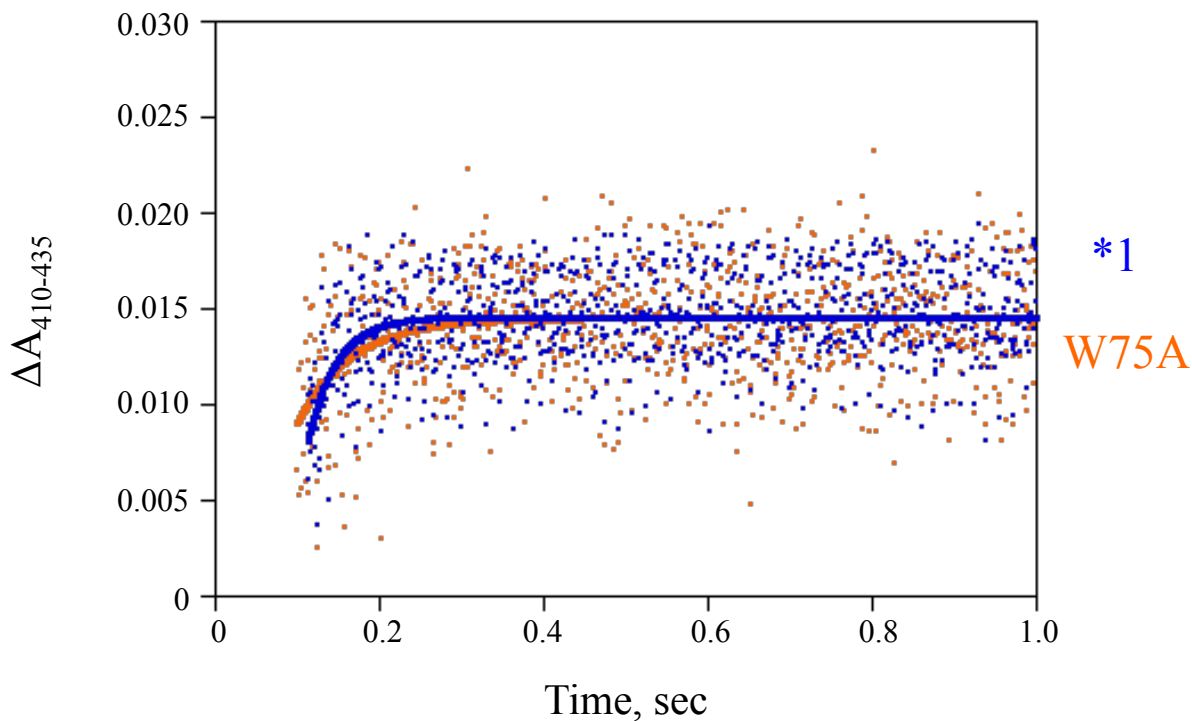


Tryptophan-75 is a low energy channel gating residue that facilitates substrate egress/access in cytochrome P450 2D6. McCarty, Ratliff, K. Furge, L. Furge. *Drug Metab Dispos*, DMD-AR-2020-000274.



**Supp Figure S5. Determination of  $k_{\text{off}}$  by stopped-flow spectroscopy.** Composite absorbance changes from absorbance maxima and minima ( $\Delta A_{410-435}$ ) over time (4 sec) were created for each reaction condition. Only the first second is shown. Four replicates of reactions with CYP2D6\*1 (blue line) and seven reactions with the Trp75Ala mutant (orange line) were averaged and fit to first order rate equation in the Olis Spectral Works software. Values for  $k_{\text{off}}$  with CYP2D6\*1 and the Trp75Ala mutant were  $28 \pm 4$  and  $14.4 \pm 0.6 \text{ s}^{-1}$ , respectively.