Supplementary information for

A Graphene Oxide-Based Fluorescent Aptasensor for Alpha-Fetoprotein Detection

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Experimental Section

Materials and reagents

Graphite oxide dispersion (2 mg ml^{-1}) was obtained from XF Nano (Nanjing, China). 5′-carboxyfluorescein (FAM)-labeled aptamers were synthesized and HPLC purified by Sangon Biotech Co. Ltd. (Shanghai, China), the sequence of aptamers was given as follows.

5′-FAM-GTGACGCTCCTAACGCTGACTCAGGTGCAGTTCTCGACTCGGTCTTGATGTGGGTCCTGTCCGTCCGAACCAATC-3′

Tris(hydroxymethyl)aminomethane (Tris) was purchased from Sigma (St. Louis, MO, USA). All other chemicals and solvents were of analytic grade and bought from commercial sources without further purification. AFP, CEA, PSA were supplied by National Institutes for Food and Drug Control (NIFDC) (Beijing, China). Human serum was produced by Zhenglong Biotech Co, Ltd. (Chengdu, China).

All work solutions were prepared with 20 mM Tris-HCl buffer solution (pH 7.4, 100 mM NaCl, 5 mM KCl, and 5mM $MgCl_2$) by using ultrapure water (18.2MΩ, Milli-Q, Millipore, USA). Proteins and aptamers were stored at −20 °C prior to experimentation.

Apparatus and characterization

Fluorescence spectra were measured using an F-2500 fluorescence spectrometer (Hitachi, Japan). Both the excitation and emission slits were set at 10.0 nm and the PMT detector voltage was 400 V. Under the excitation wavelength of 480 nm, the emission spectra from 500 to 650 nm were collected. The fluorescence intensity at 518 nm was used to evaluate the performance of the proposed assay strategy. All fluorescence detections were carried out three times under room temperature.

Fluorescence anisotropy (FA) measurement was performed on a LS 55 Fluorescence Spectrometer with an excitation wavelength at 480 nm and an emission wavelength at 518 nm (PerkinElmer, USA). The FA (*r*) of the test solution was calculatedusing following equation:

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}$$

Where the *I* factor is the fluorescence intensity, and the subscripts V and H refer to the orientation (vertical or horizontal) of polarizer, with the first subscript indicating the position of the excitation polarizer and the second for the emission polarizer. *G*, the instrumental correction factor, is defined and calculated according to the following equation:

$$G = \frac{I_{HV}}{I_{HH}}$$

Six anisotropy measurements were taken each time using an integration time of 1 s for each sample, and the resulting anisotropy values were averaged.

AFP detection

All experiments were performed in 20 mM Tris-HCl buffer (pH 7.4, 100 mM NaCl, 5 mM KCl and 5 mM $MgCl_2$). A solution containing 10 nM of FAM-ssDNA and different concentrations of AFP was incubated for 30 min at room temperature, and GO was then added into the mixture to make the final volume 1 ml. The final concentration of GO was 3.5 μg ml^{-1}. The solution was mixed well and incubated for another 15 min at room temperature. Then, the fluorescence emission spectra were recorded with an excitation wavelength of 480 nm. The same procedures were repeated in next experiments. In the selectivity experiment, concentrations of CEA and PSA both were 300 pM, and the mixed sample contained 30 pM AFP, 300 pM CEA and 300 pM PSA. Human serum samples were diluted with Tris-HCl buffer to a suitable concentration for AFP detection. The practical samples were spiked with various concentrations of AFP (0.4, 2, 5, 10 pM).

