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### **Characterization of a novel aphid prenyltransferase displaying dual geranyl/farnesyl diphosphate synthase activity**

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## Abstract

We report on the cDNA cloning and characterization of a novel short-chain isoprenyl diphosphate synthase from the aphid *Myzus persicae*. Of the three IPPS cDNAs we cloned, two yielded prenyltransferase activity following expression in *E. coli*; these cDNAs encode identical proteins except for the presence, in one of them, of an N-terminal mitochondrial targeting peptide. Although the aphid enzyme was predicted to be a farnesyl diphosphate synthase by BLASTP analysis, rMpIPPS, when supplied isopentenyl diphosphate and dimethylallyl diphosphate as substrates, typically generated geranyl diphosphate (C10) as its main product, along with significant quantities of farnesyl diphosphate (C15). Analysis of an MpIPPS homology model pointed to substitutions that could confer GPP/FPP synthase activity to the aphid enzyme.

## Introduction

Short-chain isoprenyl diphosphate synthases (scIPPSs) are a class of prenyltransferases that catalyze the condensation of isopentenyl diphosphate (IPP; C<sub>5</sub>) with an allylic co-substrate. These enzymes are distinguished by the specific chain length of the prenyl diphosphate product generated. Thus, geranyl diphosphate synthase (GPPS) catalyzes a single condensation of IPP with dimethylallyl diphosphate (DMAPP; C<sub>5</sub>) to form geranyl diphosphate (GPP; C<sub>10</sub>), whereas farnesyl diphosphate synthase (FPPS) and geranylgeranyl diphosphate synthase (GGPPS) catalyze one and two additional condensations with IPP, respectively, generating farnesyl diphosphate (FPP; C<sub>15</sub>) and geranylgeranyl diphosphate (GGPP; C<sub>20</sub>). These products are precursors to many biologically essential compounds classified in three groups: monoterpenes, sesquiterpenes and diterpenes, respectively [1] (Figure 1).

The deduced amino acid sequences of all scIPPSs display high similarity, and sequence alignments reveal seven conserved regions [2]. Regions II and VI contain the substrate-binding regions, with two characteristic aspartate-rich motifs (DDx(xx)x<sub>D</sub>; where x represents any amino acid residue). Region II, which includes the first aspartate-rich motif (FARM), is designated as the chain length determination region, whereas, the second aspartate-rich motif (SARM) in region VI is responsible for IPP binding [3].

In insects, FPPS is involved in the biosynthetic pathway of the sesquiterpenoid juvenile hormone, which has important morphogenetic and gonadotropic functions [4]. In many aphid species, FPPS is also predicted to play a key role in the biosynthetic pathway of the alarm pheromone E-β-farnesene [5].

Whereas FPPS and GGPPS occur nearly ubiquitously in plants, animals, fungi and bacteria, GPPS appears to have a more limited distribution in nature, having been identified

almost exclusively in essential oil (monoterpene)-producing plants. However, monoterpenes are also important natural products in some insects, serving as semiochemicals. Recently evidence for the existence of GPPS activity in insects was provided for scolytid [6] and chrysomelid beetles [7]. In aphids, the presence of a GPPS is also expected inasmuch as the cyclopentanoids nepetalactone and nepetalactol, which are derived from GPP, are essential components of the sex pheromone [8].

In spite of the presumed importance of scIPPSs in insect metabolism, a limited number of insect IPPS sequences have been reported to date, and characterization of the recombinant proteins has only recently begun [6,9-13].

In this paper, we report on the cloning of three scIPPS cDNAs from *M. persicae* (two long forms, MpIPPS1-L and MpIPPS2-L and a short form, MpIPPS1-S), on their heterologous expression and on the characterization of one of these isoforms, including the identification of its principal *in vitro* products, prediction of its 3D-structure, and analysis of the generated 3D models. Although MpIPPS1 isoforms were initially predicted to be FPPSs, based on their amino acid sequences, *in vitro* assays revealed a dual GPP/FPP synthase activity, generating both GPP and FPP.

## Materials and Methods

### *Biological material*

*Myzus persicae* individuals were reared on bean plants (*Vicia faba* L.) at  $20 \pm 2^{\circ}\text{C}$ , under a 16 h L:8 h D photoperiod, in a dedicated environmental chamber.

### *Cloning and sequencing of M. persicae IPPS cDNAs*

Total RNA was isolated from whole-body *M. persicae* individuals, using the Trizol reagent (Invitrogen) according to manufacturer's instructions. We constructed a cDNA library as described [14]. Briefly, 3  $\mu\text{g}$  of total RNA was reverse transcribed using an oligo dT primer with the following sequence: TTTTGTACAAGC(T)<sub>16</sub>, followed by synthesis of the second cDNA strand and ligation of an adaptor (upper primer; 5'-CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCCGGGCAGGT-3' and lower primer 5'-ACCTGCCCCGG-3'); the latter was used for amplification of the cDNA using an adaptor-specific primer (ASP; 5'-CTAATACGACTCACTATAGGGC-3') in conjunction with the oligo dT primer.

To amplify a core fragment, a set of degenerate primers were designed against highly conserved regions among IPPS proteins [11]. The primer set included the forward primers P1, 5'-TAYAAAYRYIVBIVRIGGIAARHWIAAYMGNGG-3', and P3, 5'-AYIMGIMGIGGIVMIMYITGYTGG-3', and the reverse primer P2, 5'-RTCYTGDATRTCIGTICCIWYYTTNCC-3'. Two PCR reactions were successively performed, using the primer pairs P1-P2 and P3-P2.

To amplify the 5'- and 3'-ends of the cDNA fragments, specific primers (SP) were designed against the partial sequences obtained and utilized in conjunction with ASP and oligo dT primers. For amplification of MpIPPS1 isoforms, the specific primer sequence used

for 5' RACE was SP-1, 5'-AAGCAGTCTTATATTTTACAATAGC-3', and those used for 3'-RACE were, SP-5, 5'-GACCCAGAAATCTTTCGACAAGC-3', and SP-6, 5'-GAAGATGGTAAATGTTCATGGT -3'. For amplification of the MpIPPS2-L homolog, 5'-RACE, was performed using SP-1, and for 3'-RACE the specific primer sequence was, SP-7, 5'-TATACACACATTCTGGAAGTGTTCATGACG-3'.

