Augmenting Photosynthesis through Facile AIEgen-Chloroplast Conjugation and Efficient Solar-Energy Utilization

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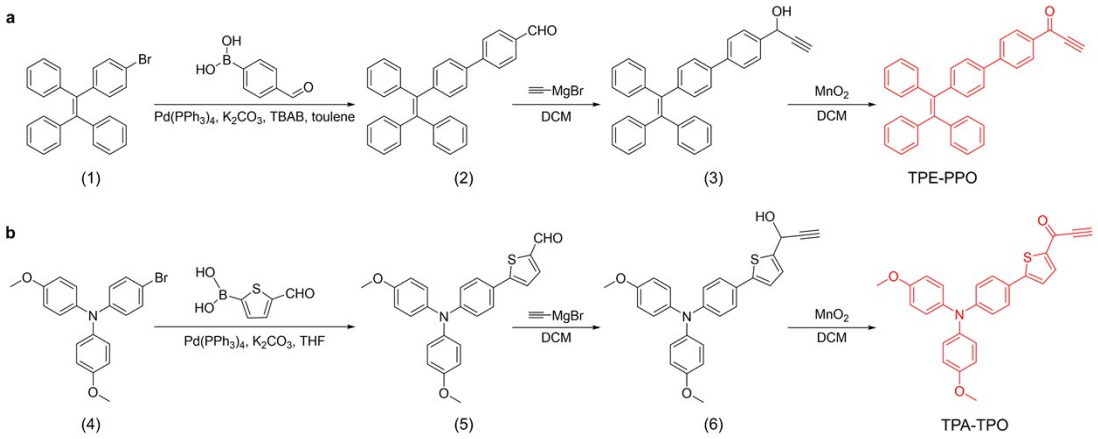
Materials and Methods

All the chemicals and organic solvents were purchased from J&K, TCI, and Sigma-Aldrich Company and used as received. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker ARX 400 NMR spectrometer using DMSO-d_6 and $CDCl_3$ as solvents. Dynamic light scattering (DLS) was performed on the Malvern ZetaSizer Nano ZS90. High-resolution mass spectra (HRMS) were measured on a Finnigan MAT TSQ 7000 mass spectrometer system operated in MALDI-TOF mode. Absorption spectra were recorded on a Perkin Elmer Lambda 20. Photoluminescence (PL) spectra were recorded on an Edinburgh Instruments spectrofluorometer FS5. Confocal Laser scanning microscope images were measured on a Zeiss laser scanning confocal microscope (LSM710) and analyzed using ZEN 2009 software (Carl Zeiss). The white light was provided by a

xenon fiber optic lamp (CXE-350, Optprco, China). The ultraviolet light was conducted with Mejiro Genossen MUA-165M-Ultra (365 nm). The optical intensity was regulated by a radiometer (Photoelectric Instrument Factory of Beijing Normal University) Chlorophyll fluorescence parameters were conducted with Handy PEA chlorophyll fluorimeter and FMS-2 pulse modulated fluorimeter.

Synthesis and characterization



Scheme S1. Synthetic route of (a) TPE-BPO and (b) TPA-TPO

Synthesis of TPE-PPO

1-(4-bromopbenyl)-l,2,2-tripbenylethene (4.11 g, 10 mmol) and 4-formylphenylboronic acid (2.25 g, 15 mmol) were dissolved in a mixture of toluene (60 mL), TBAB (0.32 g, 1.0 mmol) and 2 M potassium carbonate aqueous solution (18 mL). The mixture was stirred at room temperature for 0.5 h under nitrogen gas followed by adding $Pd(PPh_3)_4$ (0.16 g, 0.1 mmol) and then heating to 90 °C for 24 h. After that, the mixture was poured into water and extracted three times with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate. After removing the solvent under reduced pressure, the residue was chromatographed on a silica gel column with n-hexane/CH_2Cl_2 (3:1 by volume) as eluent to give TPE-PCA (2) (2.80 g, 64% yield). TPE-PCA(1.744g, 4 mmol) was dissolved in 40 mL tetrahydrofuran and placed into a 250 mL flask equipped with a magnetic stirrer and then immersed into an ice-water bath, and then 12 mmol ethynylmagnesium bromide in tetrahydrofuran was added

dropwise to the reaction system. The reaction was stirred overnight, and the obtained mixture was extracted with DCM and the saturated ammonium chloride solution. The organic layer was dried with anhydrous MgSO4 overnight and then filtered. After removing the solvent under reduced pressure, the residue was chromatographed on a silica gel column with n-hexane/CH_2Cl_2 (3:2 by volume) as eluent, the yield of the intermediate product was 71%. Then the intermediated product was dissolved in 80 mL DCM with adding 10.4 g MnO_2, the reaction was stirred for 2 hours. The remanent MnO_2 was removed by the filter, and the mixture was washed by the saturated salt solution and dried with anhydrous Na_2SO4. After removing the solvent under reduced pressure, the residue was chromatographed on a silica gel column with n-hexane/CH_2Cl_2 (1:1 by volume) as eluent, the yield of TPE-PPO was 65%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19-8.17 (d, 2H), 7.68-7.66 (d, 2H), 7.41 (d, 2H), 7.39-7.12 (m, 17H), 3.43 (s, 1H).

