



## Glow-type luminol chemiluminescence based on a supramolecular enhancer of cyclodextrin

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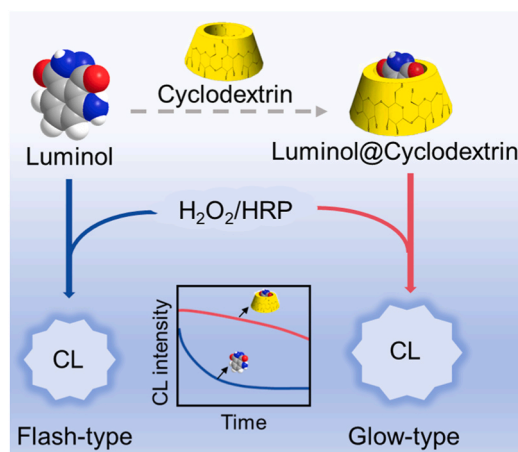
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### HIGHLIGHTS

- A glow-type chemiluminescence system of luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP was presented by using a supramolecular enhancer of cyclodextrin.
- The luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system exhibited a luminescence lifetime of 41 min.
- The luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system realized both CL macroscopic and microscopic imaging detection of HRP with high sensitivity and good precision.
- The luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system enabled point-of-care detection of 17 $\beta$ -estradiol.

### GRAPHICAL ABSTRACT



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### ABSTRACT

**Background:** Chemiluminescence (CL) bioassay is one of the most advanced and used detection method in clinical diagnosis and biomedical research because of the advantages of low background, easy operation, and wide-field imaging without a light source or microscope. The luminol/hydrogen peroxide/horseradish peroxidase (luminol/H<sub>2</sub>O<sub>2</sub>/HRP) system is the most popular CL system, but its application in high-throughput imaging detection is challenged due to its low luminescence efficiency and flash-type emission which is difficult in ensuring the reproducibility and consistency of detection results.

**Results:** We reported a glow-type CL system of luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP by using a supramolecular enhancer of cyclodextrin (CD). This luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system exhibited a luminescence lifetime of 41 min for sensitive and accurate imaging analysis. The long-lasting CL emission was attributed to the formation of a 1:1 host-guest complex between luminol and CD, which could stabilize the emitter and effectively reduce nonradiative

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relaxation. The formation of luminol@CD complex was determined through NMR experiments and theoretical analysis. Under optimum conditions, the luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system showed higher sensitivity and much better precision than classical luminol/H<sub>2</sub>O<sub>2</sub>/HRP system for imaging detection of HRP. Especially, this glow-type luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system realized CL imaging of microwell arrays on microfluidic chips. In addition, the luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system was successfully applied for point-of-care detection of 17 $\beta$ -estradiol based on a competitive mechanism of host-guest recognition.

**Significance:** An efficient CL system is crucial for obtaining reproducible and consistent results for accurate detection. Our luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system emitted strong and persistent luminescence, resulting in reliability and efficiency at both CL macroscopic and microscopic imaging detection. We expected the luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP CL system to be applied in various detection fields.

