







ARTICLE

<https://doi.org/10.1038/s41467-019-11762-0>

OPEN

Inhibition of amyloid beta toxicity in zebrafish with a chaperone-gold nanoparticle dual strategy

Ibrahim Javed ^{1,2}, Guotao Peng ², Yanting Xing³, Tianyu Yu², Mei Zhao², Aleksandr Kaminen¹, Ava Faridi¹, Clare L. Parish⁴, Feng Ding ³, Thomas P. Davis ^{1,5}, Pu Chun Ke ¹ & Sijie Lin ²

Alzheimer's disease (AD) is the most prevalent form of neurodegenerative disorders, yet no major breakthroughs have been made in AD human trials and the disease remains a paramount challenge and a stigma in medicine. Here we eliminate the toxicity of amyloid beta (A β) in a facile, high-throughput zebrafish (*Danio rerio*) model using casein coated-gold nanoparticles (β Cas AuNPs). β Cas AuNPs in systemic circulation translocate across the blood brain barrier of zebrafish larvae and sequester intracerebral A β ₄₂ and its elicited toxicity in a nonspecific, chaperone-like manner. This is evidenced by behavioral pathology, reactive oxygen species and neuronal dysfunction biomarkers assays, complemented by brain histology and inductively coupled plasma-mass spectroscopy. We further demonstrate the capacity of β Cas AuNPs in recovering the mobility and cognitive function of adult zebrafish exposed to A β . This potent, safe-to-use, and easy-to-apply nanomedicine may find broad use for eradicating toxic amyloid proteins implicated in a range of human diseases.

¹ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, VIC 3052, Australia. ²College of Environmental Science and Engineering, Biomedical Multidisciplinary Innovation Research Institute, Shanghai East Hospital, Shanghai Institute of Pollution Control and Ecological Security, Key Laboratory of Yangtze River Water Environment, Tongji University, 1239 Siping Road, Shanghai 200092, China. ³Department of Physics and Astronomy, Clemson University, Clemson, SC 29634, USA. ⁴The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, 30 Royal Parade, Parkville, VIC 3052, Australia. ⁵Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Qld 4072, Australia. Correspondence and requests for materials should be addressed to F.D. (email: fding@clemson.edu) or to T.P.D. (email: thomas.p.davis@monash.edu) or to P.C.K. (email: pu-chun.ke@monash.edu) or to S.L. (email: lin.sijie@tongji.edu.cn)

The aggregation of proteins into amyloid fibrils and plaques, under abnormal physiological conditions, is a phenomenon common to a range of human amyloid diseases including amyloid beta (A β) for Alzheimer's disease (AD), α -synuclein for Parkinson's disease (PD), and human islet amyloid polypeptide for type 2 diabetes (T2D)¹. The amyloid hypothesis regards oligomers as the most toxic species², where protofibrils or oligomers of amyloid proteins are proposed to induce local inflammation, failed autophagy, and membrane perturbation that are responsible for the further loss of neuronal or pancreatic β -cells mass^{3,4}.

AD is a primary form chronic neurodegenerative disorder and a major cause of dementia, impairing 46 million people worldwide⁵. The pathological origin of AD is highly debatable, but is believed to be associated with a range of health, genetics, environmental and lifestyle factors, as well as inflammation^{6–9}. The etiology of AD includes a number of events that precede A β plaque formation, such as autophagy or endosomal dysfunction¹⁰, endoplasmic reticulum stress¹¹, oxidative stress or hypoxia, vasculature and mitochondrial dysfunction¹², and prior history of bacterial infections¹³. A β_{42} is one of the two most abundant peptide species derived from amyloid precursor protein (APP) through proteolysis and, alongside tau, is strongly associated with the pathology of AD¹⁴. Despite much research over the past decades devoted to understanding the origin, diagnosis and prevention of AD, there is a glaring lack of success against A β amyloidosis marked by recent withdrawals of clinical trials with Eli Lilly, Pfizer, and Biogen^{15–17}. This indicates failures in current anti-amyloid therapeutic approaches, compounded by a lack of suitable in vivo models for high throughput screening^{18,19}, further justifying the urgency for developing alternative strategies against AD.

Among the common strategies against amyloidosis, peptides, small molecules, monoclonal antibodies and, more recently, engineered nanoparticles, have shown various degrees of promise as inhibitors^{20–27}. For in vivo applications, these inhibitors are designed to satisfy—partially or fully—the following criteria: minimal toxicity, good circulation/repeated dosing, good translocation efficacy across the blood brain barrier (BBB), as well as capabilities in targeting and further eliminating toxic oligomers, protofibrils, and fibrils of amyloid proteins. β casein (β Cas), a whey protein, along with α_{s1} casein, possesses a chaperone-like activity, similarly to small heat-shock proteins and extracellular clusterin. This activity of the caseins arises from the following: (1) a lack of tertiary structure and solvent-exposed hydrophobicity with well separated hydrophilic regions, (2) existence as heterogeneous oligomers, (3) dynamics and malleable protein regions, and (4) ability to bind with a wide range of partially folded proteins preventing their aggregation²⁸. One factor that attributes to these properties is the presence of a high percentage of proline residues, i.e., 18% in the case of β Cas, and no disulfide bonds that provide them with an open and flexible conformation²⁹. The chaperone-like behavior of β Cas and α_{s1} caseins shields the amyloidogenic regions and naturally prevents the amyloidosis of α_{s2} and κ -casein in mammary glands or milk while inhibiting the amyloidosis of insulin and A β_{40} in vitro^{30–32}. Structurally, monomeric caseins are mostly disordered, but tend to form micelles mediated by hydrophobic and electrostatic interactions²⁸.

Here, we devise a facile method of coating β Cas onto gold nanoparticles (AuNPs). We systemically deliver the β Cas AuNPs via intracardial administration to mitigate the toxicity of A β_{42} induced in the brain of zebrafish larvae and adults (*Danio rerio*). β Cas AuNPs sequester toxic A β_{42} in the brain of zebrafish larvae and adults through a nonspecific, chaperone-like manner. No such mitigation is obtained with caseins alone, indicating the

essential role of the AuNPs in delivering the protein. This demonstrates the inhibition potential of a chaperone protein integrated with a biocompatible nanomaterial against Alzheimer's-like symptoms. The established zebrafish model also opens the door to economically viable, high-throughput in vivo screening of emerging nanomedicines targeting a wide range of amyloid diseases.

Results and discussion

Scheme of study. Different fractions of caseins, e.g., α_{s1} and β , have a known potential for surface-assisted sequestration and colloidal inhibition of A β_{40} and insulin amyloid formation^{31,32}. Herein, β Cas with an intrinsic chaperone-like activity³³ was coated on AuNPs by NaBH₄-assisted reduction of Au. Synthesis of the β Cas AuNPs was optimized at room temperature to obtain ~5 nm in size for efficient BBB translocation while preserving the random coil structure of β Cas that is required for its chaperone activity³⁰. β Cas AuNPs were then characterized for their inhibitory activity against A β_{42} (abbreviated as A β from hereon) fibrillization in vitro. For in vivo translation, an A β toxicity model was developed in zebrafish larvae by cerebroventricular injection of A β . The biodistribution and translocation of β Cas AuNPs across the larval zebrafish BBB were then determined after introducing the nanoparticles into the bloodstream via intracardiac injection. Finally, A β and β Cas AuNPs were co-administered via cerebroventricular and intracardiac injections into zebrafish larvae, and alleviations of A β -induced behavioral symptoms were quantified. In addition, A β -induced behavioral pathology and cognitive dysfunction in adult zebrafish were rescued by β Cas AuNPs, further implicating the chaperone potential of β Cas AuNPs against A β toxicity in vivo.

In vitro interaction of β Cas AuNPs and A β . A β was fibrillized in vitro from random coils to β -sheet rich amyloid fibrils within 48 h at 37 °C. A thioflavin T (ThT) assay was used to study the fibrillization kinetics (Fig. 1a), while transmission electron microscopy (TEM) (Fig. 1b) and circular dichroism (CD) spectroscopy were employed to investigate fibril formation and secondary structural transitions of A β (Fig. 1c, d). β Cas AuNPs (Fig. 1e) often clustered together after binding with A β (Fig. 1f) and prevented β -sheet formation of the peptide (Fig. 1a). The presence of A β coronae on β Cas AuNPs was evident from TEM imaging (Fig. 1f inset). The secondary structure of β Cas AuNPs was predominantly random coils that transitioned into α helices in A β - β Cas AuNPs complex (Fig. 1g, d). β Cas formed micelles of ~100 nm in size in the aqueous medium (Fig. 1h). Upon incubation with A β , β Cas (in the absence of the AuNPs) induced an early onset of fibrillization as revealed by the ThT assay (Fig. 1a), which can be attributed to the fast nucleation of A β promoted by β Cas micelles in vicinity³⁴. However, A β fibrillization was not inhibited and random aggregates of the peptide were observed under TEM (Fig. 1i). CD spectroscopy indicated that the α -helix rich structure of β Cas was converted to β -sheets in β Cas + A β aggregates due to A β fibrillization (Fig. 1j, d). The hydrodynamic diameters of β Cas AuNPs and β Cas were increased from 7.5 ± 2.6 and 156.3 ± 34.4 nm to 39.3 ± 5.4 and 496.1 ± 114 nm ($n = 3$), respectively (Fig. 1k and Supplementary Table 1). The zeta potential of β Cas AuNPs was markedly elevated from -11.7 ± 1.8 to -33.7 ± 2.1 mV ($n = 3$), indicating adsorption of anionic A β onto the surfaces of β Cas AuNPs (Supplementary Table 1). Clusterization of β Cas AuNPs was confirmed by hyperspectral imaging (HSI), where the surface plasmon resonance (SPR) of β Cas AuNPs was red shifted from 490 ± 21 to 601 ± 24 nm ($n = 3$) upon aggregation and light illumination (Supplementary Fig. 1A–C). As oligomers/protofibrils are the main toxic species