^{13}C NMR (300 MHz, $CDCl_3$): δ 176.91, 162.14, 146.74, 144.31, 143.56, 143.51, 141.71, 140.17, 137.16, 134.87, 132.04, 131.39, 131.34, 131.32, 130,27, 127.84, 127.84, 127.79, 127.69, 127.01, 80.64, 80.42

HRMS (MALDI-TOF) m/z: [M+H] calcd for $C_{35}H_{24}O$, 461.1905, found 461.1909.

Synthesis of TPA-TPO

4-bromo-N,N-bis(4-methoxyphenyl)aniline (2 g, 5.2 mmol), (5-formylthiophen-2-yl)boronic acid (0.94 g, 6 mmol) and $Pd(PPh_3)_4$ (0.06 g, 0.052 mmol) were dissolved in degassed THF (50 mL) in two-neck round-bottom flask under nitrogen gas. And then 2 M potassium carbonate aqueous solution (10 mL) was added to the THF solution under stirring. The mixture was cooled to room temperature and filtered after reflux for 12 h. The mixture solution was extracted three times with dichloromethane and water. The organic layer was separated and dried over anhydrous sodium sulfate. The crude product was purified by silica-gel column chromatography to get the product TPA-TCA (5) (1.47 g, 68% yield).

TPA-TCA (1 g, 2.41 mmol) was dissolved in degassed THF (30 mL) in a two-neck

round-bottom flask. 0.5 M ethynylmagnesium bromide (9.6 mL, 4.82 mmol) was added dropwise to the reaction solution under 0 ℃. The mixture naturally rose to room temperature and reacted overnight. The mixture was extracted with DCM and the saturated ammonium chloride solution. The crude product was purified by silica-gel column chromatography. The product was used directly for the next reaction. The above product was dissolved in DCM (30 mL) with adding 10 g MnO_2. The mixture was stirred for 24 h. The remanent MnO_2 was removed by the filter. The crude product was purified by silica-gel column chromatography to get the product TPA-TPO.

^{1}H NMR (300 MHz, CDCl3): δ 7.90-7.89 (d, 1H), 7.47-7.45 (d, 2H), 7.23-7.22 (d, 1H), 7.12-7.09 (d, 4H), 6.91-6.89 (m, 6H), 3.82 (s, 6H), 3.33 (s, 1H).

^{13}C NMR (300 MHz, CDCl3): δ 167.79, 155.80, 155.14, 149.39, 140.18, 138.89, 136.85, 126.65, 126.53, 123.31, 121.58, 118.39.

HRMS (MOLDI-TOF) m/z: [M+H] calcd for $C_{27}H_{21}NO_3S$, 440.1320, found 440.1299.

Chloroplast isolation and determination

Intact chloroplasts were isolated from commercially available fresh spinach by the previously reported method.[1] Cleaned spinach leaves were cut to pieces and then ground in sucrose buffer (pH 7.3) containing 0.4 M sucrose, 0.01 M KCl, 0.03 M Na_2HPO_4, 0.02 M KH_2PO_4. After filtered through four layers of gauze, the mixture was centrifuged at 1500 rpm for 3 min twice to remove cell debris, and then centrifugated at 3000 rpm for 3 min to collect the precipitate. The collected sediment was resuspended with the above buffer and obtained the chloroplast suspension. All preparations were processed on the ice at a temperature of 0 ~ 4 °C and kept out of the sun. The concentration of chloroplast was determined by the concentration of chlorophyll. Mixing 2.9 mL of acetone and 100 μL of the above chloroplast suspension intensively and then centrifugated for 2 min at 7000 rpm. The concentration of the obtained chlorophyll was determined by measuring the absorption at 652 nm in acetone.[2]

The amount of chloroplasts were determined as the concentration of containing chlorophyll and calculated as the following formula:

$$C = \frac{A_{652}}{34.5} \times 30\ (mg \cdot mL^{-1})$$

C is the concentration of chloroplasts; A_{652} is the determined absorption at 652 nm; the 30 is the dilution ratio.

Fluorescence imaging

The suspension of chloroplasts was pre-treated in dark for 20 min at 4 °C. And then 20 ug·mL^{-1} chloroplasts were incubated with 5 μM TPE-PPO or TPA-TPO respectively. After 20 min in dark at 4 °C, the free TPE-PPO and TPA-TPO were moved by centrifugation for 2 min at 7000 rpm. The collected sediment was resuspended with the above buffer, and the obtained chloroplast suspension was used for the common fluorescence imaging. Meanwhile, the 20 ug·mL^{-1} chloroplasts without TPE-PPO and TPA-TPO were treated as the control group.

Photosynthetic activity measurement by DCPIP

The chloroplast suspension was pre-treated in dark for 20 min. And then the pre-treated chloroplasts were incubated with 1,2,4 μM TPE-PPO for another 20 min in dark. The 20 μg·mL^{-1} pristine chloroplasts without TPE-PPO and the free TPE-PPO through the same procedure were treated as the control groups. After incubation, all the above-tested samples were added to 96-well plates and mixed with the equal volume of DCPIP (100 μM) for 1 min. Under 0.5 mW·cm^{-2} or 1 mW·cm^{-2} UV irradiation (365 nm), the absorption of DCPIP at 600 nm was measured at certain timing (0, 1, 2, 3, 4, and 5 min, respectively) by plate reader. The TPA-TPO group was conducted as a similar procedure. The incubated concentration of TPA-TPO was 1 μM and the irradiated light source was replaced by the visible light. For the absorption spectrum measurement, the 75 μM DCPIP was mixed with 10 μg·mL^{-1} pretreated AIEgen-chloroplasts and irradiated under 2 mW·cm^{-2} UV or visible light. The final concentration of TPE-PPO was 0.5μM, and that of TPA-TPO was 1 μM.

