Supplementary Information

Insights into an Efficient Light-driven Hybrid P450 BM3 Enzyme from Crystallographic, Spectroscopic and Biochemical Studies

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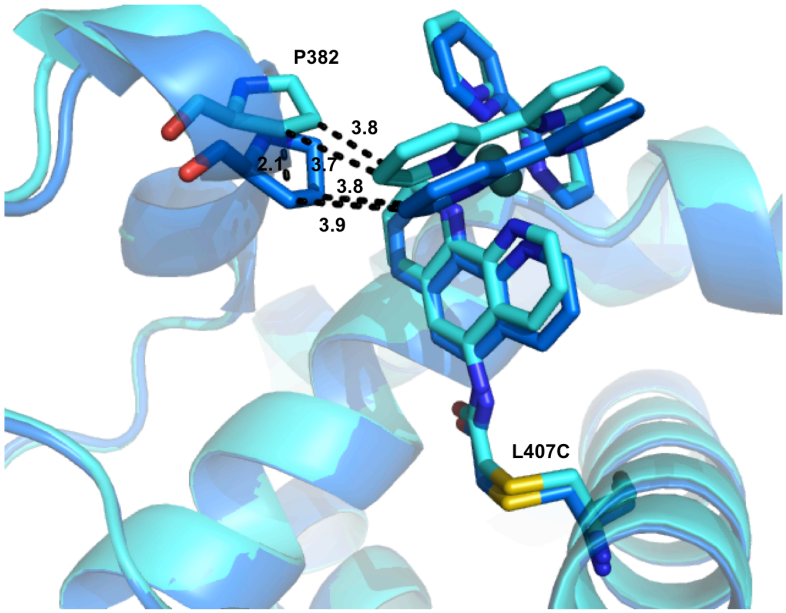

Fig. S1. Graphical representations of C-H---π interactions between the photosensitizer and the P382 residue preserved during the motion of the 3_{10} helix by 2.1 Å in the two molecules present in the asymmetric unit of the DMSO bound structure.

