Supporting Information

Ferrocene-tagged peptides as inhibitors against insulin amyloid aggregation based on Molecular Simulation

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Supplementary Figures

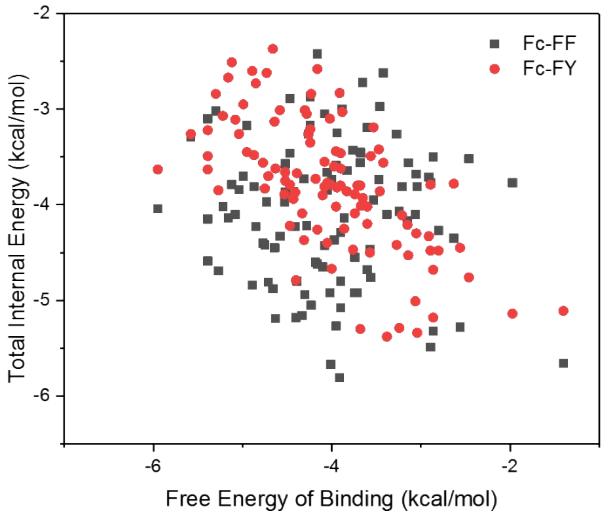

Fig. S1 Distribution of Fc-peptides on insulin, based on free energy and total internal energy. Black square dot for Fc-FF and red round dot for Fc-FY.

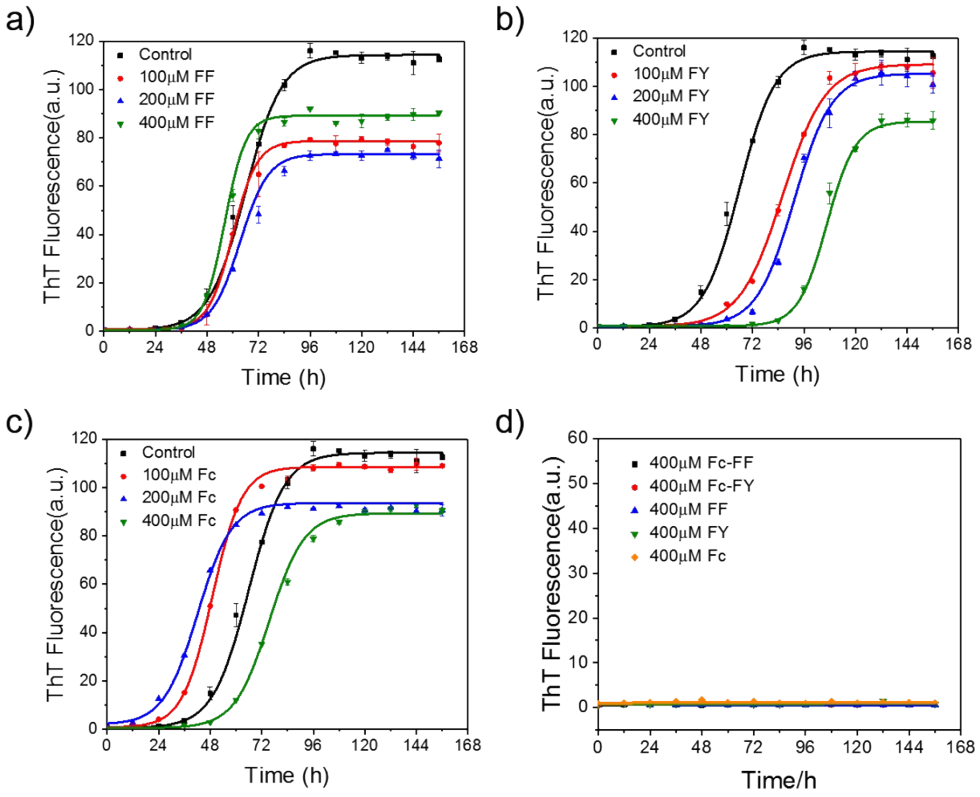

Fig. S2 The kinetics of aggregation of insulin incubated for 156h was monitored with and without FF (a), FY (b) and Fc (c) at concentration of 100, 200 and 400 μM. And the change in fluorescence intensity of Fc-FF, Fc-FY, FF, FY and Fc (d) incubated in 20% acetic acid solution at 60 °C, respectively. Error bars represent standard error of the mean (n=3).

