The Effect of Visible Light on the Catalytic Activity of PLP-Dependent Enzymes

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Author Contributions

D.R. Conceptualization:Supporting; Funding acquisition:Lead; Investigation:Supporting; Methodology:Equal; Project administration:Equal; Resources:Lead; Supervision:Equal; Validation:Equal; Writing – review & editing:Equal
T.G. Conceptualization:Lead; Data curation:Equal; Investigation:Lead; Methodology:Lead; Project administration:Lead; Supervision:Equal; Validation:Lead; Visualization:Lead; Writing – original draft:Lead; Writing – review & editing:Equal
D.N. Data curation:Equal; Investigation:Equal; Methodology:Equal; Validation:Equal; Visualization:Equal; Writing – review & editing:Supporting
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1. Expression vectors

**Plasmid sequence of pET29a_CvATA:**

```
TGGCCATGGGACCGCCCTTATCAGGCGGCGCCATTAGCAGCGCGGGTGGTGGTTGTAACCGACAGTGGAGCGCTCGGGCATGGATTTCATATACTGGGAGACCTGTGCTGGCTGGCATGGATGGGCTAGCGCGGCTGCGTACCTGATTACGTACGTAGCGCGCGCGGCGGCGGCGGCGTGCTCAGTGGTGTGCAGGCGTTACCCTGGTGAAGAACAAGGCGCCGGCGGCGACTTCAACCACGGCTTCACCTACTCCGGGACCCGGTCTGCGCCGCCGTCGCCCACGCCAACGTGGCGGCGCTGCGCGACGAGGGCATCGTCCAGCGCGTCGACCATCAGCATTTCGGCTTCCAGCCCGACCTGTTCACCGCCGCCAAGGGCCTGTCCTCCGGCTATCTGCCGATAGGCGCGGTCTTTGTCGGCAAGCGCGTGCCGAAGGCGCTGATCGTCCCGCCGGCCACCTACTGGCCGGAAATCGAGCGCATTTGCCGCAAGTACGACGTGCTGCTGGTGGCCGACGAAGTGATCT
```

**Expression vector**

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CCGCAAGGAATGGTGCATGC
ATACCGCGA
TCACC
TGCAGGCAGCTTCCACAGCAATGG
GTGACCCAATGCGACCAGAT
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CAGGCGAA
GTAT
TGCA
CCGGGTCCTCAACGACA
AAGACCATTCATGTTGTTGC
GGTTAGCGCCCTGACGGGCTTGTCT
TGATACCGCTCGCCGC
GCCAGCAACGCG
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CGTCTACCTGTGGGATTCGGAAGGCAACAAGATCATCGACGGCATGGCCGGACTGTGGTGCGTGAACGTCGGCTACGGCCGCAAGGACTTTGCCGAAGCGGCGCGCCGGCAGATGGAAAGCAACGTACGA
```

**Plasmid sequence of pET29a**

```
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TGATAAAGAAGACAGTCATAA
ACCGAACTGAGATACCTACAGCGTGAGCT
TAGGTGTTCCACAGGGTAGCCAGCAGCATCC
TCGGCTGAATTTGATTGCGAGTGAGATAT
AGCCGAACGACCGAGCGCAGCGAGTCAGT
CAAT
TATACCCATATAAATCAGCATCCA
```

**Expression vector**

