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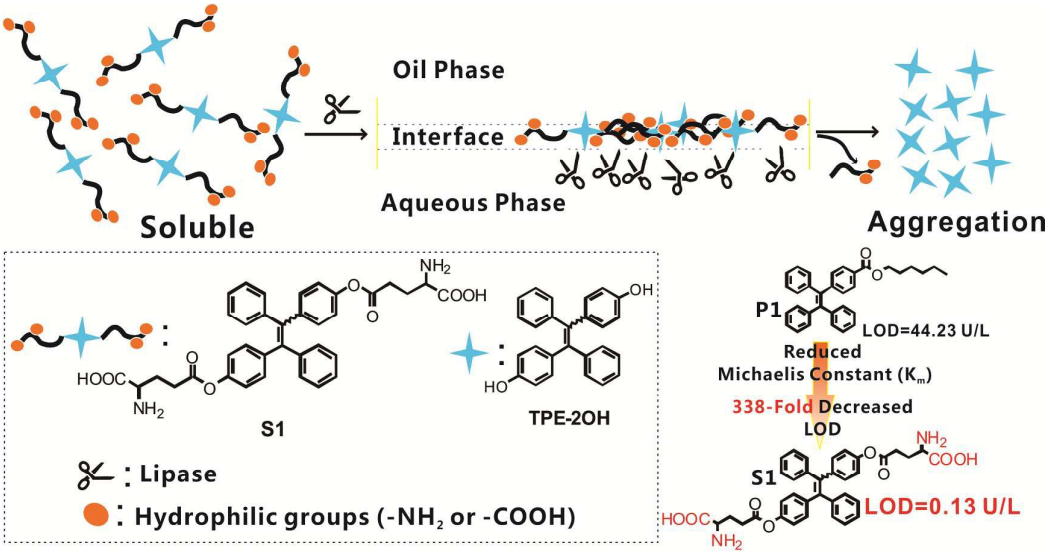

We demonstrate a sensitive, easy and fast method for fluorometric probing lipase levels as acute pancreatitis biomarker based on a novel interfacial controlled aggregation-induced emission (AIE) mechanism.

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Fluorometric probing lipase levels as acute pancreatitis biomarker based on interfacial controlled aggregation-induced emission (AIE)

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As a sudden inflammation of the pancreas, acute pancreatitis has severe complications and high mortality despite treatment. Lipase in serum is served as an essential biomarker of acute pancreatitis even pancreatic cancer. Therefore, developing robust, convenient and sensitive probing of lipase levels is greatly needed. In this work, we present glutamate functionalized tetraphenylethylene (TPE) as "turn-on" fluorescent probe (**S1**) based on an aggregation-induced emission (AIE) mechanism for lipase levels with new recognition units. In heterogeneous media, hydrophilic amino and carboxyl in probe were specifically introduced to facilitate its full access to the lipase at the oil-water interface to achieve an interfacial controlled AIE process. A linear response of fluorescence ranging from 0 to 80 U/L, which is included in the concentration range of lipase level in human serum considering the dilution factor if necessary, limit of detection as low as 0.13 U/L and a fast response time (7 min) can be determined. The value of the apparent Michaelis-Menten constant (K_m) was obtained as 4.23 μM, which indicated superior affinity between lipase and the probe molecule. On the other hand, selectivity, photostability, dynamic monitoring of enzymatic reaction, preliminary commercially enzyme activity screening were summarized. As far as we know, this is the fastest, easiest and most sensitive method for lipase levels probing in the reported literature. Finally, the first application of probing lipase levels in real human serum samples was also conducted successfully.

Introduction

Acute pancreatitis is a sudden inflammation of the pancreas.[1] In severe cases, acute pancreatitis can result in bleeding into the gland, serious tissue damage, infection, and cyst formation.[2] The mortality for severe acute pancreatitis can be 10 to 30 %, sufferers often endure pain and malnutrition, and are most likely left with higher risk of pancreatic cancer (mortality rate > 99 %).[3] Diagnosis of pancreatic problems is often difficult and treatments are therefore delayed because the organ is relatively inaccessible.[4] However, lipase in serum, which is an exocrine enzyme from pancreas, has been employed as an essential biochemical indicator for early diagnosis and condition assessment of acute pancreatitis.[5-13] Also, measuring serum lipase levels associate with pancreatic tumor marker ca 19-9 can diagnose pancreatic cancer.[14,15] Under conditions of pancreatic injury, pancreatic autolysis induces significant elevation of the serum lipase level, high level of lipase strongly suggest acute pancreatitis.[16, 17] Therefore, the development of robust, convenient and sensitive detection of lipase level is of great significance in terms of both clinical diagnoses and pathological analysis towards acute pancreatitis.

