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Fluorometric detection of volatile amines using an indanonalkene platform†

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Excessive volatile amines are considered to cause environmental pollution and harm to human health, and especially, ammonia has been reported to be associated with a variety of human diseases. In this article, we developed a gas sensor named **CA-SCH$_3$** based on an indanonalkene structure, for real-time/on-site and quantitative detection of volatile amines. The non-luminescent probe containing a bis-vinylogous thioester receptor exhibited high reactivity towards volatile amines, *e.g.*, ammonia, *via* an amine–thiol scrambling reaction. A turn-on fluorescence signal and a blue shift in the UV-vis absorbance signal were observed for this chemosensor in the presence of ammonia. A paper-based gas sensor loaded with the probe exhibited fluorescence enhancement for the quantification of ammonia gas. Volatile amines from fish spoilage were also captured and detected *via* the paper sensor. Furthermore, we prepared a polyethylene glycol film interlinked by the **CA-SCH$_3$** reagent which exhibited a dual response of macroscopic degradation and fluorescence readout towards ammonia vapors. The fluorescent probe (**CA-SCH$_3$**) with a novel amine receptor offers extensive possibilities for developing additional photoluminescent detection systems.

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Introduction

Amines are an important class of chemical substances in nature and industrial production.[1,2] Many of them are volatile, corrosive and dangerous.[3,4] Consequently, sensing and monitoring of such highly toxic and volatile amine substances are required for early-stage warning systems and to protect the environment and human health.[5,6] The detection of volatile amines is thus critical in many fields.[6–9] In particular, endogenous volatile biogenetic amines in exhaled breath and amine compounds in urine and sweat are considered to be analytes for the early diagnosis of diseases.[10] Moreover, in the process of food spoilage, microorganisms decompose free amino acids into biogenic amines such as histamine, cadaverine, putrescine, dimethylamine, trimethylamine, *etc.*, which can cause serious harm to human health.[11,12] Therefore, it is important to develop detection methods for volatile amines with high sensitivity for real world applications.

The gas sensors reported previously include electrochemical sensors, semiconductor sensors and photochemical sensors.[13–15] They are generally based on selected metal oxides, which endow them with good sensitivity but limited selectivity,[16] and instability and loss of sensitivity after exposure to moisture or long operating time.[17,18] In recent years, gas sensors based on semiconductors have been reported to detect ammonia.[18–20] However, ammonia sensors based on these oxide chemical resistors have to work at high temperatures, thus causing energy consumption and requiring thermal insulation. In addition, a variety of composite gas sensors based on conjugated polymers of metal oxide carbon materials have been reported,[21–23] and they can sense amine VOCs (volatile organic compounds) at room temperature. However, their response rates are undesirable for further practical applications.

For fluorescent chemical sensors, multiple luminescent materials have been reported for the detection of amines.[24–26] Koner *et al.*[15] synthesized a luminescent probe containing a cyclized anhydride with molecular aggregation induced emission (AIE). Tang *et al.*[27] used the *O*-acetyl bond as an amine

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†Electronic supplementary information (ESI) available: Fluorescence images for the reaction; DFT calculations; optical properties for **CA-SCH$_3$** and **CA-NH$_2$**; calculation of detection limit; synthesis of hydrogels; scanning electron microscopy (SEM) image of **CA-PEG** gel; NMR and HRMS spectra. See DOI: https://doi.org/10.1039/d2qo02023h

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molecular trap to detect the presence of amines to produce a fluorescence indicator with AIE characteristics. Although different systems have been constructed to detect amine vapors, these systems are limited by the number of reactive amine receptors available. With this in mind, the development of chemosensors using a new receptor to capture amine vapors, along with easy operation and structural simplicity, would be beneficial for further applications and could provide an alternative fluorescence detection tool.