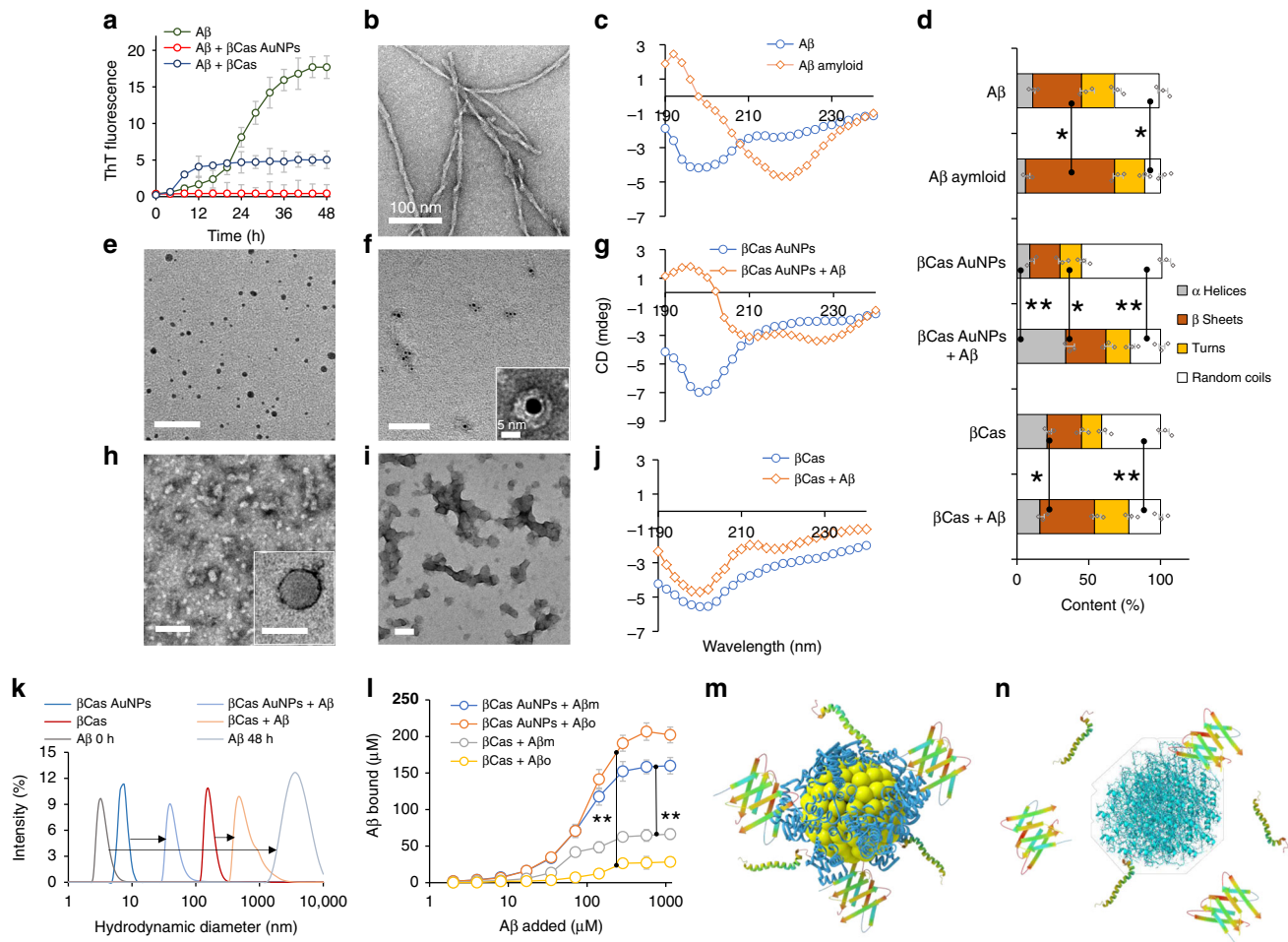


Fig. 1 In vitro inhibitory interactions between β Cas AuNPs and β Cas with $A\beta$. **a** ThT assay of $A\beta$ alone ($50 \mu\text{M}$) and in the presence of β Cas AuNPs (equivalent to $6.25 \mu\text{M}$ of β Cas) and β Cas ($6.25 \mu\text{M}$) ($n = 4$). β Cas AuNPs completely inhibited while β Cas decreased ($p < 0.005$) the lag time and plateau ThT fluorescence of $A\beta$ fibrillization. **b** TEM image of fibrillized $A\beta$. **c** CD spectra of $A\beta$ ($100 \mu\text{M}$) before and after fibrillization indicate conformation change from random coils (198 nm peak) to β -sheets (220 nm peak). **d** Secondary structure of $A\beta$ ($100 \mu\text{M}$) fibrillized with and without β Cas AuNPs or β Cas ($n = 4$). TEM images of β Cas AuNPs before (**e**) and after incubation with $A\beta$ (**f**; inset shows $A\beta$ corona on a β Cas AuNP). **g** CD spectra of β Cas AuNPs before and after incubation with $A\beta$. TEM images of β Cas before (**h**) and after (**i**) incubation with $A\beta$. **j** Appearance of a negative peak at 218 nm in CD spectra of β Cas + $A\beta$ indicates limited $A\beta$ fibrillization into β -sheets. After binding with $A\beta$, the α -helix contents of β Cas AuNPs were decreased significantly ($p < 0.05$) from 56 to 21%; while in the case of β Cas, α -helices decreased from 41 to 22% ($p < 0.005$) and β -sheets increased from 24 to 38% ($p < 0.05$). **k** Hydrodynamic radius of $A\beta$ before and after fibrillization in the presence and absence of β Cas AuNPs or β Cas. **l** Quantification of binding capacity of β Cas AuNPs or β Cas with $A\beta\text{m}$ or $A\beta\text{o}$ ($n = 4$). β Cas AuNPs ($6.25 \mu\text{M}$ β Cas equivalent) were able to bind up to 152.3 ± 13.4 and $190.7 \pm 10.9 \mu\text{M}$ of $A\beta\text{m}$ and $A\beta\text{o}$. β Cas ($6.25 \mu\text{M}$) was only able to adsorb 62.4 ± 3.4 and $26.4 \pm 4.6 \mu\text{M}$ of $A\beta\text{m}$ and $A\beta\text{o}$, indicating a significant ($p < 0.005$) increase in $A\beta$ binding capacity of β Cas in the form of β Cas AuNPs. **m, n** Enhanced binding of $A\beta\text{m}$ and $A\beta\text{o}$ with β Cas promoted by the AuNP substrate. Scale bars in TEM images is 100 nm, while F inset is 5 nm. Error bars represent the standard deviation. Source data are provided as a Source Data file

of amyloid proteins¹⁴, interactions of β Cas AuNPs with $A\beta$ monomers ($A\beta\text{m}$) and $A\beta$ oligomers ($A\beta\text{o}$) were also examined. The in vitro binding between $A\beta\text{o/m}$ and β Cas or β Cas AuNPs was further quantified by a bicinchoninic acid assay (BCA) and thermogravimetric analysis (TGA). β Cas or β Cas AuNPs were incubated with different concentrations of $A\beta\text{m}$ or $A\beta\text{o}$ for 48 h and centrifuged to remove free $A\beta$. The centrifuged pellets containing $A\beta\text{m}$ or $A\beta\text{o}$ bound to β Cas or β Cas AuNPs were subjected to analysis (Supplementary Fig. 1D). The maximum binding capacity between $A\beta$ and β Cas ($6.25 \mu\text{M}$) was quantified to be 62 and 26 μM for $A\beta\text{m}$ and $A\beta\text{o}$, respectively (Fig. 1l). However, when β Cas AuNPs (containing $6.25 \mu\text{M}$ β Cas) were exposed to $A\beta$, the maximum binding capacity was increased to 152 and 190 μM for $A\beta\text{m}$ and $A\beta\text{o}$, respectively. Similar results were obtained with TGA, where no difference in the TGA curve was observed when the concentration of $A\beta$ was increased beyond

0.3 and 0.06 mM for β Cas AuNPs and β Cas, respectively, suggesting binding saturations (Supplementary Fig. 1E). The differential binding of β Cas and β Cas AuNPs with $A\beta\text{m/o}$ is illustrated in Fig. 1m, n.

The high affinity of β Cas AuNPs for $A\beta\text{o}$ can be attributed to the ability of β Cas to bind with misfolded/molten globules of proteins²⁸. To further investigate the differential binding of β Cas AuNPs with $A\beta\text{o}$ and $A\beta\text{m}$, we incubated β Cas AuNPs with preformed $A\beta\text{o}$ and $A\beta\text{m}$ for 3 h and separated them from unbound $A\beta\text{o/m}$ via centrifugal washing. The UV-SPR spectra of β Cas AuNPs were significantly suppressed upon incubation with $A\beta\text{o}$ as compared to $A\beta\text{m}$ (Supplementary Fig. 2A). Similarly, the fluorescence of neutral red-conjugated AuNPs (NR- β Cas AuNPs) was suppressed when incubated with $A\beta\text{o}$ (Supplementary Fig. 2B). This indicates increased adsorption of $A\beta\text{o}$ than $A\beta\text{m}$ by β Cas AuNPs, as further confirmed by TEM imaging of corona

formation on the nanoparticles (Supplementary Fig. 2C). Furthermore, CD results indicated similar secondary structural distributions of A β and A β - β Cas AuNPs complex. Thus, the α helices in A β - β Cas AuNPs complex can be attributed to the A β corona on β Cas AuNPs (Supplementary Fig. 2D, E). Incubation of β Cas AuNPs with A β m did not present any difference in the secondary structure of β Cas AuNPs. That, together with the UV-SPR, fluorescence and TEM results, confirmed the high affinity of β Cas AuNPs for A β .

DMD simulations of β Cas binding with AuNP and A β monomer/oligomer. To gain a molecular insight into the adsorption of β Cas onto an AuNP surface (i.e., the formation of a β Cas AuNP “corona”) and the inhibition mechanism of β Cas AuNPs against A β aggregation, discrete molecular dynamics (DMD) simulations—an accurate and rapid molecular dynamics algorithm widely used to study the structure and dynamics of large molecular systems^{35,36}—were performed (Fig. 2). The binding of a β Cas monomer with an AuNP (4 nm in diameter), an A β monomer and an A β oligomer were examined (Supplementary Methods), and the control simulations included an isolated β Cas, an A β monomer, and an A β oligomer. We first computed secondary structure contents from equilibrium simulations (e.g., radius of gyration in Supplementary Fig. 3A and number of hydrogen bond in Supplementary Fig. 5A indicated simulations reaching steady states) and used them to estimate the expected CD spectra for different molecular systems (Fig. 2b). The predicted CD spectra agreed well with the experimental results (Fig. 1c, g, j, d) in terms of secondary structural changes. As expected, β Cas was intrinsically disordered with unstructured coils as the dominant secondary structure. Upon binding the

AuNP, β Cas exhibited increased coil and decreased helix and sheet contents both *in silico* (Fig. 2b inset) and *in vitro* (Fig. 1g, j, d). Analysis identified several specific binding sites of β Cas for the AuNP, such as residues His65, Phe67, Lys122, Met124, and His159 (the upper panel in Fig. 2a). Based on clustering analysis of the structural ensemble from multiple independent DMD simulations (Supplementary Methods), representative binding structures of β Cas monomers with the AuNP were obtained (Supplementary Fig. 3B), where individual β Cas partially covered the AuNP. To form a monolayer protein corona, at least three β Cas molecules were required to fully coat the AuNP surface (Fig. 2c, estimated by covering the NP surface with randomly selected centroid structures from top ten clusters shown in Supplementary Fig. 3B). When β Cas bound to an A β monomer, the overall contents of ordered helices and sheets increased while coils decreased (Fig. 2b), in agreement with the experiments (Fig. 1c, j, d). Residues in β Cas that had strong binding with the A β monomer did not overlap with those preferred to bind the AuNP (Fig. 2a). This result suggests that β Cas AuNP could still bind to A β monomers, as illustrated by the β Cas-AuNP complex where A β -binding residues were exposed (Fig. 2c, with the protein surface color-coded according to their binding probabilities with the A β monomer). Although we did not perform simulations for the binding of A β with β Cas AuNP, due to the prohibitively large system, we expected similar trends of secondary structure changes as observed in the experiments (Fig. 1d). Representative structures of the binding complexes obtained from the simulations (Supplementary Fig. 4A) suggest that β Cas could bind A β and form either β sheets or helices (Supplementary Fig. 4B), which in turn inhibited A β aggregation by sequestering A β in solution or capping A β fibrils from elongation. Moreover, simulations of β Cas with a preformed cross- β A β oligomer

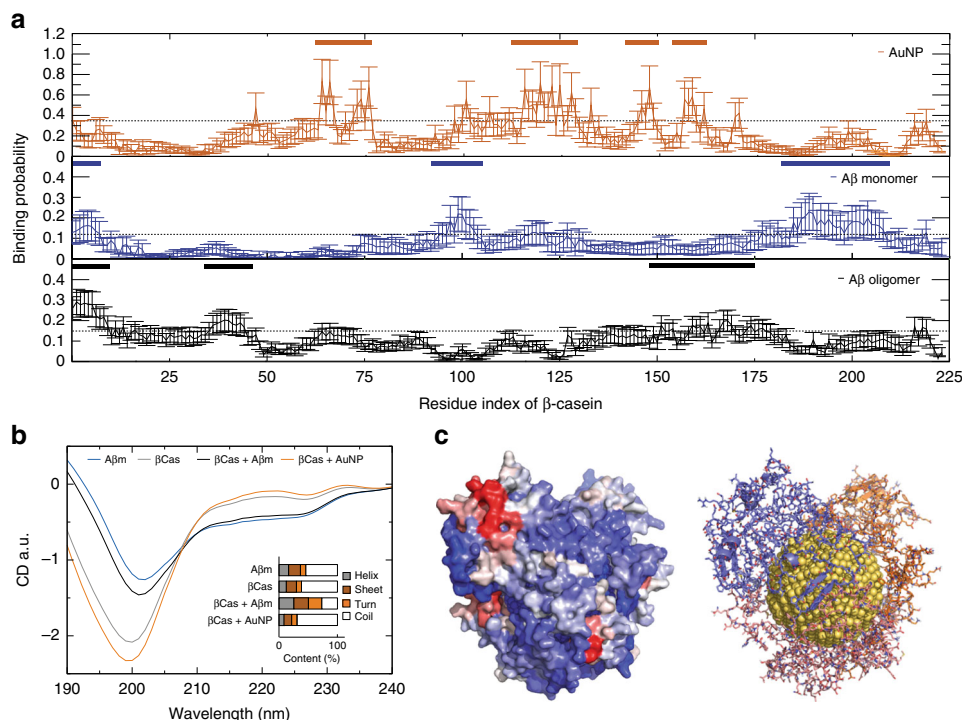


Fig. 2 DMD simulations of β Cas binding with AuNP, A β monomer, and oligomer. **a** Binding probabilities of β Cas with an AuNP, an A β monomer and an A β oligomer, where high-binding is defined as residues with binding probabilities above one standard deviation from the average (dash lines). β Cas high-binding residue regions with AuNP and with A β monomer/oligomer are highlighted with bars (inset). **b** Predicted CD spectra of secondary structure contents (inset) derived from simulations. **c** Predicted β Cas-AuNP corona structures comprised of three β Cas proteins on an AuNP surface (right) and corresponding molecular surfaces of the proteins (left) are shown to highlight their binding with an A β monomer, where each β Cas residue was colored from purple (low) to red (high) according to its binding probability with A β monomer as in panel A middle. Source data are provided as a Source Data file

indicate that the β Cas-AuNP complex could bind A β oligomer (Fig. 2a) and the strong binding between β Cas and A β oligomer (Supplementary Fig. 5D) inhibited further growth of the initial oligomer (i.e., inset of Supplementary Fig. 5E) into an extended β -sheet structure via conformational rearrangements. Taken together, our simulations were not only consistent with the ensemble measurements in vitro, but also uncovered the molecular mechanism for the formation of the β Cas AuNP complex and their inhibition of A β aggregation via either sequestering of A β monomers or capping of A β fibril elongation. In addition, the binding of A β with a bare AuNP is presented in Supplementary Fig. 6. Approximating the binding affinity and energy differences between the complex (β Cas + AuNP, A β + AuNP) and individual components (β Cas, A β) from DMD simulations (Fig. 2 and Supplementary Fig. 6) revealed that the binding of β Cas with the AuNP was significantly stronger than the binding of A β with the AuNP ($\Delta\Delta G \sim -194$ kcal mol $^{-1}$) (Supplementary Table 2). Hence, replacement of β Cas corona with A β was energetically unfavorable.

