

# Compartmental Analysis in Photophysics: Fluorescence Decay Kinetics and Identifiability Analysis of a Model for Successive Complexation

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The triple-exponential fluorescence  $\delta$ -response function is derived for the photophysical model of successive complexation between ligand and analyte. Initially, a complex with 1:1 stoichiometry between ligand and analyte is formed. Further binding leads to a complex with two analyte molecules per ligand molecule. We show that this model is uniquely identifiable. This means that all deactivation and exchange rate constants in the excited state and all spectral parameters associated with photoexcitation and fluorescence emission can be uniquely determined. The issues of controllability and observability are discussed for this photophysical system. The conditions, under which a non-controllable or non-observable system is obtained, are described.

## 1. Introduction

Time-resolved fluorescence is an essential spectroscopic technique for studying the dynamics of excited-state processes. Since the relaxation of excited states often can be described by a set of coupled, linear differential equations, excited-state systems are formally equivalent with compartmental systems.<sup>1–3</sup> Although compartmental modeling is extensively used in pharmacokinetics, ecology, engineering, and chemical reaction kinetics (see, e.g., refs 1–3), its use in photophysics (energy transfer kinetics, fluorescence decay analysis, complex association/dissociation, excited-state quenching, etc.) is rather limited. Overall, relatively little has appeared on the use of compartmental analysis of excited-state processes, probably because of the lack of first-class, user-friendly, global compartmental software for analyzing time-resolved fluorescence data. Since the first identification analysis of an intermolecular two-state excited-state process,<sup>4</sup> identifiability studies of compartmental models of intermolecular as well as intramolecular two-state and three-state excited-state processes have been reported (see ref 5 for literature data up to 2000). Lately, we have reported identifiability analyses of a model for diffusion-mediated intramolecular excited-state quenching<sup>6</sup> and of a model for intermolecular excited-state proton exchange reaction in the presence of pH buffer.<sup>7</sup> Furthermore, the identifications of models for rotational diffusion monitored by time-resolved fluorescence depolarization<sup>8–12</sup> and for fluorescence quenching in aqueous micellar systems<sup>13,14</sup> have been investigated. A review on compartmental modeling and identifiability analysis in photophysics has been published recently.<sup>15</sup>

Once a particular photophysical model is proposed for describing the excited-state dynamics, the first step in compartmental analysis should be the derivation of the mathematical expression of the fluorescence decay. Next, one should investigate if the underlying parameters defining the model can be determined unambiguously from error-free fluorescence decay

data. This is the subject of the deterministic identifiability (or identification) analysis. Such an analysis tells one which information is theoretically accessible from the fluorescence decay surface.

In this report, we investigate the photophysical model for successive association between ligand and analyte M (or co-reactant M). Compartmental modeling is ideally suited for describing the excited-state dynamics and for analyzing the deterministic identification of this kinetic photophysical model. The paper is organized as follows. In section 2, we give a compartmental description of the time-resolved fluorescence of the model of successive association. In section 3, we use similarity transformation as an identifiability analysis method to verify which model parameters can be uniquely recovered from error-free observations. For the model discussed here, the parameters that have to be identified are excited-state deactivation/exchange rate constants and spectral factors associated with photoexcitation and fluorescence emission. In section 4, we discuss the properties of controllability and observability for the investigated system. It is shown that zero values for exchange rates and spectral parameters linked to excitation and emission lead to non-controllable or non-observable systems, respectively.

## 2. Compartmental Analysis Description of Fluorescence Decay Kinetics

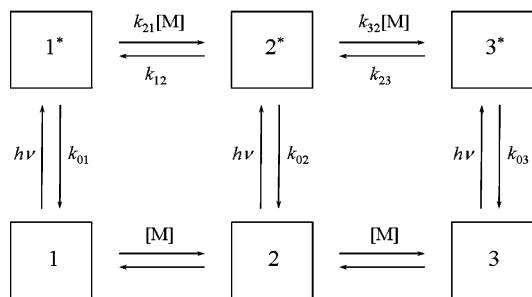
Consider a linear, time-invariant, dynamic, intermolecular photophysical system, consisting of three distinct types of ground-state species (labeled **1**, **2**, and **3**) and three corresponding excited species (labeled **1\***, **2\***, and **3\***, in that order) as depicted in Scheme 1. Ground-state species **1** can undergo a reversible association reaction with co-reactant (or analyte) M to form ground-state species **2**, which can associate further with M to form ground-state species **3**. Scheme 1 represents the reversible association–dissociation between **1** and co-reactant M with a 1:1 stoichiometry leading to **2** and the successive association with 1:2 stoichiometry between **1** and M leading to **3**. It is further understood that only species **1**, **2**, and **3** absorb light at the excitation wavelength  $\lambda_i^{\text{ex}}$ . Photoexcitation at  $\lambda_i^{\text{ex}}$  creates, in principle, the excited species **1\***, **2\***, and **3\***, which can decay by fluorescence (F) and nonradiative (NR) processes.

