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## Portable fluorogenic probe for monitoring of volatile amine vapour and food spoilage



Jian-Hao Zhao<sup>a,c,1</sup>, Wen-Xing Xu<sup>a,b,1</sup>, Bin Li<sup>a,c</sup>, Wei Xu<sup>a,b</sup>, Wu-Kun Zhang<sup>a,b</sup>,  
Ming-Shuai Yuan<sup>a,b</sup>, Hui-Zi Li<sup>a,b</sup>, Qing-Guo He<sup>a,b</sup>, Xiang Ma<sup>d</sup>, Jian-Gong Cheng<sup>a,b,\*</sup>,  
Yan-Yan Fu<sup>a,b,\*</sup>

<sup>a</sup> State Key Lab of Transducer Technology, Shanghai Institute of Microsystem and Information Technology, Chinese Academy of Sciences, Shanghai 200050, China

<sup>b</sup> Center of Materials Science and Optoelectronics Engineering, University of the Chinese Academy of Sciences, Beijing 100039, China

<sup>c</sup> School of Physical Science and Technology, ShanghaiTech University, Shanghai 201210, China

<sup>d</sup> Key Laboratory for Advanced Materials and Feringa Nobel Prize Scientist Joint Research Center Frontiers Science Center for Materiobiology and Dynamic Chemistry, School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai 200237, China

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## ABSTRACT

There are urgent needs of volatile amine gas sensors with high-performance in food quality control, disease monitoring and environmental pollution. Thin-film fluorescent probe is suitable for amine vapour sensing due to its high sensitivity, high selectivity, and no polluting analyte. Herein, a novel fluorescent probe based on indacenodithiophene structure with  $\pi$  conjugated system was designed and synthesized. The experimental results show that the films prepared by this material exhibit rapid and distinct fluorescence quenching after being exposed to saturated vapours of primary amine, secondary amine and tertiary amine represented by *n*-propylamine, diethylamine and trimethylamine, respectively. The quenching of fluorescence is 84%, 87% and 96%, respectively, within 10 s. The detection mechanism of probe for primary amine is based on specific chemical reaction, while the detection mechanism for secondary amine and tertiary amine is intramolecular charge transfer. Further experiments show that the detection limit of the fluorescent probe for trimethylamine, an important marker of food spoilage, could reach 4.610 ppt. On-site detection based on spoilage of small yellow croaker suggests the material possesses the potential for food freshness detection. This simple fluorogenic probe is an original approach to simplify real-time visual monitoring of volatile amine vapour.

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Widely used in pharmaceutical, agricultural production and food industries, amines play an important role in modern chemical industry [1–4]. However, despite the significant contribution, certain amines may endanger human life or pose threats to environment [5]. For instance, meth-amphetamine will cause great harm to human mind and body [6], small molecule organic amines such as dimethylamine and aniline, are environmental pollutants [7]. Therefore, increasing demands for detection of volatile amines can not be neglected.

Current detection methods for amine vapour mainly include chromatography-mass spectrometry [8], metal oxide sensors [9], gas chromatography [10], ion mobility spectrum [11], electrochemical methods [12], and other classic instrumental analysis methods [13,14]. Although these traditional lab-based off-site testing methods have been proven successful with high precision, they still face problems such as expensive instrumentation, complicated sample pretreatment process, and time-consuming detection procedures which cannot meet the needs of real-time on-site monitoring situations such as public security. Fluorescent probes, compared with methods above (Table S1 in Supporting information), with the advantages of simple operation, rapid response, high sensitivity, relatively stable performance, low detection limit, and simple signal with easy identification [15–23], could be more suitable for on-site detection of various amines. Amongst variety of organic amines, trimethylamine (TMA) is a gas with an odour of fishy ammonia. The presence and concentration of trimethylamine

\* Corresponding authors at: State Key Lab of Transducer Technology, Shanghai Institute of Microsystem and Information Technology, Chinese Academy of Sciences, Shanghai 200050, China.

