SUPPORTING INFORMATION for:

Solid-State Hydrogen Deuterium Exchange Mass Spectrometry: Correlation of Deuterium Uptake and Long-Term Stability of Lyophilized Monoclonal Antibody Formulations

Balakrishnan S. Moorthy[1], Isidro E. Zarraga[2], Lokesh Kumar[3], Benjamin T. Walters[4], Pierre Goldbach[5], Elizabeth M. Topp[1], Andrea Allmendinger[5,*]

[1] Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, IN

[2] Late State Pharmaceutical Development, Genentech Inc., South San Francisco, CA, USA

[3] Pharmaceutical Processing and Technology Development, Genentech Inc., South San Francisco, CA, USA

[4] Early State Pharmaceutical Development, Genentech Inc., South San Francisco, CA, USA

[5] Late-stage Pharmaceutical and Processing Development, Pharmaceutical Development and Supplies, Biologics, Europe, Hoffmann-La Roche, Basel, Switzerland

Corresponding Author:

Dr. Andrea Allmendinger, Hoffmann-La Roche
Grenzacherstr. 124
CH-4070 Basel
Switzerland
andrea.allmendinger.aa1@roche.com

The supporting information contains Table S1 – 3 and Figure S1 – 8.

Table S1. Amino acid sequence and location of 110 peptic fragments analyzed in the ssHDX-MS of mAb1 formulations.

Peptide Number	Residue Numbers	Sequence	m/z	Charge	Location
1	4-11	MTQSPSSL	850.389	1	V_L
2	10-25	SLSASVGDRVTITCSA	783.890	2	V_L
3	14-18	SVGDR	533.269	1	V_L
4	16-25	GDRVTITCSA	1022.490	1	V_L
5	34-49	NWYQQKPGKAPKVLIY	484.020	4	V_L
6	36-47	YQQKPGKAPKVL	339.955	4	V_L
7	36-48	YQQKPGKAPKVLI	368.225	4	V_L
8	36-49	YQQKPGKAPKVLIY	544.985	3	V_L
9	42-55	KAPKVLIYFTSSLH	321.591	5	V_L
10	47-53	LIYFTSS	415.708	2	V_L
11	55-70	HSGVPSRFSGSGSGTD	767.845	2	V_L
12	62-75	FSGSGSGTDFTLTI	695.330	2	V_L
13	65-97	SGSGTDFTLTISSLQPEDFATYYC QQYSTVPWT	1231.880	3	V_L
14	71-78	FTLTISSL	881.491	1	V_L
15	71-83	FTLTISSLQPEDF	749.371	2	V_L
16	74-82	TISSLQPED	989.475	1	V_L
17	75-82	ISSLQPED	888.430	1	V_L
18	90-99	QYSTVPWTFG	1185.54	1	V_L
19	91-104	YSTVPWTFGQGTKV	785.906	2	V_L
20	91-116	YSTVPWTFGQGTKVEIKRTVAAP SVF	957.166	3	V_L
21	104-114	VEIKRTVAAPS	390.889	3	C_L
22	106-115	IKRTVAAPSV	347.876	3	C_L
23	116-122	FIFPPSD	822.399	1	C_L
24	116-123	FIFPPSDE	951.439	1	C_L
25	116-125	FIFPPSDEQL	596.793	2	C_L
26	117-123	IFPPSDE	804.376	1	C_L

Table S1. continued (I)					
Peptide Number	**Residue Numbers**	**Sequence**	**m/z**	**Charge**	**Location**
27	117-125	IFPPSDEQL	1045.510	1	C_L
28	122-131	DEQLKSGTAS	518.230	2	C_L
29	136-161	LNNFYPREAKVQWKVDNALQSG NSQE	759.626	4	C_L
30	143-160	EAKVQWKVDNALQSGNSQ	1001.500	2	C_L
31	144-148	AKVQW	316.181	2	C_L
32	147-152	QWKVDN	789.388	1	C_L
33	148-157	WKVDNALQSG	1117.550	1	C_L
34	153-157	ALQSG	475.248	1	C_L
35	167-172	DSKDST	326.638	2	C_L
36	171-176	STYSLS	657.309	1	C_L
37	179-192	LTLSKADYEKHKVY	565.631	3	C_L
38	182-192	SKADYEKHKVY	342.678	4	C_L
39	187-208	EKHKVYACEVTHQGLSSPVTKS	486.444	5	C_L
40	194-212	CEVTHQGLSSPVTKSFNRG	682.995	3	C_L
41	4-18	LVESGGGLVQPGGSL	1367.720	1	V_H
42	5-17	VESGGGLVQPGGS	1143.560	1	V_H
43	5-18	VESGGGLVQPGGSL	1256.630	1	V_H
44	6-38	ESGGGLVQPGGSLRLSCAASGYTFT NYGMNWVR	859.647	4	V_H
45	11-18	LVQPGGSL	385.724	2	V_H
46	27-35	YTFTNYGMN	1110.450	1	V_H
47	36-45	WVRQAPGKGL	371.216	3	V_H
48	36-46	WVRQAPGKGLE	414.229	3	V_H
49	36-50	WVRQAPGKGLEWVGW	590.309	3	V_H
50	44-65	GLEWVGWINTYTGEPTYAADFK	630.300	4	V_H
51	50-62	WINTYTGEPTYAA	496.227	3	V_H
52	54-63	YTGEPTYAAD	1087.450	1	V_H

Table S1. continued (II)					
Peptide Number	**Residue Numbers**	**Sequence**	**m/z**	**Charge**	**Location**
53	64-72	FKRRFTFSL	401.230	3	V_H
54	65-72	KRRFTFSL	352.208	3	V_H
55	72-92	LDTSKSTAYLQMNSLRAEDTA	772.372	3	V_H
56	76-92	KSTAYLQMNSLRAEDTA	633.643	3	V_H
57	79-106	AYLQMNSLRAEDTAVYYCAKYPH YYGSS	1088.820	3	V_H
58	84-89	NSLRAE	689.357	1	V_H
59	84-93	NSLRAEDTAV	538.263	2	V_H
60	84-101	NSLRAEDTAVYYCAKYPH	421.006	5	V_H
61	103-110	YGSSHWYF	523.718	2	V_H
62	106-110	SHWYF	370.161	2	V_H
63	108-112	WYFDV	729.319	1	V_H
64	111-118	DVWGQGTL	875.419	1	V_H
65	117-131	TLVTVSSASTKGPSV	478.597	3	C_{H1}
66	118-147	LVTVSSASTKGPSVFPLAPSSKSTSG GTAA	931.490	3	C_{H1}
67	119-150	VTVSSASTKGPSVFPLAPSSKSTSGG TAALGC	984.834	3	C_{H1}
68	125-165	STKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWN	698.030	6	C_{H1}
69	152-161	VKDYFPEPVT	597.802	2	C_{H1}
70	162-180	VSWNSGALTSGVHTFPAVL	971.999	2	C_{H1}
71	163-180	SWNSGALTSGVHTFPAVL	922.456	2	C_{H1}
72	164-193	WNSGALTSGVHTFPAVLQSSGLYSL SSVVT	1022.53	3	C_{H1}
73	169-180	LTSGVHTFPAVL	621.344	2	C_{H1}
74	170-180	TSGVHTFPAVL	564.803	2	C_{H1}
75	173-199	VHTFPAVLQSSGLYSLSSVVTVPS SSL	691.360	4	C_{H1}
76	191-203	VVTVPSSSLGTQT	638.332	2	C_{H1}
77	192-198	VTVPSSS	676.349	1	C_{H1}

