Supporting Information for:

Epigallocatechin-3-gallate Inhibits Cu(II)-induced β-2-microglobulin Amyloid Formation by Binding to the Edge of its β-sheets

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Supplemental Methods:

Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) of Size Exclusion Chromatography (SEC) Fractions

SEC fractions from the SuperSW2000 column were obtained using the same SEC method described in the main text, but the effluent was collected from the column, rather than allowing it to flow through the detector. Based on matching retention times from prior SEC runs, the middle ~66% of the chromatographic peak was collected, which corresponded to approximately 1 minute. The samples (200 μM β2m, 400 μM $CuSO_4$, and 500 μM EGCG) were incubated for 2 days at 37°C prior to injection. Immediately after collection, the SEC fractions were injected on an Agilent 1260 HPLC (Santa Clara, CA), equipped with an Agilent Eclipse XDB-C8 reversed-phase column (P/N 993967-906). The mobile phase solvents consisted of (A) MilliQ H_2O with 0.1% acetic acid and (B) acetonitrile with 0.1% acetic acid. The reversed-phase LC method consisted of an initial 10 minute equilibration at 99% A, and then a gradual ramp to 99% B over the next 60 minutes. There was 5 minute hold at 99% B, followed by a drop back to 1% B, and then a 10 minute post-separation equilibration before the next sample. The flow rate was 0.2 mL/min. On separate dedicated runs, the VWD detector was set to both 214 nm for detection of protein and to 265 for the detection of (primarily) EGCG.

Reverse Phase-Liquid Chromatography-Mass Spectrometry (LC-MS) of SEC Fractions

Reverse phase LC-MS was carried out on an Agilent 1100 HPLC fitted with a OPTI-TRAP Micro column (Cat# 10-04816-TM) (Optimize Technologies, Oregon City, OR) that was interfaced with a Bruker Amazon (Billerica, MA) quadrupole ion trap mass spectrometer fitted with an electrospray ionization source. SEC fractions were as described above. Reversed-phase LC separation was performed using a binary mobile phase system that consisted of (A) water with 0.1% acetic acid and (B) acetonitrile with 0.1% acetic acid. The flow rate was 0.2 mL/min. Following injection, the column was equilibrated at 5% B for 3 minutes, then moved to 90% over the next 5 minutes. The column was then at 100% B for 5 minutes, and then returned to 5% for column equilibration. The electrospray needle voltage was kept at 3.9 kV, and the capillary temperature was set to 250°C. Mass spectra were acquired from *m/z* 300-2200.

Determination of β2m affinity for Cu(II)

In order to measure the binding affinity of Cu(II) to β2m, a fluorescence quenching method was used.[1,2] Tryptophan fluorescence was monitored via excitation at 295 nm while emission was monitored from 300 to 400 nm using a PTI Quantamaster 300 (Edison, NJ). The solution conditions were: 2.5 μM β2m, 0.1 to 75 μM $CuSO_4$, 150 mM potassium acetate, 25 mM MOPS buffer, and 290 μM EGCG at pH 7.4. Prior to the measurements, the samples were equilibrated at room temperature for 15 minutes. The fraction bound was determined by measuring the average emission intensity ($<\lambda>$) via intrinsic tryptophan fluorescence. Free Cu(II) concentrations in each samples were calculated iteratively from the nominally added concentrations using Hyperquad Simulation and Speciation (HySS) software (Protonic Software). Data were plotted using Origin (Northampton, MA) where titration data were fitted using a sigmoidal function.

Circular Dichroism (CD)

CD data were acquired on a Jasco J-1500 spectrophotometer. Data were collected by scanning from 250 to 195 nm with a data pitch of 0.5 nm using a scan rate of 20 nm/min. Three total scans were averaged to generate the final results shown here. Solution conditions were similar to the ones described above, but contained 25 μM β2m, 50 μM Cu(II), and 25 μM EGCG. Urea was omitted from the sample in order to generate a higher quality spectra. Samples were equilibrated and measured at 20°C. Data were analyzed using Spectra Analysis.

