Supplementary information

α-Synuclein Exhibits Differential Membrane Perturbation, Nucleation and TLR2 Binding through Its Secondary Structure.

Manisha Kumari[†], Pranita Hanpude[†] and Tushar Kanti Maiti[†]*

[†]Functional Proteomics Laboratory, Regional Centre for Biotechnology, NCR Biotech Science Cluster, 3rd Milestone, Faridabad-Gurgaon Expressway, Faridabad-121001, India

Figure S1.

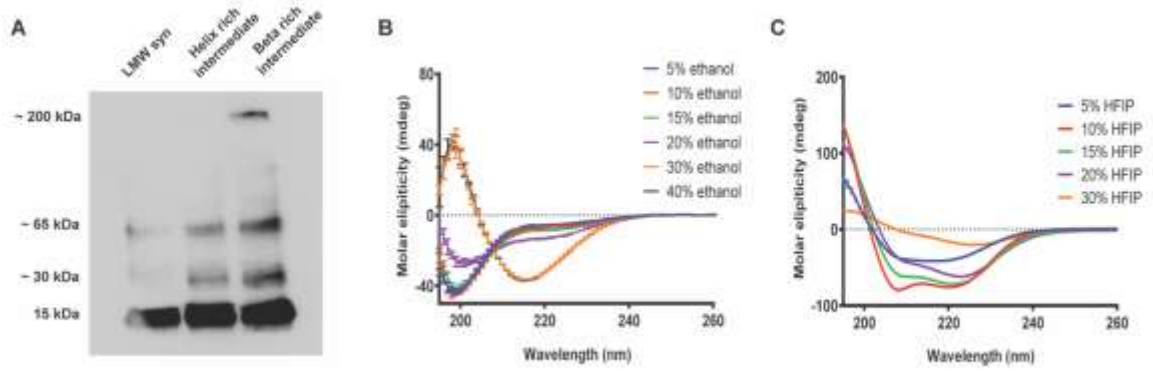

Figure S1: Standardization of condition used for generation of intermediate species of α-synuclein. (A) Western blot showing the cross linking of helix rich intermediate species and beta rich intermediate species with 0.01% glutaraldehyde. CD spectra showing change in secondary structure of α-synuclein under (B) different concentration of ethanol (5%-40%) and (C) different concentration of HFIP (5%-30%).

Table S1. Secondary structure analysis of α-synuclein species using DicroWeb Analysis software.

Samples	Before lyophilisation				After lyophilisation			
	Helix	Beta	Unordered	Turn	Helix	Beta	Unordered	Turn
LMW syn	5±0.1	15.7±0.6	68±1.7	10.7±1.1	6.3±1.1	16.7±3.2	63.7±4.7	12±1.7
Helix rich intermediate	62.3±1.1	15.7±2.3	15±1	7.3±0.6	40.7±2.3	11	32.3±0.5	14.3±1.1
Beta rich intermediate	22±1	34±1.7	26±1.7	19±1	14.7±6.8	29±2.6	34.3±6.5	21.3±1.1

Figure S2.

