Talanta 85 (2011) 1233–1237

Contents lists available at ScienceDirect

Talanta

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

Review

Perylene bisimide as a versatile fluorescent tool for environmental and biological analysis: A review

Nobuaki Soh*, Toshihisa Ueda

Faculty of Agriculture, Saga University, 1 Honjo-Machi, Saga 840-8502, Japan

ARTICLE INFO

Article history:
Received 25 April 2011
Received in revised form 6 June 2011
Accepted 7 June 2011
Available online 14 June 2011

Keywords:
Perylene bisimide (PBI)
Fluorescence
Environmental analysis
Biological analysis
Aggregation

ABSTRACT

Perylene bisimide (PBI) is a fluorescent dye which has strong emission and high photostability. Although PBI has been widely used for industrial materials, the application of PBI in analytical fields was limited mainly due to its high hydrophobicity. In recent years, however, unique and useful analytical methods based on PBI platform are being successfully developed by utilizing the characteristic features of this compound including its high hydrophobicity. In this article, the recent trend of environmental and biological analysis using PBI is reviewed.

Contents

1. Introduction

Perylene-3,4:9,10-tetracarboxylic bisimide, so-called perylene bisimide (PBI) is known as red vat dyes, and has been applied to pigments such as automotive finishes due to its light-fastness, thermal stability, and chemical inertness [1,2]. PBI is also utilized as electronic materials, for example, n-type semiconductors, in recent years. In 1959, the potential of PBI as a useful fluorescent dye with high fluorescence quantum yield and photostability was reported [3]. After that, PBI has been used as laser dyes, fluorescent light collectors, and materials for single molecule spectroscopy [4–10].

The properties of PBI are also appealing in the field of analytical chemistry. PBI would have a strong potential for achieving high-sensitive analysis because the fluorophore shows strong fluorescence with visible ranges and high photostability. In general, fluorescent dyes, which are soluble in polar solvents like water, are favorably used for environmental and biological analysis. Therefore, the insolubility of PBI to polar solvents seems to inhibit the practical use of PBI for analytical applications at first glance. However, the insolubility can be taken advantage for constructing some unique analytical techniques.

Here, the recent trend of environmental and biological analysis using PBI is described. The analytical methods presented here are classified based on the detection mechanisms.

2. Overview of field

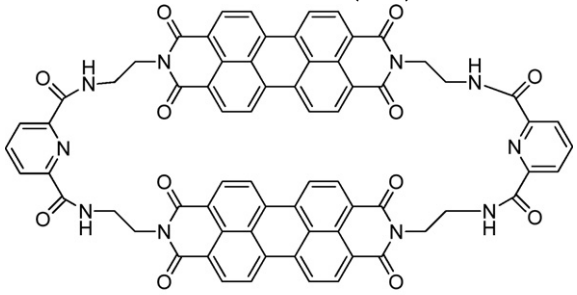
2.1. Fluorescent molecular sensors having PBI structures

PBI shows strong emission in the visible ranges. This contributes to the selective detection of analytes in the presence of vari-

* Corresponding author. Tel.: +81 952 28 8773.
E-mail address: nsoh@cc.saga-u.ac.jp (N. Soh).

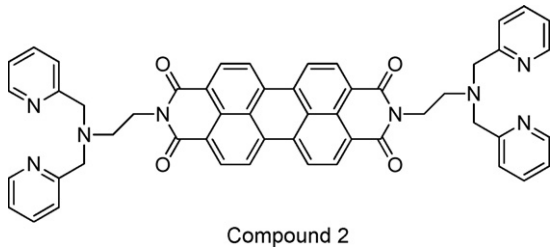
0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.06.010

