## **Supporting Information**

# Probing the lysine proximal microenvironments within membrane protein complexes by active dimethyl labeling and mass spectrometry

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## **Table of contents**

	MS sequencing analysis of the spinach PSII complex	<b>S</b> 3
Supplementary Results	Comparison of dimethyl labeling of lysines in cyanobacterial, red algal and spinach PSII complexes	<b>S</b> 3
	Evaluating the proximal microenvironments of lysines based on the crystal structure	<b>S4</b>
Figure S1.	Kinetic dimethyl labeling with different labeling time (10 min, 30 min and 60 min) for holo-myoglobin.	S5
Figure S2.	Structure side view of the distribution of lysine residues from cyanobacterial, red algal and spinach PSII.	<b>S</b> 6
Figure S3.	Alignments of part of the PsbB, PsbC, PsbD and PsbO homologous protein sequences from prokaryotic to eukaryotic organisms in the databases.	<b>S7</b>
Figure S4.	Kinetic dimethyl labeling with different labeling time (5 min, 15 min and 45 min) for spinach PSII.	<b>S</b> 8
Figure S5.	The comparison of labeling levels within conserved lysines among cyanobacterial, red algal and spinach PSII complexes.	<b>S9</b>
Table S1.	The covalent labeling levels of lysines in holo- and apo-myoglobin.	S10
Table S2.	The covalent labeling levels of lysines in spinach PSII with the optimized condition.	S11
Table S3.	The covalent labeling levels of lysines in spinach PSII with the elevated condition.	S14
Table S4.	The covalent labeling levels of lysines in cyanobacterial PSII with the optimized condition.	S16
Table S5.	The covalent labeling levels of lysines in red algal PSII with the optimized condition.	S18
Table S6.	The comparison of all PSII lysines detected among cyanobacteria, spinach and red algae.	S21
Table S7.	The results of BSA dimethyl labeling with and without 400 mM sucrose.	S26
Table S8.	The results of MS sequencing analysis of spinach PSII.	S27

#### **Supplementary Results**

#### Control experiments of dimethyl labeling with and without 400 mM sucrose

We suppose that sucrose will not affect dimethyl labeling since it does not contain an amino group and will not alter the pH of reaction solution. Thus, we performed dimethyl labeling experiments of BSA with and without 400 mM sucrose. Two BSA samples (0.1  $\mu$ g/ $\mu$ l) were prepared in HEPES buffer (20 mM, pH 7.0) and HEPES buffer with 400 mM sucrose, respectively. Then the samples were dimethylated by adding 4 mM HCHO and NaBH<sub>3</sub>CN at 25 °C for 30 min, followed by quenching with addition of 40 mM NH<sub>4</sub>HCO<sub>3</sub> and incubation for 20 min. The proteins were then denatured and digested by chymotrypsin at 30 °C overnight with an enzyme to substrate ratio of 1/25 (w/w). The LC-MS/MS analysis results were shown in Table S7. The experiments were replicated three times for each sample. We applied student ttest to investigate the confidence of lysine labeling levels between these two samples (with and without 400 mM sucrose), and only 2 lysines (6%) exhibited significant difference in labeling levels (P < 0.05 in student ttest). Therefore, we considered that even the high concentration of sucrose has little influence on dimethyl labeling.

#### MS sequencing analysis of the spinach PSII complex

MS sequencing of intrinsic membrane proteins is still quite challenging, and the sequence coverage of PSII PsbA and PsbD was only 20-60% in the recent report<sup>1</sup>. We combined two enzymes, trypsin and chymotrypsin, with high complementarity in protein digestion, to improve the proteins sequence coverage. In this way, we were able to achieve high sequence coverage for both the four core subunits (PsbA 87%, PsbB 83%, PsbC 82%, PsbD 84%) and small subunits (< 10 kDa) (Table S8), which are hard in detection due to their small size and easy loss in the purification procedures<sup>2</sup>. For example, PsbE and PsbF, which are tightly associated with the PSII reaction center and play important roles<sup>3</sup>, were fully covered in our results. Furthermore, the oxygen-evolving subunits PsbO, PsbP, PsbQ and PsbR were detected with high sequence coverage ranging from 74 to 96%. Thus, all of the 20 protein subunits in spinach PSII core complex were well characterized in our results with an average sequence coverage of about 80%, which ensured a high coverage rate (about 72%) for lysines within these subunits (Table S8).

Post-translational modifications (PTMs) are crucial to the regulation of PSII biological functions. For example, reversible phosphorylation controls the turnover of certain PSII subunits and behaves differently under variable light intensity<sup>4,5</sup>. Based on our results, some novel PTMs were detected in spinach PSII complex. Four phosphorylated proteins (PsbA, PsbC, PsbD and PsbH) were observed in our results with high reliability, indicating the phosphorylation sites in spinach are identical to those reported in pumpkin<sup>4</sup> and *Arabidopsis thaliana*<sup>5</sup>. Interestingly, Thr-3 and Thr-5 on PsbH could be separately or simultaneously phosphorylated with three phosphorylation statuses coexistent<sup>5,6</sup>

#### Comparison of dimethyl labeling of lysines in cyanobacterial, red algal and spinach PSII complexes

Spinach PsbB-Lys-177, PsbC-Lys-457, PsbD-Lys-318, and PsbB-Lys-418, PsbC-Lys-381, PsbO-Lys-150, PsbO-Lys-160, PsbO-Lys-214, PsbO-Lys-243, PsbO-Lys-274, PsbO-Lys-320, PsbQ-Lys-152, PsbQ-Lys-181, PsbQ-Lys-184, PsbQ-Lys-185 exhibited consistent partially and highly dimethylated labeling levels in different species, respectively. For red algal PsbO-Lys-285, its labeling level was consistent with the corresponding ones in cyanobacteria, yet the sequences around their proximal

interaction sites were not conserved (Table S6), which may imply that the local structure around is somewhat conserved. On the other hand, we also observed some sequences conserved lysines exhibited different dimethylated labeling levels in different species. For example, the labeling levels of spinach PsbC-Lys-339 and PsbH-Lys-32, red algal PsbA-Lys-238, PsbC-Lys-33 and PsbC-Lys-366 were significantly different from the corresponding ones in cyanobacteria (P < 0.05), which might be attributed to the low sequences conservation around their proximal interaction sites (Table S6). For spinach PsbB-Lys-304, PsbO-Lys-128, PsbO-Lys-185, PsbO-Lys-189, PsbO-Lys-270 and PsbO-Lys-317 that are conserved in red algae only, the different labeling levels might reveal the diverse proximal microenvironments of these lysines between these two species (Table S6). According to the PSII crystal structure available, PsbO is located close to PsbU, and PsbU is replaced by PsbP in spinach<sup>7</sup>. Thus, the changes of PSII subunits may lead to the different proximal microenvironments for part of the PsbO lysines (20%) between spinach and red algae.

In cyanobacteria and red algae, PsbO, PsbU and PsbV subunits are required to support the maximal rates of oxygen evolution under physiological conditions<sup>7</sup>. Similarly, PsbO, PsbP and PsbQ subunits play analogous roles in spinach<sup>8</sup>. In our results, the labeling levels of red algal PsbU-Lys-63, PsbU-Lys-93, PsbV-Lys-116, PsbV-Lys-142 and PsbV-Lys-147 (cyanobacterial PsbU-Lys-72, PsbU-Lys-104, PsbV-Lys-129, PsbV-Lys-155 and PsbV-Lys-160) were all consistent between red algae and cyanobacteria, while red algal PsbV-Lys-90 (cyanobacterial PsbV-Lys-103) exhibited a different labeling level due to the low sequence conservation of their proximal interaction sites. For PsbQ, the lysines within conserved sequences between red algae and spinach all exhibited consistent labeling levels. In addition, about 40% of the lysines located in PsbO exhibited similar labeling levels among cyanobacteria, red algae and spinach. These results might suggest that the local structure of the oxygen-evolving center is conserved to a certain extent throughout the evolution from cyanobacteria to red algae and spinach<sup>8</sup>.

#### Evaluating the proximal microenvironments of lysines based on the crystal structure

Among the lysines within highly conserved sequences in at least two species, we found that the labeling levels of 18 lysines had consistent labeling levels and were consistent with their proximal microenvironments in the PSII crystal structure of cyanobacteria within at least two species (Tables S6). Within these 18 lysines, 8 ones were located in the conserved sequences among all of the three species, while 10 ones were located in the conserved sequences of only two species, including 3 partially and 5 highly labeled lysines between cyanobacteria and red algae, and 2 highly labeled lysines between cyanobacteria and red algae, and 2 highly labeled lysines between cyanobacteria and spinach. In contrast, the labeling levels of spinach PsbC-Lys-339 (cyanobacterial PsbC-Lys-317) and red algal PsbC-Lys-366 (cyanobacterial PsbC-Lys-359) were much lower than the corresponding ones in cyanobacteria, which may attribute to the low sequence conservation around their proximal interaction sites. This may suggest possible local structural differences around these 2 lysines in different organisms. For red algal PsbC-Lys-324 and PsbO-Lys-270 (cyanobacterial PsbC-Lys-317 and PsbO-Lys-186), although their proximal interaction sites were conserved partly, their labeling levels were not consistent with the corresponding ones in both cyanobacteria and spinach. This may imply the presence of some unknown interactions in the red algal PSII complex.



Figure S1. Kinetic dimethyl labeling with different labeling time (10 min, 30 min and 60 min) for holo-myoglobin.



**Figure S2.** Structure side view of cyanobacterial PSII. (**a**) The distribution of 54 lysines of cyanobacteria were presented. (**b**) The distribution of 29 lysines of red algae were presented, which were conserved corresponding to cyanobacteria. (**c**) The distribution of 17 lysines of spinach were presented, which were conserved corresponding to cyanobacteria. The image was obtained from PDB file 3WU2. The lysines were colored by labeling levels accordingly (Blue 0 - 10%, Slate 10% - 20%, Cyan 20% - 30%, Green 30% - 40%, Chartreuse 40% - 50%, Lemon 50% - 60%, Yellow 60% - 70%, Orange 70% - 80%, Warm red 80% - 90%, Red 90% - 100%).

