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REPORT

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Identification of a CE-SDS shoulder peak as disulfide-linked fragments from common C_H2 cleavages in IgGs and IgG-like bispecific antibodies

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Introduction

- Therapeutic protein fragmentation is a critical quality attribute
- SEC is traditionally used to monitor aggregates, while CE-SDS is used to monitor fragments
- IgG1 HC CDR fragments typically appear as shoulder peaks in CE-SDS
- RPLC, intact mass, and top-down MS2 provide fundamental intrachain cysteine bonds in antibody folding domains, including the C_H2 domain and possible intrachain disulfide bond clippings
- In our study, we identify a non-reduced CE-SDS shoulder peak appearing in a bispecific antibody (bsAb-A) after heat stress
- Our study suggests that host cell proteases are the cause of C_H2 clipping in both mAb and bsAbs

Figure 1. Comparison of non-reduced and reduced CE-SDS profiles and degradation rates of three bsAb-A drug substance lots under accelerated stress conditions at 40°C up to three months. (A) nrCE-SDS overlay of heat-stressed Lot A, Lot B and Lot C; (B) rCE-SDS overlay of heat-stressed Lot A, Lot B and Lot C; (C) % purity of Lot A, Lot B and Lot C by nrCE-SDS; (D) % purity of Lot A, Lot B and Lot C by rCE-SDS

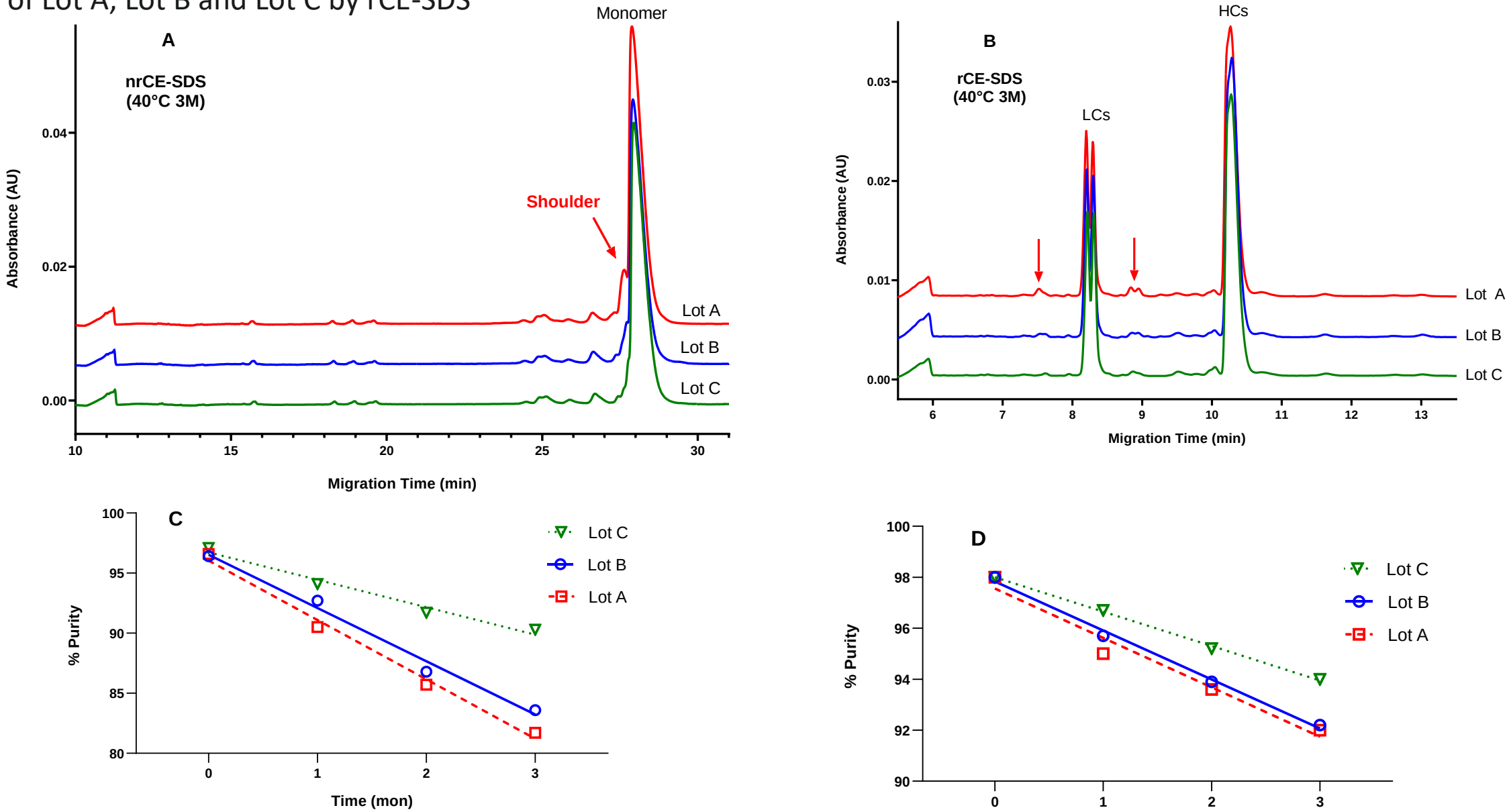


Figure 2 nrCE-SDS profiles of HIC fractions from 40°C 1 mon heat-stressed bsAb-A, in which size variant peaks are labeled from 1 to 11 (A) nrCE-SDS profiles of HIC prepeaks and main peak; (B) nrCE-SDS profiles of HIC post peaks.

