Electronic Supplementary Material (ESI) for Metallomics.

Supporting Information

for

Calprotectin influences the aggregation of metal-free and metal-bound amyloid-β by direct interaction

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Preparation and biochemical characterization of $S100B_{\Delta}$

Design of the synthetic gene for $S100B_{\Delta}$. To obtain the protein variant where the metal-binding residues are mutated to the non-coordinating Ala residues, a synthetic gene containing an *E. coli*-optimized sequence for *S100B(H16A)(H26A)(H86A)(E90A)* was designed and purchased from DNA2.0 (now ATUM). The synthetic gene contained an *N*-terminal *NdeI* restriction site and a *C*-terminal stop codon followed by a *XhoI* restriction site. The gene was ligated into the *NdeI* and *Xho*I sites of pET41a by DNA2.0.

NdeI-$S100B_{\Delta}$-STOP-XhoI

CATATGAGCGAGTTGGAGAAAGCGATGGTTGCCCTGATTGACGTTTTTGCACAGTA
CAGCGGTCGTGAGGGCGATAAGGCTAAGCTGAAGAAAAGCGAGCTGAAAGAACTG
ATCAACAACGAACTGTCCCACTTCCTGGAAGAAATCAAAGAACAGGAAGTCGTGGA
CAAAGTTATGGAAACGCTGGATAATGATGGTGACGGCGAGTGTGACTTTCAAGAATT
CATGGCGTTTGTCGCGATGGTGACCACCGCGTGCGCCGAGTTCTTCGCACACGAG
TAACTCGAG

The restriction sites are underlined; the stop codon is in bold font.

Translation

H M S E L E K A M V A L I D V F A Q Y S G R E G D K A K L K K S E L K E L I N N E L S
H F L E E I K E Q E V V D K V M E T L D N D G D G E C D F Q E F M A F V A M V T T
A C A E F F A H E **STOP** L E

The restriction sites are underlined; the stop codon is in bold font; the mutated residues are highlighted in yellow.

Preparation of S100B$_\Delta$. S100B$_\Delta$ [S100B(H16A)(H26A)(H86A)(E90A)] was overexpressed and purified as described previously for S100B.[1] The pET41a-*S100B(H16A)(H26A)(H86A)(E90A)* plasmid was obtained from DNA2.0, dissolved in 20 μL Milli-Q water, and transformed into chemically competent *E. coli* BL21(DE3) cells. The cells were incubated at 37 ºC overnight, and freezer stocks were prepared with 25% glycerol and stored at –80 ºC. Purified S100B$_\Delta$ was obtained as the apo homodimeric protein and was stored in buffer containing 5 mM DTT (20 mM HEPES, pH 8.0, 100 mM NaCl, 5 mM DTT). Protein concentrations are reported as the homodimer determined using the extinction coefficient (ε_{280} = 2980 M^{-1} cm^{-1}) from the ExPASy ProtParam online tool.

Biochemical characterization of S100B$_\Delta$. S100B$_\Delta$ was characterized by mass spectrometry, SDS-PAGE, circular dichroism (CD) spectroscopy, and analytical size exclusion chromatography (SEC). Together, the data are in agreement with expectations based on S100B and other S100 proteins, and indicate that S100B$_\Delta$ was obtained in high purity as an α-helical, homodimeric protein.

Mass spectrometry: The identity of S100B$_\Delta$ was confirmed by liquid chromatography mass spectrometry (LC-MS) using an Agilent Poroshell 300SB-C18 column on an Agilent 1260 LC system with an Agilent Jetstream ESI following a protocol as previously described.[2] The calculated masses of the S100B monomer are 10,456.8 (+Met1) and 10,325.6 Da (–Met1), and the masses 10,456.96 Da and 10,325.62 Da (–Met1) were observed.

Purity: The purity of $S100B_{\Delta}$ was evaluated by SDS-PAGE gel (15% Tris-glycine gel), which revealed one major band at *ca.* 10 kDa (Fig. S1a).

Secondary structure: CD spectroscopy was conducted using an Aviv Model 202 CD spectrometer housed in the MIT Biophysical Instrumentation Facility. CD spectra of 10 μM $S100B_{\Delta}$ in 1 mM Tris, pH 8.5, 0.5 mM EDTA in the absence and presence of $CaCl_2$ (2 mM) were collected. These spectra are consistent with α-helical secondary structure, as expected for the S100 protein (Fig. S1b).

