SUPPORTING INFORMATION

TITLE: Formation of the High-Spin S_2 State Related to the Extrinsic Proteins in the Oxygen Evolving Complex of Photosystem II

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The supporting information containing Figure S1-S7 and Table S1

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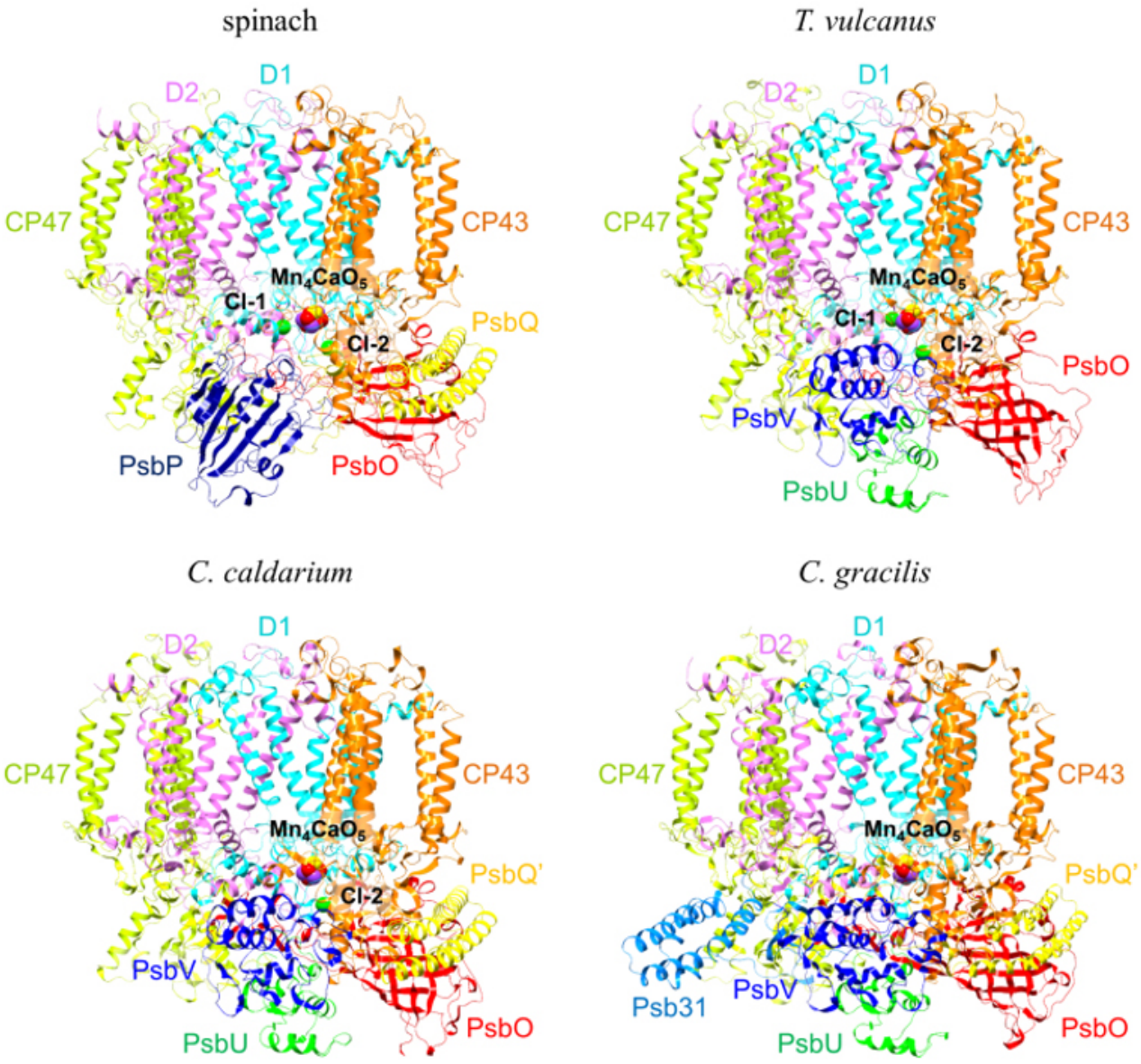

Figure S1. X-ray crystallographic and cryo-electron microscopic structures of PSII protein complexes from spinach (PDB: 3jcu[1]), *T. vulcanus* (PDB: 4ub6[2]), *C. caldarium* (PDB: 4yuu[3]), and *C. gracilis* (PDB: 6jlu[4]).