Table S1. continued (III)					
Peptide Number	**Residue Numbers**	**Sequence**	**m/z**	**Charge**	**Location**
78	192-203	VTVPSSSLGTQT	1176.600	1	C_{H1}
79	194-207	VPSSSLGTQTYICN	1469.690	1	C_{H1}
80	194-219	VPSSSLGTQTYICNVNHKPSNTK VDK	1409.210	2	C_{H1}
81	247-257	FLFPPKPKDTL	434.917	3	C_{H2}
82	248-257	LFPPKPKDTL	385.888	3	C_{H2}
83	248-258	LFPPKPKDTLM	643.851	2	C_{H2}
84	256-267	TLMISRTPEVTC	1350.660	1	C_{H2}
85	258-267	MISRTPEVTC	1136.540	1	C_{H2}
86	269-283	VVDVSHEDPEVKFNW	600.621	3	C_{H2}
87	284-306	YVDGVEVHNAKTKPREEQYN*STY	1043.710	4	C_{H2}
88	288-306	VEVHNAKTKPREEQYN*STY	748.329	5	C_{H2}
89	306-325	YRVVSVLTVLHQDWLNGKEY	605.580	4	C_{H2}
90	307-312	RVVSVL	672.438	1	C_{H2}
91	317-331	QDWLNGKEYKCKVSN	906.450	2	C_{H2}
92	325-354	YKCKVSNKALPAPIEKTISKAKGQP REPQV	662.771	5	C_{H2}
93	339-361	EKTISKAKGQPREPQVYTLPPSR	653.354	4	C_{H2}
94	340-354	KTISKAKGQPREPQV	417.494	4	C_{H2}
95	342-354	ISKAKGQPREPQV	360.201	4	C_{H2}
96	351-367	EPQVYTLPPSREEMTKN	673.662	3	C_{H3}
97	365-371	TKNQVSL	790.434	1	C_{H3}
98	371-394	LTCLVKGFYPSDIAVEWESNGQPE	1341.640	2	C_{H3}
99	375-386	VKGFYPSDIAVE	662.838	2	C_{H3}
100	383-391	IAVEWESNG	502.734	2	C_{H3}
101	387-404	WESNGQPENNYKTTPPVL	1037.490	2	C_{H3}
102	397-404	YKTTPPVL	459.766	2	C_{H3}
103	398-404	KTTPPVL	378.234	2	C_{H3}

Table S1. continued (IV)					
Peptide Number	**Residue Numbers**	**Sequence**	**m/z**	**Charge**	**Location**
104	398-415	KTTPPVLDSDGSFFLYSK	1001.520	2	C_{H3}
105	417-428	TVDKSRWQQGNV	709.353	2	C_{H3}
106	430-452	SCSVMHEALHNHYTQKSLSLSPG	632.293	4	C_{H3}
107	435-446	HEALHNHYTQKS	366.928	4	C_{H3}
108	437-452	ALHNHYTQKSLSLSPG	438.979	4	C_{H3}
109	438-444	LHNHYTQ	456.707	2	C_{H3}
110	439-452	HNHYTQKSLSLSPG	392.941	4	C_{H3}

Table S2. Relative methionine (Met) oxidation (%)in mAb1 formulations stored for 960 days at different temperatures. Solvent exposed Met residues are in bold.

Residue Number	**LC-Met4**	**HC-Met34**	**HC-Met83**	**HC-Met258**	**HC-Met364**	**HC-Met434**
Reference	0.3	0.6	0.4	**3.3**	0.3	**0.8**
F1 at 5˚C	0.4	0.5	0.4	**2.5**	0.2	**0.4**
F2 at 5˚C	0.4	0.5	0.4	**2.3**	0.3	**0.4**
F3 at 5˚C	0.3	0.5	0.4	**2.4**	0.2	**0.4**
F4 at 5˚C	0.3	0.5	0.3	**2.4**	0.1	**0.5**
F1 at 25˚C	0.3	0.6	0.4	**2.8**	0.2	**0.6**
F2 at 25˚C	0.4	0.6	0.4	**2.9**	0.2	**0.5**
F3 at 25˚C	0.3	0.8	0.5	**3.4**	0.3	**0.7**
F4 at 25˚C	0.3	0.7	0.5	**3.4**	0.2	**0.5**
F1 at 40˚C	0.3	0.8	0.4	**5**	0.3	**0.8**
F2 at 40˚C	0.3	0.8	0.5	**3.8**	0.3	**0.6**
F3 at 40˚C	0.3	1.0	0.6	**7.2**	0.6	**1.7**
F4 at 40˚C	0.3	0.9	0.5	**6.9**	0.4	**1.0**

Values obtained from average of two injections.

Table S3. Relative tryptophan (Trp) oxidation (%)in mAb1 formulations stored for 960 days at different temperatures.

Residue Number	**HC-Trp47/50**	**HC-Trp283**	**HC-Trp319**	**HC-Trp387**
Reference	0.3	0.2	0.5	0.1
F1 at 5˚C	0.2	0.1	0.5	0.1
F2 at 5˚C	0.5	0.2	0.6	0.1
F3 at 5˚C	0.3	0.2	0.6	0.1
F4 at 5˚C	0.5	0.2	0.6	0.2
F1 at 25˚C	0.7	0.2	0.7	0.1
F2 at 25˚C	0.6	0.2	0.7	0.2
F3 at 25˚C	0.6	0.2	0.7	0.1
F4 at 25˚C	0.5	0.2	0.5	0.1
F1 at 40˚C	0.4	0.2	0.6	0.1
F2 at 40˚C	0.8	0.3	0.8	0.2
F3 at 40˚C	0.7	0.2	0.7	0.2
F4 at 40˚C	0.7	0.2	0.7	0.2

Values obtained from average of two injections.

Figure S1. Overlay of XRPD spectra of crystalline mAb1 formulations (F3 and F4).

