Analytica Chimica Acta 1219 (2022) 340027

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Split-aptamer mediated regenerable temperature-sensitive electrochemical biosensor for the detection of tumour exosomes

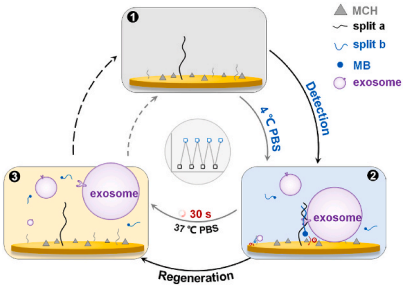
Dongfang Liu [a], Jinlu Tang [b,**], Hui Xu [a], Kun Yuan [a], Aaron Albert Aryee [a], Cuijie Zhang [a], Hongmin Meng [a], Lingbo Qu [a,c], Zhaohui Li [a,c,*]

[a] College of Chemistry, Institute of Analytical Chemistry for Life Science, Zhengzhou Key Laboratory of Functional Nanomaterial and Medical Theranostic, Zhengzhou University, Zhengzhou, 450001, PR China
[b] School of Basic Medical Sciences, Zhengzhou University, Zhengzhou, 450001, PR China
[c] Institute of Chemical Biology and Clinical Application of the First Affiliate Hospital, Zhengzhou University, Zhengzhou, 450001, PR China

HIGHLIGHTS

- A split-aptamer mediated regenerable temperature-sensitive (SMRT) biosensor was built for the first time.
- The sensitive and specific detection of SMMC-7721 exosomes was successful implementation.
- The SMRT biosensor could be regenerated simply and quickly by adjusting the temperature to 37 °C in 30 s.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Exosomes
Split-aptamer
Temperature-sensitive
Regenerable
Electrochemical biosensor

ABSTRACT

In this paper, a split-aptamer mediated regenerable temperature-sensitive (SMRT) electrochemical biosensor was constructed for the detection of exosomes. The split-aptamer used in this SMRT biosensor was composed of two fragments, one of which was immobilized on the surface of an electrode via sulfhydryl groups and named split-a and the other was labelled with methylene blue and named split-b. The two fragments could form sandwich structures at the electrode surface via target-induced self-assembly in the presence of target exosomes at 4 °C in PBS, and then realizing the detection of exosomes via voltammetry. In addition, due to the temperature sensitivity of the split-aptamer, the electrode could be regenerated through temperature-induced disassembly of the sandwich structures. Consequently, the SMRT biosensor realized sensitive and specific analysis of target exosomes with a limit of detection of 1.5×10^6 particles/mL and could be quickly and easily regenerated by washing with PBS at 37 °C for 30 s without any additives. This is the first study on the construction of a reproducible electrochemical biosensor using a split-aptamer for the specific detection of tumour exosomes, and may provide an innovative strategy for the economical and efficient design of regenerable electrochemical biosensors.

* Corresponding author. College of Chemistry, Institute of Analytical Chemistry for Life Science, Zhengzhou Key Laboratory of Functional Nanomaterial and Medical Theranostic, Zhengzhou University, Zhengzhou, 450001, PR China.
** Corresponding author.
E-mail addresses: tangjl@zzu.edu.cn (J. Tang), zhaohui.li@zzu.edu.cn (Z. Li).

https://doi.org/10.1016/j.aca.2022.340027
Received 25 March 2022; Received in revised form 11 May 2022; Accepted 29 May 2022
Available online 2 June 2022

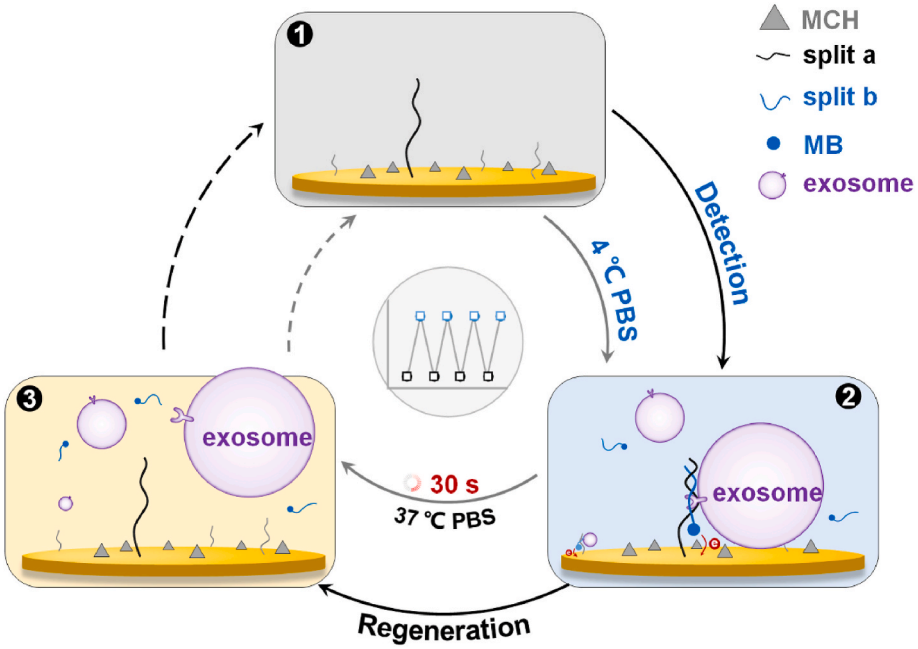
1. Introduction

Exosomes, which are double-layered vesicles with sizes of 40–160 nm, are widespread in the supernatants of cell cultures and in multiple body fluids [1]. These vesicles possess a variety of biomolecules from parental cells, including nucleic acids, proteins and lipids [2], and play significant roles in various physiological and pathological processes of the body, including tumour proliferation, metastasis, and immune modulation [3–5]. Hence, exosomes have become new and valid biomarkers for tumour screening, diagnosis and treatment [6]. However, due to their small volume and low content in biofluids at the early phases of cancer, realizing highly sensitive and selective detection of exosomes remains challenging [7].

