Supporting Information

Study on the Kinetics and Mechanism of Ferrocene-Tripeptide Inhibiting Insulin Aggregation

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1. Supplementary tables

Table S1. Effects of Fc-peptides on the kinetic parameters of insulin fibrillation in 20% (v/v) acetic acid solution. The concentration of insulin was 2 mg/mL.

	Fibril Formation		
	Lag time (h)	Fluorescence Intensity (a.u.)	Amyloidogenic extent (%)[a]
Insulin	62.9	138.0	100
Insulin + Fc-FFY			
100 μM	89.9	58.8	42.6
200 μM	135.1	23.7	17.2
300 μM	n.a.[b]	0.8	0.6
Insulin + Fc-FFF			
100 μM	98.4	52.5	38
200 μM	120.0	28.2	20.4
300 μM	n.a.	0.96	0.7
Insulin + Fc-FFD			
100 μM	90.0	67.13	48.6
200 μM	113.2	42.4	30.7
300 μM	122.0	37.4	27.1
Insulin + Fc-FFK			
100 μM	90.3	89.0	64.5
200 μM	86.0	64.9	47
300 μM	91.1	47.7	34.6

[a] The fluorescence intensity of insulin was set as a reference value. Amyloidogenic extent of a system was calculated by the final fluorescence intensity of the system divided by that of insulin.
[b] n.a. is short for not available.

Table S2. Sizes of insulin fibrils incubated with Fc-peptides for 156 h.

	Peptide inhibitors concentration (μM)		
	100	200	300
Insulin + Fc-FFY			
Hydrodynamic diameter (nm)	490.47	248.43	181.60
Full Width at Half Maximum (nm)	473.66	215.66	101.85
Insulin + Fc-FFF			
Hydrodynamic diameter (nm)	583.37	344.55	284.45
Full Width at Half Maximum (nm)	313.09	369.26	118.68
Insulin + Fc-FFD			
Hydrodynamic diameter (nm)	523.49	442.41	328.68
Full Width at Half Maximum (nm)	431.13	416.98	102.45
Insulin + Fc-FFK			
Hydrodynamic diameter (nm)	713.88	483.25	349.96
Full Width at Half Maximum (nm)	529.91	370.14	329.60

2. Supporting figures

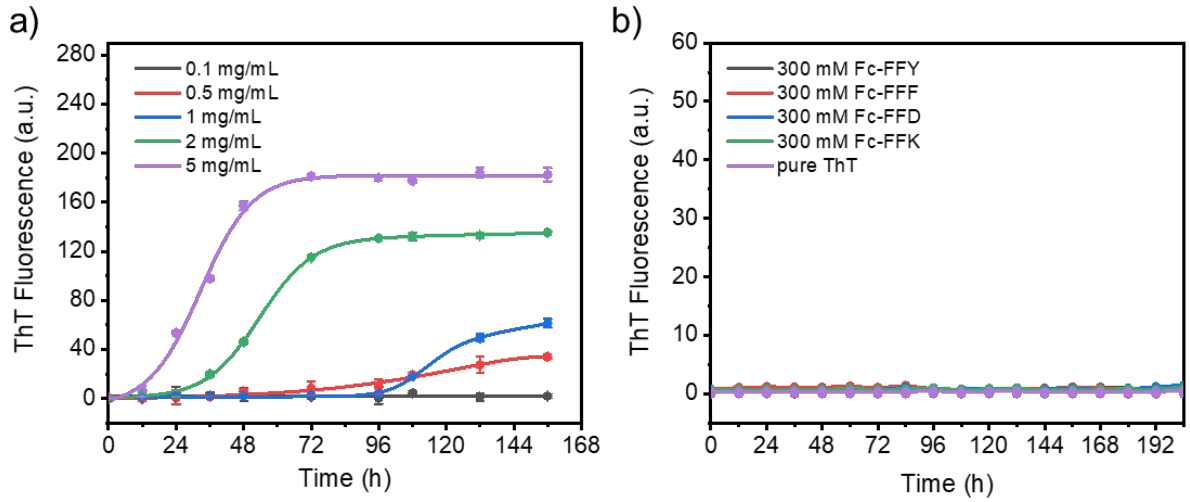

Figure S1. a) Fluorescence intensity of different concentrations of insulin incubated in 20% acetic acid solution at 60 °C. b) Fluorescence intensity of Fc-tripeptides and ThT incubated in 20% acetic acid solution at 60 °C.

