

Atom-Economic Electron Donors for Photobiocatalytic Halogenations – Experimental Section and Supporting Information

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1. General Information

Solvents used in reactions were p.a. grade. Solvents for chromatography were technical grade and distilled prior to use. Reactions were monitored by thin layer chromatography (TLC) carried out on Merck silica gel aluminum plates with F-254 indicator using UV light as the visualizing agent. Silica gel Merck 60 (particle size 0.63 – 0.2 mm) was used for flash column chromatography. Solvent mixtures are reported as volume/volume (v/v). NMR spectra were recorded on Bruker AV300, Bruker AV400 or Bruker AV500 spectrometers. The spectra were calibrated using residual undeuterated solvents as internal references (CHCl_3 @ 7.26 ppm, DMSO @ 2.50 and 3.33 ^1H NMR and CHCl_3 @ 77.00 ppm, DMSO @ 39.52 ^{13}C NMR). The following abbreviations (or combinations thereof) are used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, q = quartet, m = multiplet, br = broad. In addition, the following abbreviations are used: EtOAc = ethyl acetate, MeCN = acetonitrile, rt = room temperature, sat = saturated, MES = 2-(*N*-morpholino)-ethanesulfonic acid, NaOAc = sodium acetate, Tris = tris(hydroxymethyl)aminomethane, MOPS = 3-(*N*-morpholino)propanesulfonic acid, HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, EDTA = ethylenediaminetetraacetic acid, TFA = trifluoroacetic acid, MCD = monochlorodimedone, IPTG = isopropyl β -D-1-thiogalactopyranoside, Ni-NTA = nickel-nitrilotriacetic acid, LB = lysogeny broth, TB = terrific broth, FMN = flavin mononucleotide, RFTA = riboflavin tetraacetate, PMSF = phenylmethyl-sulfonyl fluoride, SDS = sodium dodecyl sulfate, n.d. = not determined. . RFTA^[1] and Au-TiO₂^[2] were prepared according to known literature procedures. The DA-64 dye was purchased from Ark Pharm Inc. All other chemicals were purchased (Sigma Aldrich, Acros, or TCI) at the highest commercial quality and used without further purification or synthesized as described below. Vanadium-dependent bromoperoxidase from *Corallina officinalis* (CoVHPO), glucose oxidase from *Aspergillus niger* and horseradish peroxidase were purchased from Sigma-Aldrich.

1.1 Analytical and Preparative Procedures

Mass spectra (**LC-ESI-MS**) were determined on a Thermo LCQ fleet coupled with a Dionex UltiMate 3000 HPLC. **GC-MS** measurements were conducted on a Finnigan MAT SSQ 7000 (MS-EI, 70 eV; CI, 100 eV). HR-ESI-MS mass spectra were recorded on a Thermo LTQ FT Ultra coupled with a Dionex UltiMate 3000 HPLC system. Gas chromatography (**GC**) was performed on two different instruments. **GC(1)** measurements were carried out on a HP 6890 Series GC instrument (Agilent, equipped with a FID detector and a HP-5 capillary column [length = 29.5 m]). Hydrogen was used as the carrier gas and the constant-flow mode (flow rate = 1.8 mL min⁻¹) with a split ratio of 1:20. The following temperature-program was used: 60°C for 3 min, 15°C min⁻¹ to 250°C in 12 min, and 250°C for 5 min. **GC(2)** measurements were carried out on a HP 6890 Series GC instrument (Agilent, equipped with a FID detector and a HP-5 capillary column [length = 30.0 m]). Helium was used as the carrier gas and the constant-flow mode (flow rate = 1.0 mL min⁻¹) with a split ratio of 1:40. The following temperature-program was used: 40°C for 3 min, 15°C min⁻¹ to 280°C in 16 min, and 280°C for 5 min. **Head-space CG** measurements were performed on INFICON 3000 Micro GC equipped with MS-5A column using argon as a carrier gas. High performance liquid chromatography (**HPLC**) analysis was performed on a HITACHI Chromaster system employing the following conditions: 150

x 4 mm Eurospher II (KNAUER®), 100 Å, 5 µm, C18, 25°C, flow rate: 1.0 mL min⁻¹, water (+0.01% TFA)/MeCN (+0.01% TFA) gradient: 90/10 → 0/100 in 13 min. For medium pressure liquid chromatography (**MPLC**) a Reveleris® X2 MPLC system (Grace) was used together with Reverleris® Reverse Phase (RP) C18 columns (Grace) using UV-detection at 220 nm, 254 nm, and 280 nm. **Protein concentrations** were determined using a Nanodrop 2000c spectrophotometer (Thermo Scientific), $\epsilon(AmVHPO+His) = 65780 \text{ M}^{-1}\text{cm}^{-1}$, MW = 73.13 kDa; $\epsilon(CiVCPO+His) = 91790 \text{ M}^{-1}\text{cm}^{-1}$, MW= 72.59 kDa. **UV/Vis spectra** were recorded on BioTek® Eon and Agilent Technologies Cary 100 spectrophotometers. *In situ* concentration of oxygen was monitored by PreSens Fibox 3 **oxygen sensor**.

1.2 Light Sources

Sample irradiation was performed using 455 nm (OSRAM Oslon SSL 80 royal-blue LEDs, $\lambda_{em} = 455 \text{ nm} (\pm 15 \text{ nm})$, 3.5 V, 700 mA) or 375 nm (Avonec 370 – 380 nm UV-A LEDs, $\lambda_{max} = 375 \text{ nm}$, 3.5 – 4.5 V, 750 mA) LED light sources.

2. Cloning, Protein Production and Purification (C. J. Seel, A. Frank)

The heterologous expression and purification of the *Acaryochloris marina* vanadium-dependent haloperoxidase (AmVHPO) has been reported previously.^[3] The protocol was slightly adjusted using Ni-NTA beads (Jena Bioscience) instead of a Hisrap column.

2.1 Heterologous Production of C_iVHPO (C. J. Seel)

For recombinant production of the *Curvularia inaequalis* vanadium-dependent haloperoxidase (C_iVHPO, Genbank accession number CAA59686), the codon-optimized synthetic gene (Eurofins) was introduced into a pETDuet-1 (Novagen) vector by restriction digest (PstI, NotI) and ligation. Successful insertion of the gene and the absence of undesired mutations were verified by Sanger sequencing (GATC). As a consequence of the cloning process, the VHPO open reading frame was modified by the addition of a N-terminal hexahistidine sequence.

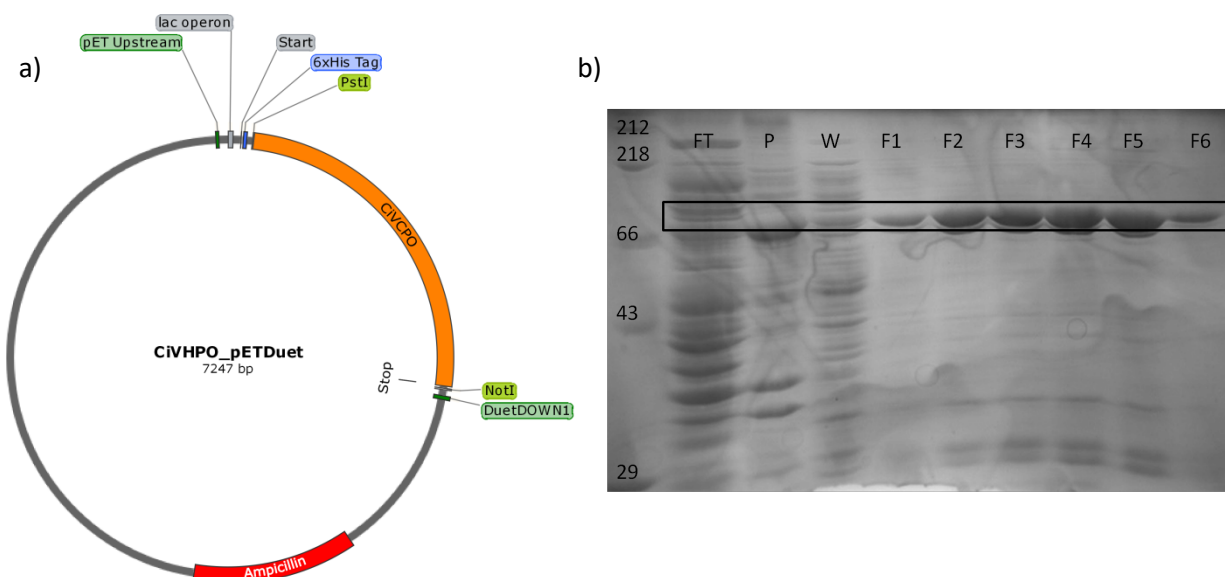


Figure S1. a) Vector map of the pETDuet::civhpo construct. b) SDS-Page of the purification of C_iVHPO; FT = flow-through; P = pellet; W = wash; F1 – F6 = elution fractions; the corresponding molecular weights of the protein marker are stated in kilodaltons (kDa); size of C_iVHPO+His approx. 73 kDa.

The obtained pETDuet::civhpo expression vector was used to transform *E. coli* BL21 (DE3) cells for protein production. Recombinant cells were grown on LB agar plates and subsequently used for inoculation of TB liquid cultures. All media contained ampicillin as a selective antibiotic at a final concentration of 100 µg mL⁻¹. Cells were grown in 1.5 – 3 liter expression cultures at 37°C while shaking at 150 – 180 rpm. At an optical density of 0.6, protein production was induced by the addition of IPTG to a final concentration of 0.1 mM and cultures were incubated overnight at 16°C. Cells were harvested by centrifugation and washed with 0.9% NaCl solution. The cells were then resuspended in lysis buffer (10 mM imidazole, 50 mM NaH₂PO₄, 300 mM NaCl, 10% glycerol) and PMSF (protease inhibitor), lysozyme and DNase were

added. The suspension was sonicated on ice and centrifuged at 14,000 *g* for 30 min. Subsequently, the soluble fraction was incubated with Ni-NTA resin (Jena Bioscience, 1 mL per 1 L culture) and gently shaken on ice for 45 min. Thereafter, the resin-containing solution was transferred to a column and the flow-through was eluted (gravity flow). After washing the bead bed with 20 mL of binding buffer (30 mM imidazole, 50 mM NaH₂PO₄, 300 mM NaCl, 10% glycerol), the His-tagged protein was eluted in fractions of 0.5 mL (500 mM imidazole, 50 mM NaH₂PO₄, 300 mM NaCl, 10% glycerol). Fractions of the highest apparent purity were pooled and then subjected to a buffer-exchange by the use of PD-10 desalting columns (GE Healthcare). The exchanged buffer was used for storage of the enzyme (50 mM Tris pH 7.0, 100 μ M Na₃VO₄). After concentration (if required), the enzyme was flash-frozen using liquid nitrogen and stored at -80°C until further use.

Alternatively, a buffer containing 50 mM MES pH 6.0, 300 mM NaCl and 100 μ M Na₃VO₄ was used in order to keep the pH of the reactions at pH 6, more accurately. The stability of *AmVHPO* in the MES storage buffer, however, was notably lower.

2.2 Heterologous Production of *AmVHPOII* (*C. J. Seel*)

The gene sequence of the isoenzyme *AmVHPOII* (Genbank accession number WP_012161787) was PCR-amplified using genomic DNA from *Acaryochloris marina* (strain MBIC 11017) as a template. The sequence was then introduced into a pETDuet-1 (Novagen) vector by restriction digest (BamHI, PstI) and ligation. Successful insertion of the gene and the absence of undesired mutations were verified by Sanger sequencing (GATC). Analogous to the above described cloning strategy for *CiVHPO*, the protein was tagged *N*-terminal with a His₆-tag. *E. coli* BL21 (DE3) was used as expression host. The heterologous

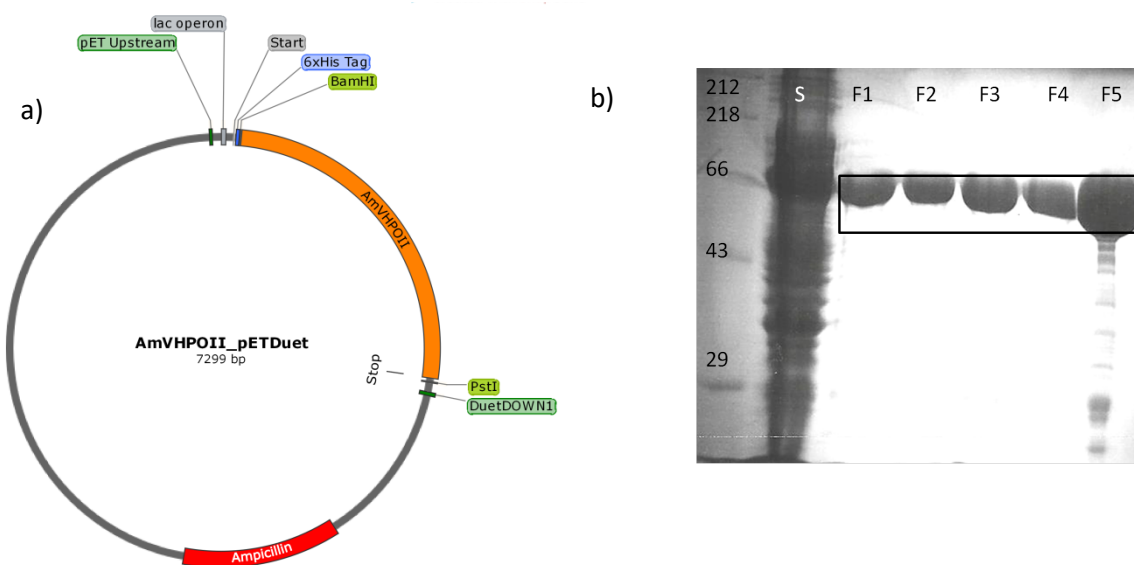


Figure S2. a) Vector map of the pETDuet::amvhpoll construct. b) SDS-Page of the purification of *AmVHPOII*; S = supernatant; F1 –F5 = elution fractions; the corresponding molecular weights of the protein marker are stated in kilodaltons (kDa); size of *AmVHPOII*+His approx. 70 kDa.

production was carried out as described in 2.1, except for the utilization of LB media instead of TB media and the induction with 1 mM IPTG final concentration. The Ni-affinity purification was followed by size-exclusion chromatography. The relatively late elution of the protein pointed to a smaller oligomeric state compared to the isoenzyme *AmVHPO*. For gel filtration a flow-rate of 1.0 – 1.6 mL min⁻¹ in a single buffer (20 mM Tris pH 7.5, 100 mM NaCl, 0.001% NaN₃) was used. For activity assays, the buffer was exchanged (to 50 mM MES pH 6.0, 100 μM Na₃VO₄) by the use of a PD-10 column.

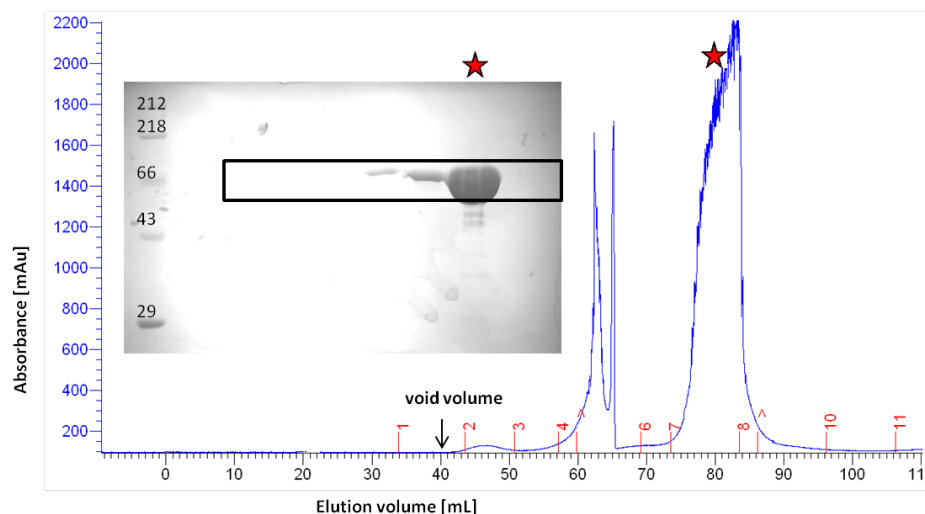


Figure S3. UV diagram of the size-exclusion chromatography of *AmVHPOII* using a Superdex S200 analytical gel filtration column (GE healthcare). The peak fraction of *AmVHPOII* (marked with a red star) eluted approx. 35 mL after the expected void volume of the column, suggesting the presence of either a dimer or a monomer. In comparison, the isoenzyme *AmVHPO* elutes shortly (approx. 10 mL) after the void volume and has been shown to form a homododecameric structure. The fractions were analyzed by SDS-Page; the corresponding molecular weights of the protein marker are stated in kilodaltons (kDa).