ous biological substances. Compound 1 (PB-BP) is a fluorescent reagent for the selective detection of mercury ion [11]. Mercury is a widespread global pollutant and this provides a motivation to the development of analytical method for monitoring Hg^{2+} in environmental and biological samples. The compound 1 is composed of two PBI flurophores and two nonmacrocycle receptors of 2,6-bis(aminoethyl)pyridine. The 2,6-bis(aminoethyl)-pyridine functions as both ions receptor and the quencher of photoinduced electron transfer (PET). The chemosensor displays a high selectivity and sensitivity for Hg^{2+}. When Hg^{2+} is recognized with the receptor, fluorescence at 365 nm is obviously enhanced and fluorescence at 557 nm is effectively quenched, simultaneously. The detection limit of the sensor with fluorescent enhancement and quenching (FEQ) property is 10 nM. It was confirmed that the chemosensor works in the presence of human serum albumin (HSA).



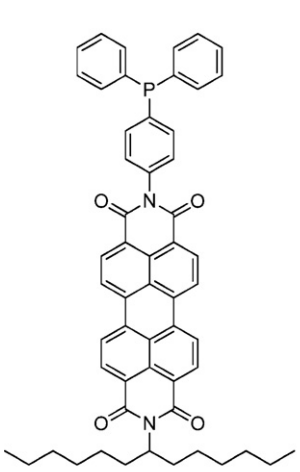
Compound 1

PBI is also applied to the construction of fluorescent reagent for other metal. Compound 2 shows the fluorescent chemosensor for the detection of Cu^{2+} [12]. Cu^{2+} is contained in abundance in the human body and plays an important role in physiological processes. Compound 2 has two dipicolylethylenediamine (DPEN) moieties for selective recognition of Cu^{2+}. The fluorescent intensity of the sensor is effectively quenched upon the addition of Cu^{2+}. In addition, the sensor shows distinguishing pink color upon the binding with Cu^{2+}. This result indicates that the compound works as a colorimetric and fluorometric dual-channel sensor for the detection of Cu^{2+}.

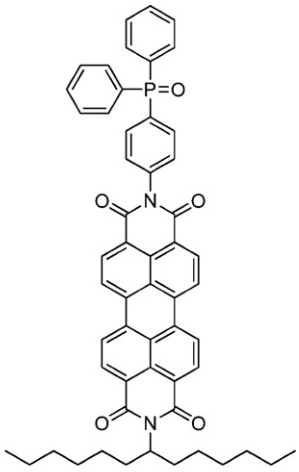


Compound 2

PBI is still useful for the detection of various biomolecules. Compound 3a (Spy-LHP) shows the fluorescent reagent for detecting lipid hydroperoxides (LOOH) [13–15]. LOOH, which is the primary product in lipid peroxidation, has attracted considerable attention as an important maker of oxidative stress. Compound 3a is composed of a triphenylphosphine for reactive moiety and a perylene derivative for fluorophore moiety. The strategy for sensing LOOH is based on an oxidation reaction of compound 3a induced by the target ROS, which leads to the formation of highly fluorescent compound 3b. The large differences of the fluorescence quantum yields between compound 3a and compound 3b are reasoned by the cancellation of PET process upon the conversion from compound 3a to compound 3b. In the experiments using methyl linoleate hydroperoxide (MeLOOH), compound 3a enhanced the fluorescence intensity with an increase in the concentration of MeLOOH. No significant fluorescence change was observed upon the addition of other ROS.



