Biomass-Derived Sulfur, Nitrogen Co-Doped Carbon Dots for Colorimetric and Fluorescent Dual Mode Detection of Silver (I) and Cell Imaging

Hongzhi Lu,1,2,† Chenchen Li,8,‡ Huihui Wang,‡ Xiaomeng Wang,*†‡ and Shoufang Xu*†‡

1School of Chemistry and Chemical Engineering and 2Laboratory of Functional Polymers, School of Materials Science and Engineering, Linyi University, Linyi 276005, China
8Tumor Precision Targeting Research Center, School of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, China

ABSTRACT: A method for green synthesis of sulfur, nitrogen co-doped photoluminescence carbon dots (S,N/CDs) originating from two natural biomass was proposed. By simple hydrothermal heating of bean pod and onion, blue emission CDs were prepared. Ag+ can effectively quench the as-prepared S,N/CDs. Under optimized conditions, the linear range of the established method for Ag+ detection was 0.1–25 μM, and the detection of limit based on 3S/N was 37 nM. More interestingly, the addition of Ag+ can induce an evident color change of S,N/CDs from yellow to brown under sunlight. The developed method was applied for detection of Ag+ in river water and tap water samples. Satisfied recoveries ranging from 96.0 to 102.0% with precision below 4.1% were obtained. S,N/CDs showed low toxicity toward 4T1 cells, which also can be extended to cellular imaging and intracellular Ag+ detection. The simple and green approach proposed here could meet the requirements for bioimaging and environmental monitoring.

INTRODUCTION

Carbon dots (CDs) are widely applied in chemical sensors and bioimaging due to their low cost, low toxicity, easy surface modification, chemical inertness, and excellent biocompatibility.1−3 CDs can be prepared by top-down approaches (laser ablation approach,4 electrochemical approach5) and bottom-up approaches (thermal pyrolysis,6 hydrothermal method,7 solvothermal treatment,8 microwave heating method,9,10 and metal–organic framework (MOF) template-based approach11). More recently, hydrothermal treatment has become the most commonly used method because of low cost and nontoxic routes. By regulating the precursor and doping elements, the emission light of CDs can be regulated from blue12,13 to yellow,16 to green,17 to red,18−21 or even dual emission CDs22−25 can be designed. Generally, element doping can increase the quantum yield (QY).26−30 Usually, doping of N atom into CDs could increase their stability and fluorescence quantum yield.31 Sulfur doping not only enhances fluorescence intensity but also causes red shift in the maximum absorption wavelength.32 Using natural biomass, such as chestnut,33 papaya,34 pineapple peel,35 potatoes,36 corn bract,37 and Tamarindus indica leaves,38 as precursor to prepare doping CDs was interesting.

Ag+ is widely used in medical, skin care, and pharmaceutical and electrical fields. However, excessive Ag+ is considered as a toxic heavy metal ion for human beings and the environment.38,39 In recent years, CDs play an important role for Ag+ detection in water environment monitoring and cell imaging, due to their facile operation, superior biocompatibility, excellent selective and low detection limits.3,4,40 Ag+ induced fluorescence quenching of CDs is common through static quenching,2,10 the inner filter effect,41 and electron transfer.2 Based on the quenching process of CDs by Ag+, many bifunctional sensing systems were developed.2,3,40,41 Therefore, it is still significant to develop an environmentally friendly and cost-effective fluorescent sensor for Ag+ detection to cope with complicated real water and biological samples.

