Supporting Information

Capillary SERS sensor based on CRISPR/Cas13a and DS Au-AgNRs for detecting miRNA-221 in serum of hepatocellular carcinoma patients

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1074 cm^{-1} was chosen as the characteristic peak of Cy5. The SERS spectra of DS Au-AgNRs capillaries and Cy5@ssDNA@DS Au-AgNRs capillaries washed three times with PBS buffer were detected. The intensity of SERS signals at 1074 cm^{-1} was significant, proving that Cy5@ssDNA was successfully connected to DS Au-AgNRs.

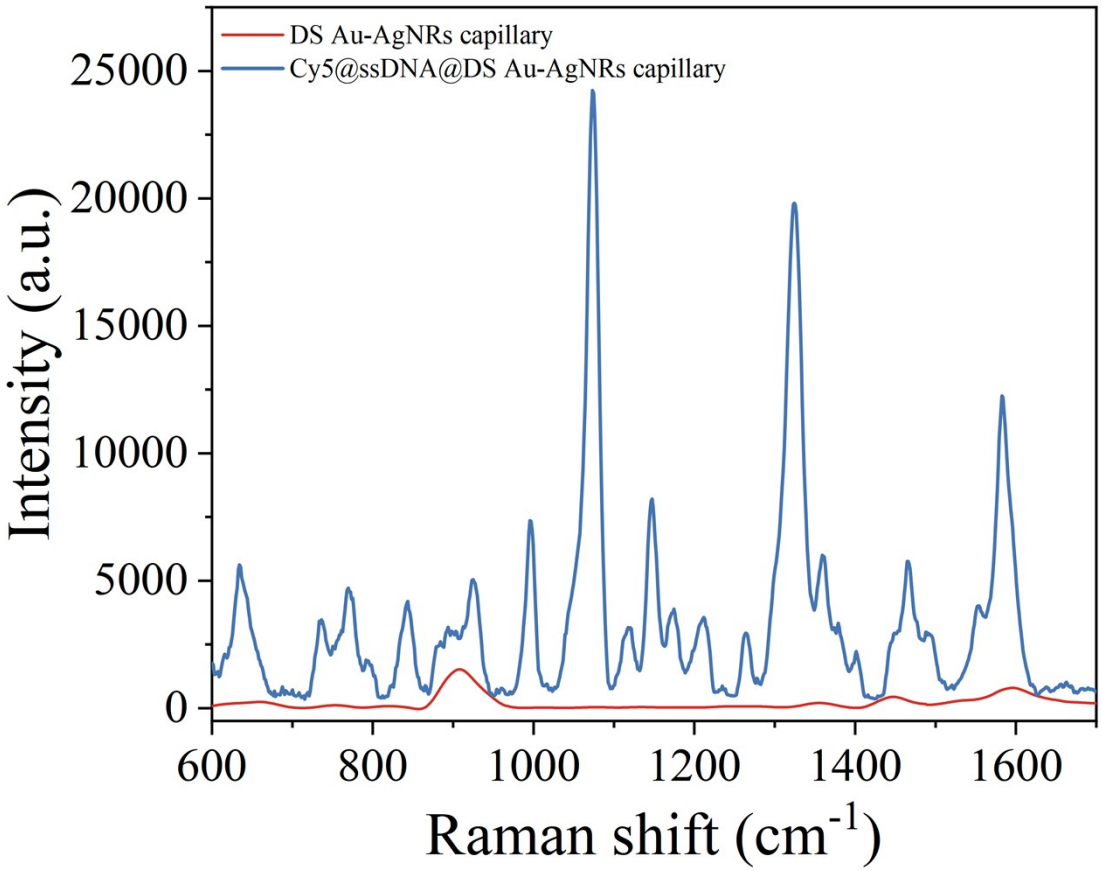

Fig.S1 (A) The SERS spectra of Cy5@ssDNA@DS Au-AgNRs capillary and DS Au-AgNRs capillary

Fig.S2 A is the electron micrograph of DS Au-AgNRs synthesised from decahedral gold seeds prepared at 84°C. Fig.S2 B is the electron micrograph of DS Au-AgNRs synthesised from decahedral gold seeds prepared at 86°C. Both morphology and size are consistent with the results of 85°C water bath reaction. It proves that the present synthesis scheme can fluctuate within the range of 85 °C ± 1 °C, and has certain temperature stability and reproducibility.

