Tetrahedron 65 (2009) 9686–9689

Contents lists available at ScienceDirect

Tetrahedron

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

Zinc-supported azobenzene derivative-based colorimetric fluorescent 'turn-on' sensing of bovine serum albumin

Jing Wang [a], Hai-Bo Liu [b], Chang-Sik Ha [a,*]

[a] Department of Polymer Science and Engineering, Pusan National University, Busan 609-735, South Korea
[b] Department of Chemical and Biochemical Engineering, Pusan National University, Busan 609-735, South Korea

ARTICLE INFO

Article history:
Received 8 September 2009
Received in revised form
24 September 2009
Accepted 24 September 2009
Available online 30 September 2009

Keywords:
Fluorescent system
Colorimetric
Zinc-supported
Azobenzene derivative
Bovine serum albumin

ABSTRACT

Fluorescence sensing with small molecular chemosensors is a versatile technique for elucidation of function of various substances. Herein, a new zinc ion supported fluorescent turn-on system for bovine serum albumin (BSA) has been demonstrated. The fluorescence intensity increases 15.4-fold and visual color changes from light reddish orange to yellow can be detected after adding BSA.

1. Introduction

The development of analytical tools to detect proteins with easy handling, rapid monitoring, high sensitivity, and good binding linearity for both qualitative and quantitative analyses is of particular importance in biochemistry.[1,2] Luminescent transition metal complexes are of increasing interest in photochemistry,[3] organic optoelectronics,[3,4] and luminescence sensors.[3,5–7] Recently, a selective luminescent switch-on probe based on an octahedral iridium (III) complex for the staining of histidine/histidine-rich proteins[1a] has been reported and luminescent cyclometalated platinum(II) complexes are a new class of protein-binding probes.[1b] In addition, azobenzene compounds have been used as biomarkers, due to their ability to undergo azo-hydrazone tautomerism.[8,9] 2-(4′-hydroxyphenylazo)benzoic acid can bind to proteins such as avidin[10] and streptavidin[11] at the same binding site as the natural ligand, biotin,[12] in spite of the structural dissimilarity of these compounds. Bearing in mind all the above information, we tested the sensing of azobenzene derivative **1** in the presence of 5 equiv of Zn^{2+} toward BSA, and found this Zn^{2+} supported system to have the following properties: i) excitation and emission wavelength in the visible spectral region, ii) high sensitivity toward BSA, iii) visual color changes from light reddish orange (in the absence of BSA) to yellow (in the presence of BSA).

Herein, **1** was readily prepared from the reaction of 2,2′-dihydroxyazobenzene and 10-undecenoyl chloride,[13a] with molecule **1** in hand, we obtained Zn^{2+} supported system easily by the addition of an aqueous solution of $ZnCl_2$ to methanolic solution of **1**. Synthesis, characterization, and photophysical properties of compound **1** have been detailed by us in a previous paper.[13a]

2. Results and discussion

2.1. Effect of solvents

1 has two tautomeric forms, the azo form and hydrazone form (see Fig. 1). There are two typical absorptions at around 325 nm and 384 nm for the azo form and hydrazone form, respectively.[13a] **1** is

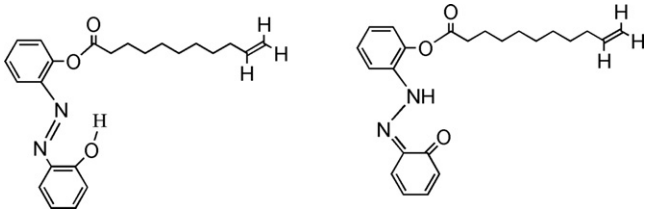


Figure 1. Two tautomeric forms (azo form (left) and hydrazone form (right)) of **1**.

* Corresponding author. Tel.: +82 51 510 2407; fax: +82 51 514 4331.
E-mail address: csha@pnu.edu (C.-S. Ha).

