Electronic Supplementary Material (ESI) for Chemical Communications


Supporting information for

# Light-Initiated Hydroxylation of Lauric Acid Using Hybrid P450 BM3 Enzymes

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Fig. S1:
FPLC chromatogram showing the separation of Ru-Q397C-BM3 and Q397C-BM3 proteins after labeling reaction using stepwise elution gradient (Solvent A: 10 mM Tris pH = 8.0; Solvent B: 10 mM Tris, 300 mM NaCl; HiTrap Q column).

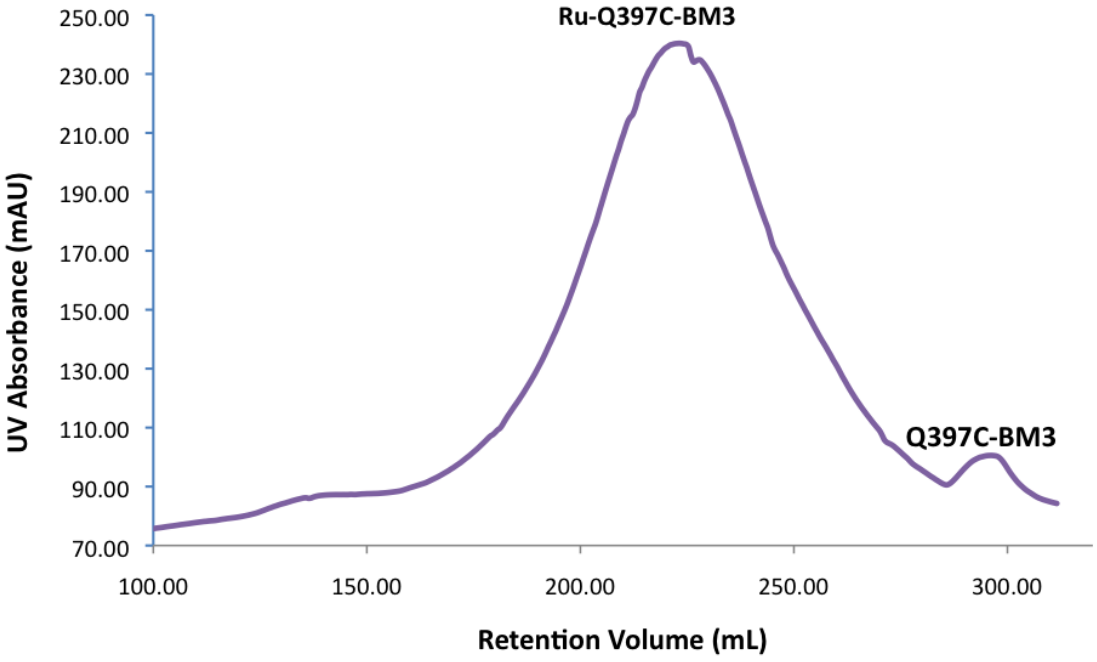


Fig. S2:
Mass Spectra of the Q397C-BM3 mutant (blue) and the Ru-Q397C-BM3 (red) displaying a mass increase of 646, indication of the covalent attachment of the Ru(II) photosensitizer to BM3 mutants.

