Supporting Information

Supplementary Table 1. Primers used to construct the CYP1A1 expression vector and for genotyping analysis.

Primer name	**nucleotide sequence 5'-3'**
NS1 LF fw LL ext	ACGCGCCCTGACGGGCTTGTCTGCTCGTTTAAACtgaagcgattggctatgatctacc
NS1 LF rv	TTCTATCGCCTTCTTGACGAGTTCTTCTGAAGATCTttttgatgggccatggtcat
Kan fw	tcagaagaactcgtcaagaaggcg
Kan rv	tggacagcaagcgaaccgga
Pcpcb fw kan ext	CAATTCCGGTTCGCTTGCTGTCCAAGATCTgttataaaataaacttaacaaatctatacc
Pcpcb rv 6H ext	TGCGGCCGCATGGTGATGGTGATGATGCATtgaattaatctcctacttgactttatg
TrrnB fw Pcpcb ext	ACTCATAAAGTCAAGTAGGAGATTAATTCAatgcatcatcaccatcaccatgc
TrrnB rv NS1 RF ext	ACGATTACCAGTGGTACCGAGGTCTAACGCcctaggagcggatacatatttgaatg
NS1 RF fw TrrnB ext	AATACATTCAAATATGTATCCGCTCCTAGGgcgttagacctcggtaccac
NS1 RF rv RR ext	GAAGATCCTTTGATCTTTTCTACGGGGTTTAAACgctcgactgcaccgttgg
NS1 seg fw	tttggatcgttggcagttgg
NS1 seg rv	tgttgacgacctgttgcatg

Bases that serve as extensions to guide recombination, which are not complementary, are shown in uppercase.

Molecular cloning. All primers used in this study are listed in Supplementary Table 1 and PCRs were run using the manufacturers cycling conditions. WT *Synechococcus* genomic DNA was used as a template to amplify genomic sequences.

Expression cassettes for *Synechococcus* were generated by *in vivo* recombination in yeast[1] and were designed to integrate into the *glpK* pseudogene (SYNPCC7002_A2842).[2] All DNA fragments used were generated by PCR or from synthetic DNA. To generate targeting flanks, two ~0.5 kb regions were amplified from *glpK* using primer pair NS1 LF fw LL ext and NS1 LF rv for the left flank and primer pair NS1 RF fw TrrnB ext and NS1 RF rv RR ext for the right flank. The kanamycin selection marker was amplified from pGFP::hph::loxP with primer pair kan fw and kan rv. The *cpcBA* promoter was amplified from WT *Synechocystis* PCC 6803 using the primer pair Pcpcb fw kan ext and Pcpcb rv 6H

ext. The *E. coli rrnB* terminator was amplified from pDF-lac[3] with primer pair TrrnB fw Pcpcb ext and TrrnB rv NS1 RF ext. The *cyp1a1* gene (NCBI reference: NM_012540.2) from *R. norvegicus* was modified to include the FLAG epitope at the C-terminus, codon-optimized for expression in *Synechococcus* and synthesized by GeneArt (Thermo Scientific). The *cyp1a1* gene and *FLAG* sequence are fused by a 4 x glycine-alanine linker. The pKU acceptor vector, into which the DNA fragments were recombined, was linearized by PCR with primer pair pKU LL rv and pKU RR fw. The amplicons generated have 30 bp extensions at the 5' and 3'-end that permit recombination with the adjacent amplicon. Amplicons consisting of targeting flanks, a selection marker, promoter, *CYP1A1* and terminator were co-transformed into yeast, along with the linearized acceptor vector pKU, for assembly via its endogenous recombination system. Yeast transformations were carried out with the lithium acetate/PEG method[4] and grown in 20 ml of SC-uracil for selection. The assembled plasmid was transferred from yeast to *E. coli*[5] and following restriction digest screening and confirmation of the correct vectors by DNA sequencing, the cassette was released from the backbone by digestion with *Pme*I and transformed into *Synechococcus* by adding the DNA (~ 1 µg) to 3 ml of cells in exponential growth phase ($OD_{730\,nm}$ 0.6-0.7). After 16-18 h under standard growth conditions cell were plated out. Single colonies from transformation plates were serially sub-cultured in liquid medium under standard growth conditions to obtain fully segregated strains. Integration and segregation was confirmed by colony PCR using primers NS1 seg fw and NS1 seg rv. Two independent transformants, Sy21a and Sy21b, were selected and cryopreserved. No differences in CYP1A1 expression were observed between these transformants (data not shown). All experiments were carried out on Sy21a, which was renamed as Sy21.

