Plant immunophilins: a review of their structure-function relationship

Dileep Vasudevan[1,2,#], Gayathri Gopalan[1,#], Ashish Kumar[2], Veder J Garcia[3], Sheng Luan[3]* and Kunchithapadam Swaminathan[1]*

[1]Department of Biological Sciences, National University of Singapore, Singapore 117543, [2]Institute of Life Sciences, Bhubaneswar, Orissa 751023, India, [3]Department of Plant and Microbial Biology, University of California at Berkeley, Berkeley, CA 94720, USA.

 # Equal first authors

***Corresponding authors**: **SL**: Phone: +1 (510) 642 6306, Fax: +1 (510) 642 4995, Email: sluan@berkeley.edu. **KS**: Phone: +65 6516 7932, Fax: +65 6779 2486, Email: dbsks@nus.edu.sg

Key words: immunophilin, FK506, FKBP, cyclophilin, cyclosporin, redox

ABSTRACT

Background: Originally discovered as receptors for immunosuppressive drugs, immunophilins consist of two major groups, FK506 binding proteins (FKBPs) and cyclosporin A binding proteins (cyclophilins, CYPs). Many members in both FKBP and CYP families are peptidyl prolyl isomerases that are involved in protein folding processes, though they share little sequence homology. It is not surprising to find immunophilins in all organisms examined so far, including viruses, bacteria, fungi, plants and animals, as protein folding represents a common process in all living systems.

Scope of Review: Studies on plant immunophilins have revealed new functions beyond protein folding and new structural properties beyond that of typical PPIases. This review focuses on the structural and functional diversity of plant FKBPs and CYPs.

Major Conclusions: The differences in sequence, structure as well as subcellular localization, have added on to the diversity of this family of molecular chaperones. In particular, the large number of immunophilins present in the thylakoid lumen of the photosynthetic organelle, promises to deliver insights into the regulation of photosynthesis, a unique feature of plant systems. However, very little structural information and functional data are available for plant immunophilins.

General Significance: Studies on the structure and function of plant immunophilins are important in understanding their role in plant biology. By reviewing the structural and functional

properties of some immunophilins that represent the emerging area of research in plant biology, we hope to increase the interest of researchers in pursuing further research in this area.

1. Introduction

Members of the immunophilin protein family function as receptors for immunosuppressive drugs and are present in a broad range of organisms, including viruses, bacteria, fungi, plants and animals [1-3]. In plants, as in other organisms, two groups of immunophilins: FK506-binding proteins (FKBPs) and cyclophilins (CYP) with various molecular weights are the principal intracellular targets for immunosuppressant drugs FK506/rapamycin and cyclosporin A (CsA) respectively [1, 4]. They catalyze peptidyl-bond isomerization preceding a proline residue [5, 6]. However, parvulins, which are also peptidyl prolyl isomerases (PPIases), do not bind to any known immunosuppressant molecule and are not classified as immunophilins [7]. PPIases are involved in protein biosynthesis and folding of proteins [8], making protein folding a key role of immunophilins and parvulins [9, 10]. Moreover PPIases interact with several intra- and extracellular targets and are involved in several biochemical processes, including protein folding, signal transduction and development.

The cellular localization and domain organization patterns, along with structural diversity, of immunophilins underlie their functional versatility. Thus, in addition to their basic roles as PPIases and binding partners for immunosuppressant drugs, immunophilins take part in a variety of cellular processes such as receptor complex stabilization [11], apoptosis [12], cell signaling [13], RNA processing [14], spliceosome assembly [15] and plant stress response [16-18], along with several others [19].

The pioneering studies on FKBP12 and cyclophilin A, and their complexes with assorted ligands, have provided significant structure-function details on immunophilins. hFKBP12 contains a central five-stranded antiparallel β-sheet around a short α-helix. The twist in the β-sheet results in the formation of hydrophobic concave and convex surfaces [20]. Cyclophilin A comprises an eight β-strand barrel and α-helices at the top and bottom [21, 22]. Although the cyclophilin barrel has some similarity to the transport proteins β-lactoglobulin and retinol-binding protein, its topology is very different. The inner side of the β-barrel is blocked by the side chains of hydrophobic residues, thereby preventing ligand binding inside the barrel [23]. It is worth mentioning here that the binding of FK506 to FKBP12, or CsA to CyPA, does not cause significant structural changes to either protein but does allow the identification and analysis of the PPIase active sites [24].

In this review we attempt to mainly focus on the structure-function relationship of plant immunophilins. While it may be beyond the scope of this article to enlist all, first we give an overview about the salient features of selected plant immunophilins, Table 1. Subsequently, we present the details of plant FKBPs and CYPs in separate sections.

2. Plant FK506 binding proteins (FKBPs)

FKBPs form a large family in eukaryotes and plants harbor the maximum. In *Arabidopsis thaliana*, at least 23 genes encode FKBPs whereas in *Oryza sativa*, 29 genes have been identified. Some of these genes are essential for regulation of plant development. Cytosol, nucleus and chloroplast are some of the cellular compartments to which plant FKBPs are targeted [75]. Surprisingly, the chloroplast harbors the largest number of FKBPs. Most of them

are localized in the thylakoid lumen and are involved in photosynthetic protein-complex assembly and redox regulation [26, 76-79].

Plant FKBPs, which may be single and multi-domain structures, are composed of either single copy or tandem copies of the FK506 binding domain (FKBD). In addition, other functional domains like the tripartite tetratricopeptide repeat (TPR) and calmodulin-binding domain may also be present [47, 80-85]. Some plant FKBPs: wheat FKBP73 and FKBP77 isoforms (TaFKBPs) [46], rice FKBP64, FKBP65, and FKBP75 isoforms (OsFKBPs) [86], maize FKBP66 (ZmFKBP66) [83], *Ricinus communis* FKBP (RcFKBP), *Populus trichocarpa* FKBP (PtFKBP), *Vitis vinifera* FKBP (VvFKBP) and *Arabidopsis thaliana* FKBP62 and FKBP65 (AtFKBPs) [81, 85, 87] possess three tandem copies of the FKBD. The 12 conserved residues that are essential for drug binding and PPIase activity are present only in the first FKBD [46, 87], similar to human FKBP51 and FKBP52, wherein only the first of the two FKBDs remains active [88].

2.1. FKBP structures

As explained above, the general architecture of FKBPs follow a central β-sheet, flanked by an α-helix. The β-structure is composed of residues 21-31, 35-38, 46-49, 71-78, and 97- 107 and an α-helix is formed by residues 57-63 (hFKBP-12 numbering). Two loops comprised of residues 39-45 (which connect the two parts of the third β-strand) and 84-91, termed the 40s and 80s loop, respectively, surround the rotamase domain and are rather disordered in the unliganded protein [24].

The structures of only three plant FKBPs have been solved until now (AtFKBP13, AtFKBP42 and TaFKBP73). Each of them provide insights into the distinct features of the

respective multi-domain plant FKBPs and serves as the beginning for detailed characterization of their complexes with physiologically relevant target proteins. Nevertheless, their explicit behavior in plants has been difficult to pin down. Sequence alignment of the FKBDs of AtFKBP13, AtFKBP43 and the three FKBDs of TaFKBP73, along with the FKBD of hFKBP12 (Fig. 1) reveals that of the 11 key active-site residues inside the hydrophobic pocket that are involved in PPIase function (Y27, F37, D38, F47, F49, V56, I57, W60, Y83, I92 and F100; hFKBP12 numbering) [89], all 11 are conserved in the first FKBD of TaFKBP73 and 10 are conserved in AtFKBP13. In TaFKBD73-3 only six out of the 11 active site residues are conserved and only 2 are conserved in TaFKBD73-2. AtFKBP42 has only 3 conserved active site residues. The following section focuses on the structural characteristics of plant FKBPs and their biological functions.

2.2. AtFKBP13 structure reveals redox regulation

AtFKBP13 localizes to the thylakoid lumen of chloroplasts, wherein it regulates the accumulation of the Rieske protein, which is an essential component of the photosynthetic electron transport chain [26]. Active site residues involved in PPIase function of AtFKBP13 are quite conserved (Fig. 1) and that explains it being an active PPIase. Unlike the FKBPs studied in animals, AtFKBP contains a unique pair of disulfide bonds at both termini, as demonstrated by the crystal structure of oxidized AtFKBP13, (Fig. 2A; PDB: 1U79; [27]), hereafter called AtFKBP13-S2. The disulfide bonds in the protein play a major role in regulating its own enzymatic activity by their redox states [90]. Under normal conditions, the protein remains in the oxidized state and displays PPIase activity. AtFKBP13 gets reduced and inactivated rapidly by thioredoxin *in vitro*.

The structure provides an important insight into the relative orientations of the disulfide bridges, which are important for the biological function of the protein. The two disulfide bonds are formed by Cys5–Cys17 and Cys106–Cys111, respectively. These disulfide bonds are not present in FKBPs from animals or yeast [27]. Structural comparison with human and *Legionella pneumophila* FKBPs (Fig. 2B) indicates that the PPIase domain of AtFKBP13 has an additional strand (β_{6a} and β_{6b}) inserted at the C-terminus, the position occupied by Cys106 and Cys111. The disulfide bond between Cys5 and Cys17 at the N-terminus is located at the β_1-strand. The disulfide bonds form extra secondary structures on either sides of the central β-sheets and reveal the intrinsic flexibility and versatility of these regions. From the analysis of AtFKBP13 structure it can be seen that both disulfides are located on the surface of the molecule, with ready access to DTT *in vitro* and thioredoxin *in vivo* [27].

Superimposition of the backbone Cα traces of reduced AtFKBP13 onto the corresponding residues of the oxidized structure (residues 5 to 129) gives an RMSD of 1.22 Å (Fig. 3). Most of the secondary structural elements of oxidized and reduced AtFKBP13 (PDB: 1Y0O) superimpose very well. The surface features of both forms also reveal no major differences. However, a substantial difference could be seen in the disulfide regions, the C-terminal loop and the PPIase catalytic domain. There are some apparent changes in the local structure in the active site region upon the change of the reduction state. Small dihedral angle differences are also observed in the region of residues 107-109, which form a loop structure that closely contacts the active site. The major contribution to this significant difference is coming from the redox active C-terminal loop [90]. The catalytic domains are significantly shifted and twisted compared with the oxidized structure and this results in differences of up to 10 Å. Superimposition of only the catalytic domains of the reduced structure and the corresponding parts of the oxidized structure

gives an RMSD of 1.48 Å for 17 Cα atoms (residues 100-116). Reduction of the disulfide bond leads to an increase in the distance between the sulfur atoms of the two cysteines: the observed S–S distance in AtFKBP13-S2 Cys5–Cys17 and Cys106–Cys111 are 2.04±0.01 and 2.01±0.01Å, respectively as expected for a disulfide bond, whereas the corresponding distance is 5.18 Å and 2.81 Å for AtFKBP13- (SH)2 [90].

One of the most striking features of the reduced (inactive) form of the protein is the way in which the C-terminal loop undergoes conformational changes. When the disulfide bond is broken, the contacts in the active site are no longer strong enough to hold the extension. The backbone conformational change that occurs to accommodate the increase in the radius of sulfur upon reduction and the breakage of the disulfide bond also causes an increase in the Cα–Cα distances between the two cysteines [90]. Furthermore, a noteworthy difference in secondary structures between oxidized AtFKBP13-S2 and AtFKBP13-(SH)2 is found in helix α2, which in oxidized AtFKBP13 includes residues 50 to 53 and is absent in AtFKBP13-(SH)2. This region is converted to a loop. Another region where there is a difference in secondary structure is helix α1, which is one residue shorter in AtFKBP13-(SH)2. Interestingly, α2 in the oxidized structure contains the conserved PPIase residues, Ser50 and Arg53. These shifts in secondary structures may account for the loss of PPIase activity in the reduced form [90].

The oxidized and reduced structures demonstrate that reduction of the reactive cysteine residues does not disturb the geometry of the PPIase site in oxidized AtFKBP13 drastically. However, it alters the accessibility of the catalytic residues involved in PPIase activity to some extent. The significant change caused by the reduction is limited to the movement of the side chain Sγ of active site Cys5 ,Cys106 and Cys111 thus opening up the structure at the N-terminus [90]. The C-terminal disulfide is involved in inactivating the enzyme. In fact, the structural study

reveals the movement of the C-terminal loop toward the active site. Mutants of AtFKBP13 lacking the C-terminal disulfide show reduced catalytic activity, suggesting a direct interaction of the C-terminal loop with the active site region. In short, the structural features of AtFKBP13 support the idea that this protein is regulated by redox mechanism and possibly link to other proteins in the thylakoid lumen as a regulator [90].

2.3. AtFKBP42 is a regulator of ABC transporters

AtFKBP42, a type II membrane protein comprising of 365 amino acid residues, also known as TWISTED DWARF1 (TWD1) due to the reduced height and disoriented growth of null mutants, consists of an FKBD, a TPR motif, a putative calmodulin-binding site and a hydrophobic C-terminal membrane anchor [31, 32, 93, 94]. A unique feature of AtFKBP42 is that it does not possess any PPIase activity and it does not display any measurable affinity for FK506. As such, out of the 11 known residues involved in PPIase function, the protein has only 3 of the active site residues conserved (Fig. 1). The putative FKBD of this protein has been shown to physically interact with AtPGP1 and AtPGP19, which are plasma membrane-localized ABC transporters [31] and is also responsible for functional association with vacuolar transporters AtMRP1 and AtMRP2 [32].

The N-terminus of AtFKBP42 (residues 1-180) containing the FKBP-like domain structure (PDB: 2F4E; [95]) and also the water-soluble portion of the molecule (residues 1-339), which contains the putative TPR modules, in addition to the FKBD (PDB: 2IF4; [94]), was determined by X-ray crystallography (Fig. 4). The N-terminal FKBP-type fold consists of a five-stranded antiparallel β-sheet wrapped around a short α-helix. The C-terminus consists of a helical bundle and the two domains are linked by a long loop. The domain interface of AtFKBP42 (1–

339) is made up of a hydrophobic network, surrounded by hydrogen bonds and electrostatic contacts. It is suggested that there could be some intrinsic freedom in the relative orientation of the two domains *in vivo*, similar to the closely related FKBP51 [96] and FKBP52 [97]. Generally, FKBP and TPR modules have been implicated in a variety of biological activities that involve numerous interaction partners, which may not be compatible with a fixed domain arrangement. The authors therefore speculate that these two domains of AtFKBP42 may exhibit different orientations depending on their physiological context [94].

