

Dual-probe-based fluorescent sensor ensemble for the pattern recognition of various flavonols and its application in tea identification

Junmei Fan¹✉, Haiyuan Ding¹, Yingjiao Wu¹, Ruitao Zhu¹, Tingting Zhao¹, Hongfei Han¹, and Liping Ding²

¹ College of Chemistry and Materials, Taiyuan Normal University, Jinzhong 030619, China

² Key Laboratory of Applied Surface and Colloid Chemistry of Ministry of Education, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an 710119, China



Cite this article: *Nano Research*, 2026, 19, 94909035. <https://doi.org/10.26599/NR.2026.94909035>

ABSTRACT: Flavonols are widely distributed in complex samples such as food matrices, and the coexistence of multiple structurally similar flavonols in such samples renders identifying them highly challenging, while also bringing difficulties to the detection of real samples. Herein, a novel dual-probe-based fluorescent sensor ensemble composed of dansyl derivative (DanBrIM) aggregates and pyrene derivative (PyTS) was constructed and explored for differentiation of flavonols. The powerful DanBrIM/PyTS ensemble exhibits turn-off responses to seven different flavonols, owing to the synergistic effect of the inner filter effect (IFE) and photo-induced electron transfer (PET). Fingerprint recognition patterns, radar plots, heat maps, linear discriminant analysis (LDA), and partial least squares-discriminant analysis (PLS-DA) results illustrate that the present sensor can effectively discriminate different flavonols, flavonol isomers and unknown samples by collecting fluorescence signals at three typical wavelengths. Moreover, it can be employed to distinguish various tea samples, demonstrating promising practical applications.

KEYWORDS: flavonols, fluorescent sensor, pattern recognition, multivariate analysis, tea

1 Introduction

Tea is one of the most popular beverages around the world and possesses a variety of biological functions, including antioxidant, anticancer, antiinflammatory, vascular wall-strengthening, and antibacterial effects, which are largely ascribed to its rich flavonoid content [1–3]. Flavonoids not only confer health benefits but also play a key role in determining the flavor and color of tea. Different processing techniques and fermentation degrees can produce diverse types of tea, resulting in variations in flavor, quality, efficacy and price [4]. The considerable economic value of tea has led to frequent adulteration for excessive profits, which seriously endangers consumer health and damages the tea trade. Therefore,

there is an urgent need to develop efficient sensing methods for tea identification. Since the content of flavonoids varies with the type and quality of tea, several sensing detection strategies based on the main indicators of flavonoids have been proposed for the identification of tea. The reported analytical techniques include mass spectrometry (MS) [5], colorimetry [6], electrochemistry [7], and fluorescence methods [4]. By comparison, fluorescence methods have emerged as promising approaches owing to their inherent high sensitivity, cost-effectiveness, and time efficiency.

Fluorescent sensor arrays, also known as optical noses or tongues, are analytical systems composed of multiple distinct nonspecific sensing elements that can generate distinctive fingerprint-like recognition patterns for each analyte by integrating response signals from all elements [8–10]. This pattern-based approach mimics the mammalian systems of smell and taste, enabling robust discrimination of structurally similar pure analytes, analyte mixtures, and analytes in complex matrices [11–13]. Various fluorescent sensor arrays have been extensively developed for the identification of diverse analytes such as explosives [14, 15],

Received: May 15, 2026; Revised: July 6, 2026

Accepted: July 17, 2026

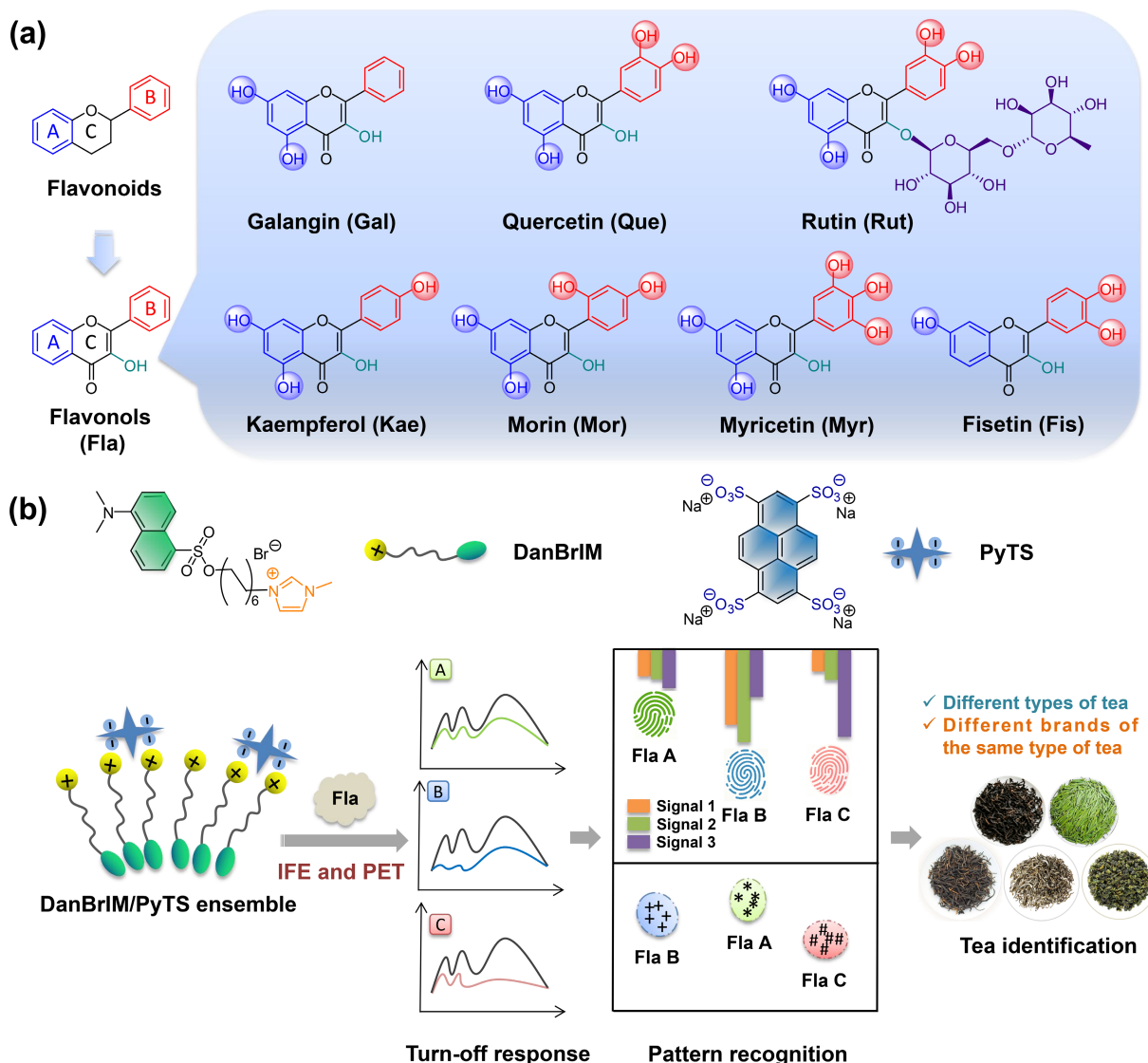
✉ Address correspondence to jmfan@tynu.edu.cn

biothiols [16, 17], and saccharides [18, 19], and have been widely employed in areas including food safety control [20, 21], biomedical diagnostics [22, 23], and environmental monitoring [24, 25].

Flavonols (Fla) are a subgroup of flavonoids characterized by a 3-hydroxyflavone backbone (Scheme 1(a)). Structurally, they feature a 15-carbon skeleton (C6-C3-C6), in which two aromatic rings (A and B) are linked via a heterocyclic oxygen-containing ring (C). This C-ring bears a carbonyl group and a double bond, with a hydroxyl group specifically attached at the 3-position [26, 27]. Different flavonols exhibit variations in the number and positions of additional hydroxyl groups and other substituents (e.g., methoxy, glycosyl) on their rings, such as galangin (Gal), kaempferol (Kae), quercetin (Que), morin (Mor), rutin (Rut), myricetin (Myr), and fisetin (Fis). In addition, they also display diverse biological activities and confer multiple benefits. For example, Que, which is abundant in fruits, vegetables, tea and wine, exhibits anti-aging, antiinflammatory, and antitumor pharmacological effects [28, 29]. However, the identification of flavonols is challenging owing to their structural diversity and high degree of similarity. Up to now, only few studies have reported the discrimination of flavonols. Peng

et al. fabricated a two-element cyclodextrin (CD)-based fluorescent sensor (Al^{3+} -CD@Cu and Mg^{2+} -CD@Cu), which realized the accurate quantitative determination of Que, Kae, and Myr in *Kaempferia galangal* L. and honey [30]. This array could well identify three flavonols, yet it still suffers from drawbacks such as relatively complicated data acquisition processes by collecting spectral responses from all sensor elements. It is an arduous undertaking to develop a facile, high-efficiency identification platform for flavonols.

