Tannic Acid-Induced Surface-Catalyzed Secondary Nucleation during the Amyloid Fibrillation of Hen Egg White Lysozyme

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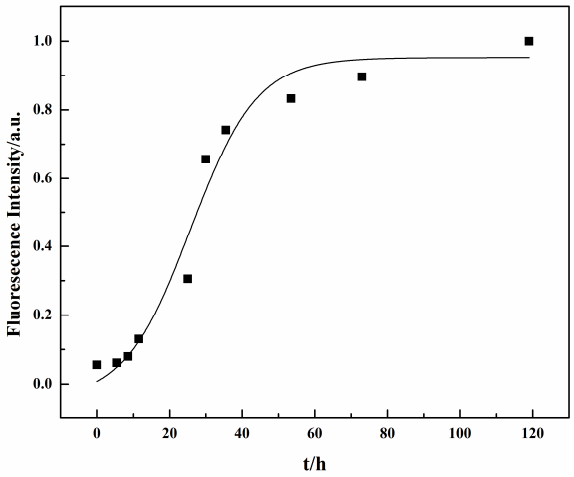

Figure S1. ThT fluorescence assay for the fibrillation kinetics of lysozyme with empirical fit using the equation of $F = F_0 + 1/\left(1+\exp\left[r_{\max}\left(\tau_{1/2}-t\right)\right]\right)$, where $\tau_{1/2}$ is the time for half completion of aggregation, r_{max} is the maximum growth rate, and $\tau_{1/2}$-$2/r_{max}$ is the lag phase time duration [1].

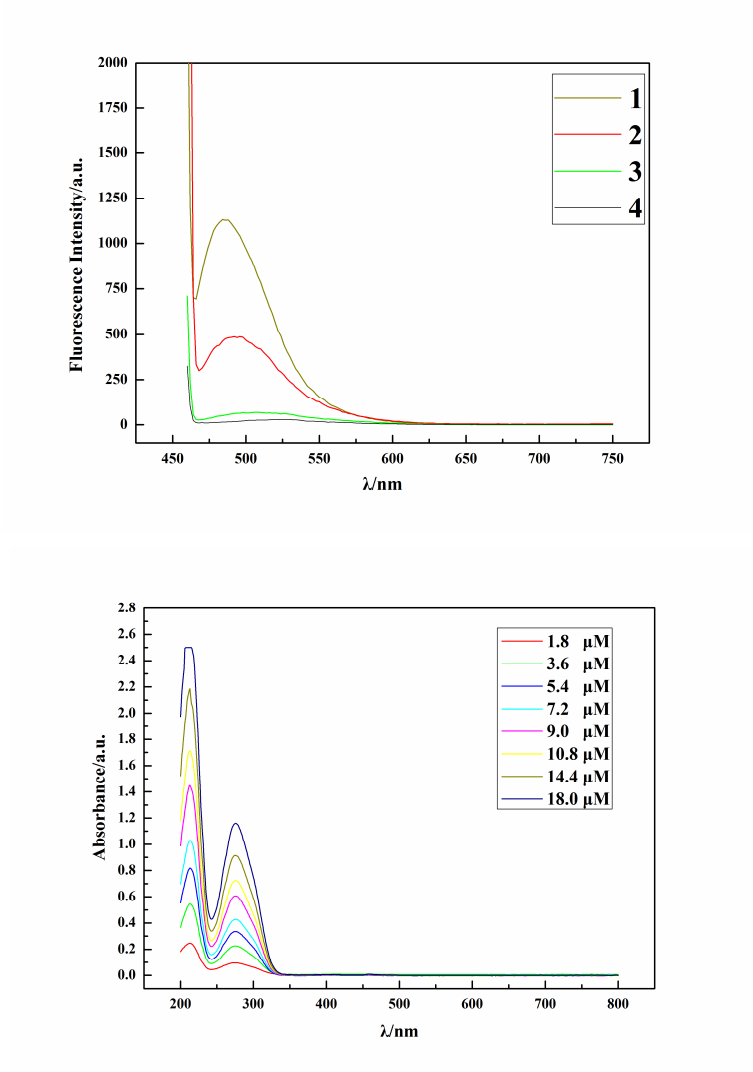

Figure S2. (**Top**) Fluorescence spectra of ThT bound to amyloid fibril. C_{ThT} =10μM; Incubation condition: $C_{lysozyme}$=5mg/mL; t=120h (1); ThT bound to non-fibrillar aggregates C_{ThT} =10μM; Incubation condition: $C_{lysozyme}$=5mg/mL; $C_{Tannic\ acid}$=1200μM; t=0h (2); the control (i.e., ThT + tannic acid), C_{ThT} =10μM (3); and the control (tannic acid alone), $C_{Tannic\ acid}$=1200μM (4). (**Bottom**) Absorption spectra of tannic acid under different concentrations.

