**Introduction**

Research on biosensors has been booming in recent years because of their wide applications in the area of healthcare and diagnosis. Among them, fluorescent biosensors have attracted much attention as they offer many outstanding advantages such as low background noise, high sensitivity and facile sample preparation. Monitoring of physiological protein concentrations or enzyme activities using fluorescent bioprobes is of clinical importance. For example, protamine, a highly positively charged polypeptide with an isoelectric point of 12–13, is widely used to reverse the anticoagulant activity of heparin after cardiovascular surgical procedures. Despite its universal use in clinical practice, protamine can induce adverse effects such as hypotension to idiosyncratic fatal cardiac arrest. On the other hand, alkaline phosphatase (ALP) is one of the most commonly assayed enzymes, which catalyses the hydrolysis of monophosphate and has broad substrate adaptability. ALP activity in blood serum is often regarded as a diagnostic indicator of several diseases such as osteoporosis, achondroplasia, cretinism, severe anemia, myelogenous leukemia and hepatic diseases. So far, a few methods have been established for protamine quantification and ALP activity assay. Umezawa and coworkers developed volumetric detection of protamine by using self-assembled monolayers of thioctic acid. Kim’s and Yu’s groups realized fluorescent biosensor for targeting protamine and ALP is essential and significant.

Fluorophores with aggregation-induced emission (AIE) characteristics have recently emerged as promising materials for biosensing and imaging applications. Unlike conventional fluorophores suffering from fluorescence quenching in high concentration owing to strong π–π interaction, AIE luminogens such as tetraphenylethene (TPE) and its derivatives enjoy high emission efficiency when their molecules are aggregated or bind to specific analytes. To date, a great number of AIE-based “turn-on” biosensors have been reported to target proteins, nucleic acids and glucose with superior sensitivity. To the best of our knowledge, most of these AIE bioprobes are monofunctional. It is more desirable to develop dual-mode or multifunctional bioprobes with advantages of material saving, less synthetic work, cost-effectiveness and the potential for multiplex monitoring. A novel dual-mode fluorescence “turn-on” probe is developed based on a phosphorylated tetraphenylethene (TPE) derivative bearing aggregation-induced emission (AIE) characteristics. The probe is weakly emissive in aqueous solution but its fluorescence is significantly enhanced in the presence of protamine or alkaline phosphatase (ALP). The cationic protamine interacted with the anionic phosphate group of the amphiphilic probe via electrostatic interaction and induced micelle formation. This micelle aggregates the hydrophobic TPE core and results in fluorescence enhancement. The detection limit for the protamine assay reached as low as 12 ng mL$^{-1}$. On the other hand, ALP hydrolysed the fluorescent probe and led to self-aggregation of insoluble fluorescent residues. The linear light-up response of the probe enables ALP quantification in the range of 10–200 mU mL$^{-1}$, which covers the physiological level of ALP activity in human serum. Moreover, the two activation modes could be differentiated by distinct responses to protamine and ALP.

A dual-mode fluorescence “turn-on” biosensor based on an aggregation-induced emission luminogen

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A novel dual-mode fluorescence “turn-on” probe is developed based on a phosphorylated tetraphenylethene (TPE) derivative bearing aggregation-induced emission (AIE) characteristics. The probe is weakly emissive in aqueous solution but its fluorescence is significantly enhanced in the presence of protamine or alkaline phosphatase (ALP). The cationic protamine interacted with the anionic phosphate group of the amphiphilic probe via electrostatic interaction and induced micelle formation. This micelle aggregates the hydrophobic TPE core and results in fluorescence enhancement. The detection limit for the protamine assay reached as low as 12 ng mL$^{-1}$. On the other hand, ALP hydrolysed the fluorescent probe and led to self-aggregation of insoluble fluorescent residues. The linear light-up response of the probe enables ALP quantification in the range of 10–200 mU mL$^{-1}$, which covers the physiological level of ALP activity in human serum. Moreover, the two activation modes could be differentiated by distinct responses to protamine and ALP.
In this work, a dual-mode fluorescence “turn-on” probe based on a phosphorylated TPE derivative (TPE-TEG-PA) has been developed for quantifying protamine concentration and ALP activities by two different activation modes (Scheme 1). With the aid of tetraethylene glycol (TEG) and phosphate (PA) groups, the probe is water-soluble and emits weakly in aqueous buffer. Owing to their amphiphilic feature, the probe molecules form micelles when interacting with protamine. The micelle aggregates the hydrophobic TPE core and results in fluorescence enhancement. On the other hand, the ALP can hydrolyse the phosphate group on the probe and release an insoluble TPE residue (TPE-TEG-OH) into the buffer solution resulting in strong emission. Through the two different pathways, the detection of protamine and ALP activity with high sensitivity and selectivity is achieved.

