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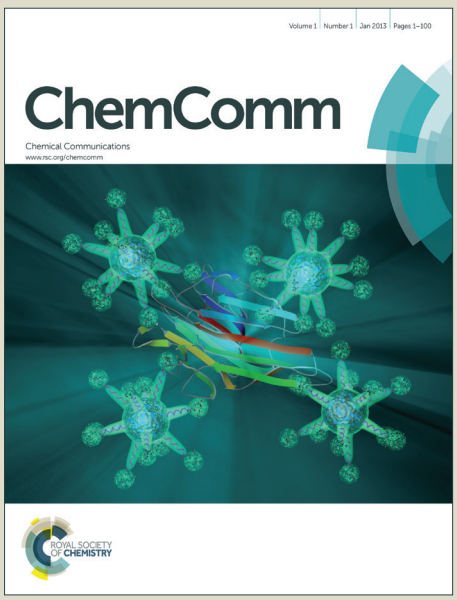

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A sensitivity tuneable tetraphenylethene-based fluorescent probe for directly indicating the concentration of hydrogen sulfide

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A novel tetraphenylethene-based fluorescent H_2S probe was designed and synthesized, which exhibited high selectivity and tuneable sensitivity, making direct indication of H_2S concentration in blood and unknown samples possible.

Hydrogen sulfide (H_2S) has been regarded as a toxic gas with unpleasant rotten egg smell for a long time. However, recent studies suggest that H_2S is also an important endogenous gasotransmitter existing in human body and other biological systems.[1] The significance of H_2S has been validated in various physiological processes, such as the regulation of cell growth,[2] cardiovascular protection,[3] the stimulation of angiogenesis,[4] antioxidative effect,[5] modulation of neuronal transmission,[6] and anti-inflammation effect.[1b] It has also revealed that the deregulation of H_2S is related to the symptoms of Alzheimer's disease and diabetes, etc.[7-10]

It is highly urgent to understand the physiological and pathological functions of H_2S, but the contribution of H_2S to human body and other biological systems, and the underlying molecular events remain unknown. Therefore, accurate and reliable detection of H_2S and direct indication of its concentration are desirable in order to provide useful information to study the function of H_2S in depth. In recent years, the detection of H_2S has become a hot research area, and a series of methods for sensing H_2S have been developed, such as colorimetry,[11] electrochemical analysis,[12] and gas chromatography.[13] Among these reported technologies, the fluorescent method has drawn much attention because of its high sensitivity, high selectivity, simplicity, and non-destructive advantage.[14]

For the design of fluorescent H_2S probe, one of the most prevalent strategies is to reduce the non-emissive azido-containing fluorophores to corresponding emissive amino derivatives, in which the sensing mechanism of fluorescence resonance energy transfer (FRET) or excited-state intramolecular proton-transfer (ESIPT) was mostly employed.[15] Generally, such measurements were carried out in aqueous solution and the traditional hydrophobic emissive amino derivatives suffer from the notorious aggregation-caused quenching (ACQ), which has greatly decreased their sensing performance.[16]

Since the detection of H_2S in the biological systems will be carried out in aqueous solution, researches have to take tedious processes to make the probes water-soluble to enhance the sensitivity. The alternative facile strategy is to take the advantage of the aggregation of the hydrophobic probes in the aqueous solution, that is, to develop the systems that the aggregation of the reduced products enhances instead of quenches the emission.

Indeed, we have observed such systems: a series of propeller-shaped non-emissive molecules in solutions, such as silole and tetraphenylethene (TPE), are induced to emit efficiently by aggregate formation.[17] This unique photophysical process has been termed as aggregation-induced emission (AIE).[18] The AIE phenomenon is exactly opposite to the ACQ effect, and the AIE-active luminogens have found wide applications in diverse areas including light-emitting devices, chemosensors and bioprobes. We anticipated that the AIE-active probes will behave differently from the traditional ACQ ones with FRET or ESIPT mechanisms. To the best of our knowledge, there is no probe used for H_2S detection based on AIE process was reported.

For the traditional H_2S detection systems, the emission of the reduced products gradually intensified with addition of H_2S and no tuneable sensitivity was reported. This linear relationship makes the direct indication of the H_2S concentration in its containing samples time-consuming because the work plot has to be first figured out. Furthermore, as far as we know, there is no fluorescent probe for detection of H_2S with tuneable threshold. With such feature, it is facile to indicate the H_2S concentration in an unknown sample by addition of it into the probe solutions with different concentration, which will be benefit to diagnose certain disease over the traditional linearly responsive probes.

