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# Finite concentration fluorescence quenching in the presence of diffusion

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A fluorophore is converted from its ground state  $A$  to an electronically excited state  $A^*$  by absorption of a photon  $h\nu_{ex}$ .  $A^*$  decays to  $A$  by fluorescence, with a lifetime  $\tau$ . It is quenched by collision with quencher  $Q$  at a diffusion controlled rate  $k[Q]$ . We suppose that  $A$  and  $Q$  are capable of reacting to form a complex  $AQ$ , even in the absence of the excitation.  $AQ$  forms at a diffusion controlled rate  $k_f[Q]$  and decays at a rate  $k_b$ . It is assumed that the quencher concentration is large enough so their depletion in the forward reaction of the final step can be neglected. The Stern–Volmer law is derived for the above four step chemical mechanism; the theory being given to all orders in the concentration of the quenchers. The results are compared with the concentration dependence proposed by Felderhof and Deutch. It is concluded that the predictions of the present work should be amenable to experimental test.

## I. INTRODUCTION

The theory of fluorescence quenching without diffusion is quite well established. Recently,<sup>1</sup> we have examined the infinite dilution diffusion controlled theory. Here, we shall provide the theory for finite concentrations. Firstly, we include the effects of complex formation in a four step chemical mechanism as follows:

(i) A fluorophore  $A$  in its ground state is converted to an electronically excited state  $A^*$  by absorption of an excitation photon  $h\nu_{ex}$ : i. e.,



The concentration of  $A$  is denoted by  $[A]$ , the absorption cross section is  $\sigma$  and the photon flux is  $\phi$ , which is taken to be time independent.

(ii) The excited state  $A^*$  decays to  $A$  by fluorescence with a lifetime  $\tau$ , through



(iii) It is quenched by collision with quencher  $Q$  at a rate  $k$ , described by



and

(iv)  $A$  and  $Q$  are assumed capable of reacting to form a complex  $AQ$



even in the absence of this excitation.  $AQ$  forms at a rate  $k_f$  and decays at a rate  $k_b$ .

Throughout it will be assumed that the quenchers are present in sufficiently large concentrations (typically  $[Q] = 0.1$  M while  $[A] = 10^{-4}$  M) so that their depletion by

the forward reaction in step (iv) can be neglected.

The implications of this mechanism for the homogeneous case where diffusion plays no role are considered in Sec. II below. The second direction in which progress is made here is to include diffusion through the above mechanism with a finite concentration of quenchers. In Sec. III, we demonstrate that step (iv) involving complex formation can play an essential part in determining the concentration dependence of the Stern–Volmer coefficient. We then discuss the relation to the work of Felderhof and Deutch<sup>2,3</sup> in the final section of this paper.

We turn, then, having summarized the chemical mechanism adopted in Eqs. (1.1)–(1.4), to the special case where there is no diffusion.

## II. FLUORESCENCE QUENCHING WITHOUT DIFFUSION

The rate equations governing the above processes are evidently

$$\frac{d}{dt} [A^*] = \sigma\phi[A] - (k[Q] + \tau^{-1}) [A^*] , \quad (2.1)$$

$$\frac{d}{dt} [A] = -\sigma\phi[A] + (k[Q] + \tau^{-1}) [A^*] - k_f[Q][A] + k_b[AQ] , \quad (2.2)$$

and

$$\frac{d}{dt} [AQ] = k_f[A][Q] - k_b[AQ] . \quad (2.3)$$

We now define quantities

$$k_1 = k_f[Q] \quad (2.4)$$

and

$$\bar{k} = k[Q] + \tau^{-1} . \quad (2.5)$$

Equations (2.1)–(2.3) can be solved by Laplace transform, taking account of the initial conditions

$$[A]_{t=0} = [A]_0, \quad [A^*]_{t=0} = 0, \quad [AQ]_{t=0} = [AQ]_0 . \quad (2.6)$$

The steady state value  $[A^*]_{t=\infty}$  is then found to be

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$$[A^*]_{\infty}^Q = \frac{\sigma\phi[A]_0^{\text{tot}} k_b}{k(k_1 + k_b) + \sigma\phi k_b}, \quad (2.7)$$

where, because of reaction (iv) represented by Eq. (1.4), all of the  $A$  initially present is represented either as free  $A$  or as  $AQ$ . Hence,  $[A]_0 + [AQ]_0$  represents this initial total amount which is written  $[A]_0^{\text{tot}}$  in Eq. (2.7).

