**Au$_{23}$(CR)$_{14}$ nanocluster restores fibril Aβ’s unfolded state with abolished cytotoxicity and dissolves endogenous Aβ plaques**

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**ABSTRACT**

The misfolding of amyloid-β (Aβ) peptides from the natural unfolded state to β-sheet structure is a critical step, leading to abnormal fibrillation and formation of endogenous Aβ plaques in Alzheimer’s disease (AD). Previous studies have reported inhibition of Aβ fibrillation or disassembly of exogenous Aβ fibrils in vitro. However, soluble Aβ oligomers have been reported with increased cytotoxicity; this might partly explain why current clinical trials targeting disassembly of Aβ fibrils by anti-Aβ antibodies have failed so far. Here we show that Au$_{23}$(CR)$_{14}$ (a new Au nanocluster modified by Cys-Arg (CR) dipeptide) is able to completely dissolve exogenous mature Aβ fibrils into monomers and restore the natural unfolded state of Aβ peptides from misfolded β-sheets. Furthermore, the cytotoxicity of Aβ$_{40}$ fibrils when dissolved by Au$_{23}$(CR)$_{14}$ is fully abolished. More importantly, Au$_{23}$(CR)$_{14}$ is able to completely dissolve endogenous Aβ plaques in brain slices from transgenic AD model mice. In addition, Au$_{23}$(CR)$_{14}$ has good biocompatibility and infiltration ability across the blood–brain barrier. Taken together, this work presents a promising therapeutics candidate for AD treatment, and manifests the potential of nanotechnological approaches in the development of nanomedicines.

**Keywords:** gold nanoclusters, Alzheimer’s disease, restores fibril Aβ’s unfolded state, abolished cytotoxicity, dissolves endogenous Aβ plaques

**INTRODUCTION**

A hallmark sequence of events in Alzheimer’s disease (AD) is the misfolding, fibrillation and accumulation of amyloid-β (Aβ) peptides, resulting in cellular dysfunction, loss of synaptic connections and brain damage [1–4]. Over the past three decades, the inhibition of Aβ fibrillation and the disassembly of deposited Aβ fibrils have been the magnets for searching promising therapeutics for AD treatment [5–9]. A number of inhibitors (including β- and γ-secretase inhibitors) for inhibiting Aβ production were discontinued in phase ii or iii clinical trials due to their low efficacy and serious side effects [10]. Anti-Aβ antibody-based immunotherapy for disassembling the mature Aβ fibrils was once expected to be the first radical treatment of AD [11]. However, prior studies have indicated that the soluble Aβ oligomers, as the most toxic species, might reappear during the disassembly process to induce more neurotoxicity (i.e. the ‘dust-raising’ effect) [12–16]. One approach to ameliorate the toxicity of soluble Aβ oligomers is to promote their aggregation by, for example, chiral silica nanoribbons and star-shaped poly(2-hydroxyethyl acrylate) nanostructures [17,18]. Also, graphene quantum dots are reported to drive the peptide fibrillation off-pathway to eliminate the toxic intermediates, which points to the potential of using zero-dimensional nanomaterials for in vivo mitigation of a range of amyloidosis types [19]. Recently, polymer-peptide conjugates and curcumin–gold nanoparticles (AuNPs) with hydrodynamic diameters of 10–25 nm have been shown to disassemble exogenous Aβ fibrils in vitro, but they failed to restore the natural unfolded state of Aβ from the misfolded β-sheets [20–22]. However, the β-lactoglobulin ‘coronae’ of the
AuNPs are reported to enable X-ray destruction of islet amyloid polypeptide (IAPP) amyloids, providing a viable new nanotechnology against amyloidogenesis [23]. The small molecule epigallocatechin gallate (EGCG) presents the capability to prevent aggregation and remodel amyloid fibrils, which could also convert mature amyloid fibrils to amorphous protein aggregates that are less toxic to cells, implying the possibility of reducing the toxicity of amyloid fibrils by remodeling their molecular structures [24–26]. Therefore, the treatment of AD needs to explore new materials that are able to dissolve endogenous Aβ plaques and abolish the proteotoxicity of Aβ fibrils by restoring their natural unfolded state from the misfolded β-sheets.

To date, nanomaterials and multifunctional nanocomposites possessing certain structural and physicochemical traits are promising candidates for mitigating amyloidosis in vitro and in vivo, indicating the use of nanoparticles as an emerging field against amyloid diseases [16,27]. Gold nanoclusters (AuNCs) (d < 3 nm) have been widely studied in biomedical fields due to their small-size effect and good biocompatibility [28–30]. Our previous study has shown that glutathione-modified AuNCs (GSH-AuNCs) can completely inhibit the fibrillation of Aβ peptides [31,32]. This study was expanded to explore whether any AuNCs including GSH-AuNCs could dissolve mature exogenous Aβ fibrils and endogenous Aβ senile plaques, and, more importantly, restore the natural unfolded state of Aβ from the misfolded β-sheets. To this end, seven kinds of AuNCs (i.e. Cys-AuNCs, CSH-AuNCs, p-MBA-AuNCs, MPA-AuNCs, GSH-AuNCs, NIBC-AuNCs and CR-AuNCs) modified by cysteine, cysteamine, 4-mercaptopentanoic acid, mercaptopropionic acid, glutathione, N-isobutyryl-L-cysteine or Cys-Arg, respectively, have been synthesized.