Recently, numerous methods that are based on employing the surface proteins of exosomes as targets have been applied for the analysis of exosomes, including fluorescence [8,9], colorimetry [10], surface plasmon resonance [11], surface-enhanced Raman spectroscopy [12], and electrochemical methods [13,14]. Among them, the electrochemical method has aroused the interest of researchers due to its various advantages, such as rapid response speed, hypersensitivity, simple device, and low cost [15]. However, electrochemical biosensors have poor reproducibility and poor stability, which hinders their wide application as diagnostic equipment [16]. This is due mainly to discrepancies in the modifications of electrodes [17]. To solve this problem, a sensor regeneration strategy was proposed, which enables a sensor to repeatedly perform multiple detections, thereby substantially reducing divergence from sensor to sensor [18]. To realize the regeneration of electrochemical biosensors, most current strategies require additional chemical reagents, such as guanidine chloride [19], glycine [20], and urea [21]. Moreover, these strategies not only affect the type and dispersion of ions on the biosensor surface but also damage the probes that have been modified onto the electrode [22]. Consequently, the design of a reliable, gentle, simple, and fast strategy for the regeneration of electrochemical biosensors is vital.

Aptamers, which are single-stranded oligonucleotides with 20–60 bases that can identify targets with high affinity and specificity, have attracted extensive attention in the development of simple and universal electrochemical sensors [23–26]. Among them, split-aptamers have shown significant advantages in constructing electrochemical sensors [27–29]. A split-aptamer originates from one intact aptamer sequence that is artificially divided into two or more fragments [30]. They are more flexible and easier to design for sensors due to their short sequences [31,32]. In addition, the split-aptamer fragments can be induced to aggregate to form a recognition configuration in the presence of the target [33,34]. The recognition configuration is mostly a sandwich structure, which is very convenient for the construction of electrochemical sensors [35–37]. However, building reproducible electrochemical biosensors using aptamers or split-aptamers under mild conditions remains challenging.

Recently, our group and Wang's group explored a few successful split-aptamer based probes with temperature sensitivity for the detection of tumour cells or exosomes [8,38]. In this work, we report a split-aptamer mediated regenerable temperature-sensitive (SMRT) electrochemical biosensor for the sensitive and selective detection of tumour exosomes for the first time. As displayed in Scheme 1, the two fragments, namely, split-a and split-b, that originate from an integrated aptamer that can specifically recognize the N-glycoprotein on the surfaces of SMMC-7721 cells [8,39], are used as recognition elements in this study. After modification with a sulfhydryl group, split-a can be immobilized on a gold electrode surface by Au–S bonds. Redox moiety methylene blue is modified onto split-b. In the presence of exosomes at 4 °C, fragments split-a and split-b move close to each other to capture the target and form a recognition configuration that can realize the specific and sensitive detection of exosomes as the methylene blue approaches the electrode, thereby generating a redox signal. The recognition configuration that is formed by the split-aptamer and exosomes can be destroyed by rinsing the electrode in PBS at 37 °C due to the split-aptamer losing its recognition ability at higher temperatures. Therefore, the electrodes can be regenerated in as little as 30 s and then used directly for subsequent testing without any additional modifications.



Scheme 1. Schematic illustration of split-aptamer mediated regenerable temperature-sensitive (SMRT) electrochemical biosensor for the detection of tumor exosomes.

2. Materials and method

2.1. Reagents

6-Mercaptohexanol (MCH) and Tris (2-carboxyethyl) phosphine (TCEP), were bought from Sigma. DNA oligonucleotides, RPMI-1640 and DMEM medium, and Dulbecco's phosphate buffered saline (D-PBS) were obtained from Sangon Biotech Co. Ltd. (Shanghai, China). Tris aminomethane hydrochloride (Tris-HCl) was purchased from Aladdin Reagents Inc. (Shanghai, China). Fetal Bovine serum (FBS) was obtained from System Biosciences (USA), bovine serum albumin (BSA) and yeast tRNA were bought from Sinopharm Chemical Reagent Co., Ltd. The chemical reagents used in this experiment were analytical grades. Milli-Q water (18 MΩ/cm resistivity) was used to prepare solutions.

The reaction buffer was Tris-HCl buffer (10 mM, pH 7.4) including 10 mM $MgCl_2$ and 500 mM NaCl. Binding buffer was prepared by adding glucose (4.5 mg/mL), BSA (2 mg/mL), yeast tRNA (1 mg/mL) and $MgCl_2$ (5 mg/mL) into D-PBS.

2.2. Sequences of oligos

All sequences of oligos were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China) and are listed in Table S1. Additional details on the aptamer were displayed in Fig. S1. The dissociation constant of the splitting of the aptamer was about 5.8 nM, the sequences were as follows:

Split-a: 5′–$SH(CH_2)_6$–TTT TTT CGT CAG GTT GAG CTG AAG ATC GTA CCG TGA AGT CCG T- 3'

Split-b: 5' -ACG GAC TAC CTG ACG TTT TTT-methylene blue- 3'

2.3. Apparatus

All Electrochemical assays were implemented on CHI-660E electrochemical workstation (Chenhua Instrument Co. Ltd, Shanghai). The experiment was carried out in a three-electrode system, including gold working electrode with a diameter of 3 mm, saturated calomel reference electrode, and platinum counter electrode. Transmission electron microscopy (FEI-Tecnai G2, USA) and scanning electron microscopy (Hitachi SU8000, Japan) were used to characterize the morphology of exosomes. Nanoparticle tracking analysis (NanoSight 300, UK) was applied to quantify exosomes. Ultracentrifuge (Himac CP100NX, Japan) was used to isolate exosomes.

