SUPPORTING INFORMATION

**Solution Structure of Psb27 from Cyanobacterial
Photosystem II**

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MATERIALS AND METHODS

Cloning and Expression. The coding sequence for residues 1 to 110 of the mature Psb27 protein was amplified by PCR from genomic *Synechocystis* sp. PCC 6803 DNA using the forward and reverse primers: 5'–ACAGGATCCTGCGACAGCGGCACAGGACTC–3' and 5'–ATAGGATCCTCACACGCCCCGTTGAATG–3', respectively, with both containing *Bam*HI recognition sites. The Psb27 cDNA was digested with *Bam*HI and ligated into the pGEX-6P-3 plasmid (GE Healthcare, Piscataway, NJ) that had been cut with the same restriction enzyme. The resulting plasmid was then used as the template for amplification using a forward primer complementary to the 3C protease recognition site from pGEX-6P-3 (5'–TCGGATCTAGAGGTTCTGTTCCAGGG–3') and a reverse primer complementary to *psb27*, and including an *Xho*I recognition site (5'–GTATCCTCGAGTCACACGCCCCGTTCAATGGA–3'). Amplified product was digested with *Xho*I and ligated into the pENTR-11 vector (Invitrogen, Carlsbad, CA) that had been digested with *Xho*I and *Xmn*I. A clone of the resulting plasmid (pGSZ-11) was isolated and the *psb27* gene was sequenced in both the forward and reverse directions. The pGSZ-11 vector was recombined in a Clonase reaction (Novagen, La Jolla, CA) with a modified pET32a vector (Novagen, La Jolla, CA) containing bacteriophage λ recombination sites (*attR*) flanking a *ccdB* gene (Gateway cassette; Novagen, La Jolla, CA). The identity of the resulting plasmid (pGSZ-32a) was confirmed by restriction digestion. This plasmid encodes thioredoxin followed by 6 histidine residues, a PreScission protease cleavage site (LEVLFQ$\times$GP) and mature Psb27 (THX-(His)₆-x-Psb27). Cleavage resulted in five residues fused to the N-terminus of the mature Psb27 protein.

Escherichia coli BL21 (DE3) cells transformed with the pGSZ-32a vector were grown at 37°C in Lysogeny broth, or in minimal medium containing ¹⁵N-ammonium chloride or ¹⁵N-ammonium chloride and ¹³C-glucose, and supplemented with 50 µg/mL of ampicillin. When cultures reached an optical density at 600 nm of 0.6, 500 µM isopropylthio-D-

galactopyranoside was added in order to induce the expression of recombinant protein. Cells were further incubated for 4 h at 37°C, harvested by centrifugation at 8,000 g for 10 min and then resuspended in buffer A (20 mM sodium phosphate pH 7.6, 500 mM sodium chloride, 20 mM imidazole).

Protein Purification. Bacteria were disrupted by sonication then centrifuged for 20 min at 8000 g to pellet cell debris. The soluble fraction was recovered and filtered (0.2 µm) before application to a 5 mL nickel immobilized metal affinity chromatography (IMAC) column (GE Healthcare, Piscataway, NJ) equilibrated with 25 mL of buffer A. After washing the column with 50 mL of buffer A, protein was eluted with 25 mL of buffer B (20 mM sodium phosphate pH 7.6, 500 mM sodium chloride, 100 mM imidazole). Dithiothreitol (DTT) (1 mM) and PreScission protease were added to the fractions that contained THX-(His)₆-x-Psb27 in order to remove the N-terminal thioredoxin hexa-histidine tag. After overnight incubation samples were diluted with 20 mM sodium phosphate pH 7.6, 500 mM sodium chloride to an imidazole concentration of 20 mM then concentrated to 5 mL using a 10 kDa molecular weight cut-off filter (GE Healthcare, Piscataway, NJ). To separate Psb27 from the hexa-histidine tagged thioredoxin, the sample was applied to a 5 mL IMAC column (GE Healthcare, Piscataway, NJ). The flow through contained Psb27 and was concentrated to 5 mL as before, and then applied to a Superdex-75 16/60 size exclusion column (GE Healthcare, Piscataway, NJ) equilibrated with degassed buffer C (20 mM sodium phosphate, pH 6.7, 50 mM sodium chloride). Following addition of 5 mM Tris (2-carboxy-ethyl)phosphine hydrochloride (TCEP) the fractions that contained Psb27 were concentrated. Samples were stored frozen at -80°C. Protein purity was checked by SDS-PAGE according to the method of Bollag and co-workers (51) using 12% acrylamide with a 37.5:1 ratio of cross-link to monomer.

