Electronic Supplementary Information

Homogeneous probing lipase and α-amylase simultaneously by the AIEgens

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Materials and instruments.

The all reagents and chemicals and were commercially available and used as received without any further purification. Diphenylmethane, 4-bromobenzophenone, p-toluenesulfonic, γ-Cyclodextrin, 2-methylbenzothiazole, iodoethane, sodium ethoxide were purchased from Sigma-Aldrich (St. Louis, USA). 4-(1,2,2-triphenylvinyl)benzaldehyde was synthesized according to previous paper. α-Amylase, lipase, alkaline phosphatase; α-chymotrypsin; typsin; pepsin; lactatedehydrogenase; thrombin; alcohol dehydrogenase; lysozyme; AChE, lipase and α-amylase activity assay kit were purchased from Sigma-Aldrich. The water used in the texts was triple distilled, treated with an ion exchange column, and then treated with Milli-Q water Purification system. NMR spectroscopic characterization were measured on a Bruker Avance 500 MHz NMR spectrometer. Tetramethylsilane (TMS) was used as internal reference. FL spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer with a xenon discharge lamp excitation. HRMS spectra were obtained by an Agilent 6530 Accurate-Mass Q-TOF LC/MS system. The particle sizes of AIE aggregates were measured on a laser light scattering instrument (Malvern ZEN3690, Malvern Instruments) at 25 °C.

Procedure for determination of optical properties.

The PBS buffer solution used consists of 51 mM NaCl, 5 mM $Ca(CH_3COO)_2$, 1.52 mM NaN_3 and 100 mM PBS with pH = 6.6. The equimolar ratio of **TPE-γ-CD** and **TPE-H** were weighted, dissolved in small amount of DMSO and well shaken, then diluted with PBS buffer to obtain a 20 μM solution. A series of lipase and α-amylase solutions with different activity gradients (18-144 U L^{-1} for lipase, 20 to 220 U L^{-1} for α-amylase) were dissolved in same PBS buffer solution (pH 6.6).

Procedure for enzyme titration and dynamic

Monitoring of enzymatic reaction. Different concentrations of lipase (0, 18, 52, 62, 72, 96, 100, 114 U L^{-1}) and α-amylase (0, 20, 28, 36, 60, 82, 100, 128, 140, 180, 220 U L^{-1}) stock solutions, 1.5 mL, were mixed with 1.5 mL of 20 μM **S4** solution in one 3.5 mL cuvette. The final concentrations of lipase and α-amylase are 0, 9, 26, 31, 36 ,48, 50, 72 and 0, 10, 14, 18, 30, 42, 50, 64, 70, 90, 110 U L^{-1}, respectively, for

probe S4, and the diluted concentration is 10 μM. The mixtures were incubated with different concentration gradients of lipase for 10 min and α-amylase for 30 min, respectively. The solution was then subjected to fluorescence measurement under an excitation wavelength of 390 nm. In order to investigate the enzymatic reaction kinetics, the hydrolysis process was measured by the fluorescence spectral measurements, which were scanned at different intervals when incubating lipase or α-amylase.

Enzyme activity assay in human serum. The biological sample used in this research was human serum collected from the Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. All experiments in this section were performed in compliance with the relevant laws and institutional guidelines. This study was approved by the Ethic Committees of the Tongji Medical College, Huazhong University of Science and Technology, Oil Crops Research Institute, Chinese Academy of Agricultural Sciences and the Shandong Normal University, which conform to the provisions of the Declaration of Helsinki, and written informed consent was obtained from all patients. Human intravenous blood samples were collected via venipuncture and then assured to clot for 1 h at room temperature. After the clot part was removed by centrifuging at 3000 rpm for 5 min, the obtained serum samples were diluted 10-fold with 0.1 M PBS buffer (pH = 6.6). The same fluorescence assay above was carried out to measure the activity of lipase and α-amylase. The final dilution factors used for the measurement of serum is 10-fold.

Molecular docking. Molecular docking studies were performed to investigate the binding mode between the compounds **S1**, **TPE-γ-CD** and the human lipase using Autodock vina 1.1.21. The three-dimensional (3D) coordinate of the lipase (PDB ID: 3PE6) was downloaded from Protein Data Bank (http://www.rcsb.org/). The 3D structure of the compounds were obtained by ChemBioDraw Ultra 14.0 and ChemBio3D Ultra 14.0 softwares. The AutoDockTools 1.5.6 package[2-3] was employed to generate the docking input files. The search grid of the lipase was identified as center_x: -10.87, center_y: 20.03, and center_z: -9.412 with dimensions

size_x: 17.25, size_y: 15, and size_z: 15. The value of exhaustiveness was set to 20. For Vina docking, the default parameters were used if it was not mentioned. The best-scoring pose as judged by the Vina docking score was chosen and visually analyzed using PyMoL 1.7.6 software (http://www.pymol.org/).