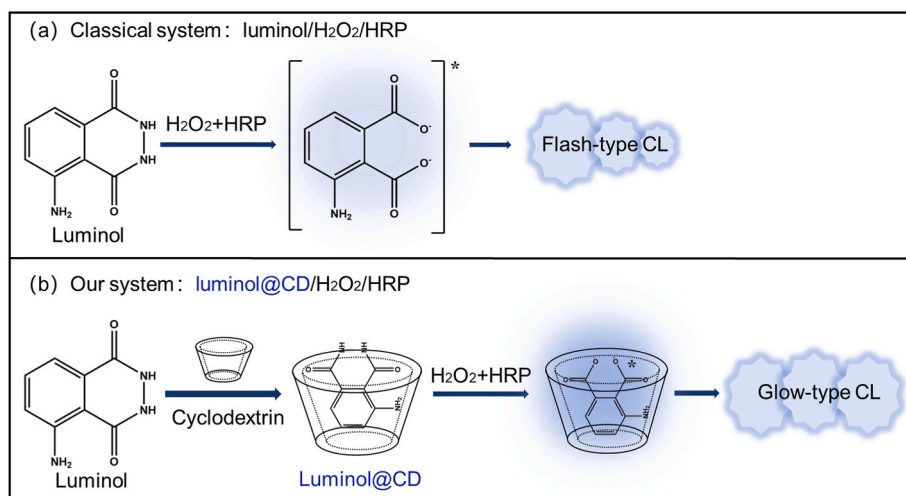
## 1. Introduction

Chemiluminescence (CL) is a kind of light radiation phenomenon accompanying the process of chemical reactions of some special substances [1]. Owing to the advantages of low background, simple instrument, easy operation and low cost, CL bioassay, especially CL immunoassay, is one of the most advanced and widely used detection methods in clinical diagnosis and biomedical research [2,3]. The common liquid-phase CL luminophores include luminol, oxalate esters and adamantylidene-dioxetane [4,5]. Among them, luminol is widely used in CL bioassay due to its ease of synthesis, low toxicity, good solubility and stability [6,7]. Horseradish peroxidase (HRP) is the most used enzyme tag in enzyme-linked biological assays, for example, in enzyme-linked immunoassay, HRP is labeled on the secondary antibody to produce amplified detection signals by catalyzing the reaction of substrates. Therefore, luminol/hydrogen peroxide/HRP (luminol/H<sub>2</sub>O<sub>2</sub>/HRP) system has always been the highly regarded CL system because it is one of the most used detection systems in clinical immunoassay [8]. In this system, HRP catalyzes the reaction between H<sub>2</sub>O<sub>2</sub> and luminol to generate excited state 3-aminophthalic acid, and during the transition from the excited state to the ground state, light with a wavelength located in the blue part of visible light is emitted. (Scheme 1a). However, the excited-state oxidation product of luminol is extremely unstable and easily quenched by the surrounding solution, resulting in weak and flash-type luminescence [9,10]. This makes the classical luminol/H<sub>2</sub>O<sub>2</sub>/HRP system not only difficult to provide reproducible and consistent results for accurate detection, but also require the automatic sampling equipment (for example, flow injection device) and highly sensitive signal reading instrument (for example, photomultiplier tubes), hindering its application in biological analysis [11,12].

Improving the classical luminol/H<sub>2</sub>O<sub>2</sub>/HRP system to achieve intensive and glow-type luminescence has always been hot research in

CL field, as strong and long-lasting emission facilitates signal collection (for example, by charge-coupled device, CCD), which is beneficial for enhancing the detection sensitivity and accuracy, as well as for developing high-throughput imaging assays [13–16]. The most commonly used strategy is to explore enhancers for luminol/H<sub>2</sub>O<sub>2</sub>/HRP system [17]. The reported enhancers include *p*-iodophenol (PIP), substituted phenols, lophine derivatives and analogues, and substituted arylboronic acid derivatives [18,19]. It is usually considered that these enhancers enhance luminol luminescence by affecting the electron density of the luminescent molecules [20]. With the addition of these enhancers, the CL intensity of luminol/H<sub>2</sub>O<sub>2</sub>/HRP system can be instantly enhanced, however, the luminescence lifetime has not been significantly improved and most of them can only extend the CL lifetime to a few minutes [21, 22], which is imperfect for CL detections, especially CL imaging assays.

In recent years, cyclodextrins (CDs) have been reported to be an effective enhancer for boosting luminescence intensity [23,24]. CDs are characterized by a hydrophilic exterior and a hydrophobic central cavity. This distinctive structure endows them with excellent host-guest complexation capabilities and notable biocompatibility. Supramolecular complexes composed of CDs have been applied in both in vitro and in vivo biological imaging detections [25]. For example, Shabat et al. reported that the light emission intensity of the phenoxy-adamantyl-1, 2-dioxane probe was enhanced by 1500 times through supramolecular interactions with 3-Me- $\beta$ -CD, and by using such supramolecular dioxetane CL probe they achieved microscopic cell imaging [26]. Ma et al. reported that the assembly of  $\beta$ -CD with luminol and fluorophores enhanced both the CL intensity of luminol/H<sub>2</sub>O<sub>2</sub>/Co<sup>2+</sup> system and the efficiency of cascade Förster resonance energy transfer [27]. In addition, some studies have also investigated the effect of CD on CL of luminol and its derivatives. It was found that CD could enhance the CL of luminol and isoluminol in alkaline solution with Co<sup>2+</sup> catalyst [28]. However, the effect of CD on luminol's CL depended significantly on the condition and catalysts used, and sometimes contradictory results were obtained [29,



**Scheme 1.** Schematic diagram of CL reaction and emission of (a) the classical system (luminol/H<sub>2</sub>O<sub>2</sub>/HRP) and (b) our system (luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP).

30].

In this work, we investigated the effect of CD on CL intensity and emission kinetics of luminol/H<sub>2</sub>O<sub>2</sub>/HRP system in neutral solution which was commonly required in biological analysis. We confirmed that in the presence of CD, luminol existed in the form of luminol@CD based on the host–guest binding between CD and luminol. This host–guest complex stabilized luminol and its excited-state product within the macrocycle host, allowing the luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system to emit intensive and persistent CL (Scheme 1b). Under optimum conditions, the luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system showed a CL lifetime of 41 min. Compared to the classical luminol/H<sub>2</sub>O<sub>2</sub>/HRP system, this luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system exhibited higher sensitivity and better precision in both macroscopic and microscopic imaging detection of HRP. In addition, based on a competitive mechanism of host–guest recognition, the luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system was used for point-of-care testing (POCT) of 17 $\beta$ -estradiol (E2).

## 2. Experiment section

### 2.1. Reagents and apparatus

HRP, luminol, PIP,  $\beta$ -CD and  $\gamma$ -CD were purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). 3-Me- $\beta$ -CD was bought from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). H<sub>2</sub>O<sub>2</sub> (30 %) and PBS (pH 7.4) were from Sangon Biotechnology Co., Ltd. (Shanghai, China). E2 was purchased from J&K Scientific Ltd., Ltd. (Shanghai, China). The luminol stock solution was self-prepared containing 5 mM luminol, 20 mM PIP and 50 mM NaOH. 5 mM H<sub>2</sub>O<sub>2</sub> aqueous solution was used as the H<sub>2</sub>O<sub>2</sub> stock solution. 20 mM CD in 1  $\times$  PBS (pH 7.4) was used as the CD stock solution. HRP at different concentrations was prepared in 1  $\times$  PBS (pH 7.4).

