**A novel fluorescent sensor based on aptamer recognition and DNA walker amplification strategy and its determination of 17β-estradiol**

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**Abstract** Based on aptamer specific recognition, DNA walker molecular machine and Exonuclease III-assisted dual cycle signal amplification technology, a fluorescent aptamer sensor was constructed and applied to the detection of 17β-estradiol. The fluorescent aptamer sensor designed in this study is mainly composed of DNA walker. The system of DNA walker consists of graphene oxide (GO) with fluorescence quenching function, signal probe double-stranded DNA (dsDNA), hairpin probe 1(HP1) with 17β-estradiol aptamer sequence, hairpin probe 2(HP2), and Exonuclease Ⅲ (Exo III). By adding 17β-estradiol of different concentrations into the system, DNA S1 solution of different concentrations can be obtained through the action of Exo Ⅲ. At the same time, as the starter chain that triggers DNA walker, it can perform the second round of signal amplification reaction with dsDNA on GO, thus realizing the double amplification of fluorescence signal. Therefore, the quantitative detection of 17β-estradiol was realized by increasing the amount of fluorescence signal. Atomic force microscopy (AFM) and polyacrylamide gel electrophoresis (PAGE) were used to verify the feasibility of DNA walker participating in the reaction. Under the optimal conditions, 17β-estradiol concentration showed a good linear relationship with fluorescence in the range of 5.0×10-10-1.8×10-8 M, and the detection limit was 5.6×10-11 M. The fluorescent aptamer sensor established in this study has good selectivity, simple operation and good specificity, and the results of its application in the determination of milk samples are satisfactory.

**KEYWORDS** Aptamer; Fluorescence sensor; DNA walker; Exonuclease Ⅲ;17β-estradiol

**Supporting Information**

**Fig.S1** Quenching of fluorescence groups under different conditions. The concentrations of FAM-DNA, dsDNA and GO were 1.0 μM, 1.0 μM and 2.0 mg/mL.

**Fig.S2** Fluorescence spectra of aptamer sensors comparing the presence and absence of E2. The concentrations of E2, HP1, HP2, HP2, Exo Ⅲ and the volume ratio of dsDNA to GO were 1.0 nM,4.0 μM,4.0 μM,20.0 U and 1:3. The capture time of target is 30 min and enzyme cycle amplification temperature and reaction time were 37 ℃ and 90 min.

**Fig.S3** Fluorescence spectra of running DNA walker reaction. The concentrations of E2, HP1, HP2, Exo Ⅲ and the volume ratio of dsDNA to GO were 1.0 nM,4.0 μM,4.0 μM,20.0 U and 1:3. The capture time of target is 30 min and enzyme cycle amplification temperature and reaction time were 37 ℃ and 90 min.

**Fig.S4** Effect of different HP1 concentrations on the change in fluorescence intensity. The concentrations of E2, HP2, Exo Ⅲ and the volume ratio of dsDNA to GO were 1.0 nM,4.0 μM,20.0 U and 1:3. The capture time of target is 30 min and enzyme cycle amplification temperature and reaction time were 37 ℃ and 90 min.

**Fig.S5** Effect of different HP2 concentrations on the change in fluorescence intensity. The concentrations of E2, HP1, Exo Ⅲ and the volume ratio of dsDNA to GO were 1.0 nM,4.0 μM,20.0 U and 1:3. The capture time of target is 30 min and enzyme cycle amplification temperature and reaction time were 37 ℃ and 90 min.

**Fig.S6** Effect of different Exo III reaction temperatures on the variation in fluorescence intensity. The concentrations of E2, HP1, HP2, Exo Ⅲ and the volume ratio of dsDNA to GO were 1.0 nM,4.0 μM, 10.0 μM,20.0 U and 1:3. The capture time of target is 30 min and enzyme cycle amplification reaction time is 90 min.

**Fig.S7** Effect of different Exo III reaction time on the variation in fluorescence intensity. The concentrations of E2, HP1, HP2, Exo Ⅲ and the volume ratio of dsDNA to GO were 1.0 nM,4.0 μM, 10.0 μM,20.0 U and 1:3. The capture time of target is 30 min and enzyme cycle amplification reaction temperature is 37 ℃.

**Fig.S8** Effects of different Exo Ⅲ enzyme dosages on changes in fluorescence intensity. The concentrations of E2, HP1, HP2 and the volume ratio of dsDNA to GO were 1.0 nM,4.0 μM, 10.0 μM and 1:3. The capture time of target is 30 min and enzyme cycle amplification temperature and reaction time were 37 ℃ and 60 min.

**Fig.S9** Effects of different volume ratios of dsDNA@GO on the change of fluorescence intensity. The concentrations of E2, HP1, HP2, Exo Ⅲ were 1.0 nM,4.0 μM,10.0 μM,15.0 U. The capture time of target is 30 min and enzyme cycle amplification temperature and reaction time were 37 ℃ and 60 min.



**Fig.S1** Quenching of fluorescence groups under different conditions. The concentrations of FAM-DNA, dsDNA and GO were 1.0 μM, 1.0 μM and 1.0 mg/mL, 2.0 mg/mL.



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**Fig.S6** Effect of different Exo III reaction temperatures on the variation in fluorescence intensity. The concentrations of E2, HP1, HP2, Exo Ⅲ and the volume ratio of dsDNA to GO were 1.0 nM,4.0 μM, 10.0 μM,20.0 U and 1:3. The capture time of target is 30 min and enzyme cycle amplification reaction time is 90 min.



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