So far, to our knowledge, very rare methods have been established for lipase probing. Traditional methods such as colorimetric or turbidimetric methods usually require sample processing and complex instrumentation and indicate various limitations. Therefore, some new methods emerged to overcome these obstacles. For instance, Tian's group developed some gold nanoparticles (GNPs) based biosensors for lipase assays, colorimetric probes by using Tween 20 functionilized GNPs and one-pot nanoparticle growth as reporters.[18, 19] Moreover, some drawbacks still exist for the reported lipase assays, some specific substrates and indicator have been used as the main screening tool, however, all of which possessed relatively complicated production process and suffer from lower sensitivity. For that, their application to the detection of lipase level in real biological samples and early warning of acute pancreatitis cannot be implemented. Up to now, fluorometric assays are receiving remarkably attention owing to their convenience, unparalleled sensitivity, simplicity, rapid implementation, noninvasive monitoring capability and usability in biological samples.[20-37] Nevertheless, fluorescent probe about lipase level detection is a virgin land area until our work published.[38] In our previous work, an aggregation-induced emission (AIE)-based fluorescence probe bearing characteristics to rapidly visualize lipase activities has been constructed with compound **P1**, unfortunately, however, the sensitivity and detection limit were significantly worse

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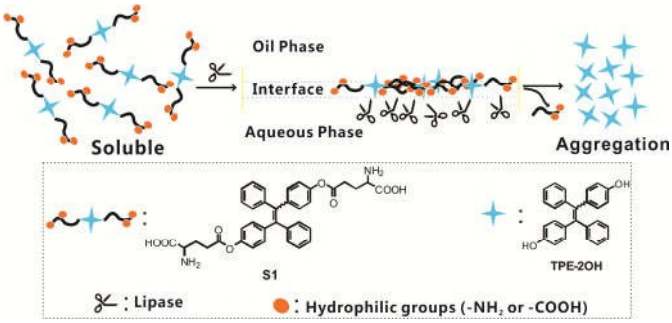

Scheme 1 Schematic illustration of sensing mechanisms and structure of probe S1 in lipase detection.

and the range of detection is outside the concentration range of lipase level in human serum. Therefore, in order to obtain a probe of excellent performance towards lipase, the affinity between probe and enzyme must be greatly improved, by considering of the interfacial reaction characteristics of lipase, the improvement of the interfacial catalytic efficiency is the key factor to the limit of detection and sensitivity of probe molecule.

Herein, we report new fluorometric "turn-on" detection for lipase level by manipulating the deaggregation and aggregation of probe **S1**. The whole design rationale is illustrated in Scheme 1 and explained as follows: (1) probe **S1** is derived from tetraphenylethylene (TPE) core with two glutamate units, TPE derivatives were known as their fantastic AIE behavior; (2) as a heterogeneous catalytic enzyme, it is well known that the best catalytic performance of lipase can be achieved at the oil-water interface, therefore, we could, by coupling the glutamate units onto TPE core, on one hand, notably improved the solubility of the AIE-active probe in hydrophobic medium as the existence of alkyl chains, on the other hand, significantly increased the hydrophilicity of **S1** with the absence of amino and carboxyl groups, facilitating its full access to the lipase in the aqueous phase at the oil-water interface, thus, an interfacial control effect can be conducted simply via the hydrophilic group. (3) as shown in Scheme 1, lipase-catalyzed cleavage of the ester group in probe **S1** will induce the transformation of **S1** into 4, 4'- dihydroxy tetraphenylethylene (see scheme S1, **TPE-2OH**), the glutamate units will be cut off, which may release an insoluble TPE residue (**TPE-2OH**) into the solvent. Accordingly, aggregation of 4, 4'- dihydroxy tetraphenylethylene in solvent will occur and resulting in strong blue emission, that is to say, turn on the fluorescence; (4) since presence of interfacial control effects, very lower lipase levels in some real biological samples can be probed, and glutamate units are the specific substrate for lipase hydrolysis reaction, **S1** could serve as a selectively probe for lipase levels in real biological samples like serum. To this end, a simple fluorescence turn-on assay for lipase probing can be established with **S1**, through this pathway, the catalytic rate of reaction can be significantly improved by this AIE-based interfacial control effect, therefore, the detection of lipase with higher sensitivity and lower detection limit are achieved. As far as we know, no reports to date have been published on the application of a fluorescent probe in testing lipase level of real human serum samples. This work solved the drawbacks of high detection limit and low sensitivity of lipase probe and built an analytical method for early warning of acute pancreatitis. We believe that the research of screening lipase level would not only broaden the spectrum of AIE luminogens-based enzyme assays but also provide a new approach on improving catalytic efficiency of the interface.

