Journal of Photochemistry & Photobiology, B: Biology 163 (2016) 105–109

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Simultaneous uptake of a Förster transfer dye pair by diatoms: Application in determination of staining density

Michael Grimann, Thomas Fuhrmann-Lieker *

Macromolecular Chemistry and Molecular Materials, Center of Interdisciplinary Nanostructure Science and Technology, University of Kassel, Heinrich-Plett-Str. 40, D-34109 Kassel, Germany

ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form 21 July 2016
Accepted 24 July 2016
Available online 26 July 2016

Keywords:
Diatoms
Biological photonic crystals
Resonant energy transfer
Fluorescence lifetime

ABSTRACT

The simultaneous uptake of PDMPO and Rhodamine B as two fluorescent dyes forming a Förster transfer pair by the diatom *Cyclotella meneghiniana* is demonstrated by *in vivo*-fluorochromation. The incorporation density in the cell walls was high enough for achieving resonant energy transfer between the two dyes as detected by fluorescence and excitation spectroscopy. The mean fluorescence lifetime of the donor is shortened in the presence of the acceptor by a factor of 0.75. By determining the mean lifetime from the fluorescence decay fitted by three eponentials, the efficiency of the energy transfer and the acceptor concentration is calculated assuming a homogenous distribution. For an initial concentration of both dyes of 5 μM in the culture medium which is at the saturation limit of incorporation, an acceptor incorporation density of 0.6 mM is obtained. In addition to such quantitative determinations, efficient emitting systems based on resonant energy transfer between two laser dyes may be useful in photonic applications of the hybrid biomineral. By achieving stimulated emission, the presence of optical modes in diatom frustules, which may act as photonic resonators due to the refractive index contrast to the environment in combination with the more or less regular pore pattern, may be characterized further.

1. Introduction

Fluorescent dyes have been frequently used for the staining of cell compartiments in which they selectively accumulate. For staining of silica in biomineralizing organisms such as diatoms and siliceous sponges, a variety of suitable organic staining dyes that cover the visible emission range have been reported, including blue emitting oxazoles [1], green emitting oxadiazoles [2,3], yellow to red emitting rhodamines [4,5] and red emitting thiadiazoles [6]. In addition of being an useful tool in biomineralization studies that enable the observation of silica deposition, more recently a second field of research evolved in which light-emitting biominerals walls may prove useful as analytical tool. This new field originated in the consideration of the silica structure as photonic resonator due to the refractive index contrast of the biosilica and the surrounding fluid. The characteristic feature of diatoms *(Bacillariophyta)* is their silicified cell wall, called frustule, with a more or less regular pore pattern depending on the species. In terms of photonics, that means that the cell wall functions as a waveguide for light, with a restriction of waveguide modes by the pore pattern. Very regular patterns as in centric diatoms of the genus *Coscinodiscus* can be regarded as two-dimensional biological photonic crystals [7], and more disordered pore patterns should be described accordingly as photonic glasses.

The modified photonic mode structure is expected to impact the emission spectrum and directionality in the stimulated emission regime, which could be tested by efficient laser dyes. From the viewpoint of dye chemistry, prerequisites for such efficient emitters are a high quantum yield of photoluminescence and low self-absorption. From research on organic solid state laser systems it is known that Förster-transfer pairs consisting of a donor dye that is pumped and a fluorescent acceptor dye that receives the excitation energy by resonance energy transfer (FRET) are ideal chromophores with high quantum yields and low thresholds [8].

In this contribution we explore the possibility of double-staining diatoms with two dyes that constitute a Förster transfer pair. Our aim is twofold: First - which is the main focus of this contribution - by measuring the change fluorescence lifetime of the donor in the presence of an acceptor it is possible to calculate the acceptor density and therefore the composition of light-emitting frustules as a new kind of hybrid biological material. Second, in view of future applications, the implementation of Förster transfer systems may lower the threshold for stimulated emission, leading to self-reproducing microlasers carrying their own resonators.

2. Material and Methods

For *in vivo*-fluorochromation, the marine centric diatom *Cyclotella meneghiniana* (Culture Collection of Algae at the University of Göttingen, strain EPSAG No. 1070-1) was cultivated using the nutrition medium described in Ref. [5]. The light-dark cycle in the incubator

* Corresponding author.
E-mail address: th.fuhrmann@uni-kassel.de (T. Fuhrmann-Lieker).

http://dx.doi.org/10.1016/j.jphotobiol.2016.07.034
1011-1344/

(Sanyo MIR 153 with irradiation by a Philips TL-D 15W/840 lamp) was set to 18:6 h at a temperature of 18 °C ± 1 °C.

For simultaneous staining, the fluorescence dyes PDMPO (Invitrogen Lysosensor DND-160) and Rhodamine B (Merck) were added to the culture medium, yielding a concentration of 5 μM for each dye (appropriate concentrations of the stock solutions were prepared by weighing and dilution). In addition, strains with only one dye were prepared for comparison. The chemical structure of the dyes is displayed in Fig. 1.

The selective staining of diatom frustules by these dyes was reported before [1,5] and was controlled for our samples by fluorescence microscopy of living cells and isolated frustules.

After staining the cell walls, the living cells were rinsed with unstained culture medium resulting in a final dye concentration of less than 10^{-12} M which is below the detection limit of the used spectrometers. This procedure ensured that only incorporated dye molecules were measured.

For optical measurements, the stained cells were suspended in unstained culture medium in order to set the external refractive index to 1.34. Fluorescence and excitation spectra were measured using a Hitachi F4500 fluorescence spectrometer. Fluorescence lifetime measurements were performed by time-correlated single photon counting with a PicoQuant FluoTime200 setup using a 372 nm laser (Picoquant LDH-P-C-375 with 59 ps pulses) as excitation source. Since the diameter of *C. meneghiniana* cells is 5–10 μm, whereas the typical spot size for irradiation is 0.5 mm, in each measurement the emission from many cells is collected.

