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Supporting information:

Examining the Inhibitory Potency of Food Additive Fast Green FCF Against Amyloid Fibrillogenesis Under Acidic Conditions

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Running title: Lysozyme fibrillogenesis is suppressed by fast green FCF

#: Both authors contributed equally to this work.

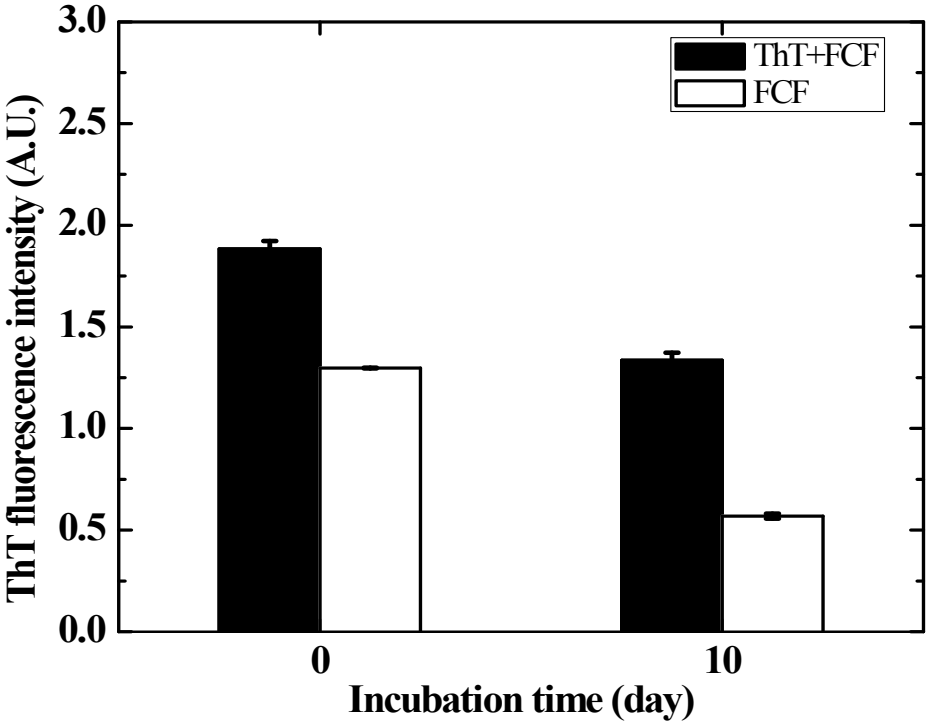


Fig. S1. Fluorescence intensities of fast green FCF without and with ThT at various incubation times (0 and 10 days) under the condition used for the ThT fluorescence measurement.

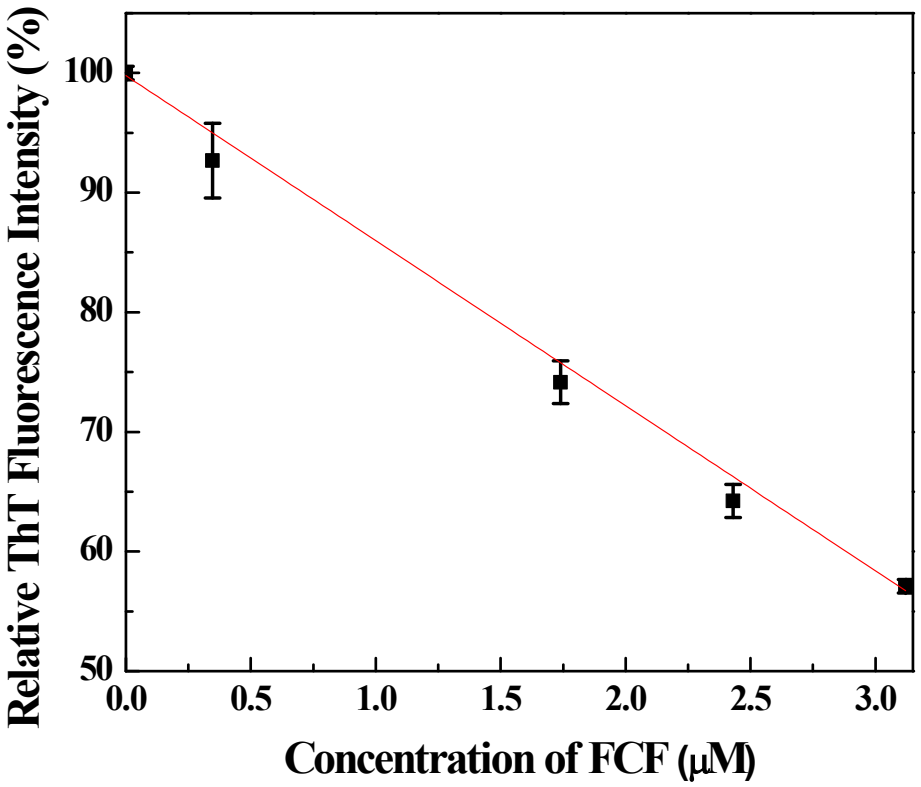


Fig. S2. Dose–response curve for the inhibition of lysozyme fibril formation by fast green FCF. The extent of lysozyme fibril formation was monitored by ThT fluorescence emission and plotted as a percentage of the untreated control (the relative ThT fluorescence intensity (%)).

