

Supporting Information

Mechanism of two styryl BODIPYs as fluorescent probes and protective agents in lipid bilayer against aqueous ClO^-

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I. Synthetic routes and structural characterization by NMR and HRMS

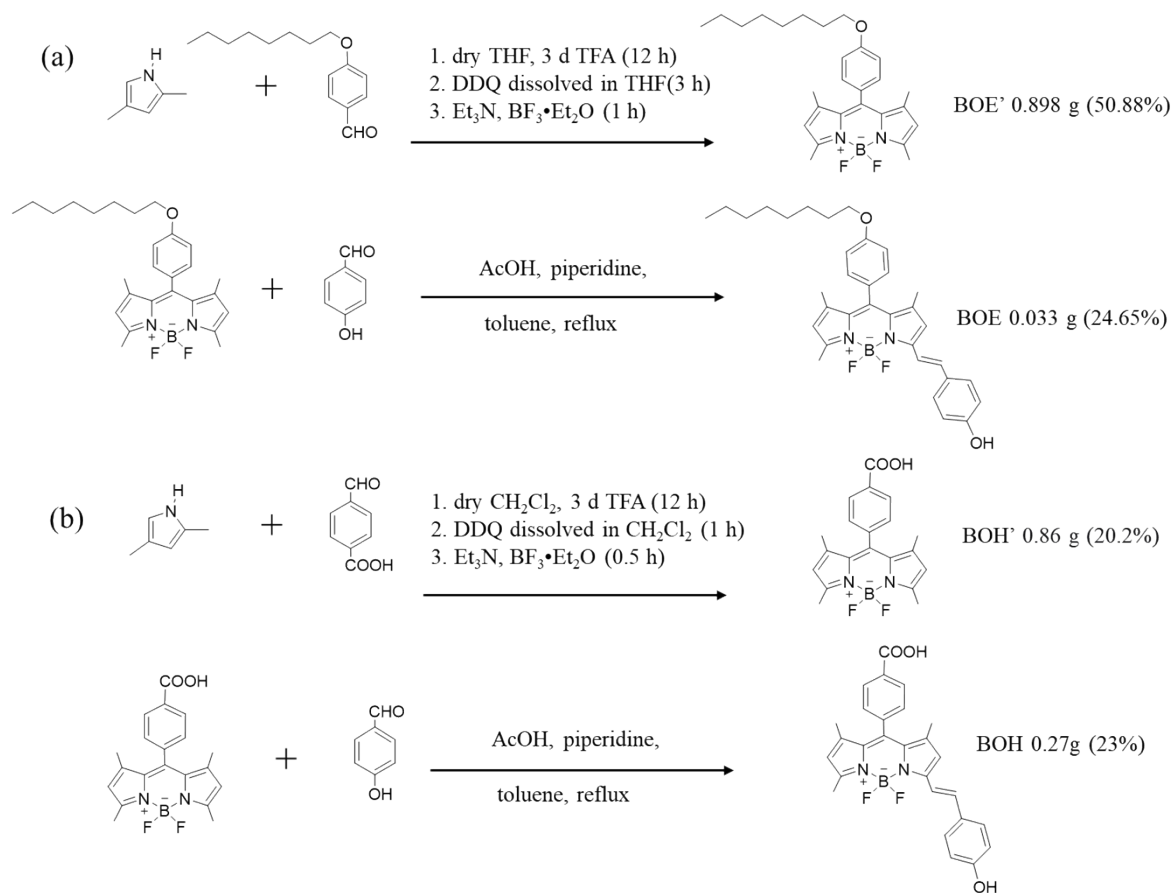


Fig. S1. Synthetic routes for BOE (a) and BOH (b). The yields were listed in parentheses.

1. Synthetic procedures of **BOE**:

4-(Octyloxy) benzaldehyde (0.8225 g, 3.51 mmol) and 2,4-dimethylpyrrole (0.6879 g, 7.23 mmol) were dissolved in dry tetrahydrofuran (THF, 90 mL) under a nitrogen atmosphere. Three drops of trifluoroacetic acid (TFA) (about 0.2 mL) were added, and the mixture was stirred at room temperature overnight. After TLC monitoring showed complete disappearance of the aldehyde, a solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.6775 g, 2.98 mmol) in anhydrous THF (75 mL) was added. This mixture was

further stirred for 3 h, then 18 mL of Et₃N and 18 mL of BF₃·OEt₂ were successively added and after reacting 1 h, the reaction mixture was washed thoroughly with water and brine, dried over anhydrous Na₂SO₄, filtered, and evaporated under vacuum. The crude product was purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate v/v ratio changing from 250/1 finally to 250/12) to obtain a shiny orange powder (0.8980 g, yield: 50.88%) as **BOE'**.

BOE' (0.1200 g, 0.27 mmol) and *p*-hydroxybenzaldehyde (0.1130 g, 0.93 mmol) were added to a 100 mL round-bottomed flask containing 16 mL toluene, piperidine (1.6 mL) and acetic acid (0.7 mL) were added to the mixed solution, and condensed at 110 °C for 17 h. After cooling to room temperature, the reaction mixture was washed thoroughly with water and brine, dried over anhydrous Na₂SO₄, filtered, and evaporated under vacuum. The crude compound was purified by silica gel column chromatography (eluent: dichloromethane/methanol v/v ratio changing from 500/1 finally to 500/4) to give a purple-black solid (0.0330 g, yield: 24.65%) as **BOE**. ¹H NMR (400 MHz, CDCl₃) δ 7.07(s, 3H), 6.92(s, 2H), 6.75(s, 2H), 6.49(s, 1H), 5.91(s, 1H), 5.21(s, 1H), 3.93(s, 2H), 2.54 (d, *J* = 39.9 Hz, 6H), 1.74(s, 3H), 1.38(m, *J* = 18.5 Hz, 10H), 0.82(s, 7H). ¹³C NMR (150 MHz, CDCl₃) δ 158.67, 156.34, 153.20, 152.41, 141.81, 141.26, 139.20, 135.37, 132.24, 131.01, 128.37, 128.16, 127.92, 125.89, 119.92, 116.42, 115.56, 114.86, 114.01, 67.17, 52.41, 39.46, 30.90, 30.79, 28.68, 28.38, 28.24, 28.21, 25.04, 21.64, 13.83, 13.65, 13.54, 13.09 ppm. HRMS (EI) *m/z* calcd for C₃₄H₃₉BF₂N₂NaO₂⁺: 579.2965; Found 579.2963.

