Supporting Information
for
Effects of Clioquinol on Metal-Triggered Amyloid-β Aggregation Revisited

Allana M. Mancino, Sarmad S. Hindo, Akiko Kochi, and Mi Hee Lim*

Department of Chemistry and the Life Sciences Institute, University of Michigan, 210 Washtenaw Avenue, Ann Arbor, Michigan, 48109

Materials and Procedures

All reagents were purchased from commercial suppliers and used as received unless stated otherwise. All solutions including buffers were prepared from Millipore water, treated with chelex overnight, and filtered through a 0.22-μm acetate filter in order to remove any traces of metals. Optical spectra were obtained on an Agilent 8453 UV-visible spectrophotometer. CD measurements were acquired with a Jasco J-810 spectropolarimeter fitted with a 150-W xenon lamp. The BCA Protein Kit from Thermo Fisher Scientific was used and measurements were collected on a SpectraMax M5 microplate reader.

Amyloid-β Peptide Experiments. Amyloid-β (1-40) peptide (1 mg, EZBiolab) was purchased, dissolved with ammonium hydroxide (NH_4OH, 1% v/v, aq), aliquoted, lyophilized, and stored at –80 °C. The stock solution (ca. 200 μM) for the reactions was made by redissolving Aβ with NH_4OH (1% v/v, aq, 10 μL) followed by dilution with ddH_2O or with buffer solutions. All Aβ samples were prepared by previously reported procedures.[1,2] For the inhibition studies, solutions of samples contained freshly prepared Aβ (10, 25, or 100 μM), metal ion ($CuCl_2$ or $ZnCl_2$, 10, 25, or 100 μM) with or without CQ (20, 50, or 100 μM; the stock solution of CQ was in DMSO with a 1% final DMSO concentration). All solutions were incubated for 24 h at 37 °C with constant agitation, and analyzed by UV-vis, TEM, or BCA protein analysis. For the disaggregation studies, CQ (20, 50, or 200 μM; the stock solution was in DMSO with a 1% final DMSO concentration) was treated for 2 h or 24 h (37 °C, constant agitation)

with Aβ fibrils, generated by reacting freshly prepared Aβ (10, 25, or 100 μM) with or without metal ions (10, 25, or 100 μM) for 24 h at 37 °C with continuous agitation. Buffer solutions (20 μM HEPES, 150 μM NaCl) at pH 6.6 and pH 7.4 were used for Cu^{II}- and Zn^{II}-containing samples, respectively.[1]

BCA Protein Analysis. Samples containing freshly prepared Aβ (100 μM) and metal ions ($CuCl_2$ or $ZnCl_2$, 100 μM) were prepared for inhibition experiments. After 2 min, CQ (200 μM, the stock solution of CQ was in DMSO with a 1% final DMSO concentration) was added into the solution followed by incubation (5 min, 15 min, 30 min, 1 h, 2 h, 4 h, and 24 h) at 37 °C with continuous agitation. For disaggregation experiments, the freshly prepared solutions of Aβ (100 μM) in the absence or presence of metal ions (100 μM) were incubated for 24 h at 37 °C with continuous agitation followed by 2 or 24 h treatment of CQ (200 μM). All solutions were centrifuged at 14,000 rpm for 20 min. The supernatants of the samples were analyzed by the BCA Protein Assay Kit.

Transmission Electron Microscopy. Samples for TEM were prepared by following previously reported methods.[3,4] Glow-discharged grids (Formar/Carbon 300-mesh, Electron Microscopy Sciences) were treated with samples of Aβ aggregates (25 μM, 5 μL) for 2 min at room temperature. Excess sample was removed using filter paper followed by washing twice with ddH_2O. Grids were incubated with uranyl acetate (1% w/v, ddH_2O, 5 μL, 1 min) and were blotted with filter paper and dried for 15 min at room temperature. Images were taken on a Philips CM-100 transmission electron microscope (60 – 80 kV, 19000 – 46000 magnification).