Circular Dichroism and Light Scattering. CD Spectra were acquired on an OLIS DCM-10 circular dichroism spectrophotometer. Pure Psb27 (0.3 mg/mL) buffered with 20 mM sodium phosphate/citrate pH 3.5, 5.5, 7.5 or Tris-phosphoric acid pH 9.0 was analyzed in a 1 mm pathlength cuvette. The molecular mass of purified Psb27 (14 mg/mL and 1 mM DTT) was determined using a Superdex-75 10/30 column connected to a DAWN8+ multiple angle light scattering (MALS) detector (Wyatt Technology, Santa Barbara, CA) and refractive index detector. Data were collected every 0.5 s at a flow rate of 0.5 mL/min. The column was equilibrated with 20 mM sodium phosphate pH 6.7, 50 mM sodium chloride. Data analysis was performed using ASTRA.

NMR Spectroscopy and Spectral Assignments. NMR experiments were performed on unlabeled, ^{15}N enriched or ^{13}C and ^{15}N enriched Psb27 samples (~ 0.5 mM) in sodium phosphate buffer (20 mM, pH 6.7), in the presence of 50 mM sodium chloride, 5 mM TCEP, 5% D_2O and 0.02% sodium azide. Spectra were recorded at 25°C on Bruker AV-500 and AV-800 spectrometers equipped with cryogenically cooled probes, operating at 500 and 800 MHz, respectively. A series of heteronuclear 3D NMR experiments were recorded using ^{13}C , ^{15}N labeled Psb27 protein (S2). Spectra were processed using TOPSPIN (Bruker AG, Karlsruhe, Germany) and analyzed using XEASY (S3).

Resonance Assignment and Structure Determination. The favorable NMR line widths and solution stability found for Psb27 and 5 additional N-terminal residues, (GPLGS, arising from cloning artifacts) allowed spectral acquisition and chemical shift assignment to proceed. The ^1H , ^{15}N , and ^{13}C resonance assignment of the backbone and side-chains of Psb27 was completed using standard multidimensional heteronuclear NMR techniques (S2). Complete backbone resonances and 99.6% of all non-exchangeable side chain resonances were assigned.

Distance and Dihedral Angle Restraints. Distance restraints were measured from the 100 ms mixing time 3D ^{15}N -edited NOESY, ^{13}C -edited NOESY and 2D NOESY spectra. Hydrogen bond constraints were applied within α -helices and the β -sheet at a late stage of the structure calculation. Both ϕ and ψ backbone torsion angles were derived using TALOS (S4). Dihedral angle restraints for ϕ and ψ angles were used as summarized in Table S1.

Structure Calculation and Analysis. Initial structures were calculated using CYANA 2.1 (S5) and then refined with Xplor-NIH (v 2.22) (S6). The coordinates of the 20 conformers that represent the solution structure of Psb27 and the experimental constraints have been deposited in the Protein Data Bank (PDB code 2KMF). Structural statistics for the final set of 20 structures, selected from the 256 calculated structures on the basis of their stereochemical energies, are presented in Table S1. They are energetically reasonable and display acceptable covalent geometry with 99.3% of residues lying in allowed areas of the Ramachandran plot. PROCHECK_NMR (S7) and MOLMOL (S8) were used for the analysis of structure quality. The final structures had no experimental distance violations greater than 0.30 Å or dihedral angle violations greater than 5.0°.

Sequence Alignment and Analysis. Sequences were found by a BLAST (S9) search of the NCBI sequence database and aligned using Clustal-W (S10). Signal sequences were identified using DOLOP (S11, S12) for the cyanobacterial sequences and a report by Peltier and co-workers (S13) for the *Arabidopsis thaliana* sequence and removed. A search for structural homologs of Psb27 in the Protein Data Bank was performed using secondary structural matching as described in (S14). Structure alignment was performed using TOP (S15).

Prediction of Interaction Sites and Docking Simulations. To identify regions on Psb27 that were likely to mediate interactions with other proteins sites SHARP2 was used (S16). Positions identified included the P- Φ -P motif (Pro86, Ile87, Pro88), three conserved residues

belonging to the polar patch (Thr70, Tyr78, Tyr82) and a number of non-conserved polar residues on helices 3 and 4 (Thr66, Ser74, Ser81, Arg85, Glu89, Lys90). A large cluster of residues at the N-terminal end of helix 1 (Thr5 to Gln13) and the adjacent C-terminal region of helix 2 (Arg52-Kys56) together with the C-terminal end of helix 4 (Thr100 to Val110) were also identified as a potential binding site.

