Global Analysis of Kinetic and Stationary Diffusion-Mediated Fluorescence Quenching Data

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The recovery of the Smoluchowski−Collins−Kimball (SCK) model parameters from simulated kinetic and stationary diffusion-mediated fluorescence quenching data is studied. A Levenberg−Marquardt least-squares optimization routine was used for the estimation of the sum of the diffusion coefficients of the fluorophore and quencher, $D = D_{F^*} + D_Q$, the sum of their radii, $R = R_{F^*} + R_Q$, and the intrinsic quenching rate coefficient k. Single-curve analysis leads to rather poor parameter estimates. Global, i.e., simultaneous, analysis of quenching decays with different time resolutions (= channel widths) improves the recovery. The best results are obtained when quenching decays are analyzed globally with stationary Stern−Volmer data. The intrinsic quenching rate coefficient k can be recovered when quenching decays are measured with sufficiently high number of counts at a peak channel and analyzed globally with stationary Stern−Volmer data.

1. Introduction

The Smoluchowski−Collins−Kimball (SCK) model is the basic theory of diffusion-mediated collisional quenching.[1] In this model, spherical quencher and fluorophore molecules diffuse in a continuum solvent and interact only upon collisions. Quenching occurs when a quencher molecule Q encounters an excited fluorophore molecule F*: $F^* + Q \rightarrow F + Q$. For fast quenching in viscous solvents, the deviations of the fluorescence decays from first-order kinetics carry information about the microscopic quantities characterizing the quenching process. Assuming the SCK model, one can determine the relative diffusion coefficient, i.e., the sum of the diffusion coefficients of the fluorophore and quencher, $D = D_{F^*} + D_Q$, the sum of the radii of the fluorophore and quencher, $R = R_{F^*} + R_Q$, and the intrinsic quenching rate coefficient k. Deterministic (i.e., assuming errorless data) identifiability analysis of the SCK model shows that one decay trace at a nonzero quencher concentration suffices to determine D, R and k, when the fluorophore fluorescence lifetime τ is known.[2] From a practical point of view, one needs to know whether noisy and sampled fluorescence quenching data allow reliable estimation of the microscopic parameters.

So far there have been only a few studies devoted to the estimation of diffusion-mediated quenching parameters from time-correlated single-photon counting (TCSPC) data.[3−6] Those studies focused on single-curve analysis, where individual decay traces were fitted to the SCK model, and showed that the quality of the parameter estimates is poor, unless optimal conditions are met. On the other hand, it has been known from studies on multiexponential decays that global, i.e., simultaneous, analysis of several decays can substantially improve the parameter recovery compared to single-curve analysis.[7] In the present paper, we describe a Monte Carlo study of parameter estimation of fluorescence quenching with diffusion-mediated transients, where global analysis of fluorescence decays is employed to improve the parameter recovery. We simulate experimental fluorescence decay traces using the SCK model and use the reference convolution method[8,9] combined with a Marquardt−Levenberg nonlinear least squares routine[10] for the estimation of fluorescence quenching parameters.

For the sake of concreteness, we focus here on parameter ranges corresponding to the recently studied quenching of S_2-xanthions by hydrocarbons in perfluorohydrocarbons.[11] This choice is motivated by the fact that quenching of S_2-xanthions was well described in terms of the SCK model and that the corresponding SCK parameters were determined. In our preliminary work, we found a large scatter of parameters estimates when single-curve analysis was performed on simulated decays for the parameter ranges covering those in ref 11. This prompted us to explore to what extent global analysis can improve the parameter recovery. To the best of our knowledge, this the first study of the parameter estimation of diffusion-mediated quenching where global analysis has been applied.

The paper is organized as follows. In the next section, we briefly recall the theory of the reference convolution method, and that of the stationary Stern−Volmer (SV) plots as applied to the SCK model. In section 3, we introduce the methods of data synthesis and parameter recovery used in this work. In section 4, we present Monte Carlo simulations of parameter estimation using single-curve analysis, global analysis of fluorescence quenching decays, and global analysis of decays combined with stationary SV data. Finally, in the last section, we summarize the present results and comment on their consequences.