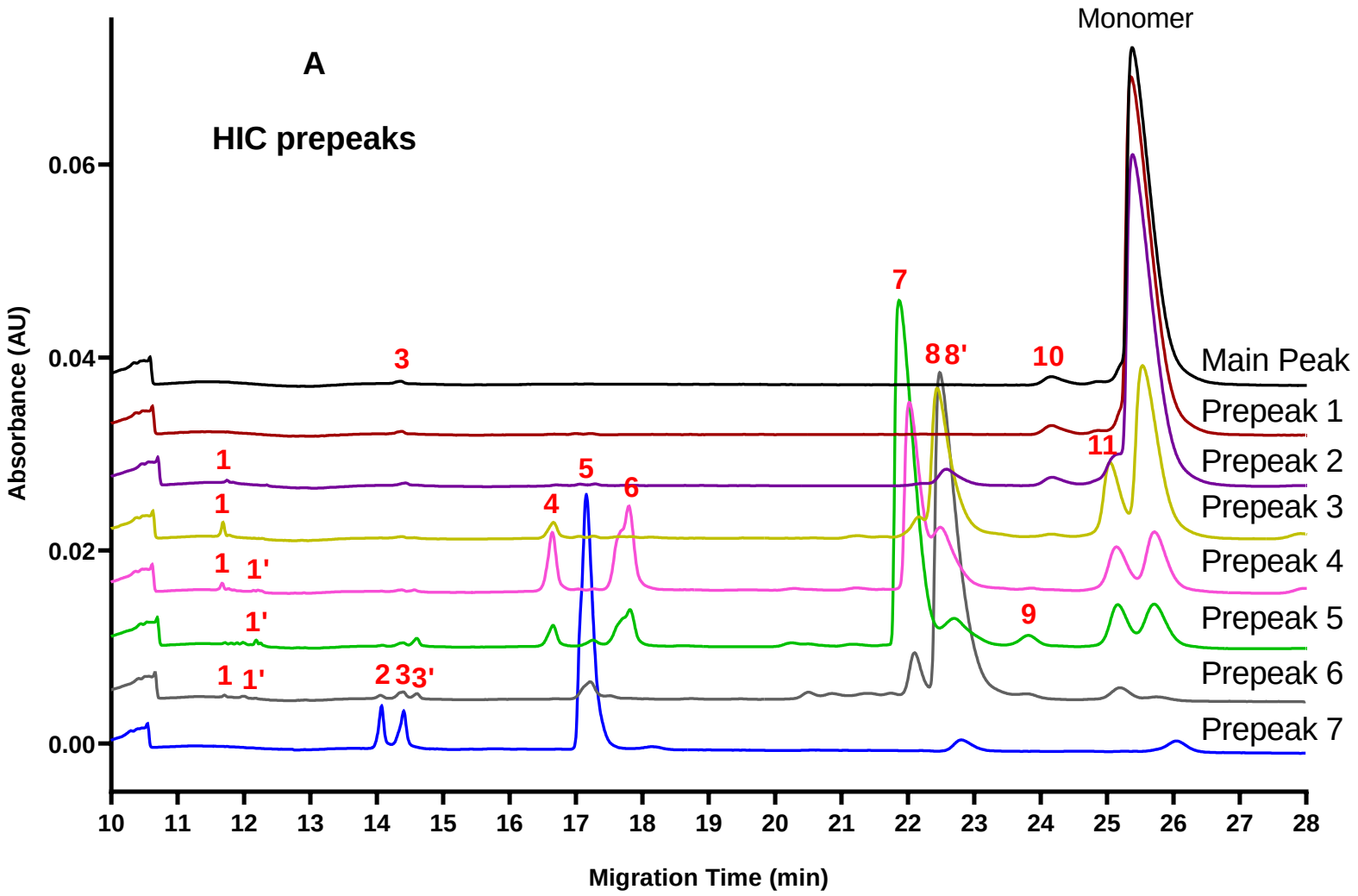


Figure 2 nrCE-SDS profiles of HIC fractions from 40°C 1 mon heat-stressed bsAb-A, in which size variant peaks are labeled from 1 to 11 (A) nrCE-SDS profiles of HIC prepeaks and main peak; (B) nrCE-SDS profiles of HIC post peaks.

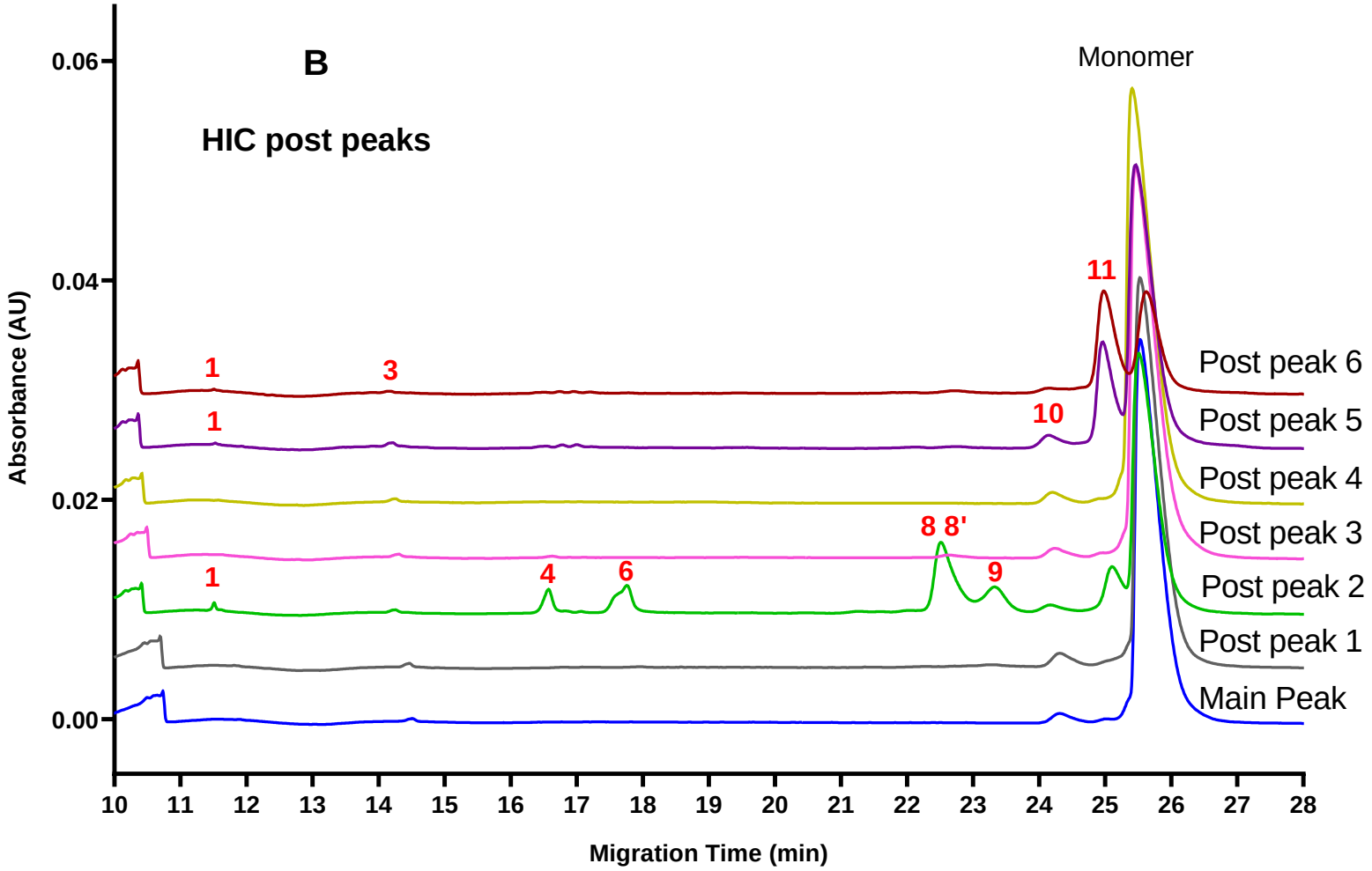


Table 1. nrCE-SDS peak assignment based on HIC fractionation study and partial reduction study (for peak 10 assignment)

