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Ultrasenstive SERS biosensor based on Zn^{2+} from ZnO nanoparticle assisted DNA enzyme amplification for detection of miRNA

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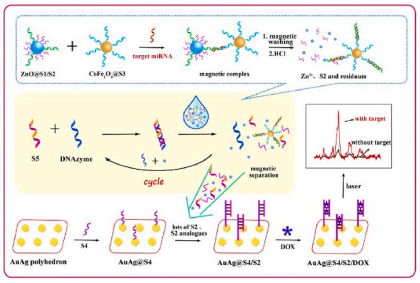
HIGHLIGHTS

- AuAg polyhedral structure can provide strong Raman enhancement capability.
- The efficient DNA utilization based on DNA enzyme induced cycle amplification can improve the sensitivity.
- Nanoparticles induced DNA enzyme amplification strategy can further improve the sensitivity.
- The introduction of $CoFe_2O_4$ nanoparticle can simplify experiment operation.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, a simple and sensitive SERS biosensor was proposed for ultrasensitive detecting miRNA 122 based on ZnO nanoparticle amplification strategy and the full utilization of DNA chain. Firstly, ZnO@S1/S2 and $CoFe_2O_4$@S3 complexes can flock together with the assistance of target miRNA. Accompanied with the incremental amount of miRNA, the quantity of ZnO@S1/S2 would increase. Therefore, a significant amplification capability can be obtained by converting ZnO complexes into Zn^{2+} with the assistance of HCl. In this case, the DNA chain S2 can be obtained by the ZnO dissolving. In addition, through a clever design, the obtained Zn^{2+} can be further utilized to induce DNA enzyme cycle amplification to cleave S5 into DNA chain which was similar with DNA S2. This step greatly avoided the waste of DNA chains and improved the utilization efficiency of DNA chains. The S2 and abundant S2 analogues can complement with S4 on the Raman sensing interface to imbed lots of Raman probe DOX for obtaining strong Raman signal. By this way, with the increased number of miRNA, the S2 and abundant S2 analogues would increase, so the amount of DOX would increase to produce strong Raman signal to quantitatively detect target miRNA. As a result, this SERS biosensor based on Zn^{+} amplification and high utilization efficiency of DNA chain can obtain a low detection limit of 6.82 aM and wide linear range from 10 aM to 10 pM, which shown great potential in the clinical application and medical diagnosis.

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1. Introduction

MicroRNAs (miRNAs) play an important role in regulating transcription and disease diagnosis, and thus rapid, accurate detection of miRNAs is indispensable [1–4]. Currently, various detection methods have been developed to detect miRNAs especially enzyme-assisted amplified method. Polymerase chain reaction (PCR) [5,6], rolling circle amplification (RCA) [7,8] and duplex-specific nuclease signal amplification (DSNSA) [9,10] were widely applied to realize quantitative detection of miRNA with good selectivity and sensitivity. However, thermal-cycling protocol with complicated handling in experimental optimization and thermo-unstable enzymes all limit the development of enzyme-assisted amplification. Based on these circumstances, enzyme-free and simple detection method should be proposed to overcome these shortcomings.

Nanoparticles-assisted amplification strategy is an emerging method which utilizes metallic oxide to convert into metal ion for achieving detection of target. Here, metallic oxide such as CuO [11,12] and ZnO [13,14] often associated with target. In this case, a certain amount of metallic oxide was positive correlation with the amount of target. Soon afterwards, with the assistance of acidic solution, metallic oxide would be dissolved into abundant metal ions. By this way, target can indirectly related to the number of metal ions. This transformation can achieve a significant magnification. However, the detection limit is needed to improve. Therefore, how to efficiently use nanoparticle amplification to achieve maximum magnification is crucial.

