ABSTRACT: Manganese dioxide (MnO₂) nanosheet-based fluorescence sensors often use oxidase-like activity or wide absorption spectrum for detection of antioxidants. In those strategies, MnO₂ nanosheets were reduced to Mn²⁺ by antioxidants. However, few strategies emphasize the role of Mn²⁺ obtained from MnO₂ reduction in the design of the fluorescence sensor. Herein, we expanded the application of a MnO₂ nanosheet-based fluorescence sensor by involving Mn²⁺ in the detection process using ascorbic acid (AA) as a model target. In this strategy, carbon dots (CDs), MnO₂ nanosheets, and tetraphenylporphyrin tetrasulfonic acid (TPPS) comprise a ternary system for ratiometric fluorescence detection of AA. Initially, CDs were quenched by MnO₂ nanosheets based on the inner filter effect, while TPPS maintained its fluorescence intensity. After the addition of AA, MnO₂ nanosheets were reduced to Mn²⁺ so that the fluorescence intensity of CDs was recovered and TPPS was quenched by coordination with Mn²⁺. Overall, AA triggered an emission intensity increase at 440 nm for CDs and a decrease at 640 nm for TPPS. The ratio intensity of CDs to TPPS (F₄₄₀/F₆₄₀) showed a good linear relationship from 0.5 to 40 μM, with a low detection limit of 0.13 μM for AA detection. By means of the alkaline phosphatase (ALP)-triggered generation of AA, this strategy can be applied for the detection of ALP in the range of 0.1−100 mU/mL, with a detection limit of 0.04 mU/mL. Furthermore, this sensor was applied to detect AA and ALP in real, complex samples with ideal recovery. This novel platform extended the application of MnO₂ nanosheet-based fluorescence sensors.

1. INTRODUCTION
Manganese dioxide (MnO₂) nanosheets, as a kind of two-dimensional nanomaterial, have received extensive investigative attention in the fields of chemical sensors, bioimaging, drug delivery, catalysts, and adsorbents owing to their simple fabrication procedures, high specific surface area, controllable size and morphologies, and good environmental compatibility. MnO₂ nanosheets have been characterized by wide UV−vis absorption and oxidase-like activity, which allowed the proposal of many “off−on”, “on−off”, or “ratiometric” fluorescence sensors. More specifically, the absorbance spectrum of MnO₂ nanosheets is from 250 to 500 nm, which overlaps with the excitation or emission spectrum of many fluorescence nanoparticles. Thus, fluorescence nanoparticles could be quenched by MnO₂ nanosheets based on the inner filter effect (IFE) or fluorescence resonance energy transfer (FRET). However, MnO₂ nanosheets can be reduced to Mn²⁺ by reductive species, such as glutathione (GSH) and ascorbic acid (AA), due to their oxidation ability, thereby losing their UV−vis absorption. Thus, the fluorescence intensity of fluorescence nanoparticles could be recovered. Based on the above mechanism, fluorescence nanoparticles and a MnO₂ binary system—such as carbon dots (CDs) and MnO₂ nanosheets,5−9 upconversion fluorescence nanoparticles and MnO₂ nanosheets,10,11 and gold nanoclusters and MnO₂ nanosheets12—were proposed for fluorescence signal “off−on” detection of GSH, AA, or H₂O₂.

MnO₂ nanosheets can oxidize a nonfluorescent substrate, such as o-phenylenediamine or dopamine, to enhance its...
fluorescence intensity. The reduction of MnO₂ resulted in the loss of the oxidase-like properties accompanied by a decrease in fluorescence intensity. On the basis of the above mechanism, many fluorescence "signal-off" sensors were proposed.15–17

The above single-emission fluorescence sensors displayed limited visualization effects or sensitivity when compared with dual-emission fluorescence sensors. Some ratiometric fluorescence sensors were proposed based on the quenching ability or oxidase-like property of MnO₂ nanosheets.18–24 For example, MnO₂ nanosheets oxidized o-phenylenediamine into 2,3-diaminophenazine with a strong fluorescence emission at 575 nm, which can quench Ag nanoclusters at 450 nm by the IFE, and based on this mechanism, AA was detected.18

