

The RNA Hydrolysis and the Cytokinin Binding Activities of PR-10 Proteins Are Differently Performed by Two Isoforms of the Pru p 1 Peach Major Allergen and Are Possibly Functionally Related^[W]

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PR-10 proteins are a family of pathogenesis-related (PR) allergenic proteins playing multifunctional roles. The peach (*Prunus persica*) major allergen, Pru p 1.01, and its isoform, Pru p 1.06D, were found highly expressed in the fruit skin at the pit hardening stage, when fruits transiently lose their susceptibility to the fungal pathogen *Monilinia* spp. To investigate the possible role of the two Pru p 1 isoforms in plant defense, the recombinant proteins were expressed in *Escherichia coli* and purified. Light scattering experiments and circular dichroism spectroscopy showed that both proteins are monomers in solution with secondary structures typical of PR-10 proteins. Even though the proteins do not display direct antimicrobial activity, they both act as RNases, a function possibly related to defense. The RNase activity is different for the two proteins, and only that of Pru p 1.01 is affected in the presence of the cytokinin zeatin, suggesting a physiological correlation between Pru p 1.01 ligand binding and enzymatic activity. The binding of zeatin to Pru p 1.01 was evaluated using isothermal titration calorimetry, which provided information on the stoichiometry and on the thermodynamic parameters of the interaction. The structural architecture of Pru p 1.01 and Pru p 1.06D was obtained by homology modeling, and the differences in the binding pockets, possibly accounting for the observed difference in binding activity, were evaluated.

In plant cells, pathogenesis-related (PR) proteins function in a wide range of processes related to signal transduction and antimicrobial activity. These proteins were originally considered as induced by different biotic and abiotic stresses but were subsequently found constitutively expressed in different plant organs during growth (van Loon et al., 2006). PR proteins are classified in 17 families on the basis of structural and functional features. For many PR protein families, the molecular mechanisms that underlie their activity in defense processes have not been clarified so far.

The PR-10 protein family is a large group of PR proteins, containing >100 members. They were first identified in parsley (*Petroselinum crispum*; Somssich et al., 1988) and later isolated from several plant species and tissues (Matton and Brisson, 1989; Crowell et al., 1992; Warner et al., 1994; Breda et al., 1996; Midoh and Iwata, 1996; Huang et al., 1997; Lo et al., 1999; Yu et al., 2000; Wu et al., 2003). PR-10 proteins are

typically small (from 155–163 residues) cytoplasmic proteins with acidic pI (Liu and Ekramoddoullah, 2006). Despite the low similarity in their primary structure, they feature a conserved three-dimensional fold, consisting of a seven-stranded β -sheet wrapped around a C-terminal α -helix and two additional small α -helices. This is revealed by the structures of a number of PR-10 proteins obtained by NMR and x-ray crystallography, such as Bet v 1 from birch (*Betula* spp.; Gajhede et al., 1996; Markovic-Housley et al., 2003), four isoforms of LIPR10 from yellow lupin (*Lupinus luteus*; Biesiadka et al., 2002; Pasternak et al., 2005; Fernandes et al., 2008), VrCSBP (cytokinin-specific binding protein) from mung bean (*Vigna radiata*; Pasternak et al., 2006), Pru av 1 from cherry (*Prunus avium*; Neudecker et al., 2001), and Ap g 1 from celery (*Apium graveolens*; Schirmer et al., 2005).

Two aspects make the biological characterization of PR-10 proteins a potentially important goal both for human health and agriculture: on one hand, some PR-10 proteins are important allergen sources from tree pollen, fruits, and vegetables. Examples are Bet v 1 from birch (Swoboda et al., 1996), Mal d 1 from apple (*Malus domestica*; Vanek-Krebitz et al., 1995), Pru ar 1 from apricot (*Prunus armeniaca*), Pru av 1 from cherry (Neudecker et al., 2001), and Dau c 1 from carrot (*Daucus carota*; Yamamoto et al., 1997). On the

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other hand, several PR-10 proteins are up-regulated upon pathogen infection (Walter et al., 1990; Breda et al., 1996; Pühlinger et al., 2000; Park et al., 2004; Liu et al., 2005), have a direct and selective antifungal activity, or accumulate in overwintering organs of tree species, suggesting a key role in selective defense mechanisms against microbes and fungi and in protecting plants from abiotic stresses (Yu et al., 2000; Flores et al., 2002; Chadha and Das, 2006). In addition, a number of PR-10 proteins are constitutively expressed at different plant developmental stages and/or in different tissues and organs, such as in seeds, roots (Sikorski et al., 1999), flowers, and senescent leaves (Breiteneder et al., 1989; Crowell et al., 1992; Barrat and Clark, 1993; Vanek-Krebitz et al., 1995; Sikorski et al., 1999), suggesting a role for PR-10 proteins also in developmental regulation.

The molecular mechanisms through which PR-10 proteins regulate these important plant processes are not yet understood. Several PR-10 protein members were reported to hydrolyze RNA (Bufe et al., 1996; Swoboda et al., 1996; Bantignies et al., 2000; Biesiadka et al., 2002; Koistinen et al., 2002; Zhou et al., 2002; Park et al., 2004; Liu et al., 2006; Yan et al., 2008). This activity can be crucial during plant defense for controlling the burst of transcription that occurs upon stress sensing. In addition, RNase activity could be important for the apoptotic processes activated in pathogen-infected cells in order to limit the pathogen invasion. Finally, this activity could be directly involved in the degradation of pathogenic virus RNA. A notable example is the case of CaPR-10 from hot pepper (*Capsicum annuum*), in which the RNase activity was shown to be directly associated to its antiviral function (Park et al., 2004). Additional roles of PR-10 proteins could be related to their capability to bind hydrophobic ligands, such as fatty acids, flavonoids, and steroids (Neudecker et al., 2001; Mogensen et al., 2002; Koistinen et al., 2005). PR-10 proteins are also capable of binding plant hormones, such as cytokinins (CKs; Fujimoto et al., 1998; Pasternak et al., 2006; Fernandes et al., 2008). CKs are signal elements that regulate plant growth and development, but which are also recently emerging as important components of the plant defense strategy repertoire (Chung et al., 2008). Indeed, their concentration is highly affected by pathogen invasion and, in turn, their high concentration induces apoptosis (Carimi et al., 2003) and expression of PR proteins, such as PR-1 (Memelink et al., 1987). Furthermore, Arabidopsis (*Arabidopsis thaliana*) mutants constitutively expressing ABR-17, a pea (*Pisum sativum*) RNase belonging to the PR-10 protein family involved in salt stress tolerance (Srivastava et al., 2007), contained up to 3-fold higher concentration of endogenous CKs and showed up-regulation of several CK-responsive genes, as compared to the wild-type plant (Krishnaswamy et al., 2008). These observations thus suggested a functional link between the RNase function and the CK binding activity of PR-10 proteins (Krishnaswamy et al., 2008). CKs are adenine

derivatives, which are normally synthesized from 5'-AMP. However, certain types of plant tRNAs contain a CK moiety adjacent to their 3'-end, which enhances their translational efficiency (Taller, 1994). Degradation of these tRNAs is believed to significantly contribute to the plant endogenous CK content (Chen and Ertl, 1994; Prinsen et al., 1997). On the basis of these data, it was suggested that PR-10 proteins, such as ABR-17, modulate the level of endogenous CKs, through yet unknown mechanisms that might possibly involve RNA degradation and lead to stress defense (Krishnaswamy et al., 2008).

These recent findings prompted us to further investigate the possible functional correlation between the RNase activity and CK binding of PR-10 proteins at the molecular level. In particular, we focused on a PR-10 protein from peach (*Prunus persica*), Pru p 1, identified as a fruit allergen homolog to Bet v 1 (Wisniewski et al., 2004). The possible role of Pru p 1 in fruit defense against pathogens as well as in fruit development was investigated by monitoring the transcript level of the genes encoding two isoforms of Pru p 1 (Pru p 1.01 and Pru p 1.06D) at different stages of the fruit ripening. The two proteins were expressed in *Escherichia coli* and purified, and their structural properties were analyzed. Their RNase activity was established, the thermodynamics of zeatin binding were elucidated, and the influence of this CK on the enzymatic activity was investigated. The structural models of Pru p 1.01 and Pru p 1.06D were calculated, and the model of a zeatin-bound Pru p 1.01 was analyzed. Based on the results, possible roles of these proteins in plant metabolism are proposed and discussed.

RESULTS

Expression of *pru p 1.01* and *pru p 1.06D* in Peach Fruit during Growth

The expression level of two isoforms of PR-10 proteins from peach, Pru p 1.01 and Pru p 1.06D, during fruit development was quantified in peach fruit skin (the tissue where typically allergens accumulate) by measuring the relative abundance of mRNA of *pru p 1.01* and *pru p 1.06D* in the four growth stages of the fruit: S1 (the early stage), S2 (the phase when fruit temporary stops growing and pit hardening occurs), S3 (the early preclimatic phase), and S4 (the fruit ripening stage; Fig. 1). During the initial S1 phase, the amount of the two mRNAs was similar. Subsequently, it increased, reaching the highest level during the S2 phase. The genes *pru p 1.01* and *pru p 1.06D* showed the same pattern of expression, with differences in the mRNAs accumulation levels. In particular, the transcript of *pru p 1.01* and *pru p 1.06D* increased by 223% and 144%, respectively, from the S1 to the S2 phase. Subsequently, during the S3 stage, mRNA decreased to 86.3% and 51.4% of the quantity observed in S2 and slightly increased again during the S4 stage to a value similar to the amount observed in S1.

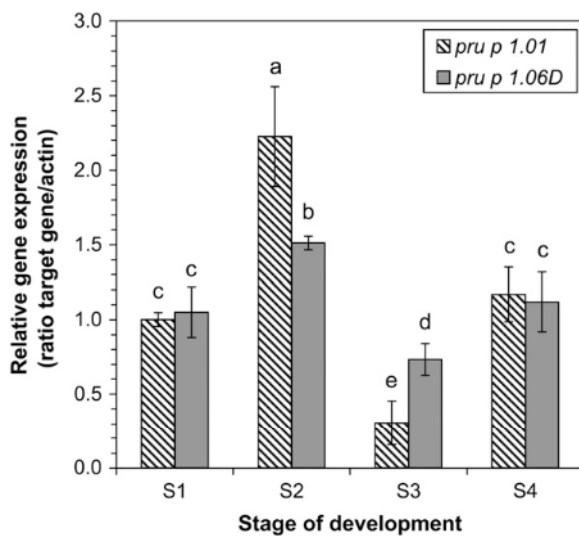


Figure 1. Expression levels of the genes encoding Pru p 1.01 and Pru p 1.06D during the four S1 to S4 growth phases of fruit development. Three independent replicates of first-strand cDNA were synthesized from three separated RNA preparations. Each independent sample was run in duplicate. The levels of *pru p 1.01* and *pru p 1.06D* cDNA were normalized to the one of actin. The relative cDNA levels are the means of the 12 values (six values $\times$ two independent experiments) obtained. Letters represent the nonsignificant ranges according to the LSD test ($P \leq 0.05$), whereas the line bars show the SE.

Purification and Structural Analysis of Pru p 1.01 and Pru p 1.06D

The His-tagged forms of Pru p 1.01 and Pru p 1.06D proteins (theoretical pI 6.22 and 5.68, respectively) were expressed in the soluble fraction of *E. coli* and purified using nickel affinity chromatography, yielding 20 and 15 mg of protein per liter of bacterial culture respectively. The purity of the isolated proteins was confirmed by SDS-PAGE, which showed a unique band at about 20 kD, in agreement with the calculated molecular masses (19,491.8 D for Pru p 1.01 and 19,151.4 D for Pru p 1.06D; Fig. 2A). No formation of inclusion bodies was observed after 3 h of protein expression at 37°C. The molecular mass and hydrodynamic radius of recombinant Pru p 1.01 and Pru p 1.06D in solution were determined using online size exclusion chromatography, multiple-angle light scattering, and quasielastic (dynamic) light scattering (Fig. 2B). The elution profile and the light scattering data show that both proteins are monomers in solution (calculated molar masses M_w [Pru p 1.01] = 21.9 ± 0.1 kD, M_w [Pru p 1.06D] = 22.8 ± 0.2 kD; hydrodynamic radii R_h [Pru p 1.01] = 1.8 ± 0.1 nm, R_h [Pru p 1.06D] = 1.5 ± 0.1 nm). The circular dichroism (CD) spectra of the two proteins showed the presence of a significant amount of both α -helices and β -strands, with a negative deflection centered on 216 nm and a positive peak at 195 nm (Fig. 2C). The spectra were quantitatively analyzed using the Dichroweb server. The results of fits averaging (normalized root mean square deviation

[RMSD] in the range 0.012–0.025) provided a secondary structure composition of 36% α -helix, 26% β -strand, 15% turns, and 23% random coil for Pru p 1.01 and of 29% α -helix, 28% β -strand, 16% turns, and 27% random coil for Pru p 1.06D. These data indicate that the two proteins are largely similar, with the presence of a small difference in the secondary structure composition that mainly concerns the α -helical content.

RNase Activity of Pru p 1.01 and Pru p 1.06D

The ribonuclease activity of Pru p 1.01 and Pru p 1.06D was tested by incubating the proteins in the presence of total RNA of peach fruit skin at different pH. Under acidic conditions (pH 3), no hydrolysis of RNA was observed (Fig. 3A). Accordingly, Pru p 1.01 was reported to be fully denatured under these conditions (Gaier et al., 2008). At pH 5, RNA appeared partially degraded when incubated with Pru p 1.06D, while in the presence of Pru p 1.01, no RNA hydrolysis was detected. On the other hand, under basic conditions (pH 9), total RNA degradation was performed effectively by Pru p 1.01 after 4 h, whereas Pru p 1.06D was not capable of full hydrolysis after this incubation time. Considering that both Pru p 1 proteins completely hydrolyzed RNA at pH 7.5 after 2 h (Fig. 3B), RNase activity was tested at this pH also at shorter incubation times. The assay showed that Pru p 1.06D completely hydrolyses RNA after 30 min and that this isoform is a more efficient RNA hydrolase than Pru p 1.01: the latter starts to hydrolyze RNA only after 1 h of incubation and completes degradation after 2 h. The RNase activity was also evaluated in the presence of a 10-fold molar excess zeatin at pH 7.5. In these conditions, the rate of enzymatic activity of Pru p 1.01 is significantly affected because degradation starts only after 2 h of incubation and is completed after 4 h (Fig. 3B; Supplemental Figs. S1–S3). On the other hand, the presence of zeatin did not affect the RNA hydrolysis rate catalyzed by Pru p 1.06D.

No RNA hydrolysis was detected when RNA was incubated with the buffer alone or with the purified lysate of *E. coli* transformed with the pHAT vector eluted from the Ni column or with the proteins thermally inactivated by incubation at 100°C for 15 min (Fig. 3C). Shorter incubation times (e.g. 5 min at 100°C) did not lead to complete inactivation of the proteins and resulted in residual RNA hydrolysis. This observation suggests the occurrence of protein refolding, consistently with results recently published indicating that the thermal denaturation of Pru p 1.01, as monitored by CD, is a reversible process (Gaier et al., 2008).

Other PR-10 protein homologs were found to increase their RNA hydrolyzing activity with the temperature (Yan et al., 2008). Therefore, the RNase activity of Pru p 1.01 and of Pru p 1.06D was also tested at 60°C, and a substantial decrease in protein activity was observed. Furthermore, RNA hydrolysis was not affected by the presence of 10 mM EDTA,

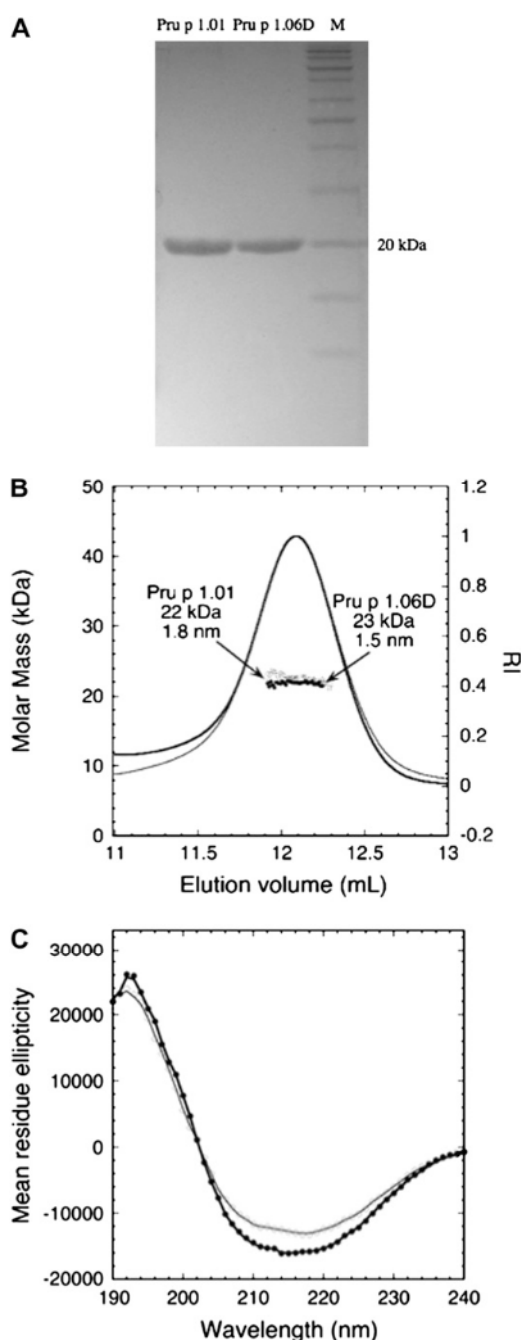


Figure 2. Pru p 1.01 and Pru p 1.06D purification and characterization of hydrodynamic and structural properties. A, SDS-PAGE of Pru p 1.01 and Pru p 1.06D purified proteins. The proteins were stained with Coomassie Brilliant Blue R-250. Bands of the protein marker lane (M) correspond to molar masses of 260, 160, 110, 80, 60, 50, 40, 30, 20, 15, and 10 kD. B, Molar mass distribution plot for Pru p 1.01 (bold line and black circles) and Pru p 1.06D (thin line and gray circles). The solid lines indicate the size exclusion elution profile monitored by the refractive index (RI) detector, and the dots are the weight-averaged molecular masses for each slice, measured every second. The average molecular mass and the hydrodynamic radius of the proteins are indicated. C, Far-UV CD spectrum of Pru p 1.01 and Pru p 1.06D. The CD data are shown as black circles (Pru p 1.01) and gray circles (Pru p

1.06D), indicating that these proteins can be classified as type II RNases (Bariola and Green, 1997).

Antifungal Activity

The antimicrobial activity of the recombinant Pru p 1.01 and Pru p 1.06D against fungal pathogens of peach fruits, such as *Monilinia laxa*, *Monilinia fructigena*, *Botrytis cinerea*, *Colletotrichum acutatum*, *Rhizopus stolonifer*, and *Penicillium expansum*, was tested on solid substrate. The proteins were placed in proximity of the edge of hyphal growth, and the latter was monitored for 12 to 24 h, depending on the fungal species (until the fungal growth reached the filter). No growth inhibition was detected for any of the tested fungi. The effect of zeatin on the antifungal activity was also assayed by placing the proteins, mixed with 10- or 20-fold molar excess of zeatin, on filter discs, and no effect on the growth of the tested fungi was observed.

Pru p 1.01 and Pru p 1.06D Interaction Properties with Zeatin followed by Isothermal Titration Calorimetry

The thermodynamics of zeatin binding to Pru p 1.01 and Pru p 1.06D were analyzed using isothermal titration calorimetry (ITC). In the case of Pru p 1.01, the occurrence of a binding event was revealed by the presence of exothermic peaks following each addition of a solution of zeatin to the protein solution (Fig. 4A). Fits of the integrated heat data (Fig. 4B) were carried out using a model involving a single binding event and yielded a stoichiometry of one equivalent of zeatin molecule bound to a Pru p 1.01 molecule with a dissociation constant $K_d = 9.4 \mu\text{M}$. This process is driven by favorable enthalpic ($\Delta H = -470 \pm 20 \text{ cal mol}^{-1}$) and entropic ($\Delta S = +21 \text{ cal mol}^{-1} \text{ K}^{-1}$) factors calculated from the fit. Analogous ITC experiments performed on Pru p 1.06D did not show any heat changes related to binding events, thus indicating that under these conditions Pru p 1.06D does not specifically interact with zeatin (Fig. 4C).

Sequence Search and Homology Modeling of Peach Pru p 1.01 and Pru p 1.06D

More than 400 PR-10 protein sequences were retrieved from a sequence database search. The analysis of the resulting sequence alignment (Supplemental Fig. S4), together with the distribution of the available structures of PR-10 proteins along the unrooted phylogenetic tree (Supplemental Fig. S5), helped us to choose the templates for the modeling of the structures of Pru p 1.01 and Pru p 1.06D proteins. In particular, the NMR structure of cherry Pru av 1 (Protein Data Bank [PDB] code 1E09) was picked, considering its proximity to the target sequences in the phylogenetic

tree. The solid lines represent the best fits calculated using the CDSSTR program available at the Dichroweb server.

