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Compartmental Modeling and Identifiability Analysis in Photophysics: A Review

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Abstract

The main objective of this review is to show how the concepts of compartmental modeling can be introduced and applied in photophysics. The term “compartment” in a photophysical context is defined as a subsystem composed of a distinct type of species that acts kinetically in a unique way. Compartments can be divided into ground and excited-state compartments depending upon the state of the composing species. In photophysics, a compartmental system is perturbed by a light pulse (photo-excitation) and its dynamics is followed via fluorescence in the time range from picoseconds to several hundred nanoseconds. In this review, we present the fluorescence δ -response functions for compartmental systems consisting of one excited-state compartment, two reversibly interconnected excited-state compartments, and their corresponding ground-state compartments. In deterministic identifiability one investigates whether the parameters of a specific model can be uniquely defined assuming perfect time-resolved fluorescence data. The identifiability is presented for the model with one excited-state compartment and three models of reversible intermolecular two-state excited-state processes in isotropic environments: (1) model without external quencher, (2) model with added quencher, (3) model with coupled species-dependent rotational diffusion described by Brownian reorientation. The parameters that have to be identified are time-invariant rate constants and parameters related to excitation and emission. It is shown under what conditions the relevant parameters can be identified. For all models, the explicit relationships between the true and alternative model parameters are shown.

1. Introduction

Compartmental modeling is frequently used in biomedicine, pharmacokinetics, analysis of ecosystems, engineering, and chemical reaction kinetics (see, for example, refs 1, 2, 3). Considering this extensive interest, it may seem rather surprising that compartmental modeling of excited-state processes in photophysics has started relatively late.^{4, 5, 6, 7, 8} Indeed, as the relaxation of excited-state processes can in many instances be described by a set of coupled first-order differential equations, excited-state systems are *formally* equivalent with compartmental systems. It will be shown in this review that modeling of excited-state processes in photophysics can conveniently be done within the framework of compartmental analysis.

Let us start by defining what the term “compartment” stands for in a photophysical context. A compartment is a subsystem composed of a distinct type of species that acts kinetically in a unique way. The concentration of the constituting species can change when the compartments exchange material through intramolecular or intermolecular processes. In the perspective of compartmental modeling of excited-state processes, compartments can be divided into ground-state and excited-state compartments depending upon the state of the composing species. The compartmental system then consists of one or more excited-state compartments and their corresponding ground-state compartments. There may be inputs from ground-state compartments into one or more of the excited-state compartments by photo-excitation. There is always output from the excited-state compartments to the ground-state compartments through emission and/or nonradiative deactivation. If the concentrations of the species in the ground state do not significantly change upon photo-excitation, it suffices to consider the excited-state

compartments only. In that case, the ground-state compartments can be lumped together as the outside environment. Compartments are usually depicted as boxes (rectangles, see Schemes), circles or ovals enclosing the composing species. Single-headed arrows pointing away from a compartment represent outflow from that compartment, whereas single-headed arrows pointing toward a compartment depict inflow into that compartment. In contrast to “classical” compartmental analysis, the terms “size” or “volume” are not relevant for compartments in photophysics.

In kinetics, one is concerned with finding the response of a compartmental system to an external perturbation, given the structure of the system (i.e., the links between the compartments) and the rate constants describing the rates of the intercompartmental transitions. Deriving the kinetic expressions is often referred to as the direct problem of compartmental analysis. In photophysics, the response of the excited-state compartments after photo-excitation (called “the external perturbation” in compartmental jargon) is commonly measured as time-resolved fluorescence. Since fluorescence decay traces can be monitored under a diversity of experimental conditions – the excitation wavelength λ_i^{ex} , the emission wavelength λ_j^{em} , the concentration of coreactant $[X]_k$, the concentration of external quencher $[Q]_l$, the temperature, the added buffer, orientation of excitation and emission polarizers, ... can all be varied independently – compartmental systems in photophysics have more experimental axes to study a given problem than in the “classical” areas of application of compartmental modeling. Moreover, a very large number (several thousands) of data points of high quality can be collected from time-resolved fluorescence measurements. This is usually not possible for the “common”

compartmental systems. Additionally, the prior knowledge available in fluorescence studies might also be different from that in “classical” compartmental systems.

An essential first stage of any identifiability analysis deals with finding solutions for the model parameters when ideal (noise-free) data are available. This identifiability with perfect observations is called *deterministic identifiability*.

In the deterministic identification (or identifiability) problem, one investigates as to whether or not the parameters of a specific model are uniquely defined under error-free observations, given that the model is completely specified including input into, output from, and exchange between the compartments.¹⁻³ The deterministic identification problem reduces to the question of whether a system of nonlinear algebraic equations has a unique solution. There are three possible outcomes to the identifiability analysis.

(1) The parameters of an assumed model can be estimated uniquely and the model is said to be *uniquely (globally) identifiable* from the idealized experiment.

(2) Any of a finite number of alternative estimates for some or all of the model parameters fits the data and the model is *locally identifiable*.

(3) An infinite number of model parameter estimates fits the data and the model is *unidentifiable* from the experiment.

For the linear, time-invariant models with a limited number of excited-state compartments that are generally encountered in photophysics, the parameters that have to be identified are the time-invariant rate constants and parameters related to excitation and emission.

Deterministic identification is thus concerned only with perfect observations and can point the way to improved experimental design. Imperfect data resulting from noisy

observations sampled over a limited time range affect the accuracy and precision with which model parameters can be estimated. This numerical parameter estimation of the model parameters and the statistical properties of the estimates are the subject of the second stage of any identifiability analysis and is called *numerical identifiability*. This topic, however, is beyond the scope of this review.

Since the first deterministic identification of an intermolecular two-state excited-state process, identifiability studies of a broad range of compartmental models of excited-state processes have been reported (see ref 9 for literature data). In the current review, we consider the compartmental analysis and the deterministic identifiability of a limited number of photophysical models. We start with the simplest model (with one excited-state compartment). Next, models for reversible *intermolecular* two-state excited-state processes in the absence^{9, 10} and presence¹¹ of quencher as monitored by the total (or “magic angle”-selected) fluorescence are discussed. Finally, the model of a reversible *intermolecular* two-state excited-state process coupled with species-dependent rotational diffusion described by Brownian reorientation is considered.^{12, 13}

The paper is organized as follows. Section 2 starts with some general concepts and definitions of the analysis of compartmental models and their identifiability. Two identification (or identifiability) approaches will be discussed in some detail. In Section 3, the fluorescence kinetics and identifiability analysis of the system with one excited-state compartment will be considered to illustrate some aspects of compartmental analysis in photophysics. In Section 4, the fluorescence dynamics and the identifiability analyses of three models of a reversible *intermolecular* two-state excited-state process without transient effects (i.e., with kinetics governed by time-invariant rate constants) are

presented. We will discuss what the effect is of the addition of quencher and of analyzing the time-resolved fluorescence anisotropy. When the reversible intermolecular two-state excited-state process is coupled with species-dependent rotational diffusion – described by Brownian reorientation – both spherically and cylindrically symmetric rotors, with no change in the principal axes of rotation in the latter, will be considered. The paper concludes with a discussion of the results of the identifiability studies and their relevance for designing experimental fluorescence decay measurements. Finally, we will refer to some applications of compartmental analysis in photophysics.

For anyone new to the field, this limited review can serve as a low threshold entry point to compartmental modeling in photophysics. To make this introduction to this field as accessible as possible, we have restricted the discussion to simple models. Furthermore, we have avoided unnecessary mathematical derivations that may cloud the essentials. A full mathematical treatment can be found in the literature.^{9–13} Those already familiar with compartmental models and their application (in the areas of biomedicine, pharmacokinetics, ecology, engineering) will, we hope, find that compartmental modeling in photophysics is an exciting rather recent development with many potential applications.

2. Fluorescence kinetics and identifiability analysis: general concepts

Given a certain photophysical model, the fluorescence δ -response function $f(t)$ (i.e., after an excitation pulse of infinitely short duration described by the Dirac delta function) is a prerequisite for tackling the important problem of identifiability – that is, whether the unknown model parameters can be recovered from the fluorescence δ -response function $f(t)$.

If a causal, linear, time-invariant compartmental system consisting of N ground-state compartments is excited with a δ -pulse of low intensity at time zero, so that the ground-state species population is not appreciably depleted, the time course of the excited-state species $\mathbf{x}^*(t)$ can be described by the following differential matrix equation:

$$\frac{d\mathbf{x}^*(t)}{dt} = \mathbf{A} \mathbf{x}^*(t), \quad t \geq 0 \quad (1)$$

with \mathbf{x}^* a $N \times 1$ vector whose elements are the concentrations of the excited-state species:

$$\mathbf{x}^* = ([1^*] \ [2^*] \ \dots \ [N^*])^T \quad (2)$$

\mathbf{A} is a $N \times N$ matrix (called ‘‘compartmental matrix’’ in compartmental parlance and ‘‘system matrix’’ in systems theory) containing the kinetic information (‘‘transfer coefficients’’) of all processes:

$$\mathbf{A} = \begin{pmatrix} -\left(k_{01} + \sum_{\substack{n=1 \\ n \neq 1}}^N k_{n1}\right) & k_{12} & k_{13} & \dots & k_{1N} \\ k_{21} & -\left(k_{02} + \sum_{\substack{n=1 \\ n \neq 2}}^N k_{n2}\right) & k_{23} & \dots & k_{2N} \\ k_{31} & k_{32} & -\left(k_{03} + \sum_{\substack{n=1 \\ n \neq 3}}^N k_{n3}\right) & \dots & k_{3N} \\ \dots & \dots & \dots & \dots & \dots \\ k_{N1} & k_{N2} & k_{N3} & \dots & -\left(k_{0N} + \sum_{\substack{n=1 \\ n \neq N}}^N k_{nN}\right) \end{pmatrix} \quad (3)$$

k_{mn} stands for the rate constant of transfer *to* compartment m^* *from* compartment n^* ; the subscript 0 denotes a ground-state compartment. In photophysics there is always deactivation [through fluorescence (F) emission and/or nonradiative (NR) processes] from the excited-state compartments to the associated ground-state compartments ($k_{0n} >$

0, $n = 1, 2, \dots, N$ with $k_{0n} = k_{Fn} + k_{NRn}$). Therefore, all excited-state compartments are said to be open. Furthermore, all rate constants k_{mn} ($m \neq n$) and x_n^* are nonnegative: $k_{mn} \geq 0$, $m \neq n$; $x_n^* \geq 0$, $n = 1, 2, \dots, N$.

