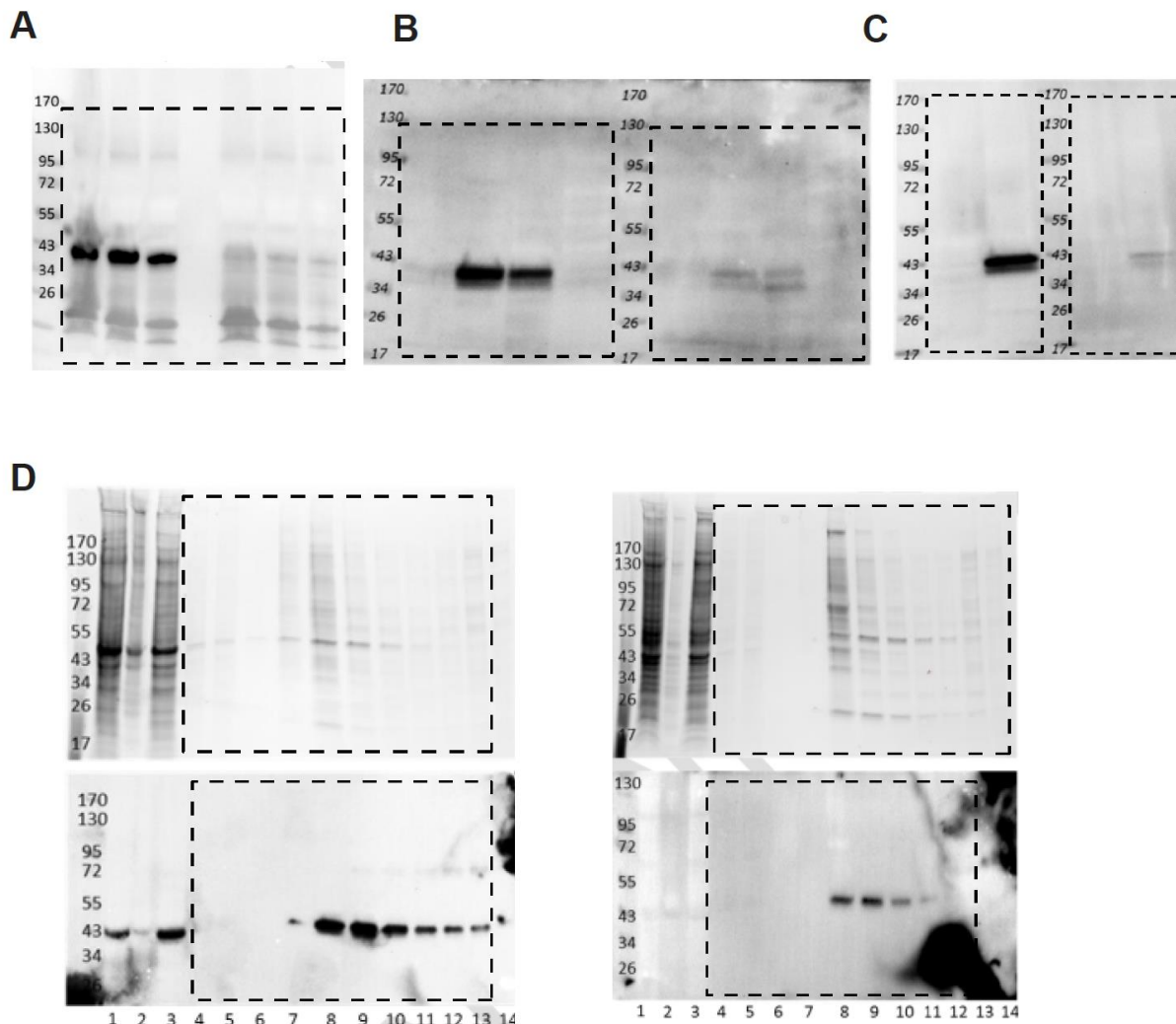


## SUPPLEMENTARY MATERIAL

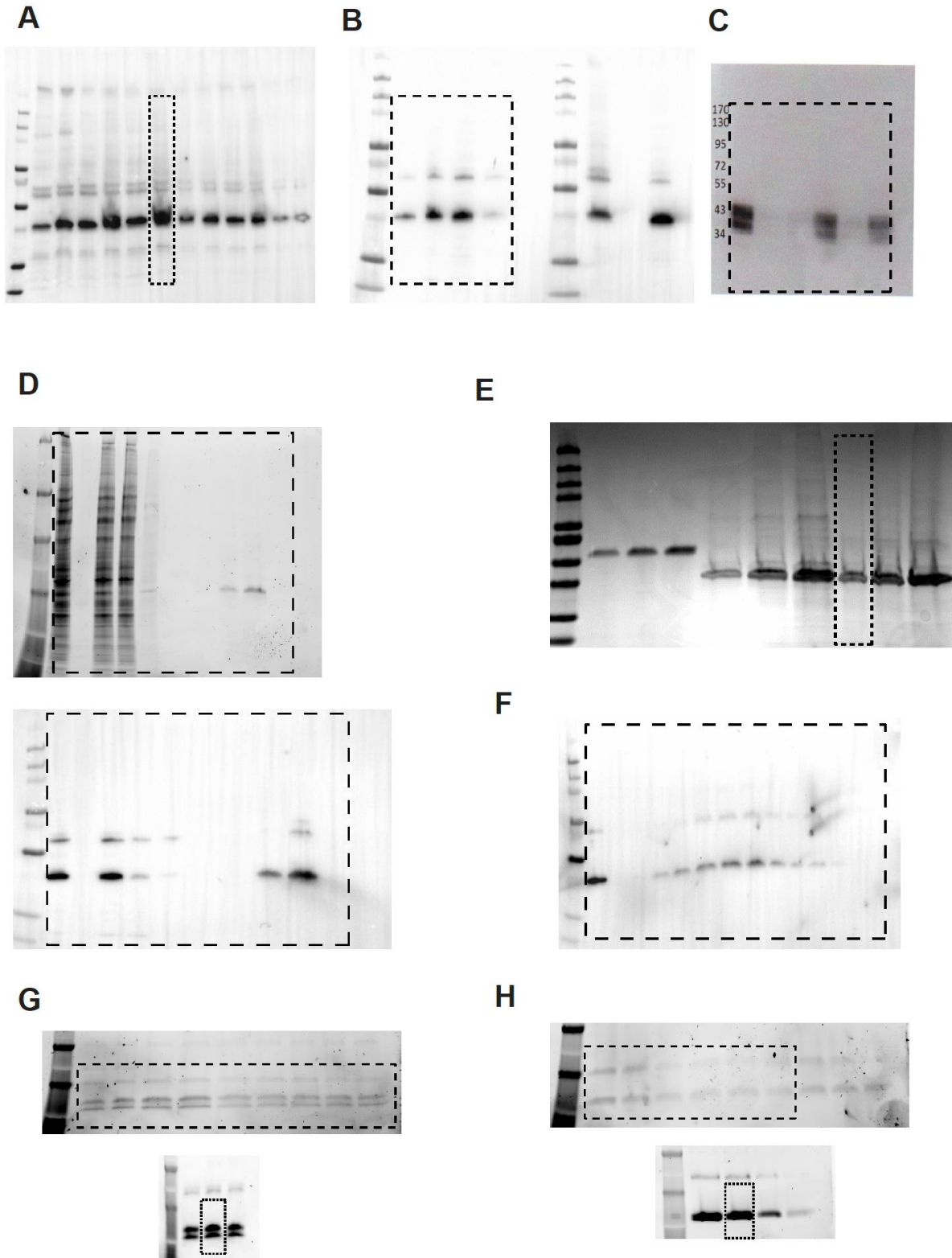
**TITLE: Biochemical and biophysical characterization of purified native CD20 alone and in complex with rituximab and obinutuzumab**

