

# An investigation of energy transfer between coumarin 35 and xanthene derivatives in liquid medium

Mahmut TOPRAK and Mustafa ARIK\*

*Faculty of Sciences, Department of Chemistry, Atatürk University,  
25240 Erzurum-TURKEY  
e-mail: marik@atauni.edu.tr*

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The energy transfer between coumarin 35 (C35) and pyronin compounds, which are xanthene derivatives, i.e. pyronin B (PyB) and pyronin Y (PyY), in methanol was investigated at room temperature by using steady-state absorption, emission, and time-resolved fluorescence spectroscopy. Fluorescence energy transfer rate constants ( $k_T$ ) and critical radius ( $R_0$ ) were determined for C35-PyB and C35-PyY molecular pairs in methanol. The obtained values of  $k_T$  and  $R_0$  indicated that the dipole-dipole interaction between C35-PyB and C35-PyY molecular pairs accounted for the energy transfer mechanism. The energy transfer efficiency and the distance between the donor and acceptor ( $r$ ) were also calculated for donor-acceptor pairs using Förster's theory.

**Key Words:** Fluorescence energy transfer; coumarin 35, pyronin compounds, fluorescence intensity quenching.

## Introduction

Coumarin derivatives are investigated due to their importance as laser dyes whose photophysical properties are dependent on molecular structure and surrounding medium.<sup>1,2</sup> Several coumarin derivatives are biologically important and used as fluorescent probes,<sup>3</sup> sensitizers for photoprocesses,<sup>4,5</sup> and anticoagulants.<sup>6</sup> Pyronin B and pyronin Y are xanthene derivatives that are sensitive to molecular environments and used as active media in dye lasers and in biological systems. Therefore, the photophysical properties of coumarin and pyronin compounds have been studied extensively in different media.<sup>7-19</sup>

The fluorescence resonance energy transfer (FRET) of some coumarin compounds has also been reported in some previous studies.<sup>20-27</sup> FRET is an important physical technique for biological systems.<sup>28</sup> FRET is

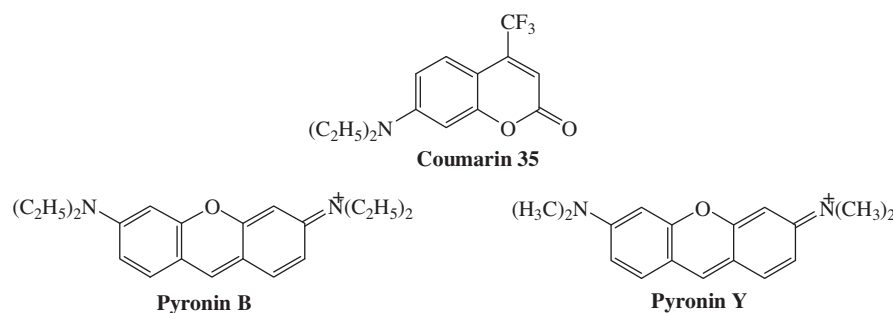
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\*Corresponding author

used to study protein folding and to examine distances between fluorescent tags to determine structural and conformational properties of proteins.<sup>29–31</sup> The fluorescence energy transfer has been discussed in detail by Förster.<sup>32</sup> According to Förster's theory, the rate of energy transfer is based on the overlap of the emission spectrum of the donor and the absorption spectrum of the acceptor, relative orientation of the donor and acceptor transition dipoles, and the quantum yield of the donor.<sup>28,32,33</sup> Although the photophysical properties of coumarin 35 (C35), pyronin B (PyB), and pyronin Y (PyY) have been investigated in different media, the energy transfer process between C35 and pyronin compounds in liquid medium has not been investigated. In this study, we report on the energy transfer from the fluorescent donor compound C35 to acceptors PyB and PyY in methanol.

## Experimental

Pyronin B, pyronin Y, and coumarin 35 (the molecular structures are shown in the Scheme) were purchased from Sigma and used without further purification. Methanol was purchased from Fluka. C35, PyB, and PyY were stored in the dark as concentrated stock solutions of 1.0 mM in methanol. Absorption spectra of the samples were recorded with a Shimadzu UV-3101PC UV–VIS–NIR spectrophotometer and fluorescence spectra were recorded with a Shimadzu RF-5301PC spectrofluorophotometer. The temperature of the samples was controlled with a Grant W14 circulating water bath during the absorption and fluorescence measurements.



