RESEARCH ARTICLE

Photosynthetic Phosphoribulokinase Structures: Enzymatic Mechanisms and the Redox Regulation of the Calvin-Benson-Bassham Cycle

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Short title: Structures of PRK and GAPDH/CP12/PRK complex

One-sentence summary: The structures of cyanobacterial and vascular plant phosphoribulokinase (PRK), alone and in complex, provide insights into the mechanisms by which the oxygenic photosynthetic organisms regulate the CO₂ assimilation in response to the redox signaling.

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ABSTRACT

The Calvin-Benson-Bassham (CBB) cycle is responsible for CO₂ assimilation and carbohydrate production in oxyphototrophs. Phosphoribulokinase (PRK) is an essential enzyme of the CBB cycle in photosynthesis, catalyzing adenosine triphosphate (ATP)-dependent conversion of ribulose-5-phosphate (Ru5P) to ribulose-1,5-bisphosphate. The oxyphototrophic PRK is redox-regulated, and can be further regulated by reversible association with both glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and oxidized chloroplast protein CP12. The resulting GAPDH/CP12/PRK complex is central in the regulation of CBB cycle; however, the PRK-CP12 interface in the recently reported cyanobacterial GAPDH/CP12/PRK structure was not well resolved, and the detailed binding mode of PRK with ATP and Ru5P remains undetermined, as only apo-form structures of PRK are currently available. Here, we report the crystal structures of cyanobacterial (Synechococcus elongatus) PRK in complex with adenosine diphosphate and glucose-6-phosphate, and of the Arabidopsis thaliana GAPDH/CP12/PRK complex, providing detailed information regarding the active site of PRK, and the key elements essential for PRK-CP12 interaction. Our structural and biochemical results together reveal that the ATP binding site is disrupted in the oxidized PRK, whereas the Ru5P binding site is...
occupied by oxidized CP12 in GAPDH/CP12/PRK complex. This structure–function study greatly advances the understanding of reaction mechanism of PRK and the subtle regulations of redox signaling for the CBB cycle.

INTRODUCTION

Oxygenic phototrophs such as cyanobacteria, algae and land plants convert carbon dioxide and water into carbohydrates, and release the by-product oxygen, a process that can be divided into the light reactions and the Calvin-Benson-Bassham (CBB) cycle (light-independent reactions). Adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) are produced in the light reaction process, before being utilized by the enzymes of the CBB cycle responsible for CO₂ assimilation (Johnson, 2016). The CBB cycle is regulated by light/dark transitions through the redox states of the chloroplast stroma (Buchanan, 1980; Geiger and Servaites, 1994; Scheibe, 1991). Chloroplast thioredoxins (TRXs), including TRXf and TRXm (Collin et al., 2003; Kamo et al., 1989; Maeda et al., 1986; Ruelland and Miginiac-Maslow, 1999), are reduced by photosystem I upon illumination through ferredoxin (Fd) and ferredoxin thioredoxin reductase (FTR) system (Buchanan, 1991; Dai et al., 2004), and further reduce and activate the enzymes of CBB cycle, including phosphoribulokinase (PRK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Buchanan and Balmer, 2005; Michelet et al., 2013; Ruelland and Miginiac-Maslow, 1999; Schurmann and Buchanan, 2008; Schurmann and Jacquot, 2000). In addition, the small chloroplast protein CP12 (Pohlmeier et al., 1996), which is central for the regulation of CBB cycle and usually possesses two cysteine (Cys) pairs at both N- and C-terminal regions, is redox-regulated by TRXs (Howard et al., 2008; Lopez-Calcagno et al., 2014; Wedel et al., 1997).

PRK catalyzes the ATP-dependent phosphorylation of ribulose-5-phosphate (Ru5P) to produce ribulose-1,5-bisphosphate (RuBP), which is required for Rubisco-dependent CO₂ fixation (Avron and Gibbs, 1974), as a result of which the CBB cycle is initiated. The PRK structure and catalytic mechanism were previously
studied in an anoxygenic bacterium, *Rhodobacter sphaeroides* (*RsPRK*) (Harrison et al., 1998; Kung et al., 1999). However, PRK from oxygenic phototrophs differs significantly from *RsPRK* in primary sequence, oligomeric state and regulatory mechanism (Buchanan, 1980; Tabita, 1980). In contrast to the octameric *RsPRK*, PRKs from oxygenic phototrophs are commonly presented as homodimers, and each monomer contains a pair of Cys residues at the N-terminal region. PRKs from plants and eukaryotic algae are redox-regulated, through reversible reduction and oxidation of this Cys pair (Brandes et al., 1996; Latzko et al., 1970; Milanez et al., 1991; Wirtz et al., 1982), which is absent in *RsPRK*. Reduced PRK represents its active state, while the oxidized form is inactive, though a previous report suggested that the latter form exhibits basal activity (Marri et al., 2005). Cyanobacterial PRKs also contain a similar Cys pair at the N-terminal region; however, earlier studies suggested that *in vivo*, cyanobacterial PRK is more resistant than plant PRK to oxidative inactivation (Kobayashi et al., 2003; Tamoi et al., 1998). Sequence alignment results revealed that cyanobacterial PRKs lack the loop between the two cysteine residues in plant-type PRKs (termed ‘clamp loop’), which is considered to participate in TRX binding (Gurrieri et al., 2019). The architecture of the dimeric PRK had not been determined until the crystal structure of PRK from the archaeon *Methanospirillum hungatei* (*MhPRK*) was reported (Kono et al., 2017). However, *MhPRK* lacks the Cys pair and is not subject to redox regulation. Recently, the structures of oxidized PRK from cyanobacterium *Synechococcus* sp. strain PCC6301 and of reduced PRK from *Arabidopsis thaliana* (*At*) and *Chlamydomonas reinhardtii* were solved (Gurrieri et al., 2019; Wilson et al., 2019). These structural data revealed the dimerization pattern of photosynthetic PRK and confirmed that the two Cys residues involved in redox regulation are positioned apart in the reduced PRK, while forming a disulfide bond in the oxidized form. As one of the Cys residues is located in the P-loop region, which usually functions in ATP binding, these findings suggested that disruption of its ATP binding site results in the inactivation of oxidized PRK (Wilson et al., 2019).
GAPDH catalyzes the conversion of D-glycerate 1,3-bisphosphate to glyceraldehyde 3-phosphate in the presence of NADPH (Melandri et al., 1968). Two types of GAPDH, namely GapA and GapB, are present in plant chloroplasts, and give rise to A_4 and A_2B_2-type enzymes (Brinkmann et al., 1989; Petersen et al., 2006; Seaglierini et al., 1998). Most algae do not contain GapB, and the A_4-type is the major form of GAPDH in eukaryotic algae and cyanobacteria (Petersen et al., 2006). GapB is almost identical to GapA but has an additional C-terminal extension (CTE) that bears a pair of Cys residues targeted by TRXs (Baalmann et al., 1996; Petersen et al., 2006). The A_2B_2 heterotetramer is subjected to redox regulation via TRX through this Cys pair (Baalmann et al., 1996; Sparla et al., 2002). Previously reported structural data showed that the CTE of oxidized GapB binds at the interface of two GAPDH monomers within one tetramer, occupying their active sites and exhibiting an autoinhibition state of A_2B_2-GAPDH (Fermani et al., 2007). The A_4-GAPDH is a stable and constitutively active tetramer (Graciet et al., 2004; Oesterhelt et al., 2007; Tamoi et al., 2005; Wedel and Soll, 1998); it is inactivated upon forming a complex with the oxidized CP12 (Clasper et al., 1991; Pohlmeier et al., 1996; Wedel et al., 1997). The C-terminal fragment of CP12 is homologous to the CTE of GapB (Baalmann et al., 1996; Petersen et al., 2006). Several crystal structures of A_4-GAPDH in complex with CP12, either the short C-terminal tail or the full-length, were previously determined (Fermani et al., 2012; Matsumura et al., 2011; McFarlane et al., 2019). These studies showed that the C-terminal region of CP12 occupies the similar position at the interface of two GAPDH monomers as CTE of oxidized GapB, and therefore inhibits GAPDH activity. Earlier reports showed that A_2B_2-GAPDH in several land plants can also be de-activated by associating with CP12 (Howard et al., 2011; Scheibe et al., 2002; Wedel and Soll, 1998).

The protein CP12 has been identified in most oxygenic phototrophic organisms (Grob en et al., 2010; Wedel and Soll, 1998). Classical CP12 proteins possess a highly conserved motif “AWD_VEE” at the central part of its sequence in addition to the N-
and C-terminal cysteine pairs (Graciet et al., 2003; Stanley et al., 2013; Wedel and Soll, 1998). Reduced CP12 is a fully intrinsically disordered protein (Groben et al., 2010; Launay et al., 2016), while the conformation of oxidized CP12 is stabilized by the two intramolecular disulfide bridges (Fermani et al., 2012; Matsumura et al., 2011). Oxidized CP12 is able to interact with the GAPDH tetramer, and further assembles with oxidized PRK dimer, forming the GAPDH/CP12/PRK complex (Graciet et al., 2003; Marri et al., 2005; Marri et al., 2008; Wedel and Soll, 1998). The enzymatic activity of both GAPDH and PRK were previously shown to be suppressed upon forming a complex, and the activity of PRK in the complex is further decreased compared with the free oxidized PRK dimer (Marri et al., 2009). The activation of PRK in the complex occurs quickly, while the transition of free oxidized PRK to its active state is slower (Marri et al., 2005), though the mechanism for this difference remained unclear. A subsequent study suggested that the GAPDH/CP12/PRK complex provides a pool of both enzymes, ready to be released with full activity (Marri et al., 2009). In addition to regulating the CBB cycle, CP12 was suggested to play wider roles, including to protect both GAPDH and PRK from oxidative stress (Marri et al., 2014), and to facilitate the stabilization of PRK during or after its synthesis in vivo (Elena Lopez-Calcagno et al., 2017).

Several models of the GAPDH/CP12/PRK complex were previously proposed based on small-angle x-ray scattering analysis (Del Giudice et al., 2015). Recently, a cryo-EM structure of cyanobacterial GAPDH/CP12/PRK complex from *Thermosynechococcus elongatus* BP-1 (*Te*GAPDH/CP12/PRK) was solved at an overall resolution of 4 Å (McFarlane et al., 2019). This work provided important information regarding the ternary complex assembly and the CP12 structure; however, the CP12-PRK interaction interfaces, with a local resolution of 6.2 Å, were not well resolved. The detailed inter-molecular interactions of the ternary complex GAPDH/CP12/PRK requires high-resolution structures for further elucidation. In addition, though the structures of PRK dimer from oxygenic phototrophs were
recently solved (Gurrieri et al., 2019; Wilson et al., 2019), the binding mode of ATP
and Ru5P with PRK remains unknown. Here, we solved the crystal structures of
ligand-bound PRK as well as the GAPDH/CP12/PRK complex, enabling us to
identify the active site of PRK, together with its regulatory mechanisms for the redox
signaling responsible for activating the CBB cycle.
RESULTS

Overall structure of PRK

Here, we determined the crystal structures of PRK from *Synechococcus elongatus* PCC7942 (SePRK) in complex with adenosine diphosphate (ADP) and glucose-6-phosphate (G6P), and from *Arabidopsis thaliana* in both their reduced (AtPRK\textsubscript{red}) and oxidized (AtPRK\textsubscript{ox}) forms, at resolutions ranging from 2.3 Å to 2.5 Å. Furthermore, we solved the crystal structure of AtGAPDH/CP12/PRK complex at 3.5 Å resolution (Table 1). In the four crystal structures, protein molecules were built almost entirely with only several terminal residues missing (Table 1).

