Oxidative Degradation of Sequence-Defined Peptoid Oligomers

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SUPPLEMENTARY INFORMATION

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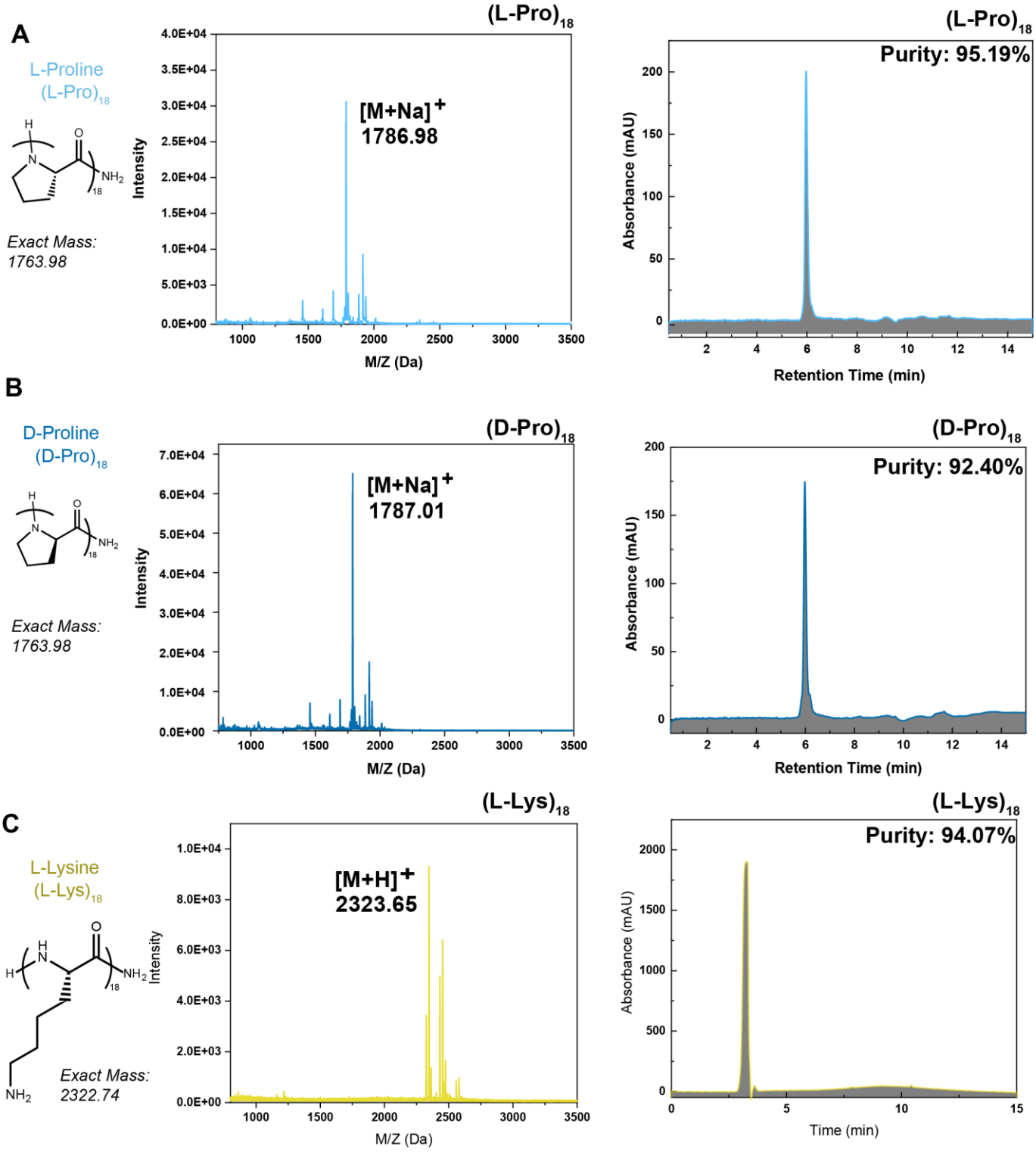
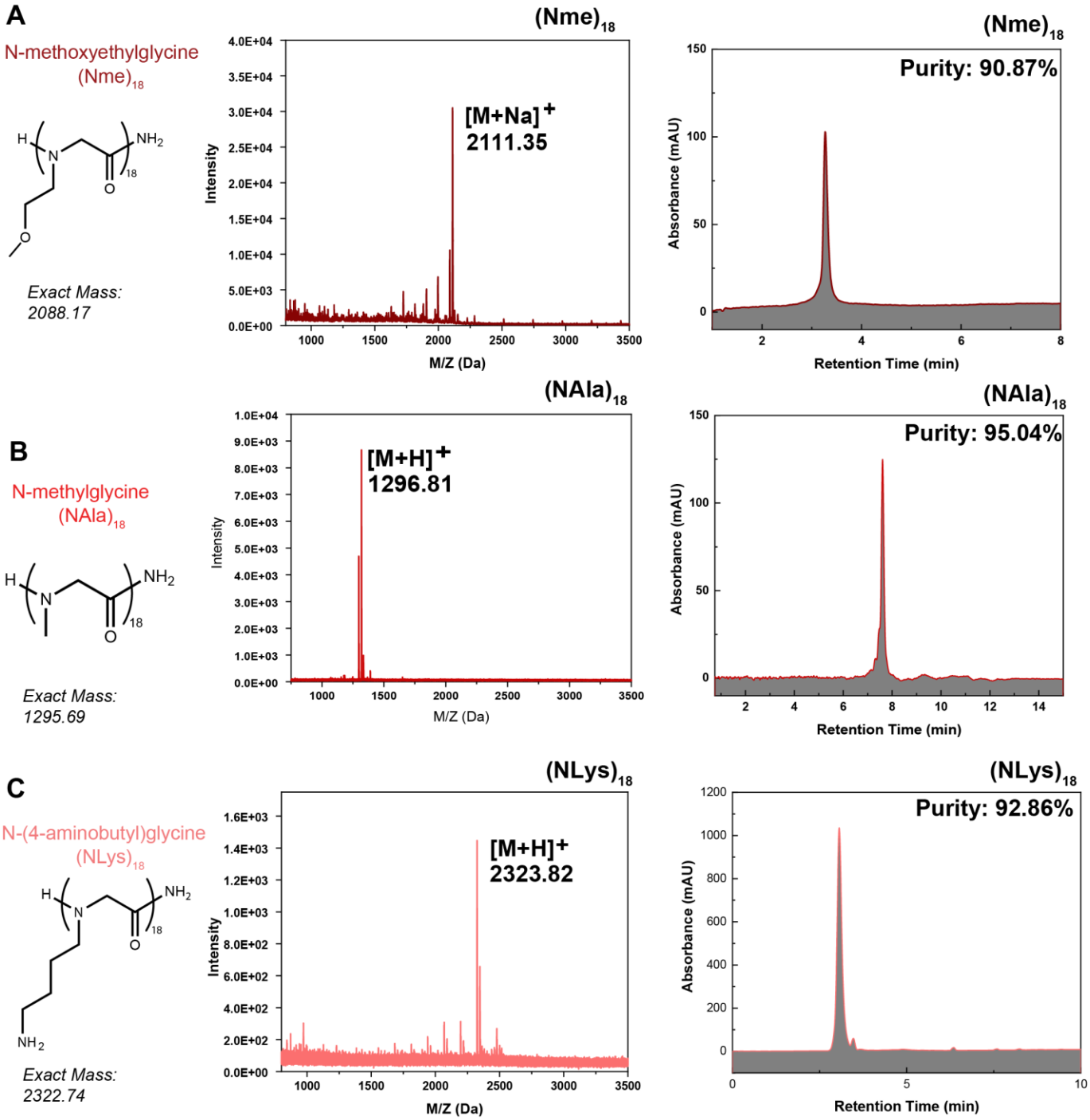
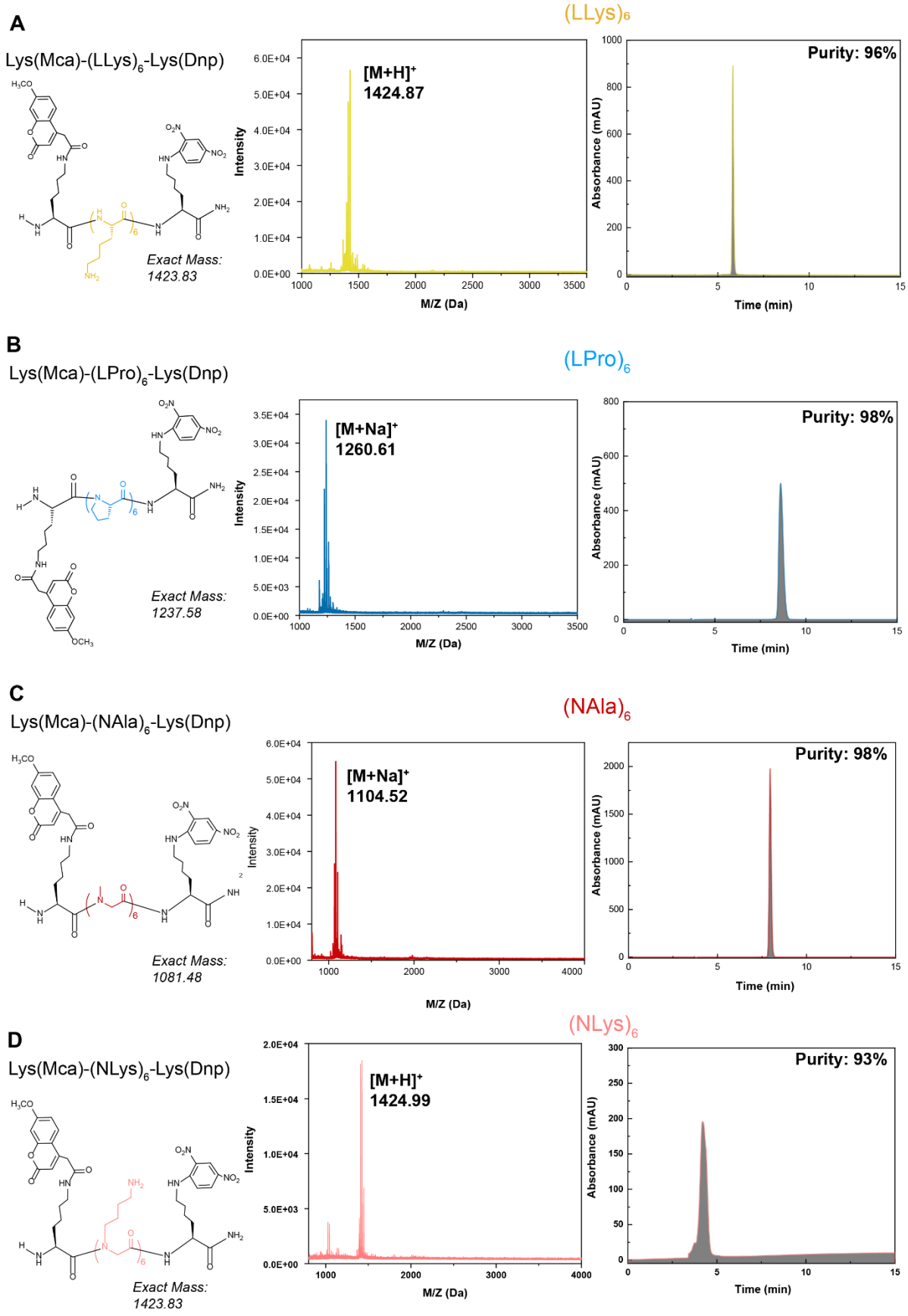


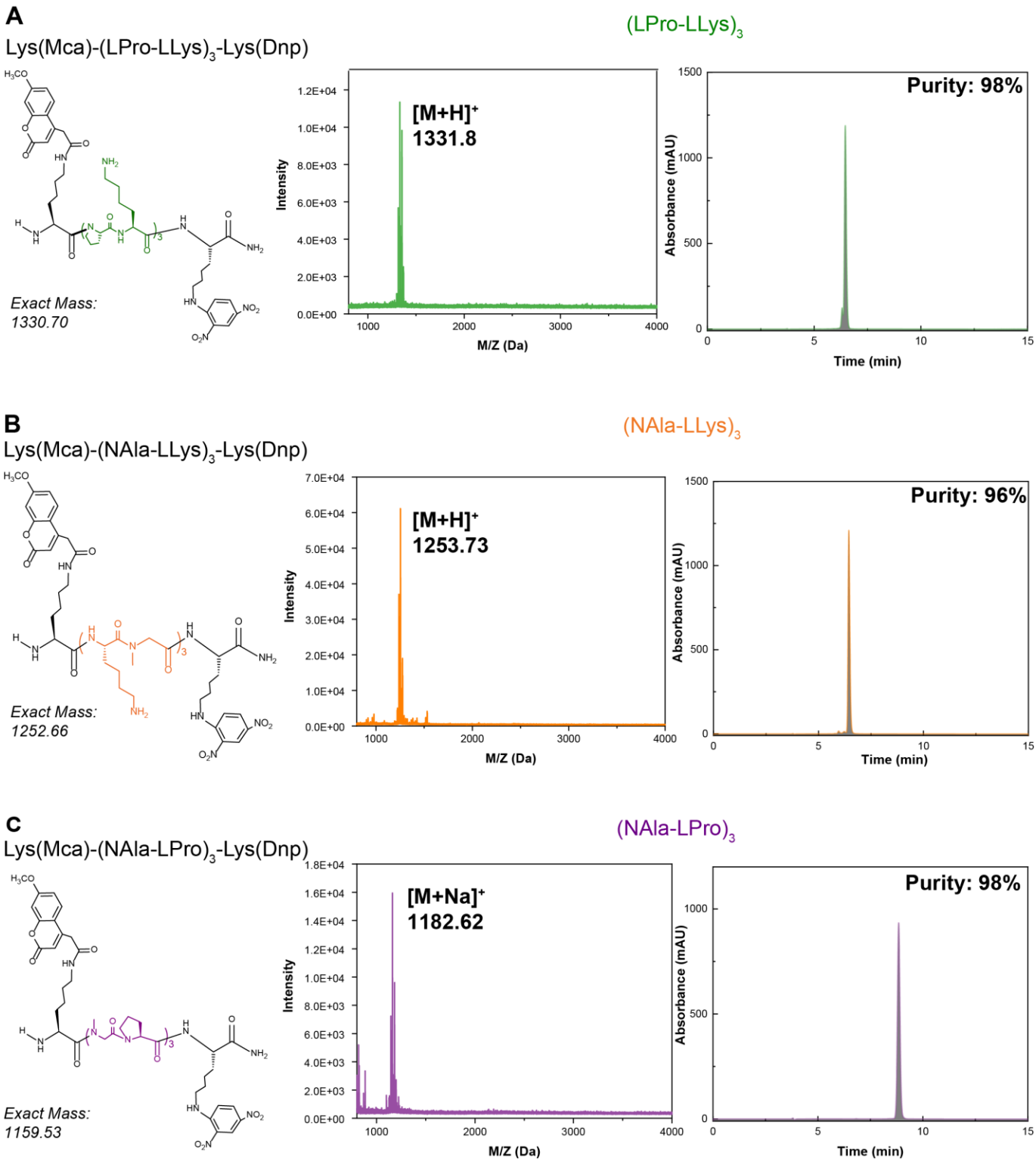
Supplementary Figure S1. MALDI and LC-MS confirming >90% purity of 18mer homopolymer peptides.



