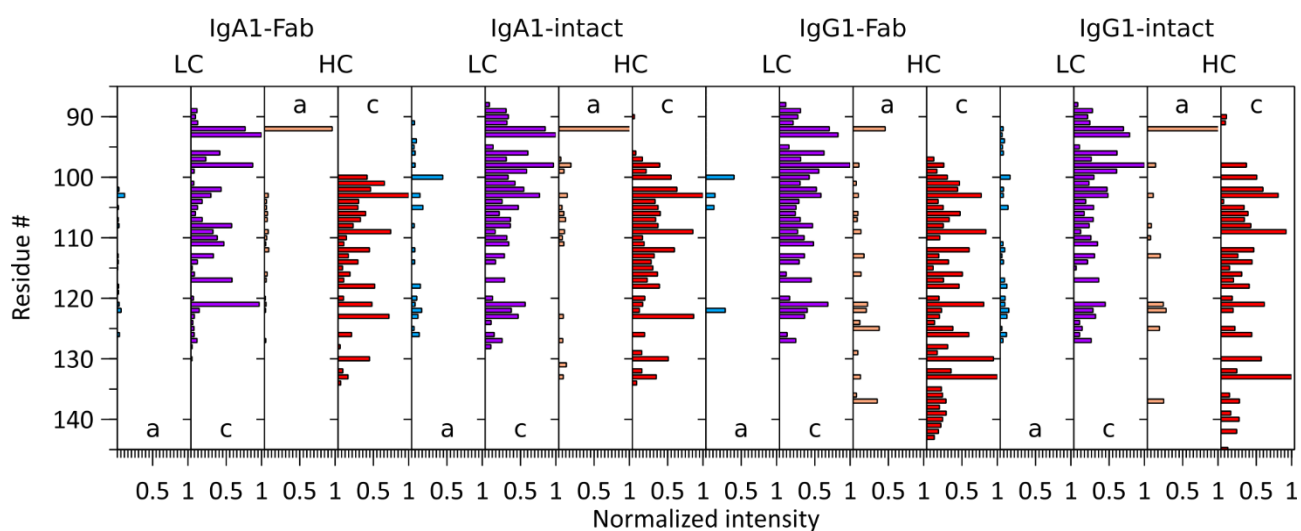

Figure S4. Bar-chart displaying normalized *c*- and *a*-ion fragment intensities for each residue in the LCs and HCs of IgA1 Fab, IgA1 intact, IgG1 Fab, and IgG1 intact. The a-ion fragments are generally of lower abundance, but for some cleavage sites, a-ions complement missing c-ions.
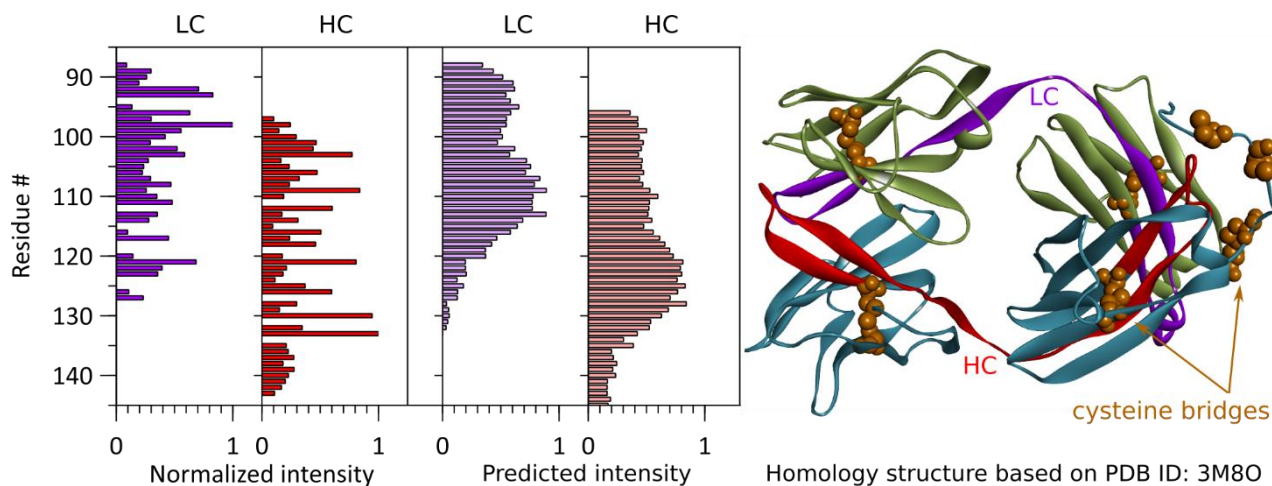
Figure S5. Correlation between fragment ion intensities and intensities predicted from the normalized interaction energies of (*c*-, bridged *z*-) ion pairs for the anti-CD20 IgG1 Fab molecule and the homology model, based on the available crystal structure (PDB ID 3BKY), used to compute the interaction energies.

