Supporting Information

Substrate Specificity-Enabled Terminal Protection for Direct Quantification of Circulating MicroRNA in Patient Serums

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1. Diameter distribution of 30T-P-templated CuNPs.

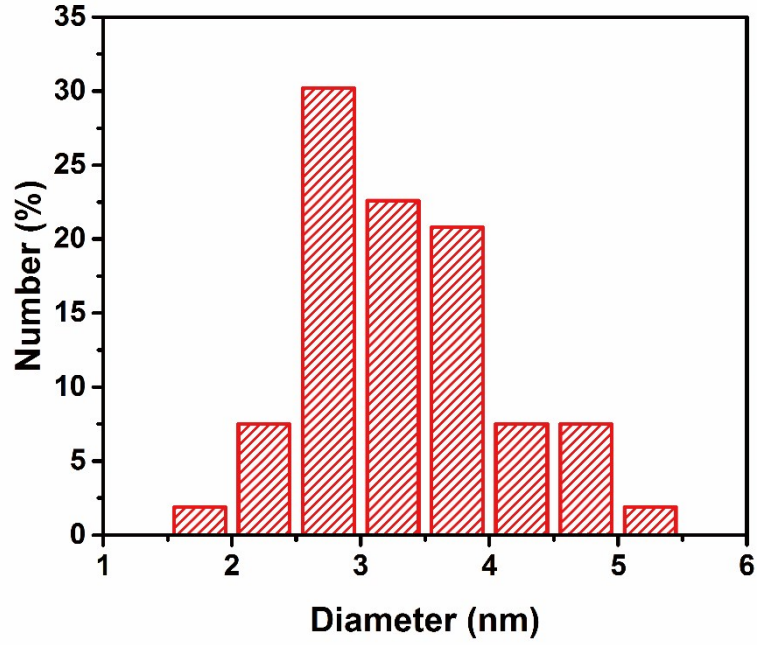

Fig. S1. Diameter distribution of 30T-P-templated CuNPs.

2. Effect of exo I on fluorescence of 30T-templated CuNPs and 30T-P-templated CuNPs.

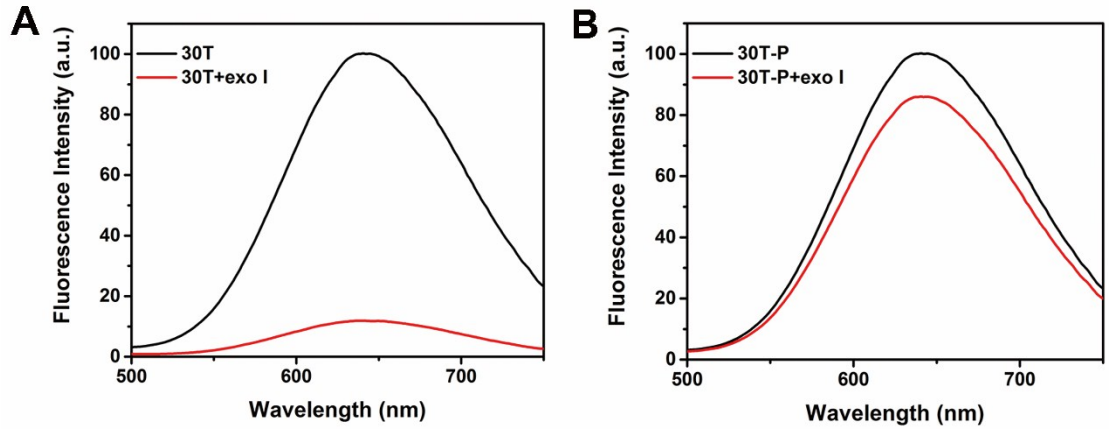

Fig. S2. (A) Fluorescence spectra of 30T-templated CuNPs with and without 10 U exo I. (B) Fluorescence spectra of 30T-P-templated CuNPs with and without 10 U exo I.

