Cyanobacterial Sfp-type phosphopantetheinyl transferases functionalize carrier proteins of diverse biosynthetic pathways

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Cyanobacteria produce structurally and functionally diverse polyketides, nonribosomal peptides and their hybrids. Sfp-type phosphopantetheinyl transferases (PPTases) are essential to the production of these compounds via functionalizing carrier proteins (CPs) of biosynthetic megaenzymes. However, cyanobacterial Sfp-type PPTases remain poorly characterized, posing a significant barrier to the exploitation of cyanobacteria for biotechnological and biomedical applications. Herein, we describe the detailed characterization of multiple cyanobacterial Sfp-type PPTases that were rationally selected. Biochemical characterization of these enzymes along with the prototypic enzyme Sfp from Bacillus subtilis demonstrated their varying specificities toward 11 recombinant CPs of different types of biosynthetic pathways from cyanobacterial and Streptomyces strains. Kinetic analysis further indicated that PPTases possess the higher binding affinity and catalytic efficiency toward their cognate CPs in comparison with noncognate substrates. Moreover, when chromosomally replacing the native PPTase gene of Synechocystis sp. PCC6803, two selected cyanobacterial PPTases and Sfp supported the growth of resulted mutants. Cell lysates of the cyanobacterial mutants further functionalized recombinant CP substrates. Collectively, these studies reveal the versatile catalysis of selected cyanobacterial PPTases and provide new tools to synthesize cyanobacterial natural products using in vitro and in vivo synthetic biology approaches.

More than 1,000 structurally diverse natural products have been isolated from cyanobacterial species over the past decades1,2. These compounds possess a wide array of bioactivities, e.g., antibacterial, antifungal, antiviral, immunomodulatory, protease inhibitory, and cytotoxic activities3–5. Evidently, monomethyl auristatin E, a derivative of cyanobacterial nonribosomal peptide/polyketide (NRP/PK) hybrid dolastatin 106,7, is a clinically valuable anti-mitotic agent. Remarkably, bioinformatic analysis of over 140 cyanobacterial genomes available in the NCBI database reveals at least three NRP synthetases (NRPSs) per genome on average, while some genomes contain more than forty natural product gene clusters8,9. These results illustrate the untapped potential of cyanobacteria as a source of new chemicals. However, this potential remains unachievable unless capable tools are available to translate cyanobacterial genetic information into compounds10,11. Indeed, only a few cyanobacterial natural products including several ribosomally synthesized and post-translationally modified peptides12,13, barbamide (a PK/NRP hybrid) and lyngbyatoxin A (an NRP) have so far been heterologously produced in E. coli or Streptomyces sp.14–16. Recently, lyngbyatoxin A was also produced in the model cyanobacterium Anabaena sp. PCC7120, achieving a yield similar to its native producer Moorea producens17.

Phosphopantetheinyl transferases (PPTases) play an essential role in the biosynthesis of fatty acids (FAs), NRPs, and PKs by functionalizing carrier protein (CPs) of their corresponding biosynthetic enzymes. They use coenzyme A (CoA) as one substrate to posttranslationally modify one conserved serine residue of the CPs with a 4′-phosphopantetheine (PPant) moiety, thereby converting inactive apo-form CPs to active holo-form proteins (Fig. S1)18. The CPs are responsible for shuttling biosynthetic intermediates among multiple catalytic domains

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within a module and delivering the substrate to the next module via the PPant arm. Based on sequence simila-
rity and substrate specificity, PPTases are categorized into three subfamilies19. AcpS from E. coli is the prototype
of the first subfamily20, whose members generally comprise about 120 amino acids and functionalize only the
CPs of FA synthases (FAs). The second subfamily of PPTases comprises integrated domains within the type I
yeast and fungal FAs, where they specifically modify the cognate CPs19. The last subfamily is known as Sfp-type
PPTases, named after the archetypal enzyme Sfp from Bacillus subtilis21. Compared to AcpSs, these enzymes are
two times longer and functionalize a broad range of substrates including noncognate CPs for the biosynthesis
of FAs, NRPs, PKs, and their hybrids21. Sfp-type PPTases can further be subdivided into the NRPS-preferred F/
KES and PKS-favored W/KEA groups based on their sequences22. Given their attractive substrate promiscuity,
Sfp-type PPTases have demonstrated many biotechnological applications, including site-specific protein labeling,
cell imaging, and the heterologous production of microbial secondary metabolites22–25.