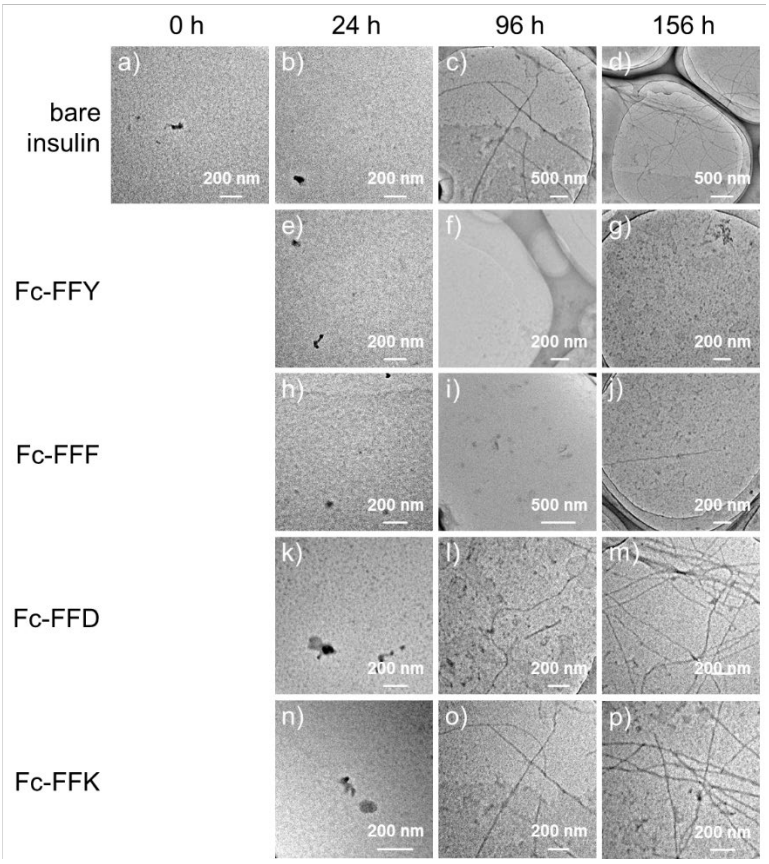

Figure S2. TEM images of insulin and insulin/Fc-tripeptides incubated for 156 h.

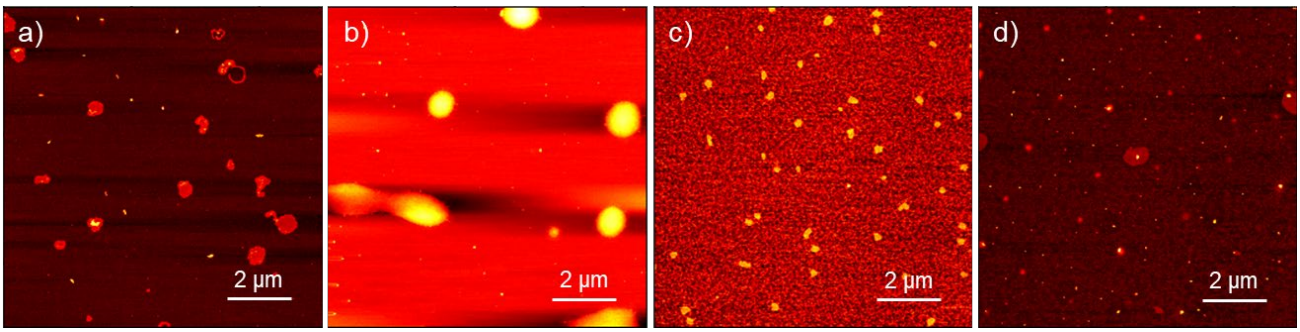

Figure S3. AFM images of Fc-tripeptides incubated for 156 h. (a) Fc-FFY, (b) Fc-FFF, (c) Fc-FFD or (d) Fc-FFK.