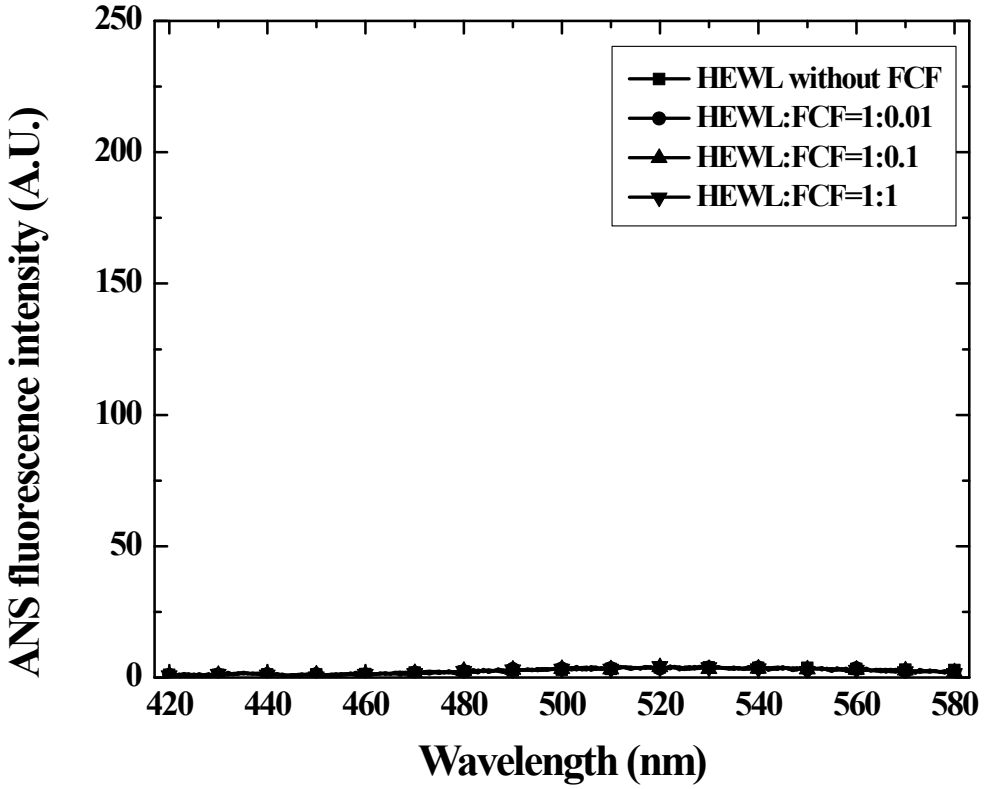


Fig. S3. The ANS fluorescence emission spectra of lysozyme samples with different molar ratios of lysozyme to fast green FCF taken at the beginning of incubation (t = 0).

