

Supplementary Information

Inhibition of Amyloid Beta Toxicity in Zebrafish with A Chaperone-Gold Nanoparticle Dual Strategy

Javed *et al.*

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Supplementary Methods

Discrete molecular dynamics (DMD) simulations

DMD is a type of molecular dynamics algorithms where conventional continuous interaction potentials are replaced by optimized step-wise potential functions.^{1,2} Comprehensive description of the DMD algorithm was published elsewhere.⁴⁹ In brief, the united-atom model was used to represent all molecules where all heavy atoms and polar hydrogen atoms were explicitly modeled. An implicit solvent model was adopted in the current simulations. The interatomic interactions included *van der Waals*, solvation, electrostatic interactions and hydrogen bond. The solvation energy was adopted by the Lazaridis-Karplus implicit solvent model, EEF1.³ The distance- and angular-dependent hydrogen bond interactions were modeled using a reaction-like algorithm.⁴ Screened electrostatic interactions were computed by the Debye-Hückel approximation. A Debye length of 1 nm was used by assuming a water dielectric constant of 80 and a monovalent electrolyte concentration of 0.1 M. The Anderson's thermostat was used to maintain constant temperature.⁵

During the synthesis of AuNP, β Cas served as the capping agent. The only one cysteine residue in β Cas was not expected to form disulfide bridge under the reducing condition during synthesis. Hence, without chemical modifications β Cas adopted its thermodynamically stable conformations on the AuNP surface and the structure of the β Cas corona remained independent of the synthesis process of AuNPs. Since our focus was to study the capping of β Cas AuNPs and the subsequent ability of β Cas corona to bind with A β , we therefore modelled only the binding of β Cas with a pre-formed AuNP.

We adopted the recently developed Au molecular mechanics force field⁶ to model a spherical AuNP with a diameter of 40 Å comprising 1,865 Au atoms. The AuNP force field included both physical and chemical absorption, aromatic and “image” charge interactions. The polarization was modelled by attaching a charged virtual particle ($-0.3e$) to each metal ($0.3e$)

atom with a fixed bond length (1.0 Å) as implemented in the GolP force field.⁶ Only electrostatic interaction was taken into account for the virtual particle.

The structural coordinates for β Cas were obtained from the protein homology prediction server (<https://zhanglab.ccmb.med.umich.edu/I-TASSER/>). The structural coordinates for A β oligomer were obtained from the protein data bank (PDB code: 2NAO), which was a β -sheet rich hexamer structure. Structural coordinates for proteins, basic and acidic amino acids were assigned charges corresponding to their titration states at physiological conditions, i.e. Arg and Lys residues were assigned +1, Asp and Glu were assigned -1, while His was neutral. Counter ions (Cl⁻) were added to maintain the net charge of the systems zero and accounted for possible counter-ion condensation.⁷ All simulations were conducted at 300 K. The periodic boundary condition was applied in all simulations. For each molecular system, 20 independent simulations were performed with different initial inter-molecular distances and orientations to avoid bias. For data analysis, an inter-atomic distance cutoff of 5.0 Å was used to define an atomic contact.

We used a hierarchical clustering program, oc (www.compbio.dundee.ac.uk/downloads/oc), to group similar protein binding poses with the AuNP. Based on an input pair-wise distance matrix that was the center-of-mass distances of the proteins on the AuNP surface in our study, a hierarchical clustering algorithm iteratively joined the two closest clusters into one cluster according to the distances between two clusters. The “cluster distance” was computed based on all pairwise distances between the elements of the two corresponding clusters, which can be the minimum, maximum, or mean of all these values. In this study, we used the mean to compute the distance between two clusters. The centroid structure of each cluster was selected as the one with the smallest average distance to other elements in the cluster.

Supplementary Tables

Supplementary Table 1. Zeta potential, hydrodynamic diameter and polydispersity index (PDI) of β Cas AuNPs and β Cas, before and after incubation with A β .

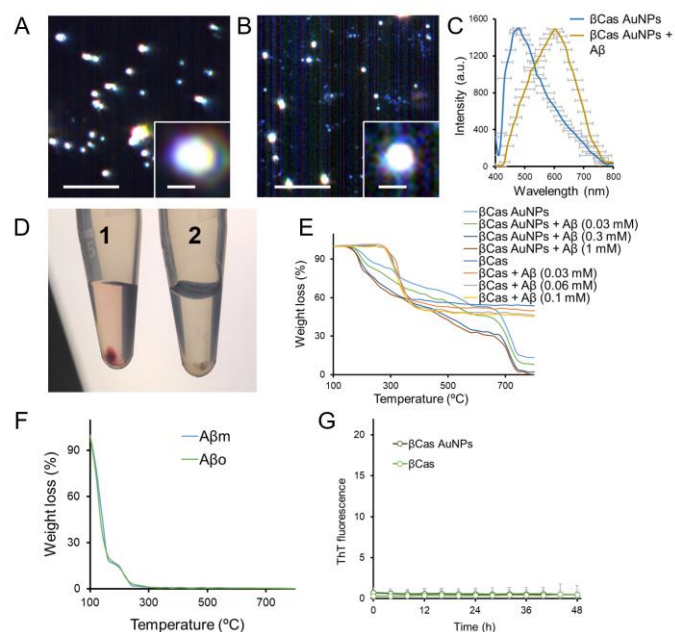
Sample	Zeta potential $\pm$ SD (mV)	Hydrodynamic diameter $\pm$ SD (nm)	PDI $\pm$ SD
A β 0 h	-43.2 ± 2.8	3.2 ± 1.6	0.261 ± 0.04
A β 48 h	-37.4 ± 2.1	3565 ± 846	0.634 ± 0.13
β Cas AuNPs	-11.7 ± 1.8	7.5 ± 2.6	0.214 ± 0.06
β Cas AuNPs+ A β	-33.7 ± 2.1	39.3 ± 5.4	0.345 ± 0.08
β Cas	-19.9 ± 2.7	156.3 ± 34.4	0.324 ± 0.07
β Cas + A β	-21.7 ± 2.0	496.1 ± 114	0.674 ± 0.15