Experimental

Materials

The All chemicals and reagents were commercially available and used as received without further purification. Superdry tetrahydrofuran (THF) were purchased from J&K Scientific. Titanium tetrachloride ($TiCl_4$), 4-dimethylaminopyridine (DMAP), 4-hydroxybenzophenone, n-butyllithium were purchased from Aldrich. Dicyclohexylcarbodiimide (DCC), p-toluene sulfonic acid (PTSA) monohydrate, Boc-L-glutamic acid 1-tert-butyl ester (Boc-Glu-OtBu) were purchased from Acros Organics. Lipases from Pseudomonas cepacia (PCL), Candida rugosa (CRL), Candida Antarctica B (CALB), Pseudomonas fluorescens (PFL), Pocine pancreas (PPL), Aspergillus niger (ANL) were all ordered from Amano Enzymes. Glucose oxidase, alkaline phosphatase, acylase, laccase and α-amylase were all purchased from Sigma-Aldrich. Lipase Assay Kit was purchased from Nanjing Jiancheng Bioengineering Institute. Human serum samples were supplied by Union Hospital, Tongji Medical College, Huazhong University of Science and Technology; and the serum samples were from both healthy people and acute pancreatitis patients. The water used in the texts was triple distilled, treated with an ion exchange column, and then treated with Milli-Q water Purification system.

Instrumentation

^1H and ^{13}C NMR spectroscopy study was conducted with a Bruker Avance 400 MHz NMR spectrometer using tetramethylsilane (TMS; δ = 0 ppm) as internal standard. Fluorescence spectra were performed on a Hitachi F-7000 fluorescence spectrophotometer with a Xe lamp as the excitation source. ESI-MS spectra were recorded with a Thermo TSQ QUANTUM ULTRA EMR system. Dynamic laser scattering (DLS) measurements were performed on a commercial laser light scattering instrument (Malvern ZEN3690, Malvern Instruments) at 25 °C.

Synthesis

Synthesis of 4,4'-(1,2-diphenylethene-1,2-diyl)diphenol (TPE-2OH, E/Z isomers) Zinc dust (1.45 g, 22.0 mmol) and 4-hydroxybenzophenone (1.0 g, 5.0 mmol) were placed in a two-necked, round-bottomed flask fixed with a condenser. The flask was evacuated under vacuum and purged with dry nitrogen for three times. After addition of 60 mL superdry THF, the mixture was cooled to -78 °C and $TiCl_4$ (1.25 mL, 11.0 mmol) was injected dropwise. After that, the mixture was slowly warmed to room temperature, stirred for 0.5 h, and then refluxed overnight. The reaction was quenched by 10% aqueous K_2CO_3 solution. The mixture was extracted with diethyl ether three times and the combined organic layer was washed with brine twice and dried over anhydrous sodium sulfate. After solvent evaporation, the crude product was

further purified by a silica gel column to obtain **TPE-2OH** (0.77 g, 84.9%, E/Z isomers) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (TMS, ppm) 6.93-7.04 (m, 10H), 6.75-6.80 (m, 4H), 6.46-6.51 (m, 4H).

Synthesis of compound TPE-BGO (E/Z isomers). To a 30 mL DCM solution, 4,4'-(1,2-diphenylethene-1,2-diyl)diphenol (2.0 mmol, 0.728 g), DCC (3.0 mmol, 0.619 g), DMAP (0.4 mmol, 0.048 g), PTSA monohydrate (0.4 mmol, 0.076 g) and Boc-L-glutamic acid 1-tert-butyl ester (Boc-Glu-OtBu) (8.0 mmol, 2.43 g) were added. The reaction mixture was stirred and refluxed for 24 h. After solvent evaporation, the crude product was further purified by a silica gel column to afford the compound 2 (0.82 g, 43.7%, E/Z isomers) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (TMS, ppm) 7.21-7.25 (m, 12H), 7.01-6.98 (m, 6H), 4.49 (d, 1H), 4.47 (d, 1H), 2.34-2.31 (m, 4H), 2.18-2.15 (m, 2H), 1.95-1.91 (m, 2H), 1.51 (s, 18H), 1.49 (s, 18H). ^{13}C NMR(100 MHz, $CDCl_3$): δ (TMS, ppm) 172.25, 171.78, 156.17, 153.36, 140.89, 139.91, 137.44, 128.92, 128.75, 128.40, 128.11, 123.46, 81.92, 79.74, 53.17, 28.71, 28.36, 27.68, 26.12. MS (ESI): m/z 935.82 $[M+H]^+$.

Synthesis of compound S1 (E/Z isomers). Compound TPE-BGO (0.47 g, 0.5 mmol) was dissolved under stirring in 10 mL DCM, then hydrogen chloride generated from the reaction of NaCl and H_2SO_4 was passed into the solution for 1 h. The organic layer was washed with water. Afterwards the solvent was evaporated under vacuum to get the product (0.29 g, 92.1%, E/Z isomers). ^{1}H NMR (400 MHz, $CDCl_3$): δ (TMS, ppm) 7.21-7.26 (m, 12H), 7.00-6.96 (m, 6H), 4.49-4.46 (m, 2H), 2.38-2.33 (m, 4H), 2.18-2.16 (m, 2H), 1.94-1.92 (m, 2H). ^{13}C NMR(100 MHz, $CDCl_3$): δ (TMS, ppm) 173.91, 172.18, 153.41, 141.54, 139.97, 136.51, 128.74, 128.26, 127.91, 127.52, 126.63, 52.98, 29.07, 27.74. MS (ESI): m/z 623.71 $[M+H]^+$.

