Chemical Physics Letters 492 (2010) 170–173

Contents lists available at ScienceDirect

Chemical Physics Letters

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

Luminescence detection of DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates on a polyaniline/ITO electrode associated with in situ electrochemical tuning

Jiangyang Shao [a], Ting Sun [b], Shibo Ji [a], Hong Li [a,*], Sheng Lan [b], Zhenghe Xu [a]

[a] Key Lab of Technology on Electrochemical Energy Storage and Power Generation in Guangdong Universities, School of Chemistry and Environment, South China Normal University, Guangzhou 510006, PR China
[b] Lab of Photonic Information Technology, School for Information and Optoelectronic Science and Technology, South China Normal University, Guangzhou 510006, PR China

ARTICLE INFO

Article history:
Received 8 January 2010
In final form 7 April 2010
Available online 10 April 2010

ABSTRACT

A new method for luminescence detection of $[Ru(bpy)_2tatp]^{2+}$-based thin layer (where bpy = 2,2′-bipyridine, tatp = 1,4,8,9-tetra-aza-triphenylene) on a polyaniline (PANI)/ITO electrode in the absence and presence of herring sperm DNA tuned by applied electrode potentials has been developed under the excitation of CW green laser. It is found that the DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates are formed either in solution or on the PANI/ITO surface, exhibiting an effective enhancement in the luminescence by DNA. More interestingly, the application of anodic potentials significantly enhances the emission intensities of both $[Ru(bpy)_2tatp]^{2+}$ and DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates on the PANI/ITO surface excited with green laser.

1. Introduction

The majority of studies on interactions of polypyridyl ruthenium complexes with DNA have been concentrating on developing hybridization indicators that can be used as electrochemical and luminescent devices for the detection of nucleotide sequences and DNA damage [1,2], as photochemical and stereo-selective probes of nucleic acid structure [3], and as anticancer drugs, which control the reproduction of DNA in the body of living organs [4]. Based on their excellent electrochemistry and efficient luminescence responses, ruthenium complexes have recently emerged as some of the most promising materials for DNA identification by electroluminescence devices [5], electrochemiluminescence detector [6] and emission spectroscopy [7]. Up to now, the molecular luminescence systems in connection with the design of electronic/photonic devices have attracted a considerable interest [8]. In such systems, external input such as photons [9], electrons [10] and protons [11] was often used to stimulate the functional units to tune the luminescence intensity, leading to the development of various kinds of molecular luminescence systems.

To simplify experimental design, part ruthenium complexes such as $[Ru(bpy)_3]^{2+}$ (bpy = 2,2′-bipyridine) have been immobilized onto solid electrode surfaces in design of the solid-state luminescent devices for detecting various biomolecules [12]. Recently, electroluminescent devices obtained by adding the ruthenium complexes into conductive polymers have been reported to reach electroluminescence efficiencies up to 3%, approaching the limit of photoluminescence efficiency [13]. In comparison, electrochemically enhanced luminescence of ruthenium complexes with laser as excitation source has received little attention, in particular for the studies on in situ electrochemically modulated photoluminescence of the ruthenium complexes conjugated with DNA.

In this Letter, we develop for the first time, a novel method for the luminescence detection of DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates under the excitation of CW green laser tuned by the applied electrode potentials. The principles of this method are schematically shown in Fig. 1. It is based on the photoluminescence behavior of DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates (bpy = 2,2′-bipyridine, tatp = 1,4,8,9-tetra-aza-triphenylene) arising from the Ru(II)-to-ligand ($d-\pi^*$) charge transition either in solution or on the PANI/ITO surface. In comparison with the electroluminescence or photoluminescence detector reported previously, the present technique has three major advantages: (i) it eliminates the contributions of the luminescent reactant in solution to the collected emission spectra; (ii) it can be fabricated into the form of solid-state electroluminescence or photoluminescence detector; and (iii) it provides a unique opportunity for determining the electroluminescence (or electrochemiluminescence) and photoluminescence properties of various materials.

2. Experimental

2.1. Chemicals and materials

Tris-hydroxy methyl amino-methane (Tris) purchased from Sigma Chemical Company was used to prepare electrolyte buffer solutions. Herring sperm DNA (Qiyun Co.) and other reagents were used as received. Unless otherwise noted, the electrolyte solution

* Corresponding author. Fax: +86 20 3931 0187.
E-mail address: lihong@scnu.edu.cn (H. Li).

