

	1	20	86
BphP1-FP	(1)	MVAGHASGSPAFGTASHSN C EEHIEHLAGSIQPHGALLVSE H DHRV I QASANAEEFLNLGS-VL-----VLGVPLAEI-DGDLLIKILPHLD-PT	
RpBphP1	(1)	MVAGHASGSPAFGTADLSN C EREEIHLAGSIQPHGALLVSEPDHRI I QASANAEEFLNLGS-VL-----VLGVPLAEI-DGDLLIKILPHLD-PT	
iRFP682	(1)	-----MAEGSVARQPDLLT C DDEPIH I PGAIQPHGLLLALAADM T IVAGSDN L PELTGLAIG-AL-----IGRSAADVFDSETHNRLTIALAEPG	
RpBphP2	(1)	-----MTEGSVARQPDLS T CDDDEPIH I PGAIQPHGLLLALAADM T IVAGSDN L PELTGLAIG-AL-----IGRSAADVFDSETHNRLTIALAEPG	
iRFP670	(1)	-----MARKVDLTS C DREP I H I PGSIQPCGCLLACDAQAV R ITRITENAGAFFG R ET-PR-----VGELLADYF-GETEHALRNALAQ	
RpBphP6	(1)	-----MPRKVDLTS C DREP I H I PGSIQPCGCLLACDAQAV R ITRITENAGAFFG R ET-PR-----VGELLADYF-GETEHALRNALAQ	
DrBphP	(5)	PLPFFPPLYLGGPE I T T EN C EREPIH I PGSIQPHGALL T ADGHSGEVLQMSLN A ATFLGQEP-TV-----LRGQTLAALLPEQWPALQALPFGC	
AtPhyA	(50)	RVTGPPVENQPPRSKVT T TYLHH I QKGK L IQPFGLLALDEK T FKV I AYSENASE L LMASHAVPSVGEHPVLG I GT I RS L FTAPSASALQKALGFGD	
AtPhyB	(84)	SQSLK T TYGSSVPEQ Q ITAYLSR I Q R GGY I Q P FGCM I VADESS F RI G YSENARE M L G IMPQ S VPTLE K PE L AMGT D VR S L F TSSSS I LLER A VF A RE	
Sy Cph1	(1)	-----MATTVQLSDQSLRQ L E T LAI H TA H L I Q P FG M V V LQ E PD L T I SQ I SAN T G L GR S PE D L-----LGR T L G EV F DS F Q I D I Q S R L T A G Q	
Cl CphA	(1)	-----MVSE F Q A Q S IN V NS L KE A A H VC S Q I Q P H G V L L V GE P EL N I L Q I SS N T S W V FG I L P ED V -----LQ K L E D L L D PF Q I E R I K A G I LE G N	
		N-terminal extension of PAS domain	PAS domain
	87		173
BphP1-FP	(87)	--AEGMPVAVRCRIGNPSTEY C GLMHRP E GG L I E LERAG P S I -----DL S GT L AP A LER I RTAG S LR A L C DD T V L LF Q Q-----CTGYDRVMVYR F D	
RpBphP1	(87)	--AEGMPVAVRCRIGNPSTEYDGLMHRP E GG L I E LERAG P P I -----DL S GT L AP A LER I RTAG S LR A L C DD T ALL F Q Q -----CTGYDRVMVYR F D	
iRFP682	(85)	AAVG- A PI T VG F TM R KD A G-F I GS W HR H D- Q L I F L E L EP P Q R D V -----A E P Q A F FR R T N S A IR R L Q A E T L S A C A A A Q E V R K I T G F D R V M I Y R F A	
RpBphP2	(85)	AAVG- A PI A VG F TM R KD A G-F V GS W HR H D- Q L V F L E L EP P Q R D V -----A E P Q A F FR R T N S A IR R L Q A E T L S A C A A A Q E V R E I T G F D R V M I Y R F A	
iRFP670	(79)	SDPKR P AL I FGWRD G L T GR T FD I SL H R H D-G T S I E F E P A A A E Q-----A D N P --L R L T R Q I A R T K E L K S L E E M A A R V P R Y L Q A M L G Y H R V M L Y R F A	
RpBphP6	(79)	SDPKR P AL I FGWRD G L T GR T FD I SL H R H D-G T S I E V E F P A A A D Q -----A D N P --L R L T R Q I A R T K E L K S L E E M A A R V P R Y L Q A M L G Y H R V M L Y R F A	
DrBphP	(94)	PDALQYR A T L D W P A A G H---L S L T V H R V G E -L L I E F E P T E A W D -----S T G P H A L R N A M F A L E S A P N L R A L A E V A T Q T ---V R E L T G F D R V M L Y K F A	
