

Supporting Information

CRISPR/Cas13a-assisted amplification-free miRNA biosensor via dark-field imaging and magnetic gold nanoparticles

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SUPPLEMENTARY FIGURES

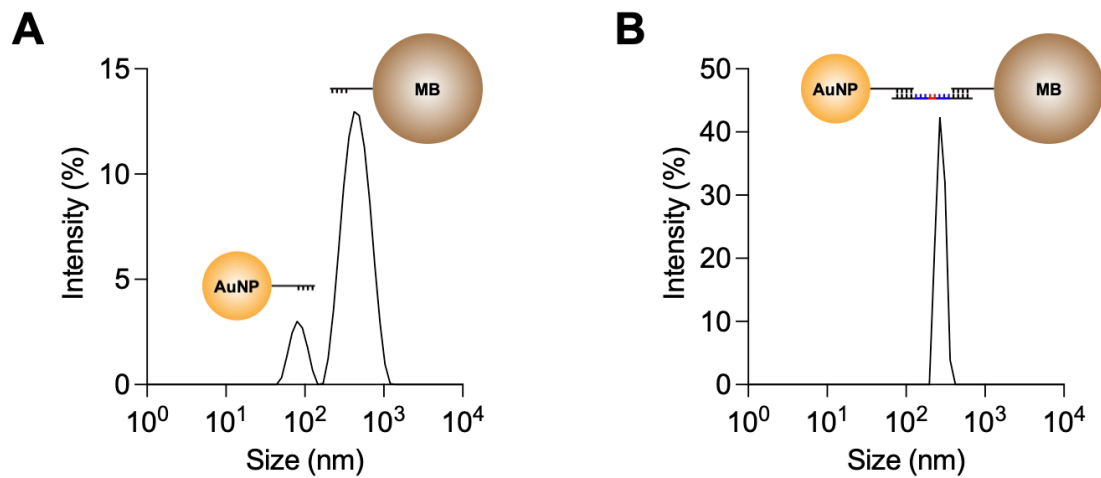


Figure S1. Dynamic Light Scattering (DLS) measurement results for mixture of magnetic beads (MBs) and gold nanoparticles (AuNPs) without (A) or with (B) a linker.

