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Additional Information

Review

Structural insights into PYR/PYL/RCAR ABA receptors and PP2Cs

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Research highlight:

The structure of the PYR/PYL/RCAR-ABA-PP2C complexes illustrates the mechanism of ABA signaling through inhibition of PP2Cs

Key words: phytohormone/ abscisic acid/ signaling/ phosphatase-2C/ receptor

Abstract

Abscisic acid (ABA) plays an essential function in plant physiology since it is required for biotic and abiotic stress responses as well as control of plant growth and development. A new family of soluble ABA receptors, named PYR/PYL/RCAR, has emerged as ABA sensors able to inhibit the activity of specific protein phosphatases type-2C (PP2Cs) in an ABA-dependent manner. The structural and functional mechanism by which ABA is perceived by these receptors and consequently leads to inhibition of the PP2Cs has been recently elucidated. The module PYR/PYL/RCAR-ABA-PP2C offers an elegant and unprecedented mechanism to control phosphorylation signaling cascades in a ligand-dependent manner. The knowledge of their three-dimensional structures paves the way to the design of ABA agonists able to modulate the plant stress response.

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1. Introduction

ABA plays a pivotal role to coordinate plant response under water stress situations as well as to regulate plant growth and development [1]. Chemically, ABA ($C_{15}H_{20}O_4$) is a sesquiterpenoid derived from isopentenyl pyrophosphate synthesized in plastids through the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway[2]. ABA contains one asymmetric carbon atom at C1', the natural form is S(+)-ABA and the side chain of the molecule is present in the 2-cis,4-trans isomeric state (Fig. 1). A plane of symmetry can be defined in the ABA molecule through the optical center, which defines two sides that differ only in that C6' carries two methyl group whereas C2' carries one and a double bond. Consequently, the non-natural (-)-enantiomer only differs structurally in these positions from the (+)-enantiomer (Fig. 1B) [3].

Plant hormone research has greatly benefited from genetic screenings aimed at the identification of key components of the hormone signaling pathways. Arabidopsis mutants showing reduced or enhanced sensitivity to a hormone usually pointed out to critical loci that, once cloned, revealed crucial components of the hormone signaling pathway [4]. However, such screenings, although highly successful for different hormone signaling pathways, failed to identify ABA receptors. Functional redundancy or pleiotropic effects including embryo or gamete lethality looked as sound arguments to justify this failure. Indeed, the first approaches to identify ABA receptors, marked by retracted data in one case [5], used biochemical techniques to identify ABA-binding proteins (CHLH/ABAR/GUN5) [6] or followed pharmacological evidence suggesting the involvement of G-protein coupled signaling in the ABA pathway (GTG1/GTG2) [7]. Finally, a chemical genetic approach using a synthetic selective ABA agonist, pyrabactin, made it possible to identify a family of soluble ABA receptors,

named PYR/PYL/RCAR for PYRABACTIN RESISTANCE1/PYR1-LIKE/ REGULATORY COMPONENTS OF ABA RECEPTORS, respectively [8, 9]. In a convergent approach, yeast-two hybrid screenings [9,10] and *in planta* interaction studies [11] identified these receptors through their capacity to bind to phosphatases type-2C (PP2Cs), which are key negative regulatory components of the pathway involved in the dephosphorylation of certain sucrose non-fermenting 1-related subfamily 2 (SnRK2) kinases [12,13,14].

The PYR/PYL/RCAR family of ABA receptors has witnessed a rapid explosion of structural data, since five independent groups have reported structural and functional mechanisms on ABA signaling [15,16,17,18,19], and even more recently, using the seed ABA-agonist pyrabactin (Fig. 1C), the structural basis for selective activation of certain receptors and the mechanism of antagonism in other members [20,21,22]. Such wealth of structural information is not available for other ABA receptors, such as the Mg-chelatase H subunit and the GTG1/GTG2 proteins, therefore we will focus this review on the recent structural data reported for the PYR/PYL/RCAR ABA receptors and their inhibitory interaction with PP2Cs. Additionally, different studies have provided a clear connection of PYR/PYL/RCAR receptors with downstream signaling components, such as PP2Cs, SnRK2s and ABA-responsive elements binding factors (ABFs/AREBs) or the SLOW ANION CHANNEL 1 (SLAC1), whose key role in ABA signaling had been previously revealed by biochemical and genetic studies. These findings have been recently reviewed [1, 23].

2. The structure of PYR/PYL/RCAR receptors

The crystal structure of three ABA receptors, PYR1, PYL1 and PYL2, has been reported to date (see Protein Data Bank codes in Table 1). As

predicted, these data confirm that the PYR/PYL/RCAR family belongs to a branch of the Bet v superfamily, which is structurally characterized by the presence of the Bet v fold or START domain [24]. This structure comprises a seven-stranded β -sheet flanked by two α -helices, which is designated as helix-grip fold. Additionally, the PYR/PYL/RCAR family contains a α -helical segment at the N-terminus, which is not present in the Bet v fold and therefore, it can be considered as a particular feature of this family of ABA receptors.

Crystal structures were obtained both in the apo form (ABA-free) and in the presence of ABA. For instance, apoPYL1 [15], apoPYL2 [15,19], ABA-bound PYL1 [16] and ABA-bound PYL2 [15,19] structures have been resolved. In the case of PYR1, in the crystallographic asymmetric unit, there is one ABA-bound and one ABA-free subunit [17,18]. A high degree of structural similarity is evident from the superposition of the three structures (Fig. 2), however, recent works using the pyrabactin molecule reveal subtle differences among the receptor binding pockets with important functional consequences, since pyrabactin is an agonist of PYR1 and PYL1, whereas it is an antagonist of PYL2 [20, 21, 22]. Different experimental data indicate that these receptors exist as dimers in solution, however, the receptor-PP2C complexes have a 1:1 stoichiometry, implying that the receptor dimers have to dissociate before interacting with the PP2C[17,18,19].

