Supporting Information

From Interface to Solution: Integrating Immunoassay with Netlike Rolling Circle Amplification for Ultrasensitive Detection of Tumor Biomarker

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Table S1. Sequences of oligonucleotides. The underlined sequences are the recognition sites of Nb. BsrDI nicking enzyme. This enzyme can nick on their complementary sequences (5'-CATTGC(nick)N-3').

Oligonucleotides	Sequence (5'-3')
Circular Probe-1 (C-1)	CAC GCG ATC CGC ATG TGG AAA ATC TCT AGC AGT CCC ACC CTC CAA CCA CCA AGG CAA TGT ACA CGA ATT CGC CGA ACG
Circular Probe-2 (C-2)	GTA TGA GTA TCT CCT ATC TTA ACC CAC GCC GAA TCC TAG ACT ATA TAT GAT GGT TAT GCT ATG GCA ATG ATC CCG CTG AT
Primer 1-1 (P1-1)	Biotin-ACT GCT AGA GAT TTT CCA CAT
Primer 1-2 (P1-2)	Biotin-CTA GGA TTC GGC GTG GGT TAA
Primer 2-1 (P2-1)	ACC AAG GCA ATG TAC ACG AAT TC
Primer 2-2 (P2-2)	GCT ATG GCA ATG ATC CCG CTG AT

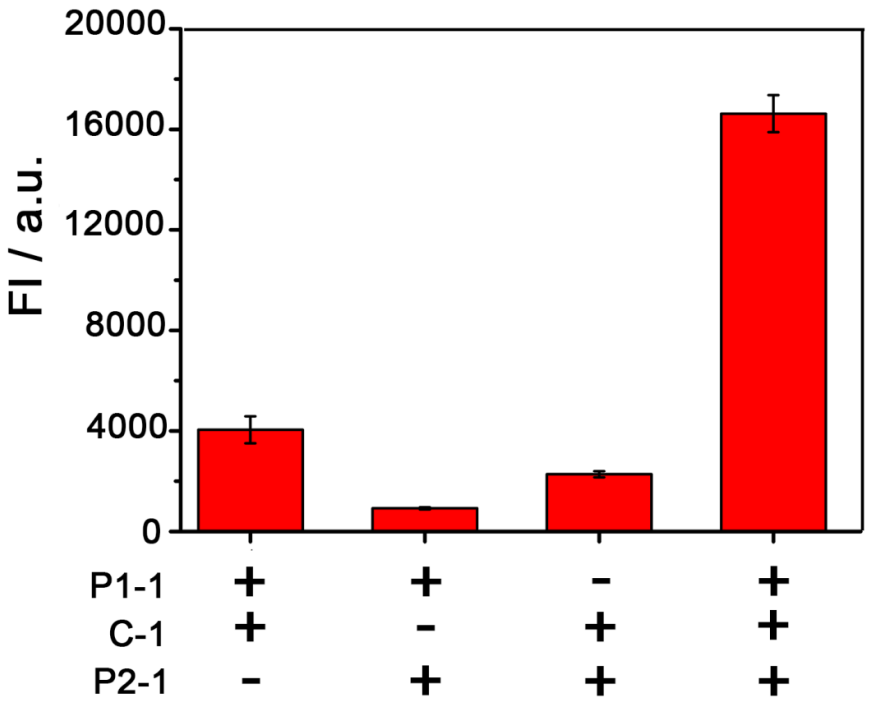

Figure S1. Fluorescent signals of immuno-NRCA in presence of the three oligonucleotides or in absence of any of them.

