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SUPPLEMENTARY INFORMATION

State of the Art Native Mass Spectrometry and Ion Mobility Methods to Monitor Homogeneous Site-Specific Antibody-Drug Conjugates Synthesis.

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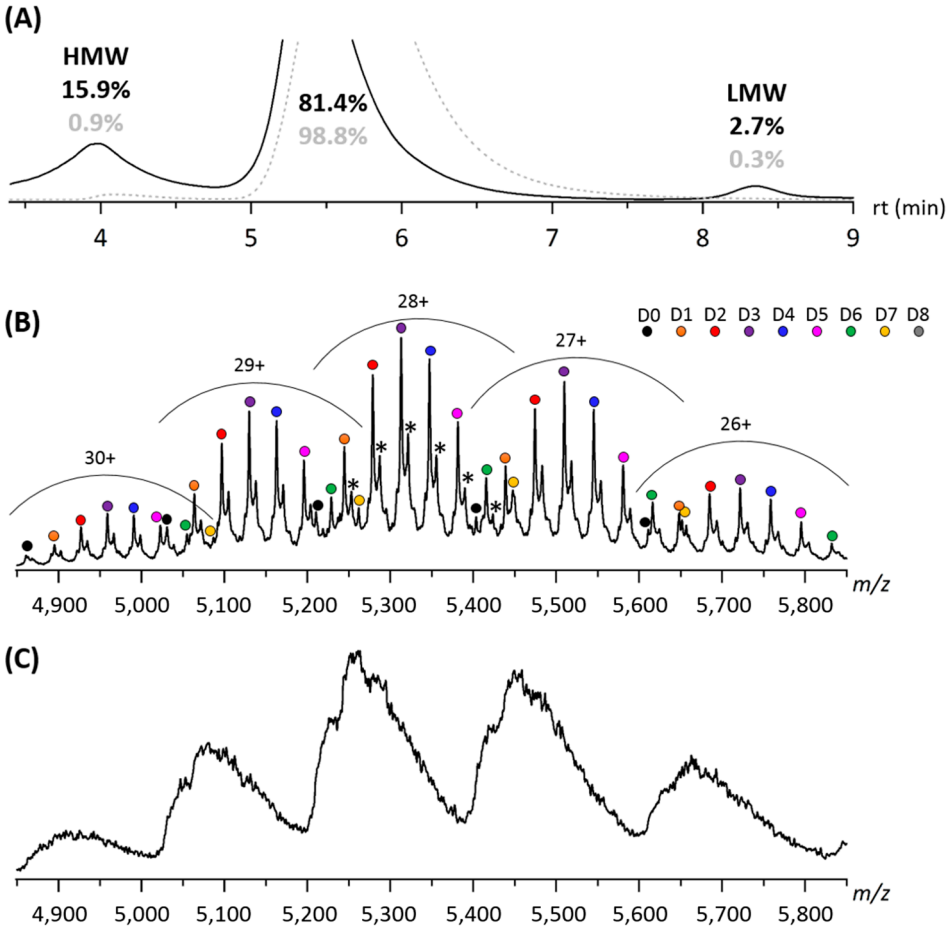

Figure S1. Online SEC-nMS analysis of T-DM1. (A) Overlaid SEC chromatograms of stressed (solid line) and non-stressed (dotted line, grey) samples. (B) SEC-nMS spectrum of intact non-stressed T-DM1 with corresponding UV chromatogram (280 nm) depicted in inset; ✱ = linker adducts (+220 Da). (C) SEC-nMS spectrum of thermally-stressed T-DM1.