2. Theory

The decay profile of the sample $d(t)$ is the convolution of the fluorescence intensity response of the sample $i(t)$ with the instrument response function (IRF) $r(t)$

$$d(t) = \int_0^t r(u) i(t-u)\, \mathrm{d}u \tag{1}$$

In the reference convolution method,[8,9] instead of measuring the IRF directly, one measures the fluorescence decay profile

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$d_r(t)$ of a reference compound

$$d_r(t) = \int_0^t r(u) i_r(t-u)\, du \tag{2}$$

where $i_r(t)$ is the reference fluorescence response. The parameters of the fluorescence response of the sample $i(t)$ are obtained from the measured decay profiles of the sample $d(t)$ and reference $d_r(t)$ through the relation

$$d(t) = \int_0^t d_r(u) \tilde{\imath}(t-u)\, du \tag{3}$$

where $\tilde{\imath}(t)$ is the modified fluorescence response of the sample. If the fluorescence response of the reference $i_r(t)$ is monoexponential

$$i_r(t) = i_r(0) \exp(-t/\tau_r) \tag{4}$$

where τ_r is the reference lifetime, then the modified fluorescence response of the sample is

$$\tilde{\imath}(t) = i(0)\, [\delta(t) + i(t)/\tau_r + di(t)/dt]/i_r(0) \tag{5}$$

For the SCK model of diffusion-mediated quenching, the fluorescence response at the quencher concentration [Q] is

$$i(t) = i(0) \exp\{-t/\tau - [\mathrm{Q}]\int_0^t k(u)\, du\} \tag{6}$$

where the time-dependent coefficient $k(t)$ reads

$$k(t) = \frac{k k_D}{k + k_D}\left[1 + \frac{k}{k_D} \exp(x^2)\, \mathrm{erfc}(x)\right] \tag{7}$$

where $k_D = 4\pi RD$ is the diffusion mediated rate constant, $\tau_D = R^2/D$ is the diffusion relaxation time, and $x = (1 + k/k_D)(t/\tau_D)^{1/2}$. For the SCK model the modified intensity $\tilde{\imath}(t)$ in eq 5 can be calculated explicitly since[1]

$$i(t) = i(0) \exp\{-t(\tau^{-1} + p) - (q/c^2)[\exp(x^2)\, \mathrm{erfc}(x) - 1 + 2x/\pi^{1/2}]\} \tag{8}$$

and

$$di(t)/dt = -i(t)[\tau^{-1} + p + q \exp(x^2)\, \mathrm{erfc}(x)] \tag{9}$$

where

$$p = [\mathrm{Q}] k k_D/(k + k_D), \quad q = [\mathrm{Q}] k^2/(k + k_D), \quad c = (k + k_D)/(k_D \tau_D^{1/2}) \tag{10}$$

In TCSPC experiments one measures the counts D_n in channels $n = 1, ..., N_{ch}$. The counts in a channel is a random quantity whose expectation value $\langle D_n \rangle$ is related to the decay profile $d(t)$ as

$$\langle D_n \rangle = \int_{t_{n-1}}^{t_n} d(t)\, dt \tag{11}$$

where the integration over (t_{n-1}, t_n) represents averaging the decay profile over channel n of length $h = t_n - t_{n-1}$. An equivalent expression used is this work to calculate the expected counts is[6]

$$\langle D_n \rangle = \int_0^{t_n} R(u) i(t_n - u)\, du \tag{12}$$

where

$$R(t) = \int_{t-h}^{t} r(u)\, du \tag{13}$$

is the average of the instrument response function $r(t)$ over the channel width h.

Stationary fluorescence intensity measurements are represented by the SV plots[1]

$$\left\langle \frac{I_0}{I} \right\rangle = \frac{\int_0^\infty i_0(t)\, dt}{\int_0^\infty i(t)\, dt} = \frac{\tau}{\int_0^\infty [i(t)/i(0)]\, dt} \tag{14}$$

where $\langle I_0/I \rangle$ is the expected ratio of the stationary fluorescence intensity I_0 in the absence of the quencher, $[\mathrm{Q}] = 0$, to the stationary intensity I at a nonzero quencher concentration; $i_0(t)$, and $i(t)$ are the corresponding fluorescence responses (6), whose initial value is $i(0)$. In actual experiments one measures the quantity I_0/I that differs from $\langle I_0/I \rangle$ by a random error. To the best of our knowledge, an explicit form of the denominator in eq 14 is not known for the SCK model, and therefore, to calculate SV plots, one needs to resort to numerical integration (see the next section).

