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# Detection of lipase activity in human serum based on a ratiometric fluorescent probe†

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Acute pancreatitis results in major mortality and morbidity. Lipase is a vital biomarker of acute pancreatitis in the early stage. Developing a simple and convenient methodology for monitoring the lipase activity is of significance to the early diagnosis of acute pancreatitis. In this work, we designed a novel ratiometric fluorescent probe (CARA) based on FRET for detecting lipase activity in human serum. The energy donor group coumarin derivative (CA) and the energy acceptor group rhodamine derivative (RA) were linked for the construction of a coumarin–rhodamine platform through an ester bond that is easily cleaved by lipase, breaking the FRET process between CA and RA. CARA shows a high selectivity to lipase over other biological substances and a good linearity between the fluorescence ratio and lipase activity in serum samples. CARA has the potential to be applied in the clinical diagnosis of acute pancreatitis.



## 1. Introduction

Acute pancreatitis is a common gastrointestinal disease with high morbidity, and nearly 20% severe acute pancreatitis is associated with organ failure, which results in high mortality. The serum lipase level of an acute pancreatitis patient increases dramatically within 24 h and is three times higher than the upper limit of normal.[1,2] Therefore, lipase is a crucial biomarker in the early diagnosis of acute pancreatitis.[3–5] Thus, the detection of serum lipase activity is necessary for the diagnosis and assessment of acute pancreatitis.[6,7]

Lipase is secreted from the pancreas for hydrolyzing triglycerides into glycerol and fatty acid. The conventional methodologies for the detection of lipase activity are colorimetry and titrimetry. These methods undergo tedious sample processing and render damage to lipase activity, affecting the measurement accuracy.[8–11] Compared with these methods, fluorescent probes have attracted widespread attention due to their convenience, rapid response, high sensitivity and noninvasiveness.[12–22] However, only several single emission fluorescent probes were developed for the measurement of lipase activity.[17,23,24] These single emission probes are vulnerable to the variations in the environment, concentration, and excitation intensity.[25–27] In addition, some probes need to be used in a two-phase (lipid and water) solution for meeting the interfacial catalysis requirement of lipase, which requires tedious operation. The lipid solution is also harmful to the environment. Therefore, there is still demand for developing an environment friendly fluorescent probe that has a built-in correction for the detection of lipase activity.[28–31]

In this paper, we designed and synthesized a novel ratiometric fluorescent probe for the detection of lipase activity based on fluorescence resonance energy transfer (FRET). The probe was named *N*-(6-(diethylamino)-9-(2-(((3-(6-(diethylamino)-2-oxo-2*H*-chromene-3-carboxamido)propanoyl)oxy)methyl)phenyl)-3*H*-xanthen-3-ylidene)-*N*-ethylethanaminium (CARA).[32,33] Energy donor group 3-(6-(diethylamino)-2-oxo-2*H*-chromene-3-carboxamido) propanoic acid (coumarin group) and energy acceptor group (*E*)-3-(diethyliminio)-*N*-ethyl-*N*-ethylidene-9-(2-(hydroxymethyl)phenyl)-3*H*-xanthen-6-aminium (Rhodamine group) are linked by an ester bond (Scheme 1). The CARA probe can monitor lipase activity in aqueous solution through hydrolyzing the ester bond to interrupt the FRET process. CARA possesses two fluorescence emission peaks at 405 nm and 585 nm, and exhibits a high selectivity to lipase and a good linearity between the fluorescence intensity ratio and the concentration of lipase. CARA also can detect the lipase activity in serum, providing a potential tool for diagnosis in pancreatic diseases.

## 2. Experimental section

### 2.1. Materials and instrument

Unless stated otherwise, all reagents were purchased from Macklin Company and used without further purification.

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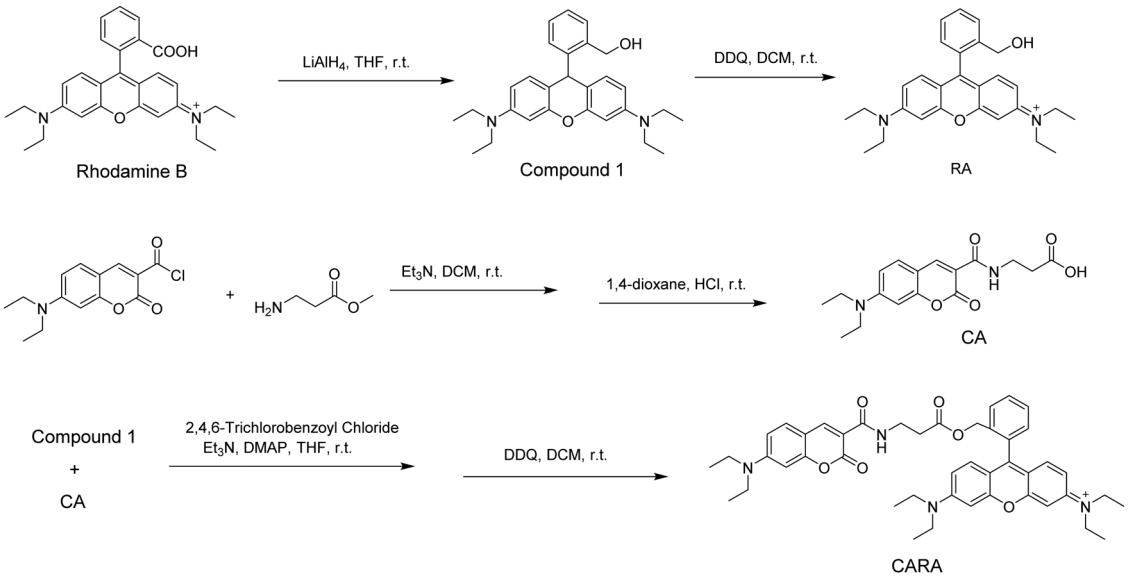


Scheme 1 Synthesis of CARA.

