

## Electronic Supplementary Information (ESI) for:

### Visible light-driven and chloroperoxidase-catalyzed oxygenation reactions

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#### 1. Experimental details

##### *Materials*

Chloroperoxidase from *Caldariomyces fumago* (EC 1.11.1.10) was purchased from BioChemika and used as received. A R<sub>z</sub> value of 0.85 was determined by UV spectroscopy and the concentration of CPO in the solution was 25 mg/mL (600 μM). Thioanisole, sulfoxide, indole, oxindole, furfuryl alcohol, furfuryl aldehyde, *tert*-butyl alcohol (<sup>t</sup>BuOH) and H<sub>2</sub>O<sub>2</sub> (wt 50%) were purchased from Aldrich in analytical grade and used without further purification.

##### *General procedure*

A typical light-driven reaction was set up as follows: A mixture of <sup>t</sup>BuOH/(50 mM phosphate buffer pH=5.1) (25/75) containing thioanisole (50 mM), EDTA (50 mM) or formate (50 mM), flavin (10-250 μM), and CPO (0.36–7.85 μM final concentration) was placed in front of a slide projector lamp (Philips Focusline 250 W) and illuminated for 1-8h under aerobic conditions. At intervals, samples were withdrawn, extracted with ethyl acetate (containing 5 mM of anisole as internal standard) and analyzed via gas chromatography (vide infra).

##### *Experimental setup*

All photoenzymatic reactions were performed in the setup shown in Figure 1.



**Figure 1: Experimental setup of the photobiocatalytic oxidation/oxygenation reactions.** Instead of the projector lamp any given source for visible light can be applied. Also sunlight can be used but is not advisable in terms of reproducibility due to the varying Dutch weather conditions.<sup>1</sup>

As light source an ordinary slide projector equipped with a 250W bulb (Philips 7748 XHP) was placed in front of a conventional glass reaction vessel. Oxygen intake was achieved by stirring the reaction mixture under ambient atmosphere.

#### *Analytical procedures*

The progress of the reactions was measured by GC.

For thioanisole oxidation, chiral GC analysis was performed on a Shimadzu GC 17A with AOC-201 automatic injector using a chiraldex G-TA column (50m\*0.25mm\*0.12 um) that was run isothermal at 160 °C for 20 minutes (column flow: 0.9 ml/min;split ratio:1/60). Retention times for thioanisole were 3.41 min, *R*-methyl phenyl sulfoxide 9.88 min and for *S*-methyl phenyl sulfoxide 12.00 min.

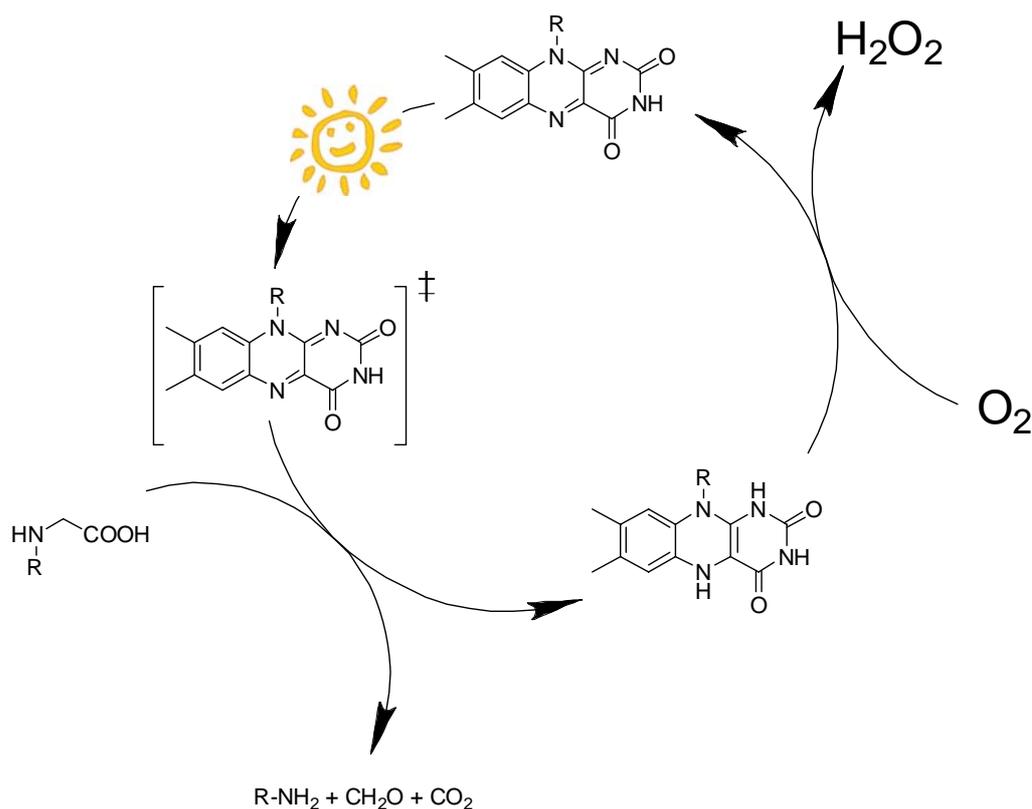
For indole oxidation, GC analysis were performed on a Shimadzu GC 2014 with AOC-20i automatic injector using a CP-SIL-5CB column (50m\*0.53mm\*2 um) that was run 80°C for 1 min, then at the rate of 30°C per minute to 200°C for 10 minutes (column flow: 23 ml/min; no split (on column injection)). Retention times for indole were 4.80 min and for oxindole 5.92 min. Both compounds were also confirmed by GC-MS Shimadzu GC 2010 with AOC-20i automatic injector using a CP WAX 52 CB (50m\*0.53mm\*2 um) that was run 50°C for 1 min, then at the rate of 10°C per minute to 280°C for 5 minutes (column flow: 0.9 ml/min; split ratio:1/10).