The PRK molecules in the four structures, either in the free form or in the complex, are highly similar, adopting an αβα fold (Fig. 1A, Supplemental Fig. 1). Four small β-strands (β1'-β4') following α3 flank at one side of the PRK molecule, while β7 is located at the other side of the molecular surface. Consistent with previously reported structures of dimeric PRK (Gurrieri et al., 2019; Wilson et al., 2019), β7 appears to be primarily responsible for the monomer-monomer interactions (Fig. 1A, B). We also found that the structure of the α5-α6 region varied considerably between the four PRK structures (Fig. 1C). The fragment between α5 and α6 was previously suggested to be a mobile “lid” in RsPRK, and may cover the active site from the aqueous environment after substrate binding (Harrison et al., 1998; Runquist et al., 1998). The lid region cannot be traced in the SePRK structure, while it forms a flexible loop in PRK in the AtGAPDH/CP12/PRK complex (Fig. 1C). By contrast, this part is stabilized by the neighboring symmetrical PRK molecule packed in the crystals, thus forming a short helix in both AtPRK structures (Fig. 1C, Supplemental Fig. 2). Comparison with other oxygenic phototrophic PRK structures showed that the α5-α6 region adopts the least conserved conformation in whole PRK molecule (Supplemental Fig. 3), indicating that the lid is internally mobile, a property that might be critical for the proper functioning of PRK.
The two Cys residues essential for the redox regulation are located at the
N-terminal region, within the P loop (C17) and at the C-terminal end of β2 (C56)
Previous studies reported structures of both reduced and oxidized forms of PRK; however, the reduced form was obtained from *Arabidopsis* and *Chlamydomonas* (Gurrieri et al., 2019), while the oxidized form was obtained from a cyanobacterium (Wilson et al., 2019). Here, we solved the structures of *At*PRK in both reduced and oxidized forms, thus allowing accurate structural comparison within the same species. Furthermore, we identified the conformational changes induced exclusively by the different redox states of *At*PRK. As shown in Fig. 2A, the folding observed for the *At*PRK\(_{\text{red}}\) structure is largely identical to the folding of *At*PRK\(_{\text{ox}}\). The only visible difference between the two structures is the orientation of the P loop (residues 14DSGCGKST21), which is flipped by approximately 180 degrees owing to the formation of C17-C56 disulfide bond in the *At*PRK\(_{\text{ox}}\) structure (Fig. 2A, B). The structural features for the P-loop region were also observed in the recently reported structures of the reduced and oxidized PRKs (Gurrieri et al., 2019; Wilson et al., 2019) (Supplemental Fig. 3). The two Cys residues are 14 Å from each other in the *At*PRK\(_{\text{red}}\) structure, whereas the flipped P loop places C17 closer to C56 in the oxidized *At*PRK (Fig. 2B), allowing the formation of a disulfide bond without affecting other parts of PRK molecule, even the position of C56. The clamp loop, which precedes β2 and was previously suggested to provide the binding site for TRX (Gurrieri et al., 2019), forms a long coil loop (Fig. 2A). The C-terminal residues of the clamp loop (T46-I48) shape as an additional β-strand, which is oriented in an anti-parallel manner relative to β2 (Fig. 2C). In comparison, the N-terminal half of the clamp loop, although interacting with β1’-β4’ region, is more flexible as characterized by their higher B-factors (Fig. 2C).

Overall structures of *At*GAPDH/CP12/PRK and *At*CP12 within the complex

The *At*GAPDH/CP12/PRK complex was earlier reported to possess a molecular weight of 500 kDa (Marri et al., 2009), allowing analysis by cryo-electron microscopy.
(cryo-EM) method. Therefore, we used both x-ray crystallography and cryo-EM.
methods to solve the complex structure. We obtained 3.5 Å resolution x-ray
diffraction data and a 4.9 Å cryo-EM reconstruction map of GAPDH/CP12/PRK
complex from *Arabidopsis* (Table 1, Supplemental Fig. 4). We further refined the
crystal structure (Fig. 3A) as its higher resolution would provide more details. We
next fitted the refined crystal structure into the cryo-EM density map, confirming that
the crystal structure corresponds to the cryo-EM reconstruction map (Fig. 3B).
However, the peripheral region of the complex, especially the PRK-CP12 interface,
displayed a flexible nature, with a weaker density (around 7 Å resolution) compared
with the other parts of the complex in the cryo-EM map (Supplemental Fig. 4D). The
flexible PRK-CP12 interface was also observed in the recently reported cryo-EM
structure of the *Te*GAPDH/CP12/PRK complex (McFarlane et al., 2019), indicating
that this region is highly dynamic. Since the cryo-EM reconstruction was of low
resolution and revealed features similar to that of the crystal structure, we did not
further refine the structure based on the cryo-EM map and used the crystal structure of
the complex for further analysis.

The structure of the *At*GAPDH/CP12/PRK complex can be described as
spindle-like, and is composed of two A4-GAPDH tetramers, two oxidized PRK
dimers and four oxidized CP12 monomers (Fig. 3A). The two A4-GAPDHs are
located at the two end points, while two PRK dimers form the side edges of the
spindle. CP12 functions as a linker and associates with both enzymes. In the complex
structure, the oxidized CP12, in a hook-like shape, is mainly composed of three
α-helices (Fig. 3C). The N-terminal helix (N-helix, residues G6-T21), together with a
long central helix (residues E30-G56), forms a hairpin structure, constituting the
N-terminal domain (NTD), which is stabilized by a disulfide bond between C22 and
C31. The conserved region of CP12 (3AWD_VEE40) (Lopez-Calcagno et al., 2014) is
located at the N-terminal half of the central helix (Fig. 3C). The C-terminal domain
(CTD, residues P59-N78) is more globular and composed of a short C-helix followed
by a loop, which are connected by the C-terminal disulfide bond (C64-C73).
Comparison of the four *At*CP12 molecules within the complex showed that, when aligned independently, their NTD and CTD can both be superposed well (Fig. 4A). However, the overall structures are considerably different because of the different conformations adopted by the loop connecting the two domains. The flexible loop between NTD and CTD of CP12 was also observed in cyanobacterial GAPDH-CP12 complex structures (McFarlane et al., 2019). In addition, the two PRK dimers within the complex exhibit slightly different conformations, and both have a twist of about 10 degrees of one monomer with respect to the other monomer when compared with the free PRK dimer (Fig. 4B). By contrast, the GAPDH tetramers in complex adopt an identical conformation to its free form (Fig. 4C). Comparison of our *At*GAPDH/CP12/PRK structure with recently reported cryo-EM structure of *Te*GAPDH/CP12/PRK showed that though their overall folds are similar (Supplemental Fig. 5A), CP12 and PRK in the two complexes adopt different conformations (Supplemental Fig. 5B, C). In addition, our crystal structure of the *At*GAPDH/CP12/PRK complex is slightly asymmetrical, presumably a result of the molecular packing of the crystals (Supplemental Fig. 5D). Together, these structural findings clearly demonstrated that CP12 monomers and PRK dimers adjust their conformations to assemble with the rigid GAPDH tetramers, allowing the formation of the ternary complexes with slightly altered conformations. This flexible feature of the complex might facilitate its formation in the crowded chloroplast environment, thus ensuring the effective regulation of the CBB cycle.

**Interactions of CP12 with GAPDH and PRK within *At*GAPDH/CP12/PRK complex**

Each GAPDH tetramer of the GAPDH/CP12/PRK complex simultaneously links with two monomers of both PRK dimers through CP12 (Fig. 3A). The C-terminal loop of the CP12-CTD is inserted into the interfacial region of two GapA monomers
Figure 4. Structural comparison of proteins within the AtGAPDH/CP12/PRK complex or with their free forms.

(A) Structural comparison of four CP12 monomers within the AtGAPDH/CP12/PRK ternary complex superposed on their NTD (upper panel) and CTD (lower panel). The four CP12 molecules are colored differently.

(B) Structural comparison of two PRK dimers within the AtGAPDH/CP12/PRK ternary complex superposed on the left monomer (upper panel), and comparison between one PRK dimer within the ternary complex structure (colored marine) and the AtPRKox structure (colored yellow) superposed on the left monomer (lower panel).

(C) Superposition of the GAPDH part in the AtGAPDH/CP12/PRK structure (blue-white), the previously reported GAPDH-CP12(CTD) structure (PDB code 3RVD, magenta) and the GAPDH tetramer structure (PDB code 1RM4, pale yellow).

(Fig. 5A), resembling the previously reported structure of GAPDH in complex with
CP12 is shown as salmon cartoon, with the conserved region (\(\omega_{W,V,E}\)) highlighted in yellow-orange.

Figure 5. The inter-molecular interactions in AGAPDH/CP12/PRK ternary complex.

(A) The CTD of CP12 is inserted into the interfacial region of two GAPDH monomers, blocking their active sites. The CP12 is shown as salmon cartoon, the two GAPDH monomers are shown in surface mode and colored in pale green and grey, respectively. The ligand NAD\(^+\) bound to the GAPDH monomers are shown as sticks.

(B) The central helix of CP12 is located in a long groove on the surface of PRK. The CP12 is shown as salmon cartoon, and the conserved region is highlighted by magenta. The PRK molecule is shown in surface mode and the groove is composed of the second helix (\(\omega_2\), magenta), the lid region (yellow) and P-loop (cyan).

(C) The conserved region (\(\omega_{W,V,E}\)) within the central helix of CP12 is located in the positively charged region of the groove in PRK. PRK is shown in electrostatic potential surface mode, red represents negative charge, and blue represents positive charge.

The NTD of CP12 contacts PRK, with its central helix...
located in a long groove on the surface of one PRK molecule (Fig. 5B). A recent report showed that an adenylate kinase (ADK3) from *Chlamydomonas reinhardtii* contains a C-terminal extension similar to the CTD of CP12. The oxidized ADK3 is able to interact with GAPDH through its C-terminal extension, but is unable to recruit PRK (Zhang et al., 2018). The C-terminal extension of ADK3 probably interacts with GAPDH in the similar way as the CTD of CP12. The long groove of PRK accommodating CP12 is formed by the P loop (D14-T21), $\alpha_2$ (D64-Q71) and the lid (R160-L169) regions, which shape the bottom and both sides of the groove, respectively (Fig. 5B). Remarkably, these regions are also involved in establishing the active site of PRK (see below). All three fragments together form a positively charged surface of the groove and surround the conserved $^{34}$AWD$\_^{40}$VEE region of CP12 (Fig. 5C). The conserved acidic residues E40 and D36 of CP12, face the groove of PRK and form hydrogen bonds with the alkaline residues R65 and R68 from $\alpha_2$ region of PRK (Fig. 5D). Mutation of either R65 or R68 to Ala in PRK prevents the formation of the ternary complex (Fig 5E), indicating that both play a critical role in stabilizing the PRK-CP12 interaction.

Moreover, we found that the conformation adopted by the P loop in the reduced form of PRK does not hinder CP12 binding (Fig. 5F), suggesting that the reduced *AtPRK* is able to form a similar complex to that of the oxidized form. To test our hypothesis, we incubated the PRK mutant C17S with GAPDH and oxidized CP12. As the mutant C17S is not able to form a disulfide bond with C56, it represents the constitutively reduced form of PRK. Our size exclusion chromatographic result showed that the mutant C17S forms a similar GAPDH/CP12/PRK complex as the oxidized wild-type PRK (Fig 5E). Our results are consistent with previous observations in pea (*Pisum sativum*) and *Chlamydomonas reinhardtii* showing that reduced PRK also exists in the GAPDH/CP12/PRK complex in vivo (Howard et al., 2008; Lebreton et al., 2003).
Active Site of PRK and Recognition of ATP and Ru5P

All previously reported structures of PRK are present in their apo-forms, and thus lack the details of their binding to either ATP or Ru5P. To identify the cofactor binding pattern, we co-crystallized PRK from Arabidopsis thaliana and Synechococcus elongatus (AtPRK and SePRK) with various ligands, including ATP/glucose-6-phosphate (G6P) and AMPPNP (a non-hydrolyzed ATP analog)/Ru5P. Ultimately, only the structure of SePRK in complex with ADP and G6P was obtained (Fig. 6A). In this structure, although we co-crystallized SePRK with ATP, an ADP molecule is clearly visible in the density map, likely because of the hydrolysis of its γ-phosphate group during the crystallization process.

