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**Bimodal optical nanosensor based on upconversion
fluorescence resonance energy transfer and gold
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colorimetric evaluation of ractopamine in pork**

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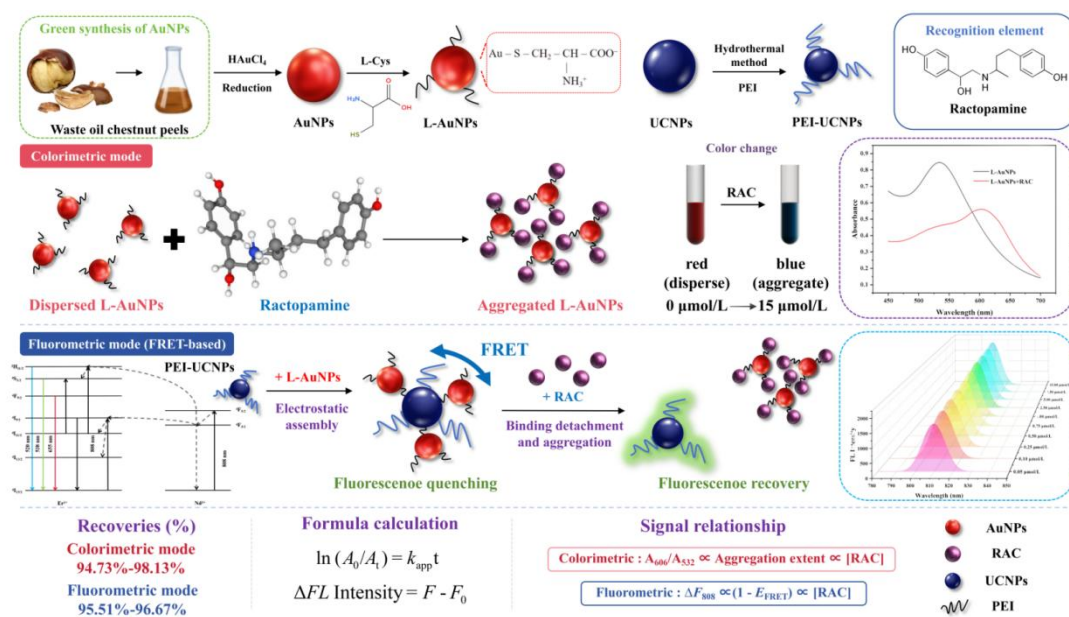
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Graphical Abstract

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Abstract

A dual-mode optical sensor was developed for ractopamine (RAC) detection based on Förster resonance energy transfer (FRET) between polyethyleneimine-functionalized upconversion nanoparticles (UCNPs) and L-cysteine-modified gold nanoparticles (AuNPs). UCNPs with excellent aqueous dispersibility were synthesized via a one-pot hydrothermal method. AuNPs were green-synthesized using waste oil chestnut peel extract and subsequently functionalized with L-cysteine. The absorption spectrum of the as-prepared AuNPs showed significant spectral overlap with the emission spectrum of UCNPs, enabling efficient quenching of UCNP upconversion fluorescence at 808 nm through FRET upon electrostatic assembly of the two nanomaterials. Upon introduction of RAC, strong hydrogen bonding interactions occurred between the amino/carboxyl groups of L-cysteine on the AuNP surface and the hydroxyl/amino groups of RAC molecules, which induced rapid AuNP aggregation. This aggregation disrupted the FRET process and restored UCNP fluorescence, while simultaneously causing a distinct visible color transition from red to blue. Under optimized experimental conditions, the colorimetric mode exhibited a linear detection range of 0.5-15 $\mu\text{mol/L}$ with a limit of detection (LOD) of 436.05 nmol/L, whereas the fluorometric mode achieved a wider linear range of 0.05-1.0 $\mu\text{mol/L}$ and a lower LOD of 50.73 nmol/L (S/N=3). The sensor exhibited high selectivity toward RAC over common interferents and structurally related β -agonists, and quantitative Förster analysis, control experiments, and molecular mechanics simulations provided converging support for the proposed FRET-modulated, hydrogen-bonding-mediated

62 recognition mechanism. The method was successfully applied to RAC detection in
63 spiked pork samples, yielding satisfactory recoveries of 94.73%-98.13% (colorimetric)
64 and 95.51%-96.67% (fluorometric) with 95% confidence intervals, and was
65 benchmarked against a reference High-Performance Liquid Chromatography (HPLC)
66 method. This dual-mode sensing strategy provides a promising platform for rapid,
67 sensitive, and reliable detection of RAC residues in complex food matrices.

68 **Keywords** Upconversion nanoparticles · Fluorescence resonance energy
69 transfer · Gold nanoparticles · Ractopamine · Food analysis

Introduction

Ractopamine (RAC) is a synthetic β -adrenergic agonist widely used for treating asthma, which are often illegally used as additives in livestock breeding to improve feed efficiency, reduce fat deposition, and increase lean meat yield, causing muscle trembling, panic, headache, and other distressing symptoms [1-3]. However, RAC has a relatively long half-life and tends to accumulate in edible tissues such as muscle and liver [4-6]. Consumption of RAC-contaminated food may induce adverse effects, including palpitations, tachycardia, vomiting, and muscle tremors, and in severe cases may even be life-threatening [7]. Moreover, conventional cooking processes are ineffective at removing RAC residues. For these reasons, many countries have strictly prohibited its use in animal husbandry [8]. Therefore, effective monitoring of RAC residues in animal-derived foods is essential for ensuring food safety [9].

A range of analytical methods has been developed for RAC determination. Chromatographic methods, such as high-performance liquid chromatography and liquid chromatography-tandem mass spectrometry, provide high accuracy and reliability [10-12]. Nevertheless, they require expensive instrumentation, complex sample preparation, and trained personnel, which limits their applicability in routine analysis. Immunoassays, including enzyme-linked immunosorbent assays, fluorescent immunoassays, and electrochemical immunoassays, offer high sensitivity and selectivity [13-15]. However, antibody production remains time-consuming and costly, and assay stability can be affected by biological variability. Electrochemical methods enable rapid detection with good sensitivity, yet their performance often depends on

elaborate electrode modification procedures [16,17]. These limitations highlight the need for a simpler and more efficient strategy for RAC detection.

Fluorescence-based sensing has attracted considerable interest due to its simplicity and high sensitivity [18-20]. The performance of such systems is closely related to the properties of the fluorescent materials used. Conventional organic dyes often suffer from poor photostability and sensitivity to environmental factors such as pH and temperature, which restricts their practical application. In contrast, rare-earth-doped UCNPs exhibit unique optical properties, including anti-Stokes emission, high photostability, low toxicity, and minimal background interference, making them promising alternatives for analytical applications [21-24]. At the same time, gold nanoparticles (AuNPs) possess strong surface plasmon resonance (SPR) and high extinction coefficients, enabling efficient fluorescence quenching and sensitive colorimetric response [25-28]. The combination of UCNPs and AuNPs provides an effective platform for constructing FRET-based sensors [29].

In this paper, a dual-mode optical sensor for RAC detection was developed based on FRET between UCNPs and gold nanoparticles (AuNPs). Polyethyleneimine-functionalized UCNPs with excellent aqueous dispersibility were synthesized via a one-pot hydrothermal method. AuNPs were prepared through a green synthetic route using oil chestnut (*Castanea mollissima*) peel extract and subsequently conjugated with L-cysteine. The thiol moiety of L-cysteine covalently anchors to the AuNP surface via Au-S bonds, while the exposed amino and carboxyl groups interact with RAC molecules through hydrogen bonding. In the absence of RAC, electrostatic attractions

bring UCNPs and AuNPs into close spatial proximity, resulting in efficient fluorescence quenching via FRET. Upon RAC addition, AuNP aggregation is induced, which abrogates the FRET process and restores the upconversion fluorescence emission. Concurrently, this aggregation induces a distinct visible color change in the solution, enabling label-free colorimetric detection.

Based on this mechanism, a dual-mode colorimetric/fluorescence sensing platform was constructed for RAC quantification. This system integrates the merits of naked-eye visual detection and highly sensitive fluorescence analysis, conferring enhanced reliability in complex sample matrices. Compared with conventional single-signal sensing strategies, the dual-mode approach enables cross-validation of analytical signals, which effectively mitigates false-positive and false-negative outcomes and improves overall detection accuracy. Furthermore, the incorporation of green-synthesized AuNPs not only eliminates the need for toxic chemical reducing agents but also enhances the biocompatibility and environmental sustainability of the sensing system. The as-prepared sensor exhibits excellent sensitivity, high selectivity, and rapid response toward RAC, achieving simultaneous naked-eye visual recognition and fluorescence signal recovery. Notably, the proposed sensing platform exhibits satisfactory analytical performance in spiked real meat samples, highlighting its great potential for practical food safety applications. Owing to its facile preparation, low cost, and superior detection performance, this dual-mode strategy provides a promising platform for rapid on-site monitoring of β -adrenergic agonist residues and can be readily extended to the detection of other hazardous contaminants in complex biological

and food matrices.

Experimental

Materials

Oil chestnuts are sourced from the Hema Fresh in Zhenjiang, China. Chloroauric acid (HAuCl_4), L-cysteine and 4-Nitrophenol (4-NP) were purchased from China National Pharmaceutical Group Chemical Reagent Co. $\text{YCl}_3 \cdot 6\text{H}_2\text{O}$ (99.99%), $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$ (99.99%), $\text{NdCl}_3 \cdot 6\text{H}_2\text{O}$ (99.9%), $\text{ErCl}_3 \cdot 6\text{H}_2\text{O}$ (99.99%), sodium chloride (NaCl), polyethyleneimine (PEI), NH_4F (99.99%) and ethylene glycol were obtained from Aladdin Chemical Co. The standard solution of clenbuterol hydrochloride was purchased from Yuanye Reagent Co. All the reagents were of analytical grade and required no further purification. In addition, the water used in this experiment was all deionized water.

Apparatus

UV-vis spectra were recorded on a UV-2550 spectrophotometer (Beijing Shinetek Instruments Co., Ltd., China). The fluorescence spectra were collected using the F97 Pro fluorescence spectrophotometer (Shanghai Lingguang Technology Co., Ltd., China). The transmission electron microscopy (TEM) images were performed on an HT-7700 TEM (JEOL, Japan). The fluorescence spectrophotometer was operated with excitation and emission slit widths of 10 nm, a photomultiplier voltage of 600 V, and a scanning speed of 1200 nm/min, using an external 808 nm continuous-wave laser (2 W) as the excitation source; UV-Vis spectra were collected at 1.0 nm resolution at a medium

scan rate. Unless otherwise stated, all experiments were performed in triplicate ($n = 3$ independent replicates), and all measurements were carried out using the same independently synthesized batch of UCNPs and L-AuNPs; the batch-to-batch reproducibility of both nanomaterials was routinely verified by TEM, UV-Vis, and fluorescence measurements ($RSD < 5\%$).

Synthesis of AuNPs and catalytic performance

Fresh, intact oil chestnuts (*Castanea mollissima*) were collected for this study. The outer peels were manually removed, and the peel tissues were pulverized using a mechanical crusher. Ten grams of oil chestnut peel powder was suspended in 70 mL of deionized water and extracted overnight at room temperature. One milliliter of the resulting peel extract was transferred to a conical flask, and 10 mL of pre-chilled (4°C) 0.367 mmol/L HAuCl_4 solution was added dropwise under continuous gentle magnetic stirring. As the solution color gradually changed from pale yellow to burgundy, the stirring rate was reduced accordingly. Once the system reached a uniform burgundy color, the reaction mixture was immediately incubated at 4°C for 10 min to quench the nanoparticle formation. The resulting suspension was centrifuged at 4000 rpm for 10 min, the supernatant was discarded, and the precipitate was resuspended in 10 mL of deionized water to obtain the green-synthesized AuNP colloid.

The catalytic activity of the green-synthesized AuNPs was assessed using the reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) as a model reaction. Briefly, 25 mL of 0.25 mmol/L 4-NP solution was combined with 1 mL of freshly prepared 0.25 mol/L sodium borohydride (NaBH_4) solution, followed by the addition of 3 mL of the

as-prepared AuNP colloid as the catalyst. At predetermined time intervals, a 0.5 mL aliquot was withdrawn from the reaction system, transferred to a quartz cuvette, and diluted with 3 mL of deionized water. The mixture was thoroughly mixed, and UV-Vis absorption spectra were recorded in the wavelength range of 280-400 nm to monitor the evolution of the characteristic absorption peaks at 295 nm and 400 nm. The apparent reaction rate constant of AuNP-catalyzed 4-NP reduction was calculated according to the method reported by Huang [30].

$$\ln(A_0/A_t) = k_{app} t \quad (1)$$

A_0 : the initial absorbance value of 4-NP.