0040-4020/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2009.09.102

solvatochromic that is sensitive to changes in the external environment. Thus, the absorption spectra, fluorescence spectra and the color of the solution exhibit dramatic changes in different solvents (see Figs. 2 and 3). In polar solvents, such as DMSO, DMF, and MeOH, a new peak at about 540 nm in absorption and new peak at approximately 575 nm in emission spectra appeared, due to the deprotonation of the hydroxyl group on **1**, the deprotonation behaviour results in an increase in electron delocalization. This delocalized π-conjugated system is energetically higher, resulting in bathochromic shifts of both absorption and fluorescence spectra.[13] As shown in Figure 3a, in all solvents investigated, **1** existed as two tautomeric forms (azo and hydrazone) with the azo form predominating except for in DMF, where the two tautomeric forms are almost identical.

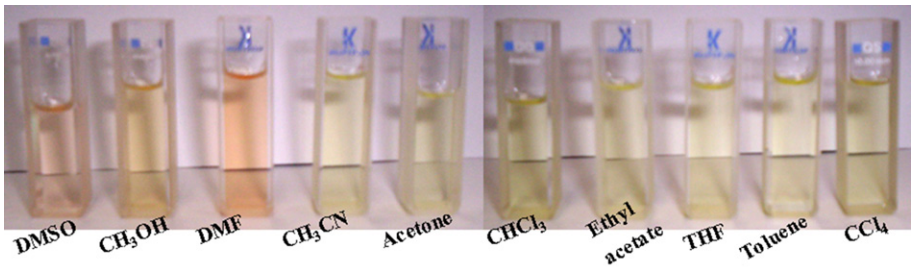


Figure 2. Images of **1** (6×10^{-5} M) in various solvents of different polarity.

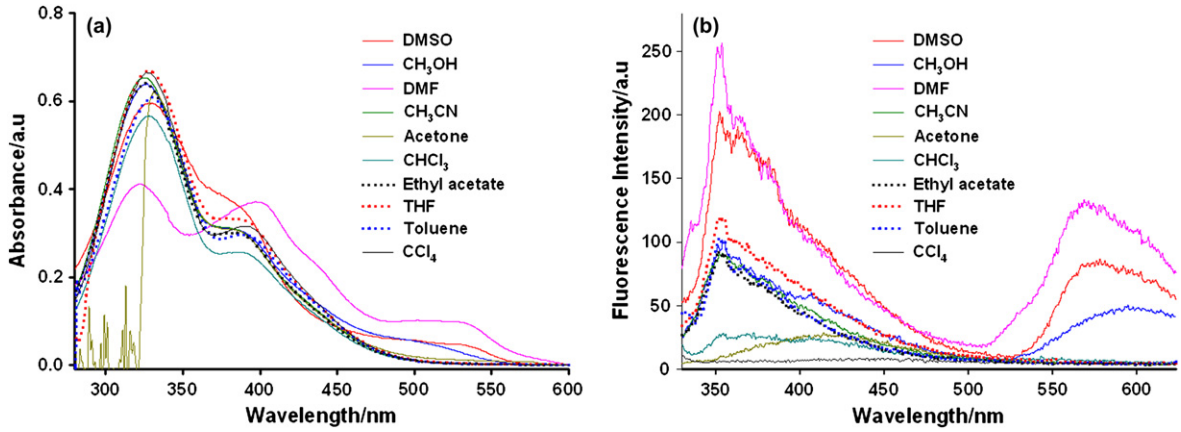


Figure 3. (a) UV–vis absorption spectra of **1** (6×10^{-5} M) in different solvents, and (b) fluorescence emission spectra of **1** (6×10^{-5} M) in different solvents.

