Supplementary Information (SI)

A New Fluorescent Probe for Monitoring Amyloid Fibrillation with High Sensitivity and Reliability

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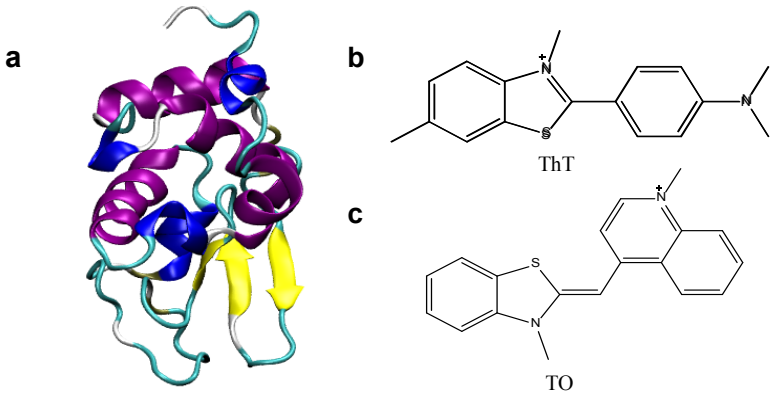

Fig. S1. Ribbon structure of lysozyme (a). Molecular structures of thioflavin T (b) and thiazole orange (c).

Atomic force microscopy (AFM) measurements were performed to examine the morphology of lysozyme aggregates (Fig. S2). It is apparent that after 14 days of incubation, lysozyme self-assembled into mature fibrils and some fibrils seem to have merged. Some small aggregates are also visible in the background of the image which are thought to evolved into fibrillar structures, although the detailed mechanism underlying the fibrillogenesis process is not yet completely understood. The line profile along the section labeled a–a′ and b–b′ in Fig. S2b are portrayed in Fig. S2c and 2d, respectively. It is clearly seen that the height of single lysozyme fibrils is about 7.0 nm (Fig. S2c), which is comparable to the average diameter of amyloid fibrils reported previously (7–10 nm).[1] Mature fibrils and single fibrils twisted into fibrils bundle are observed, while in this case the height of the fibrils measured is about 13.0 nm (Fig. S2d). These AFM results are in good agreement with the below TEM measurements.

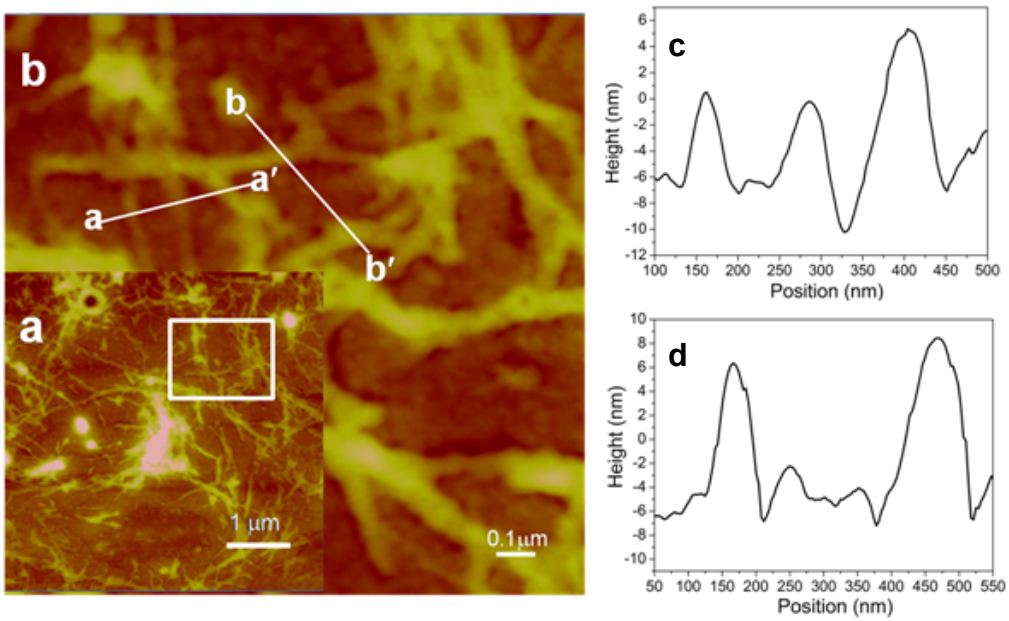

Fig. S2. Tapping-mode AFM height images of lysozyme amyloid fibrils at different magnifications (a–b); (c) the line profile of a–a′ labeled in Fig. S2b; (d) the line profile of b–b′ labeled in Fig. S2b.