### ***Construction of expression vectors***

After amplification by PCR, using specific primers to introduce *Eco*RI and *Xho*I restriction sites at the 5' and 3' ends, respectively, the coding region of each cDNA was cloned, in phase with a His-tag at the N-terminus, into the expression vector pET30b (Novagen) previously cut with the same restriction enzymes. Fidelity of the inserts was confirmed by sequencing.

### ***Expression and purification of recombinant proteins***

*Escherichia coli* strain BL21 (DE3) was transformed with each MpIPPS construct. Single colony transformants were cultured at 37°C for 3 h in 10 ml of LB medium containing 1% glucose and 30 µg/ml kanamycin, with shaking at 200 rpm. This starter culture was used to inoculate a flask containing 200 ml of the same medium, and cells were grown under the same conditions to an OD<sub>600</sub> of ~ 0.6. Induction was initiated by addition of 1 mM isopropyl-β-D-thiogalactoside (IPTG), after replacing the medium with 200 ml of LB medium containing 30 µg/ml kanamycin. The cells were incubated overnight at 15°C with shaking at 150 rpm, and then harvested by centrifugation (3000 g, 10 min, 4°C).

After resuspending the resulting bacterial pellet in 10 ml of 25 mM Tris-HCl buffer pH 8, containing 5 mM MgCl<sub>2</sub> and 2 mM β-mercaptoethanol (BME), the cells were first treated with lysozyme (1 mg) for 10 min at room temperature, and then sonicated for 3 min

using 1 sec bursts at 4°C (Branson sonicator, 80W, 20% power). The clarified sample was subjected to Co<sup>2+</sup> metal affinity chromatography using TALON Resin (1 ml, Clontech), which had been equilibrated with binding buffer (25 mM Tris-HCl pH 8, 150 mM NaCl, 5 mM MgCl<sub>2</sub>, 5 mM imidazole and 2 mM BME). The column was washed with 4 ml of binding buffer, followed by 4 ml of wash buffer (25 mM Tris-HCl pH 8, 150 mM NaCl, 5 mM MgCl<sub>2</sub>, 25 mM imidazole, 2 mM BME). Proteins were eluted with 25 mM Tris-HCl pH 8 containing 150 mM NaCl, 5 mM MgCl<sub>2</sub>, 150 mM imidazole and 2 mM BME. The protein fractions were finally placed in 50 mM Tris-HCl pH 7 containing 2 mM BME, using a PD-10 desalting column (Amersham Biosciences). The purity of each rMpIPPS was verified by SDS-PAGE and protein concentration was determined by the microassay procedure of the Bio-Rad Protein Assay, using BSA as standard. The procedure described above was also used to produce the previously characterized FPPS of *Drosophila melanogaster* (rDmFPPS) [13]. Thrombine cleavage of the His-tag was carried out as described [13].

### ***Prenyltransferase product analysis***

To identify the products formed *in vitro* by rMpIPPS and to compare them to those formed by rDmFPPS, the purified enzyme suspensions (0.5 µg) were assayed in 50 mM Tris-HCl, pH 7, containing 50 mM MgCl<sub>2</sub> and 2 mM BME, using 10 to 100 µM allylic substrate (DMAPP or GPP) and 4 µM [<sup>14</sup>C]IPP, in a total volume of 50 µl. The reactions were incubated for 40 min at 37°C. Samples were further incubated overnight at 37°C in the presence of 2 U shrimp alkaline phosphatase in 10x phosphatase buffer (Roche), 2% Triton X-100, 5% glycerol and 2.5 mg/ml BSA, to convert the diphosphates into the corresponding alcohols. Acetonitrile (final concentration of 50%) and authentic standards (geraniol, farnesol and geranylgeraniol; Sigma) were added to the samples prior to HPLC analysis. Reverse-phase HPLC was carried out on a Dionex quaternary pump equipped with a C-18 column (25

cm x 4.6 mm, 5  $\mu$ m; Supelco). Products were fractionated using an acetonitrile-water gradient (60 to 70% ACN over 10 min; 70 to 80% ACN over 20 min; held at 80% ACN for 15 min) at a flow rate of 1 ml/min, with standards detected at 210 nm. The amount of radioactivity in each 1-min fraction was assessed by liquid scintillation counting using a Beckman LS6000SC.

### ***Kinetic studies***

The  $K_m$  values for GPP, DMAPP and IPP were obtained by double reciprocal Lineweaver–Burk plots of the amount of total allylic diphosphate product formed at increasing concentrations of substrate.  $K_m$  values for GPP and DMAPP were determined in the range of 4–200  $\mu$ M, with a fixed IPP concentration of 4  $\mu$ M. The  $K_m$  value for IPP was determined for concentrations between 0.5 and 12  $\mu$ M, at 100  $\mu$ M GPP. Assays were conducted with 5.5  $\mu$ g of recombinant proteins in a final volume of 25  $\mu$ l, using 50 mM Tris-HCl pH 7 containing 50 mM  $MgCl_2$ , 2 mM BME. The reaction was incubated for 20 min at 37°C, and then quenched by the addition of 25  $\mu$ l of 2.5 M HCl, which served to hydrolyze the allylic diphosphate formed. After 10 min the reaction was partitioned between aqueous and organic phase by extraction with diethyl ether/pentane (1:1, v:v) and analyzed by liquid scintillation counting.

### ***Molecular Modelling***

The aphid IPPS displays 47% sequence identity to chicken; 3D models of the MpIPPS1-S dimer (residues 15-362) with bound DMAPP, GPP, or FPP were thus developed by homology modelling using MODELLER [15] and the appropriate PDB avian structures as template (1UBX, 1UBY, and 1UBW). Homology models were then minimized using GROMACS [16] and successfully validated with PROCHECK [17]. Models were generated

similarly for the WT sequence of the avian enzyme; the PDB files used as templates correspond to a double mutant.

All residues located at less than 5 Å from DMAPP, GPP or FPP in the avian and in the *M. persicae* 3D models were identified with PEX [18]. These interactions were determined for the dimers. These residues were then sorted out with respect to the portion(s) of the ligand involved in the interaction (OPP, C1-C5, C6-C10, and C11-C15).

