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# A Novel “Off-On” Fluorescent Probe Based on Carbon Nitride Nanoribbons for the Detection of Citrate Anion and Live Cell Imaging

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**Abstract:** A novel fluorescent “off-on” probe based on carbon nitride (C<sub>3</sub>N<sub>4</sub>) nanoribbons was developed for citrate anion (C<sub>6</sub>H<sub>5</sub>O<sub>7</sub><sup>3-</sup>) detection. The fluorescence of C<sub>3</sub>N<sub>4</sub> nanoribbons can be quenched by Cu<sup>2+</sup> and then recovered by the addition of C<sub>6</sub>H<sub>5</sub>O<sub>7</sub><sup>3-</sup>, because the chelation between C<sub>6</sub>H<sub>5</sub>O<sub>7</sub><sup>3-</sup> and Cu<sup>2+</sup> blocks the electron transfer between Cu<sup>2+</sup> and C<sub>3</sub>N<sub>4</sub> nanoribbons. The turn-on fluorescent sensor using this fluorescent “off-on” probe can detect C<sub>6</sub>H<sub>5</sub>O<sub>7</sub><sup>3-</sup> rapidly and selectively, showing a wide detection linear range (1~400 μM) and a low detection limit (0.78 μM) in aqueous solutions. Importantly, this C<sub>3</sub>N<sub>4</sub> nanoribbon-based “off-on” probe exhibits good biocompatibility and can be used as fluorescent visualizer for exogenous C<sub>6</sub>H<sub>5</sub>O<sub>7</sub><sup>3-</sup> in HeLa cells.

**Keywords:** carbon nitride; nanoribbons; fluorescent detection; citrate anion; biosensing

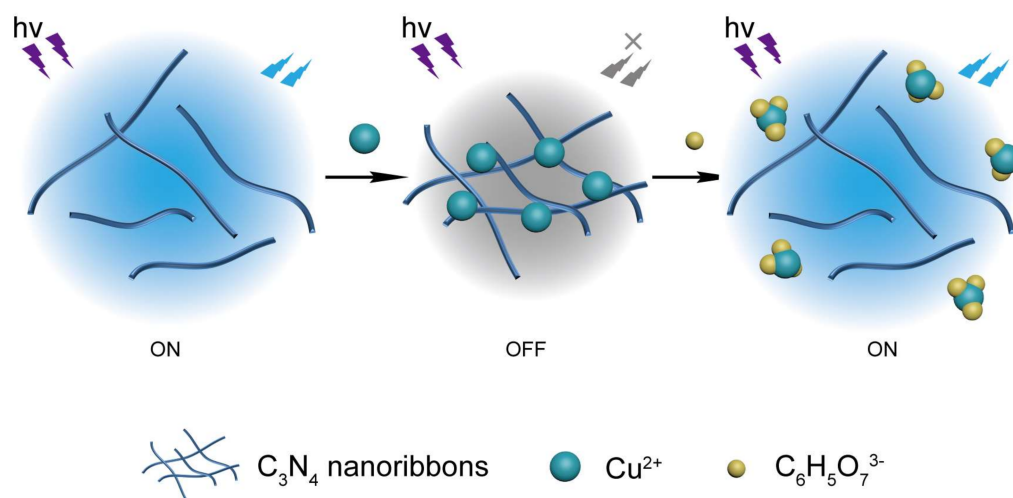
## 1. Introduction

Citrate is a critical metabolite that is involved in various biological systems, such as mitochondrial energy generation, cytosolic biomacromolecular synthesis, inflammatory response, blood coagulation, and the regulation of the size of apatite crystals in bone [1–5]. Citrate deficiency is the main reason for kidney dysfunction such as nephrocalcinosis and nephrolithiasis [6]. Recent medical research has shown that the tracking of citrate levels has become an effective method for the identification of prostate cancer [2,7]. Therefore, monitoring citrate content is of great importance in biomedical and analytical sciences. To date, many analytical methods have been used for citrate detection including electrochemistry [8], capillary electrophoresis [9], polarography [10], gas or liquid chromatography [11,12], UV-vis spectrophotometry [6,13], and spectrofluorimetry [14,15]. The spectrofluorimetry method has attracted great attention owing to its easy operation, high sensitivity, and lower equipment requirements [6,13,16].

Carbon nitride (C<sub>3</sub>N<sub>4</sub>) is a metal-free polymeric semi-conductor with a narrow bandgap of about 2.7 eV. Due to its special photoluminescence (PL), facile synthesis, and good biocompatibility [17,18], C<sub>3</sub>N<sub>4</sub> has been applied for environmental decontamination, artificial photosynthesis, biotherapy, bioimaging, and sensors [17–24]. So far, some fluorescent sensing platforms based on C<sub>3</sub>N<sub>4</sub> have been designed for the detection of various ions and molecules, including Cu<sup>2+</sup>, Fe<sup>3+</sup>, Ag<sup>+</sup>, acetylthiocholine,

biothiols, and cyanide [18,23,25,26]. However, to the best of our knowledge, there is no report of fluorescent sensors based on  $C_3N_4$  nanoribbons for citrate anion detection.

