Supporting Information

Target-Triggered and Controlled Release Plasmon-Enhanced Fluorescent AIE Probe for Conformational Monitoring of Insulin Fibrillation

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Experimental Section

Chemicals and Materials.

All reagents were purchased and used without further purification. Anthracene, paraformaldehyde, 4,4'-Bis(dimethylamino)benzophenone and potassium tert-butoxide (t-BuOK) were purchased from Alfa Aesar (Shanghai, China). Dry tetrahydrofuran (THF) and dry dichloromethane (DCM) were purchased from Sigma-Aldrich (Shanghai, China). 1,4-Dioxane, chloroform-d, dimethyl sulfoxide-d6, triethyl phosphite, ethyl acetate, methyl iodide, chloroform ($CHCl_3$), hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), polyvinylpyrrolidone (PVP), silver Nitrate ($AgNO_3$), ethylene glycol, sodium chloride (NaCl), sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) and Tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) were obtained from Innochem (Beijing) Technology Co., Ltd. Hydrochloric acid, concentrated sulfuric acid, petroleum ether (PE), and methanol were obtained from Beijing Chemical Co. (Beijing, China). Insulin was purchased from MedChemExpress LLC. 10 mM pH 7.2 phosphate buffered saline (PBS) was obtained from Solarbio (Beijing, China). All of the synthetic Oligonucleotide sequences were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). All the information relating to DNA sequence was listed in **Table S1**. Deionized water obtained from a Milli-Q water purification system (18.2 $M\Omega\ cm^{-1}$, Milli-Q, Millipore) was utilized in all the experiments.

Instrumentation.

^1H NMR spectra were measured on a JEOL 400 MHz spectrometer. Mass spectral analysis were measured on a Thermo Fisher Scientific LTQ-XL. High-resolution mass spectra (HRMS) were measured on a Triple TOF 5600 instrument (AB Sciex, USA). Fluorescence spectra were collected on a RF-6000 fluorescence spectrophotometer (Shimadzu, Japan). UV-vis spectra were recorded on a UV-2450 spectrophotometer (Shimadzu, Japan). Dynamic light scattering (DLS) were measured on a Nano-ZS Zetasizer ZEN3600 (Malvern Instruments Ltd, UK). Transmission electron microscopy (TEM) images and high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) EDS mapping images were taken by a FEI Talos F200S electron microscope (FEI, America). The morphology of insulin were measured on a SEM (FESEM, S-8010, Hitachi). CD spectra were performed on a J-810 spectropolarimeter (JASCO)

from 180 to 260 nm. Fluorescence polarization measurement were measured by a FP-8300 (JASCO, Tokyo) instrument.

Synthesis of (1) 9,10-bis(dichloromethyl)anthracene.

The vessels used in synthesis experiments were oven-dried and cooled to room temperature under a nitrogen atmosphere. Anthracene (27 g) and paraformaldehyde (22.8 g) were dissolved in 1,4-dioxane (216 ml) and added to a two-necked flask (500 mL). Then the mixture was stirred for 2 h at 110 °C under HCl atmosphere. After removing the HCl atmosphere, the reaction was continued to stir for 4 h at 110 °C. Then, the mixture was cooled to room temperature, washed with 1,4-dioxane and neutralized with water to obtain a yellow powder (43% yield). ^{1}H NMR(400MHz, $CDCl_3$) δ (TMS, ppm): 8.40-8.38 (m, 4H), 7.67-7.65 (m, 4H), 5.61 (s, 4H). m/z 274.32.

Synthesis of (2) tetraethyl anthracene-9,10-diylbis(methylene)diphosphonate.

Compound 1 (17 g) and $P(OC_2H_5)_3$ (86 ml) were added to a dried one-necked flask (250 mL). The reaction mixture was slowly stirred at 140 °C for 18 h under a nitrogen atmosphere. After cooling to room temperature, the reaction mixture was filtered and washed with petroleum ether to obtain a light yellow solid (82% yield). ^{1}H NMR (400MHz, $CDCl_3$) δ (TMS, ppm): 8.38-8.37 (m, 4H), 7.57-7.56 (m, 4H), 4.25 (s, 2H), 4.21 (s, 2H), 3.91-3.78 (m, 8H), 1.06 (t, 12H). m/z 479.27 $[M+H]^+$.