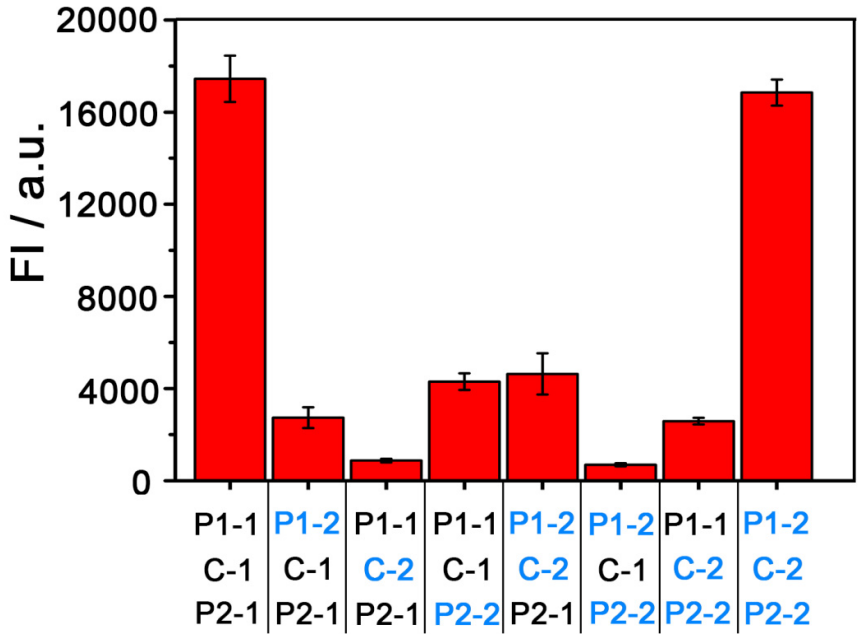

Figure S2. Fluorescent signals of immuno-NRCA in presence of different oligonucleotides sets, i.e. P1-1, C-1, P2-1 (black fonts), and P1-2, C-2, P2-2 (blue fonts), and the mashup of them.

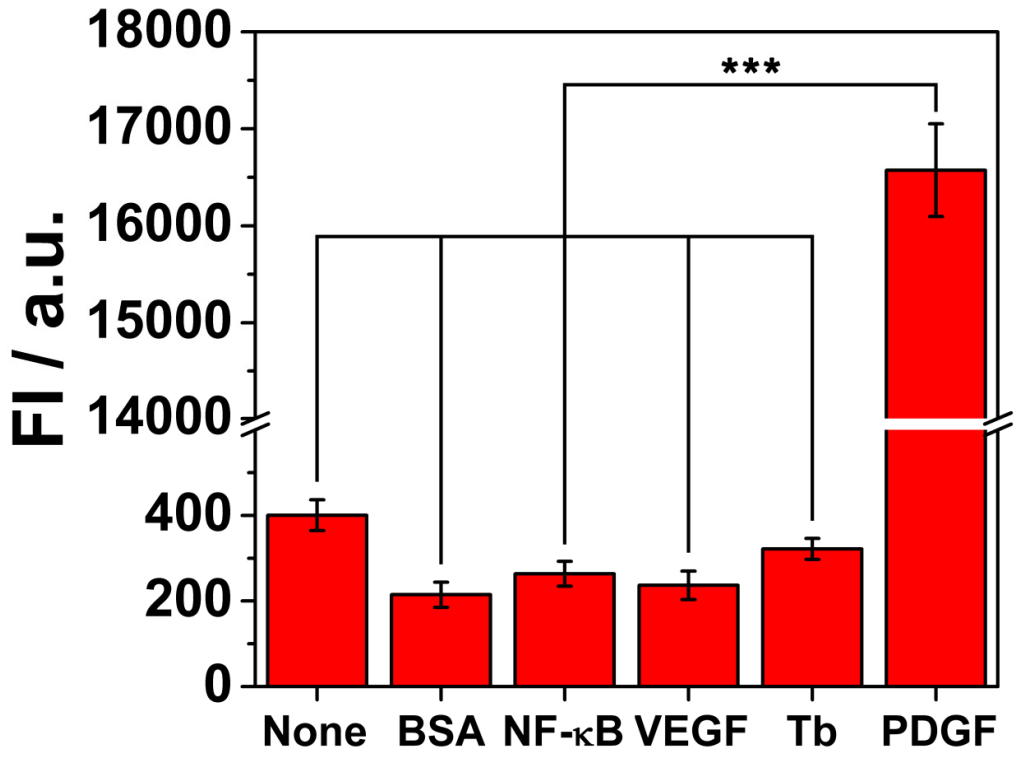

Figure S3. Detection specificity of the immuno-NRCA assay for PDGF. (BSA: bovine serum albumin, NF-κB: nuclear factor kappa-B, VEGF: vascular endothelial growth factor, Tb: thrombin).