From Eq. (1) we see that the dynamics of compartmental models are simply defined by a set of coupled first-order differential equations.

Photo-excitation with light of wavelength λ_i^{ex} thus produces N associated excited-state compartments, for which the fluorescence δ -response function $f(t)$ at emission wavelength λ_j^{em} can be expressed in matrix form as¹⁰

$$f(t) = \mathbf{c} \mathbf{x}^*(t) = \mathbf{c} e^{t\mathbf{A}} \mathbf{b} = \mathbf{c} \mathbf{U} e^{t\mathbf{\Gamma}} \mathbf{U}^{-1} \mathbf{b} \quad (4)$$

In this equation, we assume that the matrix \mathbf{A} has N linearly independent eigenvectors $\mathbf{U}_1, \mathbf{U}_2, \dots, \mathbf{U}_N$ associated with the eigenvalues $\gamma_1, \gamma_2, \dots, \gamma_N$, respectively, i.e., $\mathbf{A} = \mathbf{U}\mathbf{\Gamma}\mathbf{U}^{-1}$ with $\mathbf{U} = [\mathbf{U}_1, \mathbf{U}_2, \dots, \mathbf{U}_N]$ and \mathbf{U}^{-1} the inverse of the matrix of the eigenvectors, $\mathbf{\Gamma}$ is the diagonal matrix of N eigenvalues, $\Gamma_{mm} = \gamma_m$ and $\Gamma_{mn} = 0$ for $m \neq n$ ($m, n = 1, 2, \dots, N$), and $e^{t\mathbf{\Gamma}} = \text{diag} [\exp(\gamma_1 t), \exp(\gamma_2 t), \dots, \exp(\gamma_N t)]$. \mathbf{A} , \mathbf{U} , and $e^{t\mathbf{\Gamma}}$ are functions of the rate constants and for the intermolecular models considered in this paper also of the concentration of coreactant X. The matrix $e^{t\mathbf{A}}$ is called the “transition matrix” in compartmental terminology and is nonnegative for all t ,¹⁴

$$e^{t\mathbf{A}} = \mathbf{I} + t \mathbf{A} + \frac{(t \mathbf{A})^2}{2!} + \frac{(t \mathbf{A})^3}{3!} + \dots \quad (5)$$

with \mathbf{I} the $N \times N$ identity matrix. Hence, the fluorescence response of such a compartmental system after perturbation with a light pulse of infinitely short duration consists of a sum of (maximally) N exponentials.

$$f(t) = \sum_{n=1}^N \alpha_n \exp(\gamma_n t) \quad (6)$$

where α_n is the pre-exponential factor associated with the n th eigenvalue γ_n of \mathbf{A} . \mathbf{b} is a column vector of dimension N whose (constant nonnegative) elements are the initial (i.e., at time zero) concentrations of each excited-state compartment:

$$\mathbf{b} = (b_1 \quad b_2 \quad \dots \quad b_N)^T \quad (7a)$$

$$\mathbf{b} = \mathbf{x}^*(0) \quad (7b)$$

The elements b_n of \mathbf{b} are generally dependent on the excitation wavelength λ_i^{ex} and for the intermolecular models considered in this paper also on the concentration of co-reactant X.

\mathbf{c} is a $1 \times N$ vector related to the contribution of each compartment to the emission at λ_j^{em} :

$$\mathbf{c} = (c_{1j} \quad c_{2j} \quad \dots \quad c_{Nj}) \quad (8)$$

The emission weighting factor c_{mj} of species m^* at emission wavelength λ_j^{em} is given by

$$c_{mj} = k_{Fm} \int_{\Delta\lambda_j^{\text{em}}} \rho_m(\lambda_j^{\text{em}}) d\lambda_j^{\text{em}} \quad (9)$$

In Eq. (9), k_{Fm} stands for the fluorescence rate constant of m^* ; $\Delta\lambda_j^{\text{em}}$ is the emission wavelength interval around λ_j^{em} where the fluorescence signal is monitored; $\rho_m(\lambda_j^{\text{em}})$ is the emission density of m^* at λ_j^{em} defined by

$$\rho_m(\lambda_j^{\text{em}}) = F_m(\lambda_j^{\text{em}}) / \int_{\text{full emission band}} F_m(\lambda_j^{\text{em}}) d\lambda_j^{\text{em}} \quad (10)$$

where the integration extends over the whole steady-state fluorescence spectrum $F_m(\lambda_j^{\text{em}})$ of species m^* . The system $(\mathbf{A}, \mathbf{b}, \mathbf{c})$ that has $f(t)$ as its impulse response function is called a realization of $f(t)$.

Given the realization $(\mathbf{A}, \mathbf{b}, \mathbf{c})$, the fluorescence δ -response function $f(t)$ is uniquely determined. However, given $f(t)$, it is usually possible to find several realizations $(\mathbf{A}, \mathbf{b}, \mathbf{c})$ that generate the function $f(t)$. A general way of formulating the identification problem is as follows: is it possible to find different realizations of $f(t)$, say $(\mathbf{A}, \mathbf{b}, \mathbf{c})$ and $(\mathbf{A}^+, \mathbf{b}^+, \mathbf{c}^+)$, so that Eq. (11) holds

$$f(t, \mathbf{A}, \mathbf{b}, \mathbf{c}) = f(t, \mathbf{A}^+, \mathbf{b}^+, \mathbf{c}^+) \quad (11)$$

In other words, the fluorescence δ -response function $f(t)$ should be the same for the true $(\mathbf{A}, \mathbf{b}, \mathbf{c})$ and the alternative $(\mathbf{A}^+, \mathbf{b}^+, \mathbf{c}^+)$ model parameter set.^{2, 3} Global (unique) identifiability is attained when $\mathbf{A}^+ = \mathbf{A}$, $\mathbf{b}^+ = \mathbf{b}$, and $\mathbf{c}^+ = \mathbf{c}$ (i.e., a unique set of model parameters is obtained). The model is locally identifiable when there is a limited set of alternative \mathbf{A}^+ , \mathbf{b}^+ , and \mathbf{c}^+ . An unidentifiable model is found when there are an infinite number of alternative \mathbf{A}^+ , \mathbf{b}^+ , and \mathbf{c}^+ . The formulation as given by Eq. (11) is suitable for most systems found in biomedicine, pharmacokinetics, ecosystem modeling and engineering, but is inappropriate for photophysical systems where absolute values for \mathbf{b} and \mathbf{c} cannot be obtained. Therefore, in a photophysical context, it is more appropriate to use *normalized* vectors $\tilde{\mathbf{b}}$ and $\tilde{\mathbf{c}}$ ^{9, 10} (see Section 4).

There are several methods available for the analysis of the deterministic identifiability (i.e., identification with perfect, errorless data) of linear, time-invariant models.

In some cases, Eq. (11) can be used directly to determine the relationship between the true and alternative model parameters [see Eq. (20) in Section 3].

The approach which, in our investigations of the deterministic identifiability of time-invariant models for time-resolved fluorescence, has predominantly been used previously¹⁰⁻¹² involves Markov parameters m_m of the realization $(\mathbf{A}, \mathbf{b}, \mathbf{c})$ and elementary symmetric functions σ_n in $\gamma_1, \gamma_2, \dots, \gamma_N$. The set of eigenvalues γ_n of \mathbf{A} allows the construction of functions σ_n ($n = 1, 2, \dots, N$)

$$\sigma_1 \equiv \sum_n \gamma_n, \sigma_2 \equiv \sum_{m < n} \gamma_m \gamma_n, \dots, \sigma_N \equiv \gamma_1 \gamma_2 \dots \gamma_N \quad (12)$$

This set of nonlinear equations σ_n relates the eigenvalues γ_n or, equivalently, the relaxation times $\tau_n = -\gamma_n^{-1}$ to the elements of matrix \mathbf{A} .

The vectors \mathbf{b} and \mathbf{c} appear only in the Markov parameters m_m ($m = 0, 1, \dots, N-1$). The Markov parameters m_m are defined as the m th time derivatives of $f(t)$ at time zero:

$$m_m \equiv \frac{\partial^m f(0)}{\partial t^m}, \quad m = 0, 1, \dots, N-1 \quad (13a)$$

$$m_m = \sum_{n=1}^N \alpha_n \gamma_n^m, \quad m = 0, 1, \dots, N-1 \quad (13b)$$

where γ_n^m denotes the m th power of the eigenvalue γ_n and α_n is the associated pre-exponential factor (see Section 4). In terms of the realization $(\mathbf{A}, \mathbf{b}, \mathbf{c})$ of $f(t)$, the Markov parameters are expressed by:

$$m_m = \mathbf{c} \mathbf{A}^m \mathbf{b}, \quad m = 0, 1, \dots, N-1 \quad (14)$$

where \mathbf{A}^m represents the m th power of matrix \mathbf{A} . For an N -compartmental system, only N independent Markov parameters can be constructed for each set of experimental conditions. The identifiability analysis deals with the determination of the unknown elements of matrix \mathbf{A} and vectors \mathbf{b} and \mathbf{c} from σ_n and m_m .

An excellent method of finding a different (alternative) realization (\mathbf{A}^+ , \mathbf{b}^+ , \mathbf{c}^+) of $f(t)$ is via similarity transformation,^{2,3,15,16} yielding

$$\mathbf{T} \mathbf{A}^+ = \mathbf{A} \mathbf{T} \quad (15)$$

where \mathbf{T} is a constant invertible (or nonsingular) matrix (i.e., $\det \mathbf{T} \neq 0$) having the same dimension as \mathbf{A} .

The alternative \mathbf{b}^+ and \mathbf{c}^+ are given by

$$\mathbf{b}^+ = \mathbf{T}^{-1} \mathbf{b} \quad (16)$$

$$\mathbf{c}^+ = \mathbf{c} \mathbf{T} \quad (17)$$

The major advantage of the similarity transformation approach is that it not only offers a direct way of determining if a model is uniquely or locally identifiable or unidentifiable, but it also gives the explicit relationships between the true and alternative model parameters.

Equations (15–17) should be satisfied for each experimental condition. For the models considered, the possible experimental variables are excitation wavelength λ_i^{ex} , emission wavelength λ_j^{em} , coreactant concentration $[\text{X}]_k$, and quencher concentration $[\text{Q}]_l$. This implies that matrix \mathbf{T} should be independent of λ_i^{ex} , λ_j^{em} , $[\text{X}]_k$, and $[\text{Q}]_l$.