3. Optimization of exo I in P-induced terminal protection.

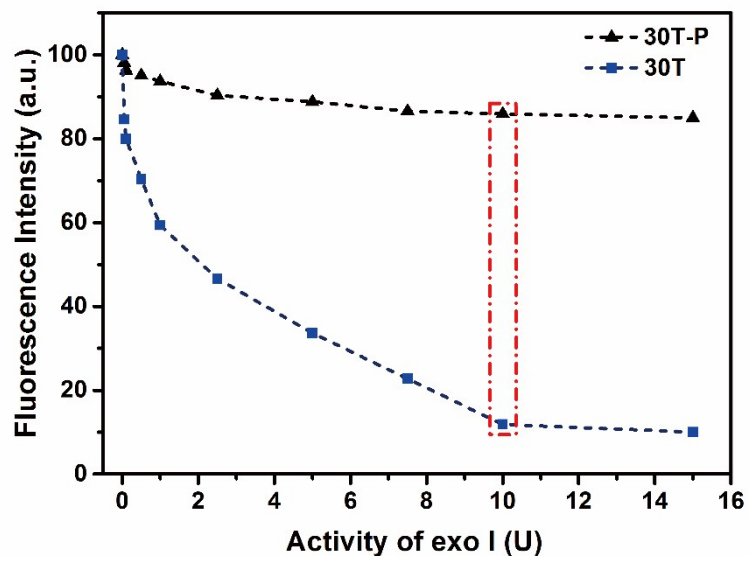

Fig. S3. Fluorescence intensity of 30T-templated CuNPs and 30T-P-templated CuNPs as a function of exo I.

4. Results of CD measurements for the ALP assay.

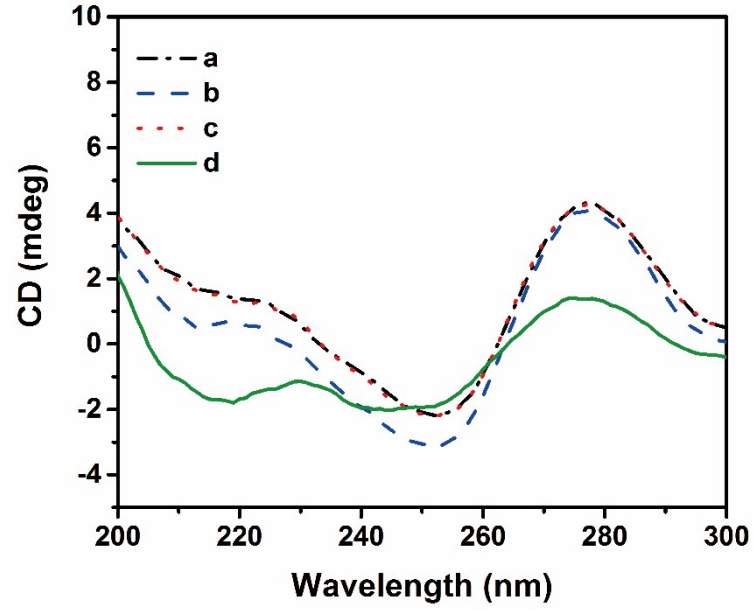

Fig. S4. CD spectra of (a) 30T-P, (b) 30T-P and ALP, (c) 30T-P and exo I, and (d) 30T-P, ALP, and exo I.

5. Comparison of different ALP biosensors (Table S1).

Table S1. Comparison of analytical performances of different ALP biosensors.

Detection method	Signal probe	Detection limit	Linear range	Ref.
Colorimetry	Upconversion nanoparticles	0.065 U/L	0.08 - 70 U/L	S1
Colorimetry	Gold nanoparticle	0.032 U/L	0.032 - 100 U/L	S2
Colorimetry	Gold/silver core/shell nanorod	3.3 U/L	5 - 100 U/L	S3
Photoelectrochemical	CdTe quantum dots	0.15 U/L	0.2 - 15 U/L	S4
Chemiluminescence	MSN@RhB@b-CD@ AMPPD	0.0748 U/L	0 - 400 U/L	S5
Electrochemistry	Nanoceria particles	20 U/L	5000 - 64000 U/L	S6
Electrochemistry	Molecular beacon	0.1 U/L	0.1 - 10 U/L	S7
Electrochemiluminescence	CdSe nanoparticles	2 U/L	2 - 25 U/L	S8
Fluorescence	Silicon nanoparticle	0.2 U/L	0.2 - 30 U/L	S9
Fluorescence	Gold nanoclusters	0.05 U/L	1 - 200 U/L	S10
Fluorescence	Upconversion nanoparticles	0.032 U/L	0.08 - 70 U/L	S1
Fluorescence	MoS_2 quantum dots	0.1 U/L	0 - 5 U/L	S11
Fluorescence	MnO_2 nanosheets	0.045 U/L	0.13 - 10 U/L	S12
Fluorescence	Copper (II)-based metal-organic frameworks	0.19 U/L	1 -34 U/L	S13
Fluorescence	Copper nanoparticles	0.03 U/L	0.067 - 665.64 U/L	This work

