Automated Target-Driven Molecular Machine-Based Engineering Process For SARS-CoV-2 Monitoring

Zhenqiang Fan
Jiangsu Institute of Nuclear Medicine
Minhao Xie
Jiangsu Institute of Nuclear Medicine
Jianbin Pan
Nanjing University
Kai Zhang (zhangkai@jsinm.org)
Jiangsu Institute of Nuclear Medicine https://orcid.org/0000-0002-7021-5395

Research Article

Keywords: SARS-CoV-2 monitoring, Ti3C2-based composites, automated molecular machine, saliva environmental testing, modular reaction

Posted Date: January 21st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1248018/v1

Abstract

Nucleic acid structured electrochemiluminescence (ECL) biosensors provide valuable, versatile, programmable support for the development of medical diagnostics. Here, we constructed an automated DNA molecular machine with just one thiol-modified DNA probe and three nucleic acid probes on the surface of Ti_3C_2-based composites to monitor SARS-CoV-2, free from the limitations of motion tracks on the DNA molecular machine and significantly decreasing the probe modification fee. In the presence of the target, the designed DNA molecular machine conducted a modular reaction to transduce the target concentration information into an enhancement of the ECL signal based on DNA hybridization calculations. Modular, scalable reactions occur on DNA automata, reducing complex bioanalytical reactions to a single nucleic acid probe unit and eliminating the tedious steps of laying down motion tracks required by traditional molecular machines. By designing three capture probes, it is possible to extend the application ranges of the protocol from single to multi-target monitoring. Moreover, the strategy implements the bioanalysis of the SARS-CoV-2 in complex environments such as saliva dilutions and serum dilutions, which is valuable in promoting public health development and evaluating the environmental hazards of SARS-CoV-2.

1. Introduction

Coronavirus disease 2019 (COVID-19) has caused the collapse of health systems in many parts of the world. Like modern infectious diseases before it, it is spreading rapidly worldwide (Yan et al 2021). Currently, countries around the globe are actively collaborating and working together to control the spread of the global outbreak. Developing and optimizing methods for rapid and accurate diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) becomes particularly important to test a country's public health capacity to rapidly identify infected individuals and take appropriate measures to contain the virus in a pandemic (Delafiori et al 2021).

Recently, nucleic acid-structured electrochemiluminescence (ECL) biosensing platforms have been widely applied in bioanalysis, food safety, and environmental contaminant monitoring due to their fast response, simple operation, compact device, and high sensitivity (Bai et al 2019; Fan et al 2021b; Qi and Zhang 2020). These nucleic acid-involved ECL sensings rely on programmed DNA probes to participate in molecular recognition and signaling (Feng et al 2017). Programmed DNA tetrahedron reveals enormous potential due to their stiffness, size programmability, which could improve the low attachment efficiency, uneven spatial distribution and reduce the aggregation of conventional DNA such as single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) (Fan et al 2021a; Fan et al 2020; Fan et al 2022; Fan et al 2021c; Li et al 2014).

Typically, the diagnosis procedure of SARS-CoV-2 consists of the following steps. Firstly, the samples to be tested are collected by oropharyngeal or nasopharyngeal swab from suspected COVID-19 patients. Secondly, the RNA of the virus extracted through an RNA extraction step (Mattioli et al 2020). Then target DNA is obtained from viral RNA by enrichment steps containing RT-PCR (reverse transcription-polymerase

chain reaction) or RT-RPA (reverse tranion-recombinase polymerase amplification) (Byrnes et al 2021; Mahas et al 2021). In the detection of SARS-CoV-2, both upstream RNA and downstream DNA can be used as targets to achieve the diagnosis of COVID-19.

Herein, we developed an interfacial DNA machine containing an inverted DNA tetrahedron using three capture probes as reaction probes to monitor the SARS-CoV-2 downstream DNA. When target DNA is introduced over the reaction system, the local DNA automata produce a correlation effect on the ECL signal output of the Ti_3C_2/PEI-Ru@Au nanocomposite on the glassy carbon electrode (GCE) based on DNA hybridization calculations, by which the concentration of the SARS-CoV-2 could be deduced.

2. Results And Discussion

2.1 Principle of the proposed ECL biosensor for SARS-CoV-2 monitoring

In our strategy, ECL emitting material (Ti_3C_2/PEI-Ru@Au) was coated onto the GCE surface, and the ECL signals were used as the signal output of the sensor. Then, we modified the automated molecular machine on the surface of Ti_3C_2/PEI-Ru@Au. The inverted tetrahedron has only one vertex modified by thiol, while possessing three capture probes where two of the strands (H and P) are partially complementarily paired with H’ and P’, respectively. The one remaining capture probe (S’-S) forms a hairpin structure by itself and its S’ end carries the quenching motif ferrocene (Fc) of the ECL signal. When the target DNA (T) binds to the exposed toehold sequence of the anticodon H’, H is released, which in turn binds to the toehold sequence of the anticodon P’, which allows P to be released. Immediately afterward, P binds to the anticodon S’-S, producing a site that is recognized and cleaved by the nuclear endonuclease (Nt.BbvCI), resulting in the release of quenched motif Fc and reuse of the P. In this process, the repeatedly generated P enables the cyclic cleavage of the S’-S and the cyclic release of the Fc, resulting in the generation of a transduction signal that allows the assessment and quantitative analysis of target concentration.

