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Detection of Amines with Fluorescent Nanotubes: Applications in the Assessment of Meat Spoilage

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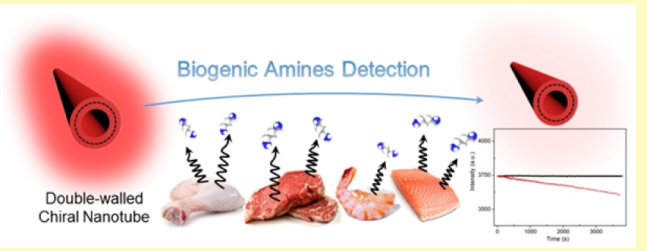
Supporting Information

ABSTRACT: Highly fluorescent nanotubes assembled from designed asymmetric perylene diimide molecules (PDIs) exhibit high sensitivity (lowering the existing detection limit to ppb levels) and selectivity to amines in the vapor phase, which renders them capable of monitoring and assessing the deterioration of meat.



KEYWORDS: *fluorescence quenching, sensors, nanotubes, meat spoilage, biogenic amines*

The safety and quality control of food, particularly meat and seafood, have attracted increasing attention due to health and economic concerns.[1,2] Given that ammonia and biogenic amines (BAs), such as trimethylamine, putrescine, and cadaverine,[3] are released during the decomposition of meat protein,[4,5] the sensitive detection of the released BAs is crucial to the assessment of the safety and quality of meat products during storage and transportation.[6] However, detection strategies for BAs have been primarily dependent on chromatography,[6,7] capillary electrophoresis (CE),[8,9] electrochemistry,[10−12] and chemiluminescence,[13−17] which are not suited to use as a simple, rapid, portable, and sensitive way for chemical detection and analysis,[3] despite the sensitivity of some technologies having reached 10 ppb levels.[6] Therefore, simple, portable, and sensitive sensing technologies enabling the real-time and in situ detection of BAs are highly desired.

Electronic sensors that employ functionalized carbon nanotubes as sensory materials have been demonstrated to constitute a simple and rapid method for the detection of organic amines and meat spoilage.[3,18] However, the sensitivity of electronic sensors remains relatively low, and they were not found to respond to meat spoilage within 1 day.[3] Optical sensors,[6,13,19−22] particularly fluorescent sensors, represent another simple and expedient detection technology for amines.[16,23−25] However, the fluorescence quantum yields of previously reported sensory materials are relatively low (less than 25%),[23,24] which limits their sensitivity. To improve the detection limits for the real-time monitoring of meat spoilage, we employ highly fluorescent nanotubes assembled from chiral asymmetric perylene diimide molecules (PDIs) (Figure S1) as sensory materials based on the following considerations: These chiral nanotubes have been shown to exhibit a fluorescence quantum yield of ca. 46%,[26] which is significantly higher than the fluorescence quantum yield of previously reported materials.[27] More importantly, these nanotubes have inherent internal hollow structures, which favor the diffusion of analytes into sensory materials and enhance their sensitivity.[28] The combination of high emission efficiency and intrinsic hollow structures is expected to allow these nanotubes to be ideal fluorescence sensors for amines. These nanotubes were found to have very high sensitivity to amines (e.g., ammonia, methylamine, dimethylamine trimethylamine, putrescine, and cadaverine) and facilitate real-time monitoring for meat freshness. The nanotubes exhibited substantial fluorescence quenching when exposed to the spoilage vapor from a 1 g meat sample placed 1.0 cm from the nanotubes within 1 h.

The fluorescent sensors were fabricated by drop-casting a suspension of the nanotubes in ethanol (30 μL, 10 μg/mL) onto approximately 1 cm^2 of polytetrafluoroethene (PTFE) film. The thickness of the formed nanotube film was determined by scanning electron microscopy (SEM) (Figure S2). As shown in Figure 1a and b, when 9 ppm cadaverine vapor was blown onto the nanotube film, the fluorescence of the nanotubes was significantly quenched by ca. 24%. Similarly, when putrescine (43 ppm) was blown onto the nanotube film, substantial fluorescence quenching of ca. 30% was observed (Figure S3). The fluorescence-quenching mechanism is ascribed to the photoinduced electron transfer from amines (electron donor) to the excited PDI in the nanotubes (electron acceptor).[24] To determine the detection limit for these BAs, the fluorescence-quenching efficiency ($1 - I/I_o$) that occurred when nanotubes were exposed to various concentrations of BA vapors was recorded under ambient conditions, as shown in Figure 1c. The fluorescent nanotubes are extremely sensitive to BA vapors at a concentration of 9 ppb, which represents a decrease in the existing detection limit for BA vapors from the