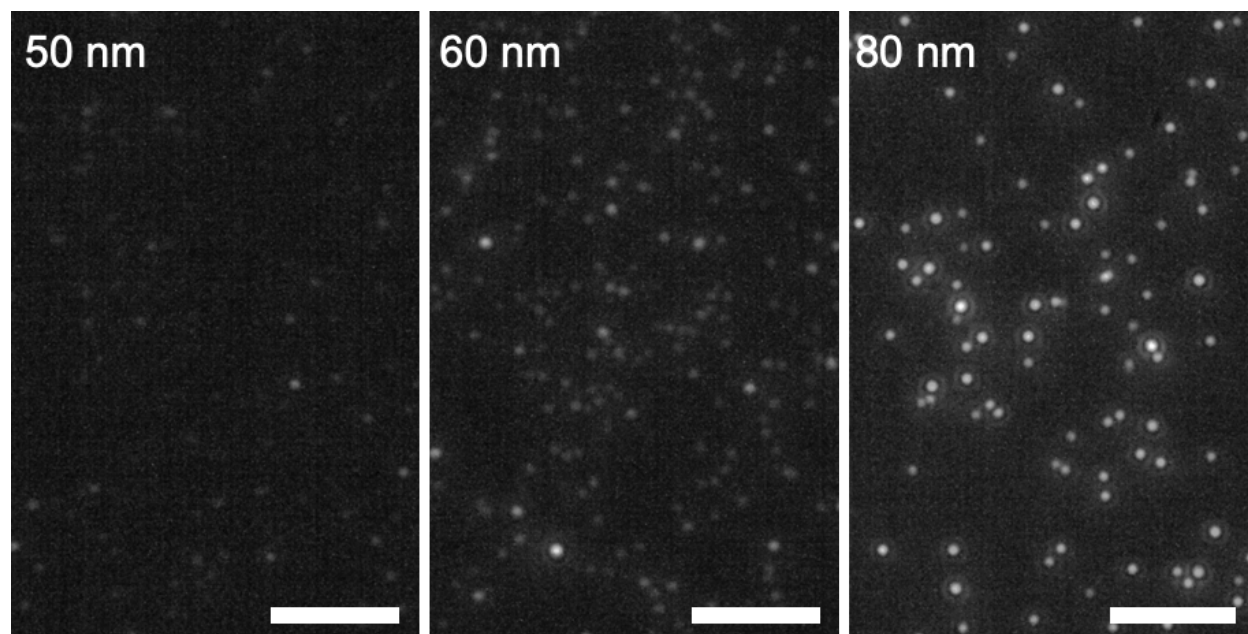


Figure S2. Dark-field (DF) images of gold nanoparticles (AuNPs) of different sizes (50 nm, 60 nm, and 80 nm) captured using a portable DF imaging system. Scale bar, 100 μm .

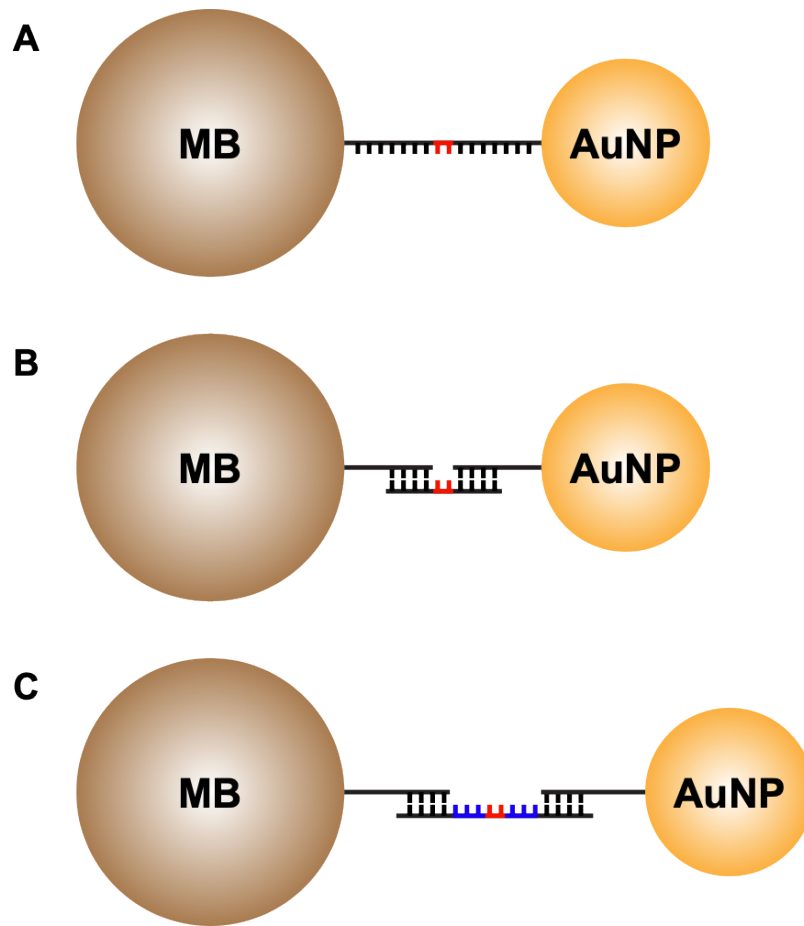


Figure S3. Hybridization strategies for linking magnetic beads (MBs) and gold nanoparticles (AuNPs) using nucleic acid linkers. **A.** Direct linking of MBs and AuNPs with a single-stranded DNA/RNA/DNA linker. **B-C.** Sandwich hybridization with bridges without (**B**) or with (**C**) poly-T. DNA and RNA sequences are denoted in black and red, respectively. Poly-T sequences are highlighted in blue.

