Supporting Information

Flaponite: recyclable heterogenous flavin-based photocatalyst employing clay as immobilisation scaffold

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Experimental procedures

Materials and methods

Commercially available reagents were purchased in the highest purity from Merck, Sigma Aldrich and VWR Chemicals. The laponite B was received as a gift from Professor Stuart Clark (Department of Chemistry, University of Cambridge). Riboflavin tetraacetate (RTA) was synthesized by Dr I. Ahmed (University of Cambridge, CEB) from (-)-riboflavin (from *Eremothecium ashbyii*, ⩾98%, Merck) as detailed by Metternich *et al*.[46] All experiments were done with ultra-high purity (UHP) water. UV-Vis absorption spectra were obtained using a custom built setup. Fluorescence emission spectra were obtained using a Varian Cary Eclipse Fluorescence Spectrophotometer.

Solid-state nuclear magnetic resonance spectroscopy

All experiments were carried out on full 4 mm zirconia rotors spinning at 10 kHz magic angle spinning (MAS) in a Bruker double-resonance MAS probe on a Bruker AVANCE Neo 600 MHz spectrometer. 13C CP-MAS: 1H 90° pulse / SPINAL64 decoupling: 100 kHz, contact time of 2 ms with a ramped pulse on 1H and square pulse on 13C, 60 kHz spin lock field strength, 2.5 s recycling delay. Chemical shifts were referenced, setting the Cα Glycine resonance to 43.1ppm.

Powder X-ray diffraction (XRD)

Powder x-ray diffraction data was collected using a Malvern PANalytical X′ Pert Pro diffractometer, equipped with an X’ celerator detector and using non-monochromated Cu-Kα radiation. The sample was placed on a glass sample mount and measured in reflection geometry with sample spinning. Data was collected over a 2θ range from 5° to 70° with a step size of 0.01° and a total collection time of 1 hour.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR (ATR) spectroscopy measurements were performed using a Shimadzu IRTracer spectrophotometer equipped with QATR 10 single reflection ATR accessory. Data were acquired between 3500–400 cm−1. FTIR of RTA was performed with a Bruker Tensor 27 FT-IR Spectrometer with KBr discs over wavenumber ranging from 4000–400 cm−1.

Transmission electron microscopy (TEM)

Electron microscopy images were taken using a Thermo Fisher Scientific Talos F200X G2, a 200 kV FEG Scanning Transmission Electron Microscope (S/TEM) equipped with a Ceta 16M camera. The samples, dispersed in water, were drop cast and left to air-dry on Agar Scientific Ltd (Stansted, UK) continuous carbon-coated copper grids. Energy dispersive X-ray spectroscopy (EDS) was performed in the same instrument using a Super-X EDS detector and Velox Software (Thermo Fisher Scientific).

Scanning electron microscopy (SEM)

2 mg of dry laponite B, FlapLP or FlapMix were spread across carbon adhesive discs, sputter-coated with a 10 nm platinum film using a Q150T ES Turbo-Pumped Sputter Coater (Quorum Technologies) and imaged using a Tescan MIRA3 FEG-SEM operated at 5 kV.

Flaponite prepared by Lake Pigment method (FlapLP)

1 g of laponite B was mixed with 20 mL of 1 mM RTA in water using a magnetic stirrer in a beaker and heated to 80 °C. Upon heating, 400 µL of 0.5 M alum (in water) was added to the suspension and mixed for 2 minutes, followed by 700 µL of saturated NaHCO3 (9.6% wt/wt in water). The mixture was stirred at rt for 30 minutes before being allowed to settle for 18 h. The precipitate was dried at 120 °C for 24 h. Yield 79.2%.

Flaponite prepared using mixing of flavin and laponite B (FlapMix)

1 g of laponite B was mixed with 20 mL of 1 mM RTA in water using a magnetic stirrer in a beaker and heated to 80 °C. The mixture was stirred at rt for 30 minutes before being allowed to settle for 18 h. The precipitate was dried at 120 °C for 24 h. Yield 83.2%.

Study of flavin leaking

The leaking experiment was carried out by mixing 10 mg of Flaponite in 1 mL of 50 mM EDTA solution for 1 h using a shaker centrifuging the mixture at 5000 rpm for 10 minutes. Then the solution was decantated, and the solid precipitate was dissolved in 1 ml of UHP water (C = 10 mg of clay per ml), sonicated in an ultrasound bath for 20 min (at 40 C), diluted to get the concentration 3 mg/ml and fluorescence spectra were recorded (Excitation wavelength 450 nm). The intensity of fluorescence at 522 nm was compared to a sample of Flaponite (10 mg) prepared by the addition of 50 mM EDTA solution, centrifuging for 10 min, decantation of supernatant, dissolving the residual clay in 1 ml of UHP water and dilution to reach C = 3 mg/ml.

Photocatalytic activity

Photocatalytic reactions were monitored using a custom-build setup, as shown in Figure S7. A DH-2000 UV-Vis-NIR light source (OceanInsight), in combination with a QE6000 spectrometer (OceanOptics) was used to obtain absorption spectroscopy measurements from samples within a cuvette. The cuvette was placed within a holder on a magnetic stirrer plate to ensure continuous mixing throughout measurements. A 450 nm LED lamp (HepatoChem, HCK1012-01-002) was situated across from the cuvette to allow side irradiation of samples without interfering with the absorption measurements. The intensity of the lamp at the position of the cuvette was measured to be 140 mWcm-1.

