

# Determination of dopamine by exploiting the catalytic effect of hemoglobin–stabilized gold nanoclusters on the luminol–NaIO<sub>4</sub> chemiluminescence system

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**Abstract** Hemoglobin–stabilized gold nanoclusters (Hb–AuNCs) were prepared by using hemoglobin as both reducing and stabilizing agents. The Hb–AuNCs display a strong catalytic effect on the chemiluminescence (CL) reaction of luminol with NaIO<sub>4</sub>. The CL mechanism is discussed and the experimental variables are examined. It is found that dopamine strongly inhibits CL. This finding is exploited in a CL–based dopamine assay that works in the range between 0.3 and 9.0 nM and has a 0.1 nM detection limit. The relative standard deviation is 3.1% at a 5 nM dopamine level (for  $n = 11$ ). The method was applied to the determination of dopamine in spiked human plasma with satisfactory results.

**Keywords** Dopamine · Chemiluminescence · Nanomaterial–amplified chemiluminescence · Gold nanoclusters · Hemoglobin · Luminol · Periodate · Inhibitory · Flow injection · Human plasma

## Introduction

Chemiluminescence (CL) has been extensively explored to analyze different trace substances in a veracity of samples [1–3]. The study of CL systems has been diverting from molecular systems to nanomaterial–assisted systems with the

purpose to improve the properties of sensitivity, selectivity, and stability owing to the rapid development of nanoscience [4–6]. The catalytic behavior of nanomaterials in CL reactions is strongly dependent on their sizes [7], surface charge [8] and morphology [9–11].

Metal nanoclusters (NCs) are composed of several to tens of atoms with sizes between atoms and small nanoparticles that have attracted enormous attention since their discovered [12]. This can be witnessed by an explosive growth in publications concerning the application of metal NCs as fluorescent probes in biosensing and bioimaging [13, 14]. Besides of their famous fluorescent property, their catalytic activity has also attracted analytical scientist's interest. For example, Chen et al. used metal NCs to catalyze the luminol CL reaction with H<sub>2</sub>O<sub>2</sub> [15, 16] or KMnO<sub>4</sub> [17] as the oxidants. Periodate (IO<sub>4</sub><sup>−</sup>) is colorless, thus avoiding emission absorption problems. Additionally, periodate is more stability than H<sub>2</sub>O<sub>2</sub> and hypohalites and related oxidants. Periodate can oxidize luminol in an alkaline solution to produce CL [18, 19]. Up to date, there is no report with regard to the periodate–luminol CL system catalyzed by metal NCs.

Dopamine (3,4-dihydroxyphenethylamine) is one of the most important neurotransmitter and plays significant roles in the function of human metabolism, central nervous, renal and hormonal system. The level of dopamine in human body is an important biomarker for the diseases of schizophrenia and Parkinson. Therefore, it is of very urgent to develop sensitive and selective method for the determination of dopamine in biological fluid [20]. A variety of techniques have been reported for the detection of dopamine, including spectrophotometry [21, 22], fluorescence [23, 24], electrochemical methods [25–27], and chemiluminescence [28–32]. Among them, CL methods have the advantages of simple and inexpensive instrumentation, high sensitivity, and wide linear dynamic range.

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In this work, hemoglobin-stabilized gold nanoclusters (Hb–AuNCs) were prepared through a biomineralization process by revisiting the previous work of Shamsipur et al. [33]. The reaction time is shortened from 30 days to 1.5 days (36 h) by increasing the reaction temperature from 37 °C to 60 °C. The prepared Hb–AuNCs exhibits a significant catalytic effect on the luminol–periodate CL reaction. This system is developed as a sensitive CL method for the determination of dopamine which strongly inhibits the CL signal. The practicability of the method is evaluated by the determination of dopamine in spiked human plasma samples.

## Experimental

### Apparatus

All CL measurements were conducted on an IFFM–D flow–injection CL analyzer (<http://www.chinaremex.com>, Xi'an Remax, China) equipped with a CR105 photomultiplier tube (<http://www.hamamatsu.com.cn>, Hamamatsu Photonics (China) Co., Ltd). Fluorescence spectra and CL spectra were obtained on a F–2700 fluorescence spectrophotometer (<http://www.hitachi-hightech.com>, Hitachi, Japan). Absorption spectra were recorded with a U–3900 UV–Visible spectrophotometer (<http://www.hitachi-hightech.com>, Hitachi, Japan). Transmission electron microscopy (TEM) was performed on a JEM–2100 transmission electron microscope (<http://www.jeol.co.jp/cn>, Japan Electronic Company, Japan) at an accelerating voltage of 200 kV. The X–ray photo electron spectrum (XPS) was measured on UltraDLD X–ray photoelectron spectrometer (<http://www.kratos.com>, Kratos, Britain) using Al–K $\alpha$  as the exciting source (1486.6 eV) and C1s at 284.8 eV for binding energy calibration.

