SUPPORTING INFORMATION FOR

Taking a Bite Out of Amyloid: Mechanistic Insights into α-Synuclein Degradation by Cathepsin L

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MATERIALS AND METHODS:

Proteins and reagents. Recombinant acetylated α-synuclein (α-syn) was expressed in *E. Coli* BL21(DE3) (Stratagene) using human α-syn (pRK172)[1] and yeast NatB genes.[2] Cell cultures were first grown in LB media at 37 ˚C. After reaching an $OD_{600\ nm}$ = 0.6, the temperature was subsequently dropped to 21˚C and the cells were induced with IPTG (1 mM) for 20 h. Cells were harvested by centrifugation using a Sorvall SLC6000 rotor at 6,000 rpm for 20 min. Purification of the acetylated α-syn was adopted from a previously described protocol for non-acetylated α-syn.[3] To lyse the cells, cells were resuspended in lysis buffer (100 mM Tris, 300 mM NaCl, 1 mM EDTA, pH 8.0 supplemented with 1 mM PMSF) and stirred under N_2 for 10 min followed by a 10-min heat treatment in a boiling water bath. The cellular debris were removed by centrifugation (Sorval SS34, 18,000 rpm for 30 min, 4 ˚C). The supernatant which contained α-syn was then titrated to pH 3.5 by the addition of concentrated HCl, stirred for 10 min at 4 ˚C, and separated from insoluble materials by ultracentrifugation using a Beckman Ti45 rotor at 30,000 rpm for 30 min at 4 ˚C. Following two changes of dialysis into a low salt buffer (20 mM Tris, pH 8), the protein was then applied to a HiPrep DEAE column (GE Healthcare) and eluted with a linear salt gradient. Fractions containing α-syn was subjected to another round of anionic exchange chromatography by using a MonoQ column (16/10, GE Healthcare). All steps were carried out at 4 ˚C. To confirm the purified protein under these conditions was monomeric, gel-filtration chromatography was used. A typical elution time of 53–59 min was obtained on a HiLoad 16/60 Superdex 75 prep grade column (GE Healthcare). Filtered buffers (0.22 μm) were used for all experiments. Purity of acetylated α-syn was assessed by SDS-PAGE (NuPAGE 4–12% Bis-Tris, Invitrogen) and confirmed by mass spectrometry (NHLBI Biochemistry Core). Based on mass spectrometry, the acetylation was 100 % with a small population of methionine oxidized species. Purified protein was stored at −80 ºC until use. Protein concentrations were determined using a molar extinction coefficient estimated on basis of amino-acid content: $\varepsilon_{280\ nm}$ = 5,120 M^{-1} cm^{-1}. Purified Cathepsin L (CtsL) (C6854-25UG) from human liver was purchased from Sigma-Aldrich (St. Louis, MO) and used as received.

Degradation reactions of α-syn. Purified α-syn was buffered exchanged into pH 5 buffer by a PD-10 column and subsequently filtered through a YM100 spin column (MWCO 100 kD, Millipore) to remove any potential aggregates. In glass vial, α-syn monomer (15 μM) was incubated with cathepsin L (15 nM) in reaction buffer (50 mM NaOAc, 20 mM NaCl, 5 mM DTT, pH 5) in a total volume of 500 μL and agitated at 600 rpm at 37 ˚C in a Mini-Micro 980140 shaker (VWR). Reactions (50 μL) were taken either every 15 min between 0 to105 min or after 23 h and terminated with 2M GuHCl final conc. For α-syn fibril experiments, α-syn monomer (15, 29 or 40 μM) was agitated at 600 rpm in glass vials for 3 d at pH 5 and 37 ˚C in a Mini-Micro 980140 shaker (VWR). Aggregated samples were centrifuged at 16,100 *g* for 20 min and the supernatant was discarded. Fibril pellets were re-suspended in reaction buffer at desired concentrations. Brief bath sonication (10 min) was performed to give a more homogenous sample. Fibril degradation was performed in sealed glass vials (with magnetic stir bar) containing 500 μl solution with 150 nM CtsL. Reactions (50 μL) were taken at 0.5, 1, 2, 3, 4 or 23 h and terminated with 2 M GuHCl final concentration.