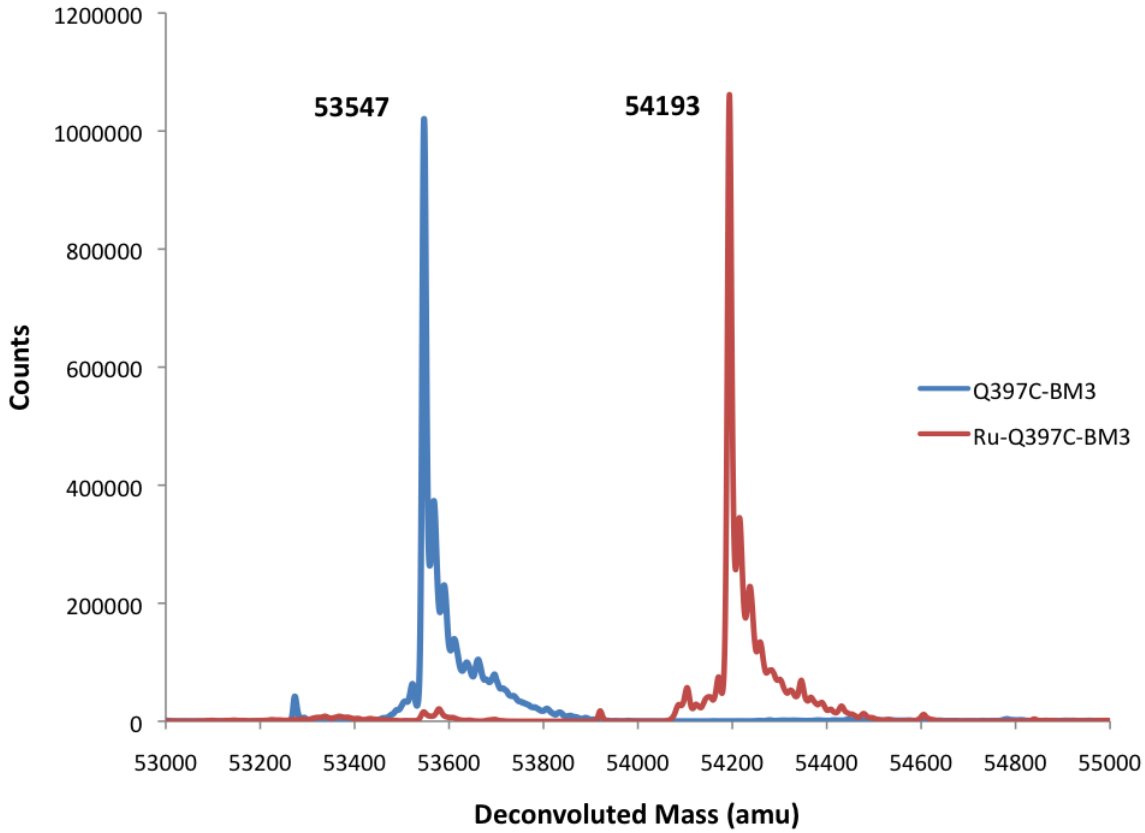

Fig. S3:
UV-Vis spectra of the Q397C-BM3 mutant (blue), Ru-Q397C-BM3 (red) and Ru(II) photosensitizer (green) at the same concentration of 3 μM.

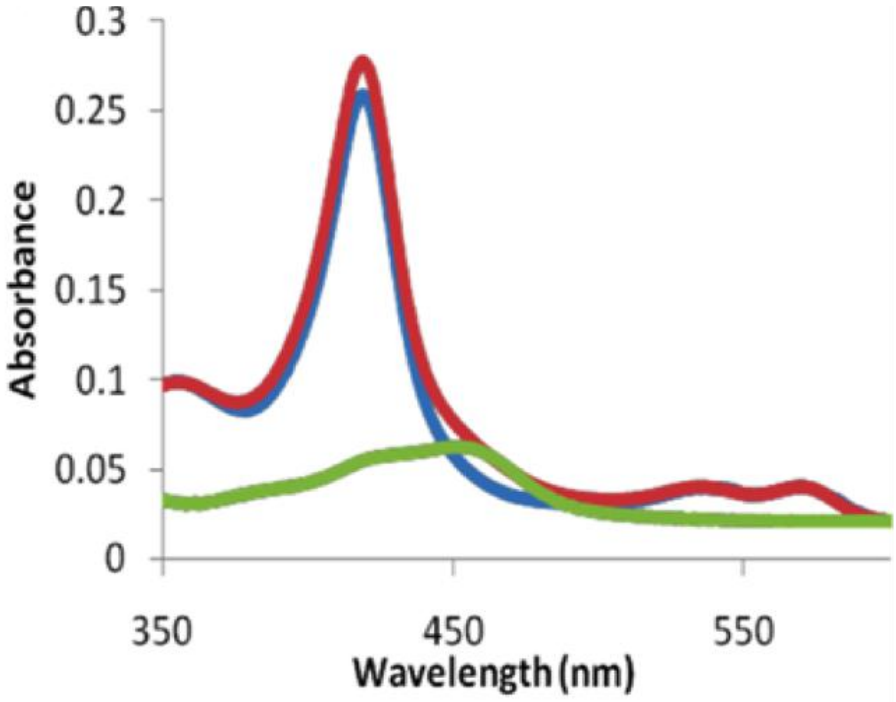


Fig. S4:
Steady state emission quenching of the Ru(II)-Q397C-BM3 with increased concentration of the reductive quencher DTC ($\lambda_{ex}$ = 455 nm).

