Supporting Information for Manuscript Entitled

"Use of *In vitro* Systems to Model *In vivo* Degradation of Therapeutic Monoclonal antibodies""

Authors:

Na Yang*, Qing (Mike) Tang*, Ping Hu and Michael J. Lewis

Large Molecule Analytical Development, Pharmaceutical Development and Manufacturing Science, Janssen Research & Development LLC, 200 Great Valley Parkway, Malvern, PA 19355, USA.

* The authors contributed equally to the work

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Table S1. Relative Quantitation Results of mAb1 Degradation in Spiked Serum

Modification Sites	Attribute	Relative Abundance (%)[a]									
		Control[b]	Day 1	Day 2	Day 4	Day 8	Day 15	Day 22	Day 29	Day 36	Day 43
HC Trp33	Oxidation	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.2	0.2
HC Met40	Oxidation	2.5	4.0	5.7	7.0	4.5	7.7	9.3	12.1	9.6	12.3
HC Met93	Oxidation	0.7	1.7	1.9	1.8	1.3	1.5	2.4	2.5	2.7	2.5
HC Trp99	Oxidation	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2
HC Met252	Oxidation	2.2	3.4	3.9	3.8	3.1	3.0	4.6	5.1	4.5	4.8
HC Met428	Oxidation	1.2	2.0	2.4	2.4	1.9	1.7	2.9	3.0	2.8	3.2
LC Trp93	Oxidation	1.0	1.1	1.1	1.1	0.9	1.1	1.3	1.1	1.2	1.4
HC Asn55	Deamidation	0.7	0.6	0.6	0.7	0.7	1.0	1.3	1.3	1.8	2.1
HC Asn286	Deamidation	0.1	0.2	0.2	0.1	0.2	0.2	0.3	0.4	0.5	0.6
HC Asn315	Deamidation	11.1	10.8	10.5	11.5	11.0	11.4	11.3	11.8	13.1	13.3
HC Asn325	Deamidation	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2
HC Asn384/Asn389	Deamidation	11.8	13.7	13.9	14.6	15.8	20.4	24.5	27.0	31.6	33.2
LC Asn53	Deamidation	0.3	0.4	0.4	0.5	0.4	0.6	0.6	0.7	0.7	0.8
HC Asp280/Asp283	Isomerization	0.5	0.4	0.5	0.5	0.5	0.7	0.8	0.9	1.1	1.3
LC Asp95	Isomerization	5.5	3.5	4.0	4.2	3.9	6.1	7.2	8.1	9.5	13.4
HC Glu1	Cyclization	1.4	1.6	1.9	2.3	3.7	5.7	7.9	9.9	11.7	12.7
LC Gln1	Cyclization	98.2	98.3	99.3	99.8	99.9	99.9	99.9	99.9	99.9	99.9
HC Lys447	CT Des-Lys	97.1	99.1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Mono-Glycated (Total)[c]	Glycation	4.5	4.5	5.2	5.7	7.8	12.2	15.3	19.0	21.5	26.9

[a] Relative abundance was calculated using integrated peak area from the selected ion chromatogram (LC-MS peptide-mapping).
[b] MAb1 was dosed in the clinical study and analyzed along with other serum samples for control.
[c] Relative percentage of total glycation was calculated using peak height intensity from intact MS.

Table S2. Relative Quantitation Results of Major mAb1 Degradation in Clinical Serum