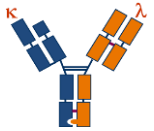
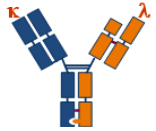
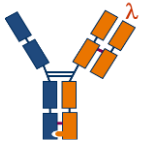
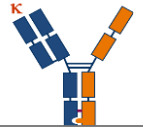
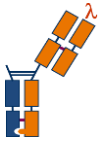
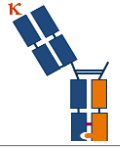
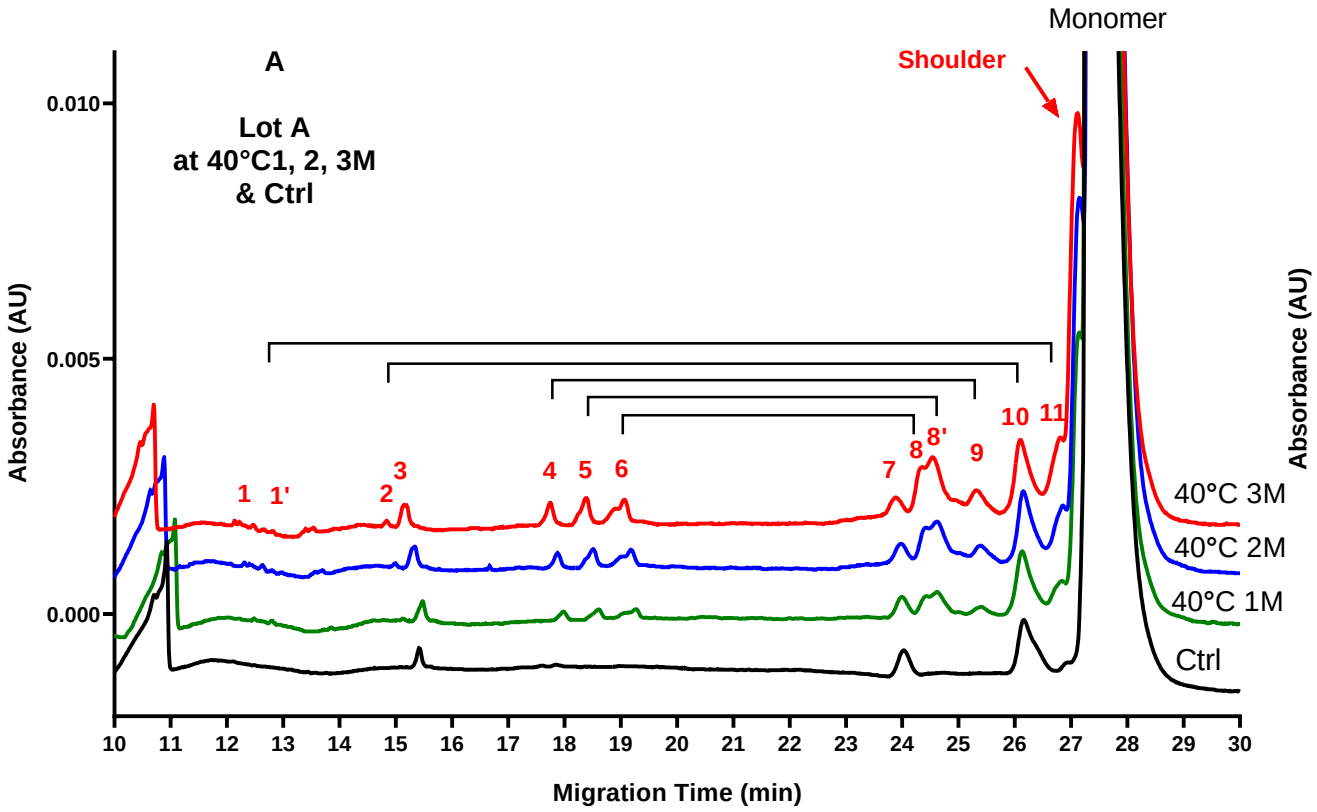
nrCE-SDS pk #	Fragment	Mass (Da) Theo	Mass (Da) Detect	HIC enrichment	Complimentary fragment	CE-SDS pk # for complimentary Frag.
1	*Hole HC 1-102* *Hole HC 1-101* *Hole HC1-109*	11,109 10,992 12,106	11,108 10,992 12,105	HIC pre-pk 2, 3, 4, 5, 6 & post-pk 2 Post-pk 5, 6 Pre-pk 4		11
1'	*Knob HC1-99* *Knob HC1-103* *Knob HC1-106* *Knob HC1-107*	11,019 11,403 11,778 11,943	11,019 11,402 11,778 11,942	All HIC fractions HIC pre-pk 4, 5, 6 HIC pre-pk 4, 5 HIC pre-pk 4, 5		
2	*κ LC2-218*	23,971	23,969	HIC pre-pk 7, 6		10
3	*κ LC + Cys*	24,304	24,304	All HIC fractions		
3'	*λ LC + Cys*	23,041	23041	HIC pre-pk 5, 6		
4	*Fab 2 fragment (λ LC-knob HC1-141, 143, 145)*	38,370 38,558 38,702	38,370 38,558 38,701	HIC pre-pk 4, 5, 3 & post-pk 2		9
5	*Fab 1 (hole ½Ab)*	48,738 48,499 48,256 48,371 48,353	48,737 48,499 48,256 48,371 48,352	HIC pre-pk 7, 6		8'
6	*Fab 2 (knob ½Ab)*	47,875 47,208 47,637	47,873 47,206 47,639	HIC pre-pk 4, 5 & Post-pk 2		8
7	*LHL mispair*	97,908 97,891(Su)	97,906 97,890	HIC pre-peak 5 HIC pre-peak 4	NA (only impacted by cell culture condition; no complimentary)	NA
8	*missing Fab 2 (LHF)*	101,903 102,207 101,537	101,905 102,208 101,537	HIC pre-pk 6, 3 & post-pk 2		6
8'	*IgG missing Fab 1 (LHF)*	101,042 100,675	101,040 100,673	HIC pre-pk 2, 3, 4 & post-pk 2		5
9	*Missing λ LC-knob HC1-141, 143, 145*	111,041 110,853 110,709	ND (by LCMS)	HIC pre-pk 5 & post-pk 2		4
10	*HHL (missing κ LC)*	125,209	125212	Enriched by partial reduction		3
		125,209	ND	ND	 *κ LC2-218*	2
	HHL (missing λ LC)	126,472	126477	Enriched by partial reduction		3'
11	*Missing hole HC 1-102* *Missing hole HC 1-101* *Missing hole HC 1-109*	138,302 138,419 137,305	ND (by LCMS)	HIC pre-pk 2, 3, 4, 5, 6 & post-pk 2 post-pk 5, 6 Pre-pk 4	 *Hole HC 1-102* *Hole HC 1-101* *Hole HC 1-109*	1

Figure 3. CE-SDS profiles of bsAb-A non-stressed control and 40°C stressed Lot A at 1, 2 and 3 mon.