Experimental section

Materials
All chemicals and reagents were commercially available and used as received without further purification. Tetrahydrofuran (THF) and dichloromethane (DCM) were distilled from sodium benzophenone ketyl and calcium hydride respectively under nitrogen immediately prior to use. 4-Dimethylaminopyridine (DMAP), 4-bromobenzophenone, n-butyllithium, protamine sulphate and trypsin were purchased from Aldrich. p-Toluene sulfonic acid (PTSA) monohydrate was obtained from Meryer. Dicyclohexylcarbodiimide (DCC), tetraethylene glycol and triethylamine were purchased from Acros Organics. Phosphorus oxychloride was purchased from International Laboratory. Alkaline phosphatase (ALP) from calf intestine was purchased from Invitrogen. Buffer solutions (pH = 1–13) were purchased from Aldrich. All the other reagents used in selectivity tests were purchased from Aldrich.

Instrumentation
$^1$H, $^{13}$C and $^{31}$P NMR spectra were recorded on a Bruker ARX 400 NMR spectrometer using CDCl$_3$, methanol-$d_4$, or acetone-$d_6$ as a solvent and tetramethylsilane (TMS; $\delta = 0$ ppm) was chosen as an internal reference. UV absorption spectra were recorded on a Biochrom spectrophotometer. Photoluminescence (PL) spectra were recorded on a Perkin–Elmer LS 55 spectrophotometer. High-resolution mass spectra (HRMS) were obtained on a GCT Premier CAB 048 mass spectrometer operated in MALDI-TOF mode. Particle sizes and zeta potential of micelles were determined using a Zeta-Plus Potential Analyser. Morphologies of micelles were examined using a JEOL 100CX transmission electron microscope (TEM).

Synthesis

4-(1,2,2-Triphenylethenyl)benzoic acid (TPE-COOH). 1-(4-Bromophenyl)-1,2,2-triphenylethene (TPE-Br) was synthesized according to a previous report.$^{14}$ To a solution of TPE-Br (6 mmol, 2.466 g) in distilled THF (80 mL), 4.1 mL of n-butyllithium solution (1.6 M in cyclohexane) was added dropwise under N$_2$ at $-78^\circ$C. The reaction mixture was stirred and kept at $-78^\circ$C for two hours. Excess dry ice was added in one portion followed by stirring over six hours at room temperature. The resulting solution was acidified with an excess amount of 1 M hydrochloric acid and extracted with DCM several times. The organic layer was combined and dried using MgSO$_4$. After simple filtration, the organic solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography with DCM-ethyl acetate (10 : 1) as eluent to afford the desired product (2.0 g, 5.3 mmol) as a white solid. Yield = 88%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ (TMS, ppm) 7.85–7.83 (d, 2H), 7.15–7.10 (m, 11H), 7.05–7.00 (m, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ (TMS, ppm) 171.39, 149.15, 142.53, 142.41, 142.39, 142.37, 142.35, 142.33, 142.31, 142.29, 142.27, 142.25, 142.23, 142.21, 142.19, 142.17, 142.15, 142.13, 142.11, 142.09, 142.07, 142.05, 142.03, 142.01, 141.99, 141.97, 141.95, 141.93, 141.91, 141.89, 141.87, 141.85, 141.83, 141.81, 141.79, 141.77, 141.75, 141.73, 141.71, 141.69, 141.67, 141.65, 141.63, 141.61, 141.59, 141.57, 141.55, 141.53, 141.51, 141.49, 141.47, 141.45, 141.43, 141.41, 141.39, 141.37, 141.35, 141.33, 141.31, 141.29, 141.27, 141.25, 141.23, 141.21, 141.19, 141.17, 141.15, 141.13, 141.11, 141.09, 141.07, 141.05, 141.03, 141.01, 140.99, 140.97, 140.95, 140.93, 140.91, 140.89, 140.87, 140.85, 140.83, 140.81, 140.79, 140.77, 140.75, 140.73, 140.71, 140.69, 140.67, 140.65, 140.63, 140.61, 140.59, 140.57, 140.55, 140.53, 140.51, 140.49, 140.47, 140.45, 140.43, 140.41, 140.39, 140.37, 140.35, 140.33, 140.31, 140.29, 140.27, 140.25, 140.23, 140.21, 140.19, 140.17, 140.15, 140.13, 140.11, 140.09, 140.07, 140.05, 140.03, 140.01, 140. Derived data followed.

