Supporting Information

Injectable Biodegradable Polymeric Complex for Glucose-Responsive Insulin Delivery

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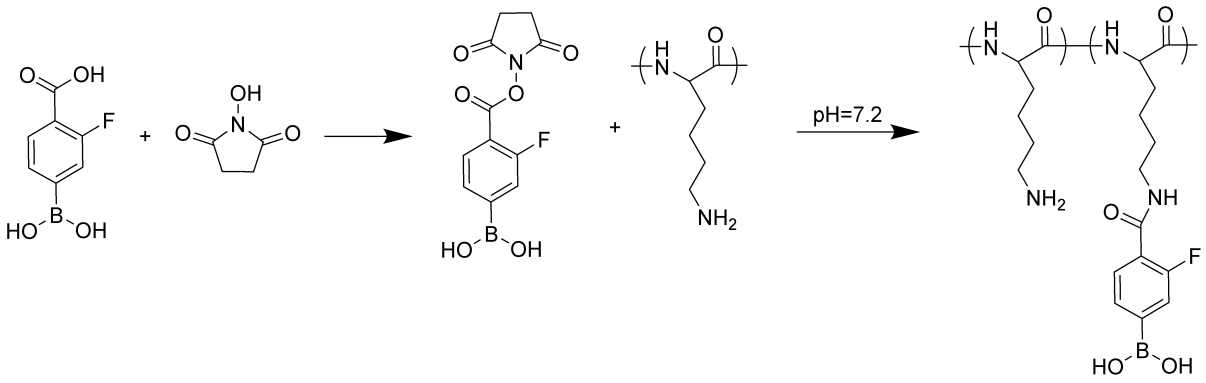

Scheme S1. Synthesis route of PLL-FPBA.

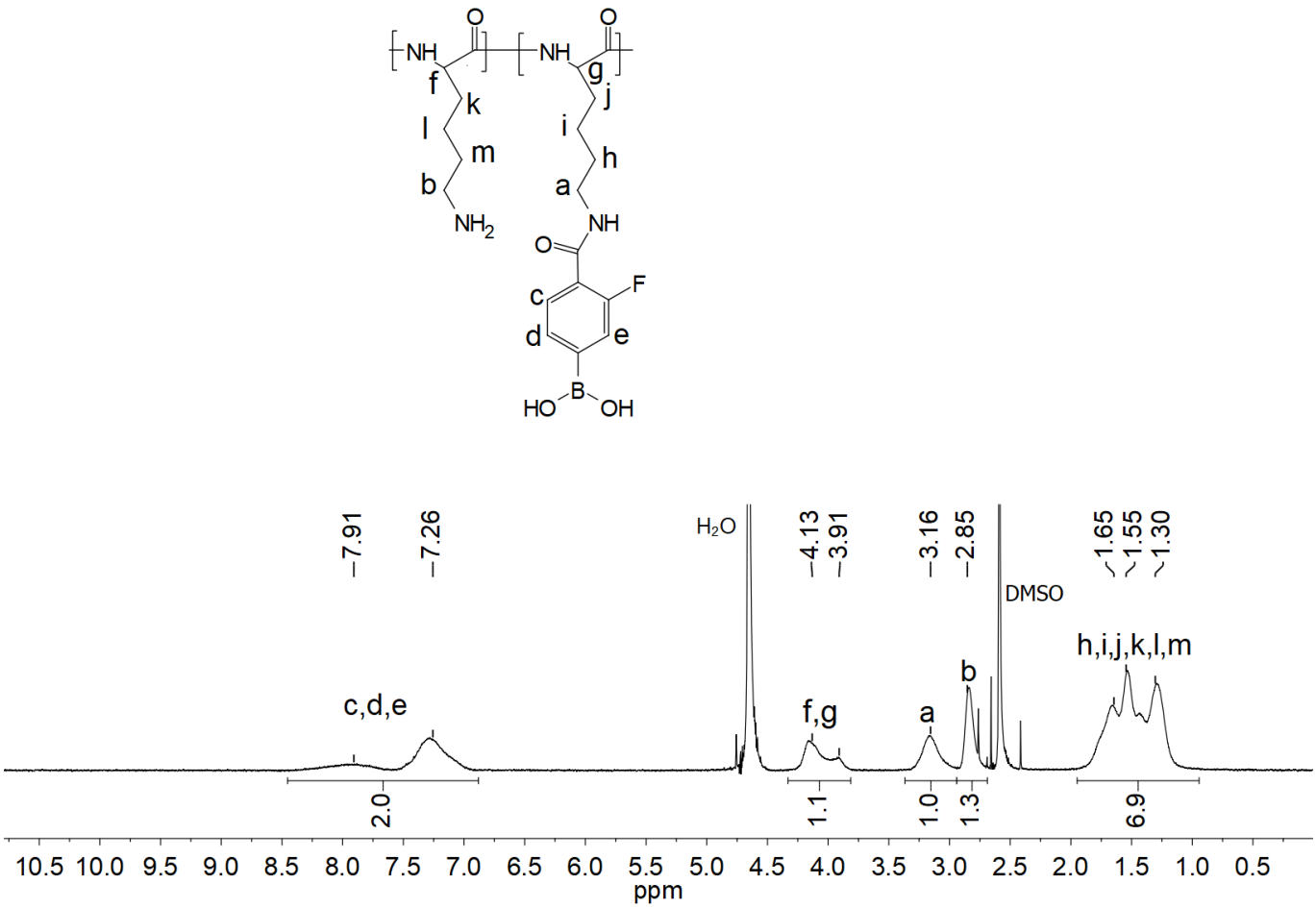

Figure S1. ^{1}H-NMR spectrum of FPBA modified $PLL_{4\text{-}15k}$ in D_2O with TFA to adjust its pH. About 43% of the amino groups in this polymer was reacted with FPBA-NHS.

