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Conformational dynamics of α-synuclein in presence of bare and surface functionalized ZnONPs

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Parkinson's disease is a progressive neurodegenerative disorder associated with aggregation of α-synuclein (αS), resulting in formation of plaques in neurons described as Lewy bodies. α-synuclein is a small, soluble, and intrinsically disordered protein that upon gaining amyloidogenic conformation forms rigid cross-β sheet structure with fibril-like morphology via cytotoxic oligomeric intermediates. In recent years, nanoparticles have gained momentum due to their nano-size and large surface to volume ratio. The binding of α-synuclein onto nanoparticle surface is likely to induce conformational rearrangements, thereby anticipated to impede them in folding pathways and affect overall bio-reactivity of nanoparticle. In this subject, our study explores the positive and negative interface interaction of zinc oxide nanoparticle (ZnONP) with α-synuclein, and its following impact on protein fibrillation kinetics and fibril mediated cytotoxicity. The interaction studies of α-synuclein monomer and ZnONPs interface at higher concentration is indicative of multi-layered adsorption of α-synuclein or significant rearrangement in protein orientation that ensures tight packaging leading to inhibition of protein fibrillation. Further, TEM micrographs of ZnONP complexed α-synuclein shows mesh like pattern as compared to fibril like structure found in wild type α-synuclein. Impressively, α-synuclein complexed with ZnONP shows remarkably lowered cytotoxicity against the SH-SY-5Y and THP-1 cells *in-vitro*, as compared to aggregated α-synuclein. Henceforth, this study provides new insight on the therapeutic potential of ZnONP in combating pathologies related to α-synuclein aggregation.

Keywords: Protein aggregation, Nanoparticle, Interaction profile, Fibrillation kinetics, Cytotoxicity

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Abstract

The work explores the positive and negative interface interaction of zinc oxide nanoparticle (ZnONP) with α-synuclein, and its following impact on protein fibrillation kinetics and fibril mediated cytotoxicity. The binding of α-synuclein onto nanoparticle surface is likely to induce conformational rearrangements, thereby anticipated to impede them in folding pathways and affect overall bio-reactivity of nanoparticle.. The interaction studies of α-synuclein monomer and ZnONPs interface at higher concentration is indicative of multi-layered adsorption of α-synuclein or significant rearrangement in protein orientation that ensures tight packaging leading to inhibition of protein fibrillation.

Results and discussion

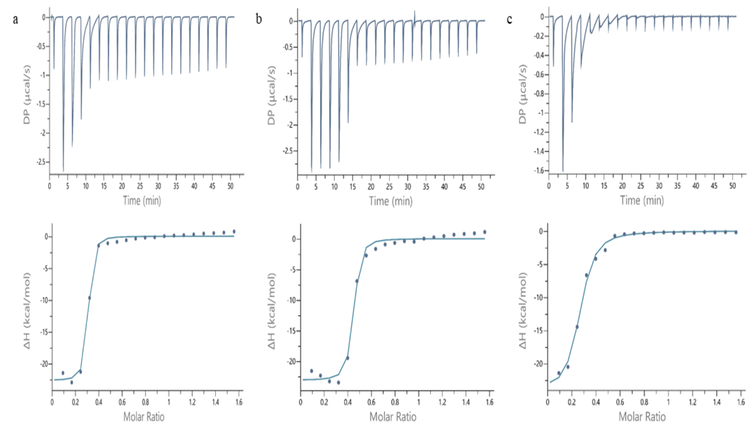

Figure 1. Thermograms depicting isothermal titration curves for 50 µM α-Synuclein titrated in presence of 30 µg/mL (a) $ZnONP_P$ (b) $ZnONP_Y$ (c) $ZnONP_W$ at 25 °C.

Table 1

Thermodynamic parameters for interaction profiling of α-synuclein complexed with different ZnONPs as analyzed by isothermal titration calorimetry.

Sl.No.	50 µM α-Synuclein and 30 µg/mL ZnONPs	Enthalpy change (ΔH), Kcal/mol	Entropy change (TΔS), Kcal/mol	Free energy change (ΔG), Kcal/mol	Binding constant (K_D), M
1	$ZnONP_P$	-22.7	-13.5	-9.22	$1.76e^{-07}$
2	$ZnONP_Y$	-23.2	-14.1	-9.09	$2.19e^{-07}$
3	$ZnONP_W$	-24.1	-16.3	-7.8	$1.93e^{-06}$