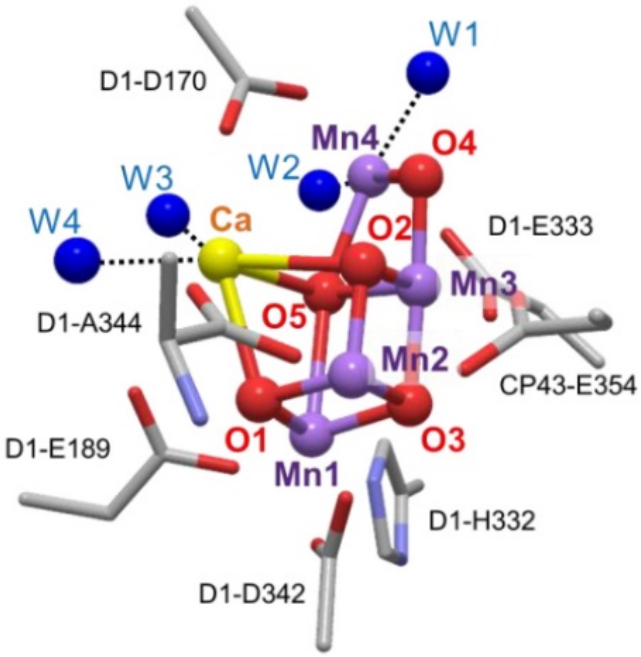

Figure S2. The structure of the water oxidizing center of photosystem II (PDB:4ub6). Mn_4CaO_5 cluster and its direct ligands, seven amino acid residues of D1 and CP43 subunits, and four water molecules are shown. Purple, yellow, red, and blue spheres represent Mn, Ca, O, and water oxygen, respectively.

