SUPPORTING INFORMATION

Table S1. Parameters obtained from the correlation of t = 0 ssHDX-MS and FTIR results with the percent loss Mb at t = 180 days.

Figure S1: Amino acid sequence of Mb showing secondary structure elements with cylinders representing the α-helices. Solid lines indicate the 13 pepsin digest fragments analyzed in this study with a total sequence coverage of 100% .

Figure S2: Kinetics of deuterium uptake for the pepsin digest fragments from MbA (closed circle), MbB (closed triangle), MbC (open circle), MbD (open square) and MbE (closed square). Plots of the time course of deuterium exchange were fitted to an equation for two phase exponential association (Graph Pad Prism software version 5 (San Diego, CA)) (n = 3, ± SE).

Figure S3: Peak width broadening at 20% peak height for 8 non redundant pepsin digest fragments from Mb in MbA (closed circle), MbB (closed triangle), MbC (open circle), MbD (open square) and MbE (closed square) (n = 3, ± SE).

Figure S4: Peak width against percentage deuterium uptake for 8 non redundant pepsin digest fragments from Mb in MbA (closed circle), MbB (closed triangle), MbC (open circle), MbD (open square), MbE (closed square); (X) represents the peak width for undeuterated peptides (n = 3, ± SE).

Figure S5: Comparison of the Z-average diameter values for prelyophilized and lyophilized Mb samples.

Figure S6: Correlation of Mb aggregation during long-term storage (at t = 180 days) with t = 0 ssHDX-MS (**A**) and FTIR (**B**) analysis. The percent loss of Mb monomer after 180 days of

storage at 25 ˚C and 40 ˚C as a function of percent deuterium uptake (**A1** and **A2**), $N_{fast}/(N_{fast}+N_{slow})$ (**A3** and **A4**), band intensity for α-helix (**B1** and **B2**) and band position for α-helix (**B3** and **B4**) in formulations, MbA (closed circle), MbB (closed triangle), MbC (open circle), MbD (open square) and MbE (closed square) (n = 3, ± SE). The data were subjected to linear regression to obtain slope, intercept and R^2 values (see Table S1).

TABLES

Table S1. Parameters obtained from the correlation of t = 0 ssHDX-MS and FTIR results with the percent loss Mb at t = 180 days.

Figure No. [a]	Parameters [b]		R^2
	Slope	Intercept	
S6A1	0.31 ± 0.08	-1.73 ± 1.95	0.8415
S6A2	0.79 ± 0.16	-4.84 ± 4.02	0.8912
S6A3	-31.26 ± 6.06	25.19 ± 3.95	0.8985
S6A4	-81.53 ± 5.13	65.02 ± 3.34	0.9883
S6B1	1735.32 ± 1096.97	19.73 ± 9.34	0.4549
S6B2	5153.19 ± 2189.41	56.04 ± 18.64	0.6487
S6B3	-1.46 ± 1.62	2381.54 ± 2686.38	0.2036
S6B4	-5.27 ± 3.40	8721.92 ± 5628.01	0.4393

[a] Refer Figure S6 for storage conditions and variables used.

[b] Parameters ± SE determined by linear fit of values obtained from FTIR and HDX-MS experiments at t=0 against Mb aggregation at t=180 days .