The FK506 molecule bound to an FKBP has been suggested to mimic the transition state of a PPIase substrate [98]. The reason for the lack of FK506 affinity and inactivity in PPIase assays in AtFKBP42 has been accounted to the absence of several of the hydrogen bonding partners at the corresponding positions. Among various multi-domain immunophilins, AtFKBP42 is unique in that both the FKBP and TPR domains interact with ABC transporters *in vivo*. The FKBD of the protein interacts with the C-terminal portions of AtPGP1 and AtPGP19 which contain a second nucleotide-binding domain (NBD2), while the TPR domain binds to the equivalent domains in AtMRP1 and AtMRP2. In order to improve the understanding about these novel protein-protein interactions, homology models for the NBD2 of AtPGP1 and AtMRP1, as representatives of these two classes of ABC transporters, were generated [94]. The models show that the NBD2 surfaces, contacted by the FKBP and TPR moieties of AtFKBP42, are closely linked to the subdomain interface; the TPR domain interacts with residues from both subdomains as well as the distal domain linker, whereas the FKBP module does not directly interact with α-subdomain, but develops an elaborate hydrogen bonding network with a short α-helix. This causes restriction to the mobility of the subsequent β-strand located at the core of the subdomain

interface. It is therefore speculated that AtFKBP42 may improve ABC transporter activity by stabilizing a conformation of NBD2 that is capable of binding nucleotides [94].

The extreme phenotypes displayed by the TWD1 and *ultracurvata 2* (UCU2) mutants of *Arabidopsis* have been attributed to disruptions in fkbp42 gene. The AtFKBP42 mutants *twd1* and *ucu2*, both show a number of pleiotropic defects such as reduced height and disoriented growth of all organs, but develop fertile flowers and seeds. Genetic and molecular analysis of the *twd1*/*ucu2* mutant indicate that FKBP42 is involved in the auxin and brassinosteroid (BR) signaling pathways [33]. For instance, by genetic manipulation of *TWD1* expression, it has recently been shown that TWD1 affects root auxin reflux towards shoot and thus, downstream developmental traits, such as epidermal twisting and gravitropism of the root [99]. Overall, several studies provide evidence that FKBP42 targets membrane transporters in plant to fulfill its essential function in the regulation of auxin-mediated plant growth and development [31, 32, 99, 100].

2.4. TaFKBP73 is a multi-domain FKBP with a regulatory function

The crystal structure of FKBP73 from wheat - TaFKBP73, also known as wFKBP73 (Fig. 5; PDB: 3JYM and 3JXV; [87]), reveals that the three FKBDs, TaFKBD73-1, TaFKBD73-2 and TaFKBD73-3, exhibit a similar fold, as can be seen in other FKBP family members [101]. However, the arrangement of the domains is unique and may have important functional implications. The TaFKBD73-2 and TaFKBD73-3 domains, which lack PPIase activity, are shown to lack the hydrophobic cavity necessary for PPIase activity and the conserved residues that are essential for binding FK506. Although there are reasonable structure and sequence identities between the three FKBDs, only the FKBD1 of all FKBPs exhibits PPIase activity,

which in turn, gets inhibited by the binding of FK506 and rapamycin [46]. This is due to the fact that only the FKBD1 of all FKBPs contains all the conserved amino acids for binding to FK506 or rapamycin and for PPIase activity [46, 102]. Out of the 11 residues important for PPIase activity of FKBPs, only two are conserved in TaFKBD73-2 and six in TaFKBD73-3, whereas TaFKBD73-1 has all 11 residues conserved (Fig. 1).

The main functions of FKBPs have been attributed to the drug binding capacity and modulatory effects on complexes. The existence of FKBPs with 3 domains offers additional interaction possibilities for these plant proteins. Multiple FKBDs of FKBPs could be the result of a duplication event. TaFKBP73, like hFKBP51/52, contains a TPR domain responsible for binding to HSP90, a calmodulin-binding domain [9, 46], and an FKBD with PPIase enzymatic activity. It has been shown that TaFKBP73 functions as a molecular chaperone *in vitro* independent of its PPIase activity [103]. Over-expression of truncated FKBP73 in plants causes male sterility [104]. It is suggested that during evolution, TaFKBD73-2 and TaFKBD73-3 may have lost their PPIase activity, but gained the ability to interact with other partners in order to fulfil certain yet unknown functions.

2.5. Redox regulation and FKBPs

In *Arabidopsis*, the identification of 16 immunophilins (11 FKBPs and 5 CYPs) in the thylakoid lumen suggests that these foldases play important roles in the assembly and upkeep of protein complexes such as the two photosystems, residing at least in part in the thylakoid lumen [9, 79, 105] AtFKBP20-2 has a unique pair of cysteine residues at the C-terminus, found to be reduced by thioredoxin (Trx), which itself gets reduced by NADPH by the action of NADP-Trx reductase. AtFKBP20-2 has been shown to be essential for the accumulation of the photosystem

II (PSII) supercomplex [106]. Understanding the mechanism underlying this function will require the identification of the immediate target(s) of FKBP20-2. The disulfide bridge of FKBP20-2 at the C-terminus which gets reduced by Trx is highly conserved in land plants and eukaryotic algae but is absent in cyanobacteria, probably suggesting that this kind of redox regulation is restricted to chloroplasts. However, the low level of PPIase activity associated with AtFKBP20-2, which contains only two of the five residues required for PPIase activity of FKBPs, was unaffected upon reduction by Trx. As such it appears that PPIase activity may not be the essential key property responsible for the accumulation (assembly or upkeep) of the photosystem complexes [106].

The AtFKBP13 structure reveals a unique pair of disulfide bonds (Cys5-Cys17 and Cys106-Cys111) that are absent in animal homologs. Furthermore, thioredoxins from both chloroplasts (*m*-type) and *Escherichia coli* could reduce both the disulfides in a reaction that was accompanied by the loss of PPIase activity. This evidence, together with similar results obtained with Cys→Ser mutants, suggests that lumenal AtFKBP13 resembles the stromal cyclophilin AtCYP20-3 in undergoing a thiol redox regulation [18]. The lumen compartment appears to differ from the stroma, however, in one fundamental aspect: activation of a resident immunophilin is achieved by oxidation (conversion of 2SH to S—S) and not reduction (S—S to 2SH), as is the case for AtCYP20-3 and many other biosynthetic enzymes.

These results show that enzymes of the thylakoid lumen, such as AtFKPB13 are activated by oxidation in the presence of light. The evidence also indicates that the response of a regulatory enzyme depends on the actual redox milieu of the host compartment.

2.6. Functional implications of other plant FKBPs

The primary function of plant FKBPs is thought to be in assisting the proper folding of substrate proteins by virtue of their PPIase activity. Their role as PPIase explains their involvement in several stress-related responses, if not all. A number of studies reveal the involvement of plant immunophilins mostly in response to different types of abiotic cues, but there are also some evidences of their participation in biotic stress response control [16, 17, 19, 107]. At the same time, some functional attributes of plant FKBPs, especially those of multi-domain forms come from the domains present in addition to the FKBDs such as TPR and calmodulin-binding domains [47, 76-79]. Presence of more than one FKBD in a single FKBP wherein only one FKBD is active as a PPIase is also common in plants, as is the case with other higher organisms [46, 87, 88].

In *Arabidopsis* FKBP family, out of the 23 members, 12 isoforms are localized to chloroplast lumen, two are predicted to be targeted to secretory pathway (AtFKBP15-1, 15-2), five to nucleus, and the remaining four seem to be cytosolic [9]. Among the 16 single-domain FKBPs, except for AtFKBP12 (cytosolic), AtFKBP15-1 (secretory), AtFKBP15-2 (secretory), AtFKBP15-3 (nuclear) and AtFKBP20-1 (nuclear), the remaining eleven (AtFKBP13, 16-1, 16-2, 16-3, 16-4, 17-1, 17-2, 17-3, 18, 19, 20-2) are predicted to be thylakoid lumen localized [9, 108].

AtFKBP12, the smallest FKBP in *Arabidopsis*, is known to interact specifically with AtFIP37 which functions primarily in the early stages of embryogenesis and endosperm formation and is thought to be an mRNA splicing factor [25]. FKBP12 from the conifer, *Picea wilsonii* (PwFKBP12) has been shown to interact with the putative CCAAT-binding transcription factor HAP5 [41]. During infection by the fungal pathogen *Fusarium graminearum,*

TaFKBP12 is targeted by the fungal lipase FGL1 which is an important fungal virulence factor [42].

The redox regulatory role of AtFKBP13 and AtFKBP20-2 has already been covered under section 2.5 of this review. The FKBP isoforms, AtFKBP15-1 and AtFKBP15-2 which are involved in plant stress response, localize to the ER and secretory pathway. AtFKBP15-1 and AtFKBP15-2 share 70% sequence identity and are upregulated by heat stress [28]. A similar set belonging to this group has been identified in maize, where ZmFKBP15-3 is overexpressed under high temperature, while ZmFKBP15-1 and ZmFKIPB15-2 are not upregulated during heat stress [43].

The chloroplast lumenal immunophilin, AtFKBP16-1 possess a poorly conserved PPIase active site and is regulated by high light intensity conditions. Overexpression of AtFKBP16-1 leads to improved tolerance to photosynthetic stress and drought. The protein plays a key role in the acclimation of plants under photosynthetic stress conditions, perhaps by interacting with and improving the stability of Photosystem I subunit L, also known as AtPsaL [29]. AtFKBP16-1 plays a significant role in early chloroplast development, architecture of photosynthetic apparatus and plant development [109]. AtFKBP16-2 is homologous to AtFKBP13 and contains four cysteine residues, suggesting it also to be a redox regulating protein [9]. AtFKBP16-2 interacts with a lumenal sub-complex of the NADPH dehydrogenase (NDH) enzyme complex, thereby stabilizing the interaction between NDH and photosystem I (PSI) [30]. TaFKBP16-3 which shares 84% sequence identity with AtFKBP16-3 has been shown to interact with potential photosystem assembly regulating proteins such as Accumulation of Photosystem One 2 (APO2) and Thylakoid Formation 1 (TaThf1) [44]. Transgenic rice plants that constitutively expressed OsFKBP16-3 exhibited increased tolerance to salinity, drought and oxidative stresses revealing a

potential role of FKBP16-3 in environmental stress response, which may be regulated by a redox relay process in the thylakoid lumen [45].

Rice isoforms OsFKBP20-1a and OsFKBP20-1b share 85% sequence identity and both are expressed in response to heat and desiccation, although their expression profiles are different. It has been shown that OsFKBP20-1a is expressed heavily in all tissues and is upregulated immediately after drought and temperature stress, while OsFKBP20-1b expression increases at a slower rate, only after 24 h of stress [17]. Both FKBPs are present in the nucleus, and OsFKBP20-1b's presence has been detected in cytosol as well [17]. Direct interaction between OsFKBP20-1a and the SUMO-conjugating enzyme (Sce) that attaches small ubiquitin-like modifier (SUMO) proteins to its client proteins is thought to mediate heat stress response [16]. However, binding partners for OsFKBP20-1b are yet to be identified. Functional characterization has not been done for several of the single-domain FKBPs that are localized into plant chloroplasts.

Among the seven multi-domain AtFKBPs, three (AtFKBP42, AtFKBP43 and AtFKBP53) possess a single FKBD, whereas AtFKBP62, AtFKBP65 and AtFKBP72 possess three copies of FKBD each and the seventh one, known as *Arabidopsis* trigger factor AtTIG has a single, very poorly conserved FKBD [9]. The cytosolic AtFKBP42 possess an N-terminal FKBD, three copies of TPR domains, a calmodulin-binding domain and a potential trans-membrane domain at the C-terminus. The cytosolic AtFKBP62 and AtFKBP65 both have a similar domain organization, wherein they possesses three N-terminal FKBDs, three TPR domains and calmodulin-binding domain. The nuclear localized AtFKBP72 shares a similar domain organization with AtFKBP62/65, but in addition possess a potential trans-membrane domain at the C-terminus. AtFKBP43 and AtFKBP53 both have a single FKBD in addition to N-

terminal RNA binding motifs rich in arginine/lysine residues and multiple nuclear localization signals [108]. The poorly conserved, chloroplast localized FKBP-like protein AtTIG has the putative FKBD sandwiched between ribosome-binding termini [9, 108]

Structure of AtFKBP42 and its functional relevance have been covered under section 2.3 of this review. AtFKBP43 and AtFKBP53 are multi-domain FKBPs which in addition to having a C-terminal FKBD, possess nuclear localization signals and highly charged domains in the N-termini, seemingly involved in nucleic acid and/or protein interactions [9]. AtFKBP53 is a histone chaperone required for repression of ribosomal RNA gene expression in *Arabidopsis*. AtFKBP53 interacts with histone H3 through its highly charged N-terminal domains and interestingly, the PPIase domain is not essential for the histone chaperone activity [34].

The *Arabidopsis* multi-domain FKBPs, AtFKBP62 (ROF1) and AtFKBP65 (ROF2) are homologous to TaFKBP73 and TaFKBP77 respectively and are composed of triple FKBDs, a TPR domain, and a calmodulin-binding domain [9]. Both AtFKBP62 and AtFKBP65 are involved in long term acquired thermotolerance although through different mechanisms [35, 36]. AtFKBP62 and HSP90/HSP70 organizing protein (HOP) have been reported to be co-chaperones to HSP90 and the interaction with HSP90 is mediated through the TPR domain of AtFKBP62 [110, 111]. Normally, AtFKBP62 binds to heat shock protein HSP90.1 via its TPR domain and localizes to the cytoplasm. However, under heat stress, the AtFKBP62-HSP90.1 complex transfers to the nucleus, dependent upon the presence of the heat stress transcription factor HsfA2 [35]. Similarly, AtFKBP65 is a heat stress protein but *fkbp65* mutant plants are resistant to heat stress and small heat shock proteins are highly expressed, contrary to what is observed in *fkbp62* mutants, indicating antagonistic functions [36]. Equally, the mammalian FKBP51/FKBP52 orthologs of AtFKBP62/AtFKBP65 have also been shown to have antagonist

functions in steroid receptor-mediated signaling [112, 113]. On a similar note, transgenic wheat overexpressing TaFKBP73 and TaFKBP77 revealed different morphological abnormalities, indicating different functional attributes for the two isoforms in plant development [114]. AtFKBP65 has been implicated in modulation of intracellular pH homeostasis in *Arabidopsis*, whereas AtFKBP62 has been reported to play an important role in osmotic/salt stress responses in germination of *Arabidopsis* seedlings through its interaction with a phosphatidylinositol-phosphate protein [37, 38]. AtFKBP62 and AtFKBP65 hint to the tangible multifunction carried out by immunophilins, depending on different stress conditions and plant developmental stages. AtFKBP65, along with the cyclophilins ACYP19-1 and AtCYP57 is involved in the plant defense response against the bacterial pathogen *Pseudomonas syringae*. AtFKBP65 overexpression is known to result in increased callose accumulation in the plant cell wall, thereby improving the overall resistance of the plant against the pathogen [39].