3. Simulations and Data Analysis

In this work, we use a model instrument response function (IRF) based on the formula

$$r(t) = w(t^2 e^{-t/\alpha} + \gamma t e^{-t/\beta}) \tag{15}$$

where the parameter $\alpha = 8.0 \times 10^{-3}$ ns defines the main peak and the parameters $\beta = 4.0 \times 10^{-2}$ ns and $\gamma = 5.0 \times 10^{-4}$ ns define the tail of the main peak. Two scaled and time-shifted "afterpulses" of shape (15) were added to the main peak (15), to mimic roughly the experimental IRF reproduced in ref 11. The parameters in eq 15 were selected so that the full width at half-maximum (fwhm) of the measured instrument response function is about 35 ps. The model IRFs were shifted to provide 92 zero-intensity leading channels (see Figure 1, panels A and B).

Simulation of the sample fluorescence counts, D_n, was done by first evaluating the expected counts $\langle D_n \rangle$ according to eq 12 in connection with integral (13) and the SCK model (6). Integral (13) was calculated analytically, whereas integral (12) was evaluated numerically using Romberg's integration method with a relative accuracy of 10^{-10}.[10] After computing $\langle D_n \rangle$ for $n = 1, ..., N_{ch}$ channels (typically $N_{ch} = 512$), the fluorescence was normalized to a peak value of D_{max} (typically $D_{max} = 10^4$, or 4×10^4). Then, to simulate the random content of a channel, the Poisson noise was added to $\langle D_n \rangle$. The Poisson noise was approximated by the Gaussian noise for the number of counts greater than 50. Simulation of the reference fluorescence counts, $D_{r,n}$, was done similarly to that of the sample fluorescence counts. The reference fluorescence was normalized to a peak value of $D_{r,max}$ (typically $D_{r,max} = 4 \times 10^4$).

Stationary Stern−Volmer data were synthesized according to eq 14, where the integral in the denominator was calculated numerically using Romberg's integration method with a relative accuracy of 3×10^{-9}.[10] A random Gaussian deviate was added to each point, with average equal to zero, and standard deviation (SD) being a fixed fraction of $(I_0/I) - 1$ (typically $\mathrm{SD} = 0.0333 \times [(I_0/I) - 1]$, which corresponds to a maximum error of about 10%, see Figure 1, panel C).

The simulated sample fluorescence counts, D_n, reference counts, $D_{r,n}$, and Stern−Volmer data, I_0/I_n, were put into an iterative reconvolution fitting procedure based on the Leven-

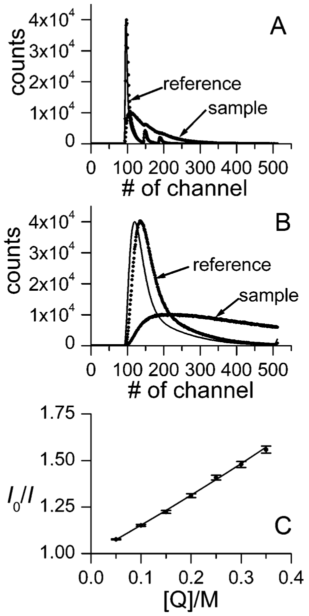

Figure 1. Averaged IRF, $R(t)$ (solid line, eqs 13 and 15), simulated reference decay with $\tau_r = 10$ ps, and simulated sample decay with $\tau = 0.6$ ns at [Q] = 0.25 M for two channel widths: $h = 4.88$ ps (panel A) and $h = 0.61$ ps (panel B). The simulation SCK parameters: $D = 1.0$ nm^2 ns^{-1}, $R = 0.8$ nm, $k = 80.0$ nm^3 ns^{-1}. Panel C shows the Stern–Volmer plot (I_0/I, eq 14, solid line) and sample SV data for [Q]/M = 0.05, 0.1, 0.15, 0.2, 0.25, 0.30, and 0.35. The error bars show the standard deviation SD = 0.0333 × [(I_0/I) − 1].

