Supporting Information

Highly Sensitive and Selective Detection of miRNA: DNase I-Assisted Target Recycling using DNA Probes Protected by Polydopamine Nanospheres

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Experimental Section

Materials and Reagents

Dopamine hydrochloride was purchased from Sangon Biotechnology Co., Ltd (Shanghai, China). DNase I, HPLC-purified RNA, RNase inhibitor, and DEPC-treated water were purchased from Takara Biotechnology Co. Ltd. (Dalian, China). The DNA probes were synthesized on a PolyGen Column 12 DNA synthesizer and the reagents were purchased from Glen Research (Sterling, VA, USA). Cell lysate from breast cancer cell MDA-MB-231 was obtained by the reported procedure adding 5mM $MgCl_2$ and 5mM $CaCl_2$.[1] All DNA/RNA sequences are listed in Tables S1. Polydopamine nanospheres (PDANSs) were synthesized using the reported procedure.[2]

Fluorescence measurements

Fluorescence measurements were carried out on a FluoroMax-4P Fluorescence Spectrophotometer (Horiba, Japan). The excitation and emission wavelengths were set at 490 and 520 nm, respectively, with a 3 nm bandwidth and 0.3s integration time. The emission spectra were obtained by exciting the samples at 490 nm and scanning the emission from 500 to 650 nm in steps of 1 nm/s. The reaction buffer contained 20 mM Tris-HCl (pH 8.0), 6% PEG, 5 mM $MgCl_2$, 5mM $CaCl_2$ and 50 mM NaCl. The amplified detection of miRNA was performed in 200 μL solution with 50 nM probe incubated with 0.09 mg/mL PDANSs for 30 min at 37°C. After the addition of 20 units of DNase I and different concentrations of miRNA, the solution was incubated at 37°C for 90 min and the fluorescence intensities were detected afterwards.

Gel electrophoresis

A 20% denaturing PAGE analysis of the products from the cyclic enzymatic amplification reaction was carried out in 1×TBE (pH 8.3) at 1W power for about 1.5hr. After Stains-All staining, gels were scanned.

Table S1. Sequences of oligonucleotides used in this study

Name	Sequence
Probe	5’- AAC TAT ACA ACC TAC TAC CTC A -FAM-3’
Let-7a	5’ - UGA GGU AGU AGG UUG UAU AGU U -3’
Let-7f	5’-UGA GGU AGU AGA UUG UAU AGU U-3’
Let-7i	5’-UGA GGU AGU AGU UUG UGC UGU U-3’
Mir122	5’- AAC GCC AUU AUC ACA CUA AAU A -3’

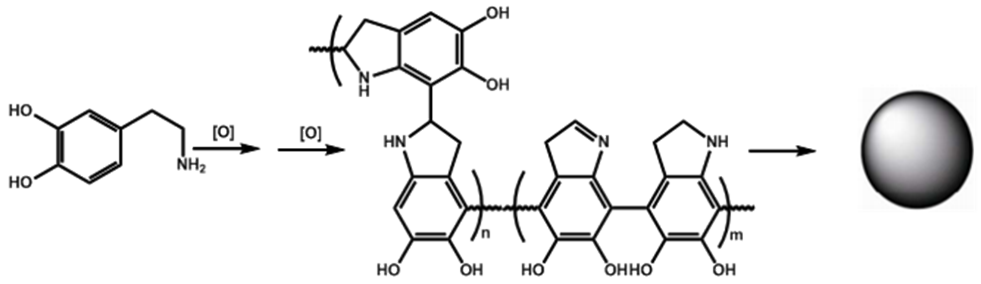

Figure S1. Simplified schematic of the oxidative polymerization of dopamine to prepare polydopamine nanospheres.

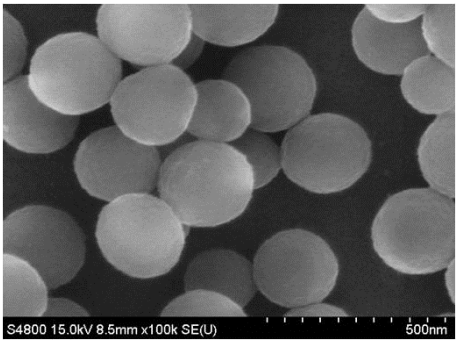

Figure S2. SEM image of PDANSs, the diameter is approximately 250 nm.

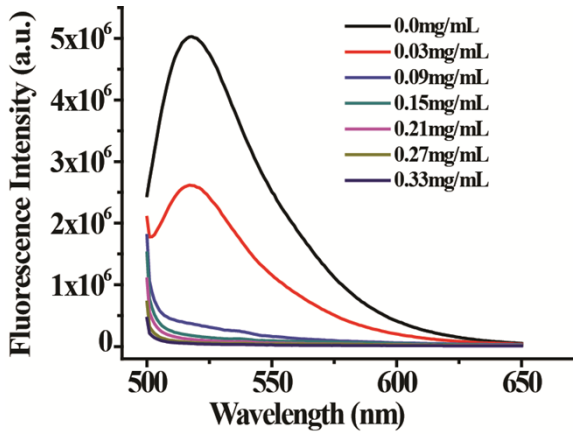

Figure S3. Fluorescence quenching of the DNA probe by different concentrations of PDANS.

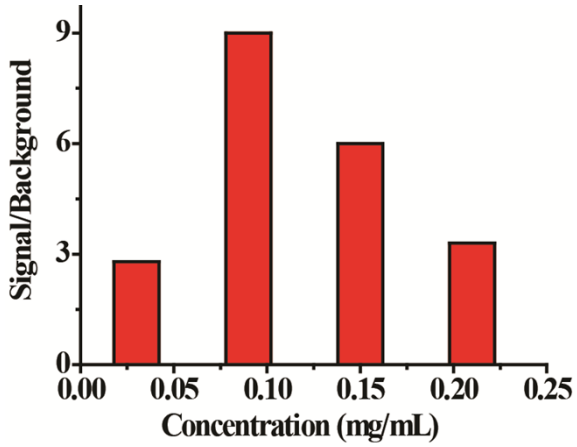

Figure S4. Signal-to-background ratio upon addition of different concentrations of PDANS.

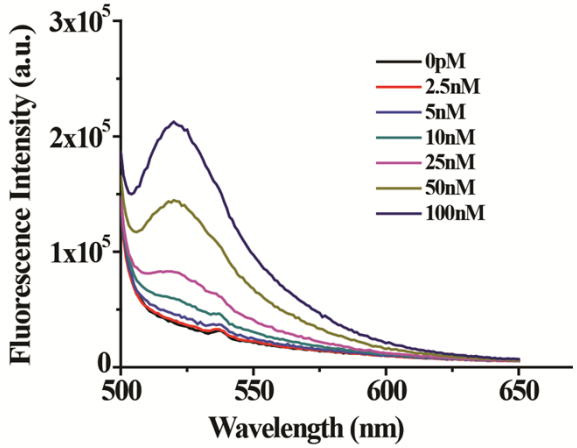

Figure S5. Response of the probe to different concentration of miRNA target in the absence of DNaseI.

Table S2. Comparison of different analysis assays for miRNA

	Sensitivity	Selectivity	Time	Conjugation	Biocompatibility	Ref
Gold Nanoparticle	5-8 pM	N.D.	5 hr	Au-Thiol	good	[3]
Graphene oxide	9 pM	Single-base mismatch	20 min	Absorption	Moderate[4,5]	[6]
PDANS	2.3 pM	Single-base mismatch	1.5 hr	Absorption	Excellent	This work

References:

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