E-mail addresses: [jgcheng@mail.sim.ac.cn](mailto:jgcheng@mail.sim.ac.cn) (J.-G. Cheng), [fuyy@mail.sim.ac.cn](mailto:fuyy@mail.sim.ac.cn) (Y.-Y. Fu).

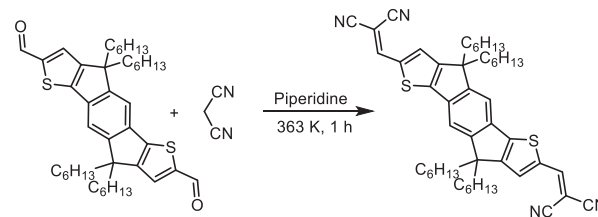
<sup>1</sup> These authors contributed equally to this work.

is an important criterion for evaluating the quality of meat and fish [24,25]. It is also the key parameter of industrial and agricultural production quality control [26]. Besides, trimethylamine vapour is an indicator that characterizes certain metabolic defects [27]. However, there are few reports of fluorescent probe for trimethylamine vapour. Thus, there is an urgent need for high-sensitivity trimethylamine vapour probes in these important areas.

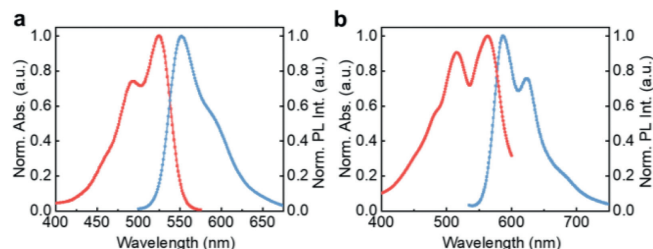
$\pi$  conjugated organic light-emitting materials have received extensive attention in many fields such as biochemical imaging [28], fluorescence sensing [29]. Their high degree of  $\pi$ -electron delocalization imparts a strong fluorescence emission, facilitating effective interactions with analytes. Furthermore, these materials offer tunable emission spectra through variations in molecular structure, providing versatility and adaptability in diverse applications. They also exhibit high photo-stability and are cost-effective. The ease of functionalization allows for the development of selective and sensitive probes, enhancing their overall utility [30,31]. Amongst these materials, 4,9-dihydro-*s*-indaceno[1,2-*b*;5,6-*b'*]dithiophene derivative, also called indacenodithiophene (IDT), is a five membered fused ring aromatic compound. Due to its large rigid planar conjugate structure, high void mobility, and easy-to-modify structure, it has become a popular structural unit in fields such as organic solar cells and organic thermoelectric materials [32,33]. Indacenodithiophene structure emits strong fluorescence in solution. However, its solid-state fluorescence is not as strong as it is in the solution phase due to aggregation induced quenching effect. To design a sensitive material for gas detection, it must exhibit high luminescence properties in the film state and good solution processability.

Therefore, in our work, we used indacenodithiophene structure with multiple alkyl chains as the parent, introduced alkenyl malonitrile structure through Knoevenagel condensation reaction. Such structure efficiently surmounts the aggregation induced quenching effect (ACQ) and improved the luminous performance of material film and introduce amine binding sites. As expected, we successfully synthesized a small-molecule fluorescent probe, IDT-CN, for real-time and on-site detection of volatile amine vapour. We investigated its luminous performance in solution and film state and its sensing properties towards three types (primary, secondary, and tertiary) of amines. Results suggest that IDT-CN shows rapid response to three types of amines with different sensing mechanisms. There are two sensing mechanisms between probe and amines: Primary amine had chemical reaction with material, while secondary amine and tertiary amine interacts with probe through intramolecular charge transfer (ICT) process. Significantly, this probe has an especially high quenching rate for TMA, which possesses valuable practical detection significance [34]. Thus, we carried out further study, exposure to TMA gas causes the film's colour change from dark pink to dim yellow together with fluorescence quenching, and material's ability of detection for TMA was measured to be as low as 3 ppb. Furthermore, the monitoring ability of this probe in actual environment was explored by evaluate the freshness of small yellow croaker, which was proved to be valid. This finding indicates a rather low-cost approach for a convenient method of highly sensitive probe for amine vapour, especially TMA.