RESULTS AND DISCUSSION

Seven kinds of AuNCs on inhibiting Aβ fibrillation

First, the effects of these seven kinds of AuNCs on inhibiting Aβ fibrillation were investigated by co-incubating 20 μmol·L⁻¹ Aβ₄₀ with each kind of AuNCs at the same concentration (25 mg·L⁻¹). The concentrations were selected based on their solubility and biological relevance from our preliminary experiments. The standard thioflavine-T (ThT) binding fluorescence assay was employed to record the fibrillation kinetics. As shown in Fig. 1A, the fibrillation kinetics of 20 μmol·L⁻¹ Aβ₄₀ without AuNCs showed a standard S-curve (black curve); the formation of preformed/mature Aβ₄₀ fibrils was confirmed by atomic force microscopy (AFM) images (Fig. 1B; Fig. S1 in the online supplementary material). Cys-AuNCs had no inhibitory effect (red curve); CSH-AuNCs (orange curve), p-MBA-AuNCs (yellow curve) and MPA-AuNCs (green curve) showed partial inhibition. Consistent with our previous studies, GSH-AuNCs showed complete inhibition of Aβ₄₀ fibrillation (blue curve). Encouragingly, NIBC-AuNCs (cyan curve) and CR-AuNCs (purple curve) were also able to completely inhibit Aβ₄₀ fibrillation, which was further verified by AFM images (no fibrils could be found in Fig. 1C–E). Moreover, in situ real-time circular dichroism (CD) spectra were used to record the conformational transition of Aβ₄₀ in the fibrillation process. As shown in Fig. 1F, in the absence of AuNCs, Aβ₄₀ had undergone a misfolding process from an unfolded state (negative peak at 198 nm) into a β-sheet structure (negative peak at 220 nm). Interestingly, GSH-AuNCs (Fig. 1G), NIBC-AuNCs (Fig. 1H) and CR-AuNCs (Fig. 1I) could maintain the unfolded state of Aβ₄₀ peptides throughout the incubation. It should be noted that the seven AuNCs used have similar particle sizes (1.6 ± 0.5 nm), and their transmission electron microscope (TEM) images and the UV-visible absorption spectra are shown in Fig. S2.

Seven kinds of AuNCs on the dissolving of mature Aβ fibrils

Since inhibition of fibrillation and dissolution of fibrils should be considered as two discrete events, then whether these AuNCs could dissolve preformed/mature Aβ₄₀ fibrils was investigated by using CD and AFM. Freshly prepared Aβ₄₀ (20 μmol·L⁻¹) were pre-incubated at 37°C for 72 h. The preformed Aβ₄₀ fibrils were then co-incubated with 50 mg·L⁻¹ individual AuNCs for 48 h. The formation and dissolution of Aβ₄₀ fibrils were recorded by CD. The data showed that the peak at 220 nm did not change between 72 h and 120 h when treated with Cys-AuNCs (Fig. 2B₁), CSH-AuNCs (Fig. 2C₁), p-MBA-AuNCs (Fig. 2D₁), MPA-AuNCs (Fig. 2E₁), GSH-AuNCs (Fig. 2F₁) and NIBC-AuNCs (Fig. 2G₁), and that the fibrils were intact (Fig. 2B₂–G₂), indicating no dissolution of the mature Aβ₄₀ fibrils. The failure of GSH-AuNCs to dissolve Aβ₄₀ fibrils confirmed that inhibition of fibrillation and dissolution of fibrils are two discrete events. Most excitingly, when treated by CR-AuNCs, the peak at 220 nm (i.e. β-sheet) disappeared and the peak at 198 nm (i.e. unfolded state) resurfaced (Fig. 2H₁). The dissolution of
In the absence (B) or presence of 25 mg co-incubated without (F) or with 25 mg presence of 25 mg AuNCs (E). (F–I) In situ real-time CD spectra monitoring of 20 μmol L−1 Aβ40 fibrils co-incubated without (F) or with 25 mg L−1 AuNCs (G), NIBC-AuNCs (H) and CR-AuNCs (I) for 72 h.