### AUTHORS:

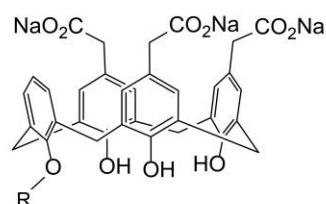
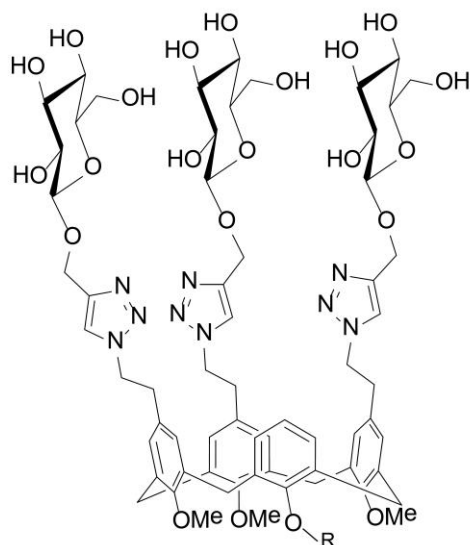
Morgane AGEZ<sup>1</sup>, Elodie DESUZINGES MANDON<sup>1</sup>, Thomas IWEMA<sup>1</sup>, Reto GIANOTTI<sup>2</sup>, Florian LIMANI<sup>2</sup>, Sylvia HERTER<sup>2</sup>, Ekkehard MÖSSNER<sup>2</sup>, Eric A KUSZNIR<sup>3</sup>, Sylwia HUBER<sup>3</sup>, Matthias LAUER<sup>3</sup>, Philippe RINGLER<sup>4</sup>, Claudia FERRARA<sup>2</sup>, Christian KLEIN<sup>2</sup> & Anass JAWHARI<sup>1¶</sup>



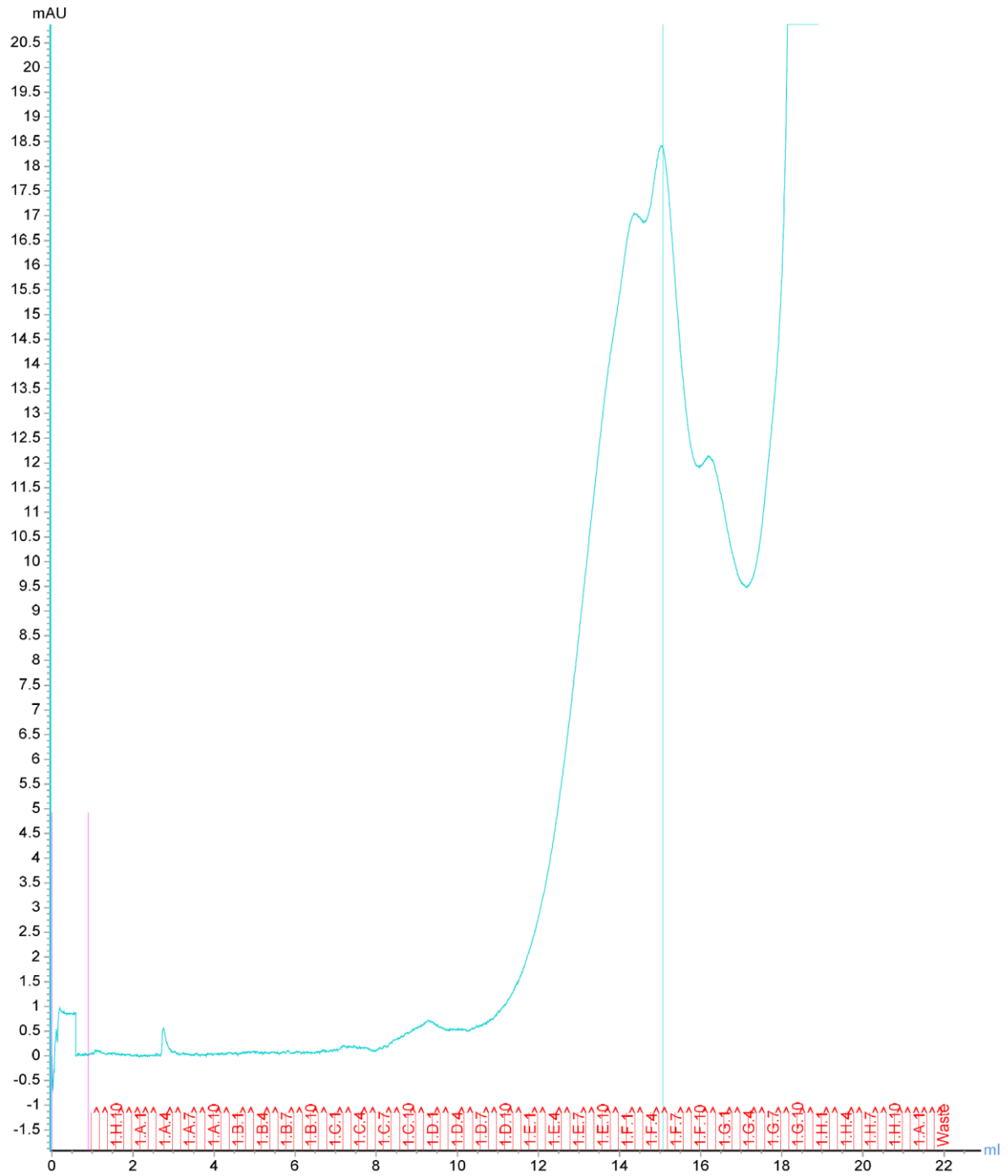
**Figure S1:** Original gels or Western-blot used for Figure 1A-D. A rectangle represents what is shown in the main figures.