2.4. Cell culture and exosome purification

The process of cell culture and exosome purification was carried out according to a previous method [40]. SMMC-7721 (human hepatocellular cancer) and MCF-7 (human mammary carcinoma) cells were seeded in DMEM. Ramos cells (human Burkitt's lymphoma, B cell line) and CCRF-CEM (human acute lymphoblastic leukaemia, T cell line) were cultured in RPMI-1640 medium. All culture media were supplemented with 10% FBS and penicillin/streptomycin (100 IU/mL). The cells were cultured in a sterile incubator with 5% CO_2 at 37 °C. The cells were washed twice with D-PBS when their density reached 70%–80%, after which they were cultured in exosome-free FBS medium for 48 h. The supernatant of the cells was collected. The collected supernatant was centrifuged at 300 g for 10 min, 2000 g for 10 min, and 10000 g for 30 min in sequence, after which it was filtered through a 0.22 μm filter. Finally, the supernatant was precipitated by ultracentrifugation at 100000 g for 70 min twice to collect exosomes. All centrifugation operations were completed at 4 °C. The collected exosomes were resuspended in PBS and stored at −80 °C.

2.5. Construction of the SMRT biosensor

The gold electrodes were pretreated according to a previously reported method [41]. For the construction of the SMRT biosensor, split-a (1 μM) was mixed with 3 mM TCEP in Tris-HCl (10 mM, pH 7.4) at room temperature for 1 h to break the disulfide bond. Subsequently, 6 μL of split-a (200 nM) was added onto the surfaces of the pre-treated electrodes and incubated in the dark overnight at room temperature. Then, the modified electrodes were treated with MCH (1 mM) at room temperature for 40 min. After that, each electrode was incubated with 6 μL of a solution that was composed of 800 nM split-b and one of various concentrations of exosomes for 30 min at 4 °C. At each step, the electrodes were rinsed with D-PBS slowly and carefully. Finally, these fabricated electrodes were immersed in D-PBS for electrochemical measurements.

2.6. Electrochemical measurements

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) measurements were performed in PBS that contained $[Fe(CN)_6]^{3-/4-}$ (5 mM) and KCl (0.1 M). The parameters for the EIS study were as follows: frequency: 0.1 Hz–100 kHz and amplitude: 5 mV. Differential pulse voltammetry (DPV) measurements were conducted in D-PBS under the following parameters: potential: −0.55–0 V; amplitude: 0.06 V; pulse width: 0.005 s; step potential: 0.004 V; and pulse period: 0.3 s.

3. Results and discussion

3.1. Characterization and quantification of exosomes

The exosomes used in this study were characterized by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). As displayed in Fig. 1, the exosomes were spherical vesicles with an average diameter of 150 nm. The concentration of exosomes (diluted 100 times) was calculated as 2.0×10^9 particles/mL using nanoparticle tracking analysis (NTA). The results were consistent with previously reported results [42,43].

3.2. Characterization of the successfully developed SMRT biosensor

Firstly, modification processes of the electrode were characterized by EIS and CV. Fig. 2A shows Nyquist plots of impedance spectra at electrodes in various modification stages. The diameter of the semicircle of the EIS curve indicated the electron transfer resistance (R_{CT}) [44,45]. The bare gold electrode exhibited a very small semicircle with a low impedance value (R_{CT} = 28.81 Ω), whereas the gold electrode that was modified with split-a displayed a larger semicircle with an R_{CT} value of approximately 1304 Ω because the modified probes on the electrode surface hindered the transfer of electrons [46]. The impedance value (R_{CT} = 2265 Ω) further increased after the electrode was sealed with MCH because MCH blocked the unmodified active sites and caused the rate of electron conduction to decrease. When the electrode was incubated with the exosomes and the aptamers, the impedance value continued to increase (R_{CT} = 3351 Ω), thereby demonstrating that exosomes were captured on the electrode by the split-aptamer. The CV results were consistent with the EIS results (Fig. 2B), thereby indicating that the SMRT biosensor was successfully constructed.

3.3. Feasibility of the constructed biosensor

To validate the feasibility of the SMRT biosensor, DPV experiments were conducted on various electrodes under the same conditions. A preliminary experiment using a complementary sequence to probe split-a demonstrated the rationality of the design (Fig. S2). Then, the feasibility of the SMRT biosensor was verified using exosomes. As shown in

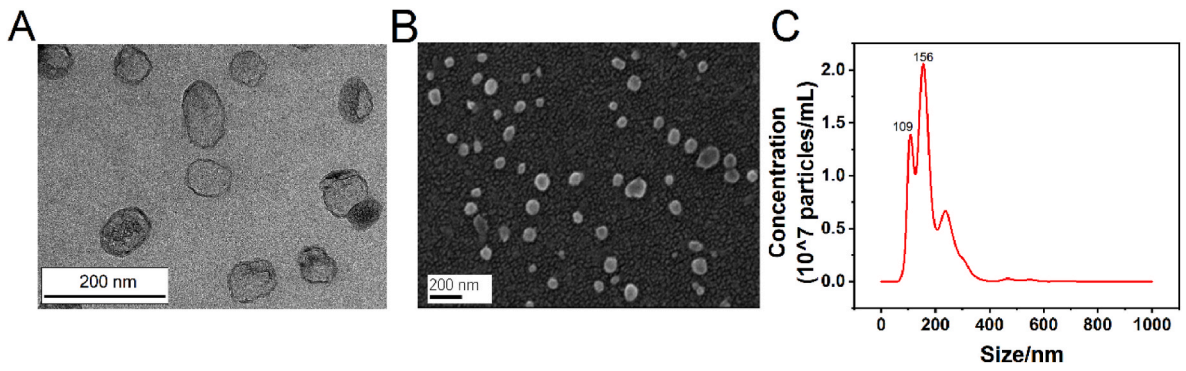


Fig. 1. The characterization of SMMC-7721 cells-derived exosomes. (A) TEM image of exosomes. (B) SEM image of exosomes. (C) Size distribution of exosomes analyzed by NTA.