Quaternary structure: Analytical SEC was conducted using an ÄKTA purification system with a Superdex 75 10/300 GL column (GE Healthcare Life Sciences), and the column was calibrated using a low-molecular-weight protein standard calibration (GE Healthcare Life Sciences) described previously.[2] The protein (100 μL, 100 μM) was analyzed by SEC in 75 mM HEPES, pH 7.5, 100 mM NaCl, 5 mM DTT in absence and presence of $CaCl_2$ (2 mM) in the running buffer. The variant elutes at 11.8 mL (–Ca(II)) and 12.0 mL (+Ca(II)), consistent with the formation of the 21.0-kDa homodimer under both low and high Ca(II) conditions (Fig. S1c and d).

References

1 M. B. Brophy, T. G. Nakashige, A. Gaillard and E. M. Nolan, Contributions of the S100A9 C-terminal tail to high-affinity Mn(II) chelation by the host-defense protein human calprotectin, *J. Am. Chem. Soc.,* 2013, **135**, 17804–17817.

2 M. B. Brophy, J. A. Hayden and E. M. Nolan, Calcium ion gradients modulate the zinc affinity and antibacterial activity of human calprotectin, *J. Am. Chem. Soc.*, 2012, **134**, 18089–18100.

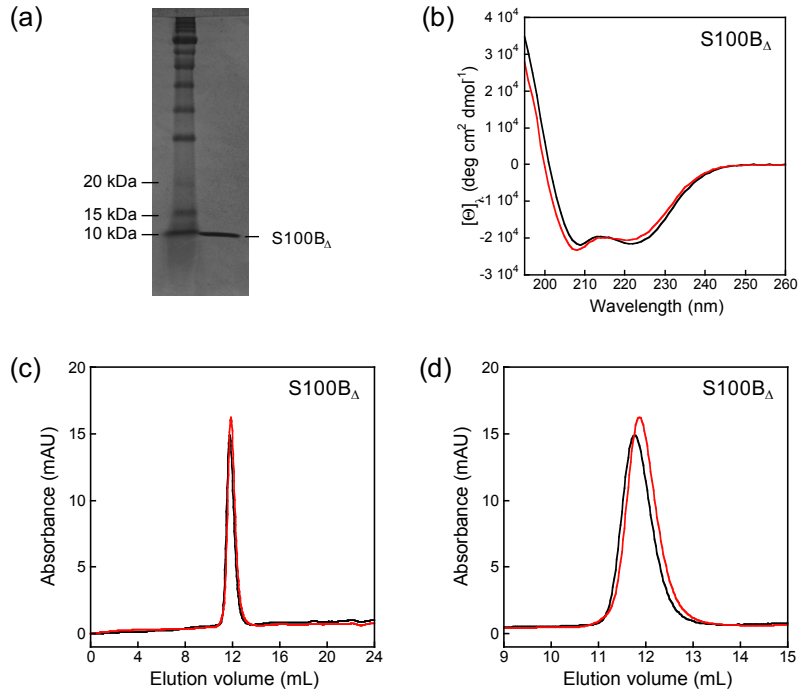

Fig. S1 Characterization of S100B$_\Delta$. (a) SDS-PAGE gel (15% Tris-glycine) of S100B$_\Delta$. (b) CD spectra of S100B$_\Delta$ (10 μM) in 1 mM Tris, pH 8.5, 0.5 mM EDTA in the absence (black line) and presence (red line) of $CaCl_2$ (2 mM). (c) Analytical SEC chromatograms of S100B$_\Delta$ (100 μL, 100 μM) in 75 mM HEPES, pH 7.5, 100 mM NaCl, 5 mM DTT in the absence (black line) and presence (red line) of $CaCl_2$ (2 mM) in the running buffer. (d) Zoom-in view of analytical SEC chromatograms.