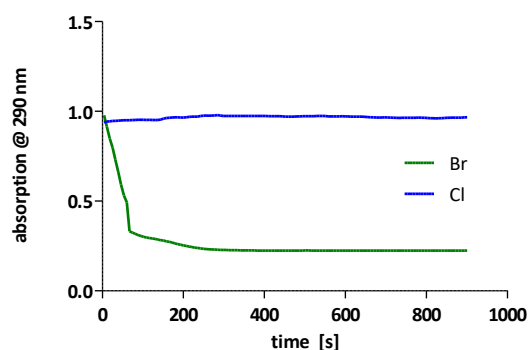


Figure S4. MCD Assay (see in 3.1.) as a proof of *ex vivo* functionality of *AmVHPOII*. Bromination activity, but no chlorination activity was observed in this standard assay for VHPOs. It is worth mentioning, however, that the MCD assay is not a fully reliable indicator for chlorination activity as selective chloroperoxidases from *Streptomyces*, for example, do not promote the chlorination of MCD.^[4]

2.3 Comparison of *Am*VHPO and *Am*VHPOII (*C. J. Seel*)

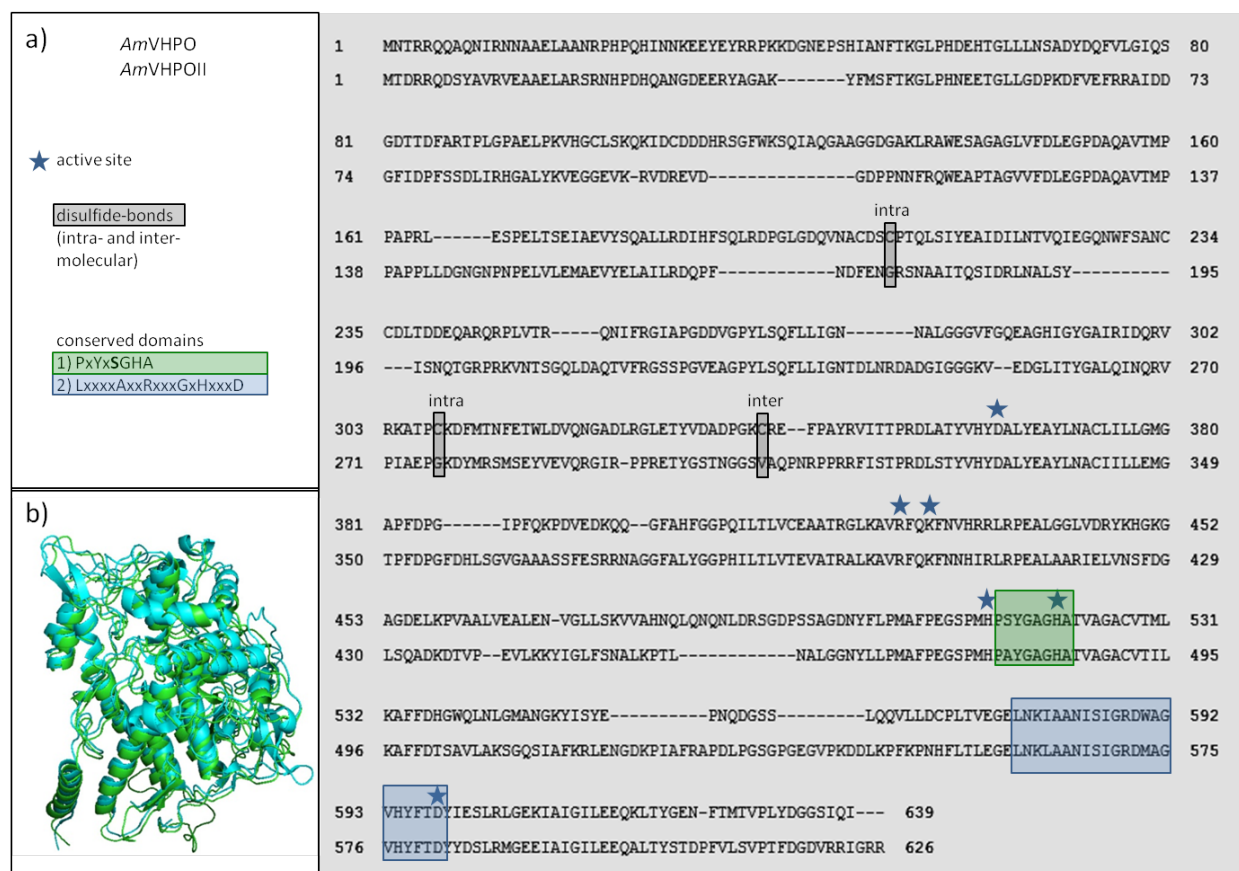


Figure S5. a) Sequence alignment of the two isoenzymes *Am*VHPO and *Am*VHPOII from *Acaryochloris marina*. Active site residues are labeled with an asterisk, intra- and intermolecular disulfide bonds present in *Am*VHPO are most likely not formed in *Am*VHPOII (no respective cysteine residues are present). Typical conserved domains in vanadium-dependent haloperoxidases^[5] are labeled with a green and blue box. Interestingly, the conserved serine (green box) is replaced by an alanine in both VHPOs from *Acaryochloris marina*. b) Superposition of the crystal structure of *Am*VHPO (PDB ID: 5LPC, cyan) and a homology model for *Am*VHPOII generated by MODELLER^[6] (green).

In addition to other striking similarities to algal VHPOs, a second isoenzyme is also present in the *A. marina* genome (a set of two VHPOs was also found in brown algae *Ascophyllum nodosum* and red seaweed *Corallina officinalis* and *Corallina pilulifera*, respectively). The two isoenzymes are not embedded in a biosynthetic gene cluster, the physiological function and natural substrate(s) thus remain unclear. Interestingly, the isoenzyme (*Am*VHPOII) seems to possess a different overall structure, lacking the characteristic disulfide bonds found in *Am*VHPO (43% sequence identity to *Am*VHPO; e-value $7e^{-146}$). We heterologously produced *Am*VHPOII and investigated its halide specificity. Just like *Am*VHPO, no chlorination activity, only bromination activity was detected in the MCD assay (Figure S4). Furthermore, we found — based on a comparably high retention time in size exclusion chromatography — that *Am*VHPOII most likely does not form a homododecamer like *Am*VHPO, but occurs rather in a dimeric or monomeric state.

2.4 Preparation of Lyophilized Lysate (*AmVHPO*) (C. J. Seel)

Lyophilized lysate was obtained from 7.4 g cell pellet which was resuspended in 80 mL lysis buffer (10 mM imidazole, 50 mM NaH₂PO₄, 300 mM NaCl). After sonication and centrifugation, the supernatant was subjected to a preliminary purification step (the supernatant was heated to 65°C for 30 min, followed by centrifugation at 40,000 g for 30 min). From the resulting supernatant, 15 mL were gathered and freeze-dried. Aliquots of 10 mg of the residual powder were used for the enzymatic reactions.

2.5 Specific Activities (C. J. Seel)

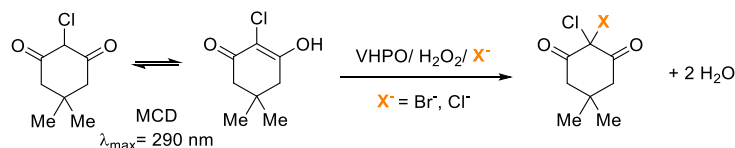
Table S1. Specific activities of the employed protein from different purification batches determined by the MCD Assay (see in 3.1).

c [mg mL ⁻¹]	A _{spec} (Br) [U mg ⁻¹]
<i>AmVHPO</i>	
3.50	6.13
1.46	3.55
1.47	n.d.
3.00	6.41
2.20	n.d.
1.50	32.54
1.30	7.69
4.50	39.51
2.90	31.14
1.62	2.71
1.71	3.07
<i>CiVHPO</i>	
0.62	6.43
<i>AmVHPOII</i>	
0.90	11.7

The specific activity of *AmVHPO* and *AmVHPOII* were determined at pH 6.0 (MES buffer), the specific activity of *CiVHPO* at pH 5.0 (MES buffer).

3. Quantitative Assays (C. J. Seel, A. Králík)

3.1 MCD Assay^[7] (C. J. Seel)

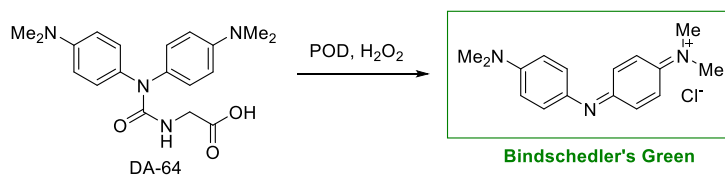


A 1 mM stock solution of MCD was prepared in 2 M aqueous NaOAc. Assays were performed in triplicate in 96-well polystyrene microplates (Brand® F-bottom, UV-transparent, pureGrade™). For initiation, H₂O₂ (30 µL, 10 mM final) was added to the assay mixture (270 µL), containing MES buffer (50 mM, pH 6.0), MCD (50 µM), KBr (200 mM) or KCl (200 mM), Na₃VO₄ (1 µM) and VHPO (1 µL of the purification batch or dilutions thereof) in an overall volume of 300 µL. The decrease in absorbance at 290 nm was monitored continually while keeping the temperature at 30°C and mixing at 410 rpm. The specific activity of each purification batch was calculated from the decrease in absorbance A₂₉₀ using the equation:

$$\text{specific activity } [\mu\text{mol MCD}/(\text{mg enzyme} \cdot \text{min})] = \frac{\Delta A_{290} [\text{s}^{-1}] \cdot 60 \cdot V_{\text{assay}} [\text{mL}]}{\epsilon_{\text{MCD}} \cdot d \cdot V_{\text{enzyme}} [\text{mL}] \cdot c_{\text{enzyme}} [\text{mg mL}^{-1}]}$$

with $\epsilon_{\text{MCD}} = 19.9 \text{ mL } \mu\text{mol}^{-1} \text{ cm}^{-1}$ and $d = 0.91 \text{ cm}$

3.2 Horseradish Peroxidase Assay for H₂O₂ Quantification (POD Assay) (C. J. Seel, A. Králík)



In order to quantify H₂O₂ concentrations, a photometric assay based on the dye DA-64 was applied. In the presence of hydrogen peroxide, the colorless dye is converted to Bindschedler's Green ($\lambda_{\text{max}} = 727 \text{ nm}$) in a reaction catalyzed by the enzyme horseradish peroxidase (POD).^[8]

A freshly pre-mixed working solution (250 µL; DA-64 1 mM (higher H₂O₂ concentrations) or 0.1 mM (lower H₂O₂ concentrations); POD 0.4 U mL⁻¹ (approx. 0.04 U mL⁻¹ for higher H₂O₂ concentrations); MES buffer pH 6.0, 50 mM) was added to the hydrogen-peroxide-containing probe or dilutions thereof (250 µL). The resulting assay solution was then incubated at 37°C for 30 min, and absorbance of the solution at 727 nm was recorded.

For quantification, a calibration curve was established by conducting the assay described above with a dilution series of defined concentrations of H₂O₂ (Figure S6).

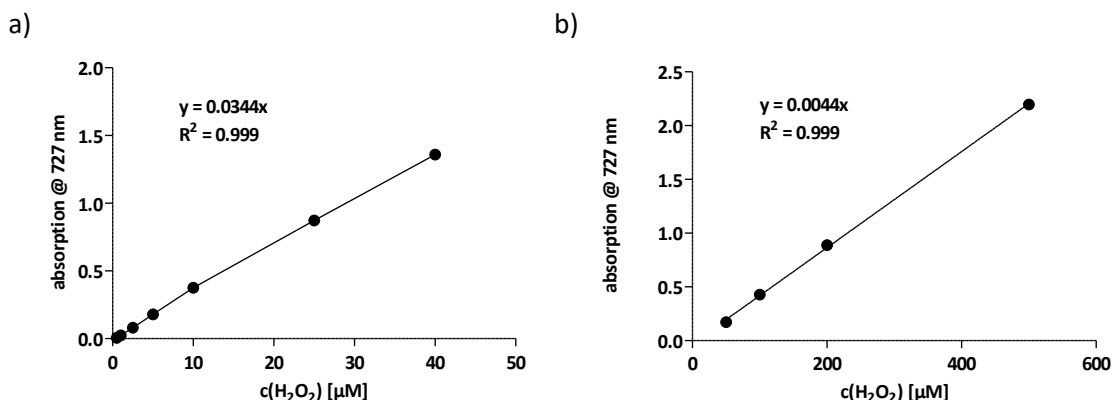


Figure S6. Calibration curve for a quantification of H_2O_2 concentrations using the POD enzymatic assay. a) Calibration for lower concentrations ranging from 0–40 μM (using 0.1 mM solution of DA-64). b) Calibration for higher concentrations ranging from 50–500 μM (using 1 mM solution of DA-64).

3.3 Kinetics of H_2O_2 Generation in MES Buffer Using FMN as Photocatalyst (A. Králík)

The reaction setup analogous to previously described photobiocatalytic reactions was used (see Section 4.2). An aqueous solution (1.6 mL) containing FMN (37.15 μM , corresponds to 0.5 mol% with regard to the substrate as described in Section 4.2) and MES buffer (100 mM, pH 6.0) in an open 5 mL crimp-top vial was irradiated with 455 nm LED for a selected amount of time. Afterwards, the reaction mixture was diluted 50-times and analyzed by the POD assay as described in Section 3.2. In parallel, we investigated if the presence of KBr influences the FMN-mediated photocatalysis (the kinetic experiment was performed as described above without KBr). No difference in the hydrogen-peroxide-production rate was observed.

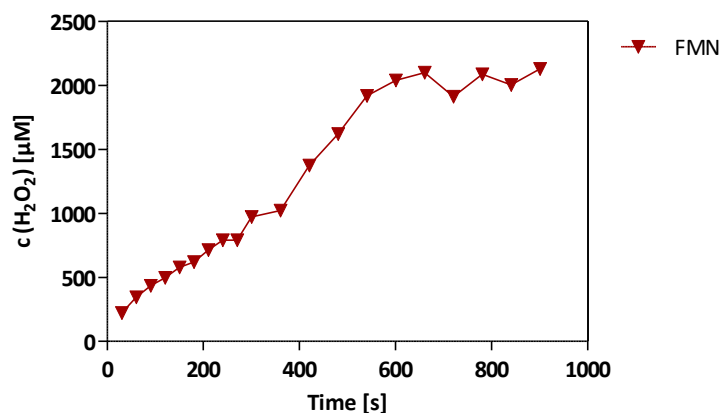


Figure S7. Kinetic of the H_2O_2 evolution by FMN in a MES-buffered solution. (cf. Figure 2, main text).

