Multifunctional fluorescent “Off-On-Off” nanosensor for Au$^{3+}$ and S$^{2−}$ employing N-S co-doped carbon–dots

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Out of the plethora of sensors being developed using carbon dots, the use of green alternatives with the potential of multi-functionality is still in its infancy. In view of this, the present work shows the use of Rosa indica derived heteroatom i.e. nitrogen and sulfur co-doped rose carbon dot (N-S@RCD) for dual fluorescence based sensing of multi-analytes. The N-S@RCD shows interesting fluorescence turn “off-on-off” response towards S$^{2−}$ and Au$^{3+}$ with limit of detection (LOD) 92.4 nM and 63.1 nM, respectively. To the best of our knowledge, this is first report on direct “Turn-on” of c-dots with S$^{2−}$ ions without any intermediate c-dot–quencher complex. Further, the bioimaging and flow cytometry studies revealed the potential of live cell imaging, intracellular sensing and cytocompatibility of N-S@RCD. The hemo-compatibility of N-S@RCD was also investigated for potential in vivo applications. Moreover, the “off-on-off” fluorescence behaviour was used to construct molecular logic gates which simulate single input logic “YES” and multi-input “INHIBIT” logic system.

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1. Introduction

Carbon-dot, the new fascinating material of carbon family, continues to gain attraction in the area of sensing, bioimaging and electronic devices. The obvious fascination is driven by the unique optical properties, non-toxic nature and facile synthesis of carbon-dots. The fluorescent nature of these quantum dots, offers new avenues for sensing and selective bio-labelling studies [1–3].

The heteroatom doping impacts the optical characteristics of carbon dots and is shown to have improved quantum yield (QY) [4–11]. The size similarity between nitrogen and carbon atoms makes it feasible to achieve good nitrogen doping which may result in better QY [12]. On the other hand, sulfur tunes the photoluminescence to longer wavelength by providing emissive traps states to photoexcited electrons [13]. Recently S and N have been used for heteroatom dopant for improving the properties of c-dots [14–17].

The detection of metal ions and anions have seen a tremendous upsurge in recent past because the traditional methods suffer from tedious experimentation and high instrument costs, hence simple and sensitive fluorescence turn-on/off methods has gained momentum [18,19].

The on-site detection of biologically and industrially important anions is of great interest. The sulphide ion (S$^{2−}$) has important biological significance due to its production in biological processes like reduction of sulfate by microbes and the presence of sulfur in various amino acids. The industrial processes like the synthesis of sulfuric acid, dye and cosmetics production has led to increased presence of sulphide in the environment. The sulphide ion is a toxic pollutant and its protonated form further increases the toxicity. A high exposure to S$^{2−}$ can cause suffocation, respiratory paralysis, irritation of mucous membrane and loss of consciousness, while its protonated ion can cause permanent brain damage or asphyxiation [20–24]. Hence, it is imperative to develop an environmentally benign, quick and sensitive sensor for S$^{2−}$ ions in aqueous media.

On the other hand, gold species are capable of binding with DNA and enzymes and are potentially toxic which might lead to damage in peripheral nervous system, liver and kidney [25]. The previously reported fluorescence based Au$^{3+}$ sensors suffers from the requirement of complicated multi-step synthesis, long incubation time, relatively high LOD and requirement of organic media for
synthesis of probe, hence development of Au3+ sensor in aqueous media still remains a challenge [26,27]. The electron transfer properties of carbon dots on gold nanorods were recently studied by Long et al. [28], which highlights the need and scope of electronic communications between metal and c-dots.

The use of organic/inorganic single molecules for molecular electronics is a growing research area since the physical constraints has pushed the conventional silicon based electronics to its limits [29,30]. The development of intelligent molecules which mimicking logic functions and provide measurable output are compelling. The molecular logical gates are found to be extremely valuable in small object recognition, sensing and diagnosis [31]. The fluorescence based sensing or the chemical triggered optical response can be exploited in making new generation molecular electronics [32].

