Electronic Supplementary Information (ESI) For:

Integrated Magneto-Fluorescence Nanobeads for Ultrasensitive Glycoprotein Detection Using Antibody Coupled Boronate-Affinity Recognition

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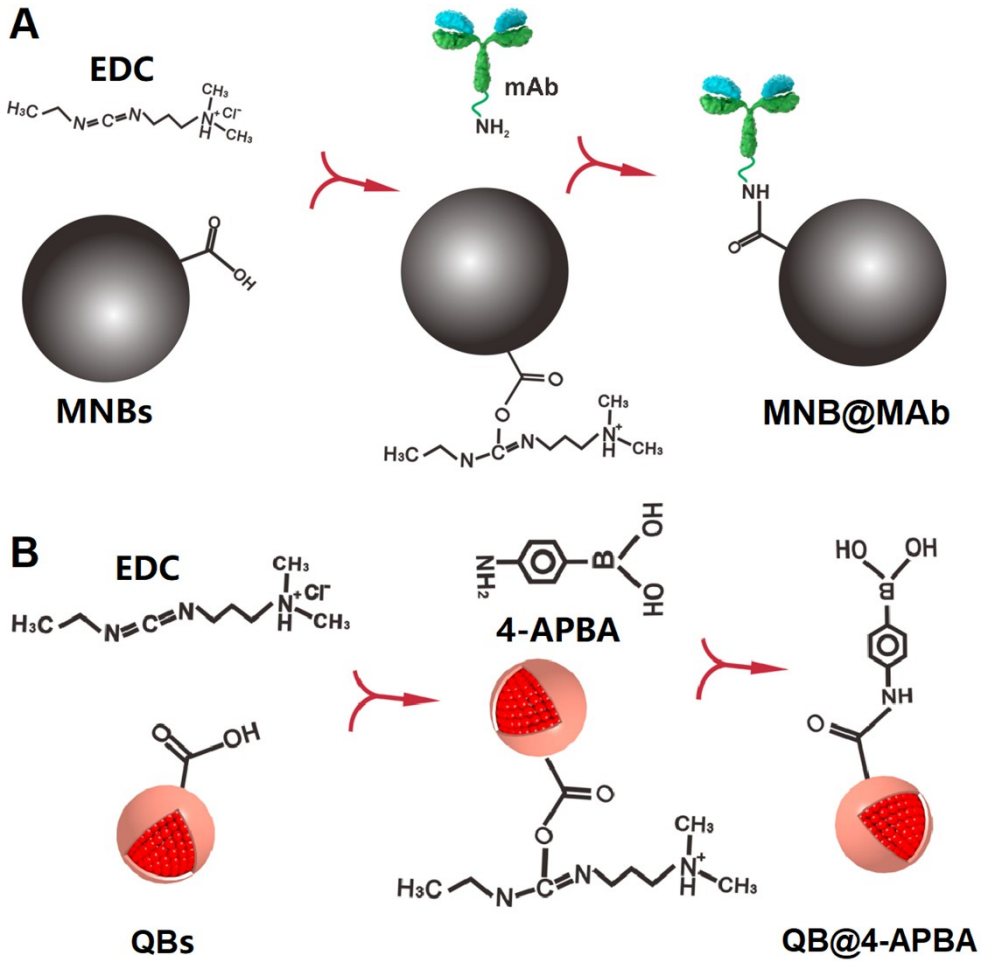

Scheme S1 Schematic representation for synthesis of MNB@mAb and QB@4-APBA using EDC method.

1. Comparison of fluorescence intensity between CdSe/ZnS QDs and the as-prepared QBs

To compare the fluorescence intensity escalation of the synthesized QBs, the fluorescence signal of the synthesized QBs and the CdSe/ZnS QDs with a maximum emission wavelength in 620 nm can be detected.[1] As shown in Figure 1B, the fluorescence intensity of the CdSe/ZnS QD (4.3×10^{-6} mol/L, dissolved in octane) was the same as that of QBs (5.96×10^{-10} mol/L, dissolved in ultrapure water). Therefore, the fluorescence intensity of QBs was presumably 7214 times higher than that of QDs on the foundation of the ratio of their concentrations under the same fluorescence signal (the concentrations of QD/the concentrations of QBs = (4.3×10^{-6} mol/L) /(5.96×10^{-10} mol/L) = 7214).

2. Measurement of the amount of antibody labeled on MNB surface

A commercial BCA kit (ThermoScientific) was used to quantify the amount of antibody loaded on the MNB surface. Results indicated that approximately 15.64 μg antibody was coupled onto MNB surface per milligram, corresponding to 3.31×10^3 antibody molecules labeled on each MNB surface.