2.1. ABA-binding pocket

The structure of the ABA-bound forms of PYR1, PYL1 and PYL2 reveals the nature of the interactions stabilizing the hormone into the receptor binding pocket [15,16,17,18,19]. The ABA sits in a deep cavity almost completely buried from the external medium (see figure 3). The walls of this cavity match perfectly the chemical character of the different

functional groups of the hormone. The cyclohexane ring and the isoprene moiety establish hydrophobic interactions with apolar PYR1 side chains, while the carboxylic, hydroxyl and ketone groups of the ABA molecule are stabilised through interactions with polar side chains. Strikingly, many of these polar interactions involve hydrogen bonds with water molecules (which are sandwiched between the hormone and the walls of the receptor cavity) rather than direct side chain hormone contacts. For instance, the carboxylate group of ABA establishes interactions with the side chains of Glu94, Glu141, Ser122 and Tyr120 through hydrogen bonds mediated by three internal water molecules (Fig. 3). Additionally, a direct polar contact is found between the amine group of Lys59 and the carboxylate group, which is buried into the ABA binding pocket, away from the loops flanking the entry. These data are in agreement with the requirement of the carboxylate group for ABA bioactivity [3], and indeed, different mutations in these residues, e.g. Glu94Lys, Glu141Lys, Lys59Gln, abolish or reduce PYR1 function. In this context, it is interesting to mention that coupling of ABA through its carboxylic group to the amino group of a 10-atom spacer arm of a Sepharose resin has been used as a tool to identify ABA binding proteins, which resulted in the identification of the Mg-chelatase H subunit as an ABA receptor [6,25]. Such approach is likely to interfere with the binding of the carboxylate group to the receptor, so the mechanism of ABA binding by Mg-chelatase has still to be explained. The addition of bulky groups to any part of the hormone molecule is likely to interfere with binding to PYR/PYL/RCAR receptors, since this kind of receptor wraps very tightly around the hormone.

The architecture of the ABA-binding pocket and the structure of ABA enantiomers suggest that both molecules can be accommodated into the pocket of at least some PYL receptors, although with different affinity. Stereospecificity would be contributed by steric constraints imposed by the

mono-methyl group at position C2' and the dimethyl group at C6'. Indeed, binding of (-)ABA to the PYL5 receptor has been measured using isothermal titration calorimetry [10], although the K_d for the natural (+)enantiomer was 20-fold lower (1.1 versus 19.1 μM), which indicates a higher affinity for ABA binding of the natural form. Additionally, (-)ABA promoted interaction of PYL2, PYL3 and PYL4 with HAB1 [8]. Structural studies on PYL2 also show that the minus enantiomer can be accommodated into the ABA-binding pocket [19], although the dimethyl group flipped in the (-)enantiomer would cause some steric hindrance with the narrow pocket that accommodates the monomethyl group [15].

2.2. ABA-induced conformational changes

Since the PYR1 dimer crystal structure contains both unbound and ABA-bound subunits, comparison of both structures made it possible to reveal the ABA-induced subunit conformational changes (Fig. 4A and 4B) [17,18]. Thus, superposition of the ABA-bound and ABA-free subunits of the PYR1 dimer revealed notable differences in two loop regions (loops β3-β4 y β5-β6) and the N-terminal part of the C-terminal α-helix. Specifically, the loop β3-β4 (S₈₅GLPA₈₉) and the loop β5-β6 (H₁₁₅RLT₁₁₈), upon ABA-binding, fold over ABA to complete ABA enclosure, in contrast, in the ABA-free subunit, these loops adopt an open conformation that allows entry of ABA into the cavity and hence they have been called the gating loops. The region comprising the loop between β7 and the N-terminal part of the last α-helix (M₁₄₇PEGNSEDDTRM₁₅₈) is also involved in the stabilization of the closed conformation of the gating loops, as well as in interaction with the PP2C. Indeed, in addition to trapping the ABA molecule into the receptor cavity, these conformational changes are crucial to generate a favourable interaction surface for the binding of the PP2C (Fig. 4C and 4D). For instance, the mutations P88S and S152L severely

reduce the capacity of PYR1 to interact with HAB1. Another key example is the flipping movement of S85 (equivalent to S89 in PYL2 and S112 in PYL1), which is exposed in the surface of PYR1 upon binding of ABA. This residue is crucial for the interaction with the active site of the PP2C (discussed below).

Similar conclusions have been obtained by comparison of the ABA-free and ABA-bound forms of dimeric PYL2 [19]. In addition, these authors have analysed the changes generated in the dimer interface of PYL2 upon ABA binding. ABA binding induces a slight change in the relative orientation of one PYL2 protomer with respect to the other. As a result, a significant rearrangement of the interface residues is generated, leading to a diminished number of van der Waals contacts and hydrogen bonds and consequently a weakening of the dimer interface. SAXS studies performed with PYR1 also reveal significant changes in the dimer assembly upon ABA-binding, which leads to a flatter, more compact form of PYR1, which also indicates an orientation change between both subunits [17].

3. Architecture of ternary complexes PYR/PYL/RCAR-ABA-PP2C

The crystal structure of two ternary complexes has been described, i.e. PYL1-ABA-ABI1 and PYL2-ABA-HAB1 [15,16,19]. It is important to note that only the catalytic core of the ABI1 (residues 125-429) and HAB1 (residues 172-511) PP2Cs has been used for these studies. Therefore, it has not yet been elucidated the structure of the N-terminal region of the PP2Cs, which is expected to play an important role either for biochemical regulation of PP2C activity or for regulation of the interaction with other partners.

