Electronic Supplementary Information

Enzyme-responsive Sulfatocyclodextrin/Prodrug Supramolecular Assembly for Controlled Release of Anticancer Drug Chlorambucil

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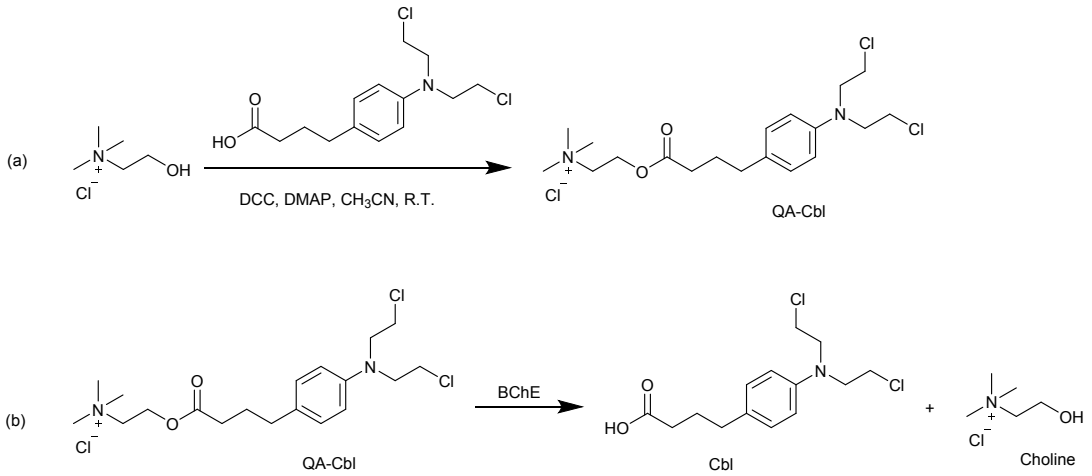
Experimental Section.

Materials. Choline chloride (Meryer), Chlorambucil (J&K), DCC (Energy Chemical), and DMAP (Amethyst) were used without further purification. BChE (from equine serum, 100U/9mg protein) was purchased from Shanghai Yuanye Bio-Technology Co. Trypsin from porcine pancreas. Glucose oxidase (GOx) was purchased from Sigma-Aldrich. Sulfato-β-cyclodextrin (SCD, sulfated sodium salt extent of labeling: 12-15 mol per mol β-CD) was purchased from Sigma-Aldrich. PBS phosphate buffer (0.01 M, pH 7.2-7.4) was purchased from Tianjin Dingguo Bio-Technology Co. All of these were used without further purification.

Preparation of the supramolecular assembly. Before each experiment, 1 mM SCD stock solution and 2 mM QA-Cbl stock solution were prepared with PBS buffer solution. Then the corresponding volume of SCD stock solution and QA-Cbl stock solution were absorbed and mixed with a pipette gun, and then PBS buffer solution was added until 3 mL. The mixing concentration for the amphiphilic assembly was 0.04 mM (120 μL) for SCD and 0.70 mM (1050 μL) for QA-Cbl.

Synthesis of QA-Cbl. Chlorambucil (915 mg, 3 mmol) and choline chloride (500mg, 3 mmol) were dissolved in dry CH_3CN (50 mL), and 4-dimethylaminopyridine (DMAP, 73 mg, 0.6 mmol) and dicyclohexylcarbodiimide (DCC, 750 mg, 3.6 mmol) were added under nitrogen atmosphere. The mixture was stirred over 24 h at room temperature. The insoluble DCU was removed by filtration, then the filtrate was removed via rotary evaporation under a reduced pressure, and yellow oil was obtained. The crude products were purified by neutral alumina column chromatography (dichloromethane/methanol = 15:1, v/v) to afford QA-Cbl as an oil. The resulting oil was dissolved in dichloromethane (200 mL) and then added dropwise to absolute ether. The lower layer of oil was collected by centrifugation. The product was dried overnight under vacuum (yield: 400 mg, 31.3%). ^{1}H NMR (400 MHz, D_2O) δ 7.09 (d, J = 8.3 Hz, 2H), 6.74 (d, J = 8.4 Hz, 2H), 4.43 (s, 2H), 3.75 – 3.54 (m, 10H), 3.15 (s, 9H), 2.51 (t, J = 7.2 Hz, 2H), 2.38 (t, J = 7.3 Hz, 2H), 1.90 – 1.78 (m, 2H). ^{13}C NMR (101 MHz, D2O): δ 172.7, 144.4, 130.0, 129.7, 112.2, 64.74, 58.1, 54.2, 53.5, 40.6, 33.8, 33.1, 26.4. HRMS: m/z 389.1761 ([QA-Cbl – Cl]$^+$, calcd for $C_{19}H_{31}Cl_2N_2O_2^+$, 389.1763).

Scheme 1. (a) Synthetic route for the prodrug QA-Cbl. (b) Butyrylcholinesterase hydrolysis QA-Cbl.



UV/Vis spectroscopy. The optical transmittance was measured in a quartz cell (light path 10 mm) on a Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller.

TEM. High-resolution TEM images were acquired using a Tecnai 20 high-resolution transmission electron microscope operating at an accelerating voltage of 200 keV. The sample for high-resolution TEM measurements was prepared by dropping the solution onto a copper grid, and the grid was then air-dried.

DLS. The sample solution for DLS measurements was prepared by filtering the solution through a 450 nm Millipore filter into a clean scintillation vial. The samples were examined on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (TurboCorr) at 636 nm at a scattering angle of 90°.

NMR Spectroscopy. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a Bruker AV400 spectrometer at 25 °C.

Cell Experiments. The A549 tumor cells were cultured in F12 medium containing 10% fetal bovine serum for 24 h. The complex ([SCD]=6.4 μM, [QA-Cbl]=112 μM) or the complex and BChE ([SCD]=6.4 μM, [QA-cbl]=112 μM, BChE with 0.032 U/mL) were added to the cell

wells. The cells were further cultured for 24 h, stained with PI (5 μg/mL) for 5 min, and observed by fluorescence microscopy. The percent of dead cells were calculated by the number of PI-positive cells divided by the number of total cells * 100%.

