Analytica Chimica Acta 1108 (2020) 21–27

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

An enzyme-free surface plasmon resonance imaging biosensing method for highly sensitive detection of microRNA based on catalytic hairpin assembly and spherical nucleic acid

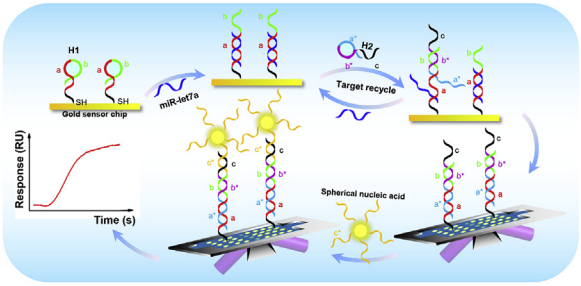
Xiaotong Wei [a, 1], Dewei Liu [b, 1], Min Zhao [a], Tiantian Yang [a], Yunpeng Fan [a], Wenqin Chen [a], Ping Liu [c], Jianbo Li [b, **], Shijia Ding [a, *]

[a] Key Laboratory of Clinical Laboratory Diagnostics (Ministry of Education), College of Laboratory Medicine, Chongqing Medical University, Chongqing, 400016, China
[b] Department of Forensic Medicine, Faculty of Basic Medical Sciences, Chongqing Medical University, Chongqing, 400016, China
[c] Bioscience (Tianjin) Diagnostic Technology CO, LTD, Tianjin, 300399, China

HIGHLIGHTS

- A simple and enzyme-free surface plasmon resonance imaging (SPRi) biosensor was developed for microRNA detection.
- The excellent signal amplification efficiency was achieved by catalytic hairpin assembly and spherical nucleic acid.
- The designed SPRi biosensor showed wide detection range of 4 orders magnitude and low limit of detection of 53.7 fM.
- The method could distinguish members of homologous microRNA families, even with single base differences.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 December 2019
Received in revised form
11 February 2020
Accepted 25 February 2020
Available online 27 February 2020

Keywords:
MicroRNA
Surface plasmon resonance imaging
Catalytic hairpin assembly
Spherical nucleic acid

ABSTRACT

MicroRNAs (miRNAs), considered as therapeutic targets and biomarkers, play important roles in biological processes. Herein, an enzyme-free surface plasmon resonance imaging (SPRi) biosensing method has been developed for miRNA detection based on catalytic hairpin assembly and spherical nucleic acid. The hairpin H1 tethered on the surface of the sensor chip is unfolded by miRNA, and then the hybridized miRNA is released through the displacement of the hairpin H2 for the successive hybridization and assembly process. The emerging DNA fragments on the sensor chip surface after hairpins assembly are further used to hybridize with spherical nucleic acid, inducing a remarkably amplified SPR signal. This biosensing method is highly sensitive to miRNA with a detection limit of 53.7 fM and a linear range of 4 orders of magnitude. Moreover, the biosensor demonstrates good specificity and has the ability to distinguish members of homologous miRNA family even with single base differences. Thus, the SPRi biosensing method may hold a great promise for further application in early clinical diagnosis.

* Corresponding author.
** Corresponding author.
E-mail addresses: lijianbo@cqmu.edu.cn (J. Li), dingshijia@163.com (S. Ding).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.aca.2020.02.055
0003-2670/

1. Introduction

MicroRNAs (miRNAs) are a large family of non-protein coding RNA molecules which play important roles in post-transcriptional regulation of genes and biological processes [1]. Evidences have suggested that the abnormal expression of miRNAs is closely related to the cancer occurrence and development [2,3]. Thus, developing highly sensitive miRNA detection method is of great significance for the early diagnosis of cancer. However, the intrinsic characteristics of miRNAs, such as small sizes, sequence homology, low concentration, and easy to degrade, seriously challenge routine techniques [4–7], therefore encouraging researchers to develop new biosensor techniques to meet the requirement of clinical diagnosis.

Surface plasmon resonance imaging (SPRi) biosensor, monitoring the change of optical refractive index near the thin metal film that occurs during the complex binding or dissociation processes of biological molecules, has been ameliorated to detect small molecules, proteins and nucleic acids [8]. Unlike other biosensors that always require additional tagging procedure, such as fluorescence [9], electrochemistry [10], and electrochemiluminescence [11]. SPRi biosensor is endowed with tremendous advantages, including label-free, real-time monitoring and high-throughput. To highly sensitive detect interesting clinical biomarkers with low abundance in complex biological samples, plenty of amplification strategies have been proposed by improving the mass of material that binds to SPRi sensor chip [12].

For one thing, isothermal amplification strategies, which achieve the goal that one target can rapidly generate numbers of products, are becoming significant approaches in facilitating the sensitivity of miRNAs detection [13]. However, enzyme-based amplification methods not only increase the cost of testing but also require strict control of reaction conditions, thus limiting the further clinical application [14]. Recently, enzyme-free amplification strategies, including catalytic hairpin assembly (CHA) [15], hybridization chain reaction (HCR) [16], and entropy-driven reaction [17], have been developed to solve the limitations brought by enzyme-based amplification methods. In particular, CHA has demonstrated to be a powerful signal amplification method due to rapid turnover rates that lead to high sensitivity and low background associated with the target-specific reactions [18]. Taking these merits together, CHA has been widely applied for the detection of various biomarkers [19]. Nevertheless, to our knowledge, integration CHA into SPRi biosensor has not been reported.

For another thing, nanomaterials have also been used to develop novel SPRi biosensor [20,21]. Among them, spherical nucleic acid has gained much more attention, due to the characteristic nanostructure of nucleic acid shell and AuNP core. AuNP core not only possess large surface areas that provide numbers of loading sites, but can protect nucleic acid from degradation in complex environments [22]. Besides, the nucleic acid shell can prevent AuNP from aggregation and improve the specificity of biosensor by accurate recognizing receptor via strict base paring [23]. Moreover, the localized surface plasmon wave of the AuNP core enables SPRi biosensor to significantly improve sensitivity [24]. Thus, spherical nucleic acid is regarded as ideal signal amplification nanomaterial, especially for SPRi biosensor.