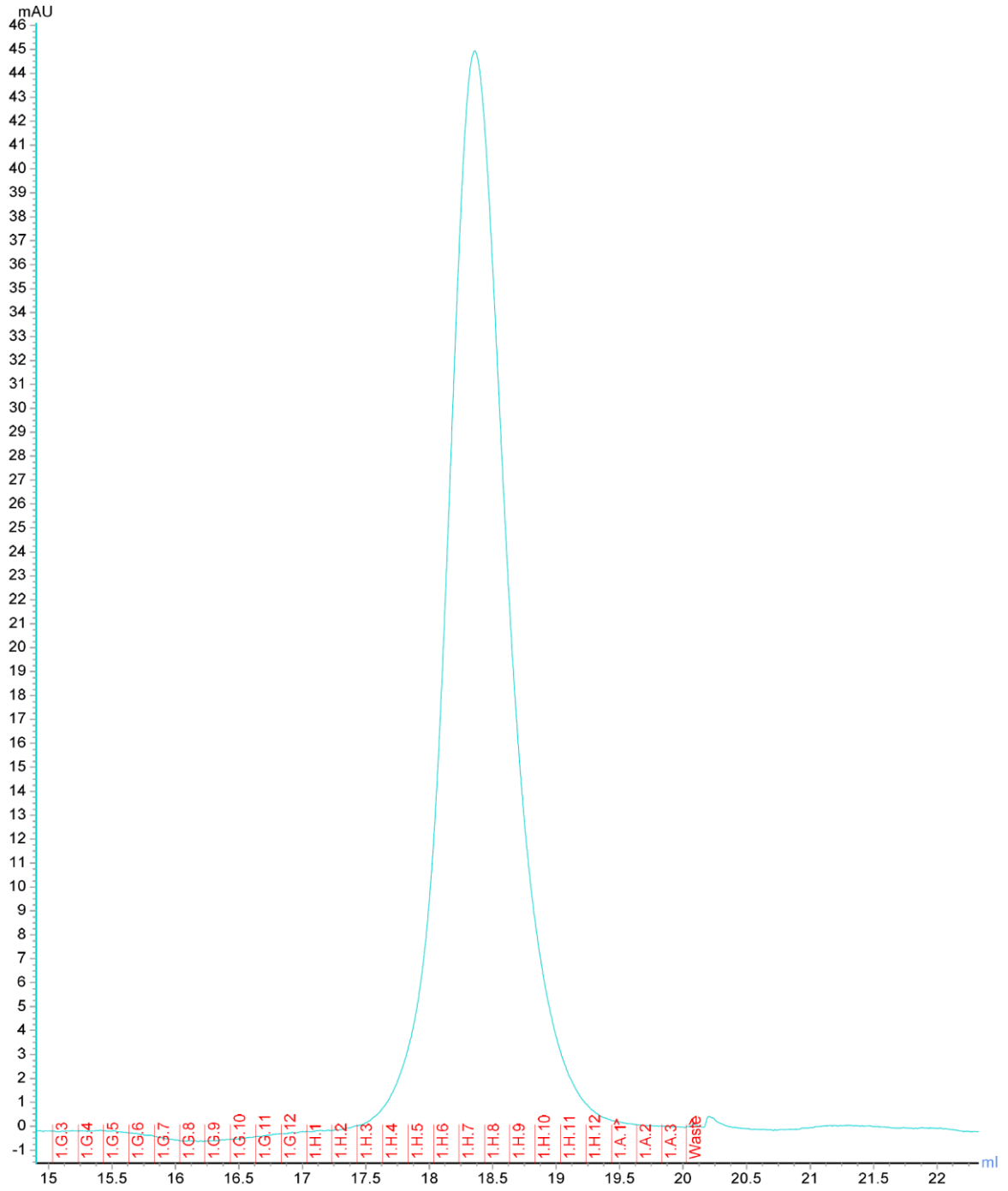
**Figure S2:** Original gels or Western-blot used for the main Figures. A-E correspond to Figure 2A-E. F corresponds to Figure 3A. G-H correspond to Figure 5A-B. A rectangle represents what is shown in the main figures.