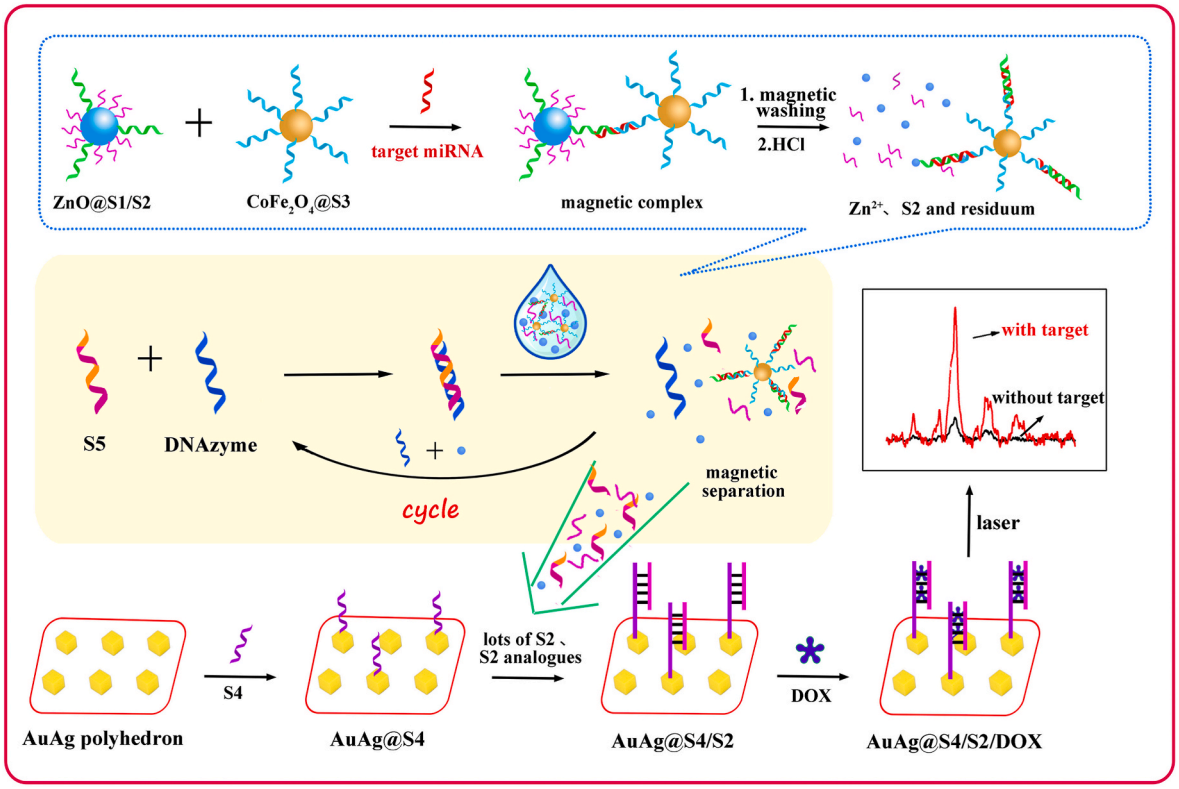
Deoxyribozyme (DNAzyme) is a single strand DNA with particular DNA structure which just like biological enzyme to catalyze plentiful reactions such as RNA/DNA cleavage, ligation or phosphorylation [15–19]. For the high catalytic activity, good stability, low cost and easy preparation, DNAzyme was often used in biosensors to instead of traditional biological enzymes. The DNAzyme is made up of two parts, including a binding site and a catalytic site. In the presence of cofactors such as Mg^{2+} [20,21], Pb^{2+} [22,23] and Zn^{2+} [24,25], the DNAzyme can carry out the catalytic activity and segment recognition ability to realize the amplification of signal. Based on this principle, we combined nanoparticle and DNAzyme-assisted amplification for ultrasensitive detection of target.