### Chemicals

All chemicals used were of analytical–grade; water was produced from Milli–Q reference ultra–pure water system (<http://www.astk.com.cn>, Beijing ASTK Technology Development Co., Ltd., China). Dopamine was purchased from Shanghai Aladdin Bio–Chem Technology Co. Ltd. (<http://www.aladdin-e.bioon.com.cn>, Shanghai, China). Chloroauric acid (HAuCl<sub>4</sub>•4H<sub>2</sub>O) was obtained from Shanghai Chemical Reagent Factory (<http://www.shiyicr.com.cn>, Shanghai, China). Hemoglobin (Hb) was offered by Beijing Solarbio Science & Technology Co., Ltd. (<https://solarbio.en.alibaba.com>, Beijing, China). Luminol was purchased from Tianjin Fuchen Chemical Reagents Factory (<http://www.tjfc.com/en>, Tianjing, China). Sodium periodate (NaIO<sub>4</sub>) was bought from Tianjing Kemiou Chemical Reagent Co., Ltd. (<http://www.tjkermel.com>, Tianjing, China). Other chemicals were

obtained from Xi'an Chemical Reagent Factory (<http://www.crc-xa.com>, Xi'an, China).

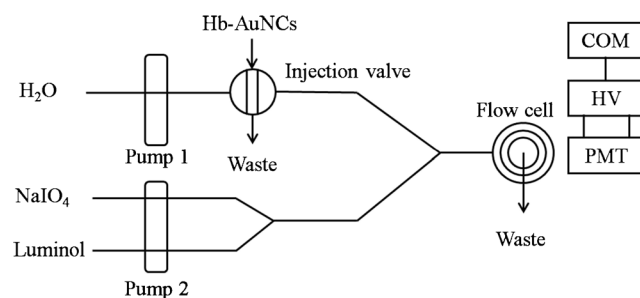
A 10.0 mM dopamine stock solution was prepared by dissolving 94.8 mg dopamine in 50 mL water. More dilution solutions of dopamine were prepared by diluting this stock solution before use. All dopamine solutions were protected from the light and stored in a refrigerator. Luminol stock solution (25 mM) was prepared by dissolving 0.2214 g luminol solid in 50 mL of 0.1 M NaOH solution. Luminol working solutions were prepared by diluting the luminol stock solution with a suitable concentration of NaOH solution. HAuCl<sub>4</sub> solution (10.0 mM) and NaIO<sub>4</sub> solution (10.0 mM) were prepared in water.

### Synthesis of Hb–AuNCs

All glassware were soaked in concentrated HNO<sub>3</sub> solution for 12 h, rinsed thoroughly with water, and then dried for use. The preparation of Hb–AuNCs was similar to the previous procedure of Shamsipur et al. [33] with some modification. In brief, aqueous HAuCl<sub>4</sub> solution (2.8 mM, 5 mL) was mixed with Hb solution (50 mg/mL, 5 mL) at 60 °C with vigorously stirring. Ten min later, 1 mL of 1 M NaOH solution was added and the reaction was performed at 60 °C for 36 h. The resultant solution was centrifuged at 12000 rpm for 10 min to remove any large size of particles. The blackish green solution (Hb–AuNCs) was collected and stored in a refrigerator for further use.

### CL measurement

Figure 1 shows the schematic diagram of CL flow system used. Hb–AuNCs solution (50  $\mu$ L) was injected into the carrier H<sub>2</sub>O via a six–way injection valve, which was then merged with the combined stream of luminol solution and NaIO<sub>4</sub> solution just prior to the flow cell. The CL signal produced in the flow cell was monitored by the photomultiplier tube biased a high voltage at 800 V and recorded as I<sub>0</sub>. In the case of dopamine, the carrier H<sub>2</sub>O was replaced by dopamine standard/sample solution and the responding CL signal was taken as I. The relative CL signal ( $\Delta$ I), calculated by



**Fig. 1** Schematic diagram of CL flow system. PMT: photomultiplier tube; HV: high voltage; COM: computer

subtracting  $I$  from  $I_0$ , was used to optimization, calibration, and quantification of dopamine.