Measurement of ATP synthesis

For experimental groups, the suspension of chloroplast was pre-treated in dark for 20 at 4 °C. Then, the pre-treated chloroplasts were mixed with TPE-PPO. The final concentration of chloroplasts was 20 μg·mL^{-1} and that of the TPE-PPO was 2 μM. After dark incubation for 20 min at 4 °C, the modified chloroplasts were divided into 4 groups equally, and irradiated under 1 mW·cm^{-2} UV light (365 nm) for 5 min respectively. For TPA-TPO, all the experimental conditions and procedures were kept the same as the TPE-PPO, yet the final concentration of TPA-TPO was 1 μM, and the light source was replaced by a visible light source. Meanwhile, 20 μg·mL^{-1} pristine chloroplasts without TPE-PPO through the same procedure with/no UV irradiation were treated as the control group. The ATP was extracted from the suspension as the previous report.[3] The amount of the extrated ATP was measured by an ATP Bioluminescent Assay Kit (Beyotime Biotechnology S0027).

The photosynthetic efficiency (quantum yield, QE) of light-to-ATP was calculated as the following formula, according to the previous reports.[4-5] The reduction of one ADP molecule to one APT molecule requires one proton.

$$ADP^{3-} + HPO_4^{2-} + H^+ \rightleftharpoons ATP^{4-} + H_2O$$

$$QE\ \% = \frac{1 \times C \times V \times N_A}{\varphi_{ph} \times t \times A} \times 100\%$$

Where t is the irradiation time to produce ATP; A is the area of illumination; C is the measured ATP concentration; V is the volume of chloroplasts; Φph is the measured photon flux, N_A is Avogadro's Number (6.02×10^{23}).

References

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[5] P. Gai, W. Yu, H. Zhao, R. Qi, F. Li, L. Liu, F. Lv and Shu Wang, *Angew. Chem. Int. Ed.* 2020, **59**, 7224 –7229

NMR and MS spectra

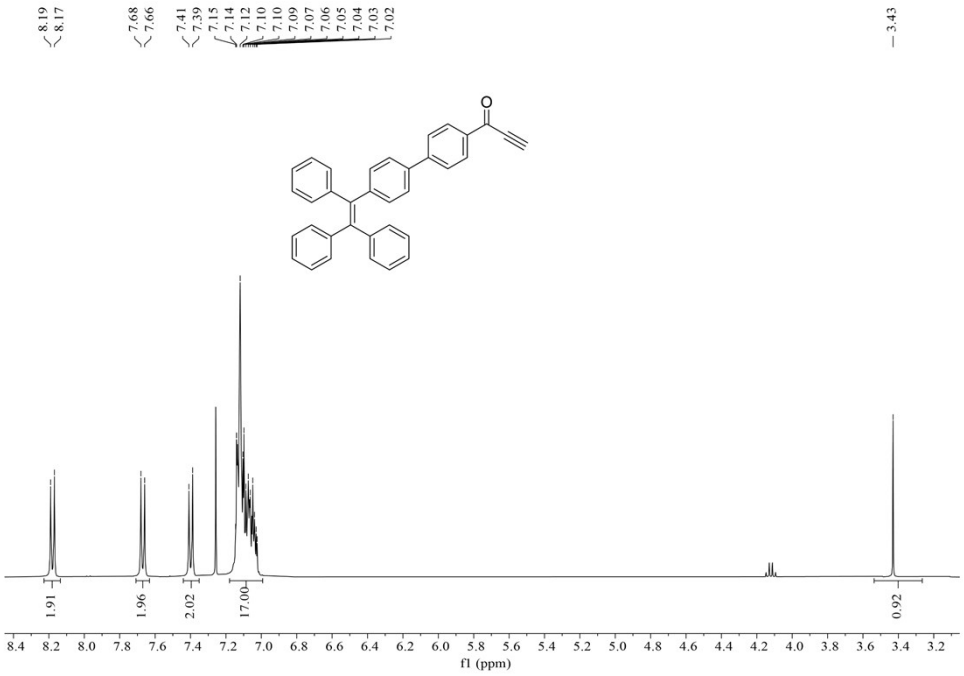

Figure S1. ^{1}H NMR spectrum of TPE-PPO in $CDCl_3$.