AtFKBP72, also known as PASTICCINO1 (PAS1) is involved in the control of cell proliferation and differentiation during plant development. Mutations in the C-terminal stretch of AtFKBP72 cause severe developmental defects throughout the growth stages [84]. The C-terminal stretch of AtFKBP72 interacts with the FKBP-associated NAC (FAN), a plant-specific NAC transcription factor [40]. It has been suggested that FKBP72 regulates the function of FAN by controlling its translocation to the nuclear compartment upon plant cell division [40].

3. Plant cyclophilins (CYPs)

Cyclophilins (CYPs) are also present in all forms of life and are known to be targeted to different subcellular compartments such as the cytoplasm, nucleus, endoplasmic reticulum, mitochondria and chloroplasts [3, 4, 9, 115-117]. Originally identified as targets for the

immunosuppressive drug cyclosporin A, CYPs were found to possess PPIase enzymatic role as well [6, 118]. Plants show the maximum diversity of cyclophilins, with *Arabidopsis* encoding 29 CYPs [105] and rice encoding 27 [17]. Expression of cyclophilin genes in plants are known to be induced by a variety of biotic and abiotic stress conditions such as light, heat and cold shock, salt stress, wound and pathogenic infections [9, 19].

Based on domain organization, CYPs are classified into single- and multi-domain forms. Single domain CYPs only possess their conserved CYP domain (PPIase domain) whereas multi-domain CYPs possess additional unique domains such as WD40 repeats, TPR repeats, U-box domain, Zn-finger domain, Leu-rich domain and helical bundle domain, in addition to the CYP domain and subcellular targeting sequences [9]. Most of these additional domains are known to be involved in protein-protein or protein-nucleic acid interactions. Although the majority of CYP proteins in plants remain to be characterized, current reports show their involvement in regulation of gene expression, heat stress and chloroplast protein-complex biosynthesis along with other functions [52, 60, 119]. Thus, CYP protein structure diversity, in addition to the diversity in subcellular localization, together with functional versatility of plant CYPs provide solid evidence that CYPs are involved in key functions regulating plant growth and development.

3.1. Cyclophilin structures

Several cyclophilin structures have been studied in detail and all of them share a common fold. The structures of mammalian cyclophilins, such as CYPA-H and CYPJ are known from human, *Mus musclus*, *Bos taurus*, *Equus caballus* and *Macaca mulatta* [89, 120, 121]. The crystal structures of apo-form, mutants and complexes with immunosuppressant drugs and peptide substrates are available for cyclophilins from *Acanthamoeba polyphaga* mimivirus,

Thermotoga, Escherichia, Mycobacterium, Azotobacter, Aspergillus, Piriformospora, Moniliophthora, Saccharomyces, Cryptosporidium, Encephalitozoon, Leishmania, Malassezia, Plasmodium, Trypanasoma, Toxoplasma, Brugia, Schistostoma and *Caenorhabditis* [89, 122, 123].

All cyclophilins have an eight stranded β-barrel structure, capped by two α-helices. The topology of this fold is rather different from other β-barrel structures that transport hydrophobic ligands in a pocket in the middle of the barrel. In a cyclophilin, the center of the barrel is filled with closely packed aromatic groups [23]. Whereas, in the case of most other β-barrel structures, the hydrophobic core is almost always open for a substrate to enter. The N- and C-termini lie close together on the same side of the protein, distal to the active site face. The X-ray structures of numerous complexes define the PPIase active site, which is mainly formed by the residues that are located on one face of the β-sheets. CsA-binding also happens in the same active site [23]

Cyclophilins are neither functionally nor evolutionally related to other eight stranded β-barrel proteins [23]. The anti-parallel β-strands of a typical cyclophilin form a right-handed β-barrel. The angle of tilt for the β-strands against the barrel axis is about 49^{o}. Eight-stranded β-barrels are all right-handed, however, their tilt angles are considerably different from that of a cyclophilin. The β-barrel of human cyclophilin A (hCYPA), the most widely studied cyclophilin, is about 14 Å in height, and 15 and 17 Å in dimension for the minor and major elliptical cross section axes of the barrel, respectively. Even though hCYPA has four cysteine residues, they do not form any disulfide bond [23].

Divergent cyclophilins form a distinct class wherein an extra loop of 4 or more amino acids is present at about residue 47 (hCYPA numbering). Also, these cyclophilins are

characterized by the presence of two reduced cysteine residues, that come close together and have been shown to get involved in a redox signaling process, and a conserved glutamate residue. The overall fold of all cyclophilins however remains the same as that of hCYPA. Most of the members of this family have only minor differences in their active site as compared to hCYPA and hence show similar CsA binding characteristics. However, the loop conformations adopted by the known divergent-loop structures seem to differ from each other. The structures of a few other divergent cyclophilins, such as *C. elegans* CYP3 [124], *B. malayi* CYP1 [125], bovine CYP40 [126], *P. falciparum* CYP [127] and human CYPH [128, 129], are also known, in addition to the structures of some plant cyclophilins.

3.2. Plant cyclophilin structures

From the family of plant cyclophilins, only four structures have been reported so far. Three of them belong to single-domain, divergent CYP family and are functional PPIases; CYPA-1 from *Triticum aestivum* (wheat plant; [130]) and CYP from *Citrus sinensis* (orange tree; [74]), and the CYP named Cat r 1 from *Catharanthus roseus* (rosy periwinkle; [131]). Among them, at least TaCYPA-1 and CsCYP are shown to bind cyclosporin A. The structural difference of the divergent CYP, CsCYP as compared to hCYPA can be seen in Fig. 7. It is to be noted that in all these three structures, the cyclophilin fold is well conserved and brings the two cysteine side chains into close proximity with the sulfur atoms less than 6 Å apart. CYP38 from *Arabidopsis thaliana* (AtCYP38) for which a crystal structure is available is a non-functional PPIase with multi-domain organization [62].

3.3. Structure and function of divergent plant cyclophilins

The crystal structures for the wheat cytosolic cyclophilin TaCYPA-1 (PDB: 4E1Q) [116] and its complex with the specific inhibitor cyclosporin A (CsA; PDB: 4HY7) [130] are available. The crystal structure of CsCYP has been solved in complex with CsA (PDB: 4JJM) [74]. The two plant cyclophilin-CsA complex structures align well with each other with an RMSD of about 0.308 Å for 992 Cα atoms (Fig. 8). The solution NMR structure of apo Cat r 1 is also available (PDB: 2MC9) [131]. CsCYP, TaCYPA-1 and Cat r 1 are all single-domain cyclophilins and members of the divergent cyclophilin family, carrying a divergent loop having the sequence 48-KSGKPLH-54, two conserved Cys residues (Cys40 and Cys168) and a conserved Glu (Glu83), based on CsCYP numbering. TaCYPA-1 is a cytosolic cyclophilin [130], whereas CsCYP gets localized into the nucleus [74]. Localization information is not available for Cat r 1, which is about 91.8% identical to CsCYP sequence-wise and the cyclophilins Cat r 1 and CsCYP are about 81.9% and 83.6% identical, respectively, to TaCYP-A-1.

CsCYP is known to be a target for the bacterial effector protein PthA, a transcription activator-like effector from *Xanthomonas citri* that causes cankers on citrus and CsTdx (a TPR domain-containing thioredoxin) [73]. The structural work on CsCYP [74] reveals that the two conserved Cys residues act as targets for CsTdx and form a disulfide bond that regulates the PPIase activity of the protein. Glu83 (CsCYP numbering), which is conserved among divergent cyclophilins, helps to bring the divergent loop towards the active site and is critical for PPIase activity as it helps stabilizing the divergent loop in a conformation that favors the active site to remain open. The divergent loop, in fact, functions as a trigger that transmits the redox status of the Cys residues to the PPIase active site via interaction with Glu83. The study also reveals the binding of CsCYP to the YSPSAP stretch of the C-terminal domain of citrus RNA polymerase II, perhaps, thereby modulating transcription progression [74].

However, not much is known about the redox regulation of PPIase activity of Cat r 1 and TaCYPA-1 and also about their PPIase substrates. With a high degree of structural and sequence similarity, it is possible that they also exhibit similar, if not the same, functional roles as CsCYP. The structure of Cat r 1 has been studied in the context of its role as a pan-allergen of plant origin [131].

16 out of 29 AtCYPs possess an additional stretch of residues corresponding to the divergent loop of known CYP structures, however, only 9 of them possess the remaining features such as conserved Cys residues and Glu residue known for divergent cyclophilins with redox regulation: AtCYP18-3, 18-4, 19-1, 19-2, 19-3, 26-1, 40, 63 and 95. Of these, CYP18-3 (ROC1), CYP18-4 (ROC5), CYP19-1 (ROC3), CYP19-2 (ROC6) and CYP19-3 (ROC2) are all cytosolic, divergent single-domain cyclophilins with all the residues needed for PPIase activity highly conserved, even though the sequence of the divergent loop varies a bit (Fig. 9). They have residues essential for redox regulation, just like the structurally characterized CsCYP and hence they may get regulated in a similar fashion. Among these, substantial functional information is available only for AtCYP18-3 which is discussed later in this review. Furthermore, extensive functional characterization is essential to understand the interaction partners and/or PPIase substrates of the remaining CYPs.

AtCYP20-2 and AtCYP20-3 (ROC4) have all the features of an active PPIase and a divergent family CYP conserved except for the fact that the divergent loop is only 4-residue long and also Glu83 (AtCYP18-3 numbering), as seen in structurally characterized divergent plant CYPs, is replaced by a Lys and Ile residues, respectively. However, redox regulation has been reported for AtCYP20-3 also [58, 73]. In the same line, the CYP domain of AtCYP63 and AtCYP40 has all the residues needed for PPIase activity conserved, except for Trp121 (hCYPA

numbering), and the proteins are expected to be functional PPIases, if not as efficient as hCYPA. The features of redox-regulated divergent CYPs are also conserved in AtCYP63 and AtCYP40. In addition to its CYP domain, AtCYP63 has a charged C-terminal domain with several nuclear localization signal (NLS) and is also thought to be involved in RNA metabolism [9].

Even though the features of redox-regulated divergent CYPs are conserved, only 3 and 4 residues essential for PPIase activity are conserved in AtCYP95 and AtCYP26-1, respectively, thereby suggesting them to be inactive PPIases. The largest CYP known till date, AtCYP95, has a highly charged C-terminal domain and several nuclear localization signals, similar to AtCYP63. Its proposed role is in RNA metabolism, as a splicing factor [9]. The cytosolic AtCYP26-1 has a potential trans-membrane domain at the C-terminus, which is thought to serve as a membrane anchor and is specifically expressed in flowers [9].

3.4. Structure and function of Arabidopsis thaliana cyclophilin 38 (AtCYP38)

Among the 29 cyclophilins encoded by the *Arabidopsis thaliana* genome, structural information is available for only one protein, AtCYP38, which is of multi-domain organization and is localized in the thylakoid lumen of chloroplasts [134-136]. The crystal structure of AtCYP38 (Fig. 10) reveals two distinct domains [62]. The N-terminus is made up of a short helix (α1), followed by four helices (α2 to α5) of varying lengths that forms a helical bundle. Even though the N-terminal domain of AtCYP38 was predicted to be a leucine zipper domain, the structure revealed it to be a helical bundle with structural similarity to the spinach photosystem protein PsbQ and *E. coli* cytochrome b562. The helical bundle is followed by a cyclophilin domain, made up of eight β-strands forming a β-barrel with an α-helix each at the top and bottom. The loop connecting the helical bundle and β-barrel is rich in negatively charged

residues. The structure clearly shows that a short N-terminal stretch of the protein gets into the C-terminal CYP domain, forming part of the β-barrel thereby auto-inhibiting the possible PPIase activity of the protein [62]. Even though the primary sequence predicted a possible PPIase active site, the structure reveals a scrambled active site, which cannot bind a substrate for PPIase activity.

A possible interaction of AtCYP38 with the E-loop of chlorophyll protein47 (CP47), a photosystem II component is also reported [62]. The overall surface of the AtCYP38 protein reveals a large number of negatively charged residues and this feature is proposed to be of significance with respect to its location within the thylakoid lumen [62] where pH is known to fluctuate dramatically during the day-night cycle [137]. However, so far no direct targets for AtCYP38 have been reported, other than the E-loop of CP47.

AtCYP38 plays a critical role in the assembly and maintenance of photosystem II [60-62]. Mutant Arabidopsis plants missing CYP38 are stunted in growth and hypersensitive to light. A further investigation into the molecular compositions of photosynthetic complexes reveals that the mutant fails to accumulate the PSII supercomplex [60, 61]. Studies suggest that CYP38 may assist in the proper folding and insertion of D1 and CP43 into the photosystem II complex and in addition, could play a role in the correct assembly of the oxygen evolving complex [62]. Intriguingly, the AtCYP38 ortholog in spinach, TLP40, regulates the activity of a photosystem II-specific protein phosphatase located in the thylakoid membrane [71, 72]. Although AtCYP38 does not function as a PPIase, spinach TLP40 (having 82% sequence identity with AtCYP38) is a functional PPIase in the thylakoid lumen. This could be true with many other plant cyclophilins that exhibit varying degrees of PPIase activity, most of which could perhaps, be attributed to

structural differences. This demands the importance and need of more structural characterization of other plant cyclophilins.

3.5. Functional implications of other plant cyclophilins

Plant cyclophilins, especially single-domain CYPs, function primarily as PPIase. Most of their functions in protein folding, signal transduction, protein degradation and stress response can be attributed to their ability to perform PPIase activity on substrate proteins. Their expression usually goes high in response to abiotic stress factors such as cold or heat shock, salinity and pathogenic infection. CYPs are also known to participate in the regulation of proper folding of proteins involved in signal transduction processes and/or stress-related responses [9, 19]. Multi-domain CYPs have functions attributable to the CYP domain as well as the unique additional domains.

