equilibrium AUC of purified, native RBC αSyn. Upper panel shows the individual experimental analyses fitting an ideal single-species model to the equilibrium data obtained at 12k, 16k, and 20k RPM for 1.1 mg/ml αSyn solution. The fitting yielded a molecular weight of 57,753 Da (SD: +/- 655.199) with a root mean square deviation of 0.004533. Lower panel shows an overlay of the residuals of data and theoretical fit for the three different speeds.

Figure 3: A: CD-spectra of native tetrameric αSyn (isolated under non-denaturing conditions from human RBC) before vs. after addition of PC/PS SUV (protein/lipid 1:500). B: CD spectra of recombinant αSyn monomer purified from E. coli alone and with addition of PC/PS SUV (protein/lipid 1:500). C: SPR sensorgram of equal protein concentrations of αSyn recombinant monomer vs. endogenous tetramer injected on a L1 chip covered with a PC/PS membrane. D: Amyloid-type aggregation kinetics of recombinant αSyn monomer vs. native RBC tetramer monitored by ThT fluorescence; average values from 3 independent experiments (error bars = SD; some SD for RBC-derived αSyn are smaller than the symbol size). AU, arbitrary units.

Materials and Methods

Materials. Recombinant human αSyn was bought from Anaspec. Recombinant human transthyretin was generously provided by Irit Rappley and Jeff Kelly (Scripps Research Institute, La Jolla, CA). HEK, COS-7 and HeLa cells were cultured in DMEM with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml) and L-glutamine (2 mM). For M17D and 3D5 human neuroblastoma cells, standard DMEM was supplemented with 400 μg/ml G418 and 1 μg/ml puromycin. Frontal cortex was obtained from wt mice aged 4-9 months. αSyn immunoblotting used antibodies C20 (1:1000, Santa Cruz), LB509 (1:400, Santa Cruz), Syn211 (1:200, Santa Cruz) and Syn1 (1:2000, BD).
**Lipid preparation.** Small (30 nm) unilamellar vesicles (SUV) of 80% 1-palmitoyl-2-oleoyl-\(sn\)-glycero-3-phosphocholine (POPC) and 20% 1-palmitoyl-2-oleoyl-\(sn\)-glycero-3-[phospho-L-serine] (POPS) (Avanti Polar Lipids) were prepared in 10 mM sodium phosphate, pH 7.4, by sonication.

**Crosslinking.** Cells were detached and incubated at RT for 30 min in DSS crosslinker (1-5 mM), then quenched with 1 M Tris buffer, pH 7.4, for 15 min at RT. Human RBC lysates were treated analogously with 1 mM BS\(^3\) (Pierce) to covalently crosslink lysine residues.

**Native PAGE.** For Blue-Native PAGE, samples were run on 4-16% Bis-Tris BN-PAGE gels (Invitrogen) at 200 V and RT. The cathode buffer was 50 mM tricine, 15 mM Bis-Tris, 0.02% Brilliant Blue G (Serva, Heidelberg, Germany), pH 7.0; the anode buffer was 50 mM Bis-Tris pH 7.0. Clear-Native PAGE was conducted identically to Blue-Native PAGE, but Coomassie Blue was omitted from the sample and the cathode buffer. Electroblotting of protein on PVDF membranes (0.45 µm pore size) was conducted at 400 mA for 2 h. For MW estimation three different molecular weight marker were loaded on each gel (Sigma Non-denaturing, 108K6408, Invitrogen Native Mark, LC0725 GE Healthcare HMW Native Marker Kit, 17-0445-01).

**Isoelectric focusing 2D PAGE.** We used the IPGphor isoelectric focusing system (GE Healthcare). Lysates were heated at 65°C overnight and brought to 200 µl with sample rehydration buffer (7 M urea, 2 M thiourea, 2% Chapso, 0.5% IPG buffer (GE Healthcare, bromophenol blue) and applied on an 11 cm 1D Ready-strip (Bio-Rad) with a pH gradient of 4–7. Sample was rehydrated for 16 hr followed by isoelectric focusing at 500 V for 30 min, then 1000 V for another 30 min, and then 8000 V for 3.5 hr. The 1D strip was then applied to a precast NuPAGE ZOOM 4-12% Bis-Tris gel (Invitrogen) and run at 200 V.

