A Significant Fluorescent Aptamer Sensor Based on Carbon Dots and Graphene Oxide for Highly Selective Detection of Progesterone

Hanyue Cui¹ · Huan Lu¹ · Jing Yang¹ · Yao Fu¹ · Yan Huang¹ · Li Li¹ · Yaping Ding¹

Received: 10 November 2021 / Accepted: 18 January 2022 / Published online: 4 February 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract
In this paper, a fluorescent aptamer sensor was constructed based on the carbon dots (CDs) and graphene oxide (GO). This sensor combines the excellent fluorescence performance of CDs with the high specificity of aptamer, which can detect progesterone (P₄) with high sensitivity and selectivity. In the absence of P₄, the CDs-aptamer system and GO form a fluorescence resonance energy transfer process (FRET), which quenches the fluorescence of the CDs. When P₄ is added, the aptamer specifically binds to it, resulting the fluorescence of the CDs is recovered. At optimal conditions, the fluorescence intensity recovered by the CDs has a linear relationship with the concentration of P₄ in the range of 0.1–120 nM and the detection limit is 3.3 × 10⁻¹¹ M. Besides, the sensor has satisfactory detection results of P₄ in milk, indicating that constructed method has enormous potential for application in food safety.

Keywords Carbon dots · Fluorescence · Progesterone · Aptamer · Graphene oxide

Introduction
Progesterone (P₄) is a kind of natural steroid, which is mostly secreted by corpus luteum [1, 2]. It plays an important role in reproductive tissue, menstrual cycle regulation, fetal growth and early pregnancy [2]. Nowadays, many clinical studies use the amount of P₄ to measure the hormone level in the body [3]. However, when the amount of P₄ is too high, it will cause weight gain, breast discomfort [4], mental depression, acne and other side effects [5]. Epidemiological studies have confirmed that the imbalance of P₄ level has an impact on the incidence of breast and cervical cancer [6]. As many scholars have studied P₄ and other hormones, they have found that the female hormones in food can also have an impact on human health. Long-term consumption of foods containing P₄, even low-concentration foods, will interfere the normal function of human body secretion and immune system [7]. Especially milk, milk contains a considerable amount of P₄ [8]. When the content of P₄ in milk is too high, it will lead to breast cancer and other diseases [9]. Therefore, the detection of P₄ content is of great practical significance. At present, the main detection methods of P₄ are high performance liquid chromatography [10], fluorescence spectrophotometry [11], electrochemical method and so on [12, 13]. Most of these methods need expensive instruments, complex pretreatment process, cumbersome analysis process and too much time, so they are not suitable for large-scale field detection. However, because of the advantages of simple operation, high sensitivity and low cost, fluorescence spectrophotometry has caught the attention of researchers.

On the basis of fluorescence spectrophotometry, fluorescent sensors based on carbon dots (CDs) have attracted more and more attention due to their fast operation, excellent fluorescence performance, less harsh storage conditions and low toxicity. CDs are carbon nanoparticles with particle size less than 10 nm, which can stably emit light [14]. At present, CDs have been used in the fields of biological imaging [15], biosensor [16], ion molecule detection [17], information anti-counterfeiting storage, and so on [18]. Trapiella et al. prepared CdSe/ZnS quantum dots as a fluorescent probe to detect P₄ in milk [19], which based on the toxicity of semiconductor quantum dots, and the relatively complex synthesis method. Cao et al. developed an “on-off” fluorescence sensor based on CDs to detect P₄, which reflects the advantages of CDs [20], but the sensitivity and selectivity...
of this sensor are not high enough. Therefore, how to design a high-sensitivity and high-selectivity fluorescence sensor based on carbon dots to detect $P_4$ needs further exploration.

According to the literature, aptamer has the advantages of high specificity, high affinity, wide target range, easy modification, short preparation time, high stability and low toxicity [21, 22]. Therefore, we tried to combine CDs with aptamer to improve the sensitivity and selectivity of the sensor. Aptamer is a small oligonucleotide sequence or short polypeptide that can specifically recognize the target obtained by systematic evolution of ligands by exponential enrichment (SELEX) technology [23–25]. As a new type of biosensor identification element, it can be applied to the detection of food [26], drugs [27], viruses and other aspects [28], and also have a broad application in the field of biological imaging [29]. This work combines CDs with aptamer, combining the excellent fluorescence performance and low toxicity of CDs with the advantages of aptamer for specific detection of the detection substance, and constructs a simple and convenient fluorescence aptamer sensor.