Full Citations of References

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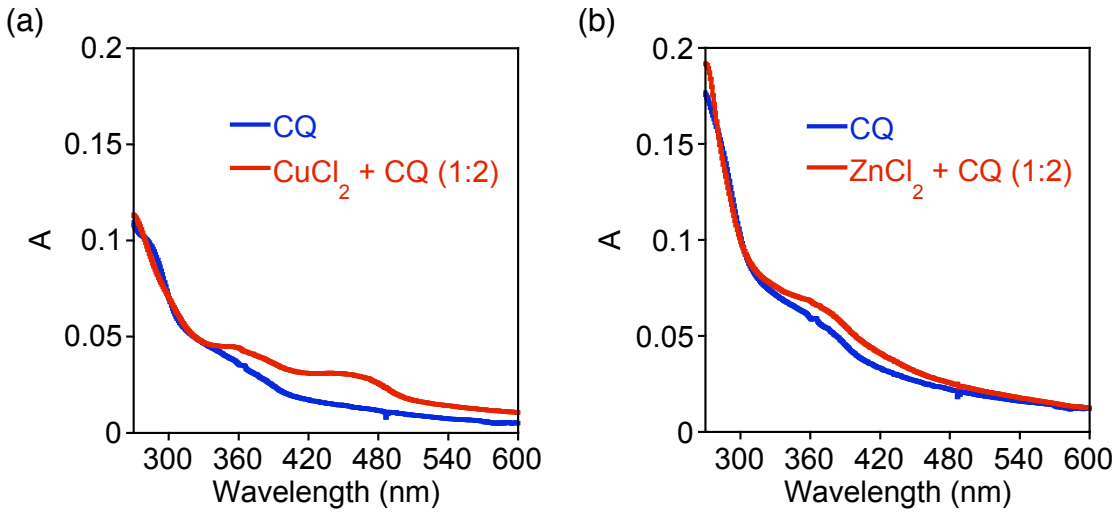

Figure S1. UV-visible spectra of solutions containing CQ (20 μM) with (a) $CuCl_2$ (10 μM) and (b) $ZnCl_2$ (10 μM) at pH 6.6 and pH 7.4, respectively.

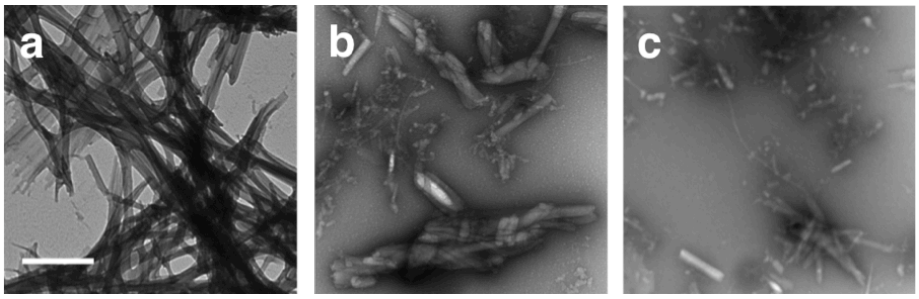

Figure S2. TEM images of Aβ samples (pH 7.4) in the (a) absence or (b) presence of CQ and (c) Aβ species (pH 6.6) incubated with the Cu complex, generated *in situ* by reacting $CuCl_2$ and CQ in a ratio of 1:2 for 30 min prior to addition to peptide ([Aβ] = 25 μM, [$CuCl_2$] = 25 μM, [CQ] = 50 μM, 24 h, 37 °C, constant agitation, scale bar = 500 nm).