Herein, we elucidate the multifunctionality of green nitrogen and sulfur co-doped carbon dots (N-S@RCD) for S2− and Au3+ fluorescence “Turn-off-on-off” sensor with added advantage of intracellular sensing and bioimaging with cyto and hemocompatibility. The sensing response mimics the logic functions “YES” and “INHIBIT” and are explored for construction of molecular logic gates.

2. Experimental section

2.1. Materials

The rose flowers were purchased from local market. L-cystein and ethylenediamine was procured from sigma-aldrich. The metal salts and anions were purchased from merck and sigma-aldrich. The chemicals were of analytical grade and used as received without further purification. Deionized water (DI) from Sartorius milli-Q system was used throughout the study.

2.2. Instrumentation

The UV-visible spectroscopic studies were performed using Varian Cary 100 Bio UV-visible spectrophotometer. The powder X-ray diffraction (PXRD) was carried out on a Rigaku Smart Lab X-ray diffractometer using CuKα radiation (1.54 Å). The fluorescence studies were performed using a Fluoromax spectrofluorometer. A Bio–Rad FTS 3000MX instrument was used to record IR spectra (4000–400 cm−1). The X-ray photoelectron spectroscopy (XPS) was performed on AXIS ULTRA. The scanning electron microscopy was performed on Carl Zeiss Supra 55 FE-SEM. A FEI Tecnai G2 F20 Transmission Electron Microscope was used for TEM studies. An Olympus confocal laser scanning microscope was used for cellular imaging studies. The absorption studies for MTT assay was performed on a Synergy H1 Biotek microplate reader. Flow cytometry imaging studies. The absorption studies for MTT assay was performed on BD LSRFortessa. The transmission electron microscope was used for TEM studies. An Olympus confocal laser scanning microscope was used for cellular imaging studies. The absorption studies for MTT assay was performed on AXIS ULTRA. The scanning electron microscopy was used for TEM studies.

2.3. Synthesis of N-S@RCD

The synthesis of N-S@RCD was carried out by hydrothermal treatment of rose petals. Typically, flower petals from Rosa indica (Family: Rosaceae; Genus: Rosa; Species: indica) was crushed for extract preparation. 10 mL of rose petal extract was mixed with and 300 μL of ethylenediamine for nitrogen doping and 300 mg of l-cysteine for sulfur doping. The homogenous mixture was hydrothermally treated at 180 °C for 5 h. The resultant suspension was centrifuged and supernatant was lyophilized to obtain N-S@RCD. An undoped sample (URCD) was also prepared by hydrothermal treatment of 10 mL rose petal extract at 180 °C for 5 h.

2.4. Calculation of quantum yield

In order to calculate the fluorescence quantum yields (Φ) of N-S@RCD, quinine sulphate was used as a standard (Φst = 0.54) and was calculated using following eqn. (1)

$$\Phi = \Phi_{st} \times \frac{S}{S_{st}} \times \frac{A/A_{st}}{n^2/n_{st}}$$

(1)

where Φ and Φst is the quantum yield of the sample and standard, respectively. A and Ast are the absorbance of the sample and standard at excitation wavelength, respectively. S and Sst are measured integrated emission band areas of the standard and the sample. The n and nst are the solvent refractive index of the standard and the sample. Quinine sulphate was dissolved in 0.1 M H2SO4 (n = 1.33) and N-S@RCD was dissolved in deionized water (n = 1.33) [33].

2.5. Au3+ and S2− ion sensing and selectivity studies

Different concentrations of Au3+ (AuCl3) and S2− (Na2S) were added systematically to 2 mL aqueous solution of N-S@RCD (20 μg/ mL). The change in fluorescence intensity was recorded using fluorescence spectrophotometer. To study the selectivity of N-S@RCD towards Au3+ and S2−, 29 other metal ion and anion were added and relative change in fluorescence intensity was plotted. The studies were performed in Milli-Q water (pH = 7.0).