0009-2614/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.cplett.2010.04.016

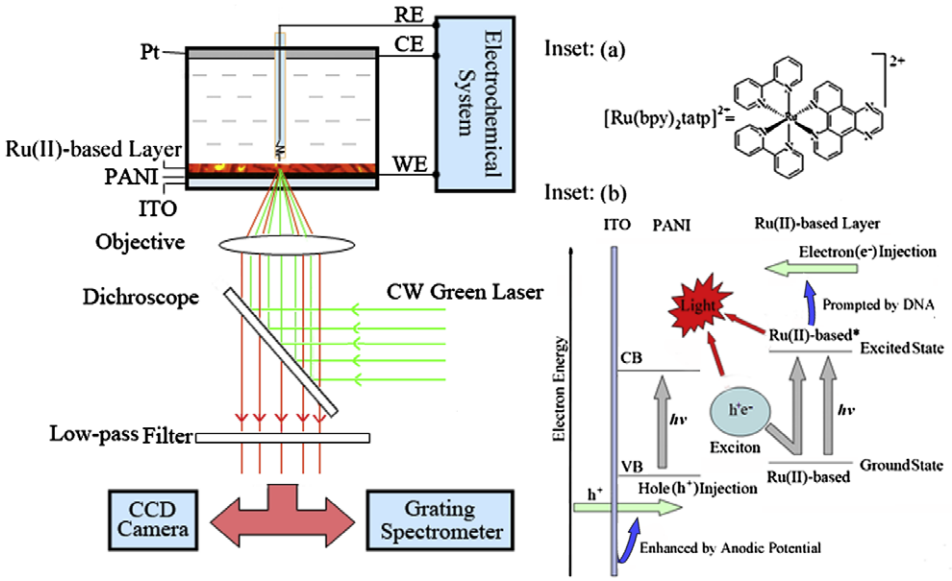

Fig. 1. Schematic diagrams of experimental setup for the luminescence detection with in situ electrochemical tuning. The insets a and b show the structure of $[Ru(bpy)_2tatp]^{2+}$ and the luminescence principle of Ru(II)-based layer on PANI/ITO electrodes associated with in situ electrochemical tuning, respectively.

was 10 mmol L^{-1} Tris/50 mmol L^{-1} NaCl of pH 7.2 adjusted by diluted HCl. Doubly distilled water was used to prepare buffer solutions.

Preparation of $[Ru(bpy)_2tatp]Cl_2$ and PANI/ITO electrode was based on the procedures reported previously [14,15]. The structure of $[Ru(bpy)_2tatp]^{2+}$ is shown in Fig. 1.

2.2. Experimental methods

Steady-state emission spectra were recorded using a RF-2500 spectrofluorimeter. The samples were excited at 450 nm. The fluorescence image was taken using a Nikon Eclipse TS100 inverted fluorescence microscope (Japan), equipped with a 50 W mercury lamp. The images were captured with a Nikon E4500 camera with blue light radiation.

The modulation of applied electrode potentials was performed on a CHI660a electrochemical system (Shanghai, China). Unless otherwise noted, a regular three-electrode system in 0.4 mL test solution was used. A polyaniline(PANI)/indium-tin oxide (ITO) modified with Ru(II)-based layer was served as working electrode (WE, sheet resistance of ITO: 20 Ω cm^{-2}, Shenzhen Nanbo Co. Ltd., China), another two electrodes were platinum counter electrode (CE) and Ag–AgCl (50 mmol L^{-1} NaCl) reference electrode (RE), respectively.

Emission spectra tuned by in situ electrochemical method were recorded using a home-built system, consisting of an optical microscope (Zeiss Axio Observer A1, Germany), a CW green laser source (532 nm, Coherent Verdi-5, USA), and an electrochemical system. As shown in Fig. 1, a laser beam was reflected by a dichroscope. The reflected beam was focused on the surface of desired depth in the working electrode through an objective lens. The emission spectra were collected using the same objective lens to direct the emitted beam from the sample through the dichroscope, followed by a low-pass filter and a grating spectrometer (7ISW3052, Beijing, China) to a photo multiplier tube (PMT 7ID101-CR131, Beijing, China). The full scale was obtained by the modulation of PMT and lock-in amplifier, operated at a biased voltage of −470 V. The collected data were transferred to a computer, and images of solid–liquid interfaces in the absence of CW green laser were synchronously captured with a CCD camera. The observed emission intensity is calibrated with fluorescent polystyrene particles [16].