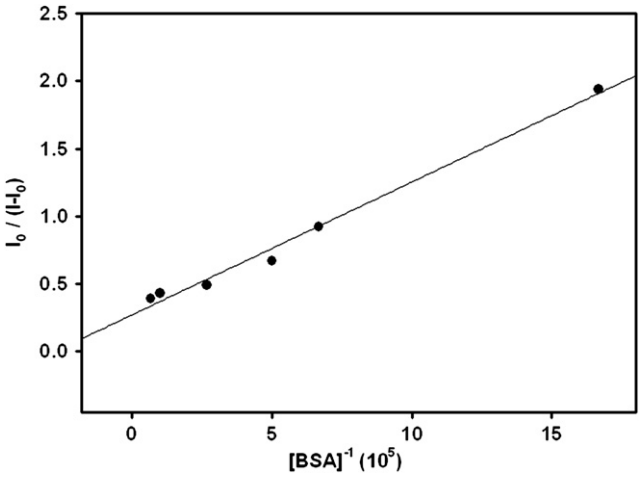


Figure 4. Plot of $I_0/(I-I_0)$ versus 1/BSA. I and I_0 are the fluorescence intensity of **1** at 355 nm in the presence and absence of BSA, respectively; The concentration of **1** was fixed at 3.0×10^{-5} M, the concentration of BSA was from 0 equiv to 0.5 equiv of **1** in a mixture of H_2O–MeOH (2/5, v/v, 10 mM HEPES buffer solution at pH 7.0). λ_{ex}=320 nm.

2.2. Sensing of BSA by 1

Bovine serum albumin (BSA), with a well characterized hydrophobic cavity, was chosen as the support protein to change the environment around **1**. The addition of BSA to the solution of **1** changed the absorption and fluorescence spectra (Fig. 4, see Supplementary data Figs. S1 and S2). As shown in Figure S1, the band at approximately 275 nm was attributed to the absorption of BSA.[14] The absorption band at 325 nm and emission band at 355 nm increased while the typical absorption peak at 325 nm and emission peak at 355 nm became undistinguishable and the emission of BSA is negligible with an excitation wavelength of 320 nm.[14] The second-order transmission replica at 640 nm in Figure S2 is due to the Rayleigh scattering of the excitation wavelength of 320 nm.[15] The fluorometric titration measurement shows a binding constant $K_{1/BSA}$ of 2.75×10^5 M^{-1} (R^2=0.989) with a 1:1 stoichiometry between **1** and BSA.[16] As **1** itself had a very weak emission at around 600 nm (excitation at 507 nm), its excitation (320 nm) and emission (355 nm) were not in the visible range, which shows disadvantage for practical application.[13a]

2.3. Zn^{2+}-supported sensing of BSA

Binding of Zn^{2+} to the serum albumin has been well elucidated in previous reports.[17] Serum albumin contains a Zn^{2+} binding motif in its core structure and it participates in transporting of Zn^{2+}. **1** had been demonstrated for the Zn^{2+} chemosensor and the excitation and emission wavelength of **1**/Zn^{2+} is in the visible range that can minimize cell and tissue damage,[13a] thus the **1**/Zn^{2+} ($K_{1/Zn}$ of

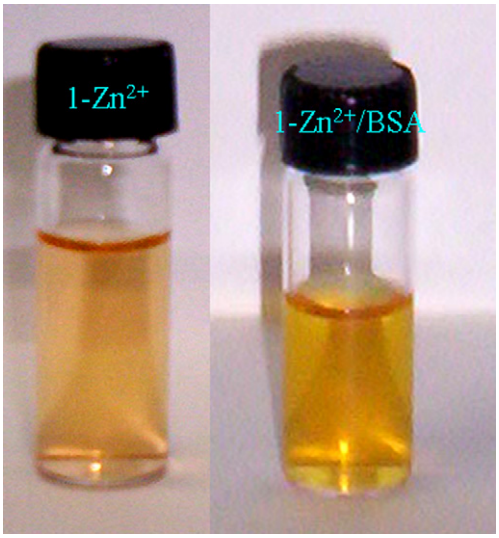


Figure 5. Photos of **1**/Zn^{2+} in the absence (left) and presence (right) of 1.0 equiv of BSA.