The above proposed fluorescence sensors were based on the reduction of MnO₂ nanosheets, and substances that can reduce MnO₂ nanosheets,13,19 inhibit MnO₂ nanosheet reduction,21 or promote MnO₂ nanosheet reduction13 were the detection targets. However, in the above strategies, Mn²⁺ derived from the reduction of MnO₂ nanosheets was not involved in these sensors, and the role of Mn²⁺ was not reflected in those strategies. Recently, the role of Mn²⁺ from MnO₂ reduction was emphasized during the design of chemical sensors. For example, Jie’s group designed a photoelectrochemical sensor for the detection of GSH based on the reduction of MnO₂ and Mn²⁺-powered DNAzyme amplification strategy.25 Similarly, Zhao reported a nanomachine for DNA imaging based on the reduction of MnO₂ nanosheets, and Mn²⁺ participated in the catalytic cleavage of the DNA hybrid.26 Tan’s group27 proposed a "turn-on" fluorometric and magnetic bifunctional strategy for AA detection via CDs–MnO₂ nanosheets based on the Mn²⁺ response to magnetic resonance imaging. To the best of our knowledge, there was no fluorescence sensor designed using Mn²⁺ from MnO₂ etching. Recently, Xian reported that Mn²⁺ can quench the red fluorescence of tetraphenylporphyrin tetrasulfonic acid (TPPS) due to its coordination with TPPS.28 AA plays an important role in human daily life as an enzyme cofactor, reducing agent, and nutritional factor.24,29 Alkaline phosphatase (ALP) has been confirmed as a crucial serum biochemical indicator in the diagnosis of various diseases.30–32 ALP can catalyze 2-phospho-L-ascorbic acid (AAP) to produce AA.

In this work, inspired by preceding work, a novel CDs–MnO₂–TPPS ternary system ratiometric fluorescence sensor was designed using AA as the model target. CDs were employed because of their green synthesis and excellent solubility. What is more important is that a fluorescence sensor based on CDs and IFE can be proposed conveniently.33,34 Initially, CDs were quenched by MnO₂ nanosheets based on the IFE, while TPPS maintained its fluorescence intensity. After the MnO₂ nanosheets were reduced by AA to Mn²⁺, the fluorescence intensity of CDs recovered, and TPPS was quenched by coordinating with Mn²⁺. With the help of ALP, AAP can transform into AA; ALP can also be detected using this strategy. In this strategy, Mn²⁺ was fully utilized.

The detection mechanism was verified in detail. First, TEM images confirmed the successful preparation of CDs (Figure 1A) and the MnO₂ nanosheet (Figure 1B). The diameter of sphere CDs was about 2.5 nm, and the prepared MnO₂ displayed typical two-dimensional sheetlike structures with good dispersion. As shown in Figure 1C, MnO₂ nanosheets displayed a strong absorption peak at 250–500 nm. The excitation and emission spectra of the prepared CDs are in the range of 300–450 nm, which coincides with the absorption peaks of MnO₂ in a wide range (Figure 1C). From Figure 1D, we can see that when different concentrations of MnO₂ nanosheet solutions were combined with 4.0 mL of 1.0 mg/L CD solution, the fluorescence intensity of CDs gradually decreased with the addition of MnO₂ nanosheets (black line). When the concentration of MnO₂ nanosheets was 80 mg/L, nearly 90% of the fluorescence intensity of CDs was quenched. Figure 1C suggests the possibility of IFE or FRET between CDs and TPPS. The quenching mechanism was further explored by fluorescence lifetime, which changed proportionally with the concentration of the quencher for dynamic quenching and kept constant for static quenching.35 Figure S1 displayed that the fluorescence lifetimes of CDs did not display an obvious change without or with the MnO₂ nanosheet. The result indicates that the fluorescence quenching might be ascribed to static quenching. Meanwhile, in the TEM images of MnO₂ nanosheets after the addition of CDs (Figure S2A), we did not find the CDs attached to MnO₂. And the XPS analysis of the MnO₂ nanosheet with or without CDs also supported the TEM images of Figure S2A. The mechanism of IFE does not require any covalent linking between the IFE acceptor and fluorophore. Even if CDs were not combined with the MnO₂ nanosheet, it did not hinder the quenching mechanism of IFE. From the fluorescence lifetime, spectrum overlap, and TEM images, fluorescence quenching was mainly attributed to the IFE. At the same time, some other quenching mechanism