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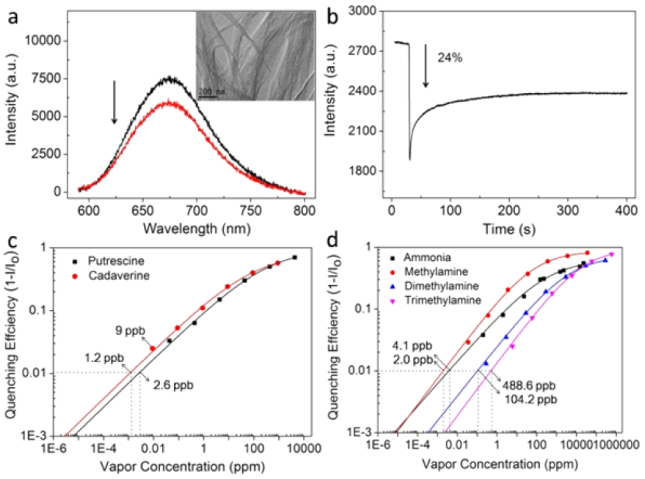

Figure 1. (a) Typical fluorescence changes in the spectra of the nanotubes assembled from chiral molecule 1 after exposure to 5 mL of blowing cadaverine vapor (9 ppm). (b) Time-dependent fluorescence quenching profile of the nanotubes after blowing cadaverine vapor (9 ppm). Fluorescence quenching efficiency ($1 - I/I_0$) of the nanotubes as a function of the vapor concentration of cadaverine and putrescine (c), as well as ammonia, methylamine, dimethyamine, and trimethymine (d). Data error ±10%.

sub-ppm level[3] to the ppb level. By fitting the fluorescence quenching results to the Langmuir equation, we determined that the detection limits of the nanotubes for putrescine and cadaverine were 2.6 and 1.2 ppb, respectively, if considering the fact that 1% fluorescence intensity change gives more than three times the S/N vs background during the measurements. Considering that other amines (including ammonia, methylamine, dimethylamine, trimethylamine, spermine, spermidine, histamine, tryamine, and tryptamine) can be released as well during meat spoilage, we also measured the sensitivity of the nanotubes to these amines. The nanotubes exhibited high sensitivity to all amines (Figure 1d and Table S1), although sensitivity differences did exist to some extent (Table S2). The sensitivity difference was further reflected by the detection limits of ammonia, methylamine, dimethylamine, and trimethylamine, which were calculated as 4.1, 2.0, 104.2, and 488.6 ppb (Figure 1d). Because the saturated pressures of other BAs (i.e., spermine, spermidine, histamine, tryamine, and tryptamine) are unavailable, we cannot determine their diluted vapor concentrations and thus their detection limit.

To highlight the significance of the unique features of the nanotubes (high emission efficiency and intrinsic hollow structures), we measured the responses of other nanostructures assembled from asymmetric PDI molecules S1−S3 (Figure S4) to amine vapors following the same method. Here, these control molecules were chosen because they either formed highly fluorescent microribbon (i.e., S1 nanoribbon with a fluorescence quantum yield of ca. 35%) or had a similar molecular structure (S2) to the molecules for the nanotube or exhibited efficient fluorescence quenching to amines (i.e., S3 nanowire).[24] As shown in Figure S5, the detection limits of these nanoribbons and nanowires for putrescine and cadaverine are much lower than that of the nanotubes, indicative of the advantages of the nanotubes over other nanostructures. For sensor applications, reversibility and stability are two additional important parameters. We observed that the fluorescence quenching by amines cannot completely recover in air (Figure 1b) because of the relatively strong interaction between BA molecules and the nanotubes. This problem can be easily addressed by heating the nanotubes at 60 °C for 10 min. The fluorescence-recovered nanotubes exhibited uncompromised sensing performance, thus enabling their repeated use (Figure S6). The negligible effect by temperature on the fluorescence intensity of the nanotubes (range from 0 to 60 °C) was also confirmed, as illustrated in Figure S7.