AtCYP40, a multi-domain cyclophilin consisting of an N-terminal CYP domain and a C-terminal tetratricopeptide repeats (TPR) triplet separated by two putative nuclear targeting signals, has been linked to post translational gene silencing (PTGS) in *Arabidopsis* [63]. *Atcyp40* (also known as *squint*, *sqn*) knock out mutants show reduced leaf number and alteration in leaf morphology associated to reduced microRNA activity [63]. It was shown that CYP40 facilitates HSP90-mediated RISC (RNA-induced silencing complex) assembly in plants by promoting or stabilizing the binding of small RNA duplexes to ARGONAUTE1, AGO1 which is involved in miRNA-directed gene repression in *Arabidopsis* [63, 138-140]. Also, like mammalian CYP40, plant CYP40 interacts with HSP90 through the TPR domains and this interaction is indispensable for AtCYP40 function *in planta* [64]. In a separate study, the TPR domain in

AtCYP40 was shown to bind to a viral replication protein and thereby inhibiting the assembly of tombusviral replicase [65].

CYPs are involved in regulation of gene expression. *Arabidopsis cyp71* mutant plants exhibit defective morphology and development of lateral organs, defective shoot apical meristem, altered flower morphology, and arrested root growth among other pleiotropic defects [141]. The nuclear-localized AtCYP71 is made up of four WD40 repeats at the N-terminal region and a CYP domain at the C-terminal region [9]. The drastic phenotypes observed in this cyclophilin mutant are correlated with the important role of CYP71 in the regulation of epigenetic gene silencing and organogenesis. Results indicate that CYP71 targets histone H3 through the WD40 repeat domain and it could also be modulating histone structure by the CYP domain. Chromatin, composed of histones and DNA, is the primary carrier of epigenetic information in higher eukaryotes, and further analysis of CYP71 function provided evidence that CYP71 is involved in chromatin assembly and histone modification in *Arabidopsis* [68]. Likewise, another nuclear immunophilin, AtFKBP53 has been identified as histone chaperone in plants that functions in chromatin remodeling as well as regulation of transcription [34]. Thus, immunophilin proteins are surging as key players in regulation of gene expression through epigenetic mechanisms.

Cyclophilins are also involved in plant hormone regulation and immunity. AtCYP18-3, also referred to as ROC1, has been shown to link phytochrome and cryptochrome to brassinosteroid (BR) sensitivity [52]. Partial loss-of-function of the cytosolic single domain cyclophilin/ROC1 mutants exhibit long hypocotyls under blue and far-red light with specific defects in phytochrome A and cryptochrome 1. BR and light signaling together control several aspects of growth and development in plants. *roc1* mutant have altered patterns of

phosphorylation of the transcription factor BES1 which is a known point of control to BR sensitivity, correlated with altered expression levels of BES1 genes targets. Furthermore, in a gain-of-function *roc1* mutant, plants exhibited reduced elongation of stem and increased branching of shoot, with these phenotypes affected strongly by photoperiod and temperature [53]. Overall, reports indicate AtCYP18-3 may be involved not only in brassinosteroid signaling but also indirectly involved in other hormone related networks, such as gibberellic acid [53]. AtCYP18-3 is known to interact with the *Agrobacterium tumefaciens* VirD2 protein thereby playing a role in the transfer of bacterial T-DNA, from its tumor inducing (Ti) plasmid into plant cell [48]. AtCYP18-3 is also known to play a role in *Pseudomonas syringae* AvRpt2 protease activation, contributing to the plant's innate immunity [49, 50]. AtCYP18-3 has been shown to catalyze the cis/trans isomerization of Pro149 residue of the RPM1-interacting protein RIN4 [51]. The PPIase activity of AtCYP18-3 on RIN4 inhibits effector-triggered immunity in Arabidopsis wherein the conformation surrounding RIN4 Pro149 gets altered due to isomerization and this in turn becomes a molecular switch for RPM1 activation [51].

A significant number of immunophilins are present in the chloroplast with studies to date indicating they play significant roles in redox and photosynthetic protein complex maintenance. The chloroplast stromal chaperone, AtCYP20-3 links light and redox signals to biosynthesis of cysteine and stress response in chloroplasts. In this context, an essential enzyme in cysteine biosynthesis within the chloroplast - serine acetyl transferase (SAT1) was found to interact with CYP20-3 [18]. AtCYP20-3 is also known to bind to the jasmonate family of phytohormones and thereby performing a signal relay during regulation of cellular redox homeostasis in response to stress [59]. A study on the role of Cys residues in AtCYP20-3 in PPIase and redox-related functions revealed the chloroplast peroxiredoxins PrxA and PrxB as its interacting partners and

also that the PPIase and disulfide-reduction activities of the cyclophilin are independent of each other, but both gets regulated by the redox status of its active site [58].

AtCYP20-2, the only known thylakoid lumenal cyclophilin with PPIase activity [142] has a role in the assembly of NDH hydrophobic domain and that makes it an important chaperone for chloroplast NAD(P)H dehydrogenase complex formation [56]. AtCYP20-2 has been shown to cause a conformational change in the brazzinazole-resistant1 (BZR1) protein [57]. This conformation change aids the binding of BZR1 to cis-elements in the flowering locus D (FLD) promoter and thereby altering the flowering pattern through modulation of FLD expression. AtCYP20-2-overexpressing plants showed earlier flowering, whereas TaCYP20-2 overexpressing plants flowered later and interestingly TaCYP20-2 is a weaker PPIase as compared to AtCYP20-2 [57]. Ectopic expression of its homolog, OsCYP20-2, has been shown to confer improved tolerance to environmental stress in tobacco and *Arabidopsis* [143].

AtCYP19-4, 20-1, 21-1, 21-2 and 23 are predicted to take secretory pathways, with AtCYP19-4 reported to get ER localized [144]. AtCYP19-4 is known to get involved in vesicle trafficking by modulating the function of the guanine nucleotide exchange factor, GNOM [54]. AtCYP20-1 (ROC7) has been reported to get involved in the regulation of the heterotrimeric protein phosphatase 2A (PP2A) by interacting with the PP2A regulatory subunit A and thereby altering auxin transport and growth response pathways [55]. The mitochondria localized CYPs 21-3 and 21-4 have highly conserved sequence and are thought to have evolved by gene duplication. Like their homologs in higher organisms, these CYPs are thought to modulate the permeability transition pore of mitochondria and play a crucial role in necrotic and apoptotic cell death [145].

AtCYP18-1, 18-2, 18-4, 19-2 and 22 are all cytosolic CYPs with very high sequence identity in their CYP domain and hence expected to have similar PPIase function within the cytoplasm of the plant cell [9]. The cytosolic AtCYP19-1 (ROC3) and cytoplasmic and nuclear localized AtCYP57 are known to be part of the defense response of the plant against the bacterial pathogen *Pseudomonas syringae* [54]. OsCYP2 (homolog of AtCYP19-2 and TaCYPA-1), when overexpressed, provides salt tolerance to rice seedlings [69] and it also takes part in the degradation of auxin-responsive proteins, thereby playing a critical role in lateral root initiation of rice plants [70]. OsCYP25, the rice homolog of AtCYP19-3 (ROC2), mediates several cellular responses under multiple abiotic stress conditions, such as salinity, heat, cold and drought [146]. The citrus cyclophilin, CsCYP interacts with the C-terminal domain of citrus RNA polymerase II, as well as to PthA and CsTdx [73].

AtCYP26-2, AtCYP28 and AtCYP37 are thylakoid lumenal cyclophilins that are highly divergent as they contain only a few (1–5) of the 12 residues important for PPIase activity [9]. The nuclear localized AtCYP59, which consists of an N-terminal PPIase domain, followed by an RNA recognition motif (RRM), zinc finger and a C-terminal stretch rich in charged amino acids like Arg and Lys, seems to play a role in transcription and pre-mRNA processing via its interaction with the serine arginine rich (SR) proteins and the C-terminal domain of RNA polymerase II [67]. AtCYP65 possesses an N-terminal U-box domain, which is a common feature of ubiquitin ligases, in addition to its C-terminal CYP domain [9].

The ectopic expression of CYP1 from the halophyte *Thellungiella halophila* (ThCYP1) has been shown to provide salt tolerance in tobacco cells [147]. A CYP isolated from Chinese cabbage exhibited antifungal activity [148]. Constitutive expression of an *Arachis diogoi* CYP in

transgenic tobacco resulted in improved resistance towards the pathogen *Phytophthora parasitica* [149].

4. Conclusion

The major role of immunophilins; both FKBP and CYP is known to be as a foldase by virtue of its ability to perform PPIase activity on protein substrates. The presence of immunophilin homologs within different sub-cellular compartments and presence of multiple immunophilins with same localization pattern suggest that the versatility of immunophilins could primarily be in their substrate specificity. The enzymes seem to have diversified to perform a variety of functions within plants, most importantly due to their PPIase catalytic role on specific substrates and also because of functions attributable to additional domains, as in the case of multi-domain forms. Our current understanding indicates that the primary function of plant FKBPs is as regulators of protein function through specific interactions with protein ligands. The differences in sequence, structure as well as subcellular localization, have aided the evolution of this diverse family of molecular chaperones.

Chloroplast has the maximum number of immunophilins - both FKBP and CYP and interestingly many of them are active PPIases; several having a possible redox regulation mechanism. Large number of single-domain immunophilins targeting to chloroplast and primarily into thylakoid lumen shows the relevance of these foldases in the assembly, maintenance and upkeep of photosystem subunits. However, only a limited number of immunophilins have been shown to directly interact with photosystem components. Many more are to be characterized both structurally and functionally.

Future studies on multi-domain FKBPs with focus on structural characterization will contribute more to our knowhow regarding how individual domains function as regulators in plants. In the case of plant CYPs, only four cyclophilins have been characterized structurally and those structures alone cannot explain the complete functional attributes of cyclophilins with a high degree of sequence variation and localization pattern. Interestingly, the available three divergent plant cyclophilin structures (CsCYPA, TaCYPA-1 and Cat r 1) are identical with respect to sequence as well as structural features and redox regulation characteristics. These proteins have strong PPIase activity as well. *A. thaliana* has 29 CYPs, with 8 multi-domain isoforms and structure is known for only one, AtCYP38. Table 2 summarizes all known plant immunophilin structures.

There are several more uncharacterized plant cyclophilins that are known to be active PPIases and/or partners for other macromolecules. The lack of knowledge regarding the three-dimensional structure for these cyclophilins, in fact, is a big void in our understanding about the molecular features of their functions within a plant cell. If at all their functional roles have been well studied, comparison has always been restricted with structures of similar cyclophilins from non-plant sources. Structural characterization of plant cyclophilins, which are highly divergent with respect to their active site amino acid residues, could provide substantial information regarding their function in plant cellular compartments. Plant hormone signaling systems such as those involving jasmonate, auxins, brassinosteroids and perhaps gibberellins which are involved in activation of plant immune system are known to interact with immunophilins. So the interaction of these hormones as well as that of the various effector proteins from plant pathogens with plant immunophilins might play a key role in regulating plant's innate immune system. More studies in this direction should be able to reveal the exact role of immunophilins in

modulating plant immune responses. On the whole, it appears that a lot more effort needs to go in order to improve our understanding about the structure-function relationships of this diverse class on immunophilins.

Acknowledgements

This project was supported by funding from the Faculty Research Council and Agency for Science and Technology Research (A*STAR), Singapore to K.S. and National University of Singapore research scholarship to G.G. and D.V. The work was also supported by a grant from the U.S. Department of Energy and the World Class University Program through the National Research Foundation of Korea to S.L.

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Table 1. Functional features and cellular localization pattern of plant immunophilins.

Immunophilin	Cellular localization	Known binding partners / functional attributes
AtFKBP12	Cytoplasm	Interacts with AtFIP37; functions as an mRNA splicing factor during early stages of embryogenesis and endosperm formation [25]
AtFKBP13	Thylakoid lumen	Interacts with Rieske [26] and involved in redox regulation [27]
AtFKBP15-1 and 15-2	ER/Secretory	Involved in heat shock response [28]
AtFKBP16-1	Thylakoid lumen	Interacts with AtPsaL; involved in photosynthetic stress and drought response [29]
AtFKBP16-2	Thylakoid lumen	Interacts with NDH enzyme complex and stabilizes NDH interaction with photosystem I [30]
AtFKBP42	Membrane-anchored	Interacts with the ABC transporters AtPGP1 and AtPGP19 [31], as well as with the vacuolar transporters AtMRP1 and AtMRP2 [32]; involved in auxin and brassinosteroid (BR) signaling pathways [33]
AtFKBP53	Nucleus	Functions as a histone H3 chaperone and thereby involved in repression of ribosomal RNA gene expression [34]
AtTIG	Chloroplast stroma	Functions as a trigger factor [9]
AtFKBP62/ROF1	Cytoplasm	Interacts with HSP90.1 (thermotolerance; [35, 36]) and phosphatidylinositol-phosphate protein (osmotic/salt stress tolerance; [37])
AtFKBP65/ROF2	Cytoplasm	Involved in thermotolerance (antagonistic to AtFKBP62; [36]) and intracellular pH homeostasis [38]; involved in plant defense response against *P. syringae* [39]
AtFKBP72/PAS1	Nucleus	Interacts with FAN, the FKBP-associated NAC transcription factor; involved in control of cell proliferation and differentiation during plant development [40]
PwFKBP12 (Picea)	Not known	Interacts with HAP5, a putative CCAAT-binding transcription factor [41]
TaFKBP12 (Wheat)	Cytoplasm	Interacts with the fungal lipase, FGL1 (a virulence factor) during infection [42]
ZmFKBP15-3 (Maize)	Not known	Involved in heat shock response [43]
TaFKBP16-3	Thylakoid lumen	Interacts with APO2 and Thf1, which are involved in photosystem assembly regulation [44]
OSFKBP16-3	Thylakoid lumen	Involved in salinity, drought and oxidative stress tolerance [45]
OsFKBP20-1a (Rice)	Nucleus	Interacts with SUMO-conjugating enzyme (heat shock response; [16]) and involved in drought response [17]
OsFKBP20-1b (Rice)	Cytoplasm and nucleus	Involved in heat shock and drought response [17]
TaFKBP73 (Wheat)	Not known	Interacts with HSP90 and calmodulin , involved in heat shock response [46]
TaFKBP77 (Wheat)	Not known	Involved in heat shock response [47]
AtCYP18-3/ROC1	Cytoplasm	Interacts with *A. tumefasciens* VirD2 protein [48], *P. syringae* AvRpt2 protease and the plant RIN4 protein (role in plant immunity; [49-51]), involved in hormone (brassinosteroid) regulation [52, 53]
AtCYP19-1/ROC3	Cytoplasm	Involved in plant defense response against *P. syringae* [39]
AtCYP19-4	ER/Secretory	Involved in vesicle trafficking by modulating the function of guanine nucleotide exchange factor (GNOM; [54])
AtCYP20-1/ROC7	ER/Secretory	Involved in protein phosphatase 2A (PP2A) regulation [55]
AtCYP20-2	Thylakoid lumen	Involved in NAD(P)H dehydrogenase complex formation [56]; causes conformational change in brazzinazole-resistant (BZR1) protein (thereby altering flowering pattern; [57])
AtCYP20-3/ROC4	Thylakoid stroma	Links light and redox signals to cysteine biosynthesis and stress response [18]; interacts with peroxiredoxins PrxA and PrxB (redox regulation; [58]), SAT1 (essential enzyme in cysteine biosynthesis; [18]) and jasmonate (phytohormones; [59])
AtCYP38	Thylakoid lumen	Involved in assembly and maintenance of PSII and oxygen evolving complexes [60-62]; PPIase auto-inhibition [62]
AtCYP40	Nucleus	Involved in vegetative shoot maturation [63] post-translational gene silencing [64], HSP90-mediated RISC assembly [65], inhibition of Tombus virus replicase assembly [66]
AtCYP57	Cytoplasm and nucleus	Involved in plant defense response against *P. syringae* [39]
AtCYP59	Nucleus	Involved in transcription and pre-mRNA processing via interaction with Serine Arginine-rich (SR) proteins and RNA polymerase II [67]
AtCYP71	Nucleus	Involved in organogenesis, epigenetic gene silencing, chromatin assembly and histone H3 modification [68]
OsCYP2 (Rice)	Cytoplasm and nucleus	Overexpression provides salt tolerance [69]; involved in degradation of auxin responsive proteins (role in lateral root initiation; [70])
SoTLP40 (Spinach)	Thylakoid lumen	Active PPIase, involved in assembly and maintenance of PSII, interaction with phosphatase in thylakoid membrane [71, 72]
CsCYP (Citrus)	Nucleus	Interacts with the bacterial effector protein PthA, the plant Tdx [73] and RNA polymerase II [74]

Table 2. Details of known plant immunophilin structures.