3. Fluorescence kinetics and identifiability analysis of the compartmental system with one excited-state compartment

The two objectives of this section are (1) to introduce as smoothly as possible the general concepts of compartmental analysis into the field of photophysics and (2) to demonstrate that even for the simplest model there are restrictions on the amount of information that can be recovered from perfect time-resolved fluorescence data.

In this section, a linear, time-invariant compartmental system consisting of one ground-state species (symbolized by 1) as shown in Scheme 1 is considered. Photo-excitation produces the excited-state species 1^* which can return to its ground state 1 with rate constant $k_{01} = k_{F1} + k_{NR1}$. This rate constant is required to be positive ($k_{01} > 0$). Scheme 1 is a graphical illustration of a photophysical system comprising one ground-state and one excited-state compartment. The system with one excited-state compartment is the *scalar* case of the compartmental model.

Insert Scheme 1 + caption

The differential equation describing the time-course of the quantity $x_1^* = [1^*]$ in the excited-state compartment after δ -pulse photo-excitation at λ_i^{ex} is

$$\frac{dx_1^*(t)}{dt} = -k_{01} x_1^*(t) \quad (18)$$

The fluorescence δ -response function $f_{ij}(t)$ is

$$f_{ij}(t) = b_1 c_1 \exp(-k_{01} t) \quad (19)$$

The subscripts i and j on $f_{ij}(t)$ indicate its dependence on λ_i^{ex} and λ_j^{em} , respectively. In photophysics, one observes the change of concentration of excited species 1^* via the measurement of the time-resolved fluorescence signal. This signal is dependent on the experimental observables $\{\lambda_i^{\text{ex}}, \lambda_j^{\text{em}}, t\}$ and the model parameters $\{k_{01}, b_1, c_1\}$.

The question one would like to answer first in identifiability analysis, is whether it is possible to obtain an alternative realization $\{k_{01}^+, b_1^+, c_1^+\}$ of $f_{ij}(t)$ different from the true model parameter set $\{k_{01}, b_1, c_1\}$, so that $f_{ij}(t)$ is the same for the true and alternative realization:

$$f_{ij}(t, k_{01}, b_1, c_1) = f_{ij}(t, k_{01}^+, b_1^+, c_1^+) \quad (20)$$

Equation (20) is the specific form of Eq. (11) for a system with one excited-state compartment. Rewriting Eq. (20) specifically for the considered model, one has

$$b_1 c_1 \exp(-k_{01} t) = b_1^+ c_1^+ \exp(-k_{01}^+ t) \quad (21)$$

Evaluating Eq. (21) at time zero gives

$$b_1^+ c_1^+ = b_1 c_1 \quad (22a)$$

and hence

$$k_{01}^+ = k_{01} \quad (22b)$$

This demonstrates that the rate constant k_{01} is uniquely identified, as is the product $b_1 c_1$. However, the individual b_1 and c_1 cannot be identified. Indeed, from Eq. (16) with $\mathbf{T} = (t_1)$ we have $b_1^+ = b_1/t_1$ and from Eq. (17) we obtain $c_1^+ = c_1 t_1$, implying that there are an infinite number of alternative b_1^+ and c_1^+ . Although the composite rate constant k_{01} is uniquely identified, the composing rate constants k_{F1} and k_{NR1} are not identifiable without extra (prior) information. This additional knowledge is available if the fluorescence quantum yield ϕ_f is known:

$$\phi_f = k_{F1}/k_{01} \quad (23)$$

The unique values of k_{F1} and k_{NR1} are then calculated according to

$$k_{F1} = \phi_f k_{01} \quad (24a)$$

$$k_{NR1} = k_{01}(1 - \phi_f) \quad (24b)$$

This simple model shows that only the rate constant k_{01} is uniquely identified. If one wants to know the contributions to k_{01} of fluorescence (k_{F1}) and nonradiative decay (k_{NR1}), prior knowledge is needed.

4. Fluorescence kinetics and identifiability analysis of models of reversible intermolecular two-state excited-state process

4A. Model without external quencher

Insert Scheme 2 + caption

In this section, we will start with the fluorescence kinetics of an intermolecular system consisting of two distinct interchanging ground-state species (1 and 2) and two corresponding excited-state species (1^* and 2^*) as depicted in Scheme 2 (i.e., two ground-state compartments and two associated excited-state compartments). Ground-state species 1 can reversibly react with coreactant X to form ground-state species 2. Photo-excitation creates the excited-state species 1^* and 2^* , which decay by rate constants k_{01} and k_{02} to their respective ground states. The rate constant describing the intermolecular transformation of 1^* into 2^* is represented by k_{21} , while the reverse process is described by k_{12} . These rate constants are required to be positive as we deal with a *reversible* excited-state process.

The fluorescence impulse response function $f_{ijk}(t)$ [Eq. (4)] for the photophysical system shown in Scheme 2 is explicitly given by

$$f_{ijk}(t) = \mathbf{c}_j \exp(t \mathbf{A}_k) \mathbf{b}_{ik} \quad (25)$$

The subscripts i , j , and k in Eq. (25) refer to the excitation wavelength λ_i^{ex} , the emission wavelength λ_j^{em} , and the coreactant concentration $[\text{X}]_k$, respectively

Matrix \mathbf{A}_k is given by

$$\mathbf{A}_k = \begin{pmatrix} -(k_{01} + k_{21}[\text{X}]_k) & k_{12} \\ k_{21}[\text{X}]_k & -(k_{02} + k_{12}) \end{pmatrix} \quad (26)$$

The 2×1 vector \mathbf{b}_{ik} contains the initial concentrations of excited species 1^* and 2^* :

$$\mathbf{b}_{ik} = (b_{1ik} \quad b_{2ik})^T \quad (27)$$

with $b_{nik} = [n^*]_0$ at λ_i^{ex} and $[X]_k$.

\mathbf{c}_j is the 1×2 vector of the emission weighting factors c_{mj} ($m = 1, 2$) at emission wavelength λ_j^{em} defined by Eq. (9).

$$\mathbf{c}_j = (c_{1j} \quad c_{2j}) \quad (28)$$

Equation (25) can be written in the common bi-exponential format:

$$f_{ijk}(t) = \alpha_1 \exp(\gamma_1 t) + \alpha_2 \exp(\gamma_2 t) \quad t \geq 0 \quad (29)$$

The eigenvalues $\gamma_{1,2}$ of the 2×2 matrix $\mathbf{A} \equiv (a_{mn})$ are

$$\gamma_{1,2} = \frac{1}{2} \left[a_{11} + a_{22} \pm \sqrt{(a_{11} - a_{22})^2 + 4 a_{12} a_{21}} \right] \quad (30)$$

For the considered model, the elements a_{mn} of \mathbf{A} are shown in Eq. (26). Since the part under the square root in Eq. (30) cannot be negative, the eigenvalues $\gamma_{1,2}$ must be real. For a *reversible* two-state excited-state process, the eigenvalues $\gamma_{1,2}$ are distinct. Repeated eigenvalues are obtained if the part under the square root in Eq. (30) is zero. For this, a_{11} and a_{22} must be equal and $a_{12}a_{21}$ must be zero; the latter implies that either a_{12} or a_{21} must be zero. In that case the excited-state process would not be reversible anymore. If a_{12} and a_{21} were both zero, the compartments would not be connected.

The pre-exponentials $\alpha_{1,2}$ corresponding to $\gamma_{1,2}$ are

$$\alpha_1 = c_1 \beta_{11} + c_2 \beta_{21} \quad (31a)$$

$$\alpha_2 = c_1 \beta_{12} + c_2 \beta_{22} \quad (31b)$$

$$\beta_{11} = \frac{b_1(\gamma_2 - a_{11}) - b_2 a_{12}}{\gamma_2 - \gamma_1} \quad (32a)$$

$$\beta_{12} = -\frac{b_1(\gamma_1 - a_{11}) - b_2 a_{12}}{\gamma_2 - \gamma_1} \quad (32b)$$

$$\beta_{21} = \frac{b_2(\gamma_2 - a_{22}) - b_1 a_{21}}{\gamma_2 - \gamma_1} \quad (32c)$$

$$\beta_{22} = -\frac{b_2(\gamma_1 - a_{22}) - b_1 a_{21}}{\gamma_2 - \gamma_1} \quad (32d)$$

To keep Eqs. (30–32) transparent, we did not use the subscripts i, j , and k on b, c, α, β , and γ to indicate their dependence on $\lambda_i^{\text{ex}}, \lambda_j^{\text{em}}$, and $[\text{X}]_k$. For the considered model the eigenvalues $\gamma_{1,2}$ [Eq. (30)] depend on the rate constants and $[\text{X}]_k$, while the pre-exponentials $\alpha_{1,2}$ [Eq. (31)] depend additionally on λ_i^{ex} (through \mathbf{b}_{ik}) and λ_j^{em} (through \mathbf{c}_j).

Now let us investigate the identifiability via similarity transformation. For the considered system with two excited-state compartments, the 2×2 matrix \mathbf{T} is

$$\mathbf{T} = \begin{pmatrix} t_1 & t_2 \\ t_3 & t_4 \end{pmatrix} \quad (33)$$

Four rate constants ($k_{01}, k_{21}, k_{02}, k_{12}$) have to be identified. The matrix multiplication in Eq. (15) with \mathbf{A} given by Eq. (26) leads to two sets of rate constant values.

Set I is the original set: $k_{01}^+ = k_{01}, k_{12}^+ = k_{12}, k_{21}^+ = k_{21}, k_{02}^+ = k_{02}$ with $\mathbf{T} = t_1 \mathbf{I}$, with \mathbf{I} the 2×2 identity matrix and where k_{mn}^+ represents the alternative value of k_{mn} .

Set II is the alternative set: $k_{01}^+ = k_{02} + k_{12}, k_{12}^+ = k_{01} - k_{02}, k_{21}^+ = k_{21}, k_{02}^+ = k_{02}$ with \mathbf{T} given by Eq. (34) with $t_3/t_1 = (k_{01} - k_{02} - k_{12})/k_{12}$:

$$\mathbf{T} = \begin{bmatrix} t_1 & 0 \\ t_3 & t_1 + t_3 \end{bmatrix} \quad (34)$$

Since k_{12}^+ must be positive, because a *reversible* excited-state process is considered, set II is possible only if $k_{01} > k_{02}$. If $k_{01} < k_{02}$, the true set I of rate constants is recovered.