FIGURES

Figure S1

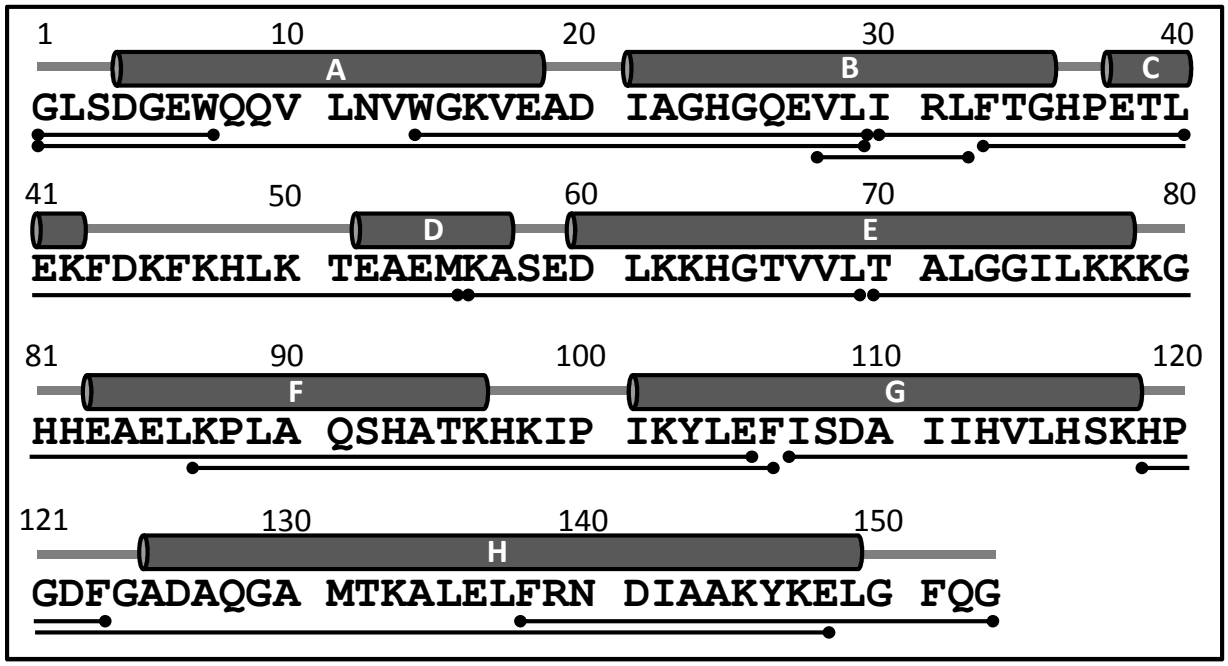
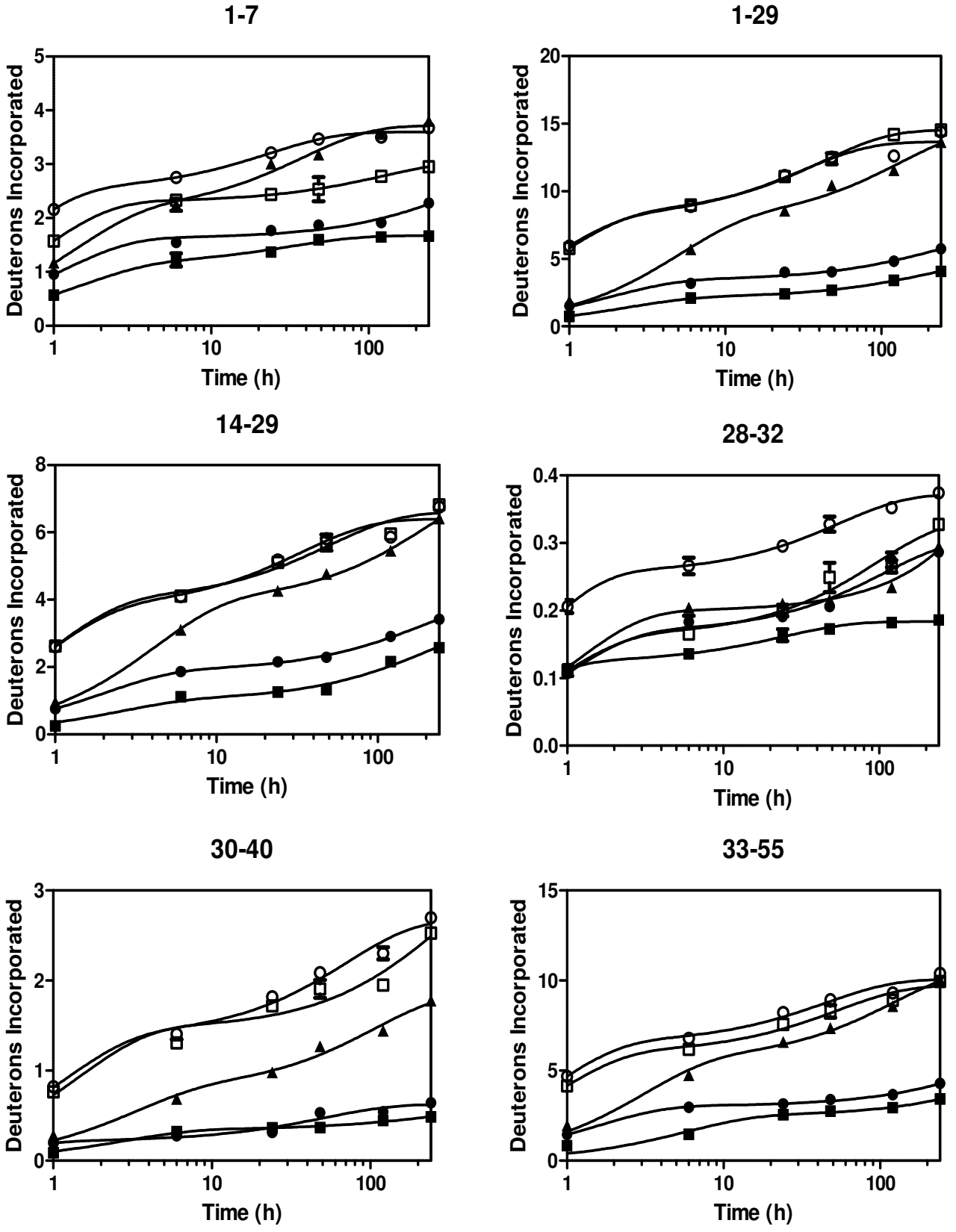