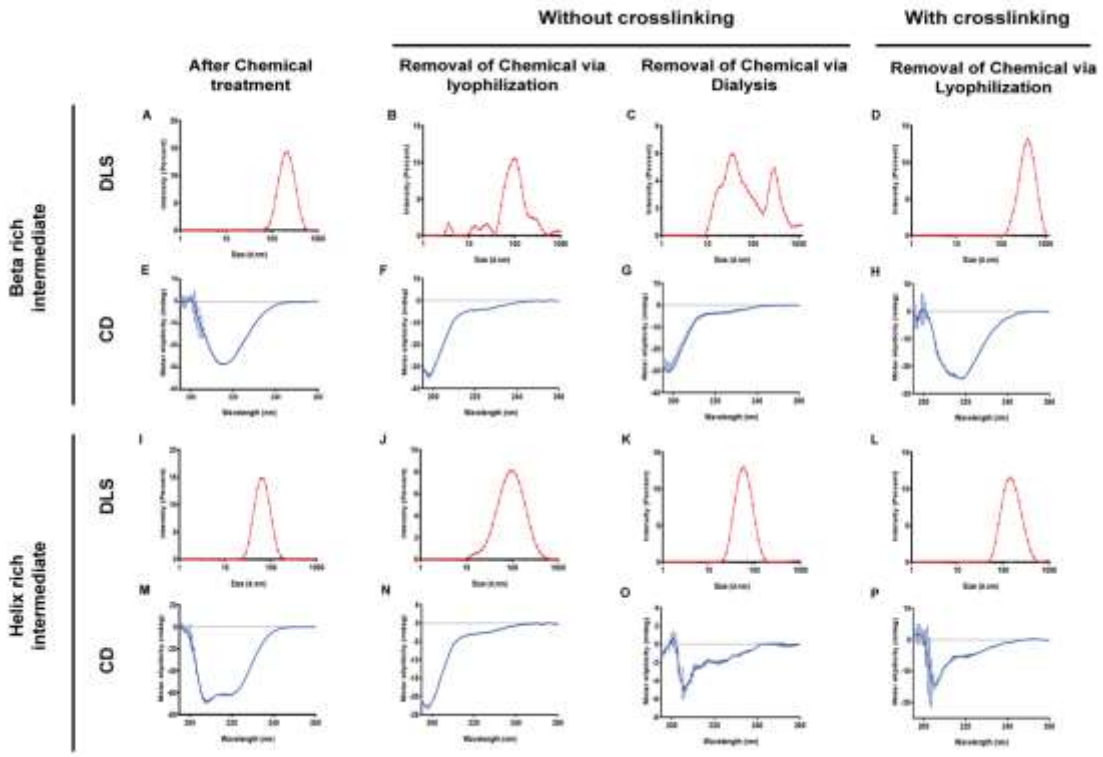

Figure S2. Characterization of intermediate species using dynamic light scattering (DLS) and circular dichroism (CD) under different conditions. DLS (A-D and I-L) and CD spectra (E-H and M-P) showing the state of species under different treatment conditions. In case of beta rich species, after treatment with 30% ethanol (A and E) showed beta rich secondary structure. B, F and C, G showing the state of species upon removal of ethanol via lyophilisation and dialysis respectively. D and H showing the nature of lyophilised cross linked species. In helix rich species, I and M showing the state of species upon treatment with 10% HFIP. J, N and K, O showing the state of species upon removal of HFIP via lyophilisation and dialysis respectively. L and P showing the nature of lyophilised cross linked species.