Despite the attractive potential of cyanobacterial natural products, only three PPTases from cyanobacteria
have so far been biochemically characterized17,26–27. The enzyme from the cyanobacterium Nodularia spumigena
NSOR10 (NspPT) is the only one activating both cognate and noncognate CPs of cyanobacteria26–27. Synechocystis
sp. PCC6803 (hereafter referred to as Synechocystis) encodes only one PPTase (SPPT) and this enzyme was
shown to activate only the CP of its cognate FAS27. In addition, biochemical characterization of the PPTase from
Oscillatoria PCC6506 (OPPT) was performed with one cognate CP28. On the other hand, the E. coli BAP1 strain
harboring a chromosomal Sfp gene is able to produce several functional cyanobacterial FASs, PK synthases (PKSs)
and NRPSs28–30, indirectly suggesting the versatility of Sfp in activating cyanobacterial CPs. Here, we report the
characterization of six cyanobacterial PPTases and Sfp toward 11 CPs of known and silent pathways from cyano-
bacteria and Streptomyces strains. Biochemical and kinetic studies indicated the varying substrate promiscuity
of these enzymes and suggested the coevolution of PPTases/cognate CP subfamilies. Two selected cyanobacterial
PPTases and Sfp further demonstrated the versatile in vivo and in vitro catalytic activity when they were tran-
siently expressed in Synechocystis. These results identify cyanobacterial PPTases with the catalytic proficiency
and efficiency in activating CPs from diverse natural product biosynthetic pathways and lay a solid foundation to
harness the biosynthetic potential of cyanobacteria via synthetic biology approaches.

Materials and Methods
Phylogenetic analysis of cyanobacterial PPTases. Sfp and E. coli AcpS were used as two query
sequences to mine currently available cyanobacterial genomes in NCBI database (up to Nov 1st, 2016) using
BLAST program. The output data of BLAST were carefully analyzed to identify the sequences with comparatively
high similarity (with e-values ≤10–5) and to eliminate redundant sequences from taxonomically close species. The
resulted selection of 39 PPTase protein sequences along with those from Streptomyces rapamycinicus NRRL5491,
Xenopus laevis and Homo sapiens was aligned by Clustal Omega and then analyzed with MEGA732 using a maxi-
mum likelihood statistical method to construct a phylogenetic tree. The confidence was evaluated with 1000
bootstraps.

Biochemical characterization of PPTase activity. Enzyme reaction solutions (100 μl) typically con-
tained 50 mM Tris-Cl, pH 8.0, 12.5 mM MgCl2, 0.5 mM coenzyme A, 5 mM dithiothreitol (DTT) and 50 μM CPs.
The reactions were initiated by adding 0.3 μM (final concentration) of PPTases, incubated at 37 °C for 20 min,
and terminated by mixing with 100 μl of 10% formic acid. To quantitatively determine the relative activity of the
enzymes, the reaction time can be up to 40 min. The quenched solutions were centrifuged at 4 °C, 16,000 × g for
15 min and clear supernatants were collected and subjected to HPLC and LCMS analysis with details shown in the
supporting information. All experiments were repeated in triplicate. For kinetic studies, the reactions were set up
as described above except that the concentrations of CPs were varied from 0.5 to 120 μM. The reactions were
performed at 37 °C for 5–10 min to ensure that ≤10% of substrates were converted. To determine the concentrations
of holo-CPs, 0.2 to 50 μM of apo-proteins were fully converted in the enzyme reactions and then quantitated in
HPLC analysis to establish standard curves of holo-CPs. The concentrations of existing holo-CPs in the substrate
solutions were subtracted in the data analysis. Data were fit into the Michaelis-Menten equation to determine
kinetic parameters using GraphPad Prism 4.0. All experiments were independently repeated three times.

