Supporting Information

A multifunctional black phosphorus nanosheets based immunomagnetic bio-interface for heterogenous circulating tumor cell capture and simultaneous self-identification in gastric cancer patients

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1. Experimental section

Synthesis of Fe_3O_4 particles. Fe_3O_4 magnetic nanoparticles were synthesized according to the previous precedures:[1] 1 g PEI and 4 mM $Fe(acac)_3$ were dissolved in 30 mL DEG, and the solutions was stirred vigorously and heated to 120°C to form a homogeneous dark red solution. Then it was heated to 220°C and kept for 2 h. After cooling to room temperature, the obtained Fe_3O_4 particles were collected by magnetic separation and rinsed three times with deionized water and ethanol. After drying, 1.0 mg Fe_3O_4 particles were re-dispersed in 1.0 mL deionized water (1.0 mg/mL) for further use.

Synthesis of AuNR. For the synthesis of AuNR, a seed solution needed to be prepared first.[2] 5 mL CTAB solution (0.1 M) and 125 μL $HAuCl_4$ solution (0.01 M) were added into a 10 mL beaker. The solution was stirred at 600 r/min for 1 min until the color of the solution turned golden brown. Quickly, 300 μL of 0.01 M $NaBH_4$ was added at 0°C and stirred at 1200 r/min for 2 min. The obtained brown-yellow seed solution was placed in a water bath at 30°C and incubated for 2 h at constant temperature. Then, 0.7 g CTAB and 0.1234 g sodium oleate were dissolved in 25 mL deionized water at 60°C. After cooling to room temperature, 100 μL of 0.01 M $AgNO_3$ was added and stirred at 600 r/min for 5 min, followed by incubating in a 30°C water bath for another 15 min. 25 mL of 1 mM $HAuCl_4$ was then added and stirred at 600 r/min on a magnetic agitator for 90 min, during which the solution was changed from golden to colorless, and the pH was adjusted by adding 2.5 mL of 1 M HCl. Then, the mixture was stirred at 400 r/min for 15 min, after which, 80 μL of 0.1 M AA was added. Afterwards, we added 25 μL of the prepared seed solution into the mixture and the solution was stirred at 700 r/min for 1 min and then transferred to a water bath at 30°C for 15-18 h. At this stage, the solution turned purple and the color gradually became darker to obtain the final wine red AuNR. After drying, it was re-dispersed in 1.0 mL deionized water to obtain the final concentration of 20 μg/mL.

Cell culture. MGC-803 (human stomach cancer cells), BGC-823 (human stomach cancer cells), THP-1 (human monocytes cells) and Hela cells were cultured in 1640 medium supplemented with 10% fetal bovine serum (FBS) and double antibody (100

U/mL penicillin, 100 U/mL streptomycin) and cultured in a 37°C, 5% CO_2 incubator. MCF-10A cells were grown in DMEM/F12 medium (1: 1, Hyclone, US). All of these cells were provided by Affiliated Hospital of Xuzhou Medical University.

Biocompatibility evaluations of BP-Fe_3O_4-AuNR/Apt probe on CTCs. Before biocompatibility evaluations, the captured cells needed to be released from the probe. Typically, after MGC-803 and BGC-823 cells were captured, complementary DNA was added for cell release. The released cells were re-collected by centrifugation and resuspended in 2 mL complete medium for reculture in a 37°C, 5% CO_2 incubator for one week. During this period, the medium was changed every two days, and the growth of the cells was observed and recorded every day.

The biocompatibility of the BP-Fe_3O_4-AuNR/Apt probe was assessed by CCK-8 assay, Calcein AM and PI stainings and apoptosis assay. Firstly, 0 (control group), 20, 40, 60, 80, and 100 μg/mL of BP-Fe_3O_4-AuNR/Apt probe were incubated with MGC-803 and BGC-823 cells for 30 min in the incubator. Then, 100 μL incomplete medium containing 10 μL CCK-8 (2.5 mM) was added and incubated for another 4 h. Finally, the absorbance value (OD) of the 96-well plate was detected at a wavelength of 450 nm using a microplate reader. Cell viability was calculated as: % = $([OD]_{measurement} - [OD]_{blank}) / ([OD]_{control} - [OD]_{blank}) \times 100\%$.