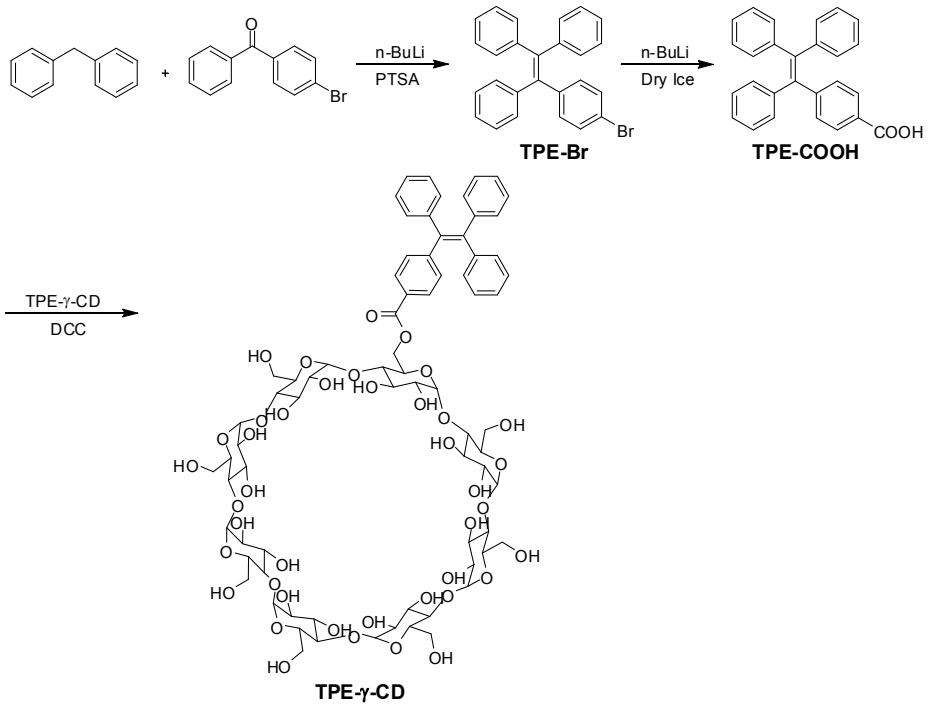
Experimental Procedures

Synthesis

1-(4-Bromophenyl)-1,2,2-triphenylethene (TPE-Br). To a solution of diphenylmethane (3.03 g, 18 mmol) in dry tetrahydrofuran (50 mL) was added 6.3 mL of a 2.4 M solution of n-butyllithium in hexane (15 mmol) at -78 °C under nitrogen. The resulting orange-red solution was stirred for 30 min at that temperature. To this solution was added 4-bromobenzophenone (3.53 g, 13.5 mmol). Then the reaction mixture was allowed to warm to room temperature and stirred for another 6 h. The reaction was quenched with the addition of an aqueous solution of ammonium chloride. The organic layer was then extracted with dichloromethane. The organic layers were combined, washed with saturated brine solution and dried over anhydrous magnesium sulphate. After solvent evaporation, the resulting crude alcohol (containing excess diphenylmethane) was subjected to acid-catalyzed dehydration without further purification. The crude alcohol was dissolved in about 80 mL of toluene in a 100 mL Schlenk flask fitted with a Dean-Stark trap. A catalytic amount of p-toluenesulfonic acid (0.51 g, 2.7 mmol) was added and the mixture was refluxed for 4 h. After the reaction mixture was cooled to room temperature, the toluene layer was washed with 10% aqueous $NaHCO_3$ solution and dried over anhydrous $MgSO_4$. Evaporation of the solvent under reduced pressure afforded the crude tetraphenylethene derivative, which was further purified by silica gel column chromatography. Yield 89%. ^{1}H NMR (500 MHz, DMSO-d_6), δ (TMS, ppm): 6.94-7.11 (m, 17H), 7.32 (d, 2H).

4-(1,2,2-Triphenylvinyl)benzoic acid (TPE-COOH). To a solution of **TPE-Br** (1.92 g, 4.67 mmol) in 30 mL dry THF was added dropwise 2.32 mL (5.67 mmol) of n-butyllithium (2.4 M in n-hexane) at -78 °C under stirring. The reaction mixture was stirred for 2 h to get a dark brown solution. To the obtained solution was then added dry ice pieces in small portions under nitrogen. The solution was allowed to warm to room temperature and stir for additional 12 h. The solvent was evaporated under reduced pressure. The crude product was purified on a silica-gel column. Yield 80%. ^{1}H NMR (500 MHz, DMSO-d_6), δ (TMS, ppm): 6.94-7.10 (m, 17H), 7.65 (d, 2H).

