Supporting Information

Light-Powered Sequential Manipulation of Microalgal Lipid Bioprocessing via an Artificial Photoactive Membrane Antenna

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Experimental Methods

Synthesis of TPyD. TPyD was synthesized referring to the previous report.[1] Briefly, TPy (100 mg, 0.20 mmol) and 1-decylbromide (80.5 mg, 0.40 mmol) were placed in a round-bottom flask. 5 mL of *N*,*N*-dimethylformamide (DMF) was added, and the mixture was heated at 80 ºC for 24 h under a nitrogen atmosphere. After evaporating the solvent, the crude product was purified by silica gel column chromatography using a gradient of eluent solvents: ethyl acetate/*n*-hexane (5:1, *V*/*V*) followed by dichloromethane/methanol (10:1, *V*/*V*). The final product was dried to get a red solid with a 75.8% yield. ^{1}H NMR (600 MHz, DMSO-d_6), δ (ppm): 8.95 (d, J = 6.5 Hz, 2H), 8.19 (d, J = 6.5 Hz, 2H), 7.93 (d, J = 16.4 Hz, 1H), 7.52 (d, J = 8.1 Hz, 2H), 7.44 (d, J = 16.3 Hz, 1H), 7.19-7.10 (m, 3H), 7.06–6.96 (m, 4H), 6.92–6.85 (m, 4H), 6.74–6.66 (m, 4H), 4.49 (t, J = 7.3 Hz, 2H), 3.68 (s, 6H), 1.93–1.85 (m, 2H), 1.30-1.20 (m, 14H), 0.84 (t, J = 6.8 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6), δ (ppm): 157.98, 157.87, 152.82, 146.29, 144.18, 143.38, 140.97, 140.46, 138.06, 135.43, 135.33, 132.94, 132.10, 132.02, 131.47, 130.79, 127.96, 127.71, 126.46, 123.68, 122.93, 113.31, 113.18, 60.23, 59.65, 54.94, 54.93, 31.24, 30.49, 28.83, 28.75, 28.61, 28.36, 25.40, 22.06, 13.93. HRMS (m/z): calculated for $C_{45}H_{50}NO_2$ $[M\text{-}Br]^+$: 636.3836, found: 636.3831.

Synthesis of TPy-C8 and TPy-C12. The synthesis procedure similar to that used for TPyD was followed. For TPy-C8: TPy (100 mg, 0.2 mmol) and 1-bromooctane (74.8 mg, 0.4 mmol) were placed into a round-bottom flask. For TPy-C12, TPy (100 mg, 0.2 mmol) and 1-bromododecane (87.6 mg, 0.4 mmol) were used. The remaining steps were same as TPyD. The corresponding characterizations are detailed below:

TPy-C8 (Yield: 80.5%). ^{1}H NMR (600 MHz, DMSO-d_6), δ (ppm): 8.98 (d, J = 6.3 Hz, 2H), 8.21 (d, J = 6.4 Hz, 2H), 7.95 (d, J = 16.3 Hz, 1H), 7.52 (d, J = 7.9 Hz, 2H), 7.45 (d, J = 16.3

Hz, 1H), 7.19–7.09 (m, 3H), 7.06–6.95 (m, 4H), 6.93-6.83 (m, 4H), 6.75–6.66 (m, 4H), 4.50 (t, J = 7.4 Hz, 2H), 3.68 (s, 6H), 1.93-1.85 (m, 2H), 1.32–1.20 (m, 10H), 0.85 (t, J = 6.8 Hz, 3H). ^{13}C NMR (151 MHz, DMSO), δ (ppm): 158.00, 157.89, 152.85, 146.31, 144.20, 143.41, 140.99, 140.49, 138.10, 135.46, 135.37, 132.98, 132.13, 132.05, 131.49, 130.82, 127.99, 127.75, 126.49, 123.72, 122.96, 113.34, 113.20, 110.39, 59.68, 54.97, 54.96, 31.13, 30.55, 28.45, 28.36, 25.45, 22.04, 13.94. HRMS (m/z): calculated for $C_{43}H_{46}NO_2$ $[M\text{-}Br]^+$: 608.3523, found: 608.3513.

TPy-C12 (Yield: 72.4%). ^{1}H NMR (600 MHz, DMSO-d_6), δ (ppm): 9.04 (d, J = 6.6 Hz, 2H), 8.24 (d, J = 6.6 Hz, 2H), 7.99 (d, J = 16.3 Hz, 1H), 7.53 (d, J = 8.2 Hz, 2H), 7.49 (d, J = 16.3 Hz, 1H), 7.16–7.08 (m, 3H), 7.03-6.94 (m, 4H), 6.90–6.82 (m, 4H), 6.73–6.65 (m, 4H), 4.53 (t, J = 7.2 Hz, 2H), 3.66 (s, 6H), 1.93–1.85 (m, 2H), 1.27–1.17 (m, 18H), 0.82 (t, J = 6.9 Hz, 3H). ^{13}C NMR (151 MHz, DMSO), δ (ppm): 157.96, 157.84, 152.85, 146.21, 144.20, 143.38, 140.92, 140.48, 138.07, 135.40, 135.34, 133.02, 132.08, 132.01, 131.41, 130.78, 127.94, 127.75, 126.44, 123.68, 122.93, 113.31, 113.16, 72.21, 60.17, 59.50, 54.96, 54.95, 31.26, 30.55, 28.97, 28.87, 28.75, 28.68, 28.37, 25.37, 22.06, 13.92. HRMS (m/z): calculated for $C_{47}H_{54}NO_2$ $[M\text{-}Br]^+$: 664.4149, found: 664.4155.

Algal culture. *C. pyre* cells were cultured in BG11 medium in an illumination incubator under the following conditions: 25 ºC, 40% illuminance, and a 12 h light/dark cycle. Culture flasks were shaken manually three times each day. The concentration of *C. pyre* suspensions was determined by measuring the optical density at 680 nm (OD_{680}).

PI staining to assess the effects of three molecules on algal membrane integrity. *C. pyre* cells were diluted to an initial OD_{680} of 0.3 with BG11 medium. 20 mL of the cell suspension was incubated with 1.0 μM TPy-C8, TPyD and TPy-C10 in an illuminated incubator for 48 h, respectively. 1 mL of the cell suspension was taken and centrifuged at 7000 rpm for 5 min. The collected cell pellet was resuspended in 500 μL of PBS and stained with PI (5 μg/mL) for 10 min. The cell suspension was then transferred to a sample tube, and the PI fluorescence signals of ~10,000 cells were collected under the PE-Texas Red-A channel using a flow cytometer.

Chlorophyll content measurement. The chlorophyll content of *C. pyre* cells was measured according to the reference.[2] Briefly, *C. pyre* cells (OD_{680} = 0.3) were incubated with 1.0 μM TPy-C8, TPyD and TPy-C10 in an illuminated incubator for 48 h, respectively. Then, 10 mL of the algal suspension was centrifuged at 7000 rpm for 5 min to harvest the cells, followed by

the addition of mixed solvent of acetone and ethanol (*V*/*V* = 1:1). After 30 min of extraction, the mixture was centrifuged (7000 rpm, 5 min), and the supernatant was collected to measure the absorbance at 645 nm (OD_{645}) and 663 nm (OD_{663}). The chlorophyll content was calculated using the following formula:

$$Concentration\ of\ chlorophyll\ a\ (mg/L) = 12.7 \times OD_{663} - 2.69 \times OD_{645}$$

$$Concentration\ of\ chlorophyll\ b\ (mg/L) = 22.9 \times OD_{645} - 4.86 \times OD_{663}$$

$$Total\ chlorophyll\ concentration\ (mg/L) = 7.84 \times OD_{663} + 20.21 \times OD_{645}$$

Flow cytometric analysis of the binding of algae with TPyD. *C. pyre* suspension (2 mL, OD_{680} = 1.0) was treated with TPyD (20 μM) in BG11 for 30 min, the cells were then collected by centrifugation (7000 rpm, 5 min). The collected cells were resuspended in 500 μL PBS and loaded into a sample tube. Fluorescence signal from about 20,000 cells was then collected using the Qdot 565 channel on a flow cytometer.

Morphology characterization of algae. The morphology of *C. pyre* cells without and with TPyD treatment was observed using SEM following the procedure described in the reference.[3] 2 mL of *C. pyre* cells were treated with TPyD (1.0 μM) in BG11 at room temperature for 30 min. After centrifugation to remove the supernatant, the harvested cells were washed with PBS, and then resuspended in 50 μL PBS. 10 μL of the samples were dropped on a silicon slice, allowed to dry naturally, and fixed by 2.5% glutaraldehyde PBS solution at 4 ºC for 6 h. The samples were then washed with PBS for three times, and gradient dehydrated using ethanol solutions at concentrations of 50%, 70%, 80%, 90%, 95% and 100%, with each step for 15 min. The prepared samples were dried under vacuum and coated with platinum before SEM observation.

Zeta potential measurements. 2 mL of *C. pyre* suspensions (OD_{680} = 2.0) were treated by different concentrations of TPyD in BG11 medium for 30 min at 25 ºC. The cells were centrifuged (7000 rpm, 5 min) and washed twice with PBS to remove unbound TPyD; the residual *C. pyre* pellets were re-suspended in 1 mL of ultrapure water. Zeta potential of the samples was then measured at 25 ºC using a disposable DTS1070 capillary cell with a He-Ne laser of 632.8 nm and a scattering angle of 173°.

Growth curve. *C. pyre* cells in the logarithmic phase were inoculated into fresh BG11 medium and diluted to an initial OD_{680} of about 0.3 for measuring growth curves. Different

concentrations of TPyD were added to the *C. pyre* suspensions. The samples were then cultured in an illumination incubator, and their OD_{680} values were recorded each day. The experiment was conducted in triplicate.

SOD activity. SOD activity in *C. pyre* cells without and with TPyD treatment was assessed using a total SOD assay kit with WST-8. Specifically, *C. pyre* cells (initial OD_{680} = 0.3) were incubated with TPyD (1.0 μM) for 14 days in an illumination incubator. 2 mL of *C. pyre* suspension was centrifuged to harvest the cells, which were then lysed with 1 mL of lysis buffer by sonication for 30 min in an ice bath. The supernatant was collected by centrifugation (10000 rpm, 10 min) at 4 ºC, and the SOD activity was tested with SOD activity assay kit by measuring the absorbance at 450 nm using a microplate reader.

MDA content. *C. pyre* cells (OD_{680} = 0.3) were incubated in the absence and presence of TPyD (1.0 μM) for 14 days in an illumination incubator. The cells were harvested and lysed, and their MDA content was tested using a lipid peroxidation MDA assay kit following the procedure described in previous report.[4]

ROS generation in algae. *C. pyre* cells (OD_{680} = 2.0) were incubated without and with TPyD (1.0 μM) in BG11 for 30 min in an illumination incubator. DCFH-DA probe (50 μM) was added into the samples with a 20 min incubation. The fluorescence emission of the probe (λ_{ex} = 488 nm) was then measured at different irradiation times under three light conditions: blue light (8.7 mW/cm^2), red light (8.7 mW/cm^2) or P-light (26 mW/cm^2).

