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Discriminatory Detection of Cysteine and Homocysteine Based on Dialdehyde-Functionalized Aggregation-Induced Emission Fluorophores

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Abstract: We demonstrate a concept-proof work of using fluorescence (FL) "turn-on" probes for the discriminatory detection of cysteine (Cys) over homocysteine (Hcy). The fluorogens are provided with aggregation-induced emission (AIE) characteristic and functionalized with two aldehyde-groups (DMTPS-ALD and TPE-ALD). All the detections were carried out in a biocompatible medium (10 mM HEPES buffer and DMSO, pH 7.4). In principle, the formation of thiazinane/thiazolidine through the chemical reaction of aldehydes on the probe molecules and the residue of Cys/Hcy determines the selective recognition of Cys and Hcy over other amino acids and glucose. The FL responses originate from the AIE property of thiazinane/thiazolidine resultants, which have low solubility and precipitate (aggregate) in the detection medium. The discrimination between Cys and Hcy comes from the difference in reaction kinetics of TPE-ALD/DMTPS-ALD with Cys and Hcy, thereby the FL responses show different time courses and intensity enhancement. It is worth noting that TPE-ALD outshined the other two probes in performance with fast response, a high FL enhancement up to 16-fold, high sensitivity, and good specificity and selectivity. Moreover, its FL response threshold at 250 μM is very close to the lower limit of the normal level of Cys in human plasma, which implies that TPE-ALD could be applied as a potential indicator of Cys deficiency.

Keywords: aggregation · aldehydes · amino acids · fluorescence spectroscopy · fluorescent probes

Introduction

Fluorogens with aggregation-induced emission (AIE) characteristics have drawn considerable research attention in the area of fluorescent materials.[1–4] As a phenomenological description, AIE gained its name from the unique fluorescent behavior of a silole derivative (hexaphenylsilole (HPS)).[2] HPS was non-emissive in dilute acetonitrile solution; however, with the addition of an adequate amount of water (non-solvent) into the solution, HPS became partially insoluble in the mixed solvents thereby aggregates began to form, and the suspension became emissive.[2a] The AIE-active fluorogens offer a kind of unique fluorescence (FL) turn-on probes with high sensitivity and contrast. Thus, a series of AIE-based fluorescent probes have recently been developed for the detection of a variety of biomolecules.[1,4]

Cysteine (Cys) and homocysteine (Hcy) are important biomolecules and play crucial roles in numerous biological processes in organisms.[5] It is of great importance, both in academic research and in clinic applications, to develop efficient methods for the detection and recognition of Cys and Hcy.[6–12] Fluorescent probes have also been explored to detect Cys and Hcy.[13] In most cases, the fluorescent detection is based on chemical reaction between the active groups on the probe and the analytes. For example, the well-known cyclization of aldehyde group with amino and thiol groups forming thiazinane/thiazolidine is a classical reaction that is widely used for Cys/Hcy detection. The detection mechanism is associated with the transformation of the aldehyde group to thiazinane/thioazolidine, which changes the electronic structure of the probe thereby alters the FL properties (intensity, color, or lifetime).[13] This strategy, however, can hardly provide discriminative and selective detection of Cys over Hcy because of the high structure similarity between the two resulting analogues. Consequently, to discriminatively and selectively detect Cys over Hcy is still a challenge.[14]

AIE-active fluorescent probes may provide new solutions. According to AIE mechanism, FL turn-on/turn-off process can be observed if the reaction between the probe and the

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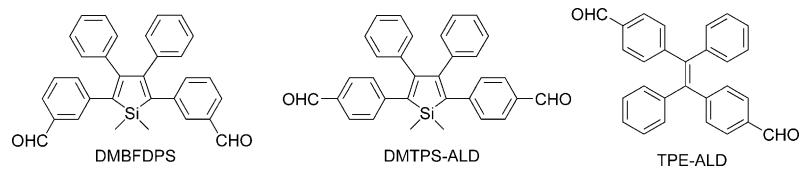


Scheme 1. Dialdehyde-substituted AIE-active molecules.

to-be-probed molecules can alter the aggregation behavior (solubility) of the fluorescent species.[4,15] These observations prompted the use of AIE luminogens as fluorescent probes to detect Cys and Hcy. Therefore, we took the first attempt at applying an aldehyde functionalized silole derivative DMBFDPS to detect Cys over other amino acids and glucose (Scheme 1).[15c] DMBFDPS can selectively react with Cys and Hcy to form thiazinane and thiazolidine derivatives in the presence of diverse amino acids, protected Cys and glucose. Relying on the differences in kinetics, Cys can be easily and discriminately detected over Hcy by the observation of FL responses. GSH shows great interference with the detection of Cys and Hcy and it can be quantitatively detected by using a FL spectroscopic titration method. By using this single fluorescent probe, the discriminate detection of Cys, Hcy, and GSH is possible by FL turn-on and turn-off strategies. The discrimination relies on difference in the solubility of adducts of the probe molecule and analytes.

This strategy is intrinsically a fluorescent titration, which combines the high sensitivity of FL spectroscopy and the reliability of precipitate titration methodology. Thus, it is assumed to be a universal property of dialdehyde-substituted AIE-active molecules. Herein, we report our concept-of-proof work by using two such molecules as shown in Scheme 1. One is an isomer of DMBFDPS, or DMTPS-ALD, which has two aldehyde groups on the *para* position instead of *meta* position of the 2,5-phenyl rings on the silole core. And the other is a dialdehyde-substituted tetraphenylethene (TPE), or TPE-ALD, in which the AIE-active tetraphenylsilole core is replaced by a TPE moiety. As expected, for discriminatively detecting Cys and Hcy, both DMTPS-ALD and TPE-ALD show sound fluorescent performance.

Results and Discussion

Responses of DMTPS-ALD to Cys and Hcy: To prove the generality of using dialdehyde-substituted AIE-active molecules for the discriminative detection of Cys and Hcy, we first studied DMTPS-ALD. The preparation of DMTPS-ALD was reported elsewhere.[15b] HEPES buffer was chosen because it is a simple buffer system with a moderate oil solubility. DMSO was selected in consideration that it is a biocompatible organic solvent. The solution with 40% volume fraction of HEPES buffer (f_b) was used as an optimized detection medium after careful examination of the fluorescent behaviors of DMTPS-ALD in the mixtures with different f_b values (Figure S1 in the Supporting Information).