## Results and discussion

### Revisiting the synthetic conditions of Hb–AuNCs

In the work of Shamsipur et al. [33] for the synthesis of Hb–AuNCs, 30 days was needed to complete the reaction. To shorten the reaction time, the synthesis conditions, including reaction temperature and reaction time, was revisited.

The effect of reaction temperature was examined in the range of 37 °C–80 °C. The reaction efficiency increased with increasing the reaction temperature as indicated by the rapid increase in the fluorescence intensity of Hb–AuNCs at 450 nm (upon excitation at 365 nm) (Fig. S1). When the reaction temperatures were above 60 °C, the fluorescence intensity remained a platform indicating the complete of the reaction.

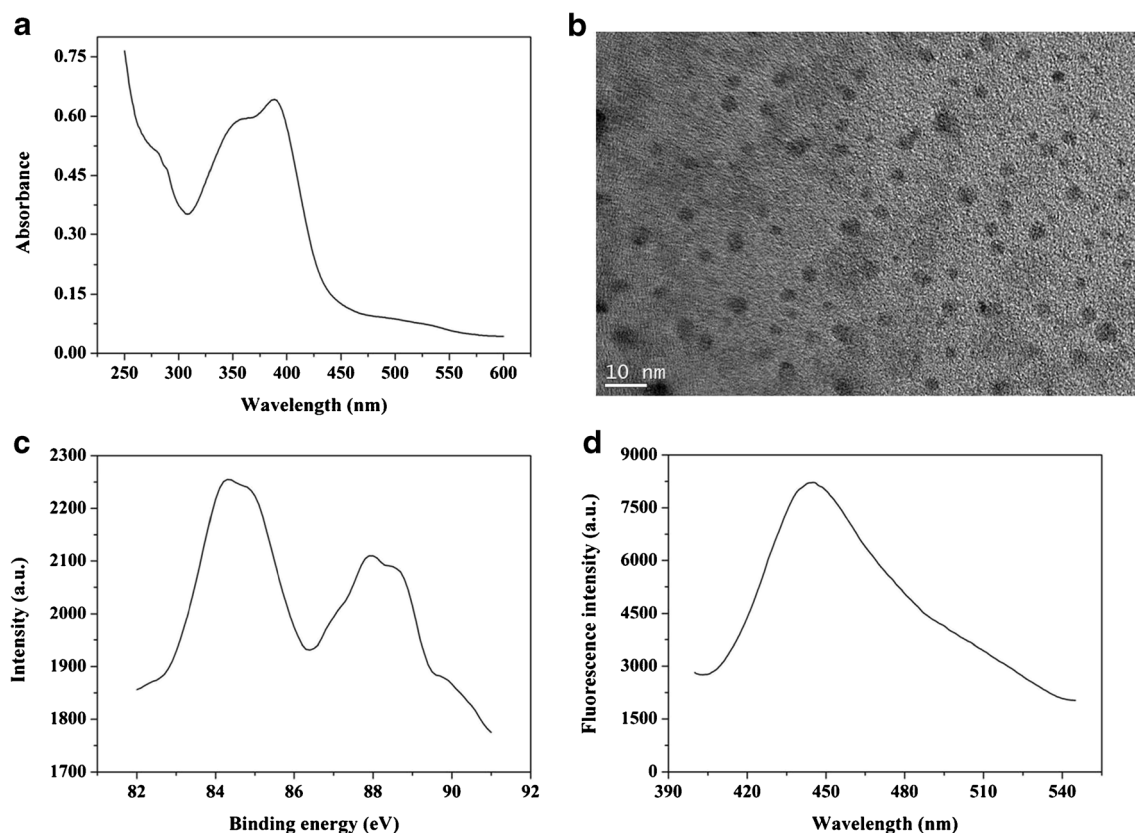
The effect of reaction time was studied from 4 h to 48 h by fixing the reaction temperatures at 60 °C. As shown in Fig. S2, the fluorescence intensity increased as the reaction time was increasing from 4 h to 36 h. When the reaction time was longer than 36 h, the fluorescence intensity remained essentially constant, indicating the complete of the reaction. Therefore, the reaction temperatures of 60 °C and the reaction

time of 36 h were employed for the preparation of the Hb–AuNCs.

