Supporting Information for

"Structural Insights into the Pre-amyloid Tetramer of β-2-microglobulin from Covalent Labeling and Mass Spectrometry"

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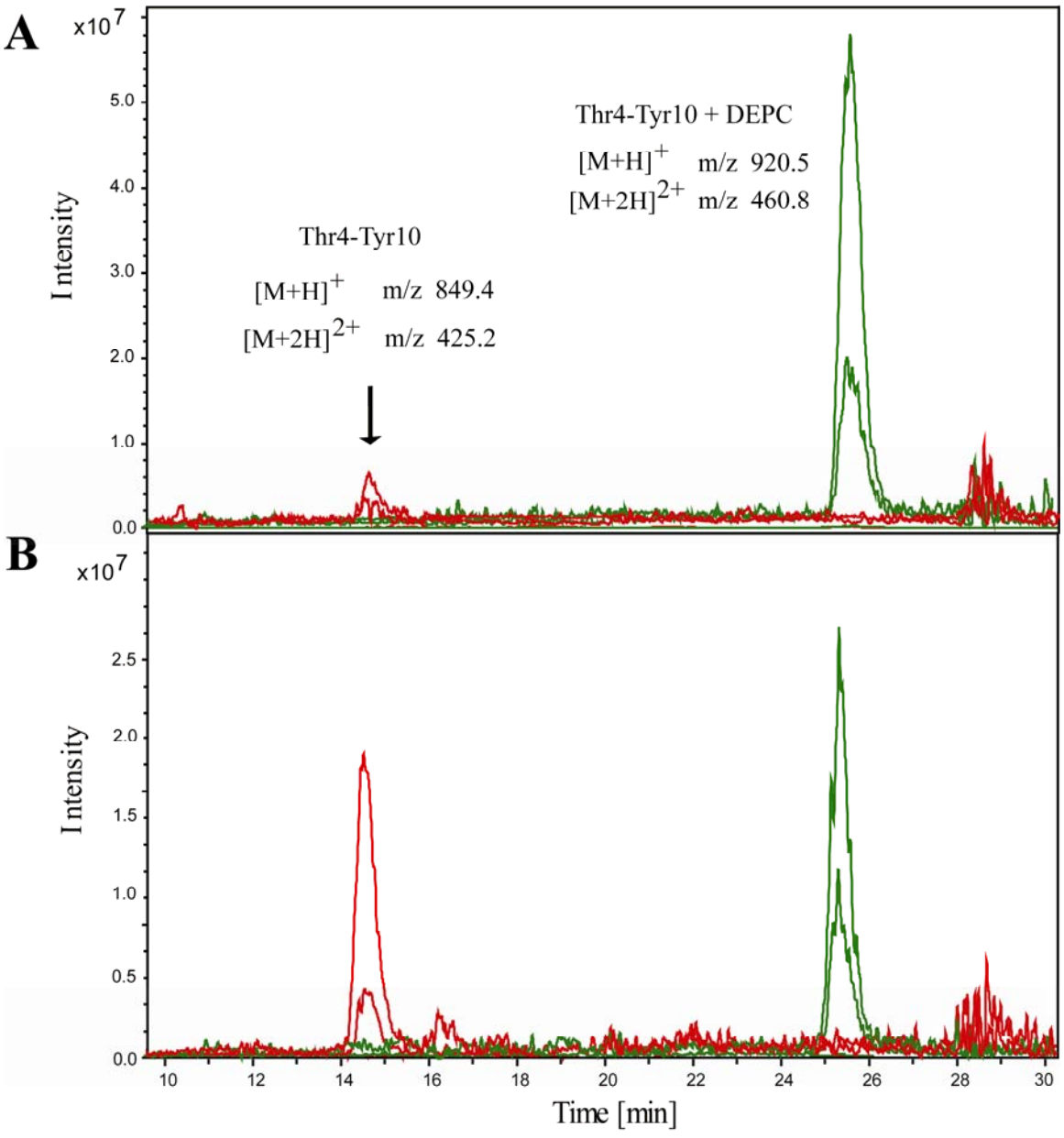


Figure S1: Extracted ion chromatograms (EIC) of the singly (m/z 849.4) and doubly charged (m/z 425.2) unmodified (red) and DEPC modified (green) forms of Thr from the proteolytic fragment Thr4-Tyr10 (m/z 920.5 and 460.8). (A) 2 hours and (B) 2 days after addition of Cu(II). The ion intensities of the unmodified and modified peptide ions were determined from these EIC plots. The peaks at ~14.5 min and ~25 min are the unmodified and Thr4-modified forms,

respectively. The changes in ion intensities 2 hours and 2 days after the addition of Cu(II) show the decrease in the DEPC modification percentage of Thr4 upon formation of the tetramer.