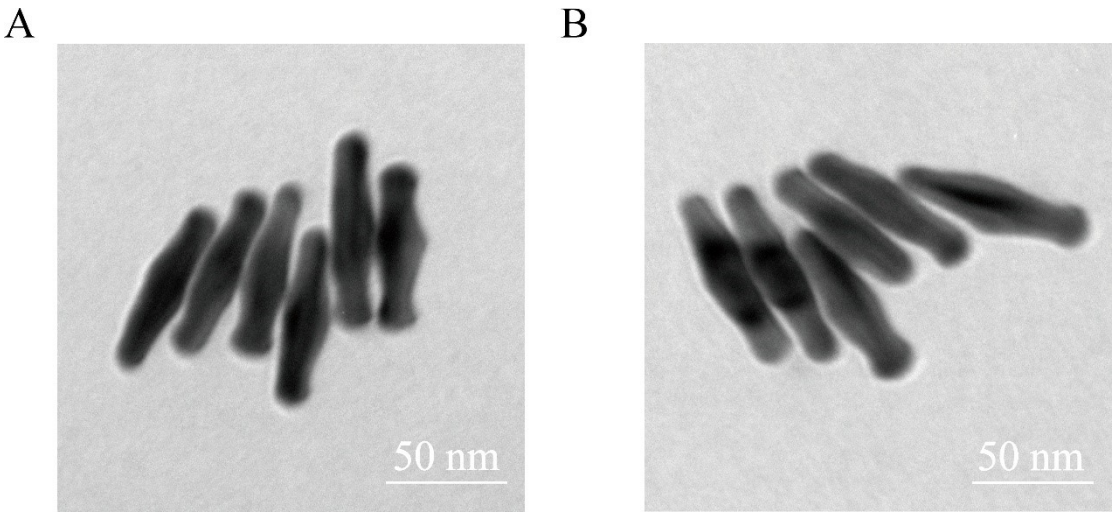

Fig.S2 (A) TEM image of DS Au-AgNRs prepared from decahedral gold nanoseeds prepared at 84°C. (B) TEM image of DS Au-AgNRs prepared from decahedral gold nanoseeds prepared at 86°C.

Table S1 SERS and qRT-PCR were used to detect the expression levels of miR-221

in blood samples from 30 healthy people

Sample	SERS (fM)	qRT-PCR (fM)	Relative error (%)
1	5.31	5.15	3.11
2	2.45	2.59	-5.41
3	2.58	2.01	28.36
4	1.62	1.67	-2.99
5	6.73	6.24	7.85
6	2.75	2.86	-3.85
7	2.83	2.93	-3.41
8	2.91	2.67	8.99
9	2.97	3.29	-9.73
10	3.02	2.84	6.34
11	3.08	3.18	-3.14
12	3.15	3.23	-2.48
13	3.59	3.27	9.79
14	3.42	3.11	9.97
15	3.25	3.36	-3.27
16	3.08	3.41	-9.68
17	3.33	2.57	29.57
18	3.37	3.52	-4.26
19	3.42	3.58	-4.47
20	3.18	3.63	-12.40
21	3.53	3.23	9.29
22	3.59	3.76	-4.52
23	3.64	3.42	6.43
24	3.09	3.12	-0.96
25	3.75	3.84	-2.34
26	3.61	3.46	4.34
27	3.87	3.65	6.03
28	3.92	4.11	-4.62
29	3.98	3.85	3.38
30	4.03	4.19	-3.82

Table S2 SERS and qRT-PCR were used to detect the expression levels of miR-221in

blood samples from 30 HCC patients

Sample	SERS (fM)	qRT-PCR (fM)	Relative error (%)
1	11.05	11.22	-1.52
2	12.18	12.56	-3.03
3	6.32	6.01	5.16
4	5.67	4.78	18.62
5	9.89	12.03	-17.79
6	5.75	5.43	5.89
7	10.76	11.34	-5.11
8	8.43	8.67	-2.77
9	12.31	12.12	1.57
10	10.15	10.89	-6.80
11	11.92	10.75	10.88
12	10.58	10.93	-3.20
13	12.84	13.61	-5.66
14	9.63	9.28	3.77
15	11.27	10.47	7.64
16	7.94	8.05	-1.37
17	12.05	10.82	11.37
18	9.97	11.16	-10.66
19	6.56	6.72	-2.38
20	10.42	10.97	-5.01
21	12.51	11.59	7.94
22	9.78	12.24	-20.10
23	11.34	10.45	8.52
24	10.29	11.31	-9.02
25	12.23	10.69	14.41
26	5.65	5.58	1.25
27	7.86	10.84	-27.49
28	10.03	10.73	-6.52
29	11.68	11.95	-2.26
30	10.81	11.63	-7.05

Table S3 Basic characteristics of HCC patients in this study.

