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Zinc oxide nanoparticle interface moderation with tyrosine and tryptophan reverses the pro-amyloidogenic property of the particle

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Zinc oxide nanoparticle with negative surface potential (ZnONP) enhances bovine insulin fibrillation. Here, we are exploring ZnONP with positive surface potential ($ZnONP_{Unc}$) and surface functionalized with tyrosine and tryptophan amino acids to observe the effects of surface potential and surface functional groups on the fibrillation. $ZnONP_{Unc}$, despite of inversed surface potential, enhances the insulin fibrillation with increase in the interface concentration at physiological pH. Whereas, the interface moderation with the amino acids mitigates the surface-mediated insulin fibrillation propensity. Additionally, the study indicates that the change in interfacial functional groups at $ZnONP_{Unc}$ significantly reverses the interface-mediated destabilization of insulin conformation. The functional groups from the amino acids, C=O, N-H and aromatic functional groups, are anticipated to further stabilize the insulin conformation by forming hydrogen bond and van der Waals interactions with the key amyloidogenic sequences of insulin, A13-A20 from A-chain and B9-B20 from B-chain. Hence, the altered interaction profile, with change in interfacial functional groups, mitigates the interface-mediated insulin fibrillation and the $ZnONP_{Unc}$-/fibril-mediated cytotoxicity.

Keywords: Insulin, Fibrillation, Nanoparticle surface functionalization, ZnONP, Cytotoxicity

Zinc Oxide Nanoparticle Interface Moderation With Tyrosine And Tryptophan Reverses The Pro-amyloidogenic Property Of The Particle

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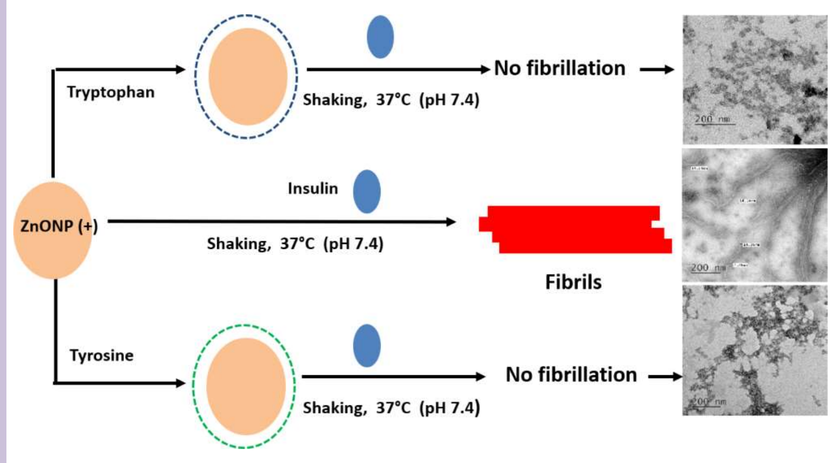
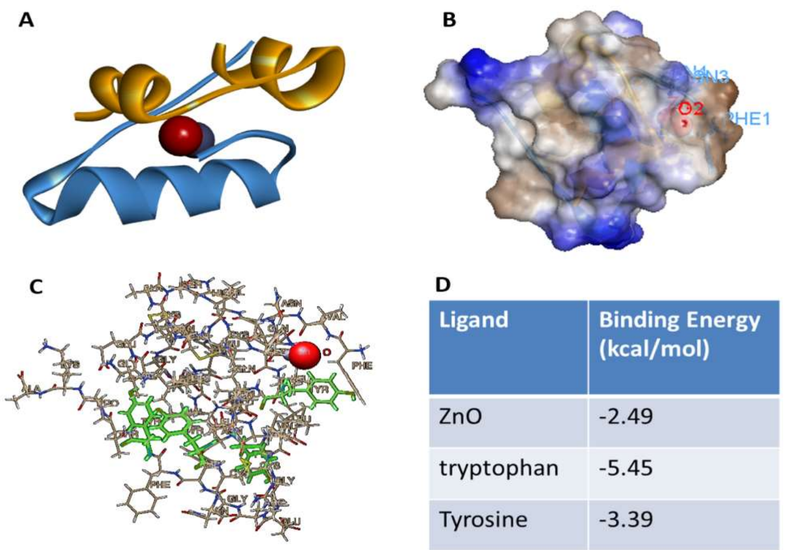
Graphical Abstract



Objective

The objective of this study was to elucidate the amyloidogenic property of bare and surface functionalised ZnONPs on Bovine Insulin.