Preparation of the solution of probe S1 and lipase

A stock solution of **S1** (1×10^{-3} M) was prepared by dissolving the **S1** solid (6.22 mg) in hexane (10 mL). The solution of **S1** (10 μM) in hexane were prepared by placing 0.1 mL of **S1** stock solution, then diluting the solution to 10 mL with hexane. For a series of commercial purchased lipase samples solutions with concentration gradients (5 to 90 U/L) and other commercial lipases for preliminary screening (80 U/L) were dissolved in 0.1 M potassium phosphate buffer (pH 7.4) which were prepared by K_2HPO_4 and KH_2PO_4.

Lipase detection and dynamic monitoring of enzymatic hydrolysis

Different concentrations of lipase stock solutions (5~90 U/L) 3 mL were mixed with 10 μM **S1** solution 3 mL in a 10 mL flask and stirred at room temperature for 20 min. Then the organic layer was taken to proceeded fluorescence measurements with an excitation wavelength of 360 nm. To examine the kinetics of the enzyme reaction, the enzymatic hydrolysis process was monitored by the fluorescence spectral measurements which scanned at intervals of 1 min from 0 to 10 min after incubation with 0, 5, 10, 30, 50, 70 and 80 U/L of lipase. To investigate the photobleaching of reaction product aggregates, the enzymatic product mixture solution was exposed to an ultraviolet lamp (254 and 365 nm simultaneously) for up to 150 min.

Lipase levels detection in real samples

For lipase detections in serum, human blood samples were obtained from Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. The writing of informed consent was obtained from human subjects in compliance with relevant laws and institutional guidelines. Human intravenous blood samples were obtained through venipuncture and allowed to clot for 1 h at room temperature. After removing the clot part by centrifuging at 3000 rpm for 5 min, the obtained serum samples were ten-fold diluted with 0.1 M potassium phosphate buffer (pH 7.4). Standard recovery experiments were performed by adding a known quantity of lipase to diluted human serum samples of healthy people. The percent recovery referred to the difference in lipase levels determined before and after the addition of lipase in comparison with the added amount. The fluorescence assay was performed as above to determine the amount of lipase. For comparison, lipase levels were determined by using a commercially lipase assay kit.

Results and discussion

Design, synthesis and sensing mechanism of probe S1

The sensing mechanism is illustrated in Scheme 1. As is known to all, lipases can catalyze the hydrolysis of ester bond, hence this mode of stimuli can activate the AIE characteristic, giving fluorescent response through a simple reaction. Soluble glutamate groups were attached onto TPE core, so as to make the probe molecules are well dissolved in some media. The hydrolytic cleavage of the ester bond in **S1** catalyzed by lipase and compound **TPE-2OH** was produced, then, the rapid aggregation of **TPE-2OH** will be triggered due to its poor solubility which caused by the loose of glutamate units, thus strong blue fluorescence can be "turn on" because of AIE effect. The probe **S1** was synthesized using the synthetic protocol described in Scheme **S1** and starting from 4-hydroxybenzophenone via a Mcmurry coupling reaction leading to compound **TPE-2OH**, which was esterificated with two equivalents Boc-Glu-OtBu to yield intermediates, after hydrogen chloride was bubbled into the DCM solution of compound **TPE-BGO** for 1 h, the desired product **S1** was obtained. The structure and purity of **S1** were confirmed by ^{1}H NMR, ^{13}C NMR and ESI-MS.

Fluorescent response toward lipase

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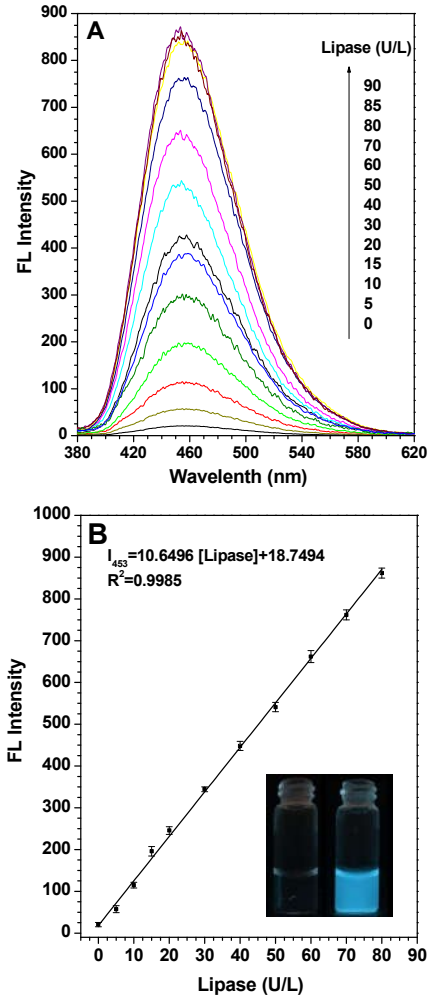

Fig. 1 (A) The fluorescence emission spectra of probe **S1** (10 μM) after incubation with different concentrations of lipase-buffer (0.1 M PBS, pH 7.4) solution at 37 °C; (B) calibration curve versus the different concentration (0-80 U/L) of lipase; inset shows the photo of the corresponding solutions of probe **S1** (10 μM) without (left) and with (right) lipase (90 U/L) after incubation at 37 °C for 15 min under UV light (365 nm) illumination. Excitation wavelength: 360 nm.