Therefore, this paper constructed a fluorescent aptamer sensor based on CDs and graphene oxide (GO) to detect $P_4$. Among them, GO acts as a quencher to form an excellent donor–acceptor pair in the fluorescence resonance energy transfer (FRET) process with CDs. Energy transfers from the surface of CDs to GO, resulting in fluorescence quenching of CDs. When $P_4$ is present, it specifically binds to the aptamer on the surface of CDs, and CDs fall off from the surface of GO, causing the fluorescence of CDs to recover. Experiments show that the sensor has many advantages such as easy operation, high selectivity, high sensitivity, wide detection range, and low detection limit.

**Experimental Section**

**Materials and Apparatus**

Apparatus: RF-5301PC Fluorescence Spectrophotometer (Shimadzu, Japan), UV-2501PC UV Visible Spectrophotometer (Shimadzu, Japan), electronic analytical balance (Shanghai Precision Instrument Factory), ultrasonic cleaning instrument (Kunshan Ultrasonic Instrument Co., Ltd.), pH meter (Shanghai Leici instrument factory), beaker (25 mL, 50 mL, 100 mL), 25 ml brown volumetric flask, stirrer, pipette (10 μL, 200 μL, 1000 μL), JEOLJEM-200CX Transmission Electron Microscope (Hitachi, Japan), AVATAR 370 Fourier Transform Infrared Spectrometer (Nicol, USA), vacuum drying oven (Shanghai Yiheng Technology Co., Ltd.), autoclave.

Materials: Citric acid was purchased from Jiangsu Qiangsheng functional Chemistry Co., Ltd, ethylenediamine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), N-hydroxysuccinimide (NHS), potassium dihydrogen phosphate, potassium dihydrogen phosphate and ascorbic acid were purchased from Sinopharm Chemical Reagent Co., Ltd, progesterone aptamers: GATTAACATTAGCCCC ACCGGCCACCCC was purchased from Shanghai Qingke biological, L-cysteine was purchased from Shanghai Blue season biological Co., Ltd, graphene oxide, progesterone, l-epinephrine, glutathione, KCl, NaCl, MnCl₂·4H₂O, CdCl₂, dopamine, estradiol, acetone and anhydrous sodium sulfate were purchased from Aladdin Reagent Co., Ltd, ultra pure water.

**Synthesis of the CDs**

CDs was prepared by one-step hydrothermal method according to the reports in the literature [30], ultrapure water was used as the reaction solvent. First, 1.2 g citric acid was weighed and poured into 20 mL ultra pure water. Then 600 μL ethylenediamine was added into above solution. The mixed solution was ultrasonically stirred for 30 min until citric acid was dissolved. The mixed solution was transferred to 30 mL PTFE autoclave, heated at 200 °C for 4 h and cooled down to ambient temperature. Finally, the carbon dots solution was centrifuged and filtered to remove impurities, and the purified carbon dots was stored at 4 °C for further use.

**Synthesis of CDs and Progesterone Aptamer Conjugates**

The synthesis of the conjugates of CDs and $P_4$ aptamer is through covalent coupling, which means aptamer can be binded to the surface of CDs through covalent bonds [31]. First, 30 μL EDC (1 mg/μL) solution and 30 μL NHS (0.25 mg/μL) solution were mixed in 1 mL PBS buffer solution (pH 7.4), and then add 100 μL CDs solution. The mixed solution is ultrasonically stirred for 30 min. Next, 20 μL $P_4$ (10 μM) aptamer was added to 10 μL above mixed solution, and ultrasonic stirring was performed for 2 h.

**Detection of Progesterone**

Fluorescence quenching: 50 μL GO (100 μg/mL) solution was added into the mixed solution of CDs and $P_4$ aptamer to quench the fluorescence of CDs. After stirring and ultrasonic for 1 h, the mixture was diluted to 25 mL with PBS solution (pH 7.4) and transferred to a brown volumetric flask for fluorescence detection. The fluorescence intensity measured was $F_0$.

