SUPPORTING INFORMATION

Enhanced Photogenerated Electron Transfer in a Semi-artificial Photosynthesis System based on Highly Dispersed Titanium Oxide Nanoparticles

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Experimental

Materials

Tetrabutyl titanate ($Ti(OC_4H_9)_4$, 99%), ethanol absolute (CH_3CH_2OH, 99%), cyclohexane (C_6H_{12}), hydrochloric acid (HCl, 36–38%), methylene blue (MB), 2,6-dichlorophenolindophenol (DCPIP), sucrose, potassium chloride (KCl), dibasic sodium phosphate (Na_2HPO_4), potassium phosphate monobasic (KH_2PO_4), and acetone were purchased from MACLIN.

Synthesis of nanoTiO_2

According to Liu et al.,[1] 36 mL $Ti(OC_4H_9)_4$ was mixed with 90 mL ethanol and 90 mL C_6H_{12} for 10 min. Then, 9.0 mL 36% HCl was added and mixed for 10 min. The mixture was placed into a 70 °C water bath and stirred refluxing for 10 h. After cooling to room temperature, 600 mL ethanol was mixed dropwise in 2 h. After setting for 10 h, the supernatant was poured out and centrifuged at 5000 rpm for 5 min. Finally, white powder was obtained by washing with anhydrous ethanol three times. The white powder is TiO_2.

Characterization

Transmission electron microscopy (TEM) and high resolution transmission microscopy (HRTEM) were collected on a JEOL-2010 electron microscope. X-ray diffraction (XRD) analysis was employed on PANalytical (Cu-Kα radiation, λ=0.154051 nm). Fourier transform infrared

spectroscopy pattern (FTIR) was carried out on Nicolet 6700. X-ray photoelectron spectroscopy (XPS) analysis was conducted on Thermo Scientific Escalab 250Xi and element mapping by scanning electron microscopy (SEM) was conducted on Hitachi SU8220. Zeta potential and DLS measurements were carried out on Malvern Nano ZSE. UV-vis DRS and UPS measurement were conducted on and Thermo Fisher Scientific Escalab 250xi respectively. Steady-state photoluminescence (PL) emission spectrum and excited-state lifetime were conducted on Edinburgh Instruments FLS 1000.

MB reduction test

Quantities of titanium dioxide powder of 0.25 mg, 2.5 mg, 5.0 mg, 25 mg, 50 mg, and 100 mg were added to 50 mL MB solution at a concentration of 10 mg L^{-1}, respectively. After 1 h of dark stirring, the mixture was stirred under a xenon lamp with a light intensity of 190 $mW \cdot cm^{-2}$ for 3 h. Then, 5 mL of mixed liquid was taken every 0.5 h, and the purified liquid was centrifuged to record the absorbance of $OD_{664.}$

Isolation of chloroplast

Lettuce leaves with variable treatment were homogenized in a buffer containing 0.40 M sucrose, 10 mM KCl, 30 mM Na_2HPO_4, and 20 mM KH_2PO_4, and then filtered through four layers of cotton gauze. The filtrates were subsequently centrifuged at 1000 rpm for 3 min. Supernatant liquid in

tube was centrifuged at 3000 rpm for another 3 min. Chloroplast were obtained from the residue in the bottom of the tube and spread in buffer solution.

Hill reaction

Concentrations of chloroplast suspension from lettuce leaves with variable treatment were measured by means of adding 0.10 mL chloroplast suspension into a 4.9 mL mixture of ethanol and acetone for measuring the absorbance at 650 nm. 4.0 mL chloroplast suspension (421.74 $mg \cdot L^{-1}$) were mixed with 4.0 mL 10, 100, 200, 1000 2000, and 4000 $mg \cdot L^{-1}$ TiO_2 NPs buffer dispersion for 2 h. 2.0 mL of 12 μM DCPIP solution was added, subsequently. Illuminated under a xenon lamp with the intensity of 7.0 mW cm^{-2} for 5 min, the absorbance spectrum was recorded at 600 nm every 1 min.

Ferricyanide reduction

According to Chandra,[2] 1.0 mL hybrid chloroplast suspension (500 $mg \cdot L^{-1}$) was added to 2.0 mM ADP and 1.0 mM ferricyanide. After 1 min of sunlight irradiance at an intensity of 73 $mW \cdot cm^{-2}$, 2% trichloroacetic acid was added. Recorded The absorbance at 420 nm of the supernatant was recorded by 10 min of centrifuge at 8000 g.