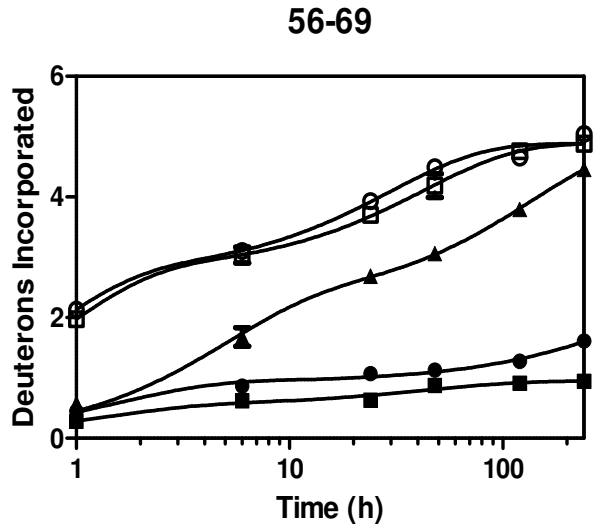
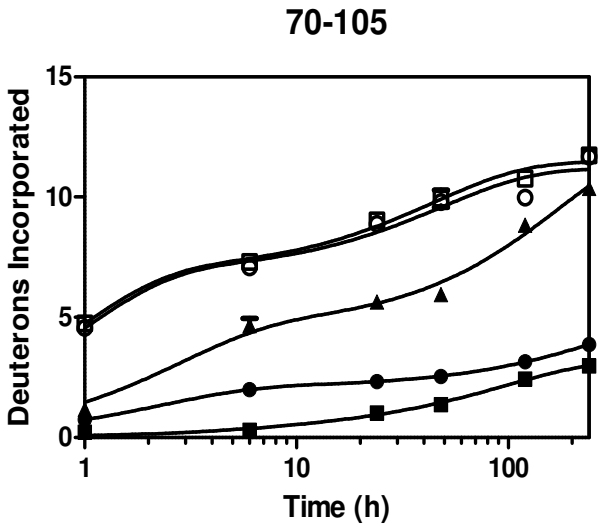