Immunoblotting and quantification of CYP1A1. Whole cell extracts of *Synechococcus* strains were prepared from 40 ml cultures. Cells were harvested by centrifugation at 3,500 *g* for 10 min at room temperature (RT; 21 °C). Approximately 100 mg of 0.1 mm zirconia beads (Biospec Products) was added to the pellet followed by the addition of an equal

volume (~ 300 µl) of SDS lysis buffer (200 mM NaCl, 25 mM EDTA, 0.5% (w/v) SDS, 200 mM Tris-Cl, pH 8.5). The cells were lysed in a Tissue lyser (Qiagen) for 2 x 30 s cycles at a frequency of 30 Hz. The tubes were then heated to 95 °C in a heat block for 10 min, cooled briefly on ice then cell debris was pelleted by centrifugation at 17,000 *g* for 10 min at 4 °C. The cleared lysate was removed and quantified for total protein using a bicinchoninic acid (BCA) protein assay (Pierce) with bovine serum albumin (BSA) as the standard. We used BAP conjugated to the FLAG peptide at the C-terminus (BAP-FLAG) as a standard for quantitative immunoblots. BAP-FLAG was quantified in the same manner as whole cell extracts and then diluted in a 1 mg ml^{-1} solution of BSA, which minimizes nonspecific binding of the protein standard to plasticware. Protein samples were prepared in LDS loading buffer (Invitrogen) containing DTT at 50 mM and heated for 10 min at 70 °C. Thirty micrograms of total protein was separated by electrophoresis on a 4-12% gradient Bis-tris NuPAGE gel in MES buffer (Invitrogen) in a Novex XCell SureLock Mini Cell (Invitrogen) for 35 min at 200 V. Where appropriate, BAP-FLAG standard was loaded in amounts ranging from 2-10 ng. Gels were then stained for total protein using SYPRO Ruby (Invitrogen), according to the manufacturer's instructions, or used for immunoblots.

For immunoblotting, gels were transferred to an Millipore Immobilon-P 0.45 µm polyvinyl fluoride (PVDF) membrane in NuPAGE transfer buffer (Invitrogen) for 60 min at 30 V in a XCell blot module (Invitrogen) and the membrane was then incubated in blocking solution (TBS-T; 20 mM Tris-Cl, 150 mM NaCl, 0.02% (v/v) Tween-20, pH 7.6 supplemented with 2% (w/v) ECL Advance blocking reagent; GE Healthcare) for 1 h. All incubation steps were carried out on a rocker table. Blocking agent was discarded and the membrane was incubated with mouse monoclonal anti-FLAG M2-peroxidase (HRP) antibody (Sigma; diluted to 1:1,000 in blocking solution). Membranes were washed for 3 x 5 min in TBS-T then incubated for 5 min in ECL substrate consisting of 0.5 ml each of SuperSignal West Dura reagent A and B (Thermo Scientific) and imaged using a Versa-Doc Imaging system (BioRad). For quantitative immunoblots, images were analysed using Image Lab 3.0 software (BioRad) for quantification of proteins.

Thylakoid membrane preparation. To prepare membranes enriched in thylakoids, cells from 40 ml cultures were harvested by centrifugation as described above and the pellet was re-suspended in 25 mM potassium phosphate buffer pH 7.4 with 100 mM NaCl and 10 mM $MgCl_2$. An equal volume of 0.1 mm zirconium beads was added and the cells were subjected to 8 pulses of bead beating for 20 s in a Mini-beadbeater-16 (Biospec Products), with 2 min intervals of incubation on ice between the pulses. The liquid fraction was centrifuged for 1 min at 2000 *g* at RT to remove contaminating beads and the cell lysate was loaded onto a step sucrose gradient made from solutions of 30 % (w/v) and of 50 % (w/v) sucrose. The gradient was made in a SW41 centrifuge tube and the cell lysate was loaded on top of the gradient and then centrifuged at 154,000 *g* in an SW41 rotor for 30 min at 4 ^{o}C. The membrane band was harvested using a peristaltic pump and analysed by immunoblotting as described above.

Dry cell weight determination. Cell density was measured with a spectrophotometer (model 7315 by Jenway) at a wavelength of 730 nm. The relationship between $OD_{730\ nm}$ and dry cell weight was determined from a 140 ml culture grown under standard conditions to $OD_{730\ nm}$ of 0.716. Three 40 ml samples were taken from this culture for dry cell weight determination. Cells were harvested by centrifugation for 10 min at 3,500 *g* at RT. The supernatant was removed and the cells were washed with 1 ml of PBS. The cell suspension was transferred to pre-weighed 1.5 ml tubes and centrifuged again for 5 min at 3,500 *g* at RT. The supernatant was aspirated and the cell pellets were dried at 80 °C for 24 h and were weighed after cooling in a desiccator. The amount of cells in a litre at one absorbance unit at 730 nm corresponds to 0.258 g_{dcw}.

