Supplementary Material

Colorimetric aptasensor for sensitive low-density lipoprotein detection based on reduced oxide graphene@molybdenum disulfide–ferrocene nanosheets with peroxidase-like activity

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1. Materials and Reagents

Graphene oxide (GO) was procured from Xianfeng NanoMaterials Technology Co., Ltd (Nanjing, China). Ascorbic acid (AA), N,N-Dimethylformamide (DMF), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), glutaraldehyde (GA) were acquired from Xilong Scientific Co., Ltd. (Guangdong, China). Molybdenum disulfide (MoS_2), N-Hydroxysuccinimide (NHS), sodium phosphate dibasic (Na_2HPO_4), sodium dihydrogen phosphate dehydrate (NaH_2PO_4), 3,3',5,5'-tetramethylbenzidine (TMB), bovine serum albumin (BSA), citric acid, sodium citrate were provided by Aladdin Reagent Co., Ltd. (Shanghai, China). Ferrocene-carboxylic acid (Fc), o-phenylene diamine (OPD) was acquired from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). β-mercaptoethylamine (β-ME) was from Changsha Nosebel Biotechnology Co., Ltd (Hunan, China). N-(3-Dimethylaminopropyl)-N'-ethylcarbodimide hydrochloride (EDAC) was from Dalian Meilun Biotechnology Co., Ltd (Dalian, China). Terephthalic acid (PTA) was acquired from Bidepharm Scientific Co., Ltd (Shanghai, China). Immunoglobulin G (IgG), alpha-fetal protein (AFP), human serum albumin (HSA), horseradish peroxidase (HRP) was from Beijing Solarbio Science&Technology Co., Ltd (Beijing, China). Acetic acid was from Guangdong Guanghua Chemical Factory Co., Ltd (Guangdong, China). Sodium acetate was from Beijing Innochem Science&Technology Co., Ltd (Beijing, China). Low density lipoprotein (LDL), and high-density lipoprotein (HDL) was from Guangzhou Yiyuan Biotechnology Co., Ltd (Guangzhou, China). Cholesterol (chol), aminated LDL aptamer (LDLapt, 5'-NH_2-ACCTCGATTTTATATTATTTCGCTTACCAACAACTGCAGA-3')[1], carboxyfluorescein modified LDLapt ((FAM)LDLapt, 5'-FAM-ACCTCGATTTTATATTATTTCGCTTACCAACAACTGCAGA-3'), Random

Sequence (Rand, 5'-NH_2-TAA CGC TGA CGC TGA CCT TAG CTG CAT TTT ACA TGT TCC A-3') were got from Shenggong Bioengineering Co., Ltd (Shanghai, China). Clinical human serum samples were collected from the Guangxi Key Laboratory of Metabolic Disease Research of the 924th Hospital of the Chinese People's Liberation Army (Guilin, China). Ultrapure water (>18 MΩ) was used throughout. The stock solution of HAc-NaAc buffer, phosphate buffer solution (PBS) and citric acid/sodium citrate buffer solution were diluted for use by ultrapure water. All reagents were analytical grade and used directly.

2. Apparatus

Transmission electron microscope (TEM) images and scanning electron microscopy (SEM) images were acquired using JEM-1200EX (JEOL, Japan) operating at an acceleration voltage of 100 kV and a Quanta 400 field environmental scanning electron microscope (FEI COMPANY, USA), respectively. Optical properties were determined using a Fourier-transform infrared spectroscopy (FT-IR, Nicolet-IS10, Nicolet, USA), UV-visible spectroscopy (UV-Vis, UH5300, HITACHI, Japan), a spectrofluorophotometer (F-4600, HITACHI, Japan), a Raman spectrometer (Raman, Renishaw, UK). X-ray photoelectron spectroscopy (XPS, ESCALAB 250xi, Thermofisher, USA) was used to analyze the elements of the nano-material. X-ray diffraction (XRD) images were from the GENESIS type energy spectrometer (EDAX, USA). Particle size potential was acquired from zeta sizer (ZS90, Melvin, UK). The conformational change of the secondary structure of protein was measured using the circular dichroism spectrum (CD, J-1500, Jasco, Japan).

