Supporting Information

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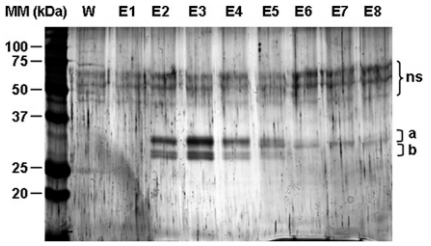

Fig. S1. Identification of PII-interacting proteins in leaf chloroplast extracts. Soluble proteins from purified chloroplasts were loaded onto a PII-affinity resin. Unbound proteins were removed by washing with 50 mL of buffer A. Lane W shows the absence of protein at the end of the wash process. Bound proteins were eluted with 5 mM 2-OG (8 × 0.5 mL fractions corresponding to lanes E1–E8). In control experiments omitting Mg-ATP or with NAGK fixed to the affinity resin, these proteins were not bound. Eluted proteins were TCA-precipitated and subjected to SDS/PAGE (12% acrylamide) and silver staining. This figure shows the complete SDS/PAGE gel from which Fig. 1*A* was taken. ns, proteins that were detected in each fraction/lane by the silver staining but did not meet selection criteria (specifically eluted by 2-OG) for LC-MS/MS analysis; a and b, protein bands analyzed by LC-MS/MS and identified (as given in Table 1).

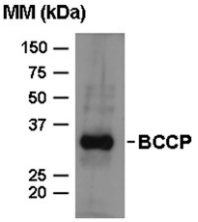

Fig. S2. Chloroplastic BCCP is fixed onto the monomeric avidin resin used to examine PII interactions. Soluble biotin-containing proteins from intact chloroplasts were fixed to immobilized avidin, and their interaction with recombinant PII was tested in the presence of Mg-ATP and 2-OG (Fig. 1*C*). Afterward, the biotin-containing proteins were removed from the monomeric avidin by elution with 10 mL of 0.1 M glycine (pH 2.8). They were TCA-precipitated and subjected to SDS/PAGE (12% acrylamide) and Western blotting using BCCP antibodies. It can be seen that BCCP was fixed to the avidin and therefore could interact with recombinant PII in an ATP/2-OG-dependent manner.