Subject	Modification Site	Attribute	Relative Abundance (%)[a]									
			Control[b]	Day 1	Day 2	Day 4	Day 8	Day 15	Day 22	Day 29	Day 36	Day 43
000001	HC Met40	Oxidation	1.7	2.3	2.2	2.1	2.5	3.7	3.7	4.4	5.0	5.9
	HC Met252	Oxidation	2.0	3.2	3.0	3.0	3.3	3.9	4.0	4.1	4.3	4.8
	HC Asn55	Deamidation	0.8	1.1	1.0	1.1	1.3	1.6	2.1	2.5	2.7	2.8
	HC Asn384/Asn389	Deamidation	12.8	17.5	15.9	17.2	20.3	25.1	29.0	32.1	32.3	37.4
	LC Asp95	Isomerization	5.1	4.8	4.7	4.6	5.1	6.3	8.6	10.0	12.2	13.2
	HC Glu1	Cyclization	1.1	1.4	1.7	2.2	2.9	4.7	6.5	8.3	9.9	11.6
	Mono-Glycated (Total)[c]	Glycation	4.3	4.3	5.4	6.3	8.7	12.3	16.3	17.8	20.4	23.7
000004	HC Met40	Oxidation	1.7	1.9	1.8	3.5	2.3	4.2	4.7	6.1	6.1	7.1
	HC Met252	Oxidation	2.0	2.6	2.5	4.9	2.9	4.2	5.3	5.2	5.9	6.2
	HC Asn55	Deamidation	0.8	0.8	0.9	1.0	1.3	1.8	2.2	1.6	2.3	2.5
	HC Asn384/Asn389	Deamidation	12.8	15.9	15.9	17.9	19.8	24.6	28.6	30.4	33.2	36.5
	LC Asp95	Isomerization	5.1	4.7	4.4	5.1	5.7	6.1	8.8	8.6	10.6	14.6
	HC Glu1	Cyclization	1.1	1.2	1.4	1.9	2.5	3.7	5.2	6.9	8.7	10.0
	Mono-Glycated (Total)[c]	Glycation	4.2	4.6	5.5	6.4	9.7	13.5	16.3	19.1	21.7	22.7
000005	HC Met40	Oxidation	1.7	1.4	1.5	1.5	1.9	2.2	3.6	5.7	4.5	5.5
	HC Met252	Oxidation	2.0	2.4	2.5	2.7	2.8	2.9	4.0	6.4	4.1	4.0
	HC Asn55	Deamidation	0.8	0.9	1.1	1.0	1.4	1.6	2.1	2.3	2.7	2.9
	HC Asn384/Asn389	Deamidation	12.8	15.6	17.5	17.5	21.1	25.1	28.1	31.7	36.1	38.5
	LC Asp95	Isomerization	5.1	4.4	4.6	3.9	5.3	5.6	8.3	6.7	10.0	12.0
	HC Glu1	Cyclization	1.1	1.5	1.7	2.2	3.2	4.8	6.4	8.0	9.6	10.9
	Mono-Glycated (Total)[c]	Glycation	4.5	4.5	5.1	6.7	9.3	12.8	16	18.6	22.1	24.2
Average	HC Met40	Oxidation	1.7	1.9	1.8	2.4	2.2	3.4	4.0	5.4	5.2	6.2
	HC Met252	Oxidation	2.0	2.7	2.7	3.5	3.0	3.7	4.4	5.2	4.8	5.0
	HC Asn55	Deamidation	0.8	0.9	1.0	1.0	1.3	1.7	2.1	2.1	2.6	2.7
	HC Asn384/Asn389	Deamidation	12.8	16.3	16.4	17.5	20.4	24.9	28.6	31.4	33.9	37.5
	LC Asp95	Isomerization	5.1	4.6	4.6	4.5	5.4	6.0	8.6	8.4	10.9	13.3
	HC Glu1	Cyclization	1.1	1.4	1.6	2.1	2.9	4.4	6.0	7.7	9.4	10.8
	Mono-Glycated (Total)[c]	Glycation	4.3	4.5	5.3	6.5	9.2	12.9	16.2	18.5	21.4	23.5

STDEV	HC Met40	Oxidation	0.0	0.5	0.4	1.0	0.3	1.0	0.6	0.9	0.8	0.8
	HC Met252	Oxidation	0.0	0.4	0.3	1.2	0.3	0.7	0.8	1.2	1.0	1.1
	HC Asn55	Deamidation	0.0	0.2	0.1	0.1	0.1	0.1	0.1	0.5	0.2	0.2
	HC Asn384/Asn389	Deamidation	0.0	1.0	0.9	0.4	0.7	0.3	0.5	0.9	2.0	1.0
	LC Asp95	Isomerization	0.0	0.2	0.2	0.6	0.3	0.4	0.3	1.7	1.1	1.3
	HC Glu1	Cyclization	0.0	0.2	0.2	0.2	0.4	0.6	0.7	0.7	0.6	0.8
	Mono-Glycated (Total)[c]	Glycation	0.2	0.2	0.2	0.2	0.5	0.6	0.2	0.7	0.9	0.8

[a] Relative abundance was calculated using integrated peak area from the selected ion chromatogram (LC-MS peptide-mapping).
[b] MAb1 was dosed in the clinical study and analyzed along with other serum samples for method control.
[c] Relative percentage of total glycation was calculated using peak height intensity from intact MS.

Table S3. Relative Quantitation Results of Major mAb1 Degradation in Spiked PBS

Modification Site	Attribute	Relative Abundance (%)[a]									
		Control[b]	Day 1	Day 2	Day 4	Day 8	Day 15	Day 22	Day 29	Day 36	Day 43
HC Met40	Oxidation	6.5	6.0	6.4	5.7	5.6	5.7	5.5	5.8	5.9	5.8
HC Met252	Oxidation	5.1	5.0	5.2	5.0	4.9	4.9	5.0	5.0	5.2	5.5
HC Asn55	Deamidation	0.8	0.8	0.8	1.0	1.0	1.4	1.4	1.6	1.9	2.1
HC Asn384/Asn389	Deamidation	9.3	8.3	9.3	10.1	12.6	16.7	20.4	24.5	29.8	31.2
LC Asp95	Isomerization	5.5	5.3	5.4	6.4	7.5	9.8	11.8	14.0	15.6	17.2
HC Glu1	Cyclization	1.2	1.3	1.5	2.2	3.2	5.3	7.4	9.4	11.4	13.3
Mono-Glycated (Total)[c]	Glycation	5.7	4.8	5.4	6.4	8.5	12.1	15.0	18.0	19.8	22.0

[a] Relative abundance was calculated using integrated peak area from the selected ion chromatogram (LC-MS peptide-mapping).
[b] MAb1 was dosed in the clinical study and analyzed along with other serum samples for method control.
[c] Relative percentage of total glycation was calculated using peak height intensity from intact MS.

