Electronic Supplementary Information

Ultrasensitive SERS sensor for simultaneous detection of multiple cancer-related miRNAs

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Table S1. A summary of the methods for detecting single type of nucleic acid biomarkers in buffer

Detection method	Target	Detection range	LOD	Ref
chronoamperometry	miRNA-21	0.1-70 pM	0.06 pM	[1]
chemiluminescence	let-7b	20 fM-5 pM	10 fM	[2]
field-effect transistor	miRNA	1 fM-100 pM	10 fM	[3]
colorimetric	Let-7a	10 fM-10 nM	7.4 fM	[4]
fluorescence	miRNA-205	3.8 pM-10 nM	3.8 pM	[5]
	let-7a	5 fM-1 pM	5 fM	[6]
	miRNA-141	100 fM-10 nM	100 fM	[7]
	let-7a	0.1 fM-0.1 μM	0.1 aM	[8]
	let-7a	0.5 pM-25 nM	0.4 pM	[9]
	miRNA-21	1 fM-1 nM	1 fM	[10]
SPR	miRNA-21	1 fM-1 nM	0.1 fM	[11]
SERS	DNA	100 fM-1μM	10 pM	[12]
	RSAD2 DNA	1 nM-100 nM	1 nM	[13]
	DNA	1 fM-100 pM	1 fM	[14]
	DNA	50-500 pM	50 pM	[15]
	let-7b	1 pM-10 nM	0.3 fM	[16]
	DNA	10 fM-100 pM	3 pM	[17]
	miRNA-21	100 aM-100 pM	15 aM	**This work**

Table S2. A summary of the methods for detecting multiple nucleic acid biomarkers in buffer

Detection method	Target	Detection range	LOD	Ref
electrochemical	miRNA-21	0.005-50 pM	3 fM	[18]
	miRNA-141		4.2 fM	
	miRNA-155	0.5-30 pM	12 fM	[19]
	miRNA-27b	0.5-1050 pM	31 fM	
electrochemiluminescence	miRNA -21	0.02-150 pM	6.3 fM	[20]
	miRNA-141	0.03-150 pM	8.6 fM	
fluorescence	miRNA-141	2-700 fM	300 aM	[21]
	miRNA-210		740 aM	
	miRNA-221		620 aM	
	miRNA-155	0.67-4 nM	23.8 nM	[22]
	miRNA-103		79.0 nM	
	miRNA-155	0.01 nM-200 nM	10 pM	[23]
	miRNA-196a			
	miRNA-210			
SERS	DNA 1	N/A	N/A	[24]
	DNA 2			
	DNA 3			
	N. meningitidis	1 nM-60 nM	45.3 pM	[25]
	S. pneumoniae		99.5 pM	
	H. inuenzae		21.7 pM	
	HAV DNA	100 nM-10 pM	0.39 pM	[26]
	HBV DNA		0.18 pM	
	HIV DNA		0.51 pM	

Table S3. A summary of the methods for detecting multiple miRNAs in human serum

Detection method	Target	Linear range	LOD	Ref
fluorescence	miRNA-155	0.67-4 nM	59.3 nM	[22]
	miRNA-103		75.9 nM	
	miRNA-20a	2-10 nM	N/A	[27]
	miRNA-20b			
	miRNA-21			
SERS	miRNA-21	1 fM-100 pM	393 aM	**This work**
	miRNA-486	1 fM-1 nM	176 aM	
	miRNA-375	1 fM-100 pM	144 aM	

Table S4. Detailed data of SERS sensor array for simultaneous analysis of miRNA-21/486/375 in human serum

Sample	Target	Added (pM)	Found (pM)	RSD (%)	Recovery (%)
Serum 1	miRNA-21	0.80	0.86	8.10	107.5
	miRNA-486	0.80	0.78	4.53	97.5
	miRNA-375	0.80	0.81	4.97	101.3
Serum 2	miRNA-21	1.50	1.46	5.91	97.3
	miRNA-486	3.00	3.23	5.13	107.7
	miRNA-375	1.50	1.62	4.94	108.0
Serum 3	miRNA-21	25.00	25.06	7.55	100.2
	miRNA-486	75.00	75.31	5.42	100.4
	miRNA-375	30.00	30.12	7.36	100.4

