Supporting Information For

Smartphone-assisted low-cost paper-based colorimetric and fluorescent dual-response sensor for visual evaluation of food freshness

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Table of contents

Experimental section

Materials and instruments

$CuSO_4$, GSH, HCy, Cys, KCl, NaCl, $NaHSO_3$, $NaSO_4$, aniline (An), triethylamine (TEA), tryptamine (Try), tyramine (Tyr), histamine (His), spermine (Spm), Spermidine (Spd), cadaverine (Cad) and putrescine (put) were purchased from Shanghai McLean Biochemical Technology Co., Ltd. Dimethyl sulfoxide (DMSO) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Acetone was purchased from Guangzhou Anjiehui Trading Co. Ultrapure water was supplied by a laboratory ultrapure water meter (Pall-cascada III). Filter paper (Jiaojie qualitative grade) with a diameter of 7 cm was used.

^{1}H NMR spectra were conducted with Bruker AVANCE III 400 and 600 spectrometer. The absorption spectra were carried out on U-3900H spectrophotometer (HITACHI). The fluorescence spectra were performed on FluoroMax-4 spectrometer. The fluorescence imaging was performed on inverted fluorescence microscope (NIKON, Ti-S, Japan). FTIR spectra were recorded on a Thermo Fisher spectrometer (iS50R, USA). A smartphone (iPhone 15 Pro) equipped with the color recognition application “Color Name Recognizer Camera” was used for image capture and analysis. Photography under UV light was performed using a ZF-23max UV dark box (Shanghai Xiniu, China).

Synthesis of compound B.

A solution of 1,1,2-Trimethyl-1H-benz[e]indole (1 g, 4.78 mmol) and iodomethane (446 μL, 7.16 mmol) in acetonitrile (5 mL) was stirred at 90 °C

overnight. The crude product was then recrystallized from anhydrous ether to afford the desired compound as a white solid (1.58 g, 94% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.37 (d, *J* = 8.4 Hz, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 8.22 (d, *J* = 8.2 Hz, 1H), 8.11 (d, *J* = 8.9 Hz, 1H), 7.82-7.77 (m, 1H), 7.75-7.71 (m, 1H), 4.10 (s, 3H), 2.88 (s, 3H), 1.76 (s, 6H).

Synthesis of compound BOH.

Compound B (600 mg, 1.71 mmol) and 4-hydroxybenzaldehyde (314 mg, 2.56 mmol) were dissolved in ethanol (5 mL) and heated under reflux at 80 °C in a sealed tube overnight. After cooling to room temperature, the solvent was removed under reduced pressure. The resulting residue was washed with anhydrous diethyl ether, and the solid collected by filtration was dried in a vacuum oven to give an orange-red solid (620 mg, 80% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.49-8.39 (m, 2H), 8.28 (d, *J* = 8.9 Hz, 1H), 8.21 (d, *J* = 8.2 Hz, 1H), 8.15 (d, *J* = 8.7 Hz, 2H), 8.07 (d, *J* = 9.0 Hz, 1H), 7.80 (t, *J* = 7.0 Hz, 1H), 7.71 (t, *J* = 7.5 Hz, 1H), 7.51 (d, *J* = 16.3 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 4.22 (s, 3H), 2.00 (s, 6H).

Optical response of probe BOH toward cadaverine (Cad)

Firstly, a 1 mM stock solution of the probe BOH was formulated in dimethyl sulfoxide (DMSO). Subsequently, a 1 mM stock solution of cadaverine was obtained by dilution with pure water. The test system consisted of DMSO: pure water = 1:1 (v: v), with a final concentration of probe BOH at 10 μM and cadaverine at 0 ~ 20 μM. The absorption and fluorescence spectra (excitation at

550 nm; slit width: 3 nm/3 nm) of the mixed solution were measured immediately after preparation. Photographs of the solution were also taken under daylight.

Fabrication of the paper-based tag

Probe BOH was dissolved in an acetone/DMSO (29:1, v/v) mixture to prepare stock solutions at concentrations of 10 μM, 100 μM, 500 μM, 1 mM, and 5 mM. Each solution (20 μL) was then spotted uniformly onto filter paper, avoiding overlapping areas, and air-dried at room temperature. Circular tags with a diameter of 1 cm were punched from the dried paper. According to the concentration of the probe solution, the resulting tags were designated as BOH1@tag (10 μM), BOH2@tag (100 μM), BOH3@tag (500 μM), BOH4@tag (1 mM), and BOH5@tag (5 mM). All tags were stored sealed under dry conditions for further use.

Smartphone-based analysis and calibration

(1) Image capture standardization: Use of a homemade light-shielding box with fixed LED lighting to eliminate ambient light interference.

(2) Color value extraction: The average RGB values of a defined circular region on each tag were obtained using the “Color Name Recognizer Camera” app (v1.9, iOS).

(3) Signal calculation: The analytical signal was defined as the B/G intensity ratio.

(4) Calibration: The mean B/G ratio (n=3 tags) at each concentration was plotted to generate the calibration curve. The LOD was calculated as 3σ/slope.

Response evaluation of the paper-based tag toward cadaverine or putrescine

1 mM stock solutions of cadaverine or putrescine were prepared by aqueous dilution, followed by serial dilution to a range of concentrations (50–350 μM). The response of the paper-based tag BOH4@tag to cadaverine or putrescine was evaluated using a titration method. Aliquots of each concentration were applied dropwise uniformly onto the BOH4@tag and allowed to react until no further color change was observed visually. After removing any excess solution with absorbent paper, the tags were photographed using a smartphone under daylight or in a UV light dark box (365 nm). The resulting images were analyzed using a color recognition application on the smartphone to extract and process the RGB values. The image analysis was performed using the 'Color Name Recognizer Camera' application (version 1.9, freely available on the Apple App Store for iOS devices). This application extracts the red (R), green (G), and blue (B) values from a user-defined region of interest in a captured image. To ensure reliability and reproducibility, all measurements were conducted under controlled lighting conditions using a homemade light-shielding box. The same smartphone (iPhone model: iPhone 15 pro) and application settings were used throughout the experiments. The consistency and linear response of the application for quantitative analysis were validated against standard color cards prior to sample testing.

Selectivity evaluation of the paper-based tag toward various analytes

For biothiol analytes, including cysteine (Cys), glutathione (GSH), and homocysteine (HCy), aqueous solutions were prepared at a final concentration of 200 μM. Various ionic interferents—namely NaCl, $CuSO_4$, KCl, $NaHSO_3$, and Na_2SO_4—were each dissolved in water to a final concentration of 1 mM. Regarding amine species, aniline, triethylamine, tryptamine, tyramine, histamine, spermine, spermidine, cadaverine, and putrescine were prepared as aqueous solutions with a final concentration of 200 μM. The response characteristics of these species toward the paper-based tag BOH4@tag were subsequently evaluated using the same titration method. Photographs of the tags were taken under daylight or in a UV light dark box (365 nm), and the RGB values of each tag were extracted and analyzed using a smartphone color recognition application.