### 2. RESULTS AND DISCUSSION

#### 2.1. Detection Mechanism

The mechanism of the CDs–MnO₂–TPPS ternary system ratiometric fluorescence sensor for the detection of AA and ALP is presented in Scheme 1. In the ternary system, CDs were quenched by MnO₂ nanosheets based on the IFE, and TPPS maintained its intensity. After the MnO₂ nanosheets were reduced by AA to Mn²⁺, the fluorescence intensity of CDs recovered, and TPPS was quenched by coordinating with Mn²⁺. With the help of ALP, AAP can transform into AA; ALP can also be detected using this strategy. In this strategy, Mn²⁺ was fully utilized.

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**Scheme 1. The Principle of the CDs–MnO₂–TPPS Ternary System for Detection of AA and ALP**

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**Figure 1.**

1A) TEM images of CDs; 1B) TEM images of MnO₂ nanosheets; 1C) Absorption spectra of MnO₂ nanosheets; 1D) Fluorescence spectra of CDs (black line), MnO₂ nanosheets (blue line), and CDs–MnO₂ nanosheets (red line).
might be coexistent in the quenching system. With the addition of AA, MnO2 nanosheets were reduced, and the sheet structure was destroyed, as shown in Figure S2B, which resulted in the decreased absorption intensity. As a result, the color of the MnO2 nanosheet solution changed from brown to colorless (Figure 1D). Correspondingly, the fluorescence intensity of the corresponding CDs−MnO2 binary system recovered with the addition of AA (Figure 1D, red line). It was confirmed that CDs can be quenched by MnO2 nanosheets, and the fluorescence intensity of CDs can be restored by the addition of AA.

The excitation and emission spectra of TPPS (listed in Figure S3A) were partly overlapped with the absorption peaks of MnO2. As shown in Figure 2A, when MnO2 nanosheets (at a fixed concentration of 100 mg/L) were combined with 4.0 mL of 100 mg/L TPPS solution, the fluorescence intensity of TPPS did not change significantly. Only 10% of the fluorescence intensity of TPPS was quenched, while the fluorescence intensity of CDs was nearly completely quenched by 100 mg/L of MnO2 nanosheets. This is because the concentration of CD is low (10 mg/L), while the concentration of TPPS is high (100 mg/L). Another reason was that the spectrum overlap of the MnO2 nanosheet and CDs was higher than that of TPPS. Additionally, the sole addition of 30 μM AA could not decrease the fluorescence intensity of TPPS. However, with the addition of AA and MnO2 simultaneously, the fluorescence intensity of TPPS decreased. For control, the fluorescence intensity of TPPS decreased with the addition of 8 μM Mn2+. It can be concluded that the quenching of TPPS was caused by Mn2+, and the quenching mechanism was the coordination of Mn2+ with TPPS, as previously reported.28 The mechanism was verified by mass spectrum and UV absorption spectrum, as shown in Figure 2. From the mass spectrum of TPPS after the addition of Mn2+, it can be found that the largest m/z was 989, which was consistent with the molecular weight of TPPS−Mn2+. The peak of m/z 934 was consistent with the molecular weight of TPPS. From the MS analysis, the formation of the TPPS−Mn2+ complex can be concluded. The absorbance of TPPS was reduced after the addition of Mn2+, as shown in Figure 2C. The results show that the ability of light absorption of the TPPS−Mn2+ complex was weakened, which led to the decrease in fluorescence efficiency.

After the detection mechanism of CDs−MnO2 and the MnO2−TPPS binary system was verified, the detection mechanism of the CDs−MnO2−TPPS ternary system was further verified. As shown in Figure 2F, after MnO2 nanosheets were added to the CDs−TPPS binary system, the fluorescence intensity of CDs decreased greatly, while that of TPPS changed slightly, as discussed above. With the addition of AA, the fluorescence intensity of CDs gradually recovered, and the fluorescence intensity of TPPS decreased with the etching of MnO2 nanosheets and the formation of Mn2+. Moreover, the fluorescence color of the solution changed significantly from pink to blue before and after the addition of AA. Based on the above fact, the detection mechanism was considered feasible. It should be noted that, in this CDs−MnO2−TPPS ternary system, the detection mechanism was IFE and Mn2+ coordination, which does not require any covalent linking between CDs, MnO2, and TPPS. From Figure S3, the TEM image, XPS spectra, and Raman spectra confirmed that the CDs−MnO2−TPPS ternary system was the mixture of CDs,
MnO$_2$ and TPPS. There was no covalent linkage among them, which was convenient for the construction of a chemical sensor.

