

Sensitive Determination of Coomassie Brilliant Blue Using a Dual-Emission Ratiometric Fluorescent Probe Composed of Sulfur Quantum Dots and Gold Nanoclusters

Yifan Xie

Shenyang University of Chemical Technology

Xiaotian zhao

Weifang University of Science and Technology

Zhihao Fang

wfsgfzh@163.com

Weifang University of Science and Technology

Wenxu Wang

Shenyang University of Chemical Technology

Haijian Liu

Weifang University of Science and Technology

Yiran Liu

Weifang University of Science and Technology

Runxiu He

Weifang University of Science and Technology

Yifei Zhu

Weifang University of Science and Technology

Chunyu Jia

Weifang University of Science and Technology

Jiaxuan He

Weifang University of Science and Technology

Hui Yao

Shenyang University of Chemical Technology

Research Article

Keywords: sulfur quantum dots, gold nanoclusters, dual-emission ratiometric fluorescent probe, Coomassie Brilliant Blue, inner filter effect

Posted Date: September 11th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10595619/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Coomassie Brilliant Blue (CBB) is widely used for protein staining, but its residues may pose environmental and biological risks, creating a need for simple and sensitive detection methods. In this work, a dual-emission ratiometric fluorescent probe was constructed from sulfur quantum dots (S-QDs; blue emission at 430 nm) and BSA-templated gold nanoclusters (Au NCs; red emission at 640 nm). CBB preferentially attenuated the Au NC emission through the inner filter effect (IFE). The fluorescence intensity ratio (F_{430}/F_{640}) exhibited a linear correlation with CBB concentration from 0.1 to 100 μM ($R^2 = 0.991$). The limit of detection was calculated to be 0.01 μM (3σ), around six times lower than that of the single-emission Au NC probe, accompanied by improved calibration sensitivity. The spectral overlap between CBB absorption and Au NC emission, together with the nearly unchanged fluorescence lifetime, supported IFE as the predominant sensing mechanism and indicated that dynamic quenching was not dominant. The probe was applied to CBB determination in a BSA model matrix and two real samples, and the results agreed well with those obtained by HPLC-MS/MS. By using the S-QD emission as an internal reference, the ratiometric strategy reduced common-mode signal fluctuations and improved measurement reliability, providing a potential approach for CBB determination in food-related and biological matrices.

1. Introduction

Coomassie Brilliant Blue (CBB) was first synthesized in the 1960s by the German chemist Coomassie^[1, 2]. Named after its inventor, CBB is primarily used for protein quantification. By the 1970s, it had become a standard dye for visualizing proteins after gel electrophoresis because of its favorable affinity and high sensitivity toward most proteins. Its widespread adoption has contributed substantially to research in biochemistry and molecular biology^[3, 4].

CBB is a triphenylmethane dye that is commercially available mainly as the *G*-250 and *R*-250 forms^[7]. CBB *G*-250 appears red in acidic solution and turns blue upon binding to proteins, with a maximum absorption wavelength of approximately 595 nm^[9]. Its molecular formula is $\text{C}_{47}\text{H}_{48}\text{N}_3\text{O}_7\text{S}_2\text{Na}$. The molecule contains three nitrogen atoms in the forms of quaternary ammonium nitrogen (N^+), a secondary amine ($-\text{NH}$), and trivalent nitrogen, together with two sulfonic acid groups, one of which is highly ionizable and associated with Na^+ . CBB *G*-250 was introduced for protein staining after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the 1970s^[1, 7]. Conventional Coomassie staining uses methanol, acetic acid, and other reagents for fixation and destaining, resulting in relatively cumbersome procedures and the use of irritant chemicals. More recent rapid-staining formulations, including methanol-free systems, have shortened staining times and reduced background interference.

Despite its extensive laboratory use, the potential hazards associated with CBB warrant attention^[6]. As a synthetic triphenylmethane dye rather than a conventional azo dye, CBB has raised concerns regarding

possible metabolic toxicity and environmental risks^[8]. Although its structure does not contain an azo group ($-N=N-$), studies have suggested that its conjugated aromatic framework and sulfonic acid substituents may generate toxicologically relevant products during biological metabolism or environmental degradation^[9]. Under reductive conditions, microbial enzymes may degrade the aromatic structure of CBB to produce phthalates and sulfonated anilines. These aromatic amines may form DNA adducts, contribute to genotoxic and carcinogenic effects, and interfere with estrogen-related endocrine pathways. CBB-derived sulfonic groups may also promote sulfate-radical formation, increase reactive oxygen species (ROS), reduce mitochondrial membrane potential, and cause cellular damage. The metabolic and excretion profiles of CBB in organisms remain insufficiently understood. Its high water solubility, enhanced by sulfonic acid groups, may retard environmental degradation and favor bioaccumulation. Direct discharge of CBB-containing waste can contaminate aquatic environments, inhibit embryonic development and enzymatic activity in aquatic organisms, induce subacute toxicity, and disrupt ecological balance.

Because CBB is widely used as both a biological stain and an industrial dye, its environmental residues and potential bioaccumulation have received increasing attention^[6, 8]. Reported concentrations are generally at trace levels in environmental water and biological samples. Nevertheless, possible toxicity associated particularly with metabolized aromatic amine derivatives may pose long-term ecological and human-health risks through food-chain biomagnification, even at low exposure levels. CBB concentrations of 5–50 ng/mL have been reported in wastewater-treatment effluents and contaminated surface water, whereas residual levels in biological fluids, including fish plasma and mammalian urine, may be as low as 1–10 ng/mL. By comparison, conventional spectrophotometric methods represented by the Bradford assay have an LOD of approximately 1 $\mu\text{g/mL}$ (1000 ng/mL), which is insufficient for trace-level analysis and may lead to underestimation or non-detection of low-level CBB exposure in practical samples.

The sensitivity of spectrophotometric determination is constrained by the limitations of the Lambert-Beer law and the instrumental signal-to-noise ratio (SNR)^[3, 4]. The molar extinction coefficient of CBB at 595 nm is approximately $4.3 \times 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. For a standard 1 cm cuvette, the theoretical minimum detectable concentration is approximately 0.1 μM , equivalent to about 0.86 $\mu\text{g/mL}$ for CBB G-250 with a molecular weight of 854. In practical measurements, background scattering and additional absorbance arising from sample turbidity and miscellaneous proteins further reduce the SNR, increasing the practical LOD to above 1 $\mu\text{g/mL}$ ^[2]. In addition, the binding kinetics of CBB with proteins may exhibit a lag phase; incomplete binding at low concentrations can cause nonlinearity in the calibration curve and compromise quantitative accuracy^[5].

Environmental and biological matrices contain diverse interferents that can substantially impair the specificity of conventional spectrophotometric measurements^[2]. Colored contaminants in industrial wastewater, such as azo dyes and humic acids, exhibit broad absorption across the visible region (400–700 nm), which may overlap with the characteristic CBB absorption at 595 nm and produce false-positive

responses. Experimental results have shown that 10 $\mu\text{g/mL}$ Congo red can increase the apparent CBB concentration by approximately 30%. High-salinity matrices, including seawater and cell lysates, may also induce aggregation of CBB-protein complexes and cause nonlinear absorbance responses. When the NaCl concentration exceeds 150 mM, the recovery of the Bradford assay reportedly decreases below 70%. Surfactants provide an additional source of interference: detergents such as Tween-20 and Triton X-100 may competitively bind CBB or alter protein conformation, thereby suppressing the chromogenic reaction.

Existing methods for CBB determination each have practical limitations. High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) provides high analytical accuracy but requires costly instrumentation, relatively complex operation, and lengthy analysis, limiting its suitability for rapid on-site screening. UV-Vis spectrophotometry is straightforward but has limited sensitivity and is susceptible to matrix interference, making trace determination difficult. Single-emission Au NC probes offer rapid responses, but their signals are more vulnerable to environmental and instrumental fluctuations, which can compromise measurement stability and accuracy^[10, 11, 14–16].