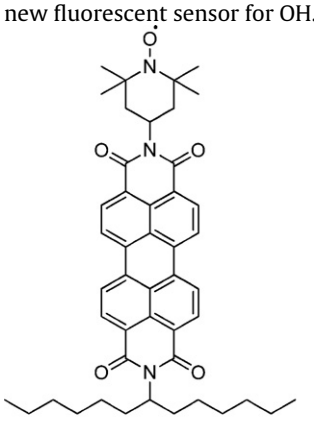
Compound 3a



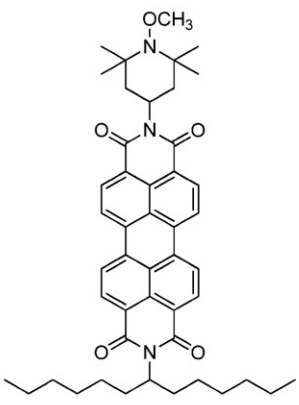
Compound 3b

Generally, the high hydrophobicity of PBI might inhibit the contribution of PBI for the biological analysis. However, from the different point of view, the hydrophobicity could contribute to accumulate a PBI-based chemosensor to hydrophobic cellular membranes. When compound 3b was incubated with living cells, ring-shaped fluorescence images along with shape of cells were observed, indicating that the fluorescent dye accumulates efficiently in cell membranes. In addition, compound 3a was successfully applied to image the production of LOOH in living cells.

Other PBI-based sensor for the detection of different ROS is also reported. Compound 4a (Spy-OH) is the chemosensor for hydroxyl radical (OH) [16]. OH is particularly high reactive and contributes to ongoing damage of biomolecules in cellular systems, thus makes the biological roles of OH the subject of considerable attention. Compound 4a can be obtained by replacing the triphenyl phosphine moiety in compound 3a with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO). Although the fluorescence intensity of compound 4a is low because of the quenching effect of nitroxyl radical of TEMPO, the compound increases the fluorescence intensity with an increase in the concentration of OH in the presence of DMSO. The mechanism of the change in fluorescence intensity would be due to the production of highly fluorescent compound 4b, which is formed by the reaction of compound 4a with methyl radical derived from DMSO upon the reaction with OH. High selectivity of compound 4a toward OH was confirmed by the experiments using other various ROS. The results indicate that compound 4a should be useful as a new fluorescent sensor for OH.



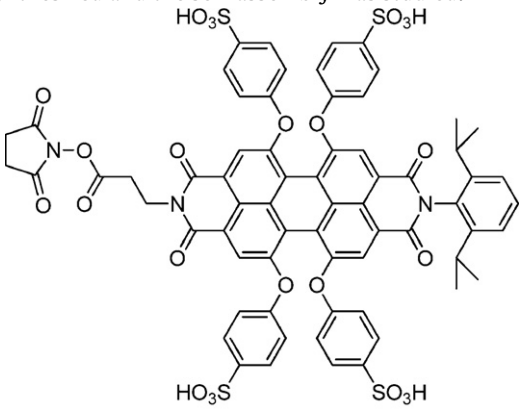
Compound 4a



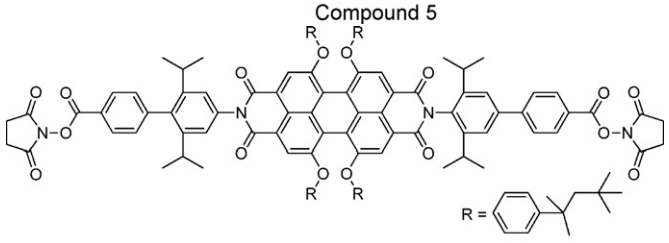
Compound 4b

PBI is also applied to the construction of chemical reagents for protein labeling. The fluorescent labeling of protein is important for the elucidation of protein functions. Compound 5 is a PBI molecule having a succinimide ester that can be used for covalent labeling of

proteins [17]. Using this compound, fluorescent imaging of phospholipase (PLA) was successfully achieved. PBI molecule in which the succinimide group is replaced by maleimide group was also synthesized [17]. Aside from these, PBI molecule having two succinimide esters (compound 6) has also reported [18]. In the report, conjugates of compound 6 with two oligodeoxyribonucleotides were synthesized and the self-assembly was studied.