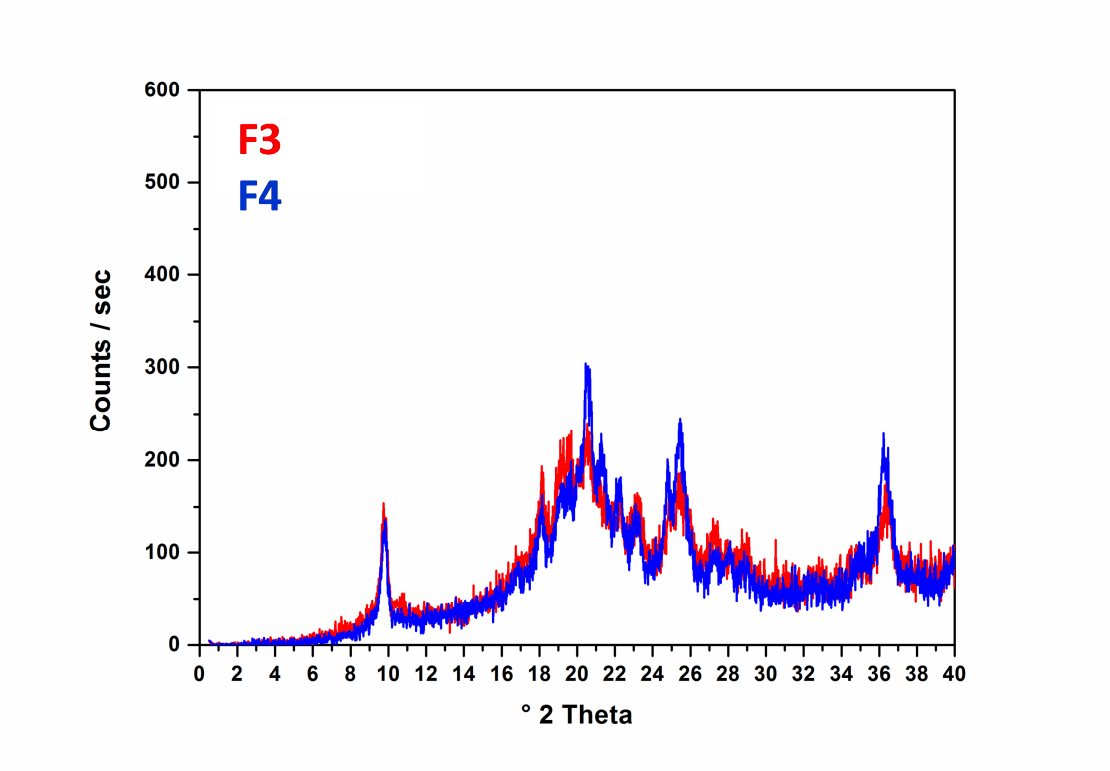

Figure S2. SEM images of lyophilized mAb1 formulations with (A-D) and without (E-H) exposure to D_2O vapor at 11% RH and 22 ˚C for 5 days. All images were collected at 500X magnification.

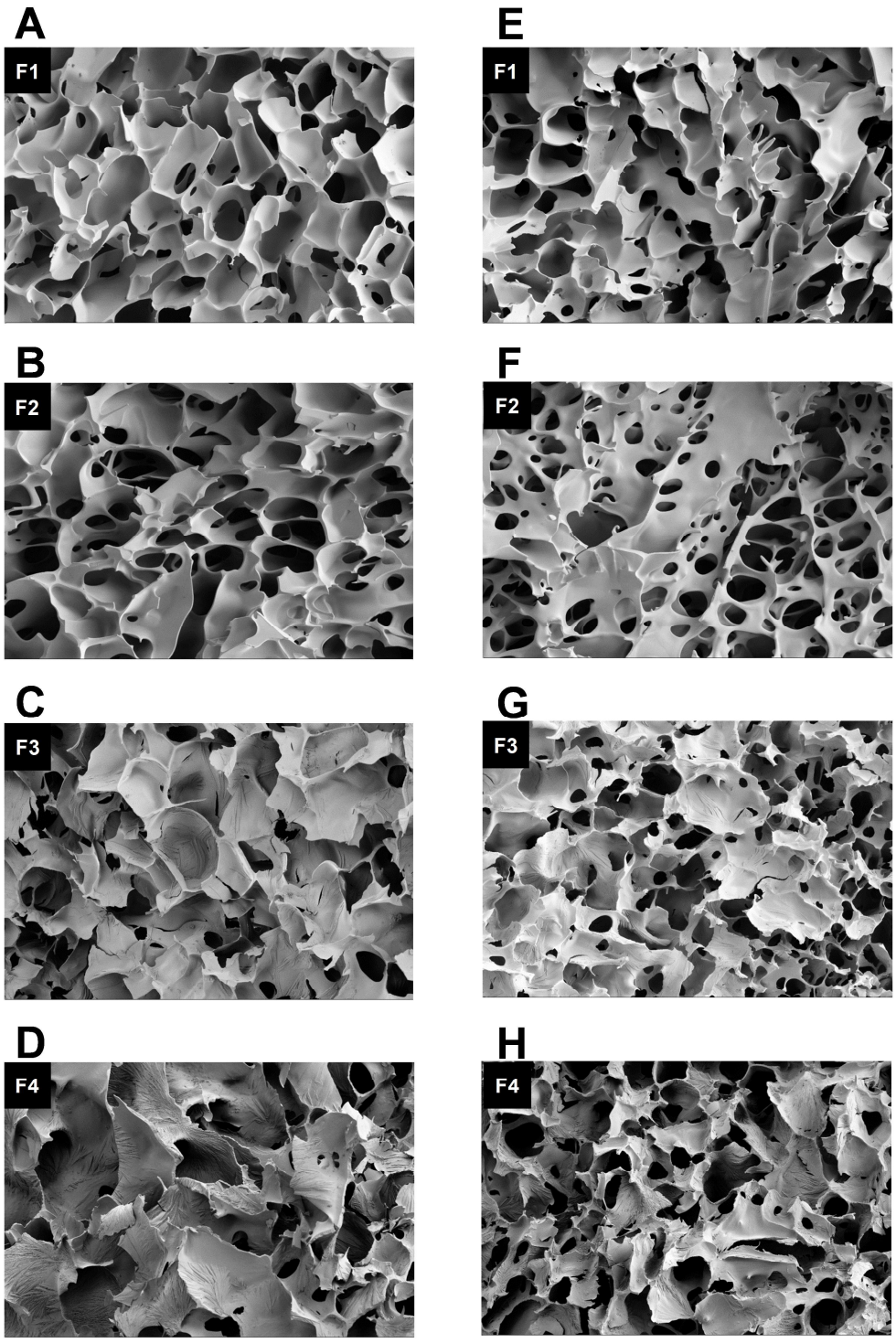

Figure S3. Physical stability of mAb1 formulations stored at 5˚C (A), 25C˚ (B), 40˚C (C) and 50˚C (D). Percent HMWs of mAb1 obtained using SEC; see text for experimental details. .