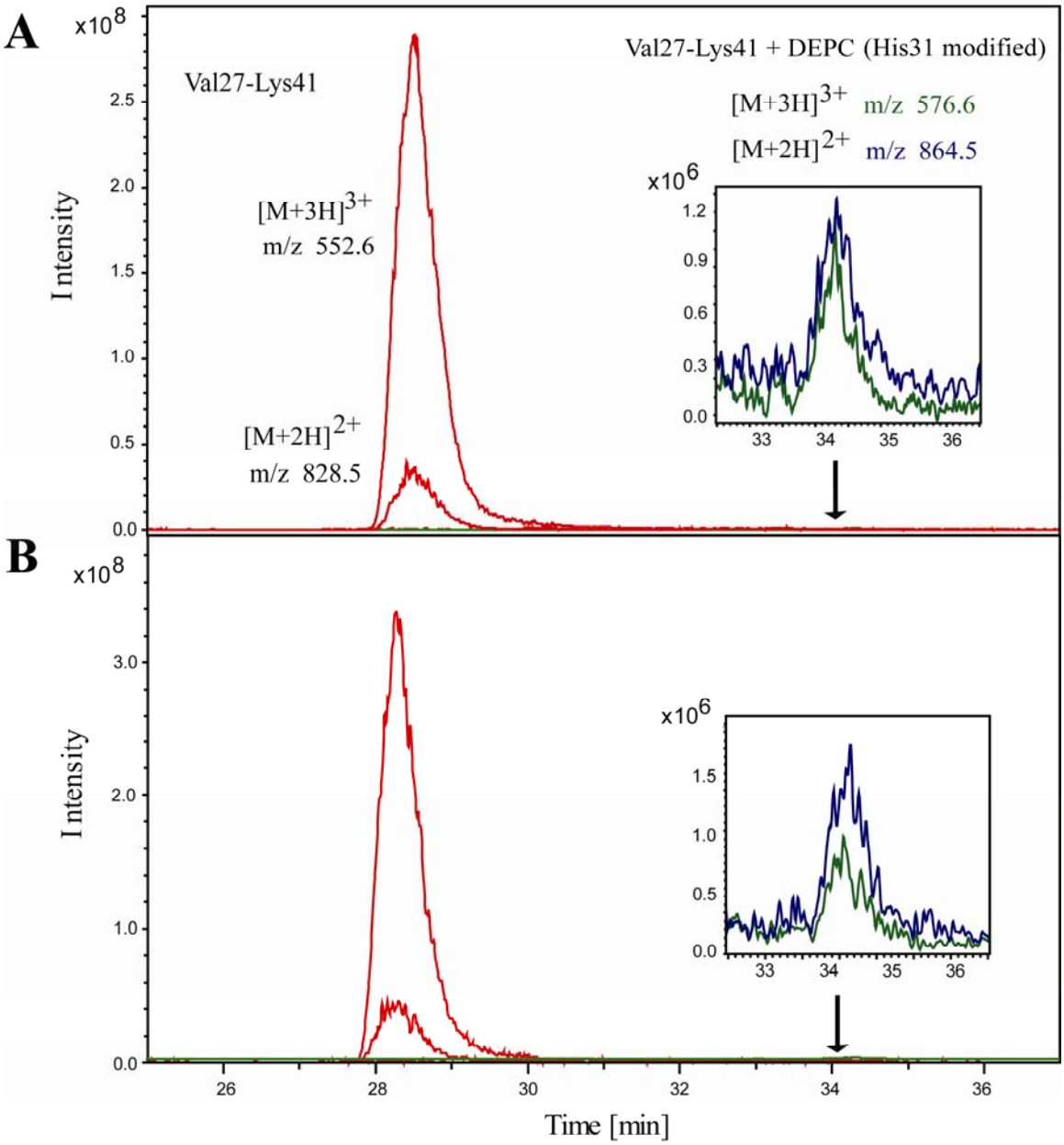


Figure S2: Extracted ion chromatograms (EIC) of the doubly (m/z 828.5) and triply charged (m/z 552.6) unmodified (red) and DEPC modified (blue and green) forms of His31 from the proteolytic fragment Val27-Lys41 (m/z 864.5 and 576.6) (A) 2 hours and (B) 2 days after addition of Cu(II). The ion intensities of the unmodified and modified peptide ions were determined from these EIC plots. The peaks at ~28 min and ~34 min are the unmodified and His31-modified forms, respectively. The ion intensities 2 hours and 2 days after the addition of Cu(II) show that there is little to no change in the percent DEPC modification of His31 upon formation of the tetramer.