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**SCHEME 1: Schematic Representation of the Kinetic Model for Successive, Reversible, Intermolecular Association between Ligand and Analyte M: Species 1 Forms with Co-reactant M the 1:1 Complex 2 and Subsequently the 1:2 Complex 3; Photoexcitation (symbolized by  $h\nu$ ) Leads to the Excited Species 1\*, 2\*, and 3\***



The composite rate constant for these processes is represented by  $k_{0m}$  ( $= k_{Fi} + k_{NRi}$ ) for species  $m$  in the excited state. The excited-state association reaction of  $1^*$  with co-reactant M is described by rate constant  $k_{21}$ , while  $k_{12}$  stands for the rate constant of dissociation of  $2^*$  into  $1^*$  and M. The further excited-state association of  $2^*$  with M to form  $3^*$  is described by rate constant  $k_{32}$ , whereas  $k_{23}$  denotes the rate constant of dissociation of  $3^*$  into  $2^*$  and M.

If the system shown in Scheme 1 is photoexcited (symbolized by  $h\nu$ ) by a  $\delta$  pulse which does not significantly alter the concentrations of the ground-state species (i.e., in the low excitation limit), the fluorescence  $\delta$ -response function,  $f_{ij}(t, [M])$ , at co-reactant concentration  $[M]$  and at emission wavelength  $\lambda_j^{\text{em}}$  due to excitation at  $\lambda_i^{\text{ex}}$  is given by<sup>16</sup>

$$f_{ij}(t, [M]) = \mathbf{c}_j e^{t\mathbf{A}} \mathbf{b}_i([M]) \quad t \geq 0^+ \quad (1)$$

It is assumed that the concentration  $[M]$  of the co-reactant is experimentally known. The  $3 \times 3$  matrix  $\mathbf{A}$  can be represented in terms of its elements:

$$\mathbf{A} = (a_{mn}) \quad (2a)$$

with  $a_{mn}$  given by

$$a_{11} = -(k_{01} + k_{21}[M]) \quad a_{12} = k_{12} \quad a_{13} = 0$$

$$a_{21} = k_{21}[M] \quad a_{22} = -(k_{02} + k_{12} + k_{32}[M]) \quad a_{23} = k_{23} \quad (2b)$$

$$a_{31} = 0 \quad a_{32} = k_{32}[M] \quad a_{33} = -(k_{03} + k_{23})$$

It is assumed that all rate constants  $k_{0m}$  and  $k_{mn}$  are positive, leading to negative diagonal and nonnegative off-diagonal elements of  $\mathbf{A}$ .

$\mathbf{b}_i([M])$  is the  $3 \times 1$  vector with elements  $b_{mi} = [m^*]_{t=0^+}$  ( $m = 1-3$ ), symbolizing the time-zero concentration of  $m$  in the excited state due to excitation at  $\lambda_i^{\text{ex}}$ :

$$\mathbf{b}_i([M]) = (b_{1i}, b_{2i}, b_{3i})^T \quad (3)$$

where  $T$  indicates transpose.

$\mathbf{c}_j$  is the  $1 \times 3$  vector with elements  $c_{mj}$  ( $m = 1-3$ ):

$$\mathbf{c}_j = (c_{1j}, c_{2j}, c_{3j}) \quad (4a)$$

The emission weighting factor  $c_{mj}$  is defined by<sup>16</sup>

$$c_{mj} = k_{Fm} \int_{\Delta\lambda_j^{\text{em}}} \rho_m(\lambda^{\text{em}}) d\lambda^{\text{em}} \quad (4b)$$

where  $k_{Fm}$  is the fluorescence rate constant of species  $m$  in the excited state and  $\Delta\lambda_j^{\text{em}}$  is the wavelength interval for monitoring the fluorescence signal around  $\lambda_j^{\text{em}}$ .  $\rho_m(\lambda^{\text{em}})$  denotes the spectral density of the emission due to species  $m$  in the excited state at  $\lambda_j^{\text{em}}$ , normalized to its complete steady-state fluorescence spectrum  $F$ , and is defined by<sup>16</sup>

$$\rho(\lambda^{\text{em}}) = F / \int_{\text{full emission band}} F d\lambda^{\text{em}} \quad (5)$$

The triple  $(\mathbf{A}, \mathbf{b}_i, \mathbf{c}_j)$  is called a realization of the fluorescence  $\delta$ -response function  $f_{ij}(t)$ . Equation 1 represents  $f_{ij}(t)$  in terms of the realization  $(\mathbf{A}, \mathbf{b}_i, \mathbf{c}_j)$  and shows that the impulse response function  $f_{ij}(t)$  is composed of three separate contributions: photoexcitation (through  $\mathbf{b}_i$ ), fluorescence emission (through  $\mathbf{c}_j$ ), and deactivation and redistribution of the excited species (through  $\mathbf{A}$ ).