In this work, blue fluorescent  $C_3N_4$  nanoribbons were prepared by alkali-catalyzed hydrolysis from bulk  $C_3N_4$  [27]. Furthermore, we demonstrated that the obtained  $C_3N_4$  nanoribbons can serve as a novel “off-on” fluorescent probe for the detection of citrate anion ( $C_6H_5O_7^{3-}$ ) with excellent sensitivity and selectivity based on fluorescence quenching by  $Cu^{2+}$  through a photoinduced electron transfer [28,29] and fluorescence recovery by the addition of  $C_6H_5O_7^{3-}$ . We hypothesized that the interaction of  $Cu^{2+}$  and  $C_3N_4$  nanoribbons is inhibited by the strong chelation between  $Cu^{2+}$  and  $C_6H_5O_7^{3-}$  [30–32]. This is the first time that  $C_3N_4$  nanoribbons have been applied for  $C_6H_5O_7^{3-}$  detection. Importantly, the turn-on fluorescent sensor using this fluorescent “off-on” probe exhibits low cytotoxicity and can be applied for  $C_6H_5O_7^{3-}$  detection in living cells. Scheme 1 illustrates the sensing principle of the  $C_3N_4$  nanoribbon-based fluorescent sensor.



**Scheme 1.** Schematic illustration of the  $C_3N_4$  nanoribbon-based fluorescent citrate sensor.

## 2. Materials and Methods

### 2.1. Materials and Reagents

Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum, and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Hyclone. HeLa cells were supplied by KeyGen Biotech Co., Ltd. (Nanjing, China). All inorganic salts were of analytical grade and obtained from Aladdin reagent (Shanghai, China). Formic acid, sodium acetate, and propionic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Ultrapure water (Milli-Q system, Millipore Corp., Billerica, MA, USA) was used throughout this study.

### 2.2. Characterization

UV–vis spectroscopy measurements were performed on a Shimadzu UV-3600 spectrophotometer. Fluorescence spectra were recorded on an RF-5301PC spectrofluorophotometer. The Fourier transform infrared (FTIR) spectrum of  $C_3N_4$  nanoribbons was measured on a Nexus 870 FTIR spectrometer. A JEOL 2010 transmission electron microscope (TEM) was used for the characterization of  $C_3N_4$  nanoribbons. The X-ray diffraction (XRD) pattern of  $C_3N_4$  nanoribbons was recorded on an X-ray powder diffractometer with graphite monochromatized  $Cu\ K\alpha$  radiation (D8 Advance; Bruker, Karlsruhe, Germany). The X-ray photoelectron spectroscopy (XPS) investigation was carried out on PHI 5000 VersaProbe system with Al cathode as the X-ray source. The  $C_3N_4$  nanoribbons was dropped on silicon slices for XPS characterization. Dynamic light scattering (DLS) and zeta potential measurements were conducted on a zeta potential analyzer (Zeta PALS, Brookhaven Instruments Corp.,

Brookhaven, NY, USA). Cell fluorescent images were recorded by confocal laser scanning microscopy (Olympus FV1000, Tokyo, Japan).

### 2.3. Preparation of $C_3N_4$ Nanoribbons

The bulk  $C_3N_4$  was prepared by the calcination of melamine at  $600\text{ }^\circ\text{C}$  ( $5.0\text{ }^\circ\text{C min}^{-1}$ ) for 4 h under an Ar atmosphere [33]. The  $C_3N_4$  nanoribbons were prepared via the alkali-catalyzed hydrolysis of bulk  $C_3N_4$  [27]. In brief, 10 mg of bulk  $C_3N_4$  was dispersed in 10 mL of sodium hydroxide solution (8.0 M) and sonicated for 2 h at  $60\text{ }^\circ\text{C}$ . After cooling to room temperature, this solution was centrifuged and washed five times with ultrapure water. The product was collected and dialyzed (the molecular weight cutoff of the dialysis bag was 1000 kDa) for further experiments.

### 2.4. Synthesis of $Cu^{2+}$ - $C_3N_4$ Nanoribbon Complex

One hundred microliters of  $CuCl_2$  (10 mM) was added to 9.9 mL of  $C_3N_4$  nanoribbons aqueous solution ( $1\text{ mg mL}^{-1}$ ). Then, the mixture was stirred for 10 min at room temperature. After that, the mixture was centrifuged and washed with ultrapure water to remove excessive copper ions. Finally, the obtained precipitate was dispersed in ultrapure water to obtain a  $Cu^{2+}$ - $C_3N_4$  nanoribbon solution.