**Scheme**

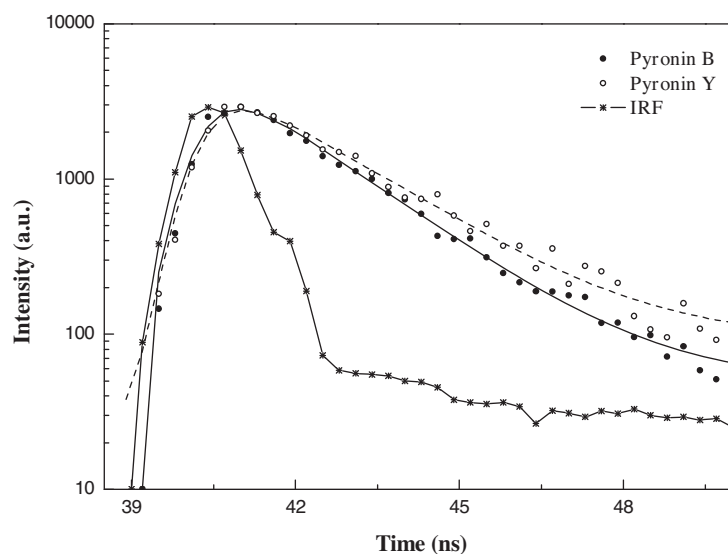
To determine fluorescence lifetime values, fluorescence decays were measured with a LaserStrobe Model TM-3 lifetime fluorometer from Photon Technology International. A more detailed description of the method is given elsewhere.<sup>17</sup> For instance, the fluorescence decays of PyB and PyY in methanol are given in Figure 1.

Fluorescence quantum yields ( $\Phi_f$ ) were determined by comparison with a reference solution. For this purpose, the following relation was used to calculate the fluorescence quantum yields:<sup>34</sup>

$$\Phi_s = \Phi_r \left( \frac{D_s}{D_r} \right) \left( \frac{n_s}{n_r} \right)^2 \left( \frac{1 - 10^{-OD_r}}{1 - 10^{-OD_s}} \right)$$

where  $D_s$  and  $D_r$  are the integrated area under the corrected fluorescence spectra for the sample and reference, and  $n_s$  and  $n_r$  are the refractive indices of the sample and reference, respectively.  $OD_s$  and  $OD_r$  are the optical densities for the sample and reference at the excitation wavelength, respectively. The reference used in

this study for quantum yield determination is quinine sulfate in 0.1 N sulfuric acid solution. This reference has a known fluorescence quantum yield of 0.55.<sup>35</sup>



**Figure 1.** Fluorescence decays for PyB and PyY in methanol. IRF: the instrument response.

The spectral data, quantum yield, and lifetime ( $\tau_f$ ) values are listed in Table 1 for C35, PyB, and PyY in methanol.

**Table 1.** Photophysical and spectral properties of C35, PyY and PyB in methanol.

Compound	$\lambda_{abs}$ (nm)	$\lambda_{fl}$ (nm)	$\tau_f$ (ns)	$\phi_f$
C35	400	505	$0.18 \pm 0.05$	$0.19 \pm 0.02$
PyB	553	572	$1.58 \pm 0.05$	$0.44 \pm 0.01$
PyY	547	568	$1.77 \pm 0.01$	$0.52 \pm 0.01$