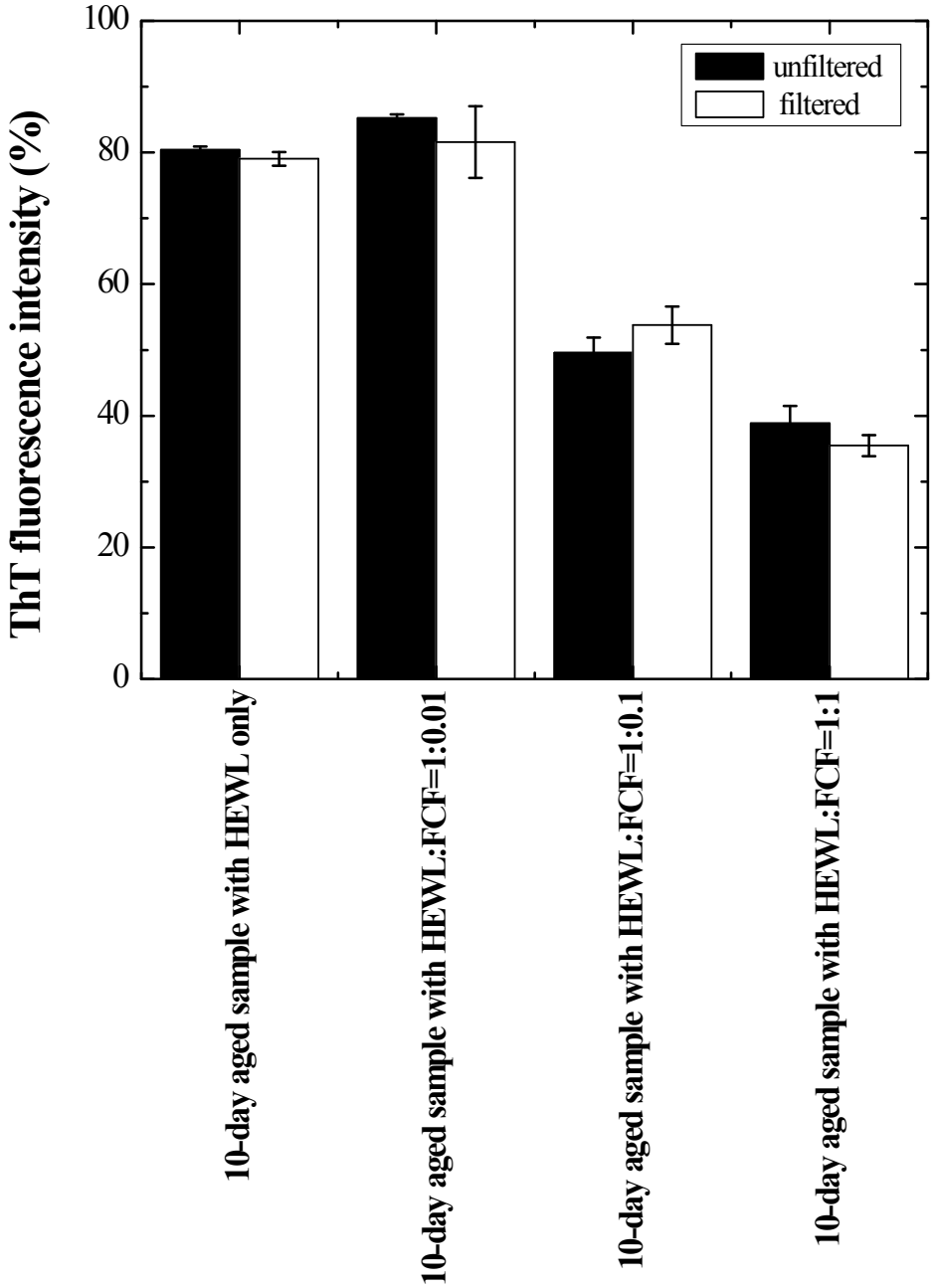
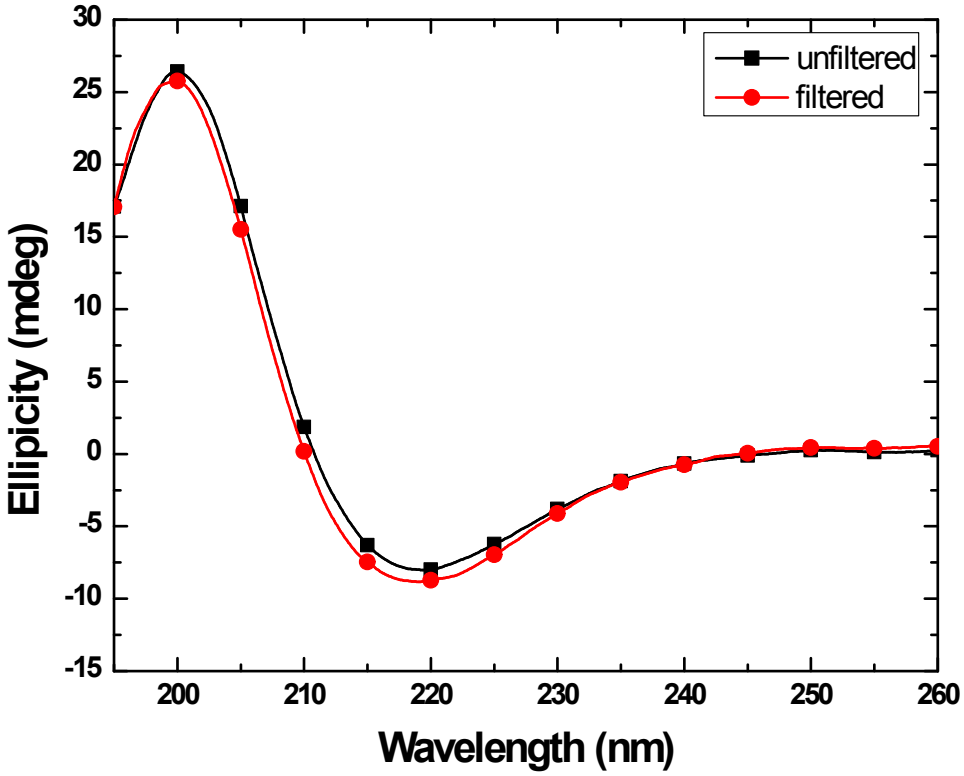


Fig. S4. Effects of acid-induced peptide fragements on the ThT fluorescence intensity of lysozyme samples. The lysozyme samples without and with fast green FCF were first freshly prepared and then incubated at pH 2.0 and 55 oC for 10 days. To remove the peptide fragments, the 10-day aged samples (without and with fast green FCF) were transferred to a Vivaspin 6 centrifugal concentrator filter unit containing the

membrane filter with MWCO of ~10 kDa (Sartorius, USA). After spinning for ~10 min at 3000 rpm, the remaining protein solution with species larger than 10 kDa was collected and diluted with glycine buffer (100 mM glycine, 100 mM NaCl, and pH 2.0) to ~0.81 mg/mL, and then further analyzed by ThT fluorescence measurement. For comparison, the unfiltered 10-day aged lysozyme samples without and with fast green FCF were properly diluted to a concentration comparable to the filtered samples and were also subjected to ThT fluorescence measurements.