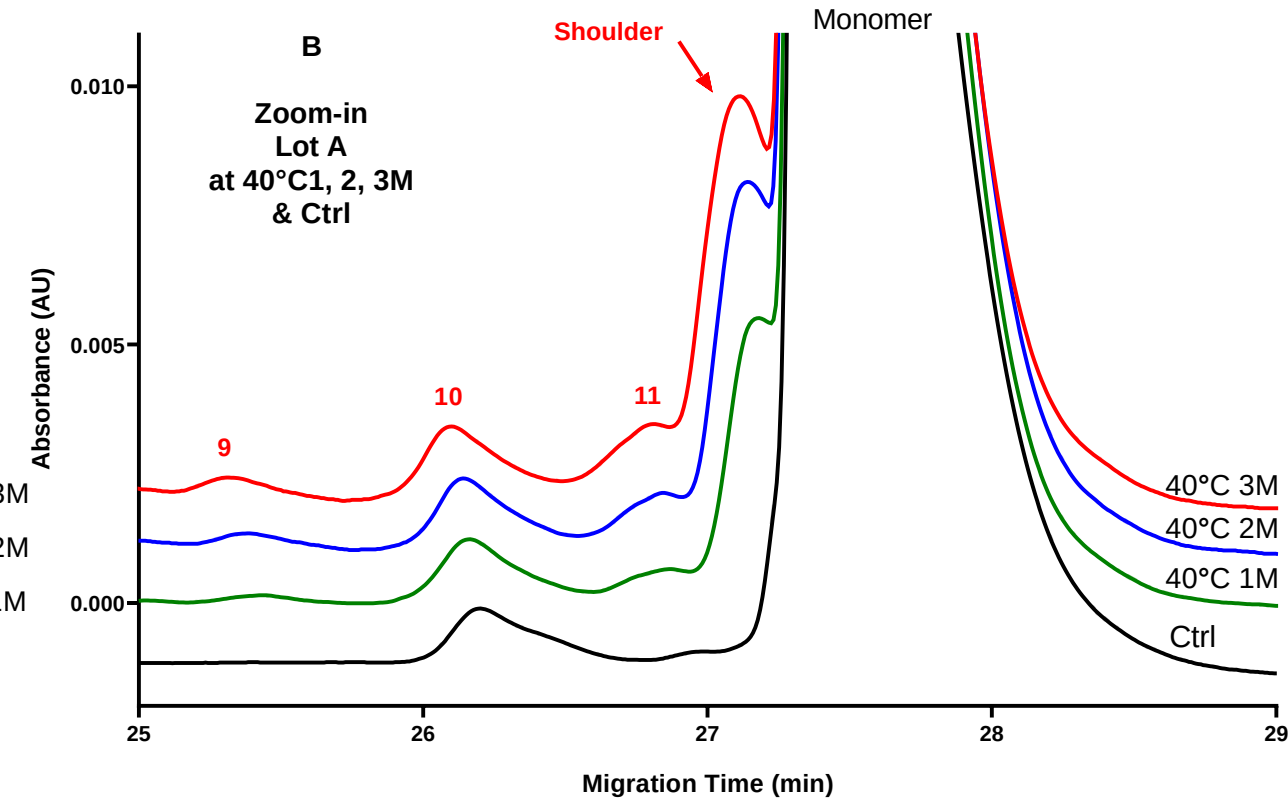
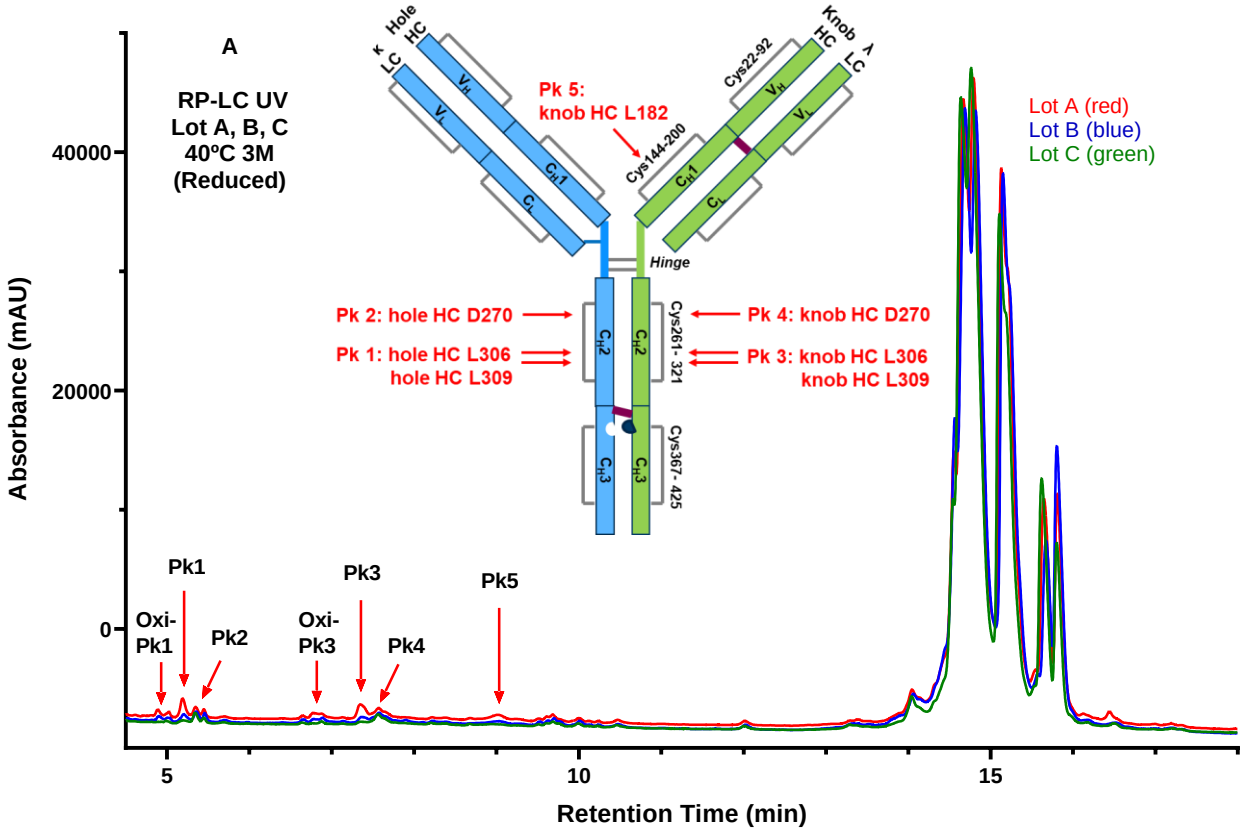
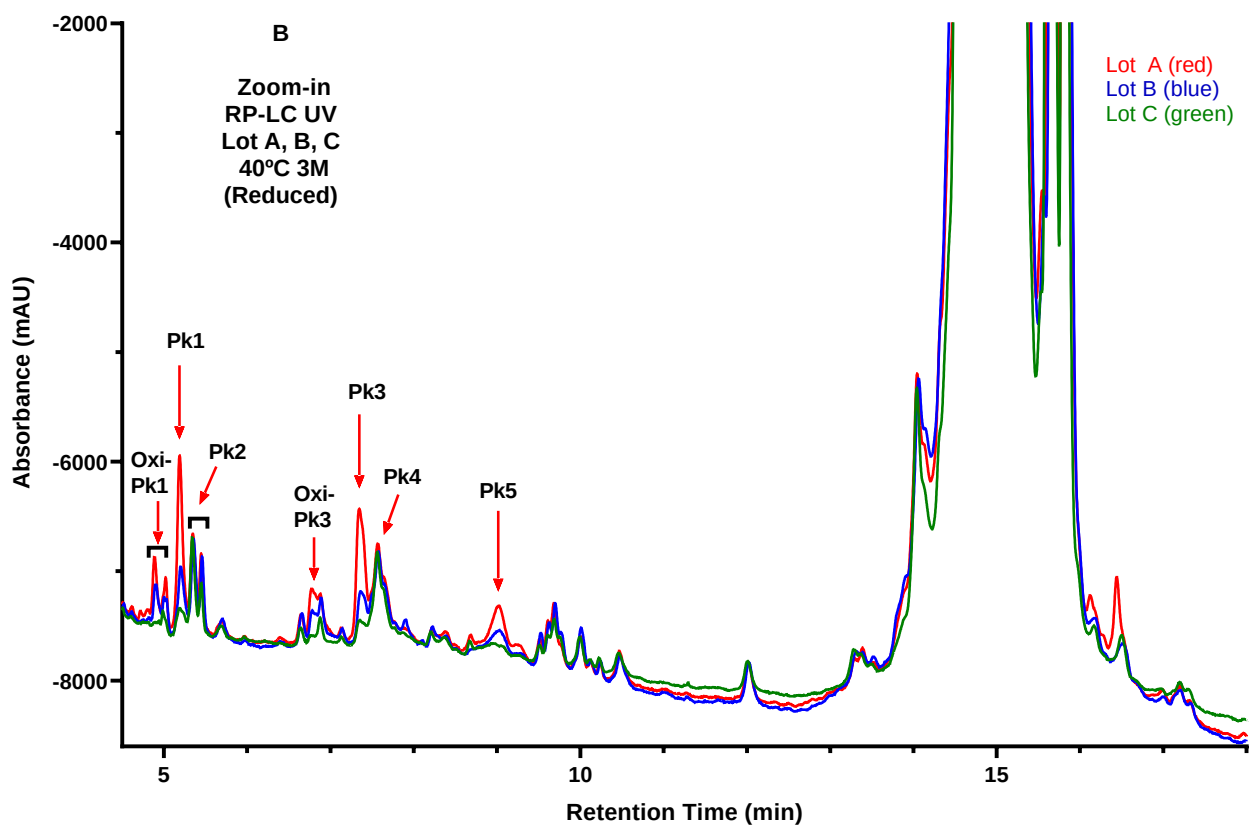
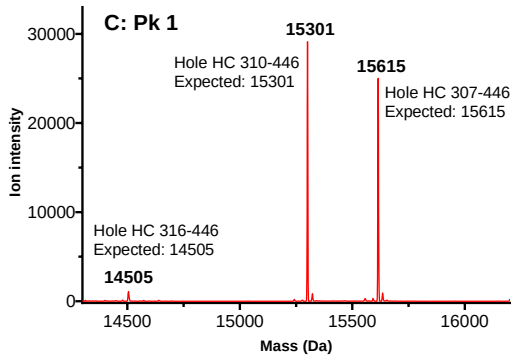
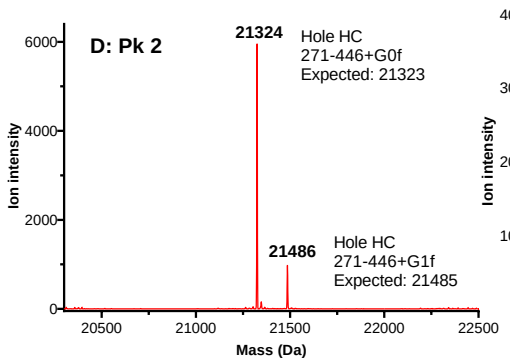