```
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CCAGGGGGAAACGCCTGGTATCTTTATAG
ACGTAGTGGGCCATCGCCCTGATAGACGG
TTTTGATTTATAAGGGATTTTGCCGATTT
TCCGGTTCCGAATCGGTGGACACCATGATCCGCATGGTGCGCCGCTACTGGGACGTGCAGGGCAAGCCGGAGAAGAAGACGCTGATCGGCCGCTGGAACGGCTATCACGGCTCCAC
AAGAGCTGCCGTTCTACAACACCTTCTTCAAGACCAC
```

**Plasmid sequence of pET29a_CvATA**

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CTTGCGATGGGACCGCCCTTATCAGGCGGCGCCATTAGCAGCGCGGGTGGTGGTTGTAACCGACAGTGGAGCGCTCGGGCATGGATTTCATATACTGGGAGACCTGTGCTGGCTGGCATGGATGGGCTAGCGCGGCTGCGTACCTGATTACGTACGTAGCGCGCGCGGCGGCGGCGGCGTGCTCAGTGGTGTGCAGGCGTTACCCTGGTGAAGAACAAGGCGCCGGCGGCGACTTCAACCACGGCTTCACCTACTCCGGGACCCGGTCTGCGCCGCCGTCGCCCACGCCAACGTGGCGGCGCTGCGCGACGAGGGCATCGTCCAGCGCGTTCATCAGCATTTCGGCTTCCAGCCCGACCTGTTCACCGCCGCCAAGGGCCTGTCCTCCGGCTATCTGCCGATAGGCGCGGTCTTTGTCGGCAAGCGCGTGCCGAAGGCGCTGATCGTCCCGCCGGCCACCTACTGGCCGGAAATCGAGCGCATTTGCCGCAAGTACGACGTGCTGCTGGTGGCCGACGAAGTGATCT
```

**Hiss-tag**

```
CVATA
```
HisVf
GACCCGACA
CTCTGACTTGAGCGTCGATTTTTGTGATGCT
AAGGGAGAAAGGCGGACAGGTATCCGG
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ACAGCGCATTAGAGCTG
GGGGAGTCAGGCA
CCGGCTGGC
CAACA
CTTTTAAAGTTCTGCTATGTGGCG
AGTAAAAGATGCTGAAGATC
CGCTTACAATTT
CAACCCTA
GATT
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CTACTGCGTCACGGATCTCCACGCG
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ATCT
CCATCGAATGGCCAGATGATTAATTCCTA

Amino acid sequence of the CvVATA:

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PIQAGVYKVRWVLAGGGDNEHNTSYGHVCAVANHANVALLREDI1QVQVDKIDGMYKQRWETFSRFREHDDVGRMVRQQATFLVKNKRELFPF
FGEIGTLRCRDIFFRRNLIMRCGDHIUVSAPPLVMTRAEDMLAVAERCLEEEFTLKARGA-

Plasmid sequence of pASK-IBA-35 _VfATA:

CCATCGAATGGCCAGATGATTAATTCCTA

VfATA gene
His6-tag

pASK
**Plasmid sequence of pET21a** *BmA*

```
TGCGCAATTGGGACACCCCTGGTACAGAAGCTGCGCGGATGGCTGCTGATGCTGACCGGCTCCGTCATGGCCTGAGCTCCGCTGCTGACCGGCTCCGTCATGGCCTGAGCT
```

