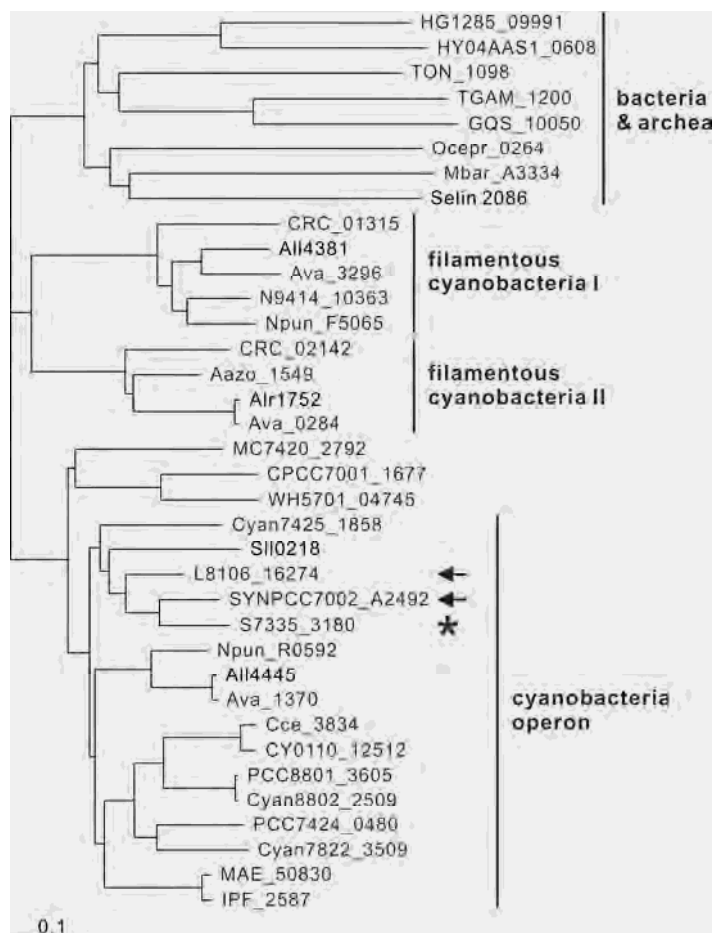
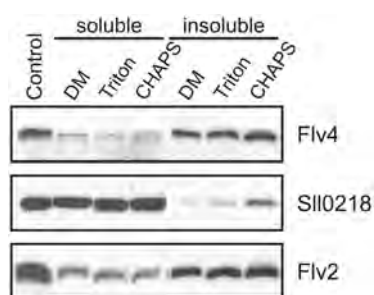


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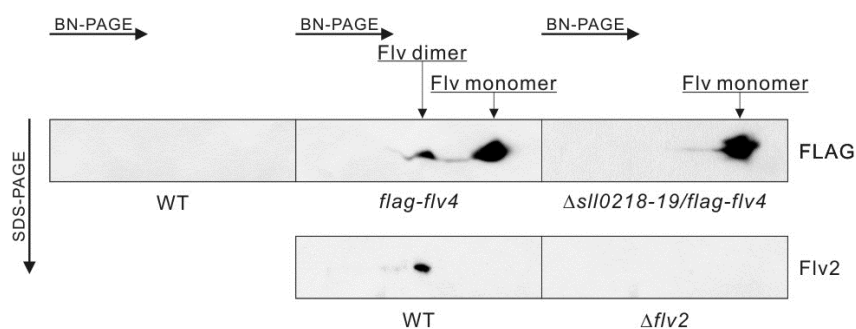
**Supplemental Figure 1.** Phylogenetic analysis of Sll0218 in cyanobacteria, bacteria and archaea.

Amino acid sequences were retrieved from CyanoBase (<http://bacteria.kazusa.or.jp/cyanobase/>) and NCBI (<http://www.ncbi.nlm.nih.gov/>), and were named according to their locus tags. The exceptions are indicated. The arrows show the sequences with high identity with Sll0218 but are missing the *flv4* and *flv2* genes. The star indicates a Sll0218 homolog, which is not located between the *flv4* and the *flv2* genes.