3.4 Kinetic Measurements of the O₂-Consumption (A. Králík)

To confirm the crucial role of oxygen in flavin-catalyzed hydrogen-peroxide evolution, the change of the oxygen concentration in an aqueous FMN solution under irradiation with blue light in a sealed vial was monitored with an oxygen probe.

A screw-top vial (4.5-cm-high) equipped with a phosphorescent dot for oxygen sensing and a stirring bar filled to the top with an aqueous solution of FMN ($37.15\ \mu\text{M} \pm 0.5\ \text{mol}\%$; $371.5\ \mu\text{M} \pm 5\ \text{mol}\%$; $743\ \mu\text{M} \pm 10\ \text{mol}\%$; $2.23\ \text{mM} \pm 30\ \text{mol}\%$) and MES buffer (100 mM, pH 6) was placed on a magnetic stirrer above a single 455 nm LED equipped with a glass rod. The solution was constantly stirred and after one minute of oxygen-concentration measurement, the light was switched on (black arrow in Figure S8) and the sample was irradiated for 4 min.

The results of these experiments can be found in Figure S8 confirming that oxygen is consumed during this reaction. The kinetic profiles were measured for all FMN concentrations and showed comparable behavior. The calculated light-penetration depth for a $37.15\ \mu\text{M}$ (0.5 mol%) solution of FMN in 100 mM MES buffer at 455 nm is 2.66 cm. These findings suggest that all light has been absorbed, indicating that the rate-determining factor of this reaction under given conditions is the intensity of the used light source.

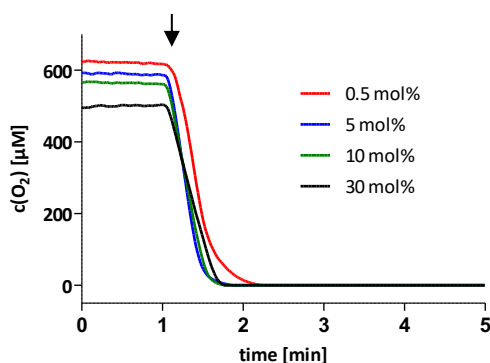


Figure S8. Change in oxygen concentration in an aqueous solution containing MES buffer and different concentrations of FMN under blue-light irradiation.

4. Photobiocatalytic Reactions

4.1 Photochemical Set-Up



Figure S9. a) Image of the photocatalytic set-up combined with a horizontal shaker. b) Schematic image of the set-up including cooling block and LED array.^[9]

FMN-catalyzed photobiocatalytic reactions were performed using 455 nm LEDs. Reaction vials (5 mL crimp-cap vials with loosely attached cap to allow oxygen supply) were illuminated from the bottom and cooled from the side using a custom-made aluminum cooling block connected to cooling-water supply. A horizontal shaker was placed below the LED array to allow for homogeneous mixing without mechanically disrupting the enzyme.

4.2 General Procedure (*C. J. Seel, M. Hacker*)

The enzymatic reactions were performed as described below and terminated by the addition of sat. NaCl solution. EtOAc (300 μ L) and 100 μ L of a stock solution of internal standard (0.06 M phenol for HPLC; 0.06 M dodecane or toluene for GC analysis) were added, the mixture was extracted with EtOAc and the organic phase was analyzed (dilution 1/10 for HPLC analysis). For NMR analysis, the mixture was extracted with EtOAc (3 $\times$ 2 mL), the organic extract was dried over MgSO₄, filtered and concentrated under reduced pressure. Subsequently, the internal standard (1,4-dibromobenzene) was added to the crude material and spectra were recorded in CDCl₃. The reaction scale was adjusted for the determination of the conversion by NMR: **1j** (25 μ mol); **1k** (12 μ mol for bromination, 7.5 μ mol for chlorination (0.5 mol% FMN), 3.7 μ mol for chlorination (H₂O₂)) and **1m** (23 μ mol).

Comparative Reactions with H₂O₂ (1.1 eq.)

The reaction mixture containing substrate (7.43 mM; 11.89 μ mol), KBr (1.1 eq; 8.13 mM or 2.2 eq; 16.26 mM), MES pH 6.0 (50 mM), Na₃VO₄ (187.5 μ M), H₂O₂ (1.1 eq; 8.13 mM or 2.2 eq; 16.26 mM) and VHPO (300 μ L, concentrations indicated) in MeCN/H₂O (1/1; total volume of 1.6 mL) was heated to 30°C and mixed at 300 rpm for 16 h.

Reactions with 0.5 mol% FMN in MES buffer

The reaction mixture in MeCN/H₂O (1/1; total volume of 1.6 mL) containing FMN (37.15 μ M $\pm$ 0.5 mol%), substrate (7.43 mM; 11.89 μ mol), KBr (1.1 eq; 8.13 mM), MES pH 6.0 (100 mM), Na₃VO₄ (187.5 μ M) and VHPO (300 μ L, concentrations indicated) was irradiated for 16 h at rt.

Reactions with 10 – 30 mol% FMN, 1.1 eq. KBr in MES buffer

Aliquots of a stock solution of FMN in water (11.89 mM; 100 μ L aliquot $\pm$ 10 mol% etc.) were added to an aqueous mixture (total volume of 1.45 mL) containing MeCN (800 μ L), substrate (11.89 μ mol), KBr (1.1 eq; 8.98 mM), MES pH 6.0 (110.6 mM), Na₃VO₄ (207.3 μ M) and VHPO (300 μ L, concentrations indicated).

Reactions with 10 – 30 mol% FMN, 2.2 eq. KBr in MES buffer

Aliquots of a stock solution of FMN in water (11.89 mM; 100 μ L aliquot $\pm$ 10 mol% etc.) were added to an aqueous mixture (total volume of 1.46 mL) containing MeCN (800 μ L), substrate (11.89 μ mol), KBr (2.2 eq; 17.81 mM), MES pH 6.0 (109.5 mM), Na₃VO₄ (205.5 μ M) and VHPO (300 μ L, concentrations indicated).

Method A: Addition of 10 – 30 mol% FMN and irradiation for 1 – 2 h in the presence of enzyme (300 μ L), total reaction time 16 – 72h.

Method B: Addition of 15 mol% FMN and irradiation for 30 min; after addition of enzyme (150 μ L) 30 min without irradiation; addition of 15 mol% FMN and irradiation for 30 min; subsequently addition of enzyme (150 μ L), total reaction time 16 h.

Method C: Addition of 5 mol% FMN every 30 min (3 h irradiation), total reaction time 16 h.

Method D: Addition of 30 mol% FMN (1 h irradiation), then addition of AmVHPO, total reaction time 16 h.

Method E: Addition of 15 mol% FMN and irradiation for 30 min; addition of 15 mol% FMN and irradiation for 30 min; subsequently addition of the substrate and enzyme (300 μ L).

Method F: Addition of 15 mol% FMN and irradiation for 30 min; then addition of the substrate and enzyme (300 μ L).

Method G (optimized procedure used for oxidation-sensitive substrates): Irradiation of 15 mol% FMN in MES-buffered solution for 15 min; addition of 15 mol% FMN and irradiation for further 15 min; subsequently addition of the substrate, enzyme (300 μ L) and all other components of the mixture.

4.3 GC and HPLC Measurements (C. J. Seel, A. Králík)

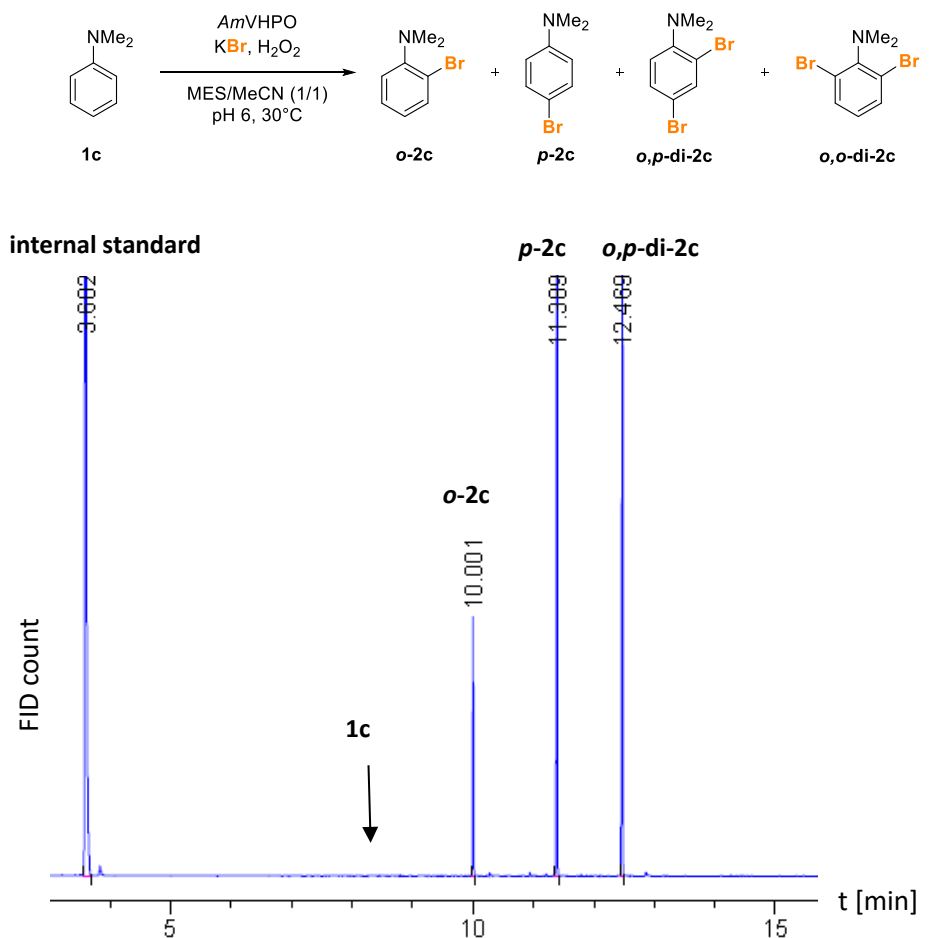


Figure S10. Example of a GC chromatogram of the enzymatic *N,N*-dimethylaniline (**1c**) bromination. The chemical structures of **o-2c**, **p-2c** and **o,p-di-2c** were confirmed by authentic references. Toluene was used as the internal standard. The formation of the dibrominated product **o,o-di-2c** was not observed.

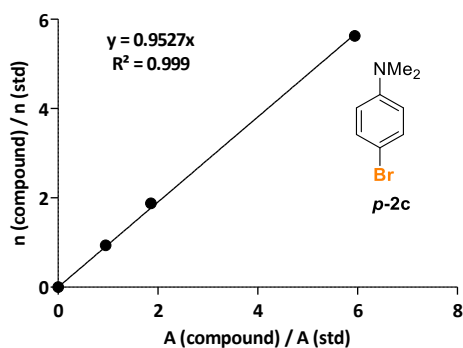
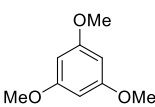
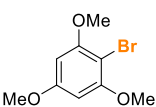
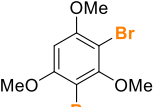
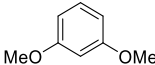
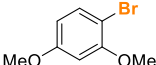
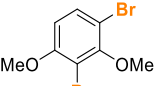
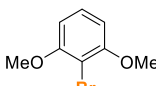
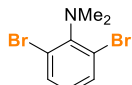
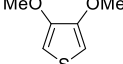
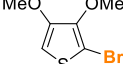
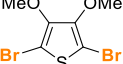


Figure S11. Example of a calibration curve using brominated reference material.

4.4 Overview of the Halogenated Products and Retention Times (C. J. Seel)

GC(1) measurements were carried out on a HP 6890 Series GC instrument (Agilent, equipped with a FID detector and a HP-5 capillary column [length = 29.5 m]). Hydrogen was used as the carrier gas and the constant-flow mode (flow rate = 1.8 mL min⁻¹) with a split ratio of 1:20. The following temperature-program was used: 60°C for 3 min, 15°C min⁻¹ to 250°C in 12 min, and 250°C for 5 min. Dodecane was used as internal standard. **GC(2)** measurements were carried out on a HP 6890 Series GC instrument (Agilent, equipped with a FID detector and a HP-5 capillary column [length = 30.0 m]). Helium was used as the carrier gas and the constant-flow mode (flow rate = 1.0 mL min⁻¹) with a split ratio of 1:40. The following temperature-program was used: 40°C for 3 min, 15°C min⁻¹ to 280°C in 16 min, and 280°C for 5 min. Dodecane was used as internal standard. **GC(3)** measurements were carried out on a HP 6890 Series GC instrument (Agilent, equipped with a FID detector and a HP-5 capillary column [length = 30.0 m]). Helium was used as the carrier gas and the constant-flow mode (flow rate = 1.0 mL min⁻¹) with a split ratio of 1:40. The following temperature-program was used: 40°C for 3 min, 15°C min⁻¹ to 280°C in 16 min, and 280°C for 5 min. Toluene was used as internal standard. High performance liquid chromatography (**HPLC**) analysis was performed on a HITACHI Chromaster system employing the following conditions: 150 x 4 mm Eurospher II (KNAUER®), 100 Å, 5 µm, C18, 25°C, flow rate: 1.0 mL min⁻¹, water (+0.01% TFA)/MeCN (+0.01% TFA) gradient: 90/10 → 0/100 in 13 min and phenol as the internal standard.

					
1a	2a	di-2a			
HPLC: 10.3 min	10.8 min	12.1 min			
					
1b	2b	di-2b	regio-2b		
HPLC: 10.2 min	11.9 min	12.7 min	10.7 min (<5 %)		
					
1c	o-2c	p-2c	o,p-di-2c	o,o-di-2c	
GC(3): 8.3 min	10.0 min	11.4 min	12.5 min	not observed	
					
1d	2d	di-2d			
HPLC: 8.9 min	11.3 min	13.5 min			

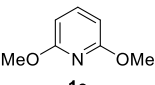
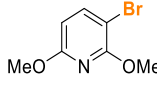
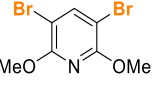
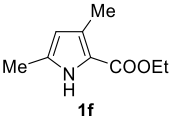
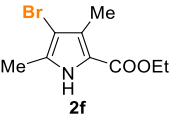
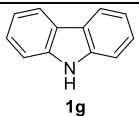
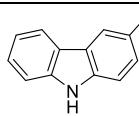
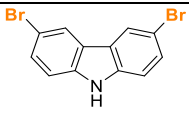
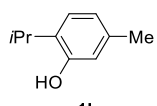
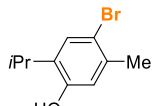
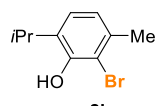
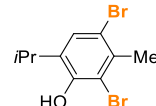
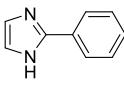
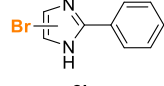
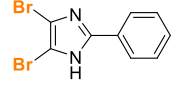
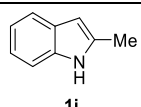
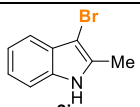
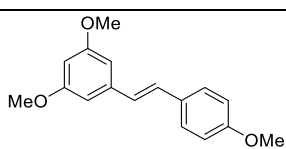
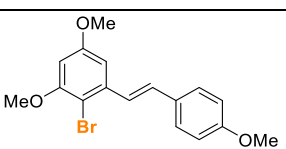
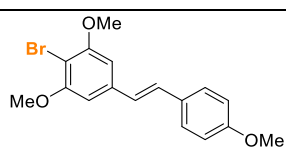
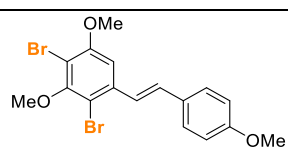
			
HPLC: 10.2 min	12.2 min	13.7 min	
			
HPLC: 10.1 min	11.9 min		
			
GC(2): 14.8 min	17.2 min	19.3 min	
			
GC(1): 8.6 min	11.2 min	9.8 min	12.0 min
			
GC(1): 9.0 min	13.8 min	14.9 min	
			
NMR: 6.22 (s, 1H)	7.47 (m, 1H)		
			
NMR: 6.65 (d, 2H)	6.42 (d, 2H)	6.70 (s, 2H) <5% in all cases	not observed

Figure S12. Substrates and brominated products. The retention times (GC) and selected signals for determination of the conversion by NMR are shown below.

