Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Highly Intense Fluorescence of Novel Carbon Nanocrystals Combined with a DNAzyme-Assisted Autocatalytic Multiple Amplification Strategy for Sensitive Detection of Thrombin

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1. Experimental section

1.1. Materials and apparatus

Chloroauric acid ($HAuCl_4$) and trisodium citrate, Tris (99%), $MgCl_2$ (98%), and $K_2S_2O_8$ were purchased from aladdin-reagent com. (Shanghai). Graphite rod was purchased from Beijing Jixing Shengan industry and trade co., LTD. G5 PAMAM was purchased from Sigma-Aldrich (Shanghai). The endonuclease (Nb.BbvCI, 10000 U/mL) and Exonuclease III (100000 U/mL) were purchased from the NEW ENGLAND Biolabs (NEB). Magnetic bead (MB) with the diameter of 4 μm and surface sulfydryl groups were obtained from Tianjin Baseline Chromatogram Technical Research Center. All the DNA sequences were synthesized and purified by SBS Genetech Co. Ltd. (China), and the DNA sequences used in this work are listed in Table 1. 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were obtained from Sigma. Human thrombin was purchased from Shanghai Bio Technology Co. Ltd. All other reagents were of analytical grade. Double distilled water was used for all experiments. 0.1 M TE buffer (pH 7.4, containing 10 mM, pH 8.0 tris-HCl, 1 mM EDTA, and 12.5 mM $MgCl_2$) was prepared according to the standard methods. Magnetic microsphere (3-4 μm) was purchased from were obtained from Tianjin Baseline Chromatogram Technical Research Center.

The recognition site of Nb.BbvCI was:

5′ …CCTCA｜GC…3′

3′ …GGAGT｜CG…5′

Table S1. The Sequences of the DNA in Scheme 1

DNA:	Sequence（5' to 3'）
HP1:	GGTTGGTGTGGTTGGAGACTAGACTATTCCAACCACT GGTGGGTTGGTGTGGTTGG (Thrombin Aptamer)
Target DNA (t1)**:**	AGACTAGACTATTCCAACCACTGGTG
HP2:	AGA CTA GAC CGC CTC AGC CTT TCG GTC TAG TCT GTC TAG TCT
Target DNA (s1):	AGA CTA GAC CGC CTC AGC CTT T
Capture DNA (MB):	H_2N-AA AGGCTGAGGTTTAGA-SH

ECL measurements were carried out on a MPI-A ECL analyzer (Xi'An Remax Electronic Science & Technology, Xi'An, China) using a three-electrode system. Cyclic voltammetry electrolysis was performed on a CHI 660C electrochemical workstation (Shanghai CH Instruments, China) using a three-electrode system, the electrodes were a graphite rod working electrode, a Ag/AgCl reference electrode, and a Pt counter electrode. Transmission electron microscopy (TEM) images were recorded with H-7650 (HITACHI, Japan) microscope. Photoluminescence (PL) spectra were obtained on an RF-540 spectro-photometer (Shimadzu). Field-emission scanning electron microscopy (FE-SEM) was carried out on a JEOL JSM-6700F instrument.

1.1.3. Preparation of the carbon nanocrystals (CNCs)

The preparation of CNCs was performed in an electrochemical cell consisting of a graphite rod (GR) working electrode, a Pt counter electrode, a Ag/AgCl reference electrode, and pH 7.0 phosphate buffer solution (PBS). The applied potential at the GR electrode was cycled between – 3.0 and 3.0 V at 0.1 V/s. After 1.5 h of electrolysis, the colourless electrolyte solution slowly turned to light yellow and finally puce. The resulting solution was filtrated by membrane filtration (0.2 μm) and ultrafiltration centrifugation to remove superfluous saline ions and solvent.

1.2. Preparation of the MB@Au conjugates

First, 100 μL of magnetic bead (MB) were transferred into a 1.5 mL Eppendorf tube and were washed three times with PBS. Second, the MB was resuspended in pH 7.4 PBS and 1 mL of Au colloid was added. After shaking gently for 16 h at room temperature, excess reagents were removed by magnetic force and the MB@Au conjugates were resuspended in 200 μL of pH 7.4 PBS.

*1.3. Preparation of the MB@Au-DNA-**CNCs** conjugates*

To form the MB@Au-DNA capture probe, 100 μL of MB@Au bead was washed three times with PBS and transferred into a 1.5 mL Eppendorf tube, then 200 μL of capture DNA (10^{-6} mol/L) was added and incubated at 37°C for 16 h. At the same time, 0.1 M EDC (100 μL) and 0.025 M NHS (100 μL) were added to 1 mL of CNCs solution for 1 h to active the CNCs. Then 500 μL of the activated CNCs solution were mixed with 100 μL of MB@Au -DNA and reacted for at least 16 h. After the unlinked CNCs were removed by magnetic separation, the precipitate was redispersed in 500 μL of PBS.