Lipase was purchased from Macklin Company and the lipase activity was determined by *p*NPB according to the reported method.[34] Twice-distilled water was used throughout all experiments. The pH values were measured with a pH meter (PHS-3C). $^1$H NMR and $^{13}$C NMR spectra were acquired on a Bruker Avance-500M and JNM ECZ 600R spectrometer, with used $CDCl_3$ an $d_6$-DMSO as solvents and tetramethylsilane (TMS) as an internal standard. Ultraviolet (UV) and fluorescence spectra were performed on an UV-2600 Spectrophotometer and F-4600 Spectrophotometer, respectively. A HPLC system (Agilent 1260 Infinity II, Agilent technologies, Germany) equipped with a Kromasil 100-5-C18 column (4.6 × 250 mm).

### 2.2. Synthesis of compound 1

Compound **1** was synthesized according to the literature methods.[35] Briefly, rhodamine B (5 g, 10.44 mmol) was dissolved in THF (50 mL), and then $LiAlH_4$ (2.5 M, 10 mL) was added slowly under the protection of nitrogen. After stirring overnight at room temperature, the reaction was quenched with $H_2O$ (20 mL) and the mixture was extracted with $CH_2Cl_2$ (50 mL × 3). The organic phase was dried with anhydrous $MgSO_4$, and concentrated *in vacuo* to get the crude product. Then, it was purified by column chromatography to get a white solid (compound **1**, 1.32 g, 26%). $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ = 7.42–7.38 (m, 1H), 7.25 (dd, $J$ = 8.6, 3.6, 3H), 6.67 (d, $J$ = 8.6, 2H), 6.38 (d, $J$ = 2.3, 2H), 6.26 (dd, $J$ = 8.5, 2.5, 2H), 5.34 (s, 1H), 4.55 (s, 2H), 3.31 (q, $J$ = 7.1, 8H), 1.15 (t, $J$ = 7.2, 12H) ppm. $^{13}$C NMR (151 MHz, $CDCl_3$) $\delta$ = 151.70, 147.82, 144.83, 138.66, 131.48, 130.01, 129.69, 128.07, 127.00, 111.46, 107.47, 98.83, 77.35, 77.14, 76.92, 63.12, 44.48, 40.12, 12.74 ppm.

### 2.3. Synthesis of RA

Compound **1** (86 mg, 0.2mmol) and DDQ (67 mg, 0.3 mmol) were dispersed in $CH_2Cl_2$ (10 mL), and then the mixture was stirred at room temperature for 4 h. The reaction solution was washed with water (50 mL × 3). The organic phase was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography to give a light pink solid (RA) (38 mg, 45%). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.39, (m, 1H), 7.25 (m, 3H), 6.67 (d, $J$ = 8.6, 2H), 6.38 (d, $J$ = 2.3, 2H), 6.26 (dd, $J$ = 8.5, 2.5, 2H), 5.34 (s, 1H), 4.55 (s, 2H), 3.31 (q, $J$ = 7.1, 8H), 1.15 (t, $J$ = 7.2, 12H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 151.70, 147.82, 144.83, 138.66, 131.48, 130.01, 129.69, 128.07, 127.00, 111.46, 107.47, 98.83, 63.12, 44.48, 40.12, 12.74 ppm. ESI$^+$-HRMS ($m/z$): 429.2534 ([M]$^+$ calculated: 429.2537).

### 2.4. Synthesis of CA

β-Alanine ethyl ester hydrochloride (154 mg, 1.49 mmol) was dissolved in dry $CH_2Cl_2$ (5 mL) and triethylamine (0.5 mL), and a solution of 7-(diethylamino)-2-oxo-2*H*-chromene-3-carbonyl chloride (334 mg, 1.19 mmol) in $CH_2Cl_2$ (2 mL) was added slowly at room temperature. The mixture was stirred for 2 h and the organic solvents were removed under reduced pressure to get the crude product. The crude product (1.0 g) was dissolved in dry 1,4-dioxane (25 mL), and then HCl (25 mL, 3M) was added into the solution, and stirred overnight. The reaction solution was extracted with $CH_2Cl_2$ (50 mL × 3) and the organic phase was concentrated under reduced pressure. The residue was purified by column chromatography to yield a yellow solid CA (350 mg, 73%).$^1$H NMR (500 MHz, $d_6$-DMSO): $\delta$ = 12.30 (s, 1H), 8.80 (t, $J$ = 5.6, 1H), 8.64 (s, 1H), 7.66 (d, $J$ = 9.0, 1H), 6.78 (d, $J$ = 9.0, 1H), 6.59 (s, 1H), 3.47 (m, 6H), 2.48 (s, 5H), 1.12 (t, $J$ = 7.0, 6H) ppm. $^{13}$C NMR (125 MHz, $d_6$-DMSO): $\delta$ = 173.59, 162.58, 162.10, 157.68, 152.89, 148.23, 132.04, 110.56, 109.62, 108.08, 96.29, 44.77, 35.30, 34.36, 12.76 ppm, ESI$^+$-HRMS ($m/z$): 333.1403 ([M + H]$^+$ calculated: 333.1406).

