Molecular Determinants Elucidate the Selectivity in Abscisic acid Receptor and HAB1 Protein Interactions

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Abstract

The abscisic acid (ABA), as a pivotal plant hormone, plays a key role in controlling the life cycle and adapting to the environmental stresses. The receptors of ABA are the Pyrabactin Resistance/Pyrabactin Resistance -Like/Regulatory Component of ABA Receptors (PYR/PYL/RCAR, PYLs for simplicity), which regulate the Protein Phosphatase 2Cs (PP2Cs) in the signal pathway. As an important ABA mimicking ligand, Pyrabactin shows the activation function to parts of members of PYLs, such as PYR1 and PYL1. Due to the antagonism of Pyrabactin to PYL2, it was used as a probe to discover a part of ABA receptors. Since then, many researchers have been trying to find out the determinants of the selective regulation of PYLs and PP2Cs interaction. However, the roles of residues on the selective regulation of PYR1/PYL2 and PP2Cs interaction induced by Pyrabactin are still ambiguous. This research investigated the selective activation mechanism of Pyrabactin through the sequence alignment, molecular docking, molecular dynamics simulation, and binding free energy calculation. Furthermore, the electrostatic and hydrophobic interaction differences induced by Pyrabactin and agonists were compared. The results indicate that Leu137/Val114, Ser85/Ser89, and Gly86/Gly90 from the pocket and gate of PYR1/PYL2 are the vital residues for the selective activation of Pyrabactin. Meanwhile, the electrostatic interaction between PP2Cs and PYLs complexed with agonists was improved. This mechanism provides strong support for the design of selective agonists and antagonists.

Keywords: abscisic acid, PYR/PYL/RCARs, Pyrabactin, selectivity, molecular dynamics
Introduction

Drought stress is a severe abiotic stress, which may lower the crop yield of the world [1, 2]. The phytohormone abscisic acid (ABA), as an anti-stress regulator, has aroused widespread concern [3]. Indeed, it not only regulates many aspects of plant growth and development, but also responds to environmental stresses (e.g. drought, salinity, cold, and UV radiation) [4]. Generally speaking, ABA-deficient plants show defects in stomatal regulation, seed dormancy, and germination [5]. Due to the essentiality of ABA in the plants, the discoveries of ABA receptor protein and the core signaling complexes may transmit cues for the understanding of subsequent molecular events and plant anti-stress phenotype [6].

The bona fide ABA receptor and the regulation mechanism were discovered and understood in 2009 [7-9]. The receptors of ABA are Pyrabactin Resistance/Pyrabactin Resistance -Like/Regulatory Component of ABA Receptors (PYR/PYL/RCARs, simplified as PYLs). There are 14 members in Arabidopsis Thaliana, consisting of PYR1, PYL1-PYL13. Besides, PYLs contain a binding pocket with loop as gate and it is closed in response to ABA [10], which will create a binding surface outside the gate loop for Protein Phosphatase 2Cs (PP2Cs), including ABI1, ABI2, HAB1, HAB2, and PP2CA. Then, SNF1-related protein kinase 2s (SnRK2s) are activated to phosphorylate downstream effectors [10, 11]. As a gate-lock mechanism, it offers a key clue for the discovery of ABA mimic molecule and plays a pivotal role in revealing some specific regulation pathways.