Scheme 1 Schematic illustration of sensing mechanisms of TPE-TEG-PA in protamine detection and ALP activity assay.
PL spectra were recorded after each addition of protamine immediately with an excitation wavelength of 330 nm. In the selectivity study, dextran, calf DNA, trypsin, cytochrome C, bovine serum albumin (BSA) and human serum albumin (HSA) with a concentration of 0.002 mg mL\(^{-1}\) in distilled water were examined under the same conditions.

**ALP activity assay and dynamic monitoring of enzymatic hydrolysis**

A 10 \(\mu\)M TPE-TEG-PA probe solution was prepared by mixing 20 \(\mu\)L of the stock solution (1 mM) with 1.98 mL of Tris–HCl buffer (10 mM, pH 9.6). Different amounts of ALP stock solutions (35 000 mU mL\(^{-1}\)) were added into the Tris–HCl buffer solution to yield final ALP from 0 to 200 mU mL\(^{-1}\). PL measurements were carried out after the prepared solutions were incubated at 25 °C for 30 min. The enzymatic hydrolysis process was monitored by the fluorescence spectral measurements which scanned at intervals of 90 s.

**Results and discussion**

**Design and synthesis of dual-mode biosensor**

To develop a dual-mode fluorescence “turn-on” biosensor, two modes of stimuli can activate the fluorescent response through different mechanisms respectively: one mode was for protamine detection and the other was for ALP activity assay. Protamine detection can be realized via electrostatic interaction between the anionic TPE derivative and cationic protamine. The design rationale is illustrated in Scheme 1. TPE-TEG-PA comprises a hydrophobic TPE core and hydrophilic TEG and PA groups. The synthesis route to TPE-TEG-PA is shown in Fig. 1. The amphiphilic feature makes the probe partially soluble in pure water or buffer solution. The probe shows weak fluorescence in the aqueous solution when the concentration is low. While increasing the concentration of TPE-TEG-PA to above its critical micelle concentration (CMC), the fluorescence is enhanced significantly due to the hydrophobic TPE core aggregated in the micelles. Similarly, when the concentration of TPE-TEG-PA is fixed below its CMC, a fluorescence enhancement could also be achieved through addition of protamine which induces the micelle formation through electrostatic interaction.

**Determination of critical micelle concentration**

A stock solution of TPE-TEG-PA (1 mM) was prepared by dissolving the gel-like solid (12.6 mg) in distilled water (20 mL) under oscillation. For a series of TPE-TEG-PA solutions with concentration gradients (0.1 to 400 \(\mu\)M), varying amounts of stock solution and distilled water were mixed with a final volume of 2 mL. PL spectra of all the samples were recorded at room temperature with an excitation wavelength of 330 nm. All samples were freshly prepared before each test.

**Fluorescence detection of protamine**

A 10 \(\mu\)M TPE-TEG-PA probe solution was prepared by mixing 20 \(\mu\)L of the stock solution (1 mM) with 1.98 mL of distilled water. Protamine solution (0.1 mg mL\(^{-1}\) in distilled water) was added intermittently to 2 mL of the probing solution and mixed well.
interaction. Because of the high density of positive charges in protamine, the formation of micelles between TPE-TEG-PA and protamine is expected. Thus, protamine detection can be realized by measuring the magnitude of fluorescence enhancement. Subsequently, the fluorescence of the protamine/probe micelles will be diminished if the micelles are degraded. On the other hand, the end group PA on the probe could be hydrolysed by ALP and converted to a hydroxyl group. Because TPE-TEG-OH has limited water solubility, the molecules would aggregate in aqueous solution to trigger the AIE response. Such fluorescence enhancement enables assay of the ALP activity with high sensitivity and selectivity.