Supplementary Figures

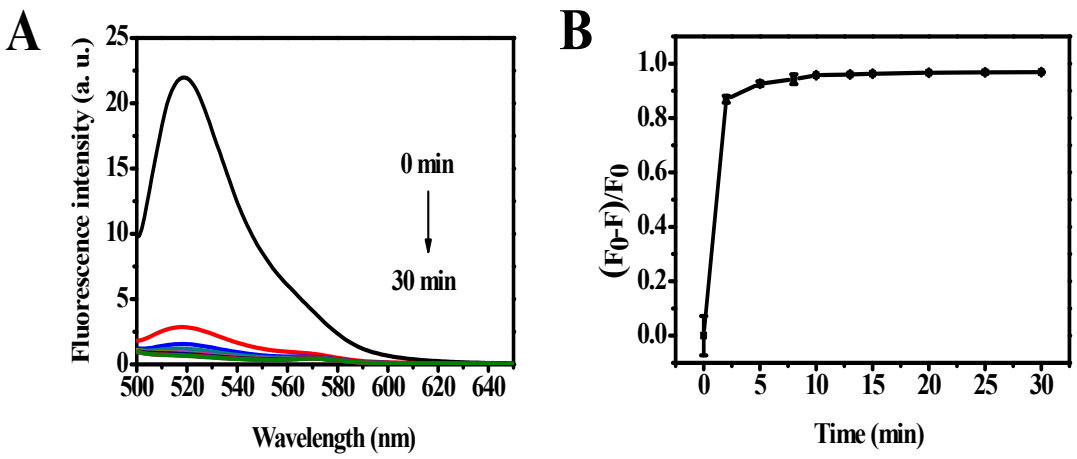

Fig. s1 (A) The fluorescence intensity of FAM-ssDNA upon addition of GO in different time (0, 2, 5, 8, 10, 13, 15, 20, 25, 30 min). (B) The (F_0-F/F_0) for the assay with the time. F_0 and F were the fluorescence intensities without and with GO. Concentrations of FAM-ssDNA and GO were 10 nM and 3.5 μg ml^{-1}, respectively. The excitation and emission wavelengths were 480 and 518 nm. Error bars indicated standard deviation for three replicates.

Table s1 Performance comparison of this work with other methods for AFP detection

Analytical method	**Sensor strategy**[a]	**Linear range**[b]	**Detection limit**	**Detection time**	**References**
Fluorescence	Aptamer/GO	0-10 pM	43 fM	45min	This work
Fluorescence	Exo III/GO/ anti-AFP	0.36-2.17 pM (25-150 pg ml^{-1})	116 fM (8 pg ml^{-1})	~3 h 10 min	1
Fluorescence	DA-functionalized CdSe/ ZnS QDs/anti-AFP	10 pM-100 nM	10 pM	~2h10min	2
Fluorescence	C-Dots labeled anti-AFP	0-5.07 nM (0-350 ng ml^{-1})	Not reported	~2h	3
Fluorescence	Silver plasmonic chips	Not reported	55 fM	6 h	4
Fluorescence	NaYF4:Yb,Tm/ NaGdF4 core-shell nanoparticles-GNRs	2.61-166 pM (0.18-11.44 ng ml^{-1})	2.32 pM (160 pg ml^{-1})	1.5h	5
Fluorescence	PLNPs/Ab-AuNPs	11.59-652 pM (0.8-45.0 ng ml^{-1})	5.94 pM (410 pg ml^{-1})	30min	6
Laser-induced fluorescence (LIF)	R6G labeled AFP antibody	0.073-14.49 pM (0.005-1.0 ng ml^{-1})	23 fM (1.6 pg ml^{-1})	2h	7
ELISA	MIPs	0-1.45 nM (0-	Not reported	52min	8

		100 ng ml^{-1})			
Chemiluminescence (CL)	PMs/ALP labeled anti-AFP	0.014-1.16 nM (1-80 ng ml^{-1})	7.54 pM (520 pg ml^{-1})	12min	9
ECL	BPE/PET	0.29-2.90 nM (20-200 ng ml^{-1})	145 pM (10 ng ml^{-1})	Not reported	10
Electrochemistry	Au–PDA-PB–GO/ anti-AFP	0.14-1159 pM (0.01-80.0 ng ml^{-1})	101 fM (7 pg ml^{-1})	14h23min	11
Electrochemistry	ZnO IOs electrode/ AFP–CdS–GOD	0.0014-7.26 nM (0.1-500 ng ml^{-1})	145 fM (10 pg ml^{-1})	Not reported	12
SERS	Ag-trimers/aptamer	0.2-20 aM	0.097 aM	~3.5h	13
SERS	3D Ag-SPCBs/ anti-AFP	1.45 fM- 14.49 nM (0.1 pg mL^{-1}-1 μg ml^{-1})	1.04 fM (0.072 pg ml^{-1})	~2h	14
Photoelectrochemical Immunosensing	TiO_2/ AFP–CdTe–GOx/ anti-AFP	7.25 fM- 145 nM (0.5 pg mL^{-1}-10 μg ml^{-1})	1.88 fM (0.13 pg ml^{-1})	14 h 55 min	15
Colorimetry	Au@Ag core-shell nanorods	Not reported	435 fM (30 pg ml^{-1})	6min	16

a Exo III, exonuclease III; DA, dopamine; QDs, quantum dots; GNRs, gold nanorods; PLNPs, persistent-luminescence nanoparticles; Ab-AuNPs, AFP-antibody-gold nanoparticle conjugates; R6G , rhodamine 6G; MIPs, molecularly imprinted polymers; PMs, paramagnetic spheres; ALP, alkaline phosphates; BPE, bipolar electrode; PET, poly(ethylene terephthalate); Au–PDA-PB–GO, gold nanoparticles–polydopamine-Prussian blue–grapheme oxide nanocomposites; IOs, inverse opals structure; GOD, glucose oxidase; Ag-SPCB, silver nanoshells silica photonic crystal bead; GOx, glucose oxidase.

b 1pM AFP is the equivalent of 69 pg ml^{-1} [17].

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