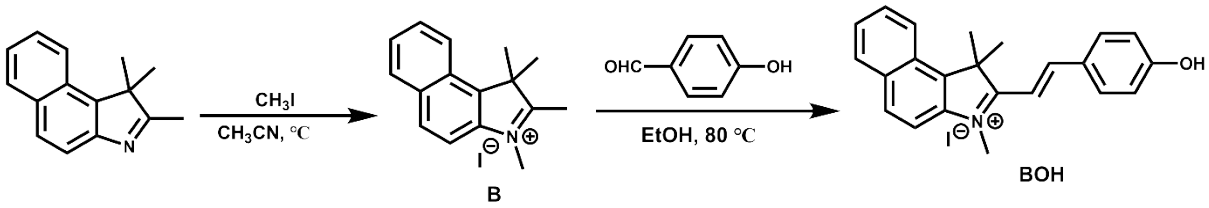
Freshness assessment of live shrimp and chicken breast using the paper-based tag

Fresh live shrimp and chicken breast were purchased from a local market. The prepared paper-based BOH4@tag was utilized to evaluate the freshness changes of these two food products under different storage conditions. Initially, the chicken breast was portioned into approximately 5 g pieces, while live shrimp of similar size and mass were selected. The samples were blotted dry, placed in clean Petri dishes, and a BOH4@tag was affixed to each dish. The dishes were then covered, sealed with sealing film, and stored at different temperatures (25 °C, 4 °C, and –20 °C) for various durations. At designated time points (0, 4,

8, 12, 24, 36, and 48 h), the samples were retrieved and photographed under daylight or in a UV light dark box. Simultaneously, the TVB-N values of each group at corresponding time points were determined according to the Chinese National Standard method for the determination of total volatile basic nitrogen in foods (GB 5009.228-2016).

Statistical analysis

All quantitative sensing experiments were performed in at least triplicate. Data are presented as the mean ± standard deviation (SD)



Scheme S1 The synthesis route of probe BOH.

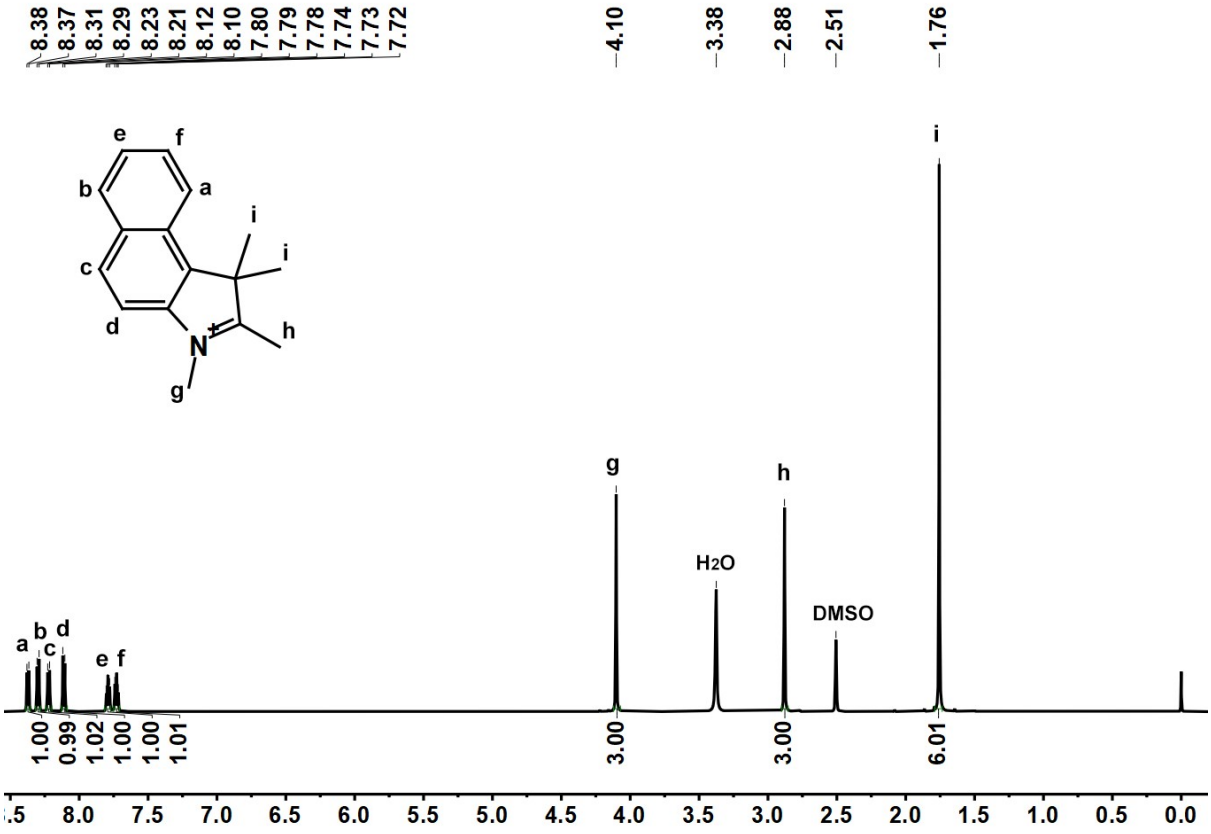


Fig. S1 ^{1}H NMR spectrum of compound B (d_6-DMSO).

