SUPPLEMENTAL DATA

STRUCTURAL AND MORPHOLOGICAL CHARACTERIZATION OF AGGREGATED SPECIES OF α-SYNUCLEIN INDUCED BY DOCOSAHEXAENOIC ACID

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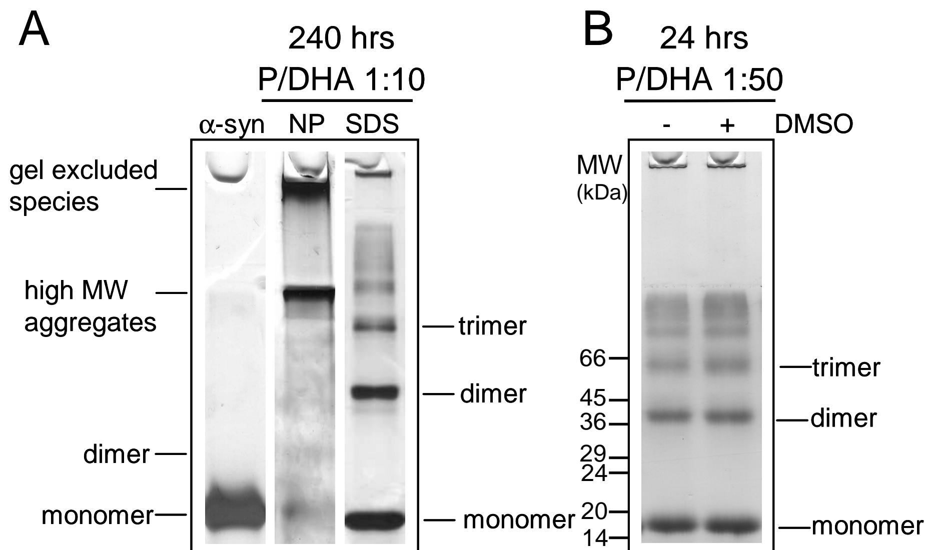


FIGURE S1. **Effect of SDS-and DMSO-treatment on the mixture of α-syn and DHA, analyzed by native- and SDS-PAGE**. (A) Aliquots (4 μg) taken from the mixture of α-syn and DHA (P/DHA 1:10) after 240 hrs of incubation were loaded into a native- (NP) and SDS- (SDS) PAGE in order to check the sensitivity of the aggregates formed in the presence of DHA to the electrophoretic denaturant conditions. α-Syn monomer was loaded as a control in the native gel. (B) SDS-PAGE analysis of the mixture of α-syn and DHA (P/DHA 1:50) after 24 hrs incubation. Aliquots (4 μg) taken from the mixture were dried in vacuum and subjected to two treatments with DMSO (95%), followed by drying. Then the samples were suspended in sample electrophoretic buffer containing SDS and loaded into a SDS-PAGE, using a standard procedure (lane +). Not treated samples are also loaded as a control (lane -). Numbers on the left indicate the molecular weight of proteins used as standard.

