

Supporting Information

Integrate Intact mass analysis and Middle-down mass spectrometry approaches to effectively characterize Trastuzumab and Adalimumab structural heterogeneity

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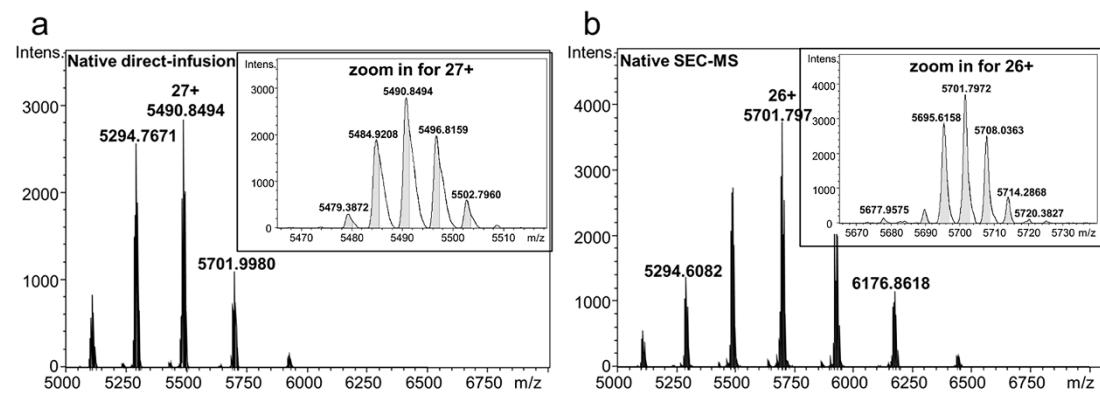


Figure S1. Averaged MS spectra acquired from intact Trastuzumab in native conditions using both (a) direct-infusion MS and (b) SEC-MS. Highest intensity species (charge state 27+ for direct-infusion MS and 26+ for SEC-MS) are highlighted as inset.

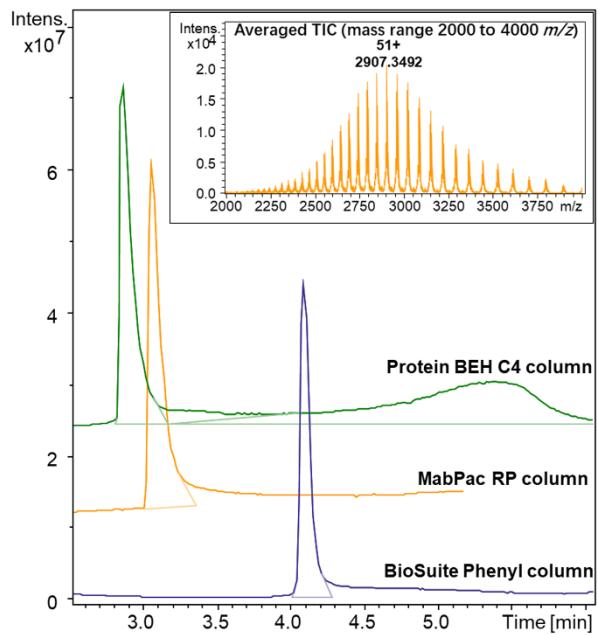


Figure S2. Denaturing RPLC-MS of Trastuzumab using the BEH C4, BioSuite Phenyl and MAbPAC RP column, respectively. Total ion chromatogram (TIC), mass spectrum acquired by averaging over the MAbPAC RP column eluted LC-MS peak is highlighted as inset.

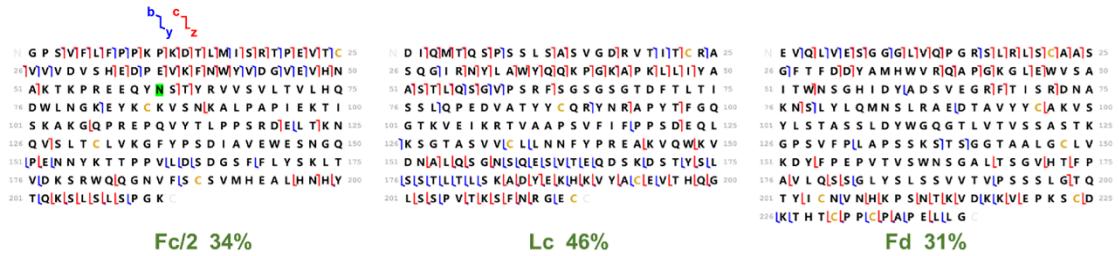


Figure S3. Fragmentation maps of Fc/2 (a), Lc (b) and Fd (c) subunits from Adalimumab. Via CID and ETD combined MS/MS, 34% sequence coverage is achieved for Fc/2, 46% sequence coverage is reached for Lc and 31% for Fd subunits. And the N-glycosylation site on the Fc/2 fragment is indicated in green.

Table S1. Relative intensity (%) heatmap of the detected glycoforms when Trastuzumab was eluted from the BEH C4, BioSuite Phenyl and MAbPac RP column separately.

