Electronic Supporting Information

A Triosmium Carbonyl Cluster that Inhibits α-Synuclein Aggregation and Disassembles Preformed Aggregates

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EXPERIMENTAL PROCEDURES

General Experimental

The cluster precursor $Os_3(CO)_{10}(NCCH_3)_2$ was prepared following a published procedure from $Os_3(CO)_{12}$, which was purchased from Strem.[1] **3HF** was purchased from Haoyuan Chemexpress. All other chemicals were purchased from other commercial sources and used as received. TLC separations were carried out on Supelco TLC Silica gel 60 F_{254} 20x20 cm^2 plates. IR spectra were recorded on a Bruker APEX FTIR spectrometer in solution IR cells fitted with KBr windows with a 0.1 mm pathlength, at 2 cm^{-1} resolution. ^1H NMR spectra were recorded on a JEOL ECA 500 spectrometer in acetone-d_6 and chemical shifts were referenced to the residual proton resonance of the solvent. High-resolution mass spectrometry (HRMS) spectra were recorded in the ESI mode on a Waters UPLC-QTOF MS mass spectrometer.

Synthesis of Cluster **2**

Cluster **2** was synthesized following the method reported for **1**.[2] To a solution of $Os_3(CO)_{10}(NCCH_3)_2$ (48 mg, 50 μmol) in dichloromethane (15 mL) was added **3HF** (16 mg, 67 μmol). After stirring overnight at room temperature, the solvent was

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[1] B. F. G. Johnson, J. Lewis, D. Pippard, *J. Organomet. Chem.*, 1978, **160**(1), 263.

[2] (a) M. Monari, R. Pfeiffer, U. Rudsander, E. Nordlander, *Inorg. Chim. Acta*, 1996, **247**, 131. (b) G. R. Crooks, B. F. G. Johnson, J. Lewis, I. G. Williams, *J. Chem. Soc. A: Inorg. Phys. Theor.*, 1969, **0**, 797. (c) E. W. Ainscough, A. M. Brodie, R. K. Coll, A. J. A. Mair, J. M. Waters, *J. Organomet. Chem.*, 1996, **509**, 259. (d) C. Li, W. K. Leong, *J. Organomet. Chem.*, 2008, **693**, 1292. (e) J. W. Kong, Z. Lam, K. H. Chan, R. Ganguly, J.-Y. Joey Lee, L.-H. Loo, R. D. Webster, Z. X. Wong, W. K. Leong, *ACS Omega*, 2021, **6**, 29045. (f) H. Z. Lee, W. K. Leong, S. Top, A. Vessières, *ChemMedChem*, 2014, **9**, 1453.

removed under reduced pressure, the residue redissolved in dichloromethane for purification by TLC with hexane/dichloromethane (v/v, 2:1) as the eluent, to yield two distinct bands. The first orange band (Rf = 0.6) afforded **2**. Yield = 13 mg, 21.3% (calculated based on $Os_3(CO)_{12}$). IR (ν_{CO}, in hexane): 2100w, 2060s, 2015vs, 1999m, 1993m, 1925w. ^{1}H NMR: δ -9.27 ((s, 1H, OsHOs), 7.73 – 7.77 (m, 3H, HAr), 7.80 – 7.84 (m, 1H, HAr), 7.98 – 8.00 (m, 1H, HAr), 8.05 – 8.08 (m, 1H, HAr), 8.20 – 8.22 (m, 2H, HAr), 8.38 – 8.40 (m, 1H, HAr). ESI-MS (m/z): 1061.9043; calc. for $C_{24}H_{11}O_{12}{}^{187}Os^{192}Os_2$ [M + H$^+$]: 1061.9038.