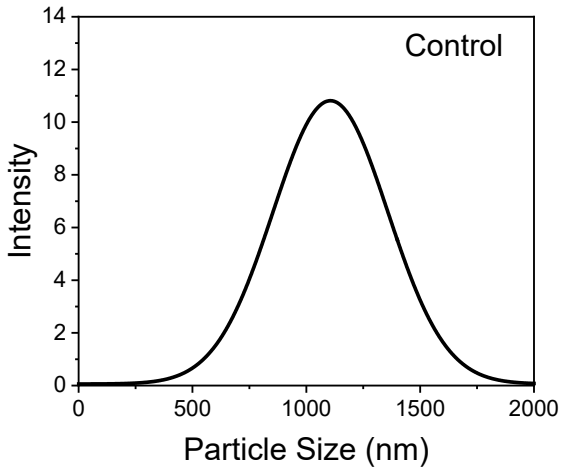

Figure S4. Size distribution of insulin incubated for 156 h.

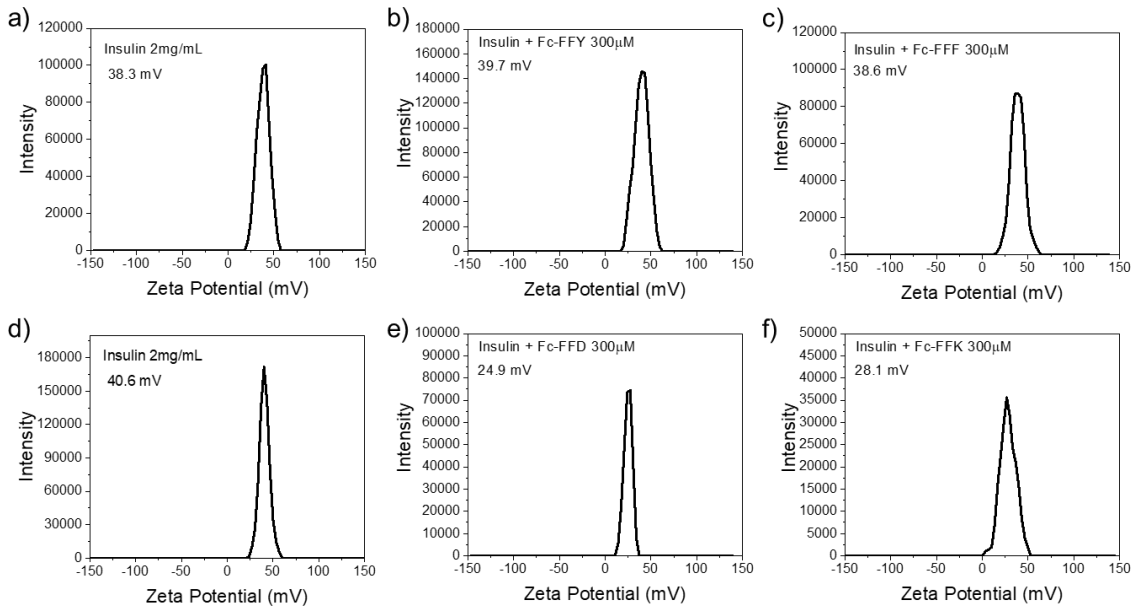

Figure S5. Zeta potential values of (a, d) insulin, (b) 300 μM Fc-FFY, (c) 300 μM Fc-FFF, (e) 300 μM Fc-FFD and (f) 300 μM Fc-FFK in water solution.