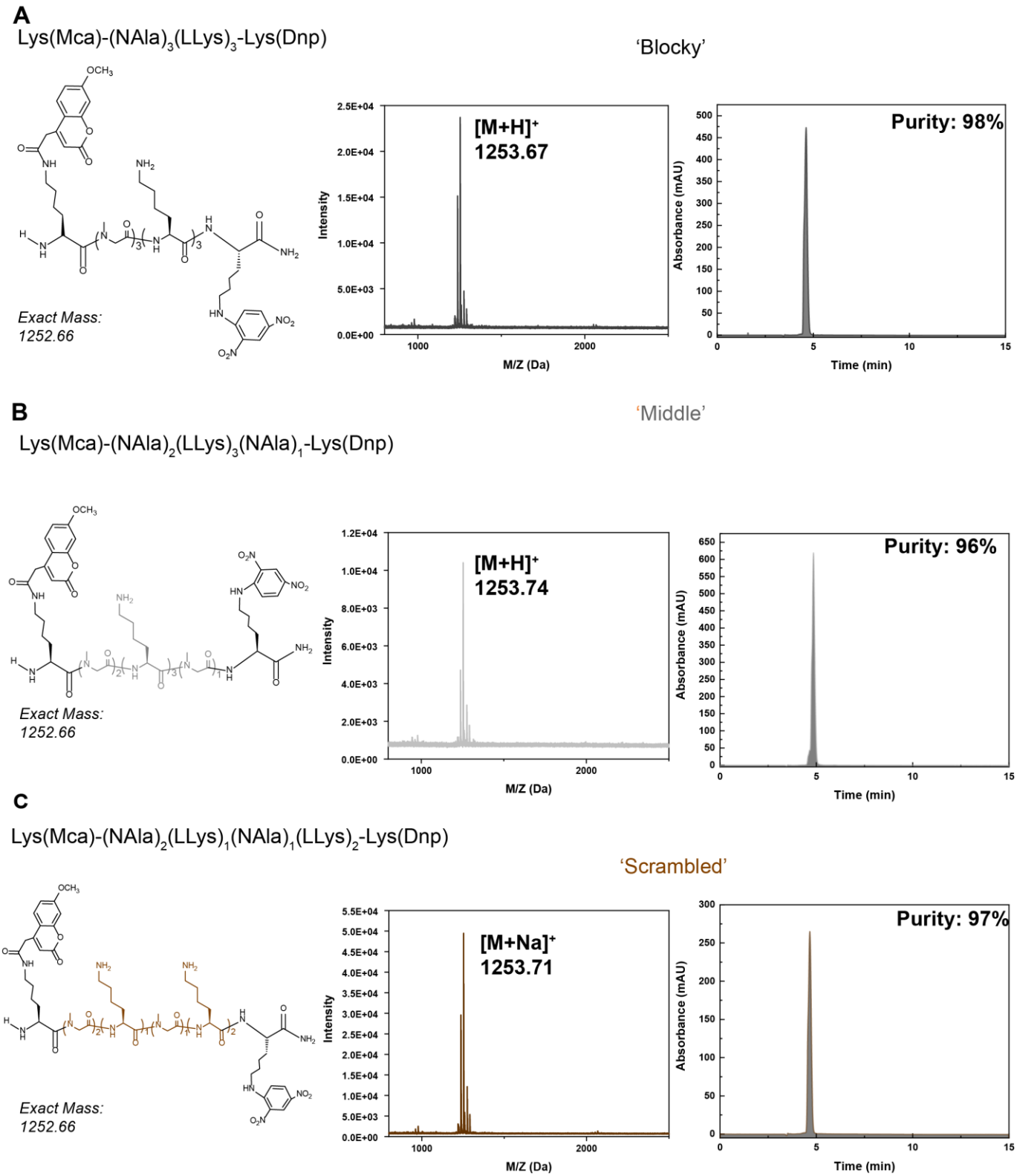
Supplementary Figure S2. MALDI and LC-MS confirming >90% purity of 18mer homopolymer peptoids.



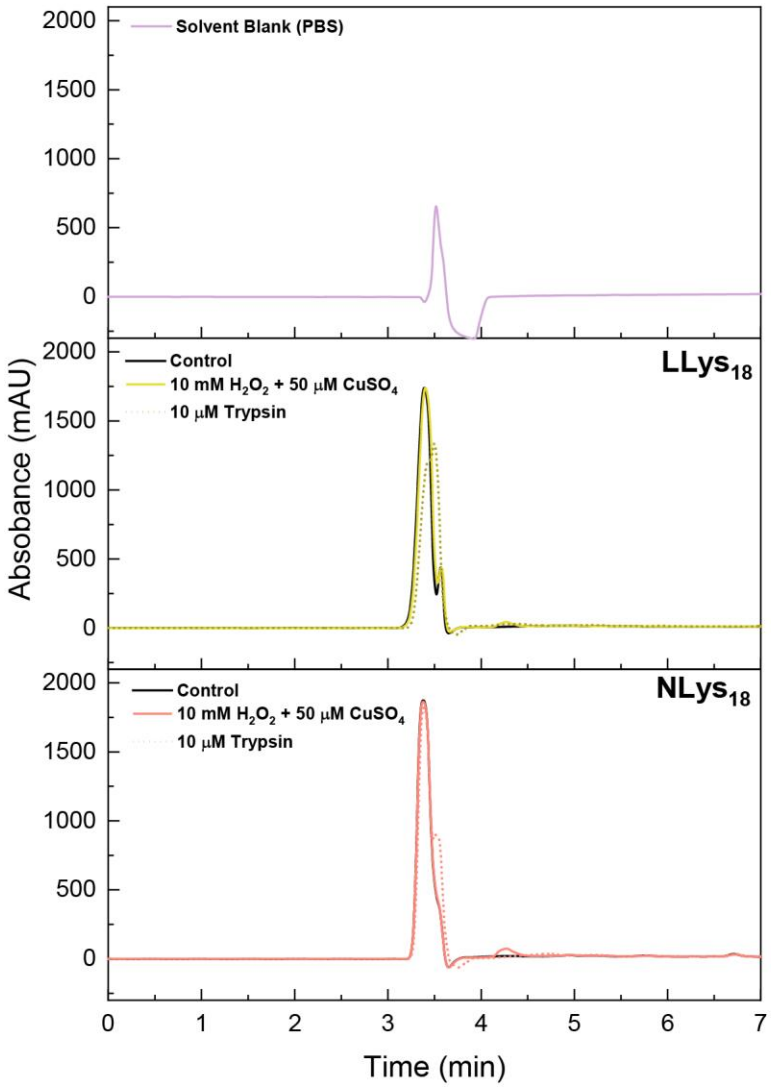
Supplementary Figure S3. MALDI and analytical HPLC confirming >95% purity of fluorescent peptide and peptoid homopolymers.



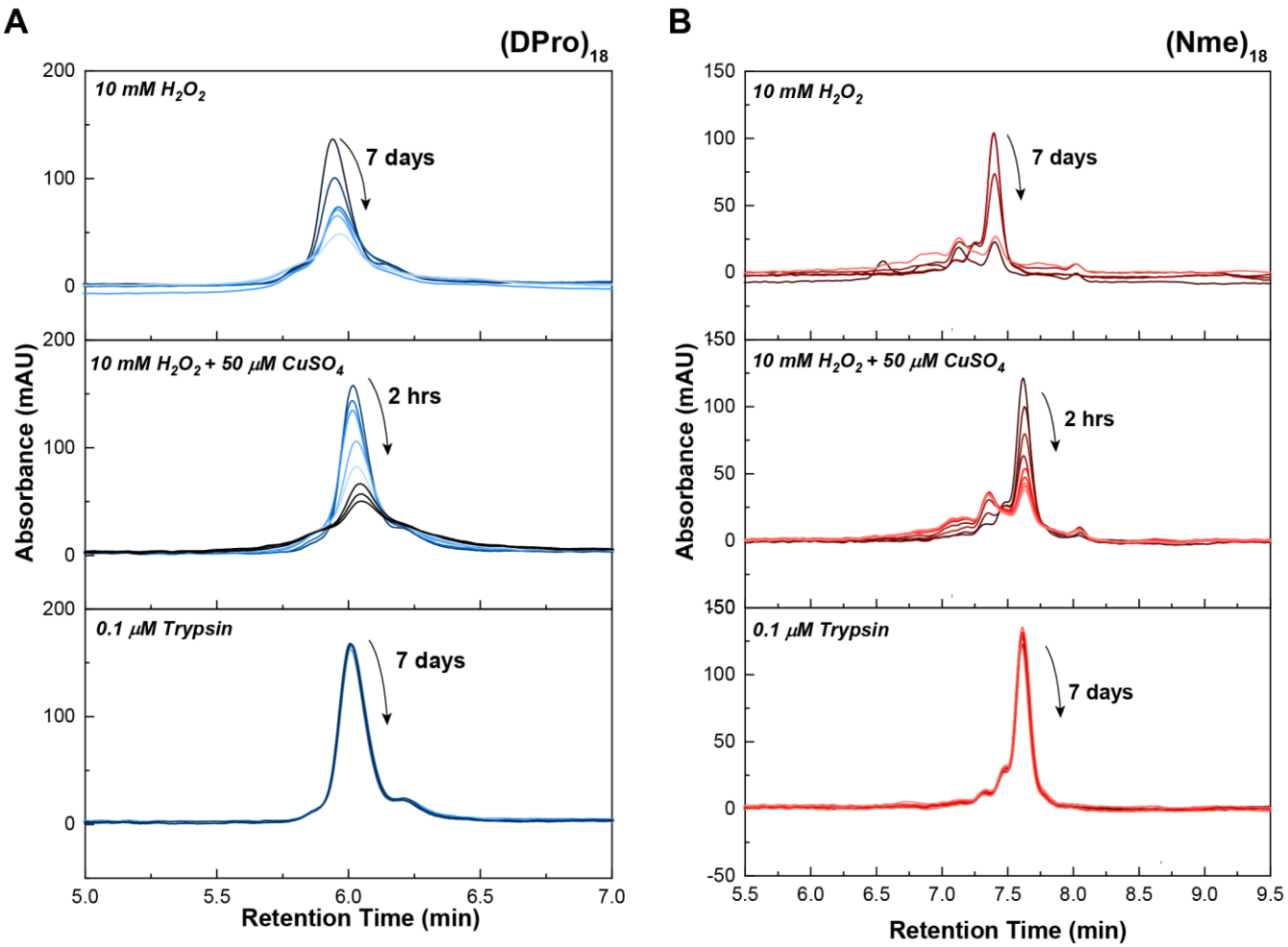
Supplementary Figure S4. MALDI and analytical HPLC confirming >90% purity of fluorescent peptomers.



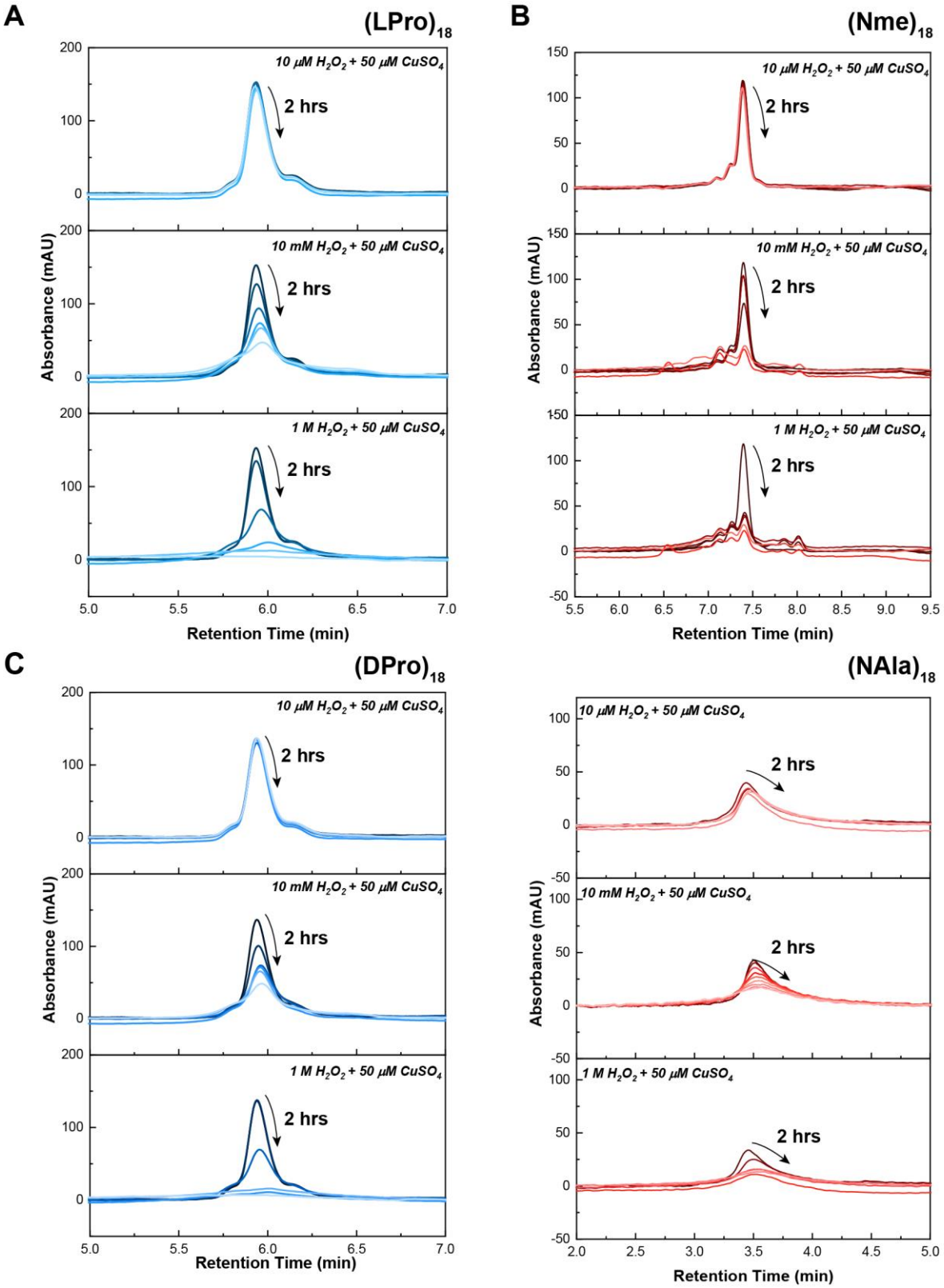
Supplementary Figure S5. MALDI and analytical HPLC confirming >90% purity of 'sequence effects' fluorescent peptomers.



