# Supporting Information

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## SI Methods

**Heterologous Expression and Metabolism.** The coding sequences of CYP6Z1 (GenBank accession no. EU153375) and CYP6Z2 (GenBank accession no. EU153376) from the G3 strain of mosquito were cloned by RT-PCR amplification of total RNA from 3- to 5-day-old adults of mixed sexes by using the CYP6Z1-specific (AG6Z1EF1, 5′-GGgaattcATGATCCTTTACACGATCGG-3′; AG6Z1NR1, 5′-GGgcggccgcTTAATGTTTAGCACGATTCTC-3′) and CYP6Z2-specific (AG6Z2EF1, 5′-GGgaattcATGTTT-GTTTACACTCTCGCGC-3′; AG6Z2NR1, 5′-GGgcggccgcT-CACTTTCTATGGTCTATCCT-3′) primers and Pfu polymerase (Stratagene). The RT-PCR fragments were directly cloned into the pFastBac1 vector (Invitrogen) by using the EcoRI and NotI sites present in the forward and reverse primers, respectively (lowercase in the primers listed above). After sequence verifications, bacmids and viral stocks were generated following the manufacturer's instructions (Invitrogen). The viral stock for *Drosophila* cytochrome b5 from 3- to 5-day-old adults (Oregon-R strain) was generated by cloning the b5 ORF (GenBank accession no. EU166920) into the pFastBac1 vector by using a forward primer (B5EF, 5′-GGgaattcATGTCGAGCGAGGAAACAAAGA-3′) with an EcoRI site and a reverse primer (B5HR, 5′-GGaagcttC-TATTGCAAGGCACCGCCAAAGAAGA-3′) with a HindIII site.

Coexpressions of CYP6Z1 and CYP6Z2 with house fly P450 reductase (HFR) and b5 were as outlined by Wen *et al.* (1) at an MOI ratio of 2:2:0.1 (P450:HFR:b5) where the yield of P450 was not significantly affected and HFR was not limiting. Whereas the procedure for CYP6Z2 coexpression with HFR and b5 followed that of CYP6B1 coexpression with HFR (1), CYP6Z1 coexpression differed slightly in two ways. First, Sf9 cells were initially infected with recombinant CYP6Z1 virus alone (MOI of 2) for 24 h at a cell density of $0.6 \times 10^6$, and then HFR and b5 virus stocks were added to MOIs of 2 and 0.1, respectively. Second, CYP6Z1 enzymes were prepared from cells after 96 h of infection with the recombinant CYP6Z1 virus rather than the more standard 72 h of infection.

Cleared cell lysates were prepared for CYP6Z1 and CYP6Z2 by collecting and centrifuging Sf9 cells expressing these P450s in 50-ml Falcon tubes at 3,000 rpm for 5 min and washing them in 0.1 M phosphate buffer lacking glycerol and at pH 7.5 as outlined in Wen *et al.* (1).

Metabolism assays for DDT, carbaryl, and xanthotoxin were conducted as described for cleared cell lysates by Wen *et al.* (1) and Sasabe *et al.* (2) with some modifications. Briefly, 500-$\mu$l reactions containing 50 pmol baculovirus-expressed CYP6Z1 or CYP6Z2 (100 nM final), 1 $\mu$l of stock solution (in methanol) of 50 mM DDT (100 $\mu$M final), 10 mM carbaryl (20 $\mu$M final) or 5 mM xanthotoxin (10 $\mu$M final) with and without 50 $\mu$l of 3 mM NADPH (0.3 mM final) in cell lysate buffer (pH 7.5) were set up in side-by-side duplicates. Reactions were initiated with the addition of NADPH, incubated at 30°C for 30 min, and stopped by adding 250 $\mu$l of acetonitrile containing an internal standard (methoxychlor for DDT, carbofuran for carbaryl). The terminated reactions were mixed by vortexing and microcentrifuged at 13,000 rpm for 10 min. Each supernatant was then passed through a 0.45-$\mu$m Alltech Microspin filter by microcentrifuging at 5,000 rpm for 5 min. The proportion of internal standard and remaining substrate were determined by reversed-phase HPLC using a 4.6- $\times$ 150-mm Waters Xterra $RP_{18}$ column with acetonitrile:$H_2O$ (70:30) as solvent at a flow rate of 1.2 ml/min for DDT or acetonitrile:$H_2O$ (40:60) as solvent at a flow rate of 1 ml/min for carbaryl. DDT and the methoxychlor internal standard were UV-detected at 220 nm with the retention time for DDT being 10.8 min and the retention time for methoxychlor being 5.0 min. Carbaryl and the carbofuran internal standard were UV-detected at 280 nm with the retention time for carbaryl being 6.0 min and the retention time for carbofuran being 4.8 min. P450 enzymatic activities were reported as substrate disappearance by comparing the substrate remaining in the NADPH-containing reactions with substrate remaining in NADPH-lacking reactions after normalization against each internal standards and are expressed as nanomoles of substrate disappearance per minute per nanomole of P450. Each experiment was replicated at least three times.