**Fluorescence property and CMC determination**

The PL property of TPE-TEG-PA was investigated using THF and hexane solvent mixtures (Fig. 2). TPE-TEG-PA is almost non-emissive in pure THF solution. With increasing the hexane content ranging from 0 to 50%, the fluorescence intensity remains at a low level. Further addition of hexane into the mixture results in a sharp and dramatic enhancement of the fluorescence intensity. At the 90 vol% hexane content, the PL intensity is about 150-fold higher than that in the pure THF solution, suggesting that TPE-TEG-PA is AIE-active.

Since the CMC value is an essential parameter for the protamine detection, the CMC value of TPE-TEG-PA was determined by a fluorometric assay. The PL spectra of the dye in aqueous solutions at different concentrations are shown in Fig. 3A. When the concentration of the dye is lower than 10 μM, only a very weak fluorescent signal is detectable. At the concentration of 20 μM, the PL intensity enhances abruptly, suggesting that the dye starts to form micelles. By further increasing the dye concentration, the PL intensity increases. The plot of peak intensity against TPE-TEG-PA concentration is shown in Fig. 3B. An inflexion at around 20 μM could be derived through the intersection point of the two tangent lines, indicating that the CMC value of the fluorescent probe is about 20 μM. To further verify the micelle formation, particle sizes and TEM measurements were carried out. When the dye concentration is below the CMC value, no detectable signal is recorded in both measurements. However, a diameter of a micelle entity around 200 nm is found above the CMC value of the dye (Fig. 3C and D).

Compared with traditional amphiphilic molecules, the AIE molecules show a self-reported CMC value through fluorescence signal change. TPE-TEG-PA could report whether the concentration reached its CMC value by the aggregation behaviour of the TPE unit. This unique feature would obviously benefit the subsequent protamine detection, making the probe solution simple and easy to prepare.

As the phosphate group of TPE-TEG-PA can be protonated under acidic conditions, such protonation could change the solubility and thus the aggregation behaviour of the dye. We thus examined the pH-dependent fluorescence of TPE-TEG-PA. As shown in Fig. S6,† the probe is highly emissive under strong acidic conditions (pH < 3). When the pH is increased to 3, the fluorescence of the probe decreased significantly. Increasing the pH from 3 to 7 further decreases the fluorescence intensity. Under basic conditions with pH > 7, the emission remains weak. Under acidic conditions, the phosphate group is protonated, which is less soluble in water and the AIE response is activated, while under basic conditions, the deprotonated ionic species is highly water-soluble and thus less emissive. Change of fluorescence intensity versus pH revealed that there are two deprotonation processes of TPE-TEG-PA involved with the increase of pH (Fig. S6B and Scheme S1†). Since the probe emits faintly with the pH range from 3–13, the probe could be used for turn-on sensing of biomolecules in this broad pH range.

**Protamine detection**

To construct a “turn-on” biosensor with excellent signal to noise ratio, the concentration of the probe was chosen to be 10 μM for

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Fig. 2 (A) PL spectra of TPE-TEG-PA (10 μM) in THF–hexane mixtures with different fractions of hexane (f_H). (B) Plot of I/I_0 at 480 nm versus f_H, where I_0 is the PL intensity in pure THF solution. λ_ex = 330 nm.

Fig. 3 (A) PL spectra of TPE-TEG-PA in aqueous solution at different concentrations. (B) Plot of peak intensity in different concentrations of TPE-TEG-PA (with logarithmic axis). (C) Particle size distribution of TPE-TEG-PA (100 μM) in pure water. (D) TEM image of TPE-TEG-PA (100 μM) in pure water. λ_ex = 330 nm.
the protamine detection. At this concentration, the background emission of the probe is low. Meanwhile, the pH of the probing solution will also affect the performance of protamine quantification due to the protonation/deprotonation processes of TPE-TEG-PA at different pHs. Under acidic conditions, the PA group of TPE-TEG-PA is protonated, which decreases the solubility and increases the background signal. Under neutral or basic conditions, the PA group is deprotonated, which increases the solubility of the dye and thus lowers the background noise for the assay (Fig. S9--S11†). The signal increment for TPE-TEG-PA to protamine is more significant in pure water than under basic conditions, probably because there is no interference from other ionic species in pure water to effect the interaction between TPE-TEG-PA and protamine. Therefore, pure water is selected as a probing medium.

