Electronic Supplementary Information (ESI) for Chem. Comm.

Gold nanoparticle-mediated rapid *in vitro* assay of anti-aggregation reagents for amyloid *β* and its validation

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Supporting Information

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Supplementary Methods

Materials. Amyloid β fragment 1-40 [Aβ (1-40)] and Amyloid β fragment 1-42 [Aβ (1-42)] were purchased from AnaSpec (Fremont, CA, USA). Albumin, (−)-epigallocatechin gallate, resveratrol, acetylcholine chloride, rutin hydrate, silibinin, curcumin from Curcuma longa, and copper (II) sulfate pentahydrate ($CuSO_4$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Caffeine, trehalose dihydrate, and glutathione (reduced form) were purchased from Wako (Osaka, Japan). Hydrochloric acid (HCl) was purchased from Samchun Pure Chemical Co. (Seoul, South Korea). All water used in our experiment was purified to 18.3 MΩ·cm.

Preparation of GNPs. GNPs (17 nm in diameter) were prepared as described previously.[1] Briefly, they were prepared by boiling 100 mL of aqueous 0.01% (w/w) hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$) in a flask connected to a water-cooling column that was maintained at 98 °C with constant vigorous stirring and then adding 3 mL of 1% (w/w) sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) all at once. When the colour of the solution began to change, the heat was turned off and the solution was allowed to cool to room temperature for about 20–30 min. The intrinsic 520-nm absorbance peak of the 17-nm GNPs was observed with ultraviolet-visible spectroscopy, and its intensity was adjusted to 1.0 by dilution with deionized water for further experiments.

Rapid colorimetric assay for screening anti-aggregation reagents. For the colorimetric screening of anti-aggregation reagents against Aβ aggregation, 10 μL of Aβ solution comprised 9:1 ratio between 10 μM Aβ (1-40) and 10 μM Aβ (1-42) was first incubated with 10 μL of each anti-aggregation reagent for 10 mins. Then, this 20 μL solution was mixed with 90 μL of GNPs solution together with 10 μL of 5 mM Cu^{2+} and 10 μL of 30 mM HCl. The volumetric ratio of the GNPs, Aβ peptides, tested anti-aggregation reagent, and aggregation-promoting agents was 9:1:1:2. By varying the concentration of each anti-aggregation reagent, concentration-dependent colorimetric responses were observed with the naked eye after 5 min, allowing the evaluation of anti-aggregation effects for all candidates.

Quantification of anti-aggregation effects of reagents. To quantify the observed colour change of each reaction, optical absorbances at 520 nm (as an intrinsic GNPs peak) and 650

nm (as an indicator peak of large fibrillar aggregation) were measured using a microplate reader (Molecular Devices LLC, Sunnyvale, CA, USA).

Dark-field microscopy. To visualize the anti-aggregation effect of each reagent, a BX-43 microscope (Olympus, Tokyo, Japan), equipped with a water-/oil-immersion dark-field condenser and charge-coupled device camera was used. An aliquot of each reaction solution was transferred onto a clean slide glass that had been pre-treated in a piranha solution (H_2SO_4:H_2O_2 = 7:3 v/v) for 1 h. A homemade polydimethylsiloxane fluidic chamber was prepared to confine the sample.

TEM imaging. To directly confirm the anti-aggregation effects of the reagents selected by the newly designed rapid *in vitro* assay, 10 μL of Aβ peptides solution comprised 9:1 ratio between 10 μM Aβ (1-40) and 10 μM Aβ (1-42) without GNPs was incubated with or without the test reagents for 24 h at 37 °C under aggregation-promoting conditions identical to those used for the rapid *in vitro* assay. Then, the samples were spotted onto TEM grids (300-mesh Cu, Ted Pella, Redding, CA, USA) overnight. Next, the grids were stained with 10 μL of 2% (v/v) filtered (0.2 μm) uranyl acetate in water for 90 s. After the staining process, the grids were washed with deionized water (*ca.* 18 MΩ) and allowed to dry at room temperature. The samples were examined using an energy-filtering transmission electron microscope (LIBRA 120, Carl Zeiss, Oberkochen, Germany) equipped with a charge-coupled device camera. During imaging, the accelerating voltage was 200 kV.