Genetic engineering of Synechocystis. Synechocystis cells (about 1 × 10⁶ cells/ml) in the exponential
phase were collected after centrifugation at 8,000 rpm for 15 min and resuspended in fresh BG11 medium at a
density of 1 × 10⁹ cells/ml. Integration constructs at a final concentration of 10 μg/ml were then incubated with the
cell solution at room temperature. After 5 h, the mixtures were spread onto BG11 agar plates supplemented
with 5 μg/ml kanamycin. The segregation of wild type with the desirable mutants was achieved by iteratively
streaking the colonies onto plates with progressively increased concentrations of kanamycin (up to 50 μg/ml).
The final stable mutants were genotyped by the colony PCR using the primers listed in Table S1. Growth curves of
the wild type and three mutant strains were determined by daily record of the OD₅₇₀ of the liquid cultures over
the period of 13 days.

Quantitative RT-PCR analysis of the integrated exogenous PPTase genes. Total RNA samples
were isolated from Synechocystis and its mutants using ZR Fungal/Bacterial RNA MiniPrep kit (Zymo Research).
The quantity and quality of the isolated RNAs were determined using Nanodrop. Synthesis of cDNAs was per-
formed with random primers following the manufacturer’s protocol (Thermo Scientific). The synthesized cDNAs
were used as templates for qPCR to detect the transcription of the integrated PPTase genes, while the isolated
RNA samples themselves were used as the templates of PCR reactions to detect any residual genomic DNAs using
primers listed in Table S1. The student’s t-test analysis was applied to determine significance difference between
the samples, and a P-value < 0.05 was considered to be statistically significant.
Preparation of cell lysates of *Synechocystis* mutants. Cells of the wild type and three *Synechocystis* mutants were harvested from 0.8 to 1.0 L culture after centrifugation at 4 °C, 4,000 × g for 15 min. Cell pellets were washed with fresh BG11 medium and then resuspended in 4 ml of lysis buffer (50 mM MES, pH 7.0, 10 mM MgCl₂, 5 mM CaCl₂, 1 mM phenylmethylsulfonyl fluoride and 10% glycerol). The solutions were frozen at −80 °C and thawed at room temperature prior to the sonication on ice with 2-s pulses. Cell homogenates were centrifuged at 4 °C, 25,000 × g for 30 min to collect clear cell lysates. The enzyme reaction mixtures were set up as described above except containing 70 μl of cell lysates. The reactions were incubated at 37 °C for 16 h, and the holo-products were detected in LCMS analysis as described above. The reactions were performed in triplicate.

**Data availability statement.** All data generated or analyzed during this study are included in this manuscript (and its Supplementary Information files).

**Results and Discussion**

Phylogenetic analysis of cyanobacterial Sfp-type PPTases. To gain an understanding of the evolutionary relationship of cyanobacterial PPTases, we mined all cyanobacterial genomes available in NCBI database using Sfp and *E. coli* AcpS as queries. A total of 39 sequences was then selected and retrieved from 39 strains of 26 genera that broadly cover all five subsections of cyanobacteria (Table S2)16. Phylogenetic analysis of these sequences led to a constructed tree comprising an AcpS-type clade, which included AcpS and eight cyanobacterial PPTases, and a Sfp-type clade containing all other enzymes (Fig. 1)16. In the Sfp-type clade, Sfp and three PPTases from *Streptomyces rapamycinicus* NRRL5491, *Xenopus laevis* and *Homo sapiens* as outgroups were separated from cyanobacterial Sfp-type PPTases. Furthermore, enzymes from the heterocystous cyanobacteria (subsections IV and V) formed a sub-clade, while those from sections I-III showed a less clear pattern. For example, the PPTases from *Gloeocapsa* sp. PCC73106 (subsection I) and *Spirulina subsalsa* (subsection III) fell into the...
same group (Fig. 1). These results indicate that cyanobacterial Sfp-type PPTases share a common ancestor and have acquired different traits over the course of evolution.