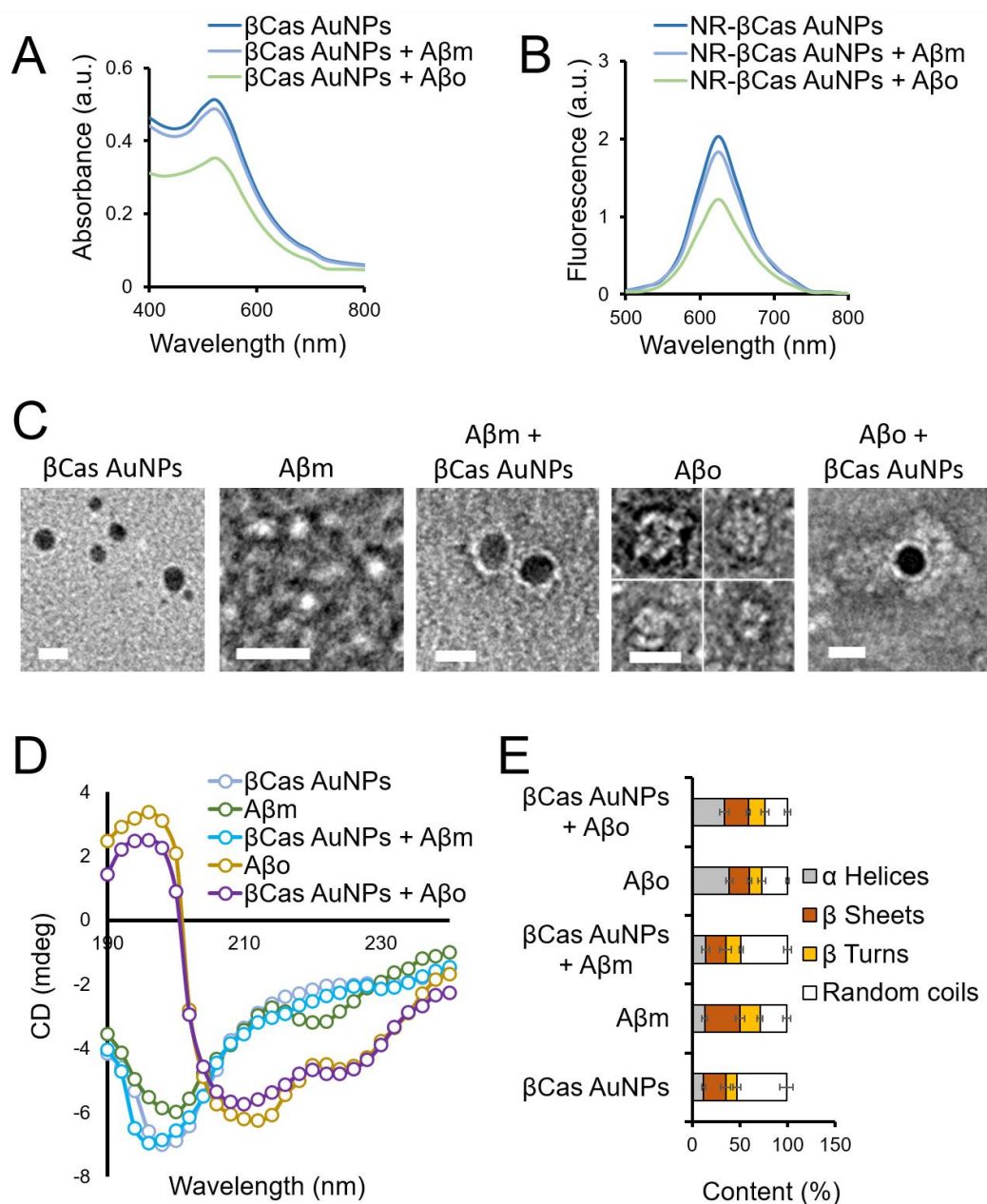
Supplementary Table 2. Estimation of protein-AuNP binding energies. The average potential energies of each molecular system were obtained from the corresponding equilibrated DMD simulations. The AuNP binding affinity difference between β Cas and A β was estimated based on the obtained average potential energies.

	$\langle E \rangle \pm \text{SD (kcal}\cdot\text{mol}^{-1})$
A β	-79.6 ± 3.5
β Cas	-595.5 ± 7.0
A β +AuNP	-97.3 ± 4.0
β Cas+AuNP	-807.1 ± 11.1