Previously, we established a photoluminescent platform based on indanonalkene luminogens (Scheme 1A).[28,29] On this basis, we employed **CA-SCH$_3$**, an indanonalkene molecule containing a bis-vinylogous thioester group, as an amine receptor for the fluorescence detection of volatile amines *via* amine–thiol scrambling (Scheme 1B).[30–32] As such, the luminescent precursor **CA-SCH$_3$** was turned on in the presence of ammonia in both solution and solid states to generate the luminophore **CA-NH$_2$**, exhibiting a strong green emission (Scheme 1B). In addition, multiple volatile amines can be detected using a paper-based probe loaded with **CA-SCH$_3$**, and further used to detect amine vapor in rotting fish. Furthermore, a non-luminescent soft material, *i.e.*, hydrogel, was prepared, which can be degraded by NH_3(g) through an amine–thiol decoupling reaction and the macroscopic degradation process can be tracked visually on spatial and time scales by following the changes in luminescence as a result of the generation of the indicator. Thus, we developed a new amine fluorescent chemosensor with ease of operation and structural simplicity, which demonstrated promise for the construction of portable devices.

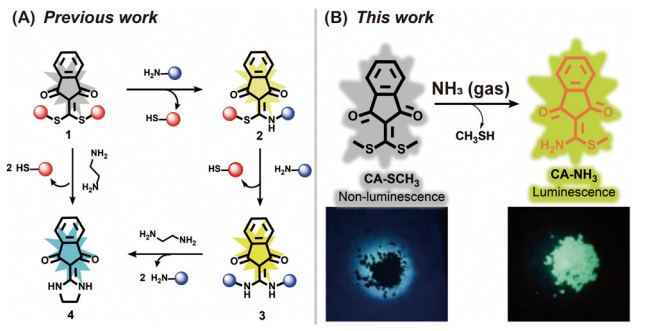
Results and discussion

UV-Vis and fluorescence responses

The luminescent precursor **CA-SCH$_3$** was prepared according to the previous method.[33] The molecule contains bis-vinylogous thioester active moieties, which are reactive towards amine derivatives.[32,34] The chemical reaction enables the design of a reaction-based chemosensor through amine–thiol scrambling, with the release of methylmercaptan. Firstly using UV-Vis absorbance spectroscopy, kinetic tracking of the photophysicochemical reaction between **CA-SCH$_3$** (10 μM) and ammonia (0.0125%) over 0–180 min resulted in a ratiometric change, with a decrease at λ_{abs} = 366 nm an increase at λ_{abs} = 336 nm and isosbestic point at 345 nm in a H_2O/DMSO (1%/99%) system (Fig. 1A and B). As can be observed by the naked eye, the test solution exhibited a colorimetric change (Fig. 1C) from yellowish to colorless. Secondly, significant fluorescence turn-on was observed for the luminescent precursor **CA-SCH$_3$** (10 μM) in the presence of ammonia (0.0125%), resulting in a more than 32-fold fluorescence enhancement at λ_{em} = 495 nm (Fig. 1D–F and S1†). To confirm the reaction product and optical properties, we prepared an independent sample of **CA-NH$_2$** and studied its absorbance and fluorescence spectra (Fig. S2 and S3†), which showed a good agreement with the absorbance and fluorescence spectra mentioned above. In addition, it was observed that the water fraction (1–99%) could affect the reaction between **CA-SCH$_3$** and $NH_3{\cdot}H_2O$ in DMSO. With an increase in the water fraction, the fluorescence was significantly quenched and the emission wavelength red shifted (Fig. S4†), which was probably due to the hydrogen bonding induced internal energy conversion of luminescent **CA-NH$_2$**.

Proposed mechanism of the optical sensing

Next, the spatial conformation optimization and energy level difference calculation of the HOMO–LUMO orbitals for **CA-SCH$_3$** and **CA-NH$_2$** were analysed using density functional theory (DFT) software. According to the calculation results (Fig. 2), the HOMO–LUMO gap of **CA-NH$_2$** was found to be



Scheme 1 Schematic illustration of the previous and current work. (A) Previous work: indanonalkene photoluminescent platform; (B) this work: fluorescence detection of volatile amines. Conjugate acceptor **CA-SCH$_3$** as the chemosensor and its reaction with NH_3 to generate luminogen **CA-NH$_2$**. Inset pictures: non-luminescent solid **CA-SCH$_3$** and luminescent solid **CA-NH$_2$**.