The SePRK structure harbors two deep narrow grooves consisting of primarily positively charged residues forming a “L-shape” (Fig. 6B). Each groove is occupied by one ligand; the ADP binding groove (named the ATP site) is mainly formed by residues from the P loop (residues 13DSGCST22) and α5 region. The α-phosphate of ADP interacts with T22, while the β-phosphate group is hydrogen bonded with S21. In addition, the adenosine ring is positioned parallel to the purine ring of W140 and is hydrogen bonded with T304 (Fig 6A). In the second pocket, the G6P molecule is stabilized by residues from the α2, α6 and β2′-β3′ regions. Its phosphate group is hydrogen bonded with R52 and R163, and the hydroxyl groups are stabilized by R49, Y88 and H90 (Fig 6A). G6P and Ru5P differ in their stereochemical structures. G6P is characterized by a cyclic glucose skeleton with six carbons, while Ru5P comprises a linear ribulose with five carbon atoms. However, both contain a phosphate group at their terminal hydroxyl group, implying that they may interact with an identical region that also accommodates the phosphate group. Hence, we hypothesized that the binding pocket occupied by G6P in our structure represents the Ru5P-binding site (named the Ru5P site).
To test our hypothesis about the position of the Ru5P site and to locate the
γ-phosphate group of ATP, we docked Ru5P and ATP into our SePRK structure.
through the Autodock Vina program (Trott and Olson, 2010) (Fig. 6C). In addition, we docked a G6P molecule into the SePRK structure to assess the docking program. We found that the docked G6P is located in a position identical to that we observed in the crystal structure (Supplemental Fig. 6), indicating that it is feasible to apply the docking method to explore the ligand binding site in our PRK structures. In the docked model of SePRK in complex with Ru5P and ATP, the phosphate group of Ru5P locates at the similar position of that of G6P, while the C1 hydroxyl group of Ru5P locates at a distance of 5.8 Å away from that of G6P (Fig. 6D). This docking result confirms the hypothesized position of the Ru5P site, and suggests that the Ru5P site is composed of residues from α2 (D42, R49 and R52) and the β2’-β3’ region (Y88, H90), as well as R163 from α6 (Fig. 7A). Moreover, docking of an ATP molecule suggests that its γ-phosphate is fixed by S16, K20 and S21 from the P loop (Fig. 7A). These residues are completely conserved among the oxygenic phototrophic PRKs (Supplemental Fig. 1), indicating that they play an essential role during catalysis.

Key Residues in Ligand Binding and Catalysis of AtPRK

Despite extensive screening for crystallization conditions, we failed to obtain the ligand-bound AtPRK structure. However, our structural superposition analysis showed that AtPRK_red exhibits an overall structure nearly identical to that of SePRK (Fig. 1B), suggesting that both enzymes bind ATP and Ru5P in same pockets with a similar manner. On the basis of these findings, we generated several AtPRK mutants (Supplemental Fig. 7) altered in the conserved residues potentially involved in catalysis and ligand binding according to our findings on SePRK structure and tested their activity. Our enzymatic assays showed that the mutant forms D58A, Y104F and H106A almost completely lost catalytic ability, while the mutant forms including
S15A, K19A, S20A, R65A and W156A showed dramatically reduced catalytic activity when compared with the wild type (Fig. 7B).

Residues S15, K19, S20 and W156 in AtPRK correspond to residues S16, K20, S21 and W140 in SePRK, which participate in ATP binding in SePRK. In agreement with the structural observation on SePRK, the binding analysis between AtPRK and ATP through surface plasmon resonance (SPR) method revealed that the two mutations K19A and W156A drastically decreased the binding affinity of PRK to ATP (Fig. 7B, Supplemental Fig. 8). However, mutation of S15A and S20A from the P loop failed to show such effect on ATP binding of AtPRK. Our docking results suggested that S15, S20 and K19 (S16, S21 and K20 in SePRK) are all involved in interacting with the \( \gamma \)-phosphate of ATP (Fig. 7A). These data suggest that K19 is essential for interacting with ATP, as its positively charged side chain favors the binding and stabilization of the negatively charged phosphate group of ATP. While the two Ser residues S15 and S20 play minor roles in binding ATP, they are critical for catalysis, presumably by ensuring the proper orientation of \( \gamma \)-phosphate of ATP.

In addition, our structural analysis suggested that residues D58, R65, Y104 and H106 (corresponding to D42, R49, Y88 and H90 in SePRK) are involved in Ru5P binding. We therefore measured the binding affinity of wild-type and mutant (D58A, R65A, Y104F and H106A) PRK with Ru5P using an Isothermal Titration Calorimetry (ITC) method. Our ITC results indicated that these four residues are indeed involved in Ru5P binding (Fig. 7B, Supplemental Fig. 9), which is in agreement with our docking results as well as a previous report showing that R64 in Chlamydomonas PRK (corresponding to R65 in AtPRK) is important for binding the substrate (Roesler et al., 1992). However, considering the relatively high concentration of Ru5P in our enzymatic assay (1.2 mM), the reduced activity of these mutants is likely to represent their different \( k_{cat} \) compared with that of the wild-type PRK. Furthermore, the results obtained with the two loss-of-function mutants D58A and H106A strongly suggest
that these two residues directly participate in the catalysis in addition to binding Ru5P. This finding is in agreement with previous analysis demonstrating that D42 in RsPRK (equivalent to D58 in AtPRK) is essential for its catalytic activity (Harrison et al., 1998). Based on our docking results obtained from the SePRK structure, D58 and H106 in AtPRK are possibly located at the crossing point of the ATP site and Ru5P site, in close vicinity with the potential positions of ATP γ-phosphate and the C1 hydroxyl oxygen of Ru5P (Fig. 7A). Together, these findings provide strong evidence that D58 and H106 function as the catalytic base that activates the nucleophilic attack.

Insights into the catalysis and regulation of PRK

Superposition of our PRK structures shows that the P loop is the only region that adopts different conformation between AtPRK\textsubscript{red} and AtPRK\textsubscript{ox} structures (Fig. 2A, B). Further comparison with the SePRK structure clearly demonstrates that the ATP site is disrupted in the AtPRK\textsubscript{ox} structure (Fig. 8A), which was also suggested previously for the oxidized cyanobacterial PRK (Wilson et al., 2019). However, the Ru5P site is located somewhat distantly from the P loop and stays unaffected in the oxidized form (Fig. 8A). These structural observations demonstrate that the inactivation of oxidized PRK is because it is unable to bind ATP, and suggest that the switch between the active (reduced) form and the inactive (oxidized) form of PRK only requires the conformational change of its P loop, without affecting the Ru5P site in PRK.

In addition to the redox regulation, another important regulatory mechanism of PRK activity involves controlling the reversible assembly of the GAPDH/CP12/PRK complex. In this ternary complex, both GAPDH and PRK remain inactive, as CP12 binds to their active sites. The central helix of CP12 is located exactly in the Ru5P site of PRK (Fig. 8B). The two acidic residues E40 and D36, interacting with R65 and R68 of PRK (Fig. 5D), insert into the Ru5P site. D36 could potentially clash with
Figure 8. The ATP-site and Ru5P-site in PRK.
(A) Structural superposition of AtPRK<sub>ox</sub> (yellow) and SePRK (pink) with docked ATP and Ru5P molecules (shown as sticks). ATP-site is disrupted in the AtPRK<sub>ox</sub> structure (yellow) as the P-loop clashes with ATP molecule, whereas the Ru5P-site is unaffected in AtPRK<sub>ox</sub> structure.
(B) Structural superposition of AtPRK in the ternary complex (slate) and SePRK (pink) with docked ATP and Ru5P molecules (shown as sticks). The Ru5P-site is at the PRK-CP12 interface of the AtGAPDH/CP12/PRK complex, and is disrupted upon binding CP12 (green), whereas ATP-site is unaffected. The two conserved residues D36 and E40 of CP12 are shown as sticks and labeled. Residue D36 of CP12 is potentially clashing with Ru5P.

Ru5P (Fig. 8B). As a result, the CP12 would interfere with the binding of PRK to
Ru5P, which explains the further decrease of PRK activity within the complex. Interestingly, we found that ATP binding is a precondition for PRK to interact with Ru5P. While we detected no available signal when titrating Ru5P into AtPRK alone using ITC (Supplemental Fig. 9A), we obtained reliable data by titrating Ru5P into a mixture of AtPRK incubated with ATP (Fig. 7B, Supplemental Fig. 9B). While the affinity of Ru5P to PRK increased in the presence of ATP, incubating PRK with Ru5P failed to affect its ATP binding (Supplemental Fig. 8C). This experimental observation suggests that binding Ru5P requires the conformational change of PRK, which is induced by ATP binding. PRK was previously proposed to follow an ordered sequential mechanism for catalysis (Lebreton and Gontero, 1999), indicating that PRK binds ATP and Ru5P simultaneously, and catalyzes the direct transfer of phosphate group from ATP to Ru5P, without forming the enzyme-phosphate intermediate. Our results presented here suggest that during the ordered sequential catalytic reactions, PRK binds ATP first before binding Ru5P.
DISCUSSION

In this study, we solved the structure of SePRK in complex with ADP and G6P, and generated a docked model of SePRK bound with ATP and Ru5P based on our crystal structure. Our results show that the ATP site is mainly formed by residues from the P loop and α5 (Fig. 6A). Two residues of PRK, K19 and W156 (numbered as in AtPRK), are crucial for ATP binding. Together, they stabilize the phosphate groups and the adenosine ring of ATP through ionic bond and π-π interaction, respectively (Fig. 7). Moreover, we found that mutation of either of the two conserved Ser residues in the P loop (S15 and S20) results in a significant decrease of PRK activity, without affecting its ATP binding ability (Fig. 7B). Since the two Ser residues are located near the γ-phosphate group of ATP in our docked model, it is possible that they facilitate the proper orientation of ATP, especially its γ-phosphate group. This suggestion explains their crucial role for the catalytic activity of PRK. The oxidized PRK fails to bind ATP, as the disulfide bond formed between C17 and C56 changes the conformation of the P loop, thus disrupting the ATP site (Fig. 8A). Except for the P-loop region, other parts of the AtPRK_red and AtPRK_ox are highly identical (Fig. 2A). Together, these observations suggest that PRK undergoes a minimum conformational change to switch between the reduced and oxidized states, with the other parts remaining relatively stable, which may facilitate the rapid activation-deactivation cycle of PRK.

Our structures show that while the ATP site is disrupted in the oxidized PRK, the Ru5P site remains unaffected by the C17-C56 disulfide bond formation. The Ru5P site is shaped by residues from α2, α6 and β2’-β3’, including D58, R65, Y104 and H106 (numbered as in AtPRK), which are pivotal for Ru5P binding (Fig. 7). The binding of Ru5P is inhibited by the assembly of the GAPDH/CP12/PRK complex, as the central helix of CP12 is located in the Ru5P site and potentially clashes with Ru5P in the groove on PRK surface. By contrast, the CP12-PRK interaction does not affect
ATP binding with PRK (Fig. 8B). This observation is in line with an earlier report showing that the GAPDH/CP12/PRK complex is able to bind ATP (Marri et al., 2005). These results indicate that ATP and Ru5P binding are independently regulated, through the reversible formation of the C17-C56 disulfide bond and the GAPDH/CP12/PRK complex, respectively.