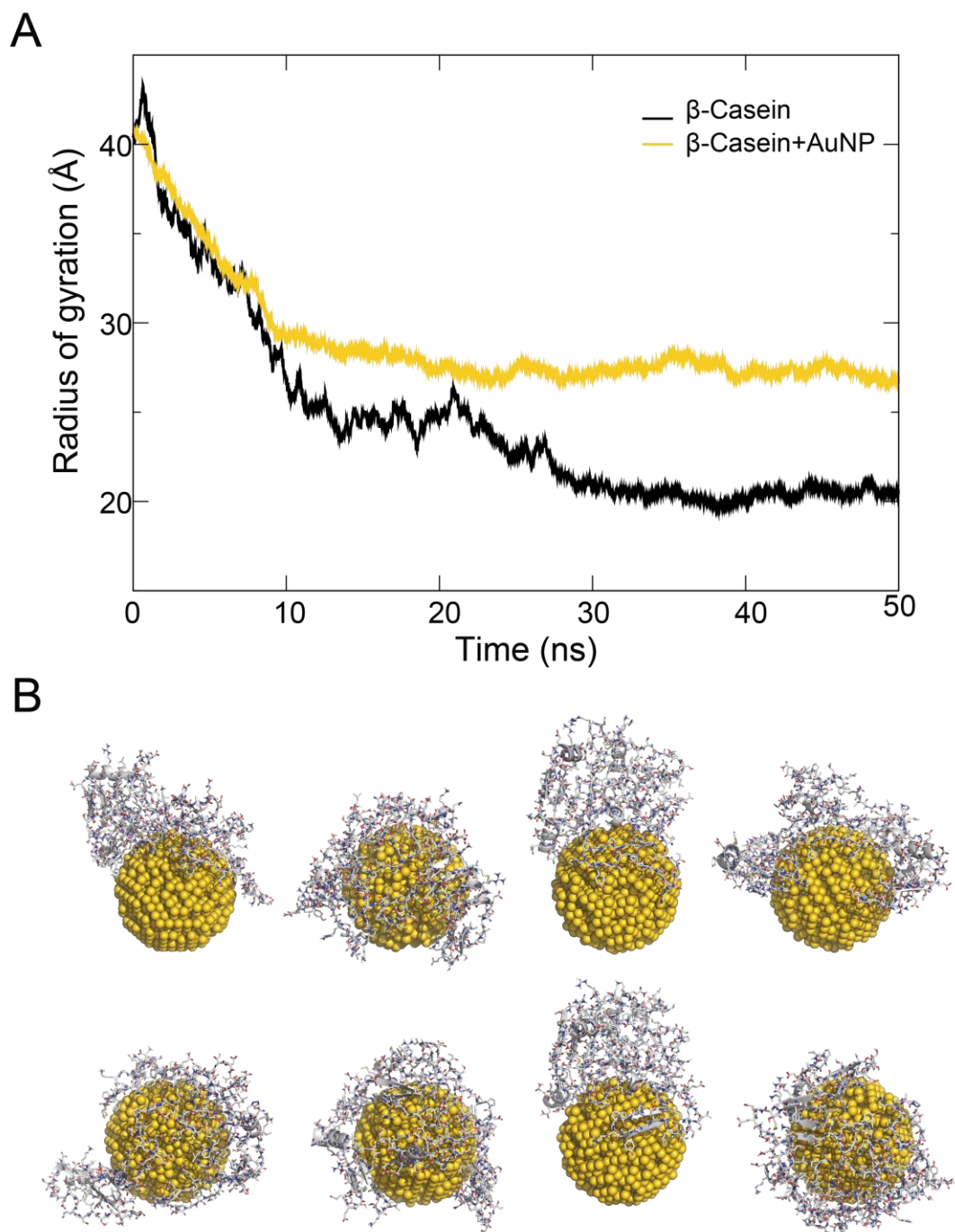
$$\Delta\Delta G \sim (\langle E_{\beta\text{Cas-AuNP}} \rangle - \langle E_{\text{A}\beta\text{-AuNP}} \rangle) - (\langle E_{\beta\text{Cas}} \rangle - \langle E_{\text{A}\beta} \rangle): -193.9 \text{ (kcal}\cdot\text{mol}^{-1})$$