Chlorophyll fluorescence kinetics and parameters. *C. pyre* suspensions (2 mL, OD_{680} = 2.0), without and with 30 min of TPyD (1.0 μM) treatment, were placed in the sample cell. After dark adaptation for 20 min, the chlorophyll fluorescence kinetics curves and dark-adapted parameters of the samples were measured using a chlorophyll fluorescence monitor. For the light-adapted chlorophyll fluorescence parameters, the samples were illuminated for 30 min under P-light (26 mW/cm^2), and then the corresponding parameters were measured on the chlorophyll fluorescence monitor.

Electron generation. 2 mL of *C. pyre* cells (OD_{680} = 2.0), without and with TPyD (1.0 μM) treatment for 30 min, were placed in a quartz cuvette. The electron acceptor DCPIP (100 μM)

was added to the samples. The absorbance at 600 nm of DCPIP was then measured at different P-light (26 mW/cm^2) irradiation time on a spectrophotometer.

ATP content. *C. pyre* cells (OD_{680} = 2.0), without and with 30 min of TPyD (1.0 μM) treatment, was irradiated under P-light (26 mW/cm^2) for 30 min. 2 mL of the samples were centrifuged (7000 rpm, 5 min) to harvest the cells. The collected cells were lysed with 200 μL lysis buffer by sonicating in an ice bath for 20 min. The supernatant was obtained by centrifugation (10000 rpm, 10 min) at 4 ºC and transferred to a black 96-well plate. The ATP content was tested with an enhanced ATP assay kit by measuring luminescence intensity on a microplate reader.

NADPH and NADP$^+$ content. Following the ATP measurement pretreatment, the obtained supernatant was transferred to a 96-well plate. NADPH and NADP$^+$ content were then tested using an enhanced NADP$^+$/NADPH assay kit, with the absorbance at 450 nm measured on a microplate reader.

Rubisco activity. *C. pyre* cells (OD_{680} = 2.0), without and with 30 min of TPyD (1.0 μM) treatment, was irradiated under P-light (26 mW/cm^2) for 30 min. 2 mL of the samples were centrifuged (7000 rpm, 5 min) to collect the cells. The collected cells were dispersed in 500 μL of extraction buffer and sonicated in an ice bath for 20 min. The supernatant was obtained by centrifugation (10000 rpm, 10 min) at 4 ºC, and assay buffer was then added. The absorbance of nicotinamide adenine dinucleotide (NADH) at 340 nm was measured every 30 s on a spectrophotometer. A blank control was set with assay buffer. Rubisco activity was then calculated based on the decrease in NADH absorbance referring to our previous report.[4]

Lipid content evaluation. *C. pyre* (OD_{680} = 0.3) were cultured without and with TPyD (1.0 μM) for 12 days in an illumination incubator. Their lipid content was then evaluated by the following three methods:

(1) Nile red imaging: 2 mL of the samples were centrifuged (7000 rpm, 5 min) to collect *C. pyre* cells. The collected cells were suspended with 1 mL PBS and stained with Nile red (5 μM) for 5 min. The supernatant was then removed by centrifugation, and the remaining cells were suspended in 50 μL PBS. 5 μL of the samples were placed on a glass slide and imaged under CLSM with λ_{ex} = 488 nm and λ_{em} = 550-600 nm.

(2) Flow cytometric analysis: 2 mL of the samples were centrifuged to harvest *C. pyre* cells. The collected cells were suspended with 1 mL PBS and stained with Nile red (5 μM) for 5 min.

The samples were then transferred to a sample tube, and the Nile red fluorescence signal from about 20000 cells was collected under the PE channel using a flow cytometer.

(3) Organic solvent extraction: 20 mL of the samples were treated according to the steps detailed in the reference.[5] Briefly, 1.5 mL methanol and 5 mL methyl tertiary-butyl ether (MTBE) were added into the samples and placed for 1 h at room temperature. Then, 1.25 mL water was added to facilitate phase separation, and the upper organic phase was collected. To ensure complete lipid recovery, 2 mL MTBE/ methanol/water (10/3/2.5, *V*/*V*/*V*) was added to the lower aqueous phase for re-extraction and repeated for three times. The collected organic phase was vacuum-dried and weighted to assess lipid content.

Lipid harvesting yield after TPyD-regulated algal lipid accumulation and release via light intensity control. *C. pyre* cells (OD_{680} = 0.3) were incubated with 1.0 μM TPyD for 12 days in an illumination incubator. 20 mL samples were taken and then irradiated with H-light for 30 min to induce cell membrane disruption for lipid release. The lipid yield was then determined following the procedure described in "Lipid extraction yield by light-driven membrane disruption" of the main text. For the control group, *C. pyre* cells (without TPyD treatment) were incubated under identical conditions for 12 days, then treated with 1.5 mL methanol for 30 min to induce cell membrane disruption for lipid release, with subsequent lipid extraction performed using the same procedure as the experimental group. All experiments were performed in triplicate.

Flocculation Efficiency of TPyD on *C. pyre*. 5 mL of *C. pyre* suspension (OD_{680} = 2.0) was treated with different concentrations of TPyD (0, 2.0, 5.0, 10.0 μM). Prior to TPyD addition, the OD_{680} value of the *C. pyre* suspension was measured and recorded as A_0. After adding the TPyD, the samples were placed in dark for static incubation over different durations. At 1 h, 2 h, 3 h, and 5 h post-TPyD treatment, the OD_{680} value of each sample supernatant was measured, recorded as A_t. The flocculation efficiency of TPyD on *C. pyre* at each time point was calculated using the following formula:

$$\eta = \frac{A_0 - A_t}{A_0} \times 100\%$$

PI staining to evaluate TPyD effect on algal membranes after flocculation. *C. pyre* cells (5 mL, OD_{680} = 2.0) with 10 μM TPyD, were placed in the dark for 5 h to allow flocculation, and then was irradiated with H-light for 5 min (10 μM TPyD (5 h) + 5 min H-light). The control

groups include: (1) *C. pyre* cells incubated in the dark for 5 h without 5 min of H-light irradiation (no TPyD, no H-light); (2) *C. pyre* cells with 10 μM TPyD incubated in the dark for 5 h without 5 min of H-light irradiation (10 μM TPyD (5 h), no H-light); (3) *C. pyre* cells incubated in the dark for 5 h with 5 min of H-light irradiation (5 min H-light, no TPyD). In addition, *C. pyre* cells with 10 μM TPyD were placed in the dark for 5 h and then directly irradiated with sunlight (85 mW/cm^2) for 5 min (10 μM TPyD (5 h) + 5 min sunlight) were also included. PI (5 μg/mL) was then added to the above samples with 10 min of staining. The samples were centrifuged (7000 rpm, 5 min), and the resulting pellets were resuspended in 500 μL PBS. Flow cytometry analysis was performed on about 20,000 cells using the PE-Texas Red-A channel and FSC-A channel.

UPLC-IM-Q-TOF MS analysis of lipid content. The lipid contents of *C. pyre* were analyzed by UPLC-IM-Q-TOF MS under different membrane disruption conditions, with sample preparation and treatment as follows: (1) conventional ultrasonic treatment: 20 mL of *C. pyre* suspension (OD_{680} = 2.0) was subjected to ultrasonic treatment (80 W, 40 kHz) for 30 min; (2) 1.0 μM TPyD and 30 min H-light irradiation: 20 mL of *C. pyre* suspension was incubated with 1.0 μM TPyD, followed by H-light irradiation for 30 min; (3) 10 μM TPyD and 5 min H-light irradiation: 20 mL of *C. pyre* suspension was incubated with 10 μM TPyD, followed by H-light irradiation for 5 min; (4) H-light irradiation alone (30 min): 20 mL of *C. pyre* suspension was exposed to H-light irradiation for 30 min without TPyD addition. Subsequently, 5 mL of MTBE was added to each treated sample, and the mixtures were allowed to stand for 10 min for phase separation. The organic phase was collected, dried, and redissolved in 1 mL of methanol. The resultant solution was filtered through a 0.22 μm membrane filter, and the filtrate was transferred to an HPLC vial. Lipid analysis was performed in positive ion mode on a C18 column (ACQUITY UPLC BEH C18 Column, 100 × 2.1 mm, 1.7 μm; Waters) with the mobile phase consisting of 40% acetonitrile (ACN, buffer A) and 10% ACN/90% isopropanol (IPA, buffer B). Detailed analytical conditions followed the literature method.[6]

TPyD recovery and residual content in Lipids. During TPyD-assisted *C. pyre* flocculation and lipid release for extraction, TPyD was mainly distributed in the post-flocculation supernatant (marked as **1**), the extraction aqueous phase (marked as **2**), the lipid-containing organic phase (marked as **3**), and the partition bound to algal membranes and retained in algal debris (marked as **4**). The concentration of TPyD in partitions **1, 2, 3** was determined by measuring the absorbance at 425 nm. 10 μM TPyD (marked as **0**) was added into 5 mL of *C.*

pyre suspension (OD_{680} = 2.5) and flocculation proceeded for 5 h. The supernatant was then collected, and its absorbance at 425 nm was recorded. The remaining algal sample was subjected to H-light irradiation for 5 min, followed by the addition of 1.5 mL water and 5 mL MTBE for lipid extraction. After phase separation, the aqueous phase and the lipid-containing organic phase were collected separately. The above extraction procedure was repeated three times. The combined aqueous and organic phases were then concentrated to 2 mL by vacuum evaporation, and their absorbance at 425 nm was measured separately. The absorbance of 10 μM TPyD in aqueous solution at 425 nm was used as a reference to quantify TPyD in the post-flocculation supernatant (**1**) and extraction aqueous phase (**2**). Similarly, the absorbance of 10 μM TPyD in MTBE at 425 nm served as a reference for determining TPyD concentration in the lipid-containing organic phase (**3**). The mass of TPyD in these three partitions was calculated from their respective concentrations, volumes, and the molar mass of TPyD. The mass of TPyD retained in algal debris (**4**) was obtained by subtracting the mass of the three partitions from the initially added TPyD mass. The mass (m) of TPyD in each of the four partitions, and their mass percentage relative to the initial addition (m/m_0, %) were calculated from 5 mL algal samples and then scaled to 1 L of algal suspension.