1. Wen Z, Pan L, Berenbaum MR, Schuler MA (2003) Metabolism of linear and angular furanocoumarins by *Papilio polyxenes* CYP6B1 coexpressed with NADPH cytochrome P450 reductase. *Insect Biochem Mol Biol* 33:937–947.

2. Sasabe M, Wen Z, Berenbaum MR, Schuler MA (2004) Molecular analysis of CYP321A1, a novel cytochrome P450 involved in metabolism of plant allelochemicals (furanocoumarins) and insecticides (cypermethrin) in *Helicoverpa zea*. *Gene* 338:163–175.

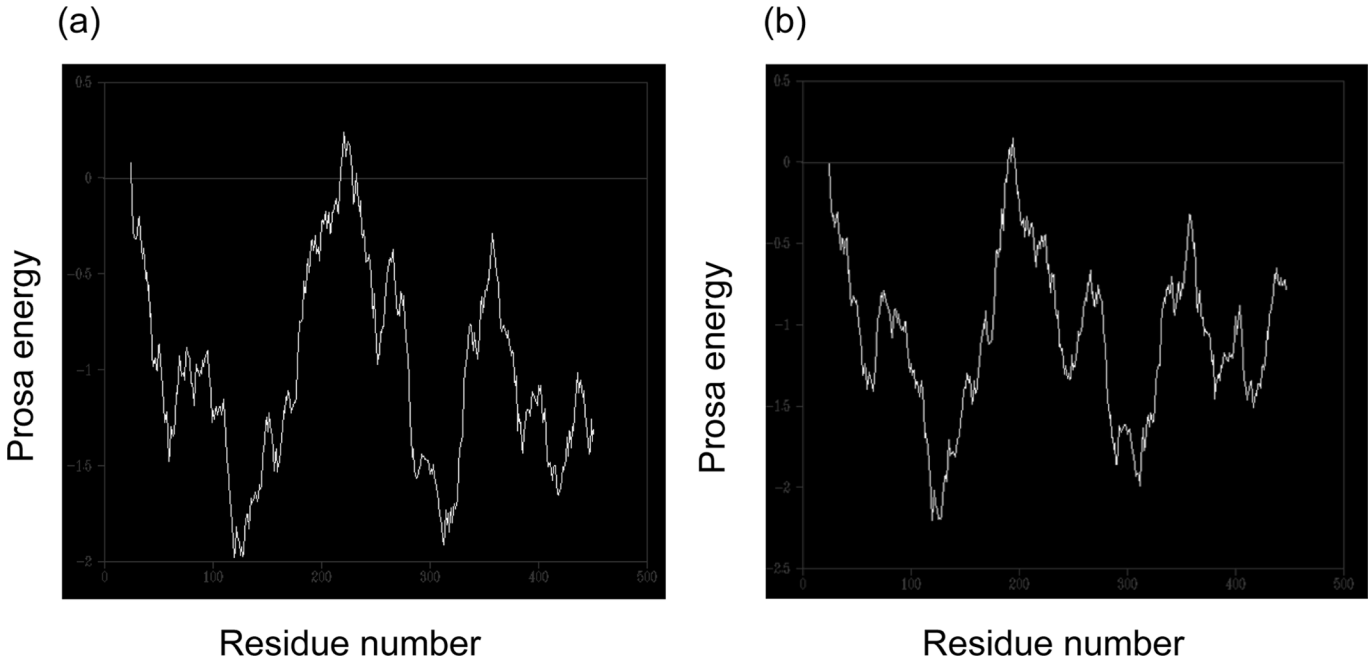


**Fig. S1.** Prosa energy profiles. The Prosa energy profiles of CYP6Z1 (*a*) and CYP6Z2 (*b*) are shown with amino acid positions corresponding to those in the full-length protein.