**Amino acid sequence**

```
- FGPMGAVILGPELSKRLETAIEAIEE
erGGETEEQMSDQTVMLSEKLVEVSPFD
- MGSHHHHHHGANKPQSWEARAETYSL
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Amino acid sequence
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Sr
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ATTT
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Plasmid sequence of pKK32_EcLDc:
TGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCC
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VKDERGLKAAAAYNVRRFT
MHHHHHHKNFRLSEKEVKTLAERFPTPFLVASLDKVEENYQFMRRHLPRAGVFYAMK
Amino acid sequence
His
Sr
AAAAGGCGGCTGTATAA
GGCACCAA
TCGGCACCAAGACCCGCGGCCCGCAGCC
CTGGTGGCCCGCAAGCTTTT
ATTT
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CCGGGCGCTATCA
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Plasmid sequence of pKK32_EcLDc:
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TCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCGACATCGTATAACGTTACTGGTTTCACATTCACCACCC
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TGA
ATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAA
GGAGGAACTATATCCGGAT
EcLDC
Hiss-tag
Amino acid sequence of the EcLDC:
MNIIAIMGPHGVFYKDEPIKELESALVAQGFOI1WFQNSVDLLKFIENHFRICGVIFDWEYSLDLCSDIQLNEYLFYAYFINTHSTMDVSQDMRMALWFYFEYALGQARQDIAIRMRQVTDEYLNDNITPTFKALFNYVERKRTFCPTPHGMHTAYQSYCVGCFLFFDFGNGTMLKAVISVTLGSLSDLHTGHELAEEXYIAIARTFGAEQSY1VTNSTGSTSN1VGMYAAPSGSTLLIDNCHKSALHLLMIMDVVPVLKFTPRLNALGILGGFRRFETFREDSEIEKVKAYATQAGQFPVHAVITSNTRYDGLY1NMDY11QDLVPSIFHPSDF1SFHYP1FTPSY1THPV1KETSQTHKMLAALSQSLINHIKBEYDEAFEMFHMTTTSPSYPIVASVETAAAMLRRNFGKRLNRSVERALHRKUEVQRRLIESDDOFWFIDIQFQVDUEACRFVPAPEGQWHFNDADAGVNYDKVITLTPGMEDQGNM5REGICPAVLWAVKELDRGIVVEKHGYNLLFLPSIDKWTAMMLGGLTETFKEPSYDLNHILKHIHLMDPLYEDPFPNNMIR1QDQ1QHIKLIRKHNLPGCLMLRAFSTLPEMTIPHTQAVQRQ1IKEVEITAEQLVLPGVSNMLILPPYGVPFLFMGEMZKESRVLDFMLM1SVQHVGPFDTINAGAQEDGVVVYRVLKMAGSAALEHHHHH1
2. Acetophenone calibration

![Calibration Curve](image)

**Figure SI.1.** Calibration curve for acetophenone determined via HPLC. The mean linear regression yields in $y = 57.400x + 2.432$ and $R^2 = 0.999$. Samples were diluted 1:20 (v/v) in a mixture of 45% acetonitrile, 55% H$_2$O and 0.1% trifluoroacetic acid (v/v). The samples were analyzed by reversed phase chromatography using a Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a diode array detector and a LiChrospher® 100 RP-18 column (Merck, Darmstadt, Germany). The analysis was carried out isocratically with a flow rate of 1.5 mL/min at 25°C for 10 min using a solvent mixture of 45% acetonitrile, 55% H$_2$O and 0.1% trifluoroacetic acid (v/v). A 10 μL sample was injected and the absorption of acetophenone was detected at 254 nm with a retention time of 4.3 min. The error bars represent the standard deviation of technical triplicates.
3. Spectrophotometric analysis of PLP solutions

![Graph showing spectrophotometric analysis of PLP solutions](image)

**Figure S1.2.** Spectrophotometric analysis of 0.1 mM PLP solutions in 100 mM HEPES buffer (pH 7.5) after 0, 5, 30, 60 or 180 min incubation in the dark (○), in normal light conditions (●) and with blue light illumination (★). The analysis was done in a 1.5 mL cuvette using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Kyōto, Japan), measuring the spectrum from 250 nm to 500 nm. 1 h blue light illumination was carried out in a stirred 1.5 mL quartz glass cuvette (Hellma GmbH & Co. KG, Müllheim, Deutschland; 600 rpm) using a blue LED (450 nm, royal blue, XP-E2 SMD-LED, Star-PCB, Cree, Durham, NC, USA; ~60 mW/cm²). The setup was cooled with ice to keep the solution at an average temperature of 22°C. Normal illumination of the respective PLP solution was done in a 2 mL glass vessel under the influence of sun and electric light on ice.
4. Blue light controls