berg–Marquardt weighted nonlinear least-squares algorithm.[10] The weights w_n of the decay data points were adjusted during the fitting procedure, because in the reference convolution method they contain adjustable parameters $i(0)$ and $i_r(0)$[8,9]

$$w_n^{-1} = D_n + [i(0)/i_r(0)]^2 D_{r,n} \tag{16}$$

Since we assumed that the relative standard deviation of the random error of the SV data points is constant, the weights of the SV data are

$$w_m^{-1} = [(I_0/I_m) - 1]^2 \tag{17}$$

where I_m is the stationary fluorescence intensity at the mth quencher concentration $[\mathrm{Q}]_m$.

The parameters were recovered by minimizing

$$\chi^2 = \sum_i \sum_n w_{ni}(D_{ni} - \langle D_{ni} \rangle_c)^2 + \sum_m w_m(I_0/I_m - \langle I_0/I_m \rangle_c)^2 \tag{18}$$

The first term on the r.h.s. of eq 18 represents the contribution to the global χ^2 from individual channels n in different decays i, and the second term stands for the contribution from Stern–Volmer data points. The measured (=simulated) data are denoted as D_{ni} (counts in channel n in decay i) and as I_0/I_m (the mth SV data point), respectively. The corresponding weights are given by eqs 16 and 17, respectively. An index c is used to indicate the calculated values for the expected $\langle D_{ni} \rangle$ and $\langle I_0/I_m \rangle$. In global analysis, parameters D, R, and k are linked over the data surface (18). The entire decay profiles were analyzed including the rising edge. In the reconvolution analysis, the trapezoid integration rule was used for the decays, and Romberg's integration was used for the SV data. For each simulation experiment, the minimization procedure was repeated 30 times with different initial parameter guesses in search for a global minimum.

The fit parameters were the linked quenching parameters D, R, and k and the local decay scaling factors. The fluorescence lifetimes of the sample, τ, and of the reference, τ_r, were assumed to be known. The initial parameter guesses of the quenching parameters D, R, and k were chosen at random from an interval around the true values of the parameters, whose lower bound was 0.1 of the true values of the parameters and the upper bound was 10 times the true values of the parameters. Each simulation experiment was repeated 10^3 times, and the fit results were recorded. For each experiment, we also calculated the statistical characteristics of the goodness of fit: the reduced chi-square, χ^2, its standard normal variate, Z_{χ^2}, the Durbin-Watson parameter, d, and the ordinary run test statistics, Z.[7] Only those fits were accepted that stayed at least 10% away from the lower and upper parameters bounds and satisfied the statistical conditions $|Z_{\chi^2}| < 3$, $1.6 < d < 2.4$, and $|Z| < 3$.

4. Results

The simulation SCK parameters used in this study, $D = 1.0$ nm^2 ns^{-1}, $R = 0.8$ nm, and $k = 80.0$ nm^3 ns^{-1}, corresponded to quenching of S_2-xanthions by hydrocarbons in perfluorohydrocarbons.[11] Following ref 11, we used the quencher concentration [Q] = 0.25 M, the number of channels $N_{\mathrm{ch}} = 512$, maximum counts per channel for the sample $D_{\max} = 10^4$, and for the reference $D_{r,\max} = 4 \times 10^4$, and channel width $h = 2.44$ ps. The results of single-curve analysis of simulated decays are presented in Table 1 and Figure 2. The accuracy of the estimated diffusion coefficient D, and quenching radius R is poor for the shortest lifetimes ($\tau = 0.1$ and 0.2 ns) but improves when the fluorophore lifetime gets longer. The maximum relative error of the D and R estimates is about 25% for lifetimes $\tau \geq 0.4$ ns. However, the estimates of the intrinsic quenching constant k remain poor for all fluorophore lifetimes τ. Interestingly, the histograms of estimates of k are bimodal, which indicates the presence of local false minima on the χ^2 surface. Thus, the

