

# Dynamic of the Fluorescence of the Complex Zn<sup>++</sup>/Bis-*N*-Carbazolyl-Distyrylbenzene

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The discrimination between similar concentrations of the different metal ions is one of the important roles of fluorescent sensors. Here we present the study of the fluorescence dynamic of the chromophore bis-*N*-carbazolyl-distyrylbenzene (BCDSB) in acetonitrile/water (mmol/L), doped with metal ions such as K<sup>+</sup>; Ca<sup>++</sup>; Mg<sup>++</sup>; Zn<sup>++</sup> (10 μmol/L). BCDSB has the fluorescence with λ<sub>max</sub> at 448 nm by excitation at λ<sub>exc</sub> = 378 nm, lifetime 1.089 ns; quantum yield of the fluorescence is 0.68. With continuation of irradiation fluorescence quenching has been registered for all investigated metal ions. However, in the presence of Zn<sup>++</sup> oscillation of the intensity was observed. The energy activation of the oscillation as much as 15 kcal/mol was estimated. We believe, that the specificity of the complex Zn<sup>++</sup>/BCDSB, is in an asymmetrical structure, formed via binding sites of Zn<sup>++</sup> with the electron-enriched binding sites of the BCDSB, excited in nπ\* state. This asymmetrical complex structure can cause the photoinduced structural fluctuation in the complex coordination.

**KEY WORDS:** fluorescent sensors; bis-*N*-carbazolyl-distyrylbenzene; Zn<sup>++</sup>; oscillating quenching.

## INTRODUCTION

Interactions of fluorescent molecules with metals molecules or ions [1–5] significantly change parameters of an emission. A number of fluorescent molecules are widely used because of that for metal sensing; especially some of them, which possess an ability building complexes with, metal cations. The general concept of such sensors is the control of photoinduced electron transfer (PET) pathway of fluorescent molecules by coordination to a close-shell metal ion or protonation of an amine [3]. A monitoring of PET might control the fluorescent quantum yield. The detailed analysis of the different fluorescent sensors, mechanism electron transfer is broadly viewed [1,4].

Recently a new class of metal sensing fluorescent probe becomes a subject of essential importance, namely, fluorescent metal sensing in living cells [5–9]. The main outcome of this topic is the possibility of imaging of the cellular processes through the metal sensing receptors. These fluorescent sensors required excitation with near-UV to prevent the damage of the living cells, limited range of dissociation constant (sub-nM), wide range of binding affinity, cell permeability [3]. Important to note, that in spite of an ability of primary amines to protonation at physiological pH, nitrogen-containing fluorescent probes play an important role as sensors on intracellular metal cations (see for e.g. [6]). Zinquin [2-methyl-8-*p*-toluenesulphonamido-6-quinolyloxy]-acetic acid] easily taken up by living cells is non-fluorescent until it complexes Zn [6]. It was suggested that because Zn is tightly bound in active sites of enzymes, Zinquin probably detected only the less tightly bound Zn. Two new fluorescent sensors Zinpyr-1 and Zinpyr-2 considerably increased intensity of emission in the presence of free Zn<sup>++</sup> [8], further modification of these fluorescent sensors Zinpyr-4 contained aniline species, makes the molecule less sensitive

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to protonation and decrease the PET thus diminishing the intensity of the background emission in the absence of  $Zn^{++}$  [9].

Interesting observations were done recently, where complex formation—metal cation—fluorescent label as a ligand—causes changes of another parameters of emission, e.g. redistribution of the dual emission intensity for novel zwitterions-like chromophore upon complexation with  $Ca^{++}$  [10], fluorescence red-shift as a result of the increasing intramolecular charge transfer in the fluorescent probes based on distyrylbenzene and 1,4-diphenyl 1,3 butadiene due to complex formation with  $Ca^{++}$  [11].

