

Supplemental Material

Table S1: Peptide Mapping Sequence List

Figure S1: ADC Purification with Protein A Magnetic Beads

Table S2: %WSD Data Table

Table S1: LC/MS analysis of trypsin digested ADCs revealed 44 conjugated lysines. The sequence corresponding to each conjugated lysine as well as the respective population percentage (%) and standard deviation for each site are shown below. Peptide mapping samples were digested and analyzed in triplicate.

Conjugated Lysine		Sequence	Population Percentage \pm SD (%)	
			Off-Bead	On-Bead
Light Chain	L39+42	VTITCRASQDVNTAVAWYQQKPGK	0.6 \pm 0.4	0.2 \pm 0.2
	L42	ASQDVNTAVAWYQQKPGKAPK	2.1 \pm 0.5	1.6 \pm 0.2
	L107	VEIKR	11.6 \pm 0.5	10.2 \pm 2.0
	L126	TVAAPSVFIFPPSDEQLK	0.2 \pm 0.2	0.3 \pm 0.2
	L145	EAKVQWK	2.9 \pm 0.4	2.1 \pm 0.1
	L188	ADYEKHK	9.3 \pm 0.4	8.6 \pm 0.8
Heavy Chain	H43	QAPGKGLEWVAR	3.2 \pm 0.0	3.3 \pm 0.8
	H65	YADSVKGR	12.6 \pm 1.2	7.7 \pm 0.1
	H76	FTISADTSKNTAYLQMNSLR	0.0 \pm 0.0	0.2 \pm 0.3
	H136	GPSVFPLAPSSKST	0.0 \pm 0.0	1.3 \pm 1.8
	H136+150	GPSVFPLAPSSKSTSGGTAALGCLVK	1.3 \pm 1.0	1.6 \pm 0.3
	H216	VDKK	2.3 \pm 0.1	2.2 \pm 0.1
	H252	THTCPPCPAPELLGGPSVFLFPPKPKDTLMISR	0.1 \pm 0.1	0.2 \pm 0.1
	H278	DTLMISRTPEVTCVVVDVSHEDPEVK	0.3 \pm 0.4	0.3 \pm 0.4
	H292	FNWYVDGVEVHNAKTKPR	4.9 \pm 2.9	2.8 \pm 2.9
	H294	TKPR	10.6 \pm 7.6	19.3 \pm 1.3
	H330	VSNKALPAPIEK	3.5 \pm 0.3	2.5 \pm 0.1
	H338	ALPAPIEKTISK	19.6 \pm 2.1	22.6 \pm 1.6
	H342	TISK	0.0 \pm 0.0	0.1 \pm 0.1
	H344	AKGQPR	4.4 \pm 0.3	3.0 \pm 0.8
	H396	GFYPSDIAVEWESNGQPENNYKTPPV	0.2 \pm 0.2	0.0 \pm 0.0
		LDSDGSFFLYSK		
	H418	LTVDKSR	10.4 \pm 1.2	10.0 \pm 1.0

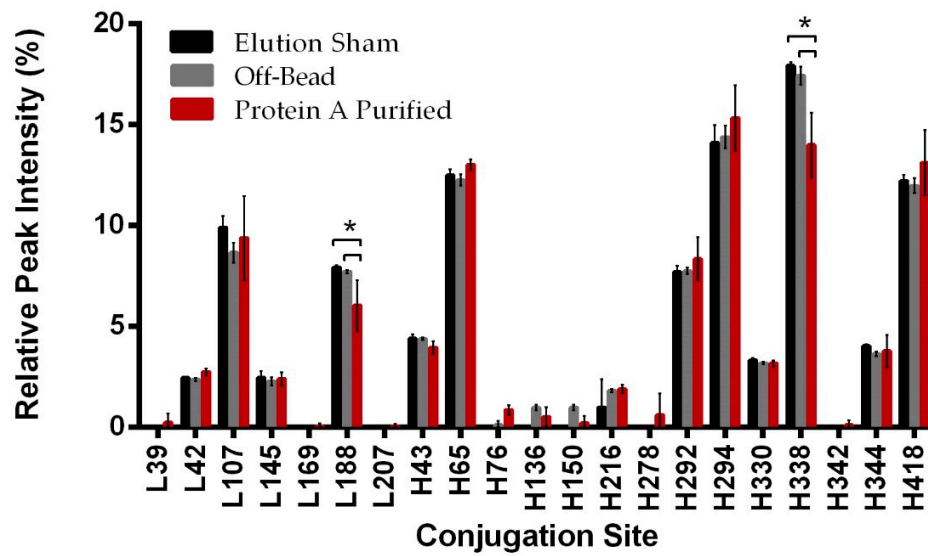


Figure S1: Relative peak intensity of conjugation sites as analyzed by LC/MS following tryptic digestion. All samples are T-DM1 Off-Bead ADCs from the same batch split into 3 groups. Off-Bead: No additional handling; Elution Sham: T-DM1 was exposed to elution buffer (50 mM glycine-HCl) for 5 minutes and then neutralized; Protein A Purified: T-DM1 ADCs were bound to Protein A magnetic beads to simulate a purification protocol then eluted and neutralized. Following treatment, all 3 groups underwent the peptide mapping digestion as described previously. LC/MS data of the digested peptides indicates statistically significant shifts in relative population between Protein A Purified and the other samples at 2 lysine conjugation sites as indicated by the *.

Table S2: Percent weighted spectral difference (%WSD) of Off-Bead and On-Bead relative to an unmodified trastuzumab antibody. Values are reported as average percent \pm SD of triplicates.

Wavenumber Range (cm ⁻¹)	Off-Bead (%WSD)	On-Bead (%WSD)
823 – 836.5	2.75 \pm 0.41	2.49 \pm 0.57
849 – 816.5	0.49 \pm 0.20	0.35 \pm 0.11
1547.5 – 1561.5	1.14 \pm 0.37	0.97 \pm 0.07
1642.5 – 1670.5	9.74 \pm 0.32	11.78 \pm 0.71
1671 – 1697	3.89 \pm 0.83	4.11 \pm 0.89