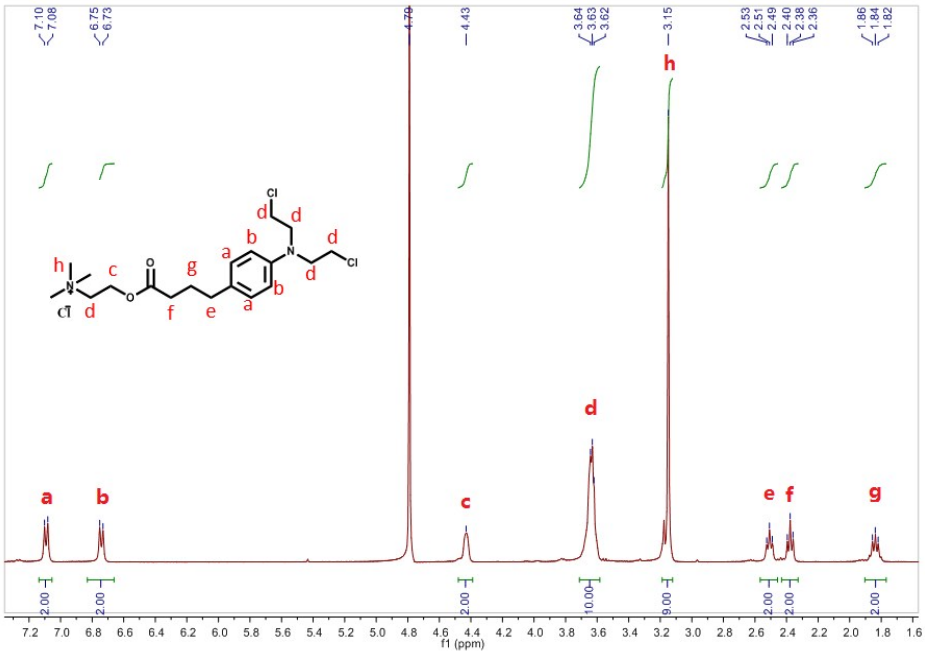


Figure S1. ^{1}H NMR spectrum (400 MHz, D_2O, 25°C) of QA-Cbl.

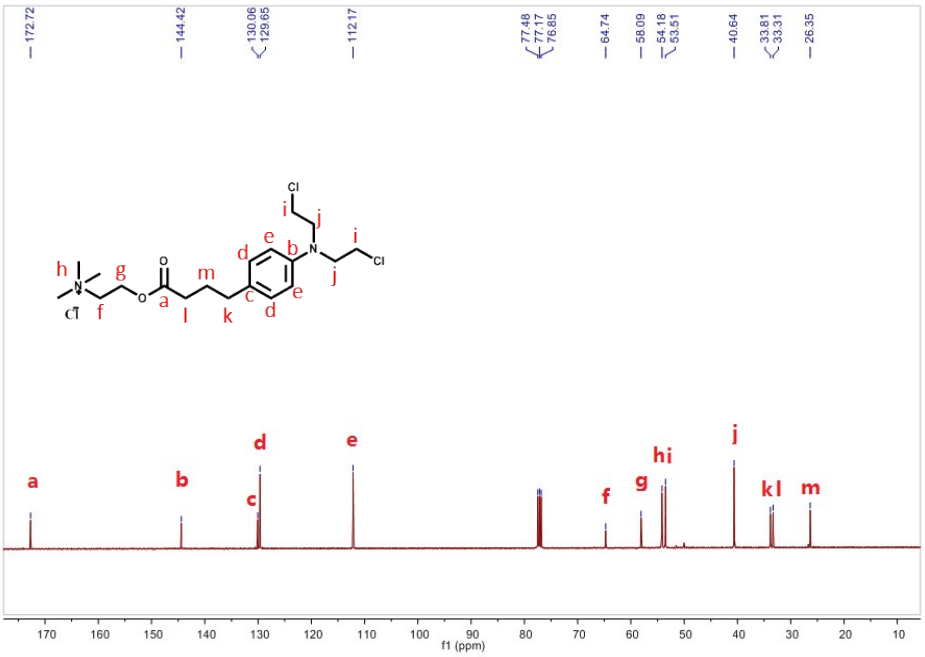


Figure S2. ^{13}C NMR spectrum (101 MHz, $CDCl_3$, 25°C) of QA-Cbl.

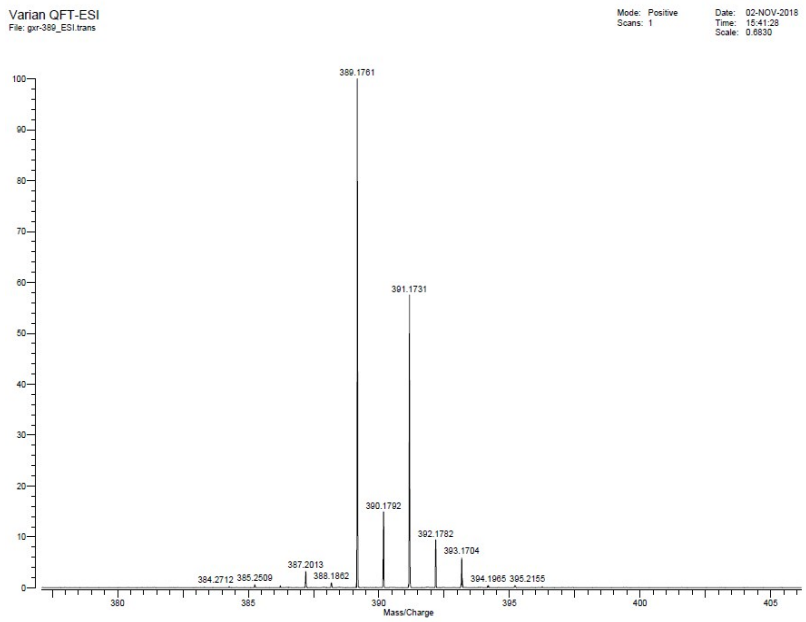


Figure S3. HRMS spectra of QA-Cbl.