Photo-oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)

Reaction mixtures containing 2.5 mg/mL Flaponite, 2.5 mM ABTS and 5 mM NaOAc pH 4 were prepared within a cuvette with a total volume of 2 mL. The cuvette was then placed within the previously described photocatalytic setup. The spectrometer was set to record data every 2.5 s with an integration time of 10 ms, and after 1 minute of measurements, the 450 nm LED lamp was switched on. After 10 minutes of irradiation, the lamp was switched off, and data was collected for a further 1 minute. Experiments involving RTA were carried out as described

above but using 0.027 mg/mL RTA instead of Flaponite. Samples were later filtered with a Minisart SFCA 0.2 µm filter (Sartorius).

RNO assay for singlet oxygen detection

Reaction mixtures containing 50 µM p-nitrosodimethylaniline (RNO), 1 mM imidazole and 2.5 mg/mL $Flap_{Mix}$ were prepared in PBS pH 7 buffer within a cuvette, with a total volume of 2 mL. Negative control samples were also prepared by omitting the $Flap_{Mix}$. The cuvette was then placed within the previously described photocatalytic setup. The spectrometer was set to record data every 5 s with an integration time of 10 ms, and after 1 minute of measurements, the 450 nm LED lamp was switched on. After 10 minutes of irradiation, the lamp was switched off, and data was collected for a further 1 minute.

Photoreduction of amaranth dye

Reaction mixtures containing 0.5 mg/mL Flaponite, 50 mM EDTA pH 6 and 50 µM amaranth were prepared within a cuvette, with a total volume of 2 mL. A magnetic stir bar was then added before the cuvette was capped with a rubber stopper and purged with nitrogen for 15 minutes to remove the presence of oxygen. Following purging, the cuvette was placed within the previously described photocatalytic setup. The spectrometer was set to record data every 0.2 s with an integration time of 10 ms, and after 30 s of measurements, the 450 nm LED lamp was switched on. After 5 minutes of irradiation, the lamp was switched off, and data was collected for a further 1 minute.

Recycling of Flaponite from reaction mixtures

Following the photo-reduction of amaranth, the recyclability of the sample was tested. The samples were transferred into an Eppendorf and centrifuged for 15 minutes at 5000 rcf. Centrifuging caused the Flaponite to pellet at the bottom of the Eppendorf, and the supernatant was then carefully removed so as not to disturb the Flaponite. The samples were then resuspended using a solution of 50 mM EDTA pH 6, and following the addition of amaranth, the photo-reduction protocol was repeated as described previously.

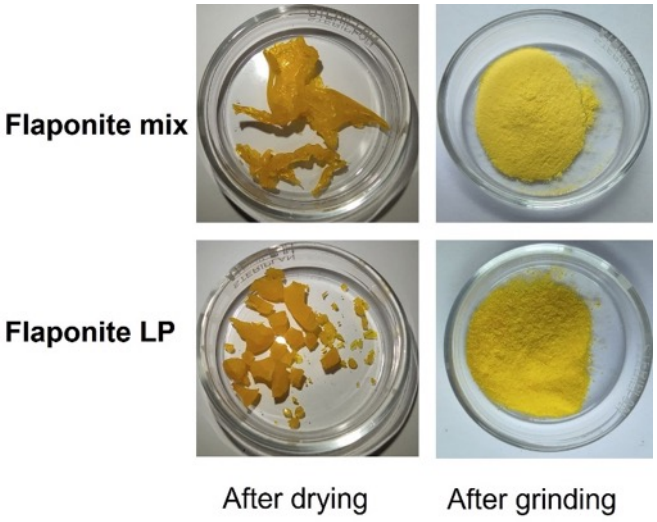


Figure S1. Photo of $Flap_{mix}$ and $Flap_{LP}$ after drying for 24 hours at 120 °C and after grinding with mortar and pestle.

TEM imaging

TEM of the clay helped to clarify the structural features of clay platelets and the chemical composition of the material. The images in Figure S2 show that the individual clay platelets, embedded in the hydrogel structure, are nanosized particles of indefinite form and are not nanodisks,although higher resolution imaging would be required to discern smaller features.

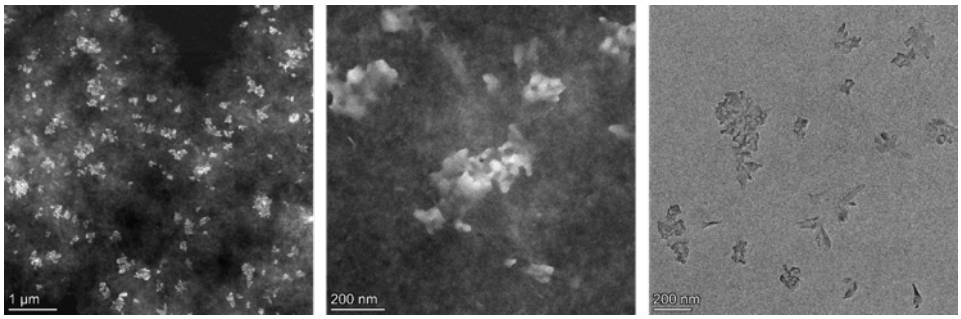


Figure S2. Laponite B TEM images.