Figure S3

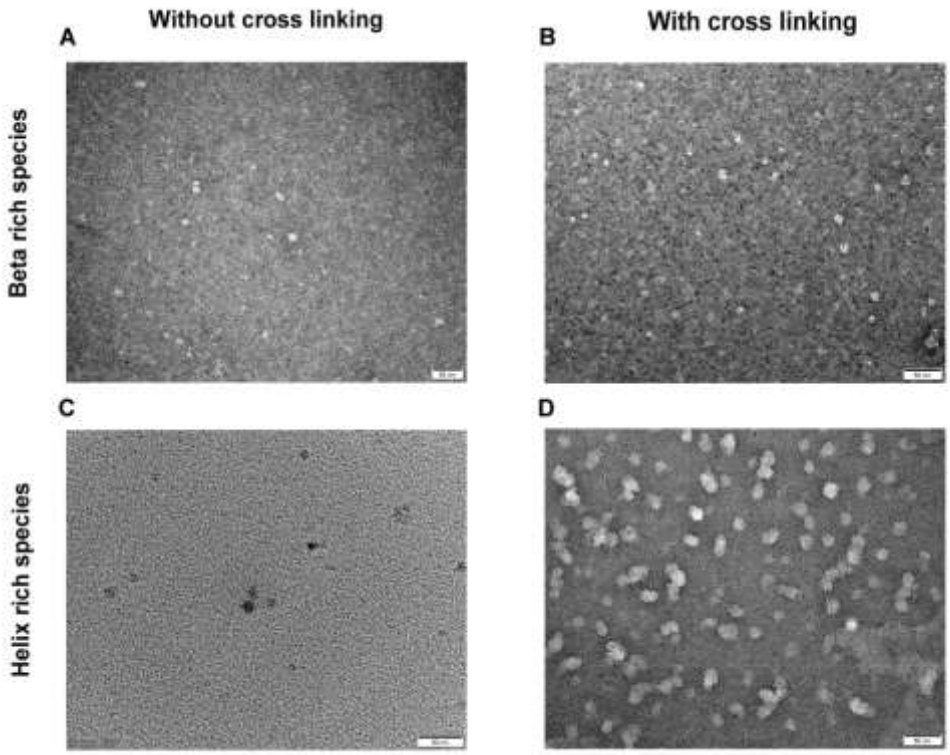

Figure S3. TEM images of intermediate species under different conditions. TEM images showing the morphology of beta rich species (A, B) and helix rich species (C, D) in absence and presence of cross linker respectively. Scale bar for all images is 50 nm.

Figure S4

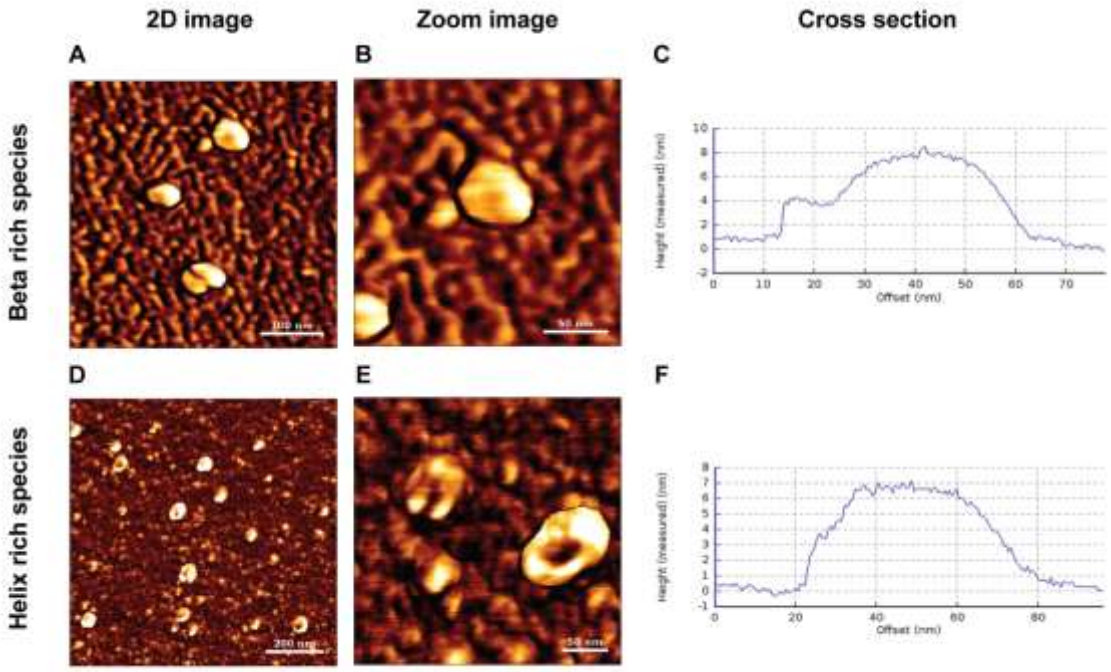

Figure S4. Atomic force microscopy of intermediate species. AFM images showing the detailed morphology of cross linked intermediate species of α-synuclein. A and D showing the 2D AFM image of beta rich and helix rich species of α-synuclein respectively. Cross section analysis (C and F) provide the detail of height of oligomers in magnified AFM images (B and E) of beta rich and helix rich species respectively. Scale bar for 2D image is 100 nm and 200 nm. Scale bar of zoom images is 50 nm.

Figure S5.