After collecting the released cells, cells were stained with 2 μM Calcein AM and 8 μM PI at room temperature for 30 min, and the number of live and dead cells was counted under microscopy.

Finally, the effect of BP-Fe_3O_4-AuNR/Apt probe on cell viability was further investigated by apoptosis assay. Well-grown MGC-803 and BGC-823 cells were seeded in a 6-well plate at a density of 5×10^5 cells per well, and incubated for 12 h to make cells adherent. The medium was discarded and cleaned twice with PBS buffer, and then a certain amount of BP-Fe_3O_4-AuNR/Apt probe was added. After mixing, 2 mL per well was added to the 6-well plate and incubated for 30 min. Trypsin without EDTA was digested for 5 min and centrifuged for 3 min. The cell pellet was washed twice with PBS and resuspended. Annexin V-FITC (5 μL, 20 μg/mL) and Annexin V-PI (5 μL, 0.1 mg/mL) were added in sequence. Then, the mixture was wrapped in foil and incubated at room temperature for 5~15 min. Apoptosis was detected by flow

cytometry within 1 h.

2. Results and discussion

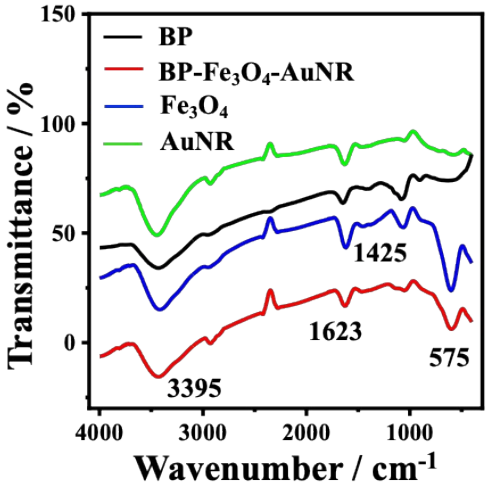

Figure S1. FT-IR absorption spectra of Fe_3O_4, BP, AuNR and BP-Fe_3O_4-AuNR.

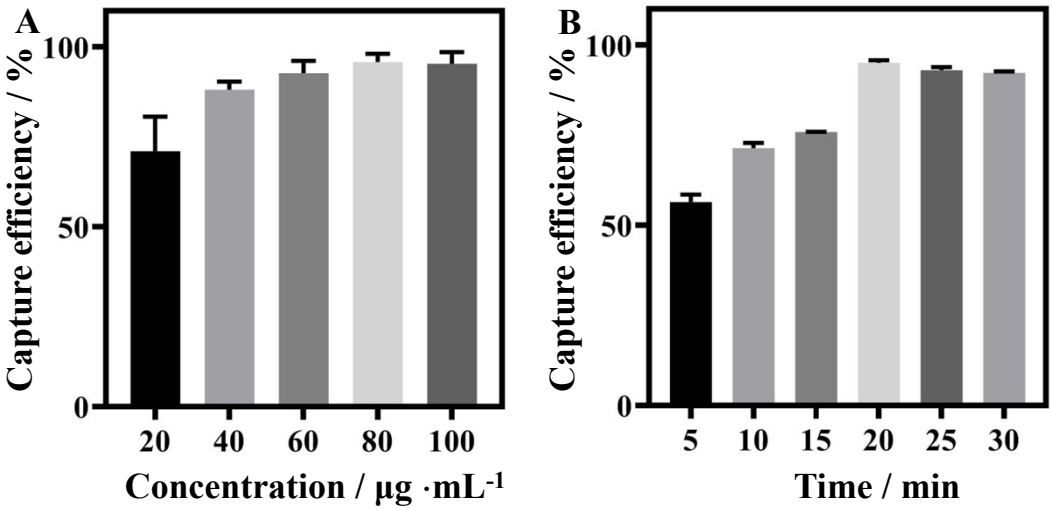

Figure S2. Effects of concentrations of BP-Fe_3O_4-AuNR/Apt probe (A) and incubation time (B) on the capture efficiency of MGC-803 cells. Cell concentration: 1000 cells/mL.