To check the feasibility of our design, firstly, we studied the photophysical properties of **TPE-2OH** when it aggregates. The results in Fig. **S1** suggest that **TPE-2OH** is an AIE-active substance. Secondly, we investigated the fluorescence change when the probe **S1** was treated with lipase-buffer solution in hexane. First, the effect of pH values and temperatures on the reaction was investigated, with the results summarized in Fig. S2 and Fig. S3, respectively. The pH value of 7.4 (PBS buffer) and 37 °C were chosen in order to ensure sensitive and rapid detection. A hexane solution of probe **S1** (10 μM) was prepared for the fluorescent spectral studies and Pocine pancreas lipase (PPL) was selected to evaluate the application of probe **S1**. In Fig. 1, as we expected, the fluorescent intensity of probe **S1** (10 μM) displays almost no emission under the testing conditions, and nearly no photoluminescence could be seen upon excitation even in a dark room. Next, we added a series of fixed concentration lipase to solution of probe **S1**, after the incubation, excitingly, the blue fluorescence emission displayed apparent enhancement. As shown in Fig. 1A, when the concentration of lipase was increased in the test system, the intensity centered at about 453 nm increased correspondingly. In fact, even at the concentration of lipase as low as 20 U/L, the emission intensity at about 453 nm increased to about 15 times the original one. When the concentration of lipase reaches 80 U/L, the fluorescence intensity has increased to the maximum by 42-fold compared to the blank. At last, lipase concentration reaches to 90 U/L, the fluorescence depicts no significant enhancement and the intensity reaches a plateau. This phenomenon verified our design idea mentioned above: by accompanying with the leakage of the alkoxy chain and hydrophilic groups of the glutamate group linked to TPE core after incubated by some lipase, its aggregation behavior changed to show detectable photophysical signal in response to the analytes. On the other hand, MS analysis (Fig. S4) also suggests that the product of the enzymatic reaction has the same molecular structure as **TPE-2OH**. Ideally, the change of a fluorometric probe should be from one extreme case to another, just like in our case, from the “off” state to “on”. According to the calibration curve (Fig. 1B), it was also remarkable that the relative fluorescence intensity at about 453 nm gives a linear increase with the concentration of lipase in the range from 0 to 80 U/L (R^2 = 0.9985). The photograph was taken from the solution of probe **S1** itself and the probe after incubation with lipase in hexane solution under UV illumination at 365 nm. It is obvious that blue fluorescence appeared after treatment with lipase, so distinct fluorescence “turn on” was observed. More importantly, the limit of detection (LOD) is as low as 0.13 U/L, which is much better compared with previous reports and sensitive enough for determining lipase levels in biological assays considering the dilution factor, given that for normal adults age 60 and younger, the normal of lipase level in blood is 10-140 U/L, and that for adult older than age 60 is 18-180 U/L.[39]

To further verify the formation of aggregates of probe **S1** was caused by lipase, particle sizes were performed by the DLS (Dynamic Laser Scattering) analysis, and the results were displayed in Fig. S5. In the stock solution of probe **S1** (10 μM) with no lipase added, the nanoparticles could be observed in the range of 3-20 nm, while the mean diameter of S1 is approximately 210 nm after incubated with determine concentration of lipase 80 U/L, which uncovered that aggregates are indeed occurred in the solution. Transmission Electron Microscopy (TEM) also confirms compound **TPE-2OH** in the reaction mixture were produced (Fig. S6), and the length of formed aggregates appeared to be 100-400 nm, which was well consistent with the DLS analysis. It suggested that the aggregation size significantly influenced the fluorescence of the solution, because the AIE effect could be triggered by the larger aggregates.