Development of A β toxicity model in zebrafish larvae. Zebrafish larvae express human orthologues of A β , APP, and γ -secretase components (PSENEN³⁷, NCTN³⁸, APH1b³⁷) 24 h after hatching³⁹. Gene knockout or chemical inhibitors may create an imbalance among these protein components to result in neurological and behavioral abnormalities^{40,41}. Here, an A β toxicity model was developed using zebrafish larvae (5 days old) by injecting A β into the cerebroventricular space (Fig. 3a and Supplementary Fig. 7). In vivo oligomerization of A β into toxic oligomeric species induced pathological features in zebrafish larvae after 5 days of A β treatment (Fig. 3b). Different concentrations of A β were injected into the larvae and no lethality was observed even with the highest concentration of A β , at 1200 fM per larva. However, reduced locomotion of the larvae was notable in a concentration dependent manner, with nonresponsive mobility and a loss of balance at higher A β concentrations (≥ 75 fM) (Fig. 3c). The nonresponsiveness of the larvae was recorded using tapping as a stimulus and loss of balance was observed as a tilt of the larvae from the normal horizontal axis to the imbalanced vertical axis (Supplementary Videos 1–3). The larvae injected with 10, 50, and 100 fM A β were characterized on an automated zebrafish behavior analysis system, to quantify total distance traveled and frequency of movement during the 1 h recording period. Observations were made on the third (Supplementary Fig. 8A) and fifth (Fig. 3d) day post treatment with A β . Significant reductions in both total distance traveled and frequency of movements were noted, in a concentration-dependent manner on the fifth day, in A β -treated larvae compared to untreated control. To visualize the presence of A β fibrils in the brain of the larvae, Congo red dye was injected in the cerebroventricular space of zebrafish larvae (Fig. 3e) on the third (Supplementary Fig. 8B) and fifth day post injection of A β (Fig. 3f). Significantly increased fluorescence was observed from the brain of the A β -injected larvae on the fifth day post injection. By comparison, Congo red dye was injected in untreated control and no fluorescence was observed (Fig. 3g). A β amyloid formation in the brain of zebrafish larvae was further confirmed by matrix assisted laser desorption/ionization (MALDI) analysis. Five days post injection with A β , the larvae heads were excised after euthanization, homogenized in phosphate-buffered saline (PBS) buffer and analyzed by MALDI. A peak corresponding to the molecular weight of A β was observed at 4538.1 m z^{-1} (Fig. 3h). A β treated larvae were further fixed, cryo-sectioned and stained with Congo red. A β plaques were observed using the red fluorescence protein (RFP) channel

of a microscope (Fig. 3i). No red fluorescence was observed in untreated control (Fig. 3j).

Biodistribution of β Cas AuNPs in zebrafish larvae. The biodistribution of β Cas AuNPs was characterized by conjugating the AuNPs with NR dye and injecting the AuNPs via the intracardiac route (Fig. 4a). Whole-mount imaging was performed under the RFP channel of a fluorescence microscope at 0.5, 6, and 12 h after injection in order to trace the biodistribution of NR- β Cas AuNPs in different regions of the larvae (Fig. 4b). No fluorescence was observed from the dorsal or lateral view of the larvae in the control (Supplementary Fig. 9). The zebrafish BBB is a double-layered membrane separating cerebral blood vessels from brain tissues. Alongside tight junctions, zebrafish BBB expresses occluding, claudins and p-glycoproteins and thus possesses a selectivity against xenobiotics^{42,43}. In the present study, upon intracardiac injection of NR- β Cas AuNPs into larvae, bright red fluorescence was observed from the brain after 0.5 h, indicating translocation of β Cas AuNPs across the BBB (Fig. 4c). At 6 h after injection, the fluorescence from the cerebral region was decreased while it was recorded in the liver. However, the fluorescence was diminished from the liver at 12 h. β Cas AuNPs were detectable by HSI and the SPR signals of the AuNPs were recorded from the brain sections of the larvae, prepared 0.5 h post injection of β Cas AuNPs (Fig. 4d). However, no AuNPs or SPR were detected from the brain of untreated control. Finally, inductively coupled plasma mass spectroscopy (ICP-MS) was performed to further quantify the presence of AuNPs in the brain. The larvae treated with intracardiac injection of β Cas AuNPs were euthanized at 0.5, 0.6, and 12 h and their heads were homogenized and quantified for Au in the brain and trunks. The concentration of Au was the highest in the brain at 0.5 h while decreased to the lowest level at 12 h (Fig. 4e). A correlation of AuNPs injected in the heart and delivered across the brain is shown in Fig. 4f. Injection of 1.5, 3, and 6 ng Au equivalent AuNPs via the heart delivered around 0.15, 0.45, and 0.5 ng of AuNPs to the brain, indicating that injection of >3 ng of AuNPs did not increase the delivery of AuNPs to the cerebral region. TEM images of microtome slices of the zebrafish larval brain also showed the presence of β Cas AuNPs in the intracellular space (Supplementary Fig. 10).

Apart from the brain, 5 nm β Cas AuNPs (conjugated with NR dye) also distributed to the fins and were imaged while circulating inside the microvasculature of zebrafish larvae (Supplementary Fig. 11). In contrast to β Cas AuNPs, NR-conjugated β Cas micelles (4.5 ng) were not able to translocate across the BBB and, instead, accumulated in the liver 6 and 12 h post injection (Supplementary Fig. 12) due to their larger sizes.

Mitigation of A β toxicity and pathological symptoms. Mitigation of A β toxicity was first assessed in vitro with SH-SY5Y neuronal cells. β Cas AuNPs were able to sequester A β toxicity against SH-SY5Y cells in the viability assay (Supplementary Fig. 13A). Helium ion microscopy (HIM) revealed morphological damage induced by A β to the SH-SY5Y cells and their recovery by β Cas AuNPs (Supplementary Fig. 13B, C). For in vivo, A β toxicity was induced in the zebrafish larvae by cerebroventricular injection of A β and was relieved by intracardiac injection of β Cas AuNPs. Specifically, β Cas AuNPs were administered at different time intervals post A β injection and the exposed larvae were studied for their behaviors 3 (Supplementary Fig. 14) and 5 days (Fig. 5a) post A β injection. β Cas AuNPs completely relieved the symptoms when treated within 2 h of A β injection, as indicated by the total distance traveled, movement frequency and trajectories during 1 h of observation. Administration of β Cas AuNPs, 6 h after A β treatment, partially alleviated the behavioral

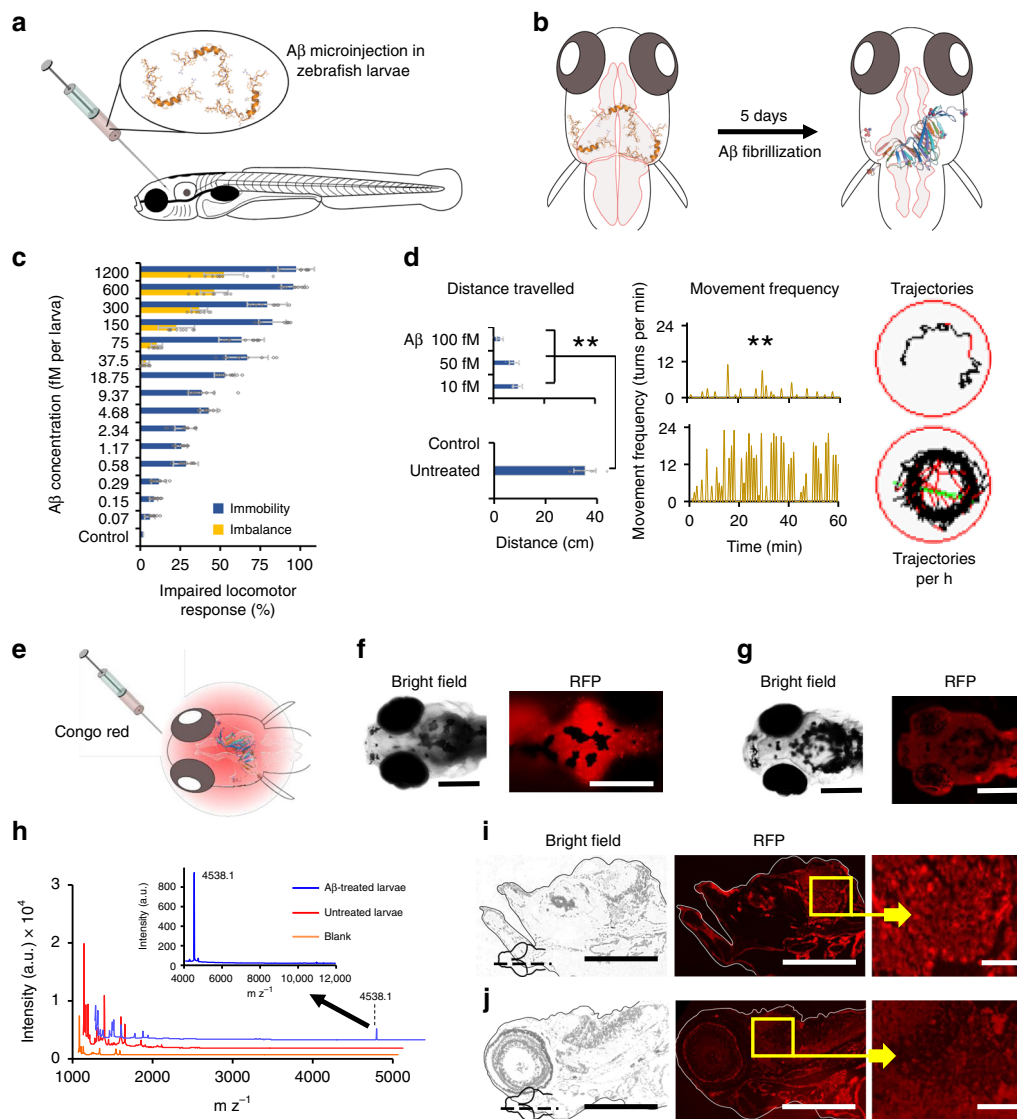


Fig. 3 Aβ toxicity in zebrafish (*Danio rerio*) larvae. **a** Five-day-old zebrafish larvae were treated with Aβ and developed pathological symptoms (**b**). **c** Disruptive locomotive behavior was recorded in terms of percentage of larvae who failed to respond upon tapping (blue bars) and unable to maintain horizontal swimming position (orange bars) ($n = 10$). **d** The behavior of the larvae was further recorded on an automated zebrafish behavior monitoring system for 1 h at 5 days post Aβ treatment ($n = 10$). Total distance travelled along with movement frequency was significantly decreased compared to untreated control ($p < 0.005$). Representative trajectories of 100 fM Aβ treated and untreated larvae inside a single well of a 96-well plate, during 1 h of observation. **e** Five days after Aβ (100 fM) treatment, larvae were further treated with Congo red (100 fM) via cerebroventricular injection. Whole mount larvae were imaged under the RFP channel 6 h after Aβ treatment. **f** Significant fluorescence was retained in the cerebral region of larvae on the fifth day post Aβ treatment. **g** Congo red injected in untreated larva was not retained in the cerebral region. **h** MALDI detection of Aβ in the brain of zebrafish larvae, 5 days post Aβ injection ($n = 10$, Mean ± SD). Five days after cerebroventricular injection of Aβ, the heads of zebrafish larvae were excised after euthanization. The heads were homogenized in Holtfreter's buffer and subjected to MALDI-TOF/TOF analysis. Peak corresponding to Aβ molecular weight was observed at 4538.1 m z⁻¹. Untreated larvae and matrix alone were used as controls. **i** Congo red-stained thin section (sagittal) of Aβ treated larvae brain tissue. Bright red spots were observed in the cerebral region of larvae, corresponding to the Aβ amyloid or plaque formation. **j** In thin sections of the brain tissue of untreated larvae (negative control), no red spots were observed. Scale bars in all images are 200 μM, while in **i** and **j** inset are 20 μM. Error bars represent the standard deviation. Source data are provided as a Source Data file

symptoms. However, treatment with βCas AuNPs 12 h after Aβ injection did not rescue the larvae from Aβ toxicity, indicating the neurotoxicity of Aβ had been initiated. This observation correlates with the nucleation and oligomerization of Aβ into toxic species around 12 h, as indicated by the ThT kinetic assay (Fig. 1a). In contrast, βCas micelles failed to rescue the larvae from Aβ toxicity even injected 2 h after Aβ administration. βCas AuNPs and βCas as controls did not induce any behavioral abnormalities in zebrafish larvae (Supplementary Fig. 15). In

addition, citrate-capped AuNPs failed to rescue the larvae from Aβ toxicity, implicating that βCas, but not AuNPs was mainly responsible for toxicity mitigation (Fig. 5a).

