PhotUPO: Switchable UPO biocatalysis by genetically encoded photosensitizers

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Unspecific peroxygenases (UPOs) can oxyfunctionalise a broad set of substrates only requiring hydrogen peroxide as a co-substrate. High turnover numbers, stabilities and excellent selectivities render UPOs exciting enzymes for C–H activations. A major challenge for UPOs is haem-bleaching by hydrogen peroxide, lowering the catalytic efficiency. The necessity to avoid this lead to the development of different systems for the *in situ* production of hydrogen peroxide.[1] Herein we report on a new approach to avoid haem-bleaching and thus inactivation of the enzyme.

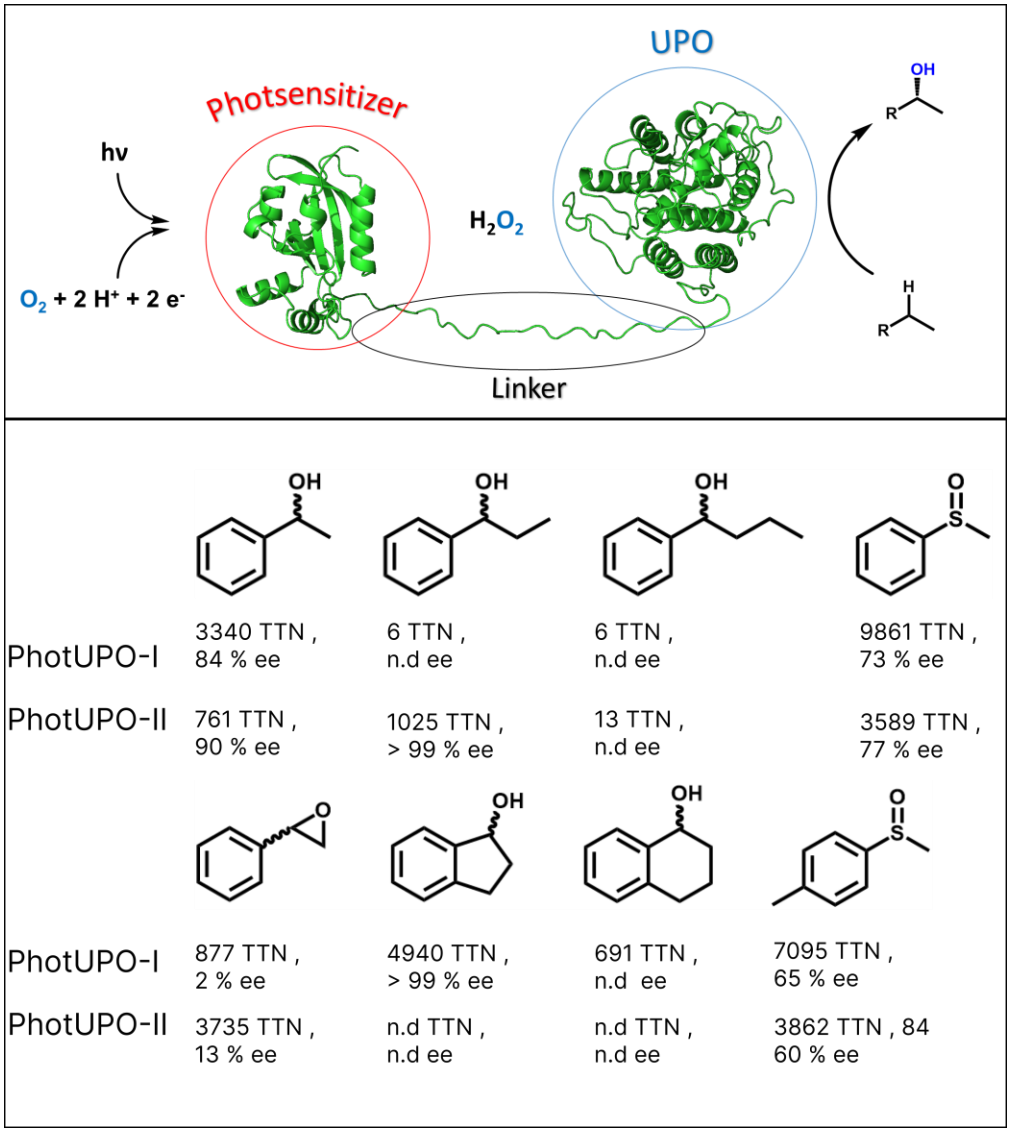

Figure 1: Principle of PhotUPO, utilizing a genetically encoded photosensitizer to fuel the UPO.

A fusion construct – photosensitizer, linker, UPO – was successfully designed and expressed in *Pichia Pastoris*. As photosensitizer a LOV photoreceptor from *D. shibae* – DsFbFP – was used. This photoreceptor can be genetically encoded and produces ROS upon excitation with the right wave length. ROS can react with the surrounding water to form hydrogen peroxide, which is then consumed by the enzyme. Photosensitizer and UPO are connected by a linker, enabling the user to express the whole construct in one run.

Photochemical as well as chemical optimisation followed and a broad substrate scope was screened. The PhotUPOs showed promising conversions with excellent ee-values (Figure 1). This system adds an easy and switchable biophotocatalytic access to oxyfunctionalised C-H bonds.

Literature

[1] a) Y. Ni, E. Fernández-Fueyo, A. G. Baraibar, R. Ullrich, M. Hofrichter, H. Yanase, M. Alcalde, W. J. H. Van Berkel, F. Hollmann, *Angewandte Chemie International Edition* **2016**, *55*, 798-801; b) S. J. P. Willot, M. D. Hoang, C. E. Paul, M. Alcalde, I. W. C. E. Arends, A. S. Bommarius, B. Bommarius, F. Hollmann, *ChemCatChem* **2020**, *12*, 2713-2716; c) W. Zhang, H. Liu, M. M. C. H. van Schie, P.-L. Hagedoorn, M. Alcalde, A. G. Denkova, K. Djanashvili, F. Hollmann, *ACS Catalysis* **2020**, *10*, 14195-14200; d) P. Gomez de Santos, S. Lazaro, J. Viña-Gonzalez, M. D. Hoang, I. Sánchez-Moreno, A. Glieder, F. Hollmann, M. Alcalde, *ACS Catalysis* **2020**, *10*, 13524-13534.