Selection of cyanobacterial Sfp-type PPTases and CP substrates. To comprehensively understand the catalytic performance of cyanobacterial Sfp-type PPTases, we next selected representative enzymes based on the result of phylogenetic analysis (Fig. 1) and predicted biosynthetic potential of cyanobacterial strains. The genomes of Fischerella sp. PCC9339 (hereafter Fischerella), Anabaena sp. PCC7120 (hereafter Anabaena) and A. variabilis ATCC29413 all possess more than 10 NRP and/or PK gene clusters and their PPTases, FPPT, APPT and AvPPT, respectively, were included in this work due to potentially broad substrate promiscuity. In addition, FPPT and APPT/AvPPT belong to two distantly related groups in the same subclade of subsections IV and V, likely representing the enzymes from a variety of heterocystous cyanobacteria (Fig. 1). We also selected the PPTases from Microcystis aeruginosa NIES843 (MPPT) carrying a rich biosynthetic potential and Synechococcus elongatus PCC7942 (SePPT) that forms a separate leaf in the phylogenetic tree (Fig. 1). S. elongatus PCC7942 encodes no PK or NRP cluster. Furthermore, SPPT was included as a control due to its reported narrow substrate scope. Finally, the paucity of biochemical characterization of Sfp in activating cyanobacterial CPs led to its selection. The six selected cyanobacterial PPTases and Sfp contain the featured W/KEA motif and together cover the broad space of the constructed phylogenetic tree (Fig. 1).

We next chose 11 CPs from multiple biosynthetic pathways of different species for biochemical characterization of the selected PPTases (Table S3, Fig. S3). They included two ACPs of FASs from Synechocystis (SFACP) and Anabaena (AFACP), one ACP of the glycolipid PKS in Anabaena (APACP)35, one ACP of the apratoxin (PK/NRP) gene cluster in Lyngbya (Moorea) bouillonii (AprACP), and the PCP of the shinorine gene cluster from Fischerella36. In addition, we included three CPs of uncharacterized gene clusters from Fischerella (FNPCP, an NRP pathway), Anabaena (APNPCP, an NRP/PK pathway) and M. aeruginosa NIES843 (MACP, an NRP/PK pathway) and one ACP from Fischerella (FNSACP) that is a homologous enzyme of previously characterized ArCP26. To thoroughly examine the versatility of selected PPTases, we also included one ACP of a putative concanamycin gene cluster from Streptomyces coelicolor A(3)2 (ScACP) and one PCP of a thaxtomin cluster from the plant pathogen S. scabiei 87.22 (SsPCP)37.

In vitro Phosphopantetheinylation of cognate and noncognate CPs by selected PPTases. Recombinant proteins (Fig. S3) were purified from E. coli by a single step Ni-NTA affinity chromatography. All purified proteins showed expected molecular weights in SDS-PAGE analysis (Fig. S4), and the CP substrates were further validated in LC-MS analysis. SFACP, AFACP and SsACP gave rise to two peaks in the LC traces with the minor peaks as the holo-proteins (Fig. 2A). The rest of CP substrates were in apo-forms (Fig. 2B, Table S3, and Fig. S5). This result suggests that E. coli AcpS activates noncognate ACPs to a low level and shows the limited promiscuity toward CPs of NPRSs and PKSs.

We next examined the catalytic activity of each PPTase toward all 11 recombinant CPs. The substrates in 69 out of all 77 reactions were fully converted into the holo-products (Figs 2 and 3). APPT, AvPPT, MPPT and
Table 1. Kinetics parameters of four selected PPTases toward 11 CPs. aThe data represented mean ± SD of three independent experiments; bUnits of $K_m$, $k_{cat}$, and $k_{cat}/K_m$ are µM, min$^{-1}$, and µM$^{-1}$ min$^{-1}$, respectively; cNo detectable activity.