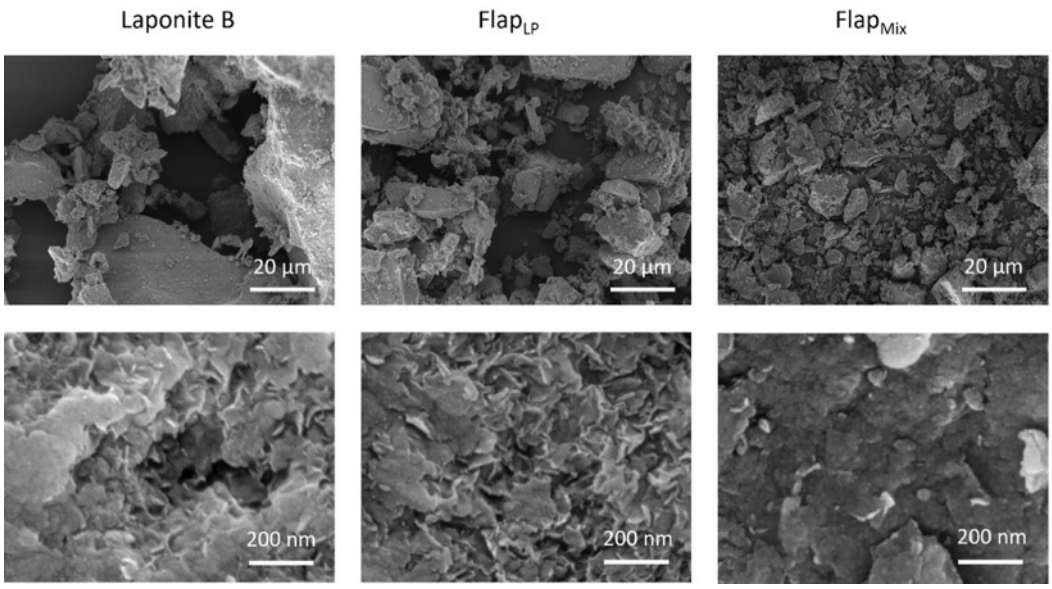
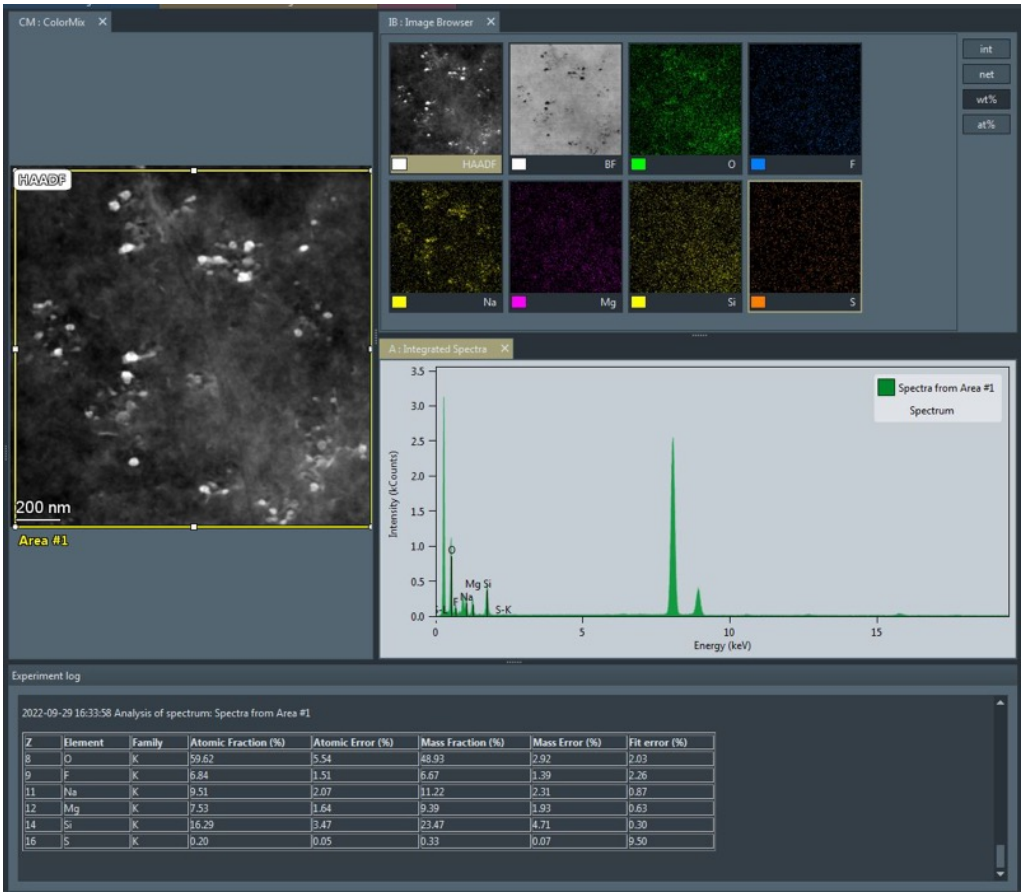


Figure S3. SEM images of Laponite B, FlapLP, FlapMix.

SEM imaging



Z	Element	Family	Atomic Fraction (%)	Atomic Error (%)	Mass Fraction (%)	Mass Error (%)	Fit error (%)
8	O	K	59.62	5.54	48.93	2.92	2.03
9	F	K	6.84	1.51	6.67	1.39	2.26
11	Na	K	9.51	2.07	11.22	2.31	0.87
12	Mg	K	7.53	1.64	9.39	1.93	0.63
14	Si	K	16.29	3.47	23.47	4.71	0.30
16	S	K	0.20	0.05	0.33	0.07	9.50

Figure S4. TEM images with EDX analysis of Laponite B, chemical composition.

TEM with EDX function showed that the present Laponite B is a sodium magnesium fluorosilicate (Figure S4).