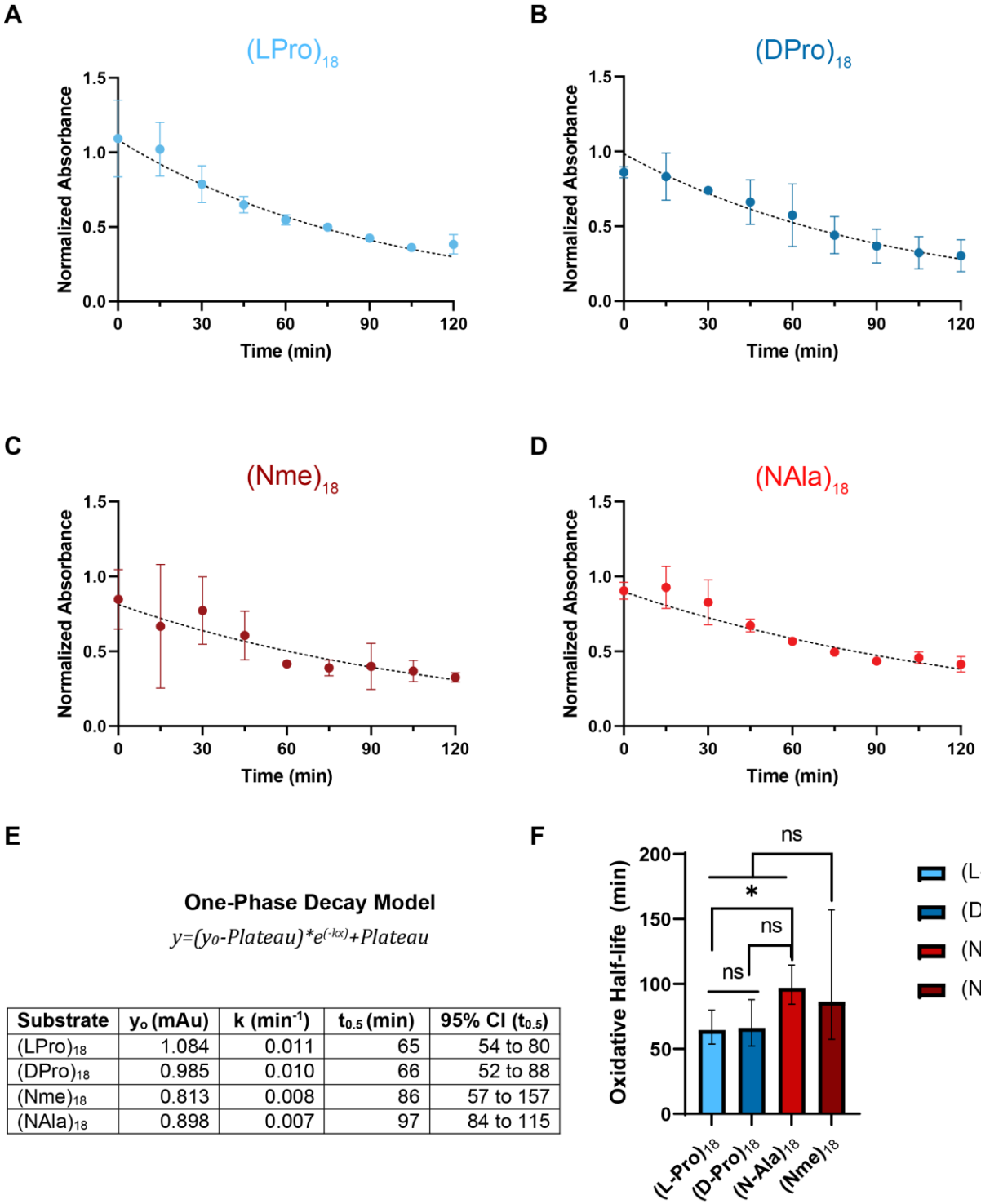
Supplementary Figure S6. The highly hydrophilic nature of the lysine oligomers ($(LLys)_{18}$ and $(NLys)_{18}$) made analysis by LC difficult given the fast elution from the C18 column. As illustrated, both oligomers elute at the same retention time as the PBS solvent peak, thus confounding degradation rate analysis results.



Supplementary Figure S7. LC traces of 18-mer peptide and peptoid molecules. A) D-proline peptide, $(DPro)_{18,}$ in comparison to B) N-methylglycine peptoid, $(NAla)_{18,}$ upon exposure to 10 mM H_2O_2 (top panel), 10 mM H_2O_2 + 50 µM $CuSO_4$ (middle panel) or 10 µM trypsin (bottom panel). As indicated by the arrows, timepoints were taken at 15-minute intervals over the course of 2 hours for H_2O_2 + 50 µM $CuSO_4$ (MCO) and at 24-hour intervals over the course of 7 days for H_2O_2 and Trypsin.



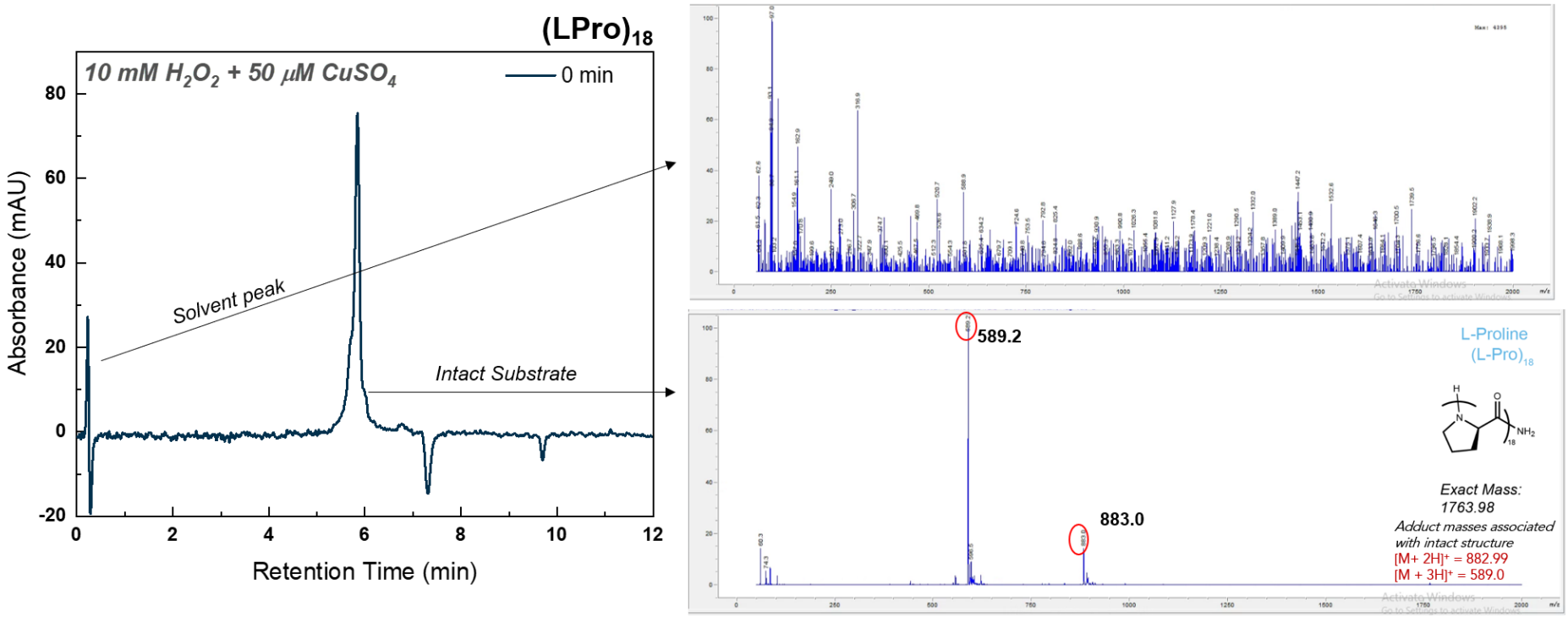
Supplementary Figure S8. Comparison of LC traces of 18-mer L-Proline, D-Proline peptides and N-Methoxyethylglycine and N-Methylglycine peptoids upon exposure to metal catalyzed oxidative (MCO) conditions (H_2O_2 + $CuSO_4$) at varying concentrations (10 µM - 1 M H_2O_2) and 50 µM $CuSO_4$. Oxidative degradation was monitored over 2 hours.



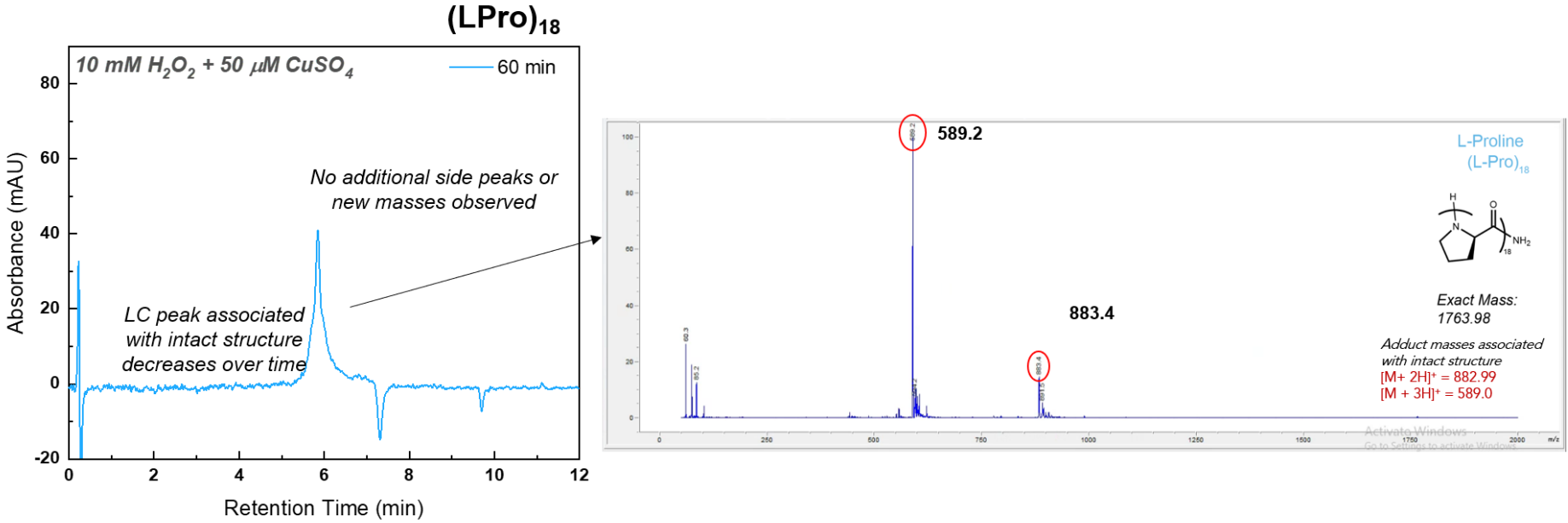
Substrate	y_o (mAu)	k (min^{-1})	$t_{0.5}$ (min)	95% CI ($t_{0.5}$)
$(LPro)_{18}$	1.084	0.011	65	54 to 80
$(DPro)_{18}$	0.985	0.010	66	52 to 88
$(Nme)_{18}$	0.813	0.008	86	57 to 157
$(NAla)_{18}$	0.898	0.007	97	84 to 115

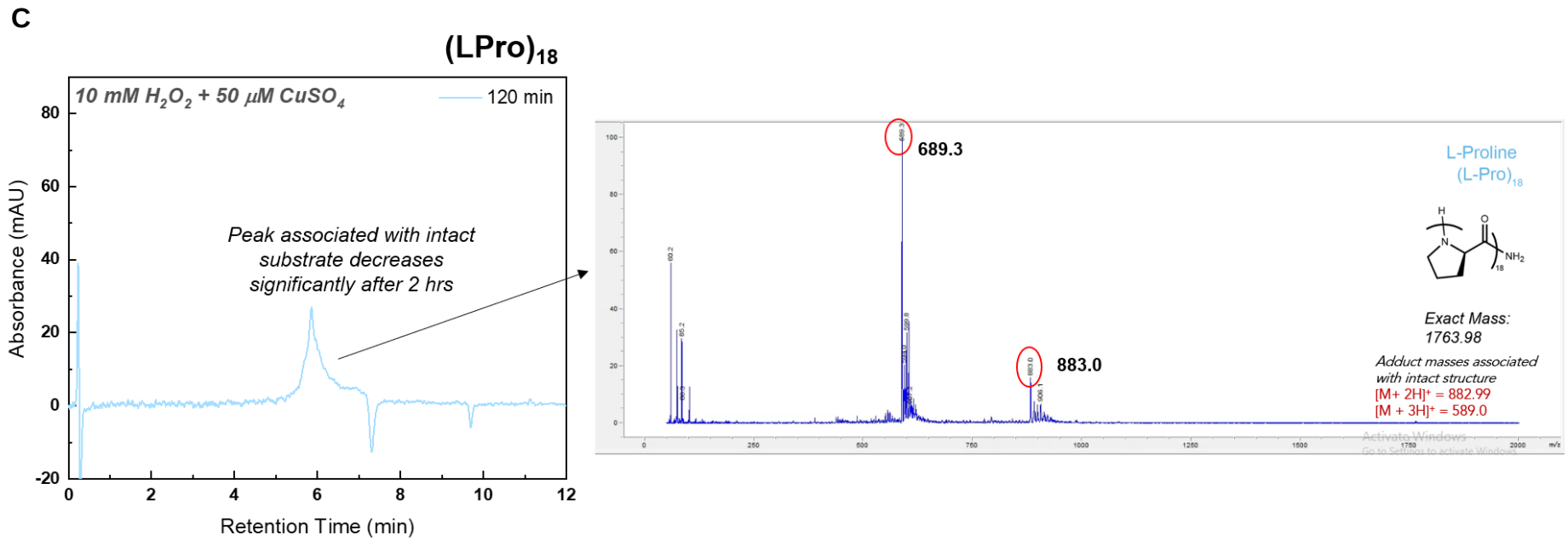
Supplementary Figure S9. Exponential decay degradation rate fits for 18mer fluorescent homopolymers. A-D) Exponential decay degradation rates were fit to the max absorbance of each sample normalized against its respective control at fixed retention times. Each point represents max absorbance of degraded substrate samples normalized to max absorbance of the intact control (n=3). The resulting exponential decay equation, $y = (y_0 - Plateua) * e^{-kx} + Plateau$ (E) was used to calculate half-lives, $t_{0.5}$, of each substrate (F). *y* is the absorbance representing intact substrate remaining, *x* is the incubation time of the substrate (in min), k is the fitted rate constant in $min^{-1}$and *Plateau* was constrained to zero. Error bars for the bar graph represent the 95% confidence interval. Significance brackets extend over all samples that are significantly different from one another such that *p ≤ 0.05 with exceptions (ns) denoted.