TABLE 1: Single-Curve Analysis[a]

	parameter estimates									
	D/nm^2 ns^{-1}			R/nm			k/nm^3 ns^{-1}			
τ/ns	min	avg (std)	max	min	avg (std)	max	min	avg (std)	max	%
0.1	0.27	1.44 (0.26)	2.34	0.518	0.650 (0.061)	1.416	49	164 (91)	634	95.5
0.2	0.38	1.089 (0.13)	1.37	0.627	0.733 (0.084)	1.379	39	210 (126)	563	99.1
0.4	0.686	1.031 (0.090)	1.232	0.652	0.755 (0.074)	1.057	45	211 (141)	684	99.4
0.6	0.759	1.026 (0.076)	1.192	0.669	0.758 (0.069)	1.000	48	206 (139)	532	99.6
0.8	0.639	1.024 (0.075)	1.176	0.676	0.757 (0.070)	1.093	45	213 (141)	613	99.3
1.0	0.742	1.021 (0.072)	1.182	0.670	0.758 (0.068)	1.002	50	214 (145)	607	99.5
1.6	0.731	1.018 (0.070)	1.153	0.679	0.759 (0.070)	1.020	47	221 (147)	615	99.3

[a] Minimum (min), average (avg), maximum (max), and standard deviation (std) of the estimated D, R, and k obtained from the reference convolution analysis of 1000 simulated sample/reference decay pairs for [Q] = 0.25 M, $\tau_r = 10$ ps, and different τ as indicated. The last column gives the percentage of the accepted fits. Simulation SCK parameters: $D = 1.0$ nm^2 ns^{-1}, $R = 0.8$ nm, $k = 80.0$ nm^3 ns^{-1}. Other simulation parameters: $N_{\mathrm{ch}} = 512$, $h = 2.44$ ps, $D_{\max} = 10^4$ (sample), and $D_{r,\max} = 4 \times 10^4$ (reference).

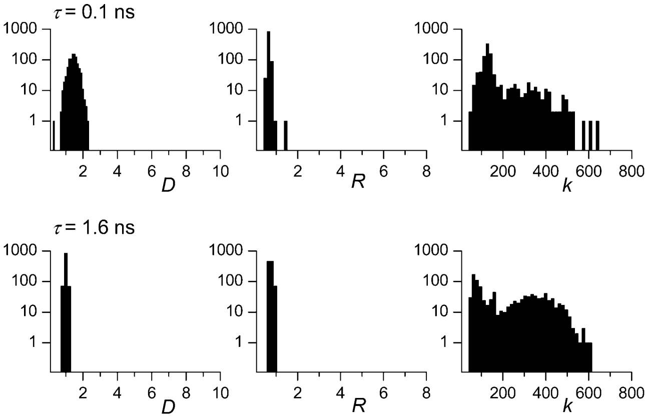

Figure 2. Single curve analysis: histograms of estimates of *D*, *R*, and *k* for the simulations in Table 1 for two fluorophore lifetimes $\tau = 0.1$ ns (top panels) and $\tau = 1.6$ ns (bottom panels).

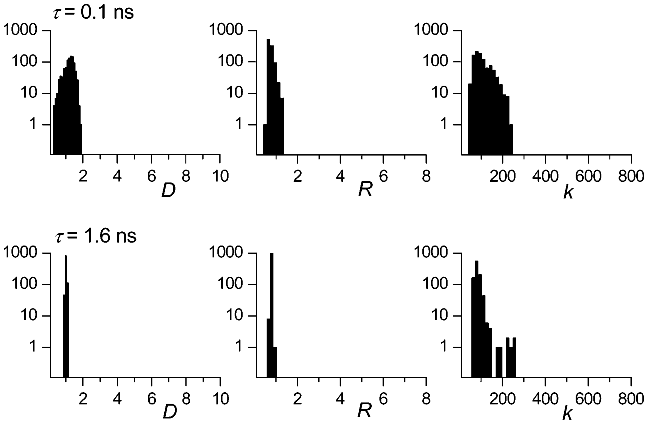

Figure 3. Global analysis of two decays with different time resolutions: histograms of estimates of *R*, *D*, and *k* for the simulations in Table 2 for two fluorophore lifetimes $\tau = 0.1$ ns (top panels) and $\tau = 1.6$ ns (bottom panels).

intrinsic quenching constant *k* cannot be recovered in single-curve analysis, unless additional data is available.