Fluorescence polarization measurement.

5′-FAM-labeled aptamers (150 nM) were heat-treated at 95 °C in 50 mM Tris-HCl buffer (100 mM NaCl, 20 mM KCl, pH 7.2) for 3 min and then gradually cooled to 25 °C over a period of 2 h. 5′-FAM-labeled aptamers incubated with insulin (10 μM) for 1 h at room temperature. The fluorescence polarization of the FAM-labeled aptamers were measured by FP-8300 (JASCO, Tokyo) at 493 nm excitation and 518 nm emission at room temperature.

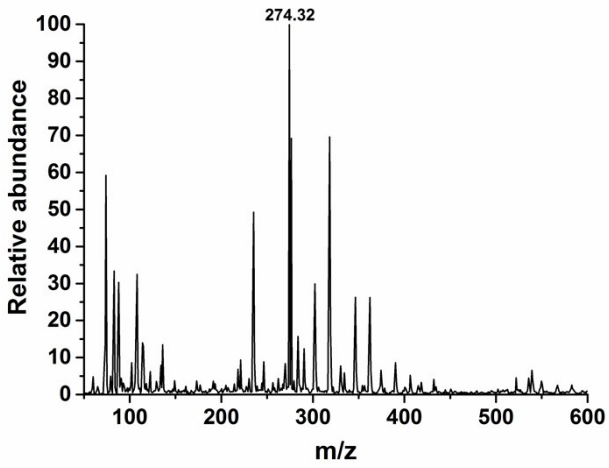

Figure S1. Mass spectra of compound 1.

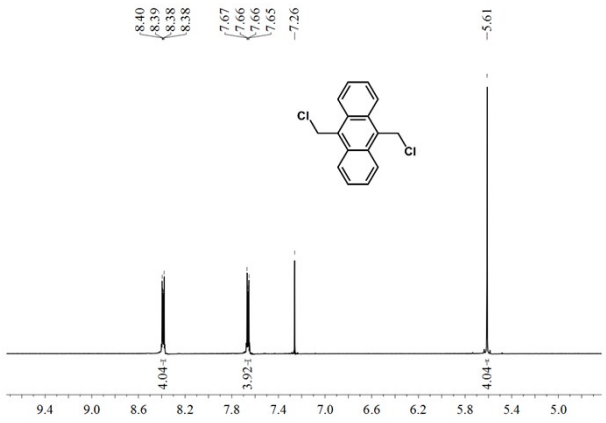

Figure S2. ^{1}H NMR of compound 1.

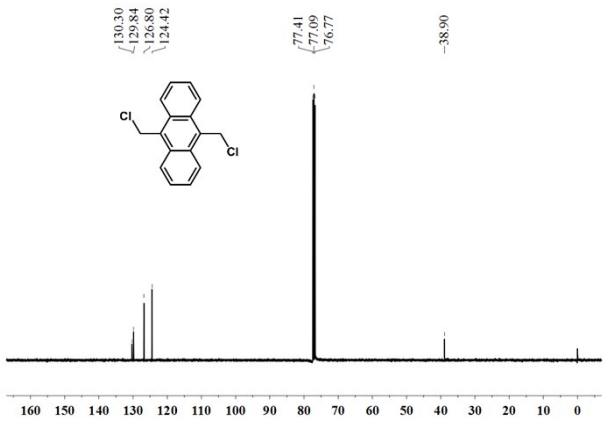

Figure S3. ^{13}C NMR of compound 1.

