Supporting information for:

Enhancing the reactivity of a P450 decarboxylase with ionic liquids

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Supplementary Materials and Methods

Characterisation

UV-Vis spectroscopy was performed using a Shimadzu UV2600i fitted with a Quantum Northwest Peltier controlled heating environment. Aqueous measurements were performed using a 10 mm quartz cuvette (Starna scientific). Measurements in ionic liquids were performed in a 2-part 0.5 mm quartz cuvette (Starna scientific). For all measurements a full spectrum was recorded (800 nm-220 nm) with a data interval of 0.5 nm.

Dynamic Light Scattering (DLS) and zeta potential experiments were performed on Anton Paar Litesizer 500 instrument with typical protein concentrations of 0.1 – 0.5 mg.mL^{-1}. DLS measurements were recorded using disposable 1 cm cuvettes at 25 °C in 18.2 MΩ water. The data was initially analysed using Anton-Paar Kalliope then the processed data was normalised and gaussians were then fit using Igor Pro. Zeta potential experiments were performed using an omega cuvette (Anton Paar) at 25 °C with an applied maximum voltage of 200 V. The data was analysed using Anton Paar Kalliope using the Smoluchowski approximation.

Circular Dichroism spectroscopy was performed using a Chirascan V100 circular dichroism spectrometer to analyse the aqueous secondary structure of the enzyme. Measurements were made using a 0.5 mm quartz cuvette with a typical protein concentration of 0.2 mg·mL^{-1}. CD data measurements were taken with a wavelength range of 180 – 280 nm, 1 nm wavelength interval and an integration time of 2 s at 25 °C. Temperature was controlled with a Quantum Northwest Peltier control unit. Temperature-dependent CD spectra of aqueous solutions of the enzyme were measured between 180 – 280 nm at temperatures ranging from 25 to 95 °C. A full spectra was taken every 5 °C with 5 min of equilibration time, an integration time of 0.5 s, and 1 nm wavelength interval. Samples were measured in quartz cuvettes with 1 cm pathlength and a typical protein concentration of 0.02 mg·mL^{-1}. Thermal

denaturation parameters were calculated following an established procedure assuming a two-state model of thermal denaturation[1].

Synchrotron radiation circular dichroism (SRCD) spectra of [C-OleTP$_{RN}$][S] in [bmpyrr][OAc], [bmpyrr][$EtSO_4$], and the solvent free state were collected on beamline B23 at the Diamond Light source. Temperature dependent SCRD experiments were performed using a Linkam thermal stage. Measurements were made with a wavelength range of 180 – 260 nm, integration time of 2 s and 1 nm wavelength interval at temperature ranging from 30 to 200 °C. Samples were cast as thin films between two quartz plates.

Chemical modification of protein

Initially, the surface of OleTP$_{RN}$ was cationised by 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-Hydroxysuccinimide mediated coupling of N,N,N′-Trimethyl-1,3-propanediamine to surface oriented aspartate and glutamate residues of the protein. The resulting C-OleTP$_{RN}$ solution filtered using a 0.22 µm syringe filter and then buffer exchanged into phosphate buffer (10 mM, pH 7) via repeated centrifugal concentration (Corning® Spin-X®, 10,000 MWCO) and resuspension in phosphate buffer. The purified C-OleTP$_{RN}$ solution was then added dropwise to a solution of Sulfated Brij-C10 (10 mg.mL^{-1} in 10 mM phosphate buffer pH 7) and shaken for 16 hrs yielding the [C-OleTP$_{RN}$][S] nanoconjugate. Excess surfactant was removed from the nanoconjugate solution by repeated centrifugal concentration (Corning® Spin-X®, 10,000 MWCO) and resuspension in phosphate buffer (10 mM, pH 7). The [C-OleTP$_{RN}$][S] solution was then lyophilised to give a dark red liquid after thermal annealing.

Aqueous enzyme activity

Enzyme activity was determined by an end point assay of the H_2O_2 concentration using horseradish peroxidase and ABTS which produces a bright blue colour change in the presence of H_2O_2 which was quantified using UV/Vis spectroscopy. Briefly, 10 µg of P450 was added to 1 mL of phosphate buffer (100 mM pH 7.2) containing 0.1 mM H_2O_2 and 0.5 mM myristic acid and was shaken for 5 minutes. The reaction was then quenched by addition of 0.2 mM ABTS and 3 U of horseradish peroxidase, then the reaction was centrifuged for 5 minutes. The concentration of oxidised ABTS (ε_{420} = 36 mM^{-1}.cm^{-1})[2] was then quantified by measuring the absorbance at 420 nm using a nanophotometer (Implen, N60). The rate of consumption of H_2O_2 was then calculated by comparing the absorbance of the reaction compared to a control reaction containing no P450 enzyme.

$$Rate\ (\mu mol.min^{-1}) = \frac{A_{420nm}(control) - A_{420nm}(reaction)}{36 \times time}$$

Synthesis of Sulfated Brij-C10

Sulfated Brij-C10 was synthesised by reacting Brij-C10 with sulfamic acid[3]. An equimolar mixture of Brij C10 (10.0 g, 14.7 mmol) and sulfamic acid (1.43 g, 14.7 mmol) was heated neat at 120 °C for 18 hrs under nitrogen producing a brown viscous liquid. The resulting liquid was cooled to room temperature and diluted with 200 mL of dichloromethane and stirred for 1 hr. The solution was subsequently filtered, and the solvent was removed from the filtrate using a rotatory evaporator yielding a viscous oil. The oil was then washed with 200 mL of hexane to remove non-polar impurities. The hexane was then decanted, and the oil was lyophilised to give ammonium Brij-C10 sulfonate ester as an off-white waxy solid (8.62 g 11.08 mmol) at a 75% yield.