1.4. DNAzyme-Assisted Autocatalytic Amplification for Protein Detection

The detailed procedure for the detection of thrombin as a model analyte of protein was as follows. First, 5 μL of thrombin aptamer was mixed with 38 μL of different concentrations of thrombin at 37 ˚C for 30 min to form the aptamer−target complex. Single-stranded target DNA s1 was released from the aptamer/DNA hybrids in the process. Second, 1 μL of MB1 (10^{-4} mol/L), 1 μL of Exo III (100000 U/mL) and 5 μL of Buffer were added and incubated at 37 ˚C for 30 min. Then the prepared MB@Au-DNA-CNCs conjugates were added to the above mixture, and 2 μL of endonuclease, 15 μL of Buffer were added and incubated at 37 ˚C for 1.5 h. After magnetic separation of CNCs from MB, the amplified CNCs fluorescence signals related to the thrombin concentrations were measured**.**

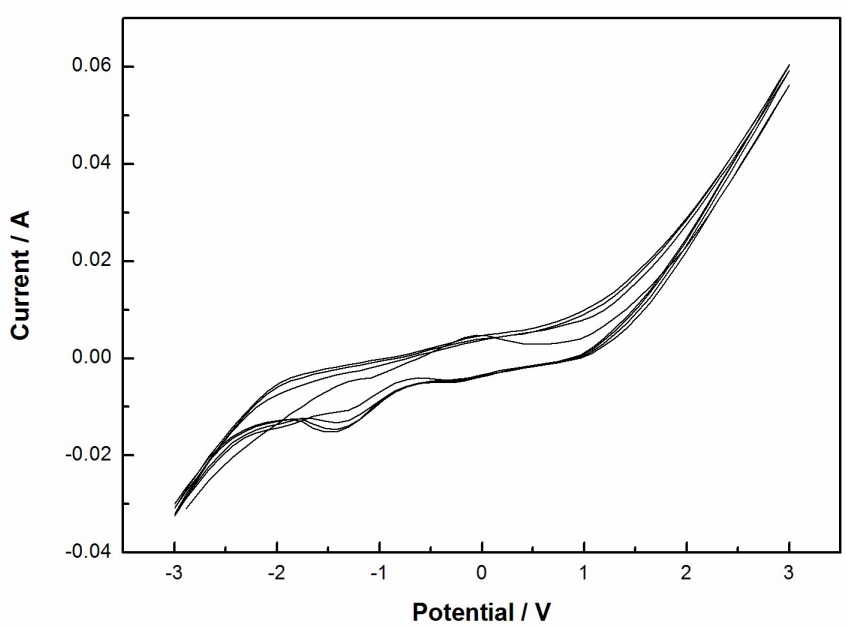

Figure S1. Continuous cyclic voltammograms (CV) obtained for a graphite rod working electrode in 0.1 mol/L pH 7.0 PBS. The applied potential was cycled between –3.0 V and 3.0 V at a scan rate of 0.1 V/s.

2. Fluorescence quantum yield (QY) of the CNCs

As the maximum emission wavelength of quinine sulfate was the same as the CQD solution, the fluorescence QY of the CNCs was tested using quinine sulfate solution (in 0.1mol L^{-1} sulfuric acid solution, QY=0.54) as a reference solution. The fluorescence intensity ratio and absorbance ratio of the CNCs and quinine sulfate solution under the same excitation wavelength (319 nm) were calculated as the following.

Qu = Qs [As/Au][n_u^2/n_s^2][Fu/Fs], Qu, Qs are the fluorescence QYs of CNCs and the reference solution, respectively; Au, As are the corresponding UV absorbance values; n_u, n_s are the corresponding refractive index; Fu, Fs are the corresponding fluorescence intensity. The fluorescence QY of the CNCs was about 21%.

Table S2. Detection limits for thrombin using different aptasensors.

Detection methods	detection limits	references
Fluorescence of Carbon Nanocrystals	0.006 pM	This work
ECL quench with ferrocene as quencher	60.0 pM	S1
DNAzyme-Based ECL Detection	0.28 fM	S2
ECL Resonance Energy Transfer	1.4 fM	S3
$Ru(phen)_3^{2+}$ as intercalator into ds-DNA	0.02 pM	S4
DNA aptamer-based QDs biosensor	2.72 nM	S5
Magnetic Composite Quantum Dot Electrochemiluminescence	0.12 pM	S6
"Off–On" Electrochemiluminescence Biosensing	0.04 pM	S7
Nanomaterial-amplified ECL aptasensors	3.0 f M	S8
Ferrocene-labeled molecular beacon aptamer	0.02 pM	S9
Sandwich type with $Ru(bpy)_3^{2+}$ as label	10.0 nM	S10
Electrochemical Detection of and Protein	5.0 pM	S11

Table S3. Recovery of Thrombin Assay at Different Concentrations Spiked into Human Serum Samples[a]

sample[b]	sample (pM)	spiked (pM)	Found (pM)	recovery (%)	RSD (%)
1	-[c]	10.0	9.6	96.0	2.9
2	-[c]	50.0	52.0	104.0	2.8
3	-[c]	100.0	105.0	105.0	3.4
4	15.0	50.0	67.5	103.8	2.7
5	60.5	100.0	155.0	96.6	2.5

a Each sample was repeated for three times and averaged to obtain the recovery and RSD values.

b Sampes 1-3 were from healthy people, and 4-5 were from patients.

c "-" represents not detectable.

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