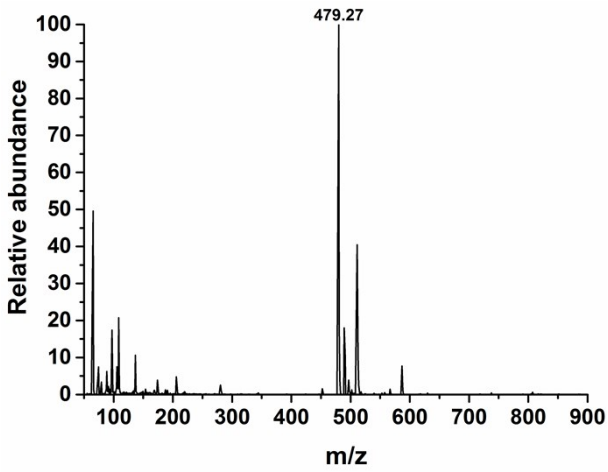

Figure S4. Mass spectra of compound 2.

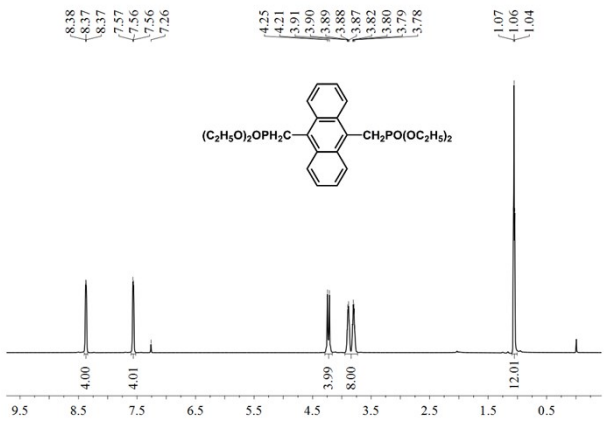

Figure S5. ^{1}H NMR of compound 2.

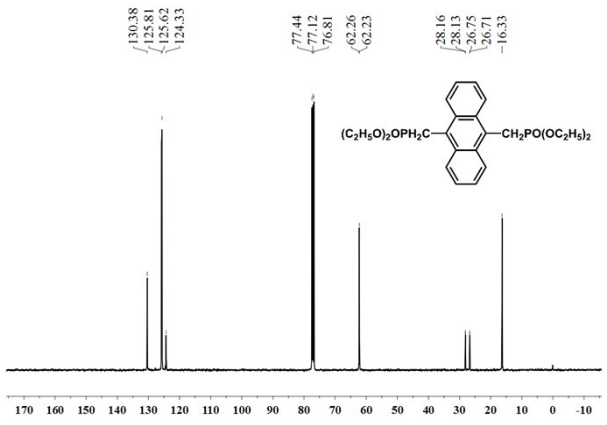

Figure S6. ^{13}C NMR of compound 2.

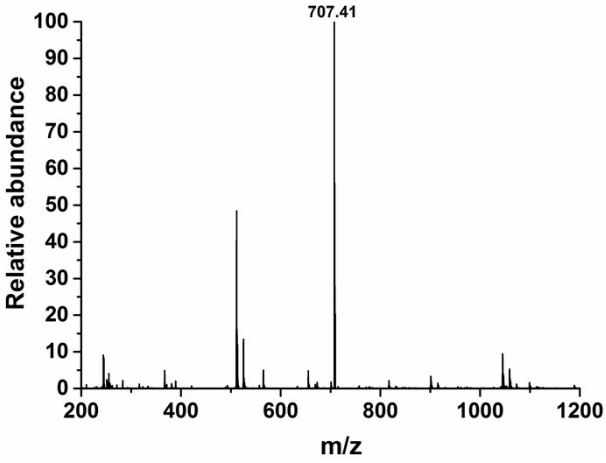

Figure S7. Mass spectra of compound 3.

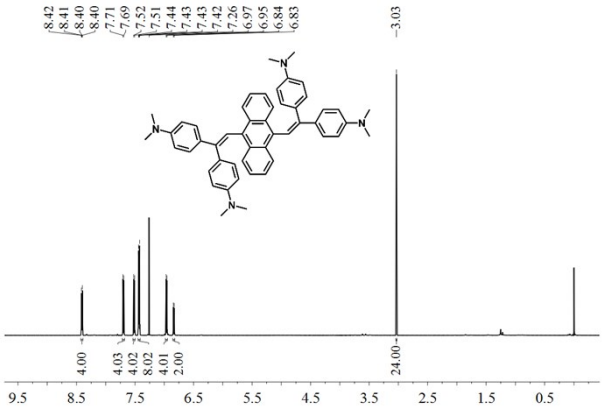

Figure S8. ^{1}H NMR of compound 3.