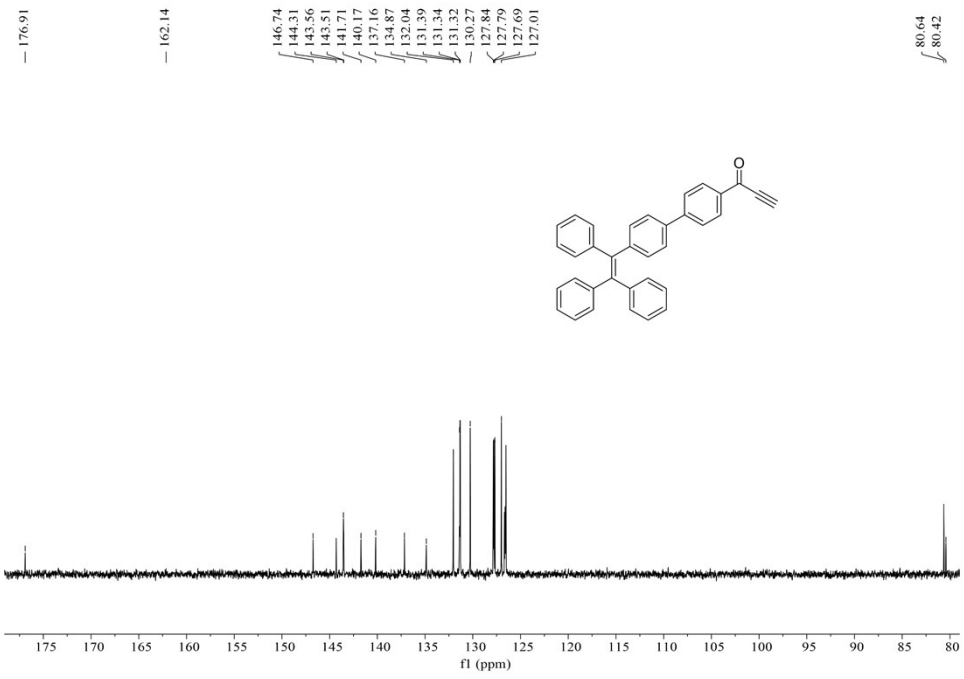

Figure S2. ^{13}C NMR spectrum of TPE-PPO in $CDCl_3$.

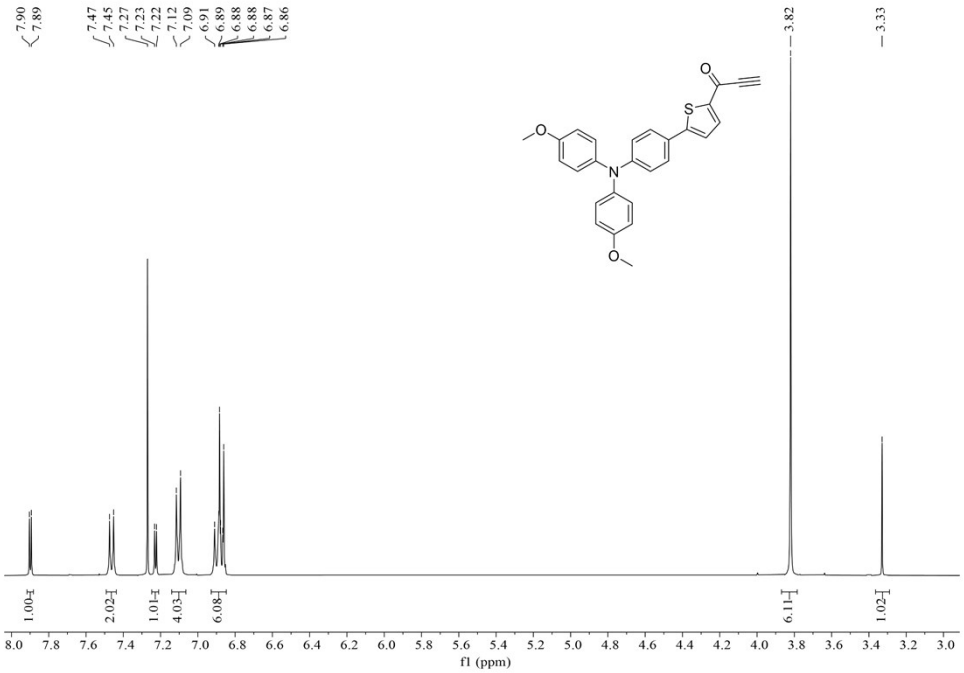

Figure S3. ^{1}H NMR spectrum of TPA-TPO in $CDCl_3$.

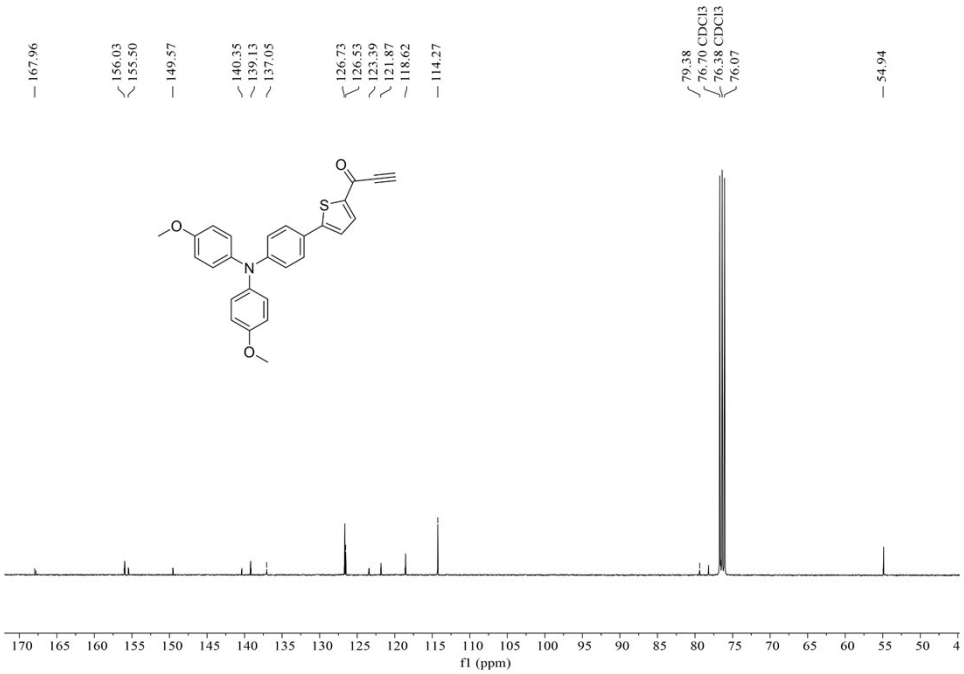

Figure S4. ^{13}C NMR spectrum of TPA-TPO in $CDCl_3$.