Results



Ligand	Binding Energy (kcal/mol)
ZnO	-2.49
tryptophan	-5.45
Tyrosine	-3.39

Fig. 1. Molecular docking studies of ZnO with insulin. (A) Binding conformation of ZnO with insulin. (B) The scale of hydrophobicity at region of ZnO binding with insulin. (C) Interacting residues with ZnO. (D) Comparative chart of binding energy for ZnO, Tyrosine and Tryptophan respectively.

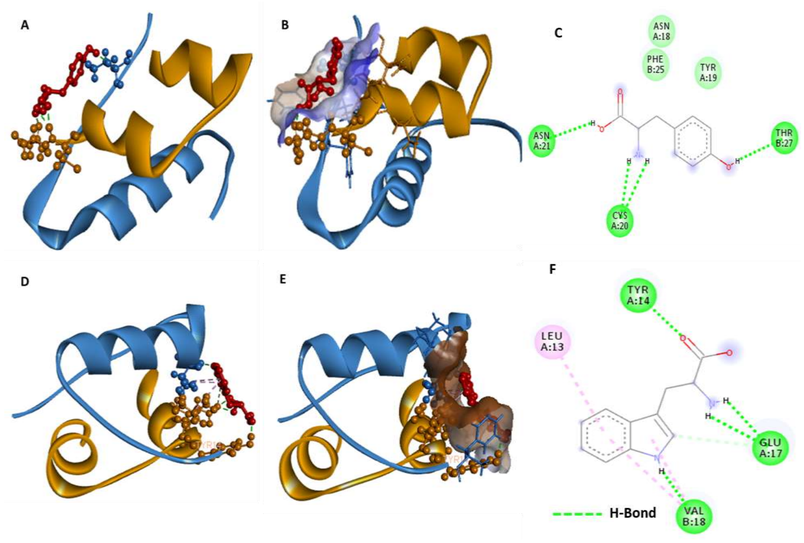


Fig. 2. Molecular docking study and analysis interactions of tyrosine ligand (A, B, C) and tryptophan ligand with insulin (D, E, F). The H-bond is indicated with green colour dashed lines and pi-interaction is indicated with pink dashed line.

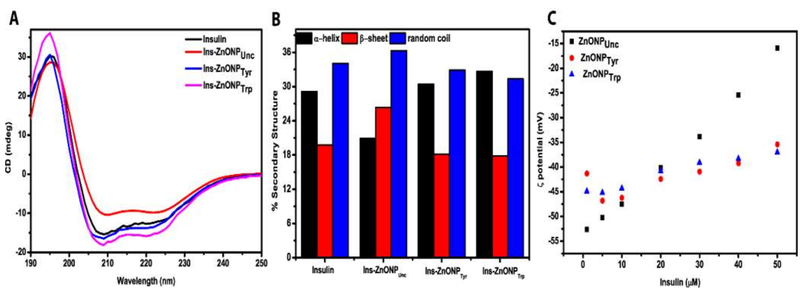


Fig. 3. (A) Far-UV CD spectra analysis of insulin in absence and presence of $ZnONP_{Unc}$, $ZnONP_{Tyr}$ and $ZnONP_{Trp}$. (B) Percentage (%) secondary structure of insulin obtained in absence and presence of $ZnONP_{Unc}$, $ZnONP_{Tyr}$ and $ZnONP_{Trp}$. (C) Charge neutralization study of $ZnONP_{Unc}$, $ZnONP_{Tyr}$ and $ZnONP_{Trp}$ with increasing concentration of insulin.