3. Specificity analysis between the LDLapt and LDL using fluorescence spectra

100 μL of 1 μM (FAM)LDLapt was mixed with 100 μL of 1.0 μg/mL LDL, and incubated in the dark at 25°C for 1 h, then the above mixture and 200 μL of 0.5 μM

(FAM)LDLapt were detected by fluorescence spectrometer, respectively. The emission peak was set at 375 nm and the excitation peak at 520 nm.

Fig.S1 shows the fluorescence spectrum of (FAM)LDLapt. (FAM)LDLapt (curve a) emits fluorescence with an intensity of 919 due to its own fluorophores. After incubation of (FAM)LDLapt with LDL (curve b), the fluorescence intensity decreases to 379, indicating that the binding of (FAM)LDLapt with LDL led to changes in the structure of (FAM)LDLapt, thus leading to fluorescence quenching, which indicates that LDLapt is specific to LDL.

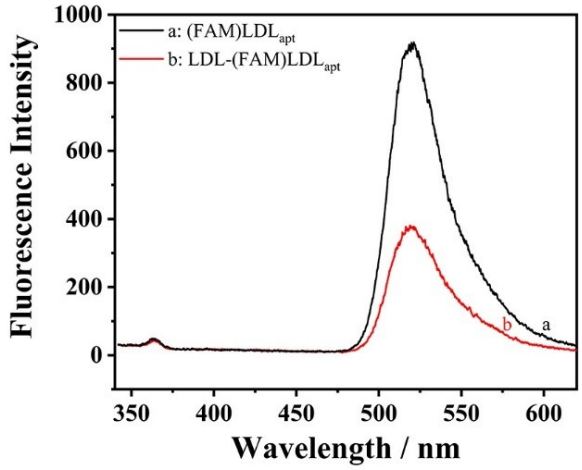

Fig.S1 Fluorescence spectrum of (FAM)LDLapt with or without LDL

4. Specificity analysis of the LDLapt and LDL using circular dichroism

To study the conformational change of the secondary structure of the LDLapt upon the LDL, a CD analysis was conducted. 200 μL of 2 μM LDLapt was mixed with 200 μL of 1.0 μg/mL LDL, and incubated at 25°C for 1 h, then the above mixture, 400 μL of 1 μM LDLapt, 400 μL of 0.5 μg/mL LDL were detected by CD, respectively. The range was 185 nm – 400 nm.

Fig.S2 shows the CD spectrum of LDLapt. LDL (curve a), as a class of lipoprotein, has a negative peak near 200 nm and a small but wide positive peak near 220 nm, indicating that LDL is an irregular curly conformation. LDLapt (curve b) has

a positive peak near 200 nm, a weak negative peak near 208 nm, a strong positive peak near 220 nm, a strong and wide negative peak near 250 nm, and a strong and wide positive peak near 275 nm, showing a B-type right-handed double helix DNA structure. After incubation of LDLapt with LDL (curve c), compared with the positive peak of LDLapt and LDL, a new weak and narrow negative peak (region ☆) appears in LDL-LDLapt between 210.4 nm – 211.4 nm. Moreover, compared with the negative peak of LDLapt and LDL, a new strong but narrow positive peak (region &) appears in LDL-LDLapt between 232.7 nm – 235.8 nm, indicating that the conformation of LDLapt is changed after LDLapt is bound to LDL, and LDLapt is specific to LDL.