Figure SI.3. Stability control for 100 mM (S)-\(\alpha\)-methylbenzylamine (A) or 100 mM acetophenone (B) in 100 mM HEPES buffer (pH 7.5) in dark conditions (■) and during 30 min of blue light illumination (▲). Blue light illumination was carried out in a stirred 1.5 mL quartz glass cuvette (Hellma GmbH & Co. KG, Müllheim, Deutschland; 600 rpm) using a blue LED (450 nm, royal blue, XP-E2 SMD-LED, Star-PCB, Cree, Durham, NC, USA; ~60 mW/cm²). The incubation temperature for all samples was at 22°C. Samples were diluted 1:20 (v/v) in a mixture of 45% acetonitrile, 55% H\(_2\)O and 0.1% trifluoroacetic acid (v/v). The samples were analyzed by reversed phase chromatography using a Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a diode array detector and a LiChrospher® 100 RP-18 column (Merck, Darmstadt, Germany). The analysis was carried out isocratically with a flow rate of 1.5 mL/min at 25°C for 10 min using a solvent mixture of 45% acetonitrile, 55% H\(_2\)O and 0.1% trifluoroacetic acid (v/v). A 10 μL sample was injected and the absorption of \(\alpha\)-methylbenzylamine was detected at 213 nm with a retention time of 1.8 min, while acetophenone was detected at 254 nm with a retention time of 4.3 min. The error bars represent the standard deviation of technical duplicates.
5. Incubation of the AsATALmut11 for 24 h

Figure SI.4. Relative activity of the AsATALmut11 for the formation of acetophenone over an incubation period of 24 h in dark (●) and in light conditions with a light intensity of ~0.25 mW/cm² (○). Enzymatic reactions contained 30 mM (S)-α-methylbenzylamine, 60 mM pyruvate and 0.1 mM PLP in 100 mM HEPES buffer (pH 7.5) at 20°C and 600 rpm. The error bars represent the standard deviation of the technical triplicates. The activity in the dark increased by 35% during 3 h of incubation and remained on that activity level over the course of 24 h. With illumination, the activity increased by 50% during 6 h of incubation, after 24 h the activity decreased to 125% relative activity. It remains unclear whether light has an additional effect on the activation of the AsATALmut11 or whether the differences compared to activation under dark conditions can be attributed to the error.
6. Incubation of the CvATA for 6 h in darkness

**Figure SI.5.** Relative activity of the CvATA for the formation of acetophenone over an incubation period of 6 h in dark conditions (●). The enzymatic reaction contained 30 mM (S)-α-methylbenzylamine, 60 mM pyruvate and 0.1 mM PLP in 100 mM HEPES buffer (pH 7.5) at 20°C and 600 rpm. The error bars represent the standard deviation of the technical triplicates. Activation over time was not observed for the CvATA, instead, activity decreased by 5%.
7. Illumination setups

Figure SI.6. (A) Incubation under dark conditions prior to enzymatic reactions was carried out on ice using an amber colored 1.5 mL reaction tube. (B) Incubation under normal light conditions prior to enzymatic reactions involved sun and electric light and was carried out on ice using a 2 mL glass vessel. (C-D) Blue light illumination of PLP solutions was carried out in a stirred 1.5 mL quartz glass cuvette (Hellma GmbH & Co. KG, Müllheim, Deutschland; 600 rpm) using a single blue LED (450 nm, royal blue, XP-E2 SMD-LED, Star-PCB, Cree, Durham, NC, USA; ~60 mW/cm²). To keep the temperature at 22°C, the setup was cooled with ice. To enable stirring, the cuvette box containing the illumination setup was placed on a magnetic stirrer (RCT, IKA, Staufen, Germany). (E-F) Blue light inactivation of the CvATA was carried out in a stirred 2 mL glass vessel (600 rpm) using blue LED strips (60 LEDs, 450 nm, X105-0200, revoART GmbH, Markkleeberg, Germany; ~12 mW/cm²). To keep the temperature at 22°C, the setup was cooled with ice. To enable stirring, the ice box containing the illumination setup was placed on a magnetic stirrer (RCT, IKA, Staufen, Germany).