To improve parameter recovery, we employed global analysis of fluorescence decays. Table 2 and Figure 3 show the results of global analysis, where two decays with different time resolutions (=channel widths) $h = 0.61$ ps and $h = 4.88$ ps are analyzed simultaneously. The other simulation parameters were the same as in single-curve analysis. The accuracy of *D* and *R* estimated in global analysis of decay pairs improves, as the fluorophore lifetime gets longer. The maximum relative error reduces to about 15% for *D* and 10% for *R*, when the lifetimes $\tau \geq 0.6$ ns. However, the estimates of the intrinsic quenching rate *k* remain poor. Thus, for a reliable recovery of *k* more data is needed.

Table 3 and Figure 4 show the results of global analysis, where two decays with different time resolutions (channel widths $h = 0.61$ ps and $h = 4.88$ ps) combined with stationary SV data (at quencher concentrations [Q]/M = 0.05, 0.1, 0.15, 0.2, 0.25, 0.30, and 0.35). Addition of the SV data to the decay data affects mostly the recovery of *k* and improves the recover of *D* and *R* only marginally. The standard deviation of *k* estimates becomes less than 20% of the true value of *k*. By comparing the histograms of the parameter estimates (Figure 2−4), we find that addition of the SV data points has the effect of removing false minima from the global χ^2 surface and, therefore, affects the recovery of *k* (note that the histograms of *k* became unimodal). However, as there only a few SV data points, the effect on parameters not suffering from false minima (*D* and *R*) is modest.

Guided by the positive effect of combining decays at different time resolutions *h*, one might expect that combining curves at different quencher concentrations [Q] may improve the recovery. We analyzed globally pairs of decays at quencher concentrations [Q]/M = 0.15 and 0.35, with other simulation parameters as in the single-curve analysis. The accuracy of *D* and *R* estimated in global analysis of decay pairs with different quencher concentrations improves, when the fluorophore lifetime gets longer. When the lifetimes $\tau \geq 0.6$ ns, the maximum relative error reduces to about 25% for *D* and 20% for *R* and further to about 20% for *D* and 15% for *R*, when two decays at different quencher concentrations are combined in a global analysis with stationary SV data (at quencher concentrations [Q]/M = 0.05, 0.1, 0.15, 0.2, 0.25, 0.30, and 0.35). However, the estimates of the intrinsic quenching rate *k* remain poor even when kinetic data are analyzed globally with stationary SV data. Interestingly, in that case, the standard deviation of the *k* estimates increases from 17.1 nm^3 ns^{-1} for the shortest lifetime $\tau = 0.1$ ns to 43.3 nm^3 ns^{-1} for the longest lifetime $\tau = 1.6$ ns. Thus, combining decays with different concentrations ([Q]/M = 0.15 and 0.35) has less effect on the recovery of *D* and *R* than combining decays at different resolutions and fails completely as a means of the recovery of *k*.

We have explored several ways to further enhance the parameter recovery. We found that increasing the number of decays analyzed globally at the same, or different quencher concentrations [Q], and/or adding more SV data points improves the accuracy of the estimates, but the improvement scales rather weakly with the number of additional data points (data not shown). It remains to be seen if that behavior is peculiar to the parameter rangers studied here or is a general feature of diffusion-mediated quenching decays.

We found, however, that the number of counts at peak channel can have a pronounced effect on the quality of the parameter estimates. For instance, we repeated the global