Supplementary Figures

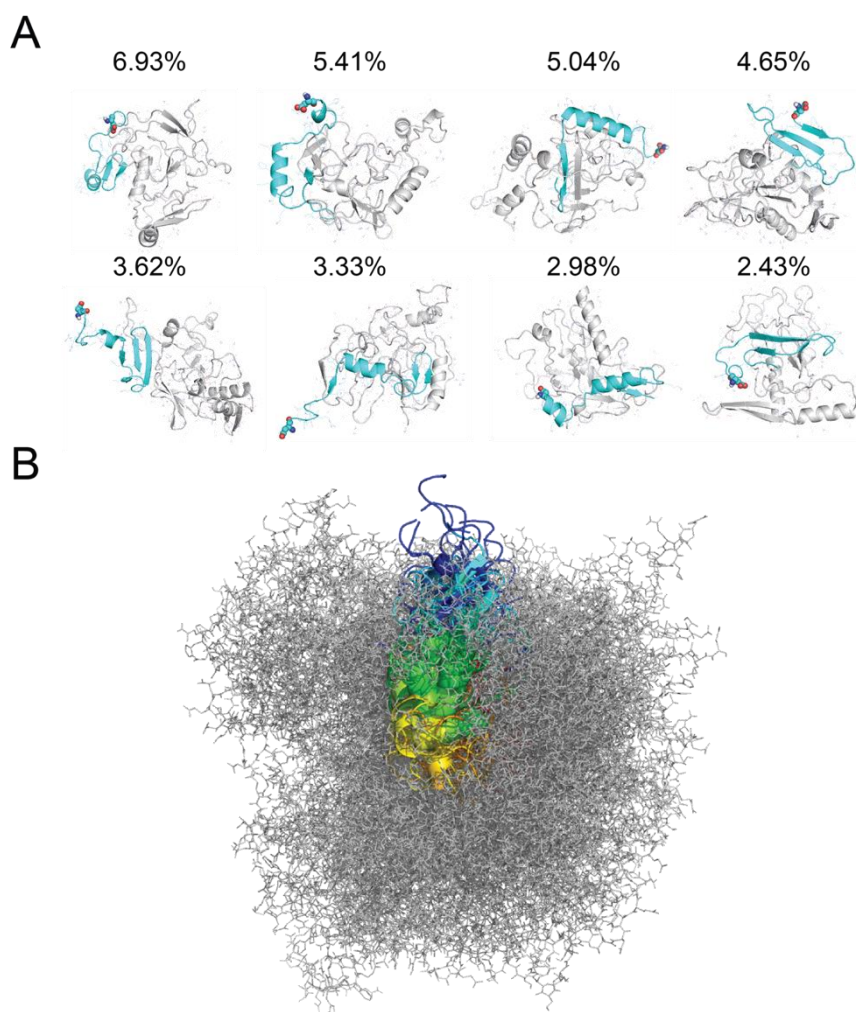




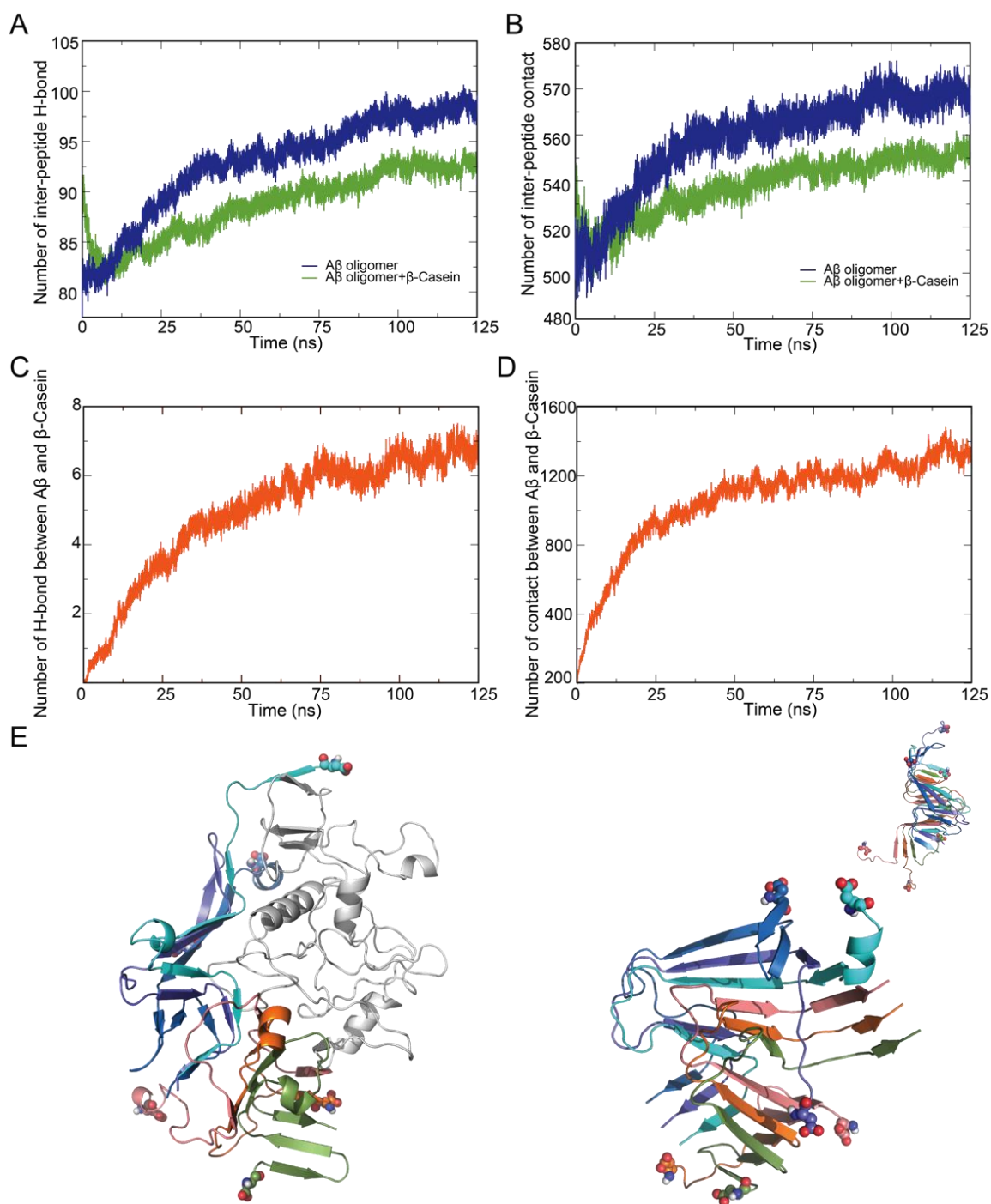
Supplementary Figure 2. Differential binding of β Cas AuNPs with A β m vs A β o. (A) The UV-SPR spectrum of β Cas AuNPs was suppressed significantly after binding with A β o as compared to A β m. (B) Similarly, the fluorescence spectrum of NR- β Cas AuNPs was significantly suppressed after binding with A β o. (C) TEM shows corona formation on β Cas AuNPs after incubation with A β o but not with A β m. (D) CD spectra and percentage secondary structure (E) of β Cas AuNPs before and after binding with A β m and A β o (n=3). β Cas AuNPs displayed a greater affinity for the A β o corona. Scale bars in TEM the images are 5 nm. Error bars represent the standard deviation. Source data are provided as a Source Data file.



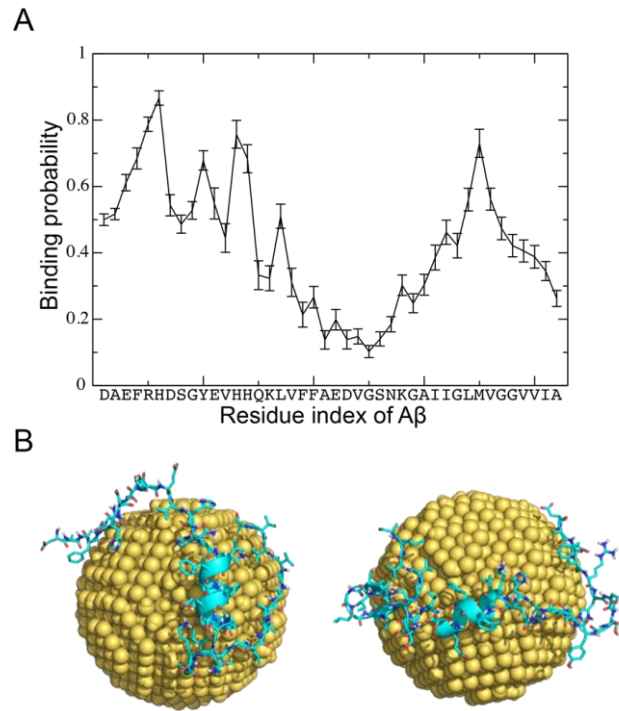
Supplementary Figure 3. DMD simulations of β Cas and AuNP. (A) Radius of gyration (R_g) of β Cas in the absence/presence of AuNP as a function of time. The thermo-fluctuations after 30 ns indicate the equilibrium of the simulation system and the larger R_g of β Cas in the presence of AuNP indicates extended conformation of β Cas. (B) Centroid structures of top 8 clusters of β Cas and AuNP complexes, obtained from multiple independent DMD simulations with clustering analysis.