2. Synthetic procedures of **BOH**:

2,4-dimethylpyrrole (22.10 mmol, 2.1026 g), 4-carboxybenzaldehyde (13.90 mmol, 2.0868 g), and 400 mL of CH₂Cl₂ degassed by argon were added to a 1 L round-bottomed flask, then a few drops of trifluoroacetic acid (TFA) were added, and the mixture was stirred under N₂ protection for 1 day. Then, add DDQ (9.90 mmol, 2.2474 g) dissolved in 100 mL CH₂Cl₂ to the mixture and continue stirring for 1 hour. Finally, 5 mL Et₃N and 5 mL BF₃·OEt₂ were added to the mixture and the reaction continued for 30 minutes. The mixed solution after the reaction was extracted, washed with water three times, dried with anhydrous Na₂SO₄, and evaporated solvent using a rotary evaporator to get the remaining product. By silica gel column chromatography and chloroform/methanol (v/v = 98/2) as eluent, the purified **BOH'** was obtained as red solid (0.8600 g, yield: 20.2%).

BOH' (2.50 mmol, 0.9229 g) and *p*-hydroxybenzaldehyde (7.50 mmol, 0.9159 g) were added to a 500 mL round-bottomed flask containing 200 mL toluene, piperidine (0.5 mL) and acetic acid (0.5 mL) were added to the mixture, and the mixture was heated at 110 °C. The water and toluene were separated with a Dean-Stark water separator, and the reaction was monitored with TLC. When the reaction material is completely consumed, the mixture is cooled to room temperature. After the reaction, the mixed solution was washed three times with water and dried with anhydrous Na₂SO₄. The residual product was purified by silica gel column chromatography. chloroform/methanol (v/v = 97/3) mixture was used as eluent to give purplish black solid (0.2700 g, yield: 23.0%). The characterization results were shown

in our previous work.

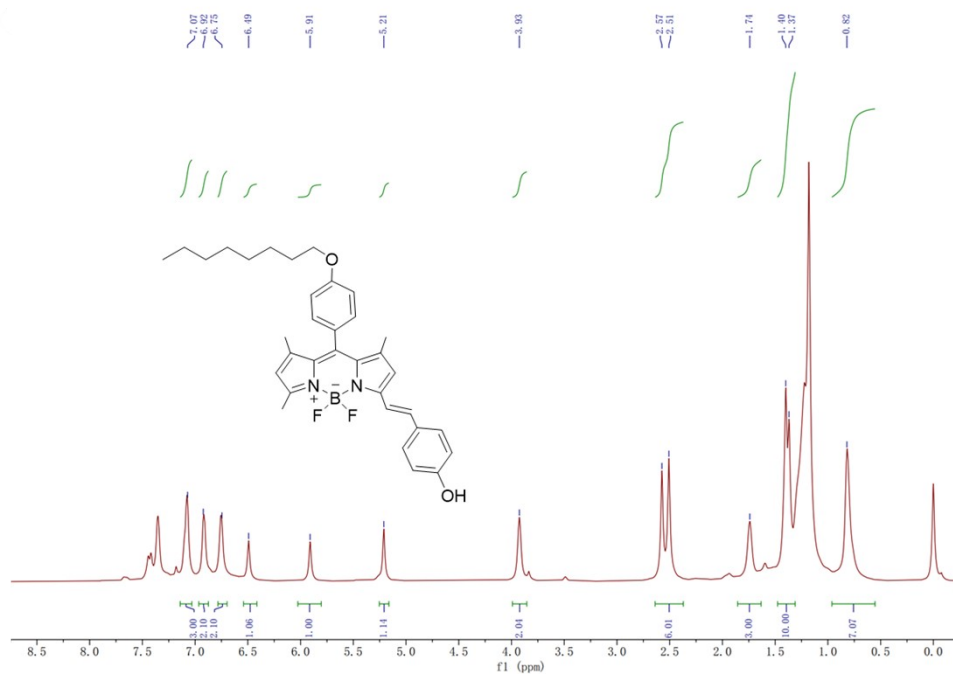


Figure S2. ¹H NMR spectrum of BOE.

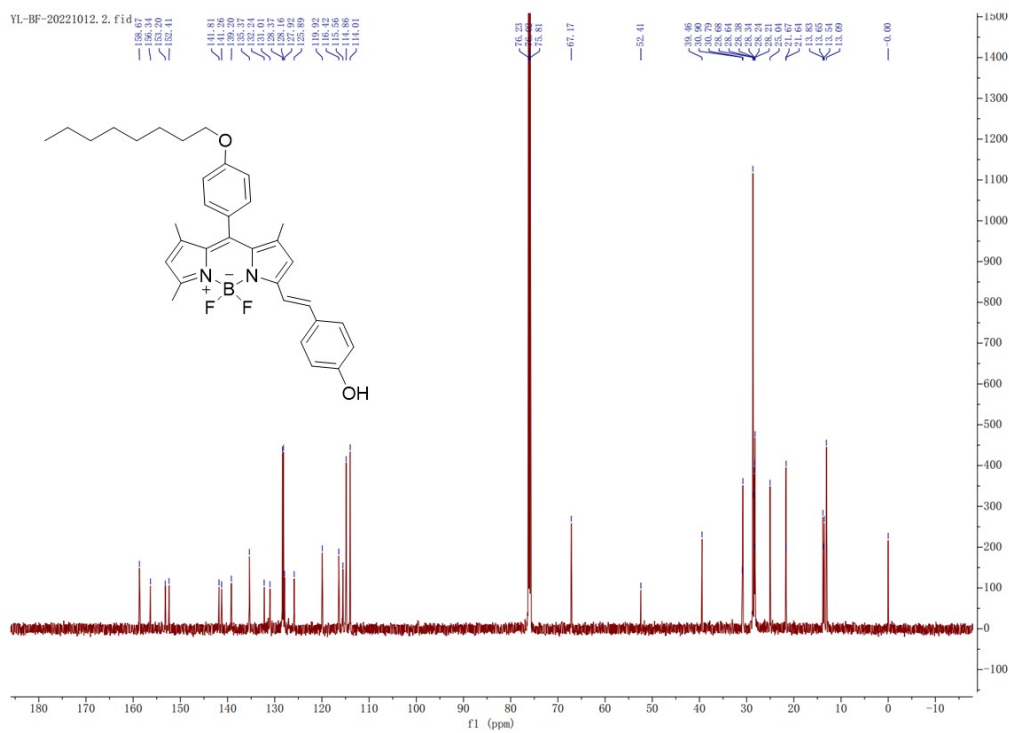


Figure S3. ¹³C NMR spectrum of BOE.