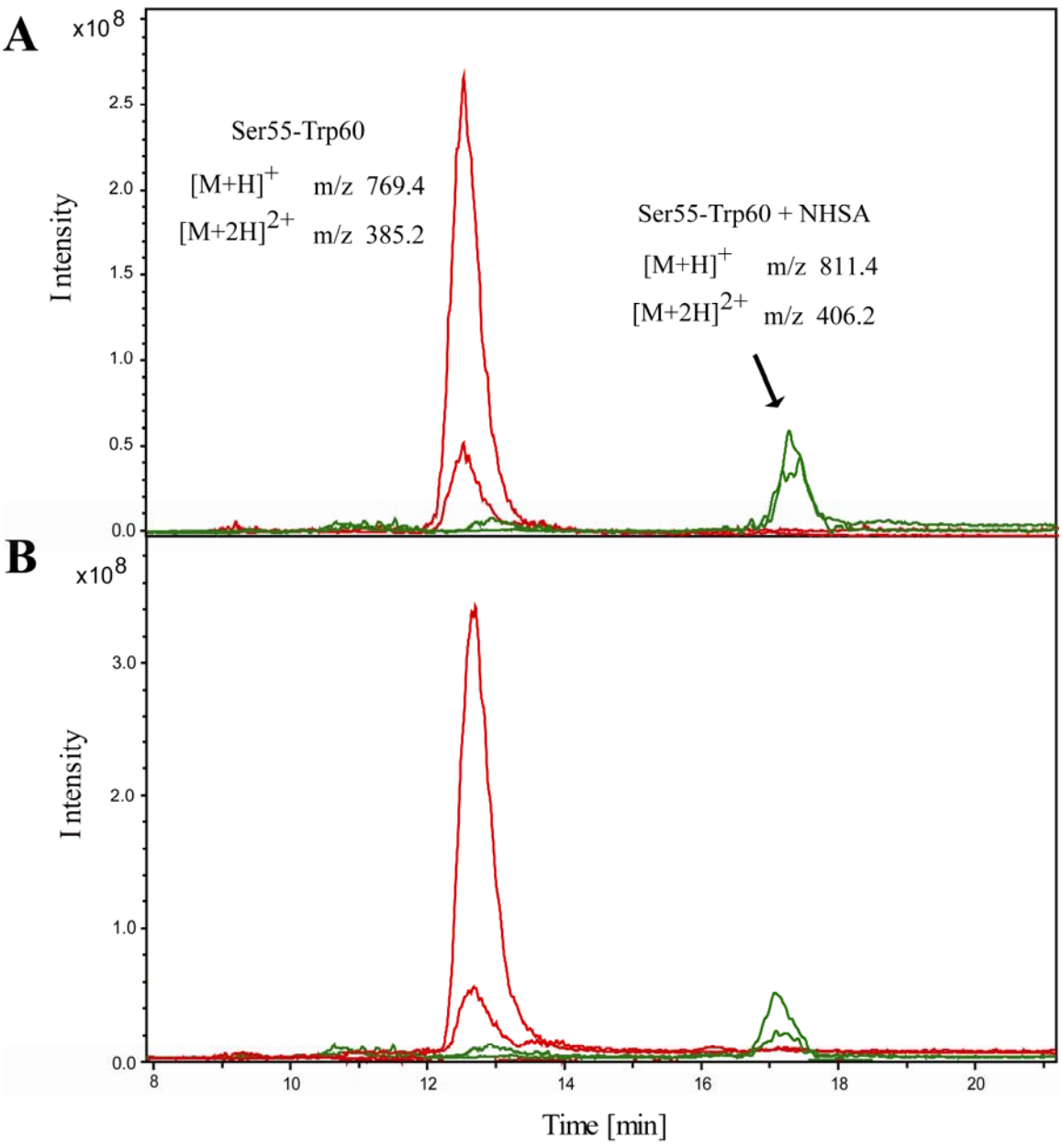


Figure S3: Extracted ion chromatograms (EIC) of the singly (m/z 769.4) and doubly charged (m/z 385.2) unmodified (red) and modified (green) forms of Lys58 from the proteolytic fragment Ser55-Trp60 (m/z 811.4 and 406.2) (A) 2 hours and (B) 2 days after addition of Cu(II). The ion intensities of the unmodified and modified peptide ions were determined from these EIC plots. The peaks at ~12.5 min and ~17 min are the unmodified and Lys58-modified forms, respectively. The changes in ion intensities 2 hours and 2 days after the addition of Cu(II) show the decrease in the NHSA modification percentage of Lys58 upon formation of the tetramer.

