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Supporting Information

Toehold-Mediated Strand Displacement Coupled with Single

Nanoparticle Dark-Field Microscopy Imaging for Ultrasensitive

Biosensing

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Reference

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Name	Sequences (5'-3')
Substrate strand	TCAACATCAGTCTGATAAGCTATAGGGCCGTAAGTTAGTGAGATT
(Sub)	$TTTTTTTT-NH_2$
P1	TCTCACTAACTTACGG
P2	CCCTATAGCTTATCAGACT
Assist strand	TAACTTACGGCCCTATAGCTTATCAGACTTTTTTTTTTT
miRNA-21	
(Target)	UAGCUUAUCAGACUGAUGUUGA
Single base	
mismatched	UAGCUUAUCAGACUGAUGU <u>A</u> GA
miRNA	
Two bases	
mismatched	UA <u>A</u> GUUAUCAGACUGAUGU <u>A</u> GA
miRNA	
miRNA-144	UAACACUGUCUGGUAAAGAUGG
miRNA-122a	UGGAGUGUGACAAUGGUGUUUG
miRNA-32	UAUUGCACAUUACUAAGUUGCA

 Table S1 DNA and RNA sequences in this work.



Figure S1 (A) The UV-Vis spectrum changes during the synthetic process. (B) TEM image of gold seeds before Au@AgNCs prepared. (C) Large scale of SEM image and (D) High resolution transmission electron microscopy image of as prepared Au@Ag NCs.



Figure S2 The SEM (left) and EDS (right) characterization of Au@AgNCs with reaction time at (A) 0 min, (B) 60 min, and (C) 120 min.



Figure S3 The effect of (A) assist concentration, (B) TSMD reaction time, (C) glucose concentration and (D) pH value of buffer solution on the Au@AgNCs scattering intensity changes, target concentration was 100 fM.

Detection	Signal Amplification	Linear Range	Detection Limit	Ref.
Method	Strategy		(fM)	
Fluorescence	nuclease assisted target recycling	0.1~100 nM	100 fM	S 1
Fluorescence	nuclease assisted target recycling	0.5~500 pM	0.4 pM	S2
Fluorescence	enzyme-free amplification	$1.0 \ pM \sim 1.0 \ nM$	1.0 pM	S3
Electrochemical	Hierarchical Flower-Like Gold Nanostructures	1.0 fM~100.0 pM	1.0 fM	S4
Electrochemical	Oligonucleotide encapsulated Ag nanoclusters	100.0 fM ~ 10.0 nM	67 fM	S5
surface-enhanced Raman scattering	catalytic hairpin assembly	10.0 fM~ 100.0 nM	3.5 fM	S6
surface-enhanced Raman scattering	nuclease assisted target recycling	0.33 fM ~ 3.3 pM	42.0 aM	S 7
Point-of-care testing	DNA-inorganic hybrid nanoflowers	$0.5 \sim 100 \ nM$	0.41 nM	S 8
Colorimetric	DNAzyme-coupled branched hybridization chain reaction	$1.0 \text{ pM} \sim 1.0 \text{ nM}$	1.0 pM	S9
Colorimetric	DNA walker, AuNPs	0.05–10.0 pM	16.7 fM	S10
DFM	Enzyme assisted TMSD	1.0 ~ 100.0 fM	1.0 fM	This worł

Table S2 The comparison of analytic performance of diverse methods for miRNA-21 detection.

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