Published in final edited form as:

Sens Actuators B Chem. 2015 December 1; 220: 1338–1345. doi:10.1016/j.snb.2015.07.051.

A ratiometric two-photon fluorescent probe for hydrazine and its applications

Shenglong Yu^{a,b}, Shuxin Wang^{a,b}, Haizhu Yu^{a,b}, Yan Feng^{a,b}, Shuting Zhang^c, Manzhou Zhu^{a,b}, Hang Yin^c, and Xiangming Meng^{a,b,*}

^aDepartment of Chemistry, Anhui University, Hefei, 230601, China.

^bCenter for Atomic Engineering of Advanced Material, Anhui University, Hefei, 230601, China.

^cDepartment of Chemistry and Biochemistry, BioFrontiers Institute, University of Colorado at Boulder, Boulder, CO 80309, USA.

Abstract

A ratiometric two-photon fluorescent probe **TNQ** was developed based on quinoline platform to detect hydrazine (N_2H_4) with high selectivity. **TNQ** exhibited large two-photon absorption cross sections at 710 nm (250 GM) and excellent ratiometric two-photon fluorescent detection signal for hydrazine. **TNQ** was also successfully applied to selectively detect hydrazine vapor even at a concentration down to 0.05%. Cell cytotoxicity and bio-imaging studies revealed that probe **TNQ** was cell-permeable and could be used to detect hydrazine in living cells with low cytotoxicity under two-photon excitation.

Graphical Abstract

A ratiometric two-photon fluorescent probe was developed based on quinoline platform to detect hydrazine (N_2H_4) with high selectivity and sensitivity.

Keywords

Ratiometric; Two-photon; Fluorescent probe; Hydrazine; Hydrazine vapor detection

*Corresponding author Fax: +86-551-63861467; Tel: +86-551-63861467, mengxm@ahu.edu.cn(Xiangming Meng).

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Introduction

Hydrazine is often used as an important reactant in various chemical industries, due to its high reducibility and alkalinity [1]. However, hydrazine has considerable toxic effects and can also cause serious damage to the liver, lungs, kidneys, central nervous system and even potentially lead to gene mutations and cancer [2]. Although there is no endogenous hydrazine in live cells, it is readily absorbed by oral, dermal or inhalation routes of exposure thus causing harm to live cells. As a result, hydrazine has been classified as possible cancer-causing environmental contaminants [3]. Therefore, selective and sensitive real-time detection methods of hydrazine in environmental and biological system are highly needed.

At present, fluorescent probes provide the optimal choice to detect biological agents due to its high sensitivity, simplicity for implementation, real-time detection, and good compatibility for bio-samples [4]. Only a few fluorescent probes for hydrazine have been reported [5]. However, they are all designed based on single-photon fluorescence technology. These probes with one-photon microscopy (OPM) require excitation with short-wavelength light (ca. 350–550 nm) that limits their application in deep-tissue imaging, owing to the shallow penetration depth (less than 80 nm) as well as to photo-bleaching, photo-damage, and cellular auto fluorescence. Recently, two-photon fluorescence (TPF) probes, which can be excited by two-photon absorption in the NIR wavelength, provided an opportunity to overcome the problems originated from the single-photon fluorescence technology [6]. On the other hand, a ratiometric fluorescent probe, measuring the ratio of fluorescence intensities at two different wavelengths can eliminate the influence such as concentration, photobleaching and background interference to realize quantitative detection [7, 8]. To the best of our knowledge, no ratiometric two-photon fluorescent probe for hydrazine has been reported in the literature to date.

Herein, we report the first ICT-based ratiometric two-photon fluorescent probe (**TNQ**, Scheme 1) for the detection of hydrazine, which is based on 6-substituted quinoline, an excellent two photon-fluorescent group for cell imaging that was previously developed by our group [9]. In order to obtain large two-photon absorption cross section, the conjugate system of 6-substituted quinolone was enlarged [10]. The hydroxyl of the compound **De-TNQ** was levulinated to corresponding levulinoyl ester which can be selectively removed by hydrazine [11]. We deem that the hydrazine induced deprotection process will enhance the intramolecular charge transfer (ICT) with the molecular system, which will result the red-shift of the fluorescence spectra. Thus, the ratiometric detection signal will be obtained with enlarged two-photon absorption cross-section [12].