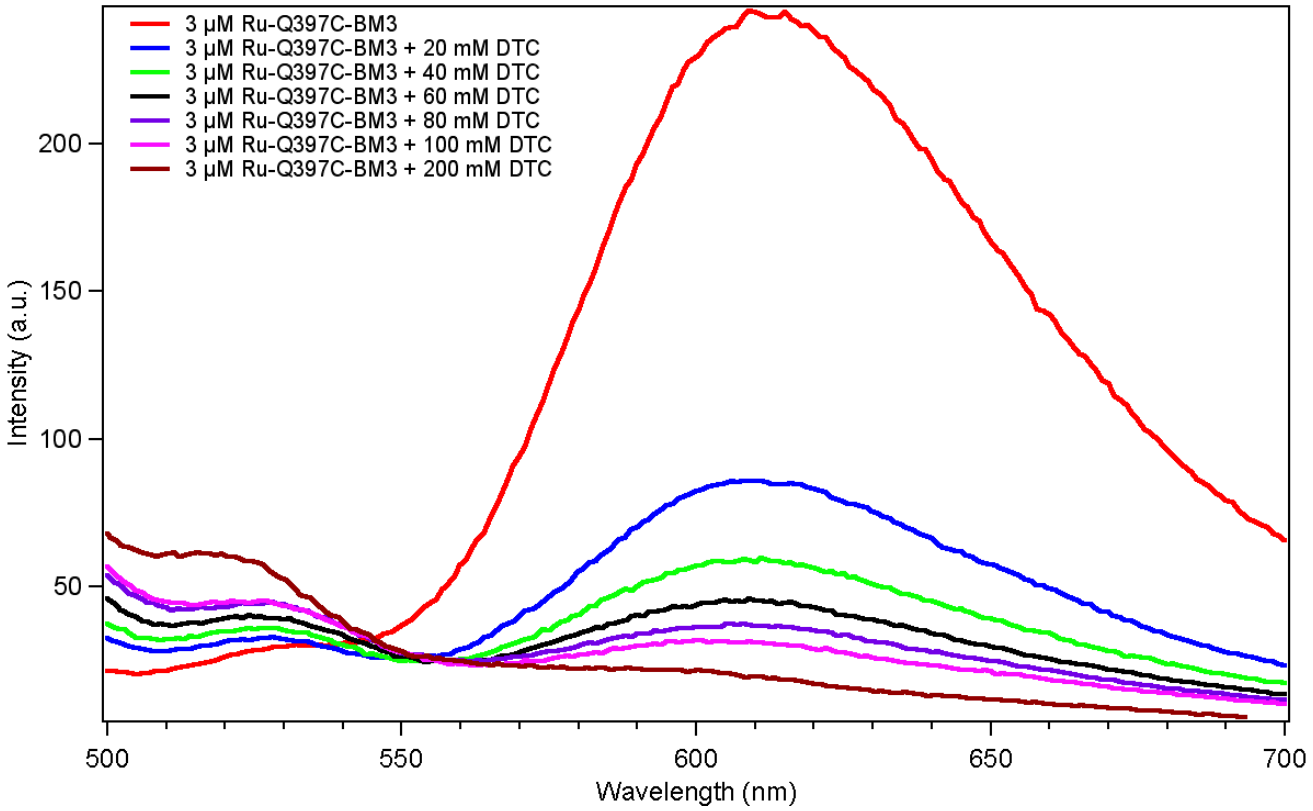

Fig. S5:
Representative curves showing formation of products over time for the Ru-Q397C-BM3 mutants (3 μM) with 0.1, 0.5 and 1 mM lauric acid concentrations under constant light irradiation.