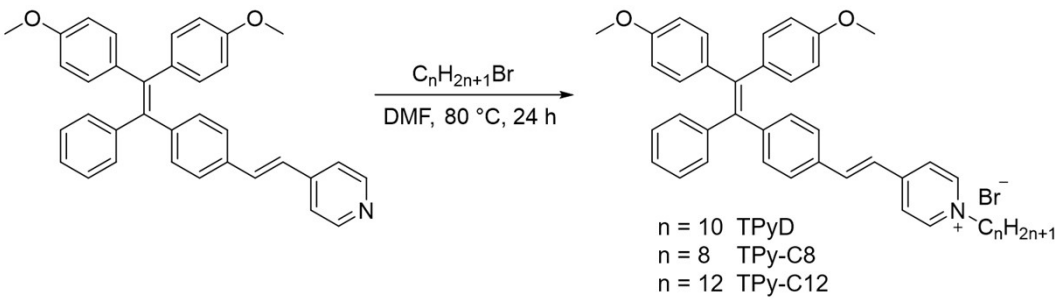
The TPyD in the post-flocculation supernatant (**1**) and extraction aqueous phase (**2**) was regarded as recoverable. Thus, the recovery rate (R) of TPyD was calculated using the following formula:

$$R = \frac{m_1 + m_2}{m_0} \times 100\%$$

The residual percentage (R_3) of TPyD in lipids was calculated based on the TPyD mass (m_3, ~0.12 mg) in the lipid-containing organic phase (**3**) and the lipid yield of 291.3 mg/L.

$$R_3 = \frac{m_3}{291.3\ mg} \times 100\%$$

Supplementary Figures



Scheme S1. Synthetic routes for the three AIE molecules.

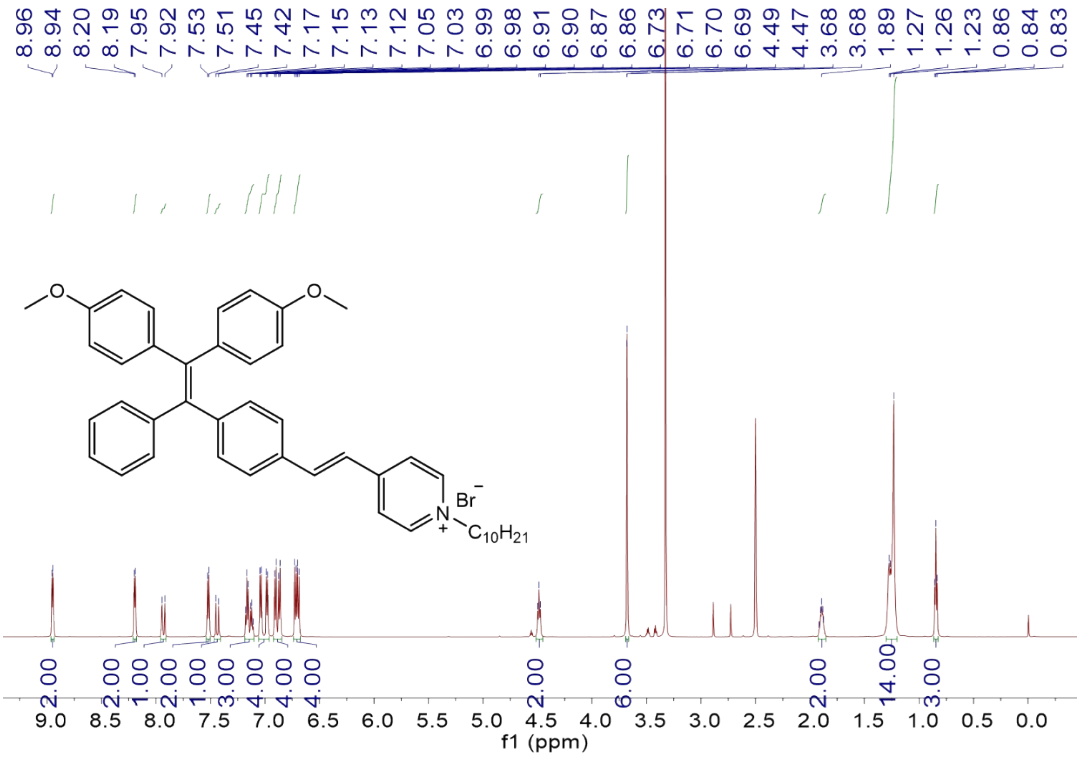


Figure S1. ^{1}H NMR spectrum of TPyD in DMSO-d_6.

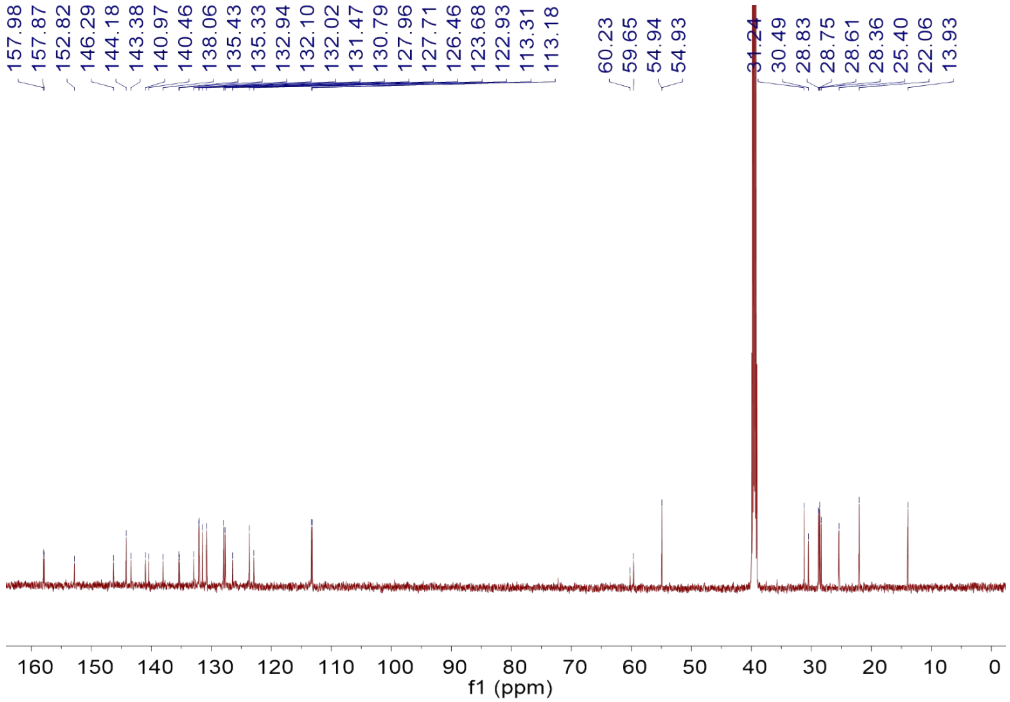


Figure S2. ^{13}C NMR spectrum of TPyD in DMSO-d_6.

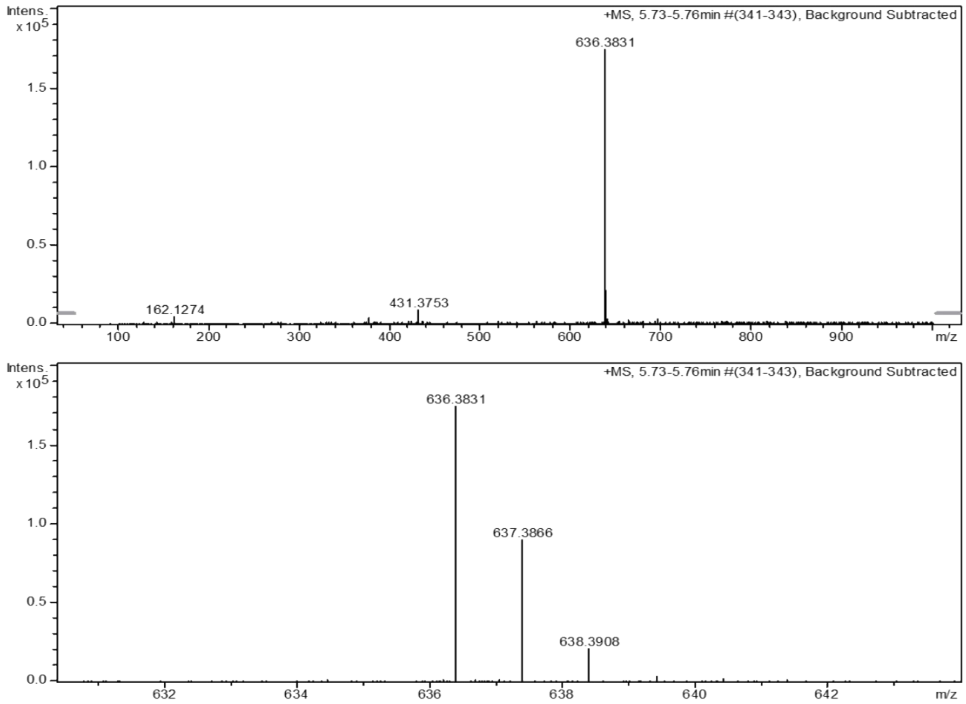


Figure S3. HRMS spectrum of TPyD.

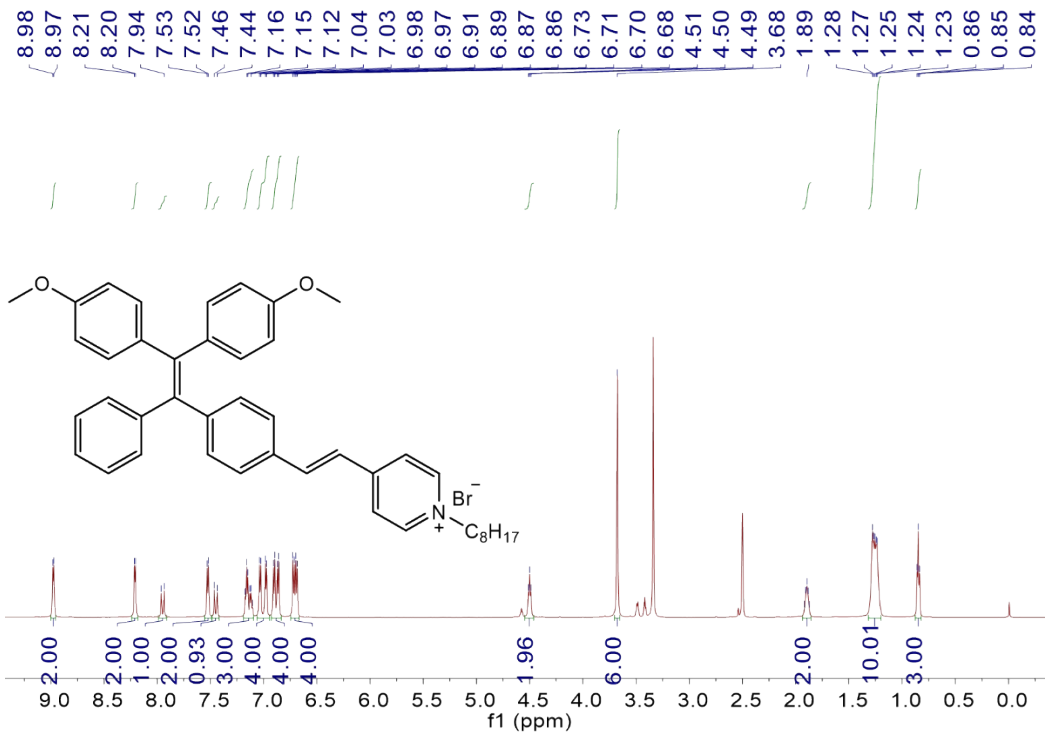


Figure S4. ^{1}H NMR spectrum of TPy-C8 in DMSO-d_6.