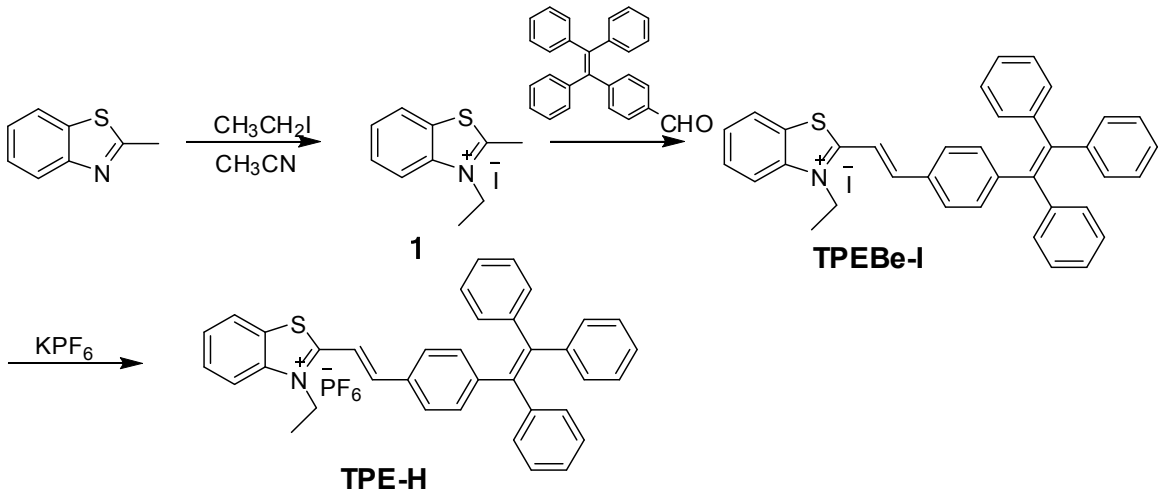
TPE-γ-CD. Into a 50 mL round-bottom flask were dissolved **TPE-COOH** (0.226 g 0.6 mmol) and γ-CD (0.78 g, 0.6 mmol) in 10 mL of anhydrous dimethylformamide (DMF) at 0 °C under nitrogen. After all the solids were dissolved, of N,N'-dicyclohexylcarbodiimide (0.103 g, 0.6 mmol) in 5 mL of anhydrous DMF was then added. The reaction mixture was allowed to warm to room temperature and stirred for 3 days under nitrogen. After filtration, the filtrate was added dropwise into copious ethyl ether under vigorous stirring. The solid was isolated by filtration and dried in vacuum overnight. Yield 70%. ^{1}H NMR (500 MHz, DMSO-d_6), δ (TMS, ppm): 7.95 (s, 2H), 5.81 (d, 7H), 4.88 (d, 7H), 4.56 (t, 6H), 3.62 (br, 7H), 3.60 (d, 12H), 3.55 (d, 7H), 3.33 (d, 7H), 3.29 (d, 7H).



Scheme S1. The synthetic route of the **TPE-γ-CD**.

3-Ethyl-2-methyl-1,3-benzothiazol-3-ium Iodide (1). A solution of 2-methylbenzothiazole (4.48 g, 30 mmol) and iodoethane 14.03 g, 90 mmol) in acetonitrile (250 ml) was heated under reflux for 24 h. After cooling, diethyl ether (200 ml) was added, the desired salt collected by filtration under reduced pressure and washed several times with diethyl ether. The process was repeated sevel times to achieve a suitable yield as white solid. Yield 64%. ^{1}H NMR (CD_3OD, 500 MHz) δ (ppm) 8.32 (d, 2H, 8.29 (d, 2H), 7.92 (dd, 2H), 7.80 (dd, 2H), 4.83 (q, 4H), 3.26 (s, 3H), 1.61 (t, 6H)

TPE-H. A solution of 4-(1,2,2-triphenylvinyl)benzaldehyde (0.2 g, 0.55 mmol) and iodide salt of 1 (0.17 g, 0.55 mmol) in dry ethanol (15 mL) was refluxed under nitrogen for 48 h. After cooling to ambient temperature, the solvent was evaporated under reduced pressure. The residue was purified by a silica gel column chromatography using dichloromethane/acetone mixture as eluent to give a yellow product. Yield 72%. ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 8.41 (d, 1H), 8.27 (d, 1H), 8.12 (d, 1H), 7.91 (d, 1H), 7.75-7.88 (m, 4H), 7.12-7.19 (m, 11H), 6.96-7.03 (m, 6H), 4.93 (q, 2H), 1.43 (t, 3H).



Scheme S2. The synthetic route of the **TPE-H**.