### 2.5. Synthesis of CARA

Compound **1** (150 mg, 0.45 mmol) and CA (232 mg, 0.54 mmol) were dissolved in THF (5 mL). Then, to this above solution,

 

2,4,6-trichlorobenzoyl chloride (343 mg, 1.4 mmol) and triethylamine (101 mg) were added sequentially. After stirring for 5 min, 4-dimethylaminopyridine (DMAP, 122 mg, 1 mmol) was added and the reaction solution was further stirred for 30 min. The organic solvents were removed under reduced pressure, and the crude product was purified by column chromatography to yield a dark purple solid. The dark purple solid (143 mg, 0.2 mmol) and DDQ (67 mg, 0.3 mmol) were dissolved in $CH_2Cl_2$ (10 mL) and stirred at room temperature for 4 h. The reaction solution was washed with water (50 mL × 3). The organic phase was dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude compound was purified by column chromatography to give a dark purple solid (CARA, 67 mg, 46.8%). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.90 (s, 1H), 8.60 (s, 1H), 7.59 (d, $J$ = 21.9, 3H), 7.41 (s, 1H), 7.31 (s, 1H), 7.09 (s, 2H), 6.85 (s, 3H), 6.65 (s, 1H), 6.46 (s, 1H), 4.84 (s, 2H), 3.62 (s, 8H), 3.45 (s, 6H), 2.37 (s, 2H), 1.31 (s, 12H), 1.23 (s, 6H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 171.12, 163.29, 162.55, 157.70, 155.68, 152.75, 148.07, 133.89, 131.64, 131.21, 130.39, 129.67, 128.92, 114.26, 113.67, 110.15, 109.50, 108.19, 96.61, 96.42, 63.81, 46.24, 45.12, 34.97, 33.99, 12.68, 12.41 ppm, ESI$^+$-HRMS ($m/z$): 743.3802 ($[M]^+$ Calculated: 743.3803).

### 2.6. Detection of hydrolysis of CARA by lipase

RA, CA, and CARA were dissolved with DMSO as a stock solution (1 mM). Then the solution was diluted with PBS to a final concentration of 100 μM. CARA solution in PBS (100 μM, 2 mL) was incubated with lipase (5 mg mL$^{-1}$) at 37 °C for 1 h. The solutions of CA, RA, CARA, and CARA + Lipase were analyzed using an Agilent 1260 Infinity II. The chromatographic conditions were as follows: Kromasil 100-5-C18 (4.6 mm × 250 mm) column; column temperature: 30 °C; mobile phase: 0.1% trifluoroacetic acid–water and acetonitrile, gradient elute; flow rate: 1 mL min$^{-1}$; detection wavelength: 254 nm.

### 2.7. Lipase activity assay in PBS

CARA was dissolved in DMSO as a stock solution (1 mM). In all the experiments, the final concentration of CARA in PBS (pH 7.2, 0.01M) was 10 μM and the mixture was incubated at 37 °C. The effect of pH was conducted as follows. CARA was added to PBS (2 mL) with different pH values ranging from 5.5 to 8 with or without lipase (5 mg mL$^{-1}$). After incubation for 1 h, the fluorescence spectra of the mixture were tested. The response time of CARA towards lipase was tested as follows. CARA in PBS (pH 7.2, 2 mL) was incubated with lipase (0.5 mg mL$^{-1}$) at 37 °C for different times, and then the fluorescence spectra at a specific time were measured respectively. The selectivity of CARA toward lipase was tested by adding lipase and other biological substances. Each of the following biological substances including $Mg^{2+}$, $Zn^{2+}$, $Br^-$, $Cu^{2+}$, $Fe^{2+}$, $NO_2^-$, $Fe^{3+}$, $Ca^{2+}$, $NH_4^+$, $S_2O_3^{2-}$, $I^-$, Phe, Pro, Glu, α-amylase, tryptone, yeast extract, BSA and lipase was separately incubated with CARA solution (2 mL), where the concentration of lipase was 5 mg mL$^{-1}$, and the levels of some ions and amino were 100 μM, and the concentrations of α-amylase, tryptone, yeast extract, and BSA were 1 mg mL$^{-1}$. The fluorescence spectra of all mixed solutions were recorded using a fluorescence spectrophotometer after incubation for an hour. The concentration dependence of lipase was measured by adding a certain amount of lipase to the CARA in PBS (pH 7.2, 2 mL). Then, the fluorescence spectra of CARA were recorded at various concentrations of lipase. The fluorescence emission spectra were recorded from 425 nm to 700 nm with excitation at a wavelength of 405 nm. The UV absorption spectroscopy spectrum was recorded from 300 nm to 800 nm.

### 2.8. Lipase activity assay in human serum (HS)

The serum from a volunteer was collected by the Affiliated Hospital of Qingdao University and informed consent was obtained for the use of human serum. All experiments were tested in buffered human serum (HS)/PBS solution (v/v = 1 : 9, pH 7.2). The response time and selectivity of CARA toward lipase were tested in the same way as the test in PBS. The concentration dependence of lipase in human serum was detected by adding a serious of concentrations of lipase to the CARA in buffered HS/PBS solution (v/v = 1 : 9, pH 7.2). Then, their fluorescence spectra were recorded after incubation at 37 °C for 1 h.

## 3. Results and discussion

### 3.1. Design and detection mechanism of the CARA probe for lipase

Lipase is an interface catalytic hydrolysis enzyme that can hydrolyze the ester bonds of fatty acid esters. According to the function of lipase, we designed and synthesized a ratiometric fluorescent probe (CARA) based on FRET for detecting lipase activity. The coumarin fluorophore (CA) and rhodamine fluorophore (RA), the classic partners of the FRET platform, were employed as the energy donor and acceptor of CARA, respectively. The two fluorophores are linked through the fatty acid ester bond that is easily broken by lipase. In addition, CA is a water-insoluble dye and RA is a water-soluble dye, so they were linked to construct amphipathic molecules. These amphipathic molecules not only enhanced the water-solubility of the CARA probe, but also easily formed a micro-interface to meet the requirement of the interface catalysis of lipase. CA shows strong blue fluorescence from 450 nm to 560 nm (Fig. 1A, blue solid line) and RA has obvious UV-vis absorbance from 490 nm to 600 nm (Fig. 1A, red solid line), so there is a great overlap between the fluorescence spectra of CA and the UV-vis absorption spectra of RA. The fluorescence of CA is absorbed well by RA to emit the red fluorescence of RA. Thus, the free probe CARA shows strong red fluorescence at 585 nm and weak blue fluorescence at 470 nm upon excitation at 405 nm, which can be attributed to the FRET process between CA and RA. The apparent FRET efficiency of the CARA probe was calculated to be 88.8% according to a reported method (Fig. S1, ESI†).[36] As lipase was added into the CARA solution, the ester bond of CARA was hydrolyzed by lipase to generate CA and RA, which increased the distance between CA and RA and weakened the FRET effect