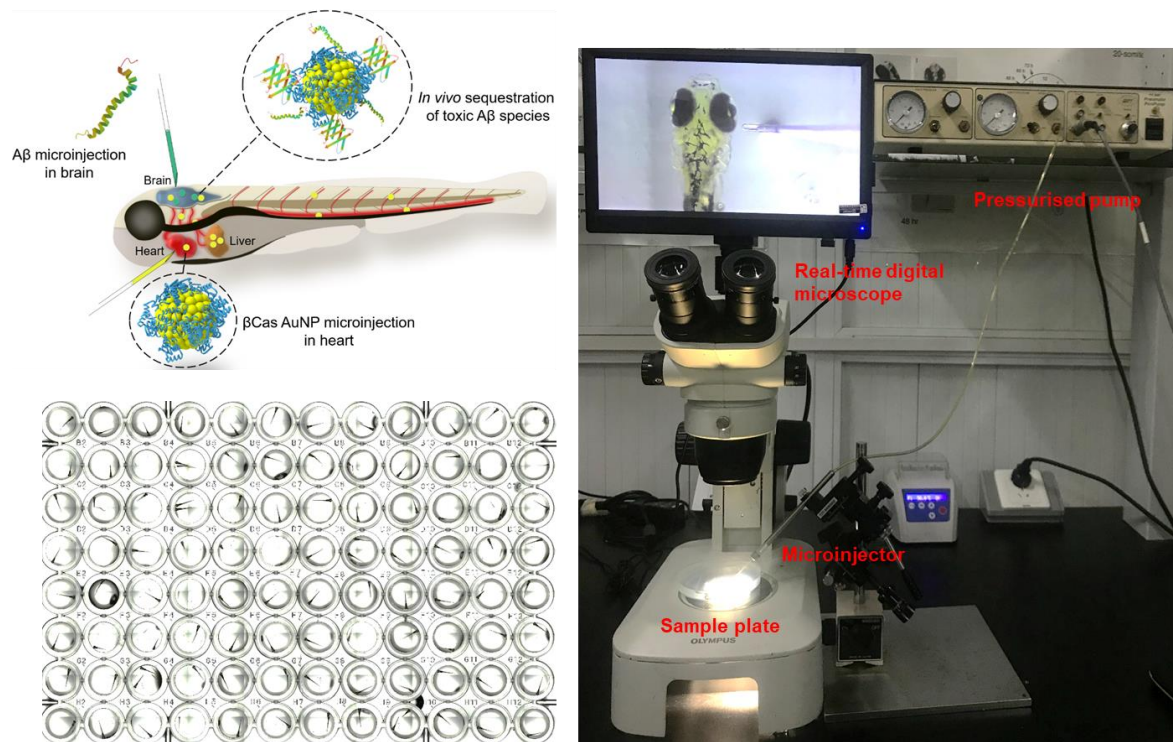
Supplementary Figure 4. DMD simulation of β Cas and A β monomer. (A) Centroid structures of top 8 clusters of β Cas and A β complexes. (B) Representative binding structures of A β (cartoon in rainbow color) and β Cas (sticks in gray) aligned according to A β . The complex structures were obtained from independent binding simulations of A β and β Cas monomers, and the centroid structures of top 50 clusters were used in the alignment.



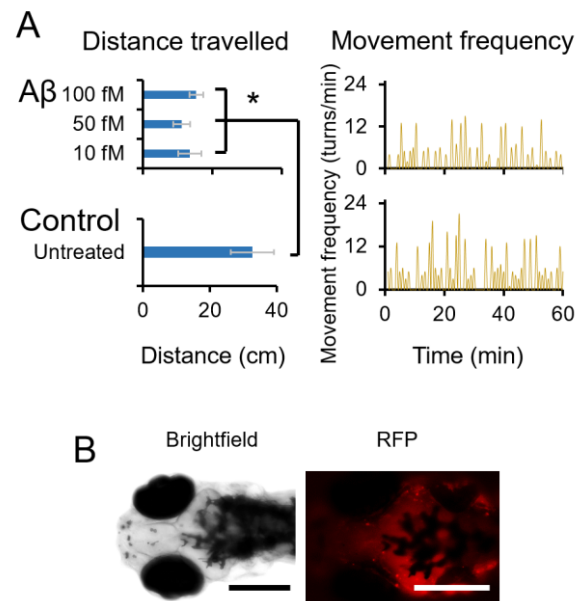
Supplementary Figure 5. DMD simulation of β Cas and A β oligomer. Number of A β inter-peptide H-bonding (A) and contact (B) ranging from residues 10 to 42 in the presence/absence of β Cas as a function of time. Number of H-bond (C) and contact (D) between A β oligomer and β Cas as a function of time. (E) Representative snapshots of A β oligomer in the presence (left) and absence (right) of β Cas (gray) after 125 ns of DMD simulations.