### 2.2. Optimization of the Detection Condition.

Considering that the fluorescence intensities of CDs and TPPS are affected by pH, the pH of the detection system was optimized. The fluorescence intensities of CDs and TPPS at different pH were measured, and the results are shown in Figure S4A. As shown in Figure S4A, the fluorescence intensity of CDs was less affected by pH and remained stable over a wide range from 5 to 8. The fluorescence intensity of TPPS was greatly affected by pH. It was lower in acidic conditions and higher in neutral and alkaline conditions. Considering the effect of pH on TPPS and CDs and the testing environment of real samples, pH 7.4 was chosen as the optimal detection condition.

To optimize the performance of the sensor for the detection of AA, the concentration of MnO$_2$ nanosheets was optimized, which mainly affected the detection sensitivity. When the amount of MnO$_2$ was too high, only a small amount of MnO$_2$ was etched after the addition of AA, and the fluorescence intensity of CDs was not effectively restored. At the same time, the fluorescence intensity of TPPS was partially quenched due to the large amount of MnO$_2$, and the fluorescence quenching effect caused by Mn$^{2+}$ was weakened. To achieve a high sensitivity, the concentration of MnO$_2$ nanosheets should be as low as possible. However, when the amount of MnO$_2$ was too low, the quenching effect of CDs was not obvious, and the background was strong. Conversely, a small amount of AA would reduce all MnO$_2$, resulting in a narrow detection range. Therefore, in the CDs–TPPS binary system, different amounts of MnO$_2$ were added, and the fluorescence spectra were investigated. As shown in Figure S4B, the fluorescence intensity of CDs decreased gradually with increasing MnO$_2$ concentration, while the fluorescence intensity of TPPS changed slightly. From Figure S4C, the concentration of MnO$_2$ was chosen at the inflection point as 70 mg/L for subsequent detection of AA, and this concentration yielded the maximum detection range and sensitivity.

The detection time was also optimized. After the addition of AA, the fluorescence intensities of CDs and TPPS were determined. Figure S4D shows that the recovery of the fluorescence intensity of CDs was very fast, which was due to the rapid etching of MnO$_2$ nanosheets. More importantly, it was due to the quenching of CDs by MnO$_2$ and based on the IFE. When MnO$_2$ was etched, the fluorescence intensity of TPPS decreased more slowly, which was mainly due to the slow coordination process between Mn$^{2+}$ and TPPS. Considering the fluorescence intensity changes of CDs and TPPS, the optimized detection time was 30 min.

### 2.3. Detection of AA.

The detection of AA by the CDs–MnO$_2$–TPPS ternary system was investigated under the above optimal conditions. As shown in Figure 3A, in the initial state, CDs were quenched by MnO$_2$ nanosheets, while TPPS maintained its fluorescence intensity. With the addition of AA, MnO$_2$ was etched, and the fluorescence intensity of CDs recovered gradually, while the fluorescence intensity of TPPS decreased significantly due to its coordination with Mn$^{2+}$. The ratio of the FL intensity ($I_{640}/I_{660}$) displayed good linearity for AA over the range of 0.5 to 40 μM, with a correlation coefficient of 0.993, as shown in Figure 2B. The detection limit was 0.13 μM at a signal-to-noise ratio of 3.

Moreover, the performance of the CDs–MnO$_2$–TPPS ternary system fluorescence sensor was compared with that of the binary system fluorescent probe. In the CDs–MnO$_2$ binary system, a single-emission "signal-off–on" fluorescence probe was constructed to detect AA, as displayed in Figure 3C. In the MnO$_2$–TPPS binary system, a single-emission "signal-off–on" fluorescence probe was constructed to detect AA, as displayed in Figure 3D. The linear range and limit of detection (LOD) of the three methods were compared and summarized in Table S1. The signal-off detection mode had the highest detection background; therefore, the detection sensitivity of the MnO$_2$–TPPS binary system was the lowest. The ratiometric fluorescence sensor, which could eliminate external interference and offers self-tuning capabilities, possessed the highest sensitivity and reliability. More importantly, visual detection of AA could be achieved based on the ternary ratiometric fluorescence sensor. As displayed in Figure 3E, with increasing AA concentrations, the fluorescence color of the ratiometric fluorescence sensor changed from orange to pink to blue. For the binary system, the fluorescence color change was not obvious.