TABLE 2: Global Analysis of Decays with Different Time Resolutions[a]

	parameter estimates									
	D/nm^2 ns^{-1}			*R*/nm			*k*/nm^3 ns^{-1}			
τ/ns	min	avg (std)	max	min	avg (std)	max	min	avg (std)	max	%
0.1	0.30	1.20 (0.28)	1.85	0.55	0.74 (0.12)	1.35	48	104 (37)	232	97.6
0.2	0.73	1.18 (0.12)	1.48	0.613	0.726 (0.057)	0.959	58	111 (39)	278	98.3
0.4	0.858	1.105 (0.058)	1.261	0.658	0.746 (0.039)	0.896	60	105 (33)	286	96.0
0.6	0.865	1.038 (0.046)	1.175	0.678	0.780 (0.034)	0.909	59	89 (24)	284	99.5
0.8	0.872	1.025 (0.041)	1.159	0.682	0.787 (0.032)	0.904	59	87 (20)	269	99.0
1.0	0.911	1.016 (0.037)	1.128	0.695	0.792 (0.029)	0.878	61	84 (14)	202	98.7
1.6	0.902	1.008 (0.035)	1.134	0.685	0.796 (0.029)	0.881	60	83 (17)	253	99.4

[a] Minimum (min), average (avg), maximum (max), and standard deviation (std) of the estimated *D*, *R*, and *k* obtained from the reference convolution analysis of 1000 simulated sample/reference decay sets, each made up of two sample decays at $h = 0.61$ ps and $h = 4.88$ ps, and [Q] = 0.25 M. The simulation SCK parameters are as in Table 1.

TABLE 3: Global Analysis of Decays with Different Time Resolutions Combined with Stationary Stern−Volmer Data[a]

	parameter estimates									
	D/nm^2 ns^{-1}			R/nm			k/nm^3 ns^{-1}			
τ/ns	min	avg (std)	max	min	avg (std)	max	min	avg (std)	max	%
0.1	0.40	1.13 (0.24)	1.82	0.561	0.772 (0.092)	1.230	51	84 (14)	167	98.1
0.2	0.82	1.12 (0.10)	1.43	0.636	0.758 (0.045)	0.912	61	88 (14)	172	98.3
0.4	0.857	1.080 (0.046)	1.221	0.670	0.762 (0.029)	0.912	58	91 (14)	205	96.4
0.6	0.902	1.032 (0.041)	1.154	0.688	0.785 (0.029)	0.882	61	85 (12)	181	98.4
0.8	0.902	1.018 (0.038)	1.147	0.683	0.792 (0.029)	0.888	59	84 (15)	272	99.3
1.0	0.893	1.001 (0.036)	1.129	0.719	0.797 (0.028)	0.887	61	82 (11)	137	99.3
1.6	0.916	1.005 (0.030)	1.105	0.718	0.799 (0.025)	0.872	61	82 (10)	154	99.4

[a] Minimum (min), average (avg), maximum (max), and standard deviation (std) of the estimated D, R, and k obtained from global analysis of 1000 simulated data sets, each made up of two sample/reference decays at $h = 0.61$ ps and $h = 4.88$ ps, and [Q] = 0.25 M, and stationary Stern−Volmer data at [Q]/M = 0.05, 0.1, 0.15, 0.2, 0.25, 0.30, 0.35. The simulation SCK parameters are as in Table 1.

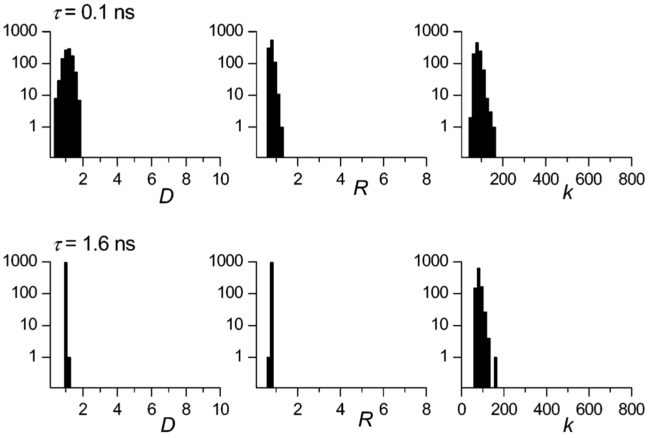

Figure 4. Global analysis of two decays with different time resolutions combined with stationary Stern−Volmer data: histograms of estimates of R, D, and k for the simulations in Table 3 for two fluorophore lifetimes $\tau = 0.1$ ns (top panels) and $\tau = 1.6$ ns (bottom panels).