In this work, a double signal amplification strategy was developed for detection of miRNA with Zn^{2+} assisted DNA enzyme amplification(as shown in Scheme 1). Firstly, ZnO and magnetic nanoparticles $CoFe_2O_4$ were respectively aminated to covalent link with DNA S1/S2 and S3 forming ZnO@S1/S2 and $CoFe_2O_4$@S3 nanoparticle complexes. In the presence of target miRNA, ZnO@S1/S2 and $CoFe_2O_4$@S3 would form a sandwich structure for the complementary sequences between S1/miRNA and S3/miRNA. At this time, the amount of miRNA was positive correlation with the amount of ZnO@S1/S2. Subsequently, the sandwich structure would be dissolved into Zn^{2+}, DNA S2 single strand and $CoFe_2O_4$@S3/miRNA/S1 by adding a certain amount of HCl. With the assistance of obtained Zn^{2+}, DNAzyme cycle can be triggered to get lots of DNA short chains whose sequence was similar with S2. By this smart design, abundant DNA S2, S2 similar chains and Zn^{2+} can be produced, and after these two amplification processes, S2 and S2 similar chains can completely complementary with DNA S4 which has already covalent linked with AuAg polyhedral on the sensing interface. The more DNA double chains was formed, the more Raman tag DOX can be embedded. In this way, a strong Raman signal can be obtained to quantify the target miRNA with the detection limit of 6.82 aM and linear range from 10 aM to 10 pM. This simple, sensitive and waste-free method may possess great potential in early diagnosis of disease and practical application.

2. Materials and methods

2.1. Materials and reagents

Zinc acetate (Zn $(CH_3COO)_2{\cdot}2H_2O$), methanol, ethylene glycol, Potassium hydroxide (KOH), APTMS, Cobalt nitrate (Co $(NO_3)_2{\cdot}6H_2O$), Sodium hydroxide (NaOH), Ferric chloride ($FeCl_3{\cdot}6H_2O$), Sodium



Scheme 1. The principle of the proposed SERS biosensor.

borohydride ($NaBH_4$), Gold chloride ($HAuCl_4$) and Ascorbic acid (AA) were obtained from Sigma Chemical Co. (St.Louis, MO). The solutions used in this work were prepared using ultrapure water.

2.2. Apparatus

The SERS spectra used in this work were tested by a Raman spectrometer (Renishaw Invia Raman spectrometer, Invia, UK). The morphologies of AuAg polyhedral and $CoFe_2O_4$ were recorded by a scanning electron microscope (SEM, S-4800, Hitachi, Japan).

2.3. Preparation of ZnO@S1/S2

ZnO nanoparticles were synthesized according to the reference. [26] Briefly, 1.23 g Zn $(CH_3COO)_2{\cdot}2H_2O$ was firstly dissolved into 55 mL methanol at 60 °C with vigorous stirring. Then 25 mL KOH solution was dropped into the above solution in 20 min. This reaction was kept 2 h at 60 °C to obtain a homogeneous and transparent solution. Subsequently, the solution was left alone with 2 h to precipitate. Finally, the precipitate was separated by centrifugation with methanol and ultrapure water, respectively.

The ZnO@S1/S2 nanocomposites were synthesized as following steps. Firstly, APTMS (50 μL 0.28 mM) was added into the ZnO solution for 2 h with magnetically stirring to functionalize ZnO. Then, 10 μL 10 μM S1, 10 μL 10 μM S2, 10 μL EDC and 10 μL NHS were added into the functional ZnO for 2 h with gentle stirring. Finally, the obtained ZnO@S1/S2 was washed twice with PBS (pH = 7.4) to clear away the unreacted DNA.

2.4. Preparation of $CoFe_2O_4$@S3

The $CoFe_2O_4$ magnetic nanoparticle was synthesized as follows [27]. Shortly, 0.88 g lysine was added into 25 mL ethylene glycol to obtain homogeneous solution. Hereafter, 0.29 g Co $(NO_3)_2{\cdot}6H_2O$ and 0.27 g $FeCl_3{\cdot}6H_2O$ were successively added into lysine solution under magnetic stirring to called A solution. On the other hand, 0.36 g NaOH was dissolved in another 15 mL ethylene glycol to form B solution. Then, solution B was dropwise added into solution A. Nextly, the mixture was constant stirring for 20 min to mixed together, transferred to an autoclave immediately with the temperature of 180 °C for 10 h. Finally, the products were washed three times for further use.

The $CoFe_2O_4$@S3 was prepared with following steps. Firstly, $CoFe_2O_4$ was functionalized with APTMS to introduce amino group. Then, 10 μL 10 μM DNA S3, 10 μL EDC and 10 μL NHS were added into $CoFe_2O_4$–NH_2 solution under magnetic stirring for 2 h. Finally, the obtained $CoFe_2O_4$@S3 was washed with PBS (pH = 7.4) for further use.