AtPhyA	(151)	V S LL N P I L V C R T S A K P---F Y A I I H R V T-G S I I D F E P V K P Y E V P M T A A G A L Q S Y K L A A K A I T R L Q S L P S G S M E R L C D T M V Q E V F E L T G Y D R V M A Y K F H	
AtPhyB	(185)	I T LL N P V W I H S K N T G K P ---F Y A L L H R I D-V G V V I D L E P A R T E D P A L S I A G A V Q S Q K L A V R A I S Q L Q A L P G G D I K L L C D T V V E S V R D L T G Y D R V M V K F H	
Sy Cph1	(86)	I S LL N P S K L W A R V M G D D F V I D G V F H R N S D GL L V C E L E P A T S D N L P F L G F Y H M A A L N R-L R Q A N---L R D F Y D V I V E --E V R R M T G F D R V M L Y R F D	
Cl CphA	(86)	L D Y I N P T K I W R K G D E Y V V F A D F H R N E GL L I L E L E P A I S Q E N I P F L S F Y H L A R A S I N Q -L E K T T N ---L R D F C Q I V Q---E V R K V T G F D R V M L Y K F D	
		PAS domain	GAF domain
	174		253
BphP1-FP	(174)	E Q GH G L V F E S C H V P G L E S Y F G N R Y P S S T V P Q M A R Q L Y V R Q R V R V L D V T Y Q P V P L E P R L S P L T G R D L D M S G C F L R S M S P C H L Q Y L K M D M G V R A T L A V S L V	
RpBphP1	(174)	E Q GH G E V F S E R H V P G L E S Y F G N R Y P S S D I P Q M A R R L Y E R Q R V R V L D V S Y Q P V P L E P R L S P L T G R D L D M S G C F L R S M S P I H L Q Y L K N M G V R A T L V S L V	
iRFP682	(175)	S D F S G V V I A E D R C A E V E S K L G L H Y P A S A V P A Q A R R L Y T I N P V R I I P D I N Y R P V P V T P D L N P V T G R P I D L S F A I L R S V S P C H L E F M R N I G M H G T M S I S I L R	
RpBphP2	(175)	S D F S G E V I A E D R C A E V E S L G L H F P A S D I P A Q A R R L Y T I N P V R I I P D I N Y R P V P V T P D L N P V T G R P I D L S F A I L R S V S P V H L E Y M R N I G M H G T M S I S I L R	
iRFP670	(169)	D D G S G M V I G E A K R S D L E S F L G Q H F P A S L V P Q A R L L Y L K N A I R V S D S R G I S S R I V E H -D A S G A A L D L S F A H L R S I S P C H L E F L R N M G V S A S M S L S I I	
RpBphP6	(169)	D D G S G K V I G E A K R S D L E S F L G Q H F P A S D I P Q A R L L Y L K N A I R V S D S R G I S S R I V P E R-D A S G A A L D L S F A H L R S V S P I H L E Y L R N M G V S A S M S L S I I	
DrBphP	(180)	P D A T G E V I A E A R E G L H A F L G H R F P A S D I P A Q A R A L Y T R H L L R L T A D T R A A A V P L D P V L N P Q T N A P T P L G A V L R A T S P M H Q Y L R N M G V S L S V S V V	
AtPhyA	(247)	E D D H G E V V E V T K P G L E P Y L G L H Y P A D I P Q A R F L M R N K V R M I V D C N A K H A R V L Q D E---K L S F D L F L C G S T L R A F H S C H L Q Y M A N M D S I A S L V M A V V	
