Metal-dependent inhibition of amyloid fibril formation: Synergistic effects of cobalt–tannic acid networks†

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Metal–phenolic networks (MPNs) have received widespread interest owing to their modular incorporation of functional metal ions and phenolic ligands. However, the interaction between MPNs and biomolecules is still relatively unexplored. Herein, we studied the effects of MPN-coated gold nanoparticles on amyloid fibril formation (which is associated with Alzheimer's disease) as a function of the metal ion in the MPN systems. All coated particles examined inhibited amyloid formation, with cobalt(II) MPN-coated particles exhibiting the highest inhibition activity (90%). Molecular dynamics simulations and quantum mechanics calculations suggested that the geometry of the exposed cobalt coordination site in the cobalt–tannic acid networks facilitates its interactions with histidine and methionine residues in the amyloid beta peptides. Furthermore, the unique structure of cobalt MPNs may enable a wider variety of biomedical applications.

Introduction

Alzheimer's disease (AD) is a global disease that primarily affects elderly people and is also the most prevalent form of dementia.¹ Currently, there is no cure for AD, and its progress cannot be stopped or reversed. Although the biogenesis of AD is poorly understood, plaques formed by amyloid protein fibrils are always found in Alzheimer-affected brain tissue and are considered to be one of the key factors for the development of AD.^{2–4} Fibrils in AD are formed by the aggregation of amyloid beta (A β) peptides, which are 40 (A β ₄₀) or 42 (A β ₄₂) residues long. A β ₄₂ is 10 times less abundant in humans but forms fibrils more easily compared to A β ₄₀.⁵ Moreover, soluble aggregates (oligomers and protofibrils) are considered to be the toxic species to brain neuronal cells.^{2–5} Therefore, inhibition of this aggregation could potentially benefit the development of effective AD therapies. In the past decade,⁶ various amyloid fibril inhibitors have been reported including peptides,^{6–8} small organic molecules,⁹ metallic compounds,^{10–12} and nanoparticles.^{13–18} Free metal ions, largely transition metals, can bind to A β peptides and promote amyloid fibril formation.^{19–23} It has also been reported that free cobalt ions can induce oxidative stress, neurotoxicity, and increased A β secretion in neuronal cells.²⁴ However, chelated metals can help inhibit fibril

formation. For example, Qu *et al.* showed that cobalt and nickel substitution in polyoxometalates can better inhibit amyloid fibril formation.¹⁰ However, the preparation of transition metal-substituted polyoxometalates generally involves complex and time-consuming preparation steps.²⁵ Moreover, coordinated transition metals can exhibit proteolytic activity toward A β peptides.²⁶ Therefore, combining metal ions and other small molecule inhibitors within a particle system, which can be prepared using a simple and time-effective approach, could potentially offer an effective synergistic approach to inhibit amyloid fibrils formation.

Recently, we reported a versatile strategy to form diverse metal–phenolic network (MPN) coatings based on the coordination between polyphenols and metal ions.^{27,28} MPNs hold promise for the fabrication of multifunctional hybrid materials, owing to their versatile and tunable nature, combining the benefits of both the organic and inorganic components. Although MPNs are interesting systems for a range of biomedical applications, *e.g.*, modular assembly,²⁹ medical imaging,^{28,30} drug delivery, and cell targeting,^{31,32} their interactions with biomolecules are not well understood, especially the role of the incorporated metal ion. Understanding the role of metal ions in bio–nano interactions could expand the applications of MPNs and offer a new route toward inhibiting amyloid fibril formation. While polyphenols have been reported as potential amyloid fibril inhibitors, *e.g.*, based on their interaction with lysine residues in A β peptides,^{9,33–36} the combination of the natural bioactivity of polyphenols with a wide range of metal ions in MPNs offers the potential for the rational development of amyloid fibril inhibitors. Herein, we systematically investigated the effect of MPN-coated gold nanoparticles (AuNPs) toward inhibiting A β fibril formation.