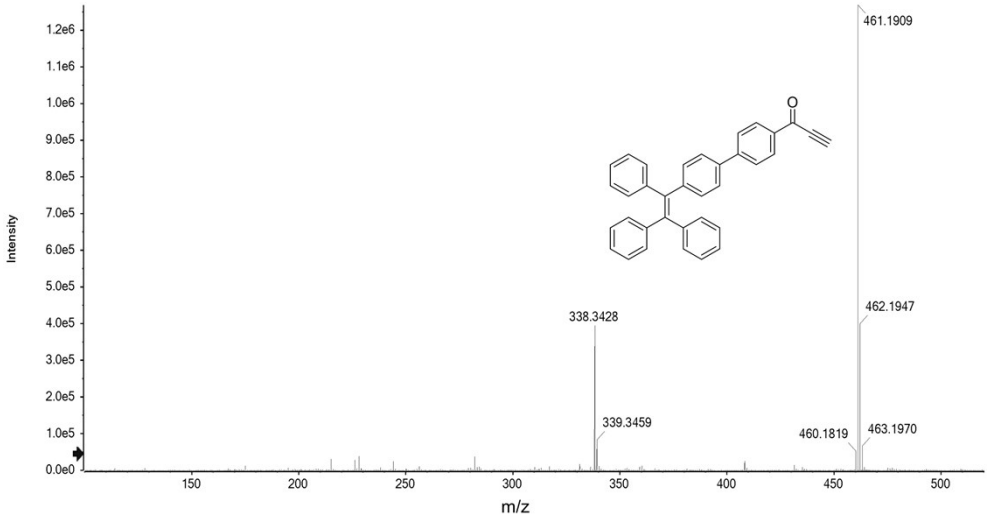

Figure S5. High Resolution Mass Spectrometry (HRMS) spectrum of TPE-PPO, [M+H] calculated for $C_{35}H_{24}O$, 461.1905.

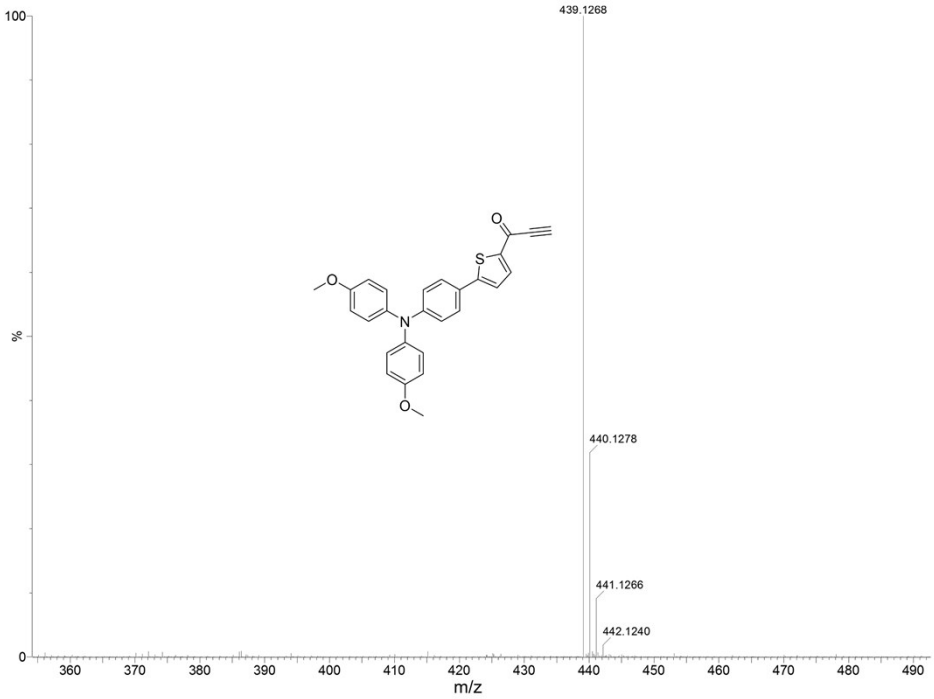

Figure S6. High Resolution Mass Spectrometry (HRMS) spectrum of TPA-TPO, [M+H] calculated for $C_{27}H_{21}NO_3S$, 439.1242.

Supplementary Figures and Tables

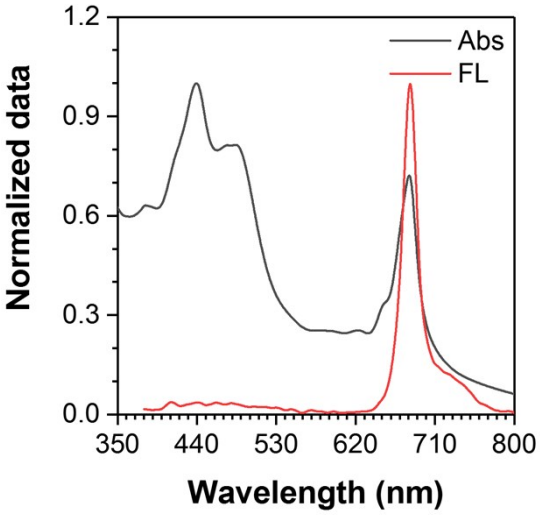

Figure S7. The normalized absorption (Abs) and emission (FL) spectra of the pristine chloroplasts.