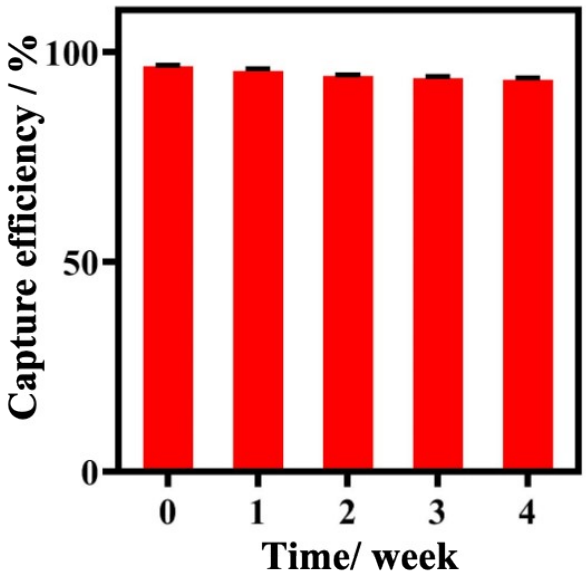

Figure S3. Trend of capture efficiency by BP-Fe_3O_4-AuNR/Apt probe within 4 weeks.

Table S1. Counted cell numbers in three mimic clinical samples (n = 3)

Sample no	Spiked cell number /mL	Counted cell number/mL			Mean	RSDs (%)
		1	2	3		
1	1	1	1	0	1	86.6
2	5	5	4	5	5	12.3
3	10	9	8	10	9	11.1

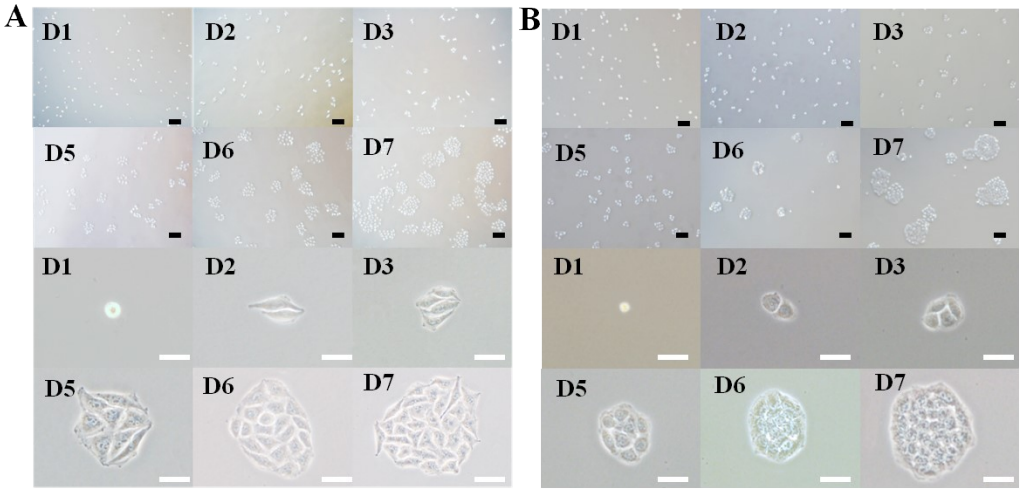

Figure S4. Cell re-culture after release. (A) MGC-803 cells; (B) BGC-823 cells. Black scale bars: 100 μm. White scale bars: 20 μm.