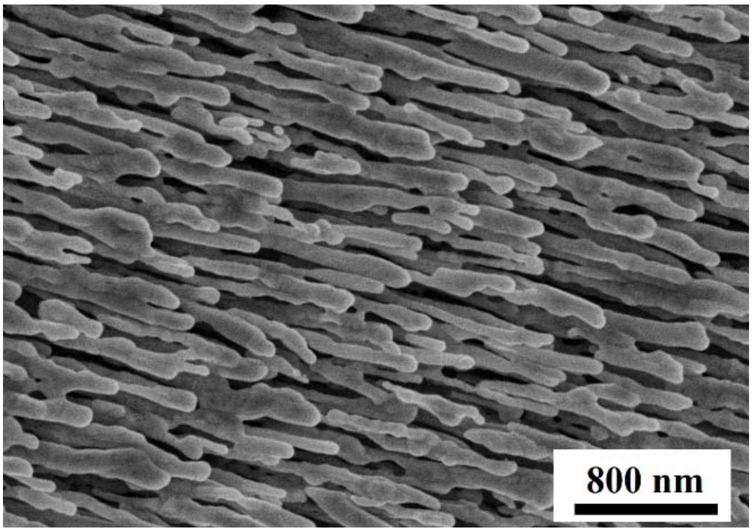

Fig. S1 FESEM image of Ag nanorod array.