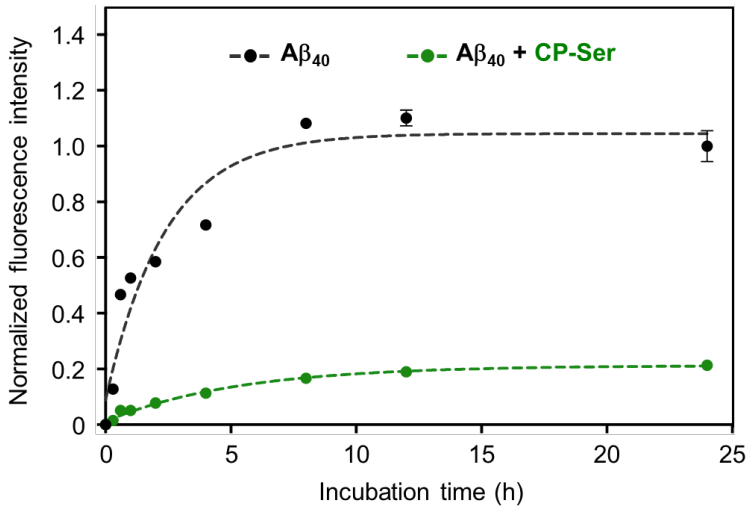

Fig. S2 Influence of CP-Ser on metal-free $A\beta_{40}$ aggregation kinetics, observed by the ThT assay. Conditions: [$A\beta_{40}$] = 20 μM; [CP-Ser] = 20 μM; [ThT] = 20 μM; pH 7.4; 37 °C; constant agitation.

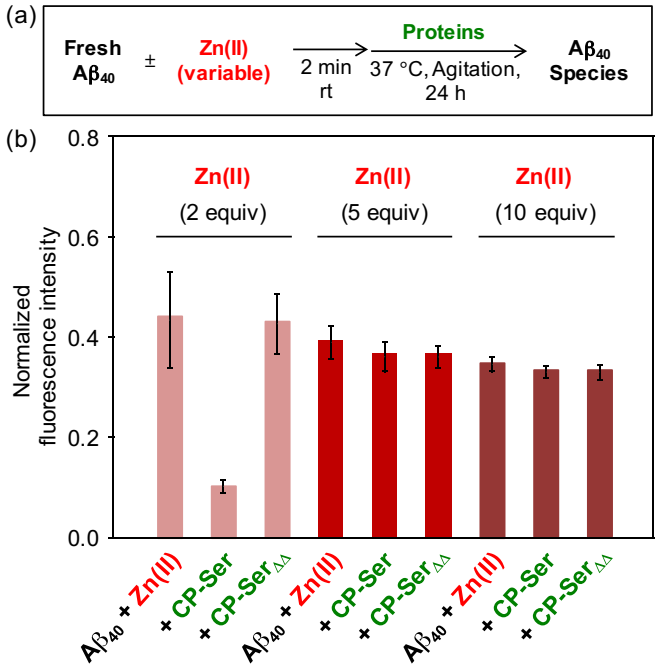

Fig. S3 Effects of CP-Ser and CP-Ser$_{\Delta\Delta}$ on Aβ_{40} aggregation in the presence of excess Zn(II). (a) Scheme of the experiment. (b) Determination of the degree of β-sheet-rich Aβ_{40} aggregates formation, observed by the ThT assay, from the 24 h incubated Aβ_{42} samples with CP-Ser and CP-Ser$_{\Delta\Delta}$ in the presence of 2, 5, and 10 equiv of Zn(II). Fluorescence intensities of ThT from the samples were normalized by comparison to the metal-free Aβ_{40} sample (shown in Fig. 1). Conditions: [Aβ_{40}] = 20 μM; [$ZnCl_2$] = 40, 100, and 200 μM; [CP-Ser and CP-Ser$_{\Delta\Delta}$] = 20 μM; pH 7.4; 24 h incubation; 37 °C; constant agitation.

