

Supplemental Information

ANALYSIS OF HETEROTROPIC COOPERATIVITY IN CYTOCHROME P450 3A4 USING α -NAPHTHOFLAVONE AND TESTOSTERONE

Daniel J. Frank, Iliia G. Denisov, Stephen G. Sligar*

Department of Biochemistry, School of Molecular and Cellular Biology,

University of Illinois, 505 S. Goodwin Avenue, Urbana, IL 61801

*Corresponding author email: s-sligar@uiuc.edu

Derivations of equations in the mixed titration experiments

The total substrate [S], is equal to the sum of the individual substrates, [A] and [T], and $\mu_A[S] = [A]$ and $\mu_T[S] = [T]$, where μ_A and μ_T represent the molar fractions of A and T respectively. Here, K_i 's represent association constants for ease of displaying the equations.

Equations for the stepwise binding constants in the mixed substrate experiments

The concentration of enzyme with one substrate bound, c_1 , is given by the sum of the concentrations of the two states of the enzyme with a single substrate molecule bound, c_{01} and c_{10} .

$$c_1 = c_{01} + c_{10} = EK_1[S] = EK_{A1}[A] + EK_{T1}[T] = E\mu_A K_{A1}[S] + E\mu_T K_{T1}[S]$$

$$K_1 = \mu_A K_{A1} + \mu_T K_{T1}$$

The concentration of enzyme with two substrates bound, c_2 , is given by the sum of the concentrations of the three states of the enzyme with a two substrate molecules bound, c_{02} , c_{20} and c_{11} .

$$c_2 = c_{02} + c_{20} + c_{11} = EK_1K_2[S] = EK_{A1}K_{A2}[A] + EK_{T1}K_{T2}[T] + EK_{T1}K_{A2}[A] + EK_{A1}K_{T2}[T]$$

$$K_1K_2[S] = \mu_A^2 K_{A1}K_{A2}[S] + \mu_T^2 K_{T1}K_{T2}[S] + \mu_T\mu_A(K_{A1}K_{T2} + K_{T1}K_{A2})[S]$$

$$K_2 = (\mu_A K_{A1} + \mu_T K_{T1})(\mu_A K_{A2} + \mu_T K_{T2})/K_1$$

$$K_2 = \mu_A K_{A2} + \mu_T K_{T2}$$

The concentration of enzyme with three substrates bound, c_3 , is given by the sum of the concentrations of the four states of the enzyme with a three substrate molecules bound, c_{03} , c_{30} , c_{21} and c_{12} .

$$c_3 = c_{03} + c_{30} + c_{21} + c_{12} = EK_1K_2K_3[S] = EK_{A1}K_{A2}K_{A3}[A] + EK_{T1}K_{T2}K_{T3}[T] + EK_{A1}K_{T2}K_{A3}[A] + EK_{A1}K_{A2}K_{T3}[T] + EK_{T1}K_{A2}K_{A3}[A] + EK_{T1}K_{T2}K_{A3}[A] + EK_{T1}K_{A2}K_{T3}[T] + EK_{A1}K_{T2}K_{T3}[T]$$

$$K_1K_2K_3[S] = \mu_A^3 K_{A1}K_{A2}K_{A3}[S] + \mu_T^3 K_{T1}K_{T2}K_{T3}[S] + \mu_A^2\mu_T(K_{A1}K_{T2}K_{A3} + K_{A1}K_{A2}K_{T3} + K_{T1}K_{A2}K_{A3})[S] + \mu_A\mu_T^2(K_{T1}K_{T2}K_{A3} + K_{T1}K_{A2}K_{T3} + K_{A1}K_{T2}K_{T3})[S]$$

$$K_3 = (\mu_A K_{A1} + \mu_T K_{T1})(\mu_A K_{A2} + \mu_T K_{T2})(\mu_A K_{A3} + \mu_T K_{T3})/K_1 K_2$$

$$K_3 = \mu_A K_{A3} + \mu_T K_{T3}$$

Equations for incorporating heterotropic cooperativity into simulated data sets

This allows for binding constants of the mixed substrate system to be evaluated according to equation 1, by combining the mixed and non-mixed states for a given number of substrates bound into a single state. In order to simulate specific heterotropic interactions, as shown in Figure 4, it is necessary to separate the mixed and non-mixed states so that:

$$K_1 = \mu_A K_{A1} + \mu_T K_{T1}$$

$$K_2 = (\mu_A^2 K_{A1} K_{A2} + \mu_T^2 K_{T1} K_{T2} + \gamma_1 \mu_T \mu_A (K_{A1} K_{T2} + K_{T1} K_{A2}))/K_1$$

$$K_3 = (\mu_A^3 K_{A1} K_{A2} K_{A3} + \mu_T^3 K_{T1} K_{T2} K_{T3} + \gamma_2 \mu_A^2 \mu_T (K_{A1} K_{T2} K_{A3} + K_{A1} K_{A2} K_{T3} + K_{T1} K_{A2} K_{A3}) + \gamma_3 \mu_A \mu_T^2 (K_{T1} K_{T2} K_{A3} + K_{T1} K_{A2} K_{T3} + K_{A1} K_{T2} K_{T3}))/K_1 K_2$$

and γ_1 , γ_2 , and γ_3 represent heterotropic parameters, which indicate favorable (or unfavorable) binding for the mixed states relative to the non-mixed states.

Equations for incorporating state specific functional amplitudes with binding constants in the mixed substrate experiments

To incorporate functional properties, such as spin transition, the mixed and non-mixed states may have different functional amplitudes, it is also necessary to for them to be considered separately:

$$a_1 K_1 = a_{A1} \mu_A K_{A1} + a_{T1} \mu_T K_{T1}$$

$$a_2 K_2 = (a_{A2} \mu_A^2 K_{A1} K_{A2} + a_{T2} \mu_T^2 K_{T1} K_{T2} + a_{M1} \mu_T \mu_A (K_{A1} K_{T2} + K_{T1} K_{A2}))/a_1 K_1$$

$$a_3 K_3 = (a_{A3} \mu_A^3 K_{A1} K_{A2} K_{A3} + a_{T3} \mu_T^3 K_{T1} K_{T2} K_{T3} + a_{M2} \mu_A^2 \mu_T (K_{A1} K_{T2} K_{A3} + K_{A1} K_{A2} K_{T3} + K_{T1} K_{A2} K_{A3}) + a_{M3} \mu_A \mu_T^2 (K_{T1} K_{T2} K_{A3} + K_{T1} K_{A2} K_{T3} + K_{A1} K_{T2} K_{T3}))/a_1 K_1 a_2 K_2$$

where a_{Ai} and a_{Ti} represent the functional amplitudes from the non-mixed states of ANF and TST respectively with i substrates bound ($i = 0, 1, 2, \text{ or } 3$), and the mixed state amplitudes a_{Mi} are given by the average of the non-mixed amplitudes:

$$a_{M1} = (a_{A2} + a_{T2})/2$$

$$a_{M2} = (2*a_{A3} + a_{T3})/3$$

$$a_{M3} = (a_{A3} + 2*a_{T3})/3$$

These equations are used to generate the models for Figures 4 and 5. The estimations of the mixed state amplitudes are necessary as they are impossible to measure independently.