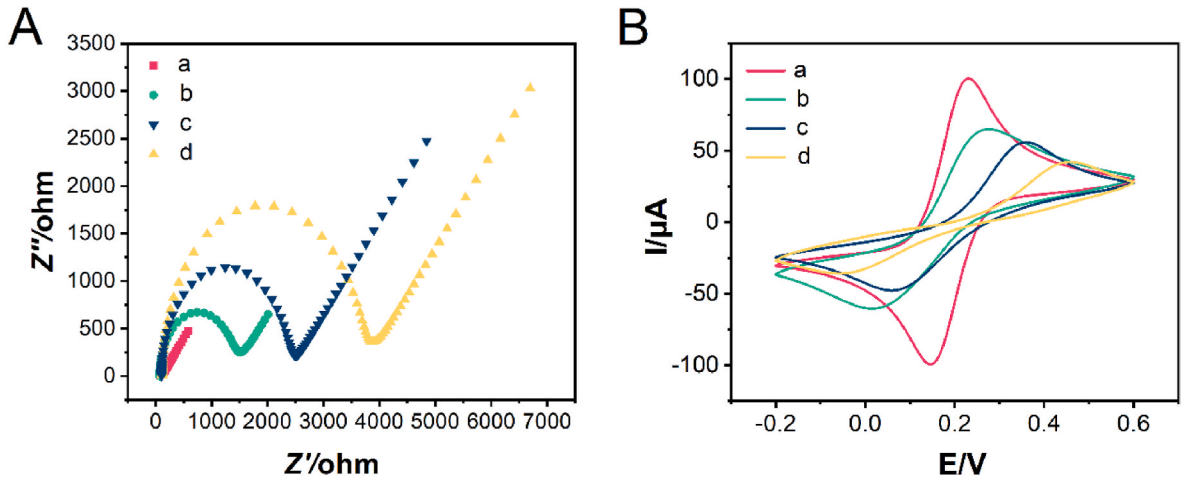


Fig. 2. (A) EIS and (B) CV responses of different electrodes in PBS containing 5 mM $[Fe(CN)_6]^{3-/4-}$. (a) bare Au, (b) split-a/bare Au, (c) MCH/split-a/bare Au and (d) exosomes/split-b/MCH/split-a/bare Au.

Fig. 3A, after incubation with split-a, split-b and exosomes (4.0×10^8 particles/mL) on the electrode, an obvious oxidation peak of methylene blue was observed at −0.266 V (curve a). In contrast, only small currents were generated in the absence of exosomes (curve b), split-a (curve d) and split-b (curve c). To further prove that the signal was generated by the split-aptamer recognizing exosomes, a control fragment, namely, split-ctrl-b, that was modified with methylene blue was used. As curve e shows, only a weak oxidation peak was obtained, which was likely due to the nonspecific absorption of split-ctrl-b. These results confirmed that the target exosomes could induce the reassembly of the split-aptamer into the recognition configuration and further promote the movement of the methylene blue that was modified onto split-b closer to the electrode to generate signals. Therefore, the constructed SMRT biosensor could be used to detect exosomes.

3.4. Analytical performance of the SMRT biosensor

To realize the best analysis performance, the experimental conditions were optimized by adjusting the molar ratio of split-a to split-b, the concentration of split-a, and the incubation time (Fig. S3). Then, the performance of the biosensor for the detection of exosomes was explored by DPV under optimal experimental conditions (using 200 nM split-a and 800 nM split-b, incubated for 30 min). As displayed in Fig. 3B, the current was enhanced as the number of target exosomes increased, and the DPV intensity showed a linear relationship with the logarithm of the exosome concentration in the range from 2.0×10^6 to 4.0×10^8 particles/mL (Fig. 3C). The linear regression equation was identified as $I = 207.7 \lg C - 1065$, with $R^2 = 0.9974$. The limit of detection (LOD) was 1.5×10^6 particles/mL, which was at the same level as the values in most previous reports (Table S2).

Next, the selectivity of the fabricated biosensor was explored using three groups of control exosomes (MCF-7, CEM, and Romas) with equal concentrations of 4.0×10^8 particles/mL. The DPV response currents of the samples are displayed in Fig. 3D. The current of the SMRT biosensor to SMMC-7721 exosomes was approximately 700 nA, and those of the other samples were all lower than 200 nA. The higher signal showed that the split-aptamer reassembled into a sandwich structure at the surface of the electrode in the presence of target SMMC-7721 exosomes, which was consistent with previous reports [8]. In summary, the results indicated that this strategy has excellent specificity for the detection of target exosomes.

The practicality of the SMRT biosensor in a complex biological environment was also tested using binding buffers that contained 10% and 20% FBS. As shown in Fig. S4, the current responses to target exosomes in 10% and 20% FBS were similar to those in PBS buffer solution. This result indicated that the SMRT biosensor has anti-interference ability and application potential for the assay of exosomes in intricate biological environments.

3.5. Regeneration performance of the SMRT biosensor

The electrode regeneration process is illustrated in Fig. 4A. In brief, the biosensor performed the detection function through exosome-induced assembly of the split-aptamer on the electrode surface at 4 °C and could be regenerated by changing the temperature to 37 °C due to the disassembly of the split-aptamer at high temperatures [47,48]. First,