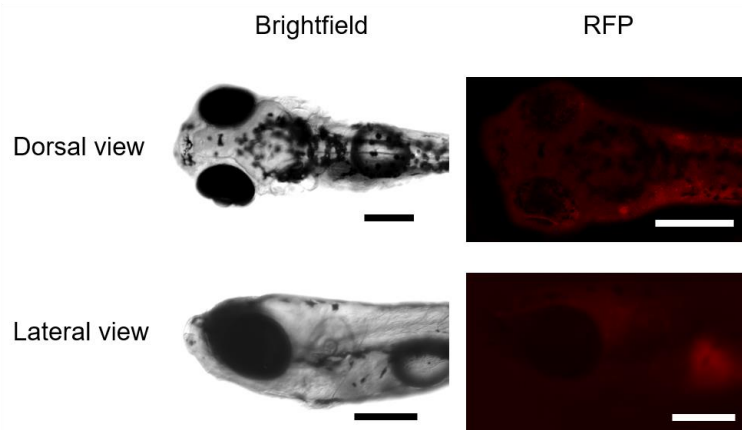
Supplementary Figure 6. binding of A β with a bare AuNP. (A) Binding probability of each A β residue with the AuNP. (B) Typical snapshots of A β binding with the AuNP.



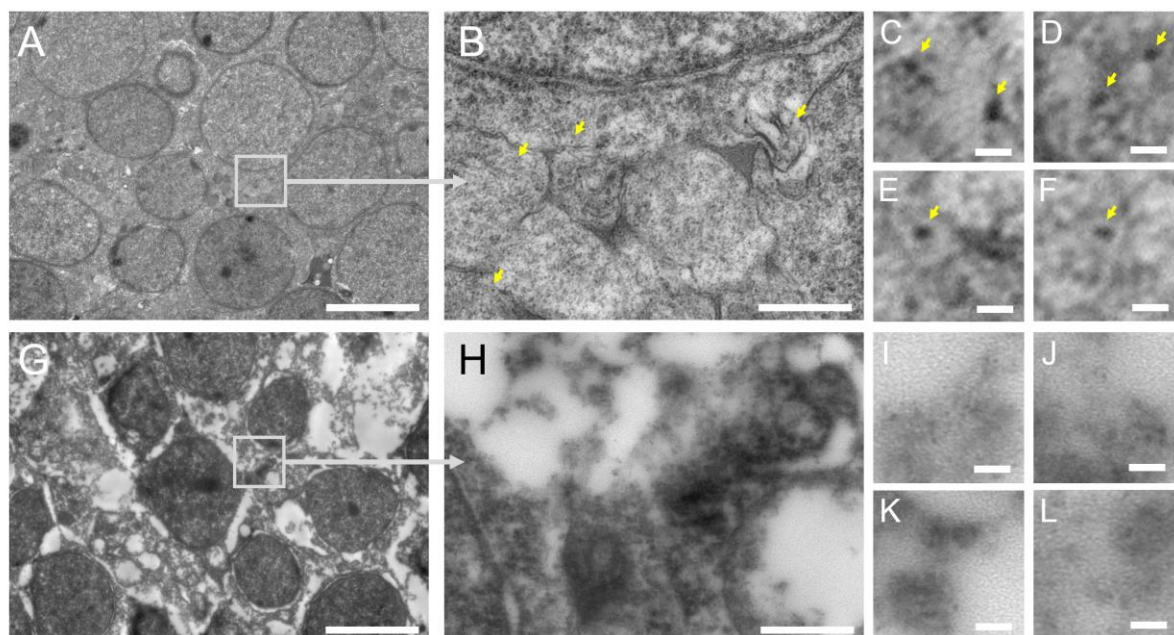
Supplementary Figure 7. High-throughput setup for A β microinjection with zebrafish larvae.



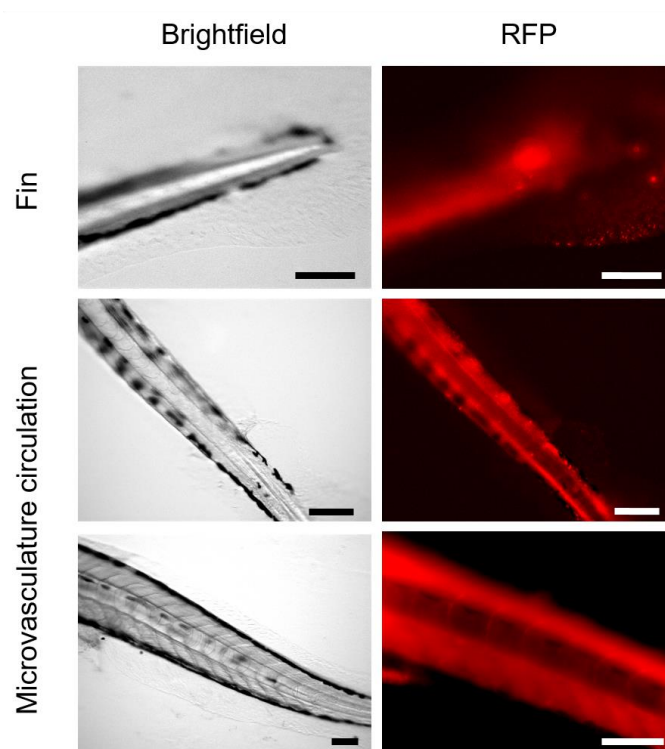
Supplementary Figure 8. Behavioral changes in zebrafish larvae on the 3rd day post A β treatment. (A) Zebrafish larvae swimming behavior was suppressed significantly ($p < 0.05$) on the 3rd day post A β treatment. Swimming distance was reduced to 13.5 ± 3.3 , 11.1 ± 2.4 and 15.4 ± 1.9 cm with 10, 50 and 100 fM A β as compared to 35.3 ± 4.3 cm of untreated control ($n=3$). (B) A β treated larvae were injected with Congo red dye on the 3rd day post A β treatment and slight retention of the dye was observed in the brain. Scale bars in the images are 300 μ m. Error bars represent the standard deviation. Source data are provided as a Source Data file.