Figure 1. (A) Fibrillation kinetics for 20 μmol L−1 Aβ40 in the absence (black) or presence of 25 mg L−1 Cys-AuNCs, CSH-AuNCs, p-MBA-AuNCs, MPA-AuNCs, GSH-AuNCs, NIBC-AuNCs or CR-AuNCs. (B–E) AFM images of Aβ40 after 72 h co-incubation in the absence (B) or presence of 25 mg L−1 AuNCs (C), NIBC-AuNCs (D) and CR-AuNCs (E). (F–I) In situ real-time CD spectra monitoring of 20 μmol L−1 Aβ40 peptides co-incubated without (F) or with 25 mg L−1 AuNCs (G), NIBC-AuNCs (H) and CR-AuNCs (I) for 72 h.

the mature Aβ40 fibrils by CR-AuNCs was further confirmed by AFM observation (Fig. 2H2). The perfect overlay of CD curves of 0 h and 120 h demonstrated that CR-AuNCs could completely dissolve the mature Aβ40 fibrils, and fully restore the unfolded state of Aβ40 peptides from β-sheet structure.

Molecular composition and structure of CR-AuNCs

To ascertain their molecular composition and structure, CR-AuNCs were characterized using various technical platforms (Fig. 3 and Fig. S3). The electrospray ionization mass spectrometry (ESI-MS) analysis showed a single distinct peak at 8397.9925, indicating that CR-AuNCs had a formula of Au23(CR)14 (Fig. 3A). The formula was further confirmed by thermogravimetric analysis (Fig. 3B). The weight loss of 46.0% meant that the CR weight ratio agrees well with the formula of Au23(CR)14 (calculated loss: 46.0%). In addition, the high resolution TEM analysis showed that the Au23(CR)14 had a spherical morphology (Fig. 3C), where the shape was regular with a clear lattice fringe (inset of Fig. 3C).

The process detail and possible mechanisms of Au23(CR)14 dissolving the preformed Aβ40 fibrils

To gain more insights into the dissolution process, preformed Aβ40 fibrils were co-incubated with 50 mg L−1 Au23(CR)14 for 48 h. The dissolution dynamics were monitored by ThT assay. The fluorescence intensity declined continuously during 48 h incubation (Fig. 4A), indicating a gradual process of dissolution. The gradual dissolution of Aβ40 fibrils had also been evidenced by AFM studies (Fig. 4B1–B6). The apparent sizes of the samples were assayed by dynamic light scattering (DLS). The DLS results showed that the apparent sizes of the samples decreased from over 1000 nm to less than 10 nm (Fig. 4C1–C6). The in situ real-time CD spectra revealed that the peak at 220 nm was continuously shifted to 198 nm (Fig. 4D), indicating that the dissolution of Aβ40 fibrils by Au23(CR)14 is a dynamic process accompanied by a conformational transition from a β-sheet structure to an unfolded state. The native PAGE results showed one band with a molecular weight less than 6.5 kDa (Fig. 4E, 48 h), directly demonstrating that Au23(CR)14 completely dissolves Aβ40 fibrils into monomers (~4.2 kDa).

To explore possible mechanisms of how Au23(CR)14, but not the other six kinds of AuNCs, could dissolve Aβ40 fibrils, the zeta potentials of Aβ40 fibrils, individual AuNCs and Aβ40 fibrils, together with individual AuNCs, were measured. The median of the zeta potential of mature Aβ40 fibrils was −41 ± 2 mV (black curves in Fig. 4F and Fig. S4). Cys-AuNCs, CSH-AuNCs, p-MBA-AuNCs, MPA-AuNCs, GSH-AuNCs, NIBC-AuNCs and Au23(CR)14 have a zeta potential of −32, +36, −49, −57, +2, −34 and +68 mV, respectively (blue curves in Fig. 4F and Fig. S4). After addition of AuNCs, while the mixtures with the other six kinds of AuNCs showed a negative zeta potential with a median value from −44 to −18 mV, the mixture with Au23(CR)14 showed a positive zeta potential with a median value of +34 mV (red curves in Fig. 4F and Fig. S4). These data suggest that Au23(CR)14 adsorb onto...
Aβ_{40} fibrils more strongly than other AuNCs. In consideration of Aβ_{40} monomers with a net charge of negative 2.7 at physiological pH (7.4) [33], and the existence of a guanidine group in the residue of CR that could be protonated in a wide range of pH [34], the strong electrostatic interaction between Aβ_{40} and Au_{23}(CR)_{14} might drive the gradual dissolution of mature Aβ_{40} fibrils. The above results strongly suggest that Au_{23}(CR)_{14} dissolve the preformed/mature Aβ_{40} fibrils from misfolded β-sheets into the unfolded monomer state through strong electrostatic interactions.