**A****B**

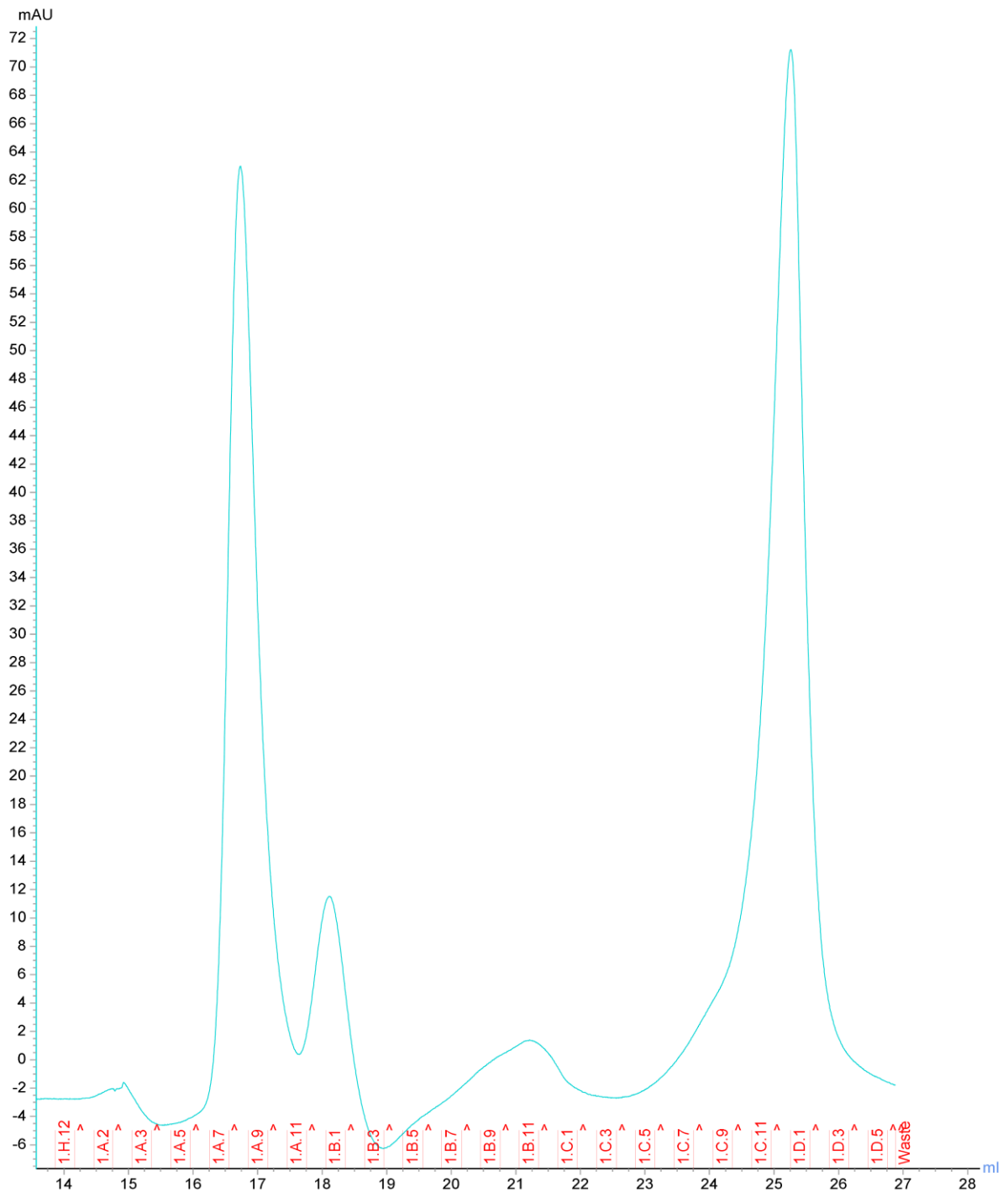
**Figure S3:** Structure of two calixarene based detergents used for solubilization and purification of CD20. **A-** 5,11,17-tris[(carboxy)methyl]-25-monoalkoxy-26,27,28-trihydroxycalix[4]arene. Also called CALX-R2 in this manuscript. **B-** 5,11,17-tris(2-[4-( $\beta$ -D-glucopyranosyl)-1H-1,2,3-triazol-1-yl]ethyl)-25-monoalkoxy-26,27,28-trimethoxycalix[4]arene; respectively, also called CALX-173-GK. R corresponds to an aliphatic tail of seven carbons.