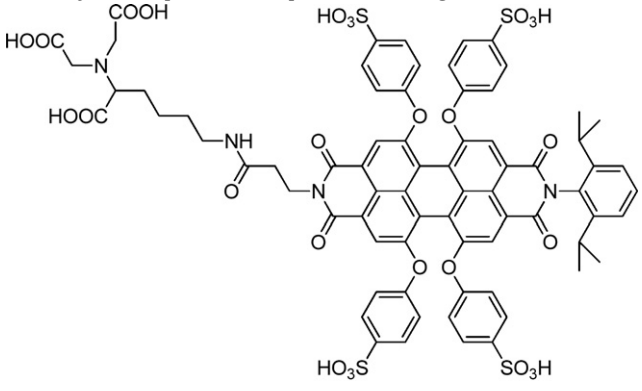


Compound 5



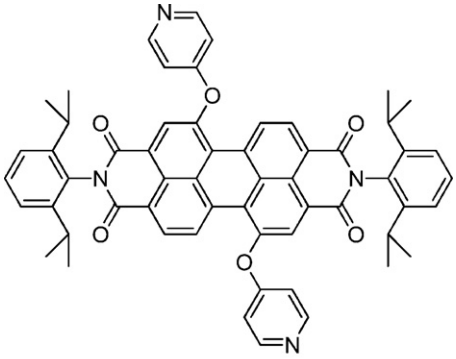
Compound 6

On the other hand, the fluorescent sensor for the selective labeling of histidine (His)-tagged protein was also reported [19]. Compound 7 consists of a water-soluble PBI derivative and a nitrilotriacetic acid (NTA) moiety. Since NTA–Ni complexes shows affinity with His-tag sequence (consecutive histidine residues), compound 7 is applicable to the selective labeling of His-tagged proteins. Mostly, fluorescent-NTA compounds show a severe loss of the fluorescence upon the binding with paramagnetic Ni^{2+}. However, the photophysical properties of the compound 7 remain unchanged upon Ni^{2+} binding. Because His-tag is one of the most widely used genetically encoded tags, it is expected that compound 7 is applied to a variety of His-tagged recombinant proteins. In this report, His_6-tagged F_1 complex of F_0F_1-ATP synthase was used to prove the suitability of compound 7 for protein labeling.



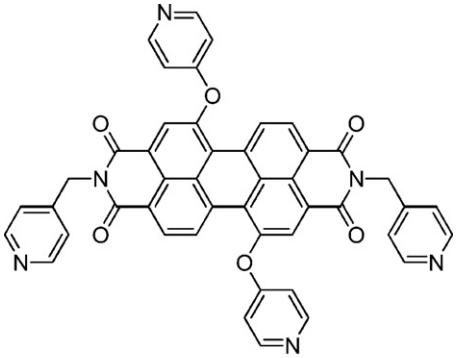
Compound 7

Other unique sensing systems based on PBI derivative complexed with gold nanoparticles (AuNPs) are also reported [20,21]. In the absence of Cu^{2+} ion, the PBI derivative containing pyridyl moieties (compound 8) coordinate to AuNPs through weak N–Au interactions [20], which causes quenching of the fluorescence of the chromophore, since AuNPs are efficient quenchers. In the presence of the Cu^{2+} ion, AuNPs are displaced from the chromophore due to the stronger coordination ability of Cu^{2+} ion with the pyridyl moiety in comparison with that of the AuNPs. Therefore, the fluorescence of the PBI derivative is turned on when it binds to the Cu^{2+} ion. The detection limit of the sensor for Cu^{2+} is ca. 1.0×10^{-6} M.



Compound 8

On the other hand, compound 9 (TPPCA) was also reported for the detection of the reduction of glutathione [21]. The fluorescence of compound 9 in the compound 9-AuNP composite was highly efficiently quenched by AuNPs. When glutathione reductase is added, oxidized glutathione (GSSG) could be reduced to reduced glutathione (GSH) rapidly. GSH coordinates with AuNPs effectively and compound 9 is displaced, and as a result, the fluorescence is turned on. Therefore, it is possible to monitor the reduction of GSSG to GSH based on the turn-on fluorescence response of compound 9 in compound 9-AuNP composite. To validate this method for biological applications, fluorescence titration experiments were performed in a physiological PBS buffer with blood serum. While 20 units of glutathione reductase were added to the solution of compound 9-AuNP/GSSG, about 3-fold enhancement of compound 9 emission intensity was observed.

