

Electronic Supplementary Information

Singlet oxygen photosensitisation by the fluorescent protein PP2FbFP L30M, a novel derivative of *Pseudomonas putida* flavin-binding PP2FbFP

Joaquim Torra,^a Andrés Burgos-Caminal,^a a Stephan Endres,^b Marcus Wingen,^b Thomas Drepper,^b Thomas Gensch,^c Rubén Ruiz-González^a and Santi Nonell*^a

a Grup d'Enginyeria Molecular, Institut Químic de Sarrià, Universitat Ramon Llull, Via Augusta 390, 08017, Barcelona, Spain.

b Institute of Molecular Enzyme Technology, Heinrich-Heine-University Düsseldorf, Forschungszentrum Jülich, 52425 Jülich, Germany.

c Institute of Complex Systems 4 (ICS-4, Cellular Biophysics), Forschungszentrum Jülich, 52425 Jülich, Germany.

Table of Contents:

Fig. S1 Pp2FbFP L30M ¹O₂ phosphorescence kinetics

Fig. S2 Effect of oxygen concentration on the rate of Pp2FbFP L30M triplet state decay

Fig. S3 Transient absorbance decays

Fig. S4 Deconvolution of ¹O₂ phosphorescence

Fig. S5 HPLC analysis of the chromophores in Pp2FbFP L30M and miniSOG

Fig. S6 Sequence alignment in LOV-based FPs.

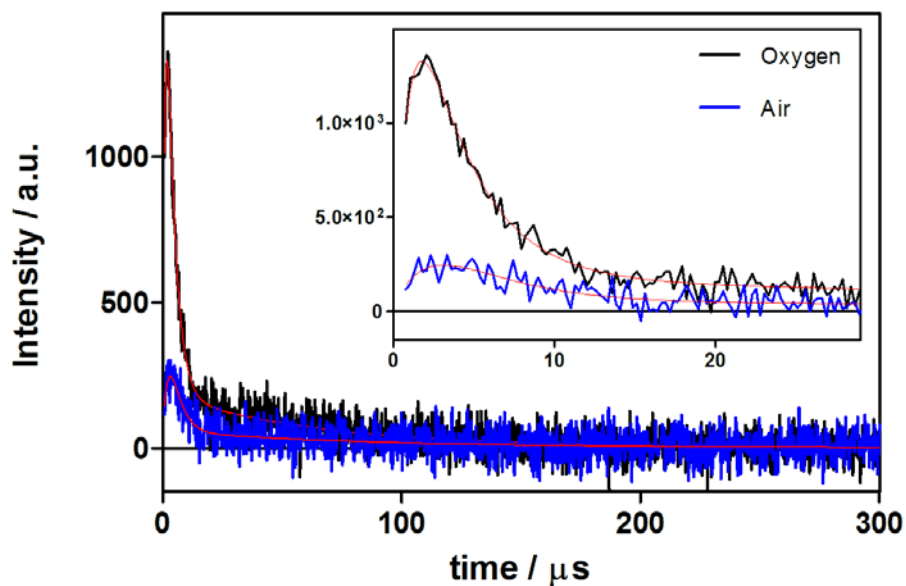


Fig. S1 $^1\text{O}_2$ luminescence at 1275 nm ($\lambda_{\text{exc}} = 355$ nm) for Pp2FbFP L30M in air- and oxygen-saturated PBS. Inset: detail of the early part of the signals.

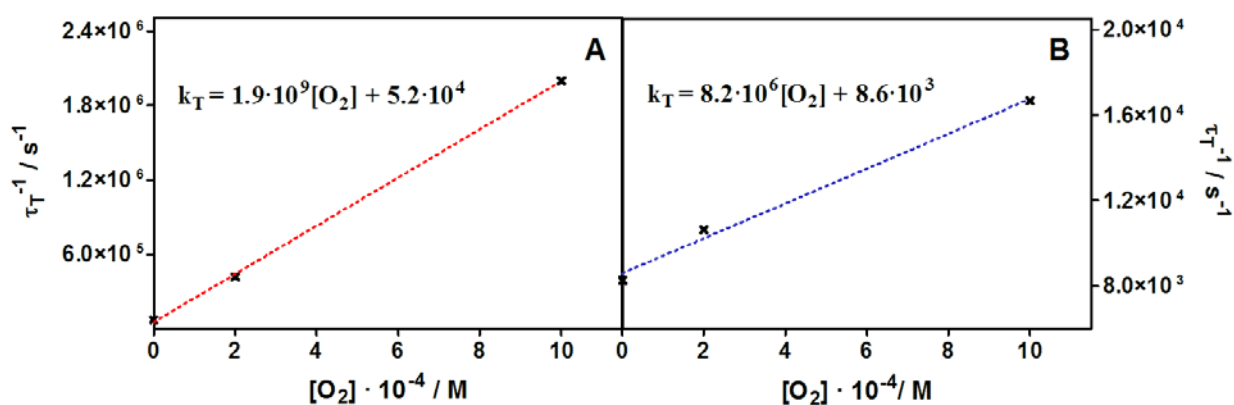


Fig. S2. Effect of oxygen concentration on the rate of Pp2FbFP L30M triplet state decay. A: Short-lived triplet. B: Long-lived triplet. The slope of the linear plots yields the corresponding quenching rate constants.