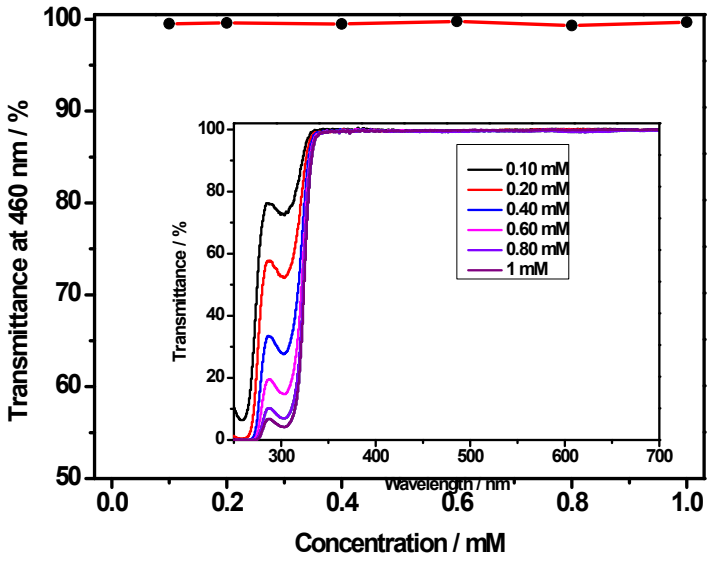


Figure S4. Dependence of the optical transmittance at 460 nm on QA-Cbl concentration. Inset: optical transmittance of QA-Cbl at different concentrations at 25 °C in PBS.

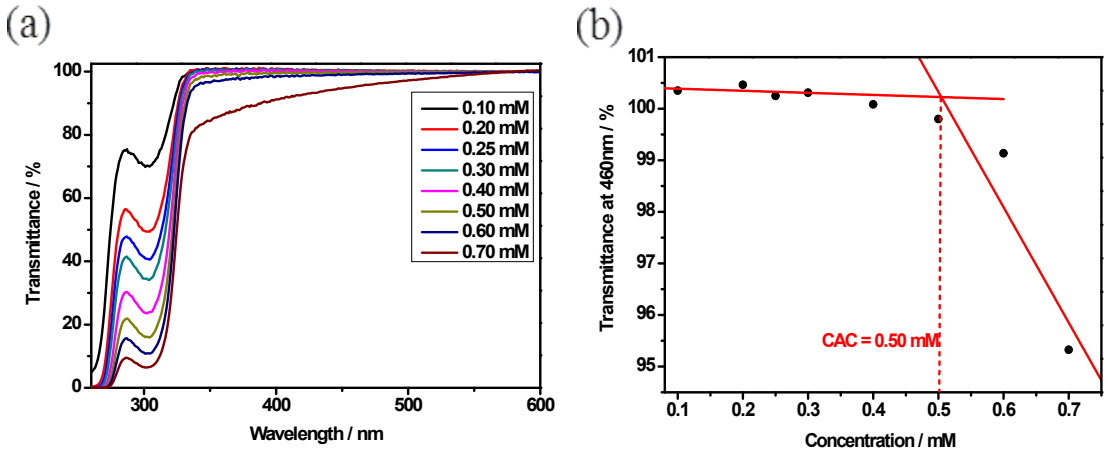


Figure S5. (a) Optical transmittance of QA-Cbl at different concentrations in the presence of SCD (0.03 mM) at 25 °C in PBS. (b) Dependence of the optical transmittance at 460 nm on QA-Cbl concentration in the presence of SCD (0.03 mM).

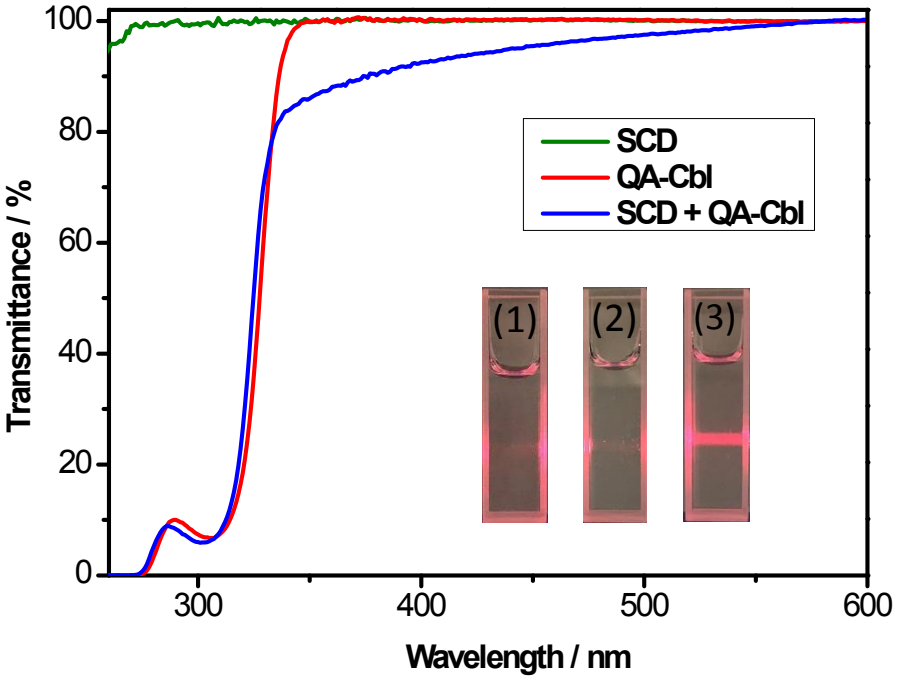


Figure S6. Optical transmmitance of SCD, QA-Cbl, and SCD+QA-Cbl assembly at 25 °C in PBS. [SCD] = 0.04 mM, [QA-Cbl] = 0.70mM. Inset: Tyndall effect of free SCD (1), free QA-Cbl (2), SCD+QA-Cbl (3).