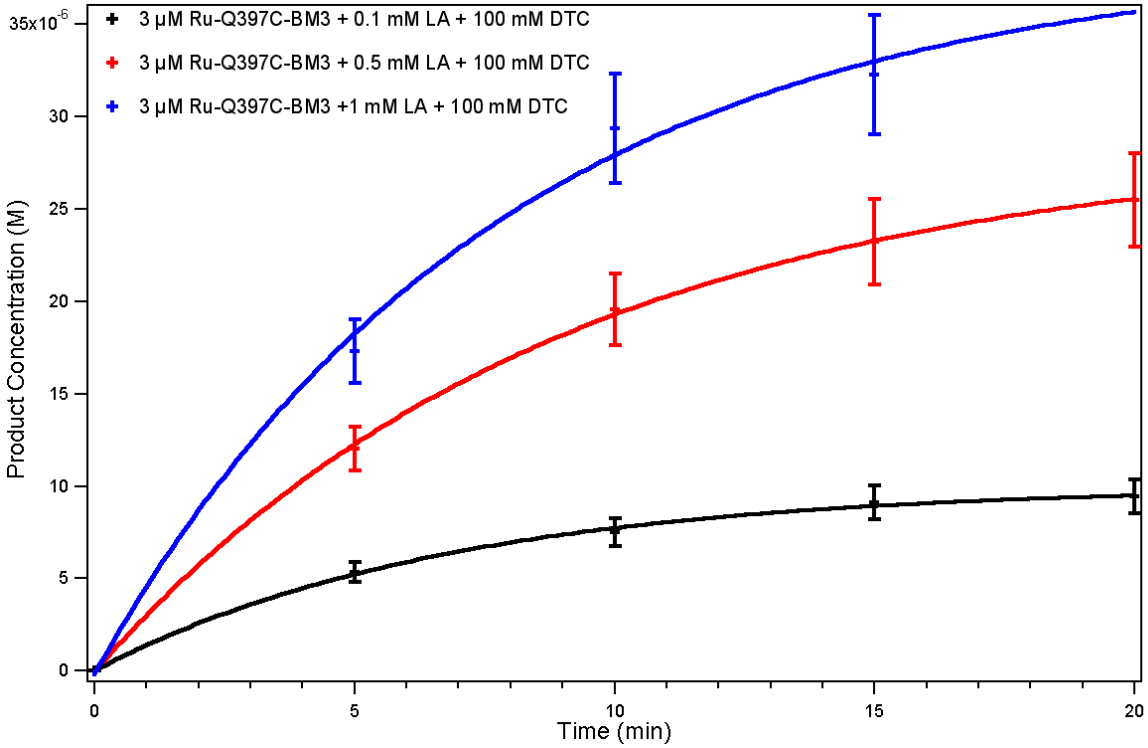


Fig. S6:
Lineweaver-Burk plot for the Ru-Q397C-BM3 and Ru-K97C-BM3 hybrid enzymes.