ABA mimic molecules, which function as agonists or antagonists of PYLs and have the potential application in agriculture, may deepen the knowledge of the signaling and promote the study of ABA receptor. Hence, there is a wide interest about the discovery of ABA mimic molecules. Pyrabactin, an early synthetic ABA mimic, functions as an agonist of PYR1 and PYL1, but as an antagonist of PYL2 [9, 12]. Another selective agonist toward part of PYLs (PYR1, PYL1-PYL3, and PYL5), Quinabactin (also known
as AM1), is a promising agrochemical to elicit stomatal closure and enhance crop drought resistance [13, 14]. After structure optimization, the fluoro-substitution compound (AMF4) was synthesized. AMF4 has a long-lasting effect to promote the stomatal closure, induce the expression of stress-responsive genes, and activate the same PYLs as AM1 [15]. Because of the significance of ABA mimic molecules for the selective activation of PYLs, a lot of research have focused on the distinct selectivity of ABA mimic molecules to PYLs. It is believed that some important amino acids, such as Val67 and Val114 of PYL2, are important for the selectivity of antagonist [12, 16]. However, the dynamics roles and the energy determinants of the residues on the PYLs-PP2Cs interface remain unclear, which are crucial for understanding the selectivity and performing precise ABA mimic molecule design.

This research found that the electrostatic (ELE) interaction between PP2Cs and PYLs complexed with agonists was improved. Moreover, the complex structures of HAB1 and PYR1/PYL2 with ABA, AM1, AMF4, and Pyrabactin binding were used to reveal the selective activation determinants of PYLs. Additionally, the sequence alignment, structures comparison, molecular dynamics (MD) simulation, and binding free energy and decomposition were performed. Beyond that, the interactions between PYR1/PYL2 and HAB1 were analyzed and decomposed. Finally, it is found that the electrostatic and hydrophobic interactions between PYR1/PYL2 and HAB1 induced by agonists are conserved. At the same time, pocket residues Leu137/Val114 and gate residues Ser85/89 and Gly86/90 of PYR1/PYL2 are the key residues for selectivity of Pyrabactin. More importantly, this finding provides guidance for the design of agonist and antagonist of PYLs.
Method

Sequences Alignment.

There are already a large number of tools available to analyze sequences and structures, such as ClustalW [17], Muscle [18], T-coffee [19], and Dialign [20]. Discovery Studio is a popular commercial software to perform combined analyses. Therefore, 14 Arabidopsis Thaliana PYLs sequences downloaded from NCBI were aligned using an automatic tool in Discovery Studio 2.5 [21].

Molecular Docking and Structures Preparation.

The crystal structures of PYR1 (ABA)-HAB1 (PDB ID: 3QN1) [22], PYL1 (Pyrabactin)-ABI1 (PDB ID: 3NMN) [23], PYL2 (ABA)-HAB1 (PDB ID: 3KB3) [10], PYL2 (AM1)-HAB1 (PDB ID: 4LA7) [24], and PYL2 (AMF4)-HAB1 (PDB ID: 5VSR) [25] downloaded from PDB database were superimposed and analyzed by Pymol software [26, 27], some of which were used for molecular docking and MD. AutoDock 4.2 was applied to molecular docking [28]. In this study, AM1, AMF4, and Pyrabactin were docked into the crystal structure of PYR1-HAB1 (PDB ID: 3QN1) to generated initial structures for MD. The Lamarckian genetic algorithm (LGA) was applied for the conformational search of the ligand [29]. The grid size was set as 40 x 40 x 40, and the grid space was set to the default value of 0.375 Å. A total of 256 runs were launched for each compound. Most of the parameters for the docking calculation were set to the default values recommended by the software.
To reveal the conformational selective mechanism of Pyrabactin to PYR1 and PYL2, the structures of PYR1 (Pyrabactin)-HAB1 from docking and crystal structure PYL2 (Pyrabactin) (PDB ID: 3NS2) were superimposed [30]. Then the Pyrabactin was extracted from two complexes and saved with the other ABA receptor to get the complexes PYR1 (Pyrabactin) and PYL2 (Pyrabactin) with the initial Pyrabactin structure from PYL2 (Pyrabactin) and PYR1 (Pyrabactin)-HAB1, respectively. Meanwhile, HAB1 was saved with the PYL2 (Pyrabactin) to build up the initial structure of PYL2 (Pyrabactin)-HAB1 for later MD.