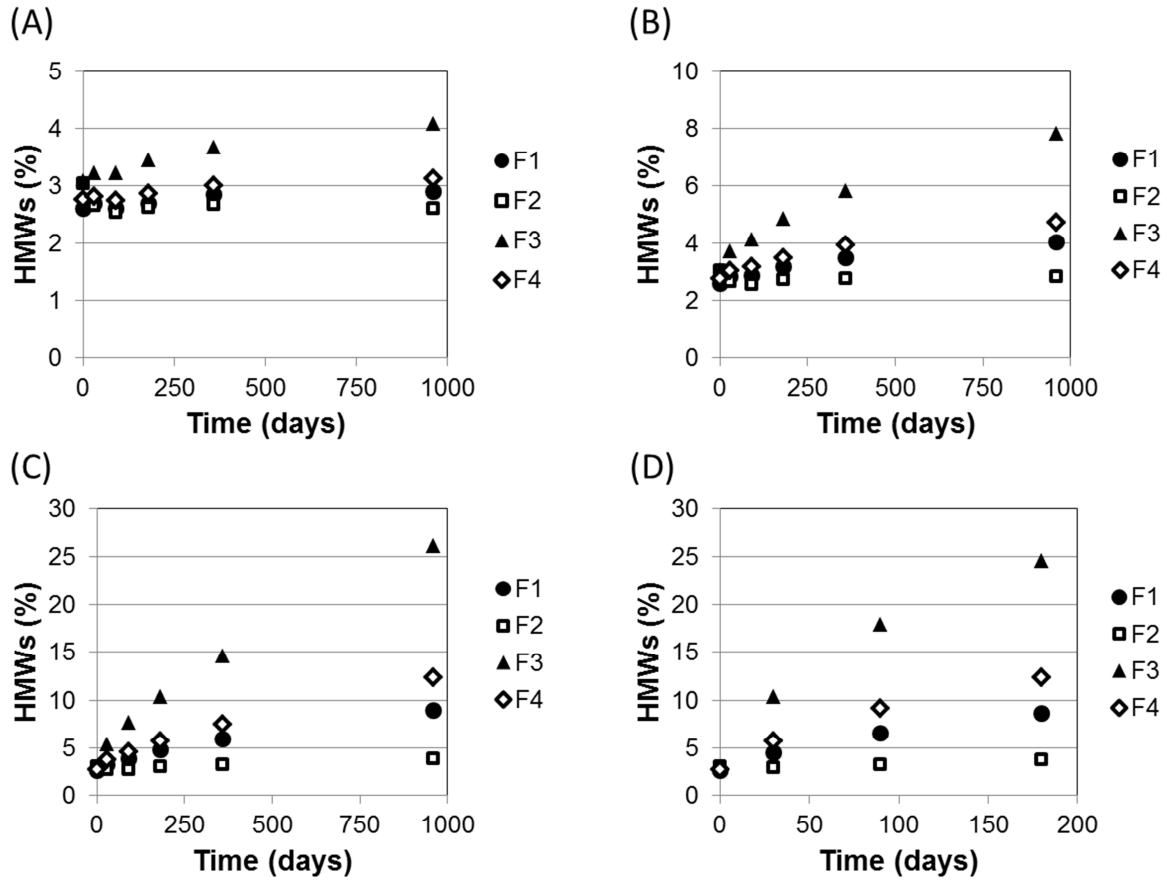

Figure S4. Percent main peak (A), basic regions (B), and acidic regions (C) measured by IEC for formulations stored at 5˚C, 25C˚ and 40˚C for 960 days.

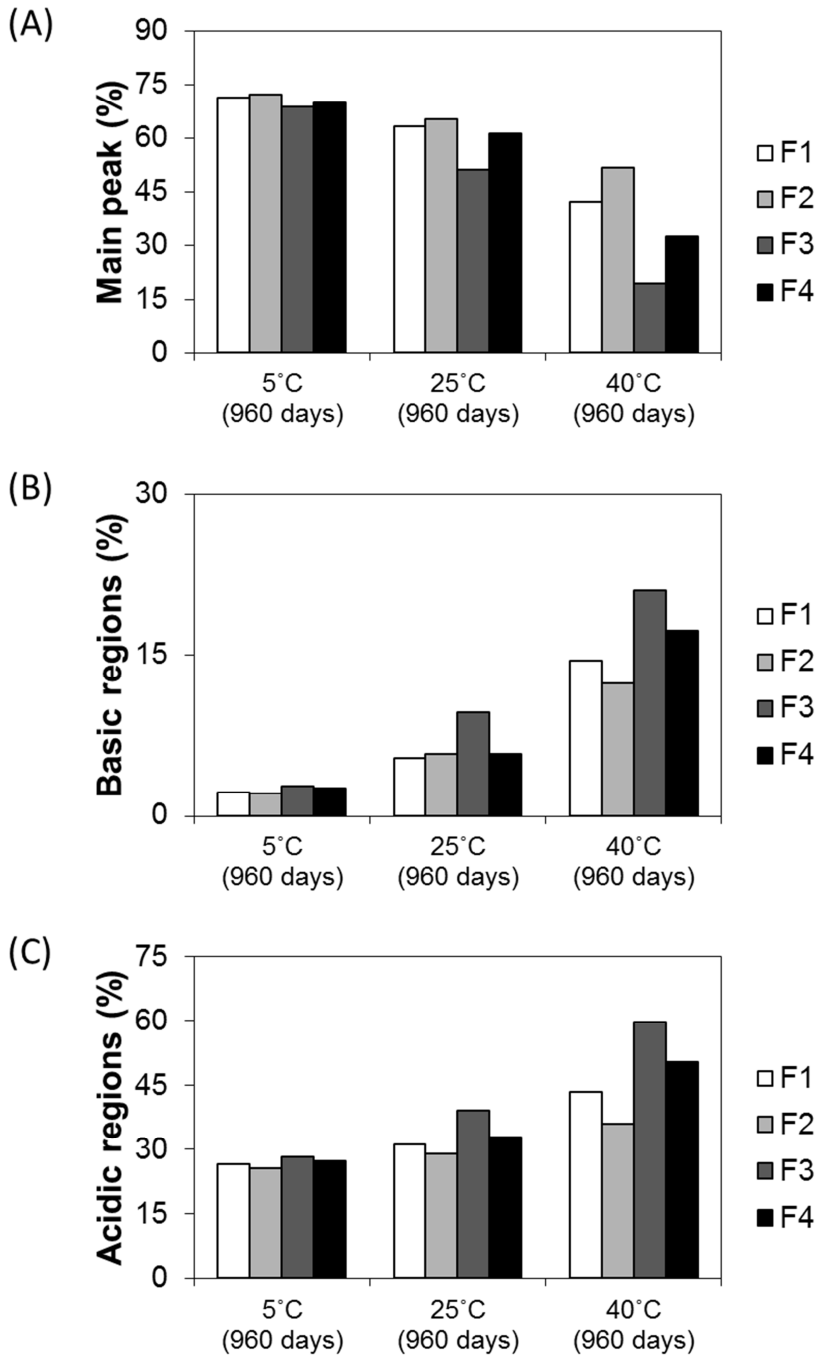

Figure S5 – A. Correlation of HMWs of mAb1 stored at 5˚C (A), for 960 days with band intensity for β-sheet (1637.5 cm^{-1}) obtained using ssFTIR (I), amount of sucrose used (II), percent residual moisture (III) and the glass transition temperature (Tg) (IV). See Figure S6-B for correlations with data at 25^{o}C storage conditions, and Figure 3 (main text) for correlations with data at 40^{o}C storage conditions.