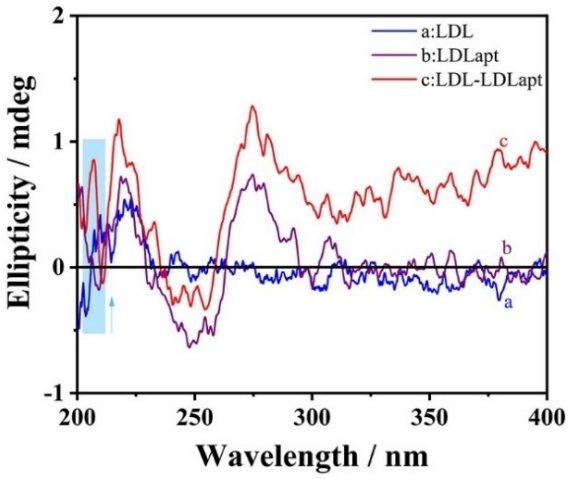

Fig.S2 Circular dichroism analysis of LDLapt with or without LDL

5. XPS analysis of C, N, O of rGO@MoS_2-Fc

Fig.S3 is the XPS analysis of C, N, O of rGO@MoS_2-Fc. FigS3A shows the XPS energy spectrum analysis of C 1s with binding energies of 284 eV, 285.8 eV, 287.8 eV for C-C, C-O, and COOH functional groups, respectively. Fig.S3B shows the XPS energy spectrum analysis of N 1s, and the C-N at 394.6 eV and the N-H bond at 399.4 eV indicate the presence of amino groups on MoS_2 and the formation of MoS_2-Fc with Fc through amide bonding. Fig.S3C shows the XPS spectra of O 1s with binding

energies of 530.7 eV, 531.7 eV, and 532.5 eV for O-C=O, C=O, and C-O-H functional groups, respectively. XPS spectra of C 1s and O 1s indicate the presence of rGO or Fc in the prepared rGO@MoS_2-Fc.

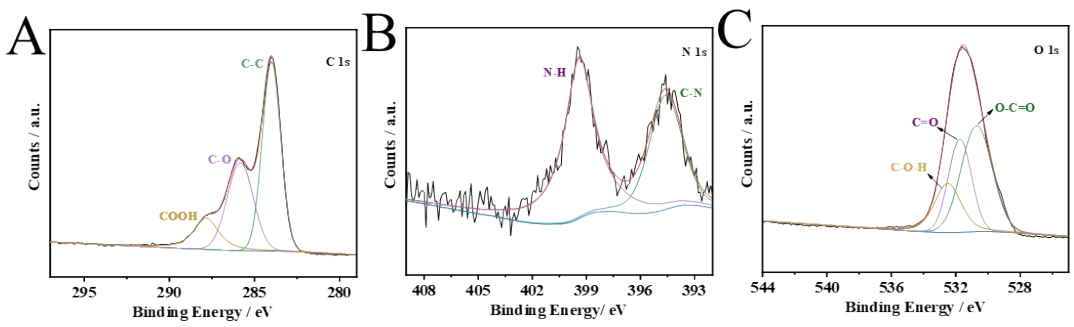

Fig.S3 (A) XPS spectra of C 1s., (B) XPS spectra of N 1s, and (C) XPS spectra of O 1s of rGO@MoS_2-Fc

6. Stability of rGO@MoS_2-Fc and HRP

The pH stability and temperature stability of rGO@MoS_2-Fc were measured under the same experimental conditions as well as that of HRP, and the two were compared. rGO@MoS_2-Fc or HRP, OPD-H_2O_2 solution, different pH (3.0 - 8.0) of PBS (125 mM) were incubated in the dark at room temperature for 1 h, then transferred for UV-Vis spectrophotometry to observe the absorbance evolution at 420 nm. The temperature stability of rGO@MoS_2-Fc was measured as well as that of HRP, and the two were compared. rGO@MoS_2-Fc or HRP, OPD-H_2O_2 solution, phosphate buffer (PBS, 125 mM, pH 5.0) were incubated in the dark at different room temperature (4°C - 80°C) for 1 h, then transferred for UV-Vis spectrophotometry to observe the absorbance evolution at 420 nm.