2.5. Preparation of AuAg polyhedral

The preparation of AuAg polyhedral was performed in following procedure [28]. Firstly, 0.6 mL 10 mM $NaBH_4$ solution was added into a 10 mL aqueous solution containing 0.25 mM $HAuCl_4$ and 100 mM CTAB to generate brownish Au seed. The seed solution was kept undisturbed for 3 h at room temperature. Secondly, 6 mL 0.5 mM $HAuCl_4$, 6 mL 200 mM CTAC solution and 4.5 mL 100 mM Ascorbic acid (AA) aqueous solution were mixed, followed by the addition of 0.3 mL Au seed which have already synthesized. The color would change from colorless into red at this time. Then the obtained CTAC-capped Au seed was collected by centrifugation with ultrapure water. Finally, 0.5 mL CTAC-Au seed and 4.5 mL 20 mM CTAC aqueous solution were mixed and heated at 60 °C for 20 min with magnetic stirring. And then 2 mL 2 mM $AgNO_3$, 2 mL 50 mM AA and 4.8 mL 40 mM CTAC was added into above solution at the same time. During this reaction, the mixture turned from red to brownish yellow. The products AuAg polyhedral were cooled in an ice-bath after 4 h and washed with ultrapure water.

2.6. SERS strategy assay process

Scheme 1 showed the detection principle of the SERS biosensor for target miRNA. Firstly, 10 μL ZnO@S1/S2, 10 μL $CoFe_2O_4$@S3 and a certain amount of target miRNA were mixed together to obtain sandwich complexes. After magnetic washing, the unreacted ZnO@S1/S2 would be removed to reduce experimental error. Subsequently, HCl solution (pH = 2) was added into above products to dissolve ZnO@S1/S2/miRNA/$CoFe_2O_4$@S3 which can produce Zn^{2+}, DNA S2, and S1/miRNA/$CoFe_2O_4$@S3. Then these complexes were added into a solution which have already reacted for 3 h at 43 °C containing 100 μL 10 μM S5 and 100 μL 10 μM DNA enzyme at 37 °C with 30 min. Finally, the above products were added on the SERS substrate with AuAg@S4 for 30 min and followed with the addition of DOX for 10 min. Then the obtained SERS substrate was washed with ultrapure water and irradiated with 633 nm HeNe laser with 20 mW to obtain Raman signal.

3. Results and discussion

3.1. Characterization of materials

Fig. 1A was the SEM image of ZnO nanoparticles, which were homogeneous spheroidal structure. The AuAg polyhedral structure can be observed clearly in Fig. 1B. Fig. 1C was the SEM image of $CoFe_2O_4$ with flower like structure.

3.2. Optimization of assay conditions

To increase the sensing performance of the double signal magnified biosensor, the detection conditions like the incubation time of the target miRNA and concentration of DOX were studied in this work (see Fig. 2). With the increasing incubation time of miRNA, the Raman intensity of 1268 cm^{-1} increased until reach a steady value of 50 min as shown in Fig. 2A. Therefore, 50 min was chosen as optimal incubation time of miRNA. Fig. 2B was the investigation of DOX concentration in this work. We can see that with the increasing concentration of DOX, the Raman intensity increased accordingly until the concentration of DOX was 0.2 μg mL^{-1}. As a result, 0.2 μg mL^{-1} was the optimal concentration in this work.

3.3. Analytical performance

Based on the optimal conditions, the analytical performance of the SERS biosensor was characterized by testing different concentrations of target miRNA for three times (see Fig. 3). As depicted in Fig. 3A, Raman signal intensity of 1268 cm^{-1} was increased with the increasing concentration of miRNA 122 with a detection range of 10 aM to 10 pM. Fig. 3B presented a good linear relationship between the Raman intensity of 1268 cm^{-1} and the logarithm of miRNA 122 concentration. The corresponding regression equation is $y = 1067\ \lg c - 829$ ($R^2 = 0.997$), where y is the Raman intensity at 1268 cm^{-1} and c is the concentration of miRNA 122. The estimated limit of detection (LOD) was 6.82 aM with IUPAC method [29,30]. Compared with other biosensors for assay of the miRNA 122, our biosensor showed better performance with a lower detection limit and wider linear range for the reason that DNAzyme induced cycle amplification can obtain lots of repetitive DNA strand and ZnO nanoparticles induced amplification can also effectively improve the sensitivity. And the resluts were shown in Table 1.