A


(LPro)18
10 mM H2O2 + 50 μM CuSO4
0 min
Absorbance (mAU)
Retention Time (min)
Solvent peak
Intact Substrate
589.2
883.0
L-Proline
(L-Pro)18
Exact Mass:
1763.98
Adduct masses associated
with intact structure
[M+ 2H]+ = 882.99
[M + 3H]+ = 589.0

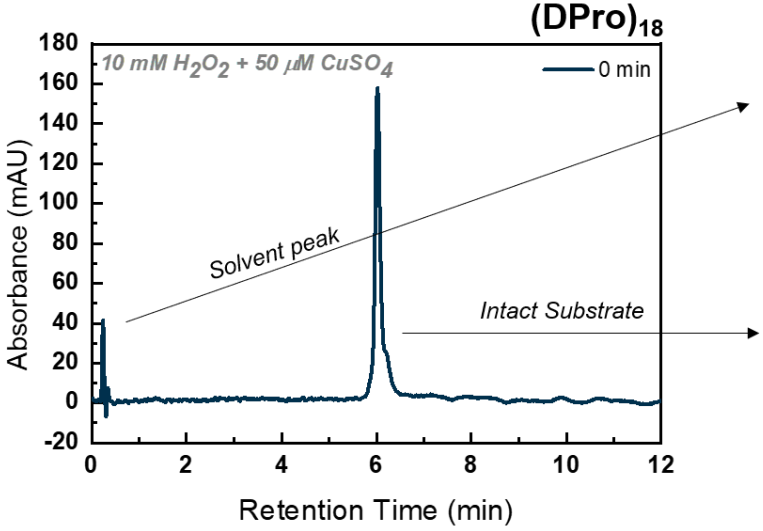
B

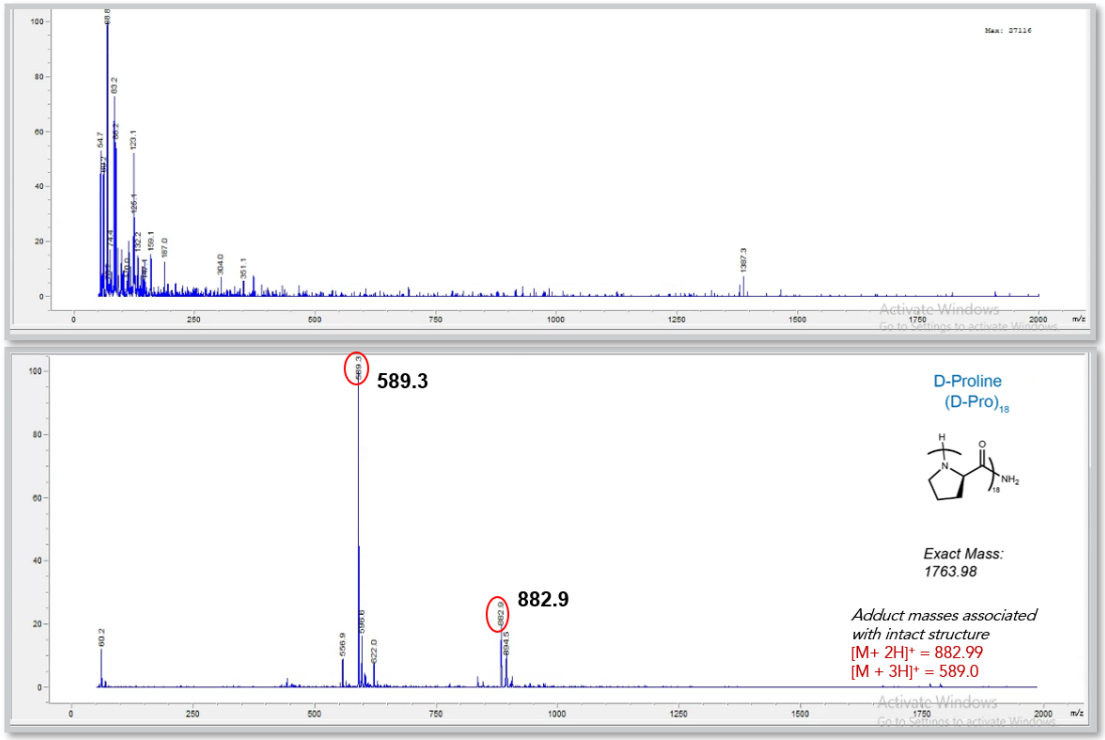

(LPro)18
10 mM H2O2 + 50 μM CuSO4
60 min
Absorbance (mAU)
Retention Time (min)
No additional side peaks or new masses observed
LC peak associated with intact structure decreases over time
589.2
883.4
L-Proline
(L-Pro)18
Exact Mass:
1763.98
Adduct masses associated with intact structure
[M+ 2H]+ = 882.99
[M + 3H]+ = 589.0



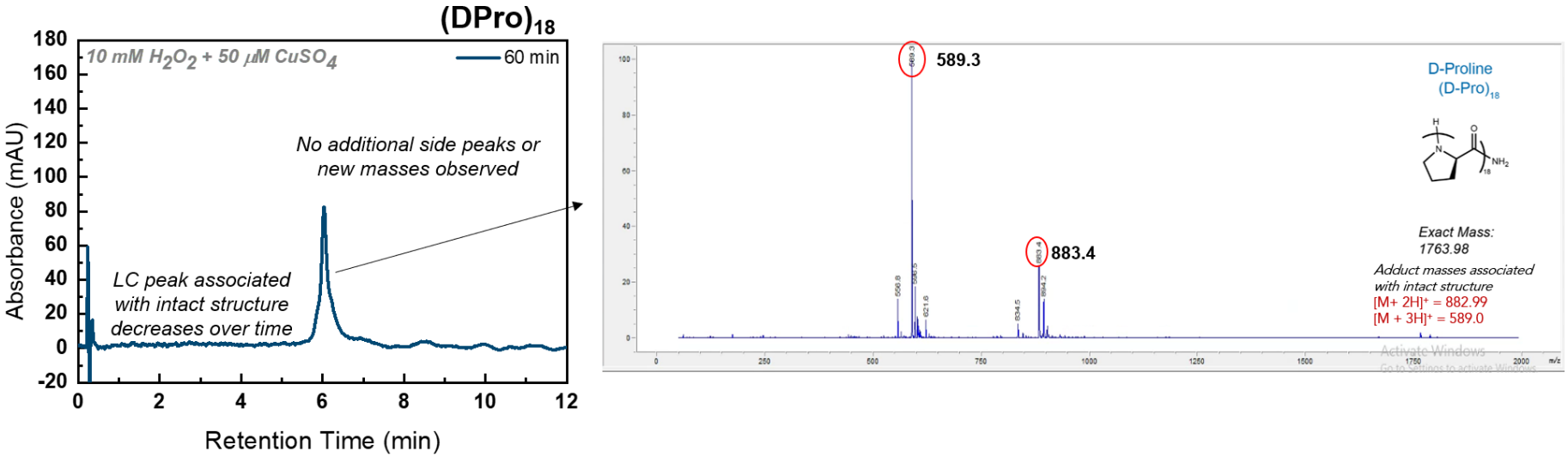
Supplementary Figure S10. Mass spectrometry data for LC chromatographs (214 nm) of $(LPro)_{18}$ after A) 0 min B) 60 min and C) 120 min of MCO exposure. Masses circled in red indicate adducts associated with intact structure. The lack of identifiable side peaks in the LC trace suggest non-specific oxidative degradation. Note that the size of the main peak significantly decreases from time 0 min to 120 min.

A

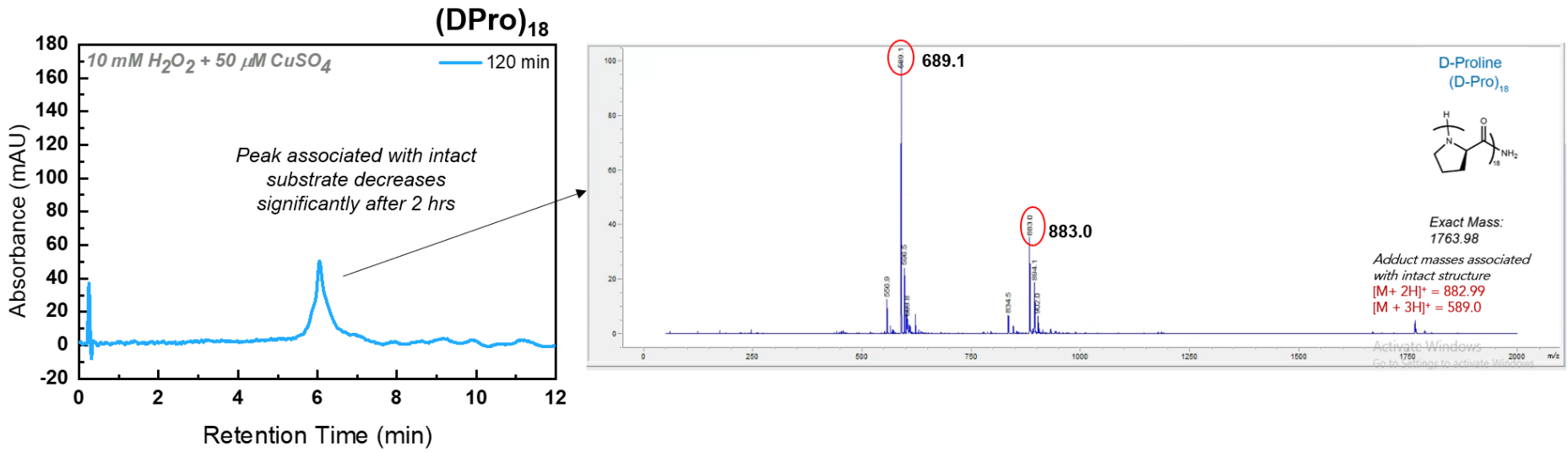

(DPro)18
10 mM H2O2 + 50 μM CuSO4
0 min
Absorbance (mAU)
Retention Time (min)
Solvent peak
Intact Substrate


589.3
882.9
D-Proline
(D-Pro)18
Exact Mass:
1763.98
Adduct masses associated
with intact structure
[M+ 2H]+ = 882.99
[M + 3H]+ = 589.0

B

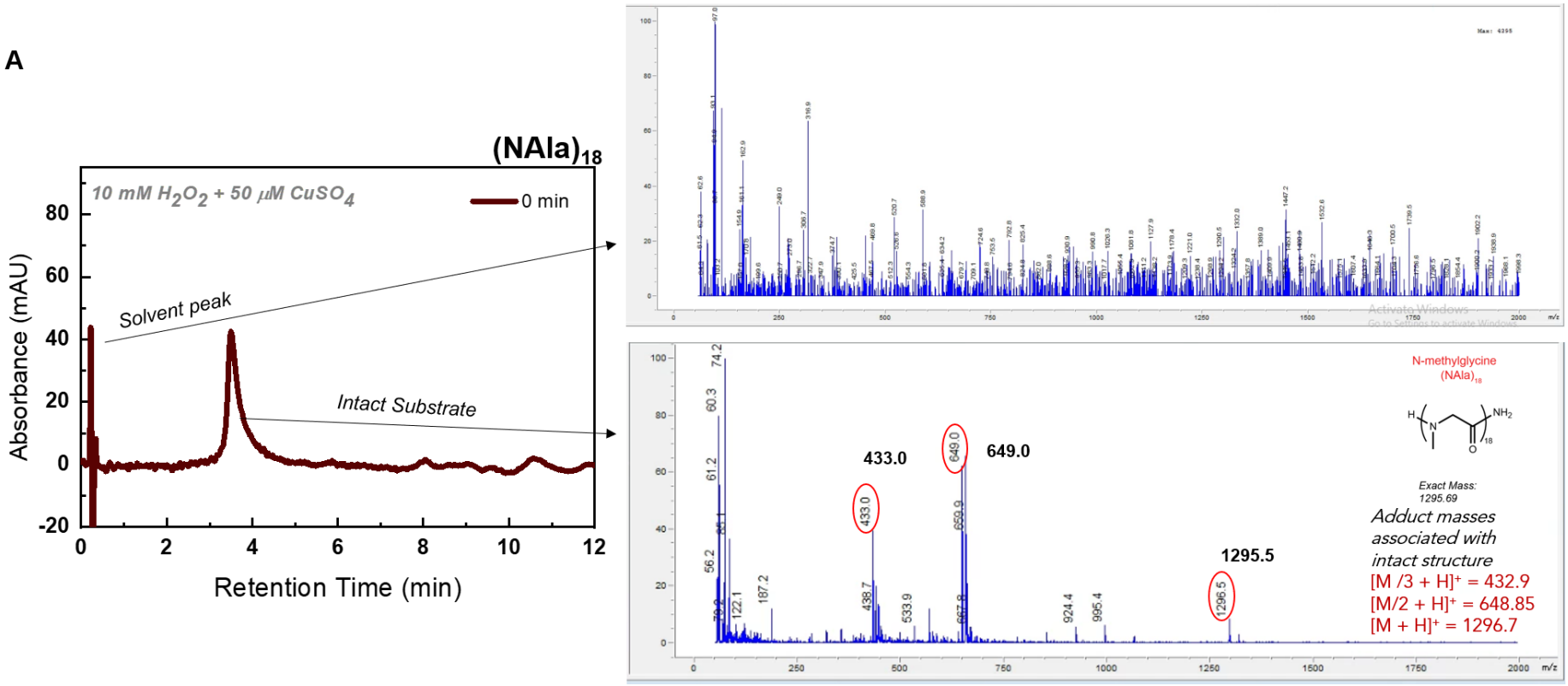

(DPro)18
10 mM H2O2 + 50 μM CuSO4
60 min
No additional side peaks or new masses observed
LC peak associated with intact structure decreases over time
Absorbance (mAU)
Retention Time (min)
589.3
883.4
D-Proline
(D-Pro)18
Exact Mass:
1763.98
Adduct masses associated with intact structure
[M+ 2H]+ = 882.99
[M + 3H]+ = 589.0
Activate Windows

C

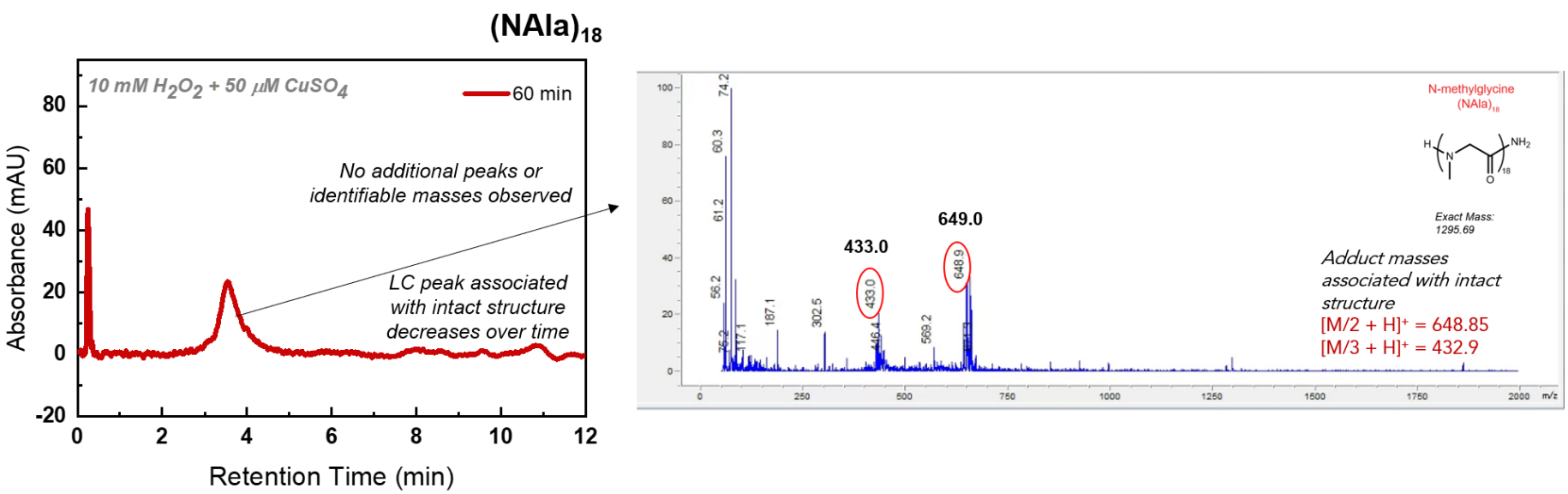


Supplementary Figure S11. Mass spectrometry data for LC chromatographs (214 nm) of $(DPro)_{18}$ after A) 0 min B) 60 min and C) 120 min of MCO exposure. Masses circled in red indicate adducts associated with intact structure. The lack of identifiable side peaks in the LC trace suggest non-specific oxidative degradation. Note that the size of the main peak significantly decreases from time 0 min to 120 min.

