## **Supporting Information**

## A "turn-on" fluorescence resonance energy transfer aptasensor based on carbon dots and gold nanoparticles for $17\beta$ -estradiol detection in sea salt

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Assembly of CDs-F1-Apt-F2-AuNPs was characterized by TEM. Hybridization of aptamer with F1 and F2 made CDs close to AuNPs. AuNPs were surrounded by CDs (Fig. S1a). When 17-estradiol was detected, aptamers were specifically bound to the target, leading to the separation of CDs and AuNPs. The distance between CDs and AuNPs was large (Fig. S1b).



Fig. S1. TEM images of CD-F1-apt-F2-AuNP in the absence (a) and presence (b) of 17b-estradiol.

To confirm the best excitation wavelength of carbon dots (CDs), fluorescence spectrum was scanned with different excitation wavelengths. As shown in Fig. S2, the best excitation wavelength of CDs was 360 nm and the best emission wavelength was 443 nm.



Fig. S2. Excitation and emission wavelength of CDs.

The coupling between CDs and oligonucleotide F1 was characterized by Fourier transform infrared (FTIR). The characteristic absorption peak of CDs-F1 conjugation appeared at 1644.5 cm<sup>-1</sup> and 1709 cm<sup>-1</sup>, showing the existence of amide vibration. It confirmed that the amide bond between CDs and oligonucleotide was successfully formed by EDC/NHS method (Fig. S3). In addition, the absorption peak at 1088 cm<sup>-1</sup> was generally attributed to the C-O stretching of the DNA main chain.



Fig. S3. FTIR spectrum of CDs and oligonucleotide conjugation.

The measurement of circular dichroism spectra was implemented to characterize the conformation change of aptamer after the binding of  $17\beta$ -estradiol. As illustrated in Fig. S4, the circular dichroism spectrum of aptamer exhibited one positive peak at ~280 nm and one negative band at ~248 nm. In the presence of  $17\beta$ -estradiol, a decrease in intensity of both the positive band and the negative band appears without shift in the band position.



Fig. S4. Circular dichroism analysis of the complex (F1-aptamer-F2) in the absence and presence of 17b-estradiol.

The phenomenon of FRET was confirmed by fluorescence lifetime measurements (Fig. S5). The excitation wavelength of the pulse laser was 360 nm and the emission wavelength of the pulse laser was 443 nm for lifetime measurement. The time-resolved fluorescence spectra of CDs, CDs-F1 and CDs-F1 after addition of 17β-estradiol showed no significant changes.



Fig. S5. Time-resolved fluorescence lifetime of CDs, CDs-F1 and CDs-F1 after addition of 17β-estradiol.

	$\tau_1$	τ <sub>2</sub>	χ <sup>2</sup>
CDs	2.01	5.79	1.222
CDs-F1	1.75	5.50	1.214
CDs-F1 17β-estradiol	1.99	6.78	1.177

Table S1 The fluorescence lifetime of CDs, CDs-F1 and CDs-F1 after addition of 17β-estradiol

To optimize the AuNPs concentrations in the FRET system, various concentrations of AuNPs were added to the CDs-F1 conjugation bound with aptamer. The fluorescence of CDs was quenched by AuNPs. When AuNPs was at the concentration of 0.056 nM, the quenching efficiency reached the maximum (Fig. S6).



Fig. S6. Quenching efficiency of various AuNPs concentrations.

In order to obtain the most suitable number of aptamers in the FRET system, the concentration of AuNPs was fixed, and aptamers were titrated. The result is shown in Fig. S7.



Fig. S7. Quenching efficiency of various aptamer concentrations.

The fluorescence spectra of CDs-F1 with addition of only AuNPs-F2 were shown in Fig. S8.



Fig. S8. The fluorescence spectra of CDs-F1 with addition of only AuNPs-F2.

The specificity experiments were conducted. The recovery efficiency of 1 nM hydrocortisone, estriol, estrone, diethylstilbestrol, and hexestrol was investigated (Fig. S9). The results indicated that this FRET aptasensor showed good specificity for  $17\beta$ -estradiol detection.



Fig. S9 Recovery efficiency comparison among estradiol, hydrocortisone and estriol with concentration of 1 nM.