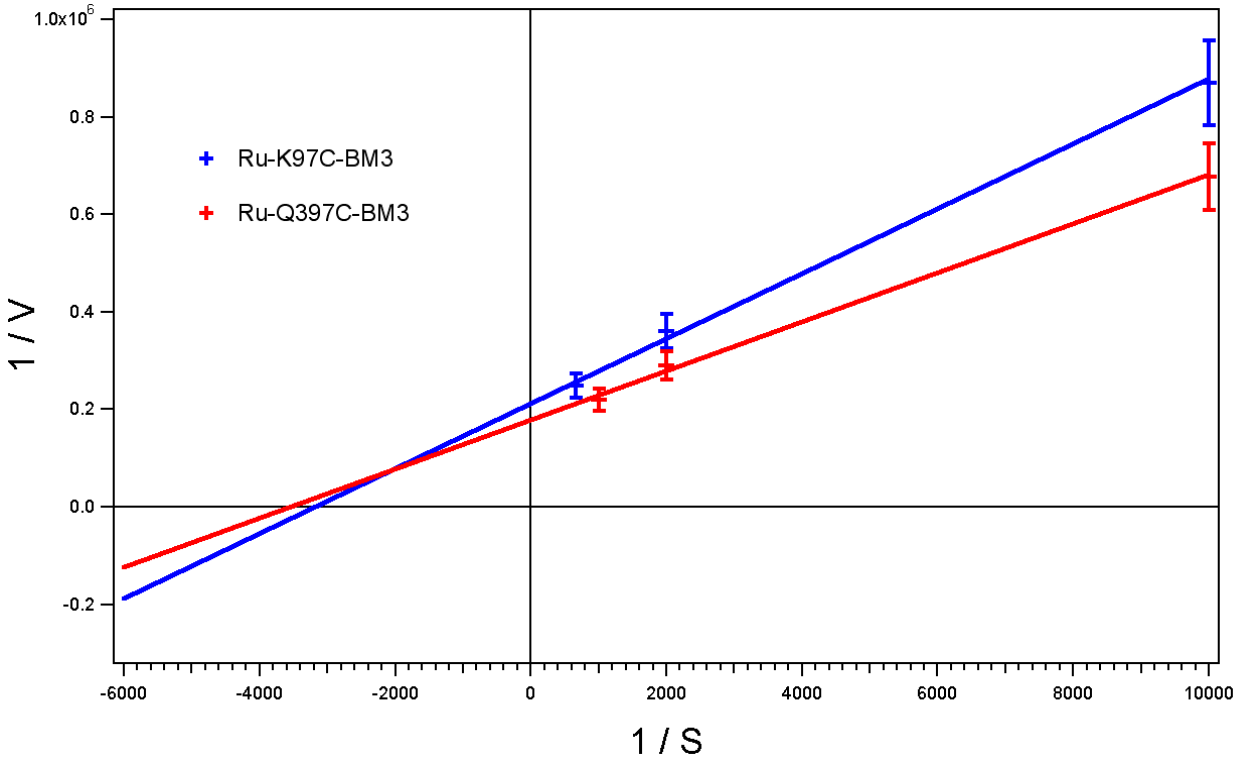

Table S1:
Initial rates of reaction for the different enzymatic systems determined in triplicates after one-minute reaction with 1.5 mM lauric acid.

| Enzymatic system | Initial reaction rate (mol product/mol enzyme/min) |
|---|---|
| 10 μM WT + 10 mM $H_2O_2$ | 0.68 ± 0.05 |
| 10 μM WT + 100 μM $Ru(bpy)_3^{2+}$ | 0.11 ± 0.05 |
| 5 μM Ru-K97C-BM3 + 100 mM DTC + hν | 1.41 ± 0.13 |
| 5 μM Ru-K97C-BM3 + 100 mM DTC + hν +10 μM catalase | 1.80 ± 0.08 |
| 5 μM Ru-Q397C-BM3 + 100 mM DTC + hν | 1.59 ± 0.16 |
| 5 μM Ru-Q397C-BM3 + 100 mM DTC + hν + 10 μM catalase | 2.65 ± 0.14 |

Fig. S7:
Difference spectrum for the BM3-WT and Ru-BM3 enzymes in the presence of CO under photoreductive conditions (100 mM DTC and constant light irradiation from a mercury lamp with UV- and IR-cutoff filters).