LC-MS. Samples (30 μL) were separated using a Vydac 218TP C_{18} reverse-phase column (2.1 x 50 mm, 5 μm, Vydac) and an Agilent 1100 series HPLC (Agilent Technologies) coupled to an Agilent G1946D mass selective detector (MSD) equipped with an electrospray ionization (ESI) interface (Agilent Technologies). Mass spectra were obtained using positive ion mode. The HPLC system and MSD were controlled and data were analyzed using LC/MSD ChemStation software (Rev. A.10.02, Agilent Technologies).

TEM. Samples (10 µL) were put on TEM grids (400-mesh formvar and carbon coated copper, Electron Microscopy Sciences) for approx. 2 min and wicked away by filter paper. An addition of 10 µL of deionized water was then applied and wicked away immediately. A solution of 1% uranyl acetate (10 µL) is placed on the grid for 2 min, wicked away, and air-dried. TEM was performed using a JEOL JEM 1200EX transmission electron microscope (accelerating voltage 80 keV) equipped with an AMT XR-60 digital camera (NHLBI EM Core Facility).

(1) Jakes, R.; Spillantini, M. G.; Goedert, M. *FEBS Letters* **1994**, *345*, 27.
(2) Johnson, M.; Coulton, A. T.; Geeves, M. A.; Mulvihill, D. P. *Plos One* **2010**, *5*.
(3) Pfefferkorn, C. M.; Lee, J. C. *J. Phys. Chem. B* **2010**, *114*, 4615.

Table S1. MS analysis taken after incubating recombinant α-syn$_m$ (15 μM) with CtsL (15 nM) for 15 and 30 min at pH 5 and 37 °C.

CtsL + α-syn$_m$ (15 min incubation)

Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
14501.72	14502.16	1–140
13835.81	13836.37	6–140
13450.33	13450.91	10–140
12667.45	12669.97	18–140
11683.00	11683.02	28–140
10345.58	10345.86	1–103
10279.72	10280.25	42–140
9313.19	9314.15	51–140
9086.43	9086.88	54–140
7603.22	7603.29	1–75
7600.30	7601.23	68–140
6915.99	6916.44	76–140
5433.12	5433.12	1–53
5205.88	5206.02	1–50
4239.57	4239.92	1–41
4182.59	4182.87	1–40
4173.77	4174.31	104–140
1851.38	1852.02	1–17

CtsL + α-syn$_m$ (30 min incubation)

Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
14501.66	14502.16	1–140
13835.93	13836.37	6–140
13450.46	13450.91	10–140
12667.82	12669.97	18–140
11682.96	11683.02	28–140
10599.08	10599.61	39–140
10345.56	10345.86	1–103
10336.26	10337.30	41–140
10279.67	10280.25	42–140
9313.34	9314.15	51–140
9086.43	9086.88	54–140
7602.45	7603.29	1–75
7600.30	7601.23	68–140
6915.92	6916.44	76–140
5433.06	5433.12	1–53
5205.84	5206.02	1–50
4767.31	4767.50	6–53
4239.52	4239.92	1–41
4182.63	4182.87	1–40
4173.77	4174.31	104–140
3920.33	3920.56	1–38
2836.19	2836.32	1–27
2665.90	2666.11	1–25
1851.90	1852.02	1–17

Table S2. MS analysis taken after incubating recombinant α-syn$_m$ (15 μM) with CtsL (15 nM) for 45 and 60 min at pH 5 and 37 °C.

CtsL + α-syn$_m$ (45 min incubation)		
Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
14501.65	14502.16	1–140
13835.95	13836.37	6–140
13450.44	13450.91	10–140
12667.82	12669.97	18–140
11682.92	11683.02	28–140
10599.09	10599.61	39–140
10345.59	10345.86	1–103
10336.29	10337.30	41–140
10279.70	10280.25	42–140
9313.45	9314.15	51–140
9086.42	9086.88	54–140
7602.52	7603.29	1–75
7600.70	7601.23	68–140
6918.64	6918.94	1–67
6915.91	6916.44	76–140
6123.55	6123.96	42–103
5433.06	5433.12	1–53
5205.78	5206.02	1–50
4767.32	4767.50	6–53
4381.91	4382.04	10–53
4239.58	4239.92	1–41
4182.60	4182.87	1–40
4173.74	4174.31	104–140
3920.14	3920.56	1–38
3708.11	3708.27	1–36
3598.75	3599.10	18–53
3381.53	3381.83	42–75
3188.38	3188.67	10–41
2836.27	2836.32	1–27
2665.90	2666.11	1–25
1851.89	1852.02	1–17