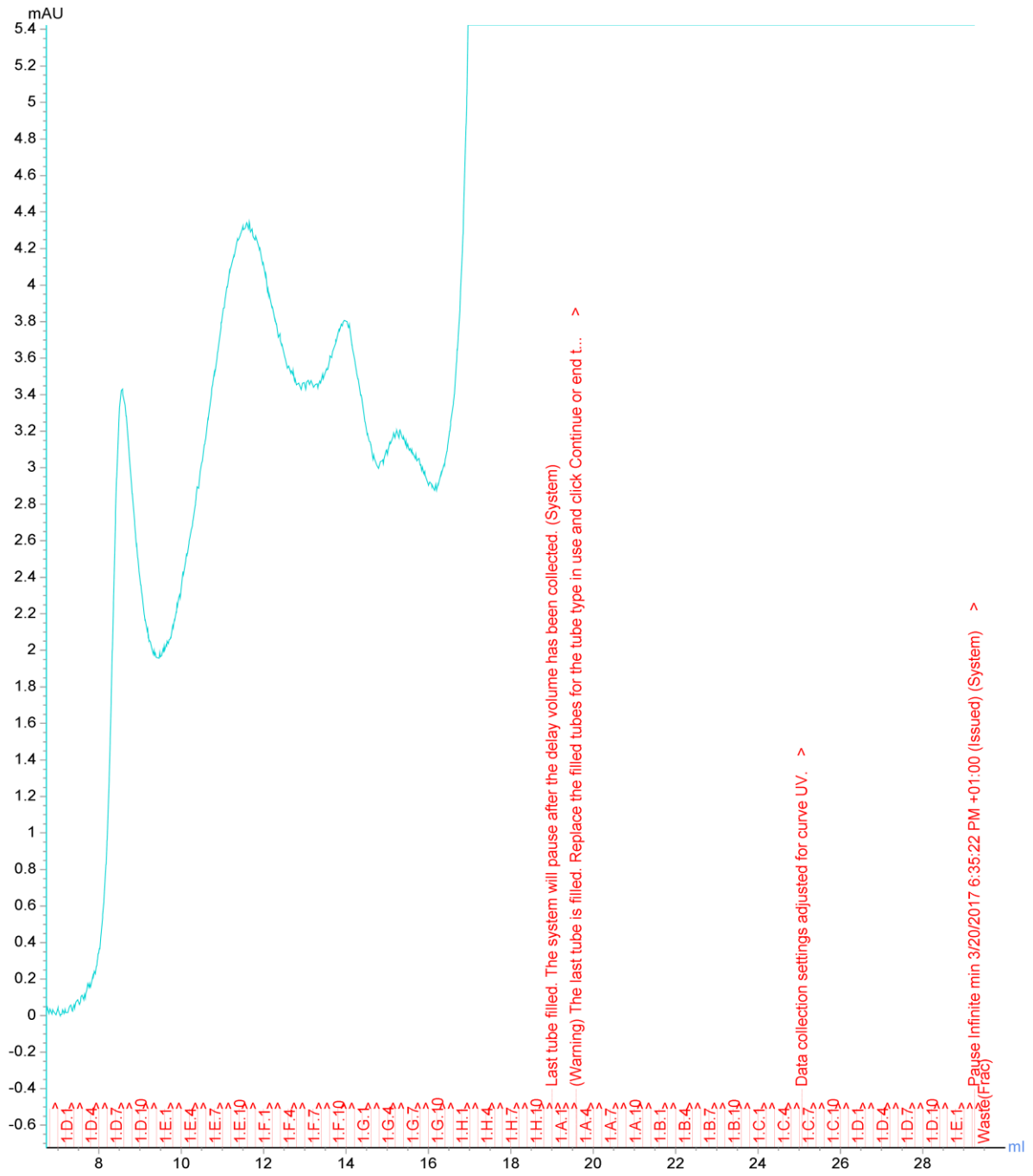
**Figure S4:** Full length size exclusion chromatogram of purified CD20.



