

Support Information for Manuscript Entitled

“Understanding the Conformational Impact of Chemical Modifications on Monoclonal Antibodies with Diverse Sequence Variation Using HDX-MS and Structural Modeling”

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Supporting information

Experimental Methods

1. LC-MS/MS method for chemical modification quantitation

The relative percentages of each chemical modification were quantified using LC-MS/MS based peptide mapping method. In brief, each treated mAb sample and control was denatured using 6M Guanidine·HCl (MP Chemicals), reduced using dithiothreitol (DTT, purchased from Fluka) followed by alkylation using sodium iodoacetate (IAA, purchased from Fluka). The reduced and alkylated samples were then digested by trypsin (purchased from Worthington) at 1:20 enzyme to substrate mass ratio at 37°C for 15 min. The tryptic digests were analyzed using Waters Acquity UPLC system interfaced with Thermo Scientific LTQ Orbitrap XL mass spectrometer. The mass spectrometry data were acquired in a data-dependent mode with one full MS scan followed by two tandem MS scans of the two most intense ions. Peptide identification was carried out using BioWorks software from Thermo Fisher Scientific. The relative percentage of chemical modifications at each site was calculated using extracted ion chromatograms (EIC) by comparing the peak area ratio of modified and unmodified peptides.

2. Differential scanning calorimetry (DSC)

DSC measurements were performed on a MicroCal VP-Capillary DSC (GE Healthcare) at 1 mg/ml protein concentration. A DSC scan rate of 1°C/min was used. All DSC data analysis was performed using the Origin 7.0 software. A buffer background was subtracted first and then the sample scan was normalized based on molar concentration of the protein. Baseline corrections using a liner function in the transition region were made to generate the final excess heat capacity thermograms. Peaks in these thermograms are reported as melting temperatures.

3. Size Exclusion Chromatography (SEC)

The control and H₂O₂-treated mAb samples were further incubated at 40°C, and aliquots were taken at 0, 3 and 7 day time points. The aggregation level for each mAb sample after incubation was measured using SEC method. The SEC analysis was conducted by injecting 10 µg of each mAb sample into a Tosoh TSK-GEL G3000SWxl column (7.8 mm × 30 cm, 5 µm in particle size), followed by separation in PBS buffer containing 100 mM sodium phosphate, 400mM sodium chloride, pH 6.8. The mobile phase flow rate is 1 mg/ml with UV detection at 280nm.

Table S1: Percentage methionine oxidation quantified using LC/MS-based peptide mapping method. Kabat numbering is used if the Met residues appeared in more than one mAbs.

		Met50	Met252	Met358	Met397	Met428
mAb1	Control	0.2	3.1	NA	NA	0.6
	Oxidized	0.4	98.8	NA	NA	98.9
mAb2-1M	Control	7.2	2.6	NA	NA	1.5
	Oxidized	93.6	98.5	NA	NA	98.5
mAb3-2M	Control	0.4	3.7	0.5	1.4	1.4
	Oxidized	0.5	98.8	90.4	98.7	99.0
mAb4-AG	Control	NA	4.3	NA	NA	0.8
	Oxidized	NA	99.3	NA	NA	99.0

NA: Met residue not present in corresponding location

Table S2: Changes in melting temperatures for the first and major transitions caused by Met oxidation. ΔT_{m1} : Melting temperature difference for the first transition before and after Met oxidation; ΔT_{m2} : Melting temperature difference for the major transition before and after Met oxidation

Melting temperature change upon oxidation	mAb1	mAb2-1M	mAb3-2M	mAb4-Ag
ΔT_{m1} (°C)	-8.2	-8.4	-8.2	-12.4
ΔT_{m2} (°C)	-0.2	-1.7	0.1	0.2

Table S3: LC-MS/MS peptide map quantification of Asn deamidation and Asp isomerization in mAb1 samples after incubation at 40 °C under different pH conditions. Only Asn and Asp residues with a modification level higher than 5% upon 14 day incubation are listed.