Fluorescence polarisation was measured with a Perkin Elmer Fusion α-FP using a 535 nm emission filter and 450 nm/485 nm excitation edge filter for PDMPO and Rhodamine B, respectively.

2.1. Theory/Calculation

If the emission spectrum of a donor molecule overlaps with the absorption spectrum of a corresponding acceptor molecule and the intermolecular distance between the two dye molecules is low (~10 nm), resonant energy transfer (FRET) can occur [9,10]. The donor emission is then quenched whereas the acceptor emission is enhanced. The respective transfer rate constant k_T shows a sixth order dependence on the intermolecular distance r.

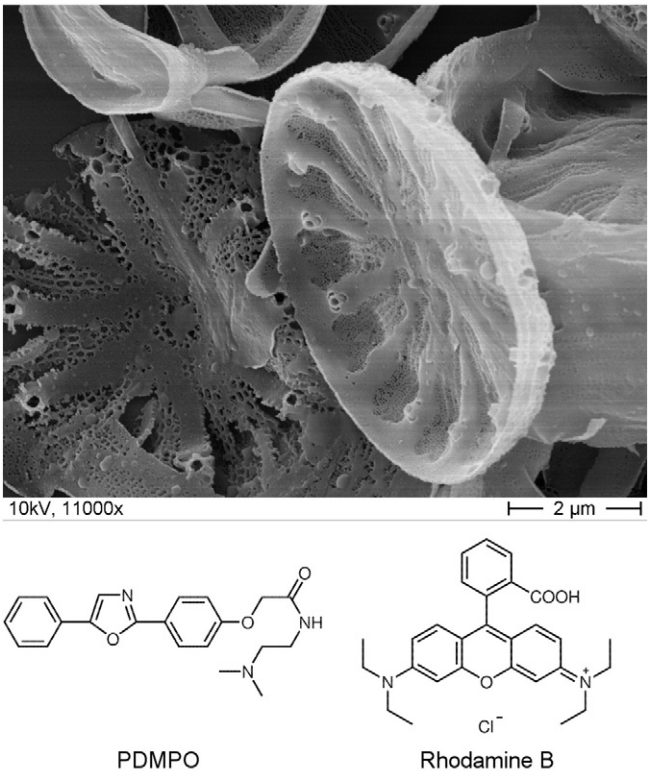

Fig. 1. Morphology of *Cyclotella meneghiniana* (SEM image) and structure of staining dyes.

$$k_T = \tau_D^{-1} R_0^{\,6} / r^6 \tag{1}$$

Herein τ_D denotes the fluorescence lifetime of the donor and R_0 the Förster distance

$$R_0^{\,6} = (9 \ln 10 J \kappa^2 \Phi_D) / (128\, \pi n^4 N_A) \tag{2}$$

which in principle is accessible through the spectral overlap integral J

$$J = \int I_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda \tag{3}$$

Here, κ denotes the orientation factor, Φ_D the quantum yield of the donor, n the refractive index, N_A Avogadro's constant, $I_D(\lambda)$ the donor emission spectrum normalized to unity area, $\varepsilon_A(\lambda)$ the molar extinction coefficient of the acceptor, λ the wavelength.

The quantum yield of the transfer Φ_T can be calculated from the ratio of steady-state donor emission intensity in presence of an acceptor F_{DA} and without its presence F_D

$$\Phi_T = 1 - F_{DA}/F_D \tag{4}$$

The ratio of steady-state intensities F_{DA}/F_D is proportional to the corresponding ratio of fluorescence lifetimes τ_{DA}/τ_D. In three-dimensional environments, where multiple molecules are randomly distributed, the fluorescence decay of the donor in presence of an acceptor is generally non-exponential even if a monoexponential decay is obtained for the pure donor dye. Thus, a distribution of lifetimes is generally taken into account. Assuming a homogenous distribution of chromophores, the temporal response of a delta-pulse excitation in presence of FRET is [10–12]:

$$I_{DA}(t) = I_0 \exp\left(-t/\tau_D - 2\gamma (t/\tau_D)^{1/2}\right) \tag{5}$$

with the concentration parameter

$$\gamma = 2/3\, \pi^{3/2} N_A R_0^{\,3} [A] \tag{6}$$

in SI-conform units. Herein $I_{DA}(t)$ is the time (t) dependent fluorescence, I_0 the initial fluorescence intensity in the donor channel, and [A] denotes the concentration of the acceptor molecule in mol/m^3. If [A] is set to zero, the monoexponential decay of the pure donor is obtained. Within this framework, Eq. (4) still holds, but the relative change in donor fluorescence intensity can be expressed by amplitude-weighted mean lifetimes and is given by

$$F_{DA}/F_D = \langle \tau_{DA} \rangle / \langle \tau_D \rangle = 1 - \pi^{1/2} \gamma \exp(\gamma^2)\, erfc(\gamma) \tag{7}$$

In this expression, erfc( ) is the complementary error function. Eqs. (6) and (7) allow a calculation of the acceptor concentration [A].

3. Results and Discussion

Before investigating the simultaneous incorporation of the two dyes it was confirmed that both dyes were incorporated separatedly into the cell wall (frustule) of *Cyclotella meneghiniana*. Fig. 2 displays the emission and excitation spectra of living cells of different strains, stained with one of the dyes, respectively. For the emission spectrum, the excitation wavelength was set near to the absorption maximum. For recording the excitation spectrum, the detecting channel was set near to the fluorescence maximum, and the excitation wavelength was scanned. A dye concentration of 5 μmol/l for each dye in the culture medium