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One- and Two-Photon Turn-on Fluorescent Probe for Cysteine and Homocysteine with Large Emission Shift

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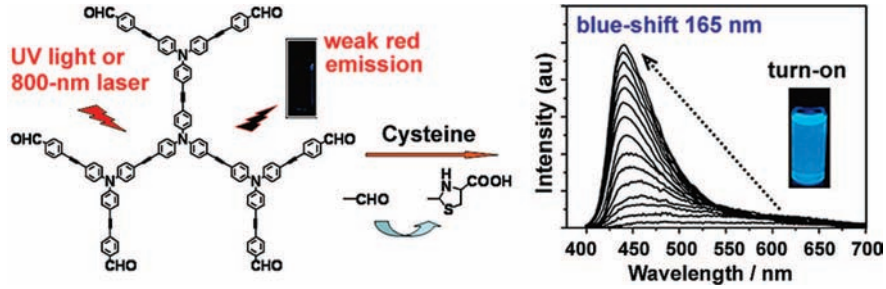
ABSTRACT



A novel dendritic chromophore with efficient intramolecular charge transfer (ICT) and strong two-photon absorption (TPA) was designed as a turn-on fluorescent probe for cysteine (Cys) and homocysteine (Hcy). The probe exhibited greatly enhanced fluorescence intensity as well as a very large emission peak shift (165 nm) upon addition of Cys/Hcy due to ICT switch off. The sensing process was also monitored by two-photon excited fluorescence (TPEF).

The detection of important biological thiols such as cysteine (Cys) and homocysteine (Hcy) is highly relevant to a variety of fundamental physiological processes in organisms.[1] For example, the levels of glutathione (GSH), Cys, and Hcy in the plasmas have been linked to human diseases such as AIDS,[2] Alzheimer's, and Parkinson's diseases.[3] Thus, the search for sensitive and selective sensors for Cys, Hcy, and related peptides has stimulated intense interest.[4,5] Some fluorescent probes have been developed recently. However, most of the fluorescent probes reported only exhibited emission intensity enhancement or decrease but with little peak shift. A major limitation of intensity-based probes is

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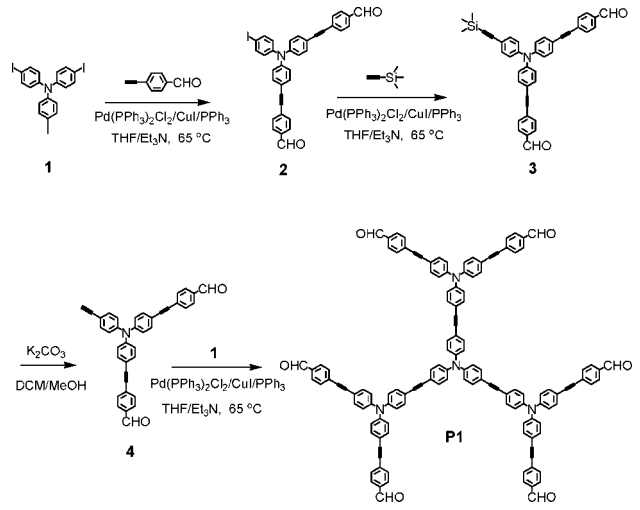
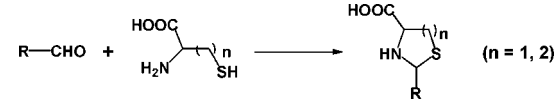
Scheme 1. Synthesis Route of Probe **P1**



that variations in sample environment and probe distribution may be problematic for utilization in quantitative measurements. On the other hand, to image the distribution of Cys/Hcy in cellular processes, suitable turn-on fluorescence chemosensors for Cys/Hcy should be developed. Thus, a probe exhibiting greatly changed emission intensity (turn-on/off) as well as large emission peak shift is ideal for sensing but leaves a great challenge for design and synthesis.

