Electronic Supplementary Material (ESI) for Analyst.

A Portable SERS Reader Coupled with Catalytic Hairpin Assembly for Sensitive MicroRNA-21 Lateral Flow Sensing

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1. Materials and Apparatus Used in This Study

Trisodium citrate ($C_6H_5Na_3O_7$ $2H_2O$), silver nitrate ($AgNO_3$), Tween-20, L-ascorbic acid (AA) and chloroauric acid ($HAuCl_4$) were purchased from Sinopharm Chemistry Reagent Co., Ltd. (Shanghai, China). Streptavidin (SA), 4-Nitrothiophenol (4-NTP, 90%), 4-mercaptobenzoic acid (4-MBA, 95%) and tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) were provided by Sigma-Aldrich (St. Louis, USA). PBS buffer (0.1 M, pH=7.4) containing 137 mM NaCl was utilized as reaction solution and lateral flow sensing running buffer. All the chemicals were used without further purification and modification with analytical grade. When dealing with RNA, DEPC-treated water was employed. NC membrane (Sartorius CN140), sample pad (GL-b02), absorption pad (H5072), adhesive backing (DB-6) and conjugate pad (Ahlstrom 8964) were all obtained from Shanghai Jieyi Biotechnology Co. Ltd. (Shanghai, China). Human serum sample that was obtained from the Huazhong Agricultural University Hospital (Wuhan, China).

The oligonucleotides were adapted from our previous study[1] and were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The modified oligonucleotides were purified by HPLC and unmodified ones were purified by UltraPAGE. All the oligonucleotides were shown in Table S1.

A Biodot cutting system (CM4000) and a dispense platform (ZX1000) were employed to prepare lateral flow strips. A JEM-2010 transmission electron microscope and a field emission scanning microscopy (SIGMA-500, Germany) were used to collect transmission electron microscopy (TEM) images and scanning electron microscopy (SEM) image, respectively. UV−Vis spectra were collected on a LAMBDA 25 UV−vis spectrometer. All Raman measurements were carried out using 785 nm laser and integration time 10 s with a portable Accuman-SR510Pro SERS reader (Ocean Optics, China).

2. Supporting Tables

Table S1 Oligonucleotides used in this study.

Name	Sequences (5' to 3')
H1	TCA ACA TCA GTC TGA TAA GCT ACG ACA TCT AAC TAG CTT ATC AGA CT
H2	ATA AGC TAG TTA GAT GTC GTA GCT TAT CAG ACT CGA CAT CTA AC
T	UAG CUU AUC AGA CUG AUG UUG A
T DNA	TAG CTT ATC AGA CTG ATG TTG A
MT1	UAG CUU AUC GGA CUG AUG UUG A
MT3	UUG CUU AUC GGA CUG AUC UUG A
Random	CAC AAA UUC GGU UCU ACA GGG UA

Note: T is the microRNA-21. T DNA is the analogue of microRNA-21. MT1 is a single-base-mismatched target RNA, while MT3 is a three-base-mismatched target RNA. Random is a non-complementary random RNA sequence.

Table S2 Comparison of the proposed method with other analytical methods for microRNA.

Method	Linear range/pM	LOD/pM	Ref.
SERS-LFA	$0.1\text{-}1.0\times10^{5}$	0.084	This work
Electrochemical	$1.0\text{-}1.0\times10^{4}$	0.26	[2]
Fluorescence	$10\text{-}1.0\times10^{3}$	7	[3]
Chemiluminiscence	$0.50\text{-}5.0\times10^{2}$	0.20	[4]
Electrochemiluminescence	$0.1\text{-}1.0\times10^{4}$	0.10	[5]
SERS	$1.0\times10^{-4}\text{-}1.0\times10^{2}$	0.070	[6]
LFA	$1.0\times10^{2}\text{-}1.0\times10^{4}$	73	[7]