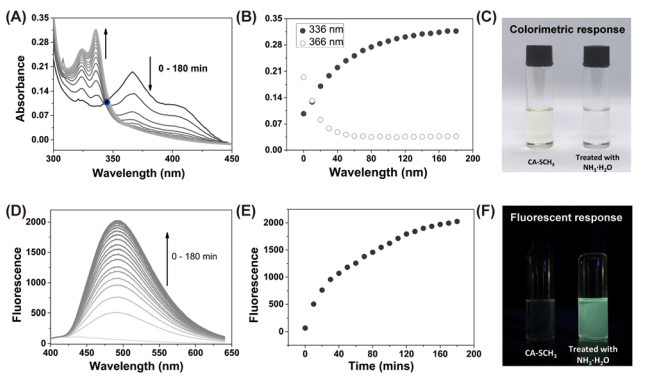


Fig. 1 UV-Vis and fluorescence responses for the **CA-SCH$_3$** probe. (A) Time-dependent UV-Vis absorbance spectra of **CA-SCH$_3$** (10 μM) upon addition of $NH_3{\cdot}H_2O$ (0.0125%) in a H_2O/DMSO = 1% system over 180 min. (B) Time course of absorbance at λ_{abs} = 336 nm (●) and λ_{abs} = 366 nm (○), respectively, referred to (A). (C) Colorimetric photograph before and after $NH_3{\cdot}H_2O$ treatment. (D) Time-dependent fluorescence spectra of **CA-SCH$_3$** (10 μM) upon addition of $NH_3{\cdot}H_2O$ (0.0125%) in a H_2O/DMSO = 1% system. λ_{ex} = 336 nm; λ_{em} = 495 nm. (E) Time course of fluorescence intensity at 495 nm, referred to as (D). (F) Fluorescence photograph before and after $NH_3{\cdot}H_2O$ treatment under 365 nm UV light.

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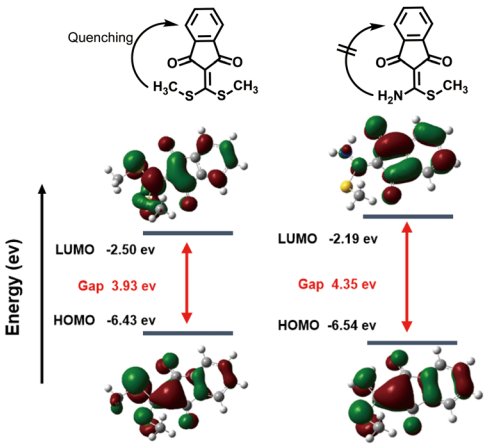

Fig. 2 Optimized DFT molecular orbital (LUMO and HOMO) for **CA-SCH$_3$** and **CA-NH$_2$** at the B3LYP/6-31G (d) level (parameter settings are shown in the ESI†).

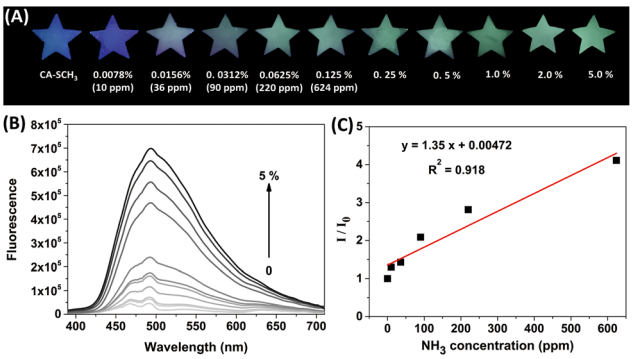

Fig. 3 (A) Photographs of paper-based sensor **CA-SCH$_3$** treated with various concentrations of $NH_3{\cdot}H_2O$ for 1 h were taken under UV light (365 nm). (B) Solid state fluorescence spectra of the filter papers in (A). (C) Linear fitting of I/I_0 with ammonia concentrations ranging from 0 to 624 ppm. I_0 and I show the fluorescence intensities of **CA-SCH$_3$** at 495 nm and that upon exposure to various concentrations of amine vapor.