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Experimental

Tannic acid-stabilized gold nanoparticle (TA-AuNP) synthesis

Vials and stir bars were washed with aqua regia before use. Then, 200 μL of HAuCl_4 solution (10 mM) was added dropwise to a solution of 4.7 mL Milli-Q water (ddH_2O) and 100 μL of TA solution (10 mg mL^{-1}) at 30 $^\circ\text{C}$ with continuous stirring, and the resulting suspension was further stirred for 15 min. The suspension was then centrifuged at 10 000 g for 10 min. The supernatant was carefully removed and the resultant TA-AuNPs were washed with ddH_2O once.

MPN coating

To achieve an MPN coating on the TA-AuNPs, 0.8 mL of TA-AuNPs solution (optical density at 520 nm (OD_{520}) ≈ 1) was mixed with 4.08 μL of TA solution (10 mg mL^{-1}) by vortex in a 1.7 mL tube for 10 s. Then, 1 μL of metal ion solution (24 mM; Al^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , or Zn^{2+}) was added and the mixed solution was vortexed for 10 s. Subsequently, 200 μL of Tris buffer (50 mM, pH = 8.0) was added to raise the pH of the suspension, which was subsequently vortexed vigorously for 10 s. The resulting MPN-coated AuNPs were washed 3 times with 0.1 mM phosphate-buffered saline (PBS) by centrifugation (10 000 g , 10 min) to remove excess metal ions and TA. After each wash, the solution was sonicated for 10 s. The final product is referred to as AuNP@MPNs.

AuNP characterization

The structure of the AuNPs was analyzed by FEI Tecnai Spirit transmission electron microscopy (TEM) at an operation voltage of 120 kV. The metal content of the different AuNP@MPNs was analyzed by energy-dispersive X-ray spectroscopy (EDS) on a FEI Tecnai TF20 transmission electron microscope at an operation voltage of 200 kV. The concentration of the AuNPs was determined by measuring the absorbance of the corresponding solution at 520 nm; the calculated molar absorption coefficient of 20 nm AuNPs was $8.8 \times 10^8 \text{ cm}^{-1} \text{ M}^{-1}$. Size distribution was determined by dynamic light scattering (Malvern Nano ZS). The concentration of the different metals in AuNP@MPNs was determined by inductively coupled plasma optical emission spectroscopy (Varian 720-ES). Zeta potentials and size were measured on a Zetasizer (Malvern Nano ZS) instrument.

Amyloid beta fibril formation

$\text{A}\beta_{40}$ and $\text{A}\beta_{42}$ peptides were dissolved to a final concentration of 0.974 and 1.016 mg mL^{-1} , respectively, using 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). The mixture was incubated at 22 $^\circ\text{C}$ and vortexed intermittently for at least 2 h to dissolve the $\text{A}\beta$ peptide. An aliquot of the $\text{A}\beta$ solution was transferred to a tube to vaporize the HFIP under N_2 flow. After HFIP was fully removed, a thin film of $\text{A}\beta$ remained. The $\text{A}\beta$ film was kept under vacuum for 2 h to remove any residual HFIP. Subsequently, $\text{A}\beta$ film was dissolved with 9 equivalents of 1 mM NaOH solution and then 1 equivalent of $10\times$ PBS to achieve a final solution of $1\times$ PBS and a final concentration of $\text{A}\beta$ of 10 μM . The final $\text{A}\beta$

solution was incubated at 37 $^\circ\text{C}$ for 7 days with and without AuNPs for fibril formation.

Thioflavin T assay

Thioflavin T (ThT) was used to analyze amyloid fibril formation. An aliquot (100 μL) of $\text{A}\beta$ peptide with or without the tested inhibitors was mixed with 3 μL of ThT solution (1 mM) in a black 96-well plate. The fluorescence spectra were determined after 10 min on a Microplate Reader Infinite M200 (Tecan) instrument at an excitation wavelength of 440 nm and the fluorescence intensity was recorded at an emission wavelength of 485 nm. Fluorescence inhibition (%) was calculated as follows:

$$\text{Fluorescence inhibition} = 1 - \frac{I_{\text{inhibitor}} - I_{\text{initial}}}{I_{\text{control}} - I_{\text{initial}}}$$

Where $I_{\text{inhibitor}}$ and I_{control} are the end-of-point ThT fluorescence intensity of $\text{A}\beta$ with or without inhibitors, respectively, and I_{initial} is the initial ThT fluorescence intensity of $\text{A}\beta$.