Fluorescence analysis is increasingly used for dye determination because it combines convenient operation with comparatively simple instrumentation, high sensitivity, and favorable selectivity^[12, 13]. In recent years, sulfur quantum dots (S-QDs) have been applied in ratiometric fluorescence sensing of analytes including tetracycline^[23], heavy-metal ions^[25, 28], ascorbic acid^[26], and Cr(VI)^[27], and have also shown utility in environmental monitoring^[31] and cellular imaging^[29, 30]. Metal nanoclusters and quantum dots have attracted broad interest because of their distinctive optical properties, low toxicity, photostability, and biocompatibility^[14]. Gold nanoclusters (Au NCs), in particular, exhibit good water solubility, high quantum yield, and low toxicity. BSA-capped Au NCs have been used for the detection of intracellular hydrogen peroxide^[37], histidine^[38], morin^[39], and copper ions in water^[40], and have also demonstrated catalytic activity^[41]. S-QDs can be prepared through relatively simple procedures and exhibit stable fluorescence. Combining S-QDs and Au NCs in a dual-emission ratiometric probe enables signal normalization and internal referencing, thereby improving measurement reliability^[11].

In this study, a dual-emission ratiometric fluorescent probe composed of S-QDs and Au NCs was developed for CBB determination^[15]. Blue-emissive S-QDs with an emission maximum at 430 nm were synthesized hydrothermally, while red-emissive Au NCs ($\lambda_{\text{em}} = 640 \text{ nm}$) were prepared using bovine serum albumin (BSA) as the template^[32]. The two materials exhibited well-separated emission bands. Meanwhile, the UV-visible absorption spectrum of CBB overlapped substantially with the Au NC emission spectrum, providing the spectroscopic basis for an inner filter effect (IFE)-mediated response^[35]. In the resulting probe, the S-QD emission served as an internal reference for the CBB-responsive Au NC signal, reducing common-mode fluctuations and supporting reliable determination of CBB residues in food-related and biological samples^[18].

2. Experimental Section

2.1 Instruments and Reagents

The instruments used in this study included a collecting-type constant-temperature magnetic stirrer (DF-10S, Shanghai Lichen Bangxi Instrument Technology Co., Ltd.); an electric thermostatic drying oven (DHG-9030, Shanghai Yiheng Scientific Instrument Co., Ltd.); an electronic analytical balance (PX224ZH, Ohaus Corporation, USA); a single-channel adjustable pipette (0.5-5 mL, Eppendorf AG, Germany); a fluorescence spectrophotometer (F-7000 FL, Hitachi, Ltd.); a four-function dark-box UV analyzer (ZF-8, Wenzhou Mingren Instrument Co., Ltd.); a benchtop high-speed centrifuge (BK-G165, Bickman Biotechnology Co., Ltd.); a hydrothermal synthesis reactor (LC-KH-50, Shanghai Lichen Bangxi Instrument Technology Co., Ltd.); a freeze-dryer (FD-1-50, Beijing Boyikang Experimental Instruments Co., Ltd., Beijing, China); and a UV-Vis spectrophotometer (UV-1801, Beijing Beifen Ruili Analytical Instrument Co., Ltd.).

The reagents were bovine serum albumin (Component V, analytical grade, Shandong Shengyang Group Co., Ltd.); sublimed sulfur (analytical grade, Sinopharm Chemical Reagent Co., Ltd.); ethylenediamine (analytical grade, Sinopharm Chemical Reagent Co., Ltd.); Coomassie Brilliant Blue G-250 (analytical grade, Tianjin Damao Chemical Reagent Factory); sodium hydroxide, chloroauric acid ($\text{HAuCl}_4 \cdot \text{H}_2\text{O}$), sodium chloride, potassium chloride, ferrous sulfate, zinc sulfate, and cadmium nitrate (all analytical grade, Sinopharm Chemical Reagent Co., Ltd.); D-fructose, ascorbic acid, Sudan I, bromocresol green, cobalt chloride, and germanium sulfate (all analytical grade, Aladdin Reagent Co., Ltd.); and ultrapure water (resistivity $\geq 18.2 \text{ M}\Omega \cdot \text{cm}$ at 25°C).

2.2 Preparation of Sulfur Quantum Dots (S-QDs)

S-QDs were synthesized by a hydrothermal method. Dried sublimed sulfur powder (0.2000 g, weighed to 0.0001 g; purity $\geq 99.9\%$) was first dried at 60°C for 2 h to remove adsorbed water and then added to 20.0 mL of ethylenediamine (EDA; purity $\geq 99.5\%$)^[32, 33]. The mixture was stirred in a fume hood at 500 rpm and room temperature for 30 min and inspected at 5 min intervals. When visible sulfur particles remained in suspension, as indicated by sediment formation upon tilting the beaker, sodium borohydride (NaBH_4 ; 0.0500 g, weighed to 0.0001 g; purity $\geq 98\%$) was added. Stirring was continued at 500 rpm for 10 min until a homogeneous pale-yellow turbid suspension without visible particles was obtained^[34–36].

The entire mixture was transferred by pipette to a 50 mL polytetrafluoroethylene-lined autoclave. Before use, the liner had been soaked in 10% nitric acid for 24 h, rinsed with deionized water to neutrality, and dried at 60°C . The reaction mixture occupied 40% of the liner volume, within the recommended filling range of 30%-80%. The sealed autoclave was placed in an electric thermostatic drying oven, heated to 170°C at $5^\circ\text{C}/\text{min}$, and maintained at this temperature for 8 h. After the reaction, the oven was switched off and the autoclave was allowed to cool naturally to room temperature for approximately 4 h; rapid cooling was avoided to prevent damage to the liner.

The reaction mixture was diluted to 50 mL with deionized water and transferred to a cellulose dialysis bag with a molecular-weight cutoff of 3500 Da, which had been activated by boiling in deionized water

for 10 min. The bag was immersed in 500 mL of deionized water and dialyzed at 25°C for 24 h. The external water was replaced every 6 h, and its pH was measured before each replacement until it stabilized at 7.0 ± 0.2 , indicating removal of free EDA.

After dialysis, the solution was transferred to a 50 mL centrifuge tube and centrifuged at 12,000 rpm and 4°C for 15 min. The clear supernatant was carefully collected without disturbing the sediment and filtered through a 0.22 μm aqueous microporous membrane that had been rinsed three times with deionized water. The resulting filtrate was collected as the purified S-QD solution and stored at 4°C in the dark until use.

2.3 Preparation of Gold Nanoclusters (Au NCs)

Protein-templated synthesis is a widely used route for preparing Au NCs. In this study, bovine serum albumin (BSA) was used as the template for the facile preparation of red-emissive Au NCs. BSA and $\text{HAuCl}_4 \cdot \text{H}_2\text{O}$ were mixed under alkaline conditions. At $\text{pH } 12.0 \pm 0.1$, reducing amino acid residues in BSA, including tyrosine, tryptophan, and phenylalanine, reduced $\text{HAuCl}_4 \cdot \text{H}_2\text{O}$ to gold atoms, yielding red-fluorescent Au NCs.

For BSA-templated synthesis, bovine serum albumin (0.2000 g; purity $\geq 98\%$) was dried at 60°C for 2 h to remove adsorbed water. After cooling, 20.0 mL of ultrapure water (resistivity $\geq 18.2 \text{ M}\Omega \cdot \text{cm}$ at 25°C) was added, and the mixture was stirred at 500 rpm for 15 min at room temperature until a homogeneous, transparent pale-yellow solution was obtained. An absorbance of approximately 0.63 at 280 nm was used to verify solution uniformity. A 10.0 mM $\text{HAuCl}_4 \cdot \text{H}_2\text{O}$ solution was prepared by dissolving 0.0358 g of $\text{HAuCl}_4 \cdot \text{H}_2\text{O}$ and diluting to 10 mL with ultrapure water; the calibration error determined by ICP-OES was controlled within 0.5%. The $\text{HAuCl}_4 \cdot \text{H}_2\text{O}$ solution (10 mL) was mixed with 10 mL of the BSA solution and stirred at 800 rpm for 2 min at room temperature. Subsequently, 0.50 mL of 1 M NaOH was added to adjust the pH to 12.0 ± 0.1 . The mixture was incubated at $80.0 \pm 0.5^\circ\text{C}$ for 120 min. During the reaction, the solution gradually changed from pale yellow to light brown and finally to dark brown.