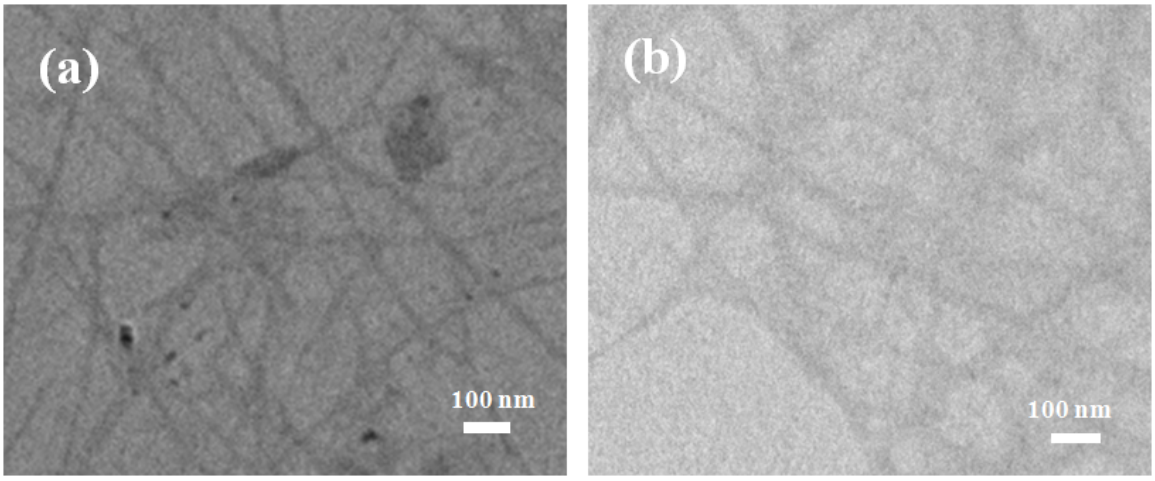

Fig. S3. High-resolution TEM images of aggregated lysozyme species formed for 14 days without (a) or with (b) TO during fibrillation.

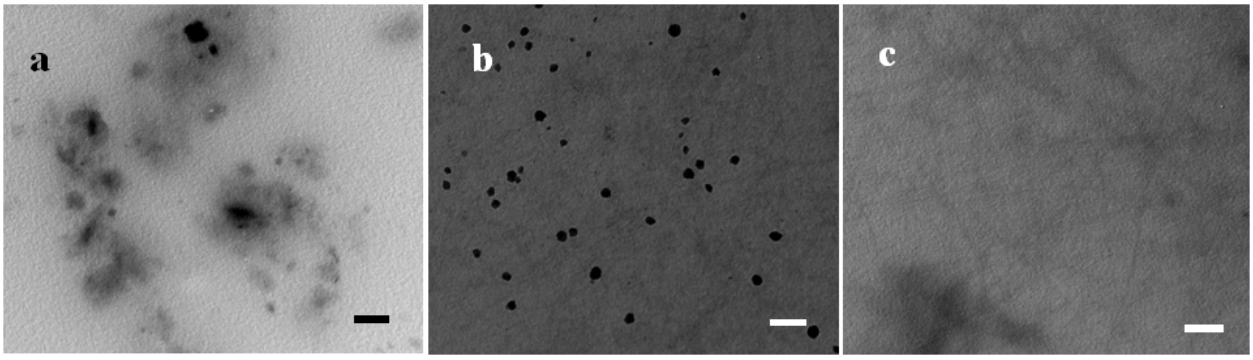

Fig. S4. TEM images of aggregated lysozyme species formed for 4 days (a), 8 days (b), and 14 days (c) with ThT during fibrillation. The scale bar represents 200 nm.

The dependence of final fluorescence value (FFV) on TO concentration for these measurements is shown in Fig. S4; the results display a linear increase of FFV from 5 to 30 □M, and then, starting from 30 M of TO, the FFV value begins to decrease by further addition of TO. The attenuation effect at higher TO concentration may be due to the fact that excess TO covered the fibrils accessible binding sites.[2] In our typical experiments, only 10 M TO was used which is not enough to cover all of the fibrils accessible binding sites. So the saturation of fluorescence intensity value measured at high protein concentration in our experiments is mainly due to lack of free dye in solution.

