SUPPORTING INFORMATION

Photophysics of a Xanthenic Derivative Dye Useful as “on/off” Fluorescence Probe.

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Theory of the global bicompartamental analysis.

When exciting the photophysical system shown in Scheme 2 with a $\delta$-pulse at time $t = 0$, that does not significantly alter the concentrations of the ground-state species, the time course of the concentrations of the excited-state species $1^*$ and $2^*$ is described by the first-order differential equation

$$\dot{x}(t) = Ax(t)$$  \hspace{1cm} (S-1)

$x(t)$ is the $2\times1$ vector function of the concentrations of the excited-state species $1^*$ and $2^*$. 
\[
\begin{bmatrix}
 x_1(t) \\
 x_2(t)
\end{bmatrix}
= \begin{bmatrix}
 [1^*](t) \\
 [2^*](t)
\end{bmatrix}
\] (S-2)

\(\dot{x}(t)\) denotes its time derivative and \(A\) is the 2×2 compartmental matrix:

\[
A = \begin{bmatrix}
 -\left(k_{01} + k_{21} + k_{21}^b[R]\right) & k_{12}[H^+] + k_{12}^b[HR] \\
 k_{21} + k_{21}^b[R] & -\left(k_{02} + k_{12}[H^+] + k_{12}^b[HR]\right)
\end{bmatrix}
\] (S-3)

The fluorescence impulse response function, \(f(\lambda_{em}, \lambda_{ex}, t)\), at emission wavelength \(\lambda_{em}\) due to excitation at \(\lambda_{ex}\) is given by eq. S-4,\(^1\) and depends on \(\lambda_{ex}, \lambda_{em}, [H^+]\), and the total buffer concentration (\(C^B = [R] + [HR]\)).

\[
f(\lambda_{em}, \lambda_{ex}, t) = \kappa c U \exp(t\Gamma) U^{-1}\tilde{b}
\] (S-4)

In this equation, we assume that the 2×2 compartmental matrix \(A\) has two linearly independent eigenvectors \(U_1\) and \(U_2\) associated with the eigenvalues \(\gamma_1\) and \(\gamma_2\), respectively, i.e. \(A = U \Gamma U^{-1}\) with \(U = [U_1, U_2]\) and \(U^{-1}\) the inverse of the matrix of the eigenvectors, \(\Gamma\) is the diagonal matrix of two eigenvalues, and \(\exp(\Gamma t) = \text{diag} [\exp(\gamma_1 t), \exp(\gamma_2 t)]\). \(U\) and \(\exp(\Gamma t)\) are functions of the rate constants \(k_{ij}\) and the concentrations \([R], [HR]\), and \([H^+]\).

The concentrations of species \(i^*\) at time zero are defined by \(x(0) = b\) where \(b\) is the 2×1 vector with elements \(b_i (i = 1, 2)\). \(b\) depends on the excitation wavelength \(\lambda_{ex}\) and \([H^+]\).

\(\tilde{b}\) is the 2×1 vector with normalized elements of \(b\). The elements \(\tilde{b}_i\) are calculated by\(^1\)

\[
\tilde{b}_i = \frac{e_i \alpha_i}{\sum_i e_i \alpha_i}
\] (S-5)

where \(e_i\) is the molar absorption coefficient of the \(i^{th}\) compartment, and \(\alpha_i\) the molar fraction of each form in the ground-state.
\( \mathbf{c} \) is the 1x2 vector of the normalized emission weighting factors \( \tilde{c}_i = c_i / \sum c_i \) of species \( i^* \) at emission wavelength \( \lambda_{em} \):

\[
e_i(\lambda_{em}) = k_{fi} \int_{\Delta \lambda_{em}} \rho_i(\lambda_{em}) d\lambda_{em}
\]

where \( k_{fi} \) is the fluorescence rate constant of \( i^* \); \( \Delta \lambda_{em} \) is the emission wavelength interval around \( \lambda_{em} \) where the fluorescence signal is monitored; \( \rho_i(\lambda_{em}) \) is the emission density of \( i^* \) at \( \lambda_{em} \) defined by

\[
\rho_i(\lambda_{em}) = \frac{F_i(\lambda_{em}, \lambda_{ex})}{\int_{\text{full emission band}} F_i(\lambda_{em}, \lambda_{ex}) d\lambda_{em}}
\]

where the integration extends over the whole steady-state fluorescence spectrum \( F_i \) of species \( i^* \).

