

Figure S1

Figure S1. Measurement of activity of native (●) and aggregated (○) BCA at different time intervals. The esterase activity of BCA was measured using p-nitrophenyl acetate as the substrate, as per the reported protocol.^{S1} 100 % activity refers to the initial activity of native BCA.

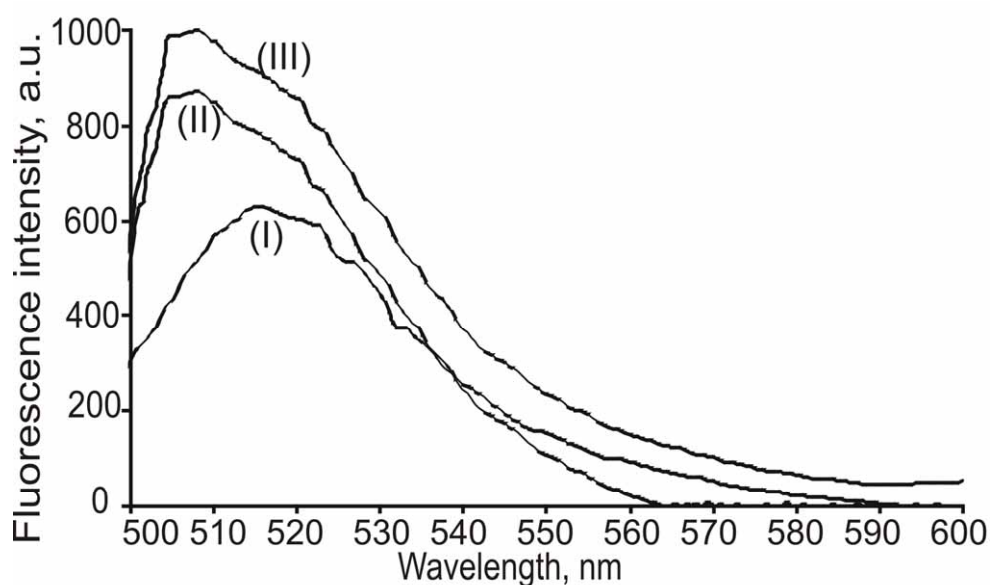


Figure S2

Figure S2. Effect of presence of Coumarin 6 during thermal aggregation of BCA on the fluorescence intensity of Coumarin 6. The fluorescence emission spectra Coumarin 6 alone (I), when added to BCA during aggregation (II) and when added to BCA after aggregation (III) are shown. The concentration of Coumarin 6 was 400 nM in each case. Samples were excited at 470 nm. Excitation slit width was kept at 5 nm and emission slit width was kept at 7.5 nm.

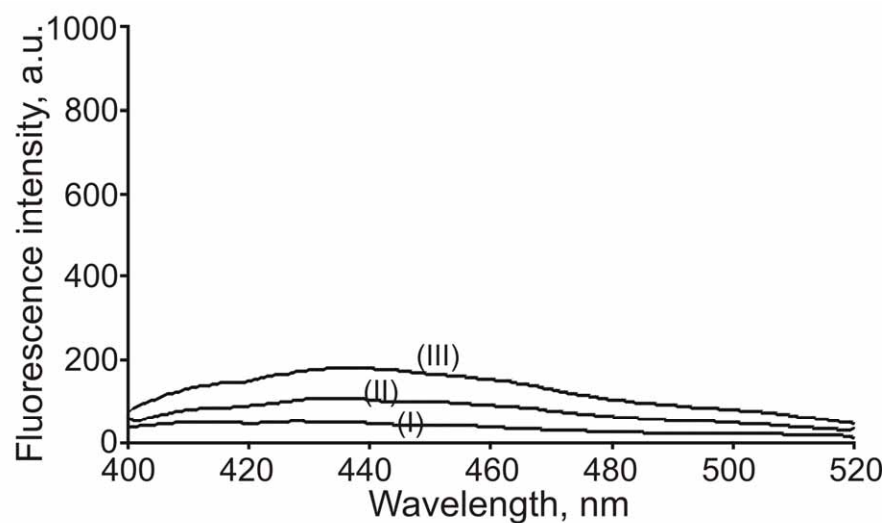


Figure S3

Figure S3. Effect of concentration of the dye on the fluorescence emission intensity of DPH. Fluorescence spectra of DPH were recorded at 5 nM (I), 500 nM (II) and 5 μ M (III) concentrations as shown. Samples were excited at 360 nm. Excitation slitwidth was kept at 5 nm and emission slitwidth was kept at 7.5 nm.