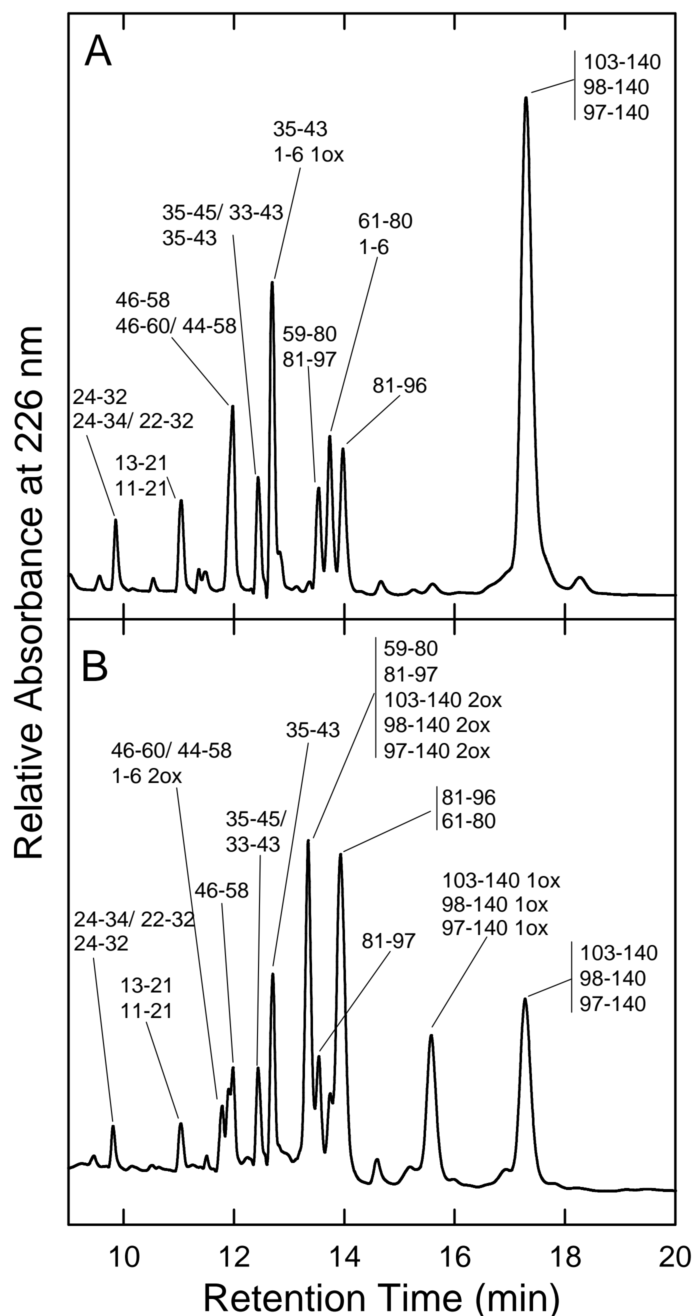


FIGURE S2. **Tryptic digests of α-syn and oxidized α-syn**. RP-HPLC of the mixtures of fragmentation of α-syn with trypsin (Promega Corporation, Madison, Wisconsin, USA). The enzymatic digestions were conducted on α-syn samples eluted by gel filtration with RT correspondent to the monomeric form (see Fig. 6A, RT 28 min)(A) and to the oligomeric one (Fig. 6C, RT 20 min) (B). The reactions were conducted, overnight, at an enzyme to protein ratio of 1:25 (by weight). The RP-HPLC was conducted on a Vydac C_{18} column (4.6 × 250 mm; The Separations Group, Hesperia, CA, USA), eluted with a gradient of acetonitrile/0.085% TFA *vs* water/0.1% TFA from 5 to 25% in 5 min, from 25 to 28% in 13 min, from 28 to 39% in 3 min and from 39 to 45% in 21 min, at a flow rate of 1 ml/min. The effluent was monitored by recording the absorbance at 226 nm. The identity of the peaks was assigned, analyzing the protein material of each fraction by ESI-mass spectrometry (Table S1).