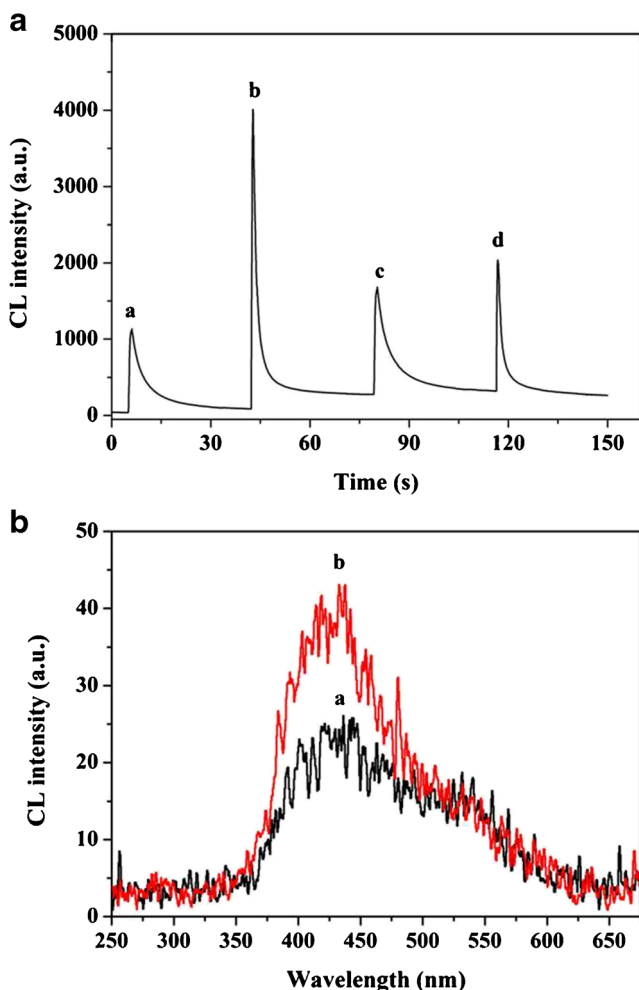
The prepared Hb–AuNCs were characterized with UV–vis, fluorescence, TEM, and XPS. The Hb–AuNCs have a wide absorption band centering at 350–400 nm and lack typical surface plasmon resonance peak at 520 nm (Fig. 2a). The TEM image confirms the Hb–AuNCs are spherical in shape with a diameter about 3 nm (Fig. 2b). The XPS suggests the presence of Au(0) and Au(I) in the Hb–AuNCs (Fig. 2c). Upon excitation at 365 nm, the Hb–AuNCs emits blue emission with a maximal emission at about 450 nm (Fig. 2d), which was consistent with that of Shamsipur et al. [33]. All of these confirmed the successful preparation of the Hb–AuNCs.

### Enhancement of the luminol–NaIO<sub>4</sub> CL system by Hb–AuNCs

The effect of the Hb–AuNCs on luminol–NaIO<sub>4</sub> CL system was investigated by a batch method. Into reaction cell, 0.5 mL of H<sub>2</sub>O, 0.5 mL of 0.5 mM luminol solution, and 0.5 mL of 1:10 dilution Hb–AuNCs solution were added successively. After homogeneous, 0.5 mL of 0.1 mM NaIO<sub>4</sub> solution was introduced to initiate the CL reaction. As shown in Fig. 3a, the CL signal of luminol–NaIO<sub>4</sub> reaction is obviously enhanced when the Hb–AuNCs is present in the system (curve a and b). To rule out possible catalytic activity from Hb, a control



**Fig. 2** a UV–vis absorption spectrum, b TEM image, c XPS and d fluorescence spectrum of the Hb–AuNCs



**Fig. 3** **a** CL profiles of the luminol–NaIO<sub>4</sub> reaction catalyzed by Hb–AuNCs and **b** the responding CL spectra. *(a)* luminol–NaIO<sub>4</sub> reaction, *(b)* luminol–NaIO<sub>4</sub>–Hb–AuNCs reaction, *(c)* luminol–NaIO<sub>4</sub>–Hb reaction and *(d)* luminol–NaIO<sub>4</sub>–Hb–AuNCs–dopamine reaction

experiment was conducted by replacing Hb–AuNCs with Hb. Hb displays weak catalytic effect on the luminol–NaIO<sub>4</sub> system (curve c), but it is much lower than that of Hb–AuNCs (curve b). Therefore, the catalytic activity originates from the Hb–AuNCs not from Hb. Further investigation indicates dopamine significantly inhibits the CL signal of luminol–NaIO<sub>4</sub>–Hb–AuNCs reaction (curve d).