Fluorescence recovery: 50 μL $P_4$ with different concentrations was added into the mixed solution of CDs—aptamer—GO to recover the fluorescence. After stirring
and ultrasonic treatment for 1 h, the mixture was diluted to 25 mL with PBS solution (pH 7.4) and transferred to a brown volumetric flask for fluorescence detection. The measured fluorescence intensity was F.

**Pretreatment of Actual Sample Milk**

According to the previous literature, the actual sample pretreatment process is as follow [32]: 2 mL fresh milk purchased from a supermarket in Shanghai was mixed with 4 mL acetonitrile to the test tube and was sonicated for 10 min. Then, 5 g anhydrous sodium sulfate was added and stirred for 30 s. The mixed solution was centrifuged at 10,000 rpm for 5 min, and the liquid supernatant was gathered for detection of actual samples.

**Results and Discussion**

**Characteristics of CDs**

Firstly, the morphology of CDs was characterized by transmission electron microscope (TEM). As shown in Fig. 1, the carbon dots are nearly spherical particles, and they are uniformly dispersed in ultrapure water. The diameters of CDs were centered at 2.5–4.5 nm.

Then, the optical properties of CDs were studied. As shown in Fig. 2a, CDs has an obvious symmetrical strong UV absorption peak at 350 nm, which represents the $\pi - \pi^*$ transition of $C=\equiv$. There is a weak UV absorption peak at 240 nm, which represents the $n - \pi^*$ transition of $C=O$.

![Fig. 1 TEM image of CDs](image1)

![Fig. 2](image2)

---

![Fig. 3 FT-IR of the CDs](image3)

---

![Fig. 4](image4)

---

![Fig. 5](image5)
[33]. Under the excitation of 355 nm, there is a strong emission peak at 450 nm. Both UV absorption and fluorescence emission spectra show that the prepared CDs have excellent optical properties. It can be seen from Fig. 2b, the fluorescence emission spectra of CDs does not change with the change of the excitation wavelength. When the excitation wavelength is increased from 350 to 410 nm, the emission wavelength is kept at 450 nm. Only the emission intensity decreases, and there is no red shift. When the excitation wavelength is 355 nm, the emission intensity of CDs is the strongest.

Next, the surface functional groups were characterized by FT-IR. We can see from Fig. 3, the broad absorption peak near 3480 cm\(^{-1}\) corresponds to the stretching vibration of O–H functional group. The absorption peak near 1643 cm\(^{-1}\) corresponds to the stretching vibration of C = O functional group, and the absorption peak near 1556 cm\(^{-1}\) corresponds to the in-plane bending vibration of N–H functional group.

**Discussion on Detection Mechanism**

Scheme 1 is the process of detecting P\(_4\) by a fluorescent aptamer sensor based on CDs and GO. The aptamer of P\(_4\) is covalently bound to the probe CDs. Due to the fluorescence emission spectra of CDs \(\cdot\) CDs + aptamer and CDs + aptamer + P\(_4\) d The color change of (a) CDs-aptamer (b) CDs-aptamer-GO (c) CDs-aptamer-GO-P\(_4\) under UV light.
resonance energy transfer (FRET) process between CDs and GO, the fluorescence intensity of CDs is effectively suppressed. After $P_4$ is added into solution, it can specifically bind to the aptamer, contributing to the release of CDs and the recovery of fluorescence intensity.

We studied the effect of GO on the intensity of fluorescence. As shown in Fig. 4a, when GO exist, the fluorescence of CDs can be effectively quenched. As shown in Fig. 4c, the fluorescence of CDs can not be quenched without GO, which indicated that aptamer do not work in the quenching process, but the FRET process between CDs and GO. In the process of fluorescence resonance energy transfer, CDs is the donator and GO is the receptor. The basic condition to realize the FRET process is that the emission spectrum of the donator overlaps the absorption spectrum of the acceptor [34]. As shown in Fig. 5, the emission spectra of CDs overlap with the excitation spectra of GO in a large area, and the fluorescence emission spectra of CDs overlaps with the UV absorption spectra of GO in a small area. This phenomenon fits the conditions for the formation of FRET.