Supplemental Results:

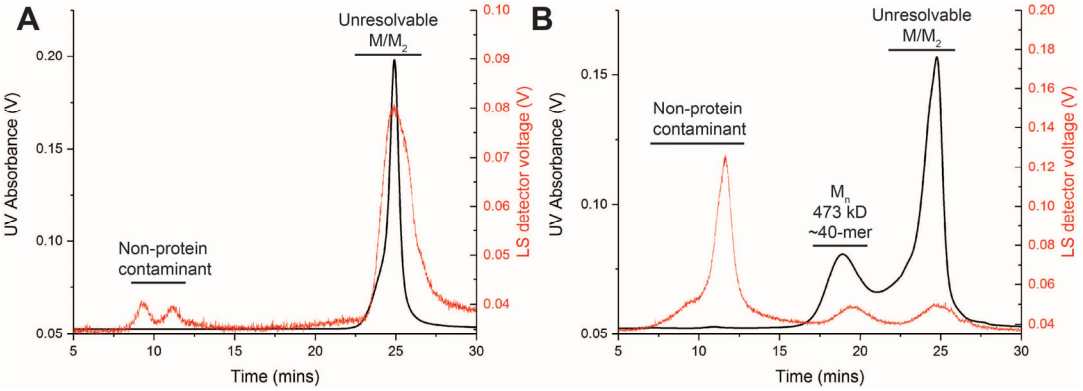

Figure S1. Comparison of SEC-MALS data for (A) β2m under amyloid forming conditions without EGCG and (B) β2m under amyloid forming conditions with a 1:1 ratio of EGCG (50 μM). The black trace is UV absorbance at 214 nm, while the red trace is light scattering data from one of the light scattering (LS) detectors.

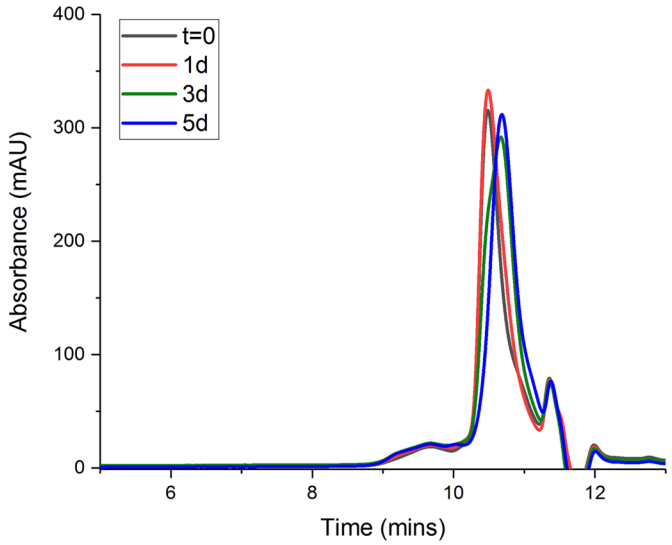

Figure S2. EGCG has no effect on β2m oligomerization in the absence of Cu(II). SEC chromatograms at increasing incubation times for incubated samples that contained 1:1 β2m and EGCG, but no Cu(II).