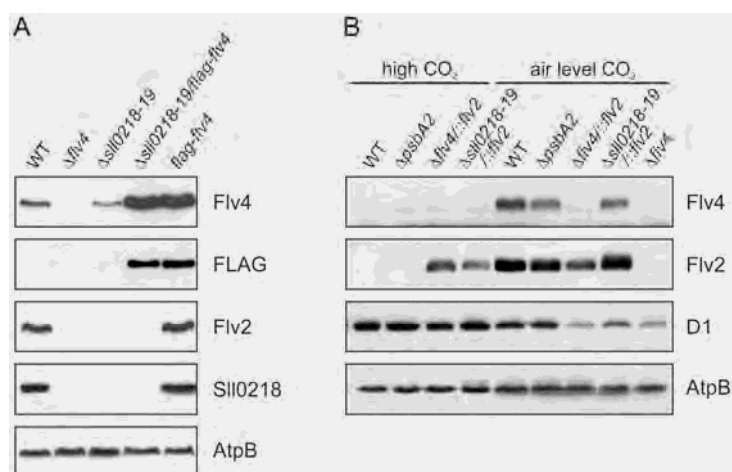
**Supplemental Figure 2.** Solubility of the Flv4, SII0218 and Flv2 proteins by nonionic and zwitterionic detergents.

Total membrane of the air level CO<sub>2</sub> grown WT cells was isolated in Buffer B and solubilized by 2% DM, 1% Triton X-100 or 2% CHAPS according to BN PAGE protocol in Methods. The soluble and insoluble proteins were separated by centrifugation. The pellet was resuspended in water. Both soluble and insoluble materials were solubilized by Laemmli sample buffer with 6 M urea, and subjected to SDS-PAGE. Control represents the total membrane directly solubilized with the Laemmli solubilization buffer. The Flv4, SII0218 and Flv2 proteins were detected by Western blot.



**Supplemental Figure 3.** BN/SDS-PAGE demonstrating the heterodimer formation by Flv2 and Flv4.

Soluble fractions were isolated from WT,  $\Delta flv2$ , *flag-flv4* and  $\Delta sll0218-19/flag-flv4$  cells, and the protein complexes were separated by BN gel. The 2D BN/SDS-PAGE and immunoblotting with the FLAG and the Flv2 antibody were subsequently performed. Only the sections of the SDS-PAGE gels corresponding to the molecular masses of the Flv2 and Flv4 proteins are shown.

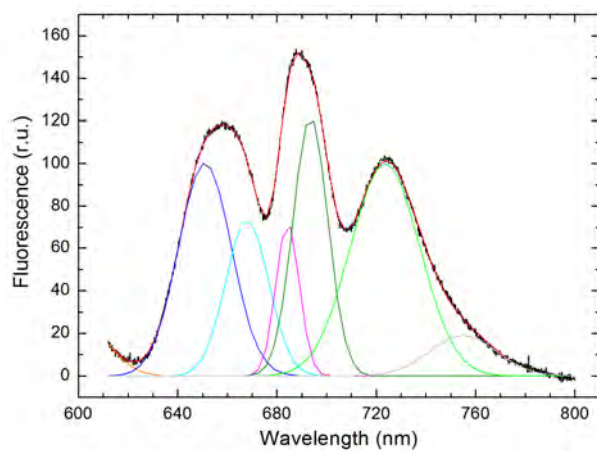


**Supplemental Figure 4.** Expression of Flv4, Sll0218 and Flv2 proteins in WT, *flv* mutants, the *flag-flv4* strains and the *flv2* complemented strains.

**(A)** Expression of the Flv4, FLAG-Flv4, Sll0218 and Flv2 proteins in WT, *flv* mutants and the *flag-flv4* strains.

**(B)** Expression of the Flv4, Flv2 and D1 proteins in WT,  $\Delta$ *psbA2*,  $\Delta$ *flv4::flv2* and  $\Delta$ *sll0218-19::flv2* strains.

Total membranes were isolated from the WT and various *flv* mutants, and proteins separated by SDS-PAGE. The expression of the Flv4, FLAG-Flv4, Flv2, Sll0218, D1, PsaB proteins was detected by specific antibodies. ATPase  $\beta$  subunit represents a loading control.