Table S4. mAb1 Concentration in Clinical Serum Samples

Clinical Subject ID	Time Point	Concentration (ug/mL)
Subject 01	Predose	0.0
	Day 1 1h	288.9
	Day 2 24h	246.2
	Day 4 72h	184.5
	Day 8 168h	120.2
	Day 15	74.1
	Day 22	59.7
	Day 29	42.1
	Day 36	33.1
	Day 43	18.3
Subject 02	Predose	0.0
	Day 1 1h	271.3
	Day 2 24h	272.7
	Day 4 72h	186.7
	Day 8 168h	120.3
	Day 15	86.9
	Day 22	62.7
	Day 29	45.6
	Day 36	36.1
	Day 43	29.9
Subject 03	Predose	0.0
	Day 1 1h	283.0
	Day 2 24h	257.4
	Day 4 72h	176.4
	Day 8 168h	108.6
	Day 15	80.2
	Day 22	62.8
	Day 29	48.4
	Day 36	34.4
	Day 43	30.4

Table S5. Recovery of mAb1 from Spiked Serum

Spike Sample ID	Recovery (%)[a,b]
Control	75
Day 1	90
Day 2	99
Day 4	103
Day 8	81
Day 15	94
Day 22	83
Day 29	78
Day 36	76
Day 43	84

[a] Recovery is assessed by A280 measurement

[b] Average recovery = 86% (± 11%)

Table S6. ANOVO Analysis Summary on mAb1 Attributes across 3 systems

ANOVA* summary	HC Asn384/389	HC Asn55	N-term PyroGlu	LC Asp95	Total Glycation
F	1.688	2.495	0.2248	2.499	0.02554
P value	0.2061	0.1037	0.8003	0.1033	0.9748
P value summary	ns	ns	ns	ns	ns
Significant diff. among means (P < 0.05)?	No	No	No	No	No
R square	0.1233	0.1721	0.01839	0.1724	0.002124

*ANOVO analysis performed on 3 systems (in vivo, in vitro-serum and in vitro-PBS) on listed attributes using GraphPad Prism7.00

Table S7. Assessing Variation of Linear Regression Slopes for mAb1 Attributes

Attributes	*k,* first rate constant (% day^{-1})			*CV (%)*
	In vivo	*In vitro (Serum)*	*In vitro (PBS)*	
Asn384/389	0.512	0.495	0.567	7.2
Asn55	0.044	0.034	0.030	20.0
IsoAsp95	0.198	0.206	0.291	22.2
N-term PyroE	0.227	0.278	0.289	12.5
Total Glycation	0.458	0.518	0.419	10.7
Met252	0.058	0.037	---	---
Met40	0.106	0.177	---	---

Table S8. 95% Confidence Interval of Linear Regression Slopes for mAb1 Attributes

	In vivo		In vitro (serum)		In vitro (PBS)	
Attribute	*k (% day^{-1})*	95% CI (%)	*k (% day^{-1})*	95% CI (%)	*k (% day^{-1})*	95% CI (%)
Asn384/389	0.512	0.4814 – 0.5418	0.495	0.4571 – 0.5337	0.567	0.5290 – 0.6053
Asn55	0.044	0.0382 – 0.0492	0.034	0.0284 – 0.0403	0.031	0.0263 – 0.0339
IsoAsp95	0.198	0.1718 – 0.2245	0.206	0.1590 – 0.2519	0.291	0.2732 – 0.3084
N-term PyroE	0.227	0.2232 – 0.2316	0.278	0.2591 – 0.2975	0.289	0.2855 – 0.2932
Total Glycation	0.458	0.4100 – 0.5050	0.518	0.4882 – 0.5474	0.419	0.3792 – 0.4585
Met252	0.058	0.0365 – 0.0802	0.037	0.0072 – 0.0645	---	
Met40	0.106	0.0892 – 0.1223	0.177	0.1011 – 0.2518	---	

*Confidence interval was calculated for each listed attribute using GraphPad Prism7.00

Table S9. Deamidation of IgG1 mAb at different pH Conditions by LysC peptide-mapping

Samples	LC41	HC57	HC206	HC318	**HC387/392 (UV)**	HC437 (UV)
Reference Std (pH8.0)	6.3	1.8	0.0	9.3	**10.5**	1.5
Ctrl pH7.0	0.4	1.5	0.0	4.2	**5.4**	1.2
Ctrl pH7.5	2.7	1.6	0.0	7.4	**6.1**	1.2
Ctrl pH8.0	6.9	1.6	0.1	10.8	**8.9**	1.6

Materials and Methods: SDS-PAGE and cSDS separation of mAb1 from serum

Materials. Invitrogen NuPAGE 4-12% Bis-Tris gel, LDS Sample Buffer, NuPAGE MOPS SDS Running Buffer and SimplyBlue SafeStain was purchased from Life Technologies (Carlsbad, CA). N-ethylmaleimide was purchased from Research Organics. Materials used for cSDS analysis were provided in the Beckman Coulter SDS-MW Analysis Kit (PN 390953), including capillary (50 µm I.D. bare-fused silica), SDS-MW gel buffer (proprietary formulation, pH 8, 0.2% SDS), SDS-MW sample buffer (100 mM Tris-HCl, pH 9.0, 1% SDS), 10 kDa internal standard (I.S., 5mg/mL), acidic wash solution (0.1 N HCl), and basic wash solution (0.1 N NaOH).