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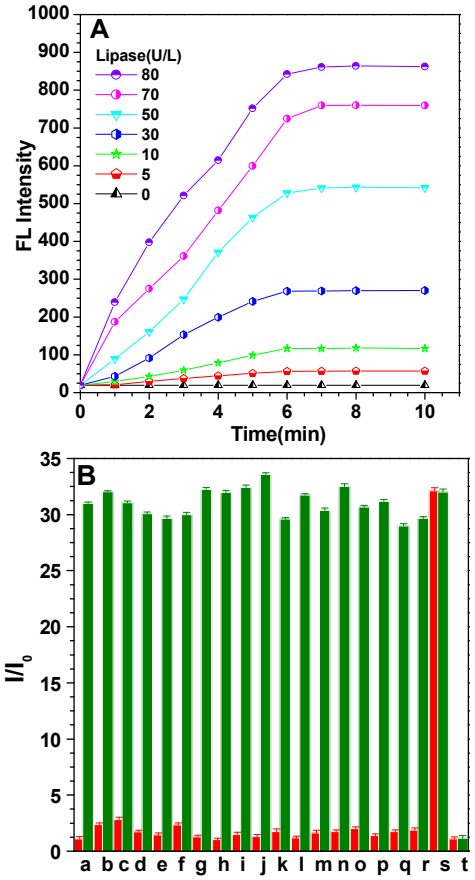

Fig. 2 (A) Time-dependent fluorescence intensity of probe S1 (10 μM) in hexane solution at 453 nm versus the hydrolysis reaction time in the presence of different concentrations of lipase in PBS (pH 7.4, 0.1 M) at 37 °C. Excitation wavelength: 360 nm. (B) Fluorescence response of 10 μM probe in PBS-buffered water (pH 7.4, 0.1 M) at 37 °C for 15 min in the presence of different biologically-relevant substances (red bars); fluorescence response of 10 μM probe in PBS-buffered water (pH 7.4, 0.1 M) at 37 °C for 15 min in the presence of 80 U/L lipase and with the addition of different biological substances respectively (green bars). (a) NaCl; (b) KCl; (c) $MgCl_2$; (d) $CaCl_2$; (e) $AlCl_3$; (f) $NaNO_3$; (g) Na_2CO_3; (h) Na_2SO_4; (i) arginine; (j) leucine; (k) glutamic acid; (l) phenylalanine; (m) cysteine; (n) glucose oxidase; (o) alkaline phosphatase; (p) acylase; (q) laccase; (r) α-amylase; (s) lipase; (t) blank; The concentration of the ions and amino acids are 100 μM, while the concentration of protein enzymes are 80 U/L. Excitation wavelength: 360 nm.

To investigate the possibility of quantitative analysis of lipase levels, we carried out the kinetic studies of enzymatic catalytic reaction. Fig. 2A displays the variation of the emission intensity at 453 nm of probe **S1** (10 μM) during incubation with different concentrations of lipase (0-80 U/L) for different times at room temperature. Evidently, the fluorescence intensity increased gradually by prolonging the incubation time, it was also found that the rate of fluorescence enhancement was directly related to the concentrations of lipase, the intensity of the probe increases gradually with different magnitudes, more rapidly fluorescence enhancement were observed when more lipase was used. This is understandable by concerning the fact that a high concentration of lipase would facilitate the hydrolysis of probe **S1**. The fluorescence changes more clearly in the initial stage for all lipase concentrations but becomes slower in the later period. The fluorescence emission reaches the plateau after about 7 min, illustrating that the test could be finished within 7 min, which was more convenient and faster than conventional turbidimetric methods. As displayed in Fig. 2A, such emission enhancement was still detectable even when the concentration of lipase was as low as 30 U/L within 1 min. It reveals that the lipase turn-on mode could be applied potentially for the real-time assay of lipase levels.

As mentioned above, in view of the fact that lipase catalytic reactions only occurred at the oil-water interface, compared to the reported probe **P1**, herein, carboxyl and amino groups which will improve the affinity of the probe towards the lipase in the aqueous phase were specifically introduced into probe **S1**. Firstly, substrates dependence of the initial degradation velocities for lipase were measured. (Fig. S7) The data of **P1** and **S1** for enzyme kinetics follow the Michaelis-Menten equation, and the kinetic parameters have been determined as V_{max} = 1.35 μM/min and K_m = 28.66 μM for **P1**, V_{max} = 1.42 μM/min and K_m = 4.23 μM for **S1**. The K_m value of **S1** is much smaller, indicating the high affinity between lipase and the probe **S1** molecule. On the other hand, as depicted in Fig. S8, the fluorescence response of **P1** began to appear when lipase level rose to 120 U/L, and the limit of detection is up to 44.23 U/L, which is 338 times higher than probe **S1**(LOD=0.13 U/L). Also, the test could be finished within 7 min, which was faster than that of probe **P1** (13 min). Therefore, this hydrophilic group modified interfacial control can successfully bring high sensitivity, low detection limit and short response time test process to us.