Herein, we synthesized a novel dansyl-based amphiphilic fluorescent probe (DanBrIM), in which dansyl serves as the fluorescent reporting moiety, 1-methylimidazolium salt as the hydrophilic head group, and dodecyl as the hydrophobic tail (Scheme 1(b)). Anionic hydrophilic pyrene derivative (PyTS) was introduced into the DanBrIM aggregates to construct a cross-reactive dual-probe-based sensor system (DanBrIM/PyTS ensemble) with multi-wavelength emission rather than multi-element cross-reactive sensor arrays. Thus, fluorescence signal variations at different emission wavelengths can be acquired via a single spectral scan, which greatly simplifies the data collection



Scheme 1 Analytes and construction of sensing platform. (a) Chemical structures of flavonoids and flavonols. (b) Representative scheme of the dual-probe ensemble for flavonol discrimination and tea identification.

procedure. Most importantly, to the best of our knowledge, no sensors based on multi-wavelength cross-reactive responses have yet been reported for the discrimination of various flavonols. In this system, we utilized response signals acquired at three distinct wavelengths to generate the characteristic fingerprint profiles for specific flavonols, and used multivariate analysis methods to realize the discrimination of seven different flavonols based on inner filter effect (IFE) and photo-induced electron transfer (PET). Moreover, this strategy can be applied to distinguish six different types of tea or five different brands of the same type of tea.

2 Experimental

2.1 Reagents and instruments

Dansyl chloride ($\geq 98\%$), 12-bromo-1-dodecanol ($\geq 98\%$), 1-methylimidazole ($\geq 99\%$), 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt ($\geq 97\%$), galangin ($\geq 98\%$), kaempferol ($\geq 97\%$), quercetin ($\geq 95\%$), morin hydrate ($\geq 95\%$), rutin hydrate ($\geq 98\%$), myricetin ($\geq 97\%$), and fisetin ($\geq 98\%$) were purchased from Aladdin company and used without further purification. Tea was commercially available and obtained from a supermarket.

The ^1H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were obtained on a Bruker Avance 400 MHz NMR spectrometer. Atmospheric pressure chemical ionization mass spectrometry (APCI-MS) and electrospray ionization mass spectrometry (ESI-MS) were acquired on a Bruker Maxis UHR-TOF Mass Spectrometer. Ultraviolet-visible (UV-vis) absorption spectra were conducted on a Specord S600 spectrometer (Germany). Steady-state fluorescence spectrum measurements were recorded on a F-7000 FL Spectrophotometer (Hitachi, Japan), and the excitation wavelength was set at 315 nm. Particle size distribution measurements were conducted using a Malvern Zetasizer Nano-ZS90. Transmission electron microscopy (TEM) images were acquired using a FEI Tecnai G2 F20 field emission transmission electron microscope. The fluorescence lifetimes of the ensemble were carried out on a single-photon-counting FLS 980 time-resolved spectrofluorometer (Edinburgh Instruments, UK) using a 300 nm laser as the excitation source.

2.2 Synthesis and characterization of compound DanBr

The synthesis route of amphiphilic target compound is shown in Scheme S1 in the Electronic Supplementary Material (ESM), and the synthesis and purification of intermediate compound DanBr is as follows: A solution of dansyl chloride (0.50 g, 1.9 mmol, 1 equiv.) in dry CHCl_3 (40 mL) was slowly added dropwise to a stirred mixture solution of 12-bromo-1-dodecanol (0.60 g, 2.3 mmol, 1.2 equiv.) and triethylamine (319 μL , 2.3 mmol, 1.2 equiv.) in dry CHCl_3 (50 mL) at room temperature under a nitrogen atmosphere. Subsequently, the resulting reaction mixture was stirred at room temperature for 24 h. After this, the light yellow reaction mixture was washed with saturated brine (5×60 mL) and the organic phase obtained was dried over anhydrous Na_2SO_4 for 4 h. Further purification was performed via silica gel column chromatography (dichloromethane:petroleum ether = 1:1, V/V) to yield DanBr as a light-yellow oil (0.76 g, 80%). ^1H NMR (400 MHz, CDCl_3 , ppm): δ 8.60 (d, $J = 8.5$ Hz, 1H), 8.35–8.22 (m, 2H), 7.66–7.49 (m, 2H), 7.20 (d, $J = 7.4$ Hz, 1H), 3.99 (t, $J = 6.4$ Hz, 2H), 3.40 (t, $J = 6.8$ Hz, 2H), 2.89 (s, 6H), 1.91–1.77 (m, 2H), 1.58 (p, $J = 6.6$ Hz, 2H), 1.47–1.02 (m, 16H). APCI-MS (m/z) ($\text{C}_{24}\text{H}_{36}\text{BrNO}_3\text{S}$), calcd for $[\text{M}+\text{H}]^+$: 498.1673, found: 498.1672.

2.3 Synthesis and characterization of compound DanBrIM

Compound DanBr (0.37 g, 0.74 mmol, 1 equiv.) and 1-methylimidazole (71 μL , 0.89 mmol, 1.2 equiv.) were dissolved in anhydrous acetonitrile (30 mL), and the resulting mixture was refluxed for 48 h under a nitrogen atmosphere. After evaporation of the solvent under reduced pressure, a greyish solid was obtained. Then, the resulting crude product was washed thoroughly with anhydrous ether and dried under vacuum, yielding a white powder (0.33 g, 77%). ^1H NMR (400 MHz, dimethyl sulfoxide (DMSO), ppm): δ 9.09 (s, 1H), 8.53 (d, $J = 8.5$ Hz, 1H), 8.16 (d, $J = 8.2$ Hz, 1H), 7.93 (d, $J = 6.6$ Hz, 1H), 7.72 (d, $J = 25.2$ Hz, 2H), 7.40 (dd, $J = 17.2, 8.7$ Hz, 2H), 7.10 (d, $J = 7.1$ Hz, 1H), 4.12 (s, 2H), 3.83 (s, 3H), 3.52 (t, $J = 6.5$ Hz, 2H), 2.79 (s, 6H), 1.93–0.96 (m, 20H). ^{13}C NMR (101 MHz, DMSO, ppm): δ 150.84, 144.90, 137.00, 130.88, 129.25, 125.88, 125.62, 124.80, 124.10, 123.05, 122.77, 114.33, 49.26, 45.62, 36.25, 35.81, 32.74, 29.90, 29.50, 29.42, 29.40, 29.34, 28.95, 28.89, 28.63, 28.03, 26.04, 26.00. ESI-MS (m/z) ($\text{C}_{28}\text{H}_{42}\text{N}_3\text{O}_3\text{SBr}$) calcd for $[\text{M}-\text{Br}]^+$: 500.2947, found: 500.2950.

2.4 Sample preparation

Stock solutions of DanBrIM (2.5 mM) and PyTS (2.5 mM) were prepared in ultrapure water. Various flavonols were prepared as 2.5 mM stock solutions in N,N -dimethylformamide (DMF), separately. Ground tea leaves (0.3 g) were extracted with 5 mL DMF, and the obtained suspension was filtered through a 0.22 μm filter membrane before the filtrate was stored at 4 $^\circ\text{C}$ for use. In each fluorescence measurement, the concentration of tea was diluted 250-fold.

3 Results and discussion

3.1 Photophysical properties and self-assembly behavior of DanBrIM in water

The absorption and fluorescence emission spectra of amphiphilic probe DanBrIM (20 μM) were measured in water to assess its basic photophysical properties. As displayed in Fig. 1(a), a distinct absorption peak is observed at approximately 315 nm, which can be assigned to the typical absorption of the dansyl unit. Fluorescence emission spectrum of the probe exhibits a strong typical emission peak of dansyl at 509 nm when excited at 315 nm. By measuring the concentration-dependent fluorescence spectra of amphiphilic DanBrIM and plotting its maximum emission intensity against probe concentration, we determined its critical aggregation concentration (CAC) in aqueous solution to be 16.8 μM from the intersection of two linear fits (Fig. 1(b)). Accordingly, DanBrIM was fixed at 20 μM (slightly higher than its CAC) to form well-defined aggregates and generate reliable fluorescent signals in subsequent studies.

The self-assembly behavior of DanBrIM in aqueous solution was further investigated via dynamic light scattering (DLS) and TEM measurements. The DLS data indicated that the average diameter of DanBrIM aggregates was approximately 252 nm (Fig. 1(c)). Meanwhile, the morphology of the aggregates measured by TEM revealed that the amphiphilic probes could self-assemble into spheroidal aggregates with an average diameter of ca. 35 nm (Fig. 1(d)). The aggregate size measured by TEM is considerably smaller than that determined by DLS. Such a discrepancy can be ascribed to distinct sample preparation processes. DLS