TEM characterization of amyloid fibrils

TEM copper grids were treated with plasma before use. An aliquot (5 μL) of $\text{A}\beta_{40}$ peptide with or without the tested inhibitors were dropped on the grids. After 10 min, excess liquid on the grids was removed and the grids were further washed with ddH_2O to remove salts. Samples were then analyzed without staining by FEI Tecnai Spirit transmission electron microscopy at an operation voltage of 120 kV.

Cell culture

PC12 cells (rat pheochromocytoma, American Type Culture Collection) were incubated at 37 $^\circ\text{C}$ in 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium containing 10% heat inactivated horse serum, 5% fetal bovine serum, and 1% penicillin-streptomycin.

Cell viability assay

Cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. PC12 cells were cultured in a 96-well plate at a density of 5 000 cells per well and incubated for 24 h. Samples of the inhibitors or controls were then added to the cells and allowed to incubate for 48 h. Filtered solutions (20 μL) of MTT (5 mg mL^{-1}) were added to each well, and the mixtures were further incubated at 37 $^\circ\text{C}$ for 4 h. The solution in each well was decanted and 150 μL dimethyl sulfoxide was added to each well. The plate was shaken, and the absorbance of the solution in the wells was measured at 570 nm on a Microplate Reader Infinite M200 (Tecan) instrument. For the $\text{A}\beta_{40}$ samples, 10 μL of the sample was added to 100 μL cell culture, achieving a final $\text{A}\beta_{40}$ concentration of 1 μM .

Matrix-assisted laser deionization/ionization time-of-flight (MALDI-ToF) analysis of $\text{A}\beta_{40}$ cleavage

$\text{A}\beta_{40}$ peptides (100 μM) were incubated with and without AuNP@Co-TA (1 nM) in PBS at 37 $^\circ\text{C}$ for 7 days. Freshly prepared amylin peptide (100 μM) was used as internal standard. The matrix, α -cyano-4-hydroxycinnamic acid, was dissolved in 40%

acetonitrile and 2% trifluoroacetic acid at a saturated concentration. The incubated A β ₄₀, matrix, and fresh amylin were mixed at a ratio of 2:3:2 and loaded on the target plate. After the samples had air dried, the mass spectra of the samples were recorded on a microFLEX MALDI-ToF (Bruker) instrument.

Terahertz/far infrared (THz/FIR) spectroscopy

The THz/FIR beamline of the Australian Synchrotron was equipped with an attenuated total reflectance accessory. Samples were analyzed in 0.1 mM PBS solution at a concentration of 1 nM. The analysis was run using the THz/FIR synchrotron beam (200 mA); the resolution was 0.00096 cm⁻¹ and the number of scans ran per measurement was 240. Quadruplicate measurements were taken and averaged with the background subtracted.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

A β ₄₀ samples (24 μ L) were mixed with 5 $\times$ loading buffer (6 μ L) and heated at 95 $^{\circ}$ C for 5 min. Samples were then loaded onto 4–20% precast polyacrylamide gel (BIO-RAD). Running conditions: running buffer with SDS, 150 V, 35 min. Gels were silver-stained using a silver stain kit (BIO-RAD).

Atomic force microscopy (AFM)

AuNP@Co-TA and AuNP@Co-TA that were incubated with A β ₄₀ peptides (A β : 10 μ M; AuNPs: 100 pM; 37 $^{\circ}$ C for 2 days) were washed with ddH₂O thrice by centrifugation (10 000 *g*, 10 min). AuNPs (2 μ L) were loaded on a silicon wafer and dried with nitrogen flow. Samples were analyzed with NanoWizard BioScience AFM (JPK).