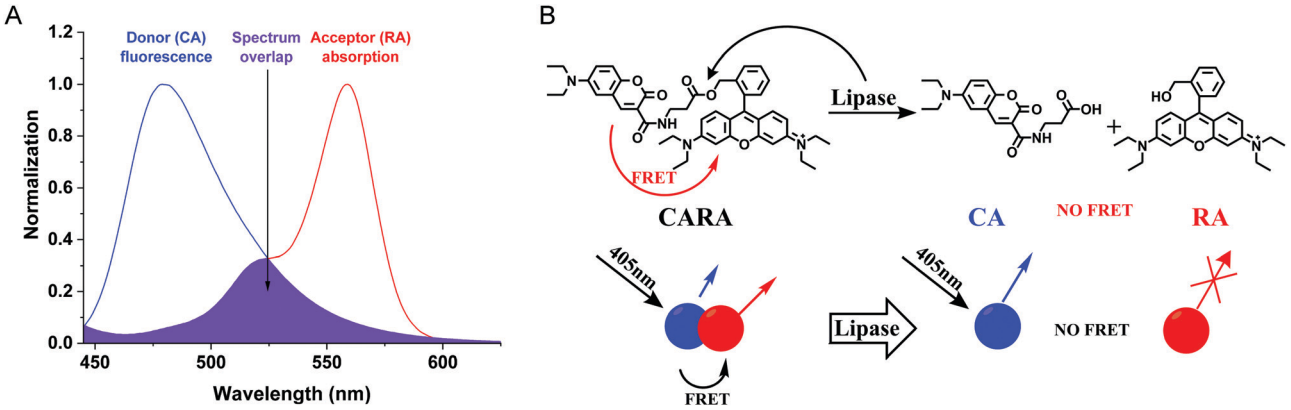


Fig. 1 (A) Normalized fluorescence spectra of a donor (CA) (blue solid line), normalized absorption spectra of an acceptor (RA) (red solid line), and the spectra overlapping domain (purple zone). (B) The proposed mechanism of CARA for detecting lipase activity.

(Fig. 1B). The fluorescence intensity increases at 470 nm and decreases at 585 nm upon excitation at 405 nm. Hence, CARA could be employed to quantify lipase activity through measuring the fluorescence intensity at 470 nm and 585 nm.

### 3.2. Effect of pH and time

Firstly, the fluorescence intensities of the CARA probe were measured in the presence or absence of lipase at different pH values (Fig. S2, ESI†). With the variation of pH from 5.5 to 8, the fluorescence intensity showed little changes at 585 nm in the presence or absence of lipase, which indicated that the fluorophore RA is stable at various pH values. Under the same conditions, the fluorescence intensity at 470 nm decreased, which may be attributed to the protonation of the amino group of *CA*. Although the fluorescence ratio ($I_{470\,nm}/I_{585\,nm}$) decreased with the increase in pH, the different values of the fluorescence