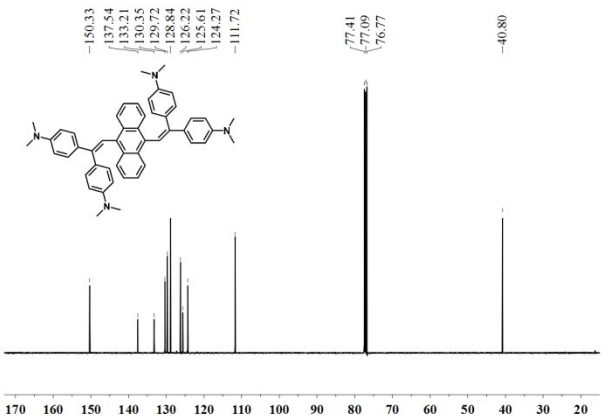

Figure S9. ^{13}C NMR of compound 3.

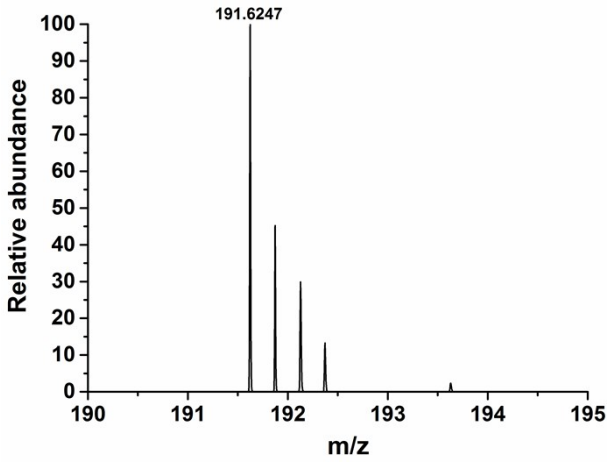

Figure S10. Mass spectra of compound BDVAI.

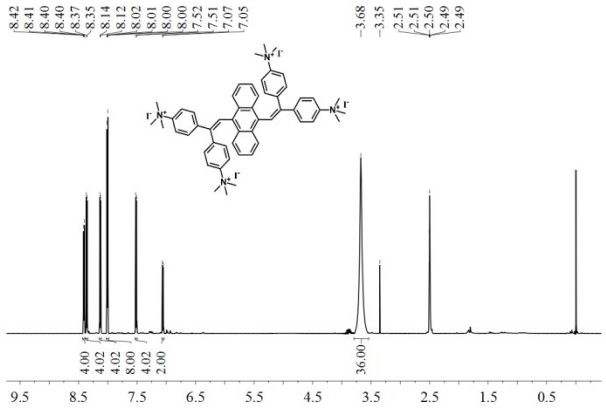

Figure S11. ^{1}H NMR of compound BDVAI.

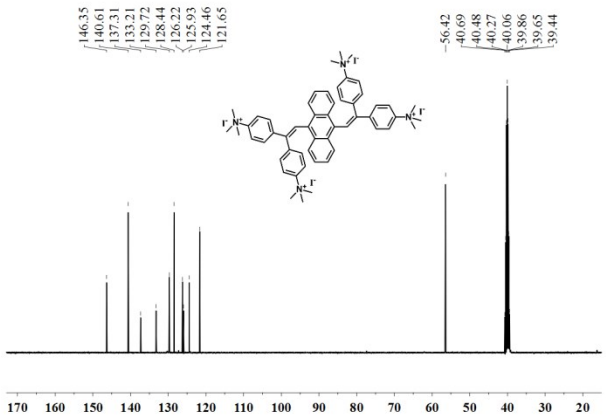

Figure S12. ^{13}C NMR of compound BDVAI.

