Quenching of Fluorescence by Light: A New Method to Control the Excited-State Lifetimes and Orientations of Fluorophores

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INTRODUCTION

The phenomenon of the quenching of fluorescence by light, or "light quenching," is the diminishing of the excited-state population by stimulated emission of atoms or molecules by external illumination. Early experiments carried out by Russian spectroscopists showed that light quenching required overlap of the quenching wavelength with the emission spectrum and displayed the same photoselection rules as does excitation. Mazurenko et al. observed depolarization at high illumination intensities, apparently due to selective stimulated decay of the vertically polarized component.

To date essentially all studies of light quenching have been performed using the intense giant pulses from Q-switched lasers and using long quenching pulses which are comparable to continuous, nonpulsed illumination. We now show that significant light quenching can occur with the lower energy pulses from a cavity-dumped picosecond dye laser. Observation of light quenching with
this commonly available laser source offers the opportunity to control the lifetime and orientation of the excited-state populations, which can have numerous applications of time-resolved fluorescence. We believe that the use of light quenching will enable a new class of fluorescence experiments, in which the sample is prepared by one or more quenching pulses prior to the time-resolved or steady-state observations.

**THEORY**

We consider a dilute solution of a fluorophore in the optically inactive solvent and assume that the absorption and emission oscillators of the fluorophore are colinear. We also assume that the viscosity of the solvent is high enough that significant rotational diffusion does not occur during the lifetime of the fluorophore's excited state. To describe the time evolution of the excited-state population during and after the excitation, we use a spherical coordinate system in which the fluorophore transition dipole makes an angle $\theta$ from the vertical axis and $\phi$ from the direction of illumination (Scheme I). We suppose that the angular distribution of the transition dipoles before excitation is uniform.

Assuming that the changes in the ground-state population are negligible, the excited-state population $n(\theta, t)$ is governed by

$$
\frac{dn(\theta, t)}{dt} = NP(t) \sigma_s \cos^2\theta - n(\theta, t) \left[ \frac{1}{\tau} + P(t) \sigma_{iq} \cos^2\theta \right]
$$

(1)

where $N$ is the ground-state population, $\sigma_s$ and $\sigma_{iq}$ the absorption and light quenching cross sections, respectively, $P(t)$ the power density [photons/(cm$^2$/s)] of the incident light, and $\tau$ the unquenched lifetime. For short pulses Eq. (1) yields

$$
n(\theta, t) = N \frac{\sigma_s}{\sigma_{iq}} \left( 1 - e^{-W_p \sigma_{iq} \cos^2\theta} \right) e^{-t/\tau}
$$

(2)

where

$$
W_p = \int_0^t P(t) \, dt
$$

(3)

is the number of photons passing the unit area of the sample during the single pulse. $W_p$ is proportional to the laser power. Importantly, it follows from Eq. (2) that for short pulses $n(\theta, t)$ does not depend on the shape of the incident pulse. The polar plots of the function $n(\theta, t)$ in the absence and presence of light quenching are shown in Fig. 1. These plots show that the extent of orientation along the $z$ axis (right) is decreased by light quenching.

![Scheme I. Coordinate system for a fluorophore. The transition moment makes an angle $\theta$ with the $Z$ axis and $\phi$ with the $X$ axis.](image)