SDS-PAGE analysis of mAb1 from serum

The NuPAGE Bis-Tris electrophoresis system is a discontinuous SDS-PAGE, pre-cast polyacrylamide mini-gel system. In this study, NuPAGE Bis-Tris 4-12% gel was used and the samples was prepared according to manual instruction. Briefly, the loading sample mixture (total volume of 10 µL) was heated for denaturing electrophoresis at 70 °C for 10 min and cooled to room temperature. 3 µg of non-reduced sample was loaded, and the gel was run using Invitrogen XCell *SureLock* Mini-Cell at 200mV in 1X MOPS SDS Running Buffer for 50 min. After separation, the gel was stained by Coomassie blue for 1 hr and washed by water according to SimplyBlue SafeStain user instructions. Precision plus protein dual color standard (BioRad, CA) was used as protein marker.

cSDS analysis of mAb1 from serum

The mAb1 samples were diluted to 10 mg/mL with water before cSDS analysis. For NR cSDS analysis, 30 µL of each sample was diluted with 156 µL of 25 mM bis-tris/citrate buffer (pH 7.0) in 1% SDS, 4 µL of 5 mg/mL 10 kDa Internal Standard, and 10 µL of 125 mM N-ethylmaleimide. The diluted samples were incubated at 70 °C for 5 min and then cooled to room temperature. NR cSDS were performed on a Beckman Coulter Proteome-Lab™ PA 800 Protein Characterization System equipped with PDA detection. Each sample was injected into the capillary for 20 seconds at 5 kV (reverse polarity), with a 10 µL injection volume, followed by separation at 15 kV (reverse polarity) for 35 minutes in the capillary containing SDS-MW gel buffer.

Figure S1. cSDS (Capillary sodium dodecyl Sulfate electrophoresis) Separation of Non-reduced mAb1 Purified from Spiked-serum.

Purity of purified mAb1: 97.3%. Peak identification: 1- internal standard with MW of 10,000 Da; 2- largest impurity peak (mAb1-$HC_{1\text{-}212,1\text{-}133}$); 3- mAb1 IgG. The analysis was performed on a Beckman PA 800(s) system with UV detection.

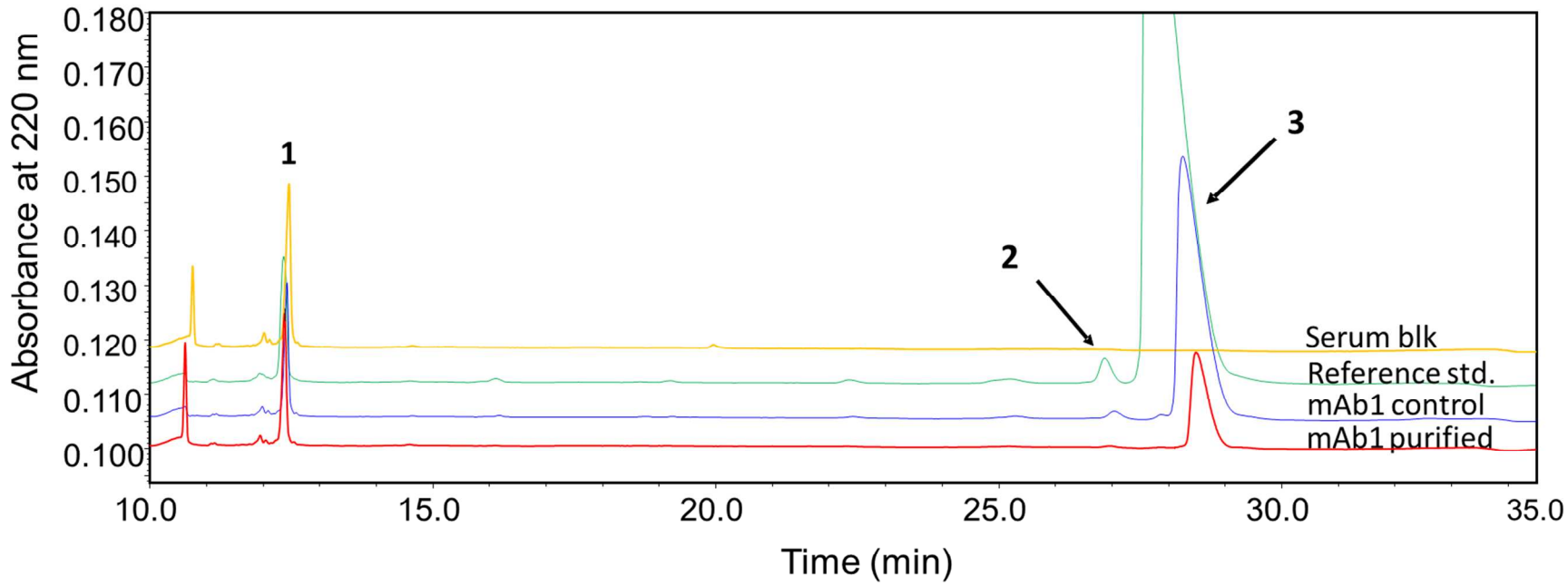


Figure S2. Deconvoluted Intact MS Profile Representative of purified mAb1 from Serum. (A)- Purified mAb1 from serum, (B)- mAb1 control.

