

# FRET between cyanine dyes in nanopores of bulk sol-gel silica matrix

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Two cyanine dyes have been embedded into SiO<sub>2</sub> sol-gel matrix with 10–20 nm pores using impregnation method. Forster resonance energy transfer (FRET) between the dyes has been implemented. Its efficiency has been revealed to be strongly dependent on dyes embedding sequence.

Два цианиновых красителя введены в SiO<sub>2</sub> золь-гель матрицу с 10–20 нм порами при помощи метода пропитки. Между данными красителями реализован ферстеровский резонансный перенос энергии. Показано, что его эффективность в значительной мере зависит от последовательности пропитки красителями.

## 1. Introduction

The sol-gel technology by which inorganic or composite organic-inorganic materials are obtained at relatively low temperature becomes very popular for production of large number of sophisticated materials [1]. Using this technology, it is possible to obtain glassy highly-porous films or bulk matrices which allow incorporation of organic and inorganic additives [1–5]. The first successful organic dye incorporation in a sol-gel matrix was reported in 1984 [3], followed by a number of successful applications of composite organic-inorganic materials obtained, such as solar concentrators, tunable lasers, and active waveguides [1–4]. The major reason for this is a significant increase in photostability of organic luminophores due to their stabilization within pores of the sol-gel matrix [6].

Cyanine dyes seem to be very promising additives for luminescent solar concentrators (LSC) based on the sol-gel matrices due to significant extinction coefficients, high luminescent quantum yield and efficient fluorescence resonance excitation energy transfer (FRET) [7, 8]. Indeed, FRET based LSC with two or three fluorophores ap-

peared to be much more efficient as compared with LSC with one fluorophore [9]. Usually, cyanine dyes are introduced into the sol-gel matrices on the synthesis stage prior to the start of polycondensation [10]. However, the matrices obtained in such a way could not be thermally treated [10], so they reveal significant water content and large pores [5]. Another way is to load dyes into pores after completion of the sol-gel matrix formation. Using the latter method, one can prepare the required matrix and embed a dye into it after that.

In the present article we report on peculiar characteristics of two cyanine dyes that are a good FRET pair [8], with interaction in nanopores of SiO<sub>2</sub> bulk sol-gel matrices. DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (Fig. 1, inset) was chosen as energy donor and DiD (1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate (Fig. 1, inset) was chosen as energy acceptor. The dyes are nearly similar in chemical structure and differ only by the polymethine bridge length: trimethine (DiI) and pentamethine (DiD). This results in spectral shift towards longer wavelengths of DiD absorption and lumines-

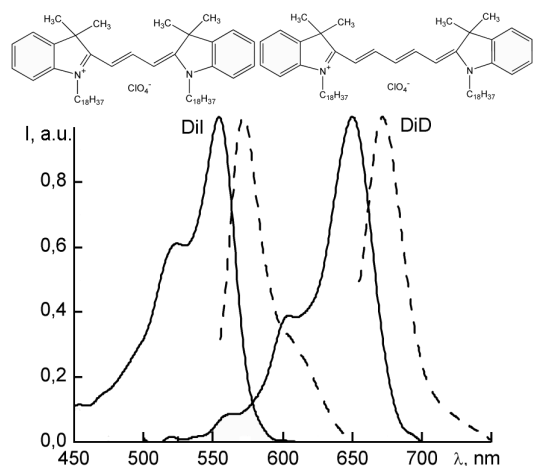


Fig. 1. Absorption (solid lines) and luminescence (dashed lines) spectra of DiI and DiD in DMF. On inset — structural formula of the dyes.

cence bands (Fig. 1). It should be noted that both dyes are characterized by extremely high extinction coefficients ( $\epsilon_{\text{DiI}} = 148\,000\text{ M}^{-1}\text{cm}^{-1}$  (in MeOH) and  $\epsilon_{\text{DiD}} = 260\,000\text{ M}^{-1}\text{cm}^{-1}$  (in MeOH)) and high luminescence quantum yields [11].

## 2. Experimental

The synthesis of  $\text{SiO}_2$  sol-gel matrices was described in [5]. Using this method of synthesis as a result, we obtained disc-shaped  $\text{SiO}_2$  transparent plates with  $\sim 2$  cm diameter and 1 mm thickness. After polycondensation, the matrices were annealed at  $750^\circ\text{C}$ . To compare different procedures of dye filling on the same matrix, each matrix was divided into several pieces. DiI and DiD dyes were obtained from Sigma-Aldrich (USA) and used as received. Each dye was dissolved in chloroform at concentration  $5 \times 10^{-5}$  M. The same total concentration was preserved in the dye mixtures. To introduce dyes into  $\text{SiO}_2$  matrix, its pieces were placed into dye solution for at least three hours. After that, the samples were dried at temperature  $60^\circ\text{C}$ .