Synthesis of [bmpyrr][OAc]

1-Butyl-1-methylpyrrolidinium acetate ([bmpyrr][OAc]) was synthesized by adding a solution of silver acetate (12.81 g, 0.085 mol, 1.0 eq.) in deionized water (50 mL) to a flask covered in aluminium foil containing [bmpyrr][Cl] (15.00 g, 0.084 mol, 1.1 eq). The resulting solution was stirred at room temperature for 3 days and then filtered. The solution was then mixed with activated charcoal for 24 hrs then filtered and tested for silver and chloride ions with aqueous hydrochloric acid and silver nitrate solutions respectively. The solution was then passed through a neutral alumina column (Thermo Scientific, HyperSep™ C18 cartridge). The resulting product was then lyophilised to give [bmpyrr][OAc] (9.3 g, 0.047 mol, 56.0 %) as a colourless liquid.

GC-MS derivatisation method

After the reaction in ionic liquids the reactions were cooled to room temperature and quenched by addition of 3 M HCl in dry methanol. The samples were then stirred at 60 °C for 1 hr to form a fatty acid methyl ester. The samples were then cooled to room temperature and extracted with hexane and the hexane layer was analysed by GC-MS.

GC-MS method

Following derivatisation and extraction, 1 µL of sample was injected into a GC-MS instrument (GCMS-QP2020 NX, Shimazdu) and analysed using the following parameters
Column: Rtx-5MS (Restek) 30 m length, 0.25 mm diameter, 0.25 µm (film thickness)
Carrier: He (CP grade), 100 kPa (constant pressure)
Inlet temperature: 250 ˚C
Injection gases: Split (50:1) split flow 30 mL min^{-1}, purge 3 mL min^{-1}
Detection: Mass Spec, EI (70 eV), source temperature 230 ˚C.
Scan range 50 – 650 amu with a scan rate of 5 Hz.
Transfer line 300 ˚C

Oven heating profile:
0 → 5 mins Hold at 50˚C
5 → 16.3 mins Ramp to 275 ˚C at 20 ˚C min^{-1}
16.3 → 18.8 mins Hold at 275 ˚C for 2.5 min

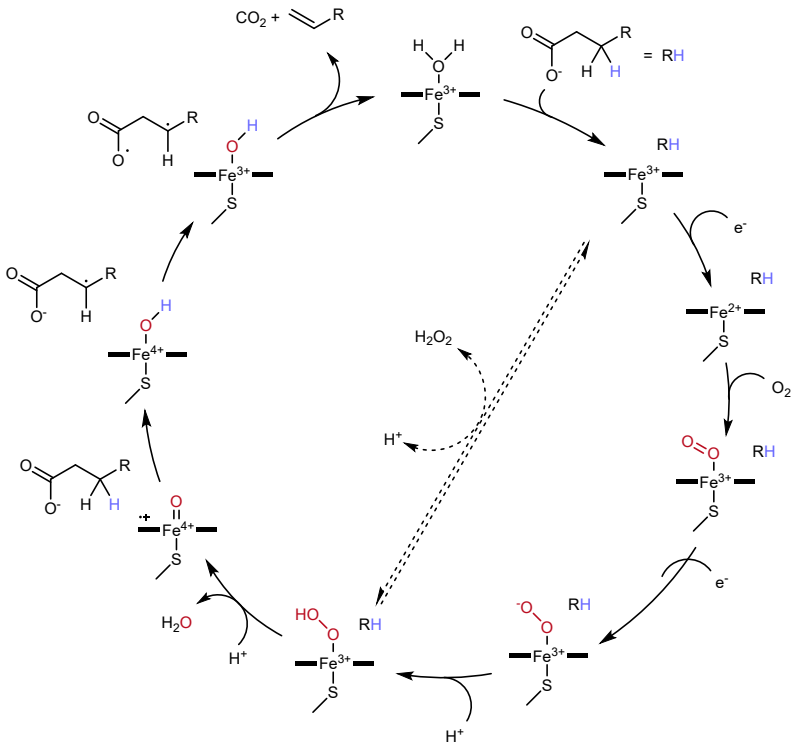

Figure S1. Catalytic cycle of P450 fatty acid decarboxylase enzymes. Dashed lines show the peroxide shunt pathway.