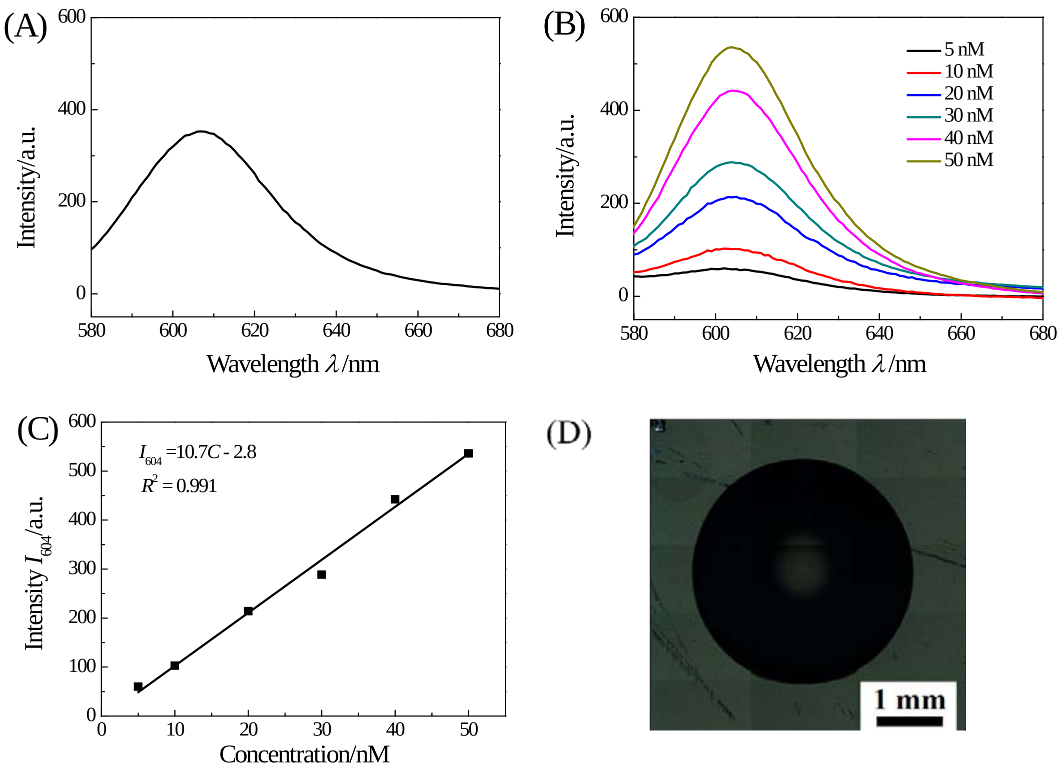

Fig. S2 (A) Fluorescence spectrum of the collected solution including the residual of the original MB solution for immobilization and washing solution. (B) Representative fluorescence spectra of MB-21-ROX at various concentrations ranging from 5 nM to 50 nM. (C) The calibration curve of the fluorescence intensity of MB-21-ROX at λ = 604 nm versus its concentration. (D) Covering area of the molecular beacons on the substrate imaged by a Renishaw InVia confocal Raman microscope under sample view mode.

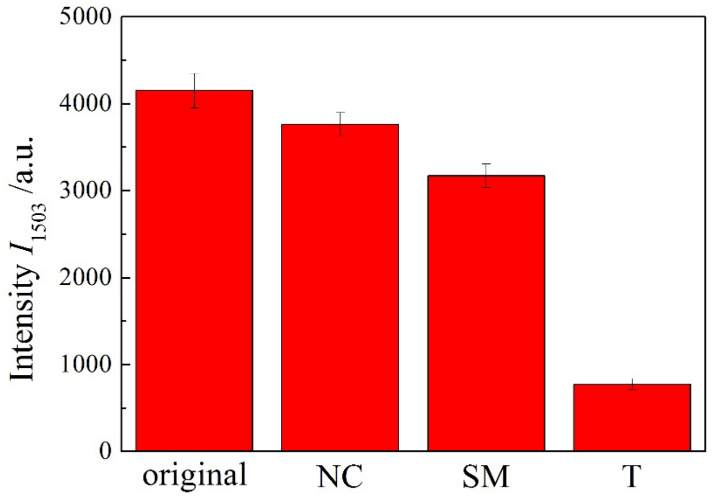

Fig. S3 Raman intensities at $\Delta\nu$ = 1503 cm^{-1} of SERS spectra obtained from SERS sensors before (original) and after incubation with complementary (T), noncomplementary (NC), and single-base mismatch miRNA (SM), corresponding to the SERS spectra shown in Figure 1D.

