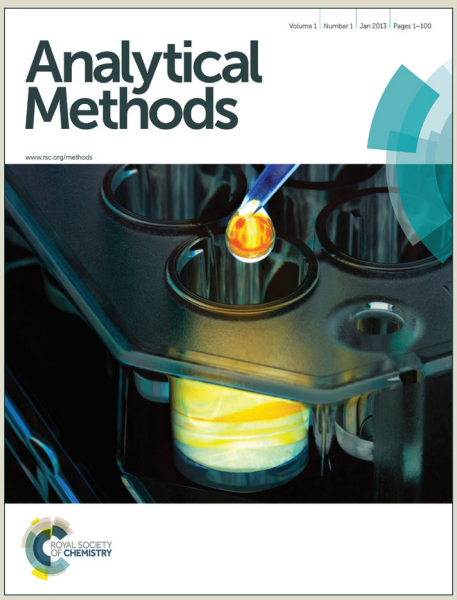
Analytical Methods

Accepted Manuscript



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Enhanced chemiluminescence of the luminol-hydrogen peroxide system by BSA-stabilized Au nanoclusters as peroxidase mimic and its application

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Abstract

In the present work, water-soluble Au nanoclusters capped with Bovine serum albumine (BSA) was synthesized. It was found that as a peroxidase mimic, Au nanoclusters could enhance the chemiluminescence (CL) emission from the luminol-hydrogen peroxide system in alkaline medium, and the enhancement mechanism of Au nanoclusters on luminol CL was discussed. The effects of the reactant concentrations and some organic compounds were also investigated. The proposed method could be used as a sensitive detection tool for hydrogen peroxide and glucose.

Keywords

chemiluminescence; Au nanoclusters; peroxidase mimic; luminol; glucose

1. Introduction

Chemiluminescence (CL) is light emission produced in a chemical reaction from the decay of chemiexcited species to the electronic ground state [1]. CL and related analytical techniques have attracted extensive interest since the CL phenomenon of luminol was first reported by Albrech [2], owing to its extremely high sensitivity along with its other advantages, such as simple instrumentation, wide calibration ranges, and suitability for miniaturization in analytical chemistry [3-6]. CL has been developed as important and powerful tools in different fields (e.g., environmental analysis, pharmaceutical analysis, food analysis, bioanalysis and immunoassay) [7-11].

Though CL has been investigated for years, study of CL was limited to some molecular systems. In recent years, much attention has been extended to the CL of nanomaterial systems, to improve the sensitivity and the stability. Many investigations have indicated that use of nanoparticles in CL reactions has provided new avenues to enhance the inherent sensitivity and expand new applications of this mode of detection [12]. Li et al. found that CeO_2 nanoparticles could enhance the CL emission of luminol–H_2O_2 system and developed a specific sandwich assay for human a-thrombin [13]. Chen et al. have made use of the active catalysis of the CuO nanoparticles to detect glucose and cholesterol [14-15]. Wei et al. reported that the chemiluminescence of the luminol–H_2O_2 system could be enhanced by ZnO nanoparticles [16]. In other cases, Cui and co-workers have reported many prominent works about noble metal nanoparticles-catalyzed CL systems, such as Au, Ag, and Pt nanoparticles, which significantly improved the inherent sensitivity and selectivity of the

traditional CL systems [17-19].

Au nanoclusters (Au NCs), owing to their ultrasmall size, biocompatibility, nontoxicity and highly fluorescent properties, have drawn wide attention [20-22]. Recently, these protein-templated Au NCs have been successfully applied to cancer-cell imaging [23], tumor imaging in vivo [24], and Hg^{2+}, CN^{-}, Cu^{2+} and H_2O_2 detection [25-28] and so forth. Wang et al. demonstrated that bovine serum albumin (BSA) stabilized Au clusters exhibited highly intrinsic peroxidase-like activity firstly [29]. Compared with other reported nanoparticles as peroxidases mimetics, BSA-Au clusters possess intrinsic peroxidase-like activity and have effective enzyme-like catalysis over a wide range of temperatures and pH values compared with nature enzymes [29-30]. However, there are no reports exploring the catalytic property of Au NCs in the luminol CL reactions, to the best of our knowledge.

Luminol is one of the earliest and most common CL reagents used in CL reaction. The luminol–H_2O_2 system still plays an important role in modern chemical analysis [31-35]. In the present study, Au NCs, the novel classes of intermediates between noble-metal atoms and nanoparticles materials, were chosen as catalysts for the luminol CL system and explored the effect of colloidal solutions of Au NCs on the CL for the first time. The possible enhancement of Au NCs mechanism was investigated. Based on the effect of Au NCs on the luminol-H_2O_2 CL system, the feasibility of using the proposed method for H_2O_2 and glucose detection was studied. Under optimum conditions, the CL intensity was linear with H_2O_2 concentration. In this work, we established a simple, low-cost sensor for glucose by coupling the highly selective enzymatic procedure with the sensitive chemiluminescence reaction catalyzed by Au NCs successfully.