Considering that bean pods are rich in nitrogen elements and onions are rich in thiol compounds, an economical green method for S,N co-doped CDs (S,N/CDs) was reported by the hydrothermal treatment of bean pod and onion in aqueous solution. The as-prepared S,N/CDs exhibited good water dispersity and strong blue photoluminescence. Furthermore, the addition of Ag+ can induce effective quenching of S,N/CDs. The quenching mechanism was discussed in detail using fluorescent lifetime, UV−vis absorption spectra, and transmission electron microscopy (TEM) images. The quenching mechanism may be ascribed to aggregation induced quenching. More interestingly, the addition of Ag+ can induce an evident color change of S,N/CDs from yellow to brown under sunlight.
sunlight, and colorimetric detection of silver ions was feasible. S,N/CDs were successfully employed to sensitively and selectively detect Ag⁺ in drinking water samples. S,N/CDs showed low toxicity toward the 4T1 cells; the probe also can be extended to cellular imaging and intracellular Ag⁺ detection, as illustrated in Scheme 1. The novelty of this work is reflected in the following aspects. First, S and N elements co-doped CDs were prepared using green raw materials, which was simple and environmentally friendly. Second, the detection of silver ions can be achieved by colorimetric and fluorescent dual modes. The detection process was convenient and fast. Third, S,N/CDs have superior biocompatibility and display high sensitivity for silver ion detection in living cells.

**RESULTS AND DISCUSSION**

**Preparation and Characterization of S,N/CDs.** S,N/CDs were prepared using bean pod and onion as raw materials via a hydrothermal method. Considering that the reaction conditions, including the mass ratios of precursor, reaction temperature, and time, would influence emission wavelength and quantum yield, the reaction conditions were optimized, and the results are listed in Table S1. First, changing the ratio of bean pod to onion, a series of CDs were prepared. It was found that as the content of onion increases, the maximum fluorescence emission wavelength of the prepared CDs moves toward the long wavelength, as shown in Figure S1. The experimental results are consistent with the literature reports: S doping causes the red shift of maximum emission wavelength. When the mass ratio of bean pods to onion was set as 0.2 (dry):10 (fresh), the highest quantum yield as 5.55% was obtained. Considering the quantum yield, the optimized mass ratios of bean pods to onion was 0.2 (dry):10 (fresh) for the following experiment. Subsequently, we explored the effect of temperature and time on the properties of the prepared CDs. The results (Table S1) show that temperature and time displayed little effect on the emission wavelength but significantly affected quantum yield. Taking quantum yield as

Scheme 1. Schematic Representation of S,N/CDs

Preparation and Fluorescence Sensing of Ag⁺ in Water and Living Cells

Figure 1. TEM images (A), particle size distribution (B), XRD pattern (C), and Fourier transform infrared (FT-IR) spectra (D) of prepared S,N/CDs. Inset are the HRTEM images of S,N/CDs.
the main indicator, the optimal experimental conditions were finally determined to be 180 °C and 8 h. The final product was freeze-dried to obtain the solid product and characterized in detail to study the elements and surface groups. It should be noted that since the precursors are fresh produce from the local market, different batches of raw materials or the seasonal changes of the produce may have an impact on the property of as-prepared CDs. So, three batches of raw materials were purchased from different supermarkets for comparison. The results show that CDs from different raw materials displayed different fluorescence intensities. However, the rules discussed above were applicable.

Figure 1A shows that the prepared particles have a uniform spherical morphology with an average size of about 6 nm calculated using 50 particles (Figure 1B), which is larger than the CDs prepared by the chemical reagents reported in the previous literature. Most particles were amorphous carbon particles, only a few number of particles displayed a lattice spacing of 0.23 nm, as shown in inset high-resolution transmission electron microscopy (HRTEM) images in Figure 1A. There was a broad diffraction peak centered at 2θ = 22.6° in the X-ray diffraction (XRD) spectra (Figure 1C), confirming the results that the prepared S,N/CDs are amorphous.37 Fourier transform infrared spectroscopy (Figure 1D) was adopted to explore the functional groups on the surface of S,N/CDs. Some typical peaks are listed as follows. The broad peak at 3300 cm⁻¹ can be attributed to the stretching vibrations of N–H and O–H groups.
and 2818 cm$^{-1}$ may be assigned to the stretching and bending vibrations of C–H groups. The strong peaks at 1820 and 1750 cm$^{-1}$ may be attributed to the stretching vibrations of C=O groups in the COOH, and the band at 1155 cm$^{-1}$ can be assigned to C–N, C–S, and C–O bonds.