As shown in Fig. 4A, the probe emits weakly with a maximum emission wavelength of 450 nm in the absence of protamine. The fluorescence intensity of the probe increases gradually upon the addition of protamine solution. When the concentration of protamine in the probe/protamine complex reaches 2.9 µg mL$^{-1}$, the fluorescence intensity has increased by 12-fold compared to the blank. This fluorescence enhancement could be attributed to the protamine-induced micelle formation and aggregation of the TPE core. To further verify the protamine-induced micelle formation, we performed particle size analysis. As shown in Fig. S12,† uniform micelles with a diameter of about 100 nm are detected when 0.002 mg mL$^{-1}$ of protamine is added to the probe. Meanwhile, it is noted that the emission peak of the complex is red-shifted to 480 nm after protamine addition, which is the typical emission wavelength for TPE aggregates.

It was also remarkable that the plot of $I/I_0$ versus the protamine concentration gives a linear curve with $R^2 = 0.9999$ in the range of 0–1000 ng mL$^{-1}$ (Fig. 4B). More importantly, the limit of detection (LOD) is as low as 12 ng mL$^{-1}$, which is much better compared with previous reports.14d To address the selectivity of TPE-TEG-PA toward protamine, a control experiment with other biomacromolecules including dextran, calf DNA, trypsin, cytochrome C, human serum albumin (HSA) and bovine serum albumin (BSA) was performed under the same experimental conditions. As shown in Fig. 5, the addition of protamine into TPE-TEG-PA causes the highest fluorescence enhancement (~13-fold). HSA and BSA present tiny interference, which may attribute to hydrophobic interactions between the large proteins and the probe.16 Other biomolecules such as dextran, DNA, trypsin and cytochrome C do not induce obvious increases in the fluorescence intensity. Based on the results, TPE-TEG-PA can work as a “turn-on” fluorescent probe for protamine quantitation with high sensitivity and selectivity.

Considering that protamine is rich in lysine and arginine residues, which are the enzymatic substrates for trypsin, we hypothesize that the decomposition of protamine by trypsin will disassemble the micelles. We investigated the effect of trypsin on the fluorescence intensity of the probe in the presence of protamine. The result reveals that the addition of trypsin to the probe/protamine complex solution almost quenches the fluorescence (Fig. 6). It further supports the protamine-induced micelle formation process.

![Fig. 4](image)

**Fig. 4** (A) PL spectra of TPE-TEG-PA (10 µM) in the presence of different concentrations of protamine. (B) Plot of $I/I_0$ versus protamine concentrations, where $I$ and $I_0$ are the PL intensities in the presence or absence of analytes. $\lambda_{ex} = 330$ nm.

![Fig. 5](image)

**Fig. 5** Change of PL intensities of TPE-TEG-PA (10 µM) at 480 nm in the presence of different biomacromolecules (0.002 mg mL$^{-1}$). $\lambda_{ex} = 330$ nm.

![Fig. 6](image)

**Fig. 6** PL spectra of the probe, probe/protamine and probe/protamine/trypsin. Concentration: probe (10 µM); protamine (2 µg mL$^{-1}$); trypsin (0.01 mg mL$^{-1}$); $\lambda_{ex} = 330$ nm. Inset: photographs of corresponding solutions (a) probe only, (b) probe/protamine and (c) probe/protamine/trypsin under UV light illumination at 365 nm.
ALP activity assay

The obvious difference in water-solubility and emission behaviours of TPE-TEG-PA and its precursor TPE-TEG-OH (Fig. S15†) inspired us to utilize the probe for ALP activity assay. ALP could catalyse the hydrolysis reaction of monophosphate to a hydroxyl group in high efficiency. As the enzymatic product TPE-TEG-OH is insoluble in aqueous buffer, it would form aggregates to turn on the fluorescence. Thus, TPE-TEG-PA can work as an ALP activity reporter. To check the feasibility of this design, we investigated the fluorescence change when the probe TPE-TEG-PA was treated with ALP in Tris–HCl buffer solution (pH 9.6).