2.2 Characterizations of the Ti_3C_2/PEI-Ru@Au

We have characterized the synthesis of Ti_3C_2/PEI-Ru@Au. Fig. 1A and Fig. 1B show the TEM morphologies of Ti_3C_2 and Ti_3C_2/PEI-Ru@Au, respectively. We can see that Ti_3C_2 shows monolayers or multilayers of nanosheets. After the modification, it can be seen that the gold nanoparticles are uniformly distributed on the Ti_3C_2 surface. The magnified Ti_3C_2/PEI-Ru@Au TEM image (Fig. 1C) and its inset show that the gold nanoparticles are uniformly distributed and the particle size is approximately 4.3 nm. To further validate the synthesis of Ti_3C_2/PEI-Ru@Au, we characterized the elemental distribution (Figs. S2A-H). The elemental distribution also shows that Au particles as well as $Ru(dcbpy)_3^{2+}$are uniformly distributed on the Ti_3C_2 nanosheets.

We have performed XPS analysis of Ti_3C_2/PEI-Ru@Au nanocomposite. The XPS survey spectrum of the complex (Fig. 1D) exhibits Ti 2p and C 1s peaks, which are the elemental peaks of Ti_3C_2. The formation process of Ti_3C_2 produces F and Cl elements; therefore, F 1s and Cl 2p peaks are observed. The complex also has Ru 3p and Ru 3d peaks, and Au 4f characteristic peaks, as a result of the introduction of $Ru(dcbpy)_3^{2+}$ and the reduction of Au^{3+} to gold nanoparticles. We also elaborated the bonding interaction between the complexes by fitting C 1s, O 1s, N 1s and Au 4f (Figs. 1E-H). Four fitted peaks appear in the C 1s peak of Ti_3C_2/PEI-Ru@Au, which mainly correspond to the characteristic bonds of O-C=O, C-O/C-N, C-C and C-Ti. Similarly, the XPS spectra at O 1s show the typical bonds of C-Ti-(OH), C-Ti-Ox and TiO_2. The XPS spectra at N 1s also show the characteristic bonds of R-NH_2, R-NH-R, R=N-R and N-Ti. The double peaks of Au at Au $4f_{7/2}$ (82.83 eV) and Au $4f_{7/2}$ (86.51 eV) also verified the presence of gold particles. All these XPS analysis data indicate the formation of the Ti_3C_2/PEI-Ru@Au complexes. We also verified the synthesis of the complex by UV-vis spectral analysis as depicted in Fig. 1I. It showed that the Ti_3C_2/PEI-Ru@Au complex has both Ru-PEI (characteristic peaks at 300 nm and 475 nm) and Au nanoparticles (characteristic absorption peak at 525 nm) peaks, also verifying the synthesis of Ti_3C_2/PEI-Ru@Au complex.

2.3 Characterization of the inverted DNA tetrahedral scaffold

We also characterized the inverted DNA tetrahedra by AFM (Fig. 2A). It can be clearly seen that the DNA tetrahedra formed self-assembled by four strands (H-T1, P-T2, S'-S-T3, T4) appear granular and uniform in size, which also confirms the successful preparation of our tetrahedral probes. Meanwhile, we characterize the heights of the tetrahedral particles relative to the silicon wafer base (control) as shown in Figs. 2B-2F. We define the silicon wafer substrate as the control, and the heights of the tetrahedra relative to the substrate as the actual height of the DNA tetrahedra. These characterization and analysis tools all demonstrate the successful synthesis of inverted tetrahedra with relatively homogeneous dimensions.

2.4 Feasibility of DNA reactions exploited by the automated molecular machine

We next verified the possibility of cascade reactions on the automated molecular machine as shown in Fig. S5A. It is shown that when T and H'-H-T1 are present together that they can be effective against P'-P-T2, eventually producing three major new bands: T-H', T1-H-P' and P-T2. However, when T is not present, H'-H-T1 and P'-P-T2 do not react with each other. Likewise, T does not act on P'-P-T2 when the H'-H-T1 intermediate is absent.

