SUPPORTING INFORMATION

Human cytochrome P450 enzymes bind drugs and other substrates using conformational selection modes

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Fig. S1

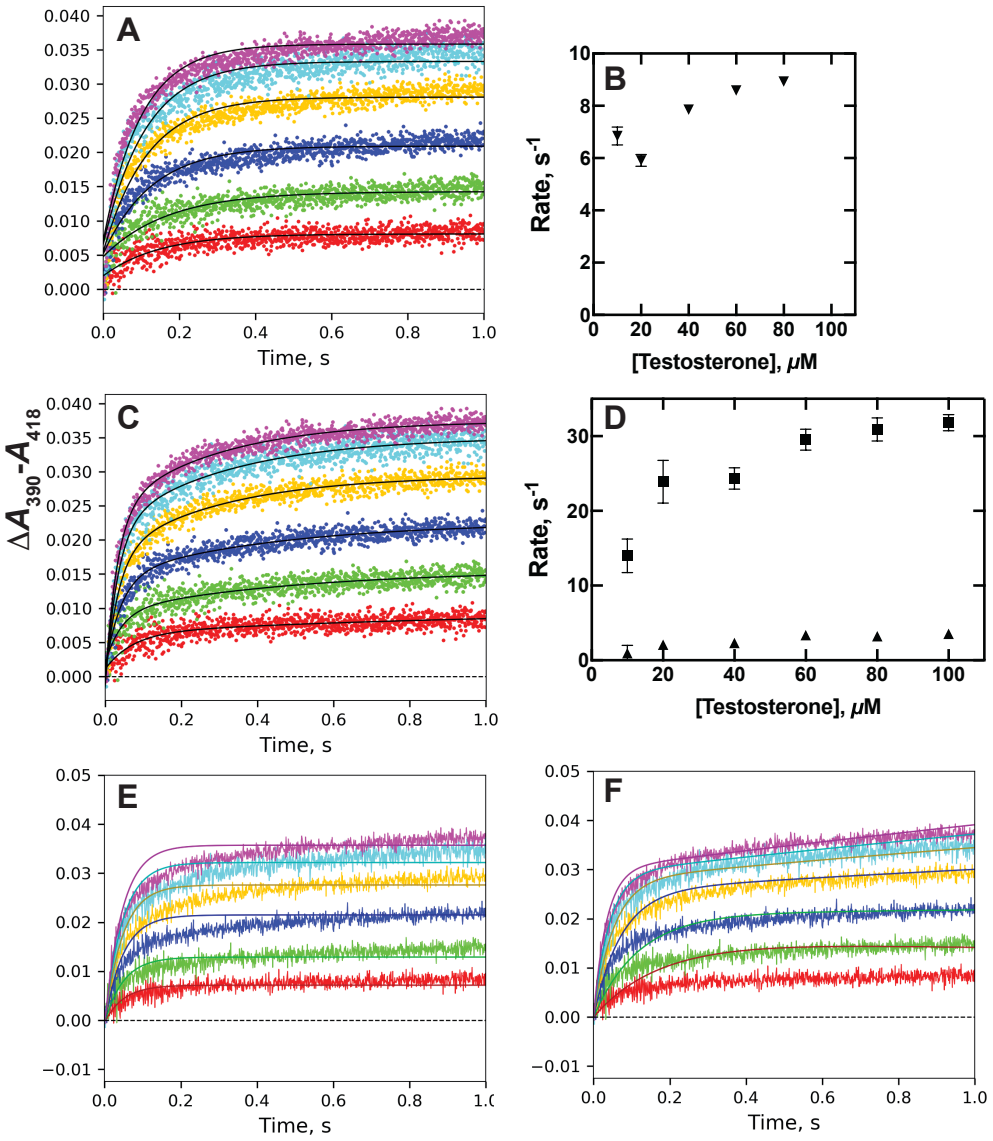

Figure S1. Binding of testosterone to P450 3A4. Data were from ref. (1). P450 3A4 was mixed with varying concentrations of testosterone (20-red, 40-green, 80-dark blue, 120-gold, 160-light blue, and 200-magenta μM). *A*, single exponential fit of data. *B*, plot of single exponential (Part *A*) rates of binding *vs*. testosterone concentration. *C*, biexponential fit of data. *D*, plots of biexponential rates of binding (from Part *C*) as a function of testosterone concentration (fast (■) and slow (▲)). *E*, fits of data to an induced fit model with $k_1 = 1.7 \times 10^6$ M^{-1} s^{-1}, $k_{-1} = 310$ s^{-1}, k_2 3.6 s^{-1}, and $k_{-2} = 20$ s^{-1} ($\varepsilon_{390\text{-}418}$ 52 mM^{-1} cm^{-1}). *F*, fits of data to a conformational selection model with $k_1 = 0.028$ s^{-1}, k_{-1} 1.4 s^{-1}, k_2 0.13 $\times 10^6$ M^{-1} s^{-1}, and $k_{-2} = 2.3$ s^{-1} ($\varepsilon_{390\text{-}418}$ 56 mM^{-1} cm^{-1}).