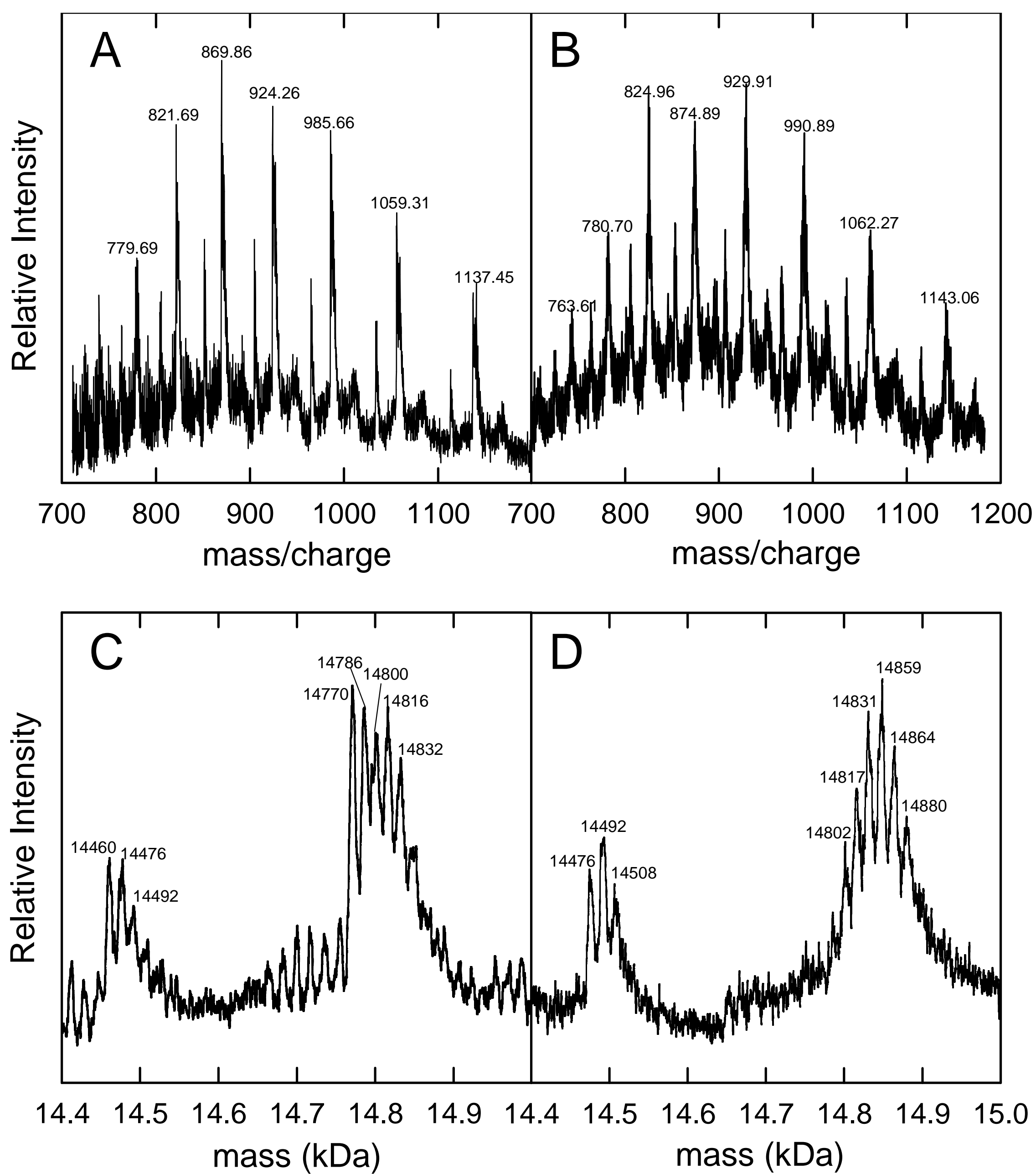
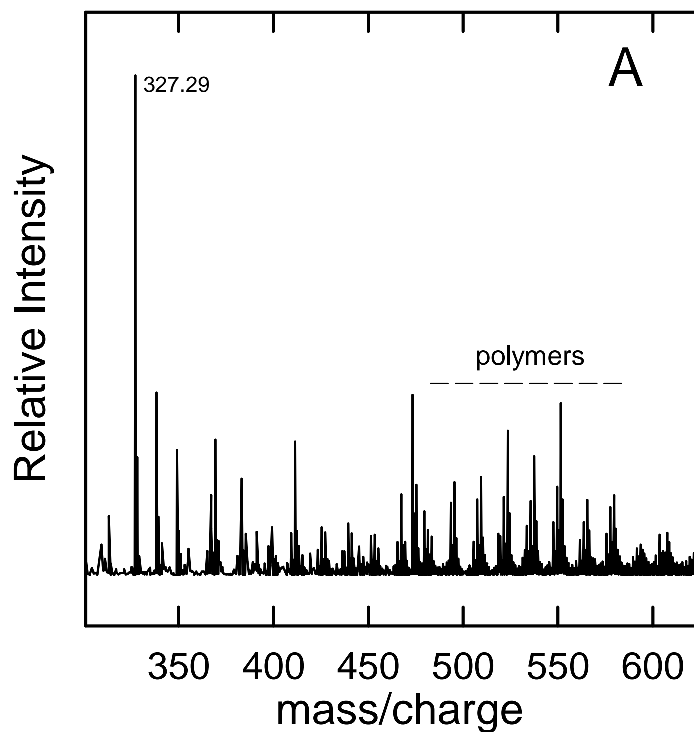