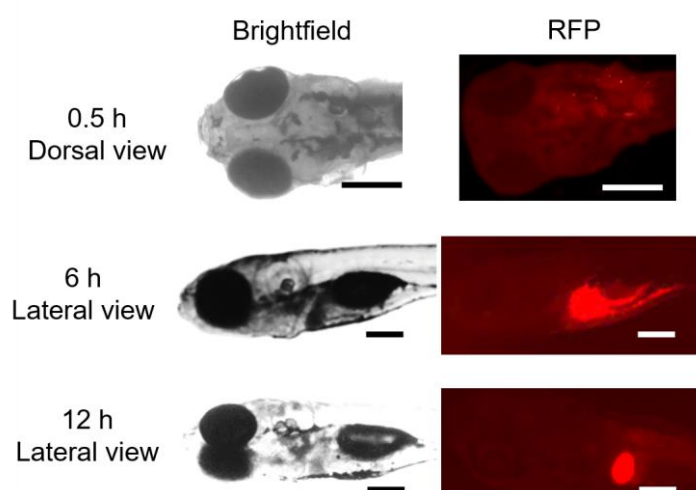
Supplementary Figure 9. Dorsal and lateral view of untreated zebrafish larvae under brightfield and RF channel of microscope. It indicates no background fluorescence in the larvae to interfere with the other fluorescence measurements. Scale bars in all images are 200 μ m.



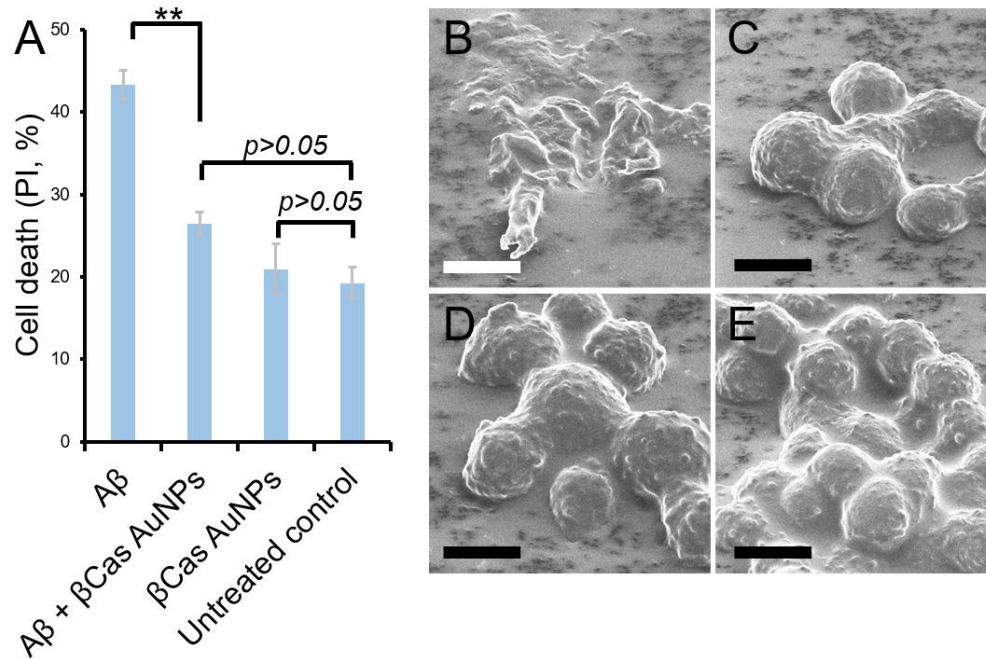
Supplementary Figure 10. TEM imaging of the brain tissues of zebrafish larvae containing β Cas AuNPs. Zebrafish larvae treated with intracardiac injection of 3 ng Au equivalent β Cas AuNPs were subjected to TEM analysis of the brain tissues for detection of AuNPs translocated across BBB. (A, B) TEM images of the brain tissues of β Cas AuNPs treated larvae. Presence of AuNPs in the interstitial spaces are highlighted and presented as zoomed images (C-F). (G, H) However, untreated control larvae did not show any presence of AuNPs in the tissue sections (I-L). Scale bars in A,G: 5 μ m; B,H: 500 nm and C-F,I-L: 10 nm.



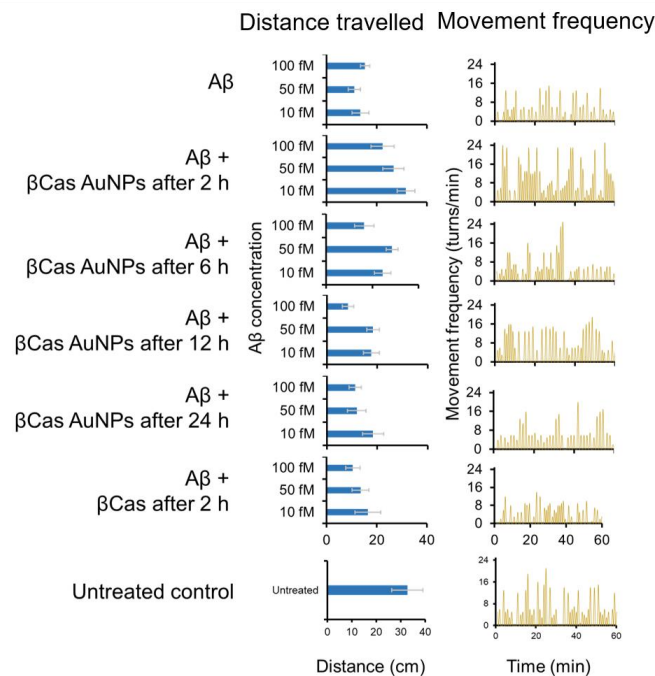
Supplementary Figure 11. Biodistribution of β Cas AuNPs. β Cas AuNPs were conjugated with neutral red and imaged for their biodistribution in zebrafish larvae. Red fluorescence of β Cas AuNPs was traced in microvasculature circulation and fins of the larvae. Scale bars in the images are 200 μ m.