**MD Simulation and Trajectory Analysis.**

MD simulations were performed using the AMBER 16 software package with the ff14SB force field [31]. The ligand electrostatic potentials were computed at the HF/6-31G* level in the Gaussian 03 program [32]. The RESP fitting technique in AMBER was used to determine the partial charges [33]. The force-field parameters for the ligands were generated with the general AMBER force field (gaff) by the Antechamber program [34]. Each complex was immersed in a cubic box of TIP3P water model with an 8.0 Å minimum solute-wall distance. Na⁺ or Cl⁻ ions were added to neutralize each complex system.

The complex systems were optimized before the simulation as follows. First, the movement was allowed only for hydrogen atoms. Next, the side chains were relaxed. Finally, all atoms were permitted to move freely. In each stage, energy minimization was executed by the steepest descent method for the first 1000 steps and the conjugate
gradient method for the subsequent 2000 steps. After that, the systems were set up to obtain stable MD trajectories. Complex systems were gradually heated from 10 K to 300 K in 200 ps and more than 500ps equilibrating calculation was executed at 1 atm and 300 K with applying periodic boundary conditions in the NPT ensemble to avoid edge effects. The 8ns MD simulation of each system was performed. The snapshots extracted at every ps of stable interval from the last 6ns production MD trajectory using the CPPTRAJ module of AMBER were used for structural and energetic analysis. Meanwhile, the snapshots from the last 6ns MD simulation processed were used to detect hydrogen bonds.

**Binding Energy and Decomposition Calculation.**

For each snapshot, the binding energy ($\Delta H$) of the protein (ligand)-protein complex was calculated by the Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) method as in the following equation [35].

$$
\Delta G_{\text{bind}} = \Delta E_{\text{MM}} + \Delta G_{\text{solv}} - T\Delta S \tag{1}
$$

$$
\Delta E_{\text{MM}} = \Delta E_{\text{ele}} + \Delta E_{\text{vdw}} + \Delta E_{\text{int}} \tag{2}
$$

$$
\Delta G_{\text{solv}} = \Delta G_{\text{GB}} + \Delta G_{\text{np}} \tag{3}
$$

The binding free energy ($\Delta G_{\text{bind}}$) equals to the changes in the molecular mechanics component in gas phase ($\Delta E_{\text{MM}}$), solvation free energy ($\Delta G_{\text{solv}}$) and entropic contribution ($-T\Delta S$). The molecular mechanics free energy ($\Delta E_{\text{MM}}$) is further split into electrostatic ($\Delta E_{\text{ele}}$), van der Waals ($\Delta E_{\text{vdw}}$), and bond, angle, dihedral ($\Delta E_{\text{int}}$) energies. The solvation free energy ($\Delta G_{\text{solv}}$) can be divided into electrostatic solvation free energy ($\Delta G_{\text{GB}}$) and a
nonpolar solvation free energy ($\Delta G_{np}$). The $\Delta G_{GB}$ to the solvation energy is computed with a GB module of the AMBER suite.

The decomposition analysis was also performed by mm_pbsa module of Amber. The detailed procedure of it was described by Gohlke et al [36].

**Result and discussion**

**Sequence and Structure Comparison of PP2Cs Binding Domain of PYLs.**

To compare the binding surfaces of different PYLs and PP2Cs, we collected the PYLs-PP2Cs complexed structures, including PYR1 (ABA)-HAB1 (PDB ID: 3QN1) [22], PYL1 (Pyrabactin)-ABI1 (PDB ID: 3NMN) [23], PYL2 (ABA)-HAB1 (PDB ID: 3KB3) [10], PYL2 (AM1)-HAB1 (PDB ID: 4LA7) [24], and PYL2 (AMF4)-HAB1 (PDB ID: 5VSR) [25]. Subsequently, the collected structures were superimposed and compared (Fig. 1a). Take PYR1-HAB1 for example, Ser85 of PYR1 forms hydrogen bond with Gly246 and Glu203 of HAB1. Meanwhile, Lys63, Gly86, and Asn151 of PYR1 form ELE interaction with Glu201, Arg389, and Gln384 of HAB1, respectively. In addition to hydrogen bonds, there is a T-π interaction between Phe61 of PYR1 and Tyr404 of HAB1 as well as a π-π interaction between Phe159 of PYR1 and Trp385 of HAB1. These interactions almost exist in all the complexes, except for the hydrogen bond between Lys90 of PYL1 (corresponding to Lys63 of PYR1) and Glu140 of ABI1 (Glu201 of HAB1). However, the long-range electrostatic interaction between them still exist. In a word, the interactions on the binding surface are very conservative.