The element contents and surface groups of the prepared S,N/CDs were further characterized using X-ray photoelectron spectroscopy (XPS). The four peaks at 166.4, 285.3, 399.2, and 531.5 eV of full-range XPS (Figure 2) spectra verified the existence of S, C, N, and O elements. There were four kinds of carbon atoms displayed in the high-resolution C 1s XPS spectrum, including carboxylic group at 288.2 eV, C=S/C=N/C=O at 286.4 eV, C=C at 285.2 eV, and C–C at 284.5 eV. Two peaks displayed in high-resolution N 1s XPS spectrum can be ascribed to N–H (399.2 eV) and C=N/N=N/S=N (399.9 eV), respectively. The peaks at 163.3 and 164.3 eV in S 2p XPS spectrum may be ascribed to C=S–C covalent bond in the thiophene-S and the peaks at 165.3 and 166.2 eV may be ascribed to the –C–SO$_2$– and –C–SO$_3$– bonds, respectively. According to the FT-IR and XPS results, we can draw the conclusion that sulfur and nitrogen were successfully doped into CDs. Some typical functional groups, such as hydroxyl, carboxyl, and amino, existed on the surface of prepared S,N/CDs. It should be noted that since two kinds of raw materials were adopted, complicated chemical reactions occurred in the preparation process to produce a plurality of functional groups. Some functional groups may not be detected due to their low content.

**Optical Properties of S,N/CDs.** The optical properties of as-prepared S,N/CDs were characterized in detail. Figure 3A displays the UV–vis absorption spectrum of N/CDs and S,N/CDs. For the light yellow N/CDs solution with different concentrations of Ag$^+$ under sunlight (E), and relationship between $A_{450}/A_{270}$ and the concentration of Ag$^+$, where $A_{450}$ and $A_{270}$ are the absorbances of S,N/CDs at 450 and 270 nm; the inset picture is the S,N/CDs solution with different concentrations of Ag$^+$ under sunlight (F).
peak at 270 nm can be observed. For the S,N/CDs, the absorption peak at 270 nm splits into two peaks at 268 and 272 nm, which may be ascribed to the n→π* transition of N=C and S=C bonds, respectively. The fluorescence spectrum of S,N/CDs was recorded. Figure 3A displays that the fluorescence emission spectrum of the S,N/CDs was excitation-wavelength-dependent. When the excitation wavelength changed from 310 to 380 nm, the emission wavelength changed from 410 to 450 nm. The fluorescence spectrum has optimal excitation and emission wavelengths at 350 and 430 nm, respectively. This excitation-dependent phenomenon may be due to the diverse emissive trap sites of the S,N/CDs.33

The photo stability and chemical stability were quite necessary for practical sensing applications, so the stability of S,N/CDs against UV irradiation, ionic strengths, and pH was investigated. Figure S2A indicates that S,N/CDs displayed tolerance to UV light. Continuous UV light irradiation for 4 h only caused a 11% fluorescence intensity change. The fluorescence intensity did not display significant change when the pH was changed from 6.0 to 9.0. Under extreme acidic or basic condition, the fluorescence intensity of S,N/CDs would decrease (Figure S2B). Hydrogen bonding is likely to occur between the hydroxyl groups on the surface of the CDs under acidic conditions, which resulted in the quenching of CDs. Under basic conditions, the electronic transition of the functional groups may be affected, thereby affecting the fluorescent properties of the CDs. At the same time, the fluorescence intensity of S,N/CDs remains stable at high salt concentrations (even at the NaCl concentration of 1 M) (Figure S2C). The stability of the CDs offers possibilities for metal ion detection in real water samples.

The response of the S,N/CDs to various ions was examined. From Figure 3B, we can see that Ag⁺ can induce the sharply fluorescent quenching of the S,N/CDs, while other metal ions, including Na⁺, K⁺, Co²⁺, Zn²⁺, Cr³⁺, Cd²⁺, Mg²⁺, Ca²⁺, Al³⁺, Fe³⁺, Pb²⁺, Hg²⁺, and Cu²⁺, does not cause significant interference with the detection of Ag⁺. The results suggest that S,N/CDs displayed high selectivity toward Ag⁺ detection when the interference of Fe³⁺ is masked using ascorbic acid. The sensitivity and the selectivity of the method guaranteed Ag⁺ detection in real water samples.