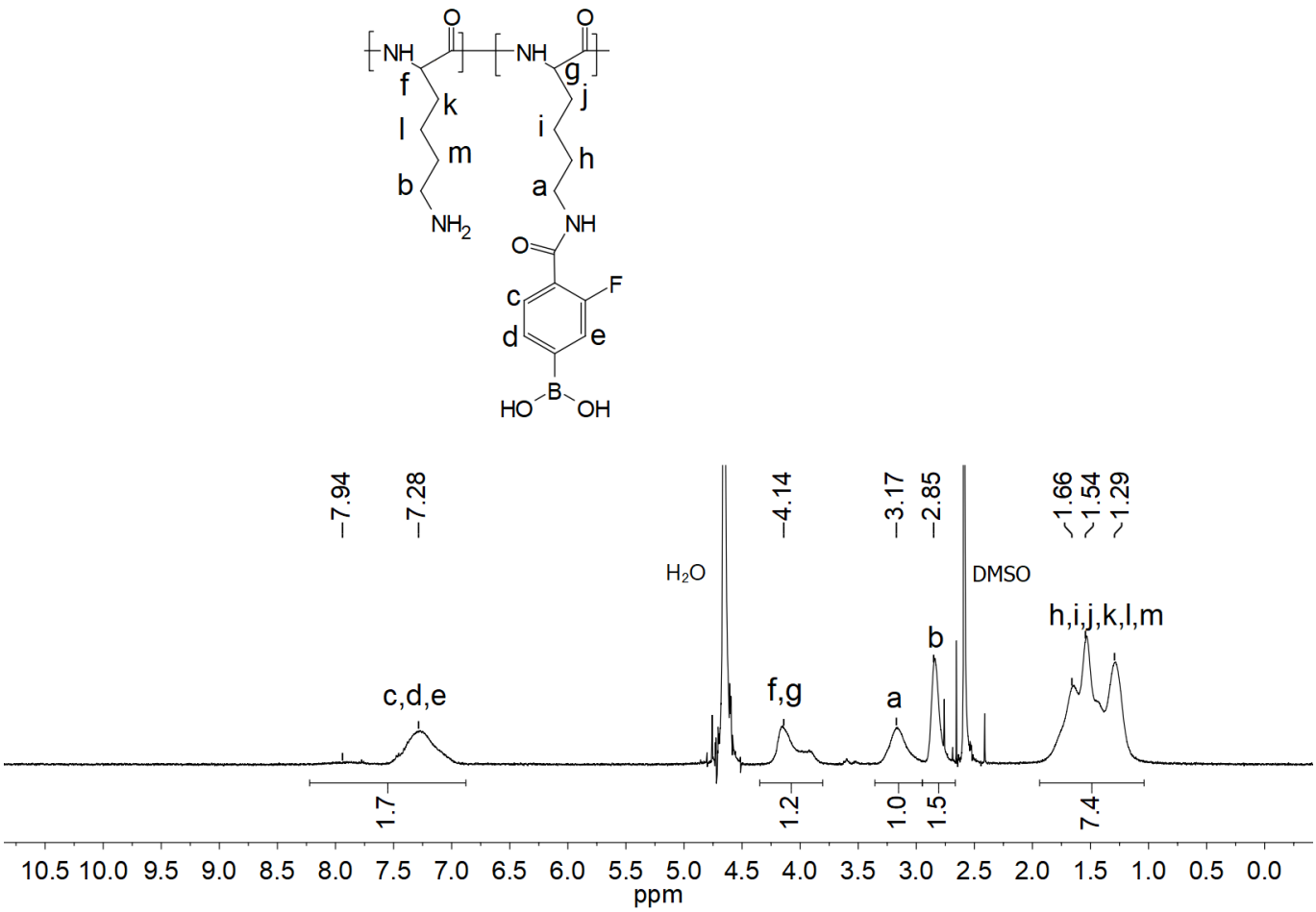

Figure S2. ^{1}H-NMR spectrum of FPBA modified $PLL_{15\text{-}30k}$ in D_2O with TFA to adjust its pH. About 40% of the amino groups in this polymer was reacted with FPBA-NHS.