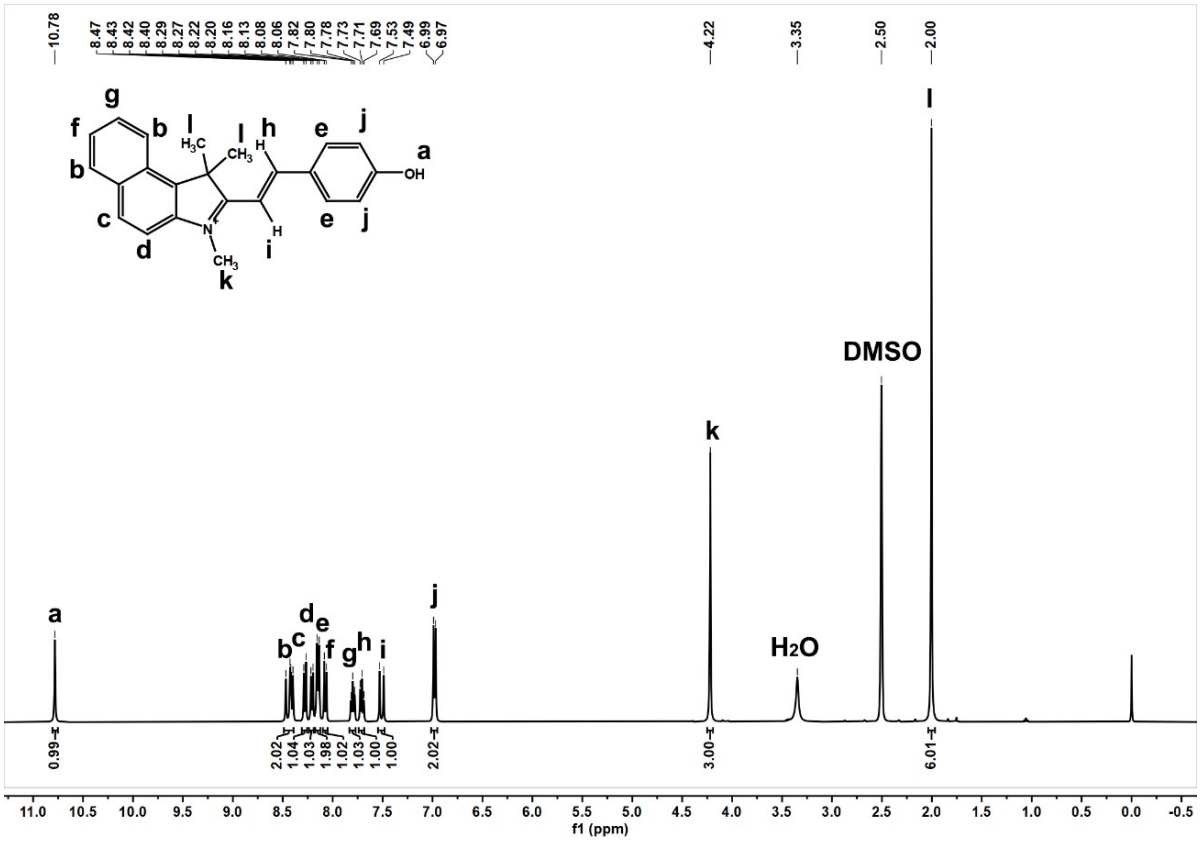


Fig. S2 ^{1}H NMR spectrum of compound BOH (d_6-DMSO).

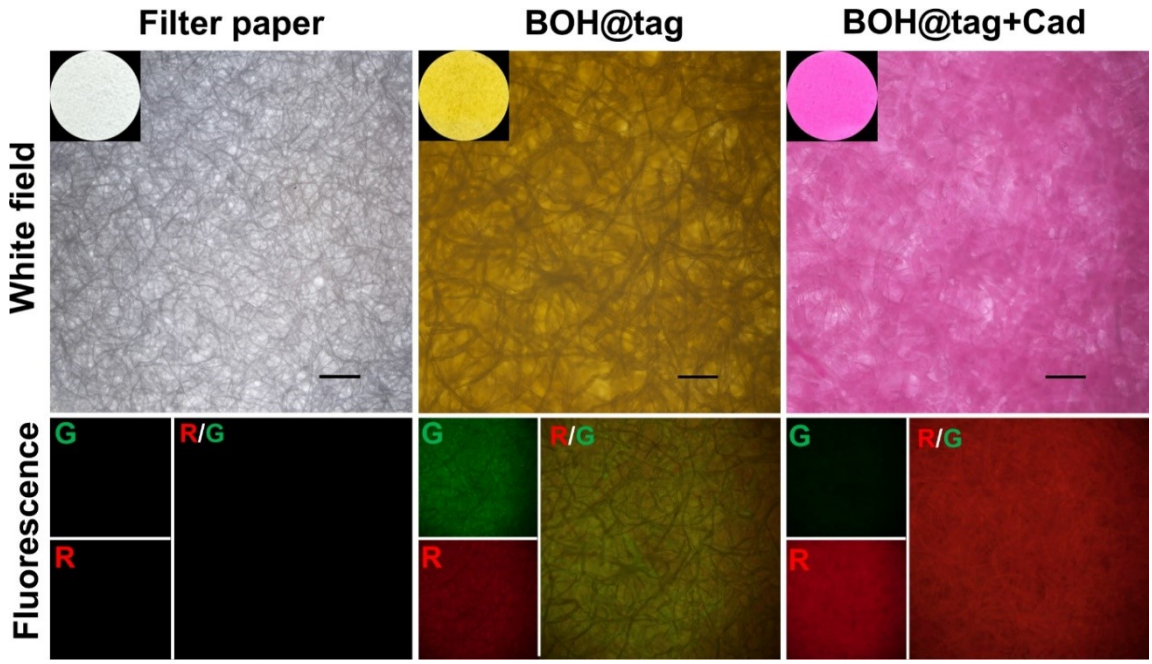


Fig. S3 Fluorescence microscopy images of blank filter paper, the BOH@tag and the BOH@tag after reaction with cadaverine. Scale bar: 200 μm. For green channel: E_x=465-495 nm, E_m=512-552 nm; for red channel: E_x=540-580 nm, E_m=600-660 nm.

