SUPPORTING INFORMATION

Curcumin modulates α-synuclein aggregation and toxicity

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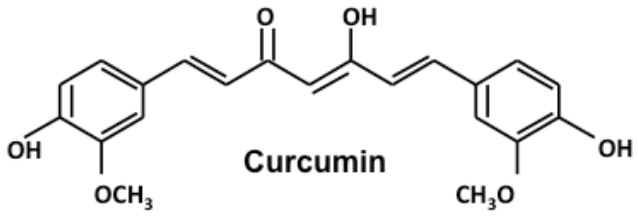
Table S1. Flow cytometry analysis showing percentage of live and dead cells in presence and absence of oligomers and oligomers+cutrcumin.

Sample name	% Dead (Q1)	% Late Apoptosis (Q2)	% Live (Q3)	% Early Apoptosis (Q4)
Buffer	0.8	0	99.2	0
Oligo	24.9	6.1	66.1	2.9
Oligo+Cur	10	1.2	86.1	2.7



M[1]DVFMKGLSK[10]AKEGVVAAAE[20]KTKQGVAEAA[30]GKTKEGV
LYV[40]GSKTKEGVVH[50]GVATVAEKTK[60]EQVTNVGGAV[70]VTGV
TAVAQK[80]TVEGAGSIAA[90]ATGFVKKDQL[100]GKNEEGAPQE[11
0]GILEDMPVDP[120]DNEAYEMPSE[130]EGYQDYEPEA[140]

α-synuclein

Figure S1. Chemical structure of curcumin and amino acid sequence of α-Syn. The structure of curcumin showing its backbone and functional groups. The human α-synuclein is 140 amino acid residue protein and is shown with one letter code.

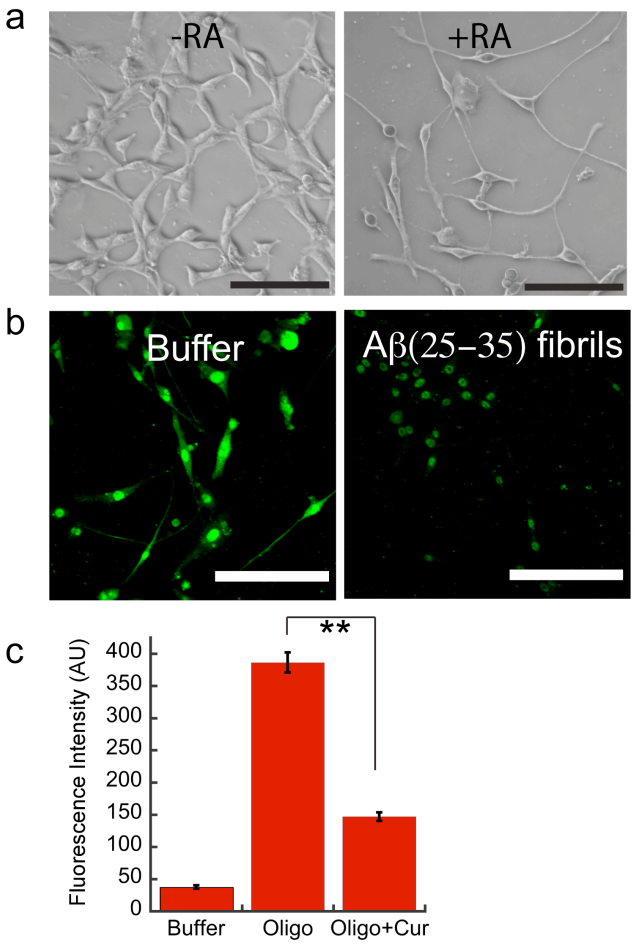


Figure S2. Differentiation of SH-SY5Y cells and toxicity measurement. SH-SY5Y cells were treated in presence (+RA) and absence (-RA) of 50 μM all-trans retinoic acid (RA) for 5 days. Phase contrast microscopy image showing differentiation of cells in presence of RA. Scale bars are 200 mm. (b) Expression of synaptophysin in SH-Sy5Y cells in presence and absence of Aβ(25-35) amyloid fibrils used as a positive control for measuring α-Syn oligomers toxicity. Scale bars is 200 μm. (c) Fluorescence intensity of oxidized hydroethidium measured in cells treated with oligomers in presence and absence of curcumin. Cells in buffer used as a control. Statistical significance **P<0.01.