The probe solution (DMTPS-ALD (25 μM) in the defined detection medium), displays an FL band peaked at 501 nm with a low intensity (Figure 1a). When it was added into Cys solution (2.5 mM in the defined detection medium), the reaction between the probe and Cys molecules took place readily at room temperature and produced a thiozalidine derivative via a Schiff base intermediate (see the Supporting Information, route I, Scheme S1, Figures S2 and S3). Since the transparent reaction solution turned turbid in a few minutes, this process could be conveniently observed by the naked eye. The FL responses provide the information in

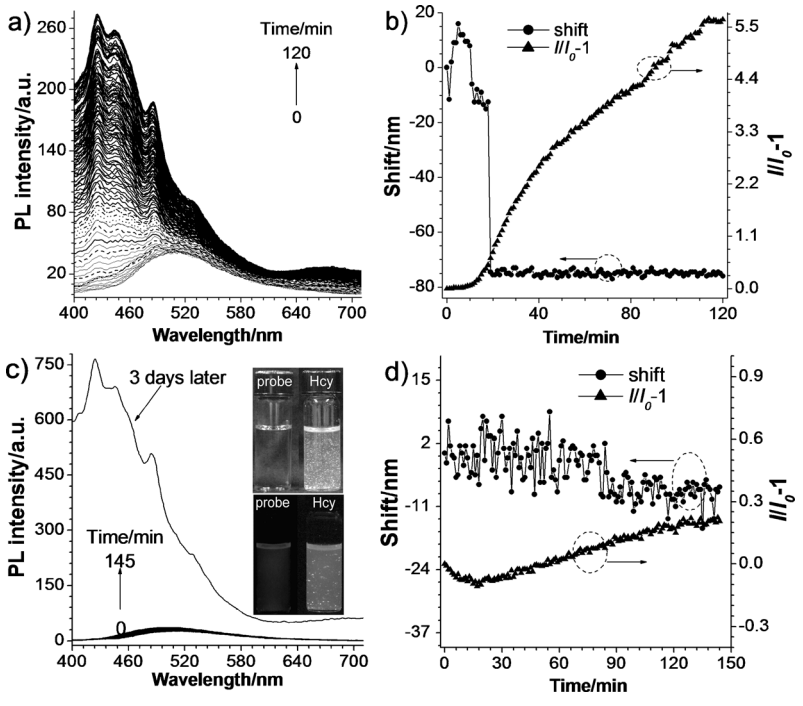


Figure 1. Time dependent FL spectra when a) Cys or c) Hcy was added into the mixture of DMSO and 10 mM HEPES buffer (6:4 v/v, pH 7.4) containing DMTPS-ALD; b) and d) are corresponding plots of the changes in FL intensity and FL peak for a and c, respectively. [DMTPS-ALD] = 25 μM, [analyte] = 2.5 mM, λ_{ex} = 377 nm. I for peak FL intensity and I_0 for the peak FL intensity of DMTPS-ALD at t = 0 min in b and d. Inset of c: photographs of DMTPS-ALD and Hcy in the above mixture taken 3 days later under ambient (upper row) and UV-light (bottom row, λ_{ex} = 365 nm).

detail. After being introduced into Cys solution, the weakly emissive probe solution gradually became intensely fluorescent and the emission color shifted from green to blue. Quantitative evaluations of the changes in FL features are depicted in Figure 1a and 1b. The FL intensity of the reaction system is enhanced immediately and levels off in about 110 min. The maximum shows an evident elevation from $\approx$41 to $\approx$273 in the absence and the presence of Cys, respectively. The FL enhancement (I/I_0-1, in which I_0 and I are the FL intensity recorded without and with analyte, respectively) is about 5.7-fold. Meanwhile, the FL band peaked at 501 nm increases gradually, and a new band peaked at 425 nm emerges ($\approx$76 nm blueshift) and its intensity grew rapidly as time went by.

Different observations were recorded when adding probe solution into Hcy solution. Little change had been observed for hours. Three days later, white precipitates appeared from the transparent solution and the suspension showed strong blue FL (Figure 1c and inset). Quantitatively, the emission peak shifted from 501 to 424 nm and the I/I_0-1 value was 26.4.

The above observations can be interpreted as following. Owing to the strong intermolecular hydrogen-bonding between the carboxylic and thiazolidine groups and the electrostatic interaction between the charged groups, molecules **1** and **2** (see the Supporting Information, Scheme S1) can easily form aggregates thereby resulting in their lower solubility. The emission enhancement can be ascribed to the resulting aggregates of thiazinane or thiazolidine. The blueshift of the emission can be associated with the transformation of the aldehyde to the thiazolidine auxochrome, which eliminates the conjugation between the silole core and the benzaldehyde group. The drastic time difference of FL turn-on exhibited in Figure 1b and 1d implies that the reaction of Hcy with the probe molecule to produce thiazolidine is quite slow in comparison with that of Cys (see the Supporting Information, Scheme S1, route II). According to the literature, upon reaction with aldehyde, Cys forms the more favored five-membered ring heterocycle, which is beneficial to the reaction proceeding, as compared with Hcy (six-membered ring formation).[13b,16] Although the substituents have changed from the *meta* to the *para* position, the mechanism of the discriminative detection of Cys over Hcy is still the same as DMBFDPS, as illustrated in Scheme S1 (the Supporting Information).

The sensitivity of the probe to Cys was investigated by a FL spectroscopic titration experiment. The changes in FL features with Cys concentration are displayed in Figures S4–S6 (see the Supporting Information). From 0 to 2.5 mM, the emission intensity increases slowly and nonlinearly. Apart from sensitivity, the specificity of a probe is a crucial parameter for potential applications. The responses of the probe to other amino acids and glucose were investigated. As shown in Figures S7 and S8 (see the Supporting Information), among all the tested analytes, Cys exhibited the highest FL response. In an hour, more than fourfold of FL enhancement and 68 nm of blueshift of the emission peak were observed for Cys. About 0.27-fold enhancement was recorded for Hcy, and below 0.2 for the rest analytes. These results indicate that although the enhancement is not very high, the probe molecule has a high specificity to Cys.