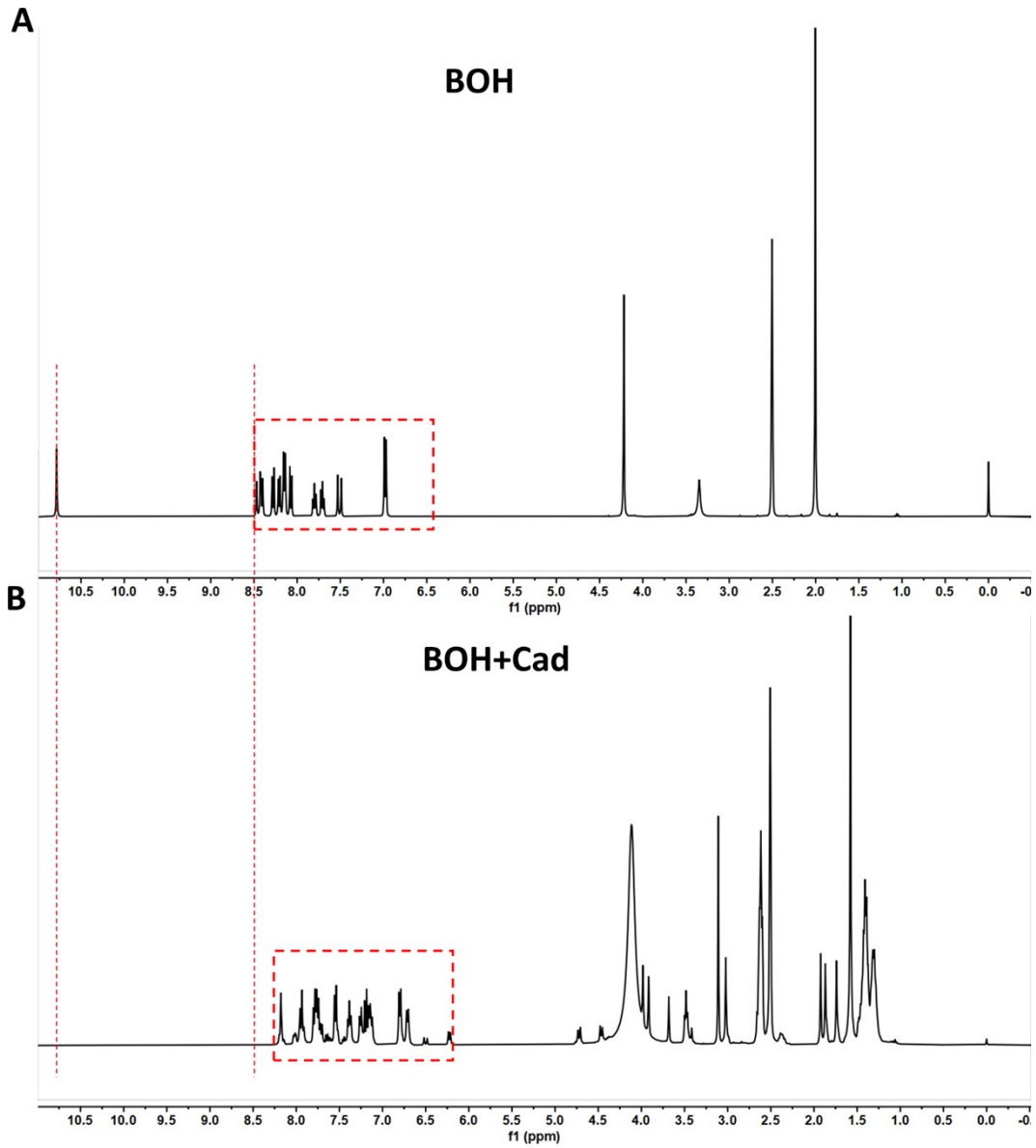


Fig. S4 ^{1}H NMR spectra of probe BOH (A) before and (B) after reaction with Cad.

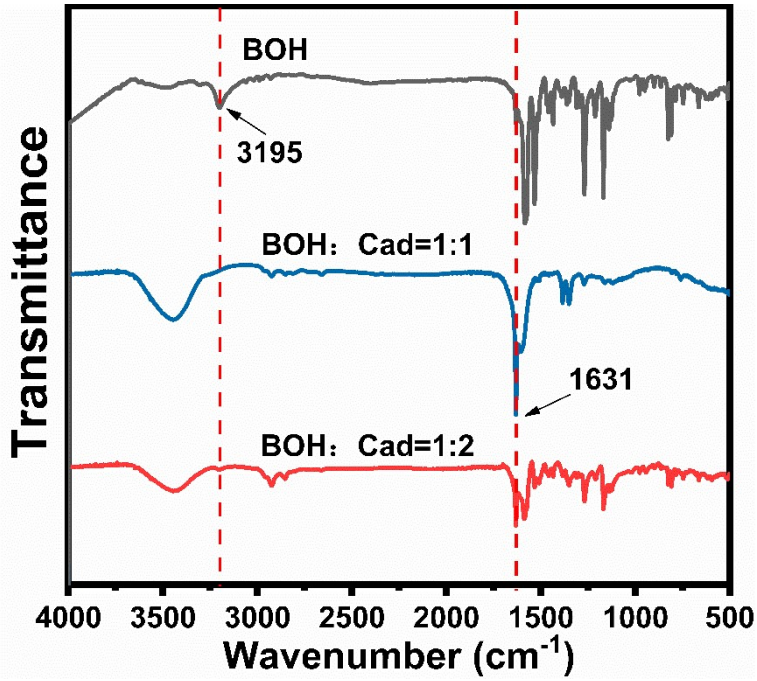


Fig. S5 Comparison of the FT-IR spectra of BOH, and BOH mixed with Cad at 1:1 and 1:2 molar ratios.

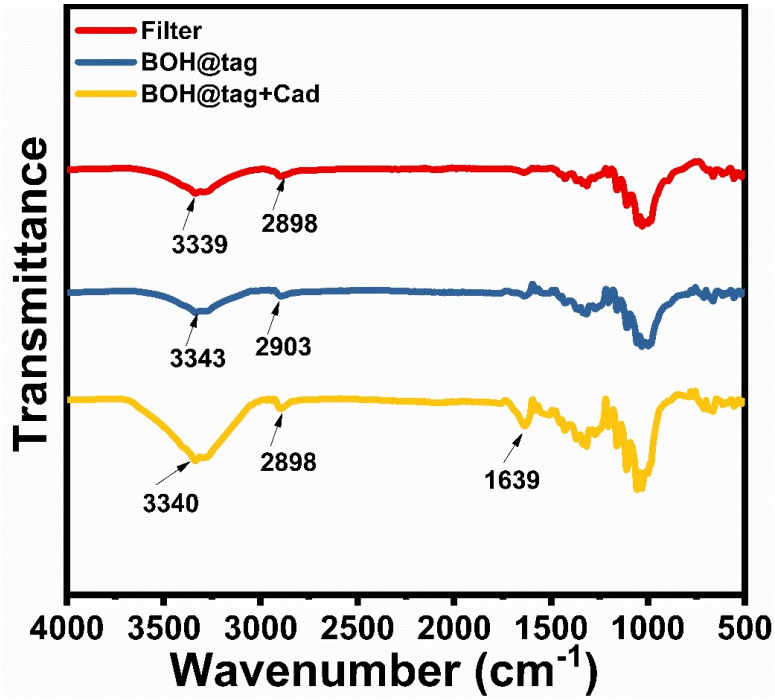


Fig. S6 Comparative FT-IR spectra of the bare BOH@tag, BOH@tag after reaction with cadaverine, and blank filter paper.