Incubation pH	Incubation Time (day)	D55		D104		N389	
		%Su55	%IsoD55	%Su104	%IsoD104	%Su389	%(D389+isoD389)
4.0	0	2.0	3.2	6.5	4.8	ND	13.0
	7	15.2	8.7	22.7	7.7	ND	13.4
	14	18.2	14.1	28.0	11.2	ND	14.9
5.5	0	1.6	3.2	3.7	2.8	ND	7.6
	7	8.7	9.3	17.0	7.4	ND	11.8
	14	10.0	15.1	21.7	12.9	ND	12.7
9.0	0	2.1	3.2	6.8	4.5	ND	13.0
	3	13.1	5.8	1.4	7.4	ND	30.9
	14	11.0	11.7	3.5	17.8	ND	52.9

ND: not detected in peptide mapping method

D: Aspartic acid; IsoD: Isoaspartic acid; Su: Succinimide

For Asn deamidation, both deamidation reaction end products aspartic acid (D) and isoaspartic acid (isoD) were quantified and combined to report the level of deamidation.

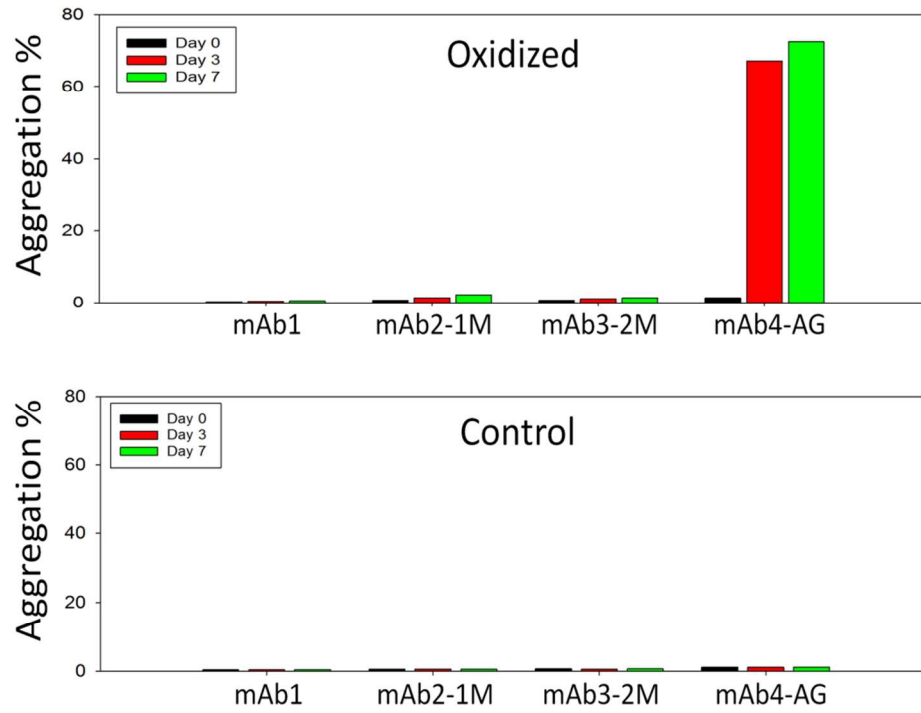
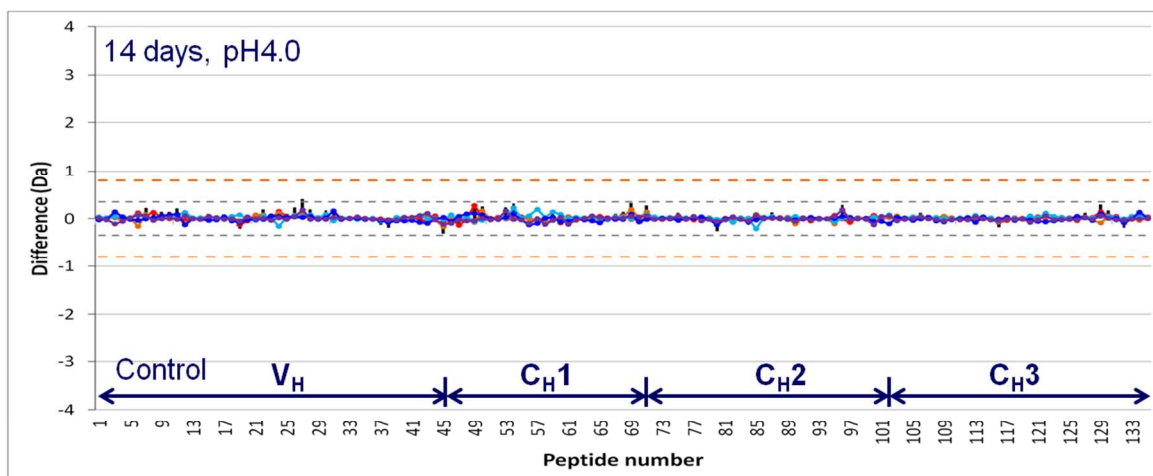