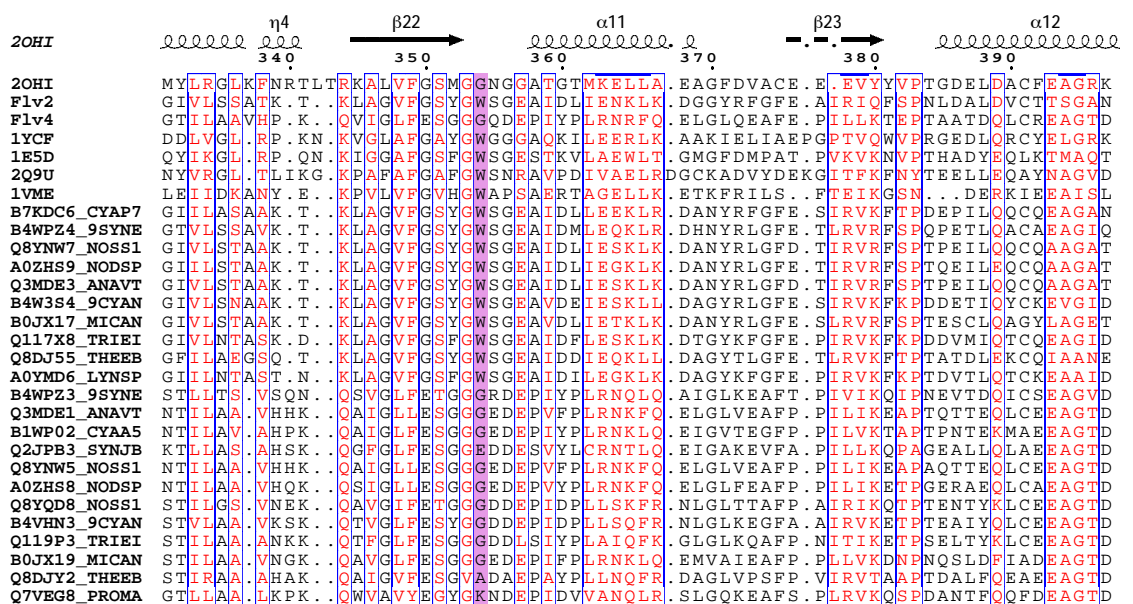
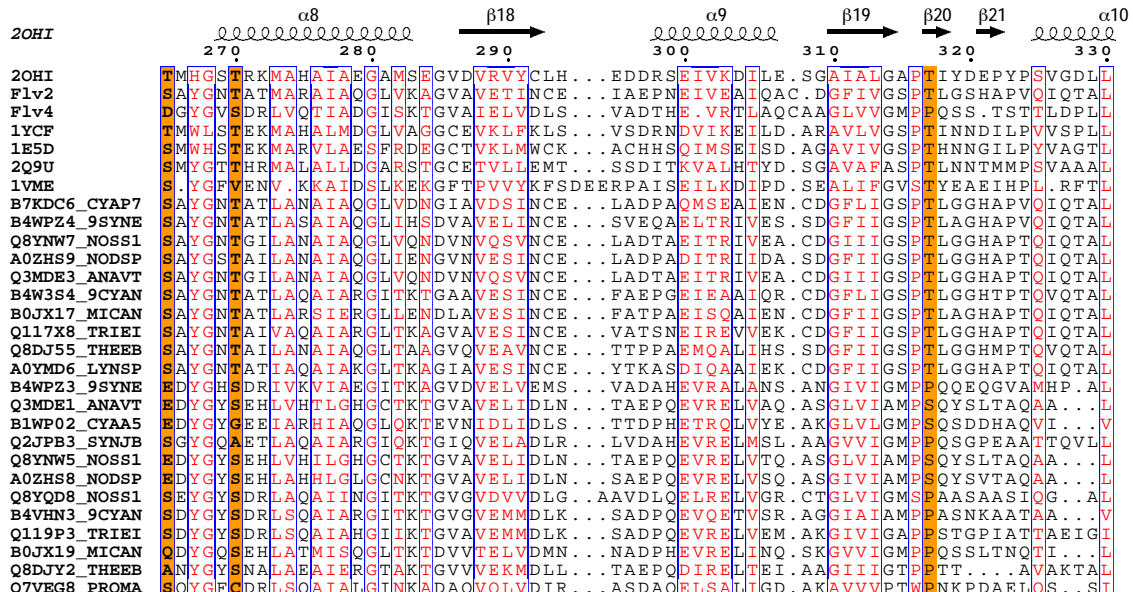