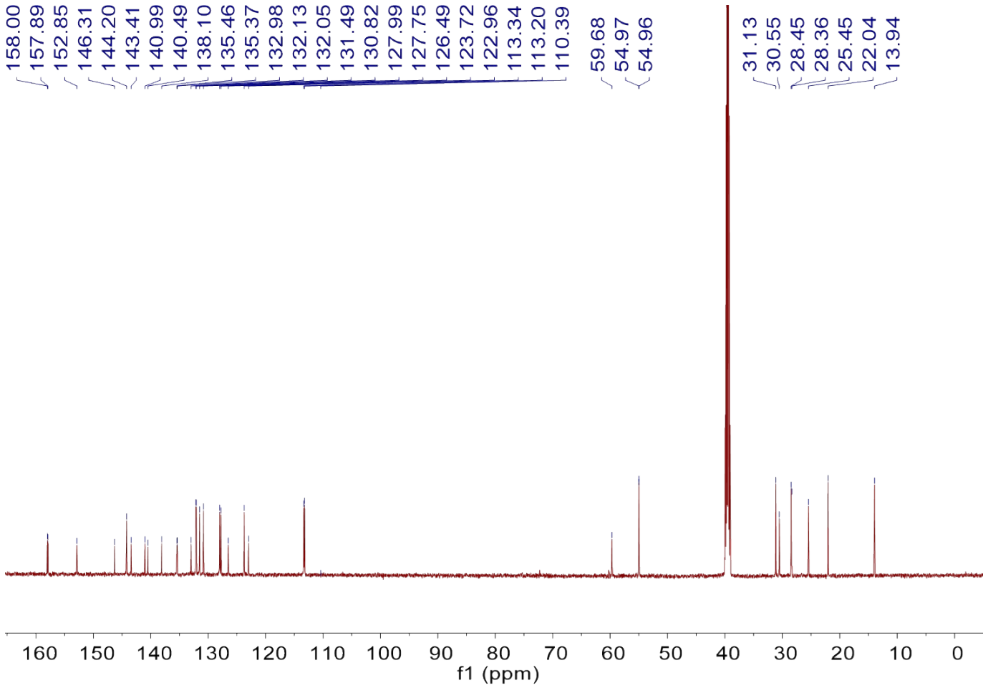


Figure S5. ^{13}C NMR spectrum of TPy-C8 in DMSO-d_6.

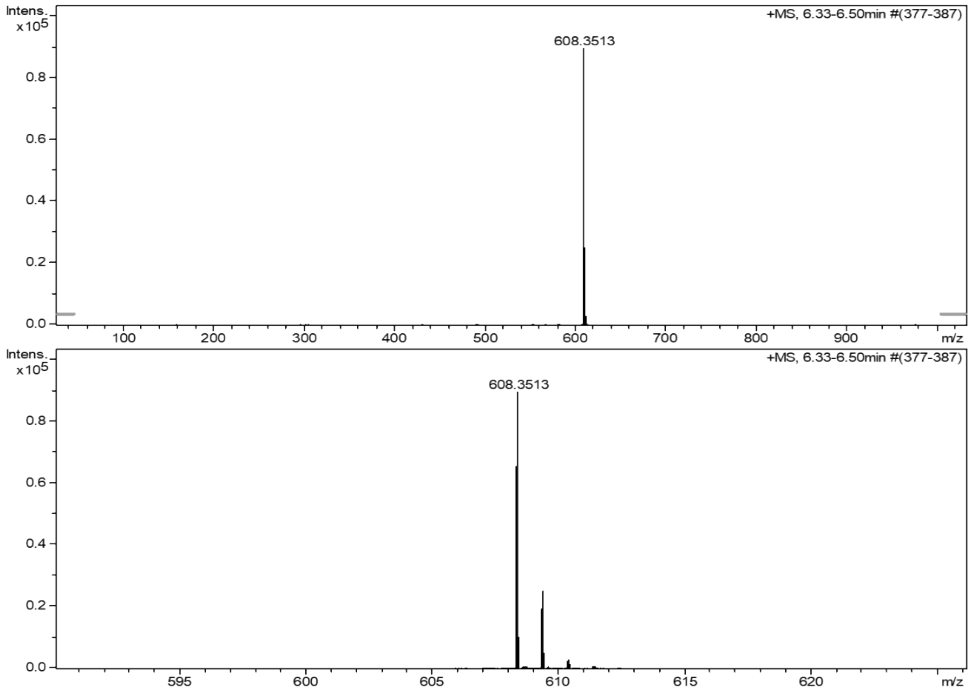


Figure S6. HRMS spectrum of TPy-C8.

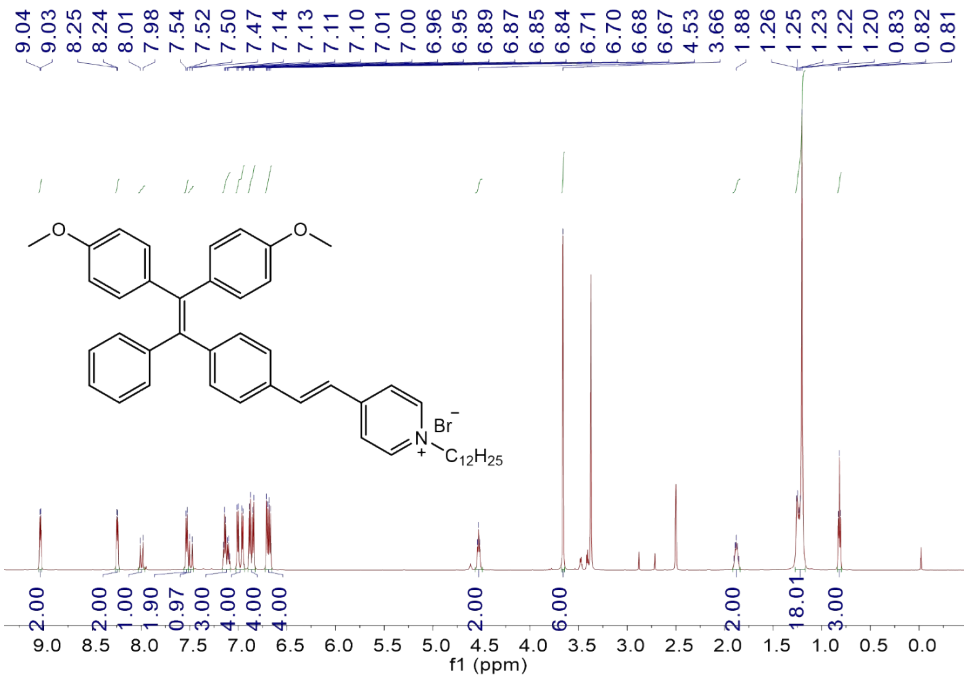


Figure S7. ^{1}H NMR spectrum of TPy-C12 in DMSO-d_6.

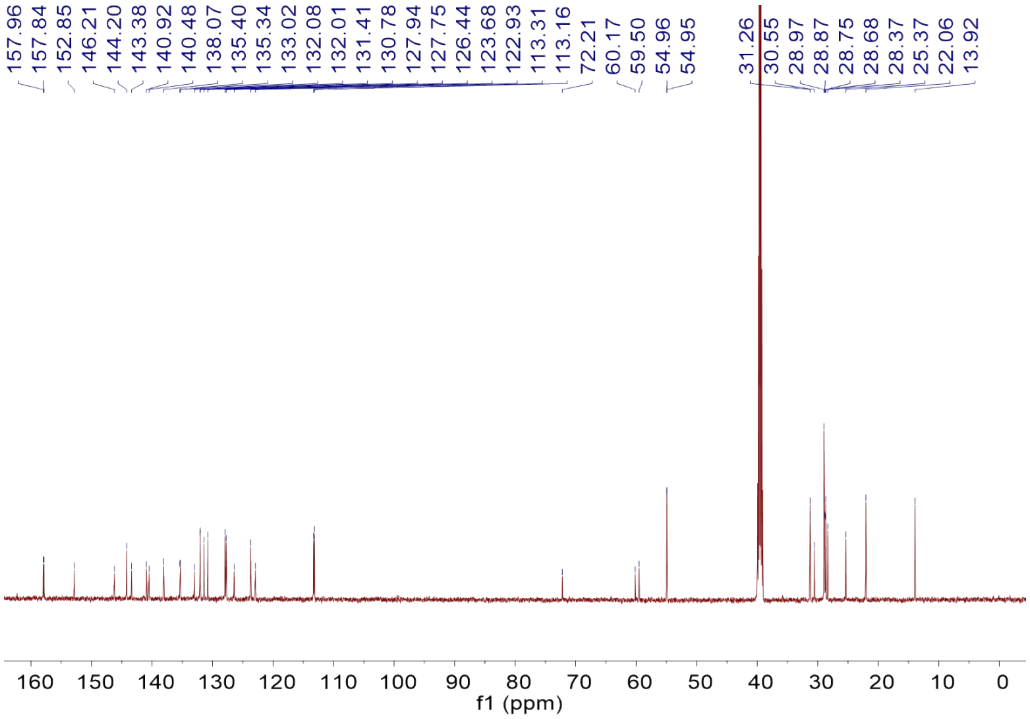


Figure S8. ^{13}C NMR spectrum of TPy-C12 in DMSO-d_6.

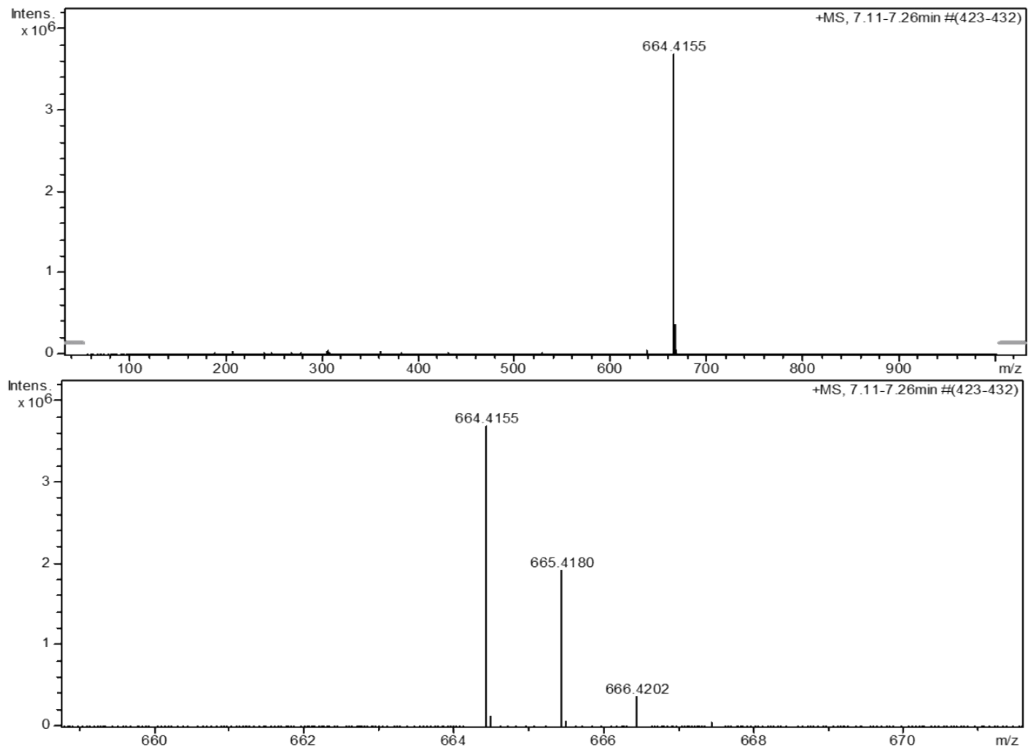


Figure S9. HRMS spectrum of TPy-C12.