The explicit expression of  $f_{ij}(t)$  (eq 1) is triple-exponential:

$$f_{ij}(t) = \sum_{k=1}^3 \alpha_k e^{\gamma_k t} \quad t \geq 0^+ \quad (6)$$

The eigenvalues  $\gamma_k$  ( $k = 1-3$ ) of the compartmental matrix  $\mathbf{A}$  are given by

$$\gamma_1 = -\frac{6u}{\sqrt[3]{\eta}} + \frac{\sqrt[3]{\eta}}{6} + \frac{h_1}{3} \quad (7a)$$

$$\gamma_{2,3} = \frac{12u}{\sqrt[3]{\eta}(1 \pm i\sqrt{3})} - \frac{\sqrt[3]{\eta}(1 \pm i\sqrt{3})}{12} + \frac{h_1}{3} \quad (7b)$$

with

$$u = -\frac{1}{3} \left( h_2 + \frac{1}{3} h_1^2 \right) \quad (8a)$$

$$\eta = 36h_1h_2 + 108h_3 + 8h_1^3 + \sqrt{12\sqrt{-12h_2^3 - 3h_2^2h_1^2 + 54h_1h_2h_3 + 81h_3^2 + 12h_1^3h_3}} \quad (8b)$$

and

$$h_1 = a_{11} + a_{22} + a_{33} \quad (8c)$$

$$h_2 = -a_{11}a_{22} - a_{22}a_{33} - a_{11}a_{33} + a_{12}a_{21} + a_{23}a_{32} \quad (8d)$$

$$h_3 = a_{11}a_{22}a_{33} - a_{12}a_{21}a_{33} - a_{11}a_{23}a_{32} \quad (8e)$$

where  $a_{mn}$  are the elements of  $\mathbf{A}$ .

The pre-exponential factors  $\alpha_k$  ( $k = 1-3$ ) are given by:

$$\alpha_k = (c_1\beta_{1k} + c_2\beta_{2k} + c_3\beta_{3k}) \prod_{\substack{m=1 \\ m \neq k}}^3 (\gamma_k - \gamma_m) \quad (9)$$

with

$$\beta_{1k} = -(a_{33} - \gamma_k) \left[ b_1 q_{1k} + a_{12} \left( b_2 - b_3 \frac{q_{3k}}{a_{32}} \right) \right] \quad (10a)$$

$$\beta_{2k} = -(a_{33} - \gamma_k)(a_{11} - \gamma_k) \left[ -b_1 \frac{q_{1k}}{a_{12}} - b_2 + b_3 \frac{q_{3k}}{a_{32}} \right] \quad (10b)$$

$$\beta_{3k} = -(a_{11} - \gamma_k) \left[ a_{32} \left( b_1 \frac{q_{1k}}{a_{12}} + b_2 \right) - b_3 q_{3k} \right] \quad (10c)$$

and

$$q_{nk} = \prod_{\substack{m=1 \\ m \neq k}}^3 (a_{mm} - \gamma_m) / (a_{11} - a_{33}) \quad n = 1, 3 \quad (10d)$$

Although the imaginary unit  $i = \sqrt{-1}$  is explicitly present in the expressions of the eigenvalues  $\gamma_2$  and  $\gamma_3$  (eq 7b), we note that these eigenvalues are real. The imaginary unit  $i$  is needed to cancel the imaginary component of the variable  $\eta$  (eq 8b). The eigenvalues  $\gamma$  are calculated as roots of the characteristic (cubic) equation of the matrix  $\mathbf{A}$  and are real. Indeed, complex eigenvalues are only possible if there are three or more compartments in a cycle<sup>1</sup> (in the current model, this means that there also would be a connection between  $\mathbf{1}^*$  and  $\mathbf{3}^*$ ), which is not the case here.

### 3. Identifiability via Similarity Transformation

In the deterministic identifiability analysis, one examines as to whether or not the parameters of a given model are uniquely defined under error-free observations, given that the model is completely specified.<sup>1-3</sup> Therefore, the identification study of a specific model for excited-state processes investigates if it is possible to find alternative realizations of the fluorescence  $\delta$ -response function  $f_{ij}(t)$ , say  $(\bar{\mathbf{A}}, \bar{\mathbf{b}}_i, \bar{\mathbf{c}}_j)$ , in addition to the true realization  $(\mathbf{A}, \mathbf{b}_i, \mathbf{c}_j)$  that satisfy eq 11.

$$f_{ij}(t, \mathbf{A}, \mathbf{b}_i, \mathbf{c}_j) = f_{ij}(t, \bar{\mathbf{A}}, \bar{\mathbf{b}}_i, \bar{\mathbf{c}}_j) \quad (11)$$

In other words, the fluorescence  $\delta$ -response function should be the same for the true  $(\mathbf{A}, \mathbf{b}_i, \mathbf{c}_j)$  and the alternative  $(\bar{\mathbf{A}}, \bar{\mathbf{b}}_i, \bar{\mathbf{c}}_j)$  model parameter sets. The realizations  $(\mathbf{A}, \mathbf{b}_i, \mathbf{c}_j)$  and  $(\bar{\mathbf{A}}, \bar{\mathbf{b}}_i, \bar{\mathbf{c}}_j)$  are said to be similar, that is, they are related via similarity transformation (see further).