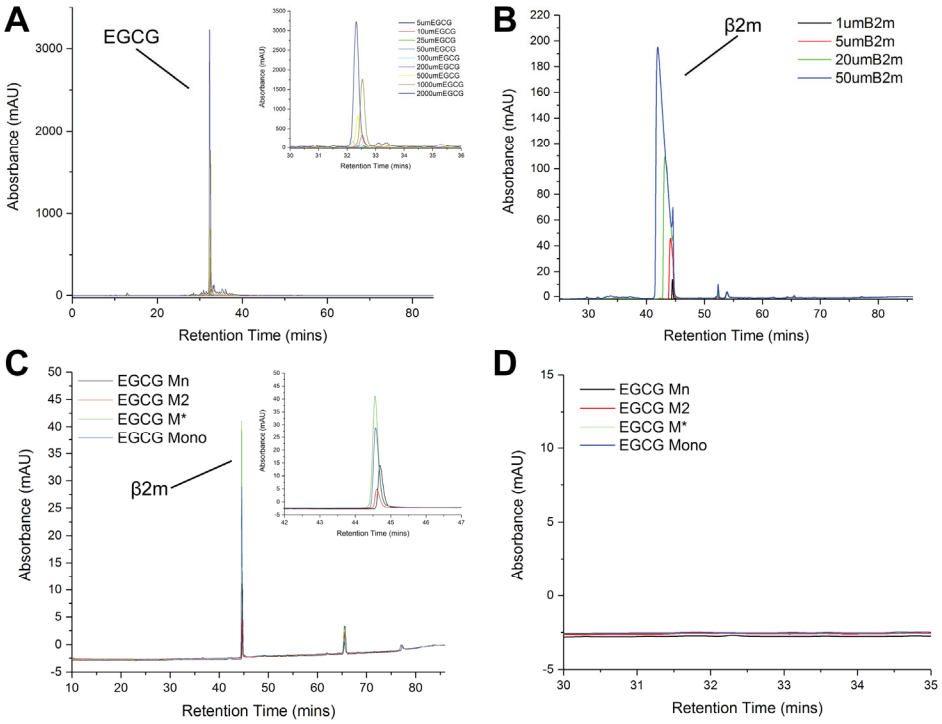

Figure S3. Reverse phase HPLC (RP-HPLC) of collected SEC fractions. Panels A and B are calibration experiments showing the retention times and abundances of (A) EGCG and (B) pure β2m dissolved in water at different concentrations. Panel C shows injections of indicated peak fractions collected from SEC after incubation for 2 days under amyloid-forming conditions. The inset shows an expanded region around the time β2m elutes. Panel D is the same experiment as C but expanded on the x-axis where EGCG elutes to show that no measurable amount of EGCG elutes with any of the collected fractions.

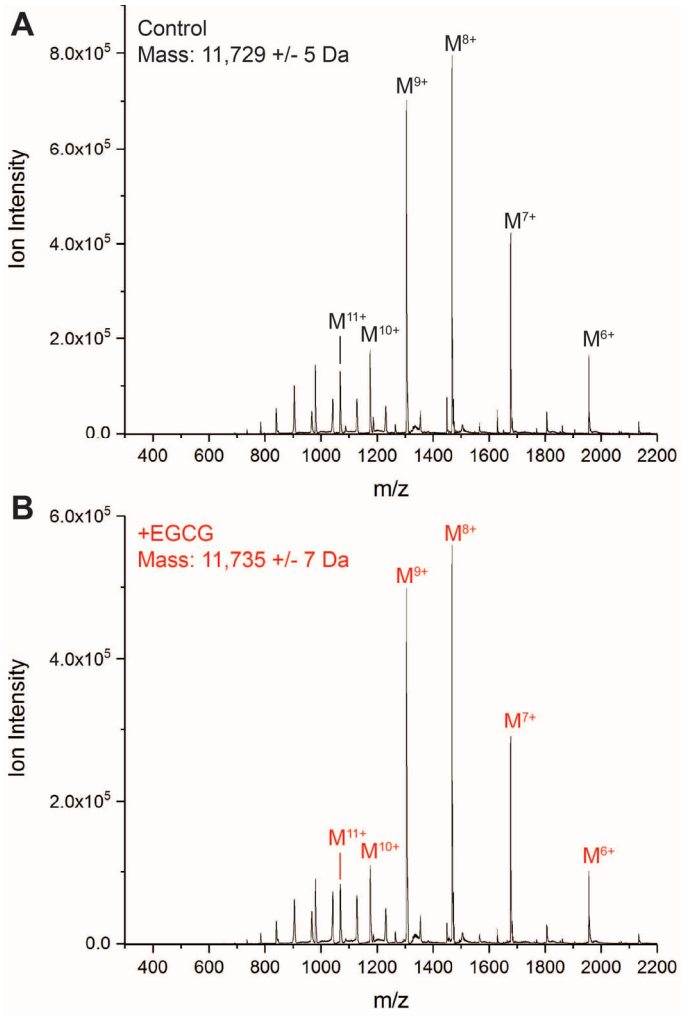

Figure S4. Reverse phase LC-MS of monomer fractions from SEC separations. Panel A is the mass spectrum for the β2m monomer under normal amyloid-forming conditions in the presence of Cu(II). Panel B is a sample incubated under identical conditions as in panel A but with EGCG (500 μM) present. The annotated peaks refer to β2m-related peaks and their associated charge states. The deconvoluted mass for each sample is shown in the upper left hand corner, ± standard deviation.