Finally, \( \kappa \) is a proportionality constant given by

\[
\kappa = \sum_{\forall i} b_i \sum_{\forall j} c_j
\]

The use of \( \kappa; \tilde{b}_i \), and \( \tilde{c}_i \) allows one to link \( \tilde{b}_i \) and \( \tilde{c}_i \) in the data analysis so that the collected decay traces are not required to be scaled. Indeed, \( \tilde{b} \) depends on \( \lambda_{ex} \) and \([H^+]\) (or pH), whereas \( \tilde{c}(\lambda_{em}) \) depends on the emission wavelength only. In the implementation of global compartmental analysis one fits directly for the rate constants \( k_{01}, k_{21}, k_{02}, k_{12}, k_{11}^b, k_{21}^b \), the normalized zero-time concentrations \( \tilde{b}_i \) of species \( 1^* \), and the normalized spectral emission weighting factors \( \tilde{c}_i(\lambda_{em}) \) of species \( 1^* \).

Eq. S-4 can be written in the common bi-exponential format (with \( t \geq 0 \)):

\[
f(t) = p_1 e^{\gamma_1 t} + p_2 e^{\gamma_2 t}
\]
The eigenvalues $\gamma_i (i = 1, 2)$ of the compartmental matrix $A$ are related to the decay times $\tau_i (i = 1, 2)$ according to

$$\gamma_i = -1/\tau_i$$  \hspace{1cm} (S-10)

and are given by

$$\gamma_i = \frac{a_{11} + a_{22} \pm \sqrt{(a_{22} - a_{11})^2 + 4a_{12}a_{21}}}{2}$$  \hspace{1cm} (S-11)

with $a_{ij}$ the $ij^{th}$ element of the compartmental matrix $A$ (eq. S-3).

The pre-exponentials, $p_i$, of eq. S-9 are related to compartmental parameters through the following equations:

$$p_1 = \kappa(c_1 \beta_{11} + \tilde{c}_2 \beta_{21})$$  \hspace{1cm} (S-12 a)

$$p_2 = \kappa(c_1 \beta_{12} + \tilde{c}_2 \beta_{22})$$  \hspace{1cm} (S-12 b)

$$\beta_{11} = \frac{[\tilde{\beta}_1 (\gamma_2 - a_{11}) - \tilde{\beta}_2 a_{12}]}{(\gamma_2 - \gamma_1)}$$  \hspace{1cm} (S-13 a)

$$\beta_{12} = -\frac{[\tilde{\beta}_1 (\gamma_1 - a_{11}) - \tilde{\beta}_2 a_{12}]}{(\gamma_2 - \gamma_1)}$$  \hspace{1cm} (S-13 b)

$$\beta_{21} = \frac{[\tilde{\beta}_2 (\gamma_2 - a_{22}) - \tilde{\beta}_1 a_{21}]}{(\gamma_2 - \gamma_1)}$$  \hspace{1cm} (S-13 c)

$$\beta_{22} = -\frac{[\tilde{\beta}_2 (\gamma_1 - a_{22}) - \tilde{\beta}_1 a_{21}]}{(\gamma_2 - \gamma_1)}$$  \hspace{1cm} (S-13 d)

Program implementation and data analysis of time-resolved fluorescence.

The global compartmental analysis of the collected fluorescence decay surfaces was implemented in a general global analysis program using Gaussian-weighted non-linear
least-squares fitting based on Marquardt-Levenberg minimization. Its first description and application was done at the system fluorescein--(±)-N-acetyl aspartic acid.

Consider the excited-state process in the presence of added buffer as depicted in Scheme 2. The parameters were linked as shown in Scheme S-1. The global (linkable) fitting parameters are $k_{01}$, $k_{02}$, $k_{21}$, $k_{12}^B$, $k_{21}^B$, $\tilde{b}_1$, and $\tilde{c}_1$. The rate constants $k_{ij}$ are independent of $\lambda_{ex}$, $\lambda_{em}$, and pH, and hence can be linked over the entire fluorescence decay data surface. The emission weighting factors $\tilde{c}_i$ only depend on $\lambda_{em}$ and therefore can be linked at the same emission wavelength. The spectral parameters $\tilde{b}_i$ are dependent on both $\lambda_{ex}$ and pH and consequently can only be linked at the same $\lambda_{ex}$ and pH. The only local (nonlinkable) fitting parameters are the scaling factors $\kappa$.