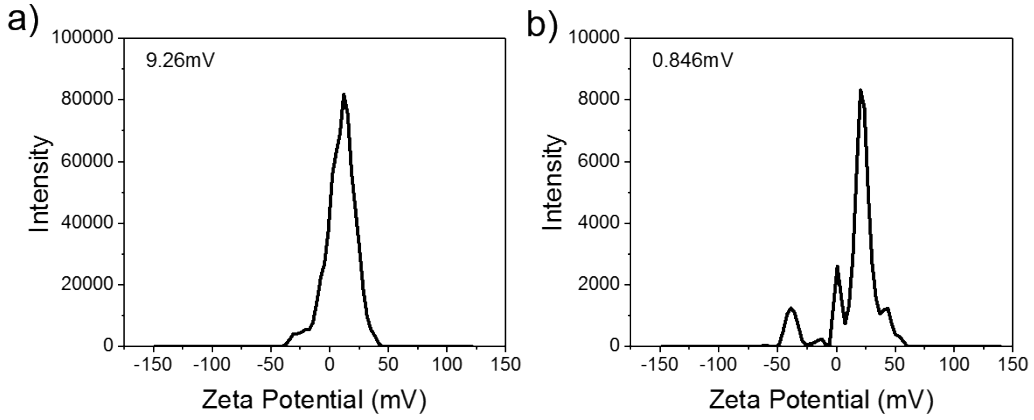

Fig. S3 Zeta potential values for Fc-FF (a) and Fc-FY (b) in 20% (v/v) acetic acid solution.

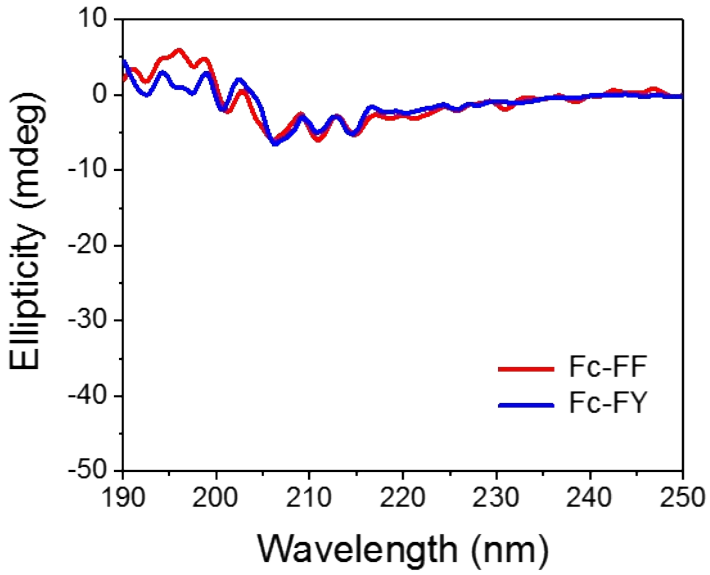

Fig. S4 Far-UV CD spectra of Fc-FF and Fc-FY after incubation for 156 h at 60 °C.