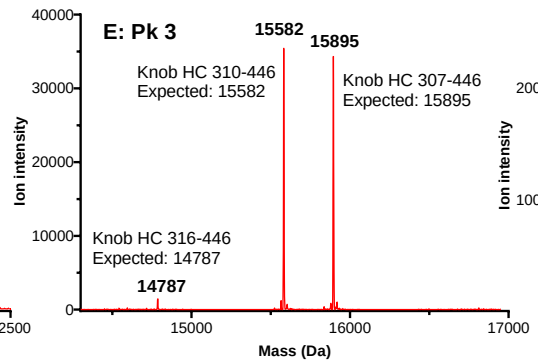
Figure 4. RP-LC UV profiles of denatured and reduced bsAb-A at 40C 3 month (A), zoom-in of RP-LC UV profiles of denatured and reduced bsAb-A at 40C 3 mon. (B); and deconvoluted spectra of CH2 and CH1 clipped reduced fragments (C to G). Red: Lot A; Blue: Lot B; Green: Lot C.

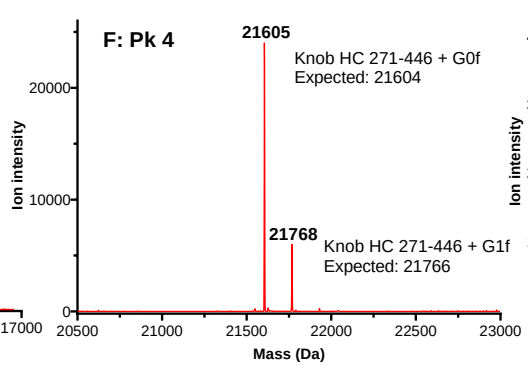












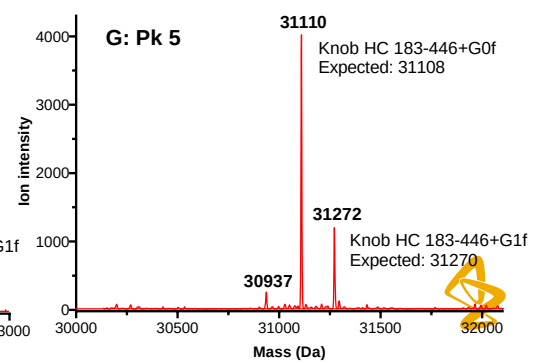


Figure 5. Tandem mass (MS/MS) spectra of peptides (L)TVLHQDWLNGK resulting from CH2 clipping at L306 (top) and (L)HQDWLNGK resulting from CH2 clipping at L309.

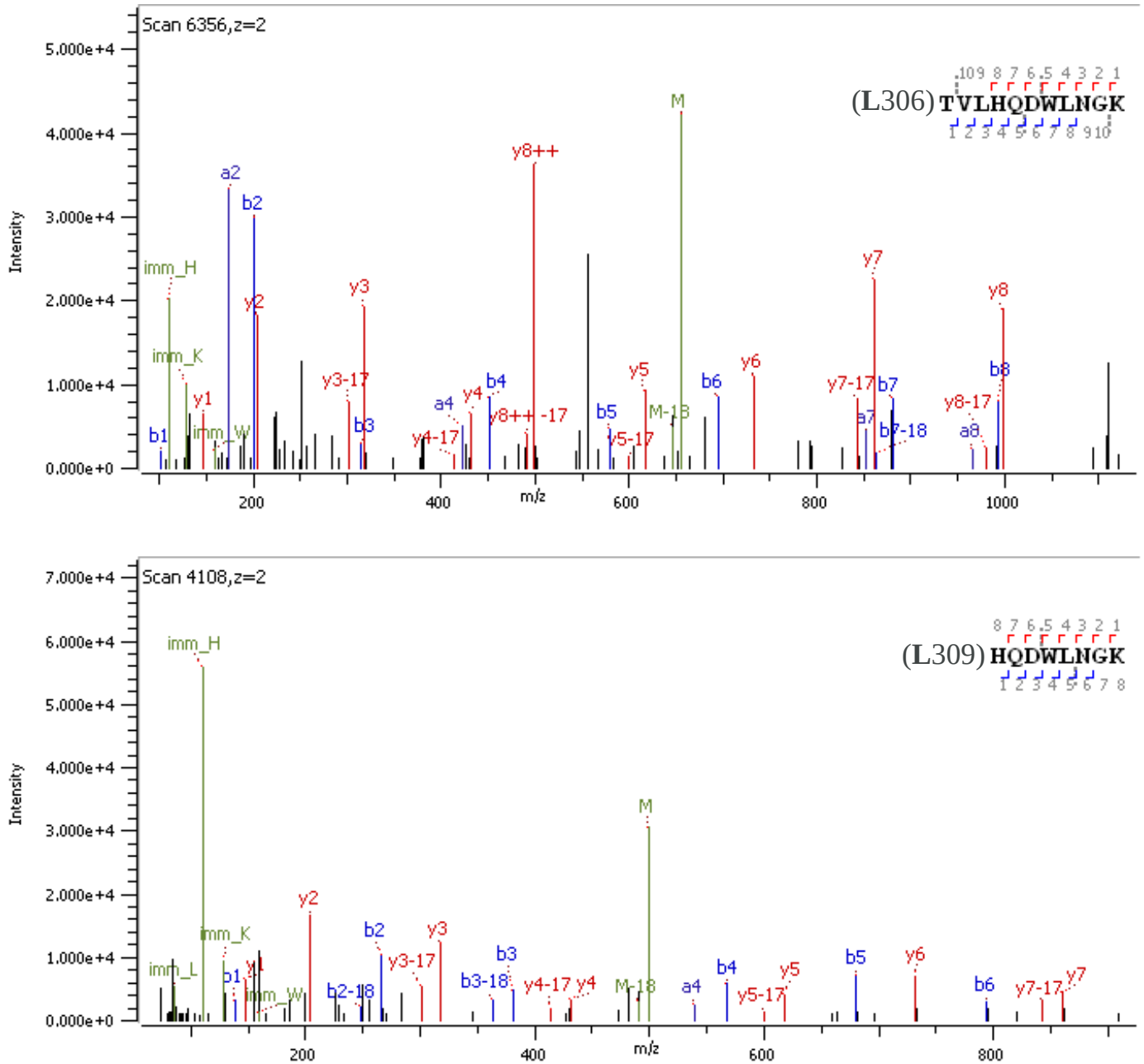


Figure 6. Deconvoluted mass spectra of Fc in bsAb-A 40°C 3 mon heat-stressed Lot A, Lot B and Lot C and unstressed control (top), and deconvoluted mass spectra of deglycosylated Fc in bsAb-A 40°C 3 mon heat-stressed Lot A, Lot B and Lot C and unstressed Control (bottom).