CtsL + α-syn$_m$ (60 min incubation)		
Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
14501.60	14502.16	1–140
13450.73	13450.91	10–140
12668.10	12669.97	18–140
11682.94	11683.02	28–140
10345.51	10345.86	1–103
10336.24	10337.30	41–140
10279.55	10280.25	42–140
9313.41	9314.15	51–140
9086.37	9086.88	54–140
7602.75	7603.29	1–75
7600.68	7601.23	68–140
6918.63	6918.94	1–67
6915.90	6916.44	76–140
6123.58	6123.96	42–103
6089.01	6089.49	84–140
5433.08	5433.12	1–53
5205.76	5206.02	1–50
4767.31	4767.50	6–53
4381.91	4382.04	10–53
4239.61	4239.92	1–41
4182.57	4182.87	1–40
4173.73	4174.31	104–140
3920.17	3920.56	1–38
3708.13	3708.27	1–36
3598.78	3599.10	18–53
3381.50	3381.83	42–75
3188.37	3188.67	10–41
2759.97	2760.14	76–103
2665.89	2666.11	1–25
2170.10	2170.54	6–27
1851.90	1852.02	1–17

Table S3. MS analysis taken after incubating recombinant α-syn$_m$ (15 μM) with CtsL (15 nM) for 75 and 90 min at pH 5 and 37 °C.

CtsL + α-syn$_m$ (75 min incubation)

Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
14501.46	14502.16	1–140
12668.40	12669.97	18–140
10345.52	10345.86	1–103
10336.14	10337.30	41–140
10279.54	10280.25	42–140
9313.51	9314.15	51–140
9086.40	9086.88	54–140
8511.17	8511.68	18–103
7602.89	7603.29	1–75
7600.71	7601.23	68–140
7402.91	7403.50	1–73
6918.63	6918.94	1–67
6915.92	6916.44	76–140
6123.58	6123.96	42–103
6089.02	6089.49	84–140
5433.03	5433.12	1–53
5205.76	5206.02	1–50
5157.40	5157.85	51–103
4957.70	4958.19	97–140
4767.34	4767.50	6–53
4416.03	4416.59	102–140
4381.91	4382.04	10–53
4239.58	4239.92	1–41
4182.60	4182.87	1–40
4173.77	4174.31	104–140
3920.17	3920.56	1–38
3708.15	3708.27	1–36
3598.73	3599.10	18–53
3381.50	3381.83	42–75
3188.34	3188.67	10–41
2836.45	2836.32	1–27
2760.00	2760.14	76–103
2665.78	2666.11	1–25
2170.10	2170.54	6–27
1851.91	1852.02	1–17

CtsL + α-syn$_m$ (90 min incubation)

Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
10345.49	10345.86	1–103
10336.31	10337.30	41–140
10279.69	10280.25	42–140
9313.46	9314.15	51–140
9086.38	9086.88	54–140
7602.95	7603.29	1–75
7600.64	7601.23	68–140
7402.91	7403.50	1–73
6918.61	6918.94	1–67
6915.89	6916.44	76–140
6674.56	6675.15	79–140
6123.58	6123.96	42–103
6088.99	6089.49	84–140
5433.05	5433.12	1–53
5205.76	5206.02	1–50
5157.42	5157.85	51–103
4957.71	4958.19	97–140
4930.28	4930.59	54–103
4767.31	4767.50	6–53
4416.02	4416.59	102–140
4381.82	4382.04	10–53
4239.57	4239.92	1–41
4208.49	4208.78	42–83
4182.59	4182.87	1–40
4173.76	4174.31	104–140
4154.74	4154.78	10–50
3920.16	3920.56	1–38
3708.13	3708.27	1–36
3598.76	3599.10	18–53
3574.12	3574.14	6–41
3381.55	3381.83	42–75
3188.41	3188.67	10–41
3181.38	3181.59	42–73
3015.09	3015.41	54–83
2836.40	2836.32	1–27
2759.95	2760.14	76–103
2665.76	2666.11	1–25
2518.57	2518.85	79–103
2415.38	2415.73	51–75
2170.08	2170.54	6–27
1976.00	1976.26	76–96
1851.90	1852.02	1–17
1211.01	1211.38	42–53

Table S4. MS analysis taken after incubating recombinant α-syn$_m$ (15 µM) with CtsL (15 nM) for 105 min and 23 h at pH 5 and 37 °C.