Characteristic	Category	Value
Age (years)	53	30
Gender	Male	17
	Female	13
TNM Stage	I	2
	II	5
	III	10
	IV	13

We introduced subject operating characteristic (ROC) curves to assess the diagnostic

performance of miR-221 and AFP and the combination of the assays in this study. As shown in Fig.S3, the AUC values were 0.888 for miR-221, 0.905 for AFP, and 0.956 for the combined assay of AFP and miR-221. although the AUC value of miR-221 was slightly lower than that of AFP, it was still at a high level, indicating its significant diagnostic value. the deficiency of AFP in early HCC with lower sensitivity. The combination of the two assays can compensate for this with better classification results, achieving diagnostic accuracy beyond that of a single biomarker and meeting the clinical needs for early HCC screening in primary care scenarios.

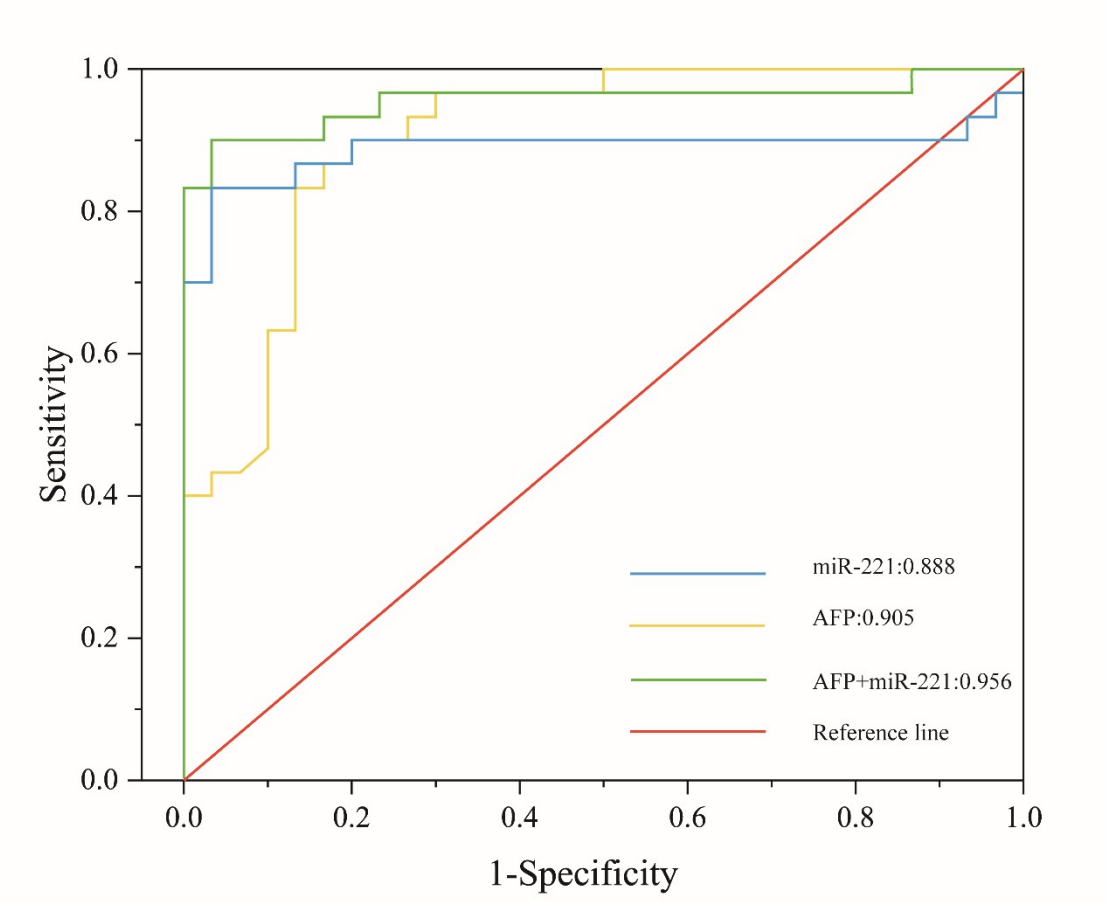

Fig.S3 ROC curves were used to assess the diagnostic accuracy of the combined AFP and miR-221 assay in differentiating healthy individuals from patients with hepatocellular carcinoma.