## Results

### *Molecular cloning of M. persicae IPPS*

The degenerate PCR cloning approach that we employed permitted amplification of three separate cDNAs, referred to here as MpIPPS1-L, MpIPPS1-S and MpIPPS2-L. These encode proteins of 395 (45.3 kDa), 363 (41.6 kDa) and 396 (45.7kDa) amino acids, with calculated pI's of 7.5, 5.83 and 8.23, respectively.

The two MpIPPS1 isoforms are identical except for the presence of an N-terminal extension of 32 amino acids in MpIPPS-L, a feature also present in MpIPPS2-L (Figure 2). As described for *Agrotis ipsilon* and *D. melanogaster* FPPSs, the N-terminal extensions of aphid isoforms have the features of a mitochondrial targeting motif [19] and display the RxxS consensus sequence, which is currently reported as a mitochondrial targeting cleavage motif [20].

On the basis of BLASTp analyses, the proteins reported here were initially identified as FPPSs. A CLUSTAL alignment revealed that the amino acid sequences show ~50% similarity to FPPSs of other organisms. The predicted translated products have the seven conserved sequence regions identified by Koyama et al. [2], including the two aspartate-rich motifs (regions II and VI) (Figure 2).

### *Functional expression and product formation*

Both the long and short isoforms of rMpIPPS1 displayed significant catalytic activity in the presence of IPP and either allylic substrate, but rMpIPPS2, with or without its N-terminal extension, failed to show any detectable activity under the same experimental conditions; as a consequence, the present report focuses on the characterization of rMpIPPS1. The specific activity of rMpIPPS1-S was approximately four times greater than that of

rMpIPPS1-L. This may result from the mitochondrial targeting peptide in MpIPPS-L affecting protein folding and catalysis; the mature mitochondrial enzyme is predicted to lose its N-terminal extension after gaining access into its subcellular target [19], suggesting that MpIPPS1-L is not the catalytically functional (i.e. mature) protein. For this reason, only rMpIPPS1-S was used in subsequent assays.

The investigation of the nature of the end-products formed *in vitro* was performed using rMpIPPS1-S and DmFPPS for comparison. Unexpectedly, the recombinant aphid enzyme generally yielded GPP as its major product, a tendency that became more pronounced with increasing concentrations of DMAPP, while DmFPPS catalyzed almost exclusively FPP formation (Figures 3A & 3B). The observed predominance of GPP is unlikely to be an artifact of our assay conditions, given that similar product profiles were obtained under long incubations and that cleavage of the His-tag altered neither the activity nor the product profile (data not shown). Under conditions where GPP was utilized as allylic substrate, only FPP was formed, with trace amounts of GGPP (Figure 3C).

Preliminary assessments of  $K_m$  values for rMpIPPS1-S ( $K_{m/GPP} = 68 \mu M$ ,  $K_{m/DMAPP} = 45 \mu M$  and  $K_{m/IPP} = 15 \mu M$ ) indicate that the  $K_{m/GPP}$  value reported here for this enzyme may be higher than that reported earlier for insect FPPSs [13,21,22], which suggests that the DMAPP substrate might compete with nascent polyprenyl chain and thus induce the abortion of the condensation reaction and the subsequent release of GPP.

### ***Prediction and analysis of MpIPPS1-S structures***

In order to identify the active-site residues that might be responsible for the end-product profile of the aphid enzyme, we compared the active site of the aphid and avian enzymes in dimeric 3D models generated by homology modelling.

Since DMAPP, GPP and FPP all contain the same diphosphate group and first five carbon atoms, we postulated that differences in the binding of allylic compounds should not be related to the substitution of residues located in the vicinity of these portions of the ligand. We thus focused our analysis on the residues that are close to the end of the allylic chain of GPP or FPP (from C6 to C15). This assumption appeared to be correct since only the Ser123/Ala117 substitution (avian/aphid numbering) is involved in interactions with the unconsidered portions of the ligands.

### ***Comparison of aphid and avian active-sites***

Seven positions (named “A” to “G” in Figure 5) of the aphid/avian sequence alignment correspond to a residue that is in direct contact with the GPP/FPP chain end but that is not conserved between the two species (Table 1 & Figure 4B). Six belong to the A chain and one is on the B chain (position E). In both enzymes, the 7 residues are all close to the FPP C11-C15 group. Most of them are also close to the C6-C10 group of GPP and FPP. Four substitutions are located in conserved region II (positions A to D). These residues are the ones whose side chains are closest to the end of the allylic chain in the aphid enzyme. The Phe113/Gln107 non-conservative substitution (position C) corresponds to the closest one in both enzymes. In the active-site of the aphid enzyme, Tyr106 from the A chain and Val141 from the B chain are further from the ligand than the corresponding Phe112 and Phe147 avian residues (positions B and E). In contrast, the aphid Leu110 residue is much closer to the FPP C11-C15 portion than the corresponding avian residue (Ala116 – position D).

### ***Analysis of a multiple alignment from various organisms***

In order to assess the significance of the substitutions reported above, we analyzed amino acid variation at the 7 positions referred to above in a multiple alignment of IPPSs

from various organisms (Figure 5). Located upstream of the first DDx(xx)xD motif, positions A to D are in a region more conserved than positions E to G. Positions F and G are the most variable with non-conservative substitutions seen even among related organisms. Residues at position E are also poorly conserved even among related organisms. Nevertheless, most of the residues at these positions are hydrophobic. The A position is always occupied by a bulky hydrophobic residue. Most of the residues at the B position are aromatic; a Tyr residue is observed in all aphid sequences as well as in those of some other organisms. Some variation is observed at the C position, where the aphid sequences display a Gln residue that contrasts with the aromatic residue generally observed in other sequences. It should be noted, however, that the aphid enzyme shares this feature with the *Choristoneura fumiferana* type-1 FPPS [11], the exact product(s) of which remain to be determined [12]. With the exception of *T. cruzi*, *E. coli*, *B. stearotheophilus* and *D. melanogaster*, the D position contains a hydrophobic residue with a side chain of variable size.