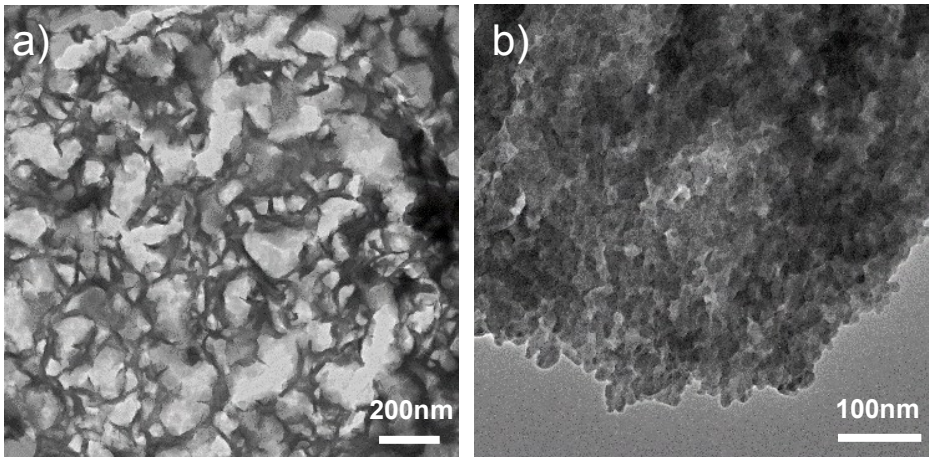

Fig. S5 Images of 400 μM Fc-FF (a) and Fc-FY (b) after incubation for 156 h at 60 °C.

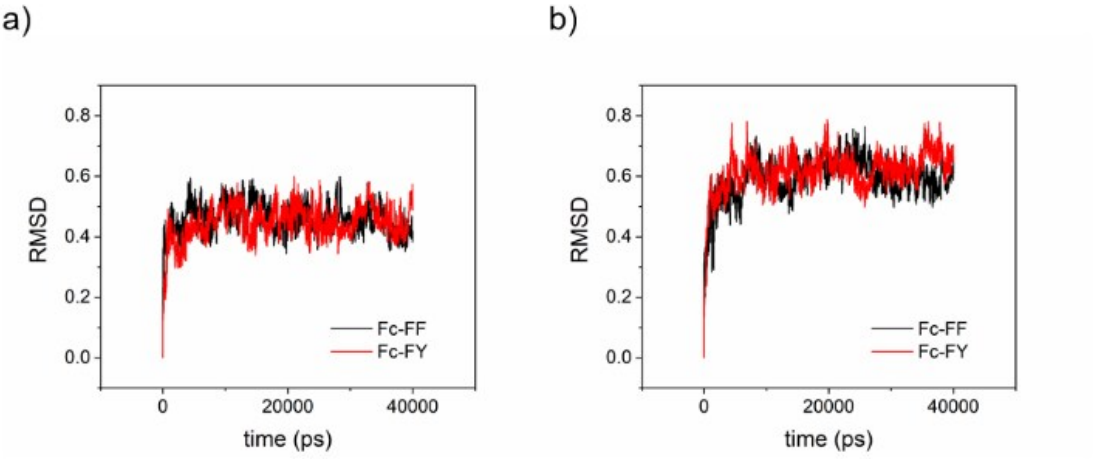

Fig. S6 RMSD of Fc-peptide/insulin-dimer (a) and Fc-peptide/insulin-monomer (b) systems during simulation.

