Electronic Supplementary Information

Conjugation of RTHLVFFARK to Human Lysozyme Creates a Potent Multifunctional Modulator for Cu^{2+}-Mediated Amyloid β-Protein Aggregation and Cytotoxicity

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Table S1. Physicochemical properties of hLys, PDEA-hLys and R-hLys at pH 7.4

Agent	Molecular weight (Da)	Size (nm)	ζ potential (mV)
hLys	14689.7	4.5 ± 0.4	5.8 ± 0.4
PDEA-hLys	15092.1	N.D.	N.D.
R-hLys	17471.3	5.3 ± 0.3	13.2 ± 0.7

N. D. not determined.

Table S2. Quantitative analysis of CD spectra of hLys and R-hLys [a]

	Helix1	Helix2	Strand1	Strand2	Turns	Unordered	Total
hLys (0 h)	0.573	0.223	0.000	0.006	0.076	0.161	1.039
R-hLys (0 h)	0.573	0.221	0.001	0.005	0.070	0.159	1.028
hLys (48 h)	0.571	0.222	0.000	0.006	0.077	0.162	1.038
R-hLys (48 h)	0.570	0.221	0.002	0.008	0.081	0.168	1.049

[a] The result was analyzed by CD Dicroweb with the self-consistent method (SELCON3) (http://dichroweb.cryst.bbk.ac.uk/html/home.shtml).[1,2]