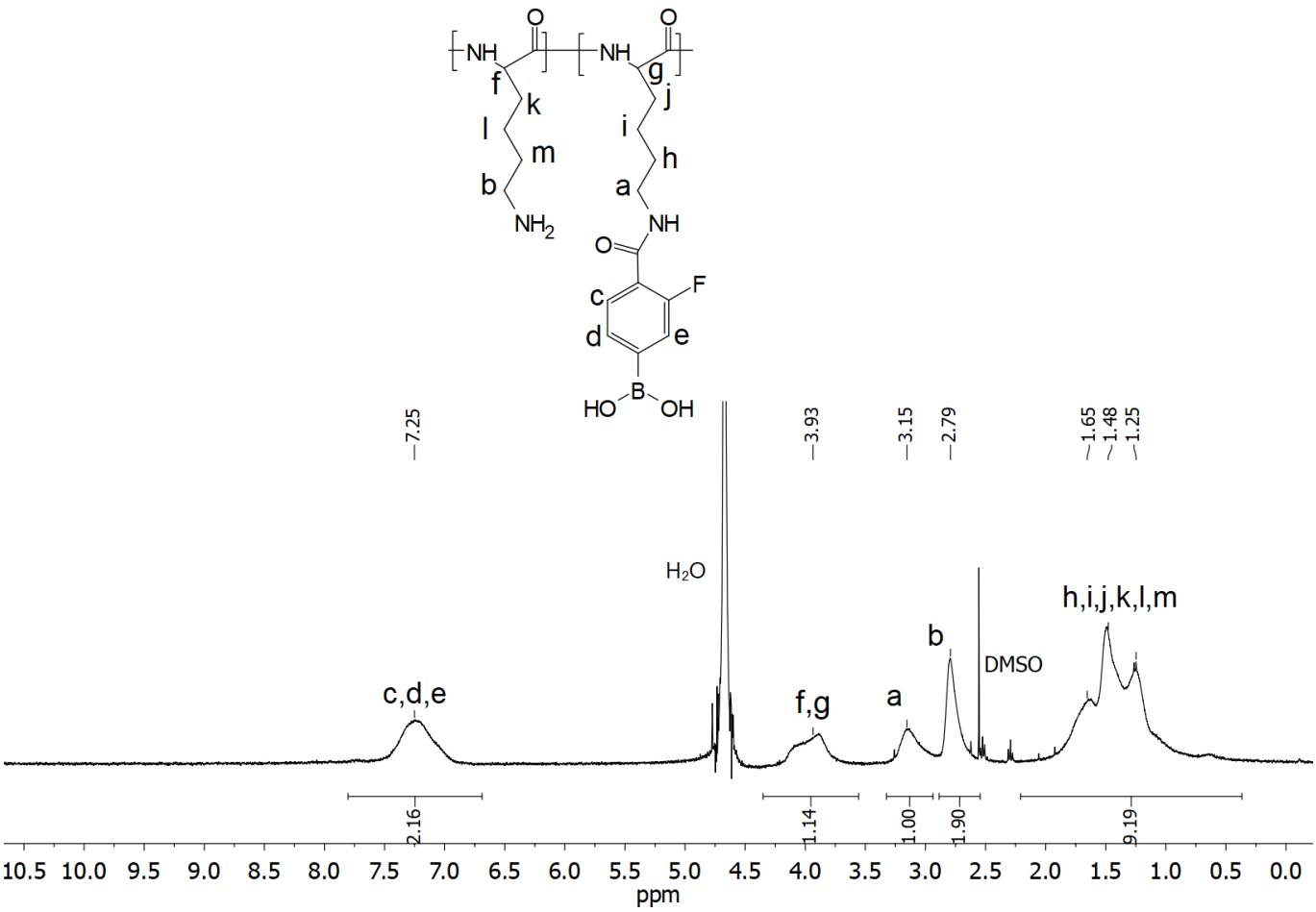

Figure S3. ^{1}H-NMR spectrum of FPBA modified $PLL_{30k\text{-}70k}$ in D_2O with TFA to adjust its pH. About 35% of the amino groups in this polymer was reacted with FPBA-NHS.

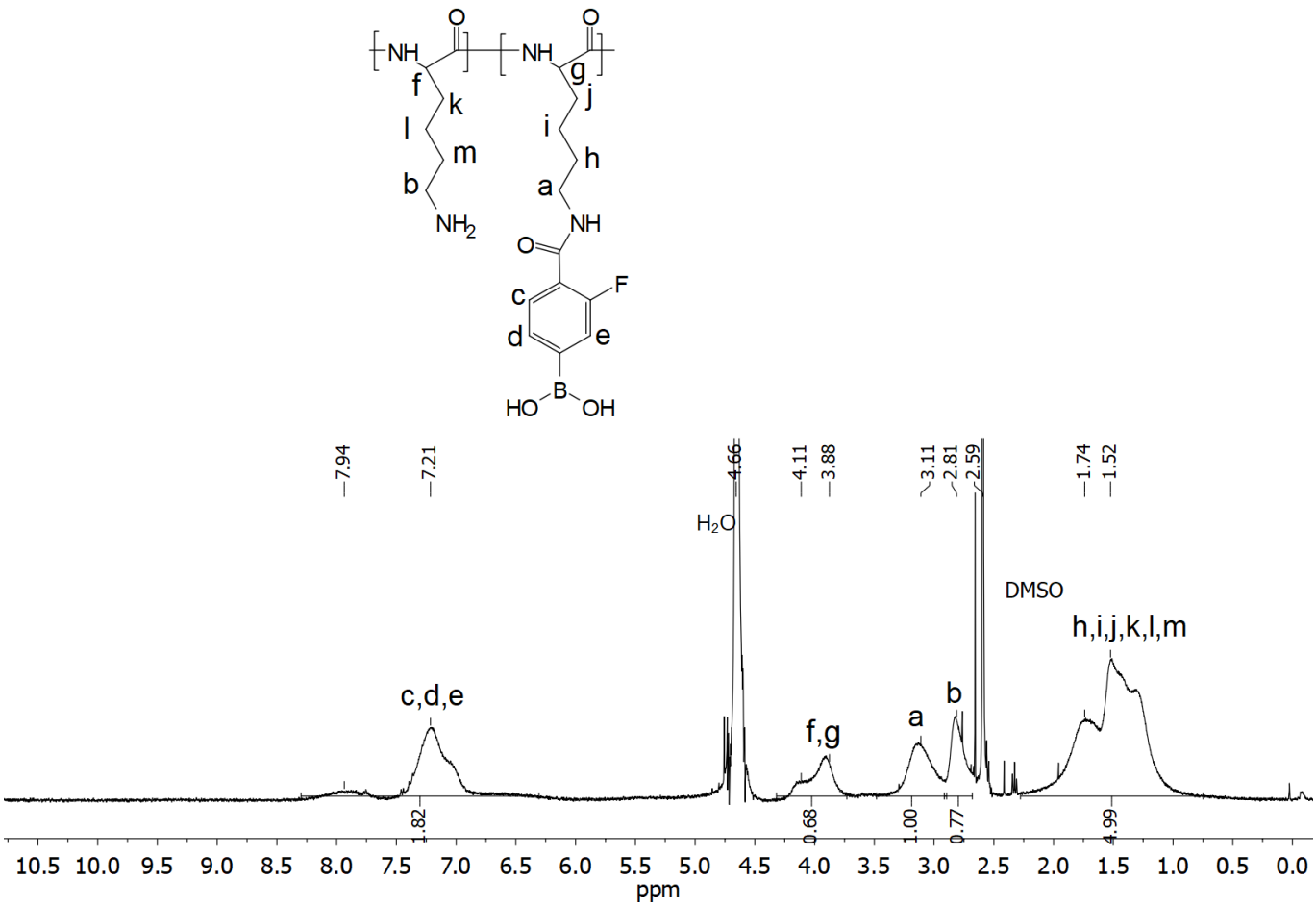

Figure S4. ^{1}H-NMR spectrum of FPBA modified $PLL_{30\text{-}70k}$ in D_2O with TFA to adjust its pH. About 60% of the amino groups in this polymer was reacted with FPBA-NHS.