First of all, these works provide the first structures of plant PP2Cs, and comparison with the pre-existing structure of human PP2C reveals that

the active-site residues are highly conserved (Fig. 5 and 6). Thus, critical active-site residues of ABI1 are Arg138, Glu142, Asp143, Asp177, Gly178, His179, Asp347 and Asp413, which correspond in HAB1 to Arg199, Glu203, Asp204, Asp243, Gly244, His245, Asp432 and Asp492, respectively. Both PP2Cs adopt a similar folding pattern as human PP2C, with two central five-stranded β -sheets sandwiched by two pairs of α -helices. The catalytic site is located at the edge of the two central β -sheets and contains 3 atoms of either Mn^{++} or Mg^{++} ions.

The structural studies of different groups have made it possible to define the molecular mechanism of the ABA-dependent inhibition of PP2C activity through PYR/PYL ABA receptors. Although a clear picture emerges from these works (see below), important questions are still open, for instance, a clear discrepancy is observed with respect to the role of the dimeric PYR/PYL proteins in the mechanism of action. Indeed, both the work of Melcher et al., [15] and Miyazono et al., [16] (2009) omit any consideration on the dimeric nature of the ABA-receptor and the role, if any, of the dimer in the regulation of PYR/PYL function. Taking into account that experimental evidence indicates that both PYR1 and PYL2 are dimers in solution, the contribution of Yin et al., [19] is particularly relevant in this context. Thus, this group postulates that PYR/PYLs exist as inactive homodimers in cells, unable to bind or inhibit PP2Cs. However, yeast two hybrid and biochemical experiments indicate that different PYLs are able to interact with PP2Cs (in a non-inhibitory manner) in the absence of exogenous ABA [8,9,10]. On the other side, experimental evidence indicates that ABA-binding induces a conformational rearrangement of the receptor, which weakens the dimerization interface of the PYLs and, in turn, generates an interaction platform to contact the PP2C [19]

3.1. Mechanism of ABA-dependent inhibition of PP2Cs by PYR/PYL/RCAR receptors

In the structure of PYL1-ABA-ABI1 and PYL2-ABA-HAB1, the ABA receptor (PYL1 or PYL2) contacts the PP2C using the gating loops that cover the ABA-binding pocket, i.e. the β 3- β 4, β 5- β 6 as well as the β 7- α 5 loop (Fig. 4C and 4D). On the other hand, the PP2C contacts the ABA receptor through its active site and a small protruding region, which has been called the flap sub-domain [26] and contains an important tryptophan residue (Fig. 6A). In the ternary complexes, access to the active-site cleft of the PP2C is blocked by the β 3- β 4 loop of PYL proteins, where the Ser112 of PYL1 or Ser89 of PYL2 establish hydrogen bonds with Gly180 of ABI1 and Gly246 of HAB1 and the metal-stabilizing residue Glu142 of ABI1 and Glu203 of HAB1, respectively (Fig. 6B) explaining the inhibitory action of receptor-hormone complexes on the activity of these phosphatases. Indeed, experiments by Melcher et al., [15] using a SnRK2.6 peptide that acts as physiological substrate of HAB1 indicate a competitive inhibitor mechanism between ABA-bound PYL2 and HAB1 in agreement with the structural data. These results however, contrast with those reported by Ma et al., [9], which using a non-peptidic PP2C substrate (methyl-umbelliferyl-phosphate) concluded that the mode of inhibition of ABI2 by RCAR1/PYL9 occurs through non-competitive inactivation of the enzyme.

In addition to the contact with the active-site cleft of the PP2C, amino acid residues Trp300 and Trp385 of ABI1 or HAB1, respectively, located at the flap sub-domain of the PP2C, are critical for the interaction with ABA-bound PYR/PYLs (Figure 6 A). This residue points into the ABA-binding pocket (through the loops that cover ABA) and establishes a water-mediated hydrogen-bond to the ketone group of ABA. This link has been interpreted for some authors as a direct proof that the PP2C serves as

a co-receptor of ABA [15,16]. An alternative point of view is that most of the ABA molecule is previously buried within the ABA-binding pocket of the ABA-receptor and only one water-mediated hydrogen bond is established between ABA and the PP2C upon interaction of the ABA-bound PYL protein with the PP2C [19]. However, the work of Melcher et al., [15] shows that upon insertion of Trp385 of HAB1 into the PYL2 ABA-binding pocket, the β 3- β 4 (SGPLA residues) and β 5- β 6 (HRL) loops undergo a conformational change, generating additional contacts with the ABA molecule. Therefore, Trp385 would act as a locking mechanism to keep the β 3- β 4 and β 5- β 6 loops in the closed conformation. Similar conclusions were reached by Miyazono et al., [16], which concluded that the docking of Trp300 of ABI1 into the PYL1 ABA-binding pocket is necessary for the β 3- β 4 loop to be properly located into the active site of ABI1. Modeling of the Trp385A mutation in the PYL2-ABA- Δ NHAB1 complex reveals the critical loss of the docking point provided by the interaction of the Trp residues with the ABA molecule (Fig. 6 D). Overall, these PP2C-induced conformational changes of PYR/PYL and ABA interaction would explain the higher ABA binding affinity measured for PYR/PYL in the presence of the catalytic core of the PP2Cs [9,10].