A

Concentration	Mock	0.1 fmole 5 pM	0.2 fmole 10 pM	0.5 fmole 25 pM
Experiment 1	362.3	457.3	446.3	521.0
Experiment 2	387.0	434.3	408.0	573.3
Experiment 3	437.7	557.3	484.3	589.0
Concentration	1 fmole 50 pM	2 fmole 100 pM	3 fmole 150 pM	5 fmole 250 pM
Experiment 1	1073.7	3027.7	4625.3	9330.3
Experiment 2	1128.3	2264.0	4203.7	9011.7
Experiment 3	1674.0	2835.3	4156.0	8424.7

$$\begin{aligned}
 \text{LOD threshold} &= \text{mean} + 3 \times \text{S.D.} \\
 &= 395.7 + 3 \times 38.4 \\
 &= \mathbf{510.9}
 \end{aligned}$$

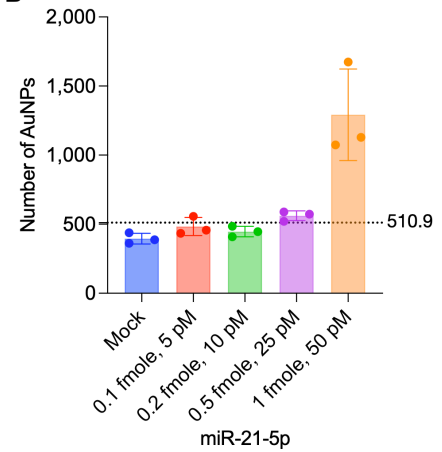
B

Figure S4. Calculation of the limit of the detection. A. Signal values for different concentrations of miR-21-5p and negative control (mock) to determine the limit of detection (LOD) threshold by the mean + 3 times the standard deviation. Based on the threshold value (510.9), we determined the LOD as 0.5 fmole (25 pM). **B.** Bar graph showing the signal values from different miR-21-5p concentrations and the LOD threshold value.

Supplemental Table S1. Sequences of oligomers used in this study.

Name	Sequence (5' to 3') ^{abc}
miR-21-5p-F	TAGCTTATCAGACTGATGTTGA
U6 snRNA-F	CAAATTCGTGAAGCGTTC CA
universal-R	AGGCAGTGGTATCAACGCAGA
miR-RT-adaptor	AGGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTV
T7-universal upper	TAATACGACTCACTATAG
T7-bottom miR-21-5p	TAGCTTATCAGACTGATGTTGAGTTTTAGTCCCCTTCATTTTTGGGGTGGTCTAAATCTATAGTGAGTCGTATTA
miR-21-5p (WT)	phospho-uagcuuau <u>cagacug</u> auguuga
miR-21-5p (SM)	phospho-uagcuuau <u>cggacug</u> auguuga
miR-21-5p (DM)	phospho- <u>u</u> gcuuau <u>cggacug</u> auguuga
miR-9-5p	phospho-ucuu <u>uggu</u> uau <u>cuagc</u> uauuga
miR-421	phospho-aucaacagacauu <u>aaugggcgc</u>
DRD probe	FAM-TA <u>uGC</u> -Quencher
Linker only	biotin-GATGGGCAGACAGGGTTAAGTTTTTTTT <u>uu</u> TAGGACTCGTGCAGCTAAGG-thiol
MB linker	biotin-TTTTTTTTTT <u>GATGGGCAGACAGGGTTAAG</u>
AuNP linker	TAGGACTCGTGCAGCTAAGTTTTTTTTTTT-thiol
Bridge without poly-T	CCTTAGCTGCACGAGTCTAT <u>uu</u> CCTTAACCTGTCTGCCCATC
Bridge with poly-T	CCTTAGCTGCACGAGTCTATTTTTTTTT <u>uu</u> TTTTTTTTTCTTAACCTGTCTGCCCATC
Capture probe	CCTTAGCTGCACGAGTCTATTTTTTTTTTTTTTTTT-biotin

^a V = A, C, or G; N = A, C, G, or T

^b Lower case letters denote RNA.

^c Underline letters indicate mismatch.