**Sensing Mechanism.** Many mechanisms have been reported to explain the quenching effect of metal ions on CDs, such as electron transfer (ET), inner filter effect (IFE), and Förster resonance energy transfer (FRET) or synergistic interaction. The average fluorescence lifetime of S,N/CDs decayed from 5.1256 (τ₁ = 2.193, τ₂ = 6.835) to 5.0645 ns (τ₁ = 2.073, τ₂ = 6.684) (Figure 4D) after the addition of Ag⁺ ion. Minor fluorescence lifetime change indicated that the quenching process belongs to static quenching. The processes of FRET and dynamic quenching reduce the fluorescence lifetime of CDs, so the possibility of FRET mechanism was excluded. Ag⁺ can chelate with CDs, which facilitate charge transfer from the excited state of the CDs to Ag⁺ and results in fluorescence quenching. The process of ET has no effect on the UV–vis spectra of CDs. However, the UV–vis spectra of S,N/CDs changed obviously after the addition of Ag⁺, as seen in Figure 4E, which can exclude the exertion of ET mechanisms. The IFE is based on the absorption of the excitation or emission light by absorbers in the detection system, and the key point is that the absorption spectra of the absorbers overlap with the fluorescence excitation or emission spectra of fluorophores. However, for silver ions, there was no obvious UV–vis absorption, and the IFE between Ag⁺ and S,N/CDs was excluded. There was an IFE possibility between Ag⁺ chelation and S,N/CDs. It is often accompanied by very obvious changes in the color of the solution. UV–vis absorption spectra of S,N/CDs before and after the addition of silver ions were recorded.

Interestingly, when Ag⁺ ions were added to the S,N/CDs buffer solution with the final concentration of 20 μM, the light yellow color of the S,N/CDs solution displayed no obvious change for the first 5 min. At about 10 min, the color of the mixture solution changed from yellow to brown. Meanwhile, a new absorbance peak at 450 nm appeared and increased too. For the S,N/CDs, the optimal emission wavelengths at 430 nm overlap with the newly appeared absorption spectrum. However, ultimately, the possibility of IFE was excluded due to the following two aspects. First, the process of IFE has no effect on the UV–vis spectra of CDs. Figure 4E shows that the absorption peak around 270 nm, which belongs to S,N/CDs, increased after the addition of Ag⁺, which can exclude the exertion of IFE mechanism. Second, when Ag⁺ coordinated with functional groups on the surface of S,N/CDs, the solution was transparent and stable, and no floccules occurred. For the solution of S,N/CDs with the addition of Ag⁺, after standing overnight, black precipitate was found at the bottom of the bottle, and the upper solution became clear and transparent, as displayed in Figure 4E (the inset photos).

Ultimately, we hypothesize that the quenching mechanism may be ascribed to aggregation induced quenching. And the aggregation stems from the formation of flocculation. As discussed above, in the solution of S,N/CDs with the addition of Ag⁺, after standing overnight, black precipitate was found at the bottom of the bottle, and the upper solution became clear and transparent. The TEM images of S,N/CDs before and after the addition of Ag⁺ were observed (Figure S3). As displayed in Figure S3, big particles were observed after the
addition of silver ions. This is the reason why the mixed solution precipitated. Similar phenomenon of flocculation has been reported by Dai’s team. In their work, Ag⁺ was reduced to silver nanoparticles by Si-CDs@DA, and the formation of silver nanoparticles can be confirmed by energy dispersive X-ray spectroscopy (EDS) analysis of the obtained flocculation, which contains silver atoms. When Ag⁺ was added to S,N/CDs, Ag⁺ can be reduced to silver nanoparticles by S,N/CDs. The formation of flocculation and the fluorescence quenching response may be attributed to the synergistic interaction between S,N/CDs and Ag⁺. When 20 μM Ag⁺ was added to N/CDs, there was no noticeable color change and flocculation was observed, even after a day. We conclude that the reduction of Ag⁺ was due to the S containing groups. The zeta potential value of S,N/CDs was −0.693 mV. After the addition of Ag⁺, the value changed to −7.70 mV, which means that some positive charge of the groups was oxidated and shielded. The functional groups of CDs prepared from natural materials are complex, so the quenching mechanism needs to be further verified in future work.