Luminescence spectra were recorded using a spectrofluorimeter based on two grating monochromators MDR-23 and xenon lamp. One of the monochromators was used to select a required wavelength (FWHM  $\sim 1$  nm), the other one was used for the luminescence collection. For luminescence measurement  $\lambda_{\text{exc}} = 480$  nm was been used. Absorption spectra were recorded using a microspectrometer USB4000 (Ocean Optics, USA) equipped with an incandescent lamp. Quartz cuvettes with optical pathway of

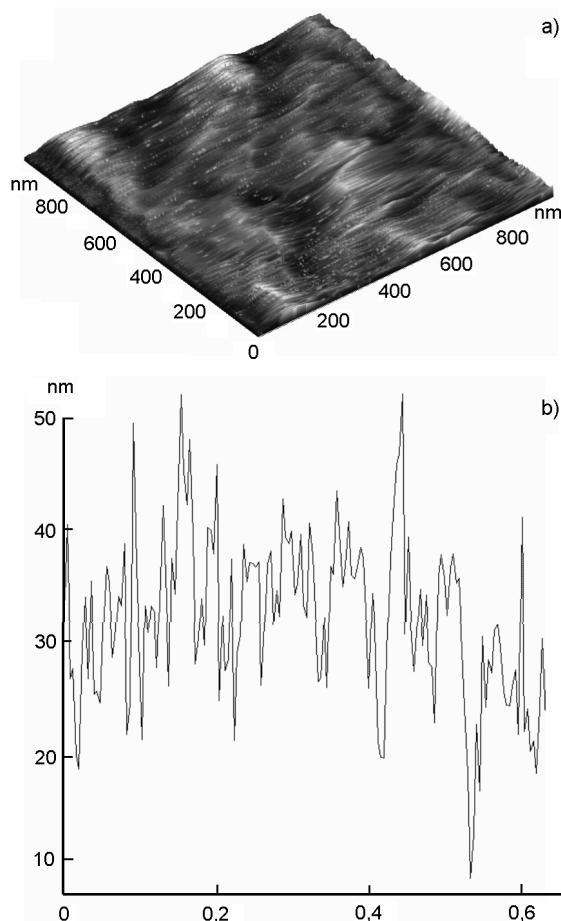


Fig. 2. AFM image (a) and surface profile (b) of sol-gel  $\text{SiO}_2$  matrix.

1 mm were used for measurements in liquid samples. For AFM measurements, a Scanning Probe Microscope Solver Pro-M (NT-MDT, Russia) was used.

## 3. Results and discussion

It is well known that for efficient FRET the distance between donor and acceptor molecules should be small [12]. So, only pores with pores of very small diameter are suitable for FRET realization. Using atomic-force microscopy (AFM), high-resolution images of the sol-gel matrix surface (Fig. 2a) and its profile (Fig. 2b) have been obtained. Thus we could conclude that diameter of the pores in  $\text{SiO}_2$  matrices obtained was about 10–20 nm, that is in good correlation with literature data [13]. The porosity of the matrices was estimated as  $\sim 50\%$  [5]. Such parameters were considered to be reasonable for our purpose.

Impregnation of silica matrices by dyes is strongly dependent on the solvent used. After testing of different solvents, chloro-

form was chosen due to such reasons: it is a good solvent for the dyes used, it is highly volatile and could be evaporated quickly, it demonstrates good matrix impregnation degree by the dyes ( $3 \pm 0.5$  mass.%). But the most interesting feature found is preferential entering of cyanine dyes into the matrices from chloroform. At low dye concentration corresponding to  $\sim 3$  mass.% of the immersed piece, almost the whole of the dye could be entered into matrix while chloroform became colorless. So,  $\text{SiO}_2$  matrices could be considered as better "solvent" compared with chloroform. To prove it we dried the dyed matrix for three hours at  $60^\circ\text{C}$  up to constant matrix mass when the whole of chloroform is evaporated and put it into pure chloroform. Surprisingly, chloroform remains colorless even after 12 h of dyed matrix staying in it. For comparison, dimethylformamide solution within 1 h get the same color as the dyed matrix, i.e. the dye freely leaves the matrix and dissolves in it.

