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Compartmental modeling in photophysics: Identifiability of models for intramolecular three-state excited-state processes

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Abstract

In this paper, we investigate if the rate constants and spectral parameters that describe models for intramolecular three-state excited-state events can be uniquely determined from ideal time-resolved fluorescence data. For the general model, in which there are reversible transformations between the three excited species, values of nine rate constants and four spectral parameters related to excitation and emission must be defined. Without prior knowledge of rate constants and spectral parameters there is an infinite number of these model parameters that fit the time-resolved fluorescence data and hence the model is not identifiable. Even simplified models leading to single exponential decay kinetics of some excited species concentrations are unidentifiable if such prior knowledge is not available. It is shown for each simplified model what kinetic information can be extracted from perfect time-resolved fluorescence data when the individual excited species concentrations can be monitored. Guidelines to the experimentalist are given for using a priori knowledge to make a distinction among the different models.

Keywords: Excited-state kinetics; Identifiability; Photophysics; Compartmental analysis; Intramolecular processes

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1. Introduction

The measurement and analysis of time-resolved fluorescence are essential tools available to the photophysicist for investigating the dynamics of excited-state processes. In order to decide on the appropriate model to describe an intricate photophysical system, fluorescence decay traces are measured under different experimental conditions (excitation wavelength λ^{ex}, emission wavelength λ^{em}, (coreactant) concentration, quencher concentration, temperature, pH, polarization, etc.), so that a multidimensional fluorescence decay data surface is generated. In the single-curve analysis, each fluorescence decay curve is analyzed separately, and a proposed model is tested by the consistency of the recovered decay parameters (most often exponential decay times τ_i and their associated amplitudes α_i). A subsequent analysis of the parameter estimates provides the relevant parameters of interest, e.g., the rate constants of the excited-state process. This conventional approach – though adequate in many cases – fails to take full advantage of relations that may exist between individual decay traces. The simultaneous or global analysis of multiple decays uses (and tests) those relationships by keeping some model parameters in common (i.e., linked in global curve-fitting analysis) between various related experiments. For the models discussed in this paper, the (three) separate decay times τ_i should be independent of the excitation wavelength λ^{ex}, the emission wavelength λ^{em}, and concentration. Experimental systems, which can be described by the models discussed here, should have decay times τ_i that can be linked in global curve-fitting analysis between experiments measured at various λ^{ex}, λ^{em}, and concentration. The advantages of the global analysis method are the improved model-discriminating capability (i.e., one can distinguish better among competing models) and the increased accuracy and precision of the parameter estimates in

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comparison to the single-curve analysis. Whenever a particular model is proposed for the description of excited-state processes, one should investigate first if the kinetic and spectral parameters defining the model can be determined from error-free fluorescence decay data. This is the subject matter of the deterministic identifiability analysis. The identification of models for *intramolecular three-state excited-state* processes constitutes the topic of this paper.

From the experimentalist's point of view, it is important to recognize possible experimental systems that can be represented by the various models for intramolecular three-state excited-state events. *Intramolecular* excited-state processes are – by definition – independent of concentration, which can be checked by the invariance of the linked fluorescence decay times τ_i in global analysis as a function of concentration. *Three-state excited-state* processes are photophysical events where three excited-states are involved with distinguishable excited-state (deactivation and exchange) kinetics. Such processes are characterized by three linked τ_i in global curve-fitting analysis as a function of λ^{ex} and λ^{em}. Potential experimental systems that lead to intramolecular processes with *three* excited species include, for example, intramolecular excimer forming molecules (locally excited state and excimer) in the presence of a fluorescent impurity. Intramolecular exciplex formation in the presence of an impurity can also lead to a three-state excited-state process. Moreover, transformations between three conformations in the excited state that happen on the timescale of fluorescence can give rise to triple exponential fluorescence decays with decay times τ_i that are independent of concentration. Other experimental systems where a locally excited state can give rise to two intramolecular excimers, or an intramolecular excimer and an exciplex with discernible excited-state deactivation and exchange kinetics may be adequately described by the presented models.

Since time-resolved fluorescence can in many instances be described by a set of coupled first-order differential equations, modeling of excited-state processes in photophysics can conveniently be done within the framework of compartmental analysis. Considering the extensive use of compartmental modeling in biomedicine, pharmacokinetics, analysis of ecosystems, etc. (see, for example, Refs. [1–3]), it is rather surprising that its use in photophysics started relatively late [4–8].

Let us define first the term "compartment" 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 constituent 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 photoexcitation. 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 photoexcitation, it suffices to consider the excited-state compartments only.

What are possible "excited-state compartments" in intramolecular three-state excited-state events? Photoexcitation of a bichromophoric molecule A–A may yield a locally excited state A^* (first excited-state compartment) and an intramolecular excimer $(A\text{–}A)^*$ (second excited-state compartment). The presence of a second molecule, B (impurity), may, upon light excitation, provide the third excited-state compartment (corresponding to B^*). A molecule A–A–B may create three excited-state compartments, corresponding to locally excited state A^*, excimer $(A\text{–}A)^*$, and exciplex or intramolecular charge transfer complex $(A\text{–}B)^*$. Photoexcitation of a single compound can lead to three excited-state compartments, if the molecule can adapt three conformations in the excited state that interconvert with distinct kinetics on a timescale, which is comparable with that of fluorescence. The time-resolved fluorescence of multichromophoric compounds and proteins can often be described by triple exponential decays, indicative of three excited-state compartments.

A first phase of compartmental modeling in photophysics is the derivation of the kinetic equations describing the time-resolved fluorescence after an infinitely short light pulse, leading to the fluorescence δ-response function $f(t)$ [see Section 2, Eq. (1)]. A second, important part of compartmental modeling is the *deterministic identifiability* (or identification) analysis, in which 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 photoexcitation into, decay from, and exchange between the excited-state compartments [1–3]. There are three possible outcomes to the deterministic 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.

A *deterministic identifiability* analysis is a prerequisite before attempting to estimate the model parameters from real experimental observations. Large uncertainties and high correlations obtained in the parameter recovery from an experimental data surface might erroneously be ascribed to numerical ill-conditioning. However, these may be