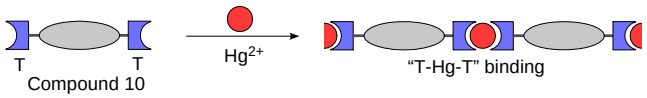


Compound 9

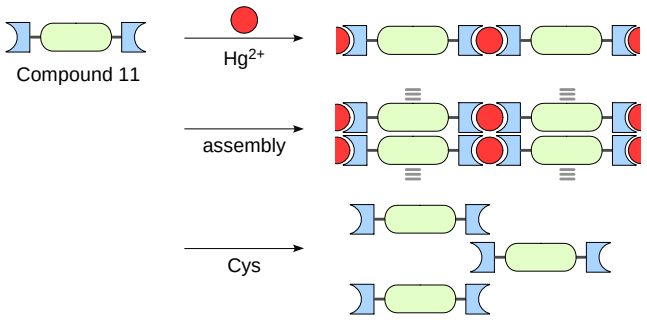
2.2. Sensing system based on the aggregation of PBIs

As already mentioned, high hydrophobicity of PBI seems to inhibit the application of PBI to environmental and biological analysis, at first glance. However, new sensing systems with a high sensitivity are being developed based on the insolubility of PBI in aqueous media in recent years.

Scheme 1 shows a schematic illustration of Hg^{2+} sensing based on PBI derivative (*N′N′*-dideoxythymidine-3,4,9,10-perylenetetracarboxylic diimide; TT-PTCDI (compound 10)) in which thymine ligand (T) exists at the ends of PBI [22]. Thymine is known to bind Hg^{2+} toughly to form T–Hg–T structure [23–25]. Compound 10 emits strong fluorescence derived from PBI in the absence of Hg^{2+}. Upon the addition of Hg^{2+}, the polymerization is induced

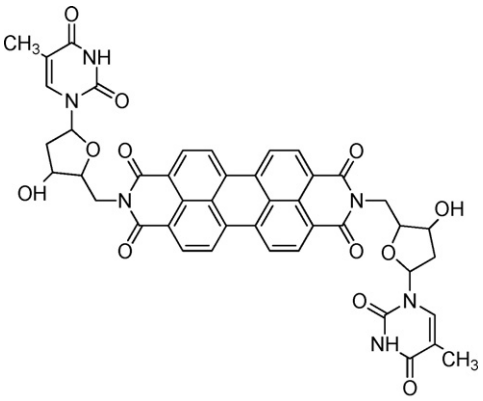


Scheme 1. Schematic illustration of Hg^{2+} sensing based on compound 10.



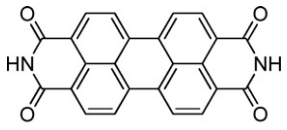
Scheme 2. Schematic illustration of Hg^{2+} and cystein sensing based on compound 11.

based on the formation of T–Hg–T structure, and the resultant aggregate does not show fluorescence. The fluorescence quenching induced by the molecular aggregation is very sharp. In addition, the presence of other metal ions did not produce fluorescence quenching because the linear coordination of T–Hg–T is extremely selective for Hg^{2+}. The detection limit of this method is as low as 5 nM (or below) of Hg^{2+}.