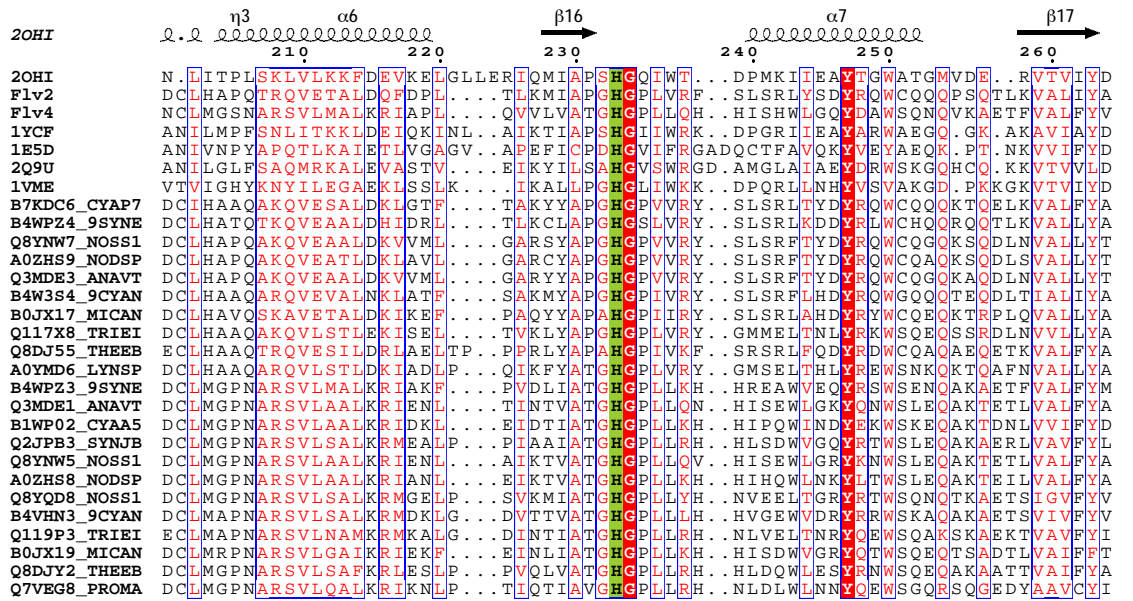