Figure 3. Relative activities of seven PPTases toward 11 CP substrates. The activity of the most active PPTase of a CP was set as 100%, and those of other PPTases toward the same substrate were normalized as shown in the heatmap. The data represented the mean of three independent experiments. Red to white color indicated the high to low relative activities of PPTases. CPs were grouped according to the biosynthetic pathways while cyanobacterial PPTases were organized by the subsections of sources.

Sfp functionalized all substrates (Table S4). Unexpectedly, SPPT also phosphopantetheinylated all CP substrates except ScACP (Fig. 3, and Table S3). In an early report, SPPT was unable to activate two ACPs from Nostoc punctiforme ATCC 29133 and one PCP from M. aeruginosa PCC7806. On the other hand, SePPT and FPPT showed no activity toward APNPCP, MACP and ScACP and the former was also inactive toward SsACP.

To quantitate the substrate preference of PPTases, the reactions were performed to allow less than 95% of substrate conversion. After setting the activity of the most active enzyme as 100%, the activities of other PPTases toward the same substrate were normalized as shown in the heatmap. The data represented the mean of three independent experiments. Red to white color indicated the high to low relative activities of PPTases. CPs were grouped according to the biosynthetic pathways while cyanobacterial PPTases were organized by the subsections of sources.
Kinetic analysis of APPT, MPPT, SPPT and Sfp. To assess the catalytic efficiency of selected PPTases, we kinetically analyzed APPT, MPPT, SPPT and Sfp in activating all 11 CP substrates (Table 1, and Fig. S6). The conversion of SsPCP by Sfp showed the highest catalytic efficiency ($k_{\text{cat}}/K_m = 2.1 \pm 0.2 \mu M^{-1} \text{min}^{-1}$) of all reactions.

Figure 4. The in vivo activity of APPT, MPPT and Sfp in Synechocystis mutants. (A) Quantitative analysis of transcriptional levels of SPPT, APPT, MPPT and Sfp genes. The signals were normalized with that of rnpB gene from each mutant and wild type. The asterisk (*) indicated statistical significance differences ($P < 0.05$). The error bars represented the standard deviations of triplicate assays. (B) Growth curve of Synechocystis wild type and mutants. OD$_{730}$ was continuously monitored for 13 days. The error bars represented the standard deviations of triplicate measurements.

Figure 5. Extracted ion chromatograms of holo-products in the reactions of cell lysate of Synechocystis APPT mutant. The products showed the expected molecular weights. Similar traces were observed in the reactions using cell lysates of two other mutants.
consistent with the overall kinetic performance of Sfp toward CPs of actinomycetes\(^{21,39}\). On the other hand, the catalytic efficiencies (\(k_{\text{cat}}/K_m\)) of Sfp toward nine cyanobacterial CPs were varied from 0.1±0.02 \(\mu\text{M}^{-1}\text{min}^{-1}\) (FisPCP) to 1.5±0.3 \(\mu\text{M}^{-1}\text{min}^{-1}\) (APACP).

Among the selected PPTases, APPT demonstrated the highest catalytic efficiencies toward AprACP, AFACP and APACP (1.6 to 1.8 \(\mu\text{M}^{-1}\text{min}^{-1}\)) (Table 1). These three cyanobacterial substrates were also favored by MPPT and SPPT (\(k_{\text{cat}}/K_m\geq1.0\ \mu\text{M}^{-1}\text{min}^{-1}\)). The catalytic efficiencies of APPT, MPPT and SPPT toward other cyanobacterial substrates were varied but both FisPCP and FNsACP were clearly disfavored (\(k_{\text{cat}}/K_m\geq0.1\text{ to }0.3\ \mu\text{M}^{-1}\text{min}^{-1}\)). Of note, we discovered that cognate CP/PPTase pairs generally demonstrate high catalytic efficiencies (\(k_{\text{cat}}/K_m\geq0.9\ \mu\text{M}^{-1}\text{min}^{-1}\), e.g., MACP/MPPT), likely indicating the potential co-evolution of biosynthetic enzymes.