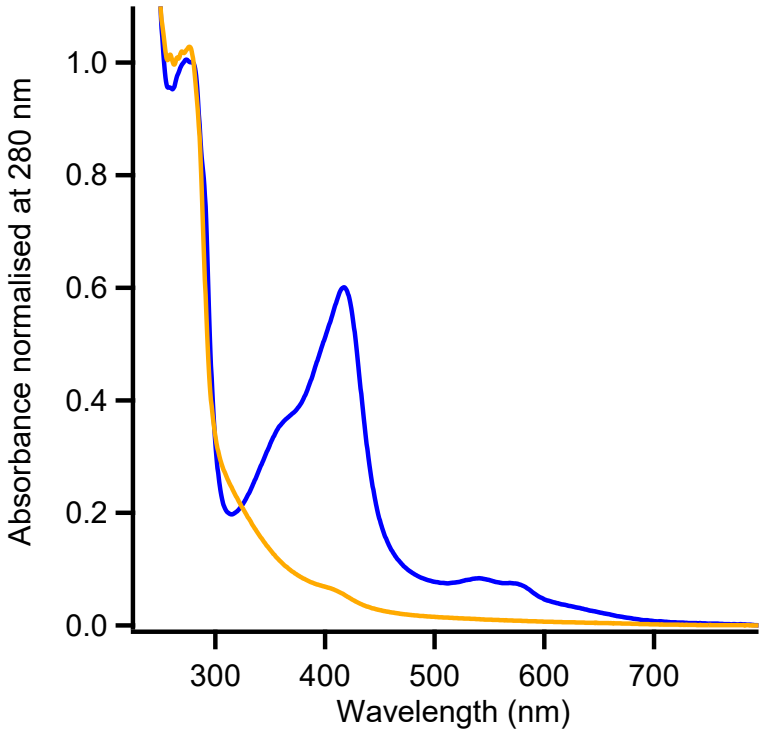

Figure S2. Normalised UV/Vis spectra of C-**OleTP$_{RN}$ before addition of** carboxylate surfactant **(bl**ue**) and after addition of** carboxylate surfactant **(**orange**)**

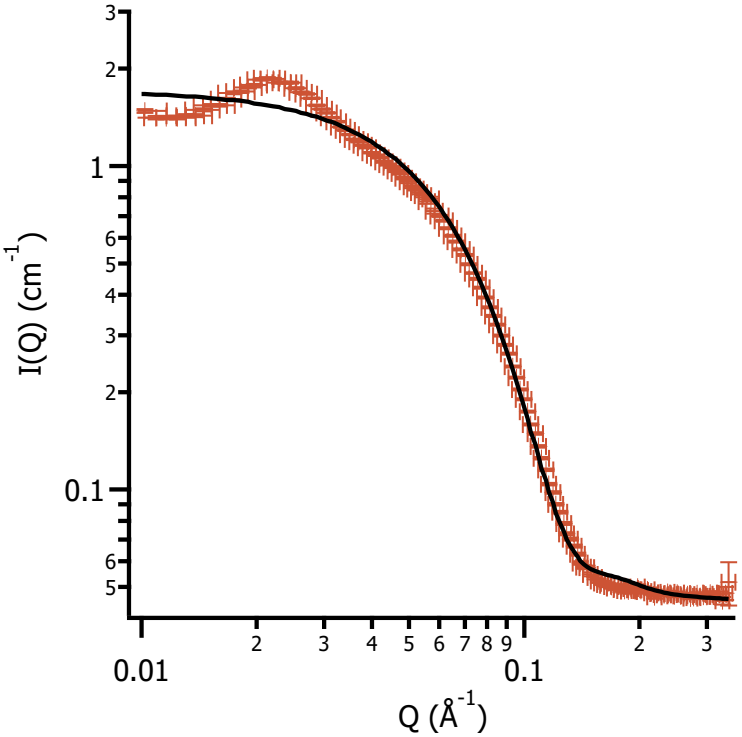

Figure S3. Neutron scattering intensity against scattering vectors for [C-OleTP$_{RN}$][S] in D_2O plot was fitted using the hard sphere model in SasView and the fit is shown as a solid black line.

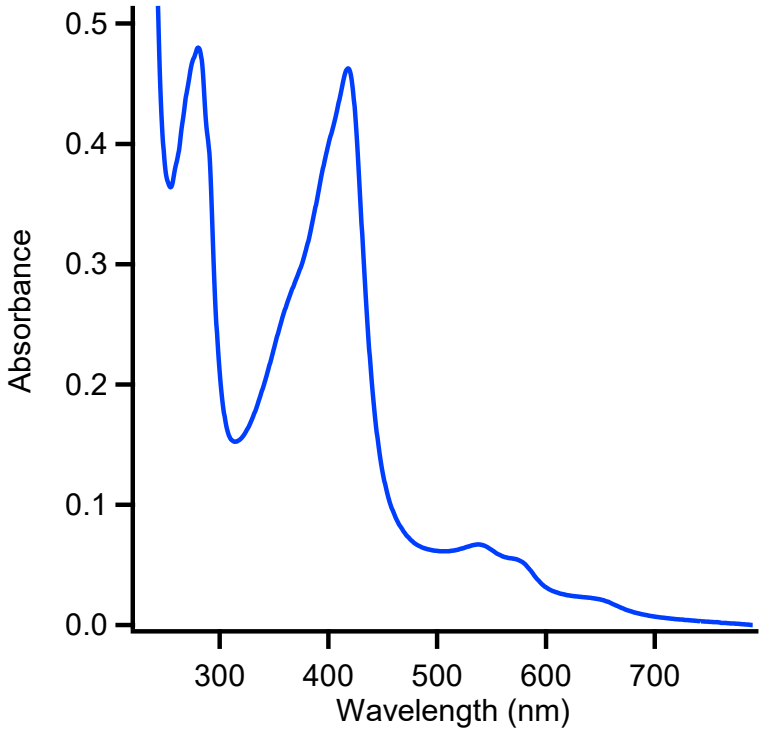

Figure S4. UV/Vis determination of surfactant:protein ratio. 2 mg.mL^{-1} of nanoconjugate was dissolved in phosphate buffer and the protein concentration was measured to determine the amount of protein in solution. The ratio in this case was found to be 168 molecules of surfactant per molecule of protein.

