Supporting Information

Regulation mechanism of human insulin fibrillation by L-lysine Carbon Dots: low concentration accelerates but high concentration inhibits the fibrillation process

Xing-Yu Liu[a], Shuai-Chen Du[a], Feng-Lei Jiang[a], Peng Jiang[a,d,*], Yi Liu[a,b,c,*]

[a]College of Chemistry and Molecular Sciences & School of Pharmaceutical Sciences, Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (MOE), Wuhan University, Wuhan 430072, P. R. China

[b]School of Chemistry and Environmental Engineering, Wuhan Polytechnic University, Wuhan 430023, P. R. China

[c]State Key Laboratory of Separation Membrane and Membrane Process & Tianjin Key Laboratory of Green Chemical Technology and Process Engineering, School of Chemistry, Tiangong University, Tianjin 300387, P. R. China

[d]Hubei Jiangxia Laboratory, Wuhan 430023, P. R. China

Corresponding Author

Peng Jiang - E-mail: jiangpeng@whu.edu.cn

Yi Liu - E-mail: yiliuchem@whu.edu.cn

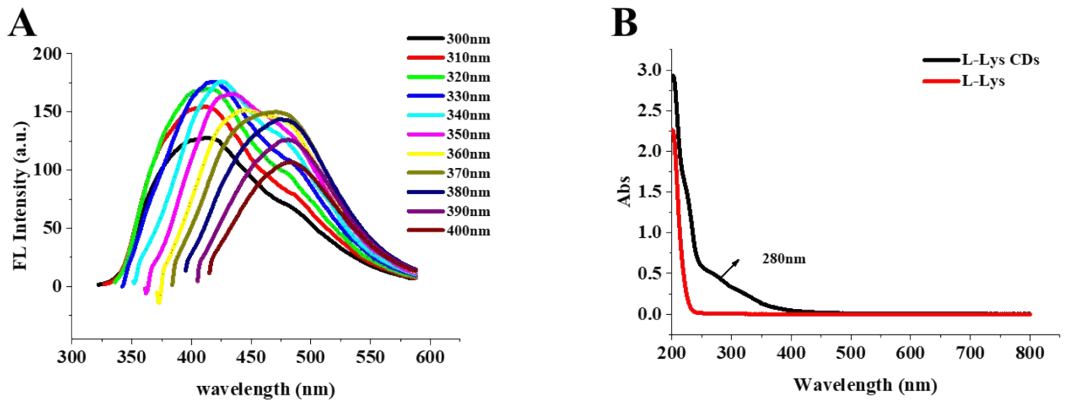

Figure S1. (A) PL spectra of L-Lys CDs; (B) Absorption spectra of L-Lys CDs

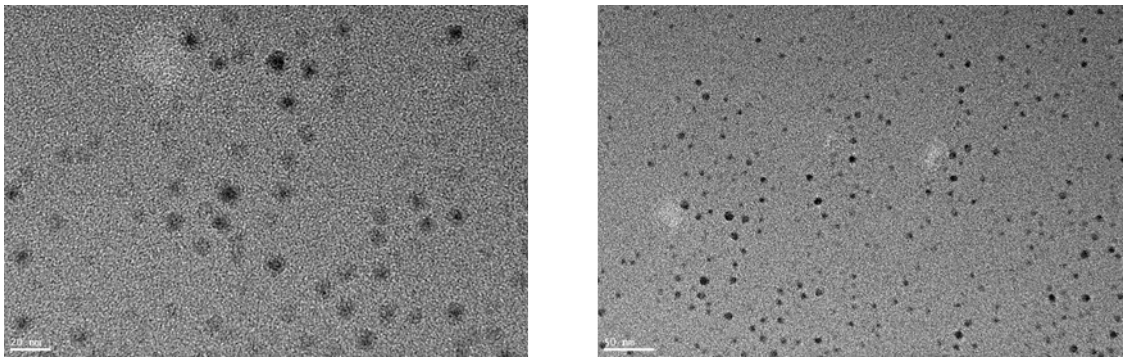

Figure S2. TEM images of L- Lys CDs.

