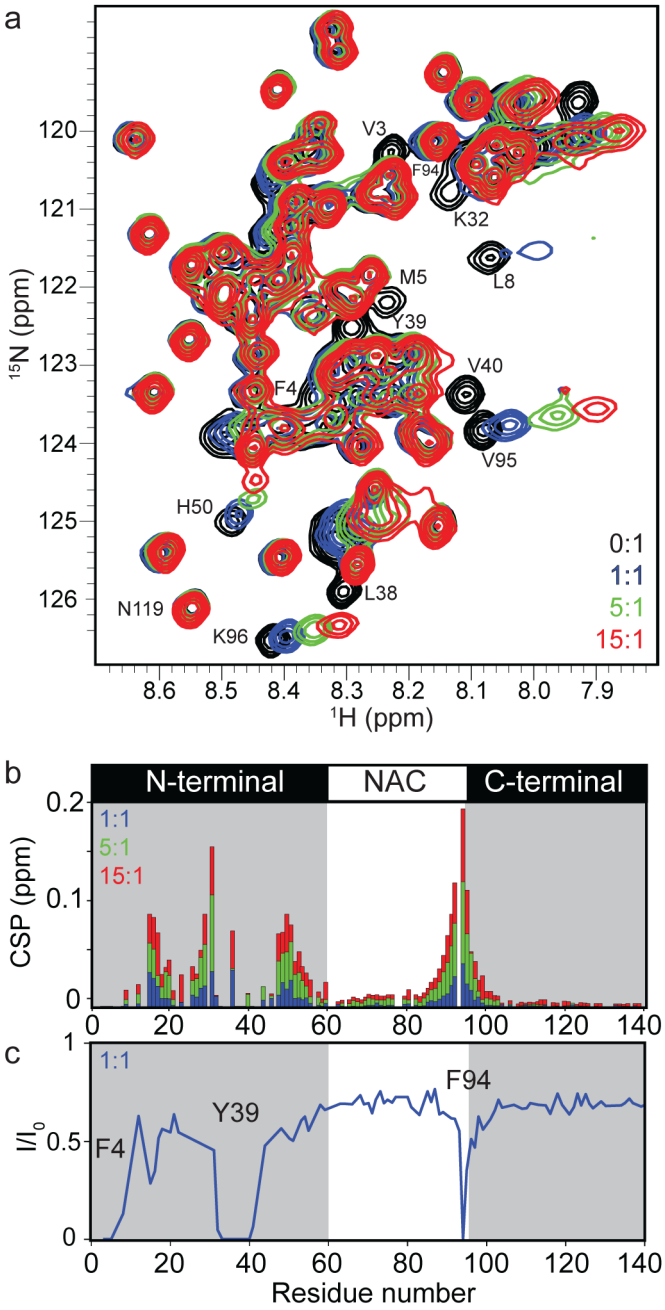

Figure S1. Interaction of PcTS with αSyn. (a) ^{1}H-^{15}N HSQC NMR spectra of 100 μM αSyn in the absence (0:1, black) and increasing equivalent concentrations of PcTS (100 μM, blue; 500 μM, green; 1.5 mM, red [1:1, 5:1, and 15:1, respectively]). Broadened and shifted crosspeaks corresponding to specific amino acids are labelled. (b) Chemical shift perturbation of αSyn crosspeaks with increasing concentrations of PcTS. (c) Intensity ratio I/I_0 (I=intensity of αSyn resonances in presence of the compound; I_0=crosspeak intensity of free αSyn).