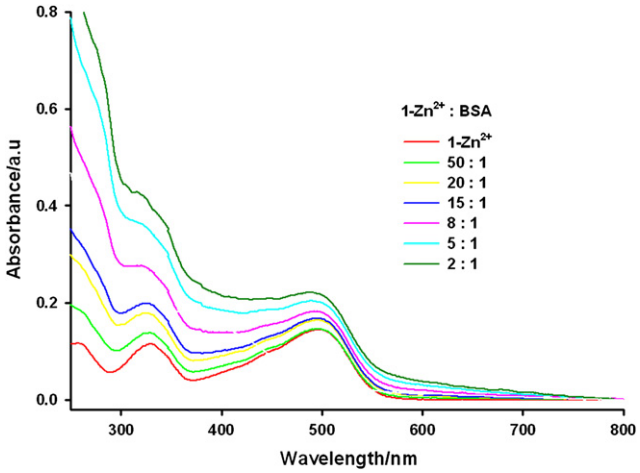
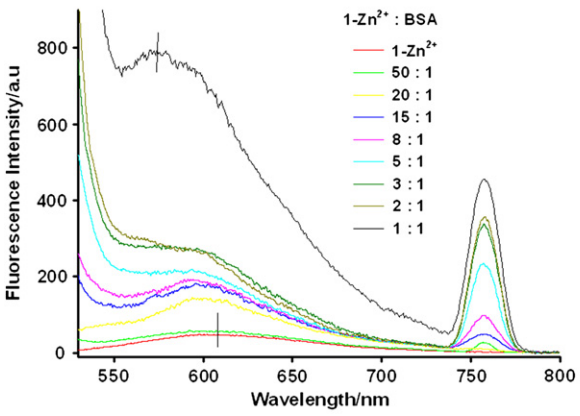


Figure 6. UV–vis absorption spectra of **1**/Zn^{2+} before and after the addition of BSA from 0 equiv to 0.5 equiv in a mixture of H_2O–MeOH (2/5, v/v, 10 mM HEPES buffer solution at pH 7.0). The concentration of **1** was fixed at 3.0×10^{-5} M and the concentration of Zn^{2+} was 3.0×10^{-5} M.

4.0×10^{5} M^{-2} [13a]) system might be superior than compound **1** alone. The addition of BSA to a solution of **1** in the presence of 5 equiv of Zn^{2+} induced changes in the absorption spectra and the fluorescence spectra, and the color of the solution changes from light orange reddish to yellow (Figs. 5–7). The addition of 1 equiv BSA to **1**/Zn^{2+} solution led to a pronounced blue shift ($\Delta\lambda_{max}$=43 nm) and a significant increase in the fluorescence emission intensity at 600 nm of about 15.4 times. These spectral changes may be caused by a complexation, in which **1**/Zn^{2+} complex is strongly bound to the hydrophobic region of the BSA through hydrophobic interaction.[1a,1b,2a,14,18] The fluorometric titration measurement shows a binding constant ($K_{1-Zn^{2+}/BSA}$) of 8.08×10^{4} M^{-1} (R^2=0.998) with a 1:1 stoichiometry between **1**/Zn^{2+} and BSA (Fig. 7). From Figure 7 and Figure S3, the detection limit of the **1**/Zn^{2+} complex for BSA sensing is estimated as 6×10^{-7} M of BSA or less.[2a] Figure 6 indicates the typical changes in the absorption spectra of **1**/Zn^{2+} system in the presence of various concentrations of bovine serum albumin (BSA). A solution of **1**/Zn^{2+} has reddish orange color and the molar extinction coefficient (ε) is 37,667 M^{-1} cm^{-1} at 325 nm and 47,667 M^{-1} cm^{-1} at 502 nm. In contract, **1**-Zn^{2+}/BSA complex forms a yellow solution, this is an obvious color change and corresponds to a dramatic increase in the absorbance at 325 nm and small increase at approximately 500 nm, which is attributed to the interaction between **1**-Zn^{2+} and BSA. The ε value of **1**-Zn^{2+}/BSA complex (**1**-Zn^{2+}/BSA, 3.0×10^{-5} M/1.5×10^{-5} M) is 138,333 M^{-1} cm^{-1} at 325 nm and 71,333 M^{-1} cm^{-1} at 502 nm. Comparison of the binding constant showing $K_{1/Zn}>K_{1/BSA}>K_{1\text{-}Zn/BSA}$ revealed that the interaction of **1** and Zn^{2+} is stronger than those of **1**-BSA and **1**-Zn/BSA and, consequently, supporting a possibility of BSA detection after the formation of a complex between **1** and Zn^{2+}.[16c] In addition, the insensitivity of **1**/Zn^{2+} system to pH values provides further advantage for sensing of BSA.[13a] Furthermore, 1/Zn^{2+} system can be repeatedly used for the sensing of BSA by renewing EDTA due to the great difference in the association constant (K_{ass}) between EDTA and **1** with Zn^{2+} ions (log K_{ass} of EDTA with Zn^{2+} is 16.4).[13a]