There are three possible outcomes to the identifiability analysis. (1) A model is uniquely (or globally) identifiable if the parameters of the assumed model can be uniquely determined from the idealized experiment. In that case, a single set of model parameters is obtained:  $\bar{\mathbf{A}} = \mathbf{A}$ ,  $\bar{\mathbf{b}}_i = \mathbf{b}_i$ , and  $\bar{\mathbf{c}}_j = \mathbf{c}_j$ . (2) If there is a finite number of alternative parameter estimates for some or all of the model parameters that fit the data, the model is locally identifiable: there is a limited set of alternative  $\bar{\mathbf{A}}$ ,  $\bar{\mathbf{b}}_i$ , and  $\bar{\mathbf{c}}_j$ . (3) An unidentifiable model is found when there is an infinite number of alternative  $\bar{\mathbf{A}}$ ,  $\bar{\mathbf{b}}_i$ , and  $\bar{\mathbf{c}}_j$ .

Here, we choose the similarity transformation approach<sup>1,2,17,18</sup> for carrying out the identification analysis because it offers an excellent method of constructing another (i.e., alternative) realization  $(\bar{\mathbf{A}}, \bar{\mathbf{b}}_i, \bar{\mathbf{c}}_j)$  of  $f_{ij}(t)$  and of determining if the model is uniquely or locally identifiable or not identifiable at all. An extra bonus of the similarity transformation approach is that the relationships between the true and the alternative model parameters are explicitly provided.

The realizations  $(\mathbf{A}, \mathbf{b}_i, \mathbf{c}_j)$  and  $(\bar{\mathbf{A}}, \bar{\mathbf{b}}_i, \bar{\mathbf{c}}_j)$  are related as in eq 12:

$$\bar{\mathbf{A}} = \mathbf{T}^{-1} \mathbf{A} \mathbf{T} \quad (12a)$$

$$\bar{\mathbf{b}}_i = \mathbf{T}^{-1} \mathbf{b}_i \quad (12b)$$

$$\bar{\mathbf{c}}_j = \mathbf{c}_j \mathbf{T} \quad (12c)$$

where  $\mathbf{T}$  is a constant, invertible (or nonsingular) matrix (i.e.,  $\det \mathbf{T} \neq 0$ ) having the same dimension (i.e., 3) as matrix  $\mathbf{A}$ :

$$\mathbf{T} = \begin{pmatrix} t_{11} & t_{12} & t_{13} \\ t_{21} & t_{22} & t_{23} \\ t_{31} & t_{32} & t_{33} \end{pmatrix} \quad (13)$$

The set of eqs 12 should be satisfied for each experimental condition<sup>5</sup> (different  $\lambda_i^{\text{ex}}$ ,  $\Delta\lambda_j^{\text{em}}$ , and analyte concentration  $[\text{M}]$ ). Therefore,  $\mathbf{T}$  should be independent of  $\lambda_i^{\text{ex}}$ ,  $\Delta\lambda_j^{\text{em}}$ , and  $[\text{M}]$ .

Performing the matrix multiplications in eq 12a leads to the following nine parts of eq 14:

$$t_{11}(\bar{k}_{01} - k_{01}) + t_{21}k_{12} = [\text{M}][t_{11}(k_{21} - \bar{k}_{21}) + t_{12}\bar{k}_{21}] \quad (14a)$$

$$t_{22}k_{12} + t_{12}(\bar{k}_{02} + \bar{k}_{12} - k_{01}) - t_{11}\bar{k}_{12} = [\text{M}](t_{12}k_{21} + t_{13}\bar{k}_{32} - t_{12}\bar{k}_{32}) \quad (14b)$$

$$t_{23}k_{12} + t_{13}(\bar{k}_{03} + \bar{k}_{23} - k_{01}) - t_{12}\bar{k}_{23} = [\text{M}]t_{13}k_{21} \quad (14c)$$

$$t_{31}k_{23} + t_{21}(\bar{k}_{01} - k_{02} - k_{12}) = [\text{M}][t_{22}\bar{k}_{21} - t_{11}k_{21} + t_{21}(k_{32} - \bar{k}_{21})] \quad (14d)$$

$$t_{32}k_{23} + t_{22}(\bar{k}_{02} + \bar{k}_{12} - k_{02} - k_{12}) - t_{21}\bar{k}_{12} = [\text{M}][t_{23}\bar{k}_{32} - t_{12}k_{21} + t_{22}(k_{32} - \bar{k}_{32})] \quad (14e)$$