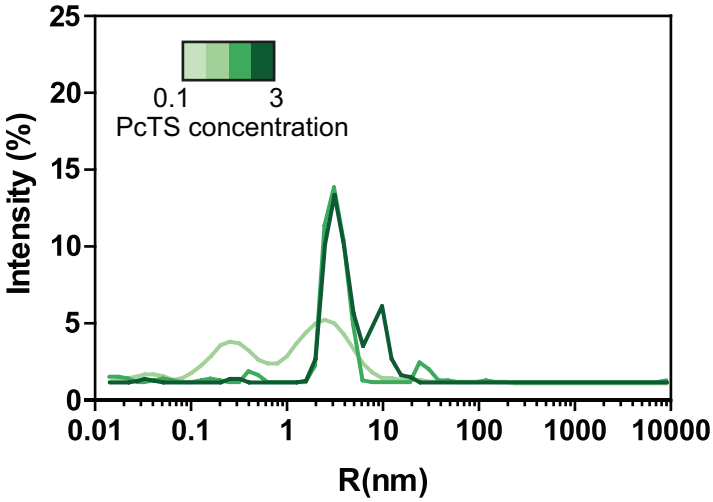

Figure S2. Increasing amounts of PcTS in the absence of any protein eventually form stacked, polydisperse aggregates, which are visible in DLS. When the PcTS concentration reached 3 mM, species from ~2 to ~15 nm in hydrodynamic radius were found. The ~40 nm wide species formed cooperatively by PcTS and αSyn was not present.

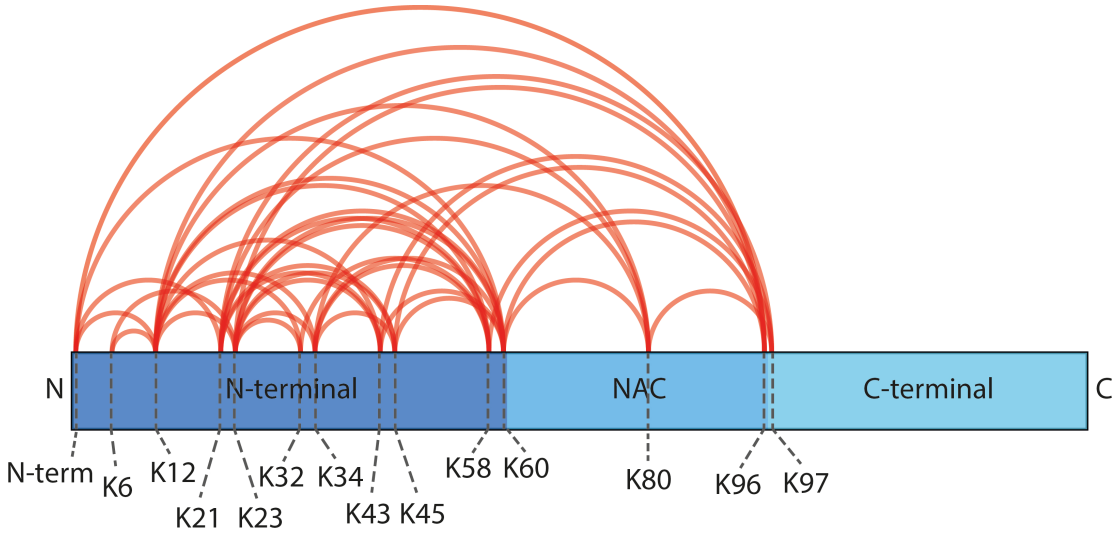

Figure S3. Intra- or inter-protein cross-links. Cross-links (red lines) and cross-linked lysine residues are shown. The protein N-terminus is also cross-linked. These cross-links cannot be assigned to inter- or intra-molecular interactions.

Table S1: Protein interactions identified by BS3 cross-linking. The αSynuclein residue numbers and the peptide sequences of cross-linked di-peptides are given. Cross-links were identified from dimer or tetramer bands using Trypsin, Chymotrypsin or both enzymes (Tryp-Chymo) for digestion of the proteins. The total number of identified spectra as well as the highest pLink identification score observed are listed. Cross-links that are specific for inter-αSynuclein interactions are highlighted in grey.