Results and discussion

Gold nanoparticles with a size of less than 50 nm have been reported to have the potential ability to cross the blood–brain barrier.^{14,37–39} TA-AuNPs were used as templates and synthesized by reducing HAuCl₄ with TA.⁴⁰ Final concentrations of 0.4 mM HAuCl₄ and 200 mg L⁻¹ TA gave uniform, well-dispersed TA-AuNPs with a diameter of 21.6 $\pm$ 2.4 nm (see Fig. S1 in the ESI). To assemble the MPN coatings on TA-AuNPs, a TA solution and a metal solution (Al^{III}, Fe^{III}, Co^{II}, Ni^{II}, Cu^{II}, or Zn^{II}) were successively added to the TA-AuNPs in Milli-Q water (Fig. 1a). Subsequently, the pH of the suspension was increased to 8.0 with Tris buffer to form the MPN coatings. After MPN coating, all divalent metal MPN-coated AuNPs (generally referred to as AuNP@MPNs or more specifically as AuNP@M-TA where M is Co, Ni, Cu, or Zn) showed similar UV–visible spectra and color compared with the uncoated AuNPs (pristine TA-AuNPs). In contrast, the trivalent metal MPN-coated AuNPs (generally referred to as AuNP@MPNs or more specifically AuNP@M-TA where M is Al or Fe) showed slightly red-shifted UV–visible spectra and displayed a different color (Fig. S2). All AuNP@MPNs were well dispersed and showed similar particle sizes (Fig. S3). TA-AuNPs and all AuNP@MPNs had comparable zeta potential values (between –30 and –40 mV). AuNP@Fe-TA

and AuNP@Co-TA respectively had the highest and lowest metal loadings (17.7 and 1.6 μ M in 1 nM AuNP@MPNs) (see Table S1 in the ESI). All AuNP@MPNs were highly dispersed after MPN coating (Fig. 1b, Fig. S4) and the MPN thickness was around 2.5 nm, which could be observed upon comparison with TA-AuNPs by transmission electron microscopy (TEM) (Fig. 1b, Fig. S5). Taking AuNP@Co-TA as an example, Co and Au element co-localization was confirmed with EDS mapping (Fig. 1c–f).

Fig. 1 (a) Scheme for AuNP@MPN preparation. (b) TEM images of AuNP@Co-TA. (c) High angular annular dark-field (HADDF) image of AuNP@Co-TA and EDS mapping of (d) Au, (e) Co, and (f) overlay.

To investigate the effects of AuNP@MPNs on amyloid fibril formation, we used the ThT assay.¹⁰ Free ThT has low fluorescence, but once it binds to β -sheet-rich structures (*e.g.*, amyloid fibrils), the fluorescence at 485 nm increases significantly. The AuNP@MPNs and pristine TA-AuNPs (100 pM) were incubated with 10 μ M A β ₄₀ (the most abundant A β peptide) at 37 $^{\circ}$ C for 7 days before measuring the fluorescence intensity of ThT. TA-AuNPs and all coated AuNPs showed fluorescence inhibition ability, which suggested inhibited amyloid fibril formation (Fig. 2a, Fig. S6). Among the AuNPs examined (including pristine TA-AuNPs), AuNP@Co-TA displayed the highest inhibition ability (90%). The other AuNP@MPNs showed inhibition similar to pristine TA-AuNPs (20–50%). It is interesting that TA-AuNPs showed only limited inhibition efficiency as TA has been reported to be an effective inhibitor.⁴¹ This might be due to the possibility that bound TA on AuNPs may have a lower “effective concentration” than free TA in solution. Additionally, under the same conditions, both AuNP@Co-TA and TA-AuNPs showed reduced ThT fluorescence inhibition to A β ₄₂ peptides (Fig. S7). This finding is consistent with reported literature findings that A β ₄₂ has a higher IC₅₀ than A β ₄₀.⁹ In contrast, free Co²⁺ at the same Co concentration (0.16 μ M) of the AuNP@Co-TA and free Co-TA complexes in solution (0.16 μ M for both Co²⁺ and TA) did not show any fluorescence inhibition toward A β ₄₀ peptides (Fig. S8). Commercial citric acid-

