Disulfide Bond Characterization of Endogenous IgG3 Monoclonal Antibodies Using LC-MS: An Investigation of IgG3 Disulfide-mediated Isoforms.

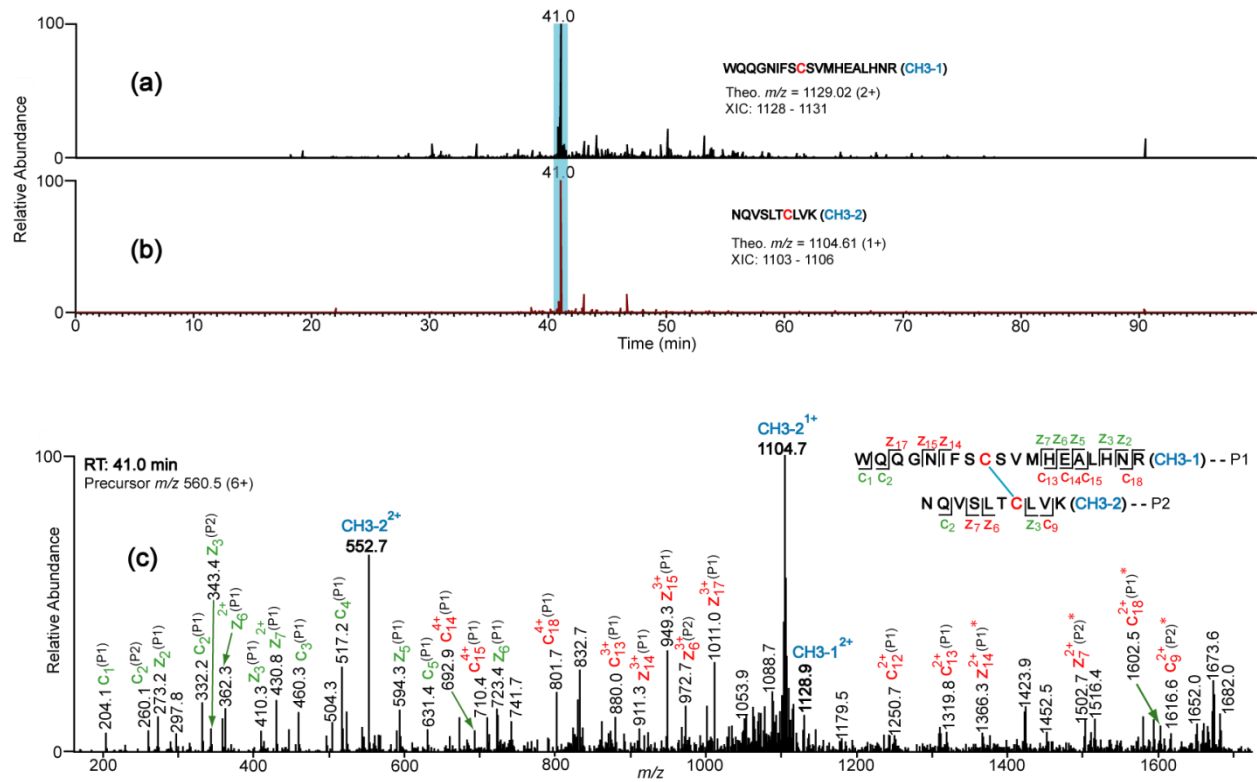Jude C. Lakbub[1], Daniel F. Clark[1], Ishan S. Shah[2], Zhikai Zhu[1], Eden