2. Experimental section

2.1 General procedures

All reagents and solvents were commercially purchased. All reactions were magnetically stirred and monitored by thin layer chromatography (TLC). The ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance spectrometer using $\text{DMSO-}d_6$ and CDCl_3 as the solvents. The one-photon absorption (OPA) spectra were

recorded on a Tech-comp UV 1000 spectrophotometer. The one-photon excited fluorescence (OPEF) spectra measurements were performed using a Hitachi F-7000 fluorescence spectrophotometer. The test solution of probe **TNQ** (5 μ M) in pH 7.4 HEPES/THF (1:9, v/v, $\lambda_{\text{ex}} = 365$ nm) was prepared. The fluorescence quantum yields were detected by HORIB FluoroMax-4P. The two-photon cross section was tested in THF with 1 mM probe **TNQ** and **TNQ** + N_2H_4 . HPLC was obtained with Agilent 1260 Infinity LC. TPEF spectra were measured using femtosecond laser pulse and Ti: sapphire system (680–1080 nm, 80 MHz, 140 fs, Chameleon II) as the light source.

2.2 Synthesis of probe TNQ

Compound 1—A mixture of 4-iodoaniline (18.6 g, 84.8 mmol) and HCl (6 N, 60 mL) was heated to 100 °C, then crotonaldehyde (11.9 g, 170 mmol) was added slowly. The mixture was refluxed until the TLC showed no raw material remains. After cooling to room temperature, 200 mL H_2O was added, and the mixture was extracted with acetic ether (100 mL $\times$ 2). The water layer was neutralized with ammonia water to about pH=9 and extracted with EtOAc (50 mL $\times$ 2). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to produce a crude residue. The residue was recrystallized in EtOAc/PE to yield 17.47 g of compound **1** (64.96 mmol, 76.6%). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 8.13 (s, 1H), 7.90 (d, $J = 8.8$ Hz, 2H), 7.73 (d, $J = 8.9$ Hz, 1H), 7.27 (d, $J = 8.4$ Hz, 1H), 2.72 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ (ppm): 159.70, 146.77, 138.08, 136.20, 134.93, 130.39, 128.23, 122.69, 90.87, 25.44.

Compound 2—The solution of compound **1** (5.80 g, 21.6 mmol) in 1, 4 - dioxane (50 mL) was heated to 60 °C. SeO_2 (26.1 mmol, 2.90 g) was added to this solution partially and the reaction temperature was increased to 80°C. After 2.5 h, the mixture was cooled to room temperature. The excess of 1, 4 - dioxane was removed by rotary evaporator. The solid was dissolved by a moderate amount of DCM and washed with water (30 mL $\times$ 2). The organic phase was combined and concentrated to yield a crude product. The crude material was purified through column chromatography (DCM as the eluent) to give 5.547 g of compound **2** (19.6 mmol, 90.9%). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 10.20 (s, 1H), 8.32 (s, 1H), 8.20 (d, $J = 8.5$ Hz, 1H), 8.05 (t, $J = 9.1$ Hz, 2H), 7.96 (d, $J = 8.9$ Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ (ppm): 193.36, 152.86, 146.82, 139.40, 136.72, 136.15, 131.84, 131.39, 118.14, 95.77.

Compound 3—The solution of compound **2** (3.1 g, 11.0 mmol) and 2-amino benzenethiol (1.39 g, 11.0 mmol) in 50 mL DMSO was stirred at 130 °C under N_2 atmosphere for 4 h. The solution was poured into water and extracted with CH_2Cl_2 . The organic layer was dried over Na_2SO_4 and evaporated in vacuum. The crude product was recrystallized from EtOH to give compound **3** as gray powder (3.54 g, 83%). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 8.55 (d, $J = 8.6$ Hz, 1H), 8.28 (s, 1H), 8.22 – 8.14 (m, 2H), 8.00 (t, $J = 7.1$ Hz, 2H), 7.93 (d, $J = 8.9$ Hz, 1H), 7.55 (t, $J = 7.3$ Hz, 1H), 7.47 (t, $J = 7.5$ Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ (ppm): 169.30, 153.89, 151.49, 146.74, 139.00, 136.57, 136.33, 135.91, 131.21, 130.51, 126.56, 126.22, 123.78, 122.10, 119.25, 93.63.