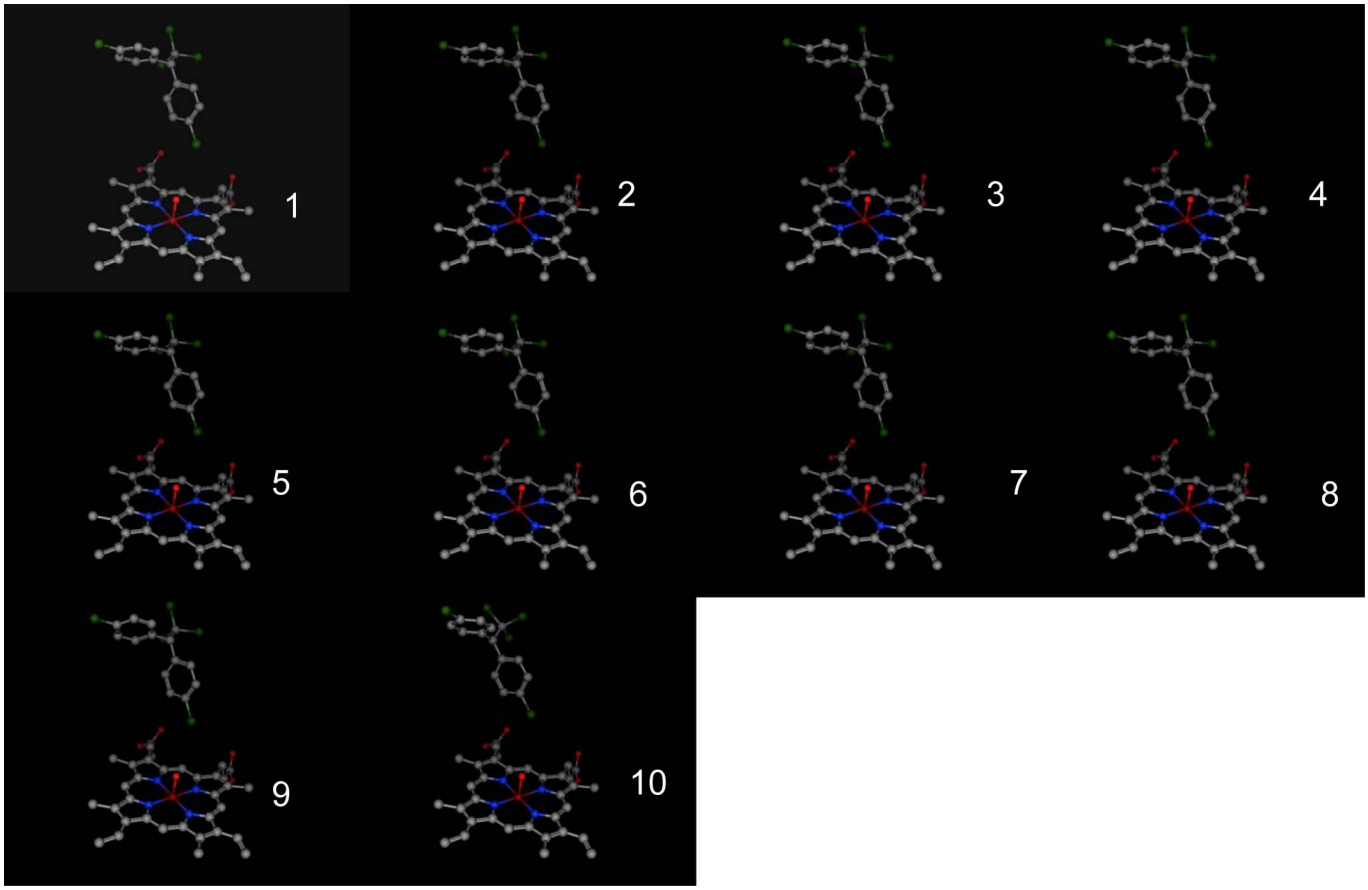


**Fig. S2.** Predicted DDT binding modes in the CYP6Z2 model. Numbers at the bottom right corner of each conformation represent the ranking of each in order of increasing predicted energies for the complex. Distances and energies for each are shown in Table S1.

**Table S1. Top-ranking DDT docking modes in the CYP6Z2 model**

| Ranking | Distance, Å | Total energy, kcal/mol |
|---|---|---|
| 1 | 5.74 | 38.30 |
| 2 | 5.63 | 39.47 |
| 3 | 5.69 | 40.45 |
| 4 | 5.25 | 40.51 |
| 5 | 5.57 | 41.09 |
| 6 | 5.65 | 42.09 |
| 7 | 4.71 | 43.76 |
| 8 | 7.28 | 44.26 |
| 9 | 8.50 | 46.49 |
| 10 | 8.20 | 46.54 |

The docked conformations of DDT in CYP6Z2 shown in Fig. S2 are ranked in order of increasing total energies and are shown with the distance between the heme oxygen and the closest atom of the docked conformation.