Figure S1: Amino acid sequence of Mb showing secondary structure elements with cylinders representing the α-helices. Solid lines indicate the 13 pepsin digest fragments analyzed in this study with a total sequence coverage of 100% .

Figure S2




56-69
Deuterons Incorporated
Time (h)
0
2
4
6
1
10
100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Deuterons Incorporated
Time (h)
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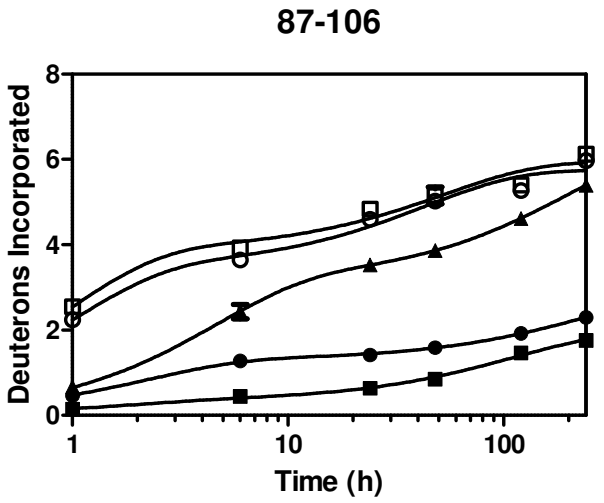
10
15
1
10
100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Deuterons Incorporated
Time (h)
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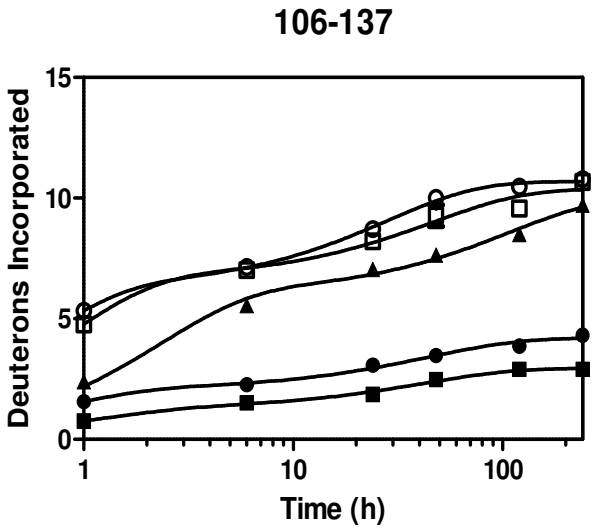
4
6
8
1
10
100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Deuterons Incorporated
Time (h)
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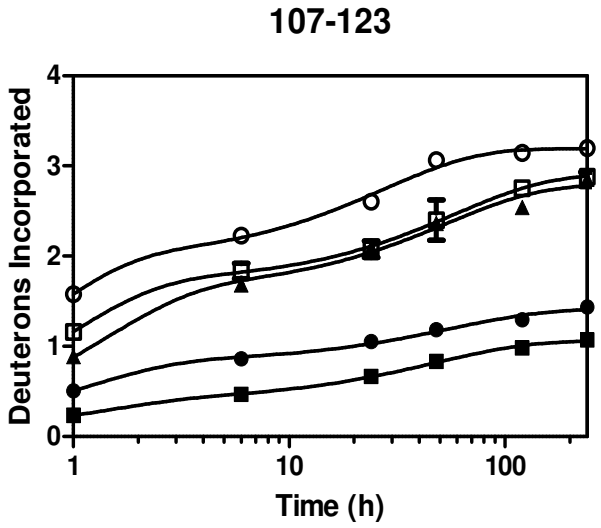
10
15
1
10
100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Deuterons Incorporated
Time (h)
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3
4
1
10
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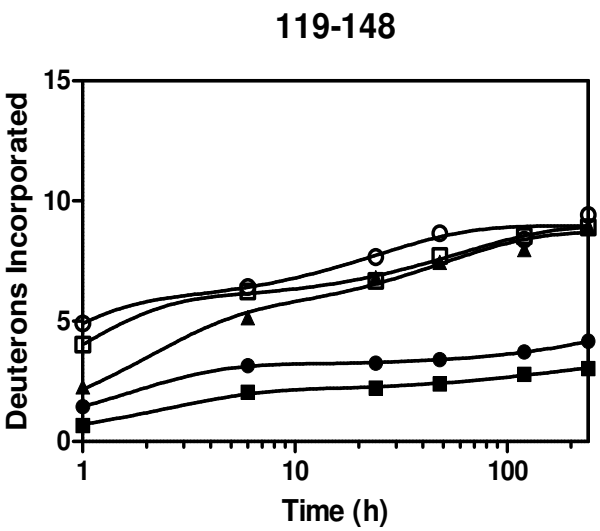