Compound De-TNQ—The mixture of compound **3** (1 g, 2.6 mmol), 4-ethynylphenol (0.31 g, 2.6 mmol), Pd(PPh₃)₂Cl₂ (0.04 mg, 0.057 mmol), CuI (0.025g, 0.13mmol), Et₃N (1.10 g, 10.8 mmol) and THF (10 mL) was stirred at 30 °C for 3 h under the anhydrous and anaerobic conditions. After cooling to room temperature, the mixture was filtered to remove salts and followed with the addition of 100 mL H₂O. The resulting mixture was extracted with EtOAc (30 mL×3). The organic phase was combined and dried with anhydrous Na₂SO₄, and evaporated to yield a crude product that was recrystallized in EtOAc/PE to give 0.875 g of compound **2** (2.314 mmol, 89%). ¹H NMR (400 MHz, DMSO-*d*₆) δ(ppm): 10.06 (s, 1H), 8.59 (d, *J* = 8.5 Hz, 1H), 8.49 (d, *J* = 8.4 Hz, 1H), 8.29 (s, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 8.17 (d, *J* = 8.1 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 1H), 7.91 (d, *J* = 8.7 Hz, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.55 (t, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 2H), 6.85 (d, *J* = 8.0 Hz, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ(ppm): 168.75, 158.45, 153.74, 150.88, 146.29, 137.54, 135.71, 133.27, 132.87, 130.66, 129.29, 128.66, 126.74, 126.36, 123.56, 122.63, 122.27, 118.66, 115.85, 111.97, 92.29, 87.12.

Compound TNQ—The mixture of compound **De-TNQ** (0.5 g, 1.32 mmol), Levulinic acid (0.23 g, 2.0 mmol), DMAP (0.04 g, 0.33 mmol), DCC (0.41 g, 2.0 mmol) and 10 mL DCM was stirred under nitrogen atmosphere at room temperature for 12 h. The precipitate of urea was removed by filtration and the filtrate was concentrated in high vacuum to give an oily residue. This residue was purified by column chromatography using silica gel (200-300 mesh size) and DCM as eluent to give a white solid compound **TNQ** (0.485 mg, 77%). ¹H NMR (400 MHz, CDCl₃) δ(ppm): 8.50 (d, *J* = 8.5 Hz, 1H), 8.24 (d, *J* = 8.6 Hz, 1H), 8.14 (dd, *J* = 8.3, 3.9 Hz, 2H), 8.03 (s, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 7.84 (d, *J* = 8.7 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.45 (t, *J* = 7.5 Hz, 1H), 7.13 (d, *J* = 8.2 Hz, 2H), 2.86 (dd, *J* = 10.6, 4.7 Hz, 4H), 2.24 (s, 3H). ¹³CNMR (101 MHz, CDCl₃) δ(ppm): 206.40, 171.22, 169.49, 154.37, 151.75, 150.82, 147.31, 136.64, 136.53, 132.93, 132.81, 130.94, 129.82, 128.73, 126.37, 126.01, 123.85, 122.45, 122.07, 121.85, 120.50, 119.04, 90.70, 89.08, 37.72, 29.73, 27.77.

2.3 Measurement of Two-photon Absorption Cross-section (δ)

Two-photon excitation fluorescence (TPEF) spectra were measured using femtosecond laser pulse and Ti: sapphire system (680~1080 nm, 80 MHz, 140 fs, Chameleon II) as the light source. All measurements were carried out in air at room temperature. TPA cross sections were measured using two-photon-induced fluorescence measurement technique. The TPA cross-sections (δ) were determined in THF with 1mM probe **TNQ** and **TNQ** + N₂H₄ by comparing their TPEF according to the following equation:

$$\delta = \delta_{ref} \frac{\Phi_{ref}}{\Phi} \frac{c_{ref}}{c} \frac{n_{ref}}{n} \frac{F}{F_{ref}}$$

Here, the subscripts ref stands for the reference molecule. δ is the TPA cross-section value, c is the concentration of solution, n is the refractive index of the solution, F is the TPEF integral intensities of the solution emitted at the exciting wavelength, and Φ is the fluorescence quantum yield. The δ_{ref} value of reference was taken from the literature [13].