ATP measurement

From hybrid chloroplast suspension, 1.0 mL was added to 2.0 mM ADP. Following 1 min of sunlight irradiance at intensity of 73 $mW \cdot cm^{-2}$, an ATP Content Assay Kit was taken to measure the ATP content.

H_2 measurement

25 mL chloroplast suspension (421.74$mg \cdot L^{-1}$) was mixed with 25 mL 500 $mg \cdot L^{-1}$ TiO_2 NPs buffer dispersion for 2 h. After 1 hour of illumination under a xenon lamp with a light intensity of 190 $mW \cdot cm^{-2}$, the hydrogen production was measured by TECHCOMP GC 7900.

MDA measurement

4.0 mL chloroplast suspension (421.74 $mg \cdot L^{-1}$) was mixed with 4.0 mL 1000 $mg \cdot L^{-1}$ TiO_2 buffer solution. After 5 min of illuminating under a xenon lamp with a light intensity of 7.0 $mW \cdot cm^{-2}$, it was centrifuged at 3000 rpm for 3 min, then 0.05 M phosphate buffer (Na_2HPO_4, NaH_2PO_4) were used to wash away the sucrose phosphate buffer three times. A MDA Content Assay Kit was taken to measure the MDA content.

SOD measurement

The SOD activity was measured by a SOD Content Assay Kit.

Spraying on lettuce leaves

Lettuce seeding was Italian Lettuce purchased from Guangdong Academy of Agricultural Sciences in China. When the seedlings were grown to three leaves, they were transplanted to the hydroponic rack. After 10 days of transplanting, 5.0, 50, and 100 $mg \cdot L^{-1}$ of as-prepared TiO_2 NP dispersion solution was sprayed on the leaves and treated every 5 days. A one-way analysis of variance (ANOVA) was used to analyse the differences.

Sample preparation for ICP-MS

The lettuce leaves were washed with pure water three times to clean TiO_2 NP covering on the surface of the leaves. After drying, 0.50 g dry sample was added to 5 mL HCl and mixed with 2.0 mL H_2O_2, placed into a microwave digestion system at 180 °C for 1 h.

Zeta potential and DLS measurement

The Zeta potential of TiO_2, CLP and the hydrate particle size of TiO_2 were measured by Molvern Zetasizer Nano ZS90.

Supplementary figures and tables

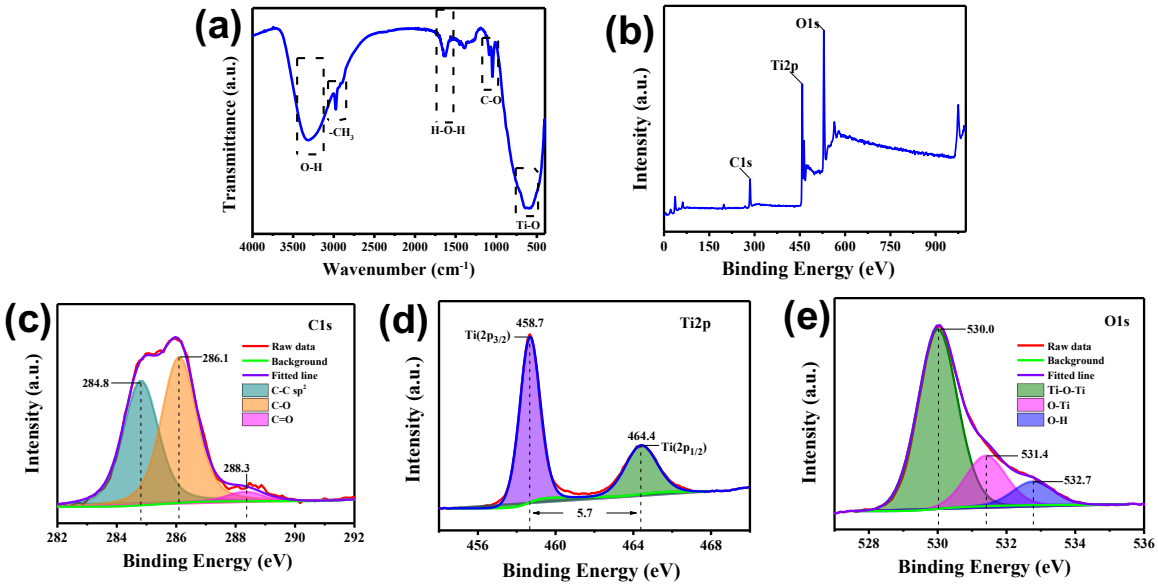

Figure S1 (a) FTIR image of TiO_2. (b) XPS image of TiO_2. (c-d) XPS peak separation images of C1s, Ti(2p), and O1s.