### 2.5. Fluorescent Detection of $C_6H_5O_7^{3-}$

Forty microliters of  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex ( $C_3N_4$ :  $1\text{ mg mL}^{-1}$ ) was added to 960  $\mu\text{L}$  ultrapure water, and then  $C_6H_5O_7^{3-}$  solution with various concentrations was added to the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex. After standing for 20 s, the fluorescence spectra were monitored at the excitation of 360 nm.

### 2.6. Selectivity of $Cu^{2+}$ - $C_3N_4$ Nanoribbon-Based Probe for $C_6H_5O_7^{3-}$ Detection

To explore the possible interference of other anions, formic acid, sodium acetate, propionic acid and the following anionic sodium/potassium salts were used in this study: 1 mM anion solutions, including  $Br^-$ ,  $C_6H_5O_7^{3-}$ ,  $Cl^-$ ,  $CN^-$ ,  $F^-$ ,  $H_2PO_4^-$ ,  $HCO_3^-$ ,  $I^-$ ,  $NO_3^-$ ,  $OH^-$ ,  $CH_3COO^-$ , and  $SO_4^{2-}$  were added to the solution of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex, respectively. After thoroughly mixing and standing for 20 s, the fluorescence measurements were carried out to investigate the selectivity of the proposed fluorescent sensor.

### 2.7. Cell Imaging and Cytotoxicity Assay

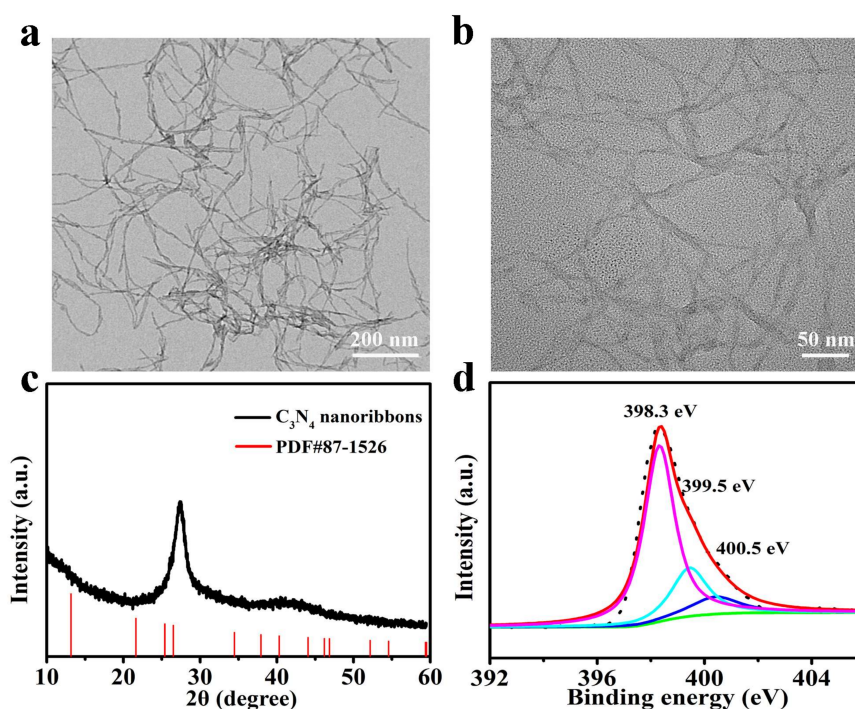
Human cervical cancer (HeLa) cells used in this study were cultured at  $37\text{ }^\circ\text{C}$  in a 5%  $CO_2$  incubator in DMEM medium, which contains fetal bovine serum (10%), streptomycin ( $100\text{ mg mL}^{-1}$ ), and penicillin ( $100\text{ U mL}^{-1}$ ). When the cells had grown to 80% confluency, the cells were digested with trypsin, collected and seeded in a confocal dish, and cultured overnight. Then the cells were pretreated with  $C_6H_5O_7Na_3$  (1 mM). After 12 h incubation, the cells were gently washed with phosphate-buffered saline (PBS) solution (10 mM, pH = 7.4) and treated with  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex for another 4 h. Finally, the resulting cells were washed with PBS solution (10 mM, pH = 7.4) and then fluorescence images were taken on a confocal microscope under UV excitation.

For cytotoxicity assay, 100  $\mu\text{L}$  of cells suspension ( $10^5\text{ cells mL}^{-1}$ ) was seeded to each well of 96-well plates. Then the medium was replaced by different concentrations of  $C_3N_4$  nanoribbons or  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex and culture for 24 h. Following this, the cells were washed with PBS solution. After that, the standard MTT assay was carried out for the determination of cell viabilities relative to the untreated cells.