2.4 HPLC detection

The concentration of both probe **TNQ** and compound **De-TNQ** were 50 μM , and the injection volumes were 10 μL . The mobile phase was $\text{CH}_3\text{CN}:\text{H}_2\text{O} = 7:3$ (v/v) and the flow rate was 1 mL/min. Detection wavelength was 360 nm. C18 chromatographic column (ZORBAX Eclipse Plus C18) was used.

2.5 Cell culture and two-photon fluorescence microscopy imaging

For two-photon bio-imaging, CHO cells were cultured in DMEM supplemented with 10% FCS, penicillin (100 $\mu\text{g}/\text{mL}$), and streptomycin (100 $\mu\text{g}/\text{mL}$) at 37 °C in a humidified atmosphere with 5% CO_2 and 95% air. More than 10 group cytotoxicity assays showed that probe **TNQ** is safe for two-photon bio-imaging at low concentrations. The cells were incubated with 10 μM probe **TNQ** at 37°C under 5% CO_2 for 30 min, washed once and bathed in DMEM containing no FCS prior to imaging and/or hydrazine addition. Then 20 μM hydrazine was added in the growth medium for 0.5 h at 37 °C, and washed 3 times with PBS buffer. Then, cells were imaged on a confocal microscope (Zeiss LSM 510 Meta NLO). Two-photon fluorescence microscopy images of labelled cells were obtained by exciting the probe with a mode-locked titanium-sapphire laser source set at wavelength 710 nm.

3. Results and discussion

3.1 UV-vis and fluorescence spectra study

As shown in Fig. 1, upon the addition of hydrazine, the maximum emission of probe **TNQ** showed a significant red shift from 414 nm to 460 nm. The UV-vis absorption spectra of probe **TNQ** (5 μM) exhibited a maximum absorption at 365 nm, upon addition of hydrazine (10 μM), the maximum absorption was shifted to 370 nm (Fig. S1). These results indicated that the hydrazine induced deprotection enhance the ICT process of the system. The ratio of fluorescent intensity ($I_{460\text{ nm}}/I_{414\text{ nm}}$) increased from 0.26 to 4.23 on the addition of hydrazine, and exhibited a good linear relationship with the concentration of hydrazine. Thus, **TNQ** can be used as ratiometric fluorescent probe for hydrazine in a wide range of the concentrations.

3.2 Hydrazine selectivity and competitive of various amines and ions

The optical responses of probe **TNQ** to various amines and ions were investigated by the emission spectroscopy. The reactions were conducted in parallel at room temperature, and then the fluorescence spectra were monitored. As shown in Fig. 2, none of the tested amines (including Cysteine [14]) induced the shift of the fluorescence of **TNQ** except N_2H_4 . However, N_2H_4 induces a significant red shift of probe **TNQ** from 414 nm to 460 nm. To further evaluate the selectivity of probe **TNQ** toward various amines, interference experiments were conducted to study the influence of other amines on N_2H_4 detection with probe **TNQ**. The detection signal (I_{460}/I_{414}) for N_2H_4 (5 equiv.) remains stable upon addition of other various amines (10 equiv.) (Fig. S2). Further selectivity test suggested that **TNQ** also exhibited high selectivity test over other metal ions and anions (Fig. S3). These results indicated that the selectively hydrazine induced deprotection of levulinoyl ester can

guarantee the high selectivity of probe **TNQ** for N_2H_4 over other competitive amines in pH 7.4 HEPES/THF (1:9, v/v, $\lambda_{\text{ex}} = 365 \text{ nm}$).

Colorimetric detection by naked eye is the simplest way to observe the selectivity of probe toward the various metal cations. **TNQ** was applied to colorimetric study to see determine whether it can be used. As shown in Fig. 3, under 365 nm UV irradiation, probe **TNQ** displayed a dramatic color change (from blue to white) upon addition of hydrazine in at room temperature, and the selectivity of probe to the various metal ions and anions as shown in Fig. S4.