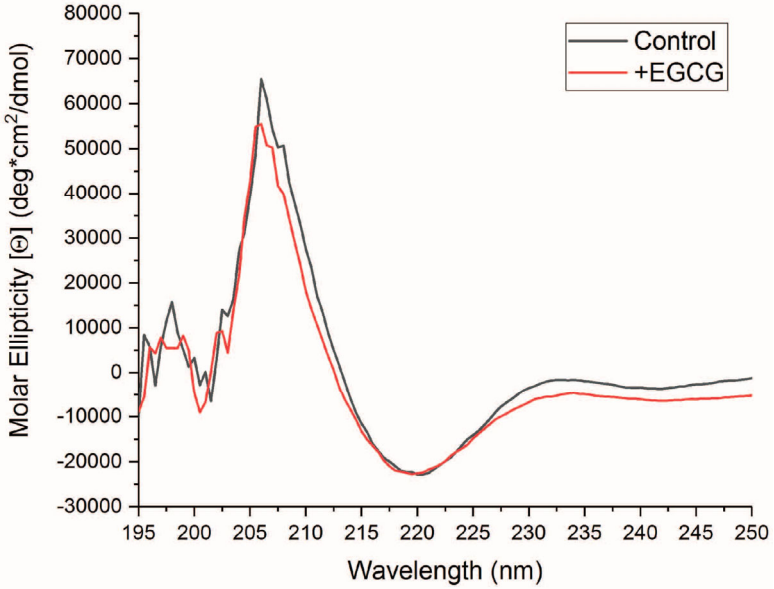

Figure S5. Circular dichroism spectra comparing β2m under amyloid forming conditions without and with EGCG after 15 minutes of incubation. The sample contained 25 μM β2m, 50 μM Cu(II), and 25 μM EGCG where applicable.

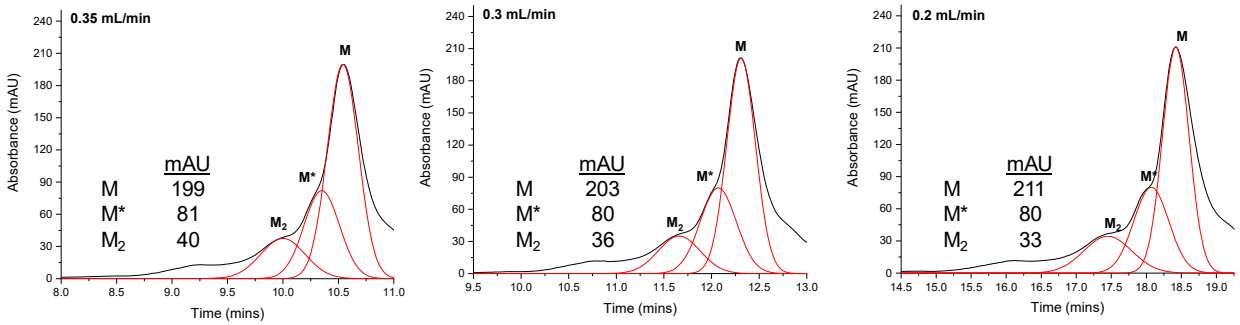

Figure S6. Effect of changing flow rate on abundances of M, M*, and M_2.

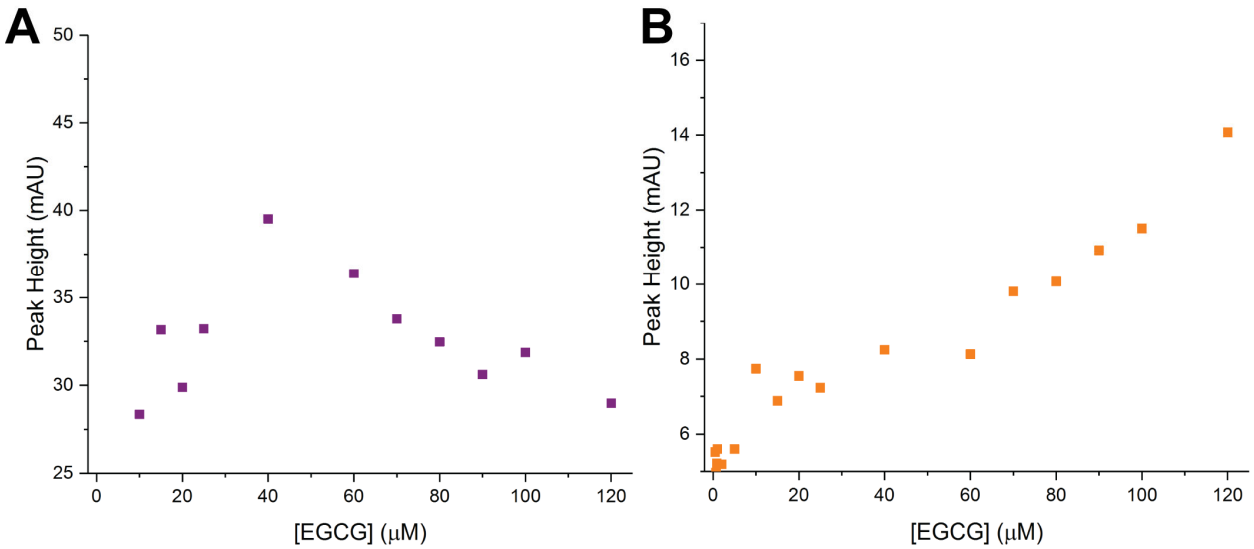

Figure S7. SEC determined abundances of the β2m (A) dimer and (B) tetramer under amyloid-forming conditions in the presence of increasing concentrations of EGCG after 5 days of incubation.