3. Conclusions

In conclusion, we have developed a new zinc supported colorimetric fluorescent turn-on probe for BSA. The detection of BSA by **1**/Zn^{2+} system can strongly impudence the emission behaviour as the fluorescence intensity at 600 nm increased 15.4-fold and the visual color changes from light reddish orange (in the absence of BSA) to yellow (in the presence of BSA). In addition, the excitation and emission wavelength of the **1**/Zn^{2+} system facilitate living cell measurements. We are currently working on synthesizing new, organic-based materials for the sensing of proteins.



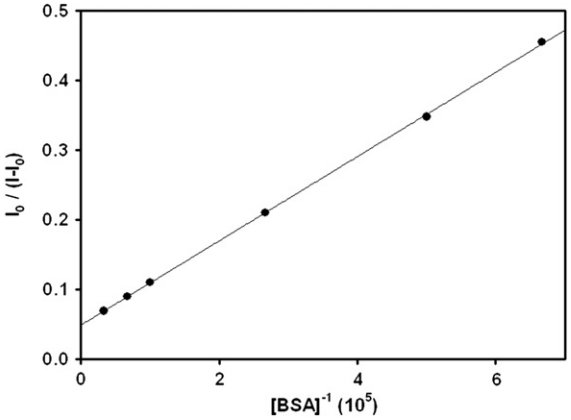


Figure 7. Fluorescence spectra of **1**/Zn^{2+} before and after the addition of BSA from 0 equiv to 1.0 equiv in a mixture of H_2O–MeOH (2/5, v/v, 10 mM HEPES buffer solution at pH 7.0). The concentration of **1** was fixed at 3.0×10^{-5} M and the concentration of Zn^{2+} was 3.0×10^{-5} M. λ_{ex}=507 nm.

4. Experimental

4.1. General

All solvents were of analytical grade. **1** was prepared by the established literature procedure.[13a] Bovine serum albumin was purchased from Sino-American Biotechnology Company (without further purification). For UV–vis spectra and fluorescence spectra, samples were dissolved by HEPES buffer (pH=7.0). The UV–vis spectra were measured using a Hitachi U-2010 spectrometer (1 cm quartz cell) at 25 °C. The fluorescence emission spectra were measured using a Hitachi F-4500 spectrometer (1 cm quartz cell) at 25 °C.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Ministry of Education, Science and Technology, Korea (MEST) (Acceleration Research Program (No. 2009-0078791)), the Korea-China Joint Research Center Program of the NRF (K20807000001-09B1200-00110), the Brain Korea 21 Project, and the MEST for the scholarship for foreign students (J.W).

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2009.09.102.