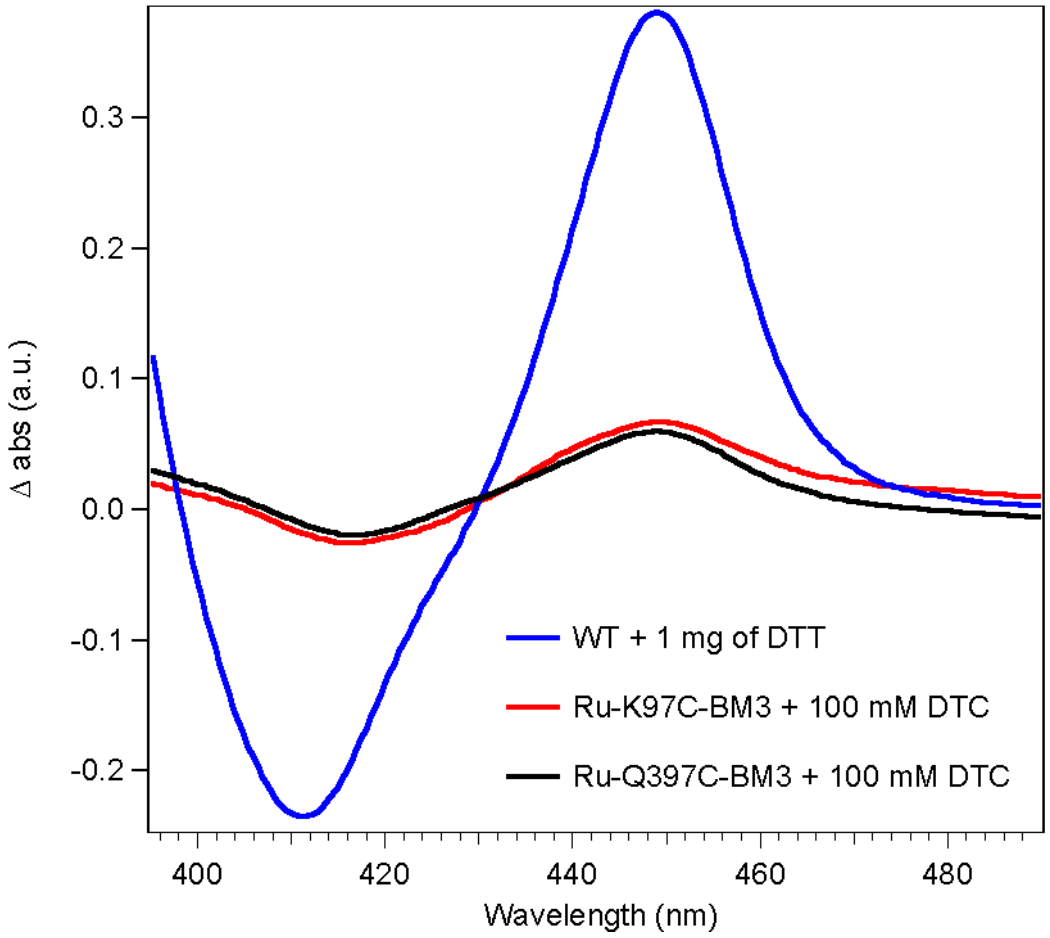

Fig. S8:
Absorption spectra showing the Ru-Q397C-BM3 protein decay over the course of the reaction (dashed lines) and with 10µM catalase (solid lines). Inset: Single exponential fit of the protein decay (rate = 0.02 $min^{-1}$ with 10µM catalase).

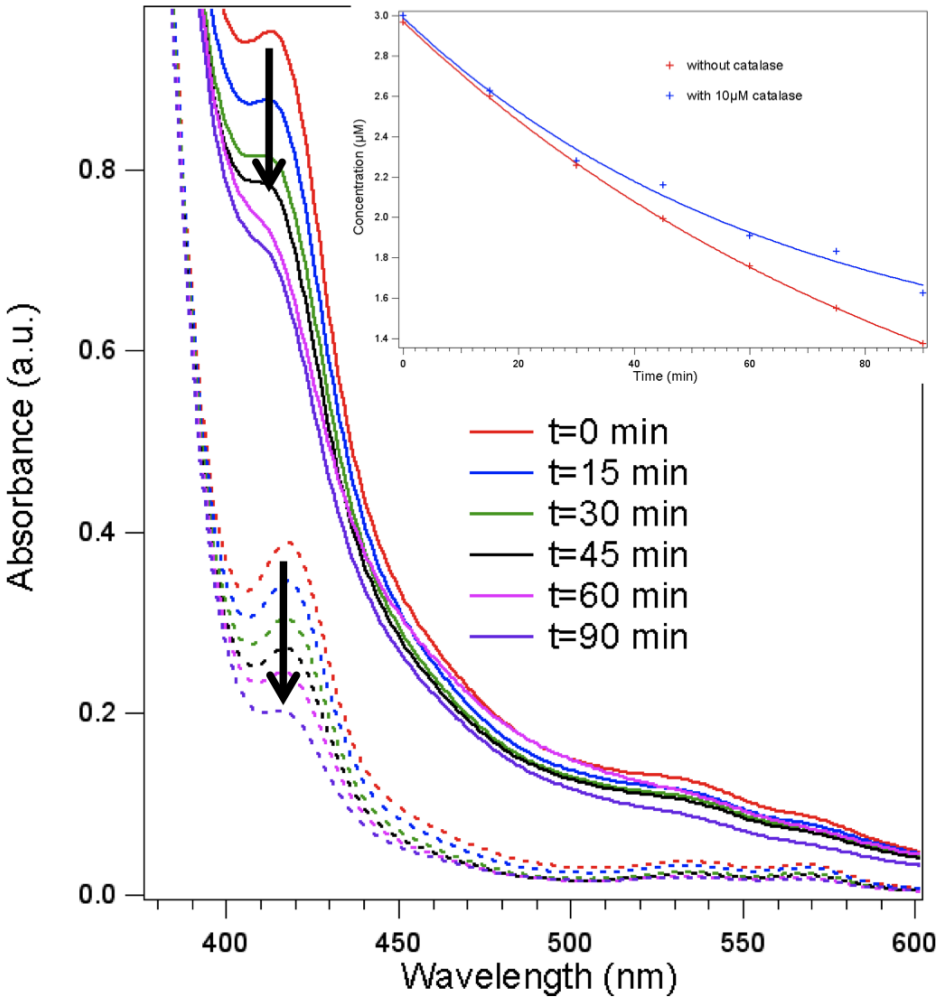


Fig. S9:
Representative curves showing formation of products over time with 3 µM BM3-WT and 10 mM $H_2O_2$ (black), 3 µM Ru-Q397C-BM3 and 10 mM $H_2O_2$ (red) and 100 mM DTC + light (blue).