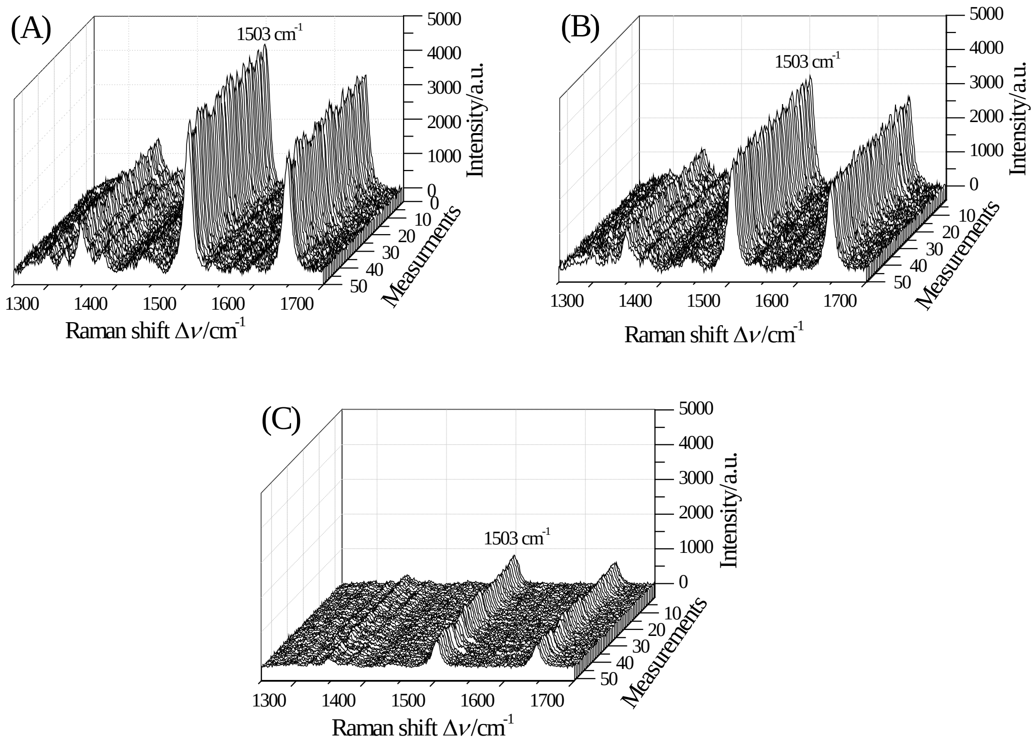

Fig. S4 SERS signals collected from 50 random points on the sensor chip before (A) and after hybridization with 100 pM single-base mismatch miRNA (B) and 100 pM complementary miRNA-21 (C).

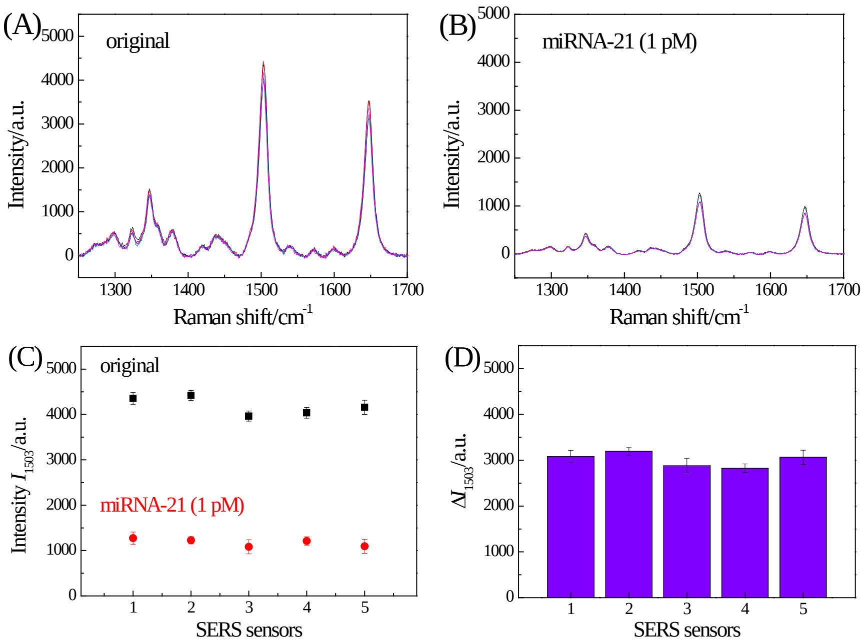

Fig. S5 Reproducibility test of the SERS sensor. (A)-(B) SERS spectra of the five sensors before (original) and after hybridization with 1 pM miRNA-21, respectively. (C) Plotting the corresponding Raman intensities at 1503 cm^{-1}. (D) SERS intensity changes ΔI_{1503} of the five sensors after sensing miRNA-21.

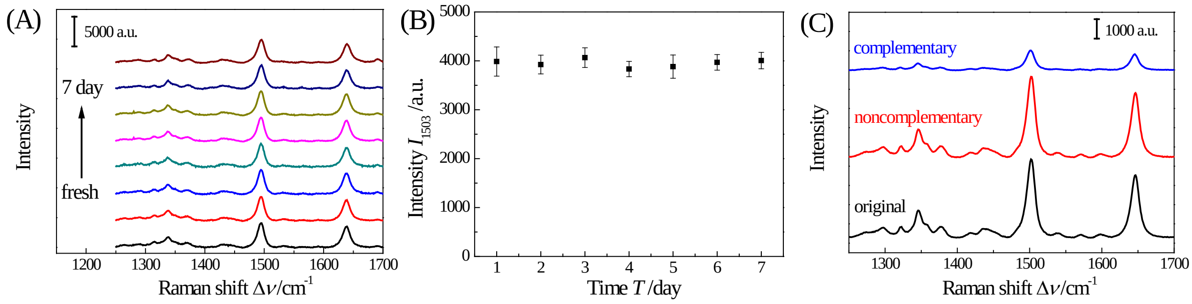

Fig. S6 Stability test of the SERS sensor stored in PBS buffer at room temperature. (A) SERS spectra collected form the fresh sensor and the sensor stored for 1 to 7 days, and (B) The plot of I_{1503} of each spectrum. (C) Performances of the one week stored sensor before (original) and after incubation with 100 pM noncomplementary and complementary miRNAs, respectively.