Herein, a simple, enzyme-free and sensitive SPRi biosensor was developed for miRNA detection based on catalytic hairpin assembly and spherical nucleic acid. The hairpin probe tethered on the sensor chip is triggered by target miRNA. With the involvement of hairpin probe 2 (H2), the target miRNA can be replaced and then released for the subsequent assembly process, resulting in the formation of numerous H1−H2 duplexes. The emerging free end of H1−H2 duplex is further utilized to hybridize with spherical nucleic acid, leading to a remarkably amplified SPR signal. Owing to the excellent signal amplification ability of catalytic hairpin assembly and spherical nucleic acid, the proposed method showed high sensitivity for miRNA detection. Moreover, this method is qualified to distinguish members of homologous miRNA families, even with single base differences. And it is successfully used for miRNA analysis in complex biological sample, proposing an alternative miRNA quantification method for early clinical diagnosis.

2. Experimental section

2.1. Reagents and materials

6-Mercapto-1-hexanol (MCH) was obtained from Sigma-Aldrich (St. Louis, USA). Tris (2-carboxyethyl) phosphine (TCEP) was purchased from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). $HAuCl_4 \cdot 4H_2O$ was obtained from Sinopharm Chem Co., Ltd (Shanghai, China). Triton X-100, bovine serum albumin (BSA), diethylpyrocarbonate (DEPC) were purchased from Sangon Biotechnology Co., Ltd (Shanghai, China). Fetal bovine serum was obtained from BBI Life Sciences Corporation (Shanghai, China). Gelred nucleic acid stain was purchased from SenBeiJia Biological Technology Co., Ltd (Nanjing, China). The gold sensor chip that possessed twenty detection spots was provided by University of California. All aqueous solutions were prepared by a Millipore Milli-Q gradient ultrapure water system (Millipore, MA).

The HPLC-purified oligonucleotide sequences were obtained from Sangon Biotechnology Co., Ltd (Shanghai, China) and listed in Table S1. DNA hybridization was conducted in 10 mM Tris-HCl (500 mM NaCl, 1 mM $MgCl_2$, pH 7.4) and the miRNA hybridization was conducted in Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The running buffer (pH 7.4) contained 30 mM sodium phosphate, 3 mM EDTA, 450 mM NaCl, and 0.25% Triton X-100. The hairpin probes were incubated at 95 °C for 10 min and then slowly cooled to room temperature.

2.2. Apparatus

A home-built surface plasmon resonance imaging (SPRi) sensing platform was used for SPRi measurements. The structure of the platform was described in Supporting Material. UV−vis absorption spectra were recorded with a UV-2550 spectrophotometer (Shimadzu, Japan). Transmission electron microscopic (TEM) image was conducted by an H-7500 transmission electron microscope (Hitachi, Japan). The gel electrophoresis was performed on the DYY-6C electrophoresis analyzer (Liuyi Instrument Company, China) and visualized on Bio-Rad ChemDoc XRS (Bio-Rad, USA).

2.3. Preparation of spherical nucleic acid

The preparation of spherical nucleic acid was performed according to the method described in the previous literature with slight modification [25]. The preparation procedures were depicted in Supporting Material.

2.4. Sensor fabrication

All detection spots of the gold sensor chip were firstly washed with acetone, ethanol and ultrapure water for 3 min, then cleaned with fresh piranha solution (H_2SO_4 and H_2O_2 at a volume ratio of 7:3) for 10 min to remove surface organic impurities, then thoroughly rinsed with ultrapure water and dried under nitrogen. The immobilization of thiolated hairpin probe on the gold sensor chip

was conducted in KH_2PO_4 (1 M) solution containing 2 μM hairpin probe at 4 °C overnight. Subsequently, the gold sensor chip was washed by ultrapure water and treated with 1 mM MCH and 1% BSA for 1 h, respectively, to prevent non-specific adsorption, finally, rinsed with ultrapure water and dried under nitrogen.

2.5. Target-induced catalytic hairpin assembly

Generally, 50 μL of different concentrations of let-7a miRNA was dropped onto the gold sensor chip for hybridization with the thiolated hairpin probe H1, and incubated at 37 °C for 2 h. Then the gold sensor chip was rinsed with ultrapure water and dried by nitrogen. After that, 50 μL Tris-HCl solution (10 mM) containing 2 μM H2 was dropped onto the gold sensor chip for target-induced catalytic hairpin assembly and incubated at 37 °C for another 2 h. Finally, the gold sensor chip was rinsed by ultrapure water and then dried under nitrogen before the SPRi measurement.

2.6. SPRi measurement

The prepared gold sensor chip was installed on the SPRi instrument. When the sensing signal reached a steady state, the spherical nucleic acid dispersed to 400 μL with running buffer was injected into the flow cell at the rate of 10 μL/min. Owing to different injection time points, the starting times of SPR response signals were discrepant. Then the gold sensor chip was rinsed with running buffer at the rate of 50 μL/min to remove the uncombined analytes. For all the experiments, the temperature was set to 25 ± 1 °C. The developed SPRi biosensor could monitor simultaneously twenty spots with same reaction in a measurement. The real-time data was recorded by a home-built SPRi platform.

2.7. Gel electrophoresis

To assess the feasibility of catalytic hairpin assembly, a 3% agarose gel electrophoresis experiment was performed at 110 mV in 1 × TBE buffer (90 mM Tris-HCl, 90 mM boric acid, 2 mM EDTA, pH 7.9) for about 39 min. The gel was imaged by Bio-Rad ChemDoc XRS.