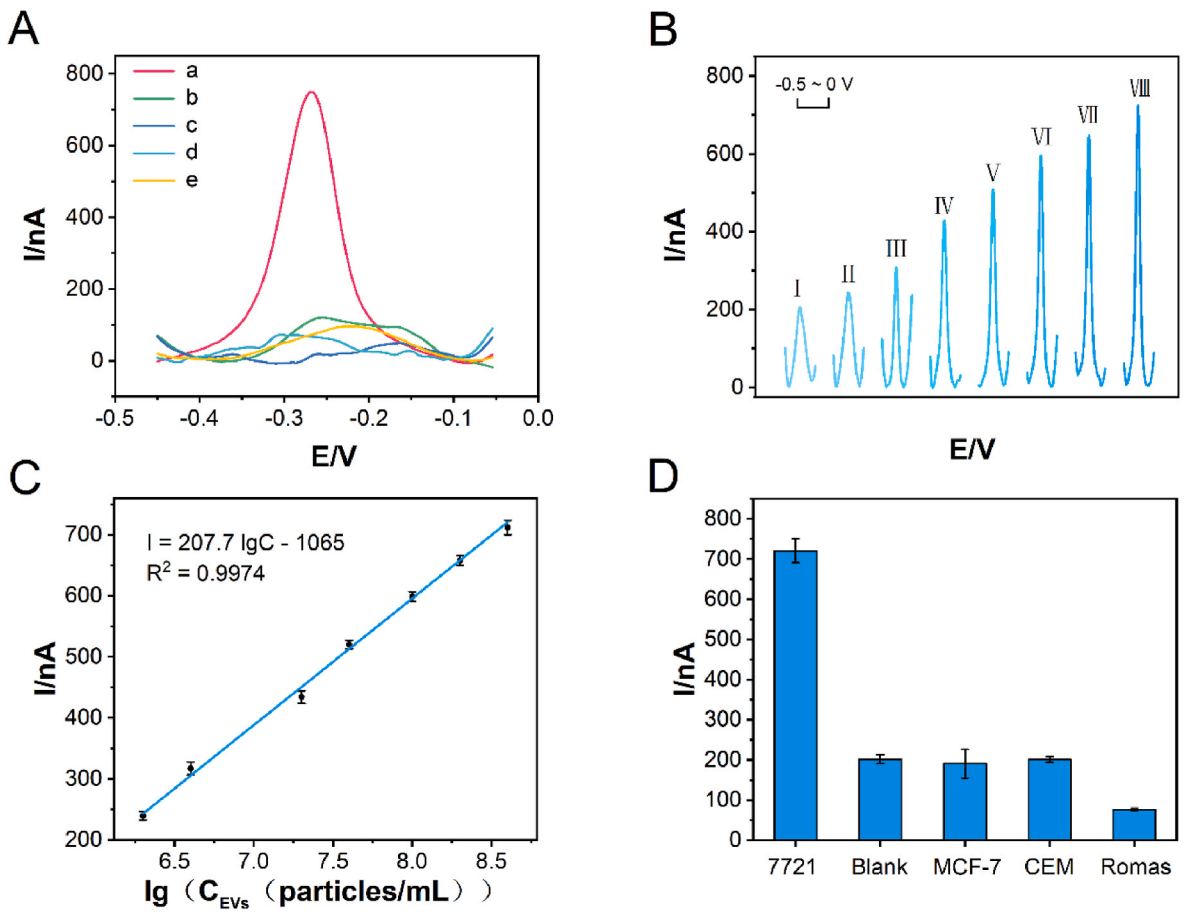


Fig. 3. (A) DPV responses of the Au electrode after incubation with (a) split-a/split-b/target exosomes, (b) split-a/split-b, (c) split-a/target exosomes, (d) split-b/target exosomes and (e) split-a/split-ctrl-b/target exosomes. The concentration of exosomes used for this process was 4.0×10^8 particles/mL. (B) DPV responses of MB under different concentrations of SMMC-7721 cells-derived exosomes. The concentration of exosomes (from I to Ⅷ) was 0, 2.0×10^6, 4.0×10^6, 2.0×10^7, 4.0×10^7, 1.0×10^8, 2.0×10^8, and 4.0×10^8 particles/mL. (C) The relationship between current intensity and the logarithm value of SMMC-7721 exosomes concentration. (D) The specificity to exosomes isolated from MCF-7 cells, CEM cells and Ramos cells. Error bars: standard deviations from three repeated experiments. The concentration of exosomes used for this process was 4.0×10^8 particles/mL.

the temperature sensitivity of the split-aptamer was explored. As shown in Fig. 4B, the value of ΔI at 4 °C was significantly greater than that at 37 °C. This was because the split-aptamer recognized exosomes effectively at 4 °C and then prompted target-induced reassembly of split-a and split-b to form a sandwich structure at the surface of the electrode, thereby resulting in a high current value. In contrast, the split-aptamer almost lost the identification ability at 37 °C; hence, there was no obvious current signal. The concentration of exosomes in this process was 4.0×10^8 particles/mL. Next, the regenerative ability of the SMRT biosensor was investigated. The time of the electrode regeneration was optimized as 30 s (Fig. S5). As shown in Fig. 4C, the electrode with a high current value (curve i) was refreshed by rinsing at 37 °C in PBS for 30 s (curve ii). Then, the refreshed electrode operated again when incubated with exosomes and split-b at 4 °C in PBS. The results indicated that the SMRT biosensor was regenerable. In addition, throughout the execution of the regeneration process for 5 cycles, the electrochemical biosensor retained its original recognition efficiency with a relative standard deviation (RSD) of between 3.0% and 10.6% (Fig. 4D, Table S3). Meanwhile, one electrode underwent constant regeneration to detect different samples, as indicated in Fig. 4E. The biosensor showed a strong signal for target exosomes but no obvious signal for control exosomes. This result demonstrated that the SMRT biosensor maintained strong binding affinity and high selectivity after regeneration. The temperature-controlled regeneration strategy that was used in this work was more effective and faster than those in most previous works (Table S4).

4. Conclusions

We constructed a split-aptamer mediated regenerable temperature-sensitive (SMRT) biosensor for the detection of exosomes for the first time. The SMRT biosensor was simply and ingeniously designed using only a split-aptamer and could detect tumour exosomes sensitively and specifically with a low limit of detection of 1.5×10^6 particles/mL. In addition, this SMRT biosensor could be regenerated simply and quickly by adjusting the temperature to 37 °C in 30 s. The main disadvantage of the biosensor is its relatively low current value, which could be improved by combining amplification strategies using enzyme or DNA nanotechnology. As a simple, effective, and reproducible biosensor for the specific detection of tumour exosomes, the development of the SMRT biosensor may provide an innovative strategy for the economical and efficient design of regenerable electrochemical biosensors.