The detailed calculation was described as follow:

(1)

$m_{(MNBs)}$ =1 mg

$\rho_{(MNBs)}$ =5.18 g/cm^3

$R_{(MNBs)}$ =250 nm

$N_{(MNBs)}$ = Number of particles in 1 mg MNBs

On Formula $\frac{m_{(MNBs)}}{\rho_{(MNBs)}} = \frac{4\pi}{3} \cdot {R_{(MNBs)}}^3 \cdot N_{(MNBs)}$

Put all values into the formula and calculate, $N_{(MNBs)}= 2.95 \times 10^9$

(2)

$m_{(mAb)}$ = 15.64 μg

$M_{(mAb)}$ = 150000 g/mol

$N_A = 6.02 \times 10^{23}$

$N_{(mAb)}$ = Number of 15.64 μg antibody

On Formula $N_{(mAb)} = \frac{m_{(mAb)}}{M_{(mAb)}} \cdot N_A$

Put all values into the formula and calculate, $N_{(mAb)} = 9.77 \times 10^{12}$

So, the antibody number loaded on each MNB: $\frac{N_{(mAb)}}{N_{(MNBs)}} = \frac{9.77 \times 10^{12}}{2.95 \times 10^{9}} = 3.31 \times 10^{3}$

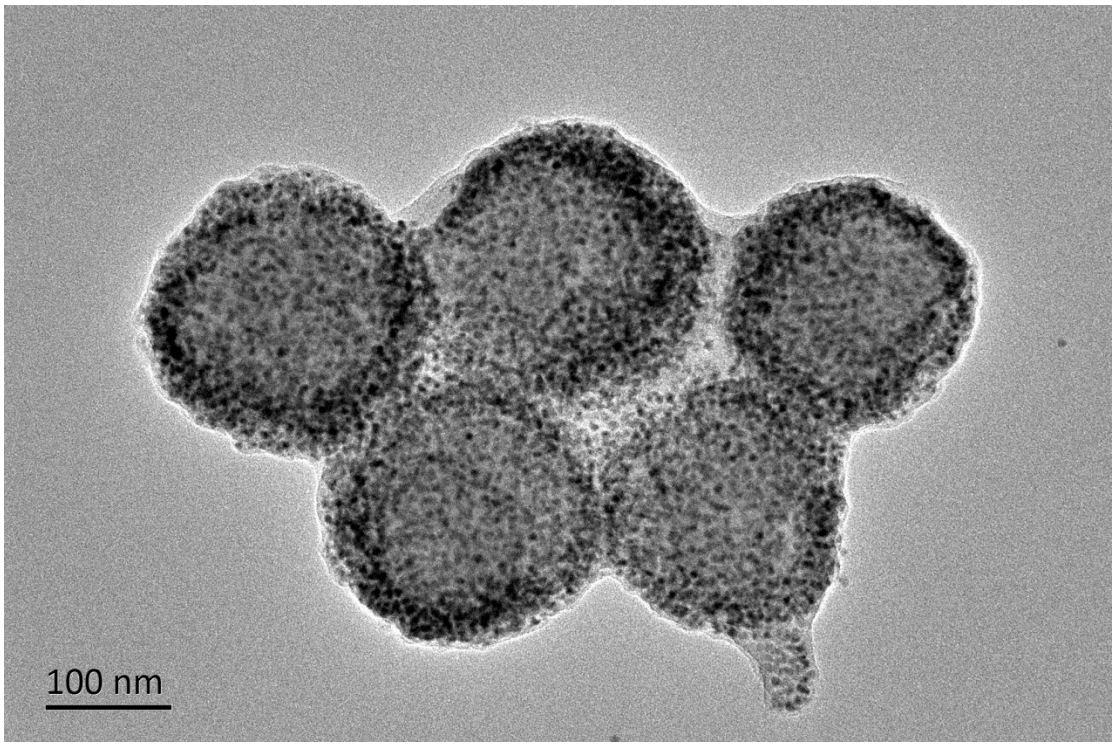

Figure S1 TEM images of QBs

Table S1 Comparison of analytical performances of our ABSTA method with the previously reported methods in detecting HCG.