UV–visible absorption spectra were recorded by a Nanodrop-2000C UV–vis spectrophotometer. Emission spectra were conducted on an F-7000 fluorescence spectrometer. The NMR spectra were obtained using a BRUKER AVANCE III 400 MHz, with D<sub>2</sub>O as the solvent. Molecular docking utilized Autodock 4.2 software. The structure of luminol, CD and their complex were manually constructed using GaussView software. Molecular dynamics simulations were conducted using Gromacs 2022.3 software. CL imaging was implemented both by the BioSpectrum 615 Imaging System (UVP, USA) and Tanon 5200 Imaging System (China).

### 2.2. CL macroscopic imaging detection of HRP

CL macroscopic imaging was implemented on the 96-well plate with 50  $\mu$ L working solution per well. The working solution was freshly prepared by sequentially mixing stock solution of luminol, CD, HRP and H<sub>2</sub>O<sub>2</sub>. In this article, unless otherwise specified, CD referred to 3-Me- $\beta$ -CD. For CL imaging by classical luminol/H<sub>2</sub>O<sub>2</sub>/HRP system, the working solution was prepared with 5  $\mu$ L luminol, 39  $\mu$ L PBS, 1  $\mu$ L HRP and 5  $\mu$ L H<sub>2</sub>O<sub>2</sub>. For CL imaging by our luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system, the working solution was prepared with 5  $\mu$ L luminol, 20  $\mu$ L 3-Me- $\beta$ -CD, 19  $\mu$ L PBS, 1  $\mu$ L HRP and 5  $\mu$ L H<sub>2</sub>O<sub>2</sub>. The CL images were collected by BioSpectrum 615 Imaging System with an exposure time of 30 s.

### 2.3. CL microscopic imaging detection of HRP

CL microscopic imaging was implemented on a polydimethylsiloxane (PDMS) microfluidic chip with a 10  $\times$  10 microwell array (200  $\mu$ m in diameter and interval). After the microfluidic chip was vacuumed, the working solution was injected automatically from the inlet. After all the microwells were filled with working solution, air was injected slowly to form independent droplets. Finally, the CL images were collected by Tanon 5200 Imaging System with an exposure time of 30 s.

### 2.4. POCT detection of E2

POCT detection of E2 was performed by sequentially mixing 5  $\mu$ L luminol stock solution, 20  $\mu$ L  $\gamma$ -CD (20 mM), 14  $\mu$ L PBS, 5  $\mu$ L E2 sample at different concentration, 1  $\mu$ L HRP (2.5  $\mu$ g mL<sup>-1</sup>) and 5  $\mu$ L H<sub>2</sub>O<sub>2</sub>, and then immediately adding this solution to the 96-well plate for CL imaging by BioSpectrum 615 Imaging System with an exposure time of 30 s. The POCT detection of E2 was completed within 1 min.

## 3. Results and discussion

### 3.1. Luminescence characterization of luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system

The CL performance of luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system in pH 7.4 PBS was investigated by continuously imaging the system at every half minute. Here, 3-Me- $\beta$ -CD was used as the model CD enhancer. Compared to classical luminol/H<sub>2</sub>O<sub>2</sub>/HRP system, our luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system exhibited significantly stronger intensity and slower kinetic emission (Fig. 1a and Fig. S1). Based on the decay curve of CL, the luminescence lifetime of luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system was calculated to be 41 min, which was 13 times longer than that of the classical luminol/H<sub>2</sub>O<sub>2</sub>/HRP system. The CD induced slow kinetic emission caused the retention of CL intensity after 10 min increased from 13 % (classical luminol/H<sub>2</sub>O<sub>2</sub>/HRP system) to 82 % (our luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system), showing the latter was more conducive for CL imaging analysis (Fig. 1b). In addition, by integrating the CL intensity of each image taken continuously within 10 min, it was found that the total CL intensity ( $I_{\text{total}}$ ) of our luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system was 3 times higher than that of classical luminol/H<sub>2</sub>O<sub>2</sub>/HRP system, indicating that CD could effectively improve the luminescence efficiency of luminol.

The CL generation of luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system was studied based on UV–visible absorption and CL spectra. Neither the absorption peaks of luminol nor the emission peak of luminol/H<sub>2</sub>O<sub>2</sub>/HRP system shifted after the addition of 3-Me- $\beta$ -CD (Fig. 1c and d), indicating that the presence of 3-Me- $\beta$ -CD did not trigger a new CL mechanism different from the luminol/H<sub>2</sub>O<sub>2</sub>/HRP system. The intensive and glow-type CL emission of luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system might be attributed to the formation of the host–guest complex between luminol and CD, which could effectively stabilize luminol and its excited-state product to reduce nonradiative relaxation.