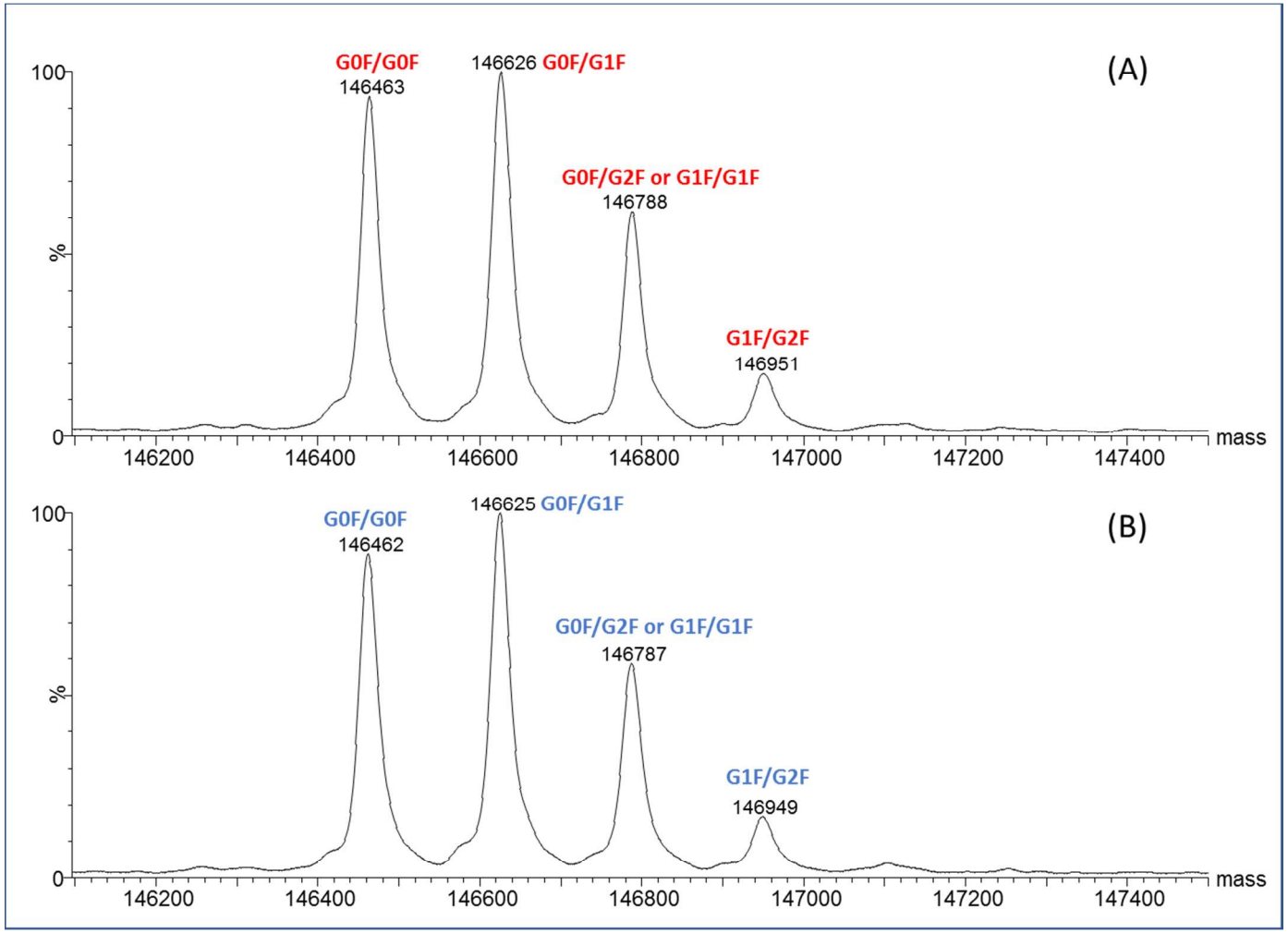
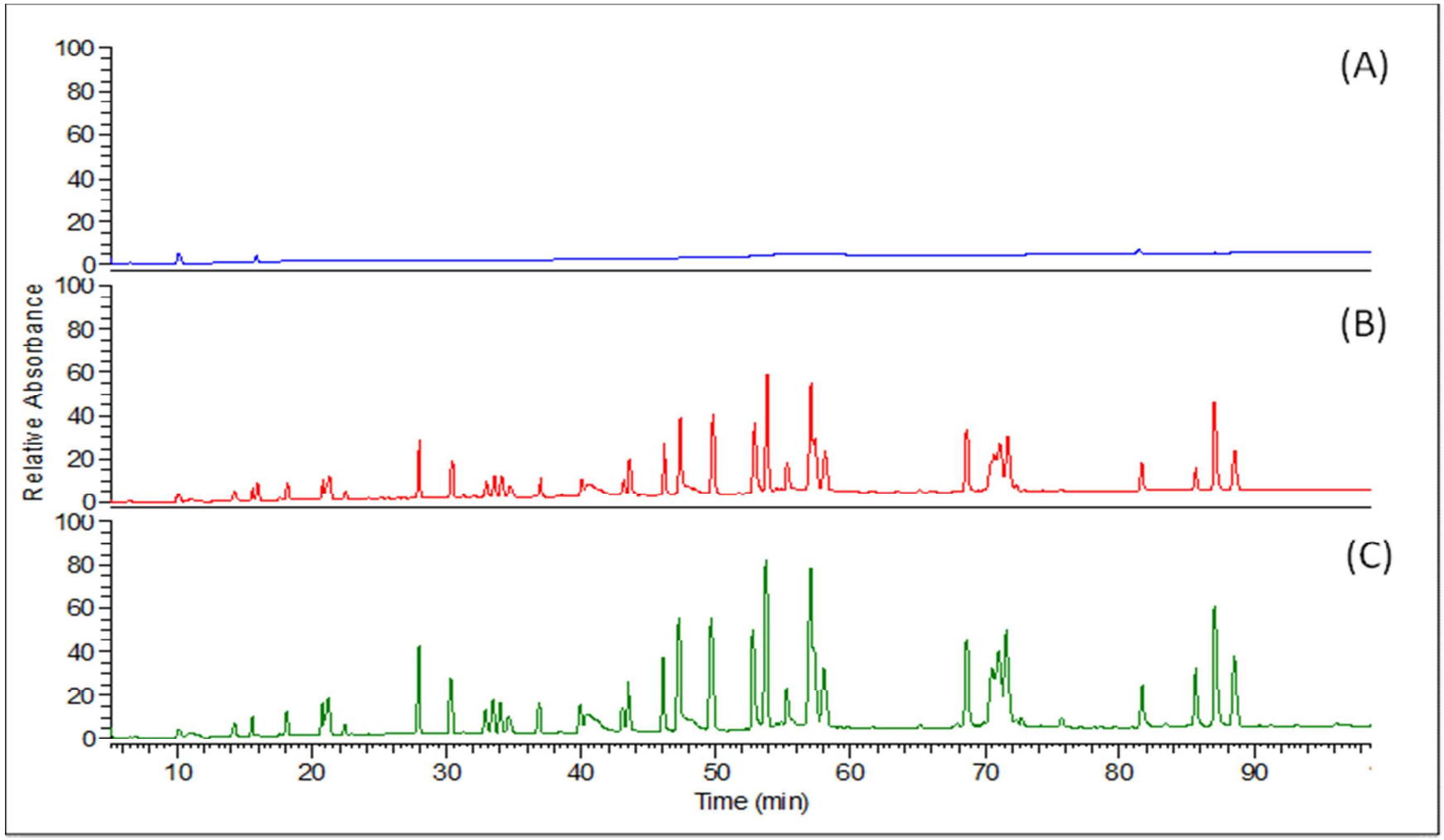