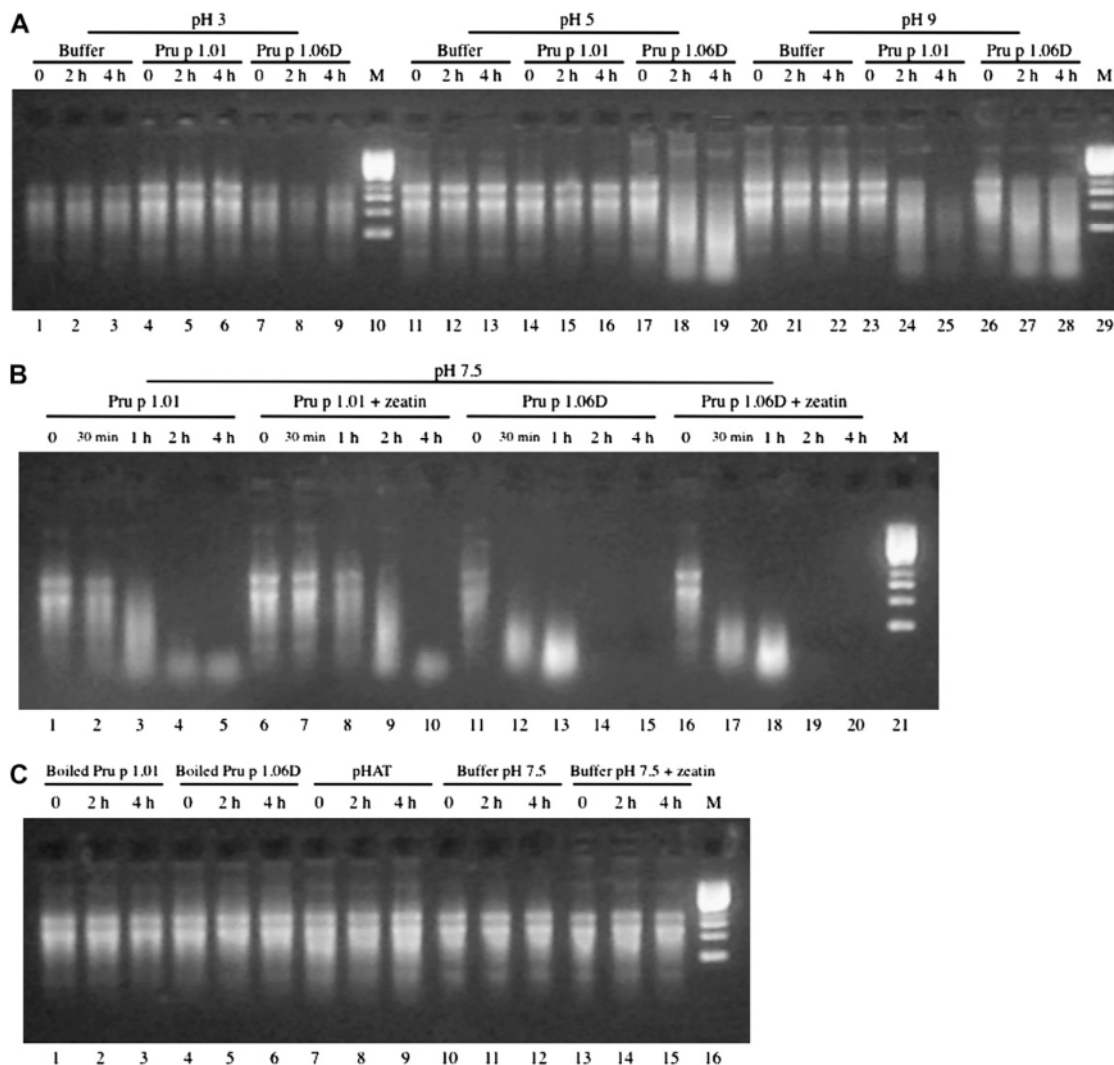


Figure 3. RNA hydrolyzing activity of recombinant Pru p 1.01 and Pru p 1.06D proteins. A, RNase activity of Pru p 1.01 and Pru p 1.06D (10 μ M) at different pH, against total peach RNA (10 μ g) at time 0 and after 2 and 4 h of incubation at 37°C. Lanes 10 and 29, DNA marker (M); lanes 1 to 3, negative control, RNA incubated with citrate buffer, pH 3, alone; lanes 4 to 6, RNA incubated with Pru p 1.01 in citrate buffer, pH 3; lanes 7 to 9, RNA incubated with Pru p 1.06D in citrate buffer, pH 3; lanes 11 to 13, negative control, RNA incubated with MES buffer, pH 5, alone; lanes 14 to 16, RNA incubated with Pru p 1.01 in MES buffer, pH 5; lanes 17 to 19, RNA incubated with Pru p 1.06D in MES buffer, pH 5; lanes 20 to 22, negative control, RNA incubated in CHES buffer, pH 9, alone; lanes 23 to 25, RNA incubated with Pru p 1.01 in CHES buffer, pH 9; lanes 26 to 28, RNA incubated with Pru p 1.06D in CHES buffer, pH 9. B, RNase activity of Pru p 1.01 and Pru p 1.06D in phosphate buffer, pH 7.5, against peach total RNA alone or in presence of 200 μ M zeatin after 30 min, 1, 2, and 4 h of incubation. Lane 21, DNA marker; lanes 1 to 5, RNA incubated with Pru p 1.01; lanes 6 to 10, RNA incubated with Pru p 1.01 in presence of zeatin; lanes 11 to 15, RNA incubated with Pru p 1.06D; lanes 16 to 20, RNA incubate with Pru p 1.06D in presence of zeatin. C, Negative controls at time 0 and after 2 and 4 h of incubation at 37°C, at pH 7.5. Lane 16, DNA marker; lanes 1 to 3, RNA incubated with boiled Pru p 1.01; lanes 4 to 6, RNA incubated with boiled Pru p 1.06D; lanes 7 to 9, RNA incubated with purified lysate of *E. coli* transformed with the pHAT vector; lanes 10 to 12, RNA incubated with buffer alone; lanes 13 to 15, RNA incubated with 200 μ M zeatin.

tree, while the yellow lupin LIPR-10.2B (2QIM) and the mung bean VrCSBP (2FLH) were chosen because of the presence, in the crystal structure, of zeatin. The alignment of peach Pru p 1.01 and Pru p 1.06D sequences with the sequences of the modeling templates, coupled with the secondary structure prediction, shows that the main structural elements of Pru p 1.01 and Pru p 1.06D are perfectly aligned with the

PR10-like fold of the template structures (Fig. 5A). The best models resulting from the structure calculation, and selected on the basis of the lowest energy score, fully complied with standard validation protocols. The backbone RMSD of peach Pru p 1.01 and Pru p 1.06D structural models with the LIPR-10.2B and VrCSBP template structures are small (1.17 and 1.18 Å for LIPR-10.2B and 1.06 and 1.01 Å for VrCSBP, respectively; Fig.

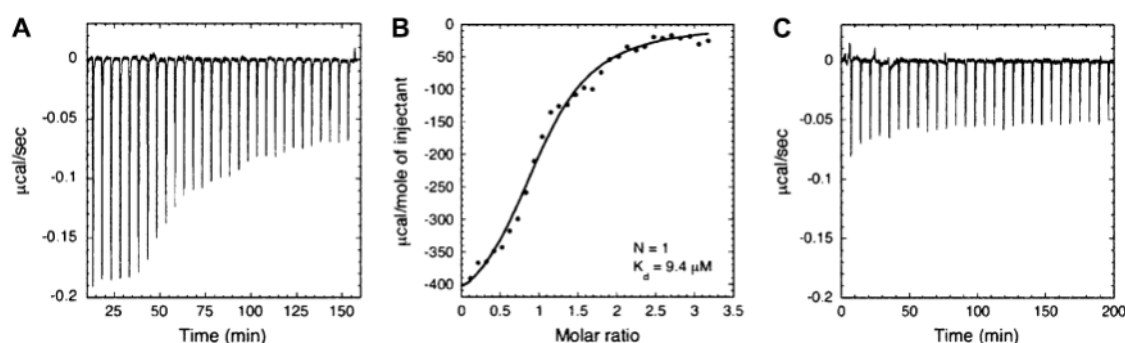


Figure 4. ITC data of zeatin binding to Pru p 1 proteins. A and C, Raw titration data representing the thermal effect of $29 \times 10 \mu\text{L}$ injections of zeatin ($750 \mu\text{M}$) onto a $50 \mu\text{M}$ solution of Pru p 1.01 (A) and Pru p 1.06D (C). B, Best fit of the integrated Pru p 1.01 data, represented as a solid line, obtained by a nonlinear least squares procedure. The calculated number of sites and the dissociation constant are indicated.

5D), indicating a substantial structural conservation. The secondary structure content of both Pru p 1.01 and Pru p 1.06D model structures (25% α -helix, 24% β -sheet, 24% turns, and 12% random coil) is in good agreement with the secondary structure found experimentally using CD (vide supra).

The calculated volumes for the internal pockets of the model structures are 1,389 and $1,498 \text{ \AA}^3$ for Pru p 1.01 and Pru p 1.06D, respectively. These volumes are comparable to that calculated, using the same protocol, for LIPR-10.2B ($1,579 \text{ \AA}^3$; Fig. 5B) but are significantly larger than that calculated for VrCSBP ($829 \text{ \AA}^3$; Fig. 5C) and Pru av 1 ($1,156 \text{ \AA}^3$). These cavities differ in shape as well as in electrostatics from the templates, with the cavities of the peach proteins bearing less negative charge (Fig. 5, B, C, E, and F). Moreover, some crucial differences are observed in the residues forming the pockets of Pru p 1.01 and 1.06D that influence their shape and electrostatics; these residues involve positions 23 (Phe versus Leu), 31 (Val versus Ile), 83 (Ser versus Asn), and 103 (Thr versus Ile) of the multiple sequence alignment (Fig. 5A). Of the terminal α -helix, only seven residues are completely buried in the model structures of peach Pru p 1.01 and Pru p 1.06D: Val-134, Gly-137, Ala-141, Phe-145, Ile-148, Glu-149, and Leu-152 (Fig. 5A). These residues are fully conserved in peach Pru p 1.01 and Pru p 1.06D and in cherry Pru av 1, while they are not conserved (with the exception of Glu-149 and Leu-152) in the other template structures. This discrepancy probably contributes to the different shape of the internal cavity of peach Pru p 1.01 and Pru p 1.06D and cherry Pru av 1 with respect to those found in yellow lupin LIPR-10.2B and mung bean VrCSBP.

Docking of Zeatin to Peach Pru p 1.01

Among the two isoforms of peach PR-10 proteins investigated in this work, only Pru p 1.01 showed the capability of binding zeatin; therefore, docking simulations were carried out using this model structure. In order to calibrate the computational methods and correctly model the zeatin orientation and conforma-

tion into the Pru p 1.01 binding pocket, two sets of benchmark calculations were carried out on yellow lupin LIPR-10.2B and mung bean VrCSBP crystal structures, in analogy to a previously published protocol (Musiani et al., 2001). This benchmarking stage further attempted to provide insights onto the different protonated forms (N7 or N9) of the zeatin molecule (details given as supplemental information; Supplemental Figs. S14 and S15). The docking of zeatin to the Pru p 1.01 binding pocket is reported in Figure 6A. The calculations performed using zeatin protonated on nitrogen N7 or N9 gave similar results of position, orientation with respect to the protein frame, and free energy of binding, suggesting that the two different protonation states of zeatin are not strongly discriminated. In both cases, the best docked structure is part of a well-resolved cluster of similar orientations. The position of the zeatin molecule (Fig. 6B) is very similar (RMSD = $1.5 \text{ \AA}$) to that found in yellow lupin LIPR-10.2B, the difference being likely due to the incomplete residue conservation inside the binding pocket (Fig. 5A). A much larger difference is instead observed when comparing the structure of zeatin docked to Pru p 1.01 with the crystal structure of zeatin bound to mung bean VrCSBP (RMSD approximately $7.5 \text{ \AA}$; blue dots in Supplemental Figs. S14 and S15). This result must derive from higher conservation within the binding pocket of Pru p 1.01 and yellow lupin LIPR-10.2B as compared to that of mung bean VrCSBP (Fig. 5A). In particular, the substitution of mung bean VrCSBP Phe-56 to residues with a less sterically hindering side chain (Ile in Pru p 1.01 and Leu in yellow lupin LIPR-10.2B) is the crucial feature leading to the different zeatin binding modes in mung bean VrCSBP on one side and in yellow lupin LIPR-10.2B and Pru p 1.01 on the other.

DISCUSSION

Pru p 1.01 is known to be a major peach allergen (Ahrazem et al., 2007) and was previously found in large amount in bark, xylem, and root of peach tree

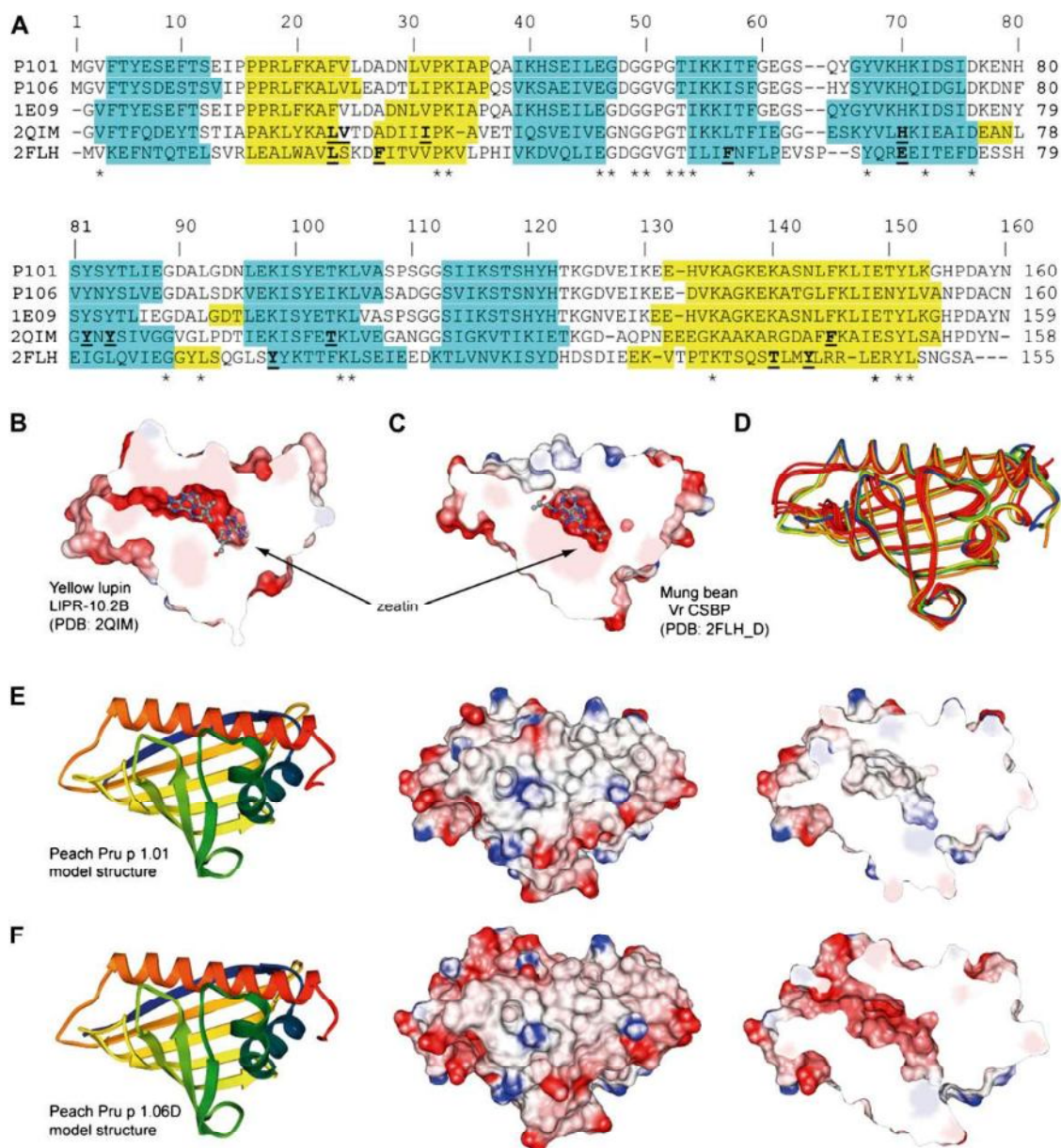


Figure 5. Pru p 1.01 and Pru p 1.06D model structure calculation. A, Multiple sequence alignment of peach Pru p 1.01 (P101), Pru p 1.06D (P106), cherry Pru av 1 (1E09), yellow lupin LIPR-10.2B (2QIM), and mung bean VrCSBP (2FLH). The secondary structure colors (α -helix, yellow; β -strand, cyan) are derived from the PDB structure for 1E09, 2QIM, and 3COV and from JPRED prediction for Pru p 1.01 and Pru p 1.06D. Underlined residues in 2QIM and 3COV are involved in the binding of the zeatin found at the end of the protein binding pocket. B and C, Solvent-exposed surface sections of yellow lupin LIPR-10.2B (2QIM) and mung bean VrCSBP (2FLH, chain D). The sections are oriented in order to expose the zeatin binding pockets and zeatin molecules found in the PDB structures. Zeatin molecules are depicted as ball-and-sticks and colored according to atom type (carbon, gray; nitrogen, blue; and oxygen, red). D, Superimposition of peach Pru p 1.01 (blue) and Pru p 1.06D (green) model structures and template structures used for the modeling (1E09, 2QIM, and 2FLH, chains A–D colored in yellow, orange, and red, respectively). E and F, Ribbons, solvent-exposed surfaces, and solvent-exposed surfaces section of peach Pru p 1.01 and Pru p 1.06D model structures. The sections are oriented in order to expose the internal cavity. All surfaces are colored according to the electrostatic potential contoured from -10.0 (intense red) to 10.0 kT/e (intense blue; where k is the Boltzmann constant, T the absolute temperature, and e the electron charge).

during the dormancy period, with a possible role in storage and/or defense (Wisniewski et al., 2004). Recent data indicated that a Pru p 1.01 isoform, named Pru p 1.06D according to its sequence identity (Gao

et al., 2005; Chen et al., 2008), is one of the most abundantly expressed genes among the ripe peach mesocarp transcriptome (Trainotti et al., 2006). Pru p 1.06D shows high sequence identity (73%) to Pru p

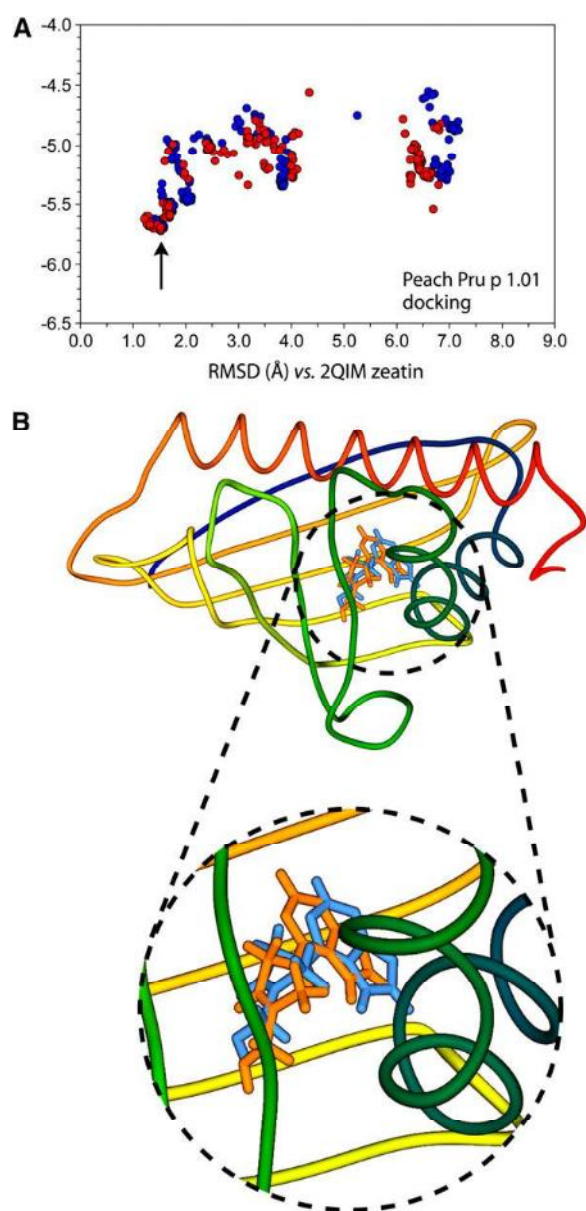


Figure 6. Simulation of zeatin docking to peach Pru p 1.01. A, RMSD versus estimated free energy of zeatin binding to peach Pru p 1.01. The RMSD is calculated with respect to the zeatin molecule found in the crystal structure of yellow lupin LIPR-10.2B (orange molecule in B). The scores for the N7 and N9 protonated forms of zeatin are shown as blue and red dots, respectively, in A. The best docked structure (light blue in B) is indicated by an arrow. B, Structure of zeatin docked to peach Pru p 1.01. The protein colors range from deep blue (N terminus) to red (C terminus).

1.01; no data were so far reported on the expression and purification of Pru p 1.06D from its biological source or using heterologous systems.

In this study, we show that the expression pattern of *pru p 1.01* and *pru p 1.06D* genes in peach fruit skin during growth is similar, with a maximum peak of expression in the S2 phase, in which pit hardening occurs (Fig. 1). A different expression pattern of *pru p*

1.01 was recently reported in the skin of nectarine fruit during growth, suggesting a different regulation of the transcription of this gene in different *P. persica* cultivars (Botton et al., 2009). During the S2 period, peach fruits transiently lose their susceptibility to brown rot, caused by the fungus *Monilinia* spp.; the fruit molecular components, which regulate this process, are not known, although various PR-10 proteins were found to have a direct antimicrobial activity against fungal pathogens (Flores et al., 2002; Chadha and Das, 2006).

In this work, both Pru p 1.01 and Pru p 1.06D were expressed and purified as recombinant proteins from *E. coli* (Fig. 2A). These proteins exist as monomers in solution, as verified by light scattering (Fig. 2B). Only few PR-10 proteins, such as AmPR-10 from the Chinese medicinal plant *Astragalus mongholicus* (Yan et al., 2008), Bet v 1 from birch (Swoboda et al., 1995; Koistinen et al., 2002), and SPE-16 from the Mexican potato *Pachyrrhizus erosus* (Wu et al., 2003), have been found to form dimers in solution. However, the functional importance of dimerization in PR-10 proteins has not been clarified. CD spectroscopy (Fig. 2C) indicated that both isolated Pru p 1.01 and Pru p 1.06D are well folded, containing significant amounts of α -helix and β -strand. The relative amount of secondary structure elements in Pru p 1.06D well matches the one calculated in the theoretical prediction based on the primary structure (Fig. 5A), while Pru p 1.01 contains a higher proportion of α -helix. In this regard, it is interesting to note that, while all PR-10 proteins contain a highly conserved and stable β -sheet, they show structural variability in the α -helical content. In particular, the conformation of helix $\alpha 3$ determines the accessibility, the size, and the shape of the hydrophobic cavity that holds zeatin or other ligands (Biesiadka et al., 2002; Pasternak et al., 2006; Fernandes et al., 2008, 2009).

At variance with other PR-10 members, Pru p 1.01 and Pru p 1.06D recombinant proteins did not show any inhibitory activity on the growth of *Monilinia* and of other peach fungal pathogens, suggesting that, if these proteins play a role in plant defense, it is not through a direct action. On the other hand, during the pit hardening stage, also the genes encoding key enzymes of the phenylpropanoid pathway were found up-regulated (P. Zubini and E. Baraldi unpublished data). This pathway controls the production of lignin precursors, which is a fundamental process during this stage. It is noteworthy that also the expression of *aoPR1*, encoding a PR-10 protein from asparagus (*Asparagus officinalis*), was shown to temporally and spatially correlate with the expression of genes coding for enzymes of the phenylpropanoid pathway. Moreover, the promoter of *aoPR1* gene contains sequence motifs similar to those found in the phenylpropanoid genes (Warner et al., 1994). These data suggest that PR-10 proteins act in concert with the phenylpropanoid pathway, which is known to be involved in several plant defense responses (Dixon et al., 2002) or that phenylpropanoid intermediates regulate PR-10 gene expression (Warner et al., 1994).

PR-10 proteins have been considered a multifunctional protein family, encompassing different functional subfamilies that share homology in tertiary structure in spite of low sequence similarity. The specific interaction of several PR-10 proteins with CKs has been so far associated with a function of storage and transport of these plant hormones (Fernandes et al., 2008) as well as with a possible regulatory role in RNase activity, CK metabolism, and stress defense (Srivastava et al., 2007). The calorimetric titration experiments described in this study showed that Pru p 1.01 binds one zeatin molecule per protein monomer with a dissociation constant of approximately 10 μM , while Pru p 1.06D does not show any binding capability under the same experimental conditions. Previous measurements using ITC indicated a specific interaction of VrCSBP with zeatin, with affinity >10 times lower than that observed for Pru p 1.01 (Pasternak et al., 2006). Such functional divergence could be due to structural differences of the binding pocket in Pru p 1.01 compared to its homolog. Indeed, the available crystal structures of PR-10 proteins in complex with zeatin show that the ligand-protein interaction is not structurally conserved: VrCSBP was crystallized with one or two zeatin molecules in the binding pocket (Pasternak et al., 2006). Conversely, the lupin LIPR-10.2B crystal structure shows three zeatin molecules bound inside the pocket (Fernandes et al., 2008). The orientation of the zeatin molecule bound deeper in the pocket is different in the two structures, indicating that proteins with the same scaffold but located in distant regions of the phylogenetic tree, and belonging to different taxonomic orders, have developed the capability of bind a different amount of zeatin molecules in different ways (Fernandes et al., 2008). This could be due to the fact that the residues important for zeatin binding are not conserved in all PR-10 proteins. Accordingly, in the case of Pru p 1.01 and Pru p 1.06D, the model structures of the two protein isoforms (Figs. 5 and 6) show a very similar general fold but a significant difference in the residues composing the binding pocket. Moreover, the electrostatics properties of the two proteins, resulting from molecular modeling, are different and vary from the other PR-10 protein structures solved so far (Fig. 5, B, C, E, and F). Docking experiments indicated that Pru p 1.01 could well accommodate a single zeatin molecule in its binding pocket (Fig. 6).