### 3.2. Characterization of luminol@CD complex

We studied the formation of the host–guest complex of luminol@CD using proton NMR chemical shifts (Fig. 2). The coexistence of luminol and 3-Me- $\beta$ -CD caused a small upfield shift of H (H<sup>4</sup>, H<sup>5</sup>, H<sup>6</sup>, H<sup>7</sup>) on the 3-Me- $\beta$ -CD and a small downfield shift of H (H<sup>1</sup>, H<sup>2</sup>, H<sup>3</sup>) on the luminol. This was attributed to the shielding of the ring current effect of aromatic systems in luminol, indicating the access of luminol to CD cavity [31].

Fig. 3a showed the 1:1 host–guest complex model of 3-Me- $\beta$ -CD and luminol, exhibiting the lowest energy docked conformation. The molecular docking analysis showed that the luminol was vertically embedded deep into the cavity of 3-Me- $\beta$ -CD, and the hydrogen bond and hydrophobic interactions were the key factors for the interaction of the complex [32]. The number of hydrogen bonds between luminol and 3-Me- $\beta$ -CD with simulation time was shown in Fig. 3b. Molecular dynamics (MD) simulations have been used to investigate the preferred host–guest binding mode of molecules and CDs [33]. Fig. 3c showed the RMSD (Root Mean Square Deviation) plot of trajectories calculated through 50 ns molecular dynamics simulation to the 1:1 host–guest complex. The RMSD curve of luminol@CD was stable and fluctuated within a fixed range, indicating the dynamic equilibrium state of luminol@CD complex.

CL enhancement was also observed in luminol/H<sub>2</sub>O<sub>2</sub>/HRP system when using other types of CDs, for example,  $\beta$ -CD and  $\gamma$ -CD (Fig. S2a). The molecular simulation analysis of 1:1 host–guest complex between

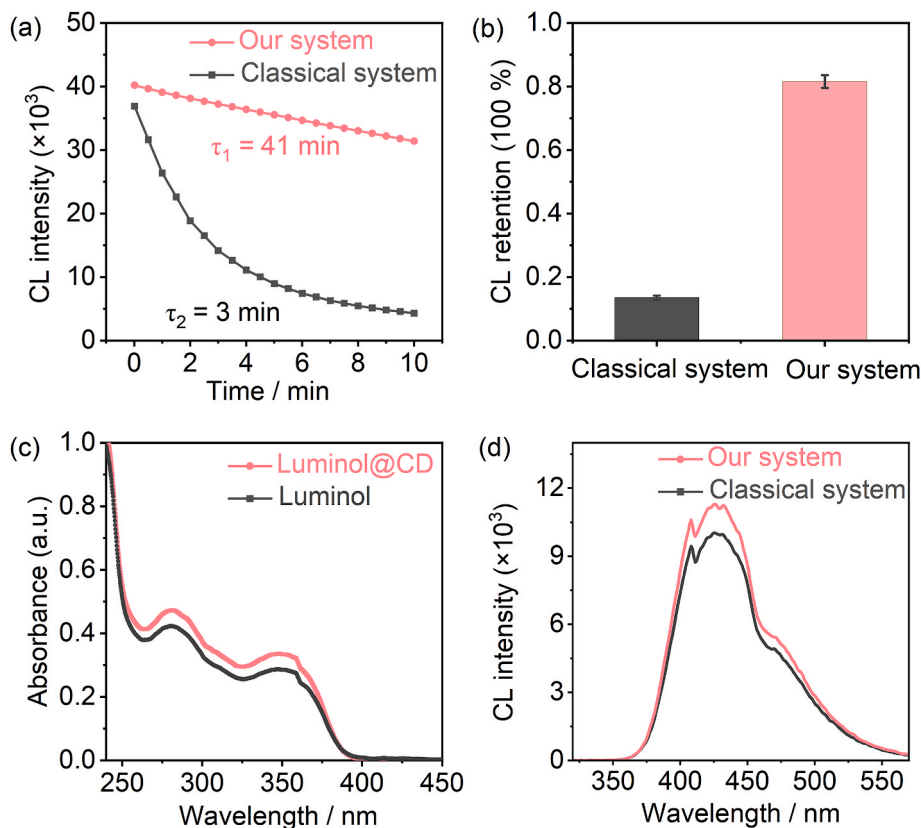


Fig. 1. (a) CL intensity over time, (b) the retention percentage of CL intensity after 10 min, (c) UV-vis absorption and (d) CL spectra of classical system (luminol/H<sub>2</sub>O<sub>2</sub>/HRP) and our system (luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP).

luminol and the three CDs showed the same number of hydrogen bonds. In addition, all the calculated binding energy was negative, suggesting that the host-guest reaction between luminol and the three CDs was thermodynamically spontaneous and active (Fig. S2b).