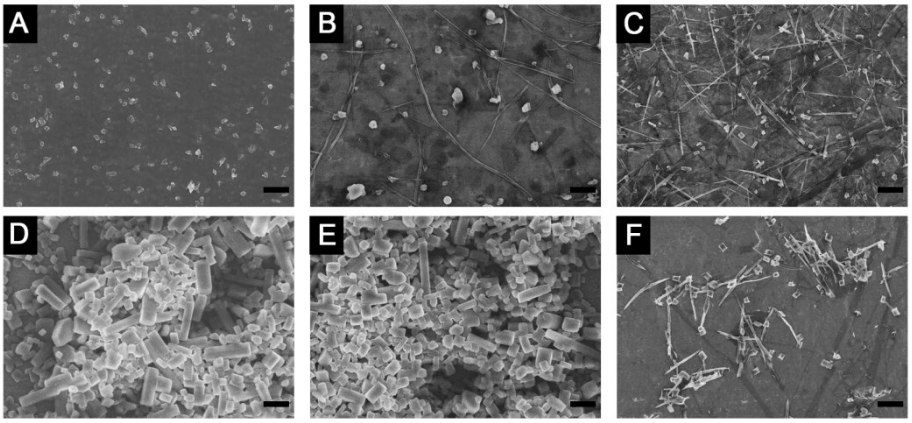

Figure S13. SEM micrographs of BDVAI generated by evaporating suspensions of water/THF mixtures with f_{THF}=0%, 20%, 40%, 60%, 80%, 90%.

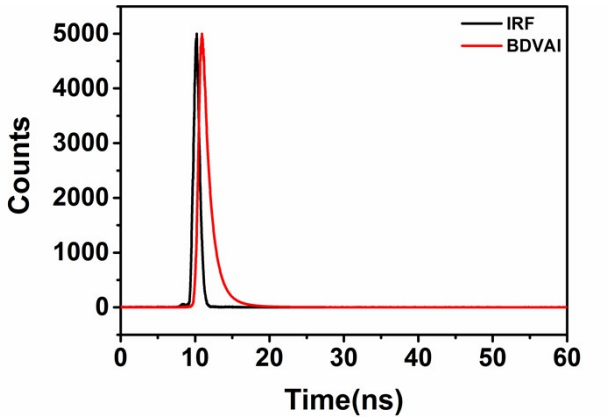

Figure S14. Decay curve of BDVAI. The black line is the instrument response function (IRF).

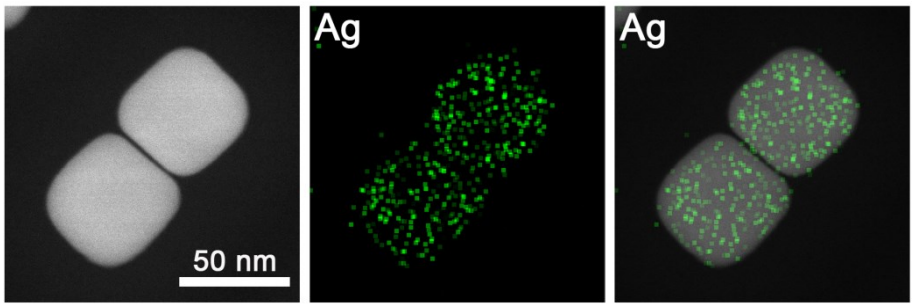

Figure S15. HAADF-STEM image and the corresponding elemental mapping images of AgNCs.