Note: The ThT assay was performed ex situ. For each ThT assay, only 10 μl of the test solution was added in to 1 mL of ThT solution. So in the test solution in ThT assay, the concentration of ThT is 10 μM; the concentration of lysozyme is 0.05 mg/ml, and the concentration of tannic acid is only 12 μM.

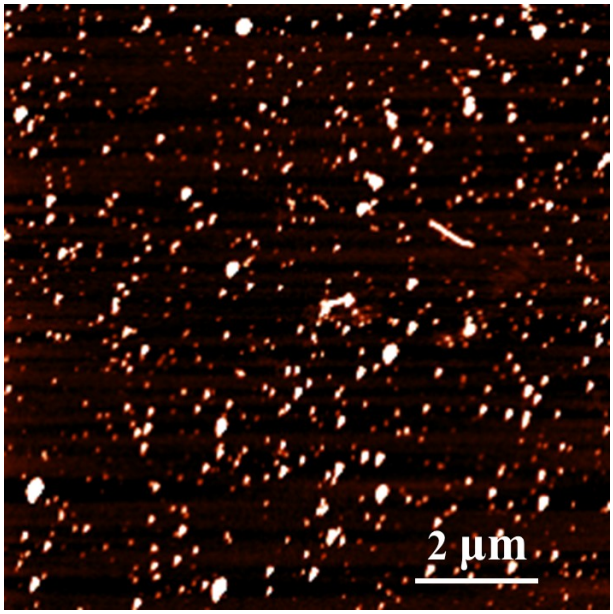

Figure S3. AFM evidence for the presence of the non-fibrillar aggregates at the end of incubation in the presence of tannic acid (i.e., t=137h). $C_{lysozyme}$=5mg/mL; $C_{Tannic\ acid}$=1200μM. This image was from the same mica surface for Figure 4F.

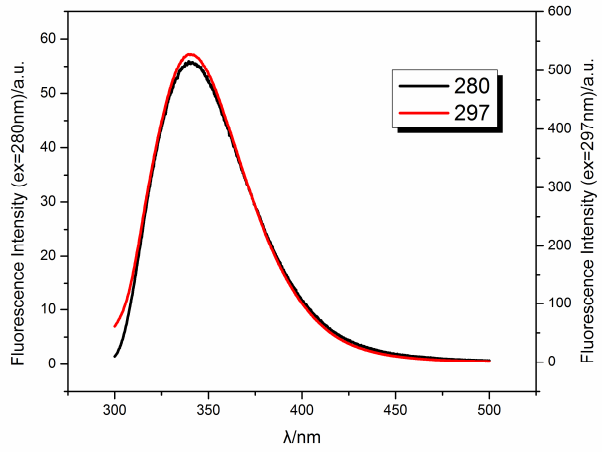

Figure S4. Fluorescence spectra of lysozyme at 5mg/mL excited with 280 nm and 297 nm.

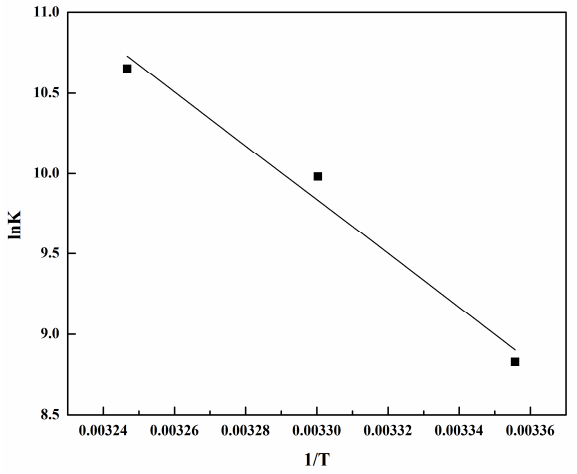

Figure S5. Van 't Hoff plot for lysozyme quenched by tannic acid at 323 K, 328 K and 333 K.

$C_{lysozyme}$=3.6×10^{-6}mol/L; $C_{Tannic\ acid}$ (1–9) = (0, 1.8, 3.6, 5.4, 7.2, 9.0, 10.8, 14.4, 18.0)×10^{-6}mol/L

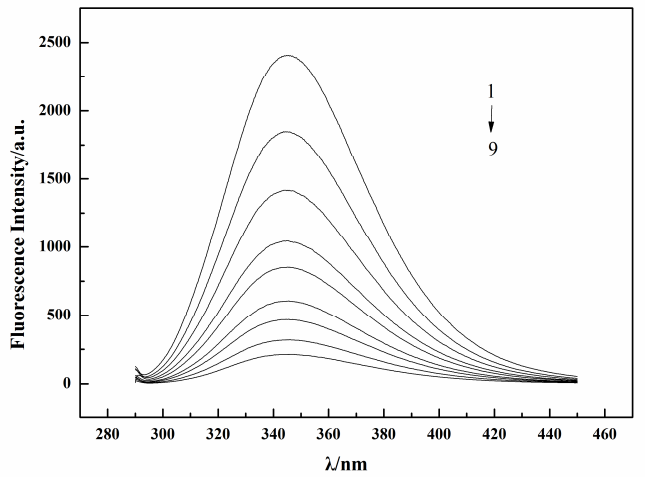

Figure S6. Fluorescence spectra of lysozyme quenched by tannic acid at 298 K.