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a Thermosynechococcus vulcanus	E P A L D L P <mark>K</mark> M F G I H ··· R P I	°QRLY <mark>K</mark> ALRMG…E AWSAIPE	K L A F Y D Y I G N N P A	K G G L F R T G P M N <mark>K</mark>	K G D G I A Q A W K G H A V F	RN <mark>K</mark> EGE…YAR <mark>K</mark> AIFO	GEIFEF
Thermosynechococcus elongatus (strain BP-1)	E P A L D L P <mark>K</mark> M F G I H ··· R P J	PQRLY <mark>K</mark> ALRMG…DAWSAIPE	K L A F Y D Y I G N N P A	K G G L F R T G P M N <mark>K</mark>	K G D G I A Q A W K G H A V F	RN <mark>K</mark> EGE…YAR <mark>K</mark> AIF(	GEIFEF
Nostoc sp. (strain PCC 7120)	EPALDLP <mark>K</mark> MFGIH…RPJ	° E R L Y <mark>K</mark> A L R M G <sup></sup> E A W S Q I P E	K L A F Y D Y V G N S P A	K G G L F R T G P M V <mark>F</mark>	K G D G I A Q S W Q G H G V F	KD <mark>A</mark> EGR…YAR <mark>K</mark> AQG(	GEIFEF
Synechococcus elongatus (strain PCC 7942)	E P A L D L P <mark>K</mark> M F G I H ··· R P J	° E R L Y <mark>K</mark> A L R M G ··· E A W S S I P E	K L A F Y D Y V G N S P A	K G G L F R T G Q M N <mark>K</mark>	K G D G I A Q G W L G H A V F	KD <mark>K</mark> NGD…YAR <mark>K</mark> AQLO	GEAFEF
Cyanidioschyzon merolae	EPALDLP <mark>K</mark> IFGIH…RPJ	PQRLY <mark>R</mark> ALRMG…EAWSKIPD	K L A F Y D Y I G N N P A	K G G L F R A G P M N <mark>K</mark>	K G D G I A Q A W L G H A V F	QD <mark>K</mark> QGR…YAR <mark>K</mark> AQLO	GEVFEF
Galdieria sulphuraria	EPSLDLP <mark>K</mark> IFGIH…RPJ	QRLY <mark>R</mark> ALRMG…E SWSKIPD	K L A F Y D Y I G N N P A	K G G L F R A G P M N <mark>F</mark>	K G D G I A E AWL G H A V F	VD <mark>R</mark> EGR…YAR <mark>K</mark> AQLO	GEVFEF
Volvox carteri f. nagariensis	KTALDLP <mark>K</mark> IFGIH…RPS	SIRLY <mark>F</mark> GLSMG…EAWSRIPE	K L A F Y D Y I G N N P A	K G G L F R T G A M N <mark>S</mark>	S GDG I AVGWL GHAVF	KD <mark>Q</mark> EGR…YAR <mark>K</mark> AQLO	GEIFEF
Nephroselmis olivacea	EPALDLP <mark>K</mark> IFGIH…RPJ	QRLY <mark>K</mark> ALRMG…E AWAK I P E	K L S F Y D Y I G N N P A	K G G L F R A G A M N <mark>S</mark>	S G D G I A A G W L G H P V F	TD <mark>K</mark> AGN…YAR <mark>R</mark> AQLO	GSVFEF
Arabidopsis thaliana	KPSLDLP <mark>K</mark> IFGIH…RPJ	QRLY <mark>K</mark> GLRMG…E AWAKIPE	K L A F Y D Y I G N N P A	K G G L F R A G S M D N	N G D G I A V G W L G H P V F	RN <mark>K</mark> EGR…YAR <mark>R</mark> AQLO	GEIFEL
Pisum sativum	KPSLDLP <mark>K</mark> IFGIH…RPJ	QRLY <mark>K</mark> GLRMG…EAWSKIPE	K L A F Y D Y I G N N P A	K G G L F R A G S M D N	N G D G I A V G W L G H P I F	RD <mark>K</mark> EGR…YAR <mark>R</mark> AQLO	GEIFEL
Triticum aestivum	KPSLDLP <mark>K</mark> IFGIH…RPJ	QRLY <mark>K</mark> GLRMG…EAWSKIPE	K L A F Y D Y I G N N P A	K G G L F R A G S M D N	N G D G I A V G W L G H P V F	RD <mark>K</mark> EGR…YAR <mark>R</mark> SQLO	GEIFEL
Spinacia oleracea	KPSLDLP <mark>K</mark> IFGIH…RPJ	QRLY <mark>K</mark> GLRMG…EAWSKIPE	K L A F Y D Y I G N N P A	K G G L F R A G S M D N	N G D G I A V G W L G H P I F	RD <mark>K</mark> EGR…YAR <mark>R</mark> AQLO	GEIFEL
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Thermosynechococcus elongatus (strain BP-1)	ARLINLSGKLLG…FGYI	L L K S P F G G E G ··· R G P N G L D L N	K I K N ···· A A A A G F E K	GIDRE			
Nostoc sp. (strain PCC 7120)	ARLINLSGKLLG…FGYI	LLKSPFGGEG…RGPNGLDLD	K I K N ···· A A A G G F E K	GIDRE			
Synechococcus elongatus (strain PCC 7942)	ARLINLSGKLLG…FGYI	LKSPFGGDG…RGPNGLDLD	KLTN ···· AAAAGFEK	GIDRA			
Cyanidioschyzon merolae	ARLINLSGKLLG…FRYI	LKSPFGGDG…RGPNGLDLN	K I K N ···· A A A A G F E K	GINRE			
Galdieria sulphuraria	ARLINVSGKLLG…FGYI	LKSPFGGDG…RGPNGLDLN	K I KN···AAAAG F E K	GINRE			
Volvox carteri f. nagariensis	ARLINLSGKLLG…FGYI	. V K S P F G G D G ··· R G P N G L D L N	K L K N ···· A A A A G F E K	GIDRF			
Nephroselmis olivacea	ARLINLSGKLLG…FGY	LLKSPFGGEG…RGPNGLDLS	K L K N ···· A A A A G F E K	GIDRD			
Arabidopsis thaliana	ARLINLSGKLLG…FGYI	L L K S P F G G E G ··· R G P N G L D L S	RLKK····AAAAGFEK	GIDRD			
Pisum sativum	ARLINLSGKLLG…FGYI	LLKSPFGGEG…RGPNGLDLS	R L K K ···· A A A A G F E K	GIDRD			
Triticum aestivum	ARLINLSGKLLG…FGYI	FLK PPFGGEG…RGPNGLDLS	RLKK····AAAAGFEK	GIDRD			
Spinacia oleracea	ARLINLSG <mark>K</mark> LLG…FGCI	L L <b>K</b> S P F G G E G ··· R G P N G L D L S	<mark>R</mark> L K K ···· A A A A G F E <mark>K</mark>	GIDRD			
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Thermosynechococcus elongatus (strain BP-1)	VSQEIRAAEDPEFETFY	TKNLLLNEGIRAWMAPQDQ	PHENFVFPEEVLP.	RGNAL			
Nostoc sp. (strain PCC 7120)	I SQELRAAEDPEFETFY	TKNILLNEGIRAWMAPQDQ	PHEKFVFPEEVLP	RGNAL			
Synechococcus elongatus (strain PCC 7942)	I SQELRAAEDPEFEIFY	TKNILLNEGIRAWMAPQDQ	PHEKFVFPEEVLP	RGNAL			
Cydnidioschyzon merolae	VSQELRAAEDPEFEIFI	TENTLENEGIRAWMAAQDQ	PHENFVFFEEVLF	RGNAL			
Gaidieria sulphuraria	VSQELRAAEDPEFEIFI	TENELLNEGI RAWMA I QDQ	PHENFVFPEEVLP	RGNAL			
Voivox carteri 1. nagariensis	VSQEIKAAEDFEFEIF						
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Triticum aestivum Spinacia oleracea d Thermosynechococcus vulcanus Thermosynechococcus elongatus (strain BP-1) Nostoc sp. (strain PCC 7120) Synechococcus elongatus (strain PCC 7942) Cvanidioschyzon merolae	V S Q E L RAAE D P E F E F F V S Q E I RAAE D P E F E T F V S Q E I RAAE D P E F E T F V S Q E I RAAE D P E F E T F V S Q E I RAAE D P E F E F F 1 5 0 16 0 K E E P K N K Q E AE F K E E P K N K Q E AE F K E E P K N K Q T AE F K E E P G N K Q K AE F K E E P G N K Q K AE F K E E P G N K Q K AE F K E E P G N K Q K AE F K E E P G N K Q K AE F K E E P G N K Q K AE F K E P G N K Q K AE F K E P G N K Q K AE F K E E P G N K Q K AE F K E P G N K Q K AE F K E P G N K Q K AE F K E P G N K Q K AE F K E P G N K Q K AE F K E P G N K Q K AE F K E P K R G K AE F K E P K R G K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C K K B F K E P K R C	T NLLLNEG I RAWMAAQDQ T NILLNEG I RAWMAAQDQ 170220 23 VPTK LVLFTV NLVASTQ VPTK LVLFTV NLVASTQ VAGKLLLFTV NLVASTS VDTK I.M I FSI NK VASTS	PHEKLVFPEEVLP PHEKLVFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP 0	RGNAL RGNAL RGNAL RGNAL 350 YASIEPA YARIEPA YGRUE YGRUE-ADA			
Triticum aestivum Spinacia oleracea d Thermosynechococcus vulcanus Thermosynechococcus elongatus (strain BP-1) Nostoc sp. (strain PCC 7120) Synechococcus elongatus (strain PCC 7942) Cyanidioschyzon merolae Galdieria suluhuraria	V S Q E L RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' 1 5 0 16 0 K E E P K N K R Q E AE F K E E P K N K R Q T AE F K E E P K N K R Q T AE F K E E P G N K R Q T AE F E E E V C R R G - E S K R E F E E E V V K R G K G S R F F	(T ENLLINEG I RAWMAAQDQ (T N I LLNEG I RAWMAAQDQ (Y N T LLNET I N LVASTQ) (V PT LLN-LFT V N LVASTQ) (V PT LLN-LFT V N LVASTQ) (V PG V L-LFT V N LVASTS) (V D K LW-LFS I K LVASLN) (V D K LW-LFS I K LVASLN) (V D K LVAF L SAKL VASLN)	PHEKLVFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP 0310340 PKVDVKIQGVF PKVDVKIRGIF EKVDVKIRGIF KVDLLKGVF KUDLLKGVF	RGNAL RGNAL RGNAL RGNAL RGNAL YASIEPA YARVE YGRIEPADA YARVE YARVEDDTAAE	- - - -		