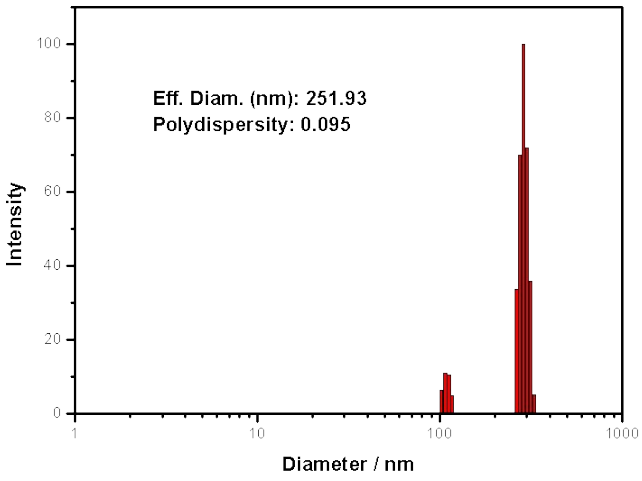


Figure S7. DLS data of the SCD/QA-Cbl assembly at 25 °C in PBS.

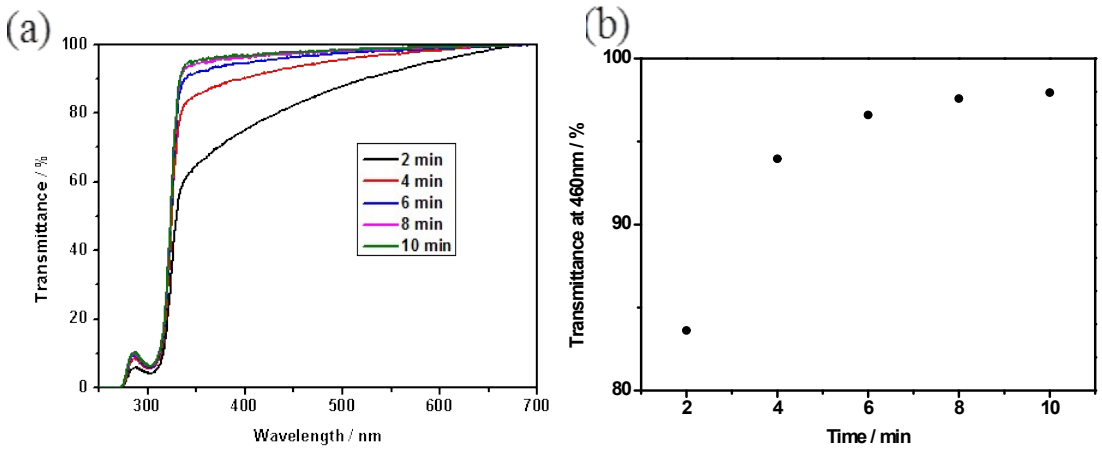


Figure S8. (a) Optical transmittance of SCD/QA-Cbl assembly at different time within 10 minutes in the presence of 0.2 U/mL BChE. (b) Dependence of the optical transmittance at 460 nm on time in the presence of 0.2 U/mL BchE.

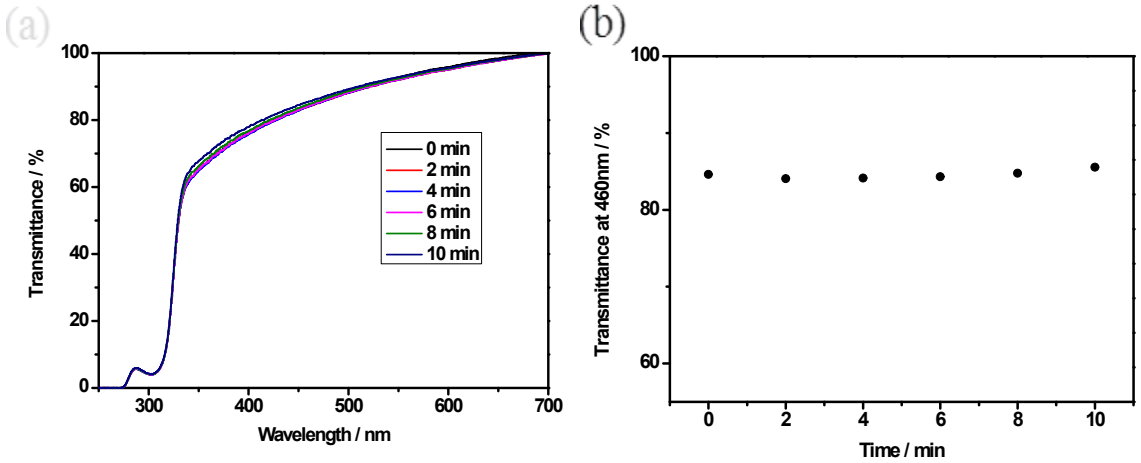


Figure S9. (a) Optical transmittance of SCD+QA-Cbl assembly in 10minutes. (b) Dependence of the optical transmittance at 460 nm on time.