Figure S3. UV Chromatograph Representative of Digested Peptides from mAb1 by Peptide-mapping.

UV absorbance at 214 nm. Purified mAb1 from unspiked serum (A), spiked serum (B), and the mAb1 control (C).

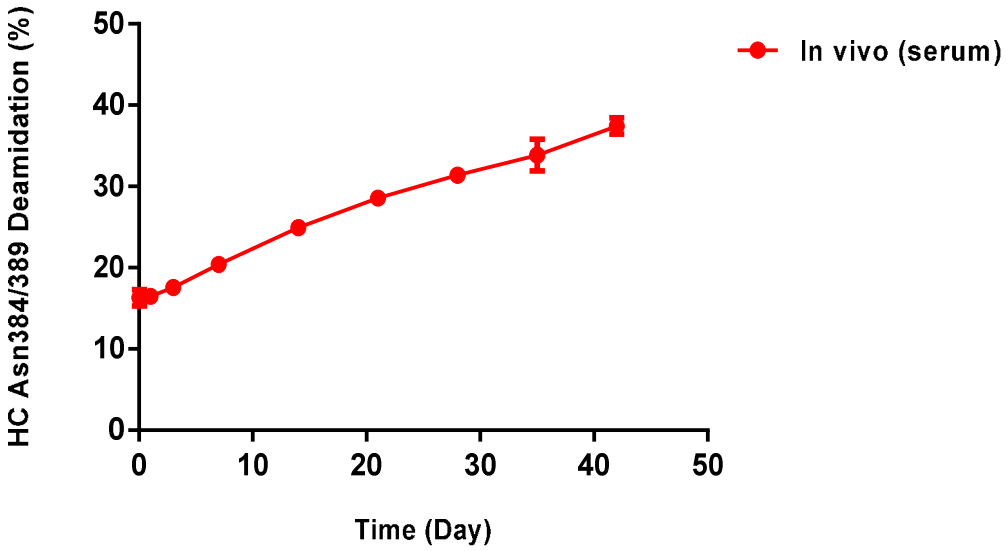


As shown in Figure S3, the UV chromatogram for mAb1 isolated from the spiked serum sample (B) was comparable to the chromatogram for the mAb1 control (C). It showed that mAb1 isolated from the spiked serum sample was also very pure. Furthermore, there was no interfering IgG peptides detected in the purified unspiked serum sample (A), which was consistent with the SDS-PAGE analysis. Together, these results indicated that the anti-id affinity purification was highly selective for mAb1, which enabled reliable relative quantitation of the extracted ion chromatograms from MS full scan. In order to evaluate any potential bias introduced by the affinity purification of mAb1, a method control (mAb1 used in clinical dosing but not subjected to purification) was performed along with other samples in peptide-mapping quantitation. The relative abundance of attributes from purified mAb1 (post-infusion) was comparable with the method control (Table S1), which suggested that isolation of mAb1 from serum samples did not induce significant bias in the MS analysis.

Figure S4. Time-course Plot of *In vivo* HC Deamidation Rates

In vivo HC Deamidation Change in Human Subjects (n=3). Error bars represent ± one standard deviation.