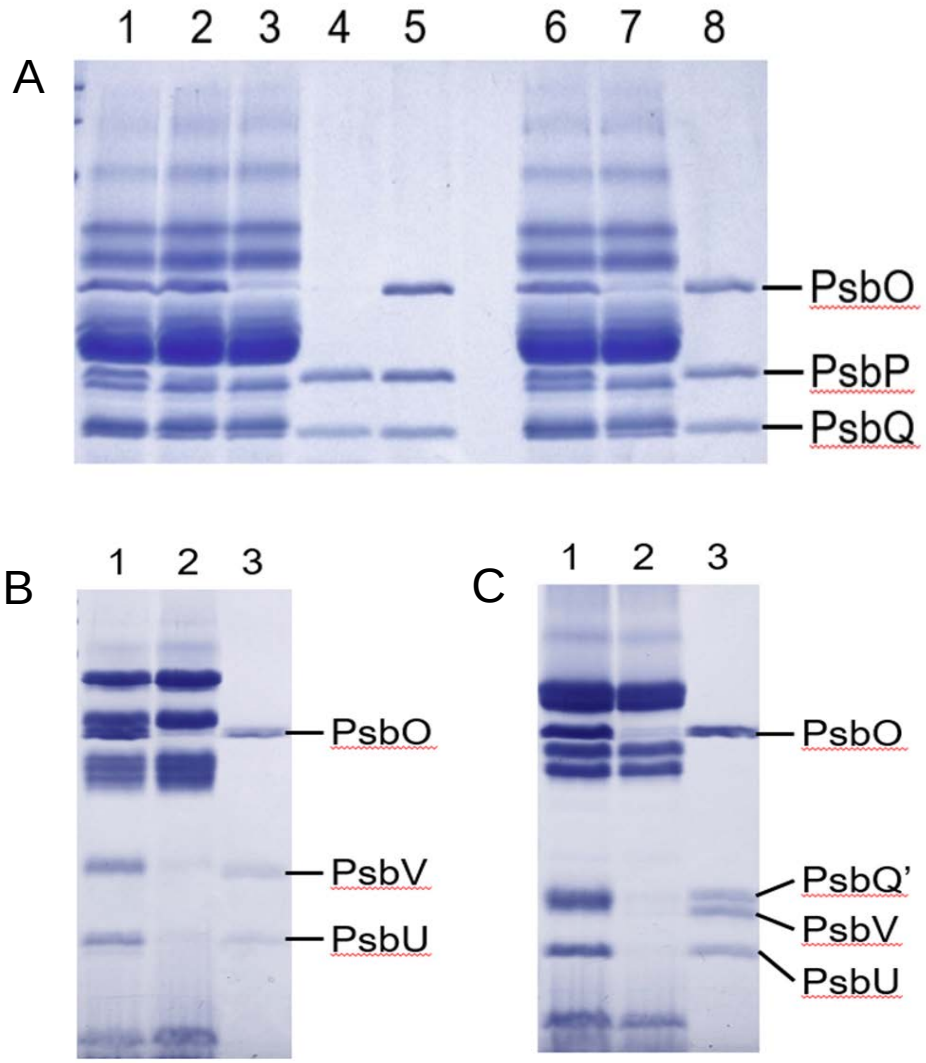

Figure S3. SDS-PAGE analysis of (A) the PSII membranes from spinach and the PSII core complexes isolated from (B) *T. vulcanus* and (C) *C. merolae*. (A) lanes 1 and 6, untreated; lane 2, NaCl-treated; lanes 3 and 7, $CaCl_2$-treated; lane 4, supernatant after NaCl treatment; lanes 5 and 8, supernatant after $CaCl_2$ treatment. (B-C) lane 1, untreated; lane 2, $CaCl_2$-treated; lane 3, supernatant after $CaCl_2$ treatment. SDS-PAGE was performed as described previously[5,6] with slight modifications. The loading buffer was 400 mM sucrose, 3% (W/V) lithium lauryl sulfate, 70 mM dithiothreitol, 60 mM Tris/HCl (pH 7.5), and bromophenol blue for spinach, or 300 mM sucrose, 3% (w/v) lithium lauryl sulfate, 70 mM dithiothreitol, 20 mM Mes/NaOH (pH6.5), and bromophenol blue for *T. vulcanus* and *C. merolae*.