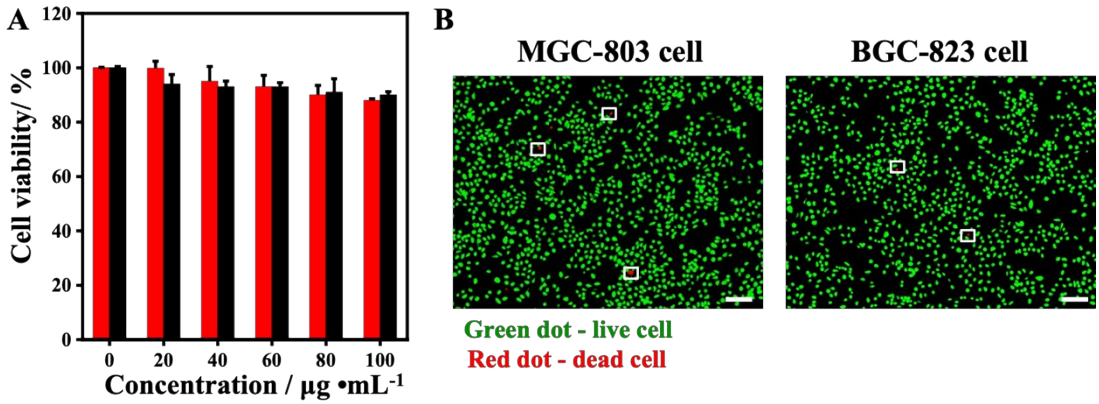

Figure S5. (A) Cell survival rate after incubated with 0 ~ 100 μg/mL BP-Fe_3O_4-AuNR/Apt probe. (B) Inverted fluorescence images of live (green dots) and dead cell (red dots) staining of MGC-803 and BGC-823 cells. Scale bars: 100 μm.

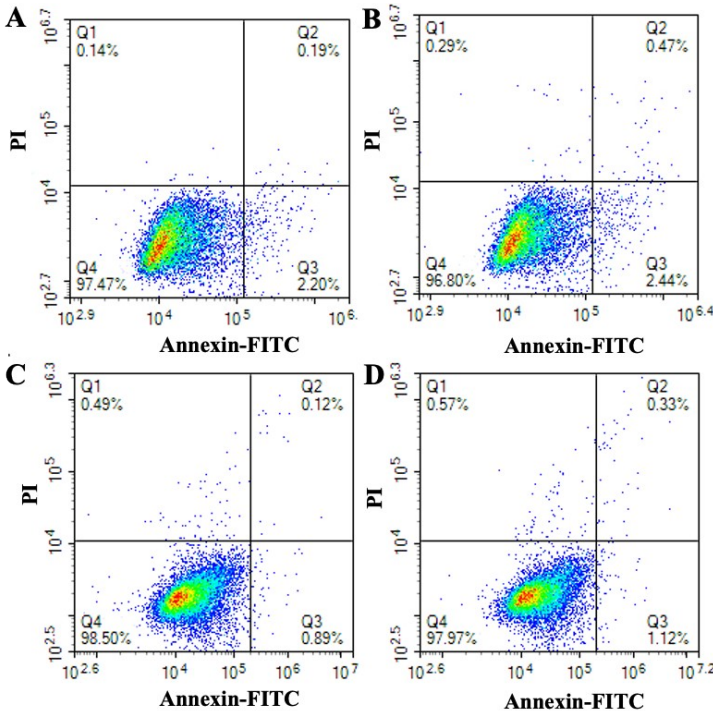

Figure S6. Apoptosis diagrams of MGC-803 (A, B) and BGC-823 (C, D) cells in control group (A, C) and experimental group (B, D).

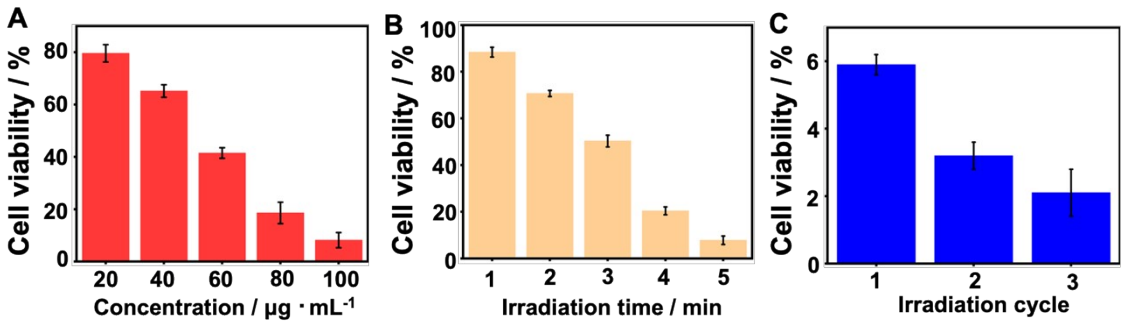

Figure S7. Investigations on MGC-803 cell viability during photothermal treatments under different conditions: (A) different concentrations of BP-Fe_3O_4-AuNR/Apt probe (20 - 100 μg/mL) and irradiated for 5 min; (B) different irradiation time (1-5 min) with 100 μg/mL probe. (C) different irradiation cycles with probe concentration of 100 μg/mL and irradiated for 5 min for each cycle. Cell concentration: 500 /mL.