We can usefully reexpress Eq. (2.7) by (a) using the definitions (2.4) and (2.5) and (b) using the equilibrium expression for reaction (iv),

$$k_f[A]^{\text{eq}}[Q] = k_b[AQ]^{\text{eq}}: K = \frac{k_f}{k_b}, \quad (2.8)$$

where  $K$  is evidently the equilibrium constant.

Then we find from Eqs. (2.7) and (2.8) that

$$[A^*]_{\infty}^Q = \frac{\sigma\tau\phi[A]_0^{\text{tot}}}{[1 + k\tau[Q]][K[Q] + 1] + \sigma\phi\tau}. \quad (2.9)$$

To obtain the Stern–Volmer law, we ought to compare  $[A^*]_{\infty}^Q$  with  $[A^*]_{\infty}^{Q=0}$ . Provided we can assume Eq. (2.9) with  $Q$  put to zero, then the result is

$$[A^*]_{\infty}^{Q=0} = \frac{\sigma\phi\tau[A]_0^{\text{tot}}}{1 + \sigma\phi\tau}. \quad (2.10)$$

The ratio: intensity of fluorescence  $I(Q)$  to its value of  $Q = 0$ ,  $I(0)$  can then be written

$$\frac{I(0)}{I(Q)} = \frac{[A^*]_{\infty}^{Q=0}}{[A^*]_{\infty}^Q} = \frac{(1 + k\tau[Q])(K[Q] + 1) + \sigma\phi\tau}{1 + \sigma\phi\tau}. \quad (2.11)$$

If one can assume the absorption is sufficiently weak to use the approximation  $\sigma\phi\tau \ll 1$ , then Eq. (2.11) reduces to the form

$$\frac{I(0)}{I(Q)} = 1 + (k\tau + K)[Q] + k\tau K[Q]^2, \quad (2.12)$$

which is the desired Stern–Volmer law in the absence of diffusion control.

Our objective in the remainder of this paper is to give the theory of the finite concentration Stern–Volmer law in the presence of diffusion.

### III. QUENCHING IN DIFFUSION LIMITED SYSTEMS AT FINITE CONCENTRATIONS

In previous work<sup>1</sup> we have given a treatment of the Stern–Volmer coefficient at infinite dilution for diffusion limited systems in the absence of the complex formation exhibited in Eq. (1.4). In this section, we first demonstrate that the infinite dilution Stern–Volmer coefficient remains valid to all orders in the concentration in the absence of complex formation between quenchers and fluorophores. We then demonstrate that in the presence of complex formation the distribution of nonexcited fluorophores  $A$  may still be taken as homogeneous when the diffusion renders the relative distribution of excited fluorophores  $A^*$  inhomogeneous with respect to the quenchers  $Q$ . With this observation an expression for the finite concentration Stern–Volmer coefficient in the presence of complex formation may be derived in terms of that at infinite dilution (in the absence of complex formation).

The starting point will be the integrodifferential equation for the distribution  $p(\mathbf{r})$  of excited fluorophores  $A^*$  about a quencher  $Q$ . We discussed the assumptions underlying this in Ref. 1; it will be useful to summarize the argument briefly below. We started<sup>1</sup> with the many-particle diffusion equation with reactive terms, as in the work of previous authors, for the joint probability density of excited fluorophores  $A_i$  and quenchers  $B_j$  as a function of time, which we denote by  $\Psi(\{\mathbf{r}_A\}, \{\mathbf{r}_B\}, t)$ . This equation reads

$$\frac{\partial \Psi}{\partial t} = \sum_i D_A \nabla_{A_i}^2 \Psi + \sum_j D_B \nabla_{B_j}^2 \Psi - k \sum_{i,j} S_{ij}(\mathbf{r}_{A_i} - \mathbf{r}_{B_j}) \Psi. \quad (3.1)$$

The diffusion described by the first two terms is free in the sense that no hydrodynamic interaction between diffusing species is included. The final reactive term on the right-hand side defines quenching as occurring between each pair of a fluorophore and quencher at a rate of magnitude  $k$  but dependent upon their relative separation vector  $\mathbf{r} = \mathbf{r}_{A_i} - \mathbf{r}_{B_j}$  via the function  $S_{ij}(\mathbf{r})$  satisfying  $\int S_{ij}(\mathbf{r}) d\mathbf{r} = 1$ . This contribution introduces an inhomogeneity into the relative distribution of excited fluorophores and quenchers, though not into that of fluorophores (excited and unexcited) and quenchers. This relative inhomogeneity may be described by a conditional density  $p(\mathbf{r}, t)$  (averaged overall quencher-fluorophore pairs) for an excited fluorophore to be in the vicinity of  $\mathbf{r}$  relative to a quencher at the origin, with an evolution equation [Eq. (2.4) of Ref. 1]