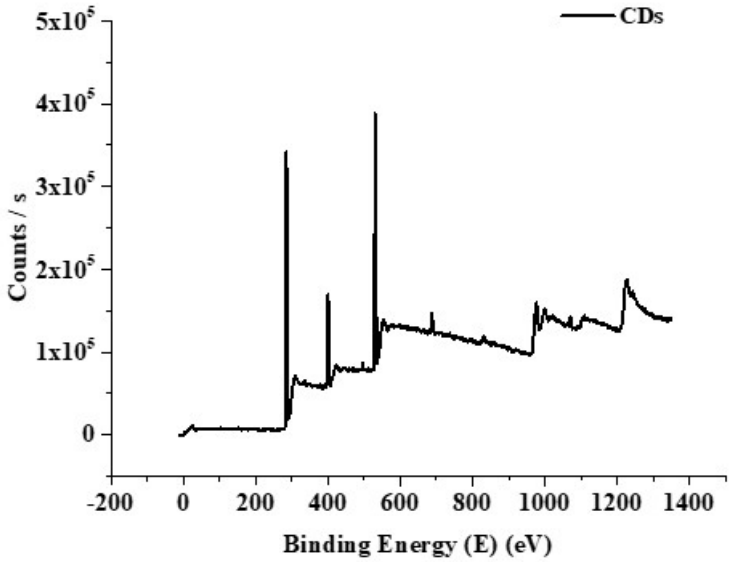

Figure S3. XPS spectra of L- Lys CDs.

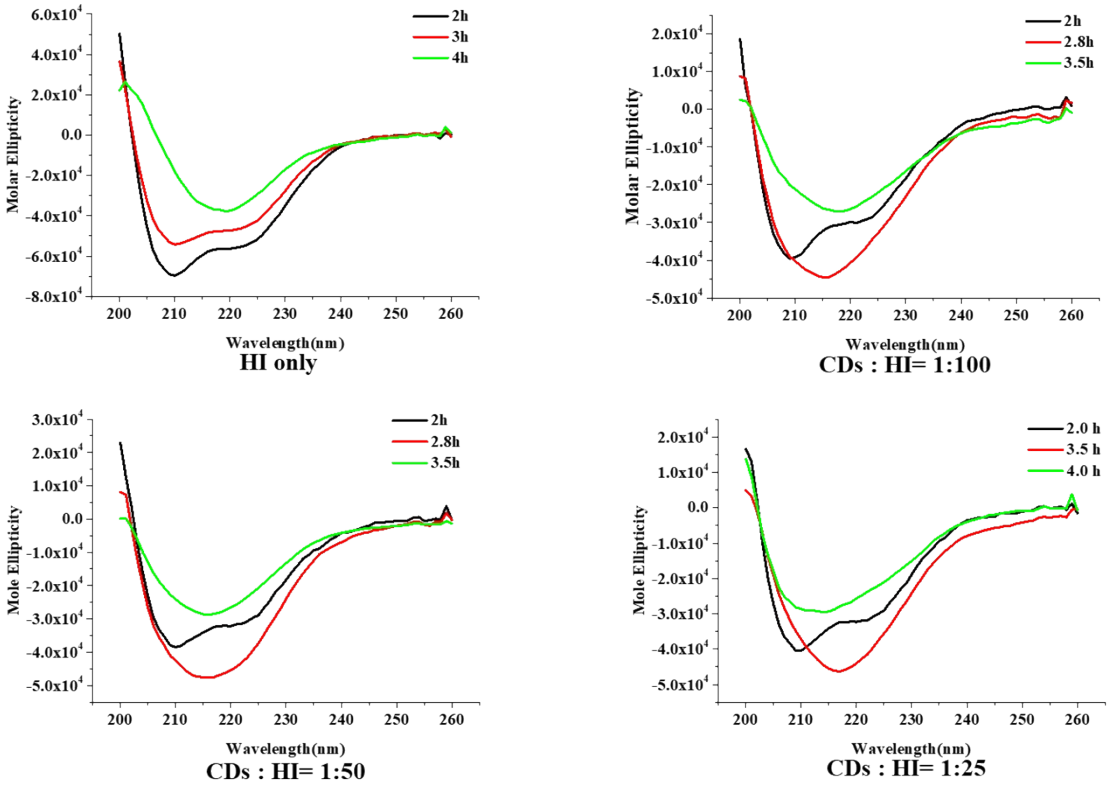

Figure S4. CD spectrum of HI at different incubation time periods.