stabilized 20 nm AuNPs (100 pM, Citrate-AuNPs) showed ~2-fold enhancement in A β ₄₀ fibril formation (Fig. S8) relative to the control. This observation was consistent with previous findings that Citrate-AuNPs could enhance the fibril formation of islet amyloid polypeptide.⁴² Tests of free Co²⁺, Co-TA complex, Citrate-AuNPs, and AuNP@MPNs with metals other than Co^{II} (*i.e.*, Al^{III}, Fe^{III}, Ni^{II}, Cu^{II}, Zn^{II}) suggest that the ThT detection of amyloid fibrils was not modified by our materials. These results indicated that Co and TA in the MPN form (*i.e.*, AuNP@Co-TA) played a synergistic role toward inhibiting amyloid fibril formation, whereas TA was the dominant inhibitor in the other AuNP@MPNs.

Furthermore, inhibition of A β ₄₀ fibril formation with AuNP@Co-TA was concentration dependent (Fig. S9)—AuNP@Co-TA concentrations ≥ 100 pM inhibited fibril formation by more than 90%. TEM images further confirmed the inhibition results (Fig. 2b, Fig. S10). In the absence of AuNPs (control), A β ₄₀ fibrils around 10 nm in width and several microns in length were formed. In contrast, in the presence of 100 pM AuNP@Co-TA, no fibrils could be observed. Fibril formation could also be inhibited in the presence of pristine TA-AuNPs at the same concentration, however, not to the same degree; some fibrils were still visibly present. Moreover, AuNP@Co-TA could gradually reduce the fluorescence intensity of ThT in preformed A β ₄₀ fibrils over 7 days (Fig. S11), implying that the β -sheet structure of the A β peptides was disrupted. We further investigated the fibril formation from the A β ₄₀ oligomers (elongation). A β ₄₀ peptides were incubated at 37 °C for 2 h and then at 4 °C for 24 h to form oligomers. The resulting oligomers were incubated with or without AuNPs at 37 °C for another 7 days. The ThT assay result showed that both TA-AuNPs and AuNP@Co-TA could inhibit fibril formation from oligomers (~20% and ~77%, respectively; Fig. S12). However, the effect of AuNP@Co-TA on fibril formation from oligomers was slightly reduced compared with the A β ₄₀ monomers. This suggested that the effect of AuNP@Co-TA on A β ₄₀ monomers or the inhibition of A β ₄₀ oligomerization may play a more important role in the overall inhibition of fibril formation.

Fig. 2 Inhibition of A β ₄₀ fibril formation. (a) Fluorescence inhibition efficiency based on ThT assay of the different AuNPs. A β ₄₀: 10 μ M; AuNPs: 100 pM. Data are shown as the mean $\pm$ standard deviation (s.d.) of at least three independent experiments. (b) TEM images of A β ₄₀ peptides incubated in the absence of AuNPs (Control) and in the presence of 100 pM TA-AuNPs or 100 pM AuNP@Co-TA.

Soluble oligomers, as well as protofibrils, are known to be cytotoxic to brain neuronal cells, which ThT fluorescence assays cannot effectively detect.^{2–4} These aggregates can accumulate outside of brain cells and disrupt cell-to-cell communication, damage brain cells, and eventually cause brain shrinkage. Therefore, the inhibition of A β -mediated cytotoxicity was investigated using the MTT assay to assess cell viability in the neuronal cell line PC12. At 100 pM, both the pristine and MPN-coated AuNPs did not show any cytotoxicity (Fig. S13). To test the inhibition of A β -mediated cytotoxicity, 10 μ M A β ₄₀ peptide was pre-incubated with either 0, 50, 100, or 200 pM AuNPs at 37 °C for 7 days before addition to PC12 cells with a volume ratio of 1:10. The final concentrations of A β ₄₀ and AuNPs were 1 μ M and either 5, 10, or 20 pM, respectively. Interestingly, only AuNP@Co-TA inhibited A β -mediated cytotoxicity after 48 h incubation, whereas the other AuNPs did not improve cell survival (Fig. 3). Moreover, cell viability recovered to ~80% at a higher AuNP@Co-TA concentration (200 pM). These results suggested that AuNP@Co-TA might be able to either inhibit the oligomerization of A β ₄₀ monomers or reduce the concentration of active oligomers in the solution. Further investigation was required to understand the inhibition mechanism of AuNP@Co-TA.

