Supporting Information

Experimental Section

Materials and measurements:

Nanopure water (18.2 MΩ; Millpore Co., USA) was used in all experiments and to prepare all buffers. Tetraethyl orthosilicate (TEOS), 3-aminopropyl trethoxysilane (APTES), NaCl were purchased form Sigma-Aldrich. Fluorescein isothiocyanate (FITC), succinic anhydride, trisodium citrate dihydrate, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and glucose were obtained from Alfa Aesar. N-hydroxysulfosucnimide sodium salt (sulfo-NHS) was purchased from Pierce Biotechnology. Human α-thrombin, bovine-serum albumin (BSA), human-serum albumin (HAS), immunoglobulin G (IgG) and lysozyme were purchased from Sigma-Aldrich. All the chemicals were used as received without further purification. The oligonucleotide used in this article was synthesized by Sangon Biotechnology Inc. (Shanghai, China). The sequence is as follows:

TBA₁₅: 5'-NH₂-TTTTTTGGTTGGTGTGGTTGG

TBA29: 5'-NH2-TTTTTAGTCCGTGGTAGGGCAGGTTGGGGTGACT

FT-IR analyses were carried out on a Bruker Vertex 70 FT-IR Spectrometer. SEM images were obtained with a Hitachi S-4800 FE-SEM. TEM images were recorded using a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV. UV-Vis spectroscopy was carried out with a JASCO V-550 UV/vis spectrometer. Fluorescence spectra were recorded with a JASCO FP-6500 spectrofluorometer.

Preparation of aptamer functionalized silica nanoparticles (silica NPs-TBA₁₅):

TEOS (1 mL) dissolved in ethanol (10 mL) was added dropwise into a mixture of

Milli-Q water (6.5 mL), ethanol (4.2 mL), and NH₃·H₂O (0.75 mL) with vigorous

stirring. After 30 min stirring at room temperature, NPs were centrifuged and washed with ethanol and Milli-Owater subsequently. In between the washing steps, the NPs were redispersed into the solution by ultrasonication. The bare NPs (6.2 mg) reacted with APTES (200 µL) in ethanol (300 µL) at room temperature and 50 °C for 2 h, subsequently. After the mixture was cooled to room temperature, the NPs were centrifuged and washed successively with ethanol and acetonitrile. The silica NPs-NH2 mg) was reacted with succinic anhydride (0.10)(5 g) in N,N-dimetylformamide solution (2 ml) under N₂ gas for 8 h with continuous stirring. By doing so, carboxyl groups were formed onto the silica NPs surface for conjugation of DNA. After a thorough water wash, the carboxylated nanoparticles (silica NPs-COOH) were activated using EDC (10 mg/ml, 15 ml) and sulfo-NHS (10 mg/ml, 15 ml) in a MES buffer (pH 6.0) for 15 min at room temperature with continuous stirring. Twenty microliters of PBS buffer (100 mM, pH 7.4) was then added in the mixture, followed by the addition of TBA₁₅ (200 µl 93.2 mM) at room temperature with continuous stirring for 6 h and washing in PBS buffer (100 mM, pH 7.4) to form the resultant DNA-conjugated nanoparticles (silica NPs- TBA₁₅).

Preparation of aptamer functionalized fluorescent carbon dots (C-Dots-TBA₂₉):

Glucose (1g) and NaCl (0.1 g) was dissovled in 10 mL water. The solution was put into a domestic microwave oven (750 W) and heated for 10 minutes. The color-changed solution was added 2 ml 2% HNO₃, and heated for 10 minutes at 50 °C with continuous stirring to add carboxyl groups onto the surface of C-Dots. C-Dots-COOH solution was purfied and diluted with water, and activated using EDC (10 mg/ml, 5 ml) and sulfo-NHS (10 mg/ml, 5 ml) in a MES buffer (pH 6.0) for 15 min at room temperature with continuous stirring. 10 ml of PBS buffer (100 mM, pH 7.4) was then added in the mixture, followed by the addition of TBA₂₉ (50 μ l 100.8 mM) at room temperature with continuous stirring for 6 h and washing in PBS buffer (100 mM, pH 7.4) to form the resultant DNA-conjugated nanoparticles (C-Dots-TBA₂₉).

Thrombin detection using carbon nanodots-based sandwich assay:

Human α -thrombin was added to the silica NPs- TBA₁₅ (10 mg/ml, 200 µl) in thrombin reaction buffer (150 µl) to yield final thrombin concentrations of 1-500 nM. The resulting mixtures were incubated for 30 min at 37 °C. Subsequently, C-Dots-TBA₂₉ (10 mg/ml, 10 µl) was added into thrombin-binding NP suspension and incubated for another 10 min incubation. The NP suspensions were then centrifuged and thoroughly washed with thrombin reaction buffer (1 ml, three times). The collected NPs were redispersed in 15 mM PBS buffer (100 mM Na⁺, pH=7.4) for fluorescence measurement. The emission spectra were recorded 360 nm,. Parallel experiments were conducted using BSA (1 mM), HAS (1 mM) IgG (1 mM) and lysozyme (1 mM) as interferences in buffer solution under the same experimental conditions.



Fig. S1 SEM images of the synthesized silica NPs with \sim 150 nm in diameter. Inset: histogram showing the particle size distribution.



Fig. S2 FTIR (A) and UV-Vis (B) of silica NPs and functionalized silica NPs.



Fig. S3 TEM images of C-Dots with \sim 1.5 nm in diameter. Inset: histogram showing the particle size distribution.



Fig. S4 FTIR (A) and UV-Vis (B) of C-Dots and functionalized C-Dots.



Fig. S5 Fluorescence spectra of C-Dots (A) and C-Dots-TBA₂₉ (B) excited at 280~500 nm.