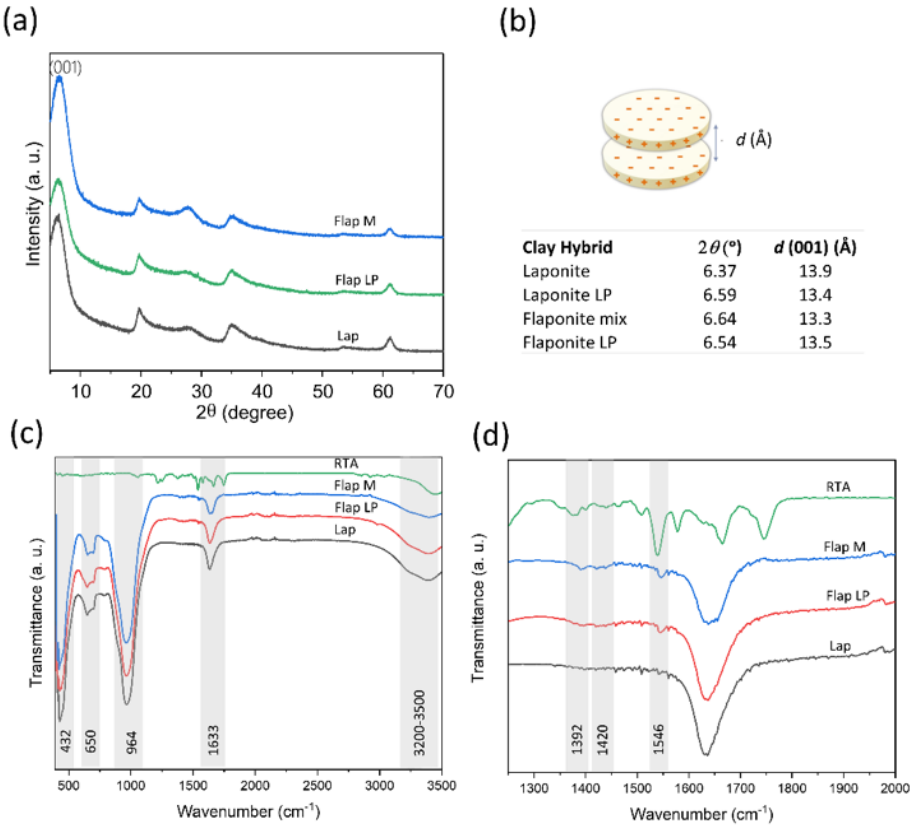


Figure S5. Characterization of unmodified laponite and Flaponite (Flap) hybrids using (a) XRD of unmodified clay and clay hybrids. (b) Table showing the *d* spacing for the clay hybrid samples. (c) FTIR of individual components (RTA and laponite) and Flaponite hybrids. (d)Enlarged area in showing a selected region of FTIR spectra, indicating weak flavin bands within hybrid structure.

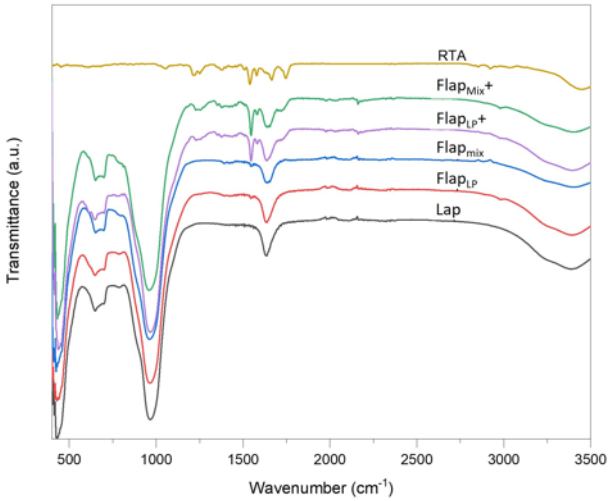
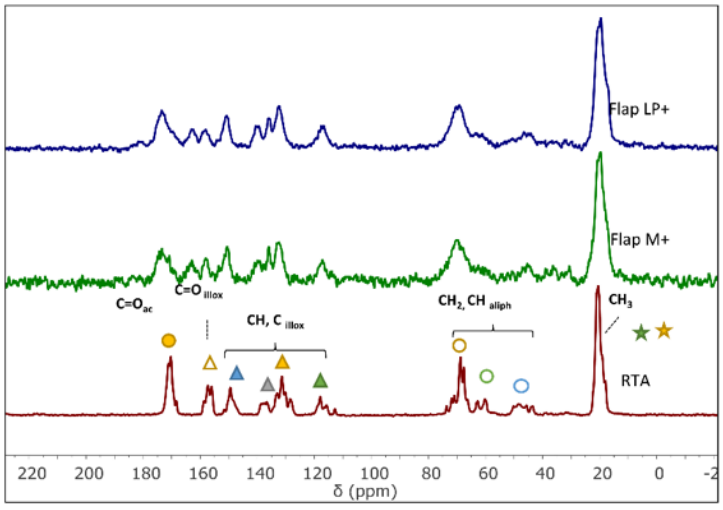


Figure S6. FTIR spectra of RTA in KBr pellet and FTIR (ATR) spectra flaponite hybrids.



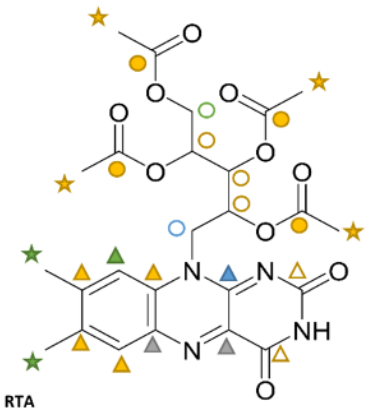


Figure S7. Solid-state ^{13}C CP/MAS NMR spectra with the molecular structure of RTA with atom labelling.