Fig. S3. **Mass spectrometry analysis**. ESI (A,B) and deconvoluted (C,D) mass spectra of samples corresponding to the peak at RT 30.5 min in the chromatograms reported in Fig. 6E (A,C) and Fig. 7E (B,D).



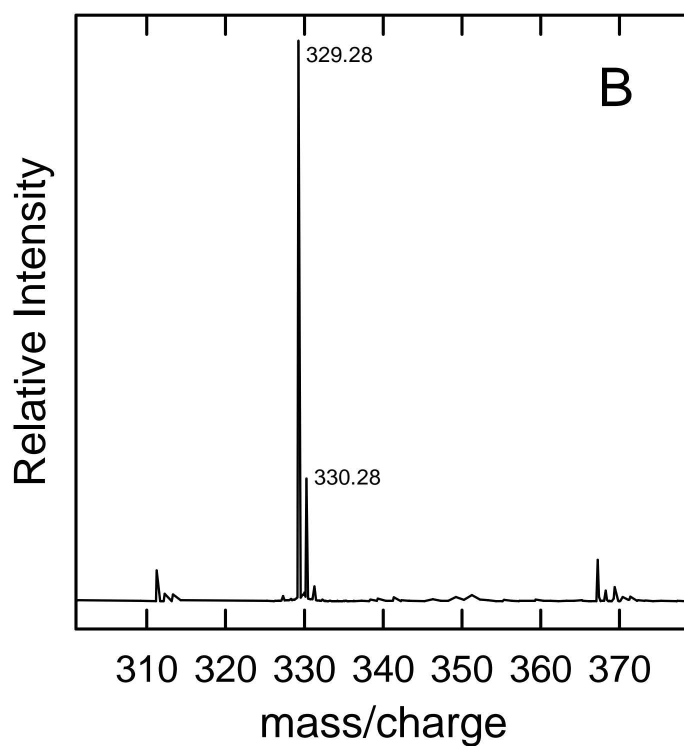


Fig. S4. **Mass spectrometry analysis**. ESI mass spectra of samples corresponding to the peaks at RT 31.9 min (A) and RT 32.8 (B) in the chromatogram reported in Fig. 7E.