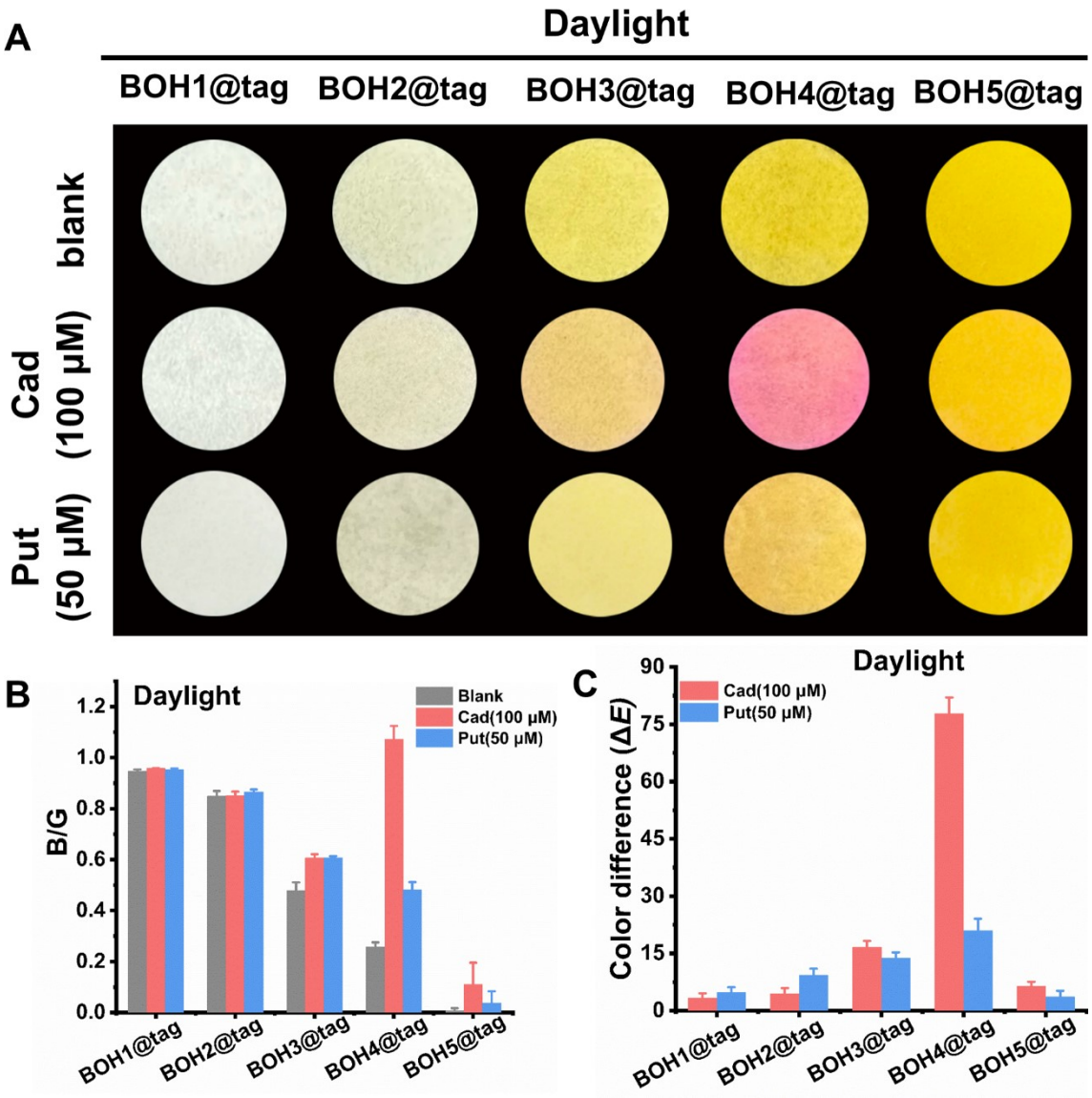


Fig. S7 Optimization of the probe concentration for the paper-based tag. (A) Photographs of filter paper deposited with different concentrations of probe BOH and after their reaction with cadaverine or putrescine under daylight. From left to right: BOH1@tag: 10 μM, BOH2@tag: 100 μM, BOH3@tag: 500 μM, BOH4@tag: 1 mM, BOH5@tag: 5 mM. (B) The blue-to-green (B/G) value of the tags with different probe concentrations before and after reaction with cadaverine or putrescine. (C) Color difference (ΔE) values of the tags with different probe concentrations after reaction with cadaverine or putrescine.

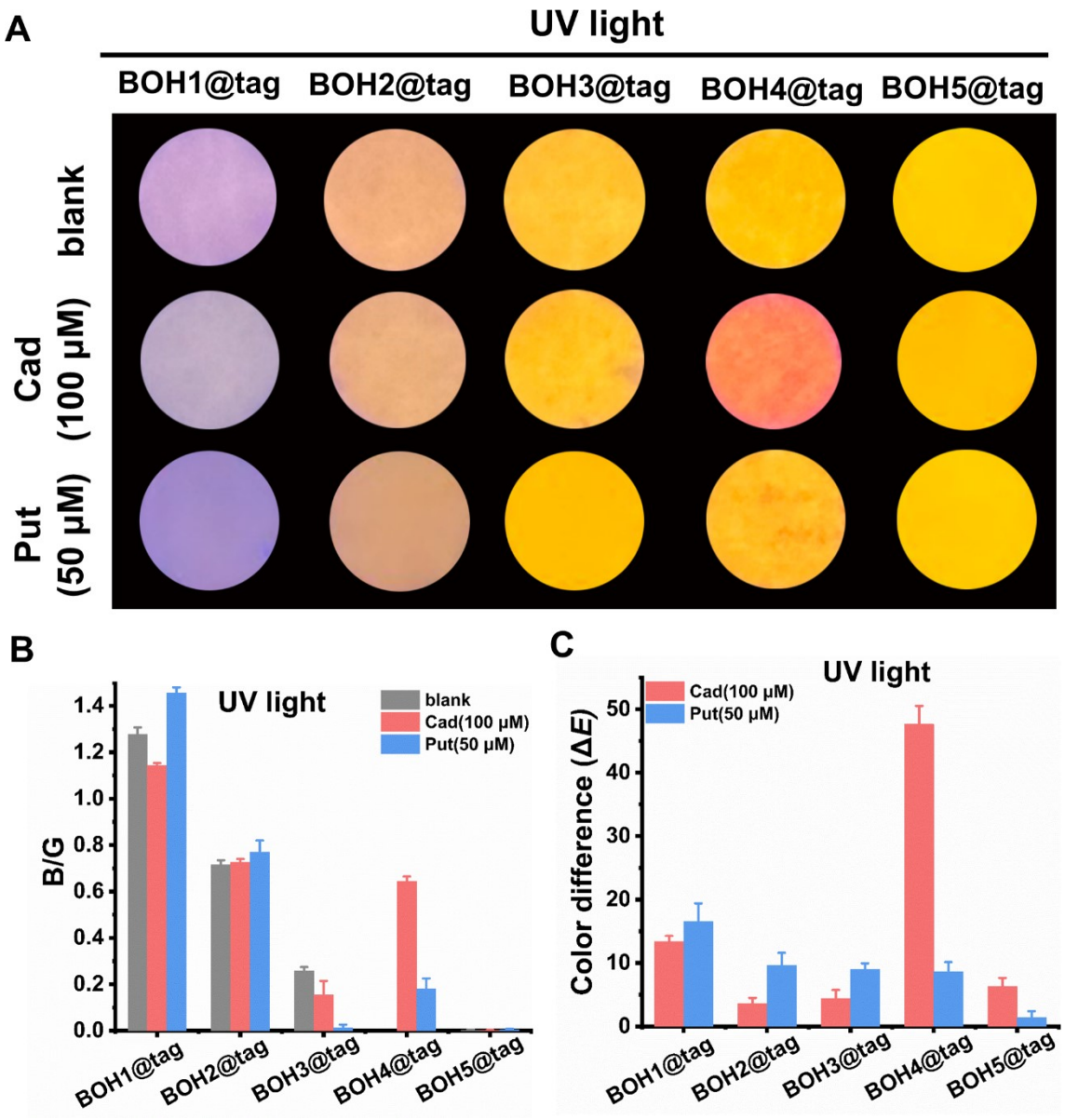


Fig. S8 Optimization of the probe concentration for the paper-based tag. (A) Photographs of filter paper deposited with different concentrations of probe BOH and after their reaction with cadaverine or putrescine under UV light. From left to right: BOH1@tag: 10 μM, BOH2@tag: 100 μM, BOH3@tag: 500 μM, BOH4@tag: 1 mM, BOH5@tag: 5 mM. (B) The blue-to-green (B/G) value of the tags with different probe concentrations before and after reaction with cadaverine or putrescine. (C) Color difference (ΔE) values of the tags with different probe concentrations after reaction with cadaverine or putrescine.