3.2. *abi1-1*^{G180D}, *abi2-1*^{G168D} and *hab1*^{G246D} hypermorphic enzymes

The ternary complex PYL1-ABA-ABI1 [16,19] provides an explanation to the dominant gain-of-function phenotype of the *abi1-ID* mutation (equivalent to *abi2-ID*), which is a missense mutation that replaces Gly180 by Asp (Gly168Asp in *abi2-1*). These mutations were isolated 25-years ago by genetic screenings in Arabidopsis, aimed to the identification of ABA-insensitive mutants [27] and the cloning of the mutant loci provided pioneering insights into ABA signaling [28,29,30,31]. Both *abi1-ID* and *abi2-ID* are strong ABA-insensitive mutants that show diminished

response to ABA in seeds and vegetative tissues, however, analysis of loss-of-function alleles indicates that ABI1 and ABI2 gene products, as well as other clade A PP2Cs, are negative regulators of ABA signaling [32,33,34,35]. Since the phenotype of the *abi1-1D* and *abi2-1D* dominant alleles is just the opposite of loss-of-function alleles, they can't represent dominant negative alleles, as it was accurately noted by Robert et al., [36]. A molecular explanation is now provided by structural studies of the PYR/PYL/RCAR receptors in complex with ABA and the PP2Cs. For instance, Gly180 of ABI1 establishes a hydrogen bond with Ser112 of PYL1, which is a key residue for the blockage of the active-site cleft of the PP2C (see above). The replacement of the glycine residue by a bulkier aspartate will likely introduce steric hindrance for the interaction of the β 3- β 4 loop with the PP2C. Indeed, it has been demonstrated that *abi1-1* protein loses the interaction and is refractory to inhibition by PYR/PYL proteins [8,13], whereas this mutation does not block interaction with downstream targets of PP2Cs, such as SnRK2s [13,14,37].

Similar considerations apply to the equivalent Gly246Asp mutation of HAB1 [14,36] and the ternary complex PYL2-ABA-HAB1 also provides a molecular explanation to the dominant nature of this mutation [15]. Thus, structural modeling of the mutated Asp246 residue in HAB1 suggests that its larger side chain compared to Gly246 will collide with the β 3- β 4 loop of the ABA-bound PYR1/PYL2 [15] (Fig. 6C). Moreover, biochemical studies performed with *hab1*^{G246D} reveal that this mutant PP2C, in contrast to HAB1 wt, escapes from ABA-dependent PYR1-mediated inhibition, and it is able to dephosphorylate OST1 in the presence of ABA and PYR1 (Rodriguez P.L., unpublished results). Therefore, it qualifies as a hypermorphic mutation in the presence of ABA and a PYR/PYL receptor, although paradoxically, *hab1*^{G246D} as well as *abi1*^{G180D}

and *abi2*^{G168D} proteins, have a lower specific activity as compared to wt in the absence of ABA.

4. Mechanism of action of pyrabactin

ABA-agonists harbour the potential to improve the yield of crop plants under drought stress or any other properties modulated by the ABA pathway in crop or ornamental plants. The identification of ABA antagonists would have also an important role in basic research, in order to manipulate ABA response in different tissues or developmental stages, which would represent a very useful tool to study ABA physiology. Recent studies with the first ABA agonist identified, pyrabactin, have provided a structural basis for understanding the selective activation of certain ABA receptors [20,21,22,38]. Additionally, these studies reveal that whereas pyrabactin is an ABA-agonist for some PYR/PYL/RCAR receptors, e.g. PYR1 and PYL1, it is an antagonist for other members of this family, e.g. PYL2. Comparison of the crystal structures of PYL1-pyrabactin-ABI1 and PYL1/PYL2-pyrabactin complexes has provided the mechanism to distinguish between productive and non-productive pyrabactin binding. In the case of PYR1 and PYL1, pyrabactin binds inside the receptor cavity and establishes interactions that stabilise the closed conformation of the gating loops, as ABA does. In the case of PYL2 however, pyrabactin also binds inside the receptor cavity but adopts a different conformation which does not promote closure of the gating loops. These findings also establish the concept of ABA receptor antagonism as well as a framework for modifying agonist selectivity.

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Table 1. PDB codes of structural data on ABA-receptors of the PYR/PYL/RCAR family:

Authors	Apo-structures	ABA-PYR/PYL/RCAR bound structures	ABA-PYR/PYL/RCAR-PP2C complex structures
<i>Nature.</i> Melcher et al.,2009	apoPYL1 3KAY apoPYL2 3KAZ	ABA-bound PYL2 3KB0	PYL2-ABA-HAB1 3KB3
<i>Nature.</i> Miyazono et al.,2009		ABA-bound PYL1 3JRS	PYL1-ABA-ABI1 3JRQ
<i>Nat. Struct. & Mol. Bio.</i> Yin et al.,2009	apoPYL2 3KDH	ABA-bound PYL2 3KDI	PYL1-ABA-ABI1 3KDJ
<i>Science.</i> Nishimura et al.,2009	PYR1 (subunit B) 3K3K	PYR1 (subunit A) 3K3K	
<i>Nature.</i> Santiago et al.,2009	PYR1 (subunit B) 3K90	PYR1(subunit A) 3K90	

Fig. 1. Chemical structure of the phytohormone ABA and the seed ABA-agonist pyrabactin. (A) 2D structure of the natural S(+) form of ABA. The red asterisk points out the C1' asymmetric carbon of the molecule. (B). 3D structure of the two ABA enantiomers. The (-) enantiomer has been rotated to illustrate the structural difference of the ring methyl groups with respect to the (+) enantiomer. (C) 2D and 3D structures of pyrabactin.

Fig. 2. Superposition of PYR1, PYL1 and PYL2 dimers. Figure generated with PyMOL (<http://www.pymol.org>) using the Protein Data Bank (PDB) codes 3KAZ (PYL2), 3KAY (PYL1) and 3K90 (PYR1).