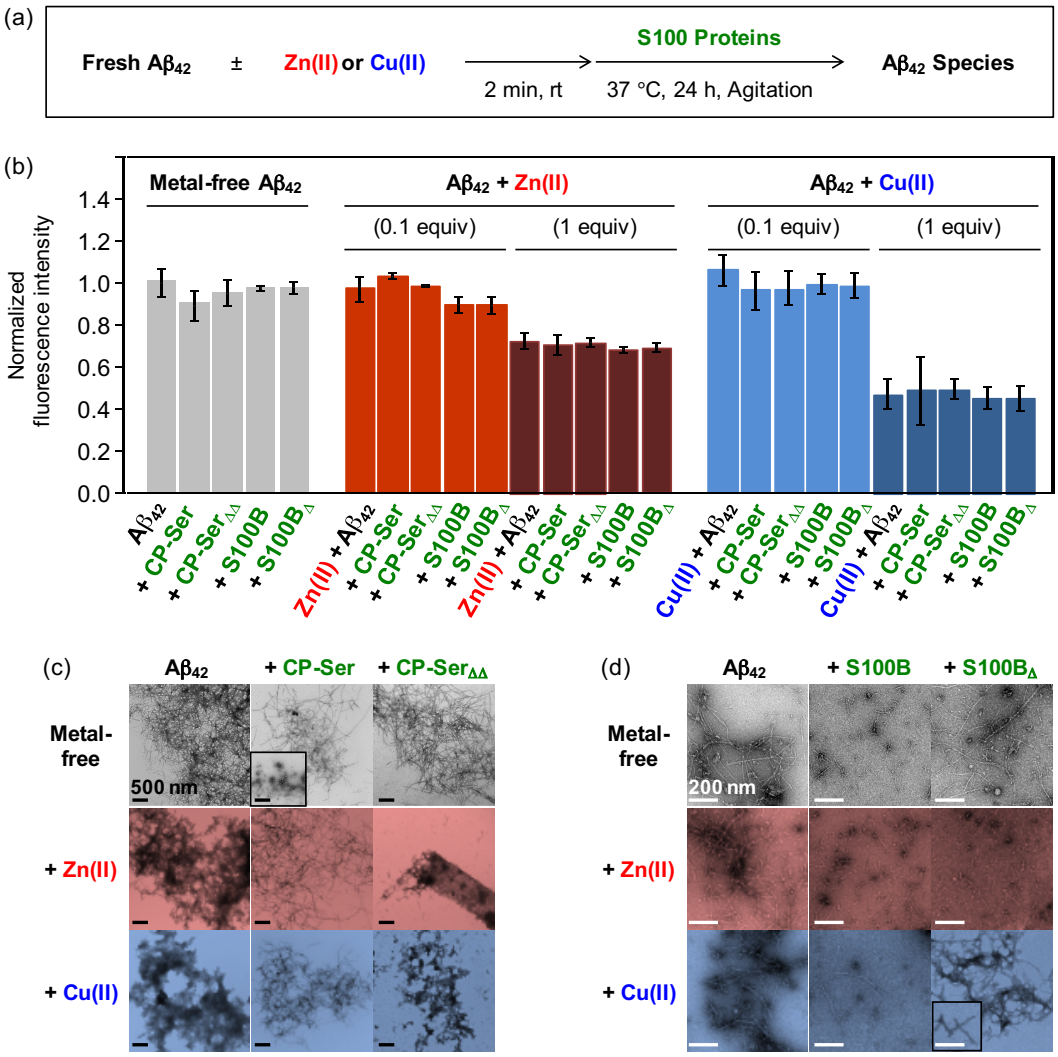

Fig. S4 Influence of S100 proteins on the aggregation of both metal-free Aβ$_{42}$ and metal–Aβ$_{42}$. (a) Scheme of the experiment. (b) Analysis of the degree of β-sheet-rich Aβ$_{42}$ aggregates formation, monitored by the ThT assay, from the 24 h incubated Aβ samples with CP-Ser, CP-Ser$_{\Delta\Delta}$, S100B, and S100B$_{\Delta}$. (c) [CP-Ser and CP-Ser$_{\Delta\Delta}$] or (d) [S100B and S100B$_{\Delta}$], monitored through TEM. The inset images represent minor species. Conditions: [Aβ$_{42}$] = 20 μM; [$ZnCl_2$ or $CuCl_2$] = 2 or 20 μM; [S100 proteins] = 20 μM; pH 7.4 (for metal-free and Zn(II) experiments) or pH 6.6 (for Cu(II) experiments); 24 h incubation; 37 °C; constant agitation. Scale bar = 500 (black) or 200 (white) nm.