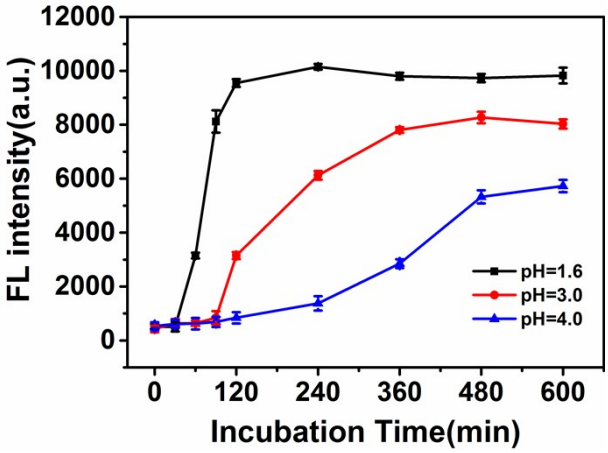

Figure S16. Fluorescence spectra of insulin incubated at acidic pH ranges 1.6-4.0 for different periods of time at 65 °C.

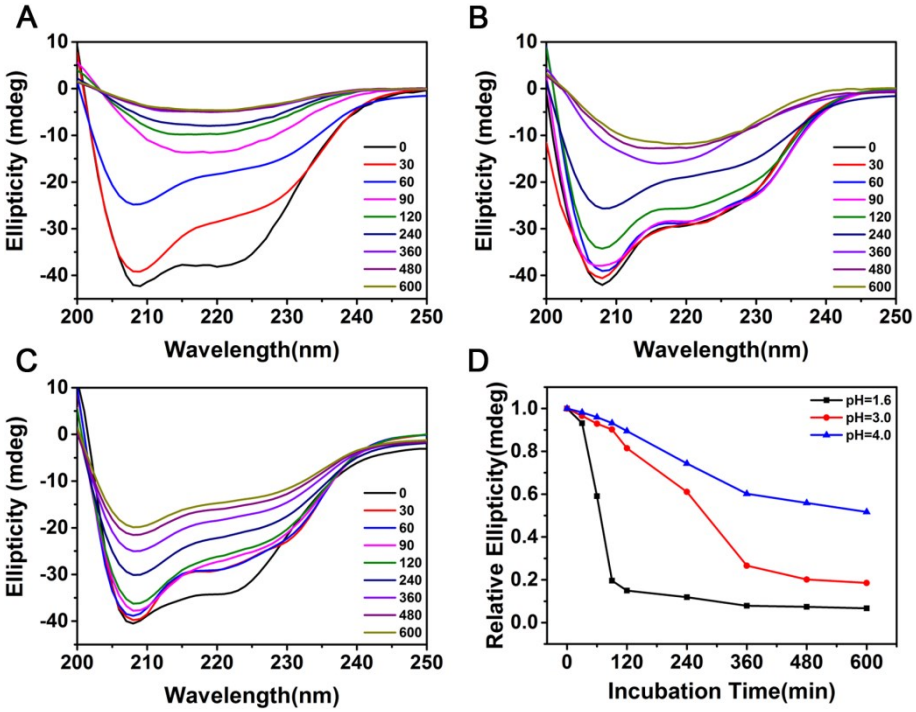

Figure S17. CD spectra of insulin incubated in (A) pH 1.6, (B) pH 3.0, and (C) pH 4.0 buffer for different periods of time at 65 °C. (D) Time-dependent change in the ellipticity recorded at 208 nm.

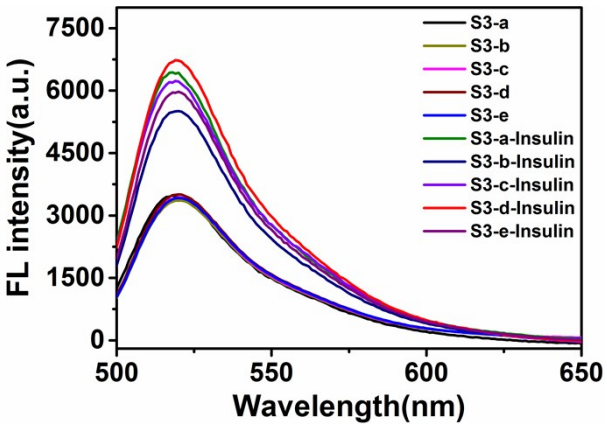

Figure S18. Binding assay of S3-a, S3-b, S3-c, S3-d, and S3-e to insulin by fluorescence measurement.