For further exploring the conservativeness of *Arabidopsis Thaliana* PYLs and PP2Cs, the multiple sequence alignment of 14 *Arabidopsis Thaliana* PYLs sequences was performed (Fig. 1b). It could be found that the residues on the position of Phe61, Ser85, and Phe159 of PYR1 are highly conservative. Gly86 and Asn151 of PYR1 are replaced by Asp and Thr in PYL12 and PYL13, respectively. Nonetheless, the side chain of Gly86 does not
influence the interaction between PYLs and PP2Cs shown by the binding mode. The least
conservative site is the position of Lys63 of PYR1, which is Lys in PYR1, PYL1-PYL4,
PYL6, and PYL11-PYL13, but Ser in PYL7-PYL10. Through comparison, we found that
the binding surface of *Arabidopsis Thaliana* PYR1 and PYL2 are in high conservation.

Comparison of the Binding Models of Pyrabactin in PYR1 and PYL2.

It has been found that the interactions on the PYLR1/PYL2-PP2Cs binding su-
rface are conservative. How does the Pyrabactin induce the selectivity? The X-ray crystal
structures show that there were two absolutely different conformations for Pyrabactin in
PYR1/PYL1 and PYL2 [12]. Apparently, PYL2 binding with Pyrabactin is insensitive to
PP2Cs. But PP2C is inhibited by PYR1 (binding with Pyrabactin) effectively. In other
words, the influence of the conformation of Pyrabactin is significant. Therefore, four
complexes, constructed based on the crystal structures PYR1 (PDB ID: 3QN1) and PYL2
(PDB ID: 3NS2) as well as the initial Pyrabactin structure from PYR1 (Conf1) and PYL2
(PDB ID: 3NS2, Conf2), were used to perform MD simulations in order to compare the
binding models of Pyrabactin in atomic level.