Microtome slices of the brain tissues of zebrafish larvae, treated with Aβ and βCas AuNPs, were prepared on the fifth day post Aβ treatment and stained with Congo red. No amyloid plaque formation was observed (Fig. 5b), indicating elimination of Aβ species by βCas AuNPs. Immunohistochemistry (IHC) (Fig. 5c) and polarized light microscopy (Fig. 5d) further confirmed

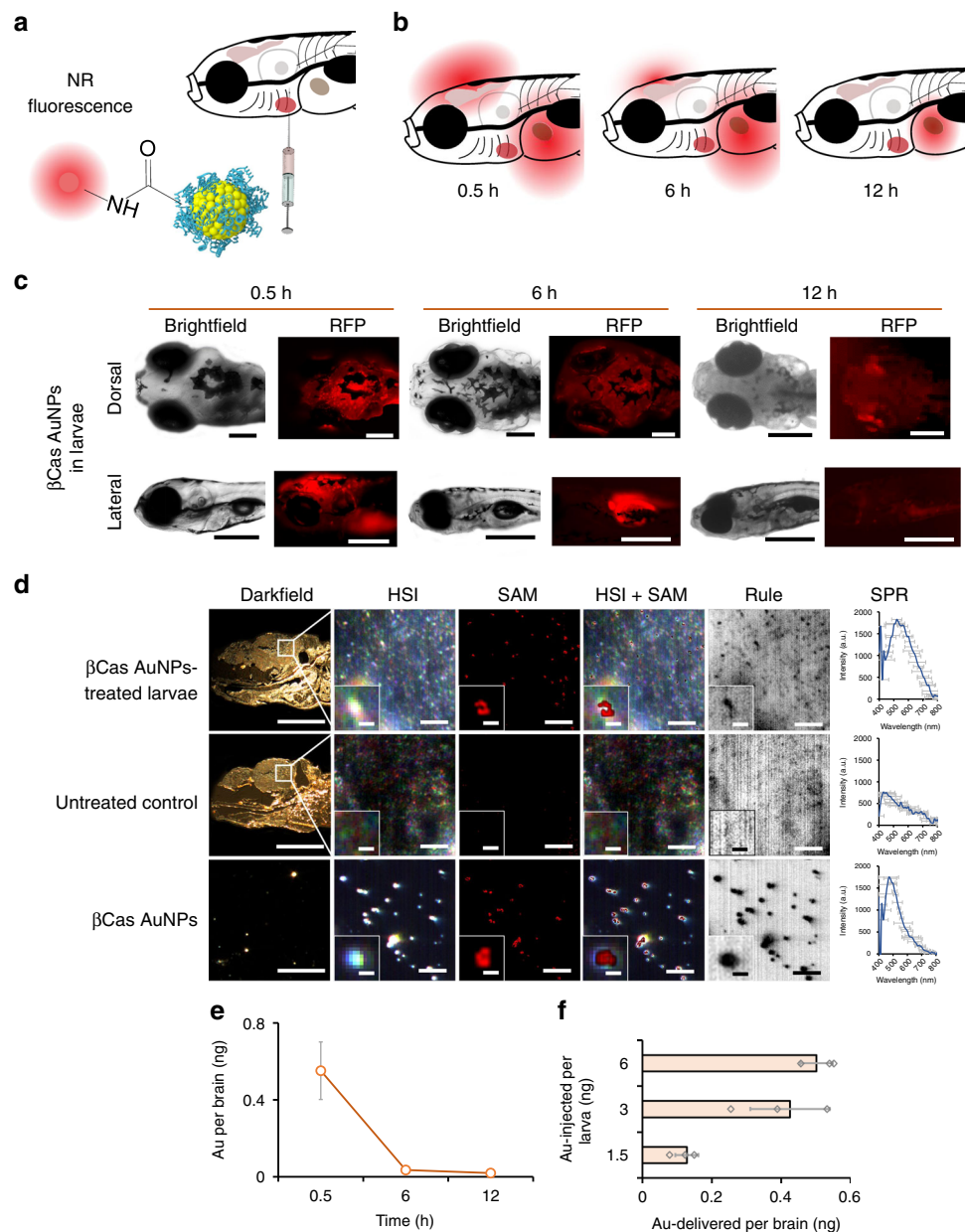


Fig. 4 In vivo biodistribution of β Cas AuNPs in zebrafish larvae. **a** β Cas AuNPs were conjugated with neutral red and injected to zebrafish larvae (5 day old) via the intracardiac route in a dose equivalent to 3 ng Au per 4.5 ng β Cas. **b** Labeled β Cas AuNPs were traced for in vivo distribution at 0.5, 6, and 12 h in dorsal and lateral positions (**c**). Bright fluorescence was observed from the brain 0.5 h after AuNP administration. However, fluorescence was eliminated from the cerebral region in 6 h, while it took 12 h to eliminate from the body (scale bars: 200 μ M). **d** Tissue microtome of zebrafish brain was subjected to HSI imaging. Spectral angular mapping (SAM) images were built from HSI by scanning against the β Cas AuNPs spectral library. SAM and Rule images colored the pixels as red and black, respectively, that have matching spectra of β Cas AuNPs. Zebrafish larvae with β Cas AuNPs in the brain presented black spots in Rule images and red pixels in SAM images. SPR spectra with peak \sim 530 nm were observed in the brain of β Cas AuNPs treated larvae. No such spectra were recorded for control larva (scale bars: darkfield 200 μ M; HSI, SAM, HSI + SAM, Rule: 10 μ M; inset scale bar: 2 μ M; scale bar for β Cas AuNPs: 10 μ M, inset scale bar: 0.5 μ M). **e** ICP MS analysis, where the AuNP concentration was the highest in the larval brain at 0.5 h, i.e., equivalent to 0.6 ± 0.1 ng of Au, and dropped to 0.05 ± 0.01 and 0.02 ± 0.008 ng at 6 and 12 h, respectively ($n = 10$). **f** Dose-response relationship between the amount of AuNPs injected vs. the amount of AuNPs delivered across the brain ($n = 10$). Significantly ($p < 0.05$) increased amount of Au was delivered when intracardiac dose of AuNPs was increased from 1.5 to 3 ng equivalent. However, increasing the dose from 3 to 6 ng did not improve AuNP delivery across the BBB, indicating a dose saturation. Error bars represent the standard deviation. Source data are provided as a Source Data file

deposition of A β amyloids in the brain tissues of zebrafish larvae, but not in β Cas AuNPs-treated or untreated control larvae. The positive controls of fibrillized A β analyzed by IHC and polarized light microscopy were shown in Supplementary Fig. 16.

In addition to the behavioral symptoms, the neurotransmitters associated with A β toxicity and reactive oxygen species (ROS)

were quantified and loss of synaptophysin was imaged (Fig. 6), to vindicate the potency of β Cas AuNPs against the toxicity of A β . Zebrafish are reported to possess cholinergic, glutamatergic and GABAergic neurotransmission that change in response to neurological dysfunction⁴⁴. The acetylcholine esterase (AChE) and glutamate (GLT) levels were therefore assayed in A β and

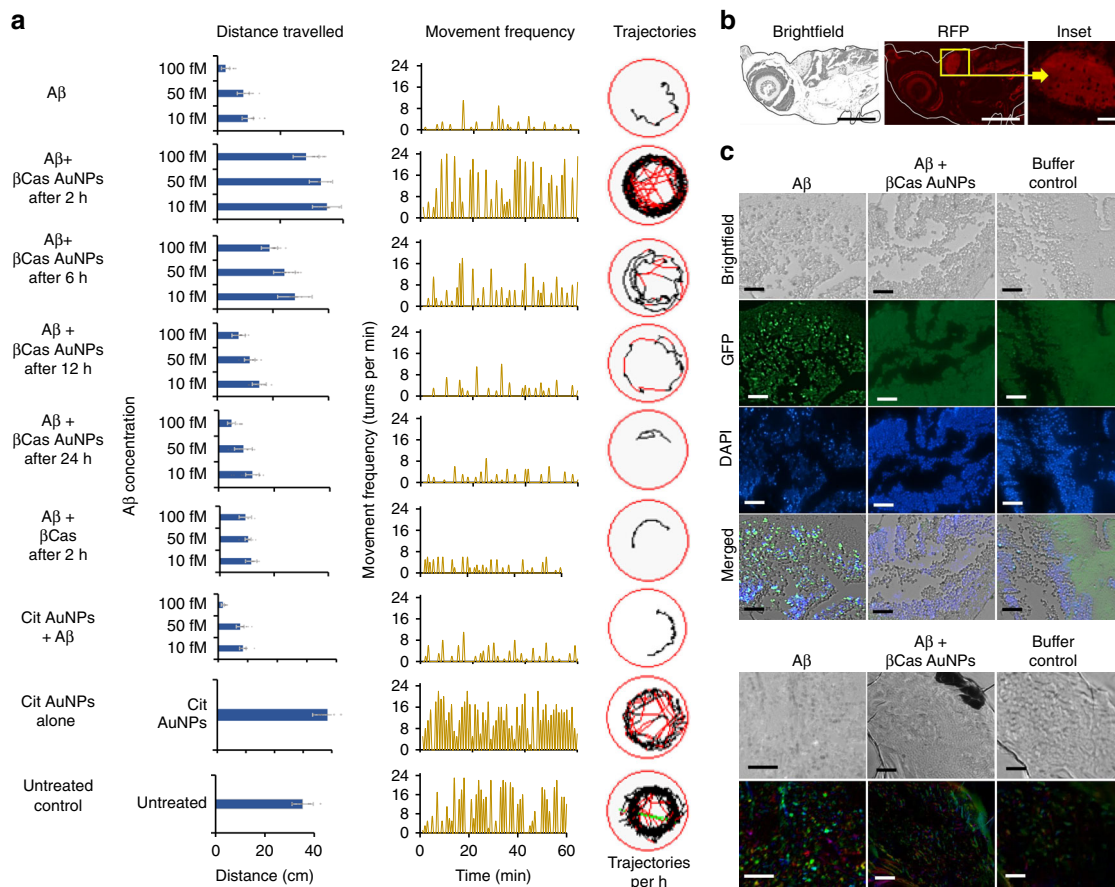


Fig. 5 Mitigation of A β toxicity in zebrafish larvae with β Cas AuNPs. **a** A β peptide was injected into the cerebroventricular space at 10, 50, and 100 fM concentrations ($n = 20$, mean \pm SD). Zebrafish larvae were monitored on an automated behavior monitoring system at fifth day post A β treatment and parameters of total distance traveled, movement frequency and trajectory path were observed for 1 h. Significant ($p < 0.005$) difference in the behavior of the larvae was observed on the fifth day post treatment. β Cas AuNPs injected, via the intracardiac route 2 and 6 h after the A β treatment, rescued the larvae from A β toxicity and from developing Alzheimer's-like symptoms. Representative trajectories of the larvae are displayed in the far-right column. Treating the larvae with β Cas AuNPs, 12 and 24 h post A β treatment, failed to protect the larvae from developing A β toxicity. **b** Zebrafish larvae, treated with β Cas AuNPs 2 h after A β treatment were fixed, sliced and stained with Congo red to image any A β fibrils that could have formed. Tissue slices of the brain section did not present any red fluorescence, indicating no A β fibril formation in β Cas AuNPs treated larvae (scale bars: 200 μ M; inset scale bar: 20 μ M). Furthermore, immunohistochemistry (IHC) (**c**) and polarized light microscopy (apple green birefringence of amyloid) (**d**) revealed deposition of aggregated A β in the larval brain while no A β deposition was observed in β Cas AuNPs or buffer treated larvae (Scale bars: IHC, 30 μ M; polarized light microscopy, 50 μ M). Error bars represent the standard deviation. Source data are provided as a Source Data file

A β + β Cas AuNPs treated larvae. The heads were separated from the euthanized larvae, homogenized and used for the assays to minimize interference from the trunks. The biomarkers were first evaluated on the fifth day post A β (6 fM per larva) treatment. AChE levels in the Alzheimer's affected brain are known to be decreased⁴⁵, however, here no significant differences in the AChE activity (0.10 ± 0.02 a.u. per brain) or GLT level (17.9 ± 1.2 nm per brain) were observed compared to untreated control (0.09 ± 0.01 a.u. for AChE and 19.7 ± 1.2 nm for GLT per brain) with 6 fM A β . As severe cases of Alzheimer's presence increased levels of AChE⁴⁶, the biomarker assay was performed with 600 fM A β and the AChE levels were found to increase twofold compared to the control, i.e., 0.22 ± 0.01 a.u. AChE per brain (Fig. 6a). AChE levels were close to the control in the larvae treated with β Cas AuNPs (3 ng Au equivalent) + A β (600 fM). β Cas AuNPs and β Cas, as controls, did not elicit any impact on the AChE levels. Similar results were observed for GLT, where β Cas AuNPs reduced the GLT level from 29.2 ± 5.08 to 18.1 ± 3.07 nm per brain (Fig. 6b). According to the literature, the A β_{42} concentration in the gray and white matter of the brain of AD patients is 1.3 and 0.25 nm mg⁻¹, respectively⁴⁷. Tg2576 mice models of AD present

~ 1600 – 1700 fM mg⁻¹ of A β_{42} after developing the disease symptoms⁴⁸. However, the wet weight of whole zebrafish larva is ~ 1 mg and its brain is 4–5 times smaller than its body weight⁴⁹. Considering this physiological relevance of the body weight, A β_{42} was injected in zebrafish larvae over a concentration range of 0.07–1200 fM per larva and based on locomotor response (Fig. 3c) 100 fM was selected for further experiments. However, 100 fM of A β concentration did not produce any difference in neurotransmitter levels that are usually disturbed in severe cases of AD⁴⁶. Therefore, A β of 600 fM was used to observe any possible fluctuations in neurotransmitters.