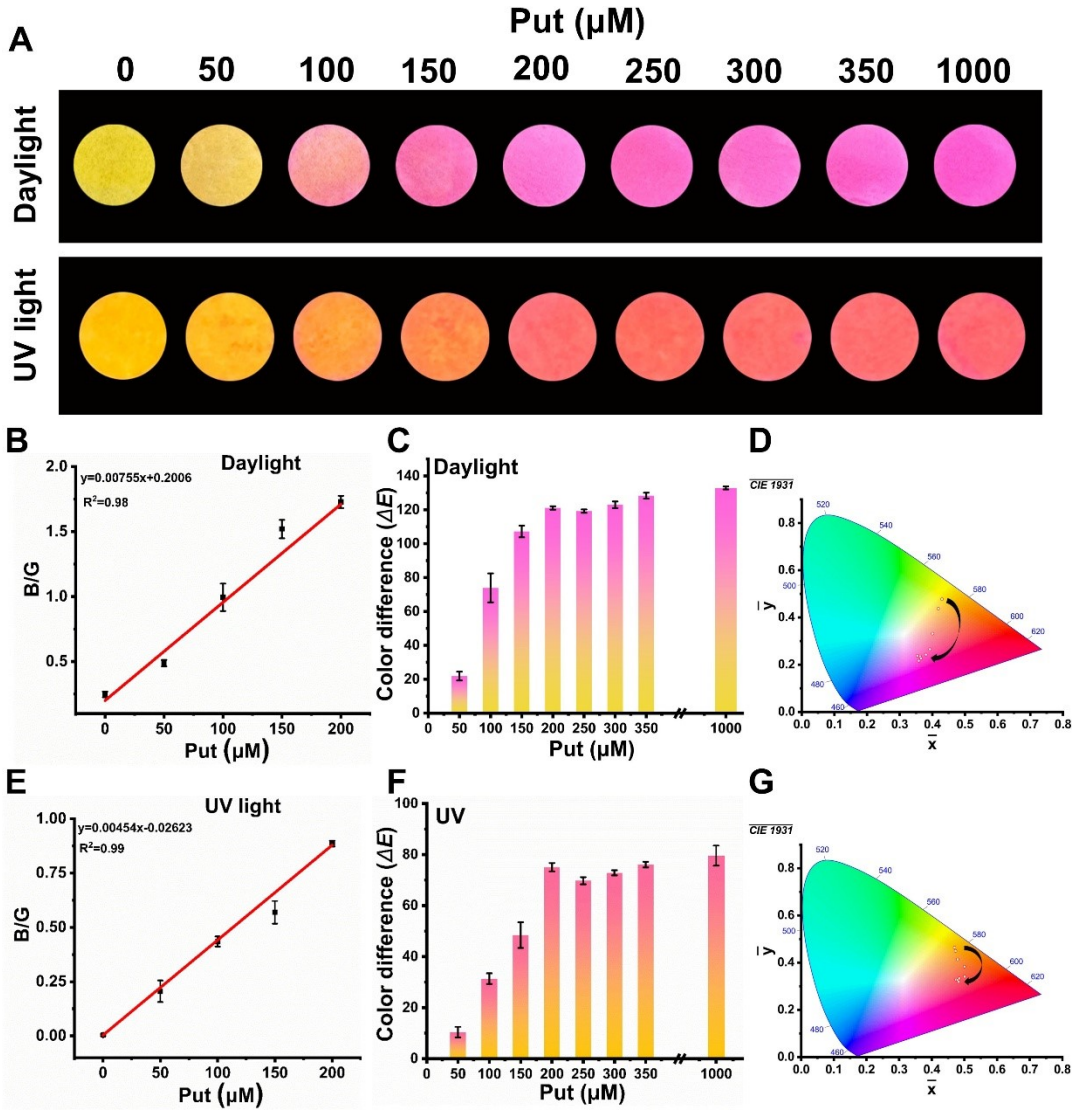


Fig. S9 Response of the BOH4@tag to putrescine. (A) Photographs of the BOH4@tag after reaction with different concentrations of putrescine, captured under daylight and UV light. The blue-to-green (B/G) intensity ratio of the BOH4@tag after reaction with different concentrations of putrescine (under daylight(B) and UV light (E)). Color difference (ΔE) values of the BOH4@tag after reaction with different concentrations of putrescine (under daylight (C) and UV light (F)). Changes in the CIE chromaticity diagram of the BOH4@tag after reaction with different concentrations of putrescine (under daylight(D) and UV light (G)).