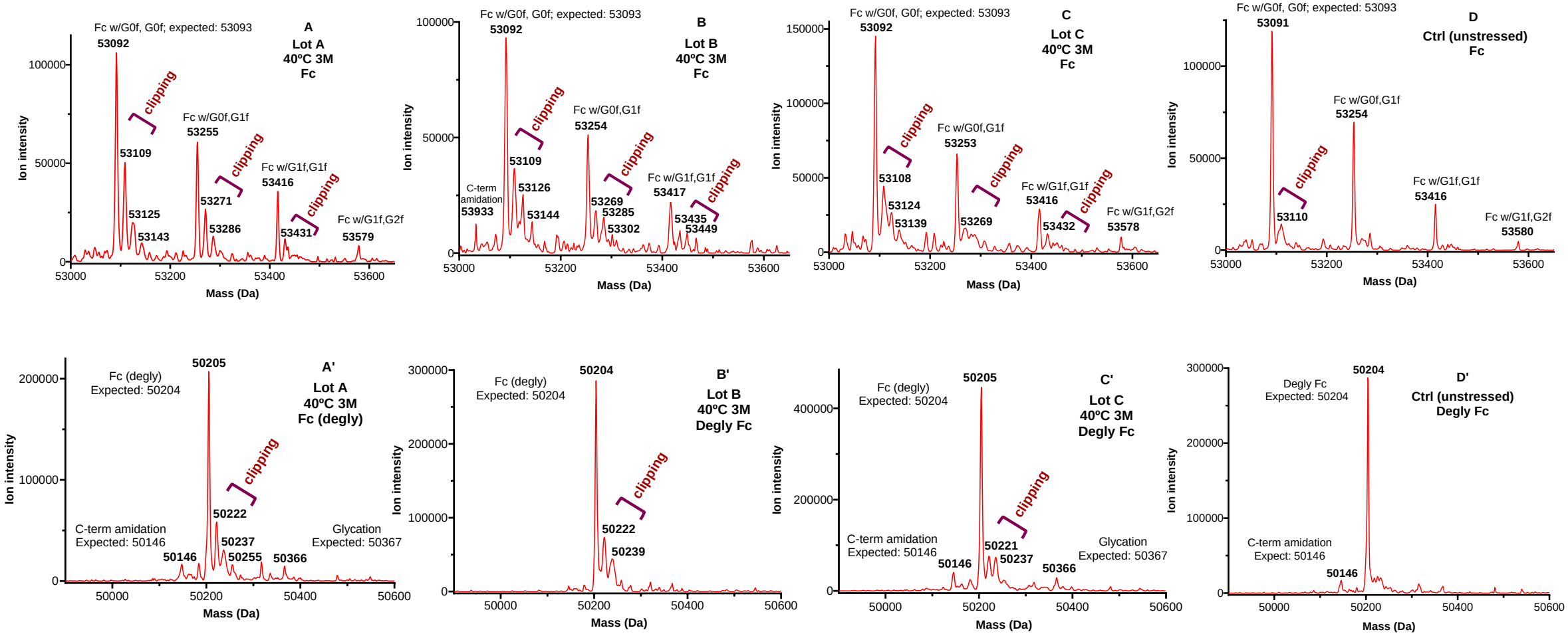


Figure 7. Deconvoluted mass spectra of Oxi-Fc and Fc in bsAb-A 40°C 3 mon heat-stressed Lot A (top), and RP-LC UV profile of bsAb-A subunits of heat-stressed Lot A at 40°C 3mon (bottom).

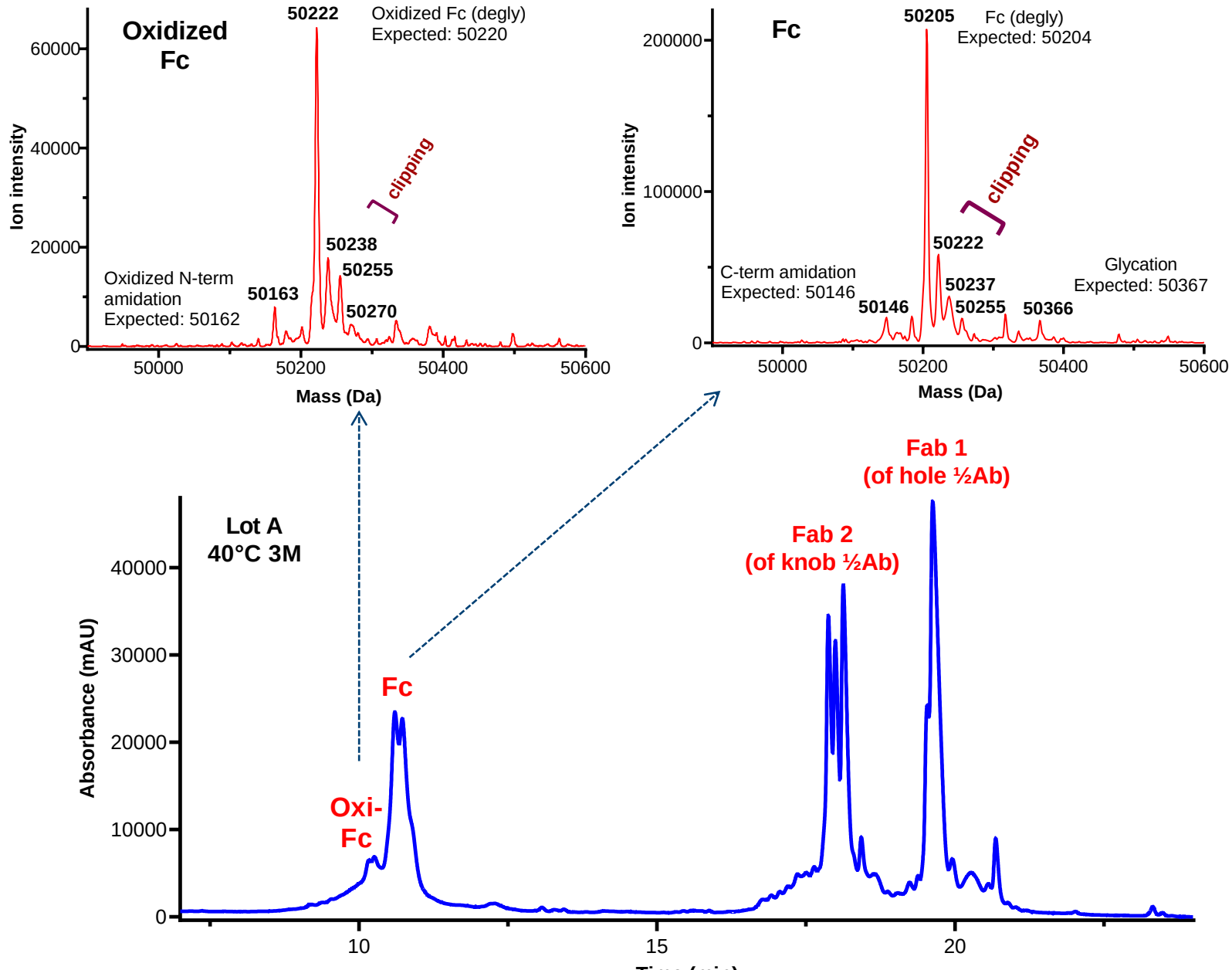


Figure 8. Deconvoluted mass spectra of Fab 1 and Fab 2 in bsAb-A 40°C 3 mon heat-stressed Lot A, Lot B and Lot C and unstressed Control. Top: Knob ½ Ab Fab; bottom: Hole ½ Ab Fab