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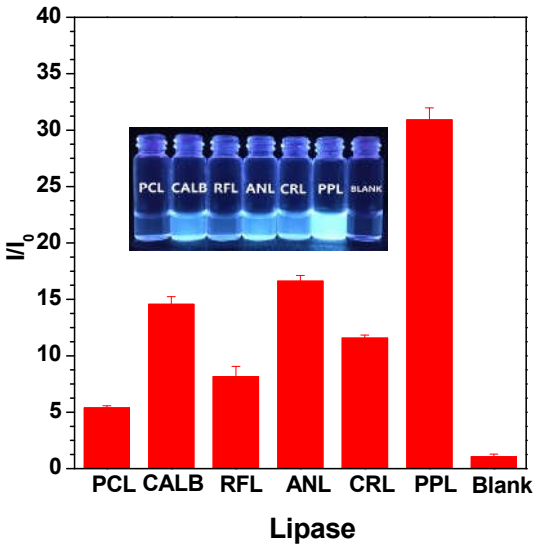

Fig. 3 Plot of I/I_0 intensity of probe **S1** upon the addition of a fixed concentration (80 U/L) of commercial lipase samples, respectively, and within a interval of 20 min. (Inset) Corresponding visual fluorescence under UV light illumination at 365 nm. Excitation wavelength: 360 nm.

Furthermore, to evaluate the selectivity of probe **S1**, the specific nature of this fluorometric assay for lipase was also tested, a control experiment with other nonspecific biological substances including ions (such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Al^{3+}, NO_3^-, CO_3^{2-} and SO_4^{2-}), amino acids (such as leucine, glutamic acid, cysteine, arginine and phenylalanine) and other enzymes (such as alkaline phosphatase, acylase, glucose oxidase, α-amylase and laccase) were performed with a fixed concentration. From the red bars in Fig. 2B, it can be seen that, only lipase induces a prominent intensity enhancement, which brings about 32-fold fluorescence enhancement of the probe was recorded with the effect of lipase, while nearly no rise in emission intensity were observed for other biological substances. Moreover, we also tested the interference of these biologically-relevant substances towards probe. The red bars tell us the presence of other background substances did not show any obvious disturbance, indicating that **S1** had superior selective response toward lipase and these potentially interfering substances show negligible interference on the enzymatic reaction between lipase and probe. To further reveal that fluorescence enhancement was caused by lipase-catalyzed hydrolysis rather than other factors, a control experiment between lipase and inactivated lipase was performed. (Fig. S9) Herein, deactivated lipase which treated by high temperature (100 °C) was selected and it is well known that high temperature environments can make the protein denaturated. According to our hypothetical sensing mechanism, inactivated lipase could not hydrolyze the probe **S1** and no fluorescence "turn on" is expected. The result in Fig. S9 indicates that inactivated lipase cannot bring the enhancement of fluorescence, which reveals that it is the catalysis effect of lipase that acting on this process, excluding other interference effects such as electrostatic interaction and hydrophobic interaction between probes and proteins. Additionally, the resistance to photobleaching for the aggregates of the enzymatic products was also discussed after a direct exposure to ultraviolet lamps (Fig. S10). The relative fluorescence intensity change ratio (I_t/I_0) remained almost unchanged upon a continuous illumination, at last fluorescence intensity of the reaction product aggregates only reduce by 2.9 %, it was indicated that the reaction product aggregates have good photostability.

We carried out a preliminary screening using the sensing mechanism to evaluate the activity of some commercial lipase samples (ANL, CALB, RFL, PPL, RNL and PCL). The fluorescence intensity in the presence of a fixed concentration (80 U/L) of lipase samples was monitored after certain time incubation (20 min). As shown in Fig. 4, the variety of fluorescence intensity was depending on the different lipase activities. We concluded that the lipase activity increases in the following order: PCL < RFL < CRL < CALB < ANL < PPL. This sequence is consistent with the results detected with a pH-stat method (Table S1), demonstrating that this fluorometric assay protocol can be used as an alternative to conventional high throughput screening technologies for lipase activity. The inserted photograph in Fig. 4 was taken from the probe **S1** after incubation with different commercial lipase under UV illumination at 365 nm. It is visible that different activity of lipases lead to the strong or weak blue fluorescence, namely, it could be easily distinguished by the naked eye under UV light.

Lipase detection in real human serum samples

Afterwards, in order to explore the operability and efficacy of the probe system in complex biological fluids, the probe were applied to detecting the lipase levels in real samples, such as human serum. Herein, we tried to detect the endogenous lipase levels in human serum samples by using the calibration curve (Fig. 1B) as standard. Firstly, a standard recovery experiment was performed by adding fixed amounts of lipase in human serum, as depicted in Table S2, the recovery value was between 95.97% and 102.44%, SD values from these two methods are also similar, suggesting the reliability and accuracy of our sensing system for lipase detection. For real serum samples, the procedures of detection were illustrate in