Fig. 3 Inhibition of A β -mediated cytotoxicity. Cell toxicity of A β_{40} (10 μ M) treated with different AuNPs at different concentrations. MTT assay was used to determine the cytotoxicity of PC12 cells. Control: cells without any treatment; A β only: cells treated with 10 μ M aged A β_{40} without any AuNPs. Data are shown as the mean $\pm$ s.d. of at least three independent experiments. ** P < 0.01, *** P < 0.001. P -values (two tailed) for difference between AuNP-treated groups and A β only are based on a t -test.

Recently, Lim *et al.* found that metal tetra- N -methylated, especially those containing Co(II), cyclam complexes were able to inhibit fibril formation by hydrolyzing A β peptides.²⁶ To investigate whether AuNP@Co-TA could also hydrolyze A β_{40} peptides, the peptides were incubated with and without AuNP@Co-TA for 7 days before analysis with MALDI-ToF spectrometry. Freshly prepared amylin peptide solutions, in comparison to the aged A β fibrils, were used as internal controls. The results revealed that there was no decrease in the number of intact A β_{40} peptides after incubating with AuNP@Co-TA, which suggested that the inhibition was not due to peptide hydrolysis (Fig. S14), and that further fundamental studies on the fibril inhibition mechanism by the Co-TA networks are necessary.

The AuNP@MPN were analyzed using synchrotron THz/FIR spectroscopy, which can provide information on metal-organic bonds and hydration structures.^{43,44} As observed from Fig. S15, all AuNP@MPNs had peaks at ~ 60 and ~ 50 cm^{-1} . However, only AuNP@Co-TA showed a strong peak at 46 cm^{-1} , which indicated a potentially unique Co-TA coordination structure. To gain further insight into the coordination characteristics of Co-TA networks, the Co-TA structure was theoretically modelled using molecular dynamics (MD) simulations and quantum mechanics (QM) calculations.

Based on the UV-visible spectra of metal ion-catecholates, for the reaction conditions used in the present study, cobalt can be expected to form 4-coordinate square planar bis complexes with the galloyl groups of tannic acid.⁴⁵ We therefore constructed all-atom models of cobalt-tannic acid complexes (Co-TA₂) and performed MD simulations of monomers and dimers of Co-TA₂ in aqueous solution. While iron forms 6-coordinate distorted octahedral tris complexes with the galloyl groups at high pH in the Fe-TA₃ complexes, cobalt exhibited uncoordinated axial positions available for coordination with