3.3 The pH stability

The pH is an important factor affecting the reaction of probe **TNQ** with hydrazine. To study the possibility of practical application in bio-system, the pH-dependence of probe **TNQ** and **TNQ** + N_2H_4 were also examined. As shown in Fig. 4, the fluorescence ratio (I_{460}/I_{414}) of probe **TNQ** and **TNQ** + N_2H_4 was almost pH insensitive in the biological relevant pH range (e.g. 6-9).

3.4 Two-photon absorption cross-sections

The two-photon absorption cross-sections of probe **TNQ** and **TNQ** + N_2H_4 were determined using the two-photon induced fluorescence measurement spectra. As shown in Fig. 5, the maximum two-photon absorption cross-section (δ_{max}) value of probe **TNQ** is 250 GM at 710 nm ($\Phi=0.1924$). Upon addition of N_2H_4 , the δ_{max} value at 700 nm was decreased to 494 GM ($\Phi=0.3698$). The large two-photon absorption cross-sections of **TNQ** and **TNQ** + N_2H_4 suggested that probe **TNQ** would be a good two-photon probe for hydrazine imaging in living systems.

3.5 Reaction mechanism studies

The proposed hydrazine induced deprotection of **TNQ** was confirmed by ^1H NMR and HPLC. From the ^1H NMR spectroscopy (Fig.6), the chemical shift of the protons at benzene ring H_h , H_k were located at 7.70 and 7.22 ppm, respectively. After the addition of hydrazine, the peaks of these protons shifted to 7.46 and 6.85 ppm, which were identical to the spectrum of **De-TNQ**. In addition, the resonances of the reaction by-product 6-methyl-4,5-dihydropyridazin-3(2H)-one was also observed (Fig. S5).

As shown in Fig. 7, HPLC analysis indicated that after addition of the hydrazine, the peak of **TNQ** ($\text{rt}=34\text{min}$) was gradually disappeared along with the arise of the peak of **De-TNQ** ($\text{rt}=22\text{min}$). These results suggested that the **TNQ** was converted to **De-TNQ** by hydrazine. ESI-TOF MS spectrum analysis provided further evidence of the reaction between hydrazine and **TNQ**. As shown in Fig. S6 and Fig. S7, **TNQ** has a peak at $m/z = 499.10$ (**TNQ** + Na^+). Upon the addition of hydrazine, the spectrum give the new peak **De-TNQ** at $m/z = 379.08$ (**De-TNQ** + H^+).

3.6 Theoretical calculation

To further understand the change of the photophysical properties, density functional theory (DFT) calculations of the energy gaps between HOMO (the highest occupied molecular

orbitals) and LUMO (the lowest unoccupied molecular orbitals) of **TNQ** and **De-TNQ** thiazolidine were performed. As shown in Fig. 8, the energy gap between HOMO and LUMO of **De-TNQ** was calculated to be 3.34 eV, while the energy gap of **TNQ** was calculated to be 3.49 eV. Thus, the smaller energy gap of **De-TNQ** arising the red shift of the UV-vis and fluorescence spectra.

3.7 The application in the detection of gas-state hydrazine

Prompted by the promising results obtained above, we further investigated whether probe **TNQ** could be applied in the detection of hydrazine vapor. The filter paper was soaked in a THF solution of probe **TNQ** (1.0 mM) and dried. Then, the filter paper was covered on the top of jars containing different concentration hydrazine solution for 1.0 min at room temperature before the observation. As shown in Fig. 9, the color of filter paper changed gradually from deep blue to white green with the increasing of the concentration hydrazine vapour under 365 nm UV lamp. The vapour of hydrazine at concentration of 0.05%-10% results in the obvious difference in the color **TNQ** on the filter paper, which is sensitive enough to detect hydrazine vapor compared with the recent developed chemo-probe for hydrazine vapor [15]. These results suggested that **TNQ** could be easily applied to detect hydrazine vapor in the environment by naked eyes.