Name	PDB ID	Domain (residues)	Resolution (Å)	Primary citation
AtFKBP13 (oxidized)	1U79	FKBD (5-129)	1.85	[27]
AtFKBP13 (reduced)	1Y0O	FKBD (5-129)	1.89	[90]
AtFKBP42	2F4E	FKBD-like domain (34-164)	2.32	[95]
AtFKBP42	2IF4	FKBD-like domain & TPR domain (35-292)	2.85	[94]
TaFKBP73	3JXV	2 FKBDs (148-386)	2.08	[87]
TaFKBP73	3JYM	3 FKBDs (33-386)	2.28	[87]
TaCYPA-1	4E1Q	CYP domain (2-171)	1.25	[130]
TaCYPA-1 (with CsA)	4HY7	CYP domain (2-171)	1.20	[130]
CsCYP (with CsA)	4JJM	CYP domain (3-172)	2.09	[74]
Cat r 1	2MC9	CYP domain (1-172)	NMR	[131]
AtCYP38	3RFY	CYP-like domain & helical bundle domain (83-433)	2.39	[62]

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                                                ^           ^^          ^ ^      ^^
hFKBP12       002 GV--QVETISPGDGRTFPKRGQTCVVHYT-GMLEDGKKFD-SSRDRNKPFKFMLGKQEVIR 058
AtFKBP13      013 GLAFCDKVV--GYGPEAVK-GQLIKAHYV-GKLENGKVFD-SSYNRGKPLTFRIGVGEVIK 068
TaFKBD73-1    042 GL--KKKLLKEGEGWDTPEVGDEVEVHYT-GTLLDGKKFD-SSRDRDDTFKFKLGQGQVIK 098
TaFKBD73-2    158 GI--FKKILKEGDKWENPKDPDEVFVKYE-ARLEDGTVVSKS-----EGVEFTVKDGHLCP 210
TaFKBD73-3    275 KI--LKKVLKEXEGYERPNEGAVVTVKIT-GKLQDGTVFLKKGHDEQEPFEFKTDEEAVIE 332
AtFKBP42      050 KV--SKQIIKEGHG-SKPSKYSTCFLHYRAWTKNSQHKFEDTWHEQQPIELVLGKEKKELA 107

                  ^                   ^           ^       ^
hFKBP12       059 GWEEGVA------QMSVGQRAKLTISPDYAYGATGH-----PGIIPPHATLVFDVELLKLE 108
AtFKBP13      069 GWDQGILGSDGIPPMLTGGKRTLRIPPELAYGDRGAGCKGGSCLIPPASVLLFDIEYIGKA 129
TaFKBD73-1    099 GWDQGIK------TMKKGENALFTIPPELAYGESGS-----PPTIPANATLQFDVELLSWT 148
TaFKBD73-2    211 ALAKAVK------TMKKGEKVLLAVKPQYGFGEMGRPAAGEGGAVPPNASLVIDLELVSWK 265
TaFKBD73-3    333 GLDRAVL------NMKKGEVALVTIPPEYAYGSTESK---QDAIVPPNSTVIYEVELVSFV 384
AtFKBP42      108 GLAIGVA------SMKSGERALVHVGWELAYGKEGNF---SFPNVPPMADLLYEVEVIGFD 159
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Fig. 1. Sequence alignment of structurally characterized plant FKBPs. hFKBP12 is aligned along with the FKBDs of structurally characterized plant FKBPs. TaFKBD73-1, TaFKBD73-2 and TaFKBD73-3 correspond to the first, second and third FKBD of TaFKBP73. As for AtFKBP42, only FKBD is used in the alignment. The eleven residues involved in the prolyl-peptide binding and PPIase activity are shown in blue and with a hat.

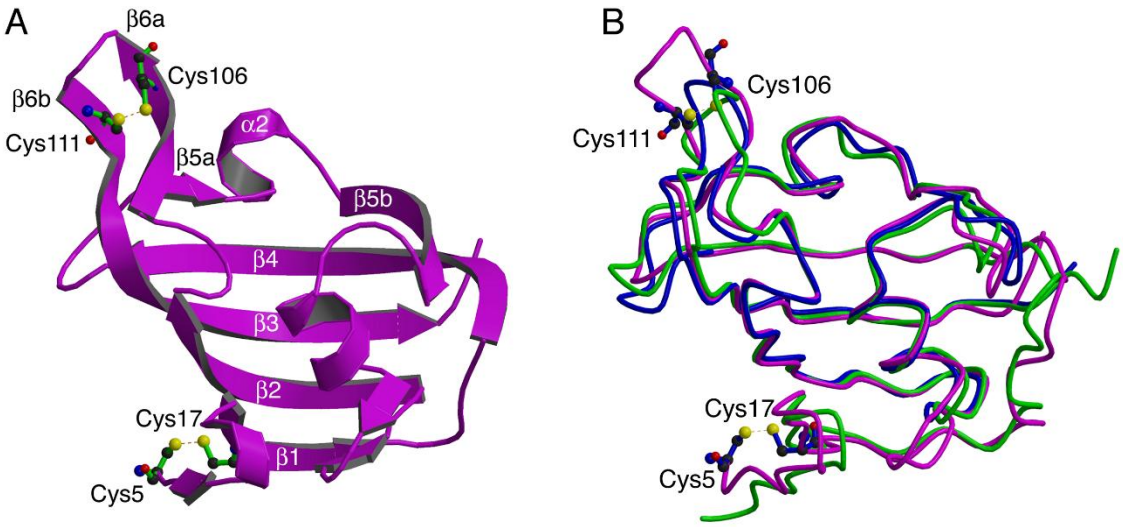

Fig. 2. Structure of AtFKBP13. The active-site disulfides are shown as a ball-and-stick representation and are yellow. (**A**) Three-dimensional structure of the AtFKBP13 monomer (PDB: 1U79; [27]). (**B**) Comparison of the Cα backbone atoms of AtFKBP13 with human (h) and *Legionella pneumophila* (Lp) counterparts. AtFKBP13 (residues 5–129), hFKBP12 (residues 1–107), and LpFKBP25 (residues 496–612) are magenta, blue, and green, respectively. The figures were prepared with Molscript [91] and Raster 3D [92].

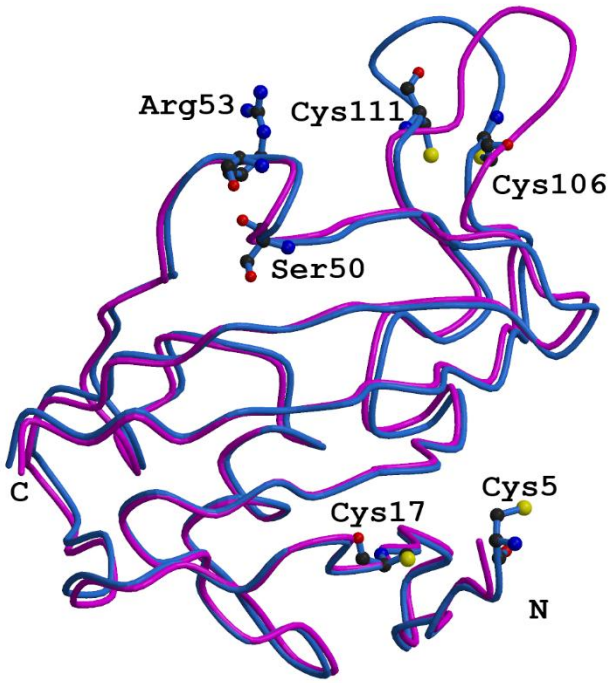

Fig. 3. Superimposition of oxidized and reduced AtFKBP13 structures. AtFKBP13-S2 (PDB: 1U79; [27]) and AtFKBP13-(SH)2 (PDB: 1Y0O; [90]) for residues 5–129, are colored magenta and blue, respectively. The structures align with an RMSD of 1.22 Å for the Cα traces. The regulatory Cys residues and the catalytic Ser50 and Arg53 residues of the reduced protein are shown. The sulfur atom is shown as a yellow sphere. The superimposition was done with the program O and figure was prepared with Molscript and Raster 3D.

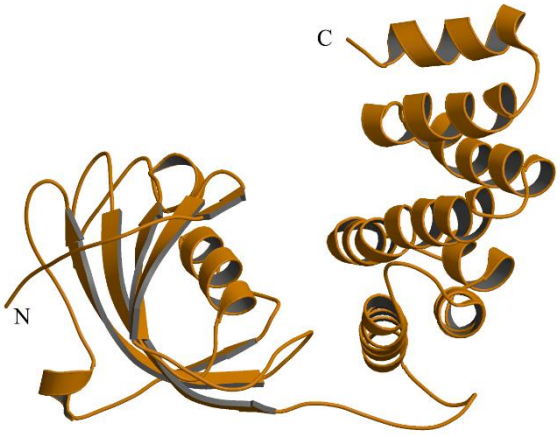

Fig. 4. Crystal structure of AtFKBP42. The FK506 binding domain is at the N-terminus and the TPR domain is at the C-terminus (PDB: 2IF4; [94]). The figure was prepared with Molscript and Raster 3D.

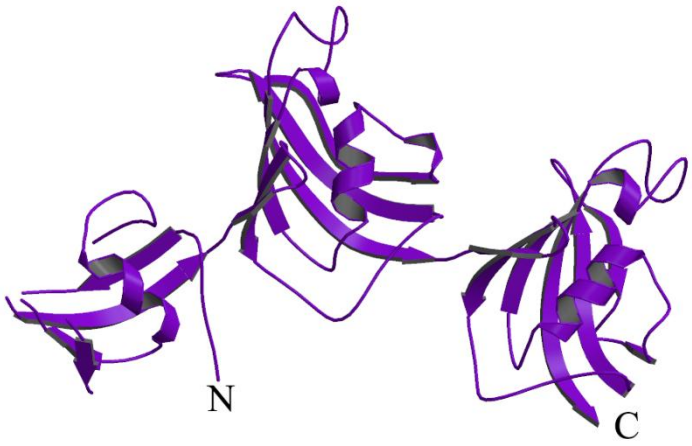

Fig. 5. Crystal structure of TaFKBP73. Note that TaFKBD73-1 is incomplete in the original structure (PDB: 3JYM; [87]). The figure was prepared with Molscript and Raster 3D.

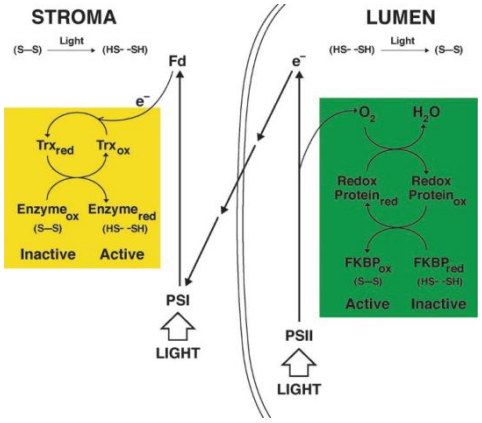

Fig. 6. Proposed mechanism for the light-mediated regulation of AtFKBP13 in the thylakoid lumen. Trx, thioredoxin; PSI, photosystem I; PSII, photosystem II; Fd, ferredoxin. Rp is an undefined redox protein.

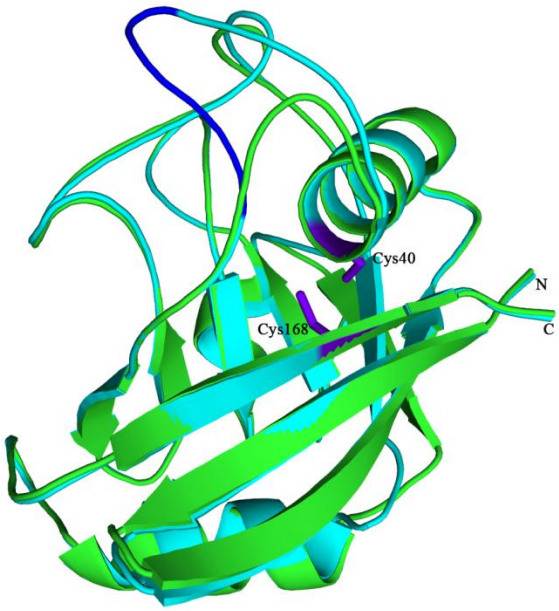

Fig. 7. Structural overlap of CsCYP and hCYPA. CsCYP (PDB: 4JJM; [74]) is shown in cyan and hCYPA (PDB: 1CWA; [132]) is shown in green. The divergent loop of CsCYP is shown in blue and its Cys residues that are involved in redox regulation are highlighted as stick in purple. The associated cyclosporin (CsA) molecule is omitted for clarity. The figure was generated using PyMOL [133].