Fig. 3. ABA binding in the PYR1 cavity. (A) ABA (in light blue) buried in the PYR1 cavity. Lys59 (orange sticks), which is located at the bottom of the cavity, establishes a direct polar contact between its amine group and the carboxylate group of ABA. Interactions of Glu94, Glu141, Ser122 and Tyr120 (red sticks) with the carboxylate group are established through hydrogen bonds mediated by water molecules. (B) 2D map of interactions between ABA and PYR1[18]. Hemispheres represent hydrophobic interactions whereas polar interactions are represented by lines. HOH indicates water molecules. The yellow ribbon around the ABA molecule indicates low solvent accessibility.

Fig. 4. Induction of conformational changes in the gating loops surrounding the PYR1 cavity upon ABA binding. Interaction of PYL2 and the catalytic core of HAB1. (A) Open conformation of the loops β 3- β 4, S₈₅GLPA₈₉, and β 5- β 6, H₁₁₅RLT₁₁₈, (in red), which maintains an open passage for ABA entry into the cavity. (B) ABA binding induces the closed conformation of the gating loops, which fold over the hormone and bury it into the cavity. In light blue, region comprising the loop between β 7 and N-

terminal part of the $\alpha 5$ (M₁₄₇PEGNSEDDTRM₁₅₈), which is also involved in stabilizing ABA into the cavity. (C) Surface representation of the complex PYL2-ABA- Δ NHAB1. In red, the PYL2 surface generated by the closed conformation of the gating loops. In light blue, the region above indicated. (D) Detail of the loops and α -helix of PYL2 involved in the contact with the PP2C (purple).

Fig. 5. Amino acid sequence and secondary structure alignment of plant PP2Cs with the catalytic core of human PP2C (residues 1-300). Figure generated with ESPript 2.2 [39]. Colour codes indicate the amino acid residues involved in the interaction with ABA receptors and contact points with phosphate, metal, ABA and hypermorphic mutations.

Fig. 6. Structural details of the PYR/PYL/RCAR-ABA-PP2C complex. (A) Overview of contact points between PYL2 and the catalytic core of HAB1. The PP2C contacts the receptor through its active site and the flap region containing the Trp385 residue. Detail of the interactions involving this residue, the PYL2 gating loops, containing Pro92 and Arg120 residues, and the ketone group of ABA. The contacts are coordinated through a water molecule (in blue) located at the narrow channel between the loops. Mn⁺⁺ ions are marked as pink dots. (B) Detail of the interaction between the PYL2 loop containing the Ser89 residue and the phosphatase active site, emphasizing Gly246 and Glu203 residues. Hydrogen bonds are indicated by dotted lines. (C). Modeling of the Gly246Asp mutation in the HAB1 active site. This amino acid substitution leads to disruption of the hydrogen bonds shown in B and steric hindrance for the interaction with the PYL2 loop. (D) Modeling of a Trp385Ala mutation in HAB1. This mutation leads to loss of the docking point provided by the interaction of the Trp residue with the ABA molecule.

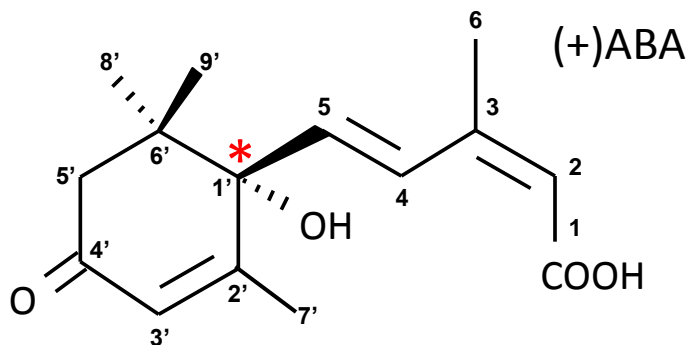
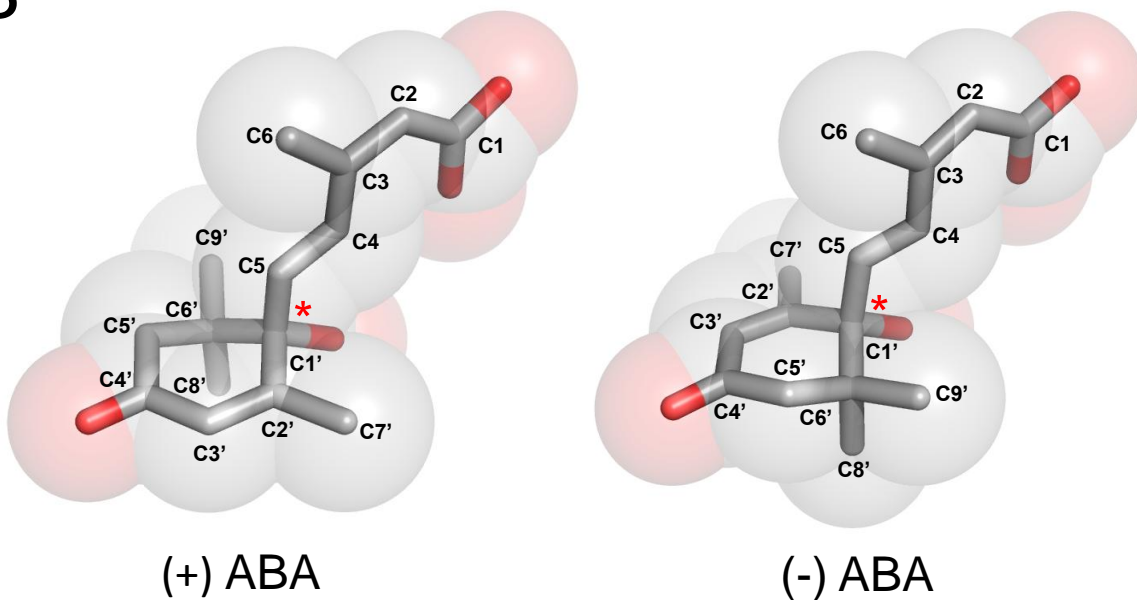
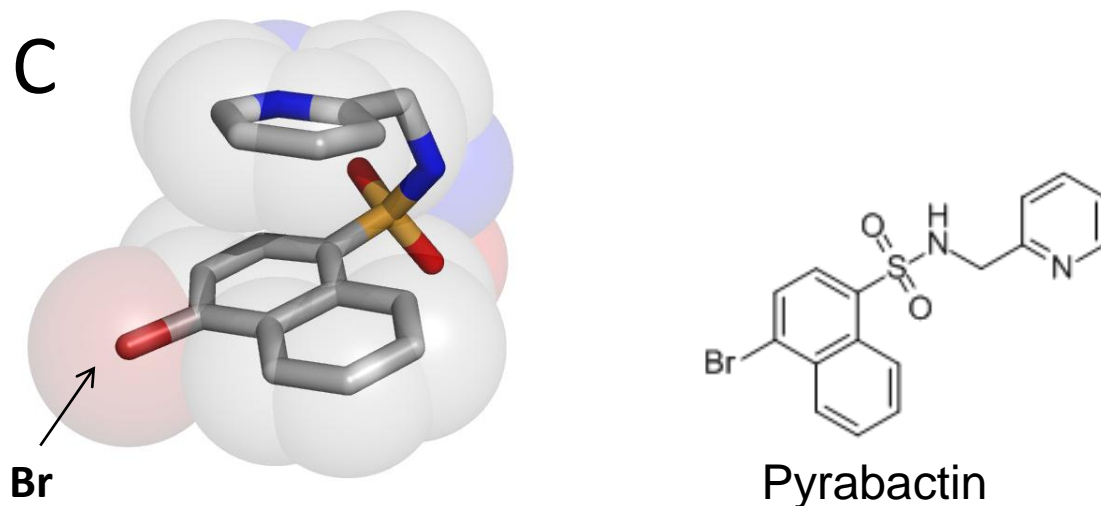
**B****C**