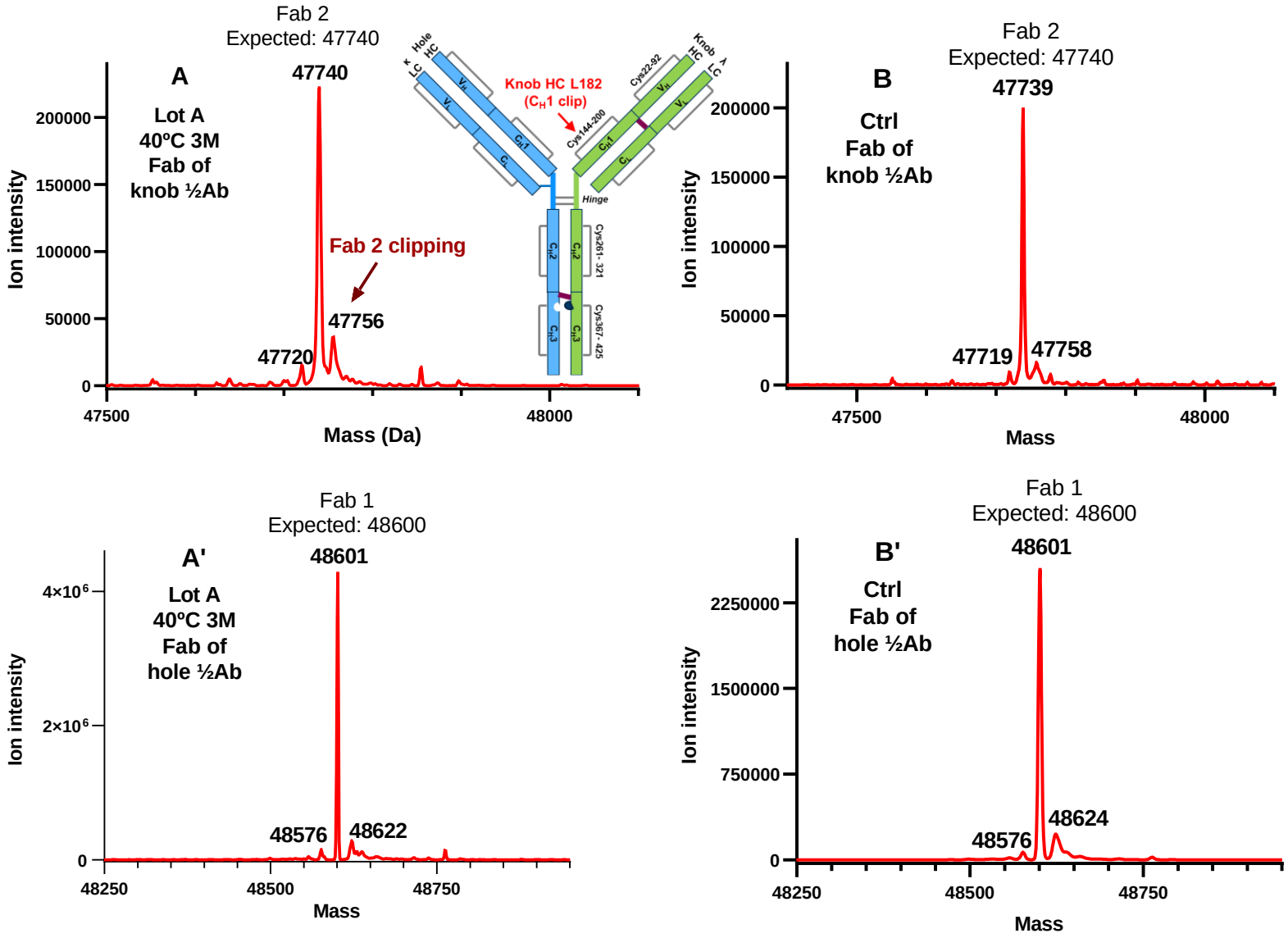
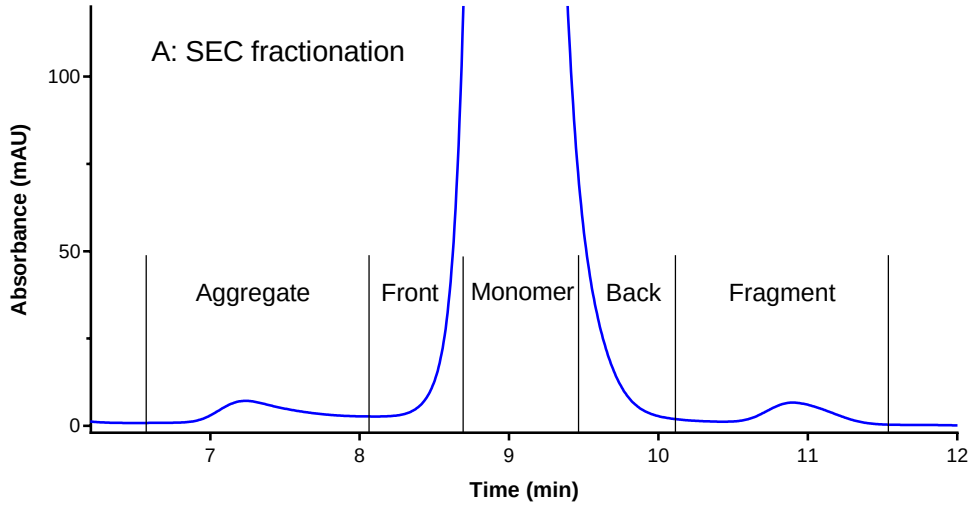
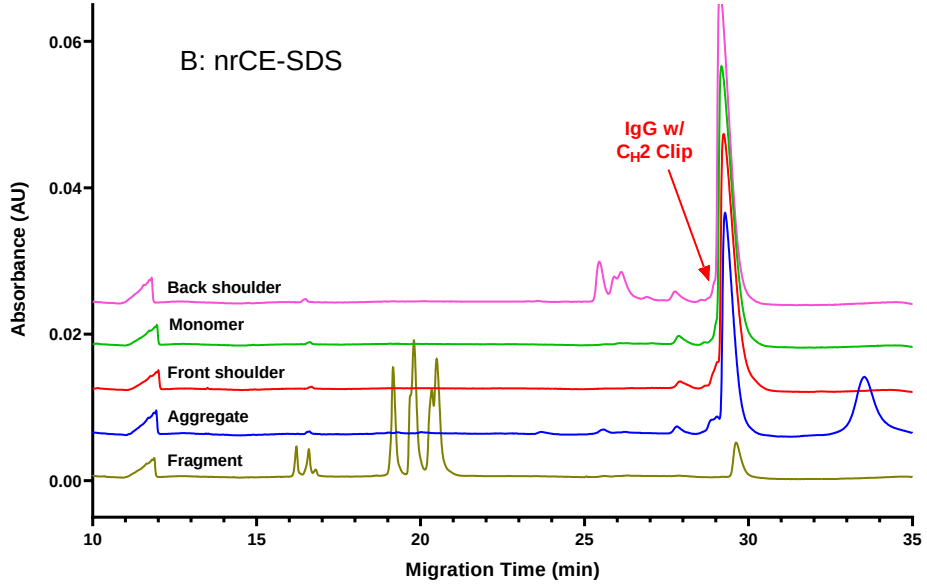
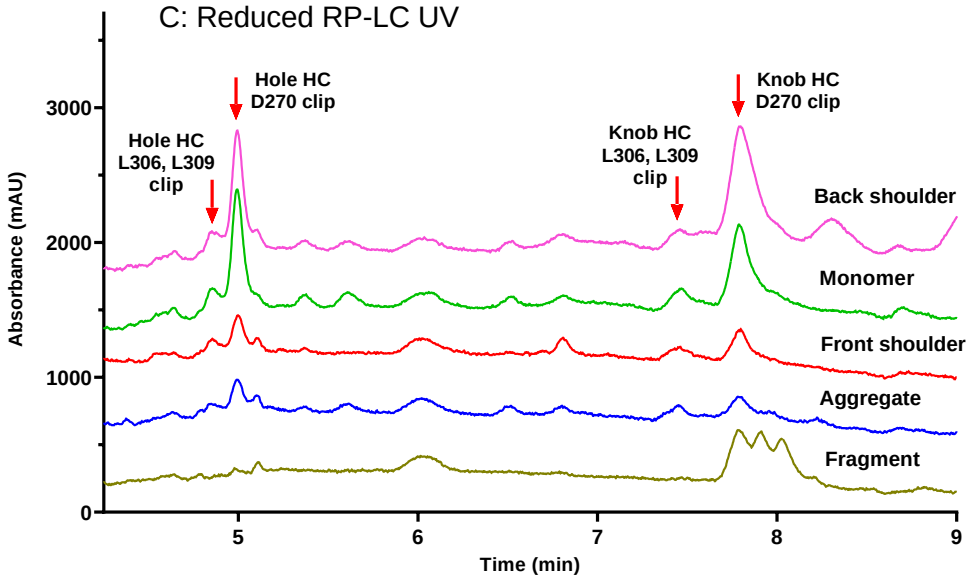