The selectivity of the sensor was demonstrated by comparing the fluorescence intensity changes of $F_{640}/F_{660}$ against other reductive substances, including GSH, Cyc glucose, oxalic acid, tartaric acid, citric acid, and malic acid. From Figure 4A, glucose, oxalic acid, tartaric acid, citric acid, and malic acid had no obvious effect on the fluorescence intensities $F_{640}/F_{660}$ and the fluorescence color was similar to the blank sample. However, the addition of GSH or Cyc...
caused significant changes in fluorescence intensities $F_{440}/F_{640}$ because GSH or Cyc can decompose MnO$_2$ nanosheets due to its considerable reducing capacity. NEM can reacted rapidly and specifically with sulphydryl groups, such as proteins, GSH, and Cyc, and the interference effects of GSH or Cyc would be blocked with the addition of NEM. As shown in Figure S5, after the addition of GSH or Cyc, the absorption of NEM at 300 nm decreased. So, in this work, to reduce the interference of GSH and Cyc, NEM was added as a masking agent. As displayed in Figure 3A, the interference effects of GSH or Cyc were blocked with the addition of NEM. Using NEM as a masking agent, AA could be selectively detected using the ternary system.

2.4. Sensitivity and Selectivity for the Detection of ALP. The feasibility of the constructed CDs–MnO$_2$–TPPS ternary system for the detection of ALP was verified. The sole addition of AAP or ALP did not cause an obvious signal change of the CDs–MnO$_2$–TPPS ternary system, indicating that AAP and ALP had little effect on the fluorescence spectra of CDs and TPPS. When AAP and ALP were simultaneously added into the CDs–MnO$_2$–TPPS ternary system, the quenched fluorescence of CDs was recovered, and TPPS was quenched due to the destruction of MnO$_2$ nanosheets. This indicates that the ternary system could be applied to detect ALP.

To optimize the detection of ALP, the amount of AAP and the incubation time were studied, and the results are displayed in Figure S6A. For an ALP concentration of 100 mU/mL, the ratio of the fluorescence intensity ($I_{440}/I_{640}$) reached the maximum when 6 mM AAP was employed. In the presence of 6 mM AAP and 100 mM ALP, the incubation time was studied, and the result is displayed in Figure S6B. The ratio of the fluorescence intensity ($I_{440}/I_{640}$) progressively increased and reached a plateau when the enzymatic reaction time reached 60 min. Thus, in the following work, 60 min was selected as the optimal reaction time. It should be noted that the assay time included (i) the generation time of AA, (ii) the reaction time between the AA and MnO$_2$ nanosheets, and (iii) the reaction time between the Mn$^{2+}$ and TPPS. Of these three steps, the generation time of AA and the reaction time between the Mn$^{2+}$ and TPPS were the time-controlled steps. Thus, the entire assay time was 90 min, which included 60 min for the production of AA and another 30 min for the coordination between Mn$^{2+}$ and TPPS.

Hence, ALP detection was performed using the above optimal conditions. As shown in Figure 5A, $F_{440}/F_{640}$ increased with increasing ALP concentration. There was a good linear relationship between $F_{440}/F_{640}$ and ALP concentration ranging from 0.1 to 100 mU/mL, with an LOD of 0.04 mU/mL. The sensitivity of the ternary system was equal to or better than that of some reported methods for ALP detection. The specificity of the CDs–MnO$_2$–TPPS ternary system toward ALP was investigated using HRP, lysozyme, GOx, thrombin, cytochrome c (Cyc), and human IgG (HigG) as control enzymes at a fixed concentration of 50 mU/mL. The results are shown in Figure S5B. The CDs–MnO$_2$–TPPS ternary system showed good sensitivity toward changes in fluorescence signal only in the presence of ALP, while the other enzymes had no obvious interference. The excellent selectivity for ALP detection stemmed from the specific reaction between ALP and AAP. The inset photo from Figure 3B shows that the addition of ALP caused an obvious color change, making visual detection possible.