In the absence of ALP, the probe emits weakly in the buffer solution. Upon addition of 200 mU mL⁻¹ ALP, the fluorescence increases gradually and the intensity reaches a plateau after 40 minutes (Fig. 7), with about 8-fold increase of the PL intensity. The photograph was taken from the solution of TPE-TEG-PA itself and the probe after incubation with ALP in buffer solution under UV illumination at 365 nm. It is obvious that green fluorescence is turned on after being treated with ALP, which is 12 nm red-shifted when compared to the emission colour induced by protamine (inset of Fig. 6). Although the difference in wavelength was not so significant, it could be distinguished by the naked eye, which was essential for a dual-mode probe.

To examine the possibility of quantitative analysis of ALP activity, we conducted the experiment of dynamic monitoring of enzymatic reaction. Fig. 8A shows the fluorescence intensity changes of the probe as a function of the ALP concentrations at different times. With different concentrations of ALP added, the PL intensity of the probe increases gradually with different magnitudes. The fluorescence changes more obviously at the beginning for all ALP concentrations but becomes slower in the later period. The fluorescence intensity reaches the plateau after about 60 min, indicating that TPE-TEG-PA is nearly completely hydrolysed by ALP. These results reveal that the ALP turn-on mode could be applied potentially for the real-time assay of ALP. As shown in Fig. 8B, the relative fluorescence intensity change (I/I₀) presents a linear increase with the activity of ALP in the range from 0 to 100 mU mL⁻¹, which covers the normal range of ALP in serum samples. To address the selectivity of TPE-TEG-PA toward ALP, a control experiment with other nonspecific enzymes including esterase, deoxyribonuclease (DNase), acetylcholinesterase (AChE) and trypsin was performed under the same conditions. As shown in Fig. 9, about 8-fold PL enhancement of the probe was recorded with the effect of ALP, while negligible changes in emission intensity were observed for other enzymes, showing that the probe is highly specific to ALP. To further demonstrate that fluorescence enhancement was due to ALP-catalyzed hydrolysis rather than other effects, a control experiment between ALP and denatured ALP was conducted. It is well known that guanidine hydrochloride is one of the traditional protein denaturants. According to our proposed mechanism, denatured ALP could not hydrolyse the probe and no fluorescence enhancement is expected. The PL result shows that denatured ALP is not able to turn on the fluorescence (Fig. S14†), which reveals that it is the catalysis effect of ALP that functions on this process, excluding

![Fig. 7](image_url) Time-dependent emission spectra of 10 μM TPE-TEG-PA in 10 mM Tris–HCl buffer solution (pH 9.6) upon addition of 200 mU mL⁻¹ ALP at room temperature; inset: photographs taken under UV light (365 nm) illumination of the corresponding solutions in the (a) absence and (b) presence of ALP incubated for 30 min. λex = 330 nm.

![Fig. 8](image_url) (A) Time-dependent PL intensity of 10 μM TPE-TEG-PA in 10 mM Tris–HCl buffer solution (pH 9.6) at 492 nm versus the hydrolysis reaction time in the presence of different concentrations of ALP at 25 °C. (B) Plot of relative fluorescence intensity (I/I₀) at 492 nm after incubation with different concentrations of ALP for 30 min. λex = 330 nm.

![Fig. 9](image_url) Variation of the PL intensity of TPE-TEG-PA (10 μM) in Tris–HCl (10 mM, pH = 7.4) buffer solution at 492 nm after incubation with esterase (200 mU mL⁻¹), DNase (200 mU mL⁻¹), AChE (200 mU mL⁻¹), trypsin (0.002 mg mL⁻¹) and ALP (200 mU mL⁻¹). λex = 330 nm.
other interference effects such as hydrophobic interaction and electrostatic interaction between proteins and probes.

Conclusions

A dual-mode fluorescence “turn-on” probe TPE-TEG-PA for protamine detection and ALP activity assay was established by making use of the AIE technology. Both TEM and particle size measurements confirmed the formation of micelles in high dye concentration as well as in the presence of protamine. The detection limit for the protamine assay was as low as 12 ng mL\(^{-1}\). On the other hand, ALP-catalysed hydrolysis of the fluorescent probe led to self-aggregation of insoluble products, resulting in strong fluorescence. The assay range of ALP was located in 10–200 mU mL\(^{-1}\). The fluorescence “turn-on” method provided a platform for the research on dual-mode biosensors and it would potentially benefit the development of multiplex monitoring using a single probe.

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Notes and references