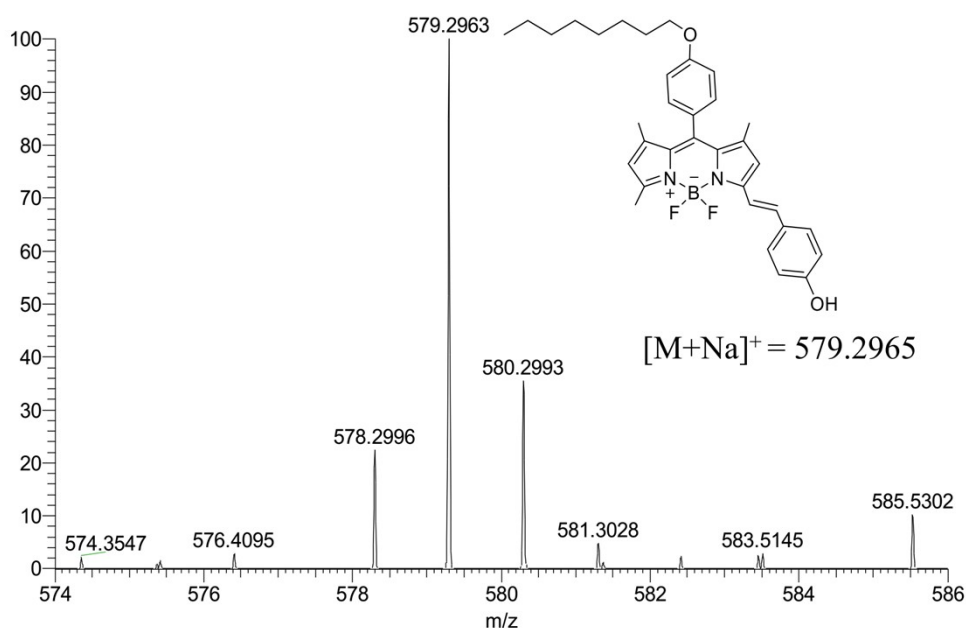


Figure S4. ESI-HRMS spectrum of BOE.

II. lipid-water partition coefficient

After thoroughly mixing 50 mL ultra-pure water and 100 mL *n*-octanol in the liquid separation funnel, set aside for 24 h (at this time the two phases have been clarified) for use. The purpose is to saturate the water phase with *n*-octanol and the organic phase with water, so as to eliminate the experimental deviation caused by the partial miscibility of the two phases in the experiment. 9 mL of BOH or BOE solution was prepared with the above organic phase, and 3 mL of the solution was taken to determine the absorption spectrum. Take another 5 mL and store it in a sealed test tube, then add 5 mL of water phase, mix it fully and place it for 4 hours until it is clarified, and measure the absorption spectrum of the upper organic phase.

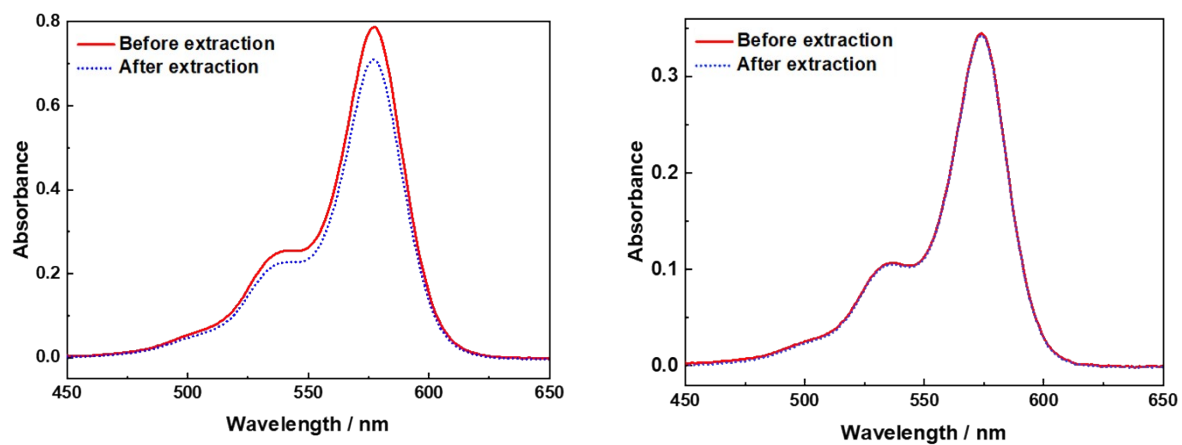
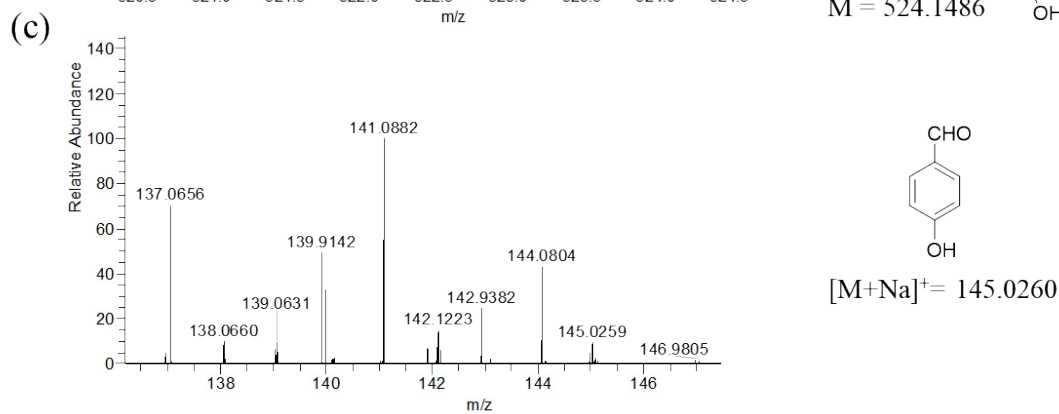
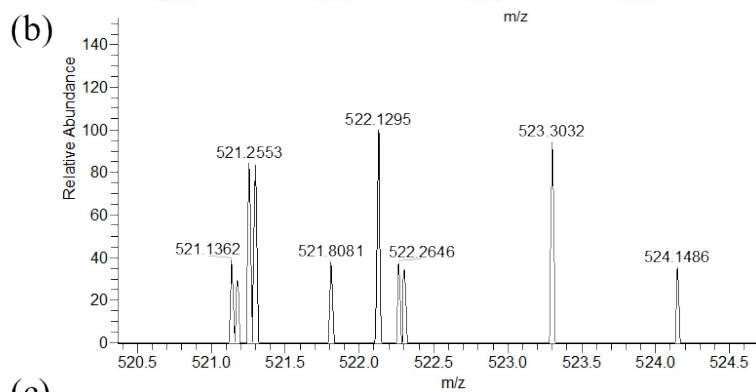
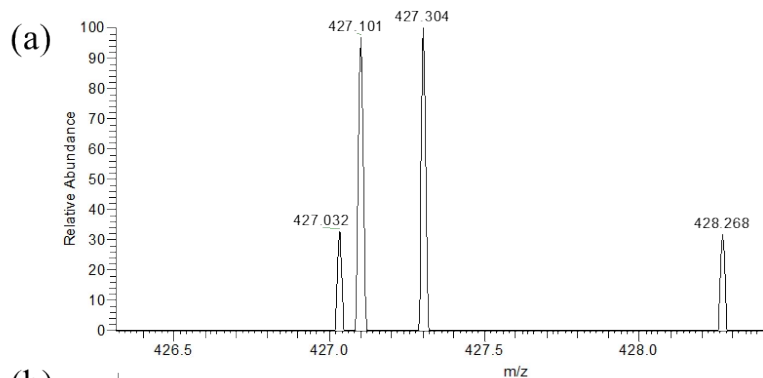