**Supplemental Figure 5.** An example of the Gaussian sub-band deconvolution of 77K fluorescence emission spectra excited at 580 nm in *Synechocystis* WT cells. The black line shows the original spectrum, the red line shows the fitted spectrum by the Gaussian deconvolution. The other color lines are the Gaussian deconvolution sub-bands from the original spectrum.

20H1	β2	β3	β4	β5	β6	β7	β8	α1
	20	30	40	50	60	70		
20H1	VYWTGVLDWDRNYHGY	TLQGT	TTYNAYLV	CGDEGV	ALIN	NSYPG	TFDELMAR	EDALQGVGMR
Flv2	TKVLRSLWDRLLKFE	VEYGRRR	TTNSNYLI	QA.DH.	TALID	PPGES	FCDLIAEL	PKYLDLA...QLD
Flv4	TTAIRCLDWRDRDFD	IEFELRH	GTTSNSFLI	RG.EK.	TALID	TSHRK	FEAVYLQ	LDLQDLV...SLD
1YCF	IYWVGAVDWNIIRYFHG	PAFSTR	HRTTNSNYLI	VD.DK.	TALVD	TVYEP	FKELIAK	LKQIKDPV...KLD
1E5D	FHLVGAIDWNSRDFH	GYTLSP	MTTNSNYLI	ED.EK.	TTLFD	TVKAE	YKGEEL	LCGLASV...IDPK...KID
2Q9U	VYWVGVLDWVIRIFH	GYHTD	GSYNYFI	DD.EC.	PTVD	SVKYP	FAEEL	LSRIAAC...CPLD...KIK
1VME	IYVLRIDDRIRYFE	AVWEIP	GTISYNYLV	KLNGA.	NVLID	GWKGN	YAKFID	ALSKIVDPK...EIT
B7KDC6_CYAP7	TTVLRSRWDRLLKFE	VEYSRQ	GTANSYLI	KS.DR.	IALID	PPGES	FTQIFL	LEELQQH...LPLN...QLD
B4WPZ4_9SYNE	TTVLRSRWDRLLKFE	VEYSRQ	GTANSYLI	QS.DR.	TALID	PPGAS	FTSIFL	QAIQPYLEET...PLD
Q8YNW7_NOSS1	TLILRSRTWDRLLKFE	VEYSRQ	RGTANSYLI	QA.DK.	KALID	PPGES	FTAIY	LEQLAQY...LDFE...TLD
A0ZHS9_NODSP	TLMLRSRTWDRLLKFE	IEYSRQ	RGTANSYLI	QA.DK.	KALID	PPGES	FTAIY	LEQLAQH...LDFI...SLD
Q3MDE3_ANAVT	TLILRSRTWDRLLKFE	VEYSRQ	RGTANSYLI	QA.DK.	KALID	PPGES	FTAIY	LEQLAQY...LDFE...TLD
B4W3S4_9CYAN	TTVLRSRTWDRLLKFE	VEYSLQ	RGTANSYLI	QA.DK.	TALID	PPGES	FTEIFL	TLQQC...LQV...QLD
B0JX17_MICAN	TWVFRSRTWDRLLKFE	IEYARQ	RGTANSYLI	CG.DK.	TALID	PPGES	FTEIY	LEELRQY...INSH...RLD
Q117X8_TRIEI	TKVLRSRTWDRLLKFE	VEYSLQ	RGTANSYLI	QG.EK.	TAVID	PPGES	FTQNY	LESELQRL...LDLH...RLD
Q8DJ55_THEEB	LVVLRSRTWDRLLKFE	VEYGRQ	GTANSYLI	QA.PQ.	PALLD	PPGES	FTIY	LELQRI...LDLN...QLR
A0YMD6_LYNP	TKVLRSRTWDRLLKFE	VEYSLQ	RGTANSYLI	QA.EH.	TALID	PPGES	FTRNY	IEALQMR...LNWS...KLN
B4WPZ3_9SYNE	TTALRCLDWRDRDFD	IEFELK	NGTTSNSFLI	RG.EK.	TALVD	TSHSK	FRQYQ	VEYLNRN...VDPQ...ALT
Q3MDE1_ANAVT	TTAIRCLDWRDRDFD	IEFGLR	NGTTSNSFLI	QG.EK.	IALVD	TSHRK	FEKLY	LEIVAGL...IDPN...TID
B1WP02_CYAA5	TTAIRCLDWRDRDFD	IEFGLR	NGTTSNSFLI	EG.EK.	TALVD	TSHGK	FRQLY	LDLCKLD...IDLS...TLD
Q2JPB3_SYNJ5	TLAIRSQDWRDRDFD	IEFALEN	GTTSNSFLI	RG.EK.	VALID	TSHK	FRQLY	LDLQGLDPA...QID
Q8YNW5_NOSS1	TTAIRCLDWRDRDFD	IEFGLR	NGTTSNSFLI	KG.EK.	IALVD	TSHRK	FEKLY	LEIVAGL...IDPN...TID
A0ZHS8_NODSP	TTAIRCLDWRDRDFD	VEFGLR	NGTTSNSFLI	QG.EK.	IALVD	TSHRK	FEELY	LEIVAGL...IDPN...KID
Q8YQD8_NOSS1	TTAIRSLDWRDRDFD	IEFGLQ	NGTTSNSFLI	RG.EQ.	IALVD	TSHK	FRQLY	LDLTKGL...INPT...EIN
B4VHN3_9CYAN	TTTIRSLDWRDRDFD	IEFGLQ	NGTTSNSFLI	RG.EK.	IALVD	TSHK	FRQLY	LDLTKGL...IEPS...QID
Q119P3_TRIEI	TMAIRSLDWRDRDFD	IEFGLQ	NGTTSNSFLI	QG.EK.	TALVD	TSHGK	FRQLY	LDLTKGL...IKPT...EID
B0JX19_MICAN	TFTLRCLDWRDRDFD	IEFGLN	GTTSNSFVI	QG.EK.	TALID	TSHRK	FRQLY	LEELTKL...ININ...HLD
Q8DJY2_THEEB	TTAIRCLDWRDRDFD	IEFALEN	GTTSNSFLI	KG.ER.	IALVD	TSHAK	FGDRY	LELQVLPNS...DLD
Q7VBG8_PROMA	TTAIRSLDWRDRDFD	IEFGLR	NGTTSNSFLI	QG.EK.	TALID	SSHVK	FRS	TWVDAITKCI...IDPK...TLD