3. Results and discussion

3.1. Design of the SPRi biosensor

The principle of the proposed SPRi biosensing method for miRNA detection is shown in Scheme 1. In this work, two hairpin probes hairpin 1 (H1) and hairpin 2 (H2) are employed. In the absence of target let-7a miRNA, due to the combination of self-complementary sequences, two hairpin probes do not interact with each other and form a stable stem loop structure. The gold sensor chip is firstly modified with the thiol group functionalized H1 by Au—S interaction. In the presence of target let-7a miRNA, target let-7a miRNA can hybridize with H1 (a, in red) and unfold the hairpin structure of H1 by a toehold mediated strand displacement reaction [26]. The exposed sequence (b, in green) of H1 can bind with H2 (b*, in purple) by the branch migration process to form a H1: H2: miRNA complex [27]. After that, the target let-7a miRNA will dissociate from the H1: H2 duplex owing to the inherently unstable state of H1: H2: miRNA complex. The liberated target let-7a miRNA is allowed to continuously trigger catalytic hairpin assembly to form the H1—H2 duplex, resulting in the generation of numerous H1—H2 duplexes on the gold sensor chip. Finally, the free end of H1—H2 duplex (c, in black) on the gold sensor chip can further hybridize with the spherical nucleic acid for signal amplification and monitored in real time by SPRi instrument.

3.2. Feasibility of the developed biosensor

The reaction pathways of catalytic hairpin assembly are confirmed by 3% agarose gel electrophoresis analysis experiments. As shown in Fig. 1B, although H1 (lane 3) and H2 (lane 4) contain complementary sequences, there is no change of the band positions in the mixture (lane 6), indicating that the hairpin probes form a sufficiently stable stem-loop structure. Similarly, the electrophoretic migration of a new band is slower than H1, indicating that H1 has a good ability to recognize the target miRNA (lane 5). When target miRNA was mixed with H1 and H2, a new band appears at shorter electrophoresis distance, which confirms the formation of H1—H2 duplexes (lane 7). These results clearly demonstrate that catalytic hairpin assembly can be initiated by the target miRNA and later on give rise to abundant assembly of H1—H2 duplexes.

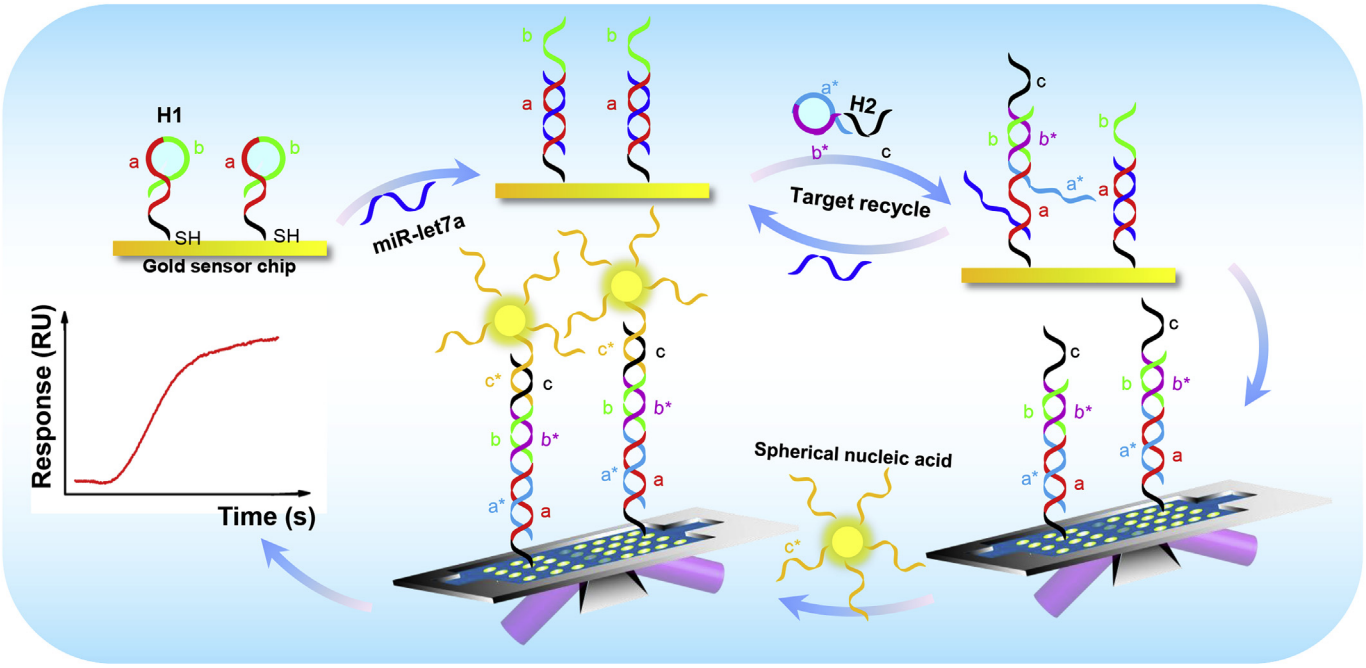
AuNPs and spherical nucleic acid were characterized by TEM and UV—vis absorption spectra, respectively (Fig. S1). To further confirm the feasibility of catalytic hairpin assembly combined with spherical nucleic acid for target miRNA detection, SPRi measurements were performed and shown in Fig. 1A. Minimal SPR sensing signal was observed in the absence of the let-7a miRNA, demonstrating that no H1—H2 duplex capable of hybridizing to spherical nucleic acid was assembled due to the hairpin structure of the hairpin probe being intact. Compared with the presence of target miRNA, with the introduction of spherical nucleic acid, the SPR sensing signal showed a significant increase, which was attributed to the formation of a great deal of H1—H2 duplex initiated by the target miRNA and the excellent SPR signal enhancement capability of the AuNPs. △RU represented the SPR total response signal which was applied for subsequent evaluation of the detection performance. These results clearly demonstrate the feasibility of the proposed enzyme-free SPRi biosensing method for let-7a miRNA detection.