### 3. Results and Discussion

#### 3.1. Characterization of $C_3N_4$ Nanoribbons

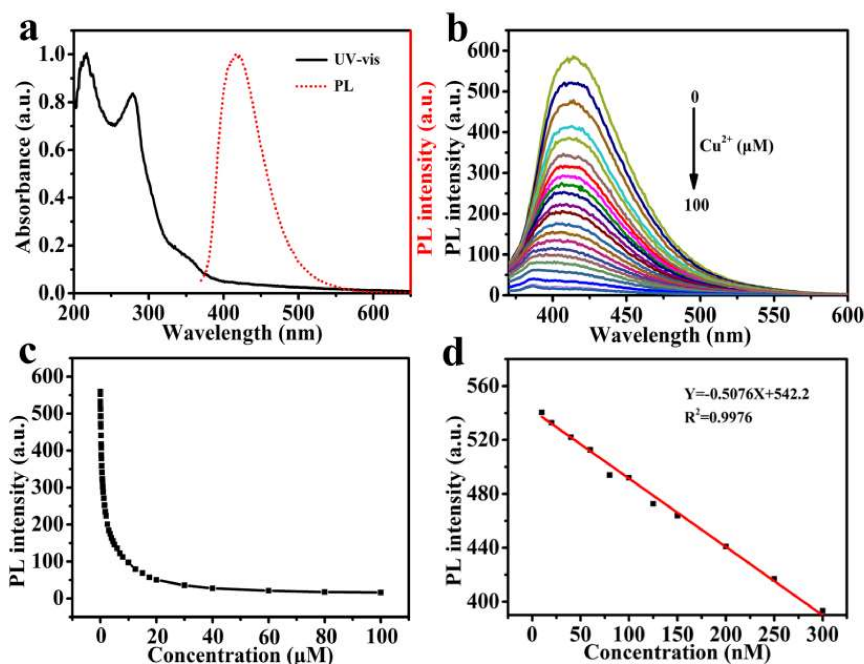
The  $C_3N_4$  nanoribbons were prepared by ultrasonic exfoliation of bulk  $C_3N_4$  in an alkaline solution. The related characterizations of bulk  $C_3N_4$  are shown in Figure S1. TEM images display the morphology and size distribution of the  $C_3N_4$  nanoribbons. As shown Figure 1a,  $C_3N_4$  nanoribbons present an average diameter of approximately 5 nm and a length of up to 200 nm. High-resolution transmission electron microscopy (HRTEM) image clearly shows that single and few-layer  $C_3N_4$  nanoribbons were obtained (Figure 1b). The XRD pattern of  $C_3N_4$  nanoribbons (Figure 1c) showed a broad distinct diffraction peak at  $27.2^\circ$ , which can be ascribed to the strong  $\pi$ -conjugated layers characteristic (002) of  $C_3N_4$  [17,34].



**Figure 1.** (a) TEM image, (b) HRTEM image, (c) X-ray diffraction (XRD) pattern and (d) N 1s X-ray photoelectron spectroscopy (XPS) spectrum of  $C_3N_4$  nanoribbons.

The composition and structure of  $C_3N_4$  nanoribbons were confirmed by XPS and FTIR measurements. The XPS survey spectrum of  $C_3N_4$  nanoribbons (Figure S2) displays binding energies of C (283 eV) and N (397 eV). The C 1s XPS spectrum of  $C_3N_4$  nanoribbons (Figure S3) can be fitted into three peaks centering at 284.6, 285.4, and 287.8 eV, which can be attributed to C-C,  $sp^2C=N$ , and  $sp^3C-N$  of  $C_3N_4$ , respectively [35–39]. The XPS spectrum of the N 1s spectrum (Figure 1d) can be fitted into three different peaks at 398.3, 399.5, and 400.5 eV, being assigned to C=N-C, (N-(C)<sub>3</sub>), and -NH<sub>2</sub>, respectively [36,40]. The FTIR spectrum of  $C_3N_4$  nanoribbons is presented in Figure S4. The broad peaks at  $3330$  and  $3182\text{ cm}^{-1}$  are ascribed to the stretching vibrations of NH<sub>2</sub> and N-H groups, respectively [39]. The peaks centered at  $1637$ ,  $1577$ ,  $1420$ ,  $1334$ , and  $1284\text{ cm}^{-1}$  indicated the typical stretching modes of CN heterocycles [41–43]. Beside the peaks mentioned above, the peak at  $810\text{ cm}^{-1}$  indicated the vibration of the s-triazine ring [34,44].

The photophysical properties of  $C_3N_4$  nanoribbons were investigated by UV-vis and PL spectra (Figure 2a). The prepared  $C_3N_4$  nanoribbons present two strong absorption peaks at 216 and 278 nm. Meanwhile, a fluorescent emission at 415 nm can be seen from fluorescence spectrum at the excitation of 360 nm (Figure 2a). The PL intensity of  $C_3N_4$  nanoribbons increases dramatically with the increasing pH of solution ranging from 9 to 12, and displays slight changes under acid conditions (Figure S5).