Fig. S2

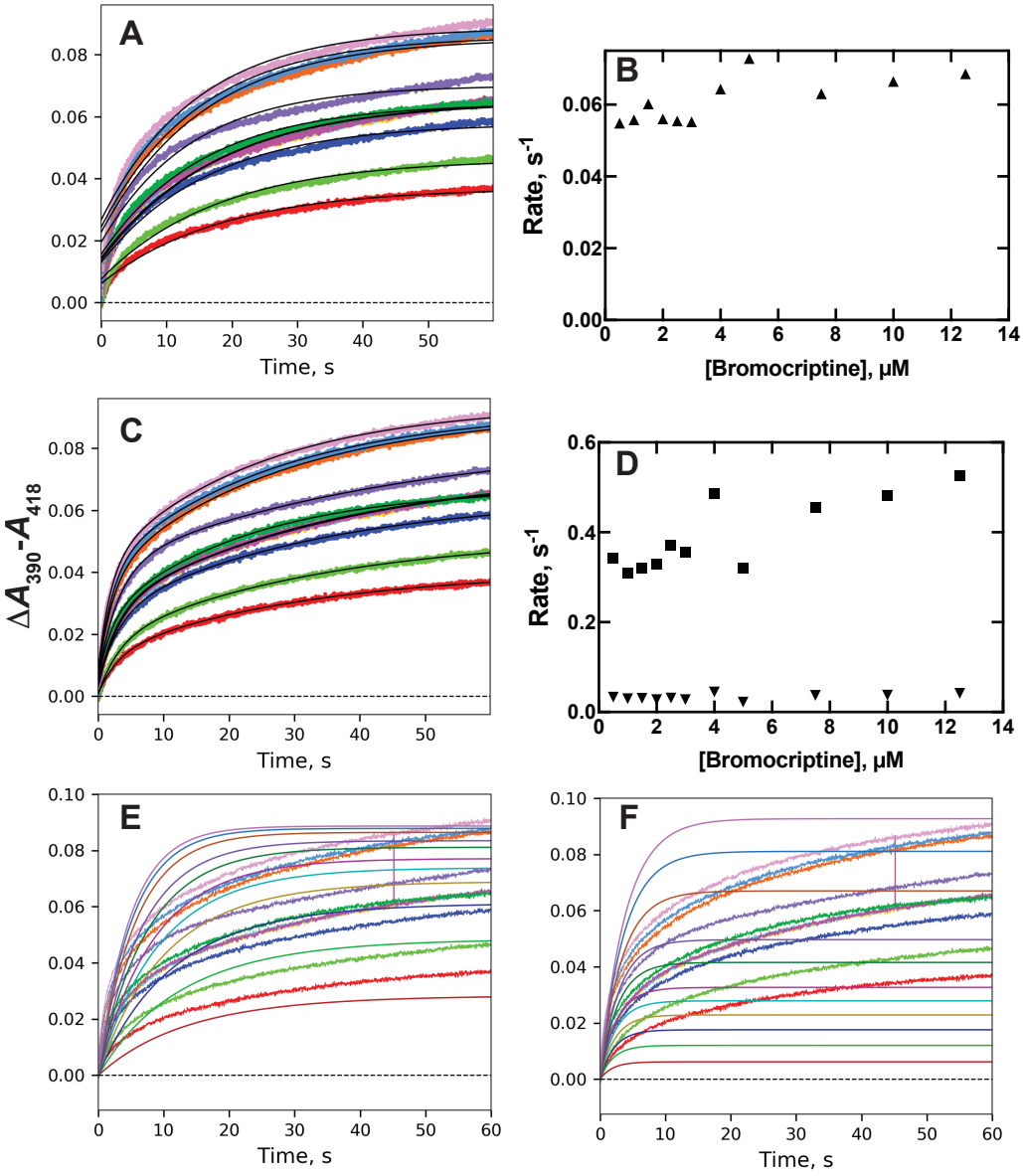

Figure S2. Binding of bromocriptine to P450 3A4. Data were from ref. (1). P450 3A4 (2 μM) was mixed with 1-red, 2-green, 3-dark blue, 4-mauve, 5-green, 6-light blue, 8-purple, 10-red, 15-orange, 20-blue, or 25-pink μM bromocriptine. *A*, single exponential fit of data. *B*, plot of single exponential (Part *A*) rates of binding *vs*. bromocriptine concentration. *C*, biexponential fit of data. *D*, plots of biexponential rates of binding (from Part *C*) as a function of bromocriptine concentration (fast (■) and slow (▼)). *E*, fits of data to an induced fit model with $k_1 = 4.1 \times 10^6$ M^{-1} s^{-1}, $k_{-1} = 36$ s^{-1}, $k_2 = 0.25$ s^{-1}, and $k_{-2} = 0.25$ s^{-1} ($\varepsilon_{390\text{-}418}$ 25 mM^{-1} cm^{-1}). *F*, fits of data to a conformational selection model with $k_1 = 0.15$ s^{-1}, k_{-1} 8.8 s^{-1}, $k_2 = 1.0 \times 10^6$ M^{-1} s^{-1}, and $k_{-2} = 0.5$ s^{-1} ($\varepsilon_{390\text{-}418}$ 26 mM^{-1} cm^{-1}).