Table S1. The value of t_0, τ, t_{lag} and υ when CDs-to-HI mass concentration ratio is different.

$y = y_0 + y_1 / (1+\exp(-(t-t_0)/\tau))$ $t_{lag} = t_0 - 2\tau$ $\upsilon = 1/\tau$	t_0 /h	τ /h	t_{lag} /h	υ /h $^{-1}$
HI only	2.42	0.13	2.16	7.69
L-Lys CDs: HI=1:100	2.79	0.08	2.63	12.5
L-Lys CDs: HI=1:50	3.00	0.06	2.88	16.66
L-Lys CDs: HI=1:25	3.31	0.11	3.07	9.09
L-Lys CDs: HI=1:20	4.78	0.12	4.54	8.33
L-Lys CDs: HI=1:10	5.93	0.17	5.59	5.88
L-Lys CDs: HI=1:5	20.01	0.18	19.65	5.55
L-Lys CDs: HI=1:2	27.02	0.19	26.64	5.26
L-Lys CDs: HI=1:1	42.42	0.21	42.00	4.76

Table S2. Distribution of HI's secondary structures calculated from CD spectrum

		α-helix	β-sheet	β-turn	Unrd
HI 2h		0.661	0.025	0.116	0.231
HI 3h		0.345	0.202	0.174	0.288
HI 4h		0.153	0.549	0.122	0.176
L-Lys CDs: HI=1:100	2.0 h	0.624	0.033	0.121	0.222
L-Lys CDs: HI=1:100	2.8 h	0.298	0.365	0.174	0.167
L-Lys CDs: HI=1:100	3.5 h	0.112	0.570	0.119	0.199
L-Lys CDs: HI=1:50	2.0 h	0.645	0.016	0.168	0.171
L-Lys CDs: HI=1:50	2.8 h	0.304	0.335	0.186	0.175
L-Lys CDs: HI=1:50	3.5 h	0.152	0.547	0.102	0.199
L-Lys CDs: HI=1:25	2.0 h	0.651	0.025	0.116	0.157
L-Lys CDs: HI=1:25	3.5 h	0.333	0.328	0.166	0.173
L-Lys CDs: HI=1:25	4.0 h	0.144	0.553	0.119	0.164

Table S3. The value of t_0, τ, t_{lag} and υ when CDs-to-HI mass concentration ratio was 1:5 with different delay time of adding CDs.

$y = y_0 + y_1 /(1+\exp(-(t-t_0)/\tau))$ $t_{lag} = t_0 - 2\tau$ $\upsilon = 1/\tau$	t_0 /h	τ /h	t_{lag} /h	S^2	υ /h $^{-1}$	S^2
Control	2.42	0.13	2.16	0.022	7.69	0.122
0 h delay	20.01	0.18	19.65	0.667	5.55	0.208
1 h delay	10.70	0.17	10.44	0.987	5.88	0.529
2 h delay	2.86	0.15	2.56	0.539	6.66	1.03

Table S4. The value of t_0, τ, t_{lag} and υ when CDs-to-HI mass concentration ratios was 1:50 with different delay time of adding CDs.

$y = y_0 + y_1 /(1+\exp(-(t-t_0)/\tau))$ $t_{lag} = t_0 - 2\tau$ $\upsilon = 1/\tau$	t_0 /h	τ/h	t_{lag} /h	S^2	υ /h $^{-1}$	S^2
Control	2.42	0.13	2.16	0.022	7.69	0.122
0 h delay	3.00	0.06	2.88	0.272	16.66	0.889
1 h delay	2.66	0.07	2.59	0.453	14.28	1.56
2 h delay	2.39	0.12	2.15	0.556	8.33	1.14

