

Supplementary information

Low dose DMSO treatment induces oligomerization and accelerates aggregation of α -synuclein

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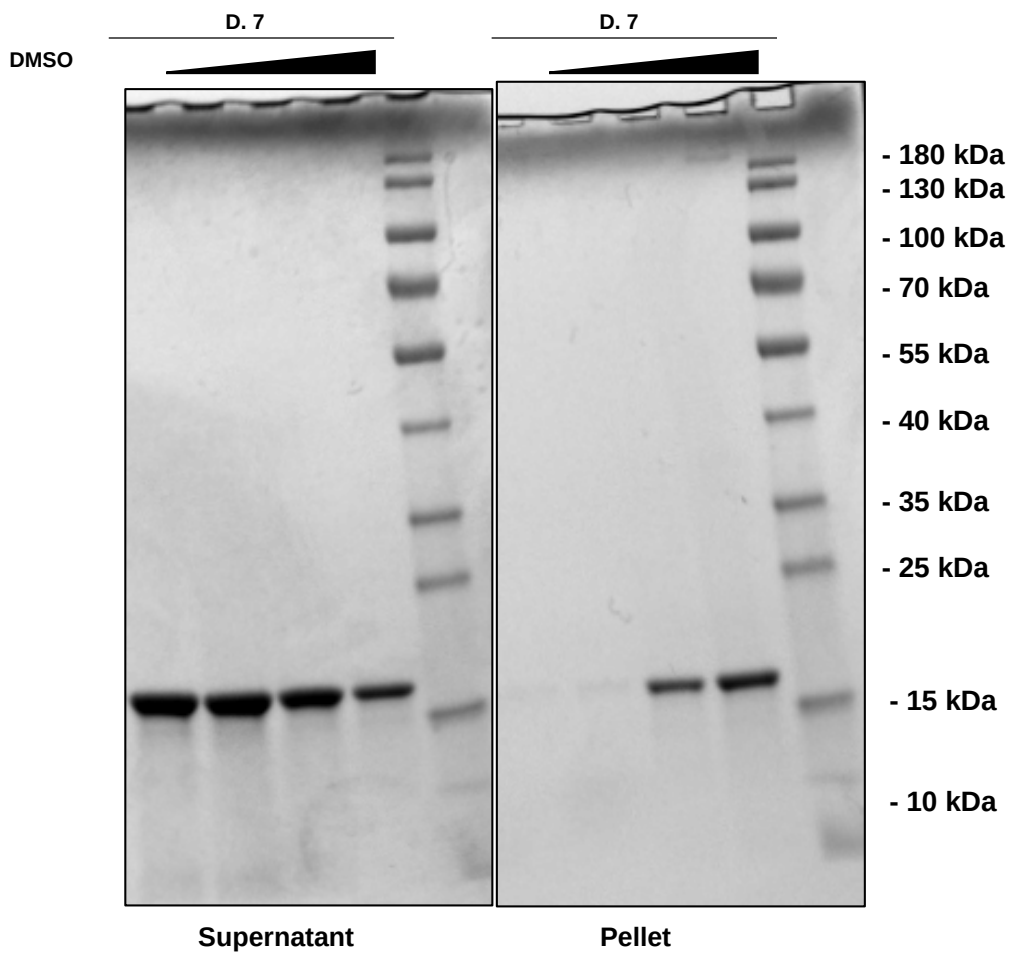