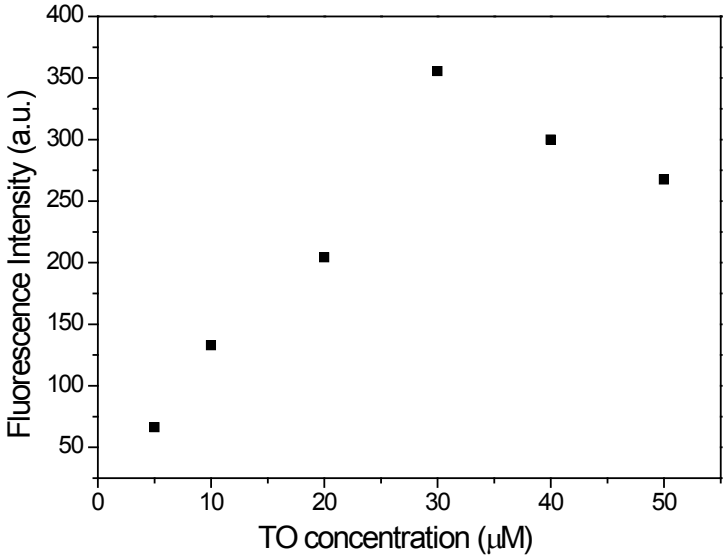

Fig. S5. Concentration dependence of the fluorescence intensity of TO with aggregated lysozyme.

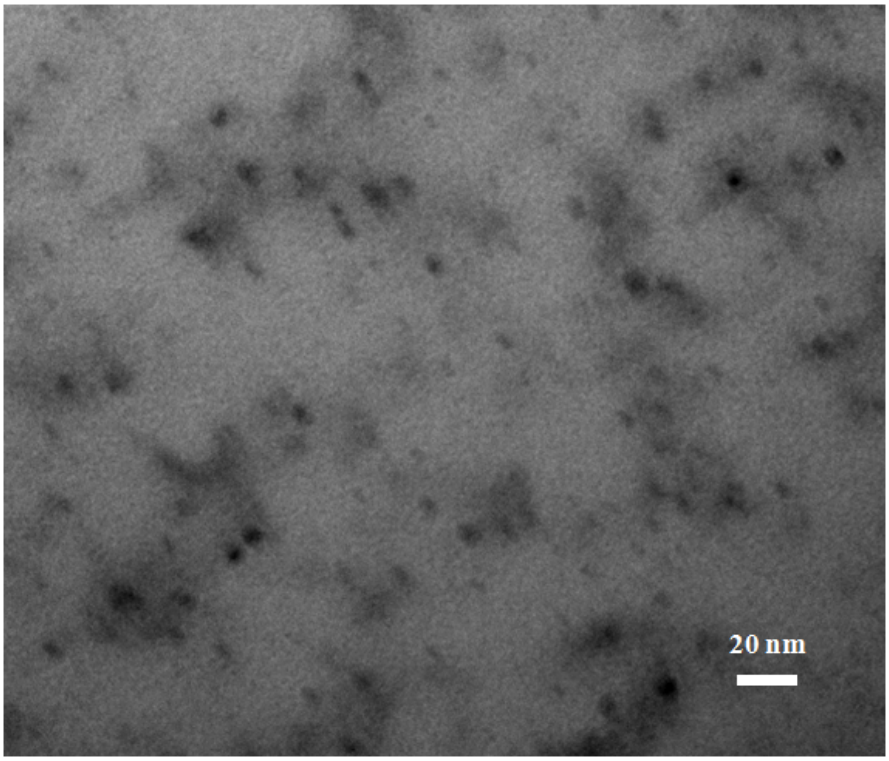

Fig. S6. High-resolution TEM images of aggregated lysozyme species formed for 14 days from 0.6 wt% lysozyme.

References:

1. C. M. Dobson, *Nature*, 2003, **426**, 884.

2. (a) M. Hirai, S. Arai and H. Iwase, *Thermochim. Acta*, 2000, **344**, 95. (b) V. Foderà, F. Librizzi, M. Groenning, M. van de Weert and M. Leone, *J. Phys. Chem. B*, 2008, **112**, 3853.