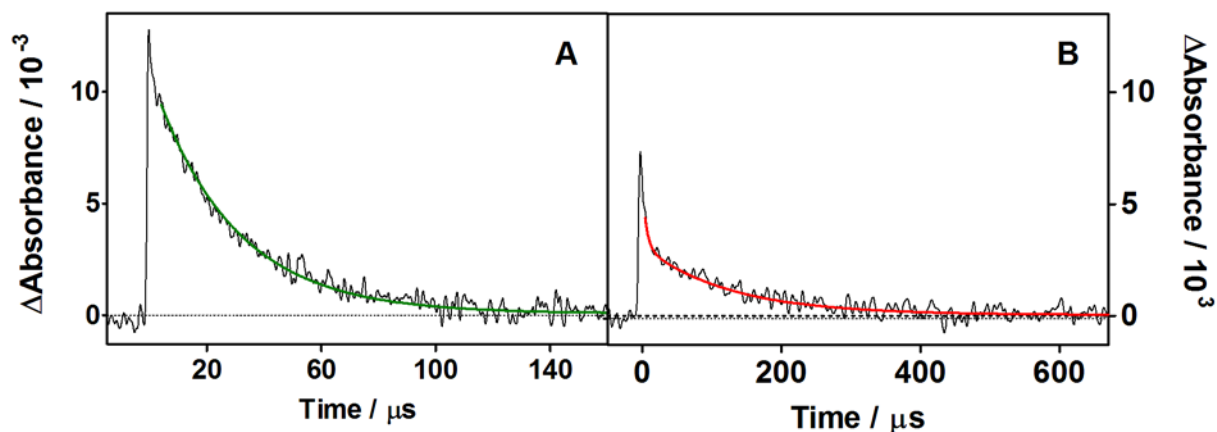


Fig. S3. Transient absorbance decays for optically-matched solutions of (A) FMN and (B) Pp2FbFP L30M in argon-saturated PBS solutions. $\lambda_{\text{exc}} = 355 \text{ nm}$ and $\lambda_{\text{obs}} = 700 \text{ nm}$.

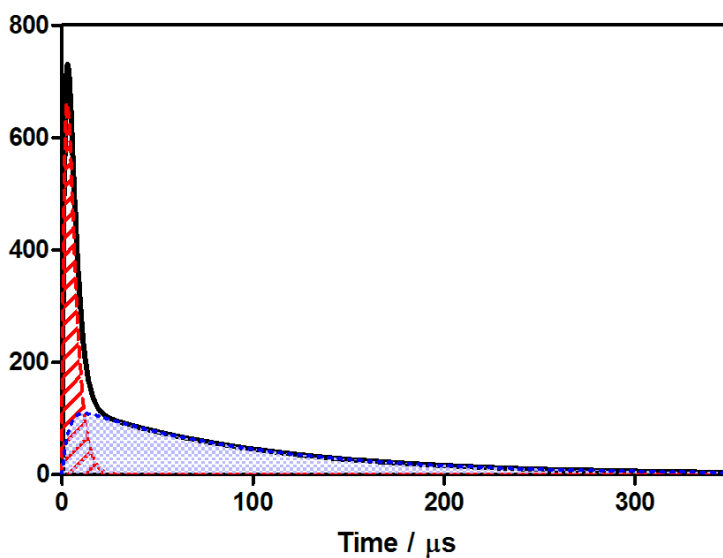


Fig. S4 Deconvolution of $^1\text{O}_2$ phosphorescence signal from Pp2FbFP L30M according to eqn (8). The fractional S_0 values are 30% for the oxygen-accessible triplet (red striped area) and 70% for the less-exposed triplet (blue dotted area).