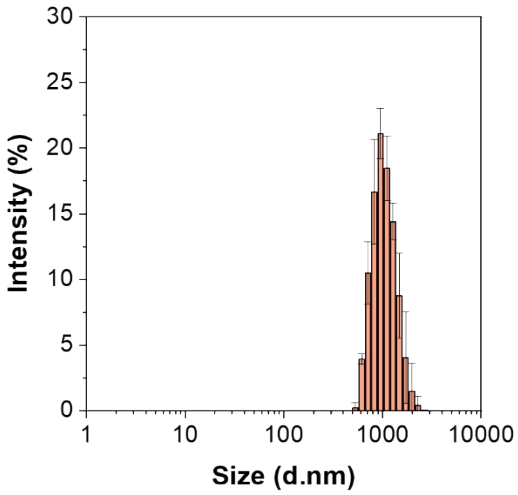


Figure S10. Size distribution of 20 μM TPyD in DMSO/water mixture with water volume fraction of 99%.

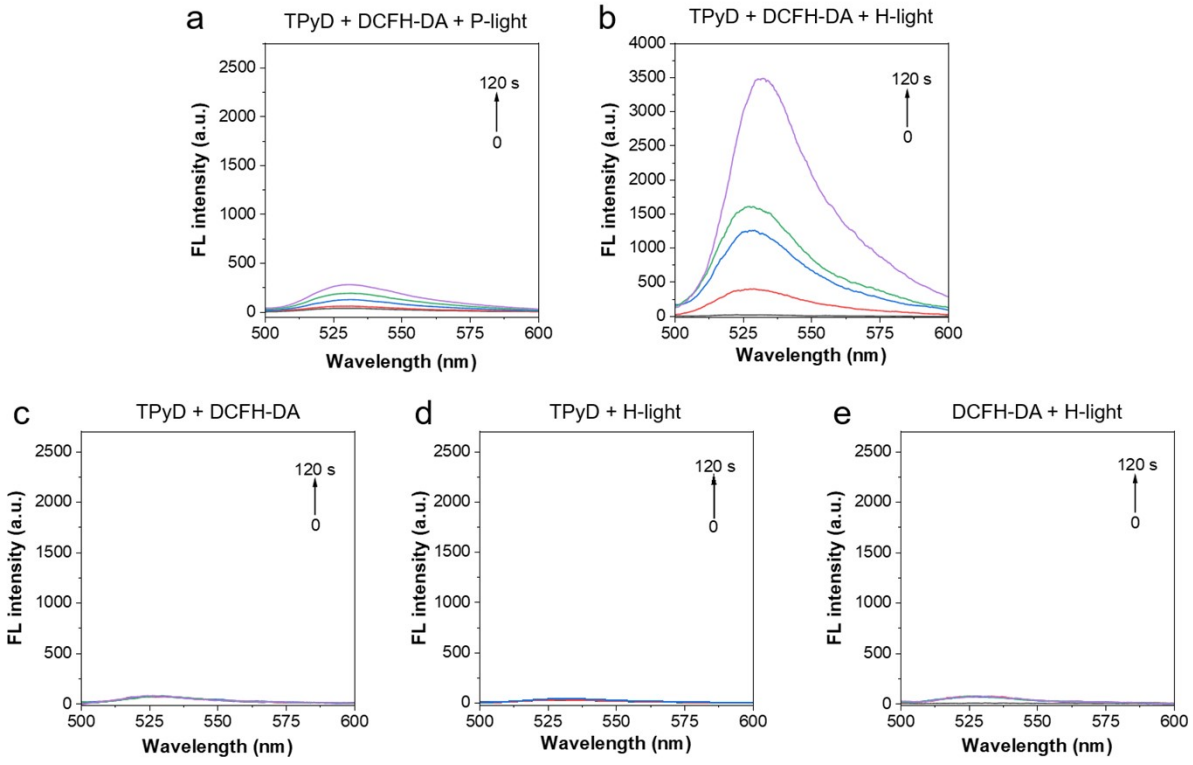


Figure S11. ROS generation by TPyD under P-light and H-light detected by the DCFH-DA probe. (a) and (b) Fluorescence spectra of DCFH-DA in the presence of TPyD at different irradiation times under P-light and H-light, respectively. (c) Fluorescence spectra of DCFH-DA with TPyD over time in the dark. (d) and (e) Fluorescence spectra of TPyD alone and DCFH-DA alone at the different irradiation times under H-light, respectively. [TPyD] = 1.0 μM and [DCFH-DA] = 50 μM.

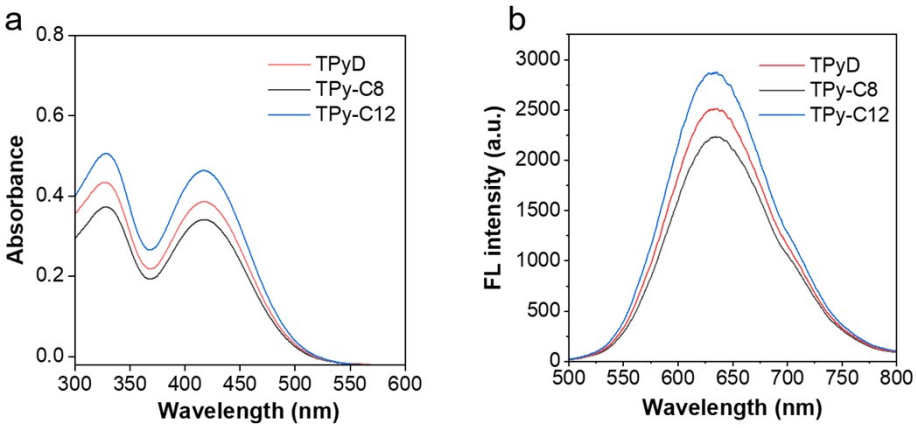


Figure S12. (a) UV-Vis absorption spectra in DMSO and (b) fluorescence spectra in BG11 solution (λ_{ex} = 440 nm) of 20 μM TPyD, TPy-C8 and TPy-C12.

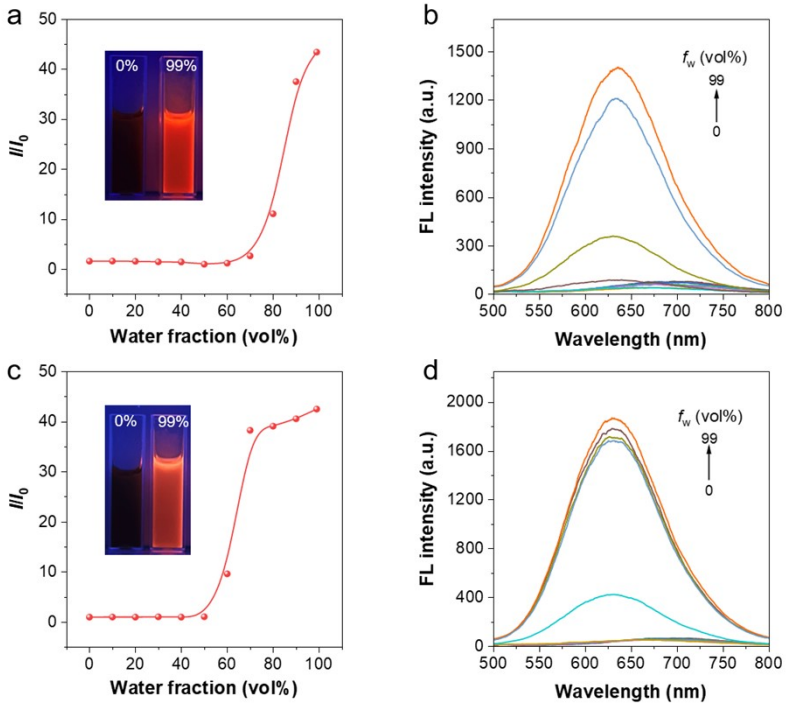


Figure S13. (a) Relative emission intensity (I/I_0) and (b) fluorescence spectra of 20 μM TPy-C8 versus water volume fraction (f_w, vol%) in DMSO/water mixtures. Inset: Photos of TPy-C8 in DMSO and DMSO/water mixture (f_w = 99%) under 365 nm irradiation. (c) Relative emission intensity (I/I_0) and (d) fluorescence spectra of 20 μM TPy-C12 versus water volume fraction in DMSO/water mixtures. Inset: Photos of TPy-C12 in DMSO and DMSO/water mixture (f_w = 99%) under 365 nm irradiation.

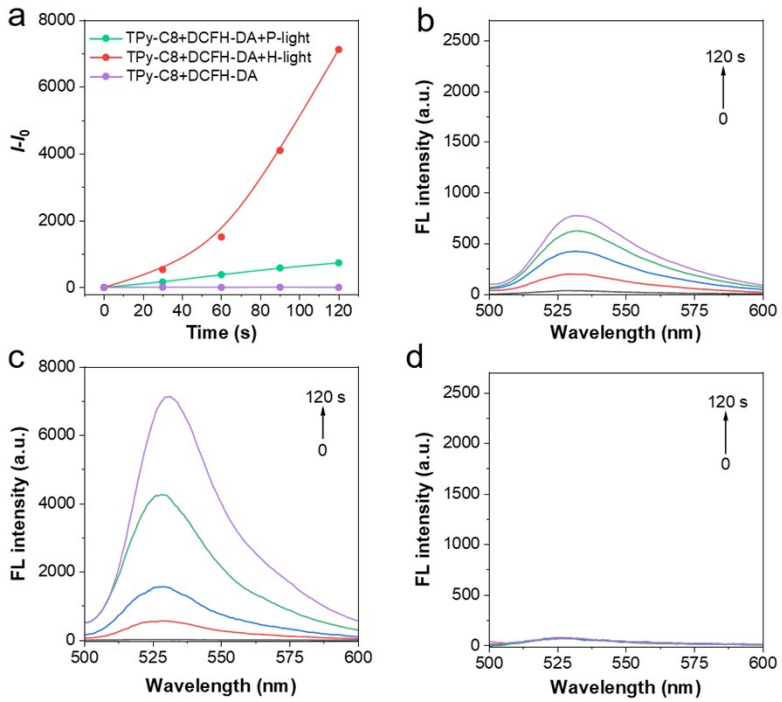


Figure S14. ROS generation by TPy-C8. (a) Fluorescence intensity changes at 530 nm and (b)-(d) fluorescence spectra of 50 μM DCFH-DA with 1.0 μM TPy-C8 over time under P-light, H-light, and dark, respectively.