ROS generation was quantified by a direct measurement of dichlorofluorescein diacetate (DCF) fluorescence from the larval brain (Fig. 6c, d). It has been shown in literature that oligomeric amyloid proteins directly interacted with cell membranes to induce cytotoxicity by membrane disruption and subsequent ROS generation¹⁴. A β were injected in the cerebroventricular space and their associated toxicity was determined by ROS generation, in comparison with the positive control of H₂O₂. The samples were mixed with DCF prior to microinjection in larvae. The corrected total (CT) fluorescence from H₂O₂ and A β treated

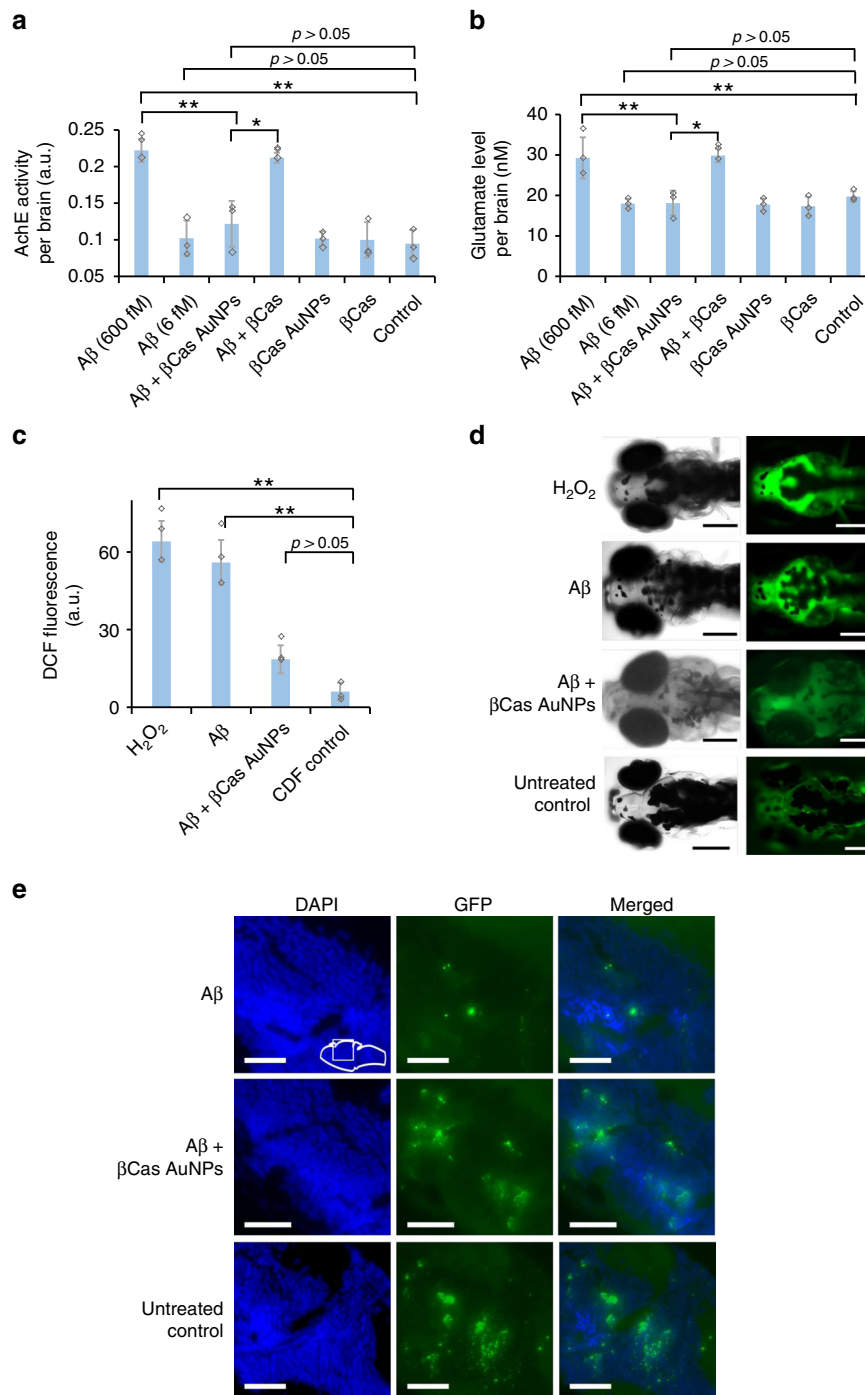


Fig. 6 Neurotransmitters and reactive oxygen species (ROS) in the brain of Aβ treated larvae. The biomarkers were measured 5 days post Aβ treatment. Cerebrovascular injection of Aβ peptide at 6 fM per larvae did not significantly ($p > 0.05$) influence the AchE (**a**) and GLT (**b**) levels in zebrafish larvae ($n = 10$). However, increasing the Aβ dose to 600 fM significantly increased ($p < 0.05$) AchE and GLT levels. βCas AuNPs (3 ng Au per 4.5 ng βCas), injected 2 h post Aβ treatment, significantly ($p < 0.005$) reduced the AchE and GLT levels on the 5th day post Aβ (600 fM) treatment. βCas micelles (dose equivalent to βCas in 3 ng βCas AuNPs), in comparison, failed to improve ($p > 0.05$) the biomarker levels. **c** ROS generation was significantly ($p < 0.005$) high in Aβ treated (600 fM) larvae. ROS generation was suppressed in βCas AuNPs treated larvae and close to control ($n = 10$). **d** Representative images of zebrafish larvae expressing DCF/ROS fluorescence when treated with H₂O₂, Aβ, Aβ + βCas AuNPs, and Aβ + βCas. **e** Larvae's brain sections were stained for synaptophysin. Aβ-treated larvae presented loss of synaptophysin indicating neurodegeneration. Scale bars in all images are 200 μM. Error bars represent the standard deviation. Source data are provided as a Source Data file

larvae were 41 ± 7.9 and 42.9 ± 8.7 , respectively. However, upon treatment with βCas AuNPs, the CT fluorescence was reduced to 14.5 ± 5.4 , comparable to DCF as negative control (8 ± 3.4). Synaptophysin-based neurodegeneration, an indicator for neuronal synapsis, was also imaged via immunostaining (Fig. 6e). Aβ

treated larvae presented a significant loss of synaptophysin as compared to Aβ + βCas AuNPs or untreated control larvae.

In this study, zebrafish larvae is developed and used as a simple, in vivo visual model to study Aβ fibrillization, toxicity, behavioral pathology, neurodegeneration, and biodistribution

and nano-chaperone activity of β Cas AuNPs. These advantages can be employed to screen or study the efficacy, pharmacokinetics and pharmacology of anti-Alzheimer's drugs, specifically nano-chaperone based therapeutic modalities. However, despite possessing a vertebrate nervous system, zebrafish larvae still develop cognitive and learning functions. Therefore, the behavioral pathology observed in this study may not be clinically equivalent to Alzheimer's symptoms. To study the $A\beta$ toxicity and chaperone activity of β Cas AuNPs, adult zebrafish was employed to offer a more advanced in vivo model with a cognitive capacity⁵⁰. Microinjection of $A\beta$ (1 μ L, 50 μ M) in adult zebrafish produced behavioral toxicity (Fig. 7a, b), $A\beta$ aggregation in brain (Fig. 7c) and clinically relevant Alzheimer's-like symptoms (Fig. 7d–f). Retro-orbital microinjection (1 μ L, 0.5 mM) of β Cas AuNPs, 2 h post $A\beta$ treatment, rescued the adult zebrafish from developing the cognitive dysfunction.

Expression of human orthologues of $A\beta$ -associated neuronal machinery at 24 h post fertilization suggests suitability of zebrafish for AD modeling^{39,51}. Macro-organization of the brain and cellular morphology of zebrafish are parallel to vertebrates and have led to studies of neurobehavioral pharmacology and stress-induced behavior⁵². In addition, exogenous microinjection or genetic overexpression of Tau in zebrafish has resulted in intracellular tangle formation and abnormalities in the animal's development and swimming behaviors^{53,54}. These, together with our observations, support $A\beta$ toxicity induction and AD modeling in zebrafish, especially within the context of cerebral deposition of $A\beta$ and their associated behavioral pathology.

Intracardiac injection of β Cas AuNPs mitigated the toxicity of cerebroventricularly injected $A\beta_{42}$ in a new, high-throughput zebrafish model. This remarkable capacity of eliminating toxic $A\beta$ and rescuing the animal from AD-like symptoms was evidenced by in vitro assays of ThT, CD, and TEM, in silico

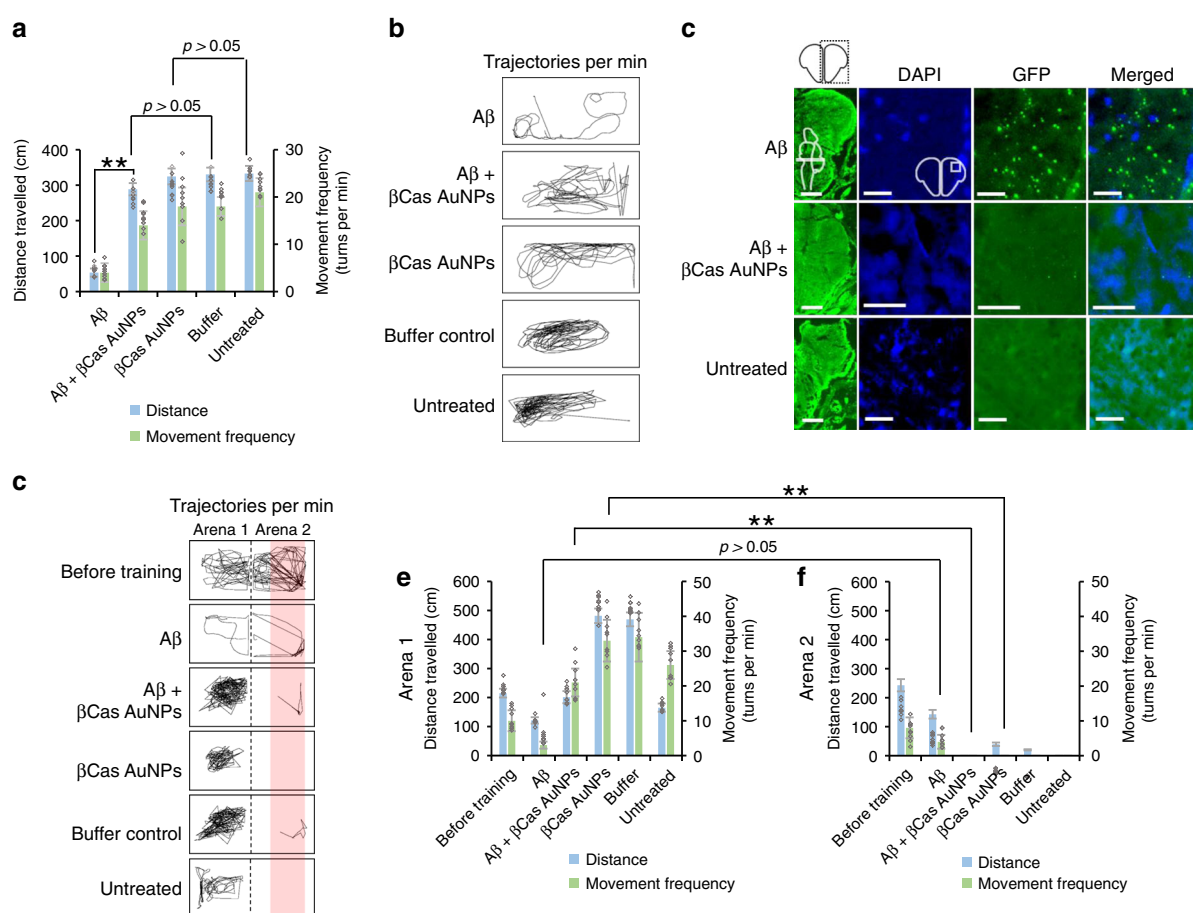


Fig. 7 Mitigation of $A\beta$ toxicity and Alzheimer's-like symptoms in adult zebrafish with β Cas AuNPs. **a** Adult zebrafish (10 months old) were microinjected (cerebroventricular) with $A\beta$ (1 μ L, 50 μ M) and observed for behavioral pathologies at 2 weeks post injection ($n = 4$, SD \pm mean). To study the mitigation with β Cas AuNPs, β Cas AuNPs were microinjected (retro-orbital, 1 μ L, 0.5 mM) 2 h prior to $A\beta$ treatment. $A\beta$ induced significant reduction in total distance traveled and movement frequency in adult zebrafish while β Cas AuNPs were able to rescue the symptoms. Movement trajectories are presented in **(b)**. Observations were made for 1 min, three times at a 2 h interval for each fish ($n = 3$). **c** IHC was performed on adult zebrafish brain sections to image the $A\beta$ deposition. The first column represents the right cerebral brain of adult zebrafish in the GFP channel (Scale bars: 200 μ M). DAPI, GFP, and merged images at higher magnifications revealed $A\beta$ plaque deposition in $A\beta$ treated but not in $A\beta + \beta$ Cas AuNPs, or untreated control (Scale bars: 20 μ M). Furthermore, cognitive behavior of adult zebrafish was analyzed. **f** Zebrafish were trained to avoid swimming into the right half (Arena 2) of the swimming tank (1 L) that was labeled red and attached with a source of electric shock (9 V) (Supplementary Fig. 17). After training, the electric source was removed and the cognitive memory of the fish to remain in arena 1 and to avoid arena 2 was assessed for a period of 2 min ($n = 3$, three times for each fish at a 2 h interval). The movement trajectories of the fish in arena 1 vs. arena 2 are presented in panel F. Comparative analysis of distance traveled and movement frequency of the fish in arena 1 **(d)** vs. arena 2 **(e)** revealed cognitive dysfunction of $A\beta$ -treated fish that were unable to avoid arena 2. However, β Cas AuNPs treated, buffer control and untreated control fish were able to avoid swimming into arena 2. Error bars represent the standard deviation. Source data are provided as a Source Data file

examination of A β - β Cas AuNP binding, ex vivo assays of microtome, HSI, ICP-MS, and MALDI analyses of A β impaired zebrafish brain, in vivo assays of ROS, behavior and neurological dysfunction biomarkers of zebrafish larvae, and cognition of adult zebrafish. The binding between A β and β Cas was mediated by nonspecific interactions via the residues of the whey protein that were free from engagement with the AuNP surface. Adsorption of β Cas onto an AuNP surface enabled trafficking of β Cas across the zebrafish larvae BBB where β Cas was then capable of efficiently binding A β for elimination. Furthermore, β Cas AuNPs induced no harmful effects on the development of healthy zebrafish, owing to the biocompatibility of both the chaperone-like protein and the AuNPs. As this nano-formulation meets all key criteria for a potent in vivo amyloidosis inhibitor, it holds the promise to be further developed into safe-to-use, preventative nanomedicines against the pathologies of AD and other debilitating human amyloid diseases. Although zebrafish lack advanced cognitive capacity as possessed by rodents, they can serve as a robust, economic, and high-throughput alternative to complement neurological mouse models that are no longer deemed sufficient¹⁹.