3.4. Selectivity of the SERS biosensor

To investigate the selectivity of the proposed biosensor, several possible interferents were tested under the same detecting condition. Here, 10 pM of miRNA 141, miRNA 21 and miRNA 155 were separately tested, and the Raman response results were shown in Fig. 4, which was similar with the blank sample (no target). However, when 10 fM target

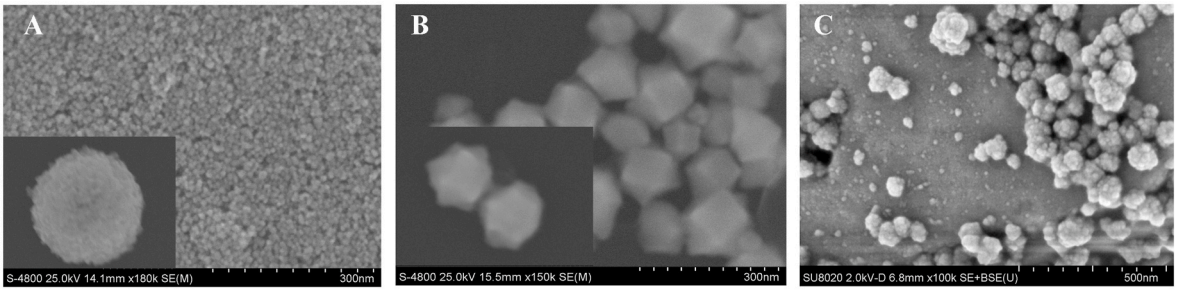


Fig. 1. SEM image of (A) ZnO; (B) AuAg polyhedral structure; (C) $CoFe_2O_4$.

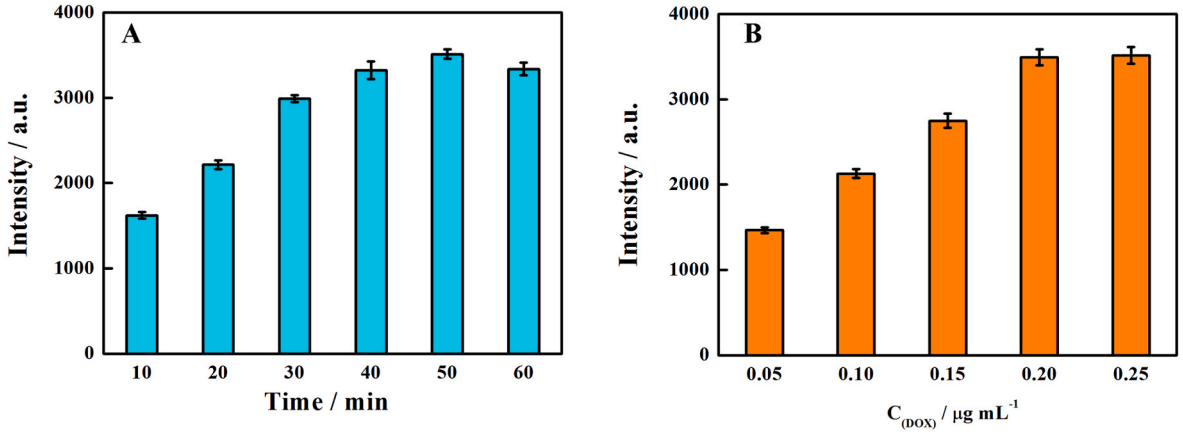


Fig. 2. SERS intensity of the proposed biosensor with different (A) incubation time of miRNA; (B) concentration of DOX.