Both Pru p 1.01 and Pru p 1.06D catalyze the hydrolysis of RNA (Fig. 3). Ribonuclease activity has been demonstrated for several PR-10 proteins, which, based on the pH dependence, were ascribed to the class II of RNases. This activity is often paralleled with the CK-binding function (Iyer et al., 2001), as we show here in the case of Pru p 1.01. However, so far, no experimental evidence on the correlation between these two functions has been provided. We show here, for the first time, that the rate of the RNA hydrolysis of a PR-10 protein, Pru p 1.01, is affected upon binding of zeatin. A zeatin inhibitory action on

the Pru p 1.01 RNase function not only supports the hypothesis that the RNA hydrolyzing activity of PR-10 could regulate the endogenous CKs concentration but also suggests for this ligand a possible regulatory role on the Pru p 1.01 enzymatic activity through a feedback mechanism. The fact that RNA hydrolysis is inhibited by zeatin during the first hour of Pru p 1.01 incubation and is restored afterward could indicate a shift in the equilibrium toward the RNA:protein complex after that time. Such a shift can be due to the experimental conditions of the reaction in which an excess of RNA is used and suggests a mechanism of zeatin/RNA alternative binding to the protein. Other PR-10 proteins have been classified as RNases, based on 2- to 4-h activity assays (Bantignies et al., 2000; Liu and Ekramoddoullah, 2006; Srivastava et al., 2006). However, our results show that the PR-10 Pru p 1.06D protein is capable of full RNA degradation in much shorter incubation time. In addition, Pru p 1.06D does not bind and is not inhibited by zeatin, suggesting that this protein plays a different role *in vivo*. A different function for the two isoforms would also explain the similar expression patterns of the *pru p 1.01* and *pru p 1.06D* genes observed during the fruit growth stage and possibly justify the large energetic cost afforded by the cell for the protein synthesis process.

In conclusion, our results show that the two isoforms of Pru p 1 protein differ in activity, in spite of the similarity in protein sequence and folding. This observation reinforces the hypothesis (Iyer et al., 2001; Radauer et al., 2008) that PR-10 proteins are multifunctional proteins that exploit a conserved tertiary structure and fine-tune their function by varying just a few key amino acid residues. Additionally, our results indicate the presence of a possible correlation between two roles of PR-10 proteins, that of RNase hydrolysis and CK binding.

MATERIALS AND METHODS

Fruit Material

Peach fruits (*Prunus persica* Batch 'K2') were weekly harvested in a local fungicide-free orchard, from the fourth week after full bloom until full maturity. The fruit growth curve showed the typical double sigmoid pattern with the four standard growth stages (S1–S4; Bregoli et al., 2002; Trainotti et al., 2006). Four samples, consisting of three replicates of five fruits each, representative of the four stages, were chosen for expression analysis. Fruits were peeled and skin was frozen in liquid nitrogen.

Gene Expression Analysis

The RNA was extracted from 2.5 g of frozen peach skin following the protocol published by Bonghi et al. (1992). The RNA pellet was air-dried and diluted in 50 μL of water pretreated with 0.1% diethyl-pyrocabonate water. DNA was removed from the samples by Turbo DNase treatment (Ambion) following the manufacturer's instructions.

The single-strand cDNA was obtained from 360 ng of total RNA with the ImProm-II Reverse Transcriptase (Promega) and oligo(dT) as primers. Real-time PCR was performed on an MX3000 instrument (Stratagene) using the Brilliant SYBR Green QPCR Master mix (Stratagene). Each reaction mixture contained 1:50 dilution of single-strand cDNA as template, 120 nM of each specific primer (5'-CCCCGATGCCTACAATAAA-3' and 5'-GCAACAA-

GAAGCCACACA-3' for *pru p 1.01*; 5'-TAAGTTGGTGGCATCTGCTG-3' and 5'-CCCAGCCTTAACATCCTCT-3' for *pru p 1.06D*), 15 nM ROX as reference dye, and 1× Brilliant SYBR Green QPCR Master mix (Stratagene). The primer annealing step was performed at 58°C. Two replicates of each reaction were run in the same experiment. Amplified products were checked by sequencing (BMR Genomics). Quantification was carried out using a standard curve, generated by the real-time PCR of serial dilutions of a single-strand cDNA. Data were analyzed using MXPro QPCR software, version 3.0 (Stratagene). Quantifications were normalized through real-time amplification of the actin housekeeping gene. Data were analyzed by ANOVA, and means were separated by the least significance difference test at the 0.05 probability level.

Expression and Purification of Recombinant Pru p 1.01 and Pru p 1.06D

The full-length coding sequences of *pru p 1.01* and *pru p 1.06D* were obtained by amplification of a single-strand cDNA, reverse transcribed from mRNA of peach skin, using 5'-TATACCATGGGTGTCTTCACATATGAGAGCGAGTT-3' and 5'-TATAAGCTTAGTTGTAGGCATCGGGGTGGCCCTTA-3' primers for *pru p 1.01* and 5'-TATACCATGGGTGTCTTCACATACTCAGACGAGTC-3' and 5'-TATAATGCATTAGTTGYAGGCATCWGG-3' primers (PCR purification grade; PRIMM) for *pru p 1.06D*. Primers introduced the *NcoI* and *HindIII* and the *NcoI* and *AvaIII* restriction sites in the *pru p 1.01* and in *pru p 1.06D* PCR amplification products, respectively. These were purified with Nucleospin Extracts II kit (Macherey-Nagel), digested with restriction enzymes (Fermentas), and ligated into a pHAT plasmid (Peränen et al., 1996) equally digested. The recombinant plasmids were purified from *Escherichia coli* DH5α cells and sequenced on both strands (BMR Genomics), before transformation into *E. coli* BL21 (DE3) strain. These cells were grown at 37°C, and the expression of the recombinant proteins, fused to a nonspecifically cleavable N-terminal tag containing six His (MNTIHHHHHNTSSAT), was induced with 0.4 mM isopropyl β-D-1-thiogalactopyranoside for 3 h. Bacteria were recovered by centrifugation and resuspended in 25 mL of 50 mM phosphate buffer, pH 7.5, containing 300 mM NaCl. Cells were disrupted using a French press (SLM AMINCO I) after addition of DNaseI (10 μg/mL) and MgCl₂ (10 mM). The soluble fraction of *E. coli* lysate was loaded onto a Ni-NTA (Ni-nickel-chelating nitrilotriacetic acid) affinity column (Qiagen). Proteins were eluted with buffer containing 200 mM imidazole and dialyzed for 16 h against 50 mM phosphate buffer, pH 7.5, containing 150 mM NaCl. Proteins were quantified using theoretical extinction coefficient at 280 nm, calculated on the basis of primary structure (13,410 M⁻¹ cm⁻¹ for Pru p 1.01 and 11,920 M⁻¹ cm⁻¹ for Pru p 1.06D) using the ProtParam server (<http://us.expasy.org/tools/protparam.html>).

Light Scattering Measurements

The oligomeric state and the hydrodynamic radius of Pru p 1.01 and Pru p 1.06D were determined using a combination of size exclusion chromatography, multiple-angle light scattering, and quasielastic light scattering. A protein sample (200 μL, 180 μM) was loaded onto a Superdex 75 HR 10/30 column (GE Healthcare), preequilibrated using 50 mM phosphate buffer, pH 7.5, containing 150 mM NaCl, and eluted at a flow rate of 0.6 mL min⁻¹. The column was connected downstream to a multiangle laser light (690.0 nm) scattering DAWN EOS photometer (Wyatt Technology) and to a quasielastic light scattering WyattQELS device. The concentration of the eluted protein was determined using a refractive index detector (Optilab DSP; Wyatt). Values of 0.185 mL g⁻¹ for the refractive index increment (dn/dc; Charlwood, 1957) and 1.321 for the solvent refractive index were used. Molecular masses were determined from a Zimm plot (Zimm, 1948). Data were recorded and processed using Astra 5.1.9 software (Wyatt Technology) following the manufacturer's instructions.

CD Spectroscopy

The secondary structure of Pru p 1.01 and Pru p 1.06D was evaluated by CD spectroscopy for protein samples (5 μM) dissolved in 50 mM phosphate buffer, pH 7.5, using a JASCO 810 spectropolarimeter and a cuvette with 0.1-cm path length. Ten spectra were accumulated from 190 to 240 nm at 0.2-nm intervals and averaged to achieve an appropriate signal-to-noise ratio. The spectrum of the buffer was subtracted. The secondary structure composition

of Pru p 1.01 and Pru p 1.06D was evaluated using the CDSSTR tool available on the Dichroweb server (Withmore and Wallace, 2004, 2008) with the reference sets 4, 7, and SP1 (<http://dichroweb.cryst.bbk.ac.uk/html/home.shtml>). The values obtained using all these reference sets were averaged to give an estimation of the relative amount of secondary structure elements.

RNase Activity Assay

Total RNA from peach fruit skin was extracted and purified as described above. Both proteins (10 μM) were incubated in the presence of RNA (10 μg) for 2 to 4 h at 37°C in 50 μL of 50 mM buffer at different pH: citrate for pH 3, MES for pH 5, phosphate for pH 7.5, and CHES (*N*-cyclohexyl-2-aminoethanesulfonic acid) for pH 9. Negative controls were performed using (1) phosphate buffer alone, (2) the protein denatured at 100°C for 15 min, or (3) the lysate of *E. coli* BL21(DE3) transformed with the pHAT vector. The latter was treated similarly to the *E. coli* lysate overexpressing the two Pru p 1 isoforms: it was induced with 0.4 mM isopropyl β-D-1-thiogalactopyranoside, loaded onto a nickel column, and eluted with 200 mM imidazole. This elution fraction was directly used in the assay to exclude any nonspecific RNA hydrolysis from components of the buffers, such as Ni cations or imidazole. At pH 7.5, the RNase activity was assayed also after 30 min and 1 h incubation time and in the presence of zeatin: RNA was incubated for 30 min, 1, 2, and 4 h with Pru p 1 proteins and 200 μM zeatin (Sigma-Aldrich) in 50 mM phosphate buffer. A negative control was performed incubating RNA with 200 μM of zeatin in 50 mM phosphate buffer. For each experiment, at least three replicates were performed. The RNA present in the resulting reaction mixtures from the assays was purified before gel analysis (1% agarose gels stained with ethidium bromide) using phenol-chloroform treatment and ethanol precipitation.

In Vitro Antimicrobial Activity of Recombinant Proteins

The antimicrobial activity of recombinant Pru p 1.01 and Pru p 1.06D was assayed against *Monilinia laxa*, *Monilinia fructigena*, *Botrytis cinerea*, *Colletotrichum acutatum*, *Rhizopus stolonifer*, and *Penicillium expansum*. The fungi were grown on potato dextrose agar plates at room temperature until the colony reached a diameter of 3.5 cm. Sterile filter paper discs of 0.6 cm diameter were placed at 0.5 cm from the growing front, and 5 or 10 μg of the recombinant protein were applied onto the discs. To test the possible effect of zeatin binding to proteins on fungal growth, antifungal tests were also performed by incubating Pru p 1 proteins with 10 or 20 molar excess (50 or 100 μM) of zeatin, 10 min before placing them on the filter discs. Negative controls were performed using the supernatant fraction of the lysate of *E. coli* BL21(DE3) transformed with pHAT vector or using the protein buffer. Fungal growth was analyzed after 12 to 24 h of incubation or until fungal growth reached the filter.

ITC Experiments

A solution of zeatin (trans-zeatin hydrochloride; Sigma-Aldrich) was prepared diluting a concentrated stock (75 mM) in 50 mM phosphate buffer, pH 7.5. Zeatin concentration was confirmed spectrophotometrically using the molar extinction coefficient $\epsilon_{272} = 16,450 \text{ M}^{-1} \text{ cm}^{-1}$ (Letham et al., 1967).

Titration was performed at 25°C using a high-sensitivity VP-ITC microcalorimeter (MicroCal). The reference cell was filled with deionized water. Before each experiment, the protein was passed through a Superdex-75 HR 10/30 column, the buffer of which was also used to dilute ligand molecule. Care was taken to start the first addition after baseline stability had been achieved. Small volumes (10 μL) of a solution containing zeatin at a concentration of 750 μM diluted in 50 mM phosphate buffer, pH 7.5, were injected into a solution of Pru p 1.01 and Pru p 1.06D (50 μM) in the same buffer, using a computer-controlled 310 μL microsyringe. In order to allow the system to reach equilibrium, an interval of 5 min between each ligand injection was applied. For each titration, a control experiment was set up, titrating the zeatin solution into the buffer alone, under the same conditions.

Integrated heat data obtained for zeatin titrations were fitted to a theoretical curve using a nonlinear least-squares minimization algorithm and the MicroCal Origin software. ΔH (reaction enthalpy change in cal mol⁻¹), K_b (binding constant in M⁻¹), and n (number of binding sites) were the fitting parameters. The reaction entropy was calculated using the relationships $\Delta G = -RT \ln K_b$ ($R = 1.9872 \text{ cal mol}^{-1} \text{ K}^{-1}$, $T = 298 \text{ K}$) and $\Delta G = \Delta H - T\Delta S$. Best results, evaluated using the reduced χ^2 parameter, were obtained using the scheme with one set of sites.

Sequence Search and Homology Modeling of Peach Pru p 1.01 and Pru p 1.06D

Sequences similar to peach Pru p 1.01 and Pru p 1.06D were retrieved by similarity search from a nonredundant sum of different protein databases (GenBank CDS translations, RefSeq Proteins, PDB, SwissProt, Protein Information Resource, and Protein Research Foundation) using the primary structures of Pru p 1.01 and Pru p 1.06D as templates. The program PSI-BLAST (Position-Specific Iterated BLAST; Altschul et al., 1997) was used for the similarity search. Multiple sequence alignments were performed on the sequences using the ClustalW program (Thompson et al., 1994). The alignment (Supplemental Fig. S4) was employed to derive an unrooted phylogenetic tree (Supplemental Fig. S5) using the program PHYLIP (Felsenstein, J., PHYLIP, PHYLogenetic Inference Package, 3.5c, Department of Genetics, University of Washington, Seattle, 1993).

An initial alignment of peach Pru p 1.01 and Pru p 1.06D sequences with the sequences of modeling templates was produced using ClustalW and manually adjusted to match up the primary and the secondary structure of the proteins as determined using the program JPRED (Cuff et al., 1998). This alignment was then used to calculate 100 structural models of Pru p 1.01 and Pru p 1.06D using the program Modeller9v4. The NMR structure of cherry (*Prunus avium*) Pru av 1 (PDB code 1E09) and the yellow lupin (*Lupinus luteus*) LIPR-10.2B and mung bean (*Vigna radiata*) CSBP crystal structures (PDB codes 2QIM and 3C0V, respectively) were used as templates. The best model was selected on the basis of the lowest value of the Modeller objective function. Results of the ProCheck (Laskowski et al., 1993) validation for the final model were fully satisfactory.

The molecular solvent-exposed surfaces were calculated using the UCSF Chimera package (Pettersen et al., 2004). All His residues were considered neutral. The electrostatic color-coding was generated using the Delphi software (Honig and Nicholls, 1995). This program solves the linearized Poisson-Boltzmann equation to obtain the electrostatic potential in and around the protein, while taking the presence of solvent into account as a high dielectric continuum. The protein internal dielectric constant was set to 4 in all calculations, and the solvent dielectric constant was 80. The salt concentration was set to physiological ionic strength of 150 mM NaCl. The electrostatic potential calculation with Delphi was done using a scale of 1 point/Å. The electrostatic potentials generated by Delphi were displayed using UCSF Chimera. The cavity volumes were calculated using the Pocket Finder server at <http://www.modelling.leeds.ac.uk/pocketfinder/help.html>.

Docking of trans-Zeatin to Yellow Lupin LIPR-10.2B Crystal Structure and to Peach Pru p 1.01 Model Structure

Docking was performed using an established procedure (Musiani et al., 2001) and AutoDock v4.0 (Morris et al., 1998; Huey et al., 2007). Yellow lupin LIPR-10.2B and mung bean VrCSBP crystal structures (PDB codes 2QIM and 2FLH chain D, respectively) from which the zeatin and water molecule were removed, as well as the Pru p 1.01 model structure, were used in the docking experiments. The receptor proteins were superimposed to have a common reference point from which to evaluate ligand displacement. The trans-zeatin 159 found in 2QIM was used as ligand starting model. Hydrogen atoms were added both to the protein receptors and to the trans-zeatin ligand using the UCSF Chimera program suite. Nonpolar hydrogen atoms were then deleted, and Gasteiger charges (Gasteiger and Marsili, 1980) were assigned. All the torsional bonds of trans-zeatin were free to rotate for flexible docking. The grid maps representing the proteins in the actual docking process were calculated with the program AutoGrid. The grids (one for each atom type in the ligand plus one for electrostatic interactions) were chosen to be sufficiently large to include the pocket inside the proteins. The dimensions of the grids were of $10 \times 10 \times 10$ Å, with a spacing of 0.200 Å between the grid points. Docking of trans-zeatin to receptor proteins was carried out using the empirical free energy function and the Lamarckian genetic algorithm, applying a standard protocol with an initial population of 150 randomly placed individuals, a maximum number of 2.5×10^6 energy evaluations, a mutation rate of 0.02, a cross-over rate of 0.80, and an elitism value of 1, where the average of the worst energy was calculated over a window of the previous 10 generations. For the local search, the so-called Solis and Wets algorithm was applied, using a maximum of 300 iterations. The probability of performing a local search on an individual in the population was 0.06, and the maximum number of consecutive successes or failures before doubling or halving the local search step size was 4. Two hundred independent docking runs were

carried out. Results were analyzed using the RMSD from the position of the trans-zeatin 159 found in the 2QIM crystal structure in the case of yellow lupin LIPR-10.2B and zeatin 706D in case of mung bean VrCSBP.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers AM493970 (*pru p 1.01*) and AM289148 (*pru p 1.06D*).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Band intensity profiles of Pru p 1.01 RNA degradation in absence of zeatin.

Supplemental Figure S2. Band intensity profiles of Pru p 1.01 RNA degradation in presence of zeatin.

Supplemental Figure S3. Comparison of the band intensities reported in Supplemental Figures S1 and S2.

Supplemental Figure S4. Multiple sequence alignment of PR-10 proteins.

Supplemental Figure S5. Unrooted phylogenetic tree of PR-10 proteins.

Supplemental Figures S6 to S8. Influence of different zeatin protonation states on yellow lupin LIPR-10.2B docking.

Supplemental Figures S9 to S11. Influence of different zeatin protonation states on mung bean VrCSBP docking.

Supplemental Figure S12. Influence of water molecules on zeatin docking to yellow lupin LIPR-10.2B.

Supplemental Figure S13. Zeatin docking simulations to yellow lupin LIPR-10.2B and mung bean VrCSBP.

Supplemental Figures S14 and S15. Influence of different zeatin protonation states on Pru p 1.01 docking.

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Figure S1. Band intensity profiles evidencing Pru p 1.01 RNA degradation in absence of zeatin at different times. Profiles were obtained from Fig. 3B lane 1 (*black* line), 2 (*blue* line), 3 (*green* line), 4 (*yellow* line), and 5 (*red* line). Band intensities have been extrapolated using the program IMAGEJ (Abramoff et al., 2004).

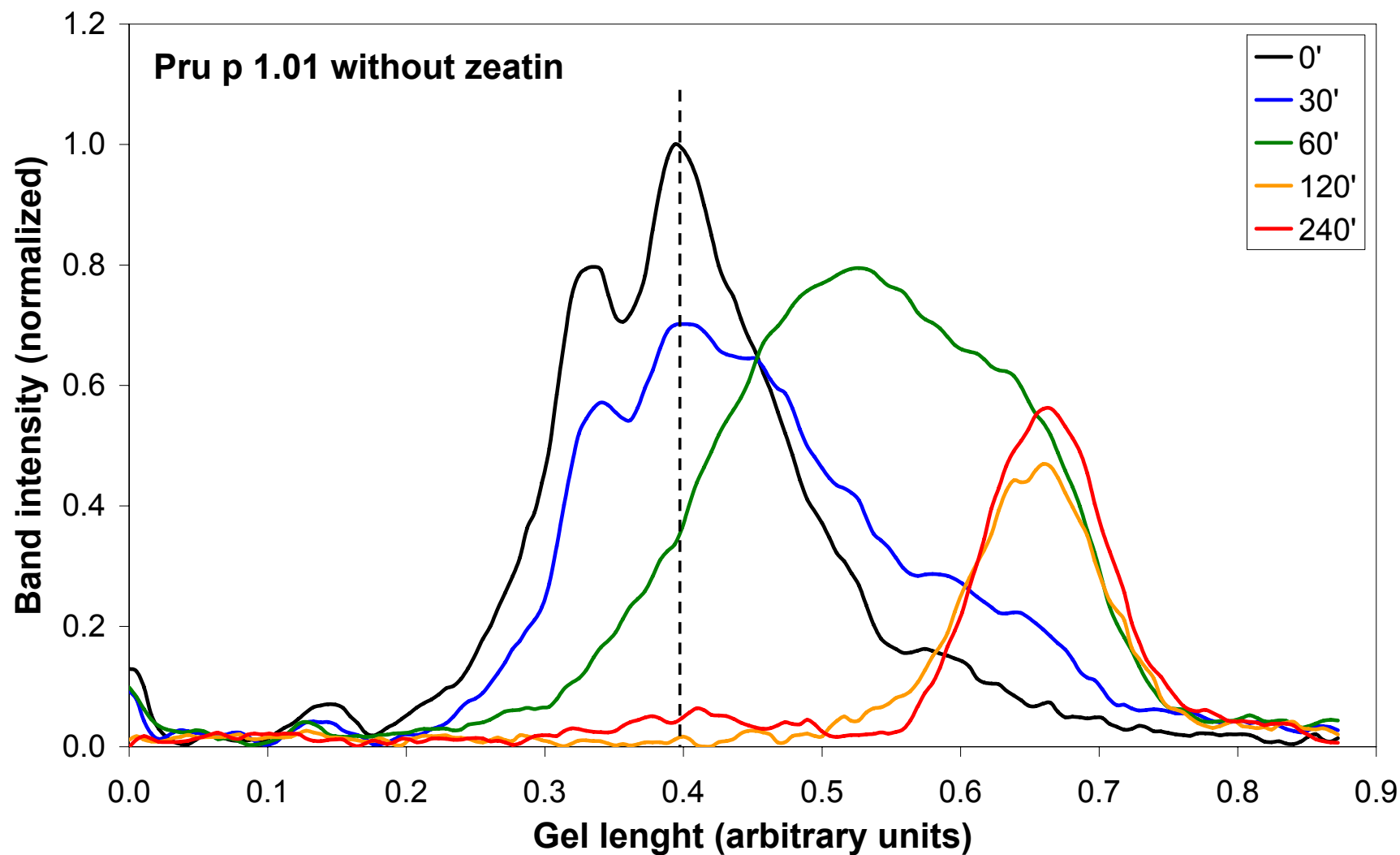


Figure S2. Band intensity profiles evidencing Pru p 1.01 RNA degradation in presence of zeatin at different times. Profiles were obtained from Fig. 3B lane 6 (*black line*), 7 (*blue line*), 8 (*green line*), 9 (*yellow line*), and 10 (*red line*). Band intensities have been extrapolated using the program IMAGEJ (Abramoff et al., 2004).