CtsL + α-syn$_m$ (105 min incubation)		
Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
10279.64	10280.25	42–140
9313.47	9314.15	51–140
9086.39	9086.88	54–140
7603.13	7603.29	1–75
7600.65	7601.23	68–140
7402.91	7403.50	1–73
6918.67	6918.94	1–67
6915.91	6916.44	76–140
6674.60	6675.15	79–140
6123.59	6123.96	42–103
6088.95	6089.49	84–140
5433.05	5433.12	1–53
5205.75	5206.02	1–50
5157.42	5157.85	51–103
4957.71	4958.19	97–140
4930.31	4930.59	54–103
4767.31	4767.50	6–53
4416.02	4416.59	102–140
4381.87	4382.04	10–53
4239.57	4239.92	1–41
4208.49	4208.78	42–83
4182.60	4182.87	1–40
4173.76	4174.31	104–140
4154.74	4154.78	10–50
3920.16	3920.56	1–38
3708.13	3708.27	1–36
3598.73	3599.10	18–53
3574.37	3574.14	6–41
3381.51	3381.83	42–75
3188.37	3188.67	10–41
3181.46	3181.59	42–73
2869.13	2869.31	10–38
2836.42	2836.32	1–27
2759.95	2760.14	76–103
2665.78	2666.11	1–25
2518.61	2518.85	79–103
2415.39	2415.73	51–75
2405.46	2405.73	18–41
2169.42	2170.54	6–27
1975.94	1976.26	76–96
1851.91	1852.02	1–17
1211.01	1211.38	42–53

CtsL + α-syn$_m$ (23 h incubation)		
Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
6089.00	6089.49	84–140
4957.74	4958.19	97–140
4415.99	4416.59	102–140
4173.79	4174.31	104–140
3708.05	3708.27	1–36
3444.64	3444.93	68–103
3086.00	3086.41	84–114
2759.94	2760.14	76–103
2660.67	2661.05	68–96
2517.23	2517.87	76–101
2470.64	2470.85	10–34
2405.45	2405.73	18–41
2387.47	2387.72	28–50
2249.35	2249.55	18–39
1987.90	1988.23	54–73
1975.97	1976.26	76–96
1932.90	1933.19	84–103
1851.91	1852.02	1–17
1690.62	1690.92	84–101
1687.25	1687.91	18–34
1614.48	1614.86	10–25
1520.42	1520.71	84–99
1503.41	1503.67	54–67
1148.93	1149.31	84–96

Table S5. MS analysis taken after incubating recombinant α-syn$_f$ (15 μM) with CtsL (150 nM) for 30 min and 1 h at pH 5 and 37 ˚C.

CtsL + α-syn$_f$ (30 min incubation)

Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
14501.57	14502.16	1–140
13797.12	13797.47	1–134
12875.07	12875.49	1–126
12382.35	12383.00	1–122
11498.42	11499.07	1–114
10345.22	10345.86	1–103
10103.21	10103.59	1–101
10832.72	10833.29	6–114
1413.11	1413.50	102–114
1393.92	1394.43	115–126
1170.34	1171.23	104–114
939.40	939.99	127–134
901.35	901.90	115–122
722.29	722.71	135–140

CtsL + α-syn$_f$ (1 h incubation)

Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
14501.57	14502.16	1–140
13797.19	13797.47	1–134
12875.04	12875.49	1–126
12382.40	12383.00	1–122
11498.40	11499.07	1–114
10345.19	10345.86	1–103
10103.28	10103.59	1–101
10832.70	10833.29	6–114
4239.69	4239.92	1–41
5205.84	5206.02	1–50
3708.10	3708.29	1–36
2665.87	2666.11	1–25
2517.47	2517.87	76–101
1413.09	1413.50	102–114
1393.92	1394.43	115–126
1170.60	1171.23	104–114
939.40	939.99	127–134
901.35	901.94	115–122
722.29	722.71	135–140

Table S6. MS analysis taken after incubating recombinant α-syn$_f$ (15 μM) with CtsL (150 nM) for 2, 3 and 4 h at pH 5 and 37 °C.