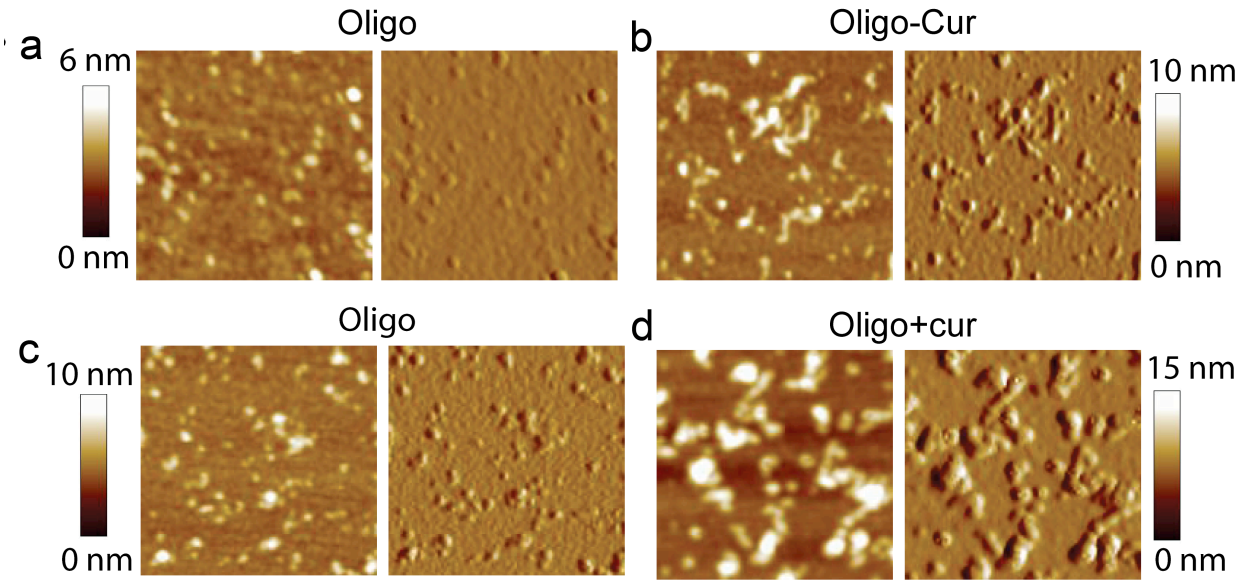


Figure S3. Curcumin alters the morphology of preformed α-Syn oligomers. AFM images of preformed oligomers isolated from SEC. 5 mg/ml solution of α-Syn in MES buffer, pH 6.0 in presence and absence of 100 μM curcumin were injected to SEC column. (a) AFM image of oligomers directly isolated from SEC. (b) Oligomers morphology by AFM of α-Syn-curcumin complex isolated from SEC. (c) AFM images of 30 mins incubated α-Syn oligomers. (d) AFM images of 30 mins incubated α-Syn oligomers in presence of curcumin. Scales are shown for the height of the corresponding height - images.