Au_{23}(CR)_{14}-mediated Aβ_{40} fibril dissolution on cell viabilities

To investigate the effect of Au_{23}(CR)_{14}-mediated dissolution of Aβ_{40} fibrils on cell viabilities, an AD cell model based on Aβ_{40} fibril-induced cell deaths of PC-12 cells was adopted [35]. First, PC-12 cells were co-incubated with freshly preformed Aβ_{40} fibrils without (Fig. 5A) or with (Fig. 5B) Au_{23}(CR)_{14}, and in situ real-time morphological changes were recorded by a Cytation 5 Cell Imaging Multi-Mode Reader. Aβ_{40} fibrils formed from
20 μmol·L⁻¹ monomers were used to cause a 50% decrease of cell viability based on our preliminary titration experiments. As shown in Fig. 5A, when treated with Aβ₄₀ fibrils alone, cell shrinkage started to appear in the 3rd hour, and then cells with reduced sizes and round shapes apparently increased from the 12th to the 48th hour. In contrast, when PC-12 cells were treated with Aβ₄₀ fibrils and 50 mg·L⁻¹ Au₂₃(CR)₁₄, no obvious morphological changes were observed (Fig. 5B). The corresponding videos are shown in the online supplementary material. Second, a CCK-8 assay was used for quantifying cell viabilities. Freshly preformed Aβ₄₀ fibrils from 20 μmol·L⁻¹ monomers were added into PC-12 cells with or without Au₂₃(CR)₁₄, the cells were cultured and sampled at the 3rd, 6th, 12th, 24th and 48th hour for assaying their viabilities. No treatment was included as the blank control. As shown in Fig. 5C, the cell viability was not affected in the blank control group (gray bars), and the addition of preformed Aβ₄₀ fibrils alone caused a gradual decrease of cell viability to 50% (red bars). In contrast, when cells were cultured with 50 mg·L⁻¹ Au₂₃(CR)₁₄ together with preformed Aβ₄₀ fibrils, the cell viability decreased initially to 70% at the 12th hour and then started to increase, reaching almost 100% (same as the blank control) at the 48th hour (blue bars). These data collectively demonstrated that Au₂₃(CR)₁₄ could fully abolish the cytotoxicity of Aβ₄₀ fibrils. As for the two phasic characteristics of cell viabilities in the Au₂₃(CR)₁₄ treatment, we speculate that the toxic oligomers [36,37] were produced during the dissolution process and the cytotoxicity was fully abolished when the oligomers were completely dissolved into non-toxic monomers.

The capacity of Au₂₃(CR)₁₄ for dissolving exogenous Aβ fibrils

The ultimate test is whether the capacity of Au₂₃(CR)₁₄ for dissolving exogenous Aβ fibrils can be translated into dissolving the endogenous Aβ plaques. We obtained brain slices derived from the resected brain tissue of an adult transgenic mouse model of AD, where the brain slices contained endogenous Aβ plaques. The brain slices were co-incubated without (Fig. 6A₁–A₃) or with (Fig. 6B₁–B₃) Au₂₃(CR)₁₄ for 24 h, and then the slices were stained with anti-Aβ antibodies for immunohistochemical analyses. Figure 6A₁–A₃ shows that the hippocampus and the neocortex were present with a large amount of endogenous Aβ plaques (yellow-brown patches indicated by the arrows). Excitingly, the treatment with 50 mg·L⁻¹ Au₂₃(CR)₁₄ eliminated all yellow-brown patches (Fig. 6B₁–B₃), demonstrating that Au₂₃(CR)₁₄ could completely dissolve the endogenous Aβ plaques in the hippocampus and the neocortex. Furthermore, our data showed that Au₂₃(CR)₁₄ did not affect cell viability at a concentration of as high as 100 mg·L⁻¹ (Fig. S5), indicating good biocompatibility. In addition, the overcoming of the blood–brain barrier is one precondition of nanomaterials in treating neurological diseases [6]. Our data showed that Au₂₃(CR)₁₄ particles were readily detected in the brain tissues when intraperitoneally injected into normal mice, demonstrating that Au₂₃(CR)₁₄ is capable of overcoming the blood–brain barrier (Fig. 6D).

CONCLUSION

In conclusion, seven kinds of AuNCs (i.e. Cys-AuNCs, CSH-AuNCs, p-MBA-AuNCs, MPA-AuNCs, GSH-AuNCs, NIBC-AuNCs and Au₂₃(CR)₁₄) were synthesized and adopted to investigate their effects on the dissolution of mature Aβ fibrils and the restoration of the unfolded state of Aβ peptides. Among the seven kinds of AuNCs tested, only Au₂₃(CR)₁₄ are able to completely dissolve exogenous mature Aβ fibrils
into monomers, and fully abolish cytotoxicity by restoring the natural unfolded state of Aβ peptides from misfolded β-sheets. Furthermore, Au23(CR)14 are able to completely dissolve endogenous Aβ plaques in the brain slices from transgenic AD model mice. In addition, Au23(CR)14 have good biocompatibility and infiltration ability across the blood–brain barrier. The biodistribution of AuNCs in vivo has been reported in our recent paper published in Nanomedicine [38]. Compared with the chaperone-gold nanoparticle in vivo test on zebrafish, similar efficacies to dissolve Aβ plaques and cross the blood–brain barrier are achieved by Au23(CR)14 based on a rodent model, further indicating the clinical potential of nanoparticles or nanoclusters against Alzheimer’s symptoms [39]. The relevant behavioral pathology and neurodegeneration would be offered in subsequent research. This study provides a compelling nanotherapeutic candidate for AD treatment.