20H1	β9	α2	α3	β10	α4	η1	β11	β12
	80	90	100	110	120	130		
20H1	YIQNHVEKDHSGVLVE	LHRRFP	EAP	IYCTE	VAVKGLL	KHYP	SLREAE	FVMVKTG
Flv2	YIVASHVNP	NRMVTL	EQLLR	RATKAK	LICSR	PAKV	LKATFP	HWEE
Flv4	YLVVNHTE	PDHSG	LIPD	LELAP	QVTVV	GSKV	AIQFLE	KLV
1YCF	YLVVNHTE	SDHAG	AFPA	IME	LCPD	AVLTC	QR	AFD
1E5D	YLVVNHTE	LELDH	AGAL	PALIE	ACQPEK	IF	TSS	LQK
2Q9U	YVVMNHAE	GDH	ASSL	KDHYH	KFTNAT	FV	CT	KK
1VME	HIIVNHTE	PDHSG	SLPAT	LKTI	GHDF	VEI	JAN	FGK
B7KDC6_CYAP7	YIILGHV	NPNRMA	TVKVL	IQOAP	QVKL	ICSK	PAK	LLKN
B4WPZ4_9SYNE	YIIVSHV	NPNRMV	TLAAL	EQQP	EAVI	ICSR	PAQA	IEA
Q8YNW7_NOSS1	YIILGHV	NPNRRV	TLQEL	LSKAP	QATL	ICSR	PAAN	ALKT
A0ZHS9_NODSP	YIILGHV	NPNRRV	TLQEL	LSKAP	QATL	ICSR	PAAN	ALKT
Q3MDE3_ANAVT	YIILGHV	NPNRRV	TLQEL	LSKAP	QATL	ICSR	PAAN	ALKT
B4W3S4_9CYAN	YVILGHV	NPNRMV	TLKAL	LELAP	QITF	CSKA	GAV	ALQN
B0JX17_MICAN	YLILSHV	NPNRIV	TLKAL	LAATH	QITL	ICSK	SAVN	TLKN
Q117X8_TRIEI	YVILGHV	NANRGG	TLKEL	LAIA	PVTF	VCSN	PSV	LNLD
Q8DJ55_THEEB	YLILSHV	NSRLA	TVKVL	LELAP	QITL	VCSN	KAG	AVTL
A0YMD6_LYNP	YIILGHV	NANRGA	TIKALL	TLPAP	QVTVV	CSN	PAAL	ALKE
B4WPZ3_9SYNE	YLIVNHTE	PDHSG	SLIED	ILWAP	HIVV	GSKV	AIQFLE	NMI
Q3MDE1_ANAVT	YLII	NHTE	PDHSG	SLVK	DILQ	LAP	SITV	GAKV
B1WP02_CYAA5	YLIVSHTE	PDHSG	SLVK	DVLK	LAP	NMTV	GSKV	AIQFLE
Q2JPB3_SYNJ5	YLIVSHTE	PDHSG	SLVK	DILQ	LAP	QVTVV	ASKV	AVQFLE
Q8YNW5_NOSS1	YLIVSHTE	PDHSG	SLVK	DILQ	LAP	NITV	GAKV	AIQFLE
A0ZHS8_NODSP	YLIVSHTE	PDHSG	SLVK	DILQ	LAP	EITV	GSKV	AIQFLE
Q8YQD8_NOSS1	YLII	NHTE	PDHSG	SLVK	DILQ	LAP	EITV	GSKV
B4VHN3_9CYAN	YLII	NHTE	PDHSG	SLVK	DILQ	LAP	EITV	GSKV
Q119P3_TRIEI	YLII	NHTE	PDHSG	SLAK	DILQ	LAP	QITV	GAKV
B0JX19_MICAN	YLII	NHTE	PDHSG	SLVK	DILQ	LAP	EITV	GAKV
Q8DJY2_THEEB	YLIVSHTE	PDHSG	SLVK	DVLK	LAP	HVTV	ASKV	AMQFLE
Q7VBG8_PROMA	FLIVSHTE	PDHSG	SLIS	DILQ	LNN	DEIV	GSKV	AIQFLE

20H1	β13	β14	β15	η2	α5
	140	150	160	170	180
20H1	...G.KT	LTFL	ETPL	LHW	PD
Flv2	...RG.HK	LQLM	ITP	TPR	WD
Flv4	...QG.HE	LQFI	ISAP	NLHW	PD
1YCF	...K.RS	LTPI	EAP	NLHW	PD
1E5D	...K.RT	VTFY	ETR	NLHW	PD
2Q9U	...K.RT	LKFI	IPV	LLHW	PD
1VME	...G.KK	FKFV	IPV	LLHW	PD
B7KDC6_CYAP7	...GG.HQ	LQFI	FVPT	PRW	AD
B4WPZ4_9SYNE	...RG.HC	LQLI	ITP	TPR	WD
Q8YNW7_NOSS1	...QG.HQ	LTFV	ITP	TPR	WD
A0ZHS9_NODSP	...QG.HH	LTPI	ITP	TPR	WD
Q3MDE3_ANAVT	...QG.HQ	LTFT	ITP	TPR	WD
B4W3S4_9CYAN	...QN.HQ	LQFI	IPV	LLHW	PD
B0JX17_MICAN	...QG.HQ	LSFIN	IPV	LLHW	PD
Q117X8_TRIEI	...SG.HK	LQLL	ITP	TPR	WD
Q8DJ55_THEEB	...GD.RQ	LMFIA	AAAT	TPR	WD
A0YMD6_LYNP	...NG.HQ	LQFI	IAV	PT	PRW
B4WPZ3_9SYNE	...KG.HE	LSFV	ISAP	NLHW	PD
Q3MDE1_ANAVT	...NG.HN	LEFV	ISAP	NLHW	PD
B1WP02_CYAA5	...QG.HE	LQFI	ISAP	NLHW	PD
Q2JPB3_SYNJ5	...KG.HV	LEFV	ISAP	NLHW	PD
Q8YNW5_NOSS1	...NG.HS	LEFV	ISAP	NLHW	PD
A0ZHS8_NODSP	...ND.HQ	LEFV	ISAP	NLHW	PD
Q8YQD8_NOSS1	...NG.HE	FEFV	ISAP	NLHW	PD
B4VHN3_9CYAN	...KG.HV	LEFV	ISAP	NLHW	PD
Q119P3_TRIEI	...NG.HV	LEFV	ISAP	NLHW	PD
B0JX19_MICAN	...NG.HI	LEFV	ISAP	NLHW	PD
Q8DJY2_THEEB	...KG.HV	LEFV	ISAP	NLHW	PD
Q7VBG8_PROMA	PSNGI	IHHK	LDFI	ISAP	NLHW