Table S2. Detection of PDGF in real serum samples using ELISA and immuno-NRCA, respectively. The values before the slash are the measured values of diluted sera (absorbance for ELISA, and fluorescent intensity for immuno-NRCA). The values after the slash are the calculated values of the concentrations of PDGF using the standard curves (referred to Fig. 4C for ELISA, and Fig. 4B for immuno-NRCA). The relative deviation is calculated using an equation as follows: ($RD = (C_{immuno\text{-}NRCA} - C_{ELISA}) / C_{ELISA}$) ($RD$: relative deviation, $C_{immuno\text{-}NRCA}$: concentration obtained using immuno-NRCA, C_{ELISA}: concentration obtained using ELISA).

Cancer group			
No.	**ELISA (O.D. / ng·L^{-1})**	**immuno-NRCA (a.u. / ng·L^{-1})**	**relative deviation**
1	1.43/342.9	4399/334.2	-2.5%
2	0.41/43.2	3073/64.9	50.2%
3	0.95/222.6	4115/235.3	5.7%
4	1.21/287.1	4320/303.1	5.6%
5	0.98/229.2	4205/263.0	14.7%
6	1.57/376.8	4520/388.1	3.0%
7	0.79/91.2	3545/116.3	27.5%
8	1.39/165.9	4006/205.6	23.9%
9	1.65/397.5	4545/400.3	0.7%
10	0.83/96.3	3588/122.7	27.4%
11	0.51/56.7	3140/70.5	24.4%
12	1.31/312.9	4384/328.1	4.9%
13	1.55/373.5	4491/374.5	0.3%
14	1.56/187.2	4093/229.0	22.3%
15	1.03/243	4283/289.6	19.2%

16	1.10/129.6	3932/187.7	44.8%
17	0.98/114.6	3869/173.6	51.5%
18	0.74/84.9	3549/116.9	37.7%
19	0.49/53.4	2964/56.7	6.2%
20	0.37/39.2	2170/21.3	-45.8%

Therapy group

No.	ELISA (O.D. / ng·L^{-1})	immuno-NRCA (a.u. / ng·L^{-1})	relative deviation
1	0.98/229.2	4067/221.7	-3.3%
2	0.27/26.4	2936/54.8	107.6%
3	1.36/162.6	3847/168.9	3.9%
4	0.80/93	3475/106.7	14.7%
5	0.29/29.2	2040/18.1	-38.0%
6	0.89/206.7	4143/243.6	17.8%
7	1.56/187.2	3761/151.9	-18.9%
8	1.16/274.5	4126/238.5	-13.1%
9	0.74/84.9	3096/66.8	-21.3%
10	1.10/129.6	3519/112.6	-13.1%
11	1.17/277.5	4237/273.6	-1.4%
12	0.79/91.5	3534/114.7	25.4%
13	0.41/43.2	2506/32.2	-25.4%
14	0.94/219.6	3776/154.7	-29.5%
15	0.49/53.7	3174/73.5	36.9%
16	0.27/26.1	2783/45.4	73.8%
17	1.15/135.9	3593/123.4	-9.2%
18	1.60/192.9	3924/185.8	-3.7%
19	0.83/96.3	2866/50.3	-47.8%
20	1.04/245.1	4078/224.8	-8.3%

Healthy group			
No.	**ELISA (O.D. / ng·L^{-1})**	**immuno-NRCA (a.u. / ng·L^{-1})**	**relative deviation**
1	1.17/138.3	3696/140.2	1.4%
2	0.27/26.1	2773/44.8	71.6%
3	0.84/96.9	3468/105.8	9.1%
4	1.08/127.5	3643/131.3	3.0%
5	0.60/67.5	3317/87.8	30.0%
6	0.58/64.8	3023/61.0	-5.8%
7	0.40/42.3	3036/62.0	46.6%
8	0.20/17.7	2579/35.2	99.1%
9	0.85/99.3	3331/89.3	-10.1%
10	1.37/163.2	3853/170.2	4.3%
11	0.42/44.4	2833/48.2	8.7%
12	0.73/84.3	3542/115.9	37.5%
13	0.20/17.4	2640/38.0	118.4%
14	1.64/197.1	3987/200.9	1.9%
15	0.49/54.3	3222/78.0	43.7%
16	0.28/27.9	2882/51.3	83.7%
17	1.12/132.9	3894/179.0	34.7%
18	1.34/159.6	3673/136.3	-14.6%
19	0.79/90.9	3099/67.0	-26.3%
20	0.86/99.6	3096/66.8	-33.0%