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# Multi-functional carbon dots-based nanoprobe for ratiometric enzyme reaction monitoring and biothiol analysis

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ABSTRACT

In this work, red-emissive carbon dots (CDs) were prepared through microwave-assisted heating and then conjugated with fluorescein isothiocyanate (FITC) to construct a ratiometric multi-functional nanoprobe (CDs-F) for both pH sensing and biothiols analysis. After coupling with FITC, the as-obtained CDs-F shows both green and red emissions. Based on the pH sensitivity of FITC, the CDs-F can be used for ratiometric pH sensing. On the other hand, the red emission of CDs can be quenched significantly by $Ag^+$. Studies of quenching mechanism demonstrate that $Ag^+$ could coordinate with the functional groups of CDs resulting in the formation of the CDs-$Ag^+$ complex and also charge transfer between the CDs and $Ag^+$. Simultaneous static and dynamic quenching processes impart the maximum quenching. Based on the strong affinity between thiol groups and $Ag^+$, the CDs-F with dual emissions can be used for ratiometric biothiols analysis with the detection limits of tens of nanomole per liter. Furthermore, the strategy has been successfully applied to monitor proton-related enzymatic catalytic reactions and to determine biothiols in fetal bovine serum samples with good recoveries.



## 1. Introduction

As a newly emerging fluorescent nanomaterial, carbon dots (CDs) have garnered great attention in biosensing, bioimaging, biomedical and opto-electronic fields for the past few years due to their fascinating properties [1–8]. As compared to traditional organic dyes, CDs exhibit improved photostability, water dispersibility and biocompatibility [9–12]. And compared with semiconductor quantum dots (QDs) composed of toxic heavy metal elements, CDs show lower toxicity which is favorable for biological applications [13–16]. Although numerous synthetic methods have been reported to obtain various CDs, most of which show blue to green emissions [17–20], therefore, it is still highly desirable to prepare CDs with remarkable fluorescence property of long-wavelength emission especially, aqueous solubility and easy functionality through simpler approaches for both biosensing and imaging applications.

Recently, ratiometric optical analysis has become a research hotspot because the measurement accuracy can be improved significantly through the evaluation of the ratio of signal intensities under two distinct channels [21–23]. So far, most ratiometric fluorescent probes are usually constructed by combining two fluorophores of different emissions together. The common components include synthetic small-molecule fluorescent probes [24] and various kinds of emissive nanomaterials, such as carbon dots [25], semiconductor quantum dots [25,26], metal nanoclusters [27], upconversion materials [28], etc. Among them, CDs have obtained more attention due to their distinctive optical properties. However, most of these ratiometric probes are for only one specific target. It is meaningful and challenging to design novel fluorescent probes for ratiometric sensing of two or more different targets simultaneously which could provide more comprehensive information for chemical and biological analysis [29–31].

Herein, a CDs-based ratiometric nanoprobe (CDs-F) for both pH sensing and biothiols analysis was constructed by combining the red-emissive CDs with fluorescein isothiocyanate (FITC). As is well-known, pH is an important parameter in all kinds of biological processes and a small fluctuation of pH can disturb the kinetics of a biochemical reaction [32]. So the accurate detection of pH is meaningful and the ratiometric strategy can improve the measurement accuracy. Meanwhile, biothiols, as a type of reactive sulfur species with free thiol group, play essential roles in maintaining cell functions [33–35]. As three representative biothiols, cysteine

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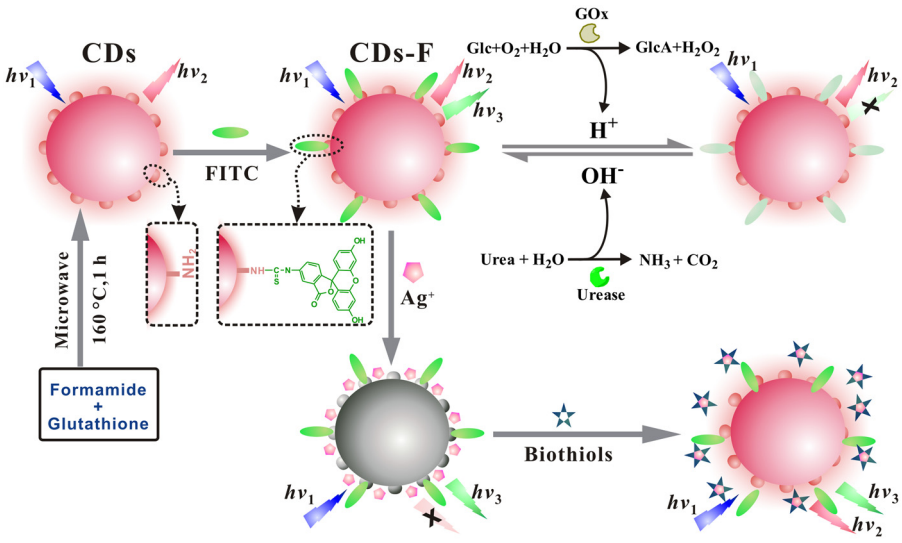


**Scheme 1.** Schematic illustration of the synthesis of the CDs and the construction of ratiometric nanoprobe for pH and biothiols analysis.