### 3.3. CL macroscopic imaging detection of HRP on 96-well plates

CCD imaging can perform high-throughput analysis and is suitable for multiplex detection on 96-well plates and chip arrays. In order to achieve reliable and accurate detection, glow-type CL system is highly required for CL imaging. In this work, we present a glow-type CL system of luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP and investigated its application in imaging detection of HRP on both 96-well plates and microfluidic chips. Previous studies reported that the CL intensity and kinetics of luminol CL systems would be affected by the concentration of both oxidants and enhancers [20]. Thus, the concentration of H<sub>2</sub>O<sub>2</sub> and 3-Me- $\beta$ -CD in our luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system were optimized firstly to ensure intensive and persistent CL (Fig. S3 and Fig. S4). As the concentration of H<sub>2</sub>O<sub>2</sub> increased, although the CL kinetics of luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system continued to slow down (Fig. S3b), the CL intensity showed a change of first increasing and then decreasing (Fig. S3c). Taking into account both the CL intensity and kinetics, 5 mM H<sub>2</sub>O<sub>2</sub> was chosen to be used in luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system. Similarly, the CL kinetics also slowed down with increasing 3-Me- $\beta$ -CD concentration (Fig. S4b), but the CL intensity decreased sharply when the concentration of 3-Me- $\beta$ -CD exceeded 20 mM (Fig. S4c). Hence, 3-Me- $\beta$ -CD concentration in luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system was optimized to 20 mM.

Under the optimized conditions, CL images of HRP at different concentrations using the proposed luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system were continuously captured every minute over a 10-min period (Fig. 4a). Obviously, CL images by luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system showed stabler and stronger luminescence than that by classical

luminol/H<sub>2</sub>O<sub>2</sub>/HRP system (Fig. 4b). This phenomenon resulted in the detection sensitivity of HRP by our system being 4 times higher than that of the classical system (Fig. 4c). More importantly, our system exhibited better consistency of the imaging detection linear curves over the 10-min period relative to the classical system. The relative standard deviation (RSD) of sensitivity of our system was 8 %, which was much lower than that of 91 % of classical system (Fig. 4c).

In addition, the practical applicability of luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system in CL imaging detection was evaluated by consecutively sampling 24 parallel samples on 96-well plates. The results showed that the RSD of our system (12 %) was much lower than that of the classical system (53 %) (Fig. 4d and Fig. S5), suggesting the better precision and reliability of our luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system than classical luminol/H<sub>2</sub>O<sub>2</sub>/HRP system in CL imaging detection.

### 3.4. CL microscopic imaging detection of HRP in microfluidics

Microfluidic chips are one of the most popular platform for single-cell analysis and highly sensitive digital bioassay due to the ease of preparing high-throughput microwell arrays [34]. However, the signal readout of microfluidic chips often relies on high-precision fluorescence microscopy. Although CL imaging device is simple and has advantages of requiring no light sources and the ability of imaging all array points with a large window at once, CL imaging is rarely used in microfluidic chips due to the lack of an effective CL system with strong and persistent luminescence [35]. Here, CL microscopic imaging performance of our luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system was investigated on a single-channel 10  $\times$  10 array chip with microwells in 200  $\mu$ m diameter and interval (Fig. 5a,b,c). In contrast to the failure of CL microscopic imaging by classical luminol/H<sub>2</sub>O<sub>2</sub>/HRP system (Fig. 5d), a clear, intact, distinguishable and high precision (RSD < 10 %) CL microscopic imaging of the microwell array was obtained by our luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP

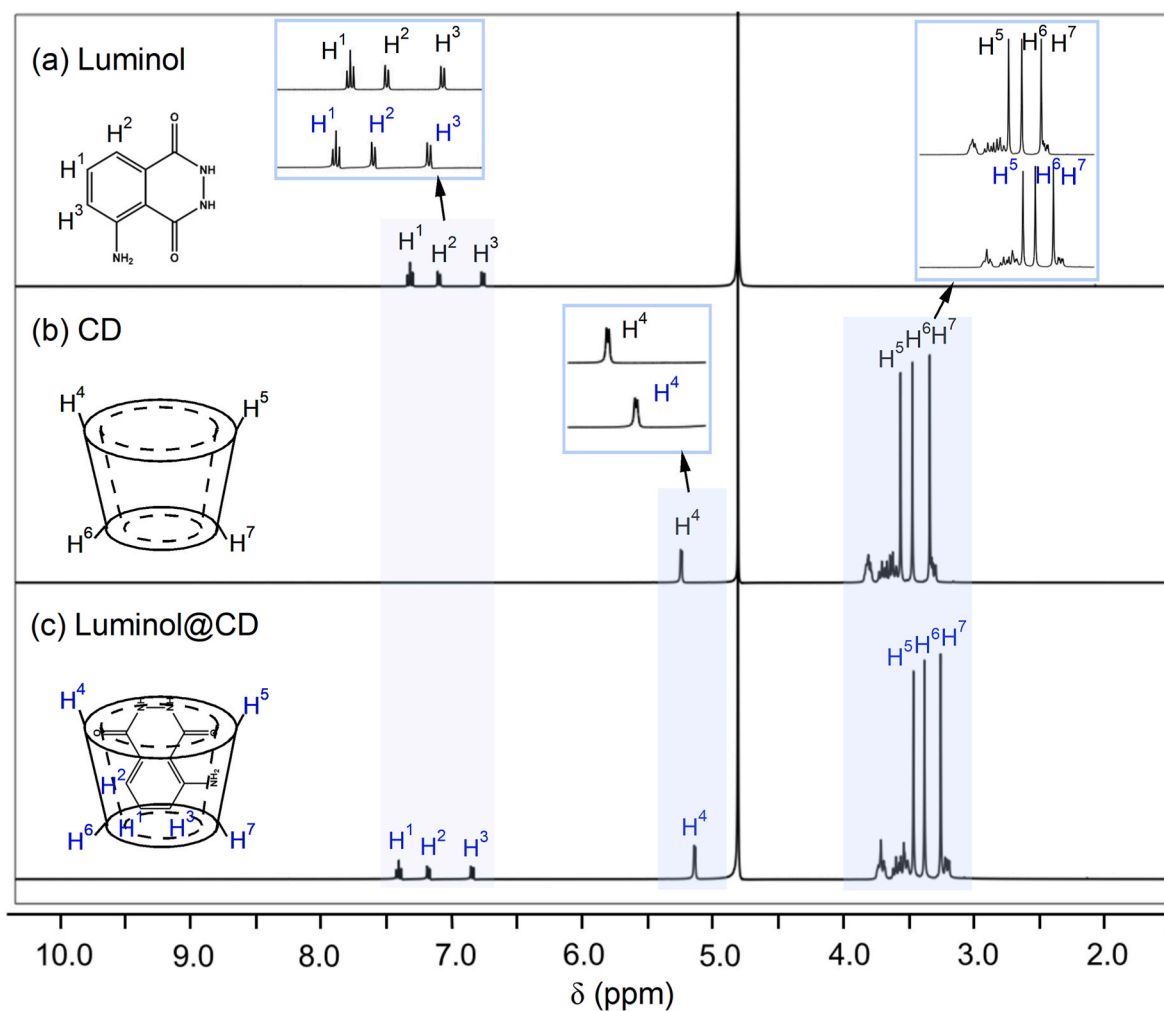


Fig. 2.  $^1\text{H}$  NMR spectra of (a) luminol, (b) CD and (c) luminol@CD in 100 %  $\text{D}_2\text{O}$ , CD referred to 3-Me- $\beta$ -CD.

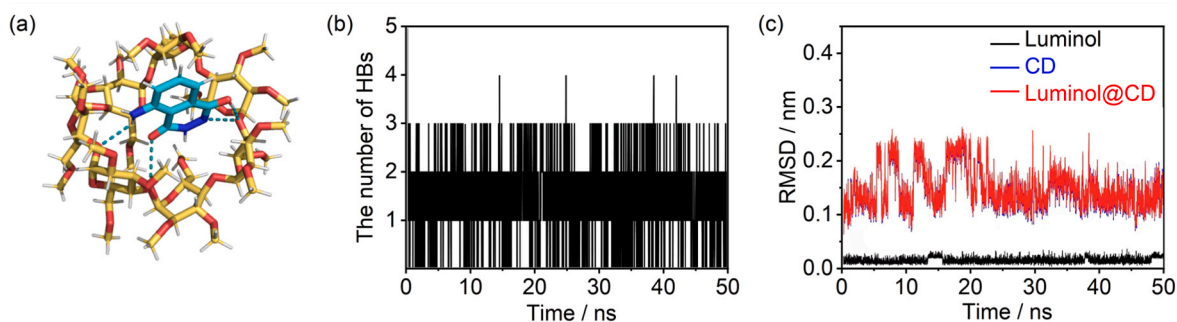


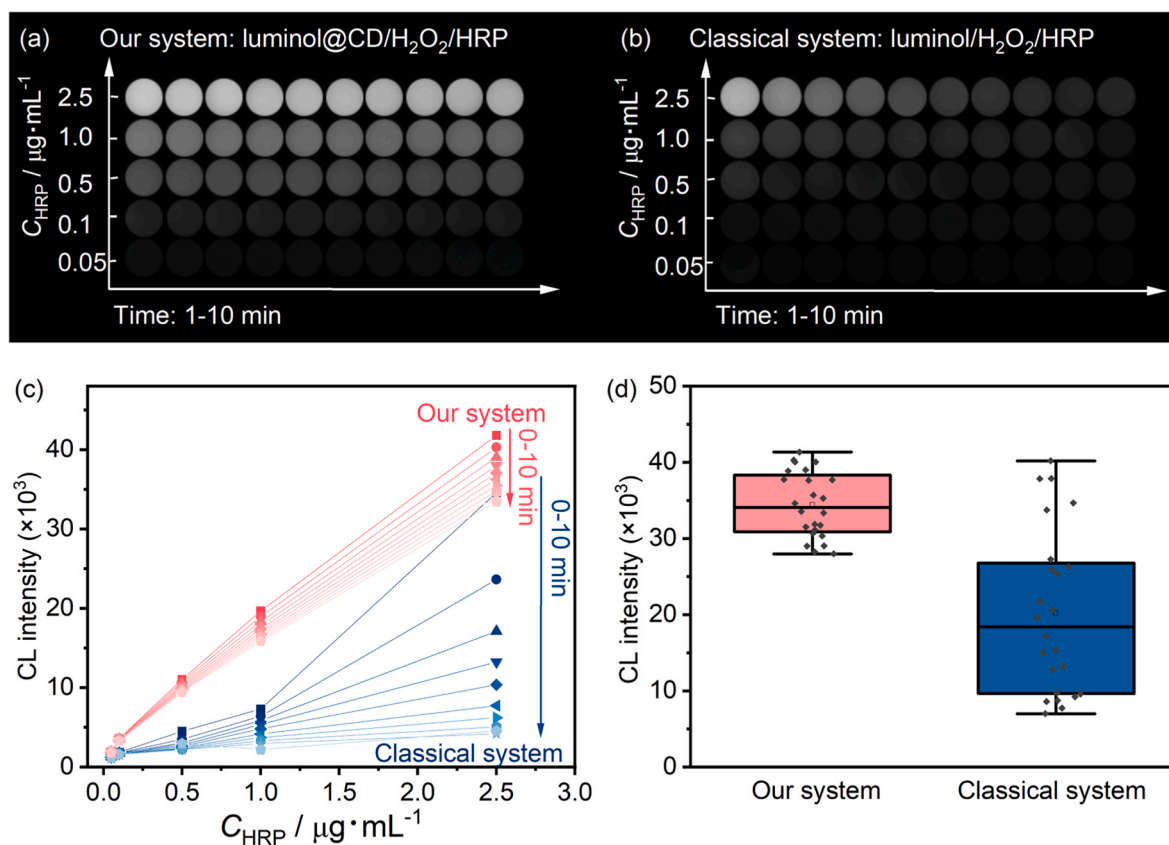
Fig. 3. (a) The lowest energy molecular docking figure of 3-Me- $\beta$ -CD (wheat sticks) and luminol (cyan sticks). (b) The number of hydrogen bonds (HBs) of luminol@CD with simulation time. (c) The RMSD curves of 3-Me- $\beta$ -CD, luminol and luminol@CD. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