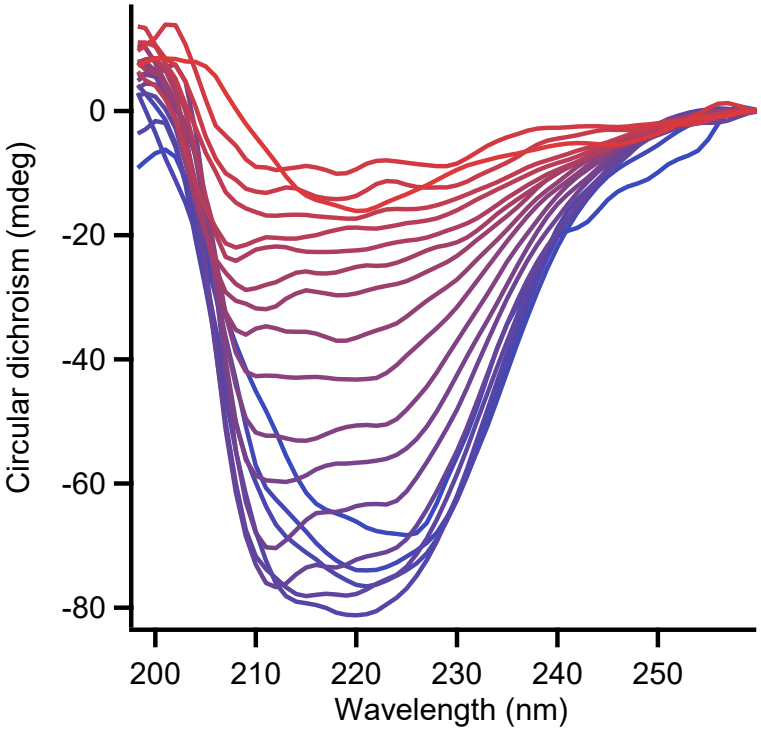

Figure S5. Temperature dependent SRCD spectra of [C-OleTP$_{RN}$][S-C10] in [bmpyrr][OAc] as it was heated from 30 °C (blue) to 200 °C (red) in ten degree increments

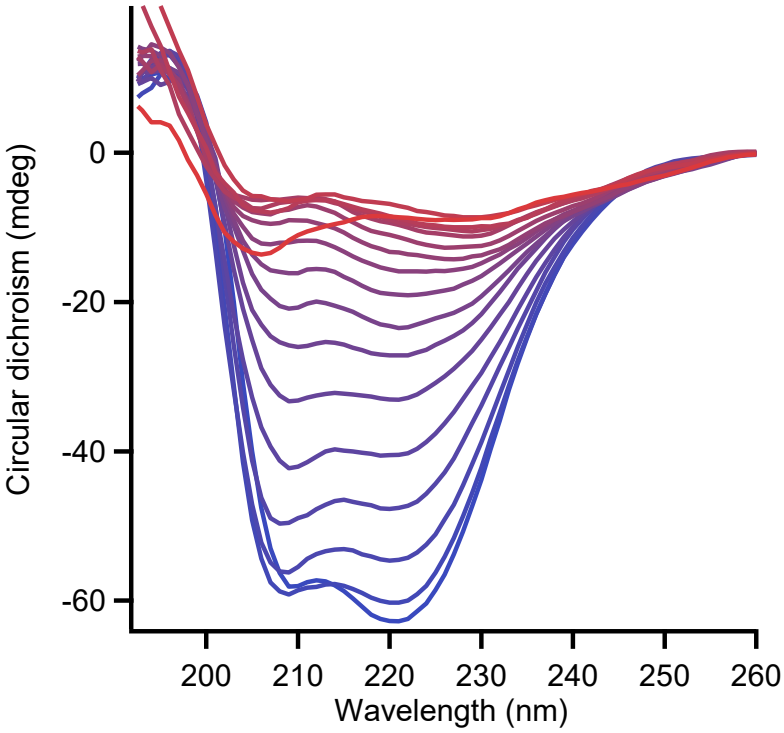

Figure S6. Temperature dependent SRCD spectra of [C-OleTP$_{RN}$][S-C10] in [bmpyrr][$MeSO_4$] as it was heated from 30 °C (blue) to 200 °C (red) in ten degree increments

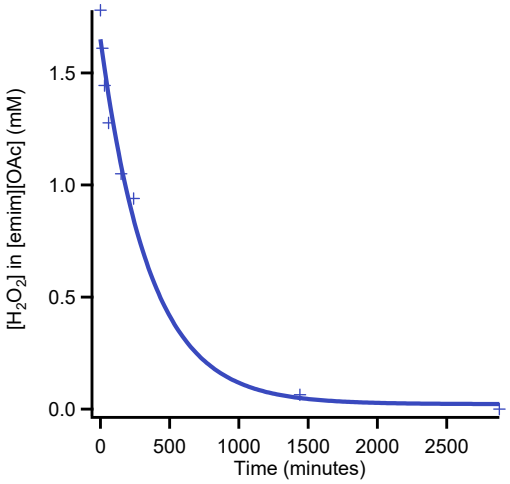

Figure S7. Plot of calculated H_2O_2 concentration against time. The loss of peroxide appears to follow an exponential decrease. An exponential was fit using IGOR Pro and shows that the half-life was approximately 4 hours.