To verify the equilibration of the systems, the atomic root-mean-square deviations
(RMSD) were calculated and the convergences of energies were analyzed [37, 38]. As
displayed in the RMSD plots, all the systems have reached the equilibrium stage (Figure
S1). It seems that there was no big conformational change of PYL2 complexed with
Pyrabactin in two starting conformations (Figure S1). However, the RMSD values of the
backbone of PYR1 and the heavy atoms of Pyrabactin with the initial structure in the
PYL2 were much higher than those of the other three systems, indicating that the
conformations of PYR1 and Pyrabactin were changed in this system (Figure S1b). In
terms of the energy, the standard deviation (STD) of binding free energy of these systems
ranged from 1.01 to 1.61 kcal•mol⁻¹, suggesting that the systems had already reached
equilibrations (Table S1). Hence, these trajectories may be used for further analysis.
For further revealing the impact of the initial structure of Pyrabactin on PYR1 and PYL2, the binding free energies and binding modes of these systems were compared. To be specific, the binding free energy of Pyrabactin in Conf1 (-20.08 kcal•mol\(^{-1}\)) was lower than that of Pyrabactin in Conf2 (-18.12 kcal•mol\(^{-1}\)) with PYR1. Meanwhile, the binding affinity of Pyrabactin in Conf2 (-13.33 kcal•mol\(^{-1}\)) is higher than the other one (-6.82 kcal•mol\(^{-1}\), Table 1) with PYL2. Therefore, the Conf1 and Conf2 were favored conformations in the pockets of PYR1 and PYL2, respectively. Beyond that, the binding free energies of Pyrabactin and PYR1 were lower than these of Pyrabactin and PYL2 (Fig. 2). Besides, the closed gate improved the $\Delta E_{\text{vdw}}$ and reduced the influence of $\Delta G_{\text{solv}}$. For Conf1, it did not undergo a big conformation change in PYL2 after the MD. There was a direct hydrogen bond between the sulfonamide of Pyrabactin and Glu94/98 of PYR1/PYL2. Furthermore, the naphthalene and pyridine of Pyrabactin formed the T-\pi and $\pi$-\pi interaction with His115/119 and Tyr120/124, respectively (Fig. 2a). The only difference was that the pyridine of Pyrabactin moved near to the Val114 of PYL2 because of the short chain of Val, which induced the naphthalene to move always from the gate. As indicated by the result, it was hard for Pyrabactin in Conf1 to induce the closure of the gate of PYL2. For the Pyrabactin in Conf2, there was a deflection in PYR1. The longer side chain of Ile110 of PYR1 conflicted with the pyridine of Pyrabactin, which was pushed near to the Asn167 and Tyr120 (Fig. 2b). This deflection made the protein outward and improved the penalty of $\Delta G_{\text{solv}}$. The hydrogen bond with Lys59/64 of PYR1/PYL2 was remained, while the hydrogen bond with Glu98 of PYL2 was replaced by Asn167 of PYR1. These hydrogen bonds improved the $\Delta E_{\text{ele}}$ of this conformation both in PYR1 and PYL2. Meanwhile, the van der Waals (VDW) interaction between the naphthalene and pyridine of Pyrabactin and Tyr120/124 and His115/119 of PYR1/PYL2 stabilized the binding modes (Fig. 1b). Apparently, the Pyrabactin in Conf2 disordered the structure of PYR1 and broke the active conformation of PYR1. At the same time,
Pyrabactin was far from the closed gate in this binding mode, indicating that it is hard to induce the active conformation of PYL2.

Therefore, the different residue Ile110/Val114 is the determinant for the conformational selectivity of Pyrabactin in PYR1 and PYL2, which is consistent with the previous experimental data. The V114I mutant of PYL2 is able to inhibit the phosphatase activity of ABI1 in the presence of Pyrabactin just like PYR1 [12, 16]. This single residue alteration influences the Pyrabactin conformation in the pockets of PYLs, directly determining the state of gate and the function of PYLs.

**Selective Activation Mechanism of PYR1 and PYL2.**

In order to obtain dynamics conformation, we applied MD simulation on eight systems, including HAB1 complexed with PYR1 and PYL2 binding with ABA, AM1, AMF4, and Pyrabactin, respectively. Moreover, RMSD value per picosecond and binding energy per nanosecond in the last 6ns were calculated to explore the dynamic stability of eight systems. In this process, the RMSD values of the backbone of PYR1/PYL2-HAB1 and the heavy atoms of ligands were lower than 2.5 Å and 0.5 Å (Figure S2). With regard to the binding energy, all the STDs were lower than 2.21 kcal•mol\(^{-1}\) (Table S2). These results revealed that all the systems reached the equilibration. Additionally, the linear relationship between the calculated and experimental binding energy was fitted to further validate the result (Fig. 3). The calculated data (\(\Delta G_{\text{cal}}, \text{ -43.58} \sim \text{-24.43 kcal•mol}^{-1}\)) is consistent with the experimental data (\(\Delta G_{\text{exp}}, \text{ -10.40} \sim \text{-6.86 kcal•mol}^{-1}\)) with the high correlation coefficient \(R^2\) (0.92), suggesting the trajectories from molecular dynamics were reliable.