Triticum aestivum Spinacia oleracea d Thermosynechococcus vulcanus Thermosynechococcus elongatus (strain BP-1) Nostoc sp. (strain PCC 7120) Synechococcus elongatus (strain PCC 7942) Cyanidioschyzon merolae Galdieria sulphuraria Volvoc catteri f. nagariensis	V S Q E L R A E D P E F E F F V S Q E I R A A E D P E F E T F V S Q E I R A A E D P E F E T F V S Q E I R A A E D P E F E T F V S Q E I R A A E D P E F E T F 150 160 K E E P K N K Q E A E F K E E P A N K Q E A E F K E E P A N K Q E A E F K E E P C N K R Q K A E F E E E V G R R G - E S K R E F E E E V V T K R G K G E S R R E F E E E V V T K G G E T F F	TT NILLINEG I RAWMAAQDQ (TNNILLINEG I RAWMAAQDQ) (TNNILLINEG I RAWMAAQDQ) (TNNIAQDQ) (TNNILLINEG I RAWMAAQDQ) (TNNILLINEG I RAWAAQD	PHERLVFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP Nork VDO-VKIQQVF' Nork VDO-VKIQQVF' Nork VDO-VKIQQVF' Nork VDO-LLKGVF' Nork KIGIF' LKVNVKITKIF	RGNAL RGNAL RGNAL RGNAL RGNAL YASIEPA YARIEPA YARVE YARILPSD YARILPSD YARVVEDDTAAE YGQLSO	- - - -		
Triticum aestivum Spinacia oleracea d Thermosynechococcus vulcanus Thermosynechococcus elongatus (strain BP-1) Nostoc sp. (strain PCC 7120) Synechococcus elongatus (strain PCC 7942) Cyanitónschyzon merolae Galdieria sulphuraria Volvox carteri f. nagariensis Nenhroselmis olivacea	VSQELRAAEDPEFETF' VSQEIRAAEDPEFETF' VSQEIRAAEDPEFETF' VSQEIRAAEDPEFETF' SQEIRAAEDPEFETF' KSQEIRAAEDPEFETF' KEEPKNKRQEAEF KEEPKNKRQEAEF KEEPGNKRQKAEF EEEVGKRGEKREF EEEVVTKRGKGESRREF KEESVFKGGETEF	T NLLLNEG I RAWMAAQDQ T NILLNEG I RAWMAAQDQ VPT KLW-LFTV NLVASTQ VPT KLW-LFTV NLVASTQ VAGKLL-LFTI NLVAQTQ VAGKLL-LFTI NLVAQTQ VDT KLM-LFSI KKLVASLAN VDAKLW-LFSI KKLVAKAAN	PHERLVFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PmKVDVKIQGVF; PmKVDVKIQGVF; PmKVDVKIQGF; NmKVDLLKGVF; NmKLDLLKGVF; NmKLDLLKGVF; NmKLDLLKGVF;	RGNAL RGNAL RGNAL RGNAL RGNAL YASIEPA YARVE YARVE YARVE YARVEDDAA YARILPSD YARVVEDDTAAE YGQLSQ	· · · ·		
Triticum aestivum Spinacia oleracea d Thermosynechococcus vulcanus Thermosynechococcus elongatus (strain BP-1) Nostoc sp. (strain PCC 7120) Synechococcus elongatus (strain PCC 7942) Cyanidioschyzon merolae Galdieria sulphuraria Volvox carteri f. nagariensis Nephroselmis olivacea Arabidowis thaliana	VSQELRAAEDPEFETF' VSQEIRAAEDPEFETF' VSQEIRAAEDPEFETF' VSQEIRAAEDPEFETF' VSQEIRAAEDPEFETF' 150 160 KEEPKNKRQEAEF KEEPANKRQTAEF KEEPANKRQKAEF EEEVYTKRGKGESRREF EEEVYTKRGKGESRREF KEESQFKAGEAEF KEESQFKAGEAEF	(T ENLLINEG I RAWMAAQDQ (T EN I LLNEG I RAWMAAQDQ (V PTELV-LFTVENLVASTQ) (V PTELV-LFTVENLVASTQ) (V PTELV-LFTVENLVASTQ) (V PGEVL-LFTVENLVASTS) (V DEKLW-LFSI EKLVASLNI) (V DAKLW-LFSI EKLVASLNI) (V KTELM-LFTI ELVAKAGN) (V NTELM-LFTI ELVAKAGN) (V NTELW-LFTI ELVAKAGN)	PHEKLVFPEEVLP PHEKLVFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP 0310340 PKVDVKIQGVF PKVDVKIQGVF PKVDVKIRGIF NKVDLLKGIF KVNILLKGIF LKVNIKITGLW PKICDVKIGGW	RGNAL RGNAL RGNAL RGNAL RGNAL YASIEPA YASIEPA YGRIEPADA YARVE YARVEDTAAE YGQLSQ YAQUK			
Triticum aestivum Spinacia oleracea d Thermosynechococcus vulcanus Thermosynechococcus elongatus (strain BP-1) Nostoc sp. (strain PCC 7120) Synechococcus elongatus (strain PCC 7942) Cyanidioschyzon merolae Galdieria sulphuraria Volvox carteri f. nagariensis Nephroselmis olivacea Arabidopsis thaliana Pisum saitvum	V S Q E L RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' K E P K K R Q E AE F K E E P K K R Q E AE F K E E P K K R Q KAE F E E E V G R R G S K R E F E E E V T K R G G E T E F K E E S Q F K G G E T E F K E E S Q F K G G E T E F K E S Q F K M G P I A F	TT NILLINEG I RAWMAAQDQ (TN NILLNEG I RAWMAAQDQ (TN NILTI NILA NILLNEG I RAWMAAQDQ (TN NILLNEG I RAWMA	PHERLVFPEEVLP PHERLVFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFFEEVLP PHENLIFFEEVLP PHENLIFPEEVLP PHENLI	RGNAL         RGNAL         RGNAL         RGNAL         RGNAL         YAS I E PA         YAS I E PA         YAR VE         YAR I L P SD         YAR I L P SD         YAR V VE DD TAAE         YGQL SQ         YAQL K         YAQL E			
Triticum aestivum Spinacia oleracea d Thermosynechococcus vulcanus Thermosynechococcus elongatus (strain BP-1) Nostoc sp. (strain PCC 7120) Synechococcus elongatus (strain PCC 7942) Cyanidioschyzon merolae Galdieria sulphuraria Volvox carteri 1. nagariensis Nephroselmis olivacea Arabidopsis thaliana Pisum sativum Triticum aestivum	V SQEL RAAED PEFETF' V SQE I RAAED PEFETF' V SQE I RAAED PEFETF' V SQE I RAAED PEFETF' V SQE I RAAED PEFETF' 150 160 KEEPKNKRQ EAEF KEEPKNKRQ EAEF KEEPGNKRQ TAEF KEEPGNKRQ KAEF EEEVGKRG ESKREF EEEVVTKRGKGESRREF KEESVFKGG ETEF KEESVFKGG EAEF KADSVSKNA PPEF KSEGGTKNT PLAF	(T ENLLINEG I RAWMAAQDQ (T EN I LLNEG I RAWMAAQDQ (T EN I LLNEF T I EN I VASTQ) (VAGKLL…LFT I EN VASTG) (VAGKLL…LFT I EN VASTG) (VATKLM…LFT I NQ FDGKGT) (VKTKLL…LFT I EN VASGK) (QNTKLM…LFT I NQ LVASGK) (QNTKLM…LFT I NQ LVASGK)	PHERLVFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PMEVDVKIQGVF PKVDVKIQGVF PKVDVKIVGUF KVDLLKGVF KVDLLKGVF KVDLLKGVF KVNIKITGLW TKVDKITGLW PKSKVKIQGVW PKSKVKIQGVW PKQQVW	RGNAL         RGNAL         RGNAL         RGNAL         RGNAL         YAS IEPA         YGR IEPADA         YAR ILPSD         YAQLSQ         YAQLES         YAQLES			
Triticum aestivum Spinacia oleracea d Thermosynechococcus vulcanus Thermosynechococcus elongatus (strain BP-1) Nostoc sp. (strain PCC 7120) Synechococcus elongatus (strain PCC 7942) Cyanidioschyzon merolae Galdieria sulphuraria Volvox carteri f. nagariensis Nephroselmis olivacea Arabidopsis thaliana Pisum sativum Triticum aestivum Spinacia oleracea	V S Q E L RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' V S Q E I RAAE D P E F E T F' 1 5 0 16 0 K E E P K N K R Q E AE E K E E P A N K R Q T AE F K E E P A N K R Q T AE F K E E P G N K R Q T AE F K E E P G N K R Q K AE F E E E V V T K R G K G E S R E F E E E V V T K R G K G E S R E F K E E S Q F K AG E T E F K E E S Q F K AG P F E F K AD S V S K N A P P E F K AE G I Q K N S P P AF K AE G I Q K N S P C F K AE G I Q K N S O F D AF	(T ENLLINEGI RAWMAAQDQ (T ENILLINEGI RAWMAAQDQ (T ENILLINEFI ENILVASTQ) (VAGK LL…LFTY GLVASTS) (VDTALLW…LFTY GLVASTS) (VT ELLW…LFTI QEDAGKGI) (VKT ELLW…LFTI QEDAGKGI) (QNTELLW…LFTY QLVASGK) (QNTELLW…LFTY QLVASGK) (QNTELLW…LFTY QLVASGK) (QNTELLW…LFTY QLVASGK) (QNTELLW…LFTY QLVASGK)	PHEKLVFPEEVLP PHEKLVFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP PHENLIFPEEVLP P····KUD····KIQQF' ·····KUD···LLKGIF' ·····KUD···LLKGF' ·····KUD···LLKGF' ····································	RGNAL         RGNAL         RGNAL         RGNAL         RGNAL         YASIEPA         YASIEPA         YASIEPA         YGRIEPADA         YARVE         YGRIEPADA         YARVEDDTAAE         YGQLSQ         YAQLK         YAQLESN			