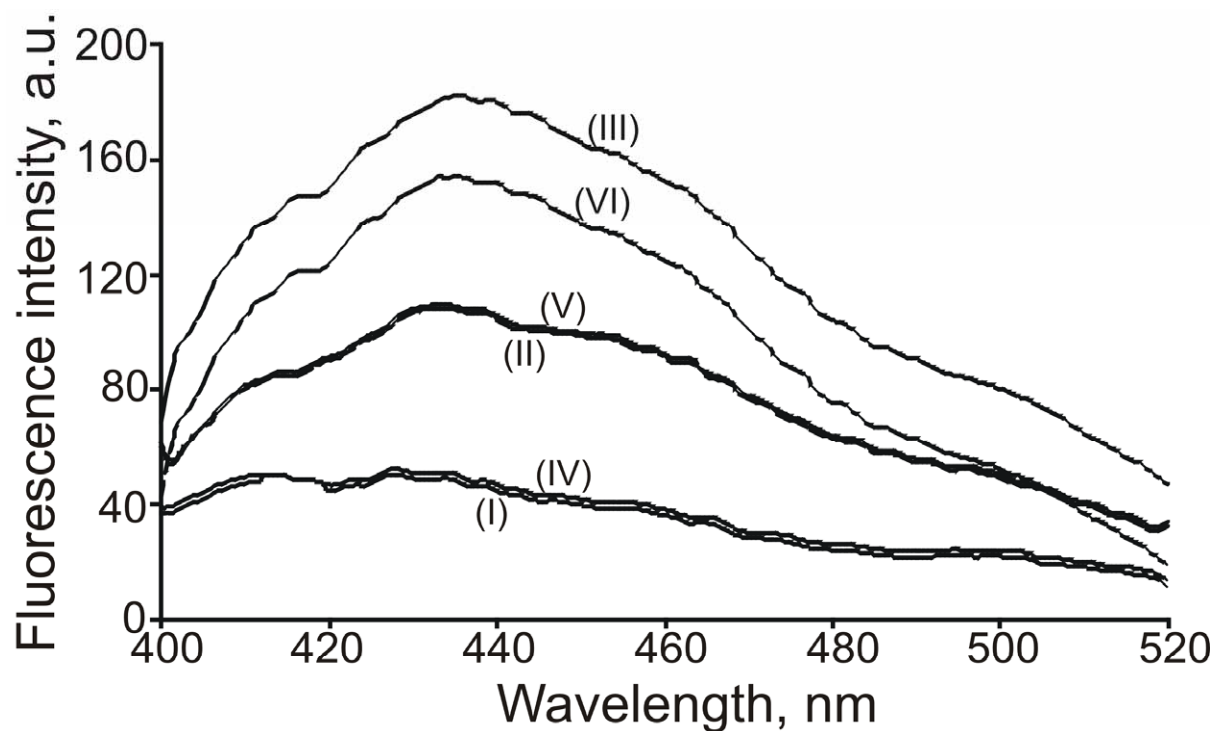


Figure S4

Figure S4. Effect of heating on the fluorescence emission intensity of DPH. Fluorescence emission spectra of DPH were recorded before (I, II, III) and after (IV, V, VI) heating at 5 nM (I, IV), 500 nM (II, V) and 5 μ M (III, VI). Samples were excited at 360 nm. Excitation slitwidth was kept at 5 nm and emission slitwidth was kept at 7.5 nm.