Table S2 Comparison of our work with other reported technologies in CTCs capture

Methods		Capture efficiency (%)	Linear range (cells/mL)	Incubation time (min)	Heterogenous	Ref
Nanomaterials	IMNs	> 90	10~300	30	Yes	[3]
	BIMNs	> 85	20~200	2	No	[4]
	Nanocage	> 92	10~4000	60	Yes	[5]
	MN@Cys@PEG2k-FA	> 90.5	20~300	15	Yes	[6]
Microfluid chip	Apt-mAb chip	>89	25~10^4	--	Yes	[7]
	AFM chip	97.3	500~5×10^4	--	No	[8]
	ISET	> 91	10~100	--	No	[9]
	VIZA chip	95	2~1200	--	Yes	[10]
Present study	BP-Fe_3O_4-AuNR/Apt	> 95	5~500	20	Yes	--

Table S3. Basic information and number of CTCs in peripheral blood of patients with gastric cancer

Number	Gender	Age	Stage	Distant metastasis	Volume of blood/mL	Number of CTCs
1	female	85	I	0	1.0	3
2	male	57	I	0	1.0	3
3	male	66	I	0	1.0	4
4	male	68	I	0	1.0	3
5	male	54	II	0	1.0	4
6	male	49	II	0	1.0	4
7	male	58	II	0	1.0	6
8	male	29	II	0	1.0	4
9	female	54	II	0	1.0	6
10	female	50	II	0	1.0	5
11	female	52	II	0	1.0	5
12	male	77	II	0	1.0	5
13	female	58	II	0	1.0	6
14	male	72	II	0	1.0	4
15	female	70	II	0	1.0	5
16	male	44	II	0	1.0	5
17	male	72	II	0	1.0	4
18	male	57	II	0	1.0	3
19	male	56	II	0	1.0	6
20	male	61	II	0	1.0	4
21	female	45	II	0	1.0	5
22	female	72	II	0	1.0	6

Number	Gender	Age	Stage	Distant metastasis	Volume of blood/mL	Number of CTCs
23	male	65	II	0	1.0	5
24	male	55	II	0	1.0	5
25	male	68	II	0	1.0	5
26	female	54	II	0	1.0	5
27	male	51	II	0	1.0	5
28	male	63	II	0	1.0	5
29	female	58	II	0	1.0	4
30	female	53	II	0	1.0	5
31	male	68	II	0	1.0	6
32	female	52	II	0	1.0	4
33	male	59	II	0	1.0	4
34	male	59	III	0	1.0	8
35	male	86	III	0	1.0	9
36	female	58	III	0	1.0	8
37	male	76	III	0	1.0	8
38	male	74	III	0	1.0	6
39	male	55	III	0	1.0	7
40	male	53	III	0	1.0	8
41	male	56	III	0	1.0	7
42	male	63	III	0	1.0	8
43	female	74	III	0	1.0	8
44	male	52	III	0	1.0	7
45	male	69	III	0	1.0	8
46	female	71	III	0	1.0	9
47	male	64	III	0	1.0	7
48	male	37	III	0	1.0	8
49	female	50	III	0	1.0	8
50	female	73	III	0	1.0	7
51	female	65	III	0	1.0	7
52	male	70	III	0	1.0	7
53	female	33	III	0	1.0	7
54	female	52	III	0	1.0	5
55	male	59	III	0	1.0	8
56	male	50	III	0	1.0	6
57	male	52	III	0	1.0	8
58	male	76	III	0	1.0	6
59	female	65	III	0	1.0	8
60	male	67	III	0	1.0	7
61	male	49	IV	1	1.0	12
62	male	50	IV	1	1.0	9
63	male	58	IV	1	1.0	10
64	male	60	IV	1	1.0	12
65	male	66	IV	1	1.0	10