## Discussion

Although prenyltransferases capable of producing both FPP and GGPP were reported earlier for two archeas [23] and more recently for the protozoan parasite *Toxoplasma gondii* [24], our study conducted on the aphid *M. persicae* led to the identification of the first prenyltransferase that can generate both GPP and FPP. In general FPPSs have been reported to yield about 3 times or more FPP than GPP [13,25-27]. Unexpectedly, rMpIPPS1-S yielded a GPP:FPP ratio varying between ~50:50 and ~70:30, depending on DMAPP concentration, while the dipteran rDmFPPS produced about 90% FPP. The latter observation suggests that rMpIPPS1 plays a predominant role in GPP formation, and that its function is not limited to FPP production, as originally hypothesized. Nevertheless, unlike other GPPSs known, the rMpIPPS1-S was shown here to be capable of using GPP as allylic co-substrate to generate significant amounts of FPP. Under our *in vitro* assay conditions, the GPP/FPP ratio was dependent on DMAPP concentration. At higher DMAPP concentration, the rMpIPPS1-S produced increasing amounts of GPP. This observation is in agreement with earlier reports indicating that the product profile of scIPPSs may be, to a certain degree, modulated by substrate concentration [28]. *In vivo*, the DMAPP concentration could be under the control of IPP isomerase, which catalyzes the isomerization of IPP to DMAPP, or could be regulated by substrate compartmentalization.

Analyses of the homology models we generated for MpIPPS1-S revealed that, with the exception of Ser123/Ala117, all the amino acid residues interacting with the diphosphate moiety are conserved between MpIPPS1-S and the avian FPPS. The amino acid residues interacting with the C6-C15 portion of the ligand are, however, less conserved, including seven substitutions among which one or several could account for the end-product distribution that we observed.

Some of the positions identified here (Phe112/Tyr106, Phe113/Gln107, Ala116/Leu110) have been shown to be involved in product chain-length selectivity in the avian enzyme, using site-directed mutagenesis [29,30].

Among these positions, the Phe113/Gln107 and the Ala116/Leu110 substitutions are the most likely to be associated with the specific product distribution observed for the aphid enzyme because of the proximity of the corresponding residues from the allylic chain, the nature of the substitution, and the variations observed in the sequence alignment of FPPSs sequence from various organisms.

The Ala116 to Leu110 substitution would reduce the size of the active-site cavity disfavoring the production of longer allylic compounds as a result of steric hindrance, according to the observations of Poulter and coworkers [29,30]. However, this explanation appears to be unlikely since several other FPPSs also have a Leu or a similar residue (Ile, Met) at this position (Figure 5). Thus, the most striking singularity in the residues lining the active-site of the aphid enzyme appears to be the Gln107 at the C position, a substitution that represents a slight decrease in steric hindrance relative to the aromatic residue typically found at this position, but a change in the nature of the residue's side chain (polar instead of hydrophobic). This modification could account for the observed aphid product distribution according to two complementary hypotheses. First, the presence of a Gln residue at this position would generate unfavorable interactions between the polar side-chain of the residue and the end of the GPP/FPP hydrophobic allylic chain of the product [31]. As a result shorter products would be favored. Second, chain elongation in the active-site would require the disruption of hydrogen bonds between the Gln107 amide group and the neighboring residues which would favor the production of products with shorter allylic chains.

Whether the Phe113/Gln107 substitution, alone or in combination with the other substitutions identified here, is responsible for the dual GPP/FPP synthase activity of the

enzyme will need to be assessed by site-directed mutagenesis coupled with appropriate enzyme assays. The possibility that other residues, outside the active site, may be involved in defining this enzyme's product selectivity also needs to be examined.

In summary, the existence of a GPP/FPP synthase in aphids is perhaps not too surprising, given that these insects require both monoterpenes and sesquiterpenes as precursors for sex pheromone, alarm pheromone and juvenile hormone biosynthesis. Whether another aphid scIPPS exists that is dedicated to FPP synthesis is not clear at this stage; MpIPPS2 is certainly a good candidate but we yet have to identify the conditions under which it is active so that its product selectivity may be characterized.

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**Figure 1.** Abbreviated biosynthetic pathways for monoterpenes, sesquiterpenes and diterpenes, including the reactions catalyzed by short-chain isoprenyl diphosphate synthases (scIPPSs).

**Figure 2.** The cDNA coding region of *M. persicae* IPPS isoforms. The RxxS mitochondrial targeting cleavage motif present at the N terminus is denoted by a blue box. The conserved regions (I–VII) are denoted by black boxes. Positions of the Asp residues within the two aspartate-rich motifs (II–VI) common to all scIPPS are denoted by red boxes.

**Figure 3.** Radio-HPLC analysis of prenyltransferase products, following their conversions to the corresponding alcohols. (A) rDmFPPS incubated in the presence of [ $^{14}\text{C}$ ]IPP and DMAPP, (B) rMpIPPS-S incubated in the presence of [ $^{14}\text{C}$ ]IPP and DMAPP, (C) rMpIPPS-S incubated in the presence of [ $^{14}\text{C}$ ]IPP and GPP. Values are the mean and standard deviation (error bars) of determinations made from three separate protein preparations.

**Figure 4:** (A) Overall structure of the *M. persicae* homodimeric model (red) with the superimposed avian homodimeric model (green). Among the amino acid residues interacting with the C6-C15 region of the FPP allylic chain, those that show differences in identity at equivalent positions between the two proteins have their side chains displayed in blue. The FPP molecule is drawn as thin lines. (B) Detailed stereo-view of the FPP C6-C15 interaction site in the A subunit (*M. persicae* residues in red, chicken in green). Residues are labeled with respect to the *M. persicae* sequence. Note that Val-141 is on the B chain (i.e. subunit). Hydrogens are omitted.

**Figure 5:** Sequence alignment of aphid IPPSs (including MpIPPS) with FPPS from different organisms and *Ips pini* GPPS. Only the regions that include the seven positions with residues that are candidates for a role in the product selectivity of the aphid enzyme are presented.

These residues are highlighted in black and designated “A” to “G” at the top of the alignment.