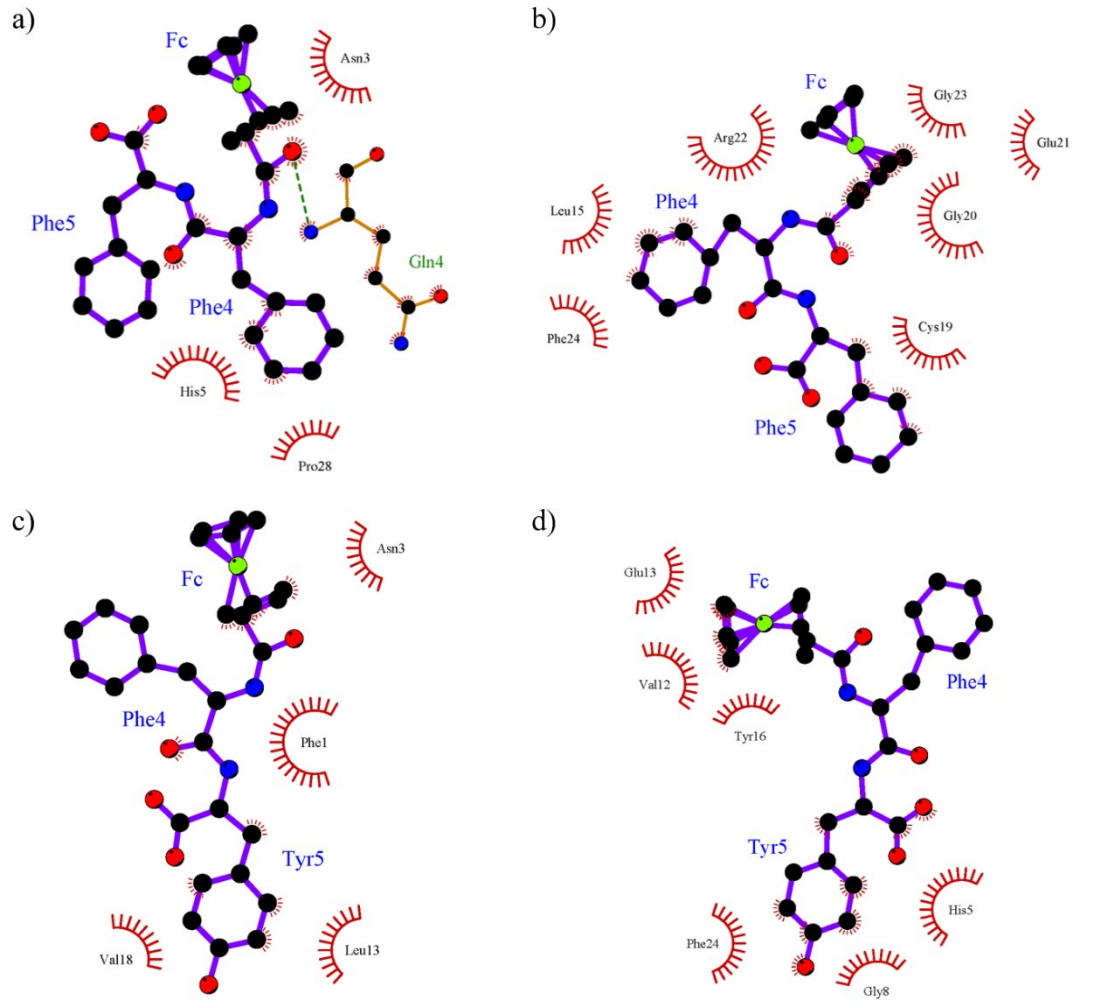

Fig. S7 Interactions between insulin and (a) Fc-FF and (b) Fc-FY in the final conformations are shown in 2-dimensional figure. Strong purple lines represent ligand bonds, orange lines represent non-ligand bond, and green dash lines are hydrogen bonds, with lengths noted above. The thin purple lines are covalent bonds among Fc group, which connected cyclopentadiene rings and Fe atom. The radial-like red lines represent hydrophobic interactions and involved residues and atoms.

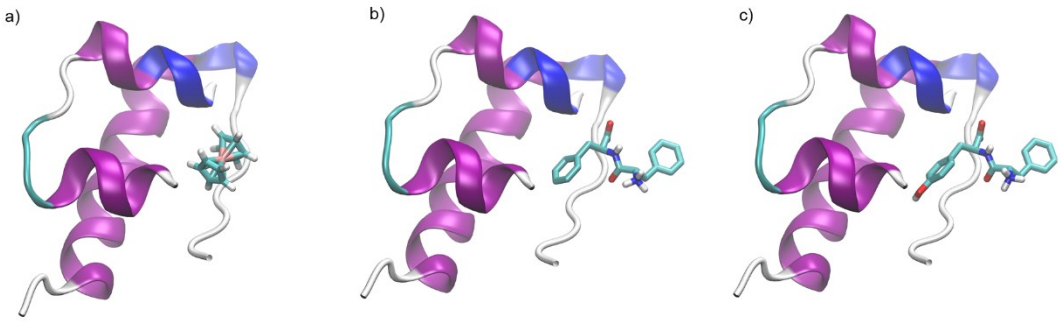

Fig. S8 The molecular docking conformation of (a) Fc, (b) FF, (c) FY with insulin.