## Results and discussion

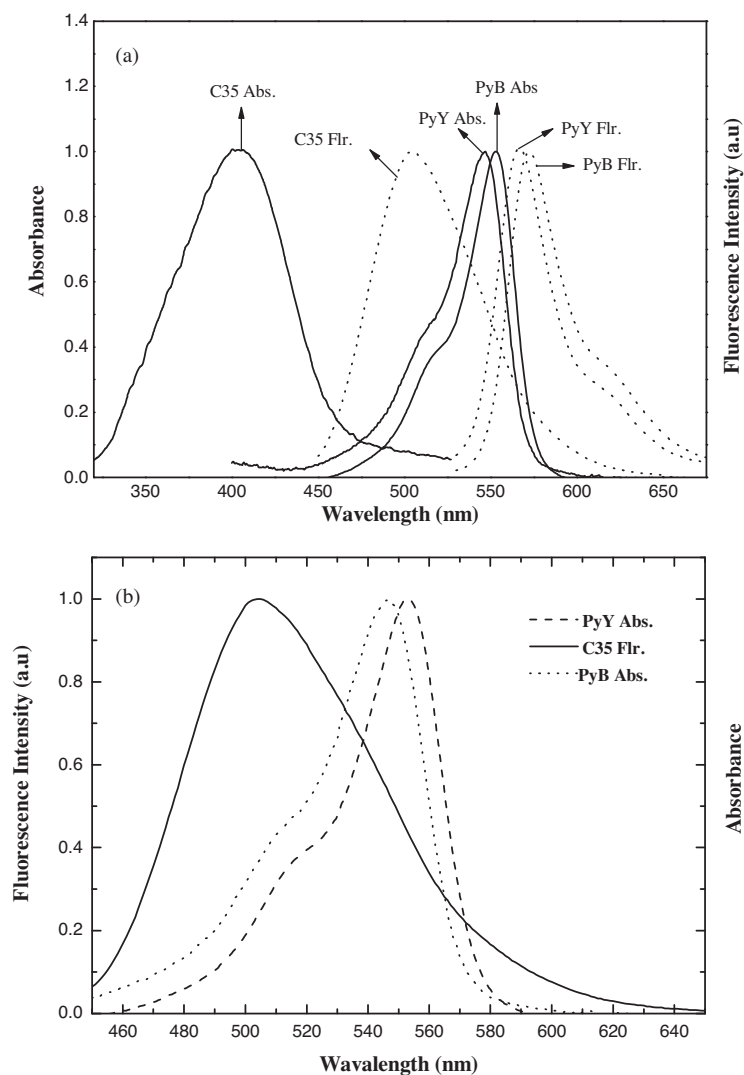
The electronic absorption and emission spectra of C35, PyB, and PyY in methanol are shown in Figure 2a. Figure 2b shows the donor emission and acceptor absorption spectra used to calculate spectral overlap integral of C35-PyB and C35-PyY molecular pairs. The spectral overlap integral ( $J$ ) was calculated by using the following formula:<sup>33</sup>

$$J = \frac{\int F_D(\bar{\nu})\varepsilon_A(\bar{\nu})\bar{\nu}^{-4}d\bar{\nu}}{\int F_D(\bar{\nu})d\bar{\nu}} \quad (1)$$

where  $\bar{\nu}$  is the wave number,  $F_D$  is the spectral distribution of donor normalized to unity, and  $\int F_D(\bar{\nu})d\bar{\nu}$  is taken to be equal to 1. Table 2 lists the values of the spectral overlap integral ( $J_{DA}$ ) for C35-PyY and C35-PyB pairs. The high value of  $J$  is not always an indicator that the highest value of the energy transfer efficiency ( $E$ ) and the rate constant of fluorescence energy transfer ( $k_T$ ) will be observed.

**Table 2.** Parameters of fluorescence energy transfer in methanol.

	$J_{DA} \times 10^{-13} \text{ (M}^{-1} \text{ cm}^3\text{)}$	$K_{SV} \times 10^4 \text{ (M}^{-1}\text{)}$	$k_T \times 10^{13} \text{ (M}^{-1} \text{ s}^{-1}\text{)}$	$R_0 \text{ (\AA)}$	$r \text{ (\AA)}$	E
C35-PyY	1.31	10.6	55.8	38.25	44.67	0.19
C35-PyB	2.50	11.5	60.5	42.60	51.48	0.18