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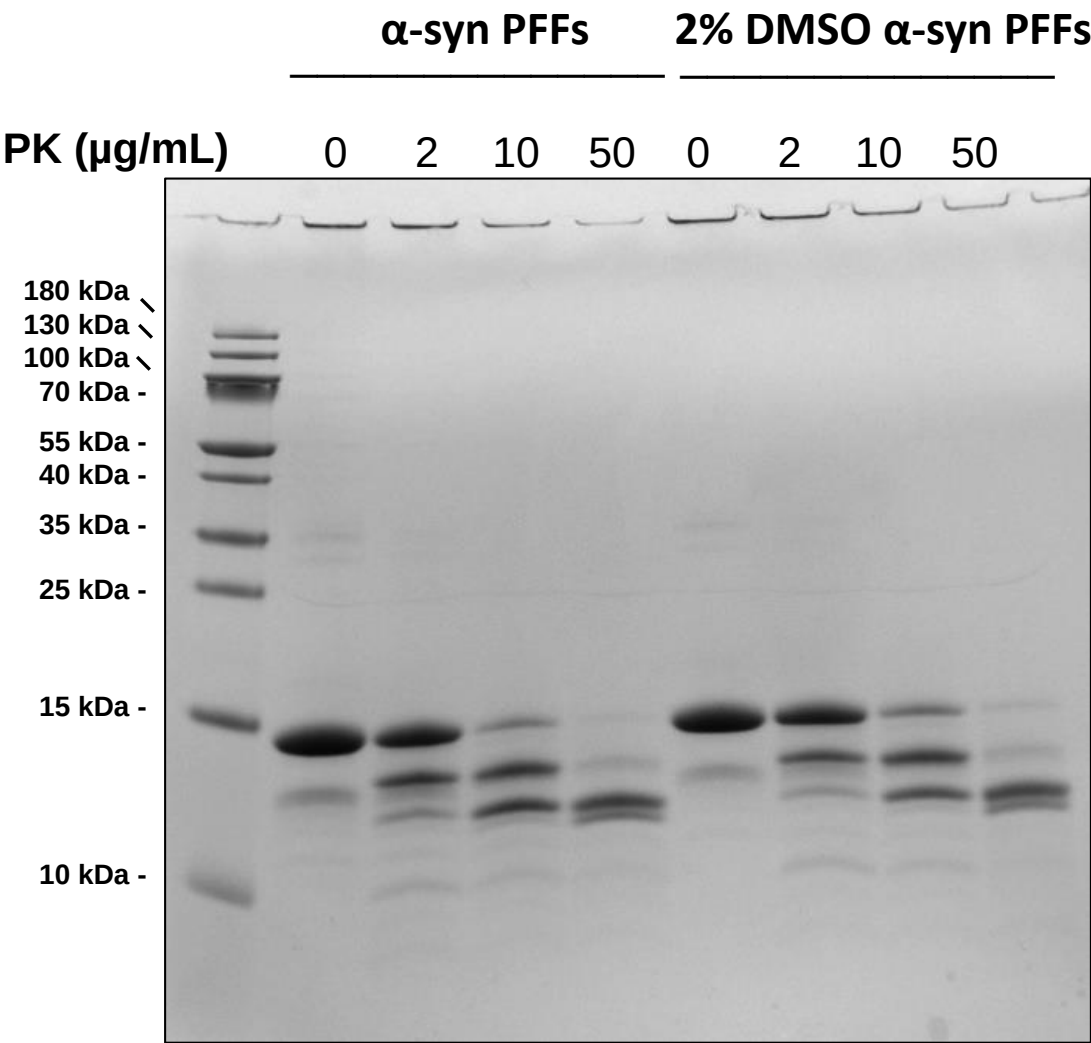
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**In vitro aggregation
(sedimentation of α -syn + DMSO)**