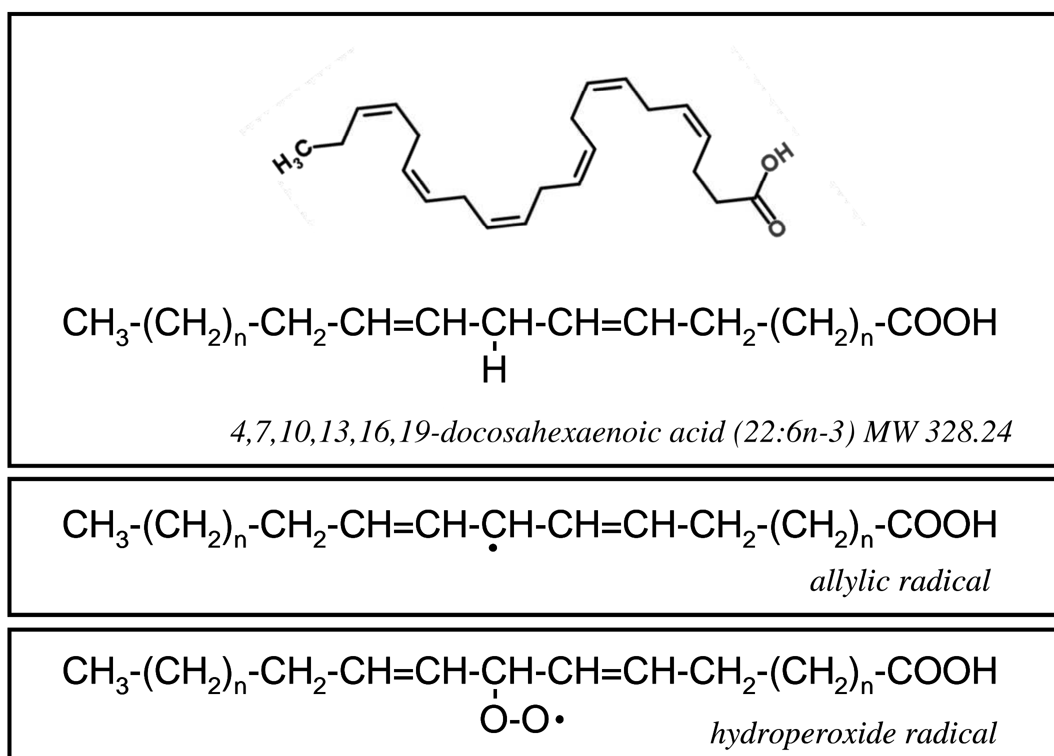


FIGURE S5. **Chemical structure of DHA.** The chemical structure of DHA was obtained from ChemSpider (Royal Society of Chemistry, Cambridge, UK). The monoisotopic mass is calculated from row formula is 328.24. The early peroxidation products of DHA are reported (Frankel, E. N. (1980) *Prog. Lipid Res*. **19**, 1–22).

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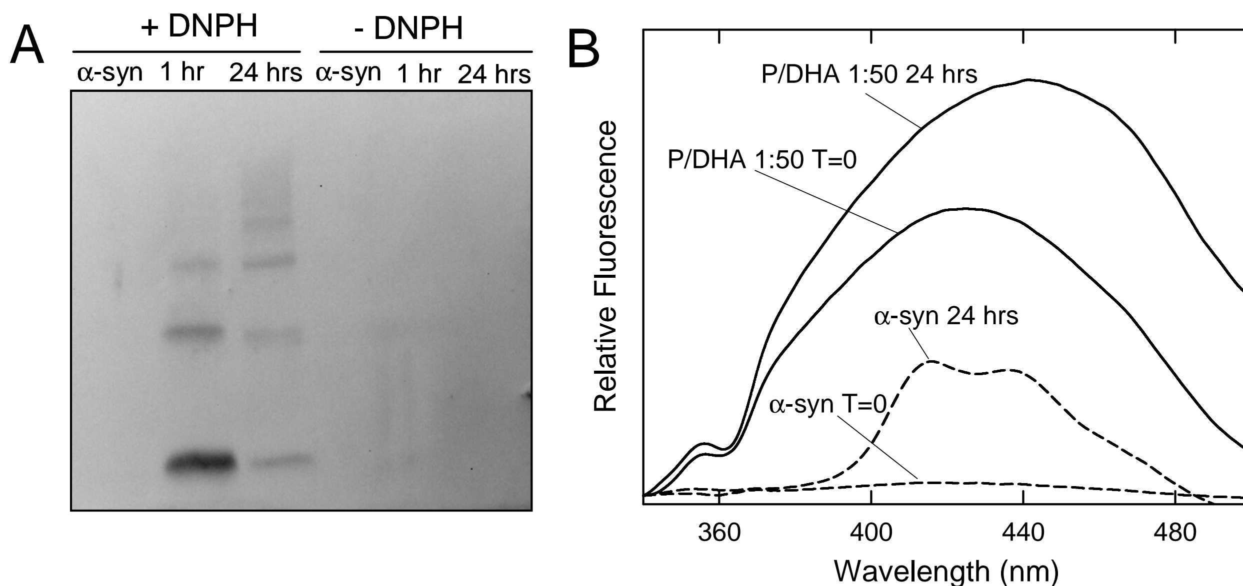