CRediT authorship contribution statement

Dongfang Liu: Methodology, Data curation, Formal analysis, Writing – original draft. **Jinlu Tang:** Conceptualization, Methodology, Visualization, Funding acquisition, Project administration, Writing – review & editing, Supervision. **Hui Xu:** Methodology, Formal analysis. **Kun Yuan:** Methodology, Formal analysis. **Aaron Albert Aryee:** Writing – review & editing. **Cuijie Zhang:** Methodology, Formal analysis. **Hongmin Meng:** Formal analysis, Supervision. **Lingbo Qu:** Project administration, Supervision. **Zhaohui Li:** Conceptualization,

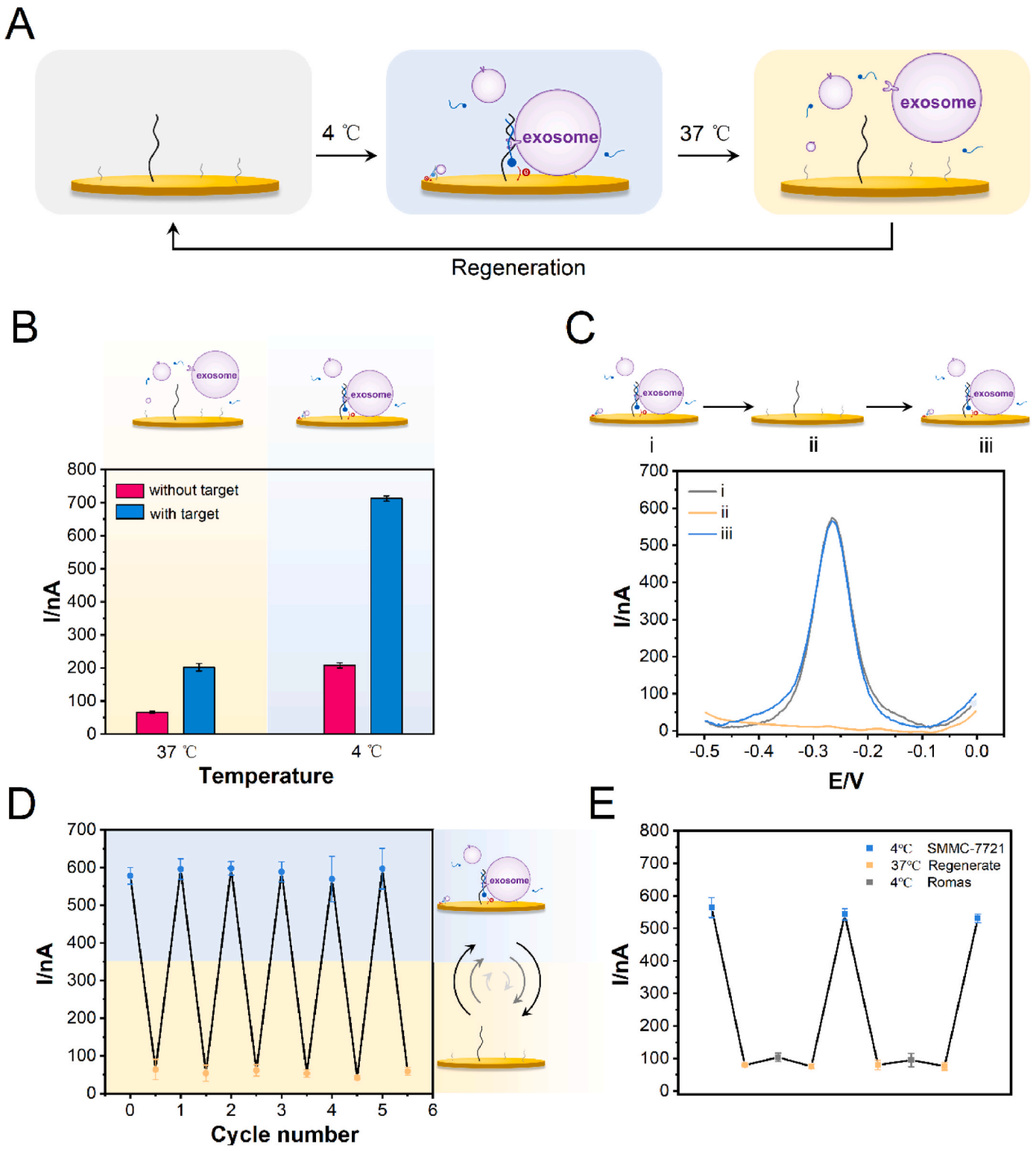


Fig. 4. (A) Schematic diagram of the electrode regeneration process. (B) The temperature sensitivity of the SMRT biosensor. The concentration of exosomes used for this process was 4.0×10^8 particles/mL. (C) The reproducibility of SMRT biosensor: (i) detection of target exosomes at 4 °C, (ii) regeneration at 37 °C for 30s, and (iii) re-detection of target exosomes using the regenerated electrode at 4 °C. The concentration of exosomes used for this process was 1.0×10^8 particles/mL. (D) Regeneration of the SMRT biosensor under stepwise temperature regulation at 4 °C and then at 37 °C for the detection of SMMC-7721 exosomes over 5 recycles, the RSD ranged from 3.0% to 10.6%. The concentration of exosomes used for this process was 1.0×10^8 particles/mL. (E) Selectivity of the regenerated SMRT biosensor for exosomes detection. The concentration of exosomes used for this process was 1.0×10^8 particles/mL. Error bars: standard deviations from three repeated experiments.

Methodology, Funding acquisition, Project administration, Writing – review & editing, Supervision.

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.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grants 21804120 and 21974125), 111 Project of Henan Province (CXJD2021001), Program for Innovative Research Team (in Science and Technology) in University of Henan Province (22TRTSTHN002) and Collaborative Innovation Project of Zhengzhou (Zhengzhou University) (18XTZX12002).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2022.340027.