Res 1	Res 2	Peptide 1	Peptide 2	Oligomer	Digest	Oligomer	Digest	# Spectra	highest pLink Score
1	1	N-term-MDVFMK	N-term-MDVFMK	Dimer	Trypsin			2	4.56e-008
1	6	N-term-MDVFMK	MDVFMKGLSK	Dimer	Trypsin	Tetramer	Trypsin	3	4.20e-015
1	12	N-term-MDVFMK	AKEGVVAAAEK	Dimer	Trypsin			3	4.86e-015
1	21	N-term-MDVFMK	EGVVAAAEKTK	Dimer	Trypsin			1	2.64e-018
1	60	N-term-MDVFMK	TKEQVTNVGGAVVTGVTAVAQK	Dimer	Trypsin			1	2.19e-014
1	96	N-term-MDVFMK	TVEGAGSIAAATGFVKK	Dimer	Trypsin	Tetramer	Trypsin	4	7.11e-023
6	12	MDVFMKGLSK	AKEGVVAAAEK	Dimer	Trypsin	Tetramer	Trypsin	7	9.70e-023
6	23	MDVFMKGLSK	TKQGVAEAAGK			Tetramer	Trypsin	1	4.39e-016
12	12	AKEGVVAAAEK	AKEGVVAAAEK	Dimer	Tryp-Chymo, Trypsin	Tetramer	Tryp-Chymo, Trypsin	17	3.05e-017
12	21	AKEGVVAAAEK	AKEGVVAAAEKTK	Dimer	Tryp-Chymo, Trypsin	Tetramer	Trypsin	8	6.30e-018
12	21	AKEGVVAAAEK	EGVVAAAEKTK	Dimer	Tryp-Chymo	Tetramer	Tryp-Chymo, Trypsin	2	7.47e-015
12	23	AKEGVVAAAEK	TKQGVAEAAGK	Dimer	Tryp-Chymo, Trypsin	Tetramer	Tryp-Chymo, Trypsin	15	2.46e-017
12	32	AKEGVVAAAEK	QGVAEAAGKTK	Dimer	Tryp-Chymo	Tetramer	Tryp-Chymo, Trypsin	1	2.80e-014
12	32	AKEGVVAAAEK	TKQGVAEAAGKTK	Dimer	Trypsin, Tryp-Chymo			2	2.16e-015
12	34	AKEGVVAAAEK	TKEGVLY	Dimer	Tryp-Chymo			1	1.19e-004
12	34	AKEGVVAAAEK	TKEGVLYVGSK	Dimer	Trypsin	Tetramer	Trypsin	7	2.52e-014
12	43	AKEGVVAAAEK	EGVLYVGSKTK	Dimer	Trypsin	Tetramer	Trypsin	1	6.23e-012
12	43	AKEGVVAAAEK	TKEGVLYVGSKTK	Dimer	Trypsin	Tetramer	Trypsin	5	1.59e-013
12	45	AKEGVVAAAEK	TKEGVVHGVATVAEK	Dimer	Tryp-Chymo	Tetramer	Tryp-Chymo	6	6.75e-020
12	58	AKEGVVAAAEK	EGVVHGVATVAEKTK	Dimer	Tryp-Chymo,	Tetramer	Trypsin	3	5.00e-019

