Supplementary material

**Conformational Changes of Cytochrome P450 2D6 upon Binding of 7-Methoxy-4-(Aminomethyl)-Coumarin Studied by Time-Resolved Fluorescence Spectroscopy**

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*Depolarization for protein/substrate mixtures*

For the reaction between a protein and a substrate $S + P \leftrightarrow SP$ the association constant $K_a = K_d^{-1}$ is given by

$$K_a = \frac{[SP]}{[P][S]} \tag{1}$$

Using initial concentrations $[P]_0$ and $[S]_0$ and supposing an amount $x$ of substrate is bound to the protein, the equilibrium concentrations can be calculated via

$$K_a = \frac{x}{([P]_0 - x)([S]_0 - x)} \tag{2}$$

The orientation dependent factor in the fluorescence signal, for a single molecule, is given by

$$\vec{e}_a\vec{e}_a : \vec{\mu}_a\vec{\mu}_a\vec{\mu}_e\vec{\mu}_e : \vec{e}_e\vec{e}_e \tag{3}$$

where $\vec{e}_a$ is the polarization direction of the absorbed field, and $\vec{e}_e$ that of the emitted field; $\vec{\mu}_a$ is the direction of the absorbing transition dipole moment, and $\vec{\mu}_e$ the direction of the emitting transition dipole moment. Assuming that the molecules have arbitrary orientations, this quantity needs to be averaged over all orientations to get the true signal. Fortunately, the result of this non-trivial averaging of rank-four tensors is known [45]:

$$\left\langle \mu_{ai}\mu_{aj}\mu_{ek}\mu_{el}\right\rangle = \frac{2}{15}\left[\mu_a^2\mu_e^2\delta_{ij}\delta_{kl} + \vec{\mu}_a \cdot \vec{\mu}_e\left(\delta_{ik}\delta_{jl} + \delta_{il}\delta_{jk}\right)\right] \tag{4}$$

Introducing this in Eq.3 gives:

$$\vec{e}_a\vec{e}_a : \left\langle \vec{\mu}_a\vec{\mu}_a\vec{\mu}_e\vec{\mu}_e\right\rangle : \vec{e}_e\vec{e}_e = \frac{2}{15}\left[\mu_a^2\mu_e^2 + 2\left(\vec{\mu}_a \cdot \vec{\mu}_e\right)^2\left(\vec{e}_a \cdot \vec{e}_e\right)^2\right] = \frac{2\mu_a^2\mu_e^2}{15}\left[1 + 2\cos^2\theta\left(\vec{e}_a \cdot \vec{e}_e\right)^2\right] \tag{5}$$

where $\theta$ is the angle between absorption and emission transition dipole moment. If the absorbing and emitting fields are parallel $\vec{e}_a \cdot \vec{e}_e = 1$, and the fluorescence intensity is proportional to:

$$I_{vv} \propto \frac{2\mu_a^2\mu_e^2}{15}\left[1 + 2\cos^2\theta\right] \tag{6}$$

If the absorbing and emitting fields are perpendicular $\vec{e}_a \cdot \vec{e}_e = 0$, and

$$I_{vh} \propto \frac{2\mu_a^2\mu_e^2}{15} \tag{7}$$

Since the anisotropy is defined as

$$r = \frac{I_{vv} - I_{vh}}{I_{vv} + 2I_{vh}} = \frac{2\cos^2\theta}{3 + 2\cos^2\theta} \tag{8}$$

its value can vary between 0 and 0.4 (for $\theta = 0$).
Supposing $N_1$ and $N_2$ are the numbers of bond and unbound substrate molecules, respectively, Eq. 3 should be written as

$$\vec{e}_a\vec{e}_a : \left[N_1\vec{\mu}_{1a}\vec{\mu}_{1a}\vec{\mu}_{1e}\vec{\mu}_{1e} + N_2\vec{\mu}_{2a}\vec{\mu}_{2a}\vec{\mu}_{2e}\vec{\mu}_{2e}\right] : \vec{e}_e\vec{e}_e \tag{9}$$

Again performing the averages gives instead of Eq. 5

$$\frac{2\mu_a^2\mu_e^2}{15}\left[N_1 + N_2 + 2\left(N_1\cos^2\theta_1 + N_2\cos^2\theta_2\right)\vec{e}_a\vec{e}_e\right] \tag{10}$$

and the anisotropy will be

$$r = \frac{2N_1\cos^2\theta_1 + N_2\cos^2\theta_2}{3\left(N_1 + N_2\right) + 2N_1\cos^2\theta_1 + N_2\cos^2\theta_2} \tag{11}$$

From Eq. 8 we can easily see that

$$\cos^2\theta_i = \frac{3r_i}{2\left(1 - r_i\right)} \tag{12}$$

Assuming the anisotropy is 0 for unbound substrate, $\cos^2\theta_2 = 0$. From the limiting anisotropy $r_0$ (i.e. $r$ when no rotation of the fluorophore is possible), $\cos^2\theta_1$ can be determined. This reduces Eq.11 to

$$r = \frac{2N_1\cos^2\theta_1}{3\left(N_1 + N_2\right) + 2N_1\cos^2\theta_1} = \frac{2\cos^2\theta_1}{3\left(1 + N_2/N_1\right) + 2\cos^2\theta_1} \tag{13}$$

and of course $N_2/N_1$ = [S]/[PS]. Assuming the initial protein and substrate concentrations are known, the dissociation constant of the reaction can be calculated using Eqs. 1 and 2.