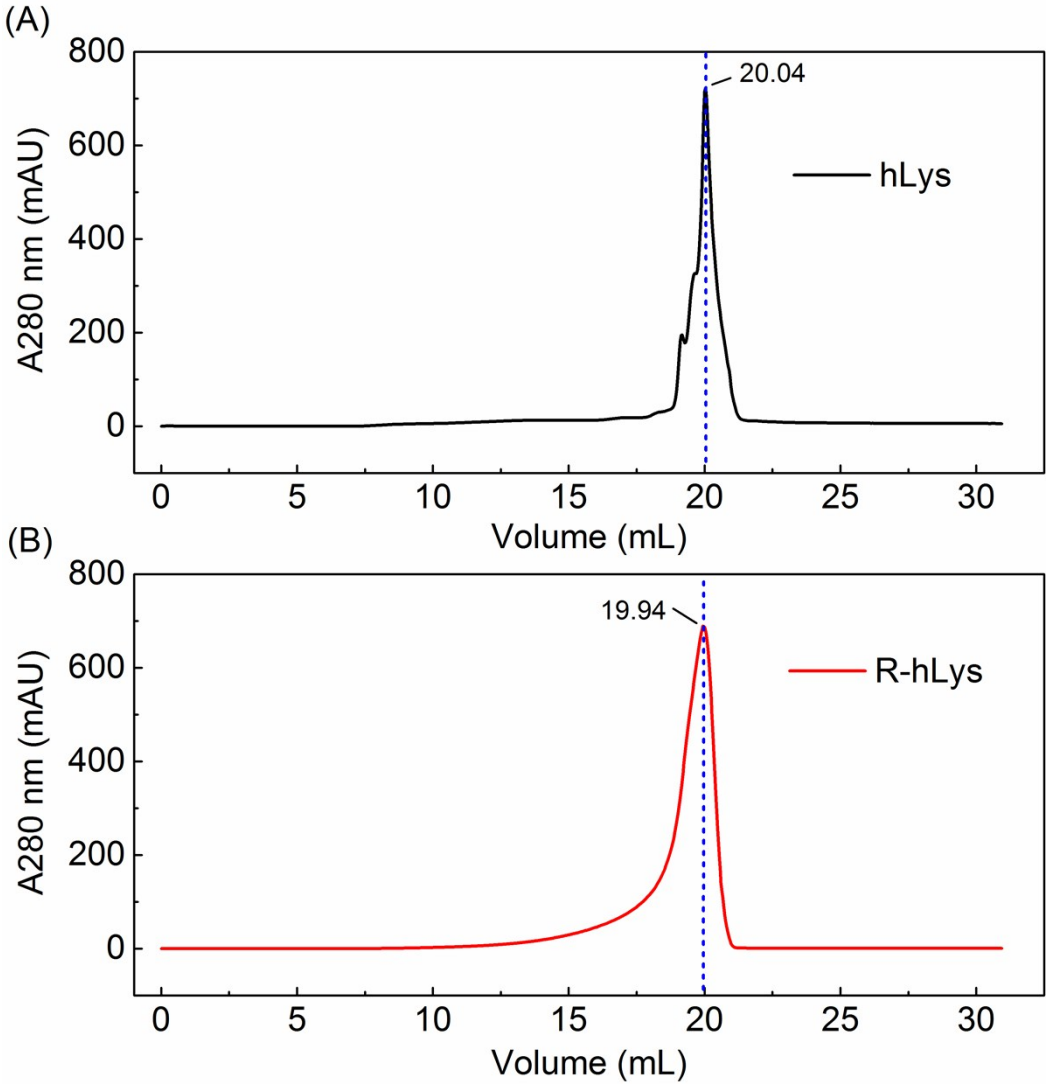

Fig. S1 Analysis of the purity of R-hLys by size exclusion chromatography (SEC). The retention volumes of hLys and R-hLys were 20.04 and 19.94 mL, respectively.

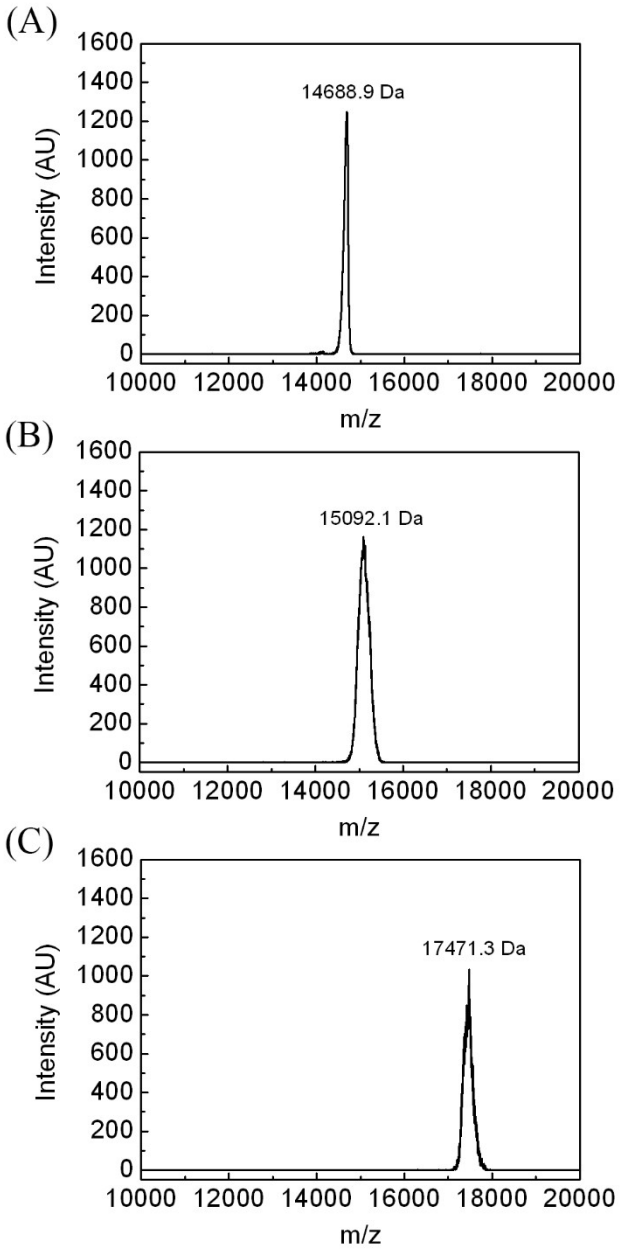

Fig. S2 Mass spectra of (A) native hLys, (B) PDEA-hLys (intermediate product, see Scheme 1) and (C) R-hLys.