6. Results of PAGE analysis for the hsa-miR-21-5p biosensor.

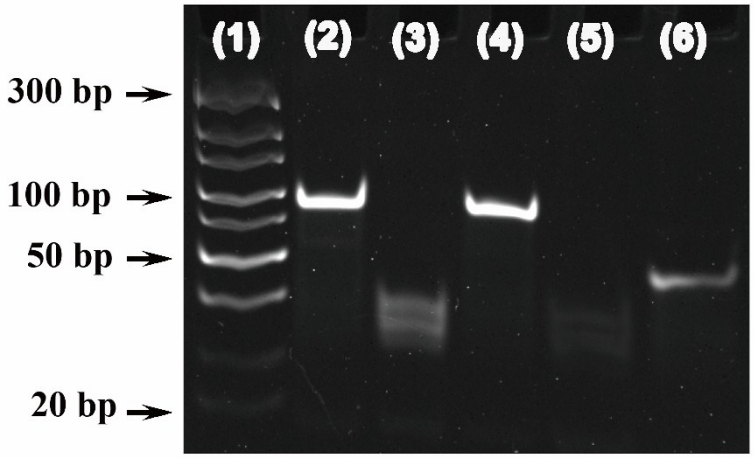

Fig. S5. PAGE image of different samples stained by CuNPs. Lane (1): DNA ladder; lane (2): 30T-antiRNA-P and hsa-miR-21-5p; lane (3): 30T-antiRNA-P, hsa-miR-21-5p, and DSN; lane (4): 30T-antiRNA-P, hsa-miR-21-5p, and exo I; lane (5): 30T-antiRNA-P, hsa-miR-21-5p, ALP, and exo I; lane (6): 30T-antiRNA-P, ALP, and exo I.

7. Comparison of different hsa-miR-21-5p biosensors.

Table S2. Comparison of analytical performances of different hsa-miR-21-5p biosensors.

Detection method	Signal probe	Detection limit	Linear range	Real sample detection	Ref.
Colorimetry	Graphene/gold-nanoparticle hybrids	3.2 nM	10 nM - 980 nM	Human serum	S14
Electrochemistry	Silver nanoparticle	400 aM	1 fM - 1 nM	Human serum	S15
Electrochemistry	Methylene blue modified DNA fuel strand	1.4 fM	5 fM - 500 pM	MCF-7 cells and Hela cells	S16
Electrochemistry	Gold nanoparticle-decorated MoS_2 nanosheet	450 aM	10 fM - 1 nM	Human serum	S17
Electrochemistry	Gold nanoparticles	100 pM	200 pM - 388 nM	Not applicable	S18
Electrochemiluminescence	Au nanoparticles functionalized g-C_3N_4 NS nanohybrid	500 aM	1 fM - 1 nM	HeLa cells	S19
Electrochemiluminescence	Gold nanoparticals functionalized reduced graphene oxide	30 aM	100 aM - 10 pM	A549 cells	S20
Impedance	Biotin-molecular beacon-gold nanoparticles	300 fM	1 pM - 1 nM	Human serum	S21
Fluorescence	Copper nanoparticles	100 fM	1 pM - 1 nM	MCF-7 cells, and A549 cells	S22
Fluorescence	Graphene oxide	47 pM	47 pM - 16 nM	Hep-2 cells, A549 cells, and HUVEC	S23
Fluorescence	AMCA dye	270 aM	1 fM - 50 pM	Human serum	S24
Fluorescence	Copper nanoparticles	16 aM	25 aM - 250 nM	Human serum	This work

