A colorimetric and fluorescent turn-on chemosensor operative in aqueous media for Zn^{2+} based on a multifunctionalized spirobenzopyran derivative†

Jian-Fa Zhu, Han Yuan, Wing-Hong Chan* and Albert W. M. Lee

Received 6th April 2010, Accepted 4th June 2010
First published as an Advance Article on the web 12th July 2010
DOI: 10.1039/c004871b

By conjugating spiropyran chloride **8** with 2-amino-*N*-(quinolin-8-yl)acetamide (**5**), multifunctionalized spirobenzopyran derivative **SPQN** was designed and synthesized as a water soluble colorimetric and fluorescent turn-on chemosensor for Zn^{2+}. In 50% aqueous ethanol buffer solution, **SPQN** displayed a selective chelation fluorescence enhancement (16-fold) at 650 nm and visible color change (from colorless to red) with Zn^{2+} among the metal ions examined. In addition, as the third channel to display the metal binding characteristics of **SPQN**, operating on an efficient FRET process between the quinoline and the merocyanine moiety of the sensor, ratiometric determination of Zn^{2+} can be realized.

Introduction

The design and synthesis of metal chemosensors with high selectivity and sensitivity is an active field of supramolecular chemistry.[1] Particular attention has been focused on targeting heavy and/or transition metal (HTM) ions for their detection in the cell environment.[2] The zinc(II) ion is the second most abundant transition metal essential for the human body with a concentration ranging from sub-nM to 0.3 mM.[3] The detection and imaging of Zn^{2+} in biological samples are of significant interest owing to its unique role in physiological functions.[4] Numerous scientific endeavours have been engaged in the development of fluorescent chemosensors for the *in vitro* and *in vivo* detection of Zn^{2+}.[5] However, some of the reported Zn^{2+} sensors have poor water solubility, and are subject to the interference of other metal ions. Furthermore, the majority of existing sensors are based on fluorophores such as anthracene, fluorescein and quinoline which emit at wavelengths of <550 nm.[6],[5c–e] Their use in cell environments may suffer from the interference of autofluorescence. Therefore, better designed and improved performance fluorescent sensors are still in great demand.

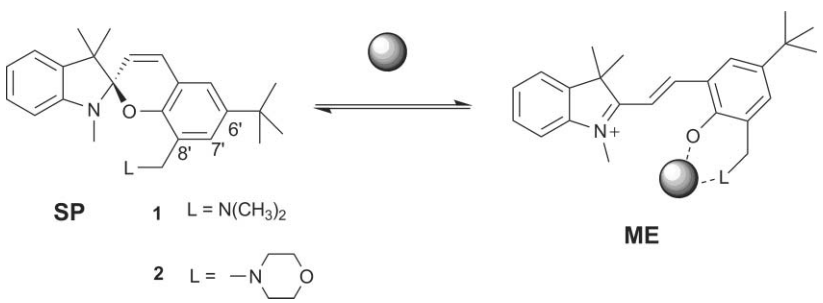
Spiropyrans (SPs) are photochromic compounds that can isomerize in response to UV/vis irradiation as a result of a reversible heterolytic cleavage of the spiro C–O bond followed by *cis–trans* isomerization, which generates a metastable merocyanine (ME).[7] By appending an additional receptive site in position 8′ on the benzopyran moiety, the isomerization of SP to ME could be modulated by specific metal ions. The metal ion-mediated ring opening of SP to ME is characterized by its color change from colorless to red, recognized readily by the naked-eye. Spiropyran-based chemosensory systems for alkali, alkaline earth, and lanthanide metals have been reported.[8] We have found that by superseding the commonly used nitro- group in the C6′-position with a *tert*-butyl group, a more photostationary closed SP form can be resulted. Such a photostationary SP derivative is an ideal molecular platform for the design of molecular/ion sensing probes. In that context, we have developed novel spiropyran-based chemosensors **1** and **2** for the colorimetric and fluorescent detection of Cu^{2+} (Scheme 1).[9] To continue our interest in the sensor development, herein we present a new colorimetric and fluorescent turn-on chemosensor **3** that responds to zinc ions in aqueous solutions.