The crude product was transferred to a 3500 Da dialysis bag that had been activated by boiling for 10 min and dialyzed against ultrapure water at 25°C for 24 h. The external water was replaced repeatedly until its conductivity decreased to $\leq 5 \mu\text{S}/\text{cm}$. The dialyzed solution was then centrifuged at 12,000 rpm and 4°C for 10 min. The dark-brown supernatant, containing the purified Au NCs, was collected and stored at 4°C in the dark.

2.4 Preparation of CBB Solutions at Different Concentrations

A 1 mM CBB stock solution was prepared accurately. Appropriate aliquots of this solution were then combined with 400 μL of the probe and diluted to obtain final CBB concentrations of 0.1, 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 μM for subsequent measurements.

2.5 Detection of CBB Using the S-QDs/Au NCs Dual-Emission Ratiometric Fluorescent Probe

Equal volumes of the S-QD and BSA-Au NC stock solutions (2.0 mL each) were mixed and vortexed for 30 s to prepare the S-QDs/Au NCs dual-emission ratiometric fluorescent probe. The probe was freshly prepared and stored at 4°C in the dark.

For each assay, 400 μ L of the probe mixture and an appropriate volume of CBB stock solution were added, and the system was diluted to a final volume of 1000 μ L with ultrapure water. In the blank, the CBB solution was replaced with an equal volume of ultrapure water. After mixing for 20 s, the samples were incubated at 25°C in the dark for 15 min to allow the fluorescence signal to stabilize.

Coexisting interferents were added at concentrations ten times that of CBB, and their effects were evaluated from changes in the fluorescence intensity ratio. Signal stability was assessed by continuously recording the ratiometric response over 0-120 min.

2.6 Pretreatment of Real Samples and Spiked-Recovery Validation

A BSA model matrix and two real samples, commercial whey-protein powder and tap water, were used to evaluate the practical applicability of the method.

For the BSA model matrix, 0.1000 g of BSA was dissolved in 10 mL of ultrapure water and vortexed for 5 min. After centrifugation at 12,000 rpm and 4°C for 10 min, the supernatant was filtered through a 0.22 μ m aqueous membrane to obtain the blank-matrix filtrate. For whey-protein powder, 0.2000 g of powder was dispersed in 10 mL of water, magnetically stirred for 30 min, and sonicated at 300 W and 25°C for 20 min. The mixture was then centrifuged at 12,000 rpm and 4°C for 15 min and filtered. Tap-water samples were allowed to stand for 30 min and were then filtered directly through a 0.22 μ m membrane.

For the spiked-recovery experiments, an appropriate volume of CBB standard solution was added to each blank-matrix filtrate. Subsequently, 400 μ L of the S-QDs/Au NCs probe and ultrapure water were added to give a total volume of 1000 μ L and final CBB concentrations of 0.1, 0.5, and 1.0 μ M. Corresponding blank samples without CBB were prepared in parallel. After incubation at room temperature in the dark for 15 min, fluorescence measurements were performed under the conditions described in Section 2.5.

3. Results and Discussion

3.1 Characterization of Gold Nanoclusters

Figure 1a presents the transmission electron microscopy (TEM) image and particle-size distribution of the Au NCs^[10, 11]. The particles were nearly spherical, uniformly dispersed, and relatively uniform in

size^[14]. Statistical analysis of the TEM image gave an average particle diameter of approximately 1.1 nm^[16].

3.2 Fluorescence Spectroscopic Analysis

The fluorescence spectra of the S-QDs, Au NCs, and their mixed system are shown in Fig. 1b^[15]. Under excitation at 360 nm, the S-QDs exhibited a blue-emission maximum at 430 nm, whereas the Au NCs displayed a red-emission maximum at 640 nm. The mixed system retained two distinct emission peaks at 430 and 640 nm and appeared pink under UV irradiation^[11].

3.3 Sensing Mechanism for CBB Detection

The inner filter effect (IFE) is an important source of signal attenuation in fluorescence analysis^[32]. It arises when the absorption spectrum of a light-absorbing species overlaps with the excitation spectrum, emission spectrum, or both, of a fluorophore. Absorption of the incident excitation light reduces the energy reaching the fluorophore, while reabsorption of emitted photons during their passage to the detector further decreases the measured fluorescence intensity. The latter process is commonly described as the emission-side inner filter effect.

IFE is typically evaluated by comparing fluorescence intensities between blank and analyte-containing samples and, when appropriate, by applying mathematical correction models. The Lambert-Beer law is commonly used with absorbance data to estimate and correct signal deviations caused by IFE.

The magnitude of IFE depends on several experimental variables^[19, 20]. An increase in the concentration of the absorbing species strengthens attenuation of both excitation and emission light. Optical path length is also important: a longer propagation distance increases the probability of photon absorption and therefore enhances the observed IFE^[21, 22].

Spectral overlap is another determining factor; a broader overlap between the absorber and the fluorophore excitation or emission band generally produces a more pronounced effect. Instrumental settings may also influence the measured response. For example, slit width controls the amount of light entering the detection system, while detector sensitivity affects signal collection efficiency. Coexisting light-absorbing substances may compete with the target analyte for excitation or emission photons and thereby interfere with IFE-based measurements. In addition, the intrinsic quantum yield of the fluorophore and changes in pH or ionic strength may alter the initial fluorescence intensity, the chemical form of the absorber, or the spectral properties of the fluorophore, indirectly affecting the observed IFE.

In the present system, the strong UV-Vis absorption of CBB overlapped with both the excitation and emission regions of the Au NCs, allowing attenuation of the Au NC fluorescence through excitation-side and emission-side IFE pathways.

For excitation-side attenuation, CBB absorbed part of the incident excitation light before it reached the Au NCs, thereby decreasing the effective excitation intensity. According to the Lambert-Beer calculation,

when the CBB concentration reached 10 μM , excitation-light transmittance decreased to 34% of its initial value, with a corresponding reduction in Au NC emission. Blank controls and the Lakowicz correction Eq. (1) were used to quantify and correct the signal deviation associated with IFE:

$$F_{\text{correct}} = F_{\text{observed}} \times 10^{(A_{\text{ex}} + A_{\text{em}})/2}$$

1

For emission-side attenuation, fluorescence photons emitted by the Au NCs at approximately 640 nm were partially reabsorbed by CBB during propagation to the detector. This emission-side inner filter effect (Em-IFE) accounted for 28% of the total signal loss according to the experimental analysis, providing an additional contribution to the observed fluorescence attenuation.

The extent of spectral overlap and the fractional contribution to total fluorescence attenuation are distinct parameters. As shown by the fluorescence and absorption spectra in Fig. 1c, the CBB absorption band overlapped substantially with the Au NC emission over 550–700 nm, supporting the occurrence of Em-IFE through reabsorption of emitted photons^[13, 14]. This overlap provides the principal spectroscopic basis for the IFE-mediated ratiometric sensing system^[15]. Nevertheless, the broad emission-band overlap did not necessarily indicate that Em-IFE dominated the overall response, because Ex-IFE produced stronger multiplicative attenuation at the excitation stage.

The Au NC fluorescence differed between the single-component and mixed systems. Au NCs alone exhibited a 640 nm emission intensity of approximately 3500 a.u., whereas the corresponding intensity in the S-QDs/Au NCs mixture decreased to approximately 3000 a.u.^[11]. Quantitative analysis using the Lakowicz equation indicated that the signal attenuation in the mixed system was governed predominantly by the overall IFE^[10, 11].

Time-resolved fluorescence measurements were used to evaluate whether other quenching pathways dominated the response^[13]. As shown in Fig. 1d, the Au NC fluorescence lifetime changed only slightly, from 1.57 μs without CBB to 1.60 μs after addition of 50 μM CBB, corresponding to a variation of less than 5%. Over the same concentration change, the fluorescence intensity decreased by more than 70%. The nearly unchanged lifetime indicates that dynamic quenching was not the dominant pathway and, together with the spectral-overlap results, supports IFE as the principal cause of fluorescence attenuation^[15]. Static quenching associated with electrostatic interactions between the Au NCs and CBB cannot be completely excluded.