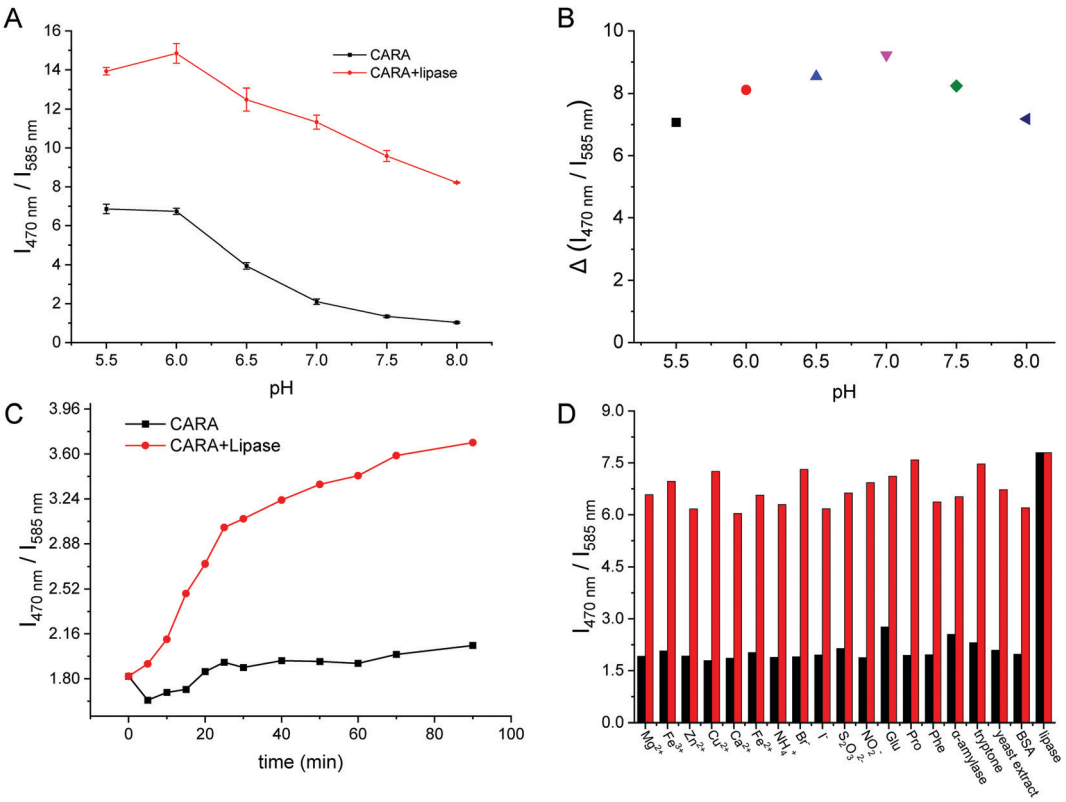


Fig. 2 (A) The ratio of the fluorescence intensity ($I_{470\,nm}/I_{585\,nm}$) of CARA (10 μM) in the presence or absence of lipase in PBS at different pH values from 5.5 to 8.0. (B) The difference value of the fluorescence ratio ($I_{470\,nm}/I_{585\,nm}$) between CARA and CARA + lipase in PBS at various pH values from 5.5 to 8.0. (C) The ratios of the fluorescence intensity ($I_{470\,nm}/I_{585\,nm}$) of CARA (10 μM) in the presence or absence of lipase in PBS (pH 7.2) at 37 °C for 0–90 min, which were obtained at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, and 90 minutes. (D) The $I_{470\,nm}/I_{585\,nm}$ ratio of CARA (10 μM) in the presence of lipase and other species including $Mg^{2+}$, $Zn^{2+}$, $Br^{-}$, $Cu^{2+}$, $Fe^{2+}$, $NO_2^{-}$, $Fe^{3+}$, $Ca^{2+}$, $NH_4^{+}$, $S_2O_3^{2-}$, $I^{-}$, Phe, Pro, Glu, α-amylase, tryptone, yeast extract, and BSA in PBS (pH 7.2).

 

ratio ($I_{470\ nm}/I_{585\ nm}$) between CARA and CARA with lipase change little (Fig. 2B). The results indicated that the CARA probe can respond to lipase activity in a wide pH range. Subsequently, the kinetics of the CARA probe to lipase was investigated through recording the fluorescence intensities of CARA incubated with lipase (3500 U $L^{-1}$) at pH 7.2 for different times (Fig. S3, ESI†). As shown in Fig. 2C, the fluorescence ratio ($I_{470\,nm}/I_{585\,nm}$) of the free probe CARA changed little within 90 minutes, indicating that the CARA probe has good stability in aqueous solution. With the addition of lipase, the fluorescence ratio ($I_{470\,nm}/I_{585\,nm}$) increased with time and changed little after 60 minutes (the red line, Fig. 2C), demonstrating that the hydrolysis of CARA by lipase reached equilibrium at 60 minutes. Thus, in the next experiments, the incubation time of CARA is 60 minutes.

### 3.3. Selectivity of CARA to lipase

Selectivity is a crucial parameter to evaluate the quality of a fluorescent probe. To test the selectivity of CARA, we measured the fluorescence of CARA in the presence of different species including lipase (35 000 U $L^{-1}$), $Mg^{2+}$, $Zn^{2+}$, $Br^{-}$, $Cu^{2+}$, $Fe^{2+}$, $NO_2^{-}$, $Fe^{3+}$, $Ca^{2+}$, $NH_4^{+}$, $S_2O_3^{2-}$, $I^{-}$, Phe, Pro, Glu, α-amylase, tryptone, yeast extract, and BSA (Fig. S4A, ESI†). As lipase was added into CARA solution, the fluorescence intensity enhanced 2 fold at 470 nm and fell by half at 585 nm upon excitation at 405 nm. The fluorescence ratio ($I_{470\,nm}/I_{585\,nm}$) of CARA with lipase increased 4 fold compared with that of free probe CARA (Fig. 2D, the black column). Upon addition of other species, both the fluorescence intensity of CARA at 470 nm and 585 nm changed slightly, and the fluorescence ratio ($I_{470\,nm}/I_{585\,nm}$) floated barely. These results exhibit that CARA permits specific response to lipase over other species. Subsequently, we evaluated the anti-interference capacity of CARA through recording the fluorescence intensity of CARA with the addition of lipase and other different species. The fluorescence intensity of CARA still increased at 470 nm and decreased at 585 nm in the presence of both lipase and other different species, which was consistent with the fluorescence change of CARA with only lipase (Fig. S4B, ESI†). The fluorescence ratio ($I_{470\,nm}/I_{585\,nm}$) of CARA with lipase was not influenced by other species (Fig. 2D, the red column). These above results indicated that CARA possessed high selectivity to lipase and excellent anti-interference capacity to other species.

### 3.4. Concentration-dependent detection of lipase

To evaluate the sensitivity and linearity of the CARA probe to lipase activity, the titration experiments were implemented in PBS solution containing 1% DMSO at pH 7.2. Various units of lipase were added into the 10 μM CARA solution, and the fluorescence spectra were recorded upon excitation at 405 nm after incubation for 60 min. As shown in Fig. 3A, the free probe CARA showed two fluorescence emission peaks at about 470 nm and 585 nm. Along with lipase concentration increased, the fluorescence emission of the donor group (CA) increased gradually at 470 nm and the fluorescence emission of the receptor group (RA) decreased at 585 nm. The fluorescence ratio ($I_{470\,nm}/I_{585\,nm}$) increased gradually from 1.77 to 9.10 with the change of lipase from 0 to 35 000 U $L^{-1}$ (0–5 mg $mL^{-1}$). The CARA probe showed a good linearity between the fluorescence intensity ratio and lipase concentration, which fitted to the equation $y = 3.30 \times 10^{-4} \times [\text{lipase}] + 2.3521$ with $R^2 = 0.999$ (Fig. 3B). The limit of detection (LOD) is estimated to be 48.5 U $L^{-1}$ based on $3\delta/k$ ($\delta$ is the standard deviation of blank measurements, $n = 10$, and $k$ is the slope of the linear equation). Thus, CARA can be used to detect lipase activity in PBS solution quantitatively. Both UV absorbances at 420 nm and 566 nm of CARA changed slightly in the presence of lipase as compared to that of free CARA, which indicated that neither the CA conjugated system nor the RA conjugated system has changed before and after adding lipase (Fig. S5, ESI†). Thus, the UV absorbance further suggested that the fluorescence change was attributed to ester bond cleavage rather than structure changes of the CA or RA moiety, which was further confirmed by HPLC and HRMS spectra (Fig. S6, ESI†).