Crystallographic

The crystallographic structural determination was carried out by Dr Yongxin Li at the Central Instrument Facilities of CCEB. A diffraction-quality crystal of **2** was obtained via hexane diffusion into a dichloromethane solution. The crystal was mounted onto a quartz fiber, cooled to 100 K, and the X-ray diffraction intensity data measured with a Bruker Kappa diffractometer equipped with a CCD detector and employing Mo Kα radiation (λ = 0.71073 Å), with the SMART suite of programs.[3] The data were processed and corrected for Lorentz and polarization effects with SAINT and for absorption effects with SADABS.[4] Structural solution and refinement were carried out with the SHELXTL suite of programs.[5] The structure was solved by direct methods followed by difference maps to complete the structure. The metal hydride was located

[3] SMART version 5.628. *Bruker AXS Inc.: Madison, WI, USA,* **2001**.
[4] Sheldrick, G. M. *SADABS* **1996**.
[5] SHELXTL version 5.1. *Bruker AXS Inc.: Madison, WI, USA* **1997**.

via a low-angle difference map and refined isotropically. All non-hydrogen atoms were refined with anisotropic thermal parameters. Organic hydrogen atoms were placed in calculated positions and refined with a riding model. Crystal data, data collection parameters, and refinement data are summarized in Table S1.

BIOLOGICAL WORK

α-Synuclein (wild-type and A53T mutant) was obtained from The Protein Production Platform in NTU. Plates used in the MTT and ThT assays were read using an Agilent BioTek Synergy H1 Multimode Reader. Confocal microscopy images were acquired using the Inverted Confocal Airyscan Microscope (LSM800) at the NTU Optical Bio-Imaging Centre (NOBIC), located in the LKCMedicine Experimental Medicine Building. Image analysis was performed using the ZEN 3.9 and ImageJ softwares. TEM images were acquired in the NTU Institute of Structural Biology using a Tecnai-12 (T12) iCorr transmission electron microscope operating at 120 kV. Statistical analysis was performed using GraphPad Prism. MTT assays followed the procedure provided by the manufacturer (MedChemExpress). Cell transfection, ThT and IFA assays were performed according to the procedures provided by the manufacturers; details of the procedures used and data analyses are given below. All biological assays were carried out in triplicates. For the statistical analysis, ordinary one-way ANOVA was performed using GraphPad Prism 9.5.1.

SH-SY5Y cells, obtained from ATCC, were cultured in complete medium consisting of Dulbecco's Modified Eagle Medium (DMEM, Gibco™, Cat# 11995065),

supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco™, Cat# 10270106) and 1% (v/v) penicillin/streptomycin (P/S, GE Hyclone, Cat# SV30010). The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. The culture medium was changed every 2–3 days to ensure optimal growth conditions. Transfected A53T SH-SY5Y cells were maintained under similar culture conditions supplemented with 200 μg/ml G418 sulfate (Gibco™, Cat# 10131035). Stock solutions of test compounds were prepared in DMSO. The final concentration of DMSO in the working solutions was kept below 1%.