Fig.S4A shows the pH stability of rGO@MoS_2-Fc and HRP. The catalytic activity of HRP is more stable between pH 3.0 - pH 8.0 and decreases with increasing pH, finally remaining above 60%, while the catalytic activity of the rGO@MoS_2-Fc is best at pH 5.0, and decreases to 20% at other pH values. Fig.S4B shows the

temperature stability of rGO@MoS_2-Fc and HRP. The catalytic activity of HRP decreases to less than 80% at low and high temperature, while the catalytic activity of rGO@MoS_2-Fc nanozyme remained above 80% between 4°C and 80°C, indicating that rGO@MoS_2-Fc has good temperature stability.

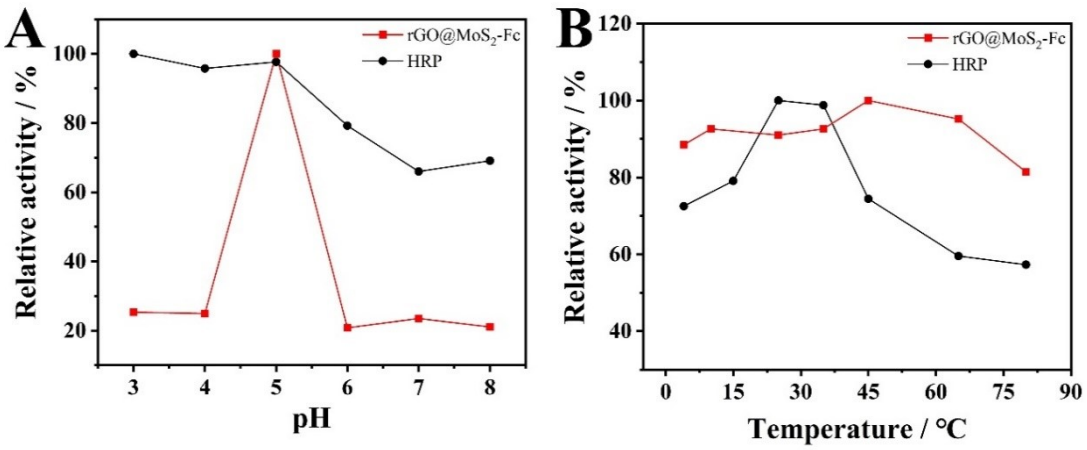

Fig.S4 (A) The pH stability and (B) temperature stability of rGO@MoS_2-Fc and HRP, respectively.

7. Study on the enzymatic catalytic activity of rGO@MoS_2-Fc over time

The enzymatic catalytic activity of rGO@MoS_2-Fc over time in HAc-NaAc buffer was studied. 10.0 μL of 50 mM OPD, 10.0 μL of 100 mM H_2O_2, and 20.0 μL of 1 mg/mL rGO@MoS_2-Fc were added into 60.0 μL HAc-NaAc buffer (125 mM, pH 5.5). The mixture was incubated in the dark at room temperature, and then the absorption curve was measured by UV-Vis every 5 minutes.

Fig.S5 shows the UV-Vis spectra of rGO@MoS_2-Fc in HAc-NaAc buffer over time (0 - 30 min). As shown in Fig.S5, the absorbance of rGO@MoS_2-Fc increases with time in 25 min, especially, the absorbance of rGO@MoS_2-Fc does not change after 30 min, thus, 25 min is regarded as the optimal reaction time of rGO@MoS_2-Fc in HAc-NaAc buffer.