Figure S5. Absorption spectra of BOH (a) and BOE (b) in organic phase (*n*-octanol) before and after aqueous phase extraction (solid line).

III. ESI-HRMS characterization of oxidative products of BOH and BOE



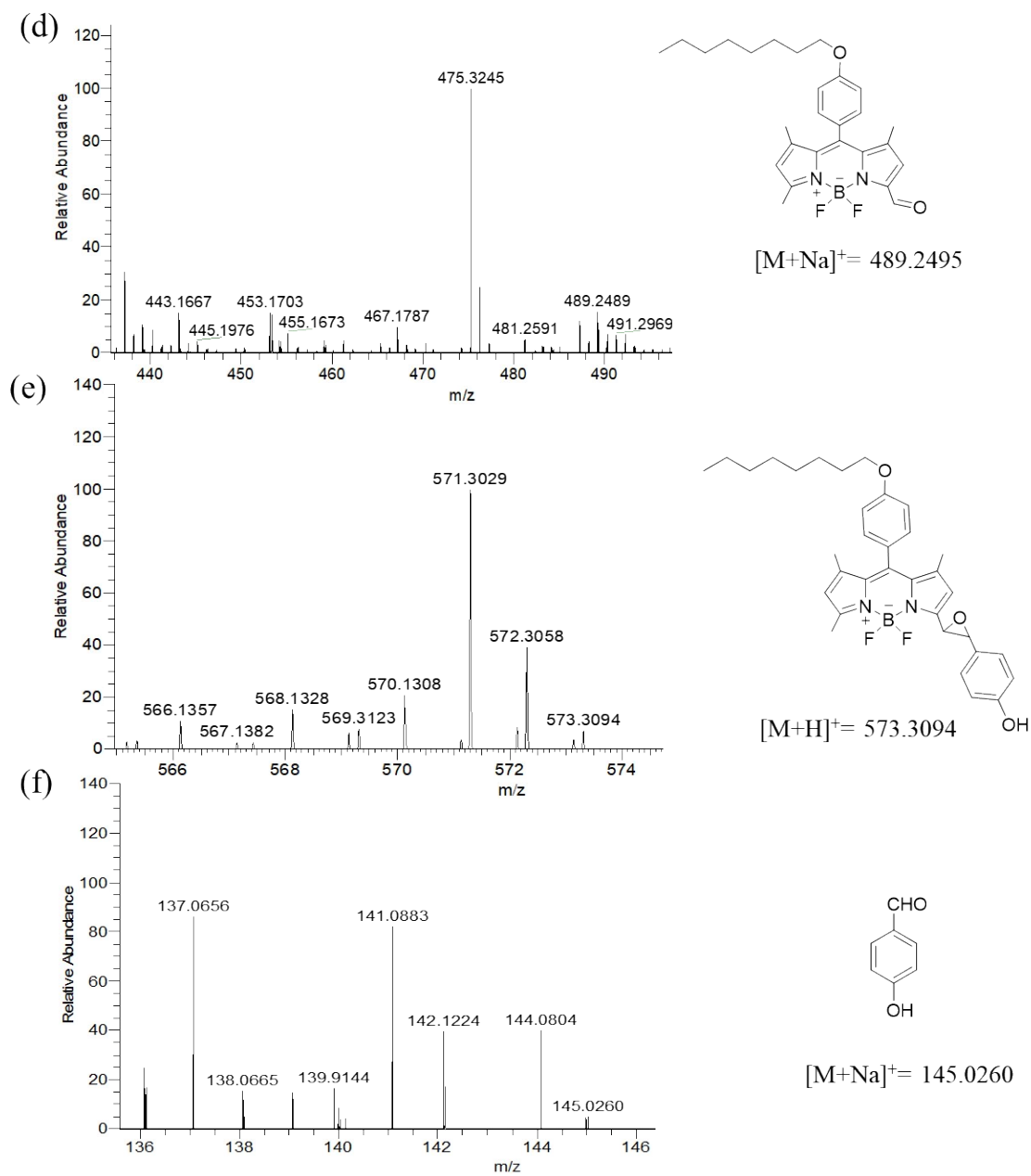


Figure S6. ESI-HRMS spectra of oxidation products of BOH (a), (b), (c) and BOE (d), (e), (f) reacting with ClO⁻/HClO, respectively.