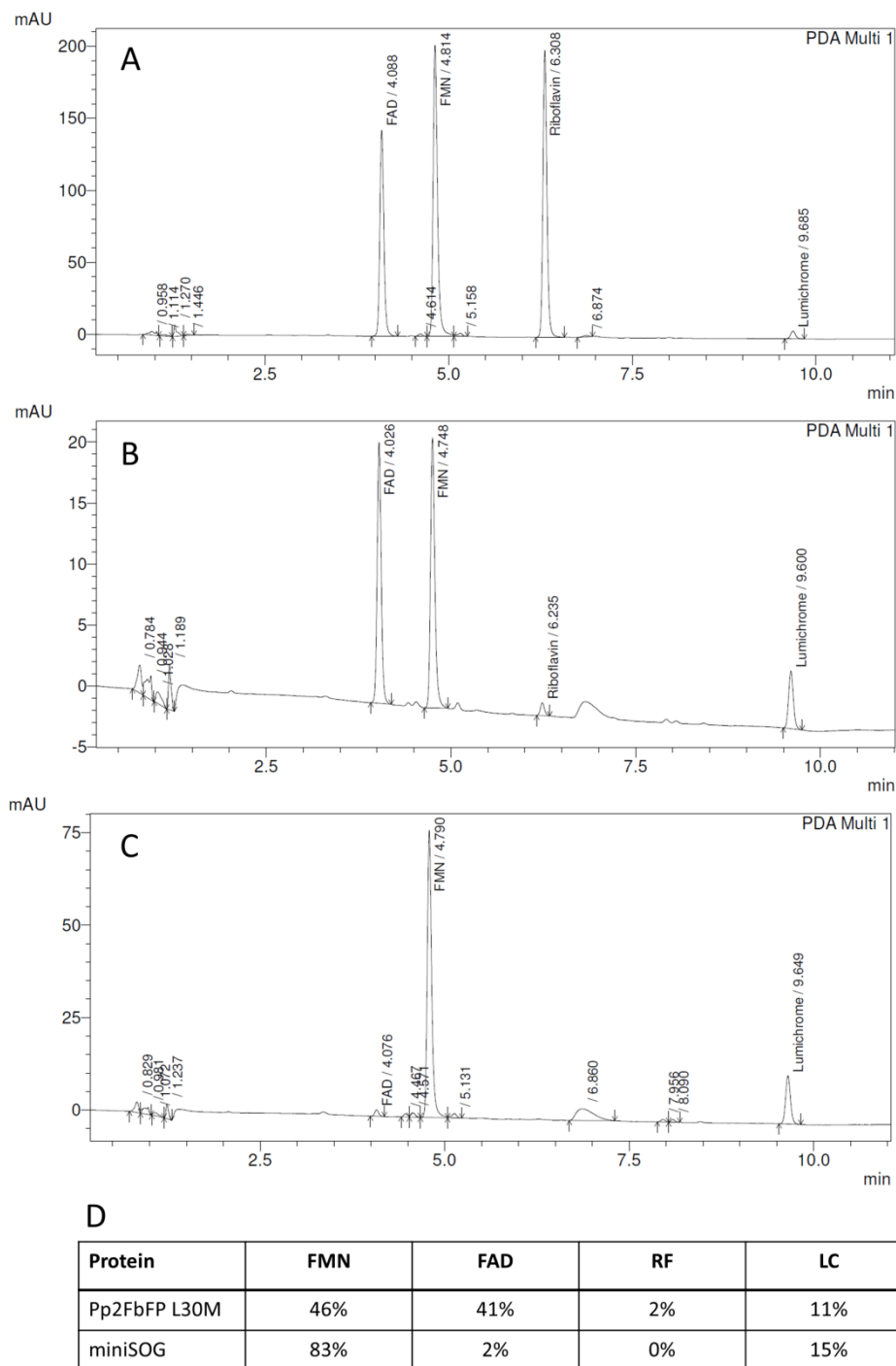


Fig. S5 HPLC chromatograms of Pp2FbFP L30M, miniSOG and the reference compounds riboflavin, FMN, FAD. Data were obtained as outlined in the materials and methods section. The absorption was measured at $\lambda = 263$ nm. A) Reference; FMN; FAD and RF of the highest available purity grade were used as references to determine the corresponding HPLC retention times. Minor amounts of LC have been identified here as well, indicating that LC may be formed during sample preparation. B) Pp2FbFP L30M; the sample contains approximately equal amounts of FAD and FMN, as well as minor amounts of LC and RF. C) miniSOG; this sample mainly contains FMN as chromophore. FAD and LC were only identified at comparatively low levels. The percentage of chromophore distribution in the LOV-based FPs is given in the Table (D).

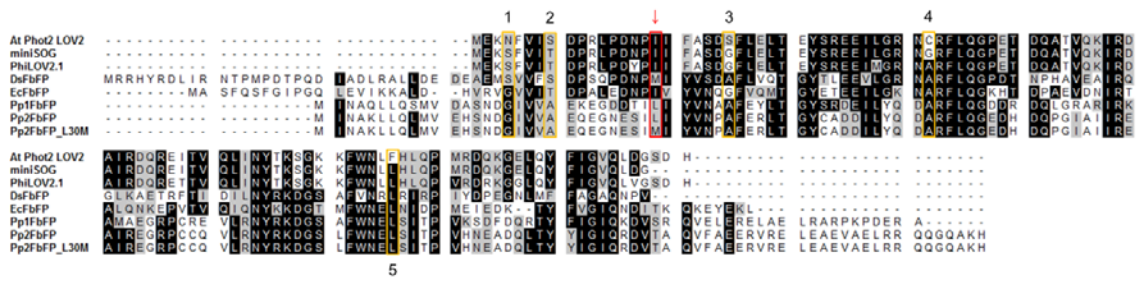


Fig. S6 Sequence alignment in LOV-based FPs.