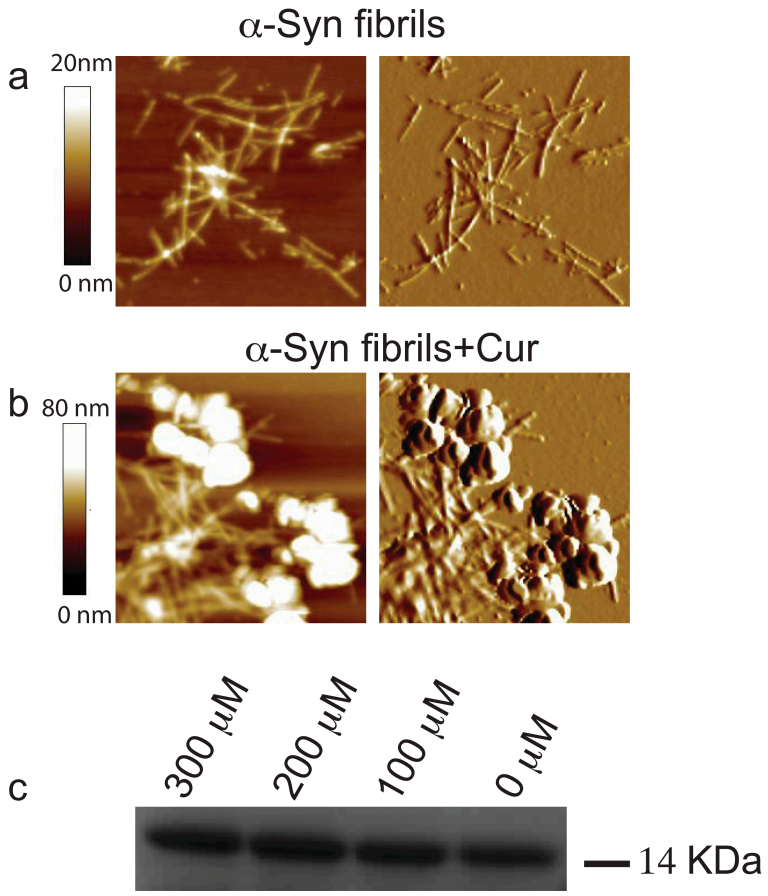


Figure S4. Curcumin alters morphology of preformed amyloids without disintegrating them into monomers. α-Syn fibrils (100 μM) were incubated without and with curcumin (100 μM) for 20 hrs and morphology were observed under AFM. AFM images of (a) α-Syn fibrils alone (b) α-Syn fibrils incubated with curcumin. Left and right images correspond to height and amplitude modes respectively and corresponding height scale bars are represented. (c) Preformed α-Syn fibrils (100 μM) were incubated in the absence and presence of 100 μM, 200 μM and 300 μM curcumin for 20 hrs. After the incubation the samples were centrifuged and supernatants were analyzed by 12% Bis-Tris NuPAGE gel (Invitrogen, USA). The intensity of monomeric fractions remained same in the absence and presence of curcumin.

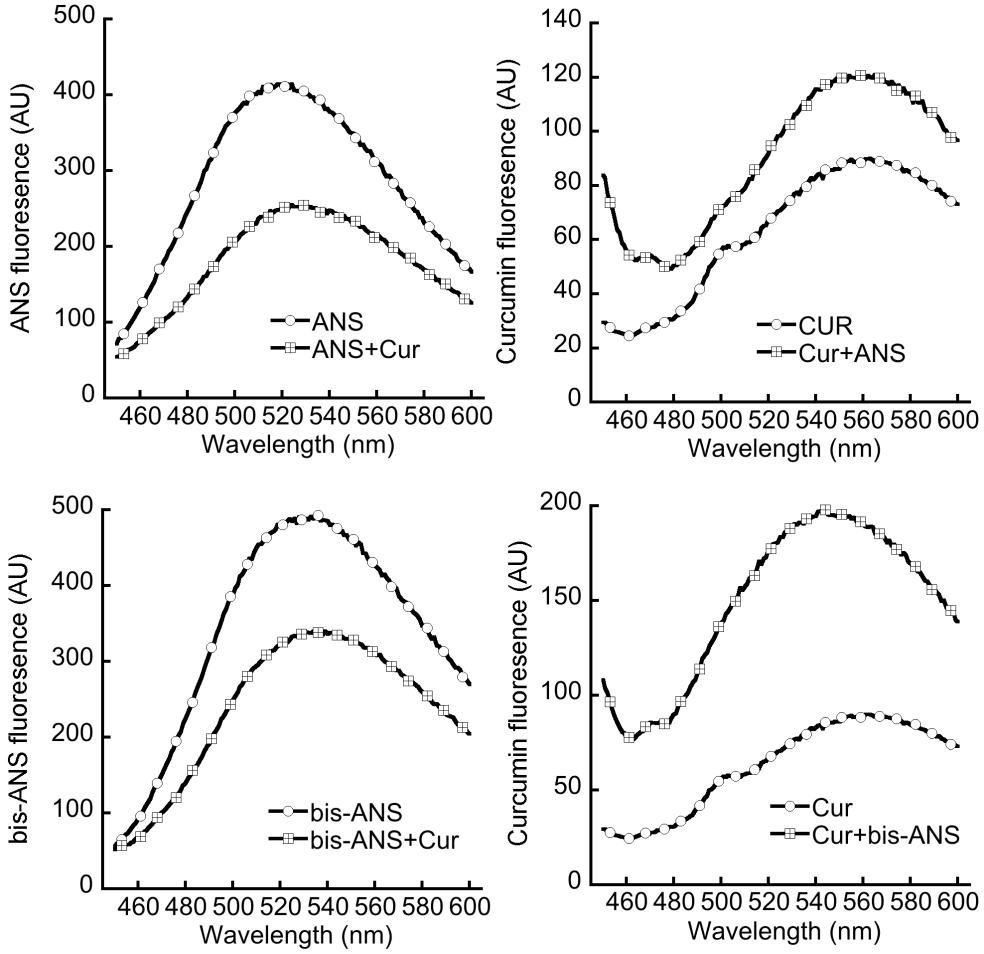


Figure S5. ANS and bis-ANS are not appropriate probes to measure the exposed hydrophobic surfaces of protein in presence of curcumin. ANS (75 μM) and bis-ANS (75 μM) fluorescence were recorded in the absence and presence of curcumin (75 μM). Similarly the fluorescence spectra of curcumin (75 μM) in absence and presence of each of ANS (75 μM) and bis-ANS (75 μM) were also recorded. The ANS/bis-ANS fluorescence intensities were decreased in presence of curcumin with concomitant increase in the curcumin fluorescence.