Fluorescence intensity measurement. To observe the anti-aggregation effect of each reagent, Aβ samples mixed as 9:1 ratio between 100 μM Aβ (1-40) and 100 μM Aβ (1-42) was incubated with or without the tested reagents for 24 h at 37 °C under aggregation-promoting conditions identical to those used for the proposed colorimetric assay. Then, 200 μL of 50 μM ThT was mixed with 20 μL of each incubated Aβ sample. The fluorescence intensity of ThT associated with Aβ aggregates was measured with an excitation maximum of 435 nm and an emission maximum of 480 nm by a SpectraMax® M2e microplate reader (Molecular Devices, Sunnyvale, CA, USA).

MTT assay. For cell viability tests, neuro-2a (N2a) cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 20 mM glutamine, and

penicillin/streptomycin. Briefly, the Aβ samples mixed as 9:1 ratio between 10 μM Aβ (1-40) and 10 μM Aβ (1-42) was pre-incubated for 24 h with each anti-aggregation reagent selected by the proposed colorimetric assay. Then, the samples were added to N2a cell cultures followed by incubation for 48 h. The MTT assay for cell viability was carried out as previously described,[2] and the converted dye was solubilized with dimethyl sulfoxide.

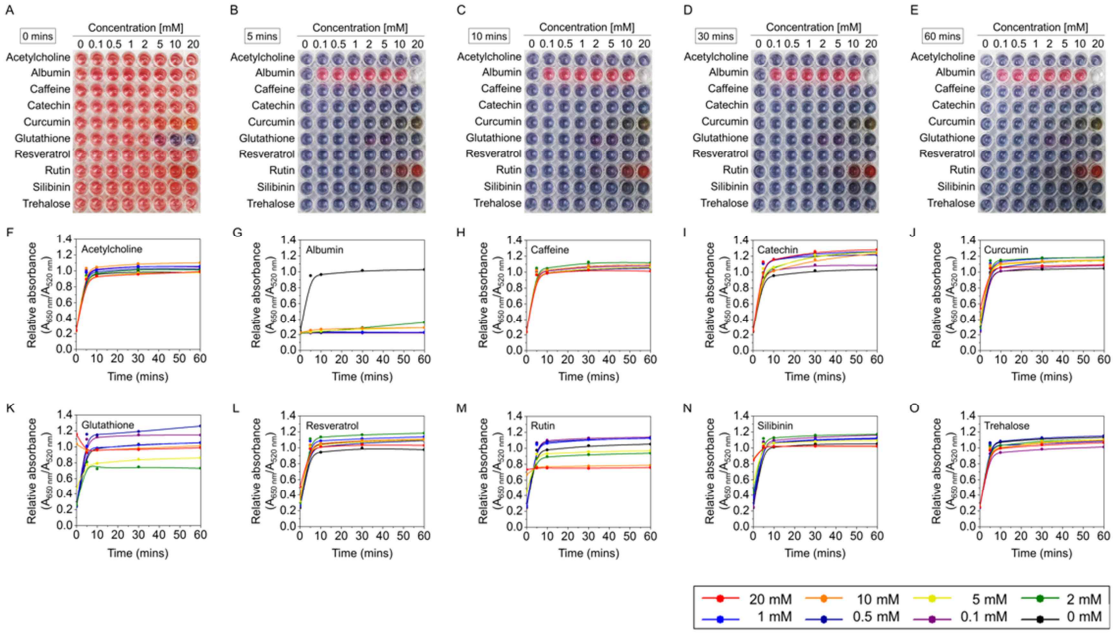

Fig. S1. Time-dependent colorimetric responses of rapid *in vitro* assay. (A-E) Time-dependent colorimetric responses, initial state (A), after 5 mins (B), after 10 mins (C), after 30 mins (D), after 1 hr (E). (F-O) Plots of relative absorbance between 520 nm and 650 nm for identifying that color transition occurs within 5 mins, acetylcholine (F); albumin (G); caffeine (H); catechin (I); curcumin (J); glutathione (K); resveratrol (L); rutin (M); silibinin (N); trehalose (O).