Because IFE depends strongly on absorbance, its characteristics should be considered when optimizing analytical performance^[14]. At excessively high CBB concentrations beyond the linear range (> 100 μM), the system absorbance would exceed the linear response range of the instrument. Such IFE overload could be reduced by sample predilution or by shortening the optical path from 1 cm to 0.1 cm, for example using a microfluidic chip. In the present design, the S-QD emission at 430 nm was introduced as an internal reference to improve analytical sensitivity. The F_{430}/F_{640} ratio mitigates interferences

originating from light-source fluctuations and matrix effects, delivering an LOD of 0.01 μM , approximately six times lower than that of the conventional single-emission Au NC probe. Since the IFE mechanism does not demand covalent conjugation between fluorophores and absorbers, it offers a relatively facile strategy for constructing ratiometric fluorescent sensors and has been widely applied for the detection of diverse analytes^[17].

Overall, CBB-induced fluorescence attenuation in this system involved coupled excitation- and emission-side inner filter effects. Characterizing these contributions and incorporating an internal reference were important for achieving sensitive and reliable CBB determination.

3.4 Effect of CBB Concentration on the Fluorescence of Au NCs

As shown in Fig. 2a, each fluorescence spectrum corresponds to a different CBB concentration^[15]. As the CBB concentration increased, the Au NC emission at 640 nm was progressively quenched, and the peak intensity decreased from approximately 6000 to 3500 a.u. This concentration-dependent attenuation indicates that CBB effectively reduces Au NC fluorescence through the proposed IFE mechanism. The 640 nm emission continued to decrease as the CBB concentration increased.

3.5 Effect of CBB Concentration on the Fluorescence Response of the S-QDs/Au NCs Probe

As shown in Fig. 2c, the short-wavelength emission band was centered at 430 nm, with an intensity of approximately 3500–5000 a.u., consistent with the characteristic blue emission of S-QDs^[2]. Hydrothermally prepared S-QDs have been reported to emit within 400–500 nm as a result of quantum-confinement effects and to exhibit relatively high quantum yields and narrow emission bands. The symmetric profile and comparatively stable intensity of the 430 nm peak further support its assignment to S-QD emission. The long-wavelength band was located in the orange-red region and exhibited a broad profile with a full width at half maximum of approximately 50 nm, consistent with Au NC emission^[4]. BSA-templated Au NCs have been reported to emit within 610–650 nm because of surface-plasmon-resonance and size-related effects^[10]. As the CBB concentration increased, the long-wavelength peak decreased from approximately 3000 a.u. for the red curve to 1000 a.u. for the blue curve, whereas the S-QD emission remained comparatively stable. These results indicate that CBB primarily modulated the Au NC response channel without substantially affecting the S-QD reference signal^[11].

3.6 Analytical Performance of the Dual-Emission Ratiometric Fluorescent Probe

3.6.1 Calculation of Probe Sensitivity

As shown in Fig. 2b, the relative fluorescence intensity of the single-emission Au NC probe (F/F_0) decreased linearly as the CBB concentration increased, with an absolute calibration slope of $0.00968 \mu\text{M}^{-1}$. At $40 \mu\text{M}$ CBB, F/F_0 reached 0.761, corresponding to a fluorescence-quenching efficiency of 23.9%. For a single-emission sensing system, calibration sensitivity is represented quantitatively by the absolute slope of the calibration curve.

By comparison, the dual-emission ratiometric probe used F_{430}/F_{640} as the analytical response, and this ratio was positively correlated with CBB concentration:

$$F_{430}/F_{640} = 0.05817 c(\text{CBB}) + 1.37699, R^2 = 0.991 \quad (2)$$

The calibration sensitivity, S , was obtained from the derivative of the analytical response with respect to CBB concentration, c :

$$S = \frac{d(F_{430}/F_{640})}{dc} = 0.05817$$

3

Accordingly, the slope of the regression equation gave a calibration sensitivity of $0.05817 \mu\text{M}^{-1}$ ^[12].

The improved analytical performance of the dual-emission probe arose mainly from normalization of the CBB-responsive Au NC signal against the S-QD reference signal. The ratio measurement mapped the broad CBB concentration range of $0.1\text{--}100 \mu\text{M}$ onto a compact response window, helping to reduce signal saturation at high analyte concentrations and improve discrimination of small fluorescence changes at trace concentrations^[14].

The comparatively stable F_{430} reference signal also compensated for common fluctuations in excitation intensity and instrumental response, making small changes in the F_{640} response easier to distinguish and thereby improving the effective signal-to-noise ratio^[13]. Related S-QD-based dual-emission ratiometric strategies have been reported for the determination of iron(II) and hydrogen peroxide in food samples^[24].

The regression equation in Fig. 2d was rearranged to express CBB concentration as a function of the measured fluorescence ratio:

$$c(\text{CBB}) = \frac{(F_{430}/F_{640}) - 1.37699}{0.05817} \quad (4)$$

The standard deviation of the blank F_{430}/F_{640} ratio was $\sigma_R = 0.0002$, calculated from replicate measurements of samples containing no CBB.

The LOD was calculated using the $3\sigma_R/k$ criterion:

$$\text{LOD}=3\sigma_{\text{R}}/k = 0.0006/0.05817 \approx 0.01 \mu\text{M} \text{ (5)}$$

These calculations demonstrate the improvement in calibration sensitivity and LOD provided by the ratiometric probe.

At 20 μM CBB, the single-emission Au NC probe exhibited a fluorescence-quenching amplitude of 0.239 ($F/F_0 = 0.761$), corresponding to a calibration sensitivity of $0.00968 \mu\text{M}^{-1}$. By comparison, the ratiometric probe produced a response change of $\Delta(F_{430}/F_{640}) \approx 1.16$, from a baseline ratio of 1.38 to 2.54 at 20 μM CBB, as calculated from the regression equation. Its calibration sensitivity was $0.05817 \mu\text{M}^{-1}$, approximately six times that of the single-emission Au NC probe.

This improvement was attributed primarily to self-calibration by the 430 nm internal-reference signal, which reduced the influence of baseline noise. The dual-wavelength ratio also enhanced the visibility of small fluorescence changes at low CBB concentrations. Across the linear range of 0.1–100 μM , the calibration sensitivity was approximately sixfold higher than that of the single-emission Au NC probe.

Unlike the single-emission Au NC probe, the ratiometric system used two emission channels for internal correction, reducing susceptibility to common instrumental and environmental fluctuations. Together with its preferential response to CBB, this feature supports the reliability of the method in the tested complex matrices.

3.6.2 Selectivity of the Detection Method

The selectivity of the method was evaluated against common potential interferents. As shown in Fig. 3a, the responses to 10 and 20 μM CBB were compared with those produced by 20 μM D-fructose, ascorbic acid (AA), Zn^{2+} , Fe^{2+} , Ge^{4+} , K^{+} , Cd^{2+} , Cl^{-} , and Sudan I. These interferents caused only minor changes in F_{430}/F_{640} , whereas CBB produced a pronounced fluorescence response. The results indicate satisfactory selectivity for CBB under the tested conditions.

3.6.3 Fluorescence-Signal Stability of the Probe

As shown in Fig. 3b, under 360 nm ultraviolet irradiation, F_{430}/F_{640} remained approximately between 1.2 and 1.4 during the 0-120 minute measurement period, with an overall fluctuation of about 0.2 and no significant upward or downward trend. The near-zero overall slope indicates that the S-QDs/Au NCs probe maintained acceptable ratiometric signal stability throughout the 120 minute test.

3.7 Comparison with Other CBB Detection Methods

A representative HPLC-UV method reported for CBB determination used a C18 reversed-phase column (4.6 mm $\times$ 250 mm, 5 μm) and gradient elution with methanol and 0.1% aqueous formic acid at a flow rate of 1.0 mL/min. The column temperature was 30°C, the injection volume was 20 μL , and UV detection was performed at 595 nm, the characteristic absorption wavelength of CBB G-250. The reported method provided a linear range of 0.1–100 μM and an LOD of $0.01 \mu\text{M}$ ^[42, 43]. In the same work, the UV-Vis

spectrophotometric method for free CBB determination was also validated as a reference, providing a linear range of 1–50 μM and an LOD of approximately 0.5 μM under optimal conditions^[42]. The key performance parameters of various CBB testing methods are compared in Table 1.