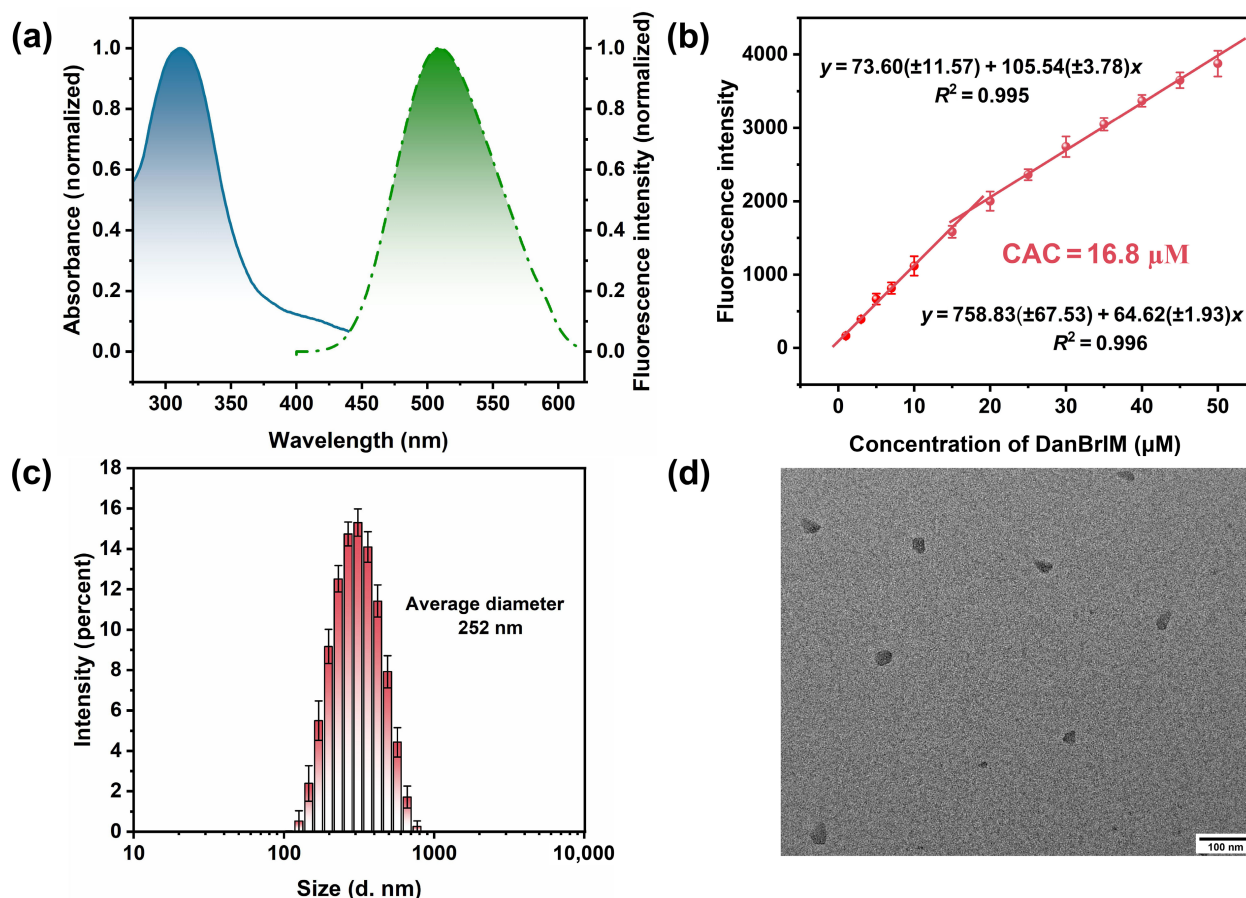


Figure 1 Photophysical properties and self-assembly behavior of DanBrIM. (a) Normalized UV-vis absorption and fluorescence emission spectra of DanBrIM (20 μM) in water. (b) Fluorescence emission intensity of DanBrIM as a function of its concentration in water. (c) Particle size distribution and (d) TEM image of DanBrIM aggregates in water.

measurements were performed in aqueous solution, while TEM characterization required vacuum dehydration. Consequently, the loosely packed DanBrIM aggregates displayed a larger hydrodynamic size in aqueous solution than in the dehydrated state. Both measurements provide unambiguous evidence that the amphiphilic DanBrIM probes form supramolecular aggregates in aqueous solution.

3.2 Sensing behaviors of DanBrIM/PyTS ensemble for flavonols

In order to construct a single water-soluble system with multi-wavelength cross-reactive response, we introduced a negatively charged pyrene derivative PyTS, which exhibits multiple emission bands, to the positively charged amphiphilic aggregates to establish a dual-probe-based fluorescent sensing platform. Fluorescence emission spectra were first recorded upon the addition of different concentrations of PyTS into a 20 μM DanBrIM solution. It was found that with the increasing concentration of PyTS, the emission peak intensity of DanBrIM at 509 nm slightly decreased, while the fluorescence intensities of PyTS at 391 and 408 nm increased significantly (Fig. S1 in the ESM). Therefore, 10 μM PyTS was chosen to construct the sensing platform, since only a mild reduction in the emission intensity of DanBrIM was observed at this concentration, and comparable co-emission intensities can be obtained for PyTS and DanBrIM. Besides, we further investigated the photostability of the DanBrIM/PyTS (20/10 μM) ensemble. As

depicted in Figs. 2(a) and 2(b), the ensemble exhibits excellent photostability.

The sensing behaviors of DanBrIM/PyTS ensemble to the seven flavonols, including Gal, Kae, Que, Mor, Rut, Myr, and Fis, were systematically examined in aqueous solution. As shown in Fig. 2(c) and Fig. S2 in the ESM, the binary ensemble exhibited turn-off responses toward all the flavonols, accompanied by a pronounced decrease in the emission intensities of both PyTS (391 and 408 nm) and DanBrIM (509 nm). By comparing the quenching intensity variations (I_0/I) of PyTS (391 nm) and DanBrIM (509 nm) as a function of flavonol concentration, we found that the fluorescence quenching effect was much more significant at 391 nm (Fig. S3 in the ESM). Consequently, the signal changes recorded at 391 nm were chosen for subsequent analysis. We also observed that the variations of quenching ratio I_0/I at 391 nm are linear over the low flavonol concentration range and the quenching behaviors follow the Stern-Volmer equation (Eq. (1))

$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

where I_0 and I are the emission intensities at 391 nm in the absence and presence of quencher concentrations (i.e., flavonols), and $[Q]$ is the concentration of flavonols. K_{SV} represents the Stern-Volmer constant, which can be obtained from the linear plot of I_0/I versus $[Q]$. As displayed in Fig. 2(d), K_{SV} values are determined to be 3.5×10^4 , 2.2×10^4 , 2.9×10^4 , 3.6×10^4 , 4.1×10^4 , 3.9×10^4 , and 4.2×10^4 for Gal, Kae, Que, Rut, Mor, Myr and Fis, respectively. The seven

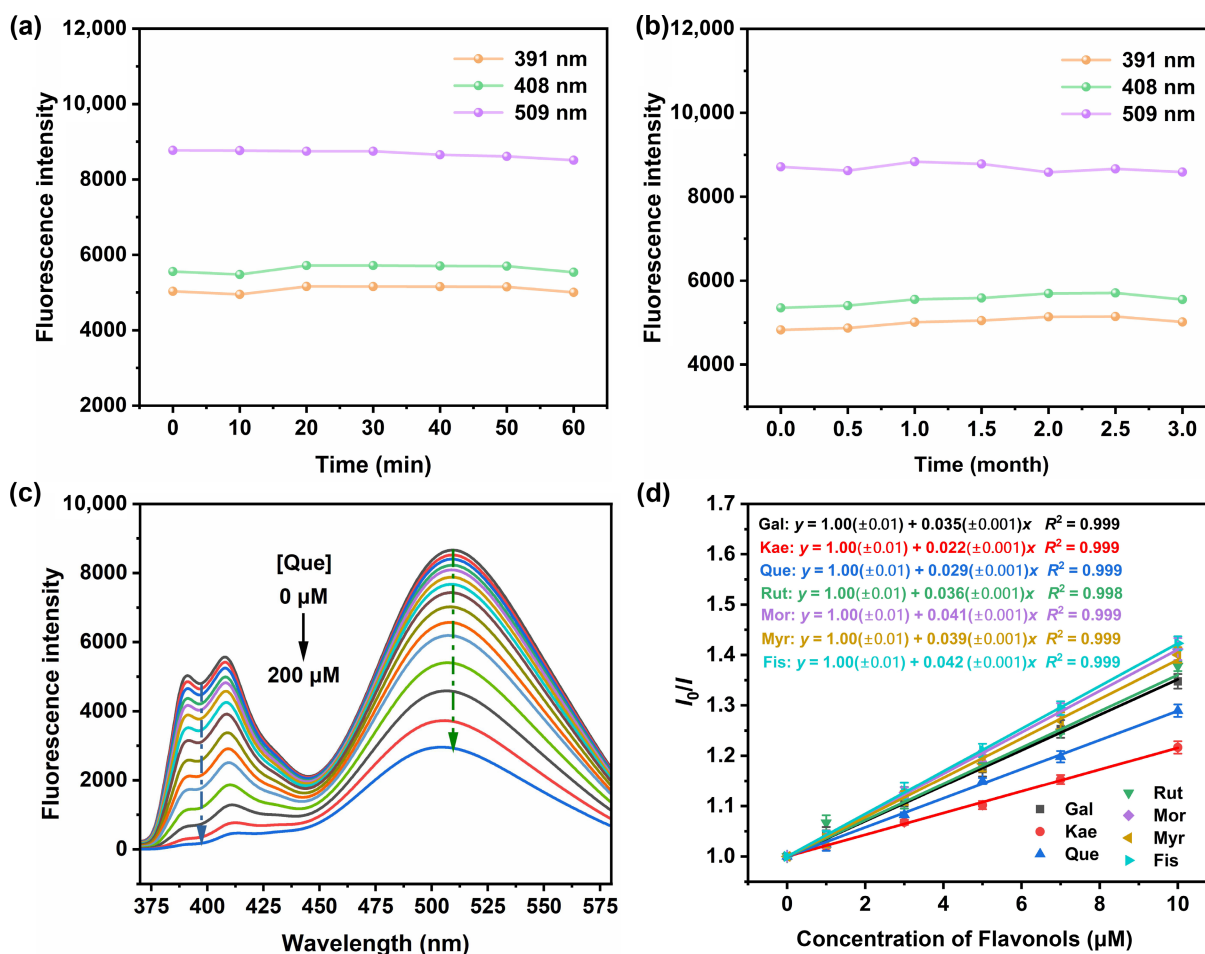


Figure 2 Photostability and sensing behaviors of the DanBrIM/PyTS ensemble. Effects of (a) 365 nm UV irradiation time and (b) storage time on the photostability of DanBrIM/PyTS (20/10 μM) ensemble in water. (c) Fluorescence emission spectra of DanBrIM/PyTS (20/10 μM) ensemble upon addition of Que from 0 to 200 μM. (d) Fluorescence quenching plots (I_0/I) of the DanBrIM/PyTS ensemble versus the concentration of seven flavonols. The error bars represent the standard deviation of three independent measurements.

analytes exhibit distinct quenching efficiencies in the order of Fis > Mor > Myr > Rut > Gal > Que > Kae.