CtsL + α-syn$_f$ (2 h incubation)		
Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
14501.55	14502.16	1–140
13797.19	13797.47	1–126
12382.40	12383.00	1–122
11498.36	11499.07	1–114
10345.19	10345.86	1–103
10103.24	10103.59	1–101
10832.81	10833.29	6–114
9051.86	9052.34	10–101
6123.50	6123.96	42–103
5262.80	5263.08	1–51
5205.91	5206.02	1–50
4687.85	4688.31	54–101
4239.62	4239.92	1–41
4182.59	4182.87	1–40
4154.62	4154.62	10–50
3708.10	3708.27	1–36
3202.27	3202.66	68–101
3188.04	3188.67	10–41
2759.90	2760.14	76–103
2665.79	2666.11	1–25
2517.47	2517.87	76–101
2347.41	2347.65	76–99
1851.86	1852.02	1–17
1413.06	1413.50	102–114
1170.55	1171.23	104–114
939.39	939.99	127–134
901.35	901.94	115–122
722.29	722.71	135–140

CtsL + α-syn$_f$ (3 and 4h incubation)		
Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
14501.57	14502.16	1–140
13797.19	13797.47	1–126
12382.40	12383.00	1–122
11498.40	11499.07	1–114
10345.17	10345.86	1–103
10103.28	10103.59	1–101
10832.72	10833.29	6–114
6448.10	6448.42	1–62
6190.94	6191.11	1–60
5262.80	5263.08	1–51
5205.81	5206.02	1–50
4687.96	4688.31	54–101
4239.72	4239.92	1–41
4211.67	4211.83	10–51
4182.62	4182.87	1–40
4154.73	4154.62	10–50
3708.11	3708.27	1–36
3574.44	3574.14	6–41
3521.96	3522.10	1–34
3444.69	3444.93	68–103
3202.30	3202.66	68–101
3188.04	3188.67	10–41
2759.91	2760.14	76–103
2665.48	2666.12	1–25
2656.68	2657.02	10–36
2517.55	2517.87	76–101
2470.62	2470.85	10–34
2387.82	2387.72	28–50
2347.46	2347.65	76–99
1975.99	1976.26	76–96
1851.89	1852.02	1–17
1690.61	1690.92	84–101
1520.43	1520.71	84–99
1413.11	1413.50	102–114
1393.92	1394.43	115–126
1170.60	1171.23	104–114
939.40	939.99	127–134
901.35	901.94	115–122
722.29	722.71	135–140

Table S7. MS analysis taken after incubating recombinant α-syn$_f$ (15 μM) with CtsL (150 nM) for 23 h at pH 5 and 37 °C.

CtsL + α-syn$_f$ (23h incubation)		
Observed Mass (Da)	Theoretical Mass (Da)	Position of α-syn sequence
6448.10	6448.42	1–62
6190.94	6191.11	1–60
5262.80	5263.08	1–51
5205.81	5206.02	1–50
4687.96	4688.31	54–101
4239.72	4239.92	1–41
4211.67	4211.83	10–51
4154.73	4154.62	10–50
3708.11	3708.27	1–36
3574.44	3574.14	6–41
3521.96	3522.10	1–34
3444.69	3444.93	68–103
3202.30	3202.66	68–101
3188.04	3188.67	10–41
3032.11	3032.49	10–39
2786.78	2787.17	27–54
2759.91	2760.14	76–103
2665.48	2666.12	1–25
2656.68	2657.02	10–36
2517.55	2517.87	76–101
2470.62	2470.85	10–34
2387.82	2387.72	28–50
2347.46	2347.65	76–99
1975.99	1976.26	76–96
1851.89	1852.02	1–17
1690.61	1690.92	84–101
1520.43	1520.71	84–99
1413.11	1413.50	102–114
1393.92	1394.43	115–126
1170.60	1171.23	104–114
939.40	939.99	127–134
901.35	901.94	115–122
722.29	722.71	135–140