Results and discussion

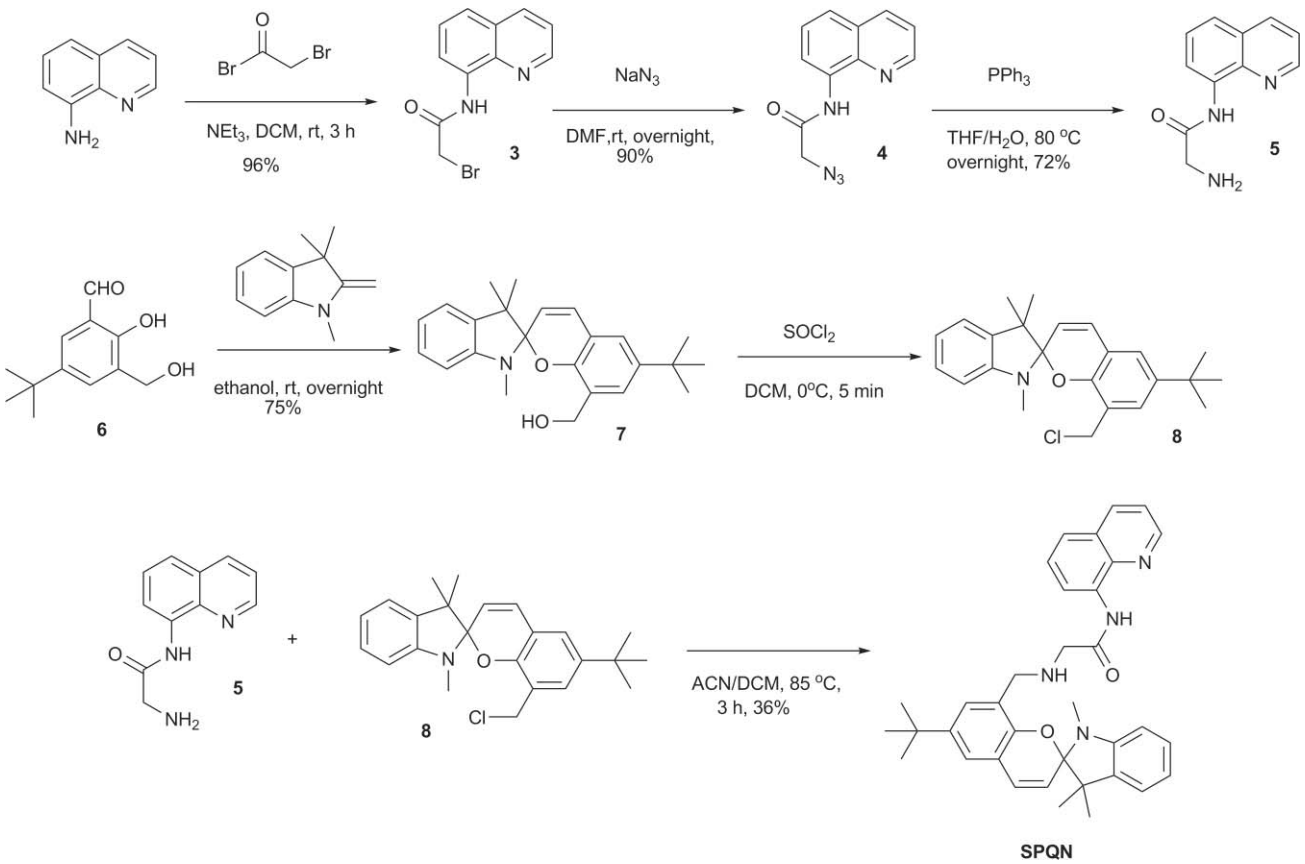
On the outset of our investigation, to confer the synthetic host with selective binding affinity to transition metal ions, we envisioned that multifunctional ligands could be appended onto the spiropyran scaffold through the C8′ carbon. In addition to the phenoxy group generated from the ring opening reaction, the C8′ pendant of the host should incorporate three additional ligating

Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, China. E-mail: whchan@hkbu.edu.hk; Fax: + 852 3411 7348; Tel: +852 3411 7076

† Electronic supplementary information (ESI) available: Supplementary data (Figs. S1–S23). See DOI: 10.1039/c004871b



Scheme 1 Metal ion-mediated ring opening of spiropyran.