3.3 Discrimination capabilities of DanBrIM/PyTS ensemble to flavonols

The quenching response of this binary DanBrIM/PyTS ensemble to flavonols may endow it with the capability of fingerprinting recognition for different concentrated flavonols. For quenched fluorescence, the logarithm data, $\lg(I/I_0)$, yield negative values that intuitively separate quenching response signals from the baseline background (zero). Besides, it can normalize the signal dynamic range, mitigate interference from spectral outliers, and extract highly discriminative features for classification. Then, $\lg(I/I_0)$ data of the sensor ensemble at three distinct wavelengths (391, 408, and 509 nm) in response to the seven flavonols at three concentrations (10, 50, and 100 μM) were collected and analyzed. As illustrated in Fig. 3(a), markedly differentiated recognition patterns can be obtained for the seven flavonols either at the same or different concentrations. Moreover, the radar plots corresponding to each output signal element display cross-reactive recognition responses toward different flavonols (Fig. 3(b) and Fig. S4 in the ESM). The relevant heat map of the present sensor can also generate differentiable color map for different flavonols (Fig. 3(c) and Fig. S5 in the ESM). To further evaluate the discrimination capability of

this sensor system, linear discriminant analysis (LDA) was employed for pattern recognition analysis.

LDA is a supervised linear dimensionality reduction method that maximizes inter-class separation and minimizes intra-class variation, thereby projecting high-dimensional data into a discriminative low-dimensional space for effective sample classification and identification [31]. Using the analysis method of LDA in OriginPro 2026b, the identification among various flavonols at the same concentration was first assessed. Subsequently, a data matrix of 1 sensor × 3 wavelengths × 7 flavonols × 5 replicates was constructed for samples at three concentrations (10, 50, and 100 μM), respectively. As shown in Fig. 3(d), the clusters of Gal, Kae, Que, Rut, Mor, Myr and Fis are well-separated from one another at both low and high concentrations. By contrast, the separation is much better for flavonols at higher concentration.

In addition, we also adopted other multivariate analysis methods including partial least squares-discriminant analysis (PLS-DA), hierarchical cluster analysis (HCA), and principal component analysis (PCA) to investigate the discrimination performance of this sensor. PLS-DA, a supervised chemometric classification tool, extracts discriminative latent variables correlated with sample categories to accurately distinguish diverse analytes from high-dimensional collinear sensing data [32]. PLS-DA was employed to identify the seven different flavonols at three different

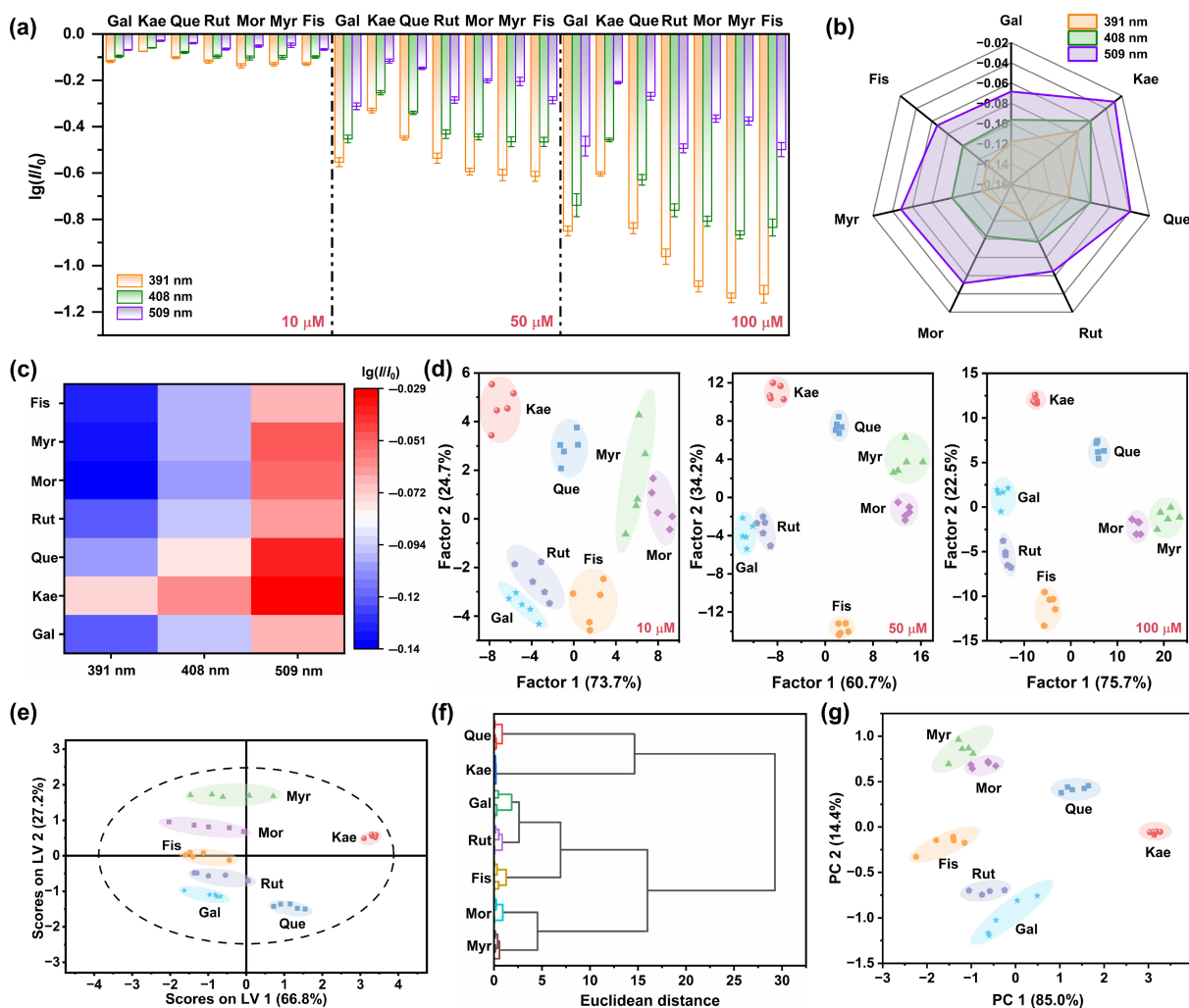


Figure 3 Discrimination of different flavonols at the same concentration. (a) Fingerprint recognition patterns of DanBrIM/PyTS ensemble to flavonols (10, 50, and 100 μM) based on fluorescence variation collected at three different wavelengths. (b) Radar plots and (c) heat map of flavonols (10 μM) obtained with the present sensor ensemble. (d) LDA score plots of the sensor ensemble to flavonols at three different concentrations (10, 50, and 100 μM). (e) PLS-DA canonical score plots based on the first two latent variables to flavonols at 10 μM. (f) HCA dendrogram and (g) PCA score plots of the sensor ensemble to flavonols at 100 μM.

concentrations. As presented in Fig. 3(e) and Fig. S6 in the ESM, all the flavonols were clearly distinguished and accurately classified into seven non-overlapping independent clusters with 95% confidence.

HCA, an unsupervised multivariate analytical method that classifies samples according to their similarity, calculates inter-sample distances usually via the Euclidean metric and constructs hierarchical data structures through linkage algorithms, which are visualized as dendrograms where samples with higher similarity (smaller inter-sample distances) are grouped into the same cluster [33]. As displayed in Fig. 3(f) and Fig. S7 in the ESM, the discriminative capability gradually enhanced as the analyte concentration rose. When the analyte concentration reached 100 μM, the HCA dendrogram illustrated that all flavonol samples were well segregated into separate clusters based on Euclidean distance, with each flavonol forming an individual cluster and exhibiting optimal discrimination performance. PCA, an unsupervised statistical dimensionality reduction technique, employs linear transformation to convert abundant highly correlated raw observed variables into several uncorrelated principal components ranked by variance contribution and achieves feature

visualization while maximally preserving the information of original data [34]. The results of PCA are analogous to those of HCA, and satisfactory discrimination of flavonols can be achieved at the concentration of 100 μM (Fig. 3(g) and Fig. S8 in the ESM). By comparing the four algorithms, we found that LDA and PLS-DA yielded more satisfactory classification results, which can be attributed to the fact that PLS-DA and LDA are supervised multivariate discrimination algorithms, while PCA and HCA correspond to unsupervised statistical methods. Accordingly, LDA and PLS-DA were adopted for discrimination in the subsequent research of this work.