Investigation of the selective detection of Cys in the presence of other biospecies is essentially necessary in practical detection. When only 2.5 mM of Cys exists in the medium (control sample), the I/I_0-1 value is 4, and the emission blueshift is about 68 nm. By measuring the fluorescent emission spectra of mixtures containing Cys (2.5 mM) and other analytes (2.5 mM), it was found that with the exception of Hcy, all other analytes exerted little impact on the detection of Cys in FL spectroscopic titration method (shown in the Supporting Information, Figures S9–S11). Whereas, when co-existing with Hcy, the I/I_0-1 value is 0.7, which is evidently lower than that of the control one. Meanwhile, the blueshift of the emission peak is also limited. The interference response can be associated with the competing reaction of the intermediate with the free thiol group of Hcy and Cys as shown Scheme S2 (the Supporting Information). The addition of thiol to the Schiff base of the intermediate led to soluble products (without aggregates) and thus decreased the emission.

Based on the above results, it is concluded that DMTPS-ALD possesses the capability to detect Cys and Hcy over other species and could discriminate Cys from Hcy with high specificity and good selectivity. The action principle is also based on AIE behavior (poor solubility) of the resultants, which attests our hypothesis that using dialdehyde-substituted silole derivatives can achieve the discriminatory detection of Cys and Hcy. The overall performance is similar to its isomer DMBFDPS, although the sensitivity and FL enhancement of DMTPS-ALD is lower than that of DMBFDPS.

Fluorescent behavior of TPE-ALD in the mixtures of buffer solution and DMSO: Although we have demonstrated the discriminative detection of Cys over Hcy with two isomers of dialdehyde-functionalized siloles, the generality of our assumed strategy to discriminatively detect Cys over Hcy by dialdehyde-functionalized AIE-active fluorogens cannot be confirmed until the same strategy is shown to be workable in other AIE systems. In addition to siloles, tetraphenylethene (TPE) derivatives are another kind of widely explored AIE-active fluorogen. Thus, we replaced the silole core with a TPE moiety to give the dialdehyde-functionalized TPE derivative (Scheme 1, TPE-ALD, or (*E*)-4,4′-(1,2-diphenylethene-1,2-diyl)dibenzaldehyde) as the fluorescent probe. TPE-ALD was obtained by the lithiation of (*E*)-1,2-bis(4-bromophenyl)-1,2-diphenylethene and the subsequent reaction with *N*-formylpiperidine.[17]

We firstly checked the fluorescent behavior of TPE-ALD in the mixtures of HEPES buffer (10 mM) and DMSO with different buffer fraction (f_b) values. As displayed in the Supporting Information (Figure S12), TPE-ALD is almost non-emissive in a solution (25 μM) of DMSO. When f_b is lower than 60%, the FL spectrum is nearly a flat line parallel to

the abscissa. As the f_b increases, the emission is intensified. When f_b reaches 90%, the emission intensity is fairly strong ($I/I_0=384$, $I=775$, $f_b=90\%$, respectively). The inset of Figure S12 clearly demonstrates the relationship between FL enhancement (I/I_0) and f_b. When f_b is lower than 60%, I/I_0 is small and remains unchanged; however, once f_b is increased up to 60%, the I/I_0 value increases abruptly and continues to rise with an increase of f_b. This characteristic curve indicates that TPE-ALD is a typical AIE active fluorophore with a threshold of f_b, at which the aggregates are produced and the emission is turned on. On the basis of our previous research, the mixture of HEPES buffer (10 mM) and DMSO with $f_b=50\%$ and pH 7.4 was chosen as the action ratio and was subsequently used as the detection medium in the following work.

Distinct fluorescent responses of the probe to Cys and Hcy: The probe solution contained TPE-ALD (25 μM) in the detection medium and displayed an emission band peaked at 501 nm with a low intensity (Figure 2a). When the probe solution was added into Cys solution (2.5 mM in the defined detection medium), the response took place immediately at RT. This process could be easily monitored by the naked eye, because the reaction solution changed from transparent to turbid and the solids accumulated in a few minutes. However, the FL responses provide more detailed information. The probe solution itself showed a quite weak green emission, however, after being introduced into Cys solution, the emission became more intense and the color shifted from green to purple/blue (Figure 2a). A quantitative evaluation of the changes in FL is illustrated in Figure 2b. The FL intensity of the reaction system is readily enhanced after the addition of probe solution into Cys solution, leveling off at about 80 min. The response time is significantly shorter than the one of DMTPS-ALD and DMBFDPS. The maximum intensity shows a remarkable augment from ≈43 in the absence of Cys to ≈675 in the presence of Cys, or about 15.7-fold enhancement (I/I_0-1). The enhancement is higher as compared to DMBFDPS, especially much higher than DMTPS-ALD. Meanwhile, the FL band peaked at 501 nm increased gradually, and a new peak at 421 nm emerges (≈80 nm blueshift) and its intensity grew rapidly with time.

As for Hcy, different observations were recorded, as shown in Figure 2c and 2d. Almost no response had been observed for hours; however, three days later, white solids precipitated out from the transparent solution with the FL enhancement increasing from about 0.2 to 3.0. As time went by, the amount of precipitates increased. When the reaction system was kept at RT for a week, the value of I/I_0-1 increased to 13.6. Finally, the suspension showed strong blue FL (inset of Figure 2c and the Supporting Information, Figure S13) and a 27-fold enhancement after ten days. Concurrently, the emission peak shifted from 501 to 422 nm. Compared with the two isomers of dialdehyde substituted silole derivative, TPE-ALD amplified the responding difference between Cys and Hcy by shortening the response time of probe to Cys and prolonging the response time of probe to Hcy.