$$\begin{aligned} \frac{dp(\mathbf{r}, t)}{dt} &= D \nabla^2 p(\mathbf{r}, t) - \tau^{-1} p(\mathbf{r}, t) - k S(\mathbf{r}) p(\mathbf{r}, t) \\ &\quad - k[Q] \int S(\mathbf{r}') p(\mathbf{r}', t) d\mathbf{r}' + \sigma\phi[A], \end{aligned} \quad (3.2)$$

where  $D = D_A + D_B$  is the relative diffusion constant. The reduction to Eq. (3.2) assumes only the neglect of three-particle correlations in the reactive distribution. However, as we shall see, this will prove sufficient to have as a consequence a concentration independent Stern–Volmer coefficient, when no complex formation is allowed.

As shown in Appendix A, the steady state integral equation for the distribution  $p(\mathbf{r})$  of excited fluorophores  $A^*$  about a quencher  $Q$  follows from Eq. (3.2), namely,

$$\begin{aligned} p(\mathbf{r}) &= -k \int d\mathbf{r}' G(\mathbf{r}\mathbf{r}', \tau^{-1}) S(\mathbf{r}') p(\mathbf{r}') \\ &\quad + \sigma\phi[A] - k[Q] \langle S \rangle \tau, \end{aligned} \quad (3.3)$$

where  $G$  is the appropriate Green function.<sup>1</sup> This Eq. (3.3) may be analyzed to different orders in  $Q$  and the expression for the weighted reaction function  $\langle S \rangle$  related to that at infinite dilution by

$$\langle S \rangle = \frac{\langle S \rangle^0}{1 + \frac{k[Q]}{\sigma\phi[A]} \langle S \rangle^0}. \quad (3.4)$$

The Stern–Volmer coefficient at finite concentration is given by

$$K_{\text{sv}} = \frac{1}{[Q]} \left( \frac{[A^*]_0}{[A^*]} - 1 \right) \quad (3.5)$$

from Eq. (1.2) of Ref. 1. Integrating Eq. (3.3) leads directly to  $[A^*]$  in terms of  $\langle S \rangle$  and substituting Eq. (3.4) for  $\langle S \rangle$  determines the result as

$$K_{SV} = K_{SV}^{(0)}, \quad (3.6)$$

i.e., the Stern–Volmer coefficient is identical with that at infinite dilution, within the basic factorization approximation discussed in Ref. 1 for the joint distribution function.

We now turn to the influence of complex formation on the diffusion limited analysis, recalling the assumption  $[AQ] \ll [Q]$ . The forward reaction (1.4) of complex formation may then be treated as a sink term as in the discussion for excited fluorophores in Ref. 1. The reverse reaction provides an inhomogeneous source term which introduces another unknown function  $S_2(r)$  into the theory. However, because only linear deviations of  $A$  from equilibrium by the incident radiation are being considered, the complex formation reaction may be treated as in equilibrium. Employing microscopic reversibility arguments then ensures a cancellation of the sink and source term and the distribution of nonexcited fluorophores is homogeneous. The analysis of Eq. (3.1) is then unaltered for the inhomogeneous steady state distribution of excited fluorophores  $[A^*]$ , except that the new equilibrium estimate of  $A$  must be employed.

In fact, the formation of complexes may influence the fluorescence analysis both through its effect on  $[A]$  and if the complex also absorbs the exciting radiation through a competition for light. The latter effect may be characterized through a weighted ratio of optical absorbances

$$\alpha = \frac{\epsilon_A[A]}{\epsilon_A[A] + \epsilon_{AQ}[AQ]} = \left(1 + \frac{\epsilon_{AQ}}{\epsilon_A} K_{AQ}[Q]\right)^{-1} \quad (3.7)$$

premultiplying  $[A]$  in Eq. (3.1). Both these effects change the expansion of the equation in powers of the concentration and in Appendix B, we demonstrate the central result of this section of the paper:

$$K_{SV} = \frac{1}{[Q]} [(1 + K_{AQ}[Q]) \alpha (1 + K_{SV}^0[Q]) - 1] \quad (3.8)$$

and setting  $\alpha = 1$ , i.e., ignoring the optical absorbance of the complex we find

$$K_{SV} = K_{SV}^0 + K_{AQ} + K_{AQ} K_{SV}^{(0)} [Q]. \quad (3.9)$$

In particular, this general equation reproduces Eq. (2.12) when  $K_{SV}^0 = k\tau$  and yields with  $K_{SV}^0 = 4\pi DR\tau$ , where  $D$  is the mutual diffusion coefficient of  $A^*$  and  $Q$ , while  $R$  is their contact radius.

$$K_{SV} = 4\pi DR\tau + K_{AQ} + K_{AQ} 4\pi DR\tau [Q], \quad (3.10)$$

when the kinetics are diffusion controlled. The status of the diffusion controlled limit  $K_{SV}^0 = 4\pi DR\tau$  was discussed in Ref. 1.