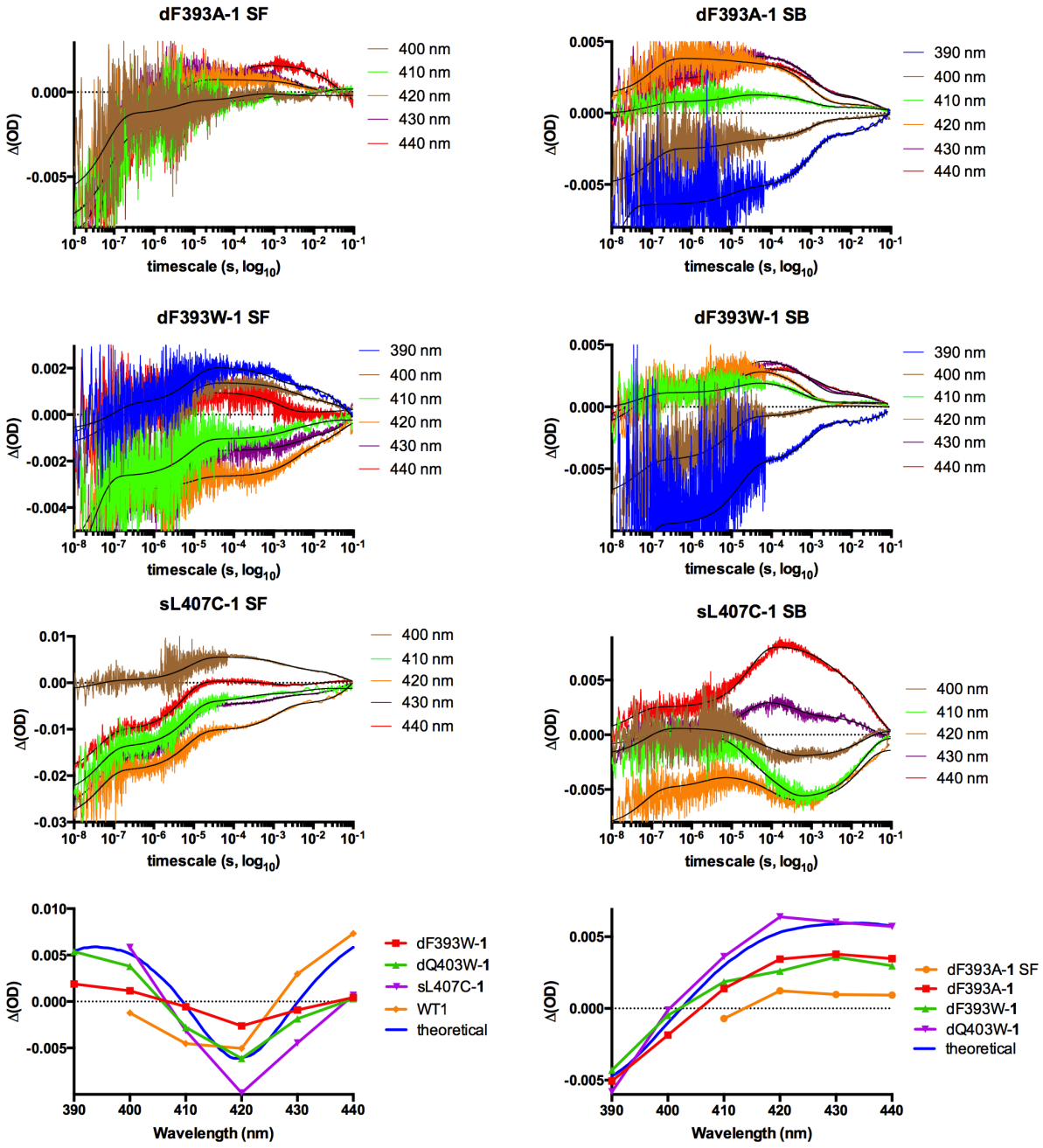

Fig. S2. Transient absorption traces for the dF393A-**1**, dF393W-**1** and sL407C-**1** hybrid enzymes and the corresponding difference spectra for the substrate free (SF) and substrate bound (SB) forms (bottom panels).

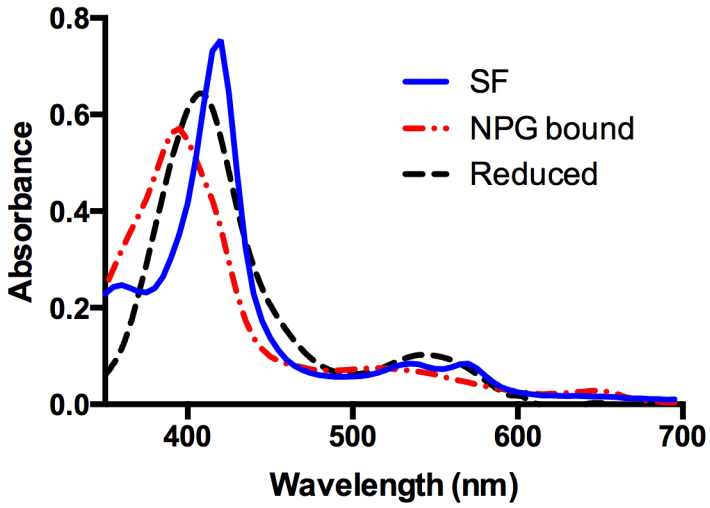

Fig. S3. UV-vis spectra of the substrate free (SF), N-palmitoylglycine bound (NPG bound) and electrochemically reduced (Reduced) of the hybrid enzymes.

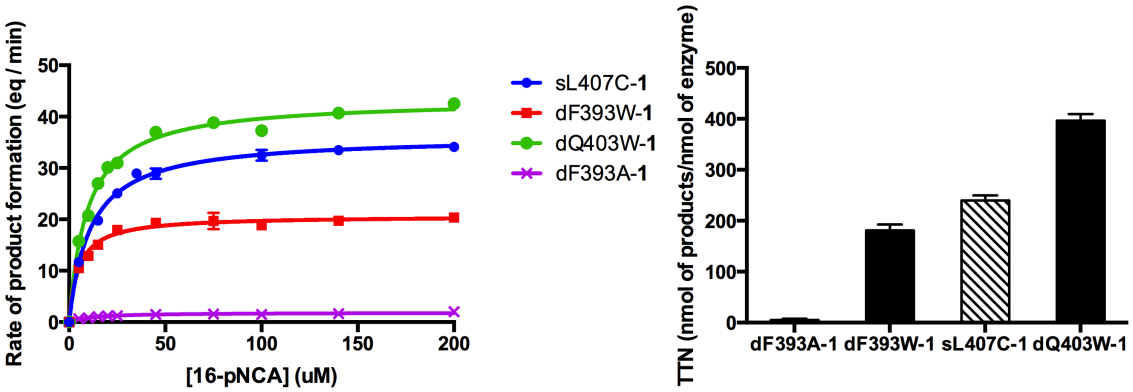

Fig. S4. A) Michaelis-Menten saturation curves for the photocatalytic activity of the four hybrid enzymes (dF393A-**1**, dF393W-**1**, sL407C-**1**, dQ403W-**1**) as a function of the 16-pNCA concentration; B) Total turnover numbers as nmol of products per nmol of enzymes at the end of the reaction.