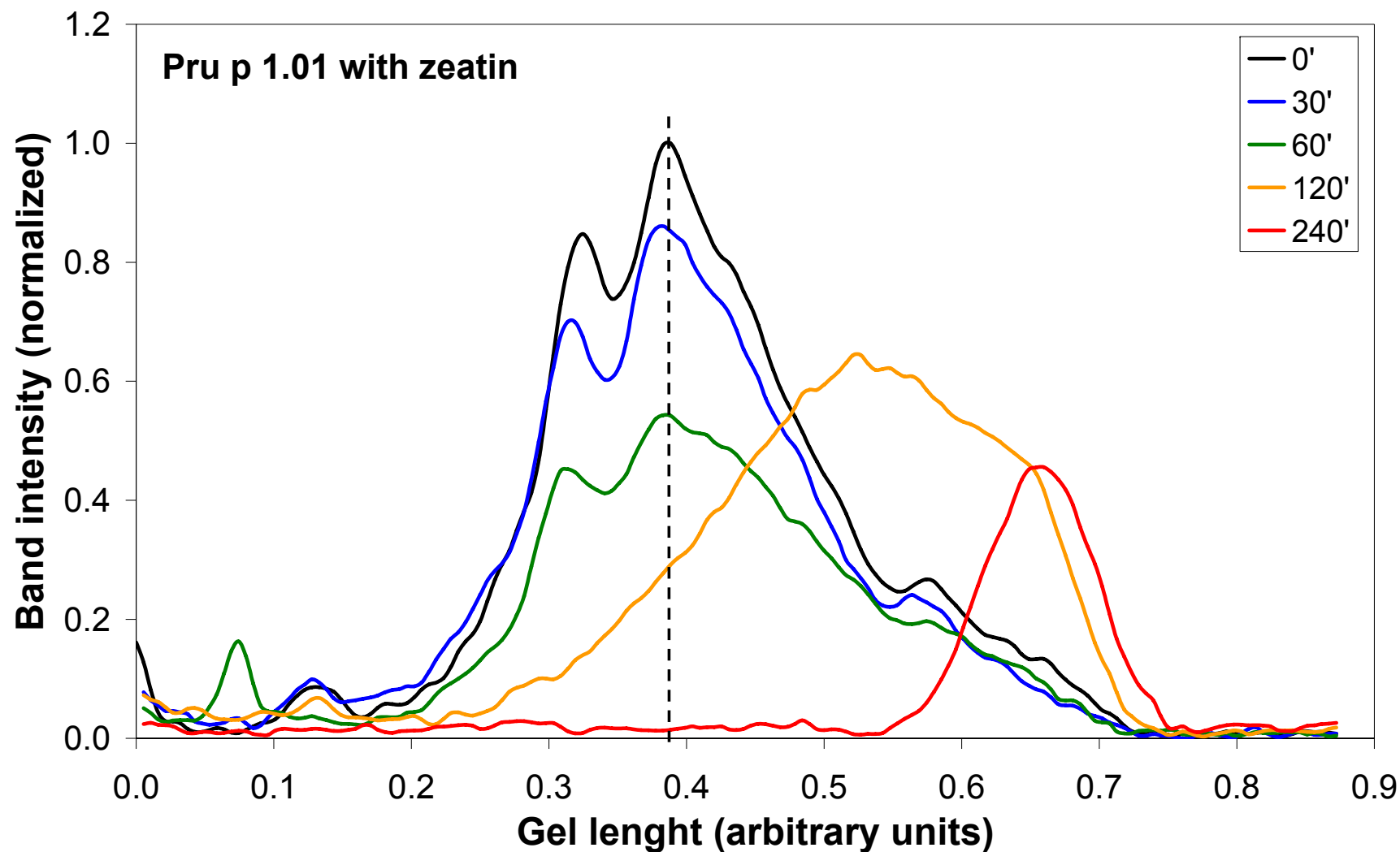


Figure S3. Band intensity, as reported in Fig. S3 and S4, taken in correspondence of the broken line in Fig. S3 and S4

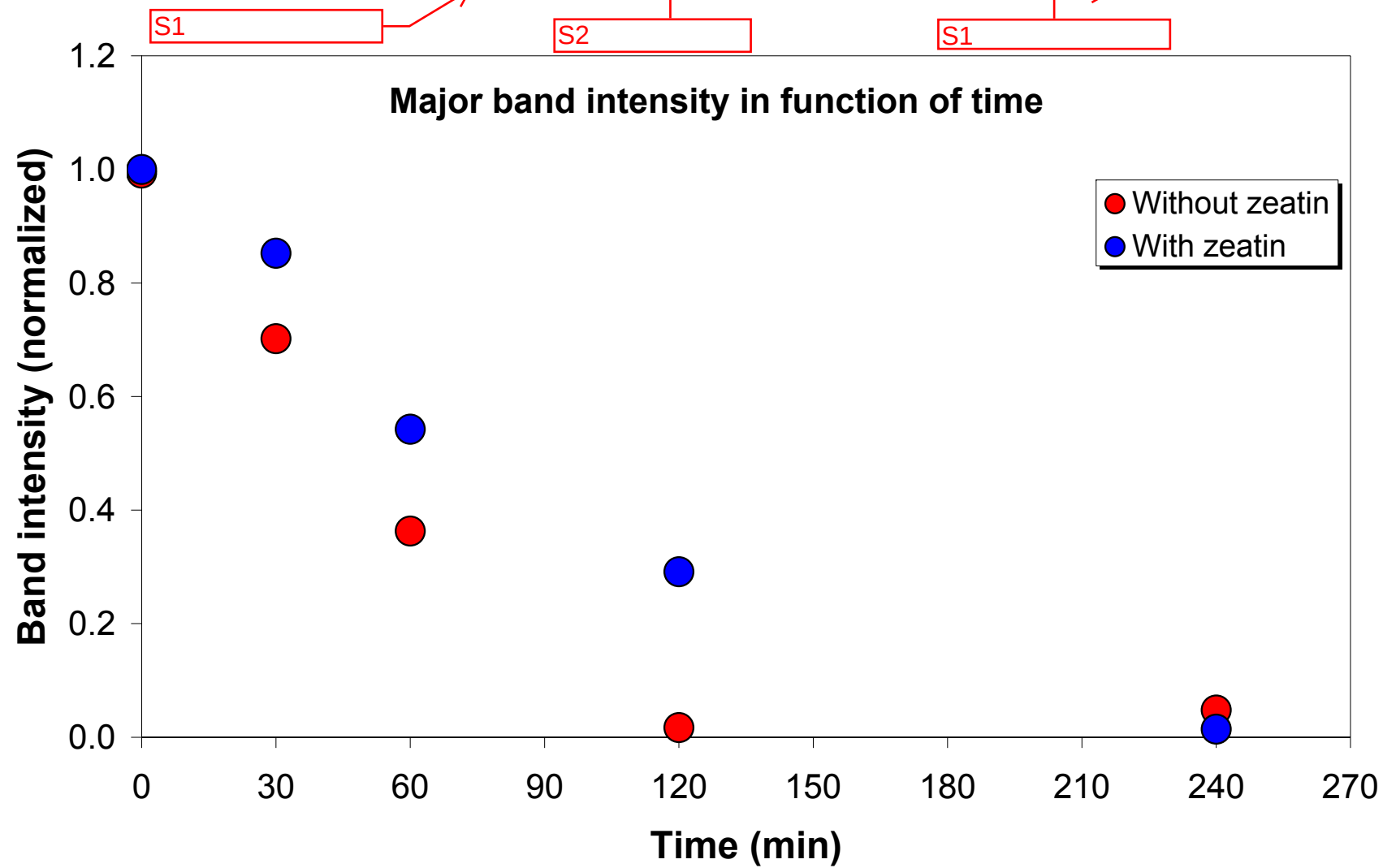


Figure S4. Multiple sequence alignment of PR-10 proteins found in protein data bases. Reported PR-10 are from the following sources: *Prunus persica* (peach), Pru p 1.01, Pru p 1.06D, 47; *Prunus avium* (cherry), 1-3, 49, 52, 54; *Malus x domestica* (apple), 4-26, 28-46, 48, 50, 51, 53, 56, 57, 59-64, 66, 68, 71, 75, 77, 78, 80-85, 88, 91, 95, 241, 244, 248, 301; *Prunus armeniaca* (apricot), 25, 55; *Fagus sylvatica* (European beech), 58; *Fragaria x ananassa* (strawberry), 65, 67, 69, 72, 74; *Pyrus communis* (pear), 70; *Vitis vinifera* (grape vine), 73, 79, 87, 89, 90, 94, 96, 99, 101, 103, 105, 109, 110, 154, 155, 173, 222, 272; *Castanea sativa* (sweet chestnut), 76; *Datisca glomerata* (Durango root), 86; *Betula pendula* (birch), 92, 98, 102, 111-113, 115, 117, 119, 121, 123-129, 131-145, 147-149, 152, 153, 156-158, 160-164, 166, 168-172, 175, 177, 178, 181, 182, 184-193, 195, 197, 201-204, 206, 207, 210-219, 223-226, 228, 232, 234; *Corylus avellana* (hazel), 93, 100, 104, 106, 107, 116, 146, 183, 194, 220; *Rubus idaeus* (raspberry), 97; *Corylus heterophylla* (Asian hazel), 108; *Vitis pseudoreticulata*, 114, 118, 122; *Vitis quinquangularis*, 120; *Morus bombycis*, 130, 150, 165; *Carpinus betulus* (hornbeam), 151, 174, 196, 198-200, 205, 209, 230, 235, 238, 242, 243; *Alnus glutinosa* (alder), 159; *Betula platyphylla* var. japonica (Japanese white birch), 167, 176, 180; *Vitis hybrid cultivar*, 179; *Solanum virginianum* (Surattense nightshade), 208; *Medicago truncatula* (barrel clover), 221, 333; *Medicago sativa* (alfalfa), 227, 236, 255, 285; *Gossypium hirsutum* (Mexican cotton), 229, 247, 263; *Gossypium arboreum* (cotton), 231; *Gossypium barbadense* (Egyptian cotton), 233, 237, 265, 315; *Vigna unguiculata* (cowpea), 239, 280, 297; *Lupinus luteus* (yellow lupine), 240, 245, 249-251, 253, 256, 283, 295, 299, 304, 313, 419; *Capsicum chinense* (chilli peppers), 246, 308; *Citrus sinensis* (orange), 252; *Pisum sativum* (pea), 254, 268, 290, 291, 293, 294, 296; *Capsicum annuum*, 257, 266, 276, 317; *Lupinus albus* (white lupine), 258, 284; *Phaseolus vulgaris* (bean), 259, 262, 282, 287; *Retama raetam*, 260; *Capsicum baccatum* (Peruvian hot pepper), 261; *Solanum tuberosum* (potato), 264, 269; *Cicer arietinum* (chickpea), 267, 278; *Salvia miltiorrhiza* (red sage), 270, 298; *Arachis hypogaea* (peanut), 271, 273, 288, 292, 308, 335, 337; *Solanum lycopersicum* (tomato), 274; *Glycine max* (soybean), 275, 305; *Vigna radiata* (mung bean), 277, 412, 413; *Vigna angularis* (Azuki bean), 279; *Oxalis tuberosa* (oka), 281; *Panax ginseng* (ginseng), 286, 323; *Pisum fulvum* (tawny pea), 289; *Trifolium repens* (white clover), 300; *Catharanthus roseus* (Madagascar periwinkle), 302, 321; *Casuarina glauca* (swamp she-oak), 303; *Petroselinum crispum* (parsley), 306, 307, 310, 312; *Hypericum perforatum* (St. John's wort), 311; *Daucus carota* (wild carrot), 314, 326-329, 332, 338, 352; *Pachyrhizus erosus* (Mexican potato), 316, 320; *Taraxacum officinale* (dandelion), 318; *Rehmannia glutinosa*, 319; *Apium graveolens* (celery), 322, 324; *Populus trichocarpa* (black cottonwood), 325; *Cichorium intybus* (chicory), 330; *Lithospermum erythrorhizon*, 331, 334; *Picea sitchensis* (Sitka spruce), 336, 343, 361, 365, 366, 371, 399-404; *Pimpinella brachycarpa*, 339; *Oryza sativa* (rice), 340, 383, 408-411, 414-416, 418, 423; *Pinus monticola* (white pine), 341, 346-351, 354-357, 359, 363, 367, 369, 377, 378, 385; *Zea mays* (maize), 342, 405, 406; *Sorghum bicolor* (sorghum), 344, 345, 353, 358, 381; *Pseudotsuga menziesii* (coast Douglas-fir), 360; *Picea glauca* (white spruce), 362, 364, 372, 374, 379, 380; *Asparagus officinalis* (asparagus), 368, 388, 392, 393, 395-398; *Zinnia elegans* (zinnia), 370; *Picea mariana* (black spruce), 373, 375; *Picea abies* (Norway spruce), 376; *Hyacinthus orientalis* (hyacinth), 382; *Lilium longiflorum* (Easter lily), 384, 386, 387, 389, 390, 394; *Pinus pinaster* (maritime pine), 391; *Triticum aestivum* (wheat), 407; *Hordeum vulgare* (barley), 417; *Sonneratia caseolaris*, 420; *Brassica rapa* (field mustard), 421; *Arabidopsis thaliana* (thale cress), 422, 424, 425.

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1      10      20      30      40      50      60      70      80      90      100     110     120     130     140     150     160
P101  -----MGVFTYESEPTSEIPPPRLF-KAFVLADADNLVPKIAPOA-----IKHSEILEGDDGV-GTIKKITFGEQS--QY--GYVKHQIDSDIKENHSYSYTLIEGD-----ALGDNLEKISYETKLVASP-----SGGSIKSTSHYHTKGDVEIKEE-HVKAGKEKASNLFK-----LIETYLKGH--PDAYN-----160
P106  -----MGVFTYSDESTSVIPPPRLF-KALVLEADTLIPKIAPOS-----VKSAEIIVGDDGV-GTIKKISFGEQS--HY--SYVKHQIDGLDKDNFVYNSYSLVEGD-----ALSKDVEKISYEIKLVASA-----DGGSVIKSTSNYHTKGDVEIKEE-DVKAGKEKATGLFK-----LIENYLVAN--PDACN-----160
1      -----MGVFTYSDESTSVIPPPRLF-KALVLEADTLIPKIAPOS-----VKTAEIIVGDDGV-GTIKKISFGEQS--HY--SYVKHRIDGLDKDNFVYNSYSLVEGD-----ALSKDVEKISYEIKLVASA-----DGGSIKSTSNYHTTGDVEIKEE-DVKAGKEKATGLFK-----LIENYLVAN--PDACN-----160
2      -----MGVFTYADESTSVIPPPRLF-KALVLEADTLIPKIAPOS-----VKSAEIIVGDDGV-GTIKKISFGEQS--HY--SYVKHRIDGLDKDNFVYNSYSLVEGD-----ALSKDVEKISYEIKLVASA-----DGGSIKSTSNYHTTGDVEIKEE-DVKAGKEKATGLFK-----LIENYLVAN--PDAYN-----160
3      -----MGVFTYADESTSVITPPRLF-KALVLEADTLIPKIAPOS-----VKGAEIIVGDDGV-GTIKKISFGEQS--HY--SYVKHRIDGLDKDNFVYNSYSLVEGD-----ALSKDVEKISYEIKLVASA-----DGGSIKSTSNYHTTGDVEIKEE-DVRAGKEKATGLFK-----LIENYLVAN--PDAYN-----160
4      -----MGVFTYETEFTSVIPAPRLF-KAFILDGDNLIPKIAPOA-----IKSTEIIEGDDGV-GTIKKVTFGEQS--QY--GYVKQVNGIDKDNFTYSYSMIIEGD-----TLDKLEKITYETKLIASP-----DGGSIKSTSHYHAKGDVEIKEE-HVKAGKEKASGLFK-----LLEAYLVAN--PDAYN-----160
5      -----MGVFTYETEFTSVIPAPRLF-KAFILDGDNLIPKIAPOA-----IKSTEIIEGDDGV-GTIKKVTFGEQS--QY--GYVKQVNGIDKDNFTYSYSMIIEGD-----TLDKLEKITYETKLIASP-----DGGSIKSTSHYHAKGDVEIKEE-HVKAGKEKASGLFK-----LLEAYLVAN--PDAYN-----160
6      -----MGVLTYTEYASVIPPAPRLF-NALVLADADNLIPKIAPOA-----VKTVEIIEGDDGV-GTIKKVSFGEQS--EY--SYVKHKVGEIDKDNFVYNSYSLIEGD-----AISDKIEKISYEIKLVASG-----SG-SIIKNISHYHTKGDVEIKEE-HVKAGKERAGHLFK-----LIENHLVAN--PDAYN-----159
7      -----MGVLTYTEYASVIPPAPRLF-NALVLADADNLIPKIAPOA-----VKTVEIIEGDDGV-GTIKKVSFGEQS--EY--SYVKHKVGEIDKDNFVYNSYSLIEGD-----AISDKIEKISYEIKLVASG-----SG-SIIKNISHYHTKGDVEIKEE-HVKAGKERAGHLFK-----LIENHLVAN--PDAYN-----159
8      -----MGVLTYTEYASVIPPAPRLF-NALVLADADNLIPKIAPOA-----VKTVEIIEGDDGV-GTIKKVSFGEQS--EY--SYVKHKVGEIDKDNFVYNSYSLIEGD-----AISDKIEKISYEIKLVASG-----SG-SIIKNISHYHTKGDVEIKEE-HVKAGKERAGHLFK-----LIENHLVAN--PDAYN-----159
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11     -----M-----

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[illegible]

[illegible]

182	MGVNYEIAETSVIPARLF-KAFILDGNLFLPKVAPQA	-ISSVENIEGNGGP-GTIKKISFPBGG	PF-KYVKDRVDEVDHNTFKFYSYSVIEGG	-PVGDTLEKISNEIKIVATP	-NGGSLILKINNKYHTKGDHEVKAE-QIKASKEKMEGTLTL	-AVESYLLAH-SDAYN	160
183	-FNYESETTSVIPARLF-KAFILLEGDTLIPKVAPOA	-ISSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-ALGDTLEKICNEIKIVATP	-DGGSLILKINNKYHTKGDHEVKAE-HMKAIKEKGEALLR	-AVESYLL	160
184	MGVNYEVEETSPVIPARLF-KSYVLGDGKILPKVAPQA	-ITSVENVNGGNGP-GTIKNITFPBGG	RY-KYVKRVEDVDHNTFTFYSYTVIEGD	-VLGDLEKVSHELKIVAAP	-DGGSLILKISKFHAKGDHEVNAE-EMKAKEMAEKLLR	-AVETVYLLAH-SAEYN	160
185	MGVNYEETSPVIPARLF-KAFILVLDGNLFLPKVAPQA	-SSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-AVGDALEKISNEIKIVAAP	-DGGSLILKINSKFHTKGDHEVNAE-QIKIEKEKAEGLK	-AVESYLLAH-SDAYN	160
186	-GVFNYEITTSVIPARLF-KAFILDGNLFLPKVAPQA	-ISSVENIEGNGGP-GTIKKINFPBGG	PF-KYVKDRVDEVDHNTFKFYSYSVIEGG	-PVGDTLEKISNEIKIVATP	-DGGCVLILKINNKYHTKGNHEVKAE-QVKASKEKMEGTLTL	-AVESYLLAH-SDAYN	159
187	-FNDEAETTSVIPARLF-KSFILDADNLSIKVAPQA	-FKSAENIEGNGGP-GTIKKITFPBGG	HF-KHLKQRIDEIDHNTFKFYSYSVIEGG	-PLGDTLEKISNEIKIVAAP	-DGGSLILKFSKSYVTGNISINQOE-QIKAEKEKGAGLFP	-AIEGYLL	150
188	-GVFNYEIAETSVIPARMF-KAFILDGNLFLPKVAPQA	-ISSVENIEGNGGP-GTIKKINFPBGG	PF-KYVKDRVDEVDHNTFKFYSYSVIEGG	-PVGDTLEKISNEIKIVATP	-DGGCVLILKINNKYHTKGNHEVKAE-QVKASKEKMEGTLTL	-AVESYLLAH-SDAYN	159
189	-GVFNYEITTSVIPARLF-KAFILDGNLFLPKVAPQA	-ISSVENIEGNGGP-GTIKKINFPBGG	PF-KYVKDRVDEVDHNTFKFYSYSVIEGG	-PVGDTLEKISNEIKIVATP	-DGGSLILKINSHRYHTKGNHEVKAE-QVKASKEKMEGTLTL	-AVESYLLAH-SDAYN	159
190	-FNYESETTSVIPARLF-KAFILLEGDTLIPKVAPOA	-ISSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-ALGDTLEKICNEIKIVATP	-DGGSLILKINNKYHTKGDHEVKAE-HMKAIKEKGEALLR	-AVESYLL	160
191	-FNYESETTSVIPARLF-KAFILLEGDTLIPKVAPOA	-ISSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-ALGDTLEKICNEIKIVATP	-DGGSLILKINNKYHTKGDHEVKAE-HMKAIKEKGEALLR	-AVESYLL	160
192	MGVNYEETTSVIPARLF-KAFILDGNLFLPKVAPQA	-ISSVENIEGNGGP-GTIKKISFPBGG	PF-KYVKDRVDEVDHNTFKFYSYSVIEGG	-PIGDTLEKISNEIKIVATP	-DGGSLILKINNKYHTKGDHEVKAE-QIKASKEKMEGTLTL	-AVESYLLAH-SDAYN	160
193	-FNDEAETTSVIPARLF-KSFILDADNLSIKVAPQA	-FKSAENIEGNGGP-GTIKKITFPBGG	HF-KHLKQRIDEIDHNTFKFYSYSVIEGG	-PLGDTLEKISNEIKIVAAP	-DGGSLILKFSKSYVTGNISINQOE-QIKAEKEKGAGLFP	-AIEGYLL	150
194	-FNYESETTSVIPARLF-KAFILDGNLFLPKVAPQA	-ISSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-ALGDTLEKICNEIKIVATP	-DGGSLILKINNKYHTKGDHEVKAE-HMKAIKEKGEALLR	-AVESYLL	160
195	MGVNYEVEETSPVIPARLF-KSYVLGDGKILPKVAPQA	-ITSVENVNGGNGP-GTIKNITFPBGG	RY-KYVKRVEDVDHNTFTFYSYTVIEGD	-VLGDLEKVSHELKIVAAP	-DGGSLILKISKFHAKGDHEVNAE-EMKAKEMAEKLLR	-AVETVYLLAH-SAEYN	160
196	-FNYESETTSVIPARLF-KAFILDGNLFLPKVAPQA	-ISSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-ALGDTLEKICNEIKIVATP	-DGGSLILKINNKYHTKGDHEVKAE-HMKAIKEKGEALLR	-AVESYLL	160
197	MGVNYEIAETSVIPARLF-KSYVLGDGKILPKVAPQA	-ISSVENVNGGNGP-GTIKNITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-ALGDTLEKICNEIKIVATP	-DGGSLILKINNKYHTKGDHEVKAE-HMKAIKEKGEALLR	-AVESYLLAH-SDAYN	160
198	MGVNYEIAETSVIPARLF-KSYVLGDGKILPKVAPQA	-SSVENVNGGNGP-GTIKKITFPBGG	PV-KYVKRVEDVDHNTFKFYSYSVIEGG	-VLGDLEKVSHELKIVAAP	-DGGSLILKISKFHAKGDHEVNAE-EMKAKEMAEKLLR	-AVESYLLAH-TAEYN	160
199	MGVNYEIAETSVIPARLF-KSYVLGDGKILPKVAPQA	-SSVENVNGGNGP-GTIKNITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-VLGDLEKVSHELKIVAAP	-DGGSLILKISKFHAKGDHEVNAE-EMKAKEMAEKLLR	-AVESYLLAH-TDEYN	160
200	MGVNYEIAETSPVIPARLF-KSYVLGDGKILPKVAPQA	-ISSVENVNGGNGP-GTIKNITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-VLGDLEKVSHELKIVAAP	-DGGSLILKISKFHAKGDHEVNAE-EMKAKEMAEKLLR	-AVESYLLAH-TDEYN	160
201	-FNYESETTSVIPARLF-KAFILDGNLFLPKVAPQA	-ISSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-ALGDTLEKICNEIKIVATP	-DGGSLILKINNKYHTKGDHEVKAE-HMKAIKEKGEALLR	-AVESYLL	160
202	-FNYESETTSVIPARLF-KAFILDGNLFLPKVAPQA	-ISSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-ALGDTLEKICNEIKIVATP	-DGGSLILKINNKYHTKGDHEVKAE-HMKAIKEKGEALLR	-AVESYLL	160
203	-FNYESETTSVIPARLF-KAFILDGNLFLPKVAPQA	-SSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-VGDTLEKICNEIKIVAAP	-DGGSLILKINNKYHTKGNHEMKEE-QIKASKEKAEALFR	-AVESYLL	150
204	-FDYEGETTSVIPARLF-KAFILDGNLFLPKVAPQA	-SSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-AVGDTLEKICNEIKIVAAP	-DGGSLILKINNKYHTKGNHEMKAE-QIKASKEKAEALFR	-AVESYLL	150
205	MGVNYEIAETSPVIPARLF-KSYVLDFDKLIPKVAPOA	-ISSVENVNGGNGP-GTIKNITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-VLGDLEKVSHELKIVAAP	-DGGSLILKISKFHAKGDHEVNAE-EMKAKEMAEKLLR	-AVESYLLAH-TAEYN	160
206	-FDYEGETTSVIPARLF-KAFILDGNLFLPKVAPQA	-SSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-AVGDTLEKICNEIKIVAAP	-DGGSLILKINNKYHTKGNHEMKAE-QIKASKEKAEALFR	-AVESYLL	150
207	-FDYEGETTSVIPARLF-KAFILDGNLFLPKVAPQA	-SSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-AVGDTLEKICNEIKIVAAP	-DGGSLILKINNKYHTKGNHEMKAE-QIKASKEKAEALFR	-AVESYLL	150
208	MGVNYTHESTISPSRFLF-KALVHFDNLPQVVPHE	-VKNITIEVBGGGA-GSIQKMNFPBGG	PI-KYLKHKIHVIDDKMLTFKYSYLIEGGV	-LGDKLESINYDIFPEASG	-DGGCVKVTITTEYHTKGDHVIETEE-EDNVGKDKAIINFLP	-AVEAHLHAN-PSVYA	160
209	-GVFNYEIAETSVIPARLF-KAFILDGNLFLPKVAPQA	-ISSVENVNGGNGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-VLGDLEKVSHELKIVAAP	-DGGSLILKISKFHAKGDHEVNAE-EMKAKEMAEKLLR	-AVESYLLAH-TAEYN	159
210	-GVFNYEGATSVIPARLF-KAFILDGNLFLPKVAPQA	-SSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG	-AVGDTLEKICNEIKIVATP	-NGGSLILKINNKYHTKGDHEVKAE-QIKASKEKAEALFR	-AVESYLLAH-SDAYN	160
211	-FDYEGETTSVIPARLF-KAFILDGNLFLPKVAPQA	-SSVENIEGNGGP-GTIKKITFPBGG	PF-KYVKRVEDVDHNTFKFYSYSVIEGG				