2.6. Biocompatibility, bioimaging and flow cytometry studies

The cytocompatibility of N-S@RCD was studied on cervical cancer cell line HeLa, using MTT assay [34]. The HeLa cells were seeded in a 96 well plate, minimum essential media (MEM, Himedia) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin streptomycin was used as nutrient media. The cells were incubated for 24 h, at 37 °C and 5% CO2 under humidified environment. The treatment of N-S@RCD was given in the range of 100–1000 μg/mL for a period of 16 h. Subsequently, the media containing N-S@RCD was replaced with MEM media containing 3- (4, 5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) (0.5 mg/mL) and incubated for 4 h. After incubation the MTT containing media was replaced with DMSO and plate was read under plate reader.

For bioimaging studies, HeLa cells were seeded in a 27 mm confocal dish, and treated with 500 μg/mL N-S@RCD for a period of 6 h. After the treatment, the cells were washed thrice with 1X PBS and imaged under confocal microscope. The flow cytometry studies were conducted using λex = 405 nm.

For flow cytometry, cells were seeded in 6-well plate. One sample was kept as control, where no treatment was given. Other samples were given N-S@RCD treatment, one of them was further treated with 500 μM Au3+. The cells were trisipnized, centrifuged and resuspended in 1X PBS and subjected to flow cytometry analysis.

2.7. Hemocompatibility studies

In order to assess the hemocompatibility of N-S@RCD, a hemolysis test was conducted in which fresh blood was treated with N-S@RCD in concentration range 0.01–50 mg/mL (in 1X PBS). A positive control was prepared by treating blood with Triton X100 (in PBS), and a negative control was set by treating RBCs with 1X PBS. The blood was incubated for 1 h. Further, the samples were centrifuged at 1000 × g for 10 min, and the supernatant was read at 540 nm using a micro plate reader. To study the morphological changes in RBC, the treated RBC pellet was fixed with 4%
glutaraldehyde and subjected to serial dehydration using ethanol (30%, 50%, 70%, 90%, 100% v/v). After air drying, the samples were coated with gold and imaged under a scanning electron microscope.

Due approval was taken from Institute Human Ethics Committee (IHEC), IIT Indore for using human blood samples. All procedures were performed following the guidelines of Indian Council of Medical Research (ICMR, New Delhi).

3. Results and discussion

The multifunctionality of nitrogen and sulfur co-doped rose carbon dots (N-S@RCD) was systematically investigated for fluorescence “Off-On-Off” response towards sulfide (S²⁻) and gold (Au³⁺) ions and construction of logic gates. Further, the cytocompatibility, hemocompatibility and bioimaging were also explored (Fig. 1).

3.1. Morphological and compositional analysis of N-S@RCD

The structural and morphological characterization of N-S@RCD was performed using PXRD, TEM, FT-IR and XPS. The PXRD spectra exhibit the amorphous nature of N-S@RCD as reflected by a broad peak centered at 24.8° (Fig. S1). The TEM micrograph (Fig. 2(a–c)) shows well dispersed ultra-small particles with average particle size of 4.6 ± 1.17 nm. The poor crystalline nature of N-S@RCD was confirmed by SAED pattern. However, some particle shows well-resolved lattice fringes, which is similar to previous reports indicating the presence of some crystalline particles in the mixture [35,36]. The obtained lattice spacing 0.26 nm, is close to (002) graphitic facet indicating distorted carbon structure [36]. The particle size distribution is calculated using 76 particles from Fig. 2(c) and shown in inset Fig. 2(c). The elemental analysis of N-S@RCD shows C 42.2 wt%; N 14.52 wt%; S 4.80 wt%; H 2.39 wt% and O (calculated) 36.09 wt%. The FTIR spectra shown in Fig. S2 exhibits broad absorption bands at 3350 cm⁻¹ and 2920 cm⁻¹ represent the stretching vibrations of O–H/N–H and C–H respectively. The peak at 1724 cm⁻¹, 1636 cm⁻¹ and 1460 cm⁻¹ represent the stretching mode of C=O, C=O and C–N, respectively. The IR bands in the range 1000–1400 cm⁻¹ can be attributed to stretching vibrations of C–O, C–S and C–H [17]. The XPS survey spectrum of N-S@RCD (Fig. S3) shows peaks corresponding to C1s, N1s, O1s S2p and S2s [37–39]. The zeta potential of N-S@RCD suspension at pH – 7 was found to be –14.98 mV indicating negative surface charge of N-S@RCD.