Synthesis procedure of IDT-CN was shown in Scheme 1, experimental detail about reagents, materials, characterizations and measurements been used, was recorded in Supporting information. Along with details about preparation of sensing films and test paper, volatile amine detection. The framework of indacenodithiophene itself has a rigid planar structure, which is easily self-association thus not beneficial to sensing. The incorporation of long alkyl chains and the alkenyl malonitrile structure played a significant and multifaceted role. Firstly, incorporating long alkyl chains into our material serves to enhance its solubility and film-forming properties. The increased nonpolar character of the mate-



**Scheme 1.** Synthesis procedure of IDT-CN.



**Fig. 1.** (a) Normalized UV-vis absorption and emission spectra of IDT-CN in solution (Solvent: tetrahydrofuran) state,  $\lambda_{\text{ex}} = 524$  nm,  $\lambda_{\text{em}} = 552$  nm; (b) Normalized UV-vis absorption and emission spectra of IDT-CN in film state,  $\lambda_{\text{ex}} = 563$  nm,  $\lambda_{\text{em}} = 588$  nm.

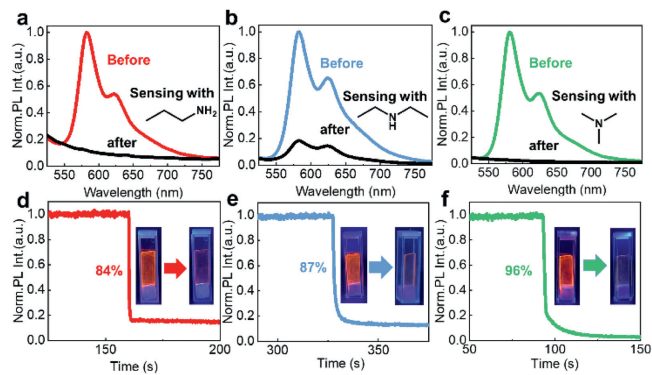
rial, along with the greater surface area for dispersion forces, facilitates solubility in nonpolar solvents.

Additionally, steric hindrance introduced by the alkyl chains prevents excessive self-association of the planar structures, thereby promoting better solubility and improved molecular organization in film formation. Secondly, allyl malonitrile had been proved to be a selective functional group for amines [35]. Most importantly, long alkyl chains together with alkenyl malonitrile structure jointly extend in three dimensions, preventing the  $\pi$ - $\pi$  stacking of molecules in solid phase and inhibiting the ACQ effect, thus improving the gas permeability and solid-state fluorescence.

IDT-CN shows excellent solubility, its absorption and fluorescence emission spectra in solution state (0.5 mg/mL tetrahydrofuran solution) are shown in Fig. 1a. The maximum absorption peak and fluorescence emission peak of IDT-CN are located at 524 nm and 552 nm respectively. Due to molecule's inhibition of  $\pi$ - $\pi$  stacking, IDT-CN can emit strong fluorescence in the film state, the maximum absorption peak and fluorescence emission peak had a slight red shift compared with the solution state, which is 563 nm and 588 nm respectively, as shown in Fig. 1b. As shown in Fig. S2 (Supporting information), the fluorescence photo quenching of IDT-CN in 60 s in air are 1.2% indicating IDT-CN's good optical stability which is important in film sensing.

All experiments were conducted at a temperature of 30 °C. The optimal concentration of IDT-CN used to form films during all sensing experiments was determined to be 0.5 mg/mL. The excitation wavelength was set at 563 nm, while the emission wavelength was 588 nm. The saturated vapour pressures for the analytes used during this experiment were 33.025 kPa for *n*-propylamine, 25.9 kPa for diethylamine, and 187 kPa for trimethylamine.