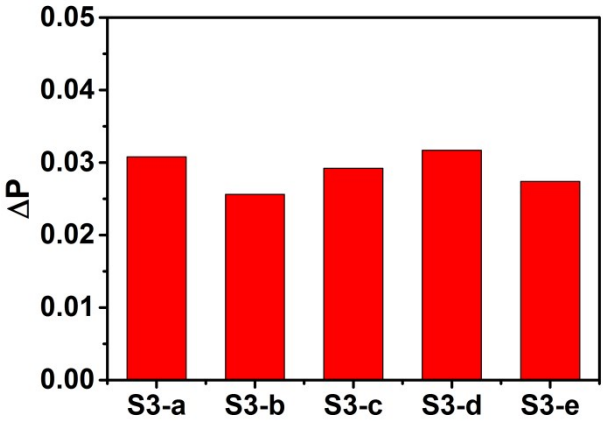

Figure S19. Binding assay of S3-a, S3-b, S3-c, S3-d, and S3-e to insulin by fluorescence polarization measurement.

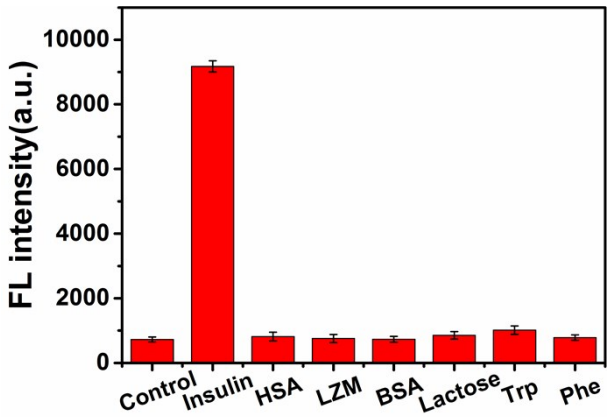

Figure S20. Specificity of the BDVAI@AuNCs-Apt sensor.

Table S1. Oligonucleotide sequences designed in the current study

Name	Detailed sequence information (from 5′ to 3′)
S1	SH-TTT TTT TTT TTT CGG ATC GAA GAC ACC C
S2	CCC CCC ACC ACC TAT ATA-SH
S3	GGT GGT GGG GGG GGT TGG TAG GGT GTC TTC GAT CCG AAA AAA AAA AAA-SH
S1-a(10nt)	SH-GAA GAC ACC C
S3-a	GGT GGT GGG GGG GGT TGG TAG GGT GTC TTC-SH
S1-b(16nt)	SH-TTT TTT GAA GAC ACC C
S3-b	GGT GGT GGG GGG GGT TGG TAG GGT GTC TTC AAA AAA-SH
S1-c(22nt)	SH-TTT TTT TTT TTT GAA GAC ACC C
S3-c	GGT GGT GGG GGG GGT TGG TAG GGT GTC TTC AAA AAA AAA AAA-SH
S1-d(28nt)	SH-TTT TTT TTT TTT CGG ATC GAA GAC ACC C
S3-d	GGT GGT GGG GGG GGT TGG TAG GGT GTC TTC GAT CCG AAA AAA AAA AAA-SH
S1-e(34nt)	SH-TTT TTT TTT TTT CGA TAC CGG ATC GAA GAC ACC C
S3-e	GGT GGT GGG GGG GGT TGG TAG GGT GTC TTC GAT CCG GTA TCG AAA AAA AAA AAA-SH

Table S2. Limit of detection of published insulin sensors

strategy	detection limit	reference
chemiluminescence biosensor	1.6 pM	1
fluorescence sensor based on aptamer composite	2.74 nM	2
fluorescence-based aptamer biosensor	9.97 nM	3
colorimetric biosensing based on graphene/aptamer	10 nM	4
optical nanosensor based on carbon nanotubes	10 nM	5
graphene-based aptameric nanosensor	35 pM	6
electrochemical aptamer-based sensor	20 nM	7
label-free sensor based on SERS	35 pM	8
PEF-AIE aptasensor	23.6 pM	our work

References

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