$$t_{33}k_{23} + t_{23}(\bar{k}_{03} + \bar{k}_{23} - k_{02} - k_{12}) - t_{22}\bar{k}_{23} = [\text{M}](t_{23}k_{32} - t_{13}k_{21}) \quad (14f)$$

$$t_{31}(\bar{k}_{01} - k_{03} - k_{23}) = [\text{M}](t_{32}\bar{k}_{21} - t_{31}\bar{k}_{21} - t_{21}k_{32}) \quad (14g)$$

$$-t_{31}\bar{k}_{12} + t_{32}(\bar{k}_{02} + \bar{k}_{12} - k_{03} - k_{23}) = [\text{M}](t_{33}\bar{k}_{32} - t_{32}\bar{k}_{32} - t_{22}k_{32}) \quad (14h)$$

$$\bar{k}_{23} - t_{33}(\bar{k}_{03} + \bar{k}_{23} - k_{03} - k_{23}) = [\text{M}]t_{23}k_{32} \quad (14i)$$

Because the set of eqs 14 must be valid for all values (including zero) of  $[\text{M}]$ , their right-hand sides should be equal to zero. From eq 14c (with  $k_{21} \neq 0$ ), it follows then that  $t_{13} = 0$ . Analogously, from eq 14i (with  $k_{32} \neq 0$ ), we have  $t_{23} = 0$ , and hence, from eq 14c, it follows that  $t_{12} = 0$ . Now, eq 14a,b,e,f,i reduces to eq 15a,b,c,d,e, respectively. Equation 14c does not contain any information anymore, whereas eq 14d,g,h remain unchanged.

$$t_{11}(\bar{k}_{01} - k_{01}) + t_{21}k_{12} = [\text{M}]t_{11}(k_{21} - \bar{k}_{21}) \quad (15a)$$

$$t_{22}k_{12} - t_{11}\bar{k}_{12} = 0 \quad (15b)$$

$$t_{32}k_{23} + t_{22}(\bar{k}_{02} + \bar{k}_{12} - k_{02} - k_{12}) - t_{21}\bar{k}_{12} = [\text{M}]t_{22}(k_{32} - \bar{k}_{32}) \quad (15c)$$

$$t_{33}k_{23} - t_{22}\bar{k}_{23} = 0 \quad (15d)$$

$$t_{32}\bar{k}_{23} - t_{33}(\bar{k}_{03} + \bar{k}_{23} - k_{03} - k_{23}) = 0 \quad (15e)$$

Element  $t_{11}$  cannot be zero, because  $t_{12}$  and  $t_{13}$  are already zero. If  $t_{11} = 0$ , then the first row of  $\mathbf{T}$  is zero leading to  $\det \mathbf{T} = 0$ . Similarly, the conditions  $t_{22} = 0$  or  $t_{33} = 0$  also lead (via eq 15b,d with  $k_{12} \neq 0$  and  $k_{23} \neq 0$ ) to a singular matrix  $\mathbf{T}$ , which is not a valid transformation matrix. Hence, all of the diagonal elements of  $\mathbf{T}$  must be different from zero:  $t_{11} \neq 0$ ,  $t_{22} \neq 0$ , and  $t_{33} \neq 0$ .

Equation 15a for  $t_{11} \neq 0$  produces  $\bar{k}_{21} = k_{21}$ . Analogously, eq 15c for  $t_{22} \neq 0$  yields  $\bar{k}_{32} = k_{32}$ .

From eq 14g, we have  $t_{31} = 0$ . Equation 14h with  $t_{31} = 0$  leads to  $t_{32} = 0$ ,  $t_{33}\bar{k}_{32} = t_{22}k_{32}$ , and hence  $t_{22} = t_{33}$ . Now eq 15d gives  $\bar{k}_{23} = k_{23}$ . Equation 15e with  $t_{32} = 0$  and  $\bar{k}_{23} = k_{23}$  yields  $\bar{k}_{03} = k_{03}$ . Equation 14d with  $t_{31} = 0$  leads to  $t_{21} = 0$ ,  $t_{22}\bar{k}_{21} = t_{11}k_{21}$ , and hence  $t_{11} = t_{22}$ . Equation 15a gives  $\bar{k}_{01} = k_{01}$  and eq 15b yields  $\bar{k}_{12} = k_{12}$ . Finally, from eq 15c follows that  $\bar{k}_{02} = k_{02}$ .