METHODS
Materials, cells and mice
Amyloid β Protein Fragment 1–40 (Aβ40) peptides powder (≥90%), chloroauric acid (HAuCl4·3H2O, 99.999%), L-cysteine (≥97%), cysteamine...
(≥98%). 4-mercaptobenzoic acid (≥99%), mercaptopyrionic acid (≥99%), glutathione (≥98%), N-isobutryrly-L-cysteine (≥97%) and 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium sodium salt (CCK-8) were purchased from Sigma-Aldrich Co. Ltd. (NJ, USA). Sodium borohydride (NaBH₄, ≥96.0%), DMEM, hydrochloric acid, anhydrous ethanol, anhydrous methanol, sodium hydroxide, glacial acetic acid and sulfuric acid were analytical grade and all from Innochem (China). Cys-Arg (97%) was purchased from ChinaPeptides (Shanghai, China). The ultrapure water used was from the Millipore Milli-Q ultrapure water system. All the reaction vessels were soaked in aqua regia (volume ratio = 3:1, HCl/HNO₃) for 24 h, washed thoroughly with ultrapure water several times and dried in an oven before use. The PC-12 cell line was purchased from the Cell Bank of Chinese Academy of Sciences (Beijing). APPswe/PSdE9 double transgenic mice (APP/PS1 Tg mice, 35 weeks of age) and C57BL/6 wild-type mice (9 weeks of age) were obtained from the Model Animal Research Center of Nanjing University (China). All animal experiments were approved by the Animal Ethics Committee, Wuhan University of Technology.

**Synthesis and characterizations of Cys-Arg dipeptide**

Cys-Arg dipeptides were synthesized by peptide synthesizer (LibertyBlue) [40]. Purity analysis was performed on high performance liquid chromatography (HPLC, Waters 2695). The stationary phase was a 4.6 × 250 mm chromatographic column (Kromasil, C18-5). The flow rate was adjusted to 1.0 mL·min⁻¹; 5 μL of sample was injected at room temperature. Gradient elution was performed using 0.1% trifluoroacetic acid in 100% acetonitrile (Solvent A) and 0.1% trifluoroacetic acid in 100% water (Solvent B) with the following gradient combination: 0.01 min, 1A/99B; 25 min, 26A/74B; 25.1 min, 100A/0B; and 30 min, stop. Detection was performed at 220 nm. The purified products (CR ligand) were characterized by ¹H NMR in DMSO-d₆ (Bruker BioSpin GmbH 500 MHz). Next, characterized by mass spectrometry (MS), the purified CR ligands were dissolved in a 50:50 (% v/v) acetonitrile/water mixture. The flow rate of electrospray for the dissolved sample was 0.2 mL·min⁻¹, using nebulizer gas (nitrogen) with a flow rate of 1.5 L min⁻¹ and block temperature of 200°C. Positive-mode ESI-MS were used for analysis with −4.5 kV probe bias.

**Synthesis of AuNCs with different ligands**

All AuNCs used in this work were synthesized on the basis of a method reported in our previous study with only a few minor changes in experimental parameters [19,30]. Take Au₂₃(CR)₁₄ for example, 0.675 mmol (187 mg) of CR was dissolved in a 100 mL mixture of ultrapure water and ethyl alcohol (v/v = 1/2). Then a freshly prepared aqueous solution (6 mL) of HAuCl₄ (2.5 mmol·L⁻¹) was slowly added into the pre-prepared mixture. The mixed solution was cooled to ~0°C in a cool bath for 18 h under a proper stirring frequency (340 rpm by mechanical agitation). Then, a fresh aqueous solution of NaOH (0.1 mol·L⁻¹, 18 mL) was added to the mixed solution. The reaction was maintained for 10 min and stirred vigorously (400 rpm). A freshly prepared aqueous solution of NaBH₄ (0.11 mol·L⁻¹, 200 μL) was cooled to 4°C and added rapidly to the mixed solution. Another 1 h was needed for the mixed
solution to react completely. The resulting mixed solution was collected and moved into an Amicon® Ultra-4 3K (MWCO: 3000) Centrifugal Filter device for centrifugal separation (RCF: 5000 × g, 30 min). Then the solution in the centrifuge tube was removed, and the solution in the filter device was washed by ultrapure water several times. Finally, the Au_{23}(CR)_{14} solution in the filter device was collected and lyophilized for further characterizations and experiments. The other six kinds of AuNCs were synthesized following similar conditions and operations.