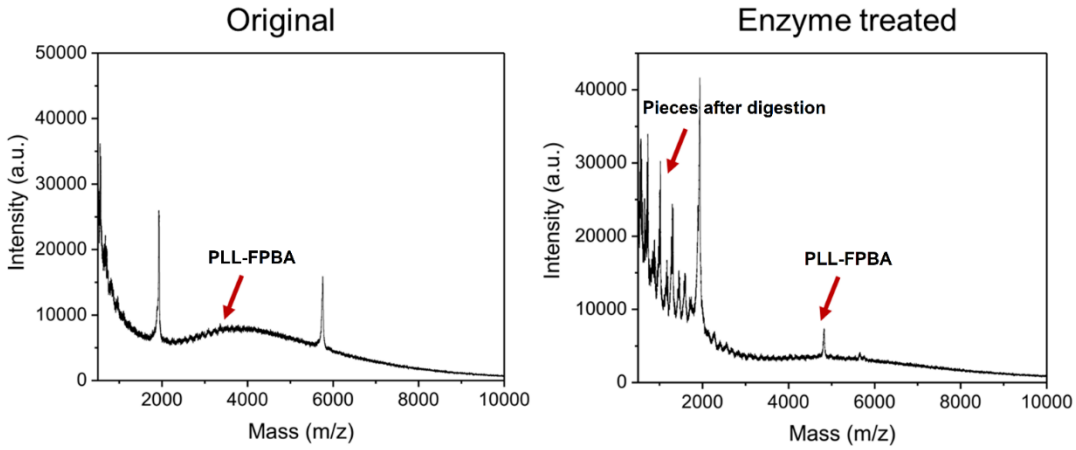

Figure S5. MALDI-TOF mass spectra of $PLL_{0.4}$-$FPBA_{0.6}$ before and after enzyme digestion. The concentration of the polymer was 20 mg/mL, while the 0.1 mg/mL of trypsin was used. The polymer was incubated with trypsin at 37 ℃ overnight on a shaker (300 rpm).

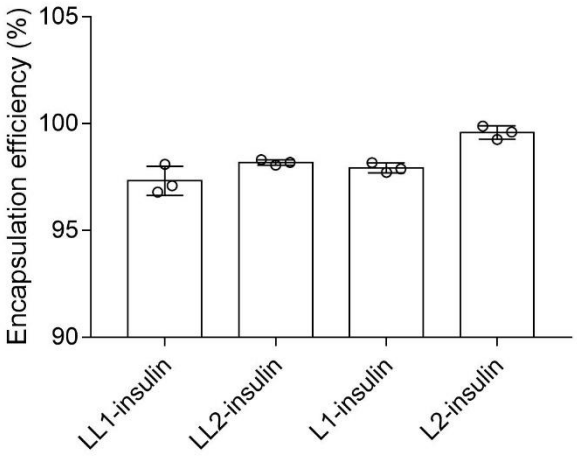

Figure S6. The encapsulation efficiency of insulin for various complexes. Data are mean ± SD (*n*=3).

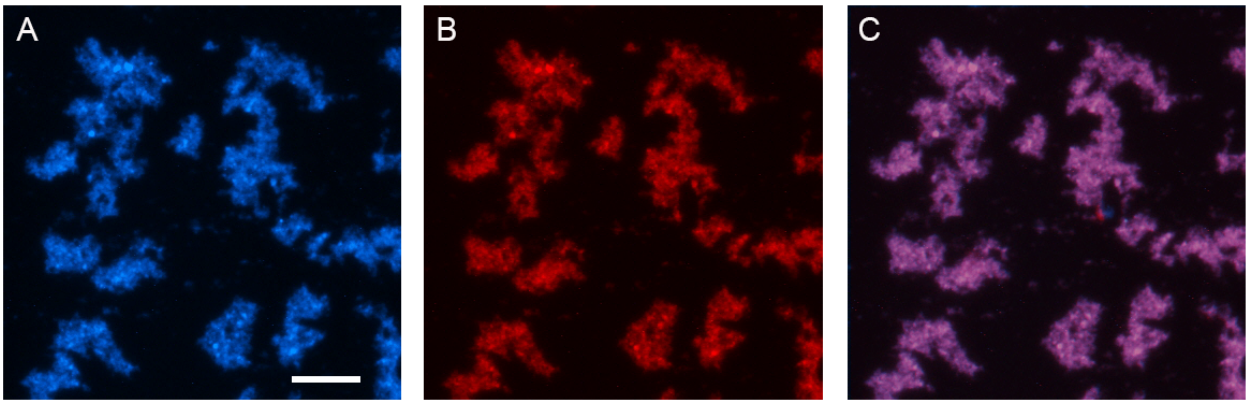

Figure S7. Fluorescence images of insulin complex. (A) Representative image of the complex observed from the channel of Cy5-labeled polymer. (B) Representative image of complex observed from the channel of Rhodamine B-labeled insulin. (C) Merge of images (A) and (B). Scale bar, 20 μm.

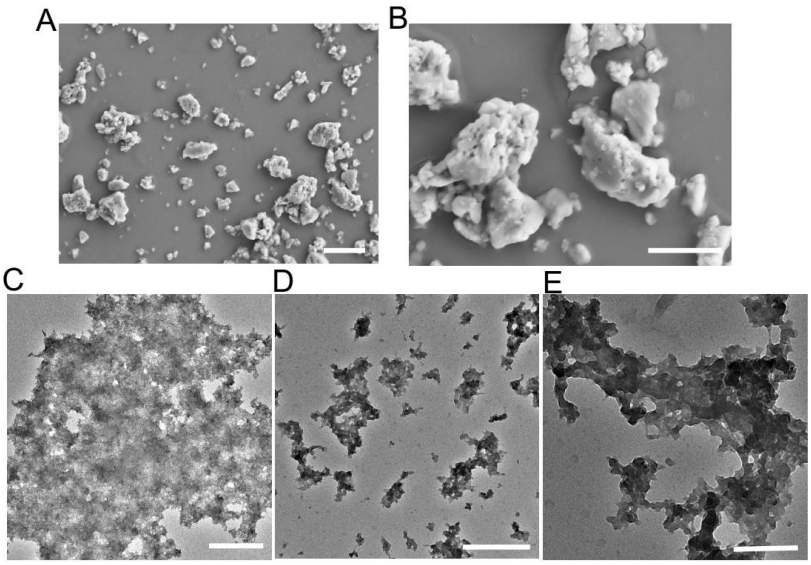

Figure S8. Representative SEM and TEM images of L2-insulin. (A-B) Representative SEM images of L2-insulin. Scale bars are 10 μm (A) and 5 μm (B), respectively. (C-E) Representative TEM images of L2-insulin. The complex was stained by phosphotungstic acid (2%). Complex particles with varied sizes were shown here. Scale bars are 1 μm (C), 0.5 μm (D), and 0.2 μm (E), respectively.