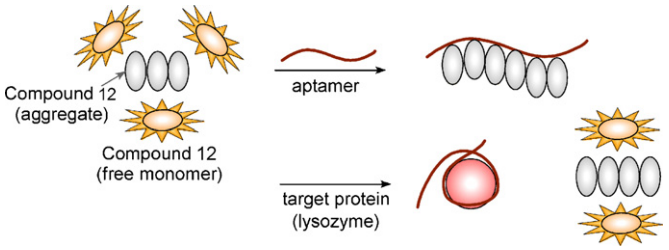
Compound 10

Similar system was also developed for the sensing of Hg^{2+} and cystein (Cys) [26]. Compound 11 contains imide groups that are similar to the binding site of thymine for Hg^{2+} for the T–Hg–T structure. In fact, Hg^{2+} induces the aggregation of compound 11 through the formation of T–Hg–T like bonding and dramatically quenches the fluorescence of compound 11. However, upon the addition of Cys, the Hg–imide interaction will be replaced by that of Hg–S, resulting in the dissociation of compound 11–Hg aggregates and the recovery of the fluorescence (Scheme 2). In this system, at least 5 nM Hg^{2+} can be detected, and the detection limit toward Cys was 9.6 nM based on the linear relationship between fluorescence intensity and [Cys] over 0.05–0.3 μM.



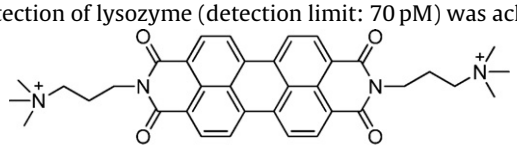
Compound 11

A detection system for protein is also reported utilizing the aggregation of PBI [27]. In the report, compound 12 and an aptamer



Scheme 3. Schematic illustration of lysozyme sensing based on the aptamer and compound 12.

for target protein (lysozyme) were used. Compound 12 tends to aggregate through the aromatic π–π stacking interaction based on the PBI structure. On the other hand, however, the compound also contains two positive charges, and the repulsive interactions decrease the tendency to aggregate. As a result, compound 12 exists in equilibrium between the aggregated form and the free monomeric form in aqueous solution at ambient temperature. Because of the existence of the free compound 12, strong fluorescence is observed at the first stage as shown in Scheme 3. However, when the anti-lysozyme aptamer is added, the repulsive electrostatic interactions among the compound 12 are greatly diminished by the negative charge of the aptamer, and a significant decrease in the fluorescence intensity is induced. Finally, upon the addition of lysozyme, selective binding of lysozyme to the aptamer weakens the binding between the aptamer and the compound 12 aggregates, and as a result, free monomeric compound 12 is recovered and a strong fluorescence signal is observed. In this method, ultrasensitive detection of lysozyme (detection limit: 70 pM) was achieved.



Compound 12

The sensing system using compound 12 and aptamers can be expanded to the detection for various analytes. In fact, Hg^{2+} was detected by this unique label-free approach [28]. In the report, compound 12 and thymine-rich oligonucleotie (oligo-M) for the specific interactions with Hg^{2+} were used. The addition of oligo-M weakened the positive charge electrostatic repulsive interactions among compound 12 and induces their aggregation. Upon the addition of Hg^{2+}, the binding of Hg^{2+} to oligo-M weakens the binding between the oligo-M and the compound 12 aggregates, and a fluorescence intensity increase derived from free monomeric compound 12 is observed. The method is highly sensitive with a limit of detection of 1 nM and also highly selective against various metal ions.

2.3. Other analytical applications of PBI

PBI can be efficiently immobilized on hydrophobic membranes due to its high hydrophobicity. An optical sensor for the detection of aldehyde was developed by immobilizing PBI derivative on the membrane of poly(vinyl chloride) (PVC) [29]. Ethanol sensor was developed by using PVC membranes on which nitroazobenzene derivative and PBI derivative are immobilized [30]. Optical sensors for vapor detection were also reported by utilizing β-cyclodextrin grafted PBI [31] or perylene monoimide derivative [32]. Aside from the sensor applications, PBI was applied to cell uptake investigations by complexed with denaturated human serum albumin having folic acids [33].