All the experiments were performed at room temperatures (23–25 °C).

3. Results and discussion

3.1. Luminescence properties of $[Ru(bpy)_2tatp]^{2+}$ enhanced by DNA

Fig. 2a shows the emission spectra of soluble $[Ru(bpy)_2tatp]^{2+}$ in the presence of DNA. In the absence of DNA, an intense emission is observed at 598 nm, arising from the Ru(II)-to-ligand (d–π*) electron transition [17]. The addition of DNA leads to a significant enhancement of the luminescence without a noticeable shift in peak position, attributed to conjugation of $[Ru(bpy)_2tatp]^{2+}$ with DNA in solution by intercalating into the bases of DNA with tatp ligand [14,18].

In order to avoid the assault of the luminescence by solvent water molecules, a given mass of $[Ru(bpy)_2tatp]^{2+}$ associated with DNA is immobilized by placing the $[Ru(bpy)_2tatp]^{2+}$-DNA solution drop-wise onto the PANI/ITO surface. As shown in Fig. 2b, the presence of DNA also enhances the photoluminescence intensity by 81.4% for $[Ru(bpy)_2tatp]^{2+}$ immobilized on PANI/ITO surfaces, illustrating that the DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates are formed either in solution or on PANI/ITO electrode surface. The fluorescence image of $[Ru(bpy)_2tatp]^{2+}$ on PANI/ITO electrodes in Fig. 2c1 shows an intense orange–red appearance under the excitation of blue light. The presence of DNA not only enhances the photoluminescence intensity of $[Ru(bpy)_2tatp]^{2+}$ on PANI/ITO surfaces, but also alters the morphology of $[Ru(bpy)_2tatp]^{2+}$, distinctly showing many long orange–red strings as shown in the image of Fig. 2c2. The morphological change of $[Ru(bpy)_2tatp]^{2+}$ on PANI/ITO surfaces in the presence of DNA reveals the aggregation of $[Ru(bpy)_2tatp]^{2+}$ with DNA by the intercalation interaction, accompanied by a significant enhancement of photoluminescence intensity. These results are in good agreement with the observations from emission spectra of $[Ru(bpy)_2tatp]^{2+}$ on PANI/ITO surfaces in the absence and presence of DNA.