A) HC Asn384/389 Deamidation (For some points, the error bars would be shorter than the height of the symbol. In these cases, the error bars do not show)
B) HC Asn55 Deamidation



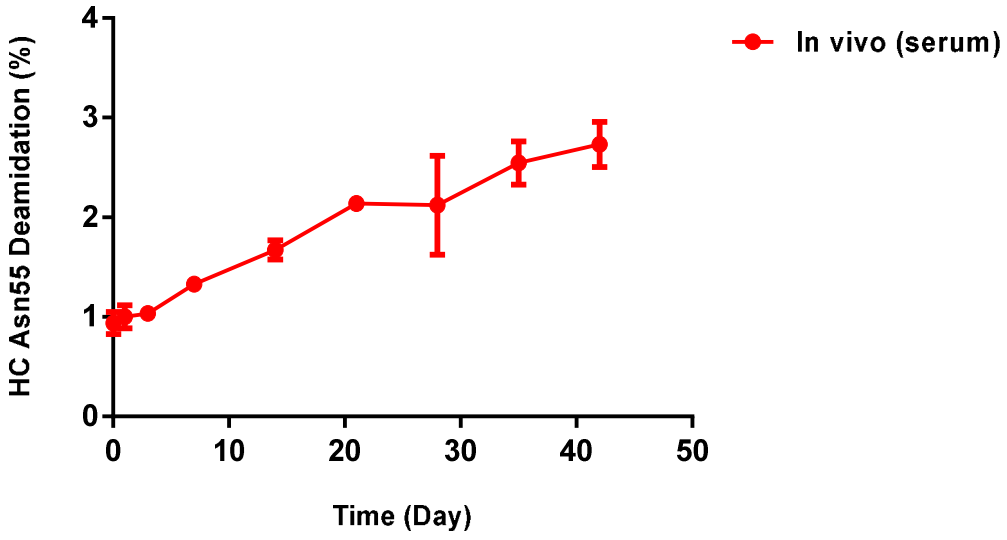


Figure S5. Time-course Plot of *In vivo* N-terminal PyroE Rates

In vivo N-terminal PyroE Change of Human Subjects (n=3). Error bars represent ± one standard deviation. For some points, the error bars would be shorter than the height of the symbol. In these cases, the error bars do not show.

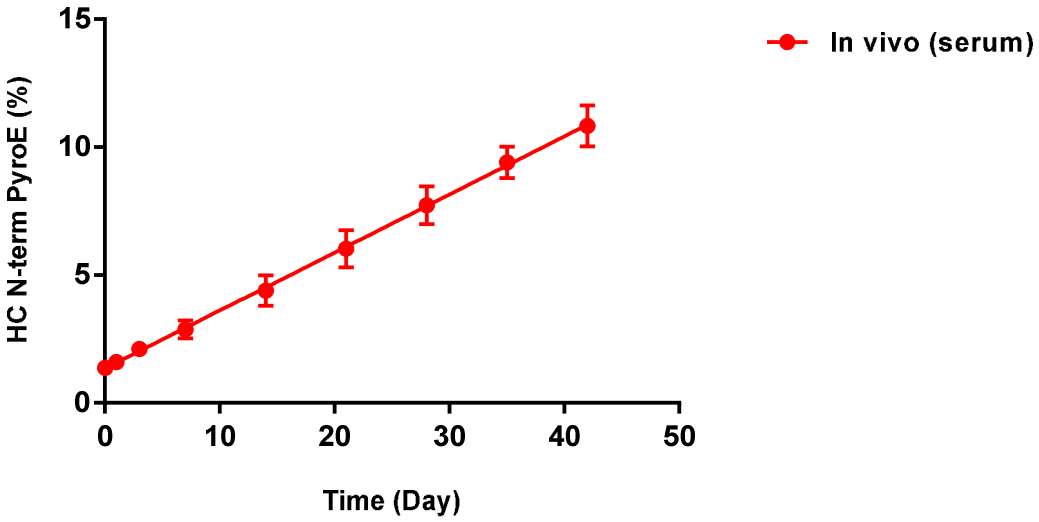


Figure S6. Time-course Plot of *In vivo* LC Asp95 Isomerization Rates

In vivo LC Asp95 isomerization change of human subjects (n=3). Error bars represent ± one standard deviation. For some points, the error bars would be shorter than the height of the symbol. In these cases, the error bars do not show.

