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Thiophenol detection using an AIE fluorescent probe through self-assembly with TPE-based glycoclusters

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We describe a novel green-emitting tetraphenylethylene-dicyanomethylene-4*H*-pyran (TPE-DCM) based fluorescent probe (**TD-1**). Conjugating TPE and DCM moieties allowed **TD-1** to display high selectivity for thiophenol with excellent AIE properties in aqueous solution. Nevertheless, the poor water-solubility from the hydrophobic structure resulted in the weak and unstable emission intensity. Non-covalent self-assembly of **TD-1** with TPE glycocluster (TPE2S) led to a largely improved water solubility producing a reliable and stable sensing system. The corresponding glyco-probe could detect sensitively exogenous thiophenol concentrations in PBS buffer or environmental water samples.

Introduction

Thiophenol (PhSH), as an important organosulfur compound, is the simplest aromatic thiol and essential starting materials extensively applied in organic synthesis of agrochemicals, pharmaceuticals and dyes.¹⁻³ Different from aliphatic thiols including cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), which play important roles in biological systems,⁴ thiophenol and its derivatives are pollutants with high toxicity for the environment. The toxicity of thiophenol is high in fish and mouse with a median lethal dose (LC50) from 0.01 to 0.04 mM or as low as 46.2 mg/kg.^{5, 6} Long-term exposure to thiophenol liquid or vapor in water or soil is detrimental to human health by triggering series of severe systemic injuries, such as damage to the central nervous system, muscle weakness and even death.⁷ Therefore, a convenient, rapid, accurate, sensitive and selective method is necessary for the detection of thiophenol pollution in the environment.

Compared with traditional methods such as HPLC, UV-vis and chromatography-mass analysis,^{8,9} fluorescence spectroscopy is widely applied for thiophenol detection due to its high sensitivity, simple processing and low detection limits. A reactive moiety is typically introduced to a fluorophore scaffold to quench its fluorescence, and then a reaction with thiophenol triggers a cascade that would release the moiety enhancing fluorescence. 2,4-Dinitrobenzene was extensively used as the

reactive group since when this group is conjugated through sulfonyl ether,^{4, 10-12} sulphonamide¹³⁻¹⁶ and sulfonate¹⁷⁻¹⁹ bonds to a fluorophore could lead to the production of fluorogenic probes for thiophenol selectively over other aliphatic thiols.²⁰ The electron-deficient 2,4-dinitrobenzene suppresses the fluorescence emission of organic dyes by the intramolecular charges transfer (ICT)^{21, 22} or photo-induced electronic transfer (PET) mechanism.^{13, 18, 23} Recently, fluorescent probes for thiophenol detection have been developed with high sensitivity, selectivity and short response time. For example, a squaraine-based NIR probe for the detection of thiophenol through colorimetric and “off-on” fluorometric response with high water-solubility was developed through conjugation with glucose.¹⁹ A dicyanomethylene-4*H*-pyran (DCM) based probe with a large Stokes shift (159 nm) and low detection limit (8.3 nM) was also constructed.²⁴ However, most fluorescent probes exhibited low reactivity and fluorescence intensity due to their poor water-solubility and the notorious ACQ (aggregation-induced quenching) effect.

Aggregated induced emission (AIE) is widely used in organic fluorescent probe design to overcome ACQ in aqueous solution²⁵⁻²⁷ based on the pioneering studies of Prof Ben Zhong Tang who coined the concept of AIE in 2001.²⁸ Tetraphenylethene (TPE) has attracted much attention due to its readily available structural modification and functionalization as well as its AIE properties and photostability.²⁹⁻³¹ However, the wavelength of the blue emission largely overlaps the auto-luminescence of biomolecules. As a consequence, fluorophores with a longer emission wavelength such as dicyanomethylene-4*H*-pyran (DCM) have been applied for the design of fluorescent probes.³²⁻³⁴

We described herein the conjugation through a C=C double bond between the DCM and TPE fluorophores to obtain a novel TPE-DCM fluorophore with yellow fluorescence emission as well as AIE properties for the detection of thiophenol. After functionalizing the electron-poor 2,4-dinitrobenzene on the fluorophore, restricted AIE effect and blocked ICT effect were