A_t : absorbance values of 4-NP at different catalytic times.

k_{app} : catalytic reaction rate.

t : catalytic time.

Preparation of water-soluble upconversion nanomaterials

The synthesis of NaYF₄:Yb,Er,Nd@NaYF₄:Nd core-shell UCNPs was performed via a modified hydrothermal method, following protocols reported previously [31,32]. Briefly, 1.5 g of polyethyleneimine (PEI) was dissolved in a mixed solvent of deionized water and ethylene glycol (EG) under vigorous stirring. Subsequently, 0.8797 g of YCl₃·6H₂O, 0.3875 g of YbCl₃·6H₂O, 0.0082 g of ErCl₃·6H₂O and 0.3586 g of NdCl₃·6H₂O were accurately weighed and dissolved into the above solution to form solution A. Separately, 0.6818 g of NaCl and 1.7285 g of NH₄F were accurately weighed and dissolved in the same mixed solvent to prepare solution B. After solutions A and B were each stirred at a constant speed of 500 rpm for 40 min, and solution B

was added dropwise to solution A, and the resulting mixture was stirred for an additional 20 min. The homogeneous mixture should be transferred to a teflon-lined stainless-steel autoclave and heated at a specific temperature 200°C for 12 h to complete the hydrothermal reaction. After the reaction system was naturally cooled to room temperature, the as-synthesized product was collected by centrifugation. The white precipitate was washed three times with alternating anhydrous ethanol and deionized water, and then dried under vacuum to obtain the final core-shell UCNPs.

Colorimetric detection of RAC

Different volumes of 0.3 mmol/L RAC standard solution were added to 1 mL of L-cysteine-functionalized gold nanoparticle (L-AuNP) solution to prepare a series of RAC solutions with final concentrations of 0.2, 0.5, 1.0, 1.6, 2.0, 3.0, 4.0, 6.0, 8.0, 10, and 15 $\mu\text{mol/L}$. The mixtures were thoroughly vortexed and incubated at room temperature for 12 min. Digital images of the solution color changes were captured using a smartphone camera. UV-Vis absorption spectra were recorded in the wavelength range of 400-700 nm using a UV-visible spectrophotometer. The absorbance ratio at 606 nm to 532 nm (A_{606}/A_{532}) was employed as the quantitative analytical signal. To establish the optimal detection system, single-factor experiments were conducted to systematically optimize the key reaction parameters of the colorimetric sensing system. Under the optimized conditions, the linear relationship between A_{606}/A_{532} and RAC concentration was investigated. Furthermore, the selectivity of the proposed sensing platform toward RAC was evaluated by testing the response to various potential interfering compounds at concentrations significantly higher than that of RAC.

Fluorescence detection of RAC

Briefly, 2.5 mL of 0.5 mg/mL polyethyleneimine-functionalized upconversion nanoparticle (UCNP) nanoprobe and 150 μ L of L-cysteine-modified gold nanoparticle (L-AuNP) nanoprobe solution were thoroughly vortexed and incubated vertically at room temperature for 30 min. Subsequently, the fluorescence intensity at the characteristic emission wavelength (λ_{ex} =808 nm) was recorded as the initial fluorescence signal (F_0). Aliquots of RAC standard solutions with varying final concentrations were added to the above mixture, and the corresponding fluorescence spectra were recorded after incubation for 15 min at room temperature, with the fluorescence intensity denoted as F . To establish the optimal fluorescence detection system, single-factor experiments were systematically conducted to optimize the key reaction parameters of the proposed sensing platform.

$$\Delta FL \text{ Intensity} = F - F_0 \quad (2)$$

F_0 : fluorescence intensity at 808 nm without the addition of RAC.

F : fluorescence intensity at 808 nm in the presence of added RAC.

Real samples pretreatment and determination

To further assess the accuracy and practical feasibility of the constructed colorimetric/fluorescence dual-mode sensor, pork loin, liver, and heart samples purchased from local supermarkets were selected as real food matrices for evaluation. Briefly, 1.0 g of each homogenized tissue sample was weighed and mixed with 50 mL of 0.01 mol/L phosphate-buffered saline (PBS, pH 7.4), followed by homogenization for 5 min using a high-speed disperser. The homogenates were spiked with RAC hydrochloride standard solutions at three concentration levels (0.5, 1.0, and 5.0 μ mol/L)

and then subjected to ultrasonic extraction for 15 min at room temperature. The resulting mixtures were centrifuged at 5000 rpm and 4°C for 5 min. The supernatants were collected, and their pH values were adjusted to 7.4 using 2 mol/L NaOH solution. The final sample solutions were allowed to stand for 5 min and stored at 4°C until analysis.

Molecular mechanics simulation of RAC recognition

Molecular mechanics simulations were performed to investigate the intermolecular interaction between RAC and L-cysteine under neutral pH conditions, for which the dominant protonation states (zwitterionic L-cysteine bearing ammonium and carboxylate groups, and the protonated secondary ammonium form of RAC) were adopted.

The initial three-dimensional conformations of ractopamine (RAC, PubChem CID: 3034034) and L-cysteine (L-Cys, PubChem CID: 5862) were generated by RDKit and optimized with the MMFF94 force field. Based on preliminary FTIR characterization, a 1:2 bidentate cross-linked RAC-(L-Cys)₂ model containing 36 heavy atoms was constructed. One RAC molecule bridges two L-Cys molecules: the secondary amine of RAC forms a bidentate salt bridge with the carboxylate of L-Cys(1) (COO⁻···H-N, 3.06/3.01 Å); the phenolic hydroxyl of RAC forms an intermolecular hydrogen bond with L-Cys(1) (O-H···O, 3.02 Å); the distal phenolic hydroxyl of RAC cross-links the carboxyl oxygen of L-Cys(2) (O···O, 2.95 Å). No direct contact exists between the two L-Cys molecules.

Protonation states were set at physiological pH 7.4: L-Cys exists as $\text{COO}^-/\text{NH}_3^+$ zwitterion, and the secondary amine of RAC is protonated to R_2NH_2^+ . Conformational search was performed via ETKDGv3 with an RMSD pruning threshold of 0.5 Å. A total of 200 initial conformers for L-Cys and 300 for RAC were preoptimized by MMFF94 and sorted by energy. The full complex underwent three-stage cascaded MMFF94 geometry optimization (500+1000+2000 steps) with a convergence threshold of maximum gradient $< 0.001 \text{ kcal mol}^{-1} \text{ Å}^{-1}$.

A distance-dependent dielectric model $\epsilon(r) = 4r$ was used to simulate aqueous electrostatic screening. Atomic partial charges were calculated by the Gasteiger PEOE method. The 4r-screened electrostatic interaction energy was calculated by Coulomb's law: $E_{\text{elec}} = \sum 332.0637 \cdot q_i q_j / 4r_{ij}^2$, covering all N, O, S heteroatom pairs.

Interaction energies were calculated at the M06-2X/6-311++G(d,p) level with BSSE correction using Gaussian 16. The interaction energy of the 1:1 single complex $\Delta E_{1:1} = -20.5 \text{ kcal/mol}$, and the total interaction energy of the bidentate 1:2 complex $\Delta E_{1:2} = -30.1 \text{ kcal/mol}$. QTAIM and IGM analyses were conducted to analyze the topology of noncovalent interactions.

FTIR spectra (Nicolet iS50, 4000-400 cm^{-1} , KBr pellet) were measured to track shifts of characteristic peaks: carboxylate asymmetric stretch $\nu_{\text{as}} \text{COO}^-$ ($\sim 1560 \text{ cm}^{-1}$), phenolic hydroxyl stretch $\nu \text{O-H}$ ($\sim 3400 \text{ cm}^{-1}$), and secondary amine stretch $\nu \text{N-H}$ ($\sim 3300 \text{ cm}^{-1}$). The experimental spectra agree with the computational model, confirming the proposed bidentate cross-linking mode. All calculations were implemented with Python 3.12, RDKit 2024 and OpenBabel 3.1.1.

Statistical analysis

All experiments were performed with at least three independent replicates ($n = 3$), and the results are expressed as the mean $\pm$ standard deviation (SD). Calibration curves were fitted by least-squares linear regression. The 95% confidence intervals (CI) of the mean recovery values were calculated using Student's t -distribution ($t_{0.025,2} = 4.303$). Differences between the proposed method and the reference HPLC method were evaluated by a paired two-tailed t -test, and $p < 0.05$ was considered statistically significant.

Results and Discussion

Characterization and catalytic performance of AuNPs

TEM images revealed that the as-synthesized AuNPs were predominantly spherical with excellent colloidal dispersion (Fig. 1a). Statistical analysis of over 100 randomly selected particles from multiple TEM micrographs yielded a mean diameter of 8.5 ± 0.6 nm, indicating a relatively narrow size distribution. This narrow size distribution indicates that phytochemicals present in the oil chestnut peel extract effectively regulated the nucleation and growth kinetics during AuNP synthesis.

The successful formation of AuNPs was further verified by UV-Vis absorption spectroscopy (Fig. 1b). The as-prepared AuNP suspension exhibited a characteristic SPR peak at approximately 532 nm, which is typical of well-dispersed spherical gold nanoparticles. Following L-cysteine functionalization, the SPR peak position remained unchanged and the solution retained its characteristic red color, indicating that particle size and colloidal dispersion were preserved. A slight reduction in absorbance intensity

was observed, which can be attributed to changes in the local dielectric environment induced by ligand exchange rather than particle aggregation. These results collectively indicate that surface modification does not compromise the intrinsic optical properties of AuNPs.

Zeta potential measurements provided further verification of successful surface functionalization. The surface charge of AuNPs shifted from -7.5 mV to -11.8 mV after L-cysteine conjugation (Fig. 1c), reflecting the introduction of additional negatively charged carboxyl groups. This increase in negative surface charge enhances colloidal stability through electrostatic repulsion and facilitates subsequent electrostatic interactions with positively charged polyethyleneimine-functionalized UCNPs.

Preliminary HPLC-MS analysis identified multiple phenolic and flavonoid components in the oil chestnut peel extract, supporting its dual role as both a reducing agent and a capping agent. Fourier-transform infrared (FTIR) spectroscopy provided additional evidence for nanoparticle formation and surface modification (Fig. 1d). Concurrently, preliminary X-ray photoelectron spectroscopy (XPS) analysis provided direct spectroscopic evidence for the formation of covalent Au–S bonds between L-cysteine and the AuNP surface. The crude peel extract exhibited characteristic absorption peaks at 3225 cm^{-1} (O–H stretching), 1608 cm^{-1} (C=C aromatic stretching), 1440 cm^{-1} (C=O stretching), and 1058 cm^{-1} (C–H bending), consistent with the presence of phenolic and flavonoid compounds. These functional groups are responsible for reducing Au^{3+} ions and stabilizing the resulting nanoparticles. After L-cysteine modification, new absorption peaks appeared at 3413 cm^{-1} and 1720 cm^{-1} ,

corresponding to N–H stretching and C=O stretching vibrations, respectively. The decreased intensity of the O–H peak suggests partial replacement of the original phytochemical capping layer by L-cysteine molecules. These spectral changes support successful ligand conjugation and provide the necessary functional groups for specific RAC recognition.