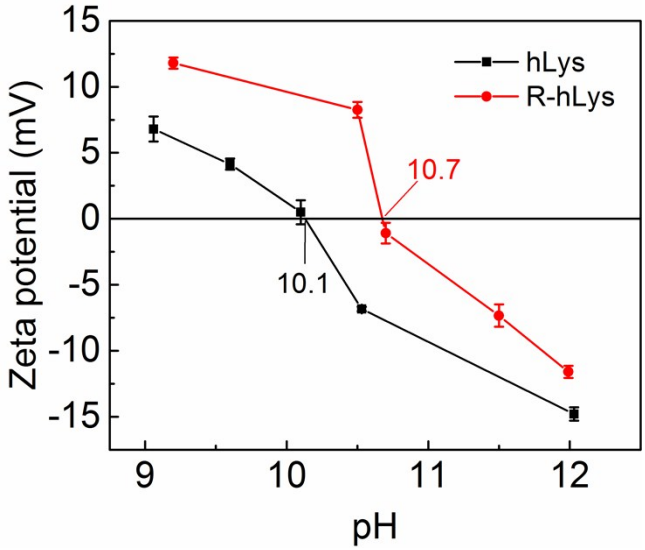

Fig. S3 Zeta potentials of hLys and R-hLys as a function of pH. Native hLys and R-hLys were both 25 μM in deionized water. Each point was the mean of three different measurements and error bars represent the standard deviations.

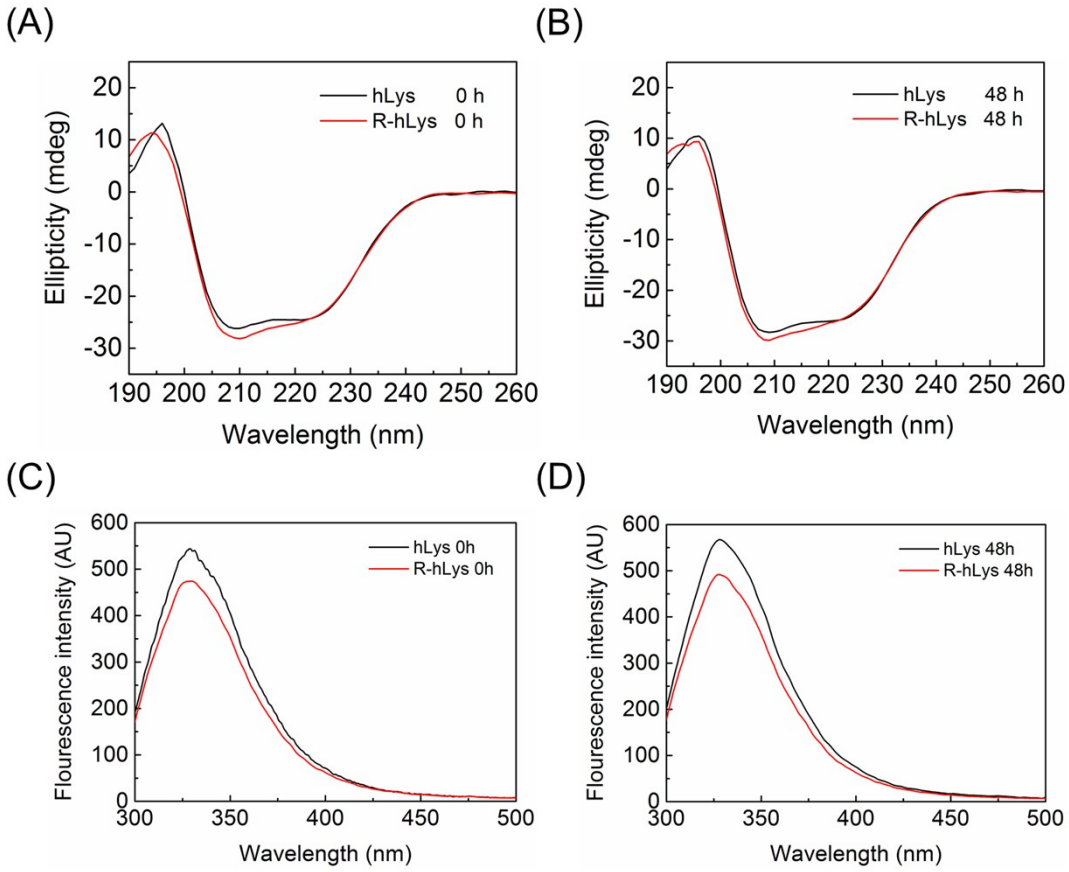

Fig. S4 Comparison of the far-UV circular dichroism spectra of native hLys and R-hLys after incubation for (A) 0 h and (B) 48 h and their fluorescence spectra after incubation for (C) 0 h and (D) 48 h. Native hLys or R-hLys was dissolved in buffer A at 25 μM and kept static at 37 °C.