After successfully identifying the seven flavonols at the same concentration, we investigated the recognition capability for individual flavonols with varying concentrations. Here, diverse concentrations of flavonols were separately analyzed with this sensor system. The results demonstrate that this sensor ensemble exhibits sufficient capability for the classification of flavonols with varying concentrations. The radar plots revealed that the fluorescence intensity exhibited distinct quenching variations ($\lg(I/I_0)$) at three wavelengths with increasing flavonol concentration (Fig. 4(a) and Fig. S9 in the ESM). Meanwhile, the

LDA score plots exhibited distinct regular patterns rather than random distribution, which could be clearly differentiated from one another at multiple concentrations (Fig. 4(b) and Fig. S10 in the ESM). Since factor 1 (the first discriminant factor) in LDA score plots accounted for more than 60% of the total variance, it could be used to quantify the concentrations of flavonols [35]. As displayed in Fig. 4(c), a good linear relationship was observed between factor 1 values and Que concentrations, corresponding to the range of 5–200 μM ($R^2 = 0.998$). Good linear relationships were also observed for other flavonols (Fig. S11 in the ESM), demonstrating that this sensor system enables the quantitative and discriminative detection of different flavonols. Besides, PLS-DA was also applied to analyze the dataset, and the resulting score plot displays 95% confidence Hotelling's T^2 ellipses with complete separation between all sample groups (Fig. 4(d) and Fig. S12 in the ESM).

Que and Mor are isomers of flavonols with the same molecular formula but different substituent positions in their structures. Thus, we attempted to distinguish these two structurally highly similar flavonols at different concentrations using this system. As depicted in Figs. 4(e)–4(g), it was found that this sensing platform could achieve effective discrimination of the two flavonols, especially at high concentrations. In addition, we also investigated the

recognition performance for identifying unknown samples. As seen from Figs. 4(h) and 4(i), the seven unknown samples were clearly and correctly clustered with their corresponding trained known samples. These results demonstrate that the present sensor ensemble has a robust capability for the accurate identification of multiple flavonols. To verify that the excellent discrimination capacity of this system originates from the synergistic effect of DanBrIM and PyTS, we further evaluated the discrimination performance of DanBrIM alone and PyTS alone toward different flavonols. As a minimum of two signals are mandatory for multivariate analysis, DanBrIM, which only provides one single response signal at 509 nm, cannot discriminate flavonols when used alone. Although the PyTS probe produces two response signals at 391 and 408 nm, it still shows unsatisfactory discrimination performance toward flavonols (Fig. S13 in the ESM). Therefore, synergistic use of the two probes is necessary to achieve effective differentiation of various flavonols.

3.4 Sensing mechanism of DanBrIM/PyTS ensemble to flavonols

Generally, the fluorescence quenching response of fluorescent sensors primarily arises from Förster resonance energy transfer

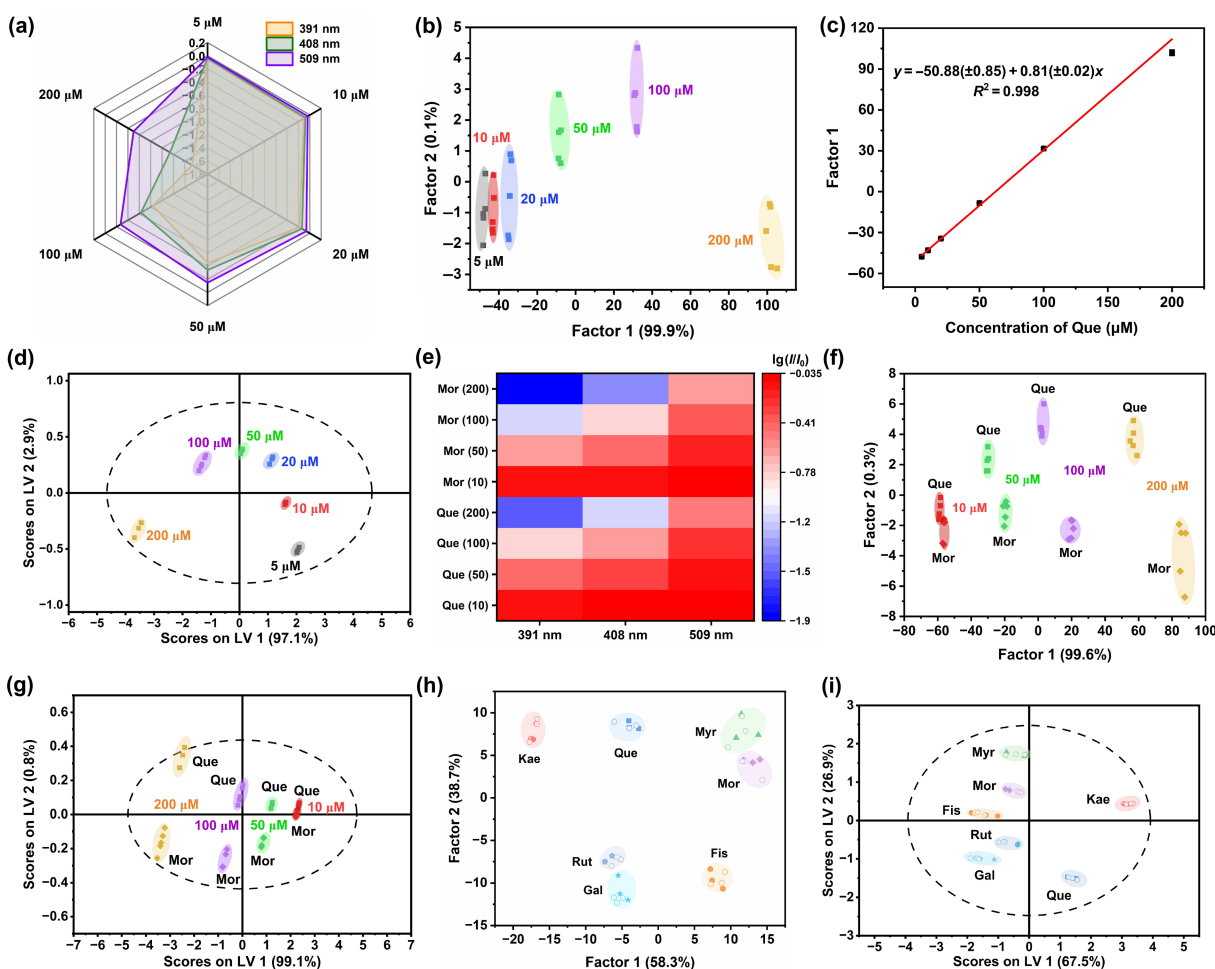


Figure 4 Discrimination of individual flavonols with varying concentrations, flavonol isomers, and unknown samples. (a) Radar plots and (b) LDA score plots of DanBrIM/PyTS ensemble to Que at different concentrations. (c) Plot of the factor 1 vs. the Que concentrations. (d) PLS-DA canonical score plots of DanBrIM/PyTS ensemble to Que at different concentrations. (e) Heat map, (f) LDA, and (g) PLS-DA canonical score plots of sensor ensemble to Que and Mor at different concentrations. (h) LDA and (i) PLS-DA canonical score plots of DanBrIM/PyTS ensemble to identify unknown flavonols at 50 μM . Filled symbols denote the trained known samples, while open circles represent unknown samples.

(FRET), IFE, dynamic quenching, static quenching, or PET. In order to elucidate the fluorescence quenching sensing mechanism of the DanBrIM/PyTS ensemble toward flavonols, the UV-vis absorption spectra of flavonols and the fluorescence excitation and emission spectra of DanBrIM/PyTS ensemble were tested. As shown in Fig. 5(a), the absorption spectra of different flavonols exhibit varying degrees of spectral overlap with the fluorescence excitation and emission spectra of the sensor ensemble. Specifically, the overlapping range with the excitation spectrum is mainly located at 270–450 nm (Fig. 5(b) and Fig. S14 in the ESM), and the overlapping region with the emission spectrum is concentrated at 370–450 nm (Fig. 5(c) and Fig. S15 in the ESM). Different degrees of spectral overlap may lead to varied quenching efficiencies, which can be attributed to either FRET or IFE [36, 37]. Notably, FRET can shorten the fluorescence lifetime, whereas IFE cannot [38]. Therefore, time-resolved fluorescence decay curves of the present sensing platform in the absence and presence of flavonols were recorded at 391 nm and the results were presented in Fig. 5(d). The decay curves were fitted to a bi-exponential function with acceptable χ^2 values, as listed in Table S1 in the ESM. And the average fluorescence lifetime remains almost unchanged before and after the addition of the analytes, indicating that the FRET

mechanism can be excluded. Meanwhile, dynamic quenching is ruled out as a potential quenching mechanism, because it generally leads to distinct changes in the fluorescence lifetime of sensing platforms.

