SUPPLEMENTARY INFORMATION

SPECIFICITY AND KINETICS OF α-SYNUCLEIN BINDING TO MODEL MEMBRANES DETERMINED WITH FLUORESCENT ESIPT PROBE*

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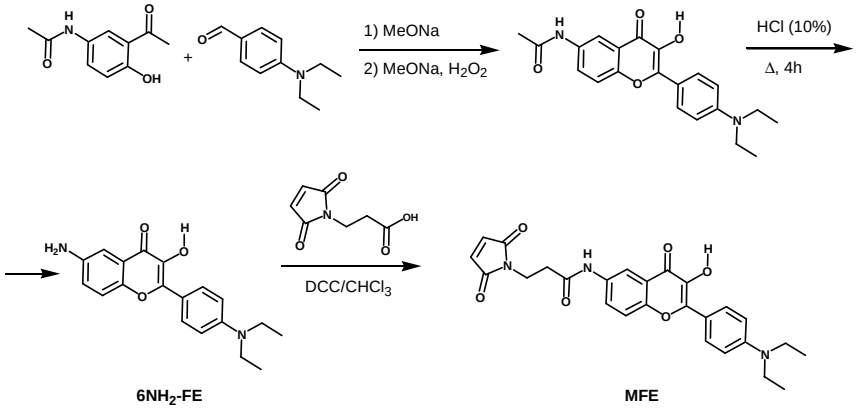
EXPERIMENTAL PROCEDURE

Protein expression and purification

Alpha-Synuclein was expressed in *Escherichia coli* using plasmid pT7-7 encoding for the protein (courtesy of the Lansbury laboratory, Harvard Medical School, Cambridge, MA). Following transformation, BL21- competent cells were grown in LB in the presence of ampicillin (100 mg/ml). Cells were induced with IPTG, cultured at 37 °C for 4 hours and harvested by centrifugation in an Avanti J25 centrifuge with a JA-20 rotor at 5000 rpm (Beckman Coulter). The cell pellet was resuspended in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 1 mM PMSF, and lysed by multiple freeze–thaw cycles and sonication. The cell suspension was boiled for 20 min and centrifuged at 13500 rpm with a Beckman JA-20 rotor. Streptomycin sulfate was added to the supernatant to a final concentration of 10 mg/ml and the mixture was stirred for 15 min at 4 °C. After centrifugation at 13500 rpm, the supernatant was collected and ammonium sulfate was added (to 0.36 g/ml). The solution was stirred for 30 minutes at 4 °C and centrifuged again at 13500 rpm. The pellet was resuspended in 25 mM Tris-HCl, pH 7.7, and loaded onto an HQ/M-column on a BIOCAD (Applied Biosystems) workstation. AS eluted at 300 mM NaCl using a salt gradient from 0-600 mM NaCl and was further purified and desalted with a Superdex 200 column (GE Healthcare) equilibrated with 25 mM Na-PO_4, pH 6.2. The protein was concentrated with an Amicon Ultracel filter (10 kDa), and its purity was assessed by PAGE and electrospray ionization mass spectrometry (ESI-MS). The protein concentration was estimated from the absorbance at 275 nm using a molar extinction coefficient of 5600 M^{-1} cm^{-1}.

The cysteine variants of AS A18C, A90C and A140C were constructed using the Quick-Change site-directed mutagenesis kit (Stratagene), and the introduced modification was verified by DNA sequencing. Cysteine mutants were prepared similarly to WT AS but with addition of 1 mM TCEP as a reducing agent to the buffer during purification and in the final buffer in order to prevent oxidation.

Synthesis of MFE label.



MFE dye, *N*-(2-[4-(diethylamino)phenyl]-3-hydroxy-4-oxo-4*H*-chromen-6-yl)-4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propanamide, was obtained as the product of the reaction of BMPA

(N-β-Maleimidopropionic acid) with 2-[4-(diethylamino)phenyl]-3-hydroxy-4-oxo-6-amino-4*H*-chromen ($6NH_2$-FE). Briefly, 25 mg (0.077 mmol) of $6NH_2$-FE and 20 mg (0.12 mmol) of BMPA were dissolved in 3 mL of $CHCl_3$ and cooled to 0 °C. To this solution 50 mg (0.25 mmol) of N,N'-Dicyclohexylcarbodiimide (DCC) was added and the reaction mixture was allowed to warm to room temperature while stirring overnight. The mixture was filtered through Celite, concentrated, and purified by column chromatography (ethyl acetate). Chemical structure of final product was confirmed by NMR (400 MHz, DMSO-d6): δ 10.24 (s, 1H, OH), 9.00 (s, 1H, NH), 8.34 (d, *J*=2.6Hz, 1H, HetH), 8.07 (d, *J*=9Hz, 1H, ArH), 7.81 (dd, *J*=2.6Hz, *J*=9Hz, 1H, HetH), 7.64 (d, *J*=9Hz, 1H, Het), 7.02 (s, 2H, CH=CH), 6.82 (d, *J*=9Hz, 2H, ArH), 3.76 (t, *J*=7.2Hz, 2H, CH_2), 3.42 (q, *J*=6.8Hz, 4H, CH_2), 2.63 (t, *J*=7.2Hz, 2H, CH_2), 1.15 (t, *J*=6.8Hz, 6H, CH_3).