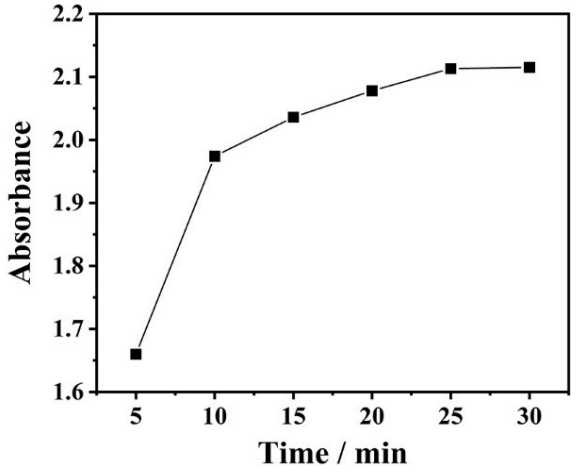

Fig.S5 UV-Vis spectra of rGO@MoS_2-Fc over time

8. Optimization of experimental conditions for the LDL colorimetric aptasensor

As shown in Fig.S6A, the absorbance values increased when the LDLapt concentration increased from 0.5 μM to 1 μM, and gradually decreased when the concentration increased from 1 μM to 6 μM, indicating that when the LDLapt concentration was 1 μM, the LDL captured by the constructed colorimetric aptamer sensor and the amount of bound rGO@MoS_2-Fc/LDLapt could make the most chromogenic substrate. Changes in the concentration of LDLapt affect the amount of LDL captured, which in turn affects the amount of rGO@MoS_2-Fc/LDLapt bound, resulting in differences in color or absorbance. Choosing the optimal concentration of LDLapt can specifically bind more LDL, and then construct more rGO@MoS_2-Fc/LDLapt/LDL/LDLapt systems, and improve the selectivity and sensitivity of the sensor. Therefore, 1.0 μM LDLapt concentration was chosen as the optimal experimental condition.

The change of LDL incubation time affects the amount of LDL captured, and insufficient time will lead to the inability of LDLapt to bind to LDL. In order to ensure the full combination of LDLapt and LDL, it is often necessary to set a long

enough reaction time. Therefore, finding the best LDL incubation time can make the detection of the sensor faster. As shown in Fig.S6B, when the incubation time of LDL was increased from 40 min to 60 min, the absorbance value increased, while when the incubation time was increased from 60 min to 120 min, the absorbance value gradually decreased and there was no significant change between the current values, indicating that the constructed colorimetric aptamer sensor was sufficient to capture LDL and bind to it when the incubation time of LDL was 60 min, so 60 min was chosen as the optimal incubation time for LDL.

The change of LDL incubation temperature will reduce the activity of LDL or make it denatured and inactivated, so that the concentration of LDL can not be accurately detected or even detected. Therefore, the reaction at the most appropriate temperature can ensure the accuracy of the sensor in detecting LDL concentration. As shown in Fig.S6C, when the incubation temperature of LDL increased from 4^{o}C to 25^{o}C, the absorbance value increased, and when the incubation temperature increased from 25^{o}C to 50^{o}C, the absorbance value gradually decreased, and the number of bound LDL and rGO@MoS_2-Fc/LDLapt decreased due to the denaturation and inactivation of LDL at 50^{o}C, indicating that the best sensor effect was achieved when the incubation temperature of LDL was 25^{o}C. Therefore, 25^{o}C LDL incubation temperature was chosen as the optimal experimental condition.

Because the change of the pH value of HAc-NaAc buffer solution affects the pH value of the whole solution reaction system, and then affects the catalytic performance of rGO@MoS_2-Fc, which makes it unable to rapidly and completely catalyze the decomposition of H_2O_2 to produce ·OH. Therefore, it is necessary to choose the best buffer solution pH to ensure the sensitivity of sensor detection. As shown in Fig.S6D, when the pH of HAc-NaAc buffer solution increased from 3.5 to

4.0, the absorbance value increased; and when it increased from 4.0 to 5.5, the absorbance value gradually decreased, indicating that the best sensor effect was achieved when the pH was 4.0, which is easier to catalyze the decomposition of H_2O_2 to produce ·OH, so pH 4.0 was chosen as the optimal experimental condition Therefore, pH 4.0 was chosen as the optimal experimental condition.