IV. GUV morphology changes over time

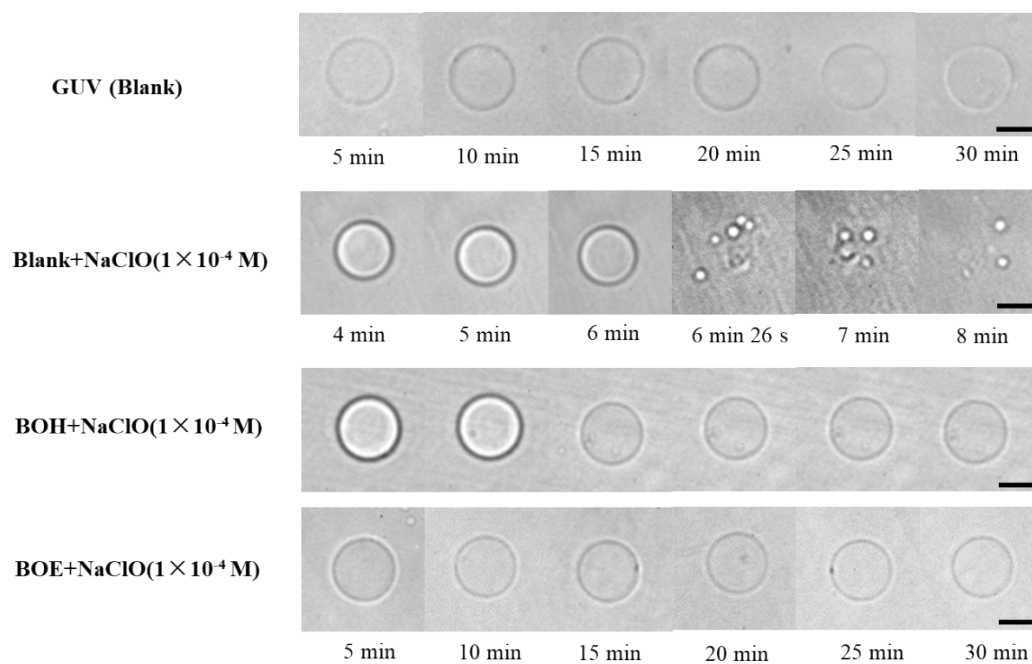
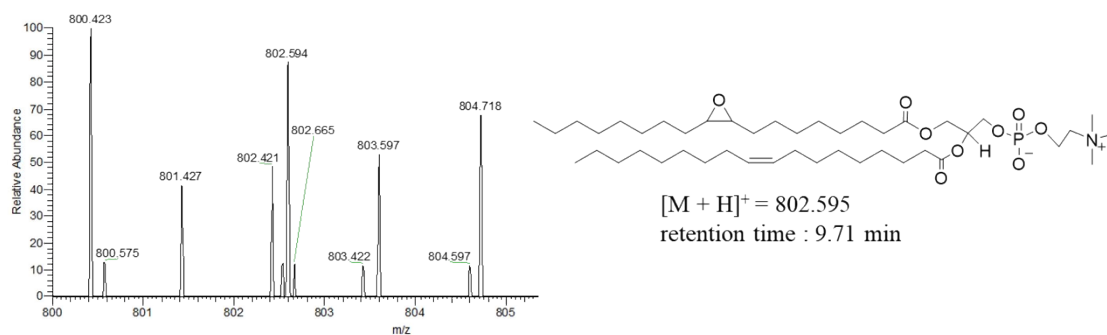
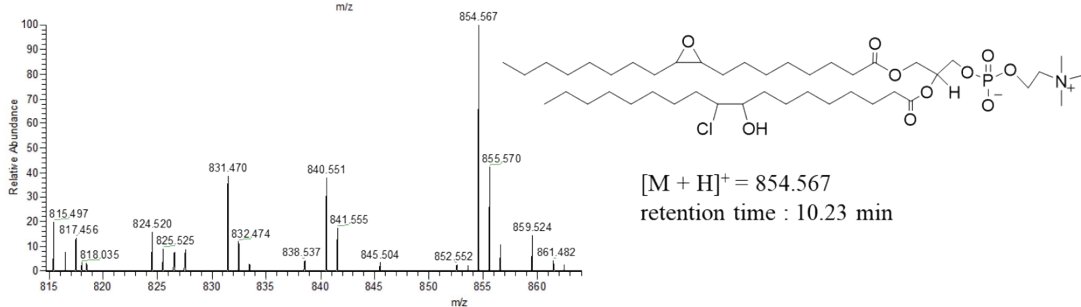
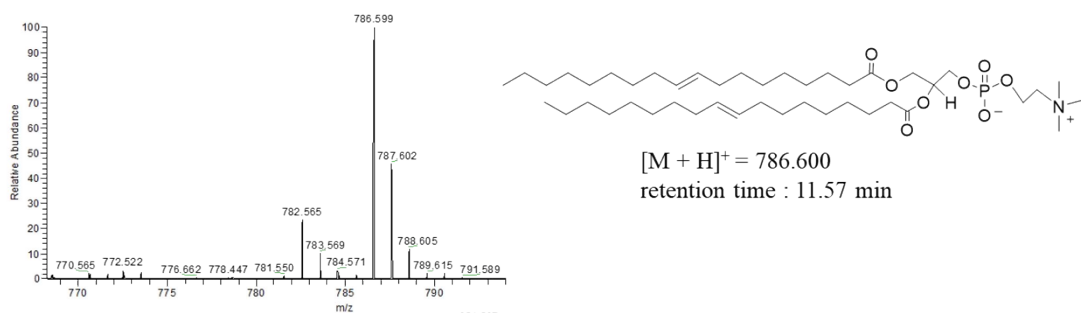
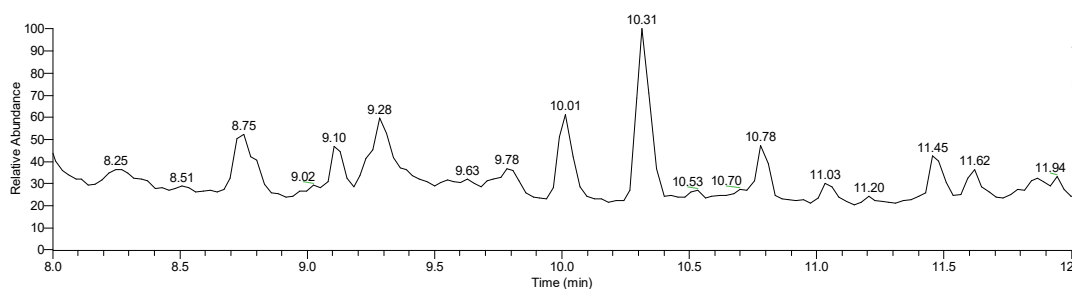
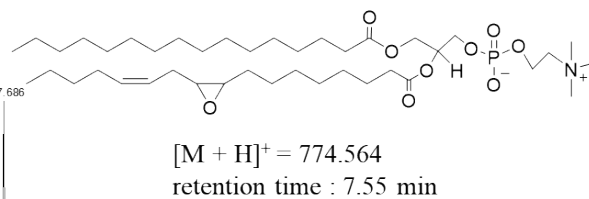
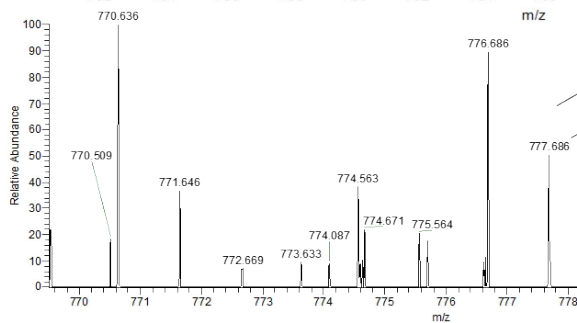
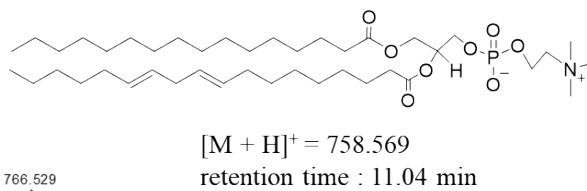
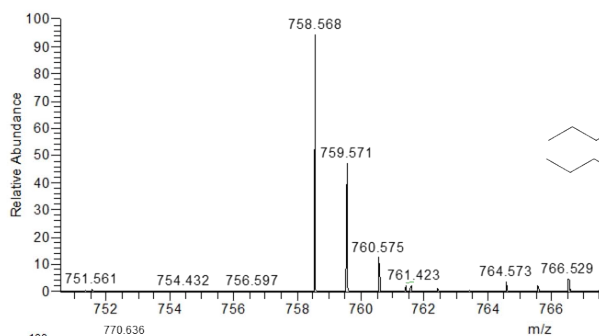
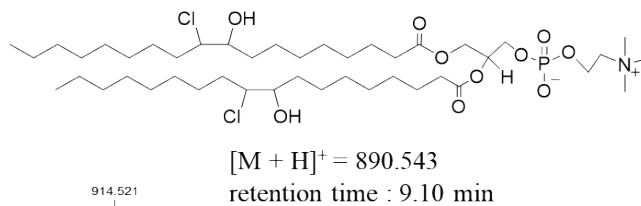