system (Fig. 5e), indicating its application in CL imaging of microfluidics.

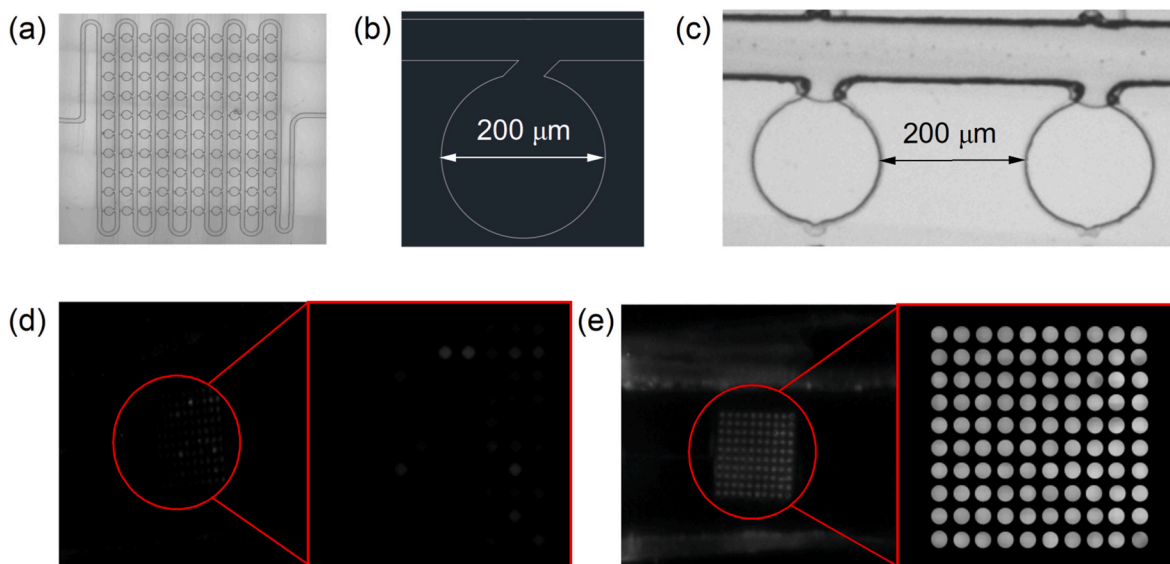
### 3.5. POCT detection of E2

E2 is a naturally occurring estrogenic steroid which has become a serious environmental problem due to its overuse in medicine and animal husbandry [36,37]. A highly reliable and convenient method for E2 analysis is needed. It has been reported that E2 can form stable inclusion complexes with CDs [38]. Here, by using the luminol@CD/ $\text{H}_2\text{O}_2$ /HRP

CL system, we proposed a POCT detection of E2 based on a competitive mechanism of host-guest recognition between luminol and E2 for CDs. In the presence of E2, E2@CD complexes were competitively formed, which caused luminol unprotected and resulted in the decrease of CL intensity (Fig. 6a).  $\gamma$ -CD was chosen for POCT detection of E2 based on its higher signal-to-noise ratio (SNR) than 3-Me- $\beta$ -CD and  $\beta$ -CD (Fig. 6b). The proposed method showed good linearity for E2 detection ranging from  $1.0 \times 10^{-7}$  to  $1.0 \times 10^{-3}$  M (Fig. 6c). Although this work exhibited a higher detection limit of E2 than the literature works (Table S1), its detection time (<1 min) was much shorter, showing unrivaled