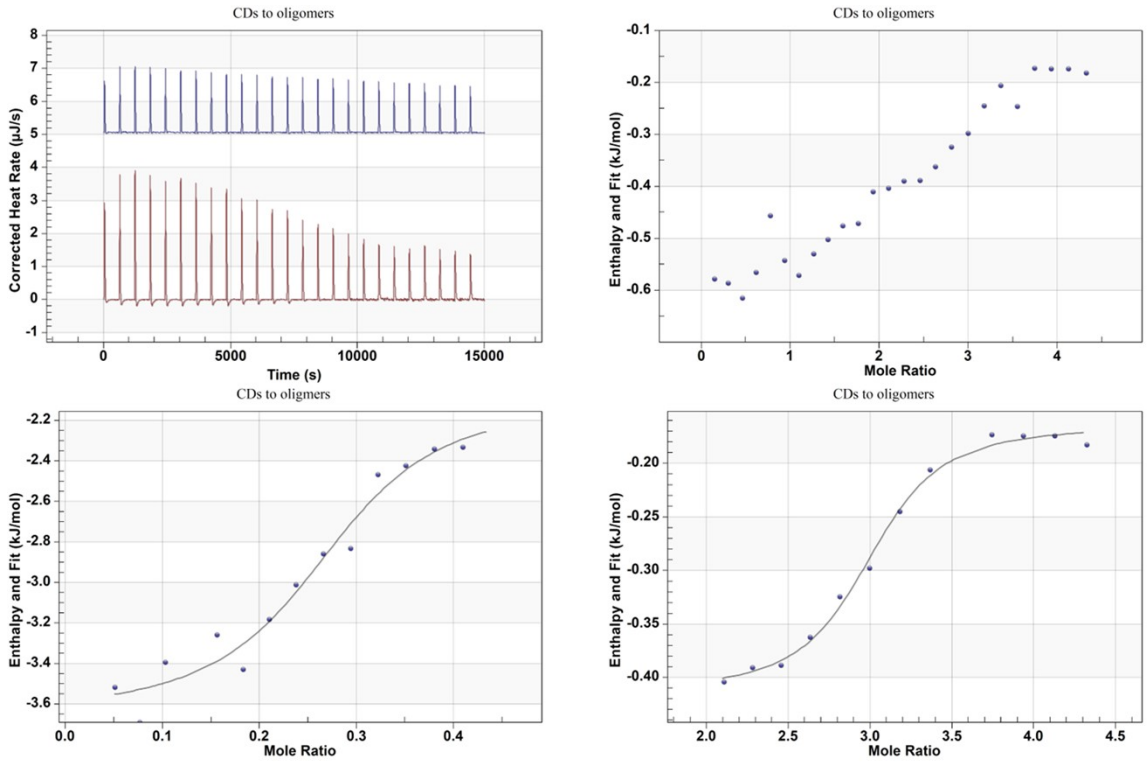

Figure S5. ITC process of CDs to HI monomers. Corrected heat rate of background and experiment; Enthalpy heat of overall process; The 1st step's enthalpy heat; The 2nd step's enthalpy heat. Molar concentration of HI was 0.53 mmol L^{-1}, molar concentration of CDs was 1.21 mmol L^{-1}. All experiments repeat for three times. The deviations of fitting parameters were shown in Table 1.