To find out the best dye entering conditions for effective FRET realization, different sequences of matrix saturation by the dyes were used. In all cases the stock dye solution was concentrated enough for maximum saturation of the silica matrix piece put in it. This leads to strong absorption of dyed matrix with optical density  $D > 2$  (not shown) unresolved with our experimental setup. First of all dye mixture Dil/DiD = 1:1 was tested. For luminescence spectra collection  $\lambda_{exc} = 480$  nm was chosen due to nearly full absence of DiD luminescence (Fig. 3b) but rather efficient Dil luminescence excitation. The luminescence spectrum revealed a weak DiD (acceptor) band, compared with intense Dil (donor) band (Fig. 3a). Luminescent excitation spectrum of DiD for Dil/DiD mixture demonstrated intense band corresponding to Dil absorption (Fig. 1). So, it could be concluded that DiD luminescence appears as a result of FRET, but its efficiency is rather small.

To enhance FRET efficiency, the procedure of matrix saturation by the dyes was changed. At first, silica matrix was put into solution of Dil or DiD for three hours. After saturation with one of the dyes, silica matrix was dried for half an hour and put into solution of the other dye. Finally, after saturation with the second dye, the silica matrix was dried for three hours for fixation of the dyes in the matrix. Surprisingly, such step-by-step dye saturation significantly increased FRET efficiency for both

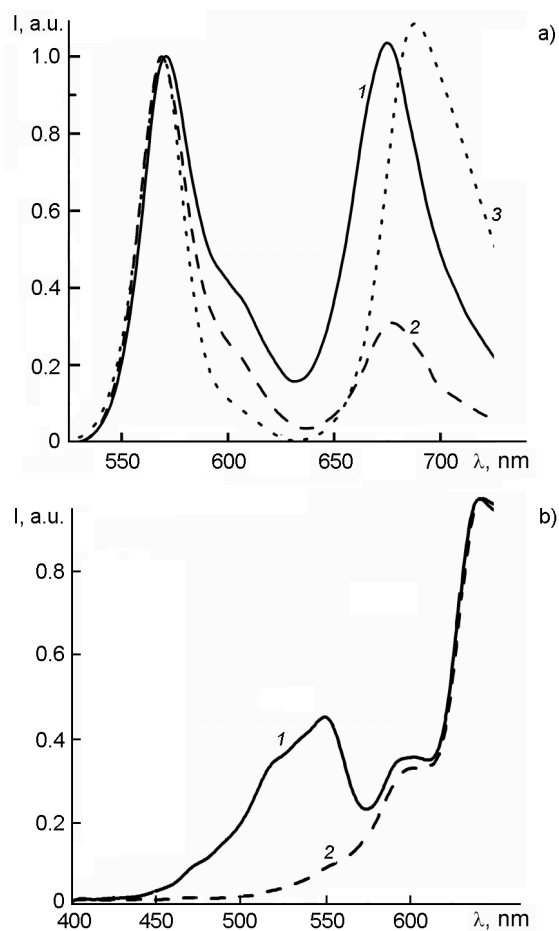


Fig. 3. (a) Luminescence spectra ( $\lambda_{exc} = 480$  nm) of silica matrix colored by both dyes: 1 — sequence Dil  $\rightarrow$  DiD, 2 — Dil + DiD mixture, 3 — sequence DiD  $\rightarrow$  Dil; (b) DiD luminescence excitation spectra ( $\lambda_{reg} = 680$  nm): 1 — in the presence of Dil, 2 — in the absence of Dil.

sequences Dil  $\rightarrow$  DiD and DiD  $\rightarrow$  Dil (Fig. 3a). Similar change of FRET efficiency depending on dye entering sequence was observed for solutions with smaller dye concentrations ( $1 \cdot 10^{-5}$  M and  $2 \cdot 10^{-5}$  M, Fig. 4). In all cases the best FRET efficiency is observed for the sequence Dil  $\rightarrow$  DiD (Fig. 3a and 4). Note that DiD luminescence band in Fig. 3a is red-shifted ( $\lambda_{max} = 688$  nm) for the case Dil  $\rightarrow$  DiD as compared with DiD  $\rightarrow$  Dil and DiD + Dil (mixture) cases ( $\lambda_{max} = 675$  nm). It could be explained by quenching due to high DiD concentration as in the case of FRET between these dyes solubilized by surfactant micelles [8].