To elucidate the enhancing mechanism of the Hb–AuNCs on luminol–NaIO<sub>4</sub> reaction, the CL spectra were investigated. To obtain the CL spectra, the CL flow cell was placed before the emission window of F–2700 fluorescence spectrophotometer with the excitation source turned off. The CL reagents were continuously driven into the flow cell by peristaltic pumps. The CL signal was recorded by the photomultiplier tube biased at high voltage at 700 V. As shown in Fig. 3b, the CL spectra of two reactions have the same spectrum profile and maximum emission wavelength, suggesting that they share the same CL emitter. The maximum emission wavelength locates at 425 nm, which is the typical emission of

luminol reaction. Therefore, the CL emitter is still the excited state 3-aminophthalate anions, the oxidation product of luminol [34]. The presence of the Hb–AuNCs does not alter the CL emitter, just enhances the CL intensity. Therefore, the enhancing mechanism is therefore attributed to the catalytic effect of the Hb–AuNCs on the luminol–NaIO<sub>4</sub> reaction.

Superoxide anion was reported to be generated from the reaction between periodate and dissolved O<sub>2</sub> in alkaline solution [35]. The superoxide anion can oxidize luminol to produce CL emission [36]. In the presence of the Hb–AuNCs, the reaction between periodate and dissolved O<sub>2</sub> is accelerated and more amounts of superoxide anion are produced. As a result, stronger CL emission is recorded. Dopamine belongs to polyhydroxy compounds. Such kind of compounds have strong reducibility and can be used as scavenging agents of reactive oxygen species. Thus, in the case of dopamine, the produced superoxide anion is eliminated and the CL signal is inhibited.