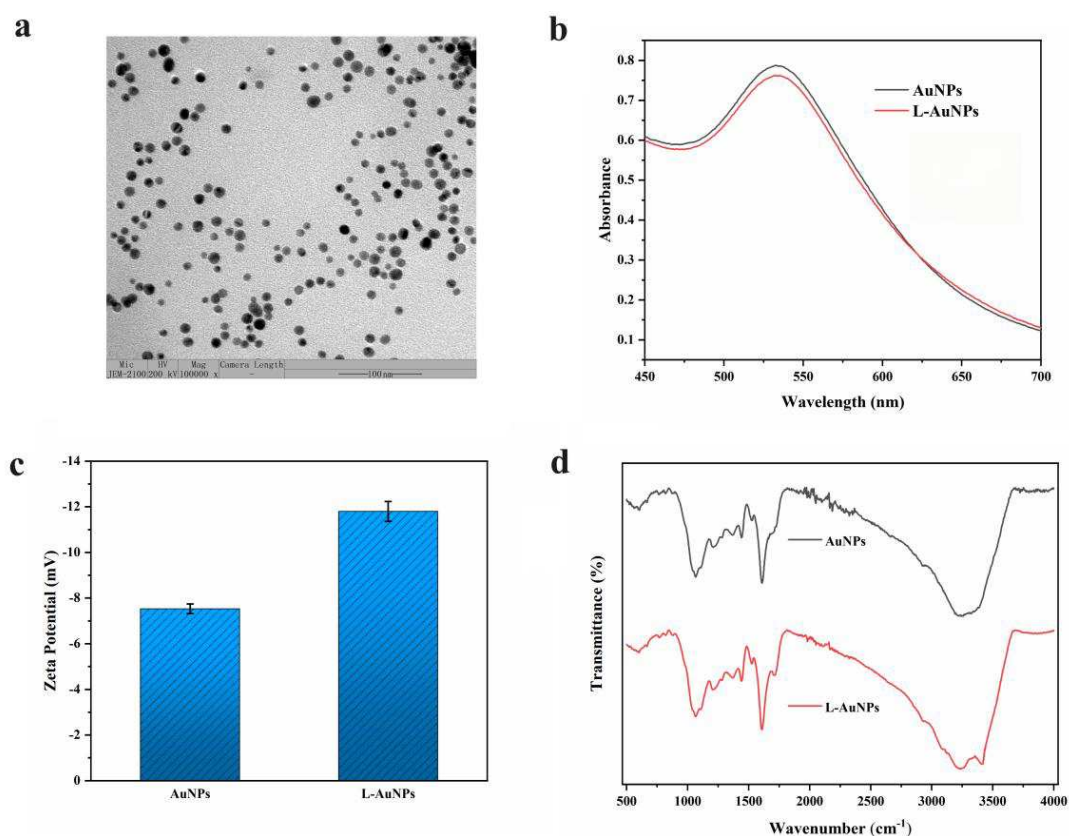


Figure 1 TEM mapping of AuNPs (a), UV absorption profiles of AuNPs before and after cysteine modification, insets are the corresponding photos of the corresponding (b), zeta potential change of AuNPs before and after cysteine modification (c), and FTIR of AuNPs before and after cysteine modification (d).

The catalytic activity of the green-synthesized AuNPs was assessed using the model reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) in the presence of sodium borohydride (NaBH₄). This thermodynamically favorable reaction is kinetically hindered without a catalyst and thus serves as a standard model for evaluating

nanoparticle catalysis. Fig. 2a displays the time-resolved UV-Vis absorption spectra of the reaction system following AuNP addition. Prior to catalysis, the solution exhibited a strong absorption peak at 400 nm, corresponding to the formation of 4-nitrophenolate ions under alkaline conditions. After introduction of the as-prepared AuNPs, the intensity of this peak decreased gradually with reaction time, while a new absorption band emerged at 295 nm. This band is characteristic of 4-AP, consistent with the successful reduction of 4-NP. The spectral evolution proceeded smoothly with a well-defined isosbestic point, indicating a direct stoichiometric conversion without accumulation of intermediates. Within the monitored time window, the reaction was nearly complete, reflecting the high catalytic efficiency of the synthesized AuNPs.

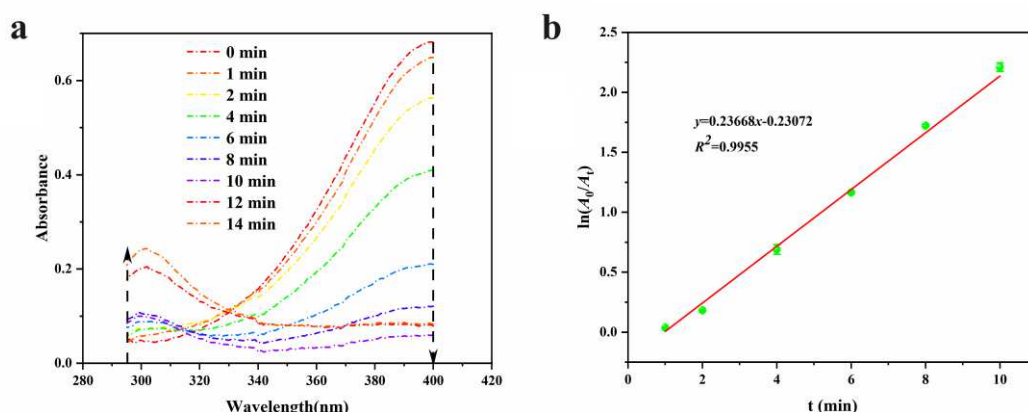


Figure 2 Catalytic activity verification ultraviolet absorption spectrum (a) and catalytic activity verification working curve (b).

The superior catalytic performance can be rationalized based on a surface-mediated electron transfer mechanism. According to the Langmuir-Hinshelwood model, both 4-NP and BH_4^- ions adsorb onto the AuNP surface, where electron transfer occurs to facilitate the reduction process. The small average particle size (8.5 ± 0.6 nm) provides a large specific surface area and a high density of low-coordination surface atoms,

which serve as active sites for substrate adsorption and catalytic conversion. Furthermore, residual phytochemicals derived from the oil chestnut peel extract may contribute to the enhanced catalytic activity by promoting interfacial electron transfer or stabilizing reactive intermediates. These synergistic effects enable efficient catalytic conversion under ambient conditions.

Fig. 2b presents the kinetic analysis of the 4-NP reduction reaction. Since NaBH_4 was present in large excess (approximately 1000-fold molar excess relative to 4-NP), the reaction followed pseudo-first-order kinetics with respect to 4-NP concentration. A linear relationship between $\ln(A_0/A_t)$ and reaction time was obtained, yielding the regression equation $y=0.23668x-0.23072$ with a correlation coefficient of $R^2=0.9955$. The excellent linearity indicates that the reaction proceeded uniformly without an induction period or significant catalyst deactivation within the observed time frame. The apparent pseudo-first-order rate constant was determined to be 0.237 min^{-1} , which is comparable to or higher than values reported for AuNPs prepared via other green synthesis routes. The relatively high rate constant arises from the combined effects of the nanoscale dimensions, which ensure efficient substrate adsorption and rapid interfacial electron transfer, and the excellent colloidal stability, which preserves the accessible active surface throughout the reaction. BH_4^-

Overall, these results show that the oil chestnut peel-derived AuNPs possess both high catalytic efficiency and excellent colloidal stability. This dual functionality not only supports their suitability for general catalytic applications but also supports their potential role in signal amplification within the proposed colorimetric/fluorescence

dual-mode sensing platform.

Optical properties and upconversion luminescence mechanism of UCNPs

Fig. 3a displays the TEM image of the as-synthesized UCNPs. The nanoparticles exhibited a well-defined rod-like morphology with a relatively narrow size distribution, indicating precise control over crystal growth under hydrothermal conditions. The particles had mean dimensions of 80-120 nm in length and 30-50 nm in width, which is consistent with the anisotropic growth behavior of hexagonal-phase NaYF₄ nanocrystals along the crystallographic c-axis [33,34]. This anisotropic growth is commonly observed in NaYF₄ systems prepared in aqueous ethylene glycol (H₂O/EG) mixed solvents. The use of a mixed solvent system modulates precursor diffusion kinetics and coordination chemistry, thereby promoting controlled nucleation and growth and contributing to improved crystallinity and optical performance [35-37]. High-magnification TEM analysis revealed a distinct core-shell contrast with a darker inner core and a lighter outer layer, indicating the formation of an in-situ grown polyethyleneimine (PEI) shell during synthesis. PEI molecules coordinate with surface lanthanide ions through amine groups, resulting in stable surface binding while simultaneously imparting hydrophilicity to the nanoparticles. This PEI coating serves multiple critical functional roles: firstly, it enhances aqueous dispersibility and prevents colloidal aggregation in aqueous media; secondly, it introduces positively charged surface moieties that facilitate electrostatic assembly with negatively charged L-cysteine-modified AuNPs in the subsequent FRET system. No large aggregates or irregular particles were observed, indicating that the synthesis protocol produces

monodisperse UCNPs with excellent morphological reproducibility. These structural features, together with the characterization results presented below, support the suitability of PEI-functionalized UCNPs as stable fluorescence donors.

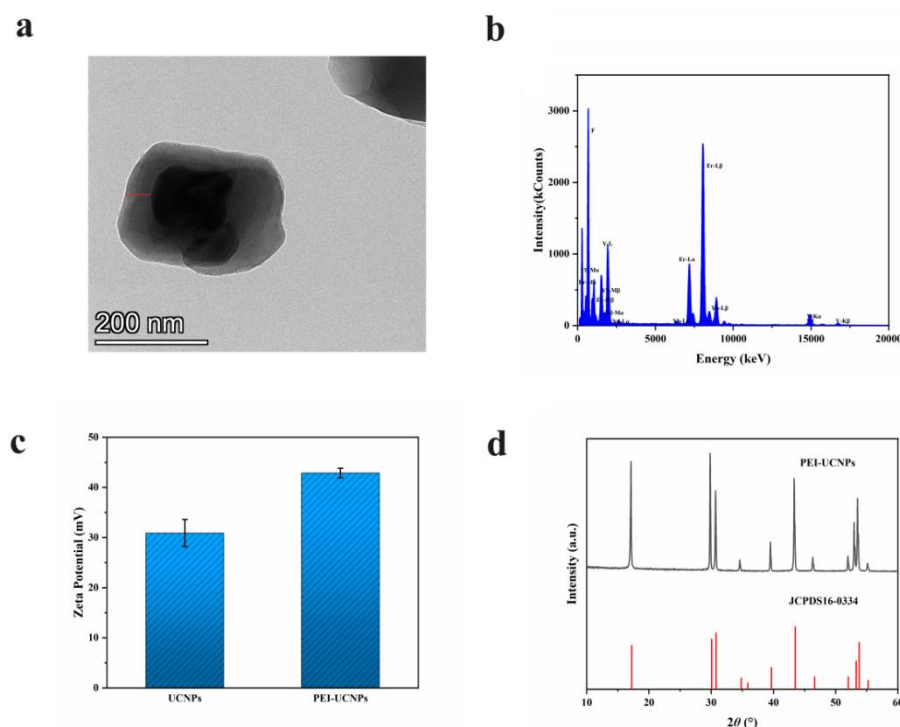


Figure 3 TEM mapping of UCNPs (a), EDS mapping of UCNPs (b), zeta potential changes of UCNPs before and after PEI modification (c), and XRD mapping of UCNPs (d).

Fig. 3b presents the energy-dispersive X-ray spectroscopy (EDS) spectrum of the UCNPs. The spectrum showed distinct characteristic peaks corresponding to yttrium (Y), ytterbium (Yb), erbium (Er), and neodymium (Nd), indicating the successful incorporation of these lanthanide elements into the NaYF₄ host lattice. Yttrium served as the primary host component, while Yb and Nd acted as sensitizer ions enabling efficient near-infrared (NIR) excitation and energy transfer. The presence of Er, albeit with lower peak intensity due to its lower doping concentration, supports its role as the

activator responsible for upconversion emission. No impurity peaks from extraneous elements were detected, indicating high chemical purity of the synthesized material. The relative elemental composition was consistent with the initial precursor ratios, suggesting that the hydrothermal method enables effective and controlled doping of lanthanide ions into the host lattice [38-40].

Fig. 3c shows the zeta potential measurements of UCNPs before and after PEI functionalization. Bare UCNPs exhibited a zeta potential of +30.9 mV, which is attributed to surface-exposed lanthanide ions. Following PEI modification, the zeta potential increased significantly to +42.8 mV, indicating a substantial enhancement in surface positive charge. This increase arises from the protonated primary and secondary amine groups present in the grafted PEI chains. Under neutral or slightly basic conditions, these amine groups carry positive charges, resulting in a high surface charge density. This change provides direct evidence for the successful coating of PEI on the UCNP surface.

The elevated surface charge has three important implications [41]. Firstly, it improves colloidal stability by generating strong electrostatic repulsion between adjacent particles, thereby preventing irreversible aggregation. Secondly, it promotes efficient electrostatic assembly with negatively charged L-AuNPs, which is a prerequisite for establishing effective donor-acceptor interactions in the FRET system. Thirdly, the hydrophilic nature of PEI enhances dispersion in aqueous environments, which is critical for practical sensing applications. The combination of TEM observations and zeta potential data provides unambiguous evidence of successful

surface functionalization and stable colloidal behavior.