#### IV. DISCUSSION

In the above analysis we have employed the steady state solution of the integral equation for the excited

fluorophore distribution about a quencher involving the Wilemski–Fixman reaction function  $S(r)$  as discussed in Ref. 1. It is of interest to compare this analysis with that of Felderhof and Deutch<sup>2</sup> concerning the equation

$$\frac{\partial c_{A^*}}{\partial t}(\mathbf{r}, t) = D^*(Q) \nabla^2 c_{A^*}(\mathbf{r}, t) + \sigma\phi[A] - \{k(Q)[Q] + \tau^{-1}\} c_{A^*}(\mathbf{r}, t) \quad (4.1)$$

by means of the time dependent differential precursor of Eq. (3.3), namely

$$\frac{\partial p(\mathbf{r}, t)}{\partial t} = D \nabla^2 p + \sigma\phi[A] - \tau^{-1}p - kS(\mathbf{r})p - [Q]k \int S(\mathbf{r}') p(\mathbf{r}', t) d\mathbf{r}'. \quad (4.2)$$

In Eq. (4.1),  $D^*(Q)$  and  $t(Q)$  are functions of  $[Q]$ . Whereas Eq. (4.1) is an equation for the concentration  $c_{A^*}(\mathbf{r}, t)$  at an arbitrary point in the solution, Eq. (4.2) is for the conditional probability or concentration at position vector  $\mathbf{r}$  relative to the quencher. When integrated over all space, both equations determine the total concentration of excited fluorophores, but the homogeneous assumption admissible in Eq. (4.1) with the consequent complexity in  $k(Q)$  is not permitted in Eq. (4.2). Integrating both equations over all space yields the identity in steady state:

$$k(Q) = k \frac{\langle S \rangle}{[A^*]} \quad (4.3)$$

and, hence, substituting the general expression implied in Ref. 1

$$[A^*] = \tau([A]\sigma\phi - k[Q]\langle S \rangle) \quad (4.4)$$

[obtainable from the steady state limit of the integral of Eq. (4.2)] and Eq. (3.2) into Eq. (4.3) yields

$$k(Q) = \frac{k \langle S^0 \rangle}{\sigma\phi[A]\tau} \quad (4.5)$$

exactly as in infinite dilution. Explicitly, in the simplest limit  $4\pi DR\tau$  for the Stern–Volmer coefficient one obtains

$$k(Q) = 4\pi DR. \quad (4.6)$$

In contrast, Felderhof and Deutch obtain a concentration dependent  $k(Q)$  without complex formation for, by considering dipoles of sinks they transcend the neglect of three-particle correlations explicit in the present work and Ref. 1. Explicitly,<sup>3</sup>

$$K_{SV} = \tau k(Q) = 4\pi D\gamma N [1 + (4\pi\gamma^3)^{1/2} N^{1/2} [Q]^{1/2} + O([Q] \ln [Q]) + \dots], \quad (4.7)$$

where

$$\gamma = R [1 + 4\pi DR/K]^{-1} \quad (4.8)$$

and  $N = 6.023 \times 10^{20} \text{ M}^{-1} \text{ cm}^{-3}$  is a factor which converts  $[Q]$  from moles per liter =  $M$  to molecules per  $\text{cm}^3$ . It would of course be of considerable interest to compare this expression and the different mechanism due to complex formation discussed in this work with experiment.