Scheme 2 Synthesis of **SPQN**.

sites so as to bind metal ions with four coordinating ligands. To be a viable probe operative in aqueous medium, the binding energy of the probe and the metal ion must be comparable to that of the hydration energy of the metal. Inspired by a recent work by Zhang *et al.* for the development of a fluorescent Zn sensor,[5d] we envision that commercially available 8-aminoquinoline would provide us with a semi-rigid molecular platform to introduce multifunctional ligating sites favoring the binding on zinc metal. Thus, treatment of 8-aminoquinoline with 2-bromoacetyl bromide in the presence of triethyl amine gave the corresponding amide **3** in 96% yield. Amide **3** underwent smooth substitution reaction with NaN_3 affording azide **4**, which was then reduced by triphenylphosphine to give rise to 2-amino-*N*-(quinol-8-yl)-acetamide (**5**) in 72% yield. It is noteworthy that **5**, possessing three nitrogen ligating groups, is a good candidate to be appended on the spiropyran scaffold. The requisite spiropyran precursor **8** was assembled from 2-hydroxy-3-hydroxymethyl-5-*tert*-butylbenzaldehyde (**6**) in two simple synthetic operations as shown in Scheme 2. Condensation reaction between **6** and *N*-methyl-2,3,3-trimethylindolenine in ethanol gave a good yield of spiropyran derivative **7**, which underwent a fast nucleophilic substitution reaction with thionyl chloride to afford the corresponding chloride **8**. The crude labile **8**, without purification, was allowed to conjugate with quinoline derivative **5** to give rise to multifunctional spiropyran-based fluorescent sensor **SPQN** in 36% yield. The purity of **SPQN** was fully confirmed by ^{1}H and ^{13}C NMR, and HRMS analysis.

In addition to bearing multifunctional ligating sites for binding metals, **SPQN** comprises two non-interacting fluorophoric systems. We anticipate that the binding characteristics and photophysical properties of **SPQN** would be quite interesting.

We have previously established that only Cu^{2+} can effectively induce the ring opening of colorless spiropyrans **1** and **2** to the corresponding red colored merocyanine open form. This unique property was exploited to develop a naked-eye detection system for copper. By synthesizing a structurally more elaborate **SPQN**, we intended to tune the metal binding property of the probe to respond to other metal ion(s). Screening tests revealed that, upon addition of common metal ions, only Cu^{2+} and Zn^{2+} can turn the colorless **SPQN** probe to red color in aqueous ethanol solution. To evaluate its potential as a "naked-eye" zinc detection probe, **SPQN** was subjected to UV-vis titration with increasing concentrations of zinc. As shown in Fig. 1, upon addition of up to 2 equiv. of Zn^{2+} to a 50% aqueous ethanol buffer solution of **SPQN**, the absorbance at 230 and 350 nm decreased with concomitant formation of new peaks at 240, 380 and 540 nm, which induced a color change from colorless to red. Clear isosbestic points at 250, 290 and 360 nm were observed, which indicate the formation of only one visible active zinc complex with the probe. Furthermore, a linear dependence of the absorbance at 520 nm as a function of Zn^{2+} concentration was observed. 10 μM of Zn^{2+} can induce an 11-fold enhancement of the absorbance at 520 nm. Similarly, the spectrophotometric titration of **SPQN** with Cu^{2+} was also conducted (Fig. S1, ESI†). On the

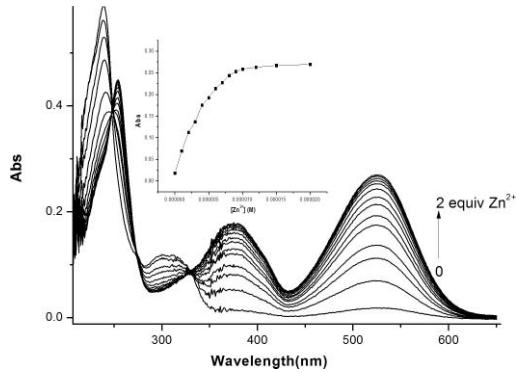


Fig. 1 UV-vis spectra of **SPQN** (10 μM) upon the titration of Zn^{2+} (0–2 equiv.) in buffer solution (50 mM HEPES, 50% ethanol, pH = 7.4). Inset: calibration curve $A_{520\,nm}$ as a function of Zn^{2+} concentration.