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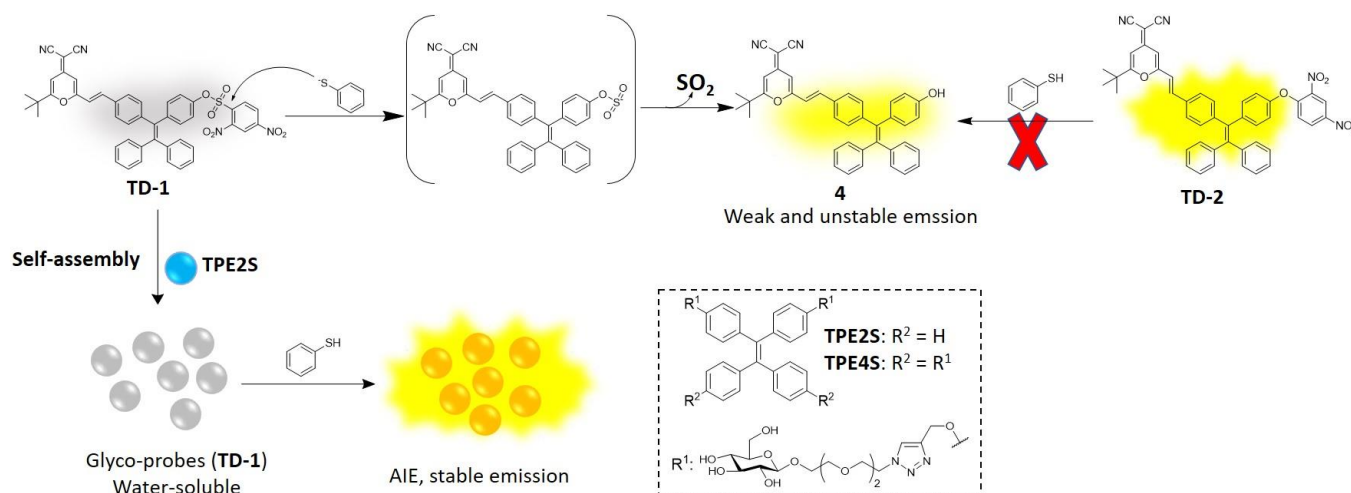
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observed to “turn-off” the fluorescence in aqueous solution. However, the poor water-solubility from the hydrophobic structure of **TD-1** led to weak and unstable fluorescence emission in aqueous solution, due to precipitation of probes from the solution. Tetraphenylethene (TPE) based glycoclusters (TPE2S) have an outstanding ability to improve the water-

solubility, fluorescence stability and sensitivity of **TD-1** for thiophenol detection after self-assembly (Scheme 1). Therefore, the self-assembled glyco-probe was explored to quantify the concentration of thiophenol in real water samples (Rhône River and Saône River, Lyon).



Scheme 1 TPE-DCM based fluorescent probe (**TD-1**) and its glycol-probes self-assembled with TPE-based glycoclusters (TPE2S) for thiophenol (PhSH) detection.

Results and discussion

Chemical synthesis

The synthesis procedures of novel TPE-DCM based fluorescent probe and TPE-based glycoclusters were shown (Scheme S1, ESI[†]). The synthesis of the conjugate started from the known TPE-aldehyde **1**³⁵ and DCM analogue **2**³⁶ through an aldolisation/crotonization sequence to afford TPE-DCM **3**. Demethylation of the phenol group with BBr₃ produced the fluorescent probe **4**. 2,4-Dinitrobenzene was functionalized on the phenol **4** to obtain two fluorescent probes **TD-1** and **TD-2** with different molecular architectures. TPE-based glycoclusters functionalized by two monosaccharides (TPE2S) was synthesized from dipropargyl TPE compound **S5**. Through CuAAC cycloaddition, acetyl protected azide-glucose were conjugated on the TPE core. Then TPE-based glycoclusters **5** was deprotected in aqueous MeOH solution with Et₃N to obtain the glycocluster TPE2S. TPE-based glycoclusters (TPE4S) was synthesized similarly according to the literature.³⁷