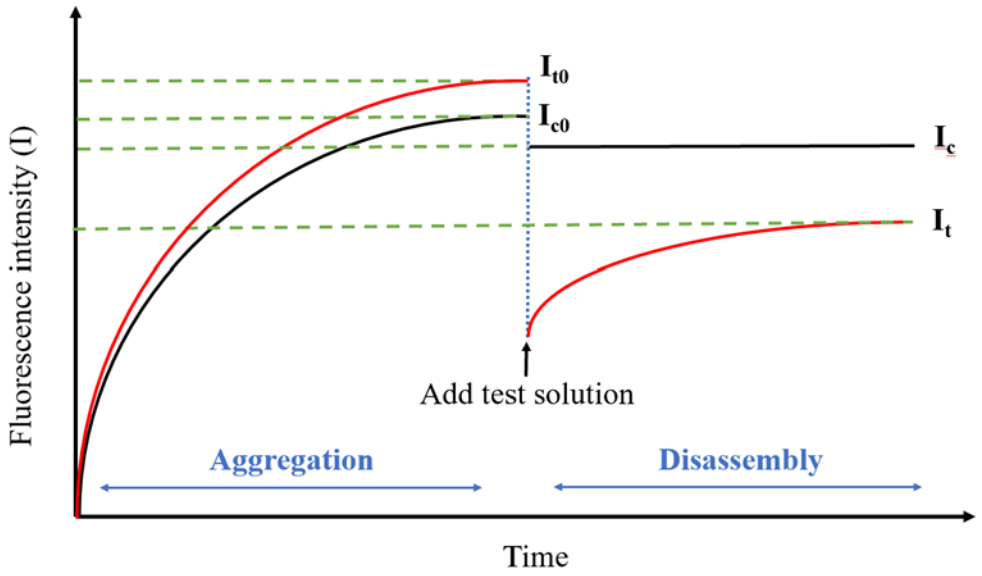
Transfection of SH-SY5Y Cells

For stable transfection, SH-SY5Y cells were cultured in complete DMEM at 37°C and 5% CO_2. Plasmid DNA (Addgene plasmid #40823) was transfected using Lipofectamine 2000™ reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. Briefly, the plasmid DNA (1.6 μg) and Lipofectamine 2000™ (8 μL) were separately diluted in Opti-MEM medium (250 μL). After a 5 min incubation, the two solutions were combined and incubated for 20 mins at room temperature. The DNA-Lipofectamine complex was then added to the cells. Following a 24 h incubation, the transfection medium was replaced with complete DMEM supplemented with 0.5 mg/mL G418 sulfate (Thermo Fisher Scientific) to select for stably transfected cells. The cells were further cultured in selective medium for 4 d, followed by fluorescence-activated cell sorting (FACS) to isolate GFP-positive cells.

Thioflavin T (ThT) assay

The ability of the compounds to inhibit α-synuclein aggregation was evaluated using ThT assays following the procedure provided by the manufacturer (Sigma Aldrich). Briefly, a solution containing α-synuclein (100 µg/mL), SDS (0.33 mM), ThT (20 µM), and the desired concentration of test compounds (2.5 µM to 100 µM) was prepared in a buffer consisting of 10% glycerol, aq NaCl (300 mM), HEPES (20 mM), and TCEP hydrochloride (2 mM). An aliquot (70 µL) of the prepared solution was then added to each well of a black 384-well, clear flat-bottom microplate (Greiner, Austria). Deionized water (70 µL) was added to each of the wells along the fringes to minimize evaporation, and the plate sealed with parafilm. The microplate was then incubated at 37 °C without agitation in a microplate reader. The fluorescence at 485 nm, at an excitation wavelength of 450 nm, was recorded from the bottom of the plate at 10 mins intervals. Fluorescence contributions from ThT and test compounds (without α-synuclein) were subtracted before graphing. Percent inhibition was calculated as relative fluorescence units (RFU) at the endpoint against that of negative control.

For the disassembly assay, a similar procedure was followed but first without the test solution until the fluorescence signal in each well reached a plateau. The test solution (0.2 µL) was then added to the designated wells; DMSO (vehicle, 0.2 µL) was used as negative control. After sealing the microplate with parafilm, it was incubated at 37 °C in a microplate reader, and the fluorescence signals recorded until a new plateau was reached.



The disassembly ability was calculated using the following formula:

$$\text{Disassembly percentage} = \frac{I_{t0} - (I_t + Icorr)}{I_{t0}} \text{x } 100 \quad \text{where } I_{corr} = I_{c0} - I_c$$

Immunofluorescence (IFA) assay

Transfected A53T SH-SY5Y cells were seeded into 8-well chamber slides (Labselect, Cat# CS-08-TC) and grown for 24 h to reach approximately 70% confluency. The cells were then treated with a solution containing the test compound and 500 nM rotenone in medium (10% FBS, 1% P/S, and 200 μg/mL G418 sulfate) for 24 h. Following treatment, the cells were washed twice with PBS (Gibco, Cat# 20012027), fixed with 4% paraformaldehyde (PFA, Nacalai Tesque, Cat# 09154-56) for 15 mins at room temperature, and permeabilized with 0.1% Triton X-100 (Sigma) for 10 mins. Blocking was performed using 5% donkey serum (Sigma) in 1x PBS for 1 h at room temperature to prevent non-specific binding. The cells were incubated overnight at 4 °C with a 1:1000 dilution of anti-α-synuclein aggregate antibody (Abcam, Cat# MJFR-14-6-4-2). After washing twice with PBS, the cells were incubated for 1 h at room temperature with Goat anti-rabbit IgG H&L AF 647 secondary antibody (Abcam, Cat# ab150079). Following three additional washes with PBS, the cells were incubated with 1 μg/mL DAPI in PBS for 10 mins at room temperature. Finally, the cells were washed twice with PBS and mounted using ProLong Glass Antifade Mountant (Thermo Fisher). Slides were allowed to dry before imaging.

Table S1. Crystallographic data for cluster **2**.

