Supplementary Information for

Megadalton-sized dityrosine aggregates of α-synuclein retain high degrees of structural disorder and internal dynamics.

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Supplementary Figure Legends:

Figure S1: Mass spectrometry and NMR analysis of PICUP HMWAs. (a) Representative fragmentation spectra of αSyn dityrosine peptide-pairs following photo-induced crosslinking and proteolytic digestion. Matched αSyn fragments were identified via automated database searches using the Xi software package. (b) Overlay of 2D 1H-15N NMR spectra of 15N isotope-labeled, N-terminally acetylated, monomeric αSyn (50 µM, black) and of PICUP-HMWAs (50 µM, red). Resonance signals of N-terminal αSyn residues 1-10 are shown in blue. HMWA resonances broadened beyond detection are labeled, NMR signals of Y39, Y125, Y133 and Y136 are highlighted in grey. NMR spectra were recorded at 283 K (10 °C).

Figure S2: HMWAs in crowded environments. (a) Overlay of 2D 1H-15N NMR spectra of 15N isotope-labeled, N-terminally acetylated, monomeric αSyn (50 µM) in the presence of 200 mg/mL BSA (black). N-terminal αSyn residues 1-10 in the reference spectrum are broadened beyond detection due to transient interactions with BSA. Y39 signal intensity of monomeric αSyn is attenuated for the same reason. The corresponding in situ NMR spectrum of HMWAs (50 µM) formed in the presence of BSA is overlaid in red. Broadened HMWA resonances are labeled, NMR signals of Y39, Y125, Y133 and Y136 are highlighted in grey. (b) Western blot analysis of RCSN3 lysate recovery times following DEPC treatment to determine compound decomposition. After incubation with DEPC, αSyn (50 µM) and H2O2 (10 mM) were added to lysates after 2 h, 4 h, 6 h and 22 h. Residual DEPC activity was monitored via peroxidative crosslinking of αSyn. (c) Overlay of 2D 1H-15N NMR spectra of 15N isotope-labeled, N-terminally acetylated, monomeric αSyn (50 µM) in DEPC-treated RCSN3 lysate (3 mg/mL total protein concentration, black) and in situ NMR spectrum of HMWAs (50 µM) formed after addition of cyt c (50 µM) and 50 mM H2O2 (red). HMWA
resonances broadened beyond detection are labeled. Chemical shift changes of N-terminal αSyn residues 1-10 are shown in blue. NMR signals Y39, Y125, Y133 and Y136 are highlighted in grey. All NMR spectra were recorded at 283 K (10 °C).

**Figure S3: αSyn and HMWA SUV-binding.** (a) Overlay of 2D $^1$H-$^{15}$N NMR spectra of $^{15}$N isotope-labeled, N-terminally acetylated, monomeric αSyn (50 µM, black) and in the presence of small unilamellar vesicles (SUV) prepared from pig-brain polar lipid extract. Non-broadened NMR signals of detectable C-terminal residues 106-140 are labeled. Y125, Y133 and Y136 are highlighted in grey. (b) Overlay of 2D $^1$H-$^{15}$N NMR spectra of $^{15}$N isotope-labeled HMWAs (50 µM, black) and upon binding to SUVs (red). Non-broadened NMR signals are labeled. Spectra were recorded at 303 K (30 °C).
Figure S1

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(a) Y39-Y39

(b) Y39-Y125

(c) Y39-Y133

(d) Y39-Y136

(e) Y133-Y133

(f) Y136-Y136
Figure S2

(a) 

(b) RCSN3 lysate + DEPC

(c) RCSN3 + DEPC

\( \alpha \text{Syn} + \text{H}_2\text{O}_2 \)}