basis of non-linear fitting of the titration curve of a 1 : 1 binding model (*vide infra*), the association constant of Zn^{2+}–**SPQN** and Cu^{2+}–**SPQN** complexes were found to be 6.18×10^6 and 3.76×10^7 M^{-1}, respectively.

Due to the intrinsic high sensitivity associated with fluorescent detection, fluorescent sensors, particularly "off-on" sensors, are desirable and very much sought after sensing devices. **SPQN**, showing very weak fluorescent emission at 650 nm upon excitation at 515 nm, met the basic requirement to confer the probe with a low fluorescence "off" state. The fluorescent intensity of **SPQN** at 650 nm was dramatically enhanced upon addition of 1 equiv. of Zn^{2+}. Zn^{2+}-mediated opening of the spiropyan ring of **SPQN** followed by formation of a tight complex with the proximate nitrogen ligands could enhance the rigidity of the complex. The observed dramatic fluorescence enhancement of 15.8-fold could be a result of the combination effect of internal charge transfer and chelation-enhanced fluorescence (CHEF).[10] It is noteworthy that other metal ions, except Pb^{2+}, did not induce any enhancement on the fluorescence of **SPQN** (Fig. 2). Furthermore, the Zn^{2+} sensing ability of **SPQN** at different pH values was examined. At a pH below 4.0, the fluorescence of **SPQN** is very weak, presumably due to the protonation of the phenoxy and the amino group leading to a weak coordination ability for Zn^{2+}. Under acidic conditions, the solution of **SPQN** turns to yellow color which is indicative of the formation of protonated phenolic compound.[11] The fluorescence of the complex increased abruptly from pH 4.0 to 6.0, then reached a plateau of a maximum under the physiological pH window (Fig. S2, ESI†). At a higher pH, Zn^{2+} may be complexed by OH^- to reduce the free Zn^{2+}, which in turn reduces its coordination with the spiropyran. Therefore, subsequent metal binding studies were carried out in HEPES buffer solution at pH = 7.4.

The kinetics of Zn^{2+}–**SPQN** complex formation were found to be highly dependent on the water content of the solvent system (Fig. 3). In pure ethanol, a stable and steady fluorescent reading was observed within one minute after one equiv. of Zn^{2+} was introduced into the sensor solution. Increasing the water content of the aqueous ethanol solution from 20% to 80%, not only did the time required for reaching the steady fluorescent reading extend from 2.5 to 6 min, but also the intensity of the fluorescence of the complex was suppressed by as much as 50%. To have a compromise on high sensitivity, short response time and practical applications of the metal sensing probe, in subsequent investigation, we chose 50% aqueous ethanol as the optimum solvent system.

To fully assess the viability of using **SPQN** as a fluorescent chemosensor for Zn^{2+}, **SPQN** (10 μM) was titrated with increasing concentrations of Zn^{2+}. Fig. 4(a) shows that the maximum fluorescence emission of the probe at 650 nm gradually increased

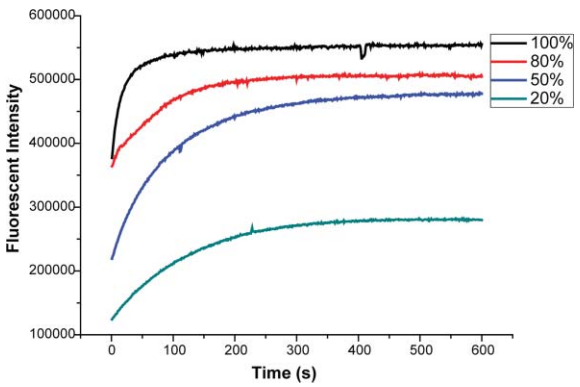


Fig. 3 Fluorescent emission intensity of **SPQN** (10 μM) at 650 nm (λ_{ex} = 515 nm) in response to the addition of Zn^{2+} (1 equiv.) as a function of time in ethanol with different contents of water.