Flavin leaking experiments

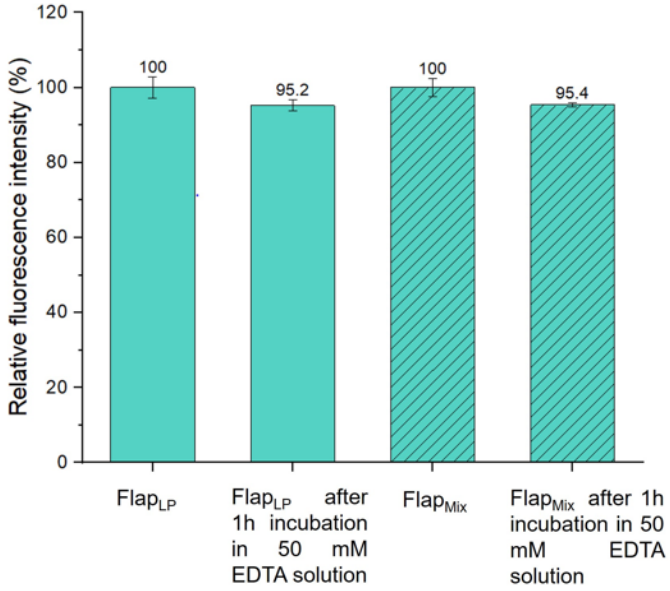


Figure S8. Leaking experiment: relative fluorescence intensity of $Flap_{LP}$ and $Flap_{Mix}$ at 522nm (excitation wavelength is 450 nm) in solution (3mg/ml in 50mM EDTA solution) after 1h of incubation of dry $Flap_{Mix}$ compared to non-incubated sample.

Optical set up for study of photocatalysis

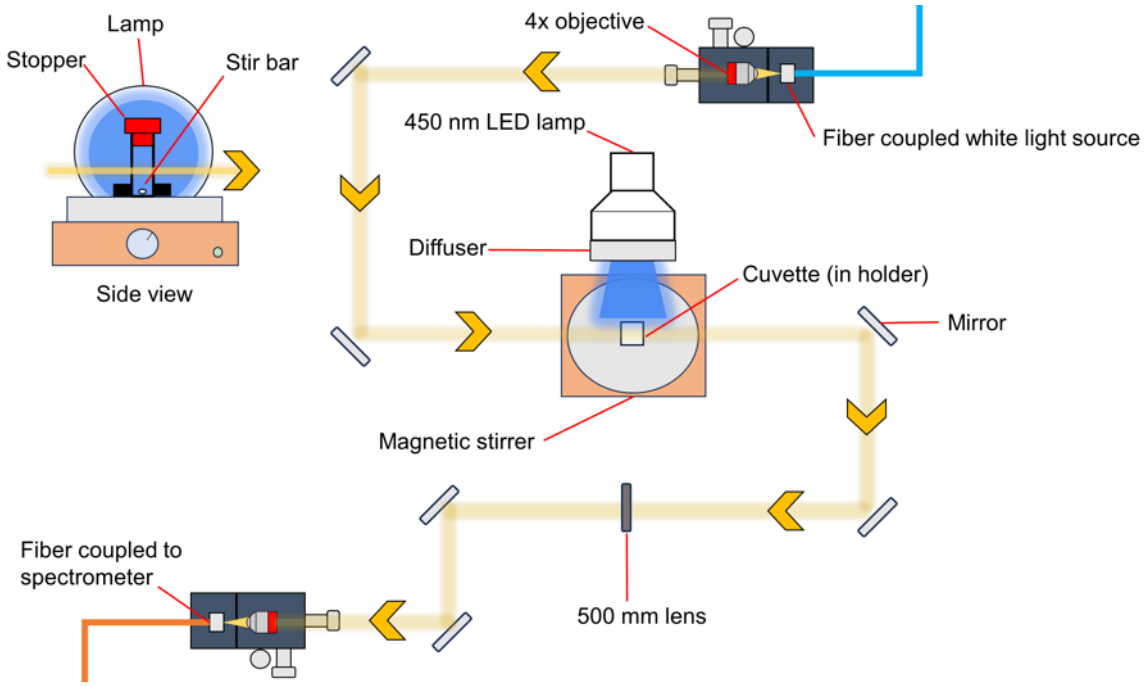


Figure S9. Schematic of the optical setup used within the photocatalysis experiments.