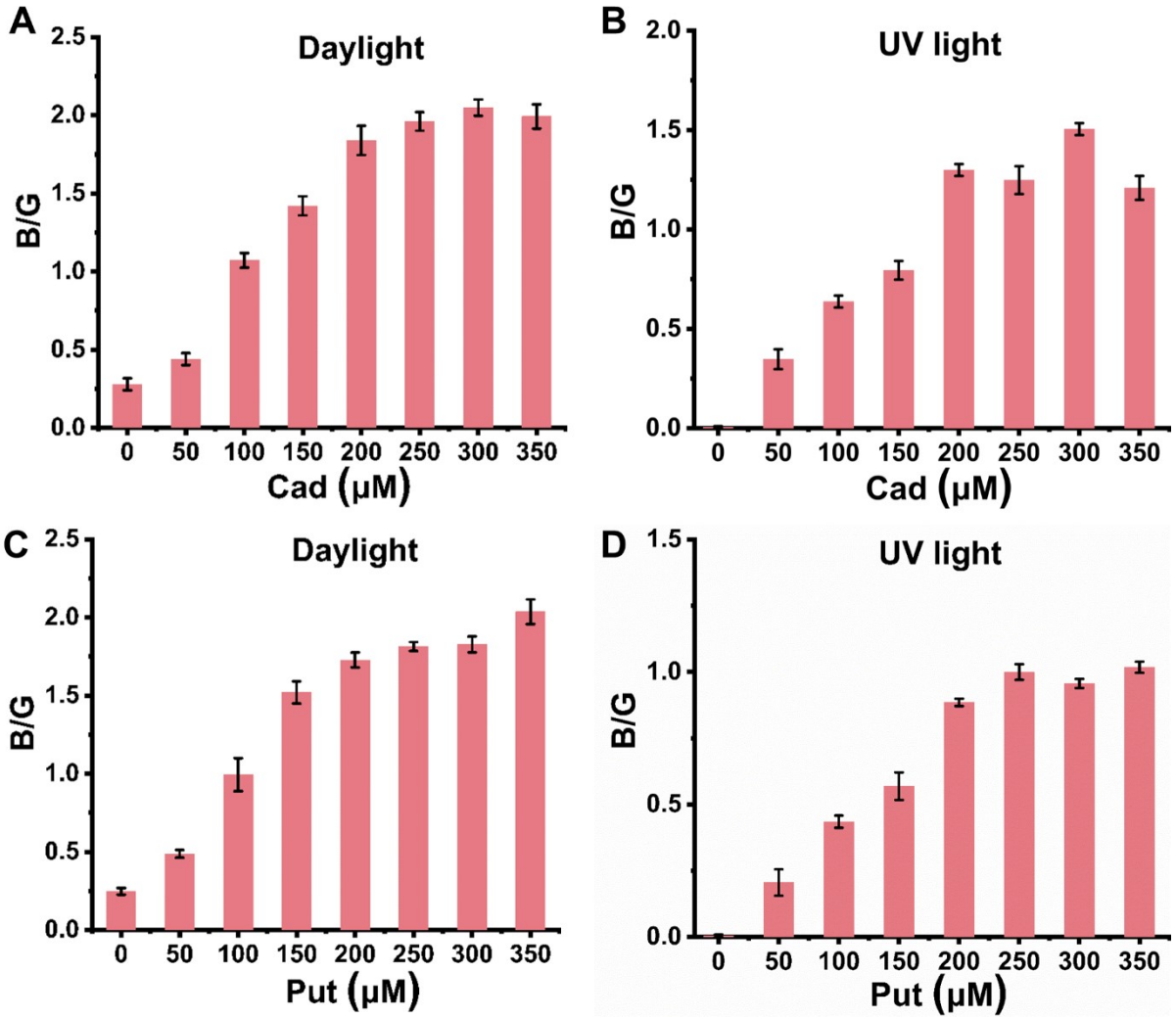


Fig. S10 (A) The blue-to-green (B/G) value of the BOH4@tag after reaction with different concentrations of cadaverine (under daylight). (B) The blue-to-green (B/G) value of the BOH4@tag after reaction with different concentrations of cadaverine under UV light. (C) The blue-to-green (B/G) value of the BOH4@tag after reaction with different concentrations of putrescine (under daylight). (D) The blue-to-green (B/G) value of the BOH4@tag after reaction with different concentrations of putrescine under UV light.

Table S1 Response equilibrium time of tag to different bases

Bases (200 μM)	Cad	$NH_3 \cdot H_2O$	NaOH
Response equilibrium time	5 min	8 min	10 min

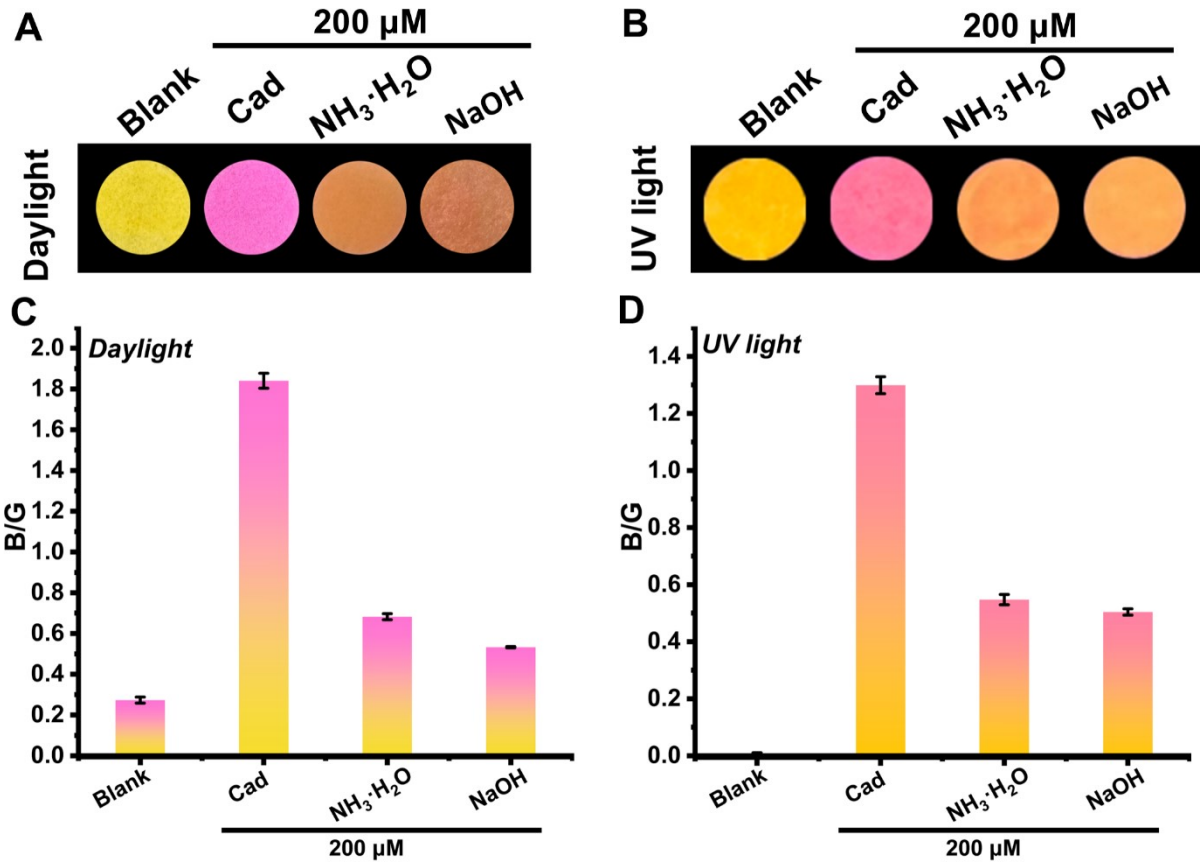


Fig. S11 Photographs and corresponding quantitative analysis of the BOH4@tag before and after exposure to different bases. (A) Photographs under daylight and (B) under UV light (365 nm) after reaction with cadaverine, ammonia solution, and sodium hydroxide solution, respectively. (C) B/G ratio calculated from the daylight images and (D) B/G ratio from the UV-light images corresponding to (A) and (B).