The binding modes were further analyzed to study the interactions on the binding surface of HAB1 and PYR1/PYL2. As for agonists, the conservative ELE interactions were kept during MD, such as the hydrogen bond of Lys63/68-Glu201, Ser85/89-Gly246, Ser85/89-Glu203, and Asn151/157-Gln384 between PYR1/PYL2 and HAB1. Meanwhile, Gly86/90 of PYR1/PYL2 contacted with Arg389 of HAB1 through hydrogen bond or
Furthermore, the T-π interaction between Phe61/66 of PYR1/PYL2 and Tyr404 of HAB1 as well as the π-π interaction between Phe159/165 of PYR1/PYL2 and Trp385 of HAB1 promoted the complexes formation (Figs. 4a-g). Due to the conservative interactions on the binding surface, there is no absolute difference between ELE (-279.65 ~ -252.25 kcal•mol⁻¹) and VDW (-92.95 ~ -86.34 kcal•mol⁻¹) contribution in the systems binding with agonists (Table 2). However, the ELE contribution on the binding surface of PYL2 (Pyrabactin) and HAB1 dropped to -197.74 kcal•mol⁻¹, which induced the low binding affinity of them directly (Table 2). Based on the binding mode and the hydrogen bond monitoring result, this research found that the conserved hydrogen bonds were broken, such as the hydrogen bond of Lys68-Glu201, Gly86-Arg389, Ser89-Glu203, and Asn151-Gln384 between PYL2 and HAB1. Nonetheless, the Ser89 of PYL2 contacted with Gly246 of HAB1 through ‘water bridge’, in which a new hydrogen bond was formed between Lys176 of PYL2 and Glu323. On the other hand, it seems that the hydrophobic interactions between PYL2 (Pyrabactin) and HAB1 is no better than those of PYR1 (Pyrabactin)-HAB1 (Fig. 4h).

To reveal protein-protein interactions influenced by Pyrabactin from the energy aspect, the energy decompositions of amino acid residues on the binding surface of HAB1 and PYR1/PYL2 complexed with Pyrabactin were performed. In comparison to PYR1, the ELE contributions of Lys68, Ser89, Gly90 in PYL2 and Glu201, Glu203, and Arg389 in HAB1, induced by the loss of hydrogen bonds or water bridges, were reduced by 15.04 kcal•mol⁻¹, 16.61 kcal•mol⁻¹, 10.18 kcal•mol⁻¹, 13.81 kcal•mol⁻¹, 6.18 kcal•mol⁻¹, and 21.17 kcal•mol⁻¹, respectively (Fig. 5a). On the other hand, the ELE contributions of Lys176 and Glu323 of PYL2 and HAB1 were improved from -0.82 kcal•mol⁻¹ and -0.56 kcal•mol⁻¹ to -79.77 kcal•mol⁻¹ and -30.31 kcal•mol⁻¹, because of the new hydrogen bond formed. The influence of VDW was smaller than that of ELE. Only the contribution of Trp385 in HAB1, which formed π-π interaction with Phe165 of PYL2, was reduced by -2.09 kcal•mol⁻¹. The decomposition analysis of the binding energy was in accordance
with the protein-protein interaction analysis results. These differences derived from the conformation of the gate determined by the Pyrabactin. In short, the closed gate formed a better binding surface of HAB1 and formed more interactions with the downstream proteins (Figs. 4g, h).

Conclusion

In this study, we studied the selective activation mechanism of PYLs through the sequence alignment, molecular dynamics simulation, and binding free energy calculation methods. Even though the residues on the binding surface of PYR1 and PYL2 are conserved, the gate conformations of PYR1 and PYL2 induced by Pyrabactin are different. The Val114 in the pocket of PYL2 leads to a rotated binding model of Pyrabactin, which leads to an opened gate. This reduces the binding free energy of PYL2 and HAB1. The energy contribution changes of Lys63/68, Ser85/89, and Gly86/90 on the binding surface elucidate the selectivity of PYR1 and PYL2 complexed with Pyrabactin to HAB1. Therefore, the gate conformation influences the functions of PYLs directly. The results elucidate molecular determinants of the selectivity of PYLs and HAB1 interactions, which may provide new ideas for further agrochemical design and drought tolerance research.
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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Not applicable.