As shown in Figs. 2a-c, IDT-CN films showed massive fluorescence quenching in fluorescence emission intensity after exposure to *n*-propylamine, diethylamine and trimethylamine which represent primary amine, secondary amine and tertiary amine, respectively. This indicates the sensing material has excellent detection performance towards all three types of amines. In addition, fluorescence response time towards three types of amines was rapid. As shown in Figs. 2d and e, the fluorescence quenching rate of the films was 84% and 87% within 10 s, and subsequently maintained equilibrium after exposure to *n*-propylamine and diethylamine, respectively. While Fig. 2f shows the fluorescence quench-

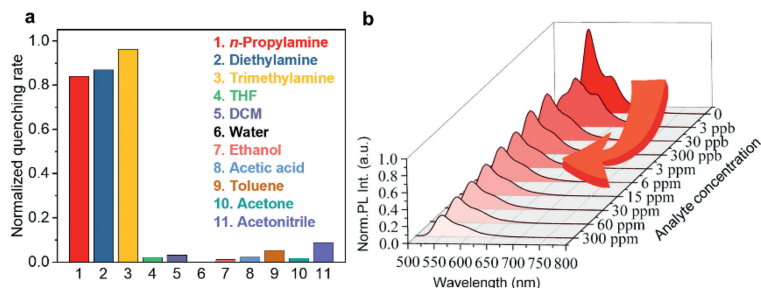


**Fig. 2.** Fluorescence emission spectra of IDT-CN films before and after exposure to saturated vapour of (a) *n*-propylamine, (b) diethylamine and (c) trimethylamine. Fluorescence intensity change of IDT-CN films (inset: fluorescence colour change of IDT-CN films) upon exposure to saturated vapour of (d) *n*-propylamine and (e) diethylamine and (f) trimethylamine.

ing rate reached 96% within 30 s (80% within 3 s) and subsequently maintained equilibrium when exposed to trimethylamine. This indicates that the probe possesses a better and faster response time for sensing trimethylamine. The same result can be obtained by naked eye observation, the quenching extent of the film exposed to trimethylamine vapour showed in Fig. 2f is significantly greater than other two amine vapours in Figs. 2d and e.

To explore the impact of temperature and humidity on the sensing response, we maintained the concentration of the analyte trimethylamine at 300 ppm and subsequently adjusted the laboratory temperature and humidity (*via* laboratory air conditioning and humidity control systems) to examine the effects of these experimental parameters on the sensing response. As depicted by the degree of fluorescence quenching in Fig. S3 (Supporting information), at equivalent analyte concentrations, an elevated temperature and reduced humidity correlate with a heightened quenching degree for IDT-CN. Nevertheless, it is noteworthy that the influence of humidity and temperature on the sensing characteristics of IDT-CN is relatively subtle, thereby suggesting that the material exhibits satisfactory stability.

The potential applicability of our fluorescent probe for monitoring and detecting industrially relevant amines warrants further exploration. For instance, *n*-propylamine serves as an intermediate in pharmaceuticals, agrochemicals, and rubber chemicals production. Diethylamine is utilized as a reagent in organic synthesis and contributes to the manufacture of dyes, plastics, and other compounds. Trimethylamine is involved in choline production, pharmaceutical neutralization processes, fish-meal preservation, and resin and dye solvation. With its sensitivity, specificity, and rapid response time, IDT-CN presents a promising opportunity for real-time monitoring and quality control in industries that employ amine compounds in their processes.



**Fig. 3.** (a) The fluorescence quenching rate of IDT-CN films after exposed to saturated vapours of *n*-propylamine, diethylamine, trimethylamine and other interferents. (b) Fluorescence intensity gradient change of IDT-CN films after sensing with different concentrations of trimethylamine vapours.

Furthermore, the selectivity of the IDT-CN film was assessed against a diverse array of common laboratory interferents. These interferents encompassed water, organic acids, alcohols, tetrahydrofuran, dichloromethane (a halogenated solvent), toluene (an aromatic hydrocarbon), acetone (a ketone), and acetonitrile (a nitrile). As depicted in Fig. 3a, the material exhibited a pronounced selectivity towards organic amine vapours, while yielding negligible responses to the other investigated interferents.