266 -----MGAYTFTDKSTASVAPSRFL-KALVIDFNMLVSKLAP-D-----VKSIEINVEGDGGG-GTIKMTTFVEBG--PI--KYMKHKHVIIDEKNLVTKYSILIESDV-----TENKASVVDYDGKFEASA-----DGGSVCTTVTVYNTKGDYVVTTEE-EHNVHKEKANOLLK-----AIEAYLLAN--PSYVV-----159

267 -----MGVTFPEQSTASVPAKLY-KAMVKDADNIVPKAV-DA-----IKSTIEVEGNGGP-GTIKKLTFVEBG--QT--LYVLHKHIEADIEANLVNYSIVGGG-----GLSEPTVERYHFPAKLCEGP-----NGGSIKGVSVKYQTKGD-AKPNKEVEQEGKAGDALFK-----AIEGYVLAN--PHYN-----158

268 -----MGVNFPEEETISIVAPATLY-KALIKDADNIIIPKAV-DA-----IKSIEIVEGNGGP-GTIKKLTFVEBG--ET--LYVLHKHIEADIEANLVNYSIVGGG-----GLPDTVEKISFEAKLSAGP-----NGGSIKGVSVKYQTKGD-AKPNKEVEQEGKAGDALFK-----ALEGYCVAN--PDYN-----158

269 -----MGVTSVTHETTTIAPATLY-KALVIDDSNLIIPKLM-Q-----VKNIIEAE-----GD-GSIKKMFVEBG--PI--KYLKHKHIVDDNKNLVTKYSIMIEGDV-----LGDKLIESISYDLKFEAAG-----NGGCVCKSITSEYHTKGDYVVKDE-EHNEQKQKAGELGR-----IVEAYLLAN--PSYVA-----155

270 -----MGVIKVSTPHKTPNRMF-KALIMDSHNLQPMFSS-----INKNVEIQTGGTGP-GTIQINFTEA--PL--NVYKHKHIDELDENVMCKYTFIEVGDV-----LMKDLRIETHVLFKEAYV-----FGGCRCKITSEYHTKENMEIKEE-DEIQGQKARVGMYE-----VVEAYLLAH--PHAYN-----160

271 -----MGVTFEDEISTLPKAKLY-NAL-KDADITPKKI-DD-----VKSVIEVEGNGGP-GTIKKLITVEBG--ET--KFLHLKHVESIDEANVYNNYSIVGGG-----ALPDTAEKITFTEKLVVEBG-----NGGSIKGLTLKYHTKGD-AKPNDEELKKGKAGELGR-----AIEGYVLAN--PSQY-----157

272 -----MGVTRLSQKYATHVTPGRMF-KALILDSHNLCPKLMFSS-----IKSIEFLEGESEV-GSIQINFTEA--PL--TYVLHKHIDAEIDKNLVTKYSILEGDV-----LMKDIETIYTDVDFEYV-----FGGVCNLTISVYKTKGDVEIREE-DIEHGKDRAGIOME-----VVEAYLLAH--PRAYT-----161

273 -----MGVTFEDEISTVPAKLY-NAM-KDADISIPKII-DD-----VKSVIEVEGNGGP-GTIKKLITVEBG--ET--KFLHLKHVESIDEANVYNNYSIVGGG-----ALPDTAEKITFTEKLVVEBG-----NGGSIKGLTLKYHTKGD-AKPNDEELKKGKAGELGR-----AIEGYVLAN--PTQY-----157

274 -----MGVTTYTHEDTSTVPAKLY-KALVIDDGNLIPKLM-P-----VKNVETE-----GD-GSIIKINFVEBG--PI--KYLKHKHIVDDNKNLVTKYSILEGDV-----LGDKLIESITHVDFEPAG-----NGGCVCKTKEYTEHTKGDYVVKDE-EHNEQKQKAGELGR-----AVEYLLAN--PSIYA-----155

275 -----MGVTFEDEINSVPAATLY-KALVIDADNIVPKAL-DS-----FKSVIEVEGNGGP-GTIKKITFLEBG--ET--KFLHLKHVESIDEANLVGYSYIVGGG-----ALPDTAEKITFDSKLVEBG-----NGGSAKGLTKVYETKGD-AEPNQDELTKGAKADALFK-----AIEAYLLAH--PDYN-----158

276 -----MGAYTFTDKSTASVAPSRFL-KALVIDFNMLVSKLAP-D-----VKSIEINVEGDGGG-GTIKMTFVDDG--PI--KYMKHKHVIIDEKNLVTKYSILIESDV-----LENKASVVDYDGKFEASA-----DGGCVCTTVTVYNTIGDYVVTTEE-EHNVHKEKANOLLK-----AIEAYLLAN--PSYVV-----159

277 -----MAVTFDDQATSPVAPATLY-NALAKDADNIIIPKAV-GS-----FQSVIEVEGNGGP-GTIKKISFVEBG--ET--KFLVHLKHVESIDEANLVGYSYIVGGV-----ALPDTAEKITIDTKISDGA-----DGGSLIKLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEAYLLAN--P-----155

278 -----MGVFSFDELHSTIVPAKLY-KALAKDADDIVPKVI-EA-----IQSVIEVEGNGGP-GTIKKLTAHVGG--KT--SYVLHKHIDAEIDANFNGYNSIVGGT-----DVEDSEKIVTFETKIVAGP-----SGGSIKVISVKYHTKGDVLSDA-VRDETAKGTGLLK-----AIEGYVLAN--PDY-----157

279 -----MAVTFEDQSTSPVAPATLY-QALVKDADNIVPKAV-DS-----FKSVIEVEGNGGP-GTIKKISFLEBG--ET--KFLVHLKHIEADIEANLVGYSYIVGGG-----ALPDTAEKITIDTNI SDGP-----NGGSLIKLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----VIEAYLLAN--P-----155

280 -----MAVTFEDEPTSPVAPATLY-KALVKDADNIVPKAV-DS-----FKSVIEVEGNGGP-GTIKKISFLEBG--ET--KFLVHLKHIEADIEANLVGYSYIVGGG-----ALPDTAEKITIDTKLSDGS-----NGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----VIEAYLLAN--A-----155

281 -----MGVFVEDEITITISIPATRV-DSFVN-ADVFLKVAAEH-----IKSVTELEGDGGV-GTIKKITFVEBG--GF--GYLKQKIEVDENKNLVQYNSIVGDV-----AIVG-SIEKILNENKIIPNA-----DGGCTVKSKSTFYTS-DESEIPAQILAEQKPKRLGMPK-----ATEAYLLAN--PEAFN-----158

282 -----MAVTFEDQSTSPVAPATLY-KALVKDADNIVPKAV-DS-----FKSVIEVEGNGGP-GTIKKISFLEBG--ET--KFLVHLKHIEADIEANLVGYSYIVGGG-----ALPDTAEKITIDTKLSDGP-----NGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----VIEAYLLAN--A-----155

283 -----MGVFPEDEHPSAVAQAKLY-KALTKDSDDIIPKVI-EQ-----IQSVIEVEGNGGP-GTVKKITASHGG--HT--SYVLHKHIDAEIDANFNGYNSIVGGT-----GLDSELEKITFESKLLSGP-----DGGSIGKIKVKFHTKGD-VLSDA-VREEAKARGTGLFK-----AVEGYVLAN--PHY-----156

284 -----MGITTFEDESTSTVPAKLY-KALVADANIIIPKAV-EA-----IQSVENVEGNGGP-GTIKKLITFVEBG--ET--KFLVHLKHIEADIEANLVNYSIVGGV-----GLPDTVEKITFTEKLVVEBG-----NGGSIKGVITKIEHTKGD-AKPNQEGKAAKARGDAFFK-----AIEYVLSAH--PDYN-----158

285 -----MGVTFDEHNVSTVPAKLY-KALAKHADIIPKVI-SA-----AQSVIEVEGNGGP-GTIKKLSMEDG--KT--KFLVHLKHIDAEIDANLVNYSIVGGT-----GLDSELEKVEPTEKITVAGS-----DGGSIKVIKTVYHTKGDAAALSDA-VRNIREPTGOLIK-----AIEGYVLAN--PHY-----157

286 -----GVQKTEVIVASSVPAKLY-KALILDDIIPKALFQA-----IKSIEFLEGGGV-GTVKLVTLGEB--QF--NTMKKQVDDIVKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKVIKTVYHTKGDAAALSDA-VRNIREPTGOLIK-----AIEGYVLAN--PHY-----157

287 -----TFEDQSTSSVPAATLY-KAVAKDADITPKALPDS-----FKSVIEVEGNGGP-GTIKKISFVEBG--ET--KFLVHLKHIEADIEANLVNYSIVGGV-----ALPDTAEKITIDTKLSDGP-----NGGSIKGLTISYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----152

288 -----MGVTFEDEISTLPKAKLY-NAM-KDADISIPKII-DD-----VKSVIEVEGSGGP-GTIKKLITVEBG--ET--KFLHLKHVEADIEANVYNNYSIVGGV-----ALPDTAEKITFTEKLVVEBG-----NGGSIKGLSVKFSHGKGD-AKPEEEMKKGKAGELGR-----AIEGYVLAN--PTQY-----157

289 -----MGVFNVEDEISTVPAATLY-KALVTDADNIIIPKVI-DA-----IKSIEIVEGNGGA-GTIKKLITVEBG--ET--KHLVHLKHVELVDANLVNYSIVGGV-----GFPTVEKISFEAKLSAGP-----NGGSIKGLSVKYQTKGD-AAPTEQLTKDAGKAGDLFK-----ALEGCLAH--PDYN-----158

290 -----MGVFNVEDEISTVPAATLY-KALVTDADNIIIPKVI-DA-----IKSIEIVEGNGGA-GTIKKLITVEBG--ET--KHLVHLKHVELVDANLVNYSIVGGV-----GFPTVEKISFEAKLSAGP-----NGGSIKGLSVKYQTKGD-AAPTEQLTKDAGKAGDLFK-----ALEGCLAH--PDYN-----158

291 -----MGVVFDEDEVYSTVAPKLY-KALAKDADNIVPKVI-KE-----AQGVEIVEGNGGP-GTIKKLSFLEBG--KT--SYVLHKHIDAEIDANFNGYNSIVGGP-----GLDSELEKIVAFETIILAAGS-----DGGSIKVISVKYHTKGDAAALSDA-VRDETAKGTGLLK-----AIEGYVLAN--PGY-----157

292 -----MGVTFEDEISTLPKAKLY-NAM-KDADISIPKII-DD-----VKSVIEVEGNGGP-GTIKKLITVEBG--ET--KFLHLKHVEADIEANVYNNYSIVGGV-----ALPDTAEKITFTEKLVVEBG-----NGGSIKGLSVKFSHGKGD-AKPEEEMKKGKAGELGR-----AIEGYVLAN--PTQY-----157

293 -----NFEEETISIVAPATLY-KALVTDADNIIIPKVI-DA-----IKSIEIVEGNGGP-GTIKKLITVEBG--ET--KHLVHLKHVELVDANLVNYSIVGGV-----GLPDTVEKISFEAKLSAGP-----NGGSIKGLSVKYQTKGD-AIPSEELKKGKAGELGR-----ALEGYCVAN--PDYN-----155

294 -----MGVFNVEDEISTVPAATLY-KALVTDADNIIIPKVI-DA-----IKSIEIVEGNGGA-GTIKKLITVEBG--ET--KHLVHLKHVELVDANLVNYSIVGGV-----GFPTVEKISFEAKLSAGP-----NGGSIKGLSVKYQTKGDAAAPTEQLKSDAKAGDLFK-----ALERYCLAH--PDYN-----159

295 -----GVFAFEDEHPSAVAQAKLY-KALTKDSDDIIPKVI-EQ-----IQSVIEVEGNGGP-GTVKKITASHGG--HT--SYVLHKHIDAEIDANFNGYNSIVGGT-----GLDSELEKITFESKLLSGP-----DGGSIGKIKVKFHTKGD-VLSDA-VREEAKARGTGLFK-----AVEGYVLAN--PHY-----155

296 -----MGVFNVEDEISTVPAATLY-KALVTDADNIIIPKVI-DA-----IKSIEIVEGNGGA-GTIKKLITVEBG--ET--KHLVHLKHVELVDANLVNYSIVGGV-----GFPTVEKISFEAKLSAGP-----NGGSIKGLSVKYQTKGDAAAPTEQLKSDAKAGDLFK-----ALERYCLAH--PDYN-----159

297 -----MAVTFEDQSTSPVAPATLY-NALAKDADNIIIPKAV-DS-----FKSVENVEGNGGP-GTIKKISFVEBG--ET--KFLVHLKHIEADIEANFNGYNSIVGGV-----GLPGECEKITIDTKLSDGP-----DGGSLIKLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--A-----155

298 -----MGVKSFFQEMKTKISSRSL-KALVTSPEVVPK-FTTS-----IKSIELIQSGGYAPGAFQTFNPFGA--HF--KYMKQRVDEIDHEKHSIKVTLIEGDM-----LGDKLEKICYDMKFEDTE-----DGGCVKVTSEYHTKGGYELADE-DLKAGKESLGMVY-----SCEDYLLAN--PHVCA-----160

299 -----MGVFSFEEETISIVAPSKLY-KALTKDSDIIPKVI-EP-----IQSVIEVEGNGGP-GTIKKITFVEBG--HT--KFLVHLKHIEADIEANFNGYNSIVGGV-----GLPGECEKITIDTKLSDGP-----DGGSLIKLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEGYVLAN--PHY-----156

300 -----MGVFAFDQSTVITAPKLY-KALAKDADIIPKVI-PV-----IQPVEIVEGNGGP-GTIKKLITVEBG--KT--TFILHLKHVEADIEANLVNYSIVGGV-----GLDSELEKVEPTEKISVAGS-----DGGCVKVTSEYHTKGGYELADE-DLKAGKESLGMVY-----SCEDYLLAN--PHVCA-----160

301 -----MGVTKISQKVFQVTPQRM-KALLLDADNIIIPKALPDS-----IKSIEFLLSGGSEV-GTIQINFTEA--PM--KYMKHIDAEIDANFNGYNSIVGGT-----DHLDKLEYITDYVDFEYV-----RGGCIHLTSTYKAKDIIQKEE-DIELGDRAGIOME-----VVEAYLLAH--PRAYV-----163

302 -----MGVTSYDMEIKSSLSAALKY-KAFVLDVGLINKALPNV-----IKSVIEIQDGGGA-GTIKLVHFGEGG--PV--PSVKHVEELDKNNMSYKYSIVDGE-----ALMPGLQISYIYKIEPSS-----HGSVCKHNTTFHFKAQSNINDE-EIKAGKERAEMIK-----AVEAYVQAN--PDY-----157

303 -----RGNSIANGGP-GTIKKITFAEBS--FT--SPVLKQKVEIDENFNTYSYISIEGG-----PVGDTXDKISYVTRMVGAG-----NGGSIKGLTI SYHTKGDVEPISEE-LKAGKQKAEALFK-----AIEYVLSAH--PDYN-----155

304 -----MGIFAFENEQSTVAPAKLY-KALTKDSDIIPKVI-EP-----IQSVIEVEGNGGP-GTIKKITASHGG--HT--SFVLHKHIDAEIDANLVNYSIVGGG-----GLDSELEKITSYESKILPGP-----DGGSIGKIKVKFHTKGD-VLSET-VRDQAKFGLGGLFK-----AIEGYVLAN--PDY-----156

305 -----MGITTFEDESTSTVPAATLY-KALVTDADNIIIPKAV-EA-----FRSVENVEGNGGP-GTIKKITFVEBG--ES--KFLVHLKHIEADIEANLVNYSIVGGV-----GLPDTVEKITFTEKLVVEBG-----NGGSIKGLSVKYQTKGD-AQNPDDLKIKGVKSDALFK-----AVEYLLAN--PHYN-----158

306 -----MGVTFDEHNVSTVPAKLY-KALILDDIIPKALFQA-----IKSIEFLEGGGA-GTVKLVTLGEB--QF--NTMKKQVDDIVKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKVIKTVYHTKGDAAALSDA-VRNIREPTGOLIK-----AIEGYVLAN--PHY-----157

307 -----MGVKSSEVSTVPAKLY-KALVTDADNIIIPKALFQA-----IKSIEFLEGGGA-GTVKLVTLGEB--QF--NTMKKQVDDIVKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKVIKTVYHTKGDAAALSDA-VRNIREPTGOLIK-----AIEGYVLAN--PHY-----157

308 -----MGVTFPEEESTVPAKLY-KATVVDGDELTPKLI-PA-----IQSVIEVEGNGGP-GTVKKVTAVEBG--KT--SFVLKQKVEIDENFNTYSYISIEGG-----CQOEILKVSFTKLEA-A-----DGGSIKVISVTFHTKGDAPLDE-VHODVKQKSGIFK-----AIEGYVLSN-----153

309 -----MGAYTFTNESTVAPASRL-KALVIDFNHIVCKLMP-D-----VESIENIEVGGGA-GTIKKVTFVEBG--PM--KYLKHKHIVIDEENLVTKYSILEGDA-----LANKADSVVDYDGFEASA-----DGGCVGTTVTVYNTIGDYVVTTEE-EHNLHARDAVIT-----AVVYLLTN--PSAVA-----159

310 -----MGVQKSEVSTVPAKLY-KGCLDILIDTLPLVLPGA-----IKSSETLEGDGGV-GTVKLVHLGDS--PF--TKMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKVISVTFHTKGDAPLDE-VHODVKQKSGIFK-----AIEGYVLSN-----153

311 -----MAAYTIVKEEESTVAPKLY-KALVLERHGVVKAQPHV-----FKSGEIEGDDGV-GTVKLVTFVDDG--PL--TYMLKHIDAEIDANFNTYSYISIEGG-----LRONIKIEVSEVLEKAGV-----DGGSGKIKTVSHPKPGCTVNEE-EVKIIEKKAEPFYK-----QVEEYLAAN--PEVFA-----159

312 -----MGVQKSEVSTVPAKLY-KGCLDILIDTLPLVLPGA-----IKSSETLEGDGGV-GTVKLVHLGDS--PF--TKMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKVISVTFHTKGDAPLDE-VHODVKQKSGIFK-----AIEGYVLSN-----153

313 -----GIFAFENEQSTVAPAKLY-KALTKDSDIIPKVI-EP-----IQSVIEVEGNGGP-GTIKKITASHGG--HT--SFVLHKHIDAEIDANLVNYSIVGGG-----GLDSELEKITSYESKILPGP-----DGGSIGKIKVKFHTKGD-VLSET-VRDQAKFGLGGLFK-----AIEGYVLAN--PDY-----155

314 -----MGVQKHEQESTVPAKLY-KGCLDILIDTLPLVLPGA-----IKSSETLEGDGGV-GTVKLVHLGDS--PF--TKMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKVISVTFHTKGDAPLDE-VHODVKQKSGIFK-----AIEGYVLSN-----153

315 -----MGVSVYDLEVTLPNCSSQGSRLFLNLIPKVMPTVALPH-----VRSFE-LEGDASP-GSIVKITFVEGL--PY--QYMKHQJGGHEDNNFSYYSIMIEGDV-----PLGDKLIESYENGFVAA-----DGGSIKSVLTKFHTKGDAPLDE-VHODVKQKSGIFK-----AIEGYVLSN-----153

316 -----GVFREDSTSSVPAKLY-KALTKDSDITIAQID-GP-----IQSVIEVEGNGGP-GTIKKITFVEBG--ET--KFLVHLKHIEADIEANFNGYNSIVGGT-----GLDSELEKITFTEKLVVEBG-----NGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

317 -----MGAYTFTDKSTASVAPSRFL-KALVIDFNMLVSKLAP-D-----VKSIEINVEGDGGG-GTIKMTFVEBG--PI--KYMKHKHVIIDEKNLVTKYSILIESDV-----LENKASVVDYDGKFEASA-----DGGCVCTTVTVYNTIGDYVVTTEE-EHNVHKEKAMTFSRPSKHTSPILLSMFKPMFTLL-----166

318 -----MAVA-EFEITSSSPSNIIF-KAFVIDDITPIAKAEPET-----YKSIIKTIEGDDGV-GTISIKYSIDGV--PI--TSKHKHVIDIDENNFNYSIVTIFEGD-----VLMDIEGTHTKFLPASA-----DGGSVKHSMPFKCKGDAKLTDE-NIKYANMKEGTLFK-----AIEYVLSAH--PEAC-----157

319 -----MGITKHIQELKLRVSAKRMF-KALVTSIESHP--LPDA-----IKSIELIHDGGA-GTIRKTNLADGS-----YKRIEIAVDINQVSKYTVIEGPM-----LGDKLIESIHYEQKFEDSS-----DGGCVAKIVCEYHTKGDILQKEE-GVKAINDQALGFT-----LSEYVLAN--PHVCA-----154

320 -----MGVFNVEDEISTVPAKLY-KALTKDSDITIAQID-GP-----IQSVIEVEGNGGP-GTIKKITFVEBG--ET--KFLVHLKHIEADIEANFNGYNSIVGGT-----GLDSELEKITFTEKLVVEBG-----NGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