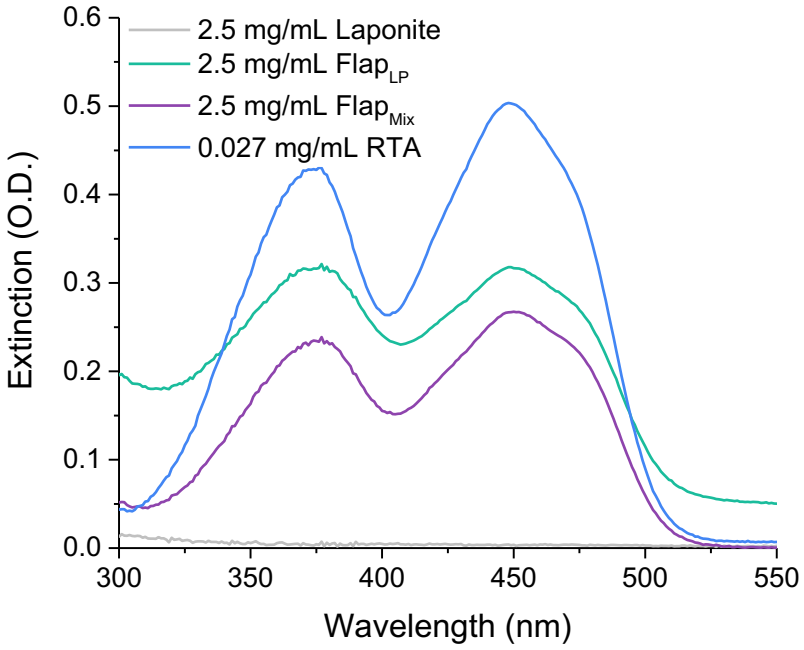


Figure S10. Extinction spectra of laponite, Flap$_{LP}$, Flap$_{Mix}$ and RTA in water. Taken using a Shimadzu UV-3600i Plus UV-VIS-NIR Spectrophotometer.

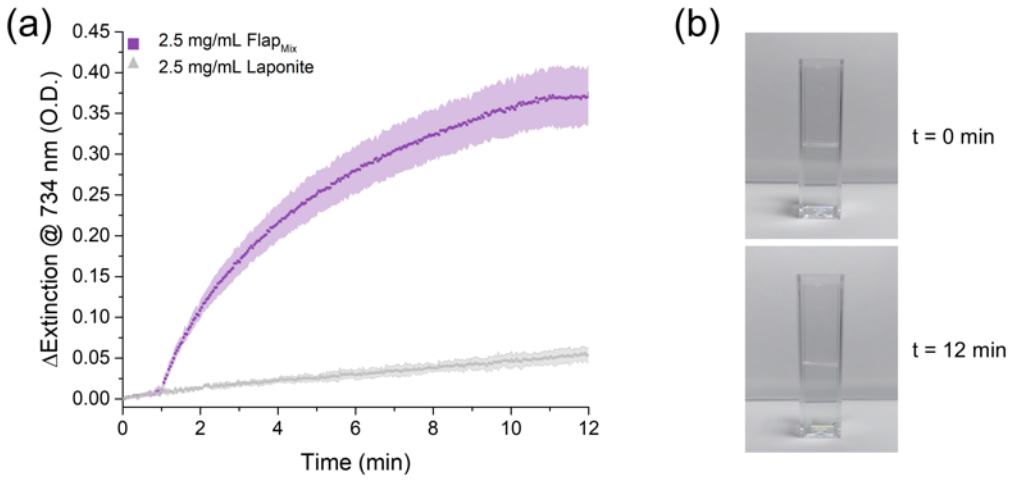


Figure S11. (a) Spectra showing the change in extinction at 734 nm of 2.5 mg/mL Flap$_{Mix}$ and laponite during ABTS photooxidation experiment. (b) Photographs of the reaction mixture used within the experiment at t = 0 min and t = 12 min.

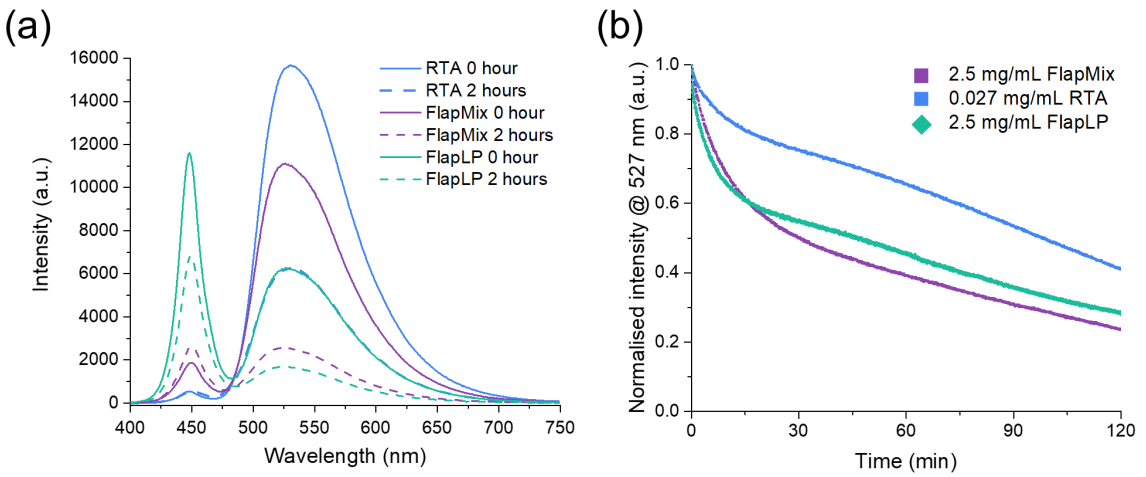


Figure S12. (a) Fluorescence spectra of RTA and $Flap_{Mix}$ and $Flap_{LP}$ at t = 0 and t = 2 hours with excitation at 450 nm. (b) The change in fluorescence at 527 nm over time during continuous light irridation of RTA and flaponite.