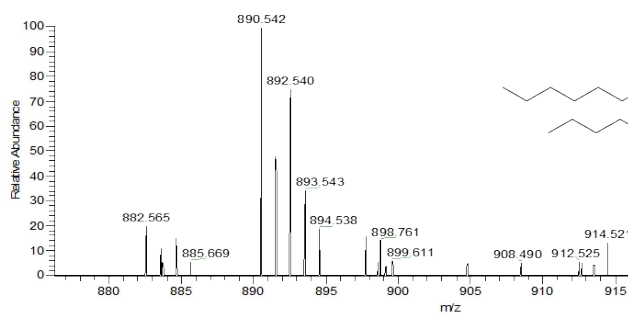
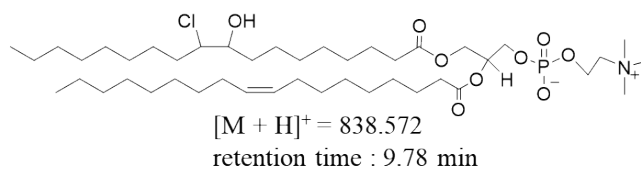
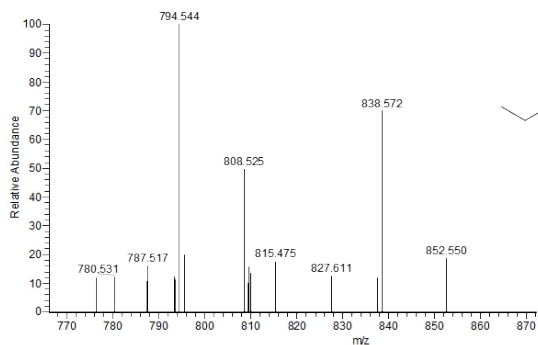
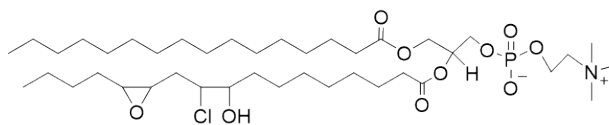
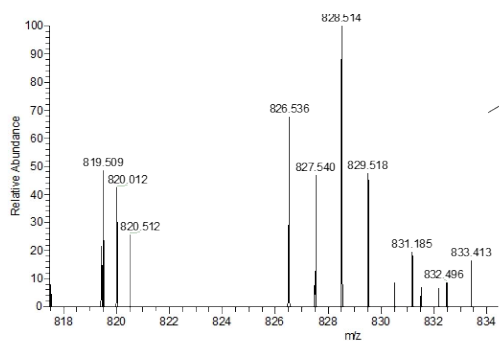


Figure S7. Image of GUV morphological change oxidized by ClO^-/HClO over time. (a) Blank GUV (control); (b) GUV with NaClO (1×10^{-4} M); (c) GUV with BOH (5×10^{-7} M) and NaClO (1×10^{-4} M); (d) GUV with BOE (5×10^{-7} M) and NaClO (1×10^{-4} M). Scale bar = 10 μm .