The above results are in perfect agreement with the results obtained via the deterministic identification analysis based on elementary functions σ_n [$n = 1, 2$, Eqs. (35, 36)]:

$$\sigma_{1k} \equiv \gamma_{1k} + \gamma_{2k} = a_{11,k} + a_{22} \quad (35a)$$

$$\sigma_{1k} = -(k_{01} + k_{21}[\mathbf{X}]_k + k_{02} + k_{12}) \quad (35b)$$

$$\sigma_{2k} \equiv \gamma_{1k}\gamma_{2k} = a_{11,k} a_{22} - a_{12} a_{21,k} \quad (36a)$$

$$\sigma_{2k} = k_{01}(k_{02} + k_{12}) + k_{02}k_{21}[\mathbf{X}]_k \quad (36b)$$

The matrix multiplication in Eqs. (16) and (17) for $\mathbf{T} = t_1\mathbf{I}$ (set I) indicates that the alternative \mathbf{b}_{ik}^+ and \mathbf{c}_j^+ are only known up to the scaling factor t_1 . This means that the photophysical system expressed in terms of \mathbf{A} , \mathbf{b}_{ik} , and \mathbf{c}_j is unidentifiable. Since the vectors \mathbf{b}_{ik} and \mathbf{c}_j are experimentally not accessible from time-resolved fluorescence experiments, it is more appropriate to use normalized vectors $\tilde{\mathbf{b}}_{ik}$ and $\tilde{\mathbf{c}}_j$. The elements \tilde{b}_{nik} of $\tilde{\mathbf{b}}_{ik}$ and \tilde{c}_j of $\tilde{\mathbf{c}}_j$ are defined as

$$\tilde{b}_{nik} \equiv b_{nik} / (b_{1ik} + b_{2ik}) \quad n = 1, 2 \quad (37)$$

$$\tilde{c}_{mj} \equiv c_{mj} / (c_{1j} + c_{2j}) \quad m = 1, 2 \quad (38)$$

Use of normalized \tilde{b}_{1ik}^+ and \tilde{b}_{1ik} in Eq. (16), and \tilde{c}_{1j}^+ and \tilde{c}_{1j} in Eq. (17) leads to $\tilde{b}_{1ik}^+ = \tilde{b}_{1ik}$ and $\tilde{c}_{1j}^+ = \tilde{c}_{1j}$.

Using normalized spectral parameters \tilde{b}_{lik} ($0 \leq \tilde{b}_{lik} \leq 1$) and \tilde{c}_{1j} ($0 \leq \tilde{c}_{1j} \leq 1$) in global compartmental analysis of related fluorescence decay traces allows one to link \tilde{b}_{lik} at the same λ_i^{ex} and $[\text{X}]_k$, whereas \tilde{c}_{1j} can be linked at the same λ_j^{em} .

Now we consider the identifiability of \tilde{b}_{lik} and \tilde{c}_{1j} associated with set II of the rate constants, where matrix \mathbf{T} is given by Eq. (34). As was the case for set I, t_1 cannot be determined for set II and consequently the model expressed in terms of \mathbf{A} , \mathbf{b}_{ik} , and \mathbf{c}_j is unidentifiable. Use of normalized \tilde{b}_{nik}^+ and \tilde{c}_n^+ and the fact that they should be restricted to the range $[0, 1]$ allows one to define ranges of physically acceptable \tilde{b}_{nik}^+ and \tilde{c}_m^+ values.

Although the similarity transformation approach expresses the explicit relationships between the true and alternative model parameters, it does not allow one to determine how many concentrations $[\text{X}]$ and emission wavelenghts λ^{em} are necessary for identifiability in terms of the rate constants and the normalized $\tilde{\mathbf{b}}_{ik}$ and $\tilde{\mathbf{c}}_j$. In the following we will use Markov parameters m_m ($m = 0, 1$) to answer this question. $m_{0,ijk}$ is given by Eq. (39), while $m_{1,ijk}$ is expressed as a function of the matrix elements of \mathbf{A}_k in Eq. (40) and as a function of the rate constants and $[\text{X}]_k$ in Eq. (41):

$$m_{0,ijk} = \mathbf{c}_j \mathbf{b}_{ik} = c_{1j} b_{lik} + c_{2j} b_{2ik} \quad (39)$$

$$m_{1,ijk} = \mathbf{c}_j \mathbf{A}_k \mathbf{b}_{ik} = b_{lik} (c_{1j} a_{11,k} + c_{2j} a_{21,k}) + b_{2ik} (c_{1j} a_{12} + c_{2j} a_{22}) \quad (40)$$

$$m_{1,ijk} = -b_{lik} c_{1j} (k_{01} + k_{21} [\text{X}]_k) + b_{lik} c_{2j} k_{21} [\text{X}]_k + b_{2ik} c_{1j} k_{12} - b_{2ik} c_{2j} (k_{02} + k_{12}) \quad (41)$$

Use of normalized $\tilde{\mathbf{b}}_{ik}$ and $\tilde{\mathbf{c}}_j$ allows one to rewrite the above equations with κ_{ijk} as a scaling constant.

$$m_{0,ijk} = \kappa_{ijk} \tilde{\mathbf{c}}_j \tilde{\mathbf{b}}_{ik} = \kappa_{ijk} (\tilde{c}_{1j} \tilde{b}_{1ik} + \tilde{c}_{2j} \tilde{b}_{2ik}) \quad (42)$$

$$m_{1,ijk} = \kappa_{ijk} \tilde{\mathbf{c}}_j \mathbf{A}_k \tilde{\mathbf{b}}_{ik} = \kappa_{ijk} [\tilde{b}_{1ik} (\tilde{c}_{1j} a_{11,k} + \tilde{c}_{2j} a_{21,k}) + \tilde{b}_{2ik} (\tilde{c}_{1j} a_{12} + \tilde{c}_{2j} a_{22})] \quad (43)$$

$$m_{1,ijk} = \kappa_{ijk} [-\tilde{b}_{1ik} \tilde{c}_{1j} (k_{01} + k_{21}[\mathbf{X}]_k) + \tilde{b}_{1ik} \tilde{c}_{2j} k_{21}[\mathbf{X}]_k + \tilde{b}_{2ik} \tilde{c}_{1j} k_{12} - \tilde{b}_{2ik} \tilde{c}_{2j} (k_{02} + k_{12})] \quad (44)$$

It has been shown via the symbolic mathematics program MAPLE V (Waterloo Maple Inc.) that six decay traces – measured at two coreactant concentrations $[\mathbf{X}]$ and three emission wavelengths λ^{em} (corresponding to different \tilde{c}_1) – are required for the model to be locally identifiable in terms of the rate constants, \tilde{b}_1 and \tilde{c}_1 . Two sets of rate constants and concomitant spectral parameters \tilde{b}_{1ik} and \tilde{c}_{1j} may be found. For set I, we have $\tilde{b}_{1ik}^+ = \tilde{b}_{1ik}$ and $\tilde{c}_{1j}^+ = \tilde{c}_{1j}$. For set II, the admissible \tilde{b}_{1ik}^+ and \tilde{c}_{1j}^+ can be expressed as a function of \tilde{b}_{1ik} , \tilde{c}_{1j} , and the rate constants.

4B. Model with added quencher

Insert Scheme 3 + caption

Consider the molecular system (Scheme 3) with an equilibrium between two different species 1 and 2 in the ground state which form upon photo-excitation the excited-state species 1^* and 2^* , respectively. The rate constants k_{01} , k_{02} , k_{21} , and k_{12} have the same meaning as in Scheme 2. By addition of an external quencher, Q, with concentration $[\mathbf{Q}]_l$, the depletion of the excited states is enhanced by $k_{q1} [\mathbf{Q}]_l$ for 1^* and $k_{q2} [\mathbf{Q}]_l$ for 2^* . It is assumed that the quencher Q has only an effect on the excited species and does not affect the ground-state equilibrium. The fluorescence δ -response function $f_{ijkl}(t)$ can be expressed in matrix notation:

$$f_{ijkl}(t) = \mathbf{c}_j \exp(t \mathbf{A}_{kl}) \mathbf{b}_{ik} \quad (45)$$

The subscripts i, j, k and l in Eq. (45) refer to λ_i^{ex} , λ_j^{em} , $[\text{X}]_k$, and $[\text{Q}]_l$, respectively.

Matrix \mathbf{A}_{kl} is given by

$$\mathbf{A}_{kl} = \begin{pmatrix} -(k_{01} + k_{21}[\text{X}]_k + k_{q1}[\text{Q}]_l) & k_{12} \\ k_{21}[\text{X}]_k & -(k_{02} + k_{12} + k_{q2}[\text{Q}]_l) \end{pmatrix} \quad (46)$$

\mathbf{b}_{ik} and \mathbf{c}_j are given by Eqs. (27) and (28), respectively. As it is assumed that Q does not affect the ground-state equilibrium, \mathbf{b}_{ik} is independent of $[\text{Q}]_l$.

$f_{ijkl}(t)$ can be expressed in the common bi-exponential format [Eq. (29)], with the eigenvalues $\gamma_{1,2}$ [Eq. (30)] dependent on the rate constants, $[\text{X}]_k$, and $[\text{Q}]_l$. The pre-exponentials $\alpha_{1,2}$ [Eq. (31)] are dependent additionally on λ_i^{ex} and λ_j^{em} .

First, we investigate the identifiability via similarity transformation. Six rate constants ($k_{01}, k_{21}, k_{02}, k_{12}, k_{q1}, k_{q2}$) have to be determined. Matrix \mathbf{T} has the same dimension as \mathbf{A} and is given by Eq. (33). If the rate constants of quenching are different ($k_{q1} \neq k_{q2}$), the matrix multiplication in Eq. (15) leads to the unique set of original rate constants (i.e., set I of Section 4A): $k_{01}^+ = k_{01}$, $k_{12}^+ = k_{12}$, $k_{21}^+ = k_{21}$, $k_{02}^+ = k_{02}$, and $k_{q1}^+ = k_{q1}$, $k_{q2}^+ = k_{q2}$ with $\mathbf{T} = t_1 \mathbf{I}$. If the quenching rate constants are identical ($k_{q1} = k_{q2}$) and additionally if $k_{01} > k_{02}$, the alternative set of rate constants is given by set II of Section 4A: $k_{01}^+ = k_{02} + k_{12}$, $k_{12}^+ = k_{01} - k_{02}$, $k_{21}^+ = k_{21}$, $k_{02}^+ = k_{02}$) and $k_{q1}^+ = k_{q2}^+ = k_{q1} = k_{q2}$.