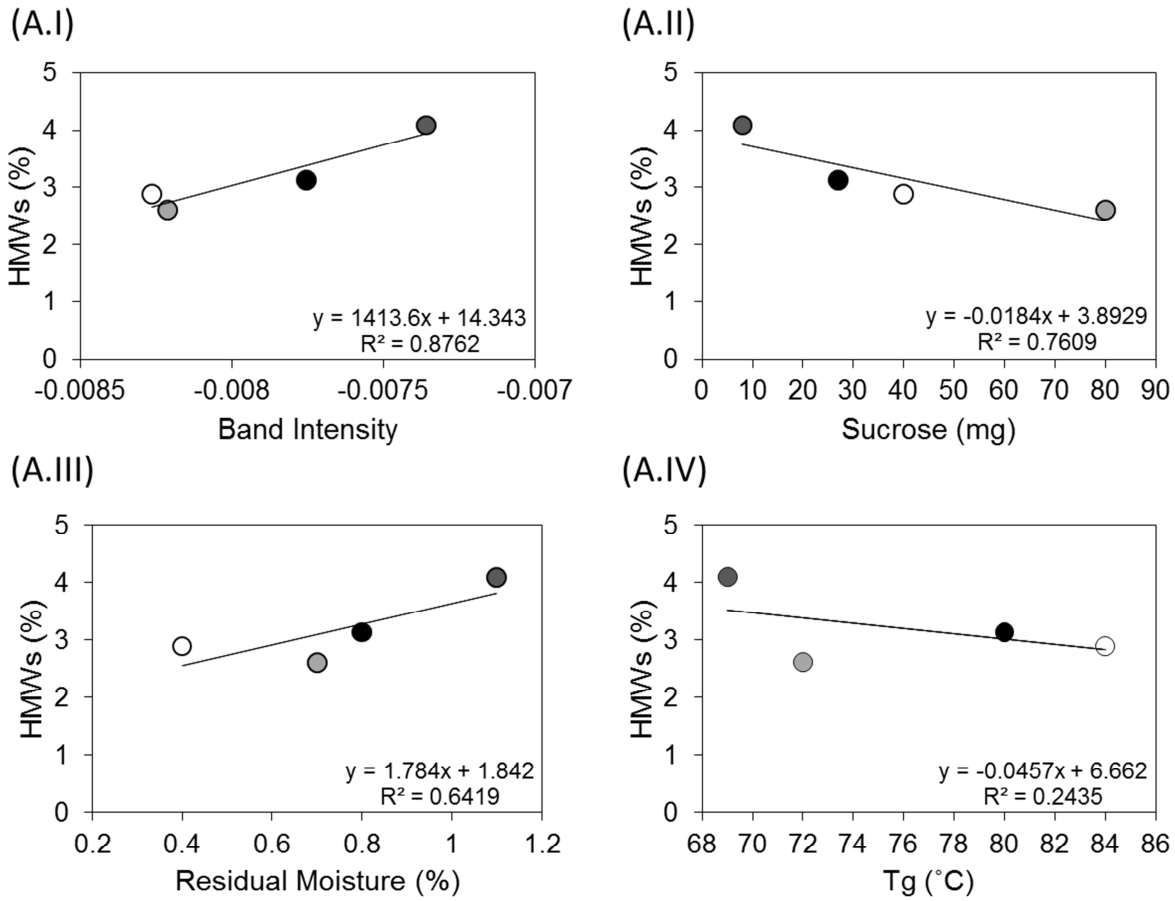

Figure S5 – B. Correlation of HMWs of mAb1 stored at 25˚C (A), for 960 days with band intensity for β-sheet (1637.5 cm^{-1}) obtained using ssFTIR (I), amount of sucrose used (II), percent residual moisture (III) and the glass transition temperature (Tg) (IV). See Figure S6-A for correlations with data at 5^{o}C storage conditions, and Figure 3 (main text) for correlations with data at 40^{o}C storage conditions.