Detection method	Assay time	Linear range (mIU/mL)	LOD (mIU/mL)	Whether matching antibodies	Reference
Assembled gold nanoparticles	2.7 h	0.1-6.4	0.03	Yes	2
Photoelectrochemical	5 h	0.5-1000	0.2	Yes	3
Chemiluminescence resonance energy transfer	3 h	0.1 -10	0.06	Yes	4
An immunonanogold resonance scattering	30 min	40-8000	13.6	Yes	5
Time-resolved fluorescence	2 h	1-8000	1	Yes	6
Surface plasmon fluorescence	48 min	0-10	0.3	Yes	7
ICA based on gold nanoflowers	15 min	9-288 288-2304	9	Yes	8
Antibody-boronate sandwich-typed assay	40 min	0.24-62.5	0.19	No	This work

Table S2 Recovery and precision of the proposed ABSTA method in HCG-spiked serum samples.

HCG concentration (mIU/mL)	Intra-assay precision			Inter-assay precision		
	Mean[b]	Recovery (%)	CV (%)	Mean[a]	Recovery (%)	CV (%)
50	49.48	98.95	0.45	49.64	99.28	0.78
25	25.36	101.45	4.96	24.39	97.58	9.19
12.5	11.85	94.77	8.48	12.22	97.80	8.32
6.25	7.00	112.04	4.19	7.18	114.90	1.92
3.13	3.20	102.10	1.42	3.18	101.57	0.41

[a] Mean value of four replicates at each diluted concentration.

[b] Assay was completed every one day for three days continuously.

Table S3 Correlation between detection results from CLIA and ABSTA analyses of 38 HCG-positive samples and 12 HCG-negative samples.

Sample	CLIA (mIU/mL)	ABSTA (mIU/mL)	Sample	CLIA (mIU/mL)	ABSTA (mIU/mL)
1	105	98.71	26	<0.1	—
2	5	5.11	27	66	60.11
3	0.1	—	28	42	61.67
4	31	26.79	29	12	9.74
5	<0.1	—	30	<0.1	—
6	30	24.84	31	24	29.69
7	63	73.57	32	4	2.76
8	14	10.52	33	9	6.94
9	<0.1	—	34	52	56.16
10	32	38.71	35	76	81.03
11	7	5.39	36	<0.1	—
12	13	12.13	37	25	18.10
13	86	97.08	38	34	34.30
14	16	22.58	39	<0.1	—
15	4	4.30	40	<0.1	—
16	<0.1	—	41	125	121.55
17	40	28.14	42	52	39.93
18	<0.1	—	43	38	44.99
19	21	16.20	44	<0.1	—
20	20	15.41	45	6	4.32
21	2	1.38	46	10	15.58
22	7	12.82	47	104	144.32
23	22	28.29	48	<0.1	—

24	<0.1	—	49	26	18.50
25	25	44.27	50	19	16.98

Table S4 Optimization of working concentrations of coating anti-HCG-α mAb and biotin- anti-HCG-β mAb in conventional HRP-based ELISA.

Biotin-anti-β-HCG	Anti-α-HCG-mAbs (μg /mL)					
-mAb (μg/mL)	20	10	5	2.5	1.25	0
10	1.960	1.719	1.637	1.455	0.669	0.467
5	1.320	1.326	1.146	1.007	0.583	0.347
2.5	1.064	1.072	0.789	0.750	0.319	0.203
1.25	1.025	0.796	0.676	0.620	0.297	0.218
0.625	0.983	0.559	0.555	0.523	0.230	0.158
0	0.182	0.169	0.147	0.117	0.170	0.118

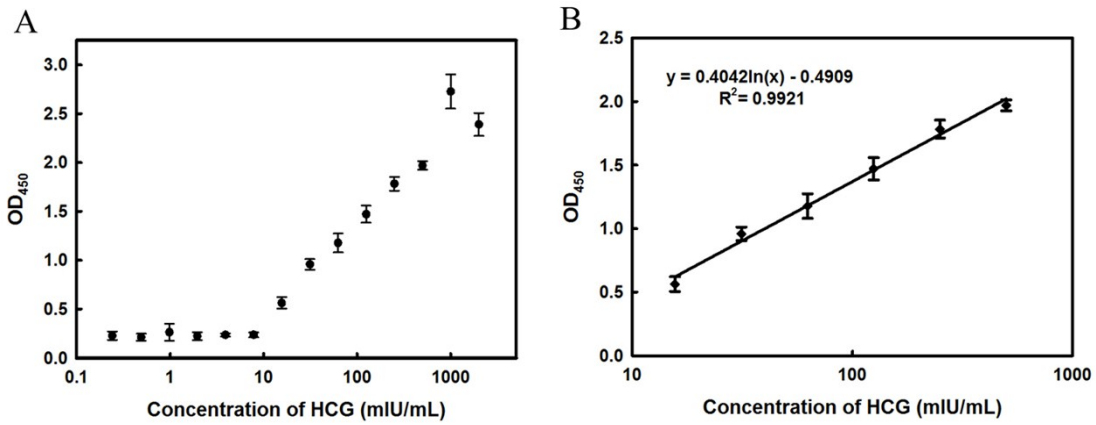

Figure S2 Calibration curve of conventional HRP-based ELISA. (A) Standard curve for HCG detection. (B) An enlarged view for HCG concentrations from 15.6 mIU/mL to 500 mIU/mL.