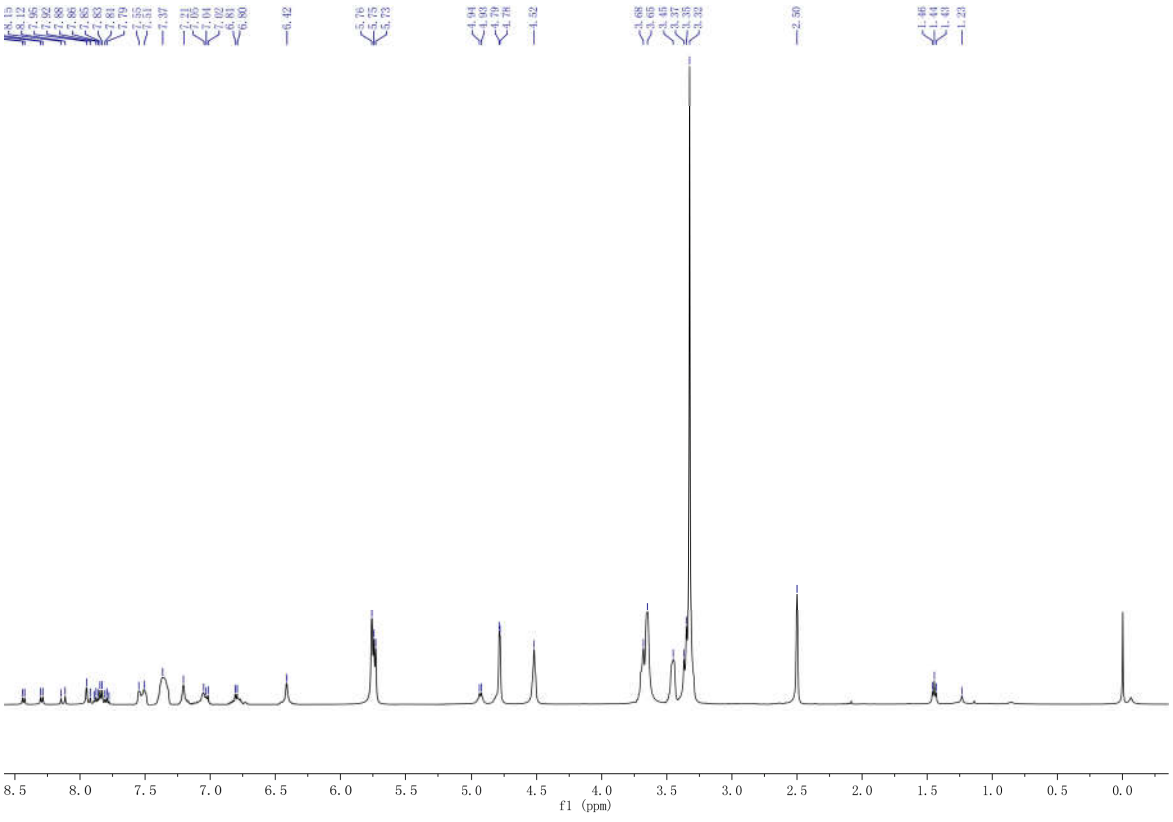


Fig. S1. ^{1}H NMR spectrum of **S4**.

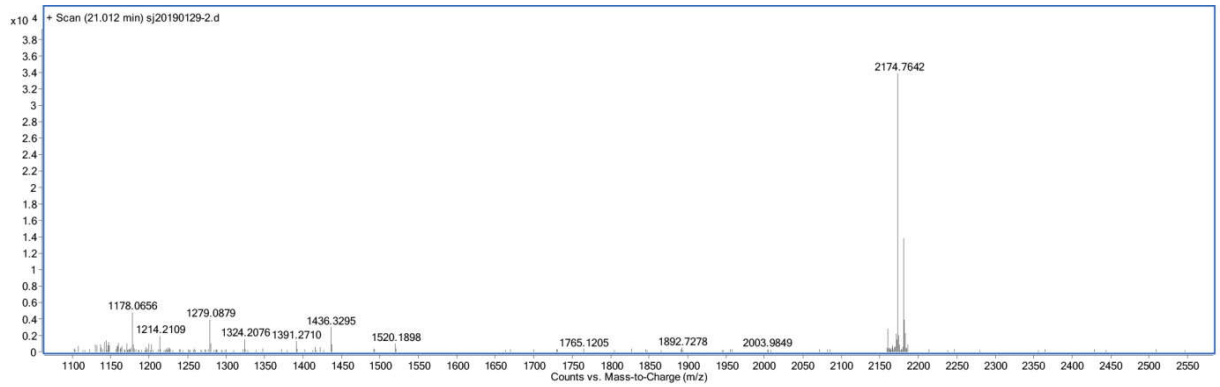


Fig. S2. HRMS spectrum of **S4**.