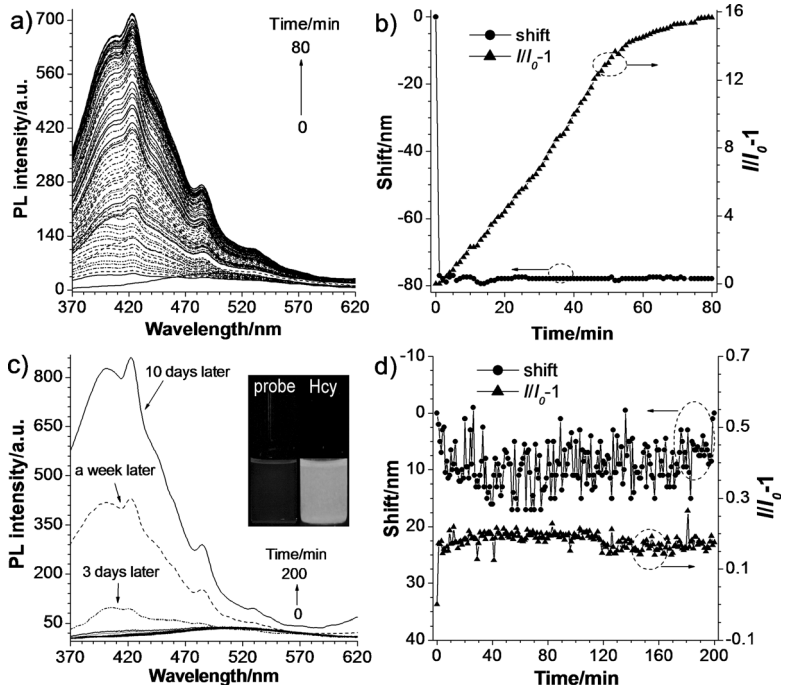
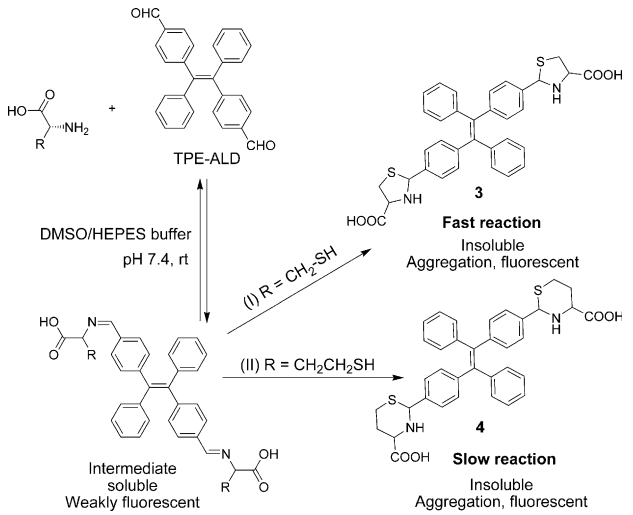


Figure 2. Time dependent FL spectra when a) Cys or c) Hcy was added into the mixture of DMSO and 10 mM HEPES buffer (5:5 v/v, pH 7.4) containing TPE-ALD; b) and d) are the corresponding plots of the changes in FL intensity and FL peak for a and c, respectively. [TPE-ALD]=25 μM, [analyte]=2.5 mM, $\lambda_{ex}=343$ nm. I for peak FL intensity and I_0 for the peak FL intensity of TPE-ALD at $t=0$ min in b and d. Inset of c: FL photographs of TPE-ALD and Hcy in the above mixture taken ten days later under UV-light ($\lambda_{ex}=365$ nm).

Mechanism for the origin of the distinction: With the resulting distinct response behaviors of the probe, our proposed mechanism of the discriminative detection of Cys over Hcy has been validated once again. The reaction between the probe and Cys or Hcy molecules takes place at RT and produces a thiozalidine derivative (molecule **3** or **4**, Scheme 2 and the Supporting Information, Figure S14) via a Schiff base intermediate. The strong hydrogen-bonding between the carboxylic and thiazolidine groups and the electrostatic interaction between the charged groups may help molecules **3** and **4** form aggregates, thereby leading to lower solubility. The emission enhancement can be ascribed to the resulting aggregates of thiazinane or thiazolidine, which have a low solubility in



Scheme 2. Mechanistic representation of the discriminative detection of Cys and Hcy.

the detection medium (aggregation-induced emission). The blueshift of the emission band can be associated with the diminished conjugation degree between the TPE core and the substituents. The drastic time differences of FL turn-on exhibited in Figure 2b and 2d imply that the reaction of Hcy with the probe molecule is quite slow in comparison with that of Cys (Scheme 2, route II); the reason for the kinetic difference is associated with the different cyclization rates of five-membered and six-membered heterocycles.

Sensitivity and Specificity: The sensitivity of the TPE-ALD to Cys was investigated by FL spectroscopic titration experiment. The changes in FL features with Cys concentration in the defined medium are exhibited in Figure 3. From 0 to 2.5 mM, FL intensity increases nonlinearly. The plot of I/I_0-1 versus Cys concentration is nearly a flat line parallel to the abscissa before the concentration of Cys increased to 250 μM (10 equiv of probe). At the mean time, the emission peak fluctuated in the range of 500 to 485 nm. Once the Cys concentration is above 250 μM, the plot takes off with a sharp inflexion, the FL peak abruptly blueshifted to around 423 nm (Figure 3b) and the transparent mixture turned turbid at this point as well. At 2.5 mM of Cys, I/I_0-1 increases to 15.4 in 1 h and white solids were clearly visible (see the Supporting Information, Figures S15 and S16). It is worth mentioning that the breaking point at 250 μM is of great signality, because the normal level of Cys is 240–360 μM in human plasma. The above results give a clue that this probe could be applied as a potential indicator of Cys deficiency.