Characterization

Nuclear magnetic resonance spectroscopy measurements

Hydrogen (\(^1\)H) nuclear magnetic resonance (H-NMR) spectra of CR dipeptide were recorded on a Bruker AVANCE III 500 MHz spectrometer. A 100 mg·L\(^{-1}\) sample solution was added to the NMR tube, and the data were analysed by MestReNova.

Infrared spectroscopy measurements

Infrared (IR) spectra of AuNCs and the corresponding ligands were recorded on a Bruker Vertex 80v Fourier transform infrared (FT-IR) spectrometer. Lyophilized AuNCs and the corresponding ligands were directly used for IR measurement with ATR mode in a vacuum atmosphere at room temperature. Scanning range: 4000–400 cm\(^{-1}\); scan times: 64; vacuum degree: <5 hPa.

UV–visible spectroscopy measurements

UV–visible spectra of AuNCs were recorded on a Shimadzu UV-1800 UV-Vis spectrophotometer with a range of 300–1000 nm at a scan rate of 0.5 nm·s\(^{-1}\). Lyophilized AuNCs were dissolved in water and then diluted to 200 \(\mu\)L with a concentration of 200 mg·L\(^{-1}\). Then the sample was transferred into a high-quality quartz glass cuvette with a black wall for spectrophotometry (volume: 600 \(\mu\)L).

Mass spectrometry measurements

ESI-MS of CR-AuNCs was performed on a Nano electrospray ionization-quadrupole time-of-flight mass spectrometer (ESI-Q-TOF MS, Bruker) operating in the negative ion mode. The sample injection rate was 8 \(\mu\)L·min\(^{-1}\). A capillary voltage of 4 kV was used for the ESI-MS (nebulizer: 1.5 bar, dry gas: 4 L·min\(^{-1}\), 120°C, \(m/z = 800–12 000\)). The ESI-MS spectra were obtained by accumulating for 5 min.

Thermal gravimetric analysis measurements

Thermal gravimetric analysis (TGA) was performed on a SETARAM TG-DSC 111 instrument. The sample was dried before TG measurement. The test was performed in flowing air with a temperature increasing rate of 1°C·min\(^{-1}\).
X-ray photoelectron spectroscopy analysis

X-ray photoelectron spectroscopy (XPS) measurements were performed by an ESCALAB 250Xi with a focused monochromatic Al Kα X-ray (1350 eV) source for excitation. The binding energy (BE) scale is calibrated by using the O 1s peak at 530.14 ± 0.05 eV, the N 1s peak at 400.06 ± 0.05 eV, the C 1s peak at 283.42 ± 0.05 eV, and the S 2p peak at 161.47 ± 0.05 eV and the Au 4f peak at 83 ± 0.05 eV for known reference foils.

TEM measurements

The TEM measurements of AuNCs were performed by using a Talos F200S TEM (Thermo Fisher, USA) with an accelerating voltage of 200 kV. The TEM images of the brain slices were performed by using a FEI Tecnai G20 TEM (FEI, USA) with an accelerating voltage of 200 kV. Blinded observation of samples with random selection of grid areas was implemented to reduce bias during imaging.

Zeta potential measurement

The zeta potentials of individual AuNCs (50 mg·L⁻¹), Aβ fibrils (pre-incubated from 20 μmol·L⁻¹ Aβ40) and Aβ fibrils (20 μmol·L⁻¹ Aβ40) together with individual AuNCs (50 mg·L⁻¹) were measured by using a Malvern Nano-ZS ZEN3600 zetasizer.

In vitro inhibition or dissolution experiments

Preparation of Aβ40 peptides

All Aβ40 peptides used in our experiments were pretreated by 1,1,1,3,3,3-hexafluoro-2-propanol (1 mg·mL⁻¹, m₉₆₆ / v₁₁₆₆) under the ultrasonic vibration in an ice bath for 2 h. Then, each solution was divided into multiple samples and dried by soft nitrogen airflow respectively. The samples after pretreatment were saved in a refrigerator at −80°C.