Glycoform	Relative abundance (%)					
	BEH C4		BioSuite Phenyl		MabPac RP	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Man5/Man5	2	3	2	2	2	2
G0F-GN/G0F-GN	2	3	2	--	2	2
G0/G0	3	3	3	3	3	3
G0F-GN/G0F	5	6	4	--	5	5
G0/G0F	6	6	7	7	7	7
G0F/G0F	22	21	24	24	24	23
G0F/G1F	25	25	28	29	26	27
G1F/G1F	19	19	19	20	19	19
G1F/G2F	9	8	7	8	7	7
G1F/G1FS	--	--	--	2	2	2
G2F/G2F	3	3	2	3	2	2
G1F/G2FS	2	2	1	1	1	1
G2F/G2FS	1	1	1	1	--	--
G1F/G2FS2	1	--	--	--	--	--

Table S2. Mass error (ppm) of the detected glycoforms when trastuzumab was eluted from the BEH C4, BioSuite Phenyl and MAbPac RP column separately.

Glycoform	Theoretical vs Observed Mass (ppm)					
	BEH C4		BioSuite Phenyl		MabPac RP	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Man5/Man5	10.8	18.5	14.9	29.8	26.7	25.3
G0F-GN/G0F-GN	-15.2	1.6	-13.1	--	6.6	9.1
G0/G0	5.7	6.5	-10.3	-0.7	-17.0	-1.7
G0F-GN/G0F	32.1	11.7	31.5	--	27.4	28.3
G0/G0F	15.3	12.5	23.5	25.5	21.9	13.0
G0F/G0F	12.3	11.9	12.1	13.7	15.3	11.8
G0F/G1F	5.6	-0.5	0.1	5.7	7.7	19.5
G1F/G1F	7.0	3.6	9.4	3.2	3.5	3.0
G1F/G2F	-2.7	-7.3	-3.0	3.5	1.7	-0.7
G1F/G1FS	--	--	--	-9.3	13.5	-10.2
G2F/G2F	-3.8	-4.0	-13.7	12.6	-18.1	-32.2
G1F/G2FS	15.8	-11.3	-0.7	22.6	9.2	9.1
G2F/G2FS	-25.0	4.3	-29.4	-27.5	--	--
G1F/G2FS2	20.4	--	--	--	--	--

Table S3. Mass error (ppm) of the detected glycoforms from native and denatured Trastuzumab.

NO.	Glycoform	Expected Mass (Da)	Mass error (ppm)			
			Native		Denatured	
			Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	Man5/Man5	147600	26.9	10.1	26.7	25.3
2	G0F-GN/G0F-GN	147650	--	--	6.6	9.1
3	G0/G0	147764	-12.0	-10.0	-17.0	-1.7
4	G0F-GN/G0F	147853	35.9	37.1	27.4	28.3
5	G0/G0F	147910	16.9	8.3	21.9	13.0
6	G0F/G0F	148057	22.7	19.3	15.3	11.8
7	G0F/G1F	148219	17.6	3.2	7.7	19.5
8	G1F/G1F	148381	13.5	13.3	3.5	3.0
9	G1F/G2F	148543	17.3	11.9	1.7	-0.7
10	G1F/G1FS	148672	18.0	-15.2	13.5	-10.2
11	G2F/G2F	148705	-9.1	-27.2	-18.1	-32.2
12	G1F/G2FS	148834	-17.7	-14.3	9.2	9.1
13	G2F/G2FS	148997	-25.7	-17.0	--	--
14	G1F/G2FS2	149126	40.2	-13.3	--	--

Table S4. Relative abundance (%) heatmap of the detected glycoforms from native and denatured Adalimumab.

NO.	Glycoform	Expected Mass (Da)	Relative abundance (%)			
			Native		Denatured	
			Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	G0F-GN/G0F-GN	147674	--	--	2	2
2	G0/G0	147788	2	1	--	--
3	G0F-GN/G0F	147877	4	4	7	8
4	G0F/G0F	148081	41	40	37	37
5	G0F/G0F+Lys	148209	17	16	17	18
6	G0F/G1F	148243	23	22	22	23
7	G1F/G1F	148405	8	8	10	10
8	G0F/G1FS	148534	4	4	--	--
9	G1F/G2F	148567	--	3	4	--
10	G1F/G2FS	148858	1	1	1	2

Table S5. Mass error (ppm) of the detected glycoforms from native and denatured Adalimumab.

NO.	Glycoform	Expected Mass (Da)	Mass error (ppm)			
			Native		Denatured	
			Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	G0F-GN/G0F-GN	147674	--	--	-8.6	16.0
2	G0/G0	147788	9.8	10.5	--	--
3	G0F-GN/G0F	147877	-33.2	-40.3	-18.6	-4.1
4	G0F/G0F	148081	-1.0	-1.2	-0.8	9.2
5	G0F/G0F+Lys	148209	4.6	-2.4	28.7	-28.4
6	G0F/G1F	148243	-11.1	-11.2	5.8	2.6
7	G1F/G1F	148405	-17.0	-1.6	-0.3	-0.3
8	G0F/G1FS	148534	-39.1	-19.2	--	--
9	G1F/G2F	148567	--	-26.6	4.8	--
10	G1F/G2FS	148858	29.4	-36.6	-28.0	20.0