Our biochemical and structural data together suggest that residues D58 and H106 (numbered as in *AtPRK*) directly participate in the catalytic reaction of PRK, since mutation of any of the two residues leads to the completely loss of PRK activity (Fig. 7B). In the docked model, the γ-phosphate group of ATP and the C1-hydroxyl group of Ru5P are positioned at the crossing point of the L-shaped groove, separated by only 5.2 Å (Fig. 6D). This distance is comparable to that between substrate/product and cofactor in other kinases reported previously (Shirakihara and Evans, 1988; Sigrell et al., 1998), and allows the direct transfer of a phosphate group from ATP to Ru5P with their slight conformational changes. Both D58 and H106 are located close to the phosphate group of ATP and the hydroxyl group of Ru5P (Fig. 7A); thus they may play an essential role in the catalytic process, such as functioning as the catalytic base that activates the nucleophilic attack.

In addition, the results of our binding assays indicated that PRK binds ATP prior to Ru5P. However, since the ATP site is solvent accessible, it is possible that the bound ATP is hydrolyzed before Ru5P binding, resulting in the waste of ATP. Nevertheless, a number of kinases have been reported to bind ATP before the substrate. The ATP binding induces the conformational change of fragments adjacent to the ATP site in these kinases, thus shielding the ATP molecule and protecting it from hydrolysis (Li et al., 2004; Schlauderer et al., 1996; Sigrell et al., 1998). It is possible that PRK uses a similar mechanism, undergoing conformational change upon ATP binding and protecting ATP. Previous observation showed that ATP binding leads to the dissociation of the GAPDH/CP12/PRK complex (Marri et al., 2005), consistent with
our suggestion that binding ATP induces the conformational change of PRK. In addition, the changed conformation of PRK may further facilitate the Ru5P-site formation, explaining our experimental observation that Ru5P only binds PRK after ATP association. Furthermore, our binding assay demonstrated that PRK has strong affinity for Ru5P (Fig. 7B, Supplemental Fig. 9B) considering the relatively high concentration of Ru5P in the chloroplast (around 25-75 µM) (Kuken et al., 2018). This strong affinity may further ensure that Ru5P binds PRK immediately after ATP binding, which may also help to avoid the non-specific hydrolysis of ATP. Possible candidates for these mobile fragments in PRK include the lid region, which is highly flexible as shown by our comparison results of different PRK structures (Fig. 1C, Supplemental Fig. 3). This mobility is a pivotal characteristic of the lid which allows this region to change conformation and perform various functions, such as covering the active site during catalysis, a commonly suggested role for lid region in many kinases, including RsPRK (Runquist et al., 1998; Schlauderer et al., 1996).

Previous studies suggested that only the oxidized AtPRK forms the ternary complex in vitro (Marri et al., 2005). However, our biochemical data showed that mutant C17S of PRK is still able to form a complex with CP12 and GAPDH under oxidized conditions (Fig. 5E). This result suggests that reduced PRK also associates with oxidized CP12 and forms the GAPDH/CP12/PRK complex. Analysis of our complex structure revealed that the reduced PRK is able to form the complex in a manner similar to the oxidized form of PRK, as the different conformation of the P loop does not interrupt PRK association with CP12 (Fig. 5F). In agreement with these observations, reduced PRK in Pisum sativum and Chlamydomonas reinhardtii were previously reported to form a GAPDH/CP12/PRK complex in vivo (Howard et al., 2008; Lebreton et al., 2003). The finding that reduced PRK can form the GAPDH/CP12/PRK ternary complex may explain the quick recovery of PRK activity when it dissociates from the complex, as it is better prepared for binding ATP than the free oxidized PRK form.
Plant-type PRK is redox-regulated mainly by chloroplast TRX f and TRX m as previously suggested, which target C56 of PRK (Brandes et al., 1996). The clamp loop between two Cys residues (C17 and C56) in PRK was suggested to provide the binding site for TRX (Gurrieri et al., 2019); however, the AtPRK structures solved in the present study and in earlier report (PDB code: 6H7H) (Gurrieri et al., 2019) together showed that the clamp loop adopts the same conformation in both oxidized and reduced forms (Supplemental Fig. 3), indicating that this is a thermodynamically stable status of AtPRK. Moreover, in our PRK structures, the pocket above C56 shaped by the clamp loop appears too narrow to accommodate the TRX protein (Fig. 9A, B). In addition, the positively charged N-terminal part of clamp loop may be unfavorable for TRX binding, for the catalytic cysteines of TRX are also located in a region containing primarily positively charged residues (Capitani et al., 2000). These structural features imply that the conformational change of the clamp loop is required for TRX binding. Structural analysis showed that the N-terminal part of the clamp loop is flexible (Fig. 2C); hence, it may swing away to make room for TRX and stabilize TRX binding. In addition, the swing of clamp loop also exposes the residue C56 that is located at the C-terminal end of β2 (Fig. 9A), which is otherwise buried under the clamp loop.

CP12 is also redox regulated by TRXs (Marri et al., 2009). Under oxidizing conditions, CP12 first forms the C-terminal disulfide bond to enable GAPDH binding, then links the N-terminal disulfide bridge and binds PRK (Fermari et al., 2012; Marri et al., 2005). Previous redox titration analysis suggested that the dissociation of the GAPDH/CP12/PRK complex mainly depends on the reduction of CP12, which is less demanding in terms of the reducing potential than GAPDH alone (Marri et al., 2009). The N-terminal part of CP12 is located at the complex surface and negatively charged (Fig. 9C), thus is likely to be the most accessible target for TRX. We propose that the disassembly of the ternary complex is initiated through the dissociation of CP12 with PRK, which is in agreement with previous reports showing that CP12 and GAPDH
form a stable binary complex (Fermani et al., 2012; McFarlane et al., 2019). Upon
reduction of the disulfide bond in CP12-NTD, the major part of CP12 adopts a disordered conformation to release PRK, and further reduction of the C-terminal disulfide bond of CP12 disassembles the entire complex. The released GAPDH and PRK expose their active sites, allowing the reduction of oxidized PRK. The complex dissociation upon CP12 reduction was suggested to be crucial for ensuring the tight coupling of GAPDH and PRK (Marri et al., 2009). PRK and GAPDH represent two key enzymes in the CBB cycle, utilizing ATP and NADPH produced through light reactions for catalysis, respectively. By controlling the activity of two key enzymes simultaneously, the GAPDH/CP12/PRK complex formation allows the regulation of the whole CBB cycle. This process represents an economic way to avoid accumulating or wasting of intermediate products. Together, the redox signal is fine-tuning the CBB cycle through regulating the activation-deactivation of PRK and the reversible formation of the ternary complex.

MATERIAL AND METHODS

Cloning

The genes encoding *At*PRK and *Se*PRK were polymerase chain reaction (PCR)-amplified using primer pairs 1 and 2 from cDNA libraries of *Arabidopsis thaliana* and *Synechococcus elongates* PCC7942, respectively. The resulting amplified products were gel-extracted and ligated into the linearized vector pET28a (Novagen) between Nco1 and Xho1 restriction sites for expression with a C-terminal His-tag. The mutants of *At*PRK were prepared by site-directed mutagenesis using overlap-PCR (Gibson, 2011). All primers for mutations were designed on the website https://www.bioinformatics.org/primers/. The PCR products were all analyzed by agarose gel electrophoresis and extracted for plasmid reconstruction. The details of all primers are listed in Supplemental Table 1.
To obtain the His tag-free proteins for the formation of AtGAPDH/CP12/PRK complex, the genes encoding AtPRK, AtGAPDH and AtCP12 were PCR-amplified with primer pairs 3, 4 and 5 from cDNA library of Arabidopsis thaliana. The resulting amplified product was gel-extracted and ligated into the linearized vector pMCSG7 through ligation-independent cloning (LIC). The expression vector pMCSG7 encodes an upstream His-tag followed by a tobacco etch virus (TEV) protease cleavage site, which allows removing of the His-tag from the mature proteins.

Escherichia coli strain Top10 was used for plasmid cloning, and the insertion of the genes were confirmed by DNA sequencing. The recombinant plasmids were transformed into Escherichia coli BL21 (DE3) for protein expression.

**Expression and purification of recombinant proteins**

The pET28a-transformed cells were cultured at 37 °C for four hours in Luria-Bertani (LB) medium supplied with kanamycin (50 μg/mL). The pMCSG7-transformed cells were cultured at 37 °C for four hours in LB medium supplied with ampicillin (100 μg/mL). The expression and purification procedure for all recombinant proteins is similar and described below.

When the optical absorption density at 600 nm (OD$_{600}$) of the Escherichia coli BL21 cells reached 0.8, the protein expression was induced by the addition of 1 mM isopropylthio-b-galactoside (IPTG). The culture was further incubated for 16 hours at 30 °C. The cells were then harvested by centrifugation at 8,000 ×g for 10 min. The harvested cells were re-suspended in buffer A (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 20 mM imidazole), and disrupted by sonication. The cell lysate was centrifuged at 18,000 ×g for 40 min at 4 °C. The supernatant containing the target protein was loaded onto a Ni$^{2+}$-affinity column (GE Healthcare) pre-equilibrated with buffer A and then washed with buffer B (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 50 mM
imidazole). The target protein was then eluted with buffer C (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 250 mM imidazole). The recombinant proteins with C-terminal His-tag were further purified by gel filtration chromatography using a Superdex 200 10/300 GL column (GE healthcare) equilibrated with buffer D (20 mM Hepes, pH 7.5, 100 mM NaCl).

The *AtGAPDH/CP12/PRK* complex formation

The recombinant *AtPRK*, *AtGAPDH* and *AtCP12* proteins with N-terminal His-tag were first digested with TEV protease at 4 °C overnight. Then the digested target protein applied to a Ni^{2+}-affinity column before gel filtration chromatography. To obtain *AtGAPDH/CP12/PRK* complex, *AtGAPDH* was first treated with 2 mM NAD\(^+\) for 5 hours at 4 °C, then *AtGAPDH*, *AtPRK* and *AtCP12* were mixed and incubated at molar ratio (subunit basis) of 8: 4: 4 with 0.01 mM CuSO\(_4\) for 12 hours at 4 °C. Finally, the *in vitro* assembled complex was isolated from free proteins by gel filtration chromatography with Superdex 200 10/300 GL column (GE healthcare) equilibrated with the buffer of 20 mM Hepes, pH 7.5, 100 mM NaCl.

Each purified protein was checked by SDS-PAGE and identified by Peptide Mass Fingerprinting on Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS).

