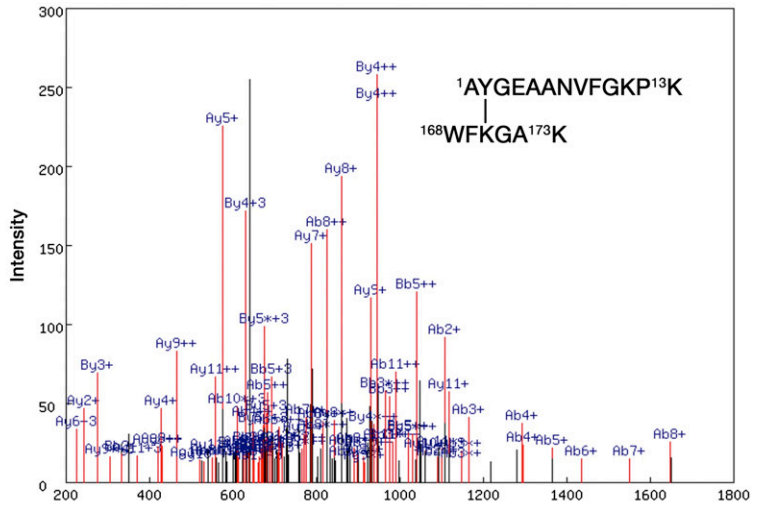
Supporting Information

Mummadisetti et al. 10.1073/pnas.1415165111

A.



B. Chain A

#	b'+3	b*+3	b+3	b'++	b*++	b++	b'+	b*+	b+	seq	y'+3	y*+3	y+3	y'++	y*++	y++	y'+	y*+	y+	#
1			24.69			36.53			72.04	A	736.39	736.72	742.40	1104.09	1104.58	1113.09	2207.16	2208.15	2225.18	M
2	364.20	364.52	370.20	545.79	546.28	554.79	**1090.57**	**1091.56**	**1108.58**	Y	712.71	713.04	**718.72**	1068.57	1069.06	1077.57	2136.13	2137.11	2154.14	12
3	383.20	383.53	389.21	574.30	574.79	583.31	**1147.59**	**1148.58**	**1165.60**	G	367.20	367.53	**373.20**	**550.30**	**550.79**	**559.30**	1099.59	**1100.57**	**1117.60**	11
4	426.22	426.54	432.22	**638.82**	**639.31**	**647.83**	1276.64	1277.62	**1294.65**	E	348.19	348.52	354.20	**521.79**	**522.28**	**530.79**	1042.57	1043.55	**1060.58**	10
5	449.90	450.22	455.90	**674.34**	**674.83**	**683.35**	1347.67	1348.66	**1365.68**	A	305.18	**305.51**	311.18	457.27	457.76	**466.27**	913.53	**914.51**	**931.54**	9
6	473.57	473.90	479.58	**709.86**	**710.35**	**718.86**	1418.71	1419.69	**1436.72**	A	281.50	281.83	287.50	**421.75**	**422.24**	**430.75**	842.49	**843.47**	**860.50**	8
7	511.59	511.92	517.59	**766.88**	**767.37**	**775.89**	1532.75	1533.74	**1550.76**	N	257.82	258.15	263.83	386.23	386.72	395.23	771.45	**772.44**	**789.46**	7
8	544.61	544.94	**550.62**	**816.41**	**816.91**	**825.42**	1631.82	1632.81	**1649.83**	V	219.81	220.14	**225.81**	329.21	329.70	338.21	657.41	658.39	**675.42**	6
9	593.63	593.96	599.64	**889.95**	**890.44**	**898.95**	1778.89	1779.87	1796.90	F	186.78	187.11	192.79	279.67	280.17	288.68	**558.34**	**559.32**	**576.35**	5
10	**612.64**	**612.97**	618.65	918.46	918.95	**927.46**	1835.91	1836.90	1853.92	G	137.76	138.09	143.77	206.14	206.63	215.14	411.27	412.26	**429.28**	4
11	655.34	655.67	**661.34**	**982.51**	**983.00**	**991.51**	1964.01	1964.99	1982.02	K	118.75	119.08	124.76	177.63	178.12	186.63	354.25	355.23	**372.26**	3
12	**687.69**	**688.02**	**693.69**	**1031.03**	**1031.53**	**1040.04**	2061.06	2062.04	2079.07	P	76.06	76.38	82.06	113.58	114.07	122.59	**226.16**	**227.14**	**244.17**	2
										K	43.71	44.03	49.71	65.05	65.55	74.06	129.10	130.09	147.11	1