Table S1: Extent of DEPC modification of a control β2m solution (i.e. no Cu(II)) at time zero, 2 min, 2 hours, 1 day, and 2 days after beginning the incubation.

Residue	**Incubation Time**				
	0 min	**2 min**	**2 hrs**	**1 day**	**2 days**
N-terminus	99 ± 5	97 ± 7	99 ± 4	98 ± 5	98 ± 6
Thr4	86 ± 1	89 ± 5	86 ± 7	87 ± 2	87 ± 6
Lys6	7.5 ± 0.5	7.2 ± 0.6	7.6 ± 0.5	7.1 ± 0.6	7.4 ± 0.4
His13	45 ± 2	43 ± 4	40 ± 4	43 ± 3	42 ± 2
Lys19	13 ± 2	11 ± 2	13 ± 1	12 ± 2	10 ± 1
Tyr26	0.7 ± 0.1	0.76 ± 0.09	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
Ser28	0.24 ± 0.02	0.26 ± 0.03	0.25 ± 0.02	0.28 ± 0.02	0.27 ± 0.03
His31	1.6 ± 0.3	1.5 ± 0.2	1.7 ± 0.2	1.8 ± 0.1	1.5 ± 0.2
Ser33	1.7 ± 0.1	1.6 ± 0.1	1.9 ± 0.2	1.7 ± 0.3	1.5 ± 0.2
Lys41	0.39 ± 0.03	0.34 ± 0.03	0.34 ± 0.03	0.37 ± 0.03	0.35 ± 0.04
His51	61 ± 3	64 ± 3	62 ± 4	61 ± 5	59 ± 4
Lys58	39 ± 3	41 ± 2	41 ± 3	37 ± 3	40 ± 2
Tyr63	6.3 ± 0.5	6.0 ± 0.5	6.9 ± 0.7	6.5 ± 0.6	6.7 ± 0.4
Tyr67	6.3 ± 0.3	6.0 ± 0.5	6.4 ± 0.5	5.9 ± 0.6	6.1 ± 0.5
Lys75	0.31 ± 0.04	0.35 ± 0.06	0.37 ± 0.04	0.35 ± 0.04	0.37 ± 0.05
Ser88	66 ± 3	62 ± 4	68 ± 5	64 ± 4	67 ± 6
Lys94	28 ± 3	30 ± 2	26 ± 3	28 ± 2	27 ± 3

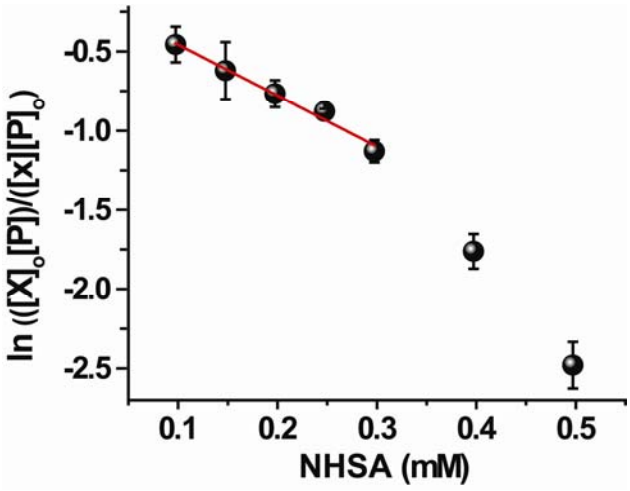


Figure S4: Dose-response plot for Lys41 after reaction with NHSA. The plot is produced from LC-MS data of the proteolytic digests of the modified protein. The $[P]/[P]_o$ ratio is obtained by dividing the peak area for the unmodified fragment by the sum of the peak areas for the modified and unmodified fragments. The difference between the [P] and $[P]_o$ values is used to determine the concentration of NHSA, [X]. Similar dose-response plots are generated for all modified peptides to confirm the maximum reagent concentration (or dose) that can be used without causing structural changes to the protein.