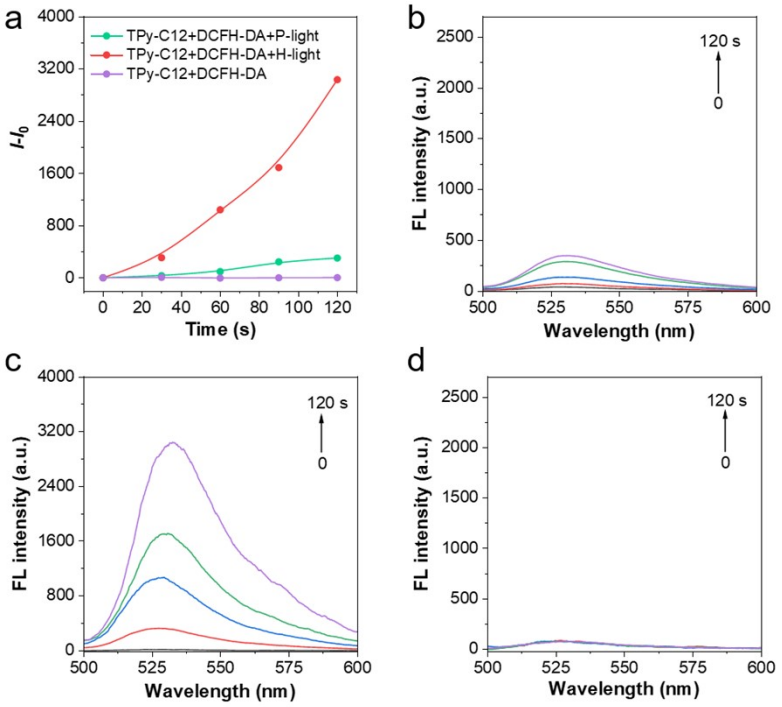


Figure S15. ROS generation by TPy-C12. (a) Fluorescence intensity changes at 530 nm and (b)-(d) fluorescence spectra of 50 μM DCFH-DA with 1.0 μM TPy-C12 over time under P-light, H-light, and dark, respectively.

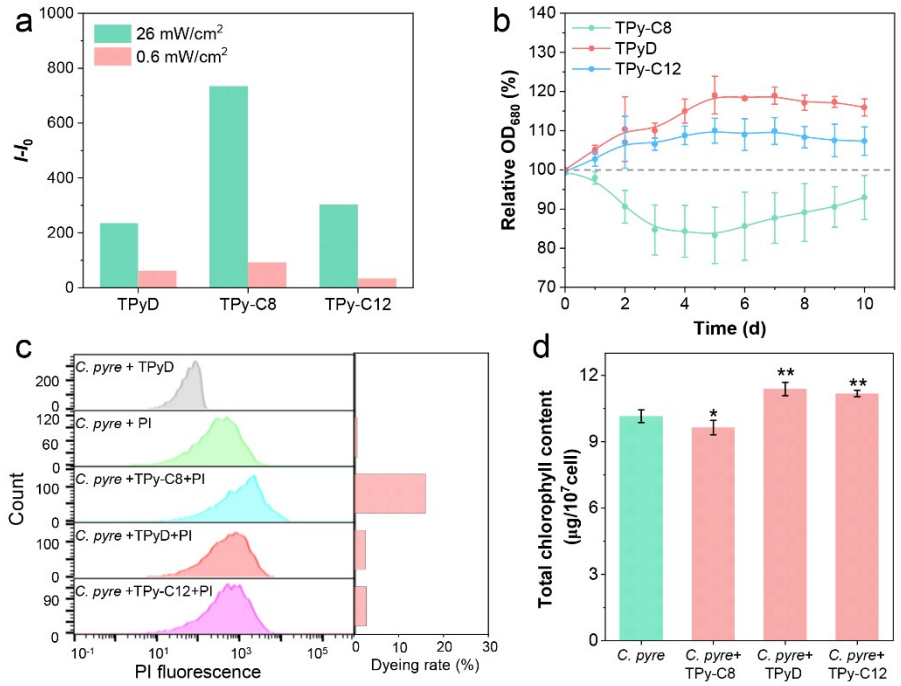


Figure S16. (a) ROS generation. DCFH-DA fluorescence intensity changes in the presence of 1.0 μM TPyD, TPy-C8 or TPy-C12 under P-light (26 mW/cm^2 and 0.6 mW/cm^2) after 120 s irradiation (λ_{ex} = 488 nm). (b) Growth curves of *C. pyre*. Data are expressed as relative OD_{680} (% of control, where the control is *C. pyre* alone) over the incubation time. Error bars: mean ± SD (*n* = 3). (c) Flow cytometry analysis of *C. pyre* cells after incubation with the three molecules for 48 h, followed by PI staining (5 μg/mL, 10 min). (d) Total chlorophyll content of *C. pyre* before and after 48 h incubation with the three molecules. Error bars: mean ± SD (*n* = 3). $^{*}P < 0.05$, significant; $^{**}P < 0.01$, highly significant.

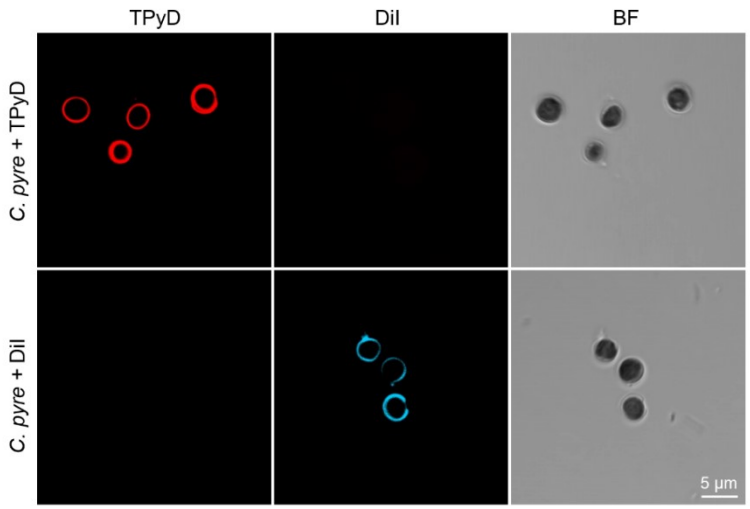


Figure S17. Fluorescence and bright field (BF) images of *C. pyre* protoplasts stained with 20 μM TPyD alone for 30 min or 10 μM DiI alone for 10 min.

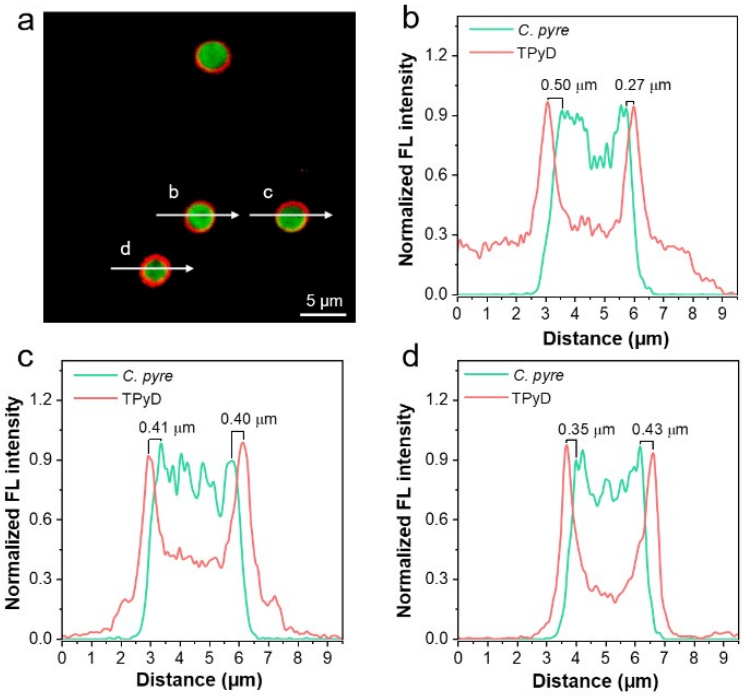


Figure S18. (a) Merged CLSM image of *C. pyre* treated with TPyD (same as Figure 2a). (b)-(d) Normalized fluorescence intensity profiles of TPyD and algal chlorophyll along the white line across three representative cells in (a). Based on these profiles, the average distance between TPyD and chlorophyll is 0.39 μm (mean of six distance data).

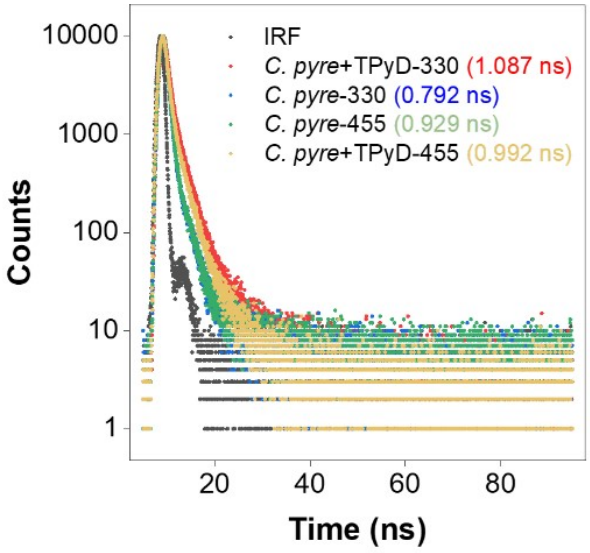


Figure S19. Fluorescence decay curves of *C. pyre* (OD_{680} = 2.0) at 685 nm without or with TPyD (20 μM) under 330 or 455 nm excitation. IRF: instrument response function.