Chain B

#	b'+3	b*+3	b+3	b'++	b*++	b++	b'+	b*+	b+	seq	y'+3	y*+3	y+3	y'++	y*++	y++	y'+	y*+	y+	#
1			63.03			94.05			187.09	W										
2			112.06			167.58			**334.16**	F	**674.37**	**674.69**	**680.37**	1011.05	1011.54	**1020.05**	2021.09	2022.07	2039.10	5
3	645.01	645.33	**651.01**	**967.00**	**967.50**	**976.01**	1933.00	1933.98	1951.01	K	**625.34**	**625.67**	**631.35**	**937.51**	**938.00**	**946.52**	1874.02	1875.00	1892.03	4
4	**664.01**	**664.34**	**670.02**	995.51	996.01	1004.52	1990.02	1991.01	2008.03	G	86.39	86.72	92.40	129.08	129.58	138.09	257.16	258.14	**275.17**	3
5	**687.69**	**688.02**	**693.69**	**1031.03**	**1031.53**	**1040.04**	2061.06	2062.04	2079.07	A	67.38	67.71	73.39	100.57	101.07	109.58	200.14	201.12	218.15	2
										K	43.71	44.03	49.71	65.05	65.55	74.06	129.10	130.09	147.11	1

C.

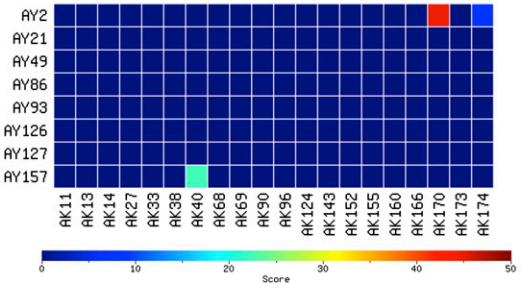


Fig. S1. Quality of the mass spectrometry used in this study. Shown are the mass spectrometry data obtained for the median *P* value of cross-linked product ^{2}Y–^{170}K (Table S1; *P* value = 7.9×10^{-11}). (*A*) Mass spectra obtained for this peptide. Identified ions are shown in red; those not identified are in black. (*B*) Table of predicted ions from this peptide. Identified ions are shown in red; those not identified are in black. Note the nearly complete Y- and B-ion series obtained. (*C*) Heat map that assists in the identification of cross-linked species. The identified peptide is shown in red. The PsbP tyrosyl residues are shown on the *y* axis, and lysyl residues are shown on the *x* axis. The additional putative cross-linked species identified (^{2}Y–^{174}K and ^{157}Y–^{40}K) exhibited *P* values $> 1 \times 10^{-3}$ and consequently were rejected.