Use of normalized \tilde{b}_{1ik} and \tilde{c}_{1j} for $\mathbf{T} = t_1 \mathbf{I}$ leads to the unique spectral parameters:

$$\tilde{b}_{1ik}^+ = \tilde{b}_{1ik} \text{ and } \tilde{c}_{1j}^+ = \tilde{c}_{1j}.$$

To conclude, if the quenching rate constants of a quencher added to a reversible intermolecular two-state excited-state process are different ($k_{q1} \neq k_{q2}$), this model is uniquely identifiable in terms of rate constants and normalized \tilde{b}_{1ik} and \tilde{c}_{1j} .

Second, the identifiability of the six rate constants can be done using σ_{1kl} and σ_{2kl} [Eq. (47)]:

$$\sigma_{1kl} = -(k_{01} + k_{02} + k_{12}) - k_{21}[X]_k - (k_{q1} + k_{q2})[Q]_l \quad (47a)$$

$$\sigma_{2kl} = k_{01}(k_{02} + k_{12}) + k_{02}k_{21}[X]_k + \{(k_{01} + k_{21}[X]_k)k_{q2} + (k_{02} + k_{12})k_{q1}\}[Q]_l + k_{q1}k_{q2}[Q]^2 \quad (47b)$$

For each combination ($[X]_k$, $[Q]_l$), one has one set of the above equations. It has been shown that four decay traces – measured at two coreactant concentrations $[X]$ and two quencher concentrations $[Q]$, namely $\{([X]_1, [Q]_1), ([X]_1, [Q]_2), ([X]_2, [Q]_1), ([X]_2, [Q]_2)\}$ – lead to the identification of all six rate constants, provided that $k_{q1} \neq k_{q2}$: $k_{01}^+ = k_{01}$, $k_{12}^+ = k_{12}$, $k_{21}^+ = k_{21}$, $k_{02}^+ = k_{02}$, $k_{q1}^+ = k_{q1}$, $k_{q2}^+ = k_{q2}$. For more details, we refer to the original paper.

4C. Model with species-dependent rotational diffusion

Insert Figure 1 + caption

The photophysical system consisting of two different interchanging species 1 and 2, each with distinct rotational characteristics – as depicted in Figure 1 – is considered. All the rate constants are assumed independent of the instantaneous orientation of the species. The rotational relaxation of each excited-state species is governed by its principal rotational diffusion constants, here D_{\perp} and D_{\parallel} for rotation, respectively, of and about the symmetry axis of each of the cylindrically symmetric rotors depicted in Figure 1. The fluorescence impulse response function $I_{\parallel ik}(t)$ for the plane-polarized component of emission of the two excited states 1^* and 2^* , having its electric vector polarized parallel to the electric vector of the plane-polarized excitation light, and the fluorescence δ -response

function $I_{\perp ij k}(t)$ for the perpendicularly polarized component can be expressed, in the case of pure transitions and *isotropic* solutions, as:¹⁷

$$I_{\parallel ij k}(t) = \frac{1}{3} S_{ikj}(t) [1 + 2r_{ikj}(t)] = \frac{1}{3} S_{ikj}(t) + \frac{2}{3} D_{ikj}(t) \quad (48a)$$

$$I_{\perp ij k}(t) = \frac{1}{3} S_{ikj}(t) [1 - r_{ikj}(t)] = \frac{1}{3} S_{ikj}(t) - \frac{1}{3} D_{ikj}(t) \quad (48b)$$

where $r_{ijk}(t)$ denotes the fluorescence emission anisotropy and with

$$S_{ijk}(t) = 3 \mathbf{c}_{j,00} \exp(\mathbf{A}_{k,00} t) \mathbf{b}_{ik,00} \quad (49)$$

$$D_{ijk}(t) = 3 \mathbf{c}_{j,2M} \exp(\mathbf{A}_{Dk} t) \mathbf{b}_{ik,2M} \quad (50)$$

As before, the subscripts i, j , and k in $I_{\parallel ij k}(t)$ and $I_{\perp ij k}(t)$ [Eq. (48)], in $S_{ijk}(t)$ [Eq. (49)] and in $D_{ijk}(t)$ [Eq. (50)] refer to λ_i^{ex} , λ_j^{em} , and $[\mathbf{X}]_k$, respectively. $S_{ijk}(t)$ corresponds to the total time-resolved emission of the photophysical system, is independent of rotational diffusion, and does not contain any information about the orientations of the transition moments. Information about rotational diffusion is contained exclusively in $D_{ijk}(t)$.

Matrix $\mathbf{A}_{k,00}$ in Eq. (49) is identical with \mathbf{A}_k defined by Eq. (26). \mathbf{A}_{Dk} in Eq. (50) is defined as:

$$\mathbf{A}_{Dk} = \begin{pmatrix} \mathbf{A}_{Dk,2-2} & 0 & 0 & 0 & 0 \\ 0 & \mathbf{A}_{Dk,2-1} & 0 & 0 & 0 \\ 0 & 0 & \mathbf{A}_{Dk,20} & 0 & 0 \\ 0 & 0 & 0 & \mathbf{A}_{Dk,21} & 0 \\ 0 & 0 & 0 & 0 & \mathbf{A}_{Dk,22} \end{pmatrix} \quad (51)$$

with blocks $\mathbf{A}_{Dk,2M}$ given by:

$$\mathbf{A}_{Dk,2M} = \begin{pmatrix} -(k_{01} + k_{21}[\mathbf{X}]_k + D_{1,2M}) & k_{12} \\ k_{21}[\mathbf{X}]_k & -(k_{02} + k_{12} + D_{2,2M}) \end{pmatrix} \quad (52)$$

with $M = -2, -1, 0, 1, 2$. $D_{n,2M}$ (n denotes either 1 or 2) is given by

$$D_{n,2M} = 6D_{\perp n} + M^2(D_{\parallel n} - D_{\perp n}) \quad (53)$$

$D_{\perp n}$ and $D_{\parallel n}$ (see Figure 1) are the components of the rotational diffusion tensor of the *cylindrically symmetric* species n in its molecular reference frame (x, y, z) , chosen such that the rotational diffusion tensor is diagonal,¹² reducing to the unique component D_n ($= D_{\perp n} = D_{\parallel n}$) in the case of the *spherically symmetric* rotor n .

For a *spherically symmetric* rotor ($D_n = D_{\perp n} = D_{\parallel n}$), the matrices $\mathbf{A}_{Dk,2M}$ [Eq. (52)] are all identical and independent of M . In this case matrix block $\mathbf{A}_{Dk,2M}$ can be written as:

$$\mathbf{A}_{Dk,2M} = \begin{pmatrix} -(k_{01} + k_{21}[X]_k + 6D_1) & k_{12} \\ k_{21}[X]_k & -(k_{02} + k_{12} + 6D_2) \end{pmatrix} \quad (54)$$

The elements of vector $\mathbf{b}_{ik,LM}$ {with $L = M = 0$ [Eq. (49)], or $L = 2$ and $M = \pm 2, \pm 1, 0$ [Eq. (50)]} are the excitation coefficients $b_{nik,LM}$. In $b_{nik,LM}$ n stands for either species 1 or 2; i and k refer to λ_i^{ex} and $[X]_k$, respectively; L and M refer to the orientation of the absorption transitions. The elements $b_{nik,LM}$ can be expressed as the product of the initial concentration of n^* , $b_{nik} = [n^*]_0$, the appropriate spherical harmonic $Y_L^M(\hat{\mathbf{a}}_n)$ ¹⁸ for the orientation of the absorption transition moment $\hat{\mathbf{a}}_n$ in the molecular frame of species n , and a scaling factor B_L :

$$b_{nik,LM} = B_L b_{nik} Y_L^M(\hat{\mathbf{a}}_n) \quad (55)$$

with $B_0 = \frac{1}{12} \sqrt{\frac{1}{\pi^3}}$ and $B_2 = \frac{1}{30} \sqrt{\frac{5}{\pi^3}}$.

For $L = M = 0$, we have $Y_0^0(\hat{\mathbf{a}}_n) = \frac{1}{\sqrt{4\pi}}$ and $b_{nik,00} = \frac{b_{nik}}{24\pi^2}$.

The 2×1 vector $\mathbf{b}_{ik,00}$ in Eq. (49) is explicitly given by:

$$\mathbf{b}_{ik,00} = (b_{1ik,00} \quad b_{2ik,00})^T \quad (56)$$

whereas the 10×1 vector $\mathbf{b}_{ik,2M}$ in Eq. (50) is expressed as:

$$\mathbf{b}_{ik,2M} = (b_{1ik,2-2} \quad b_{2ik,2-2} \quad b_{1ik,2-1} \quad b_{2ik,2-1} \quad b_{1Aik,20} \quad b_{2ik,20} \quad b_{1ik,21} \quad b_{2Bik,21} \quad b_{1ik,22} \quad b_{2ik,22})^T \quad (57)$$

The elements of vector $\mathbf{c}_{j,LM}$ {with $L = M = 0$ [Eq. (49)], or $L = 2$ and $M = \pm 2, \pm 1, 0$ [Eq. (50)]} are the corresponding emission coefficients $c_{mj,LM}$ (m represents either species 1* or 2*). The emission coefficients $c_{mj,LM}$ are given by:

$$c_{mj,LM} = C_L c_{mj} Y_L^{M*}(\hat{\mathbf{e}}_m) \quad (58)$$

where $C_0 = \frac{16}{3} \sqrt{\pi^5}$, $C_2 = \frac{16}{15} \sqrt{\frac{\pi^5}{5}}$, and $Y_L^{M*}(\hat{\mathbf{e}}_m)$ is the complex conjugate of the appropriate spherical harmonic for the orientation of the emission transition moment $\hat{\mathbf{e}}_m$ in the molecular frame.

For $L = M = 0$, we have $c_{mj,00} = \frac{8\pi^2 c_{mj}}{3}$.