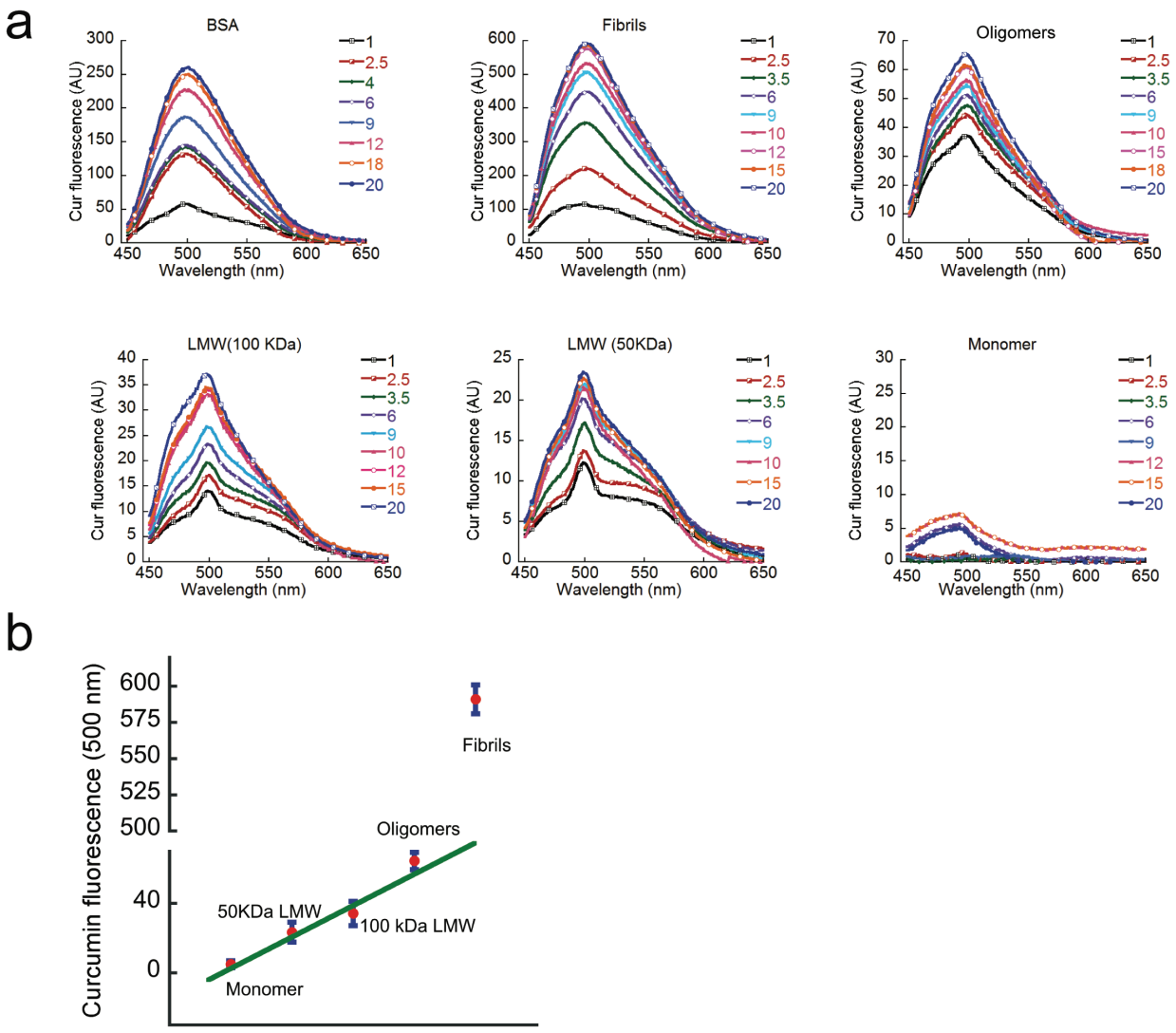


Fig S6. Curcumin fluorescence in presence of α-Syn and BSA. (a) Curcumin fluorescence of varying concentrations of curcumin (labeled) in presence of 5 μM different α-Syn and BSA. (b) The maximum fluorescence value of 20 μM curcumin at 500 nm were plotted against the individual α-Syn species showing increase in curcumin fluorescence according to the oligomers order.