119-148
Deuterons Incorporated
Time (h)
0
5
10
15
1
10
100

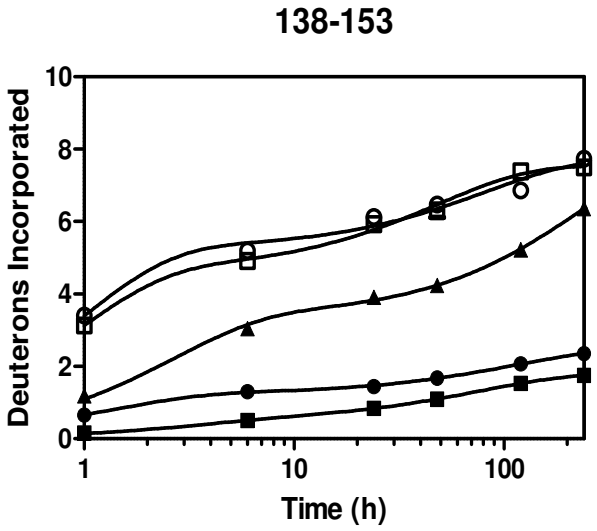
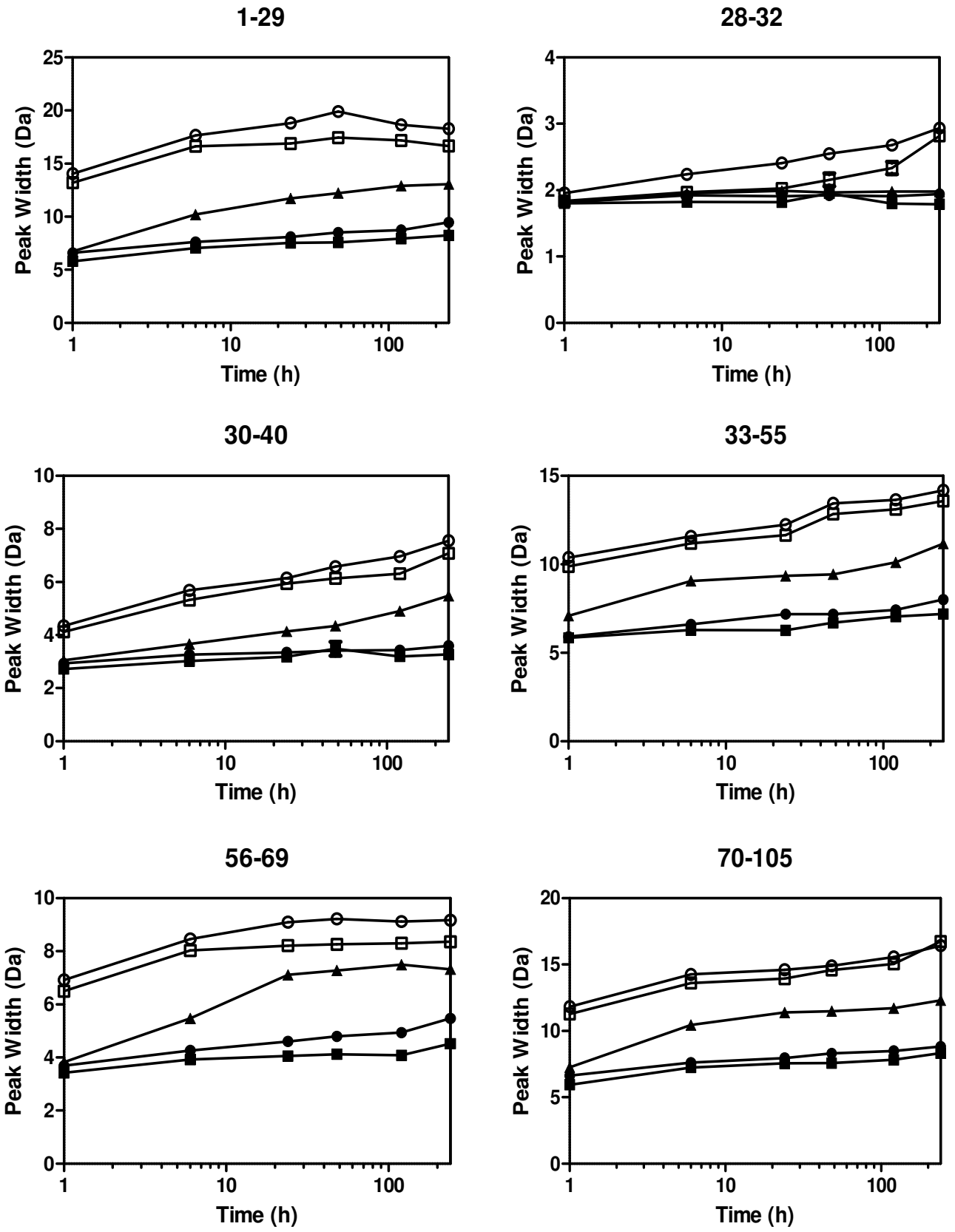