2. Experimental

2.1 Reagents and materials

Bovine serum albumin (BSA), Glucose oxidase (GOx) and Glucose were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). $HAuCl_4 \cdot 3H_2O$ and sodium borohydride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 30% (v/v) H_2O_2 and sodium hydroxide were purchased from Kelong Reagent Co., Chengdu, China.

A 1.0×10^{-2} mol/L stock solution of luminol (3-aminophthalhydrazide) was prepared by dissolving luminol (Sigma) in 0.1 mol/L sodium hydroxide solutions. Working solutions of luminol were prepared by diluting the stock solution. Working solutions of H_2O_2 were prepared fresh daily by dilution of 30% (v/v) H_2O_2. Clinical serum samples were provided by the three gorges central hospital of Chongqing. All chemicals and reagents were of analytical grade and used without further purification, and ultrapure water was used throughout.

2.2 Instrumentation

Batch model BPCL ultra weak chemiluminescence analyzer (Institute of Biophysics, Chinese Academy of Sciences, Beijing, China) was employed to study the characteristics of the CL reaction. The CL detection was conducted on a flow injection chemiluminescence system comprising of a peristaltic pump (Ruimai Company Xi'an, China), PTFE tubing (0.8 mm i.d.) which was used as connection material in the flow system. Data acquisition and treatment were performed with BPCL software running under Windows XP. The CL

spectra were obtained with an F-4500 spectrofluorimeter (Hitachi, Japan) under the model of fluorescence scan by turning off the excitation light. The pH of the solutions was detected by a PHS-3C precision pH meter (Shanghai Precision Scientific Instruments Co., Ltd., China). UV–vis absorption spectra were achieved with a Model UV-2550s Spectrophotometer (Shimadzu, Japan).

2.3 Synthesis of BSA-Au nanoclusters and BSA-Au nanoparticles

2.3.1 Synthesis of BSA-Au nanoclusters

BSA modified Au NCs was synthesized in aqueous solution following a previous publication with minor modifications [36]. In a typical experiment, All glassware was washed with AquaRegia (HCl: HNO_3 volume atio = 3:1), and rinsed with ethanol and ultrapure water. 15 mL aqueous $HAuCl_4$ solution (10 mmol/L, 37℃) was added to BSA solution (15 mL, 50 mg/mL, 37℃) under magnetic stirring. Then, 1.5 mL 1mol/L NaOH solution was introduced and the mixture was allowed to incubate at 37℃ under vigorous stirring for 24 h. The color of the solution changed from light yellow to light brown, and then to deep brown. The solution was then dialyzed in double distilled water for 48 h to remove unreacted $HAuCl_4$ or NaOH. The final solution was stored at 4℃ in refrigerator when not in use. The UV-vis and fluorescence spectra of as –prepare Au NCs are shown in Figure S2 and S3. Upon being excited 470 nm, the Au NCs showed an emission band centered at 640 nm. The features of the obtained spectra are consistent with previous studies [36].

2.3.2 Synthesis of BSA-Au nanoparticles

Au NPs were prepared according to the Liu's method [37]. In brief, 0.6700 g of BSA and 0.0125 g of $HAuCl_4 \cdot 4H_2O$ was dissolved in 100 mL of ultra-pure water, the obtained yellow solution was stirred more than 2 h. And then, 0.1530 g of $NaBH_4$ was slowly added into this solution with rapid stirring for 3 h. At last, the product was purified through centrifugation (11 000 rpm) to remove the large gold nanoparticles, leaving a clear, dark red BSA-protected gold nanoparticles solution. The UV-vis spectra of as –prepare Au NPs are shown in Figure S1.

2.4 General procedure for CL analysis

The CL intensity was measured by a flow injection CL system. The flow system employed consisted of two peristaltic pumps. One delivered luminol and Au NCs at a flow rate (per tube) of 0.8 mL/min. The other delivered the sample and carrier stream at the same flow rate. PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. Injection was made using a six-way injection valve equipped with an eight cm length sampling loop. The CL signal produced was detected by a photomultiplier tube (operated at −550 V), and was then recorded by a computer equipped with a data acquisition interface. Data acquisition and treatment were performed with BPCL software running under Windows XP.

For characterization of the chemiluminescent analysis system, aqueous standards were used. A series of working standard solutions with different concentrations was prepared by diluting a concentrated fresh standard solution of H_2O_2 or glucose with water. The net CL emission intensity ($\Delta I = I_1 - I_0$, where I_1 is the CL intensity of the sample solution and I_0 that of the blank solution) versus H_2O_2 concentration was used for the calibration. At each

H_2O_2 or glucose concentration, the injection was repeated at least three times, and the average CL signal was obtained.