P. Go[1], Thomas J. Tolbert[2], and Heather Desaire[1]

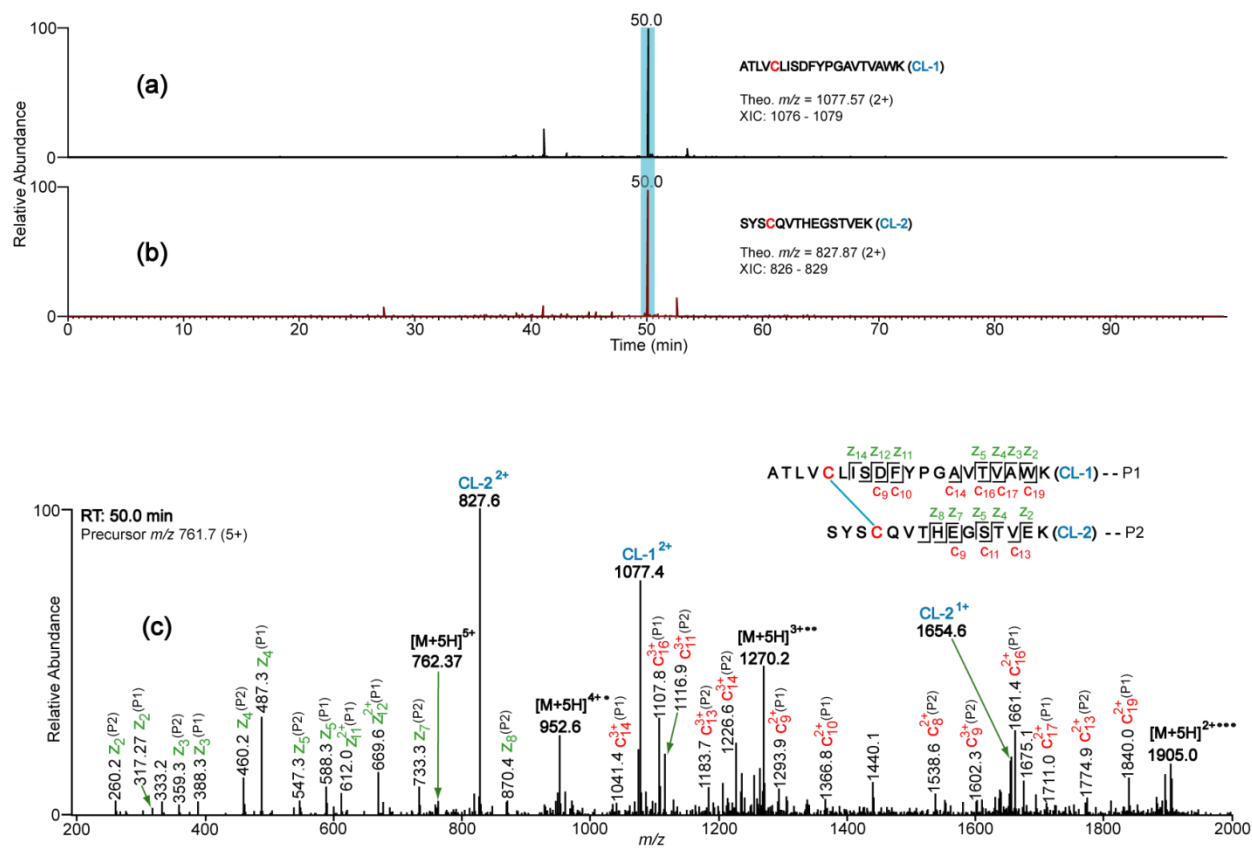[1]Department of Chemistry, University of Kansas, Lawrence, KS, 66047

[2]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, 66047
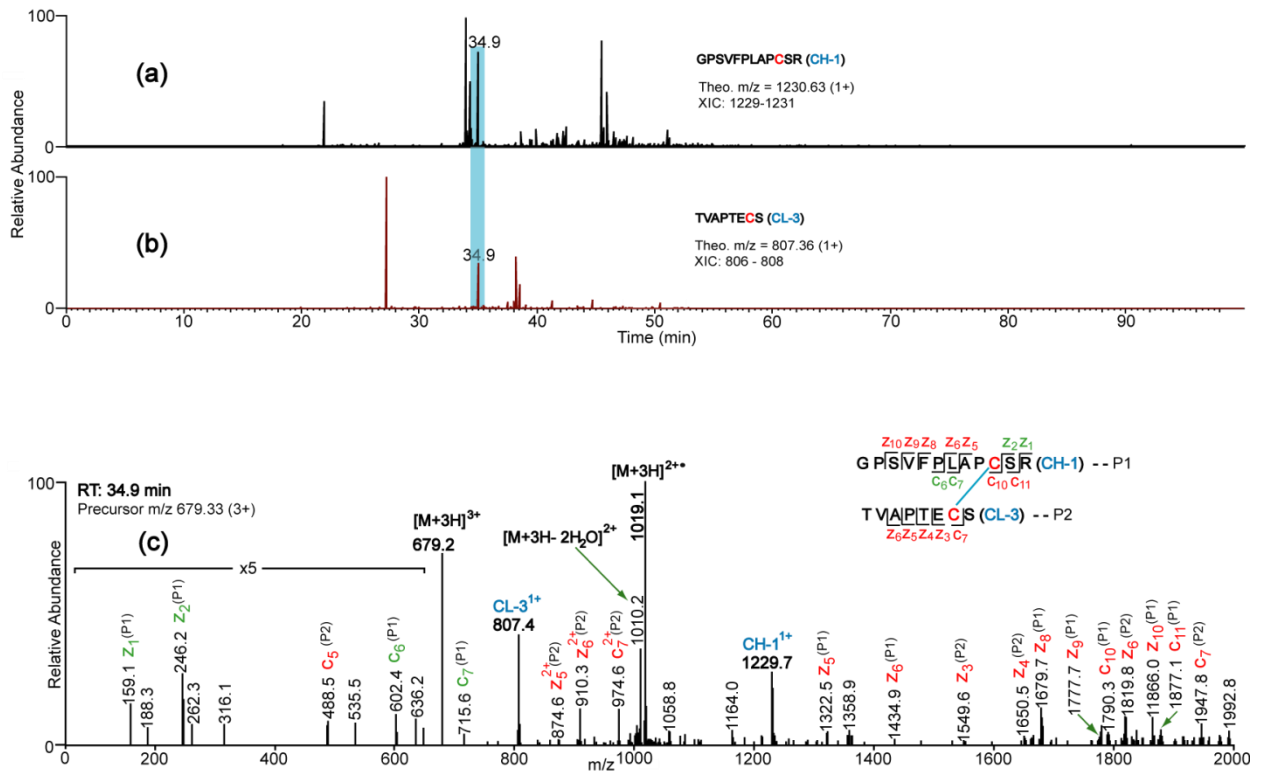
Supplementary Material

Supplementary Figures 1-6: Data supporting the assignment of disulfide bonds in the constant region of IgG3 monoclonal antibodies. Data from the Fitzgerald antibody is shown. The Sigma antibody produced the same results.