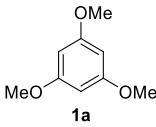
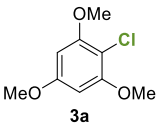
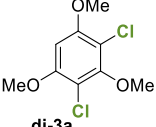
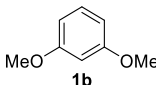
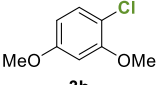
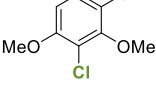
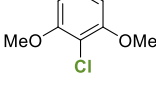
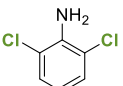
				
1a	3a	di-3a		
HPLC: 10.3 min	10.6 min	not observed		
				
1b	3b	di-3b	regio-3b	
HPLC: 10.2 min	11.0 min	not observed	not observed	
				
1l	o-3c	p-3c	o,o-di-3c	o,p-di-3c
GC(1): 5.0 min	6.9 min	7.8 min	not observed	not observed

Figure S13. Substrates and chlorinated products. The retention times (HPLC or GC) are shown below.

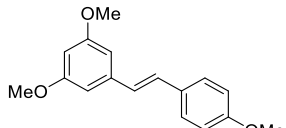
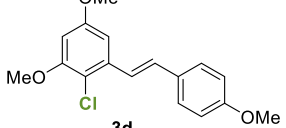
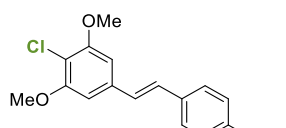
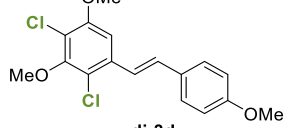
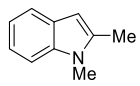
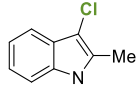
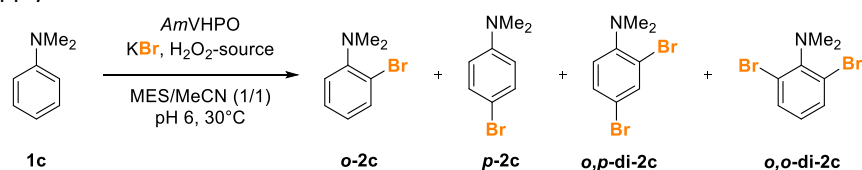
			
1k	3d	regio-3d	di-3d
NMR: 7.47-7.43 (m, 2H)	7.41 (d, 1H)	not observed	not observed
			
1m	3e		
NMR: 6.22 (s, 1H)	7.47 (m, 1H)		

Figure S14. Substrates and chlorinated products. The signals for determination of the conversion by NMR are shown below.

4.5 Bromination of *N,N*-Dimethylaniline Using Different Methods for H₂O₂ Supply (*C. J. Seel*)

Table S2. Enzymatic bromination of *N,N*-dimethylaniline (**1c**) as a model reaction to investigate the impact of different H₂O₂-supply methods.



Entry	H ₂ O ₂ supply	Conversion	Ratio (<i>o</i> -2c : <i>p</i> -2c : <i>o,p</i> -di-2c)
1	Syringe pump	81%	24 : 70 : 6
2	GOx (12 U)	10%	13 : 87 : 0
3	GOx (24 U)	18%	13 : 87 : 0
4	Urea H ₂ O ₂	64%	16 : 84 : 0
5	H ₂ O ₂ (1.1 eq.)	84%	19 : 81 : 0
6 ^a	H ₂ O ₂ (2.2 eq.)	100%	14 : 50 : 36
7	<i>t</i> BuOH one batch H ₂ O ₂	69%	25 : 63 : 12

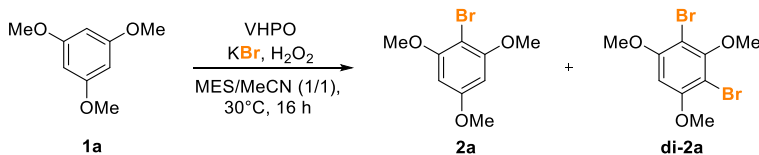
*Am*VHPO (3.0 mg mL⁻¹; 300 μL); reaction time 72 h; the formation of *o,o*-di-2c was not observed. ^aKBr (2.2 eq.)

Procedure

The reactions were performed as described in 4.2 using *Am*VHPO (300 μL $\triangleq$ ca. 6 U) pre-incubated with Na₃VO₄ (30 mM; 300 μL). The total reaction volume was 1.6 mL (MeCN/H₂O : 1/1; for Entry 7 *t*BuOH/H₂O : 1/1). The different supply methods were carried out as follows: slow supply with syringe pump (1.1 eq. H₂O₂ at a flow-rate of 0.72 μL/min, total time 3h); slow release from urea H₂O₂ (1.1 eq.; 100 mM stock solution in water, 130 μL) or addition of D-glucose (212 μL; 10 mg mL⁻¹; 1.0 eq.) and glucose oxidase (GOx was pre-weighed and calculated according to the U mg⁻¹ declaration of the supplier).

4.6 Investigations on Different Enzymes, Halides and Applicability of Lyophilized Lysate (C. J. Seel)

Table S3. Ratios and conversions of the enzymatic bromination of 1,3,5-trimethoxybenzene (**1a**).

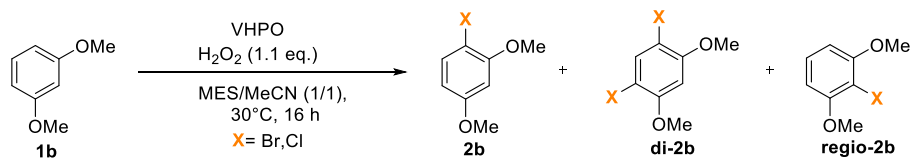


Entry	Enzyme	H ₂ O ₂ source	Conversion	Ratio (2a : di-2a)
1^a	<i>CIVCPO</i>	FMN/455 nm	87%	100 : 0
2	<i>CIVCPO</i>	H ₂ O ₂ (1.1 eq.)	40%	77 : 23
3^b	<i>AmVHPO</i>	H ₂ O ₂ (1.1 eq.)	94%	92 : 8
4	<i>AmVHPO</i>	H ₂ O ₂ (1.1 eq.)	75%	99 : 1
5^c	<i>AmVHPO</i> (lysate)	H ₂ O ₂ (1.1 eq.)	96%	94 : 6

AmVHPO (1.46 mg mL⁻¹; 300 μ L); *CIVHPO* (0.62 mg mL⁻¹; 300 μ L).^aFMN (30 mol%); method A; ^baddition of 30 mol% FMN to the reaction mixture, no irradiation and addition of H₂O₂ as oxidant; ^clyophilized lysate (10 mg).

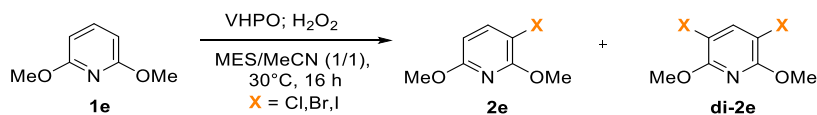
The control reaction (entry 3) indicated that the presence of FMN in the absence of light does not inactivate the enzyme.

Table S4. Ratios and conversions of the enzymatic halogenation of 1,3-dimethoxybenzene (**1b**).



Entry	Enzyme	Halogen Source	Conversion	Ratio (2b : di-2b : regio-2b)
1	<i>CIVCPO</i>	KBr	45%	100 : 0 : 0
2	<i>AmVHPO</i> (lysate)	KBr	73%	98 : 2 : 0

CIVCPO (0.62 mg mL⁻¹; 300 μ L); lyophilized lysate (10 mg).

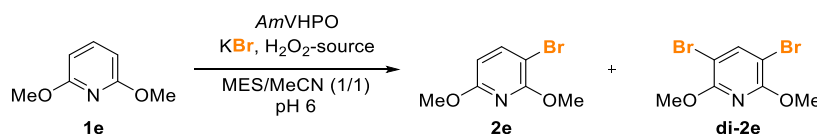
Table S5. Ratios and conversions of the enzymatic halogenation of 2,6-dimethoxypyridine (**1e**).

Entry	Enzyme	Halogen/ H_2O_2 Source	Conversion	Ratio (2e : di-2e)
1^a	<i>AmVHPOII</i>	KBr; H_2O_2 (1.1 eq.)	8%	100 : 0
2^b	<i>CIVCPO</i>	KBr; H_2O_2 (1.1 eq.)	31%	100 : 0
3^a	<i>CIVCPO</i>	KBr; H_2O_2 (1.1 eq.)	38%	100 : 0
4^a	<i>CIVCPO</i>	KBr; FMN (5 mol%)/455 nm	11%	100 : 0
5^a	<i>CIVCPO</i>	KBr; FMN (10 mol%)/455 nm	—	—
6^b	<i>CIVCPO</i>	KCl; H_2O_2 (1.1 eq.)	trace	<5 : 0
7	<i>AmVHPO</i> (lysate)	KBr; H_2O_2 (1.1 eq.)	14%	—
8	<i>AmVHPO</i> (lysate)	KI; H_2O_2 (1.1 eq.)	—	—
9^a	<i>AmVHPO</i>	KI; H_2O_2 (1.1 eq.)	—	—

AmVHPOII (0.9 mg mL⁻¹); *CIVCPO* (0.62 mg mL⁻¹); *AmVHPO* (4.5 mg mL⁻¹); lyophilized lysate (10 mg); ^a300 μL of enzyme; ^b100 μL of enzyme; entries 5 and 6: method A (1 h irradiation); total reaction time 16 h.

4.7 Optimization of Catalyst Loading and Investigations on the Inactivation of the Enzyme (C. J. Seel)

Table S6. Optimization of the Reaction conditions.



Entry	H_2O_2 -source	Conditions	Conversion	Ratio (2e : di-2e)
1^a	RFTA (30 mol%)/455 nm	method C (3 h)	2%	100 : 0
2^a	FMN (30 mol%)/455 nm	method C (3 h)	4%	100 : 0
3^a	H_2O_2 (1.1 eq.)	16 h, 30°C	53%	100 : 0
4^a	RFTA (30 mol%)/455 nm	method A (1 h)	1%	100 : 0
5^a	FMN (30 mol%)/455 nm	method A (1 h)	1%	100 : 0
6^a	H_2O_2 (1.1 eq.)	16 h, 30°C	36%	100 : 0
7^a	FMN (30 mol%)/455 nm	method D (1 h)	28%	100 : 0
8^a	RFTA (30 mol%)/455 nm	method D (1 h),	23%	100 : 0
9^a	H_2O_2 (1.1 eq.)	16 h, 30°C	57%	100 : 0
10^b	H_2O_2 (1.1 eq.)	irradiation of <i>AmVHPO</i> for 1 h, then addition of H_2O_2	64%	100 : 0
11^b	H_2O_2 (1.1 eq.)	16 h, 30°C	45%	100 : 0
12^{b,c}	FMN (30 mol%)/455 nm	irradiation in the presence of <i>AmVHPO</i> (16 h); Brj35 [®]	1%	100 : 0
13^{b,c}	H_2O_2 (1.1 eq.)	16 h, 30°C, Brj35 [®]	49%	100 : 0

^a*AmVHPO* (1.50 mg mL⁻¹; 300 μ L); ^b*AmVHPO* (2.90 mg mL⁻¹; 300 μ L); ^cBrj35[®] (11.89 μ mol; 14.45 mg; 1.1 eq.) as stabilizing additive for the enzyme.

The control reaction (Entry 10) indicated that irradiation of the enzyme in the absence of FMN does not inactivate the enzyme.

H_2O_2 (1.1 eq) is listed several times, because a control experiment was run in parallel for each set of experiments (indicated by the lines within the table).

We started our investigations with a catalyst loading of 10 to 30 mol% FMN and reached complete conversion in the enzymatic bromination of 1,3,5-trimethoxybenzene (**1a**) using *AmVHPO* (Table S7, entry 6). When turning to other (hetero-)aromatic substrates, however, the conversion dropped dramatically (Table S6, entry 4 and 5). We closely investigated the factors leading to this diminished conversion. A protocol, where H_2O_2 was pre-generated by irradiating FMN and subsequently adding the enzyme, lead to an improved turnover (Table S6, entry 7). Neither irradiation of the enzyme followed by addition of H_2O_2 (Table S6, entry 10) nor the presence of FMN without irradiation (Table S3, entry 3) influenced the

enzyme's activity indicating the high oxidative reactivity of excited FMN accounts for the inactivation of the enzyme.

Intrigued by the observed incomplete turnover of 2,6-dimethoxypyridine (**1e**), we further investigated the enzymatic bromination over time and observed a fast initial rate for both substrates, the electron-rich **1a** and the less electron-rich **1e** (Figure S15). The fast initial rate of the enzymatic bromination of **1e**, however, slows down after 30 min and a conversion of approximately 35% is not exceeded even after 16 h (neither for direct addition of the oxidant nor for *in situ* H₂O₂ generation). Both VHPOs, CVHPO and AmVHPOII showed the same incomplete turnover of **1e** as observed for AmVHPO (Table S5). Additional experiments with AmVHPO on **1e** were performed, during which a second aliquot of enzyme was added after 30 min (conversion 34%), or 10% of product **2e** were included in the reaction mixture right from the start (conversion 38%) to analyze whether product inhibition or equilibrium formation accounts for the stagnation of the conversion. These experiments, however, did not show significantly improved conversion compared to a reaction time of 30 min and addition of the enzyme in the beginning (33%). These results again address the so far unanswered question of the nature of the electrophilic halogenating species generated by VHPOs. Early work by Butler and co-workers^[10] suggested substrate binding rather than freely diffusible oxidized bromine species based on fluorescence quenching as well as based on competitive kinetic experiments. To date, no VHPO crystal structure in complex with a substrate has been reported and the selectivity of VHPOs is still under debate.

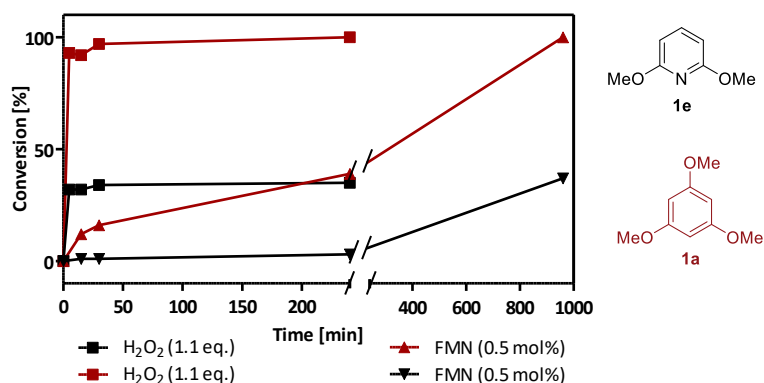


Figure S15. Kinetic monitoring of enzymatic brominations of 2,6-dimethoxypyridine (**1e**) and 1,3,5-trimethoxybenzene (**1a**). Individual reactions were run in parallel and quenched for analysis after a certain time. The total reaction time for addition of H₂O₂ in one batch was 4 h and for *in situ* H₂O₂ generation (0.5 mol% FMN) 16 h. The enzymatic reactions were performed using AmVHPO (1.47 mg mL⁻¹; 300 μL).