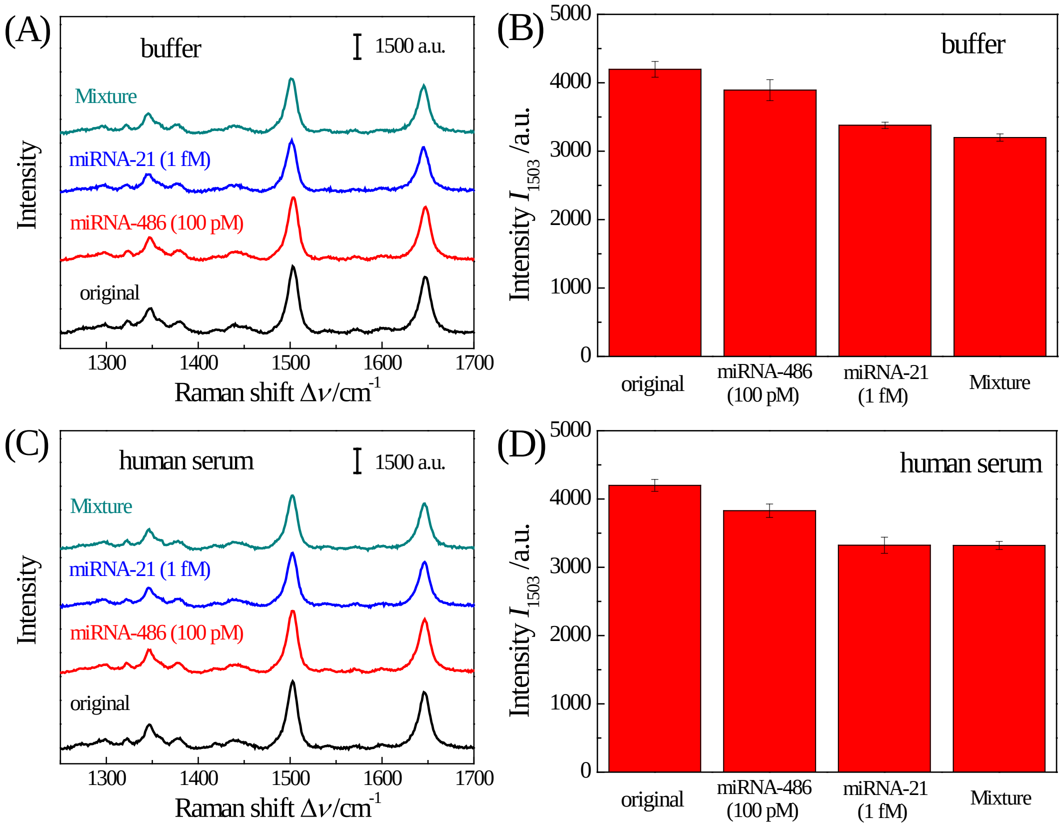

Fig. S7 The availability of the sensor for test miRNA-21 (1 fM) with high concentration of interference miRNA-486 (100 pM). (A) SERS spectra of the sensor before (original) and after incubation with miRNA-21, miRNA-486, and mixture of the two miRNAs (Mixture) in buffer. (B) The plot of I_{1503} of the spectra shown in (A). (C) SERS spectra of the sensor before (original) and after incubation with miRNA-21, miRNA-486, and mixture of the two miRNAs (Mixture) in human serum. (D) The plot of I_{1503} of the spectra shown in (C).

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