Here we present the novel observation—oscillating quenching of the emission of bis-*N*-carbazolyl distyrylbenzene (BCDSB) in the presence of  $Zn^{++}$ . BCDSB has the fluorescence with  $\lambda_{max}$  at 448 nm by excitation at  $\lambda_{exc} = 378$  nm, quantum yield of the fluorescence in acetonitrile is 0.68 [12]. The fluorescence quenching of BCDSB in acetonitrile/water solution (10  $\mu$ mol/L) doped with metal ions such as  $K^+$ ;  $Ca^{++}$ ;  $Mg^{++}$ ;  $Zn^{++}$  (mmol/L) was observed for all investigated solutions. However, only in the presence of  $Zn^{++}$  oscillation of intensity was registered. The energy activation of the oscillation 15 kcal/mol was estimated. Specificity of the complex  $Zn^{++}$ /BCDSB is in an asymmetrical structure, formed via binding sites of  $Zn^{++}$  with the binding sites of BCDSB, excited in  $n\pi^*$  state, and intramolecular interaction of BCDSB molecules. This asymmetrical structure of the complex, completed with solvent and water molecules, can cause photoinduced structural fluctuations in the complex coordination and as a consequence from that—oscillating quenching of BCDSB emission.

## EXPERIMENTAL

The following materials:  $ZnCl_2$ ,  $CaCl_2$ ,  $MgCl_2$ ,  $KCl$ ,  $ZnSO_3 \cdot 2H_2O$ , acetonitrile were purchased from Aldrich and were of the highest available purity. bis-*N*-carbazolyl-distyrylbenzene was synthesized regarding described procedure [12]. For investigated solutions preparation  $ZnCl_2$ ,  $CaCl_2$ ,  $MgCl_2$ ,  $KCl$  were dissolved in triply distilled water in concentration mmol/L. The solution of BCDSB in acetonitrile in concentration 10  $\mu$ mol/L was prepared separately. For the experiments conducting the mixture of water solutions of  $ZnCl_2$ ,  $CaCl_2$ ,  $MgCl_2$ ,  $KCl$  and acetonitrile solution of BCDSB were prepared thus the molar ratio between  $ZnCl_2$ ,  $CaCl_2$ ,  $MgCl_2$ ,  $KCl$  and BCDSB was 10:1.  $ZnSO_3 \cdot 2H_2O$  was dissolved in acetonitrile/water mixture (1:1) in concentration  $\mu$ mol/L. Afterwards this solution was mixed with the solution of BCDSB in acetonitrile

thus the molar ratio between  $ZnSO_3 \cdot 2H_2O$  and BCDSB molecules was 1:1.

Absorption spectra were recorded on a Shimadzu UV-3101PC scanning spectrophotometer. Excitation and PL spectra were taken on a Shimadzu RF-5301PC spectrofluorimeter. Samples were placed into 3.5-mm optical path quartz cuvette, and data were collected at right angles to the excitation beam.

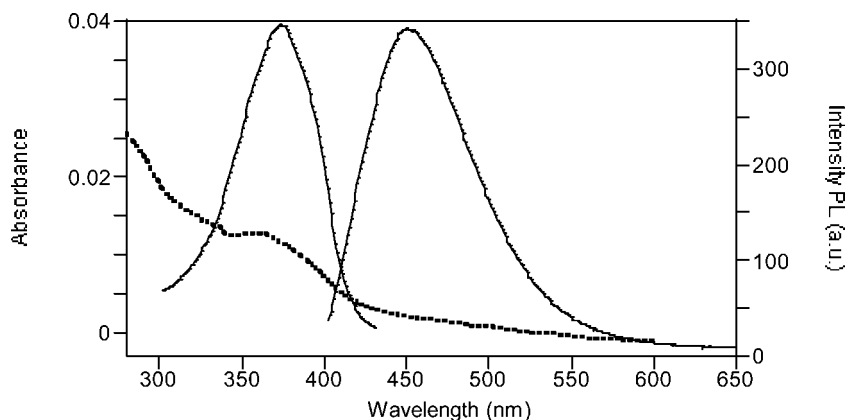
The resolution of the emission and excitation spectra was 1 nm. Quantum yields were determined by comparison of the total light emitted from the solutions to the total light emitted from a known standard quinine-sulfate solution (Aldrich) [13]. Time dependence of emission intensity on the time of the irradiation was performed by using “Time course mode” of the RF-5301 PC. This mode is used to acquire the emission intensity at the chosen wavelength over period of observation time. The stability of the light source: (Xenon lamp, USHIO) was controlled by measuring the intensity of the excited light. The intensity of the excited light had no changing during the all time of the experiments conducting. The controlled measurements of the intensity decay of emission of BCDSB in acetonitrile without addition of metal ions, demonstrate the intensity decay with a constant slope.