3. Supporting Figures

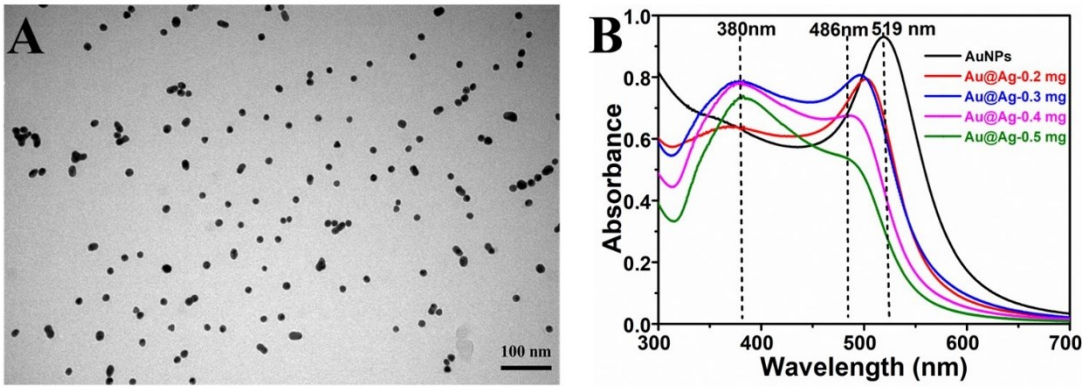

Figure S1 TEM image of 13 nm AuNPs (A) and UV-Vis spectra of Au@Ag NPs with varying $AgNO_3$ addition.

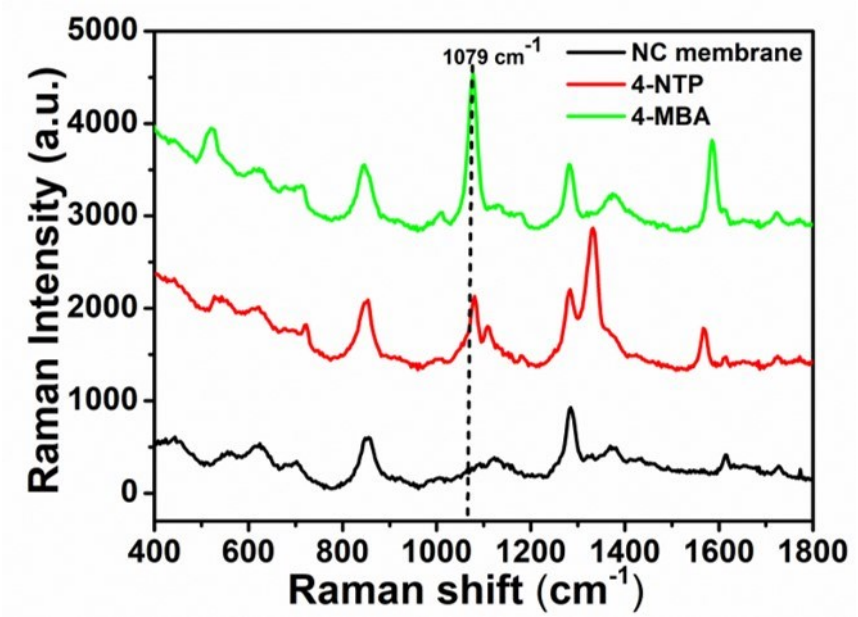

Figure S2 Comparation of Raman spectrogram characteristic peak between NC membrane, 4-NTP and 4-MBA.

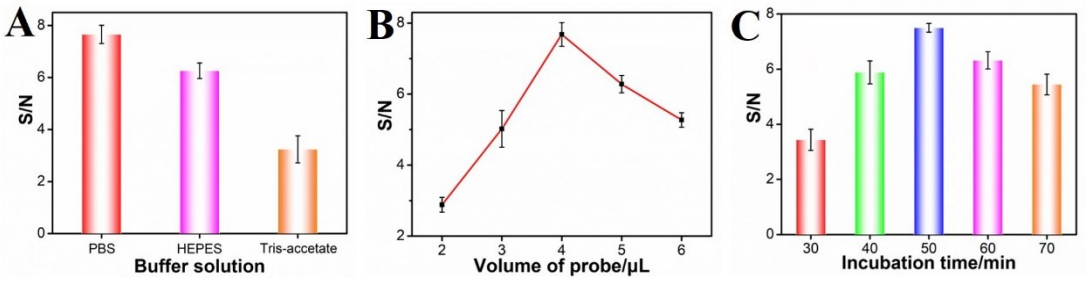

Figure S3 Optimization of experimental conditions. (A) Effect of buffer solution on microRNA-21 detection. (B) Effect of probe volume on microRNA-21 detection. (C) Effect of incubation time on microRNA-21 detection.