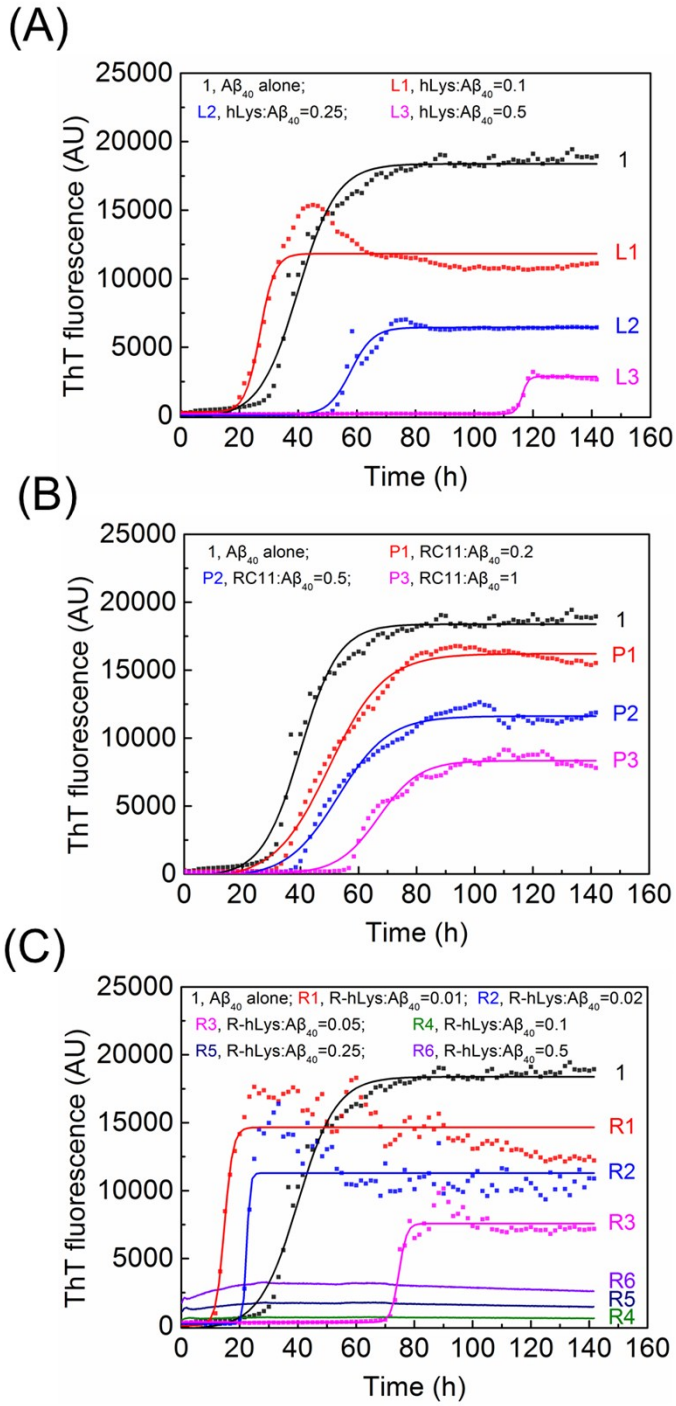

Fig. S5 Aggregation kinetics of $A\beta_{40}$ (25 μM) incubated in the absence and presence of (A) hLys, (B) RC11 and (C) R-hLys. RC11 concentrations for lines P1 to P3 in (B) were approximately equal to those of RC11 coupled to R-hLys for lines L1 to L3 in (A), respectively. All measurements were conducted in buffer A at 37 °C.