Table S1. Amino acid sequences of the anti-CD20 IgG1 and IgA1 mAbs used. The primary cleavage sites of the IgdE (IgG1) and OpeRATOR (IgA1) enzymes that produce the Fab molecules are indicated with a red forward slash in the heavy chain sequences.

IgG1 anti-CD20 (7D8)

Heavy Chain

EVQLVESGGGLVQPDRSLRLSCAASGFTFHDYAMHWVRQAPGKGLEWVSTISWNSGTIGYADSVKGRFTISRD
NAKNSLYLQMNSLRAEDTALYYCAKDIQYGNYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPK
SCDKT**/**HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
LSLSPGK

κ Light Chain

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSL
EPEDFAVYYCQQRSNWPITFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

6

IgA1 anti-CD20 (7D8)

Heavy Chain

EVQLVESGGGLVQPDRSLRLSCAASGFTFHDYAMHWVRQAPGKGLEWVSTISWNSGTIGYADSVKGRFTISRD
NAKNSLYLQMNSLRAEDTALYYCAKDIQYGNYYYGMDVWGQGTTVTVSSASPTSPKVFPLSLCSTQPDGNVVIA
CLVQGFFPQEPLSVTWSESGQGVTARNFPPSQDASGDLYTTSSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTV
PCPVPS**/T**PP**/**TPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEANLTCTLTGLRDASGVTFTWTPSSGKSAVQGP
PERDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHLLPPPSEELALNELVTLTCL
ARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFT
QKTIDRLAGKPTHVNVSVVMAEVDGTCY

κ Light Chain

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSL
EPEDFAVYYCQQRSNWPITFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Table S2. Overview of the average molecular masses of the intact Igs and Fab molecules used in this study. The theoretical mass is obtained from the provided protein sequences as shown in **Table S1**, the experimental mass is determined by native mass spectrometry.

Species	MW$_{\text{theoretical unglycosylated}}$ (Da)	MW$_{\text{experimental}}$ (Da)	Mass difference (Da)
IgG1 anti-CD20	146 228	148 858	2 630[*]
IgA1 anti-CD20	149 191	157 660 (av. glyc.)	8 469[**]
IgG1 anti-CD20 Fab	47 900	47 900	-0.8
IgA1 anti-CD20 Fab	47 956.6	47 957.5	0.9
IgA1 anti-CD20 Fab	48 618.1	48 617.9	-0.2

[*] Corresponding to the mass shift induced by G0F/G0F glycosylation and *C*-terminal lysine clipping of both HCs.

[**] Induced by extensive *N*- and *O*-glycosylation of the IgA1 HCs.

[***] Due to an additional TPP at the HC's *C*-terminus and an HexHexNAc attached (at **T**).

Table S3. ECD cell parameters.

Species	L1	L2	LM3	L4	FB	LM5	L6	L7
Immunoglobulins	2.12	-13.2	7.0	4.7	-1.5	7.0	-15.2	1.5