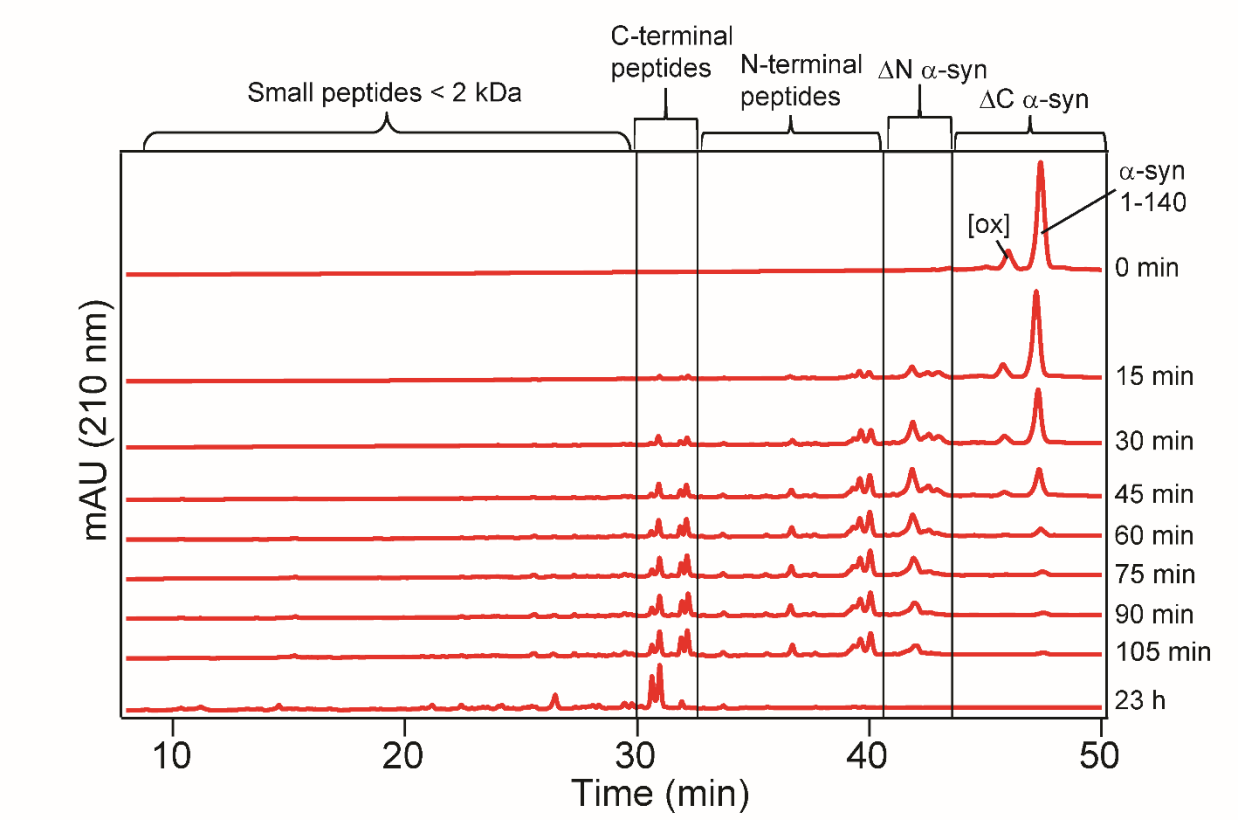

Fig S1. Purified human CtsL incubated with recombinant acetylated α-syn$_m$. LC traces taken as a function of time of α-syn$_m$ incubated with CtsL (15 nM) at pH 5 and monitored at 210 nm. A small fraction of methionine oxidized α-syn$_m$, derived from purified acetylated α-syn is shown. Peptide fragments are abbreviated as either N- or C-terminally derived peptides from full-length α-syn$_m$. For clarity, peptides derived from residues 1 to 75 are defined as N-terminal, while 76 to 140 are C-terminal. Peptide masses from each LC trace is shown in Table S1–4.