3.2. Optical properties of N-S@RCD

The absorption and fluorescence spectroscopy was performed to investigate the photophysical properties of N-S@RCD. The absorption spectrum of N-S@RCD shows peaks corresponding to π–π* and n–π* transitions at around 281 nm and 320 nm, respectively [40,41] (Fig. 3(a)). The N-S@RCD shows blue fluorescence under UV-lamp and emission maxima at 397 nm for excitation at 320 nm (Fig. 3(a)). The excitation tuned emission of N-S@RCD shown in Fig. S4, is similar to many reported c-dots [42]. The quantum yield of N-S@RCD was found to be 9.6% using quinine sulphate as a reference.

Moreover, the fluorescence stability of N-S@RCD was evaluated under different temperature, UV irradiation, high NaCl concentration and at different pH. As exhibited in Fig. 3(b), the N-S@RCD shows considerable stability at varying temperature with 80% of the initial fluorescence intensity was retained at a temperature of 80 °C. Also, the N-S@RCD shows exceptional stability under UV illumination, an exposure to a 365 nm lamp (125 W) for a period of 5 h could still retain 73% of initial fluorescence intensity (Fig. 3(c)), which is high in comparison to previous reports [18,43]. The fluorescence stability of N-S@RCD was also studied under high salt concentration up to 1 M NaCl, which shows only slight change in the fluorescence (Fig. 3(d)). The N-S@RCD also shows relatively stable fluorescence ability in pH range 2–9 (Fig. S5). These results imply high stability of N-S@RCD, which makes them potential candidate for sensing and bio-medical applications.

The undoped sample (URCD) was characterized using PXRD, FT-IR, UV-visible and XPS (Fig. S6). The URCD shows a non fluorescent nature with mainly C and O elements (C% ~ 78; O% ~ 21% by XPS). The UV-visible spectra of URCD show a hump at around 280 nm.

3.3. Fluorescence based sensing response

Interesting fluorescence properties of N-S@RCD prompted us to study the analytes detection using the fluorescence “Turn-on” and “Turn-off” behaviour. The N-S@RCD exhibit sensitive and simultaneous detection of Au³⁺ and S²⁻ as per the following fluorescence behaviour

(i) Fluorescence “Turn-on” in presence of S²⁻
(ii) Fluorescence “Turn-off” in presence of Au³⁺
(iii) Fluorescence “Turn-off-on” by serial addition of S²⁻ and Au³⁺
(iv) Recovery of quenched fluorescence by addition of S²⁻ after addition of Au³⁺

As shown in Fig. 4(a–b), the N-S@RCD shows fluorescence “Turn-on” with increasing concentration of S²⁻ in the range of 0–800 µM with a linear response in the range of 0–500 µM with an adj. R² value of 0.99. The fluorescence intensity of N-S@RCD in the presence of 500 µM S²⁻ was found to be 330% of the initial intensity indicating a tremendous rise in the fluorescence intensity of N-S@RCD as a function of S²⁻ concentration. The addition of S²⁻ also results in a significantly increased fluorescence QY of 25.3%. The limit of detection (LOD) for S²⁻ was obtained to be 92.4 nM which is calculated using the following equation [18]

\[ \text{Limit of detection (LOD)} = 3.3(s/e/S) \]

where e represents standard error and S is the slope of the calibration curve.

Interestingly, the fluorescence maxima show a clear blue shift with addition of S²⁻. Specifically, the emission maxima of N-S@RCD shift from 397 nm to 385 nm in presence of 800 µM S²⁻. The shift in
Fig. 2. TEM micrographs of N-S@RCD at different magnifications and SAED pattern. (A colour version of this figure can be viewed online.)