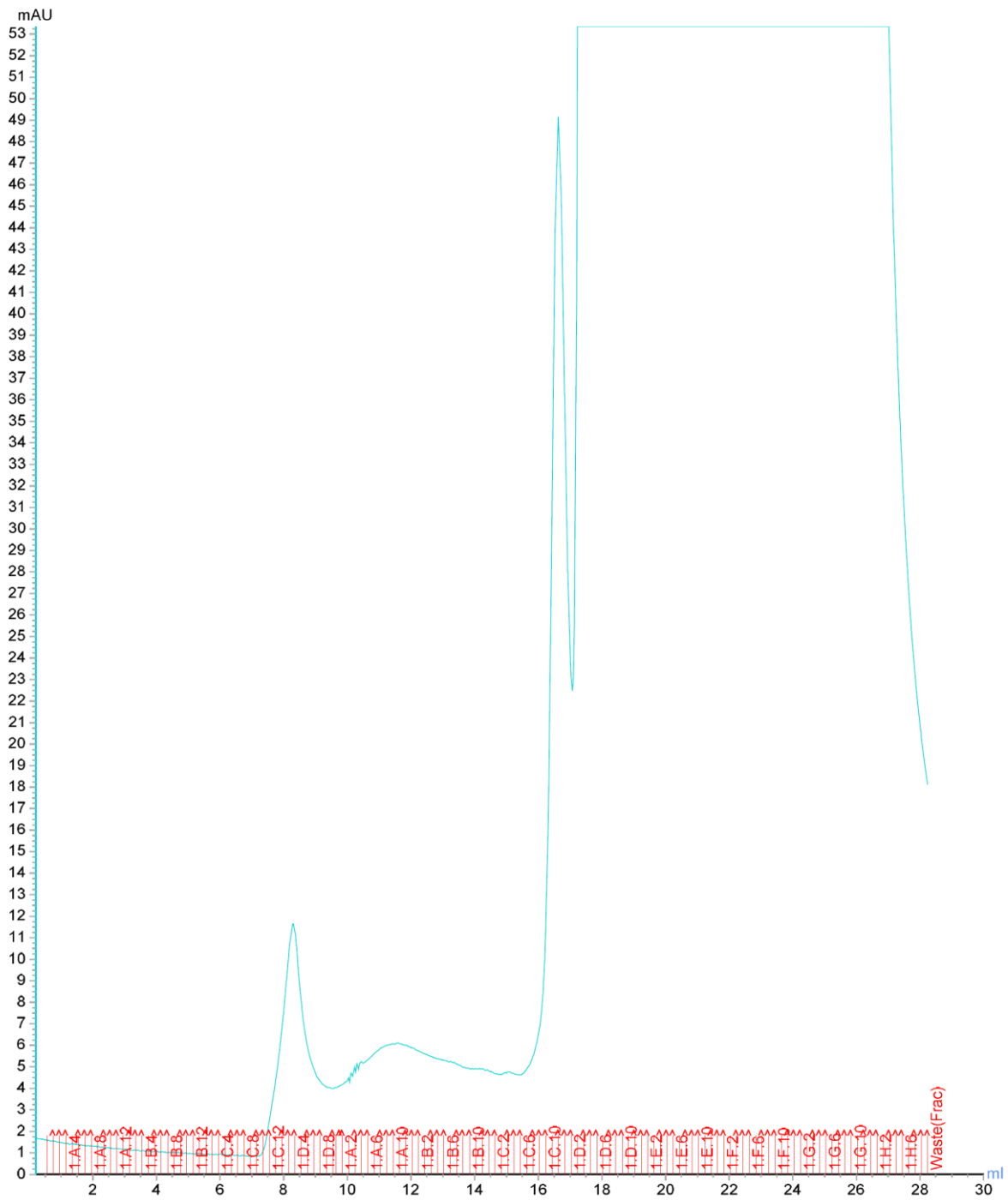
**Figure S5:** Full length size exclusion chromatogram of the rituximab.