3. Conclusions

In this article, the recent trend of analytical methods utilizing PBI was introduced. In past days, the application of PBI, which has a variety of advantages as a useful fluorophore, had been limited to partial ones such as industrial materials, and had not been toward to analytical applications. One of the main reasons of this would be the high hydrophobicity and insolubility in aqueous media of this compound. However, recent progress in the application of PBI in environmental and biological analysis is mostly indebted to unique properties of PBI containing its high hydrophobicity. Localization in cellular membranes of PBI-based sensor and PBI incorporation of hydrophobic sensor membrane should be one of the good examples for the application of hydrophobicity of PBI. It is expected that a variety of PBI-based chemosensors and new high-throughput or exhaustive sensing techniques based on the chemosensors are developed in the future.

On the other hand, new sensing system based on aggregation of PBIs is also challenging. The sensing system is high sensitive and operationally simple, and the change in fluorescent signal is sharp. Furthermore, the method is applicable to various analytes in principal. Due to these significant advantages, the system would be used for the detection of a variety of targets in environmental and biological analysis.

Finally, there would be a different demand for the solubilization of PBI in aqueous solution in the monomeric form because PBI itself has outstanding fluorescent properties for various applications. In fact, analytical method utilizing water-soluble PBI is useful for the detection of target analyte [17,19]. Therefore, solubilization of PBI in water as well as cell staining with PBI is being energetically undertaken [34–39]. Such efforts would also greatly contribute to the development of analytical techniques utilizing PBI.

Acknowledgements

We wish to thank Prof. T. Imato, Prof. M. Irie, Prof. T. Nagamura, Dr. T. Fukaminato, and Dr. F. Ito for their valuable comments and experimental supports about our research introduced in this review. We also wish to thank my co-workers for their contribution, dedication, and enthusiasm. Original work in the author's group was partially supported by Industrial Technology Research Grant Program (05A01507a) from New Energy and Industrial Technology Development Organization (NEDO) of Japan.