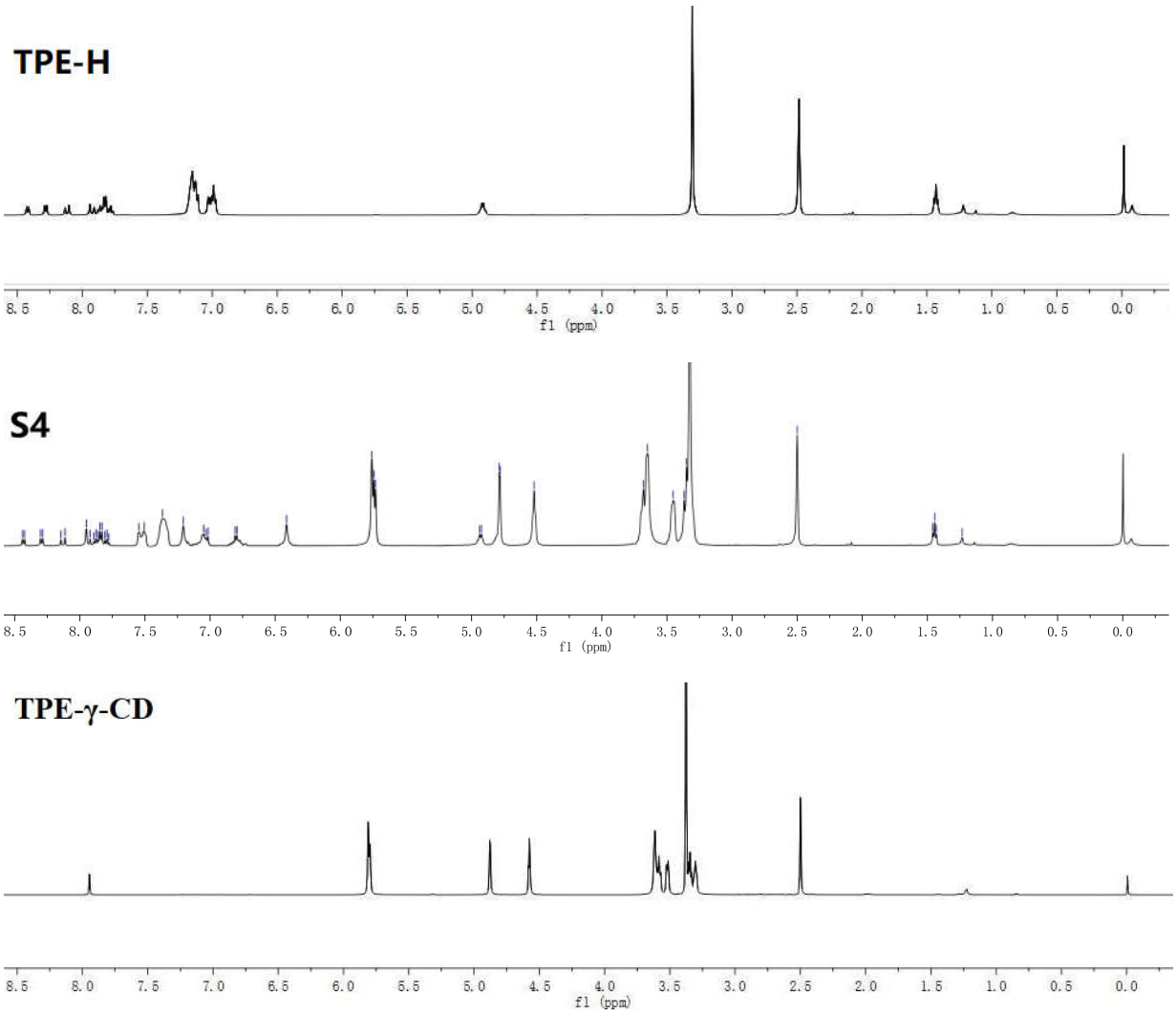


Fig. S3. Comparison of the ^{1}H NMR spectrum of **TPE-H, TPE-γ-CD** and supermolecular **S4**.

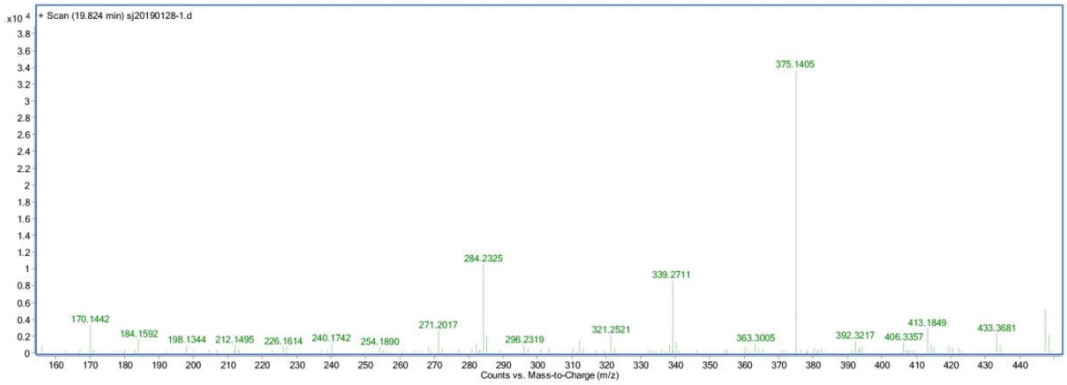


Fig. S4. HRMS spectrum of the enzymatic reaction product. The probe (10 μM) incubated with lipase (100 U L^{-1}) in PBS buffer (pH 6.6, 0.1 M). HRMS: the enzymatic reaction product m/z 375.1405 $[M-H]^-$ (calculated, 375.1463). It is indicated that the product of the enzymatic reaction has the same molecular structure as **TPE-COOH**.