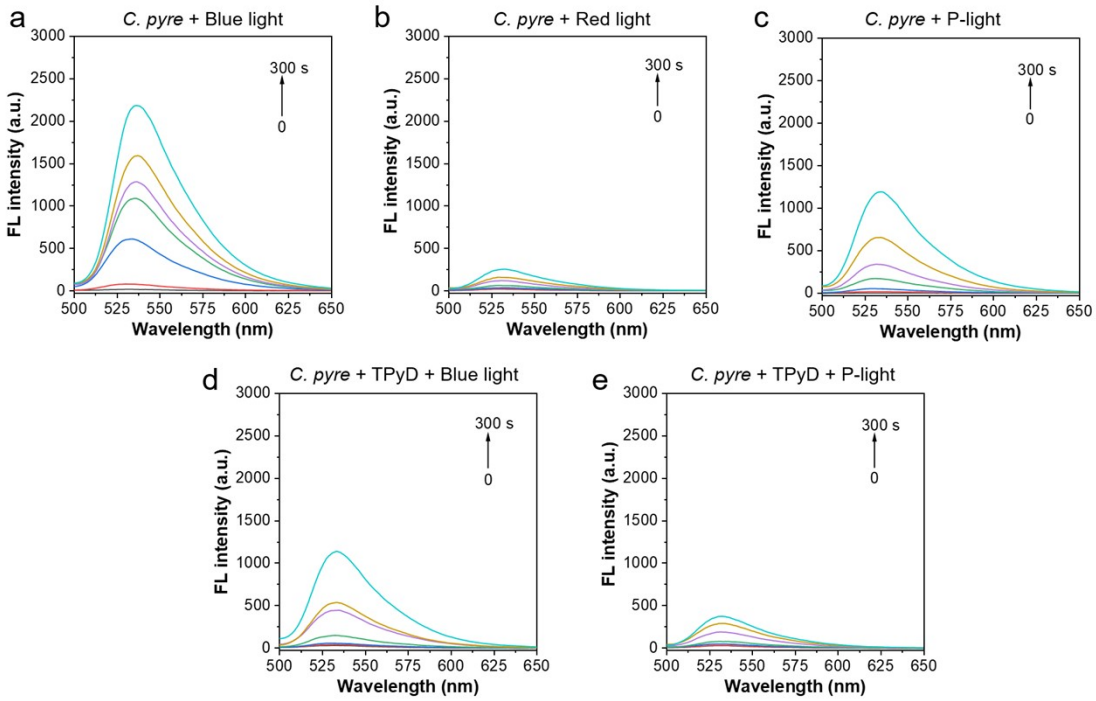


Figure S20. Intracellular ROS in *C. pyre* without and with TPyD under different light irradiation. (a)-(c) Fluorescence spectra of DCFH-DA in *C. pyre* over irradiation time under blue light, red light and P-light, respectively. (d) and (e) Fluorescence spectra of DCFH-DA in *C. pyre* with TPyD (1.0 μM) over irradiation time under blue light and P-light, respectively. λ_{ex} = 488 nm.

Table S1. Chlorophyll fluorescence characteristics of *C. pyre* under dark adaption without and with 1.0 μM TPyD.

Parameter	Definition	*C. pyre*	*C. pyre* + TPyD
F_v/F_m	Maximal quantum yield of PSII	0.645	0.670
PI_{ABS}	Performance index for energy conversion from photons absorbed by PSII to reduction of intersystem electron acceptors	1.750	3.440
ABS/RC	Absorbed energy per reaction center	1.274	1.409
TR_o/RC	Trapped energy per reaction center	0.822	0.918
ET_o/RC	Energy used for electron transfer per reaction center	0.452	0.649
N	The number of Q_A reduction from the irradiation to the maximum fluorescence in the dark	467.96	519.26

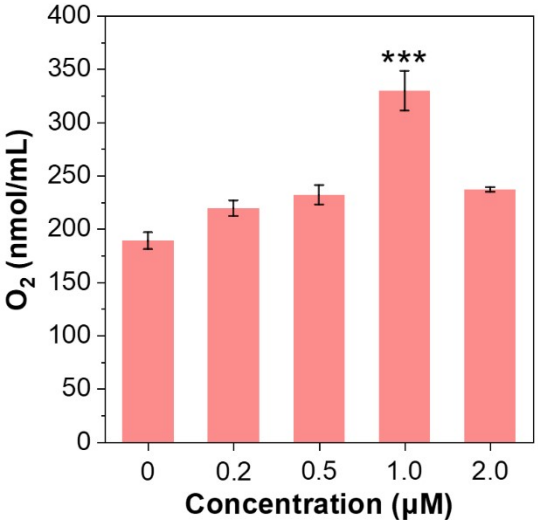


Figure S21. Oxygen evolution of *C. pyre* cells before and after cultivation with different concentrations of TPyD under illumination intensity of 26 mW/cm^2 for 1500 s. Error bars: mean ± SD (n = 3). ***P < 0.001, highly significant.

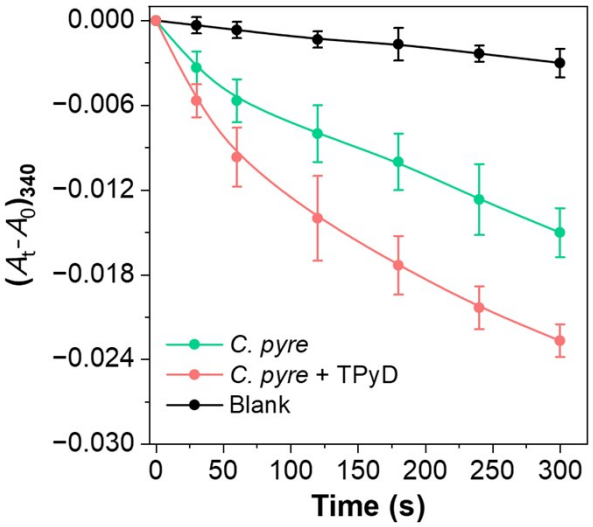


Figure S22. Absorbance changes of NADH at 340 nm in *C. pyre* suspensions without and with TPyD (1.0 μM) against the reaction time. Error bars: mean ± SD (n = 3).

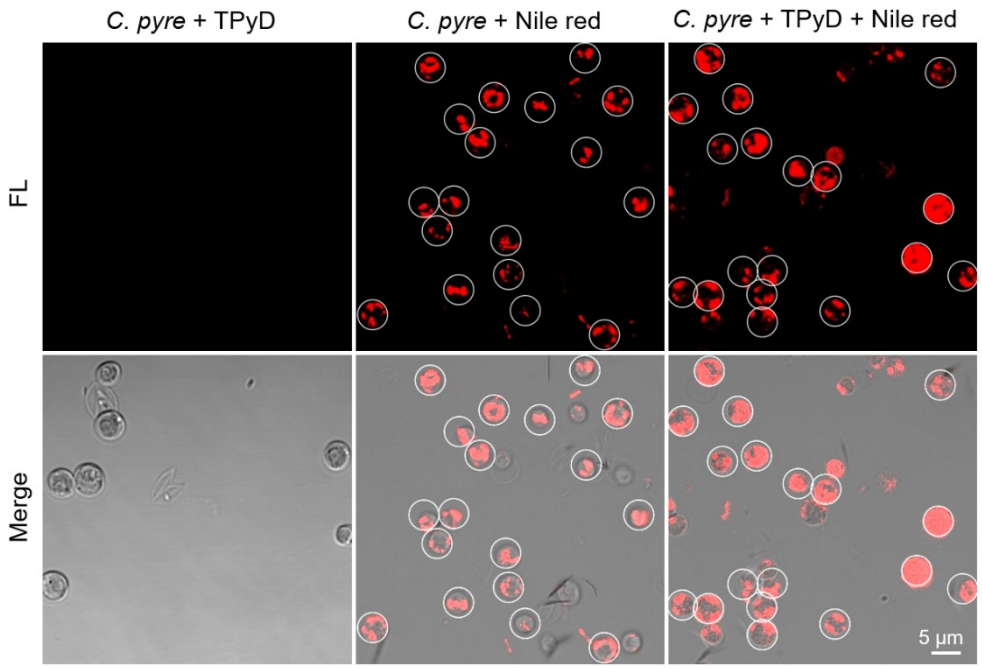


Figure S23. Fluorescence (FL) and merged images of *C. pyre* cells stained with Nile red (5 μM) for 5 min after incubated without and with TPyD (1.0 μM) for 12 days in an illumination incubator. λ_{ex} = 488 nm and λ_{em} = 550-600 nm.

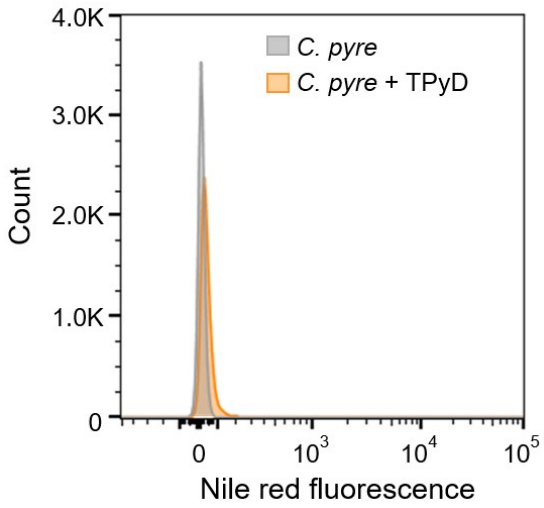


Figure S24. Flow cytometric analysis of *C. pyre* cells without Nile red staining after 12-day incubation in the absence and presence of TPyD (1 μM).

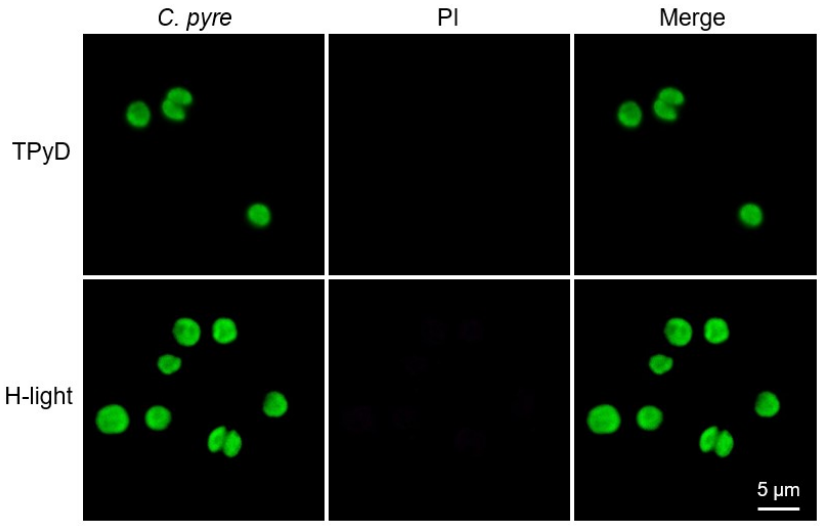


Figure S25. CLSM images of *C. pyre* stained by PI (5 μg/mL) for 10 min after pretreatment with TPyD alone (1.0 μM) for 30 min or H-light alone for 30 min. Imaging conditions: λ_{ex} = 488 nm and λ_{em} = 650-750 nm for *C. pyre*, λ_{ex} = 543 nm and λ_{em} = 600-650 nm for PI.

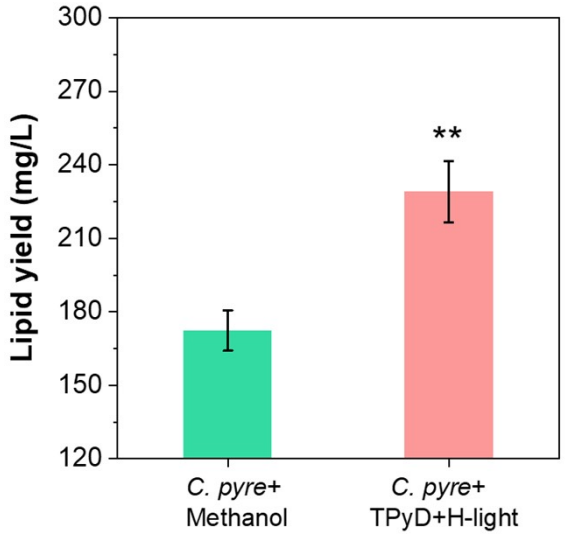


Figure S26. Lipid harvesting yield from *C. pyre* under two treatment conditions: (1) 12-day incubation followed by 30 min of methanol treatment; (2) 12-day incubation with 1.0 μM TPyD, followed by 30 min of H-light irradiation. Error bars: mean ± SD (n = 3). $^{**}P < 0.01$, highly significant.