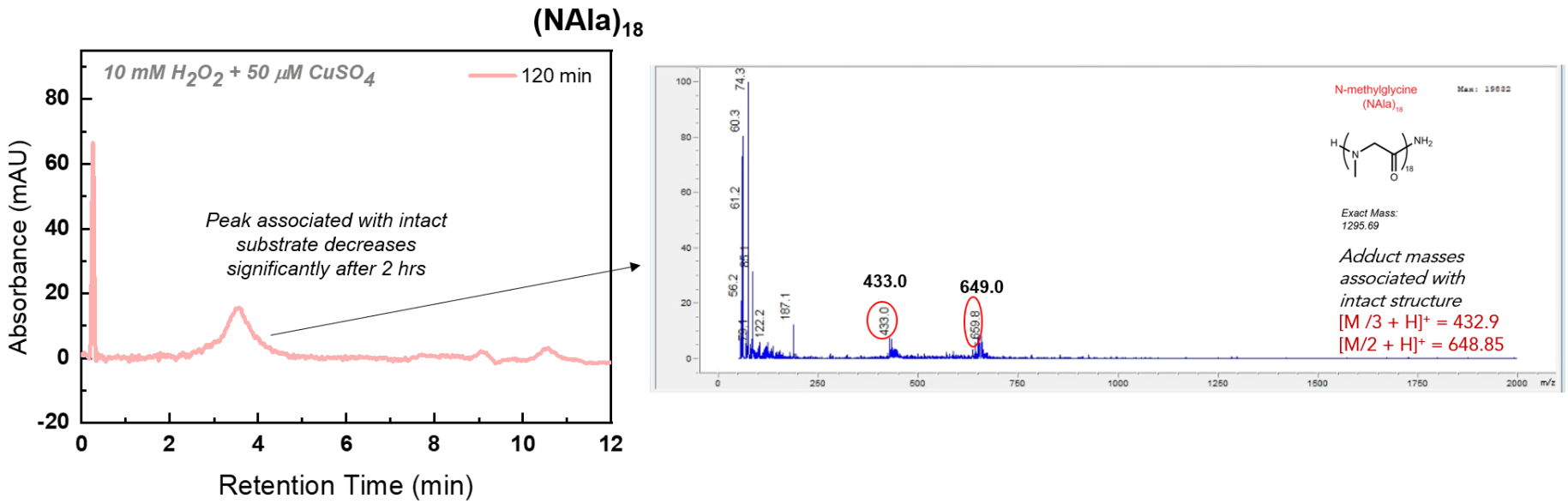
A


(NAla)18
10 mM H2O2 + 50 μM CuSO4
0 min
Solvent peak
Intact Substrate
Absorbance (mAU)
Retention Time (min)
433.0
649.0
1295.5
N-methylglycine
(NAla)18
Exact Mass:
1295.69
Adduct masses
associated with
intact structure
[M /3 + H]+ = 432.9
[M/2 + H]+ = 648.85
[M + H]+ = 1296.7

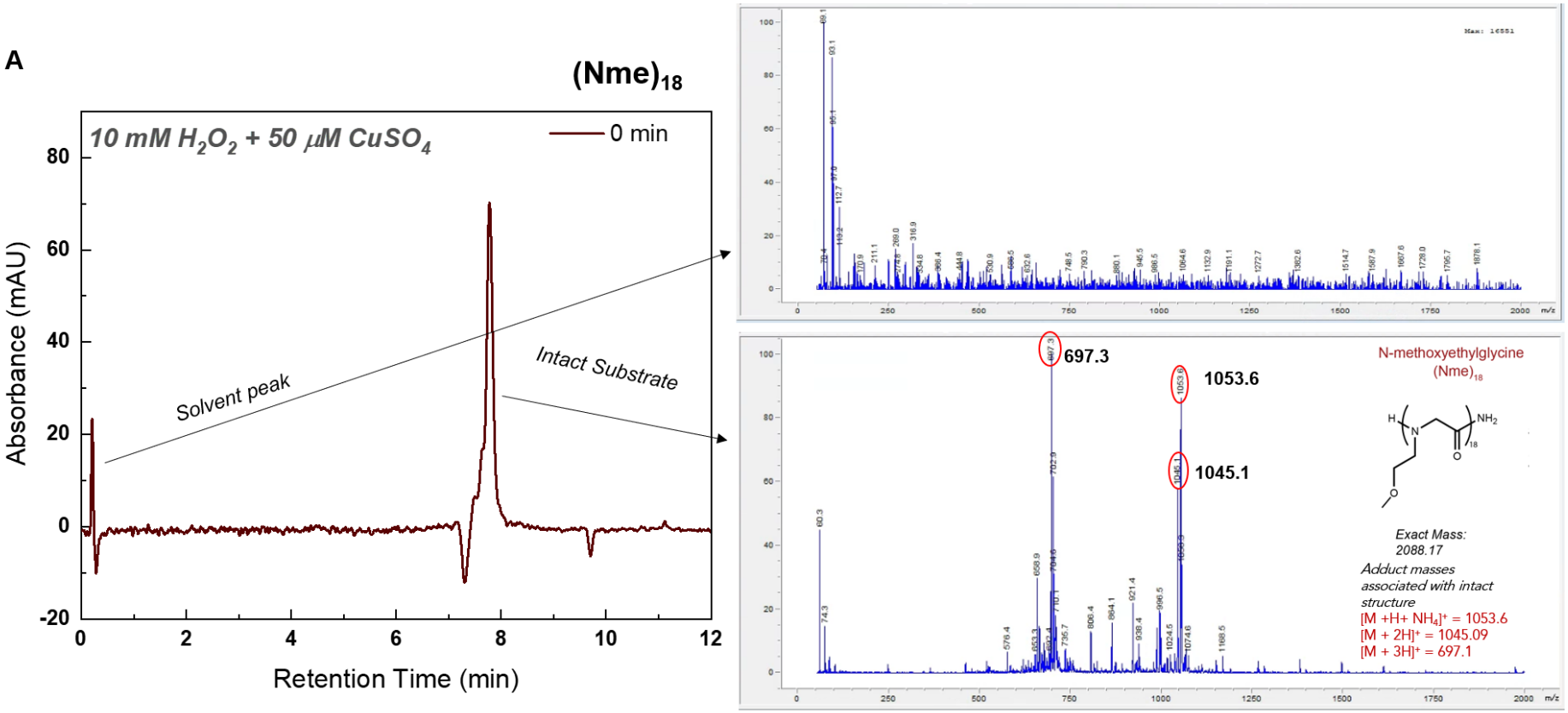
B

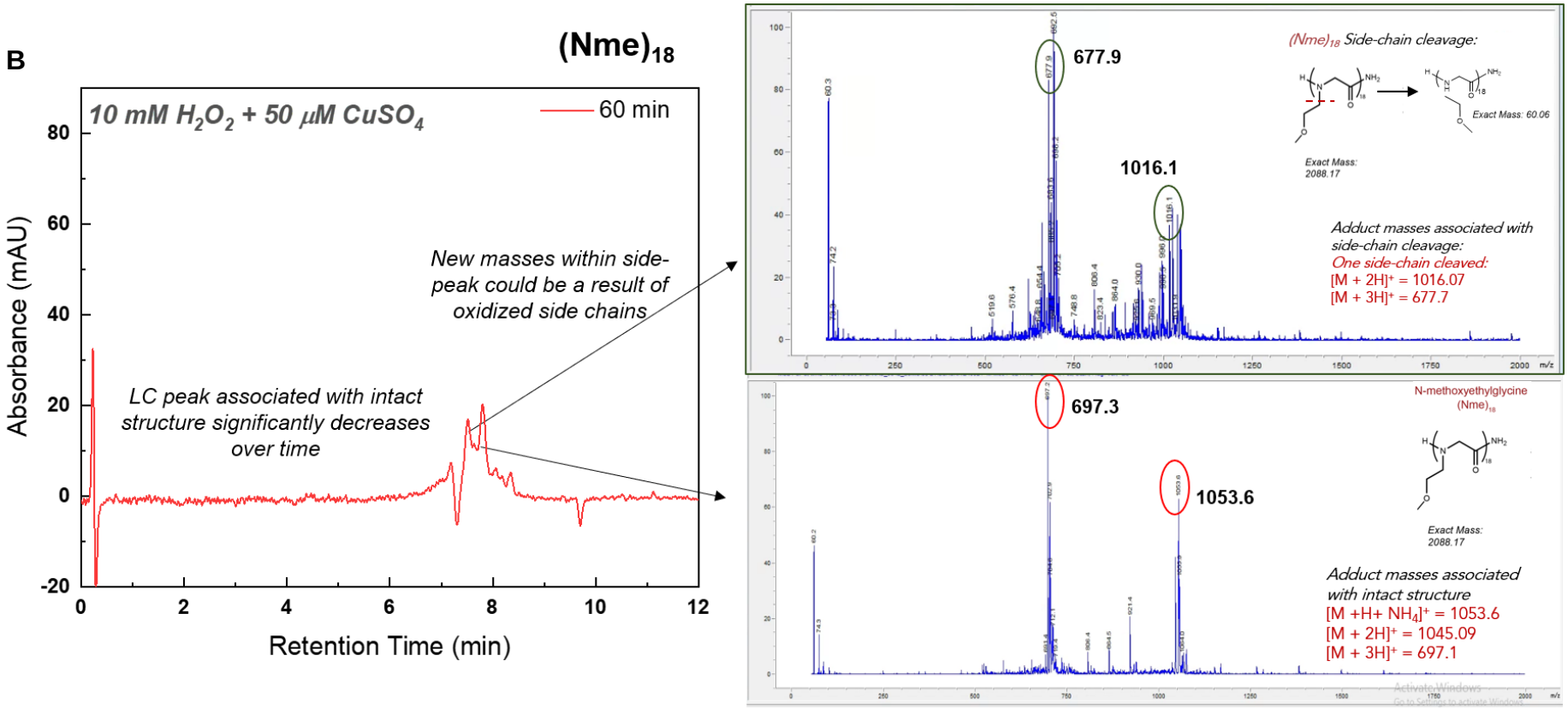

(NAla)18
10 mM H2O2 + 50 μM CuSO4
60 min
No additional peaks or identifiable masses observed
LC peak associated with intact structure decreases over time
Absorbance (mAU)
Retention Time (min)
433.0
649.0
N-methylglycine (NAla)18
Exact Mass: 1295.69
Adduct masses associated with intact structure
[M/2 + H]+ = 648.85
[M/3 + H]+ = 432.9