(A)


unfiltered
filtered
Ellipicity (mdeg)
Wavelength (nm)
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25
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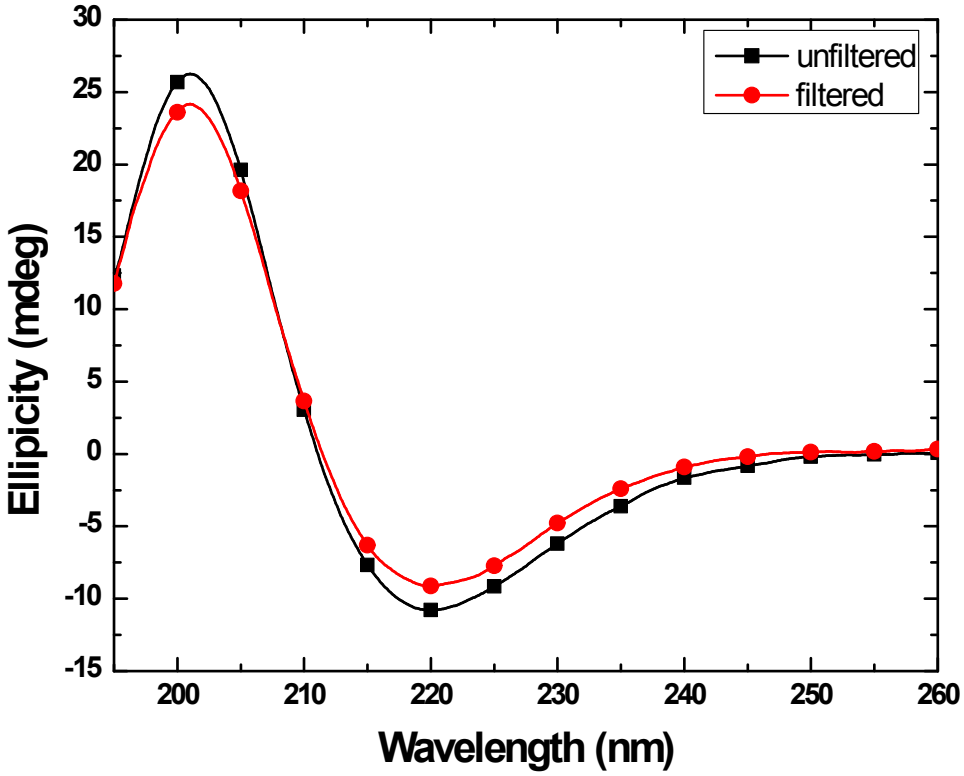
(B)


unfiltered
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Ellipicity (mdeg)
Wavelength (nm)
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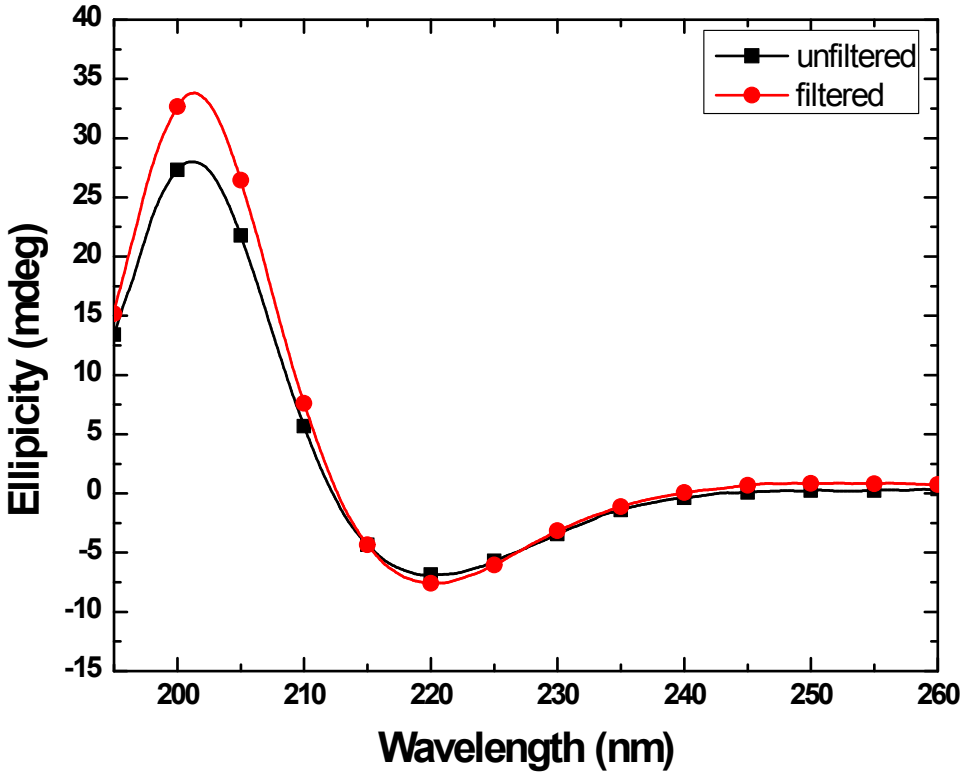
(C)