Then we studied the effect of aptamer on the intensity of fluorescence. When the aptamer was added, the specific binding of aptamer to the target led to the release of CDs and the recovery of fluorescence. CDs could not be separated from GO without aptamer, resulting in the fluorescence could not be recovered. It shows that aptamer is very important in the whole experiment. Figure 4d can visually show the detection mechanism of the experiment: (a) the blue fluorescence of CDs-aptamer under UV light; (b) the blue fluorescence of CDs-aptamer-GO under UV light is almost absent; (c) the blue fluorescence of CDs-aptamer-GO-$P_4$ under UV light.

**Optimization of Experimental Conditions**

We studied the pH value, the content of GO, the volume of CDs and the volume of aptamer to explore the optimal reaction conditions. Because CDs is sensitive to pH value, we chose pH value between 5.5 and 8.5 to study the influence of $P_4$ on fluorescence intensity. As shown in Fig. 6a, $F-F_0$ increases when pH increases from 5.5 to 7.4. This is because CDs will self assemble into larger particles rapidly under too acidic conditions, the oxygen-containing groups on the surface of the CDs will be oxidized slowly, and the fluorescence intensity of the CDs will be quenched, resulting in the decrease of F. $F-F_0$ decreases when the pH value increases from 7.4 to 8.5. The reason is that when the CDs is too alkaline, the structural tautomerism of CDs occurs rapidly, the speed of deoxidation reaction slows down, and the fluorescence intensity of the CDs will be quenched, resulting in the decrease of F [35]. Therefore, we chose a neutral environment, which is PBS buffer solution with pH 7.4.

Next, we studied the influence on the content of GO to $F_0$. It can be seen from Fig. 6b that when the content of GO increases from 20 to 100 μg/mL, the quenching degree increases greatly; When the content of GO increases from 100 to 200 μg/mL, the quenching degree decreases and then tends to be stable. This is because when the content of GO is too small, the distance between GO and CDs is too far, and it is not easy to carry FRET process. Therefore, we choose the content of GO when the quenching degree is the largest, that is 100 μg/mL.

Then, we studied the influence on the volume of CDs to $F-F_0$. As shown in Fig. 6c that when the volume of CDs increases from 5 to 18 μL, the fluorescence intensity
increases and then decreases, and reaches the peak at 10 μL. The reason is that when the volume of CDs is too small, the fluorescence intensity of CDs-aptamer conjugate is low, which leads to the low fluorescence intensity after adding P₄. When the volume of CDs exceeds the optimal value, excessive CDs will entangle or fold with the aptamer, and it is not easy to covalently couple with the aptamer. After the release of CDs, less P₄ can specifically bind with aptamer, resulting in lower fluorescence intensity of recovery. Consequently, we choose 20 μL as the optimal volume of aptamer.

Finally, we studied the influence on the volume of aptamer to F-F₀. It can be seen from Fig. 6d that when the volume of aptamer 5 increases to 35 μL, the fluorescence intensity increases and then decreases, and reaches the peak at 20 μL. This is due to the fact that when the volume of aptamer is too small, CDs are covalently coupled with too few aptamer, and less P₄ can specifically bind with aptamer after the release of CDs, resulting in lower fluorescence intensity of recovery. When the volume of the aptamer is too large, the long chain structure of the aptamer may intercept the fluorescence site of the fluorescence sensor, resulting in the low fluorescence intensity of the system. Consequently, we choose 20 μL as the optimal volume of aptamer.

**Detection of P₄**

Under the optimal experimental conditions, different concentrations of P₄ (0.1–120 nM) were added into CDs-aptamer-GO solution and diluted to 25 mL in brown volumetric flask for detection. As shown in Fig. 7a, when the concentration of P₄ increased from 0.1 to 120 nM, the fluorescence intensity of CDs-aptamer recovered, and the degree of recovery gradually increased. Figure 7b shows the linear
relationship between the recovered fluorescence intensity $F$ and $P_4$ concentration $C$ in the range of 0.1–120 nM. The linear equation is $F = 1.36728 \times C + 217.48259$ nM, the correlation coefficient $R^2$ is 0.99768, and the detection limit LOD is $3.3 \times 10^{-11}$ M. Table 1 compares the fluorescence sensor constructed for this work with other reported sensors for $P_4$ detection. The linear range of $P_4$ detection was 0.1–120 nM, and the detection limit was $3.3 \times 10^{-11}$ M. Compared with other work, our fluorescent sensor has a wider detection range and lower detection limit. Moreover, the simple synthesis method of CDs was combined with the characteristic that aptamer could specifically bind to the analytes. The sensor has the merits of convenience, high sensitivity, and can be used for the detection of $P_4$.