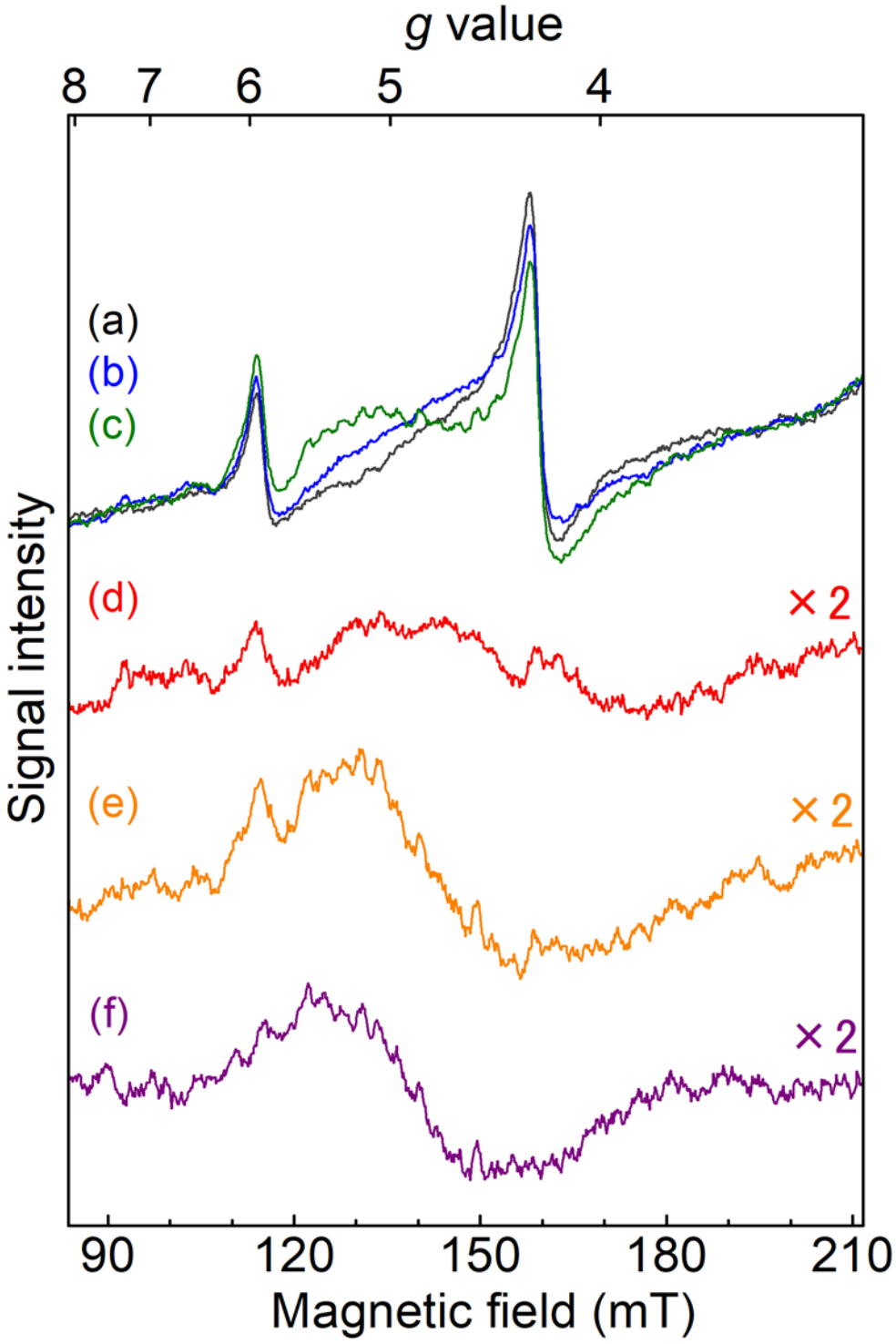

Figure S4. EPR spectra of the PsbO/V/U-depleted PS II from *T. vulcanus* in the presence of 1 M $CaCl_2$. (a, black) The S_1 state; (b, blue) illuminated at 200 K; (c, green) after subsequent annealing at 290 K. Spectra (d-f) are the difference spectra of (d) b-minus-a, (e) c-minus-a, and (f) c-minus-b. Experimental conditions: microwave frequency, 9.49 GHz; microwave power, 0.64 mW; modulation frequency, 100 kHz; modulation amplitude, 9 G.