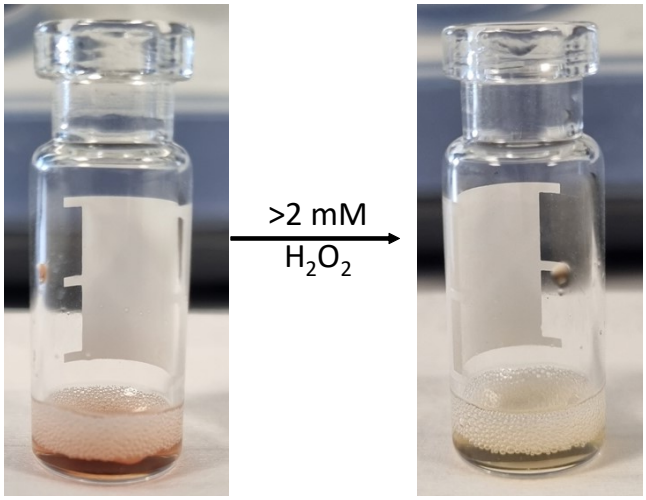

Figure S8. Image comparing an enzymatic reaction in ionic liquid before (left) and after (right) addition of 2 mM H_2O_2 which results in a loss of the red colour. The loss of colour may indicate degradation of the heme cofactor of [C-OleTP$_{RN}$][S-C10]

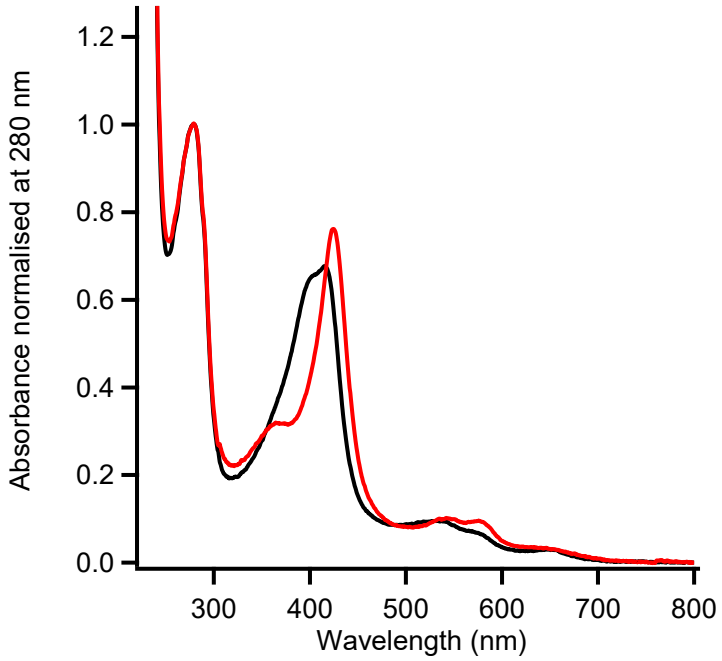

Figure S9. Normalised UV/Vis spectra of $OleTP_{RN}$ before addition of H_2O_2 (black) and after addition of 0.1 mM H_2O_2 (red)

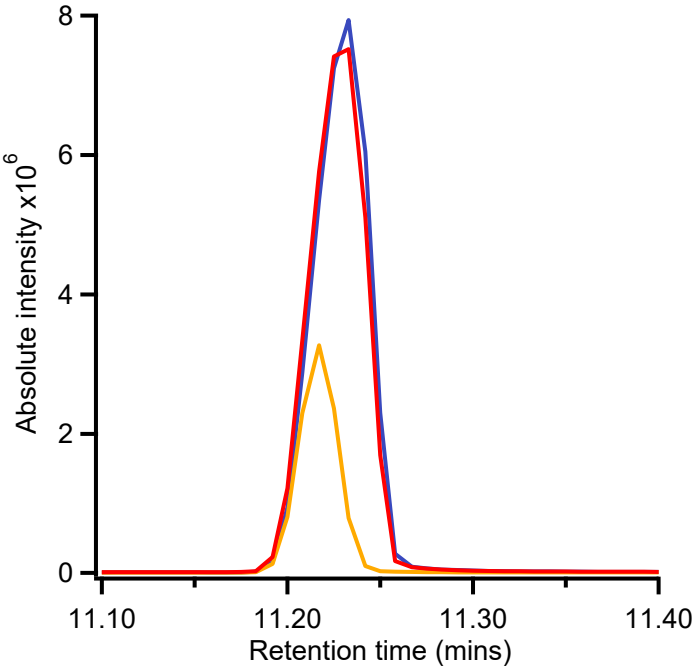

Figure S10. GCMS trace of the substrate peak after the reaction with UV light but no [C-$OleTP_{RN}$][S] (blue), No UV light but with [C-$OleTP_{RN}$][S] (red), and both UV light and enzyme (orange).