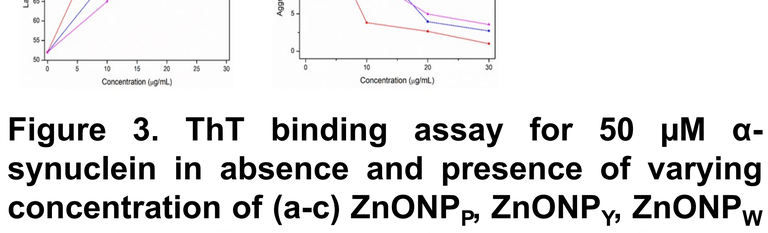

Figure 2. Steady-state fluorescence studies carried out by tyrosine excitation at 274 nm for 50 µM α-synuclein in presence of varying concentration of (a) $ZnONP_P$, (b) $ZnONP_Y$, (c) $ZnONP_W$ at 0, 24, 48, 72 and 96 h. All graphs are representative of intensity maxima at 310 nm and wavelength maxima.

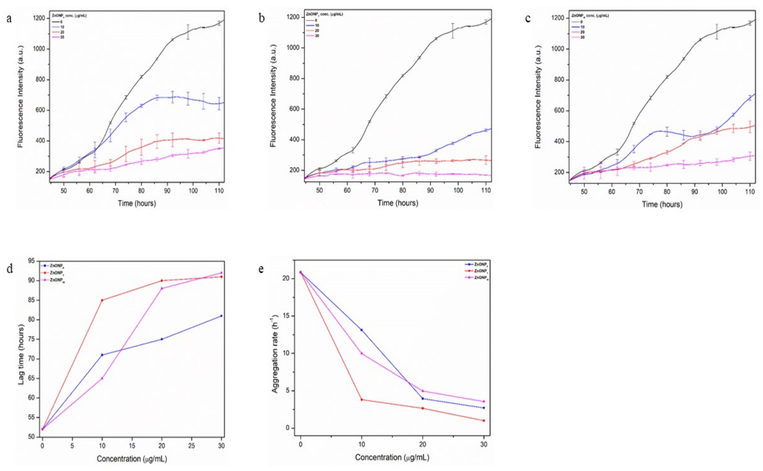

Figure 3. ThT binding assay for 50 µM α-synuclein in absence and presence of varying concentration of (a-c) $ZnONP_P$, $ZnONP_Y$, $ZnONP_W$ respectively. The comparative graphs of (d) lag time (e) aggregation rate

Results and discussion cont....

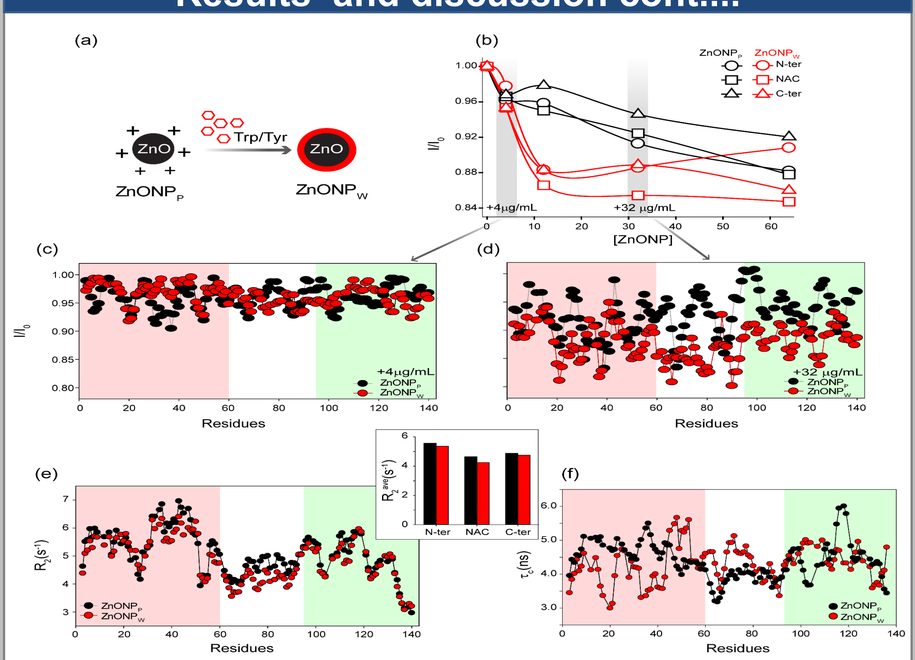

Figure 4. The molecular interactions with the surface moderated nanoparticles (a) show alteration in the protein-interaction interface. (b) Titration with increasing concentrations of $ZnONP_P$ and $ZnONP_W$. The intensity ratios for the individual resonances upon treatment with 4 µg/ml (c) and 32 µg/ml (d) of each nanoparticle variants show the higher affinity for $ZnONP_W$ in binding to the NAC region. (e) Region-specific interactions that result in modulating the dynamics of the segments in the complex formation. (f) The calculated Rotational correlation time (τ_c) corresponding to three protein segments upon nanoparticle treatment at a concentration of 64 µg/ml.