unfiltered
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Ellipicity (mdeg)
Wavelength (nm)
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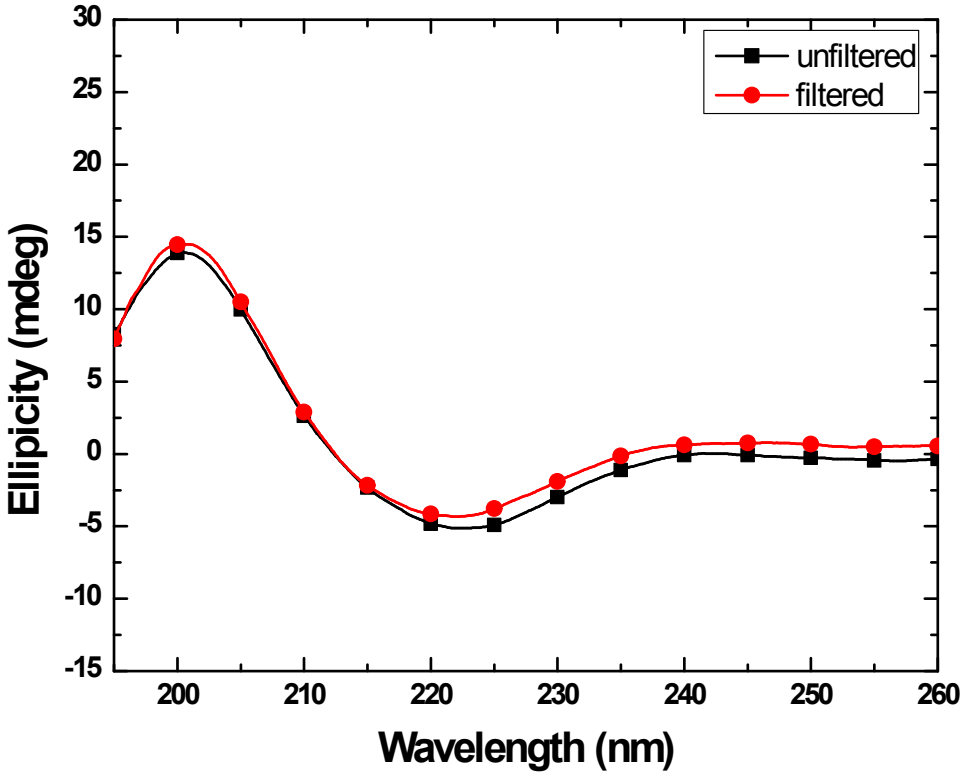
(D)


unfiltered
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Ellipicity (mdeg)
Wavelength (nm)
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(E)

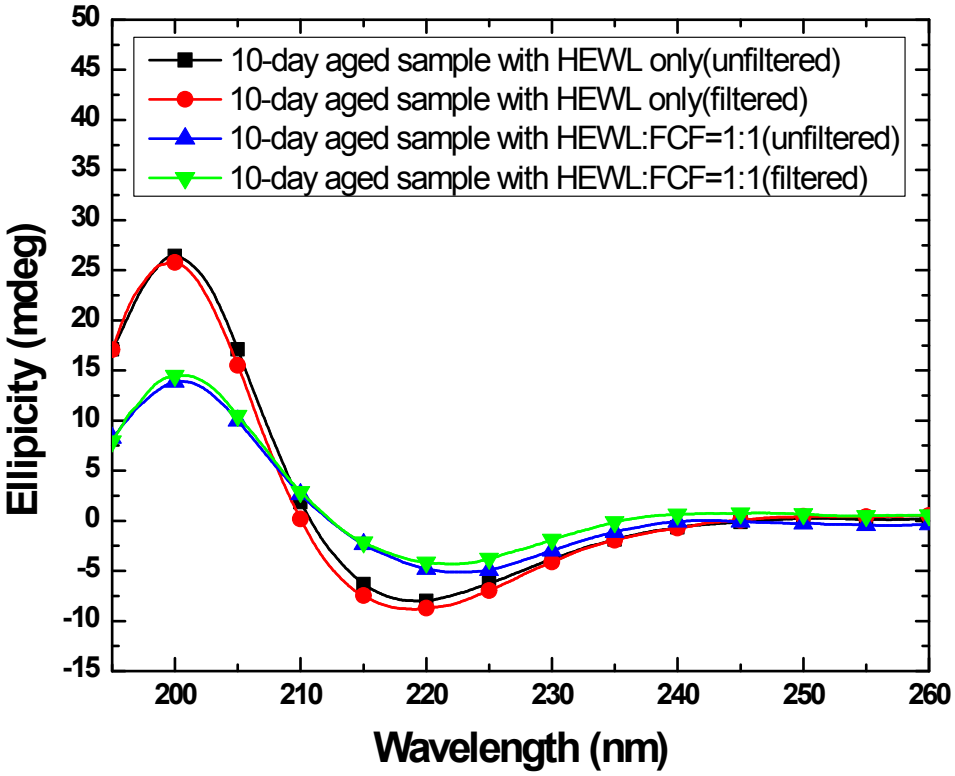


Fig. S5. Effects of acid-induced peptide fragements on the far-UV circular dichroism spectra of lysozyme samples. The lysozyme samples without and with fast green FCF were first freshly prepared and then incubated at pH 2.0 and 55 oC for 10 days. To remove the peptide fragments, the 10-day aged samples (without and with fast green FCF) were transferred to a Vivaspin 6 centrifugal concentrator filter unit containing the membrane filter with MWCO of ~10 kDa (Sartorius, USA). After spinning for ~10 min at 3000 rpm, the remaining protein solution with species larger than 10 kDa was collected and diluted with glycine buffer (100 mM glycine, 100 mM NaCl, and pH 2.0) to ~0.81 mg/mL, and then further analyzed by far-UV CD absorption spectroscopy. For comparison, the unfiltered 10-day aged lysozyme samples without and with fast green FCF were properly diluted to a concentration comparable to the filtered samples and were also subjected to far-UV CD absorption spectroscopy.