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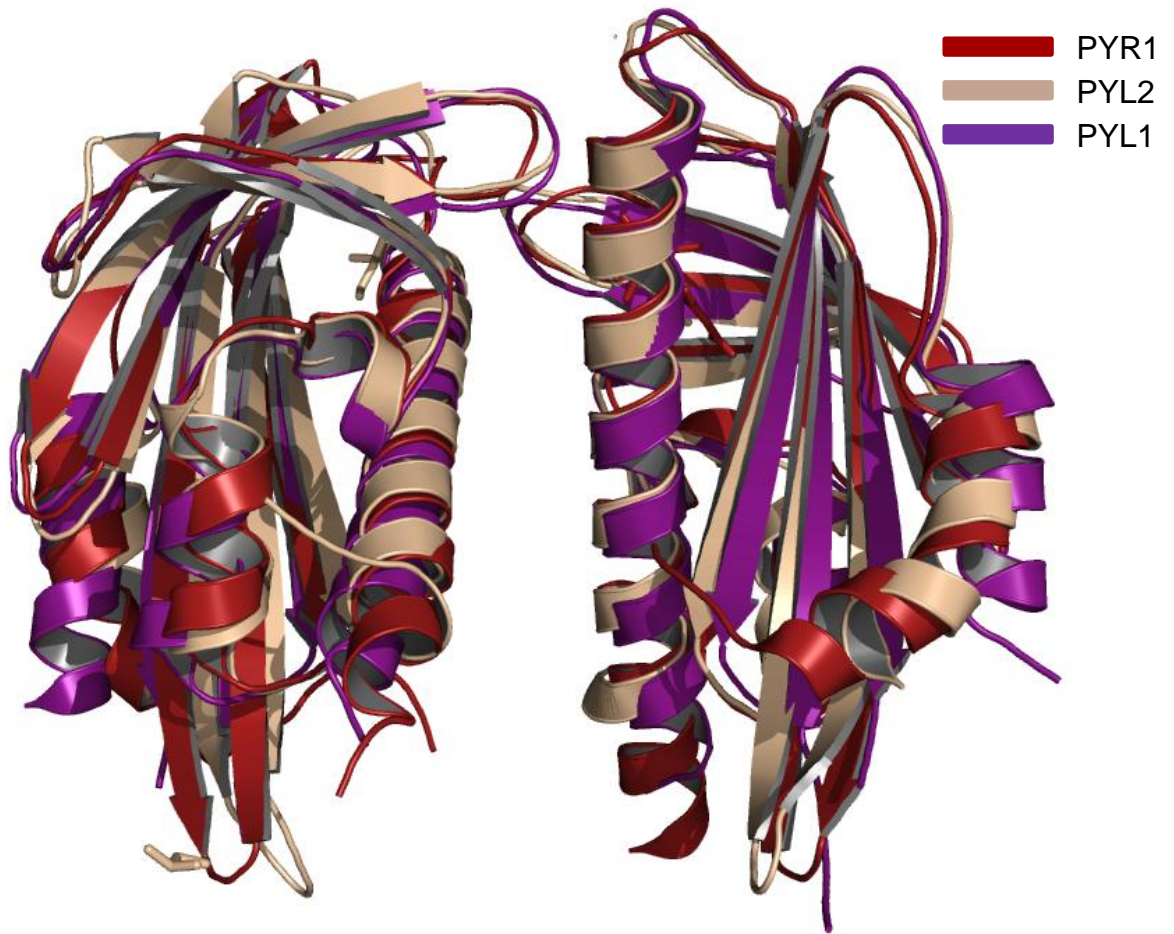