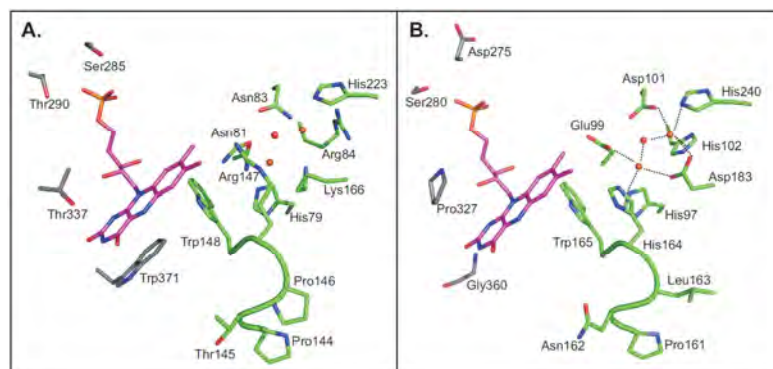
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2OHI          0000
              400
2OHI          LAEIR
Flv2          FARLR
Flv4          LGQLT
1YCF          IARIA
1E5D          IARLR
2Q9U          LKRLAI
1VME          LKKELE
B7KDC6_CYAP7 FAQLK
B4WPZ4_9SYNE FAQLR
Q8YNW7_NOSS1 FAQLK
A0ZHS9_NODSP FAQLK
Q3MDE3_ANAVT FAQLK
B4W3S4_9CYAN FAQLK
B0JX17_MICAN FARTLR
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Q8YNW5_NOSS1 IGWLT
A0ZHS8_NODSP MGWLT
Q8YQD8_NOSS1 LGWVT
B4VHN3_9CYAN MGQLT
Q119P3_TRIET AGLLS
B0JX19_MICAN LGWLS
Q8DJY2_THEEB MGWLL
Q7VEG8_PROMA LGLLN

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**Supplemental Figure 6.** Multiple structure-based alignment of *Methanothermobacter marburgensis* F<sub>420</sub>H<sub>2</sub> oxidase (PDB ID: 2OHI), *Synechocystis* Flv2, *Synechocystis* Flv4, *Moorella thermoacetica* FprA (PDB ID: 1YCF), *Desulfovibrio gigas* rubredoxin oxygen:oxidoreductase (PDB ID: 1E5D), *Giardia intestinalis* flavoprotein (PDB ID: 2Q9U), *Thermotoga maritima* flavoprotein (PDB ID: 1VME). The amino acid numbering is according to Flv2. The cyanobacterial sequences are included with UniProt Knowledgebase accession codes. The secondary structure above the alignment is according to 2OHI. The switch loop is colored cyan, the iron-binding residues are green, and Trp corresponding to Trp371 in Flv2 is pink.





**Supplemental Figure 7.** The FMN and diiron binding sites of Flv2 and Flv4 homodimers.

**(A)** *Synechocystis* Flv2/Flv2 homodimer in closed formation.

**(B)** *Synechocystis* Flv4/Flv4 homodimer in closed formation.

**Supplemental Table 1.** Ratios of low temperature (77K) fluorescence yields of PBS, PSI and PSII ( $F_{685} + F_{695}$ ) of WT and various *flv* mutants excited at 580 nm light