Colorimetric Sensing of Ag⁺ Based on the S,N/CDs. Considering that S,N/CDs solution displayed significant color changes from light yellow to deeper brown after adding Ag⁺ under sunlight, colorimetric sensing of Ag⁺ based on S,N/CDs can be carried out. When increasing the concentration of Ag⁺ up to 20 μM, the absorbance at 270 nm increased. Meanwhile, a new absorbance peak at 450 nm appeared and increased too. When further increasing the concentration of Ag⁺, the absorbance at 270 nm began to decrease, accompanied by a further increase at 450 nm. From Figure 4F, we can see that a linear relationship could be set up between A₄₅₀/A₃₇₀ calculated from the UV-absorbance spectrum and the concentration of Ag⁺ from 0.2 to 5 μM. The absorbance of Ag⁺ was determined to be 76 nM by UV–vis detection. Only the addition of silver ions causes color change of the S,N/CDs. When the concentration of silver ions exceeds 0.5 μM, the color change can be observed by the naked eye. Colorimetric detection can be used to quickly and semiquantitatively detect whether Ag⁺ exceeds the standard in water samples.

Practical Application in Real Water Sample. S,N/CDs were applied for Ag⁺ detection in river water and tap water samples. No silver ions were detected from real water samples, so water samples were spiked with standard Ag⁺ solution to verify the accuracy of the method. As shown in Table 1, recoveries higher than 96.0% and analytical precision with relative standard deviation (RSD) lower than 4.1% were obtained. Meanwhile, to verify the accuracy of this method, atomic absorption spectroscopy (AAS) was also employed to measure the concentrations of Ag⁺, and the results are listed in Table 1. It can be observed that the concentrations of Ag⁺ measured by S,N/CDs are similar to those detected by AAS. The results confirmed the reliability and feasibility of the S,N/CDs for monitoring Ag⁺ in real water samples.

Method Performance Comparison. The performance of the established method for detection of silver ions was compared with the previously reported fluorescence methods, as listed in Table S2. Compared with other CDs by green preparation method using one kind of raw material (potato, papaya or pineapple peel), in this work, the CDs were prepared by two biomass, and S and N co-doping was achieved. For the detection mode, most of the green prepared CDs were used for fluorescence single mode detection. In this work, colorimetric and fluorescent dual mode were employed for silver ion detection. In terms of detection sensitivity, all the CDs detection of metal ions displayed nanomolar sensitivity. When comparing this work with other silver ion detection methods based on CDs, it displayed similar sensitivity. However, S,N/CDs were prepared by a green method, which has the advantages of being environmentally friendly and economical. Intracellular Imaging of Ag⁺. For silver ion detection in living cells, S,N/CDs must have superior biocompatibility and high sensitivity. The cytotoxicity of the S,N/CDs was evaluated by the cck-8 assay. More than 91.2% cells survive after incubation with S,N/CDs at concentration 50 μg mL⁻¹ for 24 h, and more than 82.6% cells survive after being cultured with S,N/CDs even at concentration 200 μg mL⁻¹ for 24 h (Figure 5). The cytotoxicity study indicates that S,N/CDs have good biocompatibility and ignorable cytotoxicity. Therefore, S,N/CDs could be used in imaging and detection of sliver ions in living cells.