2.5. Detection of AA and ALP in Real Samples. To evaluate the applicability of the CDs–MnO$_2$–TPPS ternary system for AA detection in real samples, AA analysis in fruit juice beverages was performed. As presented in Table 1, the obtained AA concentration in diluted kiwi fruit juice, orange juice, and apple juice was 34.37, 36.42, and 9.75 μM, respectively, with relative standard deviations (RSDs) below 3.49%. The concentration of AA in the undiluted juice lies in the normal range of fruit, and the results are in accordance with those measured by the AA assay kit.

To evaluate the applicability of the CD–MnO$_2$–TPPS ternary system for ALP detection in real samples, detection of ALP in real human serum samples was performed. The ALP activity detected by the CDs–MnO$_2$–TPPS system in real samples was 30.5 μU/mL, while the value was 32.4 μU/mL.

Table 1. AA Concentrations (μM) in Real Samples Measured by the Developed Ternary CDs–MnO$_2$–TPPS System and Commercial Assay Kit

| sample          | CDs–MnO$_2$–TPPS sensor | commercial assay kit |
|-----------------|-------------------------|----------------------|
| orange juice    | 36.42 ± 4.32            | 37.98 ± 3.21         |
| kiwi fruit juice| 34.37 ± 3.75            | 31.43 ± 4.65         |
| apple juice     | 9.75 ± 4.65             | 10.65 ± 3.92         |
using the commercial ALP assay kit. The value was consistent with the ALP activity of normal people (normal value: 30–120 mU/mL for healthy adults\(^1\)). The spiked recovery of ALP in 100-fold diluted human serum was listed in Table 2. The obtained ALP recoveries ranged from 90.34 to 102.39%, with relative standard deviations (RSDs) below 4.25%. The detection results by the CD–MnO\(_2\)–TPPS ternary system were in accordance with those measured by the ALP assay kit when the activity of ALP was higher than 10 mU/mL. When the activity of ALP was low, such as 1 mU/mL, the recovery was still higher than 90% by the CD–MnO\(_2\)–TPPS ternary system. However, the value could not be detected by the commercial ALP assay kit with an LOD of 4 mU/mL. The results demonstrated that the proposed biosensor for ALP detection could be employed in the analysis of biological samples even if the activity was low. Compared with commercial ALP assay kits, this CD–MnO\(_2\)–TPPS ternary system has a higher sensitivity. Meanwhile, this method can realize visual detection without complicated and expensive instruments.

### 3. EXPERIMENTAL SECTION

#### 3.1. Materials and Chemicals

l-Ascorbic acid (AA), alkaline phosphatase (ALP), 2-phospho-L-ascorbic acid (AAP), glycine, urea, sodium dodecyl sulfate solution (SDS), KMnO\(_4\), TPS (85%), oxalic acid, malic acid, tartaric acid, and glucose were purchased from Aladdin Reagent Co. (Shanghai, China). N-Ethylmaleimide (NEM), GSH, horseradish peroxidase (HRP), lysozyme, and glucose oxidase (GOx) were obtained from Sigma-Aldrich (Shanghai, China). Phosphate buffer (10 mM, pH 7.0) was employed for AA and ALP detection.

#### 3.2. Characterization and Instrumentation

The fluorescence spectra in this work were recorded using an F-7000 spectrofluorometer (Hitachi) with 390 nm excitation and slit of 5.0/5.0 nm. The morphology of MnO\(_2\) nanosheets was characterized by transmission electron microscopy (TEM, JEM-2100F). UV-3600 double-beam ultraviolet spectrophotometry (Shimadzu, Japan) was employed for absorbance spectra detection.

#### 3.3. Preparation of MnO\(_2\) Nanosheets and CDs

Ultrathin MnO\(_2\) nanosheets were synthesized by the reduction of KMnO\(_4\) according to a previously reported method.\(^8\) Finally, MnO\(_2\) nanosheets were freeze-dried to a brownish black powder, and an aqueous solution at a concentration of 800 mg/L was prepared for further use. CDs were prepared by the microwave method using glycine and urea as the precursors.\(^40\) Finally, CDs were freeze-dried and prepared as a 1 mg mL\(^{-1}\) aqueous solution for further use.