3.3. Optimization of the experiment conditions

In order to improve the detection sensitivity and efficiency of the biosensor, experimental parameters such as the concentration of H1 and the reaction time of H1 and H2 were optimized. The H1—H2 duplex assembled on the gold sensor chip would hybridize with spherical nucleic acid, so its density has a great effect on the SPR sensing signal. The SPR responses of different immobilization concentrations of H1 (0.5—2.5 μM) toward 10 pM target miRNA were firstly investigated. As shown in Fig. 2A, the SPR response increased with an increase in the immobilization concentration of H1 before 2 μM. As the immobilized concentration was further increased, the SPR response decreased slightly, which may be due to the greater steric hindrance effect and limited target hybridization efficiency. Thus, 2.0 μM was used as the optimal immobilization concentration for the subsequent miRNA detection. The hybridization time of H1 and H2 was further investigated and depicted in Fig. 2B, with the reaction time increased gradually, the SPR response rose accordingly and tended to reach the plateau at 2 h. Therefore, we chose 2 h as the optimal reaction time for the detection of miRNA.

3.4. Analytical performance of SPRi biosensor

Under the optimal experimental conditions, the quantification of target miRNA based on catalytic hairpin assembly and spherical nucleic acid was investigated. As shown in Fig. 3A, the SPR response was measured at different concentrations of the target miRNA. In the absence of target miRNA (a, in black) and, spherical nucleic acid cannot bind to the gold sensor chip due to the



Scheme 1. Schematic illustration of the SPRi biosensing method.

unsuccessful formation of H1–H2 duplexes, resulting in a negligible SPR signal compared to the original stable baseline. At low level of target concentration (<0.1 pM), the SPR signal showed a negligible increase, due to the fact that the lower target could not drive catalytic hairpin assembly effectively. With the concentration of target miRNA increased (0.1 pM–1500 pM), the SPR signal response gradually increased, indicating the formation of the H1–H2 duplexes and the signal enhancement by spherical nucleic acid. In addition, the signal did not increase with further increasing concentration (>1500 pM). It may be that the materials loaded on the surface of the gold film were saturated. The SPR signal response of designed biosensor was correlated with the target miRNA concentration (Fig. 3B). The calibration curve of SPR signal response showed a good linearity with the logarithm of target miRNA concentration from 0.1 pM to 1500 pM. The linear range of the proposed method was wider than that of surface plasmon resonance based on streptavidin functionalized gold nanorods-assisted signal amplification strategy (0.1 pM–100 pM)

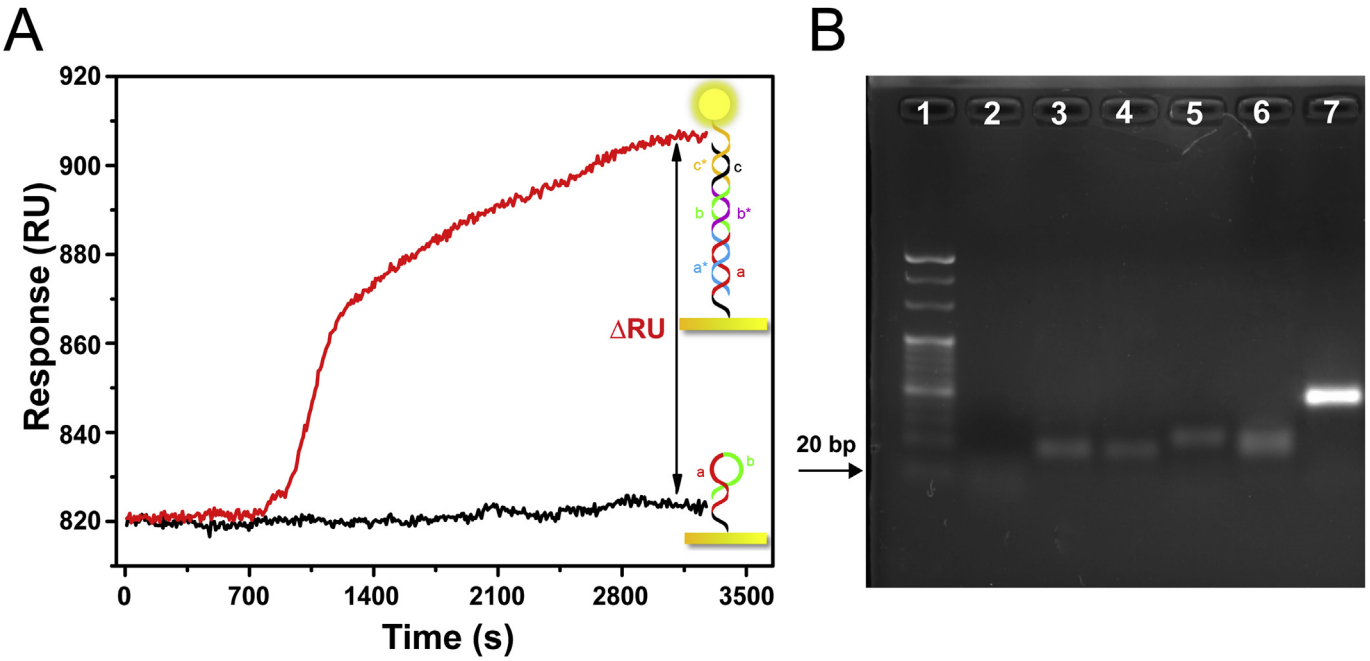


Fig. 1. A. SPR sensorgrams corresponding to a blank reaction and spherical nucleic acid-integrated catalytic hairpin assembly. B. The gel electrophoresis results of catalytic hairpin assembly. DNA ladder markers (lane 1), let-7a miRNA (lane 2), H1 (lane 3), H2 (lane 4), H1+let-7a (lane 5), H1+H2 (lane 6), H1+H2+let7a (lane 7).