(Cys), glutathione (GSH) and homocysteine (Hcy) have been the research focus because their abnormal levels are closely related to various impairments [36–39]. Several strategies based on CDs have been developed for biothiols determination [40–42]. In this work, the conjugated CDs-F could sense pH and biothiols in a ratiometric mode respectively as illustrated in Scheme 1. The initial CDs prepared by microwave assisted heating show stable red emission over a certain pH range while it can be quenched significantly by $Ag^+$. Based on the pH sensitivity of FITC and the strong affinity between thiol groups and $Ag^+$, the CDs-F with dual emissions can be used for ratiometric pH sensing and biothiols analysis respectively. The results demonstrate that the ratiometric probe could detect biothiols with the detection limits (LOD) of tens of nanomole per liter and realize the differentiation of pH variation from 5 to 8. Furthermore, the strategy has been successfully applied to monitor proton-related enzymatic catalytic reactions and to determine biothiols in fetal bovine serum (FBS) samples.

## 2. Experimental section

### 2.1. Materials and chemicals

GSH, Cys, Hcy, FITC, $AgNO_3$, 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB), HEPES, 3,3′-dithiodipropionic acid (DTDPA), 4-mercaptophenylboronic acid (MPBA), 4-nitrothiophenol (NTP), glucose oxidase (GOx), urease were obtained from Sigma-Aldrich China (Shanghai, China). NaF, NaCl, KBr, KI, NaAc, $NaH_2PO_4$, $Na_2CO_3$, $Na_2SO_4$, 2-morpholinoethanesulfonic Acid (Mes), ammonium 1-pyrrolidinedithiocarbamate (APDC), 6-mercapto-1-hexanol (MH), $H_3BO_3$, $H_3PO_4$, glucose, urea were obtained from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Formamide was purchased from Macklin (Shanghai, China). L-leucine (Leu), L-isoleucine (Ile), β-alanine (Ala), L-serine (Ser), L-threonine (Thr), L-asparagine (Asn), L-valine (Val), L-phenylalanine (Phe), glycine (Gly), L-aspartic acid (Asp), L-glutamic Acid (Glu), L-proline (Pro), L-glutamine (Gln), methionine (Met), $Na_2S$, 11-mercaptoundecanoic acid (MUA), Thioflavine S (ThS), lipoic acid, thiamazole were purchased from J&K Chemicals (Beijing, China). All chemicals were used as received without further purification. All aqueous solutions were prepared using ultrapure water deionized by Hitech laboratory water purification system (18.2 MΩ cm).

### 2.2. Apparatus

MDS-8 microwave chemical reactor (Sineo Microwave Chemistry Technology, Shanghai) was used to prepare the CDs. Transmission electron spectroscopy (TEM) images were acquired with JEM-2100F microscopy (JEOL Ltd., Tokyo, Japan). Fourier transform infrared (FTIR) spectroscopy was measured by a Thermo Nicolet 380 FTIR spectrometer (Thermo Nicolet Co., USA). X-ray photoelectron spectroscopy (XPS) results were collected by a ESCALab 220-Xi (VG, UK). Powder X-ray diffraction (XRD) was obtained on an X'Pert Philips Materials Research Diffractometer (Brook AXS, Germany). Absorption spectra were acquired with a Cary 60 UV–vis spectrophotometer (Agilent Technologies, Palo Alto, CA, USA). Fluorescence spectra were collected with a Cary Eclipse spectrofluorophotometer (Agilent Technologies, Palo Alto, CA, USA) at room temperature. The time-resolved fluorescence decay was determined with Edinburgh Instruments Model FLS980 (Edinburgh Instruments Ltd., UK).

### 2.3. Preparation and purification of the CDs and CDs-F

The CDs were prepared according to a previous report [43]. Specifically, 0.68 g GSH was dissolved in 20 mL formamide. The solution was then transferred to the Teflon-lined autoclave and heated in a microwave chemical reactor at 160 °C for 1 h. After cooling to room temperature, the mixture was diluted with water and then purified by dialysis (1000 Da of MWCO) for 3 days. The obtained green solution was filtered through 0.22 μm Millipore membrane followed by freeze-drying.

The CDs (10 mg) and FITC (1.5 mg) were dissolved in 15 mL $NaHCO_3$ solution (0.1 M) in a round bottomed flask. After stirring for 24 h, the mixture was subjected to dialysis with tube of MWCO 1000. The unreacted FITC was removed by using 0.1 M $NaHCO_3$ solution for dialysis on the first day and then deionized water was used in the next two days followed by freeze-drying.

### 2.4. Ratiometric pH sensing and enzyme reactions monitoring by CDs-F

All the sensing experiments were carried out in BR buffer (10 mM). The final concentration of the CDs-F is 5.0 μg/mL. The fluorescence spectra were obtained with the excitation wavelength of 400 and 490 nm.