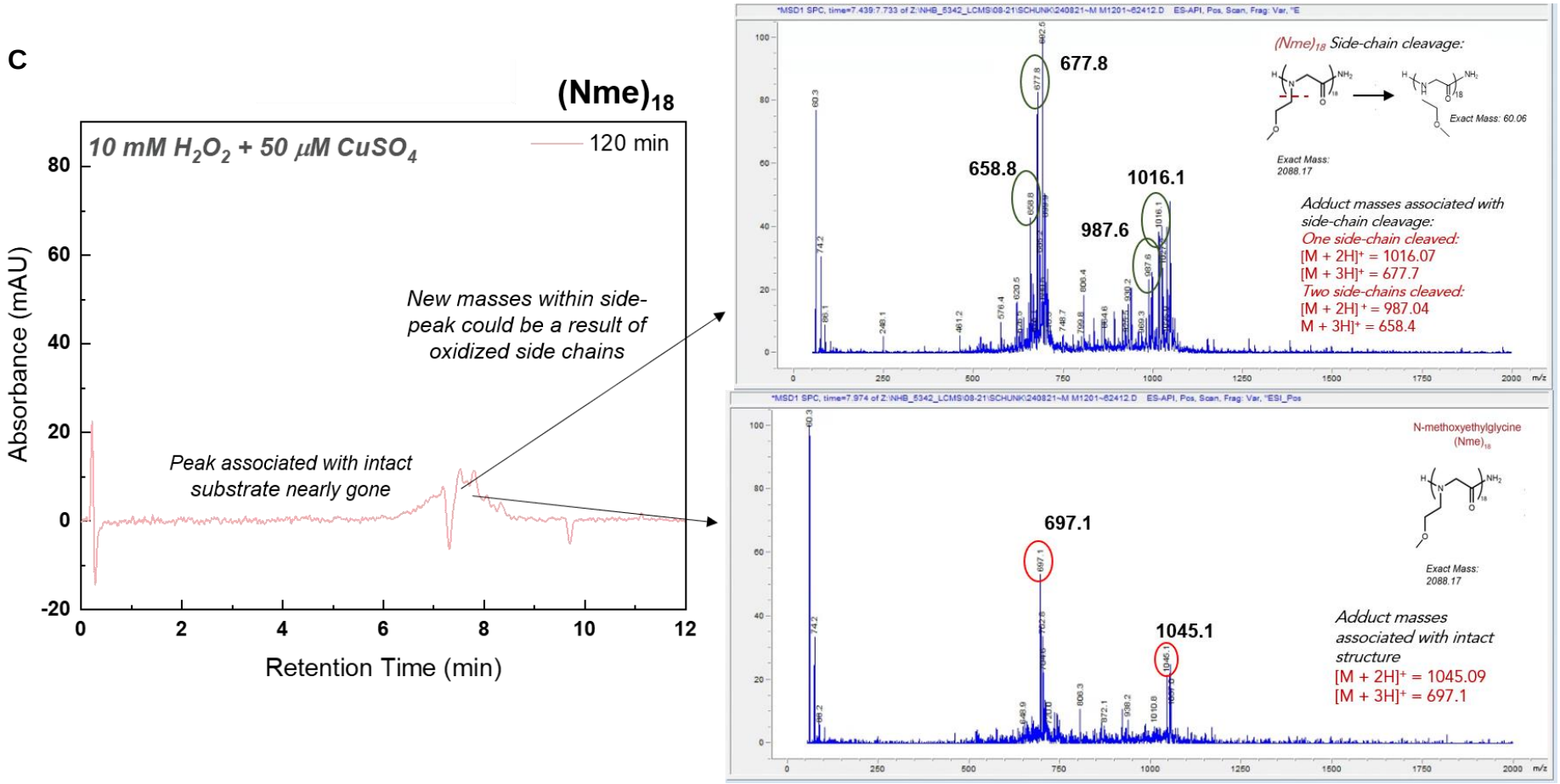
C



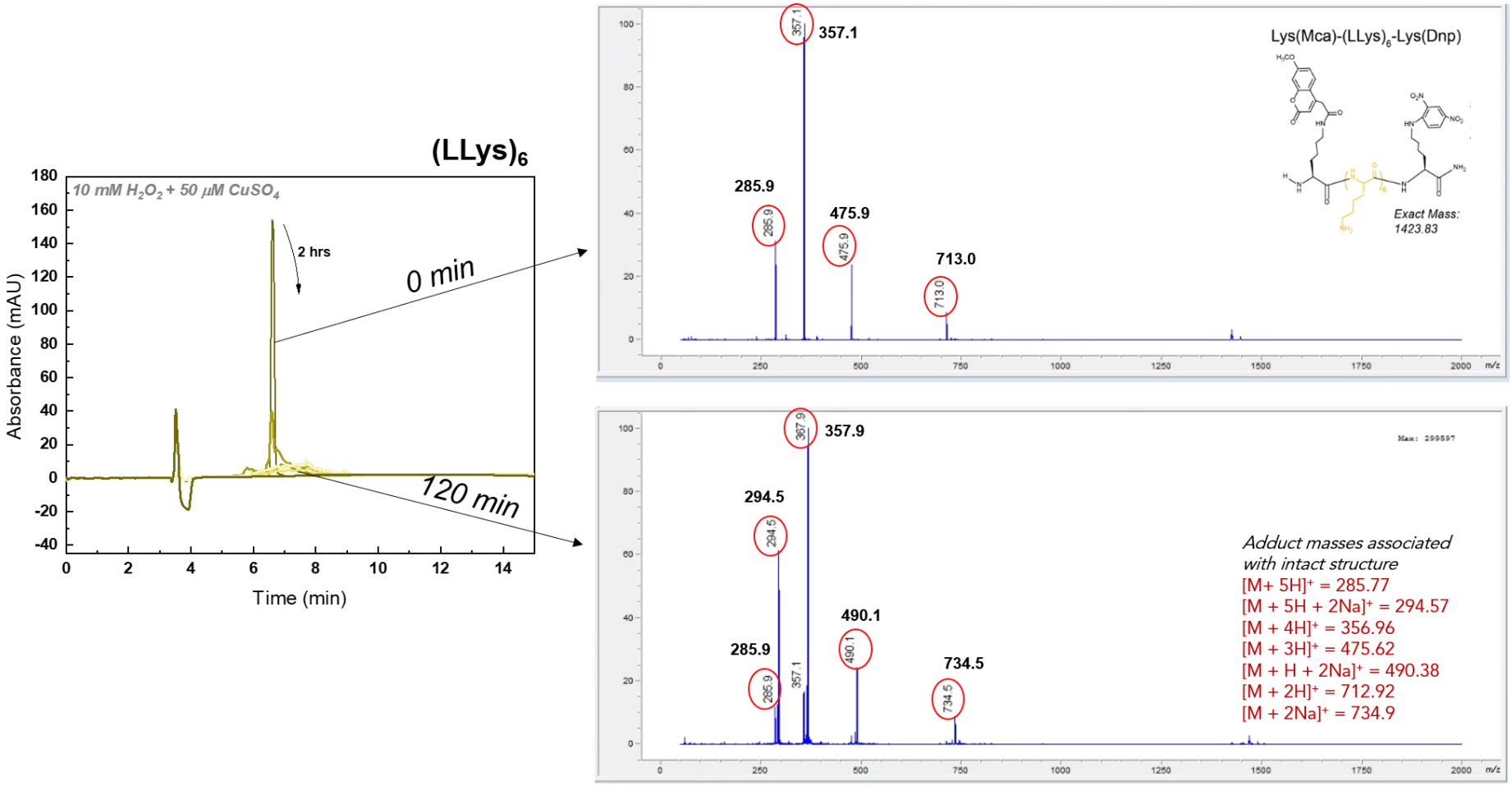
Supplementary Figure S12. Mass spectrometry data for LC chromatographs (214 nm) of $(NAla)_{18}$ after A) 0 min B) 60 min and C) 120 min of MCO exposure. Masses circled in red indicate adducts associated with intact structure. The lack of identifiable side peaks in the LC trace suggest non-specific oxidative degradation. Note that the size of the main peak significantly decreases from time 0 min to 120 min.


A
(Nme)18
10 mM H2O2 + 50 μM CuSO4
0 min
Absorbance (mAU)
Retention Time (min)
Solvent peak
Intact Substrate
697.3
1053.6
1045.1
N-methoxyethylglycine
(Nme)18
Exact Mass:
2088.17
Adduct masses
associated with intact
structure
[M +H+ NH4]+ = 1053.6
[M + 2H]+ = 1045.09
[M + 3H]+ = 697.1

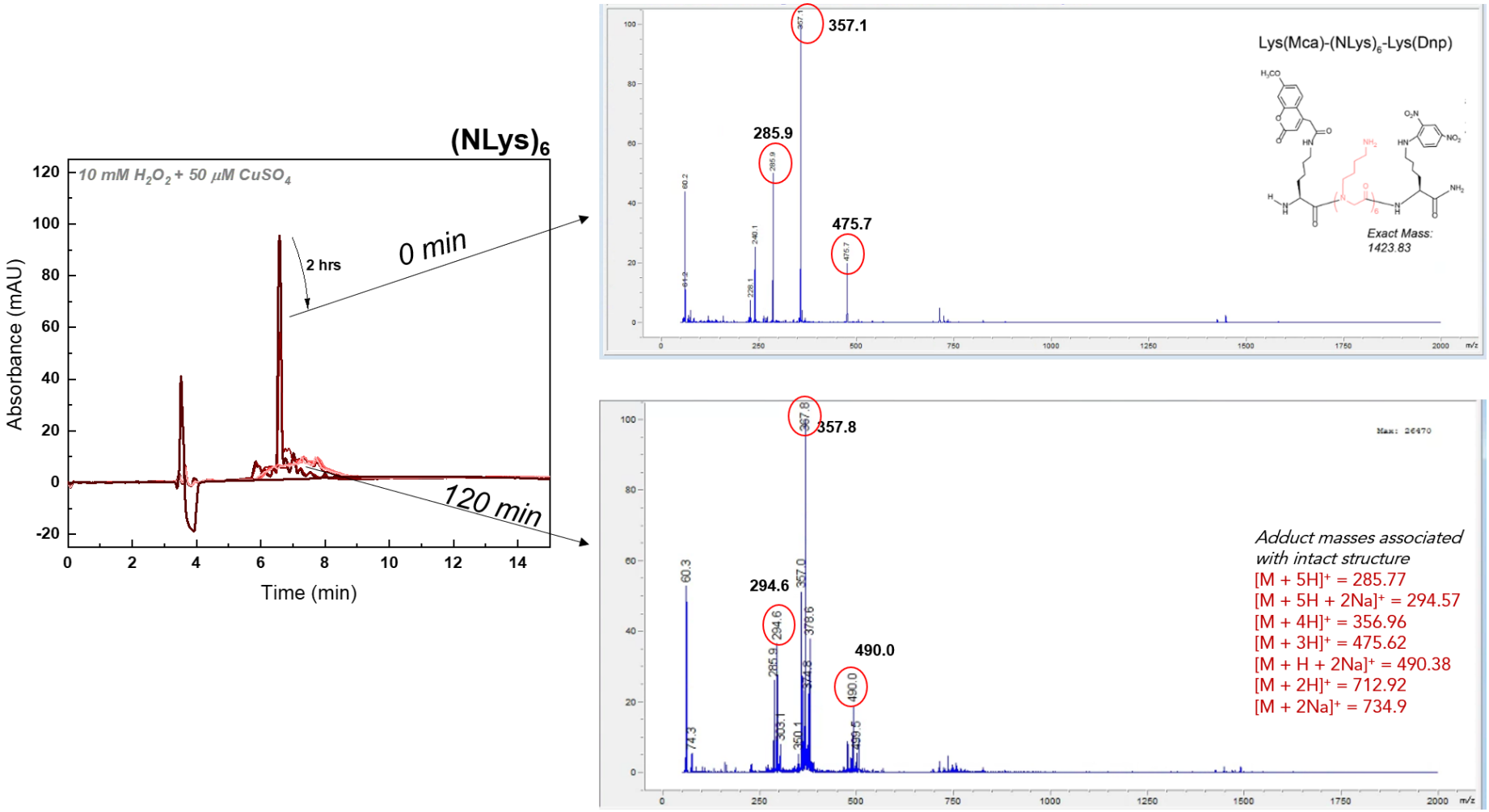

B
(Nme)18
10 mM H2O2 + 50 μM CuSO4
60 min
Absorbance (mAU)
Retention Time (min)
LC peak associated with intact structure significantly decreases over time
New masses within side-peak could be a result of oxidized side chains
677.9
1016.1
(Nme)18 Side-chain cleavage:
Exact Mass: 60.06
Exact Mass: 2088.17
Adduct masses associated with side-chain cleavage:
One side-chain cleaved:
[M + 2H]+ = 1016.07
[M + 3H]+ = 677.7
697.3
1053.6
N-methoxyethylglycine (Nme)18
Exact Mass: 2088.17
Adduct masses associated with intact structure
[M +H+ NH4]+ = 1053.6
[M + 2H]+ = 1045.09
[M + 3H]+ = 697.1



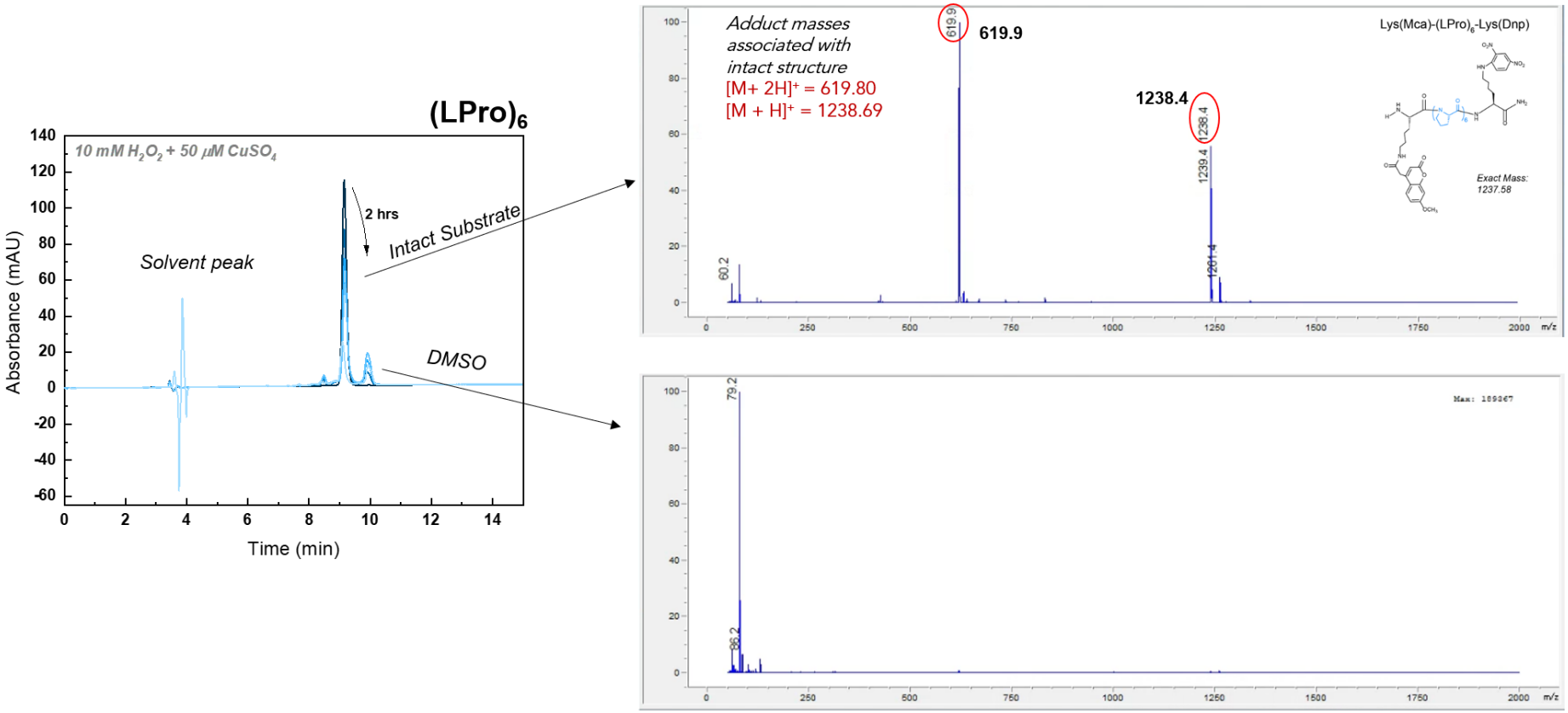
Supplementary Figure S13. Mass spectrometry data for LC chromatographs (214 nm) of $(Nme)_{18}$ after A) 0 min B) 60 min and C) 120 min of MCO exposure. Masses circled in red indicate adducts associated with intact structure. The occurrence of new masses within new side peaks (circled in green) indicate that side-chain cleavage is taking place. Note that the size of the main peak significantly decreases from time 0 min to 120 min.