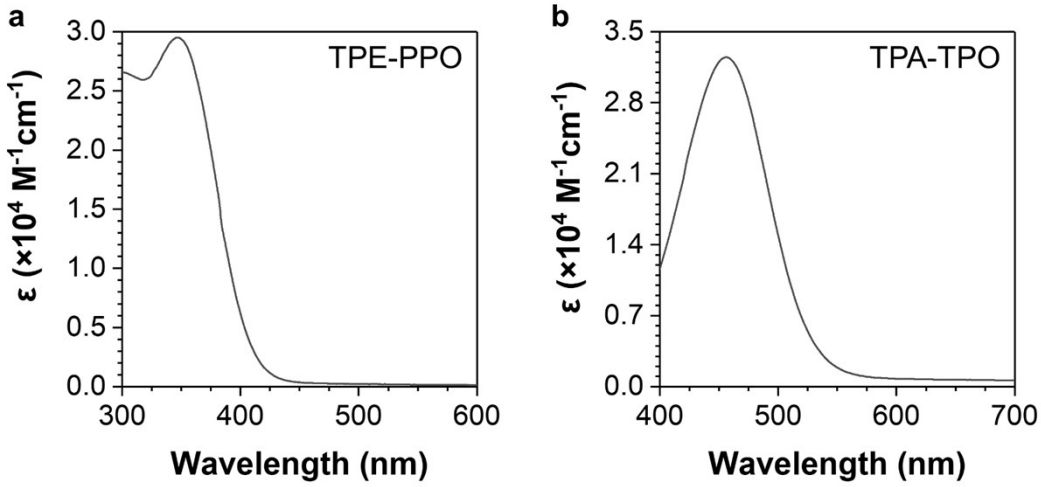

Figure S8. The molar extinction coefficient of TPE-PPO and TPA-TPO.

初稿的 **S8** 忘了添加

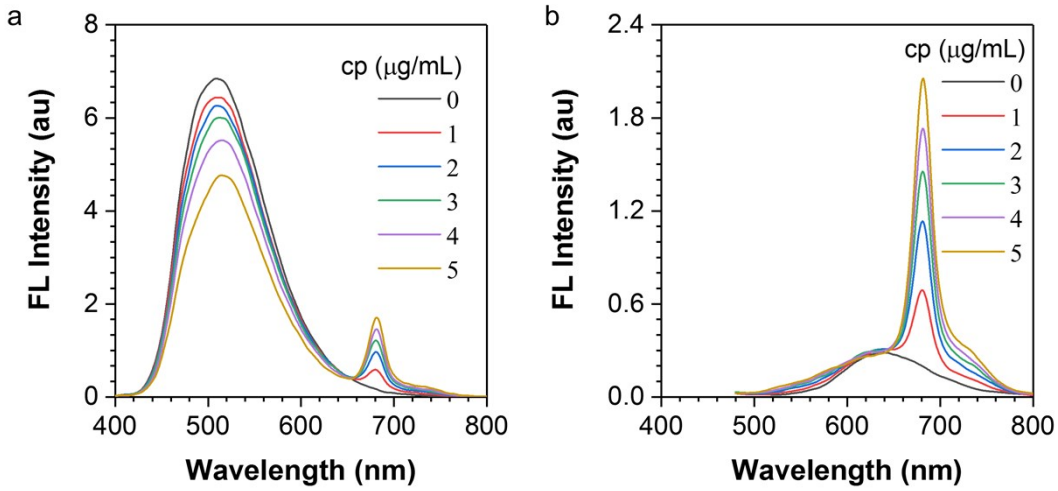

Figure S9. Fluorescence spectra of TPE-PPO (a) and TPA-TPO (b) with different amounts of chloroplasts (cp).

