Electronic Supplementary Material for

Electrochemical Detection of Zeptomolar miRNA using an RNA-Triggered Cu^{2+} Reduction Method

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Table S1. Summary of cancer-related aberrant expressions of miRNAs.

Cancer	miRNA		Reference
	Up-regulated	Down-regulated	
Brain cancer	miR-21 miR-221	miR-181	[1, 2]
Breast cancer	miR-221 miR-222 miR-328 miR-373 miR-520c	miR-21 miR-125b miR-145 miR-155	[3]
Lung cancer	miR-17-92	Let-7	[4, 5]
Gastric cancer	miR-21 miR-27a	miR-101 miR-143 miR-145	[6-8]
Colorectal cancer	miR-21	miR-127 miR-143 miR-342	[9-11]
Prostate cancer	miR-221 miR-222	miR-127 miR-449a	[12, 13]

Table S2. Oligonucleotide sequences employed in this study.

Oligonucleotide name	Sequence (5'→3')
DP for miR-141	CCA TCT TTA CCA GAC AGT GTT A [PO_4][(a)]
miR-141	UAA CAC UGU CUG GUA AAG AUG G
miR-200a	UAA CAC UGU CUG GUA AC[(b)]G AUG U
miR-200b	UAA UAC UGC CUG GUA AUG AUG A
miR-200c	UAA UAC UGC CGG GUA AUG AUG GA
miR-21	UAG CUU AUC AGA CUG AUG UUG A
DP for let-7a	AAC TAT ACA ACC TAC TAC CTC A [PO_4][(a)]
let-7a	UGA GGU AGU AGG UUG UAU AGU U
let-7b	UGA GGU AGU AGG UUG UG[(c)]U GGU U
let-7c	UGA GGU AGU AGG UUG UAU GGU U
let-7d	AGA GGU AGU AGG UUG CAU AGU U
let-7e	UGA GGU AGG AGG UUG UAU AGU U

[(a)][PO_4] indicates the modification of phosphate group at 3'-end in DP.
[(b)]The underlined sequence indicates the mismatched base within non-specific miRNA-141.
[(c)]The red-colored sequence indicates the mismatched base within non-specific let-7a.

Table S3. Comparison of this work with previous electrochemical miRNA detection strategies.

Material/Method	LOD	Practical applicability (sample type)	Limitations	Reference
Gold nanoparticles modified with biotin-labeled DNA probe	43.3 aM	Cell extract	Immobilization of capture probe on the electrode Preparation of Au NPs modified with biotin-labeled DNA probe	[14]
A nanozyme-based homogeneous electrochemical biosensor	0.25 nM	Cell extract	Low sensitivity	[15]
DNA Origami Nanostructures	79.8 fM	Human serum	Immobilization of DNA probe	[16]
DNA tetrahedron structures	0.1 fM	Human serum	Immobilization of capture probe on the electrode	[17]
A mismatched catalytic hairpin assembly (CHA) amplification	1.1 fM	Cell extract	Immobilization of hairpin probe on the electrode	[18]
Reduced graphene oxide/gold (rGO/Au) composite modified with probe DNA	1.73 pM	-	Fabrication of rGO/Au composite Immobilization of DNA probe	[19]
Popcorn-like gold nanofilms and toehold-mediated strand displacement	2.2 fM	Cell extract	Fabrication of nanofilm platform Immobilization of DNA probe	[20]
miRNA-triggered Cu^{2+} reduction	33.2 zM	Extracted total RNA	-	Present study

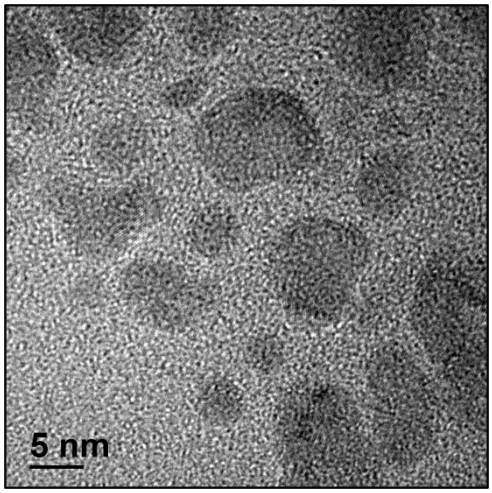

Fig. S1. TEM image of poly T-templated Cu NPs.