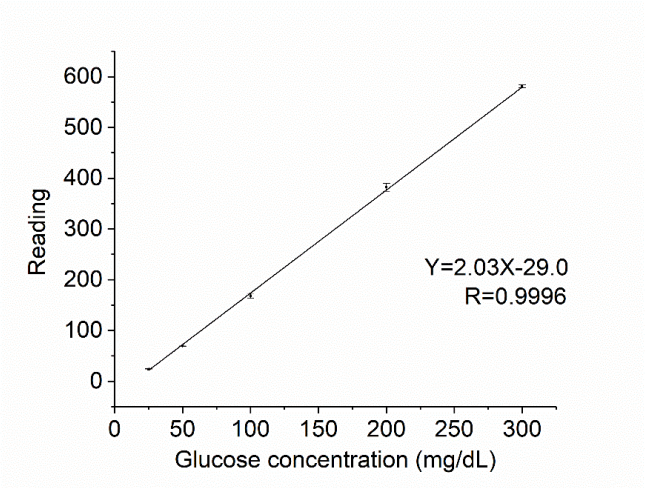

Figure S9. Glucose meter reading as a function of glucose concentration. Data are mean ± SD (*n*=3).

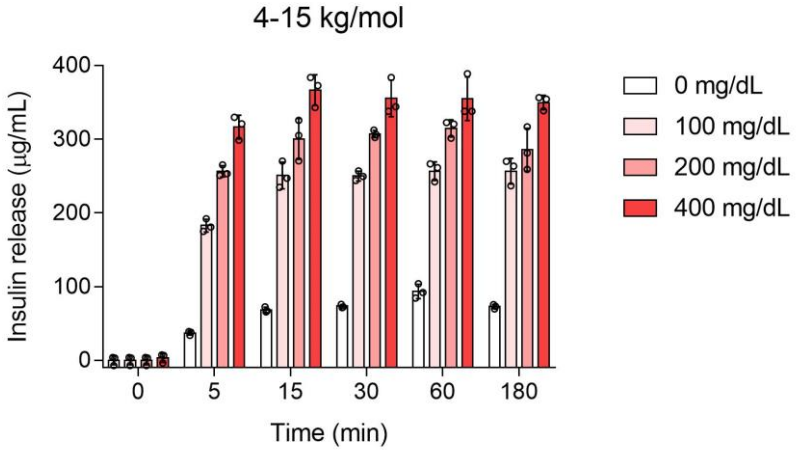

Figure S10. Glucose-responsive insulin release from complex prepared from an equal weight of insulin and $PLL_{0.57}$-$FPBA_{0.43}$ with the original PLL MW of 4-15 kg/mol. Data are mean ± SD (*n*=3).

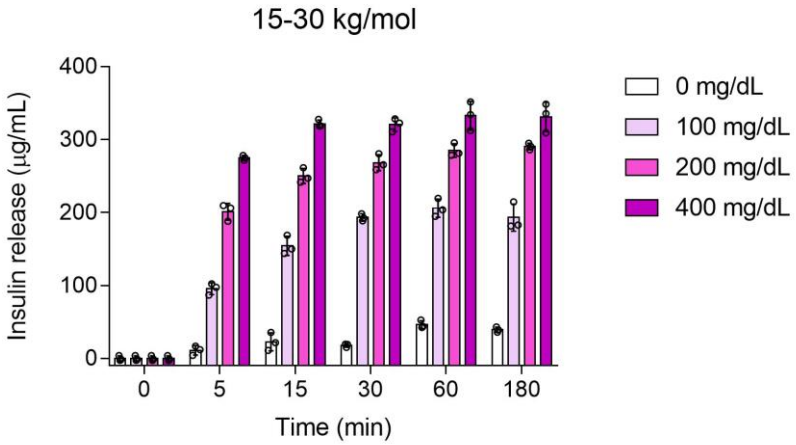

Figure S11. Glucose-responsive insulin release from complex prepared from an equal weight of insulin and $PLL_{0.6}$-$FPBA_{0.4}$ with the original PLL MW of 15-30 kg/mol. Data are mean ± SD (*n*=3).

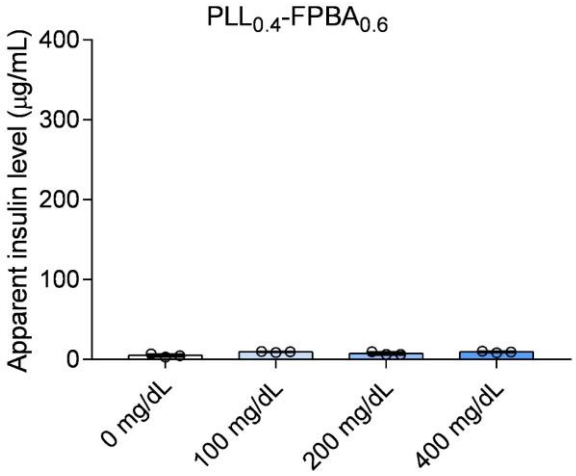

Figure S12. The glucose-dependent solubility of $PLL_{0.4}$-$FPBA_{0.6}$ in PBS at pH 7.4. The original PLL has a MW of 30-70 kg/mol. The supernatant was centrifuged, collected, and measured using a Coomassie protein assay reagent. The apparent insulin level was calculated according to the standard curve of insulin. Data are mean ± SD (n=3).