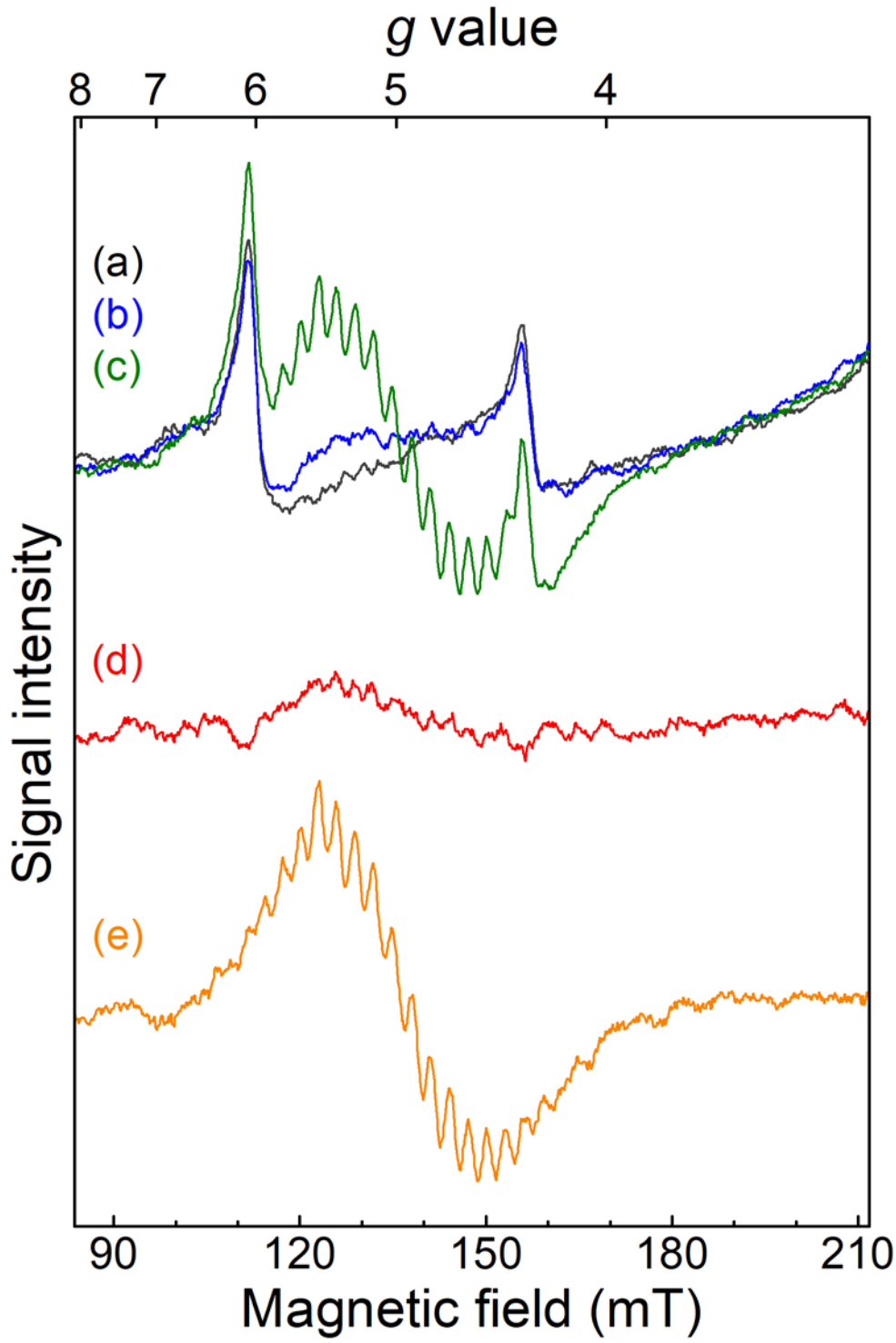

Figure S5. EPR spectra of the Psb O/V/U/Q'-depleted PS II from *C.merolae* in the presence of 1 M $CaCl_2$. (a, black) The S_1 state; (b, blue) illuminated at 200 K; (c, green) after subsequent annealing at 290 K. Spectra (d-f) are the difference spectra of (d) b-minus-a, and (e) c-minus-a. Experimental conditions are the same as those for Figure S3.