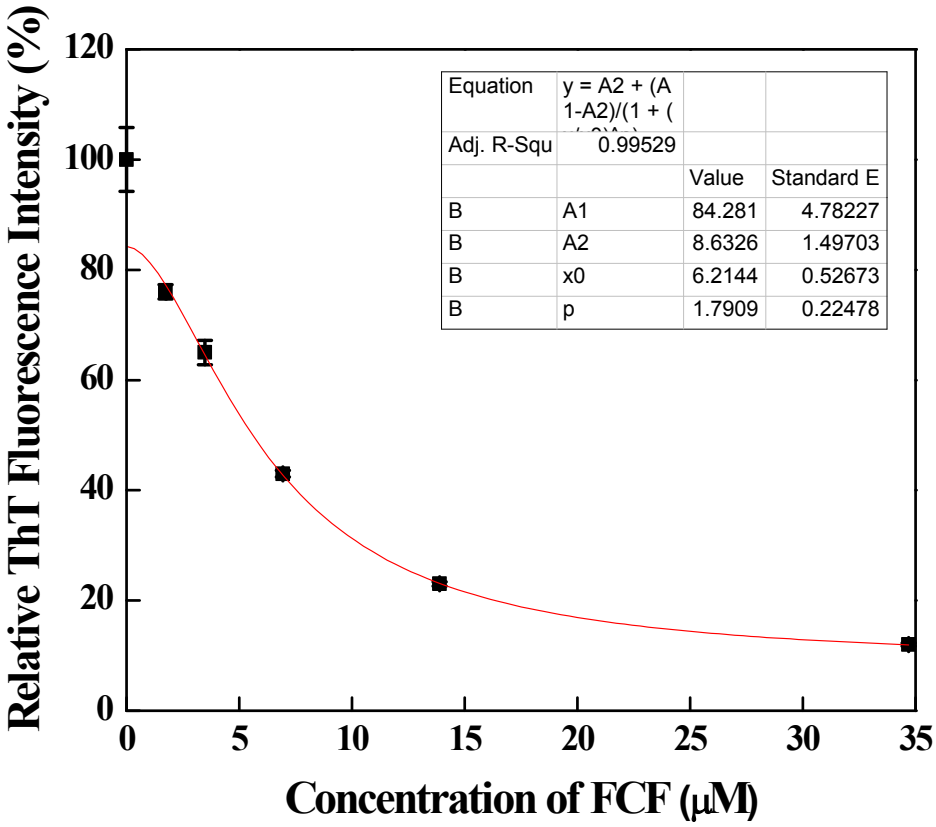


Fig. S6. Dose–response curve for the disaggregation/defibrillogenesis of preformed lysozyme fibrils by fast green FCF. The extent of fibrillogenesis in the lysozyme sample was monitored by ThT fluorescence and plotted as a percentage of the fast-green FCF-untreated control.