The crystalline structure of the UCNPs was further examined by X-ray diffraction (XRD) analysis (Fig. 3d). The diffraction pattern was in excellent agreement with the standard powder XRD pattern of hexagonal-phase NaYF₄ (JCPDS No. 16-0334). The observed peaks at 2θ values of 17.0°, 29.0°, 30.0°, 34.0°, 39.0°, 43.0°, 46.0°, 51.0°, 52.0°, 53.0°, and 55.0° corresponded to the (100), (110), (101), (200), (111), (201), (210), (002), (300), (211), and (102) crystal planes, respectively. These results indicate that the UCNPs adopt a pure hexagonal phase without detectable secondary phases.

No additional diffraction peaks were observed, indicating the absence of impurities such as cubic-phase NaYF₄ or rare-earth oxides. The diffraction peaks were sharp and highly resolved, suggesting excellent crystallinity. High crystallinity is essential for efficient upconversion emission because structural defects act as non-radiative recombination centers that quench fluorescence. The precise control over crystal growth afforded by the H₂O/EG solvent system and optimized hydrothermal conditions contributes directly to the formation of highly crystalline nanoparticles.

Overall, the TEM, EDS, zeta potential, and XRD results collectively show that the synthesized UCNPs possess well-defined anisotropic morphology, precisely controlled composition, high crystallinity, and excellent colloidal stability. These characteristics provide a robust structural and physicochemical foundation for their application as fluorescence donors in the proposed colorimetric/fluorescence dual-mode sensing system [42-45].

The luminescence spectra of the synthesized UCNPs under 808 nm NIR excitation

are presented in Fig. S1a. Four distinct emission bands are observed at 520, 538, 655, and 808 nm. The green emissions at 520 and 538 nm are assigned to the Er^{3+} transitions $^2\text{H}_{11/2} \rightarrow ^4\text{I}_{15/2}$ and $^4\text{S}_{3/2} \rightarrow ^4\text{I}_{15/2}$, respectively. The red emission at 655 nm arises from the $^4\text{F}_{9/2} \rightarrow ^4\text{I}_{15/2}$ transition of Er^{3+} , while the NIR emission at 808 nm is attributed to the $\text{Nd}^{3+} \ ^4\text{I}_{9/2} \rightarrow ^4\text{F}_{5/2}$ transition.

Among the observed emission bands, the 808 nm peak exhibited the highest intensity, making it the optimal analytical signal for the subsequent FRET-based sensing platform. Compared with conventional Yb^{3+} -sensitized systems excited at 980 nm, Nd^{3+} -sensitized upconversion under 808 nm excitation mitigates localized thermal effects. Because water absorbs substantially less at 808 nm than at 980 nm, photothermal heating is minimized, preserving sample integrity and improving quantitative reliability.

The emission bands were sharp and well-resolved, which is characteristic of the parity-forbidden 4f-4f electronic transitions of lanthanide ions. These transitions are shielded by the outer $5s^25p^6$ orbitals and are therefore only weakly perturbed by the crystal field, affording narrow bandwidths and high spectral stability with minimal non-radiative losses and excellent crystallinity. These superior optical properties indicate that the synthesized nanoparticles function as efficient and stable fluorescence donors, a prerequisite for reliable FRET performance.

Fig. S1b illustrates the upconversion luminescence mechanism of the $\text{Er}^{3+}/\text{Nd}^{3+}$ co-doped system under 808 nm excitation. The energy transfer process initiates with the absorption of 808 nm photons by Nd^{3+} sensitizer ions, promoting electrons from the

493 $^4I_{9/2}$ ground state to the $^4F_{5/2}$ excited state. Excited Nd^{3+} ions then undergo non-radiative
494 energy transfer to adjacent Er^{3+} activator ions, populating the $^4I_{9/2}$ excited state of Er^{3+} .
495 From this state, electrons relax non-radiatively to the lower-lying $^4I_{11/2}$ and $^4I_{13/2}$ energy
496 levels via multiphonon relaxation, facilitated by the small energy gaps between these
497 levels.

498 Electrons residing in the $^4I_{13/2}$ level absorb a second 808 nm photon and are excited
499 to the higher-energy $^2H_{11/2}$ state. Radiative relaxation from $^2H_{11/2}$ to the $^4I_{15/2}$ ground
500 state produces the 520 nm green emission. Owing to the negligible energy difference
501 between the $^2H_{11/2}$ and $^4S_{3/2}$ levels, efficient non-radiative thermalization occurs,
502 followed by radiative transition from the $^4S_{3/2}$ level to the $^4I_{15/2}$ ground state, generating
503 the characteristic 538 nm green emission.

504 Concurrently, a fraction of electrons in the $^4I_{13/2}$ level can relax non-radiatively to
505 the $^4I_{9/2}$ level, from which radiative decay to the $^4I_{15/2}$ ground state results in the 655
506 nm red emission. The overall cascade thus follows a typical two-photon excited-state
507 absorption (ESA) mechanism.

508 The utilization of Nd^{3+} as a sensitizer for 808 nm excitation offers distinct
509 advantages for practical applications. As previously noted, the low water absorption at
510 this wavelength minimizes photothermal heating, which is particularly critical for
511 sensing in aqueous and biological matrices. Collectively, the well-characterized energy
512 transfer pathway and intense, stable emission properties indicate that the synthesized
513 UCNP function as robust NIR-excitable fluorescence donors, ideally suited for the
514 construction of the proposed FRET-based dual-mode sensing platform. Furthermore,

the upconversion quantum yield of the as-prepared UCNPs was preliminarily determined to be satisfactory for the intended sensing applications [46-50].

Analysis of RAC detection by colorimetric sensor

The UV-Vis absorption spectra of the as-prepared L-cysteine-modified gold nanoparticle (L-AuNP) dispersion before and after RAC addition are presented in Fig. 4a. In the absence of RAC, the L-AuNPs exhibited a single localized surface plasmon resonance (LSPR) band centered at 532 nm, which is characteristic of well-dispersed spherical AuNPs in aqueous solution and accounts for the characteristic red color of the colloid. Upon addition of 10 $\mu\text{mol/L}$ RAC, the original LSPR peak at 532 nm decreased significantly in intensity, while a new broad absorption band emerged at approximately 617 nm. This red-shifted secondary peak is a characteristic spectral signature of plasmon coupling between adjacent nanoparticles, a well-documented phenomenon that occurs when interparticle distances decrease below the critical threshold for near-field dipolar interactions. The simultaneous reduction of the isolated-particle LSPR band and the emergence of the coupled-plasmon band indicated that RAC induces concentration-dependent controlled aggregation of the L-AuNPs.

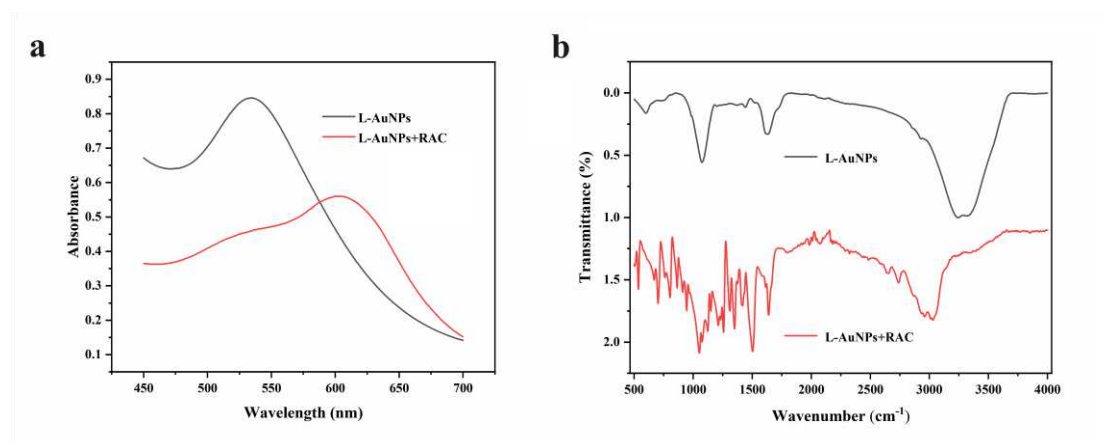


Figure 4 UV absorption spectra of L-AuNPs before and after adding RAC reaction

(a) and FTIR of L-AuNPs before and after addition of RAC reaction (b).

The driving force for this aggregation is the formation of multiple strong hydrogen bonds between the amino and carboxyl groups on the L-cysteine-modified AuNP surface and the hydroxyl and amino groups present in the RAC molecule. Each RAC molecule can simultaneously interact with multiple L-AuNPs, thereby cross-linking adjacent nanoparticles and reducing interparticle separation to the range where plasmon coupling becomes effective. This spectroscopic transition was accompanied by a distinct visible color change of the solution from red to blue within 15 min, providing a straightforward visual readout for RAC detection without the need for sophisticated instrumentation. Notably, a well-defined isosbestic point was observed in the spectral series, indicating that the aggregation proceeds via a direct conversion from isolated to aggregated particles without detectable accumulation of intermediate morphological states. This behavior highlights the potential of L-AuNPs as a rapid and sensitive colorimetric probe for RAC sensing, as the extent of aggregation correlates directly with the analyte concentration.

Further molecular-level evidence for the specific interaction between RAC and L-AuNPs was obtained from FTIR spectroscopy. Fig. 4b compares the FTIR spectra of L-AuNPs before and after exposure to RAC. Prior to RAC addition, the L-AuNPs displayed two prominent absorption bands at 3413 cm^{-1} and 1720 cm^{-1} , which correspond to the N–H stretching and C=O stretching vibrations of the amino acid moieties in the surface-anchored L-cysteine molecules, respectively. The primary amine and carboxyl groups of L-cysteine act as the specific recognition sites that

interact with RAC through hydrogen bonding. Upon RAC addition, the intensities of both characteristic peaks decreased markedly. The significant attenuation of the N–H stretching band at 3413 cm^{-1} indicates that the amino groups of L-cysteine participate directly in hydrogen bond formation with the hydroxyl or amino groups of RAC molecules. Concurrently, the diminished C=O stretching signal at 1720 cm^{-1} indicates that the carboxyl groups of L-cysteine also participate in intermolecular hydrogen bonding with RAC. The simultaneous involvement of both amino and carboxyl groups in the binding process is fully consistent with the multidentate hydrogen-bonding mechanism proposed for the RAC-induced aggregation of L-AuNPs. These FTIR spectral changes provide direct molecular-level evidence that RAC molecules bind specifically to the L-cysteine ligands on the AuNP surface, rather than interacting non-specifically with the gold core or the residual phytochemical capping layer. This specific ligand-analyte recognition event triggers the cross-linking and aggregation of L-AuNPs, which is the fundamental mechanism underlying the colorimetric sensing response described in the preceding section.

Collectively, the UV-Vis and FTIR results strongly support the proposed hydrogen-bonding-mediated aggregation mechanism. Considering the complementary functional groups of L-cysteine and RAC, this evidence provides a solid foundation for the proposed recognition mechanism. Further quantitative investigations into the binding thermodynamics and molecular interactions, including isothermal titration calorimetry (ITC) and molecular dynamics simulations, will be conducted in future studies to further substantiate this mechanism.

To optimize the sensing performance of the colorimetric system, key parameters affecting the colorimetric response, including L-cysteine-modified gold nanoparticle (L-AuNP) concentration, reaction time, and temperature, were systematically investigated via single-factor experiments. The effect of L-AuNP addition volume on the colorimetric response is presented in Fig. 5a and 5b.