Fig. 3. Optical characterization of N-S@RCD. (a) UV-vis, excitation and emission spectrum (b–d) Fluorescence stability of N-S@RCD: at different temperature (b), at varying UV illumination (c), and at high salt concentration (d). (A colour version of this figure can be viewed online.)
emission maxima shows a strong interaction between S$_2^-$ and N-S@RCD. The so-far reported turn-on sensor for c-dots first shows a Turn-off by formation of a c-dot–quencher complex and then recovery/Turn-on of fluorescence by removing the quencher by means of an analyte [22,44,45]. However, a turn-on in bare c-dots with S$_2^-$ is yet not reported and an exact mechanism for this behaviour is still not clear. The enhanced fluorescence in presence of S$_2^-$ can be attributed to the reduction in mid-gap states leading to reduced non-radiative transitions, henceforth increasing the fluorescence intensity [46]. In order to gain more evidences, the

Fig. 4. (a,c) Fluorescence spectra of N-S@RCD upon the addition of different concentrations of S$_2^-$ and Au$^{3+}$, respectively. (b,d) Relationship between F/F$_0$ and concentration of S$_2^-$ and Au$^{3+}$, respectively. (e) Fluorescence response of “N-S@RCD + S$_2^-$” system with different concentration of Au$^{3+}$. (f) Relationship between F/F$_0$ and concentration of Au$^{3+}$ in “N-S@RCD + S$_2^-$” system. (A colour version of this figure can be viewed online.)
radiative ($K_r$) and non-radiative transition rate ($K_{nr}$) was calculated before and after $S^{2-}$ addition. As shown in Table S1, $S^{2-}$ addition results in a decrease in the non-radiative transition rate from $3.67 \times 10^8$ s$^{-1}$ to $3.05 \times 10^8$ s$^{-1}$. The relative details are mentioned in SI.

On the other hand, the N-S@RCD exhibits a “Turn-off” response with increasing concentration of Au$^{3+}$ in the range of 0–1000 μM with a linear response for 50–750 μM having an adj. R$^2$ value of 0.99 as shown in Fig. 4(c–d). The remaining fluorescence intensity at a concentration of 750 μM Au$^{3+}$ was found to be approx. 24% and the LOD for Au$^{3+}$ was determined to be 63.1 nM. In contrary to $S^{2-}$, the addition of Au$^{3+}$ causes a red shift in emission maxima from 397 nm to 407 nm at a concentration of 750 μM Au$^{3+}$. The turn-off in bare c-dots is due to the non-radiative electron transfer from N-S@RCD to Au$^{3+}$ [26].

To understand the electronic communication in N-S@RCD system with Au (III), theoretical calculations were performed on the basis of density functional theory. In theoretical calculation two types of basis set were used. The LanL2DZ basic set for Au atom and 6-31G basis set for carbon (C), Hydrogen (H), Oxygen (O), Nitrogen (N) and Sulfur (S) were used [47,48]. The HOMO and LUMO energy for N-S@RCD was found to be $-4.0278$ eV and $-2.7124$ eV respectively. The LUMO energy of N-S@RCD was found higher than that of standard reduction potential of Au(III) i.e. +1.40 V, which

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**Fig. 5.** (a) Representative sensing response of N-S@RCD towards $S^{2-}$ and Au$^{3+}$, (b) Fluorescence lifetime decay of N-S@RCD in the absence and presence of $S^{2-}$ and Au$^{3+}$, (c) Selectivity in the sensing response. (A colour version of this figure can be viewed online.)
studied by serial addition of $S_2^-$ and $Au^3+$.

Moreover, the HOMO and LUMO energy of N-S@RCD $Au^3+$ was calculated which was found to be 3.5690 eV, 6.4 $×$ 10$^{-8}$ M and hence the quenched fluorescence is previously reported [20, 49].