**Figure 2.** (a) UV-vis and photoluminescence (PL) spectra of C<sub>3</sub>N<sub>4</sub> nanoribbons. (b) The PL spectra of C<sub>3</sub>N<sub>4</sub> nanoribbons (40 μg mL<sup>-1</sup>) in the presence of different concentrations of Cu<sup>2+</sup>. (c) PL intensity responses of C<sub>3</sub>N<sub>4</sub> nanoribbons with varying concentrations of Cu<sup>2+</sup> in an aqueous solution. (d) The linear calibration of PL intensity versus the concentrations of Cu<sup>2+</sup>.

### 3.2. The Influence of Metal Ions on the Fluorescence of C<sub>3</sub>N<sub>4</sub> Nanoribbons

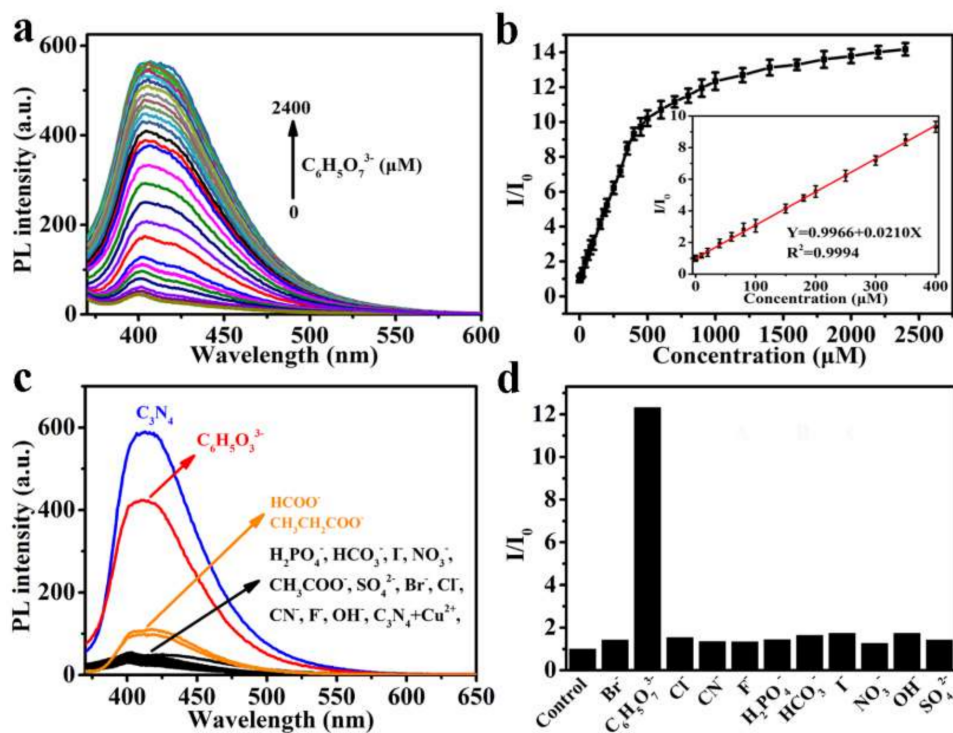
A previous study indicated that Cu<sup>2+</sup>, Fe<sup>3+</sup>, and Ag<sup>+</sup> can quench the fluorescence of C<sub>3</sub>N<sub>4</sub> due to the photoinduced electron transfer from C<sub>3</sub>N<sub>4</sub> to metal ions [23,25,45]. In this experiment, the fluorescent responses of C<sub>3</sub>N<sub>4</sub> nanoribbons towards different metal ions were investigated. As shown in Figure S6, C<sub>3</sub>N<sub>4</sub> nanoribbons display a slight change of the PL intensity in the presence of Al<sup>3+</sup>, Ba<sup>2+</sup>, K<sup>+</sup>, Li<sup>+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, Pb<sup>2+</sup>, Sn<sup>2+</sup>, and Zn<sup>2+</sup> (100 μM). However, the PL intensity of C<sub>3</sub>N<sub>4</sub> nanoribbons dramatically decreases with the addition of Cu<sup>2+</sup>, Ag<sup>+</sup>, Fe<sup>3+</sup>, Co<sup>2+</sup>, Mn<sup>2+</sup>, and Ni<sup>2+</sup>, especially for Cu<sup>2+</sup> and Ag<sup>+</sup>. Cu<sup>2+</sup> was chosen as the quencher in this work [36]. A decrease of PL intensity of C<sub>3</sub>N<sub>4</sub> nanoribbons is observed as the concentration of Cu<sup>2+</sup> increases (Figure 2b,c), and it is almost completely quenched after the addition of 40 μM Cu<sup>2+</sup>. Figure 2d shows that the PL intensity of C<sub>3</sub>N<sub>4</sub> at 415 nm versus the concentrations of Cu<sup>2+</sup> exhibits a linear relationship ranging from 10 to 300 nM ( $R^2 = 0.9976$ ).