References

[1] F. Würthner, Chem. Commun. (2004) 1564–1579.
[2] H. Langhals, Heterocycles 40 (1995) 477–500.
[3] G. Geissler, H. Remy (A.G. Hoechst) Ger. Pat. Appl. (1959), DE 1130099 (Chem. Abstr. 57 (1962) P11346f).
[4] H.-G. Löhmannsröben, H. Langhals, Appl. Phys. B 48 (1989) 449–452.
[5] R. Reisfeld, G. Seybold, Chimia 44 (1990) 295–297.
[6] W.E. Ford, P.V. Kamat, J. Phys. Chem. 91 (1987) 6373–6380.
[7] G. Seybold, G. Wagenblast, Dyes Pigm. 11 (1989) 303–317.
[8] R. Gvishi, R. Reisfeld, Z. Burshtein, Chem. Phys. Lett. 213 (1993) 338–344.
[9] S. Mais, J. Tittel, T. Basché, C. Bräuchle, W. Göhde, H. Fuchs, G. Müller, K. Müllen, J. Phys. Chem. A 101 (1997) 8435–8440.
[10] H. Langhals, H. Jaschke, U. Ring, P. von Unold, Angew. Chem. Int. Ed. 38 (1999) 201–203.
[11] L. Feng, Z. Chen, Sens. Actuators B 122 (2007) 600–604.
[12] Y. Liwei, Y. Li, L. Jingbo, Y. Jingsong, Sci. China Ser. B: Chem. 52 (2009) 518–522.
[13] N. Soh, T. Ariyoshi, T. Fukaminato, K. Nakano, M. Irie, T. Imato, Bioorg. Med. Chem. Lett. 16 (2006) 2943–2946.
[14] N. Soh, T. Ariyoshi, T. Fukaminato, H. Nakajima, K. Nakano, T. Imato, Org. Biomol. Chem. 5 (2007) 3762–3768.
[15] F. Ito, T. Ariyoshi, N. Soh, T. Kakiuchi, T. Inoue, T. Imato, T. Nagamura, Chem. Lett. 37 (2008) 1202–1203.
[16] T. Maki, N. Soh, T. Fukaminato, H. Nakajima, K. Nakano, T. Imato, Anal. Chim. Acta 639 (2009) 78–82.
[17] K. Peneva, G. Mihov, F. Nolde, S. Rocha, J. Hotta, K. Braeckmans, J. Hofkens, H. Uji-i, A. Herrmann, K. Müllen, Angew. Chem. Int. Ed. 47 (2008) 3372–3375.
[18] M.A. Abdalla, J. Bayer, J.O. Rädler, K. Müllen, Angew. Chem. Int. Ed. 43 (2004) 3967–3970.
[19] K. Peneva, G. Mihov, A. Herrmann, N. Zarrabi, M. Börsch, T.M. Duncan, K. Müllen, J. Am. Chem. Soc. 130 (2008) 5398–5399.
[20] X. He, H. Liu, Y. Li, S. Wang, Y. Li, N. Wang, J. Xiao, X. Xu, D. Zhu, Adv. Mater. 17 (2005) 2811–2815.
[21] X. He, Z. Zhong, Y. Guo, J. Lv, J. Xu, M. Zhu, Y. Li, H. Liu, S. Wang, Y. Zhu, D. Zhu, Langmuir 23 (2007) 8815–8819.
[22] Y. Che, X. Yang, L. Zang, Chem. Commun. (2008) 1413–1415.
[23] Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami, A. Ono, J. Am. Chem. Soc. 128 (2006) 2172–2173.
[24] Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima, A. Ono, J. Am. Chem. Soc. 129 (2007) 244–245.
[25] A. Bagno, G. Saielli, J. Am. Chem. Soc. 129 (2007) 11360–11361.
[26] Y.-B. Ruan, A.-F. Li, J.-S. Zhao, J.-S. Shen, Y.-B. Jiang, Chem. Commun. 46 (2010) 4938–4940.
[27] B. Wang, C. Yu, Angew. Chem. Int. Ed. 49 (2010) 1485–1488.
[28] B. Wang, F. Wang, H. Jiao, X. Yang, C. Yu, Analyst 135 (2010) 1986–1991.
[29] G.J. Mohr, U.E. Spichiger, W. Jona, H. Langhals, Anal. Chem. 72 (2000) 1084–1087.
[30] G.J. Mohr, Anal. Chim. Acta 508 (2004) 233–237.
[31] Y. Liu, K.-R. Wang, D.-S. Guo, B.-P. Jiang, Adv. Funct. Mater. 19 (2009) 2230–2235.
[32] Y. Che, X. Yang, S. Loser, L. Zang, Nano Lett. 8 (2008) 2219–2223.
[33] K. Eisele, R. Gropeanu, A. Musante, G. Glasser, C. Li, K. Muellen, T. Weil, Macromol. Rapid Commun. 31 (2010) 1501–1508.
[34] T. Cordes, J. Vogelsang, M. Anaya, C. Spagnuolo, A. Gietl, W. Summerer, A. Hermann, K. Müllen, P. Tinnefeld, J. Am. Chem. Soc. 132 (2010) 2404–2409.
[35] J. Qu, C. Kohl, M. Pottek, K. Müllen, Angew. Chem. Int. Ed. 43 (2004) 1528–1531.
[36] T. Weil, M.A. Abdalla, C. Jatzke, J. Hengstler, K. Müllen, Biomacromolecules 6 (2005) 68–79.
[37] M. Yin, J. Shen, R. Gropeanu, G.O. Pflugfelder, T. Weil, K. Müllen, Small 4 (2008) 894–898.
[38] M. Yin, C.R.W. Kuhlmann, K. Sorokina, C. Li, G. Mihov, E. Pietrowski, K. Koynov, M. Klapper, H.J. Luhmann, K. Müllen, T. Weil, Biomacromolecules 9 (2008) 1381–1389.
[39] M. Yin, J. Shen, G.O. Pflugfelder, K. Müllen, J. Am. Chem. Soc. 130 (2008) 7806–7807.