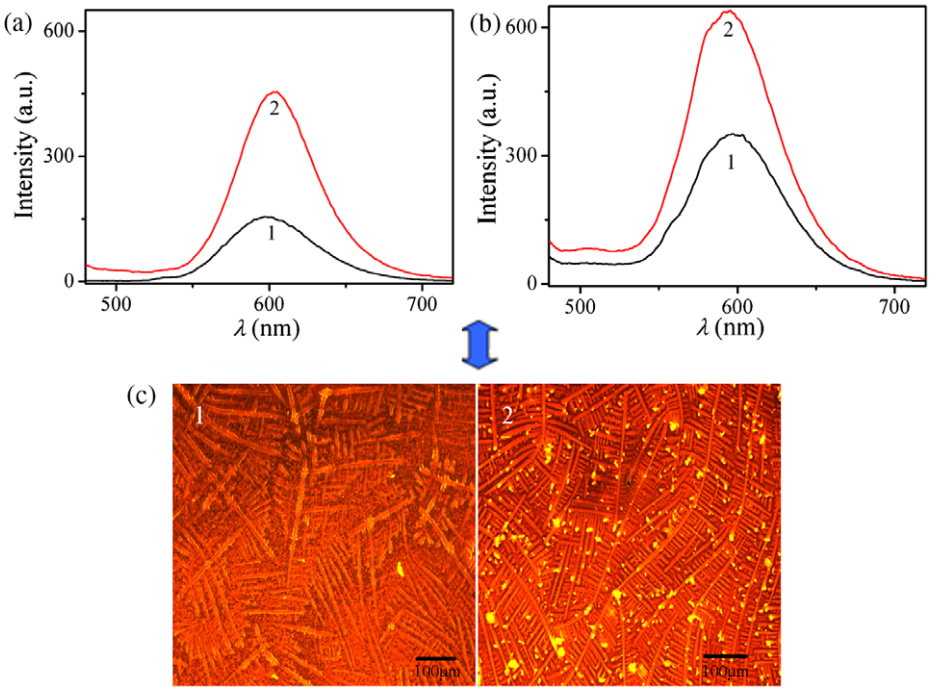

Fig. 2. (a) Emission spectra of 0.01 mmol L^{-1} $[Ru(bpy)_2tatp]^{2+}$ in buffer solution in the absence (1) and presence of 0.1 mmol L^{-1} DNA (2). (b) Emission spectra and (c) fluorescence microscopic images of $[Ru(bpy)_2tatp]^{2+}$ (1) and DNA-$[Ru(bpy)_2tatp]^{2+}$ (2) on the PANI/ITO electrode.

3.2. Emission spectra of DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates with in situ electrochemical tuning

The results above show that the presence of DNA enhances photoluminescence intensity of $[Ru(bpy)_2tatp]^{2+}$ either in solution or immobilized on PANI/ITO electrode surfaces. It is therefore interesting to investigate whether the photoluminescence of DNA-Ru(II) conjugates could be controlled by the applied electrode potentials.

In this study, the measuring system with in situ electrochemical tuning shown in Fig. 1 is used to investigate the impact of anodic potentials on laser-induced luminescence. The electrochemical cell was placed in the middle of an optical microscopic stage, in which the luminescent signals of a given plane at a desired depth were measured and images of corresponding surfaces were recorded. Fig. 3a shows a typical image of the resulting thin film on PANI/ITO electrodes in the presence of DNA compared with the picture of $[Ru(bpy)_2tatp]^{2+}$ in Fig. 3b. Long strings of DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates are observed, which are similar to those reveled by the fluorescence microscopy. The emission spectra of the Ru(II)-based thin film under the excitation of CW green laser are determined, and the results are shown in Fig. 3c. A broad emission peak is observed at 612 nm, showing a 14 nm red-shift in contrast to the value from the steady-state emission spectroscopy as depicted in Fig. 2b. The presence of broad peak and red-shift confirms that the luminescent system with in situ electrochemical tuning could be a powerful tool for investigating the electroluminescence (or electrochemiluminescence) and photoluminescence properties of various materials. Upon application of anodic potentials, no noticeable shift in maximal peak position is observed, implying that the excited species is identical, independent of external electric field. However, the luminescence intensity is significantly enhanced with increasing applied electrode potentials. For example, an applied electrode potential of 1.2 V above the open circuit potential (OCP) could increase the luminescence intensity by 442.9%. In the absence of DNA, as shown in Fig. 3d, the emission intensity at OCP shows a decrease of 32.9% in contrast to DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates. The result is analogous to the observation from emission spectra of $[Ru(bpy)_2tatp]^{2+}$ and DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates on PANI/ITO surfaces. In addition, the application of anodic potentials can linearly enhance the luminescence intensity of $[Ru(bpy)_2tatp]^{2+}$, and the regression coefficient is 0.993. An applied potential of 1.2 V above OCP increased the luminescence intensity by 67.2%. These findings reveal that not only the presence of DNA increases the luminescence of $[Ru(bpy)_2tatp]^{2+}$, but also the photoluminescence of both $[Ru(bpy)_2tatp]^{2+}$ and DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates can be finely tuned by the applied electrode potentials.

More interestingly, when the green laser is turned off, as shown by the dotted line of Fig. 3, the application of 1.2 V anodic potential does not lead to a detectable luminescent signal for $[Ru(bpy)_2tatp]^{2+}$ and DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates on PANI/ITO electrodes, even with excessively high biased voltages from −470 to −900 V at PMT. It is evident that the contribution of electroluminescence (or electrochemiluminescence) under 1.2 V anodic potential in the absence of laser is too low to be detected by our PMT. It is interesting to note that for a given system, as shown in the inset of Fig. 3, the luminescence intensity excited by green laser increases linearly with increasing the anodic potentials and the regression coefficient is 0.993. This finding indicates the presence of synergy between the irradiation of green laser and the anodic potential. Clearly, the luminescent system proposed in this study provides a very different luminescence mechanism from that of traditional organic light emitting diodes or electroluminescence [19,20]. When anodic potentials are added to the working electrode, as depicted in Fig. 1, holes generated from ITO surfaces are injected into the Ru(II)-based thin layer via hole movement of the acid-doped PANI. Under the irradiation of green laser, the Ru(II) excited state releases electrons [21,22]. These transferred electrons can recombine with the holes from PANI/ITO surfaces to yield excitons, which emit the red-shift luminescence [23]. Synchronously, the resulting excitons may in turn stimulate the formation of plentiful Ru(II) excited species, resulting in stronger luminescence.