To evaluate the selectivity of the nanotube film, responses to the vapors of common organic solvents and gases were also investigated. As illustrated in Figure 2, fluorescence responses

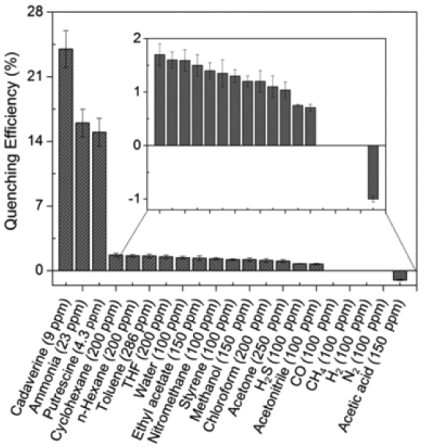

Figure 2. Fluorescence responses of the nanotubes to various vapors of common organic solvents and gases (with concentration in ppm given in parentheses) under ambient conditions.

toward these solvents are negligible, especially considering their relatively high vapor concentrations. This result is related to the higher oxidation potentials of these organic solvents and gases, which cannot transfer electrons to the excited PDI in the nanotubes and cause negligible fluorescence quenching. These observations allow us to conclude that the sensory materials based on fluorescent nanotubes exhibit high sensitivity and selectivity to amines over interferents (organic solvents and other gases). Note that the fluorescent nanotube response to all amines and thereby the fluorescence quenching cannot be used to discriminate specific amines from each other. Given that the exotic amines are usually away from meat and seafood, the fluorescence nanotubes are suitable for sensing amines emitted from food.

Having determined the very high sensitivity of the nanotubes to amines, we employed our sensor to monitor the real-time emissions of amines from meat products. We selected four types of raw meat, pork, chicken, fish, and shrimp, which were purchased from the supermarket. Prior to meat spoilage measurements, 1 g meat samples were placed in a sealed jar (20 mL) for various durations to achieve emitted amine vapor for measurements. We simultaneously prepared two samples of each meat: the first was placed in a refrigerator (5 °C), and the second was stored at 25 °C (RT). Figure 3a and b displays the time-dependent fluorescence quenching profiles of the nanotubes when blown by the vapors emitted from the shrimp stored at room temperature and in a refrigerator (5 °C) for 24 h, respectively. The fluorescence of the nanotubes was significantly quenched by ca. 11% when exposed to the vapor generated at room temperature (Figure 3a). When exposed to the vapor generated from the same shrimp sample at room temperature after 4 days, the fluorescence quenching increased

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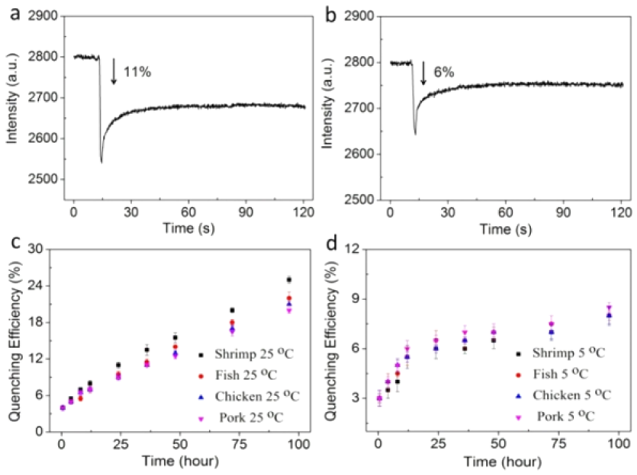

Figure 3. (a) Time-dependent fluorescence quenching profile of the nanotubes when blowing vapors generated from shrimp (1 g) stored at room temperature (25 °C) for 1 day. (b) Time-dependent fluorescence quenching profile of the nanotubes when blowing vapors generated from shrimp (1 g) stored in a refrigerator (5 °C) for 1 day. (c) Fluorescent response of the nanotubes to the selected meat samples (1 g) stored at 25 °C for 0–4 days. (d) Fluorescent response of the nanotubes to the selected meat samples (1 g) stored at 5 °C for 0–4 days.