Response mechanism

The performance of two probes for thiophenol detection was evaluated. The fluorescence of **TD-2** remained unquenched and displayed only a limited fluorescence emission decrease after incubation with thiophenol (Fig. S1, ESI[†]). In contrast, **TD-1** displayed a much weaker initial fluorescence emission ($\Phi = 0.003$), and an obvious fluorescence enhancement at 570 nm ($\Phi = 0.083$, $\lambda_{\text{ex}} = 480$ nm) was observed probably through recovering of the ICT after reaction with thiophenol, along with a slight red-shift of the absorption peak (Fig. S1, ESI[†]). The mechanism of fluorescence enhancement was proposed as

follows: the negative thiophenol (PhS⁻) ion was nucleophilic enough to add to the highly electrophilic *ipso*-carbon atom of the 2,4-dinitrophenyl group. Cleavage of the S-O bond and release of SO₂ generated the phenol derivative **4** (Scheme 1). Mass spectrometry analysis indicated that **TD-1** after responding to thiophenol generated phenol derivative **4** (m/z 573.3). On the opposite, incubation of **TD-2** with thiophenol did not produce phenol **4** and hence this compound was not further studied herein (Fig. S2a, ESI[†]). To further prove 2,4-dinitrobenzene was the leaving group, HPLC analysis was performed to identify **TD-1**, **4** and thiophenol. After incubating a sub-stoichiometric amount of thiophenol with the probe, the corresponding peaks of **TD-1** and **4** were observed. Then **TD-1** was totally converted into compound **4** when using excessive thiophenol (Fig. S2b, ESI[†]).

Spectral properties of TD-1

The sensing performances of **TD-1** (10 μM) for thiophenol in a phosphate buffered saline (PBS) solution (10 mM with 10% THF) were measured. The fluorescence enhancement of **TD-1** was measured after addition of an excess of thiophenol at different pH. We found that the fluorescence intensity kept increasing at pH 3-7 and reached equilibrium above pH 7, which suggests that the thiophenate (PhS⁻) required at higher pH values to activate fluorescence. As a result, the probe **TD-1** is capable of sensing thiophenol in neutral or basic solutions (Fig. 1a).

Then, we measured the AIE properties of **TD-1** after reaction with thiophenol (50 μM) in PBS solution (pH 7.3) with different ratio of THF. The stronger emission of **TD-1** in 50% PBS solution indicated that 2,4-dinitrobenzene could not quench the fluorescence completely probably due to the electron rich moieties on the TPE moiety. The weakening of fluorescence

intensity for **TD-1** upon addition of PBS into THF was caused by the ACQ effect. We speculate that the bulky 2,4-dinitrobenzene group restricted the rotation of TPE-DCM, hence blocking the AIE effect. Thereby, the fluorescence of **TD-1** was switched-off by ICT effect and ACQ effect in aqueous buffer. The fluorescence emission was then largely enhanced by AIE effect after incubating with thiophenol (leading to fluorophore **4**) when increasing PBS ratio from 50% to 80% (Fig. 1b). The phenol **4** recovered the AIE properties hence displayed enhanced emission intensity. Further addition of PBS buffer led to a decrease of fluorescence emission, suggesting an insufficient water-solubility of the probe to function in a pure aqueous medium. We supposed that aggregation of fluorophore **4** resulted in precipitation and a strong decrease of soluble material leading to a decreased signal.