Table S1 DLS data of TiO_2

Sample	PDI	Size (d.nm)	Volume (%)	St Dev.
1	0.249	13.04	100	6.754
2	0.264	10.68	100	6.485
3	0.262	11.72	100	6.823
4	0.247	9.738	100	6.578
Average	0.255	11.2945	100	

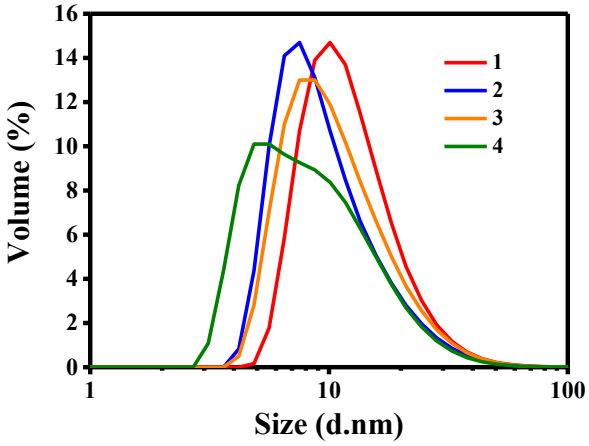

Figure S2 Volume/Size distribution of TiO_2 NPs (1, 2, 3, 4 represent the repetition times)

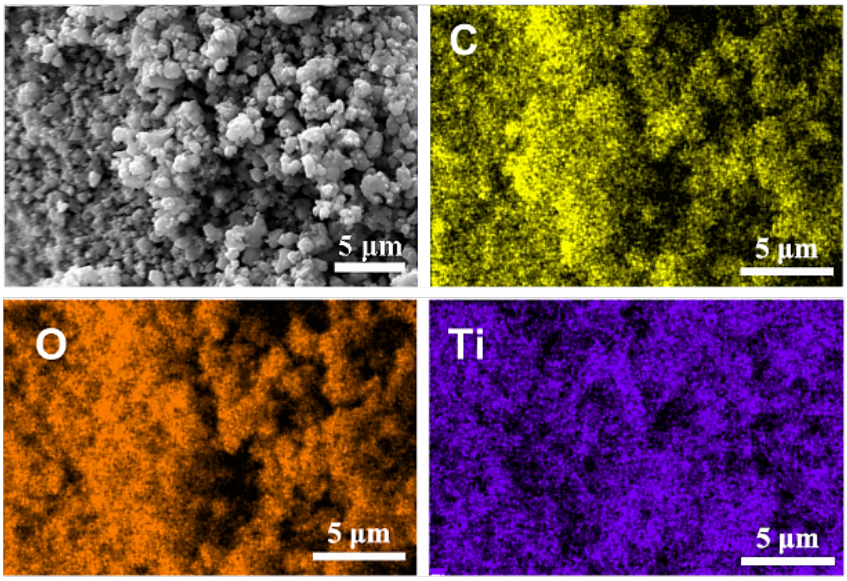

Figure S3 Element mapping of as-prepared TiO_2 powder.

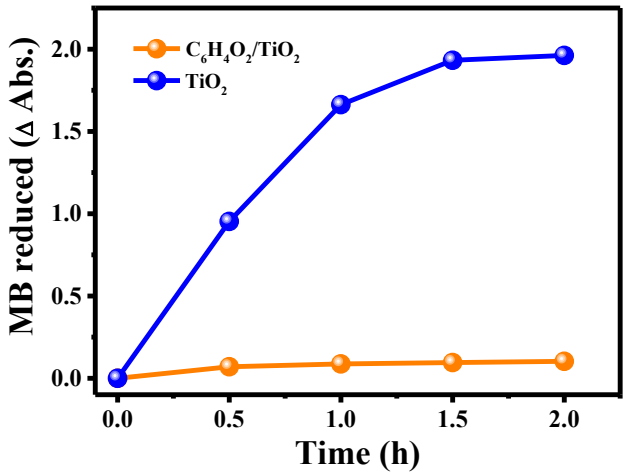

Figure S4 MB reduction by TiO_2 and with $C_6H_4O_2$ present. (The MB reduction experiments were carried out under a xenon lamp with the intensity of 190 $mW \cdot cm^{-2}$, expressed as absolute difference in absorbance.)