The coefficient c_{mj} is defined by Eq. (9). Vector $\mathbf{c}_{j,00}$ in Eq. (49) is explicitly given by:

$$\mathbf{c}_{j,00} = (c_{1j,00} \quad c_{2j,00}) \quad (59)$$

while vector $\mathbf{c}_{j,2M}$ in Eq. (50) is expressed as:

$$\mathbf{c}_{j,2M} = (c_{1j,2-2} \quad c_{2j,2-2} \quad c_{1j,2-1} \quad c_{2j,2-1} \quad c_{1j,20} \quad c_{2j,20} \quad c_{1j,21} \quad c_{2j,21} \quad c_{1j,22} \quad c_{2j,22}) \quad (60)$$

Since both $S_{ijk}(t) = I_{\parallel ij}k}(t) + 2I_{\perp ij}k}(t)$ [Eq. (49)] and $D_{ijk}(t) = I_{\parallel ij}k}(t) - I_{\perp ij}k}(t)$ [Eq. (50)] can be expressed in matrix form, the identification analysis via similarity transformation is carried out using the $S_{ijk}(t)$ and $D_{ijk}(t)$ functions.

1. Kinetic parameters

We start first with the identification via similarity transformation involving $S_{ijk}(t)$. For $f(t, \mathbf{A}, \mathbf{b}, \mathbf{c}) = S_{ijk}(t, \mathbf{A}_{k,00}, \mathbf{b}_{ik,00}, \mathbf{c}_{j,00})$, $\mathbf{A}_{k,00}$ in Eq. (49) is identical with \mathbf{A}_k defined by Eq. (26), $\mathbf{b}_{ik,00}$ and $\mathbf{c}_{j,00}$ are given by Eqs. (56) and (59), respectively, and \mathbf{T} is defined by Eq. (33).

As $S_{ijk}(t)$ reflects the time dependence of the total fluorescence and contains information only on the excited states, the identifiability analysis will be the same as that derived for a reversible intermolecular two-state excited-state process without external quencher^{9, 10} (Section 4A). As shown previously, two sets of rate constants are obtained: set I contains the original rate constants: $k_{01}^+ = k_{01}$, $k_{12}^+ = k_{12}$, $k_{21}^+ = k_{21}$, $k_{02}^+ = k_{02}$ with $\mathbf{T} = t_1 \mathbf{I}$; set II is the alternative set: $k_{01}^+ = k_{02} + k_{12}$, $k_{12}^+ = k_{01} - k_{02}$, $k_{21}^+ = k_{21}$, $k_{02}^+ = k_{02}$ with \mathbf{T} given by Eq. (34), provided that $k_{01} > k_{02}$.

Now we consider the identification involving $f(t, \mathbf{A}, \mathbf{b}, \mathbf{c}) = D_{ijk}(t, \mathbf{A}_{Dk}, \mathbf{b}_{ik,2M}, \mathbf{c}_{j,2M})$ in which we will use the results of the identifiability analysis involving $S_{ijk}(t)$. We assume that the similarity transformations for $S_{ijk}(t)$ and $D_{ijk}(t)$ are independent.

Kinetic parameters of the cylindrically symmetric rotor

Now \mathbf{A}_{Dk} is defined by Eq. (51) with blocks $\mathbf{A}_{Dk,2M}$ given by Eq. (52). $\mathbf{b}_{ik,2M}$ is given by Eq. (57) with $b_{nik,2M}$ defined by Eq. (55), $\mathbf{c}_{j,2M}$ is given by Eq. (60) with $c_{mj,2M}$ defined by Eq. (58). Also the transformations of the various blocks $\mathbf{A}_{Dk,2M}$ in \mathbf{A}_{Dk} [Eq. (51)] are independent of each other. Matrix \mathbf{T} is a block-diagonal matrix:

$$\mathbf{T} = \begin{bmatrix} \mathbf{T}_{-2} & 0 & 0 & 0 & 0 \\ 0 & \mathbf{T}_{-1} & 0 & 0 & 0 \\ 0 & 0 & \mathbf{T}_0 & 0 & 0 \\ 0 & 0 & 0 & \mathbf{T}_1 & 0 \\ 0 & 0 & 0 & 0 & \mathbf{T}_2 \end{bmatrix} \quad (61)$$

with the matrices \mathbf{T}_M ($M = -2, -1, 0, 1, 2$) expressed as

$$\mathbf{T}_M = \begin{bmatrix} t_{M,1} & t_{M,2} \\ t_{M,3} & t_{M,4} \end{bmatrix} \quad (62)$$

Because \mathbf{T} and \mathbf{A}_{Dk} are both block-diagonal matrices, the matrix multiplication of Eq. (15) is split into five separate matrix multiplications (two of those are identical; $M = -2$ and $M = +2$; $M = -1$ and $M = +1$). It is straightforward to show that the matrix multiplication [Eq. (15)] involving $\mathbf{A}_{Dk,2M}^+$ and $\mathbf{A}_{Dk,2M}$ [for $M = \pm 2, \pm 1, 0$] leads to two sets of parameters: set III ($k_{01}^+ + D_{1,2M}^+ = k_{01} + D_{1,2M}$, $k_{12}^+ = k_{12}$, $k_{21}^+ = k_{21}$, $k_{02}^+ + D_{2,2M}^+ = k_{02} + D_{2,2M}$) corresponding to $\mathbf{T}_M = t_{M,1} \mathbf{I}$, and set IV ($k_{01}^+ + D_{1,2M}^+ = k_{02} + k_{12} + D_{2,2M}$, $k_{12}^+ = k_{01} - k_{02} + D_{1,2M} - D_{2,2M}$, $k_{21}^+ = k_{21}$, $k_{02}^+ + D_{2,2M}^+ = k_{02} + D_{2,2M}$) corresponding to \mathbf{T}_M given by Eq. (63) with $t_{M,3}/t_{M,1} = (k_{01} - k_{02} - k_{12})/k_{12}$:

$$\mathbf{T}_M = \begin{bmatrix} t_{M,1} & 0 \\ t_{M,3} & t_{M,1} + t_{M,3} \end{bmatrix} \quad (63)$$

To solve for the individual k_{01}^+ , k_{12}^+ , k_{21}^+ , k_{02}^+ , $D_{\perp 1}^+$, $D_{\parallel 1}^+$, $D_{\perp 2}^+$, and $D_{\parallel 2}^+$, one should combine the equations describing sets I and II with the equations describing sets III and IV. This produces two possible solutions. (1) The original set I of rate constants is combined with the original set of rotational diffusion coefficients: $D_{\perp 1}^+ = D_{\perp 1}$, $D_{\parallel 1}^+ = D_{\parallel 1}$, $D_{\perp 2}^+ = D_{\perp 2}$, $D_{\parallel 2}^+ = D_{\parallel 2}$. (2) If the rotational diffusion coefficients of both species are equal ($D_{\perp 1} = D_{\perp 2}$ and $D_{\parallel 1} = D_{\parallel 2}$), an alternative set of rate constants (i.e., set II of Section 4A)

and the true rotational diffusion coefficients ($D_{\perp 1}^+ = D_{\perp 2}^+ = D_{\parallel 1} = D_{\perp 2}$ and $D_{\parallel 1}^+ = D_{\parallel 2}^+ = D_{\parallel 1} = D_{\parallel 2}$) may be obtained.

The same result can be obtained by considering the elementary functions σ_n [Eq. (12)] for the matrix blocks $\mathbf{A}_{Dk,2M}$, i.e. $\sigma_{D1k,2M}$ and $\sigma_{D2k,2M}$. The six eigenvalues of \mathbf{A}_{Dk} can be arranged into three pairs, each pair corresponding to a different value of M , and each pair can be assigned to the proper M .¹² Therefore, the functions $\sigma_{D1k,2M}$ and $\sigma_{D2k,2M}$ can be obtained unambiguously.

It can be shown that

$$\sigma_{D1k,2M} - \sigma_{1k} = -D_{1,2M} - D_{2,2M} \quad (64a)$$

$$(\sigma_{D2k,2M} - \sigma_{2k}) - (\sigma_{D2l,2M} - \sigma_{2l}) = k_{21}([\mathbf{X}]_k - [\mathbf{X}]_l)D_{2,2M} \quad (64b)$$

from which $D_{1,2M}$ and $D_{2,2M}$ are immediately recovered. $D_{1,2M}$ and $D_{2,2M}$ considered for two values of M then provide the required rotational diffusion coefficients $D_{\perp 1}, D_{\parallel 1}, D_{\perp 2}$, and $D_{\parallel 2}$.

To summarize, the identifiability analysis involving both $S_{ijk}(t)$ and $D_{ijk}(t)$ shows that the model for reversible intermolecular two-state excited-state processes with coupled rotational diffusion for a *cylindrically symmetric* ellipsoid is uniquely identifiable in terms of rate constants and rotational diffusion coefficients when the rotational diffusion of the two species is different. If the rotational characteristics of the two species are identical, a second set of rate constants (set II) is possible.

Kinetic parameters of the spherically symmetric rotor

An identification analysis based on similarity transformation for a *spherically symmetric* rotor similar to that for the *cylindrically symmetric* ellipsoid also gives two solutions. (1)

If the rotational diffusion coefficients of both species are different ($D_1 \neq D_2$), the unique set of true rate constants (i.e., set I of Section 4A) and true rotational diffusion coefficients ($D_1^+ = D_1, D_2^+ = D_2$) are obtained. (2) When the rotational diffusion coefficients of both species are identical ($D_1 = D_2$), an alternative set of rate constants (i.e., set II of Section 4A) may be obtained together with the true rotational diffusion coefficients ($D_1^+ = D_2^+ = D_1 = D_2$). The same conclusions can be arrived at by considering the elementary functions σ_n [Eq. (12)] for matrix $\mathbf{A}_{Dk,2M}$ [Eq. (54)],

$$\sigma_{D1k} = \sigma_{1k} - 6(D_1 + D_2) \quad (65a)$$

$$\sigma_{D2k} = \sigma_{2k} + 6(k_{01} + k_{21}[X]_k)D_2 + 6(k_{02} + k_{12})D_1 + 36D_1D_2 \quad (65b)$$

and by evaluating these expressions at two concentrations of coreactant X.

2. Parameters related to excitation and emission

For $S_{ijk}(t)$, use of normalized $\tilde{b}_{1ik,00}$ ($\tilde{b}_{2ik,00} = 1 - \tilde{b}_{1ik,00}$) and \tilde{c}_{1j} ($\tilde{c}_{2j} = 1 - \tilde{c}_{1j}$) for $\mathbf{T} = t_1 \mathbf{I}$ leads to unique normalized parameters: $\tilde{b}_{1ik,00}^+ = \tilde{b}_{1ik}$ and $\tilde{c}_{1j,00}^+ = \tilde{c}_{1j} \cdot \tilde{b}_{1ik}$ and \tilde{c}_{1j} are defined by Eqs. (37) and (38), respectively.