Supplementary Information 1)
Full-length SDS-PAGE gels of data presented in
original figure 1F.

Proteinase K digestion



Supplementary Information 2)
Full-length SDS-PAGE gels of data presented in original figure 2B. Please note that α -syn migrates slightly different relative to the 15kDa from the Prestained Protein Ladder on this 16% Tricine Gel compared to regular SDS-PAGE gels.

Supplementary figure legends:

Supplementary Figure 1

A and B) Dynamic light scattering analysis of α -syn (0.5 mg/mL, 35 μ M) and carbonic anhydrase (0.5 mg/mL, 17 μ M) incubated for 1 h with increasing concentrations of DMSO (0%, 2%, 5% and 10%). The figure demonstrates an intensity% distribution based on the scattering intensity, with log scaled hydrodynamic radius depicted on the X-axis. Representative figure of three independent replicates ($n = 3$).

Supplementary Figure 2

Dot blot of 100 ng of recombinant α -syn protein either as monomer, oligomer, cross-linked oligomer or fibrils. α -syn was visualized using total α -syn (SYN-1) and MJFR-14-6-4-2 antibodies. Representative figure of three independent replicates ($n = 3$).

Supplementary Figure 3

A) Human α -syn expressing OLN-AS7 cells were exposed to PBS (Control) or 14 μ g/mL (1 μ M) sonicated S129A α -syn PFFs prepared in PBS alone (S129A α -syn PFFs) or in the presence of 2% DMSO (2% DMSO S129A α -syn PFFs). After 24h of PFF treatment, the cells were washed to remove excess PFF and subsequently incubated for another 24h before being fixed and visualized using DAPI (blue), α -tubulin (purple), total α -syn (red) or MJFR-14-6-4-2 α -syn antibody (green). Scale bar = 20 μ m. Representative images of three biological replicates with 10 measurements in each experiment. **B)** Quantification of area of MJFR-14-6-4-2 α -syn signal relative to number of DAPI stained nuclei. Data are shown as mean of three independent experiments ($n = 3$, *** $p < 0.001$, one-way ANOVA followed by Tukey's multiple comparison test).

Supplementary Figure 4

A) Human α -syn expressing OLN-AS7 cells were exposed to PBS (Control) or 14 μ g/mL (1 μ M) sonicated α -syn PFFs prepared in PBS alone (α -syn PFFs) or in the presence of 2% DMSO (2% DMSO α -syn PFFs). After 24h of PFF treatment, the cells were washed to remove excess PFF and subsequently incubated for another 24h before being fixed and visualized using DAPI (blue), α -tubulin (purple), total α -syn (red) or anti-phospho-S129 α -syn (green). Scale bar = 20 μ m. Representative images of three biological replicates with 10 measurements in each experiment. **B)** Quantification of area of anti-phospho-S129 α -syn signal relative to number of DAPI stained nuclei. Data are shown as mean of three independent experiments ($n = 3$, * $p < 0.05$, ** $p < 0.01$, one-way ANOVA followed by Tukey's multiple comparison test).

Supplementary Figure 5

SH-SY5Y ASYN cells were treated with dox to suppress α -syn expression (- α -syn) or relieved from dox treatment to induce α -syn overexpression (0% DMSO) for 7 days prior to fixation. To stop mitosis, the SH-SY5Y ASYN cells were treated with retinoic acid (10 μ M final concentration) during the experiment. Quantification of area of MJFR-14-6-4-2 signal (**A**) or anti-phospho-S129 α -syn signal (**B**) from individual coverslips relative to area of α -Tubulin signal ($n = 4$, with ~10 images for each condition in each experiment, ** $p < 0.01$, **** $p < 0.0001$, student t-test).

Supplementary Figure 6

Free floating 30 μ m sections of brain tissue from striatum or substantia nigra or paraffin embedded sections of duodenum and ileum from transgenic F28 mice, overexpressing human α -syn under the control of the endogenous mouse α -syn promoter. Twelve transgenic F28 mice were divided into three groups with four in each and treated with water (0% DMSO), 10% DMSO (1 g/kg bodyweight) or 30% DMSO (3 g/kg bodyweight) for 14 days prior to sacrificing and tissue collection. Striatum or substantia nigra sections from all animals were stained with an anti-tyrosine hydroxylase antibody and with the MJFR-14-6-4-2 antibody ($n = 4$ for each condition). The duodenum and ileum samples from all animals were stained with the MJFR14-6-4-2 antibody. $n = 4-6$ sections per segment (s2, s5, s8) were analyzed for the intestinal samples and one series of brain sections spanning the entirety of the brain from OB to beginning of cerebellum (30 μ m sections, 10 series = 300 μ m distance between the sections for each series) for each animal. Scalebar = 20 μ m for striatum, duodenum and ileum and 50 μ m for substantia nigra.