**Purification of \(\alpha\)Syn from human RBCs.** Freshly collected and washed RBC were resuspended in 3-fold volume of ACK lysing buffer (Lonza, Walkersville MD, USA). \((NH_4)_2SO_4\) to a final concentration
of 25% was added and incubated at 4°C for 30 min. The lysate was centrifuged (20,000g, 20 min), and the supernatant brought up to 50% (NH₄)₂SO₄. The pellet was washed several times in 55% (NH₄)₂SO₄ to remove excess hemoglobin. The sample was centrifuged at 20,000 g for 20 min and the pellet resolubilized in 50-fold volume of 50 mM phosphate buffer, pH 7.0, 1 M (NH₄)₂SO₄. Five ml of the resultant solution were injected onto a 5 ml HiTrap phenyl hydrophobic interaction column (GE Healthcare) equilibrated with 50 mM phosphate buffer, pH 7.0, 1 M (NH₄)₂SO₄. αSyn was eluted with a 1 M to 0 M (NH₄)₂SO₄ gradient in 50 mM phosphate buffer, pH 7.0 (αSyn eluted at ~0.75 M (NH₄)₂SO₄). For anion exchange purification of RBC αSyn, we used the protocol employed for neuroblastoma cells (below), but the first run of RBC lysate sometimes showed low binding of αSyn and contamination by plasma transthyretin. In these cases we discarded the first eluate and used the flow-through for a second run, which showed significantly higher binding and subsequent purity. As a third alternative to HIC and AX, an XK 16/100 column packed with activated thiolpropyl Sepharose 6B gel media was employed (Fig S5B) (binding buffer: PBS, flow rate 0.2 ml/min). In this case, the flow-through contained αSyn and was processed further. The column was regenerated by eluting bound protein with 5 column volumes of binding buffer with 25 mM dithiothreitol, and reactivated with 1.5 mM dipyridyl sulfide in 50 mM borate buffer pH 8.0. The final solution was concentrated (Amicon Ultra centrifugal filter units, MWCO 10,000, Millipore) and further purified via gel filtration.

**Purification of αSyn from human neuroblastoma cells.** 3D5 cells (αSyn stables) and their parental M17D cells were scraped from the plates, washed in PBS and lysed by sonication. A (NH₄)₂SO₄ precipitation was conducted as described above, the 50% (NH₄)₂SO₄ pellet was taken up in 20 mM tris buffer, pH 8.0, 25 mM NaCl. The sample was injected onto a 5 ml HiTrap Q HP anion exchange column, equilibrated with 20 mM tris buffer, pH 8.0, 25 mM NaCl. αSyn was eluted from the column with a 25-500 mM NaCl gradient in 20 mM tris buffer, pH 8.0. αSyn eluted at ~300 mM NaCl. The column was regenerated with 1 M NaCl in 20 mM tris buffer, pH 8.0. The final solution was
concentrated (Amicon Ultra centrifugal filter units, MWCO 10,000, Millipore) and further purified via gel filtration. For the addition (“spiking”) of exogenous recombinant αSyn monomers, bacterially-expressed αSyn was added to the scraped M17D cell pellet and the purification scheme conducted as just described.

**Gel filtration.** Aliquots (250 µl) were injected onto either a Superdex 75 (10/300 GL), Superdex 200 (10/300 GL) or a Superose 12 (10/300 GL) column (GE Healthcare) at 4 °C and eluted with 50 mM ammonium acetate, pH 8.5. For size estimation, a gel filtration standard (Bio-Rad, cat. no. 151-1901) was run on each column, and the calibration curve was obtained by semi-logarithmic plotting of molecular weight vs. elution volume divided by void volume.