Number	Gender	Age	Stage	Distant metastasis	Volume of blood/mL	Number of CTCs
66	male	61	IV	1	1.0	9
67	female	46	IV	1	1.0	9
68	male	57	IV	1	1.0	8
69	male	69	IV	1	1.0	9
70	female	80	IV	1	1.0	12

Table S4. Basic information and number of CTCs in peripheral blood of patients with gastric adenocarcinoma (GA) before and after one cycle of chemotherapy

Number	Cancer	Gender	Age	Volume of blood/mL	Number of CTCs	
					Before treatment	After one cycle of treatment
1	GA	female	50	1.0	8	7
2	GA	male	70	1.0	7	5
3	GA	male	68	1.0	6	4
4	GA	male	59	1.0	4	5
5	GA	female	53	1.0	5	3
6	GA	female	80	1.0	12	6
7	GA	male	68	1.0	5	3
8	GA	male	76	1.0	6	5
9	GA	male	63	1.0	5	3
10	GA	male	55	1.0	5	4
11	GA	male	59	1.0	8	6
12	GA	female	73	1.0	7	5
13	GA	female	54	1.0	5	4
14	GA	female	52	1.0	4	3
15	GA	male	65	1.0	5	4
16	GA	female	33	1.0	7	5
17	GA	male	51	1.0	5	3
18	GA	male	66	1.0	4	3
19	GA	male	69	1.0	9	7
20	GA	female	65	1.0	8	6
21	GA	female	65	1.0	7	6
22	GA	female	52	1.0	5	4
23	GA	male	50	1.0	6	5
24	GA	male	52	1.0	8	6
25	GA	female	58	1.0	4	3
26	GA	male	68	1.0	3	2

27	GA	male	67	1.0	7	4

Table S5. Basic information and number of CTCs in peripheral blood of patients with gastric adenocarcinoma (GA) before and after two cycle of chemotherapy

Number	Cancer	Gender	Age	Volume of blood/mL	Number of CTCs	
					Before treatment	After two cycles of treatment
1	GA	female	33	1.0	7	5
2	GA	male	52	1.0	8	4
3	GA	male	51	1.0	5	2
4	GA	male	66	1.0	4	3
5	GA	male	69	1.0	9	7
6	GA	female	65	1.0	8	4
7	GA	male	68	1.0	3	2
8	GA	female	65	1.0	7	6
9	GA	female	58	1.0	4	3
10	GA	male	50	1.0	6	4
11	GA	female	52	1.0	5	3

Table S6. Basic information of patients with benign gastric disease and the number of CTCs in peripheral blood

Number	Disease	Gender	Age	Volume of blood/mL	Number of CTCs
1	chronic atrophic gastritis	male	77	1.0	0
2	chronic atrophic gastritis	male	65	1.0	0
3	chronic atrophic gastritis	male	64	1.0	0
4	chronic atrophic gastritis	male	21	1.0	0
5	chronic atrophic gastritis	female	38	1.0	0
6	chronic atrophic gastritis	female	58	1.0	0
7	chronic atrophic gastritis	female	76	1.0	0
8	chronic atrophic gastritis	female	57	1.0	0
9	chronic atrophic gastritis	female	64	1.0	0
10	chronic atrophic gastritis	male	64	1.0	0
11	chronic atrophic gastritis	male	51	1.0	0
12	chronic atrophic gastritis	male	64	1.0	0
13	chronic atrophic gastritis	male	58	1.0	0
14	chronic atrophic gastritis	male	70	1.0	0
15	chronic atrophic gastritis	male	62	1.0	0

Table S7. Basic information of healthy persons and the number of CTCs in peripheral blood

Number	Gender	Age	Volume of blood/mL	Number of CTCs
1	male	43	1.0	0
2	male	25	1.0	0
3	female	26	1.0	0
4	female	48	1.0	0
5	male	50	1.0	0

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