Table 1
Comparison of representative methods for CBB determination.

Method	Linear range (μM)	Limit of detection (μM)	Ref
Spectrophotometry	1–50	0.5	[42]
HPLC-UV	0.1–100	0.01	[42]
Probe method	1-100	0.062	This work
This method	0.1–100	0.01	This work

3.8 Real-Sample Analysis

The practical applicability of the method was evaluated by spiked-recovery experiments using a BSA model matrix, commercial whey-protein powder, and tap water. As summarized in Table 2, recoveries for the BSA model matrix ranged from 98.5% to 102.6%, with RSD values below 2.8%. Recoveries for whey-protein powder ranged from 97.3% to 103.8%, with RSD values below 4.1%, while those for tap water ranged from 98.0% to 101.6%, with RSD values no greater than 2.6%.

HPLC-MS/MS was used as the reference method for CBB quantification in the real samples, following a general protocol for triphenylmethane dyes^[44]. Separation was performed on a C18 column (4.6 mm×250 mm, 5 μm) using gradient elution with methanol and 0.1% aqueous formic acid at a flow rate of 1.0 mL/min, a column temperature of 30°C, and an injection volume of 15 μL . Mass-spectrometric detection was conducted in positive electrospray-ionization multiple-reaction-monitoring (ESI + MRM) mode. The $[\text{M-Na}]^+$ parent ion of CBB G-250 was fragmented, and characteristic product ions were selected for qualitative and quantitative analysis after optimization of the collision energy, sheath-gas temperature, and capillary voltage.

The CBB concentrations determined with the probe agreed closely with the HPLC-MS/MS results for all three sample types. A t-test showed no significant difference between the two methods, supporting the accuracy and reliability of the proposed probe in the tested matrices^[45, 46].

Table 2
Determination of CBB in the BSA model matrix and real samples

Real Sample	Spiked Amount(μM)	Detected Amount(μM)	Recovery(%)	Relative Standard Deviation(%) (n = 3)	HPLC-MS/MS Detected Amount (μM)
BSA matrix sample	0.1	0.0985	98.5	2.1	0.0978
	0.5	0.5130	102.6	1.8	0.5090
	1.0	0.9970	99.7	2.5	0.9920
Commercially available whey protein powder	0.1	0.0973	97.3	3.2	0.0968
	0.5	0.5190	103.8	2.8	0.5150
	1.0	0.9890	98.9	4.1	0.9820
Tap water	0.1	0.0980	98.0	2.3	0.0970
	0.5	0.5080	101.6	2.0	0.5050
	1.0	0.9950	99.5	2.6	0.9900

4. Conclusion

A dual-emission ratiometric fluorescent probe composed of sulfur quantum dots (S-QDs) and BSA-templated gold nanoclusters (Au NCs) was developed for sensitive CBB determination. The response was based primarily on IFE: the CBB absorption band overlapped strongly with the Au NC emission spectrum but only minimally with the S-QD emission. Consequently, CBB preferentially attenuated the red Au NC fluorescence, while the blue S-QD emission served as an internal reference for ratiometric normalization. Under the optimized conditions, the F_{430}/F_{640} ratio exhibited a linear response to CBB ranging from 0.1 to 100 μM ($R^2 = 0.991$), with a calculated LOD of 0.01 μM . Compared with the single-emission Au NC probe, the ratiometric platform achieves an approximately sixfold enhancement in calibration sensitivity and a corresponding reduction in the limit of detection. The probe also showed satisfactory selectivity toward CBB in the presence of the tested metal ions, biomolecules, and food dyes.

The S-QDs/Au NCs probe demonstrated potential for CBB determination in food-related and biological matrices. Its broad linear range, low LOD, and resistance to the tested interferents supported rapid screening of trace CBB in the evaluated samples. Future work may integrate the IFE-based probe with fluorescent films, test strips, or microfluidic devices. Coupling such formats with smartphone-assisted image acquisition and data processing could enable portable, visual, and rapid quantitative analysis of dye contaminants. Further validation in a wider range of matrices will be required before routine applications in food-safety monitoring or biomedical analysis.

Declarations

Acknowledgements

We gratefully acknowledge the financial support provided by the Fundamental Research Funds of Weifang University of Science and Technology (KJRC2022012)

Author Contributions

X.Y.F. and Z.X.T. designed the research, synthesized S-QDs and Au NCs, and completed related fluorescence, UV-Vis and fluorescence lifetime characterization. W.W.X., H.R.X., L.Y.R. and Z.Y.F. prepared CBB gradient solutions, performed linearity, selectivity and stability tests, and analyzed the IFE sensing mechanism. L.H.J., J.C.Y. and H.J.X. carried out spike recovery experiments in real samples (a BSA model matrix, whey protein powder and tap water) and verified the results via HPLC-MS/MS comparison. Y.H. and F.Z.H. oversaw the whole project, guided data analysis and revised the manuscript. X.Y.F. drafted the manuscript and organized all figures and tables. All authors read and approved the final manuscript.

Data Availability

All data generated or analyzed during this study are included in this article. Additional data are available from the corresponding author upon reasonable request.

Ethical Approval

This study did not require ethical approval.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

References

1. Korzhan L, Kulichenko S, Lelyushok S, Klovak V (2024) Coomassie Brilliant Blue G for Smart Colorimetric Determination of the Ionic Surfactants in Triton X-100 Solutions. *Appl Spectrosc* 78(10):1105–1114
2. Zanini L, Zaltron A, Turato E, Zamboni R, Sada C (2022) Opto-Microfluidic Integration of the Bradford Protein Assay in Lithium Niobate Lab-on-a-Chip. *Sensors* 22(3):1144
3. Chen J, Luo H, Tao M, Liu Z, Wang G (2021) Quantitation of nucleoprotein complexes by UV absorbance and Bradford assay. *Biophys Rep* 7(6):429–436

4. Filgueiras MF, Borges EM (2022) Quick and Cheap Colorimetric Quantification of Proteins Using 96-Well-Plate Images. *J Chem Educ* 99(4):1778–1787
5. Löptien J, Vesting S, Dobler S, Mohammadi S (2024) Evaluating the efficacy of protein quantification methods on membrane proteins. *Open Biology* 14(12):240082
6. Fakhar N, Khan SA, Siddiqi WA, Khan TA (2022) Investigating the sequestration potential of a novel biopolymer-modified ceria/montmorillonite nanocomposite for chromium and coomassie brilliant blue from the aqueous phase: equilibrium and kinetic studies. *Environ Science: Adv* 1(4):558–569
7. Pariary R, Dolui S, Shome G, Mohid SA, Saha A, Ratha BN, Maiti NC (2023) Coomassie brilliant blue G-250 acts as a potential chemical chaperone to stabilize therapeutic insulin. *Chem Commun* 59(52):8095–8098
8. Al-Shehri AS, Zaheer Z, Alsudairi AM, Kosa SA (2021) Photo-oxidative Decolorization of Brilliant Blue with AgNPs as an Activator in the Presence of K₂S₂O₈ and NaBH₄. *ACS Omega* 6(41):27510–27526
9. Rashid MH, Karn R, Emerson IA, Khan JM, Malik A, Jain A, Sen P (2025) Interaction between Coomassie Brilliant Blue and hen egg white lysozyme: Insights from molecular docking, simulation, fluorescence quenching, and FTIR. *Int J Biol Macromol* 321(Pt 1):146140
10. Wang S, Ding Y, Zhang L, Cheng Y, Deng Y, Jiang Q, Ye J (2023) Combination of colorimetry, inner filter effect-induced fluorometry and smartphone-based digital image analysis: A versatile and reliable strategy for multi-mode visualization of food dyes. *J Hazard Mater* 445:130563
11. Wang S, Wang H, Yuan Z, Li M, Gao H, Shan L, Ye J (2022) Colorimetry Combined with Inner Filter Effect-Based Fluorometry: A Versatile and Robust Strategy for Multimode Visualization of Food Dyes. *ACS Appl Mater Interfaces* 14(51):57251–57264
12. Shi T, Zhang J, Gao F, Cai D, Zhang Y (2024) A sharp and selective fluorescence paper-based sensor based on inner filter effect for ratiometric detection of four Sudan dyes in food matrix. *Food Chem* 444:138528
13. Lin Y, Ye S, Tian J, Leng A-Q, Deng Y, Zhang J, Zheng C (2023) Paper-assisted ratiometric fluorescent sensors for on-site sensing of sulfide based on the target-induced inner filter effect. *J Hazard Mater* 459:132201
14. Yue X, Zhou Z, Li M, Jie M, Xu B, Bai Y (2022) Inner-filter effect induced fluorescent sensor based on fusiform Al-MOF nanosheets for sensitive and visual detection of nitrofurantoin in milk. *Food Chem* 367:130763
15. Nan H, Liu Y, Gong W, Peng H, Wang Y, Zhang Z, Cao X (2022) An inner-filter-effect based ratiometric fluorescent sensor for the detection of uranyl ions in real samples. *Anal Methods* 14(5):532–540
16. Kumar H, Obrai SR (2024) Ratiometric fluorescent sensing of melatonin based on inner filter effect and smartphone established detection. *Spectrochim Acta Part A Mol Biomol Spectrosc* 304:123309
17. Le T-H, Kim J-H, Park S-J (2022) Turn on fluorescence sensor of glutathione based on inner filter effect of co-doped carbon dot/gold nanoparticle composites. *Int J Mol Sci* 23(1):190