The productivity of an enzyme can be directly compared when calculating the turnover number and turnover frequency:

$$\text{turnover number (TN)} = \frac{\text{mol (product)}}{\text{mol (catalyst)}} [\text{mol} \cdot \text{mol}^{-1}]$$

$$\text{turnover frequency (TF)} = \frac{\text{TN}}{\text{time}} [\text{h}^{-1}]$$

For substrate **1a** a conversion of 93% (H₂O₂ addition in one batch) after 5 min was observed, this corresponds to a TN of 1833 and TF of 21996 h⁻¹. The TN (631) and TF (7572 h⁻¹) for substrate **1e** is significantly lower (32% conversion after 5 min), indicating that the electronic properties of the substrate predominantly influence the TN. The turnover for the photobiocatalytic protocol is reduced due to the slow release of H₂O₂, the maximal productivity of the enzyme can thus not be deduced from these experiments.

Table S7. Investigations on the applicability of FMN and RFTA using 1,3,5-trimethoxybenzene (**1a**) as a substrate.

Reaction scheme: 1,3,5-trimethoxybenzene (**1a**) reacts with AmVHPO, KBr, and H₂O₂-source in MES/MeCN (1/1) at pH 6 to produce 2-bromo-1,3,5-trimethoxybenzene (**2a**) and 3,5-dibromo-1,3,5-trimethoxybenzene (**di-2a**).

Entry	RFTA (mol%)	Time	Conversion	Ratio (2a : di-2a)
1^b	20	72 h, method A (2h)	—	—
2^c	H ₂ O ₂ (1.1 eq.)	72 h	34%	66 : 35
3^c	10	16 h, method A (1h)	29%	100 : 0
4^{c,e}	20	48 h	48%	100 : 0

Entry	FMN (mol%)	Time	Conversion	Ratio (2a : di-2a)
5^c	H ₂ O ₂ (1.1 eq.)	16 h	71%	75 : 25
6^c	10	16 h, method A (1h); shaking 300 rpm	93%	100 : 0
7^c	10	16 h, method A (1h); stirring	79%	100 : 0

The irradiation time is given in brackets. ^aAmVHPO (2.2 mg mL⁻¹; 300 μL); ^bno addition of enzyme; ^cAmVHPO (3.5 mg mL⁻¹; 300 μL); ^d2.2 eq. KBr; ^eaddition of FMN (10 mol%), 1h irradiation then 16 h at rt without irradiation followed by addition of FMN (10 mol%), 1h irradiation and another 16 h at rt.

Entry 1 shows that the presence of enzyme is essential for a successful halogenation.

As it can be seen from entries 6 and 7, the special set-up with horizontal shaker (shaking 300 rpm) is superior to mechanical stirring of the enzyme-containing mixture.

Table S8. Ratios and conversions of the enzymatic halogenation of 1,3-dimethoxybenzene (**1b**).

COc1cccc(OC)c1 **1b**
 $\xrightarrow[\text{MES/MeCN (1/1), pH 6}]{\text{AmVHPO; H}_2\text{O}_2}$
COc1cc(X)ccc(OC)c1 **2b** +
 COc1cc(X)cc(OC)c(X)c1 **di-2b** +
 COc1ccccc1OC **regio-2b**

X = Br, Cl

Entry	Time	RFTA (mol%)	Conversion	Ratio (2b : di-2b : regio-2b)
1	16 h	H ₂ O ₂ (1.1 eq.)	25%	92 : 8 : 0
2^a	16 h	10	9%	100 : 0 : 0

Entry	Time	FMN (mol%)	Conversion	Ratio (2b : di-2b : regio-2b)
3	16 h	H ₂ O ₂ (1.1 eq.)	75%	98 : 2 : 0
4^a	16 h	20	26%	98 : 2 : 0
5^{a,b}	16 h	20	26%	99 : 1 : 0

^amethod A, irradiation for 1 h; AmVHPO (300 μL, 3.5 mg mL⁻¹); ^b2.2 eq. KBr.

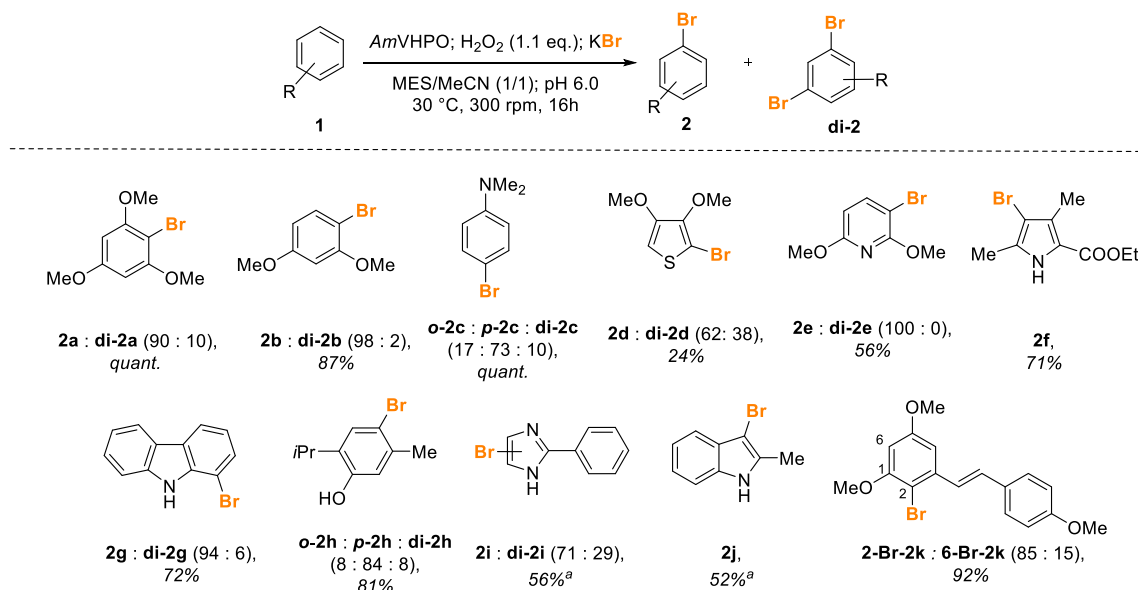
Table S9. Influence of the FMN concentration investigated for the photo-enzymatic bromination of thymol (**1h**).

CC1=C(C)C(O)C(C)C1 **1h**
 $\xrightarrow[\text{MES/MeCN (1/1), pH 6}]{\text{AmVHPO, KBr, H}_2\text{O}_2\text{-source}}$
CC1=C(C)C(O)C(Br)C1 **p-2h** +
 CC1=C(C)C(O)C(C)C1Br **o-2h** +
 CC1=C(C)C(O)C(Br)C1Br **di-2h**

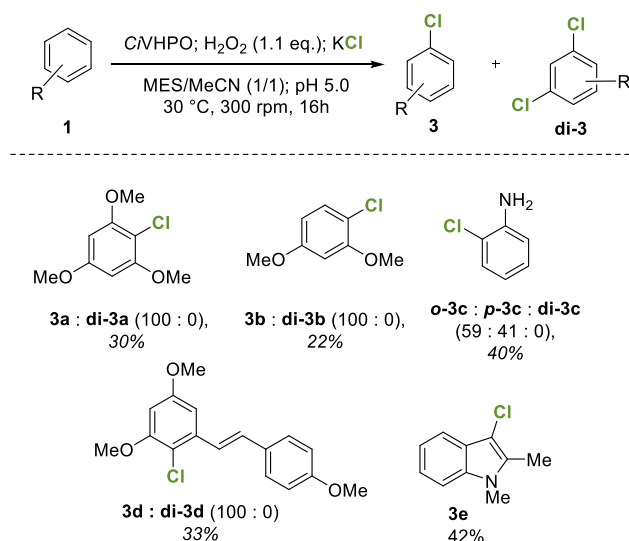
Entry	Time	FMN (mol%)	Conversion	Ratio (p-2h : o-2h : di-2h)
1^a	16 h	0.5	88%	88 : 10 : 2
2^b	16 h	1	29%	88 : 11 : 1
3^b	16 h	5	10%	88 : 12 : 0
4^c	16 h	10	17%	53 : 47 : 0
5^d	16 h	30	83%	88 : 10 : 2
6^e	16 h	30	73%	88 : 11 : 1
7	16 h	H ₂ O ₂ (1.1 eq.)	84%	87 : 11 : 2

^aIrradiation for 16 h; ^bmethod A, irradiation for 2 h; ^cMES/MeCN (9/1); ^dmethod B; ^emethod D; AmVHPO (300 μL; 1.46 mg mL⁻¹).

4.8 Comparative Reactions with H₂O₂ (1.1 eq.) (C. J. Seel)



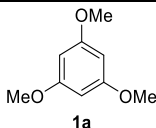
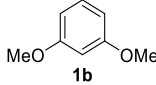
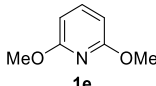
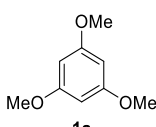
Scheme S1. Comparative enzymatic brominations with direct addition of the oxidant in one batch (1.1 eq. H₂O₂). Reactions contained MES buffer (100 mM, pH 6.0), [substrate] = 7.43 mM, [H₂O₂] = 8.13 mM, [AmVHPO] = 3.77 μM (1.47 mg mL⁻¹; 300 μL), [KBr] = 8.13 mM, [Na₃VO₄] = 187.5 μM and were run at rt. ^aAmVHPO (1.46 mg mL⁻¹; 300 μL). Conversions and ratios were determined by GC, HPLC or NMR. The respective major mono-brominated products are depicted.



Scheme S2. Comparative enzymatic chlorinations with direct addition of the oxidant in one batch (1.1 eq. H₂O₂). Reactions contained MES buffer (100 mM, pH 5.0), [substrate] = 7.43 mM, [H₂O₂] = 8.13 mM, [CVHPO] = 1.60 μM (0.62 mg mL⁻¹; 300 μL), [KCl] = 8.13 mM, [Na₃VO₄] = 187.5 μM and were run at rt. Conversions and ratios were determined by GC, HPLC or NMR. The respective major mono-chlorinated products are depicted.

4.9 Control Reactions Regarding FMN-Independent H₂O₂ Evolution (C. J. Seel, A. Králík)

Table S10. Control reactions without photocatalyst, enzyme, or H₂O₂, and dark reaction in MES buffer.

Entry	Substrate	Conditions	Conversion	Ratio (mono : di)
1	 1a	no FMN	60%	100 : 0
2		no AmVHPO	<5%	–
3	 1b	no FMN	<5%	–
4		no AmVHPO	<5%	–
5	 1e	no FMN	12%	100 : 0
6		no AmVHPO	0%	–
7	 1a	dark reaction FMN, AmVHPO	0%	–
8		AmVHPO, no H ₂ O ₂	0%	–
9		H ₂ O ₂	100%	96 : 4

AmVHPO (300 μ L; 1.71 mg mL⁻¹); FMN 0.5 mol%; irradiation for 16 h.

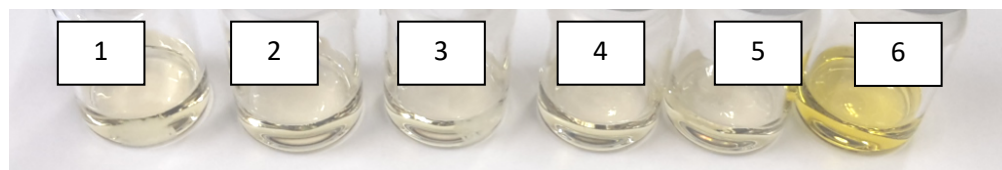


Figure S16. Color changes of the irradiated reaction mixtures after 16 h; the numbering corresponds to the entries of table S10. FMN-containing mixtures show photo-bleaching of the catalyst (entries 2, 4 and 6). A slight yellow discoloration was observed for substrate-containing mixtures (entries 1, 3 and 5).

Table S11. Control reactions using 1,3,5-trimethoxybenzene (**1a**) as a substrate in the absence of photocatalyst in phosphate buffer.

Entry	Light	KBr (8.13 mM)	Vanadate (187.5 μ M)	Conversion
1	455 nm	+	+	decomp.
2	–	+	+	0 %
3	455 nm	–	+	decomp.
4	455 nm	+	–	decomp.
5	455 nm	–	–	decomp.

trimethoxybenzene (7.43 mM); AmVHPO (80 μ L; 1.62 mg mL⁻¹); overall volume 1.6 mL; O₂ atmosphere; irradiation for 22 h.

Entries 1, 3, 4, 5 showed an overall GC yield below 5%. This suggests an oxidative decomposition of 1,3,5-trimethoxybenzene (**1a**) upon irradiation, **1a** likely serves as a source of electrons for the formation of

hydrogen peroxide. The lack of hydrogen peroxide in entries 1, and 3 of table S13 suggests decomposition of H_2O_2 under the given conditions.

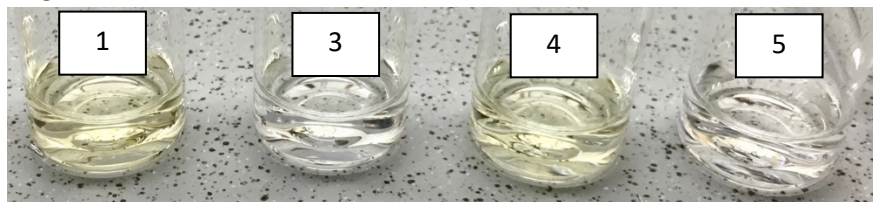


Figure S17. Color change of 1,3,5-trimethoxybenzene (**1a**) solutions after 22 hours of irradiation with blue light (originally all colorless solutions). The numbering corresponds to the entries in table S11.

Table S12. Investigation of the H_2O_2 evolution in a MES-buffered solution of 1,3,5-trimethoxybenzene (**1a**) in the absence of photo-catalyst.

Entry	Vanadate ^a	Substrate 1a	MeCN	c(H_2O_2) [mM]
1	+	+	+	4.59
2	+	–	+	0
3	–	+	+	6.72
4	+	–	–	0

trimethoxybenzene (7.43 mM); no AmVHPO; overall volume 1.6 mL; irradiation for 16 h; ^avanadate (187.5 μM) and vanadate containing storage buffer (300 μL , 50 mM Tris pH 7.0, 100 μM Na_3VO_4)

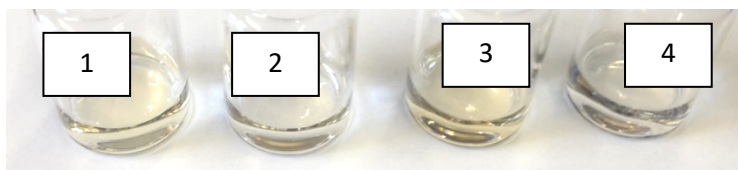


Figure S18. Color changes of the reaction mixtures; the numbering corresponds to the entries in table S12. A slight yellow discoloration is only observed for substrate-containing reaction mixtures (Entries 1 and 3).

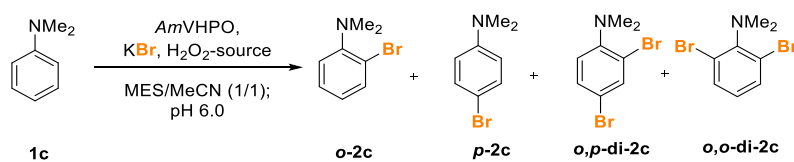
Table S13. Investigation of H_2O_2 evolution in 1,3,5-trimethoxybenzene (**1a**) solution in an absence of photocatalyst in phosphate buffer.

Entry	Light	KBr (8.13 mM)	Vanadate (187.5 μM)	c(H_2O_2) [mM]
1	455 nm	+	+	0
2	–	+	+	0
3	455 nm	–	+	0
4	455 nm	+	–	5.19
5	455 nm	–	–	6.19

trimethoxybenzene (7.43 mM); no AmVHPO; overall volume 1.6 mL; O_2 atmosphere; irradiation for 22 h.

4.10 Investigations on Decomposition of the Substrate by Photooxidation (*C. J. Seel*)

Table S14. Photobiocatalytic bromination of *N,N*-dimethylaniline (**1c**) as a model reaction to investigate the impact of photooxidation on oxidation-sensitive substrates/products.