Results cont…

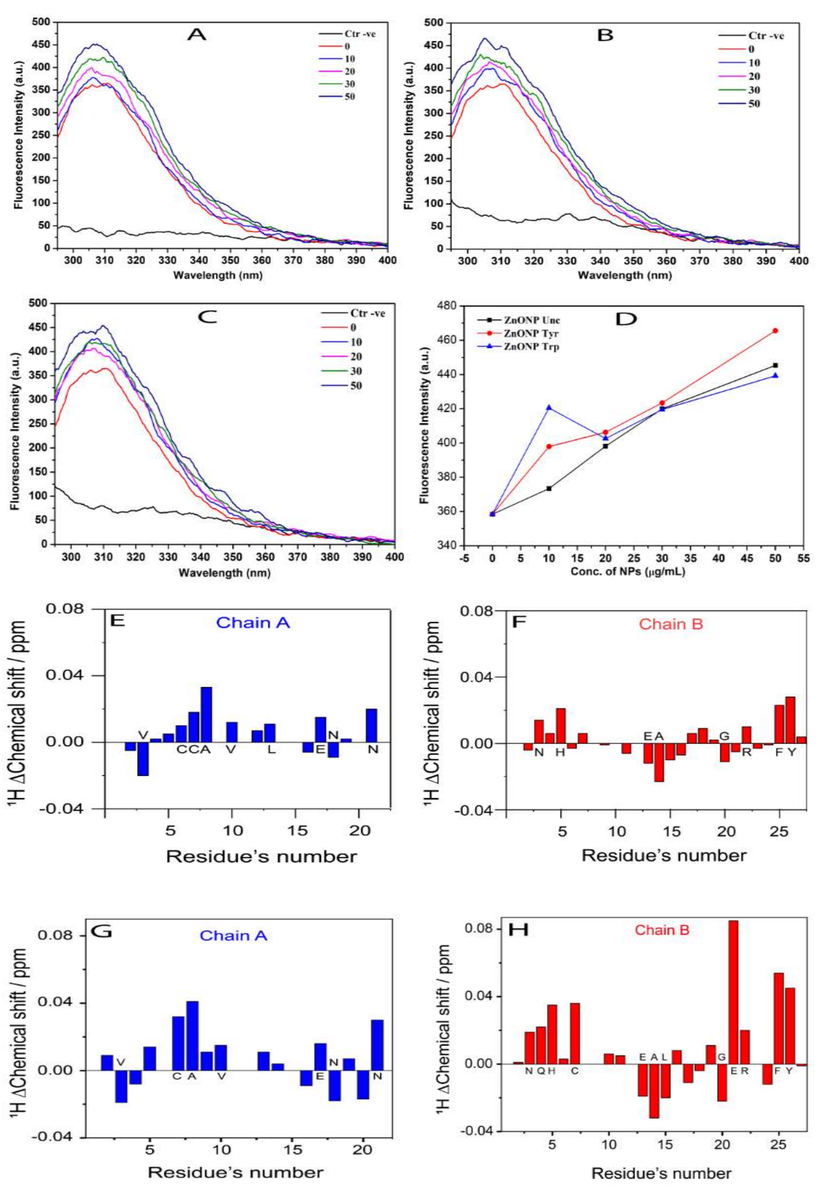


Fig. 4. Steady state fluorescence emission spectra of insulin-tyrosine in presence of nanoparticle interfaces. Fluorescence intensity of insulin-tyrosine in a dose dependent manner in presence of $ZnONP_{Unc}$ (A), $ZnONP_{Tyr}$ (B), and $ZnONP_{Trp}$ (C). The steady state fluorescence intensity at 305 nm for insulin-tyrosine in presence of increasing concentrations of bare and functionalized nanoparticles (D). Interaction site of ZnONP interface in insulin by two dimensional NOESY NMR. Chemical shift changes for insulin A-chain (E) and insulin B chain (F) residues in presence of $ZnONP_{Unc}$. Chemical shift changes for insulin A-chain (G) and insulin-B- chain (H) residues in presence of $ZnONP_{Trp}$.