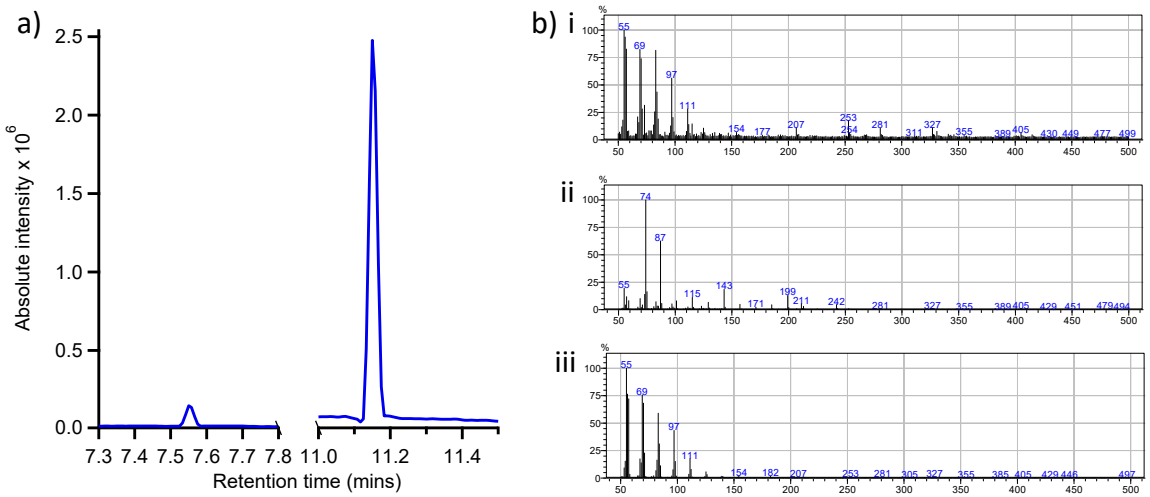

Figure S 11a. GC chromatogram of a reaction containing [C-OleTP$_{RN}$][S] and myristic acid in [emim][OAc] after illumination with UV light.. **b.i.** the mass spectrum of the peak at 7.55 minutes **ii.** The mass spectrum of the peak at 11.15 minutes. **iii.** The mass spectrum of a standard of 1-tridecene

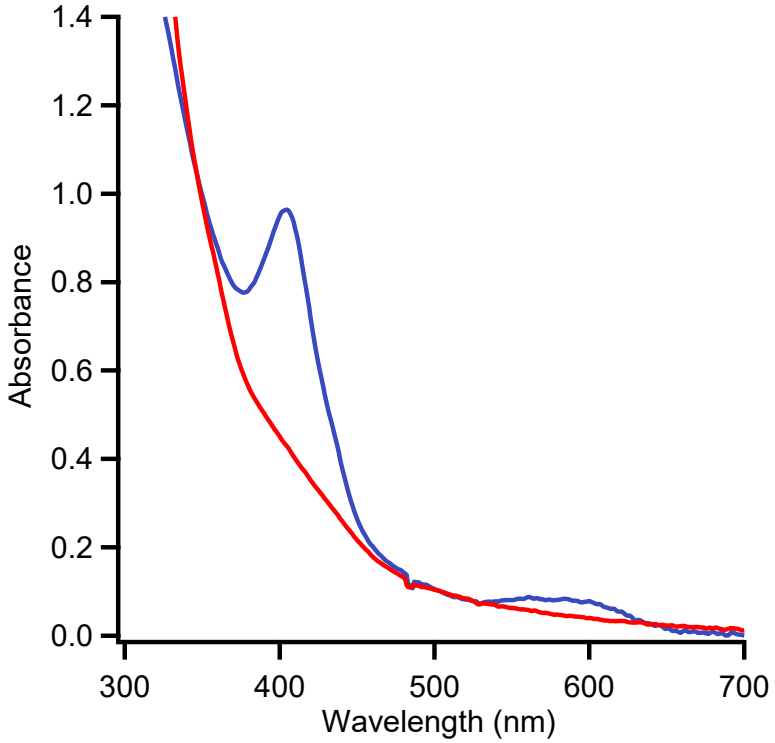

Figure S12. UV/Vis spectra of [C-OleTP$_{RN}$][S] in [emim][OAc] at 80 °C (blue) and at 90 °C (red).