In the case of sequential addition of $Au^3+$ and $S_2^-$, the quenched fluorescence by $Au^3+$ could be restored after addition of $S_2^-$ in the “N-S@RCD + Au$^{3+}$” system as shown in Fig. 56. The strong interaction between $Au^3+$ and $S_2^-$ can lead to removal of $Au^3+$ from N-S@RCD and hence the quenched fluorescence is restored. As shown in Fig. 58, approx. 93% of initial fluorescence intensity could be restored upon successive addition of $S_2^-$ in “N-S@RCD + Au$^{3+}$” system. In other words, more than 3 fold increase could be obtained by sufficient addition of sulfide ion in “N-S@RCD + Au$^{3+}$” system. Similar restoration/Turn-on of quenched fluorescence is previously reported [19, 49].

A relatively simple representation of sensing behaviour of N-S@RCD is exhibited in Fig. 5(a). Further, in order to investigate the kinetics of fluorescence response of N-S@RCD, time-correlated single photon counting (TCSPC) was performed using a diode laser of 375 nm. The addition of $S_2^-$ does not alter the lifetime and shows a similar average lifetime of 2.45ns while the addition of $Au^3+$ reduces the average lifetime to 1.81ns indicating a fast electron transfer process between N-S@RCD and Au$^{3+}$ and dynamic quenching of fluorescence. Fig. 5(b) and Table S2 shows the relevant data.

Moreover, to evaluate the selectivity in sensing response of N-S@RCD, various metal ions and anions were investigated for their impact on the fluorescence behaviour of N-S@RCD. As shown in Fig. 5(c), N-S@RCD shows selective sensing of Au$^{3+}$ and $S_2^-$ without being affected by other interfering species. It is worth mentioning that the sensing process of $S_2^-$ and $Au^3+$ by N-S@RCD is very fast and the sensing response time was less than 2 s. The comparison of ‘Rosa indica’ derived c-dots with other green precursors.

Table 2

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Green Precursor</th>
<th>Sensing</th>
<th>Bioimaging</th>
<th>Logic gate</th>
<th>Intracellular Sensing</th>
<th>Ref.</th>
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<td>X</td>
<td>X</td>
<td>[55]</td>
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<td>2</td>
<td>Onion Peel</td>
<td>X</td>
<td>✓</td>
<td>X</td>
<td>X</td>
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<td>Cu$^{2+}$ and Hg$^{2+}$</td>
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<td>X</td>
<td>X</td>
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<td>4</td>
<td>Papaya</td>
<td>Fe$^{3+}$</td>
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<td>X</td>
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<td>X</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>[60]</td>
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<td>8</td>
<td>‘Rosa indica’</td>
<td>Au$^{3+}$ and $S_2^-$</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>This Work</td>
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Fig. 6. “INHIBIT” logic function using N-S@RCD. (a) Truth table (b) Fluorescence response of N-S@RCD under different inputs (c) Symbol of INHIBIT logic. (A colour version of this figure can be viewed online.)