References

[1] R. Kalluri, V.S. LeBleu, The biology, function, and biomedical applications of exosomes, Science 367 (2020), eaau6977.
[2] J. Skog, T. Würdinger, S. van Rijn, D.H. Meijer, L. Gainche, W.T. Curry, B.S. Carter, A.M. Krichevsky, X.O. Breakefield, Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers, Nat, Cell Biol. 10 (2008) 1470–1476.
[3] C. Jiang, N. Zhang, X. Hu, H. Wang, Tumor-associated exosomes promote lung cancer metastasis through multiple mechanisms, Mol. Cancer 20 (2021) 117.
[4] P. Qiu, J. Zhou, J. Zhang, Y. Dong, Y. Liu, Exosome: the regulator of the immune system in sepsis, Front. Pharmacol. 12 (2021) 880.
[5] L. Xi, M. Peng, S. Liu, Y. Liu, X. Wan, Y. Hou, Y. Qin, L. Yang, S. Chen, H. Zeng, Y. Teng, X. Cui, M. Liu, Hypoxia-stimulated ATM activation regulates autophagy-associated exosome release from cancer-associated fibroblasts to promote cancer cell invasion, J. Extracell. Vesicles 10 (2021), e12146.
[6] V.S. LeBleu, R. Kalluri, Exosomes as a multicomponent biomarker platform in cancer, Trends Cancer 6 (2020) 767–774.
[7] W. Yu, J. Hurley, D. Roberts, S.K. Chakrabortty, D. Enderle, M. Noerholm, X. O. Breakefield, J.K. Skog, Exosome-based liquid biopsies in cancer: opportunities and challenges, Ann. Oncol. 32 (2021) 466–477.
[8] J. Chen, J. Tang, H.-M. Meng, Z. Liu, L. Wang, X. Geng, Y. Wu, L. Qu, Z. Li, Recognition triggered assembly of split aptamers to initiate a hybridization chain reaction for wash-free and amplified detection of exosomes, Chem. Commun. 56 (2020) 9024–9027.
[9] X. Zhao, C. Luo, Q. Mei, H. Zhang, W. Zhang, D. Su, W. Fu, Y. Luo, Aptamer-cholesterol-mediated proximity ligation assay for accurate identification of exosomes, Anal. Chem. 92 (2020) 5411–5418.
[10] Y. Zhang, Q. Su, D. Song, J. Fan, Z. Xu, Label-free detection of exosomes based on ssDNA-modulated oxidase-mimicking activity of CuCo2O4 nanorods, Anal. Chim. Acta 1145 (2021) 9–16.
[11] W. Chen, J. Li, X. Wei, Y. Fan, H. Qian, S. Li, Y. Xiang, S. Ding, Surface plasmon resonance biosensor using hydrogel-AuNP supramolecular spheres for determination of prostate cancer-derived exosomes, Microchim. Acta 187 (2020) 590.
[12] X. Zhang, C. Liu, Y. Pei, W. Song, S. Zhang, Preparation of a novel Raman probe and its application in the detection of circulating tumor cells and exosomes, ACS Appl. Mater. Interfaces 11 (2019) 28671–28680.
[13] Y. Guo, Q. Cao, Q. Feng, Catalytic hairpin assembly-triggered DNA walker for electrochemical sensing of tumor exosomes sensitized with Ag@C core-shell nanocomposites, Anal. Chim. Acta 1135 (2020) 55–63.
[14] J. Jiang, Y. Yu, H. Zhang, C. Cai, Electrochemical aptasensor for exosomal proteins profiling based on DNA nanotetrahedron coupled with enzymatic signal amplification, Anal. Chim. Acta 1130 (2020) 1–9.
[15] W. Wang, H. Tang, Y. Wu, Y. Zhang, Z. Li, Highly electrocatalytic biosensor based on Hemin@AuNPs/reduced graphene oxide/chitosan nanohybrids for non-enzymatic ultrasensitive detection of hydrogen peroxide in living cells, Biosens, Bioelectron 132 (2019) 217–223.
[16] Y. Sheng, T. Zhang, S. Zhang, M. Johnston, X. Zheng, Y. Shan, T. Liu, Z. Huang, F. Qian, Z. Xie, Y. Ai, H. Zhong, T. Kuang, C. Dincer, G.A. Urban, J. Hu, A CRISPR/Cas13a-powered catalytic electrochemical biosensor for successive and highly sensitive RNA diagnostics, Biosens. Bioelectron. 178 (2021), 113027.
[17] H. Li, P. Dauphin-Ducharme, G. Ortega, K.W. Plaxco, Calibration-free electrochemical biosensors supporting accurate molecular measurements directly in undiluted whole blood, J. Am. Chem. Soc. 139 (2017) 11207–11213.
[18] J.A. Goode, J.V.H. Rushworth, P.A. Millner, Biosensor regeneration: a review of common techniques and outcomes, Langmuir 31 (2015) 6267–6276.
[19] T. Wang, E. Viennois, D. Merlin, G. Wang, Microelectrode miRNA sensors enabled by enzymeless electrochemical signal amplification, Anal. Chem. 87 (2015) 8173–8180.
[20] G. Zeng, C. Zhang, D. Huang, C. Lai, L. Tang, Y. Zhou, P. Xu, H. Wang, L. Qin, M. Cheng, Practical and regenerable electrochemical aptasensor based on nanoporous gold and thymine-Hg2+-thymine base pairs for Hg2+ detection, Biosens. Bioelectron. 90 (2017) 542–548.
[21] L. Wang, Y. Wen, X. Yang, L. Xu, W. Liang, Y. Zhu, L. Wang, Y. Li, Y. Li, M. Ding, S. Ren, Z. Yang, M. Lv, J. Zhang, K. Ma, G. Liu, Ultrasensitive electrochemical DNA biosensor based on a label-free assembling strategy using a triblock polyA DNA probe, Anal. Chem. 91 (2019) 16002–16009.
[22] J.-Y. Chen, L.-Y. Yang, Z.-J. Liu, Q.-X. Wei, Y. Zhang, B. Wu, G.-X. Zhong, L.-X. Fu, X.-H. Lin, X.-H. Weng, X.-W. Xu, DNA nanosieve-based regenerative electrochemical biosensor utilizing nucleic acid flexibility for accurate allele typing in clinical samples, ACS Sens. 6 (2021) 1348–1356.
[23] M.R. Dunn, R.M. Jimenez, J.C. Chaput, Analysis of aptamer discovery and technology, Nat. Rev. Chem 1 (2017), 0076.
[24] T. Hermann, D.J. Patel, Adaptive recognition by nucleic acid aptamers, Science 287 (2000) 820–825, https://doi.org/10.1126/science.287.5454.820.