In addition, UV-vis absorption spectra of the DanBrIM/PyTS ensemble upon titration of different flavonols were measured. As depicted in Fig. 5(e) and Fig. S16 in the ESM, the addition of flavonols caused no obvious shift in the absorption spectrum of the sensing platform, suggesting that the analytes did not alter the aggregation state of the DanBrIM assembly, and no static quenching occurred between flavonols and sensor ensemble [39]. To verify the feasibility of electron transfer from excited-state DanBrIM or PyTS (electron donors) to the LUMO (lowest unoccupied molecular orbital) of electron-deficient flavonols (electron acceptors) via PET mechanism, we compared the HOMO (highest occupied molecular orbital) and LUMO energy levels of the probes (calculated using B3LYP/6-31G(g,d)) with those of flavonols. As shown in Fig. 5(f), the LUMO energy level of DanBrIM is higher than those of Gal, Kae, Que, Mor, Myr and Fis, and the LUMO energy level of PyTS is higher than those of Gal and Myr, allowing efficient PET to proceed. Accordingly, electrons readily transfer from excited-state of the probes to the LUMO of

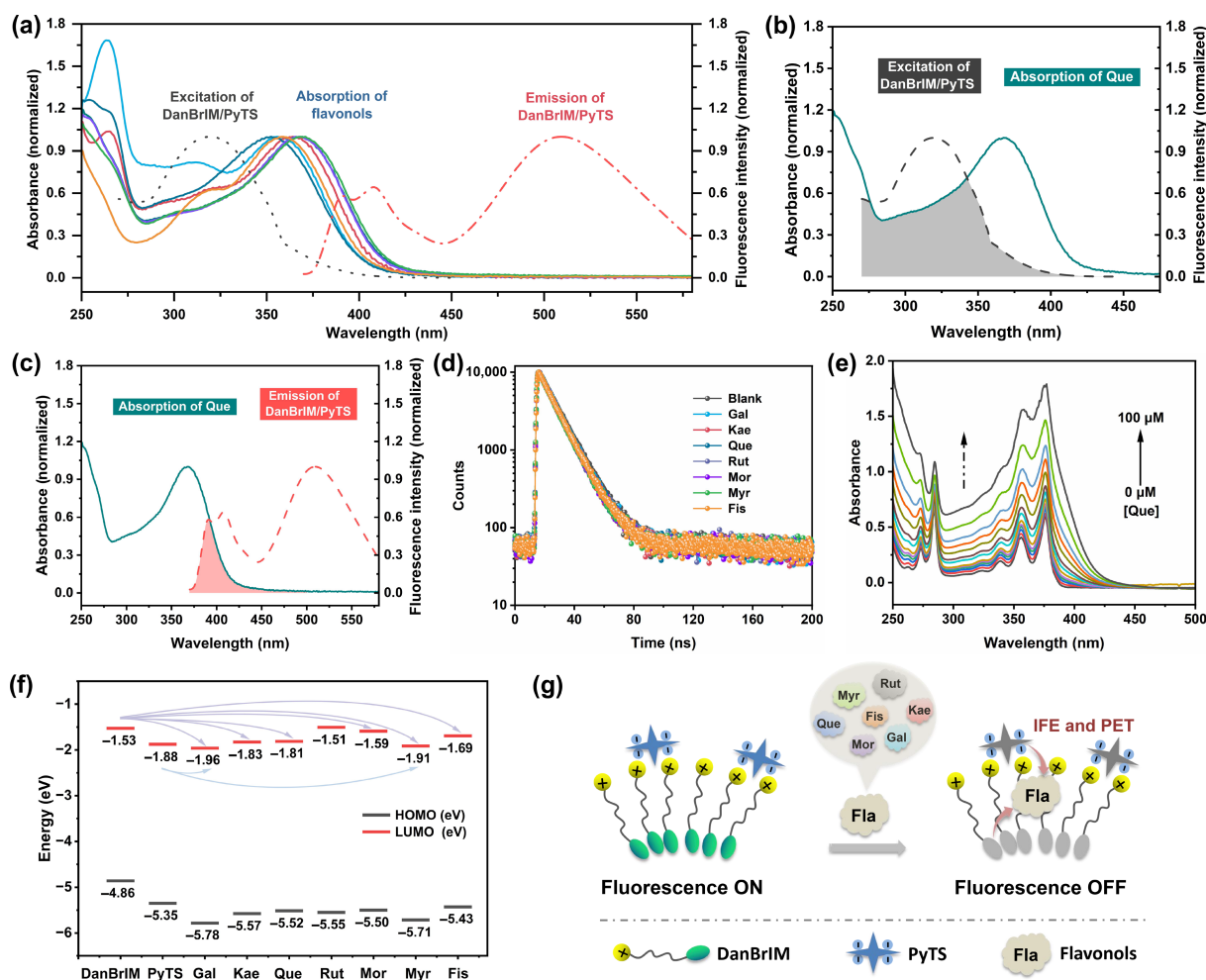


Figure 5 Sensing mechanism of the sensor ensemble to flavonols. (a) Normalized UV-vis absorption spectra of flavonols (10 μM), and normalized excitation and emission spectra of DanBrIM/PyTS ensemble. Spectral overlap between the absorption spectrum of Que (10 μM) and (b) the fluorescence excitation, and (c) emission spectrum of DanBrIM/PyTS ensemble. (d) Time-resolved fluorescence decay curves of DanBrIM/PyTS ensemble before and after addition of various flavonols (10 μM). (e) UV-vis absorption spectra of DanBrIM/PyTS ensemble upon titration of Que. (f) Frontier orbital levels of DanBrIM, PyTS, and flavonols. (g) Schematic representation of the DanBrIM/PyTS ensemble for sensing of flavonols.

flavonols, thereby quenching the fluorescence emission of probes [40, 41]. On the basis of the above results and discussion, the fluorescence quenching response of DanBrIM/PyTS ensemble to flavonols arises from the synergistic action of IFE and PET, and a proposed sensing process is illustrated in Fig. 5(g). Compared with previously reported flavonoid sensing systems, our proposed method possesses the merits of easy data collection and broad discrimination of various flavonols (Table S2 in the ESM).

3.5 Practical application in identifying various actual tea samples

As it is well known, the content and types of flavonols in tea vary among different tea types, which is critical for tea quality control and monitoring. To evaluate the practical application of the proposed sensor ensemble, its discrimination capability toward flavonols was further employed to identify various actual tea samples. First, the ability of the sensing platform to distinguish six different types of tea, including dark tea, white tea, medicinal herbal tea, black tea, oolong tea and green tea, was investigated (Table S3 in the ESM). Then, the recognition capability of the sensing platform for five different brands of the same type of tea (green tea) was tested (Table S4 in the ESM). As shown in Fig. 6(a), both

different types of tea and different brands of the same type of tea displayed significant fluorescence differences across the three wavelengths of the sensor. The fluorescence response patterns of these tea samples were similar to those of flavonols, with distinct fluorescence quenching observed at 391, 408, and 509 nm. Besides, the difference among different tea at the three wavelengths could be well displayed by the polar heat map (Figs. 6(b) and 6(c)). Furthermore, the acquired fluorescence signals were arranged in a matrix and processed by LDA and PLS-DA to derive canonical scores for the classification and identification of the tea samples. For the six different types of tea, the resulting LDA and PLS-DA score plots demonstrated that each group of tea was well-clustered and free of overlap, indicating that they could be thoroughly distinguished from one another (Figs. 6(d) and 6(e)). Similarly, the proposed sensor also exhibited excellent discrimination capability for green tea from five different brands (Figs. 6(f) and 6(g)). Then, cross-validation confusion matrix of the training set based on LDA was utilized to assess the classification accuracy. As shown in Tables S5 and S6 in the ESM, all tea samples were correctly classified with 100% discrimination accuracy. Meanwhile, we found that the PyTS probe alone exhibited weak discrimination capability toward tea samples (Fig. S17 in the ESM), further demonstrating the necessity of the dual-probe strategy. These results imply that the proposed