Compared to traditional ratiometric sensing, two-photon excited fluorescence (TPEF) microscopy offers many advantages in the biology community. Two-photon absorption (TPA) is a process by which two photons are simultaneously absorbed to an excited-state in a medium via a virtual state.[6] TPA has the advantage of high transmission at low incident intensity for incident light with an optical frequency below the band gap frequency. In recent years, materials with a large TPA cross-section have received considerable attention because of their interesting frequency up-conversion mechanism and potential applications in three-dimensional fluorescence imaging, optical data storage, lithographic microfabrication, and photodynamic therapy.[7−9] Owing to their ability to image at an increased penetration depth in tissue with reduced photodamage, two-photon active fluorescent chromophores have received increasing attention.[8,9] However, creation of efficient turn-on/off TPEF probes is still in its infancy. To address these limitations, our strategy in this work is to design a turn-on probe with both greatly enhanced emission intensity and large peak shift, which can be monitored by both one- and two-photon excitations. We selected triphenylamine as donor and high electron affinity peripheral group −CHO as acceptor (Scheme 1) to construct dendritic molecule **P1**. The electronic and vibronic coupling between the D−π−A dipolar branches is anticipated to increase the two-photon absorption (TPA) efficiency.[10] The efficient intramolecular charge transfer (ICT) favors the formation of highly polar emissive state (with red-shifted weak emission in polar medium).[11,12] The selective reaction of the aldehyde end group with N-terminal cysteines to form thiazolidines[13] (Scheme 2), for example, may alter the ICT characteristics in the molecule, which can manifest enhanced fluorescence. For example, after reaction with target molecules, the ICT switch off and the highly polar emissive state disappears leading to blue-shifted and greatly enhanced emission in polar medium.

Scheme 2. Reaction Mechanism between Aldehyde and Cysteine



Six-branched molecule **P1** was synthesized by successive Sonogashira coupling (Scheme 1). The yield of compound **2** is moderate (34%) because it is difficult to limit the reaction to the formation of only two branches. Compounds **3**, **4**, and **P1** were achieved in the following steps with high yields. Two-photon absorption cross-sections were measured by the two-photon induced fluorescence method.[14] To avoid possible complications due to the excited-state excitation, we have used femtosecond (fs) laser pulses. TPA intensity is characterized by the TPA cross-section (δ). The TPA spectrum of **P1** is shown in Figure 1, from which we can see it exhibits very strong TPA with δ_{max} of 938 GM (1 GM $= 10^{-50}$ cm^4 s photon^{-1} molecule^{-1}) at around 800 nm. The log−log dependence of two-photon excited emission power on the incident power (Figure 1, inset, at 800 nm as an example) with slope of 1.96 indicates the occurrence of nonlinear absorption. It is important that **P1** exhibit high emission yield in toluene (Φ = 93%) but show marked difference in their emission yields in polar solvents (Φ = 3.8% in dichloromethane and 1.6% in DMSO). This medium-dependent emission is due to strong ICT. A polar emissive state with weak emission intensity and red-shifted peak position is favored in polar solvents.

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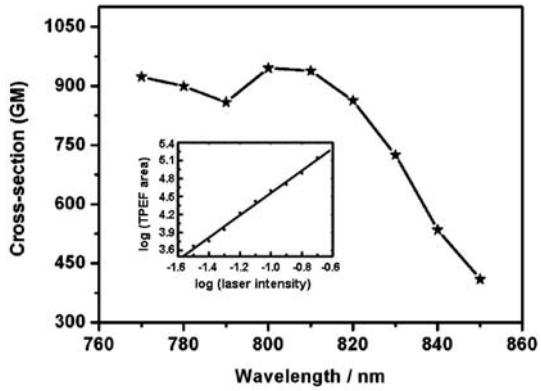

Figure 1. Two-photon cross-section spectrum of **P1**. Inset: variation of fluorescence power with incident power in a flowing condition on a log−log scale.

If the chromophore bonds with target molecules via its aldehyde group, the electron-withdrawing effect of the aldehyde group will be diminished and this will result in the quenching of the dipolar emissive state, thereby forming the basis of a probe in polar medium. We hypothesized that the reaction of **P1** with Cys and Hcy could be readily monitored by colorimetric as well as fluorescence responses.