FIGURE S6. **Characterization of the chemical modifications of α-syn.** (A) The overall oxidation state of α-syn in the presence of DHA was assessed by immunoblot detection (Oxy-Blot kit, Millipore Corporation, Cork, Ireland) of carbonyl groups modified by with 2,4-dinitrophenylhydrazine (DNPH). The analyzed samples (1 μg) are as follows: α-syn with (+) and without (-) DNPH as a control (lanes 1,4); α-syn in the presence of DHA in molar ratio 1:50 after 1 hr of incubation with (+) and without (-) DNPH (lanes 2,5) and after 24 hrs with (+) and without (-) DNPH (lanes 3,6). The blot was developed following the manufacture procedure. (B) The detection of dityrosine formation was conducted by recording the fluorescence emission spectra from 350 to 500 nm, after excitation at 325 nm of α-syn samples in the absence (dashed lines) and in the presence (continuous line) of DHA (P/DHA 1:50).

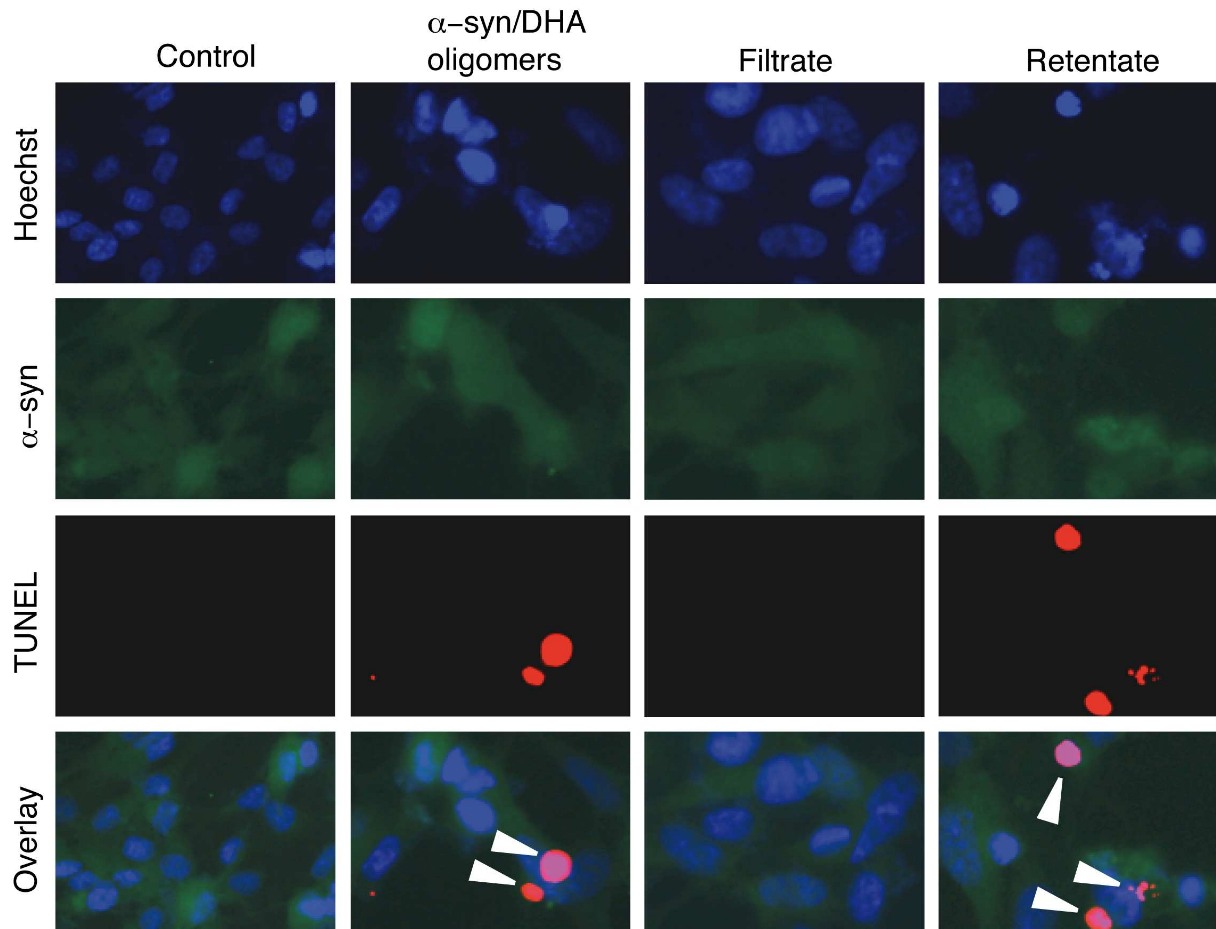

FIGURE S7. **Fluorescence microscopy analysis.** SH-SY5Y cells were treated with α-syn aggregates in the presence of DHA (P/DHA 1:50). After 24 hours, cells were fixed with 4% paraformaldehyde and stained for DNA double strand breaks using a Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) Cell Death Detection Kit (Roche Diagnostics GmbH, Mannheim, Germany). Cells were also stained with monoclonal anti-α-syn antibodies (BD Biosciences, NJ, USA) and counterstained with Hoechst 33242. Representative images of cells from selected conditions (untreated control; syn/DHA samples after 48 hour incubation; filtrate fraction of syn/DHA samples after 48 hour incubation; retentate fraction of syn/DHA samples after 48 hour incubation) are reported. Examples of cells counted as non-viable are shown with arrows in the P/DHA oligomers and retentate panels.