Figure S1: Aggregation comparison of control and oxidized samples for four mAbs.

mAb1 HC



mAb1 LC

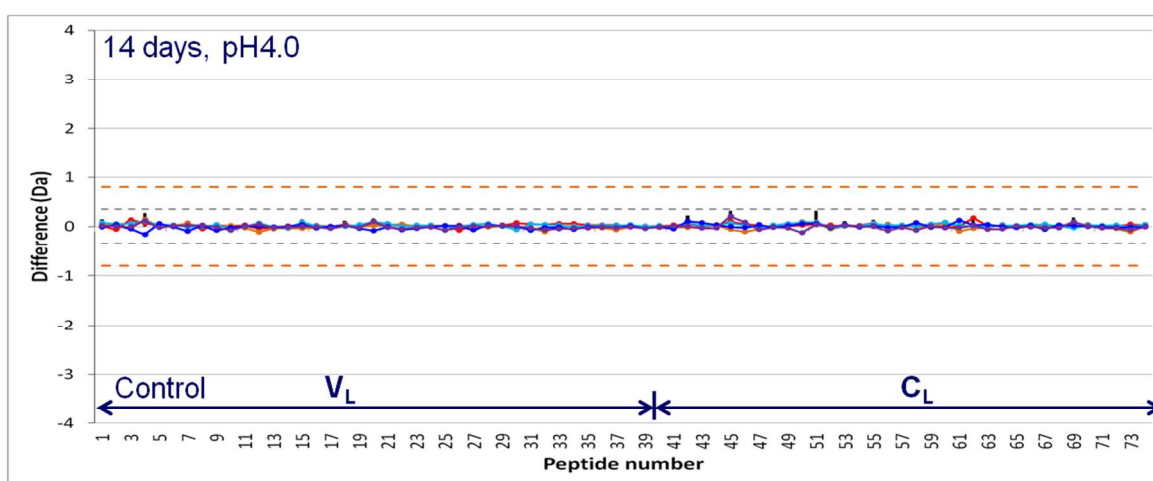


Figure S2. HDX differential plots of mAb1 heavy chain (A) and light chain (B) between the control sample and the sample incubated at pH 4.0 and 40 °C for 14 days. In each panel, deuterium labeling between pH stressed sample (top) vs. control sample (bottom) are compared. The HDX labeling time is 10 sec (red), 1 (orange), 5 (cyan), 30 (blue) and 180 min (purple). The gray and orange dotted lines represent the two criteria for significant HDX difference at one single labeling time point and total HDX difference from five time points respectively.