Another interesting aspect is focused on the comparison of DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates with $[Ru(bpy)_2tatp]^{2+}$ in the luminescence enhanced by anodic potentials. As shown in the inset

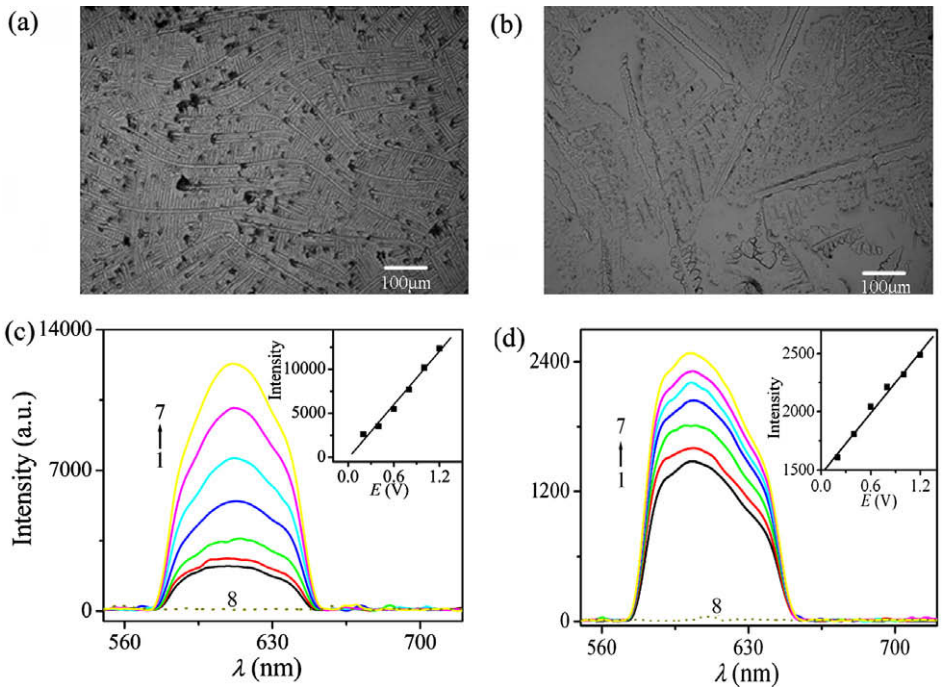

Fig. 3. Optical microscopic images of DNA-$[Ru(bpy)_2tatp]^{2+}$ (a) and $[Ru(bpy)_2tatp]^{2+}$ (b) on the PANI/ITO electrode; emission spectra of DNA-$[Ru(bpy)_2tatp]^{2+}$ (c) and $[Ru(bpy)_2tatp]^{2+}$ (d) on the PANI/ITO electrode in buffer solution at the excitation of CW green laser tuned by anodic potentials (E, V): (1) open circuit potential, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8, (6) 1.0 and (7) 1.2. PMT biased at −470 V. The inset shows the luminescence intensity as a function of E. The dotted line 8 corresponds to the emission spectroscopy in the absence of CW green laser at an applied potential of 1.2 V. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of Fig. 3, in the presence of DNA, the slope of the regression line is 10115.0 a.u. V^{-1}, which much larger than the value in the absence of DNA (871.1 a.u. V^{-1}). This finding reveals that application of anodic potentials can more effectively enhance the photoluminescence of DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates in contrast to $[Ru(bpy)_2tatp]^{2+}$. DNA molecules in the Ru(II)-based thin film may be regarded as electron donors, as shown in Fig. 1, the injection of electrons from Ru(II)-based excited states is therefore prompted by DNA.