					Trypsin				
12	58	AKEGVVAAAEK	EGVVHGVATVAEKTKEQVTNVG GAVVTGVTAVAQK	Dimer	Tryp-Chymo	Tetramer	Trypsin	3	3.71e-014
12	58	AKEGVVAAAEK	TKEGVVHGVATVAEKTK	Dimer	Tryp-Chymo	Tetramer	Trypsin	5	9.11e-019
12	60	AKEGVVAAAEK	EGVVHGVATVAEKTKEQVTNVG GAVVTGVTAVAQK	Dimer	Tryp-Chymo	Tetramer	Tryp-Chymo, Trypsin	2	2.68e-012
12	60	AKEGVVAAAEK	TKEQVTNVGGAVVTGVTAVAQK	Dimer	Tryp-Chymo, Trypsin	Tetramer	Tryp-Chymo, Trypsin	15	1.07e-021
12	80	AKEGVVAAAEK	TKEQVTNVGGAVVTGVTAVAQK TVEGAGSIAAATGF	Dimer	Tryp-Chymo, Trypsin			5	6.29e-014
12	96	AKEGVVAAAEK	TVEGAGSIAAATGFVKK	Dimer	Trypsin	Tetramer	Trypsin	5	2.26e-015
21	21	AKEGVVAAAEKTK	EGVVAAAEKTK	Dimer	Tryp-Chymo	Tetramer	Trypsin	3	5.34e-018
21	21	EGVVAAAEKTK	EGVVAAAEKTK	Dimer	Tryp-Chymo, Trypsin	Tetramer	Trypsin	7	9.63e-020
21	23	AKEGVVAAAEKTK	TKQGVAEAAGK	Dimer	Trypsin	Tetramer	Tryp-Chymo, Trypsin	7	1.97e-014
21	43	EGVVAAAEKTK	EGVLYVGSKTK			Tetramer	Trypsin	1	3.42e-016
21	43	EGVVAAAEKTK	TKEGVLYVGSKTK			Tetramer	Trypsin	1	2.10e-008
21	45	AKEGVVAAAEKTK	TKEGVVHGVATVAEK	Dimer	Tryp-Chymo	Tetramer	Trypsin	3	2.15e-014
21	45	EGVVAAAEKTK	TKEGVVHGVATVAEK	Dimer	Tryp-Chymo	Tetramer	Tryp-Chymo, Trypsin	5	1.15e-016
21	58	AKEGVVAAAEKTK	TKEGVVHGVATVAEKTK	Dimer	Tryp-Chymo			3	4.20e-019
21	58	EGVVAAAEKTK	TKEGVVHGVATVAEKTK	Dimer	Tryp-Chymo			3	4.20e-019
21	60	AKEGVVAAAEKTK	TKEQVTNVGGAVVTGVTAVAQK	Dimer	Tryp-Chymo	Tetramer	Tryp-Chymo	2	1.49e-014
21	60	EGVVAAAEKTK	TKEQVTNVGGAVVTGVTAVAQK	Dimer	Tryp-Chymo, Trypsin	Tetramer	Tryp-Chymo, Trypsin	10	3.13e-015
21	80	EGVVAAAEKTK	TKEQVTNVGGAVVTGVTAVAQK TVEGAGSIAAATGFVK	Dimer	Trypsin			1	2.21e-013
21	97	AKEGVVAAAEKTK	KDQLGK	Dimer	Tryp-Chymo			2	5.88e-011
23	23	TKQGVAEAAGK	TKQGVAEAAGK	Dimer	Tryp-Chymo, Trypsin	Tetramer	Tryp-Chymo, Trypsin	8	4.48e-014
23	32	TKQGVAEAAGK	QGVAEAAGKTK	Dimer	Tryp-Chymo, Trypsin	Tetramer	Trypsin	5	4.61e-015

23	34	TKQGVAEAAGK	TKEGVLYVGSK	Dimer	Trypsin	Tetramer	Trypsin	5	4.82e-015
23	43	TKQGVAEAAGK	TKEGVLYVGSKTK	Dimer	Trypsin	Tetramer	Trypsin	5	5.35e-018
23	45	TKQGVAEAAGK	TKEGVVHGVATVAEK			Tetramer	Tryp-Chymo	1	9.24e-016
23	58	TKQGVAEAAGK	EGVVHGVATVAEKTK	Dimer	Trypsin	Tetramer	Trypsin	3	2.27e-022
23	58	TKQGVAEAAGK	EGVVHGVATVAEKTKEQVTNVG GAVVTGVTAVAQK	Dimer	Trypsin	Tetramer	Trypsin	2	6.50e-019
23	60	TKQGVAEAAGK	TKEQVTNVGGAVVTGVTAVAQK	Dimer	Tryp-Chymo, Trypsin			2	6.28e-014
23	96	TKQGVAEAAGK	TVEGAGSIAAATGFVKK	Dimer	Trypsin	Tetramer	Trypsin	6	7.67e-017
32	58	QGVAEAAGKTK	EGVVHGVATVAEKTK	Dimer	Tryp-Chymo			1	5.09e-019
34	34	TKEGVLYVGSK	TKEGVLYVGSK			Tetramer	Trypsin	2	1.65e-009
34	45	TKEGVLYVGSK	TKEGVVHGVATVAEK	Dimer	Trypsin	Tetramer	Trypsin	6	4.96e-023
34	58	TKEGVLYVGSK	EGVVHGVATVAEKTK			Tetramer	Trypsin	2	3.44e-014
34	58	TKEGVLYVGSK	TKEGVVHGVATVAEKTK			Tetramer	Trypsin	1	6.50e-020
34	60	TKEGVLY	TKEQVTNVGGAVVTGVTAVAQK	Dimer	Tryp-Chymo, Trypsin	Tetramer	Trypsin	4	2.39e-018
34	80	TKEGVLYVGSK	TKEQVTNVGGAVVTGVTAVAQK TVEGAGSIAAATGFVK	Dimer	Trypsin			3	1.56e-011
43	43	VGSKTK	VGSKTK	Dimer	Tryp-Chymo			1	9.18e-007
43	60	EGVLYVGSKTK	TKEQVTNVGGAVVTGVTAVAQK	Dimer	Trypsin, Tryp-Chymo			4	1.34e-007
43	97	TKEGVLYVGSKTK	KDQLGK			Tetramer	Trypsin	1	1.27e-012
45	43	TKEGVVHGVATVAEK	TKEGVLYVGSKTK			Tetramer	Trypsin	1	1.12e-014
45	45	TKEGVVHGVATVAEK	TKEGVVHGVATVAEK	Dimer	Tryp-Chymo, Trypsin	Tetramer	Trypsin	6	2.91e-013
45	58	TKEGVVHGVATVAEK	EGVVHGVATVAEKTK	Dimer	Tryp-Chymo			2	5.12e-015
45	60	TKEGVVHGVATVAEK	TKEQVTNVGGAVVTGVTAVAQK	Dimer	Tryp-Chymo			2	1.28e-019
45	96	TKEGVVHGVATVAEK	TVEGAGSIAAATGFVKK	Dimer	Trypsin			3	6.73e-014
58	58	EGVVHGVATVAEKTK	EGVVHGVATVAEKTK	Dimer	Tryp-Chymo			1	5.06e-011
58	58	TKEGVVHGVATVAEKTK	TKEGVVHGVATVAEKTK	Dimer	Tryp-Chymo			1	9.91e-007
58	60	EGVVHGVATVAEKTK	TKEQVTNVGGAVVTGVTAVAQK	Dimer	Trypsin			2	1.11e-016
58	97	TKEGVVHGVATVAEKTK	KDQLGK	Dimer	Tryp-Chymo,			7	5.94e-013