Figure 5. Cellular cytotoxicity of the S,N/CDs.

| Table 1. Recoveries and RSDs for Detection of Ag⁺ in Spiked Samples by S,N/CDs Fluorescence Systems and AAS (n = 3) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| sample          | added (μM)      | found (μM)      | recovery (%) + RSD (%) | found (μM)      | recovery (%) + RSD (%) |
| river water     | 0.50            | 0.49            | 98.0 ± 2.9            | 98.0 ± 4.3      |
|                 | 1.00            | 1.02            | 102.0 ± 3.1           | 99.0 ± 4.2      |
|                 | 10.00           | 9.86            | 98.6 ± 3.7            | 98.2 ± 2.4      |
| tap water       | 0.50            | 0.48            | 96.0 ± 3.5            | 104 ± 3.0       |
|                 | 1.00            | 0.96            | 96.0 ± 4.1            | 102 ± 4.8       |
|                 | 10.00           | 9.91            | 99.1 ± 3.9            | 98.6 ± 4.3      |
fluorescence of S,N/CDs was effectively quenched by Ag⁺ in living cells. To locate the distribution of S,N/CDs in cells, we labeled the cell lysosomes with LysoTracker Deep Red, as shown in the first line of Figure 6. The Pearson’s correlation and overlap coefficient from three independent experiments are shown in Table S3. As a result, the S,N/CDs could enter into cells, and some of them distributed in lysosomes (Table S3). As we expected, the results proved that S,N/CDs could be used as the effective probe for semiquantitative detection of Ag⁺ in living cells.

■ CONCLUSIONS

In summary, a simple, economical, and environmentally friendly strategy for preparation of S,N/CDs using bean pods and onion as precursors was developed. S,N/CDs could be selectively quenched by Ag⁺ ion. The as-prepared S,N/CDs could be used for the fluorescence turn-off detection of Ag⁺ in the range of 0.1–25 μM with a limit of detection of 37 nM. S,N/CDs also can be used for on-site fast colorimetric detection of Ag⁺ based on the color change under sunlight. Furthermore, S,N/CDs showed superior biocompatibility and high sensitivity in the semiquantitative detection of Ag⁺ in living cells.

In general, the present work provides a green approach for the production of fluorescent CDs for metal ion detection.

■ EXPERIMENTAL SECTION

Materials and Apparatus. Bean and onion were purchased from the local market. Na₂CO₃, HgCl₂, FeCl₃, FeSO₄, CuCl₂, AgNO₃, MgCl₂, AlCl₃, Pb(NO₃)₂, CrCl₃, CdCl₂, CoCl₂, ZnCl₂, and KCl were received from Sinopharm Chemical Reagent Company. Fetal bovine serum (FBS, 10%) was received from Shanghai Life iLab Biotechnology Co., Ltd. cck-8 was received from Dojindo Molecular Technologies, Inc., Shanghai. LysoTracker Deep Red (635 nm excitation) and Cell Mask Orange plasma membrane stain were received from Thermo Fisher.

UV-3600 double beam ultraviolet spectrophotometry (Shimadzu, Japan) was employed for absorbance spectra detection. The morphology and lattice of the S,N/CDs were recorded by transmission electron microscopy (JEM-2100F). FT-IR spectrometer (Thermo Nicolet Corporation) and ESCALAB 250 X-ray photoelectron spectrometer were employed for functional groups and elemental analysis. All of the fluorescence analyses were performed on F-7000 Spectrofluorometer (Hitachi). X-ray diffraction (XRD) spectra was obtained using Rigaku MiniFlex 600 with a Cu Kα radiation source. The optical density (OD) of each well at 450 nm was recorded on a Microplate Reader (Molecular Devices, SpectraMax iD3). Laser scanning confocal microscope (Olympus, FV3000, Japan) was used for relative cell imaging.

Preparation of S,N/CDs. Hydrothermal method was adopted for green synthesis of S,N/CDs using bean pod and onion as precursor. Fresh bean pods were dried and ground into powder and fresh onion was ground into a slurry. Then, 0.2 g of the bean pod powder and 2 g of fresh onion slurry were dispersed in 30 mL of ultrapure water and heated at 180 °C for 8 h in a Teflon-lined autoclave. The resultant brown solution was centrifuged and dialyzed to get a transparent clear solution and then stored at 4 °C for further use. For control, N/CDs were prepared using only bean pod as precursor.
prepared CDs were freeze-dried and weighed to determine the concentration of the CDs aqueous solution.