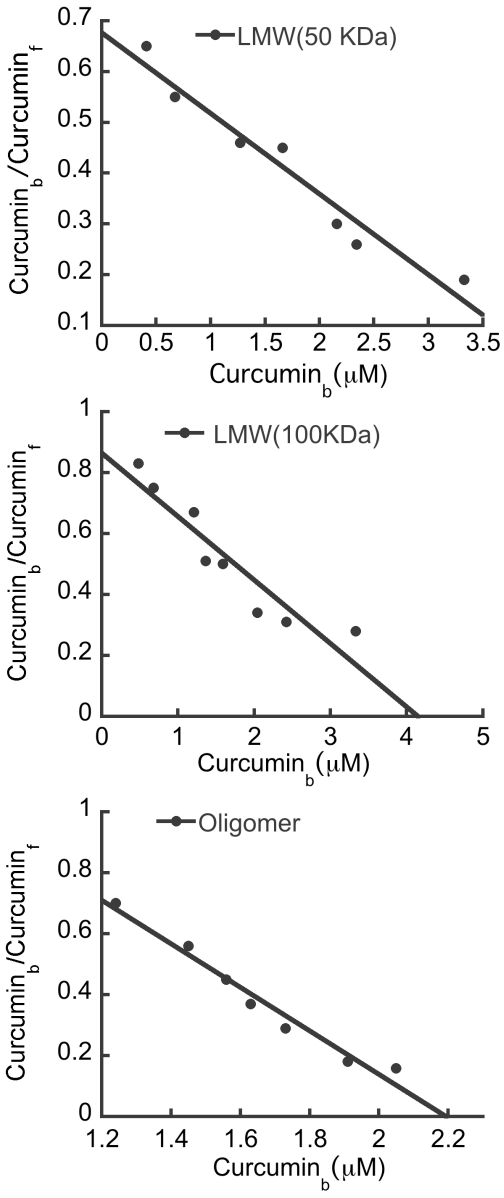


Figure S7. Scatchard plots for various α-Syn species. Scatchard plots for LMW 50 kDa, LMW 100 kDa and oligomers.

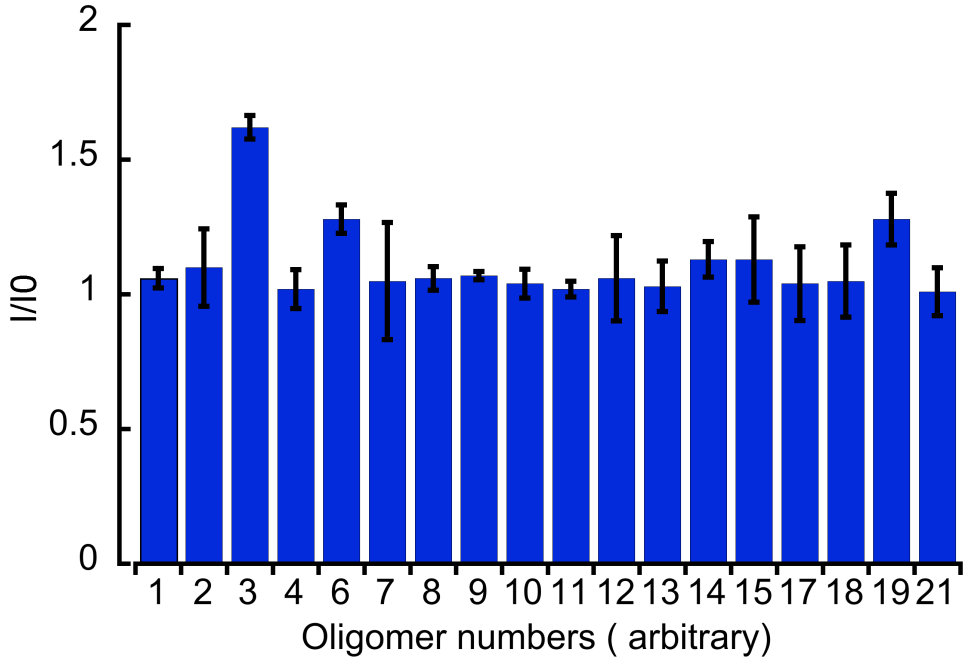


Figure S8. Relative ^{1}H-^{15}N HSQC intensity plot (I/I_0) for α-Syn oligomers. The additional peaks that were appeared in HSQC spectra of 100 kDa LMW in presence and absence of curcumin were assigned arbitrarily. The relative intensities of these peaks were calculated in the presence (I) and absence of curcumin (I_0). The I/I_0 values were plotted against the corresponding oligomers number. Significant increase in the peak intensities in the presence of curcumin was observed.