					Ttrypsin				
60	60	EGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQK	TKEQVTNVGGAVVTGVTAVAQK	Dimer	Tryp-Chymo	Tetramer	Trypsin	8	1.99e-017
60	60	TKEQVTNVGGAVVTGVTAVAQK	TKEQVTNVGGAVVTGVTAVAQK			Tetramer	Trypsin	3	9.80e-012
60	80	TKEQVTNVGGAVVTGVTAVAQK	TKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVK	Dimer	Trypsin			2	8.15e-022
60	96	TKEQVTNVGGAVVTGVTAVAQK	TVEGAGSIAAATGFVKK			Tetramer	Tryp-Chymo	1	4.17e-010
96	97	TVEGAGSIAAATGFVKK	KDQLGK	Dimer	Trypsin	Tetramer	Trypsin	9	1.18e-015
97	80	KDQLGK	TKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVK	Dimer	Trypsin			2	1.42e-013
97	97	KDQLGK	KDQLGK	Dimer	Tryp-Chymo	Tetramer	Trypsin	2	4.49e-009
97	97	TVEGAGSIAAATGFVKKDQLGK	KDQLGK			Tetramer	Trypsin	2	3.94e-008

Table S2: Protein interactions identified by $Ru(bpy)_3^{2+}$ cross-linking. The αSynuclein residue numbers and the peptide sequences of cross-linked di-peptides are given. Cross-links were identified from dimer or tetramer bands using Trypsin or Chymotrypsin for digestion of the proteins. The total number of identified spectra as well as the highest pLink identification score observed are listed. All cross-linked identified contain overlapping peptide sequences and therefore represent inter-molecular interactions.