Upon the addition of H-T1 with P'-P-T2 to S'-S-T3 with NE, we observed the disappearance of S'-S-T3 and the appearance of three new bands: P-T2, T1-H-P' and cleaved S'-S-T3, which indicates the successful linkage of the nicking reaction to the previous one proved by Fig. S5B. Owing to the essential H-T1

linkage, the modular cascade reaction on the DNA molecular machine proceeds smoothly. At the same time, the newly generated P-T2 is available for the continuous action of S'-S-T3 until its depletion. These evidences provided by the two images suggest the possibility that the reaction occurs as expected on the automated molecular machine.

2.5 Monitoring performance, specificity and stability of this automated molecular machine for target monitoring

In our testing experiments, the automated molecular machine exhibits highly sensitive ECL variations for target concentrations as shown in Fig. 3A and 3B. The increased ECL intensity (ΔECL intensity) increases with the logarithmic value of the target concentration in the range from 1 pM to 1 nM. The linear equation of the calibration plot is $y = 431.5 + 1255.9\lg C_{target}$, where y represents ΔECL intensity. The calculated limit of detection (LOD) was 0.68 pM according 3σ method. In Table S2, we compared our strategy in terms of detection methods, sensitivity and detection range with other viral DNA detection methods. It can be noted that our detecting method performs well in these three aspects. Besides, this method as a fundamental strategy in combination with other signal amplification techniques such as hybridization chain reaction (HCR), loop-mediated isothermal amplification (LAMP), catalytic hairpin assembly (CHA) could achieve higher sensitivity. We further evaluated the specificity of DNA automated molecular machine for the target monitoring. The sensing performances of three non-specific strands with 10-fold target concentration, including Bat SARS-related CoV isolate bat-SL-CoVZC45 target (M1), BM48-31/BGR/2008 target (M2), and SARS-CoV target. As shown in Fig. 3C, we can see that the ECL intensity of the target is higher than the DNA intensity of the various mismatched strands, which also illustrates that our automated molecular machine has superb accuracy. Also, our automated molecular machine has excellent reproducibility as illustrated in Fig. 3D. The automated molecular machine exhibits extremely high signal stability by performing continuous potential scans for 15 cycles, with a relative standard deviation (RSD) of only 2.54% and 2.13% for 50 pM and 0.5 nM, respectively.

2.6 Saliva environmental and clinical human serum dilution sample determinations

Since viral DNA are usually obtained from viral RNA by techniques such as RT-PCR or RT-RPA during actual virus detection, there are some non-specific substances that interfere with the diagnosis of COVID-19. Therefore, we discussed the feasibility of the assay under complex environments and clinical specimens. The pharyngeal swabs method usually collects viral or bacteria samples in saliva. To verify the ability of the DNA automated molecular machine to monitor targets in the saliva environment, we added targets with different concentrations to 20-fold or 50-fold human saliva dilutions and verified their feasibility. As shown in Table S3, after repeated experiments (n=3), we obtained recoveries between 96.4 and 104.8%, which further validates the reliability of our DNA automated molecular machine platform for target monitoring. During the experiments, targets in the range of 5 pM to 5 nM were successfully monitored, demonstrating the universality of our DNA automated molecular machine in the complex saliva environments.

3. Conclusions

Herein, we have developed an inverted tetrahedron-based DNA molecular machine for SARS-CoV-2 target monitoring, a proposal with excellent interference resistance that performs well in a saliva dilution environment. The inverted tetrahedron has three reaction probes and a thiol group for immobilization on the Ti_3C_2/PEI-Ru@Au nanocomposite, which significantly reduces the experimental fee due to nucleic acid modifications. Moreover, the complex biochemical analysis reactions are zoomed in on a single DNA tetrahedral probe with programmability and higher controllability. Meanwhile, for molecular motions that occur on a fixed tetrahedral probe, the complex procedure of constructing molecular motion tracks is avoided. Our work enables the detection of contaminants in the environment and clinical specimen dilutions, which is of great value in advancing public health development and assessing their hazards in the environment.

Declarations

CRediT authorship contribution statement

Zhenqiang Fan: Data curation, Formal analysis, Writing - review & editing. Minhao Xie: Validation. Jianbin Pan: Supervision. Kai Zhang: Conceptualization, 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.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (21705061), the Jiangsu Provincial Key Medical Discipline (Laboratory) (ZDXKA2016017).