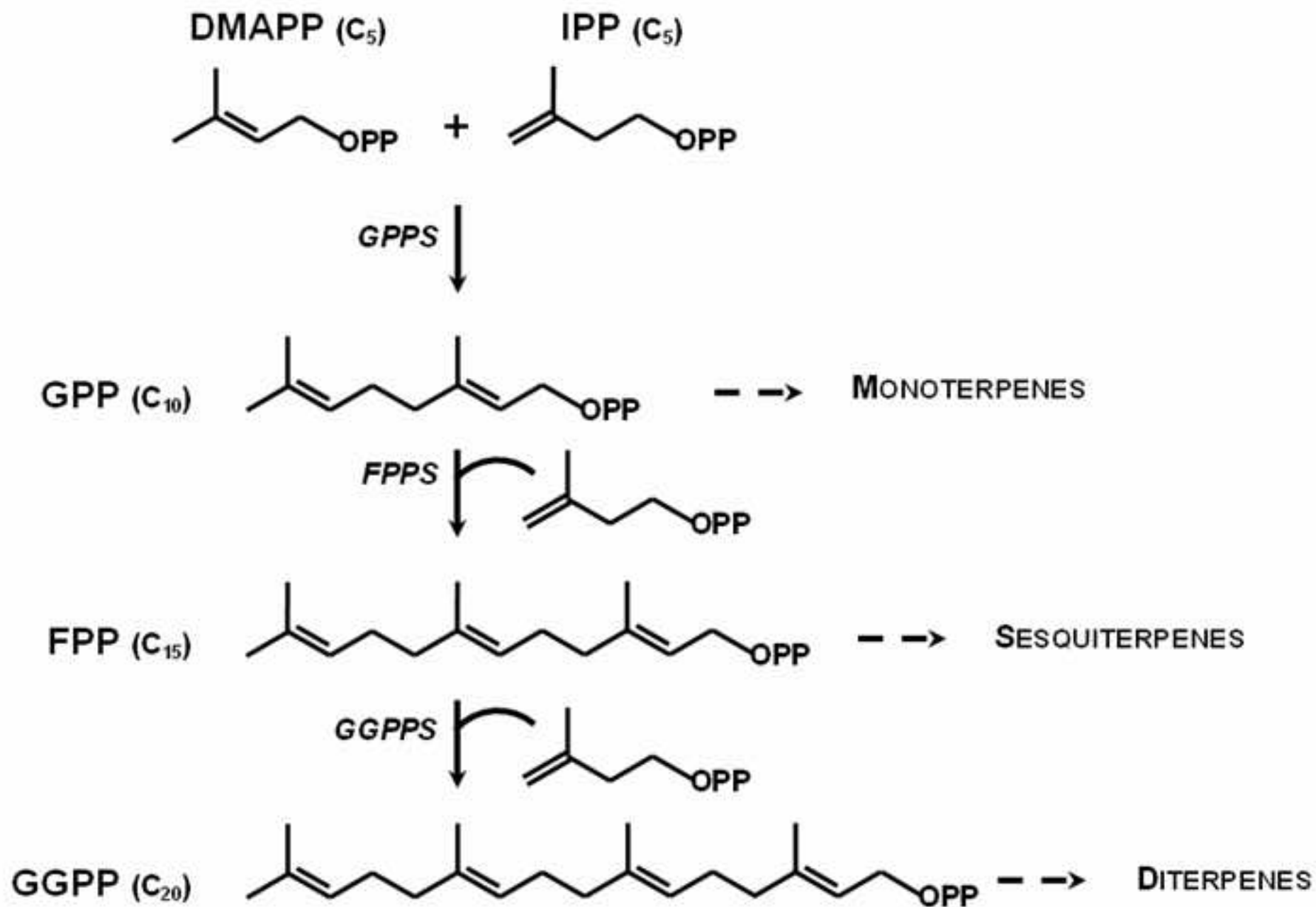
Sequence sources: (1) ZP\_01700053; (2) Q08291; (3) AAK71861; (4) P08524; (5) AAH10004; (6) NP\_114028; (7) P08836 ; (8) NP\_493027; (9) AAB07248; (10) AY968586; (11) EU447786; (12) AY968585; (13) AY968583; (14) AY968584; (15) AAD27853; (16) XP\_308653; (17) XP\_396224; (18) AY966009; (19) AY953507; (20) AY954920; (21) AY954919; (22) AAX55632.

**Table 1:** Distances (in Å) between the side chains of selected amino acid residues and GPP and FPP allylic ligands in the aphid homodimeric IPPS active site, as compared to distances determined for residues at equivalent positions in the avian structure. Residues at an identical position in the sequence alignment are presented on the same line. The chain (i.e., subunit) bearing the residue is indicated in the left-most column. The distances correspond to the distance between the mass center of the nearest atoms of the corresponding groups. Distances above the 5 Å cutoff are omitted. Results are presented only for one ligand because of the symmetry of the dimer.

**Table 1:**

	APHID					AVIAN				
	RESIDUE	C6-C10		C11-C15	RESIDUE	C6-C10		C11-C15		
		GPP	FPP	FPP		GPP	FPP	FPP		
Chain A	LEU 103			2.5	PHE 109	3.4		3.3		
	TYR 106	4.7	4.5	2.3	PHE 112	2.9	2.7	2.2		
	GLN 107	2.1	2.1	1.5	PHE 113	2.1	1.7	1.8		
	LEU 110	2.0	2.3	2.3	ALA 116	2.6	2.1	4.4		
	THR 172	3.8	4.7	2.6	ALA 178	3.6		2.4		
	ALA 176	2.7	3.2	3.1	GLU 182	2.0	2.9	2.3		
Chain B	VAL 141	4.1	4.4	3.7	PHE 147	2.4	3.2	2.6		