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 of insulin was 2 mg/mL. The fluorescence intensity of insulin alone was set as a reference value.

	Peptide inhibitors concentration (μM)					
	0 (Control)	25	50	100	200	400
Insulin + Fc-FF						
Fluorescence	109.7	91.9	71.5	18.4	9.5	0.6
Inhibition rate (%)	-	16.2	34.8	83.3	91.4	99.4
Insulin + Fc-FY						
Fluorescence	109.7	99.9	78.3	23.6	14.6	0.8
Inhibition rate (%)	-	8.9	28.6	78.6	86.7	99.2

Table S2 Sizes of insulin fibrils incubated with and without Fc-peptides for 156 h.

	Peptide inhibitors concentration (μM)			
	0 (Control)	100	200	400
Insulin + Fc-FF				
Hydrodynamic diameter (nm)	1106	398.07	324.70	223.04
Full Width at Half Maximum (nm)	505.2	463.96	321.38	128.68
Insulin + Fc-FY				
Hydrodynamic diameter (nm)	1106	521.10	382.91	106.98
Full Width at Half Maximum (nm)	505.2	550.16	229.06	101.63

Table S3 Secondary structures contents of insulin in 20% acetic acid solution.

	Peptide inhibitors concentration (μM)			
	0 (Control)	100	200	400
Insulin + Fc-FF				
α-helix	0.128	0.216	0.27	0.306
β-sheet	0.435	0.151	0.101	0.045
unordered	0.309	0.325	0.356	0.394
Insulin + Fc-FY				
α-helix	0.128	0.239	0.255	0.28
β-sheet	0.435	0.188	0.142	0.052
unordered	0.309	0.321	0.339	0.379

Table S4 Summary on the IC50 values of some amyloid aggregation inhibitors

Amyloid	Inhibitor	IC50 value scale	Reference
Insulin (500 μM)	BSPOTPE	25-50 μM	Hong et al., *Journal of the American Chemical Society*, 2012 [1]
HIAPP (10 μM)	G3 PAMAM-OH	10-100 μM	Gurzov et al., *Small*, 2016 [2]
Human insulin (1 mg/mL)	C-Dots	2-4 mg/mL	Yang et al., *Journal of Materials Chemistry B*, 2017 [3]
Human insulin (20 μM)	VVVVV and VITYF	250-500 μM	Siddiqi et al., *Frontiers in chemistry*, 2018 [4]
Insulin (2 mg/mL, 344 μM)	Fc-FF and Fc-FY	50-100 μM (26-54 μg/mL)	This study

1. Y. Hong, L. Meng, S. Chen, C. W. T. Leung, L.-T. Da, M. Faisal, D.-A. Silva, J. Liu, J. W. Y. Lam and X. Huang, *Journal of the American Chemical Society*, 2012, **134**, 1680-1689.
2. E. N. Gurzov, B. Wang, E. H. Pilkington, P. Chen, A. Kakinen, W. J. Stanley, S. A. Litwak, E. G. Hanssen, T. P. Davis and F. Ding, *Small*, 2016, **12**, 1615-1626.
3. Q. Yang, J. Jin, Z. Xu, J. Zhang, B. Wang, F. Jiang and Y. Liu, *Journal of Materials Chemistry B*, 2017, **5**, 2010-2018.
4. M. K. Siddiqi, P. Alam, T. Iqbal, N. Majid, S. Malik, S. Nusrat, A. Alam, M. R. Ajmal, V. N. Uversky and R. H. Khan, *Frontiers in chemistry*, 2018, **6**, 311.