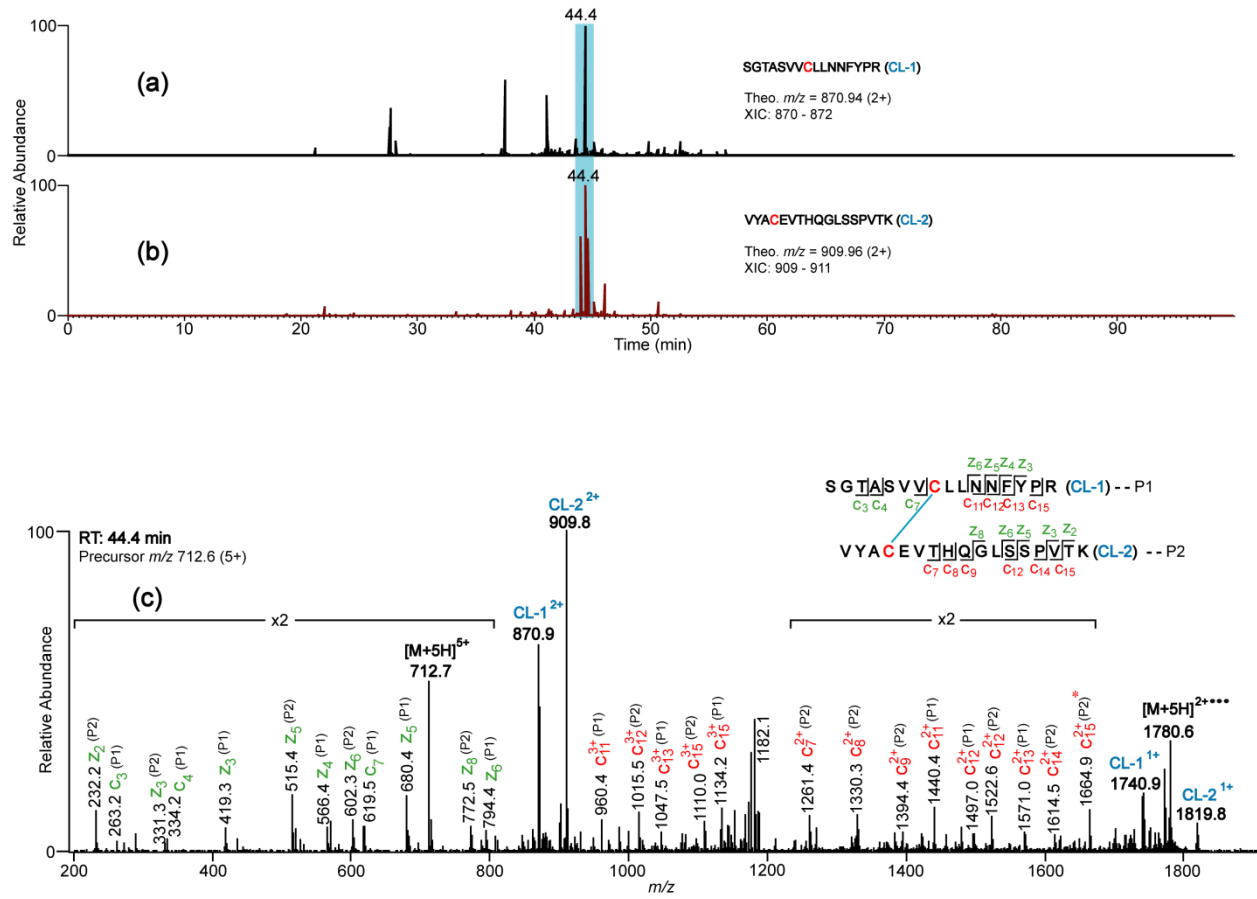
Supplementary Figure 1. XIC's (a and b) and ETD spectrum (c) supporting the assignment of the CH3 domain disulfide bond. Asterisks indicate z and c ±1 ions
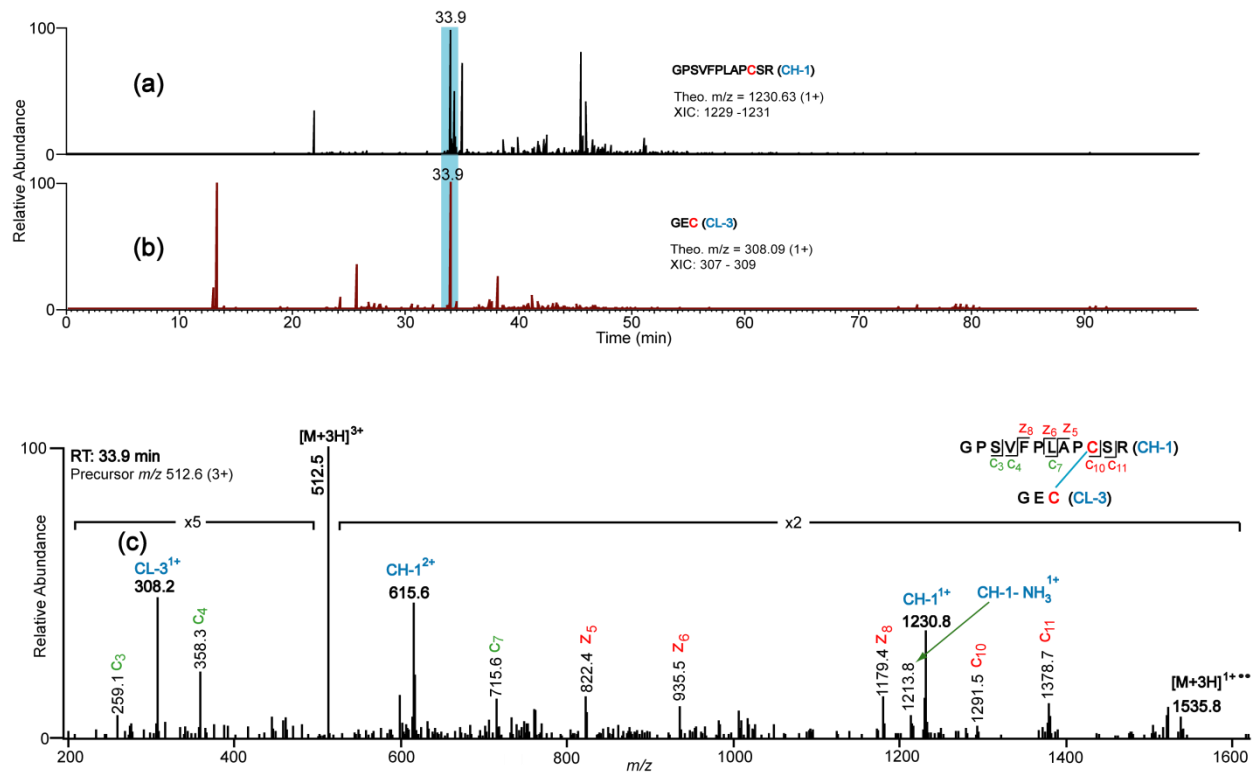
Supplementary Figure 2. XIC's (a and b) and ETD spectrum (c) supporting the assignment of the lambda constant light chain disulfide bond

Supplementary Figure 3. XIC's (a and b) and ETD spectrum (c) supporting the assignment of the disulfide bond between the heavy chain and the lambda light chain