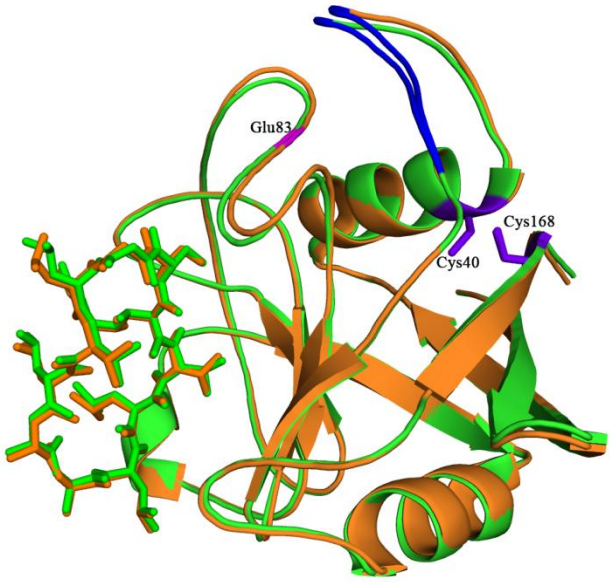

Fig. 8. Structure overlap of CsCYP with TaCYPA-1. CsCYP (PDB: 4JJM; [74]) is shown in orange and TaCYPA-1 (PDB: 4HY7; [130]) is shown in green. The associated CsA molecule is also shown in the respective colors. The

conserved divergent loop is shown in blue, the conserved Cys residues in purple and the conserved Glu83 in magenta. The figure was generated using PyMOL.

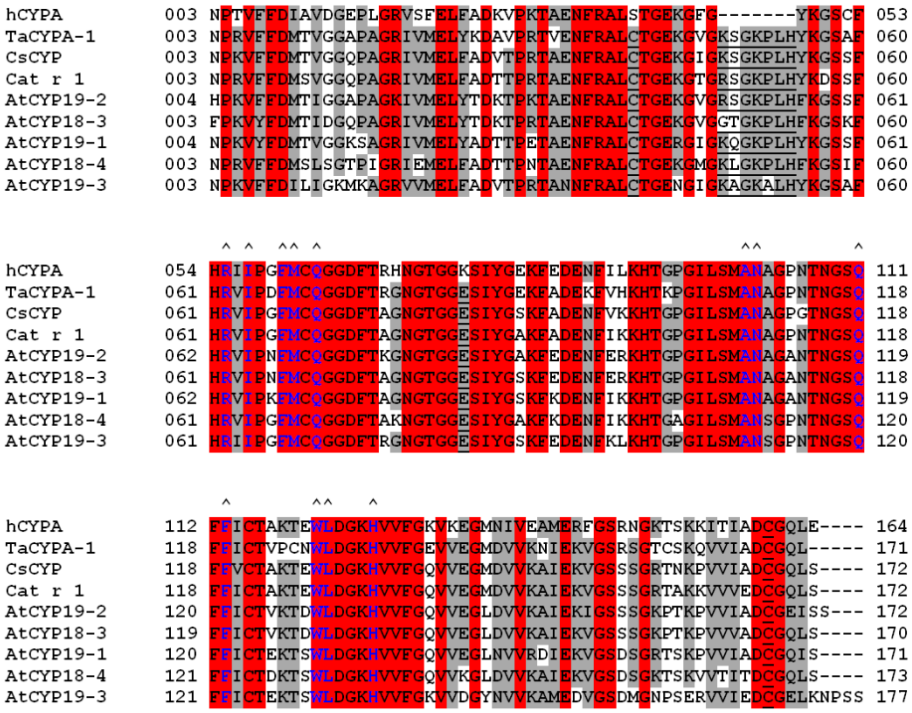

Fig. 9. Sequence alignment of single-domain, divergent plant cyclophilins. hCYPA is aligned along with the single-domain, divergent plant CYPs. Features of divergent CYPs such as the divergent loop and conserved Cys residues and Glu residue are underlined. The twelve residues involved in the prolyl-peptide binding and PPIase activity are shown in blue and with a hat.

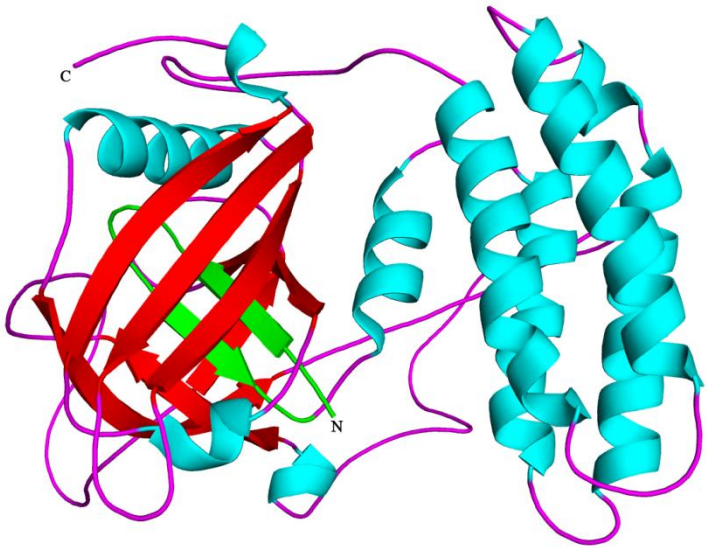

Fig. 10. Structure of AtCYP38: In this crystal structure of AtCYP38 (PDB: 3RFY; [62]), α-helices are shown in cyan, β-strands in red and loops in magenta. The N-terminus that forms part of the C-terminal cyclophilin (CYP) domain is shown in green. This structure illustrates the multi-domain organization and highlights the auto-inhibition mechanism of the PPIase function via intramolecular interaction. The figure was generated using PyMOL.

Table-1

Immunophilin	Cellular localization	Known binding partners / functional attributes
AtFKBP12	Cytoplasm	Interacts with AtFIP37; functions as an mRNA splicing factor during early stages of embryogenesis and endosperm formation [25]
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AtFKBP15-1 and 15-2	ER/Secretory	Involved in heat shock response [28]
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AtFKBP16-2	Thylakoid lumen	Interacts with NDH enzyme complex and stabilizes NDH interaction with photosystem I [30]
AtFKBP42	Membrane-anchored	Interacts with the ABC transporters AtPGP1 and AtPGP19 [31], as well as with the vacuolar transporters AtMRP1 and AtMRP2 [32]; involved in auxin and brassinosteroid (BR) signaling pathways [33]
AtFKBP53	Nucleus	Functions as a histone H3 chaperone and thereby involved in repression of ribosomal RNA gene expression [34]
AtTIG	Chloroplast stroma	Functions as a trigger factor [9]
AtFKBP62/ROF1	Cytoplasm	Interacts with HSP90.1 (thermotolerance; [35, 36]) and phosphatidylinositol-phosphate protein (osmotic/salt stress tolerance; [37])
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AtCYP38	Thylakoid lumen	Involved in assembly and maintenance of PSII and oxygen evolving complexes [60-62]; PPIase auto-inhibition [62]
AtCYP40	Nucleus	Involved in vegetative shoot maturation [63] post-translational gene silencing [64], HSP90-mediated RISC assembly [65], inhibition of Tombus virus replicase assembly [66]
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AtCYP59	Nucleus	Involved in transcription and pre-mRNA processing via interaction with Serine Arginine-rich (SR) proteins and RNA polymerase II [67]
AtCYP71	Nucleus	Involved in organogenesis, epigenetic gene silencing, chromatin assembly and histone H3 modification [68]
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CsCYP (Citrus)	Nucleus	Interacts with the bacterial effector protein PthA, the plant Tdx [73] and RNA polymerase II [74]

Table-2

Name	PDB ID	Domain (residues)	Resolution (Å)	Primary citation
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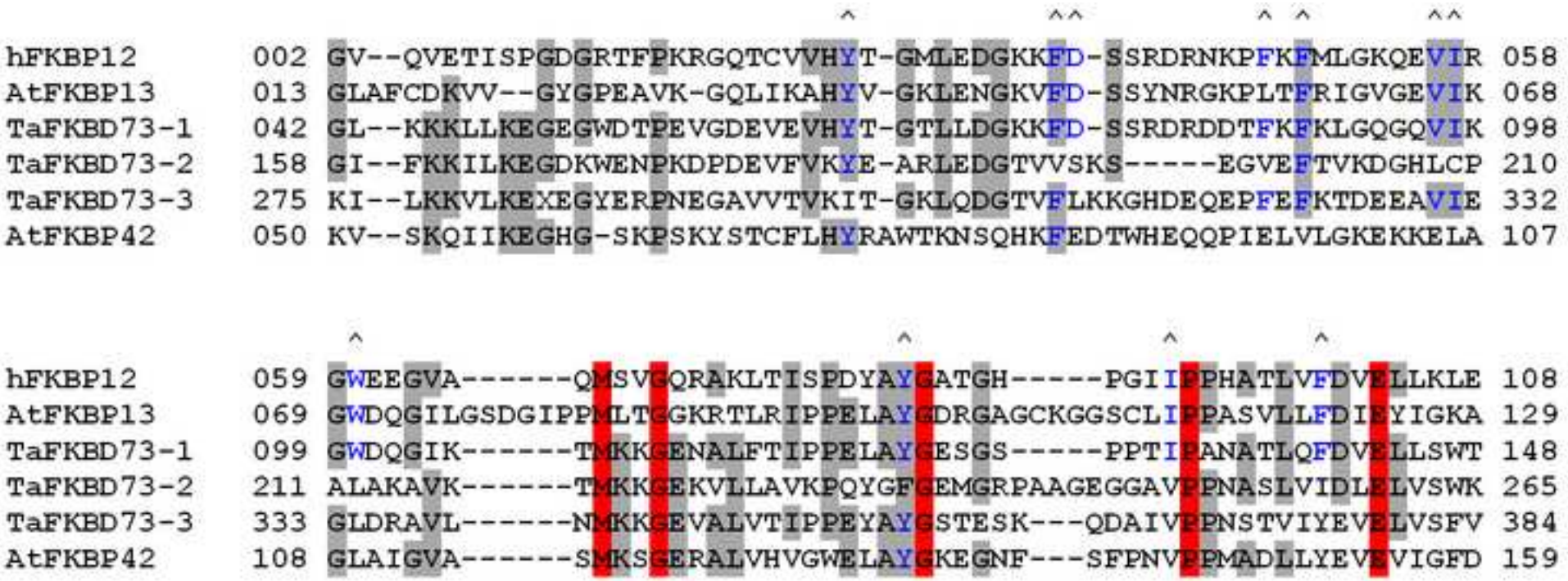
Cat r 1	2MC9	CYP domain (1-172)	NMR	[131]
AtCYP38	3RFY	CYP-like domain & helical bundle domain (83-433)	2.39	[62]

Figure-1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TaFKBD73-1 042 GL--KKKLLKEGEGWDTPEVGDEVEVHYT-GTLLDGKKFD-SSRDRDDTFKFKLGQGQVIK 098
TaFKBD73-2 158 GI--FKKILKEGDKWENPKDPDEVFVKYE-ARLEDGTVVSKS-----EGVEFTVKDGHLCP 210
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TaFKBD73-1 099 GWDQGIK------TMKKGENALFTIPPELAYGESGS-----PPTIPANATLQFDVELLSWT 148
TaFKBD73-2 211 ALAKAVK------TMKKGEKVLLAVKPQYGFGEMGRPAAGEGGAVPPNASLVIDLELVSWK 265
TaFKBD73-3 333 GLDRAVL------NMKKGEVALVTIPPEYAYGSTESK---QDAIVPPNSTVIYEVELVSFV 384
AtFKBP42 108 GLAIGVA------SMKSGERALVHVGWELAYGKEGNF---SFPNVPPMADLLYEVEVIGFD 159

Figure-2

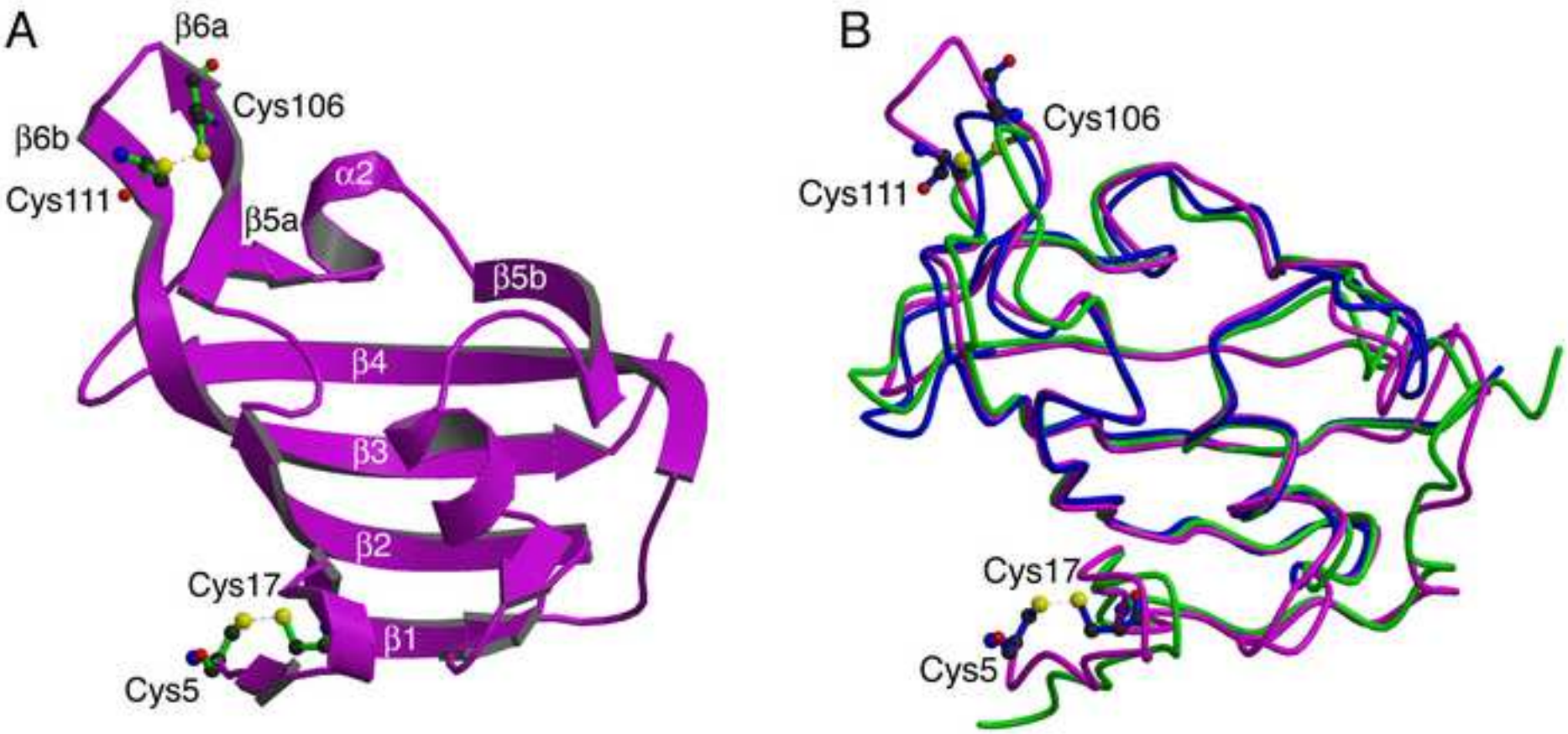

A
β6a
Cys106
β6b
Cys111
α2
β5a
β5b
β4
β3
β2
Cys17
β1
Cys5
B
Cys106
Cys111
Cys17
Cys5