321 -----KVF-KAFVLDVGLINKALPNV-----IKSVIEIQDGGGA-GTIKLVHFGEGG--PV--PSVKHVEELDKNNMSYKYSIVDGE-----ALMPGLQISYIYKIEPSS-----HGSVCKHNTTFHFKAQSNINDE-EIKAGKERAEMIK-----AVEAYVQAN--PDY-----140

322 -----MGVQTHVLEISTVSAEKIF-KGQFIDVDTVPKPAAPGA-----YKSVIEIK-GDGGV-GTLKIIITLDPGG--PV--TMTVTRDANVKEALTYDSTVIGD-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

323 -----GVQKTEVIVASSVPAKLY-KGSLDMDITVPKPAFEG-----IKSVQVLEGGGV-GTIKNVTLGDAT--PF--NTMKTRIDAEIDANFNTYSYISIEGG-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

324 -----MGVQKTVVEAPSTVSAEKMY-KGFLDMDITVPKLPQL-----IKSVIELEGDGGV-GTVKLVHLGEAT--EY--TFMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

325 -----MEVLTTFEESTVPAKLY-KAMVLEADITLIPKLPQA-----VKSIEITKNGGP-GTIKKLTFVEBG--ET--KFLVHLKHIEADIEANLVNYSIVGGV-----GLPDTVEKITFTEKLVVEBG-----NGGSIKGLSVKYQTKGD-AQNPDDLKIKGVKSDALFK-----AVEYLLAN--PHY-----158

326 -----MGVQKSEVSTVPAKLY-KGCLDILIDTLPLVLPGA-----IKSIEFLEGGGA-GTVKLVTLGEB--QF--NTMKKQVDDIVKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKVIKTVYHTKGDAAALSDA-VRNIREPTGOLIK-----AIEGYVLAN--PHY-----157

327 -----MGVQKSEVSTVPAKLY-KGCLDILIDTLPLVLPGA-----IKSIEFLEGGGA-GTVKLVTLGEB--QF--NTMKKQVDDIVKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKVIKTVYHTKGDAAALSDA-VRNIREPTGOLIK-----AIEGYVLAN--PHY-----157

328 -----LIFLLILSNLILNMAQSHSLEISTVSAEKIF-KGCLDILIDTLPLVLPGA-----IKSIEFLEGGGA-GTVKLVTLGEB--QF--NTMKKQVDDIVKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKVIKTVYHTKGDAAALSDA-VRNIREPTGOLIK-----AIEGYVLAN--PHY-----157

329 -----MGVQKSEVSTVPAKLY-KGCLDILIDTLPLVLPGA-----IKSIEFLEGGGA-GTVKLVTLGEB--QF--NTMKKQVDDIVKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKVIKTVYHTKGDAAALSDA-VRNIREPTGOLIK-----AIEGYVLAN--PHY-----157

330 -----MAVTVIEVSSSTVPAKLY-KVPS-DFDITLAPKVEPET-----YKAVNIIEGDDGV-GTISIKYTDGV--PF--TSKHKHVIDIDENNFNYSIVTIFEGD-----VLMDIEGTHTKFLPASA-----DGGSVKHSMPFKCKGDAKLTDE-NIKYANMKEGTLFK-----AIEYVLSAH--PEAC-----157

331 -----MAVFEKDVFTSTLPKLY-KAMFIDSNLTLPIAPDH-----YKSVIEIK-GDGGV-GTIRNITLDPGG--PV--TMTVTRDANVKEALTYDSTVIGD-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

332 -----MGVQKTVVEAPSTVSAEKMY-KGFLDMDITVPKLPQL-----IKSVIELEGDGGV-GTVKLVHLGEAT--EY--TFMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

333 -----MGVTFEESTVPAKLY-KALTKDSDITIAQID-GP-----IQSVIEVEGNGGP-GTIKKITFVEBG--ET--KFLVHLKHIEADIEANFNGYNSIVGGT-----GLDSELEKITFTEKLVVEBG-----NGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

334 -----MAVVDNDVFTSTVPAKLY-KAMFIDCDTLPIAPDH-----YKAVNIIEGDDGV-GTISIKYTDGV--PF--TSKHKHVIDIDENNFNYSIVTIFEGD-----VLMDIEGTHTKFLPASA-----DGGSVKHSMPFKCKGDAKLTDE-NIKYANMKEGTLFK-----AIEYVLSAH--PEAC-----157

335 -----MGVHTFEESTVPAKLY-KATVVDGDELTPKLI-PA-----IQSVIEVEGNGGP-GTVKKVTAVEBG--KT--SFVLKQKVEIDENFNTYSYISIEGG-----CQOEILKVSFTKLEA-A-----DGGSIKVISVTFHTKGDAPLDE-VHODVKQKSGIFK-----AIEGYVLSN-----153

336 -----MVTGTTTTERVSKVAKRLMSATKDNH-FLPKIWPEI-----FSSVTFLEGG-GAGGTIKQFNFTPAATKEF--SYVKERVDEIDHEKHSIKVTLIEGDM-----LGDKLEKICYDMKFEDTE-----DGGCVKVTSEYHTKGGYELADE-DLKAGKESLGMVY-----SCEDYLLAN--PHVCA-----160

337 -----VPKAKLY-NAM-KDADISIPKII-DD-----VKSVIEVEGNGGP-GTIKKLITVEBG--ET--KFLVHLKHIEADIEANFNGYNSIVGGV-----ALPDTAEKITFTEKLVVEBG-----NGGSIKGLTLKYHTKGD-AKPNDEELKKGKAGELGR-----AIEGYVLAN--PTQY-----157

338 -----MGVQKSEVSTVPAKLY-KGCLDILIDTLPLVLPQA-----IKSVIELEGDGGV-GTVKLVHLGEAT--EY--TFMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

339 -----MGVQKSEVSTVPAKLY-KGCLDILIDTLPLVLPQA-----IKSVIELEGDGGV-GTVKLVHLGEAT--EY--TFMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

340 -----MASTNSWTHIESPVAPARLFRAMVMDHT-LAPKIASHI-----VASAHVDDG-GVSGVVRQFNFT--SF-MPF--SFMKERVDEIDHEKHSIKVTLIEGDM-----LGDKLEKICYDMKFEDTE-----DGGCVKVTSEYHTKGGYELADE-DLKAGKESLGMVY-----SCEDYLLAN--PHVCA-----160

341 -----MVSSTSEVSTVPAKLY-KGCLDILIDTLPLVLPQA-----IKSVIELEGDGGV-GTVKLVHLGEAT--EY--TFMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

342 -----MASANSWTHIESPVAPARLFRAMVMDHT-LAPKVASHI-----VASAHVDDG-GVSGVVRQFNFT--SF-MPF--SFMKERVDEIDHEKHSIKVTLIEGDM-----LGDKLEKICYDMKFEDTE-----DGGCVKVTSEYHTKGGYELADE-DLKAGKESLGMVY-----SCEDYLLAN--PHVCA-----160

343 -----MVTGTTTTERVSKVAKRLMSATKDNH-FLPKIWPEI-----FSSVTFLEGG-GAGGTIKQFNFTPAATKEF--SYVKERVDEIDHEKHSIKVTLIEGDM-----LGDKLEKICYDMKFEDTE-----DGGCVKVTSEYHTKGGYELADE-DLKAGKESLGMVY-----SCEDYLLAN--PHVCA-----160

344 -----MASANSWTHIESPVAPARLFRAMVMDHT-LAPKVASHI-----VASAHVDDG-GVSGVVRQFNFT--SF-MPF--SFMKERVDEIDHEKHSIKVTLIEGDM-----LGDKLEKICYDMKFEDTE-----DGGCVKVTSEYHTKGGYELADE-DLKAGKESLGMVY-----SCEDYLLAN--PHVCA-----160

345 -----MAS-NSTWTHIESPVAPARLFRAMVMDHT-LAPKVASHI-----VASAHVDDG-GVSGVVRQFNFT--SF-MPF--SFMKERVDEIDHEKHSIKVTLIEGDM-----LGDKLEKICYDMKFEDTE-----DGGCVKVTSEYHTKGGYELADE-DLKAGKESLGMVY-----SCEDYLLAN--PHVCA-----160

346 -----MVSSTSEVSTVPAKLY-KGCLDILIDTLPLVLPQA-----IKSVIELEGDGGV-GTVKLVHLGEAT--EY--TFMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

347 -----MVSSTSEVSTVPAKLY-KGCLDILIDTLPLVLPQA-----IKSVIELEGDGGV-GTVKLVHLGEAT--EY--TFMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

348 -----MVSSTSEVSTVPAKLY-KGCLDILIDTLPLVLPQA-----IKSVIELEGDGGV-GTVKLVHLGEAT--EY--TFMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

349 -----MVSSTSEVSTVPAKLY-KGCLDILIDTLPLVLPQA-----IKSVIELEGDGGV-GTVKLVHLGEAT--EY--TFMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

350 -----MVSSTSEVSTVPAKLY-KGCLDILIDTLPLVLPQA-----IKSVIELEGDGGV-GTVKLVHLGEAT--EY--TFMKQKVDIDKAGLTIYDVTISIGGT-----LLDILIESYNMHTVPT-----DGGSIKGLTI SYHGKGD-APPNEDELKAGKAKSDALFK-----AVEYLLAN--P-----155

351 -----MVSQTSSEEVQVQEAARLWNATKDSHN-FLPKVLPEV-----FTSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTAIEGG-----PLGKKLSASACFEVKLVPRK-----EGGCVARWTCNYETLPGVQPDGE-KFKEIKEDSFGLK-----KVEQYLLSN-PNLVC-----161

352 -----MLGQKHQEQIESSSLAEKIF-NGLIVDVDTFFPKAAGPA-----YKXNVEIG-GDGGV-GTIIKHITLPDGS-PV-----FTMLRLTDALDKCACTVEYSI1DGD-----VLGLLDIKVETHLVVVPNA-----DGGSTTKTTT1FHTKGDVAVPEE-NIKYSEEQNISVFKE-----AAEAYLLAN-----154

353 -----MASANSWTLEIPSPVAAPRLFRAAVIDWHT-LAPKVAHVH-----VSAHPEVGD-GGVGSVRQFNFT-SF-MFP-----SFMKERLDLFDVOKCECKNTLVEGG-----GTSAHTEIATAVAGQLFKAALDWHN-LGPKLPEI-----VSSASVVAVD-GGVGSVRQFNFT-SA-MFP-----PYLKERLDFVDEANFECKSSLIIEGG-----DLGTTKLSASSHFVKLVPSK-----NGGCVVKLEGIFKALPGVETTD-EVAKGKEMTMNAIK-----AAEAYLLAN-PTAYL-----159

354 -----MVSQTSSEEVQVQEAARLWNATKDSHN-FLPKVLPEV-----FTSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTAIEGG-----PLGKLSASACFEVKLVPRK-----EGGCVARWTCNYETLPGVQPDGE-KLKEIKEDSFGLK-----KVEQYLLSN-PNLVC-----161

355 -----MVSQTSSEEVQVQEAARLWNATKDSHN-FLPKVLPEV-----FTSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTAIEGG-----PLGKLSASACFEVKLVPRK-----EGGCVARWTCNYETLPGVQPDGE-KFKEIKEDSFGLK-----KVEQYLLSN-PNLVC-----161

356 -----MVSQTSSTEDVQVQEAARLWNATKDSHN-FLPKVLPEV-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTAIEGG-----PLGNKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KIKEIKEDSLALLK-----KVEQYLLSN-PNLVC-----161

357 -----MVSQTSSEEVQVQEAARLWNATKDSHN-FLPKALPEI-----FTSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTAIEGG-----PLGKLSASACFEVKLVPRK-----EGGCVARWTCNYETLPGVQPDGE-KFKEIKEDSFGLK-----KVEQYLLSN-PNLVC-----161

358 -----MASANSWTLEIPSPVAAPRLFRAAVIDWHT-LAPKVNHSV-----VASAHPVEGD-GGVGSVRQFNFT-SF-MFP-----SFMKERLDLFDVOKCECKNTLVEGG-----NIGVAIETATAVAGQLFKAALDWHN-LGPKLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTAIEGG-----PLGKLSASACFEVKLVPRK-----EGGCVARWTCNYETLPGVQPDGE-KFKEIKEDSFGLK-----KVEQYLLSN-PNLVC-----161

359 -----MVSQTSSEEVQVQEAARLWNATKDSHN-FLPKVLPEV-----FTSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTAIEGG-----PLGKLSASACFEVKLVPRK-----EGGCVARWTCNYETLPGVQPDGE-KFKEIKEDSFGLK-----KVEQYLLSN-PNLVC-----161

360 -----MVSQTSSEEVQVQEAARLWNATKDSHN-LLPKVLPEI-----FASVTCHQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTAIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

361 -----MVSQTAITEDVQVQEAARLWNATKDSHN-LLPKVLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTAIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

362 -----TEDLCEVEAARLWNAMVKDGHN-LFPKIFPEF-----FSSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SYAKERVDEDEEMVFKYTTIEGG-----LLGKNLSALNLFELFKVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

363 -----MVGATSTSEEVQVQEAARLWNATKDSHN-FLPKVPEF-----FTSVTLTQGD-GGVGSVKQFNITPGK-KDF-SFIKERVDELDEENFVYKYTAIEGG-----PLGKLSASACFEVKLVPRK-----EGGCVARWTCNYETLPGVQPDGE-KLKEIKEDSFGLK-----KVEQYLLSN-PNLVC-----161

364 -----MVSRTAITEDVQVQEAARLWNATKDSHN-LLPKLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

365 -----MVGATATTEDLCEVEAARLWNAMVKDGHN-LFPKIFPEF-----FSSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SYAKERVDEDEEMVFKYTTIEGG-----PLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

366 -----MVGATATTEDLSEVEAARLWNAMVKDGHN-LFPKIFPEF-----FSSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SYAKERVDEDEEMVFKYTTIEGG-----LLGKNLSALNLFELFKVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

367 -----MASGATATEDLSEVEAARLWNALVKDSHN-LFPKIFPEF-----FSSVTLTQGD-GGVGTIKELNFTPGK-KDF-SYAKERVDELDEENFVYKYTTIEGG-----LLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLAGAPVDEG-KAKEIKENSIGMLK-----KIEQYLLSN-PSLYC-----161

368 -----MSSASVSHIESSEVSAARLFKMAITWNN-LAPKILPEI-----VSSASVVAVD-GGVGSVRQFNFT-SA-MFP-----PYLKERLDFVDEANFECKSSLIIEGG-----DLGTTKLSASSHFVKLVPSK-----NGGCVVKLEGIFKALPGVETTD-EVAKGKEMTMNAIK-----AAEAYLLAN-PTAYL-----159

369 -----MVGATSTSEEVQVQEAARLWNATKDSHN-FLPKVPEF-----FTSVTLTQGD-GGVGSVRQFNITPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----PLGKLSASACFEVKLVPRK-----EGGCVARWTCNYETLPGVQPDGE-KLKEIKEDSFGLK-----KVEQYLLSN-PNLVC-----161

370 -----MVSUTLEVEVSSQIPPEKVE-KVFS-DFDNIAKVNQVQ-----FKSIETVEGDDGV-GSVKLFPTFGDGV-PF-----TSQCKVDALDVSINYSITFTFEGD-----SLFGVLDSDINHNHVKVPSP-----NGGCVFKQITVYCNCKGKDEKPSB-LIKQKQKTIVENTFK-----AIEAYIAVH-ETIT-----158

371 -----MVSQTAITEDVQVQEAARLWNALVKDSHN-LGPKLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

372 -----TEDVCAVEAARLWNAMVKDGHN-LFPKVLPEF-----FSSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SYAKERVDELDEENFVYKYTTIEGG-----PLGKLSALNLFELFKVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

373 -----TEDLCEVEAARLWNAMVKDGHN-LFPKIFPEF-----FSSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SYAKERVDEDEEMVFKYTTIEGG-----LLGKNLSALNLFELFKVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

374 -----TEDVCAVEAARLWNAMVKDGHN-LFPKVLPEF-----FSSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SYAKERVDELDEENFVYKYTTIEGG-----LLGKLSALNLFELFKVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

375 -----TEDVCAVAKARLWNAMVKDGHN-LFPKVLPEF-----FSSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SYAKERVDELDEENFVYKYTTIEGG-----PLGKLSALNLFELFKVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

376 -----TEDLCEVEAARLWNAMVKDGHN-LFPKIFPEF-----FSSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SYAKERVDELDEENFVYKYTTIEGG-----LLGKLSALNLFELFKVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

377 -----MASGATATEDLSEVEAARLWNALVKDSHN-LFPKIFPEF-----FSSVTLTQGD-GGVGTIKELNFTPGK-KDF-SYAKERVDELDEENFVYKYTTIEGG-----LLGKLSAMNLFELFKVPRK-----EVGCVSWTCNYETLAGAPVDEG-KAKEIKENSIGMLK-----KIEQYLLSN-PSLYC-----161

378 -----MVSQTAITSEHFSEVEAARLWNALVKDSHN-LPKLPEF-----FSSATFTQGD-GGVGTIKELNFTPGK-KDF-SYAKERVDELDEENFVYKYTTIEGG-----LLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

379 -----MVSQTPVTEDEVQVQEAARLWNAMVKDGHN-LFPKVLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

380 -----TEDLCEVEAARLWNAMVKDGHN-LFPKIFPEF-----FSSVTLTQGD-GGVGTVKQLNFTPGK-KDF-SYAKERVDELDEENFVYKYTTIEGG-----LLGKLSALNLFELFKVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

381 -----MASANSWTLEIPSPVAAPRLFRAAVIDWHT-LAPKILPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

382 -----MSSQGSWSHEIATAVAPAGQLFKAALDWHN-LGPKLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

383 -----MASTNSWTEIESPVAAPRLFRAAVIDWHT-LAPKILPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

384 -----MPSLEIESSEVSAARLFWNL-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

385 -----MVGATVTTEVYSQVETKRLWNAMVKDGHN-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

386 -----MPSLEIESSEVSAARLFWNL-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

387 -----MPSLEIESSEVSAARLFWNL-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

388 -----GMSWHEAVNVAAARLFWNL-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

389 -----MPSLEIESSEVSAARLFWNL-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

390 -----MPSLEIESSEVSAARLFWNL-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

391 -----MVSQ-----SKELMERFQGGQ-FALPKACAGL-----VSDIVBESGAGV-GTITMKNLPAAGVGS-----TYKTRVAVCDASHVLSKDSVLEAESK-----VGKLSHSSTETKLEATG-----DGSVAKLKVEYE-LEDGSSLSPEKEDVLDGVYGLMK-----MIEDYLVAH-PTAYL-----153

392 -----MSSGWSHEIATAVAPAGQLFKAALDWHN-LGPKLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

393 -----MSSGWSHEIATAVAPAGQLFKAALDWHN-LGPKLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

394 -----MSSGWSHEIATAVAPAGQLFKAALDWHN-LGPKLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

395 -----MSSGWSHEIATAVAPAGQLFKAALDWHN-LGPKLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

396 -----MSSGWSHEIATAVAPAGQLFKAALDWHN-LGPKLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

397 -----MSSGWSHEIATAVAPAGQLFKAALDWHN-LGPKLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

398 -----MSSGWSHEIATAVAPAGQLFKAALDWHN-LGPKLPEI-----FASVTLTQGD-GGVGTVKQLNFTPGK-KDF-SFIKERVDELDEENFVYKYTTIEGG-----SLGKLSASACFEVKLVPRK-----EGGCVASWTCNYETLPGVQPDGE-KAKEIKENSIGMLK-----KIEQYLLSN-PNLVC-----161

399 -----MAQSVSVEIDLKVPQAKAW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

400 -----MKSVMNEIDLKVPQAKAW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

401 -----MKSVMNEIDLKVPQAKAW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

402 -----MAQSVSVEIDLKVPQAKAW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

403 -----MAQSVSVEIDLKVPQAKAW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

404 -----MARAVTVELDLRVPQAKAW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

405 -----MASKVELVVEVKSADKLW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

406 -----MASKVELVVEVKSADKLW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

407 -----MASKVELVVEVKSADKLW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

408 -----MAPACVSDHVAIVSAARLFWNL-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

409 -----MRGSSQHELETDVASELMKVIYGTIRLAAELPELLPHI-----LAKVELVTGDDGV-GTIVRLTFFPGIP-----GLQSYKEKFIKVNKNVYKAEAAVEGD-----ILKGLFSLYMIFFELIRK-----ANTSVIRSTIYEIGDEHPELQAMVSTASLAATAEFAK-----EYIKQKQVQAQNT-----151

410 -----MAPACVSDHVAIVSAARLFWNL-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

411 -----MAPACVSDHVAIVSAARLFWNL-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

412 -----MVKENQOTELSVRLALM-AVLKSKFITVVPKVLPHI-----VKGDLQIEGDDGV-GTILIFNPLFEVPS-----SYQREKIEITEPSSHEIGLQVIEGG-----YLNQGLSYRKTFTKLEIE-----EDKTLVNVKISYDHDSDIEEK-VTPTKTSQSTLMLR-----LERVLSNG-SA-----155

413 -----MVKENQOTELSVRLALM-AVLKSKFITVVPKVLPHI-----VKGDLQIEGDDGV-GTILIFNPLFEVPS-----SYQREKIEITEPSSHEIGLQVIEGG-----YLNQGLSYRKTFTKLEIE-----EDKTLVNVKISYDHDSDIEEK-VTPTKTSQSTLMLR-----LERVLSNG-SA-----155

414 -----MASKVELVVEVKSADKLW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

415 -----MASKVELVVEVKSADKLW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

416 -----MAPVCISDERSAVSAARLFWNL-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

417 -----MVAGCVITEQCALAVSTERLWNVKVFSGEDTAAALPKACAGL-----IDVVEBEGDDGV-GSVFTMTLSAAELSGSGVMRSVMARNAARVFKTELLEGGV-----SQGLKSQVEMKLEAAG-----DGACVAKLRVEYERLDGGGALSADQAAALAGVLDQL-----MVEAYLVAH-PAEY-----166

418 -----MAPAFVSDERSAVSAARLFWNL-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

419 -----MIKEPITQOELSVGLLEILW-QAMSKDLNVTQKIPNI-----VKGDLQIEGDDGV-GTILIFNPLFEVPS-----SYQREKIEITEPSSHEIGLQVIEGG-----YLNQGLSYRKTFTKLEIE-----EDKTLVNVKISYDHDSDIEEK-VTPTKTSQSTLMLR-----LERVLSNG-SA-----155

420 -----DAEVEVSSADKFW-TDLRESTLFPKIFPEF-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