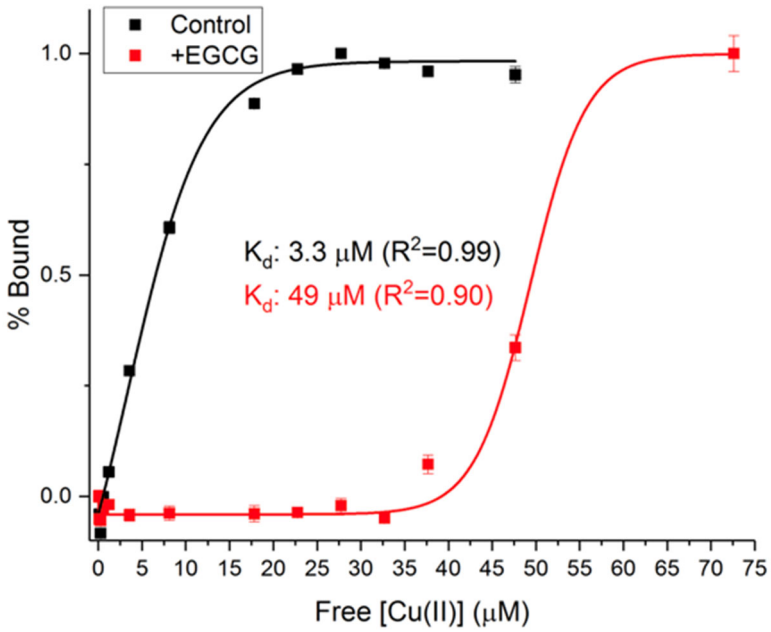

Figure S8: Fluorescence-based measurement of β2m binding affinity for Cu(II) in the presence (red) or absence (black) of EGCG. The measured K_d value in the absence of EGCG is similar to previously obtained values.[1,2] Analyses were performed in triplicate, and the error bars represent standard deviations.

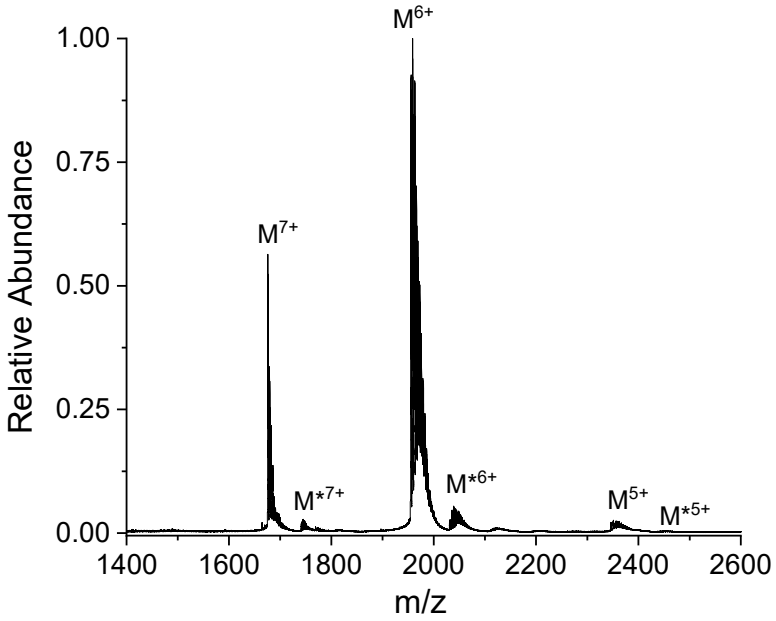

Figure S9. Native electrospray ionization spectrum, showing EGCG bound to the β2m monomer. This spectrum was obtained from a solution containing 10 μM β2m, 20 μM Cu(II), 10 μM EGCG, and 100 mM ammonium acetate at a pH of 6.9. The charge states labeled with an asterisk (*) have EGCG bound.

Supplemental References:

(1) Marcinko, T. M., Dong, J., LeBlanc, R., Daborowski, K. V., and Vachet, R. W. (2017) Small molecule-mediated inhibition of β-2-microglobulin-based amyloid fibril formation. *Journal of Biological Chemistry 292*, 10630–10638.
(2) Blaho, D. V., and Miranker, A. D. (2009) Delineating the Conformational Elements Responsible for Cu^{2+}-Induced Oligomerization of β-2 Microglobulin. *Biochemistry 48*, 6610–6617.