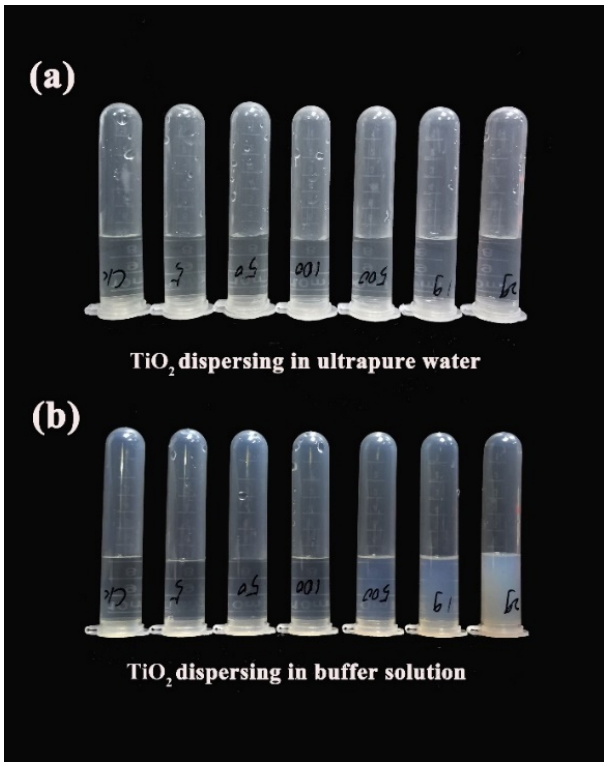

Figure S5 (a) Variable concentrations of TiO_2 dispersed in ultrapure water. (b) Variable concentrations of TiO_2 dispersed in buffer solution.

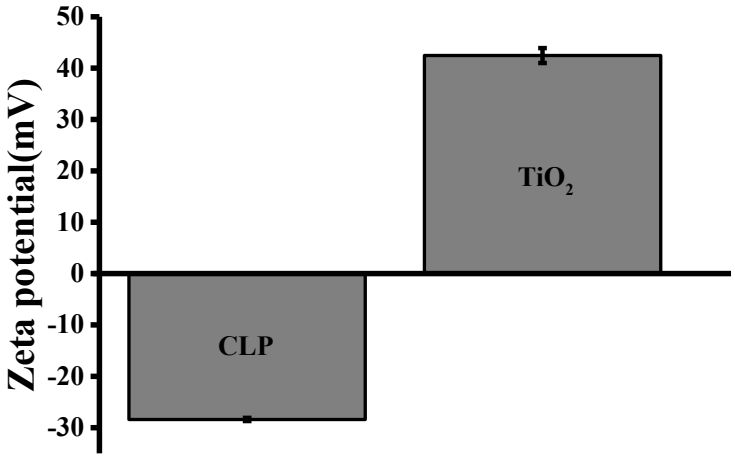

Figure S6 Zeta potential of TiO_2 and CLP.