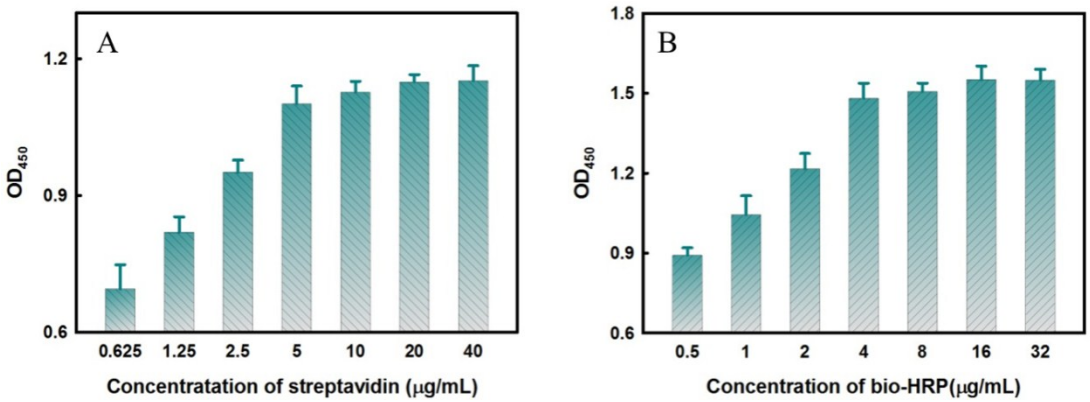

Figure S3 Parameter optimization for conventional HRP-based ELISA. (A) Streptavidin concentration. (B) Biotin-HRP concentration. The error bars represent the standard deviation of three independent measurements (n=3).

2 Experimental sections

2.1 Materials

N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl), bovine serum albumin (BSA), biotin-N-hydroxysuccinimide ester, streptavidin, casein acid hydrolysate, poly(maleic anhydride-alt-1-octadecene) (PMAO, MW=30,000–50,000), poly(methyl methacrylate) (PMMA, MW=50,000), sodium dodecyl sulfonate (SDS), and hexamethylenediamine were purchased from Sigma-Aldrich Chemical Co. Ltd. (St. Louis, MO, USA). 4-Aminophenylboronic hydrochloride (4-APBA) was purchased from Aladdin reagent Co. LTD (Shanghai, China). (6-Aminopyridin-3yl) boronic acid hydrochloride (6-APBA) was purchased from Shanghai jizhi biochemical technology Co. Ltd. (Shanghai, China). Goat anti-mouse antibody, anti-HCG-α monoclonal antibody (anti-HCG-α mAb), and anti-HCG-β monoclonal antibody (anti-HCG-β mAb) were obtained from Chongqing Xinyuanjiahe Biotechnology Inc. (Chongqing, China). Carboxylated MNBs of size 500 nm were bought from Tianjin Baseline ChromTech Research Centre (Tianjin, China). Carboxylated QBs of size 195 nm were synthesized by encapsulating octadecylamine-coated CdSe/ZnS quantum dots with an emission wavelength at 620 nm and then characterized in our laboratory as previously described. HCG standards were purchased from Wanhua Biochem Products Co. Ltd. (Nanchang, China). HCG-positive and HCG-negative samples were collected from the People's Hospital in Jiangxi Province (Nanchang, China). All other chemicals used without further

purification were of analytical grade and purchased from Sinopharm Chemical Corp. (Shanghai, China). Double-distilled water was obtained from a Milli-QA apparatus (Molsheim, France).

2.2 Preparation of QB@4-APBA conjugates

The synthesis of QB@4-APBA complex was performed through EDC method. In a typical procedure, 40 μL prepared QBs (12.5 mg/mL) was dispersed in 4.0 mL 0.01 M phosphate buffer (PB, pH 6.0) solution containing 56 μmol 4-APBA and 56 μmol hexamethylenediamine. EDC (40 μL; 1 mg/mL) was added into the above mixture under shaking at 220 rpm for 2 h. To remove excess 4-APBA, the resulting QB@4-APBA conjugates were collected via centrifugation at 14000 rpm for 15 min. The precipitates were re-suspended in 4 mL PB solution (pH 7.4) and then stored in 4 °C until further use.