Docking simulations were used to determine whether the existing crystal structures of PSII present surfaces complementary to the solution structure of Psb27. The structure of Psb27 presented here was docked to the PSII crystal structure using the PDB entry 3BZ1 (*S17*). Residues 5 to 110 of Psb27 were used in the search. A monomer from the PSII structure was used, and for most searches the extrinsic proteins PsbO, PsbU and PsbV were omitted. GrammX and PatchDock (*S18*, *S19*) were used for the docking calculations. The majority of positions ranked as favorable binding sites in these calculations were on the cytoplasmic rather than luminal side of PSII which is inconsistent with previously published biochemical data (*S20*). Two of the top ten solutions from PatchDock identified a site on the luminal surface of PSII, and this overlapped the PsbV binding site. However, GrammX produced no solutions with Psb27 on the luminal side of PSII. The lack of any biochemical evidence, particularly mutational data, defining specific residues on PSII or Psb27 as potential interaction sites and an insufficient understanding of the components or oligomeric state of PSII in its Psb27 binding mode limits the accuracy of any model.

Table S1. Summary of restraints and structural statistics for the 20 lowest energy structures of Psb27 in aqueous solution at pH 6.7 and 25°C

Experimental constraints

Total	3141
Intraresidue	516
Sequential ($ i-j = 1$)	606
Short range ($1 < i-j < 5$)	909
Long range ($ i-j \geq 5$)	771
Hydrogen bonds	57
Dihedral angles (ϕ , 84; ψ , 84)	168
Rmsd from experimental distance restraints (Å)	0.0018 ± 0.001
Rmsd from experimental dihedral restraints (°)	0.386 ± 0.118

Rmsd from idealized covalent geometry

Bonds (Å)	0.003 ± 0.0001
Angles (°)	0.495 ± 0.013
Impropers (°)	0.345 ± 0.010

Measures of structural quality

E_{LJ} (kcal mol ⁻¹)	-523.3 ± 7.4
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Procheck percentage residues in region of Ramachandran plot
(residues with $S(\phi)$ and $S(\psi) \geq 0.9$: total, 102)

Most favourable	94.6 (96.3)
Additionally allowed	3.9 (2.8)
Generously allowed	0.9 (0.4)
Disallowed	0.7 (0.5)
Angular order: residues with $S(\phi) \geq 0.9$	105
With $S(\psi) \geq 0.9$	104

Overall G-factor	0.44 ± 0.02
Bad contacts/ 100 residues	3.85 ± 1.22

Violations

Experimental distance constraints > 0.30 Å	0
Experimental dihedral constraints $> 5.0^\circ$	0

Coordinate precision

Mean pairwise RMSD (Å)	C ^α , C, N	All heavy atoms
Residues 1-110	1.17 ± 0.39	1.61 ± 0.27
Regular secondary structure ($\alpha 1$ - $\alpha 4$)	0.36 ± 0.07	1.16 ± 0.10

FIGURE S1. Purification of THX-(His)₆-x-Psb27. Fractions collected at key steps of the purification were analyzed by SDS-PAGE. Lanes are: clarified lysate (L); eluate from IMAC column (E); PreScission protease cleavage products (C), and fractions eluting at 72 - 88 mL from the Superdex-75 16/60 column (SE).

FIGURE S2. Psb27 is a monomer. Purified Psb27 was analysed by MALS following separation on a Superdex-75 size exclusion column. The refractive index (black line) as a function of elution volume and the derived molecular mass of the peak is shown (blue points). The calculated molecular mass of monomeric and dimeric recombinant Psb27 is indicated with dashed lines.

FIGURE S3. Circular dichroism analysis of Psb27. Spectra of Psb27 were acquired at 20°C in 20 mM sodium citrate/phosphate pH 3.5 (♦), 5.5 (■) 7.5 (●) or Tris-phosphoric acid pH 9.0 (▲). Mean residue ellipticity (MRE $\text{deg cm}^2 \text{dmol}^{-1}$) was measured as a function of wavelength every 2 nm. Each curve is the baseline subtracted mean of five scans.