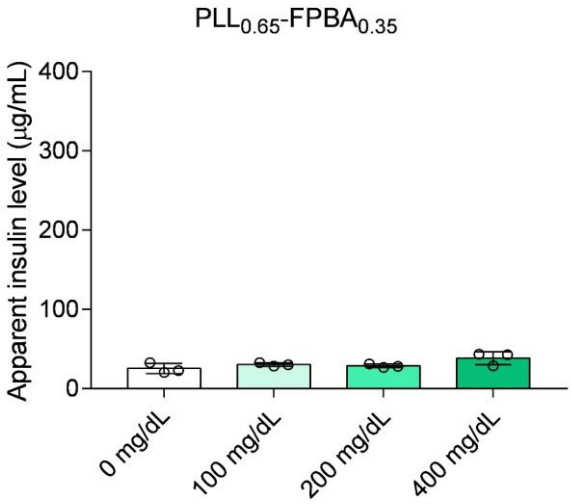

Figure S13. The glucose-dependent solubility of $PLL_{0.65}$-$FPBA_{0.35}$ in PBS at pH 7.4. The original PLL has a MW of 30-70 kg/mol. The supernatant was centrifuged, collected, and measured using a Coomassie protein assay reagent. The apparent insulin level was calculated according to the standard curve of insulin. Data are mean ± SD (*n*=3).

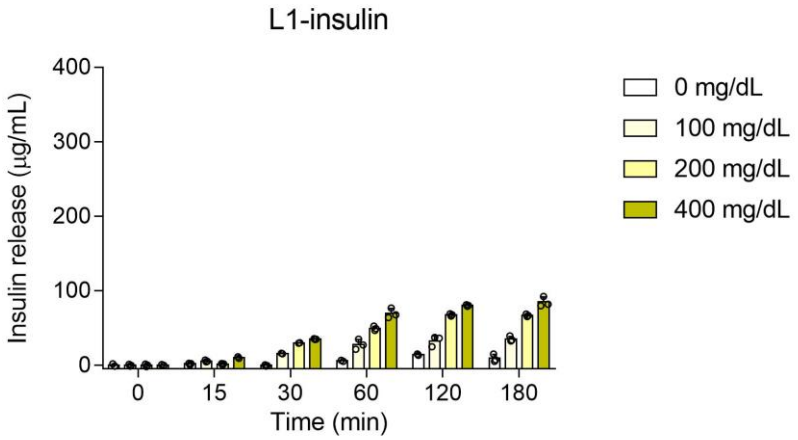

Figure S14. Glucose-dependent insulin release from bulk L1-insulin. L1-insulin was centrifuged (21, 000 G, 10 min) to the bottom of Eppendorf tubes. The complex stayed as a bulk at the bottom during the whole experiment. Data are mean ± SD (*n*=3).

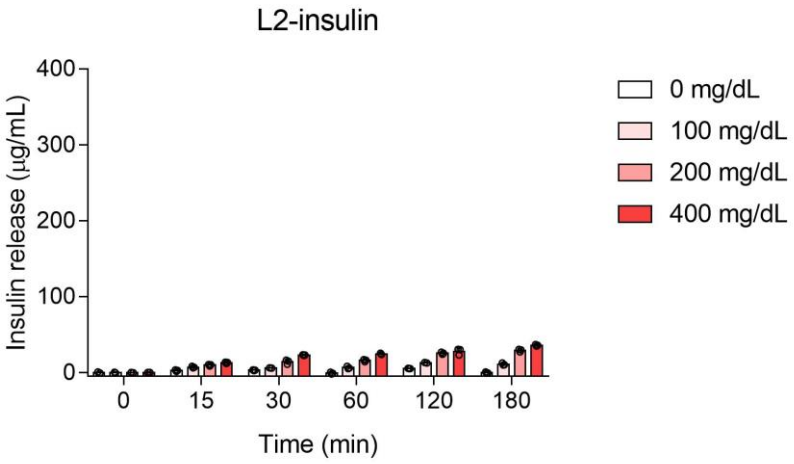

Figure S15. Glucose-dependent insulin release from bulk L2-insulin. L1-insulin was centrifuged (21, 000 G, 10 min) to the bottom of Eppendorf tubes. The complex stayed as a bulk at the bottom during the whole experiment. Data are mean ± SD (*n*=3).

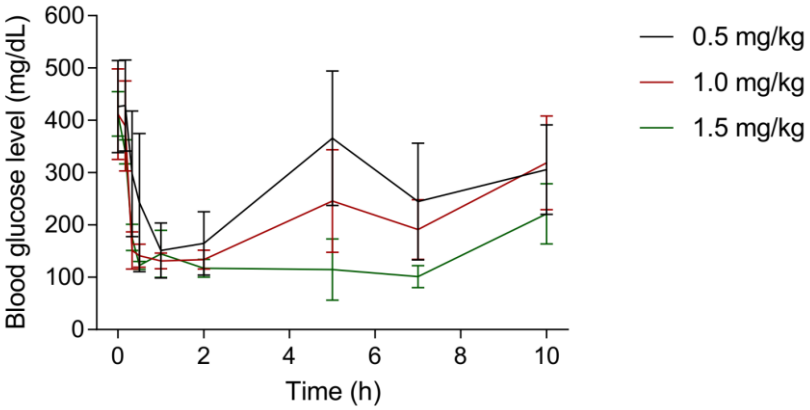

Figure S16. Dose-dependent blood glucose regulation ability of L1-insulin. Data are mean ± SD (*n*=5).

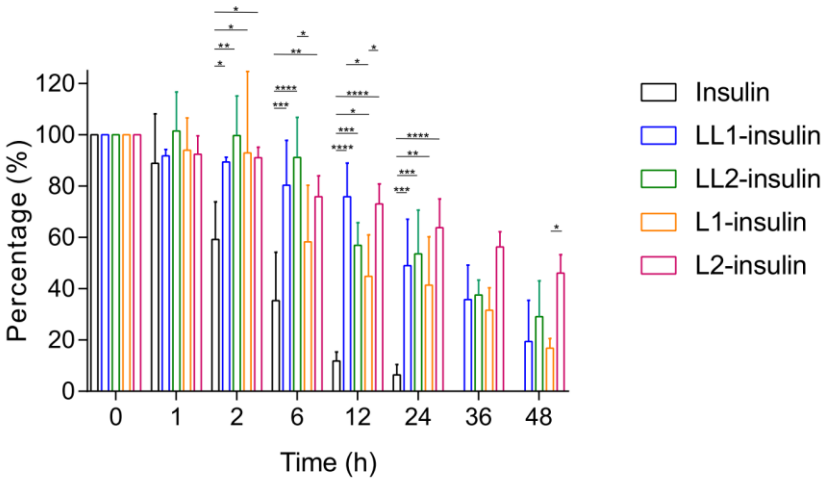

Figure S17. The statistical analysis of fluorescence intensity. Data are mean ± SD (n=3). Two-way ANOVA was used to calculate the difference among difference groups. Only P values with significant difference were shown. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