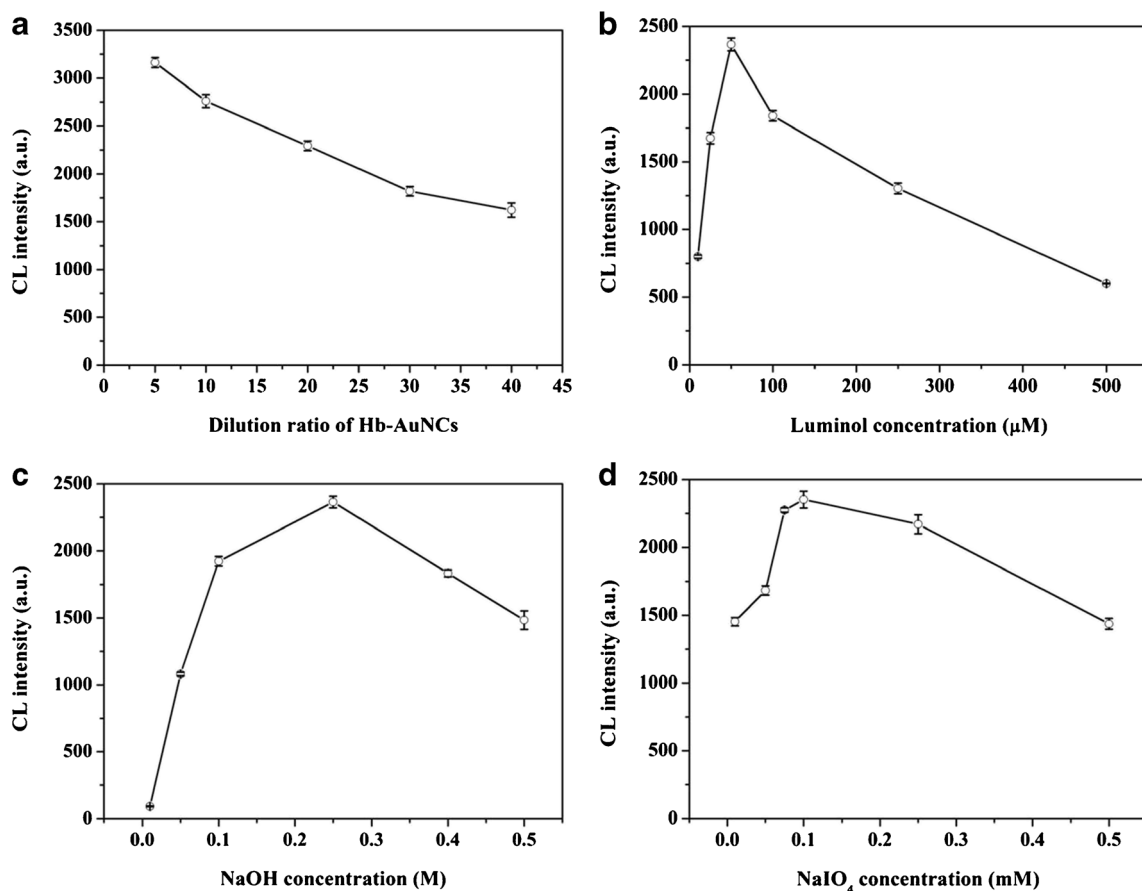
### Optimization of CL conditions

Hb–AuNCs concentration has a critical influence on the CL reaction. The Hb–AuNCs concentration is expressed as the dilution ratio of the Hb–AuNCs with water. Figure 4a shows the relationship between the dilution ratio and the relative CL intensity. The relative CL intensity continues to decrease with the increase in the dilution ratio of the Hb–AuNCs. Considering the sensitivity and reagent consumption, the Hb–AuNCs in a 1:20 dilution ratio is employed.

As the CL reagent, the concentration of luminol has an important influence on the sensitivity. The effect of luminol concentration on the CL reaction was studied in the range of 10–750 μM and the results are shown in Fig. 4b. A maximum relative CL intensity is observed at 50 μM luminol. Higher or lower concentrations of luminol cause a decrease in the relative CL intensity. Thus, 50 μM luminol is selected as the optimum.

Luminol CL reaction occurs in an alkaline condition. The alkalinity of the reaction is controlled by NaOH added into the luminol solution. The relative CL intensity increases as the concentration of NaOH increases from 0.01 M to 0.25 M, (Fig. 4c). The relative CL intensity decreases with the increase in the concentration of NaOH above 0.25 M. The relative CL intensity has a maximum at 0.25 M NaOH and this concentration of NaOH is employed.

Figure 4d shows the influence of the concentration of NaIO<sub>4</sub> on the relative CL intensity. The relative CL intensity increases with increasing the concentration of NaIO<sub>4</sub> up to 0.1 mM. Further increase in the concentration of NaIO<sub>4</sub> results in a decrease in the relative CL intensity. Therefore, 0.1 mM NaIO<sub>4</sub> is selected.



**Fig. 4** Effect of **a** dilution ratio of Hb–AuNCs, **b** luminol concentration, **c** NaOH concentration, and **d** NaIO<sub>4</sub> concentration on relative CL intensity

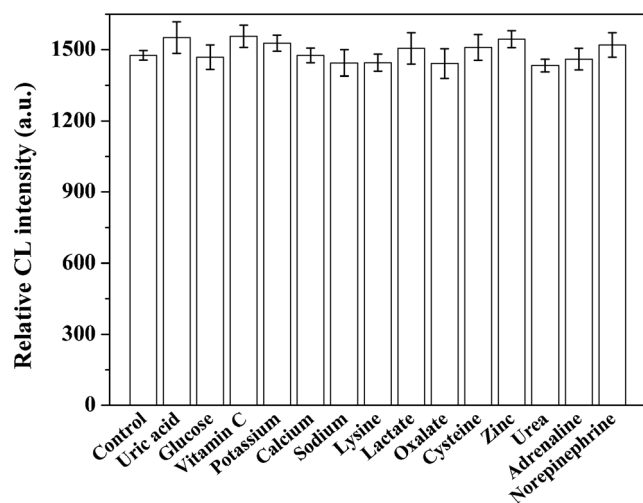
**Response of the system to dopamine**

Under above selected conditions, the response of the system for different concentrations of dopamine was investigated. The logarithm of the relative CL intensity ( $\log \Delta I$ ) was found

to be linear related with the logarithm of concentrations of dopamine ( $\log c$ ) from 0.3 to 9.0 nM (Fig. S3). The linear equation can be expressed  $\log \Delta I = 2.96 + 0.69 \log c$  (nM) ( $r^2 = 0.9921$ ). The limit of detection is 0.1 nM. In comparison with other previously reported methods for the detection of