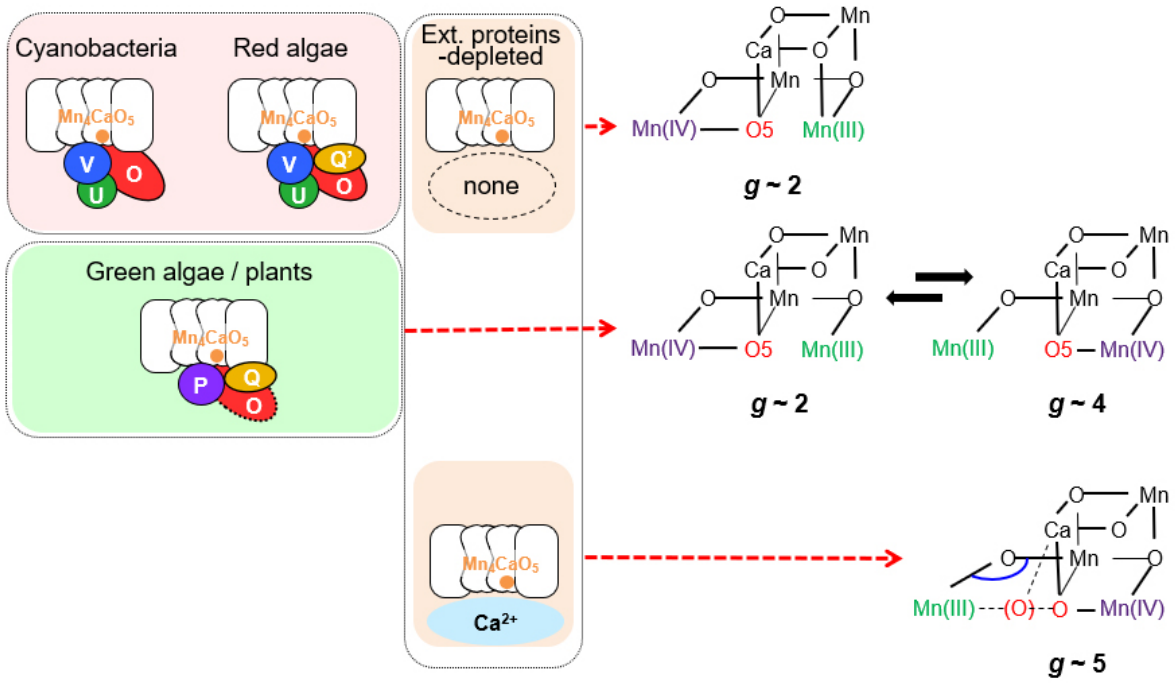

Figure S6. The model of the relationship between the manganese cluster and the extrinsic proteins.

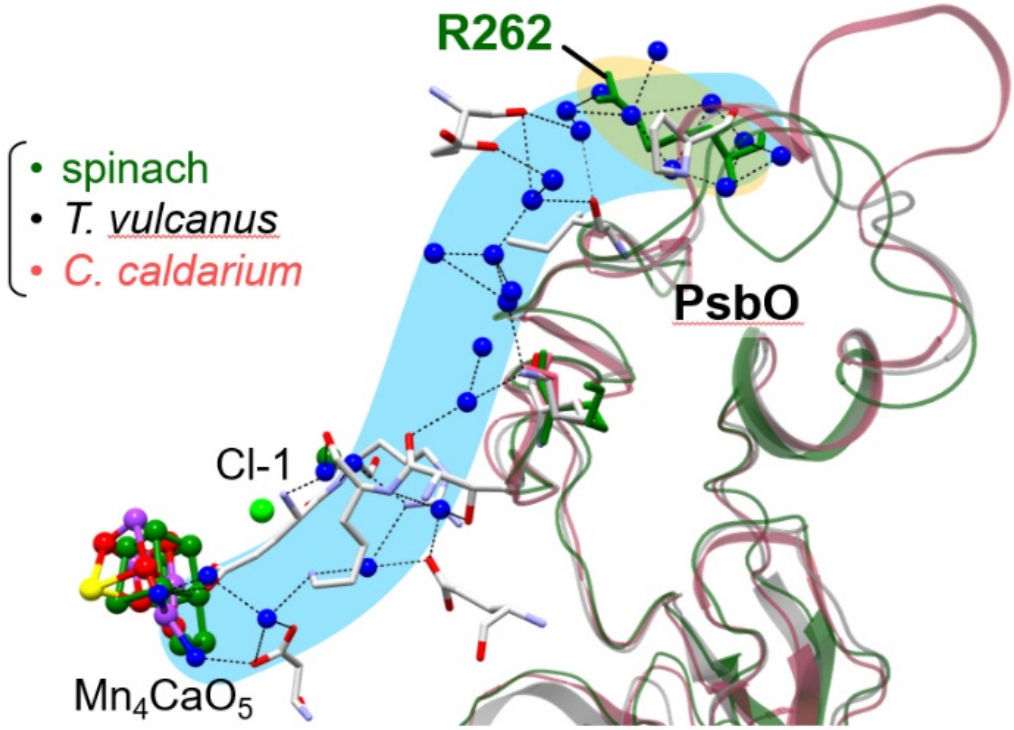

Figure S7. Protein structure of PsbO around the CP47-binding region, illustrated based on the structure of *T. vulcanus* (PDB: 4ub6), overlapped with the structures of spinach (PDB: 3jcu, green) and *C. caldarium* (PDB: 4yuu, salmon). This region includes a hydrogen-bonded network near Cl-1 (highlighted in blue).