For furfuryl alcohol oxidation, GC analysis were performed on a Shimadzu Varian star 3400 CX with Varian CP-8200 automatic injector using a CP WAX 52 CB (50m\*0.53mm\*2 um) that was run 80°C for 1 min, then at the rate of 10°C per minute to 230°C for 7 minutes (column flow: 11.2 ml/min, no split (on column injection)). Retention times for furfuryl alcohol were 12.37 min and for furfuryl aldehyde 9.98 min.

Hydrogen peroxide was quantified according to the method of Saito et al.<sup>2</sup>

## 2. Proposed H<sub>2</sub>O<sub>2</sub> formation mechanism(s)

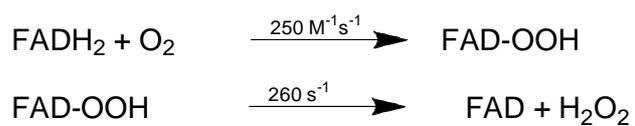
In 1959 Frisell et al.<sup>3</sup> reported on the light-promoted and flavin-catalyzed oxidation of N-alkylated amino acids. According to the proposed mechanism, electronically excited flavins catalyze a 2 electron transfer from the sacrificial electron donor to the excited, oxidized flavin (Figure 2).



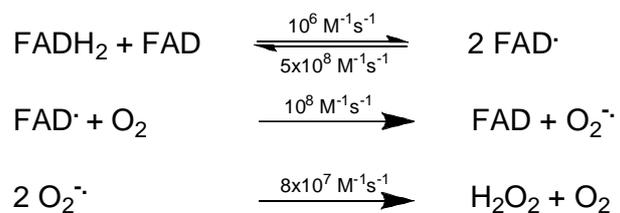
**Figure 2:** Schematic mechanism for the light-driven *in situ* generation of H<sub>2</sub>O<sub>2</sub>. Adapted from Frisell et al.<sup>3</sup>

For the aerobic reoxidation of reduced flavins, 2 pathways are principally possible (Peroxo- and Semiquinone pathway, Figure 3).<sup>4</sup> Considering the very high reaction constants, the so-called semiquinone-pathway appears to be predominant in the reported setup.

(1) Peroxoflavin pathway



(2) Semiquinone pathway



**Figure 3: Aerobic reoxidation pathways for reduced flavins.**<sup>4</sup> (For sake of simplicity, the protonation equilibrium for reduced flavins is omitted)

### 3. Enantioselectivity of CPO in the presence of $\text{Cl}^-$ and $\text{HCO}_2^-$

To further investigate the decreased enantioselectivity of CPO in the presence of formate, control reactions were performed using stoichiometric  $\text{H}_2\text{O}_2$  in the presence of  $\text{NaHCO}_2$  and  $\text{NaCl}$ , each. The results are compared in the following

| Conditions  | conversion [%] | ee [%] |
|---|----------------|--------|
| $[\text{H}_2\text{O}_2]=50$ mM, dark reaction                           | 39             | 99     |
| $[\text{H}_2\text{O}_2]=50$ mM, $[\text{NaHCO}_2]=50$ mM, dark reaction | 36             | 56     |
| $[\text{H}_2\text{O}_2]=50$ mM, $[\text{NaCl}]=50$ mM, dark reaction    | 12             | 63     |
| $[\text{FMN}]=100$ $\mu\text{M}$ , $[\text{EDTA}]=50$ mM, hv            | 100            | 99     |
| $[\text{FMN}]=100$ $\mu\text{M}$ , $[\text{NaHCO}_2]=50$ mM, hv         | 100            | 78     |

Conditions: 10 mL scale at ambient temperature in  $\text{tBuOH}/(50$  mM phosphate buffer  $\text{pH}=5.1)$  (25/75),  $T=25$  °C,  $t=3$ h,  $[\text{CPO}]=3.9$   $\mu\text{M}$ ,  $[\text{thioanisole}]=50$ mM.

These results are most straightforward rationalized by assuming a coordinative interaction between  $\text{Cl}^-$  and  $\text{HCO}_2^-$  with the heme iron which is also supported by the crystallographic data provided by Schlichting and coworkers.<sup>5</sup> However, at present time we cannot entirely exclude the possibility of (putatively formed) hypochlorite and performate to interfere with the CPO-catalyzed sulfoxidation.

### 4. References

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