2.3 Preparation of MNB@mAb conjugates

The conjugation of carboxylated MNBs with anti-HCG-β mAb was conducted through the EDC method. In brief, 200 μL carboxylated MNBs (5% w/v) was diluted in 4.8 mL 0.01 M PB solution (pH 7.4) containing 2.5 μL anti-HCG-β mAb (10 mg/mL) and 25 μL EDC (1 mg/mL) under stirring. After 1.5 h of incubation at 25 °C, 2% casein acid hydrolysate was added into the above solution to block the unreacted carboxyl groups. After 1 h of incubation at 25 °C, the resultant MNB@mAb conjugates were collected via an external magnetic field and were dissolved in 0.01 M PB solution. To remove the nonspecific binding of QB@4-APBA with the

carbohydrate chain of anti-HCG-β mAb, the as-prepared MNB@mAb conjugates were further blocked with the 6-APBA solution under stirring at 220 rpm for reaction of 1 h. The MNB@mAb conjugates were collected by an external magnetic field and then washed thrice with PB (pH 7.4) to remove excess 6-APBA. Finally, the MNB@mAb was re-suspended in 2 mL PB (pH 7.4) and stored at 4 °C until further use.

2.4 Procedure of ABSTA for HCG detection

Magnet-mediated sandwich-typed assay was conducted for sensitive and specific HCG detection. The detailed detection procedure was described as follows: 100 μL MNB@mAb compound (0.5 mg/mL) was added and mixed to 1 mL sample solutions containing different concentrations of HCG ranging from 0 mIU/mL to 1000 mIU/mL in glass tube, and the mixtures were incubated at room temperature for 10 min. The formed MNB@mAb-HCG immunocomplexes were washed thrice with PB solution (pH 7.4) via an external magnetic field, and then re-suspended in 300 μL QB@4-APBA solution (32 μg/mL). After reaction at room temperature for 30 min, the formed MNB@mAb-HCG-QB@4-APBA complexes were washed thrice with PB solution (pH 7.4) to remove the unbound QB@4-APBA probes by an external magnetic field, and then re-suspended in 120 μL ultrapure water. Finally, 100 μL of the suspensions was pipetted into a 96-well black microplate for subsequent fluorescence measurement on a Multiskan GO multimode reader (Thermo Fisher, Vantaa, Finland).

2.5 Validation analysis of the proposed ABSTA

The practicability and feasibility of our developed ABSTA method for HCG determination was evaluated in real serum. Fifty real serum samples, including 38 HCG-positive samples 12 HCG-negative samples were provided by Jiangxi Provincial People's Hospital (Nanchang). All samples were analyzed simultaneously using our prepared ABSTA method and a commercial chemiluminescence immunoassay (CLIA) kit (Roche Molecular Systems, Inc). A correlation analysis of the results obtained using the two methods was completed to estimate the reliability and availability of the developed ABSTA method.

2.6 Detection procedures of conventional HRP-based ELISA

The 96-well polystyrene plates were coating with 100 μL of anti-α-HCG mAbs (10 μg/mL) per well in PBS (pH 8.6) at 4°C for overnight. After washing the plates three times with PBST, the plates were blocked with blocking agent (5% bovine serum albumin (BSA) in PBS) for 1 h at 37°C.Afterthat, the plates were washed three times by PBST, and 100 μL, 200 mIU/mL of HCG were added into the plates. After 1 h incubation at 37°C, the plates were washed three times via PBST, and then 100 μL of bio-anti-β-HCG mAb (5μg/mL) was added for 1 h incubation at 37 °C. After washing the plates five times through PBST, 100 μL of streptavidin (5 μg/mL) was added. After 30 min incubation at 37 °C, unreacted streptavidin was washed away by PBST. Then 100 μL of biotin-HRP (4 μg/mL) was added for 45 min incubation. After the excess biotin-HRP was washed away, 100 μL of fresh prepared substrate TMB

solution was added into each well for 10 min incubation at 37 °C. Then the reaction was stopped by adding 50 μL 10% H_2SO_4 solution for each well. Finally, the absorbance value at 450 nm for each well was recorded immediately with a SpectraMax M5 plate reader (Molecular Devices).

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