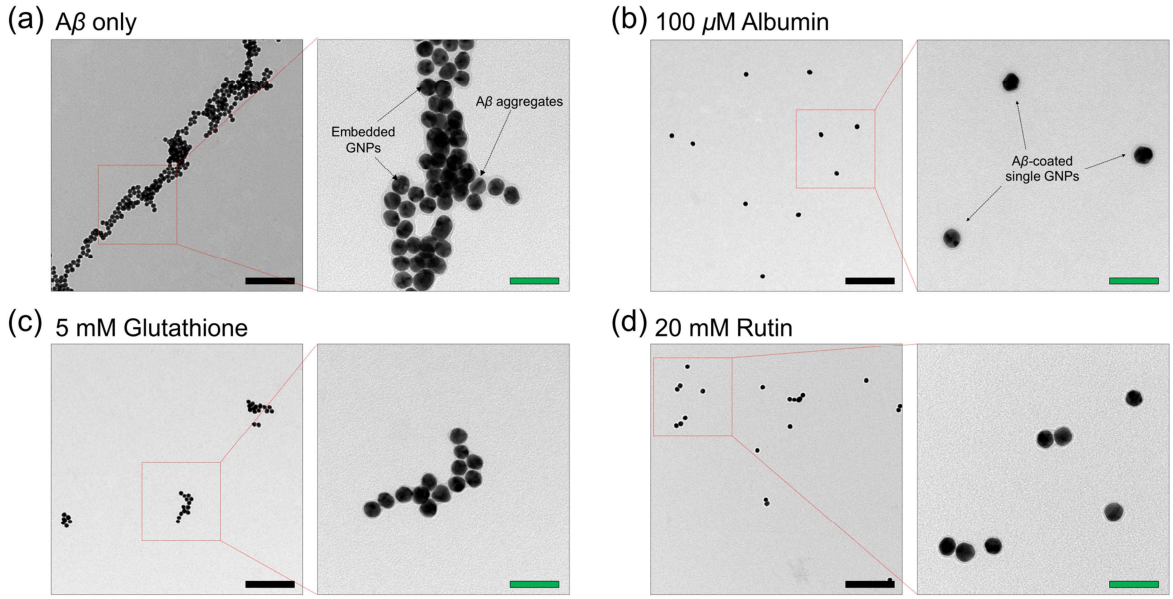

Fig. S2. TEM images of the GNPs incubated with Aβ peptides treated without and with anti-aggregation reagents under aggregation-promoting conditions. (a) GNPs embedded along the Aβ aggregates incubated without an anti-aggregation reagent. (b–d) GNPs incubated with Aβ peptides treated with anti-aggregation reagents; 100 μM albumin (b), 5 mM glutathione (c), 20 mM rutin (d). Right images are magnified images of the left images. Scale bars correspond to 200 nm (black) and 50 nm (green), respectively.