Entry	H_2O_2 -source	Conversion	Ratio (<i>o</i> -2c : <i>p</i> -2c : <i>o,p</i> -di-2c)
1	H_2O_2 (1.1 eq.)	99%	23 (<i>o</i>) : 71 (<i>p</i>) : 6
2 ^a	FMN (30 mol%)/455 nm; (D)	99%	23 (<i>o</i>) : 43 (<i>p</i>) : 35
3 ^a	FMN (30 mol%)/455 nm; (B)	99%	13 (<i>o</i>) : 70 (<i>p</i>) : 17
4	FMN (30 mol%)/455 nm; (E)	1%	62 (<i>o</i>) : 38 (<i>p</i>) : 0
5	FMN (30 mol%)/455 nm; (F)	2%	54 (<i>o</i>) : 46 (<i>p</i>) : 0
6	FMN (30 mol%)/455 nm; (G)	98%	26 (<i>o</i>) : 63 (<i>p</i>) : 11

^adecomposition of the substrate/product(s) was observed; *AmVHPO* (300 μL ; 1.46 mg mL^{-1}); Methods B – G are described in chapter 4.2; conversions were determined by GC.

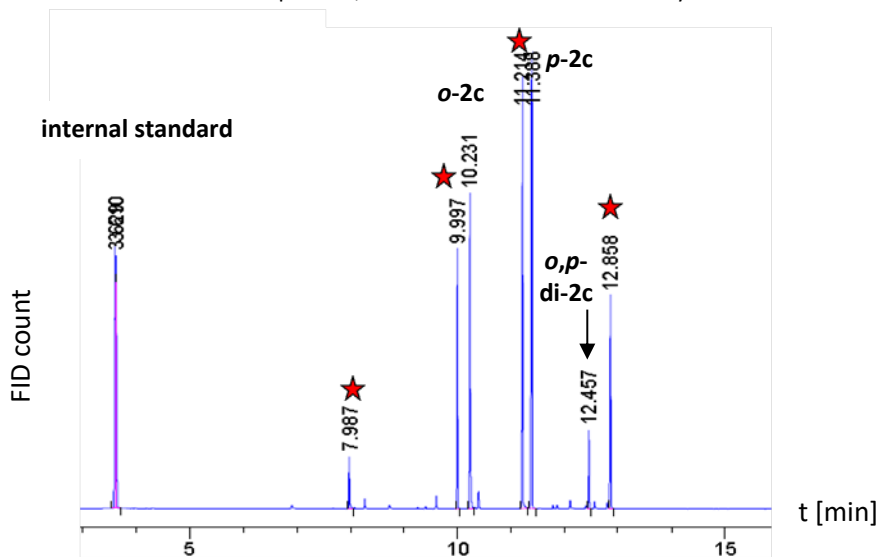
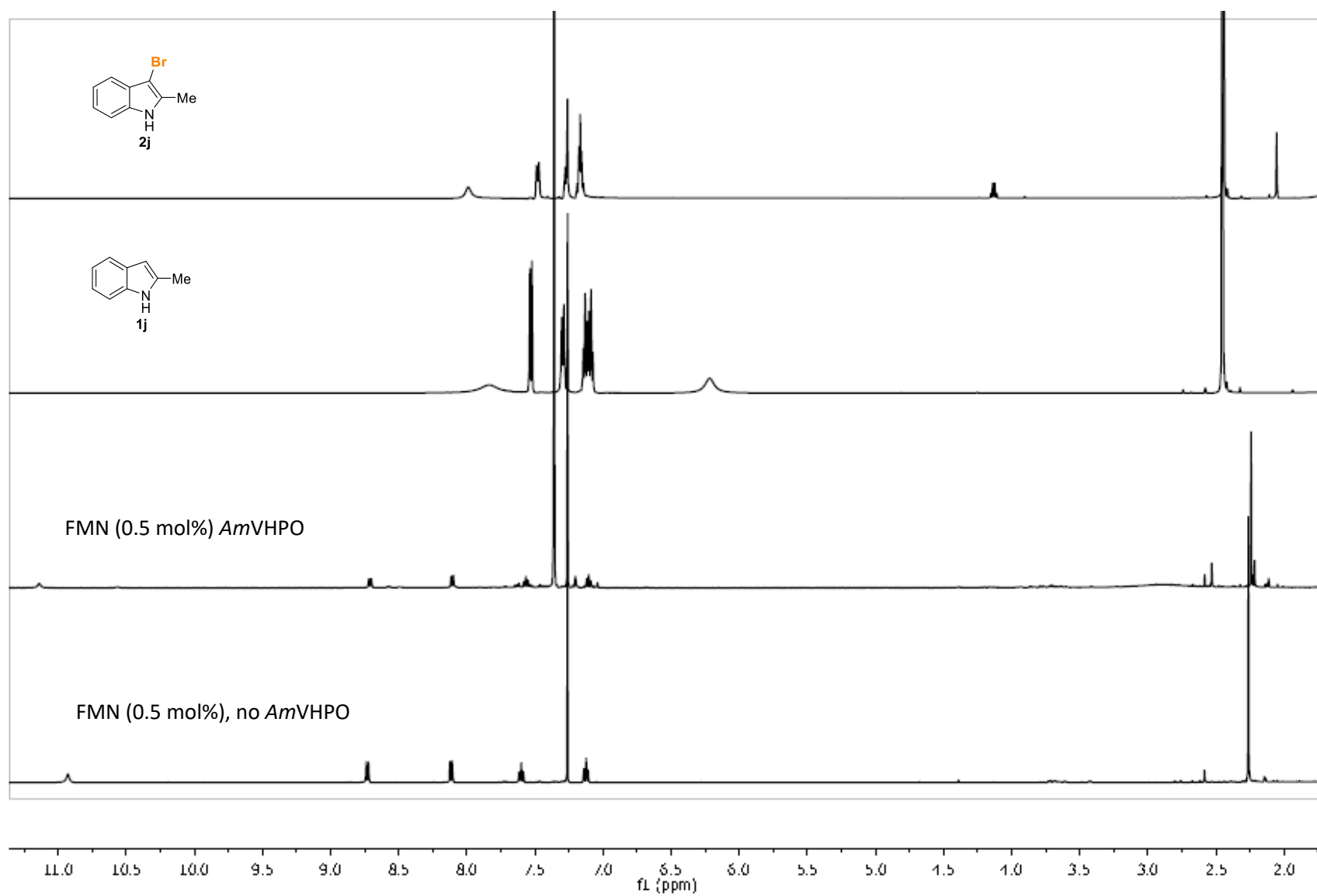


Figure S19. GC chromatogram of the enzymatic *N,N*-dimethylaniline (**1c**) bromination using photochemical *in situ* H_2O_2 generation (30 mol% FMN, entry 3). Toluene was used as the internal standard. The formation of product *o,o*-di-2c was not observed. The substrate is fully consumed, several peaks, which do not correspond to the products are observed indicating photochemical decomposition of the substrate/product(s) (labeled with an asterisk).

An additional example of photooxidation of the substrate is given below as comparative NMR spectra.



4.11 Investigations on the Buffer Concentration (C. J. Seel)

In order to examine the influence of MES-buffer concentrations lower than 100 mM on the pH change of the reaction mixture and the conversions of the enzymatic bromination reaction, we performed a series of experiments using the electron-source in lower concentrations. The reactions were performed as described in chapter 4.2 (0.5 mol% FMN, 16 h irradiation, **1a** as a substrate).

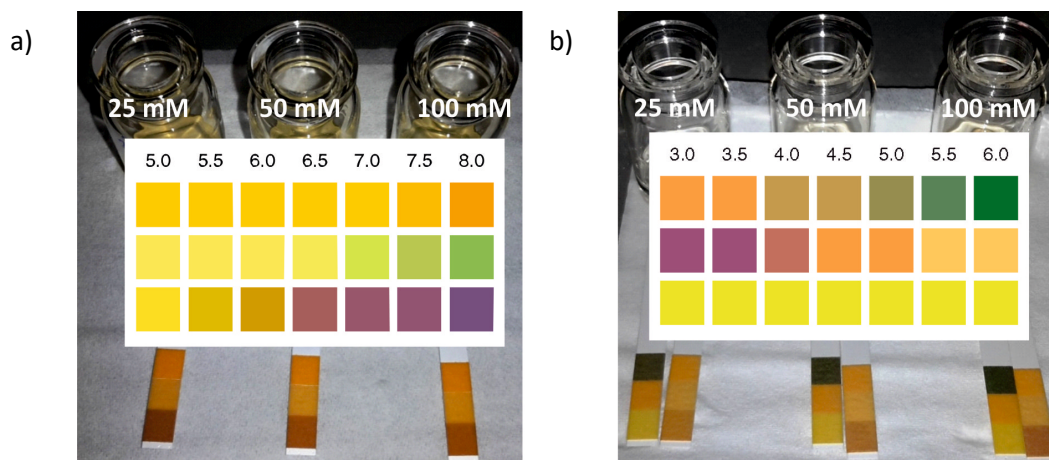
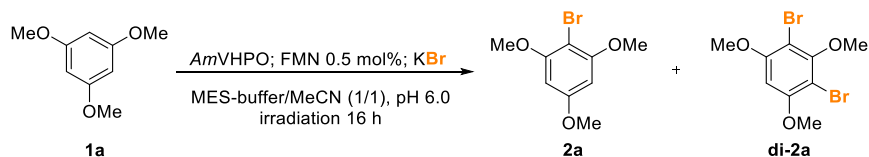


Figure S20. Monitoring of the pH of the reaction mixtures (0.5 mol% FMN, summarized in Table S15). a) The pH was determined before irradiating the reaction mixture by the use of pH-indicator strips (MColorpHast™, pH 5.0 – 10.0, MerckMilipore). b) The pH was determined again after 16 h of irradiation (MColorpHast™, pH 5.0 – 10.0 and pH 0 – 6.0, MerckMilipore).

The pH was monitored prior to irradiation and after completion of the reaction and neither a significant pH change (Figure S20) nor an influence on the conversion (Table S15) of the substrate was observed for the reaction of **1a** using of 25 – 100 mM MES as electron donor.

Table S15. Influence of the buffer concentration on the photobiocatalytic bromination of 1,3,5-trimethoxybenzene (**1a**).



Entry	MES	pH (start)	pH (end)	Conversion	Ratio (2a : di-2a)
1	25 mM	6.0	5.5 - 6.0	99%	92 : 8
2	50 mM	6.0	6.0	99%	93 : 7
3	100 mM	6.0	6.0	98%	95 : 5

AmVHPO (2.28 mg mL⁻¹; 300 μL)

5. Flavin Properties (C. J. Seel, A. Králík)

5.1 Degradation of the Enzyme by Excited FMN in High Catalyst Loadings (C. J. Seel)

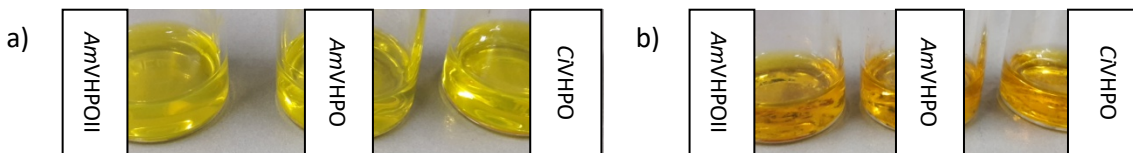


Figure S21. Impact of photooxidation on the enzymes, the mixtures contained FMN (30 mol%). a) Reaction mixtures before irradiation, the VHPOs are soluble. b) Reaction mixtures after irradiation for 15 min; FMN (30 mol%) showing obvious precipitation of the enzymes.

5.2 Self-Oxidation of FMN (A. Králík)

When irradiated under inert nitrogen atmosphere in a non-oxidizable buffer without any other source of electrons, FMN is capable of self-oxidation forming a small portion of the reduced form of flavin, which can release hydrogen peroxide upon subsequent reoxidation with oxygen. This can be achieved by irradiation with green LEDs, because low absorbance of FMN in this region prevents quick photobleaching.

A solution of FMN (2.25 mM) in phosphate buffer (10 mM, pH 6) was irradiated with 535 nm LEDs for 5.5 hours in a sealed crimp-top vial under nitrogen atmosphere. Afterwards, oxygen was bubbled through the solution for 30 minutes and the concentration of hydrogen peroxide was measured using the POD assay. The resulting H_2O_2 concentration was 353 μM , which shows a decomposition of a majority of the photocatalyst. When this process was repeated with the same reaction mixture, no increase in H_2O_2 concentration was observed.

5.3 Absorption Spectra of FMN in Different Buffers (A. Králík)

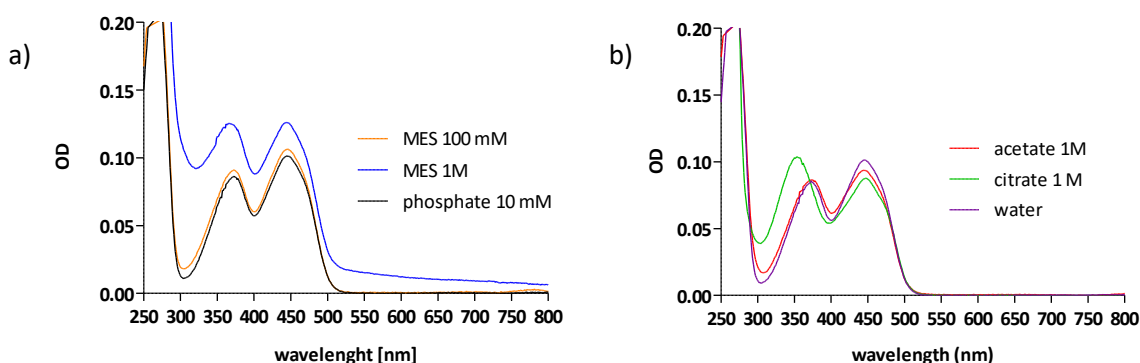


Figure S22. UV-Vis spectra of 10 μM FMN in different buffers at pH 6. a) Influence of different MES-concentrations on the absorbance. b) Shifted absorption maximum in citrate buffer.

Figure S22 illustrates that the absorption characteristics of FMN in citrate and MES buffer differ from other buffers at pH 6 or in water. Spectral differences can be explained by an interaction of ions with FMN in aqueous solutions.^[11] The second absorption maximum at 370 nm could account for the protonated species of FMN.^[12] This maximum is slightly blue-shifted in citrate buffer.

6. H₂O₂-Generation by Oxidative Water Splitting Using Au-TiO₂ (A. Králík)

6.1 Evaluation of Different Light Sources for H₂O₂ Generation via Water Oxidation (A. Králík)

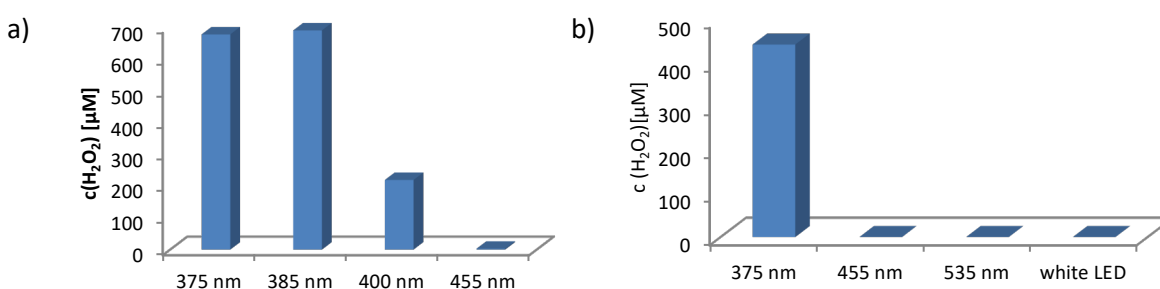


Figure S23. Activity of Au-TiO₂ in terms of H₂O₂ production as a function of the wavelength. a) Short-term irradiation. The concentration of hydrogen peroxide was determined after 4 min of irradiation of an Au-TiO₂ (Degussa P25) suspension in pure water under oxygen atmosphere using different light sources. b) Long-term irradiation. The concentration of hydrogen peroxide was determined after 1 h of irradiation of an Au-TiO₂ (pure rutile) suspension in pure water under oxygen atmosphere using different light sources.