References and notes

1. (a) Ma, D. L.; Wong, W. L.; Chung, W. H.; Chan, F. Y.; So, P. K.; Lai, T. S.; Zhou, Z. Y.; Leung, Y. C.; Wong, K. Y. *Angew. Chem., Int. Ed.* **2008**, *47*, 3735; (b) Wu, P.; Wong, E. L. M.; Ma, D. L.; Tong, G. S. M.; Ng, K. M.; Che, C. M. *Chem.—Eur. J.* **2009**, *15*, 3652; (c) Suzuki, Y.; Yokoyama, K. *Angew. Chem., Int. Ed.* **2007**, *46*, 4097; (d) Strunk, J. J.; Gregor, I.; Becker, Y.; Lamken, P.; Lata, S.; Reichel, A.; Enderlein, J.; Piehler, J. *Bioconjugate Chem.* **2009**, *20*, 41.
2. (a) Suzuki, Y.; Yokoyama, k. *J. Am. Chem. Soc.* **2005**, *127*, 17799; (b) Yan, J. X.; Devenish, A. T.; Wait, R.; Stone, T.; Lewis, S.; Fowler, S. *Proteomics* **2002**, *2*, 1682; (c) Zeng, Q.; Zhang, L. Y.; Zhen, L.; Qin, J. G.; Tang, B. Z. *Polymer* **2009**, *50*, 434.
3. (a) Sauvage, J. P.; Collin, J. P.; Chambron, J. C.; Guillerez, S.; Coudret, C. *Chem. Rev.* **1994**, *94*, 993; (b) Szaciłowski, K.; Macyk, W.; Drzewiecka-Matuszek, A.; Brindell, M.; Stochel, G. *Chem. Rev.* **2005**, *105*, 2647; (c) Demas, J. N.; DeGraff, B. A. *Coord. Chem. Rev.* **2001**, *211*, 317; (d) Lai, S. W.; Che, C. M. *Top. Curr. Chem.* **2004**, *241*, 27; (e) Wong, K. M. C.; Yam, V. W. W. *Coord. Chem. Rev.* **2007**, *251*, 2477; (f) Yamaguchi, Y.; Ding, W.; Sanderson, C. T.; Borden, M. L.; Morgan, M. J.; Kutal, C. *Coord. Chem. Rev.* **2007**, *251*, 515; (g) Nishihara, H. *Coord. Chem. Rev.* **2005**, *249*, 1468; (h) Filenko, A.; Demchenko, M.; Mustafaeva, Z.; Osada, Y.; Mustafaev, M. *Biomacromolecules* **2001**, *2*, 270.
4. (a) Chan, C. W.; Cheng, L. K.; Che, C. M. *Coord. Chem. Rev.* **1994**, *132*, 87; (b) Hissler, M.; McGarrah, J. E.; Connick, W. B.; Geiger, D. K.; Cummings, S. D.; Eisenberg, R. *Coord. Chem. Rev.* **2000**, *208*, 115; (c) Bakker, B. H.; Goes, M.; Hoebe, N.; van Ramesdonk, H. J.; Verhoeven, J. W.; Werts, M. H. V.; Hofstraat, J. W. *Coord. Chem. Rev.* **2000**, *208*, 3; (d) Li, X. H.; Wu, L. Z.; Zhang, L. P.; Tung, C. H.; Che, C. M. *Chem. Commun.* **2001**, 2280; (e) Furuta, P. T.; Deng, L.; Garon, S.; Thompson, M. E.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 15388; (f) Lu, W.; Mi, B. X.; Chan, M. C. W.; Hui, Z.; Che, C. M.; Zhu, N.; Lee, S. T. *J. Am. Chem. Soc.* **2004**, *126*, 4958; (g) Amendola, V.; Fabbrizzi, L.; Foti, F.; Licchelli, M.; Mangano, C.; Pallavicini, P.; Poggi, A.; Sacchi, D.; Taglietti, A. *Coord. Chem. Rev.* **2006**, *250*, 273; (h) Evans, R. C.; Douglas, P.; Winscom, C. J. *Coord. Chem. Rev.* **2006**, *250*, 2093; (i) Forrest, S. R.; Thompson, M. E. *Chem. Rev.* **2007**, *107*, 923.