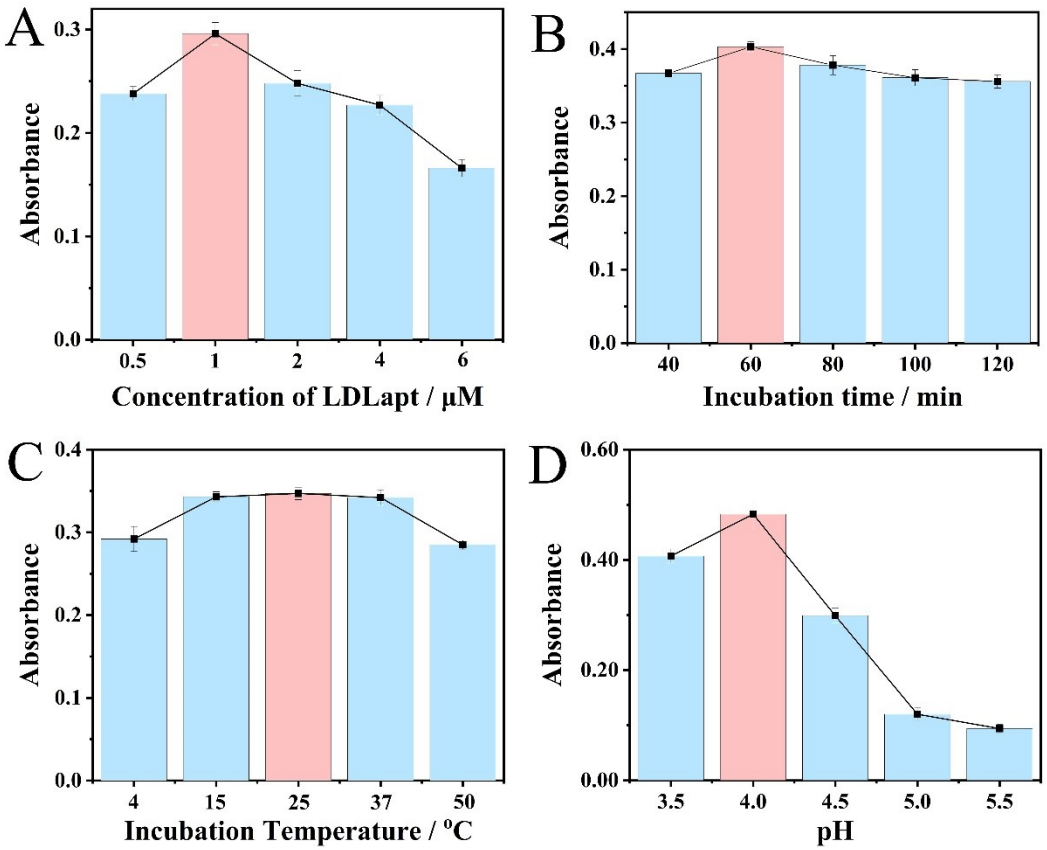

Fig.S6 Optimization of experimental conditions. (A) Concentration of LDLapt. (B) Incubation time. (C) Incubation temperature. (D) pH of HAc-NaAc buffer

9. Cost-benefit analysis of rGO@MoS_2-Fc colorimetric aptamer sensor

In the process of material preparation, the cost of GO and other materials and reagents used in each synthesis is about 1.15 yuan. In the construction of sensor system, the cost of using LDLapt is about 6.21 yuan each time, and the cost of using materials and reagents such as OPD is about 0.1 yuan each time.

The main instruments used for testing are ultraviolet-visible spectrophotometer, model UH5300, HITACHI, Japan. The price is about 20.00 w~40.00 w.

References

[1] D. Klapak, S. Broadfoot, G. Penner, A. Singh, E. Inapuri, PLOS ONE, 2018, 13, 1-16. https://doi.org/10.1371/journal.pone.0205460.