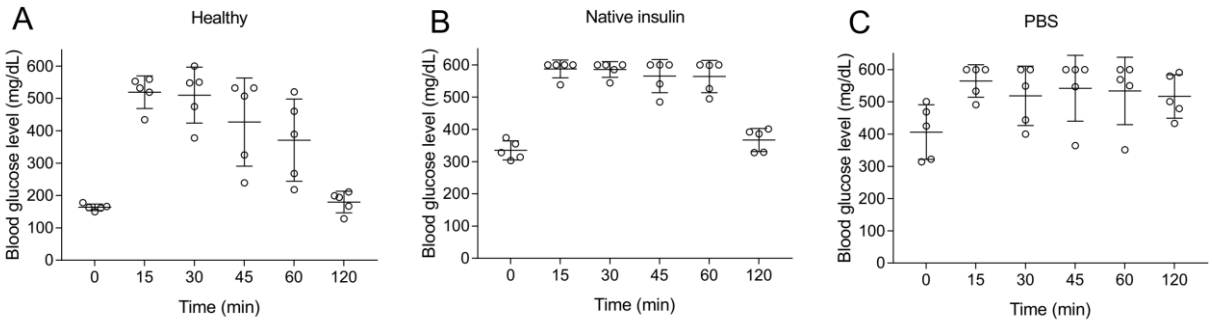

Figure S18. Intraperitoneal glucose tolerance tests. Healthy mice (A) and diabetic mice receiving native insulin (B) or PBS (C) were used as control groups. The insulin-equivalent dose was set to 1.5 mg/kg. The glucose (3 g/kg) was given at 8 hours posttreatment with complexes. Data are mean ± SD (*n*=5).

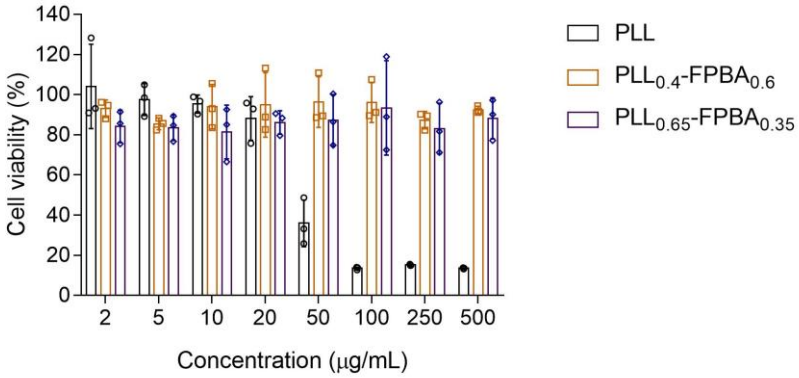

Figure S19. The dose-dependent cytotoxicity of $PLL_{30\text{-}70k}$, $PLL_{0.4}$-$FPBA_{0.6}$, and $PLL_{0.65}$-$FPBA_{0.35}$. The cytotoxicity was evaluated on L929 murine fibroblast cells. Data are mean ± SD (*n*=3).

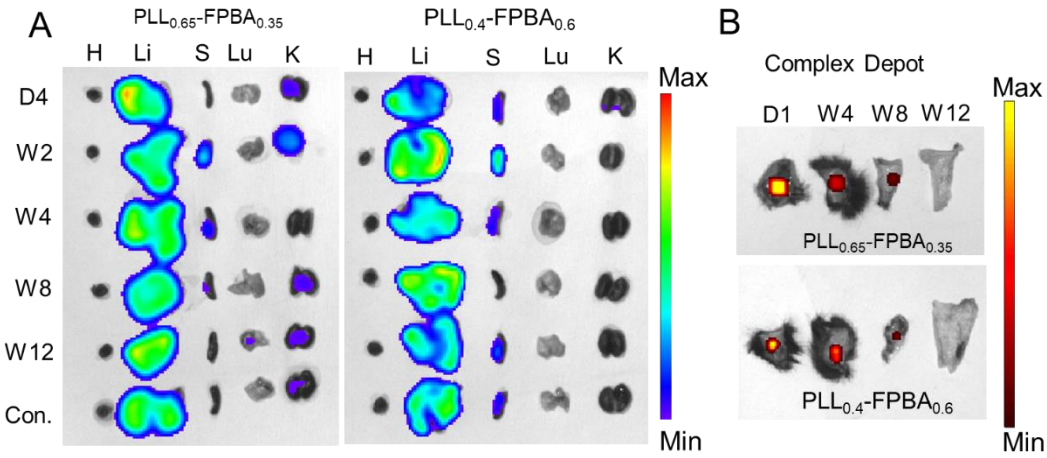

Figure S20. Biodistribution of the polymer after subcutaneous injection. Polymers were labeled with Cy5 and formed complexes LL2-insulin and L2-insulin before injection (1.5 mg/kg insulin-eq. dose). The organs (A) and the skins (B) were obtained between time intervals. IVIS spectrum was used to measure the fluorescence of each organ. H, heart; Li, liver; S, spleen; Lu, lung; K, kidney. Dn, n^{th} day posttreatment; Wn, n^{th} week posttreatment; Con., control group without treatment.