5. (a) Keefe, M. H.; Benkstein, K. D.; Hupp, J. T. *Coord. Chem. Rev.* **2000**, *205*, 201; (b) Drew, S. M.; Janzen, D. E.; Buss, C. E.; MacE-wan, D. I.; Dublin, K. M.; Mann, K. R. *J. Am. Chem. Soc.* **2001**, *123*, 8414; (c) Rogers, C. W.; Wolf, M. O. *Coord. Chem. Rev.* **2002**, *233–234*, 341; (d) Tsukube, H.; Shinoda, S. *Chem. Rev.* **2002**, *102*, 2389; (e) Wadas, T. J.; Wang, Q. M.; Kim, Y. J.; Flaschenreim, C.; Blanton, T. N.; Eisenberg, R. *J. Am. Chem. Soc.* **2004**, *126*, 16841; (f) Kruppa, M.; König, B. *Chem. Rev.* **2006**, *106*, 3520; (g) Lo, K. K. W.; Hui, W. K.; Chung, C. K.; Tsang, K. H. K.; Lee, T. K. M.; Li, C. K.; Lau, J. S. Y.; Ng, D. C. M. *Coord. Chem. Rev.* **2006**, 1724.
6. (a) Liu, H. Q.; Peng, S. M.; Che, C. M. *J. Chem. Soc., Chem. Commun.* **1995**, 509; (b) Ma, D. L.; Che, C. M. *Chem.—Eur. J.* **2003**, *9*, 6133; (c) Siu, P. K. M.; Lai, S. W.; Lu, W.; Zhu, N.; Che, C. M. *Eur. J. Inorg. Chem.* **2003**, 2749; (d) Siu, P. K. M.; Ma, D. L.; Che, C. M. *Chem. Commun.* **2005**, 1025; (e) Kui, S. C. F.; Chui, S. S.-Y.; Che, C. M.; Zhu, N. *J. Am. Chem. Soc.* **2006**, *128*, 8297; (f) Ma, D. L.; Che, C. M.; Yan, S. C. *J. Am. Chem. Soc.* **2009**, *131*, 1835.
7. Gonzalez, V.; Wilson, T.; Kurihara, I.; Imai, A.; Thomas, J. A.; Otsuki, J. *Chem. Commun.* **2008**, 1868.
8. (a) Gabor, G.; Frei, Y. F.; Fischer, E. *J. Phys. Chem.* **1968**, *72*, 3266; (b) Jacques, P.; Strub, H.; See, J.; Fleury, J. P. *Tetrahedron* **1979**, *35*, 2071; (c) Kurita, N.; Nebashi, S.; Kojima, M. *Chem. Phys. Lett.* **2005**, *408*, 197; (d) Lycka, A.; Snobl, D.; Machacek, V.; Vecera, M. *Org. Magn. Reson.* **1981**, *16*, 17.
9. (a) Lycka, A.; Snobl, D.; Machacek, V.; Vecera, M. *Org. Magn. Reson.* **1981**, *15*, 390; (b) Reetz, M. T.; Ostarek, R. *J. Chem. Soc., Chem. Commun.* **1988**, 213; (c) Zollinger, H. *Color chemistry: Syntheses, Properties and Applications of Organic Dyes and Pigments*; VCH: Weinheim, 1991; (d) Patel, A. B.; Srivastava, S.; Phadke, R. S. *J. Biol. Chem.* **1999**, *274*, 21755.
10. Livnah, O.; Bayer, E. A.; Wilchek, M.; Sussman, J. L. *FEBS Lett.* **1993**, *328*, 165.