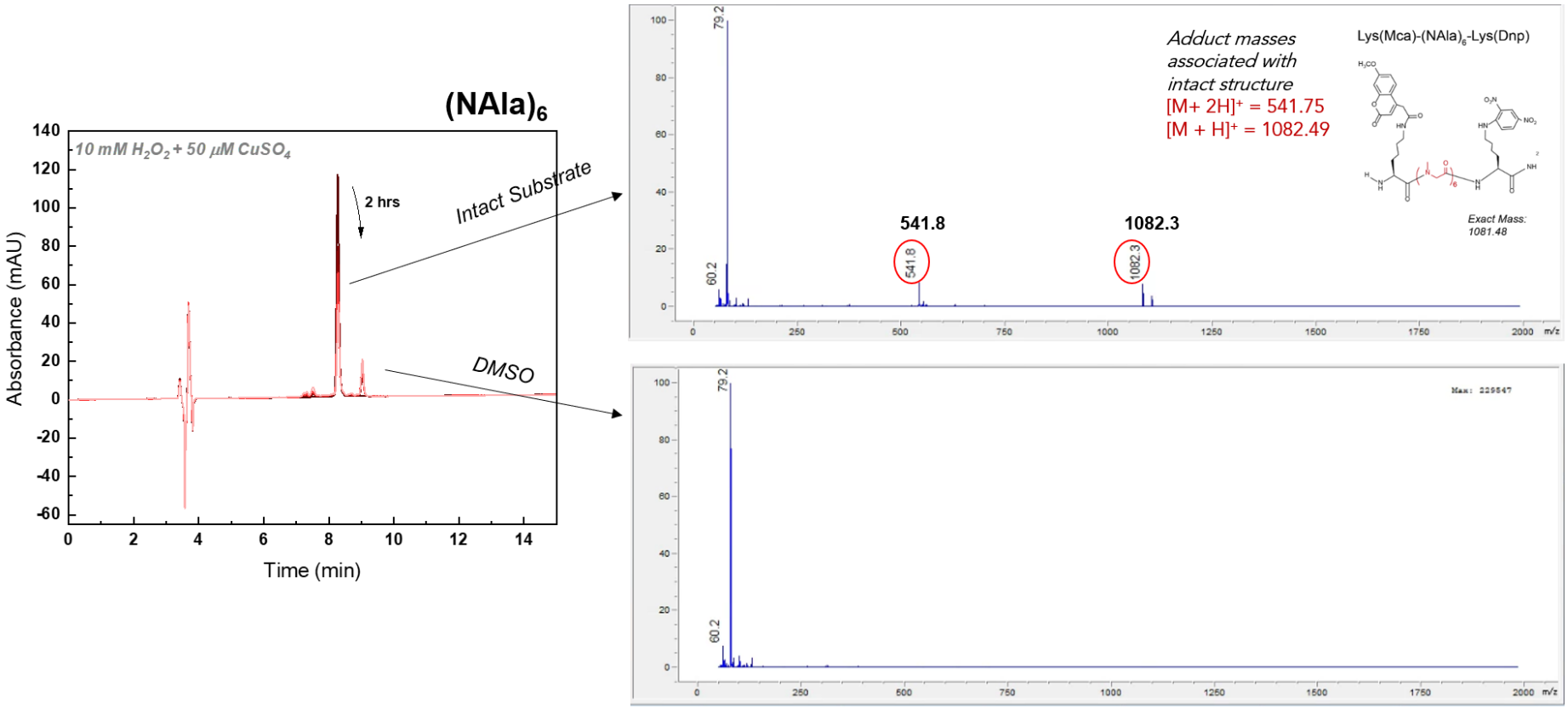
Supplementary Figure S14. Mass spectrometry data for HPLC chromatographs (214 nm) of $(LLys)_6$ spanning 120 min of MCO exposure. Following HPLC analysis, the eluted peak was analyzed with MS. Masses circled in red indicate adducts associated with intact structure. Note that the size of the main peak significantly decreases over time. The first peak was identified as a solvent peak.



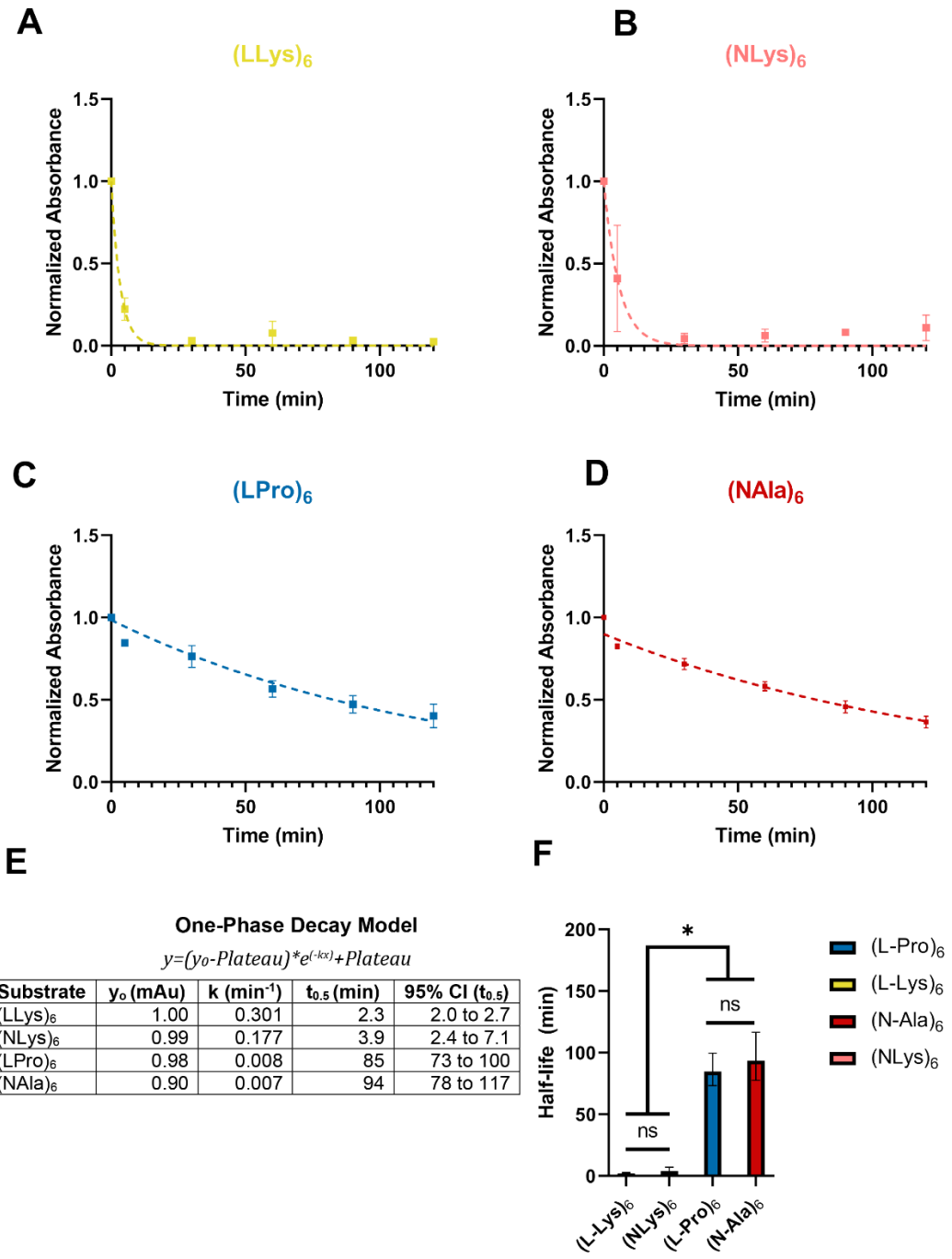
Supplementary Figure S15. Mass spectrometry data for HPLC chromatographs (214 nm) of $(NLys)_6$ spanning 120 min of MCO exposure. Following HPLC analysis, the eluted peak was analyzed with MS. Masses circled in red indicate adducts associated with intact structure. Note that the size of the main peak significantly decreases over time. The first peak was identified as a solvent peak.



Supplementary Figure S16. Mass spectrometry data for HPLC chromatographs (214 nm) of $(LPro)_6$ spanning 120 min of MCO exposure. Following HPLC analysis, the eluted peak was analyzed with MS. Masses circled in red indicate adducts associated with intact structure. Note that the size of the main peak significantly decreases over time. The small side peak was identified to be residual DMSO from fluorescent substrate stocks. The first peak was identified as a solvent peak.



Supplementary Figure S17. Mass spectrometry data for HPLC chromatographs (214 nm) of $(NAla)_6$ spanning 120 min of MCO exposure. Following HPLC analysis, the eluted peak was analyzed with MS. Masses circled in red indicate adducts associated with intact structure. Note that the size of the main peak significantly decreases over time. The small side peak was identified to be residual DMSO from fluorescent substrate stocks. The first peak was identified as a solvent peak.

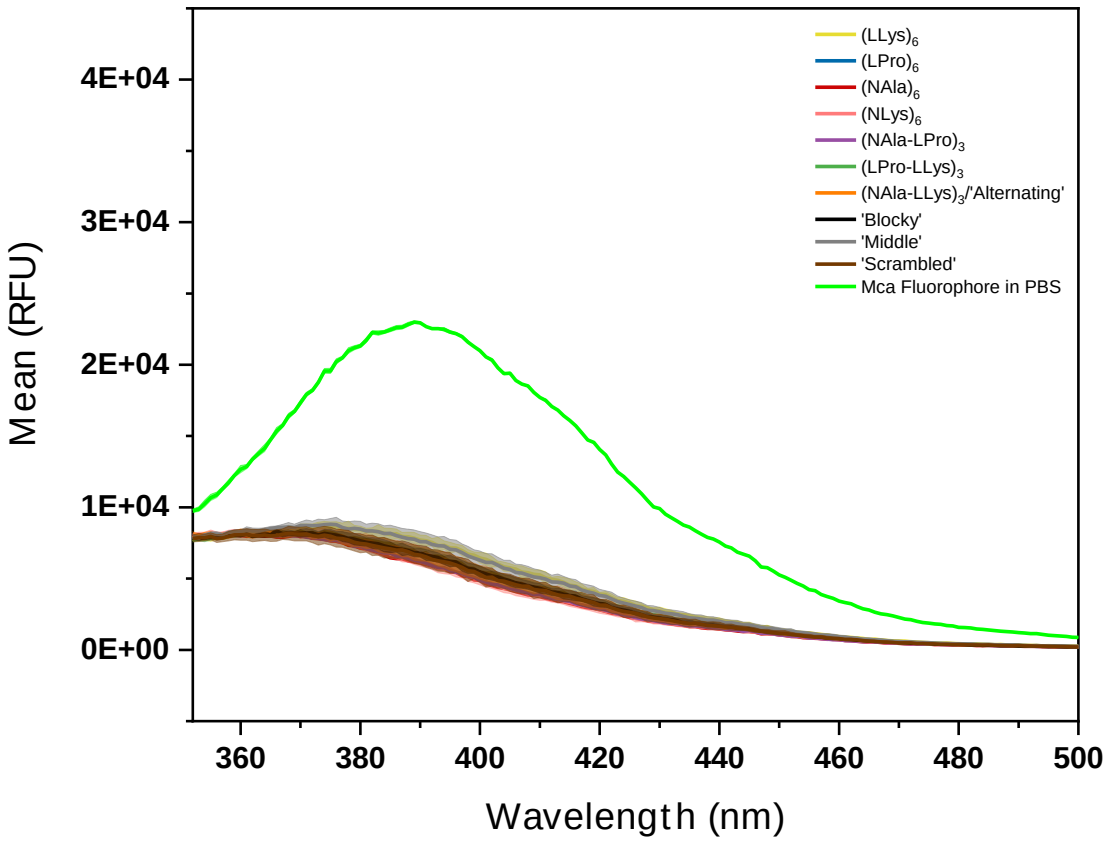


One-Phase Decay Model

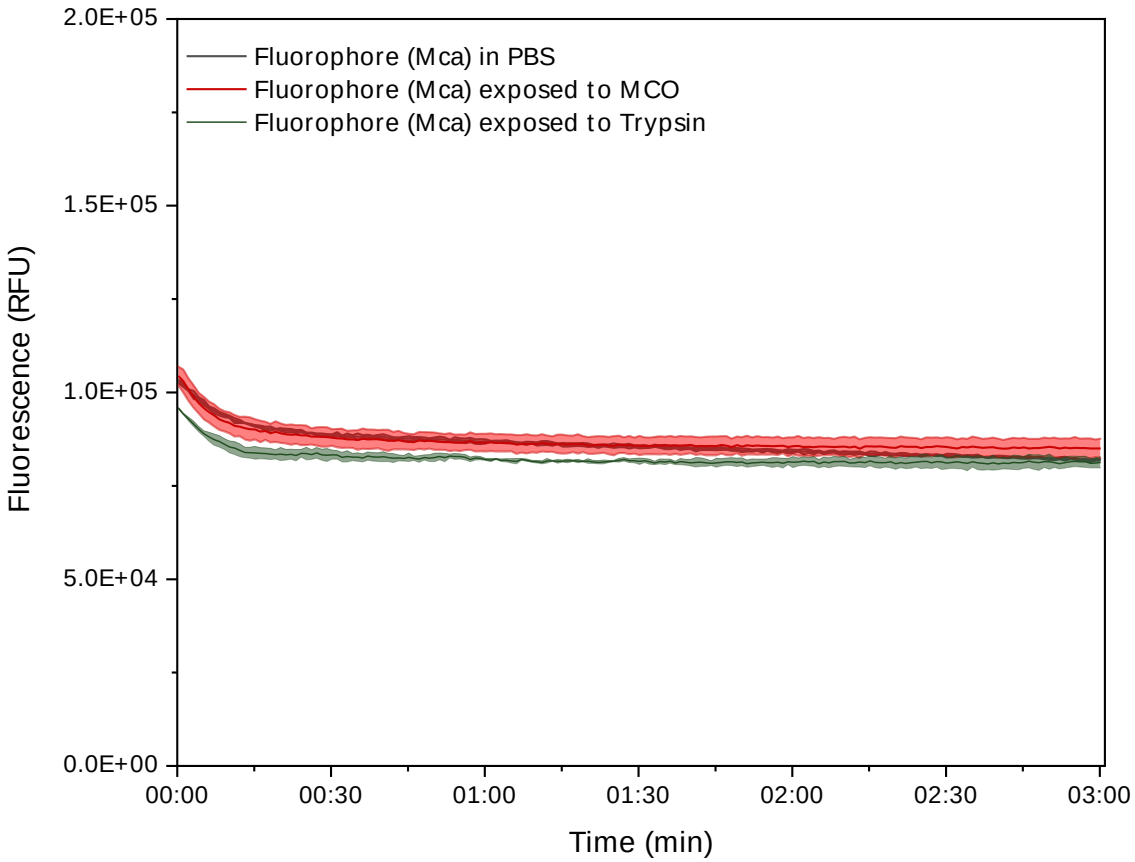
$y=(y_0\text{-}Plateau)*e^{(-kx)}+Plateau$

Substrate	y_o (mAu)	k (min^{-1})	$t_{0.5}$ (min)	95% CI ($t_{0.5}$)
$(LLys)_6$	1.00	0.301	2.3	2.0 to 2.7
$(NLys)_6$	0.99	0.177	3.9	2.4 to 7.1
$(LPro)_6$	0.98	0.008	85	73 to 100
$(NAla)_6$	0.90	0.007	94	78 to 117

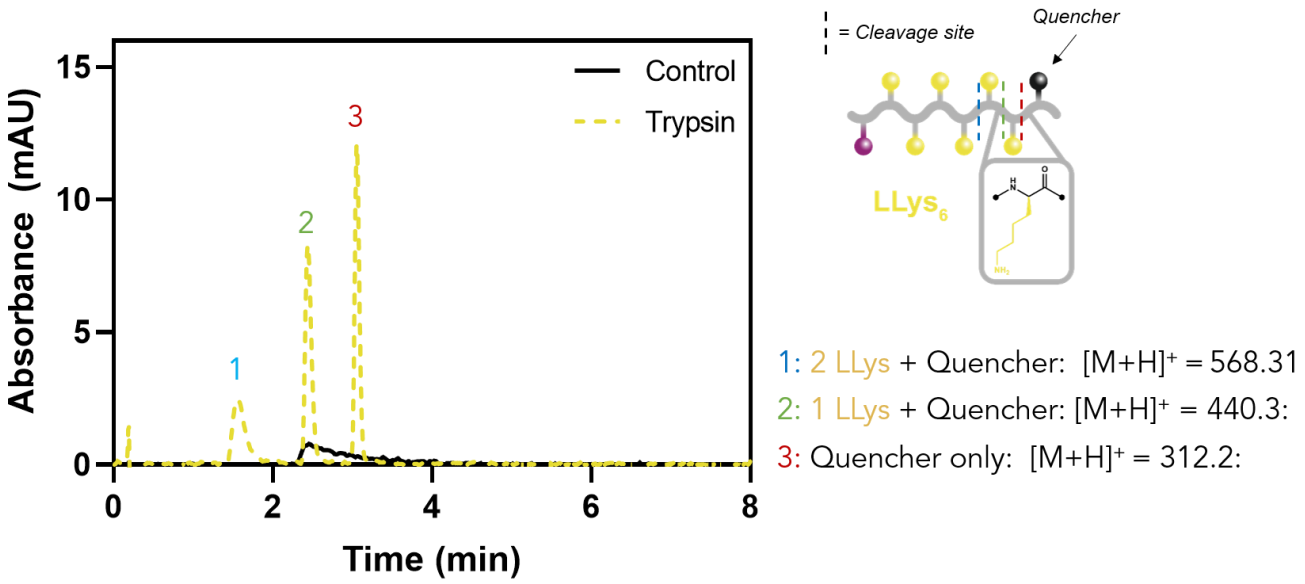
Supplementary Figure S18. Exponential decay degradation rate fits for 6mer fluorescent homopolymers. A-D) Exponential decay degradation rates were fit to the max absorbance of each sample normalized against its respective control at fixed retention times. Each point represents max absorbance of degraded substrate samples normalized to max absorbance of the intact control (n=3). The resulting exponential decay equation, $y = (y_0 - Plateua) * e^{-kx} + Plateau$ (E) was used to calculate half-lives, $t_{0.5}$, of each substrate (F). *y* is the absorbance representing intact substrate remaining, *x* is the incubation time of the substrate (in min), k is the fitted rate constant in $min^{-1}$and *Plateau* was constrained to zero. Error bars for the bar graph represent the 95% confidence interval. Significance brackets extend over all samples that are significantly different from one another such that *p ≤ 0.05 with exceptions (ns) denoted.