Methods

Animal husbandry and ethics statement. The AB wild-type zebrafish (*Danio rerio*) was maintained in a fish breeding circulatory system (Haisheng, Shanghai, China) at 28 \pm 0.5 °C with a 14 h light: 10 h dark cycle. The embryos were produced by adult spawning. For spawning, two pairs of male and female were placed in tank with shallow water. The male and female were separated by a removable partition and kept overnight. The spawning was triggered by removing the partition with first light in the morning and embryos were collected 2 h later, washed with 0.5 ppm methylene blue and placed in petri dish with Holtfreter's buffer. The healthy embryos at the same developmental stage were selected and developed to 5-day-old larvae for further measurement. All the experiments were performed with larvae in Holtfreter's buffer⁵⁵. Tricaine (0.4% in Holtfreter's buffer) was used for anesthesia. When required, larvae were euthanized by placing in 0.01% tricaine in Holtfreter's buffer for 10 min that was pre-chilled at 4 °C. The excision of head from the trunk was performed under an optical stereomicroscope with a sharp surgical blade. All zebrafish experiments were performed in accordance to the ethical guidelines of Tongji University and the protocols were approved by the Animal Center of Tongji University (Protocol #TJLAC-019-113). All in vitro and other experiments were performed in compliance with the relevant ethics, laws, and institutional guidelines of Monash University Occupational Health & Safety.

Synthesis of β Cas AuNPs. HAuCl₄ (1 mM, PBS pH 6) was heated at 40 °C and while stirring at 1000 rpm, added with an equal volume of β Cas in PBS (pH 6) at the concentration of 1 mg mL⁻¹. The heating continued for 10 min and 100 μ L of 0.5 mM NaBH₄ was added dropwise to the reaction mixture. The solution turned wine red indicating the formation of β Cas AuNPs. Heating was stopped after 2 h while the reaction was kept on stirring for overnight. β Cas AuNPs were purified by centrifugal filtration with 100 kDa spin filters. AuNPs were washed 3 \times with deionized water, transferred to PBS (pH 7.4) and stored at 4 °C for further experimentation. After purification, the β Cas concentration in β Cas AuNPs was determined by the BCA method⁵⁶. Citrate-capped AuNPs (Cit AuNPs) were synthesized via a reported method⁵⁷. Briefly, a solution of HAuCl₄ (10 mL, 1 mM) was brought to boiling at 120 °C and then 1 mL (10 mM) trisodium citrate was added dropwise. The solution turned to wine red, indicating the formation of Cit AuNPs. The solution was brought to room temperature, purified via centrifugal washing with deionized water and stored in dark for further use.

Neutral red (NR) dye was conjugated to β Cas and β Cas AuNPs for tracing their biodistributions in zebrafish larvae. The dye was conjugated via 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling. Briefly, 2 mL of 6.25 μ M of β Cas or equivalent concentration of β Cas AuNPs was stirred overnight with 18 μ M of EDC, 23 μ M of N-hydroxysuccinimide (NHS), and 14 μ M of the NR dye. The NR-conjugated β Cas or β Cas AuNPs were purified via centrifugal filtration, transferred to PBS (pH 7.4) and stored for in vivo assays.

ThT kinetic assay. A β was treated with hexafluoro-2-propanol (HFIP) to break-down pre-existing small aggregates. Specifically, 0.5 mg of A β (Anaspec Inc., purity \geq 95%) was dissolved in 500 μ L of HFIP, incubated for 3 h, aliquoted to different concentrations and dried to evaporate the HFIP. The dried A β was dissolved in 0.01% NH₄OH for dissolution purpose and left in the open for 20 min to evaporate NH₄OH, leaving behind the aqueous solution of A β that was used for further experiments. For the thioflavin T (ThT) assay, a 50 μ L aqueous solution of 50 μ M A β and 100 μ M of ThT were incubated with or without β Cas (6.25 μ M) or equivalent concentration of β Cas AuNPs in a 96-well plate. The ThT fluorescence

was recorded with excitation at 445 nm and emission at 488 nm, at 30 min intervals for 48 h.

Transmission electron microscopy. A β was mixed with β Cas or β Cas AuNPs in the same ratio as for the ThT assay and incubated for 48 h. After incubation, a drop of each sample was placed on a glow discharged, carbon coated copper grid and blotted after 1 min. The sample-coated grid was negatively stained with 1% uranyl acetate that was blotted after 30 s. The grid was dried in vacuum and visualized with a Technai F20 transmission electron microscope operated at a voltage of 200 kV.

CD spectroscopy, hydrodynamic size, and zeta potential. The secondary structural contents of β Cas, β Cas AuNPs, A β m, A β o, A β fibrillized w/o β Cas or β Cas AuNPs were determined by CD spectroscopy. In all, 200 μ L of the sample was pipetted into a CD cuvette and the concentration of A β was 100 μ M while β Cas and β Cas AuNPs were 12.5 μ M β Cas equivalent in all CD measurements. The incubation time was 48 h at 37 °C and the incubation medium was deionized water. CD spectra were recorded from 190 to 240 nm with a 1 nm step size. The acquired data were presented in the unit of millidegrees (mdeg). Percentage secondary structure contents were determined by analyzing the CD data via Dichroweb with Contin/reference set 4⁵⁸. The hydrodynamic diameter and zeta potential of the AuNPs were measured with dynamic light scattering under ambient conditions (Malvern Instruments). The concentrations and incubation conditions were the same as for the CD experiments.

Binding capacity (BCA, TGA, TEM, CD, UV-SPR, and fluorescence microscopy). The binding capacity between A β and β Cas or β Cas AuNPs was determined via a BCA assay⁵⁶ and TGA. Briefly, 6.25 μ M of β Cas or equivalent β Cas AuNPs were incubated with varying concentrations of A β m (monomers) or A β o (oligomers) ranging from 2 to 1137 μ M. Incubation time was 48 h at 37 °C in deionized water. HFIP-treated A β was considered as A β m while A β m incubated in water for 12 h at 4 °C was considered as A β o. After incubation, the samples were centrifuged at 17,300 \times g for 30 min and the pellets were redispersed in 10 μ L of deionized water and subjected to BCA protein content quantification. The BCA binding efficacy was presented in terms of the amount of A β bound to β Cas or β Cas AuNPs. For TGA analysis, the pellet dispersed in 10 μ L water was placed as a drop on a platinum pan. The samples were held at 80 °C for 30 min and then scanned from 80 to 800 °C at a scanning rate of 10 °C min⁻¹ under a constant flow of nitrogen of 1 mg mL⁻¹. For A β m and A β o, 10 μ L of 1 mg mL⁻¹ of the peptide was placed on a TGA pan and scanned under the same conditions.

To assess binding affinity, β Cas AuNPs or NR- β Cas AuNPs (12.5 μ M β Cas equivalent) were incubated with preformed A β o or A β m (100 μ M) for 3 h at 37 °C and unbound A β m/o was removed via centrifugal washing thrice (25,000 \times g for 10 min at 4 °C). CD and TEM were performed as described above in the respective sections. UV-SPR for β Cas AuNPs and fluorescence spectra (NR- β Cas AuNPs, excitation at 470 nm) were recorded with a microplate reader.

Cellular toxicity. SH-SY5Y (ATCC® CRL-2266™) human bone marrow neuroblastoma cells were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) with 10% fetal bovine serum (FBS). A 96-well plate (Costar black/clear bottom) was coated with 70 μ L of poly-L-lysine (Sigma, 0.01%) and incubated at 37 °C for 30 min. After removing poly-L-lysine, the wells were washed by PBS thrice. Cells (~50,000 cells per well per 200 μ L medium) were added to the wells and incubated at 37 °C with 5% CO₂ for 24 h to reach ~70–80% of confluency. The cell culture medium was then refreshed with 1 μ M propidium (PI) dye in DMEM/F12 with 10% FBS and incubated for another 30 min. A β was freshly dissolved in 0.005% NH₄OH buffer, in the presence or absence of β Cas AuNPs and added to the wells with final concentration of 20 and 50 μ M for A β and β Cas AuNPs, respectively. Cellular toxicity was recorded by Operetta (PerkinElmer, 20 \times PlanApo microscope objective, numerical aperture: 0.7) in a live cell chamber (37 °C, 5% CO₂) after 15 h of treatment. The percentage of dead cells (PI-positive) to total cell count was determined by a built-in bright-field mapping function of Harmony High-Content Imaging and Analysis software (PerkinElmer). The measurement was performed in triplicate and conducted at five reads per well. Untreated cells were recorded as control.

Helium ion microscopy. SH-SY5Y neuronal cells were incubated with A β in the presence or absence of β Cas AuNPs as described for the cellular toxicity assay. The incubation was performed for 2 h at 37 °C and then stabilized by 2.5% paraformaldehyde. The samples were incubated at 4 °C overnight. The paraformaldehyde/medium was replaced with gradient concentrations of ethanol in the five steps of 20%, 40%, 60%, 80%, and 95%, respectively, with ~2 h of rest time at each gradient. In all, 30 μ L suspension of cells was air-dried on a carbon tape and the morphologies of the cells were visualized by HIM (Orion NanoFab, Zeiss, USA). Untreated cells were used as control.

Microinjection of A β , β Cas, β Cas AuNPs, and Cit AuNPs in zebrafish larvae. HFIP-treated A β (10 μ g) was dissolved in PBS (pH 7.4) to make a stock solution of

100 μM . Dilutions of 0.07–1200 fM of A β per 5 nL were made in PBS and injected (5 nL injection volume) into the cerebroventricular space of 5 days old zebrafish larvae. PBS alone was used as negative control. For microinjection, zebrafish larvae were anesthetized by adding 2 drops of 0.4% tricaine in petri dish and waited until the larvae stopped moving in response to tapping on the table. The larvae were positioned on a 1% agarose gel plate and microinjected with A β peptide. Microinjections were performed with a fine calibrated needle of a pneumatic microinjection system (PV830 Pneumatic Picopump, WPI) operated under 20 psi of injection pressure. The tip of the glass capillary needle was inserted in the ventricular space, across the dorsal soft skin tissue. The tip was ensured not to penetrate more than 0.1–0.3 mm across the center meeting point of left and right telencephalon (Supplementary Video 4). βCas and βCas AuNPs were administered under similar conditions via intracardiac microinjection (Supplementary Video 5). The original as-synthesized βCas AuNPs solution contained 1 and 1.5 ng of Au and βCas per 5 nL. The original βCas AuNPs solution was concentrated 3 \times and redispersed in PBS. A 5 nL of this solution was microinjected into zebrafish larvae via the intracardiac route and each 5 nL contained 3 ng of Au per 4.5 ng (37.5 μM mL $^{-1}$) of βCas in the form of βCas AuNPs. βCas solution of equivalent concentration was prepared in PBS for microinjection. For dose dependent delivery of βCas AuNPs in cerebral tissues, the original βCas AuNPs solution was concentrated to 1.5, 3, and 6 \times , and dispersed in PBS prior to intracardiac microinjection to zebrafish larvae. Cit AuNPs were concentrated to equal concentration as βCas AuNPs and microinjected into the larvae with the same protocol.

Zebrafish larvae behavioral pathology. Larval response to tapping stimuli in a 96-well plate was observed. The 96-well plate was tapped gently at the rate of 1 per sec and the larvae unable to move after five consecutive stimuli were counted as nonresponsive. Furthermore, the larvae losing their horizontal swimming position at higher doses of A β were also counted and the percentage of the larvae losing response to stimuli and their swimming position was calculated. The swimming behavior of zebrafish larvae was observed with an automated zebrafish behavior recording system ZebraBox (Viewpoint) and characterized in terms of total distance traveled by the larvae in a 96-well plate and range of the movement that was >90°, clockwise or counter clockwise, were counted. Representative trajectories of the movement were also recorded by built-in sensors. The observation period was 1 h. The number of larvae in each group was 20 and 3 groups were used for each sample. The larvae treated with βCas or βCas AuNPs, 2 h after A β treatment, were monitored for behavioral pathology with the same method.