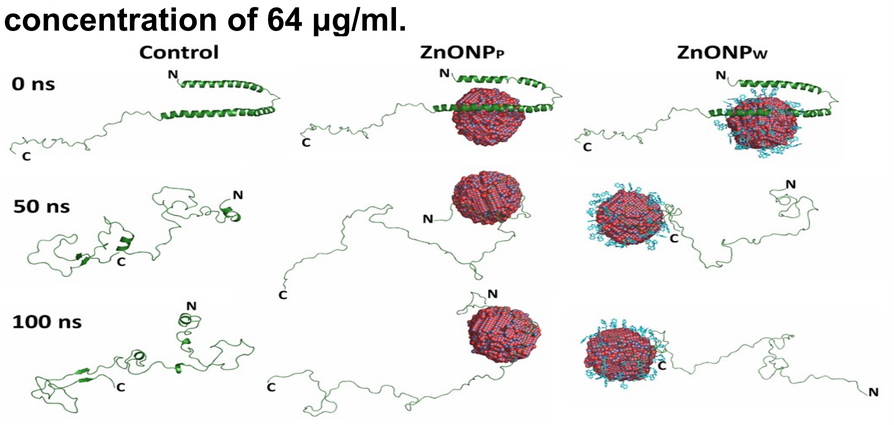

Figure 5. shows snapshots of αS protein at 0, 50 and 100 ns and it can be observed that there are no traces of any other secondary structure (other than random coil) in the systems where ZnONPs are present.

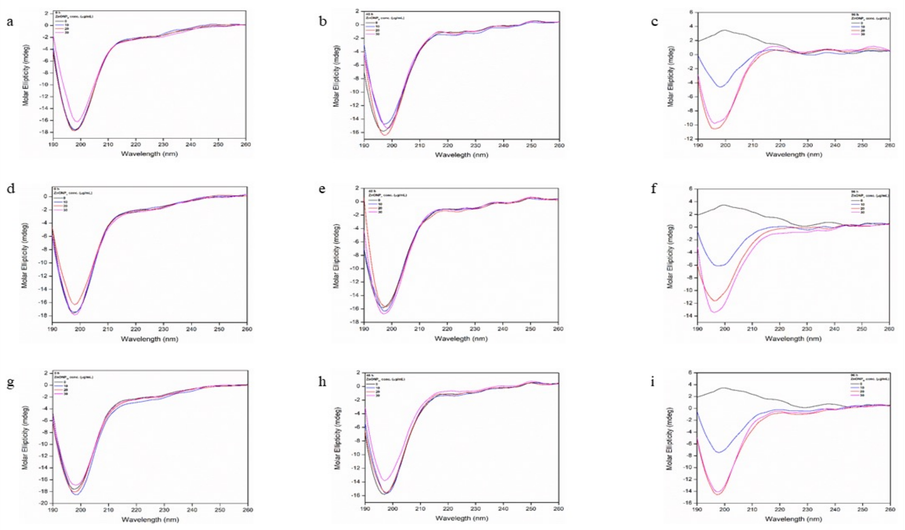

Figure 6. Changes in far-UV CD spectra of 10 µM α-synuclein in presence of varying concentrations of (a-c) $ZnONP_P$, (d-f) $ZnONP_Y$ and (g-i) $ZnONP_W$ at 0, 48, and 96 h respectively.