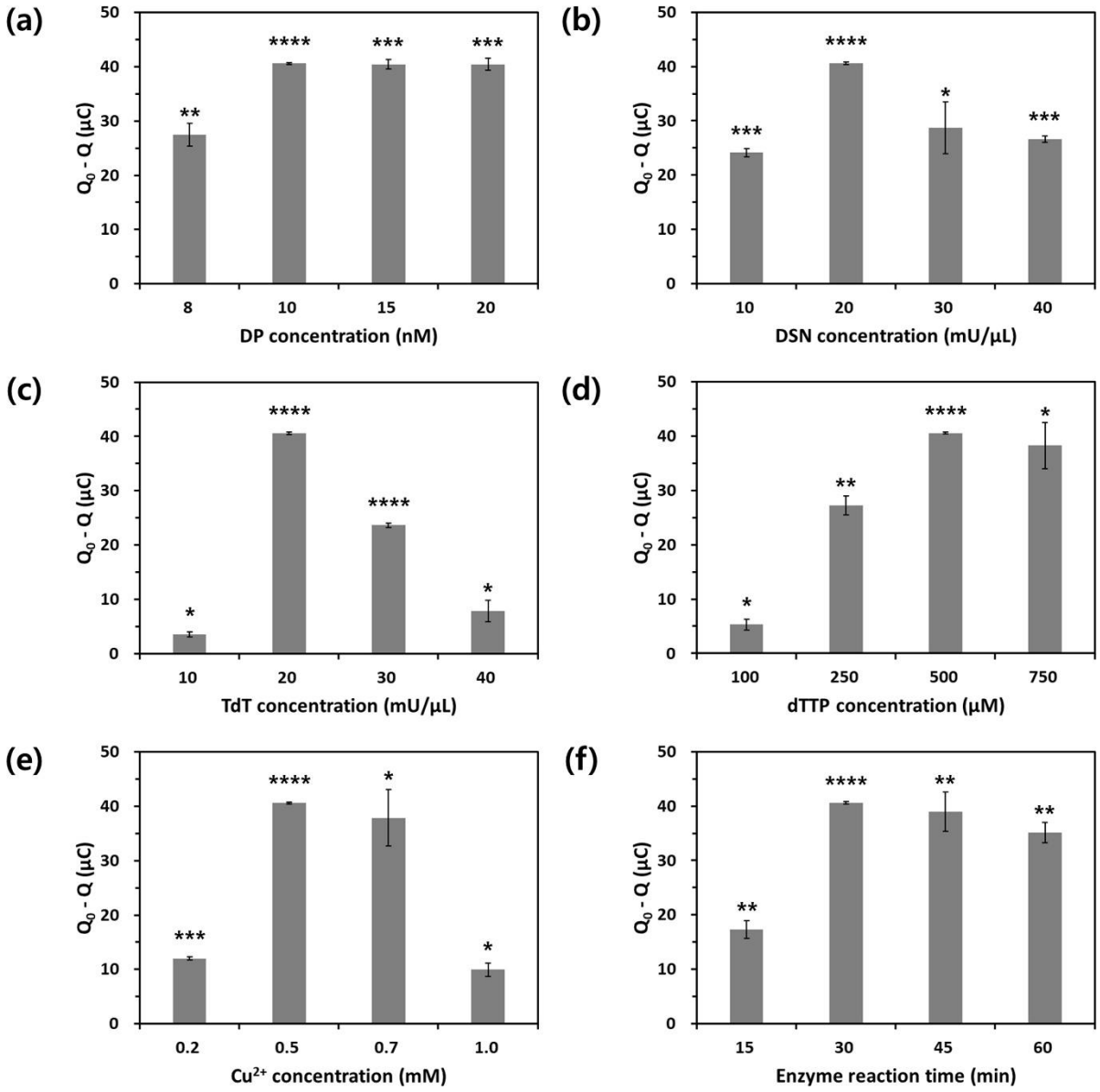

Fig. S2. Plots of $Q_0 - Q$ as functions of the concentrations of (a) DP, (b) DSN, (c) TdT, (d) dTTP, and (e) Cu^{2+} and (f) the enzyme reaction time. Q_0 and Q are charge intensities obtained at 1.5 s after the initiation of chronocoulometric signal measurement in the absence and presence of miRNA, respectively (n = 3, error bar = standard deviation). The asterisks indicate the RSD values (* ≥ 10%, 10% > ** ≥ 5%, 5% > *** ≥ 2%, and **** < 2%).

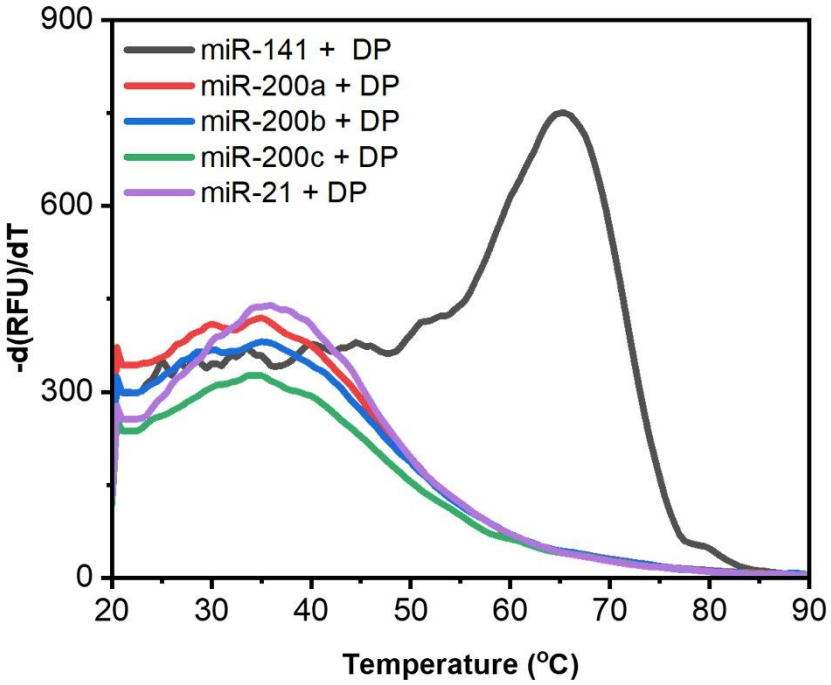