**Detection of Silver (I) Ions.** S,N/CDs were dispersed in phosphate-buffered saline (PBS) buffer solution (25.0 mM, pH 7.0) with a final concentration of 50 μg L⁻¹. Ag⁺ standard solutions (10 μL) with different concentrations were added to 4.0 mL of S,N/CDs buffer solution. Silver ions and CDs were fully reacted for 5 min, and then the fluorescence detection was performed. To study the specificity of the sensing system, the effect of other ions (including Na⁺, K⁺, Co²⁺, Zn²⁺, Cr³⁺, Cd²⁺, Mg²⁺, Ca²⁺, Al³⁺, Fe³⁺, Fe²⁺, Pb²⁺, Hg²⁺, and Cu²⁺) on the fluorescence intensity of S,N/CDs was also investigated. All fluorescence detection was performed at room temperature.

**Real Sample Detection.** River water from Yi river (Linyi, Shandong) and tap water from our laboratory were collected to explore the feasibility of the proposed method in real samples. After filtration with a 0.22 μm membrane to remove the sediments, silver ions were spiked into the river water sample. Then, S,N/CDs solution was mixed with 4.0 mL of spiked river sample with a final concentration of 50 μg L⁻¹. The concentration of Ag⁺ in water samples was detected using the above developed method. Tap water was used without further purification.

**Cytotoxicity Assay.** The cytotoxicity of S,N/CDs for 4T1 cells in vitro was performed by the cck-8 assay. 4T1 cells were cultured in the culture medium of Dulbecco’s modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) in a humidified 5% CO₂ incubator at 37 °C. When the cells proliferate to around 70% of the cell culture flasks, the cells were harvested to seed in 96-well plates and cultured in the medium containing the S,N/CDs with various concentrations for 24 h. The wells containing cells without S,N/CDs served as the control. After 24 h, each well was washed with D-Hanks twice before the test. Then, each well was treated by the addition of 100 μL of the mixed solution (90 μL of fresh culture medium and 10 μL of cck-8) and incubated for an additional 1 h at 37 °C. Finally, the optical density (OD) of each well at 450 nm was recorded.

**Cell Imaging.** The 4T1 cells were harvested to seed in culture dishes with approximately 2 × 10⁵ 4T1 cells/2 mL and then cultured overnight. Afterward, S,N/CDs were added to the dishes with a final concentration of 200 μg mL⁻¹ in the culture medium for further 24 h culture. To understand the biodistribution of CDs in cells, the cell lysosomes were stained by LysoTracker Deep Red for co-location. The biodistribution of CDs in cells, the cell lysosomes were stained by LysoTracker Deep Red for co-location. The fluorescence quenching of Ag⁺ was detected by adding 200 μg mL⁻¹ S,N/CDs combined with Ag⁺ of different concentrations (1 and 10 μM) to culture dishes. Before the cells were imaged by the laser scanning confocal microscope, the cell membrane was stained by Cell Mask Orange plasma membrane stain.

**ASSOCIATED CONTENT**

- Supporting Information
  - The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03198.

- **Effect of mass ratios of precursor, reaction temperature, and time on the emission wavelength and QY of CDs;**
- **photo stability, pH, and salt tolerance; TEM images of S,N/CDs;**
- **performance comparison; colocalization-correlation analysis (Figures S1–S3 and Tables S1–S3) (PDF)**

**AUTHOR INFORMATION**

- **Corresponding Authors**
  - E-mail: wangxiaomeng1979@163.com (X.M. Wang).
  - E-mail: shfxu1981@163.com (S.F. Xu).

- **ORCID**
  - Xiaomeng Wang: 0000-0002-2289-838X
  - Shoufang Xu: 0000-0002-6410-3258

- **Author Contributions**
  - H.L. and C.L. contributed equally.

- **Notes**
  - The authors declare no competing financial interest.

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