The \(K_m\) values of four PPTases toward 11 CPs were in the \(\mu\text{M}\) range (Table 1). SFACP showed relatively tight interactions with all PPTases (\(K_m\leq1.5\) to 3.2 \(\mu\text{M}\)), while APACP was a relatively weak binder (\(K_m\geq10.0\) to 26.5 \(\mu\text{M}\)). Conversely, the PPTases showed the higher activity toward APACP (\(k_{\text{cat}}\geq14.6\pm1.3\ \text{min}^{-1}\)) compared to SFACP (\(k_{\text{cat}}\leq2.2\pm0.1\ \text{min}^{-1}\)). Interestingly, CP substrates demonstrated the lowest \(K_m\) values toward their cognate PPTases in comparison with other enzymes, indicating the potential co-evolution. In this regard, SPPT showed higher \(K_m\) values toward the majority of noncognate CPs than APPT and MPPT, presumably because of the lack of any PK or NRP cluster in *Synechocystis*. In line with this observation, the interactions of Sfp with the majority of cyanobacterial CPs were also relatively weak.

**In vivo and in vitro activity of transiently expressed APPT, MPPT and Sfp in *Synechocystis***

To explore the *in vivo* catalytic performance of APPT, MPPT and Sfp, we chromosomally replaced the SPPT gene, the only known *PPTase* gene in *Synechocystis*, with their genes (Figs S7 and S8). The expression of the integrated PPTase genes was controlled by a constitutive strong promoter Ptrc\(^{40}\). After homologous recombination and multiple rounds of segregation, three stable *Synechocystis* mutants were identified by the PCR diagnosis as the loss of the *SPPT* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A). The transcription of these *PPTase* genes was confirmed in RT-PCR analysis (Fig. S8B). Furthermore, their transcription levels were quantitated in qRT-PCR analysis after being normalized with that of the *rnpB* gene and the presence of foreign *PPTase* gene (Fig. S8A).

**Conclusions**

PPTases are essential enzymes of all three domains of life as they functionalize CPs of FAs, PKSs, and NRPs. The past two decades have witnessed significant advances of PPTase research, particularly about structure-function-relationship and biotechnological and biomedical applications\(^{85}\). Here, we characterized the phylogenetic relationships of cyanobacterial PPTases and then rationally selected six Sfp-like cyanobacterial enzymes along with Sfp to characterize their substrate scope and catalytic activity toward 11 CPs of FAs, PKSs, and NRPs from cyanobacteria and Streptomyces strains. APPT, MPPT and Sfp demonstrated high catalytic activity and kinetic performance toward the majority of cyanobacterial CPs. Interestingly, PPTases favored cognate CP substrates in their reactions, suggesting the use of proper (e.g., cognate) PPTase for the heterologous production of natural products. This work used standalone CP substrates to characterize the catalytic performance of PPTases and previous studies suggest that they can demonstrate similar activity profiles toward CP domains within intact NRPs, PKS or their hybrids\(^{33,34}\). Furthermore, the validated *in vivo* and *in vitro* functions of transiently expressed APPT, MPPT and Sfp in the *Synechocystis* mutants suggest the availability of the novel, capable cyanobacterial synthetic biology chassis for the production of primary and secondary metabolites of cyanobacteria.

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Author Contributions
G.Y. and Y.D. conceived and designed experiments. G.Y. performed all major experiments, while Y.Z., N.K.L., M.A.C., and S.E.K. provided additional experimental assistance. G.Y., H.L. and Y.Z. wrote the manuscript. All authors critically reviewed the paper.

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