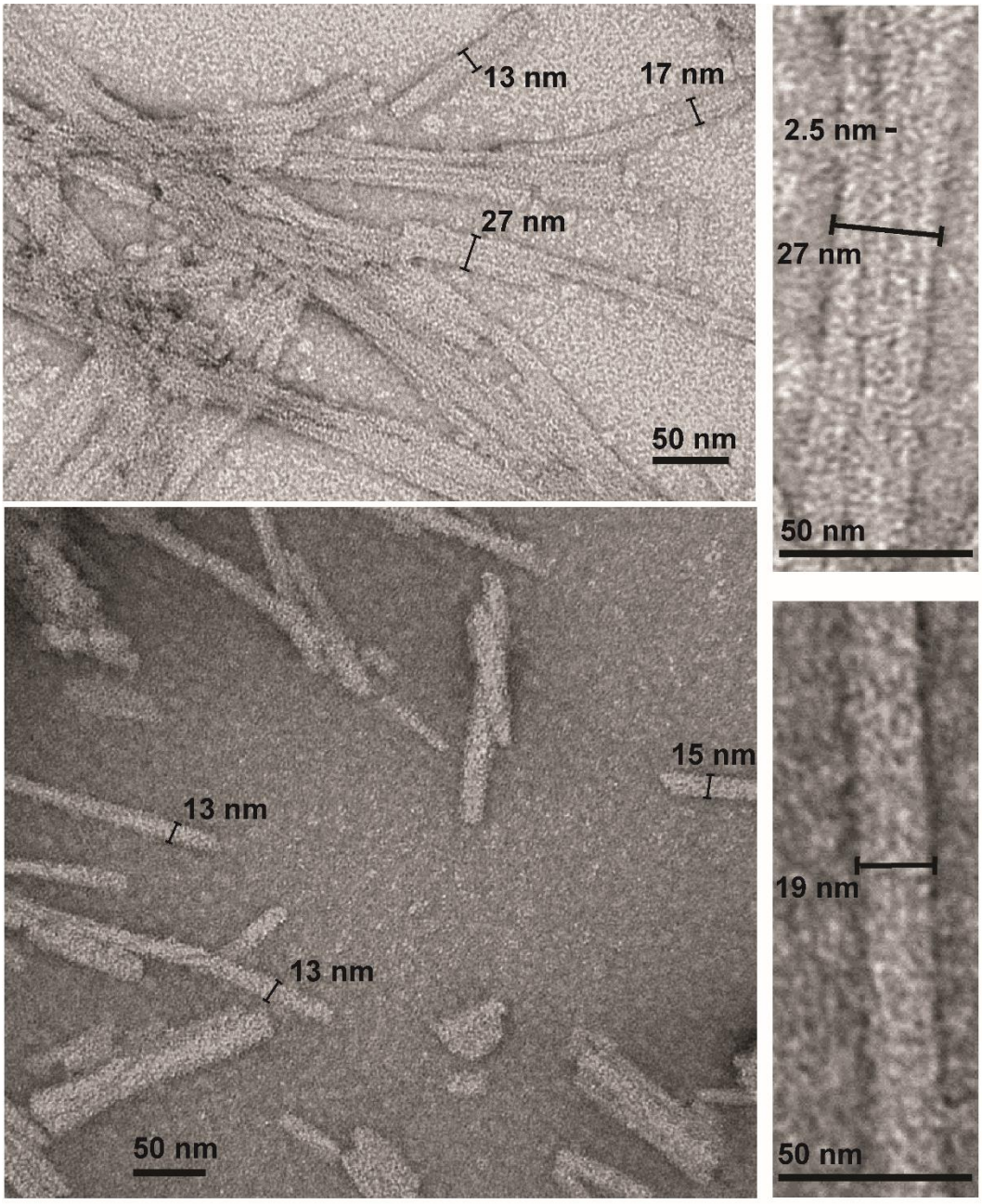

Figure S2. TEM images of α-syn fibrils (15 µM) at pH 5.