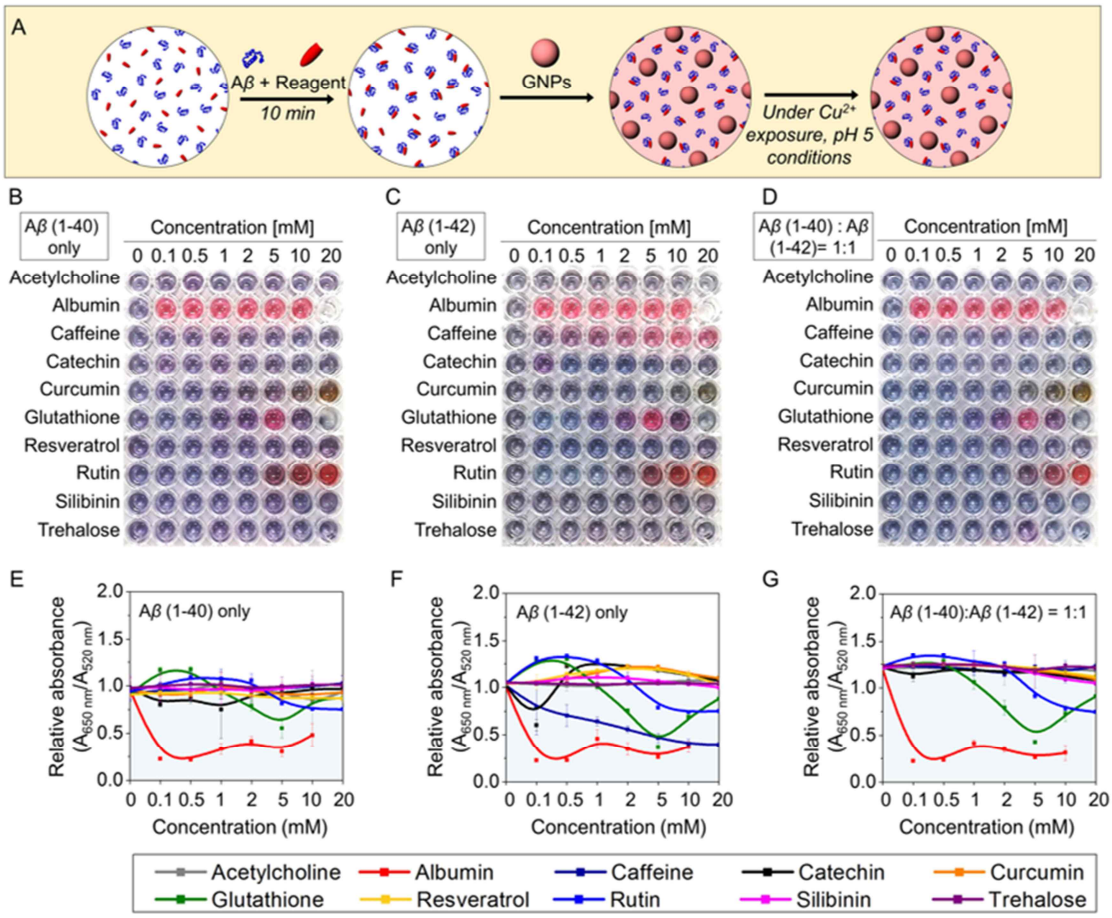

Fig. S3. Rapid colorimetric screening for concentration-dependent anti-aggregation effects of the test reagents on Aβ aggregation. (A) Schematic illustration of screening for anti-aggregation effect under 5 mM Cu^{2+}, and pH 5 conditions. (B, C, and D) Colorimetric screening for cases of 10 μM Aβ (1-40) only (B), 10 μM Aβ (1-42) only (C), and an equimolar mixture of 10 μM Aβ (1-40) and 10 μM Aβ (1-42) (D) with varying reagents at different concentrations. (E, F, and G) Plots showing changes in relative absorbances after treatment with the reagents corresponding to the colorimetric response in (B, C, and D).

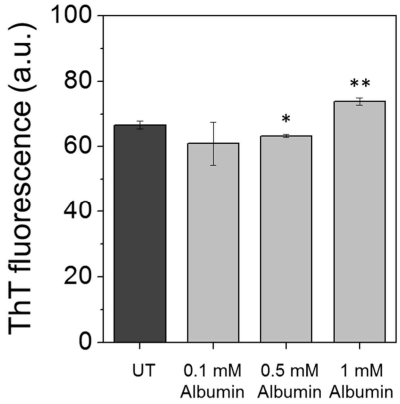

Fig. S4. Thioflavin T (ThT) fluorescence assay for samples treated only high concentration of albumin. UT indicates Aβ aggregates formed under destabilizing conditions (5 mM Cu^{2+}, pH 5) without treatment of anti-aggregation agents. *P < 0.05 and **P < 0.005 compared to untreated Aβ peptide solution (UT) incubated without test reagents under the same destabilizing conditions.

Supplementary References

1. S. Chah, M. R. Hammond and R. N. Zare, *Chem. Biol.,* 2005, **12**, 323-328.

2. K. Y. Ryu, R. Maehr, C. A. Gilchrist, M. A. Long, D. M. Bouley, B. Mueller, H. L. Ploegh and R. R. Kopito, *EMBO. J.,* 2007, **26**, 2693-2706.