421 -----MGLSGVLHVEVKSADKLW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

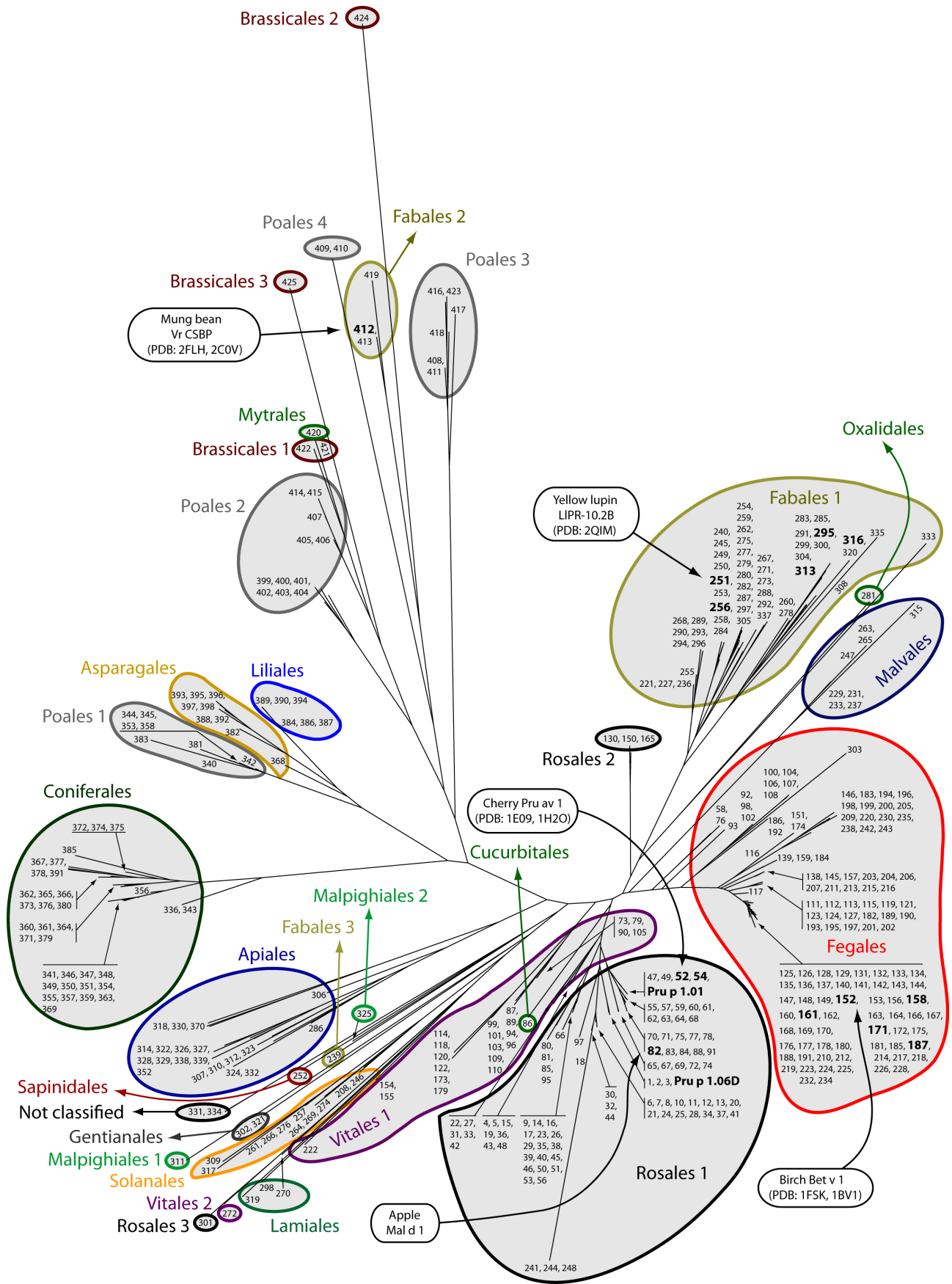
422 -----MGLSGVLHVEVKSADKLW-DAIRDSASLFPKIMPSH-----FKSIEVI-DGG-NVGTIRRIKYGEAMK-----GATHASEKIEVLDEENMTVYTVIEGE-----LLSIVYKVPRTFLVLPAGADAN-----SCSVSWTEFEFEPAGNTPPSD-----PIKEAATINTFK-----AMEAYLLTA-----151

423 -----MAPVCISDERSAVSAARLFWNL-LGPKLPEI-----LLSGSIVBESGAVGGIRQLNFS-SV-MFP-----SYVKEKRLDFIHEKFECKVSAVGEV-----HLGTILLESASAHFVKVPNS-----SGGCVVKVTESKLPKGVAPGD-DEAKAKEMVLMFK-----AAEAYLLAN-PDAYL-----156

424 -----MKSQSGVCHGVTVQTNAPLPLWMLRRFPNPKFKHFVKTKCLRSDDGGE-GSVRETVVSDLE-----SFTKLRLDELDEEDSHVYSI1DGD-----HLRVNVQSVYVDVPEGNTTEETTLFADTVIYCNGLNSLAK-----LSKEMMLT-----160

425 -----MAEASSLVIGVETTVELKSSVEKFIH-DLLVGRPHHMSNATPSN-----IQASLEQEGEMGGQVAVILWNVHGD-----EAKSAKORIESLOPEKRNITVYRVEGD-----LLKEVTSFYTTQVTPKEGE-----PGSVAHWHFEYKINEEVAHPE-----TILQLATEVSK-----DMEHLLSEE-----159

Figure S5. Unrooted phylogenetic tree of PR-10 proteins calculated from the alignment reported in the Supplemental Figure S4. Reported PR-10 proteins are from the following sources: *Prunus persica* (peach), Pru p 1.01, Pru p 1.06D, 47; *Prunus avium* (cherry), 1-3, 49, 52, 54; *Malus x domestica* (apple), 4-26, 28-46, 48, 50, 51, 53, 56, 57, 59-64, 66, 68, 71, 75, 77, 78, 80-85, 88, 91, 95, 241, 244, 248, 301; *Prunus armeniaca* (apricot), 25, 55; *Fagus sylvatica* (European beech), 58; *Fragaria x ananassa* (strawberry), 65, 67, 69, 72, 74; *Pyrus communis* (pear), 70; *Vitis vinifera* (grape vine), 73, 79, 87, 89, 90, 94, 96, 99, 101, 103, 105, 109, 110, 154, 155, 173, 222, 272; *Castanea sativa* (sweet chestnut), 76; *Datisca glomerata* (Durango root), 86; *Betula pendula* (birch), 92, 98, 102, 111-113, 115, 117, 119, 121, 123-129, 131-145, 147-149, 152, 153, 156-158, 160-164, 166, 168-172, 175, 177, 178, 181, 182, 184-193, 195, 197, 201-204, 206, 207, 210-219, 223-226, 228, 232, 234; *Corylus avellana* (hazel), 93, 100, 104, 106, 107, 116, 146, 183, 194, 220; *Rubus idaeus* (raspberry), 97; *Corylus heterophylla* (Asian hazel), 108; *Vitis pseudoreticulata*, 114, 118, 122; *Vitis quinquangularis*, 120; *Morus bombycis*, 130, 150, 165; *Carpinus betulus* (hornbeam), 151, 174, 196, 198-200, 205, 209, 230, 235, 238, 242, 243; *Alnus glutinosa* (alder), 159; *Betula platyphylla* var. *japonica* (Japanese white birch), 167, 176, 180; *Vitis hybrid cultivar*, 179; *Solanum virginianum* (Surattense nightshade), 208; *Medicago truncatula* (barrel clover), 221, 333; *Medicago sativa* (alfalfa), 227, 236, 255, 285; *Gossypium hirsutum* (Mexican cotton), 229, 247, 263; *Gossypium arboreum* (cotton), 231; *Gossypium barbadense* (Egyptian cotton), 233, 237, 265, 315; *Vigna unguiculata* (cowpea), 239, 280, 297; *Lupinus luteus* (yellow lupin), 240, 245, 249-251, 253, 256, 283, 295, 299, 304, 313, 419; *Capsicum chinense* (chilli peppers), 246, 308; *Citrus sinensis* (orange), 252; *Pisum sativum* (pea), 254, 268, 290, 291, 293, 294, 296; *Capsicum annuum*, 257, 266, 276, 317; *Lupinus albus* (white lupin), 258, 284; *Phaseolus vulgaris* (bean), 259, 262, 282, 287; *Retama raetam*, 260; *Capsicum baccatum* (Peruvian hot pepper), 261; *Solanum tuberosum* (potato), 264, 269; *Cicer arietinum* (chickpea), 267, 278; *Salvia miltiorrhiza* (red sage), 270, 298; *Arachis hypogaea* (peanut), 271, 273, 288, 292, 308, 335, 337; *Solanum lycopersicum* (tomato), 274; *Glycine max* (soybean), 275, 305; *Vigna radiata* (mung bean), 277, 412, 413; *Vigna angularis* (Azuki bean), 279; *Oxalis tuberosa* (oka), 281; *Panax ginseng* (ginseng), 286, 323; *Pisum fulvum* (tawny pea), 289; *Trifolium repens* (white clover), 300; *Catharanthus roseus* (Madagascar periwinkle), 302, 321; *Casuarina glauca* (swamp she-oak), 303; *Petroselinum crispum* (parsley), 306, 307, 310, 312; *Hypericum perforatum* (St. John's wort), 311; *Daucus carota* (wild carrot), 314, 326-329, 332, 338, 352; *Pachyrhizus erosus* (Mexican potato), 316, 320; *Taraxacum officinale* (dandelion), 318; *Rehmannia glutinosa*, 319; *Apium graveolens* (celery), 322, 324; *Populus trichocarpa* (black cottonwood), 325; *Cichorium intybus* (chicory), 330; *Lithospermum erythrorhizon*, 331, 334; *Picea sitchensis* (Sitka spruce), 336, 343, 361, 365, 366, 371, 399-404; *Pimpinella brachycarpa*, 339; *Oryza sativa* (rice), 340, 383, 408-411, 414-416, 418, 423; *Pinus monticola* (white pine), 341, 346-351, 354-357, 359, 363, 367, 369, 377, 378, 385; *Zea mays* (maize), 342, 405, 406; *Sorghum bicolor* (sorghum), 344, 345, 353, 358, 381; *Pseudotsuga menziesii* (coast Douglas-fir), 360; *Picea glauca* (white spruce), 362, 364, 372, 374, 379, 380; *Asparagus officinalis* (asparagus), 368, 388, 392, 393, 395-398; *Zinnia elegans* (zinnia), 370; *Picea mariana* (black spruce), 373, 375; *Picea abies* (Norway spruce), 376; *Hyacinthus orientalis* (hyacinth), 382; *Lilium longiflorum* (Easter lily), 384, 386, 387, 389, 390, 394; *Pinus pinaster* (maritime pine), 391; *Triticum aestivum* (wheat), 407; *Hordeum vulgare* (barley), 417; *Sonneratia caseolaris*, 420; *Brassica rapa* (field mustard), 421; *Arabidopsis thaliana* (thale cress), 422, 424, 425. Bold sequence number refers to crystal structures available in the PDB data base.



Description of the phylogenetic tree

All the PR-10 protein structures available in the PDB belong to the same SCOP class (Murzin et al., 1995), namely α and β proteins, TATA Binding Protein-like (TBP-like), Bet v 1-like, Pathogenesis-related protein 10 (PR-10)-like. However, such proteins are located in different and distant branches of the phylogenetic tree. For instance, cherry Pru av 1 has the same fold as mung bean Vr CSBP (seq. 412) but low sequence conservation. Branches of the phylogenetic tree are in most cases representative of the taxonomic order of the protein source. Rosales, Fegales, Fabales, Vitales and Coniferales orders are well represented and sequences from those orders are well grouped in large branches of the tree. Small clusters of sequences belonging to particular orders are grouped far away from the main ensemble of the same order (i.e. “Rosales 2” branch is near but distinguishable from “Rosales 1” group and sequences belonging to the Poales order are divided into three clusters), but in general, sequences belonging to the same order are located in the same region of the tree. The available structures for PR-10 proteins are representative of several branches of the phylogenetic tree. Cherry Pru av 1 structures (PDB codes 1E09 and 1H20, corresponding to the wild type and the E45W mutant of the protein, respectively) represent the “Rosales 1” cluster, and are close to Pru p 1.01 and Pru p 1.06D sequences (>70% identity). Birch PR-10 structures (1B6F, corresponding to Bet v 1a; 1FSK, corresponding to Bet v 1 in complex with a Fab fragment of a monoclonal IgG antibody; 1QMR, corresponding to the N28T/K32Q/E45S/P108G mutants of Bet v 1; 1LLT, corresponding to E45S mutant of Bet v 1; and 1FM4, corresponding to Bet v 1l) represent the Fegales order. Yellow lupin (2QIM, 1XDF, 1IFV, and 1ICX, corresponding to LIPR-10.2B in complex with cytokinin, LIPR-10.2A, LIPR-10.1B and LIPR-10.1A, respectively) and Mexican potato structures (1TW0 and 1TXC, corresponding to native SPE16 and to the complex crystal structure of SPE16 with ANS, respectively), represent the “Fabales 1” cluster. The “Fabales 2” small cluster is represented by mung bean structures (2FLH and 3C0V, corresponding to Vr CSBP in complex with cytokinin and Vr CSBP in complex with cytokinin and Ta₆Br₁₂, respectively). Finally, celery Api g 1 structure (2BK0) is representative of the Apiales order.

Description of zeatin binding in the available structures of the templates

Different binding modes of zeatin are found in the structural templates. For example, in the case of yellow lupin LIPR-10.2B, three zeatin molecules are found in the binding cavity: two of these molecules bind deeply inside the pocket, whereas a third molecule is located in the proximity of the cavity opening. Conversely, in all four different chains found in the crystal structure of Vr CSBP, one zeatin molecule is found at the bottom of the binding cavity, always in the same orientation with respect to the molecular frame (chains A-D). Additionally, a second zeatin molecule is found in two of the four chains (A and D), bound head-to-head with respect to the first zeatin molecule, while in another chain (B) a second zeatin molecule is bound head-to-tail with respect to the deeply bound zeatin. All zeatin molecules found in the crystal structure of mung bean Vr CSBP are found in the same conformation of the molecular skeleton, with the only exception of the zeatin bound to chain B, because of the head-to-tail interaction with the deeply buried zeatin molecule described before. In spite of the overall similarity between the structural fold of yellow lupin LIPR-10.2B and mung bean Vr CSBP, the orientation of the zeatin molecule found at the bottom of the binding pocket is not conserved (Fig. 5B and 5C). This difference is likely due to mutations occurring in key residues within the binding pocket, which are shown as underlined in the multiple sequence alignment (Fig. 5A). In particular, the Leu residue in position 143 of Vr CSBP sequence is replaced by Phe in the LIPR-10.2B homologue, thus changing the topology of the binding pocket in the proximity of the zeatin aromatic ring (Fig. 5A). Since the ITC data obtained in this study revealed that Pru p 1.01 binds zeatin in solution at equilibrium with a stoichiometry of 1:1, only the zeatin molecule found at the bottom of the cavity in LIPR-10.2B, considered to be the first to bind, is included in our model. Furthermore, because of the ambiguity in the orientation of the bound zeatin, as described above, the peach Pru p 1.01 and Pru p 1.06D complex structures were modeled in the apo form, and a single zeatin molecule was introduced subsequently using docking algorithms.

Figure S6. RMSD vs. estimated free energy of binding on yellow lupin LIPR-10.2B. Zeatin is protonated on nitrogen N9.

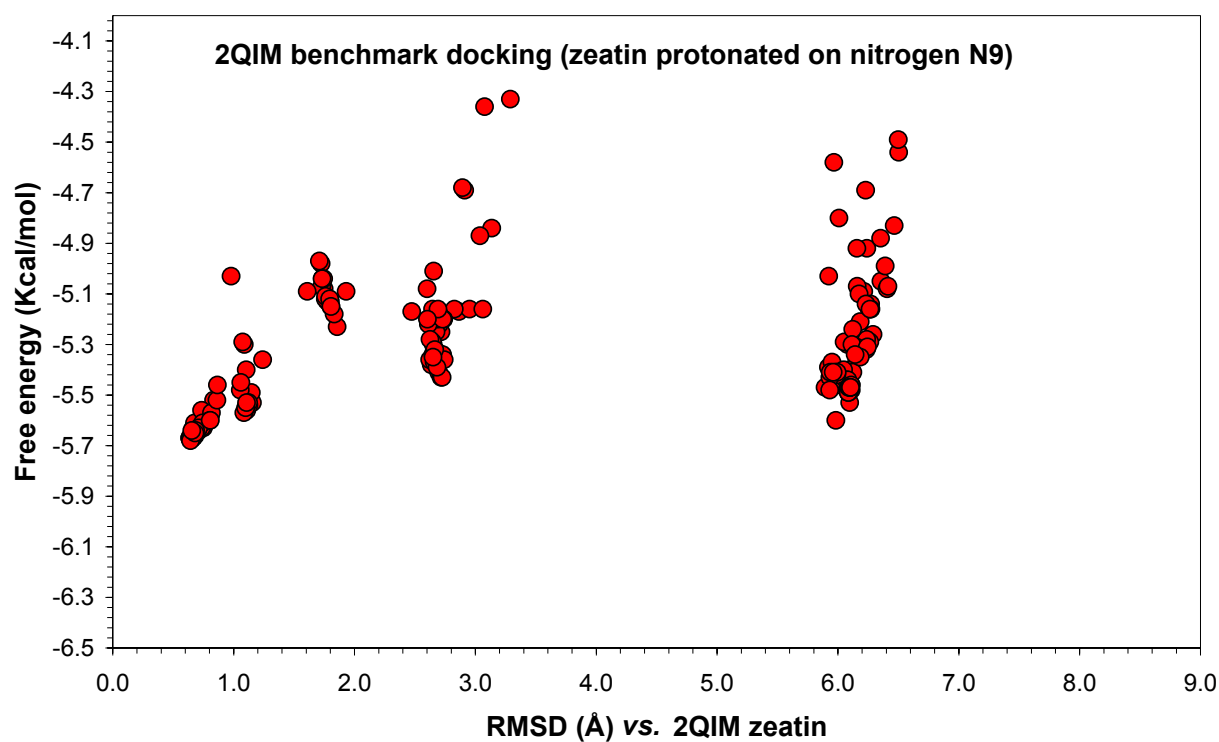


Figure S7. RMSD vs. estimated free energy of binding on yellow lupin LIPR-10.2B. Zeatin is protonated on nitrogen N7.

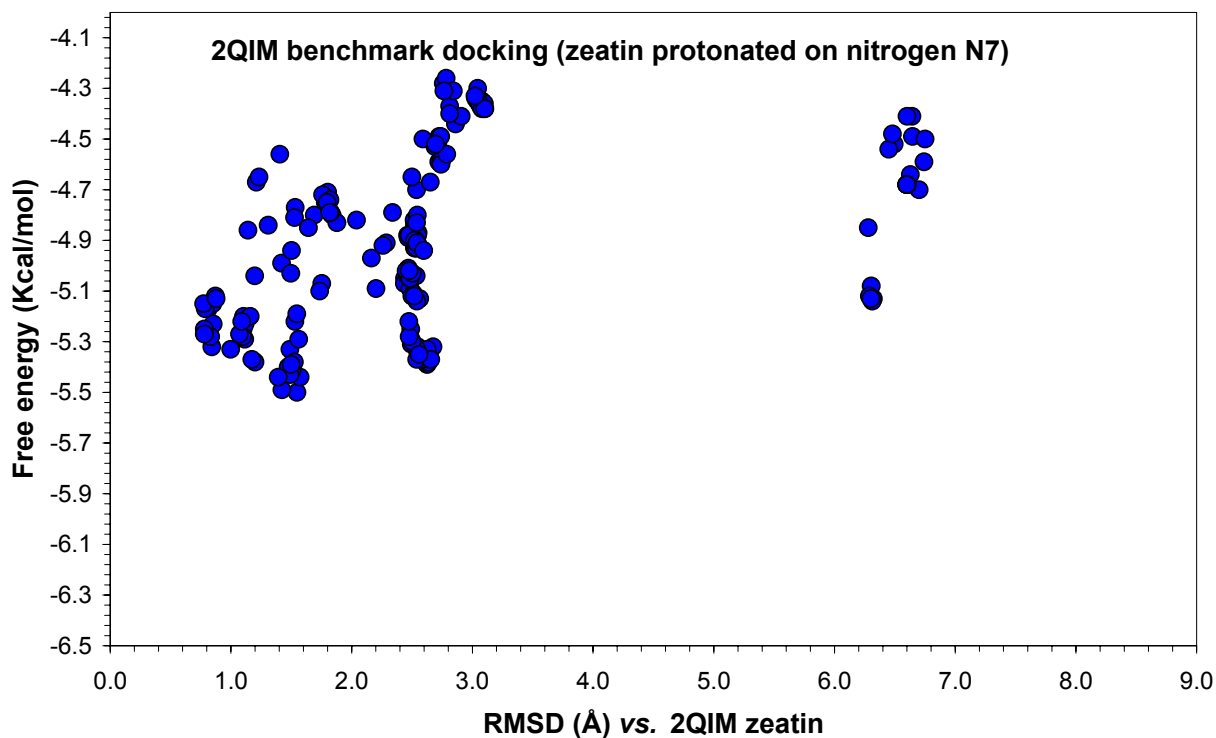


Figure S8 RMSD vs. estimated free energy of binding on yellow lupin LIPR-10.2B. Zeatin is protonated on nitrogen N9 (*red* dots) or on nitrogen N7 (*blue* dots).

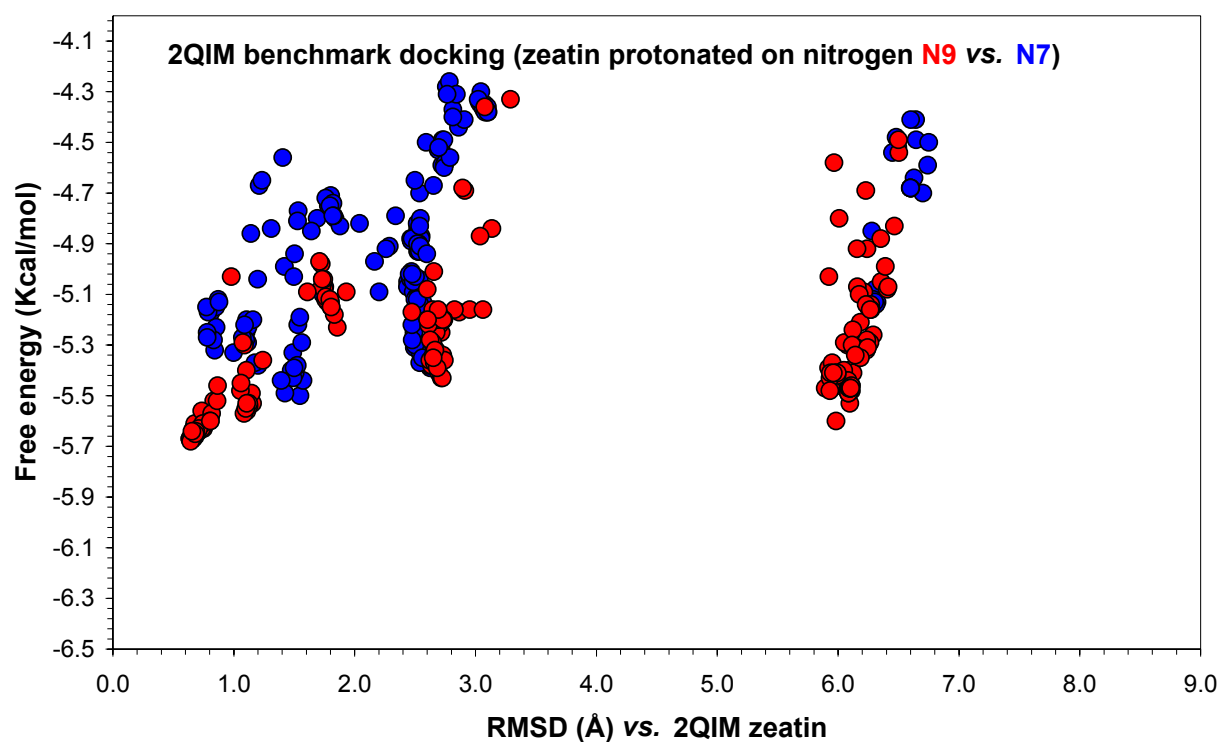


Figure S9. RMSD vs. estimated free energy of binding on mung bean Vr CSBP. Zeatin is protonated on nitrogen N9.