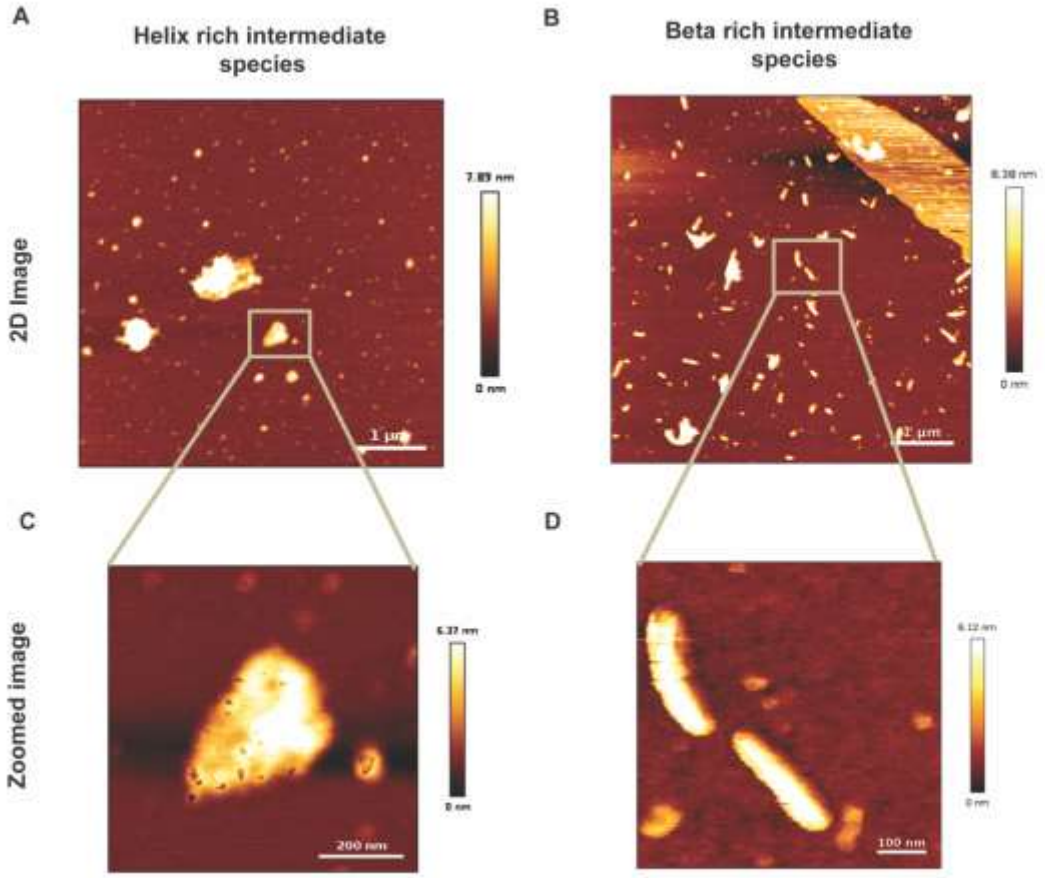

Figure S5. Atomic force microscopy showing morphology of intermediate species under aggregation condition. After incubation at 37° C for 32h under shaking condition, intermediate species form aggregates. (A) and (C) showing amorphous aggregates of helix rich intermediate. (B) and (D) showing short fibre like structures of beta rich intermediate species.

Table S2. Kinetic parameters of binding of intermediate species of α-synuclein with lipid membrane.

Samples	K_D (M)	k_{on} (1/Ms)	k_{dis} (1/s)
LMW synuclein	8.34E-05±9.2	1.55E+02±13	6.91E-03±3
Helix rich intermediate	6.23E-06±2.1	5.86E+01±17	4.13E-04±0.2
Beta rich intermediate	6.92E-06±2.9	7.18E+01±37	4.41E-04±0.1