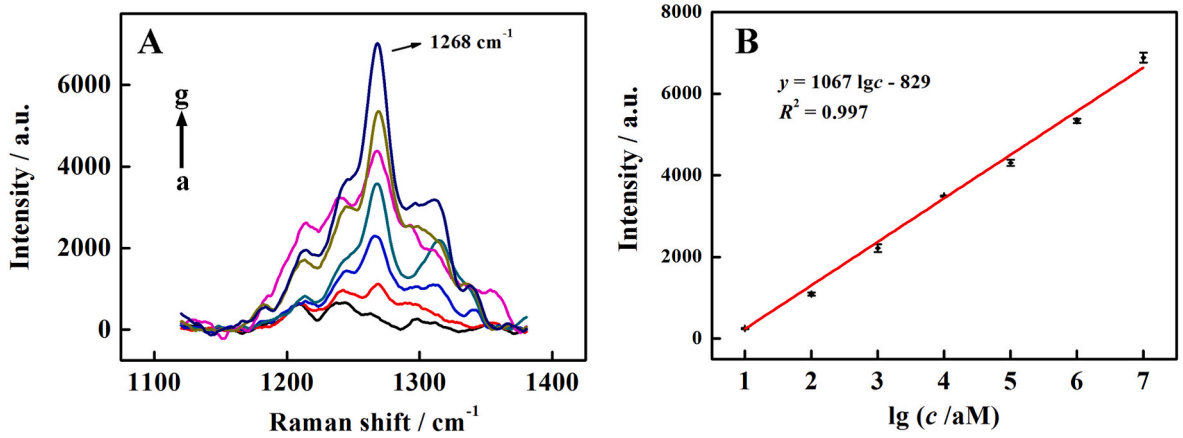


Fig. 3. (A) SERS spectra of the proposed biosensor after incubating miRNA 122 with concentration of (a–g): (a) 10 aM, (b) 100 aM, (c) 1 fM, (d) 10 fM, (e) 100 fM, (f) 1 pM, (g) 10 pM. (B) The calibration plot of Raman intensity at 1268 cm^{-1} *vs* lgc.

Table 1
Sensing performance of the proposed SERS biosenosr compared with other biosensors for miRNA 122 detection.

Analytical method	Detection limit	Linear range	refs
Chemiluminescence	0.82 fM	1 fM-100 pM	[31]
resonance light scattering	98 pM	200 pM-200 nM	[32]
Fluorescence	5 nM	5–1000 nM	[33]
Electrochemistry	0.1 fM	0.1 fM-500 pM	[34]
Electrochemistry	1.73 pM	10 μM-10 pM	[35]
Electrochemiluminescence	36 aM	0.1 fM-100 pM	[36]
SERS	0.24 pM	1 pM-1 nM	[37]
SERS	6.82 aM	10 aM-100 pM	This work

miRNA 122 coexisted with those interferences (each concentration was 10 pM), the Raman intensity was almost the same with that only containing miRNA 122. These results indicated that our proposed SERS biosensor owned high specificity for miRNA 122 detection.

3.5. Application of the SERS biosensor in serum

To investigate the analytical reliability of our biosensor, the recovery experiment was tested with a diluted healthy human real serum sample. The results shown in Table 2 were presented acceptable recoveries which was in the range of 100.1%–107.8%, and the RSD values (n = 3) were from 0.8% to 2.6%. These results also demonstrate our proposed SERS biosensor has a promising potential application in clinical analysis.

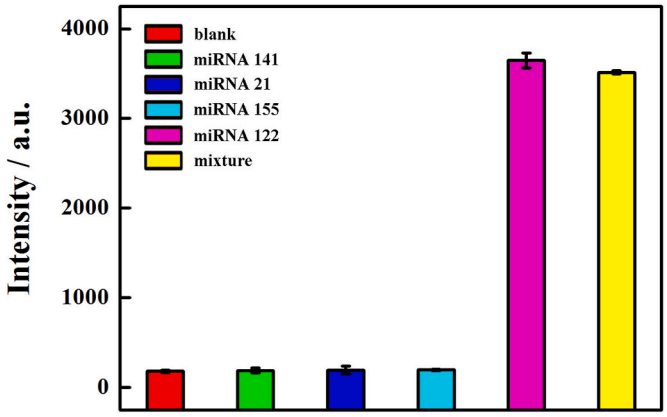


Fig. 4. Selectivity of the proposed SERS biosensor. The concentration of miRNA 141, miRNA 21 and miRNA 155 were 10 pM. The mixture contains miRNA 141 (10 pM), miRNA 21 (10 pM), miRNA 155 (10 pM), and miRNA 122 (10 fM).