Strain	$F_{\text{PBS}}/F_{\text{PSII}}$	$F_{\text{PBS}}/F_{\text{PSI}}$	$F_{685}/F_{695}$	$F_{695}/F_{\text{PSI}}$	$F_{\text{PBS}}/F_{695}$
WT	$1.50 \pm 0.03$	$1.24 \pm 0.02$	$0.38 \pm 0.03$	$0.60 \pm 0.02$	$2.06 \pm 0.04$
$\Delta flv2$	$1.30 \pm 0.04$	$1.24 \pm 0.03$	$0.85 \pm 0.05$	$0.51 \pm 0.02$	$2.45 \pm 0.09$
$\Delta sll0218-19$	$1.12 \pm 0.03$	$1.25 \pm 0.03$	$0.96 \pm 0.04$	$0.57 \pm 0.01$	$2.18 \pm 0.06$
$\Delta flv4$	$1.12 \pm 0.04$	$1.26 \pm 0.05$	$1.02 \pm 0.07$	$0.57 \pm 0.02$	$2.22 \pm 0.13$
$\Delta sll0218-19/flag-flv4$	$1.18 \pm 0.03$	$1.24 \pm 0.04$	$0.83 \pm 0.01$	$0.58 \pm 0.01$	$2.15 \pm 0.04$
<i>flag-flv4</i>	$1.68 \pm 0.10$	$1.19 \pm 0.08$	$0.44 \pm 0.05$	$0.49 \pm 0.02$	$2.42 \pm 0.13$

The quantification was performed from four independent measurements. The results are shown as mean value  $\pm$  SD. Peaks are indicated in Supplemental Figure 4.

**Supplemental Table 2.** Conserved metal binding sites on the Flv2/Flv4 heterodimer surface

Metal binding site	Conserved residues in Flv2/Flv4 heterodimer	Template	Corresponding residues in template
Ca <sup>2+</sup>	Asp404, Glu400 in Flv4	<i>Synechococcus sp.</i> flavodoxin-like domain	Asp 397, Glu393
Zn <sup>2+</sup>	Glu80, Asp84 in Flv2 Glu148 in Flv2 Asp289 in Flv4	<i>M. thermoacetica</i> FprA	Glu55, Glu59 Asp120 His271, Asp275
putative	His246, His247, His 250 in Flv4 His27, His153, Asp173 in Flv4 His143, Glu145 in Flv2	- - -	- - -

**Supplemental Table 3.** Kinetic data of flash induced fluorescence relaxation components of  $\Delta psbA2$  and two *flv2* complemented strains grown at high and air level of CO<sub>2</sub>.

	Fast		Middle		Slow	
	T1 (ms)	A1 (%)	T2 (ms)	A2 (%)	T3 (s)	A3 (%)
High CO <sub>2</sub>	No addition					
$\Delta psbA2$	0.443 ± 0.009	56 ± 0.8	3.4 ± 0.17	28 ± 1.0	17.3 ± 1.0	16 ± 0.3
$\Delta flv4::flv2$	0.450 ± 0.025	57 ± 1.8	3.1 ± 0.22	28 ± 1.4	14.0 ± 0.4	15 ± 1.0
$\Delta ssl0218-19::flv2$	0.394 ± 0.006	57 ± 1.4	2.9 ± 0.19	27 ± 1.1	17.1 ± 1.6	16 ± 0.7
	+ DBMIB					
$\Delta psbA2$	1.059 ± 0.094	46 ± 0.4	31.9 ± 1.3	36 ± 0.7	3.4 ± 0.4	18 ± 0.6
$\Delta flv4::flv2$	1.135 ± 0.082	49 ± 1.3	34.9 ± 0.4	32 ± 2.0	4.7 ± 0.8	19 ± 0.7
$\Delta ssl0218-19::flv2$	1.129 ± 0.131	51 ± 1.0	32.5 ± 1.2	30 ± 1.5	4.5 ± 0.4	19 ± 0.5
Air level of CO <sub>2</sub>	No addition					
$\Delta psbA2$	0.634 ± 0.010	67 ± 1.3	9.3 ± 1.7	17 ± 1.6	5.0 ± 0.5	16 ± 0.4
$\Delta flv4::flv2$	0.762 ± 0.042	54 ± 2.1	8.7 ± 0.7	19 ± 1.1	4.1 ± 0.8	27 ± 1.3
$\Delta ssl0218-19::flv2$	0.482 ± 0.032	67 ± 0.3	7.2 ± 1.0	18 ± 1.2	2.4 ± 0.4	15 ± 1.0
	+ DBMIB					
$\Delta psbA2$	0.757 ± 0.016	53 ± 1.4	29.1 ± 1.3	29 ± 1.0	1.3 ± 0.1	18 ± 0.4
$\Delta flv4::flv2$	0.995 ± 0.025	39 ± 1.2	27.1 ± 0.3	29 ± 1.3	0.7 ± 0.1	32 ± 2.0
$\Delta ssl0218-19::flv2$	0.637 ± 0.007	48 ± 1.2	23.4 ± 1.0	30 ± 1.8	0.5 ± 0.1	22 ± 1.4