Table 1

Performance comparison of various fluorescence based Au$^{3+}$ and S$^-2$ sensors.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sensing Element</th>
<th>Green Sensor</th>
<th>Method</th>
<th>Sensor</th>
<th>Linear Range</th>
<th>LOD</th>
<th>Ref.</th>
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<td>1</td>
<td>GO-PVA</td>
<td>No</td>
<td>Flu. “Turn Off”</td>
<td>Au$^{3+}$</td>
<td>0–300 μM</td>
<td>-275 ppb</td>
<td>[25]</td>
</tr>
<tr>
<td>2</td>
<td>Rhodamine/BODIPY</td>
<td>No</td>
<td>Flu. “Turn On”</td>
<td>Au$^{3+}$</td>
<td>0–10 equi.</td>
<td>ND</td>
<td>[27]</td>
</tr>
<tr>
<td>3</td>
<td>Phosphine-decorated Fluorescein</td>
<td>No</td>
<td>Flu. “Turn On”</td>
<td>Au$^{3+}$</td>
<td>0–500 nM</td>
<td>76 nM</td>
<td>[50]</td>
</tr>
<tr>
<td>4</td>
<td>N doped C-dots</td>
<td>Yes</td>
<td>Flu. “Turn Off”</td>
<td>Au$^{3+}$</td>
<td>0–18 μM</td>
<td>53 nM</td>
<td>[51]</td>
</tr>
<tr>
<td>5</td>
<td>N doped C-dots</td>
<td>Yes</td>
<td>Flu. “Turn Off”</td>
<td>Au$^{3+}$</td>
<td>0–50 μM</td>
<td>6.4 $×$ 10$^{-8}$ M</td>
<td>[26]</td>
</tr>
<tr>
<td>6</td>
<td>Rhodamine-PAA-ITO</td>
<td>No</td>
<td>Flu. “Turn On”</td>
<td>Au$^{3+}$</td>
<td>ND</td>
<td>0.10 μM</td>
<td>[52]</td>
</tr>
<tr>
<td>7</td>
<td>MoS$_2$-Pb$^{2+}$</td>
<td>No</td>
<td>Flu. “Turn Off”</td>
<td>$S_2^-$</td>
<td>0.5–12 μM</td>
<td>0.42 μM</td>
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<td>$S_2^-$</td>
<td>0–8 μM</td>
<td>53 nM</td>
<td>[54]</td>
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<td>9</td>
<td>C-dots</td>
<td>No</td>
<td>Flu. “Turn Off”</td>
<td>S$^-2$</td>
<td>ND</td>
<td>0.78 μM</td>
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<td>Flu. “Turn Off”</td>
<td>S$^-2$</td>
<td>0.5–80 μM</td>
<td>0.38 μM</td>
<td>[24]</td>
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<td>11</td>
<td>C-dots</td>
<td>Yes</td>
<td>Flu. “Turn On”</td>
<td>S$^-2$</td>
<td>0.1–8 μM</td>
<td>0.06 μM</td>
<td>[22]</td>
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<tr>
<td>12</td>
<td>N-S co-doped c-dots</td>
<td>Yes</td>
<td>Flu. “Turn On”</td>
<td>Au$^{3+}$</td>
<td>50–750 μM</td>
<td>63.1 nM</td>
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</table>
| 13     | N-S co-doped c-dots | Yes | Flu. “Turn Off” | Au$^{3+}$ | 0–400 μM | with a remaining 13.2% of initial fluorescence intensity at 400 μM Au$^{3+}$. The strong interaction between Au$^{3+}$ and $S_2^-$ leads to loss of the enhanced fluorescence and co-existence of non-radiative electron transfer from N-S@RCD to Au$^{3+}$ leads to efficient quenching at relatively low Au$^{3+}$ concentration in this case.

*ND- Not Determined.
than 10 s as shown in Fig. S9. The performance comparison of N-S@RCD with other reported fluorescence based Au$^{3+}$ and S$^{2-}$/C$_0$ sensors reveals its superiority in terms of green synthetic approach, broad linear range and better LOD (Table 1).

The role of *Rosa indica* derived c-dots in this multifunctional behaviour is compared with other green precursors in terms of sensing, bioimaging and bioelectronics and the comparison is summarized in Table 2 which show the superiority of *Rosa indica* as a green precursor. Since the properties of c-dots are greatly affected by the composition of precursor, it is evident that the role of *Rosa indica* in dual Au$^{3+}$ and S$^{2-}$ sensing in extremely important and certain.

3.4. Molecular logic gates using N-S@RCD

The interesting fluorescence switching of N-S@RCD can be used as multiple molecular logic gates, exploiting the Boolean algebraic logic operations. The simple single input molecular logic gate “YES”, in which the output signal is same as the input signal, can be constructed using S$^{2-}$ as single input signal (Fig. S10). The multiple input logic gate “INHIBIT” can be realized by taking the presence and absence of S$^{2-}$ and Au$^{3+}$ as input 1 or 0 respectively. Only in the presence of S$^{2-}$ and absence of Au$^{3+}$ i.e. input (1, 0), the system generates a marked enhancement in fluorescence and provide output as 1, while in case of other input signals (0/0, 0/1, 1/1), the output remains 0. The logic symbol, output fluorescence intensity at 397 nm and the truth table is shown in Fig. 6.