Figure-3

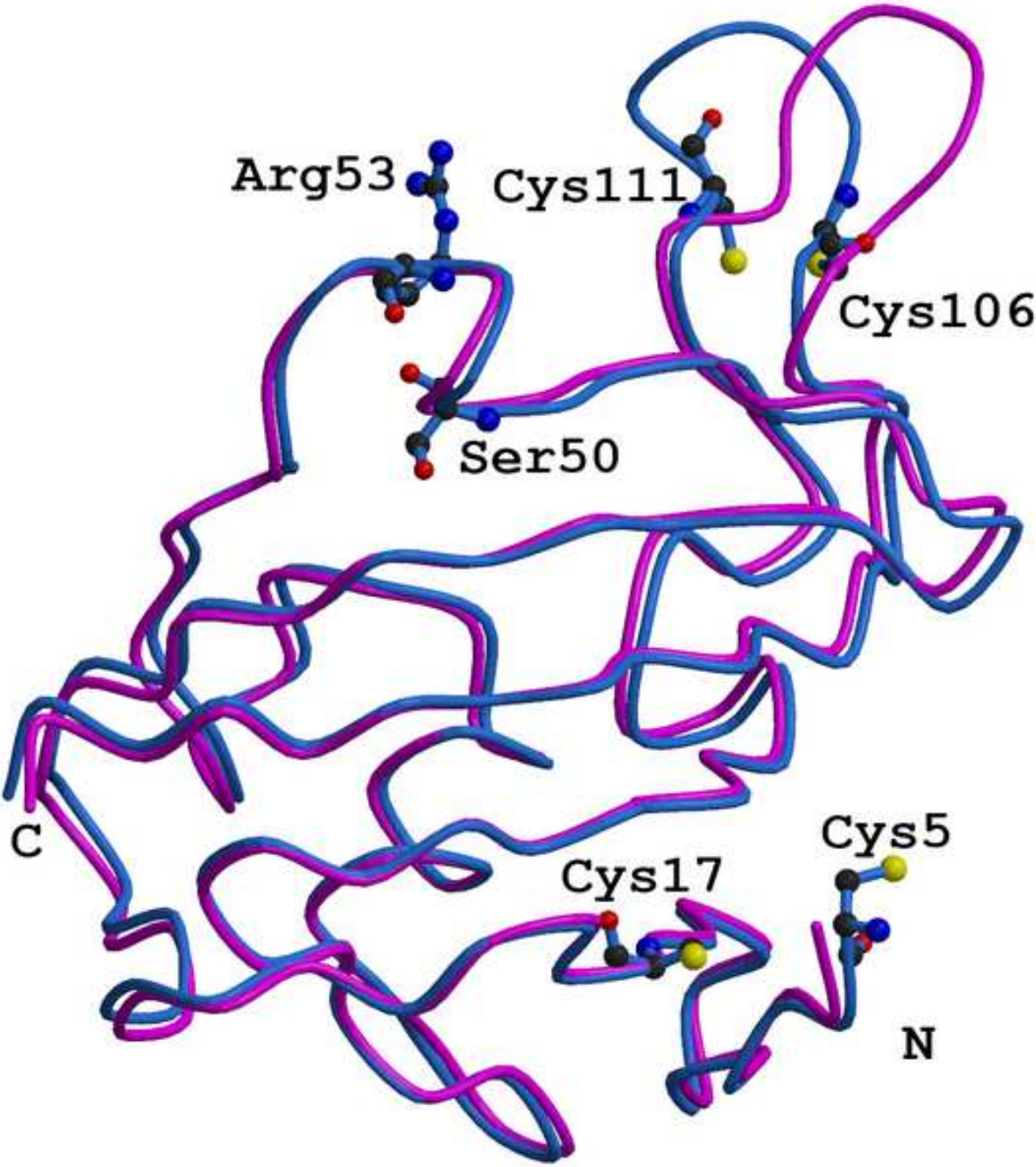


Figure-4

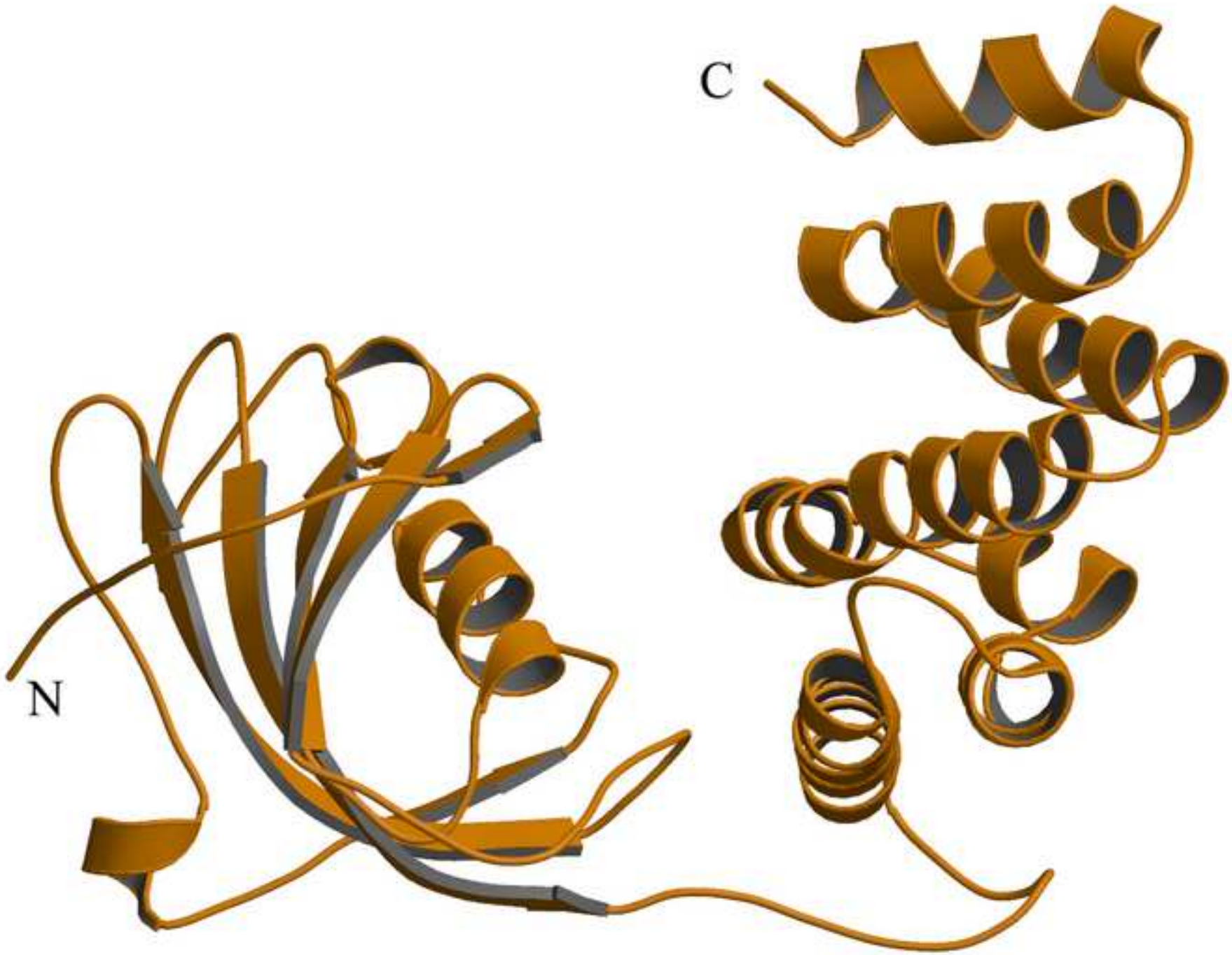


Figure-5

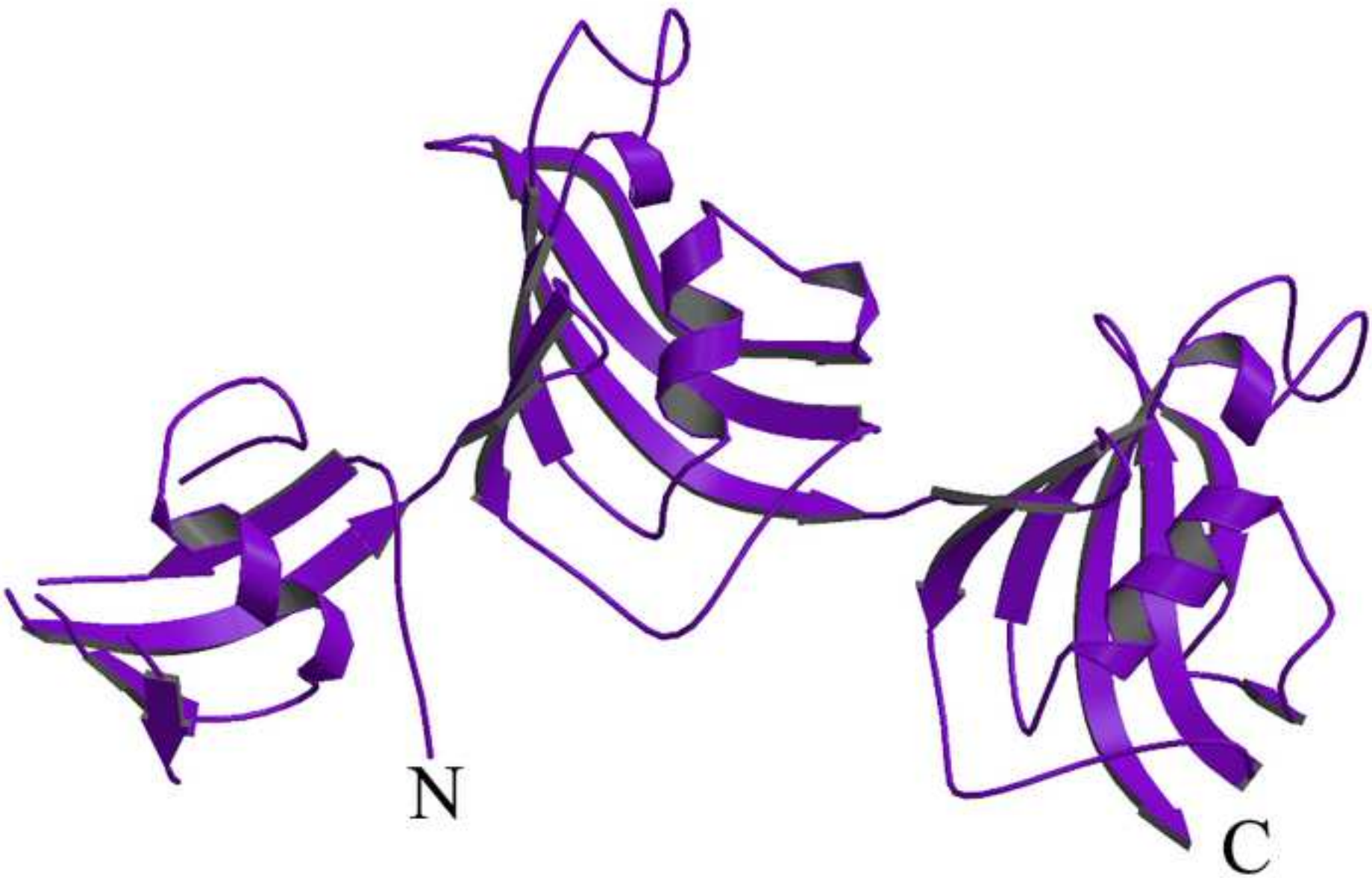


Figure-6

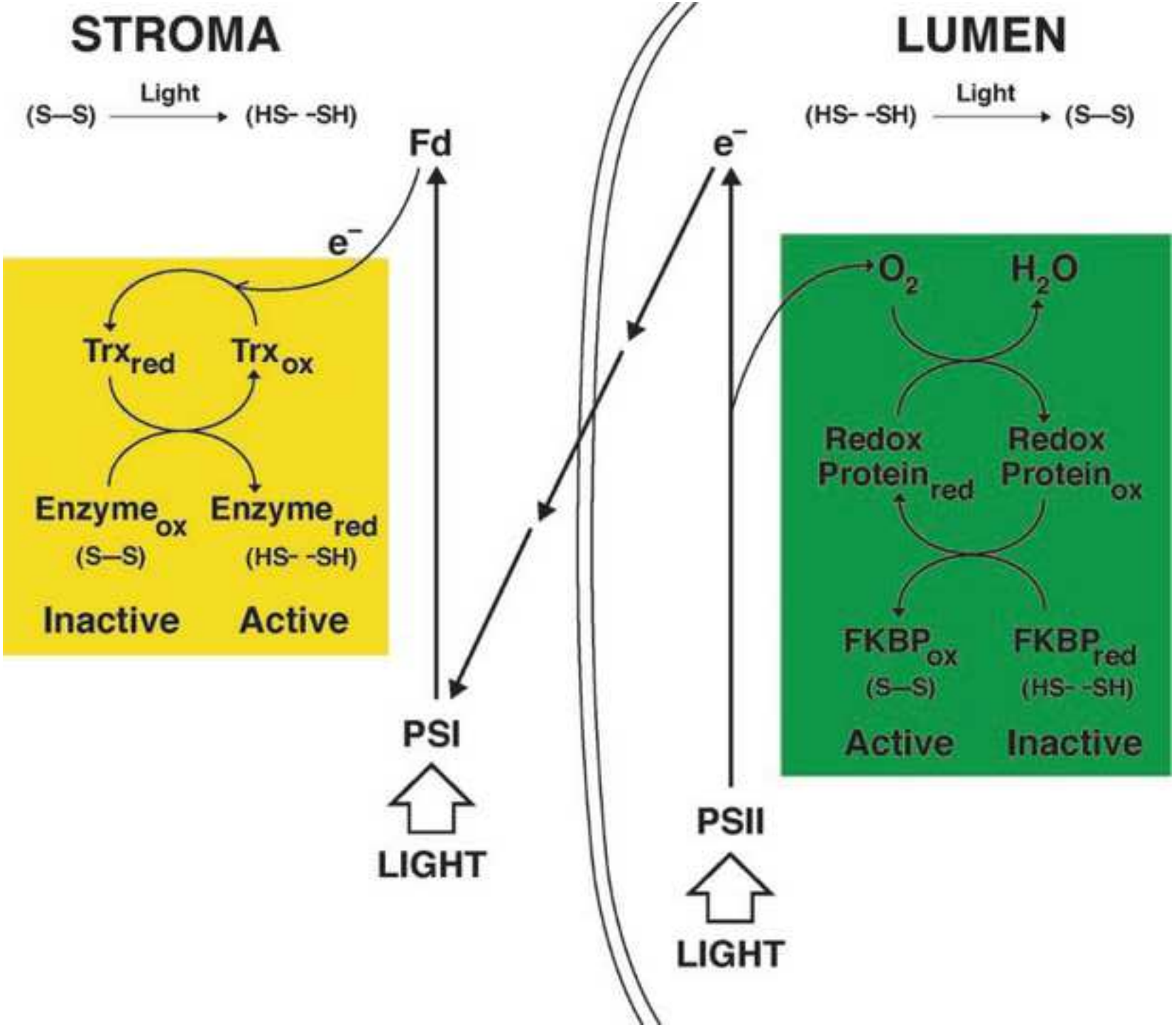

STROMA
LUMEN
(S—S) Light (HS- -SH)
(HS- -SH) Light (S—S)
Fd
e⁻
e⁻
Trx red
Trx ox
Enzyme ox
(S—S)
Enzyme red
(HS- -SH)
Inactive
Active
O2
H2O
Redox Protein red
Redox Protein ox
FKBP ox
(S—S)
FKBP red
(HS- -SH)
Active
Inactive
PSI
LIGHT
PSII
LIGHT