2.5 Glucose determination in real serum samples

For glucose determination in blood, the serum samples from a local hospital were firstly treated by centrifugation at 3000 rpm for 30 mins. Then 0.10 mL of the supernatant was diluted into 10 mL using 1×10^{-2}mol/L PBS buffer (pH 7.0) for the following work. Glucose determination was carried out by adding 0.1 mL of the diluted serum sample and 0.1 mL of 1 mg/mL GOx in 0.50 mL of 10 m mol/L PBS buffer (pH 7.0), the mixture was incubated at 37 °C for 30 mins, and then the resulting mixture was diluted to 10 mL by 1×10^{-2}mol/L PBS buffer solution (pH 7.0), and then used for glucose determination. The calibration curve for glucose detection was realized as follows: (a) 0.1 mL of 1 mg/mL GOx and 0.1 mL of glucose of different concentrations in 0.50 mL of 1×10^{-2}mol/L M PBS buffer (pH 7.0) were incubated at 37 °C for 30 mins, then the resulting solutions were diluted to 10 mL with 1×10^{-2}mol/L PBS buffer , leading to the final glucose concentration of $0.05–10\times10^{-6}$mol/L; (b) the produced mixed solution was used to prepare the calibration curve for glucose by the proposed CL method. The results were compared with those by the conventional method. The comparison study was carried out by a One Touch Ultra glucose meter (Johnson and Johnson Medical Ltd., Shanghai, China). All experiments on glucose analysis in blood were performed in compliance with the relevant laws and institutional guidelines.

3. Results and discussion

3.1 Enhancement of luminol CL

In alkaline media, the oxidation of luminol by H_2O_2 generates weak CL. Figure 1 shows the kinetic curves of the Au NCs enhanced CL system, which indicated that Au NCs could highly enhance CL systems. In order to explore the CL enhancing phenomena, the CL spectra for AuNCs mixed with luminol–H_2O_2 was acquired. Figure 2 is the CL spectra of luminol–H_2O_2–Au NCs system. The maximal emission was at ~425 nm, revealing that the luminophor was still the excited state 3-aminophthalate anions. Therefore, the addition of AuNCs did not lead to the generation of a new luminophor for this CL system. The enhanced CL signals were thus ascribed to the possible catalysis from AuNCs.

3.2 Optimization of the reaction conditions

The reaction conditions were optimized for the luminol–H_2O_2–AuNCs CL system shown in Figure. 3. The pH of luminol solution played an important role in the CL reaction. The effect of pH on the CL was studied in the range of pH 10.0–12.5. The experimental results in Figure.3a indicated that the maximum CL intensity was obtained at pH 11.3 in sodium hydroxide solution. When the pH of luminol solution was lower than 11.3, the CL intensity increased with increasing the pH. When the pH of luminol solution was higher than 11.3, the CL intensity decreased with increasing the pH. The effect of luminol concentration on the CL was studied in the range from 1.0×10^{-5} to 3.5×10^{-5} mol/L. The

result is shown in Figure.3b. As can be seen, the CL intensity increased with increasing luminol concentration in the range of 1.0×10^{-5} to 2.7×10^{-5} mol/L. An increase of the CL signal intensity was observed when the concentration of luminol was lower than 2.7×10^{-5} mol/L. However, when the concentration of luminol was above 2.7×10^{-5} mol/L, only slight changes in the light intensity were observed. Therefore, 2.7×10^{-5} mol/L was chosen as the optimal luminol concentration in the present study. Different concentrations of Au NCs were added to the CL system, they enhanced the CL intensity in different degrees. The effect of Au NCs concentration was investigated over the range 4.0–20.0 mg/L (Figure 3c). It was found that the maximum CL intensity was obtained at 16.0 mg/L, above which the CL intensity decreased, probably due to the self-absorption of the emission by Au NCs. Therefore, 16.0 mg/L of Au NCs was chosen for the next experiments.

3.3 Mechanism Discussion

The CL–generation mechanism for luminol oxidation in aqueous solution has been extensively studied. Merényi et al. had summarized the major CL–generating mechanism for luminol oxidation in aqueous solution to occur in three basic steps, as shown in Scheme 1: (1) oxidation of luminol to the luminol radical; (2) oxidation of the luminol radical to hydroxy hydroperoxide, the key intermediate; (3) decomposition of hydroxyl hydroperoxide with or without the emission of CL, among which step 1 was supposed to be the rate-determining step of luminol CL [38–39]. The presence of oxygen-related radicals (for example, OH•, $O_2^{\bullet-}$, and other radical derivatives) as oxidants was expected to occur, during the luminol oxidation processes. As for the luminol–H_2O_2 system, the CL reaction

of superoxide radical with luminol in alkaline solution was catalyzed in the presence of H_2O_2.

In order to explore the possible mechanism, the UV–visible absorption spectra were recorded. As shown in Figure. 4, it could be seen that Au NCs had no maximum absorption peak in the ranges 280–600 nm, and the luminol–H_2O_2 system had two absorption peaks at 304 and 346 nm. When mixed with luminol–H_2O_2 system, the UV-visible peak was not changed, indicating no change occured after the reaction. Therefore, the enhancement of CL signals may have originated from the catalytic effects of Au NCs.