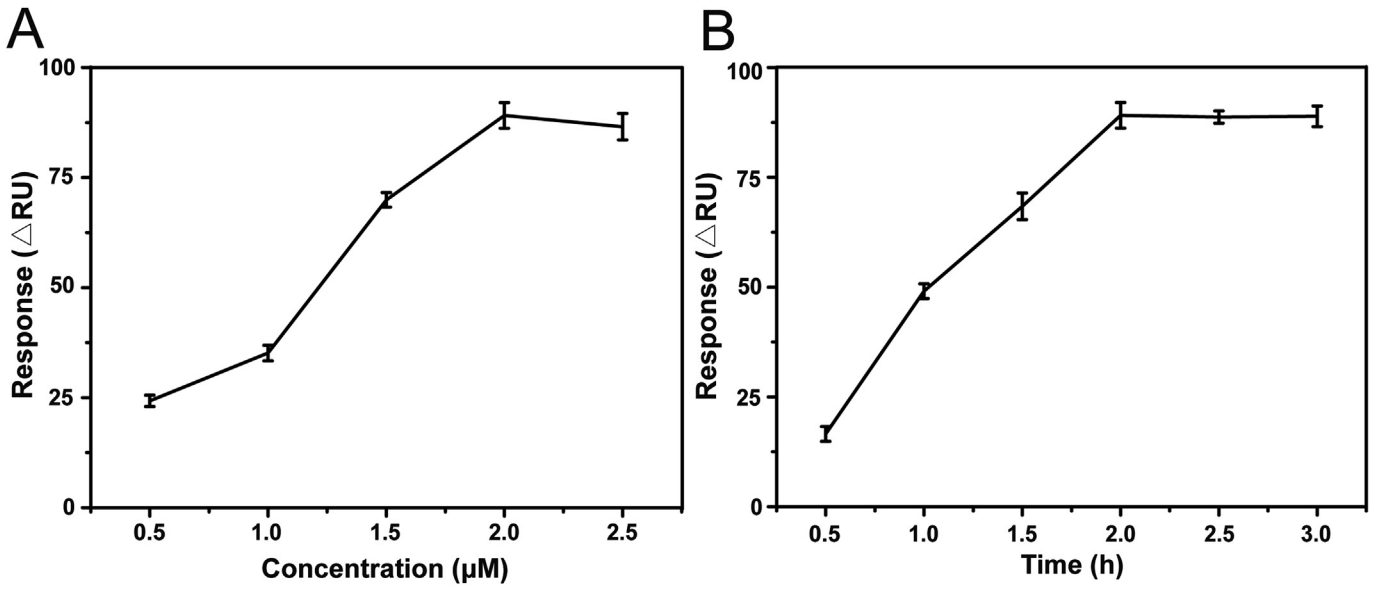


Fig. 2. Optimization of experimental parameters. A. Evaluation of the concentration of H1. B. Evaluation of the hybridization time of H1 and H2. All data expressed as mean ± standard variation (n = 3).

[28]. The regression equation was $Y = 34.917 \log C$ (fM) - 55.663 and the correlation coefficient was 0.991, where Y represents the SPR response signal and C represents the concentration of let-7a miRNA. The detection limit was calculated to be 53.7 fM, which was much lower than 45 pM of functional nucleic acid-based amplification machine SPRi method and 0.5 pM of enzyme-free single nanostructured enhancer SPRi method [29,30]. Compared with other reported methods for miRNA detection (Table 1), the proposed method showed high sensitivity and desirable linear range for miRNAs detection, attributing to the high amplification ability of catalytic hairpin assembly and excellent SPR signal enhancement by spherical nucleic acid.

3.5. Specificity and reproducibility of SPRi biosensor

The specificity assessment of the proposed method is essential due to the sequence homology among different miRNAs in the same family. To evaluate the specificity of the designed SPRi biosensing method, a number of let-7 family members (let-7a-f) with homologous sequences of only 1–2 nucleotide differences were tested. Fig. 4 shows the comparison of the SPR signal responses of the let-7a miRNA, the blank and the other let-7 family members with the same concentration of 10 pM. Only let-7a causes significantly increased SPR signal response and other let-7 family members can be clearly discriminated from let-7a even with only 1–2 nucleotide differences. Therefore, the proposed SPRi biosensor