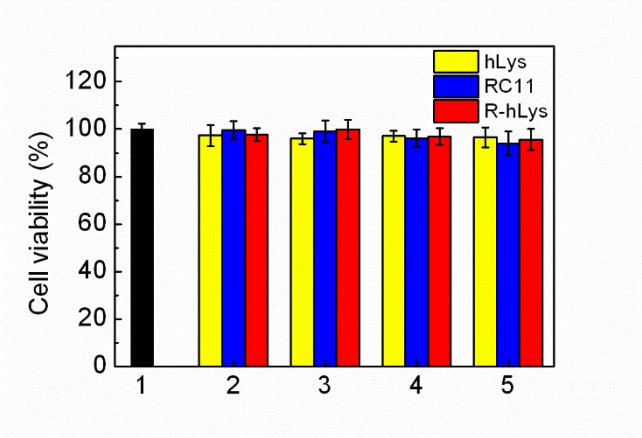

Fig. S6 Cell viability assay at different (yellow) hLys, (blue) RC11 or (red) R-hLys concentrations using MTT assay. Lanes denote the following conditions: lane 1, buffer A; lane 2, 0.5 μM hLys/R-hLys, 1 μM RC11; lane 3, 1.25 μM hLys/R-hLys, 2.5 μM RC11; lane 4, 2.5 μM hLys/R-hLys, 5 μM RC11; lane 5, 5 μM hLys/R-hLys, 10 μM RC11. All concentration data represent those in the cell culture medium.

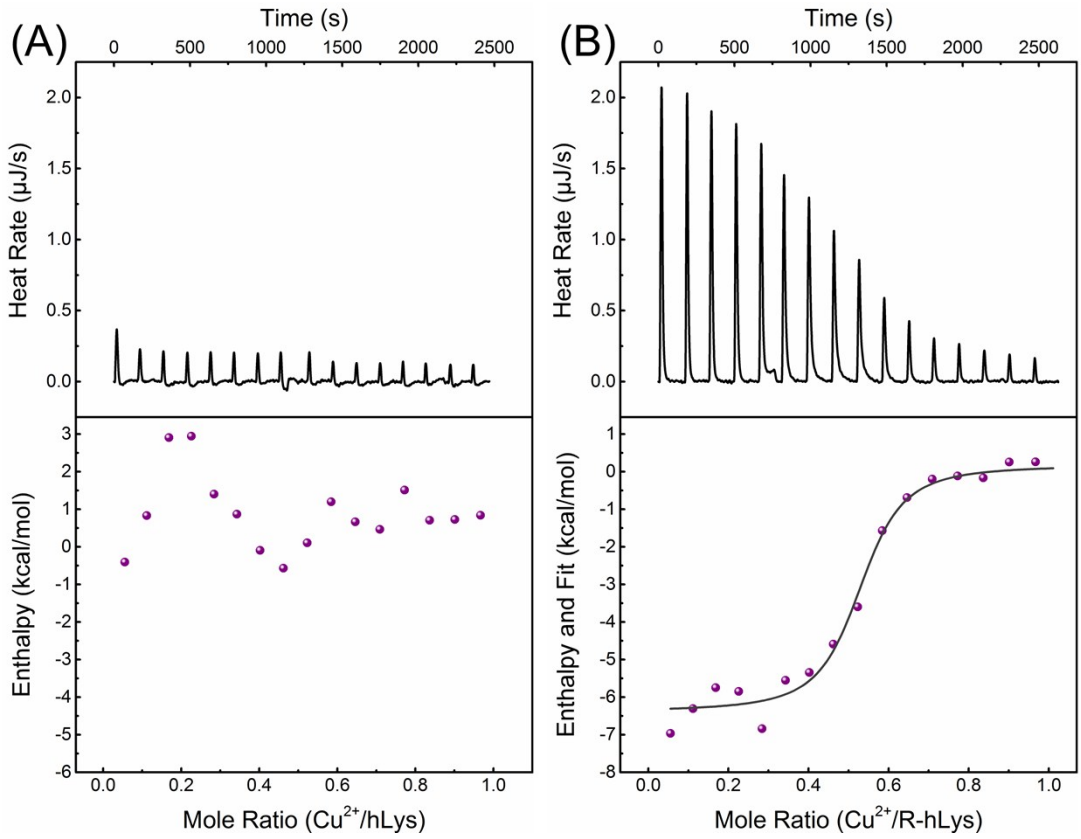

Fig. S7 ITC analysis of the interactions between Cu^{2+} and (A) hLys, (B) R-hLys. The concentrations of Cu^{2+}, glycine and chelator were 0.5 mM, 2 mM and 0.1 mM in buffer A, respectively. The top panel shows the heat rate for each injection and the positive peaks indicate an exothermic process; the bottom panel shows the integrated heat for each injection.