Based on the above discussion, it can be concluded that when added in the luminol-hydrogen peroxide system, Au NCs may interact with the reactants or the intermediates of the reaction of luminol with hydrogen peroxide. It is possible that AuNCs as the catalysts could catalyze the decomposition of H_2O_2 to yield active intermediates such as $OH^{\bullet}$, $O_2^{\bullet-}$. The hydroxyl radical reacted with luminol to form luminol radical ($L^{\bullet-}$), then the produced $L^{\bullet-}$reacts with superoxide anion, yielding an unstable endoperoxide and an electronically excited 3–aminophthalate anion (3–APA*), leading to enhanced light emission shown in Scheme 2.

In order to prove the correctness of the mechanism, we investigated the effect on CL of luminol in the presence of Au NPs @BSA and Au NCs@BSA. As shown in Figure5, the CL intensity was enhanced greatly by Au NCs, probably because that quantum size effects began to function with an increase in band gap energy, leading to a higher activation energy that was needed for electron transfer, which would be disadvantageous for the partial electron transfer from Au NPs to H_2O_2, and then decrease the catalytic efficiency of Au

NPs for luminol CL reaction [40].

3.4 Inhibition effects of organic compounds

Organic compounds containing hydroxyl (OH), amino (NH2), or mercapto (SH) groups have been reported to compete with luminol for active oxygen intermediates, leading to a decrease in CL intensity. On the other hand, these compounds may interact with AuNCs to reduce the active surface area, interrupting the formation of luminol radicals and hydroxyl radicals taking place on the surface of nanoparticles. Therefore, the effects of such organic compounds on the CL system were investigated. The results are listed in Table 1. As is expected, all the tested compounds with the concentration of 1×10^{-5} g/mL inhibited the CL signal of the luminol–H_2O_2–Au NCs system. The results demonstrate that the luminol–H_2O_2–Au NCs system system has a wide application for the determination of such compounds. However, the usefulness of this technique in terms of selectivity may be limited. If it combined with separation techniques, this CL detection will not be problematic. Therefore, it is ideal for the design of a CL detector in HPLC and high-performance capillary electrophoresis by use of this CL system for the simultaneous detection of numerous compounds.

3.5 Analytical applications

Under the optimum conditions described above, the calibration graph of the relative CL intensity versus H_2O_2 concentration was linear in the range from 2.0×10^{-8} to 5.0×10^{-6} mol/L Figure 6**.** The limit of detection (LOD) for H_2O_2 was 6.0×10^{-9} mol/L. The RSD of

the method was 3.86% for 1.0×10^{-7} mol/L H_2O_2 (n = 7). The regression equation is ΔI =850.09+1624.99 [H_2O_2] (μM), R^2 = 0.9969 (n = 7).

Because H_2O_2 is the main product of the glucose oxidase (GOx)–catalyzed reaction, therefore, when combined with glucose oxidase (GOx), the proposed CL method could be used for the determination of glucose, which is an important indicator for the diagnosis of diabetes mellitus in clinical medicine. The linear range for glucose was from 5.0×10^{-7} to 1.0×10^{-5} mol/L and the limit of detection was 1×10^{-7} mol/L (Figure.7). The RSD was 2.2% for 5.0×10^{-6} mol/L glucose (n = 7). The regression equation is ΔI =527.54+383.77 [glucose] (μM), R^2 = 0.9957 (n = 7). The present method was compared to the analytical methods previously published in the literature [14, 34, 41–44] using the luminol–H_2O_2 CL system for H_2O_2 and glucose analysis in terms of LODs (the detection limits). The LODs are listed in Table 2. As can be seen, the proposed method shows high sensitivity for H_2O_2 and glucose analysis.

The selectivity of the proposed method was also studied. The selectivity experiments were carried out by using a series of solutions containing 5×10^{-6} mol/L glucose plus various amounts of maltose, lactose, fructose or sucrose. It was found that negligible CL change was observed even after adding 5×10^{-6} mol/L maltose, lactose, fructose or sucrose. This indicates that the present biosensing system has high selectivity for glucose.

To evaluate the feasibility of the sensing system for analysis of glucose in biological samples, the proposed method was used to detect glucose in blood samples, and the results were compared with those obtained by the conventional method. The comparison study was carried out by a One-Touch Ultra glucose meter (Johnson and Johnson Medical Ltd.,

Shanghai, China). The detailed procedure is described in the Experimental section. The results are listed in Table 3, it can be seen that the results obtained by the proposed method were in good agreement with those measured by glucose oxidase endpoint method. The practical applicability of the proposed method was further verified through standard addition experiments, with the recoveries of glucose in three serum samples ranging from 96.5% to 102.7%. Therefore, the proposed method is suitable and satisfactory for glucose analysis of real samples.