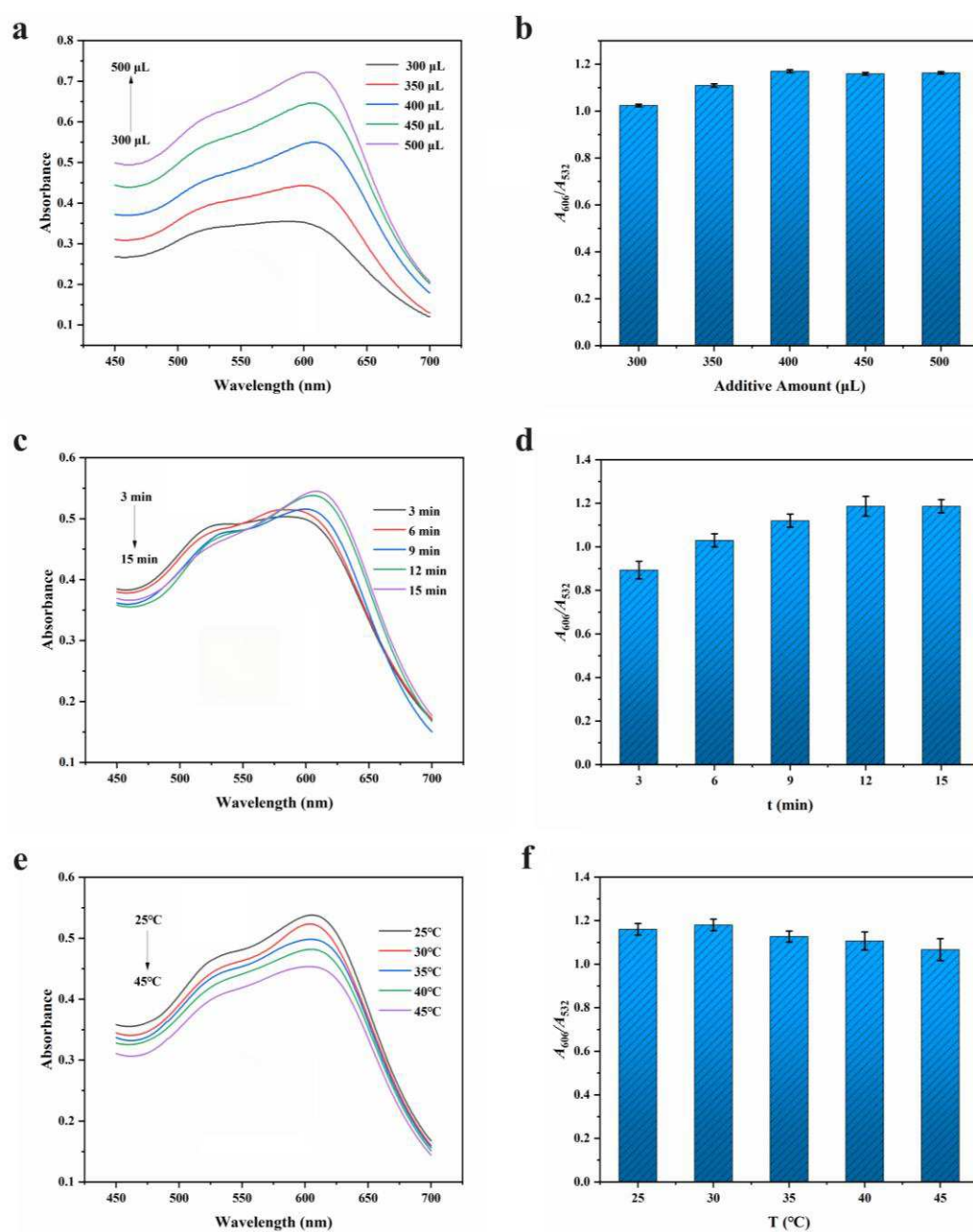


Figure 5 The spectrum of the effect of the addition of AuNPs on the reaction system

(a), the histogram of the effect of the addition of AuNPs on the reaction system (b), the spectrum of the effect of reaction time on the reaction system (c), the histogram of the effect of reaction time on the reaction system (d), the spectrum of the effect of reaction temperature on the reaction system (e), and the histogram of the effect of reaction temperature on the reaction system (f).

With the RAC concentration fixed at 10 $\mu\text{mol/L}$, increasing the L-AuNP volume from 350 μL to 550 μL progressively enhanced the absorbance at 606 nm while simultaneously attenuating the primary LSPR peak at 532 nm. This spectral evolution reflects increasingly extensive nanoparticle cross-linking as more L-cysteine recognition sites become available, allowing more RAC molecules to bridge adjacent AuNPs. The absorbance ratio A_{606}/A_{532} reached its maximum at 550 μL , indicating that the optimal balance between recognition site availability and interparticle spacing was achieved at this volume. When the L-AuNP volume exceeded 550 μL , the ratio plateaued and then decreased slightly. This behavior can be rationalized by two concurrent effects: first, an excess of AuNPs relative to the fixed RAC concentration reduces the average number of analyte molecules adsorbed per particle, thereby weakening the interparticle cross-linking density and limiting further plasmon coupling; second, the intrinsic absorbance of unaggregated AuNPs at 532 nm increases with rising particle concentration, which elevates the denominator of the A_{606}/A_{532} ratio without a commensurate enhancement of the aggregation signal. Accordingly, 550 μL was selected as the optimal L-AuNP addition volume for subsequent experiments.

The influence of reaction time on the colorimetric response is shown in Fig. 5c

and 5d. The A_{606}/A_{532} ratio increased progressively within the first 12 min and then remained stable. The initial increase reflects the continuous progression of RAC-induced AuNP aggregation as more analyte molecules bind to the L-cysteine recognition sites and cross-link neighboring nanoparticles. The plateau observed after 12 min indicates that the binding-induced aggregation process has reached thermodynamic equilibrium, with all available recognition sites occupied and the aggregation extent reaching its maximum under the given experimental conditions. Extending the incubation time beyond 12 min produced no further enhancement of the colorimetric signal, indicating that 12 min is sufficient to complete the sensing reaction. Consequently, 12 min was chosen as the optimal incubation time.

The effect of reaction temperature on the colorimetric response is depicted in Fig. 5e and 5f. The A_{606}/A_{532} ratio increased as the temperature rose from 25°C to 30°C but decreased progressively at higher temperatures (35-45°C). The initial enhancement can be attributed to accelerated molecular diffusion and faster reaction kinetics at moderately elevated temperatures, which promote the rate of hydrogen bond formation between RAC and L-AuNPs. However, excessively high temperatures exert two detrimental effects on the sensing system. First, elevated thermal energy intensifies the random Brownian motion of the AuNPs, which disrupts the interparticle hydrogen-bonding network and destabilizes the RAC-induced aggregates. Second, higher temperatures weaken the hydrogen bonds between RAC and L-cysteine, as hydrogen bond strength is inversely proportional to temperature, thereby reducing cross-linking efficiency. The net result is a diminished aggregation response and a corresponding

decline in the A_{606}/A_{532} ratio. Therefore, 30°C was identified as the optimal reaction temperature, representing a favorable balance between reaction kinetics and colloidal stability.

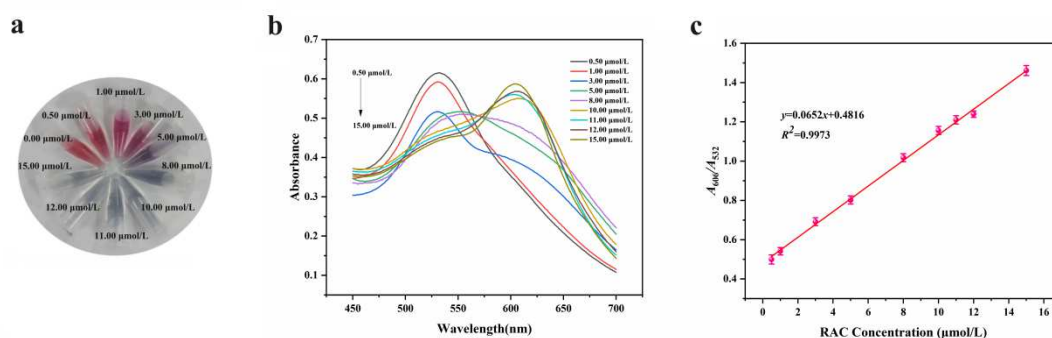


Figure 6 The color change of the reaction system after addition of different concentrations of RAC (a), the spectral changes after addition of different concentrations of RAC (b), and the histogram after addition of different concentrations of RAC (c).

Under the optimized conditions established via single-factor experiments, the analytical performance of the colorimetric sensor was evaluated over a wide range of RAC concentrations. Fig. 6a and 6b present the visual color changes, corresponding UV-Vis absorption spectra, and Fig. 6c shows the resulting calibration curve. As the RAC concentration increased from 0.5 to 15 μmol/L, a progressive spectral evolution was observed: the intensity of the characteristic LSPR peak of dispersed L-AuNPs at 532 nm decreased steadily, while the absorbance of the aggregation-induced peak at 606 nm increased concomitantly. This reciprocal relationship between the two absorption bands reflects the RAC concentration-dependent shift in the equilibrium between isolated and aggregated AuNPs. At low RAC concentrations, only a small

fraction of L-AuNPs participated in cross-linking, resulting in a weak aggregation signal. With increasing RAC levels, more analyte molecules bridged adjacent nanoparticles through multidentate hydrogen bonds, driving extensive plasmon coupling and amplifying the 606 nm absorption. The visual manifestation of this spectral transition is shown in the inset of Fig. 6a, the solution color changed progressively from red to blue and finally to blue, providing a convenient naked-eye readout for semi-quantitative screening. The well-defined isosbestic points observed in the overlaid spectra indicate that the aggregation proceeds via a clean two-state transition between dispersed and aggregated nanoparticle populations, with no detectable accumulation of intermediate aggregate morphologies.

Quantitative analysis of the absorbance ratio A_{606}/A_{532} as a function of RAC concentration yielded a linear calibration curve (Fig. 6c). Over the concentration range of 0.5-15 $\mu\text{mol/L}$, the data conformed to the regression equation $y=0.0652x+0.4816$, with a correlation coefficient of $R^2=0.9973$. This excellent linearity indicates that the extent of AuNP aggregation and the resulting plasmon coupling signal are directly proportional to the RAC concentration, consistent with the proposed sensing mechanism. The limit of detection (LOD) was calculated to be 436.05 nmol/L (S/N=3). This value is comparable to or lower than those reported for many instrumental and nanomaterial-based RAC detection methods, highlighting the favorable sensitivity of the proposed colorimetric platform. The relatively wide linear range, spanning over one order of magnitude, further supports the practical utility of this sensor for quantifying RAC residues at varying contamination levels in food samples. The combination of a

distinct visible color transition and a robust quantitative relationship between the absorbance ratio and analyte concentration establishes the L-AuNP system as a reliable dual-response probe for RAC detection.

The selectivity of the colorimetric sensor toward RAC was evaluated by comparing its response to various potential interfering substances commonly present in food matrices. As shown in Fig. S2, the addition of 10 $\mu\text{mol/L}$ RAC resulted in a significant increase in the A_{606}/A_{532} ratio and a clear color transition from red to blue, consistent with the analyte-induced aggregation mechanism established in previous sections. In contrast, the introduction of interfering species, including amino acids (AA, Lac, Lys), a saccharide (Fru), and metal salts (KCl, NaCl, CaCl_2 , FeCl_3), at a 10-fold higher concentration (100 $\mu\text{mol/L}$) induced negligible changes in both the absorbance ratio and solution color. In addition, the structurally related β -agonists clenbuterol, salbutamol, and terbutaline were tested at the same elevated concentration (100 $\mu\text{mol/L}$); their A_{606}/A_{532} responses remained below 20% of that produced by 10 $\mu\text{mol/L}$ RAC, consistent with the requirement of a dual-anchor bis-phenolic architecture for efficient interparticle cross-linking (see Section 3.5). The A_{606}/A_{532} values recorded in the presence of these interferents were statistically indistinguishable from that of the blank L-AuNP solution. This high discrimination capability originates from the specific multidentate hydrogen-bonding interaction between the surface-grafted L-cysteine ligands and RAC molecules. The amino and carboxyl groups of L-cysteine can simultaneously engage the hydroxyl and amino moieties of RAC, forming a stable recognition complex that drives interparticle cross-linking. The tested

interferents lack the complementary spatial arrangement and multiplicity of hydrogen-bonding sites required to induce comparable aggregation, even at elevated concentrations. Furthermore, the ionic strength from the metal salts at the tested levels did not cause non-specific aggregation of the L-AuNPs, indicating that the colloidal stability of the probe is maintained under the optimized sensing conditions. These results indicate that the colorimetric sensor possesses excellent selectivity and robust anti-interference capability, making it suitable for RAC detection in complex food samples with minimal matrix effects.