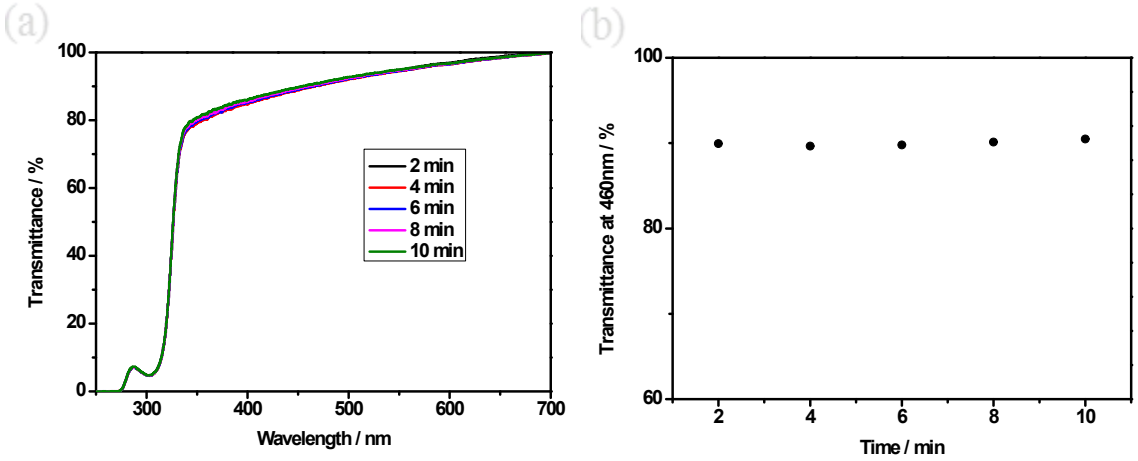


Figure S10. (a) Optical transmittance of SCD+QA-Cbl assembly at different time within 10 minutes in the presence of 0.2 U/mL denatured BChE. (b) Dependence of the optical transmittance at 460 nm on time in the presence of 0.2 U/mL denatured BChE.

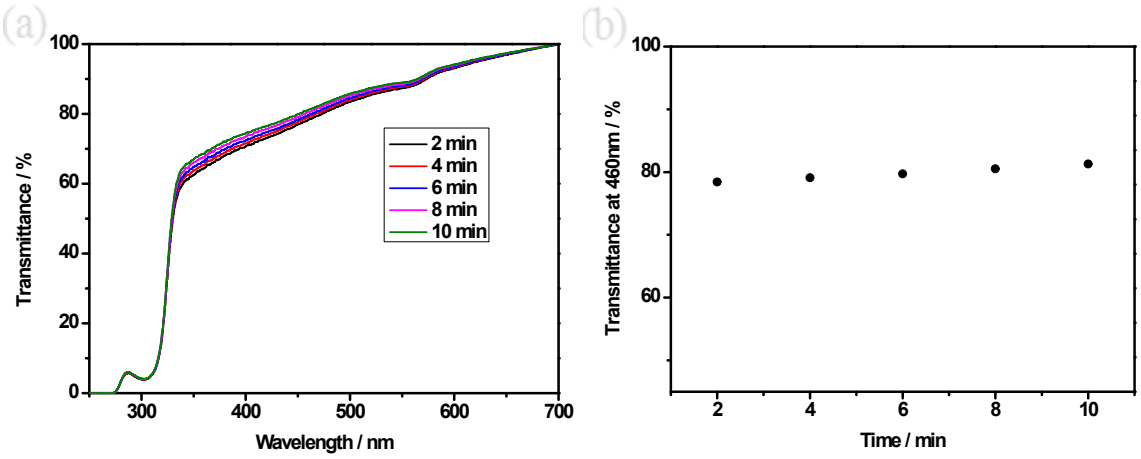


Figure S11. (a) Optical transmittance of SCD+QA-Cbl assembly at different time within 10 minutes in the presence of trypsin. (b) Dependence of the optical transmittance at 460 nm on time in the presence of trypsin.

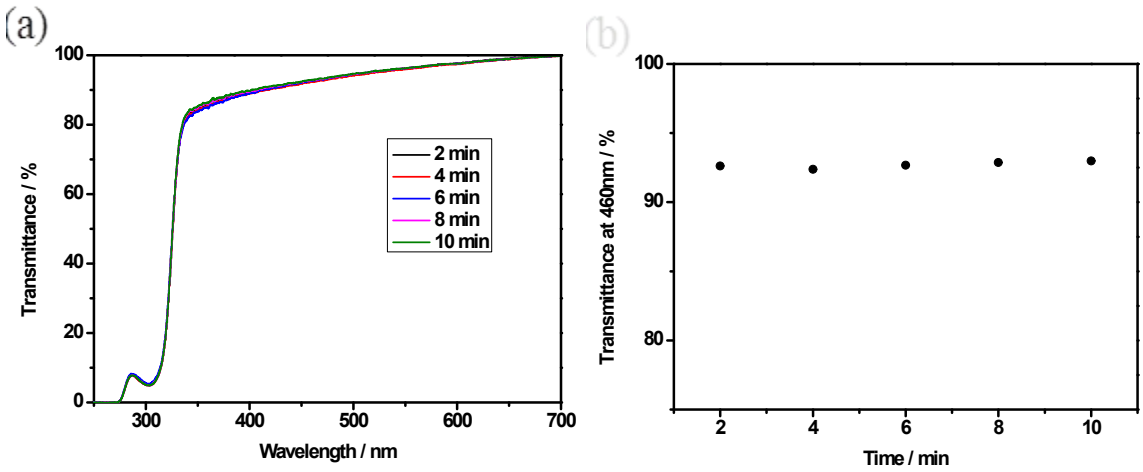


Figure S12. (a) Optical transmittance of SCD+QA-Cbl assembly at different time within 10 minutes in the presence of 0.2 U/mL GOx. (b) Dependence of the optical transmittance at 460 nm on time in the presence of 0.2 U/mL GOx.