**Crystallization**

To obtain the reduced and oxidized form of *AtPRK*, we treated *AtPRK* protein with 5 mM DTT and 0.01 mM CuSO\(_4\), respectively. Before crystallization, *SePRK*, reduced and oxidized *AtPRK* were concentrated to 10 mg/mL using 50 kDa MCWO centrifugal filter units (Millipore) and GAPDH/CP12/PRK complex was concentrated to 15 mg/mL using 100 kDa MCWO centrifugal filter units (Millipore). All crystals
were grown by the sitting-drop vapor diffusion method at 291K. Crystals of reduced and oxidized PRK were grown in the solution containing 4% v/v Tacsimate (Hampton research) pH 5.0, 12% w/v polyethylene glycol 3350. The heavy atom derivative of the *At*PRK crystal was obtained by soaking the crystals in the presence of 1 mM ethylmercuricthiosalicylic acid for 16 h at 291K. SePRK crystals were grown in solution containing 0.02 M magnesium chloride hexahydrate, 0.05 M MES monohydrate pH 6.0, 15% v/v 2-propanol with 10 mM ATP and 10 mM G6P. The condition for *At*GAPDH/CP12/PRK crystallization was 100 mM Hapes, pH 7.5, 100 mM KCl, 15% w/v polyethylene glycol 6000. To improve the diffraction quality of *At*GAPDH/CP12/PRK complex crystals, glutaraldehyde was added to reservoir solution with a final concentration of 0.1% w/v after the crystals appeared. Moreover, the crystals were soaked in solution containing PEG 6000 with increasing concentration, from 15% w/v to 40% w/v gradually before data collection. The post crystal treatment followed the previously reported method (Heras and Martin, 2005).

**Data collection and structure determination**

Five diffraction data sets for crystals of native *At*PRK$_{\text{red}}$, native *At*PRK$_{\text{ox}}$, *At*PRK$_{\text{red}}$ with heavy atom (*At*PRK-Hg), SePRK and the *At*GAPDH/CP12/PRK complex were collected. The x-ray data of *At*PRK-Hg was collected at beamline BL5A in Photon Factory, Japan. Other diffraction data were all collected at Shanghai Synchrotron Radiation Facility in China. *At*PRK$_{\text{ox}}$ and GAPDH/CP12/RPK data were collected at beamline BL17U (Wang et al., 2018). *At*PRK$_{\text{red}}$ data was collected at beamline BL18U. SePRK data was collected at beamline BL19U (Zhang et al., 2019). These data sets were indexed, integrated and scaled by HKL2000 (Otwinowski and Minor, 1997). Phase calculation and automatic model building of *At*PRK-Hg was performed with Autosol and Autobuild in PHENIX (Adams et al., 2002). The model of *At*PRK-Hg was not further refined due to low resolution. Structure of *At*PRK$_{\text{red}}$ was
solved by molecular replacement of PHENIX, using \textit{At}PRK-Hg structure as an initial model. The structures of \textit{At}PRK\textsubscript{ox} and \textit{Se}PRK were solved using the molecular replacement method with the structure of \textit{At}PRK\textsubscript{red}. The structure of \textit{At}GAPDH/CP12/PRK was solved using molecular replacement with the structures of \textit{At}PRK\textsubscript{ox} and a previously reported structure of GAPDH in complex with CP12(CTD) (PDB code: 3RVD) (Fermani et al., 2012) as searching models. The structural models were manually built with COOT (Emsley and Cowtan, 2004) and further refined with PHENIX (Afonine et al., 2018).

The first four residues were not built in all \textit{At}PRK structures. In the \textit{At}PRK\textsubscript{red} structure, there are four chains in an asymmetric unit. Chain A was built from E5 to the last residue A351 and plus two residues from the vector (L352-E353), while Chain B, C and D were built to A347 (chains B, C) and A351 (chain D). In the \textit{At}PRK\textsubscript{ox} structure, two molecules are in an asymmetric unit and were built from E5 to R346 (chain A) and E5 to A347 (chain B). In the \textit{Se}PRK structure, only one chain is in an asymmetric unit, which is built from M1 to Q143, S152 to Q325, with the last eight residues missing in the final model. An ADP and a G6P molecule were manually built based on the electron density map. In \textit{At}GAPDH/CP12/PRK structure, an asymmetric unit contains one complex. The two GAPDH tetramers were completely built (from K1 to K336) except for one chain in which the last residue is missing. Two PRK dimers were all built from E5 to R346, and four CP12 monomers were built from G6 to the last residue N78 in two chains and from I7 to N78 in the other two chains.

\textbf{Cryo-EM sample preparation, data acquisition and image processing}

Approximately 3.0 μL of the GAPDH/CP12/PRK complex was added to glow discharged holey carbon grid (GIG-C31213). The grid was flash-frozen in liquid ethane at around 100 K using a semi-automatic plunge device (Thermo Fisher...
Scientific Vitrobot IV) with a blotting time of 3 s and blotting force of level 4 at 100% humidity, 16 °C.

A total of 1,621 dose-fractionated super-resolution movies were collected on a 300-kV Titan Krios electron microscope (Thermo Fisher Scientific) equipped with K2 direct electron detector (Gatan), with a magnification of 130,000×, yielding a physical pixel size of 1.06 Å (0.53 Å super-resolution pixel size). Each exposure of 8 s was dose fractionated into 32 movie frames, leading to a total dose of ~ 50 e− Å⁻².

Movie stacks were binned, leading to a pixel size of 1.06 Å, before being subjected to beam-induced motion correction. The motion correction procedure produced two averaged images from each movie stack with or without dose weighting. The two images were subjected to anisotropic magnification distortion correction. The contrast transfer function (CTF) parameters of each image without dose weighting were determined by program CTFFIND4 (Rohou and Grigorieff, 2015). Images with dose weighting were used for particle picking, two dimensional (2D) classification, 3D classification and refinement in RELION-3.0 (Scheres, 2012). A subset of GAPDH/CP12/PRK complex particles was manually picked and processed with reference-free 2D classification, and three 2D class averages were selected as references for automatic particle selection of the whole dataset. A total of 674,628 particles were picked and processed by reference-free 2D classification, and 226,376 particles were kept for further 3D classification. The selected particles were classified into five classes. The best two classes contained about 147,996 particles, were selected for further 3D auto-refinement, which resulted in a 4.9 Å reconstruction, estimated on the basis of the gold-standard Fourier shell correlation with 0.143 criterion. The local resolution of the final maps was calculated using ResMap (Kucukelbir et al., 2013).
Enzymatic assay and binding affinity analysis

The enzyme kinetic assay was performed according to a procedure reported previously with slight modification utilizing the ADP formation coupled to the NADH oxidation (Kobayashi et al., 2003; Racker, 1957). The enzymatic activity was quantified by measuring the reduction of NADH over time through monitoring the decrease in absorbance at 340 nm ($A_{340}$). The activity of the wild types and mutants of PRK was measured at 298K in the reaction solution of 100 mM Tris-HCl, pH 8.0, 4 mM MgCl$_2$, 10 mM KCl, 10 mM DL-Dithiothreitol (DTT), 0.3 mM NADH, 5 U pyruvate kinase (PK), 5 U Lactate dehydrogenase (LDH), 0.5 mM phosphoenolpyruvate (PEP) and 1 mM ATP. PRK was added to the reaction solution at final concentration of 40 ng/mL and incubated for 10 min at 298K. Ru5P was added to a final concentration of 1.2 mM to initialize the reaction and then the $A_{340}$ was measured for 10 minutes.

The interaction between ATP and PRK was investigated by a Surface Plasmon Resonance instrument (Biacore 8K, GE Healthcare) at 298K with running buffer containing 20 mM HEPES, pH 7.5, 100 mM NaCl. A sensor chip SA (Series S GE Healthcare) flow cell was activated with 1 M NaCl and 5 mM NaOH for 4 min. PRK proteins were incubated with biotin in a molar ratio of 1:1 for 30 min. Spare biotin was removed by repeated concentrating and diluting for 3 times. Then proteins were diluted to 40 μg/ml and immobilized to sensor chip.

Calorimetric experiments were conducted utilizing a MicroCal iTC200 instrument (Malvern) at 298K. To analyze the affinity between PRK and Ru5P, 1 mM ATP and 2 mg/mL PRK in the buffer containing 100 mM Tris-HCl, pH 8.0, 4 mM MgCl$_2$, 10 mM KCl, 2 mM DTT were incubated for 10 min in the cell and then titrated with 0.5 mM Ru5P in the same buffer.
All measurements of enzymatic activity of PRKs and the binding affinity with ATP and Ru5P were repeated two to three times, with similar results obtained.

**Sequence alignment**

Multiple sequence alignment (Supplemental Fig. 1) was performed with ClustalW2 (https://www.ebi.ac.uk/Tools/msa/clustalo/) and displayed with ESPript 3.0 followed by manual adjustment (http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi).

**Accession Numbers**

The genes encoding *At*PRK, *At*GAPDH and *At*CP12 used in this study are AT1G32060, AT3G26650 and AT3G62410 in Arabidopsis Information Resource (www.arabidopsis.org/). The gene encoding *Se*PRK protein is synpcc7942_0977 in Uniprot (www.uniprot.org/). Coordinates and structure factors have been deposited in the RCSB Protein Data Bank under accession codes 6KEX (*At*PRK<sub>red</sub>), 6KEW (*At*PRK<sub>ox</sub>), 6KEV (*Se*PRK) and 6KEZ (*At*GAPDH/CP12/PRK complex). The accession codes of sequences for multiple sequence alignment in Supplemental Figure 4 are P25697 (*At*PRK), Q31PL2 (*Se*PRK), P93681 (*Ps*PRK), P09559 (*So*PRK), P19824 (*Cr*PRK) and P12033 (*Rs*PRK) in Uniprot.

**Supplemental Data**

**Supplemental Figure 1.** Multiple sequence alignment of phosphoribulokinase.

**Supplemental Figure 2.** The crystal packing of *At*PRK<sub>red</sub> and *At*PRK<sub>ox</sub>.

**Supplemental Figure 3.** Structural comparison of PRK structures from different species.
Supplemental Figure 4. Single particle cryo-EM analysis of AtGAPDH/CP12/PRK ternary complex.

Supplemental Figure 5. Structure and comparison of GAPDH/CP12/PRK complexes.

Supplemental Figure 6. Superposition of the docked G6P with the G6P molecule bound in SePRK structure.

Supplemental Figure 7. Characterization of the wild type and mutants of PRK.

Supplemental Figure 8. Measurements of ATP binding affinity of wild type and mutants of AtPRK as well as wild-type SePRK through the SPR method.

Supplemental Figure 9. Measurements of Ru5P binding affinity of wild type and mutants of AtPRK as well as wild-type SePRK through the ITC method.

Supplemental Table 1. The primers and vectors used for the constructs of GAPDH, CP12, and PRK (wild type and mutants)

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Author contributions

M.L. and W.C. conceived the project; A.Y. and Y.X. did the expression, purification, crystallization, data processing and structure determination of PRK proteins. A.Y. prepared GAPDH/CP12/PRK complex and performed the crystallization, data processing and structure determination of the complex; A.Y. performed the activity and affinity assays. X.P., H.Z. and P.C. helped in diffraction data collection; X.S. assisted in cryo-EM data collection; M.L., A.Y. and Y.X. analyzed the structures and wrote the manuscript; all authors discussed and commented on the results and the manuscript.

Conflict of Interest

The authors declare they have no conflict of interest
Figure legends

Figure 1. Overall structure of PRK

(A) Crystal structure of the AtPRK<sub>red</sub> colored in rainbow. The N- and C-termini and the structural elements important for PRK function are labeled.

(B) Structural superposition of PRK dimers in the crystal structure of AtGAPDH/CP12/PRK ternary complex (marine), AtPRK<sub>red</sub> (cyan), AtPRK<sub>ox</sub> (yellow) and SePRK (pink). The β7 is responsible for dimerization.

(C) Structural comparison of PRK monomers in the four crystal structures, color codes are the same as in (B). The lid region is highlighted in cartoon mode.

Figure 2. The P loop and clamp loop regions of the AtPRK

(A) Superposition of reduced (cyan) and oxidized (yellow) AtPRK. The clamp loop of reduced and oxidized AtPRK are highlighted in blue and orange, respectively.

(B) Comparison of the P-loop region between reduced (cyan) and oxidized (yellow) AtPRK structures. The P loop is flipped approximately 180° between the two AtPRK structures. Residues C17 and C56 are shown in ball-and-stick mode.