4.35 eV while that of **CA-SCH$_3$** was 3.93 eV, which matched the experimental blue shift in absorbance. As reported in our previous work,[28] the fluorescence quenching due to vibrational energy loss from the bis-vinylogous thioester of **CA-SCH$_3$** accounted for the initial state of the probe. When the thiol group was substituted by the amine, the quenching factor was diminished which truned on the photoluminescence output. The mechanism to explain the fluorescence performance of **CA-NH$_2$** was proposed as excited state electronic charge distribution from the double bond that was stabilized by the amine, which led to the UV-Vis absorbance shifting to a shorter wavelength with higher energy and fluorescence enhancement.

Paper-based sensor for the detection of ammonia vapors

Next, we prepared test papers loaded with **CA-SCH$_3$** as a portable sensor for the real-time detection of amine vapors. A solution of **CA-SCH$_3$** (10 mM) in CH_2Cl_2 was dropped on to star-shaped paper and dried by evaporation. As shown in Fig. 3A, the paper-based sensor **CA-SCH$_3$** was non-emissive under 365 nm UV irradiation, while treatment with ammonia vapor (produced by $NH_3{\cdot}H_2O$) led to luminescence turn-on over 1 h in a dose-dependent manner. As the concentration of ammonia vapor ranged from 0 to 624 ppm, the emission brightness and fluorescence intensity of the paper enhanced. The solid fluorescence emission spectra of the **CA-SCH$_3$**-loaded paper after exposure to ammonia vapor are shown in Fig. 3B. A 16.5-fold signal enhancement in the presence of ammonia vapor produced from 5% ammonia at λ_{em} = 336 nm was observed. With the concentration of ammonia vapor increasing from 0 to 624 ppm ($NH_3{\cdot}H_2O$ concentrations from 0 to 0.125%), a moderate linear relationship (R^2 = 0.918) between I/I_0 at 495 nm and analyte concentration was observed (Fig. 3C), which can be potentially used as a calibration curve for the detection of unknown samples. Based on the signal-to-noise method, the detection limit ($3\sigma/S$) for ammonia vapor was calculated to be 36 ppm (see the ESI†). These experimental results demonstrate that **CA-SCH$_3$**-loaded filter paper can be potentially used as a portable real-time sensor for the tracking and quantification of volatile amines.

Paper-based sensor for the detection of multiple amines

In addition to ammonia, diverse amine species can be found in nature, as well as in industrial manufacturing. Thus, the capability of the sensor to detect various amine vapors was then investigated. As shown in Fig. 4B, the fluorescence emission of the paper sensors was turned on and enhanced in the presence of different amine vapors, including ammonia (2.34-fold), hydrazine (2.31-fold), aliphatic amine (benzylamine, 4.65-fold), $EtNH_2$ (2.58-fold), putrescine (1.57-fold), 1,3-propylene diamine (1.48-fold), cadaverine (1.39-fold), trimethyl-