Preparation of preformed Aβ40 fibril solution

For ThT kinetics and fluorescence imaging, the buffer ThT solution, Aβ₄₀ solution (50 μmol·L⁻¹, 100 μL), and ultrapure water were mixed in wells in a certain ratio so that each well contained 60 μmol·L⁻¹ ThT, 10 mmol·L⁻¹ PBS and 20 μmol·L⁻¹ Aβ₄₀. The above solution was pre-incubated for 72 h to form mature fibrils, then different samples (AuNCs or H₂O) were added to each well for further experiments. For CD detection, 200 μL freshly prepared 40 μmol·L⁻¹ Aβ₄₀ were pre-incubated in a quartz cuvette at 37°C for 48 h to grow mature fibrils. The quartz cuvette was kept in a CD spectrometer, covered with a cap and sealed by sealing film. After the incubation, a 200 μL solution of 100 mg·L⁻¹ AuNCs was injected in the quartz cuvette; the concentration of preformed Aβ₄₀ fibrils would be diluted twice. Then, the quartz cuvette was sealed again and incubated for further experiments. For cell experiments, a 96-well plate was used to incubate Aβ₄₀ fibrils solution. Each well containing buffered Aβ₄₀ solution was incubated at 37°C for 48 h to grow mature fibrils. Then, Aβ₄₀ fibrils solution, DMEM and AuNCs were mixed in a certain ratio and added into another 96-well plate containing cells. The concentration of preformed Aβ₄₀ fibrils would be diluted twice.

The kinetics monitored by ThT assay

The mixtures for ThT assays were incubated at 37°C. All fluorescence data were recorded by using a Synergy™ MX Multi-Mode Microplate Reader with a bottom-reading mode in 96-well flat bottom (Costar) plates sealed with a platemax film. Plates were shaken at medium intensity for 10 s before reading fluorescence data with an excitation wavelength of 445 nm and emission wavelength of 485 nm.

The inhibition effect of different AuNCs on Aβ₄₀ fibrillation monitored by ThT assays

A series of working solutions (250 μL) were prepared containing 20 μmol·L⁻¹ Aβ₄₀ peptides, 20 μmol·L⁻¹ ThT in 10 mmol·L⁻¹ phosphate buffer (PBS) at pH 7.4 without or with 25 mg·L⁻¹ of seven kinds of AuNCs. Fluorescence data were recorded every 10 min. Each experiment was run in sextuplicate in a 96-well plate.

The effect of Au_{23}(CR)_{14} on the disassembly of Aβ₄₀ fibrils monitored by ThT

A series of sample solutions (250 μL) were prepared containing 20 μmol·L⁻¹ Aβ₄₀ peptides, 20 μmol·L⁻¹ ThT in 10 mmol·L⁻¹ PBS, pH 7.4, at 37°C. The samples were incubated until the fluorescence intensity reached platform (Aβ₄₀ fibrils were formed). Then, 10 μL solutions of different mixtures were injected in parallel groups. The samples containing Aβ₄₀ fibrils were co-incubated without or with 50 mg·L⁻¹ Au_{23}(CR)_{14} for the next 72 h. Fluorescence data were recorded every 10 min.

AFM measurement track morphology change during the dissolution of Aβ₄₀ fibrils

AFM experiments were carried out on a FastScan Scanning Probe Microscope (Bruker) with ScanAsyst in air mode and mechanical properties
mode at room temperature. A sample was prepared by dripping 5.0 μL of a solution of the mixture onto freshly cleaved mica and allowing it to dry in the air. Images were conducted with a force constant of 0.225 N·m⁻¹ and processed by NanoScope analysis software. For AFM track morphology change during the dissolution of Aβ₄₀ fibrils, mixtures of 20 μmol·L⁻¹ Aβ₄₀ were pre-incubated in 96-well plates at 37°C (in an incubator chamber) for 72 h. Then, 20 μL mixtures of Au₂₃(CR)₁₄ were injected into the pre-incubated mixtures of Aβ₄₀. After the incubation of Aβ₄₀ fibrils in the presence of 50 mg·L⁻¹ Au₂₃(CR)₁₄ for 0, 3, 6, 12, 24 or 48 h, samples for AFM studies were prepared by dripping 5.0 μL of a solution of the mixture onto freshly cleaved mica.

In situ real-time circular dichroism spectroscopy
CD spectra were recorded in the far-UV region from 190 to 250 nm by a JASCO J-1500 Spectrometer, using a setup containing a step of 0.5 nm, a bandwidth of 1.0 nm, a speed of 50 nm·min⁻¹, a time per point of 1.0 s, an ultrasonic vibration of 600 rpm and an incubation temperature of 37°C. The sample for experiment was collected in a quartz cuvette with a 1 mm optical path length; the cuvette was covered with a cap and sealed by sealing film. Each spectrum calibrated after subtraction of background signal was processed with a smoothing function of 30 points. The spectra data were recorded every 3 h.

DLS tracks apparent size change during the dissolution of Aβ₄₀ fibrils
DLS measurement was performed on a Malvern Nano-ZS ZEN3600 zetasizer. Mixtures of 20 μmol·L⁻¹ Aβ₄₀ were pre-incubated in a high-quality quartz glass cuvette at 37°C (in an incubator chamber) for 72 h. Then, 20 μL mixtures of Au₂₃(CR)₁₄ were injected into the pre-incubated mixtures of Aβ₄₀ and incubated for another 48 h. The apparent size of the sample was recorded at the 0, 3rd, 6th, 12th, 24th and 48th hour.