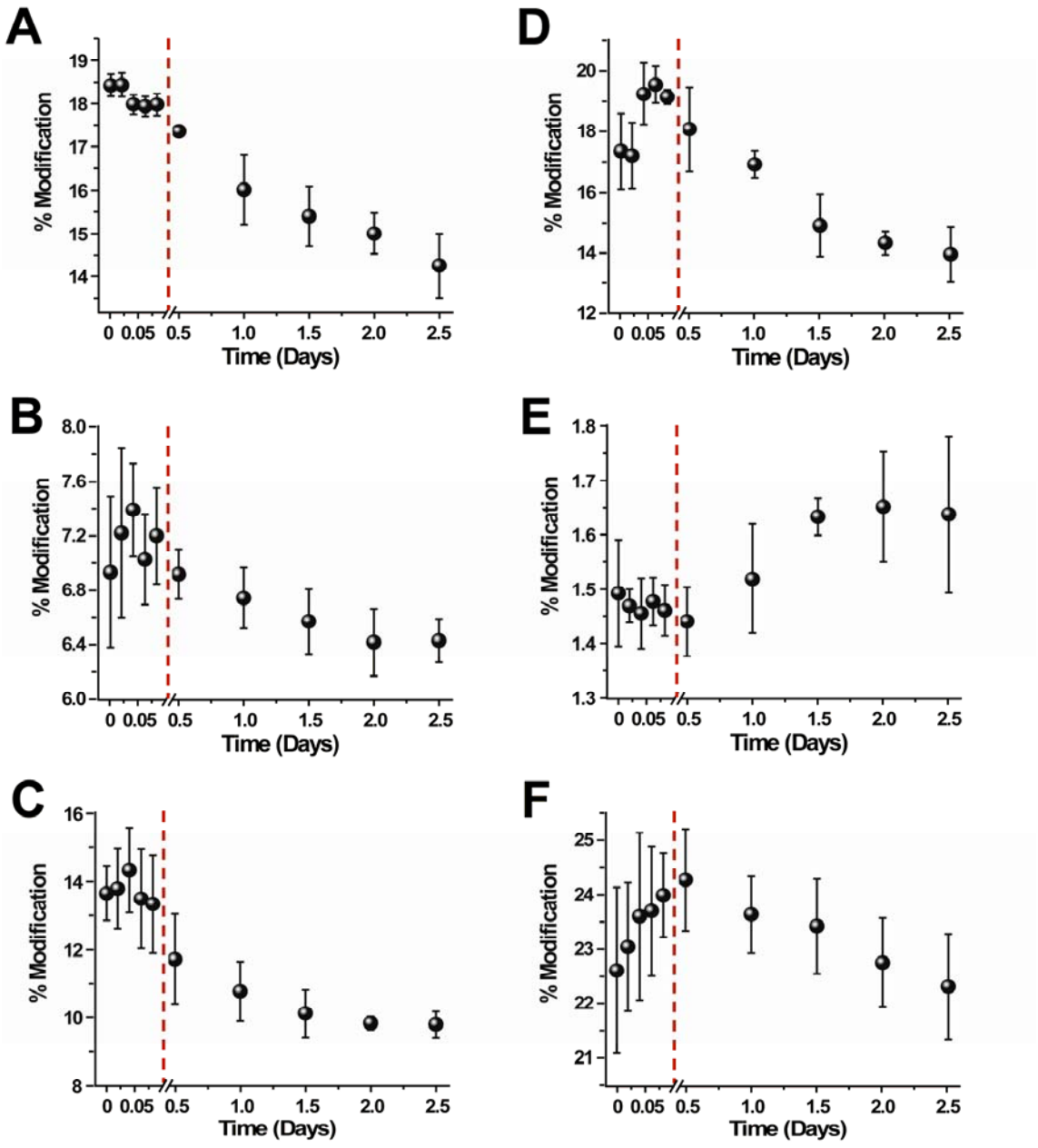