Fluorescence imaging, IHC, and polarized light microscopy. Whole mount larval imaging was performed under the brightfield (BF) and RFP channels of a stereomicroscope (Olympus MVX10). For imaging of A β in the cerebral region, the A β treated larvae (100 fM, cerebroventricular microinjection) was microinjected with 100 fM of Congo red on the third and fifth day post A β treatment. The larvae were placed in Holtfreter's buffer for 6 h to allow staining of the amyloids. The larvae were anesthetized immediately prior to imaging with 0.4% tricaine, and positioned in dorsal or lateral view in a drop of 1% low-melting agarose gel. The cerebral region, fin and mid-vascular region of the larvae were imaged with the same method. The biodistribution of NR-conjugated βCas or βCas AuNPs was imaged by following the same method 0.5, 6, and 12 h post- βCas or βCas AuNPs treatment via intracardiac microinjection. The microinjection volume was 5 nL with a dose of 4.5 ng for βCas (37.5 μM) or βCas AuNPs equivalent to 4.5 ng βCas .

For Congo red staining of the sliced sections of the zebrafish larvae, A β (100 fM) treated larvae were first fixed in 2.5% paraformaldehyde for 12 h at 4 °C. Microtome slices of zebrafish larvae were prepared by embedding the larvae into paraffin. The paraffin-embedded larvae were cut into thin slices of 5 μM via microtome and slices were placed in a hot water bath (40 °C) for removal of any wrinkles. The slices were then mounted on glass slides and dried. Slide mounted slices were dewaxed by treating with (1) xylene for 2 h, (2) absolute ethanol for 15 min, and (3) 75% ethanol for 5 min and then rinsed with deionized water. The slices were treated with 0.5% Congo red stain in 50% ethanol for 20 min, rinsed with water, differentiated with 1% NaOH solution in 50% ethanol (5–10 dips) and again rinsed with water. The slices were then dehydrated with 95% ethanol (3 min) and 2 dips in 100% ethanol (each 3 min) and cleared with 2 dips in xylene (each 3 min). The samples were finally sealed with neutral gum and imaged with an optical microscope.

For IHC, 5 μM thick sections of zebrafish larvae were mounted on glass slides as for Congo red staining. The dried sections were washed in PBS (pH 7.4) and TritonX-100 (0.05% in PBS) for 5 min each. A drop (50 μL , 2 μg mL $^{-1}$) of primary antibody (Anti-amyloid β 42, mouse monoclonal, Anaspec, AS-55922) was placed on each section on the glass slides and incubated at 4 °C overnight. Primary antibodies were washed away from the sections by dipping in PBS and TritonX-100 (0.05% in PBS) for 5 min. Sections were incubated with a drop (50 μL , 2 μg mL $^{-1}$) of secondary antibody (Goat anti-mouse HiLyte™ Fluor 488—labeled, Anaspec, AS-61057-05-H488) for 6 h at room temperature. The secondary antibodies were washed away by dipping the slides in TritonX-100 (0.05% in PBS) for 5 min. Slides were dried, mounted with a cover slip using a drop of 50% glycerol and imaged under a fluorescence microscope (Nikon Ti-Eclipse). The larvae's brain sections were immunostained for synaptophysin with the same method using primary (50 μL , 2 μg mL $^{-1}$ of anti-Synaptophysin antibody, abcam, Cat# ab32594) and

secondary antibodies (50 μL , 2 μg mL $^{-1}$ of Goat Anti-Rabbit IgG H&L, Alexa Fluor® 488, abcam, Cat# ab150081).

Polarized light microscopy was performed on the Congo red-stained larvae tissue sections. The slides were imaged for birefringence under an Abrio polarization microscope. A drop (50 μL , 20 μM) of fibrillized A β was placed and dried on a glass slide. The dried sample was processed same as for larva tissue sections for immunostaining and polarized light microscopy and used as control.

Darkfield HSI. Zebrafish larvae at 0.5 h post treatment with intracardiac microinjection of βCas AuNP (3 ng Au per 4.5 ng βCas equivalent) were euthanized and fixed in 2.5% paraformaldehyde for 12 h and then sliced to thin sections, dehydrated and mounted on glass slides with the same procedure as described for fluorescence microscopy. HSI was performed with a CytoViva darkfield microscope equipped with a pixelFly CCD camera. ENVI 4.8 software was used to capture and process the images and to acquire AuNPs spectra. The darkfield images were captured and then scanned for HSI for βCas AuNPs treated larvae, untreated control larvae and βCas AuNPs alone. βCas AuNPs alone as control were used to acquire the spectral library of AuNPs by selecting ~1500 pixels with region of interest function of ENVI 4.8. Spectral libraries obtained from βCas AuNPs and a mean spectral signature was generated. The mean spectral signature of βCas AuNPs was used to filter against the selected pixels (~1000) from βCas AuNPs treated and untreated control larval images to generate spectral angular mapping images. Rule images were obtained by matching the pixel spectra from βCas AuNPs against βCas AuNPs or untreated larval images. Rule images darkened the pixels with matching spectra of βCas AuNPs. All images were normalized against lamp spectra. For HSI imaging of βCas AuNPs and βCas AuNPs incubated with A β , the AuNPs were incubated with A β at the same ratio as for ThT for 48 h. A drop of each sample was placed on a glass slide covered with a slip. Darkfield images and SPR spectra of the AuNPs were obtained by scanning ~1500 pixels for each sample.

TEM imaging of brain tissues. For TEM analysis of tissues, zebrafish larvae were injected with 3 ng Au per 4.5 ng βCas equivalent βCas AuNPs via intracardiac microinjection as described above, euthanized and fixed in 2.5% glutaraldehyde 0.5 h post treatment. The larvae were treated with 1% osmium tetroxide for 4 h (4 °C) and then washed three times with 0.1 M PBS buffer (pH 7.4), 15 min each. The larvae were then treated with 1% citrate in 0.1 M PBS buffer (pH 7.4, 20 °C) for 2 h and again washed three times with 0.1 M PBS (pH 7.4), at 15 min each. After that the larvae were dehydrated by treating with 50, 60, 70, 80, 90, and 100% ethanol, at 15 min each. Following that the larvae were treated with acetone: 812 embedding agent (1:1) and then with 812 embedding agent, both for overnight and then baked at 60 °C for 4 h for polymerization of embedding resin. The larvae were finally sliced into ultra-thin sections of 60–80 nm with Diatome ultra 45°. The sections were double stained with 2% solutions of uranyl acetate and lead citrate, 15 min each, dried overnight and then imaged with a transmission electron microscope (Technei G2 20 TWIN) operated at 80 kV.

Inductively coupled plasma-mass spectrometry. Delivery of βCas AuNPs across the larvae's brain was quantified with ICP-MS analysis. Zebrafish larvae were microinjected with 1.3, 3, and 6 ng Au equivalent βCas AuNPs via the intracardiac route with the same method as described above. The larvae were euthanized 0.5, 6, and 12 h post microinjection and their heads were excised and homogenized in PBS (pH 7.4) in a Teflon-glass homogenizer (70 Hz for 1 min). Brain or trunk homogenate was made up to 1 mL with PBS (pH 7.4), added with 9 mL of 68% HNO $_3$ and digested by stepwise heating at 100, 150, 170, and 190 °C for 30, 30, 30, and 90 min, respectively. The dried and digested layer was dissolved in 1 mL of 4% HNO $_3$ and analyzed with ICP-MS (Agilent 7700) for quantification of Au. The instrument was operated under 0.75 MPa Ar pressure and a standard calibration was made with Au spiked PBS samples, digested in the same way as the larval samples. Untreated larvae and PBS alone were used as negative controls. The number of larvae in each group was 20 and 3 groups per sample were used for analysis.

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI TOF MS). Saturated solution of sinapinic acid (SA) was prepared in ethanol and 1 μL of the sample was dried on a ground steel MALDI plate. Another saturated solution of SA was prepared in acetonitrile and trifluoroacetic acid (30:70, v/v) and mixed with the zebrafish larvae head homogenate at a 1:1 ratio. In all, 0.5 μL of this mixture was applied to previously dried SA layer. The dried layer was analyzed by a Bruker ultraflexxtreme MALDI-TOF/TOF in the linear positive mode. The instrument was calibrated using protein calibration standards I and II. A total of 8000 shots were gathered across the sample spots using Flexcontrol software (3.4) in the range of 1–20 kDa. The acquired spectra were processed by baseline subtraction and peak picking using Flexanalysis software (3.4).

Biomarkers and ROS assay. Acetylcholine esterase (AChE) and glutamate (GLT) levels were measured as biomarkers for neurodegeneration. Briefly, zebrafish larvae treated with A β , A β + βCas , and A β + βCas AuNPs were euthanized and their heads were excised from the trunks. The heads were homogenized in PBS (pH 7.4)

and AchE and GLT levels were estimated using assay kits according to reported literature^{59,60}.

For ROS assay, A β (100 fM) were injected to the cerebroventricular space of zebrafish larvae and 2',7'-DCF (5 nL of 2 μ M) was injected 5 days post A β treatment. The whole mount larvae were imaged under the green fluorescence protein channel of an optical microscope 1 h after DCF treatment. For β Cas AuNPs, the nanoparticles were injected 2 h post A β treatment followed by the same procedure. H₂O₂ (5 nL of 0.1%) was used as positive control and injected 3 h prior to DCF microinjection in positive control larvae. DCF fluorescence was quantified by excising the head from the trunk of euthanized larvae, homogenizing in PBS buffer (50 μ L, pH 7.4) and reading the DCF fluorescence with a microplate reader with excitation/emission at 495 nm/529 nm.

Microinjection in adult zebrafish. Adult zebrafish at the age of 10 mth were used for the cognition experiment. The fish was maintained, before and during the experiment, as described in the “Animal husbandry and ethics statement” section. For microinjection, adult fish were anesthetized with ice chilled tricaine (0.01% in Holtfreter's buffer for 20 s). Cerebroventricular microinjection of A β (1 μ L, 50 μ M) was performed via 1 μ L Hamilton glass syringes. A β peptide was injected in between the right and left telencephalon and the needles did not penetrate more than 1 mm (Supplementary Video 6). The fish were held in place via a forcep. The syringes were washed with 70% ethanol and 1 \times PBS twice, in between the injections. For the group with A β with β Cas AuNPs, β Cas AuNPs were injected 2 h prior to the injection of A β . β Cas AuNPs (1 μ L, 0.5 mM, 20 psi injection pressure) were slowly introduced into the systemic circulation of the fish via retro-orbital microinjection (7 o'clock position), using a sharp glass capillary needle (Supplementary Video 7). The fish was placed back into the tank for recovery. The adult zebrafish were grouped ($n = 3$) and injected with A β , A β with β Cas AuNPs, β Cas AuNPs alone and PBS. Buffer injected and untreated fish were considered as controls.

Behavioral pathology and cognitive function test of adult zebrafish. The adult zebrafish microinjected with the above described samples were monitored on a daily basis for any apparent change to their swimming activity. The swimming activity of the fish started to change 1-week post treatment and became significantly apparent at 2 weeks post treatment. The behavioral pathology of the fish was recorded with ZebraBox (Viewpoint), using a 1 L fish tank, and characterized for total distance traveled and movement frequency. The recording was performed for 1 min, 3 times for each fish at a 2-h interval.

Cognitive function test was performed by hypothetically dividing the 1 L fish tank into two halves, i.e., arenas 1 and 2 (Supplementary Fig. 17). A red colored paper was attached to the bottom of the tank to associate a color with arena 2. Fish were allowed to freely swim in the whole tank for 30 min and then trained for 20 min to avoid swimming into arena 2 by using an electric shock punishment. Whenever the fish swam into the red arena 2, it was punished by dipping the electrodes of 9 V in electric potential. After 20 min of training, the electric source was removed and the cognitive ability of the fish to avoid arena 2, while swimming in arena 1 was recorded for 2 min. The comparative distance traveled and movement frequency of the fish in arena 1 vs. 2 were recorded simultaneously. The recordings were made 3 times for each fish ($n = 3$) at 2 h intervals. The results were analyzed via EthnoVision X1. The analysis parameters were as follows; animal: adult zebrafish, arena: open field square template (divided into two-halves for the cognitive function test), tracking feature: central point, sample rate: 5 per second, detection setting level: sensitive enough to track the fish in the whole tank, threshold for movement frequency: 50° turn (clockwise or counter clockwise) and minimum 0.5 cm of travel.

IHC was performed on adult zebrafish brain sections. Adult zebrafish at 2 weeks post A β or A β + β Cas AuNPs treatment were euthanized by placing the animals in ice chilled tricaine (1% in Holtfreter's buffer for 1 min). The heads were separated from the bodies, at pectoral fin, with a sharp scalpel and fixed in 2.5% paraformaldehyde overnight. The heads were treated with 20% sucrose overnight, fixed in Tissue Trek OCT mounting medium at -20 °C and sectioned into 20 μ M thick sections via cryostat. The sections were mounted on gelatinized glass slides, immunostained for A β as described above in the “Fluorescence imaging, IHC, and polarized light microscopy” section and imaged via a fluorescence microscope.

Statistical analysis. All the experiments in the manuscript were repeated three times, unless specified, and data was presented as mean \pm SD. For the zebrafish experiments, 20 larvae per group and 3 group per sample were used to minimize experimental error. The significance of results was determined by one-way ANOVA followed by Turkey's test and p values less than 0.05 were considered significant (presented with * in figures) while p values less than 0.005 were considered as highly significant (presented with ** in figures).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Data supporting the findings of this paper are available from the corresponding authors upon reasonable request. Reporting summary of this article is available as Supplementary Information file. The raw data underlying the respective main text (Figs. 1–7) and Supplementary figures (Supplementary Figs. 1, 2, 8, 13, 14 and 15) are provided as Source Data File.