A

	1	10	20	30	40	50	
<i>Synechocystis</i> PCC6803	-----	CDSGTGLTGNYSQDTLTVIATLREAI	DLPPQDAPNRQEVQDTARGQINDYISRYRR	55			
<i>P. marinus</i> MIT9313	-----	NGSANAIEIQLSGNYVDDTVNVAHNLQDAI	ALSSDDEGFSAAQEEARLSDNYISRYRR	59			
<i>P. marinus</i> MIT9515	-----	FNSPSYAAKTSMTGQDYTDITISVVKTLQI	AVDTPKDSPNKNEVRDESALITDYISRYRN	60			
<i>P. marinus</i> SS120	-----	FGQSLAAAKPFMSGDFAKDTSVVAQSLKETI	ALSDDDGQLSESKDEALALITAYISRYRN	60			
<i>Synechococcus</i> WH8102	---	CSGDAEARLTGQDYVEDTIAVAHNLREVID	LPQDAANRGEAESEARALINDYMSRYRP	57			
<i>Synechococcus</i> WH7803	---	CGDSTTSLLSGQDYVEDTVAVVHMLQNTL	ALPADAESLQDSEHEAHLINDYMSRYRP	57			
<i>Synechococcus</i> RCC307	---	CGGTPSGLTGTQYVDDTVSVAKSLLSII	AP-EDGQASSEQQQARDLIYNYIARYRP	55			
<i>Anabaena</i> PCC7937	---	CGSPDSLGTGQDYRQDTLAVVNTLRQALE	ITEDSPDKAALQAEARQKINDFSARYQR	55			
<i>Synechococcus</i> PCC7942	---	CTGGGDSAISGNRYRQDTLAVVNTSLRNA	ITLPDDAPEKSAAQAEARQLINDFAARYRR	57			
<i>T. erythraeum</i> IMS1	---	CSN-PSTLSGNYSQDTLTLINSLKTAIEL	PENTEAKSEAQAIAARQAINDYASRYRR	55			
<i>T. elongatus</i> BP-1	---	CANVPTGLTNFREDTLALISSLREAI	ALPENDPNKKAQAARKKLNDFALYRR	56			
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	60	70	80	90	100	110	
<i>Synechocystis</i> PCC6803	KGDAGGLKSFTTMQTALNSLAGYYTSYG-	ARPIPEKLLKRLQLEFTQAERSIERGV--	110				
<i>P. marinus</i> MIT9313	RSKVNNLQSFTTMQTALNSLAGHYNNFA-	NRPLPEELQNRITKELAKAEQTVLRGR--	114				
<i>P. marinus</i> MIT9515	RGMVNKTQSFTTMQTALNAMAGHYKNFA-	TRPLPDKLKERLTKEFSLAEKMLVRES--	115				
<i>P. marinus</i> SS12	RPQVNGTSSFTTMQTALNAMAGHYKTF-	NRPLPENLKERLNKELTRAEKVVAKEI--	115				
<i>Synechococcus</i> WH8102	QPRVNGLSSTFTTMQTALNSLAGHYASYA-	NRPLPEALHDRIAKELGKAESAVRGS--	112				
<i>Synechococcus</i> WH7803	RPQVNGLSSTFTTMQTALNSLQGHYNTYT-	NRPVPEALKTRVEKELSKAEKALRGT--	112				
<i>Synechococcus</i> RCC307	NSRVNGLASFTTMQTALNSLAHYASYN-	NRPLSDDLRLARLEKELHKAELSVVRGS--	110				
<i>Anabaena</i> PCC7937	VNSVSGLSNFTTMRTALNSLAGHYSSYP-	NRVPVQKLKSRLEQEFKQVESALKRGA--	110				
<i>Synechococcus</i> PCC7942	DSRVSGLSSTFTTMQTALNSLAGHYSSYP-	NRVPPEKLLKRLKEKFRMVLEALNREA--	112				
<i>T. erythraeum</i> IMS1	DNSVSSLSSTFTTMRTALDSLQGHYSSYP-	NRVPVQKLKRLAQEFKQVELALKRGT--	110				
<i>T. elongatus</i> BP-1	DDSLRSLSSFTTMQTALNSLAGHYSSYP-	NRPLPEKLLKRLKEKFRMVLEALDREAKS	113				
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B