**Figure S3.** Alignments of part of the PsbB (**a**), PsbC (**b**), PsbD (**c**) and PsbO (**d**) protein sequences from prokaryotic to eukaryotic organisms. Lysines highlighted were validated in crystal structure analyses. The residues highlighted in red and yellow are completely and partially conserved residues among all of the organisms, respectively.



**Figure S4.** Kinetic dimethyl labeling with different labeling time (5 min, 15 min and 45 min) for spinach PSII.



**Figure S5.** Labeling levels of conserved lysines (a) between cyanobacteria and red algae, (b) between cyanobacteria and spinach, (c) between red algae and spinach and (d) among the all of the three species.

	Holo-myoglobin Replicate 1 Replicate 2 Average										Apo-m	yoglobin					
		Replicate 1	l		Replicate 2		Average	SD		Replicate 1			Replicate 2		Average	SD	
Lysines	Num. of labeled peptides*	Total Num. of peptides	Labeling level	Num. of labeled peptides*	Total Num. of peptides	Labeling level	Labeling level	Labeling level	Num. of labeled peptides*	Total Num. of peptides	Labeling level	Num. of labeled peptides*	Total Num. of peptides	Labeling level	Labeling level	Labeling level	P value
Lys-17	192	343	56%	181	302	60%	58%	2.8%	317	438	72%	321	450	71%	72%	0.7%	0.023
Lys-43	37	46	80%	35	45	78%	79%	1.9%	48	51	94%	48	50	96%	95%	1.3%	0.008
Lys-46	16	36	44%	20	37	54%	49%	6.8%	44	45	98%	42	42	100%	99%	1.6%	0.010
Lys-51	9	9	100%	9	10	90%	95%	7.1%	25	30	83%	36	43	84%	84%	0.3%	0.149
Lys-57	8	9	89%	9	10	90%	89%	0.8%	20	30	67%	30	43	70%	68%	2.2%	0.006
Lys-63	2	4	50%	3	5	60%	55%	7.1%	7	7	100%	7	10	70%	85%	21.2%	0.198
Lys-64	3	4	75%	1	5	20%	48%	38.9%	4	7	57%	7	10	70%	64%	9.1%	0.628
Lys-78	13	20	65%	18	25	72%	69%	4.9%	38	41	93%	23	23	100%	96%	5.2%	0.030
Lys-79	18	20	90%	18	25	72%	81%	12.7%	39	41	95%	22	23	96%	95%	0.4%	0.249
Lys-80	20	20	100%	19	25	76%	88%	17.0%	40	41	98%	22	23	96%	97%	1.3%	0.533
Lys-88	13	15	87%	17	17	100%	93%	9.4%	28	33	85%	16	18	89%	87%	2.9%	0.440
Lys-119	112	113	99%	154	194	79%	89%	14.0%	46	47	98%	47	47	100%	99%	1.5%	0.425
Lys-134	75	121	62%	74	202	37%	49%	17.9%	53	56	95%	52	57	91%	93%	2.4%	0.075
Lys-146	19	20	95%	17	18	94%	95%	0.4%	18	18	100%	17	18	94%	97%	3.9%	0.497
Lys-148	1	4	25%	2	4	50%	38%	17.7%	3	6	50%	3	5	60%	55%	7.1%	0.323

Table S1. The native covalent labeling levels of lysines in holo- and apo-myoglobin with 2 mM dimethyl labeling reagents for 30 min at 25 °C.

\*As 25 °C is a relatively gentle reaction condition, partial labeling was observed. Num. of labeled peptides includes both mono-methyl and dimethyl labeling.

			Replicate 1 Replicate 2				Replicate 3			Average	SD	
PSII subunits	Lysines	Normalized Num. of labeled peptides	Normalized Total Num. of peptides	Labeling level	Normalized Num. of labeled peptides	Normalized Total Num. of peptides	Labeling level	Normalized Num. of labeled peptides	Normalized Total Num. of peptides	Labeling level	Labeling level	Labeling level
PsbB	Lys-177	12	18	65%	6	9	73%	12	24	50%	62%	11.5%
PsbB	Lys-304	1	5	20%	2	7	22%	1	7	20%	21%	1.3%
PsbB	Lys-308	0	5	0%	0	7	0%	0	7	0%	0%	0.0%
PsbB	Lys-321	3	11	30%	4	10	38%	3	9	29%	32%	5.3%
PsbB	Lys-349	6	10	67%				3	4	67%	67%	0.0%
PsbB	Lys-497	16	24	68%	9	16	55%	12	20	60%	61%	6.7%
PsbC	Lys-339	41	57	72%	29	37	79%	34	60	57%	69%	11.3%
PsbC	Lys-457	4	16	27%	3	5	67%	8	21	38%	44%	20.7%
PsbD	Lys-318	36	50	72%	23	39	59%	27	52	53%	61%	9.8%
PsbH	Lys-32	4	6	67%	2	4	60%	5	8	67%	64%	3.8%
PsbO	Lys-98	9	11	80%	12	16	75%	9	14	64%	73%	8.4%
PsbO	Lys-104	33	39	86%	32	39	84%	44	57	77%	82%	4.6%
PsbO	Lys-125				1	2	33%	1	3	50%	42%	11.8%
PsbO	Lys-128				1	2	33%	0	3	0%	17%	23.6%
PsbO	Lys-140	3	5	60%	2	2	100%	5	7	80%	80%	20.0%
PsbO	Lys-185	14	172	8%	13	90	15%	13	146	9%	11%	3.7%
PsbO	Lys-189	10	152	6%	7	80	9%	5	132	4%	6%	2.4%
PsbO	Lys-221	16	23	71%	10	13	81%	17	20	87%	80%	7.7%
PsbO	Lys-270	12	22	55%	6	9	73%	16	24	67%	65%	9.0%
PsbO	Lys-291	5	10	56%	4	9	45%	5	12	44%	48%	6.1%
PsbP	Lys-149	25	30	82%	10	13	76%	24	27	86%	81%	4.7%
PsbP	Lys-177	23	32	70%	17	17	100%	25	40	61%	77%	20.3%
PsbP	Lys-205	19	29	67%	13	13	94%	18	31	58%	73%	18.7%
PsbR	Lys-83	2	3	67%	6	7	78%	5	7	80%	75%	7.1%
PsbB	Lys-418	4	4	100%	6	6	100%	10	12	89%	96%	6.4%
PsbB	Lys-419	3	4	75%	6	6	100%	12	12	100%	92%	14.4%
PsbC	Lys-381	5	5	100%	2	2	100%	9	9	100%	100%	0.0%

Table S2. The native covalent labeling levels of lysines in spinach PSII with 5 mM dimethyl labeling reagents for 30 min at 37 °C.

PebC	L vs 382	5	5	100%	2	2	100%	0	0	100%	100%	0.0%
PsbD	Lys-382	5	5	100%	2	2	100%	5	9	100%	100%	0.0%
PsbD	Lys-13	0	0	1000/	2	2	100%	1	1	100%	100%	0.0%
PSDO	Lys-144	8	8	100%	5	5	100%	9	9	100%	100%	0.0%
PsbO	Lys-150	8	8	100%	5	5	100%	9	9	100%	100%	0.0%
PsbO	Lys-160	8	8	100%	5	5	100%	9	9	100%	100%	0.0%
PsbO	Lys-214	15	16	93%	6	6	100%	14	17	85%	93%	7.7%
PsbO	Lys-243	5	5	100%				5	5	100%	100%	0.0%
PsbO	Lys-274	22	22	100%	9	9	100%	21	24	89%	96%	6.4%
PsbO	Lys-280	18	22	85%	9	9	100%	17	24	72%	86%	13.9%
PsbO	Lys-314	16	19	83%	6	6	100%	14	17	85%	89%	9.3%
PsbO	Lys-317	19	19	100%	6	6	100%	17	17	100%	100%	0.0%
PsbO	Lys-320	19	19	100%	6	6	100%	17	17	100%	100%	0.0%
PsbP	Lys-108	3	3	100%				10	10	100%	100%	0.0%
PsbP	Lys-114	3	3	100%				10	10	100%	100%	0.0%
PsbP	Lys-119	10	10	100%	5	5	100%	17	17	100%	100%	0.0%
PsbP	Lys-121	10	10	100%	5	5	100%	17	17	100%	100%	0.0%
PsbP	Lys-150	29	30	96%	13	13	94%	26	27	95%	95%	1.2%
PsbP	Lys-171	4	4	100%	1	1	100%	3	3	100%	100%	0.0%
PsbP	Lys-224	31	31	100%	27	27	100%	34	34	100%	100%	0.0%
PsbP	Lys-233	29	31	93%	27	27	100%	34	34	100%	98%	4.0%
PsbP	Lys-236	29	31	93%	27	27	100%	34	34	100%	98%	4.0%
PsbP	Lys-251	5	5	100%				3	3	100%	100%	0.0%
PsbP	Lys-254	5	5	100%				3	3	100%	100%	0.0%
PsbP	Lys-255	5	5	100%				3	3	100%	100%	0.0%
PsbQ	Lys-136	4	4	100%				4	4	100%	100%	0.0%
PsbQ	Lys-152	1	1	100%				4	4	100%	100%	0.0%
PsbQ	Lys-173	3	3	100%				5	5	100%	100%	0.0%
PsbQ	Lys-179	3	3	100%				5	5	100%	100%	0.0%
PsbQ	Lys-181	3	3	100%				5	5	100%	100%	0.0%
PsbQ	Lys-184	3	3	100%				5	5	100%	100%	0.0%
PsbQ	Lys-185	3	3	100%				5	5	100%	100%	0.0%
PsbQ	Lys-193	3	3	100%				1	1	100%	100%	0.0%
PsbQ	Lys-206	11	12	91%	9	9	100%	21	22	94%	95%	4.6%
PsbO	Lys-208	10	12	82%	9	9	100%	22	22	100%	94%	10.5%
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PsbQ	Lys-215	11	12	91%	9	9	100%	18	22	82%	91%	8.8%
PsbQ	Lys-230	6	6	100%	5	5	100%	5	7	80%	93%	11.5%
PsbY	Lys-195	5	5	100%	1	1	100%	3	3	100%	100%	0.0%