### 3.3. Sensitivity and Selectivity of the Fluorescent “Off-On” Probe Based on C<sub>3</sub>N<sub>4</sub> Nanoribbons for C<sub>6</sub>H<sub>5</sub>O<sub>7</sub><sup>3-</sup> Detection

The influence of incubation time on the fluorescence recovery of C<sub>3</sub>N<sub>4</sub> nanoribbons was measured. As Figure S7 displayed, the PL intensity of Cu<sup>2+</sup>-C<sub>3</sub>N<sub>4</sub> nanoribbon complex increases rapidly with the time after the addition of C<sub>6</sub>H<sub>5</sub>O<sub>7</sub><sup>3-</sup>, and maintains a plateau after 20 s. Therefore, an incubation time of 20 s was selected for subsequent experiments.

As is well known, sensitivity is a critical parameter to assess the sensing performance [46,47]. The sensitivity of the fluorescent sensor using the C<sub>3</sub>N<sub>4</sub> nanoribbon-based “off-on” probe was evaluated. The PL intensity of the Cu<sup>2+</sup>-C<sub>3</sub>N<sub>4</sub> nanoribbon complex with different concentrations of C<sub>6</sub>H<sub>5</sub>O<sub>7</sub><sup>3-</sup> was recorded. As illustrated in Figure 3a,b, the PL intensity of the Cu<sup>2+</sup>-C<sub>3</sub>N<sub>4</sub> nanoribbon complex was obviously recovered as the concentration of C<sub>6</sub>H<sub>5</sub>O<sub>7</sub><sup>3-</sup> increased. The PL intensity of the Cu<sup>2+</sup>-C<sub>3</sub>N<sub>4</sub> nanoribbon complex was completely recovered with the addition of C<sub>6</sub>H<sub>5</sub>O<sub>7</sub><sup>3-</sup> (2.25 mM). The fluorescent sensor using the C<sub>3</sub>N<sub>4</sub> nanoribbon-based “off-on” probe shows a linear range from 1 to 400 μM and the calculated detection limit is 0.78 μM ( $S/N = 3$ ) (inset in Figure 3b). Compared with previously reported

fluorescent probes, such as coumarin [6], rhodamine [13], diketoprrrolopyrrole [15], TPIOP-boronate [48], and CdTe quantum dots [14], the fluorescent sensor using the  $C_3N_4$  nanoribbon-based “off-on” probe exhibits better sensing performance for  $C_6H_5O_7^{3-}$  detection with a broader linear range and faster response time (Table S1) [6,14,15,49].



**Figure 3.** (a) Fluorescence spectra of  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex with increasing concentrations of  $C_6H_5O_7^{3-}$ . (b) Plot of the fluorescence enhancement ( $I/I_0$ ) of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex after the addition of different concentrations of  $C_6H_5O_7^{3-}$ . The linear calibration range from 1 to 400  $\mu M$  is shown as an inset. (c) Fluorescence spectra of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex in the presence of  $Br^-$ ,  $C_6H_5O_7^{3-}$ ,  $Cl^-$ ,  $CN^-$ ,  $F^-$ ,  $H_2PO_4^-$ ,  $HCO_3^-$ ,  $I^-$ ,  $NO_3^-$ ,  $OH^-$ ,  $SO_4^{2-}$ ,  $HCOO^-$ ,  $CH_3COO^-$ , and  $CH_3CH_2COO^-$  (1 mM). (d) The value of fluorescent enhancement ( $I/I_0$ ) of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex after the addition of  $Br^-$ ,  $C_6H_5O_7^{3-}$ ,  $Cl^-$ ,  $CN^-$ ,  $F^-$ ,  $H_2PO_4^-$ ,  $HCO_3^-$ ,  $I^-$ ,  $NO_3^-$ ,  $OH^-$ , and  $SO_4^{2-}$  (1 mM).  $I_0$  and  $I$  stand for the fluorescence intensities of  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex at 415 nm in the absence and presence of different anions, respectively.