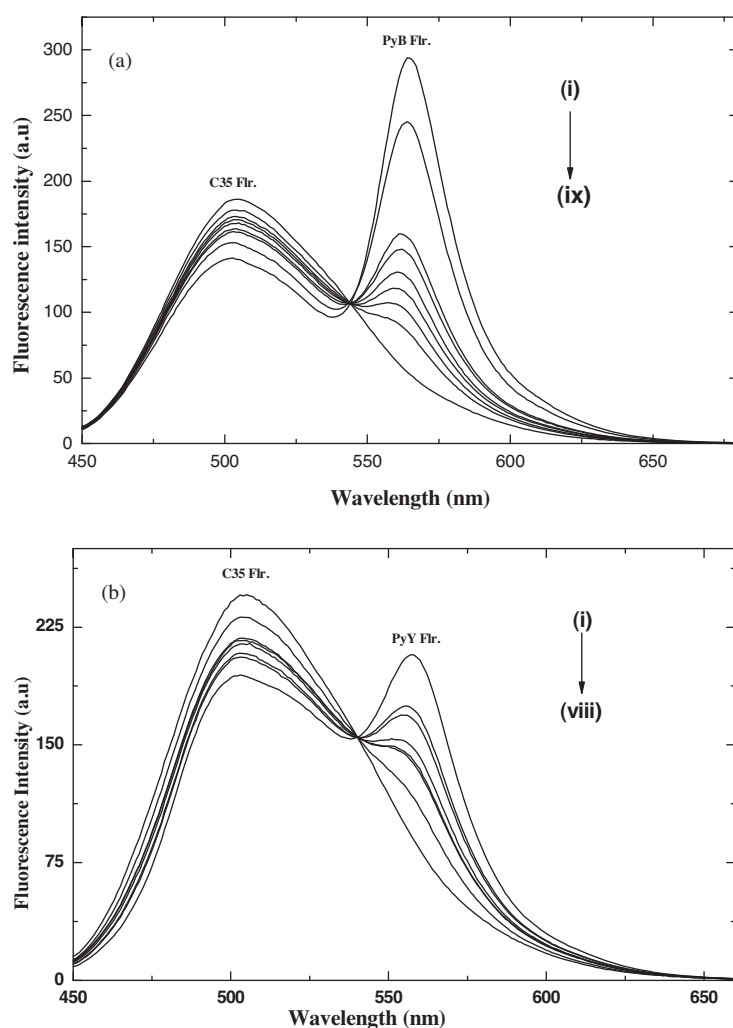


**Figure 2.** (a) Normalized absorption and emission spectra of C35, PyB, and PyY in methanol. (b) Spectral overlap for C35-PyY and C35-PyB molecular pairs in methanol.

$R_0$  is an important parameter in the Förster model and is known as the critical distance when the energy transfer efficiency is 50%.  $R_0$  is calculated according the following equation:

$$R_0 = 9.79 \times 10^3 [\kappa^2 n^{-4} \Phi_D J]^{1/6} \text{ (\AA)} \quad (2)$$

where  $\kappa^2$  is the orientation factor determined by the angle between the donor and acceptor dipoles and is equal to  $2/3$  for isotropic media.  $\Phi_D$  is the fluorescence quantum yield of the donor in the absence of the acceptor and  $n$  is the refractive index of the solvent.  $R_0$  is the average distance between donor and acceptor molecule at which the probability of energy transfer equals the probabilities of the de-excitation process of the excited state donor. Table 2 summarizes the values of  $R_0$  for C35-PyY and C35-PyB molecular pairs in methanol. These values indicate that the mechanism responsible for energy transfer results from the long-range dipole-dipole interactions between excited donor and ground-state acceptor molecules, which are considerably greater than those normally obtained for collisional energy transfer in which  $R_0$  value is in the range of 4-6 Å.<sup>36-38</sup>



**Figure 3.** (a). Steady-state fluorescence intensity quenching spectra of C35 with varying PyB concentration in methanol: (i) 0  $\mu\text{M}$ , (ii) 2.0  $\mu\text{M}$ , (iii) 3.0  $\mu\text{M}$  (iv) 4.0  $\mu\text{M}$ , (v) 5.0  $\mu\text{M}$ , (vi) 6.0  $\mu\text{M}$ , (vii) 8.0  $\mu\text{M}$ , (viii) 12.0  $\mu\text{M}$ , (ix) 20.0  $\mu\text{M}$ . (b) Steady-state fluorescence intensity quenching spectra of C35 with varying PyY concentrations in methanol: (i) 0  $\mu\text{M}$ , (ii) 4.0  $\mu\text{M}$ , (iii) 6.0  $\mu\text{M}$  (iv) 8.0  $\mu\text{M}$ , (v) 10.0  $\mu\text{M}$ , (vi) 14.0  $\mu\text{M}$ , (vii) 16.0  $\mu\text{M}$ , (viii) 20.0  $\mu\text{M}$ .