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Contributions
Guang-Fu Yang and Ge-Fei Hao conceived of the research plan; Jing-Fang Yang, Chun-Yan Yin, and Di Wang performed the project; Guang-Fu Yang, Ge-Fei Hao, Jing-Fang Yang, and Chen-Yang Jia wrote the manuscript.

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Abbreviations
ABA: abscisic acid
PYR/PYL/RCARs: The Pyrabactin Resistance/Pyrabactin Resistance -Like/Regulatory Component of ABA Receptors
PP2Cs: Protein Phosphatase 2Cs
SnRK2s: SNF1-related protein kinase 2s
ELE: electrostatic
MD: molecular dynamics
MM/GBSA: the Molecular Mechanics/Generalized Born Surface Area
RMSD: the atomic root-mean-square deviations
STD: the standard deviation
VDW: van der Waals.

Supplementary information
Table S1 The binding free energy (kcal/mol) of Pyrabactin in Conf1 and Conf2 and PYLs (PYR1 and PYL2) for per nanosecond in the last 6 nanoseconds.
Table S2 The calculated binding free energy (kcal/mol) of HAB1 and PYLs (PYR1 and PYL2) complexed with ligands per nanosecond in the last 6 nanoseconds.

Table S3 The hydrogen analysis of the binding surface of HAB1 and PYLs (PYR1 and PYL2) complexed with different ligands in the last 3 nanoseconds.

Figure S1 The RMSD of the CA atoms of receptors and heavy atoms of Pyrabactin with respect to the starting structure. The RMSD of heavy atoms of Pyrabactin in the PYR1 and PYL2 respect to the starting conformation of it in the PYR1 (Conf1, a) and PYL2 (Conf2, b) complexes are shown in black and red. The backbone RMSD of PYR1 (PDB ID: 3QN1) and PYL2 (PDB ID: 3NS2) are shown in green and blue.

Figure S2 The RMSD of the CA atoms of receptors and heavy atoms of ligands with respect to the starting structure. The RMSD of heavy atoms of ligands in the PYR1 and PYL2 respect to the starting conformation of them in the complexed structures are shown in black and red. The backbone RMSD of PYR1 and PYL2 complexed with HAB1 are shown in green and blue.

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Figure and Table Legend

Fig. 1 The interactions on the binding surface of PYLs and HAB1 (a) and the multiple sequence alignment of 14 Arabidopsis Thaliana PYLs (b). The crystal structures of PYR1 (ABA)-HAB1 (PDB ID: 3QN1), PYL1 (Pyrabactin)-ABI1 (PDB ID: 3NMN), PYL2 (ABA)-HAB1 (PDB ID: 3KB3), PYL2 (AM1)-HAB1 (PDB ID: 4LA7), and PYL2 (AMF4)-HAB1 (PDB ID: 5VSR) were superimposed (a). The PYLs and PP2Cs are colored in yellow and blue, respectively. The residue numbers of PYLs and PP2Cs are labeled according to them in PYR1 and HAB1. The important residues of PYLs on the binding surface are labeled with red stars (b).

Fig. 2 The comparison of the binding modes of Pyrabactin in PYR1 and PYL2 after MD. The starting structures of Pyrabactin in the PYR1 (Conf1) and PYL2 (Conf2) complexes were used. The PYR1 and PYL2 are colored in blue and cyan, and the corresponding Pyrabactin are colored in yellow and magentas.

Fig. 3 The correlation of calculated ($\Delta G_{cal}$) and experimental ($\Delta G_{exp}$) binding free energy.