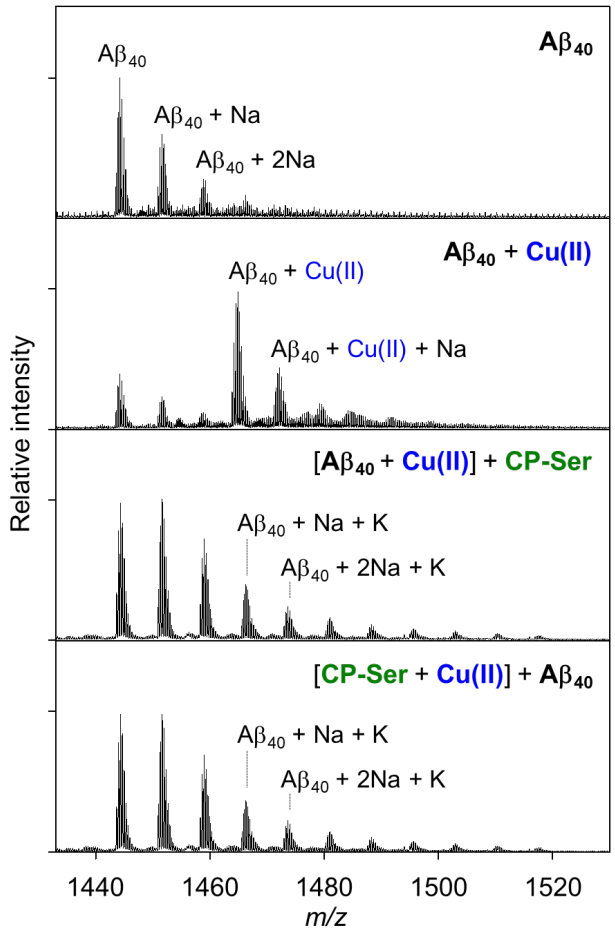

Fig. S5 ESI-MS analyses of the samples containing $A\beta_{40}$, Cu(II), and CP-Ser. The ESI-MS spectra for +3-charged monomers of $A\beta_{40}$ with and without treatment of CP-Ser and Cu(II). Two different addition orders of $A\beta_{40}$, Cu(II), and CP-Ser: (i) $A\beta_{40}$ treated with Cu(II) followed by addition of CP-Ser; (ii) CP-Ser treated with Cu(II) followed by $A\beta_{40}$. Conditions (final concentrations): [$A\beta_{40}$] = 5 μM; [$CuCl_2$] = 5 μM; [CP-Ser] = 5 μM; pH 7.5; 1 h incubation; 37 °C; no agitation.