Supplementary Figure 4. XIC's (a and b) and ETD spectrum (c) supporting the assignment of the kappa constant light chain disulfide bond
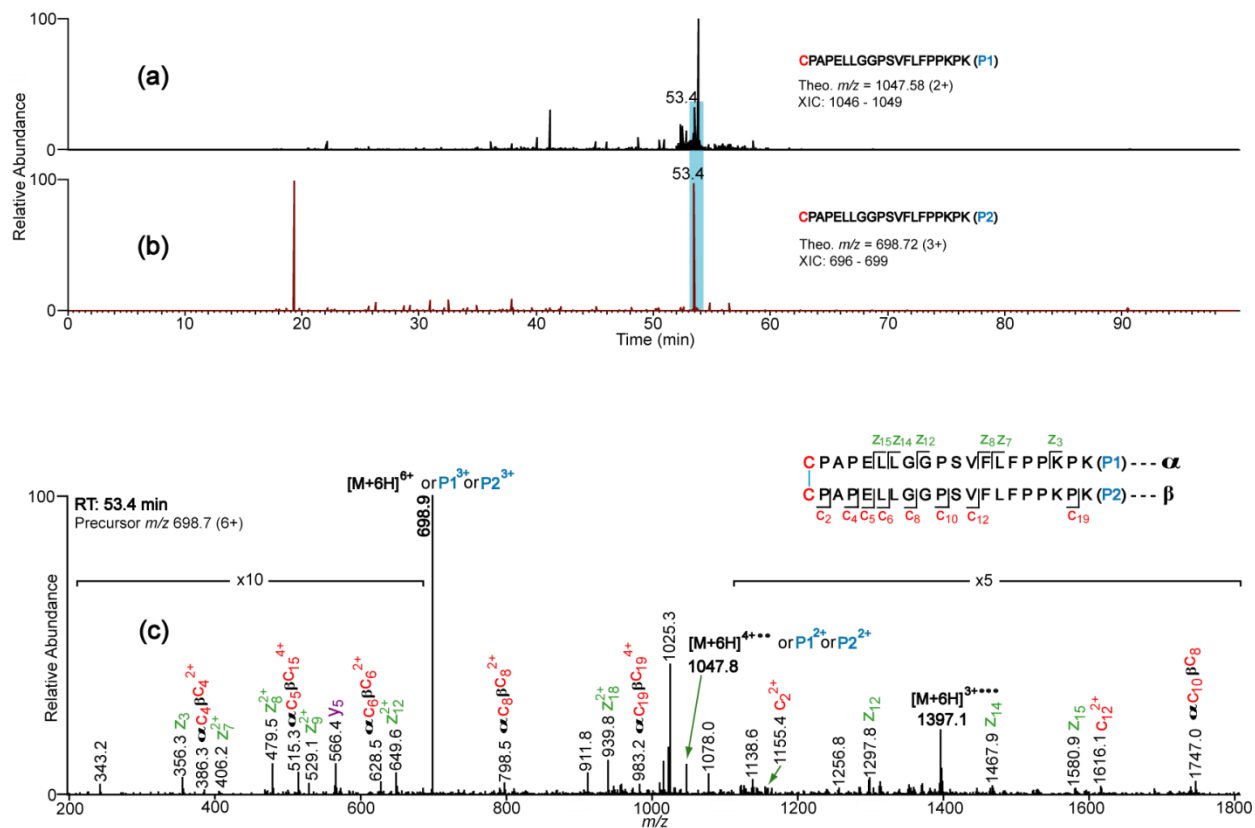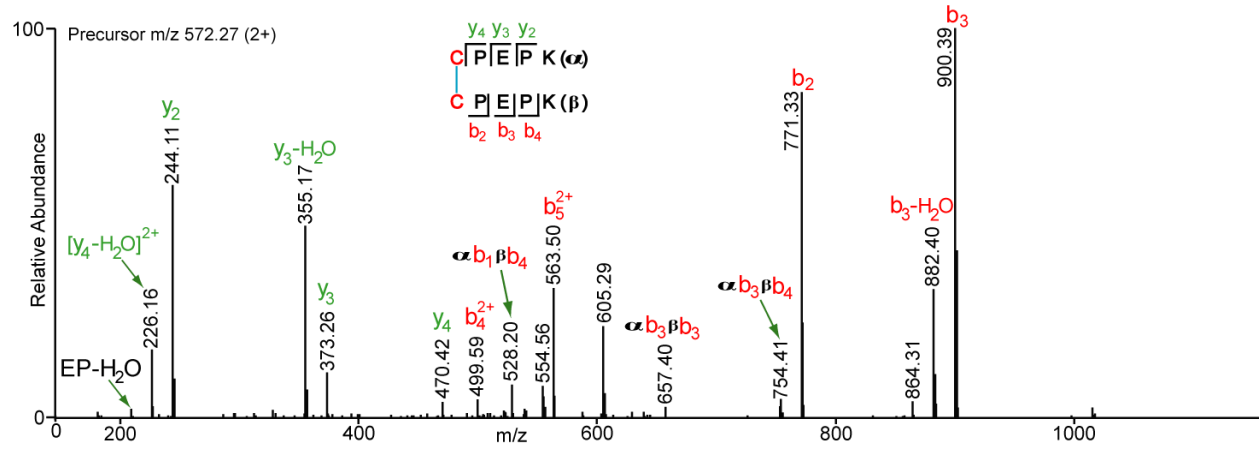
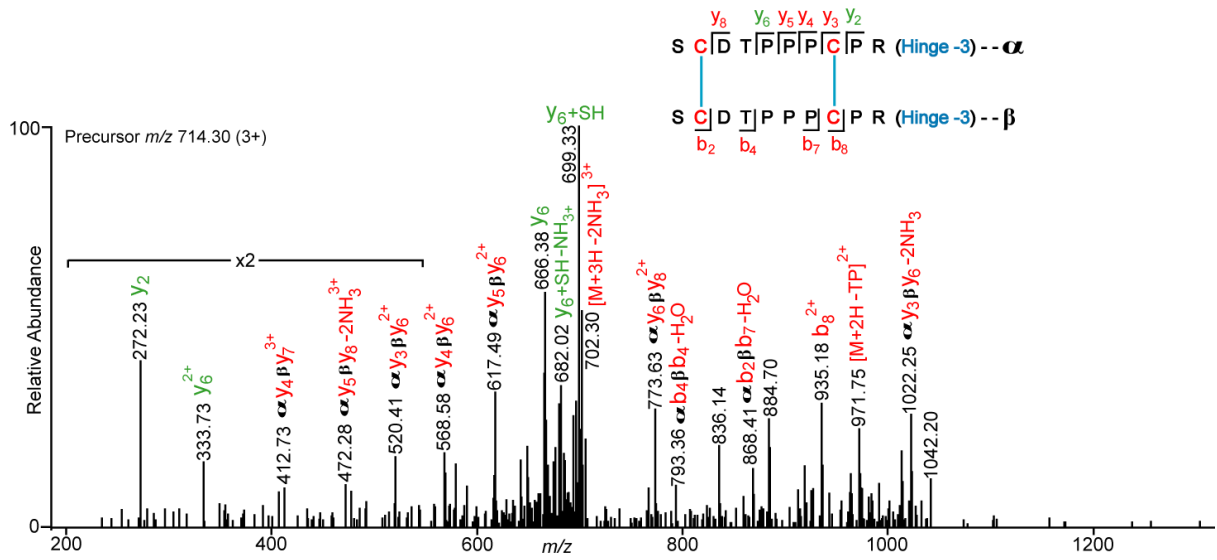**Supplementary Figure 5.** XIC's (a and b) and ETD spectrum (c) supporting the assignment of the disulfide bond between the heavy chain and the kappa light chain