8. Effect of RNA length on fluorescence response of CuNPs.

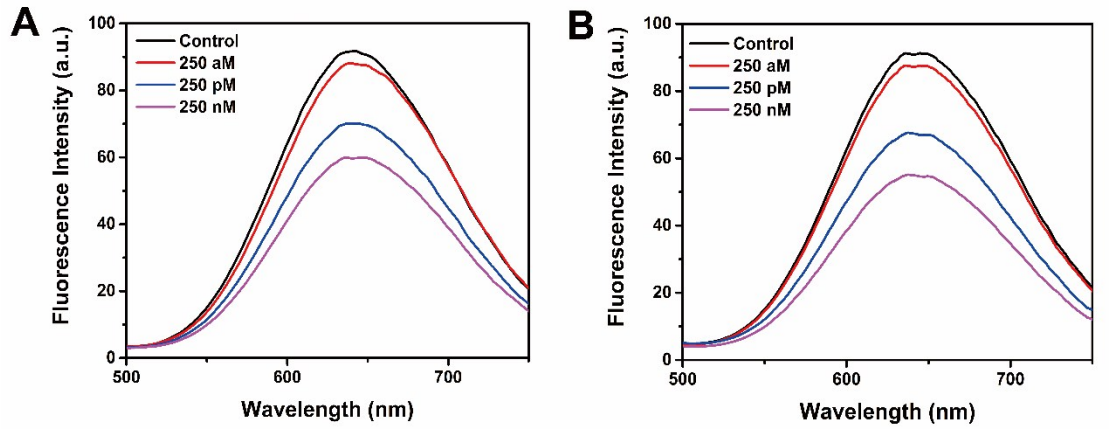

Fig. S6. Fluorescence spectra of (A) 30T-anti10R-P-templated CuNPs and (B) 30T-anti10R-P-templated CuNPs. 30T-anti10R-P is treated with the different concentrations of 10-R in the presence of DSN and exo I, while 30T-anti40R-P is treated with the different concentrations of 40-R.

9. Selectivity of the proposed method in the presence of isoforms.

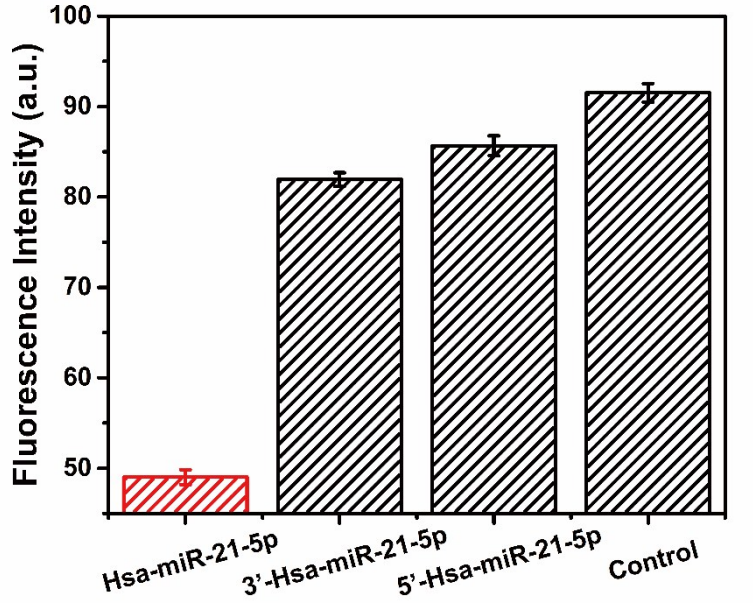

Fig. S7. F_{650nm} of 30T-anitiRNA-P-templated CuNPs. 30T-anitiRNA-P is treated with hsa-miR-21-5p, 3'-hsa-miR-21-5p and 5'-hsa-miR-21-5p in the presence of DSN and exo I. The concentration of a single miRNA in this assay is the same (250 nM).

10. References

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