FluoTime 200 (PicoQuant GmbH) with a laser LDH-C-2-077  $\lambda = 410$  nm, pulse energy 21 pJ and FWHM 54 ps; repetition frequency 20 MHz with Data Analysis Software FluoFit (PicoQuant GmbH) were used for lifetime measurements. For pulse control the solution of Ludox was used. Lifetime was estimated as average value from not less than 15 measurements. The deviation for the every each measurement was in the range  $0.0675 \pm 0.005$ .

An energy activation of the photoinduced processes has been determined according the Arrhenius equation [14]. Period of time between two neighboring minima of an intensity of emission decay has been chosen as the parameter of the reaction. The temperature range was 10–40°C. The temperature cell—tempcontrol—37 (ZEISS) was used for temperature control during experiments. The molecular modeling was done with application Software Material Studio 2.2 Accelrys. The nanolayer of  $Zn^{++}$ /BCDSB was prepared on the Si substrate, Si's native oxide. For the nanofilm preparation the substrate was kept in solution overnight at room temperature, than the layer was thoroughly washed in acetonitrile and dried.

## RESULTS AND DISCUSSION

Figure 1 presents spectra absorption, excitation and emission of BCDSB in acetonitrile. Maximum of the

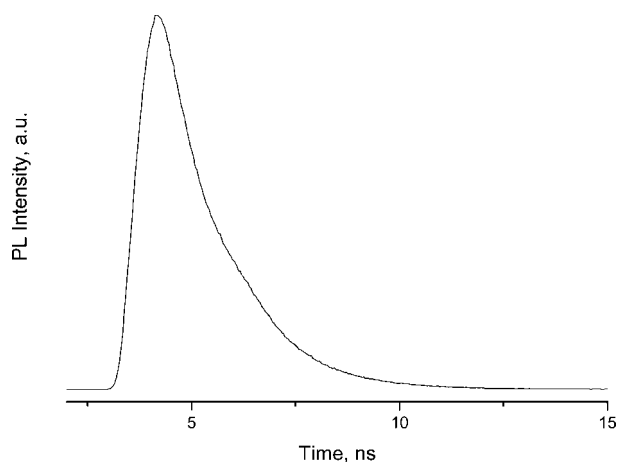


**Fig. 1.** UV-absorption (left traces, dotted line), excitation (2) spectra of the (solid line, left traces)  $\lambda_{exc} = 378$  nm, emission (solid line, right traces)  $\lambda_{em} = 448$  nm spectra of BCDSB in acetonitrile at room temperature, concentration  $10 \mu\text{mol/L}$ .

emission spectrum is located at  $\lambda_{em} = 448$  nm by excitation  $\lambda_{ex} = 378$  nm.

The maximum of the excitation spectrum strongly coincides with the maximum of the absorption spectrum of BCDSB, which means that interaction with the solvent molecules does not affect the photoprocess. The quantum yield equal 0.68 was estimated. These data are in agreement with the lifetime measurements (Fig. 2). The lifetime of the fluorescence of BCDSB 1.089 ns was found.