to 25% (Figure 3c). A significantly smaller increase in fluorescence quenching (from 3% to 11%) was observed when exposed to the vapor emitted from the shrimp sample in a refrigerator (5 °C) within 4 days (Figure 3d). Similar time-dependent fluorescence quenching profiles were also observed with the vapors emitted from other meat samples (Figure 3c and d and Figure S8). The magnitude of the fluorescence quenching by the emitted vapors from meat stored at the same time at room temperature follows the order of shrimp > fish > chicken > pork (Figures 3c). The different magnitudes of fluorescence quenching should be related to the protein levels within the four meat products that determine the amount of emitted amines. A closer analysis of the fluorescence quenching of the nanotubes by meat spoilage shows that the fluorescence quenching (ca. 10%) by the vapors generated after 4 days of meat storage at 5 °C approached the fluorescence quenching caused by the vapors generated over 1 day of meat storage at 25 °C. Given that a foul smell was emitted when the meat samples were stored at 25 °C for 1 day, we conclude that meats are "safe food" when stored at 5 °C for less than 4 days or at room temperature for less than 1 day. Using our monitoring procedure, the emitted vapor from the safe meat causes less than 10% fluorescence quenching of the nanotubes, whereas the unsafe meats result in more than 10% fluorescence quenching of the nanotubes. To determine the amine composition in the vapors emitted from the meat sample, we run the gas chromatography (GC) and identified five amines in the emitted vapors, i.e., ammonia, dimethylamine, trimethylamine, as well as putrescine and cadaverine (Figure S9). Considering that our nanotubes are sensitive to all these amines, the nanotubes are appropriate sensing materials for the practical applications in monitoring meat spoilage that are interesting to meat providers and consumers.

To explore the real-time sensitivity of the nanotubes to meat spoilage, fluorescence-quenching-based in situ monitoring of meat samples was performed. Figure 4 shows the time-dependent fluorescence quenching profile of the nanotubes

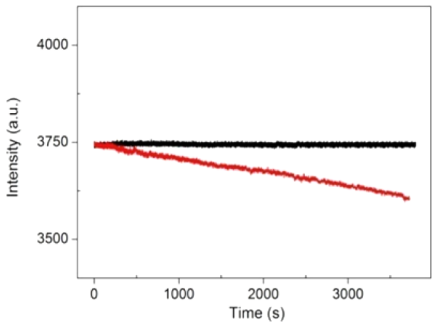

Figure 4. Time-dependent fluorescence quenching profile of the nanotubes assembled from chiral molecule 1 at room temperature (25 °C) for 1 h: blank (black) and with 1 g shrimp placed beside the nanotubes (red).

when exposed to the emitted vapor from 1 g of fresh shrimp that was placed approximately 1.0 cm from the nanotubes. The fluorescence intensity of the nanotubes linearly decreased with time and exhibited ca. 6% fluorescence quenching after 1 h. The fluorescence intensity of the same nanotubes remained unchanged in the absence of the meat sample, which indicated that the sensory material is very stable under ambient conditions. These results reveal that the nanotubes are highly sensitive to the amine vapors emitted from meat spoilage and are suitable for the real-time monitoring of meat safety.

The highly fluorescent nanotubes assembled from designed asymmetric PDI molecules exhibit very high sensitivity to amines, which lowered the existing detection limit from the sub-ppm level to the ppb level. The nanotubes are highly sensitive to the real-time vapors emitted from meat samples and can be employed to assess meat safety. The nanotube-based fluorescent sensor is capable of in situ responses to the amine vapors emitted from a meat sample within 1 h. Therefore, the nanotube-based fluorescent sensor is a highly sensitive, portable, and expedient technology that is suitable for the real-time and in situ monitoring and assessment of the deterioration of meat freshness.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.5b00040.

Table S1: The fluorescent responses of the nanotubes to the other five BAs. Table S2: Relative selectivity of the responses of the nanotubes to different amines. Figure S1: Molecular structure of chiral molecule 1. Figure S2: SEM image of the peeled nanotube film, showing the thickness of 140 ± 30 nm. Figure S3: Typical fluorescence quenching changes (a) and time-dependent fluorescence quenching profile (b) of the nanotubes upon being blown 5 mL putrescine vapor. Figure S4: The nanostructures assembled form molecules S1–S3. Figure S5: Fluorescence quenching efficiency ($1 - I/I_o$) of S1 nanoribbons (a), S2 nanoribbons (b), and S3 nanwires (c) as a function of the vapor concentration of putrescine and cadaverine. Figure S6: Quenching-recovery cycles of the nanotubes. Figure S7: Negligible temperature effect on the fluorescence intensity of the nanotubes. Figure S8: Details of the fluorescent response of the nanotubes to the four selected types of meat samples. Figure S9: GC

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separation of vapors released from the meat samples. (PDF)

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Notes

The authors declare no competing financial interest.

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