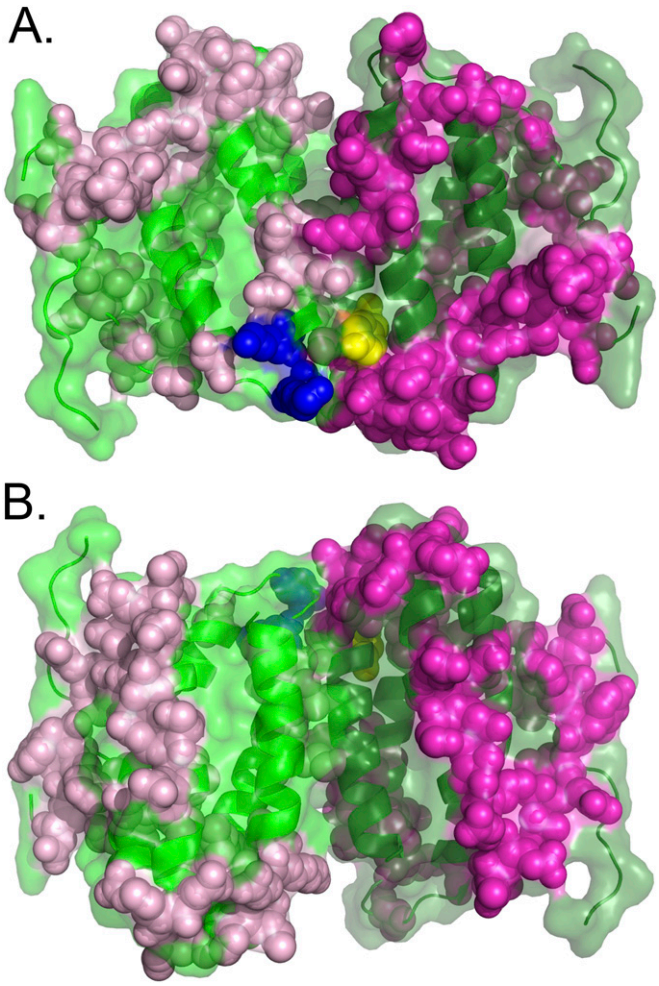


Fig. S2. Radiolytic mapping of a PsbQ dimer. (*A*) View of face I of the PsbQ protein dimer, which is proposed to be more exposed to the bulk solvent. Two unmodified domains, however, are evident and may interact with unidentified PS II components. (*B*) View of face II of the PsbQ protein dimer, which is proposed to face the core components of PS II. Labeling is primarily observed at the periphery of the dimer. The two PsbQ molecules are shown as light and dark green. Oxidatively modified residues are shown in light pink and magenta spheres. The lysyl residues ^{98}K and ^{101}K are shown as blue spheres, and the tyrosyl residue ^{133}Y is shown as yellow spheres.

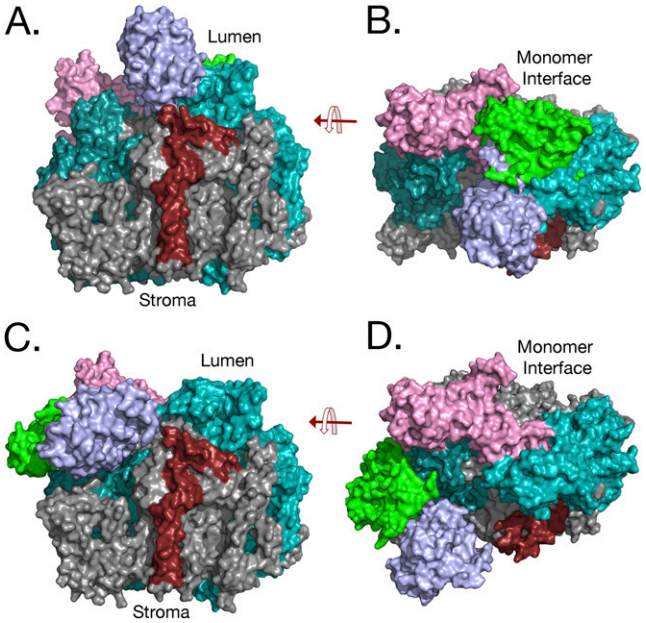


Fig. S3. Models for the interaction of PsbP and PsbQ with higher plant PS II. These represent two of the many possible models consistent with our findings regarding the interactions of these components. Single PS II monomers are shown. (*A* and *B*) This model is based on the proposal of Liu et al. (1), which positions PsbQ at the lumenal surface of the PS II dimer, proximal to the PS II monomer–monomer interface. (*C* and *D*) This model is based on the proposal of Ido et al. (2), which positions PsbQ at the periphery of the PS II dimer, adjacent to CP43. It should be noted that in our model the position of PsbQ had to be significantly altered compared with the positioning proposed in ref. 2 to satisfy distance constraints imposed by our cross-linking experiments. *A* and *C* are views within the plane of the membrane, facing the PS II monomer. *B* and *D* are views of the lumenal surface of the PS II monomer. The PS II monomer–monomer interface is indicated. CP43, dark teal (to the left); CP47, light teal (to the right); PsbE, red; PsbO, pink; PsbP, blue; PsbQ, green; all other PS II subunits are shown in gray. The location of PsbR is not illustrated.

1. Liu H, et al. (2014) MS-based cross-linking analysis reveals the location of the PsbQ protein in cyanobacterial photosystem II. *Proc Natl Acad Sci USA* 111(12):4638–4643.
2. Ido K, et al. (2014) Cross-linking evidence for multiple interactions of the PsbP and PsbQ proteins in a higher plant photosystem II supercomplex. *J Biol Chem* 289(29):20150–20157.

Table S1. Cross-linked residues of the PsbP and PsbQ proteins associated with the PS II complex

Observed crosslinked products	Cross-linked residues	*P* value*	Crystal structure distance†
Internal PsbP cross-links	^{1}A–^{174}K	7.0×10^{-5}	NA
	^{1}A–^{170}K	3.9×10^{-13}	NA
	^{1}A–^{173}K	1.5×10^{-11}	NA
	^{2}Y–^{170}K	7.9×10^{-11}	NA
	^{11}K–^{13}K	1.2×10^{-13}	NA
	^{11}K–^{14}K	1.0×10^{-8}	NA
	^{11}K–^{174}K	2.5×10^{-4}	NA
	^{13}K–^{174}K	6.3×10^{-5}	NA
	^{14}K–^{174}K	5.0×10^{-16}	NA
	^{33}K–^{174}K	6.3×10^{-9}	9.3 Å
	^{40}K–^{155}K	1.9×10^{-10}	14.4 Å
PsbP–PsbQ cross-links	PsbP:^{93}Y–PsbQ:^{1}E	2.0×10^{-13}	NA
	PsbP:^{96}K–PsbQ:^{1}E	1.9×10^{-18}	NA
Internal PsbQ cross-links	^{53}K–^{96}K	3.9×10^{-9}	8.5 Å
	^{98}K–^{133}Y	3.9×10^{-8}	33.1 Å
	^{101}K–^{133}Y	1.0×10^{-15}	30.0 Å