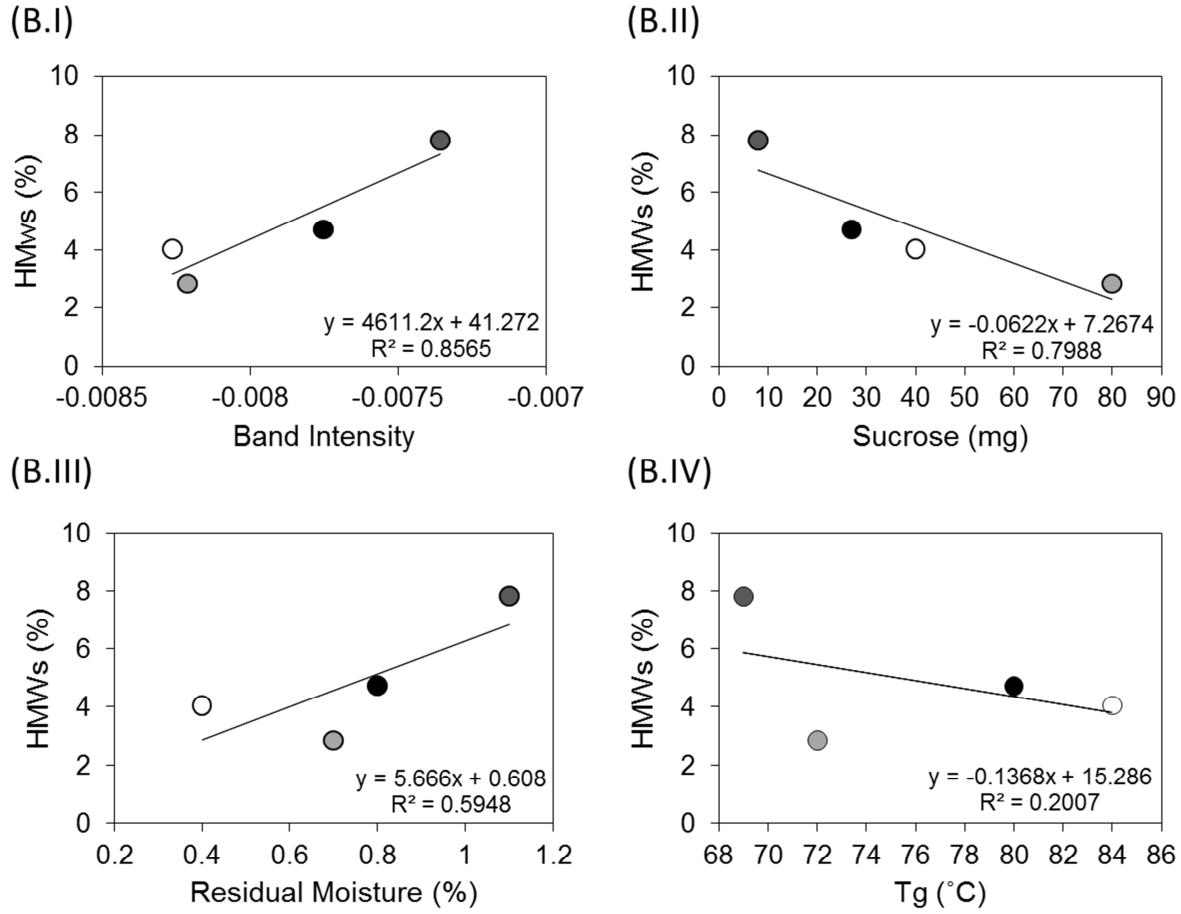

Figure S6 - I. Correlation of band intensity for β-sheet (1637.5 cm^{-1}) obtained using ssFTIR (I) with acidic regions measured by IEC at 5˚C (A), 25˚C (B) and 40˚C (C) and Met oxidation (HC Met258 + HC Met434) at 40˚C (D) after 960 days.

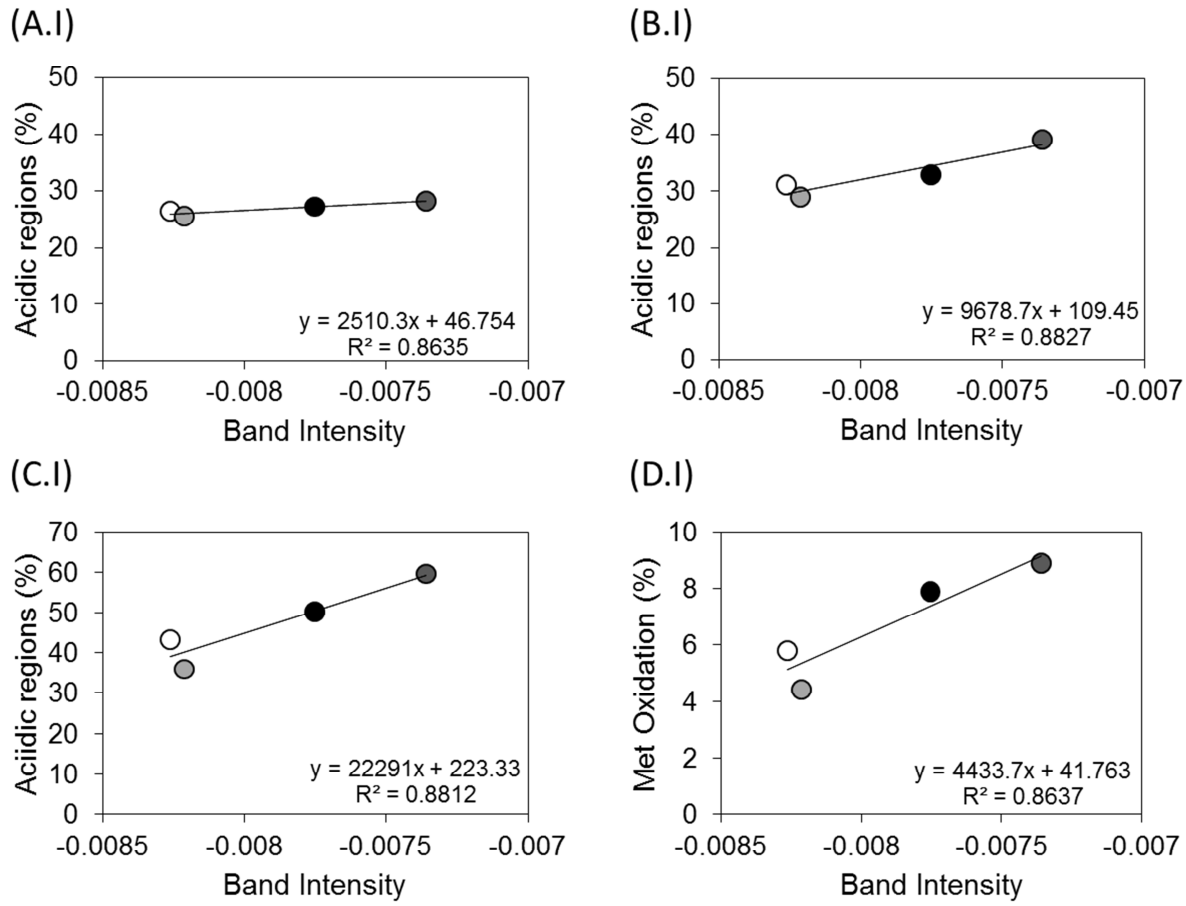

Figure S6 - II. Correlation of amount of sucrose in formulations (II) with acidic regions measured by IEC at 5˚C (A), 25˚C (B) and 40˚C (C) and Met oxidation (HC Met258 + HC Met434) at 40˚C (D) after 960 days.