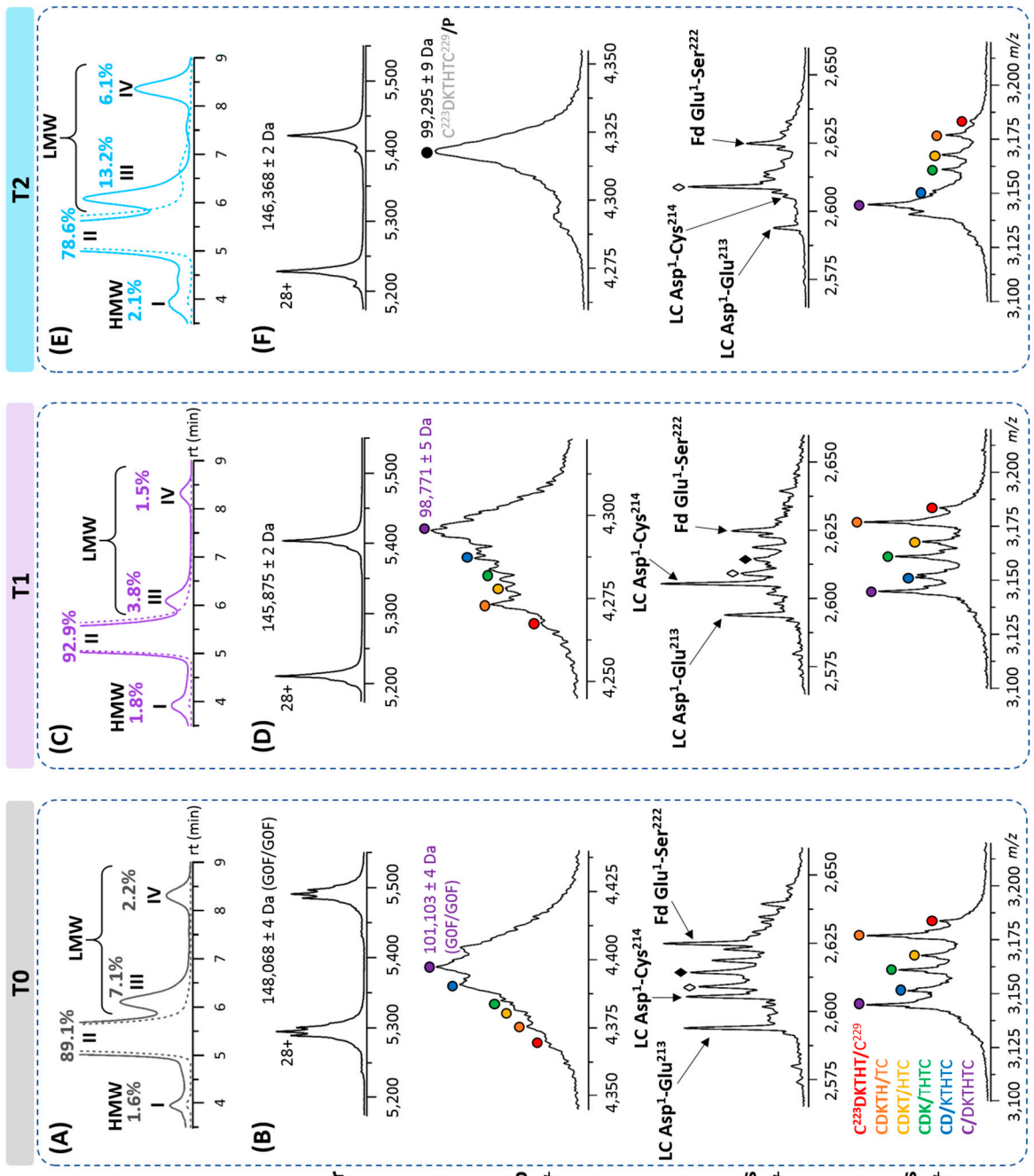

Figure S2. Online SEC-nMS analysis of T0, T1 and T2 after thermal stress. (A, C, E) Overlaid SEC chromatograms of stressed (solid line) and non-stressed (dotted line) samples. Relative amounts of HMWS and LMWS are given for the stressed sample; I = HMW dimers, II = main product, III = Fc-Fab fragments and IV = LC, Fd and Fab fragments. (B, D, F) SEC-nMS spectra of monomers and hinge-related fragments generated upon thermal stress. ◇ = sulfurized Cys^{214} (+32 Da compared to LC Asp^{1}-Cys^{214}). ◆ = +SO_xH on Cys^{214} (+80 Da compared to LC Asp^{1}-Cys^{214}).