18. Kayani KF, Mohammed SJ, Mohammad NN, Abdullah AM, Tofiq DI, Mustafa MS, Aziz SB (2024) Sulfur quantum dots for fluorescence sensing in biological and pharmaceutical samples: a review. *Mater Adv* 5(16):6351–6367
19. Ruan H, Zhou L (2022) Synthesis of Fluorescent Sulfur Quantum Dots for Bioimaging and Biosensing. *Front Bioeng Biotechnol* 10:909727
20. Ning K, Sun Y, Liu J, Fu Y, Ye K, Liang J, Wu Y (2022) Research Update of Emergent Sulfur Quantum Dots in Synthesis and Sensing/Bioimaging Applications. *Molecules* 27(9):2822
21. Arshad F, Sk MP, Maurya SK, Siddique HR (2021) Mechanochemical Synthesis of Sulfur Quantum Dots for Cellular Imaging. *ACS Appl Nano Mater* 4(4):3339–3344
22. Lei J, Huang Z, Gao P, Sun J, Zhou L (2021) Polyvinyl Alcohol Enhanced Fluorescent Sulfur Quantum Dots for Highly Sensitive Detection of Fe^{3+} and Temperature in Cells. *Part Part Syst Charact* 38(4):2000332
23. Lu H, Zhang H, Li Y, Gan F (2021) Sensitive and selective determination of tetracycline in milk based on sulfur quantum dot probes. *RSC Adv* 11(37):22960–22968
24. Lu C, Wang Y, Xu B, Zhang W, Xie Y, Chen Y, Wang X (2022) A colorimetric and fluorescence dual-signal determination for iron(II) and H_2O_2 in food based on sulfur quantum dots. *Food Chem* 366:130613
25. Wang X, Guo W, Wang X, Hua Q, Tang F, Li X, Zhao L (2022) Dual recognition strategy for ultra-sensitive fluorescent detection of Hg^{2+} at femto-molar level based on aptamer functionalized sulfur quantum dots. *Arab J Chem* 15(9):104080
26. Wei Z, Lu W, Pan C, Ni J, Zhao H, Huang G, Wang C (2022) Manipulating time-dependent size distribution of sulfur quantum dots and their fluorescence sensing for ascorbic acid. *Dalton Trans* 51(26):10290–10297
27. Deng S, Long J, Dai X, Wang G, Zhou L (2023) Simultaneous detection and adsorptive removal of Cr(VI) ions by fluorescent sulfur quantum dots embedded in chitosan hydrogels. *ACS Appl Nano Mater* 6(3):1817–1827
28. Kumar JV, Tammina SK, Rhim J-W (2024) One-step hydrothermal synthesis of sulfur quantum dots for detection of Hg^{2+} ions and latent fingerprints. *Colloids Surf A* 690:133682
29. Li S, Fan W, Chen Q, Zhang X (2024) Facile Light-Driven Synthesis of Highly Luminous Sulfur Quantum Dots for Fluorescence Sensing and Cell Imaging. *ACS Appl Mater Interfaces* 16(31):41281–41292
30. Zhao L, Sha T, Liu Y, Mei Q, Li H, Sun P, Cai H (2024) Rapid and Large-Scale Synthesis of Chiral and Fluorescent Sulfur Quantum Dots for Intracellular Temperature Monitoring. *Chem Biomedical Imaging* 2(12):817–824
31. Kayani KF, Shatery OBA, Mohammed SJ, Aziz SB, Mohammad NN, Abdullah GH, Hassan J (2025) Fluorescent sulfur quantum dots for environmental monitoring. *Nanotechnol Reviews* 14(1):20240138

32. Li T, Li Z, Chen F, Zhu L, Tang H, Wang D, Tang Z (2024) Impact of BSA and Au³⁺ concentration on the formation and fluorescence properties of Au nanoclusters. *RSC Adv* 14(27):19284–19293
33. Yang L, Hou P, Wei J, Li B, Gao A, Yuan Z (2024) Recent Advances in Gold Nanocluster-Based Biosensing and Therapy: A Review. *Molecules* 29(7):1574
34. Niazi S, Khan IM, Akhtar W, ul Haq F, Pasha I, Khan MKI, Wang Z (2024) Aptamer functionalized gold nanoclusters as an emerging nanoprobe in biosensing, diagnostic, catalysis and bioimaging. *Talanta* 268:125270
35. Van de Looij SM, Hebelers ER, Viola M, Hembury M, Oliveira S, Vermonden T (2022) Gold Nanoclusters: Imaging, Therapy, and Theranostic Roles in Biomedical Applications. *Bioconjug Chem* 33(1):4–23
36. Zhong K, Hao C, Liu H, Yang H, Sun R (2021) Synthesis of dual-emissive ratiometric probe of BSA-Au NCs and BSA-Cu NCs and their sensitive and selective detection of copper and mercury ions. *J Photochem Photobiol A* 408:113100
37. Jain V, Bhagat S, Singh S (2021) Bovine serum albumin decorated gold nanoclusters: A fluorescence-based nanoprobe for detection of intracellular hydrogen peroxide. *Sens Actuators B* 327:128886
38. Al-Mashriqi HS, Cai M, Qi S, Zhai H (2023) BSA Capped Gold Nanoclusters Modulated by Copper Ion for Sensitive and Selective Detection of Histidine in Biological Fluid. *J Fluoresc* 33(2):697–706
39. Zhang Y, Tang C, Zhang M, Huang Z, Cai Z (2022) Gold Nanoclusters as a Fluorescent Probe for the Sensitive Determination of Morin and Sensing of Temperature. *ChemistrySelect* 7(43):e202203005
40. Zhuang H, Jiang X, Wu S, Wang S, Pang Y, Huang Y, Yan H (2022) A novel polypeptide-modified fluorescent gold nanoclusters for copper ion detection. *Sci Rep* 12(1):6624
41. Swain S, Lin T-Y, Chou I-H, Liu S-C, Mallick BC, Lin H-Y, Huang C-H (2024) Photoactive nanocatalysts as DTT-assisted BSA-AuNCs with enhanced oxidase-mimicking ability for sensitive fluorometric detection of antioxidants. *J Nanobiotechnol* 22(1):585
42. Chakraborty A, Jayaseelan K (2023) Environmentally sustainable analytical quality by design aided RP-HPLC method for the estimation of brilliant blue in commercial food samples employing a green-ultrasound-assisted extraction technique. *Green Process Synthesis* 12(1):20230178
43. Khan KA, Shah A, Nisar J (2024) Electrochemical detection and removal of brilliant blue dye via photocatalytic degradation and adsorption using phyto-synthesized nanoparticles. *RSC Adv* 14(4):2504–2517
44. Wu J (2023) Rapid and Sensitive Determination of Residues of Triphenylmethane Dyes and Their Metabolites in Animal Tissues by LC-MS/MS. *LCGC North Am* 41(5):186–193
45. Wang W, Yang Z, Xiao L, Guan T, Gong X, Hu Q (2023) Supersensitive detection of auramine O in food and drug samples by using carbon dots as sensing reagents. *J Food Meas Charact* 17(5):5360–5370
46. Salman BI (2024) An Innovative Selective Fluorescence Sensor for Quantification of Hazardous Food Colorant Allura Red in Beverages Using Nitrogen-Doped Carbon Quantum Dots. *J Fluoresc*