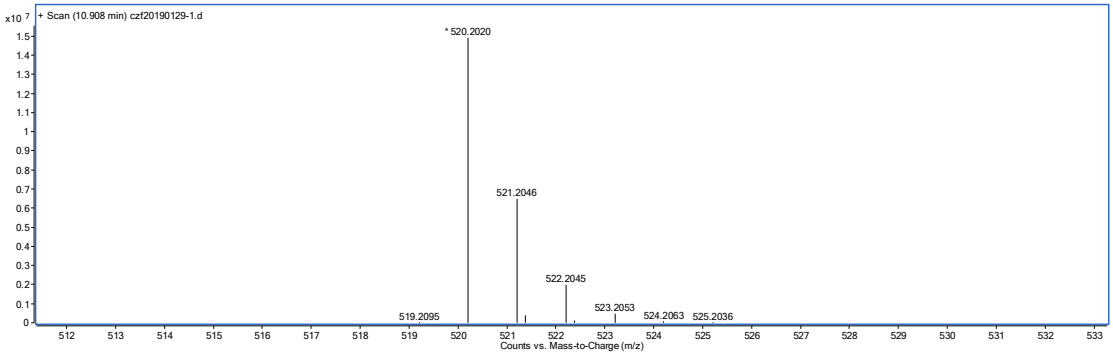


Fig. S5. HRMS spectrum of the enzymatic reaction product. The probe (10 μM) incubated with α-amylase (150 U L^{-1}) in PBS buffer (pH 6.6, 0.1 M). HRMS: the enzymatic reaction product m/z 520.2020 $[M\text{-}PF_6]^+$ (calculated, 520.2093). It is indicated that the product of the enzymatic reaction has the same molecular structure as **TPE-H**.

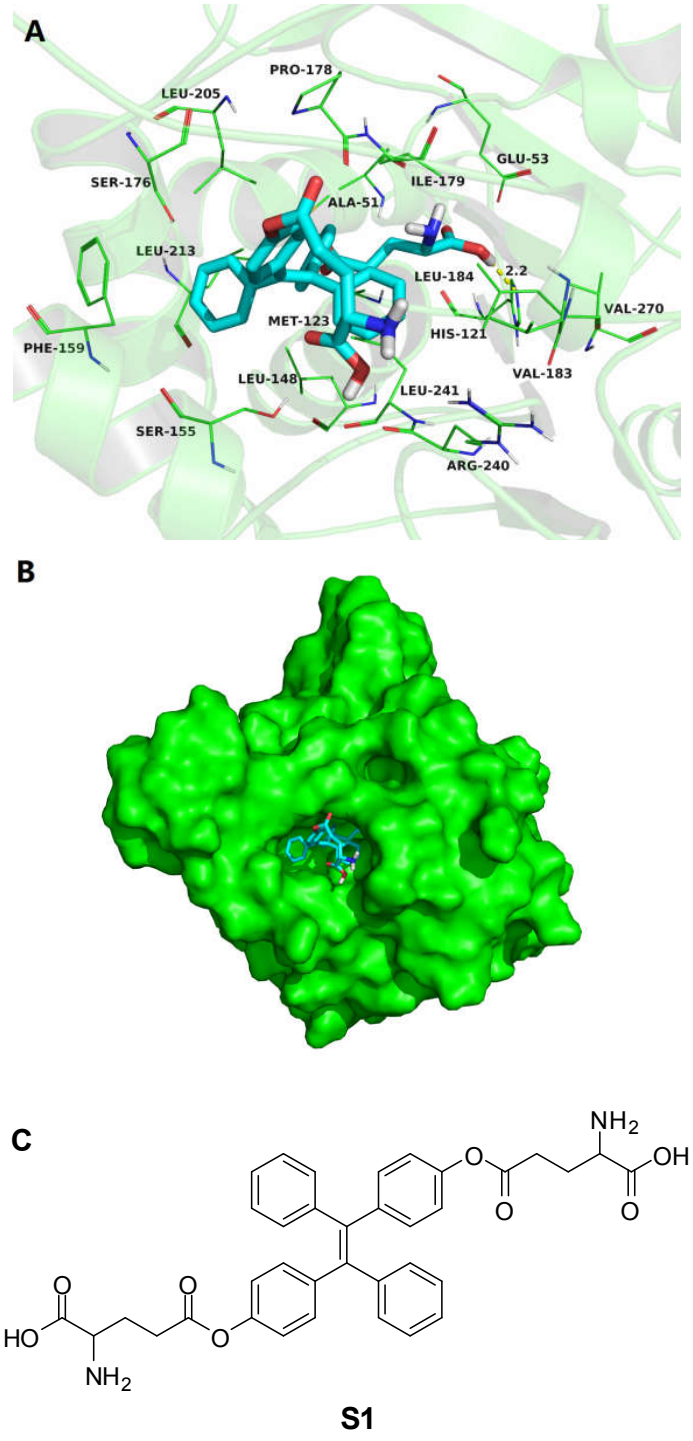


Fig. S6. Compound **S1** was docked to the binding hydrophobic pocket of the lipase: A) Detailed view; B) Total view; C) Chemical structure of **S1**.

Description: Fig. S6 shows the theoretical binding mode between **S1** and lipase. Compound **S1** adopted an "Y-shaped" conformation in the pocket of the lipase (Fig. S6B). The **S1** located at the big hydrophobic pocket, surrounded by the residues

Ala-51, Met-123, Leu-148, Phe-159, Pro-178, Ile-179, Val-183, Leu-184, Leu-205, Leu-213, Leu-241 and Val-270, forming a strong hydrophobic binding (Fig. S6A). Detailed analysis showed that one of the phenyl group of **S1** formed a CH-π interaction with the residue Phe-159. It was shown that the carboxyl group of **S1** at the entrance of the pocket formed the electrostatic interaction with the residue Arg-240. The residue His-121 (bond length: 2.2 Å) formed a hydrogen bond with **S1**, which was the main interaction between **S1** and the lipase (Fig. S6).