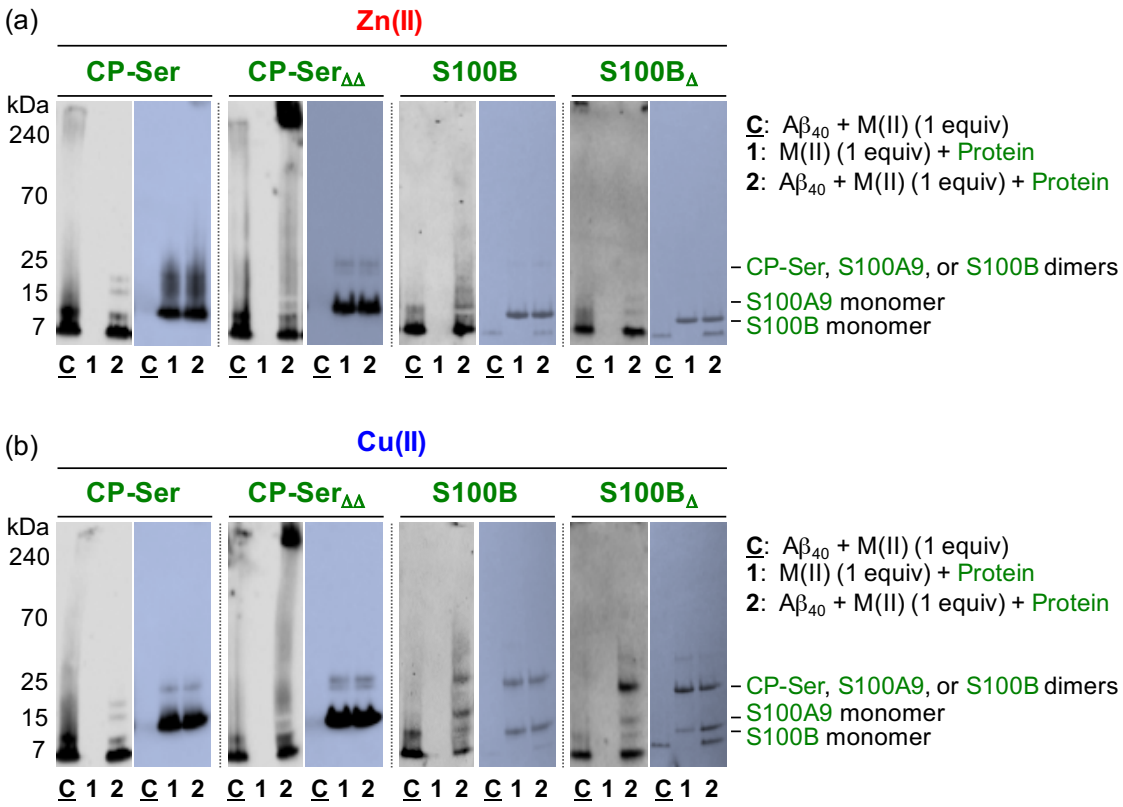

Fig. S6 Analysis of the resultant Aβ$_{40}$ species upon treatment with S100 proteins in the presence of metal ions. Aβ$_{40}$ species produced after 24 h incubation with or without the proteins with 1 equiv of (a) Zn(II) or (b) Cu(II), visualized by the gel/Western blot using an anti-Aβ antibody (6E10; gray gels) or an anti-S100A9 antibody (blue gels; CP-Ser and CP-Ser$_{\Delta\Delta}$) as well as Coomassie blue staining (blue gels; S100B and S100B$_{\Delta}$). Lanes: (Control, C) [Aβ$_{40}$ + M(II)]; (1) [M(II) + S100 proteins]; (2) (C) + S100 proteins. Conditions: [Aβ$_{40}$] = 20 μM; [$ZnCl_2$ or $CuCl_2$] = 20 μM; [S100 proteins] = 20 μM; pH 7.4 (for metal-free and Zn(II) experiments) or pH 6.6 (for Cu(II) experiment); 24 h incubation; 37 °C; constant agitation.