4. Conclusion

Au NCs were found to enhance the luminol−H_2O_2 CL signals in this work. The enhancement of CL was suggested to attribute to the peroxidase-like activity of AuNCs, which effectively catalyzed the decomposition of hydrogen peroxide into hydroxyl radicals. Some organic compounds containing hydroxyl, amino, or mercapto groups were observed to inhibit the CL signals of the luminol–H_2O_2–AuNCs system at the experimental conditions, which could be potentially applied in the analysis of these compounds. In addition, a novel AuNCs-based enzyme nano-mimic CL method was used successfully for H_2O_2 and glucose detection. This work is of great benefit to the insight of the enzyme nano-mimics and their potential applications in CL and bioanalysis.

Acknowledgement

We thank Prof. H. Z. Zheng and Prof. Y. M. Huang for measurements.

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Figure captions:

Figure1. Kinetic curves of chemiluminescence systems: (a) luminol-H_2O_2; (b) luminol-H_2O_2-Au NCs. Luminol: 2.7×10^{-5} mol/L; H_2O_2: 1×10^{-7} mol/L; Au NCs: 5 mg/L.

Figure2. Chemiluminescence spectra for luminol-H_2O_2-Au NCs system. (a) Au NCs; (b) luminol-H_2O_2; (c) luminol-H_2O_2-Au NCs. Luminol: 2.7×10^{-5} mol/L; H_2O_2: 1×10^{-7} mol/L; Au NCs: 5 mg/L; pH 11.3 (sodium hydroxide solution).

Figure3. Effects of the reaction conditions on the luminol-H_2O_2-Au NCs CL system. (a) Effects of pH of luminol: Luminol: 2.7×10^{-5} mol/L; H_2O_2: 1×10^{-7} mol/L; Au NCs: 5 mg/L (b) Effect of luminol concentration: pH: 11.3; H_2O_2: 1×10^{-7} mol/L; Au NCs: 5 mg/L (c) Effect of Au NCs: Luminol: 2.7×10^{-5} mol/L; H_2O_2: 1×10^{-7} mol/L; pH: 11.3.

Figure4. UV-visible absorption spectra of (a) Au NCs; (b) luminol-H_2O_2; (c) luminol-H_2O_2-AuNCs

Scheme1. Schematic CL-generating mechanism for the oxidation of luminol with three major steps

Scheme2. Possible mechanism for the luminol-H_2O_2-Au NCs CL system.

Figure5 Comparison of CL intensity in luminol-catalyzed reaction in pH 11.3, 2.7×10^{-5} mol/L luminol and 1×10^{-7} mol/L H_2O_2. Inset: Comparison of CL intensity in luminol-catalyzed reaction for Chemiluminescence spectra

Figure6. Calibration curves for H_2O_2. Inset: CL intensity dependence of H_2O_2 concentrations.

Figure7. Calibration curves for glucose. Inset: CL intensity dependence of glucose concentrations.

Table captions

Table 1 Inhibition effects of organic compounds (1.0×10^{-5} mol/L) on luminol-H_2O_2-Au NCs CL system.

Table 2 Comparison LOD of this work with some established methods using luminol-based CL for hydrogen peroxide and glucose

Table 3 Analytical results of glucose in human serum.

Table 1 Inhibition effects of organic compounds (1.0×10^{-5} mol/L) on luminol-H_2O_2-Au NCs CL system.

Organic compounds	Quenching,a %	Organic compounds	Quenching,a %
phenol	82.4	l-histidine	5.1

catechol	4.7	l-alanine	6.1
phloroglucinol	50.3	l-glutamine	4.9
ascorbic acid	8.4	glycine	6.7
gallic acid	10.0	l-serine	14.3
adrenaline	4.6	l-phenylalanine	12.8
dopamine	25.8	l-cystine	8.9
chlorogenic acid	12.2	glutathione	6.7

a The percentage of quenching was calculated as I/I_0. The blank CL signal I_0 was obtained by luminol-H_2O_2-Au NCs CL system without the tested organic compounds

Table 2 Comparison LOD of this work with some established methods using luminol-based CL for hydrogen peroxide and glucose

System	H_2O_2 (10^{-6} mol/L)	Glucose (10^{-6} mol/L)	Ref
CuO nanoparticles–luminol–H_2O_2	0.01	2.9	14
β -CD/$CoFe_2O_4$ MNPs–luminol–H_2O_2	0.02		34
Au nanoparticles–Hb/PMMA–luminol–H_2O_2	0.2		41
Gold NPs–luminol–H_2O_2–HRP		5	42
HRP–MNP–luminol–H_2O_2	0.5	50	43
1-Ethyl-3-methylimidazolium ethylsulfate/Cu^{2+}–luminol–H_2O_2	5	4	44
Au NCs–luminol–H_2O_2	0.006	0.1	This work

Table 3 Analytical results of glucose in human serum (n=3).