For $D_{ijk}(t)$ and $\mathbf{T}_M = t_{M,1} \mathbf{I}$ (corresponding to set I and the true rotational diffusion coefficients), one has

$$b_{nik,2M}^+ c_{mj,2M}^+ = b_{nik,2M} c_{mj,2M} \quad (66)$$

with $m, n = 1, 2$ and $M = \pm 2, \pm 1, 0$.

The products of the spherical harmonics implicitly contained in Eq. (66) can be summed via the addition theorem, yielding the second-order Legendre polynomial $P_2(\hat{\mathbf{a}}_n \cdot \hat{\mathbf{e}}_m)$ of the cosine of the angle between transition moments $\hat{\mathbf{a}}_n$ and $\hat{\mathbf{e}}_m$,

$$\sum_{M=-2}^2 b_{nik,2M} c_{mj,2M} = \frac{5}{4\pi} B_2 C_2 b_{nik} c_{mj} P_2(\hat{\mathbf{a}}_n \cdot \hat{\mathbf{e}}_m) \quad (67)$$

This theorem in combination with $b_{lik}^+ c_{mj}^+ = b_{lik} c_{mj}$ yields

$$P_2(\hat{\mathbf{a}}_n^+ \cdot \hat{\mathbf{e}}_m^+) = P_2(\hat{\mathbf{a}}_n \cdot \hat{\mathbf{e}}_m) \quad (68)$$

with $n, m = 1, 2$ and where $\hat{\mathbf{a}}_n^+$ and $\hat{\mathbf{e}}_m^+$ denote alternative transition moments. This

implies that all $P_2(\hat{\mathbf{a}}_n \cdot \hat{\mathbf{e}}_m)$ can be uniquely determined.

Use of normalized $\tilde{b}_{lik,2M}$ ($\tilde{b}_{2ik,2M} = 1 - \tilde{b}_{lik,2M}$) and $\tilde{c}_{1j,2M}$ ($\tilde{c}_{2j,2M} = 1 - \tilde{c}_{1j,2M}$) for $\mathbf{T}_M =$

$t_{M,1} \mathbf{I}$ leads to unique normalized parameters:

$$\tilde{b}_{lik,2M}^+ = \tilde{b}_{lik,2M} \quad (69)$$

$$\tilde{c}_{1j,2M}^+ = \tilde{c}_{1j,2M} \quad (70)$$

Substitution of Eq. (55) in Eq. (69) gives

$$\frac{Y_2^M(\hat{\mathbf{a}}_1^+)}{Y_2^M(\hat{\mathbf{a}}_2^+)} = \frac{Y_2^M(\hat{\mathbf{a}}_1)}{Y_2^M(\hat{\mathbf{a}}_2)} \quad (71)$$

so that the *ratio* of the spherical harmonics for the orientation of the absorption transition moments $\hat{\mathbf{a}}_1$ and $\hat{\mathbf{a}}_2$ is uniquely identified.

Similarly, substitution of Eq. (58) in Eq. (70) yields

$$\frac{Y_2^{M*}(\hat{\mathbf{e}}_1^+)}{Y_2^{M*}(\hat{\mathbf{e}}_2^+)} = \frac{Y_2^{M*}(\hat{\mathbf{e}}_1)}{Y_2^{M*}(\hat{\mathbf{e}}_2)} \quad (72)$$

implying that the *ratio* of the spherical harmonics for the orientation of the emission transition moments $\hat{\mathbf{e}}_1$ and $\hat{\mathbf{e}}_2$ is uniquely determined.

Information about the relative orientation of the transition moments can also be obtained

from the Markov parameters $m_{Dn,ikj}$. On introducing the scaling factor κ_{ijk} , one has that

$$m_{Dn,ikj} = \frac{8\pi}{25} \kappa_{ikj} \sum_{M=0}^2 \sum_{l,m=1}^2 [\mathbf{A}_{Dk,2M}^n]_{lm} \tilde{b}_{lik} \tilde{c}_{mj} \Delta_{lm,2M} \quad (73)$$

where $\Delta_{lm,2M}$ are geometrical factors given, for $M = 0$, by

$$\Delta_{lm,20} = Y_{20}^*(\hat{\mathbf{e}}_m) Y_{20}(\hat{\mathbf{a}}_l) \quad (74)$$

and, for $M = 1, 2$:

$$\Delta_{lm,2M} = Y_{2-M}^*(\hat{\mathbf{e}}_m) Y_{2-M}(\hat{\mathbf{a}}_l) + Y_{2M}^*(\hat{\mathbf{e}}_m) Y_{2M}(\hat{\mathbf{a}}_l) \quad (75)$$

Since the $m_{Dn,ikj}$ are all linear in $\Delta_{lm,2M}$, and since \tilde{b}_{lik} , \tilde{c}_{mj} , the rate constants, $D_{\perp l}$, and $D_{\parallel l}$ are already determined, $\Delta_{lm,2M}$ can readily be found from the set $m_{Dn,ikj}$, where $n = 0, 1, \dots, 5$, considered for two excitation wavelengths λ_i^{ex} in the same absorption band or for two values of the coreactant concentration $[X]$. Since, according to the addition theorem,

$$P_2(\hat{\mathbf{a}}_l \cdot \hat{\mathbf{e}}_m) = \frac{4\pi}{5} \sum_{M=0}^2 \Delta_{lm,2M} \quad (76)$$

the angles between all pairs $\hat{\mathbf{a}}_l$ and $\hat{\mathbf{e}}_m$ can be determined.

In conclusion, if the rotational diffusion coefficients of the two rotors are different, rotational diffusion coupled to a reversible intermolecular two-state excited-state process makes this model uniquely identifiable in terms of rate constants, rotational diffusion constants and normalized $\tilde{b}_{1ik,2M}$ and $\tilde{c}_{2j,2M}$.

5. Discussion and conclusions

In this review we have shown that the compartmental analysis approach is excellently suited to describe the kinetics and identifiability of models frequently encountered in photophysics. The term ‘‘compartment’’ in a photophysical context is defined as a subsystem composed of a distinct type of species that acts kinetically in a unique way.

The compartmental systems considered here consist of one excited-state compartment, two reversibly interconnected excited-state compartments, and their corresponding ground-state compartments. Three models of reversible intermolecular two-state excited-state processes in isotropic environments are discussed: (1) model without external quencher, (2) model with added quencher, (3) model with coupled species-dependent rotational diffusion described by Brownian reorientation. In a first step, we applied the similarity transformation approach to provide us with the explicit relationships between the true and alternative model parameters. The other identifiability approach based on Markov parameters and elementary functions $\sigma_{1,2}$ of the eigenvalues $\gamma_{1,2}$ was subsequently used to find the experimental conditions under which the model parameters can be recovered. Global compartmental analysis with linked model parameters can be used to estimate these parameters.

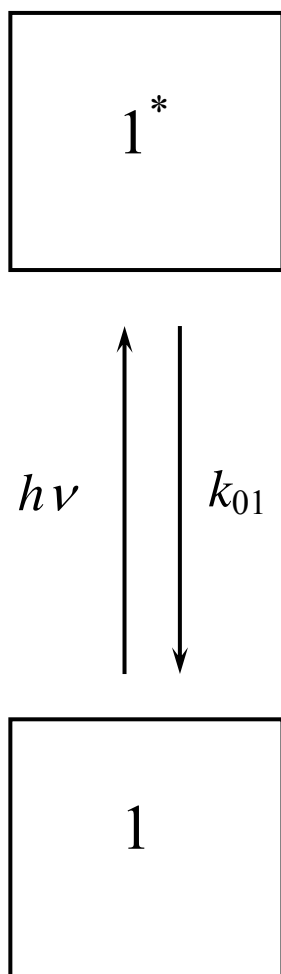
Since the deterministic identification analysis assumes by its very own nature that the decay data are error-free (the analysis is based on algebraic equations), the analysis gives the necessary and sufficient requirements to extract the model parameters. However, in real experiments, where the decays do have systematic errors and different parameter sets can sometimes produce fits of similar quality, it is advantageous to collect and analyze more fluorescence decay curves than is required according to the deterministic identification.

Finally, numerical identifiability via global compartmental analysis¹⁹ has been used to estimate rate constants and parameters related to excitation and emission for a number of reversible intermolecular two-state excited-state processes using fluorescence decay surfaces measured at various excitation and emission wavelengths and coreactant

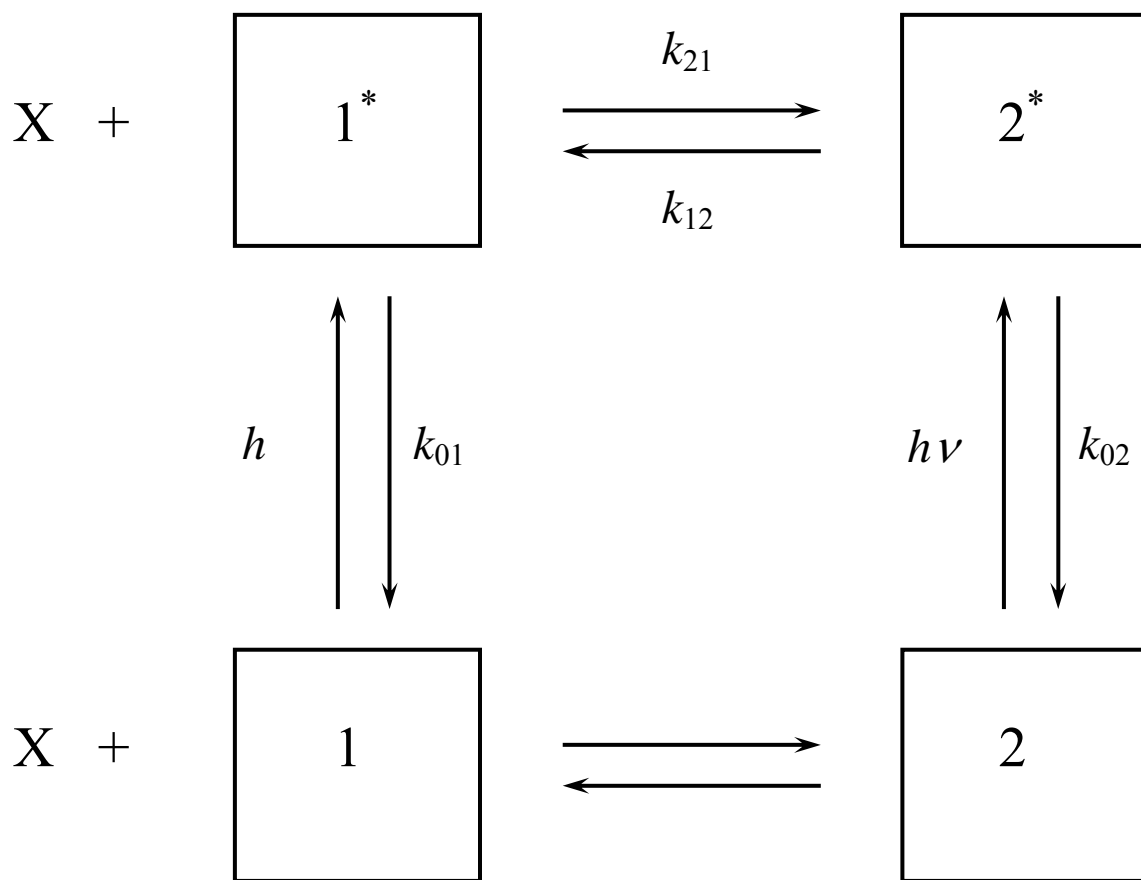
concentrations. Global compartmental analysis has the advantage that the parameters of interest are determined directly from the complete decay data surface in a single step. Applications of global compartmental analysis of reversible intermolecular two-state excited-state processes comprise excimer formation of pyrene,²⁰ exciplex formation between 1-methylpyrene and triethylamine,²¹ photophysics of 2-naphthol,²² photophysics of the binding of Ca²⁺ by Fura-2,²³ Quin-2,²⁴ APTRA-BTC,²⁵ and Thio-H,²⁶ photophysics of the fluorescent K⁺ indicator PBFI,²⁷ the fluorescent probe SBFO²⁸ for Na⁺ and Mag-fura-2²⁹ for Mg²⁺.