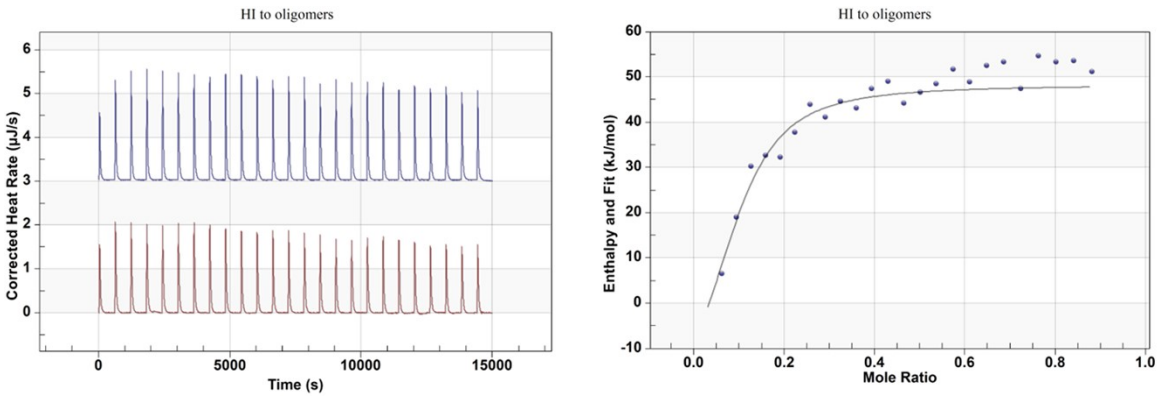

Figure S6. ITC process of HI monomers to HI oligomers. Corrected heat rate of background and experiment and Enthalpy heat of overall process. Molar concentration of HI was 0.53 mmol L^{-1}, molar concentration of CDs was 1.21 mmol L^{-1}. All experiments repeat for three times. The deviations of fitting parameters were shown in Table 1.

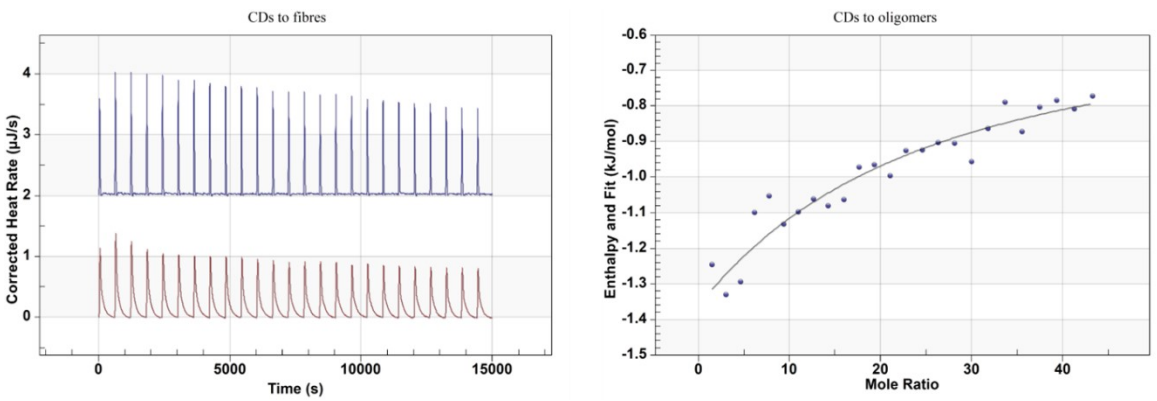

Figure S7. ITC process of CDs to HI fibres. Corrected heat rate of background and experiment and Enthalpy heat of overall process. Molar concentration of HI was 0.53 mmol L^{-1}, molar concentration of CDs was 1.21 mmol L^{-1}. All experiments repeat for three times. The deviations of fitting parameters were shown in Table 1.

Table S5. The ratio of combination (*n*) of ITC group (CDs to HI). The concentration of both compounds was 1 mg mL^{-1}