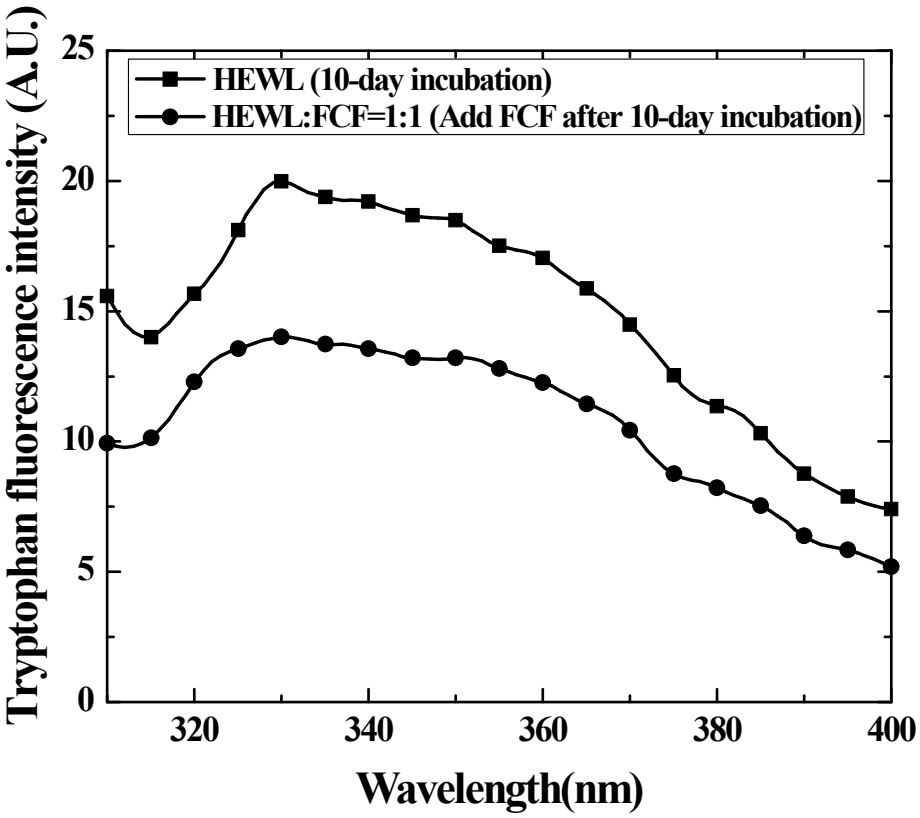
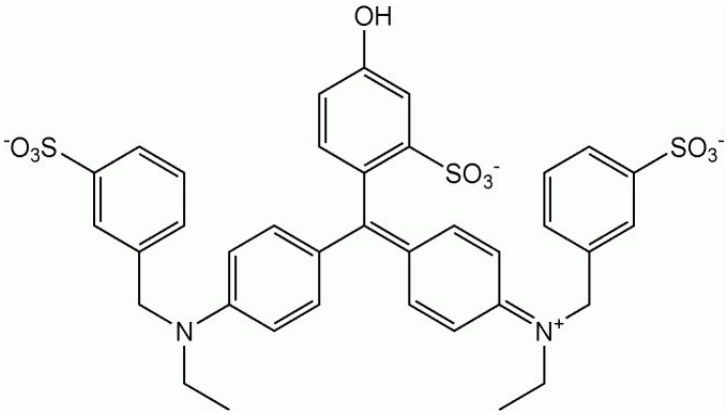
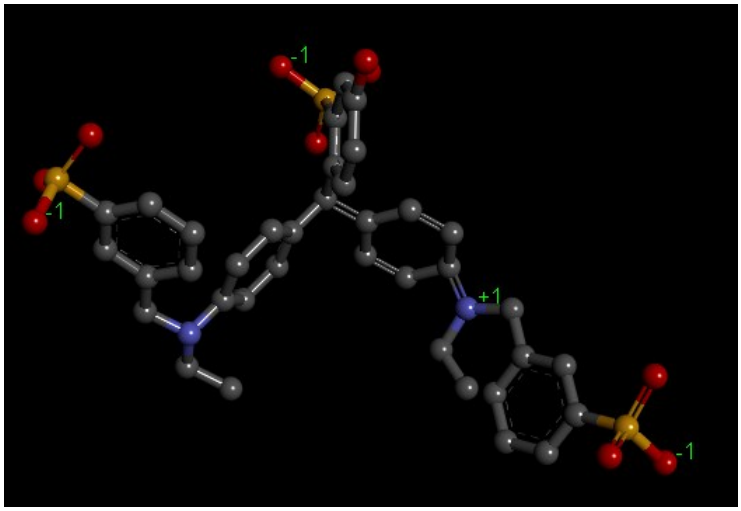


Fig. S7. Effects of fast green FCF on preformed lysozyme fibrils as evaluated by tryptophan fluorescence spectroscopy. Fast green FCF was added to the 10-day aged lysozyme sample. The molar ratio of lyszoyme to fast green FCF used was 1 to 1.

(A)

pH 7.4





(B)