Because these are cross-linked with BS3, the distances between the cross-linked atoms are ≤11.4 Å. *P* values were evaluated as described in *Materials and Methods*. NA, not applicable, as residues were not resolved in the crystal structure.

*Probability that the peptide match is a random occurrence.

†Distances reported for the crystal structure of isolated PsbP (PDB ID code 2VU4) and PsbQ (PDB ID code 1VYK) are measured between the putative cross-linked atoms (amine nitrogens or hydroxyl oxygens).

Table S2. Radiolytically oxidatively modified residues of PsbP and PsbQ

Modified protein residues

PsbP: ^{14}K+ca*, ^{15}N+go*, ^{17}E+gam, ^{19}M+go, ^{20}P+ca, ^{21}Y+go, ^{22}N+go, ^{24}D+gam, ^{27}K+ca, ^{39}E+go/ca, ^{40}K+ca, ^{41}E+gam, ^{49}Y+go, ^{50}E+gam, ^{51}D+ca/gam, ^{52}N+go, ^{65}P+ca/go, ^{66}T+gam/go, ^{67}D+gam, ^{68}K+ca, ^{69}K+go, ^{77}P+ca, ^{78}E+gam, ^{79}D+go, ^{98}D+ca/gam*, ^{100}E+ca/gam*, ^{103}F+go*, ^{120}V+ca/go, ^{121}V+go, ^{122}D+gam, ^{124}K+ca/go, ^{125}K+ca, ^{139}D+ca*, ^{140}E+gam*, ^{143}K+go, ^{168}W+to, ^{172}A+go, ^{173}K+ca, ^{174}K+ca/go, ^{175}F+go, ^{176}V+ca/go, ^{177}E+gam, ^{179}A+go, ^{181}S+go, ^{182}S+go

PsbQ: ^{1}E+gam, ^{2}A+go, ^{3}R+ca/go, ^{4}P+go, ^{5}I+go, ^{36}D+gam, ^{37}R+ca, ^{38}F+go, ^{39}Y+go, ^{40}L+ca, ^{41}Q+go, ^{43}L+go, ^{45}P+ca, ^{46}T+go, ^{47}E+gam, ^{48}A+go, ^{50}Q+go, ^{51}R+ca, ^{54}V+ca, ^{55}S+stcb, ^{56}A+go, ^{57}S+go/stcb, ^{58}E+gam, ^{59}I+ca, ^{60}L+go, ^{61}N+go, ^{62}V+ca, ^{67}D+go, ^{68}R+go, ^{77}D+gam, ^{78}L+go, ^{79}R+ca, ^{92}V+ca, ^{94}S+go, ^{101}K+go, ^{103}S+go, ^{104}L+go, ^{105}Q+ca/gam/go, ^{106}E+gam, ^{107}L+ca, ^{108}T+go, ^{109}S+go, ^{110}K+ca, ^{118}L+ca/go, ^{119}D+gam, ^{120}H+go, ^{121}A+go, ^{122}A+go, ^{123}K+ca, ^{124}I+ca, ^{125}K+ca, ^{124}I+ca, ^{125}K+ca, ^{126}S+go, ^{127}P+ca/go, ^{128}T+go, ^{129}E+gam, ^{130}A+go, ^{131}E+gam, ^{132}K+ca, ^{133}Y+go

Individual residues are listed along with the modifications observed. In some instances, different modifications were observed for the same residue on different peptides. Data were collected for 0, 4, 8, and 16 s of irradiation. The unions of these sets of data are shown in the table. For a complete list of oxidative modification types, the amino acids targeted, and mass modifications searched for in this study, see ref. 1. ca, carbonyl addition (+14 amu); gam, Glu/Asp modification (–30 amu); go, general oxidation (+16 amu); stcb, serine/threonine carbonyl (–2 amu); to, triple oxidation (+48 amu).
*Residue not resolved in the crystal structure of spinach PsbP (PDB ID code 1VYK).

1. Takamoto K, Chance MR (2006) Radiolytic protein footprinting with mass spectrometry to probe the structure of macromolecular complexes. *Annu Rev Biophys Biomol Struct* 35:251–276.