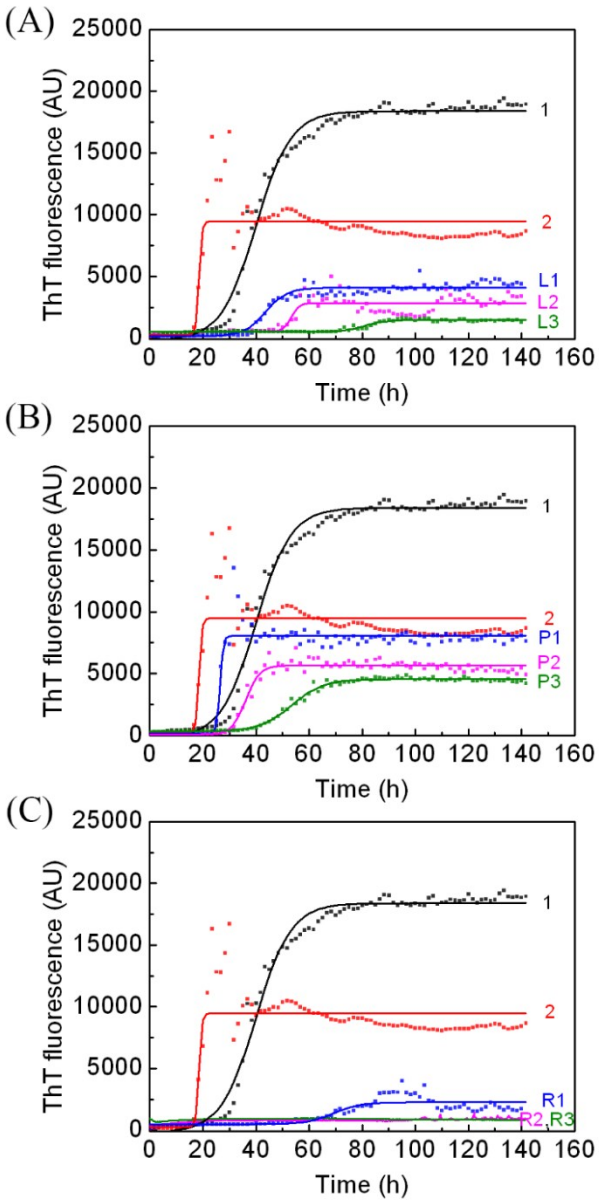

Fig. S8 Aggregation kinetics of Cu^{2+}-mediated $A\beta_{40}$ aggregation incubated in the absence and presence of (A) hLys, (B) RC11 and (C) R-hLys. The concentration of $A\beta_{40}$ was 25 μM. Lines denote the following conditions: (A) line 1, $A\beta_{40}$ alone; line 2, Cu^{2+}:$A\beta_{40}$ = 0.4:1; line L1, hLys:Cu^{2+} :$A\beta_{42}$ = 0.1:0.4:1; line L2, hLys:Cu^{2+} :$A\beta_{42}$ = 0.25:0.4:1; line L3, hLys:Cu^{2+} :$A\beta_{42}$ = 0.5:0.4:1. (B) line 1, $A\beta_{40}$ alone; line 2, Cu^{2+}:$A\beta_{40}$ = 0.4:1; line P1, RC11:Cu^{2+} :$A\beta_{42}$ = 0.2:0.4:1; line P2, RC11:Cu^{2+} :$A\beta_{42}$ = 0.5:0.4:1; line P3, RC11:Cu^{2+} :$A\beta_{42}$ = 1:0.4:1. (C) line 1, $A\beta_{40}$ alone; line 2, Cu^{2+}:$A\beta_{40}$ = 0.4:1; line R1, R-hLys:Cu^{2+}:$A\beta_{40}$ = 0.1:0.4:1, line R2, R-hLys:Cu^{2+}:$A\beta_{40}$ = 0.25:0.4:1; line R3, R-hLys:Cu^{2+}:$A\beta_{40}$ = 0.5:0.4:1. All measurements were conducted

in buffer A at 37 °C.