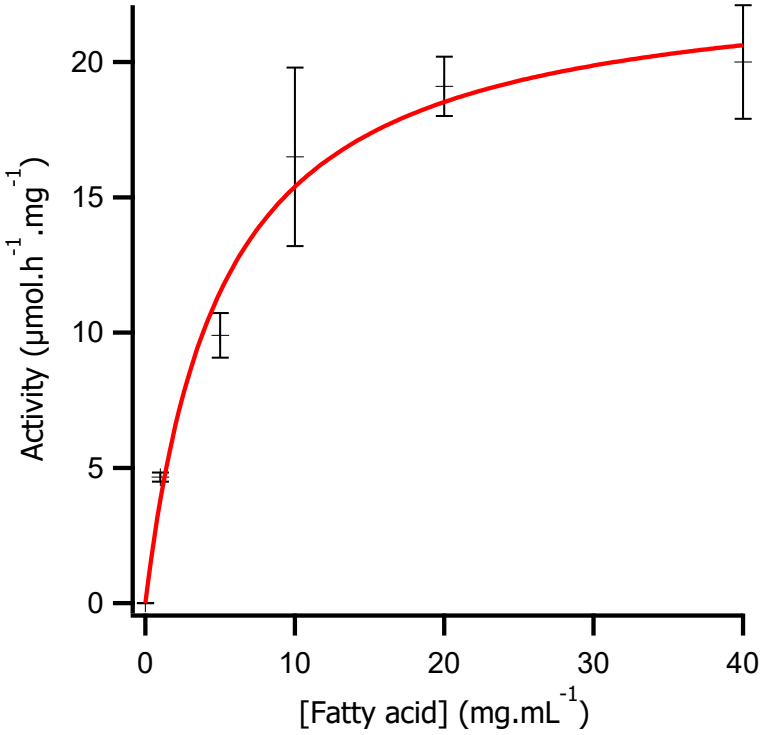

Figure S 13. Plot of activity against concentration of myristic acid for 4 hour UV driven reactions at 80 °C (black markers) and the Michaelis-Menten model fitting (red line).

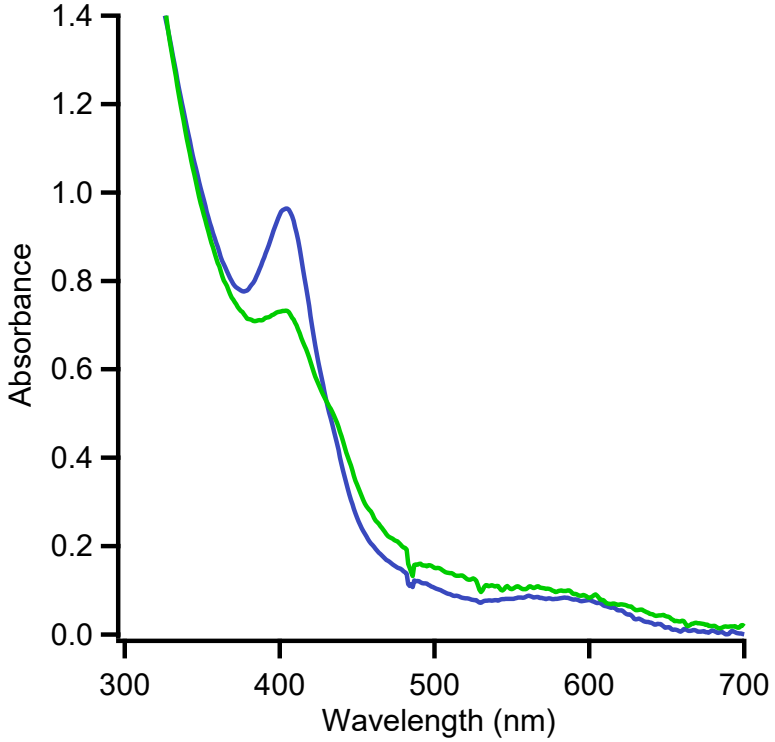

Figure S14. UV/Vis spectra of [C-OleTP$_{RN}$][S] in [emim][OAc] at 80 °C before illumination (blue) and after 4 hours of illumination with UV light (green).

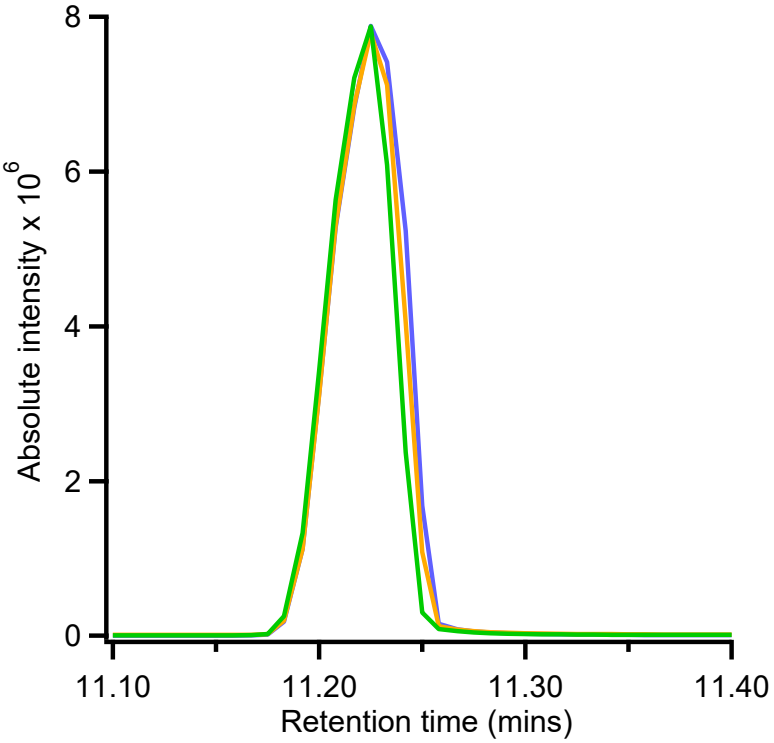

Figure S15. GCMS chromatograms showing the substrate peak at the end point of reactions in [bmpyrr][Oac] with UV light but no enzyme (blue), in [bmpyrr][Oac] with [C-OleTP$_{RN}$][S] and UV light (orange), and in [emim][EtSO$_4$] with [C-OleTP$_{RN}$][S] and UV light (green).