**Table 1** Comparison with different methods for dopamine determination

Methods	Chemicals used	Linear range (nM)	Detection limit (nM)	Reference
Colorimetry	AgNPRs/chloride	0.5–100	0.16	21
	AuNPs/melamine	33–3.33 × 10 <sup>6</sup>	33	22
Fluorescence	Resorcinol	10–2.0 × 10 <sup>4</sup>	1.8	23
	l-cys ZnS:Mn QDs	150–3000	7.8	24
Electrochemical	RNA aptamer	100–1000	62	25
	ET-SDBS-NPPy/RGO	100–1.0 × 10 <sup>5</sup>	20	26
	Imprinted silica/poly(aniline boronic acid)	50–5.0 × 10 <sup>5</sup>	18	27
	KMnO <sub>4</sub> /HCHO	31–1.7 × 10 <sup>4</sup>	10	28
	Ce(IV)/thiosulfate/C–dots	2.5–2.0 × 10 <sup>4</sup>	1.0	29
	Luminol/H <sub>2</sub> O <sub>2</sub> /HKUST–1	10–700	2.3	30
	Lucigenin/thiourea dioxide	20–800	14.7	32
	K <sub>3</sub> Fe(CN) <sub>6</sub> /g–CNQDs	10–2000	4.7	31
	Luminol/NaIO <sub>4</sub> /Hb–AuNCs	0.3–9.0	0.1	This work



**Fig. 5** The relative CL intensities of dopamine alone and in the presence of interfering species. From left to right: control (dopamine alone), uric acid, glucose, vitamin C,  $K^+$ ,  $Ca^{2+}$ ,  $Na^+$ , lysine, lactate, oxalate, cysteine,  $Zn^{2+}$ , urea, adrenaline and norepinephrine

dopamine, this method has higher sensitivity (Table 1). The 11 consecutive measurements of 5.0 nM dopamine solution produced a relative standard deviation of 3.12%, indicating the precision of the method is acceptable.

### Interference study

In order to apply the method to the determination of dopamine in human plasma, the effect of some potential interfering species was investigated on the determination of 5.0 nM dopamine. The interfering species include 5.0  $\mu M$   $K^+$ ,  $Ca^{2+}$ ,  $Na^+$ , glucose, uric acid, ascorbic acid, 0.5  $\mu M$  lysine, lactate, oxalate, cysteine, ascorbic acid,  $Zn^{2+}$ , 10.0 nM adrenaline and norepinephrine. Figure 5 shows the relative CL intensities of dopamine alone and of the mixture of dopamine with interfering species. In contrast to the CL intensity of dopamine alone, the CL intensities changed negligibly in the presence of interfering species, indicating the present CL system has good selectivity for dopamine. It should be indicated that 1000-fold is the highest tolerable ratio that we examined. The actual tolerable ratios for some interfering species are possible higher than this tolerable ratio.

### Determination of dopamine in spiked human plasma samples

In order to verify the practicality, the method was applied to the determination of dopamine in spiked human plasma samples. Human plasma samples from three health persons were obtained from Xi'an Community Hospital. The protein in plasma samples was removed by centrifuging at 12000 rpm for 10 min. The supernatant was transferred into a test tube and used as sample. Into 0.5 mL of sample, a known amount

of dopamine standard was added and diluted to 10.0 mL with water for detection. The content of dopamine in the sample was determined by standard addition method. As shown in Table S1, the method gave rise to good recoveries and acceptable precision, thus demonstrating the practicality of this method in the determination of dopamine in practical samples.

### Conclusions

In summary, Hb–AuNCs were prepared by incubating  $HAuCl_4$  and Hb at 60 °C for 36 h through a biomineralization process. The Hb–AuNCs significantly catalyze the CL reaction of luminol with periodate and dopamine strongly inhibits the signal. The method allows to the measurement of dopamine in the concentration range of 0.3–9.0 nM. The method is very sensitive and can analyze dopamine in spiked human plasma. Beside adrenaline and norepinephrine also inhibit the CL signal. Further work is in progress aiming at on the determination of other catecholamine with this CL system.

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**Compliance with ethical standards** The author(s) declare that they have no competing interests.

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