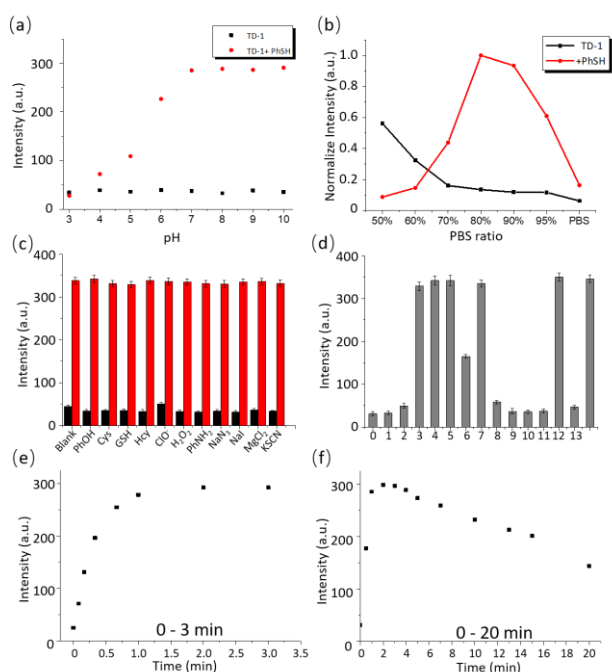


Figure 1 (a) Fluorescence variations of **TD-1** (10 μ M) before and after responding to thiophenol (100 μ M) during 3 min in PBS buffer (pH 7.3) with 10% THF (pH was adjusted with HCl 0.6M or NaOH 2M). (b) ACQ and AIE effect influenced **TD-1** response to thiophenol (100 μ M) with addition of PBS buffer (pH 7.3) in THF during 10 min. (c) The fluorescence variation of **TD-1** (10 μ M) response to potential interfering species (50 μ M) without (black bar) or with (red bar) thiophenol (100 μ M) during 3 min. (d) **TD-1** (10 μ M) response to aliphatic and aromatic thiols (100 μ M) during 3 min, Reagents: (0) Blank, (1) 1,2-ethanedithiol, (2) cyclohexanethiol, (3) *m*-NH₂C₆H₄SH, (4) *p*-NH₂C₆H₄SH, (5) *p*-MeOC₆H₄SH, (6) *p*-NO₂C₆H₄SH, (7) *p*-MeC₆H₄SH, (8) 2-methyl-2-propanethiol, (9) pyrimidinethione, (10) α -thioglycerol, (11) 2-mercaptobenzo-thiazole, (12) 4,4'-thiobisbenzenethiol, (13) 2-aminoethanethiol hydrochloride, (14) PhSH. (e) Fluorescence intensity increase of **TD-1** (10 μ M) incubating with thiophenol (100 μ M) during 3 min. (f) Fluorescence intensity decrease of **TD-1** (10 μ M) incubating with thiophenol (100 μ M) after 3-20 min due to probe separation from solution.

To test the selectivity of **TD-1** toward thiophenol and the possible interference from other analytes, the probe was incubated with a number of potential interfering species, aliphatic thiols and thiophenol derivatives. The fluorescence signal increased only in the presence of aromatic thiols (Fig. 1d). Other species were shown to not react with the probe and did not

interfere with the sensing system when co-incubated with thiophenol (Fig. 1c).

The time-dependent fluorescence enhancement was measured by incubating thiophenol with **TD-1** in PBS buffer. An incubation of excessive analyte was required to ensure that all **TD-1** probes are transformed into fluorophore **4**. After addition of thiophenol, the fluorescence intensity displayed a remarkable enhancement to maximum in 2-3 min (Fig. 1e). However, the fluorescence intensity was not stable and decreased slowly when stirring the solution for more than 3 min (Fig. 1f). We hypothesized that the poor water-solubility of compound **4** would lead to aggregated species that would separate from the solution, hence decreasing the fluorescence signal intensity. In our previous studies, TPE-based glycoclusters have excellent water solubility and similar structure as **TD-1**. For improving the water-solubility of **TD-1**, we self-assembled TPE-based glycoclusters³⁷ (TPE2S and TPE4S) and **TD-1** to construct a glyco-probe, which was used to detect thiophenol (Scheme 1). We speculated that a better aqueous dispersibility could stabilize the fluorescence emission signal, allowing us to decrease the ratio of THF in PBS buffer.

Spectral properties of glycol-probe

To improve the water-solubility and fluorescence stability of **TD-1**, we self-assembled TPE2S with **TD-1** and explored the fluorescence changes after incubating with thiophenol. The results indicated that TPE2S could enhance the fluorescence intensity probably due to an improvement of water solubility (Fig. 2a). Therefore, we compared the fluorescence enhancement efficiency of the two TPE-based glycoclusters (TPE2S and TPE4S) after self-assembly with **TD-1** (Fig. 2b). The TPE2S assembly lead to a higher fluorescence intensity than that of the TPE4S assembly with probe **TD-1**, enabling thiophenol sensing in PBS aqueous buffer without the addition of THF. As a result, the TPE2S assembly was used for further investigation. To prove the assembly process, the molecular sizes of TPE2S, **TD-1** and glyco-probe were measured by dynamic light scattering (DLS, Fig. 2c). TPE2S (blue) and **TD-1** (green) in aggregated state displayed a large diameter (200-300 nm), which indicated that two molecules were in aggregated state. The size of the assembled glyco-probe (red) was smaller than TPE2S and similar to the size of **TD-1**. We supposed that the molecules of **TD-1** were dispersed and inserted in the hydrophobic core of glyco-ball conjugating from several TPE2S, therefore the size of glyco-probe was similar to the aggregated **TD-1**. The optimal ratio between TPE2S and **TD-1** was also investigated. A large excess of TPE2S was necessitated for achieving an optimal response to thiophenol with a 1:100 molar ratio of **TD-1** to TPE2S (Fig. S7, ESI[†]).