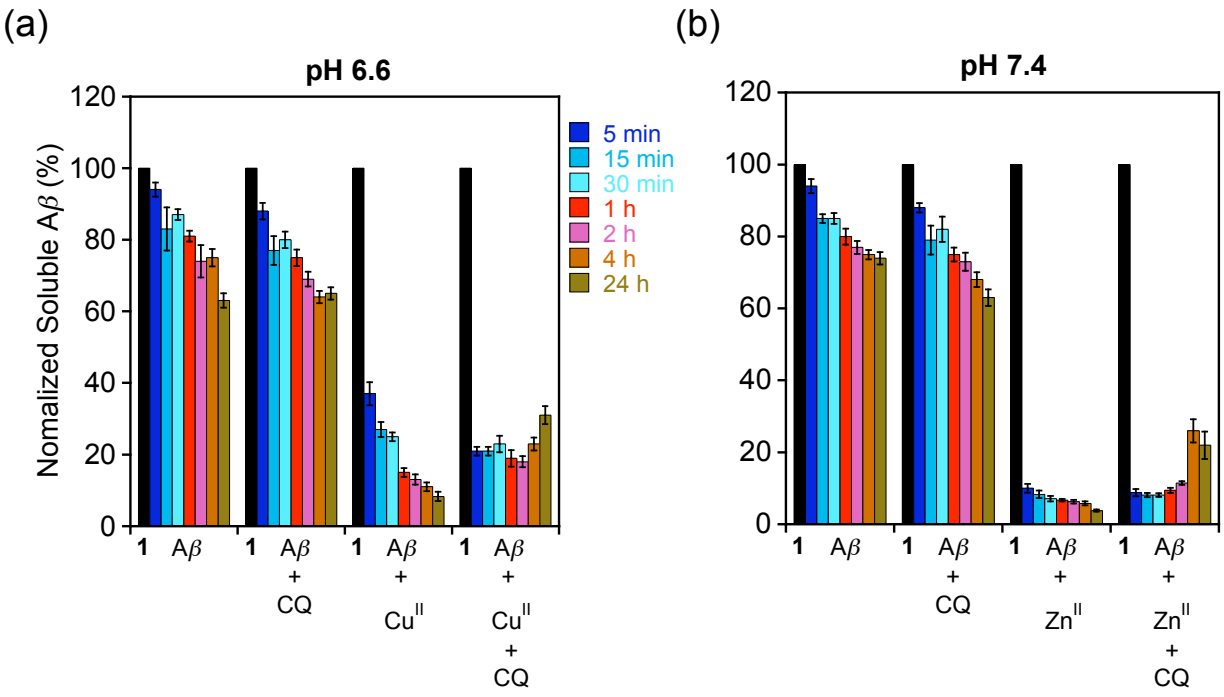

Figure S3. Analysis of soluble Aβ (%) in the samples of inhibition experiments (Aβ and metal-Aβ in the absence and presence of CQ) incubated for 5 min – 24 h ([Aβ] = 100 μM, [$CuCl_2$ or $ZnCl_2$] = 100 μM, [CQ] = 200 μM, 37 °C, constant agitation). The freshly prepared solution of Aβ (**1**, black) was depicted in the figures. The absorbance was measured at 580 nm.

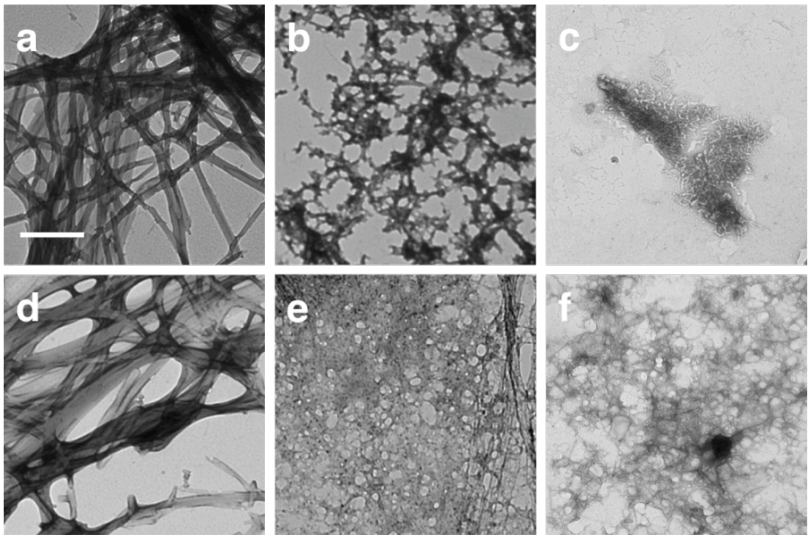

Figure S4. TEM analysis of Aβ species upon treatment of CQ. (a) Aβ aggregates (pH 6.6, 24 h); Aβ aggregates incubated with CQ (b) for 2 h and (c) for 24 h; (d) Aβ aggregates (pH 7.4, 24 h); Aβ aggregates incubated with CQ (e) for 2 h and (f) for 24 h ([Aβ] = 25 μM, [CQ] = 50 μM, 37 °C, constant agitation, scale bar = 500 nm).