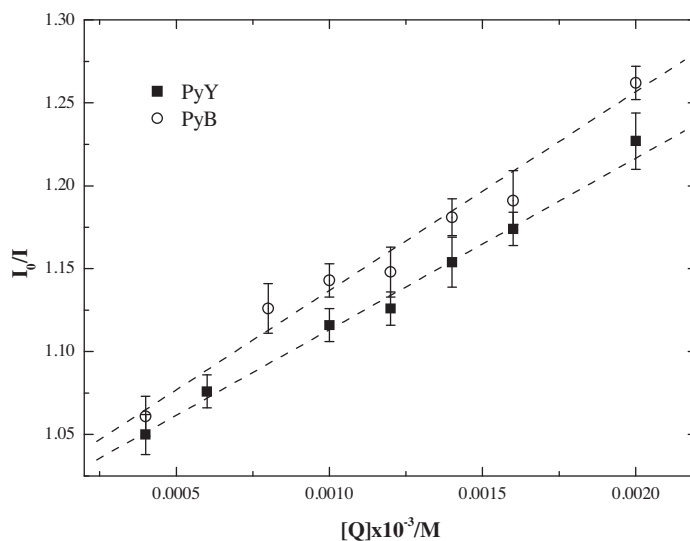
Figure 3 shows the fluorescence intensity quenching of C35 with different concentrations of PyB and PyY in methanol. During the measurements, concentration of C35 was kept constant at  $1.0 \times 10^{-6}$  M. The fluorescence intensity of C35 decreased when the quencher, PyB, concentration increased in the solution. In this case, the fluorescence intensity of PyB started to increase with an emission maximum around 568 nm. A similar observation was also obtained for the PyY quencher used in solution. An isosbestic point was observed at 544 nm and 540 nm for C35-PyB and C35-PyY molecular pairs in methanol, respectively, which is evidence for the absence of exciplex formation between the excited donor and ground state acceptor.

Förster demonstrated that the energy transfer can be regarded as a bimolecular process. The rate constant of fluorescence energy transfer ( $k_T$ ) can be calculated by using the Stern-Volmer (SV) relation:<sup>33</sup>

$$\frac{I_0}{I} = 1 + K_{SV}[Q] = 1 + k_T\tau_D[Q] \quad (3)$$

where  $I_0$  and  $I$  are the fluorescence intensities of the donor in the absence and in the presence of acceptor, respectively.  $[Q]$  is the acceptor concentration,  $\tau_D$  is the fluorescence lifetime of the donor, and  $K_{SV}$  is the quenching rate constant.

A plot of  $I_0/I$  vs.  $[Q]$  should yield a straight line with a slope having  $K_{SV}$ . Figure 4 shows the SV plots of fluorescence intensity quenching of C35 by using PyB and PyY quenchers in methanol. The values of  $K_{SV}$  and  $k_T$  for C35-PyB and C35-PyY molecular pairs are listed in Table 2. The lifetime value of the donor in Table 1 was used to determine  $k_T$  values.