samples	Proposed method (10^{-3} mol/L)	Glucose meter (10^{-3} mol/L)

Serum1	0.43±0.01	0.46
Serum2	0.84±0.02	0.85
Serum3	1.12±0.01	1.09

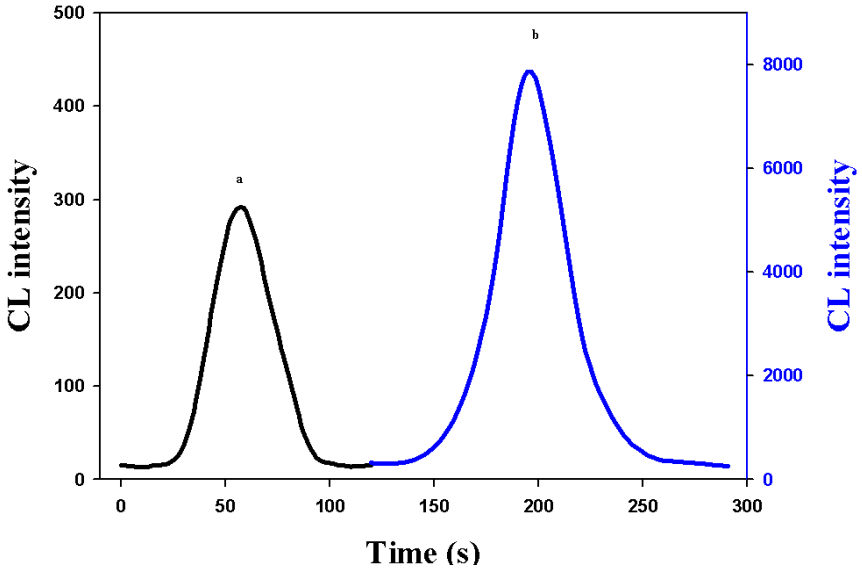


Figure1. Kinetic curves of chemiluminescence systems: (a) luminol-H_2O_2; (b) luminol-H_2O_2-Au NCs. Luminol: 2.7×10^{-5} mol/L ; H_2O_2: 1×10^{-7} mol/L; Au NCs: 16 mg/L; pH :11.3 (sodium hydroxide solution).

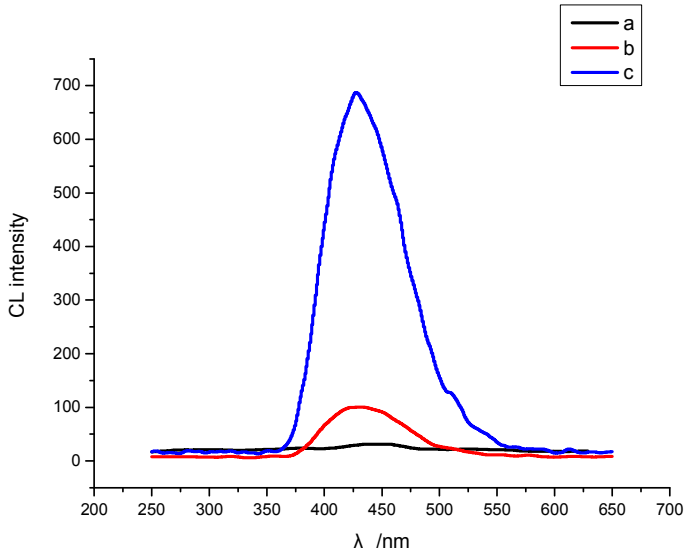
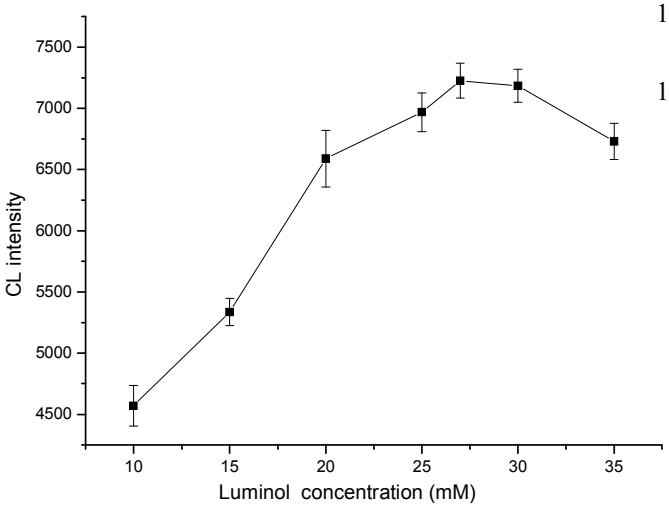


Figure2. Chemiluminescence spectra for luminol-H_2O_2-Au NCs system. (a) Au NCs; (b) luminol-H_2O_2; (c) luminol-H_2O_2-Au NCs. Luminol: 2.7×10^{-5} mol/L ; H_2O_2: $1 \times$

10^{-7} mol/L; Au NCs: 16 mg/L; pH 11.3 (sodium hydroxide solution).