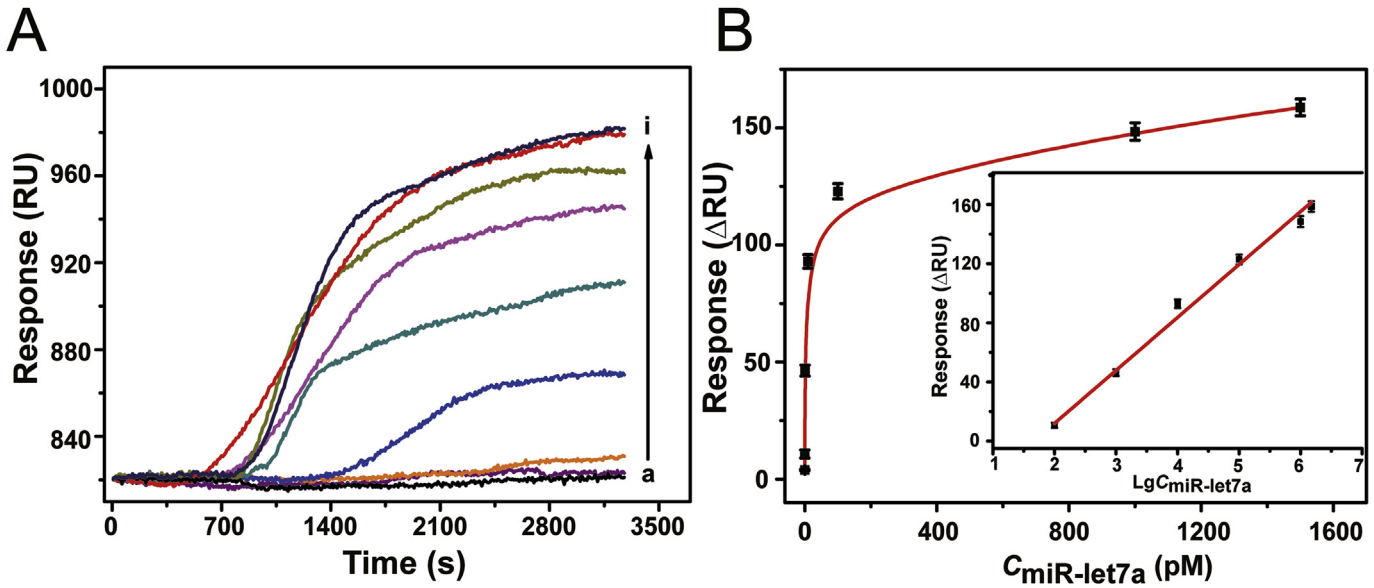


Fig. 3. A. SPR sensorgrams for detection of let-7a at 0, 0.01, 0.1, 1, 10, 100, 1000, 1500, 5000 pM (from a to i). B. Plot of SPR signal response versus let-7a concentration. Inset: calibration curve. All data expressed as mean ± standard variation (n = 3).

Table 1
Comparison of our strategy with other methods in miRNA detection.

Detection platform	With/without enzyme	Amplification strategy	Linear range	Testing time	LOD
SPRi	Enzyme-based amplification	Functional nucleic acid-based amplification	0.02 nM–10 nM	75 min	45 pM [29]
	This work	**Catalytic hairpin assembly and spherical nucleic acid**	**0.1 pM-1.5 nM**	**50 min**	**53.7 fM**
Fluorescence	Enzyme-based amplification	Duplex-specific nuclease signal amplification	1 pM-100 nM	30 min	100 fM [31]
	Enzyme-free amplification	FRET between silver nanoclusters and CdTe	5.0 pM-50 nM	60 min	1.2 pM [32]
Electrochemistry	Enzyme-based amplification	Two-stage cyclic enzymatic amplification	1 fM-100 pM	180 min	0.36 fM [33]
		MNAzyme-mediated rolling circle amplification	10 fM-1 nM	210 min	1.66 fM [34]
SPR	Enzyme-free amplification	graphene oxide-gold nanoparticles composites amplification	0.1 pM - 2.0 nM	60 min	0.1 fM [35]
		multiple signal amplification strategy		205 min	0.6 fM [36]
		gold nanoparticles-decorated molybdenum sulfide		110 min	0.5 fM [37]
		Streptavidin functionalized gold nanorods	0.1 pM–100 pM	45 min	45 fM [28]

FRET: fluorescence resonance energy transfer; MNAzyme: multi component nucleic acid enzyme.

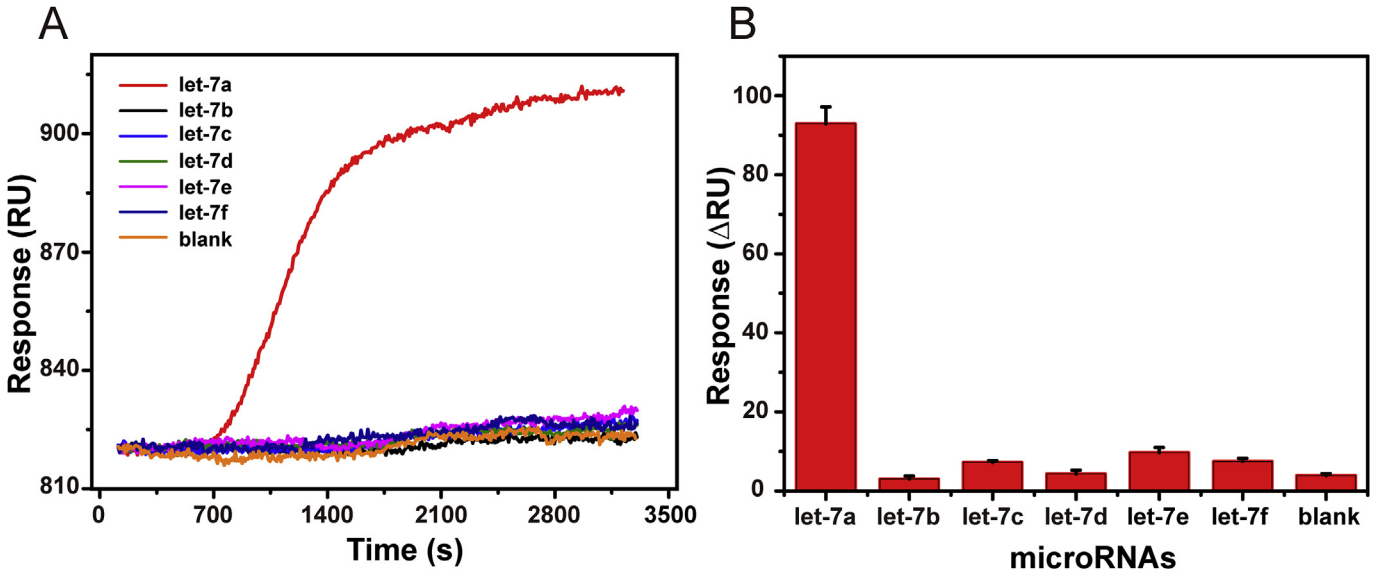


Fig. 4. SPR sensorgrams and SPR signal response corresponding to various miRNAs (10 pM). All data expressed as mean ± standard variation (n = 3).

exhibited good specificity for let-7a miRNA detection. This is because the sufficiently stable stem-loop structure of H1 and H2 which can only be unfolded by the target miRNA to form the H1–H2 duplex. To further assess the reproducibility of the proposed SPRi biosensing method, five replicate measurements were conducted for 1 pM target let-7a miRNA. The coefficient of variations was 8.71%, which demonstrated good reproducibility of this method.