### Interference Analysis

Selectivity is a vital criterion to measure the performance of sensors. The most important characteristic of aptamer is that they can specifically identify with the detected substance. Therefore, in this work, we choose to use aptamer and CDs covalent coupling to detect $P_4$, for the purpose of improve the selectivity of the sensor. With the purpose of evaluating the anti-interference ability of the constructed fluorescent sensor, we measured the interference of many potential substances to the sensor. We measured the difference $F-F_0$ between the fluorescence intensity of the system quenched and recovered before and after the addition of 120 nM $P_4$ and $3 \times 120$ nM potential interfering substances. Interfering substances include amino acids and peptides (L-cysteine, glutathione), hormones (L-adrenaline, estrone, estradiol) and other substances (ascorbic acid, KCl, NaCl, MnCl$_2$, CdCl$_2$, dopamine). As shown in the Fig. 8, the value of $F-F_0$ is very small after adding the interfering substance, which means that the interfering substance hardly

### Table 1

Comparison with other reported methods for the detection of progesterone

| Methods     | Linear range(nM) | LOD(nM) | Ref |
|-------------|------------------|---------|-----|
| Electrochemical | 0.5–180          | 0.17    | [36]|
| Fluorescence            | $0–2 \times 10^5$ | 10.25   | [20]|
| Fluorescence            | 0.95–46.11       | 0.32    | [19]|
| FO-SPR                  | 3.18–31.8        | 1.59    | [37]|
| SWV                     | 0.25–22.26       | 0.25    | [38]|
| Fluorescence            | 0.1–120          | 0.033   | This work|

Fig. 7  
(a) Emission spectrum of the CDs-aptamer-GO in the addition of different concentration of $P_4$ from 0.1 nM to 120 nM(0.1, 5, 8, 10, 20, 40, 60, 80, 100 and 120 nM) b linear calibration curve of the aptasensor for $P_4$

Fig. 8  
Selective determination of 120 nM progesterone and $3 \times 120$ nM other potential interfering substances (Other potential interfering substances include (a) progesterone, (b) L-cysteine, (c) L-adrenaline, (d) ascorbic acid, (e) glutathione, (f) KCl, (g) NaCl, (h) MnCl$_2$, (i) CdCl$_2$, (j) dopamine, (k) estrone, (l) estradiol)
interferes with the sensor, indicating that our sensor has good selectivity.

Detection of $P_4$ in Real Samples

With the purpose of verifying the practicability of the constructed fluorescence sensor, we used standard addition method to detect the concentration of $P_4$ in real milk samples. Under the best experimental conditions, we added the known concentration of $P_4$ standard solution into the milk. As shown in Table 2, the actual concentration, recovery and relative standard deviation of $P_4$ can be obtained. The recovery was in the range of 97.2–101.2%, and the relative standard deviation was 1.81%. It can be concluded that our fluorescent sensor has a more accurate detection of $P_4$ in practical application.

Table 2 Determination of different amounts of $P_4$ in Real Samples

| Sample | Added (mol·L⁻¹) | Found (mol·L⁻¹) | Recovery (%) | M (%) | RSD (%) |
|--------|----------------|----------------|--------------|-------|---------|
| Milk 1 | 5.0×10⁻⁹       | 4.86×10⁻⁹      | 97.2         | 99.8  | 1.81    |
| 2      | 6.0×10⁻⁸       | 6.05×10⁻⁹      | 100.8        |       |         |
| 3      | 8.0×10⁻⁸       | 8.02×10⁻⁹      | 100.2        |       |         |
| 4      | 1.0×10⁻⁷       | 1.02×10⁻⁷      | 101.2        |       |         |

Conclusion

In conclusion, we synthesized CDs by a simple hydrothermal method, covalently coupled CDs with $P_4$ aptamer, and constructed a fluorescent aptamer sensor. The sensor combines the low toxicity and excellent optical properties of carbon dots and the excellent affinity of aptamer. The constructed method is simple and convenient, and conforms to the concept of environmental friendliness in green chemistry. Under the best experimental conditions, the detection range is 0.1–120 nM, and the detection limit is as low as 3.3×10⁻¹¹ M. Other substances have little interference with this sensor. The recoveries of the actual samples are 97.2–101.2%, and the relative standard deviation is 1.81%. Compared with other published sensors, our fluorescent aptamer sensor has high sensitivity and high selectivity, it also has good accuracy and recovery in the detection of actual milk samples, which verifies its potential in practical application.