3.5. Biocompatibility and bioimaging

The optical superiority of N-S@RCD has to accompany a good biocompatibility for live cell applications. The cytotoxicity of N-S@RCD was evaluated against HeLa cell lines and the results are shown in Fig. S11. It can be seen that more than 80% cells were live at a concentration of 1 mg/mL for a 16 h treatment. In order to study long term toxicity, N-S@RCD treatment upto 1 mg/mL for a period

![Fig. 7. Bioimaging of HeLa cells using N-S@RCD. (A colour version of this figure can be viewed online.)](image)

![Fig. 8. Intracellular sensing of Au$^{3+}$ using N-S@RCD. (A) Confocal microscopy of HeLa cells treated with N-S@RCD and N-S@RCD + Au$^{3+}$ (B) Flow cytometry analysis of relative intracellular fluorescence intensity of control, N-S@RCD treated and N-S@RCD + Au$^{3+}$ treated HeLa cells. (A colour version of this figure can be viewed online.)](image)
of 5 days was given and the MTT results shows that more than 75% cells were live (Fig. S12). The morphology of control and treated cells was monitored every 24 h and the results summarised in Fig. S13 shows no significant morphological alterations and indicated good biocompatibility of N-S@RCD, which enables its testing as a bioimaging probe.

To investigate the bioimaging applicability of N-S@RCD, HeLa cells were treated with N-S@RCD for 6 h. The wavelength tuned behaviour can also be visualized in the intracellular fluorescence where blue and green signal was obtained at different excitation wavelength using confocal microscope (Fig. 7). At higher magnification, the cellular localisation of N-S@RCD could be visualized. A closer observation revealed that the fluorescence signal is confined to extranuclear region, primarily in the cytoplasm. However, the multicolour emission of N-S@RCD put limitations to a counter staining study for revealing exact subcellular distribution.

In order to check the autofluorescence of untreated cells, control cells were also analysed under confocal microscope and shows no intracellular fluorescence (Fig. S14).

3.6. Intracellular sensing

Moreover, to investigate the intracellular sensing of Au^{3+}, N-S@RCD treated HeLa cells were incubated with 500 μM Au^{3+}, and observed under confocal microscope and flow cytometer. As shown in Fig. 8(A), a clear intracellular quenching of fluorescence was observed after addition of Au^{3+} which corroborates spectroscopic results. The flow-cytometric investigation also revealed similar quenching of fluorescence as shown in Fig. 8(B). More investigations are required for intracellular quantification using flow cytometry. However, a detectable intracellular “Turn-On” could not be observed after addition of S^{2−} (data not shown).

3.7. Hemocompatibility

In order to evaluate potential in vivo applicability, the effect of N-S@RCD on red blood cells was studied. Human RBC was treated with N-S@RCD in the concentration range 0.01–50 mg/mL. The results of hemolysis study indicates no obvious effect on RBCs. The quantification of hemolysis shown in Fig. 9(A) shows less than 5% hemolysis which is ASTM E2524-08 standard for haemolytic nanoparticles [61]. Fig. 9(B) shows the precipitated RBCs after hemolysis experiment and the blank N-S@RCD at different concentration. The hemolysed positive control sample shows clear red color due to lysis of RBC while the treated sample shows color similar to corresponding blank N-S@RCD. In order to further investigate the change in morphology of RBCs due the interaction with N-S@RCD, SEM of control and treated RBC were performed and results summarized in Fig. 9(C) shows no significant effect on RBC morphology, validating the hemocompatible nature of N-S@RCD upto a concentration of 50 mg/mL.
4. Conclusion

The multifunctionality of N and S co-doped carbon–dots synthesized using rose petals is investigated. The as-synthesized carbon dots shows high photostability and a highly selective and ultrafast turn “off-on-off” response towards dual S2– and Au3+ sensing which also mimic “YES” and “INHIBIT” logic function hence, explored for construction of molecular logic gates. The N-S@URCD shows hemo and biocompatible nature along with the potential of bioimaging and intracellular sensing. The promising behaviour of these c-dots may open new direction for multifunctional applications of green c-dots in optical, biological and electronics applications.

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Appendix A. Supplementary data

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References