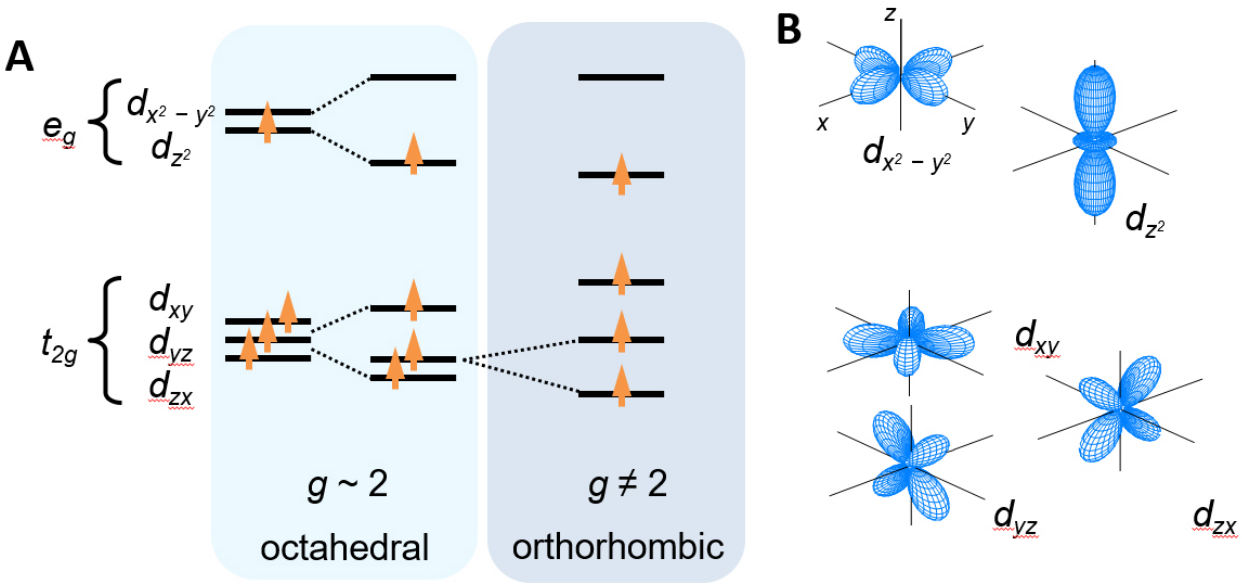

Figure S8. The relationship between *d* electrons in the crystalline field and the *g*-factor. (A) Energy levels of electron spins in the 3d orbitals of a Mn(III) ion and its ligand field splitting leading to the shift of the g-factor. (B) Shapes of the real functions of the 3d orbitals.

Table S1. Alignment of the protein sequences of PsbO in the CP47-binding region in different species.

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S. oleracea	P	A	G	–	–	–	G	R	G	D	E	E	E	L
P. sativum	P	A	G	–	–	–	G	R	G	D	E	E	E	L
C. reinhardtii	P	A	–	–	–	–	–	R	A	D	A	E	E	L
Synechocystis sp. PCC6803	P	–	–	–	–	–	–	–	–	S	A	A	D	K
T. vulcanus	P	–	–	–	–	–	–	Q	A	K	E	E	E	L
T. elongatus	P	–	–	–	–	–	–	Q	A	K	E	E	E	L
C. merolae	P	A	L	E	A	D	G	A	V	G	Q	E	V	L
C. gracilis	P	A	L	Q	L	–	G	E	E	G	D	A	E	L
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