Acknowledgments

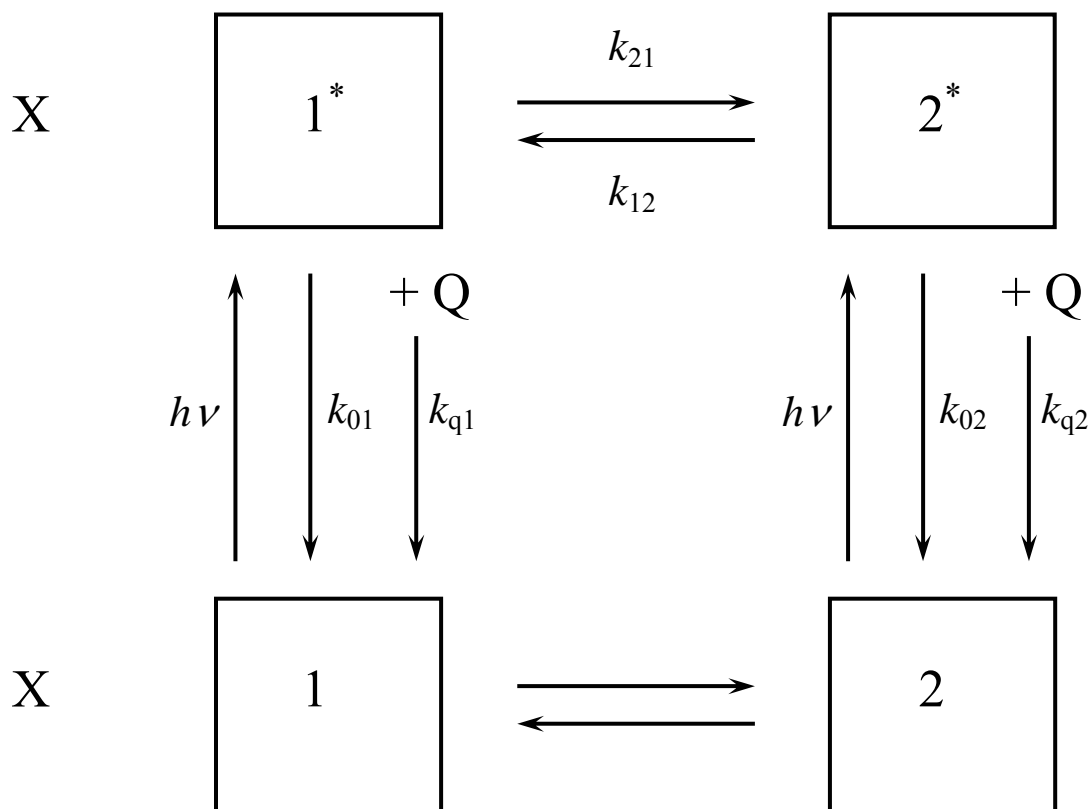
Special thanks are due to all our coworkers who have contributed to the development of the compartmental modeling approach in photophysics. NB thanks Prof. F. C. De Schryver (K.U.Leuven) for his interest and support. The *Fonds voor Wetenschappelijk Onderzoek – Vlaanderen* (FWO) and DWTC (Belgium) through IAP-V-03 are acknowledged for continuing support.



Scheme 1. Scheme representing a one-state excited-state process described by the deactivation rate constant k_{01} .



Scheme 2. Scheme representing a reversible intermolecular two-state excited-state process. The excited-state processes are described by the deactivation rate constants k_{01} and k_{02} , and the excited-state exchange rate constants k_{21} and k_{12} . The transformation of species 1 and 1^* into, respectively, 2 and 2^* is mediated by coreactant X.



Scheme 3. Scheme representing a reversible intermolecular two-state excited-state process with external quencher. It is assumed that the quencher Q has only an effect on the excited species and does not affect the ground-state equilibrium. The excited-state processes are described by the deactivation rate constants k_{01} and k_{02} , and the excited-state exchange rate constants k_{21} and k_{12} as in Scheme 2. The transformation of species 1 and 1^* into, respectively, 2 and 2^* is mediated by coreactant X. The additional deactivation of 1^* and 2^* due to the external quencher Q is described by the rate constants k_{q1} and k_{q2} , respectively.

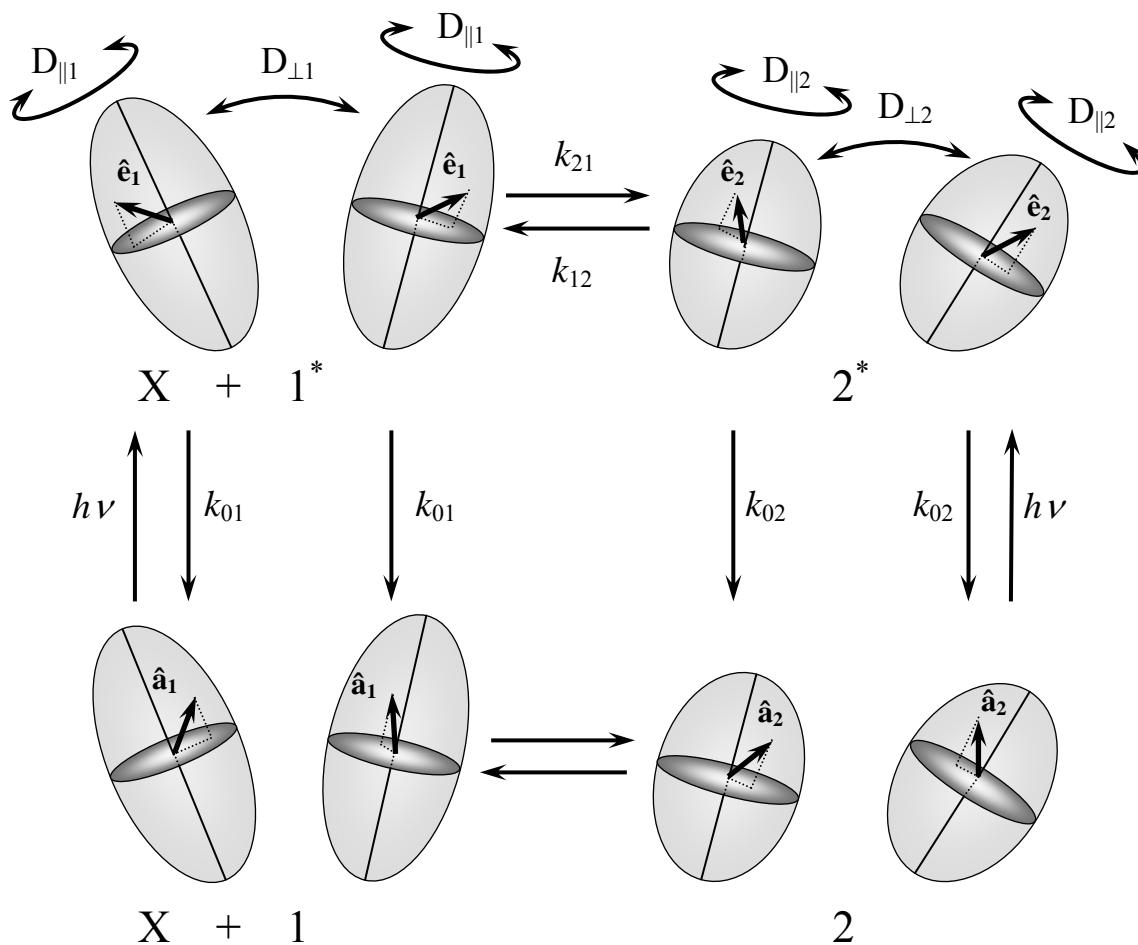


Figure 1. Graphic representation of a reversible intermolecular two-state excited-state process, including rotation. Species 1^* and 2^* are pictured as being initially excited from their ground states 1 and 2 by a short linearly polarized light pulse at wavelength λ_i^{ex} in a unique absorption band. The excited-state processes are described by the deactivation rate constants k_{01} and k_{02} , and the excited-state exchange rate constants k_{21} and k_{12} as in Scheme 2. The transformation of species 1 and 1^* into, respectively, 2 and 2^* is mediated by co-reactant X . Simultaneously the species rotate with rate constants determined by the corresponding rotational diffusion tensors which may differ between the species. The polarized emission of each species depends on the relative orientation of its emission

transition moment (with unit vector $\hat{\mathbf{e}}_1$ or $\hat{\mathbf{e}}_2$) at the instance of emission with respect to the absorption moment (with unit vector $\hat{\mathbf{a}}_1$ or $\hat{\mathbf{a}}_2$) in the species initially excited.

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University (Minsk, Belarus). For information about conditions of availability, contact Prof. F. C. De Schryver at Frans.DeSchryver@chem.kuleuven.be.

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