3.6. Recovery experiment

The ability to be applied in complex matrix of the method was investigated by testing miRNA in 10% diluted fetal bovine serum. Briefly, Different concentrations of the let-7a miRNA were mixed into 10% diluted fetal bovine serum for detection. The recovery was evaluated to be 102–109% (Table S2), indicating that the analytical performance of the SPRi biosensing method was not undermined in complex matrix. Therefore, the designed SPRi biosensing method may provide a potential tool for miRNA detection in biological samples.

4. Conclusions

In summary, an enzyme-free and label-free SPRi biosensing method was established for highly sensitive and specific detection of target miRNA based on catalytic hairpin assembly and spherical nucleic acid. The combination of catalytic hairpin assembly and spherical nucleic acid significantly enhanced the detection sensitivity of the method to let-7a miRNA with a limit of detection at 53.7 fM. The catalytic hairpin assembly provided a highly amplified efficiency and excellent specificity to distinguish members of homologous miRNA families without the participation of enzymes. Furthermore, the developed biosensing method could also be applied to detect let-7a miRNA in complex matrix. Therefore, this SPRi biosensor strategy offers a potential alternative for the quantification of miRNA in biomedical research and early clinical diagnosis.

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.

CRediT authorship contribution statement

Xiaotong Wei: Conceptualization, Methodology, Writing - original draft. **Dewei Liu:** Data curation, Software. **Min Zhao:** Software. **Tiantian Yang:** Visualization. **Yunpeng Fan:** Investigation. **Wenqin Chen:** Methodology. **Ping Liu:** Conceptualization. **Jianbo Li:** Data curation, Validation. **Shijia Ding:** Data curation, Writing - review & editing, Funding acquisition.

Acknowledgements

This work was funded by the National Natural Science Foundation of China (81873980, 21804015) and the National Science and Technology Major Project of the Ministry of Science and Technology of China (No. 2018ZX10732202).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2020.02.055.