Fig. 4 The interactions on the binding surface of PYLs and HAB1 after MD. The important residues of PYR1 (a, c, e, and g) and PYL2 (b, d, f, and h) are colored in magentas. The residues of HAB1 are colored in cyan. The ligands ABA (a, b), AM1 (c, d), AMF4 (e, f), and Pyrabactin (g, h) are colored in blue.

Fig. 5 The comparison of the interactions of HAB1 and PYR1/PYL2 complexed with Pyrabactin. The electronic (ELE, a) and van der Waals (VDW, b) contributions of the important residues on the binding surface of PYR1 and PYL2 are shown in red and blue.

Table 1 The binding free energy (kcal/mol) of Pyrabactin in Conf1 and Conf2 and PYLs (PYR1 and PYL2).

Table 2 The binding free energy (kcal/mol) of HAB1 and PYLs (PYR1 and PYL2) complexed with ligands from calculation and experiment.
Table 1 The binding free energy (kcal/mol) of Pyrabactin in Conf1 and Conf2 and PYLs (PYR1 and PYL2).

|               | $\Delta E_{\text{ele}}$ | $\Delta E_{\text{vdw}}$ | $\Delta E_{\text{MM}}$ | $\Delta G_{\text{solv}}$ | $\Delta G_{\text{cal}}$ |
|---------------|-------------------------|--------------------------|-------------------------|--------------------------|--------------------------|
| PYR1-Pyrbactin (Conf1) | -16.18                  | -39.75                   | -55.93                  | 35.86                    | -20.08                   |
| PYR1-Pyrbactin (Conf2) | -20.12                  | -39.51                   | -59.63                  | 41.51                    | -18.12                   |
| PYL2-Pyrbactin (Conf1) | -15.00                  | -36.12                   | -51.12                  | 44.30                    | -6.82                    |
| PYL2-Pyrbactin (Conf2) | -23.57                  | -37.09                   | -60.66                  | 47.34                    | -13.33                   |

Table 2 The binding free energy (kcal/mol) of HAB1 and PYLs (PYR1 and PYL2) complexed with ligands from calculation and experiment.

|               | $\Delta E_{\text{ele}}$ | $\Delta E_{\text{vdw}}$ | $\Delta E_{\text{MM}}$ | $\Delta G_{\text{solv}}$ | $\Delta G_{\text{cal}}$ | $IC_{50}$ (nM) | $\Delta G_{\text{exp}}$ |
|---------------|-------------------------|--------------------------|-------------------------|--------------------------|--------------------------|----------------|--------------------------|
| PYR1-ABA      | -276.76                 | -92.95                   | -369.71                 | 330.44                   | -39.27                   | 307.0         | -8.9                     |
| PYL2-ABA      | -266.80                 | -91.13                   | -357.94                 | 316.92                   | -41.02                   | 151.0         | -9.4                     |
| PYR1-AM1      | -279.65                 | -91.12                   | -370.78                 | 328.87                   | -41.90                   | 103.0         | -9.6                     |
| PYL2-AM1      | -252.25                 | -89.87                   | -342.12                 | 301.48                   | -40.64                   | 267.0         | -9.0                     |
| PYR1-AMF4     | -252.33                 | -90.69                   | -343.02                 | 300.04                   | -42.98                   | 119.1         | -10.2                    |
| PYL2-AMF4     | -267.41                 | -86.34                   | -353.75                 | 310.17                   | -43.58                   | 85.7          | -10.4                    |
| PYR1-Pyrbactin| -257.12                 | -89.83                   | -346.95                 | 310.16                   | -36.79                   | 656.0         | -8.5                     |
| PYL2-Pyrbactin| -197.74                 | -79.48                   | -277.22                 | 252.80                   | -24.43                   | $>10000$      | -6.9                     |
Fig. 1
Fig. 2
\[ y = 5.3463x + 9.8689 \]

\[ R^2 = 0.92 \]
Fig. 4
Fig. 5