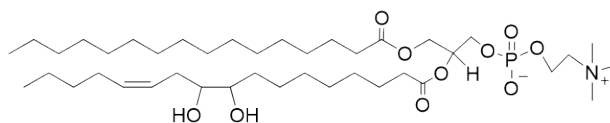
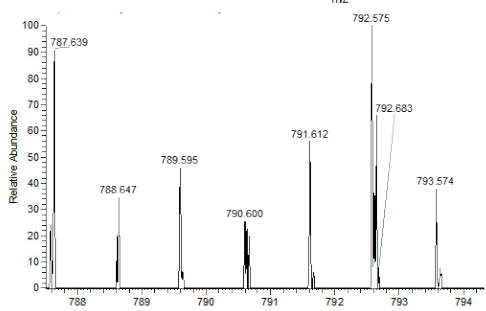
V. HPLC-HRMS results of the oxidized products of PLPC and DOPC



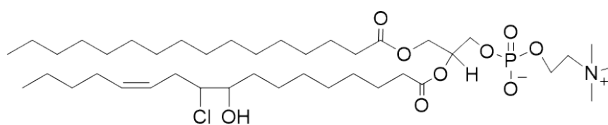
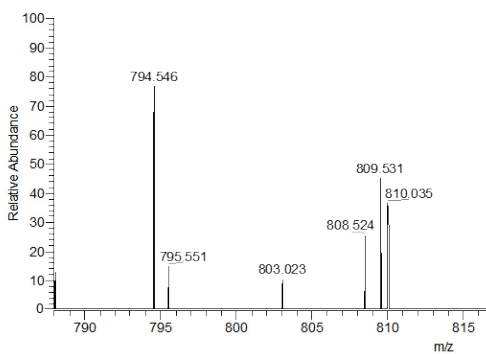




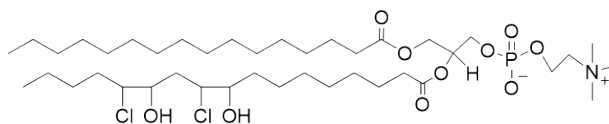
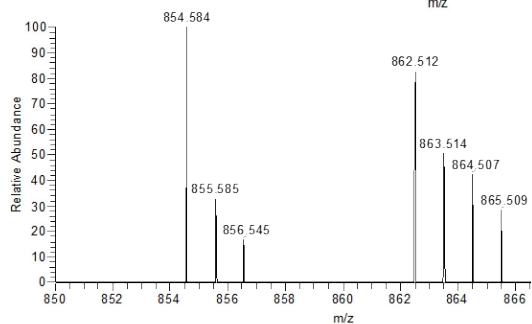
$[M + H]^+ = 826.535$
retention time : 8.27 min



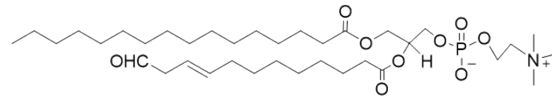
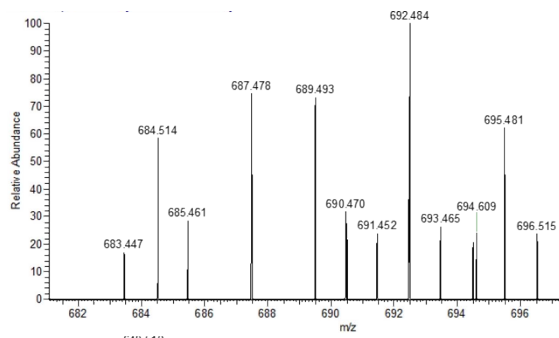
$[M + H]^+ = 792.574$
retention time : 8.29 min



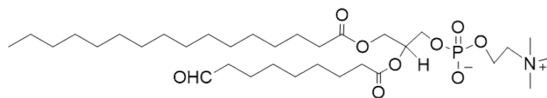
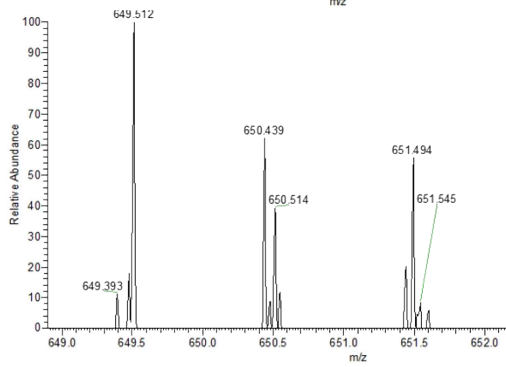
$[M + H]^+ = 809.532$
retention time : 9.65 min



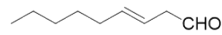
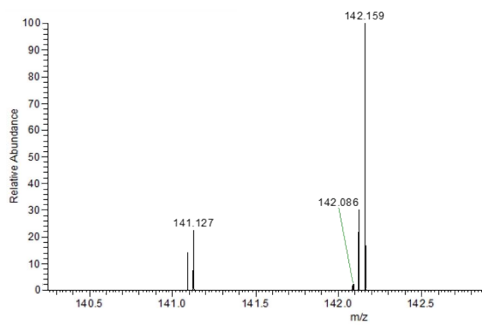
$[M + H]^+ = 862.512$
retention time : 8.25 min