Fig. S3

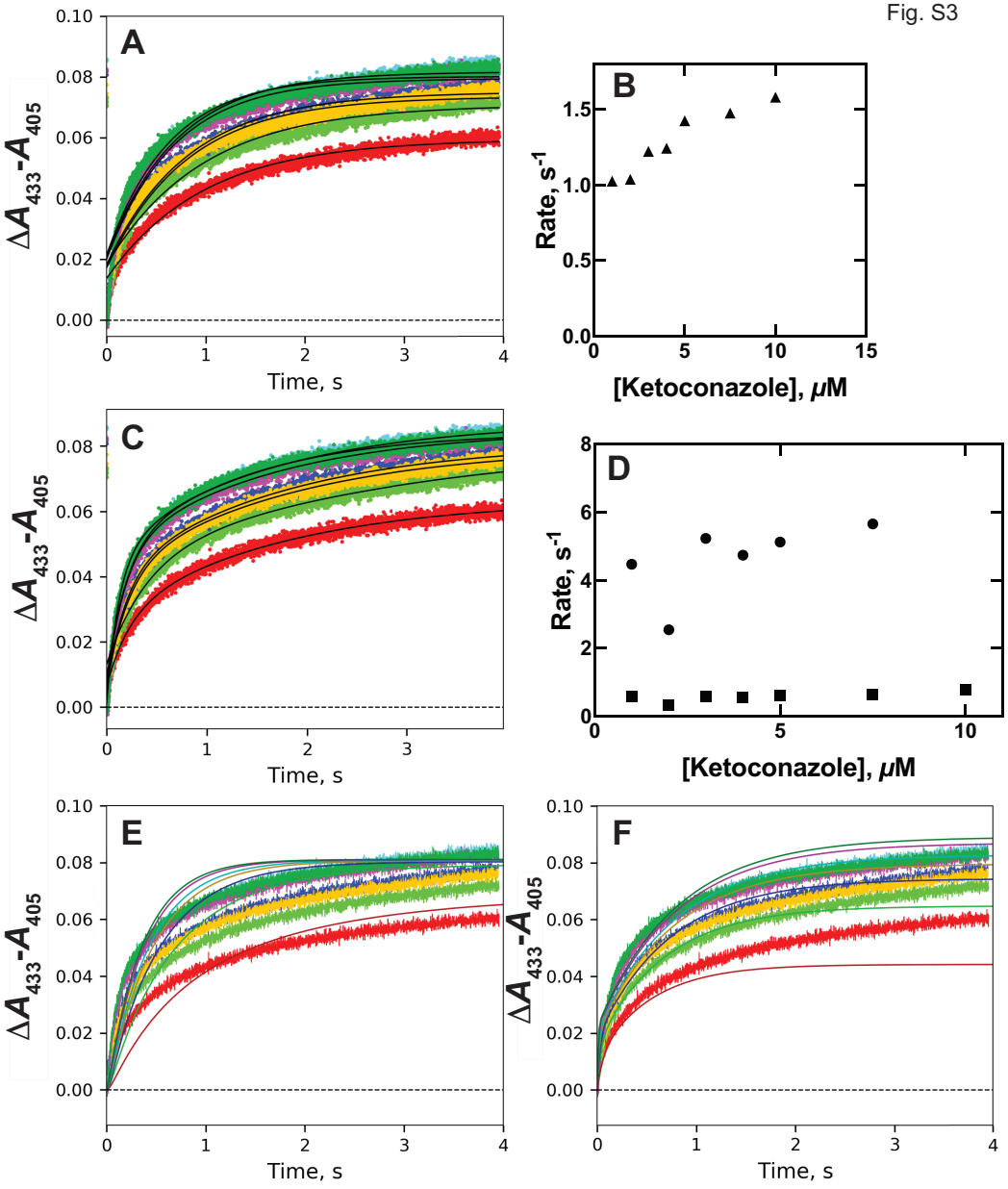

Figure S3. Binding of ketoconazole to P450 3A4. Data were from ref. (2). P450 3A4 (4 μM) were mixed with varying concentrations of ketoconazole (2-red, 4-green, 6-gold, 8-blue, 10-mauve, 15-green, or 20-blue μM). *A*, single exponential fit of data. *B*, plot of single exponential (Part *A*) rates of binding *vs*. ketoconazole concentration. *C*, double-exponential fits of data. *D*, plots of biexponential rates of binding (from Part *C*) as a function of ketoconazole concentration (fast (●) and slow (■)). *E*, fits of data to an induced fit model with $k_1 = 2.4 \times 10^6$ M^{-1} s^{-1}, $k_{-1} = 15$ s^{-1}, $k_2 = 3.2$ s^{-1}, and $k_{-2} = 0.05$ s^{-1} ($\varepsilon_{433\text{-}405}$ 10.5 mM^{-1} cm^{-1}). *F*, fits of data to a conformational selection model with $k_1 = 1.2$ s^{-1}, $k_{-1} = 3.6$ s^{-1}, $k_2 = 2.7 \times 10^6$ M^{-1} s^{-1}, and $k_{-2} = 1.7$ s^{-1} ($\varepsilon_{405\text{-}433}$ 16 mM^{-1} cm^{-1}).