The precursor $6NH_2$-FE was obtained as a product of hydrolysis in HCl of the corresponding acetamide which was synthesized according to Algar-Flynn-Oyamada oxidative cyclization of the 5'-acetamido-2'-hydroxyacetophenone and 4-diethylaminobenzaldehyde as described elsewhere (54).

RESULTS

Competitive binding of AS to SUVs of different acyl chain composition.

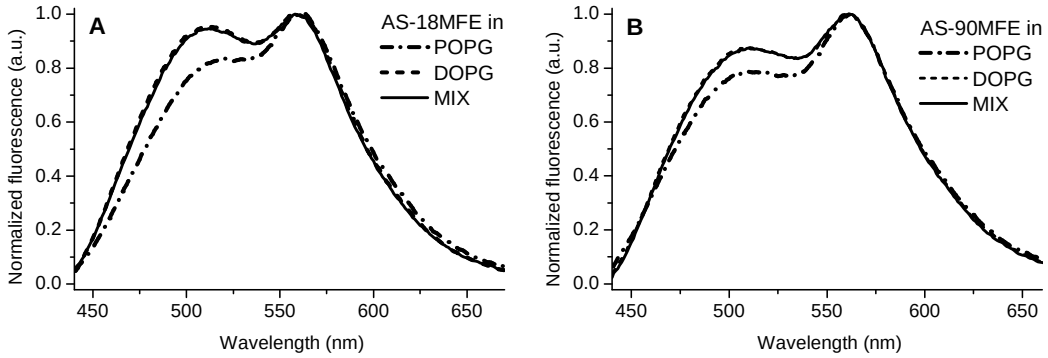


Fig. S1. Comparison of AS affinity to POPG and DOPG SUVs. Normalized fluorescence emission spectra of AS-18MFE (panel **A**) and AS-90MFE (panel **B**) in the presence of excess of POPG (-·-·) and DOPG (- - -) SUVs or both of them (—). Experiments performed at 37 °C in 25 mM Na-PO_4 pH 6.5, 150 mM NaCl. Excitation at 420 nm. Protein and lipid concentration were 0.2 μM and 40 μM respectively. Results shows that AS-MFE spectra in the presence of both types of vesicles are identical to those in the presence of only DOPG, evidencing that preferred binding of AS to the lipids with higher content of double bonds.

Effect of AS on lipid vesicles.

At high concentration AS can disrupt vesicles [Varkey, J., Isas, J. M., Mizuno, N., Jensen, M. B., Bhatia, V. K., Jao, C. C., Petrlova, J., Voss, J. C., Stamou, D. G., Steven, A. C. & Langen, R. (2010). Membrane curvature induction and tubulation are common features of synucleins and apolipoproteins. *J Biol Chem* **285**, 32486-93]. We checked if binding of AS leads to lipid aggregation/tabulation using dynamic light scattering (DLS). In the presence of excess lipid (lipid/protein>100) AS does not perturb the vesicle size distribution, excluding the formation of tubular vesicle aggregates (Fig. S2).

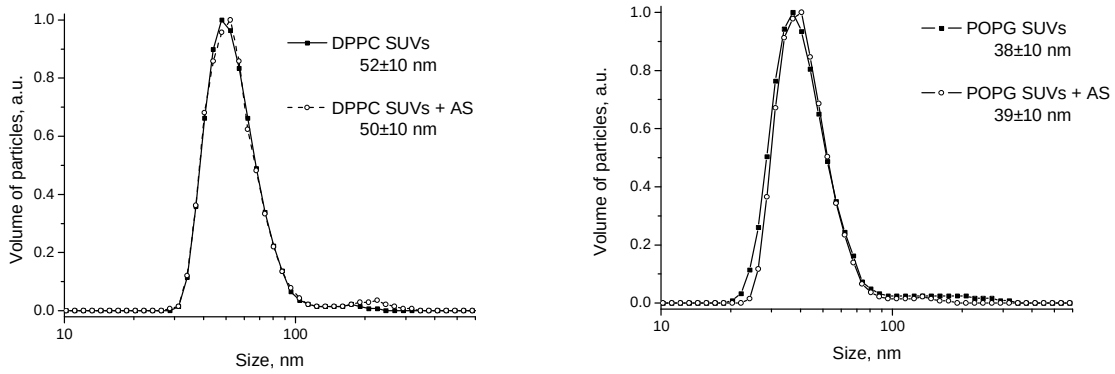


Fig. S2. Changes of size distribution of SUVs upon addition of AS. Lipid and protein concentration were 100 μM and 0.5 μM respectively. Measurements were performed at 25 °C in 25 mM Na-PO_4, pH 6.2, 150 mM NaCl. The changes in static light scattering upon addition of AS to the vesicles was less than 20%.

The effect of AS on vesicle structure was observed only when negatively charged SUVs (POPG or DOPG) were mixed at low lipid-to-protein ratio (L/P=3-15). DLS showed formation of particles with a size in the range 100-1000 nm (no sharp peak in size distribution), whereas static light scattering increased ~5 fold. We avoided this lipid-to-protein ratio range in our titration experiments.