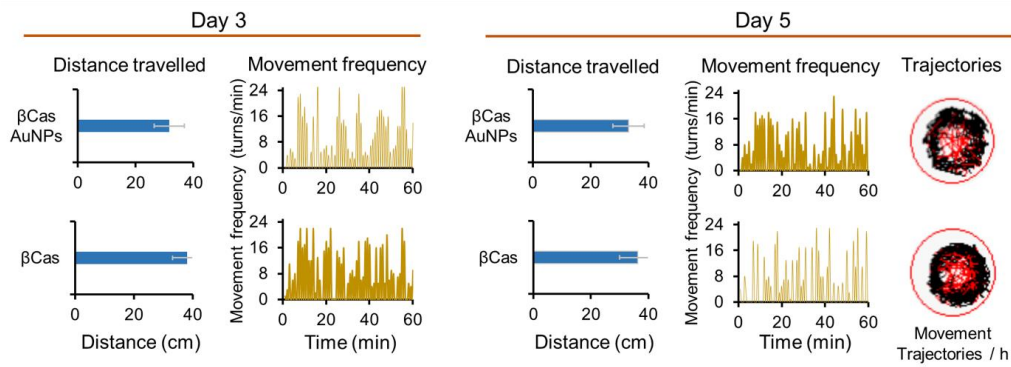
Supplementary Figure 12. Biodistribution of β Cas micelles in zebrafish larvae. β Cas micelles were conjugated with neutral red and imaged for their biodistribution in zebrafish larvae. No fluorescence was observed from the larval cerebral region in 0.5 h or later, indicating inability of large β Cas micelles ($\sim$ 100 nm) to translocate across the BBB. Red fluorescence was observed from the hepatic region of the larvae at 6 and 12 h, indicating slow elimination of β Cas micelles directly from the liver. Scale bars in images are 200 μ m.



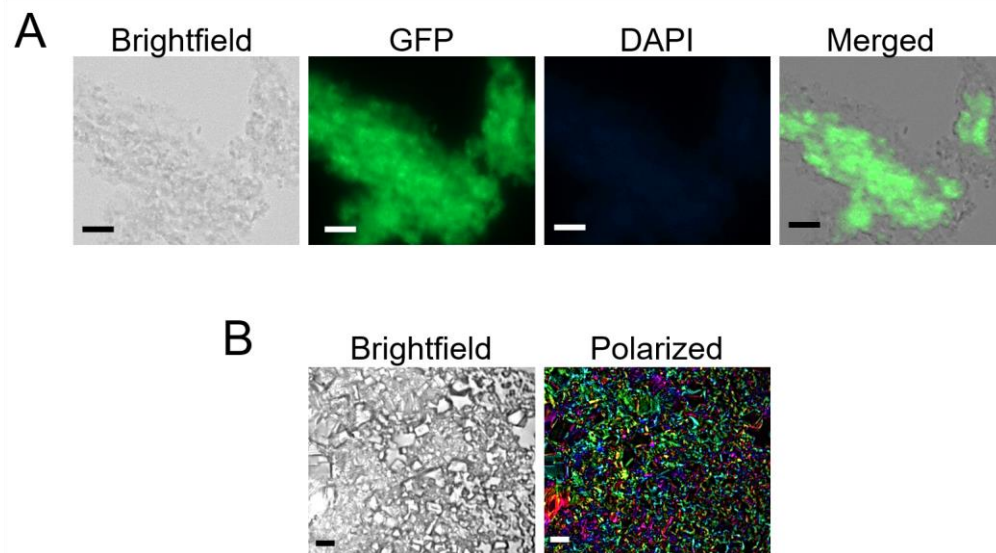
Supplementary Figure 13. Mitigation of A β -induced SH-SY5Y cytotoxicity with β Cas AuNPs. (A) Cell viability assay (n=3) and helium ion microscopy (HIM) images of (B) A β treated (20 μ M), (C) A β (20 μ M) with β Cas AuNPs (50 μ M), (D) β Cas AuNPs alone (50 μ M) and (E) untreated control cells. Scale bars in TEM images are 4 μ m. Error bars represent the standard deviation. Source data are provided as a Source Data file.



Supplementary Figure 14. Mitigation of A β induced behavioral abnormalities in zebrafish larvae by β Cas AuNPs, on the 3rd day post A β treatment (n=10). Error bars represent the standard deviation. Source data are provided as a Source Data file.

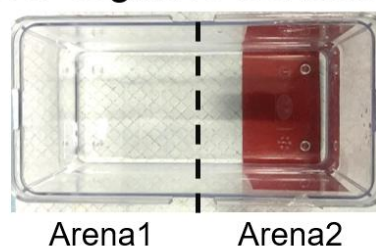


Supplementary Figure 15. β Cas AuNPs and β Cas did not influence the behavior of zebrafish larvae (n=10). Error bars represent the standard deviation. Source data are provided as a Source Data file.



Supplementary Figure 16. Immunohistochemistry IHC (A) and polarized light microscopy (B) of fibrillized A β deposited on glass slides, performed as positive controls for IHC and polarized light microscopy. Scale bars in all images are 50 μ m.

Fish tank for cognitive behavior analysis



Supplementary Figure 17. Fish tank (1L) used for cognitive behavior analysis of adult zebrafish. The tanks were hypothetically divided into two arenas. Whenever, fish swims into arena 2, electrodes of 9V battery were dipped in the arena 2 to shock the fish and train it to remain in arena 1. Arena 2 was labelled with red color to associate with the punishment. 20 min training was performed, followed by 2 min of observation period when the electric source was removed. Observations were performed 3 times for each fish, 2 h apart (n=3).

Supplementary References

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