Chemical formula	$C_{24}H_{10}O_{12}Os_3$.½hexane
Formula weight, g/mol	1104.01
Temperature, K	100(2)
Wavelength, Å	0.71073
Crystal size, mm^3	0.010 x 0.060 x 0.140
Crystal habit	yellow block
Crystal system	monoclinic
Space group	$P2_1/n$
a, Å	12.3904(9)
b, Å	14.3475(11)
c, Å	16.4480(13)
a, deg	90
b, deg	96.858(3)
g, deg	90
Volume, $Å^3$	2903.1(4)
Z	4
Density (calculated), g/cm^3	2.526
Absorption coefficient, mm^{-1}	13.160
F(000)	2012
Theta range, deg	1.889 to 31.008
Index ranges	-16<=h<=17, -20<=k<=20, -23<=l<=23
Reflections collected	47413
Independent reflections	9237 [R(int) = 0.1280]
Completeness to theta	99.9
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.51 and 0.34
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	9237 / 79 / 386
Goodness-of-fit on F^2	1.017
Final R indices, I>2σ(I)	R1 = 0.0494, wR2 = 0.0750
Final R indices, all data	R1 = 0.1178, wR2 = 0.0927

Largest diff. peak and hole, eÅ^{-3}	1.657 and -1.531

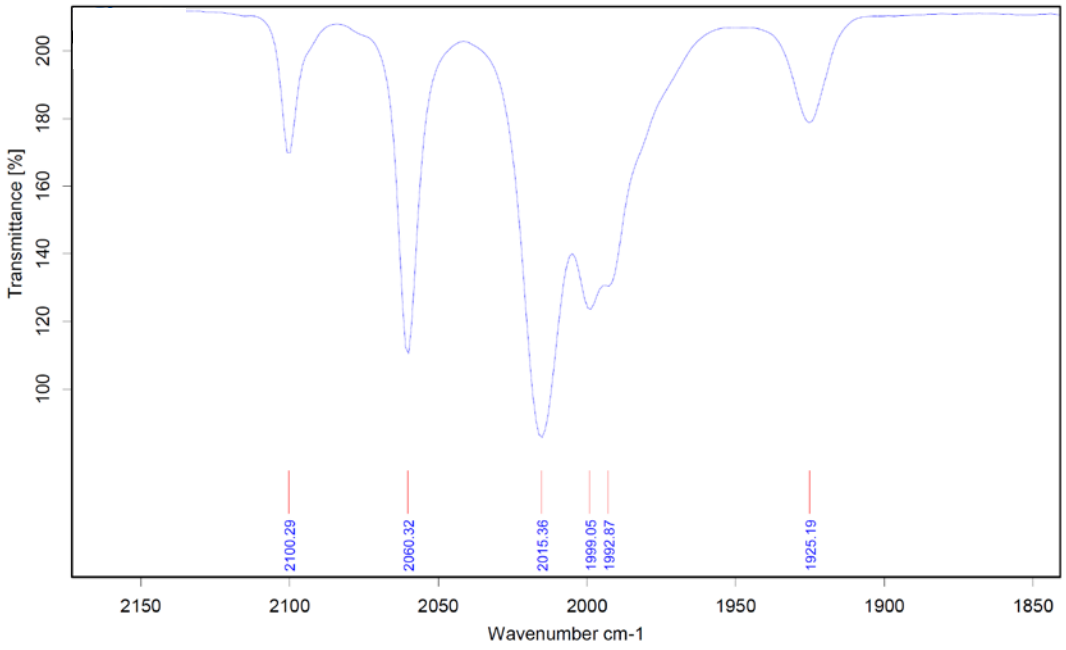


Figure S1. Solution IR spectrum (DCM) of cluster **2**.