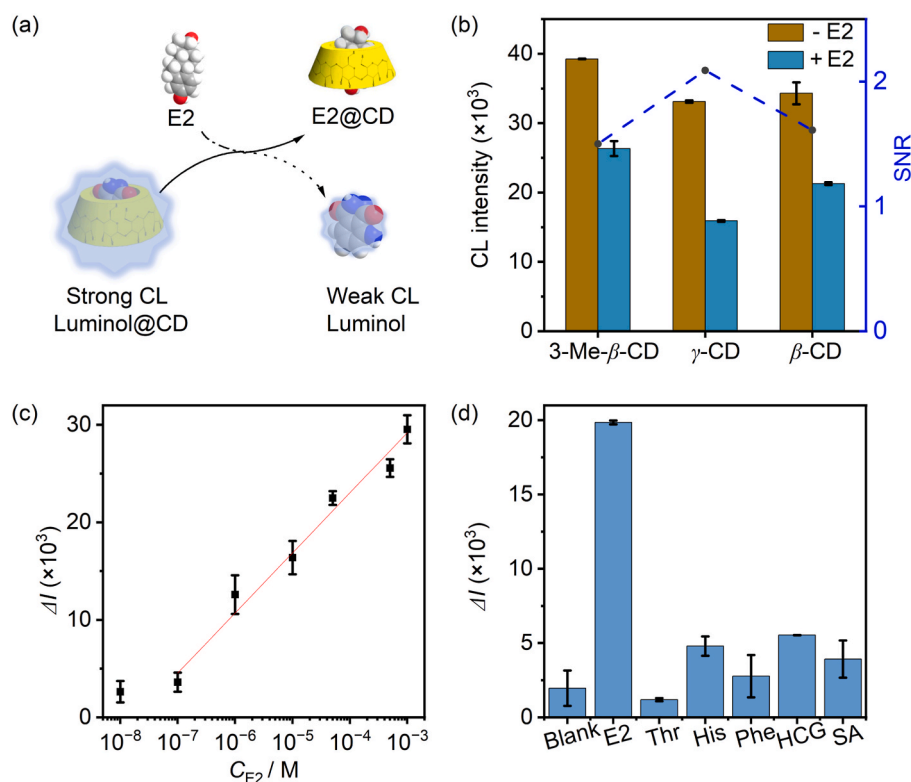
**Fig. 4.** CL images of (a) our and (b) classical systems against different concentrations of HRP. (c) Linear relationship between CL image intensity and HRP concentration at different times. (d) Box plot of CL intensity for 24 parallel samples, with 3 samples added each time in 8 consecutive batches, within 3 min. The sample solution was prepared with 2.5 μg mL<sup>-1</sup> HRP.



**Fig. 5.** (a) Microscopic graph of the microwell array on microfluidic chip. (b) Vector image of a microwell. (c) Microscopic image of microwells filled with CL reaction solution. CL imaging of the microwell array with classical (d) and our (e) CL systems.

advantage in POCT detection. The specificity of POCT detection of E2 was investigated with substances in farm effluents, for example, phenylalanine (Phe), histidine (His), threonine (Thr), human chorionic gonadotropin (HCG), stearic acid (SA). Besides E2, no obvious signal was observed by adding these interference substances in luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP CL system, showing the good specificity of this

POCT E2 detection method (Fig. 6d). Based on the competitive mechanism of host-guest recognition, we expected that the luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP CL system would be applied for POCT detection of other substances that could form stable inclusion complexes with CD, for example, cholesterol [17], dopamine [24], tetracycline [39], and nicotine [40].



**Fig. 6.** (a) Schematic representation of the direct detection of E2 with luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP CL system. (b) CL imaging detection of E2 with different types of CDs. (c) The linear relationship between the absolute change in CL intensity ( $\Delta I$ ) and E2 concentration. (d) Specificity evaluation of the CL imaging detection of E2. The concentrations of E2 and interfering substances were both 20  $\mu$ M.

#### 4. Conclusions

In this study, we presented a glow-type CL system of luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP by using CD as a supramolecular stabilizer of luminol to reduce its nonradiative relaxation and enhance the CL emission. The formation of the 1:1 host-guest complexes between luminol and CD were characterized by NMR experiments and theoretical analysis. The CD enhancer could extend the lifetime of classical luminol/H<sub>2</sub>O<sub>2</sub>/HRP system from 3 to 41 min. The luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system exhibited high sensitivity and good precision in both CL macroscopic and microscopic imaging detection of HRP. Moreover, based on a competitive host-guest recognition mechanism, the luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP system realized POCT detection of E2. Overall, the proposed glow-type CL system of luminol@CD/H<sub>2</sub>O<sub>2</sub>/HRP showed strong and persistent luminescence, providing a reliable and efficient CL imaging system for clinical diagnostics and biomedical research.

#### CRedit authorship contribution statement

**Wenrui Hu:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Hang Ao:** Validation, Investigation, Formal analysis, Data curation. **Ziyi Lv:** Validation, Formal analysis, Data curation. **Wencheng Xiao:** Formal analysis, Data curation. **Wei Li:** Formal analysis. **Jianping Lei:** Writing – review & editing. **Jie Wu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization. **Huangxian Ju:** Writing – review & editing, Funding acquisition.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aca.2024.343182>.

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