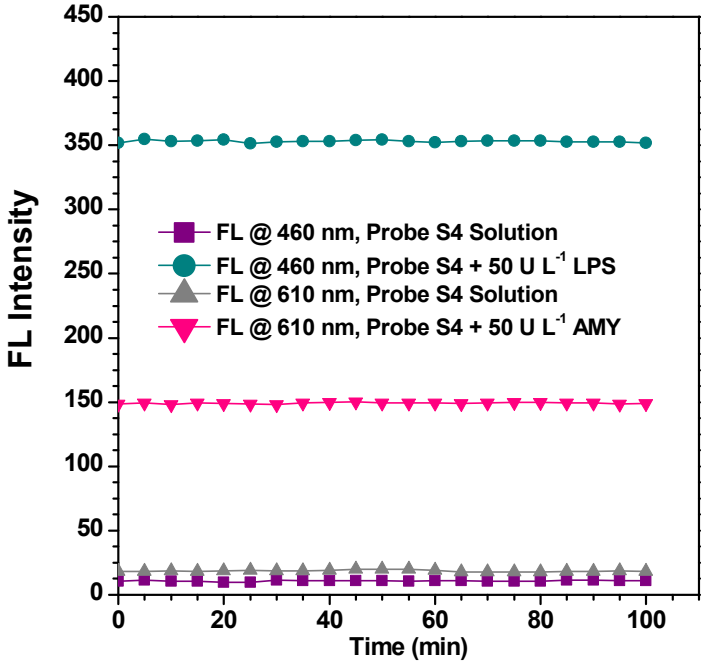


Fig. S7. Stability of the reaction product aggregates.

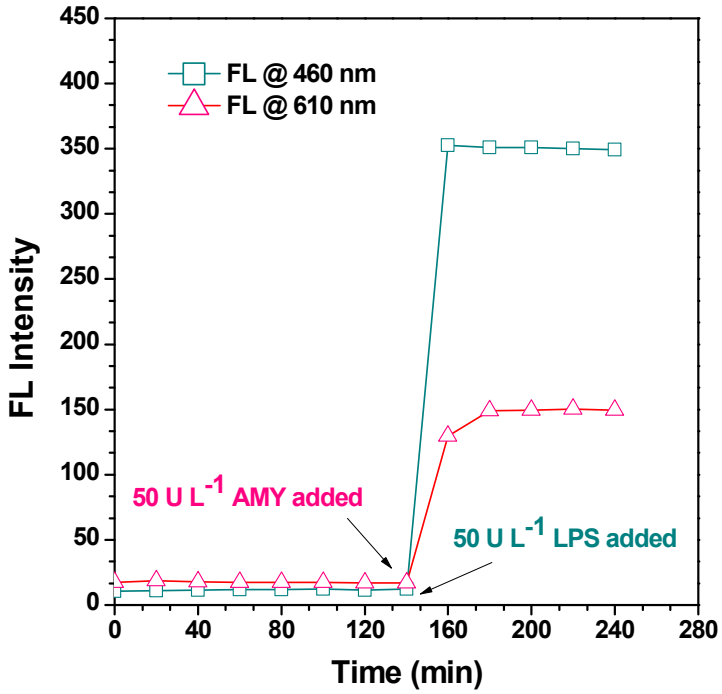
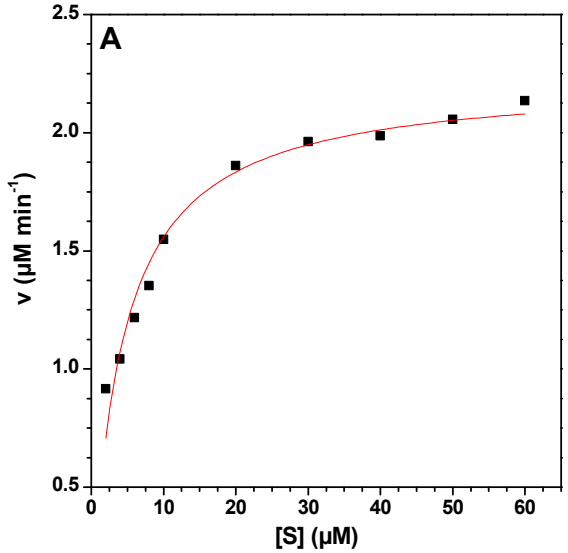
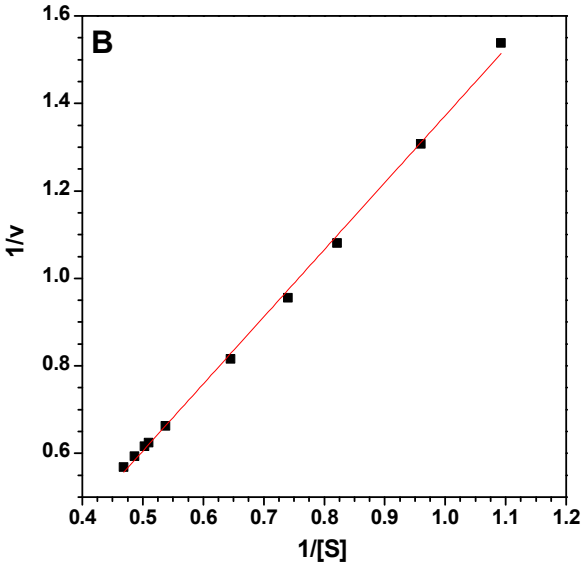


Fig. S8. Stability of the probe **S4** solution.