Figure S2: Kinetics of deuterium uptake for the pepsin digest fragments from MbA (closed circle), MbB (closed triangle), MbC (open circle), MbD (open square) and MbE (closed square). Plots of the time course of deuterium exchange were fitted to an equation for two phase exponential association (Graph Pad Prism software version 5 (San Diego, CA)) (n = 3, ± SE).

Figure S3



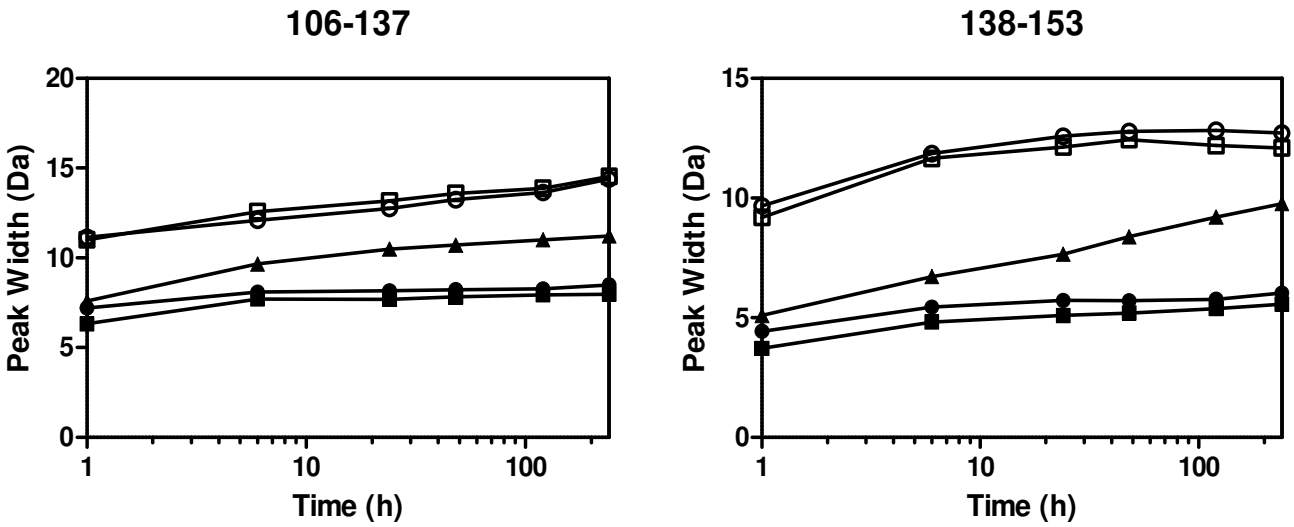
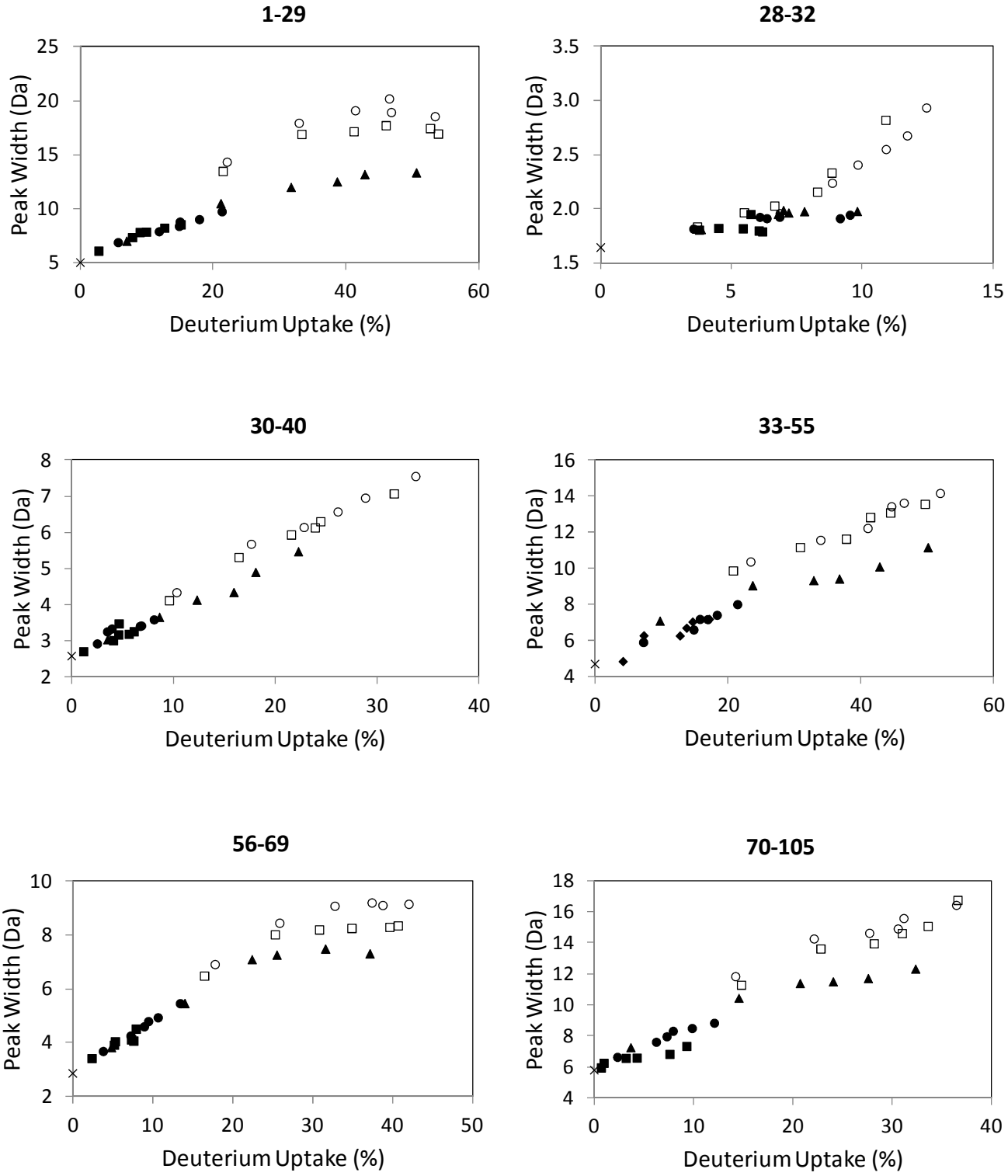


Figure S3: Peak width broadening at 20% peak height for 8 non redundant pepsin digest fragments from Mb in MbA (closed circle), MbB (closed triangle), MbC (open circle), MbD (open square) and MbE (closed square) (n = 3, ± SE).