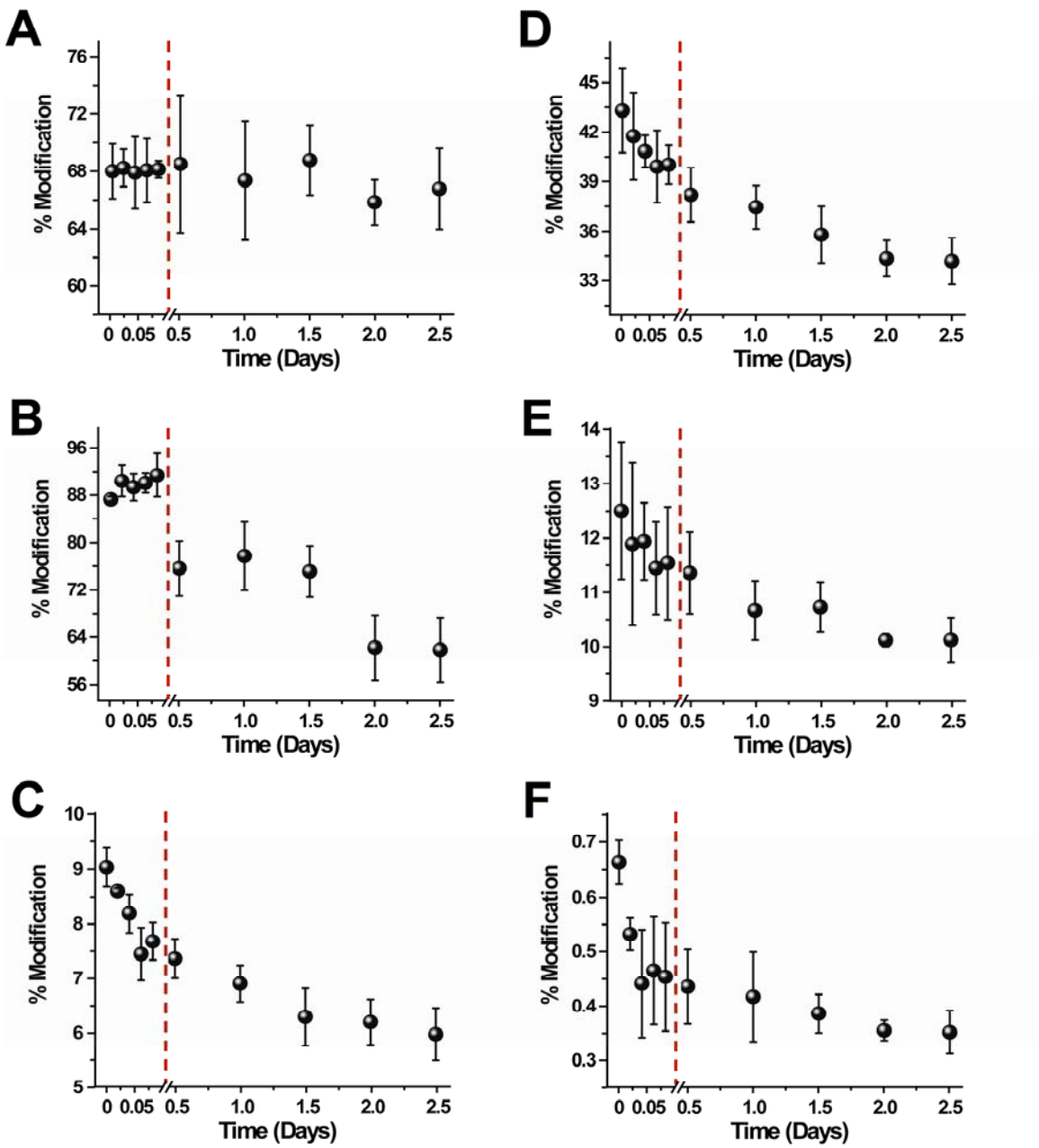