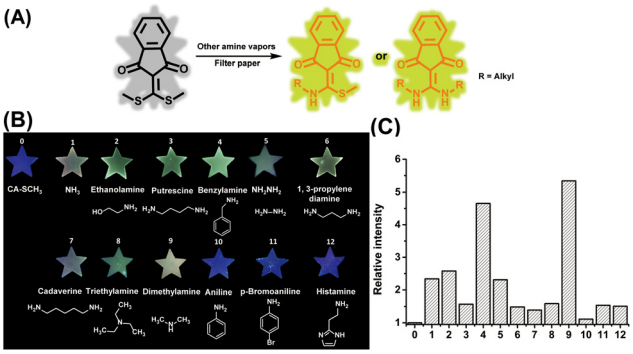

Fig. 4 (A) Schematic diagram of the reaction of **CA-SCH$_3$** with volatile amines. (B) Selectivity of **CA-SCH$_3$** for a variety of volatile amine compounds (numbers 1 to 12 represent NH_3, ethanolamine, putrescine, benzylamine, NH_2NH_2, 1,3-propylene diamine, cadaverine, triethylamine, dimethylamine, aniline, *p*-bromoaniline and histamine respectively). The test paper was exposed to a variety of amine vapors released from an aqueous or methanol solution (0.08 M) of amine compounds for 2 h and then photographed under 365 nm UV light irradiation. (C) Relative fluorescence intensity at 495 nm of the paper-based probe **CA-SCH$_3$** after exposure to the 12 amine compounds in (B). λ_{ex} = 336 nm.

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amine (1.59-fold), and dimethylamine (5.35-fold) vapors (produced by using an aqueous or methanol solution (0.08 M) of amine compounds), respectively over the initial **CA-SCH$_3$** (Fig. 4C). The signal enhancement indicated that these amine vapors led to emissive amine substituted products after amine–thiol scrambling with **CA-SCH$_3$** (Fig. 4A). However, aromatic amine derivatives, *i.e.* aniline and *p*-bromoaniline, did not exhibit any fluorescence signal response, probably due to reduced nucleophilicity.[27] In addition, histamine, as a kind of biogenic amine, may not produce fluorescence response most probably due to low volatility.[35] Therefore the paper-based **CA-SCH$_3$** sensor is capable of selective luminescence detection of volatile amine vapors, and is promising for future applications (see below).

Real-world application of the amine sensor

The generation of biogenic amines by microbial fermentation in food is used to help evaluate freshness.[36] As such, the development of a simple portable detector for reliable, fast, and real-time assessment of food freshness would be particularly beneficial. Therefore, we decided to evaluate food spoilage utilizing the newly developed chemosensor. When a cod fillet was sealed in a plastic box and stored at 4 °C for 5 days, almost no fluorescence enhancement was observed for the paper sensor after placing it with the cod fillet for 2 h (Fig. 5A). However, a strong fluorescence emission was observed by the naked eye when the cod fillet was kept at room temperature (25 °C) for 5 days (Fig. 5A), indicating that the cod fillet has seriously deteriorated, and was not suitable for consumption. These results demonstrate that the paper-based **CA-SCH$_3$** sensor can be employed for food spoilage detection by reporting the release of biogenic amine vapor from rotting food.