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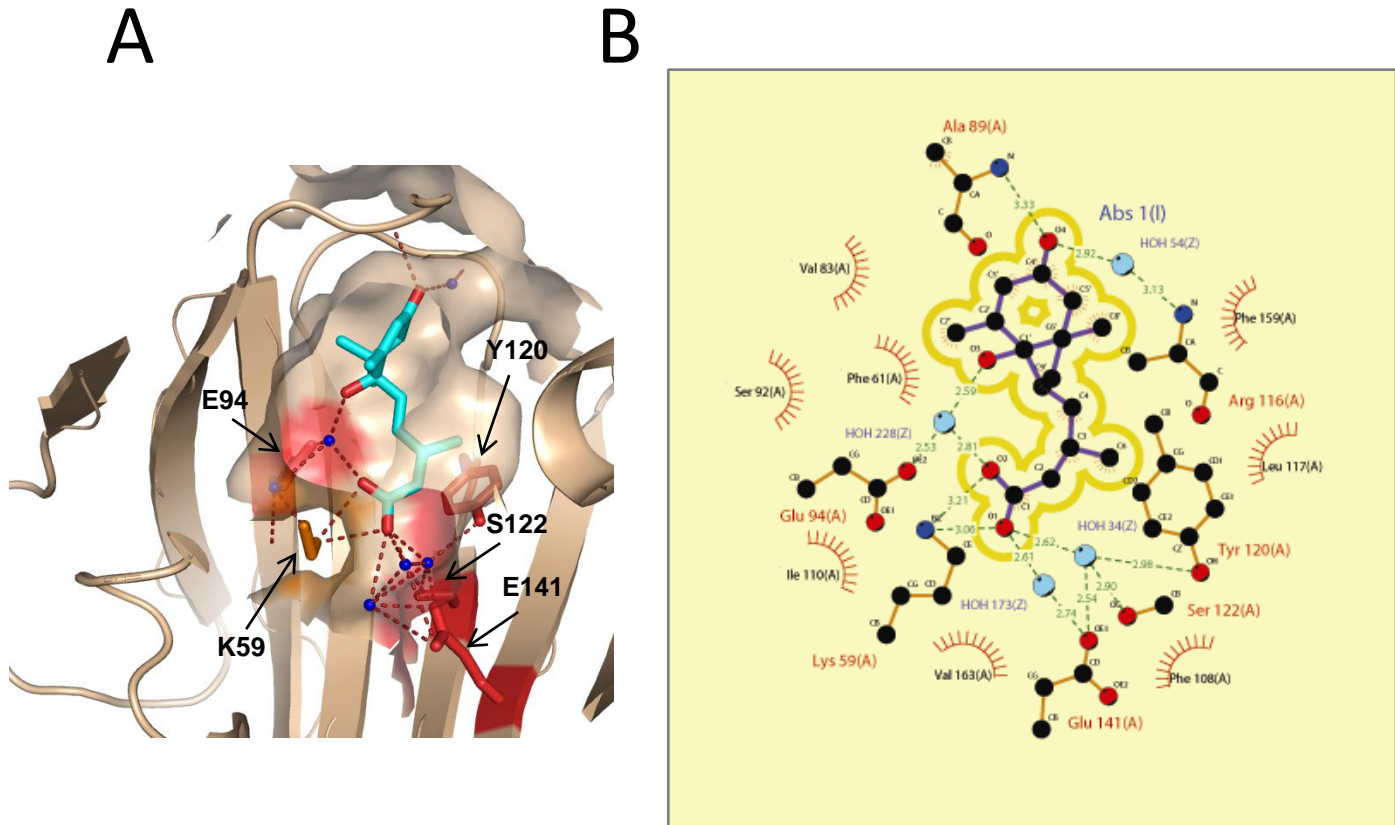
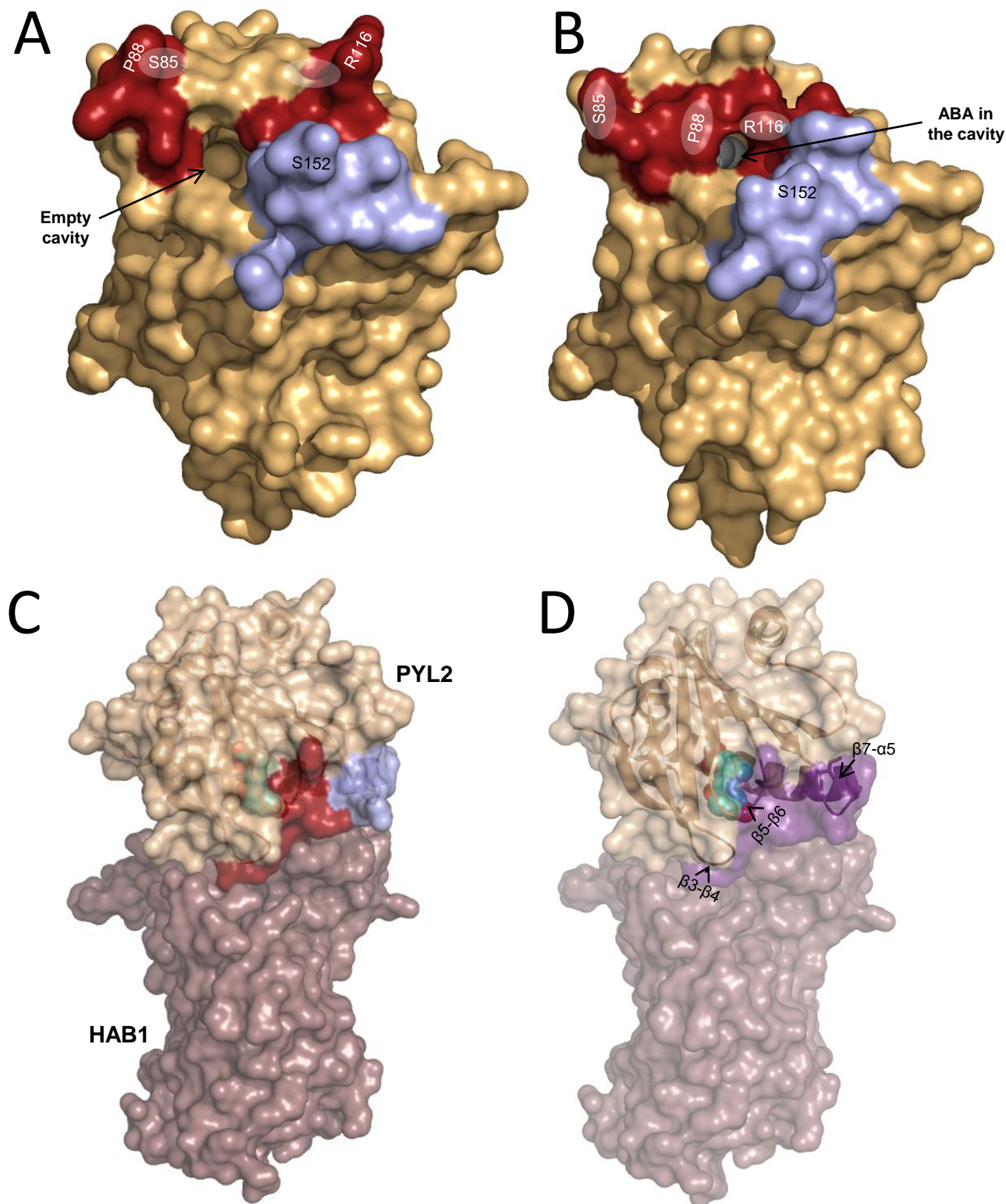