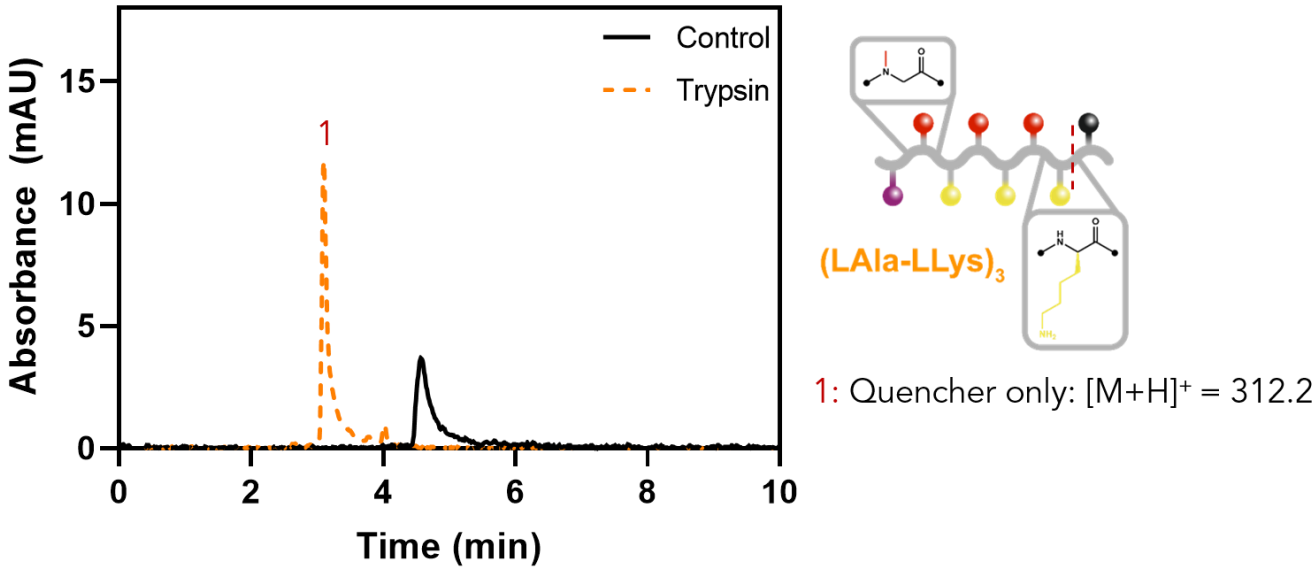
Supplementary Figure S19. Fluorescence spectral scan of intact oligomers indicate successful quenching of fluorophore, as compared to the spectra of the free fluorophore in solution (neon green).



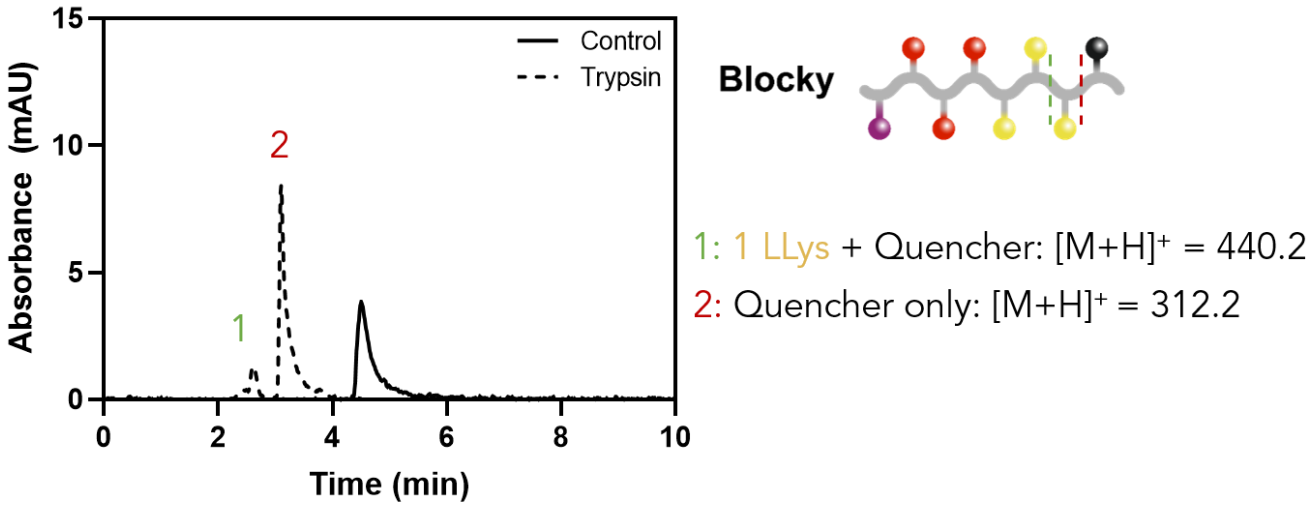
Supplementary Figure S20. Fluorescence tracking of 7-methoxycoumarin (Mca) fluorophore when exposed to MCO (10 mM H_2O_2 + 50 µM $CuSO_4$) stimuli and trypsin (0.1 µM) stimuli. The constant signal indicates the fluorophore is stable to both stimuli.



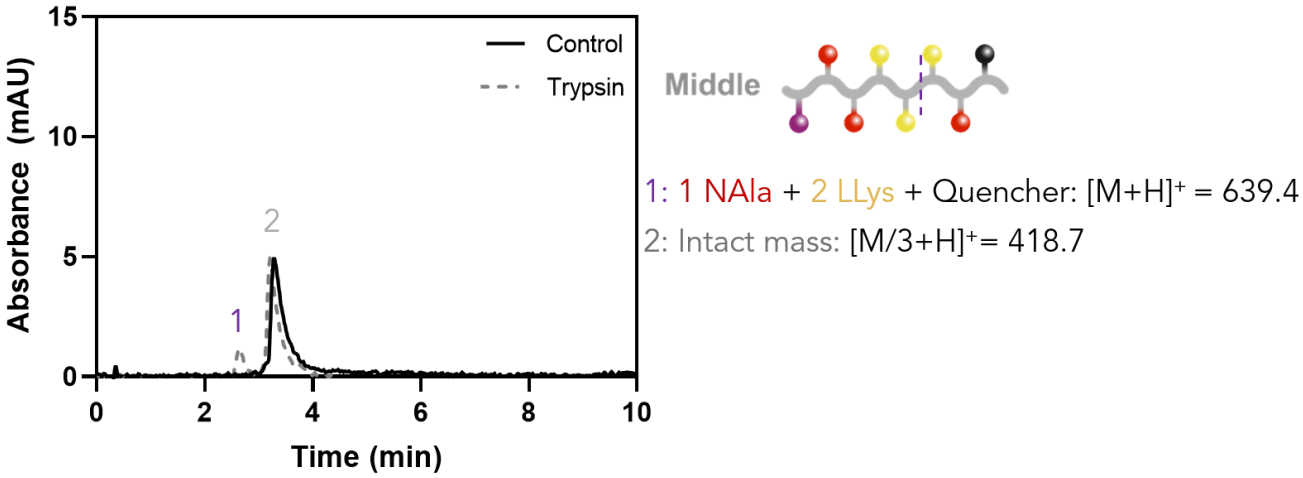
Supplementary Figure S21. LC/MS chromatographs of fluorescent $LLys_6$ oligomer collected 3 hours after exposure to either PBS (control) or trypsin (0.1 µM). Traces were collected at 400 nm to detect the absorbance of the dinitrophenyl quencher. Thus, there was only one peak corresponding to a single fragment from each cleavage event. Those peaks were matched to corresponding extracted ion chromatographs generated at specific m/z ratios according to the expected mass fragments to determine their identity. Cleavage sites observed are indicated by dashed lines and coordinated by color. Peaks are numbered and labeled based on their respective cleavage fragment and corresponding masses observed. Traces below followed the same procedure.



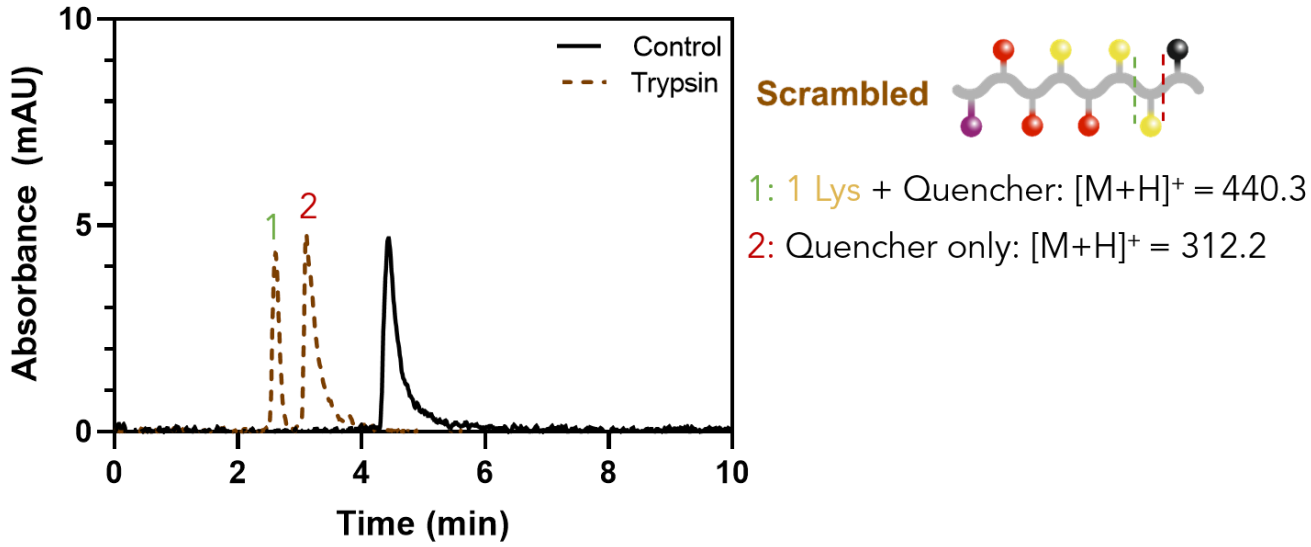
Supplementary Figure S22. LC/MS chromatographs of fluorescent $(NAla\text{-}LLys)_3$ or 'alternatig' oligomer collected 3 hours after exposure to either PBS (control) or trypsin (0.1 µM).



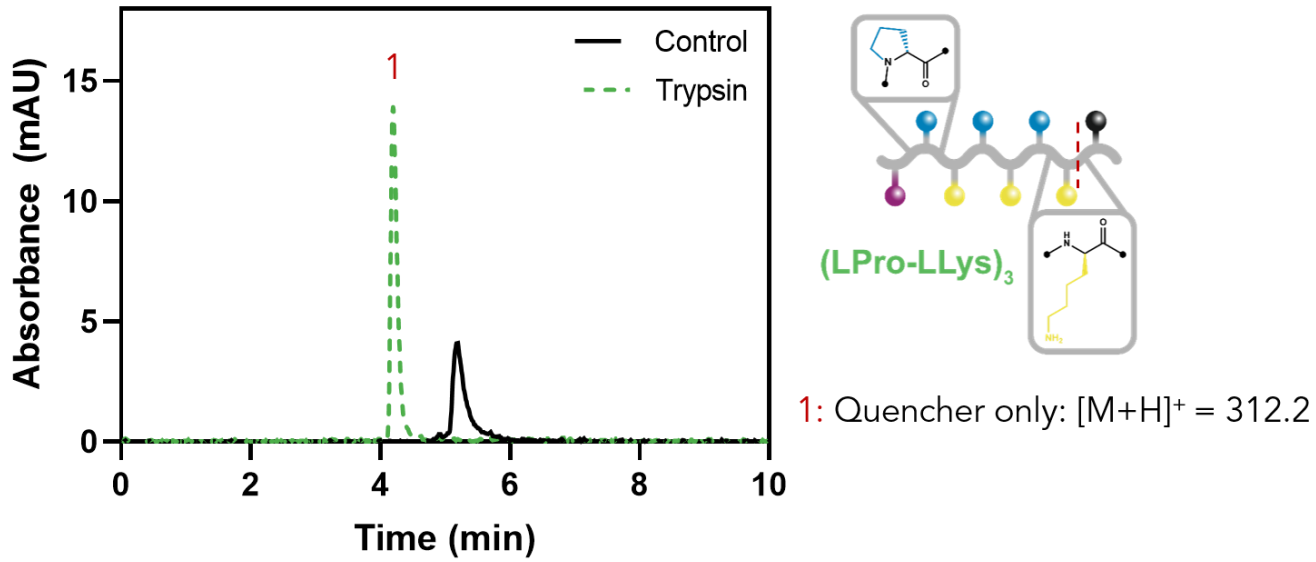
Supplementary Figure S23. LC/MS chromatographs of fluorescent 'Blocky' oligomer collected 3 hours after exposure to either PBS (control) or trypsin (0.1 µM).



Supplementary Figure S24. LC/MS chromatographs of fluorescent 'Middle' oligomer collected 3 hours after exposure to either PBS (control) or trypsin (0.1 µM).



Supplementary Figure S25. LC/MS chromatographs of fluorescent 'Scrambled' oligomer collected 3 hours after exposure to either PBS (control) or trypsin (0.1 µM).



Supplementary Figure S26. LC/MS chromatographs of fluorescent $(LPro\text{-}LLys)_3$ oligomer collected 3 hours after exposure to either PBS (control) or trypsin (0.1 µM).