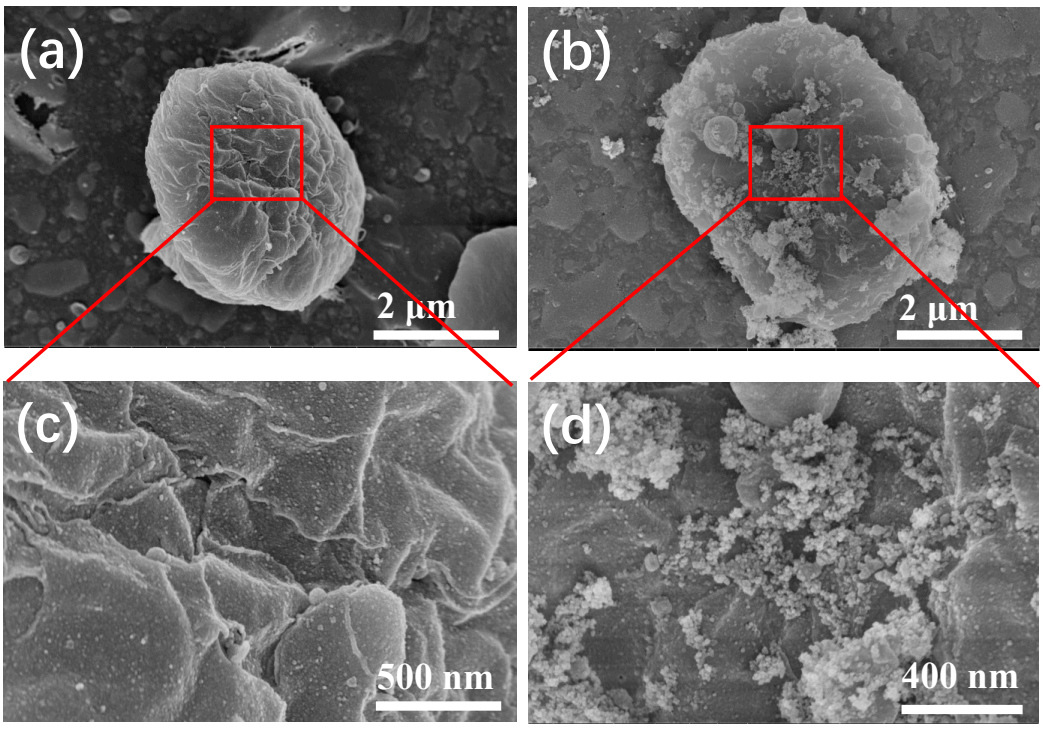

Figure S7 (a) FESEM image of pure CLP; (b) FESEM image of TiO_2/CLP; (c) Enlarged FESEM image of pure CLP; (d) Enlarged FESEM image of TiO_2 NPs distributed on CLP surface.

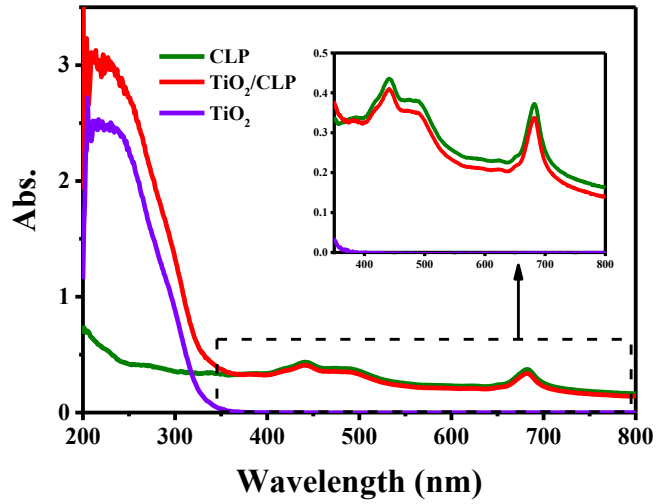

Figure S8 UV-vis absorption spectrum of TiO_2, chloroplast, and TiO_2/CLP.

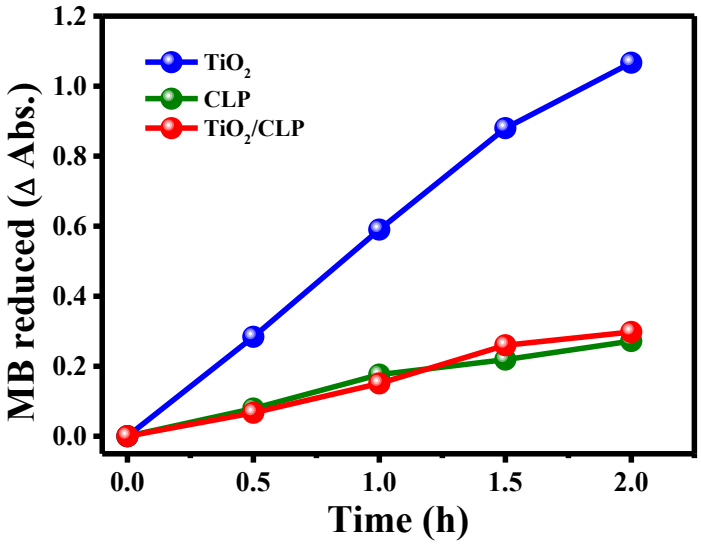

Figure S9 MB reduction by TiO_2 and TiO_2/CLP in buffer solution. (The MB reduction experiments were carried out under a xenon lamp with the intensity of 190 $mW \cdot cm^{-2}$, expressed as absolute difference in absorbance.)