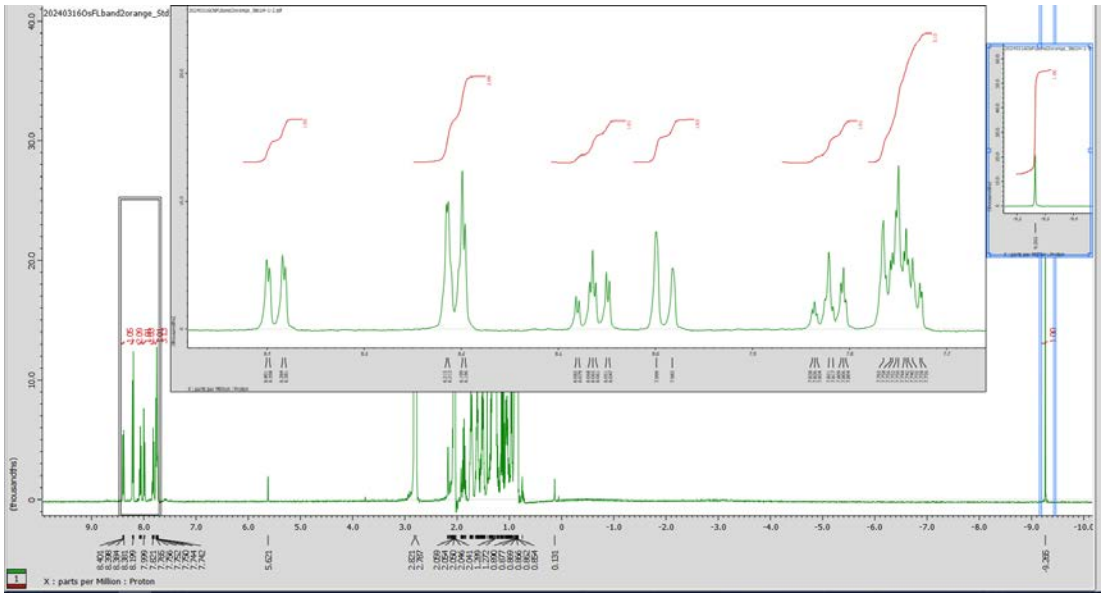


Figure S2. ^{1}H NMR spectrum (d_6-acetone) of cluster **2**.

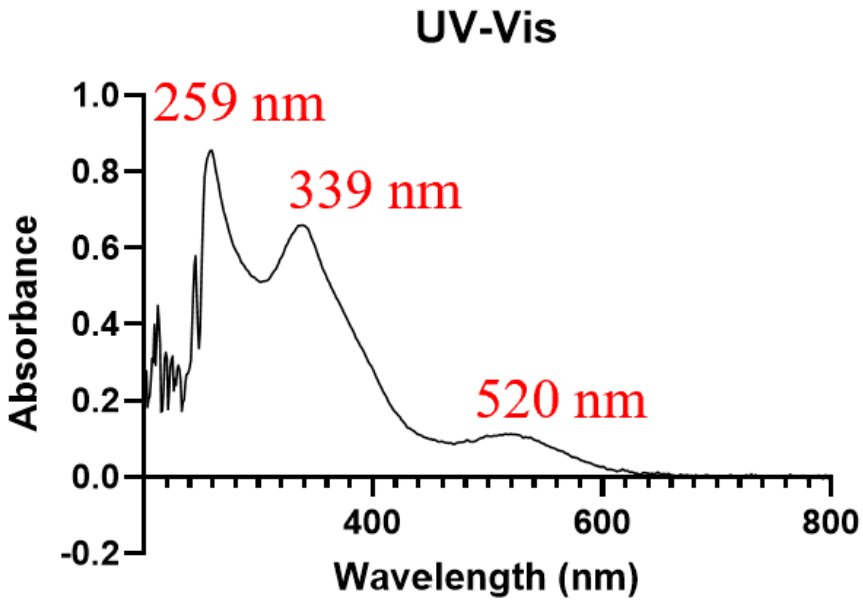


Figure S3. UV-Vis absorption spectrum of cluster **2**.