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## APPENDIX A

Let  $p^{(n)}(\mathbf{r})$  and  $\langle S^{(n)} \rangle$  be the expression for  $p(\mathbf{r})$  and  $\langle S \rangle$  correct to  $n$ th order in  $[Q]$ . Then Eq. (3.3) reads

$$\begin{aligned} p^{(0)}(\mathbf{r}) &= -k \int d\mathbf{r}' G(\mathbf{r}\mathbf{r}'; \tau^{-1}) S(\mathbf{r}') p^{(0)}(\mathbf{r}') + \sigma\phi\tau[A], \\ p^{(1)}(\mathbf{r}) &= -k \int d\mathbf{r}' G(\mathbf{r}\mathbf{r}'; \tau^{-1}) S(\mathbf{r}') p^{(1)}(\mathbf{r}') \\ &\quad - \tau k[Q] \langle S^{(0)} \rangle + \sigma\phi\tau[A], \\ p^{(2)}(\mathbf{r}) &= -k \int d\mathbf{r}' G(\mathbf{r}\mathbf{r}'; \tau^{-1}) S(\mathbf{r}') p^{(2)}(\mathbf{r}') \\ &\quad - \tau k[Q] \langle S^{(1)} \rangle + \sigma\phi\tau[A], \end{aligned} \quad (\text{A1.1})$$

where from Ref. 1,

$$G(\mathbf{r}\mathbf{r}'; s) = \frac{1}{4\pi D(2)^{1/2} |\mathbf{r} - \mathbf{r}'|} \exp\left(-|\mathbf{r} - \mathbf{r}'| \sqrt{\frac{s}{D}}\right). \quad (\text{A1.2})$$

We note that each member of the above set of equations differs only from the others in the  $\tau$ -independent final term, which we denote by  $C^{(0)}$ ,  $C^{(1)}$ ,  $C^{(2)}$ , ... and, hence, the solution of linear response to the driving field has the functional form

$$\langle S^{(0)} \rangle = C^{(0)} f(G, S, k), \quad (\text{A1.3})$$

where  $f$ , of course, contains the heart of the problem but, being independent of the constant term, retains its functional form to all orders, i.e.,

$$\langle S^{(n)} \rangle = C^{(n)} f(G, S, k). \quad (\text{A1.4})$$

Evidently we can rewrite this using Eq. (A1.3) as

$$\langle S^{(n)} \rangle = (C^{(n)} - C^{(0)}) f(G, S, k) + \langle S^{(0)} \rangle$$

$$\begin{aligned} &= \langle S^{(0)} \rangle - \tau k[Q] \langle S^{(n-1)} \rangle f(G, S, k) \\ &= \langle S^{(0)} \rangle \left(1 - \frac{k[Q] \langle S^{(n-1)} \rangle}{\sigma\phi[A]}\right), \end{aligned} \quad (\text{A1.5})$$

and proceeding to the limit  $n \rightarrow \infty$ , we obtain

$$\langle S^\infty \rangle = \frac{\langle S^{(0)} \rangle}{1 + \frac{k[Q]}{\sigma\phi[A]} \langle S^{(0)} \rangle}. \quad (\text{A1.6})$$

## APPENDIX B

If Eq. (3.3) is modified by replacing  $[A]$  by

$$\begin{aligned} \alpha[A]_{\text{eq}} &= \alpha(1 + K_{AQ}[Q])^{-1}[A], \\ &= (1 + K_{AQ}[Q]) \left(1 + \frac{\epsilon_{AQ}}{\epsilon_A} K_{AQ}[Q]\right)^{-1} [A], \end{aligned} \quad (\text{A2.1})$$

then the analysis of Appendix A requires only rather minor changes. In particular, Eq. (A1.5) becomes

$$\begin{aligned} \langle S^{(n)} \rangle &= \left\{ \left[ \frac{1}{1 + K_{AQ}[Q]} \frac{1}{1 + \frac{\epsilon_{AQ}}{\epsilon_A} K_{AQ}[Q]} \right]^{(n)} \right. \\ &\quad \left. - \frac{k[Q]}{[A]_{\text{eq}} \phi} \langle S^{(n-1)} \rangle \right\} \langle S^{(0)} \rangle \end{aligned} \quad (\text{A2.2})$$

and

$$\langle S^\infty \rangle = \frac{1}{1 + K_{AQ}[Q]} \frac{1}{1 + \frac{\epsilon_{AQ}}{\epsilon_A} K_{AQ}[Q]} \frac{1}{1 + \frac{k[Q]}{\sigma\phi[A]_{\text{eq}}}} \langle S^{(0)} \rangle. \quad (\text{A2.3})$$

Substituting Eqs. (4.4) and (A2.3) into Eq. (3.3) leads directly to Eq. (3.8) for  $K_{SV}$ .

<sup>1</sup>J. K. Baird, J. S. McCaskill, and N. H. March, J. Chem. Phys. **74**, 6812 (1981).

<sup>2</sup>B. U. Felderhof and J. M. Deutch, J. Chem. Phys. **64**, 4551 (1976).

<sup>3</sup>J. K. Baird and S. P. Escott, J. Chem. Phys. **74**, 6993 (1981).