Supporting Information

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/TOF MS) analysis was performed on a Microflex MALDI-TOF MS system (Bruker Daltonics Inc., Billerica, USA) in the positive linear mode with delayed extraction. The α -cyano-4-hydroxycinnamic acid (CHCA) matrix solution was prepared by adding 10 mg CHCA to 1 mL 50% acetonitrile in water and 0.1% trifluoroacetic acid. A 1 µL aliquot of the OPCs sample was mixed with 10 µL CHCA matrix solution. The MALDI sample was prepared by depositing 0.5 µL of the mixture solution on the stainless steel sample target and then letting the sample air-dry. The measurement was performed using the LP-ClinProt method.



Figure S1. MALDI-TOF MS spectrum of OPC: m/z 578.36 Da (M+H)⁺; 691.49 Da (M+CF₃COOH)⁺; 804.63 Da (M+2CF₃COOH)⁺.



Figure S2. AFM images of OPC–INS nanoclusters formed on the mica surface. Samples were incubated for 6 h at pH 7.0. [OPC] = 100μ M.



Figure S3. The elution profiles of insulin aggregates and OPC–INS nanoparticles prepared from insulin (2 mg/1 mL) in 20 mM SP buffer at pH 6.5 (A, B), pH 7 (C, D), and pH 8 (E, F), respectively. The molecular weight was calculated using ASTRA software.



Figure S4. The elution profiles of OPC-INS nanoparticles prepared from insulin (2 mg/1 mL) in 20 mM SP buffer (pH=7) in the presence of 10 μ M (A), 100 μ M (B), and 1 mM (C) OPC, respectively. The molecular weight was calculated using ASTRA software.



Figure S5. Photographs of OPC-INS nanoparticles suspension at different pH.



Figure S6. TEM images of insulin incubated for (A) 6 h, (B) 15 h, (C) 30 h, and (D) 70 h

at pH 7.0.



Figure S7. TEM image of OPC-INS nanoparticles incubated for 6 h at pH 7.0. [OPC] =

100 μ M (A), and AFM image of insulin aggregates (INSAs) incubated for 70 h at pH 7.0.



Figure S8. In vitro release profiles of insulin from native insulin (INS), insulin aggregates (INSAs), and OPC–INS nanoparticles with different OPC concentrations in PBS buffer (pH 7) at 37 °C under the stirring rate of 60 rpm. Each data presented as mean \pm s.d. (n = 3).

	α-Helix (%)	β-Sheet (%)	Turn (%)	Random Coil (%)
INS	0.30	0.20	0.22	0.28
OPC-INS	0.16	0.30	0.23	0.32

Table S1. Secondary structure contents of INS and OPC-INS nanoparticles