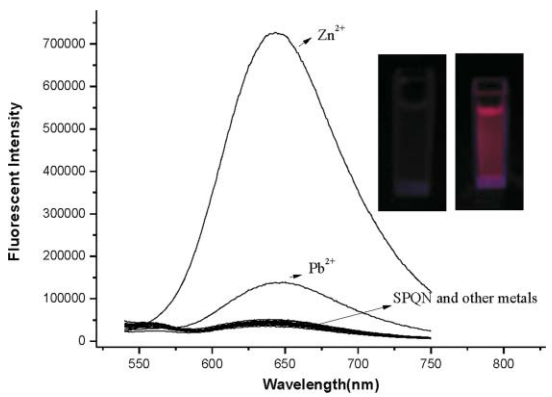


Fig. 2 Fluorescence spectra (λ_{ex} = 515 nm) of **SPQN** (10 μM) in the presence of various metal ions (1 equiv. of Mg^{2+}, Ca^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}, Hg^{2+}, Pb^{2+}, Li^+, Ag^+, Na^+, K^+, Zn^{2+}) in buffer solution (50 mM HEPES, 50% ethanol, pH = 7.4). Inset: visible emission (irradiated by 365 nm light) observed from **SPQN** in the absence and presence of Zn^{2+} (1 equiv.).

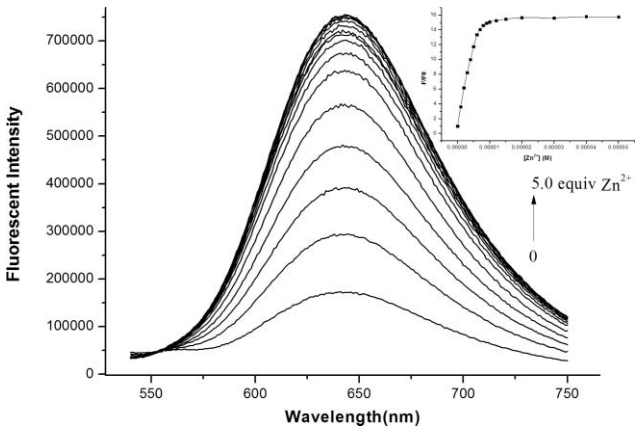


Fig. 4 Fluorescence spectra (λ_{ex} = 515 nm) of 10 μM **SPQN** upon the titration of Zn^{2+} (0–5.0 equiv.) in buffer solution (50 mM, HEPES, 50% ethanol, pH = 7.4). Inset: fluorescence intensity ratio as a function of Zn^{2+} concentration.