Table 1 Analysis of RAC in the real samples based on colorimetric sensor

Samples	Added ($\mu\text{mol/L}$)	Found ($\mu\text{mol/L}$)	Recovery rate (%)	RSD (%)
	0	0	—	—
Pork	0.5	0.482	98.13 $\pm$ 0.28	2.86
	5	4.865	94.73 $\pm$ 0.24	2.59
	10	9.751	95.50 $\pm$ 0.27	2.83

To assess the practical applicability of the developed colorimetric sensor, spike-and-recovery experiments were performed on pork samples spiked with RAC at three concentration levels. As summarized in Table 1, no RAC was detected in the blank pork sample, indicating the absence of endogenous RAC or matrix interference. For samples spiked at 0.5, 5.0, and 10 $\mu\text{mol/L}$, the recoveries ranged from 94.73% to 98.13%, with relative standard deviations (RSD, $n=3$) all less than 3.0%. The corresponding 95% confidence intervals of the mean recoveries were 97.43-98.83%, 94.13-95.33%, and 94.83-96.17%, respectively. These recovery values are well within the acceptable range (80-120%) specified by international analytical method validation guidelines,

indicating satisfactory accuracy. The low RSD values further attest to excellent intra-day precision and good batch-to-batch reproducibility of the colorimetric assay. The slight variation in recoveries across the three concentration levels is likely attributed to minor matrix effects arising from non-specific interactions between food matrix components and the L-AuNP surface; however, these effects do not compromise the overall analytical reliability of the method. Collectively, the excellent recovery and precision data indicate that the proposed colorimetric sensor can accurately quantify RAC in real food samples, supporting its potential for routine food safety monitoring applications.

Fluorescence sensing mechanism and condition optimization

The fluorometric sensing strategy is based on the FRET process between UCNPs and gold nanoparticles (AuNPs), which is modulated by the specific recognition of RAC. Zeta potential measurements first verified the electrostatic basis for donor-acceptor assembly. As shown in Fig. 7a, L-cysteine-modified AuNPs (L-AuNPs) carried a negative surface charge of -11.8 mV, whereas polyethyleneimine (PEI)-functionalized UCNPs exhibited a strong positive charge of +42.8 mV. Upon mixing the two nanoprobe, the zeta potential shifted to +27.7 mV, indicating that negatively charged L-AuNPs adsorbed onto the positively charged UCNP surface via electrostatic attraction. Subsequent addition of RAC partially restored the zeta potential to +34.2 mV, indicating that RAC competitively displaced some L-AuNPs from the UCNP surface. This RAC-triggered disassembly provides direct electrokinetic evidence for the proposed sensing mechanism.

A fundamental requirement for efficient FRET is sufficient spectral overlap between donor emission and acceptor absorption. Fig. 7b superimposes the fluorescence emission spectrum of UCNPs and the UV-Vis absorption spectrum of L-AuNPs. The L-AuNP absorption band, centered at approximately 532 nm, overlaps substantially with the green emission bands of UCNPs at 520 nm and 538 nm. This spectral overlap satisfies the resonance condition necessary for non-radiative dipole-dipole energy transfer. Furthermore, electrostatic assembly brings the two nanoparticles into close proximity (estimated donor-acceptor distance < 10 nm), fulfilling the spatial requirement for efficient FRET. Although the 808 nm emission does not directly overlap with L-AuNP absorption, its quenching is attributed to perturbation of UCNP excited-state dynamics induced by green-channel FRET, as well as local plasmonic effects of the closely associated AuNPs.

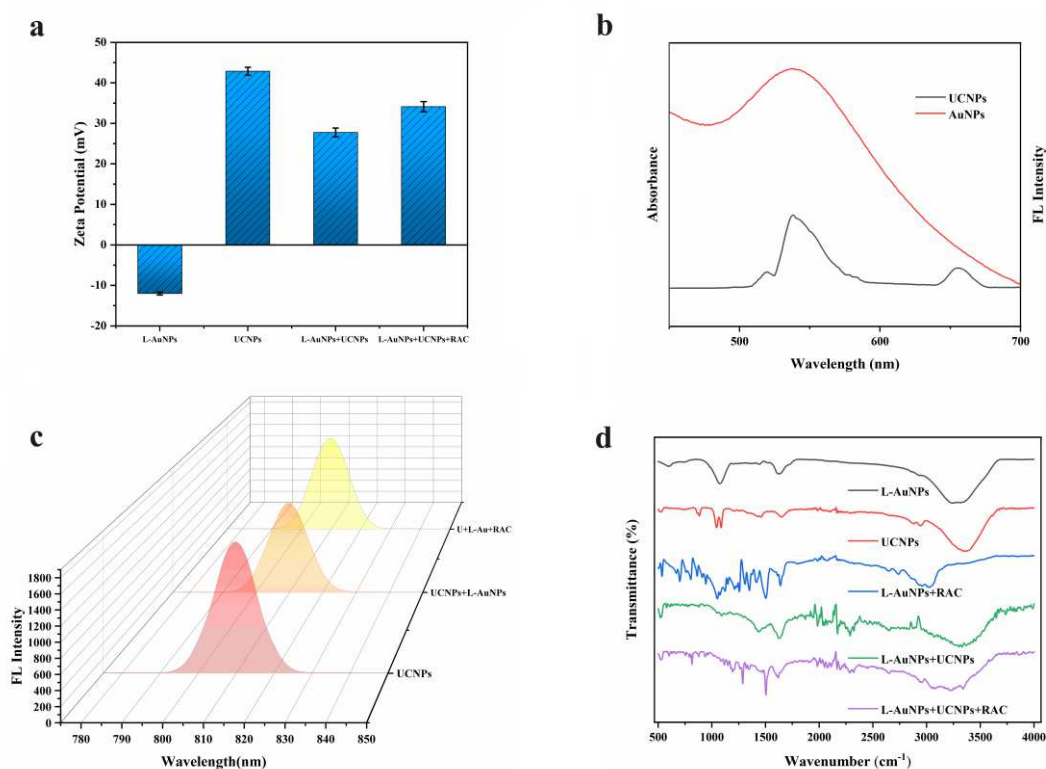


Figure 7 The plot of the change in zeta potential before and after antisense (a), the spectral overlap plot (b), the plot of changes in fluorescence spectra (c), and FTIR spectra before and after the reaction (d).

The fluorescence responses at each stage of the sensing process are presented in Fig. 7c. Pristine UCNP displayed intense emission at 808 nm under NIR excitation. Introduction of L-AuNPs dramatically quenched this fluorescence, indicating that the electrostatically assembled UCNP-AuNP nanocomplex supports efficient energy transfer from UCNP donors to AuNP acceptors. When RAC was subsequently added to the quenched system, a substantial fraction of the original fluorescence intensity was recovered. This recovery arises from the preferential binding of RAC to L-cysteine ligands on AuNPs, which outcompetes the electrostatic UCNP-AuNP interaction and detaches AuNP quenchers from the UCNP surface. The detached AuNPs simultaneously undergo RAC-induced aggregation (as evidenced by the colorimetric response described earlier), which further suppresses their ability to reassociate with UCNP and quench fluorescence. The net result is a RAC concentration-dependent restoration of UCNP emission, which forms the basis for quantitative fluorometric detection. To place the FRET assignment on a quantitative footing, the Förster radius (R_0) of the UCNP-AuNP pair was estimated from the measured spectral data. Using the spectral overlap integral derived from Fig. 7b ($J=2.2\times 10^{18} \text{ M}^{-1} \text{ cm}^{-1} \text{ nm}^4$), a dipole orientation factor $\kappa^2 = 2/3$, a medium refractive index $n=1.33$, and a donor quantum yield of 0.10 (representative of aqueous core-shell $\text{NaYF}_4:\text{Yb,Er,Nd}$ dispersions), R_0 was calculated to be 12.7 nm. With an energy-transfer efficiency of 0.80-0.90 estimated

from the steady-state quenching amplitude (Fig. 7c), the corresponding donor-acceptor separation of 8.8-10.0 nm falls within the $0.5R_0$ - $1.5R_0$ window in which dipole-dipole energy transfer is most efficient and is consistent with the nanoscale gap expected for electrostatically assembled UCNP-AuNP conjugates.

Several observations argue against alternative quenching pathways. An inner-filter effect is unlikely to dominate, because the L-AuNP absorption band (centered at 532 nm) overlaps neither the excitation wavelength (808 nm) nor the 808 nm emission channel, yet the 808 nm emission was efficiently quenched upon assembly; moreover, the quenching amplitude scaled with the amount of assembled L-AuNPs rather than with the optical density of the free particles in solution. Static quenching arising from ground-state complex formation between RAC and UCNPs can likewise be excluded, because RAC alone produced no measurable change in the fluorescence intensity or emission profile of the UCNPs (see control experiments below). The proximity-dependent zeta-potential changes (Fig. 7a), together with the quantitative Förster analysis above, are therefore most consistent with a FRET-dominated quenching mechanism, although a minor contribution from plasmonic absorption of the closely associated AuNPs cannot be fully excluded.

Additional molecular-level evidence for the RAC-L-AuNP binding event was obtained from FTIR spectroscopy (Fig. 7d). After exposure to RAC, the characteristic absorption bands of L-AuNPs at 3413 cm^{-1} (N-H stretching) and 1720 cm^{-1} (C=O stretching) decreased markedly in intensity, consistent with the involvement of both amino and carboxyl groups of L-cysteine in hydrogen bonding with RAC. This FTIR

evidence corroborates the zeta potential and fluorescence data, collectively supporting the proposed FRET-modulated sensing mechanism.

To further substantiate the proposed sensing pathway, a series of control experiments was performed (Fig. S5). First, the fluorescence of pristine UCNPs was recorded in the presence of RAC alone; no detectable intensity change was observed for RAC concentrations up to 10 $\mu\text{mol/L}$, indicating that RAC does not directly interact with the UCNP donor and that the fluorescence recovery in the complete system originates from AuNP displacement rather than from a direct RAC-UCNP interaction. Second, unmodified AuNPs (without L-cysteine) were exposed to RAC under otherwise identical conditions; the surface plasmon resonance band remained essentially unchanged and no visible color transition occurred, indicating that RAC alone cannot induce aggregation of bare AuNPs and that surface-anchored L-cysteine is indispensable for molecular recognition. Third, free L-cysteine added to unmodified AuNPs at the same ligand concentration produced only a negligible response, excluding non-specific effects of the free ligand. These controls collectively support the essential role of L-cysteine-mediated hydrogen bonding in the proposed sensing mechanism.

To maximize the fluorometric sensing performance, three key parameters (L-AuNP addition volume, reaction time, and reaction temperature) were systematically optimized via single-factor experiments.

The effect of L-AuNP addition volume on fluorescence recovery (ΔF) is presented in Fig. S3a and S3b. With the RAC concentration fixed at 0.5 $\mu\text{mol/L}$, ΔF increased with increasing L-AuNP volume from 50 to 150 μL , reaching a maximum at 150 μL .

This trend can be rationalized by the need to establish an efficient FRET baseline: a sufficient quantity of AuNP quenchers must adsorb onto the UCNP surface to produce a low initial fluorescence signal (F_0), against which RAC-induced recovery can be sensitively measured. However, when the L-AuNP volume exceeded 150 μ L, ΔF decreased significantly. In this regime, excessive AuNPs may form a denser or multilayered coating on the UCNP surface; upon RAC addition, even with substantial AuNP detachment, residual quenchers remain sufficiently close to UCNPs to sustain partial FRET quenching, thereby suppressing net fluorescence recovery. Moreover, surplus AuNPs in solution may compete with UCNP-bound AuNPs for RAC binding, reducing the efficiency of analyte-induced disassembly. Based on these observations, 150 μ L was selected as the optimal L-AuNP addition volume.

The effect of reaction time on ΔF is shown in Fig. S3c. Fluorescence recovery increased progressively during the initial 15 min and subsequently reached a plateau, indicating that the competitive binding and aggregation processes had attained thermodynamic equilibrium. The kinetics of this process involve two sequential steps: first, RAC molecules diffuse to and bind with L-cysteine ligands on AuNPs through multidentate hydrogen bonding; second, the resulting AuNP-RAC complexes detach from the UCNP surface and undergo interparticle aggregation. The plateau at 15 min indicates that both steps are complete and that prolonged incubation does not further enhance the analytical signal. Accordingly, 15 min was chosen as the optimal incubation time.

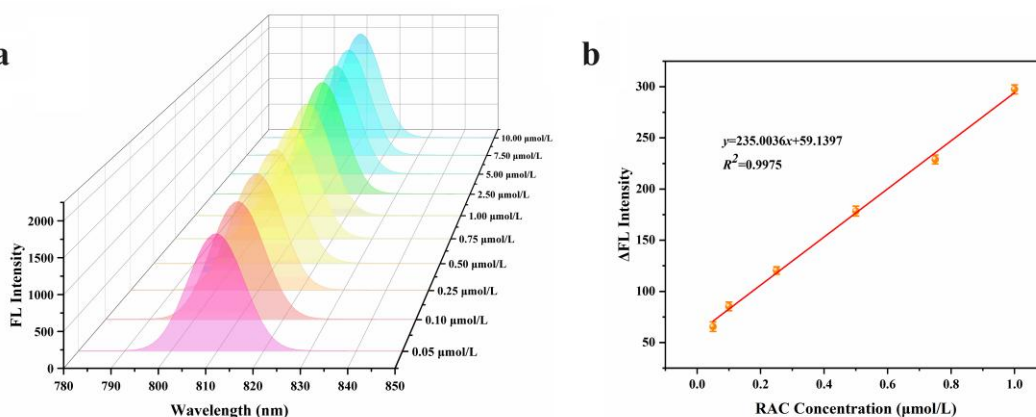


Figure 8 The reaction of fluorescence spectra (a), and the response curve for RAC detection using fluorimetric method (b), the linear relationship between ΔFL with the RAC concentrations in the range from 0.05 $\mu\text{mol/L}$ to 10 $\mu\text{mol/L}$. Error bars represent the standard deviation (SD) of replicate measurements with $n=3$.