In addition to the sensitivity, the specificity is of great significance for an effective probe. The responses of the probe to other amino acids, and glucose were investigated and the details are shown in Figure 4, and the Supporting Information, Figures S17 and S18. Among all the tested analytes, Cys exhibited the highest and positive FL response. In an hour, more than 15-fold FL enhancement and a 76.5 nm blueshift of the FL peak were observed for Cys, with which Cys was definitely recognized from other biospecies. About 0.92-fold enhancement was recorded for lysine. And as for the rest analytes, the FL is slightly quenched with an approximately 10 nm redshift. The order of the sensitivity is Cys >

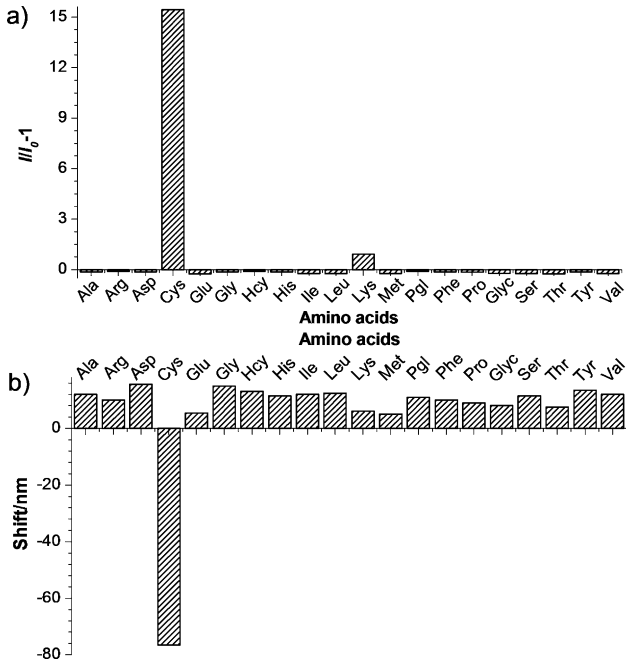


Figure 4. FL response of TPE-ALD to diverse amino acids in the mixture of DMSO and 10 mM HEPES buffer (5:5 v/v, pH 7.4), [probe] = 25 μM, [analyte] = 2.5 mM, λ_{ex} = 343 nm. All measurements were conducted at RT in 60 min later. Probe = TPE-ALD, analyte = amino acid.

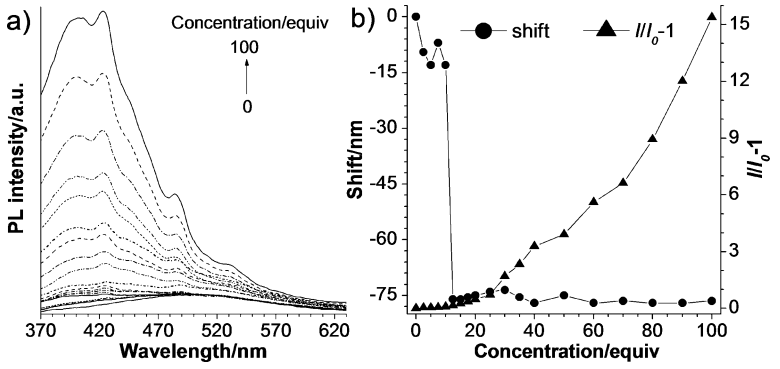


Figure 3. a) FL spectra of TPE-ALD and Cys in the mixture of chromatographically pure DMSO and 10 mM HEPES buffer (5:5 in volume, pH 7.4) with different Cys concentrations. [TPE-ALD] = 25 μM, λ_{ex} = 343 nm. b) Plot of FL enhancement versus Cys concentration. All were measured at RT 60 min later.

Lys > other amino acids and glucose. As a result of distinct kinetics, the structurally similar Cys and Hcy could be discriminated. These results indicate that the probe molecule has an extremely high specificity to Cys.

Selectivity: In practical detection, Cys may be interfered by other biospecies. Investigation of the selective detection of Cys in the presence of other analytes thus is essentially necessary. When only 2.5 mM of Cys exists in the medium (control sample), the I/I_0-1 value is 16, and the emission blueshift is about 78 nm. By measuring the FL spectra of mixtures containing Cys (2.5 mM) and other analytes (2.5 mM), it was found that with the exception of Hcy, all other analytes exerted little impact on the detection of Cys in FL spectroscopic titration method (shown in Figure 5 and the Supporting Information S19 and S20); because of the addition of the other species (2.5 mM), the solubility of the probe and the resultant is decreased. Therefore, some amino acids that co-existed with Cys showed higher FL enhancements than simple Cys. Whereas, in the presence of Hcy, the I/I_0-1 value is 1.4, which is evidently lower than that of the control one. However, the blueshift of the emission peak is still the same as other species. The interference response can be associated with the competing reaction of the intermediate with the free thiol group of Hcy and Cys as shown in Scheme S3 (see the Supporting Information). The addition of thiol to the Shiff base of the intermediate led to soluble resultants (without aggregates) and thus decreased the emission. These intermediates and the final adducts themselves, on one hand, are soluble in the reaction solution, but on the other hand, they interrupt the production of insoluble resultants (aggregates). Thus the aggregation-induced emission can hardly been observed.

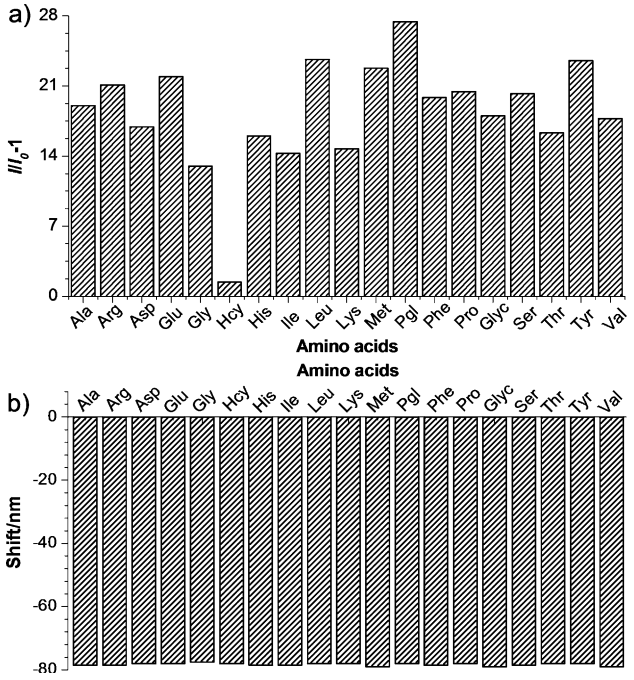


Figure 5. FL response of TPE-ALD to the mixture of Cys in the presence of various analytes (Ala, Arg, Asp, Glu, Gly, Hcy, His, Ile, Leu, Lys, Met, Pgl, Phe, Pro, Glyc, Ser, Thr, Tyr, Val) in the defined buffer, [TPE-ALD] = 25 μM, [analyte] = 2.5 mM, λ_{ex} = 343 nm. All measurements were conducted at RT after incubation for 60 min.