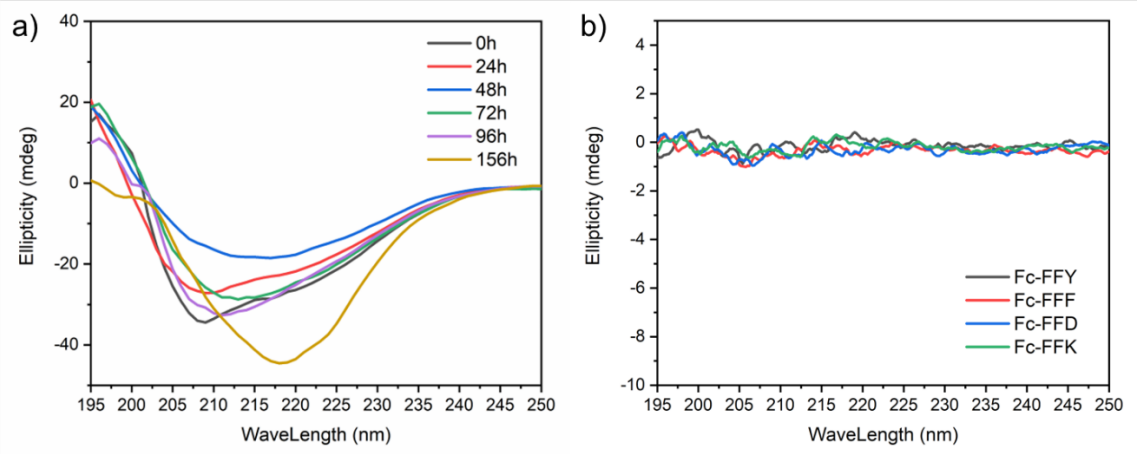

Figure S6. (a) CD spectrum of insulin in 20% (v/v) acetic acid solution after incubation for up to 156 h. (b) CD spectrum of the Fc-tripeptides.

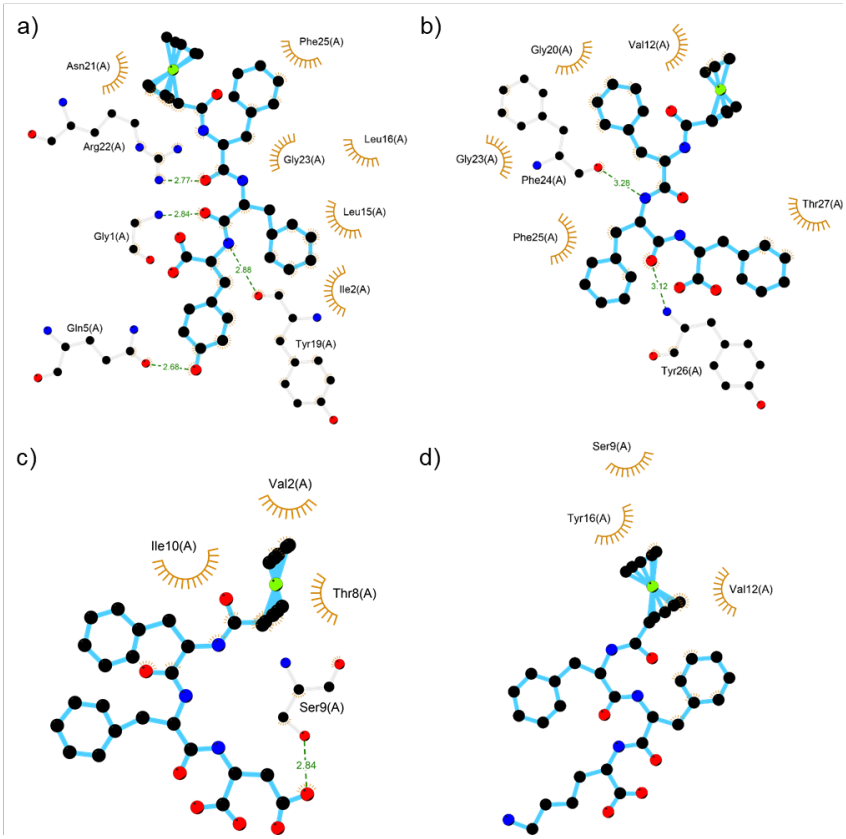

Figure S7. Intermolecular interactions between insulin and (a) Fc-FFY, (b) Fc-FFF, (c) Fc-FFD or (d) Fc-FFK were drawn with Ligplot+ program[1]. The atoms were color-coded as follows: C, black; O, red; N, blue. Bonds in Fc-tripeptides were colored cyan and those in insulin residues were colored gray. Hydrophobic interactions between Fc-tripeptides and insulins were shown in arc-shaped brown lines.

3. Supplementary methods

Transmission electron microscopy (TEM)

The morphology of insulin samples incubated in the absence and presence of Fc-tripeptides were represented on a JEOL 100CX-II transmission electron microscope (JEOL Ltd, Japan) operated at an accelerating voltage of 80 kV. Each sample was diluted and dropped 10 μL onto a carbon-coated copper grid and air-dried at room temperature. Then the grids with samples were negatively stained with 1% phosphotungstic acid solution for 4 min and examined.

References

1. R. A. Laskowski and M. B. Swindells, *J. Chem. Inf. Model.*, 2011, **51**, 2778-2786.