11. (a) Weber, P. C. *Acta Crystallogr., Sect. D* **1995**, *51*, 590; (b) Weber, P. C.; Pantoliano, M. W.; Simons, D. M.; Salemme, F. R. *J. Am. Chem. Soc.* **1994**, *116*, 2717; (c) Weber, P. C.; Wendoloski, J. J.; Pantoliano, M. W.; Salemme, F. R. *J. Am. Chem. Soc.* **1992**, *114*, 3197.
12. Weber, P. C.; Ohlendorf, D. H.; Wendoloski, J. J.; Salemme, F. R. *Science* **1989**, *243*, 85.
13. (a) Wang, J.; Ha, C. S. *Tetrahedron* **2009**, *65*, 6959; (b) Men, Y.; Korupoju, S. R.; Kurihara, M.; Mizutani, J.; Nishihara, H. *Chem.—Eur. J.* **2005**, *11*, 7322; (c) Kuwabara, T.; Shiba, K.; Nakajima, H.; Ozawa, M.; Miyajima, N.; Hosoda, M.; Kuramoto, N.; Suzuki, Y. *J. Phys. Chem. A* **2006**, *110*, 13521; (d) Zhang, L.; Dong, S.; Zhu, L. *Chem. Commun.* **2007**, 1891.
14. Wu, T. Q.; Wu, Q.; Guan, S. Y.; Su, H. X.; Cai, Z. J. *Biomacromolecules* **2007**, *8*, 1899.
15. Stella, L.; Capodilupo, A. L.; Bietti, M. *Chem. Commun.* **2008**, 4744.
16. (a) Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 2703; (b) Wong, W. L.; Huang, K. H.; Teng, P. F.; Lee, C. S.; Kwong, H. L. *Chem. Commun.* **2004**, 384; (c) Ma, L. J.; Li, Y.; Li, L.; Sun, J.; Tian, C. J.; Wu, Y. Q. *Chem. Commun.* **2008**, 6345; (d) Li, H. W.; Li, Y.; Dang, Y. Q.; Ma, L. J.; Wu, Y. Q.; Hou, G. F.; Wu, L. X. *Chem. Commun.* **2009**, 4453.
17. (a) Masuoka, J.; Saltman, P. *J. Biol. Chem.* **1994**, *269*, 25557; (b) Ohyoshi, E.; Hamada, Y.; Nakata, K.; Kohata, S. *J. Inorg. Biochem.* **1999**, *75*, 213; (c) Stewart, A. J.; Blindauer, C. A.; Berezenko, S.; Sleep, D.; Sadler, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3701; (d) Ding, F.; Pan, H.; Li, Z. Y.; Liu, F.; Sun, Y. *J. Lumin.* **2009**, *129*, 650.
18. (a) Vilar, R. *Eur. J. Inorg. Chem.* **2008**, 357; (b) Ojida, A.; Nonaka, H.; Miyahara, Y.; Tamaru, S.; Sada, K.; Hamachi, I. *Angew. Chem., Int. Ed.* **2006**, *45*, 5518; (c) Rhee, H. W.; Choi, H. Y.; Han, K.; Hong, J. I. *J. Am. Chem. Soc.* **2007**, *129*, 4524; (d) Jose, D. A.; Mishra, S.; Ghosh, A.; Shrivastav, A.; Mishra, S. K.; Das, A. *Org. Lett.* **2007**, *9*, 1979; (e) Zhao, Q.; Liu, S.; Shi, M.; Li, F.; Jing, H.; Yi, T.; Huang, C. *Organometallics* **2007**, *26*, 5922; (f) O'Neil, E. J.; Smith, B. D. *Coord. Chem. Rev.* **2006**, *250*, 3068; (g) Beer, P. D.; Bayly, S. R. *Top. Curr. Chem.* **2005**, *255*, 125; (h) Beer, P. D.; Hayes, E. J. *Coord. Chem. Rev.* **2003**, *240*, 167.