Authors’ Contributions The first author HYC performed the design, operation and data processing of the experiment, and wrote the manuscript. HL carried out the assistance of the experiment and the inspection and modification of the manuscript. YF completed the inspection and modification of the manuscript. All authors read and approved the final manuscript.

Funding The authors thank the financial support by the National Nature Science Foundation of China (No. 21671132).

Availability of Data and Material Unpublished data.

Code Availability Not applicable.

References

1. Ynsa MD, Ager FJ, Millan JC, Gomez-Zubelbia MA, Pinheiro T (2004) Effect of hormone replacement therapy on the elemental contents of uterine tissue. Biol Trace Elem Res 101(1):37–46. https://doi.org/10.1385/bter:101:1:37
2. Lishko PV, Botchkina IL, Kirichok Y (2011) Progesterone activates the principal Ca²⁺ channel of human sperm. Nature 471(7338):387–391. https://doi.org/10.1038/nature09767
3. Ricanyova J, Gadzala-Kopciuch R, Reiffova K, Bazel Y, Buszewski B (2010) Molecularily imprinted adsorbents for preconcentration and isolation of progesterone and testosterone by solid phase extraction combined with HPLC. Adsorption J Int Adsorption Soc 16(4–5):473–483. https://doi.org/10.1007/s10450-010-9265-7
4. Sherwin BB (1999) Progestogens used in menopause - Side effects mood and quality of life. J Reprod Med 44(2):227–232.
5. Greendale GA, Reboussin BA, Hogan P, Barnabei VM, Shumaker S, Johnson S, Barrett-Connor E, Estrogen P, Postmenopausal Estrogen/Progestin Interventions Trial Investigators (1998) Symptom relief and side effects of postmenopausal hormones: Results from the postmenopausal estrogen/progestin interventions trial. Obstet Gynecol 92(6):982–988. https://doi.org/10.1016/s0029-7844(98)00305-6
6. Key TJ, Appleby PN, Reeves GK, Roddam AW, Helzlsouer KJ, Alberg AJ, Rollison DE, Dorgan JF, Brinton LA, Overvad K, Kaaks R, Trichopoulou A, Clavel-Chapelon F, Panico S, Duell EJ, Peeters PHM, Rinaldi S, Riboli E, Fentiman IS, Dowsett M, Manjer J, Lenner P, Hallmans G, Baglietto L, English DR, Giles GG, Hopper JL, Severi G, Morris HA, Koenig K, Zeleniuch-Jacquotte A, Arslan AA, Toniolo P, Shore RE, Krogh V, Micheli
A. Berrino F, Muti P, Barrett-Connor E, Laughlin GA, Kabuto M, Akiba S, Stevens RG, Nerishei K, Land CE, Cauley JA, Lui LY, Cummings SR, Gunter MJ, Rohan TE, Strickler HD, Endogenous Hormones and Breast Cancer Collaborative Group (2011) Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. Br J Cancer 105(5):709–722. https://doi.org/10.1038/bjc.2011.254

7. Andersson AM, Skakkebaek NE (1999) Exposure to exogenous estrogens in food: possible impact on human development and health. Eur J Endocrinol 140(6):477–485. https://doi.org/10.1530/eje.0.1400477

8. Roelofs JB, Van Eerdenburg F, Hazeleger W, Soede NM, Kemp B (2006) Relationship between progesterone concentrations in milk and blood and time of ovulation in dairy cattle. Anim Reprod Sci 91(3–4):337–343. https://doi.org/10.1016/j.anireprosci.2005.04.015

9. Farlow DW, Xu X, Veenstra TD (2010) Consumption of Cow’s Milk and Possible Risk of Breast Cancer. Breast Care 5(1):44–46. https://doi.org/10.1159/000277938