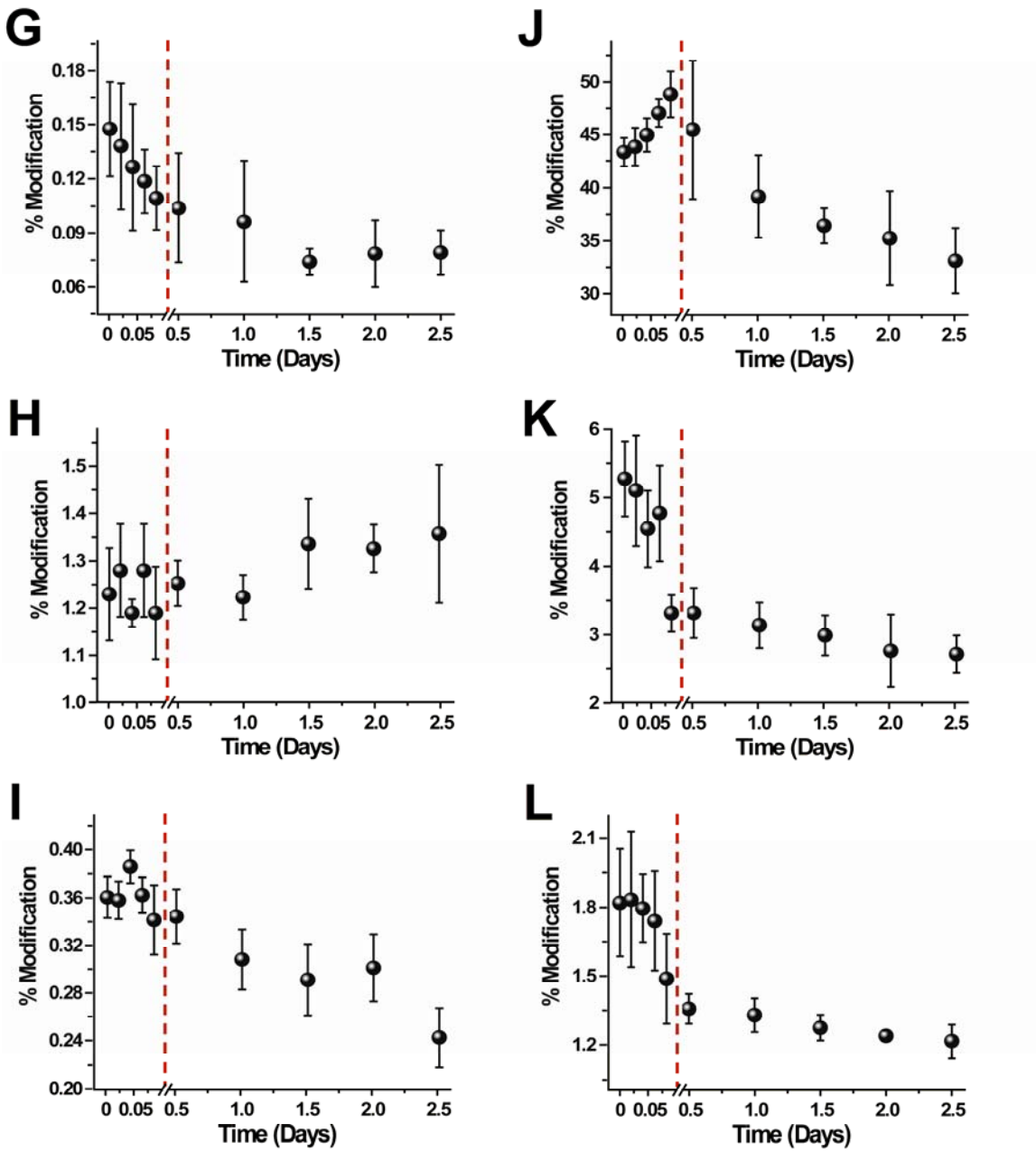