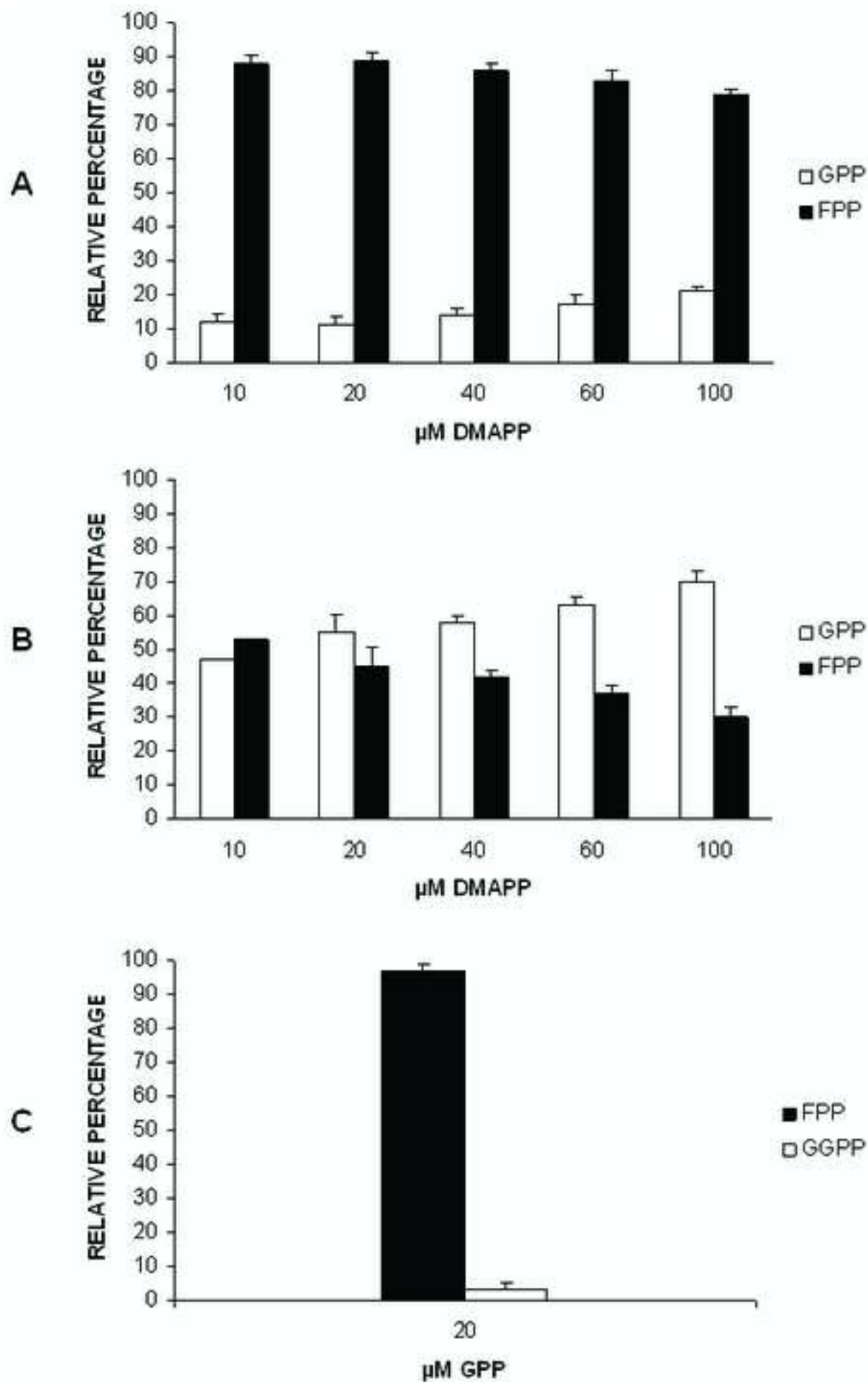
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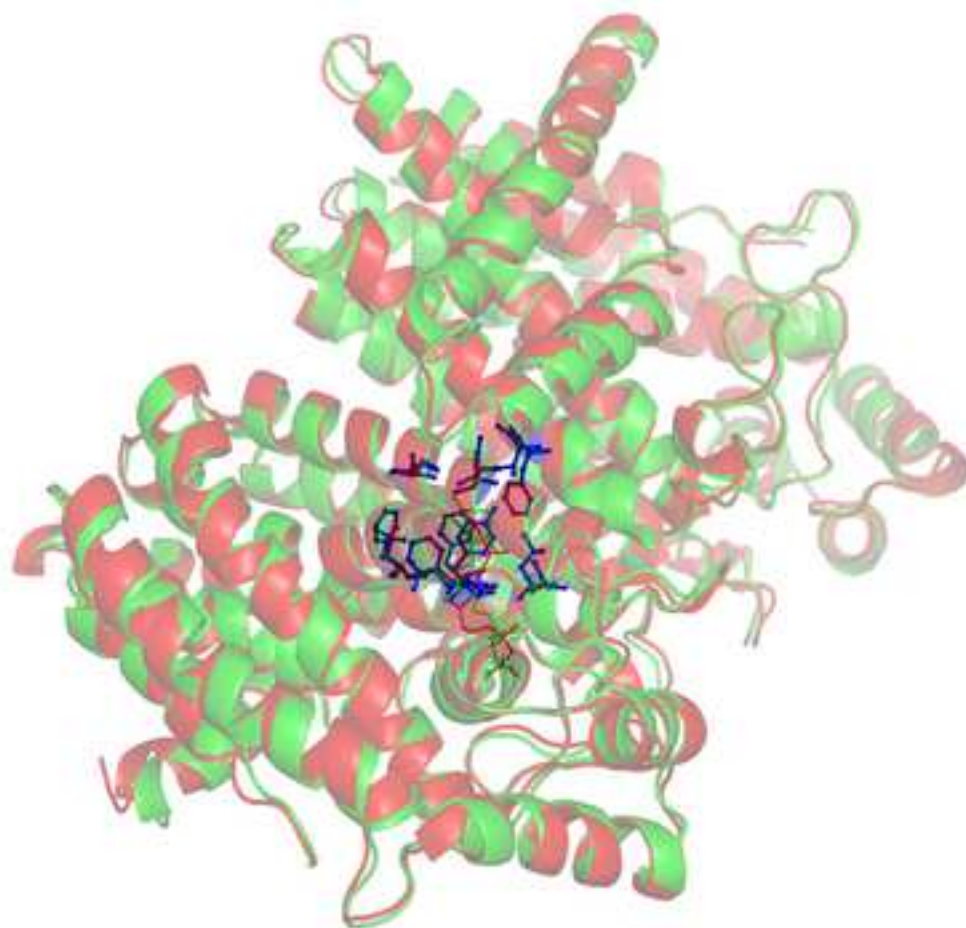
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					<b>RxxS</b>								
MpIPPS1-L	MNKMLT-FTR	ALSRRSAFLL	CDSAVV-RNN	NE <b>RAMS</b> TVRA	PFVPPVITGT	AVSKDETRDF	MAVF	PDVVRD	LTDTGRNLDV				
MpIPPS1-S	-----	-----	-----	----MSTVRA	PFVPPVITGT	AVSKDETRDF	MAVF	PDVVRD	LTDTGRNLDV				
MpIPPS2-L	MYKTLTTFTR	ALSRRATFSL	CPAVAAGREN	HE <b>RSMS</b> VILP	PFVTSVITGA	LVS KDELGNE	MAVF	PDIVKD	LTDTSLQLNK				
			<b>I</b>						<b>II</b>				
MpIPPS1-L	PDVTKWLAKL	LQYNVE <b>GGKK</b>	NRGLALVLSY	KMLSSPSDQT	DENLKLSYIL	GWCV <b>VEILQAY</b>	QLVL <b>DD</b>	IMDN	AITRGRPCW				
MpIPPS1-S	PDVTKWLAKL	LQYNVE <b>GGKK</b>	NRGLALVLSY	KMLSSPSDQT	DENLKLSYIL	GWCV <b>VEILQAY</b>	QLVL <b>DD</b>	IMDN	AITRGRPCW				
MpIPPS2-L	PDVTKWLENL	LQYNVE <b>GGKN</b>	NRGLILVSSF	KMLSSPSYLT	DENLRLSYIL	GWCV <b>VEILQAY</b>	QLVM <b>DD</b>	LMDN	AITRGRPCW				
		<b>III</b>				<b>IV</b>							
MpIPPS1-L	YRHNDIGLMA	VNDGV <b>LLEQA</b>	IYQLIKKYFK	DKPYYTHILE	LFFDVTMKTA	MGQ <b>CLDMLTA</b>	NSFKSKKLEK	YTMENYTAIV					
MpIPPS1-S	YRHNDIGLMA	VNDGV <b>LLEQA</b>	IYQLIKKYFK	DKPYYTHILE	LFFDVTMKTA	MGQ <b>CLDMLTA</b>	NSFKSKKLEK	YTMENYTAIV					
MpIPPS2-L	YRHNDVGLMA	VNDG <b>LLEQA</b>	IYQLLKKYFK	DKPYYTHILE	LFYDVTMKSA	MGQ <b>CLDMLTA</b>	KSFKSKKLEK	YTMENYKAIV					
	<b>V</b>					<b>VI</b>							
MpIPPS1-L	KY <b>K</b> TAYYSFF	LPVCLAMRMT	NINDPEIFRQ	AKTILLEM <b>GH</b>	FFQVQ <b>DDFLD</b>	CYGD <b>PDVMGK</b>	IGTD <b>I</b> EDGKC	SWLAVVALQK					
MpIPPS1-S	KY <b>K</b> TAYYSFF	LPVCLAMRMT	NINDPEIFRQ	AKTILLEM <b>GH</b>	FFQVQ <b>DDFLD</b>	CYGD <b>PDVMGK</b>	IGTD <b>I</b> EDGKC	SWLAVVALQK					
MpIPPS2-L	KY <b>K</b> TAYYSFV	LPVCLAMRMT	NVNDQEIFRQ	AKVILLDM <b>GQ</b>	FFQIQ <b>DDYLD</b>	CYGN <b>PEITGK</b>	IGTD <b>I</b> EDGKC	SWLAVKALQK					
									<b>VII</b>				
MpIPPS1-L	VNSEQKKIME	DNYGIDDPAN	VAIIKDLYAQ	LKLADTFHLY	EEESYKLICT	HIQQLSRGLS	QDMFFKELEK	IYKRTL					
MpIPPS1-S	VNSEQKKIME	DNYGIDDPAN	VAIIKDLYAQ	LKLADTFHLY	EEESYKLICT	HIQQLSRGLS	QDMFFKELEK	IYKRTL					
MpIPPS2-L	VTTEQKKIME	DNYGIDDQFN	VAVIKDLYQQ	LKLQNTFLLY	EEESYNLIRT	CIQNFSPGLS	QDMFFKLLEK	IYKRTL					