### 3.5. Application in the detection of serum lipase activity

Serum lipase activity is a key biological indicator for diagnosing acute pancreatitis in the early stage, so we evaluated the feasibility of the CARA probe for detecting lipase activity in

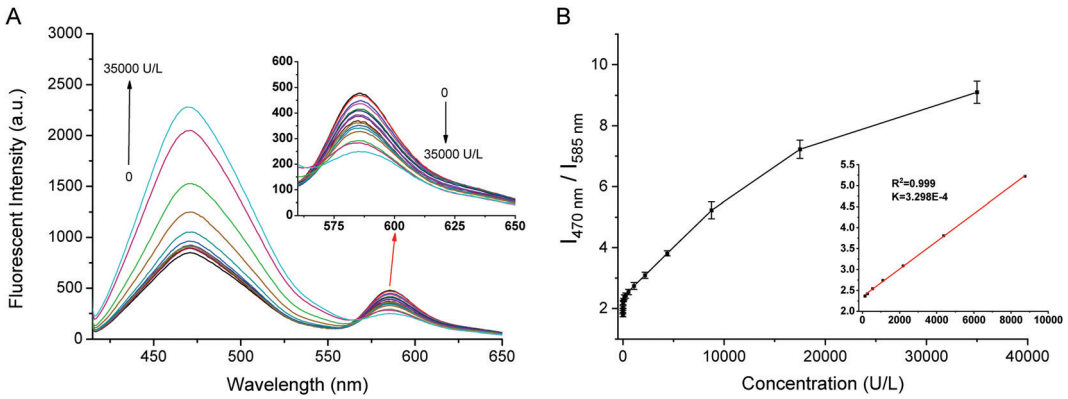


Fig. 3 (A) Fluorescence spectra of CARA (10 μM) in PBS buffer (pH 7.2, 0.01M) with different concentrations of lipase. (B) Relationship between $I_{470\,nm}/I_{585\,nm}$ of CARA and the concentrations of lipase from 0 to 35 000 U $L^{-1}$ and a linear relationship between $I_{470\,nm}/I_{585\,nm}$ and [lipase] from 0 to 8750 U $L^{-1}$ (inset). The results are presented as means ± SD with three replicates ($n$ = 3).

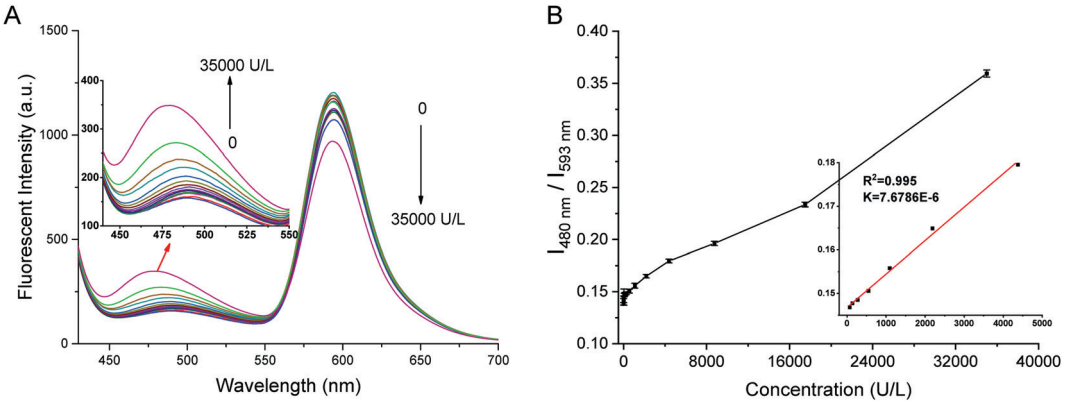


Fig. 4 (A) Fluorescence spectra of CARA (10 μM) in buffered HS/PBS solution (v/v = 1 : 9, pH 7.2) with different concentrations of lipase. (B) Relationship between $I_{480\,nm}/I_{593\,nm}$ of CARA and the concentrations of lipase from 0 to 35 000 U $L^{-1}$ and a linear relationship between $I_{480\,nm}/I_{593\,nm}$ and [lipase] from 0 to 4375 U $L^{-1}$ (inset). The results are presented as means ± SD with three replicates ($n$ = 3).

human serum samples. The fluorescence intensity of CARA was measured in the mixture of human serum (HS) and phosphate buffer solution (HS/PBS, containing 10% HS) in the absence or presence of exogenous lipase. As shown in Fig. 4A, when the CARA probe was incubated with various concentrations of lipase at 37 °C for 1 h in HS/PBS solution, the fluorescence intensity enhanced gradually at 480 nm and weakened obviously at 593 nm with the concentration of lipase, and a good linear relationship was built between the fluorescence intensity ratio ($I_{480\,nm}/I_{593\,nm}$) and lipase concentration ranging