The presence of the metal cations does not change much maximum of the emission spectrum and lifetime, however the character of the decay of the BCDSB emission in the presence of metals ions differs. With continuation of irradiation by  $\lambda_{ex} = 378$  nm intensity of the

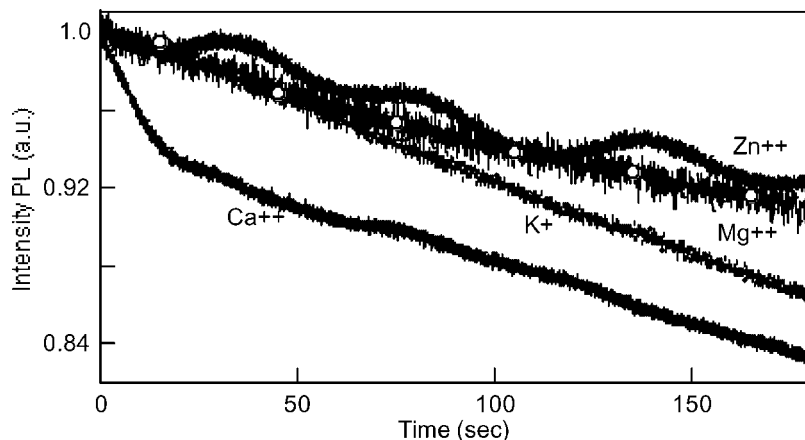


**Fig. 2.** Lifetime decay of the BCDSB emission  $\lambda_{em} = 448$  nm by excitation  $\lambda_{ex} = 410$  nm.

emission at  $\lambda_{em} = 448$  nm undergoes quenching for all investigated solutions. Figure 3 presents the time dependence of the intensity of BCDSB emission in acetonitrile/water solution doped with  $ZnCl_2$ ,  $CaCl_2$ ,  $MgCl_2$ ,  $KCl$  (decay of BCDSB emission does not shown because has similarity with the decay of the emission in the solution doped with  $MgCl_2$  and overlapped with the latter). The rate of the emission quenching has some deviations, depending on the metal cation. Nevertheless, completely different character of the emission decay is observed for the solution doped with  $ZnCl_2$ . The decay has obviously oscillating character. From our knowledge this is the first observation of the periodical manners of the decay.

We investigated the dependence of oscillating on the temperature of the solution. Figure 4 shows the decay of the emission of BCDSB in acetonitrile/water doped with  $ZnCl_2$  at  $13^\circ\text{C}$ ;  $24^\circ\text{C}$ ;  $30^\circ\text{C}$ . Clearly dependence of the period of the oscillation on the sample temperature can be observed. We evaluate the period of the oscillation as an average time between two neighboring minima of the emission intensity. The values of the period of the oscillation were obtained:  $34^\circ\text{C}$ —21 s;  $30^\circ\text{C}$ —28 s;  $24^\circ\text{C}$ —47 s;  $18^\circ\text{C}$ —83 s;  $13^\circ\text{C}$ —137 s. Regarding these data and following the Arrhenius equation [14] we estimate the energy activation of the oscillating quenching. The energy activation of the oscillating quenching 15 kcal/mol has been found.

At the present stage the mechanism of the phenomenon under consideration. However, we believe, that weakly bounded, face-to-face, bis-molecular complexes with bivalent  $Zn^{++}$  and with intramolecular interaction between BCDSB molecules, most probably formed in solution under irradiation (the model of such arrangement is