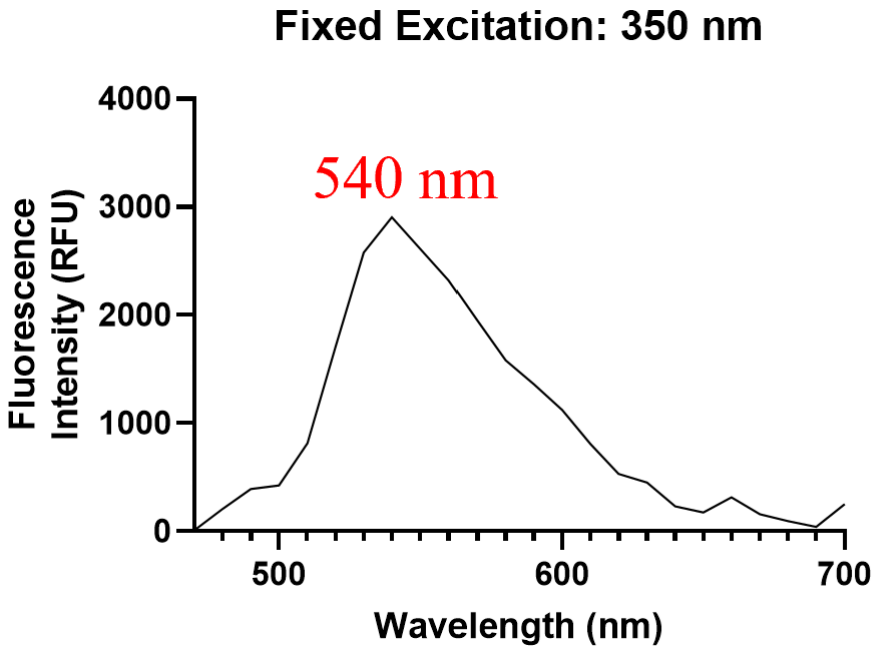


Figure S4. Fluorescence spectrum of cluster **2** with excitation at 350 nm.

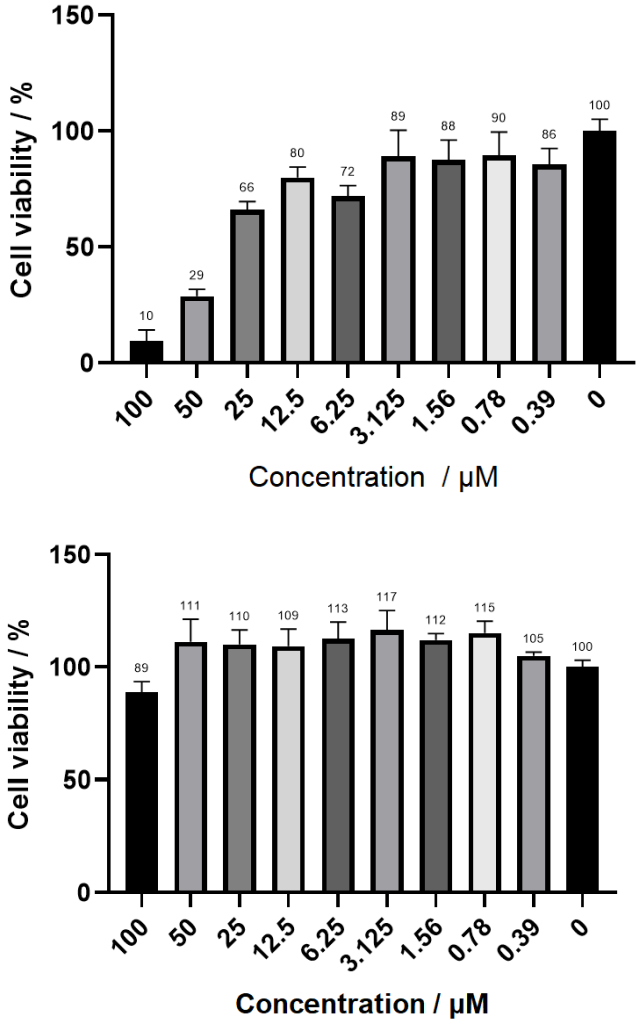


Figure S5. Cell viability study on **1** (top) and **2** (bottom) against the SH-SY5Y cell line, 24-hour treatment.

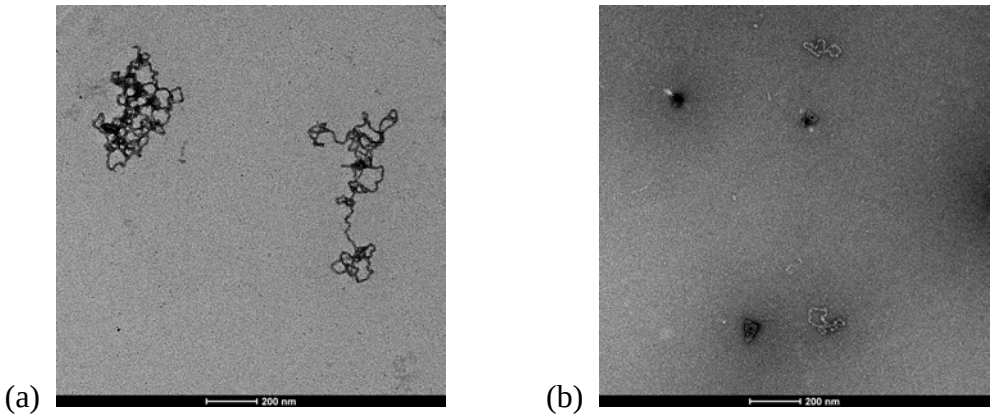


Figure S6. TEM images of aggregated wild-type α-synuclein samples, after staining

with uranyl acetate: (a) control, and (b) after treatment with cluster **1** (60 μM).