[25] T. Mo, X. Liu, Y. Luo, L. Zhong, Z. Zhang, T. Li, L. Gan, X. Liu, L. Li, H. Wang, X. Sun, D. Fan, Z. Qian, P. Wu, X. Chen, Aptamer-based biosensors and application in tumor theranostics, Cancer Sci. 113 (2022) 7–16.
[26] T.T.-Q. Nguyen, E.R. Kim, M.B. Gu, A new cognate aptamer pair-based sandwich-type electrochemical biosensor for sensitive detection of Staphylococcus aureus, Biosens. Bioelectron. 198 (2022), 113835.
[27] A. Chen, M. Yan, S. Yang, Split aptamers and their applications in sandwich aptasensors, Trac. Trends Anal. Chem. 80 (2016) 581–593.
[28] L. Feng, Z. Lyu, A. Offenhäusser, D. Mayer, Multi-level logic gate operation based on amplified aptasensor performance, Angew. Chem. Int. Ed. 54 (2015) 7693–7697.
[29] X. Zuo, Y. Xiao, K.W. Plaxco, High specificity, electrochemical sandwich assays based on single aptamer sequences and suitable for the direct detection of small-molecule targets in blood and other complex matrices, J. Am. Chem. Soc. 131 (2009) 6944–6945. High Specificity, X. Zuo, Y. Xiao, K.W. Plaxco.
[30] A.D. Kent, N.G. Spiropulos, J.M. Heemstra, General approach for engineering small-molecule-binding DNA split aptamers, Anal. Chem. 85 (2013) 9916–9923.
[31] M. Debiais, A. Lelievre, M. Smietana, S. Müller, Splitting aptamers and nucleic acid enzymes for the development of advanced biosensors, Nucleic Acids Res. 48 (2020) 3400–3422.
[32] X. Qi, X. Yan, Y. Zhao, L. Li, S. Wang, Highly sensitive and specific detection of small molecules using advanced aptasensors based on split aptamers: a review, TrAC Trends Anal. Chem. (Reference Ed.) 133 (2020), 116069.
[33] G. Mayer, The chemical biology of aptamers, Angew. Chem. Int. Ed. 48 (2009) 2672–2689.
[34] J. Li, G. He, B. Wang, L. Shi, T. Gao, G. Li, Fabrication of reusable electrochemical biosensor and its application for the assay of α-glucosidase activity, Anal. Chim. Acta 1026 (2018) 140–146.
[35] H.B. Seo, M.B. Gu, Aptamer-based sandwich-type biosensors, J. Biol. Eng. 11 (2017) 11.
[36] Z. Zhang, X. Zhao, J. Liu, J. Yin, X. Cao, Highly sensitive sandwich electrochemical sensor based on DNA-scaffolded bivalent split aptamer signal probe, Sensor. Actuator. B Chem. 311 (2020), 127920.
[37] M.A. Nameghi, N.M. Danesh, M. Ramezani, M. Alibolandi, K. Abnous, S. M. Taghdisi, An ultrasensitive electrochemical sensor for 17β-estradiol using split aptamers, Anal. Chim. Acta 1065 (2019) 107–112.
[38] J. Tang, Y. Lei, X. He, J. Liu, H. Shi, K. Wang, Recognition-Driven remodeling of dual-split aptamer triggering in situ hybridization chain reaction for activatable and autonomous identification of cancer cells, Anal. Chem. 92 (2020) 10839–10846.
[39] B. Yuan, Y. Sun, Q. Guo, J. Huang, X. Yang, Y. Chen, X. Wen, X. Meng, J. Liu, K. Wang, High signal-to-background ratio detection of cancer cells with activatable strategy based on target-induced self-assembly of split aptamers, Anal. Chem. 89 (2017) 9347–9353.
[40] Q. Liu, X. Yue, Y. Li, F. Wu, M. Meng, Y. Yin, R. Xi, A novel electrochemical aptasensor for exosomes determination and release based on specific host-guest interactions between cucurbit [7]uril and ferrocene, Talanta 232 (2021), 122451.
[41] P. Li, Y. Zhang, P. Gong, Y. Liu, W. Feng, H. Yang, Photoinduced atom transfer radical polymerization combined with click chemistry for highly sensitive detection of tobacco mosaic virus RNA, Talanta 235 (2021), 122803.
[42] H. Xiong, Z. Huang, Z. Yang, Q. Lin, B. Yang, X. Fang, B. Liu, H. Chen, J. Kong, Recent progress in detection and profiling of cancer cell-derived exosomes, Small 17 (2021), 2007971.
[43] M. Colombo, G. Raposo, C. Théry, Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles, Annu. Rev. Cell Dev. Biol. 30 (2014) 255–289.
[44] Z. Liu, S. Lei, L. Zou, G. Li, B. Ye, Grafting homogenous electrochemical biosensing strategy based on reverse proximity ligation and Exo III assisted target circulation for multiplexed communicable disease DNA assay, Biosens. Bioelectron. 167 (2020), 112487.
[45] J.A. Lee, S. Hwang, J. Kwak, S.I. Park, S.S. Lee, K.-C. Lee, An electrochemical impedance biosensor with aptamer-modified pyrolyzed carbon electrode for label-free protein detection, Sensor. Actuator. B Chem. 129 (2008) 372–379.

[46] C. Feng, J. Guo, G. Li, B. Ye, L. Zou, Novel multiple strand displacement reaction coupled hybridization chain reaction for label-free and ultrasensitive electrochemical Type b3a2 biosensing, Sensor. Actuator. B Chem. (2020), 128972.
[47] J. Tang, X. He, Y. Lei, H. Shi, Q. Guo, J. Liu, D. He, L. Yan, K. Wang, Temperature-responsive split aptamers coupled with polymerase chain reaction for label-free and sensitive detection of cancer cells, Chem. Commun. 53 (2017) 11889–11892.
[48] J. Tang, H. Shi, X. He, Y. Lei, Q. Guo, K. Wang, L. Yan, D. He, Tumor cell-specific split aptamers: target-driven and temperature-controlled self-assembly on the living cell surface, Chem. Commun. 52 (2016) 1482–1485.