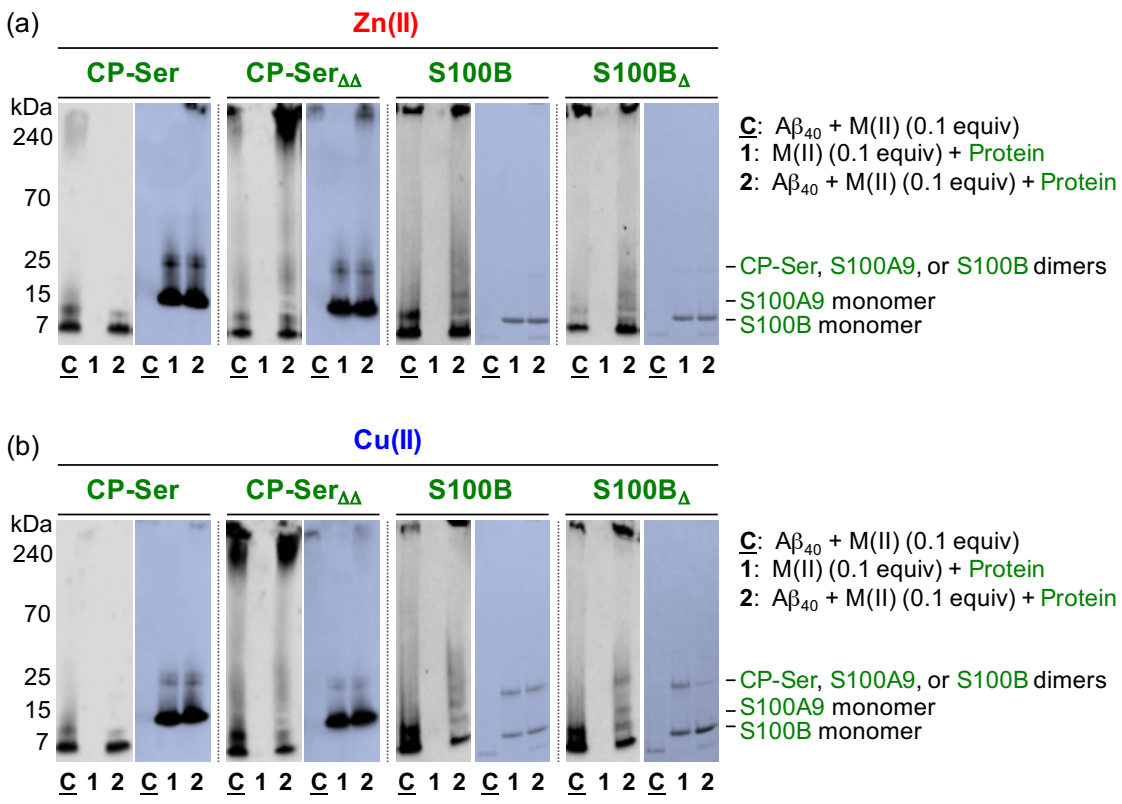

Fig. S7 Analysis of the resultant $A\beta_{40}$ species upon addition of S100 with sub-stoichiometric amounts of metal ions. $A\beta_{40}$ species generated after 24 h incubation with or without the proteins with 0.1 equiv of (a) Zn(II) or (b) Cu(II), visualized by the gel/Western blot using an anti-Aβ antibody (6E10; gray gels) or an anti-S100A9 antibody (blue gels; CP-Ser and CP-Ser$_{\Delta\Delta}$) as well as Coomassie blue staining (blue gels; S100B and S100B$_{\Delta}$). Lanes: (Control, C) [$A\beta_{40}$ + M(II)]; (1) [M(II) + S100 proteins]; (2) (C) + S100 proteins. Conditions: [$A\beta_{40}$] = 20 μM; [$ZnCl_2$ or $CuCl_2$] = 2 μM; [S100 proteins] = 20 μM; pH 7.4 (for metal-free and Zn(II) experiments) or pH 6.6 (for Cu(II) experiment); 24 h incubation; 37 °C; constant agitation.

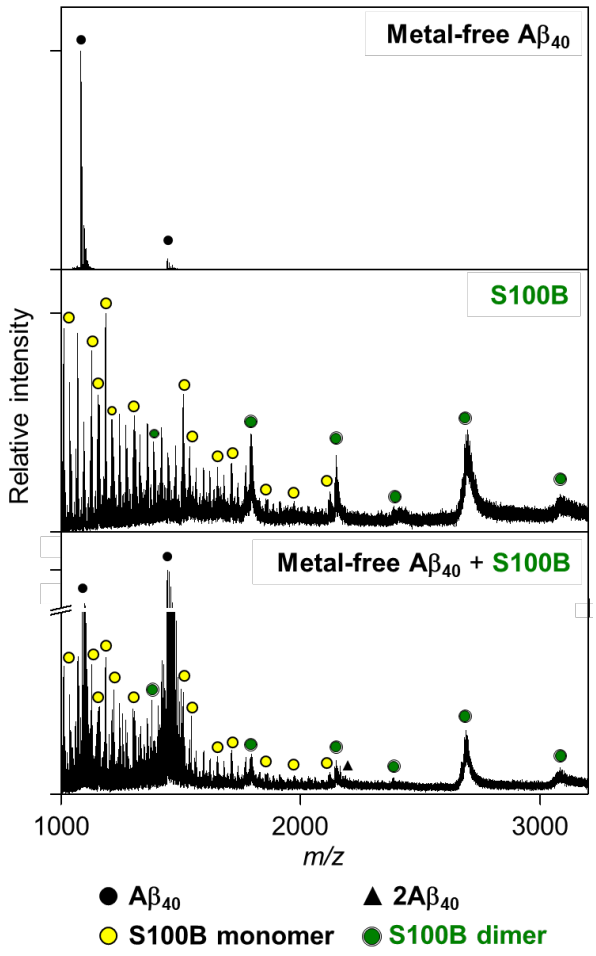

Fig. S8 ESI-MS analyses of metal-free $A\beta_{40}$ (top), S100B (middle), and metal-free $A\beta_{40}$ treated with S100B (bottom) for monitoring the interaction between S100B and $A\beta_{40}$. Conditions (final concentrations): [$A\beta_{40}$] = 5 μM; [S100B] = 5 μM; pH 7.5; 24 h incubation; 37 °C; no agitation.

