

SUPPLEMENTAL DATA

Biochemistry (2007) **46**, 000-000. Bin Zhao, David C. Lamb, Li Lei, Steven L. Kelly, Hang Yuan, David L. Hachey, and Michael R. Waterman

Different Binding Modes of Two Flaviolin Substrate Molecules in Cytochrome P450 158A1 (CYP158A1) Compared to CYP158A2

FIG. S1. Flaviolin binding to CYP158A1. CYP158A1 was present at 2.5 μM ; see Experimental Procedures for details. Additions included 1 to 50 μM of flaviolin (1 mM). The K_d value was estimated to be 10.5 μM .

FIG. S2. Identification of product (P1) by LC-MS/MS compared with corresponding product characterized from CYP158A2 activity. *A*, Extracted base peak and chromatograms; *B*, MS full scan of P1 from CYP158A1; *C*, MS full scan of P1 from CYP158A2; *D*, MS/MS scan of 411 m/z (P1) in CYP158A1; *E*, MS/MS scan of 411 m/z (P1) in CYP158A2.

FIG. S3. Identification of product (P2) by LC-MS/MS compared with corresponding product characterized from CYP158A2 activity. *A*, Extracted base peak and chromatograms; *B*, MS full

scan of P2 from CYP158A1; *C*, MS full scan of P2 from CYP158A2; *D*, MS/MS scan of 411 m/z (P2) in CYP158A1; *E*, MS/MS scan of 411 m/z (P2) in CYP158A2.

FIG. S4 Superimposition of CYP158A1 (orange) and CYP158A2 (green) indicating the different orientation of key residue Arg92 (CYP158A1) and Arg90 (CYP158A2). The Arg92 contacts the G helix and pushes it out of the active site in CYP158A1.

FIG. S5 Binding of imidazole and flaviolin molecules in CYP158A1. F1 and I1 indicate the positions of both molecules associated with the heme. F2 and I2 indicate the positions of both molecules in a cleft near the entry to the substrate access channel.

FIG. S6 Electron density maps of imidazole in the structure of inhibitor imidazole-bound CYP158A1. The electron density map was calculated using σ_A -weighted $2|F_o| - |F_c|$ coefficients and is contoured at 1.0 σ shown in red. The two electron densities above the heme are from two imidazole molecules. The proximal imidazole and distal imidazole molecules are denoted I1 and I2, respectively. A water molecule 649 is in green. The side chain atoms, imidazole, and heme are rendered as stick figures (grey, carbon; blue, nitrogen; yellow, sulfur; red, oxygen and iron).

FIG. S7 Crystal packing of CYP158A1 flaviolin-bound structure. The protein structures are represented in light blue, green, cyan, and light purple. Heme is colored in red and flaviolin molecules are in orange.

FIG. S1:

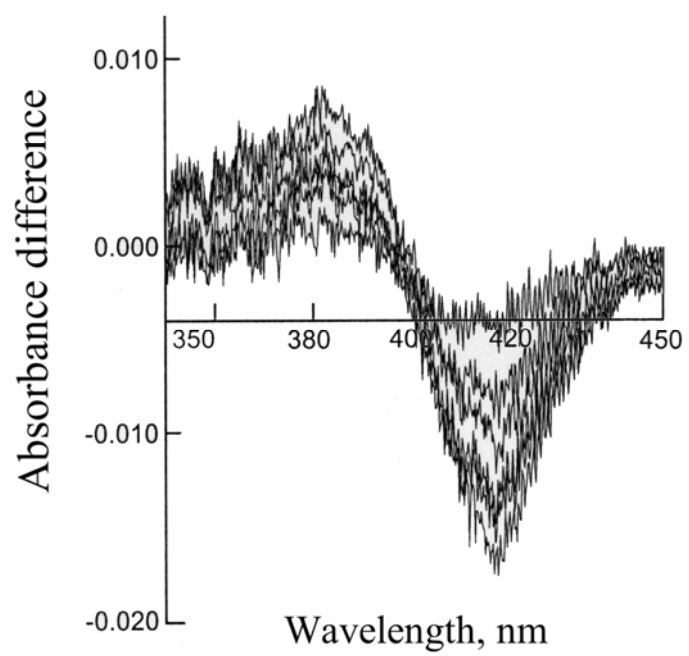


FIG. S2:

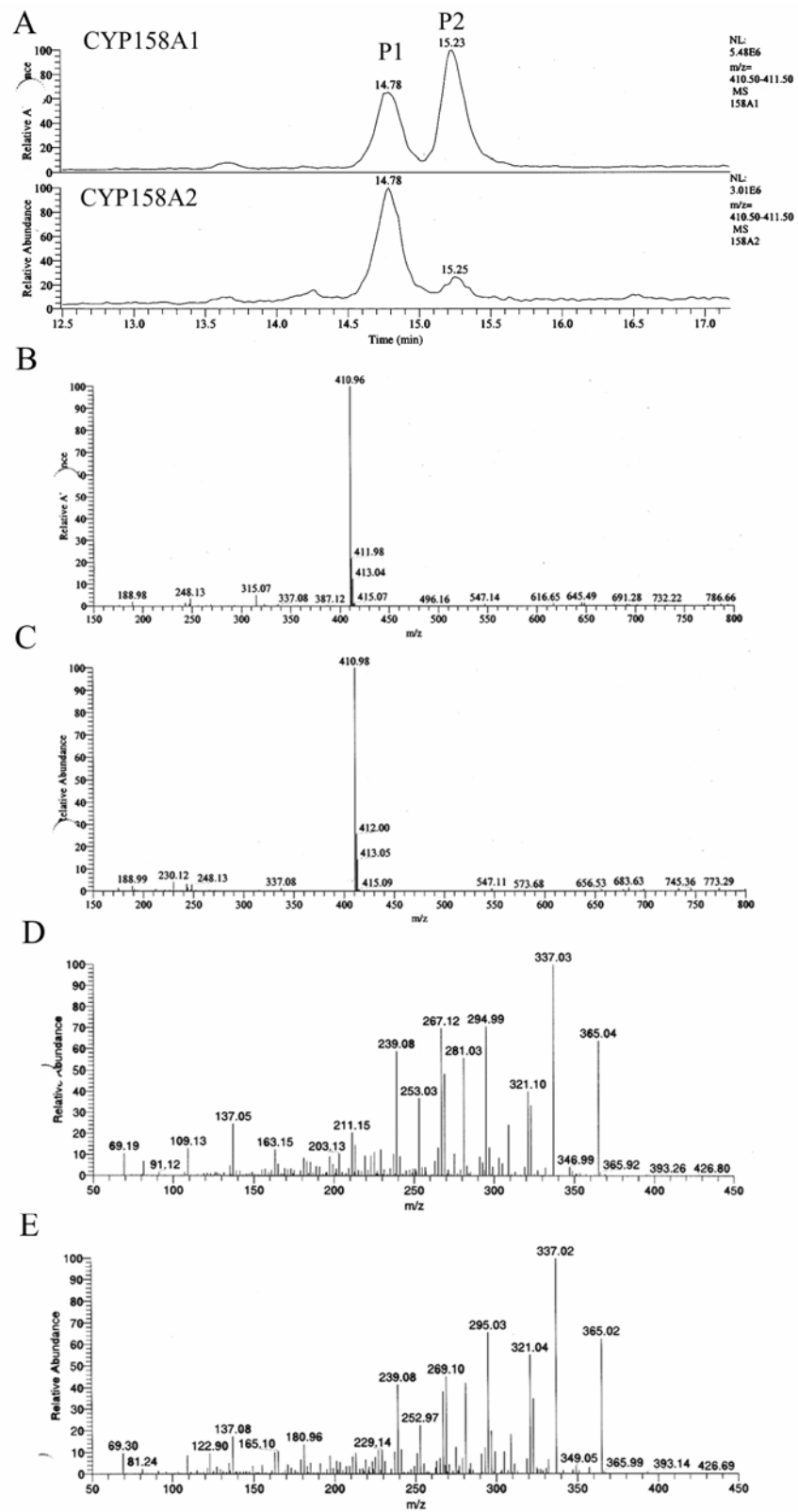


FIG. S3:

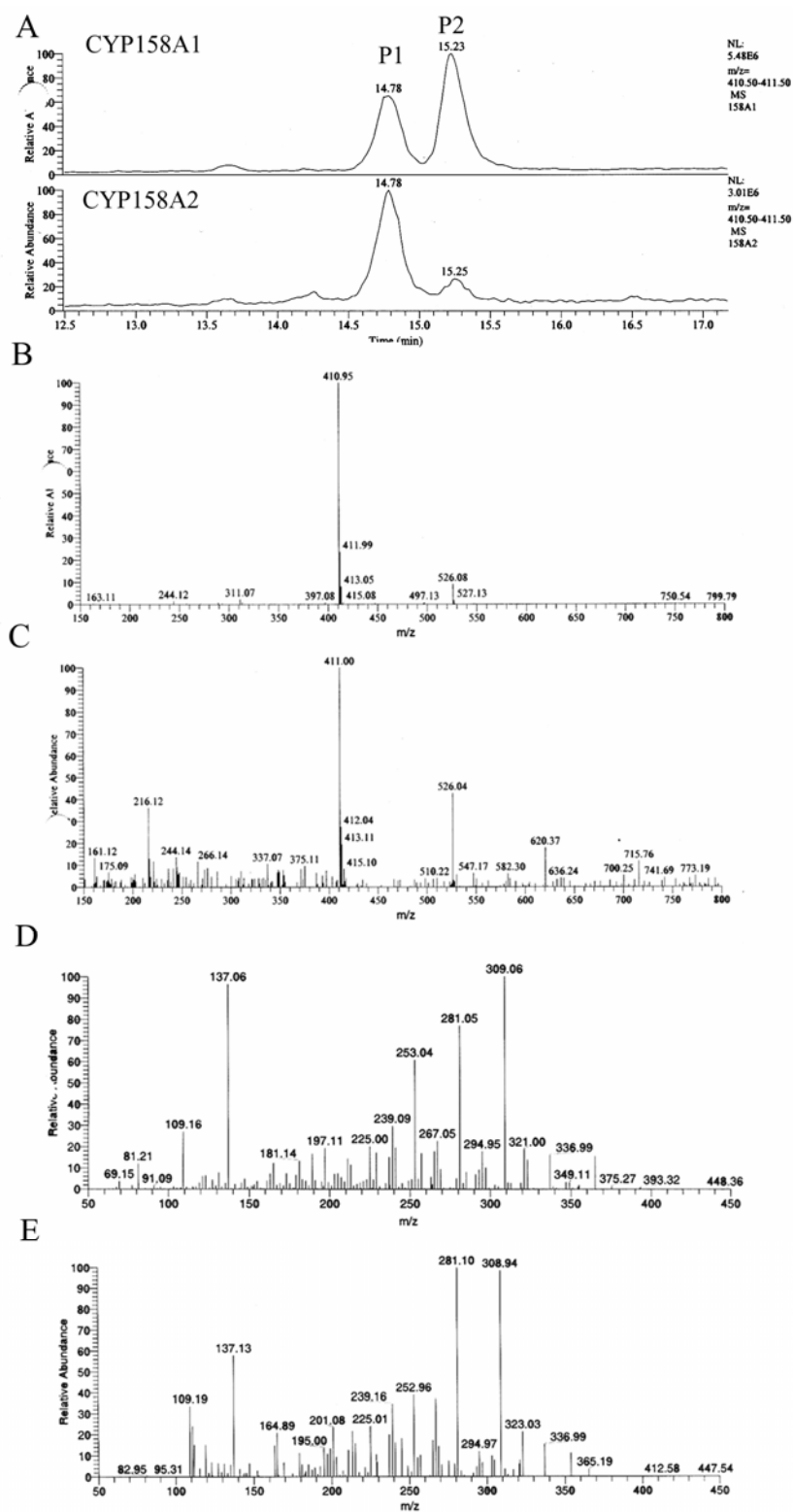


FIG. S4:

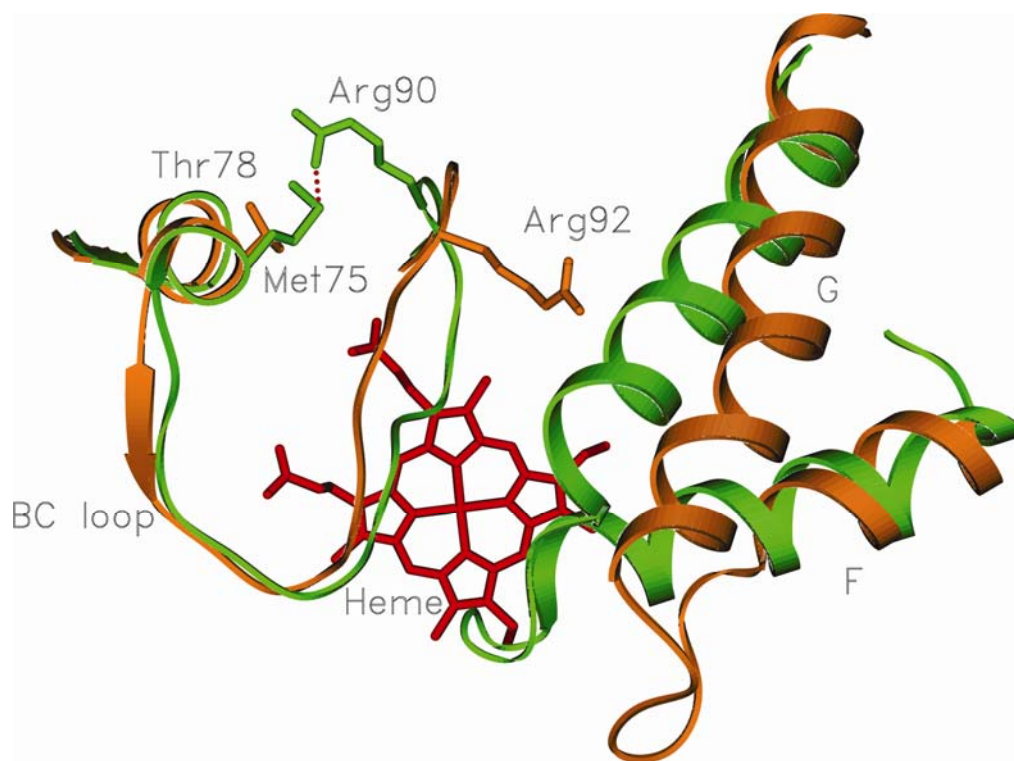


FIG. S5:

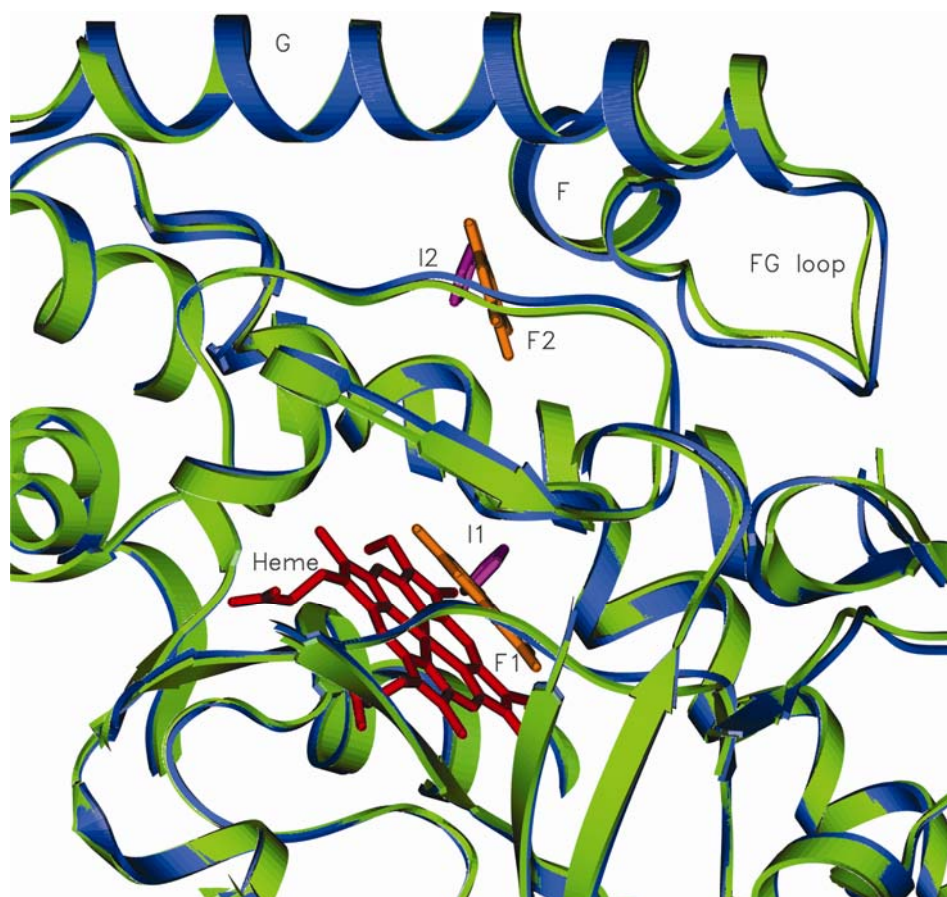


FIG. S6:

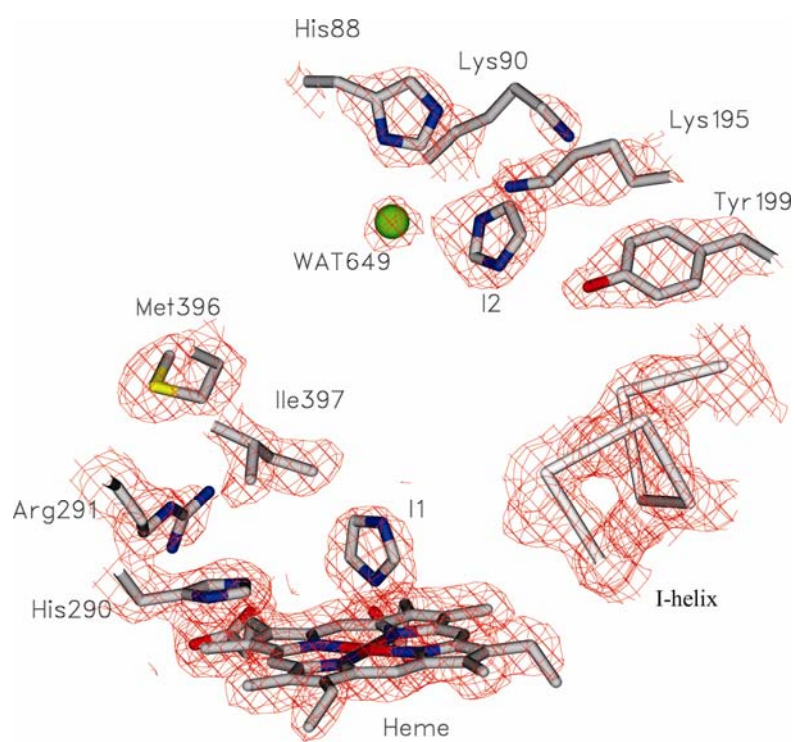


FIG. S7