	1	10	20	30	40	50	
<i>Synechocystis</i> PCC6803	-----	CDSGTGLTGNYSQDTLTVIATLREAI	DLPPQDAPNRQEVQDTARGQINDYISRYRR	55			
<i>P. trichocarpa</i>	-----	TPAFAGSDEEYVKDTEVDINKVRTTVNMD	KNDPNVADAVANLRETSNSWVAKYRR	55			
<i>R. communis</i>	---	PVVPALASDEEYVKETGEVIDKIRSTIN	MEKNDPNVATAVAELRETSNSWVAKYRR	57			
<i>V. vinifera</i>	-----	AQAVAANDEEYVKETADVINKVRTTINMD	KTDPDVATAVADLRDASNSWVAKYRR	55			
<i>A. thaliana</i>	-----	AEDEEYIKDTSAVISKVRSTLSMQKTD	PNVADAVAEALREASNSWVAKYRK	50			
<i>Z. mays</i>	-----	GAARAA--DEEYVSETKEVIGKVRSTIN	LDKSDPSVADAVAEALRELSNSWVAKYRR	54			
<i>O. sativa</i>	-----	GAARAA--DEEYVSETKDVIGKVRSTIN	MDRADPGVADAVAEALRELSNSWVAKYRR	55			
<i>P. sitchensis</i>	-----	APAKASLEEDYSKETKEVINQVKSTID	MEKSNPNTSAAVAALRETSNSWVAKYRR	55			
<i>P. patens</i>	-----	DEDYKSETQDMIGQVRSTLAMEKTD	PNKAAAVAKLRQSGNEWVAKYRK	48			
		: .:* :	:	:			: : *:
	60	70	80	90	100	110	
<i>Synechocystis</i> PCC6803	KGDAGGLKSFTTMQTALNSLAGYYTSYG-	ARPIPEKLLKRLQLEFTQAERSIERGV--	110				
<i>P. trichocarpa</i>	EKALLGRASFRDMYSALNAVTHGYVSFG	PTAPIPSKRRARILEEMDTAEKALSGR--	111				
<i>R. communis</i>	EKTLGRPSFRDIYSALNAVSGHYISFG	PTAPIPAKRKARILEEMDSAEKALLRGR--	113				
<i>V. vinifera</i>	EKALLGRPSFRDMYSALNAVSGHYISFG	PTAPIPAKRKARILEEMDTAEKALLRGR--	111				
<i>A. thaliana</i>	EKALLGKASFRDIYSALNAVSGHYVSFG	PTAPIPAKRKARILEEMDTAEKALLRGR--	106				
<i>Z. mays</i>	EKSLGRPSFREMYSALNAVSGHYISFG	PTAPIPAKRKARILEEMDTAEKALLRGR--	110				
<i>O. sativa</i>	EKSLGRPSFREMYSALNAVSGHYISFG	PTAPIPNKRKARILEEMDTAEKALLRGR--	111				
<i>P. sitchensis</i>	EKQLAGKPSFSNMYSVLNAISGHYISFG	PTAPIPAKRKARILEEMDVAEKALSGR--	111				
<i>P. patens</i>	AKKVAGKPSFSNMYSVLNAISGHYVSFG	ATYPIPAKRKDRILDEVKDAEKALNRGR--	104				
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FIGURE S4. Comparison of Psb27 protein sequences. Combined alignment of plant and cyanobacteria Psb27 sequences was made to determine the sequence conservation between phyla. The N-terminal cysteine of Psb27 from *Synechocystis* sp. PCC 6803 is the first residue of the solution structure and has been assigned as residue 1 in the alignments. (A) Comparison of Psb27 protein sequences from cyanobacteria. (B) Comparison of Psb27 amino acid sequences from plants. Alignments were made using CLUSTAL-W (*S10*) and the

numbering is according to the *Synechocystis* sp. PCC 6803 sequence. Below each alignment, residues completely conserved in both part A and B are indicated (*), residues with only conservative changes are indicated (:), and less-conservative substitutions are indicated by (.). The cyanobacteria Psb27 alignment is comprised of sequences (accession codes are given in brackets) from *Synechocystis* sp. PCC 6803 (P74367), *Prochlorococcus marinus* MIT9313 (Q7TUU3), *Prochlorococcus marinus* MIT9515 (A2BVG9), *Prochlorococcus marinus* SS12 (AE017126), *Synechococcus* sp. WH8102 (Q7U5D5), *Synechococcus* sp. WH7803 (A5GJD2), *Synechococcus* sp. RCC 307 (CT978603), *Anabaena variabilis* PCC 7937 (Q3MFN4), *Synechococcus elongatus* PCC 7942 (Q31RE4), *Trichodesmium erythraeum* IMS101 (Q114S4), and *Thermosynechococcus elongatus* BP1 (Q8DG60). In (B) the N-terminus of processed *Arabidopsis thaliana* Psb27 was taken from Ref (S13). The alignment is comprised of sequences from *Populus trichocarpa* (XP_002306756), *Ricinus communis* (EEF50160), *Vitis vinifera* (XM_002277753), *Arabidopsis thaliana* (Q9LR64), *Zea mays* (NM_001154154), *Oryza sativa* (Q10LV7), *Picea sitchensis* (ABK23991), and *Physcomitrella patens* subsp. *patens* (XM_001770867).

FIGURE S5. Surface exposed charged and hydrophobic residues. Red, acidic; blue, basic; yellow, hydrophobic (Phe, Ile, Leu, Met, Pro, Val). The views are the same as Figure 3.

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