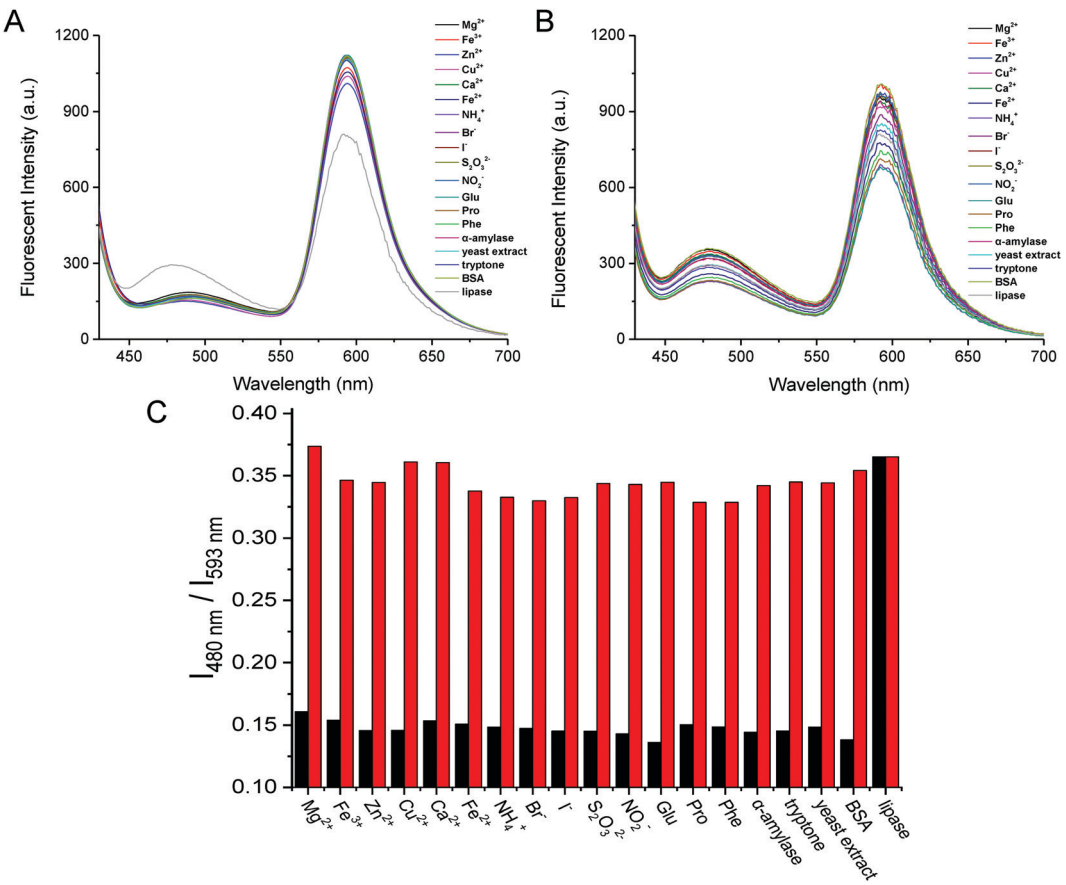


Fig. 5 (A) Fluorescence spectra of CARA (10 μM) toward lipase and other biological substances including $Mg^{2+}$, $Zn^{2+}$, $Br^{-}$, $Cu^{2+}$, $Fe^{2+}$, $NO_2^{-}$, $Fe^{3+}$, $Ca^{2+}$, $NH_4^{+}$, $S_2O_3^{2-}$, $I^{-}$, Phe, Pro, Glu, α-amylase, tryptone, yeast extract, and BSA in HS/PBS (v/v = 1 : 9). (B) Fluorescence spectra of CARA (10 μM) toward the coexistence of lipase and other biological substances including $Mg^{2+}$, $Zn^{2+}$, $Br^{-}$, $Cu^{2+}$, $Fe^{2+}$, $NO_2^{-}$, $Fe^{3+}$, $Ca^{2+}$, $NH_4^{+}$, $S_2O_3^{2-}$, $I^{-}$, Phe, Pro, Glu, α-amylase, tryptone, yeast extract, and BSA in HS/PBS (v/v = 1 : 9). (C) The relative ratio of $I_{480\,nm}/I_{593\,nm}$ of CARA (10 μM) in the presence of lipase and other species including $Mg^{2+}$, $Zn^{2+}$, $Br^{-}$, $Cu^{2+}$, $Fe^{2+}$, $NO_2^{-}$, $Fe^{3+}$, $Ca^{2+}$, $NH_4^{+}$, $S_2O_3^{2-}$, $I^{-}$, Phe, Pro, Glu, α-amylase, tryptone, yeast extract, and BSA in HS/PBS (v/v = 1 : 9).

 

from 0 to 4500 U $L^{-1}$ with $R^2$ = 0.995 (Fig. 4B). The LOD based $3\delta/k$ is estimated to be 69.88 U $L^{-1}$. The CARA probe with a low LOD has potential for distinguishing normal and acute pancreatitis patient serum, because the serum lipase activity of acute pancreatitis patients is three times higher than that (190 U $L^{-1}$) of normal people. Then, we tested the selectivity of CARA toward lipase in serum samples. As shown in Fig. 5A and the black column of Fig. 5C, the fluorescence ratio ($I_{480\,nm}$/$I_{593\,nm}$) of CARA with lipase increased about 2 fold compared with that of free CARA or CARA with other species. In addition, the results of the interference experiments (Fig. 5B and the red column of Fig. 5C) showed that the fluorescence ratio of CARA with lipase was hardly influenced by the co-existent species. These results indicate that CARA also permits a good selectivity to lipase and show a high anti-interference capacity in the sample containing serum. Compared with other fluorometric methods for lipase activity (Table S1, ESI†), the novel probe CARA that is a ratiometric fluorescent probe can effectively alleviate the interference from the variations in environments such as probe concentration and excitation intensity. These results suggest that probe CARA can be used to detect serum lipase activity and may have potential usage for clinical diagnosis of pancreatitis.

## 4. Conclusions

In this study, we developed a novel FRET probe CARA for the detection of lipase activity based on the coumarin–rhodamine platform. CARA that works in an almost aqueous solution is an environmentally friendly approach for the detection of lipase activity, and it can be directly used to measure human serum lipase activity without further processing. CARA exhibits an excellent linearity between the fluorescence ratio ($I_{480\,nm}/I_{593\,nm}$) and lipase concentration, and shows higher selectivity to lipase than other species. Because CARA also can detect serum lipase activity, it has potential for the clinical diagnosis of pancreatitis.

## Conflicts of interest

The authors declare no competing financial interest.