Chlorophyll-*a* measurement. One millilitre of a cell suspension at an absorbance of 1 at $OD_{730\ nm}$ was pelleted by centrifugation at 3,500 *g* for 10 min at RT. Cells were re-suspended in 100 µl of H_2O then incubated in 900 µl of acetone overnight at 4 °C in the dark. The

extract was centrifuged at 17,000 *g* for 5 min at RT to pellet debris, the supernatant was removed and fluorescence was then measured according to Welschmeyer et al (1994).[6]

References

[1] Oldenburg, K. R., Vo, K. T., Michaelis, S., and Paddon, C. (1997) Recombination-mediated PCR-directed plasmid construction *in vivo* in yeast. *Nucleic Acids Res. 25*. 451-452.

[2] Begemann, M. B., Zess, E. K., Walters, E. M., Schmitt, E. F., Markley, A. L., and Pfleger, B. F. (2013) An organic acid based counter selection system for cyanobacteria. *PLoS One 8*. e76594.

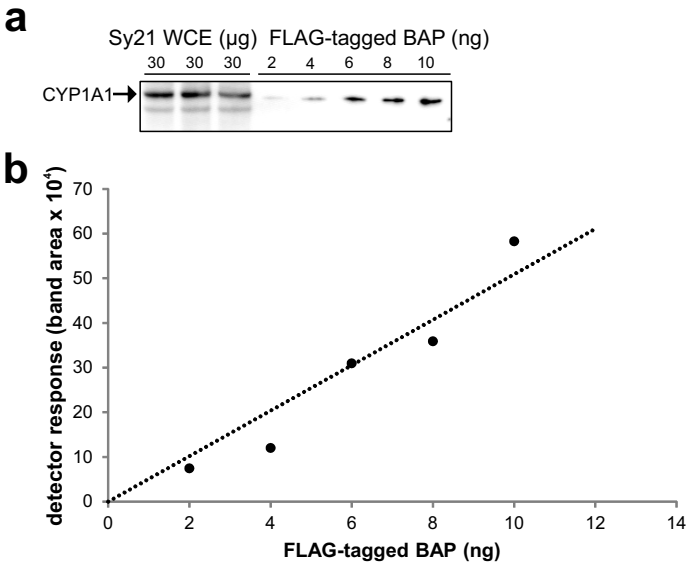
[3] Guerrero, F., Carbonell, V., Cossu, M., Correddu, D., and Jones, P. R. (2012) Ethylene synthesis and regulated expression of recombinant protein in *Synechocystis* sp. PCC 6803. *PLoS One 7*. e50470.

[4] Knop, M., Siegers, K., Pereira, G., Zachariae, W., Winsor, B., Nasmyth, K., and Schiebel, E. (1999) Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. *Yeast 15*. 963-972.

[5] Robzyk, K., and Kassir, Y. (1992) A simple and highly efficient procedure for rescuing autonomous plasmids from yeast. *Nucleic Acids Res. 20*. 3790.

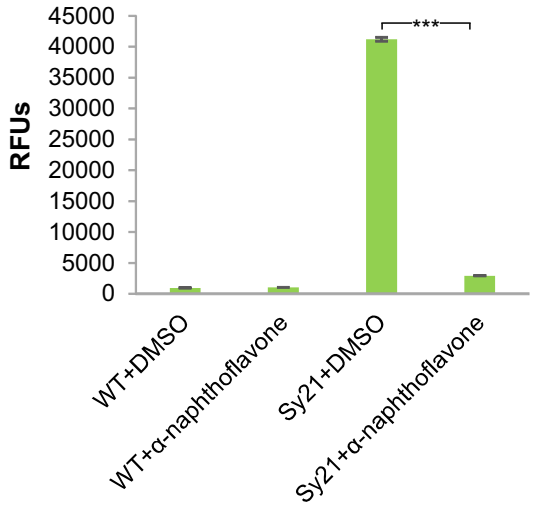
[6] Welschmeyer, N. A. (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnol. Oceanogr. 39*. 1985-1992.

Supplementary Figure 1



Supplementary figure 1. Quantification of CYP1A1. (a) Immunoblot with 30 µg of total protein from Sy21 in triplicate and the BAP-FLAG standard at different concentrations. (b) Plot of BAP-FLAG signal from panel A to generate standard curve for CYP1A1 quantification. The concentration of CYP1A1 was determined to be 6.2 pmol mg^{-1} total protein.

Supplementary Figure 2



Supplementary figure 2. The inhibitor α-naphthoflavone abolishes CYP1A1 activity. CYP1A1 activity was measured via an EROD (ethoxyresorufin O-deethylation) assay in live cells using a microplate reader. Measurements were made on three biological replicates 1 h after the addition of 5 μM of the substrate ethoxyresorufin. The experiment was repeated three times and results from a typical experiment are shown. Error bars represent the standard error of triplicate measurements. Statistical significances were inferred by the Student's *t* test; ***$P < 0.001$. Addition of 10 μM α-naphthoflavone reduced EROD by 92.9%.