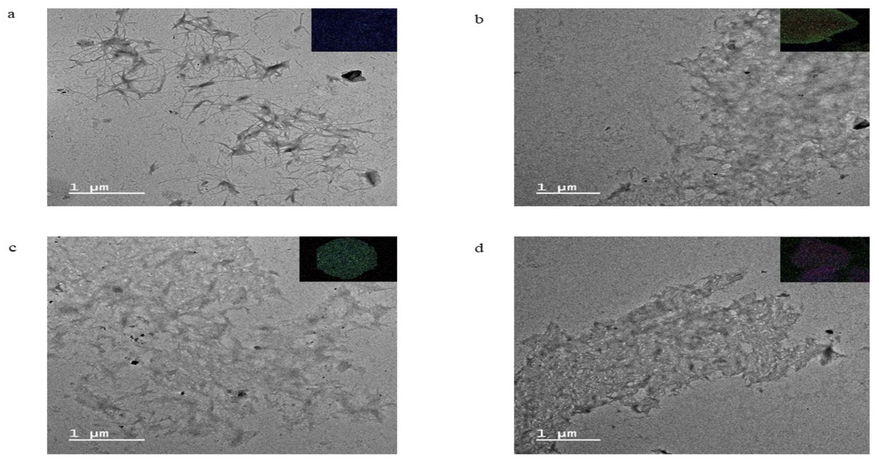

Figure 7. TEM micrographs of (a) 50 µM α-synuclein only, α-synuclein complexed with 30 µg/mL (b) $ZnONP_P$ (c) $ZnONP_Y$ (d) $ZnONP_W$ at 1 µm resolution. The inset images are HAADF-STEM map indicating C (blue), N (green), and O (red) elements for α-synuclein only and Zn (red), N (blue), and O (green) for α-synuclein complexed with ZnONPs.

Results and discussion cont....

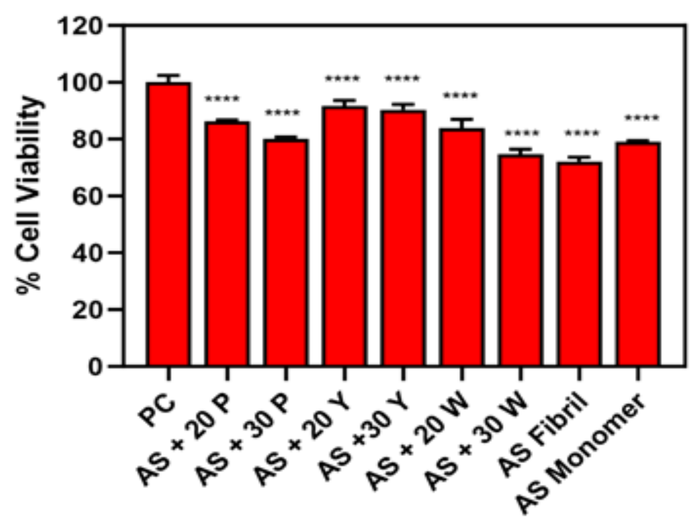

Figure 8. Alamar Blue dye reduction assay for SH-SY5Y neuroblastoma cell line treated with 72 h incubated α-synuclein only or complexed with 20 or 30 µg/mL of $ZnONP_P$, $ZnONP_Y$, $ZnONP_W$

Discussion

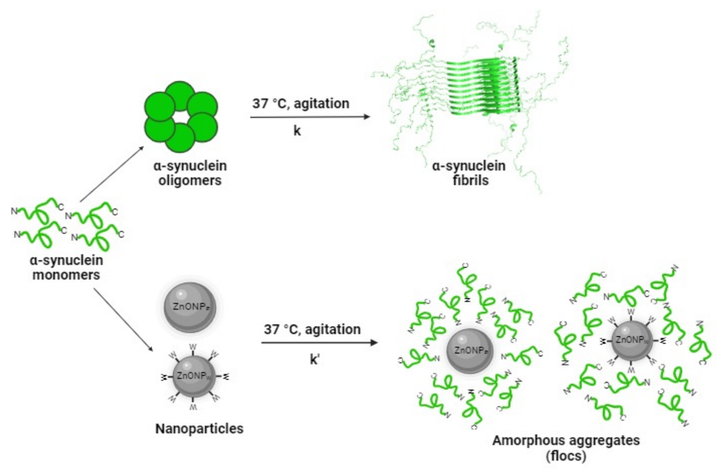

Figure 9. Illustration depicting conformational transition of α-synucelin monomers into amyloid fibrils at 37 °C under shaking conditions. However, in presence of ZnONPs under similar conditions, protein monomers adsorb onto NP interface ensuring formation of kinetically trapped amorphous aggregates (flocs).

Inferences

- **Interaction studies of α-synuclein and nanoparticle is indicative of multi-layered adsorption of α-synuclein or significant rearrangement in protein orientation that ensures tight packaging.**
- **Adsorption of protein onto nanoparticle surface is a composite factor resulting from endowment of enthalpic factors (electrostatic interaction and hydrogen bonding) and entropic factors (hydrophobic interaction and release of water molecules).**
- **NMR data suggests involvement of N-terminus and NAC region of α-synuclein in case of adsorption on bare ZnONP whereas majority of NAC region and C-terminus is involved for adsorption on functionalized ZnONP.**
- **Binding of protein monomer on to ZnONP interface at higher concentration render rigidity to protein structure which is leading to inhibition of protein fibrillation.**
- **Due to formation of stable protein-nanoparticle complexes, fewer monomers are available that can undergo fibrillation.**
- **Altogether, the data suggests favourable nanoparticle interface interaction with protein monomer that leads to formation of high entropy aggregates that are amorphous in nature.**

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