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## References

1 R. J. Vissers, R. B. Abu-Laban and D. F. McHugh, *J. Emerg. Med.*, 1999, **17**, 1027–1037.
2 A. W. Lowe, R. E. Luthen, S. M. E. Wong and J. H. Grendell, *Gastroenterology*, 1994, **107**, 1819–1827.
3 P. K. Garg and V. P. Singh, *Gastroenterology*, 2019, **156**, 2008–2023.
4 M. Avanesov, A. Loser, S. Keller, J. M. Weinrich, A. Laqmani, G. Adam, M. Karul and J. Yamamura, *Eur. J. Radiol.*, 2017, **95**, 278–285.
5 W. M. Steinberg, J. B. Buse, M. L. M. Ghorbani, D. D. Orsted and M. A. Nauck, *Diabetes Care*, 2017, **40**, 966–972.
6 R. C. Smith, J. Southwell-Keely and D. Chesher, *Aust. N. Z. J. Surg.*, 2005, **75**, 399–404.
7 C. Furey, J. Buxbaum and A. B. Chambliss, *Clin. Biochem.*, 2020, **77**, 54–56.
8 A. Hoppe and R. R. Theimer, *Phytochemistry*, 1996, **42**, 973–978.
9 M. J. Haas, D. Esposito and D. J. Cichowicz, *J. Am. Oil Chem. Soc.*, 1995, **72**, 1405–1406.
10 M. Panteghini, R. Bonora and F. Pagani, *Ann. Clin. Biochem.*, 2001, **38**, 365–370.
11 J. Zheng, X. Fu, X. Ying, Y. Zhang and Z. Wang, *Anal. Biochem.*, 2014, **452**, 13–15.
12 Y. Choi, S. H. Shin, H. Jung, O. Kwon, J. K. Seo and J. M. Kee, *ACS Sens.*, 2019, **4**, 1055–1062.
13 L. Dong, S. Shen, H. Lu, S. Jin and J. Zhang, *ACS Sens.*, 2019, **4**, 1222–1229.
14 L. Feng, Y. Yang, X. Huo, X. Tian, Y. Feng, H. Yuan, L. Zhao, C. Wang, P. Chu, F. Long, W. Wang and X. Ma, *ACS Sens.*, 2018, **3**, 1727–1734.
15 S. Y. Liu, H. Xiong, J. Q. Yang, S. H. Yang, Y. Li, W. C. Yang and G. F. Yang, *ACS Sens.*, 2018, **3**, 2118–2128.
16 J. Ou-Yang, Y. F. Li, P. Wu, W. L. Jiang, H. W. Liu and C. Y. Li, *ACS Sens.*, 2018, **3**, 1354–1361.
17 Z. Qiao, H. Zhang, Y. Zhang and K. Wang, *iScience*, 2020, **23**, 101294.
18 Z. Shen, J. Xia, Q. Ma, W. Zhu, Z. Gao, S. Han, Y. Liang, J. Cao and Y. Sun, *Theranostics*, 2020, **10**, 9132–9152.
19 S. Ma, G. Chen, J. Xu, Y. Liu, G. Li, T. Chen, Y. Li and T. D. James, *Coord. Chem. Rev.*, 2021, **427**, 213553.
20 L. Zhu, L. Shan, J. Zhu, L. Li, S. Li, L. Wang, J. Wang, S. Zhang, H. Zhou, W. Zhang and H. Li, *Sci. China: Life Sci.*, 2020, **63**, 1016–1025.
21 A. Jiang, Y. Liu, G. Chen, Y. Li and B. Tang, *Chem. Sci.*, 2020, **11**, 1964–1974.
22 B. An, H. Zhang, J. Peng, W. Zhu, N. Wei and Y. Zhang, *New J. Chem.*, 2020, **44**, 20253–20258.
23 J. Shi, Q. Deng, C. Wan, M. Zheng, F. Huang and B. Tang, *Chem. Sci.*, 2017, **8**, 6188–6195.
24 J. Shi, S. Zhang, M. M. Zheng, Q. C. Deng, C. Zheng, J. Li and F. H. Huang, *Sens. Actuators, B*, 2017, **238**, 765–771.
25 X. M. Hou and W. Cheng, *Biomed. Opt. Express*, 2012, **3**, 340–353.
26 O. I. Kolenc and K. P. Quinn, *Antioxid. Redox Signaling*, 2017, **30**(6), 875–889.
27 O. N. Shilova, E. S. Shilov and S. M. Deyev, *Cytometry, Part A*, 2017, **91**, 917–925.
28 D. Song, S. Cho, Y. Han, Y. You and W. Nam, *Org. Lett.*, 2013, **15**, 3582–3585.
29 C. Gao, S. Zang, L. Nie, Y. Tian, R. Zhang, J. Jing and X. Zhang, *Anal. Chim. Acta*, 2019, **1066**, 131–135.

30 S. Yang, C. Guo, Y. Li, J. Guo, J. Xiao, Z. Qing, J. Li and R. Yang, *ACS Sens.*, 2018, **3**, 2415–2422.
31 L. Yuan, W. Y. Lin, Z. M. Cao, J. L. Wang and B. Chen, *Chem. – Eur. J.*, 2012, **18**, 1247–1255.
32 A. S. Khadria and A. Senes, *Pept. Sci.*, 2015, **104**, 247–264.
33 L. Yuan, W. Y. Lin, K. B. Zheng and S. S. Zhu, *Acc. Chem. Res.*, 2013, **46**, 1462–1473.
34 T. Tsujita, H. Ninomiya and H. Okuda, *J. Lipid Res.*, 1989, **30**, 997–1004.
35 A. L. Luo, Y. J. Gong, Y. Yuan, J. Zhang, C. C. Zhang, X. B. Zhang and W. Tan, *Talanta*, 2013, **117**, 326–332.
36 T. Pinkert, D. Furkert, T. Korte, A. Herrmann and C. Arenz, *Angew. Chem., Int. Ed.*, 2017, **56**, 2790–2794.



 