References

1. Bai W, Cui A, Liu M et al (2019) Signal-off electrogenerated chemiluminescence biosensing platform based on the quenching effect between ferrocene and $Ru(bpy)_3^{2+}$-functionalized metal–organic frameworks for the detection of methylated RNA. Anal Chem 91(18):11840–11847. https://doi.org/10.1021/acs.analchem.9b02569
2. Byrnes SA, Gallagher R, Steadman A et al (2021) Multiplexed and extraction-free amplification for simplified SARS-CoV-2 RT-PCR tests. Anal Chem 93(9):4160–4165. https://doi.org/10.1021/acs.analchem.0c03918
3. Delafiori J, Navarro LC, Siciliano RF et al (2021) Covid-19 automated diagnosis and risk assessment through metabolomics and machine learning. Anal Chem 93(4):2471–2479. https://doi.org/10.1021/acs.analchem.0c04497

4. Fan Z, Ding Y, Yao B et al (2021a) Electrochemiluminescence platform for transcription factor diagnosis by using CRISPR–Cas12a trans-cleavage activity. Chem Commun 57(65):8015–8018. https://doi.org/10.1039/D1CC03071J
5. Fan Z, Lin Z, Wang Z et al (2020) Dual-wavelength electrochemiluminescence ratiometric biosensor for NF-κB p50 detection with dimethylthiodiaminoterephthalate fluorophore and self-assembled DNA tetrahedron nanostructures probe. ACS Appl Mater Interfaces 12(10):11409–11418. https://doi.org/10.1021/acsami.0c01243
6. Fan Z, Yao B, Ding Y et al (2021b) Electrochemiluminescence aptasensor for Siglec-5 detection based on MoS_2@Au nanocomposites emitter and exonuclease III-powered DNA walker. Sensors Actuat B Chem 334:129592. https://doi.org/https://doi.org/10.1016/j.snb.2021.129592
7. Fan Z, Yao B, Ding Y et al (2022) Rational engineering the DNA tetrahedrons of dual wavelength ratiometric electrochemiluminescence biosensor for high efficient detection of SARS-CoV-2 RdRp gene by using entropy-driven and bipedal DNA walker amplification strategy. Chem Eng J 427:131686. https://doi.org/https://doi.org/10.1016/j.cej.2021.131686
8. Fan Z, Yao B, Ding Y et al (2021c) Entropy-driven amplified electrochemiluminescence biosensor for RdRp gene of SARS-CoV-2 detection with self-assembled DNA tetrahedron scaffolds. Biosens Bioelectron 178:113015. https://doi.org/https://doi.org/10.1016/j.bios.2021.113015
9. Feng Q-M, Guo Y-H, Xu J-J et al (2017) Self-assembled DNA tetrahedral scaffolds for the construction of electrochemiluminescence biosensor with programmable DNA cyclic amplification. ACS Appl Mater Interfaces 9(20):17637–17644. https://doi.org/10.1021/acsami.7b04553
10. Li Z, Zhao B, Wang D et al (2014) DNA nanostructure-based universal microarray platform for high-efficiency multiplex bioanalysis in biofluids. ACS Appl Mater Interfaces 6(20):17944–17953. https://doi.org/10.1021/am5047735
11. Mahas A, Wang Q, Marsic T et al (2021) A novel miniature crispr-cas13 system for SARS-CoV-2 diagnostics. ACS Synth Biol 10(10):2541–2551. https://doi.org/10.1021/acssynbio.1c00181
12. Mattioli IA, Hassan A, Oliveira ON et al (2020) On the challenges for the diagnosis of SARS-CoV-2 based on a review of current methodologies. ACS Sensors 5(12):3655–3677. https://doi.org/10.1021/acssensors.0c01382
13. Qi H, Zhang C (2020) Electrogenerated chemiluminescence biosensing. Anal Chem 92(1):524–534. https://doi.org/10.1021/acs.analchem.9b03425
14. Yan L, Yi J, Huang C et al (2021) Rapid detection of COVID-19 using maldi-tof-based serum peptidome profiling. Anal Chem 93(11):4782–4787. https://doi.org/10.1021/acs.analchem.0c04590

Scheme

Scheme 1 is available in the Supplemental Files section

Figures

Figure 1

TEM image of Ti_3C_2 nanosheets (A) and Ti_3C_2/PEI-Ru@Au nanocomposite (B). The magnified Ti_3C_2/PEI-Ru@Au nanocomposite (C) and the inset is the size distribution of Au nanoparticles in Fig. 1C. XPS survey (D) and high-resolution C 1s (E), O 1s (F), N 1s (G), Au 4f (H) and UV-vis adsorption (I) spectra of Ti_3C_2/PEI-Ru@Au nanocomposite.

Figure 2

(A) 2D AFM image of inverted DNA tetrahedron. (B)-(F) are height maps plotted along the tangents at points a-e in Fig. 2A.

Figure 3

(A) ECL-Time and ECL-Potential (the inset of Fig. 3A) curves of the strategy when monitoring target with different concentrations (1 pM, 10 pM, 20 pM, 50 pM, 0.1 nM, 0.2 nM, 0.5 nM and 1 nM). (B) The variation of ΔECL intensity with target concentration and logarithm of target concentration (the inset of Fig. 3B) in the detection region from 1 pM to 1 nM. (C) Specificity of strategy at 0.1 nM target and 1 nM non-targets. (D) Stability of the sensor in the presence of 50 pM (blue curve) and 0.5 nM target (red curve).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme1.png
- SI.docx