The running buffer solution strongly affects the migration of SERS nanotags. In addition, the choice of appropriate buffer solution would increase the hybridization efficiency between target to complementary DNA. To increase the sensitivity of our method, the reaction buffer is optimized. As shown in Figure S3A, the hybridization efficiency in phosphate buffered solution is stronger than those for other buffer solution. On the basis of this result, a phosphate buffered solution is used in subsequent assays. Besides, the probe volume which may contribute to the background signal as another important factor was also investigated. As depicted in Figure S3B, a series of probe volumes from 2 to 6 μL are used, the S/N ratio increases at first and then decreases with the further increase of probe volume, with highest S/N ratio at 4 μL. This is because the increased volume of probe enhances the target-responsive signal as well as the background signal. Less than 4 μL of probe contributes more to the target-responsive signal increasement, while further increase of probe volume more than 4 μL contributes more to the background signal. Therefore, 4 μL is adopted as the optimal probe volume in the following experiments. The incubation time is also taken into account. As seen from Figure S3C, the S/N ratio increases with the incubation time, and reached the maximum at 50 min. Therefore, the incubation time is selected as 50 min.

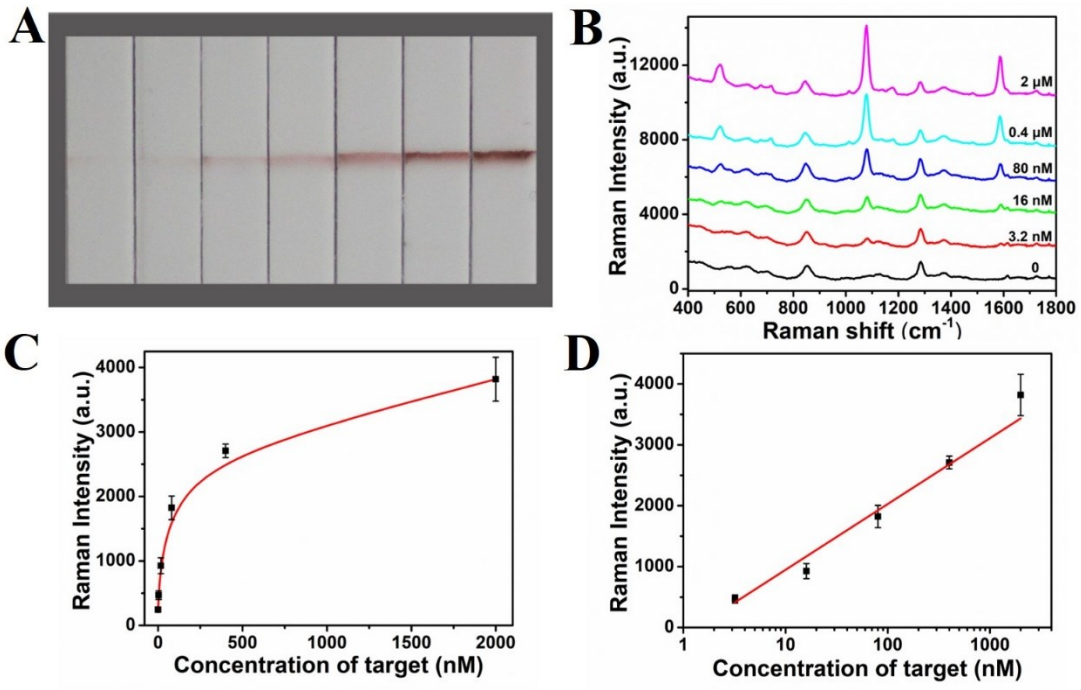

Figure S4 Performance of the SERS-based LFA platform in response to various concentrations of target DNA without CHA signal amplification. (A) Digital photograph images of lateral flow strips for: 0, 3.2×10^{-9} M, 1.6×10^{-8} M, 8.0×10^{-8} M, 4.0×10^{-7} M, 2.0×10^{-6} M, 1.0×10^{-5} M target concentration. (B) SERS spectra with different concentrations of target. (C) Calibration curve of SERS intensity of 4-MBA at 1079 cm^{-1} vs target concentration. (D) Linear relationship of SERS intensity of 4-MBA at 1079 cm^{-1} vs logarithm of target concentration.

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