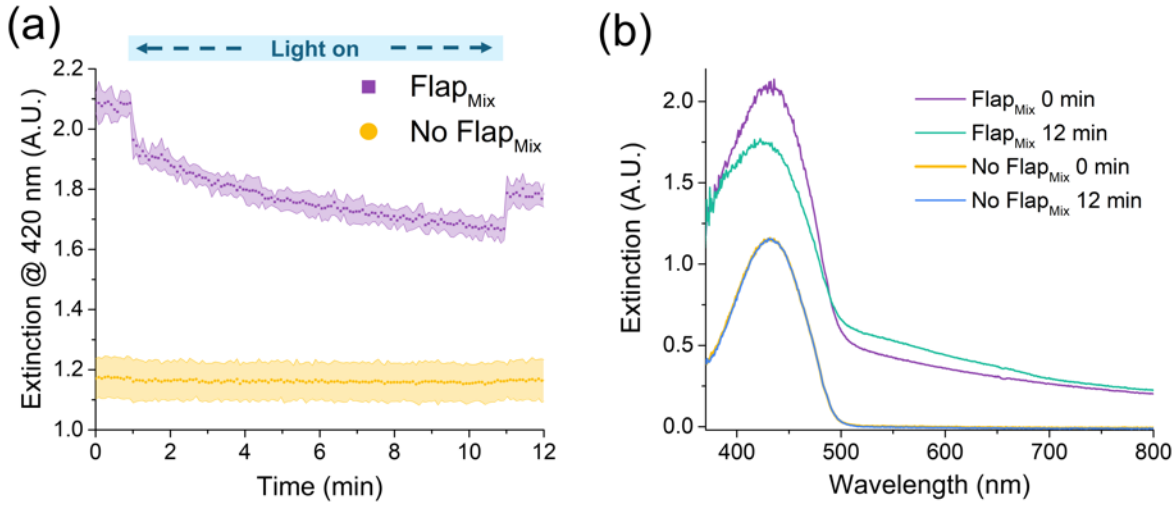


Figure S13. (a) Change in extinction at 420 nm over time during the RNO assay, with and without 2.5 mg/mL $Flap_{Mix}$. (b) Absorbance spectra of the samples at times 0 min and 12 min.

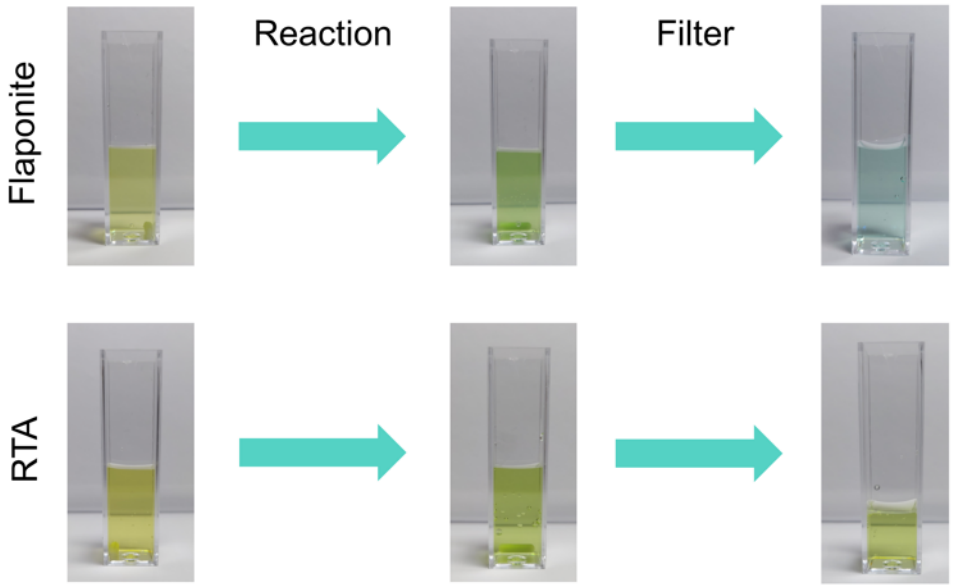


Figure S14. Photographs showing the various stages of ABTS photooxidation using both flaponite and RTA. From left to right, before light irradiation, after light irradiation and then after filtration.

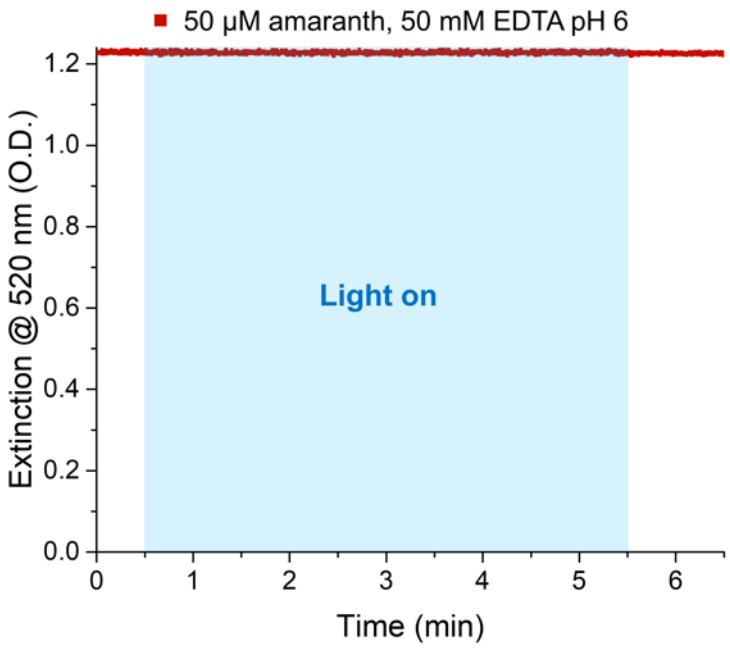


Figure S15. The extinction at 520 nm over time during the photoreduction assay without presence of any catalyst.