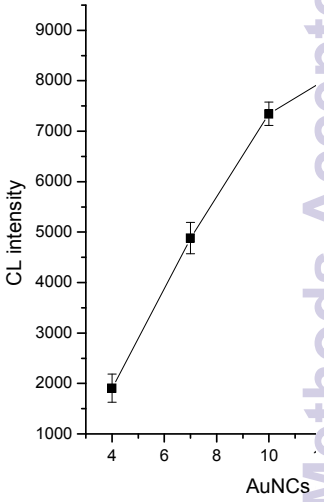


Figure3. Effects of the reaction conditions on the luminol-H_2O_2-Au NCs CL system. (a) Effects of pH of luminol: Luminol: 2.7×10^{-5} mol/L; H_2O_2: 1×10^{-7} mol/L; Au NCs: 5 mg/L (b) Effect of luminol concentration: pH:11.3; H_2O_2: 1×10^{-7} mol/L; Au NCs: 5 mg/L (c) Effect of Au NCs: Luminol: 2.7×10^{-5} mol/L; H_2O_2: 1×10^{-7} mol/L; pH: 11.3.

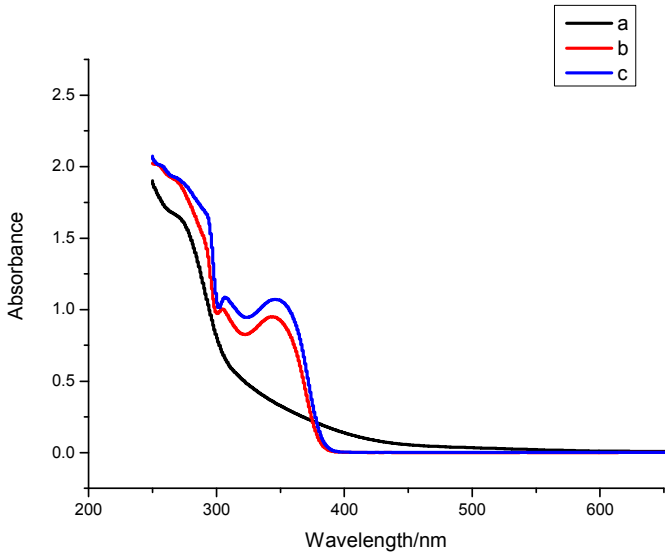
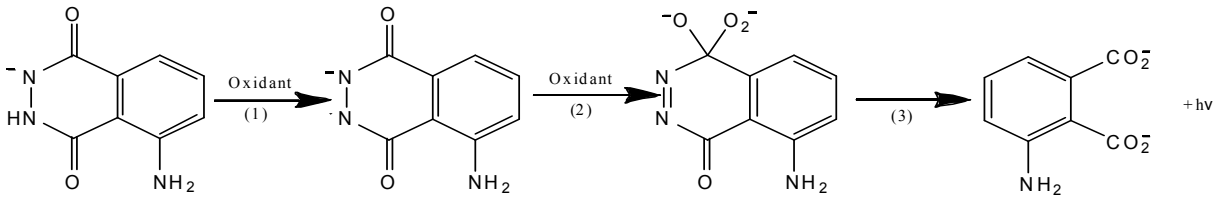
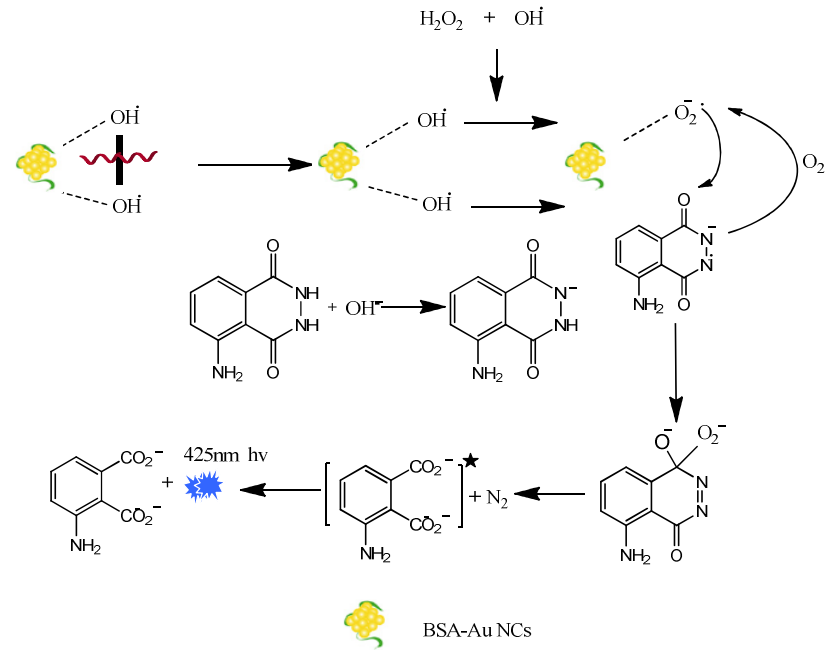


Figure4. UV-visible absorption spectra of (a) Au NCs; (b) luminol-H_2O_2; (c) luminol-H_2O_2-AuNCs



Scheme 1. Schematic CL-generating mechanism for the oxidation of luminol with three major steps



Scheme2. Possible mechanism for the luminol-H_2O_2-Au NCs CL system.