Fig. S3. Plots of −d(RFU)/dT as a function of temperature acquired for melting temperature (T_m) analysis.

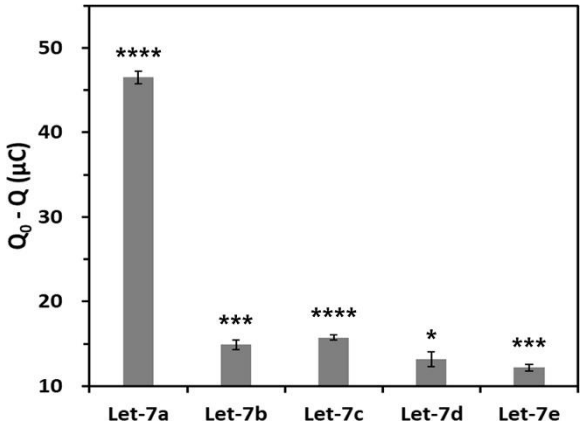

Fig. S4. Plot of $Q_0 - Q$ as a function of the miRNAs (Let-7a, Let-7b, Let-7c, Let-7d, and Let-7e) (n = 3, error bar = standard deviation). The asterisks indicate the RSD values (* ≥ 5%, 5% > ** ≥ 4%, 4% > *** ≥ 3%, and **** < 3%). The concentrations of miRNA, DP, DSN, and TdT were 10 nM, 10 nM, 20 mU/μL, and 20 mU/μL, respectively.

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