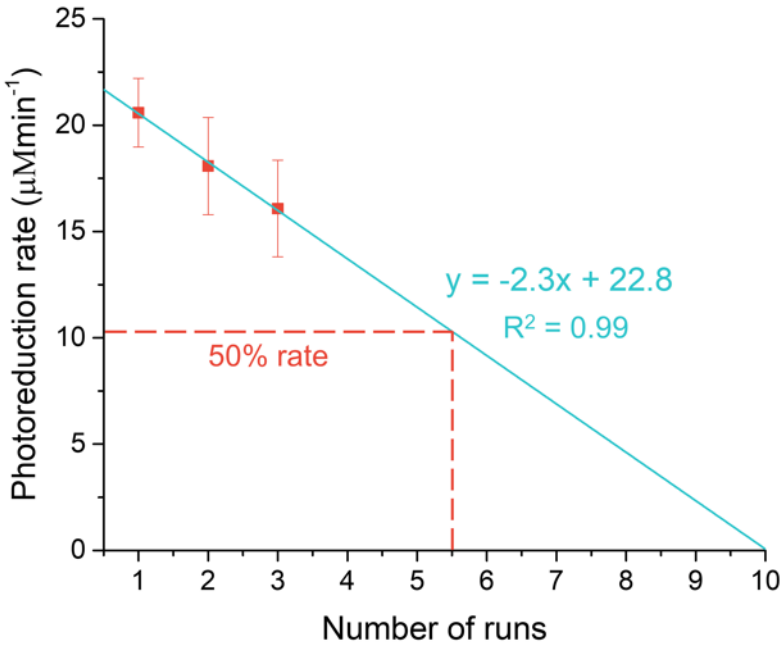


Figure S16. Linear fit added to the rate of photoreduction at each run.

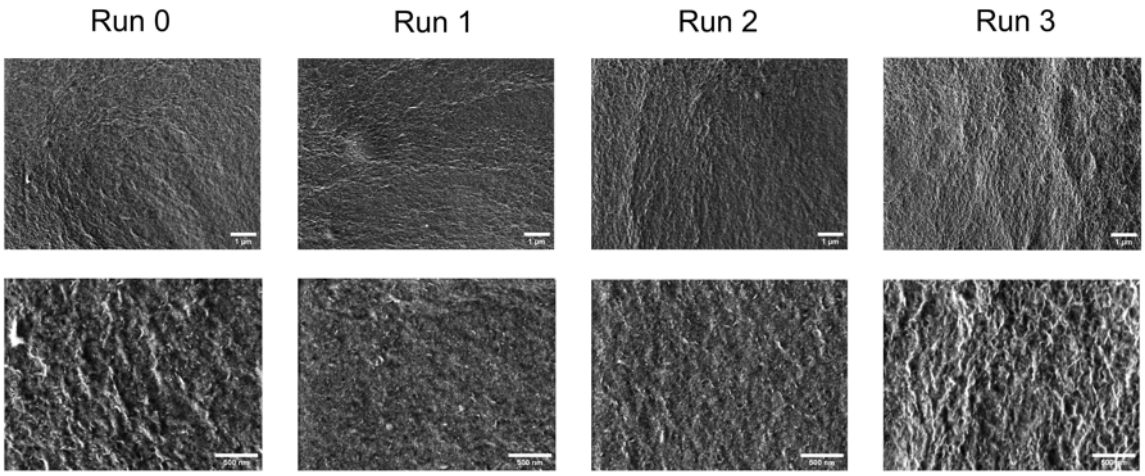


Figure S17. SEM images of $Flap_{Mix}$ (pelleted by centrifugation from a solution of 1 mg/mL $Flap_{Mix}$, 50 mM EDTA pH 6) before and after each run of amaranth photoreduction.

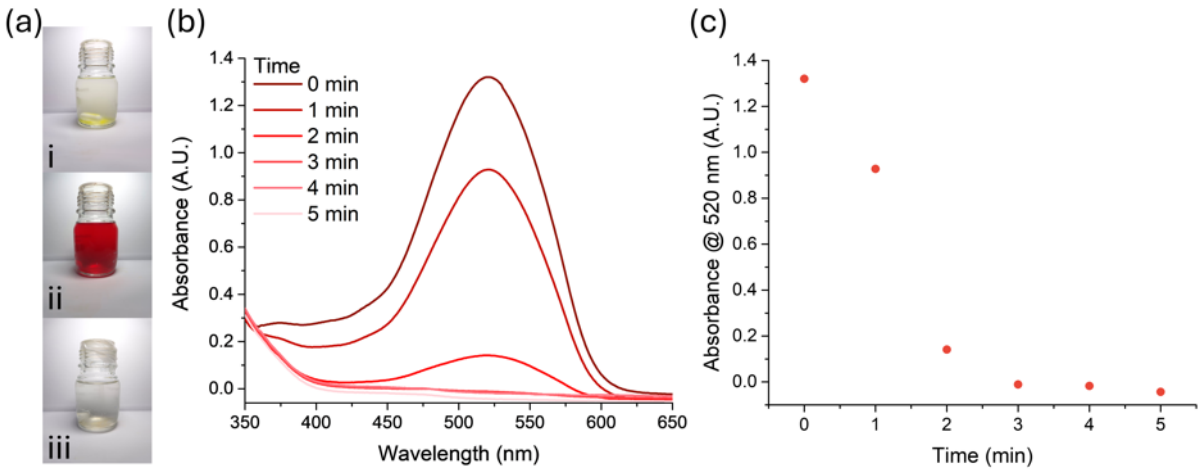


Figure S18. (a) Photographs of a scaled up reaction mixture (i) before adding amaranth, (ii) after adding amaranth and (iii) following 5 minutes of irradiation (450 nm, 100 mW/cm). (b) Absorbance spectra of samples taken at 1 minute intervals. (c) A plot of the absorbance at 520 nm at each time point.