TABLE S1. Molecular masses of material contained in the fractions of the RP-HPLC chromatograms reported in Fig. S2 (A,B).

	RP-HPLC (RT, min)	Molecular Mass (Da) Found[a]	Calculated[b]	Peptide species
A	9.8	829.48	829.43	24-32
		1058.64	1058.57	24-34/22-32
	11.2	872.53	872.46	13-21
		1071.68	1071.59	11-21
	11.9	1294.84	1294.69	46-58
		1524.03	1523.83	46-60/44-58
	12.5	1179.84	1179.65	35-45/33-43
	12.8	950.71	950.51	35-43
		785.48	785.35	1-6 + 1ox
	13.6	2157.56	2156.18	59-80
		1606.18	1605.87	81-97
	13.8	1928.44	1927.04	61-80
		769.52	769.35	1-6
	14.4	1478.10	1477.78	81-96
	17.4	4288.19	4288.43	103-140
		4830.60	4830.03	98-140
		4958.18	4958.20	97-140
B	9.9	829.42	829.43	24-32
		1058.56	1058.57	24-34/22-32
	11.1	872.5	872.46	13-21
		1071.58	1071.59	11-21
	11.8	801.28	801.34	1-6 + 2ox
		1523.82	1523.83	46-60/44-58
	12.1	1294.72	1294.69	46-58
	12.5	1179.68	1179.65	35-45/33-43
	12.8	950.48	950.51	35-43
	13.4	1605.89	1605.87	81-97
		2156.19	2156.18	59-80
		4319.74	4320.42	103-140 + 2ox
		4862.40	4862.03	98-140 + 2ox
		4990.20	4990.2	97-140 + 2ox
	13.6	1605.8	1605.87	81-97
	14.0	1477.8	1477.78	81-96
		1927.18	1927.04	61-80
	15.6	4303.45	4303.41	103-140 + 1ox
		4846.24	4846.03	98-140 + 1ox
		4974.20	4974.41	97-140 + 1ox
	17.3	4288.21	4288.43	103-140
		4829.84	4830.03	98-140
		4958.45	4958.20	97-140

[a]Experimental molecular masses determined by ESI-MS.
[b]Molecular masses calculated from the amino acid sequence of α-syn.