Figure 9. nrCE-SDS and reduced RP-LC UV overlay of SEC fractions from 40C 1mon heat-stressed bsAb-A and relative amount of the CH2 clippings at L306, L309 or D270 in SEC fractions. A: nrCE-SDS overlay; B: Reduced RP-LC UV overlay; C: Relative amount of the CH2 clippings at L306, L309 or D270 in SEC fractions.







SEC Fractions	Protein loading ratio in RP-LC
Back shoulder	3.1
Monomer	3.7
Front shoulder	1.0
Aggregate	1.3
Fragment	0.8

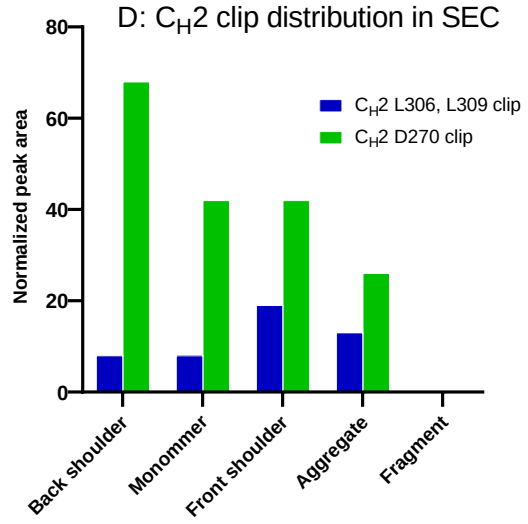
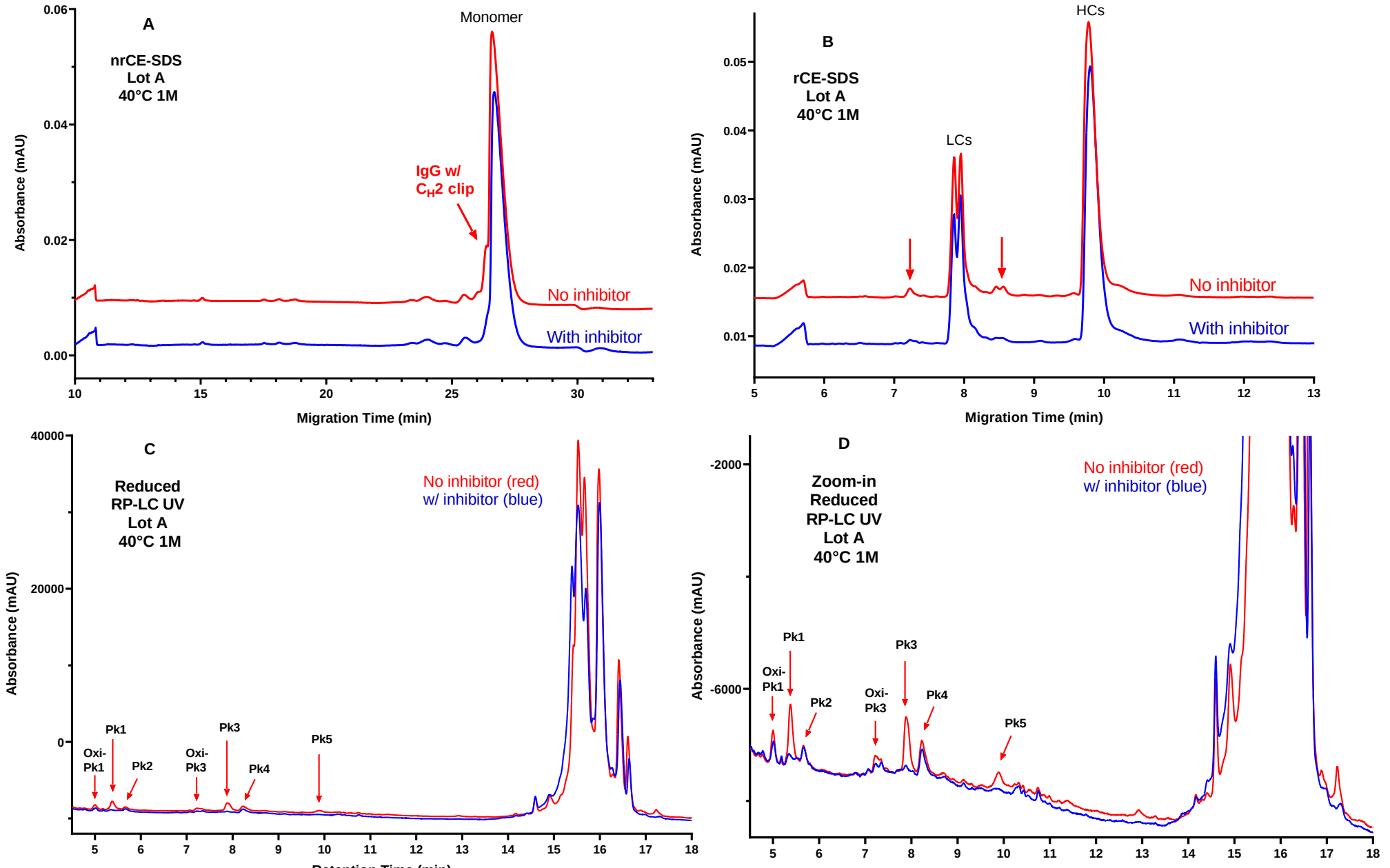


Table 2. Sequences in the vicinity of CH2 cleavage sites L306 and L309

EU #	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317
IgG1	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K
IgG2	R	V	V	S	V	L	T	V	V	H	Q	D	W	L	N	G	K
IgG4	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K

Figure 10. Overlays of nrCE-SDS (A), rCE-SDS (B) and reduced RP-LCUV (C) and zoom-in overlay of reduced RP-LC UV (D) of 40°C 1Mon bsAb-A Lot A incubated with protease inhibitors and without protease inhibitors.



Discussion

- Shoulder peak is related to protease activity in mAbs and bsAbs, and can be separated from the previously reported IgG missing HC N-terminal 100 amino acids
- C_H2 clipping can be resolved in non-reduced CE-SDS profiles as a shoulder peak on the intact IgG
- The C_H2 domain of IgGs is the most cleaved region and understanding these cleavages could help us better understand cleavages in other subclasses
- Identification of these clipping sites can lead to improve manufacturing and cell culture practices

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- Mingyan Cao
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Questions