SUPPORTING INFORMATION

Obtaining equation 8

Consider a solution containing two (or more) receptors that can interact with a fluorophore that can be quenched by a given species (quencher) present in the solution. These receptors in the present case are cyclodextrins, but the following treatment can be applied to other receptors, such as surfactant monomers and micelles, for example.

In the absence of a quencher, the intensity of emission of the fluorophore, in the presence of two cyclodextrins, CD_1 and CD_2, would be:

$$(I_{em})_0 = \frac{(I_{em})_f + (I_{em})_1 K_1[CD_1] + (I_{em})_2 K_2[CD_2]}{1 + K_1[CD_1] + K_2[CD_2]} \qquad \text{S-1}$$

In this equation $(I_{em})_f$ corresponds to the emission intensity of the probe in the absence of receptors, $(I_{em})_1$ to the situation in which all the probe molecules are bound to CD_1 and $(I_{em})_2$ to CD_2. This equation follows from the fact that the concentrations of free fluorophore and fluorophore bound to CD_1 and CD_2 are given by:

$$[R_f] = \frac{1}{1 + K_1[CD_1] + K_2[CD_2]}[R]$$

$$[R_1] = \frac{K_1[CD_1]}{1 + K_1[CD_1] + K_2[CD_2]}[R] \qquad \text{S-2}$$

$$[R_2] = \frac{K_2[CD_2]}{1 + K_1[CD_1] + K_2[CD_2]}[R]$$

In the presence of a quencher, $(I_{em})_Q = (I_{em})_{Qf} + (I_{em})_{Q1} + (I_{em})_{Q2}$ and consequently,

$$\frac{(I_{em})_0}{(I_{em})_Q} = \frac{(I_{em})_{0f} + (I_{em})_{01} + (I_{em})_{02}}{(I_{em})_{Qf} + (I_{em})_{Q1} + (I_{em})_{Q2}} \qquad \text{S-3}$$

In this equation $(I_{em})_{0f}$, $(I_{em})_{01}$ and $(I_{em})_{02}$ represent the emission intensities of R_f, R_1 and R_2 in the absence of the quencher and $(I_{em})_{Qf}$, $(I_{em})_{Q1}$ and $(I_{em})_{Q2}$ in the presence of the quencher.

Of course,

$$\frac{(I_{em})_{0i}}{(I_{em})_{Qi}} = 1 + (K_{SV})_i[Q] \qquad i = f, 1, 2 \qquad \text{S-4}$$

Thus

$$(I_{em})_{0i} = (I_{em})_{Qi} + (I_{em})_{Qi}(K_{SV})_i[Q] \qquad i = f, 1, 2 \qquad \text{S-5}$$

Introducing equation S-5 in equation S-3,

$$\frac{(I_{em})_0}{(I_{em})_Q} = 1 + \frac{(I_{em})_{Qf}(K_{SV})_f + (I_{em})_{Q1}(K_{SV})_1 + (I_{em})_{Q2}(K_{SV})_2}{(I_{em})_{Qf} + (I_{em})_{Q1} + (I_{em})_{Q2}}[Q] \qquad \text{S-6}$$

Now, $(I_{em})_{Qi}$ can be written as:

$$(I_{em})_{Qi} = \varepsilon_i l i_0 [R_i] \varphi_i \qquad \text{S-7}$$

In this equation ε_I is the molar extinction coefficient of species i, l is the light path length of the cuvette, i_0 is the intensity of the incident beam on the sample and φ_I is the quantum yield for the emission of species i, in the presence of the quencher. Of course, $[R_i]$ are given by equations S-2.

Introducing equations S-2 and S-7 in equation S-6 one obtains:

$$\frac{(I_{em})_0}{(I_{em})_Q} = 1 + \frac{\frac{l\varphi_f \varepsilon_f i_0 (K_{SV})_f}{1+K_1[CD_1]+K_2[CD_2]} + \frac{l\varphi_1 \varepsilon_1 i_0 (K_{SV})_1 K_1[CD_1]}{1+K_1[CD_1]+K_2[CD_2]} + \frac{l\varphi_2 \varepsilon_2 i_0 (K_{SV})_2 K_2[CD_2]}{1+K_1[CD_1]+K_2[CD_2]}}{\frac{l\varphi_f \varepsilon_f i_0}{1+K_1[CD_1]+K_2[CD_2]} + \frac{l\varphi_1 \varepsilon_1 i_0 K_1[CD_1]}{1+K_1[CD_1]+K_2[CD_2]} + \frac{l\varphi_2 \varepsilon_2 i_0 K_2[CD_2]}{1+K_1[CD_1]+K_2[CD_2]}}[Q] =$$

$$= 1 + \frac{(K_{SV})_f + \left(\frac{a_1}{a_f}\right)(K_{SV})_1 K_1[CD_1] + \left(\frac{a_2}{a_f}\right)(K_{SV})_2 K_2[CD_2]}{1 + \left(\frac{a_1}{a_f}\right)K_1[CD_1] + \left(\frac{a_2}{a_f}\right)K_2[CD_2]}[Q]$$

S-8

with $a_i = \varphi_i \varepsilon_i$.

Thus, as

$$\frac{(I_{em})_0}{(I_{em})_Q} = 1 + (K_{SV})_{obs}[Q] \qquad \text{S-9}$$

it results in

$$(K_{SV})_{obs} = \frac{(K_{SV})_f + \left(\frac{a_1}{a_f}\right)(K_{SV})_1 K_1[CD_1] + \left(\frac{a_2}{a_f}\right)(K_{SV})_2 K_2[CD_2]}{1 + \left(\frac{a_1}{a_f}\right)K_1[CD_1] + \left(\frac{a_2}{a_f}\right)K_2[CD_2]} \qquad \text{S-10}$$

or

$$(K_{SV})_{obs} = \frac{(K_{SV})_f + (K_{SV})_1 (K_{app})_1 [CD_1] + (K_{SV})_2 (K_{app})_2 [CD_2]}{1 + (K_{app})_1 [CD_1] + (K_{app})_2 [CD_2]} \qquad \text{S-11}$$

$$\left((K_{app})_i = \left(\frac{a_i}{a_f}\right) K_i \right)$$

This is equation 8.