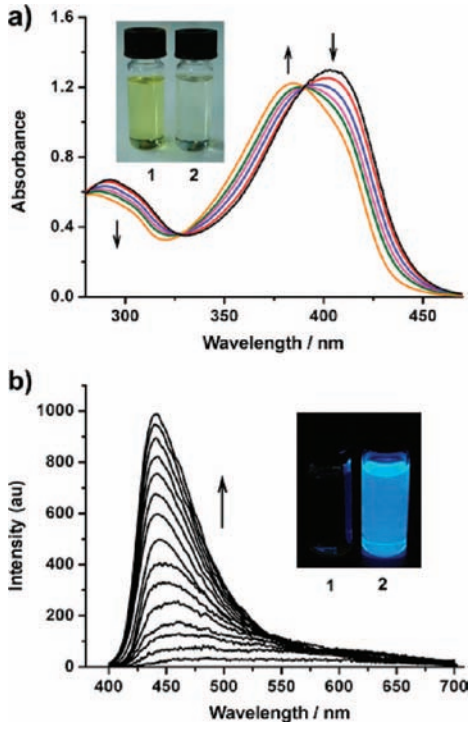

Figure 2. (a) Absorption spectra of **P1** in DMSO (5×10^{-6} M) after addition of Cys (3×10^{-4} M, measured each 10 min). Inset: photograph of before (**1**) and after (**2**) addition of Cys. (b) Emission spectra of **P1** after addition of Cys, taken every 5 min. Inset: emission photographs of **P1** (**1**) and **P1**-Cys (**2**) under UV shine (photos taken 2 h after addition of Cys).

In addition, the large two-photon cross-section of **P1** may afford sensing via TPEF.

As shown in Figure 2a, after the addition of Cys, the UV−vis absorption of **P1** slightly decreases and blue shifts gradually. The color of the solution changes from yellow to nearly colorless. This direct visualization of color change makes the detection straightforward. The photoluminescence of the mixture enhanced gradually (Figure 2b) with blue-shifts. After about 1 h, the **P1**-Cys mixture exhibits sky-blue emission under illumination by UV light, while the control **P1** solution in DMSO shows very weak emission. This emission enhancement in polar solvent is due to the removal of the aldehyde group (which is responsible for ICT and the polar emissive state) and the formation of thiazolidines. Such a structural change switches off the ICT and renders the emission behavior of the product less sensitive to micropolarity.

The mechanism of Cys binding with **P1** was also monitored by ^{1}H NMR (Figure 3). After reaction, the aldehyde resonance at 10.02 ppm in DMSO-d_6 solution disappeared. Two new peaks centered at 5.51 and 5.71 ppm can be assigned to the methane protons of the thiazolidine diastereometer.[5b]

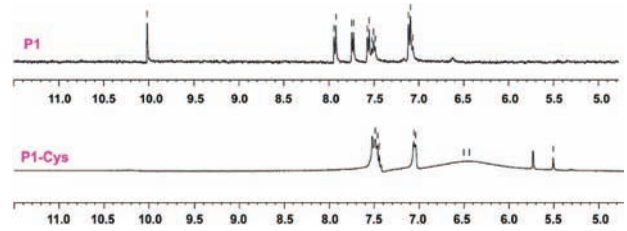

Figure 3. ^{1}HNMR of **P1** and after sensing of Cys in DMSO-d_6.

Control experiments in the presence of other common amino acids (with identical concentrations) reveal that **P1** exhibits good selectivity to cysteine and homocysteine, as demonstrated by both the color changes and striking emission enhancements (Figure 4). The sensing of Cys/Hcy can also

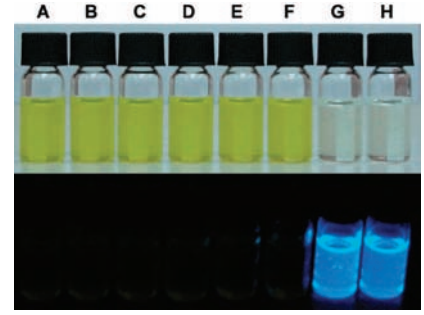

Figure 4. Color changes of **P1** solutions (5×10^{-5} M) and different amino acids (in DMSO, 3×10^{-3} M) under visible light (top row) and UV illumination (bottom row): A, no analytes; B, glycine; C, glutathione; D, *N*-acetylcysteine; E, *b*-alanine; F, L-alanine; G, cysteine; H, homocysteine.