CDs to HI	1st	2nd
n (CDs/HI)	4.12±0.11	4.35±0.14

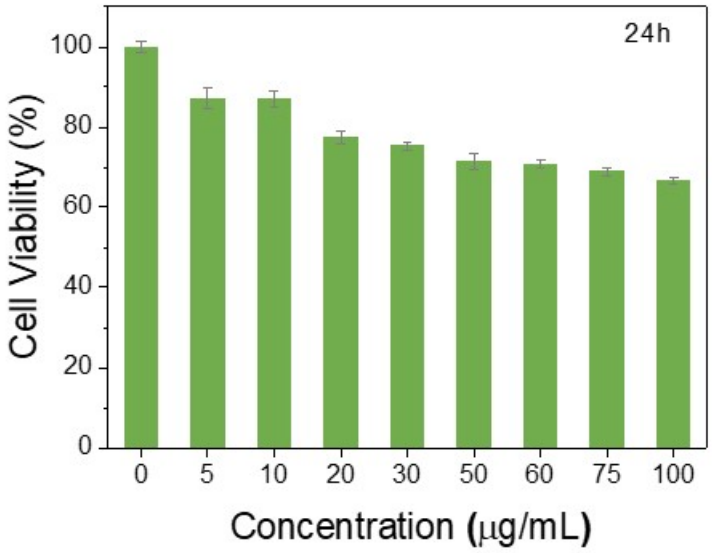

Figure S8. Viability of the Hela cells after 24h incubation with the CDs

Table S6. The zeta potential values of CDs and insulin. The pH was same as that of fibrillation process. The concentration of both compounds was 1 mg mL^{-1}

	CDs	Insulin
Zeta potential	22.6±2.7	-31.4±1.8

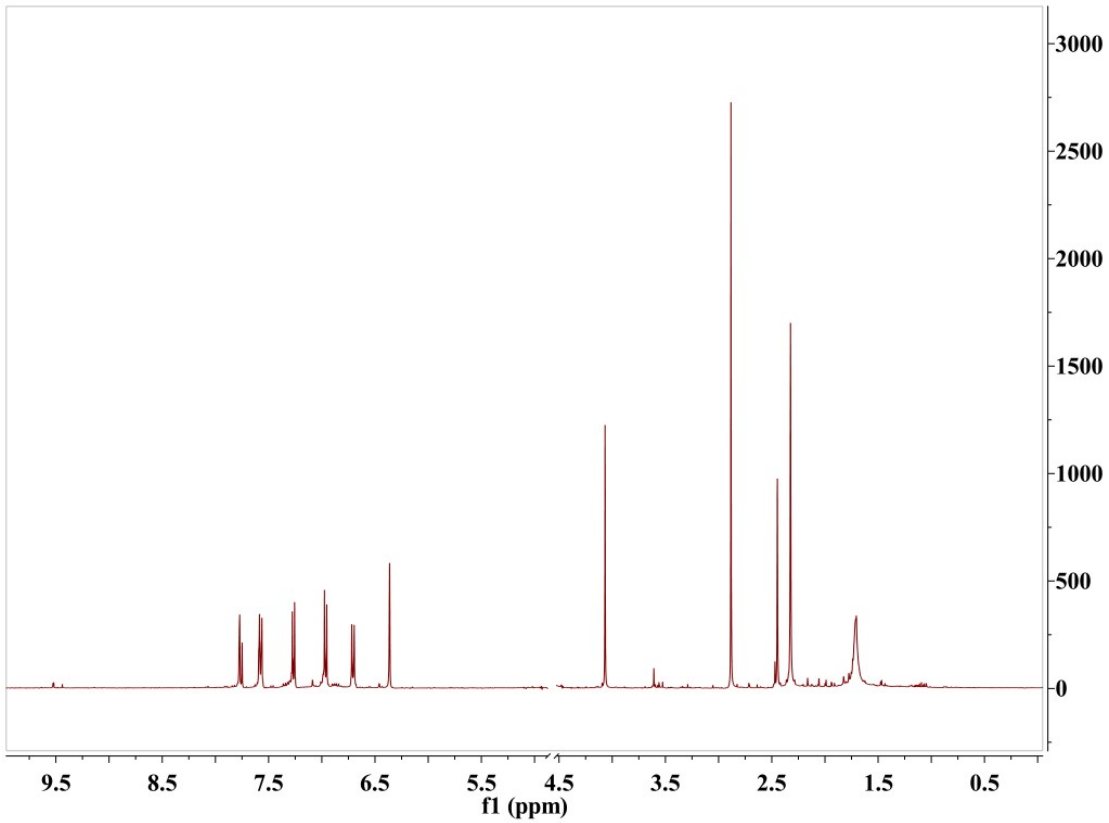

Figure S9. ^{1}H NMR spectrum of L-Lys CDs. 5 mg CDs were dissolved in 0.3 mL D_2O, the NMR spectrometer was 400 MHz.

