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Synthesis and energy-transfer properties of fluorescence sensing bichromophoric system based on Rhodamine 6G and 1,8-naphthalimide

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ABSTRACT

This paper reports on the synthesis and investigation of a novel fluorescence sensing bichromophoric system **1** based on Rhodamine 6G and 1,8-naphthalimide, designed as a wavelength-shifting FRET chromophore. A similar donor–acceptor system **2**, not containing photoinduced electron transfer (PET) receptor, was synthesized as a reference compound and investigated as well. The overlap between the emission of the donor (1,8-naphthalimide) and the absorbance of the acceptor (rhodamine) was more than 90%. Due to the efficient donor–acceptor energy transfer (96–97%) the acceptor fluorescence of the systems, excited in the maximal absorption of the donor chromophore, was enhanced more than 33 times with respect to the fluorescence intensity of the reference Rhodamine 6G. It was also found that the novel bichromophoric system **1** displayed sensitive acceptor fluorescence signaling over a wide pH scale, which has been ascribed to a PET nature of 4-(*N*-methylpiperazinyl)-1,8-naphthalimide donor in this system. The fluorescence enhancement of dyad **1** in water-DMF (4:1, v/v) between *ca.* pH 9 and 2 was remarkable (FE = 43.37). These changes, attributed to the protonation of the piperazine amine receptor, are of such magnitude that they can be considered as representing two different “off-on” states. This indicates the high potential of the novel wavelength-shifting chromophore **1** as efficient pH chemosensing material.

1. Introduction

Molecular systems capable of light harvesting and efficiently transfer of absorbed radiation unidirectionally over nanometer distances are currently of great interest. Fluorescence resonance energy transfer (FRET) is a distance-dependent interaction in the 1–10 nm range between the electronic excited states of two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule without emission of a photon [1,2]. The efficiency of FRET is dependent on the inverse sixth power of the intermolecular separation [3], making it useful over distances comparable with the dimensions of biological macromolecules. Thus, FRET is an important technique for investigating a variety of biological phenomena that produce changes in molecular proximity [4,5]. Most of FRET applications are focused on recognizing biomacromolecules [6–8], and only few FRET-based probes have been used to monitor low-weight molecular species in living samples [9–14].

Among the different fluorescent probes, we were interested in developing new wavelength-shifting bichromophores with fluorescence sensing properties, based on Rhodamine 6G and 1,8-naphthalimide. Because of their excellent fluorescence properties and good photostability, 1,8-naphthalimide dyes were used extensively in a number of areas, including chemosensing materials [15–30]. On the basis of the spirolactam (non-fluorescent) to ring-open amide (fluorescent) equilibrium of rhodamine [31], series of rhodamine-based dyes with excellent “off-on” switching of fluorescence upon encountering the correct target have been synthesized [32–43].

The photoinduced electron transfer (PET) system using the “fluorophore-spacer-receptor” format, developed by de Silva et al. [44], is one of the most popular approaches to the design of fluorescent sensors and switchers [45,46]. Recently we have synthesized new yellow–green emitting 1,8-naphthalimides, containing *N*-methylpiperazine unit in C-4 position, designed as a highly efficient fluorescence sensors for metal cations and protons [47]. Since the naphthalimide moiety can act as a good energy pumping antenna for rhodamine molecules [48], our efforts were directed towards design and synthesis of a novel fluorescence sensing dyad comprising a Rhodamine 6G acceptor and a 1,8-naphthalimide donor, C-4 substituted with *N*-methylpiperazine moiety as a cation receptor. Thus we expected the distinguishing features of FRET wavelength-shifting chromophores that affords simultaneous recording of two emission intensities at different wavelengths in the presence and absence of analyte and provides a facile method for visualizing complex biological processes at the molecular level to be combined with the properties of classical PET systems.

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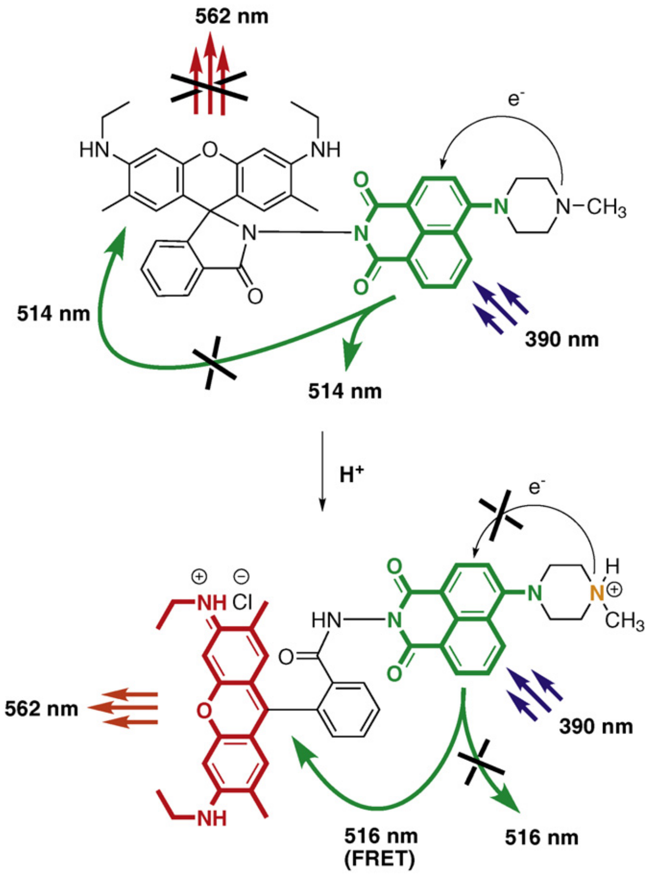

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Scheme 1.