analysis in Table 3 and Figure 4 with the number of counts at maximum increased from $D_{\max} = 10^4$ to $D_{\max} = 4 \times 10^4$. A 4-fold increase in $D_{\max}$ reduced the standard deviation of the parameter estimates by a factor 1.5−2 compared to those reported in Table 3 and Figure 4. The maximum relative error of k was reduced to about 40% at the fluorescence lifetime $\tau = 0.6$ ns and further to about 25% at $\tau = 1.6$ ns. We conclude that the intrinsic quenching rate coefficient k can be recovered when quenching decays are measured with a sufficiently high number of counts at peak channel and analyzed globally with stationary SV data. Interestingly, the percentage of accepted fits decreased to about 85% for the shortest lifetime $\tau = 0.1$ ns, compared to 98.1% at $D_{\max} = 10^4$. This indicates that, at short lifetimes, high precision decays, i.e., decays with larger number of counts at a peak channel, bring out the discretization error associated with the trapezoid rule used in the reconvolution analysis.

5. Summary and Conclusions

In this paper, we have explored the parameter estimation of diffusion-mediated fluorescence quenching assuming collisonal quenching according to the SCK model. We simulated experimental fluorescence decay traces and used the reference convolution method for the estimation of the linked fluorescence quenching parameters: the sum of the diffusion coefficients of the fluorophore and quencher D, the sum of their radii R, and the intrinsic quenching rate coefficient k.

We focused on parameter ranges corresponding to fluorescence quenching of S_2-xanthions by hydrocarbons in perfluorohydrocarbons.[11] Our results show that the standard (single-curve) analysis of decay traces offers rather poor estimates of the relative diffusion coefficient D and the quenching radius R and can be insufficient for the estimation of the intrinsic quenching rate coefficient k. Thus, the results presented in ref 11 may be overly optimistic as to the accuracy of the parameter estimates.

Global analysis of several decays improves the performance of the estimation procedure for D and R but may still be insufficient for the determination of k. Only when additional information is available, such as independent steady-state measurements, the recovery of k becomes possible. However, an accurate recovery of k may require not only that the fluorescence decay data be combined with stationary Stern−Volmer data, but also that the decays be measured with sufficiently high number of counts at the peak channel (say 4×10^4).

The present work indicates that kinetic experiments on the nanosecond and picosecond time scales require careful optimization of the experimental conditions. In the case of rapid diffusion-mediated quenching, good fits to the fluorescence decays do not necessarily entail that the recovered parameters are close to the true ones. Monte Carlo analysis can reveal whether the applied conditions allow reliable parameter estimation. For the accurate recovery of rapid quenching parameters, one may need to combine kinetic and stationary fluorescence data.

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References and Notes

(1) Szabo, A. *J. Phys. Chem.* **1989**, *93*, 6929.
(2) Molski A.; Boens, N.; Ameloot, M. *J. Phys. Chem. A* **1998**, *102*, 807.
(3) Scully, A. D.; Hirayama, S. *J. Fluoresc.* **1995**, *5*, 107.
(4) Das R.; Perisamy, N. *Chem. Phys.* **1989**, *136*, 361.
(5) Sikorski, M.; Augustyniak, W.; Khmelinskii, I. V.; Wilkinson, F. *J. Lumin.* **1996**, *69*, 217.
(6) Kłos, J.; Molski, A. *J. Chem. Phys.* **2003**, *119*, 12077.
(7) Janssens, L. C.; Boens, N.; Ameloot, M.; De Schryver, F. C. *J. Phys. Chem.* **1990**, *94*, 3564.
(8) Van den Zegel, M.; Boens, N.; Daems, D.; De Schryver, F. C. *Chem. Phys.* **1986**, *121*, 331.
(9) Boens, N.; Ameloot, M.; Yamazaki, I.; De Schryver, F. C. *Chem. Phys.* **1988**, *121*, 73.
(10) Press, W. H.; Teukolsky, S. A.; Vetterling, W. T.; Flannery, B. P. *Numerical Recipes in C*; Cambridge University Press: New York, 1992.
(11) Krystkowiak, E.; Maciejewski, A. *J. Chem. Phys.* **2002**, *117*, 2246; 5802.