Figure S6

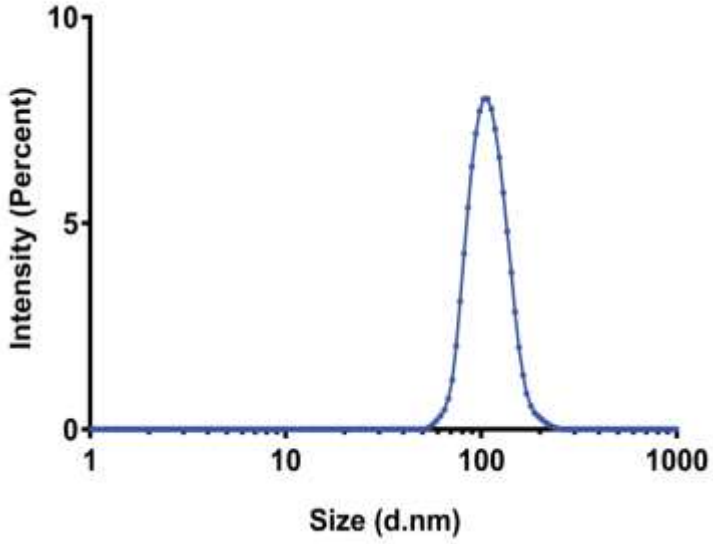

Figure S6. DLS of lipid membrane. Dynamic light scattering showing size of the LUV used to study the interaction of intermediate species with lipid membrane.

Figure S7

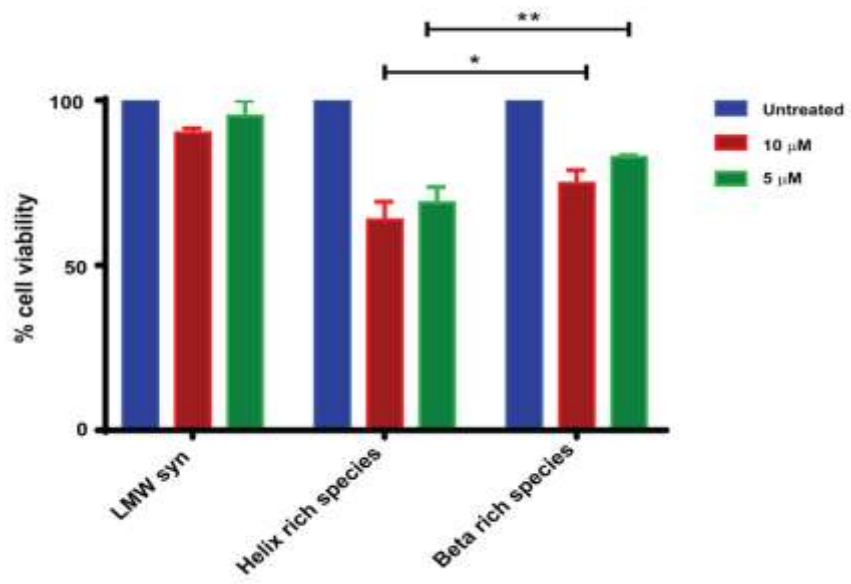

Figure S7. Cellular toxicity using MTT assay. SH-SY5Y cell viability was determined by MTT assay upon treatment with intermediate species at different concentration (10 μM and 5 μM) for 24 h.

Figure S8.

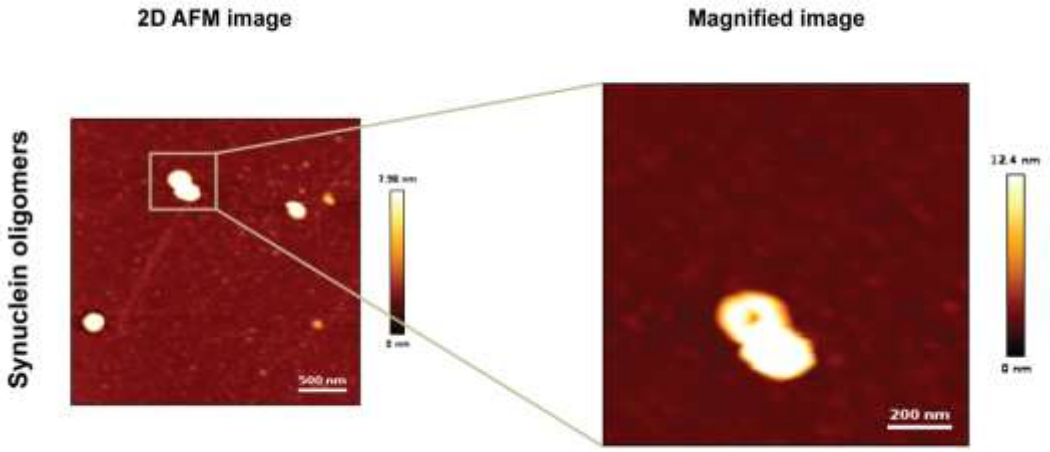

Figure S8: AFM image of α-synuclein oligomers. Atomic force microscopy showing the morphology of the α-synuclein oligomers used as positive control for the study.