water molecules, ions, or functional groups (*e.g.*, amino acid) when present in the Co-TA₂ complexes (Fig. 4, Fig. S16). MD simulations revealed that the cobalt ions were fully exposed to solution with little occlusion by the galloyl groups of TA. We found an average of 3 ± 1 water oxygens within a 4 Å radius of the cobalt ion, regardless of the partial atomic charge on the cobalt (Table S2, Fig. S17). Similar results were observed for the Co-TA₂ dimer systems, with an average of 6 ± 2 water oxygens positioned within a 4 Å radius of cobalt. The Co-TA₂ dimers had a larger contact area ($20 \pm 1\%$) when compared to the Fe-TA₃ dimers ($13 \pm 3\%$, as determined by our previous study;⁴⁶ Table S4). Additionally, the Co-TA₂ dimers had relatively more aromatic contacts and fewer intermolecular hydrogen bond occupancies when compared to the Fe-TA₃ dimers (13 ± 5 and $3 \pm 1\%$ compared to 9 ± 3 and $9 \pm 2\%$, respectively; Table S4). However, the most critical difference between the modeled systems is the geometry of the metal ions within the metal-phenolic network and their presentation to the environment (solvent exposure). Specifically, in the Co-TA₂ dimers, the average distance between the cobalt ions was found to be 13 ± 6 Å compared to 22 ± 6 Å for the Fe-TA₃ dimers (Table S4). Additionally, the Co-TA₂ dimers could form a “stepped” configuration where the two cobalt ions are not only in close proximity, but also orientationally aligned (Fig. 4d, Fig. S16d), exposing to the solvent a flat surface available for potential interactions with dissolved species. This elucidation of the internal and surface structure of MPNs led to the theoretical investigation of the interactions between the exposed surface of the Co-TA complex and the functional groups of the amyloid peptide.

Fig. 4 Representative structures of (a) Fe-GA₃, (b) Co-GA₂, (c) Co-TA₂ monomers, and (d) Co-TA₂ dimer. Iron, cobalt, oxygen, and carbon are colored yellow, pink, red, and silver, respectively. Coordinate bonds are displayed as dotted lines. Hydrogens on GA/TA and the non-coordinating solvent molecules are not shown. GA, gallic acid.

QM calculations were performed to evaluate the interaction enthalpy of the complex with water, ions, and functional groups present in A β peptides to determine the interactions between the A β peptides and the exposed cobalt ions of the Co-TA₂ complex. Cobalt-gallic acid (Co-GA₂) complexes were used for

simplicity, and the initial geometries of water molecules coordinating at the axial positions were obtained from a QM-optimized water cluster surrounding the Co–GA₂ complex (Fig. 5a). Interestingly, the interaction enthalpy for these water molecules was unfavorable (3.35 kcal mol^{−1} for the first water molecule and 1.44 kcal mol^{−1} for the second water molecule), suggesting that water molecules at the axial positions of cobalt in Co–GA₂/Co–TA₂ may be stabilized by the hydrogen bonding network of water interacting with the oxygens of GA/TA that coordinate the cobalt. Among the functional groups present in A β peptides, only the imidazole nitrogen of histidine (interaction enthalpies of −10.93 kcal mol^{−1}) and the sulfur of methionine (−5.10 kcal mol^{−1}) could favorably coordinate with the cobalt (Fig. 5b,c). Aliphatic and aromatic hydroxyl groups formed hydrogen bonds with the coordinating oxygens rather than interacting directly with the cobalt, whereas carbonyl and carboxylate groups did not coordinate with the cobalt complex (Table S5, Fig. S18). Negatively charged ions such as chloride and hydroxide also did not coordinate favorably with the cobalt of Co–GA₂. Additionally, compared with other transition metals (Fe^{III}, Ni^{II}, Cu^I, Zn^{II}), Co^{II} have been reported to exhibit a higher binding specificity to histidine residues.^{47,48} Though other possible effects such as Au–MPN interactions, the spacing of cobalt ions on the MPN surface, and competition between different peptides for available coordination sites are not included in the model, our calculations provide a rationale for the amyloid fibril inhibition observed for the Co–TA MPNs when compared to the MPNs with other metal ions or to TA alone.

Fig. 5 Optimized structures of Co–GA₂ with (a) a water cluster, (b) an imidazole group of histidine, and (c) a dimethyl sulfide group of methionine. Cobalt, oxygen, sulfur, nitrogen, and carbon are colored pink, red, yellow, blue, and silver, respectively. Coordinate bonds are displayed as black dotted lines and hydrogen bonds as orange dotted lines. Hydrogens on GA, imidazole, and dimethyl sulfide are not shown.