Figure S4


1-29
Peak Width (Da)
Deuterium Uptake (%)
28-32
Peak Width (Da)
Deuterium Uptake (%)
30-40
Peak Width (Da)
Deuterium Uptake (%)
33-55
Peak Width (Da)
Deuterium Uptake (%)
56-69
Peak Width (Da)
Deuterium Uptake (%)
70-105
Peak Width (Da)
Deuterium Uptake (%)

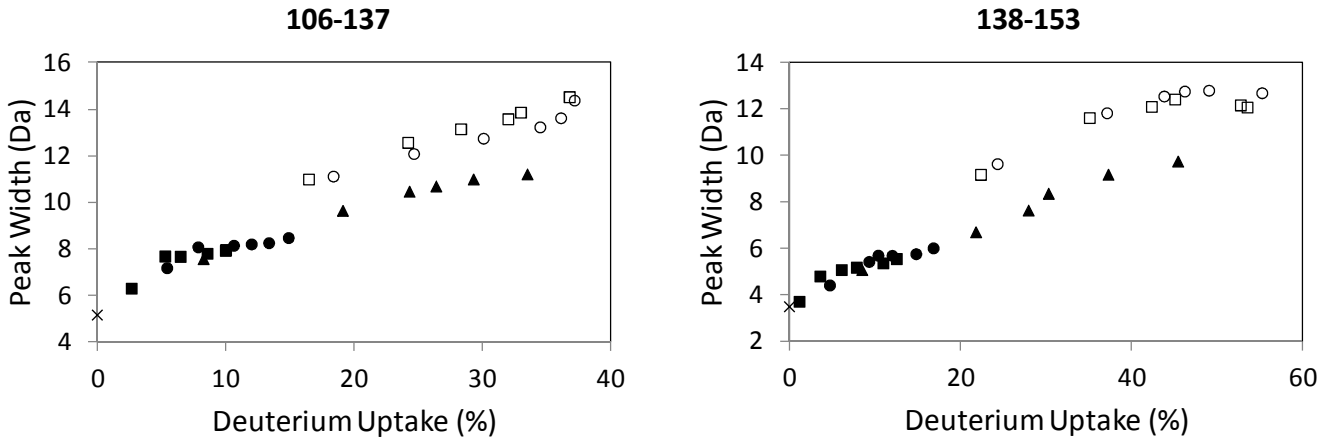


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Figure S5

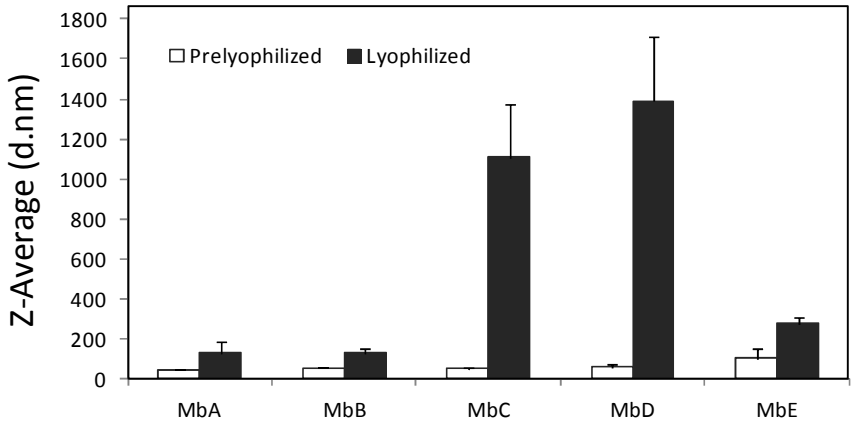
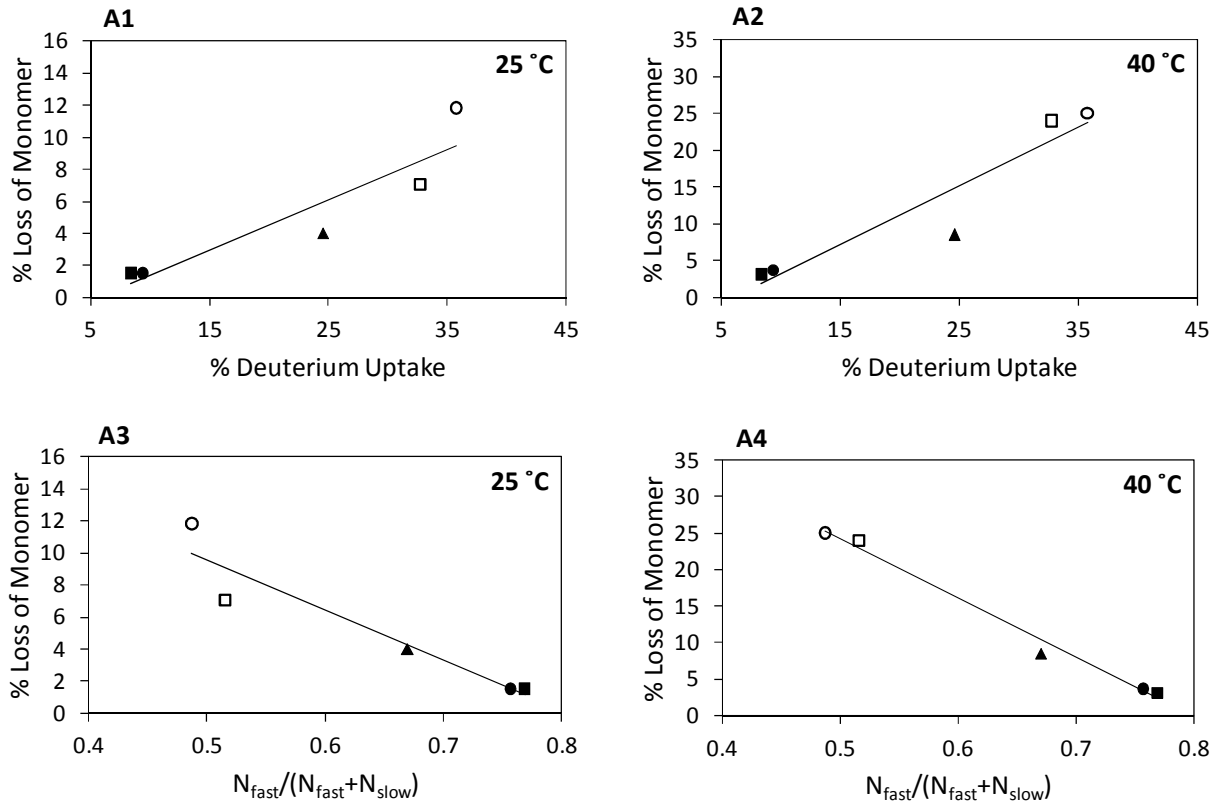