AtPhyB	(281)	E D E H G E V V A E S K R D D L E P Y I G L H Y P A D I P Q A S R F L K Q N R V R M I V D C N A T P V L V V Q D D---R L T Q S M C L V G S T L R A P H G C H S Q Y M A N M G S I A S L A M A V I	
Sy Cph1	(180)	E N N H G D V I A E D K R D D M E P Y L G L H Y P E S D I P Q A R R L F I H N P I R V I P D V Y G V A V P L T P A V N P S T N R A V D L T E S I L R S A H C H L T Y L K N M G V G A S L T I S L I K	
Cl CphA	(180)	D D G H G S V I A E E K L D S M E P Y L G L H Y P E S D I P K P A R K L F A S N F I R L I P D A H A E P Q I L P I N H P S Q Q P I D L T N S I L R T A A N C H L E Y L H N M G V G A S L T I S L I K	
		GAF domain	
	274		315
BphP1-FP	(274)	G G -----K L W G L V C H H Y L P R F I R F E L R A I C K R L A E R I A T R I T A L E S--	
RpBphP1	(274)	G G -----K L W G L V A C H H Y L P R F I H F E L R A I C E L L A E A I A T R I T A L E S --	
iRFP682	(275)	G E -----R L W G L I V C H H R T P Y Y V D L D G R Q A C E L V A Q V L A W I G V M E E--	
RpBphP2	(275)	G E -----R L W G L I A C H H R K P N Y V D L D G R Q A C E L V A Q V L A W I G V M E E--	
iRFP670	(268)	D G -----T L W G L I C H H Y E P R A V F M A Q R V A E M F A D F L S L H F T A A H H Q R	
RpBphP6	(268)	D G -----T L W G L I A C H H Y E P R A V F M A Q R V A E M F A D F F S L H F T A A H H Q R	
DrBphP	(280)	G G -----Q L W G L I A C H H Q T P Y V L P D L R T L E S L R G L L S L Q V Q V K E A	
AtPhyA	(345)	N E E D G E D A P D A T T Q P K R K R L W G L V V C H H T T P R F V F P L R Y A C E F L A Q V F A I H V N K E V E--	
AtPhyB	(379)	N G N E D D G S ---N V A S G R S S M R L W G L V V C H H T S S R C I P F P L R Y A C E F L M Q A F L Q L N M E L Q --	
Sy Cph1	(280)	D G -----H L W G L I A C H H Q T P K V I P F E L R K A C E F F G R V V F S N I S A Q E D --	
Cl CphA	(280)	D G -----K L W G L I A C H H Q T P K Y V S Y E F R K A C E F L G R V I F T E I S T R E E --	
		GAF domain	

Supplemental Data File, related to Figure 1. Sequence alignment of the blue-shifted NIR FPs, their BphP templates and representative bacterial, plant and cyanobacterial phytochromes in the regions of the PAS and GAF domains containing bilin binding Cys residues (in red). BphPs (*Rhodospseudomonas palustris* RpBphP1, RpBphP2 and RpBphP6 and *Deinococcus radiodurans* DrBphP) covalently bind BV via Cys in the PAS domain, whereas plant phytochromes (*Arabidopsis thaliana* AtPhyA and AtPhyB) and cyanobacterial phytochromes (*Synechocystis* Cph1 and *Calothrix* CphA) covalently bind P₆₈₀ and P₆₈₅ via Cys in the GAF domain. BphP1-FP differs from the PAS-GAF domains of RpBphP1 by the twenty-four amino acid residues in total (marked in blue).