Figure-7

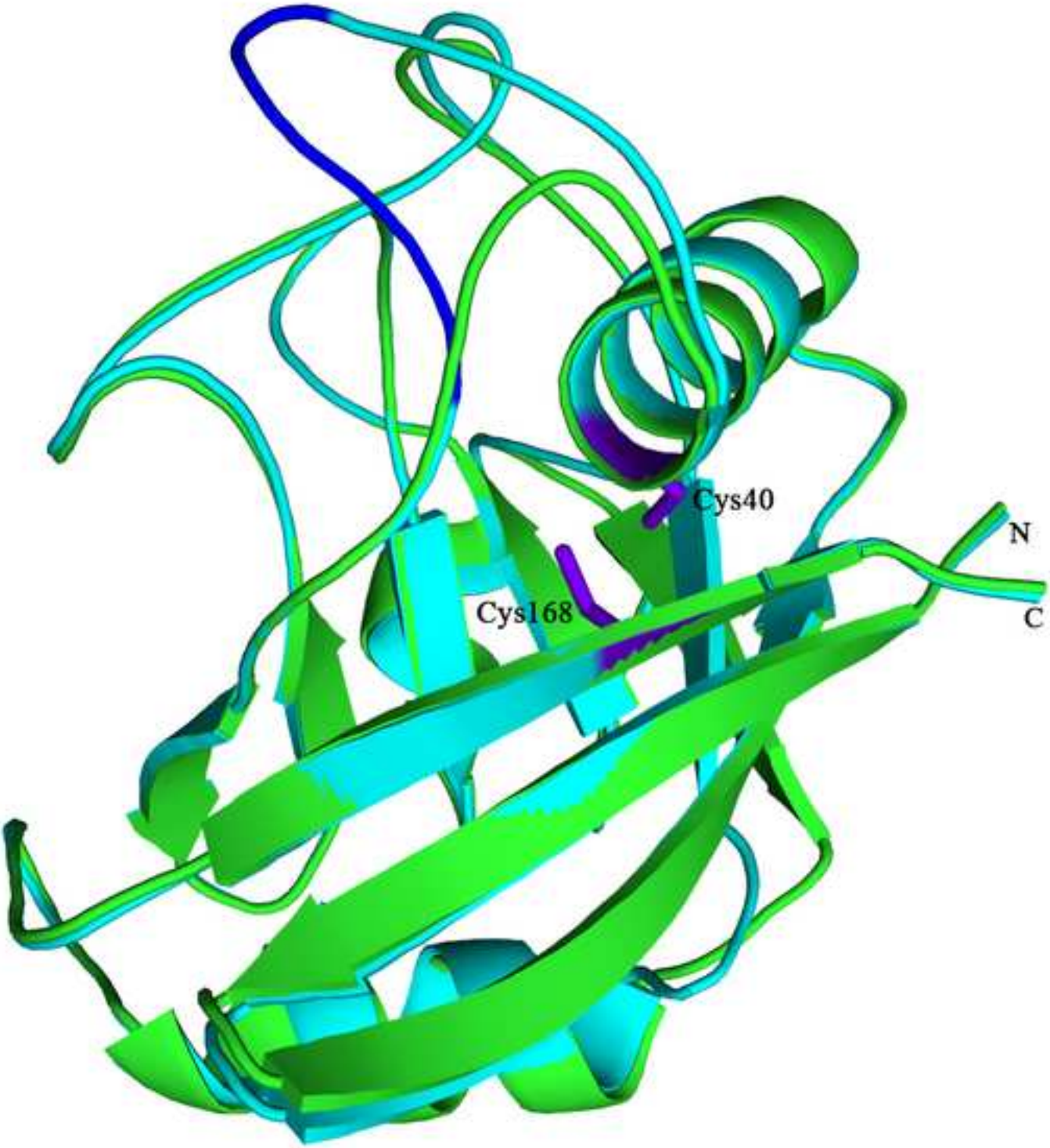


Figure-8

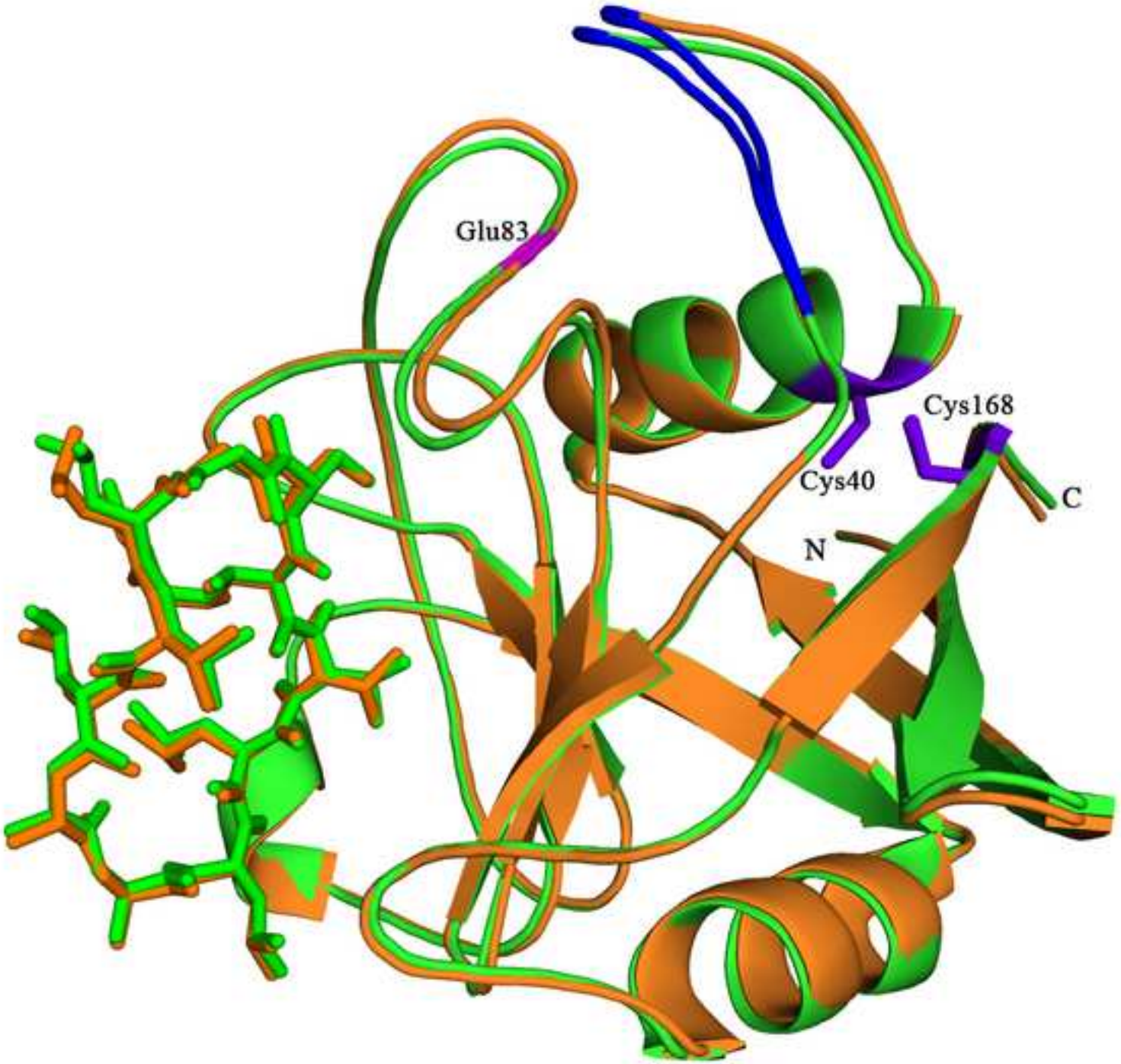
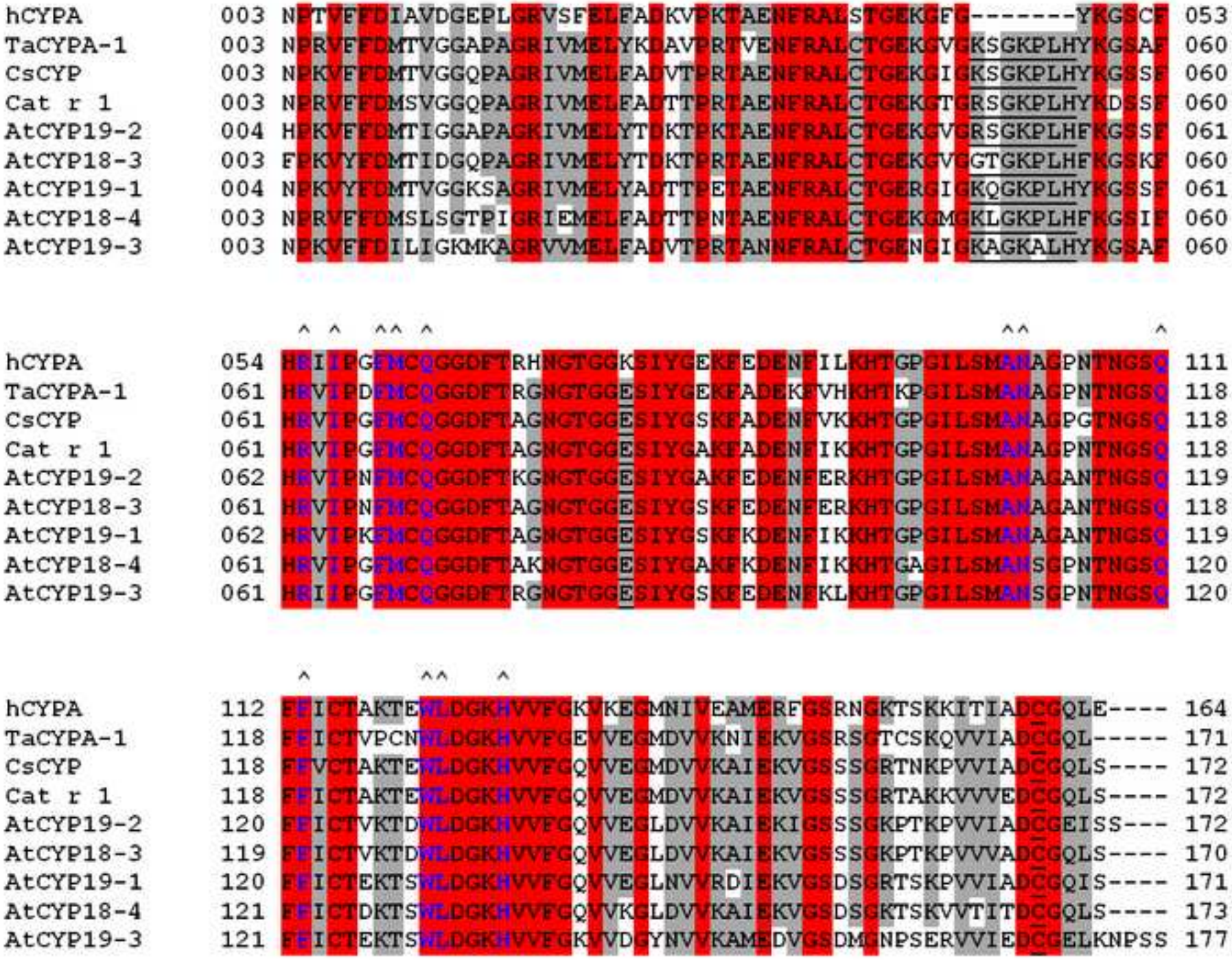


Figure-9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Cat r 1 003 NPRVFFDMSVGGQPAGRIVMELFADTTPRTAENFRALCTGEKGTGRSGKPLHYKDSSF 060
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AtCYP18-3 003 FPKVYFDMTIDGQPAGRIVMELYTDKTPRTAENFRALCTGEKGVGGTGKPLHFKGSKF 060
AtCYP19-1 004 NPKVYFDMTVGGKSAGRIVMELYADTTPETAENFRALCTGERGIGKQGKPLHYKGSSF 061
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Cat r 1 061 HEVIPGFMCQGGDFTAGNGTGGESIYGAKFADENFIKKHTGPGILSMANAGPNTNGSQ 118
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TaCYPA-1 118 FFICTVPCNWLDGKHVVFGEVVEGMDVVKNIEKVGSRSGTCSKQVVIADCGQL----- 171
CsCYP 118 FFVCTAKTEWLDGKHVVFGQVVEGMDVVKAIEKVGSSSGRTNKPVVIADCGQLS---- 172
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AtCYP19-3 121 FFICTEKTSWLDGKHVVFGKVVDGYNVVKAMEDVGSDMGNPSERVVIEDCGELKNPSS 177

Figure-10