A
v (μM min-1)
[S] (μM)
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
60


B
1/v
1/[S]
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2

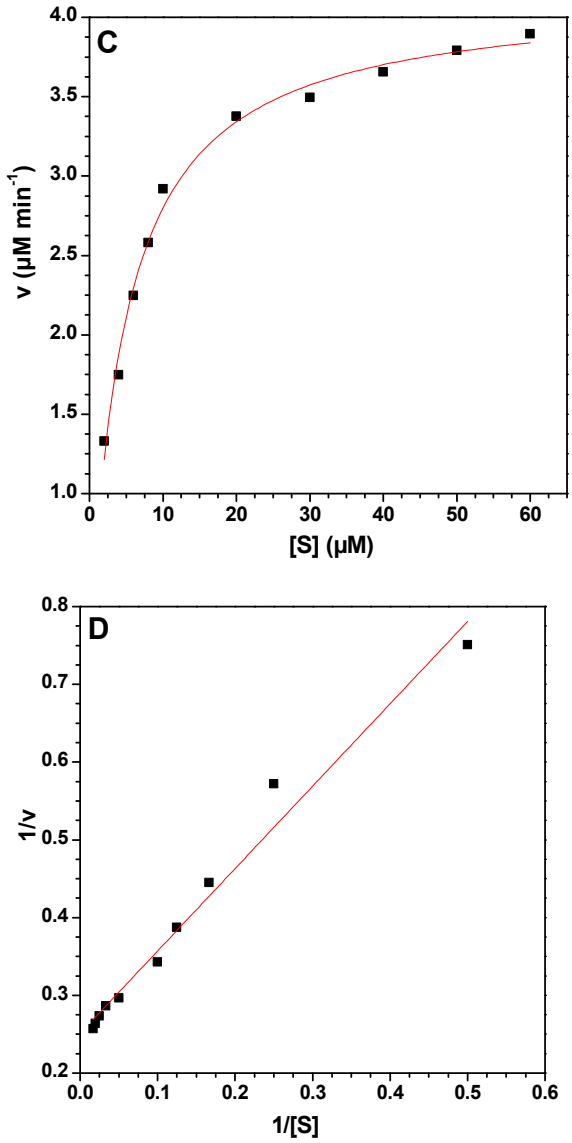


Fig. S9. Substrate dependence of the initial degradation velocities for **S4** towards lipase (A and B) and α-amylase (C and D). Initial velocities were plotted against the substrate concentrations and fit to the Michaelis-Menten model.

Table S1. Endogenous lipase and α-amylase activity in human serum samples calculated from fluorescence intensity at 460 and 610 nm by using the calibration curve (Figure 1B, D) as standard.

Subjects	Serum LPS (U L^{-1})	Serum AMY (U L^{-1})
Normal people	118.15±5.84	55.82±4.51
	125.57±6.52	108.58±3.82
	168.57±4.28	88.83±7.67
	88.49±3.34	111.64±5.89
	138.91±7.25	93.60±6.23
	121.12±7.98	141.25±2.59
	150.78±8.42	99.73±9.60
	165.61±7.27	68.68±5.24
	106.29±3.59	37.23±4.31
Acute pancreatitis patients	460.13±7.03	576.58±6.29
	525.39±5.22	515.31±7.98
	498.10±4.12	420.01±8.65
	702.16±9.74	597.00±6.84
	638.10±6.51	702.52±9.09
	652.33±5.71	729.75±7.55
	556.23±4.86	586.79±6.23
	613.18±10.07	682.09±9.74
	697.42±8.44	426.82±5.07

The serum samples were 10-fold diluted for measurement; and the values (mean ± SD) herein represent enzyme activity in the undiluted serum samples, which are calculated based on the measurements for the diluted samples.

Table S2. Results of endogenous lipase and α-amylase activity in human serum samples that obtained by the commercial lipase assay kit and α-amylase assay kit.

Subjects	Serum LPS (U L^{-1})	Serum AMY (U L^{-1})
Normal people	126.24±3.79	63.57±5.02
	145.33±6.20	107.33±6.91
	174.05±5.33	88.83±5.13
	100.29±5.81	119.28±7.52
	138.63±4.95	96.05±2.16
	129.37±7.54	152.27±6.42
	161.42±6.46	94.38±4.11
	180.59±4.08	79.72±8.07
	121.91±4.37	39.85±4.21
Acute pancreatitis patients	488.27±9.12	602.61±6.82
	519.30±5.42	537.34±7.45
	505.75±8.23	426.46±5.69
	715.39±5.78	599.06±9.76
	655.61±7.29	745.37±11.84
	659.82±7.60	764.51±8.49
	564.11±9.12	598.62±9.90
	622.97±9.45	688.53±6.41
	721.86±10.81	474.07±7.22