Table 2
Determination of miRNA 122 added in human blood serum (n = 3) with the proposed biosensor.

Serum sample	Concentration of miRNA 122 added/ fM	Concentration obtained with biosensor/fM	Recovery/ %	RSD/ %
1	1	1.001	100.1	1.8
2	10	10.71	107.1	2.6
3	100	100.6	100.6	1.7
4	1000	1077.5	107.8	0.8

4. Conclusion

In conclusion, a simple and double amplification strategy was proposed to sensitively detect miRNA 122. This SERS biosensor can detect target with a wide linear range from 10 aM to 10 pM and low detection limit of 6.82 aM. The excellent performance may be owing to the following reasons. First, DNA enzyme induced cycle amplification can obtain lots of repetitive DNA strand which can greatly improve the utilization efficiency of DNA and improve sensitivity of the biosensor. Second, ZnO nanoparticles induced amplification can effectively improve the sensitivity of the proposed biosensor for the reason of that ZnO nanoparticles can convert into abundant Zn^{2+} to induce the DNA enzyme amplification. Lastly, the AuAg polyhedral structure can further enhance the sensitivity of the biosensor. Based on these distinct advantages, our proposed SERS biosensor has great potential for future clinic application.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Acknowledgment

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References

[1] Y. Li, C.X. Qiu, J. Tu, B. Geng, J.C. Yang, T.Z. Jiang, Q.H. Cui, Nucleic Acids Res. 42 (2014) 1070–1074.
[2] I. Alevizos, G.G. Illei, Nat. Rev. Rheumatol. 6 (2010) 391–398.
[3] X.K. Niu, C.T. Lu, D. Su, F. Wang, W.H. Tan, F.L. Qu, Anal. Chem. 93 (2021) 13727–13733.
[4] C. Pothipor, J. Jakmunee, S. Bamrungsap, K. Ounnunkad, Analyst 146 (2021) 400–4009.
[5] C.F. Chen, D.A. Ridzon, A.J. Broomer, Z.H. Zhou, D.H. Lee, J.T. Nguyen, M. Barbisin, N.L. Xu, V.R. Mahuvakar, M.R. Andersen, K.Q. Lao, K.J. Livak, K. J. Guegler, Nucleic Acids Res. 33 (2005) e179–188.
[6] P. Mestdagh, N. Hartmann, L. Baeriswyl, D. Andreasen, N. Bernard, C.F. Chen, D. Cheo, P.D. Andrade, M. DeMayo, L. Dennis, S. Derveaux, Y. Feng, F.S. Stephanie, B. Gerstmayer, J. Gouffon, C. Grimley, E. Lader, K.Y. Lee, S. J, P. Mouritzen, A. Narayanan, S. Pate, S. Peiffer, S. Rüberg, G. Schroth, D. Schuster, J.M. Shaffer, E. J. Shelton, S. Silveria, U. Ulmanella, V. Veeramachaneni, F. Staedtler, T. Peters, T. Guettouche, Nat. Methods 11 (2014) 809–815.
[7] X. Tang, R.J. Deng, Y.P. Sun, X.J. Ren, M.X. Zhou, J.H. Li, Anal. Chem. 90 (2018) 10001–10008.
[8] W.M. Tian, P.J. Li, W.L. He, C.H. Liu, Z.P. Li, Biosens. Bioelectron. 128 (2019) 17–22.