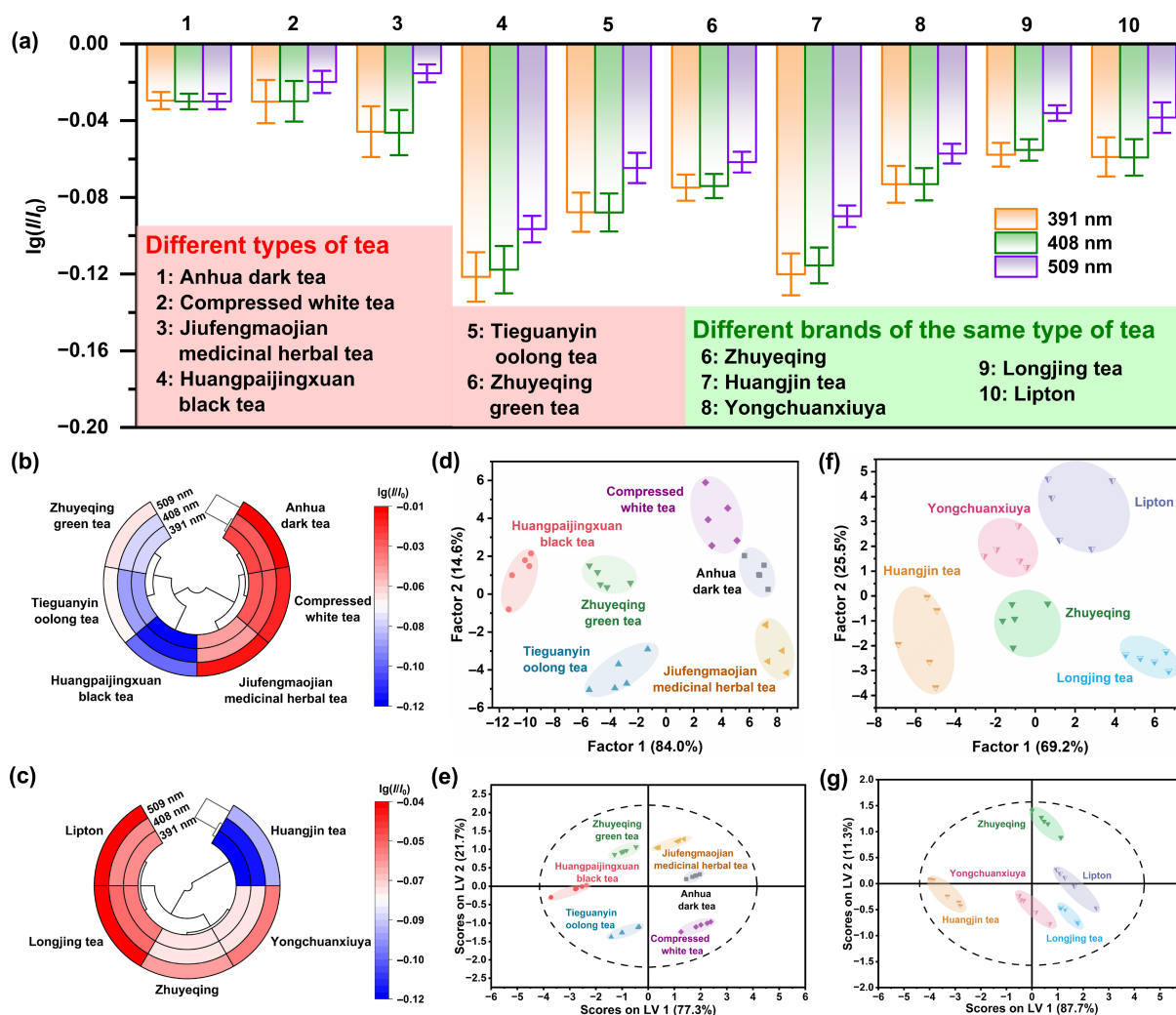


Figure 6 Discrimination of different tea samples. (a) Fingerprint recognition patterns of DanBrIM/PyTS ensemble to different tea. Polar heat map of sensor ensemble to (b) different types of tea and (c) different brands of the same type of tea. (d) LDA and (e) PLS-DA score plots of sensor ensemble to different types of tea. (f) LDA and (g) PLS-DA score plots of sensor ensemble to different brands of the same type of tea.

simple dual-probe-based sensing system possesses a strong capability for analyzing real-world samples such as commercially available tea. Therefore, this sensor exhibits great potential for applications in food testing and analysis, for example, in distinguishing counterfeit products from genuine ones.

4 Conclusions

In summary, a facile dual-probe sensor ensemble based on DanBrIM aggregates and PyTS was successfully constructed, which exhibited multi-wavelength cross-reactive responses toward flavonols. This binary DanBrIM/PyTS ensemble could discriminate seven structurally similar flavonols as well as their unknown samples by LDA and PLS-DA. The spectral overlap between the fluorescence excitation and emission spectra of the sensor system, and the UV-vis absorption spectra of the flavonols, fluorescence lifetime measurements, and frontier molecular orbital level calculations revealed that the fingerprint sensing mechanism may arise from IFE and PET processes. This proposed cost-effective sensor ensemble was further applied to identify various actual tea samples, including different types of tea and different brands of the same type of tea. This work provides a flexible method to fabricate discriminative sensors, which is expected to promote the development of efficient detection techniques for complex samples in both laboratory and food-related fields.

Electronic Supplementary Material: Supplementary material (synthesis route, fluorescence emission spectra, radar plots, heat maps, LDA, PLS-DA, HCA, PCA and other test data) is available in the online version of this article at <https://doi.org/10.26599/NR.2026.94909035>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon request.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 22302144 and 22572115), Scientific and Technological Innovation Programs of Higher Education Institutions in Shanxi (No. 2023L251), Laboratory of Protein-based Functional Materials (No. 2022P010), and College Student's Innovation Fund of Taiyuan Normal University (No. CXCY26029).

Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

J. M. F.: Supervision, writing – review & editing, funding acquisition. H. Y. D.: Data curation, investigation, writing – original draft. Y. J. W.: Data curation, investigation. R. T. Z.: Supervision, formal analysis. T. T. Z.: Investigation, data curation. H. F. H.: Supervision, funding acquisition. L. P. D.: Writing – review & editing, funding acquisition.

Informed consent

Not applicable.

Ethics statement

Not applicable.

Use of AI statement

None.

References

- [1] Bondonno, N. P.; Dalgaard, F.; Kyrø, C.; Murray, K.; Bondonno, C. P.; Lewis, J. R.; Croft, K. D.; Gislason, G.; Scalbert, A.; Cassidy, A. et al. Flavonoid intake is associated with lower mortality in the Danish diet cancer and health cohort. *Nat. Commun.* **2019**, *10*, 3651.
- [2] Wu, X. X.; Haruna, S. A.; Lv, N. Z.; Jiang, F. L.; Xia, Y.; Cai, Q. G.; Chen, Q. M.; Chen, X. M.; Jiao, T. H.; Chen, Q. S. Rapid identification of white tea based on colorimetric indicator displacement assay (IDA) sensor array. *Food Control* **2025**, *168*, 110884.
- [3] Yin, K. P.; Wu, S. Q.; Zheng, H.; Gao, L.; Liu, J. F.; Yang, C. L.; Qi, L. W.; Peng, J. J. Lanthanide metal-organic framework-based fluorescent sensor arrays to discriminate and quantify ingredients of natural medicine. *Langmuir* **2021**, *37*, 5321–5328.
- [4] Ni, W. W.; Yu, Y.; Gao, X.; Han, Y.; Zhang, W. H.; Zhang, Z. R.; Xiao, W. Q.; Hu, Q.; Zhang, Y. L.; Huang, H. et al. Multilocus distance-regulated sensor array for recognition of polyphenols via machine learning and indicator displacement assay. *Anal. Chem.* **2024**, *96*, 301–308.
- [5] He, G. Y.; Hou, X.; Han, M.; Qiu, S. T.; Li, Y.; Qin, S. D.; Chen, X. Discrimination and polyphenol compositions of green teas with seasonal variations based on UPLC-QTOF/MS combined with chemometrics. *J. Food Compos. Anal.* **2022**, *105*, 104267.
- [6] Gomes, J. S.; De Sousa, R. M. F.; Da Silveira Petruc, J. F. Paper-based colorimetric sensor array for the rapid and on-site discrimination of green tea samples based on the flavonoid composition. *Anal. Methods* **2022**, *14*, 2471–2478.
- [7] Ren, Z. M.; Deng, Q. X.; Wang, Y.; Yang, Y. J.; Wang, H. B.; Liu, F. F.; Jing, W. J. Machine learning assisted nanozyme sensor array for accurate identification and discrimination of flavonoids in healthy tea. *Food Chem.* **2025**, *486*, 144612.
- [8] Su, Z. P.; Chen, Y. X.; Ren, Y. J.; Deng, C. M.; Wei, D. L.; Zhao, R. J.; Zhang, Z. Sensor array based on luminescent metal-organic frameworks for rapid identification of multiple antibiotics. *Microchem. J.* **2026**, *222*, 117095.
- [9] Chi, J.; Qin, Y. X.; Ni, L. X.; Chen, X. Z.; Pan, X. X.; Liu, Y. W.; Wang, S. J.; Song, Y. Y.; Feng, L. Engineered single nanozyme-based time-encoded virtual sensor array for high-throughput detection of antioxidants. *Chem. Eng. J.* **2026**, *535*, 175630.
- [10] Qin, S. X.; Zhu, L. Y.; Zhu, J.; Li, F.; Li, F. L.; Lin, H. N.; Qian, H. Covalent-organic frameworks with cross-combined features as fluorescence sensor array for the discrimination and detection of multigeneration energetic materials. *Sens. Actuators B: Chem.* **2026**, *452*, 139444.
- [11] Yan, Z.; Zhang, R. R.; Qiao, M.; Ma, M.; Liu, T. H.; Ding, L. P.; Fang, Y. Single-probe-based sensor array for fingerprint recognition of trivalent metal ions and application in water identification. *Anal. Chem.* **2024**, *96*, 13801–13810.
- [12] Barimah, A. O.; Chao, M. J.; Tai, S. M.; Sun, C. D.; Agyekum, A. A.; Peng, C. F.; Wang, Z. P.; Addotey, T. N. A. Fluorescent sensor array based on emission enhanced gold nanoclusters for detecting and discriminating pesticides in vegetables. *Food Chem.* **2026**, *499*, 147405.