To understand luminescence spectra, an analysis of absorption spectra is helpful. Unfortunately, the latter is unavailable in the case of dyed matrix due to excessively

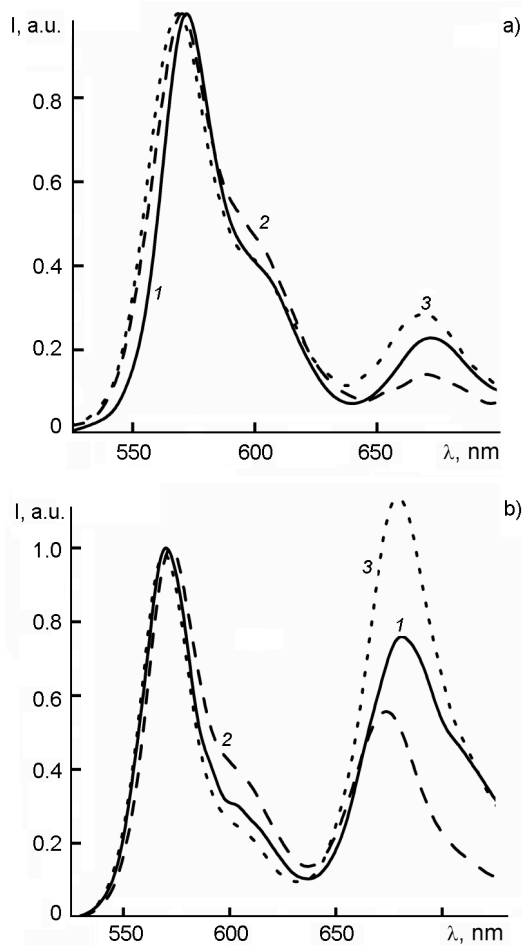


Fig. 4. Luminescence spectra ( $\lambda_{exc} = 480$  nm) of silica matrix colored by both dyes at lower dyes concentrations in stock solution: (a)  $C_{dyes} = 1 \cdot 10^{-5}$  M and (b)  $C_{dyes} = 2 \cdot 10^{-5}$  M. Labeling: 1 — sequence DiI·DiD, 2 — DiI + DiD mixture, 3 — sequence DiD → DiI.

strong absorption. But we could use the spectra of the dye solutions before and after matrix saturation. Two facts should be kept in mind while doing further analysis: 1) DiD has much larger extinction coefficient  $\varepsilon_{DiD}/\varepsilon_{DiI} \sim 1.76$ ; 2) DiI absorption band has almost no effect on DiD maximum, but the DiD absorption band shoulder coincides with DiI maximum (Fig. 1).

Under matrix saturation by the dye mixture both dyes entered into matrix in equal proportions, as one might expect, and absorption bands in chloroform solution decreased equally (Fig. 5a). At matrix saturation by one of the dyes, absorption maxima were decreasing with full matrix saturation (~ 3 mass.%) by the corresponding dye (Fig. 5b,c — curves 2). But the most interesting changes could be revealed under the satura-