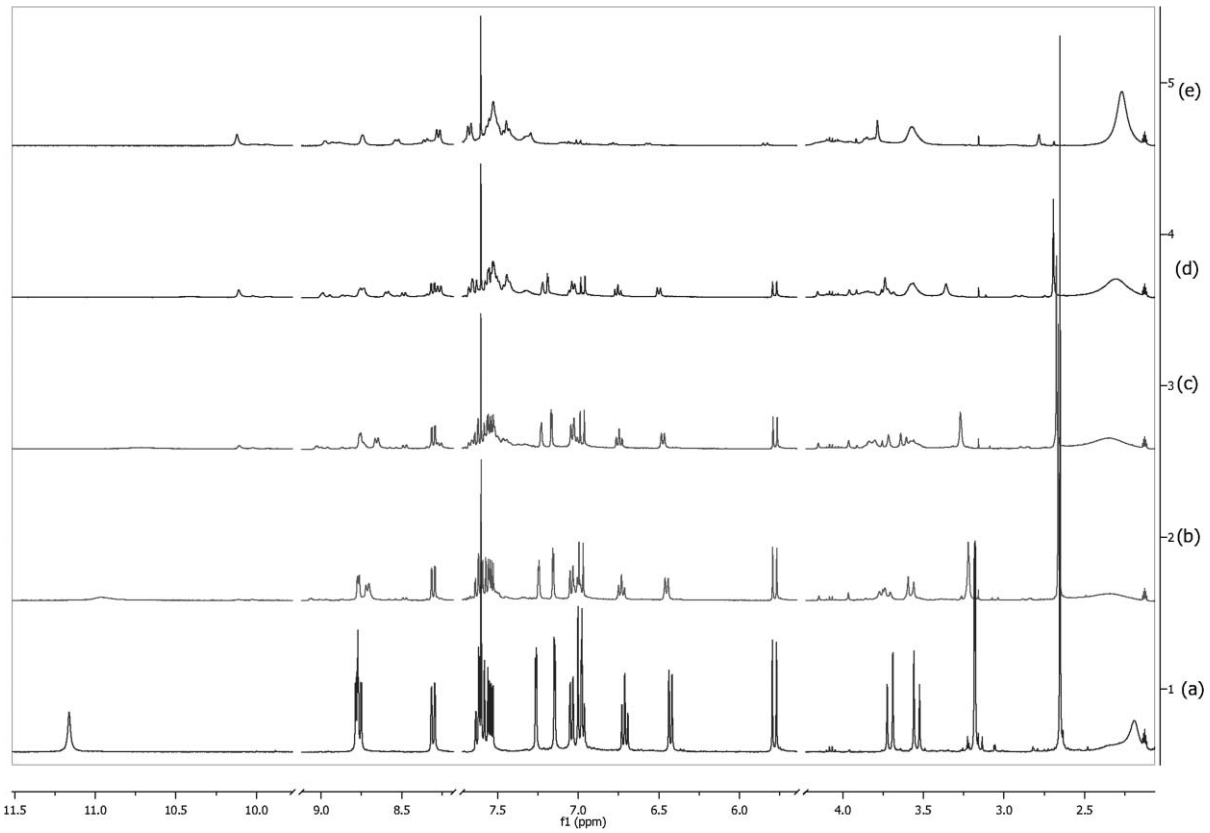


Fig. 5 Partial ^{1}H NMR spectra (400 MHz) of **SPQN** (5 mM) in CD_3CN-d_3: (a) free **SPQN**; (b) **SPQN** + 0.1 equiv. of Zn^{2+}; (c) **SPQN** + 0.2 equiv. of Zn^{2+}; (d) **SPQN** + 0.3 equiv. of Zn^{2+}; (e) **SPQN** + 0.4 equiv. of Zn^{2+}.

to almost 16-fold when 1 equiv. of Zn^{2+} was added, implicating the formation of a tight complex between **SPQN** and Zn^{2+}. The fluorescence intensity increased linearly (linear dependency coefficient $R^2 = 0.997$) with the concentration of Zn^{2+} up to a molar ratio Zn^{2+}/**SPQN** of 1 : 1, which was confirmed by Job's plot (Fig S3, ESI†). The corresponding binding constant of Zn^{2+}–**SPQN** estimated from non-linear fitting of the titration curve was found to be 7.23×10^6 M^{-1}, which is in good agreement with that obtained from UV-visible titration (*vide supra*). Although Pb^{2+} can also induce fluorescence enhancement of **SPQN** at 650 nm, on the basis of non-linear fitting of the titration curve (Fig. S4, ESI†), the corresponding binding constant of the Pb^{2+}–**SPQN** complex was found to be smaller (*i.e.* 3.70×10^6 M^{-1}).

To evaluate the binding mode of **SPQN**–Zn^{2+}, ^{1}H NMR titration was carried out. Upon gradual addition of 0.4 equiv. of Zn^{2+} to the CD_3CN-d_3 solution of **SPQN**, a large upfield shift of the amide proton from δ11.15 to 10.10 was observed, indicating the involvement of the carbonyl oxygen in complexing the metal ion.[12] At the same time, the C2 hydrogen of the quinoline moiety at δ8.80 experienced a substantial upfield shift (8.55 ppm). In contrast, the two singlets assigned to the N–H (2.22 ppm) and the CH_2 adjacent to the amide group (3.22 ppm), respectively, underwent a slight downfield shift to 2.32 and 3.35 ppm, respectively, upon Zn^{2+} coordination due to the shielding from the coordinated secondary nitrogen and carbonyl oxygen. All these observations suggested the direct interaction between the ligating groups of the probe and Zn^{2+} (Fig. 5). Consistent with the ring opening event of the spiropyran moiety triggered by the metal ion, the two doublets at δ5.78 and 6.99, ascribed to the vinyl protons of the dihydropyran ring of **SPQN**, gradually disappeared during the titration. When more than 0.4 equiv. of Zn^{2+} was added into the sensor solution, due to extensive line broadening, the resolution of the resultant ^{1}H NMR spectra became deteriorated.