Figure S9.

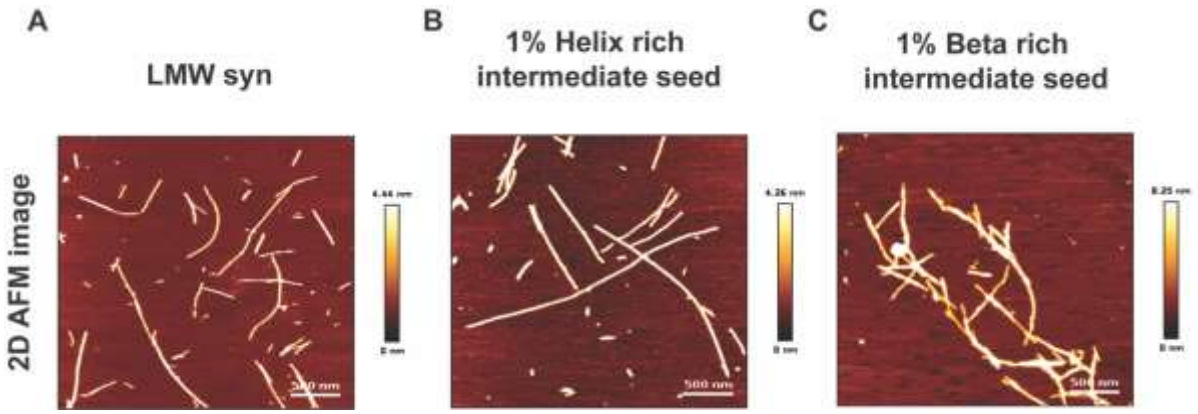

Figure S9. 2D AFM image of seeded fibres of α-synuclein. AFM image showing fibres formed in the presence of seed of LMW syn (A), 1% helix rich intermediate species (B) and 1% beta rich intermediate species.

Figure S10.

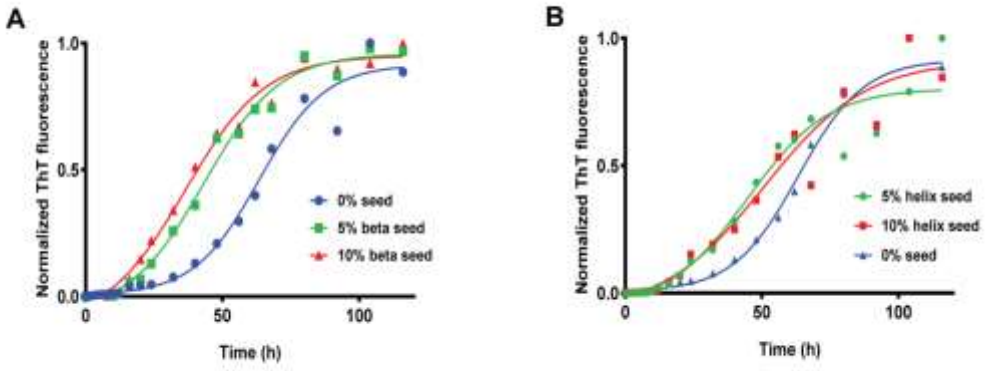

Figure S10. Seeded α-synuclein aggregation kinetics. ThT fluorescence graph representing the seeded aggregation kinetic of monomeric α-synuclein in the presence of (A) higher seed concentration (5% and 10%) of beta rich intermediate and (B) similar seed concentration of helix rich intermediate. All experiments were performed under quiescent conditions.

Table S3. Kinetic parameters of binding of intermediate species of α-synuclein and LPS with TLR2.

Samples	K_D (M)	K_{on} (1/ms)	K_{dis} (1/s)
LMW	2.29E-06±2.7	5.79E+02±2.7	1.45E-04±1.3
Helix rich intermediate	2.29E-07±1	7.73E+04±6.4	1.33E-02±4.7
Beta rich intermediate	5.97E-08±4.4	1.40E+04±2.0	2.4E-03±1.6
LPS	6.30E-09±1.8	3.43E+04±2.0	2.03E-04±9.3