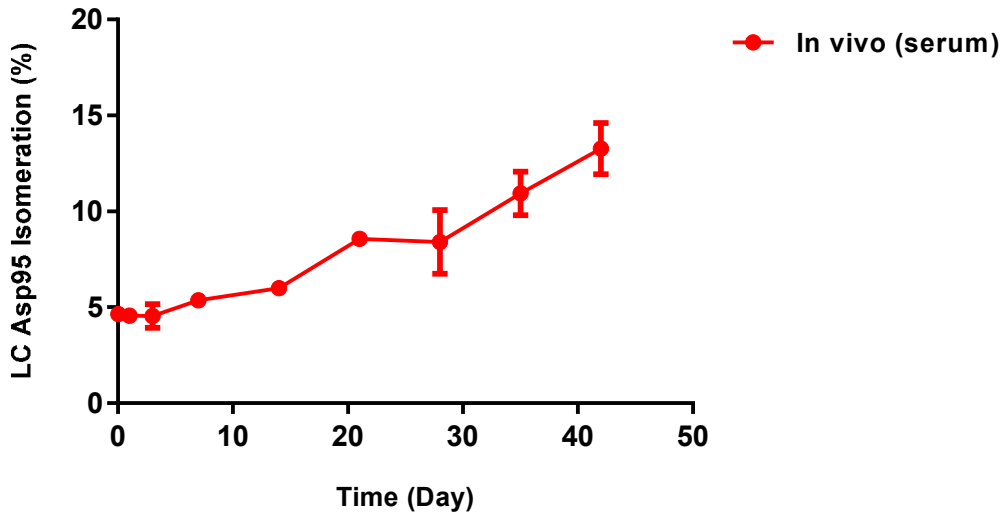


Figure S7. Time-course Plot of *In vivo* Glycation Rates

In vivo total glycation change from human subjects (n=3). Error bars represent ± one standard deviation. For some points, the error bars would be shorter than the height of the symbol. In these cases, the error bars do not show.

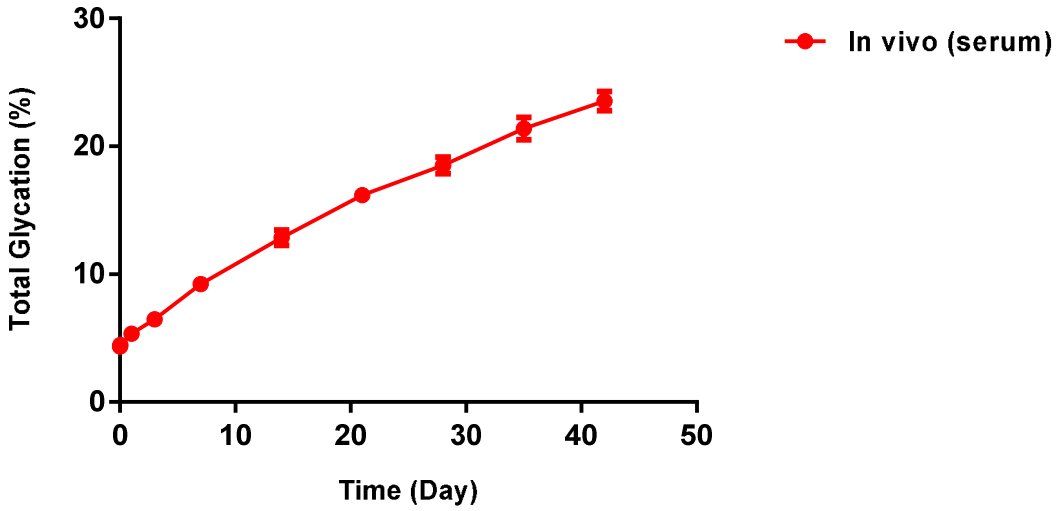


Figure S8. N-terminal Glu Cyclization Rates of mAb2

The levels of pyroE are presented for mAb2 isolated from clinical serum, spiked serum and spiked PBS samples. The *in vivo* data represent the average results from two human subjects. Error bars represent ± one standard deviation. For some points, the error bars would be shorter than the height of the symbol. In these cases, the error bars do not show.

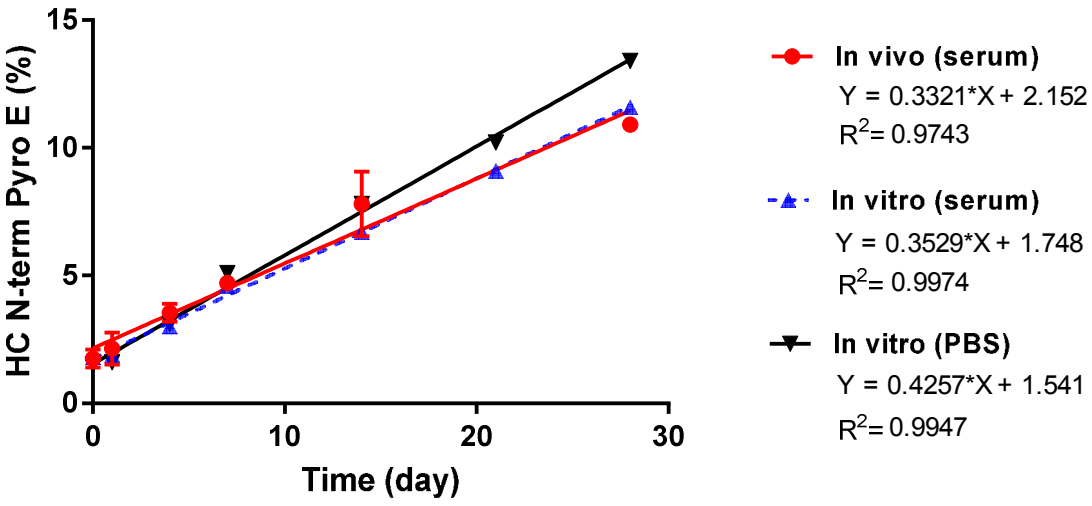


Figure S9. N-terminal Glu Cyclization Rates of mAb3

The levels of pyroE are presented for mAb2 isolated from clinical serum and spiked serum samples. The *in vivo* data represent the average results from four human subjects. Error bars represent ± one standard deviation. For some points, the error bars would be shorter than the height of the symbol. In these cases, the error bars do not show.