< Scheme S-1 >

At each pH and $C^B$, the values of [R] and [RH] of the buffer with acidity constant $K_a^B$ were computed according to:

$$[R] = \frac{K_a^B C^B}{K_a^B + [H^+]} \quad (S-14 \text{ a})$$

$$[RH] = \frac{[H^+] C^B}{K_a^B + [H^+]} \quad (S-14 \text{ b})$$

Assigning initial guesses to the rate constants $k_{01}$, $k_{02}$, $k_{21}$, $k_{12}^B$, and $k_{21}^B$ allows one to construct the compartmental matrix $A$ (eq. S-3) for each decay trace. The eigenvalues $\gamma$ and the associated eigenvectors of this matrix are determined using routines from EISPACK, Matrix Eigensystem Routines. The eigenvectors are then scaled to the initial conditions $\tilde{b}$. The fluorescence $\delta$-response of the sample, $f(\lambda_{ex}, \lambda_{em}, t)$ is
calculated according eq. S-4. Then \( f(\lambda^{\text{ex}}, \lambda^{\text{em}}, t) \) is convoluted with the experimental instrument response function and the adjustable parameters of this calculated curve are optimized to fit the experimental fluorescence decay of the sample. Using this approach, experiments done at different excitation/emission wavelengths, at multiple timing calibrations, and at different pH are linked by all rate constants defining the system. The starting value for all rate constants \( k_{ij} \) was \( 1 \times 10^9 \text{ (M}^{-1}\text{ s}^{-1}) \); for \( \tilde{b}_i \) and \( \tilde{c}_i \), the initial guesses were 0 and/or 1. The generalized global mapping table approach described previously allows one to analyze simultaneously experiments done at different \( \lambda_{\text{ex}} \) and \( \lambda_{\text{em}} \), at multiple timing calibrations, and at different pH and \( C^B \) values.

The fitting parameters were determined by minimizing the global reduced chi-square \( \chi^2_g \):

\[
\chi^2_g = \sum_l \sum_i w_{li} \left( y_{li}^o - y_{li}^c \right)^2 / \nu
\]  

(S-15)

where the index \( l \) sums over \( q \) experiments, and the index \( i \) sums over the appropriate channel limits for each individual experiment. \( y_{li}^o \) and \( y_{li}^c \) denote respectively the experimental and fitted values corresponding to the \( i \)th channel of the \( l \)th experiment, and \( w_{li} \) is the corresponding statistical weight. \( \nu \) represents the number of degrees of freedom for the entire multidimensional fluorescence decay surface.

The goodness-of-fit was judged for each fluorescence decay trace separately as well as for the global fluorescence decay surface. The statistical criteria to assess the quality of the fit comprised both graphical and numerical tests, and have been described elsewhere.\(^{85}\)
Details on the fitting of the fluorescence decay surface of TG-I.

As has been indicated in the article text, 171 selected decay traces at different pH values within the range 5.00-9.00 were recorded. Excitation wavelength was 488 nm, and emission wavelengths were 505, 515, and 535 nm. The fitting process finished with a $\chi^2 = 1.08$, and the final results did not depend on the initial guesses of the parameters. Visual adjustment of calculated decay functions, weighted residuals plot, and autocorrelation function plots were also used as goodness-of-fitting criteria. Figure S1 shows two experimental decay traces, and the fitting curves provided by the GCA results (convoluted with the instrumental profile, shown as well in the figures).

REFERENCES


[S2] Program developed jointly by the Technology Institute of the Belarusian State University (Minsk, Belarus) and the Division of Photochemistry and Spectroscopy of the K. U. Leuven (Leuven, Belgium).


Scheme S-1. Linking scheme for the global compartmental analysis. Decays recorded at different emission wavelengths ($\tilde{c}_i(\lambda_{em}^p)$, $\tilde{c}_i(\lambda_{em}^q)$), due to a single excitation wavelength ($\lambda_{ex}$), and in the absence ($C_0^b = 0$) and the presence ($C^b$) of buffer. Boxed and/or connected parameters in the same line type indicate linked parameters, whereas $\kappa$ denotes the local scaling factors. The rate constants $k_{ij}$ are linked over the entire surface, whereas $k_{ij}^b$ are only linked over decays in the presence of proton acceptor/donor. Excitation parameters $\tilde{b}_i$ are linked at the same pH value, and emission parameters $\tilde{c}_i$ are linked at the same emission wavelength.
Figure S1. Fluorescence decay traces (—) of TG-I aqueous solutions (λex = 488 nm) at (a) pH = 5.00, C^B = 0.02 M, λem = 515 nm, χ2 = 1.062; (b) pH = 6.74, C^B = 0.10 M λem = 515 nm, χ2 = 1.056. The instrument response function (—), the fitting from GCA (—), the autocorrelation function, and the residuals, are also shown.