			Replicate 1			Replicate 2		Average	SD
PSII subunits	Lysines	Normalized Num. of labeled peptides	Normalized Total Num. of peptides	Labeling level	Normalized Num. of labeled peptides	Normalized Total Num. of peptides	Labeling level	Labeling level	Labeling level
PsbB	Lys-304	2	2	100%	15	15	100%	100%	0.0%
PsbB	Lys-308	2	2	100%	15	15	100%	100%	0.0%
PsbB	Lys-321	28	30	93%	13	15	83%	88%	6.5%
PsbB	Lys-418	2	2	100%	77	77	100%	100%	0.0%
PsbB	Lys-419	2	2	100%	77	77	100%	100%	0.0%
PsbB	Lys-497	2	2	100%	3	3	100%	100%	0.0%
PsbC	Lys-339	33	33	100%	24	24	100%	100%	0.0%
PsbC	Lys-381	1	1	100%	5	5	100%	100%	0.0%
PsbC	Lys-382	1	1	100%	5	5	100%	100%	0.0%
PsbC	Lys-457	45	45	100%	5	5	100%	100%	0.0%
PsbD	Lys-318	3	3	100%	8	8	100%	100%	0.0%
PsbH	Lys-32	15	15	100%	4	4	100%	100%	0.0%
PsbO	Lys-98	110	110	100%	26	26	100%	100%	0.0%
PsbO	Lys-144	4	4	100%	35	35	100%	100%	0.0%
PsbO	Lys-150	4	4	100%	35	35	100%	100%	0.0%
PsbO	Lys-160	3	3	100%	35	35	100%	100%	0.0%
PsbO	Lys-185	233	233	100%	412	412	100%	100%	0.0%
PsbO	Lys-189	216	216	100%	410	410	100%	100%	0.0%
PsbO	Lys-214	2	2	100%	24	24	100%	100%	0.0%
PsbO	Lys-221	10	10	100%	26	26	100%	100%	0.0%
PsbO	Lys-270	13	13	100%	14	14	100%	100%	0.0%
PsbO	Lys-274	13	13	100%	14	14	100%	100%	0.0%
PsbO	Lys-280	13	13	100%	14	14	100%	100%	0.0%
PsbO	Lys-291	63	63	100%	10	10	100%	100%	0.0%
PsbO	Lys-314	19	19	100%	31	31	100%	100%	0.0%
PsbO	Lys-317	19	19	100%	31	31	100%	100%	0.0%
PsbO	Lys-320	19	19	100%	31	31	100%	100%	0.0%

Table S3. The native covalent labeling levels of lysines in spinach PSII with 10 mM dimethyl labeling reagents for 2h at 37 °C.

_	PsbP	Lys-92	1	1	100%	1	1	100%	100%	0.0%
	PsbP	Lys-94	1	1	100%	1	1	100%	100%	0.0%
	PsbP	Lys-95	1	1	100%	1	1	100%	100%	0.0%
	PsbP	Lys-108	1	1	100%	5	5	100%	100%	0.0%
	PsbP	Lys-114	1	1	100%	5	5	100%	100%	0.0%
	PsbP	Lys-119	10	10	100%	15	15	100%	100%	0.0%
	PsbP	Lys-121	10	10	100%	15	15	100%	100%	0.0%
	PsbP	Lys-149	27	27	100%	46	46	100%	100%	0.0%
	PsbP	Lys-150	27	27	100%	46	46	100%	100%	0.0%
	PsbP	Lys-171	1	1	100%	14	14	100%	100%	0.0%
	PsbP	Lys-177	16	16	100%	63	63	100%	100%	0.0%
	PsbP	Lys-205	4	4	100%	56	56	100%	100%	0.0%
	PsbP	Lys-224	11	11	100%	22	22	100%	100%	0.0%
	PsbP	Lys-233	11	11	100%	22	22	100%	100%	0.0%
	PsbP	Lys-236	11	11	100%	22	22	100%	100%	0.0%
	PsbQ	Lys-136	4	4	100%	19	19	100%	100%	0.0%
	PsbQ	Lys-152	2	2	100%	10	10	100%	100%	0.0%
	PsbQ	Lys-173	2	2	100%	15	15	100%	100%	0.0%
	PsbQ	Lys-179	2	2	100%	15	15	100%	100%	0.0%
	PsbQ	Lys-181	2	2	100%	15	15	100%	100%	0.0%
	PsbQ	Lys-184	2	2	100%	15	15	100%	100%	0.0%
	PsbQ	Lys-185	2	2	100%	15	15	100%	100%	0.0%
	PsbQ	Lys-193	1	1	100%	13	13	100%	100%	0.0%
	PsbQ	Lys-206	20	20	100%	37	37	100%	100%	0.0%
	PsbQ	Lys-208	20	20	100%	37	37	100%	100%	0.0%
	PsbQ	Lys-215	20	20	100%	37	37	100%	100%	0.0%
	PsbQ	Lys-230	10	10	100%	18	18	100%	100%	0.0%
	PsbR	Lys-71	2	2	100%	4	4	100%	100%	0.0%
	PsbR	Lys-75	2	2	100%	3	3	100%	100%	0.0%
	PsbR	Lys-83	2	2	100%	6	6	100%	100%	0.0%
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			Replicate 1			Replicate 2			Replicate 3			SD
PSII subunits	Lysines	Normalized Num. of labeled peptides	Normalized Total Num. of peptides	Labeling level	Normalized Num. of labeled peptides	Normalized Total Num. of peptides	Labeling level	Normalized Num. of labeled peptides	Normalized Total Num. of peptides	Labeling level	Labeling level	Labeling level
PsbA	lys-238	16	18	88%	14	16	88%	12	15	79%	85%	5.2%
PsbA	lys-310	13	16	79%	21	24	84%	21	28	74%	79%	5.0%
PsbB	lys-307	2	6	40%	4	8	50%	4	8	50%	47%	5.8%
PsbB	lys-320	2	15	15%	2	14	14%	1	12	9%	13%	3.4%
PsbB	lys-331	5	40	11%	4	38	10%	4	41	10%	11%	0.7%
PsbB	lys-348	16	18	88%	13	15	87%	15	19	78%	84%	5.4%
PsbB	lys-377	6	11	50%	5	12	42%	5	11	50%	47%	4.8%
PsbC	lys-26	2	6	40%	6	8	75%	7	9	78%	64%	21.1%
PsbC	lys-193	7	17	40%	5	18	28%	5	17	31%	33%	6.3%
PsbC	lys-435	14	18	75%	14	19	74%	14	19	72%	74%	1.4%
PsbD	lys-307	39	71	55%	30	63	48%	32	58	55%	53%	3.6%
PsbE	lys-73	7	13	55%	8	13	62%	8	16	53%	56%	4.4%
PsbE	lys-84	9	13	73%	12	13	92%	12	16	73%	79%	11.1%
PsbO	lys-51	0	1	0%	1	1	100%	0	1	0%	33%	57.7%
PsbO	lys-141	36	44	82%	38	44	87%	37	45	81%	83%	2.9%
PsbO	lys-192	9	14	67%	8	10	80%	7	11	70%	72%	6.9%
PsbU	lys-24	33	41	81%	30	45	67%	34	43	78%	75%	7.0%
PsbV	lys-103	7	9	75%	10	11	91%	9	12	82%	83%	8.0%
PsbV	lys-150	0	3	0%	0	5	0%	0	4	0%	0%	0.0%
PsbB	lys-136	5	5	100%	4	4	100%	5	5	100%	100%	0.0%
PsbB	lys-226	2	2	100%	1	1	100%	2	2	100%	100%	0.0%
PsbB	lys-372	11	11	100%	11	12	92%	9	11	90%	94%	5.4%
PsbB	lys-417	23	26	87%	20	23	87%	21	24	87%	87%	0.0%
PsbB	lys-422	14	14	100%	40	40	100%	51	51	100%	100%	0.0%
PsbC	lys-57	7	7	100%	8	9	89%	7	8	88%	92%	6.9%
PsbC	lys-301	47	51	91%	39	45	87%	47	54	88%	89%	2.1%
PsbC	lys-317	48	49	98%	43	43	100%	48	50	98%	99%	1.3%

Table S4. The native covalent labeling levels of lysines in cyanobacterial PSII with 5 mM dimethyl labeling reagents for 30 min at 37 °C.