Because this probe demonstrated superior performance on trimethylamine, which is also the main source of the smell from fish, meat, and other fresh food after corruption. Therefore, we studied detection ability of the probe for trimethylamine by configuring different concentrations of trimethylamine gas and performed gradient detection test. As showed in Fig. 3b, even if measured concentration reached as low as 3 ppb, the fluoresce change is still obvious. This value is far lower than the pathogenic concentration of trimethylamine (from statistics of America National Institute for Occupational Safety and Health (NIOSH): Immediately dangerous to life and health (IDLH) 2000 ppm; Time weight average (TWA) 200 ppm; Short term exposure limit (STEL) 250 ppm). The limit of detection (LOD) could be calculated from the linear relationship between the quenching rate of IDT-CN and the concentration of trimethylamine. As shown in Fig. S4 (Supporting information), the changes of fluorescence intensity of IDT-CN films in seven concentrations of trimethylamine vapour (60, 30, 15, 6, 3, 0.3 and 0.03 ppm) well-fitted to Langmuir adsorption equation. Assuming that the triple signal-to-noise ratio of the fluorometer is 1%, the LOD of IDT-CN is around 4.610 ppt. Obviously, IDT-CN has excellent performance as a fluorescent probe for trimethylamine vapour.

Thin-layer chromatography monitoring (TLC) results showed that there was chemical reaction between IDT-CN and *n*-propylamine. The formation of a new *n*-benzylidene propylamine structure was observed in HRMS (Fig. S5 in Supporting information) of the product between IDT-CN molecule and *n*-propylamine. Our previous work also supports this conclusion [35].

Nevertheless, diethylamine and trimethylamine differ from former. No chemical products were observed in TLC monitoring neither with model molecule nor IDT-CN. Thus, dynamic simulations were conducted by randomly placing probe and analyte molecules in a cell to verify the interaction between IDT-CN against diethylamine and trimethylamine (parameters are shown in Section 6 in Supporting information). The upper portion of Fig. 4 shows that the distance between the carbon atom of IDT-CN and the hydrogen atom of diethylamine is significantly shortened from 13.032 Å to 2.735 Å after a 50 ps dynamic process, Table S2 (Supporting information) shows system potential energy raising from 187.173 kcal/mol to 219.905 kcal/mol, and system kinetic energy dropping from 112.812 kcal/mol to 80.723 kcal/mol. Similar simulation result between the sulfur atom of IDT-CN and the hydrogen atom of trimethylamine was also shown in the lower portion of Fig. 4, original distance was randomly set to 58.640 Å, after 50 ps dynamic process the distance was distinctly shortened to 3.092 Å.

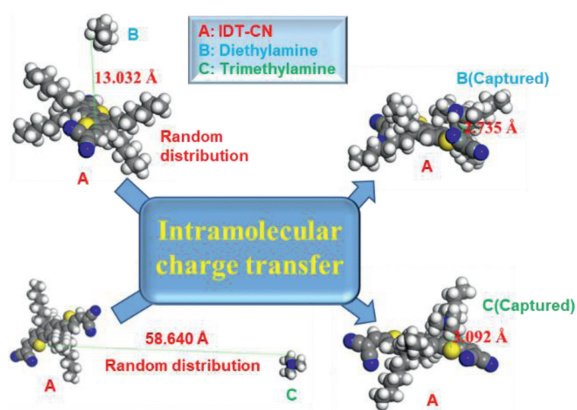


Fig. 4. Dynamic simulation of molecular interaction between IDT-CN with diethylamine and trimethylamine.

Table S3 (Supporting information) shows system potential energy raise from 141.082 kcal/mol to 191.043 kcal/mol, and system kinetic energy drop from 110.147 kcal/mol to 61.285 kcal/mol.

Both dynamic simulations illustrated strong combined molecule pair with strong interaction was formed. Both systems show a rise in potential energy and a decrease in kinetic energy, which indicates that the movement of the measured molecules was limited and then captured. Because the active region formed by thiophene group with malonitrile and alkyl chain constitutes a molecular trap. In a word, ICT process suggests the two amines are easier to bind with electron donors in indacenodithiophene, decreasing the electron donating ability of the system, decreases the conjugation structure of the system, and affects the intramolecular charge transfer, resulted a blue shift of the UV spectrum (Fig. S6 in Supporting information).