When the silole core was replaced by a TPE moiety, the detection behaviors of TPE-ALD are generally the same as DMBFDPS and DMTPS-ALD. Moreover, TPE-ALD excelled relative to the other two probes with a better performance as faster response, higher FL enhancement and sensitivity, and better specificity and selectivity. This is attributed to the most hydrophobic molecule structure of TPE-ALD among all the three dialdehyde-functionalized AIE fluorophores, which results in the poorest solubility of the resultant products and further triggers the most significant FL "turn-on" effect (precipitation- or aggregation-induced emission).

Conclusion

In contrast to the reported principles of the fluorescent probes such as intramolecular charge transfer, proton transfer, FL resonance energy transfer, and changes in FL life time,[13] the present work takes advantage of the FL responses originated from the aggregate formation of the reactive fluorogens. Specifically, we have shown a proof-of-concept study by using AIE-active fluorogens (DMTPS-ALD and TPE-ALD) as a probe to detect Cys and Hcy. The action principles for the present probes are consistent to be the precipitates (aggregation)-induced emission. The formation of thiazinane/thiazolidine by reaction of aldehyde groups on the probe molecule and the residue of Cys/Hcy determines the selective recognition of Cys and Hcy over other amino acids and glucose. The discriminatory detection of Cys over Hcy is kinetically dependent. All the detections were carried out in a biocompatible medium (10 mM HEPES buffer and DMSO, pH 7.4), which is propitious to practical application. What merits our attention is that TPE-ALD outshined the other two probes in performance with fast response, considerably high FL enhancement up to 16-fold, high sensitivity, and fairly good specificity and selectivity. Moreover, its response point at 250 μM meets coincidently with the normal level of Cys in human plasma, which gives a clue that TPE-ALD could be applied as a potential indicator of Cys deficiency. Combining all the investigations, we could come to the conclusion that the capability of discriminatorily detecting Cys over Hcy is a common attribute to all the dialdehyde-substituted AIE-active molecules and the response performance could be modulated by the molecular structures and the hydrophobicity.

Experimental Section

Chemicals and materials: Dimethyl sulfoxide (DMSO) is chromatographically pure purchased from Alfa Aesar. HEPES, alanine (Ala), arginine (Arg), aspartic acid (Asp), cysteine (Cys), glutamic acid (Glu), glycine

(Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), serine (Ser), threonine (Thr), tyrosine (Tyr), valine (Val), proline (Pro), homocysteine (Hcy), phenylglycine (Pgl) and glucose (Glyc) were purchased from Aldrich Chemical Co. All other chemicals and regents were commercially available and used as received without further purification.

General characterization: FTIR spectra were recorded on a Bruker Vector 22 spectrometer. The mass spectrum was carried out by an Esquire3000plus with an ion source type of ESI. UV/Vis absorption spectra were measured on a Varian CARY 100 Bio UV/Visible spectrophotometer. FL spectra were recorded on a Perkin–Elmer LS 55 spectrofluorometer.

Preparation of the nanoaggregates: Stock solution of DMTPS-ALD or TPE-ALD in DMSO with a concentration of 0.25 mM was prepared respectively. Aliquots (1 mL) of the stock solutions were transferred to 10 mL volumetric flasks. After adding appropriate amounts of DMSO, HEPES buffer solution (10 mM, pH 7.4) was added dropwise under vigorous stirring to furnish solutions (25 μM) with defined fractions of buffer (f_b = 0–80 or 0–90 vol%). Spectral measurements of the resultant solutions or aggregate suspensions were performed immediately.

Detection of Cys in DMSO/10 mM HEPES (6/4 and 5/5 in volume, pH 7.4): The appropriate amount of Cys was dissolved in HEPES (10 mM, 4 mL) and then chromatographically pure DMSO (5 mL) was added into the mixture, at last DMSO solution (1 mL) of DMTPS-ALD (0.25 mM) was added under vigorous stirring to afford the detection system with 25 μM of probe. The mixture was stood at RT for 60 min and underwent the necessary measurement. Similarly, the appropriate amount of Cys was dissolved in 10 mM HEPES (5 mL) and then chromatographically pure DMSO (4 mL) was added into the mixture, at last DMSO solution (1 mL) of TPE-ALD (0.25 mM) was added under vigorous stirring to afford the detection system with 25 μM of probe. The mixture was stood at RT for 60 min and underwent the necessary measurement.

Acknowledgements

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[1] a) Y. Hong, J. W. Y. Lam, B. Z. Tang, *Chem. Commun.* **2009**, 4332–4353; b) M. Wang, G. Zhang, D. Zhang, D. Zhu, B. Z. Tang, *J. Mater. Chem.* **2010**, *20*, 1858–1867; c) Y. Hong, J. W. Y. Lam, B. Z. Tang, *Chem. Soc. Rev.* **2011**, *40*, 5361–5388.

[2] a) J. Luo, Z. Xie, J. W. Y. Lam, L. Cheng, H. Chen, C. Qiu, H. S. Kwok, X. Zhan, Y. Liu, D. Zhu, B. Z. Tang, *Chem. Commun.* **2001**, 1740–1741; b) Y. Q. Dong, J. W. Y. Lam, Z. Li, H. Tong, Y. P. Dong, X. D. Feng, B. Z. Tang, *J. Inorg. Organomet. Polym. Mater.* **2005**, *15*, 287–291.