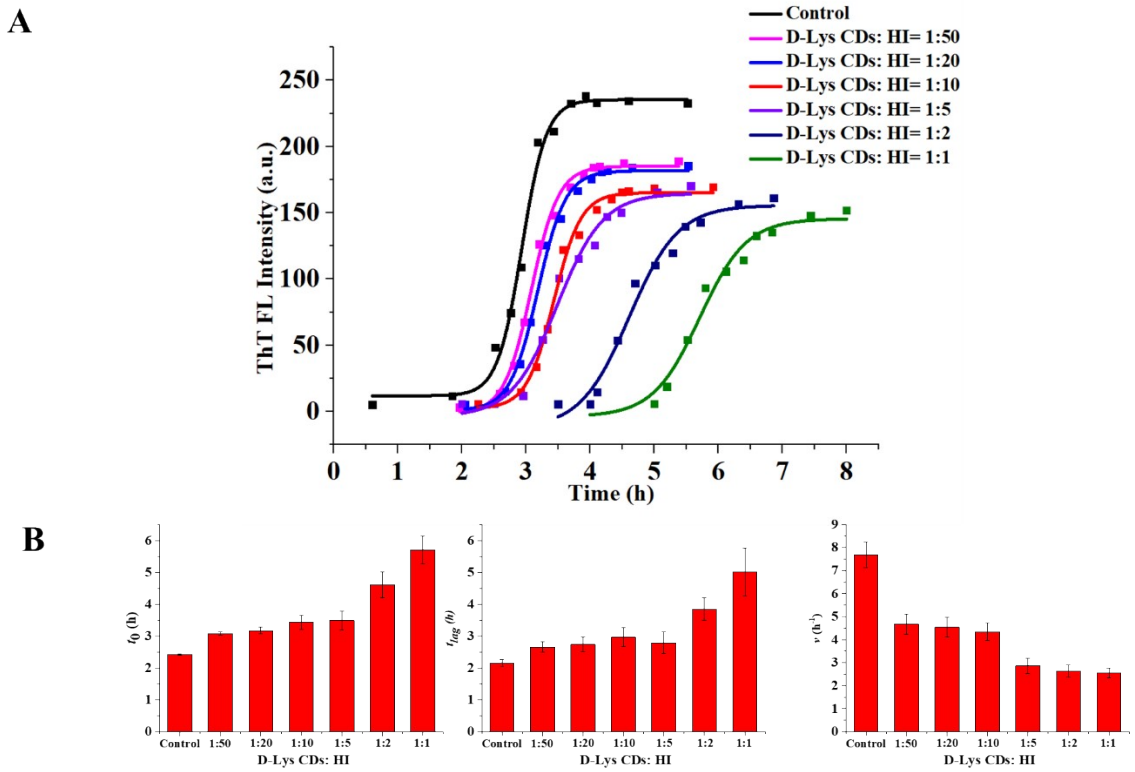

Figure S10. Kinetics of insulin fibrillation process. The concentration of HI was 1 mg mL^{-1}. The dissolvent of HI was HCl-NaCl (100 mM, pH = 2.0). The change of ThT fluorescence intensity was acquire by the samples of different incubation times (A). The column of t_0, t_{lag} and υ when CDs-to-HI mass concentration ratios of 1:50, 1:20, 1:10, 1:5, 1:2 and 1:1 (B). All experiments repeat for three times. The error bars of fitting parameters were shown in Figure 10B.

Table S7. The value of t_0, τ, t_{lag} and υ when D-Lys CDs-to-HI mass concentration ratio is different.

$y = y_0 + y_1 / (1+\exp(-(t-t_0)/\tau))$ $t_{lag} = t_0 - 2\tau$ $\upsilon = 1/\tau$	t_0 /h	τ /h	t_{lag} /h	υ /h $^{-1}$
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D-Lys CDs: HI=1:50	3.08	0.21	2.66	4.76
D-Lys CDs: HI=1:20	3.18	0.22	2.74	4.54
D-Lys CDs: HI=1:10	3.43	0.23	2.97	4.35
D-Lys CDs: HI=1:5	3.49	0.35	2.79	2.86
D-Lys CDs: HI=1:2	4.61	0.38	3.85	2.63
D-Lys CDs: HI=1:1	5.71	0.39	5.01	2.56