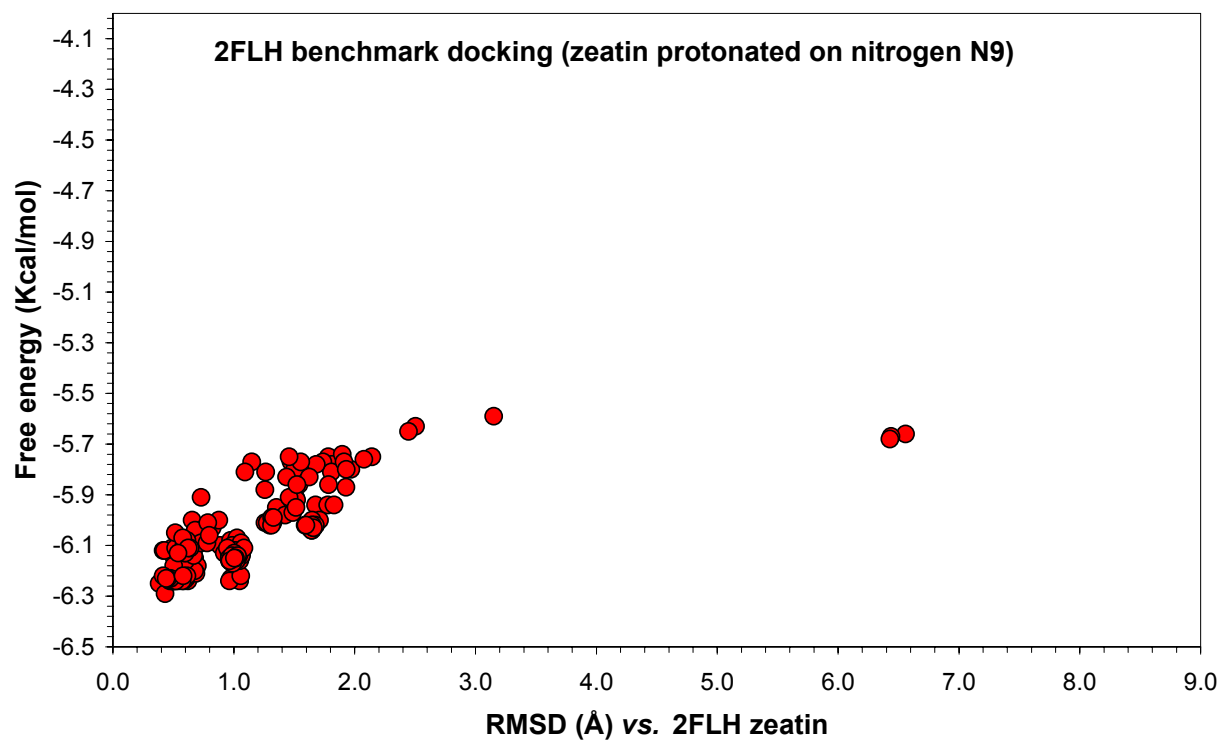


Figure S10. RMSD vs. estimated free energy of binding on mung bean Vr CSBP. Zeatin is protonated on nitrogen N7.

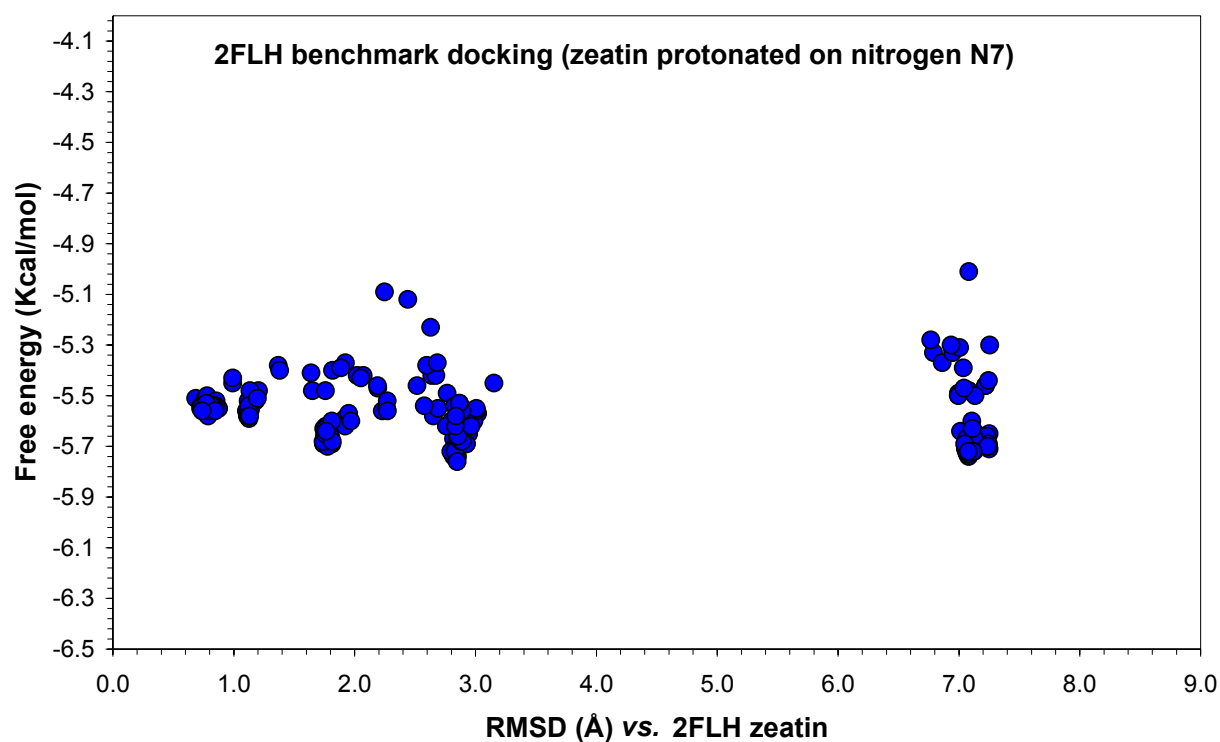


Figure S11. RMSD vs. estimated free energy of binding on mung bean Vr CSBP. Zeatin is protonated on nitrogen N9 (*red* dots) or on nitrogen N7 (*blue* dots).

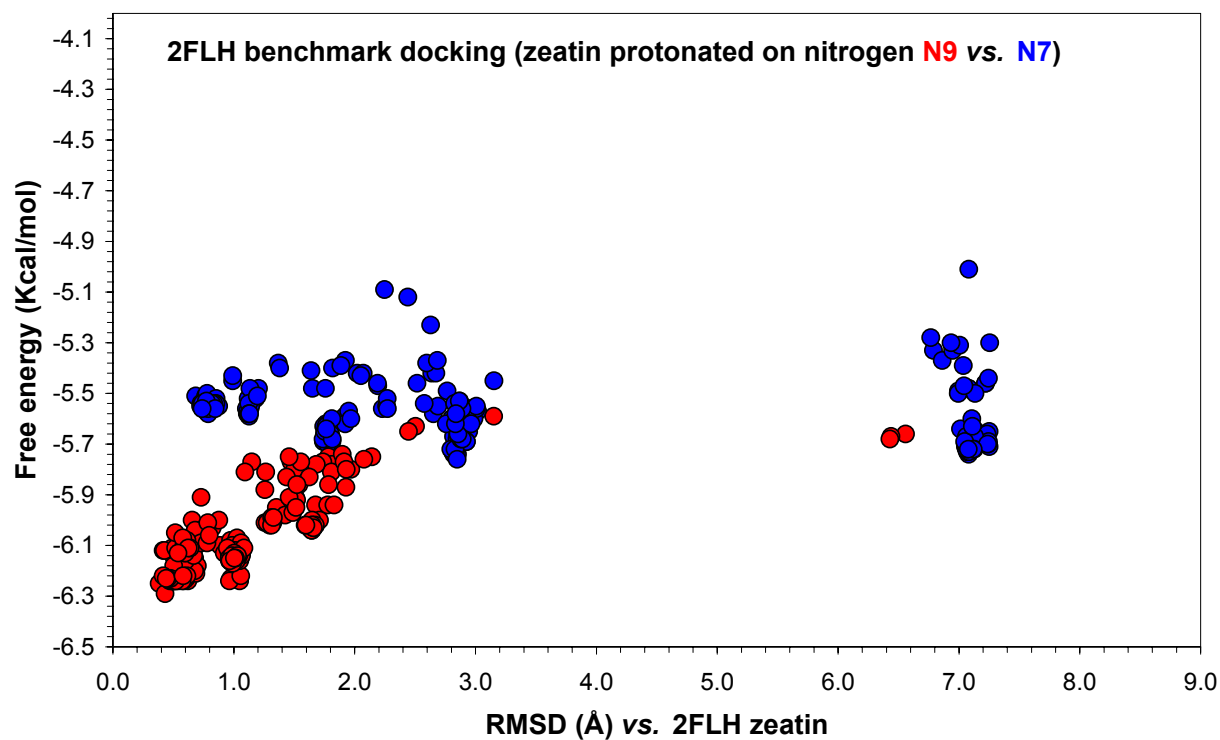


Figure S12. RMSD vs. estimated free energy of binding on yellow lupin LIPR-10.2B. Zeatin is protonated on nitrogen N9. The receptor (2QIM structure) was simulated including (*blue* dots) or not including (*red* dots) structurally relevant water molecules.

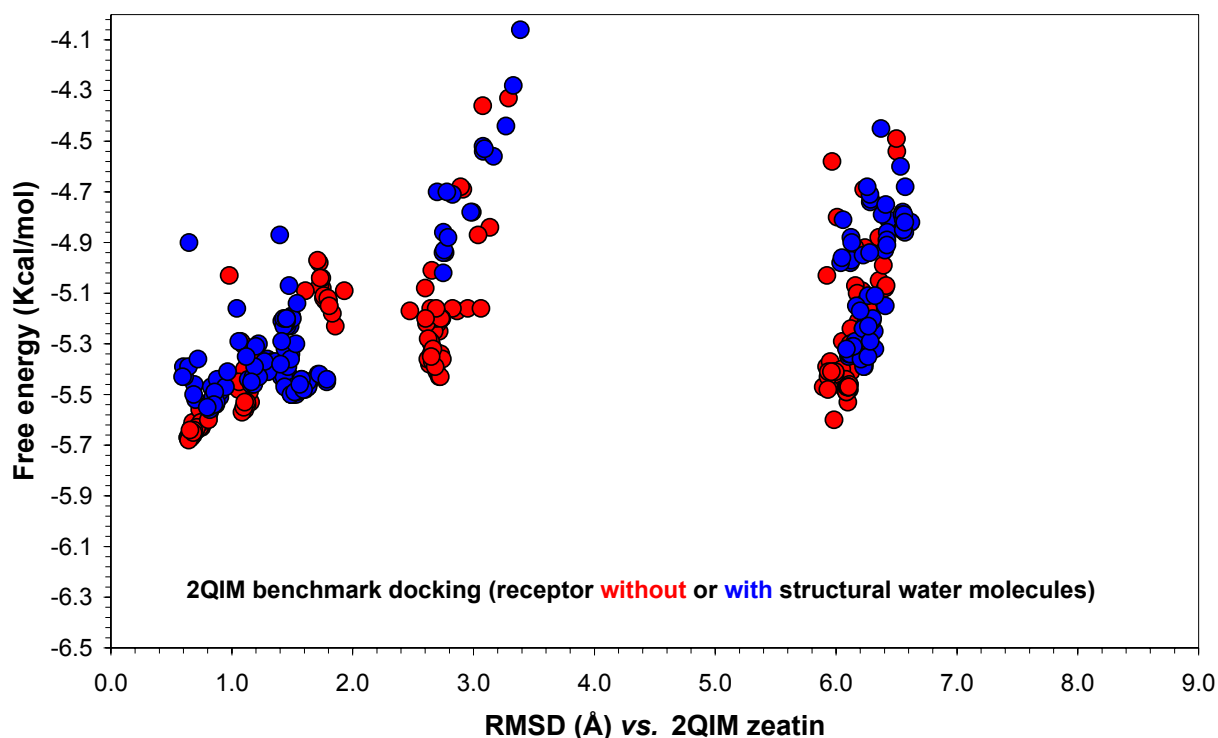
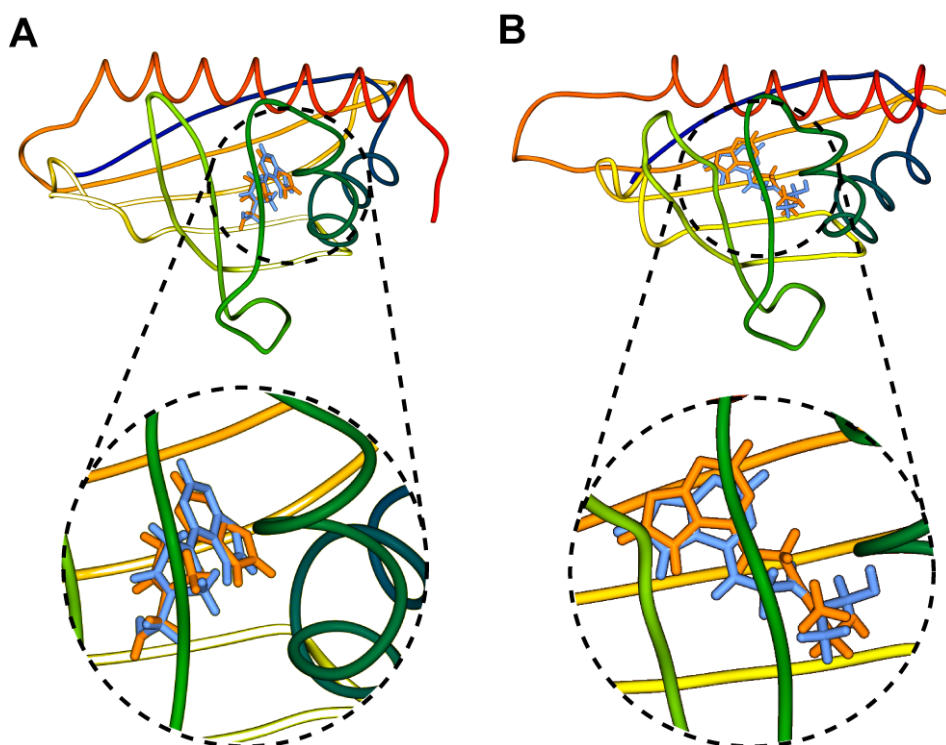


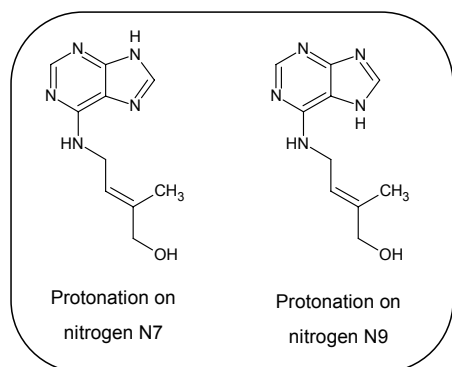
Figure S13. Best score structure of zeatin docking simulations to yellow lupin LIPR-10.2B (**A** panels) and mung bean Vr CSBP (**B** panels). The protein colors range from deep blue (N-terminus) to red (C-terminus). Trans-zeatin from the crystal structure is coloured in *orange*, while trans-zeatin from docking simulation is colored in *light blue*.



Investigation of the influence of zeatin N7 or N9 protonation form on the docking simulations

In order to obtain information on relative stability of the two possible forms of zeatin (protonated at nitrogen N7 or N9, see Scheme I) both forms were employed in our calculations.

Scheme I



In the case of yellow lupin LIPR-210.B, a distinct family of twelve zeatin molecules protonated at N9 is observed, with small RMSD with respect to the crystallographic structure (Fig. S6). The most stable modeled structure is shown in Fig. S13. In this structure, the overall position of zeatin is in good agreement with the crystallographic result (RMSD = 0.64 Å). The same simulation carried out using zeatin protonated at N7 provided docked structures significantly distant from the crystallographic result (RMSD = 1.54 Å), and with a similar free energy of binding with respect to the case of zeatin protonated at N9 (-5.50 vs. -5.68 Kcal/mol, respectively, Supplemental Fig. S7 and S8).

Mung bean Vr CSBP benchmark docking simulations carried out using zeatin protonated at N9 are reported in Fig. S9. The family of structures showing the lowest free energy of binding is well represented (33 structures) and is located at about 0.5 Å RMSD with respect to the crystallographic result. A second family of structures is found with RMSD ca. 1.0 Å with a higher free energy of binding. The simulation conducted on the same crystal structure, but using zeatin protonated at N7, gave the results reported in Supplemental Fig. S10 and S11. In this case, the best docked structure is part of a well represented family located at RMSD ca. 2.6-2.9 Å, in poor agreement with the crystallographic result. Moreover, the estimated free energy of binding in the N7 case is significantly larger than in the N9 case (-5.76 vs. -6.29 Kcal/mol, respectively). The best docked structure of zeatin protonated at N9 is reported in Fig. S13. The position and orientation of the aromatic ring is well reproduced, while the aliphatic chain shows some conformational differences even though its overall position is maintained. These results suggest that zeatin found at the bottom of the binding pocket of mung bean Vr CSBP is protonated at nitrogen N9. On the other hand, in the yellow lupin LIPR-10.2B case it is not possible to discriminate between the two zeatin protomers at this degree of approximation.

Influence of water molecules in the docking results

Docking simulations were also performed including yellow lupin LIPR-10.2B structurally relevant water molecules (Supplemental Fig. S12, namely water molecules #170, 176 and 181 in 2QIM crystal structure). Such simulation is in good agreement with the experimental result. However, when comparing with the same simulation executed in absence of water molecules (Fig. S12) a poorer correspondence with the experimental data is achieved. Such result is probably due to the difficulty of modeling the correct orientation of hydrogen atoms of the water molecules. In mung bean Vr CSBP crystal structure, the water molecules close to the zeatin bound at the bottom of the pocket are not conserved among the four chains of the crystal structure. On the basis of these considerations, we decided to perform all docking simulations without including water molecules.

Figure S14. RMSD vs. estimated free energy of binding on Pru p 1.01. Zeatin is protonated on nitrogen N7. RMSD was calculated taking zeatin found in 2QIM (*red* dots) or 2FLH (*blue* dots).

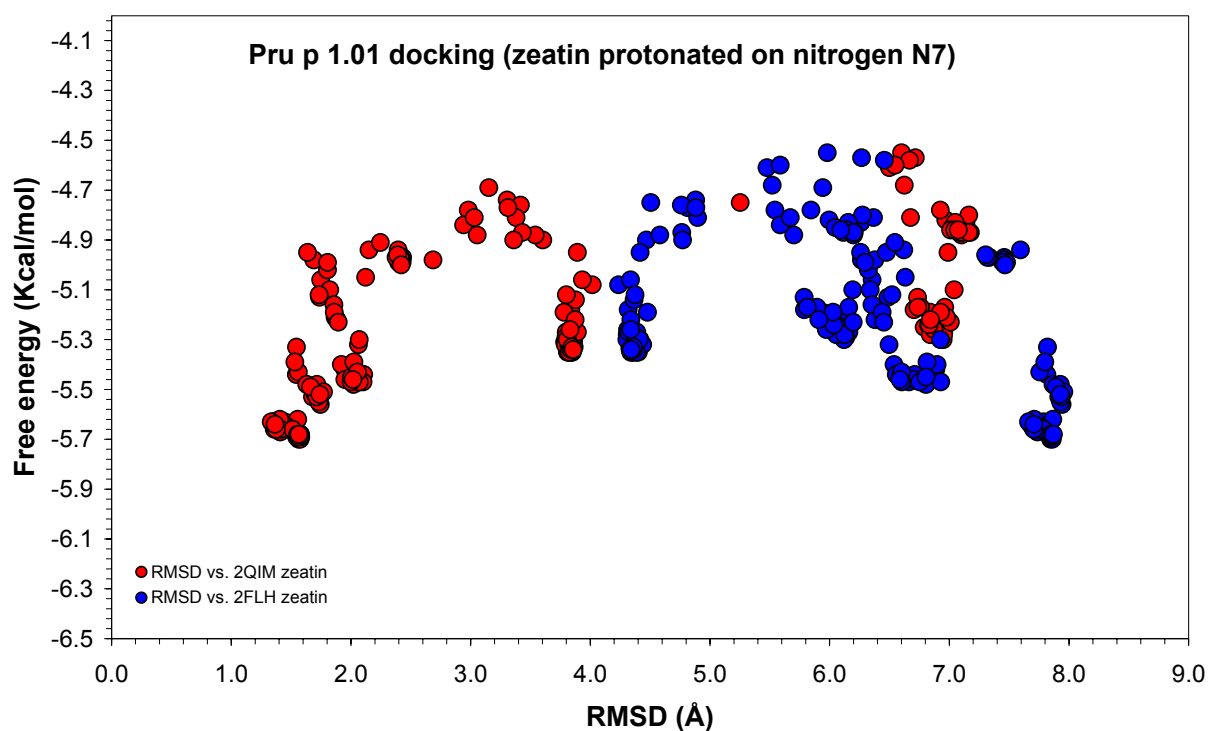
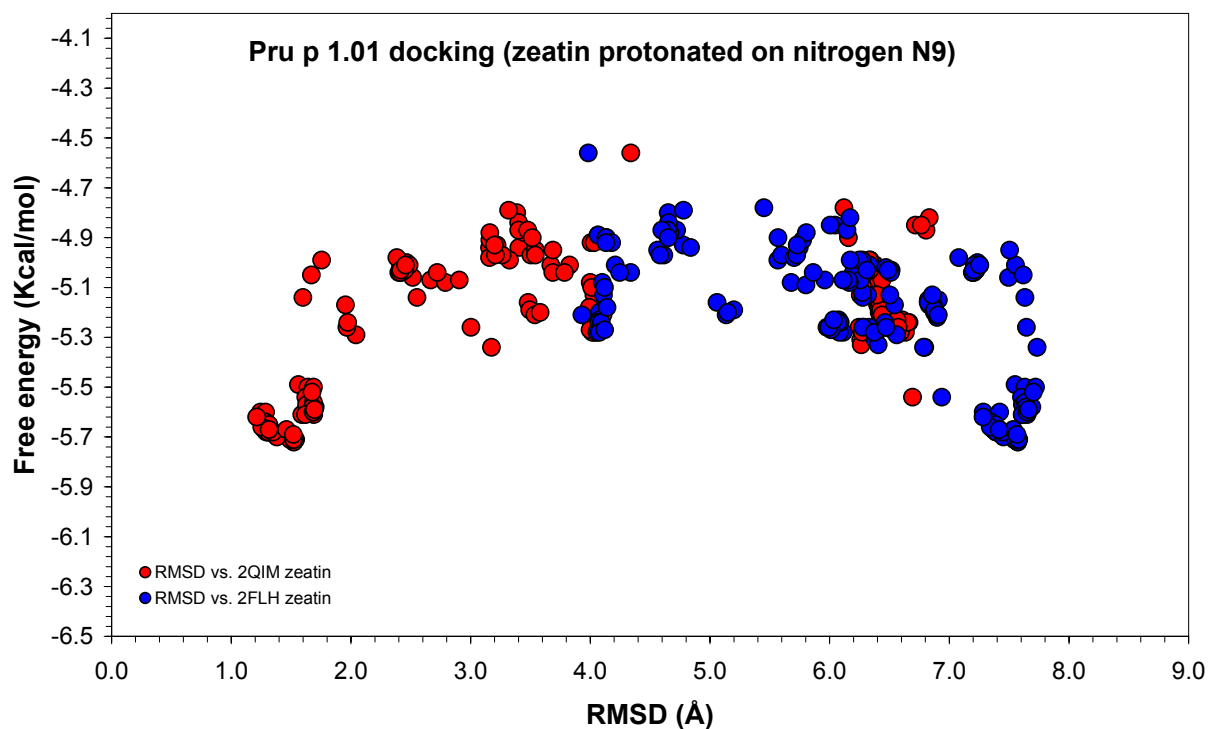


Figure S15. RMSD vs. estimated free energy of binding on Pru p 1.01. Zeatin is protonated on nitrogen N9. RMSD was calculated taking zeatin found in 2QIM (*red* dots) or 2FLH (*blue* dots).



Supplemental Data References

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