Our groups have been working on the development of new polymerization reactions based on triple-bond building blocks.[19] During the course, we have succeeded in establishing the powerful azide-alkyne click polymerization,[20] by which the AIE-active polytriazoles have been prepared from TPE-functionalized diazides. Interestingly, although TPE is AIE-active, the azido functionalized TPE (TPE-Az) is non-emissive in both solution and aggregate states, while the amino group functionalized TPE (TPE-Am) features the AIE effect and is emissive in its aggregate state (Fig. S1 in electronic

supplementary information, ESI †).[21] Combining the fact that H_2S could reduce the azido group to amino one, we thus applied TPE-Az for the detection of H_2S. The results showed that the non-emissive (off) of DMSO solution of TPE-Az became luminescent (on) upon incubation with NaHS (a commonly used hydrogen sulfide source) and generation of aggregates by adding 80 vol% 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES, (pH = 7.4)) butter into the solution. More importantly, thanks to the AIE feature of TPE-Am, the detection threshold could be facilely fine-tuned by varying the concentration of TEP-Az, which is rarely reported too.

The probe of 1-(4-azidophenyl)-1,2,2-triphenylethene (named TPE-Az), whose structure is shown in Fig. 1, was prepared according to our previously published procedures.[22] For comparison, the reduced product of TPE-Am *i.e.* 1-(4-aminophenyl)-1,2,2-triphenylethene was also prepared by cross-coupling of benzophenone and 4-aminobenzophenone via McMurry reaction in 51.2% yield.[21] The detailed synthesis procedures was given in ESI † and their structures were fully characterized using ^1H, ^{13}C NMR, FT-IR, and element analysis, and satisfactory data were obtained (Fig. S2-S9, ESI †).

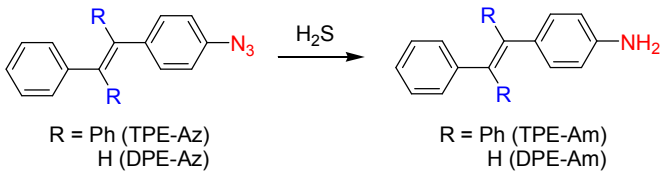

Fig. 1. Schematic illustration of detection of hydrogen sulphide by the azido-containing fluorophores.

We first measured the photoluminescence (PL) spectra of TPE-Az and TPE-Am (10 μM) in DMSO/HEPES butter mixtures (v/v = 2:8), in which the molecules become aggregates. The results confirmed our previous observation that the TPE-Az is non-emissive and its spectrum is almost a flat line parallel to the abscissa and the quantum yield is calculated as 0.36% due to the quenching effect of azido groups (Fig. 2), while remarkable increase emission (ca. 60-fold) peaked at 493 nm was recorded upon incubating with 5 equivalents of NaHS for 5 min. Moreover, the emission profile is almost same with that of TPE-Am, which has a quantum yield of 3.20%, further manifesting that TPE-Az could be converted efficiently into TPE-Am (Fig. 2A) by H_2S.

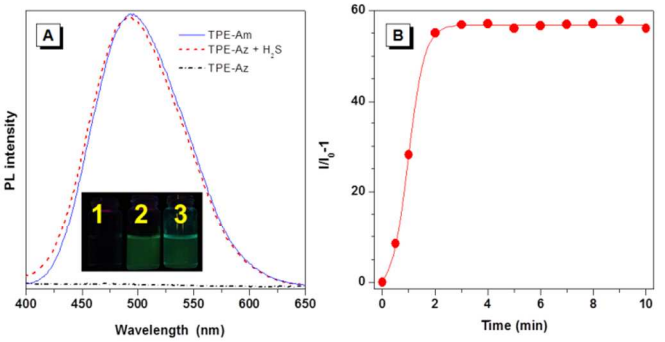

Fig. 2. (A) PL spectra of TPE-Az, TPE-Am, and TPE-Az after incubation with 50 μM H_2S in DMSO/HEPES buffer (10 mM, pH = 7.4) mixtures with buffer fraction of 80%. Inset: photograph of 1) TPE-Az 2) TPE-Az + H_2S and 3) TPE-Am under UV-light (λ_{ex}=365 nm) in DMSO/HEPES buffer mixtures with buffer fraction of 80%. (B) Time-dependent PL change of DPE-Az upon addition 50 μM H_2S in DMSO/HEPES buffer mixtures with buffer fraction of 80%. Concentration of TPE-Az and TPE-Am: 10 μM; λ_{ex} = 340 nm.