(C) B-factor representation of AtPRK<sub>red</sub> structure. The thicker ribbon indicates the higher B-factor. In addition to the terminal loops and P-loop region, the N-terminal part of clamp loop (clamp loop-N, colored marine) is characterized by higher B-factors. By contrast, the C-terminal part of clamp loop (clamp loop-C, colored grey) is stabilized by the second β-strand (β2, colored pink) and shows lower B-factors. The zoom-in view of the detailed interactions between clamp loop-C and β2 is shown in the right box. Residues are shown as sticks and labeled. The hydrogen bond interactions are represented as black dashed lines.

Figure 3. Overall structures of the AtGAPDH/CP12/PRK ternary complex and AtCP12 within the complex.
(A) Cartoon representation of *At*GAPDH/CP12/PRK complex. Two GAPDH tetramers are shown in green and orange. Two PRK dimers are shown in teal and marine. Four CP12 monomers are shown in magenta.

(B) The Cryo-EM density map of *At*GAPDH/CP12/PRK at 4.9 Å resolution (left) and the fitting of the crystal structure of *At*GAPDH/CP12/PRK at 3.5 Å resolution into the Cryo-EM map (right).

(C) The structure of oxidized CP12 in the *At*GAPDH/CP12/PRK complex. CP12 is shaped like a hook. The N-helix (magenta) and the central helix (salmon) constitute the N-terminal domain (NTD). The C-terminal domain (CTD, sand) contains the C-helix. The conserved region (34AWDEVEE40) within the central helix of CP12 is highlighted in red. Two pairs of disulfide bonds are shown in sticks.

**Figure 4.** Structural comparison of proteins within the *At*GAPDH/CP12/PRK complex or with their free forms.

(A) Structural comparison of four CP12 monomers within the *At*GAPDH/CP12/PRK ternary complex superposed on their NTD (upper panel) and CTD (lower panel). The four CP12 molecules are colored differently.

(B) Structural comparison of two PRK dimers within the *At*GAPDH/CP12/PRK ternary complex superposed on the left monomer (upper panel), and comparison between one PRK dimer within the ternary complex structure (colored marine) and the *At*PRKox structure (colored yellow) superposed on the left monomer (lower panel).

(C) Superposition of the GAPDH part in the *At*GAPDH/CP12/PRK structure (blue-white), the previously reported GAPDH-CP12(CTD) structure (PDB code 3RVD, magenta) and the GAPDH tetramer structure (PDB code 1RM4, pale yellow).

**Figure 5.** The inter-molecular interactions in *At*GAPDH/CP12/PRK ternary complex.
(A) The CTD of CP12 is inserted into the interfacial region of two GAPDH monomers, blocking their active sites. The CP12 is shown as a cartoon in salmon; the two GAPDH monomers are shown in surface mode and colored in pale green and grey, respectively. The NAD\(^+\) ligands bound to the GAPDH monomers are shown as sticks.

(B) The central helix of CP12 is located in a long groove on the surface of PRK. The CP12 is shown as a cartoon in salmon, and the conserved regions are highlighted in magenta. The PRK molecule is shown in surface mode and the groove is composed of the second helix (\(\alpha_2\), magenta), the lid region (yellow) and P loop (cyan).

(C) The conserved region (34AWD_VEE40) within the central helix of CP12 is located in the positively charged region of the groove in PRK. PRK is shown in electrostatic potential surface mode; red represents negative charge, and blue represents positive charge. CP12 is shown as a cartoon in salmon, with the conserved region (34AWD_VEE40) highlighted in yellow-orange.

(D) The detailed interaction between CP12 and PRK in the AtGAPDH/CP12/PRK ternary complex. CP12 (salmon) and PRK (teal) are shown as cartoon. Residues crucial for the interaction are shown as sticks and labeled. The hydrogen bonds are represented as black dashes. The conserved region (34AWD_VEE40) within the central helix of CP12 is highlighted in yellow-orange.

(E) Characterization of the ternary complex through size exclusion chromatography (SEC) and sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). The SEC profiles (left) of wild type (WT) as well as three mutants R65A, R68A and C17S of AtPRK incubated with NAD\(^+\)-bound GAPDH and oxidized CP12 are shown in black, blue, green and magenta lines, respectively. Peak 1 is the GAPDH/CP12/PRK ternary complex, and the peak 1 fraction in the black line (wild-type of PRK incubated with GAPDH and CP12) is identified through SDS-PAGE (right). The WT and C17S mutant of AtPRK are able to form GAPDH/CP12/PRK ternary complex.
(F) Structural superposition of AtPRK$_{red}$ (lime) and the oxidized PRK (light blue) within the AtGAPDH/CP12/PRK ternary complex. The P-loop region in reduced AtPRK does not interfere its binding with CP12 (shown in salmon cartoon). The P-loop regions are circled by black dashed line and indicated.

**Figure 6. The active site of PRK.**

(A) The structure of SePRK bound with ADP and G6P. Fragments involved in ADP and G6P binding are labeled in blue. The ligands ADP, G6P and the coordinated residues are shown as sticks and labeled in black. The $2|F_o| - |F_c|$ electron density maps of ADP and G6P contoured at 1.0 σ are shown as blue mesh.

(B) The electrostatic potential surface of SePRK. Red represents negative charge, and blue represents positive charge. The ADP and G6P molecules, occupying two positively charged grooves on PRK surface, are shown as sticks and circled with green line.

(C) The electrostatic potential surface of SePRK with docked ligands ATP and Ru5P. Red represents negative charge, and blue represents positive charge. The ATP and Ru5P are shown as sticks and circled with orange line.

(D) Superposition of bound ADP and G6P molecules (green for carbon atoms) with docked ATP and Ru5P molecules (yellow for carbon atoms) in SePRK. The ligands are shown as sticks. The distance between the C1 hydroxyl group of Ru5P and G6P is 5.8 Å, shown by black dashed line (left panel). The distance between the C1 hydroxyl group of Ru5P and the γ-phosphate group of ATP is 5.2 Å, shown by black dashed line (right panel).

**Figure 7. The key residues for catalysis of PRK.**

(A) The Ru5P site and ATP site in SePRK and AtPRK. PRK structures are shown in cartoon mode. Reduced AtPRK structure (cyan) is superposed with the SePRK structure (pink). The docked Ru5P and ATP as well as the residues potentially
involved in their interactions are shown as sticks. Residues are labeled with their
numbers in both \textit{At}PRK (before the slash) and \textit{Se}PRK (after the slash). The color of
the residue labels is consistent with that in (B).

\textbf{(B)} Enzymatic activity and binding assays of wild types of \textit{At}PRK and \textit{Se}PRK, and of
\textit{At}PRK mutants. The catalysis activity is calculated from an ATPase reporter assay.
The activity of \textit{At}PRK mutants and \textit{Se}PRK wild-type are shown in relative percentage
of \textit{At}PRK wild-type activity. The $K_D$ values of ATP were measured using SPR
methods. The $K_D$ values of Ru5P were measured using ITC, and by titrating Ru5P
into the ATP-incubated \textit{At}PRK. Mean values and standard deviation are calculated
from two repeated measurements. Mutant forms with activity completely lost and
dramatically decreased are shown in red and blue, respectively. The mean values of
activity and affinity were calculated from two independent measurements.

\textbf{Figure 8. The ATP site and Ru5P site in PRK.}

\textbf{(A)} Structural superposition of \textit{At}PRK\textsubscript{ox} (yellow) and \textit{Se}PRK (pink) with docked ATP
and Ru5P molecules (shown as sticks). The ATP site is disrupted in the \textit{At}PRK\textsubscript{ox}
structure (yellow) as the P loop clashes with the ATP molecule, whereas the Ru5P site
is unaffected in the \textit{At}PRK\textsubscript{ox} structure.

\textbf{(B)} Structural superposition of \textit{At}PRK in the ternary complex (slate) and \textit{Se}PRK (pink)
with docked ATP and Ru5P molecules (shown as sticks). The Ru5P site is at the
PRK-CP12 interface of the \textit{At}GAPDH/CP12/PRK complex, and is disrupted upon
binding CP12 (green), whereas the ATP site is unaffected. The two conserved
residues D36 and E40 of CP12 are shown as sticks and labeled. Residue D36 of CP12
is potentially clashing with Ru5P.

\textbf{Figure 9. Potential TRX binding site on \textit{At}PRK and \textit{At}CP12.}

\textbf{(A)} Electrostatic potential surface of \textit{At}PRK\textsubscript{red}. The clamp loop is shown in cartoon
mode, highlighted in blue and indicated by black arrow. The residue C56 is shown in
sticks and indicated. The potential binding pocket of TRX is circled by a green dashed line.

(B) Electrostatic potential surface of TRX from Spinach (PDB code:1FAA). The two Cys residues are shown in sticks and indicated.

(C) Electrostatic potential surface of AtCP12. The disulfide bond between C22 and C31 at the NTD is shown in sticks and indicated. The potential binding pocket of TRX is marked by a black box.
Table 1
Data collection and refinement statistics

| Parameter | Hg-ATPRK | ATPRK<sub>red</sub> | ATPRK<sub>ox</sub> | SePRK | ATGAPDH/CP12/PRK |
|-----------|----------|------------------|------------------|-------|------------------|
| PDB code  | -<sup>a</sup> | 6KEX             | 6KEW             | 6KEV             | 6KEZ             |
| Beamline  | PF-BL5A  | SSRF-BL18U       | SSRF-BL17U       | SSRF-BL19U1      | SSRF-BL17U       |
| Wave length | 0.98    | 0.98             | 0.98             | 0.98             | 0.98             |
| Space group | P3;21   | P1               | C2               | P22;2<sub>1</sub> | P2<sub>1</sub>   |
| Cell dimensions |  |                  |                  |                  |                  |
| \(a\) (\(\text{Å}\)) | 146.8, 146.8 | 103.1 | 90.0 | 90.0 | 120.0 |
| \(b\) (\(\text{Å}\)) | 49.8, 40.4 | 70.1 | 90.0 | 90.0 | 89.9 |
| \(c\) (\(\text{Å}\)) | 80.8, 80.4 | 90.0 | 90.0 | 90.0 | 90.0 |
| \(\alpha\) (°) | 90.0, 90.0 | 90.0 | 90.0 | 90.0 | 90.0 |
| \(\beta\) (°) | 103.1, 90.0 | 94.4 | 94.4 | 94.4 | 94.4 |
| \(\gamma\) (°) | 194.8, 90.0 | 157.0 | 157.0 | 157.0 | 157.0 |
| Resolution (\(\text{Å}\)) | 42.7-2.3, 29.14-2.5 | 42.7-2.3, 29.14-2.5 | 42.7-2.3, 29.14-2.5 | 42.7-2.3, 29.14-2.5 | 42.7-2.3, 29.14-2.5 |
| Completeness (%) | 95.6 (95.2) | 95.1 (98.9) | 100 (100) | 97.8 (97.5) | 97.8 (97.5) |
| Redundancy | 2.54-2.50 | 2.54-2.50 | 2.54-2.50 | 2.54-2.50 | 2.54-2.50 |
| \(R_{\text{merge}}\) | 0.188 (0.590) | 0.146 (0.763) | 0.146 (0.763) | 0.146 (0.763) | 0.146 (0.763) |
| \(R_{\text{meas}}\) | 0.193 (0.986) | 0.092 (0.601) | 0.092 (0.601) | 0.092 (0.601) | 0.092 (0.601) |
| \(R_{\text{sym}}\) | 0.124 (0.619) | 0.036 (0.232) | 0.036 (0.232) | 0.036 (0.232) | 0.036 (0.232) |
| CC1/2 | 0.958 (0.742) | 0.996 (0.978) | 0.996 (0.978) | 0.996 (0.978) | 0.996 (0.978) |