To summarize: the photophysical system depicted in Scheme 1 is uniquely identifiable in terms of the rate constants:  $\{\bar{k}_{01} = k_{01}, \bar{k}_{02} = k_{02}, \bar{k}_{03} = k_{03}, \bar{k}_{12} = k_{12}, \bar{k}_{23} = k_{23}, \bar{k}_{21} = k_{21}, \text{ and } \bar{k}_{32} = k_{32}\}$  with  $\mathbf{T} = t_{11}\mathbf{I}_3$ , where  $\mathbf{I}_3$  stands for the unit matrix of order three. Now the alternative  $\tilde{\mathbf{b}}_i$  and  $\tilde{\mathbf{c}}_j$  can be calculated from eq 12b,c, respectively, with  $\mathbf{T} = t_{11}\mathbf{I}_3$ . It is straightforward to show that

$$\tilde{\mathbf{b}}_i = \tilde{\mathbf{b}}_i \quad (16a)$$

$$\tilde{\mathbf{c}}_j = \tilde{\mathbf{c}}_j \quad (16b)$$

In eq 16a,  $\tilde{\mathbf{b}}_i$  is the  $3 \times 1$  vector containing the normalized true  $\tilde{b}_{mi}$  ( $m = 1-3$ ) defined by

$$\tilde{b}_{mi} = b_{mi} / \sum_{m=1}^3 b_{mi} \quad (17a)$$

$\tilde{\mathbf{b}}_i$  is the  $3 \times 1$  vector with the normalized alternative  $\tilde{b}_{mi}$  ( $m = 1-3$ ):

$$\tilde{b}_{mi} = \tilde{b}_{mi} / \sum_{m=1}^3 \tilde{b}_{mi} \quad (17b)$$

Analogously, in eq 16b,  $\tilde{\mathbf{c}}_j$  and  $\tilde{\mathbf{c}}_j$  represent the  $1 \times 3$  vectors with the normalized true  $\tilde{c}_{mj}$  and alternative  $\tilde{c}_{mj}$  ( $m = 1-3$ ), respectively:

$$\tilde{c}_{mj} = c_{mj} / \sum_{m=1}^3 c_{mj} \quad (18a)$$

$$\tilde{c}_{mj} = \tilde{c}_{mj} / \sum_{m=1}^3 \tilde{c}_{mj} \quad (18b)$$

To conclude, the photophysical system with successive complexation as shown in Scheme 1 is uniquely identifiable in terms of the rate constants of de-activation and exchange in the excited state and the normalized spectral factors related to excitation ( $\tilde{\mathbf{b}}_i$ ) and emission ( $\tilde{\mathbf{c}}_j$ ).

#### 4. Controllability and Observability

It is known that the identifiability analysis based on similarity transformation (section 3) leads to reliable conclusions only for controllable and observable systems.<sup>19,20</sup>

The time-invariant system described by eq 1 is controllable if and only if the  $3 \times 3$  controllability matrix  $\mathbf{R}(\mathbf{A}, \mathbf{b}_i)$  of  $\mathbf{A}$  and  $\mathbf{b}_i$ ,<sup>2,21</sup> defined in eq 19, is of full rank 3 (requiring that  $\det \mathbf{R}(\mathbf{A}, \mathbf{b}_i) \neq 0$ ).

$$\mathbf{R}(\mathbf{A}, \mathbf{b}_i) = (\mathbf{b}_i, \mathbf{A}\mathbf{b}_i, \mathbf{A}^2\mathbf{b}_i) \quad (19)$$

The controllability matrix  $\mathbf{R} = \mathbf{R}(\mathbf{A}, \mathbf{b}_i)$  can be represented in terms of its elements:

$$\mathbf{R} = \begin{bmatrix} b_{1i} & b_{1i}a_{11} + b_{2i}a_{12} & b_{1i}(a_{11}^2 + a_{12}a_{21}) + b_{2i}a_{12}(a_{11} + a_{22}) + b_{3i}a_{12}a_{23} \\ b_{2i} & b_{1i}a_{21} + b_{2i}a_{22} + b_{3i}a_{23} & b_{1i}a_{21}(a_{11} + a_{22}) + b_{2i}(a_{12}a_{21} + a_{22}^2 + a_{23}a_{32}) + b_{3i}a_{23}(a_{22} + a_{33}) \\ b_{3i} & b_{2i}a_{32} + b_{3i}a_{33} & b_{1i}a_{21}a_{32} + b_{2i}a_{32}(a_{22} + a_{33}) + b_{3i}(a_{23}a_{32} + a_{33}^2) \end{bmatrix} \quad (20)$$

where  $a_{mm}$  are the elements of matrix  $\mathbf{A}$  (eq 2) and  $b_{mi}$  are the elements of vector  $\mathbf{b}_i$  (eq 3). To determine the rank of matrix  $\mathbf{R}$ , we calculate its determinant:

$$\det \mathbf{R} = a_{21}^2 a_{32} b_{1i}^3 - [(d_{12} + d_{13})a_{32}b_{2i} + (p_{12} - 2p_{23} - d_{13}d_{23})b_{3i}]a_{21}b_{1i}^2 - [(p_{12} + p_{23} - d_{12}d_{13})a_{32}b_{2i}^2 - (d_{12}p_{12} + d_{23}p_{23} - d_{12}d_{13}d_{23})b_{3i}b_{2i} + (2p_{12} - p_{23} + d_{12}d_{13})a_{23}b_{3i}^2]b_{1i} + [d_{13}a_{32}b_{2i}^3 + (p_{12} + p_{23} - d_{13}d_{23})b_{3i}b_{2i}^2 - (d_{13} + d_{23})a_{23}b_{2i}b_{3i}^2 - a_{23}^2 b_{3i}^3]a_{12} \quad (21a)$$

with

$$d_{12} = a_{11} - a_{22}, d_{13} = a_{11} - a_{33}, d_{23} = a_{22} - a_{33}, p_{12} = a_{12}a_{21}, p_{23} = a_{23}a_{32} \quad (21b)$$