We constructed a crRNA that is reverse complementary to miR-21 and detected it by

titrating miR-21 at a concentration of 1 pM in ribonuclease-free water and using pure ribonuclease-free water as a blank control. The signal intensity of the target sample was significantly lower than that of the blank sample in the experiment, thus verifying the multifunctional feasibility of the sensor.

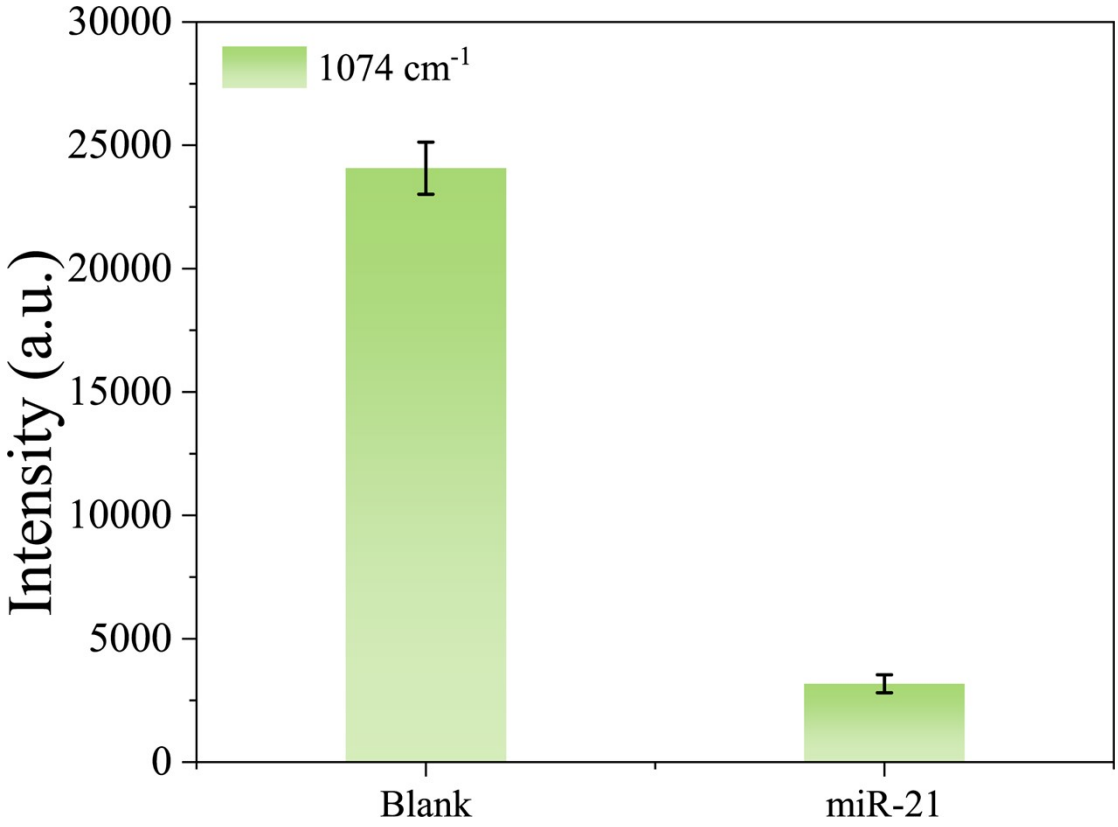

Fig.S4 Initial detection of miR-21 by redesigning crRNA

We combed other recently published CRISPR-SERS miRNA detection methods and

performed comparative analyses in Table S4. The comparative data show that the capillary SERS sensor constructed in this study has a lower detection limit, is superior to other detection methods, and exhibits excellent advantages in sensitivity.

Table S4 Comparative analysis of recently reported CRISPR/Cas13a-SERS biosensing platforms for miRNA detection.