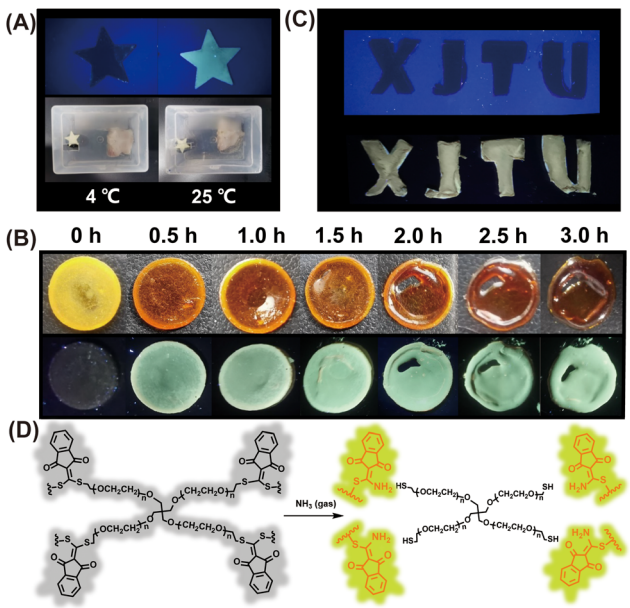

Fig. 5 (A) Paper-based sensor **CA-SCH$_3$** was placed in a box for 2 hours for the detection of amine vapors generated from cod fillets, stored at 4 °C and 25 °C for 5 days, respectively. (B) Fluorescence responses and degradation of the **CA-PEG** gel by ammonia vapor produced by 2% $NH_3{\cdot}H_2O$ for 3 hours. (C) Fluorescence responses of the gel pattern treated with ammonia vapor (from 5% $NH_3{\cdot}H_2O$) under 365 nm UV light. (D) Sensing mechanism of the gel constructed from the reaction of **CA-SCH$_3$** with 4-arm PEG thiol in the presence of ammonia (gas). Fluorescence turn-on and gel degradation after amine–thiol replacement.

Hydrogel film sensor for volatile amine detection

In addition, typical fluorescent chemosensors mainly work in solution states.[37,38] Solid-state fluorescence sensory systems, such as test papers and polymeric films, are attractive because of their portability, operational simplicity, and stability.[39] The recognition mode or sensitivity between the chemosensor and the target relies on chemical reactivity or binding affinity.[40] Nevertheless, they suffer from restricted sensitivity and relatively long equilibrium response time because of the hindered diffusion of the analyte within low-porosity dense solid systems.[41] As such, polymeric hydrogels could be a promising alternative as chemical sensing systems owing to their high porosity, 3D network, and tissue-like mechanical properties, which in principle facilitate analyte absorption and diffusion.

As such we designed and developed a polymeric gel film named **CA-PEG** (Fig. S5†), through linking four-arm poly(ethylene glycol) thiol with **CA-SCH$_3$** through thiol–thiol scrambling and high cross linking.[29] The scanning electron microscopic (SEM) images of **CA-PEG** are given in Fig. S6.† The pore diameter distribution of the **CA-PEG** gel was analyzed using pore diameter analysis software, and the average pore size was determined to be *ca.* 53 μm. On this basis, when the non-luminescent gel film (radius × thickness = 5 mm × 1 mm) was exposed to amine vapor (produced by 2% $NH_3{\cdot}H_2O$), **CA-PEG** exhibited fluorescence enhancement within 30 min (Fig. 5B), and after 1 h exposure, the hydrogel underwent macroscopic degradation with the appearance of wrinkles (Fig. 5B). Then after 2 h, more degradation and further enhanced fluorescence could be observed. The mechanism of degradation was attributed to the amine–thiol scrambling reaction between the amines and **CA-SCH$_3$** linkers (Fig. 5D) while the fluorescence enhancement was due to the generation of **CA-NH$_2$** derivatives as the indicator. Moreover, as shown in Fig. 5C, the letters "XJTU" were carved out of the gel and were non-fluorescent under a UV lamp. Then, exposure to ammonia vapors led to a significant fluorescence enhancement, which indicated that the hydrogel film can be applied to dynamic anti-counterfeiting patterns by fluorescence turn-on of the hydrogel film when it encounters volatile amine gases.

Conclusions

In summary, based on the indanonalkene photoluminescent platform and an active amine receptor, we developed a fluorescent chemosensor, which produced a fluorescence indicator after an amine–thiol substitution reaction, thereby generating an optical signal suitable for the detection of volatile amines.

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The chemosensor was then used to prepare portable paper-based sensors and a polymeric sensor film. When exposed to volatile organic amine compounds, the physical adsorption and chemical reaction led to enhanced fluorescence emission. Furthermore, the real-time quantitative detection of amines was achieved. The sensor also exhibits high sensitivity towards aliphatic amine vapors over aromatic amines. Due to its easy preparation, portability, simple operation and good sensitivity, the sensor and field-deployable device could be potentially used for the detection of volatile environmental amines.

Author contributions

XLS conceptualization, supervision and writing – review & editing. TDJ and JZ checked the validation and manuscript writing – review & editing. HC collaborated in funding acquisition. THW supported the experimental methodology. PZ performed the experiment, collected all the data and wrote the original draft. All authors have given approval to the final version of the manuscript.

Conflicts of interest

The authors declare no competing conflicts of interest.

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