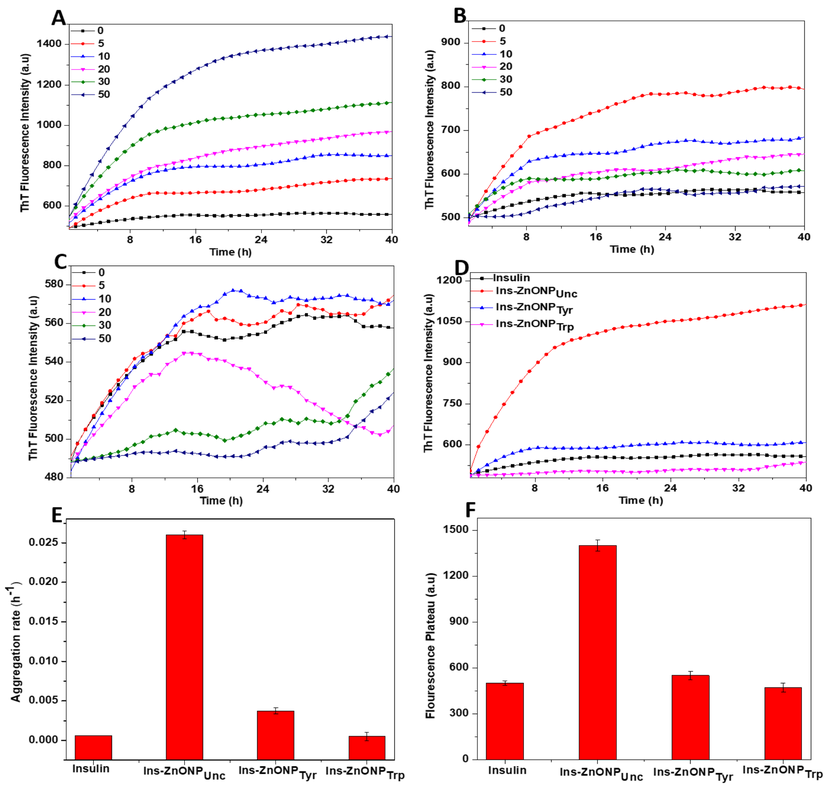


Fig. 5. Time dependent ThT dye binding assay of fibrillation in absence and presence of different concentrations of uncoated ZnONP ($ZnONP_{Unc}$) (A), tyrosine coated ZnONP ($ZnONP_{Tyr}$) (B) and tryptophan coated ($ZnONP_{Trp}$) (C) interface at physiological pH. A comparative graph showing the inhibitory effect of surface functionalization on bare ZnONP induced insulin fibrillation (D). The kinetic parameters obtained from the curves in fig. D gives the aggregation rates (E) and fluorescence plateau (F) for native insulin, insulin at $ZnONP_{Unc}$, $ZnONP_{Tyr}$ and $ZnONP_{Trp}$ interface, respectively.

Results cont....