Based on these insights, the suggested potential effects of Co–TA networks on A β peptides are manifold. First, A β peptides contain three histidine residues (His6, His13, and His14) and one

methionine (Met35). With the unique “stepped” configuration of cobalt shown in Fig. 4d, the adjacent cobalt ions can bind His13 and His14 simultaneously, which would inhibit side-chain packing between His13, Glutamine-15 (Gln15), and Valine-40 observed in the β -sheet structure of A β peptides, and the β -turn in A β oligomers.⁴⁹ Second, direct interactions between His6 and cobalt could disrupt the β -turn predicted for residues 6–8 in some fibrils.⁵⁰ Third, interactions between cobalt and Met35 could interfere with the oxidation of Met35 by Alanine-42 in A β ₄₂,⁵¹ as well as the intermolecular contacts between Met35 and Lysine-17 and Gln15 in A β monomer pairs within fibrils.⁵² Moreover, Met35 and a chelating domain comprising Aspartic acid-1, His6, His13, and His14 are key binding sites for A β peptides to form redox complexes with Fe, Zn, and Cu with oxidative stress properties.⁵⁰ Complexation of the Met and His residues by cobalt in the Co–TA networks may reduce the effects of oxidative stress, the stabilization of oligomeric and amorphous aggregates, and the lipid peroxidation associated with neuronal membrane disruption. As the A β peptides are not cleaved by the AuNP@Co–TA, the overall effect of the coordination between cobalt ions in the Co–TA networks and His and Met residues is likely an effective reduction of the concentration of A β peptides available for oligomerization in solution, where the monomers form a “A β peptide corona” and/or off-pathway A β aggregates on the AuNP@Co–TA surface.^{3,18} To support this, A β ₄₀ peptides that were incubated with TA–AuNPs and AuNP@Co–TA for 2 days were analyzed by SDS–PAGE. A smeared oligomer band was observed for A β ₄₀ peptides when incubated alone (Fig. S19, lane 2), whereas no band was observed for A β ₄₀ peptides when incubated with TA–AuNPs (Fig. S19, lane 3) or AuNP@Co–TA (Fig. S19, lane 4). In addition, a reduced monomer band in lane 4 was observed. Mature fibrils were retained at the top of the gel for lanes 1 and 2, whereas AuNPs were retained at the top for lanes 3 and 4. It was noticeable that unlike TA–AuNPs, which penetrated marginally into the gel, AuNP@Co–TA showed a narrow band at the very top of the gel. This indicated an increased size of AuNP@Co–TA after incubation with A β ₄₀ peptides, which was further confirmed by AFM (Fig. S20) and dynamic light scattering (Fig. S21), further suggesting that monomers adsorb onto the AuNP@Co–TA from solution.

Conclusions

We systematically examined the inhibition of A β ₄₀ amyloid fibrils with MPN-coated AuNPs containing different metal ions (Al^{III}, Fe^{III}, Co^{II}, Ni^{II}, Cu^I, Zn^{II}). All AuNP@MPNs along with pristine TA–AuNPs were tested for their inhibition potential toward amyloid fibril formation. We found that AuNP@Co–TA showed synergistically enhanced inhibition of the A β ₄₀ fibril formation, whereas other AuNP@MPNs showed activity similar to pristine TA–AuNPs. Mechanistic insights were revealed by theoretical all-atom simulations and electronic structure calculations, which demonstrated that the Co–TA structure in the MPN was different from that of the Fe–TA structure, with tuned coordination characteristics favoring the binding of imidazole and dimethyl sulfide functional groups. In the model, the cobalt

center presented a flat geometry to the solvent, where the exposed cobalt could potentially coordinate with the histidine and methionine peptide residues. These interactions possibly induced the formation of “A β peptide corona” and/or off-pathway A β aggregates on the AuNP@Co-TA surface and inhibited the formation of free A β oligomers in solution. Our study demonstrated that metal–phenolic networks could synergistically inhibit amyloid fibril formation, with the unique metal-exposed structure of Co–TA networks being promising candidate for future development. This distinctive feature of Co–TA networks could encourage future research regarding biomolecule–MPN interactions.

Conflicts of interest

There are no conflicts to declare.

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