Next, we investigated several basic fluorescence properties of the resulting glyco-probes for thiophenol detection. Compared with the AIE properties of **TD-1**, the glyco-probe displayed a better fluorescence increase with a varying PBS percentage of 50%-90% in THF. Importantly, the glycol-probe also displayed a satisfying emission intensity in complete PBS buffer (Fig. 2d). This result suggests that the glyco-probe with excellent water-solubility avoided the excessive intermolecular aggregation of

compound **4**, and hence was more suitable for thiophenol sensing in aqueous solution. Then, we compared the rate of reactivity of glyco-probe for thiophenol in PBS buffer (Fig. 2e) or a 95% PBS buffer with 5% THF (Fig. 2f). The glyco-probe displayed a rapidly increased fluorescence signal after incubating with thiophenol, and a stable fluorescence intensity when the probe responded completely over time. These results indicated that conjugation of TPE2S with **TD-1** improved the water-solubility to prevent the probe precipitation. The longer response time (30 min) in PBS buffer in comparison to the mixture of PBS/THF (95:5) can be rationalized by the better solubility of thiophenol in THF, leading to a faster reaction time of 5 min.

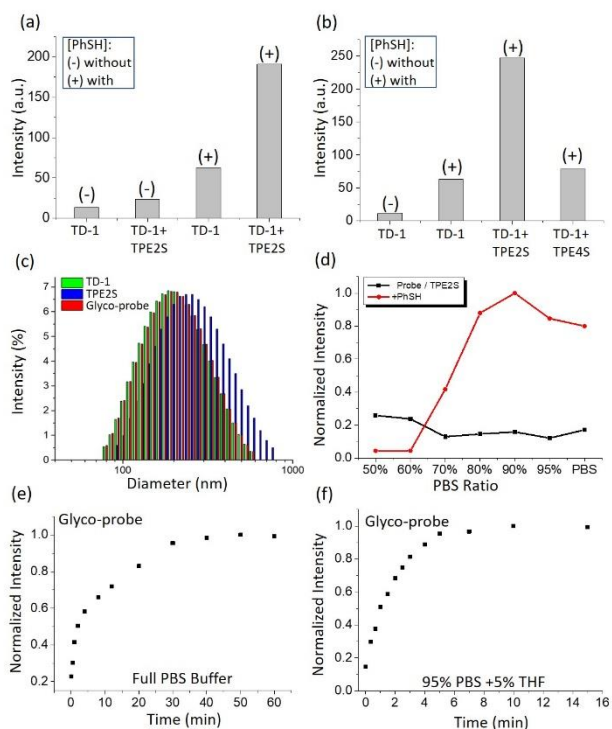


Figure 2 (a) Fluorescence changes of **TD-1** (10 μM) and glyco-probe after conjugation with TPE2S (10 mM) improved the fluorescence intensity for thiophenol (100 μM) detection in 95% PBS (pH 7.3 10 mM) with THF. (b) Fluorescence enhancement abilities of TPE2S and TPE4S (10 mM) conjugating **TD-1** (10 μM) and detect thiophenol (100 μM) in PBS buffer (pH 7.3, 10 mM). (c) DLS measurement of **TD-1**, TPE2S and glyco-probe. (d) AIE effect influenced the glyco-probe response to thiophenol (100 μM) with PBS buffer (pH 7.3, 10 mM) in THF during 5 min. (e) and (f) Response time of glyco-probe to thiophenol (100 μM) in PBS buffer or PBS buffer with 5% THF.