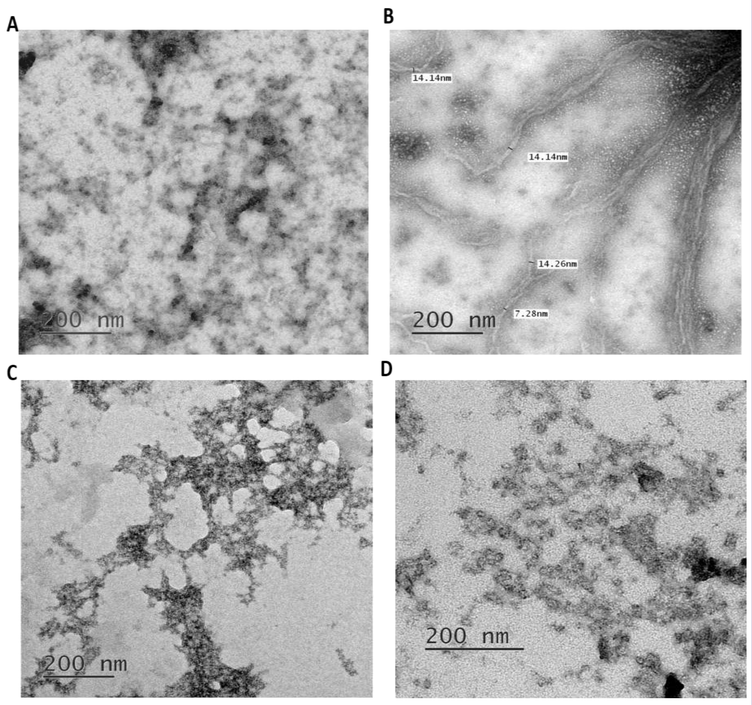


Fig. 6. TEM micrographs of insulin incubated in absence (A) and presence of $ZnONP_{Unc}$ (B), $ZnONP_{Tyr}$ (C), $ZnONP_{Trp}$ (D) interfaces, at 37°C for 40 h.

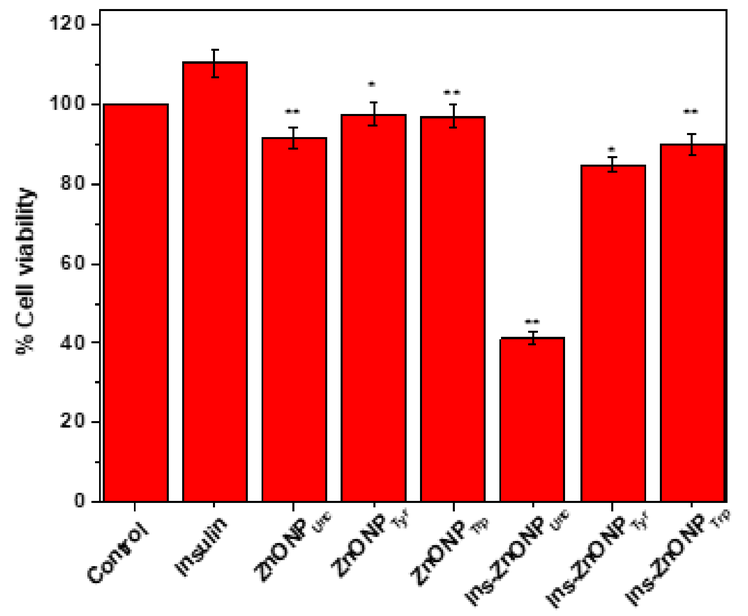


Fig. 7. Alamar blue dye reduction assay by viable IMR-32 cells. The first column is the untreated cells (control), the second column represent insulin only treated cells, column 3-5 represents the $ZnONP_{Unc}$, $ZnONP_{Tyr}$, $ZnONP_{Trp}$ only treated cells respectively, and column 6-8 represents 40 h aged insulin-nanoparticle samples treated cells, respectively. The data represents the significance value, *: P < 0.05, **: P < 0.01, with respect to the control, obtained from three independent experiments.

Conclusion

In this study, we demonstrated the effect of ZnONP with positive surface potential interface on insulin conformational dynamics. The interfacial moderation of ZnONP with tyrosine and tryptophan not only efficiently impedes the bare ZnONP-mediated insulin fibrillation, but also enhances the insulin structural stability along with reduced amyloidogenic and cytotoxic propensities. The structural stability is mainly mediated by the hydrogen bond interaction between aggregation prone sequence of insulin with $ZnONP_{Tyr}$ or $ZnONP_{Trp}$ interface. This brings us to conclude that the role of nanoparticle interfaces in protein fibrillation is mainly governed by the protein intrinsic stability and interfacial interaction pattern of the nanoparticle- protein complex.

Reference

Yadav, K. K., Ojha, M., Pariary, R., Arakha, M., Bhunia, A., & Jha, S. (2022). Zinc oxide nanoparticle interface moderation with tyrosine and tryptophan reverses the pro-amyloidogenic property of the particle. *Biochimie*, *193*, 64-77.