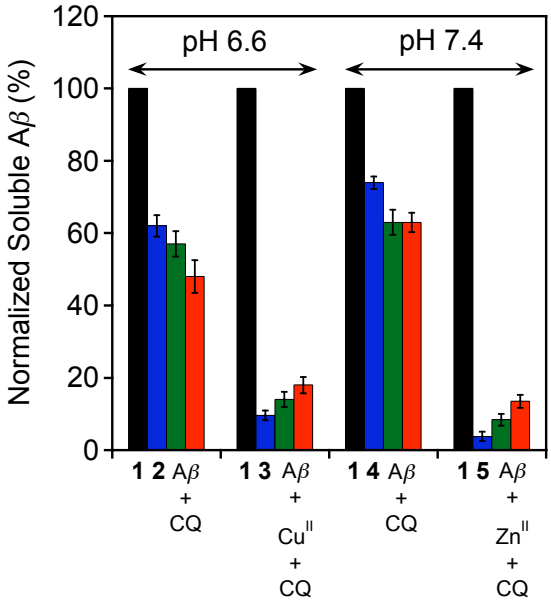

Figure S5. BCA protein analysis of samples from the disaggregation studies. The contents of soluble Aβ species from the 24 h incubated samples of Aβ (**2** and **4**), Aβ + Cu^{II} (**3**), and Aβ + Zn^{II} (**5**) were presented in blue. Solutions of Aβ aggregates were treated with CQ for 2 h (green) or 24 h (red) ([Aβ] = 100 μM, [$CuCl_2$ or $ZnCl_2$] = 100 μM, [CQ] = 200 μM, 37 °C, constant agitation). The absorbance was measured at 580 nm. The freshly prepared solution of Aβ (**1**) was also analyzed.

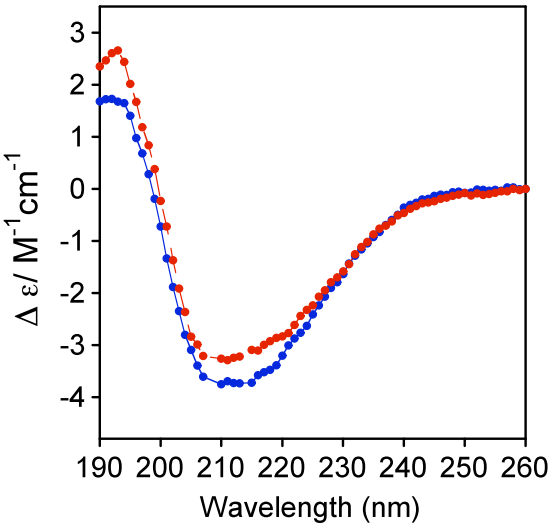

Figure S6. CD spectrum of the sample prepared by incubation of Aβ (50 μM) with $ZnCl_2$ (50 μM) at 37 °C with constant agitation for 24 h (blue line) in 10 mM sodium phosphate buffer, pH 7.4, 100 mM NaF. Upon 5 h treatment of CQ (100 μM) with Zn(II)-induced fibrils, the spectrum (red line) was obtained. All spectra were measured at 1 nm intervals from 190 – 260 nm with a scanning speed of 50 nm/min and a bandwidth of 2.0 nm. Spectra were reported as the average of 20 scans after the subtraction of measurements in the absence of peptide. All measurements were taken using a 1 mm cuvette at 37 °C.

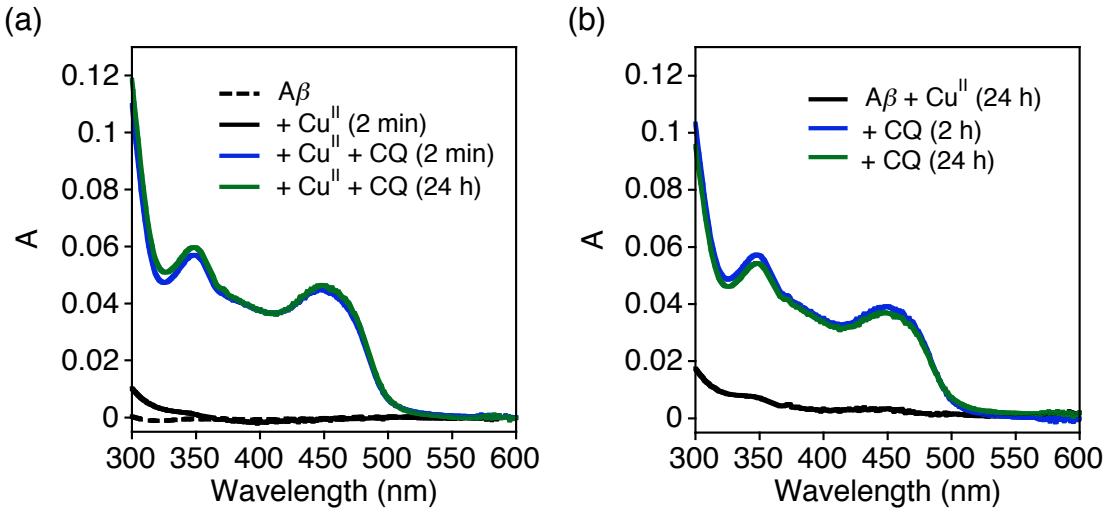

Figure S7. UV-visible spectra of the samples from the (a) inhibition and (b) disaggregation experiments. Reactions were carried out using Aβ (10 μM), $CuCl_2$ (10 μM), and CQ (20 μM) at pH 6.6.