Trimethylamine oxide (TMAO) is the reason why fish is exceptional delicious. However, it is extremely unstable. Under bacteria affects, TMAO in fish could easily reduce to TMA. Therefore, the concentration of TMA directly reflects the freshness of fish.

To verify the detection effect of IDT-CN on trimethylamine gas in on-site environment, we evaluate the freshness of small yellow croaker. We put a fresh small yellow croaker together with a filter paper of  $5 \times 20$  mm infiltrated with the IDT-CN in two clasped glass dishes. For comparison, we recorded the original colour of IDT-CN filter paper under irradiation of daylight lamp as shown in Fig. 5a. The sample dishes were then stored in a darkroom at

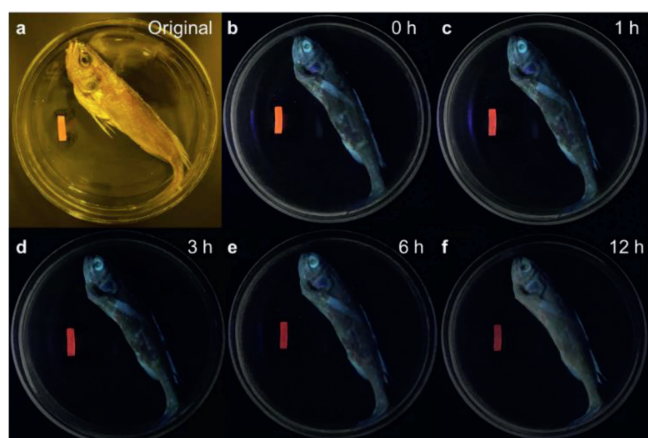


Fig. 5. Photographs of paper-based IDT-CN probe placed with fish (in two clasped glass dishes): (a) Original colour of IDT-CN filter paper under irradiation of daylight lamp. Fluorescence colour change of IDT-CN filter paper under irradiation of 365 nm UV light, after placed within darkroom for (b) 0 h; (c) 1 h; (d) 3 h; (e) 6 h; (f) 12 h.

a temperature of 30 °C, and periodically observed under a 365 nm UV lamp. As shown in Fig. 5b, the filter paper exhibited bright orange fluorescence under the irradiation of 365 nm UV light. The small yellow croaker rapidly appeared putrefaction under the effects of high temperature, and dimming of the filter paper fluorescence could be observed after just 1 h. As time progressed, the gradual quenching process of fluorescence was shown in Figs. 5c–f. After 12 h the fluorescence colour change of filter paper is significant by showing a dreary colour. We also demonstrated an example of IDT-CN used as visual indicator, after infiltrated with IDT-CN, filter paper shows pink to naked eye. To simulate the on-site outdoor environment, instead of placing in the darkroom, we placed it inside the container with small yellow croaker then put under direct sunlight, which will produce more spoilage gases. After 12 h, we found that the filter paper turned nearly white and the pink faded noticeably (Figs. S7 in Supporting information). The results of our test suggest that IDT-CN has potential for application in visual monitoring of food freshness.

In summary, a practical portable and responsive fluorogenic probe IDT-CN for on-site detection of volatile organic amine vapour was designed and synthesized. The probe has excellent optical properties in film state due to inhibit of aggregation induced quenching effect of molecules. Rapid fluorescence response within a few seconds and significant quenching rate shows the probe has a sensitive sense towards organic amines. For trimethylamine, which is more difficult to detect and less frequently reported, the probe can not only achieve 96% quenching response within 10 s, but also reach detect concentration down to 4.610 ppt, far lower than the pathogenic concentration of exposure to trimethylamine. More importantly, in actual environment test of fish corruption, the probe was able to fulfil the task of verify the presence of trimethylamine by naked eyes and fluorescence, which is significant for on-site detection. This method based on organic fluorescent sensitive materials provides an economical and facile way for the detection of volatile amine vapour and freshness of food such as fish.

#### Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ccl.2023.108579.

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