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Supporting information for article:

Expression, purification and crystallization of the photosensory module of phytochrome B (phyB) from *Sorghum bicolor*

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Construct	Protein sequence
NPGP-H ₆	MASGSRATPTRSPSSARPEAPRHAHHHHHHHSQSSGGSTSRAGGGGGGGGGGGGGGAATATATATES
	VSKAVAQYTLDARLHAVFEQSGASGRSFDYSQSLRAPPTPSSEQQIAAYLSRIQRGGHIQPFGCTLAV
	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$
	eq:plwihsrvsskpfyailhridvgvvidlepartedpalsiagavqsqklavraisrlqalpggdikllc
	${\tt DTVVEHVRELTGYDRVMVYRFHEDEHGEVVAESRRDNLEPYLGLHYPATDIPQASRFLFRQNRVR}$
	MIADCHATPVRVIQDPGMSQPLCLVGSTLRAPHGCHAQYMANMGSIASLVMAVIISSGGDDEQTGR
	GGISSAMKLWGLVVCHHTSPRCIPFPLRYACEFLMQAFGLQLNMELQLAHQLSEKHILRTQTLLCD
	MLLRDSPTGIVTQSPSIMDLVKCDGAALYYHGKYYPLGVTPTESQIKDIIEWLTVCHGDSTGLSTDSL
	A DAGYLGAAALGDAVCGMAVAYITPSDYLFWFRSHTAKEIKWGGAKHHPEDKDDGQRMHPRSSF
	KAFLEVVKSRSLPWENAEMDAIHSLQLILRDSFRDAAEGTSNSKAIVNGQVQLGHHHHHH
PGP-H ₆	MAAYLSRIQRGGHIQPFGCTLAVADDSSFRLLAFSENAADLLDLSPHHSVPSLDSAAPPPVSLGADA
	RLLFSPSSAVLLERAFAARE ISLLNPLWIHSRVSSKPFYAILHRIDVGVVIDLEPARTEDPALSIAGAVQ
	${\tt SQKLAVRAISRLQALPGGDIKLLCDTVVEHVRELTGYDRVMVYRFHEDEHGEVVAESRRDNLEPYL}$
	GLHYPATDIPQASRFLFRQNRVRMIADCHATPVRVIQDPGMSQPLCLVGSTLRAPHGCHAQYMANM
	${\tt GSIASLVMAVIISSGGDDEQTGRGGISSAMKLWGLVVCHHTSPRCIPFPLRYACEFLMQAFGLQLNM}$
	ELQLAHQLSEKHILRTQTLLCDMLLRDSPTGIVTQSPSIMDLVKCDGAALYYHGKYYPLGVTPTESQI
	${\tt KDIIEWLTVCHGDSTGLSTDSLADAGYLGAAALGDAVCGMAVAYITPSDYLFWFRSHTAKEIKWG}$
	${\it GAKHHPEDKDDGQRMHPRSSFKAFLEVVKSRSLPWENAEMDAIHSLQLILRDSFRDAAEGTSNSKAI}$
	VNGQVQLGHHHHHH
PG-H ₆	MAAYLSRIQRGGHIQPFGCTLAVADDSSFRLLAFSENAADLLDLSPHHSVPSLDSAAPPPVSLGADA
	RLLFSPSSAVLLERAFAARE ISLLNPLWIHSRVSSKPFYAILHRIDVGVVIDLEPARTEDPALSIAGAVQ
	${\tt SQKLAVRAISRLQALPGGDIKLLCDTVVEHVRELTGYDRVMVYRFHEDEHGEVVAESRRDNLEPYL}$
	GLHYPATDIPQASRFLFRQNRVRMIADCHATPVRVIQDPGMSQPLCLVGSTLRAPHGCHAQYMANM
	${\tt GSIASLVMAVIISSGGDDEQTGRGGISSAMKLWGLVVCHHTSPRCIPFPLRYACEFLMQAFGLQLNM}$
	ELQLAHQLSEKHHHHHHH
H ₆ -TEV-PG	MHHHHHHENLYFQGAAYLSRIQRGGHIQPFGCTLAVADDSSFRLLAFSENAADLLDLSPHHSVPSLD
	SAAPPPVSLGADARLLFSPSSAVLLERAFAARE ISLLNPLWIHSRVSSKPFYAILHRIDVGVVIDLEPAR
	${\tt TEDPALSIAGAVQSQKLAVRAISRLQALPGGDIKLLCDTVVEHVRELTGYDRVMVYRFHEDEHGEV}$
	VAESRRDNLEPYLGLHYPATDIPQASRFLFRQNRVRMIADCHATPVRVIQDPGMSQPLCLVGSTLRA
	$\label{eq:phi} PHGCHAQYMANMGSIASLVMAVIISSGGDDEQTGRGGISSAMKLWGLVVCHHTSPRCIPFPLRYAC$
	EFLMQAFGLQLNMELQL

Table S1List of protein sequences used in the present study

Direction	Sequence (5'- 3')	Purpose
F1	CCCGGTACC <u>GAATTC</u> ATTAAAGAGGAGAAATTAACCATG GCTTCTGGTTCT	Addition of EcoRI site for NPGP-H ₆
F2	CGG <u>GAATTC</u> ttaaagaggagaaaTTAACCA TG GetGCTGCTTACC TGTCTCGTA	Addition of EcoRI site, ribosome binding site and start codon for PGP- H ₆ and PG-H ₆
R1	GTAGGG <u>AAGCTT</u> TTAgtgatgatgatgatgatgACCCAGCTGAACC TGACC	Addition of HindIII site, stop codon and the 3'6xHis tag for NPGP-H $_6$ and PGP-H $_6$
R2	GTAGGG <u>AAGCTT</u> TTAgtgatgatgatgatgatgAGCCAGCTGCAGT TCCAT	Addition of HindIII site, stop codon and the 3'6xHis tag for PG-H ₆
F3	TAATTAACCTAGGCTGCTGCCACC	Removal of the 3' 6xHis tag
R3	CAGCTGCAGTTCCATGTTCAGC	
F4	GAAAATCTGTATTTTCAAGGCGCTGCTTACCTGTCTCGTA TCCAG	Addition of the 5' 6xHis tag and TEV site for H ₆ -TEV-PG
R4	GTGATGGTGATGGTGATGCATGGTATATCTCCTTATTAAA	

Table S2	List of prin	ners used in	the present	study.
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Figure S1 Representative difference spectra of the soluble fractions following the induction of PGP-H₆-P Φ B holoprotein from pPROLar vector in conjunction with p183, across different temperatures, inducer concentrations, and induction OD values as part of the Box-Behnken optimization process.