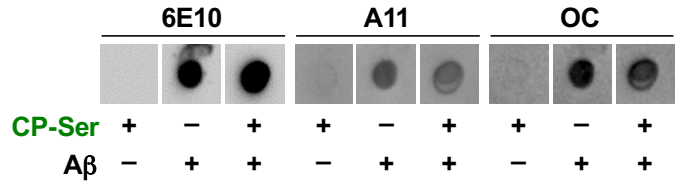

Fig. S9 Dot blot assay of the pre-incubated (for 8 h) CP-Ser with and without $A\beta_{40}$ before incubation with SH-SY5Y (5Y) cells. The resultant $A\beta_{40}$ species were monitored by an anti-Aβ antibody (6E10), an anti-amyloid oligomer antibody (A11), and an anti-amyloid fibril antibody (OC). Conditions: [$A\beta_{40}$] = 20 μM; [CP-Ser] = 20 μM; pH 7.4; incubation for 8 h; 37 °C.

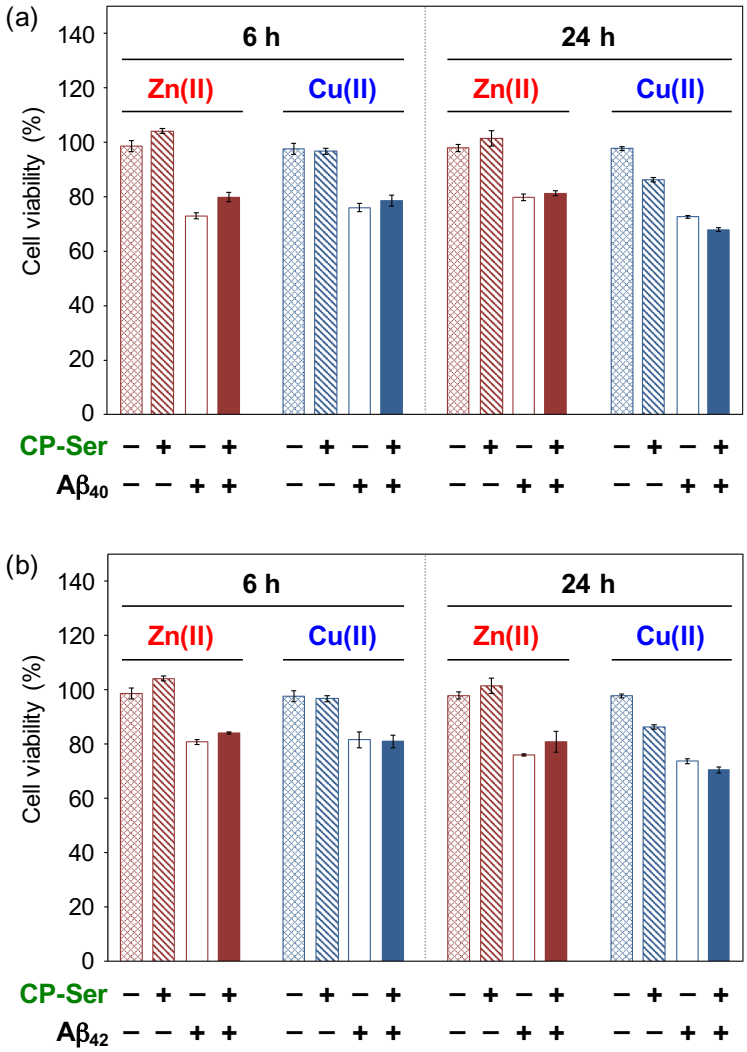

Fig. S10 Viability of SH-SY5Y (5Y) cells incubated with CP-Ser, Aβ, and/or metal ions. CP-Ser pre-treated with or without (a) $A\beta_{40}$ and (b) $A\beta_{42}$ in both the absence and presence of metal ions for 8 h was incubated with 5Y cells for 6 and 24 h. Cytotoxicity was measured by the MTT assay. The viability values were calculated by comparison to cells treated with a volume of H_2O equal to the protein added. Error bars represent the standard error from three independent experiments. Conditions (final concentrations): [CP-Ser] = 5 μM; [Aβ] = 5 μM; [$ZnCl_2$ or $CuCl_2$] = 5 μM.