Table S1. $^{TW}CCS_{N2}$ measurements of intact and IdeS-digested reaction products. ¶ Mass-based estimation of CCS, CCS = 2.435 × $MW^{2/3}$ according to Ruotolo *et al.* (*Nat Protoc* **2008**, 3(7), 1139-1152).

		$^{TW}CCS_{N2}$ (nm²)			
		T0	**T1**	**T2**	**T-GlyCLICK-DM1**
Intact	**Predicted¶**	68.1	67.5	67.6	68.4
	23+	73.8 ± 0.2	73.4 ± 0.2	73.6 ± 0.2	74.3 ± 0.2
	24+	75.3 ± 0.2	74.9 ± 0.1	75.1 ± 0.1	75.9 ± 0.1
Fc fragment	**Predicted¶**	33.3	32.3	32.5	33.6
	12+	34.1 ± 0.1	33.2 ± 0.1	33.4 ± 0.1	34.6 ± 0.2
	13+	35.2 ± 0.1	34.4 ± 0.1	34.7 ± 0.1	35.9 ± 0.1
F(ab')$_2$ fragment	**Predicted¶**	51.6	51.6	51.6	51.6
	20+	56.8 ± 0.1	56.8 ± 0.1	56.6 ± 0.2	57.0 ± 0.1
	21+	58.2 ± 0.1	58.2 ± 0.1	58.0 ± 0.2	58.2 ± 0.1

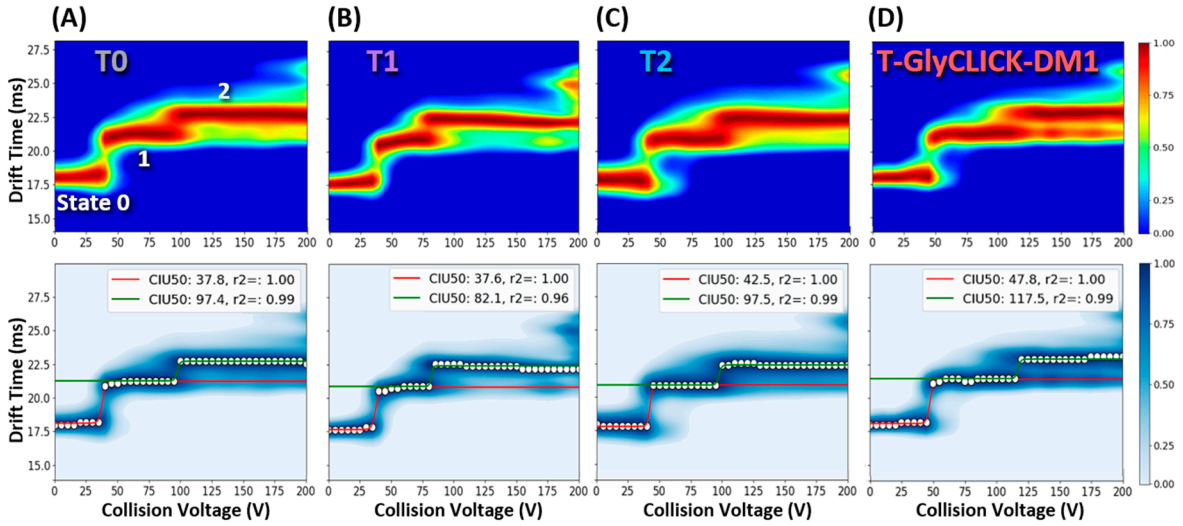

Figure S3. CIU experiments at the intact level for the 23+ charge state. CIU fingerprints (upper panel) and CIU50 analysis (lower panel) were acquired to compare the resistance to gas-phase unfolding of the reaction compounds (A) T0, (B) T1, (C) T2 and (D) T-GlyCLICK-DM1.

Table S2. RMSDs between technical triplicates for CIU fingerprints at the intact level for 23+ and 24+ charge states.

	RMSD between technical replicates (n = 3)			
	T0	**T1**	**T2**	**T-GlyCLICK-DM1**
23+	13.3	7.3	11.2	8.4
24+	9.7	5.1	11.2	7.9