LOD	Detection range	Sample type	Total assay Time	Ref.
53.16 aM	100 aM-1 nM	exosome	80 min	(1)
8.55 aM	100 aM-1 nM	serum	60 min	(2)
1.25 pM	0 -100 nM	serum	140 min	(3)
4.17 aM	100 aM-10 pM	serum	120 min	This work

To quantitatively assess the accuracy of the capillary SERS sensor constructed in this study in complex serum matrices and to analyse potential matrix effects, we implemented recovery experiments. We spiked 1 fM, 10 fM and 100 fM miRNA-221 in triplicate into pre-treated blank serum to compare the spiked concentration with the assayed concentration, and calculated the recovery rate. The results are shown in Table S5: the recoveries were high, ranging from 85.47% to 102.77%, and the relative errors (RSD) were all less than 5%, confirming the validity of the pretreatment protocol and the accuracy of the present sensor for the detection of miR-221.

Table S5 Recovery of miR-221 spiked into human serum

Spiked Concentration (fM)	Measured Concentration (fM)	Recovery Rate (%)	RSD (%)
1	1.17	85.47	3.35
10	9.73	102.77	4.78
100	106.86	93.58	3.95

We stored the same batch of Cy5@ssDNA@DS gold-silver nanorod capillary tubes at

room temperature for 1, 5, 10 and 15 days, and titrated miRNA-221 in PBS buffer at a concentration of 1 pM and assayed it, and examined the Raman intensities of the pre-assay and post-assay and compared them. Fig.S5A shows the surface-enhanced Raman scattering spectra of Cy5@ssDNA@DS Au-AgNRs capillary stored at room temperature for 1, 5, 10 and 15 days before assaying, with an RSD value of 4.35%, and Fig.S5B shows the surface-enhanced Raman scattering spectra of Cy5@ssDNA@DS Au-AgNRs capillary stored at room temperature for 1, 5, 10 and 15 days after assaying. The RSD value was 3.23%. It can be seen that the Cy5@ssDNA@DS Au-AgNRs capillaries have a certain storage stability.

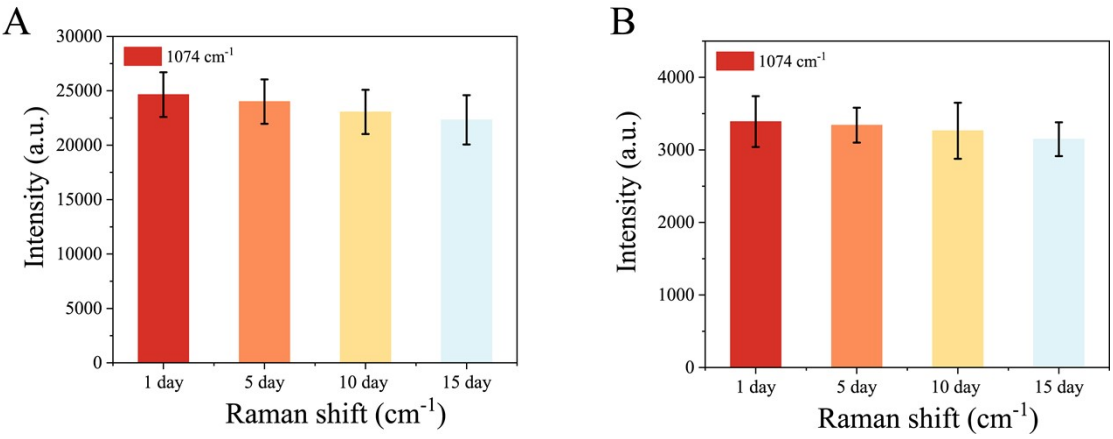

Fig.S5 (A) the SERS spectra of Cy5@ssDNA@DS Au-AgNRs capillary stored at room temperature for 1, 5, 10 and 15 days. (B) the SERS spectra of Cy5@ssDNA@DS Au-AgNRs capillary stored at room temperature for 1, 5, 10 and 15 days after assaying.

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2.Wang Z, Qin H, Liu S, Sheng J, Zhang X. Precision diagnosis of hepatocellular carcinoma. Chinese medical journal. 2023;136(10):1155-65.
3.Joung Y, Han DK, Jang H, Kang T, Chen L, Choo J. Dual-Pathway Lateral Flow Assay for Rapid and Sensitive SARS-CoV-2 RNA Detection via CRISPR/Cas13a-Mediated SERS. ACS sensors. 2025;10(8):6253-62.