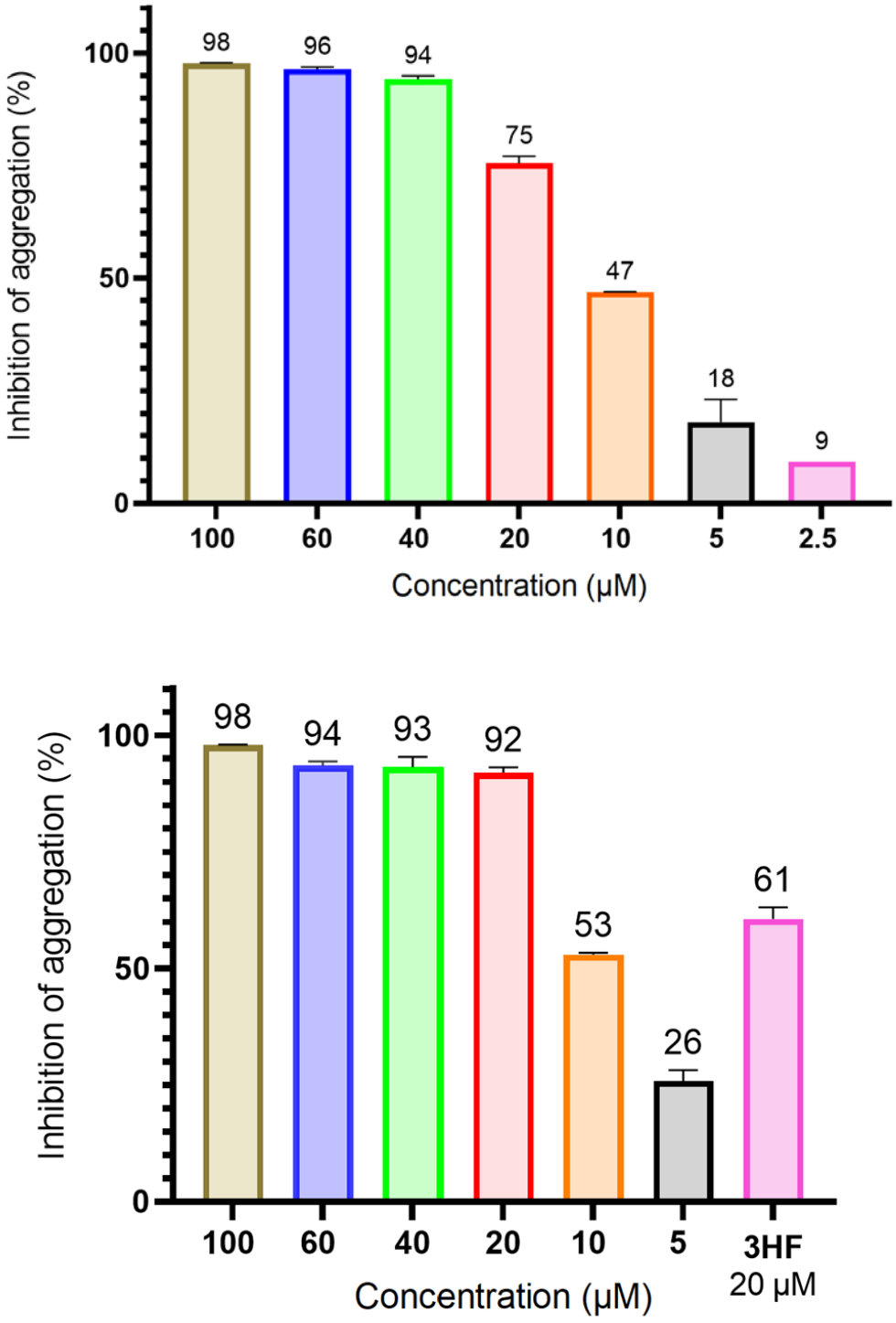


Figure S7. Percentage inhibition of wild-type α-synuclein aggregation after treatment with varying concentrations of clusters **1** (top) and **2** (bottom). Bottom plot includes inhibition with 20 μM **3HF** (positive control).