- [13] Yang, X. R.; Ma, L.; Wang, S. Q.; Hu, X. Y.; Li, B.; Li, W.; Wang, D. S.; Dai, Z. H. Electrochemiluminescence-based multiplex immunoassays for multianalyte bioanalysis: Signal encoding strategies and analytical perspectives. *Electrochem. Commun.* **2026**, *186*, 108130.
- [14] Gao, W. X.; Liu, W. F.; Huang, S. J.; Wang, L.; Jian, Y. Y.; Li, Y. T.; Wu, W. W.; Shang, L. Portable hydrogel-based tri-channel fluorescence sensor array for visual detection of multiple explosives. *Nano Res.* **2024**, *17*, 6483–6492.
- [15] Gao, W. X.; Wang, Z. B.; Li, Q.; Liu, W. F.; Guo, H.; Shang, L. Deep learning-assisted sensor array based on host-guest chemistry for accurate fluorescent visual identification of multiple explosives. *Anal. Chem.* **2025**, *97*, 11249–11258.
- [16] Yin, M. Y.; Qiu, D. F.; Wang, M. Q.; Wang, Z. D.; Han, L. R.; Li, L. S.; Tong, J.; Nie, H. L.; Wu, Y.; Qiao, X. Q. Fluorescence sensor array for highly sensitive pattern recognition of biothiols in food based on tricolor upconversion luminescence metal-organic frameworks. *J. Nanobiotechnol.* **2024**, *22*, 719.
- [17] Qian, Z. N.; Jiang, C. Y.; Liu, C.; Liu, X. Y.; Zhang, X. Y.; Leng, Y. M.; Li, K.; Chen, Z. B. A dual-channel sensor array for discrimination of biothiols based on manganese dioxide nanosheets. *Microchim. Acta* **2023**, *190*, 294.
- [18] Ding, Y. B.; Wang, J. X.; Wang, R. N.; Xie, Y. S. Development of porphyrin-based fluorescent sensors and sensor arrays for saccharide recognition. *Chin. Chem. Lett.* **2024**, *35*, 109008.
- [19] Pushina, M.; Penavic, A.; Farshbaf, S.; Anzenbacher, P. Jr. Fluorescent sensor array for quantitative determination of saccharides. *ACS Sens.* **2021**, *6*, 4001–4008.
- [20] Zhang, S. T.; Zhu, W. W.; Zhang, X.; Mei, L. H.; Liu, J.; Wang, F. B. Machine learning-driven fluorescent sensor array using aqueous CsPbBr₃ perovskite quantum dots for rapid detection and sterilization of foodborne pathogens. *J. Hazard. Mater.* **2025**, *483*, 136655.
- [21] Yin, M. Y.; Jing, C.; Li, H. J.; Deng, Q. L.; Wang, S. Surface chemistry modified upconversion nanoparticles as fluorescent sensor array for discrimination of foodborne pathogenic bacteria. *J. Nanobiotechnol.* **2020**, *18*, 41.
- [22] Tan, H. L.; Zeng, M. Z.; Fang, C.; Zhu, X. H.; Liu, F.; Long, Y.; Deng, R. J.; Yin, P.; Liu, M. L.; Li, H. T. et al. Pattern-based rapid and robust profiling of gut microbiota and its metabolism with a single fluorescent probe. *Sci. China Chem.* **2025**, *68*, 781–788.
- [23] Xiao, Y.; Zhang, M.; Lu, N. Fluorescent fingerprint identification of protein structural changes and disease-specific amyloid beta aggregates based on a single-nanozyme sensor array. *Anal. Chem.* **2025**, *97*, 4978–4986.
- [24] Chen, M. M.; Zhang, Y. Q.; Cheng, L. C.; Zhao, F. J.; Wang, P. Machine learning-powered fluorescent sensor arrays for rapid detection of heavy metals and pesticides in complex environments. *Biosens. Bioelectron.* **2025**, *287*, 117706.
- [25] Wei, D. L.; Zhang, H.; Tao, Y.; Wang, K. X.; Wang, Y.; Deng, C. M.; Xu, R. F.; Zhu, N. F.; Lu, Y. Y.; Zeng, K. et al. Dual-emission single sensing element-assembled fluorescent sensor arrays for the rapid discrimination of multiple surfactants in environments. *Anal. Chem.* **2024**, *96*, 4987–4996.
- [26] Huang, W.; Seehafer, K.; Bunz, U. H. F. Discrimination of flavonoids by a hypothesis free sensor array. *ACS Appl. Polym. Mater.* **2019**, *1*, 1301–1307.
- [27] Yang, M. H.; Zhang, M.; Jia, M. Y. Optical sensor arrays for the detection and discrimination of natural products. *Nat. Prod. Rep.* **2023**, *40*, 628–645.
- [28] Wang, Y. R.; Sun, X. L.; Zhou, Y. F.; Liu, J. C.; Zhu, H. Y.; Jiang, R.; Miao, Y. J.; Fu, Y. Q. A ratiometric fluorescent probe based on UiO-66-TCPP for selective and visual detection of quercetin in food. *Food Chem.* **2024**, *457*, 140198.
- [29] Zhang, L. L.; Ma, J. Y.; Yang, F.; Li, S. S.; Ma, W. R.; Chang, X.; Yang, L. Neuroprotective effects of quercetin on ischemic stroke: A literature review. *Front. Pharmacol.* **2022**, *13*, 854249.
- [30] Li, M.; Wang, D. Y.; Peng, C. F.; Wang, Z. P. Simultaneous qualitative and quantitative analysis of flavonols in Kaempferia galangal L. and honey by machine learning-based fluorescence sensor array. *Sens. Actuators B: Chem.* **2023**, *378*, 133183.
- [31] Zhao, S. P.; Zhang, B.; Yang, J.; Zhou, J. H.; Xu, Y. Linear discriminant analysis. *Nat. Rev. Methods Primers* **2024**, *4*, 70.
- [32] Santonocito, R.; Parlascino, R.; Cavallaro, A.; Puglisi, R.; Pappalardo, A.; Aloï, F.; Licciardello, A.; Tuccitto, N.; Cacciola, S. O.; Trusso Sfrassetto, G. Detection of plant pathogenic fungi by a fluorescent sensor array. *Sens. Actuators B: Chem.* **2023**, *393*, 134305.
- [33] Kumar, U.; Singh, G.; Kaur, N.; Singh, N. π -conjugated supramolecular self-assembled fluorescent sensor array for biogenic amines: A machine learning approach. *Org. Lett.* **2025**, *27*, 6931–6936.
- [34] Gao, R. R.; Zhao, W.; Qiu, Q.; Xie, A. M.; Cheng, S. Y.; Jiao, Y. Z.; Pan, X. H.; Dong, W. Fluorescent conjugated microporous polymer (CMP) derived sensor array for multiple organic/inorganic contaminants detection. *Sens. Actuators B: Chem.* **2020**, *320*, 128448.
- [35] Lin, Z. Y.; Qu, Z. B.; Chen, Z. H.; Han, X. Y.; Deng, L. X.; Luo, Q. Y.; Jin, Z. W.; Shi, G. Y.; Zhang, M. The marriage of protein and lanthanide: Unveiling a time-resolved fluorescence sensor array regulated by pH toward high-throughput assay of metal ions in biofluids. *Anal. Chem.* **2019**, *91*, 11170–11177.
- [36] Huang, T. T.; Li, K.; Shen, X. Q.; Quan, X. P.; Yan, B. A novel dual-centers fluorescent sensor based on the composite of COF and HOF for the detection of aristolochic acid and flavonoids. *Colloids Surf. A: Physicochem. Eng. Aspects* **2025**, *725*, 137656.
- [37] Marimuthu, M.; Xu, K.; Song, W.; Chen, Q.; Wen, H. Safeguarding food safety: Nanomaterials-based fluorescent sensors for pesticide tracing. *Food Chem.* **2025**, *463*, 141288.
- [38] Mu, X. W.; Wu, M. X.; Zhang, B.; Liu, X.; Xu, S. M.; Huang, Y. B.; Wang, X. H.; Song, D. Q.; Ma, P. Y.; Sun, Y. A sensitive “off-on” carbon dots-Ag nanoparticles fluorescent probe for cysteamine detection via the inner filter effect. *Talanta* **2021**, *221*, 121463.
- [39] Bo, Y.; Fan, J. M.; Yan, S. S.; Ding, M.; Liu, J.; Peng, J. X.; Ding, L. P. Surfactant modulation effect on the fluorescence emission of a dual-fluorophore: Realizing a single discriminative sensor for identifying different proteins in aqueous solutions. *Sens. Actuators B: Chem.* **2019**, *295*, 168–178.
- [40] Zhang, M. Y.; Li, J. Y.; Cao, W.; Ren, X. B.; Wang, L.; Li, J. Y.; Liu, S. L.; Wang, Z. Y.; Wang, D. S.; Dai, Z. H. Individual single-crystalline irregular In₂O₃ microcavity for ultrasensitive semiconductor-based SERS biosensor. *Adv. Mater.* **2026**, *38*, e15510.
- [41] Sriwastav, S.; Malik, S. Luminescent mesoporous squaraine copolymers: Probing the structural, optical, and sensing behaviors. *J. Polym. Sci.* **2025**, *63*, 1398–1406.



This is an open access article under the terms of the Creative Commons Attribution 4.0 International License (CC BY 4.0, <https://creativecommons.org/licenses/by/4.0/>).

© The Author(s) 2026. Published by Tsinghua University Press.



清华大学出版社
Tsinghua University Press

SciOpen

94909035 (11 of 11)

Nano Research, 2026, 19, 94909035