Fig. S4

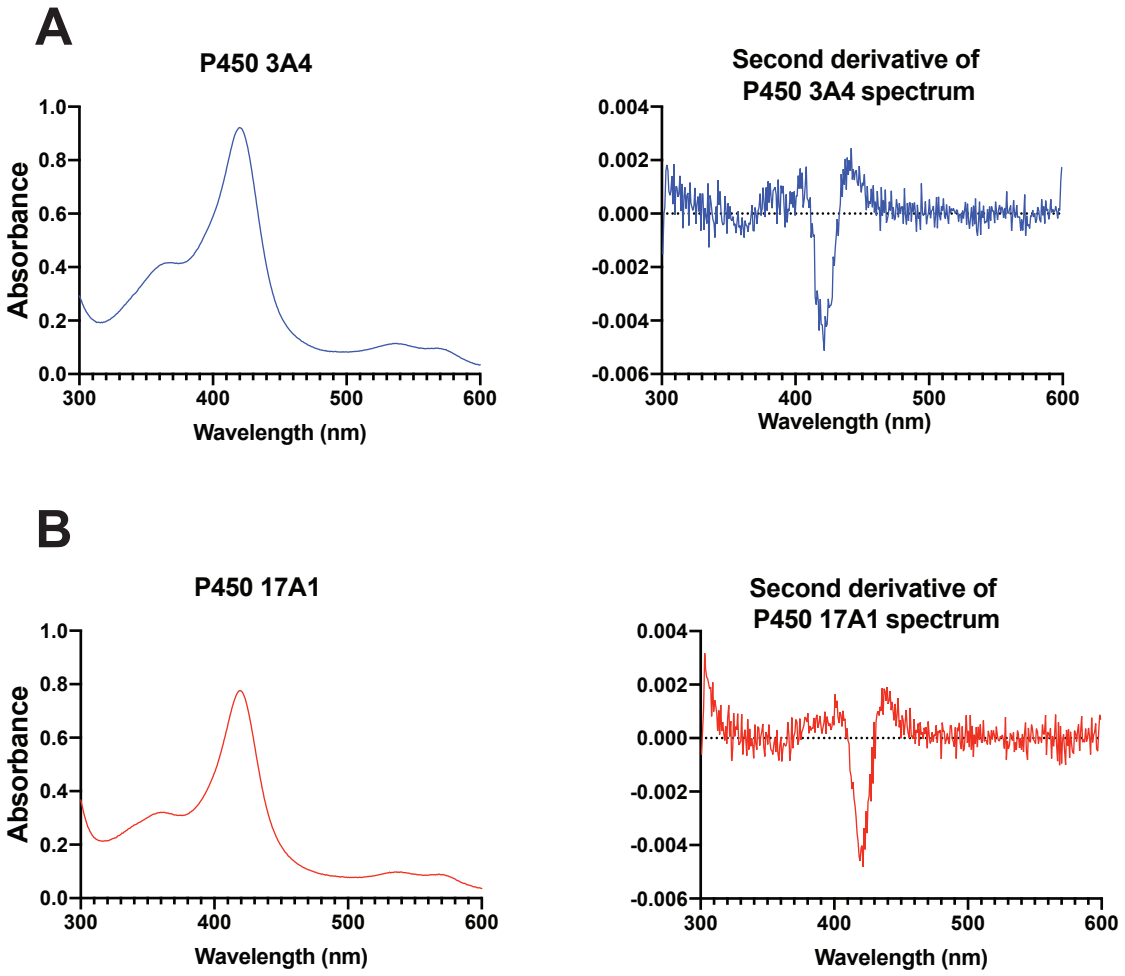

Figure S4. Spin state analysis of P450s 3A4 (Part *A*) and 17A1 (Part *B*). The estimates (>95% low-spin) were made by analyzing the negative peaks (418 and 390 nm for low- and high-spin iron, respectively) generated in second derivative analysis of spectra recorded in the absence of ligand (3). *A*, P450 3A4; *B*, P450 17A1.

References

1. Isin, E. M., and Guengerich, F. P. (2006) Kinetics and thermodynamics of ligand binding by cytochrome P450 3A4. *J. Biol.Chem.* **281**, 9127-9136

2. Isin, E. M., and Guengerich, F. P. (2007) Multiple sequential steps involved in the binding of inhibitors to cytochrome P450 3A4. *J. Biol.Chem.* **282**, 6863-6874

3. O'Haver, T. C., and Green, G. L. (1976) Numerical error analysis of derivative spectrometry for the quantitative analysis of mixtures. *Anal. Chem.* **48**, 312-318