Supporting Information for:

Surface Plasmon Resonance Analysis of Antifungal Azoles Binding to CYP3A4 with Kinetic Resolution of Multiple Binding Orientations

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Kinetic Equivalence of Heteroanalyte model in the BIAevaluation software and the parallel binding trajectory model used here.

The concentrations of [L], and hence L_1 and L_2, are constant, unless mass transfer is rate limiting, which it is not.

Heteroanalyte Model, $L_1 \neq L_2$

$$L_1 + P \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [L_1 \bullet P] \qquad \frac{\partial([L_1 \bullet P])}{\partial t} = [L_1][P]k_1 - [L_1 \bullet P]k_{-1}$$

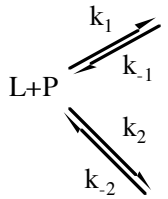
$$L_2 + P \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} [L_2 \bullet P] \qquad \frac{\partial([L_2 \bullet P])}{\partial t} = [L_2][P]k_2 - [L_2 \bullet P]k_{-2}$$

$$\text{total response} = \frac{\partial(RU)}{\partial t} = \frac{\partial([L_1 \bullet P])}{\partial t} + \frac{\partial([L_2 \bullet P])}{\partial t} = [L_1][P]k_1 - [L_1 \bullet P]k_{-1} + [L_2][P]k_2 - [L_2 \bullet P]k_{-2}$$

assuming $L_1 = L_2$, and $k_1 \neq k_2$

$$\frac{\partial(RU)}{\partial t} = [L][P](k_1 + k_2) - [L \bullet P]k_{-1} - [P \bullet L]k_{-2} \qquad \text{equation 1}$$

Parallel Pathway Model, $[L \bullet P] \neq [P \bullet L]$



$$\frac{\partial([L \bullet P])}{\partial t} = [L][P]k_1 - [L \bullet P]k_{-1}$$

$$\frac{\partial([P \bullet L])}{\partial t} = [L][P]k_2 - [P \bullet L]k_{-2}$$

$$\text{total response} = \frac{\partial(RU)}{\partial t} = \frac{\partial([L \bullet P])}{\partial t} + \frac{\partial([P \bullet L])}{\partial t} = [L][P]k_1 - [L \bullet P]k_{-1} + [L][P]k_2 - [P \bullet L]k_{-2}$$

$$= [L][P](k_1 + k_2) - [L \bullet P]k_{-1} - [P \bullet L]k_{-2} \quad = \text{equation 1}$$

The kinetic equivalence of the parallel trajectory model (scheme 1) and the heteroanalyte model is only valid when the concentration of drug is time-independent. The analysis, as in nearly all SPR experiments, assumes that the concentration of free drug does not change during the binding phase. This is true in SPR experiments only if mass transfer is not rate limiting, as we have verified experimentally with standard protocols. Also, our model will only be valid if rebinding of drug is negligible during the dissociation phase,

yielding apparent irreversible dissociation. This is also a common assumption in SPR experiments and has been verified experimentally in many cases.

Figure S1.

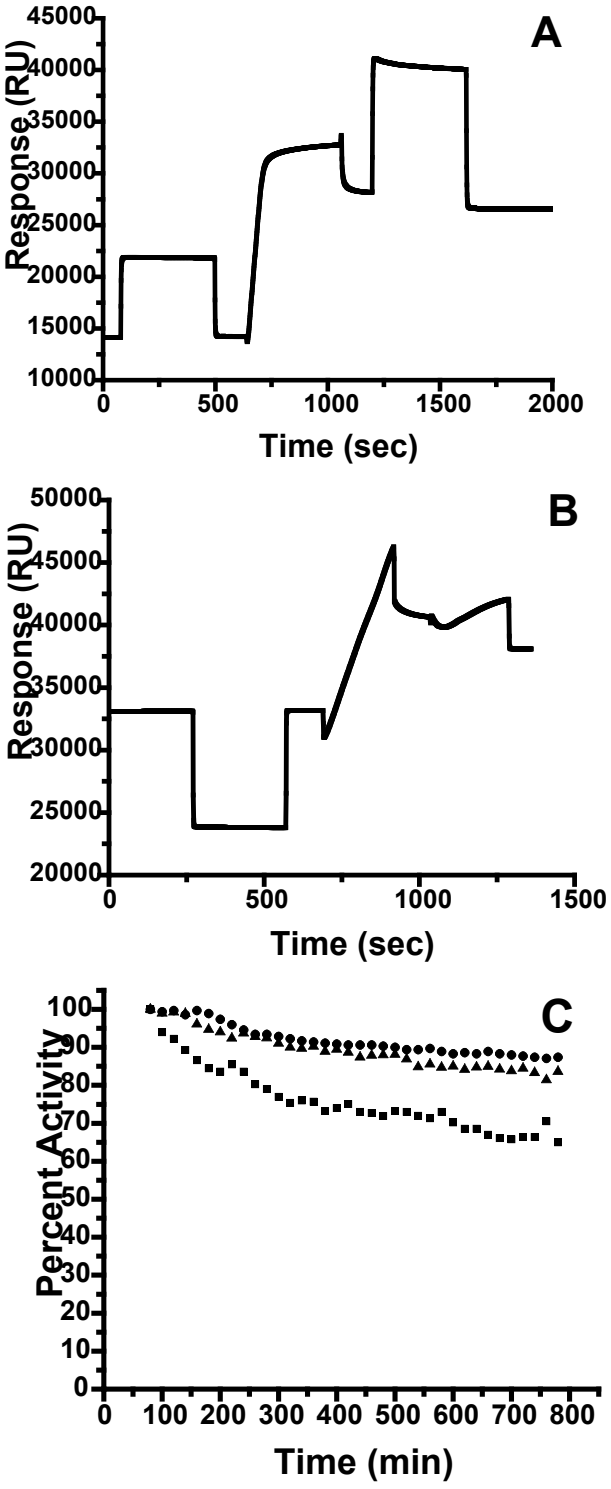


Figure S1. Sensorgrams of immobilization via amine coupling of CYP3A4 to research grade CM5 surfaces in two different buffering solutions; (A) HBS-EP buffer and (B) 10% glycerol, 100mM KPi, 7.4. (C) Measure of the stability of the CYP3A4 surface

under various conditions; (_) 3% MeOH, 100mM KPi, 7.4 at 25°C, (_) 3% MeOH, 100mM KPi, 7.4 at 10°C, (_) 1% MeOH, 10% glycerol, 100mM KPi, 7.4 at 10°C. Ketoconazole at a concentration of 20 μM was injected every twenty minutes for 10 hours. The first three injections showed irreproducibility believed due to settling of the CYP3A4 surface. The fourth injection (at the 80 minute mark) was taken as 100% binding so percent remaining activity could be plotted versus time.

Figure S2.

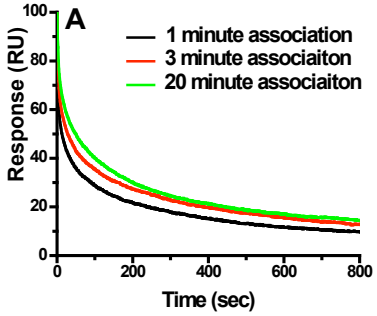


Figure S2. A 'linkage test' using 'pulsed SPR' of (2S,4R)-KTZ binding to CYP3A4. (A) Sensorgrams for dissociation of (2S,4R)-KTZ at 17 μM at 10 °C in 1% MeOH, 10% glycerol, 100 mM potassium phosphate 7.4 after 1, 3, or 20 minute association pulses. Shorter pulses of association yield faster off-rates. This is consistent with several models including the parallel trajectory model.