4. Conclusions

A novel method for the luminescence detection with in situ electrochemical tuning under the excitation of CW green laser has been developed based on the investigation of photoluminescence properties of $[Ru(bpy)_2tatp]^{2+}$ in the absence and presence of DNA on PANI/ITO surfaces. The photoluminescence of DNA-$[Ru(bpy)_2tatp]^{2+}$ conjugates is tuned by the applied electrode potentials. Most importantly, the results from this study provide a new methodology for quantitatively evaluating the contributions from electroluminescence and photoluminescence of luminescent materials in the solid–liquid interface.

Acknowledgement

This work was supported by a grant from the Specialized Research Fund for the Doctoral Program of Higher Education of China (No. 20 094 407 120 008).

References

[1] A.C. Ontko, P.M. Armistead, S.R. Kircus, H.H. Thorp, Inorg. Chem. 38 (1999) 1842.
[2] J. Huang, T. Li, Z. Chen, X. Liu, S. Liu, Electrochem. Commun. 10 (2008) 1198.
[3] H.M. Chen, J.A. Parkinson, R.E. Morris, P.J. Sadler, J. Am. Chem. Soc. 125 (2003) 173.
[4] A. Dorcier, C.G. Hartinger, R. Scopelliti, R.H. Fish, B.K. Keppler, P.J. Dyson, J. Inorg. Biochem. 102 (2008) 1066.
[5] S. Bernhard, J.A. Barron, P.L. Houston, H.D. Abruña, J.L. Ruglovksy, X. Gao, G.G. Malliaras, J. Am. Chem. Soc. 124 (2002) 13624.
[6] L. Dennany, R.J. Forster, J.F. Rusling, J. Am. Chem. Soc. 125 (2003) 5213.
[7] K.D. Copeland, A.M.K. Lueras, E.D.A. Stemp, J.K. Barton, Biochemistry 41 (2002) 12785.
[8] P.A. Liddell, G. Kodis, A.L. Moore, T.A. Moore, D. Gust, J. Am. Chem. Soc. 124 (2002) 7668.
[9] D.S. Tyson, C.A. Bignozzi, F.N. Castellano, J. Am. Chem. Soc. 124 (2002) 4562.
[10] T. Terai, K. Kikuchi, S. Iwasawa, T. Kawabe, Y. Hirata, Y. Urano, T. Nagano, J. Am. Chem. Soc. 128 (2006) 6938.
[11] Y. Pei, J. Ma, J. Am. Chem. Soc. 127 (2005) 6802.
[12] H. Wei, Y. Du, J. Kang, E. Wang, Electrochem. Commun. 9 (2007) 1474.
[13] H. Rudmann, S. Shimada, M.F. Rubner, J. Am. Chem. Soc. 124 (2002) 4918.
[14] Q.X. Zhen, B.H. Ye, J.G. Liu, L.N. Ji, L. Wang, Chem. J. Chin. Univ. 20 (1999) 1661.
[15] X.X. Li, Electrochim. Acta 54 (2009) 5634.
[16] S. Matsushita, M. Shimomura, Colloid Surf. A 284–285 (2006) 315.
[17] K.K.W. Lo, T.K.M. Lee, Inorg. Chim. Acta 360 (2007) 293.
[18] H. Li, X.Y. Le, D.W. Pang, H. Deng, Z.H. Xu, Z.H. Lin, J. Inorg. Biochem. 99 (2005) 2240.
[19] B.C. Krummacher, S. Nowy, J. Frischeisen, M. Klein, W. Brütting, Org. Electron. 10 (2009) 478.
[20] S. Bernhard, J.A. Barron, P.L. Houston, H.D. Abruna, J.L. Ruglovksy, X. Gao, G.G. Malliaras, J. Am. Chem. Soc. 124 (2002) 13624.
[21] J. Xia, S. Yanagida, Sol. Energy, in press, doi:10.1016/j.soleer.2009.10.005.
[22] M. Pellnor, P. Myllyperkiö, J. Korppi-Tommola, A. Yartsev, V. Sundström, Chem. Phys. Lett. 462 (2008) 205.
[23] B. Li, L. Wang, B. Kang, P. Wang, Y. Qiu, Sol. Energ. Mat. Sol. C 90 (2006) 549.