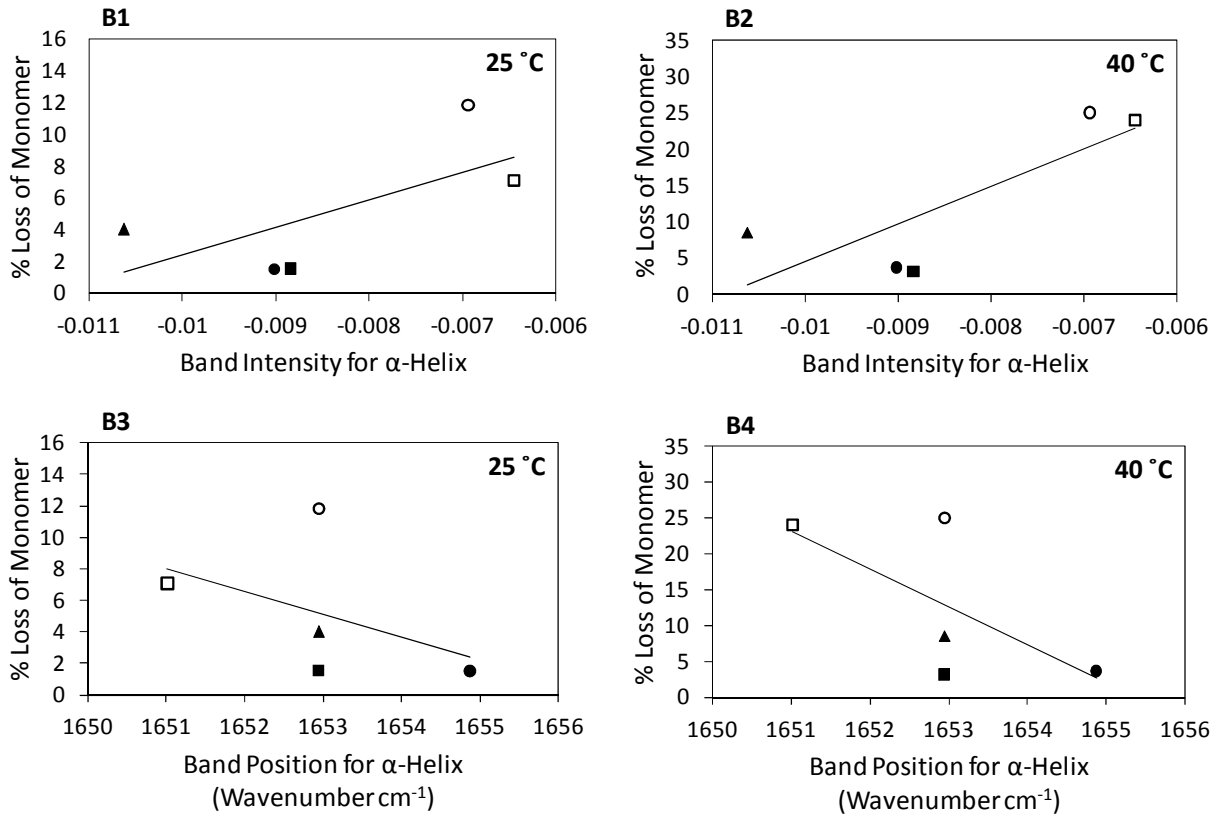
Figure S5: Comparison of the Z-average diameter values for prelyophilized and lyophilized Mb samples.

Figure S6

S6A



S6B



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