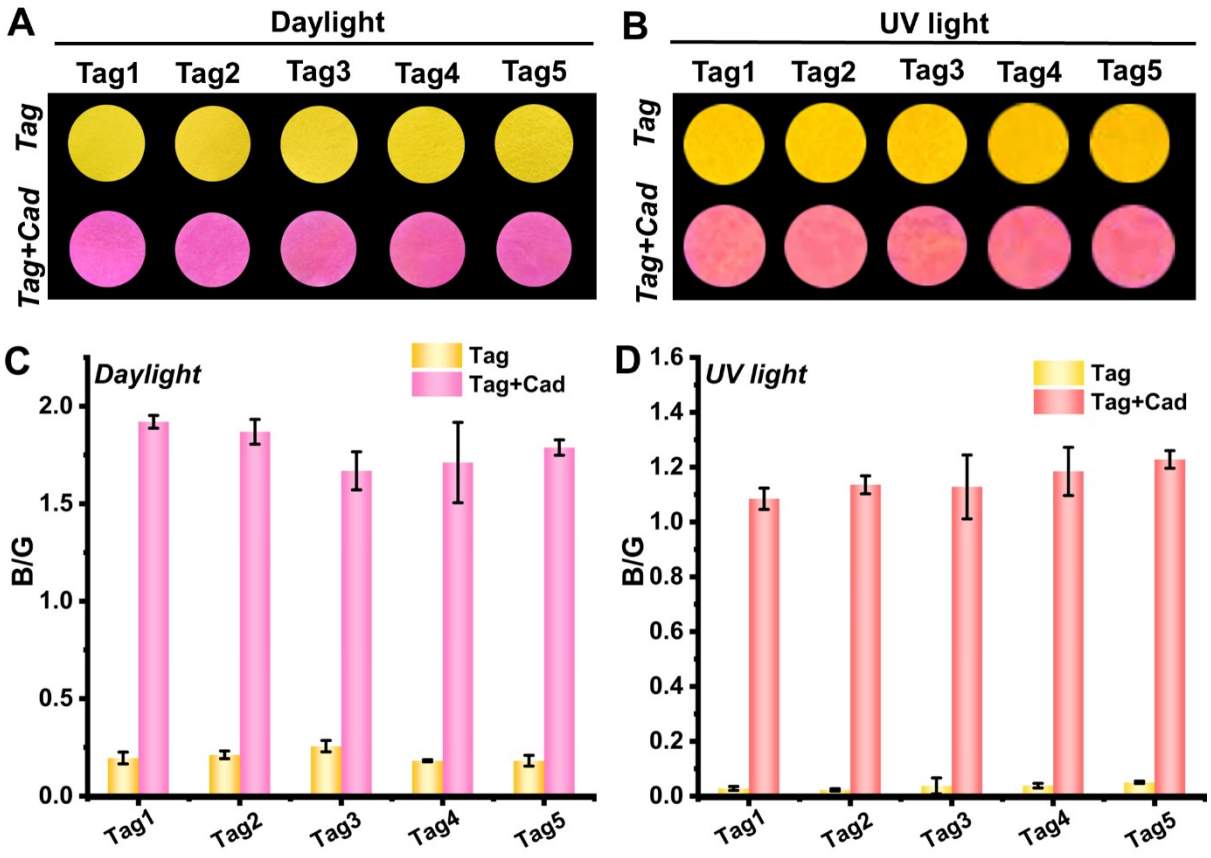


Fig. S12 Batch-to-batch reproducibility assessment of the paper-based tags. (A) Photographs under daylight and (B) under UV light (365 nm) of five independently prepared tags (Tag 1-5) before and after reaction with 200 μM cadaverine. (C) B/G ratio from the daylight images and (D) B/G ratio from the UV-light images corresponding to the tags shown in (A) and (B).

Table S2 Reference Values for TVB-N and Food Freshness Grades

TVB-N value	Food Freshness Grade
＜12 mg/100 g	Fresh
12~20 mg/100 g	Slightly rotten
20~25 mg/100 g	Approaching Spoilage
＞25 mg/100 g	Not for human consumption/Spoiled

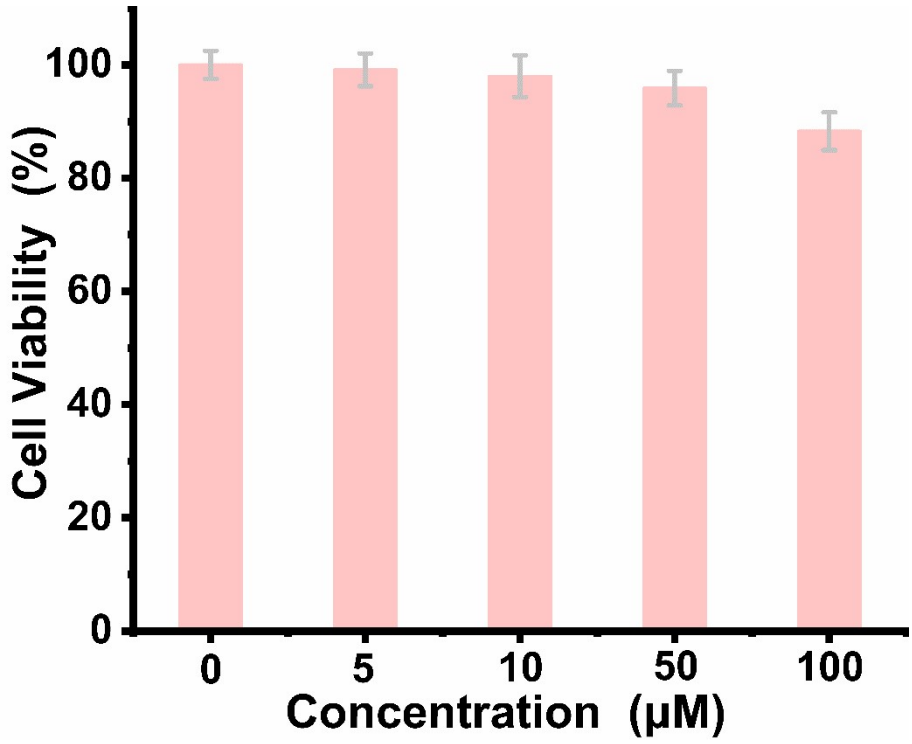


Fig. S13 Cell viability for L929 cells in the presence of the probe BOH at varied concentrations. Three independent experiments were conducted, and for each independent experiment, the assays were conducted in eight replicates. Data represent mean ± SD. Error bars represent the standard deviation (SD).

Table S3 ICT-based sensors for the detection of food freshness

Ref	Probe	Targets	Detection mode	Fluorescence	Colorimetric	Substrate
1	ASQ	ammonia	film	yes	yes	paper-based
2	SC-MPC	ammonia	film	no	yes	aerogel
3	T1/T2	cadaverine	film	yes	no	PMMA-based
4	PAA-FP	ammonia	film	no	yes	paper-based
5	HDXM	cadaverine	film	yes	yes	PVA-based
6	O17	ammonia	film	yes	no	Ethyl cellulose
7	SWJT-42	cadaverine	film	yes	yes	paper-based
8	SWJT-36	cadaverine	film	yes	yes	paper-based
9	HBT-NO_2	ammonia	film	yes	no	PAN
This work	BOH	cadaverine	film	yes	yes	paper-based

Reference

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