General Procedure

A crimp-top vial (5 mL) equipped with a magnetic stirring bar containing a suspension of Au-TiO₂ (pure rutile, 5 mg) in distilled water (1 mL) was sealed with a septum and the head-space atmosphere was exchanged with oxygen using a balloon. The vial was placed in a tempered (25 °C) aluminum block (see Section 4.2) and irradiated from the bottom with an LED of a specific wavelength (see Figure S23) for 1 h under slow stirring. Subsequently, the heterogeneous catalyst was removed via centrifugation, the supernatant was diluted 5-times with distilled water and the concentration of hydrogen peroxide was determined using the POD assay.

6.2 Kinetics of the H₂O₂ Evolution (A. Králík)

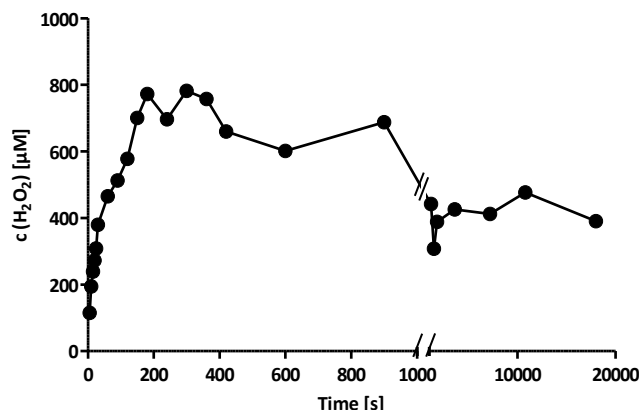


Figure S24. Extended profile of H₂O₂ generation via irradiation (375 nm LEDs) of an Au-TiO₂ suspension in water under oxygen atmosphere.

Figure S24 shows an extended kinetic measurement of hydrogen-peroxide generation via water splitting with Au-TiO₂. After reaching a maximal concentration of 800 μM within ca. 4 min, a first, short-term plateau around 600 μM is formed. After 30 min the concentration stabilizes at 400 μM forming a final, long-term plateau, which lasts for at least 5 h. In this case, a stable concentration represents an equilibrium between a photocatalytic formation of H₂O₂ and its quick decomposition in the presence of heterogeneous material.^[13] Therefore, the drop in equilibrium concentration suggests a decrease in activity of Au-TiO₂ during continuous irradiation. In addition, a color change can be observed, where the originally grey or slightly purple suspension turns pink after 1 hour of UV irradiation and completely white after 24 hours. This indicates that gold nanoparticles are released from the semiconductor surface and eventually decompose, which can explain the decrease in activity. The color change seems to be dependent on the amount of oxygen in the reaction mixture (see Figure S25). To prove the crucial role of gold nanoparticles in H₂O₂ generation, pure TiO₂ (P25) was used instead of Au-TiO₂ in the negative-control samples – no hydrogen peroxide was detected in those samples.

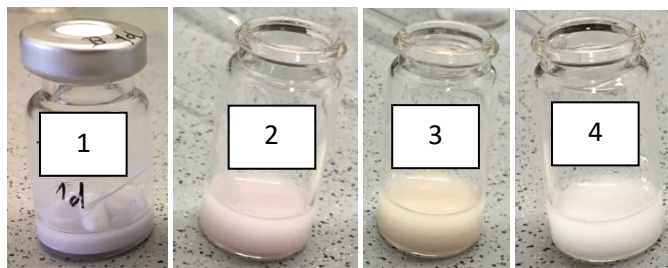


Figure S25. Suspension of Au-TiO₂ after 24 hours of 375 nm irradiation under different atmosphere: 1) before irradiation; 2) nitrogen; 3) air; 4) oxygen.

General Procedure

A crimp-top vial (5 mL) equipped with a magnetic stirring bar containing a suspension of Au-TiO₂ (pure rutile, 5 mg) in distilled water (1 mL) was sealed with a septum and the head-space atmosphere was exchanged with oxygen using a balloon. The vial was placed in a tempered (25 °C) aluminum block (see Section 4.2) and irradiated from the bottom with a 375 nm LED for a selected time under slow stirring. Subsequently, the heterogeneous catalyst was removed via centrifugation, the supernatant was diluted 5-times with distilled water and the concentration of hydrogen peroxide was determined using the POD assay.

Reaction under Inert Atmosphere

A crimp-top vial (5 mL) equipped with a magnetic stirring bar containing a suspension of Au-TiO₂ (pure rutile, 5 mg) in distilled water (1 mL) was sealed with a septum. The suspension was degassed by bubbling with nitrogen for 20 min and the head-space was evacuated and flushed with nitrogen five times. To confirm that no oxygen is present, the gas phase was analyzed with head-space GC prior to the irradiation. The vial was then placed in a tempered (25 °C) aluminum block (see Section 4.2) and irradiated from the bottom with a 375 nm LED for 1 h under slow stirring. Afterwards, the gas phase was analyzed again with head-space GC, the vial was unsealed, the heterogeneous catalyst was removed via centrifugation and the concentration of hydrogen peroxide in the supernatant was determined using the POD assay.

No detectable oxygen was present in the gas phase after irradiation and H₂O₂ concentration in the reaction mixture was 0.89 µM.

6.3 Comparison of the Activity of Different Forms of TiO₂ (A. Králík)

The rutile form of TiO₂ has been chosen for photobiocatalytic experiments due to its recently described improved biocompatibility compared to the anatase form. The hydrophobic surface of rutile TiO₂ prevents hydrophilic enzymes from adsorption and this spatial separation protects the enzyme from highly reactive — but short-lived — hydroxyl radicals formed by titanium dioxide in aerated aqueous solution upon irradiation.^[13]

A possible influence of the form of TiO₂ — used as a precursor of the water-oxidation catalyst Au-TiO₂ — on its activity in terms of H₂O₂ generation was investigated. For this purpose, results obtained with rutile-based Au-TiO₂ were compared to an Au-TiO₂ catalyst prepared from Degussa P25 TiO₂, which consists of 75–85 % anatase.^[14] Data presented in Figures S23, S26, and S27 show that the activity of both rutile- and P25-based catalysts rely on the same wavelength dependency, produce a comparable amount of hydrogen peroxide in one-point activity measurements, and have a similar kinetic profiles of H₂O₂ generation. These results suggest that there is no difference in activity between anatase and rutile TiO₂ in terms of photocatalytic generation of hydrogen peroxide via water oxidation in oxygenated aqueous solutions.

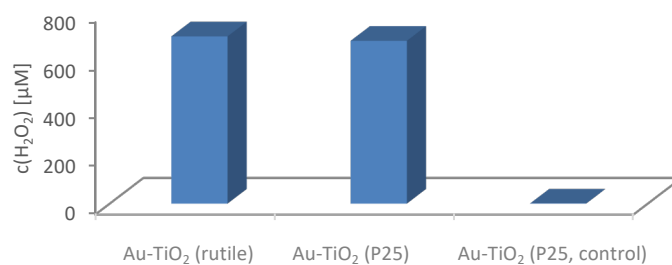


Figure S26. One-point-activity comparison between Au-TiO₂ based on rutile, Degussa P25 TiO₂ and a negative-control experiment using pure Degussa P25 TiO₂. The concentration of hydrogen peroxide was determined after 4 min of irradiation (375 nm LEDs) of a catalyst suspension in pure water.

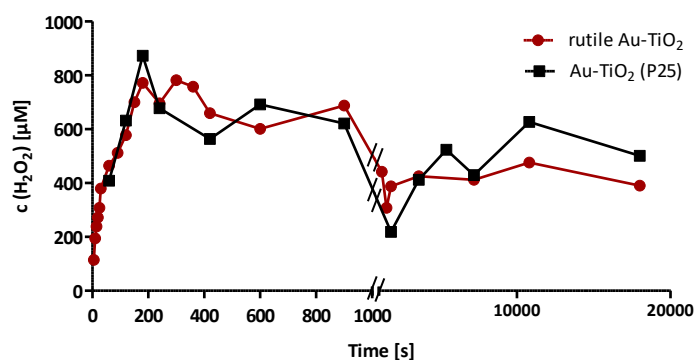


Figure S27. Comparison of extended kinetic profiles of H₂O₂ generation between rutile- (red) and P25-based (black) Au-TiO₂ catalysts via irradiation (375 nm LEDs) of a catalyst suspension in pure water under oxygen atmosphere.

6.4 Water-Splitting Coupled to *Am*VHPO Catalyzed Bromination (*A. Králík*)

Control Reactions

Table S16. Set of control reactions for bromination of 2,6-dimethoxypyridine (**1e**) using H₂O₂ generated via photo-catalytic water splitting with Au-TiO₂.



Entry	Light	Au-TiO ₂	Atmosphere	Enzyme	Conversion	Ratio (2e : di-2e)
1	375 nm	+	O ₂	+	74 %	(100 : 0)
2	–	+	O ₂	+	0 %	–
3	375 nm	–	O ₂	+	0 %	–
4	375 nm	+	Air	+	9 %	(100 : 0)
5	375 nm	+	N ₂	+	4 %	(100 : 0)
6	375 nm	+	O ₂	–	7 %	(100 : 0)

dimethoxypyridine (7.43 mM); *Am*VHPO (1.16 mg mL⁻¹; 80 μL); KBr (8.13 mM); Na₃VO₄ (187.5 μM); overall volume 1.6 mL

General Procedure

Prior to the reaction, *Am*VHPO was transferred from the storage buffer (containing Good's buffers) to phosphate buffer (10 mM, pH 6) using a PD-10 Desalting column (GE Healthcare). Solutions of **1e** (100 μL, 118.9 mM in MeCN), Na₃VO₄ (10 μL, 30 mM in water), KBr (13 μL, 1 M in water), and *Am*VHPO (80 μL, 1.62 mg mL⁻¹ in 10 mM aqueous phosphate buffer pH 6) were added in a 5 mL crimp-top vial together with aqueous phosphate buffer (1.397 mL, 10 mM, pH 6). The vial was sealed with a rubber septum, its head-space atmosphere was exchanged with oxygen using a balloon and it was placed into the irradiation setup (see Section 4.2) where it was irradiated with 375 nm LEDs at 25 °C for 24 h. Subsequently, the heterogeneous catalyst was removed via centrifugation and the supernatant was mixed with MeCN (800 μL), brine (2 mL), and ethyl acetate (2 mL) and extracted. The organic phase was analyzed by HPLC.

7. Synthesis of the Halogenated Reference Material (*C. J. Seel*)

The synthesis of the brominated references **2a**, **di-2a**, **3a**, **di-3a** and **o-2h**, **p-2h** and **di-2h** has been reported previously.^[3]

7.1 General Procedure for the Synthesis of the Halogenated Products (*C. J. Seel*)

N-Bromosuccinimide or *N*-chlorosuccinimide (1.1 eq. or 2.2 eq.) was added slowly to a cooled solution of the starting material (200 – 500 mg scale) in MeOH/dichloromethane (1/9; 10 mL). The resulting reaction mixture was stirred at 0°C for 2 h and the solvent was removed under reduced pressure. Subsequently, water (25 mL) was added and the mixture was extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with brine, dried over MgSO₄, filtered and the solvent was evaporated under reduced pressure. The crude mixture was purified by reversed phase chromatography on C18 silica (MPLC, H₂O/MeCN gradient: 70/30 → 0/100 in 18 min).

7.2 Synthesis of Ethyl 4-bromo-3,5-dimethyl-1*H*-pyrrole-2-carboxylate (**2f**) (*C. J. Seel*)

N-Bromosuccinimide (1.05 eq.) was added slowly to a mixture of 3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (1 eq.) and potassium carbonate (1.05 eq.) in acetonitrile (0.85 M) cooled in an ice bath. The mixture was warmed to room temperature, diluted with water and then stirred for 30 min. The precipitate was collected by vacuum filtration, washed with ethanol/ water (1/2) and dried in vacuo.

7.3 Up-Scaled Enzymatic Reaction on (*E*)-1,3-Dimethoxy-5-(4-methoxystyryl)benzene (**1k**) (*C. J. Seel*)

The reaction mixture containing substrate (7.43 mM; 36.99 μmol), KBr (1.1 eq; 8.13 mM), MES pH 6.0 (50 mM), Na₃VO₄ (187.5 μM), H₂O₂ (1.1 eq; 8.13 mM) and VHPO (1 mL; 4.5 mg mL⁻¹; specific activity 39.51) in MeCN/H₂O (1/1; total volume of 5.0 mL) was heated to 30°C and mixed at 300 rpm for 72 h. The reaction was stopped by addition of sat. NaCl solution and extraction with EtOAc (3 × 10 mL). The combined organic phases were washed with brine, dried over MgSO₄, filtered and the solvent was evaporated under reduced pressure. The crude mixture was purified by semi-preparative HPLC controlled by a Jasco HPLC system consisting of an UV-1575 Intelligent UV/VIS Detector, two PU-2068 Intelligent prep. pumps, a M IKA 1000 Dynamic Mixing Chamber (1000 μL Portmann Instruments AG Biel-Benken), a LC-NetII/ ADC), and a Rheodyne injection valve. The system was controlled by the Galaxie-Software. A Eurosphere II 100-5 C18 A (KNAUER® 250 x 16 mm) column with pre-column (KNAUER® 30 x 16 mm) was used as the stationary phase (H₂O (0.05% TFA)/MeCN (0.05% TFA) gradient: 95/5 → 0/100 in 43 min). The collected fractions were combined and the MeCN was removed under reduced pressure. The remaining aqueous phases were freeze-dried in liquid nitrogen and the water was removed by lyophilization (Alpha 2-4 Christ with Chemistry-Hybrid-Pump-RC6 pump).

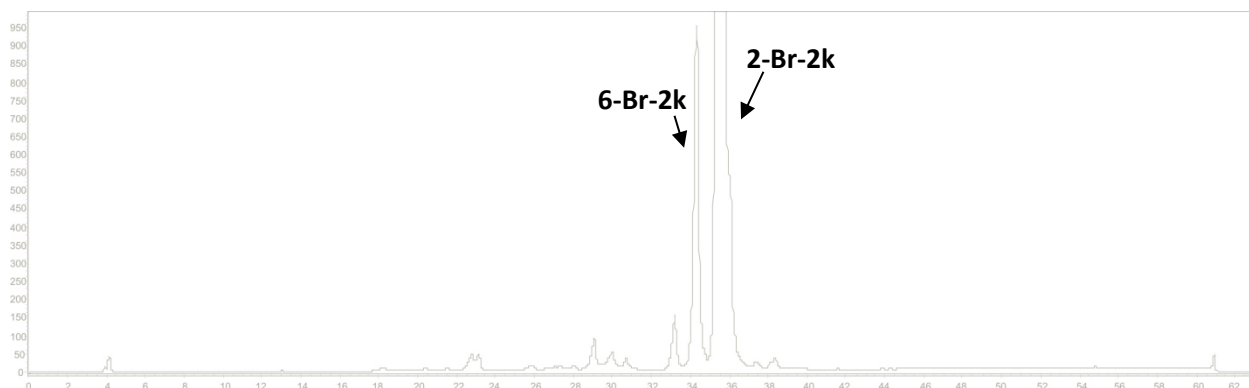


Figure S28. Chromatogram of the preparative HPLC purification of the up-scaled enzymatic reaction.