Received: 10 December 2018 Accepted: 4 August 2019

Published online: 22 August 2019

References

- Knowles, T. P., Vendruscolo, M. & Dobson, C. M. The amyloid state and its association with protein misfolding diseases. *Nat. Rev. Mol. Cell Biol.* **15**, 384 (2014).
- Hardy, J. A. & Higgins, G. A. Alzheimer's disease: the amyloid cascade hypothesis. *Science* **256**, 184–186 (1992).
- Sakono, M. & Zako, T. Amyloid oligomers: formation and toxicity of A β oligomers. *FEBS J.* **277**, 1348–1358 (2010).
- Benilova, I., Karran, E. & De Strooper, B. The toxic A β oligomer and Alzheimer's disease: an emperor in need of clothes. *Nat. Neurosci.* **15**, 349–357 (2012).
- Cummings, J. et al. Drug development in Alzheimer's disease: the path to 2025. *Alzheimers Res. Ther.* **8**, 39 (2016).
- Javed, I. et al. Probing the aggregation and immune response of human islet amyloid polypeptides with ligand-stabilized gold nanoparticles. *ACS Appl. Mater. Interfaces* **11**, 10462–10471 (2019).
- Rogers, J., Strohmeier, R., Kovelowski, C. & Li, R. Microglia and inflammatory mechanisms in the clearance of amyloid β peptide. *Glia* **40**, 260–269 (2002).
- Marsh, S. E. et al. The adaptive immune system restrains Alzheimer's disease pathogenesis by modulating microglial function. *Proc. Natl Acad. Sci. USA* **113**, E1316–E1325 (2016).
- What Causes Alzheimer's Disease? <https://www.nia.nih.gov/health/what-causes-alzheimers-disease> (2017).
- Peric, A. & Annaert, W. Early etiology of Alzheimer's disease: tipping the balance toward autophagy or endosomal dysfunction? *Acta Neuropathol.* **129**, 363–381 (2015).
- Gerakis, Y. & Hetz, C. Emerging roles of ER stress in the etiology and pathogenesis of Alzheimer's disease. *FEBS J.* **285**, 995–1011 (2018).
- Selfridge, J. E., Lezi, E., Lu, J. & Swerdlow, R. H. Role of mitochondrial homeostasis and dynamics in Alzheimer's disease. *Neurobiol. Dis.* **51**, 3–12 (2013).
- Itzhaki, R. F. et al. Microbes and Alzheimer's disease. *J. Alzheimers Dis.* **51**, 979–984 (2016).
- Ke, P. C. et al. Implications of peptide assemblies in amyloid diseases. *Chem. Soc. Rev.* **46**, 6492–6531 (2017).
- Steenhuysen, J. Biogen Scraps Two Alzheimer Drug Trials, Wipes \$18 Billion from Market Value. <https://www.reuters.com/article/us-biogen-alzheimers/biogen-scraps-two-alzheimer-drug-trials-wipes-18-billion-from-market-value-idUSKCN1R213G> (2019).
- Doody, R. S. et al. Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *N. Engl. J. Med.* **370**, 311–321 (2014).
- Salloway, S. et al. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *N. Engl. J. Med.* **370**, 322–333 (2014).
- The Lancet. Alzheimer's disease: expedition into the unknown. *Lancet* **388**, 2713 (2016).
- Reardon, S. Frustrated Alzheimer's researchers seek better lab mice. *Nature* **563**, 611–612 (2018).
- Ke, P. C. et al. Mitigation of amyloidosis with nanomaterials. *Adv. Mater.* **1901690** (2019). <https://doi.org/10.1002/adma.201901690>.
- Luo, Q. et al. A self-destructive nanosweeper that captures and clears amyloid β -peptides. *Nat. Commun.* **9**, 1802 (2018).
- Lansbury, P. T. & Lashuel, H. A. A century-old debate on protein aggregation and neurodegeneration enters the clinic. *Nature* **443**, 774 (2006).
- Kim, D. et al. Graphene quantum dots prevent α -synucleinopathy in Parkinson's disease. *Nat. Nanotech.* **13**, 812 (2018).
- Javed, I. et al. In vivo mitigation of amyloidogenesis through functional–pathogenic double-protein coronae. *Nano Lett.* **18**, 5797–5804 (2018).
- Gao, N. et al. Transition-metal-substituted polyoxometalate derivatives as functional anti-amyloid agents for Alzheimer's disease. *Nat. Commun.* **5**, 3422 (2014).
- Faridi, A. et al. Mitigating human IAPP amyloidogenesis in vivo with chiral silica nanoribbons. *Small* **14**, 1802825 (2018).
- Bieschke, J. et al. Small-molecule conversion of toxic oligomers to nontoxic β -sheet-rich amyloid fibrils. *Nat. Chem. Biol.* **8**, 93 (2012).

28. Thorn, D. C., Ecroyd, H. & Carver, J. A. The two-faced nature of milk casein proteins: amyloid fibril formation and chaperone-like activity. *Aust. J. Dairy Technol.* **64**, 34 (2009).
29. Guha, S., Manna, T. K., Das, K. P. & Bhattacharyya, B. Chaperone-like activity of tubulin. *J. Biol. Chem.* **273**, 30077–30080 (1998).
30. Thorn, D. C. et al. Amyloid fibril formation by bovine milk κ -casein and its inhibition by the molecular chaperones α S- and β -casein. *Biochemistry* **44**, 17027–17036 (2005).
31. Librizzi, F., Carrotta, R., Spigolon, D., Bulone, D. & San Biagio, P. L. α -Casein inhibits insulin amyloid formation by preventing the onset of secondary nucleation processes. *J. Phys. Chem. Lett.* **5**, 3043–3048 (2014).
32. Carrotta, R. et al. Inhibiting effect of α S1-casein on A β 1–40 fibrillogenesis. *Biochim. Biophys. Acta* **1820**, 124–132 (2012).
33. Zhang, X. et al. Chaperone-like activity of β -casein. *Int. J. Biochem. Cell Biol.* **37**, 1232–1240 (2005).
34. Gladysz, A., Abel, B. & Risselada, H. J. Gold-induced fibril growth: the mechanism of surface-facilitated amyloid aggregation. *Angew. Chem. Int. Ed.* **55**, 11242–11246 (2016).
35. Proctor, E. A. & Dokholyan, N. V. Applications of discrete molecular dynamics in biology and medicine. *Curr. Opin. Struct. Biol.* **37**, 9–13 (2016).
36. Javed, I. et al. Cofibrillation of pathogenic and functional amyloid proteins with gold nanoparticles against amyloidogenesis. *Biomacromolecules* **18**, 4316–4322 (2017).
37. Francis, R. et al. *aph-1* and *pen-2* are required for Notch pathway signaling, γ -secretase cleavage of β APP, and presenilin protein accumulation. *Dev. Cell* **3**, 85–97 (2002).
38. Team, M. G. C. P. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proc. Natl Acad. Sci. USA* **99**, 16899–16903 (2002).
39. Newman, M., Ebrahimie, E. & Lardelli, M. Using the zebrafish model for Alzheimer's disease research. *Front. Genet.* **5**, 189 (2014).
40. Geling, A., Steiner, H., Willem, M., Bally-Cuif, L. & Haass, C. A γ -secretase inhibitor blocks Notch signaling in vivo and causes a severe neurogenic phenotype in zebrafish. *EMBO Rep.* **3**, 688–694 (2002).
41. Joshi, P., Liang, J. O., DiMonte, K., Sullivan, J. & Pimplikar, S. W. Amyloid precursor protein is required for convergent-extension movements during Zebrafish development. *Dev. Biol.* **335**, 1–11 (2009).
42. Xie, J., Farage, E., Sugimoto, M. & Anand-Apte, B. A novel transgenic zebrafish model for blood-brain and blood-retinal barrier development. *BMC Dev. Biol.* **10**, 76 (2010).
43. Fleming, A., Diekmann, H. & Goldsmith, P. Functional characterisation of the maturation of the blood-brain barrier in larval zebrafish. *PLoS ONE* **8**, e77548 (2013).
44. Santana, S., Rico, E. P. & Burgos, J. S. Can zebrafish be used as animal model to study Alzheimer's disease? *Am. J. Neurodegener. Dis.* **1**, 32 (2012).
45. Garcia-Ayllón, M.-S. et al. Altered levels of acetylcholinesterase in Alzheimer plasma. *PLoS ONE* **5**, e8701 (2010).
46. Sáez-Valero, J., Sberna, G., McLean, C. A. & Small, D. H. Molecular isoform distribution and glycosylation of acetylcholinesterase are altered in brain and cerebrospinal fluid of patients with Alzheimer's disease. *J. Neurochem.* **72**, 1600–1608 (1999).
47. Roher, A. E. et al. Amyloid beta peptides in human plasma and tissues and their significance for Alzheimer's disease. *Alzheimer's Dement.* **5**, 18–29 (2009).
48. Pacheco-Quinto, J. et al. Hyperhomocysteinemic Alzheimer's mouse model of amyloidosis shows increased brain amyloid β peptide levels. *Neurobiol. Dis.* **22**, 651–656 (2006).
49. Avella, M. A. et al. *Lactobacillus rhamnosus* accelerates zebrafish backbone calcification and gonadal differentiation through effects on the GnRH and IGF systems. *PLoS ONE* **7**, e45572 (2012).
50. Lau, B. Y., Mathur, P., Gould, G. G. & Guo, S. Identification of a brain center whose activity discriminates a choice behavior in zebrafish. *Proc. Natl Acad. Sci. USA* **108**, 2581–2586 (2011).
51. Howe, K. et al. The zebrafish reference genome sequence and its relationship to the human genome. *Nature* **496**, 498 (2013).
52. Mathuru, A. S. & Jesuthasan, S. The medial habenula as a regulator of anxiety in adult zebrafish. *Front. Neural Circuits* **7**, 99 (2013).
53. Tomaszewicz, H. G., Flaherty, D. B., Soria, J. & Wood, J. G. Transgenic zebrafish model of neurodegeneration. *J. Neurosci. Res.* **70**, 734–745 (2002).
54. Van Bebber, F., Paquet, D., Hruscha, A., Schmid, B. & Haass, C. Methylene blue fails to inhibit Tau and polyglutamine protein dependent toxicity in zebrafish. *Neurobiol. Dis.* **39**, 265–271 (2010).
55. Lin, S. et al. High content screening in zebrafish speeds up hazard ranking of transition metal oxide nanoparticles. *ACS Nano* **5**, 7284–7295 (2011).
56. Smith, P. E. et al. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76–85 (1985).
57. Javed, I. et al. Lecithin-gold hybrid nanocarriers as efficient and pH selective vehicles for oral delivery of diacerein—in-vitro and in-vivo study. *Colloids Surf. B* **141**, 1–9 (2016).
58. Whitmore, L. & Wallace, B. A. Protein secondary structure analyses from circular dichroism spectroscopy: methods and reference databases. *Biopolymers: Original Research on Biomolecules* **89**, 392–400 (2008).
59. Pan, Y., Chatterjee, D. & Gerlai, R. Strain dependent gene expression and neurochemical levels in the brain of zebrafish: focus on a few alcohol related targets. *Physiol. Behav.* **107**, 773–780 (2012).
60. Richetti, S. K. et al. Acetylcholinesterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure. *Neurotoxicology* **32**, 116–122 (2011).

Acknowledgements

This work was supported by ARC Project No. CE140100036 (Davis), the NSFC grant #21607115 and #21777116 (Lin), NSF CAREER CBET-1553945 (Ding), and NIH MIRA R35GM119691 (Ding). Javed acknowledges the support of Monash International Postgraduate Research Scholarship (MIPRS) and Australian Government Research Training Program Scholarship. TEM imaging was performed at Bio21 Advanced Microscopy Facility, University of Melbourne and polarized light microscopy was performed at Monash Micro Imaging, Monash University. Javed and Lin thank Shanghai Science and Technology Commission "Belt and Road" initiative program, Grant no. 17230743000. The authors acknowledge the support from Profs. Ting Xu and Daqiang Yin on the Zebrafish instrument.

Author contributions

P.C.K., I.J., S.L. and T.P.D. designed the project. I.J. and P.C.K. wrote the paper. I.J. performed AuNP synthesis, TEM, CD, UV-SPR, fluorescence characterizations, larval, and adult zebrafish behavioral and cognitive assays, histology, and polarized light microscopy. I.J., M.Z. and T.Y. performed in vivo microinjection. I.J. and G.P. performed ICP-MS analysis. Y.X. and F.D. conducted DMD simulations. A.F. performed cell viability assay and HIM imaging. C.L.P. and A.K. provided inputs to the discussion of the paper. All authors agreed on the presentation of the paper.

Additional information

Supplementary Information accompanies this paper at <https://doi.org/10.1038/s41467-019-11762-0>.

Competing interests: The authors declare no competing interests.

Reprints and permission information is available online at <http://npg.nature.com/reprintsandpermissions/>

Peer review information: *Nature Communications* would like to thank Morgan Newman, George Perry, and other, anonymous, reviewers for their contributions to the peer review of this work. Peer review reports are available.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2019