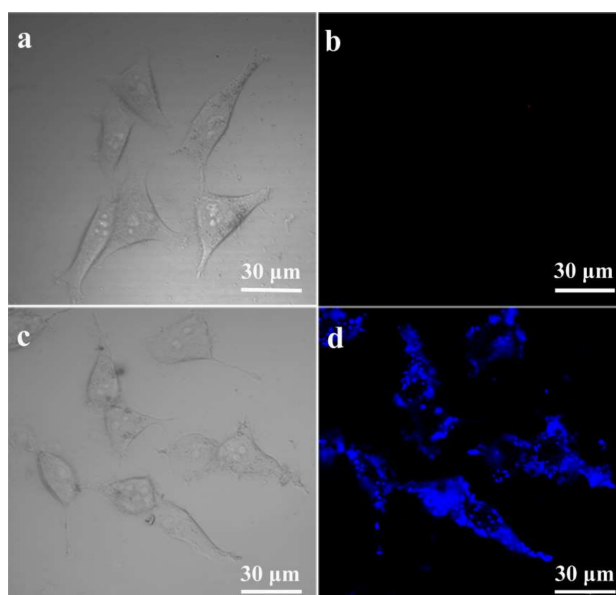
To investigate the selectivity of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon-based probe towards  $C_6H_5O_7^{3-}$ , the fluorescence responses of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex towards other anions ( $Br^-$ ,  $Cl^-$ ,  $CN^-$ ,  $F^-$ ,  $H_2PO_4^-$ ,  $HCO_3^-$ ,  $I^-$ ,  $NO_3^-$ ,  $OH^-$ , and  $SO_4^{2-}$ ) was measured. As shown in Figure 3c,d, most of the tested anions almost do not affect the PL intensity of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex, while  $C_6H_5O_7^{3-}$  can recover the fluorescence of  $C_3N_4$  nanoribbons effectively. Moreover, formic acid, sodium acetate, and propionic acid were also applied for the selectivity analysis of the proposed detection method (Figure S8), showing that the fluorescence presented the highest recovery in the presence of  $C_6H_5O_7^{3-}$ . Furthermore, the PL responses of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon-based probe towards  $C_6H_5O_7^{3-}$  were also investigated under different pH levels and metal ions environments. As shown in Figure S9a, the fluorescence intensity of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon-based probe with or without  $C_6H_5O_7^{3-}$  did not show obvious change in the pH range from 4 to 8. More importantly, almost all of the cations (10  $\mu M$ ) did not affect the fluorescence response of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon-based probe, except for  $Ag^+$  (Figure S9b). Further, cell lysate ( $1 \times 10^6$  cell  $mL^{-1}$ ) and biological molecules (10  $\mu M$ ), including glutamic acid, ascorbic acid, glutathione, and DNA, were introduced to examine the probe's stability. As Figure S9c shows, except for glutathione, the  $Cu^{2+}$ - $C_3N_4$  nanoribbon-base probe exhibited no obvious fluorescence

change upon the addition of  $C_6H_5O_7^{3-}$  and a biological molecule (10  $\mu$ M) mixture, even in cell lysate. These results indicate that the  $Cu^{2+}$ - $C_3N_4$  nanoribbon-based probe can be used for  $C_6H_5O_7^{3-}$  detection in more complex environments.

The mechanism of the fluorescence quenching and recovering process were studied by DLS and zeta-potential for this system (Figure S10). The average hydrodynamic size of  $C_3N_4$  nanoribbons increased from 230 to 1523 nm in the presence of  $Cu^{2+}$  along with fluorescence quenching. A change of the zeta-potential from  $-20.6$  mV to  $-10.4$  mV was observed after the  $C_3N_4$  nanoribbons interacted with  $Cu^{2+}$ . This result indicates that a non-fluorescent complex ( $Cu^{2+}$ - $C_3N_4$  nanoribbon) was formed [50]. With the addition of  $C_6H_5O_7^{3-}$ , the hydrodynamic size decreased because of the chelation between  $Cu^{2+}$  and  $C_6H_5O_7^{3-}$ , indicating the release of  $Cu^{2+}$  from  $C_3N_4$  nanoribbons and resulting in the restoration of the fluorescence of  $C_3N_4$  nanoribbons [18,19,40]. In order to rule out the effect of nonspecific binding, the PL intensity of the  $C_3N_4$  nanoribbons was monitored after the addition of different anion solutions. The result shows that, except for  $OH^-$ , the other anions did not dramatically affect the PL intensity of the  $C_3N_4$  nanoribbons (Figure S11).

### 3.4. Intracellular Imaging of $C_6H_5O_7^{3-}$

For further biological applications, the cytotoxicity of the  $C_3N_4$  nanoribbons and  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex to HeLa cells was assessed through MTT assays. As shown in Figure S12, the viability of HeLa cells showed no obvious change after incubation with the  $C_3N_4$  nanoribbons or  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex for 24 h, indicating their good biocompatibility. Since the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex showed a highly selective and sensitive response towards  $C_6H_5O_7^{3-}$ , this novel fluorescent “off-on” probe based on  $C_3N_4$  nanoribbons might be potentially applied for the intracellular detection of  $C_6H_5O_7^{3-}$ . As shown in Figure 4, bright blue fluorescence was observed in  $C_6H_5O_7^{3-}$ -overloaded HeLa cells incubated with the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex, whereas no fluorescence was detected in the control HeLa cells incubated with the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex. Besides, Z-scan fluorescent images of living HeLa cells further confirmed that the  $Cu^{2+}$ - $C_3N_4$  nanoribbon-based probe can be taken up by the cells (Figure S9d). These results demonstrate that the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex is membrane permeable and could be used as a fluorescent visualizer for the intracellular imaging of  $C_6H_5O_7^{3-}$ .