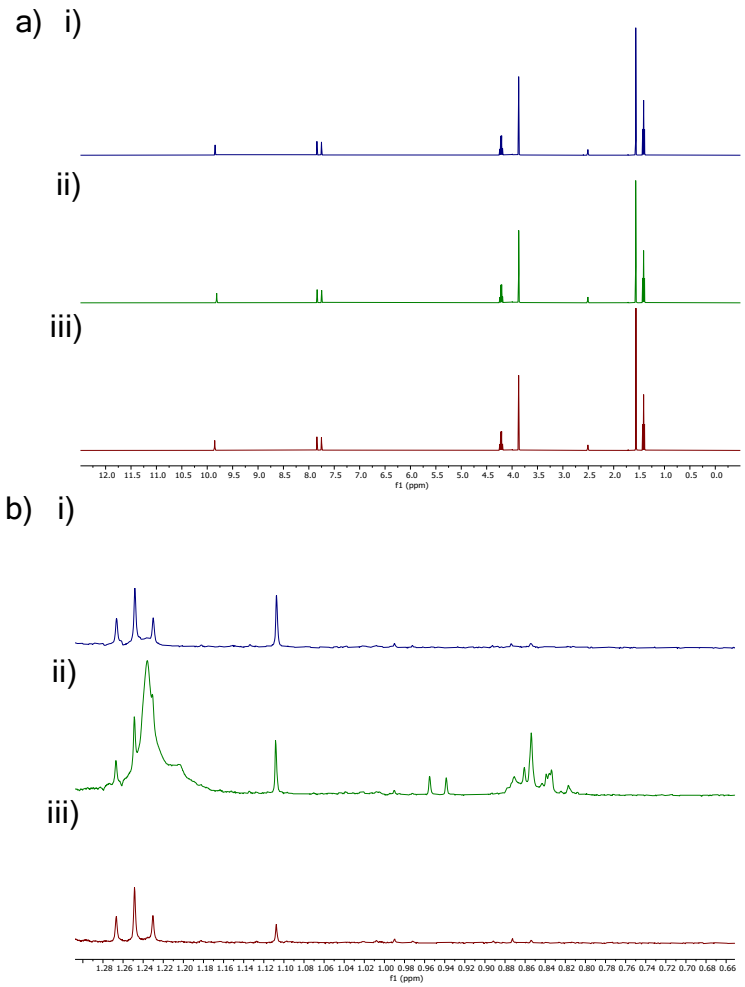

Figure S16a. ^{1}H NMR spectra of [emim][OAc] in dimethyl sulfoxide-d_6**. i.** before addition of substrate. **ii.** After the photochemical reaction. **iii.** After being washed with hexane. **b.** Expanded substrate region of the NMR spectra **i.** before addition of substrate. **ii.** After the photochemical reaction. **iii.** After being washed with hexane.

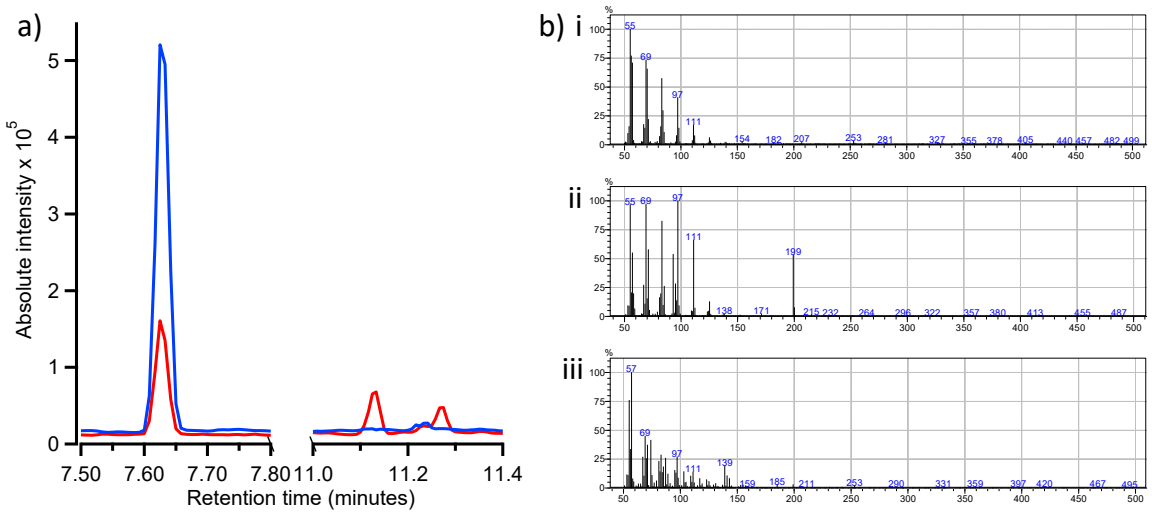

Figure S17a. GC chromatograms comparing a control reaction containing no enzyme (blue) and a reaction containing [C-OleTP$_{RN}$][S] (red). The reactions consisted of 10 mg.mL^{-1} 1-tridecene in [emim][OAc] and were illuminated with UV light for 4 hours. In the reaction containing the enzyme there was a decrease in the concentration of the alkene and there were various other peaks detected in the chromatogram, the 2 largest new peaks are shown. **b.i.** the mass spectrum of the peak at 7.6 minutes which is the retention time of the 1-tridecene standard. **ii.** The mass spectrum of the peak at 11.1 minutes. **iii.** The mass spectrum of the peak at 11.25 minutes.

References

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