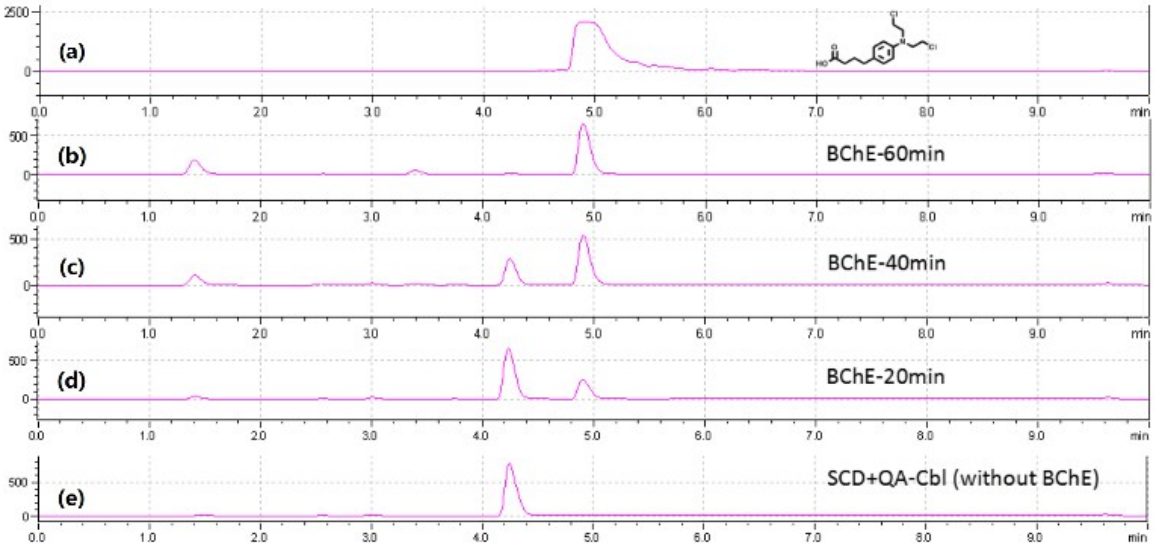


Figure S13. Typical HPLC chromatogram of Cbl (a), the SCD/QA-Cbl assembly after addition of 0.2 U/mL BChE at 60min (b), 40min (c), 20min (d) and the SCD/QA-Cbl assembly without BChE (e). Peaks in the chromatograms were detected by monitoring the absorption at 254 nm.

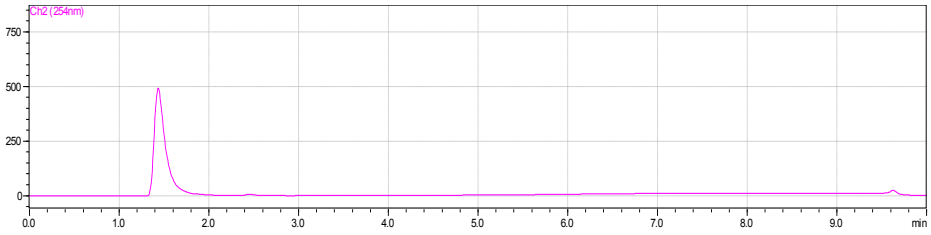


Figure S14. Typical HPLC chromatogram of the aqueous solution of the guest molecule QA-Cbl after stand for three days at room temperature. Peaks in the chromatograms were detected by monitoring the absorption at 254 nm.

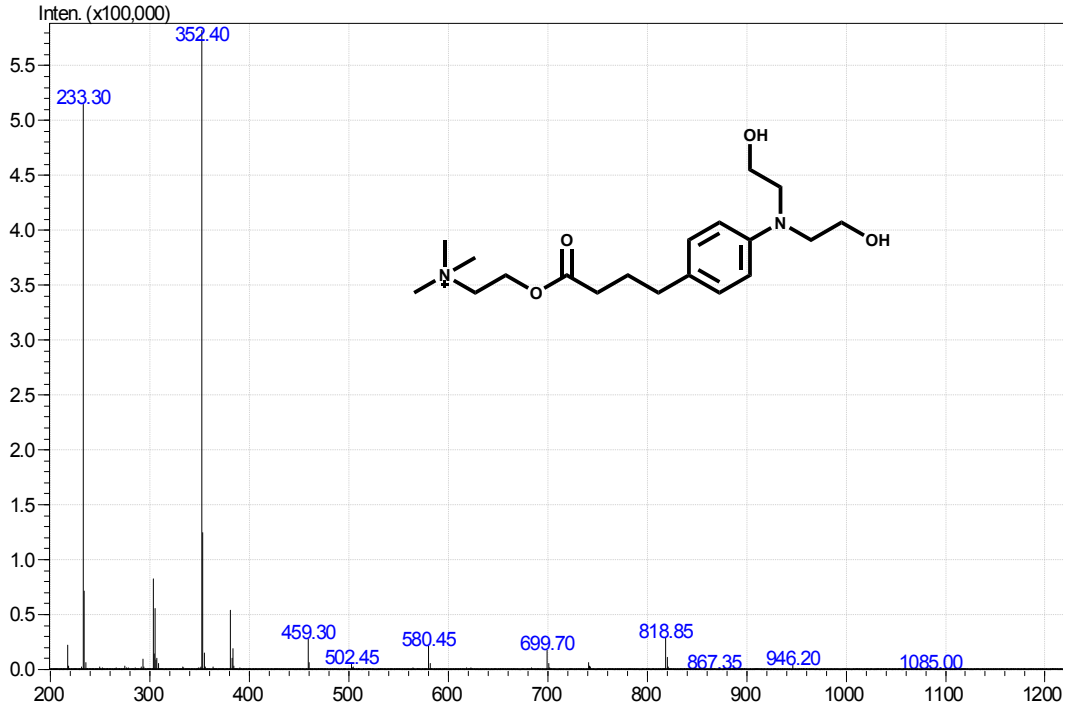


Figure S15. MS spectra of the aqueous solution of the guest molecule QA-Cbl after stand for three days at room temperature. Assignment of the main peak: m/z 352.4 ($[M - Cl]^+$, calcd for $C_{19}H_{33}N_2O_4^+$, 353.24).