Res 1	Res 2	Peptide 1	Peptide 2	Oligomer	Digest	Oligomer	Digest	# Spectra	Highest pLink score
34	39	T**K**EGVLYVGSK	TKEGVL**Y**VGSK	Dimer	Trypsin			3	4.83e-012
39	39	EGVL**Y**VGSK	EGVL**Y**VGSK	Dimer	Trypsin	Tetramer	Trypsin	5	1.98e-013
39	45	TKEGVL**Y**VGSKTK	T**K**EGVVHGVATVAEK	Dimer	Trypsin			1	1.09e-006
133	133	EMPSEEG**Y**QDYEPEA	EMPSEEG**Y**QDYEPEA	Dimer	Chymotrypsin			1	2.18e-002
133	136	EMPSEEG**Y**QDYEPEA	EMPSEEGYQD**Y**EPEA	Dimer	Chymotrypsin			6	2.53e-011
136	136	EMPSEEGYQD**Y**EPEA	EMPSEEGYQD**Y**EPEA	Dimer	Chymotrypsin			3	1.46e-009

Supplementary Experimental Section

NMR spectroscopy

NMR spectroscopy was performed on samples containing either 400 or 100 μM αSyn in a 50 mM phosphate buffer, 100 mM NaCl, pH 6.8 and 90% H_2O/10% D_2O. NMR experiments were recorded on a Bruker Avance 600 MHz spectrometer. The temperature was set to 15 °C. Data processing was performed using the software packages Topspin (Bruker) and CCPN Analysis.[48] ^{15}N-^{1}H HSQC amide cross-peaks affected during compound addition were identified by comparison of their chemical shift values with those of the same cross-peaks in the data set of samples lacking the compound. Perturbations in the chemical shift values for ^{1}H and ^{15}N were calculated as $[(\Delta\delta^{1}H)^2 + (\Delta\delta^{15}N/10)^2]^{1/2}$. ^{15}N TROSY experiments for R_2^T measurement (modified from [49]) were recorded at 303 K on a Bruker 600 MHz spectrometer as interleaved experiments with six T_2 delays (10, 40, 80, 150, 250 and 400 ms). Samples contained 300 μM protein in 50 mM HEPES buffer, 100 mM NaCl at pH 7.4 with 10% D_2O in the absence and presence of 3 mM concentration of PcTS (1:10 protein to compound ratio). Single exponential decay curves were fitted to the intensity of each cross-peak throughout the different delay times with the function $y=Ae^{(-Bx)} + C$, where B is the R_2^T value. Error bars represent the fitting errors.

Mass spectrometry

Typical MS conditions were: spray voltage of 2.3 kV; capillary temperature of 275°C; collision energy of 30 %, activation Q of 0.25. The Orbitrap Fusion Tribrid Mass Spectrometer was operated in data-dependent mode. Survey full scan MS spectra were acquired in the orbitrap (m/z 350–2000) with a resolution of 120,000 and an automatic gain control (AGC) target at 500,000. The top 20 most intense ions were selected for HCD MS/MS fragmentation in the orbitrap at a resolution of 30,000 and an AGC target of 50,000 and with a first m/z of 110. Previously selected ions within were dynamically excluded for 30 s. Only ions with charge states 2-7 were selected. Singly charged ions, as well as ions with unrecognized charge state, were excluded. Internal calibration of the orbitrap

was performed using the lock mass option (lock mass: m/z 445.120025).[1] Raw files were converted into mgfs using pXtract tools (http://pfind.ict.ac.cn/software/pXtract/index.html). Mgfs were searched against a reduced database containing αSyn sequence using pLink software.[2] Search parameters were: instrument spectra, HCD; enzyme, trypsin or chymotrypsin; max. missed cleavage sites, 3; variable modifications, oxidation (methionine) and carbamidomethylation (cysteine); cross-linker, BS3 (lysine-lysine) or $Ru(bpy)_3^{2+}$ (Tyrosine-Tyrosine, Tyrosine-Lysine, Tyrosine-Histidine, Tyrosine-Serine, Tyrosine-Threonine); min. peptide length, 4; max. peptide length, 100; min. peptide mass, 400 Da; max. peptide mass, 10,000 Da; FRD, 1%. Potential cross-linked di-peptides were evaluated by their spectral quality.

Supplementary References

[1] J. V. Olsen, L. M. F. de Godoy, G. Li, B. Macek, P. Mortensen, R. Pesch, A. Makarov, O. Lange, S. Horning, M. Mann, *Mol. Cell Proteomics* **2005**, *4*, 2010–2021.

[2] B. Yang, Y.-J. Wu, M. Zhu, S.-B. Fan, J. Lin, K. Zhang, S. Li, H. Chi, Y.-X. Li, H.-F. Chen, et al., *Nat. Methods* **2012**, *9*, 904–906.