[9] Y. Zhang, X.Y. Wang, X. Wei, C.Y. Zhang, Chem. Commun. 55 (2019) 6827–6830.
[10] S.Y. Xue, Q.Q. Li, L. Wang, W.B. You, J. Zhang, R.C. Che, Anal. Chem. 91 (2019) 2659–2666.
[11] R.D. Li, Q. Wang, B.C. Yin, B.C. Ye, Biosens. Bioelectron. 77 (2016) 995–1000.
[12] F. He, W. Jing, B.C. Yin, B.C. Ye, Anal. Chem. 90 (2018) 8072–8079.
[13] L.L. Xie, X. Yang, Y. He, R. Yuan, Y.Q. Chai, ACS Appl. Mater. Interfaces 10 (2018) 15200–15206.
[14] J. Wang, H.M. Wang, H. Wang, S.Z. He, R.M. Li, Z. Deng, X.Q. Liu, F. Wang, ACS Nano 13 (2019) 5852–5863.
[15] R.R. Breaker, Chem. Rev. 97 (1997) 371–390.
[16] R.R. Breaker, Nat. Biotechnol. 15 (1997) 427–431.
[17] D.S. Wilson, J.W. Szostak, Annu. Rev. Biochem. 68 (1999) 611–647.
[18] S. Khan, B. Burciu, D.M.C. Filipe, Y.F. Li, K. Dellinger, T.F. Didar, ACS Nano 15 (2021) 13943–13969.
[19] J. Jouha, H. Xiong, Small 17 (2021) 2105439–2105462.
[20] L.D. Yu, Y. Chen, H. Lin, S.S. Gao, H.R. Chen, J.L. Shi, Small 14 (2018) 1800708–1800723.
[21] L.W. Zeng, D.H. Zhou, J.Y. Gong, C.S. Liu, J.H. Chen, Anal. Chem. 91 (3) (2019) 1724–1727.
[22] W. Cai, S.B. Xie, J. Zhang, D.Y. Tang, Y. Tang, Biosens. Bioelectron. 117 (2018) 312–318.
[23] Y.J. Yu, C. Yu, Y.Z. Niu, J. Chen, Y.L. Zhao, Y.C. Zhang, R.F. Gao, J.L. He, Biosens. Bioelectron. 101 (2018) 297–303.
[24] J.C. Zhao, D. Shu, Z.F. Ma, Biosens. Bioelectron. 127 (2019) 161–166.
[25] H.B. Si, R.J. Sheng, Q.L. Li, J. Feng, L. Li, B. Tang, Anal. Chem. 15 (2018) 8785–8792.
[26] W.J. Beek, M.M. Wienk, M. Kemerink, X. Yang, R.A. Janssen, J. Phys. Chem. B 109 (2005) 9505–9516.
[27] M.Y. Dong, Q. Lin, D. Chen, X.N. Fu, M. Wang, Q.Z. Wu, X.H. Chen, S.P. Li, RSC Adv. 3 (2013) 11628–11633.
[28] Y.Y. Ma, W.Y. Li, E.C. Cho, Z.Y. Li, T. Yu, J. Zeng, Z.X. Xie, Y.N. Xia, ACS Nano 4 (2010) 6725–6734.
[29] IUPAC, Pure Appl. Chem. 45 (1976) 105–123.
[30] G.L. Long, J.D. Winefordner, Anal. Chem. 55 (1983) 712A–724A.
[31] S. Bi, J.Y. Ye, Y. Dong, H.T. Li, W. Cao, Chem. Commun. 52 (2016) 402–405.
[32] F. Chen, F. Zhang, Y. Liu, C.Q. Cai, Talanta 186 (2018) 473–480.
[33] R. Liao, K. He, C.Y. Chen, X.M. Chen, C. Cai, Q. Anal. Chem. 88 (2016) 4254–4258.
[34] T.Y. Wang, E. Viennois, D. Merlin, G.L. Wang, Anal. Chem. 87 (2015) 8173–8180.
[35] S. Kasturi, Y.J. Eom, S.R. Torati, C. Kim, J. Ind. Eng. Chem. 93 (2021) 186–195.
[36] N. Zhang, Z.Y. Zhao, H. Gao, Y. Yu, J.B. Pan, H.Y. Chen, J.J. Xu, J. Electroanal. Chem. 900 (2021), 115743.
[37] W. Zhou, Y.F. Tian, B.C. Yin, B.C. Ye, Anal. Chem. 89 (2017) 6120–6128.