The photophysical system studied is controllable if  $\text{rank } \mathbf{R} = 3$  and, hence, if  $\det \mathbf{R} \neq 0$ . Conversely, if  $\det \mathbf{R} = 0$ , the photophysical system is non-controllable.

The criterion for observability in terms of the matrices of the system is analogous to that of controllability. The same photophysical system is observable if and only if the  $3 \times 3$  observability matrix  $\mathbf{O}(\mathbf{A}, \mathbf{c}_j)$  of  $\mathbf{A}$  and  $\mathbf{c}_j$ ,<sup>2,21</sup> defined in eq 22, is of full rank 3 (requiring that  $\det \mathbf{O}(\mathbf{A}, \mathbf{c}_j) \neq 0$ ).

$$\mathbf{O}(\mathbf{A}, \mathbf{c}_j) = \begin{pmatrix} \mathbf{c}_j \\ \mathbf{c}_j\mathbf{A} \\ \mathbf{c}_j\mathbf{A}^2 \end{pmatrix} \quad (22)$$

The observability matrix  $\mathbf{O} = \mathbf{O}(\mathbf{A}, \mathbf{c}_j)$  can be written in terms of its elements:

$$\mathbf{O} = \begin{bmatrix} c_{1j} & c_{1j}a_{11} + c_{2j}a_{21} & c_{1j}(a_{11}^2 + a_{12}a_{21}) + c_{2j}a_{21}(a_{11} + a_{22}) + c_{3j}a_{21}a_{32} \\ c_{2j} & c_{1j}a_{12} + c_{2j}a_{22} + c_{3j}a_{32} & c_{1j}a_{12}(a_{11} + a_{22}) + c_{2j}(a_{12}a_{21} + a_{22}^2 + a_{23}a_{32}) + c_{3j}a_{32}(a_{22} + a_{33}) \\ c_{3j} & c_{2j}a_{23} + c_{3j}a_{33} & c_{1j}a_{12}a_{23} + c_{2j}a_{23}(a_{22} + a_{33}) + c_{3j}(a_{23}a_{32} + a_{33}^2) \end{bmatrix}^T \quad (23)$$

with  $c_{mj}$  defined in eq 4. The determinant of matrix  $\mathbf{O}$  is

$$\det \mathbf{O} = a_{12}^2 a_{23} c_{1j}^3 - [(d_{12} + d_{13}) a_{23} c_{2j} + (p_{12} - 2p_{23} - d_{13} d_{23}) c_{3j}] a_{12} c_{1j}^2 - [(p_{12} + p_{23} - d_{12} d_{13}) a_{23} c_{2j}^2 - (d_{12} p_{12} + d_{23} p_{23} - d_{12} d_{13} d_{23}) c_{3j} c_{2j} + (2p_{12} - p_{23} + d_{12} d_{13}) a_{32} c_{3j}^2] c_{1j} + [d_{13} a_{23} c_{2j}^3 + (p_{12} + p_{23} - d_{13} d_{23}) c_{3j} c_{2j}^2 - (d_{13} + d_{23}) a_{32} c_{2j} c_{3j}^2 - a_{32}^2 c_{3j}^3] a_{21} \quad (24)$$

with  $d_{12}$ ,  $d_{13}$ ,  $d_{23}$ ,  $p_{12}$ , and  $p_{23}$  given by eq 21b.

The photophysical system is observable if  $\text{rank } \mathbf{O} = 3$  and, hence, if  $\det \mathbf{O} \neq 0$ . Conversely, if  $\det \mathbf{O} = 0$ , the photophysical system is non-observable.

Note that  $\det \mathbf{O}$  (eq 24) is “symmetrical” compared with  $\det \mathbf{R}$  (eq 21): one has to substitute all  $b_i$  for  $c_j$  and invert all indices for  $a$  (i.e.,  $a_{mn}$  becomes  $a_{nm}$ ). Hence, the conditions for non-observability are symmetrical with those for non-controllability (switch the indices  $m$  and  $n$  in  $a_{mn}$  and substitute  $\mathbf{b}$  for  $\mathbf{c}$ ).