7.4 Preparation of (*E*)-2-Chloro-1,5-dimethoxy-3-(4-methoxystyryl)benzene (**3d**): (*C. J. Seel*)

The reaction mixture containing (*E*)-1,3-Dimethoxy-5-(4-methoxystyryl)benzene (**1k**) (3.8 mg, 1.0 eq.) and *N*-chlorosuccinimide (1.9 mg, 1.0 eq.) in MeOH/dichloromethane (1/9; 1 mL) was stirred at 0°C for 2 h. Subsequently the solvent was removed under reduced pressure, water (10 mL) was added and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with brine, dried over MgSO₄, filtered and the solvent was evaporated under reduced pressure. The crude mixture was purified by semi-preparative HPLC as described in chapter 7.3.

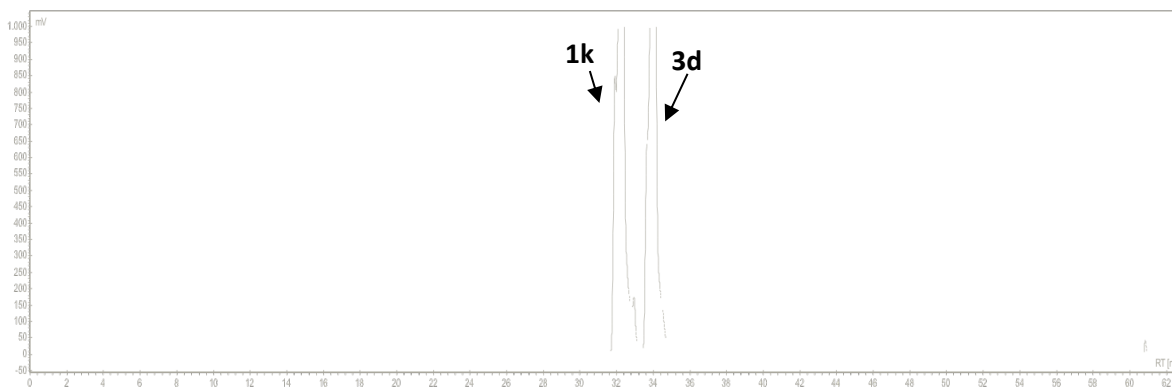
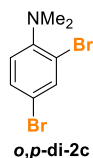
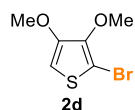


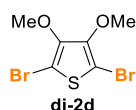
Figure S29. Chromatogram of the preparative HPLC purification of the chemical chlorination of **1k**.



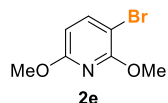
2,4-Dibromo-*N,N*-dimethylaniline (*o,p*-di-2c):^[15] yellowish oil; ¹H NMR (500 MHz, CDCl₃) δ = 7.68 (d, *J* = 2.3 Hz, 1H), 7.36 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 2.78 (s, 6H) ppm; MS (EI, 70 eV): *m/z* (%) = 278 [M]⁺ (100), 276 [M]⁺ (54), 280 [M]⁺ (53), 263 (8), 198 (10), 118 (10).



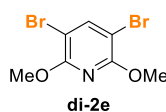
2-Bromo-3,4-dimethoxythiophene (2d):^[16] greenish oil; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ = 6.17 (d, J = 21.8 Hz, 1H), 3.90 (d, J = 2.0 Hz, 3H), 3.84 (d, J = 21.1 Hz) ppm; **MS** (EI, 70 eV): m/z (%) = 224 $[\text{M}]^+$ (100), 222 $[\text{M}]^+$ (94), 209 $[\text{M-Me}]^+$ (68), 207 $[\text{M-Me}]^+$ (66).



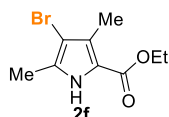
2,5-Dibromo-3,4-dimethoxythiophene (di-2d):^[16] red oil; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ = 3.91 (s, 6H) ppm; **MS** (EI, 70 eV): m/z (%) = 304 $[\text{M}]^+$ (52), 302 $[\text{M}]^+$ (100), 300 $[\text{M}]^+$ (52), 289 $[\text{M-Me}]^+$ (30), 287 $[\text{M-Me}]^+$ (61), 285 $[\text{M-Me}]^+$ (30).



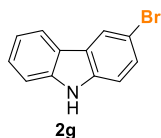
3-Bromo-2,6-dimethoxypyridine (2e):^[17] yellowish oil; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ = 7.64 (d, J = 8.3 Hz, 1H), 6.23 (d, J = 8.3 Hz, 1H), 4.00 (s, 3H), 3.90 (s, 3H) ppm; **MS** (EI, 70 eV): m/z (%) = 219 (70) $[\text{M}]^+$, 218 (96) $[\text{M}]^+$, 217 (100) $[\text{M}]^+$, 216 (65), 202 (17) $[\text{M-Me}]^+$, 188 (57), 108 (22).



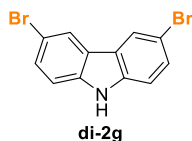
3,5-Dibromo-2,6-dimethoxypyridine (di-2e):^[18] white solid; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ = 7.86 (s, 1H), 3.99 (s, 6H) ppm; **MS** (EI, 70 eV): m/z (%) = 299 (46), 297 (100) $[\text{M}]^+$, 295 (57), 268 (45), 158 (23).



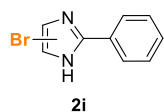
Ethyl 4-bromo-3,5-dimethyl-1H-pyrrole-2-carboxylate (2f):^[19] rose solid; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ = 9.02 (s br, 1H), 4.31 (q, J = 7.1 Hz, 2H), 2.28 (s, 3H), 2.26 (s, 3H), 1.36 (t, J = 7.1 Hz, 3H) ppm; **MS** (EI, 70 eV): m/z (%) = 247 $[\text{M}]^+$ (70), 245 $[\text{M}]^+$ (74), 218 $[\text{M-Et}]^+$ (9), 216 $[\text{M-Et}]^+$ (10), 201 $[\text{M-Et-CO}_2]^+$ (100), 200 $[\text{M-Et-CO}_2]^+$ (95), 173 (18), 92 (44), 65 (38).



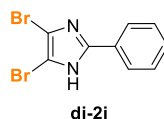
3-Bromo-9H-carbazole (2g):^[20] beige solid; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ = 8.19 (d, J = 1.9 Hz, 1H), 8.08 (s br, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.50 (dd, J = 8.6, 1.9 Hz, 1H), 7.47 – 7.42 (m, 3H), 7.32 (d, J = 8.5 Hz, 1H), 7.25 (t, J = 7.9 Hz, 1H) ppm; **MS** (EI, 70 eV): m/z (%) = 245 $[\text{M}]^+$ (100), 246 $[\text{M}]^+$ (17), 247 $[\text{M}]^+$ (92), 248 $[\text{M}]^+$ (13), 166 (50).



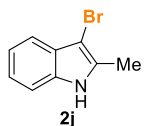
3,6-Dibromo-9H-carbazole (di-2g):^[20] beige solid; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ = 8.13 (d, J = 1.9 Hz, 2H), 8.11 (br s, 1H), 7.52 (dd, J = 8.6, 1.9 Hz, 2H), 7.31 (d, J = 8.6 Hz, 2H) ppm; **MS** (EI, 70 eV): m/z (%) = 327 $[\text{M}]^+$ (54), 325 $[\text{M}]^+$ (100), 323 $[\text{M}]^+$ (55), 246 (22), 164 (27).



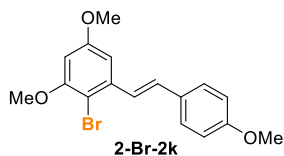
4(5)-Bromo-2-phenyl-1H-imidazole (2i):^[21] white solid; **m. p.** = 212°C; **R_f** = 0.69 (silica gel, pentane/EtOAc 50:50); **¹H NMR** (500 MHz, DMSO-*d*₆) δ = 12.86 (s, 1H), 7.88 – 7.83 (m, 2H), 7.42 (t, *J* = 8.4, 7.3 Hz, 2H), 7.38 (s, 1H), 7.34 (tt, *J* = 7.3 Hz, 1.2 Hz, 1H) ppm; **¹³C NMR** (100 MHz, DMSO-*d*₆) δ = 145.8, 129.6, 128.8, 128.5, 124.7, 117.3, 114.7 ppm; **IR** (film) $\tilde{\nu}_{max}$ = 1500, 1456, 1398, 1122, 777, 709, 670 cm⁻¹; **MS** (EI, 70 eV): *m/z* (%) = 225 (13), 224 (96), 223 (7), 222(100) [M]⁺, 143 (79), 116 (83); **HRMS** (ESI) calcd. for C₉H₈BrN₂ [M+H]⁺ 222.9871, found 222.9864.



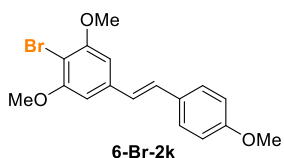
4,5-dibromo-2-phenyl-1H-imidazole (di-2i):^[22] beige solid; **m. p.** = 175°C (decomp.); **R_f** = 0.71 (silica gel, pentane/EtOAc 50:50); **¹H NMR** (400 MHz, DMSO-*d*₆) δ = 7.92 – 7.85 (m, 2H), 7.50 – 7.43 (m, 2H), 7.42 – 7.36 (m, 1H) ppm; **¹³C NMR** (100 MHz, DMSO-*d*₆) δ = 146.9, 129.0, 129.0, 128.9, 124.7 ppm; **IR** (film) $\tilde{\nu}_{max}$ = 1502, 1457, 1398, 1004, 968, 768, 702, 685 cm⁻¹; **MS** (EI, 70 eV): *m/z* (%) = 304 (50), 302 (100), 300 (50) [M]⁺, 223 (38), 221 (42), 196 (38), 194 (42) ; **HRMS** (ESI) calcd. for C₉H₇Br₂N₂ [M+H]⁺ 300.8976, found 300.8969.



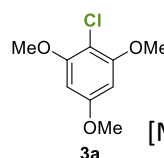
3-Bromo-2-methyl-1H-indole (2j):^[23] red solid; **¹H NMR** (500 MHz, CDCl₃) δ = 7.99 (s br, 1H), 7.51 – 7.44 (m, 1H), 7.31 – 7.22 (m, 1H), 7.22 – 7.09 (m, 2H), 2.44 (s, 3H) ppm; **MS** (EI, 70 eV): *m/z* (%) = 211 [M]⁺ (100), 209 [M]⁺ (100), 130 [M-HBr]⁺ (100).



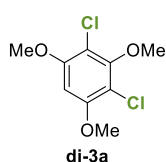
(E)-2-Bromo-1,5-dimethoxy-3-(4-methoxystyryl)benzene (2-Br-2k):^[24] white solid; **¹H NMR** (500 MHz, CDCl₃) δ = 7.50 (d, *J* = 8.7 Hz, 2H), 7.40 (d, *J* = 16.1 Hz, 1H), 6.97 (d, *J* = 16.1 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 1H), 6.80 (d, *J* = 2.7 Hz, 1H), 6.42 (d, *J* = 2.6 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H) ppm; **MS** (ESI, positive): *m/z* = 349.0; 351.4 [M+H]⁺.



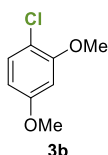
(E)-2-Bromo-1,3-dimethoxy-5-(4-methoxystyryl)benzene (6-Br-2k):^[25] white solid; **¹H NMR** (500 MHz, CDCl₃) δ = 7.46 (d, *J* = 8.7 Hz, 2H), 7.07 (d, *J* = 16.2 Hz, 2H), 6.92 (d, *J* = 16.9 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 2H), 6.70 (s, 2H), 3.95 (s, 6H), 3.84 (s, 3H) ppm; **MS** (ESI, positive): *m/z* = 349.0; 351.4 [M+H]⁺.



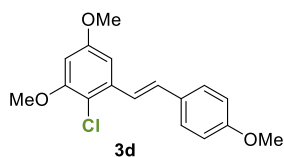
2-Chloro-1,3,5-trimethoxybenzene (3a):^[26] white solid; **¹H NMR** (500 MHz, CDCl₃) δ = 6.17 (s, 2H), 3.87 (s, 6H), 3.80 (s, 3H) ppm; **MS** (EI, 70 eV): *m/z* (%) = 202 [M]⁺ (100), 203 [M]⁺ (13), 204 [M]⁺ (35), 205 [M]⁺ (4), 159 (35).



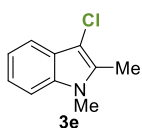
2,4-Dichloro-1,3,5-trimethoxybenzene (di-3a):^[27] white solid; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ = 6.38 (s, 1H), 3.91 (s, 6H), 3.89 (s, 3H) ppm; **MS** (EI, 70 eV): m/z (%) = 236 $[\text{M}]^+$ (100), 237 $[\text{M}]^+$ (10), 238 $[\text{M}]^+$ (69), 239 $[\text{M}]^+$ (8), 240 $[\text{M}]^+$ (13), 193 (50), 195 (33).



1-Chloro-2,4-dimethoxybenzene (3b):^[26] yellow oil; $^1\text{H NMR}$ (300 MHz, CDCl_3) δ = 7.29 (d, J = 1.0 Hz, 1H), 6.54 (d, J = 2.7 Hz, 1H), 6.46 (dd, J = 8.7, 2.7 Hz, 1H), 3.91 (s, 3H), 3.83 (s, 3H) ppm; **MS** (EI, 70 eV): m/z (%) = 172 $[\text{M}]^+$ (100), 173 $[\text{M}]^+$ (8), 174 $[\text{M}]^+$ (31), 175 $[\text{M}]^+$ (3), 129 (50).



(E)-2-Chloro-1,5-dimethoxy-3-(4-methoxystyryl)benzene (3d): white solid; **m. p.** = 85°C; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ = 7.50 (d, J = 8.7 Hz, 2H), 7.41 (d, J = 16.2 Hz, 1H), 7.26 (s, 3H), 7.01 (d, J = 16.2 Hz, 1H), 6.91 (d, J = 8.6 Hz, 2H), 6.80 (d, J = 2.6 Hz, 1H), 6.44 (s, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H) ppm; $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ = 159.8, 158.8, 156.2, 137.3, 131.2, 129.9, 128.3, 123.1, 114.4, 114.3, 101.9, 99.0, 56.4, 55.7, 55.5 ppm; **IR** (film) $\tilde{\nu}_{\text{max}}$ = 1583, 1511, 1453, 1245, 1202, 1174, 1162, 1088, 1035, 827 cm^{-1} ; **MS** (ESI, positive): m/z = 305.1; 307.1 $[\text{M}+\text{H}]^+$; **HRMS** (ESI) calcd. for $\text{C}_{17}\text{H}_{18}\text{ClO}_3$ $[\text{M}+\text{H}]^+$ 305.0944, found 305.0938.



3-Chloro-1,2-dimethyl-1H-indole (3e): yellow solid; **m. p.** = 60°C; $^1\text{H NMR}$ (500 MHz, CDCl_3) δ = 7.56 (d, J = 7.8 Hz, 1H), 7.30 – 7.20 (m, 2H), 7.16 (t, J = 7.4 Hz, 1H), 3.69 (s, 3H), 2.44 (s, 3H) ppm; $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ = 135.5, 132.4, 125.4, 121.8, 119.9, 117.6, 109.0, 102.5, 30.1, 10.1 ppm; **IR** (film) $\tilde{\nu}_{\text{max}}$ = 1705, 1470, 1323, 1238, 1172, 857, 728 cm^{-1} ; **MS** (EI, 70 eV): m/z (%) = 179 $[\text{M}]^+$ (100), 181 $[\text{M}]^+$ (33), 144 $[\text{M}-\text{HCl}]^+$ (54); **HRMS** (ESI) calcd. for $\text{C}_{10}\text{H}_{11}\text{ClN}$ $[\text{M}+\text{H}]^+$ 180.0580, found 180.0574.

The spectroscopic data are in agreement with those reported in the literature.

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9. NMR Spectra (C. J. Seel)