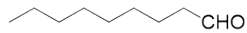
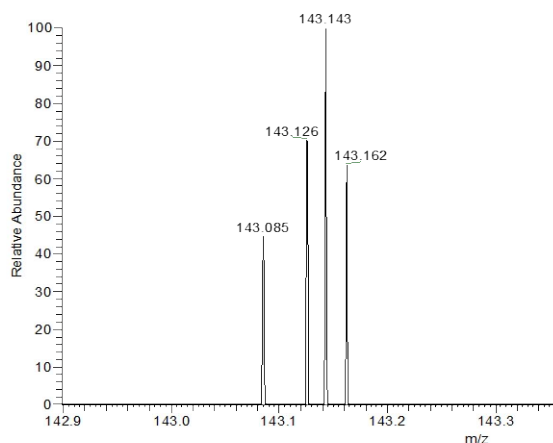
$[M+H]^+ = 690.470$
retention time : 2.89 min



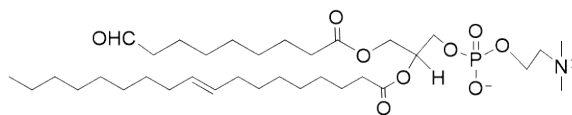
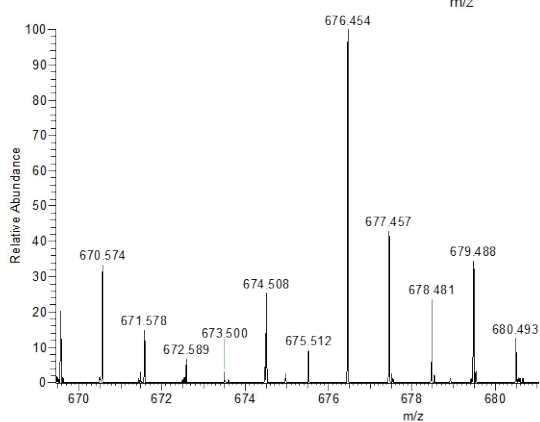
$[M+H]^+ = 650.439$
retention time : 6.31 min



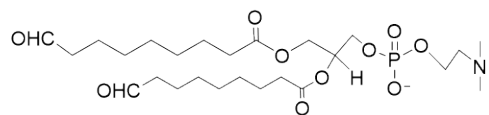
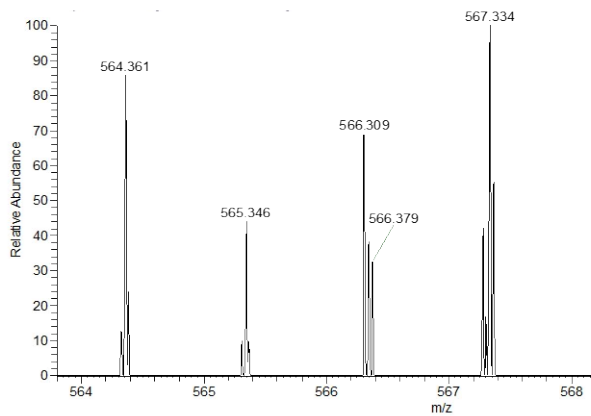
$[M+H]^+ = 141.127$
retention time : 1.65 and 6.77 min



$[M+H]^+ = 143.143$
retention time : 2.73 min



$[M+H]^+ = 676.455$
retention time : 6.55 min



$[M+H]^+ = 566.309$
retention time : 1.12 min

Figure S8. HPLC profile and the HRMS of the oxidized products of PLPC and DOPC reacting with NaClO (10^{-4} M) for 40min and their chemical structure formula.

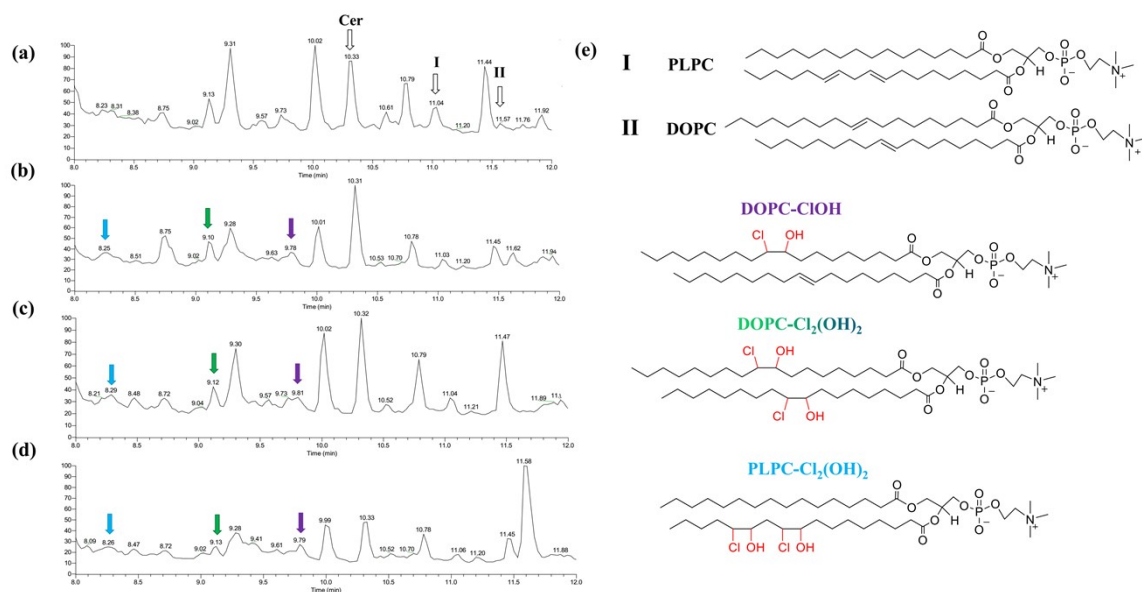


Figure S9. HPLC elution profiles of different GUUV preparations. (a) Blank GUUV (control). (b) Blank GUUV, (c) GUUV containing BOH (5×10^{-7} M) and (d) GUUV containing BOE (5×10^{-7} M), reacting with HClO/ClO⁻ (5×10^{-5} M) for 40 min, respectively. The measured contents of PLPC-Cl₂(OH)₂, DOPC-Cl₂(OH)₂ and DOPC-ClOH are 1.06%, 0.87% and 0.93% in (b), 0.42%, 0.34% and 0.46% in (c), and 0.30%, 0.14% and 0.19% in (d), respectively. Internal label Cer is Ceramide.