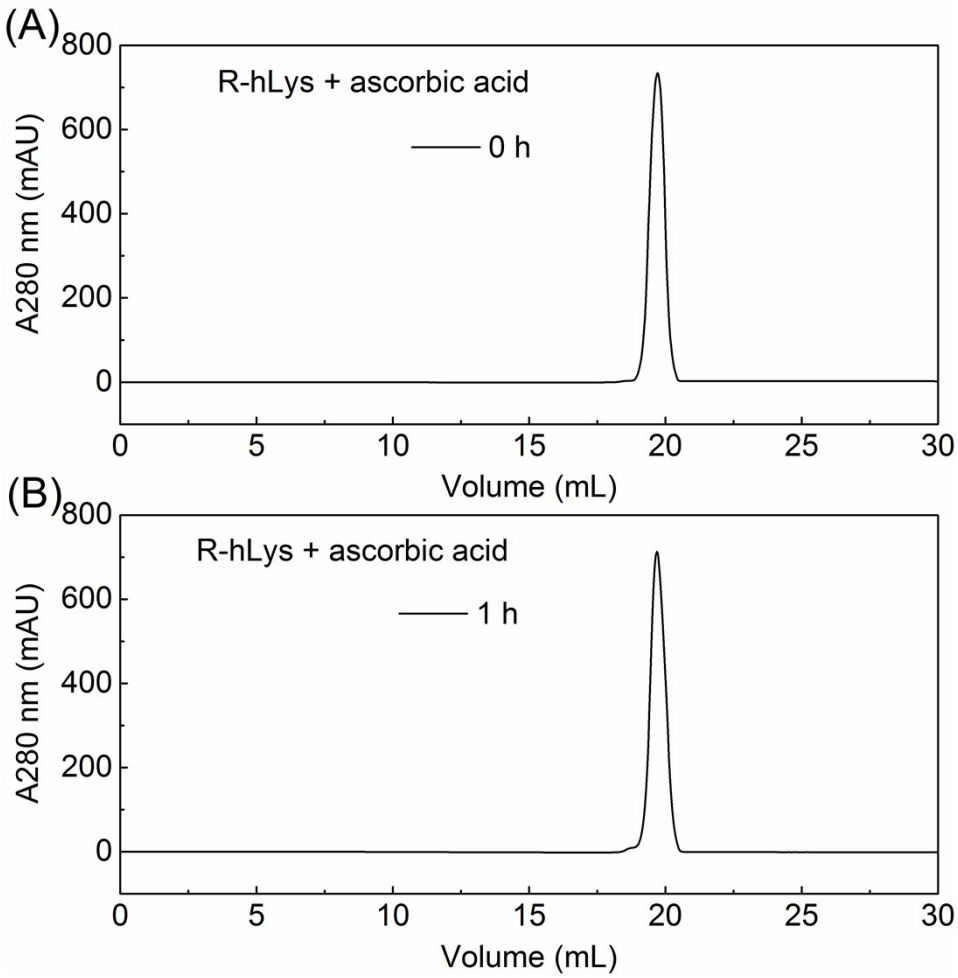

Fig. S9 Analysis of the stability of R-hLys in the presence of ascorbic acid by SEC. The concentrations of R-hLys and ascorbic acid were 25 μM and 500 μM, respectively.

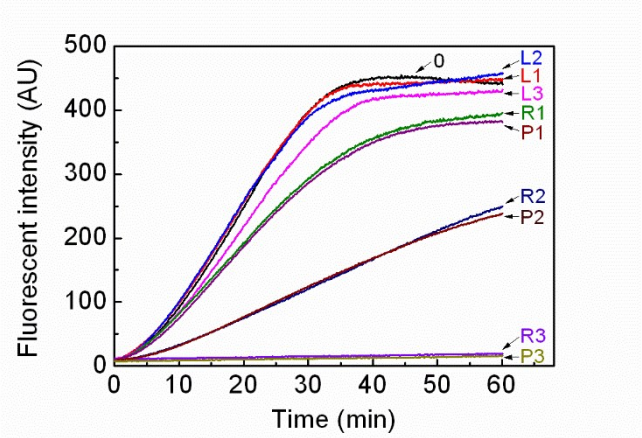

Fig. S10 Time-dependent ROS production measured by CCA fluorescence assays under a reducing environment. Cu^{2+} incubated with hLys/RC11/R-hLys in the absence of $A\beta_{42}$. Lines denote the following conditions: line 0, Cu^{2+}; line L1, Cu^{2+} + 2.5 μM hLys; line L2, Cu^{2+} + 6.25 μM hLys; line L3, Cu^{2+} + 12.5 μM hLys; line P1, Cu^{2+} + 5 μM RC11; line P2, Cu^{2+} + 12.5 μM RC11; line P3, Cu^{2+} + 25 μM RC11; line R1, Cu^{2+} + 2.5 μM R-hLys; line R2, Cu^{2+} + 6.25 μM R-hLys; line R3, Cu^{2+} + 12.5 μM R-hLys. The final concentration of Cu^{2+} was 10 μM.