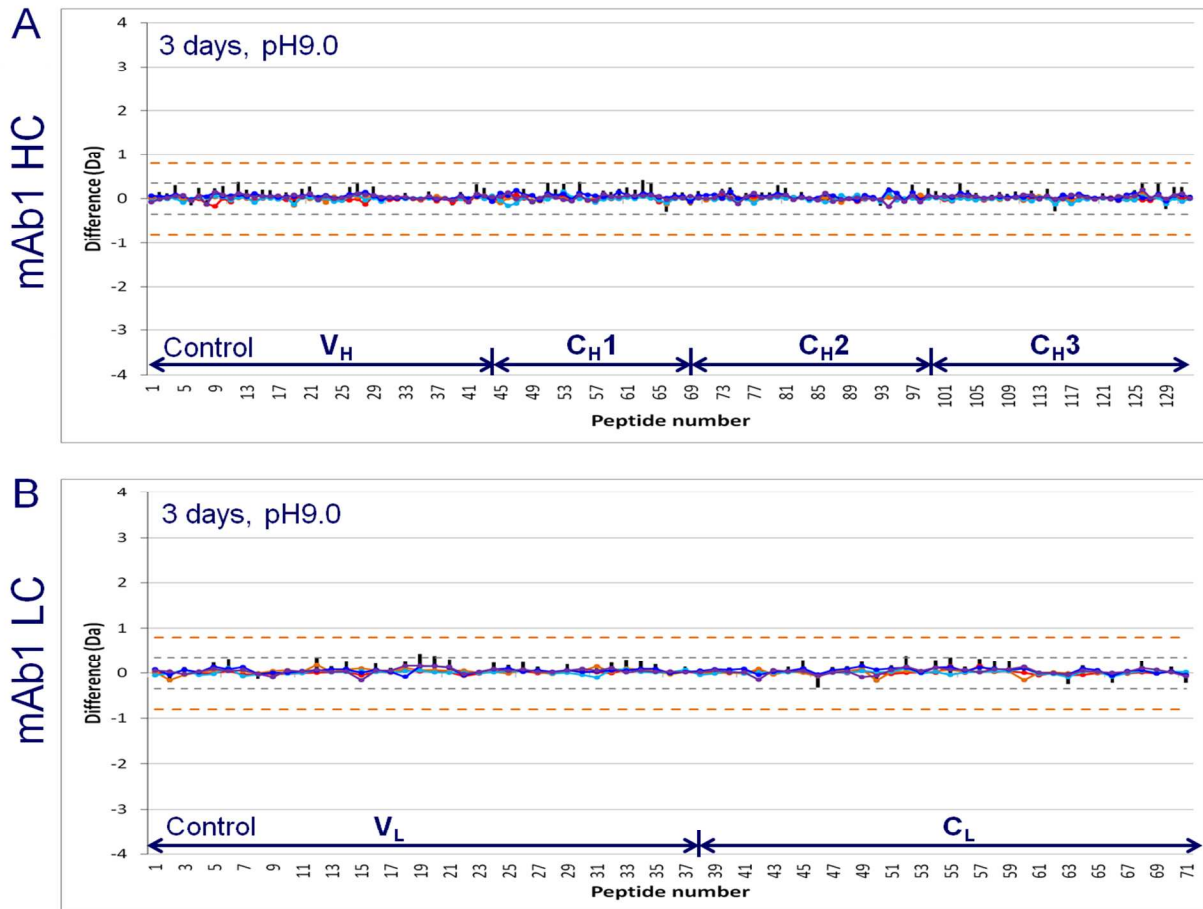


Figure S3. HDX differential plots of mAb1 heavy chain (A) and light chain (B) between the control sample and the sample incubated at pH 9.0 and 40 °C for 3 days. In each panel, deuterium labeling between pH stressed sample (top) vs. control sample (bottom) are compared. The HDX labeling time is 10 sec (red), 1 (orange), 5 (cyan), 30 (blue) and 180 min (purple). The gray and orange dotted lines represent the two criteria for significant HDX difference at one single labeling time point and total HDX difference from five time points respectively.