[3] a) J. Chen, C. C. W. Law, J. W. Y. Lam, Y. Dong, S. M. F. Lo, I. D. Williams, D. B. Zhu, B. Z. Tang, *Chem. Mater.* **2003**, *15*, 1535–1546; b) J. H. Lee, Q. D. Liu, D. R. Bai, Y. J. Kang, Y. Tao, S. N. Wang, *Organometallics* **2004**, *23*, 6205–6213; c) A. J. Boydston, B. L. Pagenkopf, *Angew. Chem.* **2004**, *116*, 6496–6498; *Angew. Chem. Int. Ed.* **2004**, *43*, 6336–6338; d) L. Aubouy, P. Gerbier, N. Huby, G. Wantz, L. Vignau, L. Hirsch, J. M. Janot, *New J. Chem.* **2004**, *28*, 1086–1090; e) Y. Ren, Y. Dong, J. W. Y. Lam, B. Z. Tang, K. S. Wong, *Chem. Phys. Lett.* **2005**, *402*, 468–473; f) B. Mi, Y. Dong, Z. Li, J. W. Y. Lam, M. Häußler, H. H. Y. Sung, H. S. Kwok, Y. Dong, I. D. Williams, Y. Liu, Y. Luo, Z. Shuai, D. Zhu, B. Z. Tang, *Chem. Commun.* **2005**, 3583–3585; g) L. Peng, M. Wang, G. Zhang, D. Zhang, D. Zhu, *Org. Lett.* **2009**, *11*, 1943–1946; h) Z. Zhang, B. Xu, J. Su, L. Shen, Y. Xie, H. Tian, *Angew. Chem.* **2011**, *123*, 11858–11861; *Angew. Chem. Int. Ed.* **2011**, *50*, 11654–11657; i) J. Wang, J. Mei, R. Hu, J. Z. Sun, A. Qin, B. Z. Tang, *J. Am. Chem. Soc.* **2012**, *134*, 9956–9966.

[4] a) M. Wang, D. Zhang, G. Zhang, Y. Tang, S. Wang, D. Zhu, *Anal. Chem.* **2008**, *80*, 6443–6448; b) M. Wang, X. Gu, G. Zhang, D. Zhang, D. Zhu, *Anal. Chem.* **2009**, *81*, 4444–4449; c) Y. Liu, C. Deng, L. Tang, A. Qin, R. Hu, J. Z. Sun, B. Z. Tang, *J. Am. Chem. Soc.* **2011**, *133*, 660–663; d) Y. Yu, C. Feng, Y. Hong, J. Liu, S. Chen, K. M. Ng, K. Q. Luo, B. Z. Tang, *Adv. Mater.* **2011**, *23*, 3298–3302; e) Y. Yu, J. Liu, Z. Zhao, K. M. Ng, K. Q. Luo, B. Z. Tang, *Chem. Commun.* **2012**, *48*, 6360–6362; f) W. Qin, D. Ding, J. Liu, W. Z. Yuan, Y. Hu, B. Liu, B. Z. Tang, *Adv. Funct. Mater.* **2012**, *22*, 771–779; g) Q. Zhao, K. Li, S. Chen, A. Qin, D. Ding, S. Zhang, Y. Liu, B. Liu, J. Z. Sun, B. Z. Tang, *J. Mater. Chem.* **2012**, *22*, 15128–15135; h) H. Shi, J. Liu, J. Geng, B. Z. Tang, B. Liu, *J. Am. Chem. Soc.* **2012**, *134*, 9569–9572.

[5] a) H. Refsum, P. M. Ueland, O. Nygård, S. E. Vollset, *Annu. Rev. Med.* **1998**, *49*, 31–62; b) S. Shahrokhian, *Anal. Chem.* **2001**, *73*, 5972–5978; c) S. Seshadri, A. Beiser, J. Selhub, P. F. Jacques, I. H. Rosenberg, R. B. D'Agostino, P. W. F. Wilson, *New Engl. J. Med.* **2002**, *346*, 476–483; d) K. G. Reddie, K. S. Carroll, *Curr. Opin. Chem. Biol.* **2008**, *12*, 746–754; e) E. Weerapana, C. Wang, G. M. Simon, F. Richter, S. Khare, M. B. D. Dillon, D. A. Bachovchin, K. Mowen, D. Baker, B. F. Cravatt, *Nature* **2010**, *468*, 790–795.

[6] a) A. R. Ivanov, I. V. Nazimov, L. Baratova, *J. Chromatogr. A* **2000**, *895*, 157–166; b) K. Amarnath, V. Amarnath, K. Amarnath, H. L. Valentine, W. M. Valentine, *Talanta* **2003**, *60*, 1229–1238; c) O. Nekrassova, N. S. Lawrence, R. G. Compton, *Talanta* **2003**, *60*, 1085–1095; d) W. Chen, Y. Zhao, T. Seefeldt, X. M. Guan, *J. Pharm. Biomed. Anal.* **2008**, *48*, 1375–1380; e) X. F. Guo, H. Wang, Y. H. Guo, Z. X. Zhang, H. S. Zhang, *J. Chromatogr. A* **2009**, *1216*, 3874–3880.

[7] M. J. MacCoss, N. K. Fukagawa, D. E. Matthews, *Anal. Chem.* **1999**, *71*, 4527–4533.

[8] A. Zinellu, S. Sotgia, B. Scanu, M. F. Usai, A. G. Fois, V. Spada, A. Deledda, L. Deiana, P. Pirina, C. Carru, *Amino Acids* **2009**, *37*, 395–400.

[9] a) H. Jiang, H. X. Ju, *Anal. Chem.* **2007**, *79*, 6690–6696; b) W. Wang, L. Li, S. F. Liu, C. P. Ma, S. S. Zhang, *J. Am. Chem. Soc.* **2008**, *130*, 10846–10847; c) M. Wen, H. Q. Liu, F. Zhang, Y. Z. Zhu, D. Liu, Y. Tian, Q. S. Wu, *Chem. Commun.* **2009**, 4530–4532; d) A. A. Ensafi, S. Dadkhah-Tehrani, H. karimi-Maleh, *Analyti. Sci.* **2011**, *27*, 409–414.