References

[1] L. Natarelli, C. Geißler, G. Csaba, Y. Wei, M. Zhu, A. di Francesco, P. Hartmann, R. Zimmer, A. Schober, MiR-103 promotes endothelial maladaptation by targeting lncWDR59, Nat. Commun. 9 (2018) 2645.
[2] M. Konno, J. Koseki, A. Asai, A. Yamagata, T. Shimamura, D. Motooka, et al., Distinct methylation levels of mature microRNAs in gastrointestinal cancers, Nat. Commun. 29 (2019) 3888.
[3] J. Zheng, D.D. Ma, M.L. Shi, J.H. Bai, Y.H. Li, J.F. Yang, et al., A new enzyme-free quadratic SERS signal amplification approach for circulating microRNA detection in human serum, Chem. Commun. 51 (2015) 16271–16274.
[4] K. Cissell, S. Shrestha, S. Deo, MicroRNA detection: challenges for the analytica chemist, Anal. Chem. 79 (2007) 4754–4761.
[5] É. Várallyay, J. Burgyán, Z. Havelda, MicroRNA detection by northern blotting using locked nucleic acid probes, Nat. Protoc. 3 (2008) 190–196.
[6] C.Y. Yu, B.C. Yin, B.C. Ye, A universal real-time PCR assay for rapid quantification of microRNAs via the enhancement of base-stacking hybridization, Chem. Commun. 49 (2013) 8247–8249.
[7] X.M. Li, W. Cheng, D.D. Li, J.L. Wu, X.J. Ding, Q. Cheng, et al., A novel surface plasmon resonance biosensor for enzyme-free and highly sensitive detection of microRNA based on multi component nucleic acid enzyme (MNAzyme)-mediated catalyzed hairpin assembly, Biosens. Bioelectron. 80 (2016) 98–104.
[8] J.B. Fasoli, R.M. Corn, Surface enzyme chemistries for ultrasensitive microarray biosensing with SPR imaging, Langmuir 31 (2015) 9527–9536.
[9] J. Huang, L. Zhu, H. Ju, J. Lei, Telomerase triggered DNA walker with a superhairpin structure for human telomerase activity sensing, Anal. Chem. 91 (2019) 6981–6985.
[10] H. Zhang, M. Fan, J. Jiang, Q. Shen, C. Cai, J. Shen, Sensitive electrochemical biosensor for MicroRNAs based on duplex-specific nuclease-assisted target recycling followed with gold nanoparticles and enzymatic signal amplification, Anal. Chim. Acta 1064 (2019) 33–39.
[11] L. Zhu, J. Ye, S. Wang, M. Yan, Q. Zhu, J. Huang, X. Yang, Dual amplification ratiometric biosensor based on a DNA tetrahedron nanostructure and hybridization chain reaction for the ultrasensitive detection of microRNA-133a, Chem. Commun. 55 (2019) 11551–11554.
[12] J.L. Wu, Y. Huang, X.T. Bian, D.D. Li, Q. Cheng, S.J. Ding, Biosensing of BCR/ABL fusion gene using an intensity-interrogation surface plasmon resonance imaging system, Optic Commun. 377 (2016) 24–32.
[13] Y.X. Zhao, F. Chen, Q. Li, L.H. Wang, C.H. Fan, Isothermal amplification of nucleic acids, Chem. Rev. 115 (2015) 12491–12545.
[14] H. Tian, Y.Y. Sun, C.H. Liu, X.R. Duan, W. Tang, Z.P. Li, Precise quantitation of microRNA in a single cell with droplet digital PCR based on ligation reaction, Anal. Chem. 88 (2016) 11384–11389.
[15] Y. Li, C. Yu, C. Zhao, C. Ren, X. Zhang, Catalytic hairpin assembly induced dual signal enhancement for rapid detection of miRNA using fluorescence light-up silver nanocluster, Anal. Chim. Acta 1084 (2019) 93–98.
[16] Z. Chu, S. Xiao, Y. Liu, G. Xiong, D. Huang, S. Wang, X. Zhao, Z. Zhang, Rapid and sensitive detection of the IS6110 gene sequences of Mycobacterium tuberculosis based on hybridization chain reaction and reusable magnetic particles, Sensor. Actuator. B Chem. 282 (2019) 904–909.
[17] D. Li, X. Li, B. Shen, P. Li, Y. Chen, S. Ding, W. Chen, Aptamer recognition and proximity-induced entropy-driven circuit for enzyme-free and rapid amplified detection of platelet-derived growth factor-BB, Anal. Chim. Acta 1092 (2019) 102–107.
[18] X. Chen, N. Briggs, J.R. McLain, A.D. Ellington, Stacking nonenzymatic circuits for high signal gain, Proc. Natl. Acad. Sci. Unit. States Am. 110 (2013) 5386–5391.
[19] B. Li, A.D. Ellington, X. Chen, Rational, Modular adaptation of enzyme-free DNA circuits to multiple detection methods, Nucleic Acids Res. 39 (2011) e110.
[20] F.C. Hu, J.Y. Xu, Y. Chen, Surface plasmon resonance imaging detection of subfemtomolar microRNA, Anal. Chem. 89 (2017) 10071–10077.
[21] W.Y. Nie, Q. Wang, L.Y. Zou, Y. Zheng, X.F. Liu, X.H. Yang, et al., Low-fouling surface plasmon resonance sensor for highly sensitive detection of microRNA in a complex matrix based on the DNA tetrahedron, Anal. Chem. 90 (2018) 12584–12591.
[22] J.L. Rouge, T.L. Sita, L. Hao, F.M. Kouri, W.E. Briley, A.H. Stegh, C.A. Mirkin, Ribozyme-spherical nucleic acids, J. Am. Chem. Soc. 137 (2015) 10528–10531.
[23] D. Li, Y. Xu, L. Fan, B. Shen, X. Ding, R. Yuan, X. Li, W. Chen, Target-driven rolling walker based electrochemical biosensor for ultrasensitive detection of circulating tumor DNA using doxorubicin@tetrahedron-Au tags, Biosens. Bioelectron. 148 (2020) 111826.
[24] C. Zhong, L. Yi, H. Mei, J. Dai, W. Hua, Z. Lu, Y. Liu, C. Li, H. Lu, Chip architecture-enabled sensitivity enhancement of oblique-incidence reflectivity difference for label-free protein microarray detection, Sensor. Actuator. B Chem. 294 (2019) 216–223.
[25] J.W. Liu, Y. Lu, Preparation of aptamer-linked gold nanoparticle purple aggregates for colorimetric sensing of analytes, Nat. Protoc. 1 (2006) 246–252.
[26] D.Z. Wang, W. Tang, X.J. Wu, X.Y. Wang, G.J. Chen, Q. Chen, et al., Highly selective detection of single-nucleotide polymorphisms using a quartz crystal microbalance biosensor based on the toehold-mediated strand displacement reaction, Anal. Chem. 84 (2012) 7008–7014.
[27] B.L. Li, Y. Jiang, X. Chen, A.D. Ellington, Probing spatial organization of DNA strands using enzyme-free hairpin assembly circuits, J. Am. Chem. Soc. 134 (2012) 13918–13921.
[28] K.H. Hao, Y. He, H.T. Lu, S.T. Pu, Y.N. Zhang, H.F. Dong, et al., High-sensitive surface plasmon resonance microRNA biosensor based on streptavidin functionalized gold nanorods-assisted signal amplification, Anal. Chim. Acta 954 (2017) 114–120.
[29] K. Zeng, H.Y. Li, Y.Y. Peng, Gold nanoparticle enhanced surface plasmon resonance imaging of microRNA-155 using a functional nucleic acid-based amplification machine, Mikrochim. Acta 184 (2017) 2637–2644.
[30] A. Sguassero, A. Artiga, C. Morasso, R.R. Jimenez, R.M. Rapun, R. Mancuso, et al., A simple and universal enzyme-free approach for the detection of multiple microRNAs using a single nanostructured enhancer of surface plasmon resonance imaging, Anal. Bioanal. Chem. 411 (2019) 1873–1885.
[31] B.C. Yin, Y.Q. Liu, B.C. Ye, One-step, multiplexed fluorescence detection of microRNAs based on duplex-specific nuclease signal amplification, J. Am. Chem. Soc. 134 (2012) 5064–5067.
[32] Y.S. Borghei, M. Hosseini, M.R. Ganjali, Fluorometric determination of microRNA via FRET between silver nanoclusters and CdTe quantum dots, Microchim Acta 184 (2017) 4713–4721.
[33] B.C. Li, F. Liu, Y.Y. Peng, Y.L. Zhou, W.X. Fan, H.S. Yin, et al., Two-stage cyclic enzymatic amplification method for ultrasensitive electrochemical assay of microRNA-21 in the blood serum of gastric cancer patients, Biosens. Bioelectron. 79 (2016) 307–312.
[34] J.R. Yang, M. Tang, W. Diao, W.B. Cheng, Y. Zhang, Y.R. Yan, Electrochemical strategy for ultrasensitive detection of microRNA based on MNAzyme-mediated rolling circle amplification on a gold electrode, Microchim Acta 183 (2016) 3061–3067.
[35] Q. Li, Q. Wang, X.H. Yang, K.M. Wang, H. Zhang, W.Y. Nie, High sensitivity surface plasmon resonance biosensor for detection of microrna and small molecule based on graphene oxide-gold nanoparticles composites, Talanta 174 (2017) 521–526.
[36] R.J. Liu, Q. Wang, Q. Li, X.H. Yang, K.M. Wang, W.Y. Nie, Surface plasmon resonance biosensor for sensitive detection of microrna and cancer cell using multiple signal amplification strategy, Biosens. Bioelectron. 87 (2016) 433–438.
[37] W.Y. Nie, Q. Wang, X.H. Yang, H. Zhang, Z.P. Li, L. Gao, et al., High sensitivity surface plasmon resonance biosensor for detection of microrna based on gold nanoparticles-decorated molybdenum sulfide, Anal. Chim. Acta 993 (2017) 55–62.