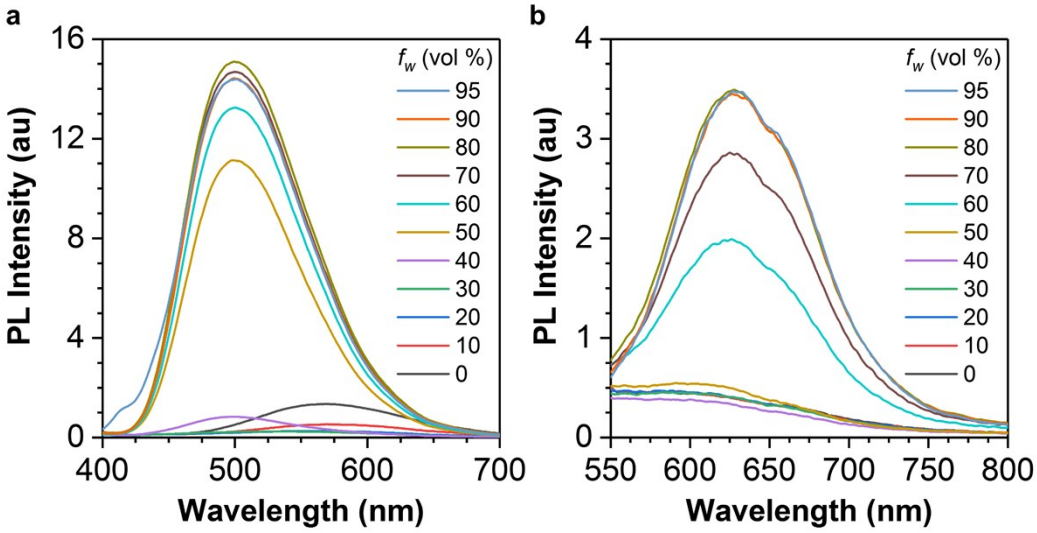

Figure S10. Fluorescence spectrum of TPE-PPO (a) and TPA-TPO (b) in DMSO/H_2O mixtures with different water fractions (*f*w).

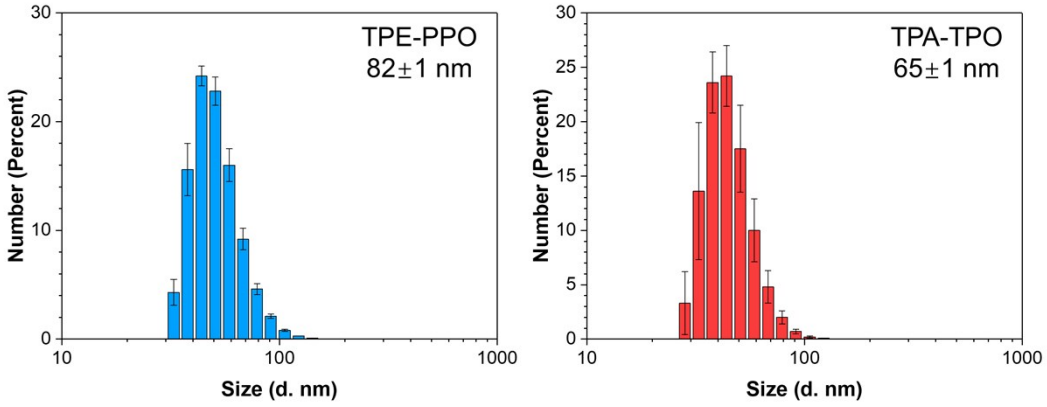

Figure S11. The size distribution of TPE-PPO and PTA-TPO (10 μM) in H_2O by DLS.

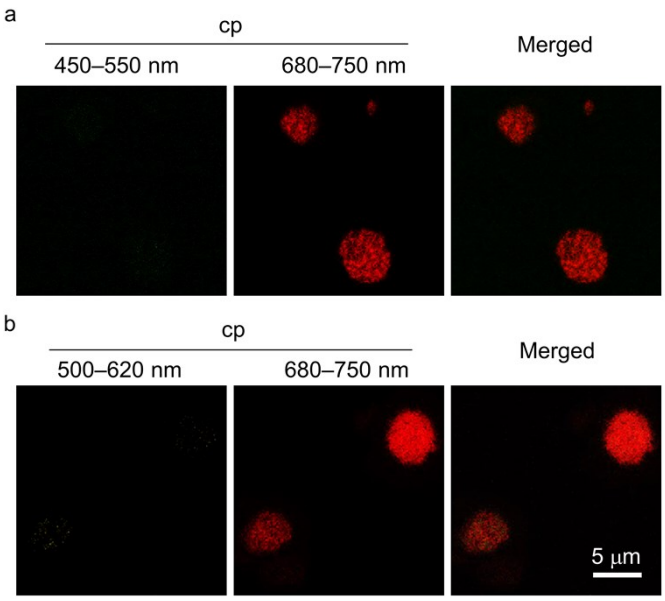

Figure S12. Confocal images of chloroplast (cp) without any treatments as the control group for Figure 3c; a is for TPE-PPO group and b is for TPA-TPO group.

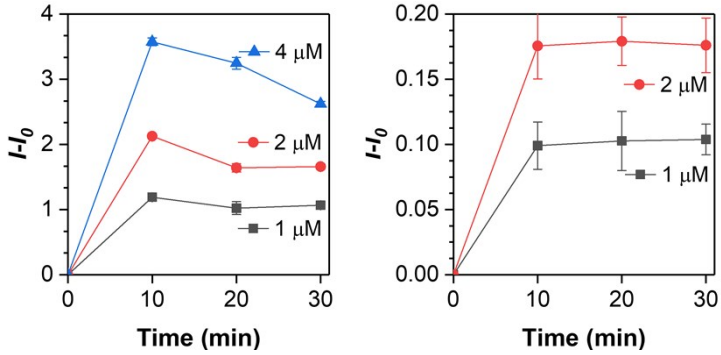

Figure S13. The fluorescent intensity change ($I-I_0$) of cp conjugated with different amount of AIEgen at different reaction time; TPE-PPO (a): λex = 360 nm, λem = 515 nm; TPA-TPO (b): λex = 460 nm, λex = 625 nm.