Scheme 1

Scheme 1 is available in the supplementary files section

Figures

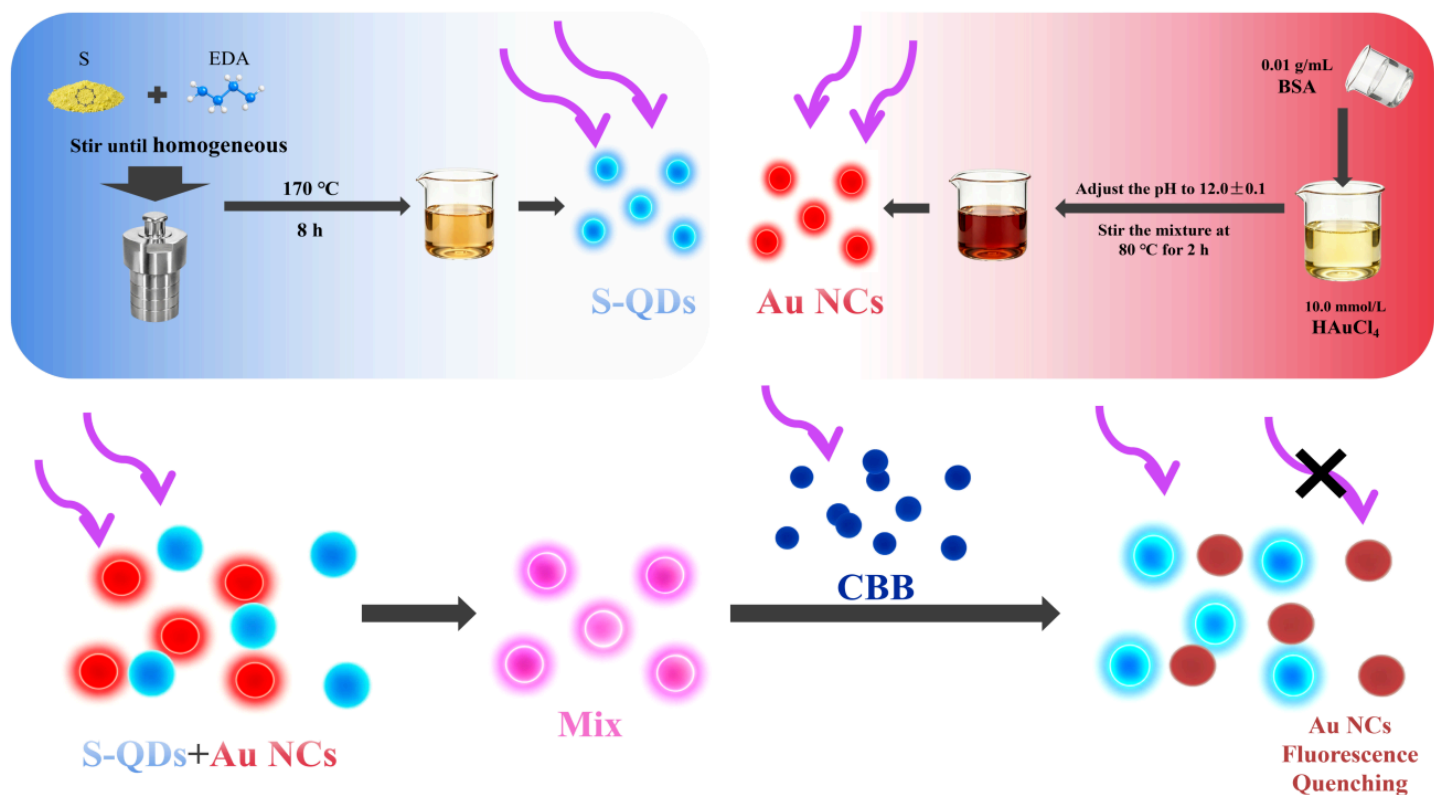


Figure 1

(a) TEM image and particle-size distribution of Au NCs. **(b)** Fluorescence spectra of the S-QDs, Au NCs, and S-QDs/Au NCs mixed system. **(c)** Spectral evidence for the inner filter effect in the S-QDs/Au NCs system. **(d)** Fluorescence-lifetime decay of the Au NC emission before and after CBB addition.

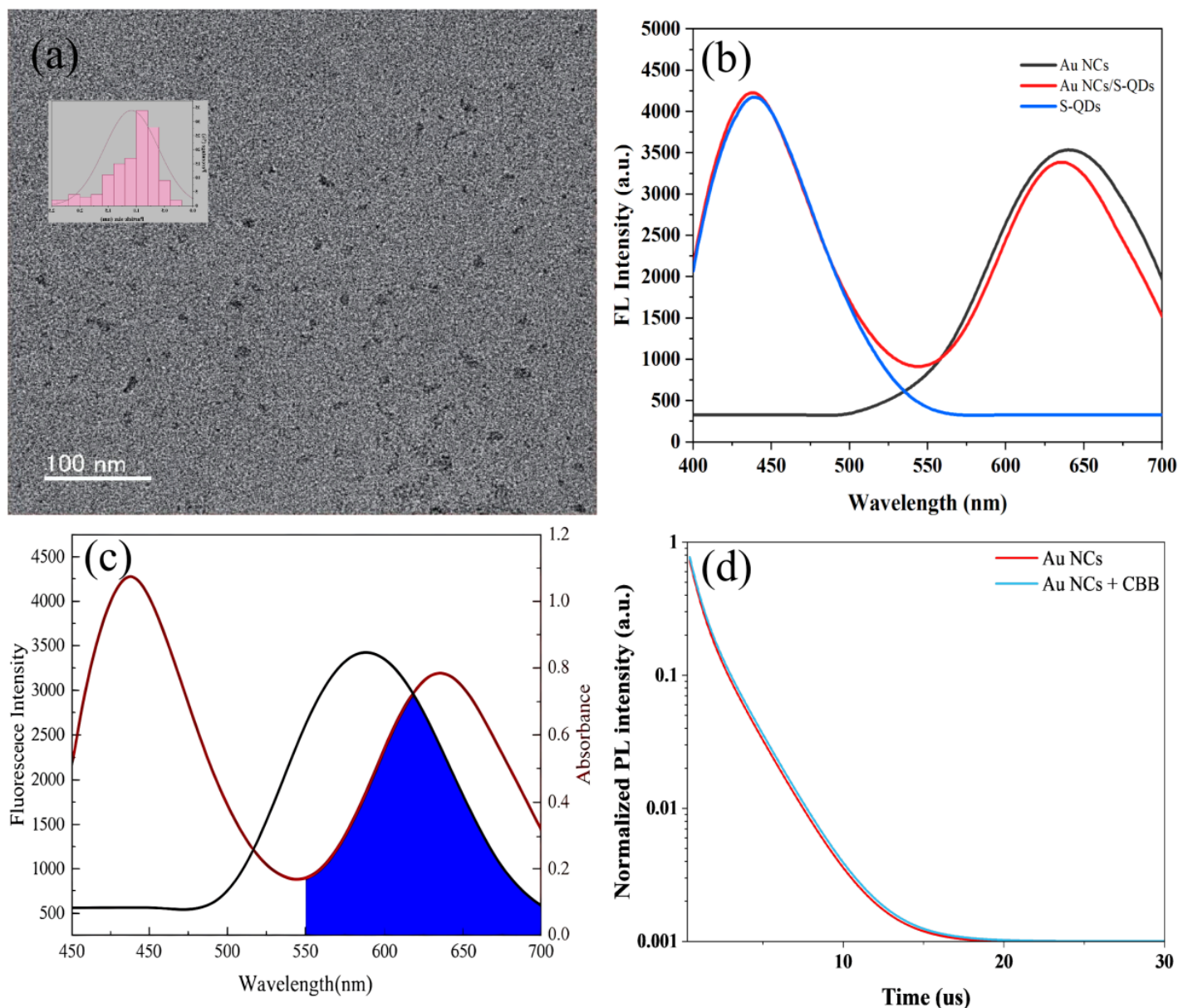


Figure 2

(a) TEM image and particle-size distribution of Au NCs. **(b)** Fluorescence spectra of the S-QDs, Au NCs, and S-QDs/Au NCs mixed system. **(c)** Spectral evidence for the inner filter effect in the S-QDs/Au NCs system. **(d)** Fluorescence-lifetime decay of the Au NC emission before and after CBB addition.