Figure S5: Extent of NHSA modification throughout the course of the tetramer formation reaction. (A) Lys6. (B) Lys19. (C) Lys41. (D) Lys58. (E) Asn83. (F) Lys91.


A
% Modification
76
72
68
64
60
0 0.05 0.5 1.0 1.5 2.0 2.5
Time (Days)
D
% Modification
45
42
39
36
33
0 0.05 0.5 1.0 1.5 2.0 2.5
Time (Days)
B
% Modification
96
88
80
72
64
56
0 0.05 0.5 1.0 1.5 2.0 2.5
Time (Days)
E
% Modification
14
13
12
11
10
9
0 0.05 0.5 1.0 1.5 2.0 2.5
Time (Days)
C
% Modification
10
9
8
7
6
5
0 0.05 0.5 1.0 1.5 2.0 2.5
Time (Days)
F
% Modification
0.7
0.6
0.5
0.4
0.3
0 0.05 0.5 1.0 1.5 2.0 2.5
Time (Days)


G
% Modification
0.18
0.15
0.12
0.09
0.06
0 0.05 0.5 1.0 1.5 2.0 2.5
Time (Days)
J
% Modification
50
45
40
35
30
0 0.05 0.5 1.0 1.5 2.0 2.5
Time (Days)
H
% Modification
1.5
1.4
1.3
1.2
1.1
1.0
0 0.05 0.5 1.0 1.5 2.0 2.5
Time (Days)
K
% Modification
6
5
4
3
2
0 0.05 0.5 1.0 1.5 2.0 2.5
Time (Days)
I
% Modification
0.40
0.36
0.32
0.28
0.24
0.20
0 0.05 0.5 1.0 1.5 2.0 2.5
Time (Days)
L
% Modification
2.1
1.8
1.5
1.2
0 0.05 0.5 1.0 1.5 2.0 2.5
Time (Days)

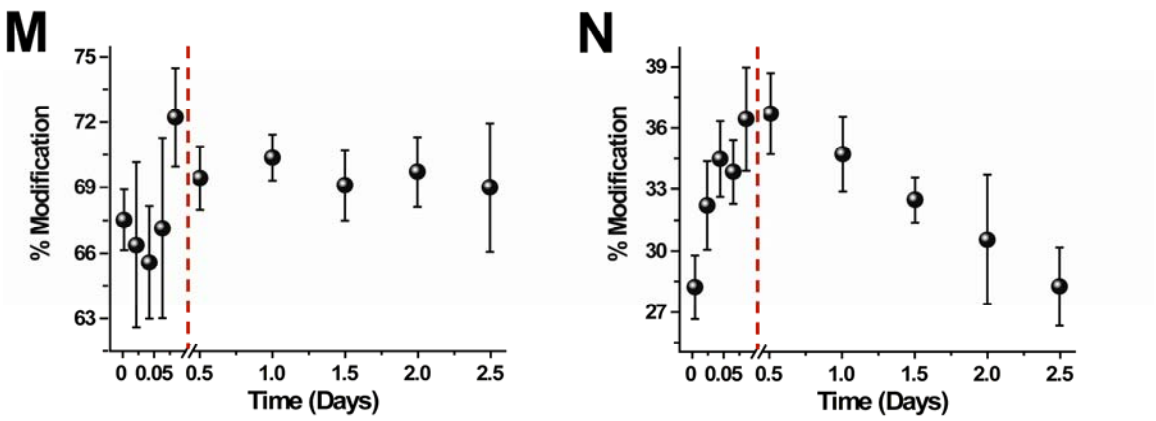


Figure S6: Extent of DEPC modification throughout the course of the tetramer formation reaction. (A) N-terminus. (B) Thr4. (C) Lys6. (D) His13. (E) Lys19. (F) Tyr26. (G) Ser28. (H) Ser33. (I) Lys41. (J) Serr57/Lys58. (K) Tyr63. (L) Tyr67. (M) Ser88. (N) Lys94.

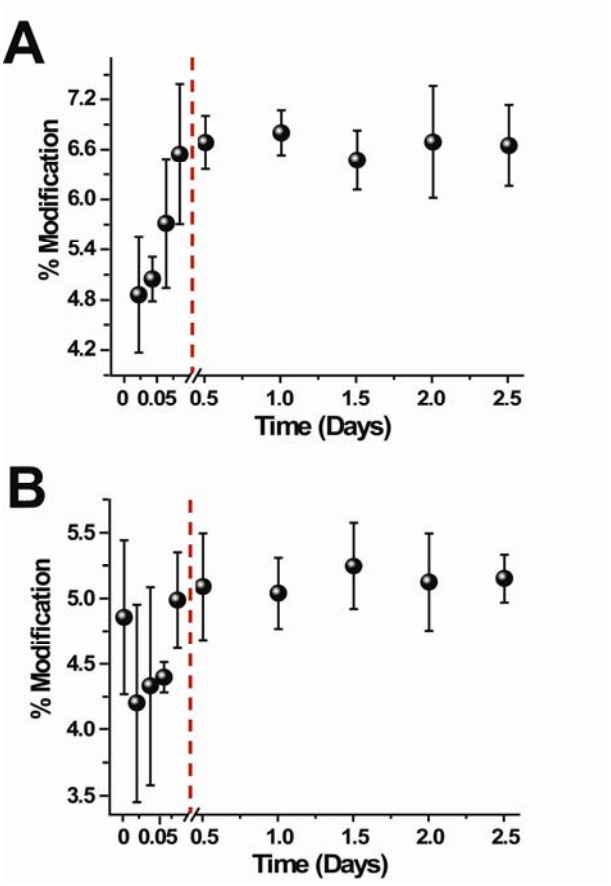


Figure S7: Extent of BD modification throughout the course of the tetramer formation reaction. (A) Arg45. (B) Arg97.