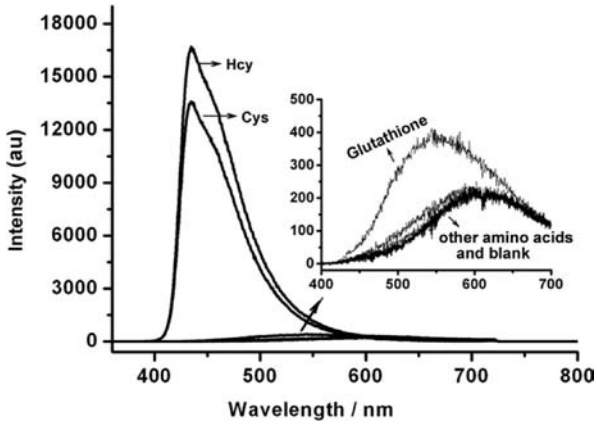

Figure 5. Two-photon excited fluorescence spectra (ex: 800 nm) of **P1** (5×10^{-6} M) in DMSO with various amino acids (3×10^{-4} M). Inset: enlarged view of emission spectra of other amino acids and blank.

be monitored by two-photon excited fluorescence. **P1** exhibits very weak TPEF in DMSO (peaked at about 600 nm), due to the very low emission quantum yield in polar solvents. After reacting with Cys/Hcy, the TPEF was enhanced by ~30 times and the peak wavelengths blue-shifted to 435 nm (with 165 nm shifts), which is comparable with the one-photon emission wavelength. This is much larger than the recent one-photon probes[15] reported by Lin, Duan, and co-workers, which exhibited large emission peak shifts after sensing. Figure 5 showed the TPEF spectra excited with an 800 nm fs-laser. Most of the amino acid controls did not show TPEF enhancement except for glutathione, which displayed a 2× enhancement of the intensity along with about 50 nm blue shift (Figure 5, inset), this is insignificant compared to the ~30× enhancement and 165 nm blue shifts of the emissions for Cys and Hcy. This remarkable TPEF enhancement and emission peak shifts enable **P1** to be used as an effective TPEF sensor for Cys/Hcy, especially at a practical wavelength (800 nm) for the fs-laser.

The multipolar chromophore **P1** affords a powerful multisignal detection strategy. First, visual detection by eye is possible even at a concentration of 5×10^{-6} M because of the large extinction coefficient ($\varepsilon = 260000\ M^{-1}\ cm^{-1}$) and the fact that efficient ICT red-shifts the absorption into the visible region (Figure 2a, inset). Second, the very weak emission of **P1** in polar solvents is enhanced greatly after reaction with Cys, which can be readily monitored by fluorescence spectra and also visualized under UV light. In addition, the large TPA cross-section of **P1** affords effective TPEF sensing.

In conclusion, we have designed a dendritic fluorescent probe exhibiting strong two-photon absorption and efficient sensing due to ICT presence/absence before and after reaction with Cys/Hcy. Upon binding to Cys/Hcy, the probe exhibited both turn-on fluorescence and large peak shift (165 nm) due to the ICT switch off. To the best of our knowledge, this is the first report that TPEF probe exhibits both greatly enhanced emission intensity and large peak shift. Owing to the potential reactivity of aldehyde toward other nucleophiles, this work should be a general guideline for the design of novel multisignal one- and two-photon turn-on probes, based on ICT switch on/off, with various sensing applications. Further modification, for example, functionalization of the branch using short poly(ethylene glycol) (PEG), would make it water soluble and biocompatible for in vitro application.

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Supporting Information Available: Synthesis, characterization, and measurement details of **P1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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