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CYP102A1    EEFRPERF--ENPSAIPQHAFKPFGNGQRACIGQQFALHEATLVLGMMLKH
CYP3A4      EKFLPERFSKKNKDNIDPYTYTPFGSGPRNCIGMRFALMNMKLALIRVLQN
CYP2C9      EMFDPHHFLDEGGNFKKSKYFMPFSAGKRICVGEALAGMELFLFLT
CYP2C8      NIFDPGHFLDKNGNFKKSDYFMPFSAGKRICAGEGLARMELFLFLT
CYP2E1      EKFKPEHFLNENGKFKYSDYFKPFSTGKRVCAGEGLARMELFLLLCAILQH
CYP1A2      RPERFLTADGTAINKPLSEKMMLFGMGKRRCIGEVLAKWEIFLFLAILLQ
CYP2A6      QDFNPQHFLNEKGQFKKSDAFVPFSIGKRNCFGEGLARMELF
CYP2D6        FHPEHFLDAQGHFVKPEAFLPFSAGRRACLGEPLARMELFLFFTSLLQH
CYP2B6      DAFNPDHFLDANGALKKTEAFIPFSLGKRICLGEGIARAELFLF
CYP2C19     EMFDPRHFLDEGGNFKKSNYFMPFSAGKRICVGEGLARMELFLFLT
CYP3A5       EEFRPERFSKKKDSIDPYIYTPFGTGPRNCIGMRFALMNMKLALIRVLQN
CYP2J2       DTFNPDHFLENGQFKKREAFMPFSIGKRACLGEQLARTE
CYP1A1      FLPERFLTPDGAIDKVLSEKVIIFGMGKRKCIGETIARWEVFLFLAILLQ
CYP1B1      FDPARFLDKDGLINKDLTSRVMIFSVGKRRCIGEELSKMQLFLFISI
CYP4V2      EEFQPERFFPENAQGRHPYAYVPFSAGPRNCIGQKFAVMEEKTILSCILRH
CYP17A1     FMPERFLNPAGTQLISPSVSYLPFGAGPRSCIGEILARQELFLIMAWLLQ
CYP46A1         FNPDRFGPGAPKPRFTYFPFSLGHRSCIGQQFAQMEVKVVMAKLLQ
CYP4A11       EVFDPSRFAPGSAQHSHAFLPFSGGSRNCIGKQFAMNELKVATALTL
CYP4F12     EVYDPFRFDPENSKGRSPLAFIPFSAGPRNCIGQAFAMAEMKVVLALMLLH
CYP24A1       QFRPERWLQEKEKINPFAHLPFGVGKRMCIGRRLAELQLHLAL
CYP5A1      TFNPERF-TAEARQQHRPFTYLPFGAGPRSCLGVRLGLLEVKLTL
CYP4B1      EVFDSLRFSTENASKRHPFAFMPFSAGPRNCIGQQFAMSEMKVVTAMCL
CYP4A22      LEVFDPSRFAPGSAQHSHAFLPFSGGSRNCIGKQFAMNQLKVARALTL
CYP2S1      EEFNPDRFLDADGRFRKHEAFLPFSLGKRVCLGEGLAKAEVFLFFTTILQ
CYP46A          FNPYRFGPGAPKPRFTYFPFSLGHHSCIGQQFAQMEVKVVMAKLLQ
CYP4X1        FDPLRFSQENSDQRHPYAYLPFSAGSRNCIGQEFAMIELKVTIALILLH
CYP4Z1        FNPLRFSRENSEKIHPYAFIPFSAGLRNCIGQHFAIIECKVAVALTL
CYP2W1       QFNPGHFLDANGHFVKREAFLPFSAGRRVCVGERLARTELFLLFAGLLQ
CYP27A1     PHRWLRNSQPATPRIQHPFGSVPFGYGVRACLGRRIAELEMQLLLARLIQ
CYP2AC1P    DTFNPEHFLNSKEKFIKREAFLPFQWGRRMCAGESFARKELFLFFTSLLQ
CYP3A7      EKFLPERFSKKNKDNIDPYIYTPFGSGPRNCIGMRFALVNMKLALVRVLQN
CYP4F23P        FDPENLQKTSPL---AFIPFSAVPRNCIGQTFAMAEMKVVLALTL
CYP11B1      ERYNPQRWLDIRGSGRNFYHVPFGFGMRQCLGRRLAEAEMLLLLHHVLKH
CYP51A       DFNPDRYLQDNPASGEKFAYVPFGAGRHRCIGENFAYVQIKTIWSTMLR
CYP26C1     DPERFGAAREDSRGASSRLHYIPFGGGARSCLGQELA
CYP2F1      QEFNPEHFLDANQSFKKSPAFMPFSAGRRLCLGELLARMELFLYLTAILQ
CYP4F11     EVYDPFRFNQENIKERSPLAFIPFSAGPRNCIGQAFAMAEMKVVLALTLLH
CYP11B2                           PFGFGMRQCLGRRLAEAEMLLLLHHVLKH
CYP4F3      VYDPFRFD-PKNIKERSPLAFIPFSAGPRNCIGQAFAMAEMKVVLGLTL
CYP4F9P     EVYDPFRFDPENSKERSPLAFIPFSAGSXNCIGQAFAMAEMKVVLALTL
CYP2R1      EVFHPERFLDSSGYFAKKEALVPFSLGRRHCLGEHLARMEMFLFFTALLQ
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Fig. S5. Partial sequence alignment indicating that the highlighted Q403 residue of the P450 BM3 enzyme (three residues away from the ligating cysteine) is highly conserved among human cytochrome P450 heme domains.