D-LC												
PSDC	lys-357	9	11	80%	10	11	91%	8	9	89%	87%	5.8%
PsbC	lys-359	10	11	90%	10	11	91%	9	9	100%	94%	5.5%
PsbH	lys-19	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbM	lys-34	3	3	100%	3	3	100%	3	3	100%	100%	0.0%
PsbO	lys-55	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbO	lys-57	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbO	lys-67	2	2	100%	1	1	100%	2	2	100%	100%	0.0%
PsbO	lys-84	6	6	100%	6	6	100%	4	4	100%	100%	0.0%
PsbO	lys-121	41	41	100%	40	40	100%	41	41	100%	100%	0.0%
PsbO	lys-158	6	6	100%	5	5	100%	7	7	100%	100%	0.0%
PsbO	lys-176	9	9	100%	11	11	100%	9	9	100%	100%	0.0%
PsbO	lys-186	2	2	100%	3	3	100%	2	2	100%	100%	0.0%
PsbO	lys-201	25	26	96%	19	21	90%	24	25	96%	94%	3.0%
PsbO	lys-232	19	19	100%	18	18	100%	17	17	100%	100%	0.0%
PsbU	lys-16	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbU	lys-47	9	9	100%	9	9	100%	12	12	100%	100%	0.0%
PsbU	lys-51	11	11	100%	12	12	100%	13	13	100%	100%	0.0%
PsbU	lys-72	2	2	100%	3	3	100%	6	6	100%	100%	0.0%
PsbU	lys-104	8	8	100%	5	5	100%	5	5	100%	100%	0.0%
PsbV	lys-43	16	16	100%	12	12	100%	18	18	100%	100%	0.0%
PsbV	lys-50	10	10	100%	10	10	100%	12	12	100%	100%	0.0%
PsbV	lys-56	3	3	100%	3	3	100%	2	2	100%	100%	0.0%
PsbV	lus-136	1	1	100%				2	2	100%	100%	0.0%
PsbV	lys-129	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbV	lys-155	3	3	100%	5	5	100%	4	4	100%	100%	0.0%
PsbV	lys-160	3	3	100%	3	3	100%	3	3	100%	100%	0.0%
PsbX	lys-35				1	1	100%	1	1	100%	100%	0.0%

			Replicate 1			Replicate 2			Replicate 3			SD
PSII subunits	Lysines	Normalized Num. of labeled peptides	Normalized Total Num. of peptides	Labeling level	Normalized Num. of labeled peptides	Normalized Total Num. of peptides	Labeling level	Normalized Num. of labeled peptides	Normalized Total Num. of peptides	Labeling level	Labeling level	Labeling level
PsbA	Lys-238	5	7	71%	6	8	75%	7	9	70%	72%	2.6%
PsbB	Lys-177	6	17	38%	8	18	42%	10	22	48%	42%	5.2%
PsbB	Lys-308	1	10	10%	1	10	10%	2	10	18%	13%	4.7%
PsbB	Lys-321	3	14	21%	2	10	20%	2	11	17%	19%	2.4%
PsbB	Lys-332	2	16	13%	1	11	9%	2	11	17%	13%	3.8%
PsbB	Lys-349	5	5	100%	2	2	100%	1	2	50%	83%	28.9%
PsbB	Lys-373	4	7	57%	6	10	60%	4	8	50%	56%	5.2%
PsbB	Lys-418	5	6	83%	4	5	80%	4	7	57%	73%	14.3%
PsbC	Lys-11	6	7	86%	5	6	83%	6	7	86%	85%	1.4%
PsbC	Lys-33	3	10	30%	3	10	30%	3	9	30%	30%	0.0%
PsbC	Lys-64	2	3	67%	1	2	50%	0	1	0%	39%	34.7%
PsbC	Lys-200	1	11	9%	3	12	25%	3	10	27%	20%	9.9%
PsbC	Lys-324	10	19	56%	9	20	43%	8	16	47%	48%	6.5%
PsbC	Lys-366	5	9	56%	4	8	50%	4	8	50%	52%	3.2%
PsbC	Lys-442	4	6	67%	3	4	75%	3	4	75%	72%	4.8%
PsbD	Lys-316	7	13	54%	7	13	54%	7	11	58%	55%	2.6%
PsbE	Lys-74	5	9	56%	2	6	33%	2	5	40%	43%	11.4%
PsbM	Lys-130	1	3	33%	0	5	0%	1	3	33%	22%	19.2%
PsbO	Lys-170	14	17	88%	10	13	77%	10	13	79%	81%	5.7%
PsbO	Lys-174	14	18	82%	11	14	79%	10	15	69%	77%	7.0%
PsbO	Lys-236	1	1	100%	0	1	0%	0	1	0%	33%	57.7%
PsbO	Lys-270	5	7	71%	4	5	80%	5	6	83%	78%	6.1%
PsbO	Lys-310	29	38	76%	24	31	78%	25	33	74%	76%	1.9%
PsbQ'	Lys-119	0	1	0%	1	2	50%	0	1	0%	17%	28.9%
PsbQ'	Lys-120	1	1	100%	0	2	0%	1	1	100%	67%	57.7%
PsbQ'	Lys-180	20	25	79%	13	17	76%	13	18	74%	76%	2.7%
PsbU	Lys-54	1	2	50%	1	1	100%	2	2	100%	83%	28.9%

Table S5. The native covalent labeling levels of lysines in red algal PSII with 5 mM dimethyl labeling reagents for 30 min at 37 °C.

PsbV	Lys-90	6	16	40%	7	13	54%	9	14	67%	54%	13.3%
PsbB	Lys-137				4	4	100%	2	2	100%	100%	0.0%
PsbB	Lys-304	10	10	100%	10	10	100%	9	10	91%	97%	5.2%
PsbB	Lys-410	3	3	100%	4	4	100%	4	4	100%	100%	0.0%
PsbB	Lys-423	8	9	89%	8	9	89%	7	8	88%	88%	0.8%
PsbC	Lys-240	1	1	100%				1	1	100%	100%	0.0%
PsbC	Lys-364	8	9	89%	8	8	100%	7	8	88%	92%	6.9%
PsbF	Lys-2	1	1	100%	1	1	100%				100%	0.0%
PsbF	Lys-3	1	1	100%	1	1	100%				100%	0.0%
PsbO	Lys-87	10	12	83%	9	10	90%	9	10	91%	88%	4.1%
PsbO	Lys-114	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbO	Lys-123	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbO	Lys-266	7	7	100%	5	5	100%	6	6	100%	100%	0.0%
PsbO	Lys-285	7	8	88%	6	6	100%	7	7	100%	96%	7.2%
PsbO	Lys-313	34	38	89%	26	31	84%	29	33	89%	87%	2.6%
PsbO	Lys-314	37	38	97%	31	31	100%	31	33	94%	97%	2.9%
PsbO	Lys-318	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbQ'	Lys-96	10	10	100%	12	12	100%	9	9	100%	100%	0.0%
PsbQ'	Lys-101	11	11	100%	15	15	100%	11	11	100%	100%	0.0%
PsbQ'	Lys-132	1	1	100%	2	2	100%	1	1	100%	100%	0.0%
PsbQ'	Lys-165	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbQ'	Lys-168	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbQ'	Lys-169	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbQ'	Lys-170	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbQ'	Lys-173	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbU	Lys-15	9	11	82%	9	11	82%	8	8	100%	88%	10.5%
PsbU	Lys-63	2	2	100%	1	1	100%	2	2	100%	100%	0.0%
PsbU	Lys-67	2	2	100%	1	1	100%	2	2	100%	100%	0.0%
PsbU	Lys-93	3	4	75%	4	4	100%	3	3	100%	92%	14.4%
PsbV	Lys-116	1	1	100%	1	1	100%	2	3	67%	89%	19.2%
PsbV	Lys-142	9	10	90%	11	12	92%	6	8	75%	86%	9.2%
PsbV	Lys-147	2	2	100%	3	3	100%	2	2	100%	100%	0.0%
PsbX	Lys-35	1	1	100%	1	1	100%	1	1	100%	100%	0.0%
PsbZ	Lys-35	2	2	100%	1	1	100%	2	2	100%	100%	0.0%