$C_{lysozyme}$=3.6×10^{-6}mol/L; $C_{Tannic\ acid}$ (1–9) = (0, 1.8, 3.6, 5.4, 7.2, 9.0, 10.8, 14.4, 18.0)×10^{-6}mol/L

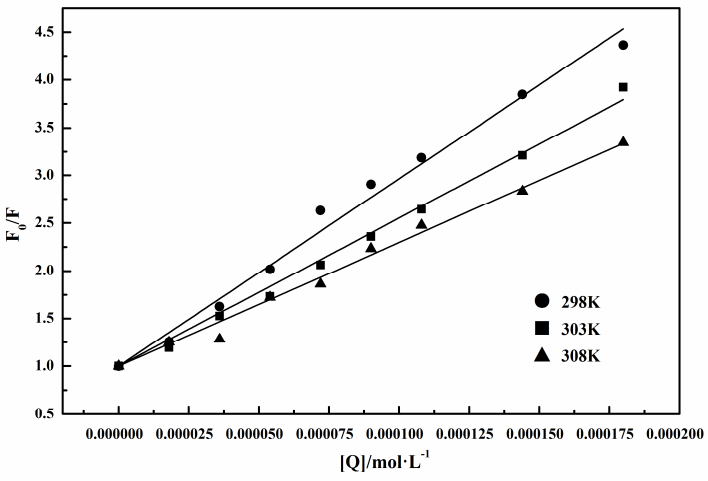

Figure S7. Stern-Volmer plots for lysozyme quenched by tannic acid at 298 K, 303 K and 308 K.

Table S1. The Stern-Volmer parameters of lysozyme-tannic acid system at 298 K, 303 K and 308 K.

T/K	K_{sv}/(L·mol^{-1})	K_q/(L·mol^{-1}·s$^{-1)}$	r*
298	(1.65±0.28)×10^4	(1.83±0.31)×10^{12}	0.9897±0.0081
303	(1.47±0.08)×10^4	(1.63±0.09)×10^{12}	0.9944±0.0030
308	(1.34±0.13)×10^4	(1.49±0.14)×10^{12}	0.9917±0.0039

*r is correlation coefficient.

Table S2. Binding parameters of lysozyme-tannic acid system at 298 K, 303 K and 308 K.

T/K	K_A/(L·mol^{-1})	n	r
298	(1.17±0.86)×10^5	1.20±0.08	0.9931±0.0051
303	(6.20±4.54)×10^4	1.13±0.10	0.9936±0.0036
308	(3.27±1.03)×10^4	1.10±0.03	0.9866±0.0114

Table S3. Parameters of *E, J, R_0, r* of the lysozyme-tannic acid system at 298 K, 303 K and 308 K.

T/K	E/%	J/(cm^3·L·mol^{-1})	R_0/nm	r/nm
298	32.43±8.52	(5.96±0.01)×10^{-15}	2.34±3.27×10^{-4}	2.65±0.17
303	31.48±3.02	(5.99±0.04)×10^{-15}	2.34±2.34×10^{-3}	2.67±0.06
308	26.61±4.28	(5.96±0.06)×10^{-15}	2.34±4.18×10^{-3}	2.78±0.11

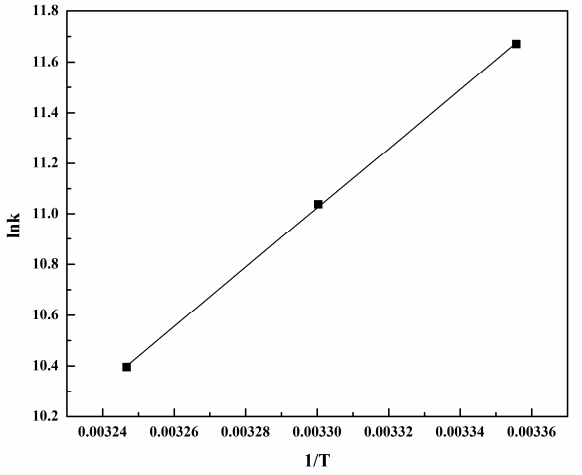

Figure S8. Van 't Hoff plot for lysozyme quenched by tannic acid at 298 K, 303 K and 308 K.

$C_{lysozyme}$=3.6×10^{-6}mol/L; $C_{Tannic\ acid}$ (1–9) = (0, 1.8, 3.6, 5.4, 7.2, 9.0, 10.8, 14.4, 18.0)×10^{-6}mol/L

1. Abelein, A.; Jarvet, J.; Barth, A.; Graslund, A.; Danielsson, J. Ionic strength modulation of the free energy landscape of a beta(40) peptide fibril formation. *J. Am. Chem. Soc.* **2016,** *138*, 6893-6902, 10.1021/jacs.6b04511