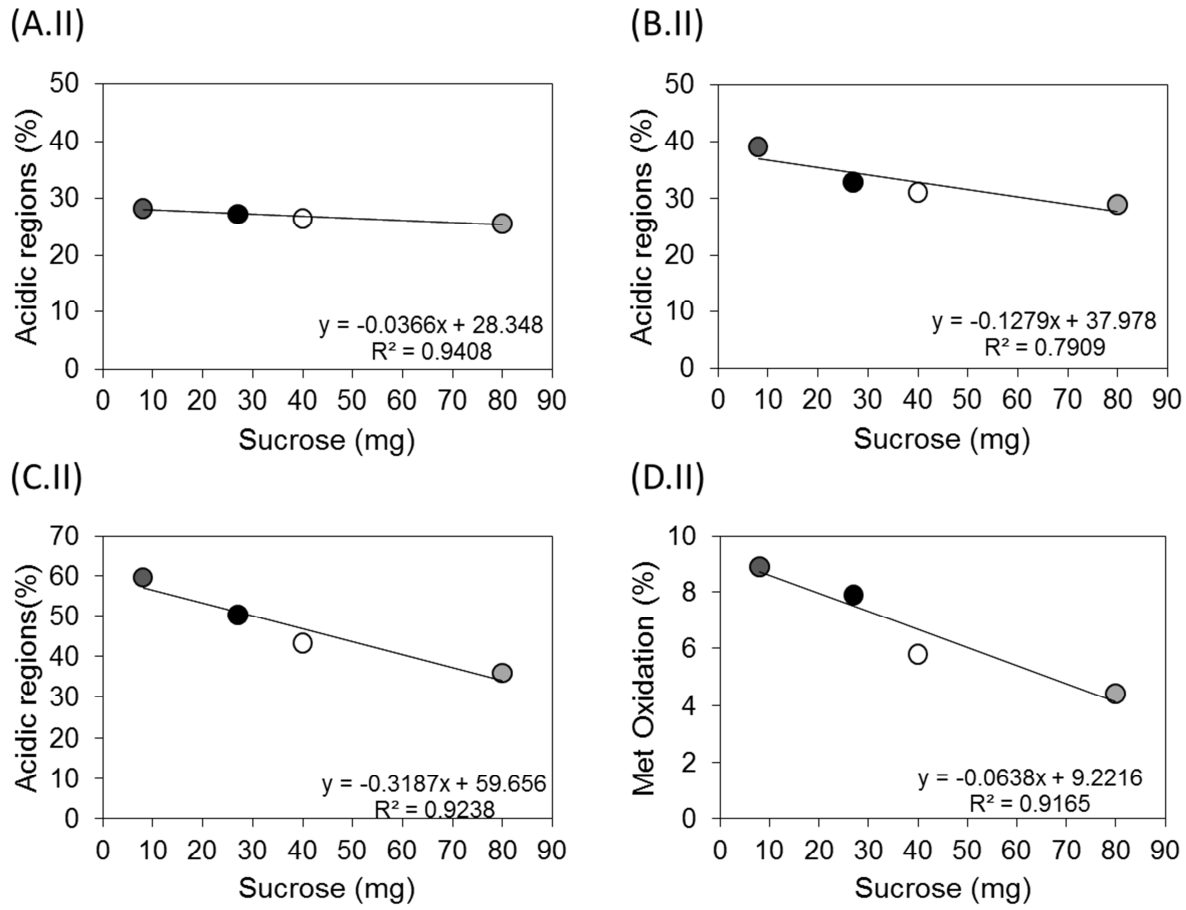

Figure S6 - III. Correlation of percent initial residual moisture in the formulations (III) with acidic regions measured by IEC at 5˚C (A), 25˚C (B) and 40˚C (C) and Met oxidation (HC Met258 + HC Met434) at 40˚C (D) after 960 days.

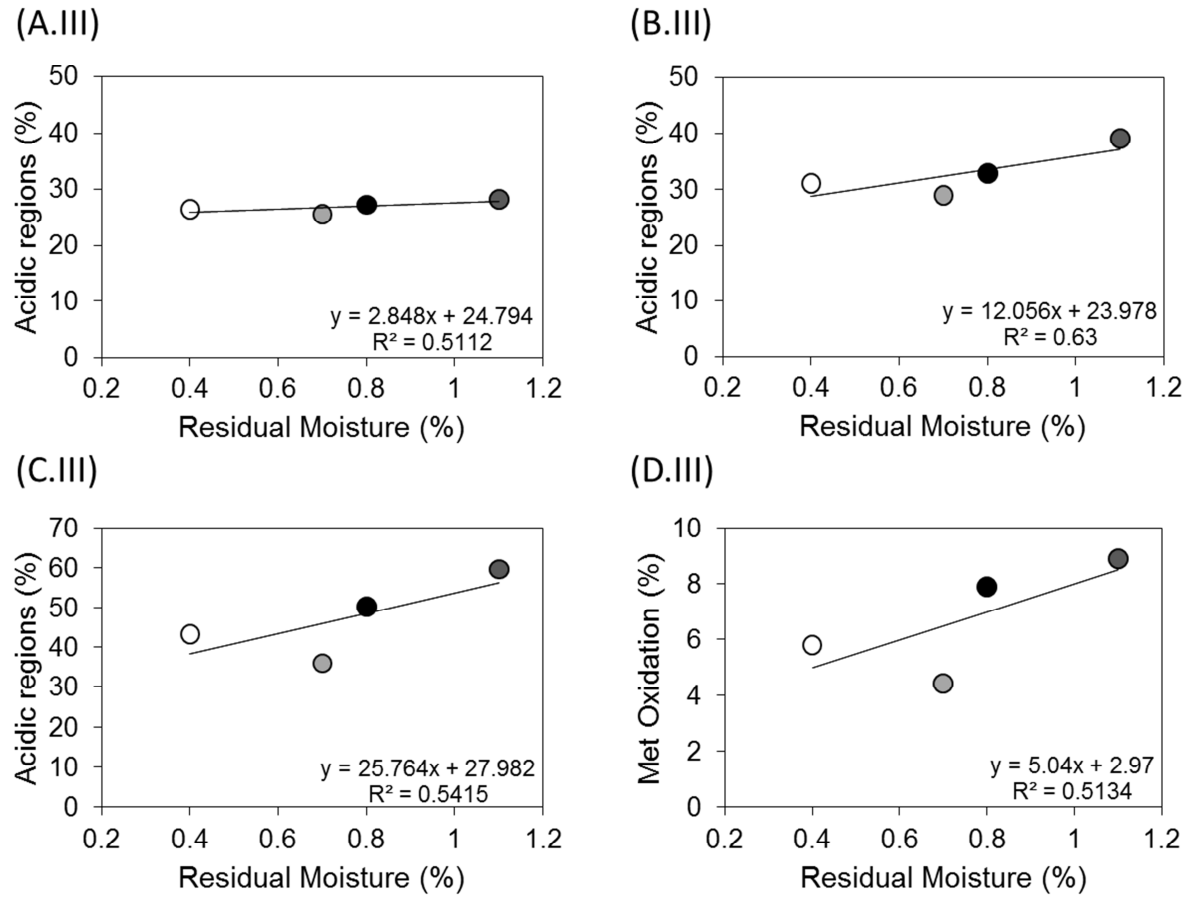

Figure S6 - IV. Correlation of glass transition temperature (Tg) (IV) with acidic regions measured by IEC at 5˚C (A), 25˚C (B) and 40˚C (C) and Met oxidation (HC Met258 + HC Met434) at 40˚C (D) after 960 days.