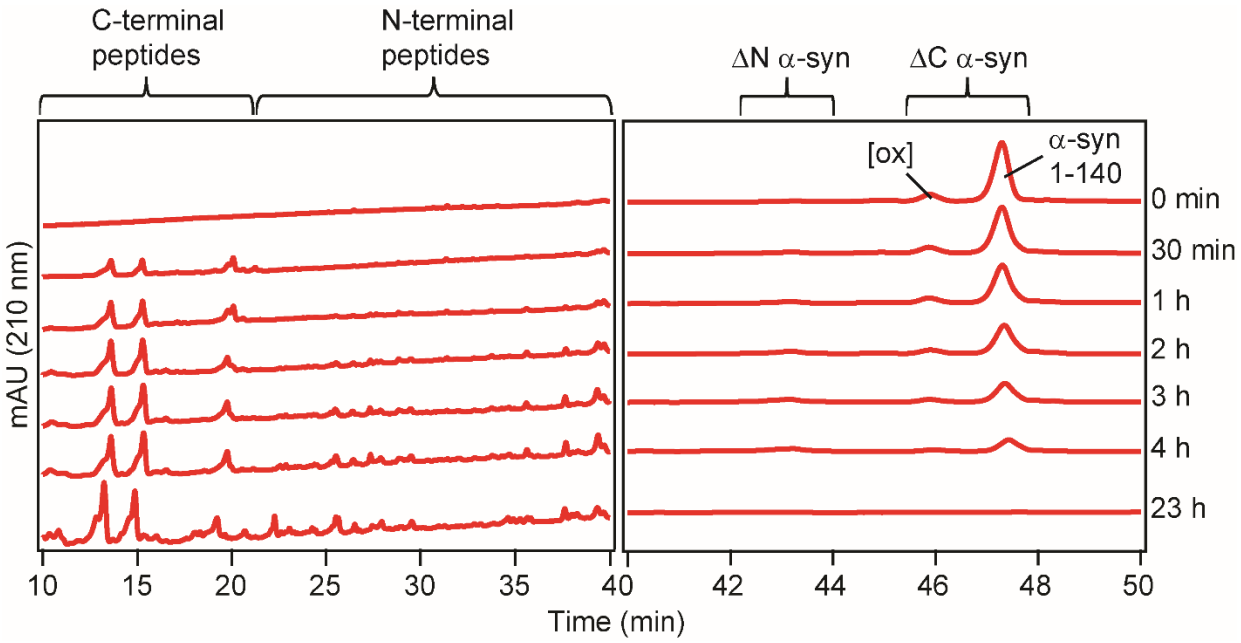

Fig S3. Purified human CtsL incubated with acetylated recombinant α-syn$_f$. LC traces taken as a function of time of α-syn$_f$ (15 µM) incubated with CtsL (150 nM) at pH 5 and monitored at 210 nm. A small fraction of methionine oxidized α-syn$_f$, derived from purified acetylated α-syn is shown. Peptide fragments are abbreviated as either N- or C-terminally derived peptides from full length α-syn$_m$. Cleavage sites between residues 1 to 75 are defined as N-terminal, while 76 to 140 is C-terminal. Peptide masses from each LC trace is shown in Table S5–7.

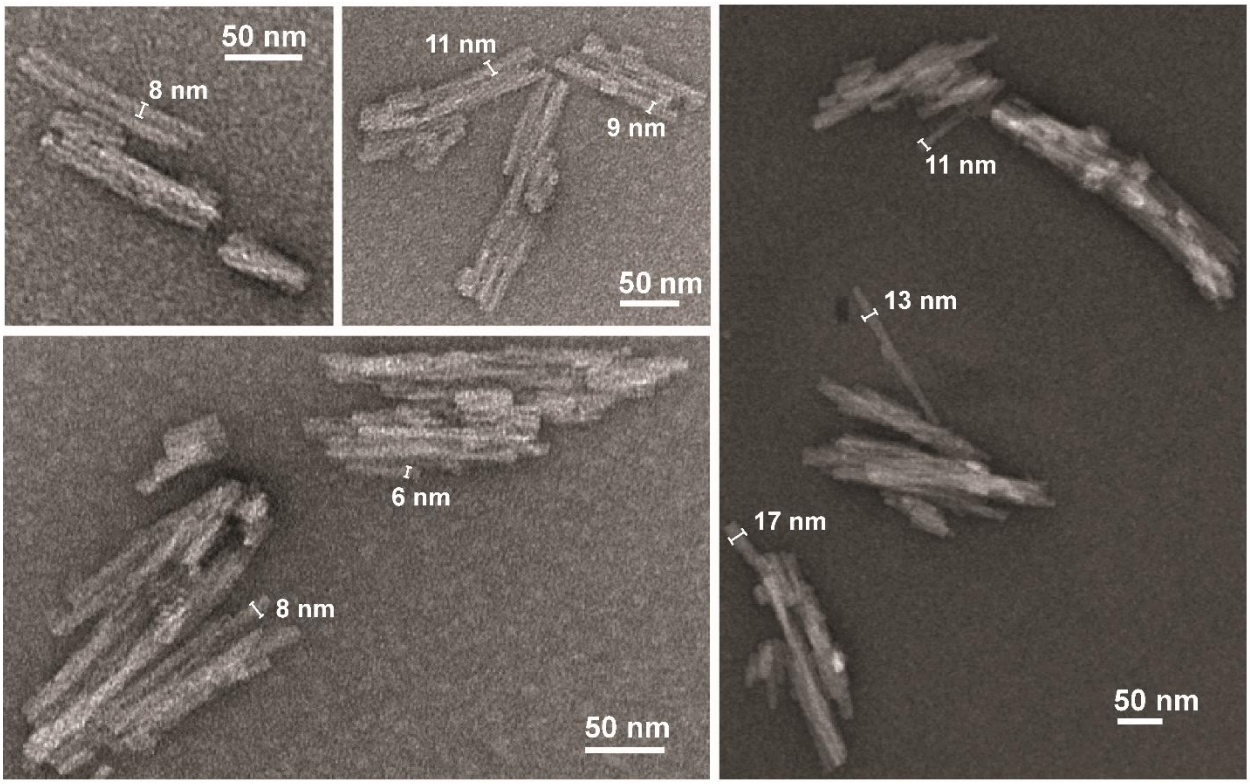

Figure S4. TEM images of α-syn fibrils (15 μM) incubated with CtsL (150 nM) for 30 min at pH 5.