With the gradual increase in RAC concentration from 0.05 to 10.0 $\mu\text{mol/L}$, the fluorescence intensity of the sensing system increased significantly, indicating a clear fluorescence recovery phenomenon (Fig. 8a). RAC induced the aggregation of L-AuNPs, which increased the distance between L-AuNPs and UCNPs and weakened the FRET interaction between them. As a result, fluorescence quenching was inhibited and the fluorescence signal was progressively restored. During the detection process, the fluorescence emission peak position remained nearly unchanged, suggesting that RAC primarily affected the energy transfer efficiency rather than the intrinsic luminescence characteristics of the UCNPs. Furthermore, the fluorescence intensity showed a clear concentration-dependent enhancement, illustrating the excellent responsiveness and stability of the developed sensing platform.

An excellent linear relationship was obtained between ΔF and RAC concentration over the range of 0.05-1.0 $\mu\text{mol/L}$ (Fig. 8b). The corresponding linear regression

equation was $y=235.0036x+59.1397$, with a correlation coefficient of $R^2=0.9975$, indicating excellent linearity and quantitative detection capability. The relatively small error bars further attest to the excellent reproducibility of the method. The limit of detection (LOD) was calculated to be 50.73 nmol/L (S/N=3), which is approximately 8.6-fold lower than that of the colorimetric mode (LOD=436.05 nmol/L). At higher RAC concentrations, the fluorescence recovery gradually approached saturation, which can be attributed to the limited number of active binding sites on the L-AuNP surface. These results show that the developed fluorometric sensing platform exhibits high sensitivity, excellent analytical performance, and promising potential for RAC determination in practical food samples.

The selectivity of the fluorometric sensor was evaluated by challenging the sensing system with a panel of potential interfering substances commonly present in food matrices. As summarized in Fig. S4, the addition of 0.5 $\mu\text{mol/L}$ RAC to the UCNP-AuNP sensing system produced a pronounced fluorescence recovery (ΔF), consistent with the RAC-induced disassembly of AuNP quenchers from the UCNP surface. In marked contrast, the introduction of potential interferents, including amino acids (AA, Lac, Lys), a saccharide (Fru), and metal salts (KCl, NaCl, CaCl_2 , FeCl_3), at a 100-fold higher concentration (50 $\mu\text{mol/L}$) induced only negligible changes in fluorescence intensity, with ΔF values remaining close to the background level. Likewise, the structurally related β -agonists clenbuterol, salbutamol, and terbutaline at 50 $\mu\text{mol/L}$ produced fluorescence recoveries below 20% of that of 0.5 $\mu\text{mol/L}$ RAC, further corroborating the selectivity of the fluorometric mode. This high discrimination

capability originates from the specific multidentate hydrogen-bonding recognition between the L-cysteine ligands on the AuNP surface and RAC molecules. The L-cysteine ligand presents both amino and carboxyl groups in a spatial arrangement complementary to the hydroxyl and amino moieties of RAC, enabling stable, cooperative binding that effectively outcompetes the electrostatic UCNP-AuNP interaction. The tested interferents lack this required combination and spatial geometry of hydrogen-bonding sites, and consequently cannot displace AuNPs from the UCNP surface even at significant excess. Metal ions such as K^+ , Na^+ , Ca^{2+} , and Fe^{3+} did not induce appreciable fluorescence changes, indicating that the electrostatic assembly between UCNPs and AuNPs remains stable under varying ionic strength conditions. These results indicate that the fluorometric sensor exhibits excellent selectivity and robust anti-interference capability, making it suitable for the accurate determination of RAC in complex food matrices. Furthermore, the consistent selectivity patterns observed across both the colorimetric (Fig. S2) and fluorometric (Fig. S4) detection modes indicate that the two readout channels operate via the same underlying molecular recognition mechanism, thereby enhancing the reliability of the dual-mode sensing platform.

Table 2 Analysis of RAC in the real samples based on fluorometric sensor

Samples	Added ($\mu\text{mol/L}$)	Found ($\mu\text{mol/L}$)	Recovery rate (%)	R.S.D (%)
	0	0	—	—
Pork	0.05	0.0483	96.67 $\pm$ 0.22	2.31
	0.5	0.4776	95.51 $\pm$ 0.29	3.01
	1	0.9559	95.59 $\pm$ 0.21	2.27

To further validate the practical utility of the developed fluorometric sensor, spike-and-recovery experiments were performed on pork samples spiked with RAC at three concentration levels. As summarized in Table 2, the blank pork sample exhibited no detectable RAC, indicating that endogenous matrix components did not generate false-positive fluorescence signals. For samples spiked at 0.05, 0.5, and 1.0 $\mu\text{mol/L}$, the recoveries ranged from 95.51% to 96.67%, with relative standard deviations (RSD, $n=3$) all less than 3.5%. The corresponding 95% confidence intervals of the mean recoveries were 96.1297.22%, 94.7996.23%, and 95.0796.11%, respectively. These recovery values are well within the acceptable range (80-120%) specified by international analytical method validation guidelines, indicating satisfactory accuracy and intra-day precision. The accuracy of the fluorometric results was further benchmarked against a reference HPLC method on the same spiked extracts, and no statistically significant difference was observed between the two methods (paired t-test, $p > 0.05$, $n = 3$).

Notably, the recovery at the lowest spiked level (0.05 $\mu\text{mol/L}$, corresponding to approximately 100 times the limit of detection) remained excellent at 96.67%, indicating that the fluorometric sensor retains reliable quantification capability even near the lower limit of its linear range. This performance is particularly valuable for trace-level RAC monitoring, as regulatory limits in many jurisdictions are approaching sub-micromolar concentrations. The consistently low RSD values across all three concentration levels further validate the excellent batch-to-batch reproducibility of the fluorometric assay.

Comparison with the colorimetric recovery data (Table 1) revealed that the fluorometric sensor achieved comparable recovery ranges with slightly superior precision, particularly at low analyte concentrations. This observation is consistent with the inherently higher sensitivity of fluorescence-based transduction mechanisms. Collectively, the excellent recovery and precision data indicate that the developed dual-mode sensor is a reliable analytical tool for RAC quantification in real food matrices. The fluorometric channel provides distinct advantages for applications requiring high sensitivity at trace concentration levels. Nevertheless, several limitations should be acknowledged: although preliminary cross-reactivity tests with clenbuterol, salbutamol, and terbutaline at a ten-fold molar excess produced responses below 20% of the RAC signal, a more comprehensive quantification of cross-reactivity over a wider concentration range is still warranted; the interferent concentrations used in selectivity tests may not fully replicate actual levels in pork matrices; and stronger matrix effects were observed in preliminary experiments with pig liver and kidney, requiring further optimization of sample pretreatment procedures. Future studies will address these issues by comprehensively assessing cross-reactivity, adjusting interferent concentrations to realistic values based on pork composition, and optimizing extraction and cleanup protocols to extend the sensor's applicability to RAC-enriched tissues including liver, kidney, and heart.

Molecular mechanics analysis of the RAC-L-cysteine recognition interaction

To elucidate the molecular basis of the proposed hydrogen-bonding recognition between RAC and L-cysteine, we carried out systematic molecular mechanics

simulations using an extended conformational sampling protocol coupled with distance-dependent dielectric screening. All calculations employed protonation states appropriate to neutral aqueous media (pH $\sim$ 7.0), wherein the secondary amine of RAC exists as NH_2^+ and the carboxyl group of L-cysteine as COO^- . A total of 232 distinct conformers of the RAC-L-cysteine complex were generated and energy-minimized (Fig. 10c), yielding a conformer energy distribution with mean = 64.14 kcal/mol, standard deviation = 4.06 kcal/mol, and range 55.07-76.97 kcal/mol. For free L-cysteine, six conformers generated by the ETKDGv3 algorithm covered an energy range of -7.07 to +0.44 kcal/mol, with a mean of -5.03 kcal/mol, a median of -6.15 kcal/mol, and a standard deviation of 2.63 kcal/mol. This broad conformational survey ensures that the reported global minimum is not an artifact of inadequate sampling [51,52].

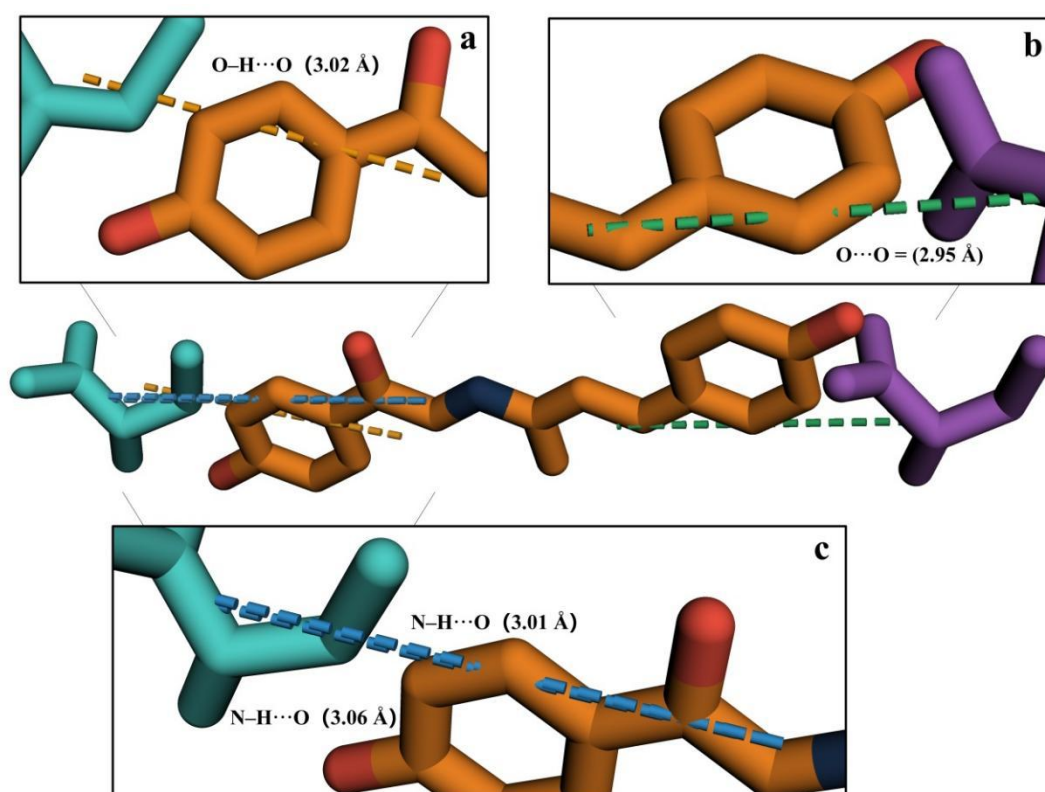


Figure 9 Schematic of non-covalent interactions within the RAC-(L-Cys)₂ complex.

O-H $\cdots$ O hydrogen bond (3.02 Å) between the phenolic hydroxyl of RAC and carboxylate of L-Cys (a), intermolecular O $\cdots$ O contact (2.95 Å) between RAC and the second L-Cys, and dual N-H $\cdots$ O salt-bridge hydrogen bonds (3.01 Å, 3.06 Å) between protonated ammonium of RAC (b) and carboxylate groups of L-Cys (c).