3.8 Cell cytotoxicity and fluorescence microscopy imaging

To further demonstrate the potential application of probe **TNQ** in living cells, cytotoxic assays of probe **TNQ** in CHO cells were performed. As shown in Fig. S8, the cell viability remained 98 % after treated with 10 μ M probe **TNQ** for 24 h. The low cytotoxicity of probe **TNQ** will guarantee the probe's application in bio-imaging.

Finally, **TNQ** was used to imaging of the hydrazine in CHO cells. TPM images were obtained by exciting the samples with a mode-locked titanium-sapphire laser source set at wavelength of 710 nm. As shown in Fig. 10, before the addition of N_2H_4 the optical window at 400–440 nm showed strong blue fluorescence, whereas the optical window at 440–480 nm exhibited weak fluorescence. After incubated with N_2H_4 (20 μ M) for 0.5 hour, the fluorescence intensity of the optical window at 440–480 nm increased dramatically, whereas that at 400–440 nm decreased significantly. Thus, the ratiometric fluorescence images generated from the above optical windows demonstrated that the new probe is able monitor the variation of the intracellular hydrazine under two-photon excitation. To clarify the esterase activity, we have added cell culture results from other three types of cells (PC-9, A431 and HeLa) in Fig. S9. As shown in Fig. S9(a), the fluorescence of the cells in both blue and green channels almost remained unchanged after treated with **TNQ** alone for 30 min. However, after treated with hydrazine for another 30 min, the fluorescence of the blue channel reduced while the fluorescence of green channel enhanced. These results suggested that the ratiometric detection signal was caused by hydrazine. The esterase activity does not affect the fluorescence of **TNQ**.

4. Conclusions

In summary, we have developed ratiometric two-photon fluorescent probe (**TNQ**) for selective hydrazine based on quinolone platform. **TNQ** showed excellent two-photon fluorescent detection signal for hydrazine under physiological conditions. Selective ratiometric detection signal based on the efficient deprotection of the levulinate group was confirmed by $^1\text{H-NMR}$, HPLC and MS spectra analysis. Moreover, the newly developed probe can be used to selectively detect hydrazine vapour at low concentration down to 0.05%. The cell imaging results showed that the new probe is cell-permeable and can be used for monitoring N_2H_4 in living cells under two-photon excitation with a ratiometric signal at low cytotoxicity. Therefore, our results proved the great potential of **TNQ** for environmental or biological detection of hydrazine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by National Natural Science Foundation of China (21102002, 21372005), NIH R01 GM101279, 211 Project of Anhui University.

Biographies

Shenglong Yu is studying for master degree in Anhui University. He received his bachelor of science in chemistry from the department of chemistry at Anhui University of Chinese Medicine.

Shuxin Wang is studying for PHD in Anhui University. He received his master degree of science in chemistry from the department of chemistry in Anhui University.

Haizhu Yu is an associate professor in the department of chemistry at Anhui University. Her current research interests are chemosensors and theoretical calculation.

Yan Feng is an associate professor in the department of chemistry at Anhui University. Her current research interests are chemosensors and organometallic chemistry.

Shuting Zhang is post-doctor fellow in the department of Chemistry and Biochemistry, BioFrontiers Institute, University of Colorado at Boulder. Her current research interests are biochemistry.

Manzhou Zhu is a professor in the department of chemistry at Anhui University. His current research interests are chemosensors and nanoclusters.

Hang Yin is an associate professor in the department of Chemistry and Biochemistry and BioFrontiers Institute, University of Colorado at Boulder. His current research interests are biochemistry.

Xiangming Meng is an associate professor in the department of chemistry at Anhui University. His current research interests are chemosensors and nanoclusters.

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Highlights

1. The first ratiometric two-photon fluorescent probe for hydrazine was developed.
2. The new probe exhibited large two-photon absorption cross-section (250 GM at 710 nm).
3. The new probe can be applied to detect hydrazine vapor even at low concentration
4. The new probe was applied in live cell imaging under two-photon excitation.

Scheme 1.

The design of probe **TNQ** for N_2H_4 detection.

Scheme 2.
Synthetic route of probe **TNQ**.

Fig. 1.