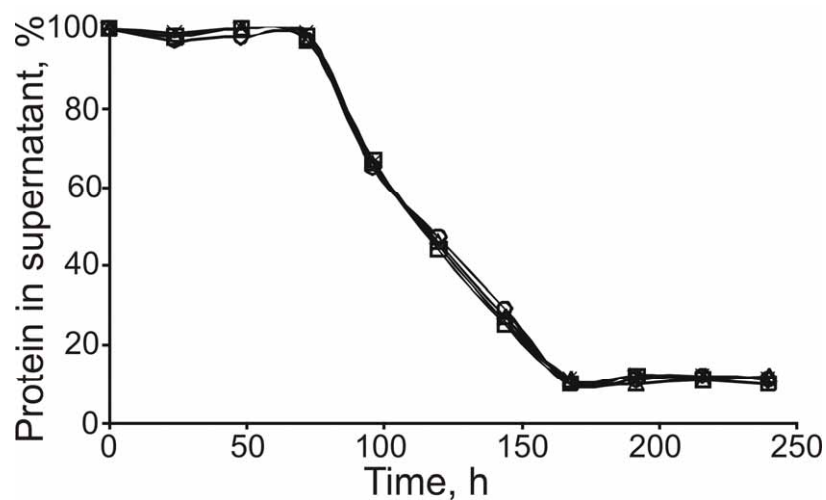


Figure S5

Figure S5. Effect of dye on the folding/unfolding equilibrium of α -synuclein. Samples were centrifuged after addition of 2 μ M thioflavin T (○), 50 nM Coumarin 6 (△) and 50 nM DPH (□) in separate experiments at different time intervals. The amount of protein in the supernatant was estimated by the dye binding method.³⁸ The amount of protein in the supernatant obtained in the absence of any dye (×) is shown for comparison. 100 % protein refers to the amount of protein in the starting sample in each case.

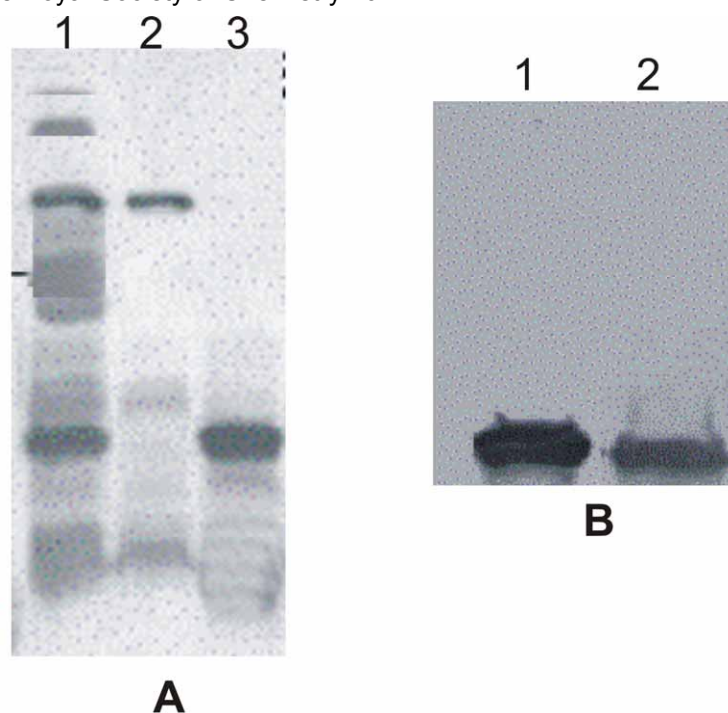


Figure S6

Figure S6. Purification of α -synuclein expressed in *E. coli* BL21 cells. Purification of α -synuclein was carried out by acid treatment and anion exchange chromatography, as per the reported protocol.⁴² The purity of the samples was checked by (A) SDS-PAGE and (B) Western blotting. Samples were loaded on 15 % crosslinked denaturing polyacrylamide gel. After the electrophoretic run, the gel was silver stained. For (A), Lane 1: Bacterial lysate, lane 2: flowthrough; lane 3: purified protein. Protein load was equal in all the lanes. For western blotting, proteins were transferred electrophoretically to nitrocellulose membrane (0.45 μ m). After blocking overnight, the membrane was incubated with anti- α -synuclein monoclonal antibody followed by anti-mouse horseradish peroxidase (HRP)-conjugated antibody as the secondary antibody. The presence of the bands was visualized by adding 1% (v v⁻¹) tetramethyl benzidine/hydrogen peroxide (TMB/H₂O₂). For (B), Lane 1: Bacterial lysate; lane 2: Purified protein.

References

S1. Y. Pocker, and J. T. Stone, *Biochemistry* 1967, 6, 668-678.