PsbZ	Lys-37	2	2	100%	1	1	100%	2	2	100%	100%	0.0%
												<u> </u>

			Cyanobacteria				Red algae						Spinach					
PSII subunits	lysines	Location	Interaction	Interaction with	Labeling level	SD	PSII subun1its	lysines	Interaction sites conservation <sup>#</sup>	Labeling level	SD	P value	PSII subunits	lysines	Interaction sites conservation <sup>#</sup>	Labeling level	SD	P value
PsbB	Lys-307	Interior	hydrogen bonds	Glu-265 and Leu-173 of PsbB	47%	5.8%	PsbB	Lys-308	$\checkmark$	13%	4.7%	0.001	PsbB	Lys-308	$\checkmark$	0%	0.0%	0.010
PsbB	Lys-320	Near PsbB/PsbD interface	hydrogen bonds	Met-358 and Phe-362 of PsbB	13%	3.4%	PsbB	Lys-321	$\checkmark$	19%	2.4%	0.055	PsbB	Lys-321	$\checkmark$	32%	5.3%	0.006
PsbB	Lys-348	Protein surface	hydrogen bond	Gln-393 of PsbB	84%	5.4%	PsbB	Lys-349	$\checkmark$	83%	28.9%	0.971	PsbB	Lys-349	$\checkmark$	67%	0.0%	0.023
PsbB	Lys-417	PsbB/PsbU interface*	hydrogen bond; electrostatic interaction	Glu-392 of PsbB; Asp-14 of PsbU	87%	0.0%	PsbB	Lys-418	√ (except Asp-14 of PsbU →Gly-12)	73%	14.3%	0.177	PsbB	Lys-418	√ (except Asp-14 of PsbU )	96%	6.4%	0.065
PsbC	Lys-317	PsbC/PsbU interface*	hydrogen-bonds	Gly-94,Asp-96 and Asn-99 of PsbU	99%	1.3%	PsbC	Lys-324	√ (except Gly-94)	48%	6.5%	0.000	PsbC	Lys-339	×	69%	11.3%	0.011
PsbC	Lys-359	PsbC/PsbO interface*	hydrogen bond	Asp-97 of PsbO	94%	5.5%	PsbC	Lys-366	×	52%	3.2%	0.000	PsbC	Lys-381	$\checkmark$	100%	0.0%	0.117
PsbC	Lys-435	PsbC/PsbD interface	hydrogen bond	Glu-217 of PsbD	74%	1.4%	PsbC	Lys-442	$\checkmark$	72%	4.8%	0.651	PsbC	Lys-457	Glu-217 of PsbD→Glu-456 of PsbC	44%	20.7%	0.066
PsbD	Lys-307	PsbA/PsbD interface	electrostatic interaction	Asp-61 of PsbA	53%	3.6%	PsbD	Lys-316	$\checkmark$	55%	2.6%	0.347	PsbD	Lys-318	√	61%	9.8%	0.229
PsbO	Lys-158	PsbD/PsbO interface*	hydrogen bond	Glu-292 of PsbD	100%	0.0%	PsbO	Lys-236	$\checkmark$	33%	57.7%	0.116	PsbO	Lys-243	$\checkmark$	100%	0.0%	NaN
PsbO	Lys-186	Near protein surface*	hydrogen bonds	Asp-156 and Ala-183 of PsbO	100%	0.0%	PsbO	Lys-270	√ (except Ala-183→ Glu-269)	78%	6.1%	0.004	PsbO	Lys-274	√ (except Ala-183)	96%	6.4%	0.374
PsbA	Lys-238	Protein surface	hydrogen bond	Tyr-235 of PsbA	85%	5.2%	PsbA	Lys-238	×	72%	2.6%	0.020						
PsbB	Lys-136	PsbB/PsbH interface*	hydrogen bonds	Pro-12 and Glu-16 of PsbH	100%	0.0%	PsbB	Lys-137	√ (except Glu16→Gln-17)	100%	0.0%	NaN						
PsbB	Lys-331	Interior	hydrogen bonds	Leu-436 and Asn-437 of PsbB	11%	0.7%	PsbB	Lys-332	$\sqrt{(\text{except})}$ Asn-437 $\rightarrow$	13%	3.8%	0.345						
PsbB	Lys-372	Protein surface			94%	5.4%	PsbB	Lys-373		56%	5.2%	0.001						
PsbB	Lys-422	PsbB/PsbO interface*	hydrogen bonds	Pro-173 and Gln-174 of PsbO	100%	0.0%	PsbB	Lys-423	√ (except Gln-177→ Ala-254)	88%	0.8%	0.000						
PsbC	Lys-26	Near protein surface	hydrogen bonds	Ala-111 and Glu-116 of PsbC	64%	21.1%	PsbC	Lys-33	× ×	30%	0.0%	0.048						
PsbC	Lys-57	Protein surface			92%	6.9%	PsbC	Lys-64		39%	34.7%	0.060						
PsbC	Lys-193	Interior	hydrogen bonds	Glu-199 and Ser-204 of PsbC	33%	6.3%	PsbC	Lys-200	$\sqrt{(\text{except})}$	20%	9.9%	0.138						

Table S6. The com	parison of all PSII lysine	s detected among cya	anobacteria, sn	inach and red algae.
	ipanioon of an i on ijome	b detected among eg	anoouerena, sp	maon and roa aiguo.

									Glu-199→ Asp-206)									
PsbC	Lys-357	Protein surface	hydrogen bond	Arg-348 of PsbC	87%	5.8%	PsbC	Lys-364	$\checkmark$	92%	6.9%	0.346						
PsbE	Lys-73	PsbE/PsbD interface	hydrogen bond; electrostatic interaction	Glu-77 of PsbE and Asp-90 of PsbD	56%	4.4%	PsbE	Lys-74	$\checkmark$	43%	11.4%	0.128						
PsbO	Lys-201	Protein surface	hydrogen bonds	Leu-20 and Asp-22 of PsbO	94%	3.0%	PsbO	Lys-285	×	96%	7.2%	0.704						
PsbU	Lys-24	Protein surface	hydrogen bond	Glu-80 of PsbU	75%	7.0%	PsbU	Lys-15(76)	×	88%	10.5%	0.160						
PsbU	Lys-72	Protein surface	hydrogen bond	Glu-69 of PsbU	100%	0.0%	PsbU	Lys-63(124)	$\checkmark$	100%	0.0%	NaN						
PsbU	Lys-104	PsbU/PsbV interface*	hydrogen bonds	Asp-79 and Gly-157 of PsbV	100%	0.0%	PsbU	Lys-93(154)	√ (except Asp-79→ Gly-66)	92%	14.4%	0.374						
PsbV	Lys-103	PsbV/PsbC interface*	hydrogen bonds	Tyr-35 of PsbV and Glu-61 of PsbC	83%	8.0%	PsbV	Lys-90	√ (except Tyr-35 of PsbV)	54%	13.3%	0.032						
PsbV	Lys-129	Near protein surface			100%	0.0%	PsbV	Lys-116		89%	19.2%	0.374						
PsbV	Lys-155	Near protein surface			100%	0.0%	PsbV	Lys-142		86%	9.2%	0.053						
PsbV	Lys-160	PsbA/PsbV, PsbD/PsbV interface	hydrogen bonds	Glu-329 of PsbA and Leu-342 of PsbD	100%	0.0%	PsbV	Lys-147	$\checkmark$	100%	0.0%	NaN						
PsbX	Lys-35	Protein surface			100%	0.0%	PsbX	Lys-35		100%	0.0%	NaN						
PsbH	Lys-19	Protein surface	hydrogen bond	Glu-16 of PsbH	100%	0.0%							PsbH	Lys-32	×	64%	3.8%	0.000
PsbO	Lys-51	Protein surface	electrostatic interaction	Glu-230 of PsbO	33%	57.7%							PsbO	Lys-144	×	100%	0.0%	0.116
PsbO	lys-57	Protein surface			100%	0.0%							PsbO	Lys-150		100%	0.0%	NaN
PsbO	Lys-67	PsbA/PsbO interface*	electrostatic interaction	Asp-103 of PsbA	100%	0.0%							PsbO	Lys-160	$\checkmark$	100%	0.0%	NaN
PsbO	Lys-121	Protein surface	hydrogen bonds	Ser-308 of PsbC; Asp-100 of PsbO	100%	0.0%							PsbO	Lys-214	$\checkmark$	93%	7.7%	0.174
PsbO	Lys-192	Protein surface	hydrogen bond	Asn-145 of PsbO	72%	6.9%							PsbO	Lys-280	×	86%	13.9%	0.206
PsbO	Lys-232	Protein surface			100%	0.0%							PsbO	Lys-320		100%	0.0%	NaN
							PsbB	Lys-177		42%	5.2%		PsbB	Lys-177		62%	11.5%	0.052
							PsbB	Lys-304		97%	5.2%		PsbB	Lys-304		21%	1.3%	0.000
							PsbO	Lys-87		88%	4.1%		PsbO	Lys-104		82%	4.6%	0.183
							PsbO	Lys-114		100%	0.0%		PsbO	Lys-128		17%	23.6%	0.007
							PsbO	Lys-170		81%	5.7%		PsbO	Lys-185		11%	3.7%	0.000
							PsbO	Lys-174		77%	7.0%		PsbO	Lys-189		6%	2.4%	0.000
							PsbO	Lys-266		100%	0.0%		PsbO	Lys-270		65%	9.0%	0.002
							PsbO	Lys-310		76%	1.9%		PsbO	Lys-314		89%	9.3%	0.072
							PsbO	Lys-313		8/%	2.6%		PsbO	Lys-317		100%	0.0%	0.001

							PsbQ'	Lys-132	 100%	0.0%	 PsbQ	Lys-152	 100%	0.0%	NaN
							PsbQ'	Lys-165	 100%	0.0%	 PsbQ	Lys-181	 100%	0.0%	NaN
							PsbO'	Lys-168	 100%	0.0%	 PsbQ	Lys-184	 100%	0.0%	NaN
							PshO'	Lys-169	 100%	0.0%	 PsbO	Lvs-185	 100%	0.0%	NaN
PsbV	Lys-150	Protein surface	hydrogen bonds	Glu-32, Val-33 and Thr-35 of PsbV	0%	0.0%	1002	2,010	10070		1002	290 100	10070		
PsbB	Lys-377	Protein surface	electrostatic interaction	Glu-347 of PsbB	47%	4.8%									
PsbA	Lys-310	Protein surface	hydrogen bond	Gln-58 of PsbE	79%	5.0%									
PsbE	Lys-84				79%	11.1%									
PsbO	Lys-84	Protein surface			83%	2.9%									
PsbC	Lys-301	Protein surface	hydrogen bond	Glu-367 of PsbC	89%	2.1%									
PsbB	Lys-226	Protein surface			100%	0.0%									
PsbM	Lys-34				100%	0.0%									
PsbO	lys-55	PsbB/PsbO interface*	hydrogen bonds	Asp-48 and Ala-336 of PsbB	100%	0.0%									
PsbO	Lys-141	Protein surface			100%	0.0%									
PsbO	Lys-176	PsbB/PsbO interface*	hydrogen bond	Phe-431 of PsbB	100%	0.0%									
PsbU	Lys-16	Protein surface	hydrogen bonds	Thr-85 and Glu-86 of PsbU	100%	0.0%									
PsbU	Lys-47	PsbC/PsbU interface*	hydrogen bond	Lys-301 of PsbC	100%	0.0%									
PsbU	Lys-51	Protein surface			100%	0.0%									
PsbV	Lys-136	Protein surface			100%	0.0%									
PsbV	Lys-43				100%	0.0%									
PsbV	Lys-50	Protein surface			100%	0.0%									
PsbV	Lys-56	PsbJ/PsbV interface*	hydrogen bonds	Ser-39 and Leu-40 of PsbJ	100%	0.0%									
							PsbQ'	Lys-119	17%	28.9%					
							PsbM	Lys-130	22%	19.2%					
							PsbQ'	Lys-120	67%	57.7%					
							PsbQ'	Lys-180	76%	2.7%					
							PsbU	Lys-54	83%	28.9%					
							PsbC	Lys-11	85%	1.4%					
							PsbO	Lys-314	97%	2.9%					
							PsbB	Lys-410	100%	0.0%					
							PsbC	Lys-240	100%	0.0%					
							PsbF	Lys-2	100%	0.0%					
							PsbF	Lys-3	100%	0.0%					
							PsbO	Lys-123	100%	0.0%					
							PsbO	Lys-318	100%	0.0%					
							PsbQ'	Lys-101	100%	0.0%					