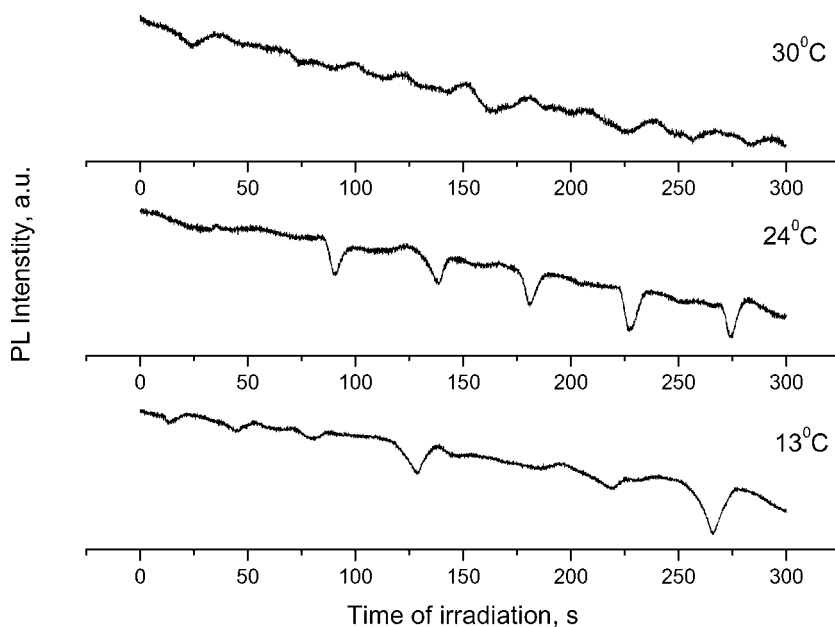


**Fig. 3.** Dependence of the intensity of BCDSB emission  $\lambda_{em} = 448$  nm by excitation  $\lambda_{exc} = 378$  nm on the duration of the time irradiation in solution doped with  $ZnCl_2$  ( $Zn^{++}$ );  $MgCl_2$  ( $Mg^{++}$ );  $KCl$  ( $K^+$ );  $CaCl_2$  ( $Ca^{++}$ ) at room temperature.

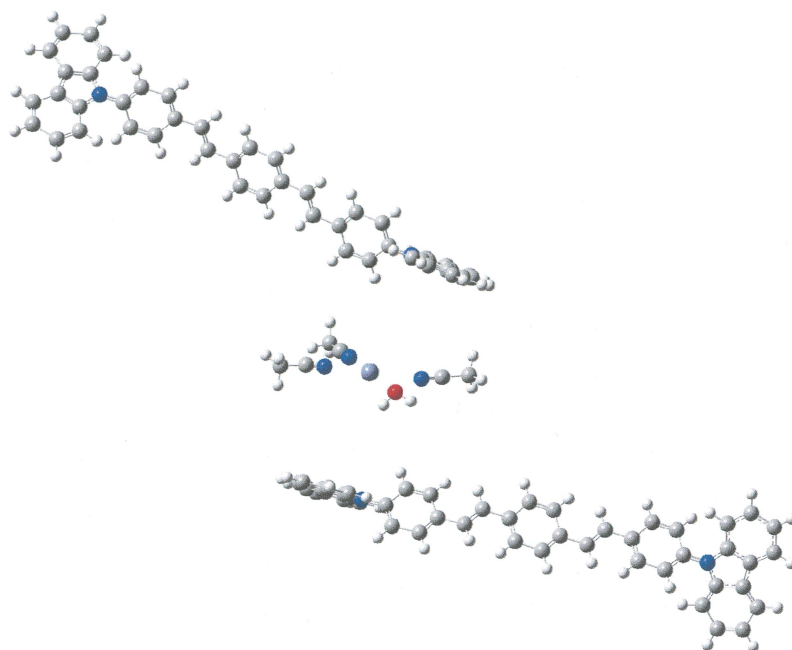
shown on the Fig. 5). The complex coordination is completed by solvent and water molecules.

The proposed model is based on the following. BCDSB molecule in acetonitrile as well as in acetonitrile/water solution accepts fully extended *trans*-conformation (*cis*-conformation has very low intensity emission maximum of the emission is located at 520 nm by excitation at 480 nm and was not observed in these experiments). This distance between two nitrogen's is around

20 Å, and it is too large to coordinate  $Zn^{++}$ . The coordination distance Zn—N in the complex Zn/bis(dipyridyl-diimine) was shown 2.105–2.183 Å [15]. As it was demonstrate recently, a basicity of heterocyclic compounds is increasing under excitation in  $n\pi^*$  electronic state [16]. An increase of basicity of BCDSB in excited  $n\pi^*$  electronic state assists spontaneous discrete complexes formation and BCDSB–BCDSB molecular interactions through the metal center.



**Fig. 4.** Dependence of the intensity of BCDSB emission  $\lambda_{em} = 448$  nm by excitation  $\lambda_{exc} = 378$  nm on the duration of the time irradiation in solution doped with  $ZnCl_2$  under different temperature 13°C; 24°C; 30°C.