Since the catabolism of H_2S is very fast in vivo but it was reported that most of the H_2S probes display slow response time (more than 20 min) in vitro,[15a] we thus performed the time-dependent PL measurement to study the kinetics of our probe. As shown in Fig. 2B and S10 (ESI †), the emission intensity was enhanced swiftly within 2 min and saturated afterward when the TPE-Az with concentration of 10 μM was reacted with 50 μM of H_2S in DMSO and then aggregated by addition of 80 vol% of the HEPES butter. The shorter response time suggests that TPE-Az is more suitable for real time detection for H_2S. To enable the azido group to be completely reduced to amino group, a time scale of 5 min was used after addition of H_2S in our experiments.

Encouraged by the distinct PL changes of TPE-Az upon treated with H_2S, we further evaluate the specific nature of this probe by co-incubation with various anions (CO_3^{2-}, $S_2O_3^{2-}$, SO_4^{2-}, Br^-, Cl^-, HCO_3^-, NO_3^-, OAc^-, OH^-, NO_2^-, PO_4^{3-}, $S_2O_4^{2-}$, and SO_3^{2-}; 50 μM), cellular signaling molecule (NO) and biothiols (cysteine, 1 mM; glutathione, 5 mM; and homocysteine, 50 μM). As can be seen from Fig. 3 and S11 (ESI †), these competitive species did not cause any PL changes of the aggregates of TPE-Az, and only could H_2S lead to pronounced PL enhancement. The excellent selectivity for H_2S over other relative analytes shows that the hydrophobic TPE-Az is promising for the detection of H_2S in a complex biological environment by taking the advantage of AIE feature of its reduced product of TPE-Am.

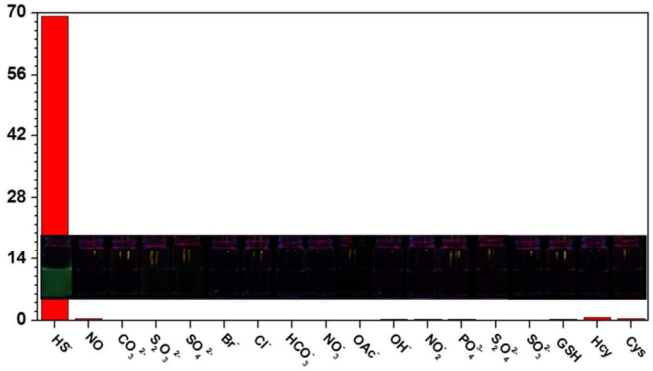

Fig. 3. Selectivity of TPE-Az with other reactive anions, cellular signaling molecule, and biothiols in DMSO/HEPES buffer mixtures with buffer fraction of 80%. Concentration: probe: 10 μM, analytes: 1 mM for Cys, 5 mM for GSH and 50 μM for others, HEPES buffer (10 mM, pH=7.4), λ_{ex} = 348 nm. Cys = cysteine; Hcy = homocysteine; GSH = glutathione. Inset: photographs of TPE-Az with these analytes incubated for 5 min and then generated aggregates (observed under UV lamp with wavelength of 365 nm).

Having demonstrated the selectivity of TPE-Az for the detection of H_2S, we next investigated its ability for quantifying H_2S concentration. TPE-Az with concentration of 10 μM was first incubated with different amount of H_2S (0 - 50 μM) in DMSO for 5 min and then added 80 vol% of HEPES buffer (Fig. S12, S13, and 4, ESI †). To our surprise, no obvious PL signal was recorded when the concentration of added NaHS was below that of the probe (10 μM) (Fig. 4). Afterward, the emission intensity increased swiftly and reached the plateau when 3 equivalents of NaHS was added.

Attracted by this abnormal phenomenon, we varied the concentration of TPE-Az from 10 to 20 and 50 μM and added NaHS into them under the same experimental conditions (Fig. S14-S17, ESI †). As expected, the detection thresholds for H_2S also varied from 10 to 20 and 50 μM. These results clearly demonstrate that the detection of H_2S by the TPE-Az has a threshold concentration in the aggregate state, below which no PL signal was observed, whereas,

above which the emission intensity increases swiftly. Furthermore, this threshold is as same as the concentration of TPE-Az and is tuneable in a wide range by simply varying the concentration of TPE-Az, which covers the typical H_2S concentration in blood. In addition, this probe is believed to be applicable in fast indicating the H_2S concentration in an unknown sample.