**Figure 4.** (a) Bright field and (b) fluorescent images of HeLa cells incubated with the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex for 4 h. (c) Bright field and (d) fluorescent images of HeLa cells pretreated with  $C_6H_5O_7Na_3$  (1 mM) for 12 h and then incubated with the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex for 4 h.

#### 4. Conclusions

In summary, a novel fluorescent “off-on” probe based on  $C_3N_4$  nanoribbons was developed for  $C_6H_5O_7^{3-}$  detection. The fluorescence of  $C_3N_4$  nanoribbons can be quenched by  $Cu^{2+}$  and then recovered by the addition of  $C_6H_5O_7^{3-}$ . The sensor using this fluorescent “off-on” probe showed a good detection linear range (1–400  $\mu M$ ) with a low detection limit (0.78  $\mu M$ ) as well as high selectivity in aqueous solutions. More importantly, this “off-on” probe based on  $C_3N_4$  nanoribbons exhibited good biocompatibility and low cytotoxicity in cell environments and can be utilized for intracellular imaging of  $C_6H_5O_7^{3-}$ .

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1424-8220/18/4/1163/s1>. Figure S1: (a) SEM image, (b) XPS survey spectrum, (c) XRD pattern and (d) FTIR spectrum of the bulk  $C_3N_4$ . Figure S2: XPS survey spectrum of  $C_3N_4$  nanoribbons. Figure S3: C 1s XPS spectrum of  $C_3N_4$  nanoribbons. Figure S4: FTIR spectrum of  $C_3N_4$  nanoribbons. Figure S5: Effect of the pH value on the PL intensity of  $C_3N_4$  nanoribbons. Figure S6: The fluorescence responses of  $C_3N_4$  nanoribbons to various metal ions ( $Cu^{2+}$ ,  $Al^{3+}$ ,  $Ba^{2+}$ ,  $Co^{2+}$ ,  $Ag^+$ ,  $Fe^{3+}$ ,  $K^+$ ,  $Li^+$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Na^+$ ,  $Ni^{2+}$ ,  $Pb^{2+}$ ,  $Sn^{2+}$ , and  $Zn^{2+}$ ) at a concentration of 100  $\mu M$  in an aqueous solution. Figure S7: The fluorescent changes of  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex as a function of interaction time after the addition of  $C_6H_5O_7^{3-}$  (1 mM). Figure S8: The value of the fluorescent enhancement ( $I/I_0$ ) of  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex after the addition of  $C_6H_5O_7^{3-}$ , formic acid, sodium acetate, and propionic acid.  $I_0$  and  $I$  are the fluorescence intensities of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex at 415 nm in the absence and presence of different anions, respectively. Figure S9: (a) Effect of the pH value on the fluorescence responses of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex after the addition of  $C_6H_5O_7^{3-}$  (1 mM). (b) Fluorescence responses of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex upon the addition of  $C_6H_5O_7^{3-}$  and metal ions (10  $\mu M$ ) mixture. (c) Fluorescence responses of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex upon the addition of  $C_6H_5O_7^{3-}$  and biological molecule (10  $\mu M$ ) mixture. (d) Z-scan images of living HeLa cells that were preincubated with 1 mM  $C_6H_5O_7^{3-}$  for 12 h and then stained with the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex for 4 h. Figure S10: (a) Hydrodynamic size of  $C_3N_4$  nanoribbons. (b) Hydrodynamic size of the  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex. (c) Hydrodynamic size of  $C_3N_4$  nanoribbons after the  $C_6H_5O_7^{3-}$  was added into the  $Cu^{2+}$ - $C_3N_4$  nanoribbon solution. Figure S11: The fluorescence responses of  $C_3N_4$  nanoribbons in an aqueous solution upon the addition of different anions ( $Br^-$ ,  $C_6H_5O_7^{3-}$ ,  $Cl^-$ ,  $CN^-$ ,  $F^-$ ,  $H_2PO_4^-$ ,  $HCO_3^-$ ,  $I^-$ ,  $NO_3^-$ ,  $OH^-$ ,  $CH_3COO^-$ , and  $SO_4^{2-}$ ) (final concentration: 1 mM). Figure S12: Cell viability of HeLa cells incubated with various concentrations of  $C_3N_4$  nanoribbons (grey) or  $Cu^{2+}$ - $C_3N_4$  nanoribbon complex (black) for 24 h. Table S1. Comparison of fluorescent citrate sensors.

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