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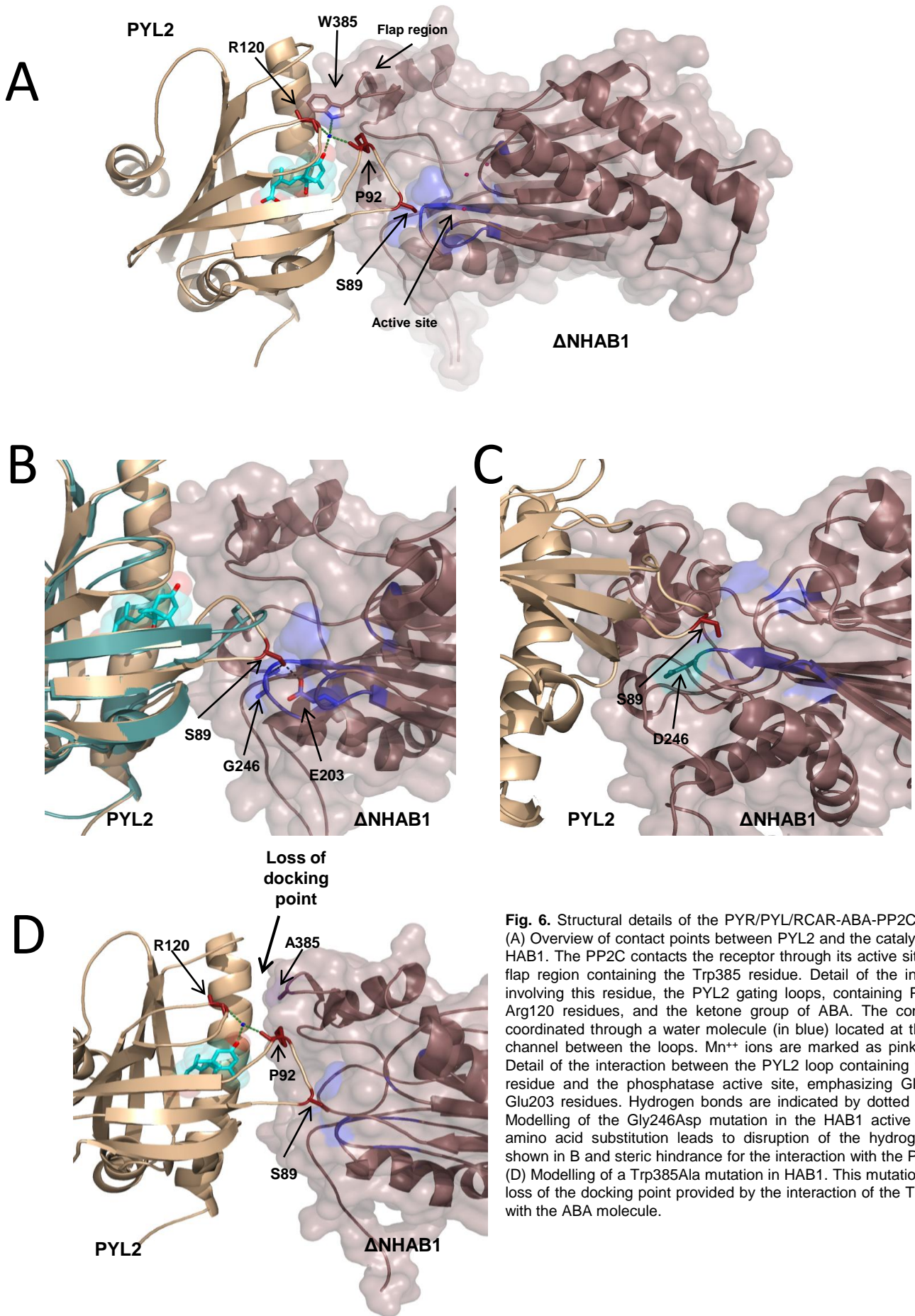


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