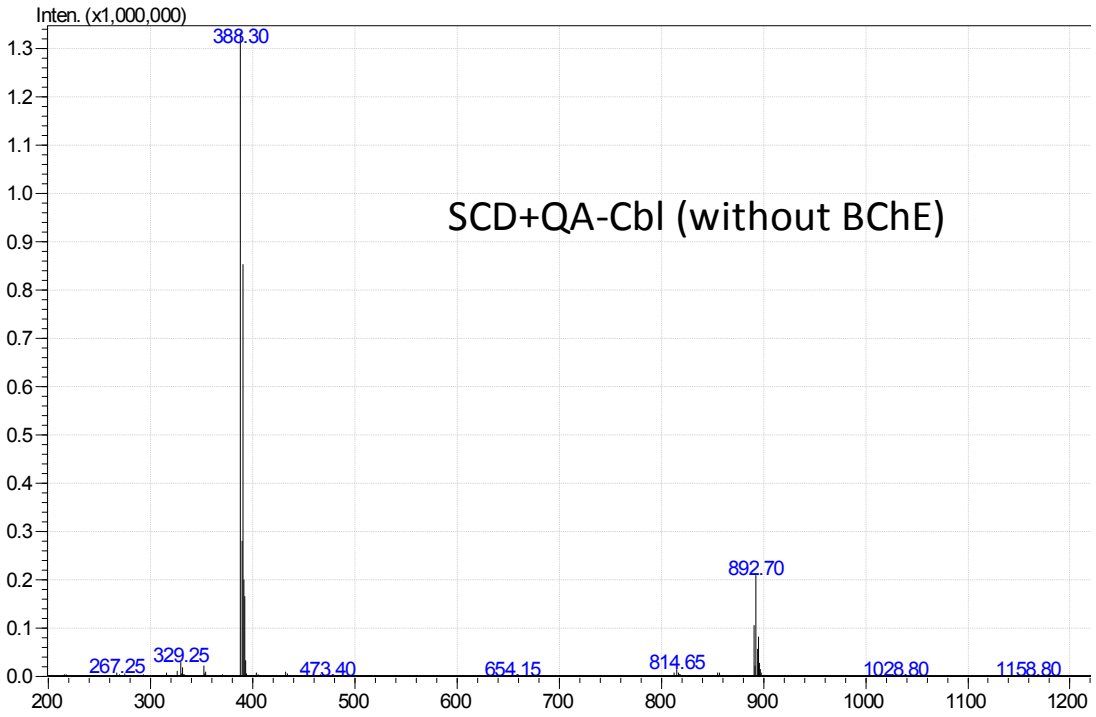
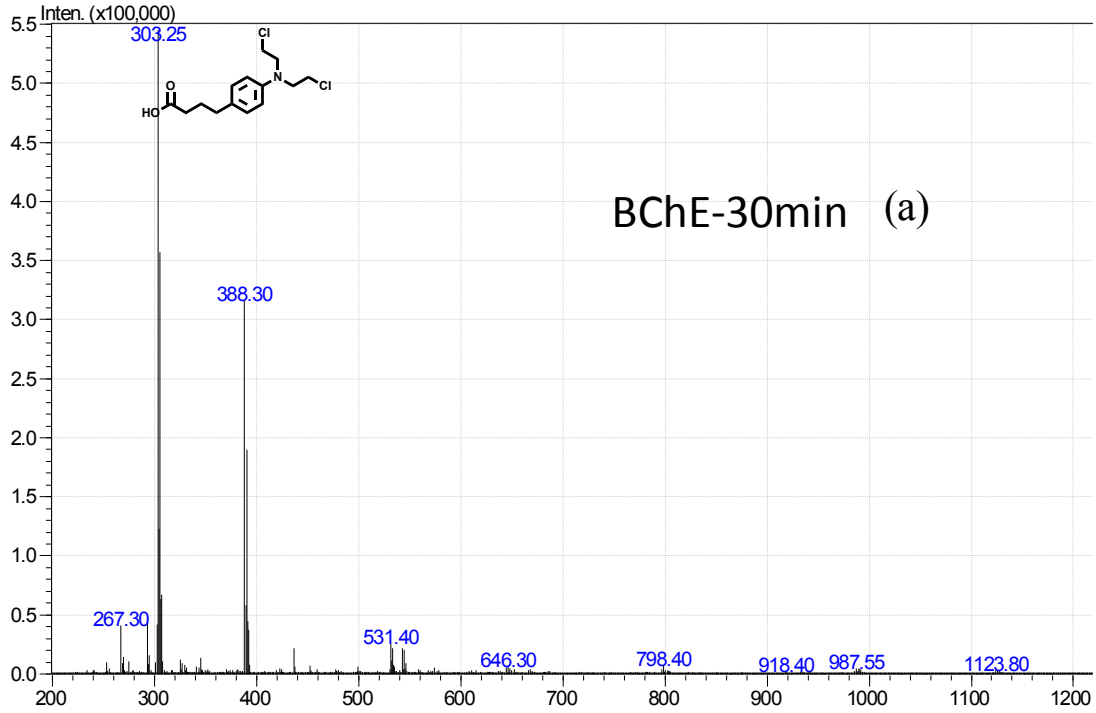


Figure S16. MS spectra of the SCD+QA-Cbl assembly without BChE.