**Refinement**

| Parameter | Hg-ATPRK | ATPRK<sub>red</sub> | ATPRK<sub>ox</sub> | SePRK | ATGAPDH/CP12/PRK |
|-----------|----------|------------------|------------------|-------|------------------|
| No. reflection | 49,034 | 23,784 | 12,042 | 63,151 |
| \(R_{\text{work}} / R_{\text{free}}\) | 0.21/0.26 | 0.24/0.25 | 0.19/0.22 | 0.25/0.29 |
| No. Protein atoms | 10,938 | 5,431 | 2,540 | 33,429 |
| No. Ligands | 3 | 8 |
| No. Water | 178 | 174 | 60 | 46 |
| \(B\) factors (\(\text{Å}^2\)) | 53.4 | 42.6 | 47.3 | 59.1 |
| Proteins | 53.4 | 42.6 | 47.3 | 59.1 |
| Ligand | 76.5 | 44.5 | 44.5 | 44.5 |
| Water | 47.2 | 37.2 | 44.1 | 38.9 |
| RMSD<sup>c</sup> | 0.005 | 0.003 | 0.004 | 0.003 |
| Bond length (\(\text{Å}\)) | 0.005 | 0.003 | 0.004 | 0.003 |
| Bond angle (°) | 0.75 | 0.62 | 0.68 | 0.64 |
| Ramachandran plot<sup>d</sup> (%) | 98.1 | 97.8 | 98.7 | 96.8 |

<sup>a</sup> Data collection and refinement for Hg-ATPRK were performed on PF-BL5A, while the other proteins were collected on SSRF-BL18U, SSRF-BL17U, and SSRF-BL19U1. The resolutions for the data sets are given in parentheses. completeness (%) and redundancy are presented in parentheses.**
| Allowed   | 1.9 | 2.2 | 1.3 | 3.2 |
|-----------|-----|-----|-----|-----|
| Outliers  | 0.00| 0.00| 0.00| 0.00|

The content of an asymmetric unit:
- four PRK monomers
- two PRK monomers
- one PRK monomer
- two GAPDH tetramers,
- two PRK dimers,
- four CP12 monomers

The percentage of model completion:
- GAPDH, 99.7%-100%
- PRK, 97.7%
- CP12, 92.3%-93.6%

| a | No PDB code for Hg-AtPRK |
| b | Highest resolution shell is shown in parentheses |
| c | r. m. s. deviation, root mean square deviation |
| d | Ramachandran plot was calculated using the program Molprobity (Chen et al., 2010) |
Adams, P.D., Grosse-Kunstleve, R.W., Hung, L.W., Ioerger, T.R., McCoy, A.J., Moriarty, N.W., Read, R.J., Sacchettini, J.C., Sauter, N.K., and Terwilliger, T.C. (2002). PHENIX: building new software for automated crystallographic structure determination. Acta Crystallogr D Biol Crystallogr 58, 1948-1954.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Afonine, P.V., Poon, B.K., Read, R.J., Sobolev, O.V., Terwilliger, T.C., Urzhumtsev, A., and Adams, P.D. (2018). Real-space refinement in PHENIX for cryo-EM and crystallography. Acta Crystallogr D Struct Biol 74, 531-544.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Avron, M., and Gibbs, M. (1974). Properties of Phosphoribulokinase of Whole Chloroplasts. Plant Physiol 53, 136-139.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Baalmann, E., Scheibe, R., Cerff, R., and Martin, W. (1996). Functional studies of chloroplast glyceraldehyde-3-phosphate dehydrogenase subunits A and B expressed in Escherichia coli: formation of highly active A4 and B4 homotetramers and evidence that aggregation of the B4 complex is mediated by the B subunit carboxy terminus. Plant Mol Biol 32, 505-513.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Brandes, H.K., Larimer, F.W., and Hartman, F.C. (1996). The molecular pathway for the regulation of phosphoribulokinase by thioredoxin f. Journal of Biological Chemistry 271, 3333-3335.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Brinkmann, H., Cerff, R., Salomon, M., and Soll, J. (1989). Cloning and Sequence-Analysis of Cdnas Encoding the Cytosolic Precursors of Subunits Gapa and Gapb of Chloroplast Glyceraldehyde-3-Phosphate Dehydrogenase from Pea and Spinach. Plant Mol Biol 13, 81-94.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Buchanan, B.B. (1980). Role of Light in the Regulation of Chloroplast Enzymes. Annu Rev Plant Phys 31, 341-374.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Buchanan, B.B. (1991). Regulation of CO2 assimilation in oxygenic photosynthesis: the ferredoxin/thioredoxin system. Perspective on its discovery, present status, and future development. Arch Biochem Biophys 288, 1-9.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Buchanan, B.B., and Balmer, Y. (2005). Redox regulation: A broadening horizon. Annu Rev Plant Biol 56, 187-220.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Capitan, G., Markovic-Housley, Z., DelVal, G., Morris, M., Jansonius, J.N., and Schurm ann, P. (2000). Crystal structures of two functionally different thioredoxins in spinach chloroplasts. J Mol Biol 302, 135-154.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chen, V.B., Arendall, W.B., 3rd, Headd, J.J., Keedy, D.A., Immormino, R.M., Kapral, G.J., Murray, L.W., Richardson, J.S., and Richardson, D.C. (2010). MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr D Biol Crystallogr 66, 12-21.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Clasper, S., Easterby, J.S., and Powls, R. (1991). Properties of two high-molecular-mass forms of glyceraldehyde-3-phosphate dehydrogenase from spinach leaf, one of which also possesses latent phosphoribulokinase activity. Eur J Biochem 202, 1239-1246.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Collin, V., Issakidis-Bourguet, E., Marchand, C., Hirasawa, M., Lancelin, J.M., Knaff, D.B., and Miginiac-Maslow, M. (2003). The Arabidopsis plastidial thioredoxins: new functions and new insights into specificity. J Biol Chem 278, 23747-23752.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Dai, S., Johansson, K., Miginiac-Maslow, M., Schurm ann, P., and Eklund, H. (2004). Structural Basis of Redox Signaling in Photosynthesis: Structure and Function of Ferredoxin:thioredoxin Reductase and Target Enzymes. Photosynth Res 79, 233-248.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Del Giudice, A., Pavel, N.V., Galantini, L., Falinti, G., Trost, P., Fermani, S., and Sparla, F. (2015). Unravelling the shape and structural assembly of the photosynthetic GAPDH-CP12-PRK complex from Arabidopsis thaliana by small-angle X-ray scattering analysis. Acta
Elena Lopez-Calcagno, P., Omar Abuzaid, A., Lawson, T., and Anne Raines, C. (2017). Arabidopsis CP12 mutants have reduced levels of phosphoribulokinase and impaired function of the Calvin-Benson cycle. J Exp Bot 68, 2285-2298.

Emnsley, P., and Cowtan, K. (2004). Coot: model-building tools for molecular graphics. Acta Crystallogr D Biol Crystallogr 60, 2126-2132.

Fermani, S., Sparla, F., Falini, G., Martelli, P.L., Casadio, R., Pupillo, P., Ripamonti, A., and Trost, P. (2007). Molecular mechanism of thioredoxin regulation in photosynthetic A2B2-glyceraldehyde-3-phosphate dehydrogenase. Proc Natl Acad Sci U S A 104, 11109-11114.

Fermani, S., Trivelli, X., Sparla, F., Thumiger, A., Calvaresi, M., Marri, L., Falini, G., Zerbetto, F., and Trost, P. (2012). Conformational selection and folding-upon-binding of intrinsically disordered protein CP12 regulate photosynthetic enzymes assembly. J Biol Chem 287, 21372-21383.

Geiger, D.R., and Servaites, J.C. (1994). Diurnal Regulation of Photosynthetic Carbon Metabolism in C-3 Plants. Annu Rev Plant Phys 45, 235-256.

Gibson, D.G. (2011). Enzymatic assembly of overlapping DNA fragments. Methods Enzymol 498, 349-361.

Graciet, E., Gans, P., Wedel, N., Lebreton, S., Camadro, J.M., and Gontero, B. (2003). The small protein CP12: a protein linker for supramolecular complex assembly. Biochemistry 42, 8163-8170.

Graciet, E., Lebreton, S., and Gontero, B. (2004). Emergence of new regulatory mechanisms in the Benson-Calvin pathway via protein-protein interactions: a glyceraldehyde-3-phosphate dehydrogenase/CP12/phosphoribulokinase complex. J Exp Bot 55, 1245-1254.

Groben, R., Kaloudas, D., Raines, C.A., Offmann, B., Maberly, S.C., and Gontero, B. (2010). Comparative sequence analysis of CP12, a small protein involved in the formation of a Calvin cycle complex in photosynthetic organisms. Photosynth Res 103, 183-194.

Gurrrieri, L., Del Giudice, A., Demtri, N., Falini, G., Pavel, N.V., Zaffagnini, M., Polentarutti, M., Crozet, P., Marchand, C.H., Henri, J., et al. (2019). Arabidopsis and Chlamydomonas phosphoribulokinase crystal structures complete the redox structural proteome of the Calvin-Benson cycle. Proc Natl Acad Sci U S A.

Harrison, D.H., Runquist, J.A., Holub, A., and Miziorko, H.M. (1998). The crystal structure of phosphoribulokinase from Rhodobacter sphaeroides reveals a fold similar to that of adenylate kinase. Biochemistry 37, 5074-5085.

Heras, B., and Martin, J.L. (2005). Post-crystallization treatments for improving diffraction quality of protein crystals. Acta Crystallogr D Biol Crystallogr 61, 1173-1180.

Howard, T.P., Lloyd, J.C., and Raines, C.A. (2011). Inter-species variation in the oligomeric states of the higher plant Calvin cycle enzymes glyceraldehyde-3-phosphate dehydrogenase and phosphoribulokinase. J Exp Bot 62, 3799-3805.

Howard, T.P., Metodiev, M., Lloyd, J.C., and Raines, C.A. (2008). Thioredoxin-mediated reversible dissociation of a stromal multiprotein complex in response to changes in light availability. Proc Natl Acad Sci U S A 105, 4056-4061.

Johnson, M.P. (2016). Photosynthesis. Essays Biochem 60, 255-273.
Kam o, M., Tsugita, A., Wessner, C., Wedel, N., Bartling, D., Herrmann, R.G., Aguilar, F., Gardet-Salvi, L., and Schurmann, P. (1989). Primary structure of spinach-chloroplast thioredoxin f. Protein sequencing and analysis of complete cDNA clones for spinach-chloroplast thioredoxin f. Eur J Biochem 182, 315-322.

Kobayashi, D., Tami, M., Iwaki, T., Shigeoka, S., and Wadano, A. (2003). Molecular characterization and redox regulation of phosphoribulokinase from the cyanobacterium Synechococcus sp PCC 7942. Plant Cell Physiol 44, 269-276.

Kono, T., Mehrotra, S., Endo, C., Kizu, N., Matusda, M., Kimura, H., Mizohata, E., Inoue, T., Hasunuma, T., Yokota, A., et al. (2017). A RuBisCO-mediated carbon metabolic pathway in methanogenic archaea. Nat Commun 8, 14007.

Kucukelbir, A., Sigworth, F.J., and Tagare, H.D. (2013). Quantifying the local resolution of cryo-EM density maps. Nature Methods 11, 63-65.