**Figure S6:** Full length size exclusion chromatogram of the obinutuzumab.



**Figure S7:** Full length size exclusion chromatogram of CD20/rituximab complex.



**Figure S8:** Full length size exclusion chromatogram of CD20/ obinutuzumab complex.



Sample	conc. [mg/ml]	Detergent in buffer	Detection system	Peak	sw [S]	f/f0	Area [%]	vbar [ml.g <sup>-1</sup> ]	MW <sub>exp.</sub> [kDa]	MW <sub>calc.</sub> [kDa]	Interpretation
CALX-173-GK alone	5,000	CALX-173-GK	Interference	1	2,9	1,18	100	0,764	no	38	detergent micelle
CD20	0,500	CALX-173-GK	Absorbance	1	3,5	1,35	30	0,741	34	~ 54	probably monomeric CD20
				2	4,6		70				
				3	5,8						
				4	7,7						
				5	10,0						
CD20	0,500	CALX-173-GK	Interference	1	3,5	1,35	27	0,741	34	~ 61	probably monomeric CD20
				2	4,4		73				
				3	5,8						
				4	7,4						
				5	8,6						
				6	9,9						
DDM alone	20,000	DDM	Interference	1	2,9	1,00	100	0,836	50	53	detergent micelle
CD20	0,044	CALX-173-GK	Absorbance	1	3,6	1,35	46	0,741	34	~ 57	probably monomeric CD20
				2	5,8		17				
				range	6-12		37				
CD20	0,044	DDM	Absorbance	1	3,1	1,42	72	0,741	34	~ 48	probably monomeric CD20
				2	5,0		28				

**Table S9:** Summary of AUC calculations of the molecular mass of CD20 species.

Expected molecular mass (MW<sub>exp.</sub>) was calculated from protein amino acid sequence. The partial specific volume (vbar) of the protein alone was taken into account as it is impossible to determine exact detergent/protein ratio due to simultaneous absorbance of the protein and CALX-173-GK detergent. Therefore, the calculated molecular masses should be interpreted as “estimates”.