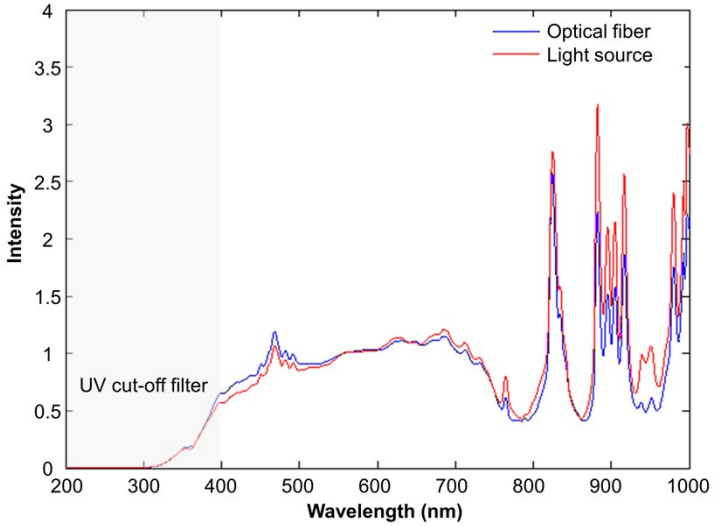

Figure S14. The spectra of the white light source.

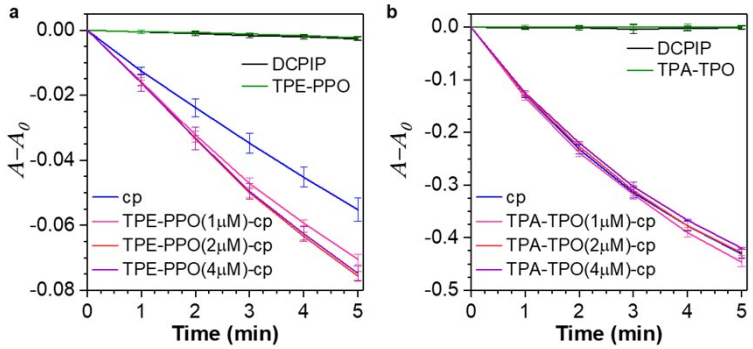

Figure S15. The absorption reduction of DCPIP at 600 nm for AIEgen-conjugated cps with different amounts of TPE-PPO (a) under 0.5 mW·cm^{-2} UV light and TPA-TPO (b) under 1 mW·cm^{-2} white light irradiation.

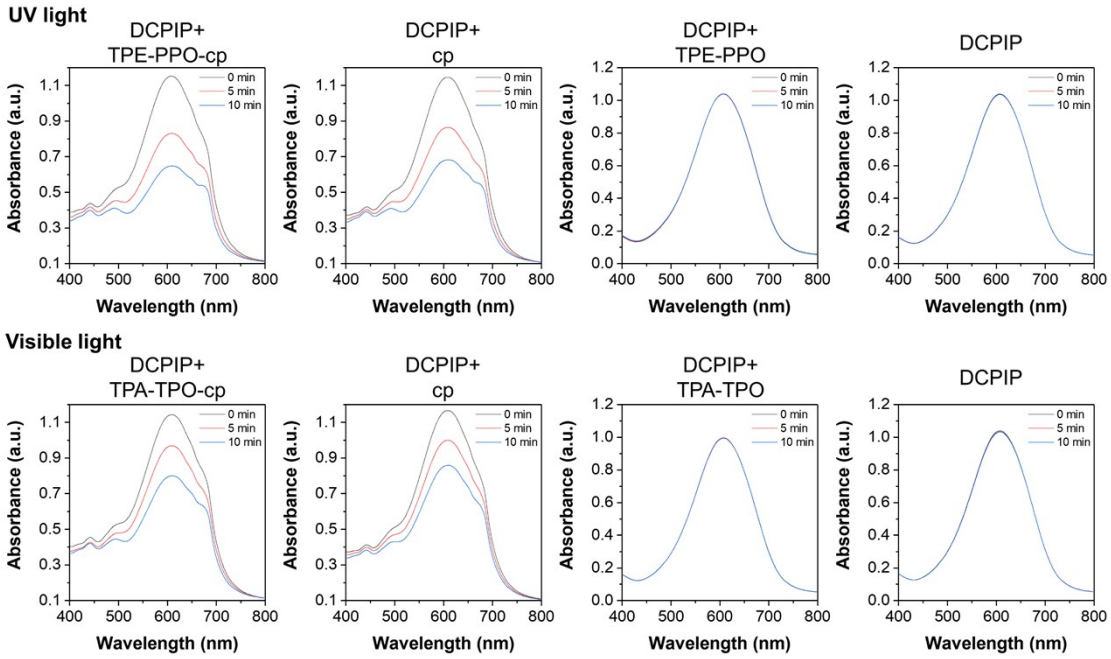

Figure S16. The UV-vis spectrum of DCPIP mixed with AIEgen-chloroplasts, chloroplast, and control groups under 2 mW·cm^{-2} UV light or visible light irradiation.

	F_v/F_m	ABS/RC	TR_0/RC	ET_0/RC	ΔR_0
cp	0.794	1.74	1.38	0.626	0.486
TPE-BPO-cp	0.790	1.79	1.42	0.657	0.483
TPA-TPO-cp	0.794	1.79	1.42	0.652	0.505

Table S1. Chlorophyll fluorescence parameters; *F*v/*F*m: maximal PSII quantum yield after dark adaptation; ABS/RC: absorbed energy per reaction center; TR_0/RC: trapped energy per reaction center; ET_0/RC: energy used for electron transfer per reaction center; ΔR_0: the efficiency of electron transfer to PSI.