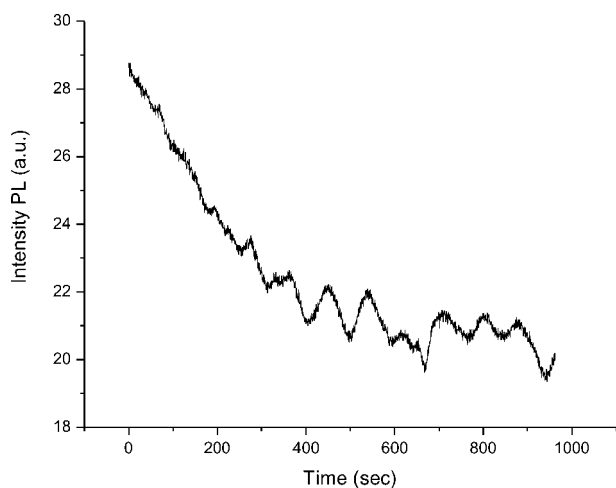


**Fig. 5.** The scheme of the model of the  $Zn^{++}$ /BCDSB complex (grey colored—C; blue colored—N; red colored—O).

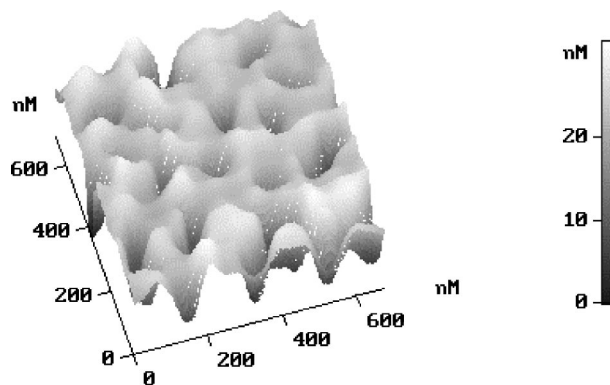
The parameters of an oscillating—time scale is in the range of the seconds, comparatively high value of the activation energy 15 kcal/mol anticipate conformational changes of BCDSB as a reason of the unusual behavior of the decay. We suppose that reversible conformation changes of BCDSB followed by a rearrangement of  $Zn^{++}$  complex, probably, “octahedral–tetrahedral.” In such case the rearrangement of the complex could lead to the changes of the distance between metal ion and BCDSB

or water molecule and BCDSB, causing the oscillating character of the decay.

To confirm our model, acetonitrile solution of BCDSB was doped with  $Zn\ SO_3 \cdot 2H_2O$ . The decay of the emission is presented on the Fig. 6. The oscillating decay of BCDSB emission also was observed. Anyhow, the character of the process was significantly different, namely, the oscillating process during prolonged time of irradiation does not reach the stable, periodical character, and the amplitude of the intensity alternated. A combination  $Zn^{++}$  coordinated structure with intramolecular interaction can stimulate an ability of the material



**Fig. 6.** The decay of the emission quenching of BCDSB in acetonitrile  $\lambda_{em} = 448\text{ nm}$  by excitation  $\lambda_{ex} = 378\text{ nm}$  in the presence of  $Zn\ SO_3 \cdot 2H_2O$ .



**Fig. 7.** The AFM image of the nanolayer of  $Zn^{++}$ /BCDSB formed from  $Zn^{++}$ /BCDSB/acetonitrile/water solution.

to polymerization. Figure 7 presents the AFM imaging of the nanolayer of Zn<sup>++</sup>/BSN prepared on Si substrate, Si's native oxide. The nanolayer of the material exhibits a similarity with polymeric structure, containing charged groups [17].

## CONCLUSION

Unusual periodical character of emission decay bis-*N*-carbazoyl-distyrylbenzene exhibits in acetonitrile/water solution doped with bivalent zinc. The time scale of such oscillation is in the seconds, energy activation of the oscillation—15 kcal/mol. The interaction of bivalent zinc ion and bis-*N*-carbazoyl-distyrylbenzene excited in  $n\pi^*$  electronic state afforded formation of intramolecular arrangement through the bimolecular complex. We believe that photoinduced reversible structural changes in Zn<sup>++</sup>/bis-*N*-carbazoyl-distyrylbenzene complex cause the oscillating character of the emission decay of the chromophore.

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