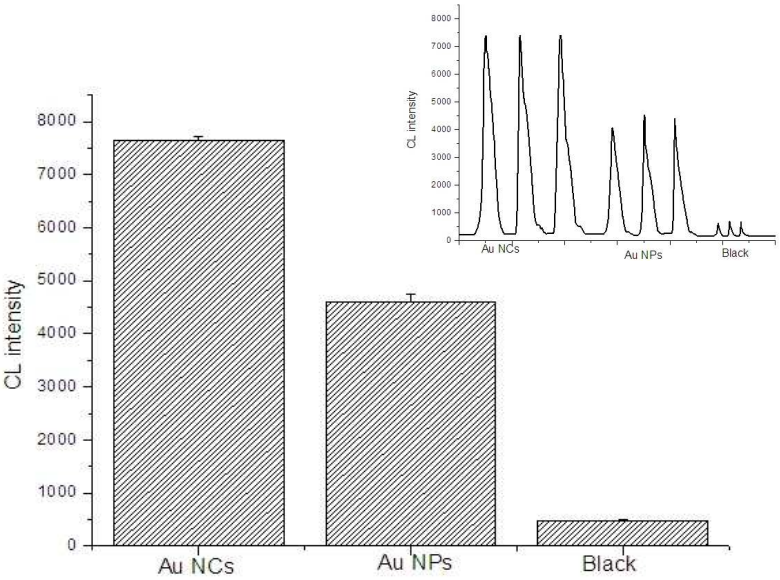


Figure5 Comparison of CL intensity in luminol-catalyzed reaction in pH 11.3, 2.7×10^{-5} mol/L luminol and 1×10^{-7} mol/L H_2O_2. Inset: Comparison of CL intensity in luminol-catalyzed reaction for Chemiluminescence spectra.

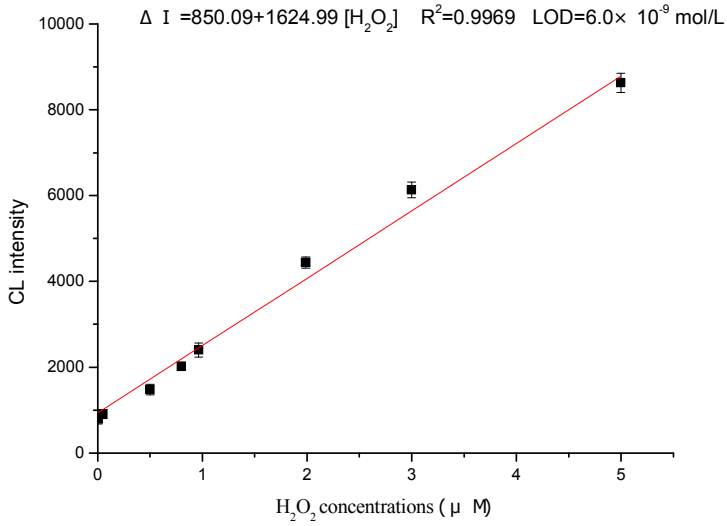


Figure 6. Calibration curves for H_2O_2.

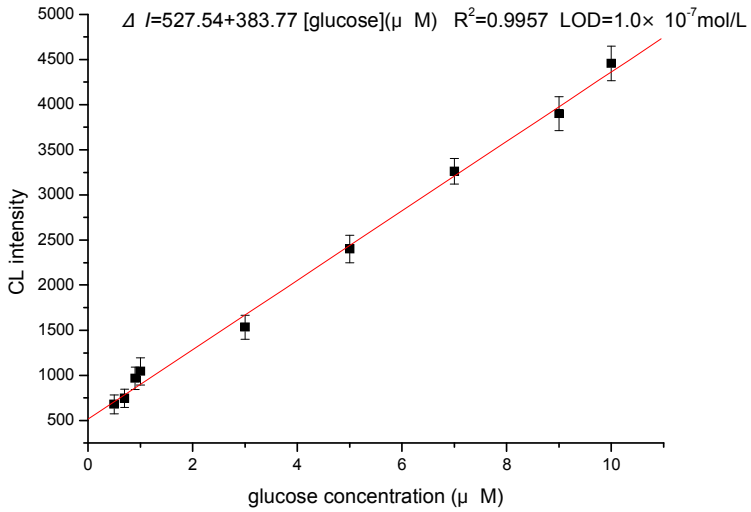


Figure 7. Calibration curves for glucose.