The emission spectra of probe **TNQ** (5 μM) upon the addition of increasing concentrations of hydrazine (0-2 equiv.) in pH 7.4 HEPES/THF (1:9, v/v, $\lambda_{\text{ex}} = 365 \text{ nm}$). **Insert:** Ratiometric calibration curve (I_{460}/I_{414}) as a function of the hydrazine concentration.

Fig. 2.

Fluorescence responses (I_{460}/I_{414}) of probe **TNQ** ($5\mu\text{M}$) to hydrazine (5 equiv.) and various amines and ions (10 equiv.) (1-21: **TNQ** only, N_2H_4 , Cys, Glu, GSH, 1,6-Diaminohexane, pyridine, diethanolamine, Chlorinated hydroxylamine, urea, piperidine, piperazine, ammonium hydroxide, triethylamine, ethylenediamine, acetamide, n-butyl amine, Cu^{2+} , Hg^{2+} , N_3^- , SO_3^{2-}) in pH 7.4 HEPES/THF (1:9, v/v, $\lambda_{\text{ex}} = 365\text{ nm}$). **Insert:** Fluorescence spectra of **TNQ** ($5\mu\text{M}$) in the presence of hydrazine (5 equiv.) and various amines and ions (10 equiv.) in pH 7.4 HEPES/THF (1:9, v/v, $\lambda_{\text{ex}} = 365\text{ nm}$).

Fig. 3.

Top: color of probe **TNQ** and **TNQ** with various amines and ions. Bottom: fluorescence ($\lambda_{\text{ex}} = 365 \text{ nm}$) change upon addition of different metal ions and anions in HEPES/THF (1:9, v/v) (1-21: **TNQ** only, N_2H_4 , Cys, Glu, GSH, 1,6-Diaminohexane, pyridine, diethanolamine, Chlorinated hydroxylamine, urea, piperidine, piperazine, ammonium hydroxide, triethylamine, ethylenediamine, acetamide, n-butyl amine , Cu^{2+} , Hg^{2+} , N_3^- , SO_3^{2-}).

Fig. 4.

I_{460}/I_{414} of probe **TNQ** and **TNQ** + N_2H_4 at various pH values in HEPES/THF (1:9, v/v, λ_{ex} = 365 nm).

Fig. 5.
Two-photon excitation spectra of probe TNQ and TNQ + N₂H₄.

Fig. 6. Partial ^1H NMR spectra of (a) **TNQ** only, (b) **TNQ + N_2H_4** and (c) **De-TNQ** only in $\text{DMSO}-d_6$.

Fig. 7. HPLC chromatograms of probe **TNQ**, **TNQ** + N_2H_4 and compound **De-TNQ** (Sample 1: probe **TNQ** only; Sample 2: probe **TNQ** + 0.5 equiv N_2H_4 ; Sample 3: probe **TNQ** + 1.5 equiv N_2H_4 ; Sample 4: probe **TNQ** + 2 equiv N_2H_4 ; Sample 5: compound **De-TNQ** only). Liquid phase conditions: mobile phase (ACN-water (7:3, v/v)); current velocity (0.2 ml/min); chromatographic column (ZORBAX Eclipse Plus C18).

Fig. 8. HOMO-LUMO energy levels and the interfacial plots of the molecular orbitals for **TNQ** and its reacted form with hydrazine **De-TNQ**.

Fig. 9.

FL color changes of probe **TNQ** (1.0 mM) coated filter paper after exposure to different concentrations of hydrazine vapors solution for 1.0 min at atmospheric pressure. The FL color changes were collected using a hand-hold UV lamp with an excitation at 365 nm.

Fig. 10.

(a) Two-Photon image of CHO cells exposed with 10 μ M probe **TNQ** after 30 min of incubation, washed by PBS buffer. $\lambda_{\text{ex}} = 710$ nm (emission wavelength from 400 nm to 440 nm); (b) Emission wavelength from 440 nm to 480 nm; (c) Bright-field image of CHO cells; (d) The overlay of (a), (b,) and (c); (e) Two-photon image followed by a 30 min hydrazine (20 μ M) treatment, washed with PBS buffer. Emission wavelength from 400 nm to 440 nm; (f) Emission wavelength from 440 nm to 480 nm; (g) Bright-field image of CHO cells; (h) The overlay of (e), (f), and (g).