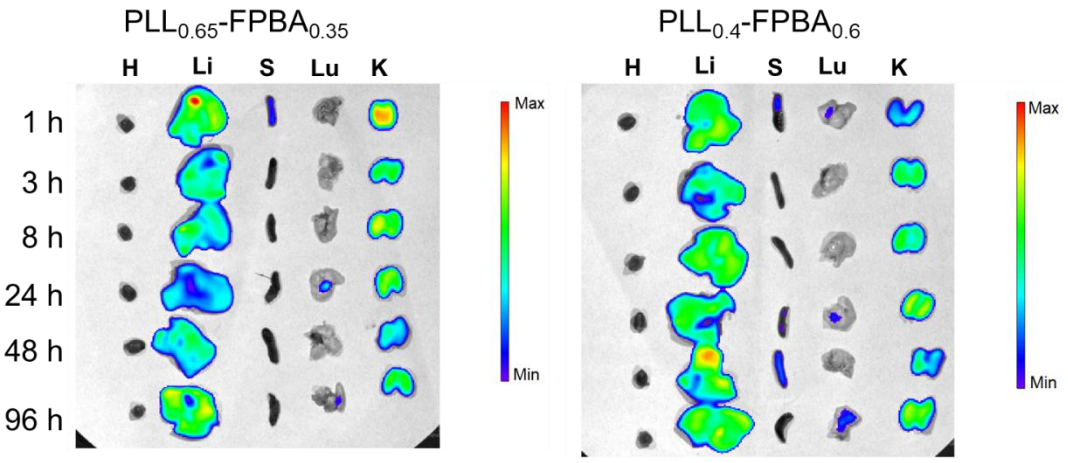

Figure S21. Biodistribution of the polymer after subcutaneous injection. Polymers were labeled with Cy5 and formed LL2-insulin and L2-insulin complexes before injection (1.5 mg/kg insulin eq. dose). The main organs were obtained between time intervals. IVIS spectrum was used to measure the fluorescence of each organ. H, heart; Li, liver; S, spleen; Lu, lung; K, kidney.

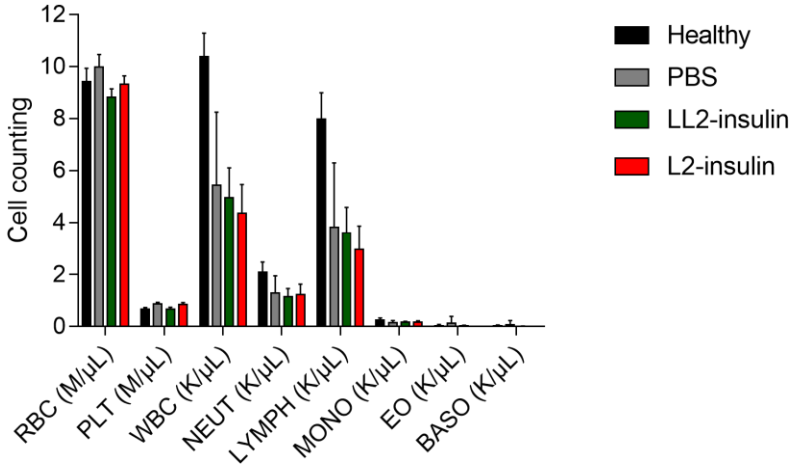

Figure S22. The effect of complex treatment on blood cell count. Diabetic mice were treated with LL2-insulin and L2-insulin every two days at a dose of 1.5 mg/kg. Diabetic mice receiving PBS and healthy mice were used as control groups. Data are mean ± SD (*n*=5). RBC, red blood cell; PLT, platelet; WBC, white blood cell; NEUT, neutrophil; LYMPH, lymphocyte; MONO, monocyte; EO, eosinophil; BASO, basophil.

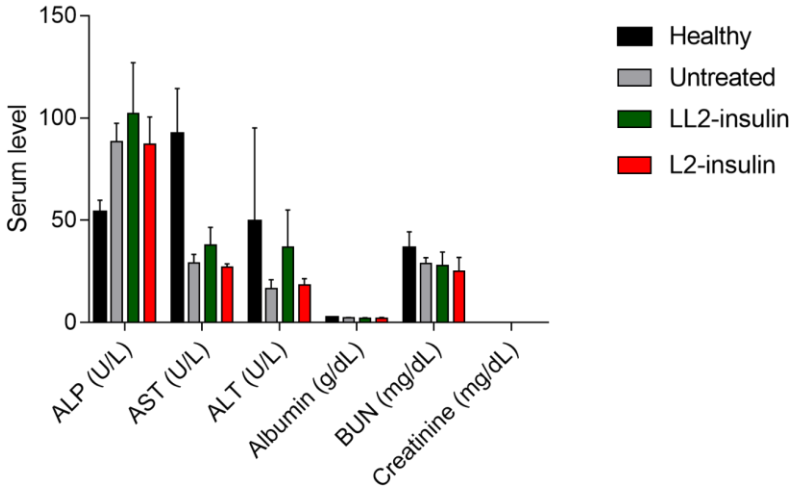

Figure S23. The effect of complex treatment on serum biochemical parameters indicating main organ healthiness. Diabetic mice were treated with LL2-insulin and L2-insulin every two days at a dose of 1.5 mg/kg for one week. Diabetic mice receiving PBS and healthy mice were used as control groups. Data are mean ± SD (*n*=5). ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine transaminase; BUN, blood urea nitrogen.

Table S1. General features of complexes.

Polymer	Abbreviation	Polymer to-insulin ratio (wt/wt)	N/C ratio (N, amine; C, carboxylic acid)	Z-averaged size (nm)	Zeta-potential (mV) in glucose solution (mg/dL)		
					0	100	400
$PLL_{0.65}$-	LL1-insulin	1	3.5	4569 ± 632	16.2 ± 4.2	10.0 ± 4.1	6.9 ± 1.0
$FPBA_{0.35}$	LL2-insulin	2	6.3	2527 ± 1217	20.9 ± 9.1	11.0 ± 2.1	7.2 ± 6.5
$PLL_{0.4}$-	L1-insulin	1	2.1	3295 ± 1476	-2.5 ± 5.0	-7.0 ± 1.1	-11.9 ± 2.2
$FPBA_{0.6}$	L2-insulin	2	3.6	2451 ± 1470	-1.6 ± 4.7	-10.0 ± 2.6	-16.1 ± 1.2

Note: Each insulin molecule has six carboxylic acid groups, three amino groups, and one guanidino group, which were all included in the calculation of N/C ratio. Phenylboronic acid groups were not included in the calculation even though they may carry negative charges. Data are presented as mean ± SD (n=3).