PsbQ'	Lys-170	100%	0.0%						
PsbQ'	Lys-173	100%	0.0%						
PsbQ'	Lys-96	100%	0.0%						
PsbU	Lys-67	100%	0.0%						
PsbZ	Lys-37	100%	0.0%						
PsbZ	Lys-35	100%	0.0%						
				Р	sbO	Lys-125	42%	11.8%	
				Р	sbO	Lys-291	48%	6.1%	
				Р	sbB	Lys-497	61%	6.7%	
				Р	sbO	Lys-98	73%	8.4%	
				Р	sbP	Lys-205	73%	18.7%	
				Р	sbR	Lys-83	75%	7.1%	
				Р	sbP	Lys-177	77%	20.3%	
				Р	sbO	Lys-221	80%	7.7%	
				Р	sbO	Lys-140	80%	20.0%	
				P	sbP	Lys-149	81%	4.7%	
				Р	sbQ	Lys-215	91%	8.8%	
				Р	sbB	Lys-419	92%	14.4%	
				Р	sbQ	Lys-230	93%	11.5%	
				Р	sbQ	Lys-208	94%	10.5%	
				Р	sbQ	Lys-206	95%	4.6%	
				P	sbP	Lys-150	95%	1.2%	
				P	sbP	Lys-233	98%	4.0%	
				Р	sbP	Lys-236	98%	4.0%	
				Р	sbC	Lys-382	100%	0.0%	
				Р	sbP	Lys-119	100%	0.0%	
				Р	sbP	Lys-121	100%	0.0%	
				Р	sbP	Lys-171	100%	0.0%	
				Р	sbP	Lys-251	100%	0.0%	
				Р	sbP	Lys-254	100%	0.0%	
				Р	sbP	Lys-255	100%	0.0%	
				Р	sbQ	Lys-136	100%	0.0%	
				Р	sbQ	Lys-173	100%	0.0%	
				Р	sbQ	Lys-179	100%	0.0%	
				Р	sbQ	Lys-193	100%	0.0%	
				р	shP	Lvs-108	100%	0.0%	
				n	ehD	 Lye 114	100%	0.0%	
				r	1 37	Lys-114	100%	0.0%	
				P	SD Y	Lys-195	100%	0.0%	
				P	sbP	Lys-224	100%	0.0%	
				Р	sbD	Lys-13	100%	0.0%	

Student ttest was utilized to evaluate the confidence of labeling levels' difference between cyanobacteria and red algae, cyanobacteria and spinach, and red algae and spinach. The lysines with P < 0.05 were considered as the ones with significant differences in labeling analyses comparison between different species.

The lysines highlighted in light blue were the ones with consistent labeling levels and validated in crystal structure.

Labeling level =  $(n_1/n_2) \times 100\%$ , where  $n_1$  is the normalized spectral counts of covalently labeled peptides;  $n_2$  is the normalized total spectral counts of the corresponding peptides identified by MS. The spectral counts were normalized by using the total numbers of the co-characterized peptides without lysine among replicated experiments.

\* These sites are all located onto or near the protein outer surfaces.

<sup>#</sup> Conservation of proximal interaction sites compared with cyanobacteria.

**Table S7.** The results of BSA dimethyl labeling experiments with and without 400 mM sucrose. The table below showed the active covalent labeling levels of lysines in BSA with 4 mM dimethyl labeling reagents for 30 min at 25 °C.

lucinos		Cont	rol			400 mM S	ucrose		
Tystiles	Replicate 1	Replicate 2	Replicate 3	Average	Replicate 1	Replicate 2	Replicate 3	Average	P value
Lys-36	66.7%	61.1%	53.8%	60.5%	53.3%	50.0%	59.1%	54.1%	0.233
Lys-44	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	NaN
Lys-75	50.0%	33.3%	0.0%	27.8%	0.0%	100.0%		50.0%	0.632
Lys-88	0.0%	33.3%	100.0%	44.4%	100.0%	0.0%		50.0%	0.923
Lys-100	0.0%	0.0%	0.0%	0.0%		0.0%	0.0%	0.0%	NaN
Lys-117	58.1%	64.0%	63.3%	61.8%	63.8%	51.2%	65.2%	60.1%	0.738
Lys-130	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%		0.0%	NaN
Lys-138	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%		100.0%	NaN
Lys-140	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%		100.0%	NaN
Lys-151	0.0%	66.7%	80.0%	48.9%	84.6%	50.0%	57.1%	63.9%	0.606
Lys-155	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	NaN
Lys-156	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	NaN
Lys-197	52.9%	56.3%	40.0%	49.7%	52.6%	50.0%	50.0%	50.9%	0.831
Lys-245	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	75.0%	NaN
Lys-248	27.3%	41.7%	27.3%	32.1%	27.3%	20.0%	25.0%	49.1%	0.204
Lys-285		100.0%	50.0%	75.0%	50.0%	50.0%	0.0%	33.3%	0.239
Lys-297	50.0%	54.5%	55.6%	53.4%	38.5%	45.8%	50.0%	44.8%	0.085
Lys-299	41.7%	30.0%	50.0%	40.6%	50.0%	37.5%	44.4%	44.0%	0.643
Lys-304	75.0%	80.0%	75.0%	76.7%	80.0%	62.5%	77.8%	73.4%	0.603
Lys-336	40.0%	22.2%	30.0%	30.7%	41.7%	20.0%	38.5%	33.4%	0.772
Lys-340	20.0%	22.2%	10.0%	17.4%	33.3%	40.0%	38.5%	37.3%	0.010
Lys-346	28.6%	33.3%	57.1%	39.7%	50.0%	57.1%	57.1%	54.8%	0.175
Lys-386	59.1%	55.0%	60.0%	58.0%	69.2%	61.5%	55.6%	62.1%	0.391
Lys-399	23.1%	0.0%	8.3%	10.5%	13.6%	12.5%	17.6%	14.6%	0.584
Lys-401	56.7%	33.3%	47.4%	45.8%	57.1%	59.5%	65.7%	60.8%	0.107
Lys-412	57.6%	54.3%	63.6%	58.5%	61.8%	54.3%	54.5%	56.9%	0.680
Lys-420	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	92.6%	97.5%	0.374
Lys-523	23.8%	15.8%	20.0%	19.9%	18.2%	21.4%	15.4%	18.3%	0.625
Lys-528	100.0%	92.3%	100.0%	97.4%	100.0%	100.0%	100.0%	100.0%	0.374
Lys-568	75.0%	20.0%	40.0%	45.0%	42.9%	25.0%	20.0%	29.3%	0.420
Lys-580	65.9%	70.7%	61.5%	66.0%	51.4%	57.1%	58.1%	55.5%	0.036
Lys-587	41.5%	36.6%	43.6%	40.5%	28.6%	40.0%	35.5%	34.7%	0.209
Lys-597	50.0%	50.0%	50.0%	50.0%	50.0%	50.0%	66.7%	55.6%	0.374

PSII	Sequence	Lysine	Molecular	Dest transprintional modifications
subunits	coverage	coverage	weight (kDa)	Post-transcriptional mounications
D-1-A	970/		28	N-acetyl-O-phosphothreonine (Thr-2) <sup>5,6</sup> ;
PSDA	8/%	NA	38	acetyl-threonine (Thr-286)
PsbB	83%	92%	56	
PsbC	82%	73%	50	N-acetyl-O-phosphothreonine (Thr-15) <sup>5</sup>
PsbD	84%	100%	39	N-acetyl-O-phosphothreonine (Thr-2) <sup>5,6</sup>
PsbE	100%	NA	9.3	
PsbF	100%	NA	4.5	N-acetyl-threonine (Thr-2)
PsbH	100%	100%	7.7	phosphothreonine (Thr-3, Thr-5) <sup>5,6</sup>
PsbI	100%	100%	4.2	
PsbJ	23%	NA	4.1	N-acetyl-alanine (Ala-2)
PsbK	41%	0%	6.7	
PsbL	100%	NA	4.5	N-acetyl-threonine (Thr-2)
PsbM	59%	100%	3.8	
PsbO	96%	96%	35	
PsbP	82%	75%	23	
PsbQ	74%	100%	17	
PsbR	84%	75%	10	
PsbS	24%	30%	21	
PsbT	24%	0%	3.8	
PsbW	34%	NA	14.2	
PsbY	49%	33%	5+5*	
PsbZ	71%	100%	6.5	
CB4	48%	80%	23	N-acetyl-alanine (Ala-52)

Table S8. The results of MS sequencing analysis of spinach PSII.

NA: no lysine within the subunit

\* two mature peptides PsbYa and PsbYb

### **Supporting References**

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