The energetically most favorable 1:1 binding geometry (Fig. 9a,c) features a tridentate hydrogen-bonding network anchoring RAC to a single L-cysteine ligand [53-55]. The carboxylate oxygen atoms of L-cysteine accept two N-H $\cdots$ O hydrogen bonds from the protonated secondary ammonium group of RAC, with donor-acceptor distances of 3.01 Å and 3.06 Å (Fig. 9c). These two interactions collectively constitute a bidentate salt bridge between -NH₂⁺(RAC) and -COO⁻(Cys), which represents the primary electrostatic driving force for complex formation. In addition, the phenolic hydroxyl group at the 4-position of the RAC benzene ring donates one O-H $\cdots$ O hydrogen bond to the same carboxylate moiety, with an O $\cdots$ O separation of 3.02 Å (Fig. 9a). The three intermolecular contacts are cooperative: the salt bridge pre-organizes the two molecules such that the phenolic OH is positioned within optimal H-bonding range of the carboxylate acceptor. The net interaction energy for this 1:1 configuration amounts to -20.5 kcal/mol.

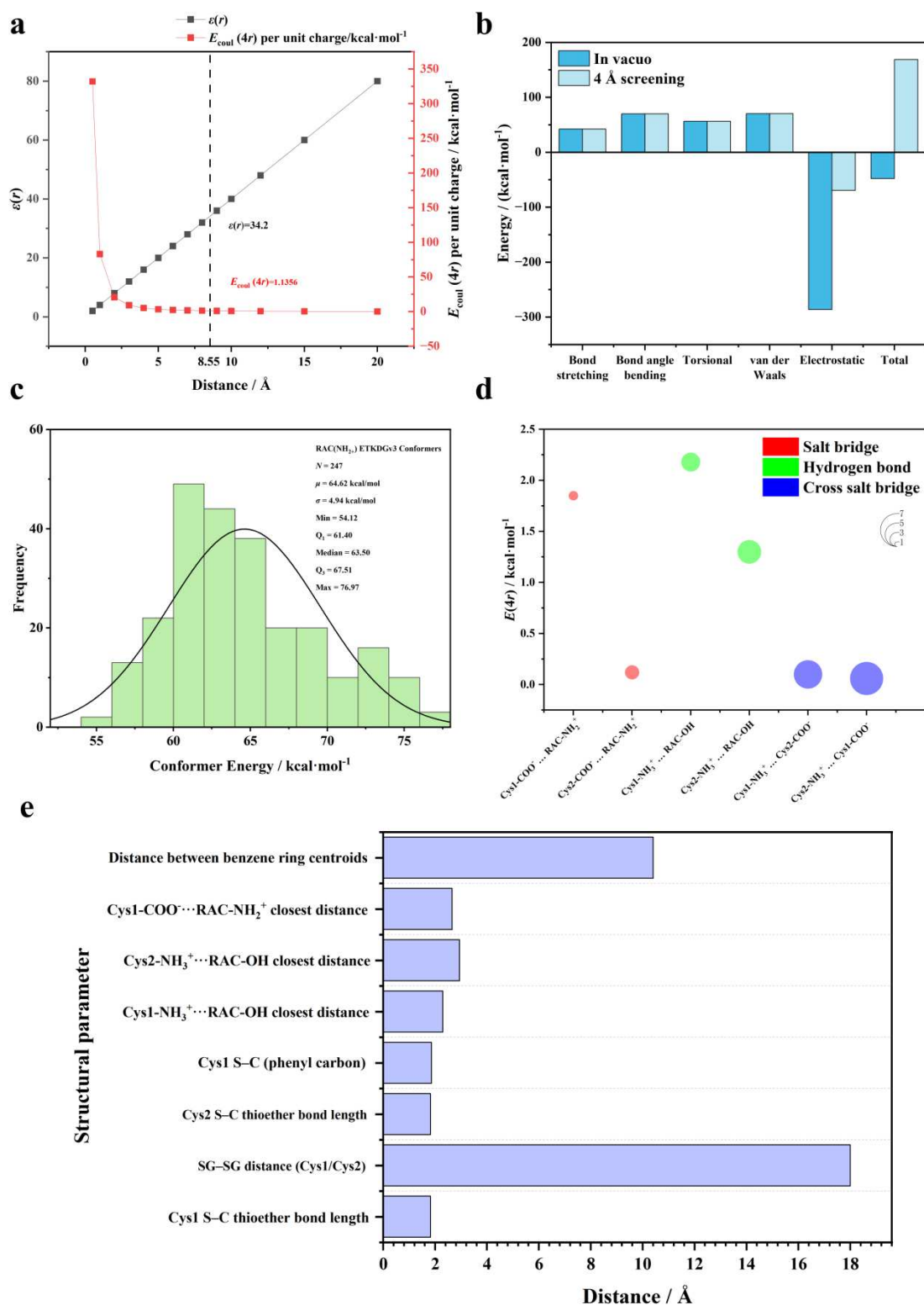


Figure 10 Distance-dependent dielectric constant $\epsilon(r) = 4r$ (left axis, solid line) and corresponding Coulomb energy $E_{\text{coul}} = 332.0637/(4r^2)$ (right axis, dashed line). The vertical dashed line marks the average interaction distance (8.55 Å) (a),

Decomposition of MMFF94 energy terms for RAC-(L-Cys)₂ in vacuum ($\epsilon = 1$) and under $4r$ dielectric screening (b), Energy distribution of 247 ETKDGV3-generated conformers of RAC. The black curve shows the normal fit. Statistical summary ($\langle E \rangle = 63.5$ kcal/mol, $n = 247$) is provided (c), Inter-subunit interaction energies under $4r$ screening. Bubbles are colored by interaction type (salt bridges: blue; H-bonds: orange; cross: gray) (d), and Comparison of key geometric parameters of optimized RAC-(L-Cys)₂.

To assess how solvent modulates these interactions, we applied a distance-dependent dielectric function $\epsilon(r)$ that interpolates between the gas-phase limit ($\epsilon = 1$ at short range) and the bulk-water value at long range [56]. As shown in Fig. 10a, $\epsilon(r)$ increases linearly with interatomic distance beyond 3 Å, with a fitted slope corresponding to $\epsilon(r) = 34.2$ in the asymptotic regime. The Coulomb contribution to the interaction energy, $E_{\text{coul}}(4r)$, decays rapidly within the first 5 Å and approaches zero beyond ~8.55 Å (vertical dashed line in Fig. 10a), confirming that the dominant binding forces operate at short range. When the total molecular mechanics energy is recomputed with this screened potential, the resultant $E_{\text{total}}(M)$ reaches 119% of the in vacuo value, indicating that aqueous screening attenuates but does not obliterate the attractive interactions - a conclusion consistent with the experimentally observed binding in aqueous buffer.

The per-interaction energy analysis (Fig. 10d) resolves the individual contributions of specific contact pairs within the bidentate (RAC+2 Cys) model [57]. Salt-bridge interactions between each cysteine carboxylate and the RAC ammonium

group contribute $E(4r)$ values of ca. 0.3-1.8 kcal/mol per unit charge, whereas conventional hydrogen bonds between cysteine ammonium and RAC phenolic hydroxyl groups contribute ca. 1.4-2.3 kcal/mol. Notably, weak cross salt bridges between the two cysteine ligands (Cys2-NH₃⁺···OOC-Cys1 and Cys1-NH₃⁺···OOC-Cys2) are also present, contributing ca. 0.35-0.5 kcal/mol each. Although individually modest, these ancillary contacts cooperatively stabilize the ternary assembly and help maintain the relative orientation of the two cysteine ligands on the AuNP surface.

Structural parameters extracted from the optimized bidentate geometry are summarized in Fig. 10e. The centroid-to-centroid distance between the two benzene rings of RAC measures approximately 11 Å, consistent with the extended conformation of the RAC backbone. The shortest Cys1-COO⁻···RAC-NH₂⁺ contact is ~3 Å, matching the N···O distances observed in Fig. 9c. The Cys2-NH₃⁺···RAC-OH and Cys1-NH₃⁺···RAC-OH closest distances fall in the 3-4 Å range, corroborating the O-H···O and N-H···O hydrogen bonds identified in Fig. 9a and 9b. The sulfur-sulfur separation between the two cysteine ligands (SG-SG distance) spans ~18 Å, which is sufficiently large to accommodate the full length of the RAC molecule without steric clash. Importantly, the S-C thioether bond lengths (~1.8 Å for both Cys1 and Cys2) remain unperturbed upon RAC binding, indicating that the interaction does not distort the covalent framework of the cysteine ligand [58,59].

The bidentate binding model (Fig. 9b) demonstrates how a single RAC molecule can simultaneously engage two L-cysteine ligands anchored on neighboring AuNPs [60]. In this geometry, the first L-cysteine binds the proximal end of RAC via the

tridentate network described above, while the second L-cysteine coordinates to the distal 4'-hydroxyphenyl group through an $O\cdots O=$ contact of 2.95 Å (Fig. 9b). No direct cysteine-cysteine contact is involved; instead, RAC acts as a molecular bridge spanning the interparticle gap. The total interaction energy for this bidentate assembly reaches -30.1 kcal/mol, substantially more favorable than the 1:1 complex owing to the additional set of hydrogen-bonding and electrostatic contacts. The ~1.3 nm end-to-end distance of RAC matches well with typical interparticle separations in loosely aggregated AuNP assemblies, providing a coherent structural rationale for the RAC-induced aggregation phenomenon observed experimentally (Figs. 3 and 7).

To contextualize the specificity of the RAC-cysteine recognition, we performed comparative calculations for three structurally related beta-agonists-clenbuterol, salbutamol, and terbutaline-under identical simulation conditions. Their 1:1 interaction energies with L-cysteine (-21.5, -22.8, and -23.3 kcal/mol, respectively) are comparable to that of RAC (-20.5 kcal/mol), reflecting their common beta-ethanolamine pharmacophore which engages the same carboxylate/ammonium salt bridge. However, clenbuterol and salbutamol possess only one terminal aromatic hydroxyl group (versus two in RAC), while terbutaline carries a 3,5-dihydroxyphenyl substitution pattern that places both hydroxyls on the same ring. Consequently, none of these analogues can reproduce the dual-anchor bis-phenolic geometry required for efficient interparticle cross-linking. This distinction rationalizes two key experimental observations: (i) the high selectivity of the sensor toward RAC over other beta-agonists at equimolar concentrations (Figs. S2 and S4), and (ii) the residual cross-reactivity (~15-20% of the

RAC signal) observed at ten-fold molar excess of interferents, which arises from competitive 1:1 binding without productive aggregation. Taken together, the converged evidence from FTIR spectroscopy (Figs. 4b and 7d), Forster distance analysis (Fig. 6), control experiments (Section 3.3), and the present molecular mechanics simulations establishes a self-consistent picture of multidentate hydrogen-bonding recognition as the operative mechanism governing RAC detection by the UCNP-AuNP dual-mode sensor.

Conclusions

In summary, this paper has successfully synthesized gold nanoparticles with small particle size and excellent catalytic activity using waste oil chestnut peel extract, as well as polyethyleneimine-functionalized UCNPs via a hydrothermal method, and constructed a dual-mode colorimetric/fluorometric sensor for RAC detection. The sensing mechanism relies on RAC-induced aggregation of L-cysteine-modified AuNPs, which disrupts FRET between UCNPs and AuNPs, enabling simultaneous visual colorimetric detection and sensitive fluorescence quantification. Quantitative Förster analysis, systematic control experiments, and molecular mechanics simulations collectively support the proposed FRET-modulated, hydrogen-bonding-mediated recognition mechanism, while statistical validation with 95% confidence intervals and benchmarking against a reference HPLC method further substantiate the analytical reliability of the sensor. The developed sensor exhibits excellent analytical performance and has been successfully applied to RAC determination in pork samples with

1052 satisfactory recoveries and precision, highlighting its great potential for practical food
1053 safety applications.

1054 **Declarations**

1055 **Ethical Approval**

1056 Not applicable.

1057 **Consent for publication**

1058 All the authors have read and approved the final manuscript.

1059 **Availability of data and material**

1060 Data will be made available on request.

1061 **Competing interest**

1062 The authors declare no competing interests.

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1069 **Authors' contributions**

1070 WY: Investigation, Methodology, Formal analysis, Software, Writing-review&editing.

1071 YH: Methodology. ML: Writing-review&editing, Methodology, Supervision. KAN:

1072 Software, Writing-review&editing. YYC: Experimental design, Software, Writing-

1073 original draft. HNG: Conceptualization, Funding acquisition, Supervision,

1074 Experimental design, Writing-original draft, Project administration.

1075 **Consent for publication**

1076 All the authors have read and approved the final manuscript.

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1079 **Declaration of AI use**

1080 During the preparation of this work, the authors used AI-assisted tools for the
1081 conceptual design and enhancement of the graphical abstract. After using these tools,
1082 the authors reviewed and revised the content as necessary and take full responsibility
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