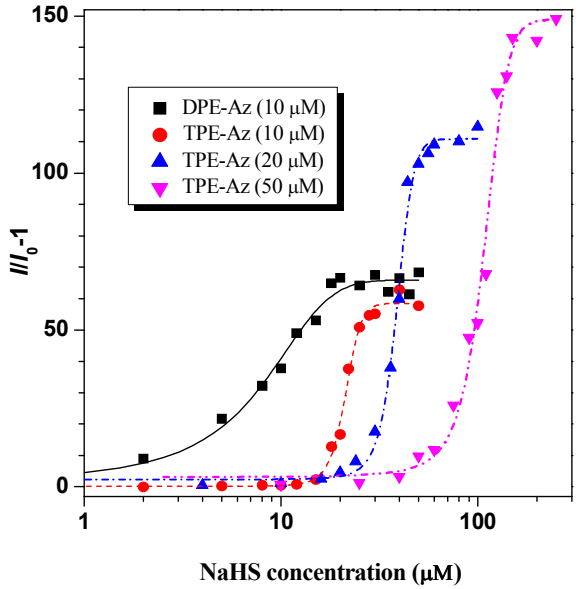

Fig. 4. Relative PL intensity of TPE-Az and DPE-Az vs the concentration of H_2S in DMSO/HEPES buffer mixtures with buffer fraction of 80%. λ_{ex} = 348 nm for DPE-Az and 340 nm for TPE-Az.

At first, we proposed that without reaching the equivalent of NaHS, TPE-Az is partially reduced. The remaining azido groups in the random mixed aggregates still could quench the emission of formed TPE-Am, thus making the whole system non-emissive. When equivalent of NaHS was added, all the azido groups should theoretically be reduced to amino ones, which should make the aggregates of TPE-Am highly emissive. But, according to our experiments, the emission of the system could not be recorded until more than equivalent of NaHS was added. Thus, we proposed another more reasonable pathway as shown in Fig. S20. TPE-Az could probably be first reduced to a nonemissive intermediate TPE-AzS by equivalent of NaHS, which could be further converted to TPE-Am upon addition of anther batch of NaHS.[23] This hypothesis could be confirmed by following experiments. At first, when mixing the TPE-Az with NaHS in equivalent, no PL signal from the aggregates was observed even after 20 min. Meanwhile, when the NaHS reaches 2 equivalents, all the TPE-Az could be reduced to TPE-Am but the reaction rate is not high enough to accomplish in 5 min due to the fact that the emission of the system could be further intensified. The reaction could finish by addition more NaHS, such as 3-5 equivalents or prolonging the time from 5 to 11 min. It is worth noting that these two strategies led to the plateau with nearly the same emission intensity (Fig. S21).

To have a better illustration of the advantage of our probe based on AIE feature, we designed and synthesized an azide-containing diphenylethene (named DPE-Az) and AIE-inactive DPE-Am, the reduced product of the former (their structures are shown in Fig. 1, and the synthetic procedure is provided in ESI †).[24] Similar to TPE-Az, DPE-Az is also non-emissive in its solution and aggregated state. Upon addition of NaHS to the system of DPE-Az under exactly the same experimental conditions as TPE-Az, the emission intensity almost linearly increased in the concentration range of 0-20 μM (Fig. 4 and Fig. S18 and S19 in ESI †). Meanwhile, the emission was immediately appeared even only was 0.1 equivalent of NaHS added into the system and no threshold was recorded, which is well coincident with the reported traditional probes.

Conclusions

In summary, different from the reported traditional probes for the detection H_2S, which are dominated by the mechanism of the FRET and ESIPT processes, we present here a unique probe based on the unique AIE feature in the aqueous solution. TPE-Az is non-emissive in its solution and aggregate states but could be reduced to the AIE-active TPE-Am by NaHS, a commonly used hydrogen sulfide source. The response time for TPE-Az for the detection of H_2S could be as short as 2 min, which are much faster than the reported probes. More important, the TPE-Az has a detection threshold and could indicate the concentration of H_2S simply by the lighting up of its emission when TPE-Am formed aggregates. Furthermore, the detection threshold could be fine-tuned in a wide range by varying the concentration of TPE-Az. Thus, this method is promising to be developed into a facile tool for direct indication of the H_2S concentration in blood or unknown samples.

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Notes and references

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† Electronic Supplementary Information (ESI) available: synthetic details of probes, IR, ^1H and ^{13}C NMR spectra of probes, PL spectra of probes upon addition of H_2S. See DOI: 10.1039/c000000x/

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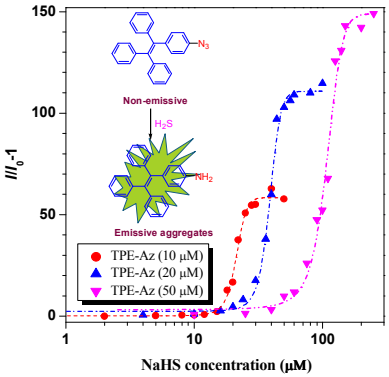

A selective tetraphenylethene-based fluorescent H_2S probe was designed and synthesized, which exhibits tuneable sensitivity and could directly indicate the concentration of H_2S.