Native PAGE
The Aβ₄₀ states after co-incubation with or without Au₂₃(CR)₁₄ were analysed via Native PAGE using 4–20% Tris-glycine gradient gels (BeyoGel). Native PAGE (no addition of β-mercaptoethanol and SDS) was adopted here for maintaining non-covalent bonds of samples. Samples of Aβ fibrils with or without Au₂₃(CR)₁₄ (50 mg·L⁻¹) were added to native loading buffer. Equal volumes of each sample (20 μL) were loaded onto gels along with BeyoColor (Beyo) prestained molecular weight mark-

ers and electrophoretically separated at 150 V. Gels were stained for total protein using a hypersensitivity Coomassie blue (BeyoBlue) according to the manufacturer’s protocol. After incubation with decoloring solution three times (each for 3 h), the gel was detected by the gel imaging analysis system.

Cell experiments
PC-12 cells were incubated in DMEM medium supplemented with 10% fetal bovine serum (FBS) at 37°C with 5% CO₂. The cells were regularly sub-cultured to maintain them in logarithmic phase of growth. The cells were seeded in 96-well plates at a cell population of ~10 000 cells per well and incubated for 24 h at 37°C before further treatment. The viability of PC-12 cells was assessed by CCK-8 assay. Before being examined by using a Synergy™ MX Multi-Mode Microplate Reader at a wavelength of 450 nm, cells were treated with 100 μL DMEM contained 10% CCK-8 solution for ~2 h.

Cytotoxicity test of Au₂₃(CR)₁₄
Cells of the blank controls were incubated with fresh DMEM, the cells of the experiment group were incubated with DMEM containing different doses of Au₂₃(CR)₁₄ (1, 10, 25 and 50 mg·L⁻¹) for 24 h. The viability of PC-12 cells was assessed by CCK-8 assay.

Preformed Aβ₄₀ fibril-lesioned PC-12 cell model
To investigate the effect of the 50 mg·L⁻¹ Au₂₃(CR)₁₄-mediated dissolution process of Aβ₄₀ fibrils on cell viabilities, the PC-12 cells of the experiment group were incubated with DMEM containing preformed Aβ₄₀ fibrils and 50 mg·L⁻¹ Au₂₃(CR)₁₄ for 3, 6, 12, 24 or 48 h, respectively. Five 96-well plates containing PC-12 cells, preformed Aβ₄₀ fibrils and 50 mg·L⁻¹ Au₂₃(CR)₁₄ were prepared for the five time points cell experiments. The PC-12 cells of the five experiment groups were incubated for 3, 6, 12, 24 or 48 h, respectively. Each 96-well plate was examined once. Cells of the blank controls were incubated with fresh DMEM. The viabilities of PC-12 cells were assessed by CCK-8 assay.

In situ cell imaging monitoring
In situ bright field cell images were recorded by using a Cytation™ 5 Cell Imaging Multi-Mode Reader (BioTek). The PC-12 cells were co-incubated with preformed Aβ₄₀ fibrils in the absence or presence of 50 mg·L⁻¹ Au₂₃(CR)₁₄ in 96-well plates at 37°C with 5% CO₂ for 48 h. The in situ real-time cell images were recorded every 1 h.
**Immunohistochemical analyses of brain slices**

The APP/PS1 Tg mice were anesthetized with 7% chloral hydrate, and transcardially perfused with phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in PBS. After perfusion fixation, the mouse brains were removed and fixed overnight in 4% paraformaldehyde at 4°C. Then the brains were dehydrated with 30% sucrose in PBS solution. Coronal slices (8 μm) containing both the neocortex and hippocampus were cut on a CM 1950 (Leica) freezing microtome. The brain slices were co-incubated without (blank control) or with Au23(CR)14 for 48 h. Then the slices were incubated with the primary mouse anti-Aβ antibody (Abcam, 1:100) followed by a horseradish peroxidase Goat Anti-Rabbit secondary antibody (Abcam). After washing, the slices were stained by using diaminobenzidine and counter-stained by hematoxylin, and observed by using an BX53 biological microscope (Olympus).

**Au23(CR)14 across the blood–brain barrier**

To investigate whether the Au23(CR)14 could cross the blood–brain barrier, 20 mg·kg⁻¹ Au23(CR)14 (dissolved in normal saline) was slowly injected into the caudal vein of the laboratory mice for a period of less than 20 s. The mice were sacrificed by cervical dislocation 6 h later. The brains were quickly collected on ice and then fixed immediately by 2.5% glutaraldehyde fixative. Subsequently, 10% osmium tetroxide was used to further fix the brains. Then the brains were embedded in paraffin and sectioned into 80–100 nm slices by using an EM UC7 (Leica) ultramicrotome. Finally, the brain slices were used for analysis of the presence of Au23(CR)14 by TEM.

**SUPPLEMENTARY DATA**

Supplementary data are available at NSR online.

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