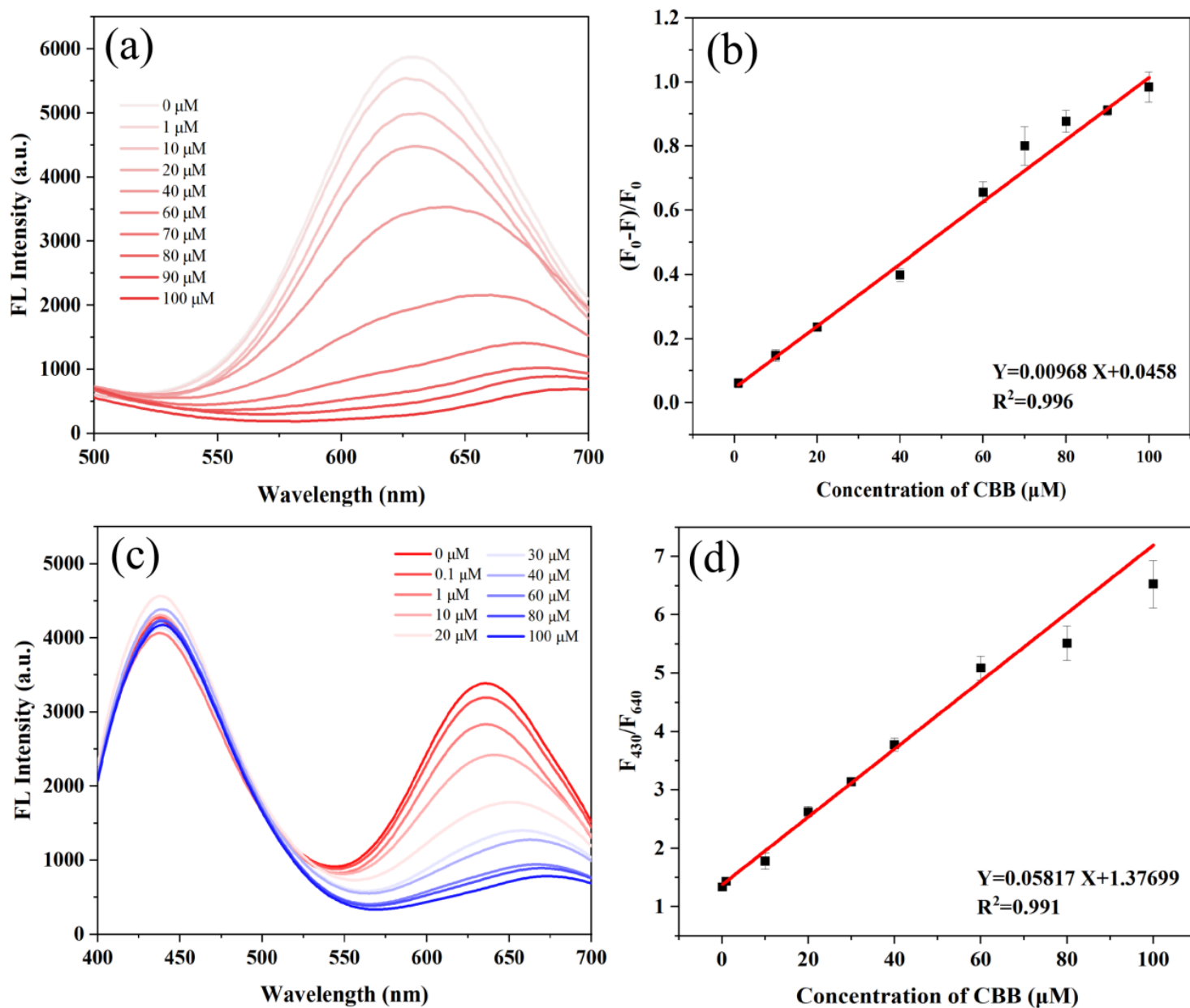


Figure 3

(a) Au NC fluorescence spectra at different CBB concentrations. (b) Calibration curve for Au NC fluorescence quenching. (c) S-QDs/Au NCs fluorescence spectra at different CBB concentrations. (d) Calibration curve of F_{430}/F_{640} versus CBB concentration.

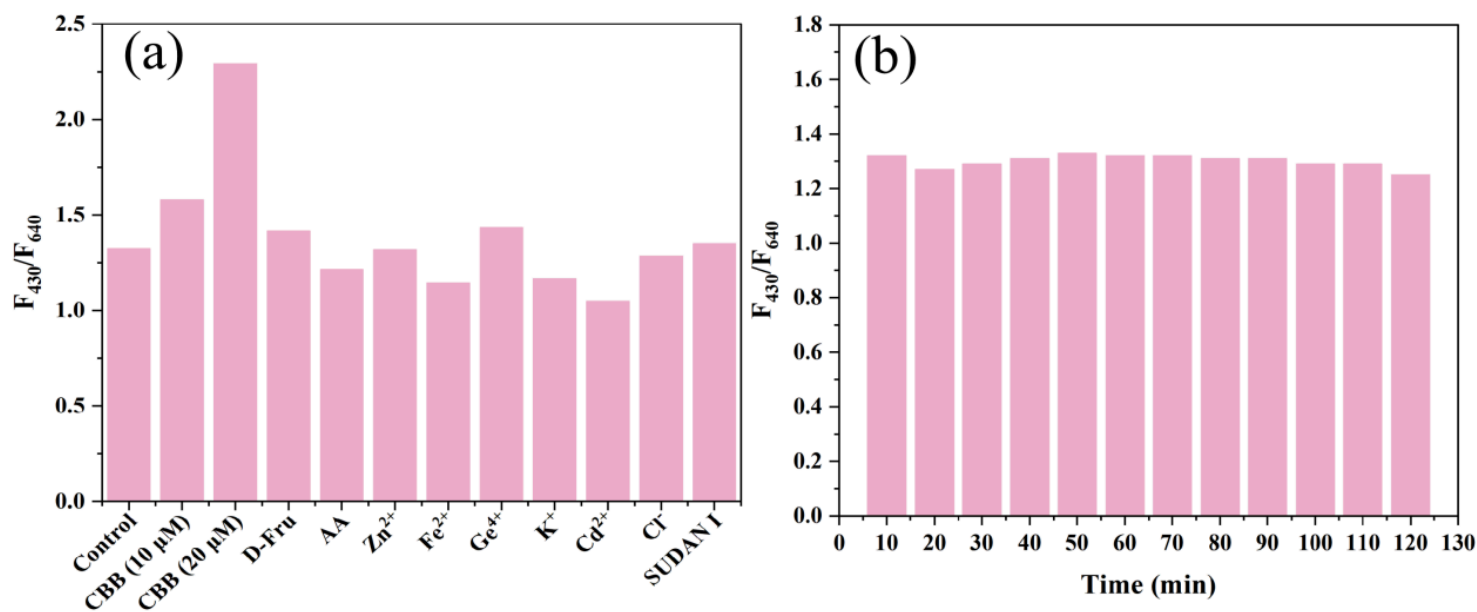


Figure 4

(a) Selectivity of the S-QDs/Au NCs probe toward CBB in the presence of potential interferents. **(b)** Stability of F_{430}/F_{640} signals under 360 nm UV light for 120 min.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Scheme1.png](#)