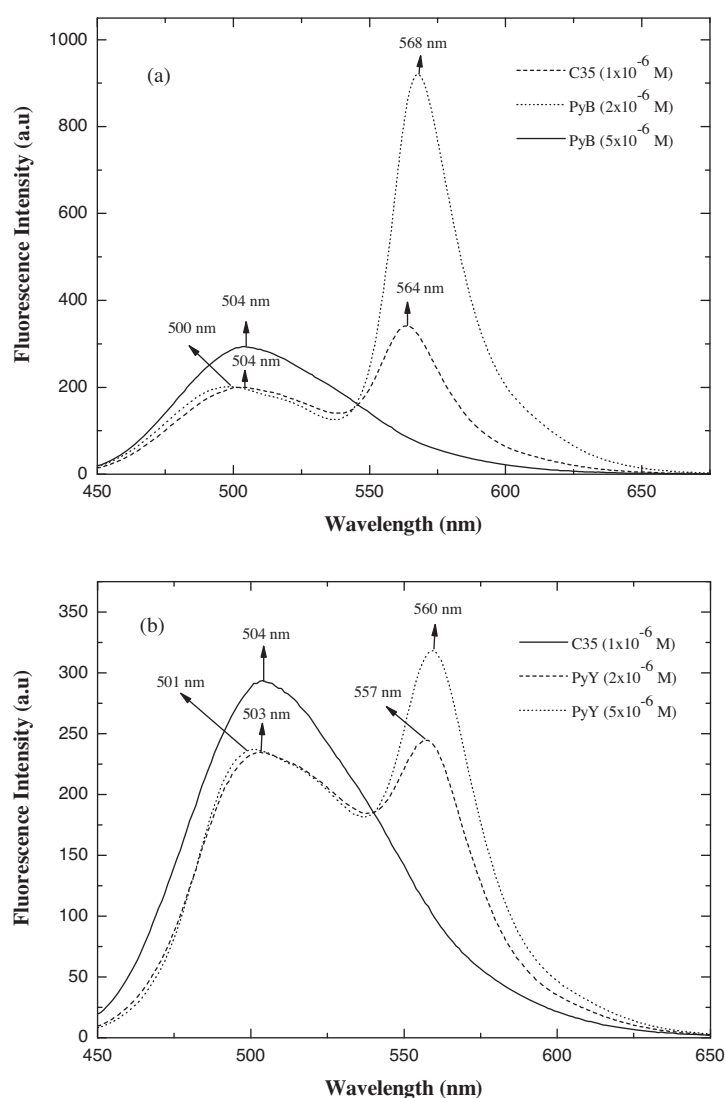


**Figure 4.** The Stern-Volmer plots of fluorescence intensity quenching of C35 by PyY and PyB.

Another parameter useful in energy transfer is the energy transfer efficiency, which is expressed as <sup>32,33</sup>

$$E = 1 - \frac{\tau_{DA}}{\tau_D} = 1 - \frac{I_{DA}}{I_D} \quad (4)$$

where  $I_{DA}$  and  $I_D$  are the donor fluorescence intensities in the presence and in the absence of acceptor, and  $\tau_{DA}$  and  $\tau_D$  are the donor lifetimes in the presence and in the absence of acceptor, respectively.



**Figure 5.** (a) Steady-state fluorescence intensity quenching spectra of C35 with varying PyB concentrations in methanol: 0  $\mu\text{M}$ , 20.0  $\mu\text{M}$ , 50.0  $\mu\text{M}$ . (b) Steady-state fluorescence intensity quenching spectra of C35 with varying PyY concentrations in methanol: 0  $\mu\text{M}$ , 20.0  $\mu\text{M}$ , 50.0  $\mu\text{M}$ .

The distance between the donor and acceptor ( $r$ ) can be calculated from the value of  $E$  and  $R_0$ :

$$E = \frac{R_0^6}{R_0^6 + r^6} \quad (5)$$

All energy transfer parameters calculated for the C35-PyB and C35-PyY molecular pairs in methanol are reported in Table 2. The high values of  $K_{SV}$ ,  $k_T$  and  $R_0$  in Table 2 indicate that the fluorescence energy transfer between molecular pairs investigated in this study results from the long-range dipole-dipole interactions between the excited donor and the ground state acceptor molecules in methanol. Das et al. studied the FRET from TX-100 to 3-acetyl-4-oxo-6,7-dihydro-12 H-indolo-[2,3-a] quinolizine in micellar medium and determined

high values of Stern-Volmer constant and energy transfer efficiency (E) and that a long-range dipole-dipole interaction is responsible for the energy transfer mechanism.<sup>39</sup> When PyB and PyY concentrations exceeded certain values, the energy transfer efficiency declined. In addition, a red shift was observed in the emission spectrum of the acceptor as its concentration increased (Figure 5). This can be attributed to the reabsorption and radiative migration. Moreover, a blue shift was observed for the donor fluorescence spectrum with increasing acceptor concentration, which could be attributed to the radiative transfer.<sup>40</sup>

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