10. Decheng S, Xia F, Zhiming X, Shulin W, Shi W, Peilong W (2021) Trace analysis of progesterone and 21 progestins in milk by ultra-performance liquid chromatography coupled with high-field quadrupole-orbitrap high-resolution mass spectrometry. Food Chem 361:130115. https://doi.org/10.1016/j.foodchem.2021.130115

11. Tschmelak J, Kappel N, Gauglitz G (2005) TIRF-based biosensor for sensitive detection of progesterone in milk based on ultrasensitive progesterone detection in water. Anal Bioanal Chem 382(5):1895–1903. https://doi.org/10.1007/s00216-005-3261-x

12. Contreras-Jimenez G, Eissa S, Ng A, Alhadrami H, Zourob M, Siaj M (2015) Aptamer-based label-free impedimetric biosensor for detection of progesterone. Anal Chem 87(2):1075–1082. https://doi.org/10.1021/ac503639s

13. Kreuzer MP, McCarthy R, Pravda M, Guibault GG (2007) Development of Electrochemical Immunosensor for Progesterone Analysis in Milk. Anal Lett 37(5):943–956. https://doi.org/10.1081/AL-120030289

14. Zan GT, Wu T, Zhang ZL, Li J, Zhou JC, Zhu F, Chen HX, Wen M, Yang XC, Peng XJ, Chen J, Wu QS (2022) Biosimulated Nanocomposites with Self-Adaptive Stress Dispersion for Super-Foldable Electrodes. Adv Sci 9(3):2103714. https://doi.org/10.1002/advs.202103714

15. Sun Y-P, Zhou B, Lin Y, Wang W, Fernando KAS, Patash K, Merzian MJ, Harruff BA, Wang X, Wang H, Luo PG, Yang H, Kose ME, Chen B, Vca LMc, Xie SY (2006) Quantum-sized carbon dots for bright and colorful photoluminescence. J Am Chem Soc 128(24):7756–7757. https://doi.org/10.1021/ja062677d

16. Zan GT, Wu T, Zhu F, He PF, Cheng YP, Chai SS, Wang Y, Huang XF, Zhang WX, Wan Y, Peng XJ, Wu QS (2021) A Bimimetic Conductive Super-foldable Material. Matter 4(10):3232–3247. https://doi.org/10.1001/j.matte.2021.07.021

17. Zhang Y-F, Maimaiti H, Zhang B (2017) Preparation of cellulose-based fluorescent carbon nanoparticles and their application in trace detection of Pb(II). RSC Adv 7(5):4284–2850. https://doi.org/10.1039/c6ra26684c

18. Chai SS, Zan GT, Dong KZ, Wu T, Wu QS (2021) Approaching Superfoldable Thickness-Limit Carbon Nanofiber Membranes Transformed from Water-Soluble PVA. Nano Lett 21(20):8831–8838. https://doi.org/10.1021/acs.nanolett.1c02241

19. Trapellia-Alfonso L, Costa-Fernandez JM, Pereiro R, Sanz-Medel A (2011) Development of a quantum dot-based fluorescent immunosassay for progesterone determination in bovine milk. Biosens Bioelectron 26(12):4753–4759. https://doi.org/10.1016/j.bios.2011.05.044

20. Cao L, Yu L, Yue J, Zhang Y, Ge M, Li L, Yang R (2020) Yellow-emissive carbon dots for “off-and-on” fluorescent detection of progesterone. Mater Lett 271:127760. https://doi.org/10.1016/j.matlet.2020.127760

21. Ma H, Liu J, Ali MM, Mahmood MAI, Labanieh L, Lu M, Iqbal SM, Zhang Q, Zhao W, Wan Y (2015) Nucleic acid aptamers in cancer research, diagnosis and therapy. Chem Soc Rev 44(5):1240–1256. https://doi.org/10.1039/c4cs00357h

22. Mairal T, Oezalp VC, Sanchez PL, Mir M, Katakis I, O’Sullivan CK (2008) Aptamers: molecular tools for analytical applications. Anal Bioanal Chem 390(4):989–1007. https://doi.org/10.1007/s00216-007-3146-4

23. Ellington AD, Szostak JW (1990) In vitro selection of RNA molecules that bind specific ligands. Nature 366(6287):818–822. https://doi.org/10.1038/366818a0

24. Li J, Yan H, Wang K, Tan W, Zhou X (2007) Hairpin fluorescence DNA probe for real-time monitoring of DNA methylhation. Anal Chem 79(3):1050–1056. https://doi.org/10.1021/ac06169i

25. Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249(4968):505–510. https://doi.org/10.1126/science.2200121

26. Siriangkhawut W, Sittichan P, Ponhong K, Chantiratikul P (2017) Stripping voltammetric determination of trace cadmium and lead in Thai organic unpolished rice after ultrasound-assisted digestion. J Food Compos Anal 59:145–152. https://doi.org/10.1016/j.jfca.2017.02.018

27. Yao D, Liang A, Yin W, Jiang Z (2014) Resonance light scattering determination of trace bisphenol A with signal amplification by aptamer-nanogold catalysis. Luminescence 29(5):516–521. https://doi.org/10.1002/bio.2578

28. Li P, Zhou L, Wei J, Yu Y, Yang M, Wei S, Qin Q (2016) Development and characterization of aptamer-based enzyme-linked aptasorbent assay for the detection of Singapore grouper iridovirus infection. J Appl Microbiol 121(3):634–643. https://doi.org/10.1111/jam.13161

29. Wu X, Zhao Z, Bai H, Fu T, Yang C, Hu X, Liu Q, Champmanac C, Teng IT, Ye M, Tan W (2015) DNA Aptamer Selected against Pancreatic Ductal Adenocarcinoma for in vivo Imaging and Clinical Tissue Recognition. Theranostics 5(9):985–994. https://doi.org/10.7150/thno.11938

30. Zhu S, Meng Q, Wang L, Zhang J, Song Y, Jin H, Zhang K, Sun H, Wang H, Yang B (2013) Highly photoluminescent carbon dots for multicolor patterning, sensors and bioimaging. Angew Chem Int Ed Engl 52(14):3953–3957. https://doi.org/10.1002/anie.201300519

31. Chu BC, Wahl GM, Orgel LE (1983) Derivatization of unprotected polynucleotides. Nucleic Acids Res 11(18):6513–6529. https://doi.org/10.1093/nar/11.18.6513

32. Gao F, Zhang Q, Li X, Zhang Q, Mao T, Lu Y, Zhang W, Li H (2016) Comparison of standard addition and conventional isotope dilution mass spectrometry for the quantification of endogenous progesterone in milk. Accreditation Qual Assur 21(6):395–401. https://doi.org/10.1007/s00769-016-1236-6

33. Dong Y, Pang H, Yang HB, Guo C, Shao J, Chi Y, Li CM, Yu T (2013) Carbon-based dots co-doped with nitrogen and sulfur for high quantum yield and excitation-independent emission. Angew Chem Int Ed Engl 52(30):7800–7804. https://doi.org/10.1002/anie.201300519

34. Dong H, Gao W, Yan F, Ji H, Ju H (2010) Fluorescence Resonance Energy Transfer between Quantum Dots and Graphene Oxide for Sensing Biomolecules. Anal Chem 82(13):5511–5517. https://doi.org/10.1021/ac100852z

35. Xu Z-Q, Lan J-Y, Jin J-C, Gao T, Pan L-L, Jiang F-L, Liu Y (2015) Mechanistic studies on the reversible photophysical properties of carbon nanodots at different pH. Colloids Surf B 130:207–214. https://doi.org/10.1016/j.colsurfb.2015.04.012
36. Zhu Y, Xu Z, Gao J, Ji W, Zhang J (2020) An antibody-aptamer sandwich cathodic photoelectrochemical biosensor for the detection of progesterone. Biosens Bioelectron 160:112210. https://doi.org/10.1016/j.bios.2020.112210

37. Daems D, Lu J, Delport F, Marien N, Orbie L, Aernouts B, Adriaens I, Huybrechts T, Saeys W, Spasic D, Lammertyn J (2017) Competitive inhibition assay for the detection of progesterone in dairy milk using a fiber optic SPR biosensor. Anal Chim Acta 950:1–6. https://doi.org/10.1016/j.aca.2016.11.005

38. Jimena Monerris M, Javier Arevalo F, Fernandez H, Alicia Zon M, Gabriela Molina P (2012) Integrated electrochemical immunosensor with gold nanoparticles for the determination of progesterone. Sens Actuators B Chem 166:586–592. https://doi.org/10.1016/j.snb.2012.03.015

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.