PhSH Quantification

To evaluate the sensitivity of the glyco-probe to thiophenol, the fluorescence signals at 560 nm of **TD-1** and glyco-probe were measured with addition of thiophenol in PBS/THF (95:5) solution. **TD-1** exhibited an unstable fluorescence intensity probably due to the poor water-solubility (Fig. 3a-b). In contrast, the glyco-probe showed a linear fluorescence enhancement with increasing thiophenol concentration (Fig. 3c). The linearity was verified from 0 to 14 μM thus demonstrating that the glyco-probe could be used to detect thiophenol quantitatively (Fig. 3d). After adding more than 14 μM thiophenol, the fluorescence intensity slowly reached a maximal emission intensity probably due to a complete conversion of the probes to compound **4**. The limit of

detection (LOD) was calculated to be as low as 9.4 nM, which suggests that the probe could be applied for the determination of trace thiophenol in aqueous solution.

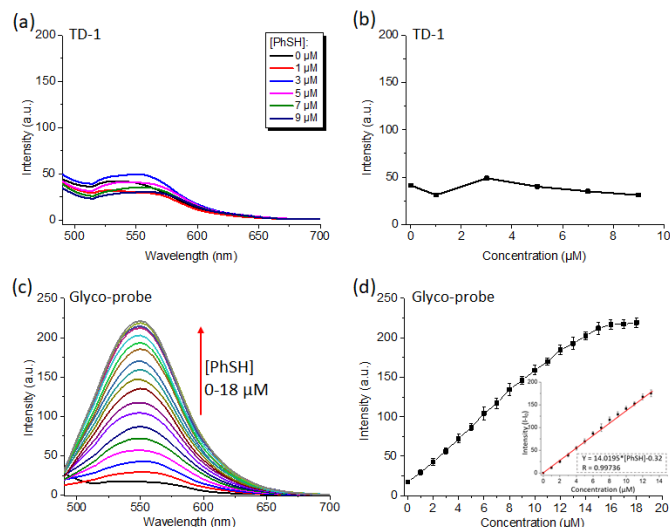


Figure 3 (a) and (b) Unstable fluorescence variation of **TD-1** (10 μM) response to thiophenol (0-9 μM). (c) Fluorescence enhancement and (d) linear relationship of glyco-probe response to thiophenol (0-18 μM) in 95% PBS buffer (pH 7.3, 10 mM) with THF during 5 min. ($\lambda_{\text{exc}} = 480 \text{ nm}$)

Next, the glyco-probe was evaluated to quantify thiophenol in two real water samples from Saône River and Rhône River (Lyon, France). The glyco-probe provided homogenous fluorescence enhancements upon addition of thiophenol (0-3 μM). The fluorescence increase ($I-I_0$) was similar to the results measured in PBS solution (Fig. S10, ESI†). We have also injected exogenously known concentrations of thiophenol (0-3 μM) into the real water samples (Table 1). The fluorescence signal recovery was always near 100% from the expected intensities through analysis by the titration curve. Therefore, the self-assembled glyco-probe between TPE2S and **TD-1** could be used for thiophenol quantification in real water samples.

Table 1 Analysis of Thiophenol Concentrations in Real Water Samples

Sample	PhSH spiked (μM)	PhSH recovered (μM)	Recovery (%)
Saône River water	0	Not detect	0
	1.00	0.97±0.09	97
	2.00	1.84±0.12	92
Rhône River water	0	Not detect	0
	1.00	1.05±0.06	105
	2.00	2.06±0.08	103
	3.00	3.07±0.14	102

Conditions: The glyco-probe self-assembled from **TD-1** (10 μM) and TPE2S (10 mM) responded to thiophenol in 95% water samples with THF during 5 min.

Conclusion

In a summary, detection of thiophenol is of interest as this toxic compound can pollute drinkable or recreative water. The design of a new fluorescent probe with a reactive 2,4-dinitrosulfonate

moiety was achieved by the conjugation between a TPE AIEgen and a DCM core. The resulting probe could react with thiophenol to release a phenol group on the TPE-DCM moiety, thus enhancing the fluorescence emission at 570 nm. Nevertheless, the poor water solubility of the resulting phenol did not allow stable measurements. We thus proposed a self-assembly strategy with water-soluble TPE-based glycoclusters, leading to a new glyco-probe which for the quantitative and selective detection of thiophenol in PBS solution as well as environmental water samples.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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