In this paper, it is not our aim to give a detailed analysis of all cases where the investigated photophysical system is non-controllable or non-observable. It suffices to note that the photophysical system becomes non-controllable if some transfer coefficients  $a_{mn}$  and some spectral excitation factors ( $b_{1i}$ ,  $b_{2i}$ ,  $b_{3i}$ ) in eq 20 are equal to zero. For example, non-controllable systems are found when (i)  $b_{1i} = 0$  and  $a_{12} = 0$ , (ii)  $b_{3i} = 0$  and  $a_{32} = 0$ , (iii)  $b_{2i} = 0$  and  $a_{21} = a_{23} = 0$ , (iv)  $b_{1i} = b_{2i} = 0$  and  $a_{23} = 0$ , (v)  $b_{2i} = b_{3i} = 0$  and  $a_{21} = 0$ , and (vi)  $b_{1i} = b_{3i} = 0$  and  $a_{12} = a_{32} = 0$ . Similarly, non-observability can be achieved if some transfer coefficients  $a_{mn}$  and some spectral emission factors ( $c_{1j}$ ,  $c_{2j}$ ,  $c_{3j}$ ) are set to zero in eq 23. For example, non-observable systems are found when (i)  $c_{1j} = 0$  and  $a_{21} = 0$ , (ii)  $c_{3j} = 0$  and  $a_{23} = 0$ , (iii)  $c_{2j} = 0$  and  $a_{12} = a_{32} = 0$ , (iv)  $c_{1j} = c_{2j} = 0$  and  $a_{32} = 0$ , (v)  $c_{2j} = c_{3j} = 0$  and  $a_{12} = 0$ , and (vi)  $c_{1j} = c_{3j} = 0$  and  $a_{21} = a_{23} = 0$ . A non-controllable or non-observable photophysical system always leads to a single- or biexponential  $f_{ij}(t)$  instead of the triple-exponential  $f_{ij}(t)$

anticipated for the controllable and observable system under investigation.

## References and Notes

- (1) Godfrey, K. *Compartmental Models and Their Application*; Academic Press: New York, 1983.
- (2) Anderson, D. H. *Compartmental Modeling and Tracer Kinetics*. In *Lecture Notes in Biomathematics*, Vol. 50; Springer-Verlag: Berlin, 1983.
- (3) Jacquez, J. A. *Compartmental Analysis in Biology and Medicine*, 3rd ed.; BioMedware: Ann Arbor, MI, 1996.
- (4) Ameloot, M.; Beechem, J. M.; Brand, L. *Chem. Phys. Lett.* **1986**, *129*, 211–219.
- (5) Boens, N.; Szubiakowski, J.; Novikov, E.; Ameloot, M. *J. Chem. Phys.* **2000**, *112*, 8260–8266.
- (6) Novikov, E.; Molski, A.; Boens, N. *J. Chem. Phys.* **2000**, *109*, 5348–5352.
- (7) Boens, N.; Basarić, N.; Novikov, E.; Crovetto, L.; Orte, A.; Talavera, E. M.; Alvarez-Pez, J. M. *J. Phys. Chem. A* **2004**, *108*, 8180–8189.
- (8) Szubiakowski, J. P.; Dale, R. E.; Boens, N.; Ameloot, M. *J. Chem. Phys.* **2004**, *121*, 7829–7839.
- (9) Boens, N.; Novikov, E.; Ameloot, M. *J. Phys. Chem. A* **2005**, *109*, 7024–7032.
- (10) Boens, N.; Novikov, E.; Szubiakowski, J. P.; Ameloot, M. *J. Phys. Chem. A* **2005**, *109*, 11655–11664.
- (11) Boens, N.; Novikov, E.; Ameloot, M. *ChemPhysChem* **2006**, *7*, 2559–2566.
- (12) Szubiakowski, J. P.; Dale, R. E.; Boens, N.; Ameloot, M. *Chem. Phys. Lett.* **2007**, *438*, 113–118.
- (13) Boens, N.; Van der Auweraer, M. *ChemPhysChem* **2005**, *6*, 2353–2358.
- (14) Boens, N.; Novikov, E.; Van der Auweraer, M. *Math. Biosci.* **2007**, *209*, 624–643.
- (15) Boens, N.; Ameloot, M. *Int. J. Quantum Chem.* **2006**, *106*, 300–315.
- (16) Ameloot, M.; Boens, N.; Andriessen, R.; Van den Bergh, V.; De Schryver, F. C. *J. Phys. Chem.* **1991**, *95*, 2041–2047.
- (17) Vajda, S.; Rabitz, H. *J. Phys. Chem.* **1988**, *92*, 701–707.
- (18) Chappell, M. J.; Godfrey, K. R.; Vajda, S. *Math. Biosci.* **1990**, *102*, 41–73.
- (19) Vajda, S.; Godfrey, K. R.; Rabitz, H. *Math. Biosci.* **1989**, *93*, 217–48.
- (20) Walter, E. *Identifiability of Parametric Models*; Pergamon Press: Oxford, 1987.
- (21) Sontag, E. D. *Mathematical Control Theory: Deterministic Finite Dimensional Systems*, 2nd ed.; Springer: New York, 1998.