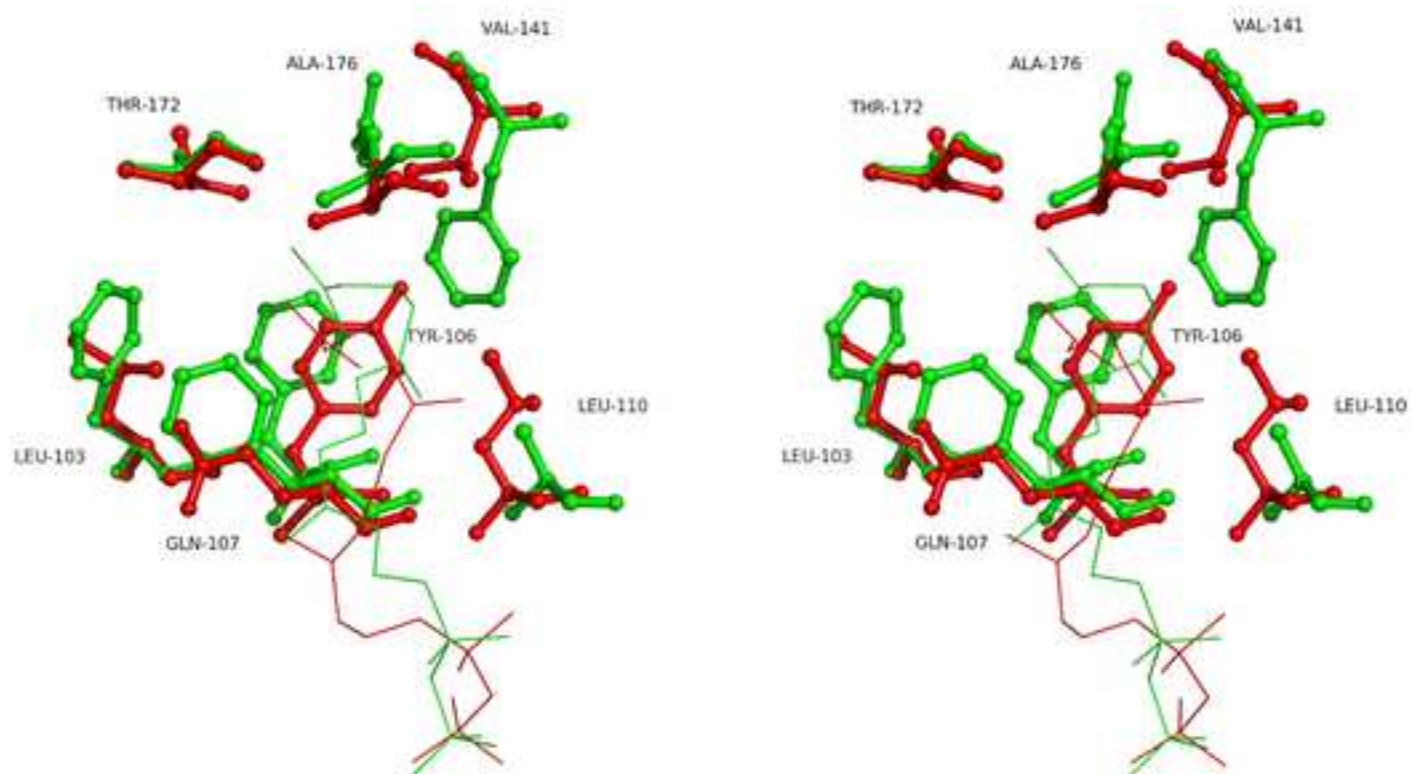
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A