Supplementary Figure 6. XIC's (a and b) and ETD spectrum (c) supporting the assignment of the Hinge-4 disulfide bond which links two identical tryptic peptides

Supplementary Figure 7. CID spectrum supporting the assignment of the Hinge-2 disulfide bond

Supplementary Figure 8. CID spectrum supporting the assignment of the Hinge-3 disulfide bond

Supplementary Table 1. Summary of Mass Assignments of Disulfide Bonds in Fitzgerald and Sigma IgG3 mAbs Using High Resolution MS Data.

Tryptic dipeptides	Position	Theor. m/z	CS	Fitzgerald IgG3		Sigma IgG3	
				Exptl. m/z	Mass Error (ppm)	Exptl. m/z	Mass Error (ppm)
DYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGT QTYTCNVNHKPSNTK **STSGGTAALGCLVK**	CH1	1581.9839	5+	1581.9953	5	1581.9974	9
		1318.4878	6+	1318.4954	6	1318.4954	5
		1130.2763	7+	1130.2854	8	1130.2886	11
		989.1177	8+	989.1251	7	989.1251	8
		879.3276	9+	879.3309	4	879.3309	8
CPEPK **CPEPK**	Hinge-2	572.2623	2+	572.2564	5	572.2602	4
SCDTPPPCPR **SCDTPPPCPR**	Hinge-3	1070.4394	2+	1070.4419	2	1070.4434	4
		713.9620	3+	713.9645	4	713.9641	< 1
		535.7234	4+	535.7264	6	535.7250	3