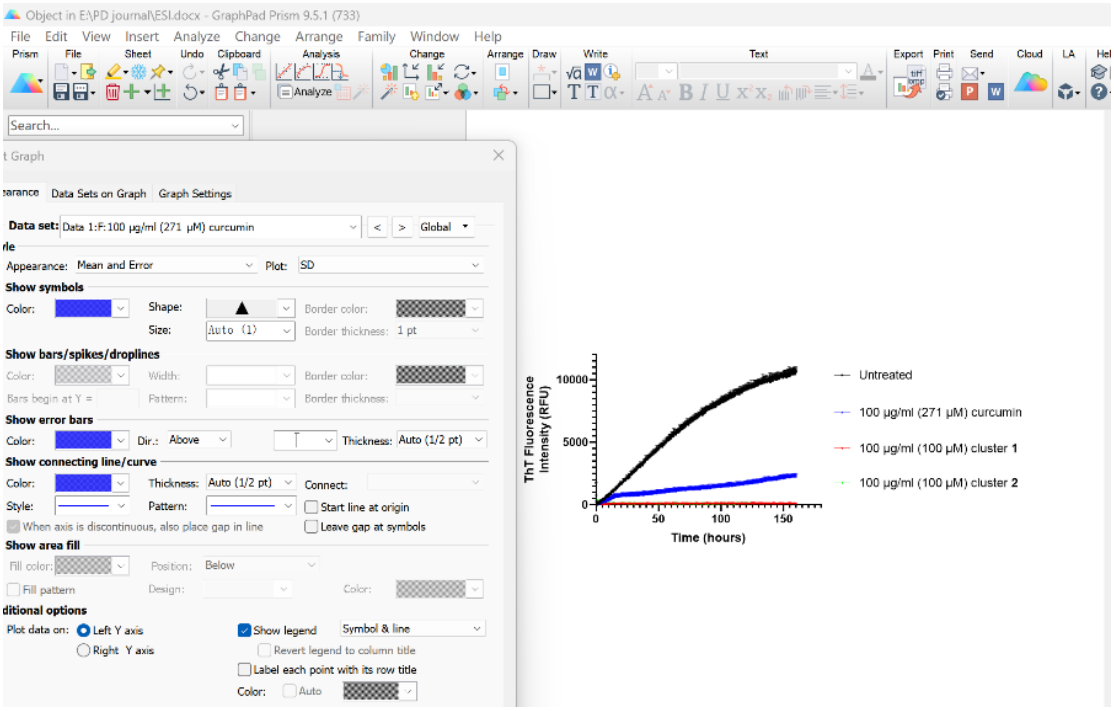


Figure S8. Details of plot parameters for Figure 3.

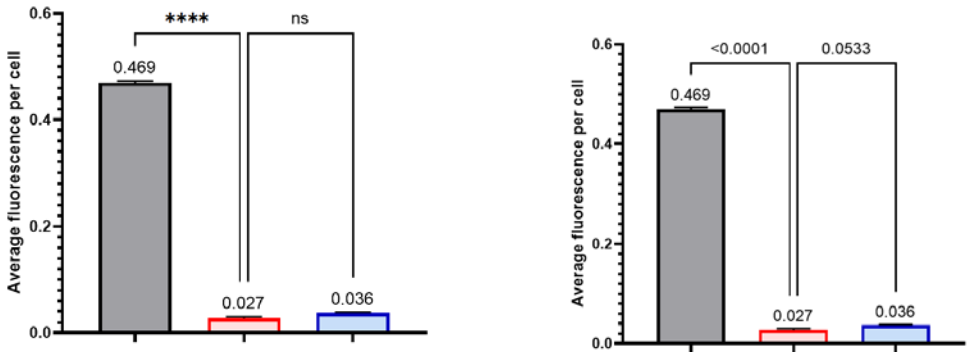


Figure S9. Statistical analysis for Figure 5.