**Scanning transmission electron microscopy.** STEM was carried out at the Brookhaven National Laboratory STEM user facility with 100 µl of sample at a concentration of 300 µg/ml in 50 mM ammonium acetate, pH 7.4, and diluted to find the appropriate concentration for a homogenous particle distribution. Tobacco mosaic virus (TMV) rods were included during specimen preparation as an internal sizing standard.

**Circular dichroism spectroscopy.** CD spectra were obtained using an Aviv Biomedical spectrometer (model 410) in the presence or absence of 4 mM POPC/POPS SUV. The spectral contributions of buffer and SUVs were subtracted. Data are reported as mean residue ellipticities measured at 20 °C and a pathlength of 0.1 mm.

**Lipidex 1000 treatment.** 10% (w/v) Lipidex 1000 (Perkin Elmer) was washed with 50% methanol-ultra pure water and added to a 100 µM solution of purified αSyn from RBC. The samples were stirred overnight at 37°C, and αSyn was purified from that mixture via size exclusion chromatography.

**Surface plasmon resonance.** All lipid binding experiments were performed at 20°C on a BIACORE 3000 apparatus using the L1 sensor chip (Biacore AB, Uppsala, Sweden). The running buffer was 10
mM sodium phosphate, pH 7.4. SUV were applied to the sensor chip surface at a flow rate of 10 µL/min in the presence of 0.1 mM NaCl. Injections were done at a flow rate of 10 µL/min with 50 µl sample volume. Apparent $K_D$ values were calculated from equilibrium data of several dilution series, collected at 300-320 s.

**Thioflavin T (ThT) binding.** To detect amyloid fibril growth, a discontinuous assay was used. Aliquots (10 µL) were removed from each purified αSyn sample (lyophilized from 50 mM ammonium acetate, pH 7.4, and agitated at 37°C at a concentration of 75 µM in 20 mM Bis-Tris propane, 100 mM LiCl, pH 7.4) and added to 2 mL of a 10 µM Thioflavin T (ThT) solution in 10 mM glycine buffer, pH 9. Fluorescence was directly quantified on a Varian Eclipse fluorescence spectrophotometer at 20°C by exciting at 444 nm and scanning the emission wavelengths from 460 to 550 nm with slit widths set at 5 nm (PMT at 750V).

**Quantitative phosphate analysis.** Samples (2 x 15 µl and 2 x 30 µl of 1 mg/ml αSyn in 50 mM ammonium acetate, pH 7.4) were placed at the bottom of glass test tubes, 225 µl of 8.9 N H$_2$SO$_4$ (in deionized water) was added, and the mixture was heated for 25 min at 200-215°C. Next, 75 µl H$_2$O$_2$ was added to all tubes at RT. After heating for 30 min at 200-215°C, 1.95 ml deionized water and then 0.25 ml 2.5% ammonium molybdate(VI) tetrahydrate solution (in deionized water) were added at RT. After addition of 0.25 ml 10% ascorbic acid solution (in deionized water), the tubes were heated for 7 min at 100°C, and samples were allowed to cool to RT. Absorbance at 820 nm was measured, and phosphate concentration calculated using a calibration curve obtained from 7 phosphate standard solutions ranging from 0-50 nmol phosphate (Sigma-Aldrich).

**Analytical ultracentrifugation (sedimentation equilibrium analysis).** AUC experiments were performed in a Beckman Optima XL-I analytical ultracentrifuge. Sedimentation equilibrium experiments were carried out at purified αSyn protein concentrations of 1.6, 1.1 and 0.6 mg/ml in 50 mM ammonium acetate, pH 8.5. The experiments were performed at 20°C at 12,000, 16,000 and 20,000
rpm (AN-60 Ti rotor), and data were collected at 278 nm. The software SEDPHAT (version 6.5) was used to calculate the M and s of the species present in equilibrium in the samples. For MW analysis, we used the model “Species Analysis” available in the SEDPHAT program with RI noise baseline correction. Analysis was performed for each protein concentration separately, and the MW determined from the average obtained for the analyses of the 3 protein concentrations. The average errors and standard deviations were calculated using Monte-Carlo simulation, with 1000 iterations and a confidence level of 0.68.