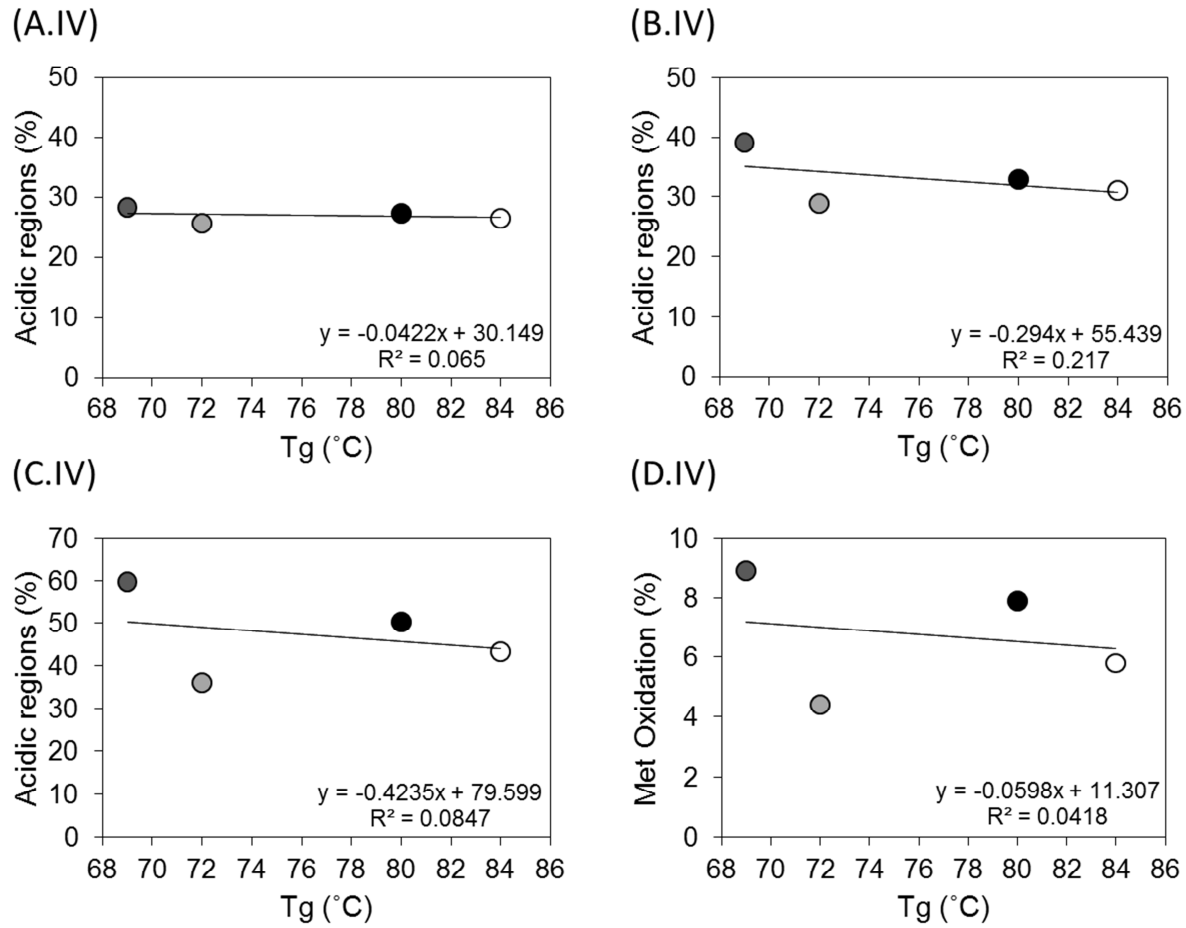

Figure S7. Correlation of deuterium uptake (% Dmax) with percent main peak (1) and basic regions (2) measured by IEC for mAb1 formulations stored at 5 ˚C (A), 25˚C (B) and 40˚C (C) for 960 days. See Figure 5 (main text) for similar correlations with acidic regions.

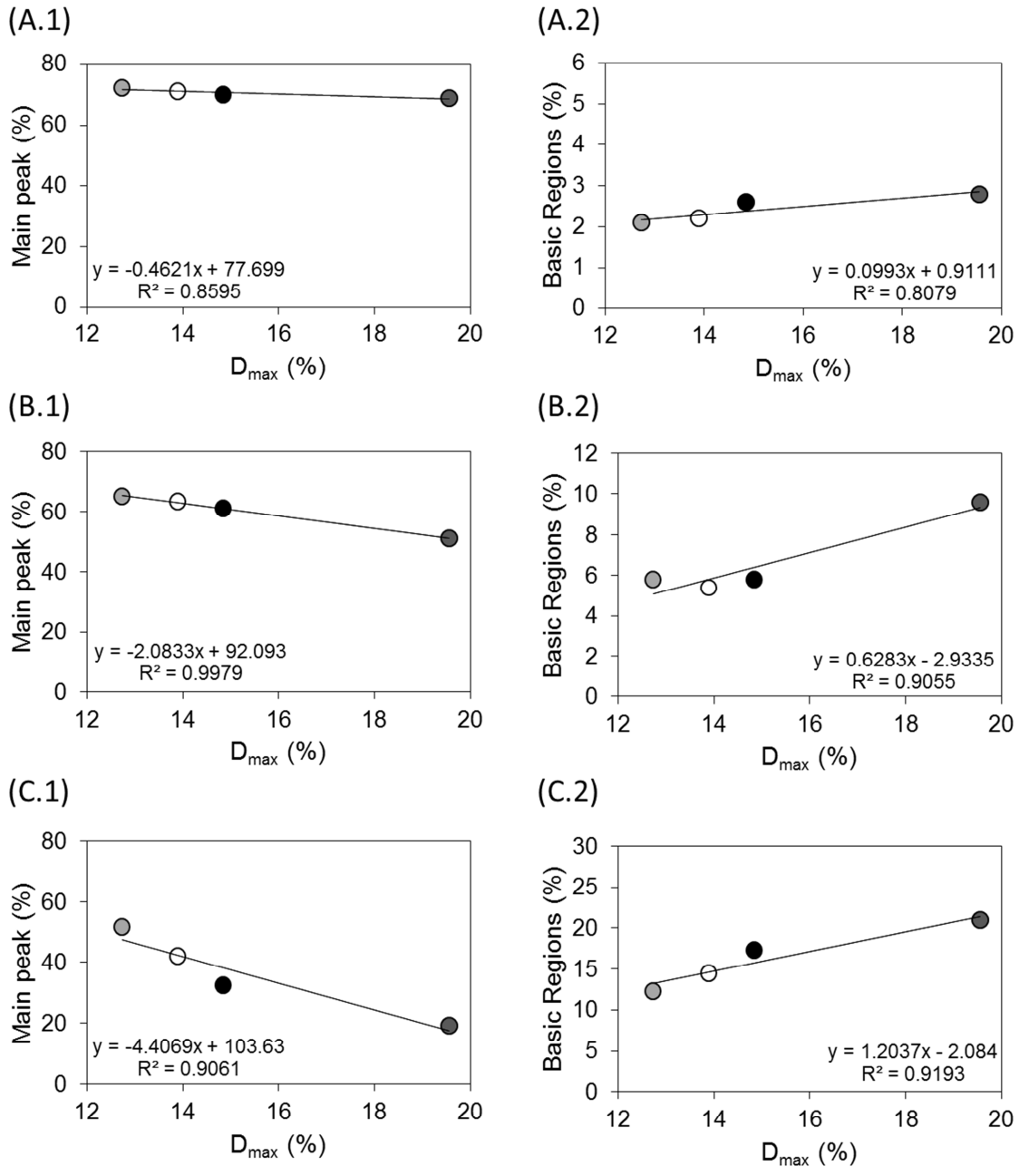

Figure S8. Time course of deuterium uptake for light chain (A) and heavy chain (B) from lyophilized mAb1 formulations. Data were fitted to a one-phase exponential model using GraphPad Prism software version 6 (San Diego, CA) (n = 3, ±SE).