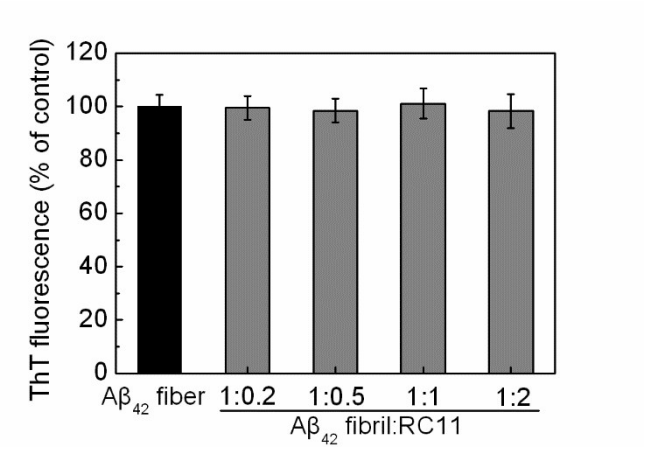

Fig. S11 Normalized ThT fluorescence of $A\beta_{42}$ fibrils incubated in the absence and presence of various concentrations of RC11 for 48 h. The final concentration of $A\beta_{42}$ was 25 μM.

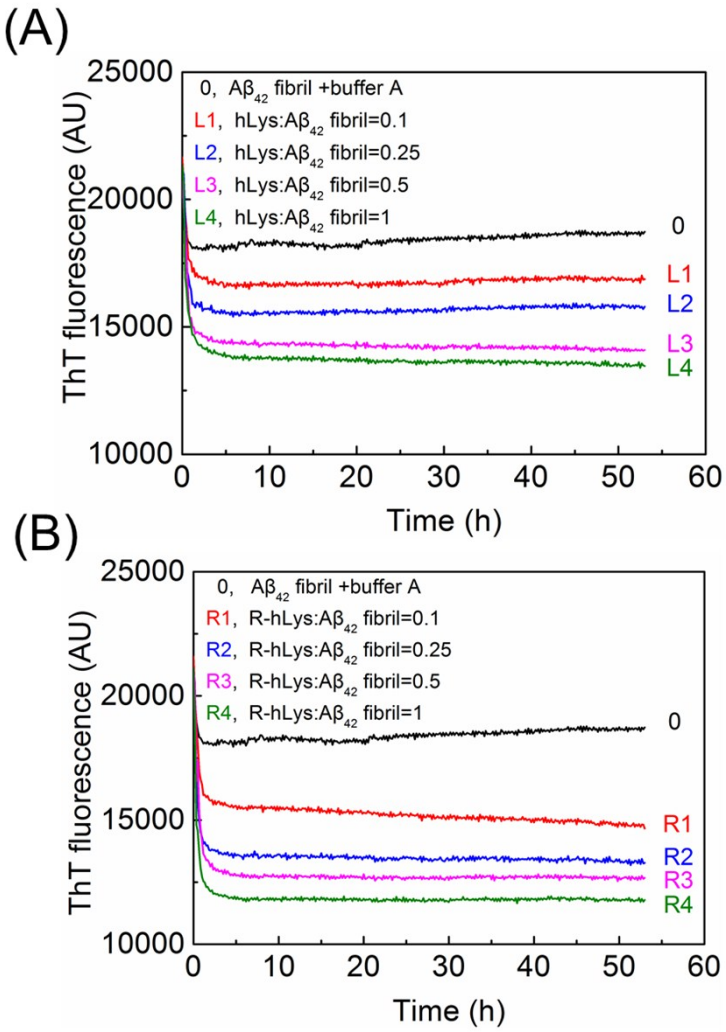

Fig. S12 ThT fluorescence kinetics for the remodeling of $A\beta_{42}$ fibrils with or without (A) hLys or (B) R-hLys at different concentrations. The final concentration of $A\beta_{42}$ fibrils was 25 μM. $A\beta_{42}$ fibrils were obtained by incubation in buffer A at 37 °C for 24 h.

References

1 L. Whitmore and B. A. Wallace, *Biopolymers*, 2008, **89**, 392-400.

2 N. Sreerama and R. W. Woody, *Anal. Biochem.* 2000, **287**, 252-260.