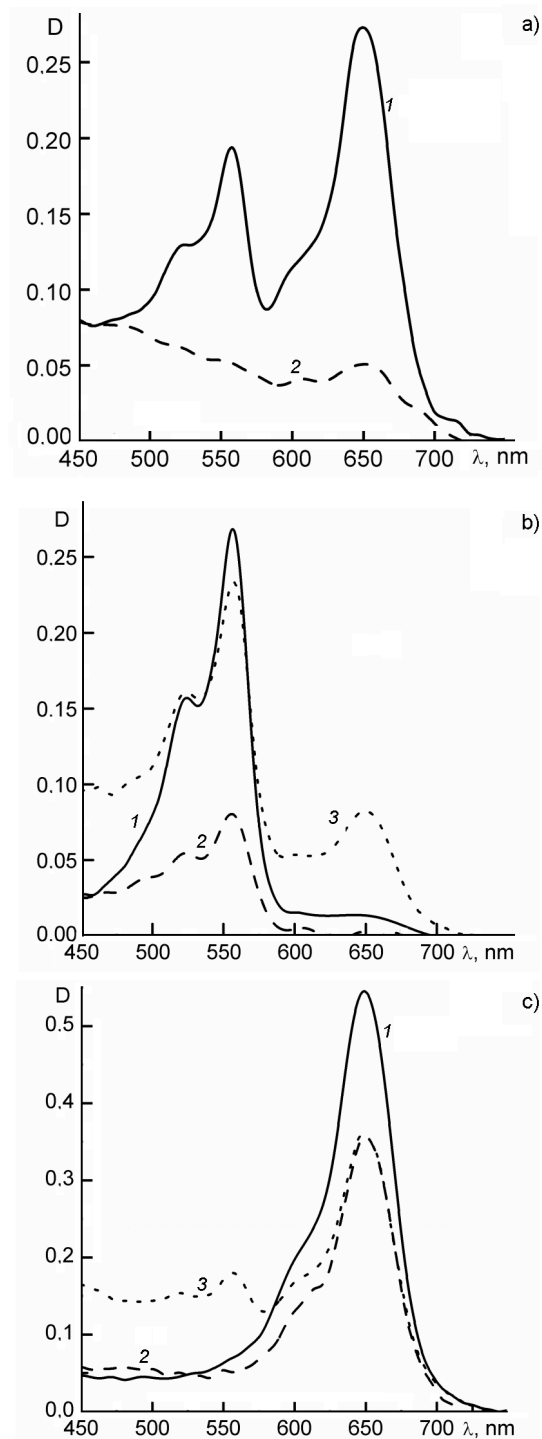


Fig. 5. Absorption spectra of the dyes chloroform solutions: (a) DiI + DiD mixture, (b) DiI, (c) DiD. Labeling: 1 — stock solution, 2 — after clear matrix impregnation, 3 — after impregnation of matrix colored by the other dye.

tion by the second dye (Fig. 5b,c — curves 3). On both absorption spectra, bands of the first dyes appear while intensities of the second dye bands decrease (Fig. 5b,c —

curves 3). Comparison of the band intensity decreasing for the second dye with intensities of the first dye bands appeared, accounting for necessary corrections, shows that they are the same within experimental accuracy. At the same time the weights of the matrix pieces remain the same after saturation by the second dye as after saturation by the first dye. Note that molecular weights of Dil and DiD dyes are almost equal. So, to enter the matrix, the second dye should displace the same amount of the first dye.

However the question still remains open whether Dil and DiD dyes concentrations are equal in the case of successive dye entering (Fig. 5b,c) as in the case of matrix saturation in the dye mixture (Fig. 5a). To answer this question we should compare absorption intensities of the first dyes entered into the matrices (Fig. 5b,c — curves 2) with intensities of the first dyes that leaved matrices under saturation by the second dyes (Fig. 5b,c — curves 3). Calculations demonstrate that in the sequence Dil → DiD only 29 % of Dil remained in matrix while other 71 % was occupied by DiD, but in the sequence DiD → Dil only 37 % of DiD was displaced by Dil. Surprisingly, but in the both cases nonuniform dye distribution is observed with Dil/DiD ratio approximately equals to 1:2. Such result could explain the FRET efficiency enhancement at successive dye entering compared to the dye mixture case (Fig. 3a). Moreover, as DiD amount in the silica matrix appeared larger in the case of Dil → DiD sequence as compared to DiD → Dil sequence, FRET in the former case appeared to be more efficient than in the latter case (Fig. 3a). But the reason for such nonuniform distribution of very similar dyes is still not clear, and further detailed studies are required.

#### 4. Conclusions

Fluorescence resonant energy transfer has been realized between two amphiphilic cyanine dyes embedded into nanopores of bulk sol-gel SiO<sub>2</sub> matrix. FRET efficiency dependence on matrix saturation sequence has been found. The weakest FRET was ob-

served at equal dye entering into matrix (donor/acceptor = 1:1). FRET efficiency increases at successive matrix saturation in sequences donor → acceptor and acceptor → donor, with the largest efficiency obtained for the former sequence. Dye entering into matrix has been analyzed using absorption spectra of the dyes remaining in the chloroform solution after the matrix impregnation. Unequal dye entering into the matrix under successive saturation (donor/acceptor = 1:2) has been found, that results in increased FRET efficiency.

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## **FRET між ціаніновими барвниками у нанопорах об'ємної силікатної золь-гель матриці**

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Два ціанінових барвника введено до  $\text{SiO}_2$  золь-гель матриці з 10–20 нм порами за допомогою методу просочення. Між даними барвниками реалізовано ферстеровський резонансний переніс енергії. Показано, що його ефективність значною мірою залежить від послідовності просочення барвниками.