B



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	A	BC	D		E		F	G				
<i>Gallus gallus</i> <sup>1</sup>	107	ELFQAF	FLVA	ADDI--MD 121	141	DAIN-DS	FLLESS	VYRV 156	176	QTAYQT--	ELGQMLD 188	
<i>Homo sapiens</i> <sup>2</sup>	159	ELLQAF	FLVA	ADDI--MD 173	193	DAIN-DAN	LLEACI	YRL 208	228	QSSYQT--	EIGQTLD 240	
<i>Rattus rattus</i> <sup>3</sup>	93	ELLQAF	FLV	LDDI--MD 107	127	DAIN-DAL	LLEAAI	YRL 142	162	QSSYQT--	EIGQTLD 174	
<i>Caenorhabditis elegans</i> <sup>4</sup>	89	EIIQSF	YLIA	ADDI--MD 103	123	SAIN-DAF	IMDSF	VEDI 138	158	KSKQKT--	LIGQFLD 170	
<i>Trypanosoma cruzi</i> <sup>5</sup>	88	EFLQAF	HYLV	EDDI--MD 102	122	QCAIND	GIILKS	WTQIM 138	158	KVDYAT--	AVGQMYD 170	
<i>Saccharomyces cerevisiae</i> <sup>6</sup>	90	ELLQAF	FLVA	ADDM--MD 104	124	IAIN-DAF	MLEAAI	YKL 139	159	EVTFTQT--	ELGQLMD 171	
<i>Arabidopsis thaliana</i> <sup>7</sup>	83	EWLQAF	FLV	LDDI--MD 97	117	IAIN-DGI	LLRNHI	HRI 132	152	EVEFQT--	ACGQMID 164	
<i>Escherichia coli</i> <sup>8</sup>	74	ECIHAY	SLIH	DDL	PAMD 90	110	AILA-GDA	LQTL	AFSIL 125	147	SASGIAGM	CGGQALD 161
<i>Bacillus stearothermophilus</i> <sup>9</sup>	76	EMINTY	SLIH	DDL	PSMD 92	112	AILA-GD	GLLT	YAPQLI 127	149	KAAGPEGM	VAGQAAD 163
<i>Myzus persicae</i> IPPS1 <sup>10</sup>	101	EILQAF	YQLV	LDDI--MD 115	135	MAVN-DG	VILLE	QAIYQL 150	170	DVTMKT--	AMGQCLD 182	
<i>Myzus persicae</i> IPPS2 <sup>11</sup>	135	EILQAF	YQLV	MODL--MD 149	169	MAVN-DG	ILLE	QAIYQL 184	204	DVTMKS--	AMGQCLD 216	
<i>Acyrtosiphon pisum</i> <sup>12</sup>	101	EILQAF	YQLV	LDDI--MD 115	135	MAVN-DG	VILLE	QAIYQL 150	170	DVTMKT--	AMGQCLD 182	
<i>Aphis fabae</i> <sup>13</sup>	101	EILQAF	YQLV	LDDI--MD 115	135	MAVN-DG	ILLE	QTIYQL 150	170	DVTMKT--	SMGQCLD 182	
<i>Megoura viciae</i> <sup>14</sup>	101	EILQAF	YQLV	LDDI--MD 115	135	MAVN-DG	ILLE	QTIYQL 150	170	DVAMKT--	AMGQCLD 182	
<i>Drosophila melanogaster</i> <sup>15</sup>	161	EMLS	FFII	SDDV--MD 174	194	TAIN-DAL	MIENAM	YAI 209	229	EITYIT--	TCGQSLD 241	
<i>Anopheles gambiae</i> <sup>16</sup>	186	EMLH	SMFL	IMDDV--MD 200	220	SGVN-DAI	MIEAAI	AHL 235	255	EMKFIT--	TIGQSLD 267	
<i>Apis mellifera</i> <sup>17</sup>	92	EIMQAF	HTM	IDD--LD 106	124	GAIN-DGL	ILET	CIYKL 139	159	KVSYNTKA	AHGECLD 173	
<i>Dendroctonus jeffreyi</i> <sup>18</sup>	168	EMVHAY	VLIL	LDDI--MD 182	202	TAVN-DAV	MIENAV	YLL 217	237	EGNLKT--	TLGQSLD 249	
<i>Ips pini</i> <sup>19</sup>	174	EMLHTY	FLIH	DDI--ID 188	208	TAVY-DAV	MMENG	VYLL 223	243	DMALKT--	SLGQSLD 255	
<i>Choristoneura fumiferana</i> (FPPS1) <sup>20</sup>	168	EMFHTH	QLIL	NDI--ME 182	202	SSIN-DAI	LVQSAM	YST 217	237	EMLLKC--	STGQFLE 249	
<i>Choristoneura fumiferana</i> (FPPS2) <sup>21</sup>	137	EILQGF	LV	LDDI--MD 151	171	AAVN-DS	SLMFSS	IFYV 186	206	ESLMHT--	SIGQHLD 218	
<i>Ips pini</i> (GPPS) <sup>22</sup>	147	EIIQAF	YFC	LDDI--MD 161	181	NAV	TDV	CLMEMTFEL 196	216	NLLFLT--	HMGQGYD 228	