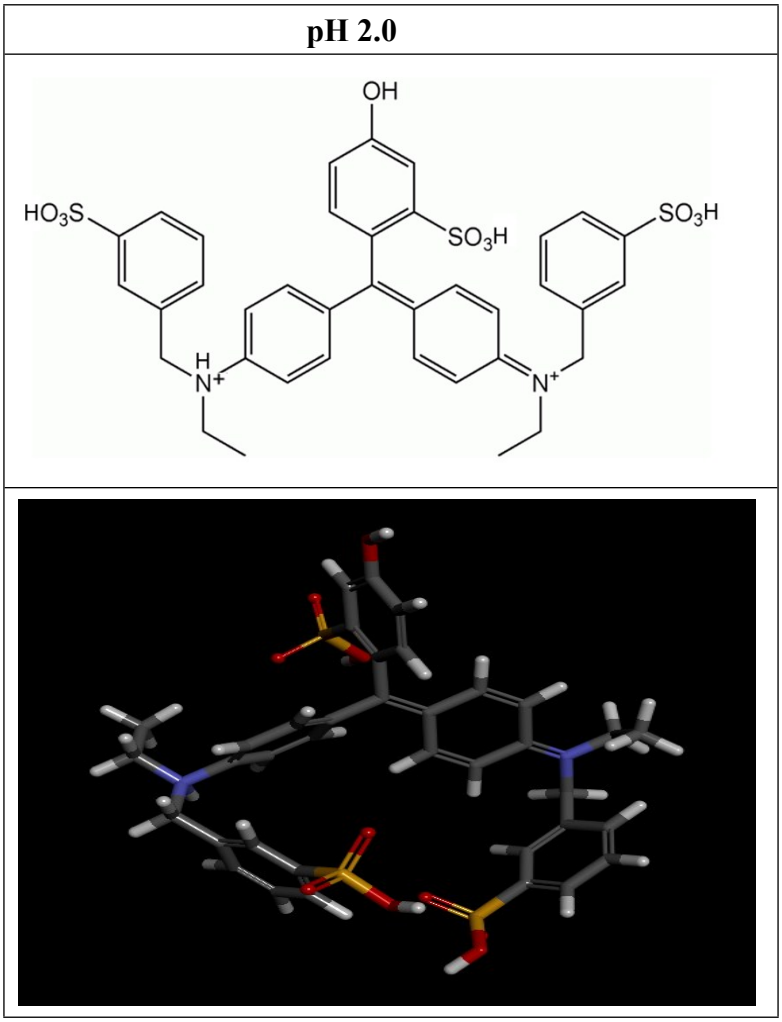


Fig. S8. Effects of pH on the structure of fast green FCF. (A) The structure of fast green FCF at pH 7.4 and (B) the structure of fast green FCF at pH 2.0.

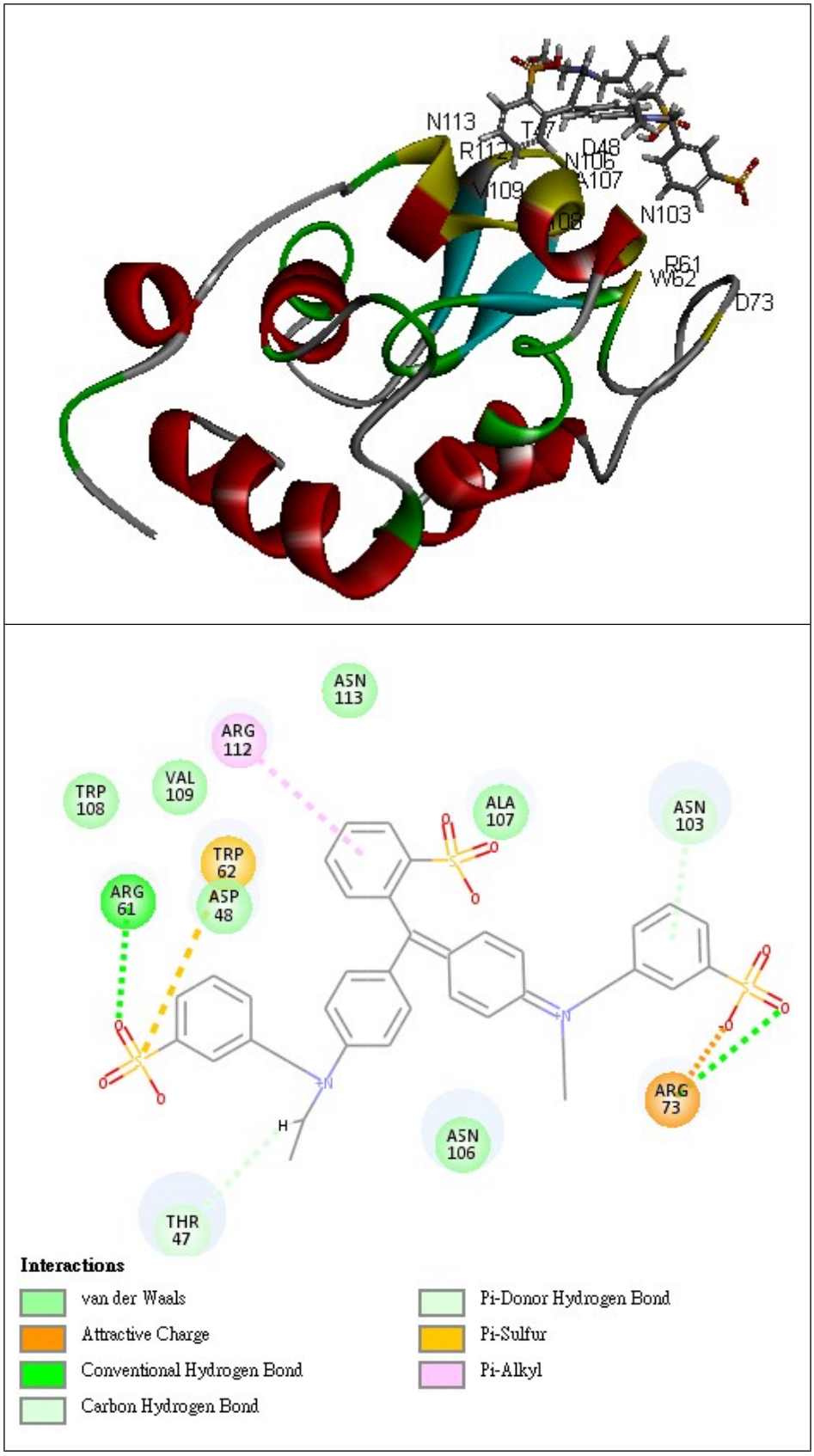


Fig. S9. (A) Best docking pose of fast green FCF on hen lysozyme at pH 2.0 and 55

^{o}C and (B) Schematic representation of the top 1 potential interaction mode (pose 1) for fast green FCF.