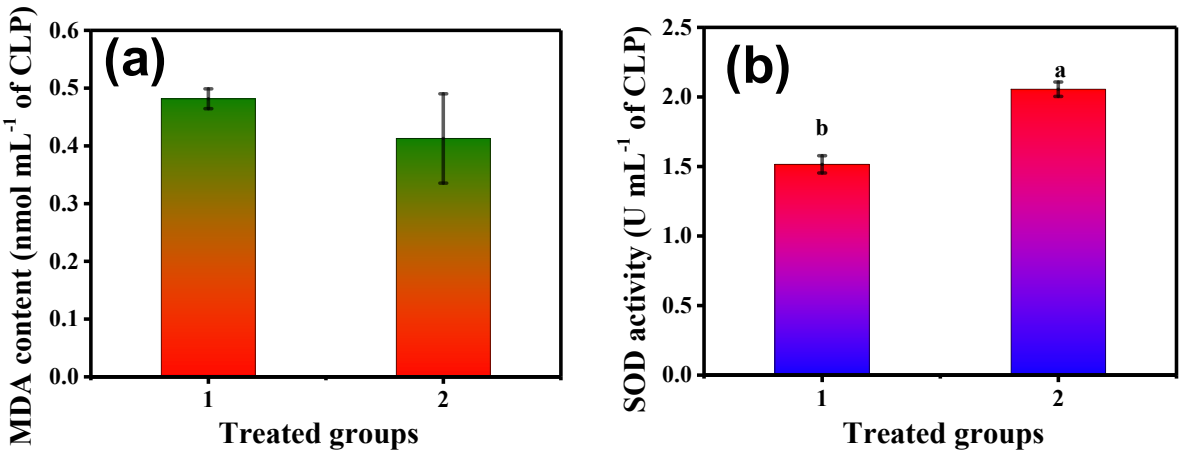

Figure S10 (a) MDA content of CLP and TiO_2/CLP after illuminating. (b)SOD activity of CLP and TiO_2/CLP after illuminating. (The illumination was under a xenon lamp with the light intensity of 7.0 mW cm^{-2}; "1" represents the CLPs and "2" represents the TiO_2/CLPs. $P < 0.05$, "a" represents the highest grade number while "b" represents the lower grade number, the different letters mean they have significant difference.)

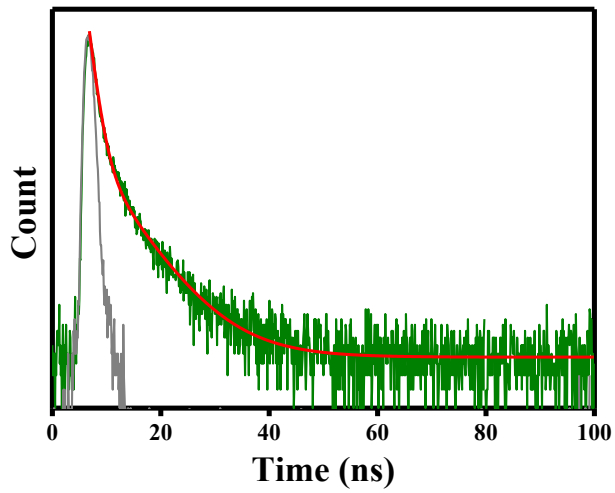

Figure S11 TRPL decay profiles of CLP monitored at 450 nm emission.

Table S2 Multi-exponential fitting of TiO_2 and TiO_2/CLP

Sample	τ_1	A_1	τ_2	A_2	τ	χ^2
TiO_2	2.14126	11043.79959	10.65794	287.58052	3.118465477	0.99019
TiO_2/CLP	1.23236	104668.5925	7.65501	313.39647	1.349632711	0.99404
CLP	1.31985	161119.68573	7.58672	371.58545	1.401841857	0.99261

Computational formula: $\tau = (A_1\ \tau_1^2 + A_2\ \tau_2^2) / (A_1\ \tau_1 + A_2\ \tau_2)$

REFERENCES

(1) Liu, X.; Chen, Y.; Rao, S. H.; Pang, G. S. One Step Preparation of Highly Dispersed TiO_2 Nanoparticles. *Chem. Res. Chinese U.* **2015**, *31*, 688-692. DOI: 10.1007/s40242-015-5300-2

(2) Chandra, S.; Pradhan, S.; Mitra, S.; Patra, P.; Bhattacharya, A.; Pramanik, P.; Goswami, A. High throughput Electron Transfer from Carbon Dots to Chloroplast: A Rationale of Enhanced Photosynthesis. *Nanoscale* **2014**, *6*, 3647-3655. DOI: 10.1039/c3nr06079a