Kuken, A., Sommer, F., Yaneva-Roder, L., Mackinder, L.C., Hohne, M., Geimer, S., Jonikas, M.C., Schroda, M., Stitt, M., Nikoloski, Z., et al. (2018). Effects of microcompartmentation on flux distribution and metabolic pools in Chlamydomonas reinhardtii chloroplasts. Elife 7.

Kung, G.S., Runquist, J.A., Mizioro, H.M., and Harrison, D.H.T. (1999). Identification of the allosteric regulatory site in bacterial phosphoribulokinase. Biochemistry 38, 15157-15165.

Latzko, E., von Garnier, R., and Gibbs, M. (1970). Effect of photosynthesis, photosynthetic inhibitors and oxygen on the activity of ribulose 5-phosphate kinase. Biochem Biophys Res Commun 39, 1140-1144.

Launay, H., Barre, P., Puppo, C., Manneville, S., Gontero, B., and Receveur-Brechot, V. (2016). Absence of residual structure in the intrinsically disordered regulatory protein CP12 in its reduced state. Biochem Biophys Res Commun 477, 20-26.

Lebreton, S., and Gontero, B. (1999). Memory and imprinting in multienzyme complexes. Evidence for information transfer from glyceraldehyde-3-phosphate dehydrogenase to phosphoribulokinase under reduced state in Chlamydomonas reinhardtii. J Biol Chem 274, 20879-20884.

Lebreton, S., Graciet, E., and Gontero, B. (2003). Modulation, via protein-protein interactions, of glyceraldehyde-3-phosphate dehydrogenase activity through redox phosphoribulokinase regulation. J Biol Chem 278, 12078-12084.

Li, M.H., Kwok, F., Chang, W.R., Liu, S.Q., Lo, S.C., Zhang, J.P., Jiang, T., and Liang, D.C. (2004). Conformational changes in the reaction of pyridoxal kinase. J Biol Chem 279, 17459-17465.

Lopez-Calacagno, P.E., Howard, T.P., and Raines, C.A. (2014). The CP12 protein family: a thioredoxin-mediated metabolic switch? Front Plant Sci 5, 9.

Maeda, K., Tsugita, A., Dalzoppo, D., Vilbois, F., and Schurmann, P. (1986). Further characterization and amino acid sequence of m-type thioredoxins from spinach chloroplasts. Eur J Biochem 154, 197-203.

Marri, L., Thieulin-Pardo, G., Lebrun, R., Puppo, R., Zaffagnini, M., Trost, P., Gontero, B., and Sparla, F. (2014). CP12-mediated protection of Calvin-Benson cycle enzymes from oxidative stress. Biochimie 97, 228-237.
Marri, L., Trost, P., Pupillo, P., and Sparla, F. (2005). Reconstitution and properties of the recombinant glyceraldehyde-3-phosphate dehydrogenase/CP12/phosphoribokinase supramolecular complex of Arabidopsis. Plant Physiol 139, 1433-1443.

Marri, L., Trost, P., Trivelli, X., Gonnelli, L., Pupillo, P., and Sparla, F. (2008). Spontaneous assembly of photosynthetic supramolecular complexes as mediated by the intrinsically unstructured protein CP12. J Biol Chem 283, 1831-1838.

Marri, L., Zaffagnini, M., Collin, V., Issakidis-Bourguet, E., Lemarié, S.D., Pupillo, P., Sparla, F., Miginiac-Maslow, M., and Trost, P. (2009). Prompt and easy activation by specific thioredoxins of Calvin cycle enzymes of Arabidopsis thaliana associated in the GAPDH/CP12/PRK supramolecular complex. Mol Plant 2, 259-269.

Matsumura, H., Kai, A., Maeda, T., Tamoi, M., Satoh, A., Tamura, H., Hirose, M., Ogawa, T., Kizu, N., Wadano, A., et al. (2011). Structure basis for the regulation of glyceraldehyde-3-phosphate dehydrogenase activity via the intrinsically disordered protein CP12. Structure 19, 1846-1854.

McFarlane, C.R., Shah, N.R., Kabasakal, B.V., Echeverria, B., Cotton, C.A.R., Bubeck, D., and Murray, J.W. (2019). Structural basis of light-induced redox regulation in the Calvin-Benson cycle in cyanobacteria. Proc Natl Acad Sci U S A 116, 20984-20990.

Melandri, B.A., Baccarini, A., and Pupillo, P. (1968). Glyceraldehyde-3-Phosphate Dehydrogenase in Photosynthetic Tissues - Kinetic Evidence for Competitivity between Nadp and Nad. Biochem Bioph Res Co 33, 160-+.

Michelet, L., Zaffagnini, M., Morisse, S., Sparla, F., Perez-Perez, M.E., Francia, F., Danon, A., Marchand, C.H., Fermani, S., Trost, P., et al. (2013). Redox regulation of the Calvin-Benson cycle: something old, something new. Front Plant Sci 4, 470.

Milanez, S., Mural, R.J., and Hartman, F.C. (1991). Roles of Cysteinyl Residues of Phosphoribulokinase as Examined by Site-Directed Mutagenesis. Journal of Biological Chemistry 266, 10694-10699.

Oesterhelt, C., Klocke, S., Holtgrefe, S., Linke, V., Weber, A.P., and Scheibe, R. (2007). Redox regulation of chloroplast enzymes in Galdieria sulphuraria in view of eukaryotic evolution. Plant Cell Physiol 48, 1359-1373.

Otwinowski, Z., and Minor, W. (1997). Processing of X-ray diffraction data collected in oscillation mode. Methods Enzymol 276, 307-326.

Petersen, J., Teich, R., Becker, B., Cerff, R., and Brinkmann, H. (2006). The GapAB gene duplication marks the origin of streptophyta (Charophytes and land plants). Mol Biol Evol 23, 1109-1118.

Pohlmeyer, K., Paap, B.K., Soll, J., and Wedel, N. (1996). CP12: A small nuclear-encoded chloroplast protein provides novel insights into higher-plant GAPDH evolution. Plant Mol Biol 32, 969-978.

Racker, E. (1957). The reductive pentose phosphate cycle. I. Phosphoribulokinase and ribulose diphosphate carboxylase. Arch Biochem Biophys 69, 300-310.

Roesler, K.R., Marcotte, B.L., and Ogren, W.L. (1992). Functional Importance of Arginine 64 in Chlamydomonas reinhardtii Phosphoribulokinase. Plant Physiol 98, 1285-1289.
Ruelland, E., and Miginiac-Maslow, M. (1999). Regulation of chloroplast enzyme activities by thioredoxins: activation or relief from inhibition? Trends Plant Sci 4, 136-141.

Runquist, J.A., Harrison, D.H., and Miziorko, H.M. (1998). Functional evaluation of invariant arginines situated in the mobile lid domain of phosphoribokinase. Biochemistry 37, 1221-1226.

Scagiarini, S., Trost, P., and Pupillo, P. (1998). The non-regulatory isoform of NAD(P)-glyceraldehyde-3-phosphate dehydrogenase from spinach chloroplasts. Journal of Experimental Botany 49, 1307-1315.

Scheibe, R. (1991). Redox-Modulation of Chloroplast Enzymes - a Common Principle for Individual Control. Plant Physiol 96, 1-3.

Scheibe, R., Wedel, N., Vetter, S., Emmelrich, V., and Sauermann, S.M. (2002). Co-existence of two regulatory NADP-glyceraldehyde 3-P dehydrogenase complexes in higher plant chloroplasts. Eur J Biochem 269, 5617-5624.

Scheres, S.H. (2012). RELION: implementation of a Bayesian approach to cryo-EM structure determination. J Struct Biol 180, 519-530.

Schlauderer, G.J., Proba, K., and Schulz, G.E. (1996). Structure of a mutant adenylate kinase ligated with an ATP-analogue showing domain closure over ATP. Journal of Molecular Biology 256, 223-227.

Schurmann, P., and Buchanan, B.B. (2008). The ferredoxin/thioredoxin system of oxygenic photosynthesis. Antioxid Redox Sign 10, 1235-1273.

Schurmann, P., and Jacquot, J.P. (2000). Plant thioredoxin systems revisited. Annu Rev Plant Phys 51, 371-400.

Shirakihara, Y., and Evans, P.R. (1988). Crystal structure of the complex of phosphofructokinase from Escherichia coli with its reaction products. J Mol Biol 204, 973-994.

Sigrell, J.A., Cameron, A.D., Jones, T.A., and Mowbray, S.L. (1998). Structure of Escherichia coli ribokinase in complex with ribose and dinucleotide determined to 1.8 angstrom resolution: insights into a new family of kinase structures. Structure 6, 183-193.

Sparla, F., Pupillo, P., and Trost, P. (2002). The C-terminal extension of glyceraldehyde-3-phosphate dehydrogenase subunit B acts as an autoinhibitory domain regulated by thioredoxins and nicotinamide adenine dinucleotide. Journal of Biological Chemistry 277, 44946-44952.

Stanley, D.N., Raines, C.A., and Kerfeld, C.A. (2013). Comparative Analysis of 126 Cyanobacterial Genomes Reveals Evidence of Functional Diversity Among Homologs of the Redox-Regulated CP12 Protein. Plant Physiol 161, 824-835.

Tabita, F.R. (1980). Pyridine nucleotide control and subunit structure of phosphoribulokinase from photosynthetic bacteria. J Bacteriol 143, 1275-1280.

Tabita, F.R. (1980). Pyridine nucleotide control and subunit structure of phosphoribulokinase from photosynthetic bacteria. J Bacteriol 143, 1275-1280.
Tamoi, M., Murakami, A., Takeda, T., and Shigeoka, S. (1998). Lack of light/dark regulation of enzymes involved in the photosynthetic carbon reduction cycle in cyanobacteria, Synechococcus PCC 7942 and Synechocystis PCC 6803. Biosci Biotech Bioch 62, 374-376.

Trott, O., and Olson, A.J. (2010). Software News and Update AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. J Comput Chem 31, 455-461.

Wang, Q.-S., Zhang, K.-H., Cui, Y., Wang, Z.-J., Pan, Q.-Y., Liu, K., Sun, B., Zhou, H., Li, M.-J., Xu, Q., et al. (2018). Upgrade of macromolecular crystallography beamline BL17U1 at SRF. Nuclear Science and Techniques 29, 68.

Wedel, N., and Soll, J. (1998). Evolutionary conserved light regulation of Calvin cycle activity by NADPH-mediated reversible phosphoribulokinase/CP12/glyceraldehyde-3-phosphate dehydrogenase complex dissociation. Proc Natl Acad Sci USA 95, 9699-9704.

Wedel, N., Soll, J., and Paap, B.K. (1997). CP12 provides a new mode of light regulation of Calvin cycle activity in higher plants. P Natl Acad Sci USA 94, 10479-10484.

Wilson, R.H., Hayer-Hartl, M., and Bracher, A. (2019). Crystal structure of phosphoribulokinase from Synechococcus sp. strain PCC 6301. Acta Crystallogr F Struct Biol Commun 75, 278-289.

Wirtz, W., Stitt, M., and Heldt, H.W. (1982). Light Activation of Calvin Cycle Enzymes as Measured in Pea Leaves. Febs Lett 142, 223-226.

Zhang, W.-Z., Tang, J.-C., Wang, S.-S., Wang, Z.-J., Qin, W.-M., and He, J.-H. (2019). The protein complex crystallography beamline (BL19U1) at the Shanghai Synchrotron Radiation Facility. Nuclear Science and Techniques 30, 170.

Zhang, Y., Launay, H., Liu, F., Lebrun, R., and Gontero, B. (2018). Interaction between adenylate kinase 3 and glyceraldehyde-3-phosphate dehydrogenase from Chlamydomonas reinhardtii. FEBS J 285, 2495-2503.