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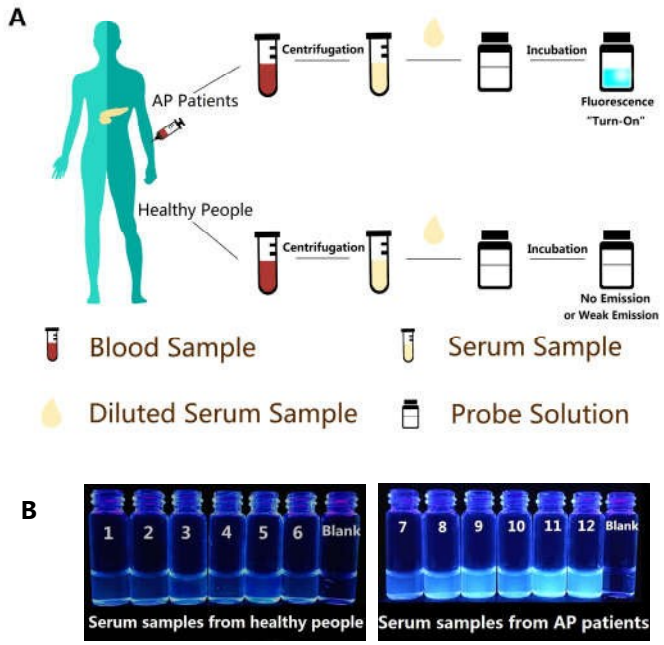

Fig. 4 (A) Procedure schematic illustration of this simple technique for lipase levels detection in real human serum samples. (B) Corresponding visual fluorescence of probe **S1** after incubation with 10-fold diluted real human serum samples under UV light illumination at 365 nm.

Fig. 4A, since the linear response of probe is ranging from 0 to 80 U/L, the dilution factor of the serum sample must be taken into account. Serum samples were obtained from human venous blood via centrifugation, and then the diluted serum was incubated with the probe solution for a while, the fluorescence emission of **S1** probe will experience off-to-on change, which can be monitored by FL spectra, as well as naked eye under UV light illumination at 365 nm. The serum the endogenous lipase levels of both healthy people (sample no.1~no.6) and acute pancreatitis (AP) patients (sample no.7~no.12) measured by this probe are close to those measured by lipase assay kit. The results from probe **S1** in Table 1 indicate that, the lipase level in the serum samples of no.1 to no.6 was in the range of 96.51-171.35 U/L, which were within the normal level of serum lipase, however, for AP patients, their serum lipase level were significantly higher in the range of 425.59-581.05 U/L. The inserted photograph (Fig. 5B) was taken from the probe S1 after incubation with diluted real human serum samples under UV illumination at 365 nm to conduct eyeball detection. It is clear that, for serum samples from healthy people (no.1~no.6), very weak fluorescence is observed, and that for AP patients serum samples (no.7~no.12), significant fluorescence emission enhancement can be noticed. In addition, the determination results were well consistent with those obtained by the lipase assay kit, moreover, compared with commercial lipase assay kit, our probe has the advantages of much lower limit of detection (0.13 U/L), which is 38-fold lower than assay kit (5.0 U/L),

Table 1 Endogenous lipase levels detection in real human serum samples by using lipase assay kit and probe **S1**.

People	Serum no.	Detected by assay kit (U/L)	Detected by probe **S1** (U/L)
Healthy People	1	129.79±2.09	134.06±1.38
	2	119.36±3.17	123.64±2.67
	3	152.18±1.63	157.97±1.85
	4	87.81±2.44	96.51±2.41
	5	176.87±2.59	171.35±3.62
	6	106.01±3.81	104.83±2.74
AP Patients	7	422.69±1.68	425.59±3.26
	8	471.14±5.06	479.87±3.82
	9	517.07±3.47	510.20±2.11
	10	441.89±2.84	443.43±4.97
	11	584.41±3.31	581.05±2.06
	12	549.62±4.01	557.03±3.45

Note: The serum samples were 10-fold diluted for measurement; and the values (mean ± SD) herein represent lipase levels in the undiluted serum samples, which are calculated based on the measurements for the diluted samples. Samples no.1 to no.6 were obtained from healthy people; samples no.7 to no.12 were obtained from patients with acute pancreatitis.

relatively simple test process of samples except the pre-treatment, and shorter test time (7 min) than assay kit (25 min). It's indicated that our fluorescence probe could act as a quite convenient one-step assay for an accurate detection of lipase levels in human serum and had great potential as an effective detection method for lipase levels in diagnostic related applications.

Conclusions

In summary, for lipase fluorescent probes, the interfacial catalytic efficiency determines its sensitivity, we designed a novel fluorescence turn-on probe for lipase detection, which functions through lipase-induced transformation of soluble to insoluble features and the corresponding AIE effect. We adjust the reaction behavior of AIE probe in the interface by the introduction of hydrophilic groups, the highest sensitivity, lowest detection limit and shortest response time in reported literature were obtained. The probe is more suitable for a linear detection of lipase levels ranging from 0 to 80 U/L, which fits into the concentration range of lipase in human serum considering the dilution factor if necessary, the aggregates product also shows a good photostability against a direct exposure to ultraviolet, few interference by other biological components and good agreement with assay kits results. To the best of our knowledge, this is the first report on the detection of lipase levels in real human serum samples using a fluorescent probe. Therefore, the probe would provide a helpful approach for further elucidating the physiological

roles of lipase as well as for conducting pathological analysis for diseases involving lipase.

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