#### 3.4. Detection of AA and ALP in Aqueous Buffer

For AA detection, 30 \(\mu\)L of the CD solution (1 mg mL\(^{-1}\)), 200 \(\mu\)L of the TPPS solution (1 mg mL\(^{-1}\) in water), and 200 \(\mu\)L of the MnO\(_2\) nanosheet solution (800 mg/L) were combined with PBS buffer (10 mM, pH = 7). Then, 2.0 to 200 \(\mu\)L of AA solutions (0.5 mM) were added, and a final volume of 2.0 mL was obtained. The mixtures were incubated for 30 min at room temperature before fluorescence spectra determination.

For ALP detection, 750 \(\mu\)L of ALP at different activities was first reacted with 250 \(\mu\)L of AAP (6 mM) at 37 °C for 60 min to produce AA. Then, 20 \(\mu\)L reaction products were added to the mixture of MnO\(_2\) nanosheets, CDs, and TPPS ternary system. The following procedure was the same as that of AA detection above.

#### 3.5. Selectivity of the Detection Assay

The selectivity of the CD–MnO\(_2\)–TPPS ternary system for AA detection was evaluated using other antioxidant substances, such as GSH, cysteine, citric acid, glucose, oxalic acid, tartaric acid, and malic acid, at a fixed concentration of 30 \(\mu\)M, respectively. The selectivity of the CD–MnO\(_2\)–TPPS ternary system for ALP detection was evaluated using other enzymes, including HRP, lysozyme, GOx, Cyc, HlgG, and thrombin, at fixed activity of 50 \(\mu\)U mL\(^{-1}\), respectively. The analysis procedure was the same as that of AA detection above.

#### 3.6. Detection of AA and ALP in Real Samples

The practical applicability of the CD–MnO\(_2\)–TPPS probe was investigated by detection of AA in juice and ALP in human serum samples. Fresh juices from orange, kiwi fruit, and apple were centrifuged at 8000 rpm for 10 min to obtain aqueous samples. After being neutralized, the kiwi fruit juice, orange juice, and apple juice were diluted 200-, 50-, and 150-fold, respectively, to ensure that the AA concentrations fell in the range of the calibration curve. Then, the samples were analyzed using the procedure described above. For control, the AA concentrations were compared with those measured by commercial Amplitude Fluorimetric AA assay kits.

For the detection of ALP in human serum samples, human serum samples were provided by a local hospital. All procedures in this study were approved by the Human Ethics Committee at Linyi University and were performed in accordance with the approved guidelines. The serum samples were treated with trichloroacetic acid and centrifuged at 12,000 rpm for 20 min, the supernatant was adjusted to pH 7.4 and diluted 100-fold. Then, ALP solutions at different activities were spiked into the treated serum samples and detected using the above-mentioned method. For control, the test results of ALP activities were compared with those measured by commercial ALP ELISA assay kits from Shenzhen Ziker Biological Technology Co., Ltd.

#### 4. CONCLUSIONS

In summary, the CD–MnO\(_2\)–TPPS ternary system was developed for the detection of AA and ALP based on the reduction of MnO\(_2\) nanosheets. With the addition of AA, MnO\(_2\) nanosheets were reduced to Mn\(^{2+}\), which resulted in the recovery of CDs and quenching of TPPS by coordination with Mn\(^{2+}\). The ratio of the fluorescence intensity of CDs to TPPS \((F_{CD}/F_{TPPS})\) was linear for AA concentrations ranging from 0.5 to 40 \(\mu\)M, with an LOD of 0.13 \(\mu\)M. In addition, this sensor could be expanded to the detection of ALP in the range of 0.1–100 mU/mL, with an LOD of 0.04 mU/mL. In this...
strategy, Mn$^{2+}$ plays an important role in the ratiometric fluorescence sensor, and this platform extends the application of MnO$_2$ nanosheet-based fluorescence sensors.

**ASSOCIATED CONTENT**

1. Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c01828.

Preparation of MnO$_2$ nanosheets and CDs; condition optimization for the CD$\text{-}\text{MnO}_2$−TPPS ternary system fluorescence sensor; comparison of the analytical performance of the CD$\text{-}\text{MnO}_2$−TPPS ternary system with the CD$\text{-}\text{MnO}_2$ binary system and MnO$_2$−TPPS binary system for detection of AA; and method performance comparison (PDF)

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**Notes**

The authors declare no competing financial interest.

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