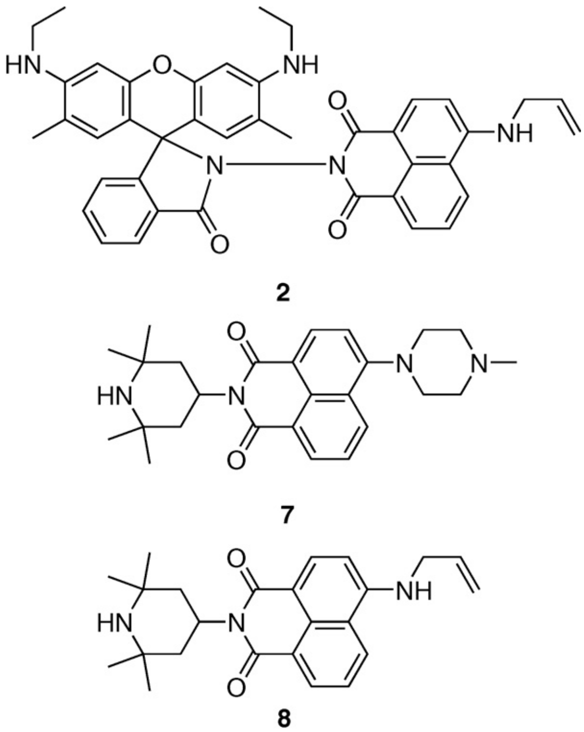
This paper takes on added significance given the growing body of sensors and other optical devices which employ 1,8-naphthalimide and rhodamine fluorophores. Hence, the novel bichromophoric FRET system **1** – modified Rhodamine 6G with 1,8-naphthalimide antenna (Scheme 1) – was synthesized and investigated by electronic absorption and emission spectroscopy as a potential PET sensor for cations.

In order to receive a more complete comparative picture for the influence of the *N*-methylpiperazine moiety on the photophysical properties of a bichromophoric system **1**, a newly synthesized dyad **2**, not containing a *N*-methylpiperazine fragment, as well as the previously synthesized 4-(*N*-methylpiperazinyl)-1,8-naphthalimide **7** [47] and 4-allylamino-1,8-naphthalimide **8** [49], were involved in the present study as reference compounds (Scheme 2).

2. Experimental

2.1. Materials

The starting 4-nitro-1,8-naphthalic anhydride **5** [25] and reference 1,8-naphthalimides **7** [47] and **8** [49] were prepared according to the reported procedure. Commercially available Rhodamine 6G **3**, hydrazine monohydrate (>98%), allyl amine and *N*-methylpiperazine (Aldrich, Merck) were used without purification. All solvents (Fluka, Merck) were pure or of spectroscopy grade. To adjust the pH, very small volumes of sulphuric acid and sodium hydroxide were used. Thin layer chromatography (TLC) was performed using silica gel plates, Merck 60 F254, 20 × 20, 0.2 mm.



Scheme 2.

2.2. Methods

IR spectra were recorded on a Specord 71 IR spectrometer using KBr pellets. The ^{1}H NMR spectra in $CDCl_3$ were recorded on a Bruker DRX-250 spectrometer, operating at 250.13 MHz. The chemical shifts (given as δ in ppm) were referenced to tetramethylsilane (TMS) standard. The UV–vis spectra were recorded on a Hewlett Packard 8452A spectrophotometer with 2 nm resolution at room temperature. The corrected excitation and fluorescence spectra were taken on a Perkin Elmer LS55s spectrofluorimeter. The melting points were determined by means of a Kofler melting point microscope. The fluorescence quantum yields (Q_F) were measured relatively to Rhodamine 6G ($Q_{ref} = 0.95$ in ethanol [50]) or Coumarin 6 ($Q_{ref} = 0.78$ in ethanol [51]) as standards.

2.3. Syntheses

2.3.1. Synthesis of Rhodamine 6G hydrazide (4)

To a solution of Rhodamine 6G (2.4 g, 5 mmol) in 40 ml of absolute ethyl alcohol, 1.46 ml of hydrazine monohydrate (30 mmol, $d = 1.032$) was added drop wise at room temperature over a period of 30 min. The resulting solution was stirred at reflux for 5 h. After cooling to room temperature the solid precipitated was filtered off, washed with water and dried to give 1.91 g (89%) of **4** as pale pink crystals ($R_f = 0.57$ in a solvent system: toluene:ethylacetate:ethanol = 10:2.5:1).

IR (KBr) cm^{-1}: 3280, 3204 ((νNH and νNH_2); 2920, 2810 (νCH); 1662 (νC=O); 1600, 1568 and 1496 (νAr=CH). ^{1}H NMR ($CDCl_3$-*d*, 250.13 MHz) ppm: 7.96 (m, 1H, 9-Ph H-3); 7.45 (m, 2H, 9-Ph H-4 and 9-Ph H-5); 7.06 (m, 1H, 9-Ph H-6); 6.39 (s, 2H, Rhodamine H-4 and H-5); 6.26 (s, 2H, Rhodamine H-1 and H-8); 3.58 (s, 2H, NH_2); 3.52 (br.s, 2H, 2 × NH); 3.21(q, 4H, $J = 3.5$ Hz, $2 \times \underline{CH_2}CH_3$); 1.92 (s, 6H, $2 \times Ar{-}CH_3$); 1.32 (t, 6H, $J = 7.1$ Hz, $2 \times CH_2\underline{CH_3}$).