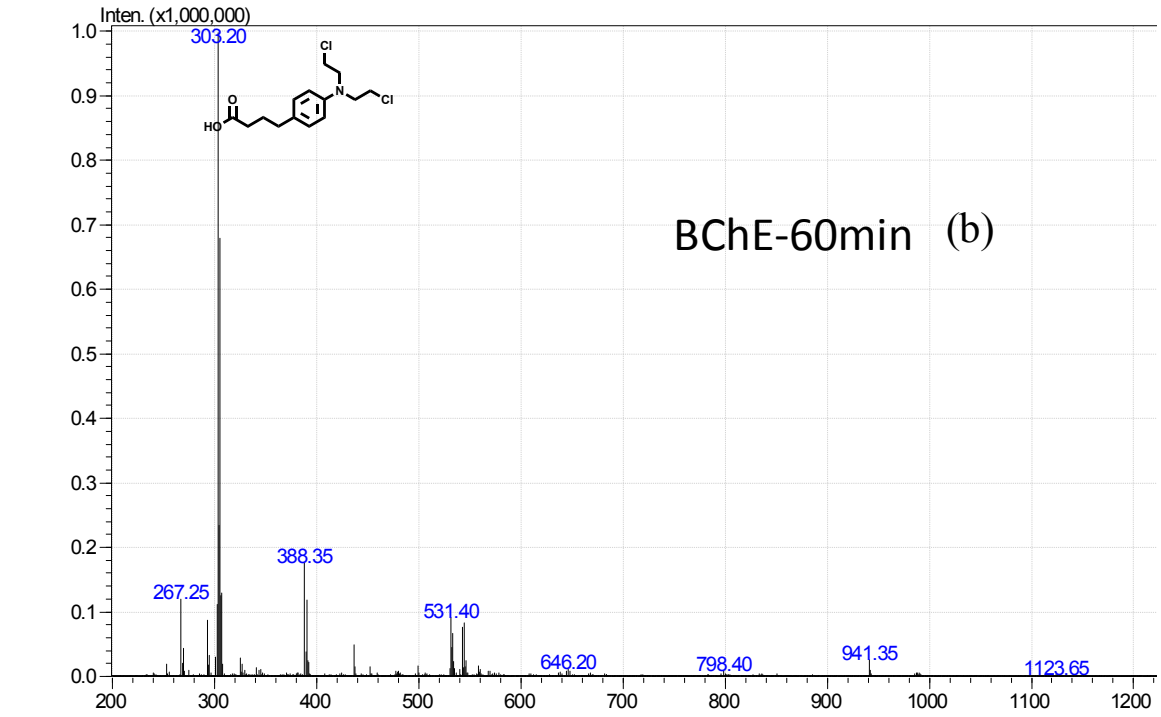


Figure S17. MS spectra of the SCD+QA-Cbl assembly at different time after addition of BChE (30 min for (a), 60 min for (b)).

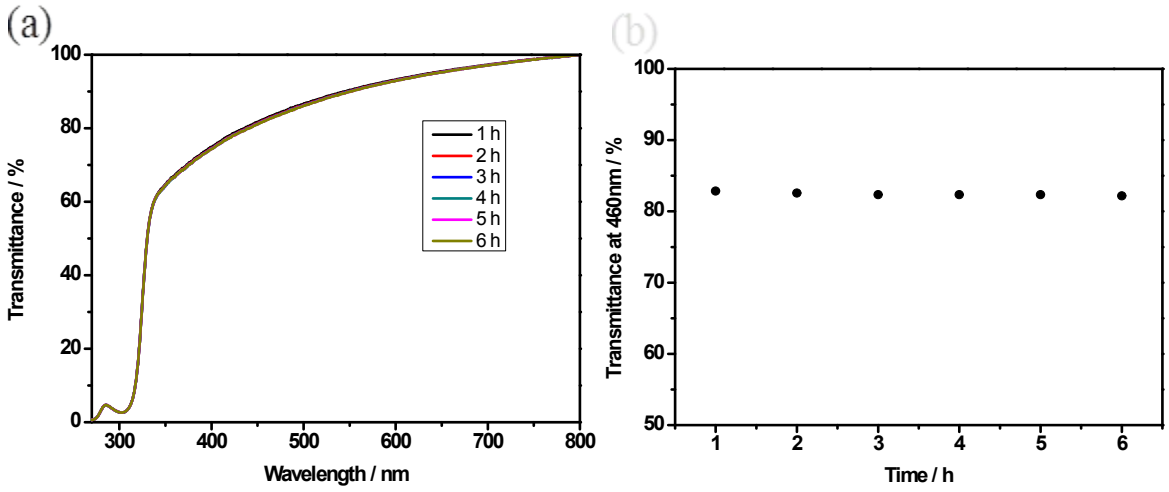


Figure S18. (a) Optical transmittance of SCD/QA-Cbl assembly within 6 hours in PBS buffer solution. (b) Dependence of the optical transmittance at 460 nm on time, [SCD] = 0.04mM, [QA-Cbl] = 0.70 mM.

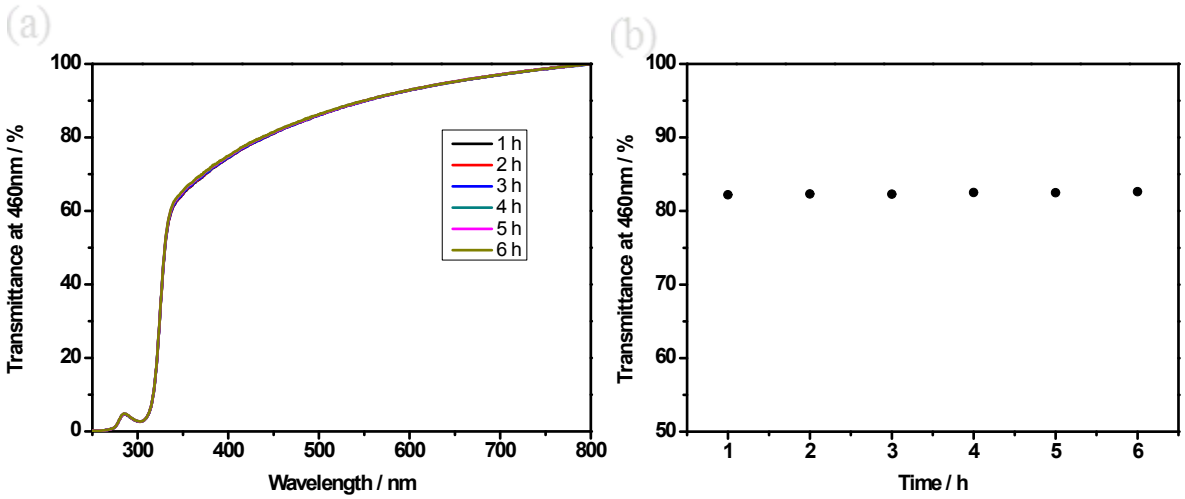


Figure S19. (a) Optical transmittance of SCD/QA-Cbl assembly within 6 hours in PBS containing 10% FBS. (b) Dependence of the optical transmittance at 460 nm on time, [SCD] = 0.04mM, [QA-Cbl] = 0.70 mM.

Drug loading efficiency (DLE). The assembly was placed in PBS buffer for dialysis until the dialysate had no UV absorption (molecular weight cutoff 3500), and then the assembly was taken out for UV-vis spectroscopy. According to the standard curve of QA-Cbl, the concentration of loaded drug was calculated as 0.54 mM. The concentration of feeding drug is 0.7 mM. Therefore, the DLE (DLE (wt. %) = (weight of loaded drug/weight of feeding drug) × 100%) was calculated as 77.14%.