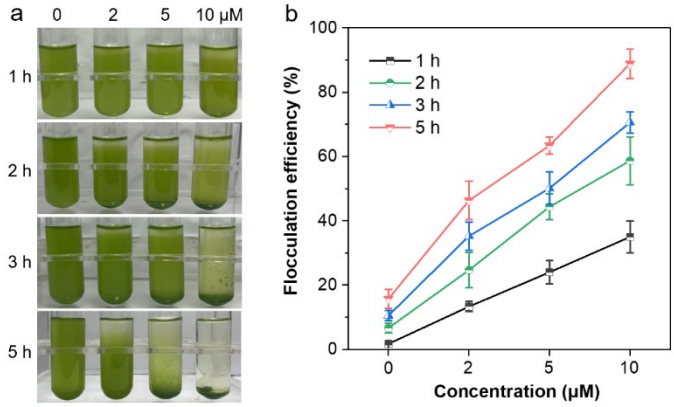


Figure S27. (a) Photos and (b) flocculation efficiency of *C. pyre* induced by different concentrations of TPyD over different incubation times.

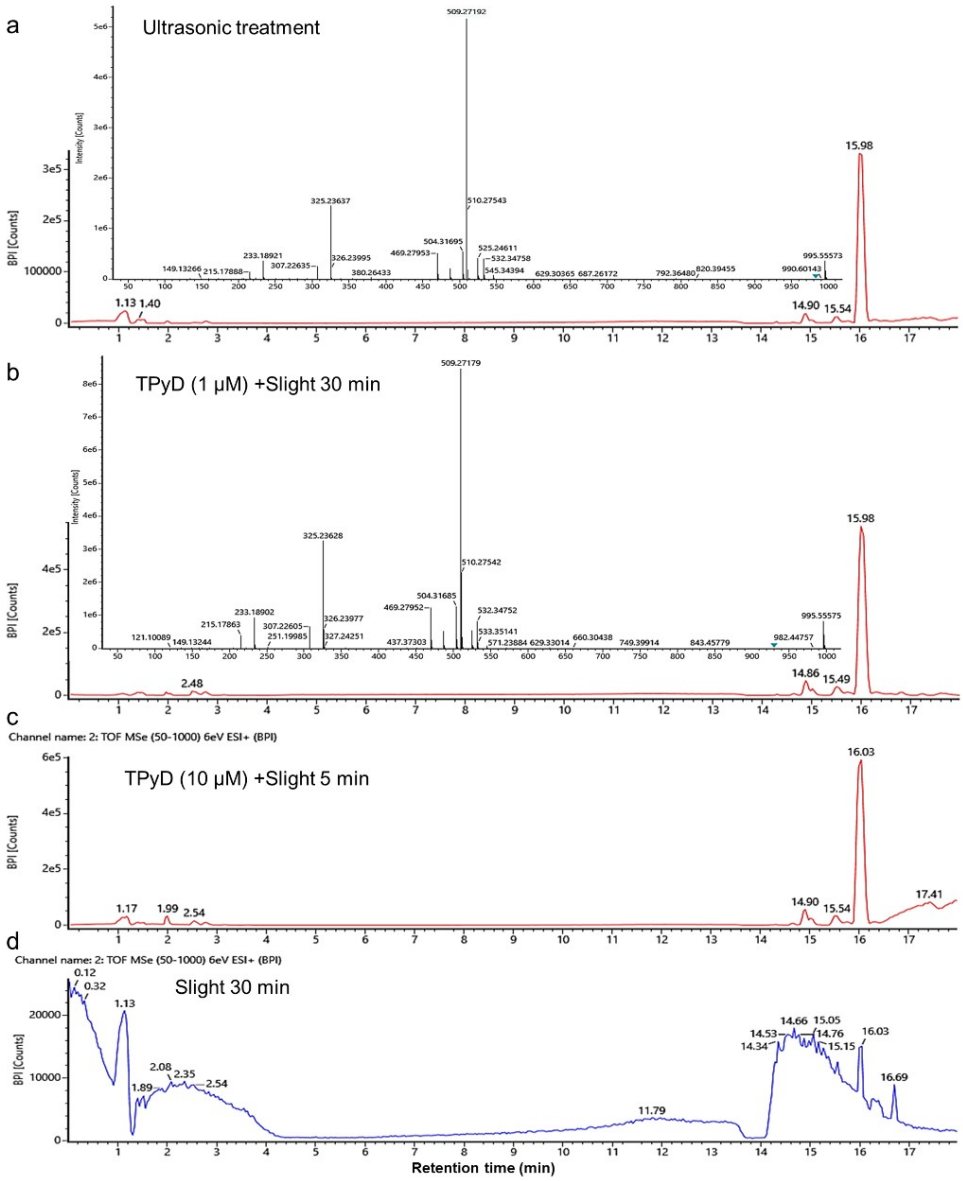


Figure S28. UPLC-IM-Q-TOF MS analysis (positive ion mode) of lipids produced by *C. pyre* under different membrane disruption conditions: (a) The base peak intensity (BPI) profile of

lipids under ultrasonic treatment, with the MS spectrum of the dominant peak at 16 min as insert; (b) BPI profile of lipids under 1.0 μM TPyD and 30 min H-light irradiation, with the MS spectrum of the peak at 16 min as insert; (c) BPI profile of lipids under 10 μM TPyD and 5 min H-light irradiation; (d) BPI profile of lipids under 30 min H-light irradiation alone.

In Figure S28a, the MS spectrum at ~16 min showed a precursor ion at m/z 995.55573 ($[M+Na]^+$), corresponding to a neutral molecular mass of 972.57 Da, which falls within the typical mass range of TAGs.[7, 8] Characteristic diacylglycerol (DAG)-like fragment ion (m/z 509.27) was observed, consistent with the fragmentation pattern of TAGs in positive ion mode. Additionally, fragment ions in the low-mass region (m/z 215.17888, 307.22635, and 325.26367) indicate that these TAGs contain unsaturated fatty acyl chains.[9]

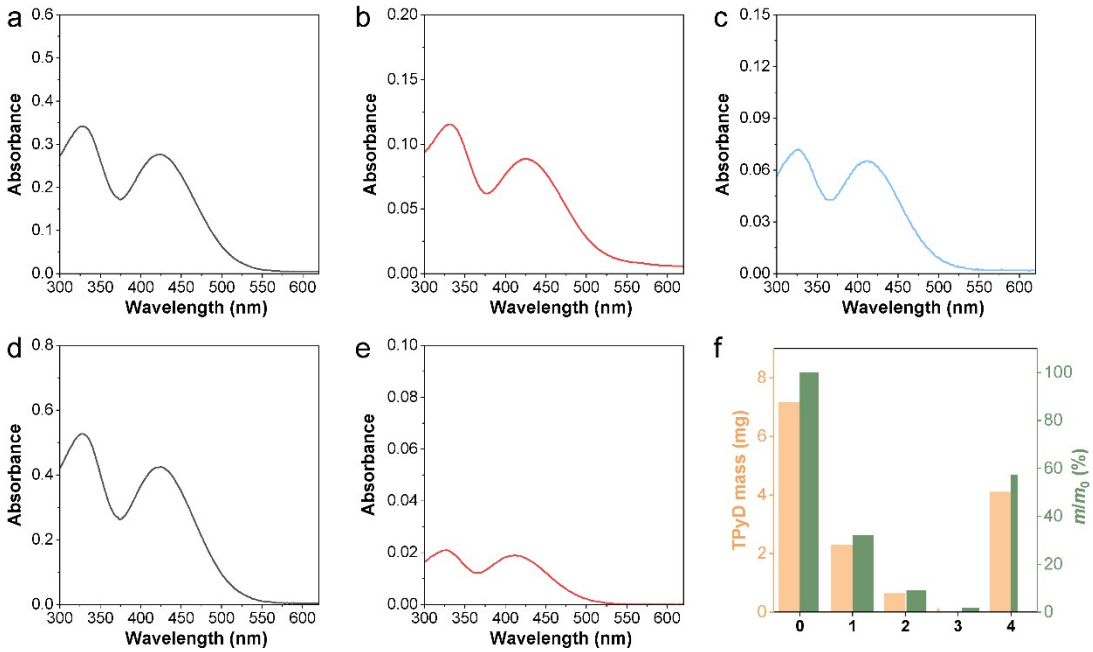


Figure S29. (a)-(e) UV-Vis absorption spectra of TPyD: (a) 10 μM TPyD in aqueous solution; (b) TPyD in supernatant after 5 h flocculation of *C. pyre* with 10 μM TPyD; (c) TPyD in aqueous phase from lipid extraction; (d) 10 μM TPyD in MTBE; (e) TPyD in lipid-containing organic phase from lipid extraction. (f) Mass distribution and mass percentage relative to the initial addition (m/m_0, %) of TPyD after treating 1 L algal suspension, converted from the data of 5 mL samples, where **0** Initial TPyD (10 μM), **1** TPyD in post-flocculation supernatant; **2** TPyD in extraction aqueous phase, **3** TPyD in lipid-containing organic phase, **4** TPyD in algal debris.

References

1. F. Hu, Y. Yuan, D. Mao, W. Wu and B. Liu, *Biomaterials*, 2017, **144**, 53-59.
2. X. Xu, W. Li, C. Hu, B. Lei, X. Zhang, Y. Li, Q. Zhan, Y. Liu and J. Zhuang, *ACS Sustainable Chem. Eng.*, 2020, **8**, 9751-9762.
3. X. Zhou, Y. Zeng, Y. Tang, Y. Huang, F. Lv, L. Liu and S. Wang, *Sci. Adv.*, 2020, **6**, eabc5237.
4. Y. Huang, B. Huang, Y. Shen, Z. Ding, H. Yao, X. Zhou, C. Zhou, L. Han and B. Z. Tang, *ACS Appl. Mater. Interfaces*, 2024, **16**, 66706-66717.
5. V. Matyash, G. Liebisch, T. V. Kurzchalia, A. Shevchenko and D. Schwudke, *J. Lipid Res.*, 2008, **49**, 1137-1146.
6. K.-J. Lu, C.-W. Hsu, W.-N. Jane, M.-H. Peng, Y.-W. Chou, P.-H. Huang, K.-C. Yeh, S.-H. Wu and J. C. Liao, *Science*, 2025, **389**, eadp3528.
7. A. Cutignano, E. Luongo, G. Nuzzo, D. Pagano, E. Manzo, A. Sardo and A. Fontana, *Algal Res.*, 2016, **17**, 348-358.
8. K. M. MacDougall, J. McNichol, P. J. McGinn, S. J. B. O'Leary and J. E. Melanson, *Anal Bioanal Chem*, 2011, **401**, 2609-2616.
9. F.-F. Hsu and J. Turk, *J Am Soc Mass Spectrom*, 2010, **21**, 657-669.