[10] P. K. Sudeep, S. T. S. Joseph, K. G. Thomas, *J. Am. Chem. Soc.* **2005**, *127*, 6516–6517.

[11] a) W. A. Zhao, M. A. Brook, Y. F. Li, *Chembiochem* **2008**, *9*, 2363–2371; b) R. Gill, M. Zayats, I. Willner, *Angew. Chem.* **2008**, *120*, 7714–7736; *Angew. Chem. Int. Ed.* **2008**, *47*, 7602–7625; c) J.-S. Lee, P. A. Ulmann, M. S. Han, C. A. Mirkin, *Nano Lett.* **2008**, *8*, 529–533; d) W. Zhong, *Anal. Bioanal. Chem.* **2009**, *394*, 47–59; e) S. Huang, Q. Xiao, R. Li, H.-L. Guan, J. Liu, X.-R. Liu, Z.-K. He, Y. Liu, *Anal. Chim. Acta* **2009**, *645*, 73–78.

[12] a) W. Wang, J. O. Escobedo, C. M. Lawrence, R. M. Strongin, *J. Am. Chem. Soc.* **2004**, *126*, 3400–3401; b) J. V. Ros-Lis, B. García, D. Jiménez, R. Martínez-Máñez, F. Sancenón, J. Soto, F. Gonzalvo, M. C. Valldecabres, *J. Am. Chem. Soc.* **2004**, *126*, 4064–4065; c) X. Chen, Y. Zhou, X. Peng, J. Yoon, *Chem. Soc. Rev.* **2010**, *39*, 2120–2135; d) W. Wei, X. Liang, G. Hu, Y. Guo, S. Shao, *Tetrahedron Lett.* **2011**, *52*, 1422–1425; e) Z. Yan, S. Guang, H. Xu, X. Liu, *Analyst* **2011**, *136*, 1916–1921; f) N. Y. Kwon, D. Kim, G. Jang, J. H. Lee, J. H. So, C. H. Kim, T. H. Kim, T. S. Lee, *ACS Appl. Mater. Interfaces* **2012**, *4*, 1429–1433; g) H. S. Jung, J. H. Han, T. Pradhan, S. Kim, S. W. Lee, J. L. Sessler, T. W. Kim, C. Kang, J. S. Kim, *Biomaterials* **2012**, *33*, 945–953.

[13] a) F. Tanaka, N. Mase, C. F. Barbas, *Chem. Commun.* **2004**, 1762–1763; b) O. Rusin, N. N. St. Luce, R. A. Agbaria, J. O. Escobedo, S. Jiang, I. M. Warner, F. B. Dawan, K. Lian, R. M. Strongin, *J. Am. Chem. Soc.* **2004**, *126*, 438–439; c) K.-S. Lee, T.-K. Kim, J. H. Lee, H.-J. Kim, J.-I. Hong, *Chem. Commun.* **2008**, 6173–6175; d) S. Sree-

jith, K. P. Divya, A. Ajayaghosh, *Angew. Chem.* **2008**, *120*, 8001–8005; *Angew. Chem. Int. Ed.* **2008**, *47*, 7883–7887; e) Y.-B. Ruan, A.-F. Li, J.-S. Zhao, J.-S. Shen, Y.-B. Jiang, *Chem. Commun.* **2010**, *46*, 4938–4940; f) Y.-K. Yang, S. Shim, J. Tae, *Chem. Commun.* **2010**, *46*, 7766–7768; g) W. Hao, A. McBride, S. McBride, J. P. Gao, Z. Y. Wang, *J. Mater. Chem.* **2011**, *21*, 1040–1048; h) Z. Yao, H. Bai, C. Li, G. Shi, *Chem. Commun.* **2011**, *47*, 7431–7433; i) X. D. Zeng, X. L. Zhang, B. C. Zhu, H. Y. Jia, Y. M. Li, *Dyes Pigm.* **2012**, *94*, 10–15; j) Z. Yang, N. Zhao, Y. Sun, F. Miao, Y. Liu, X. Liu, Y. Zhang, W. Ai, G. Song, X. Shen, X. Yu, J. Z. Sun, W.-Y. Wong, *Chem. Commun.* **2012**, *48*, 3442–3444; k) S. Vallejos, P. Estevez, S. Ibeas, F. C. Garcia, F. Serna, J. M. Garcia, *Sensors* **2012**, *12*, 2969–2982; l) J. Y. Shao, H. Y. Sun, H. M. Guo, S. M. Ji, J. Z. Zhao, W. T. Wu, X. L. Yuan, C. L. Zhang, T. D. James, *Chem. Sci.* **2012**, *3*, 1049–1061.

[14] a) X. Yang, Y. Guo, R. M. Strongin, *Angew. Chem.* **2011**, *123*, 10878–10881; *Angew. Chem. Int. Ed.* **2011**, *50*, 10690–10693; b) C. M. Yu, F. Zeng, M. Luo, S. Z. Wu, *Nanotechnology* **2012**, *23*, 305503.

[15] a) J. Mei, J. Wang, A. Qin, H. Zhao, W. Z. Yuan, Z. Zhao, H. H. Y. Sung, C. Deng, S. Zhang, I. D. Williams, J. Z. Sun, B. Z. Tang, *J. Mater. Chem.* **2012**, *22*, 4290–4298; b) J. Mei, J. Wang, J. Z. Sun, H. Zhao, W. Z. Yuan, C. Deng, S. Chen, H. H. Y. Sung, P. Lu, A. Qin, H. S. Kwok, Y. Ma, I. D. Williams, B. Z. Tang, *Chem. Sci.* **2012**, *3*, 549–558; c) J. Mei, J. Tong, J. Wang, A. Qin, J. Z. Sun, B. Z. Tang, *J. Mater. Chem.* **2012**, *22*, 17063–17070.

[16] R. G. Kallen, *J. Am. Chem. Soc.* **1971**, *93*, 6236–6248.

[17] R. Hu, J. L. Maldonado, M. Rodriguez, C. Deng, C. K. W. Jim, J. W. Y. Lam, M. M. F. Yuen, G. Ramos-Ortiz, B. Z. Tang, *J. Mater. Chem.* **2012**, *22*, 232–240.

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