To rule out the possibility that the fluorescence enhancement observed is not due to a chemical reaction (*i.e.* chemodosimeter),[13] the reversible binding of Zn^{2+} and the sensor must be established. The reversibility experiment revealed that the 16-fold fluorescence enhancement of **SPQN** caused by the addition of 1 equiv. of zinc ions can be removed completely by adding 1 equiv. of EDTA. The same extent of fluorescence enhancement can be recovered when an additional one equiv. of Zn^{2+} was introduced (Fig. S5, ESI†). Convincing evidence of the 1 : 1 binding mode of the complex of **SPQN** and Zn^{2+} was obtained by the MALDI-TOF HRMS spectroscopic data. When the complex was subjected to mass spectral measurement, a clear peak of m/z 609.2233 corresponding to $[M + Zn - H]^+$ was observed (Fig. S6, ESI†). On the basis of the combined spectroscopic information, the binding mode of **SPQN**–Zn^{2+} is proposed in Fig. 6.

The selectivities of **SPQN** to various metal ions were examined systematically. Only Zn^{2+} can induce a dramatic fluorescence enhancement of **SPQN**, while Pb^{2+} is the only second metal ion which also responds to the probe, but to a much less extent (*vide supra*). Common competing metal ions in Zn^{2+} determination, such as Mg^{2+}, Ca^{2+} and Cd^{2+}, did not respond to **SPQN**. The

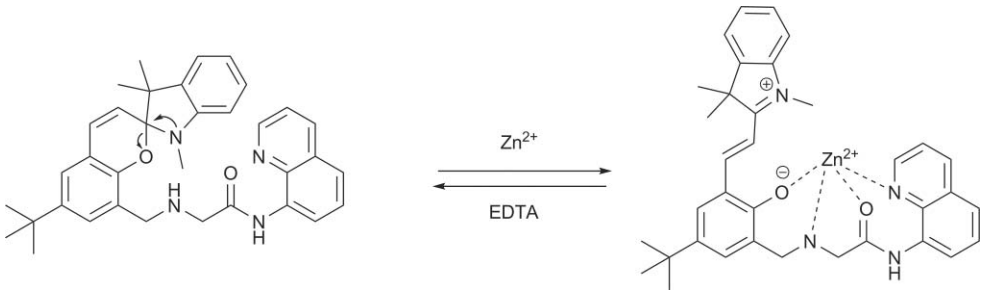


Fig. 6 Proposed binding mode of **SPQN**–Zn^{2+} complex.

titration of sensor **SPQN** with Zn^{2+} in the presence of potential competing metal ions was further determined. The results revealed that even Pb^{2+} did not show any interference effect on Zn^{2+} detection. On the other hand, Cu^{2+} was found to be a quencher to the sensing probe.

In our design of the metal ion fluorescent sensing probe, we have conjugated two separate fluorophoric moieties for the construction of **SPQN**. Would these two moieties communicate with each other when **SPQN** is allowed to interact with zinc metal? To shed light on this issue, the metal binding properties of quinoline derivative **5**, which can serve as the control compound for sensor **SPQN**, must be first investigated. Due to structural similarity between **5** and the fluorescent zinc sensor **AQZ** reported by Zhang *et al.*,[5d] compound **5**, coordinating with Zn^{2+}, displays similar fluorescence responses as those of **AQZ**. Upon addition of 0.5 equiv. of Zn^{2+}, about 8-fold fluorescence signal enhancement and a 90 nm red-shift from 398 to 488 nm of the fluorescence emission of **5** were observed (Fig. 7).[5d]

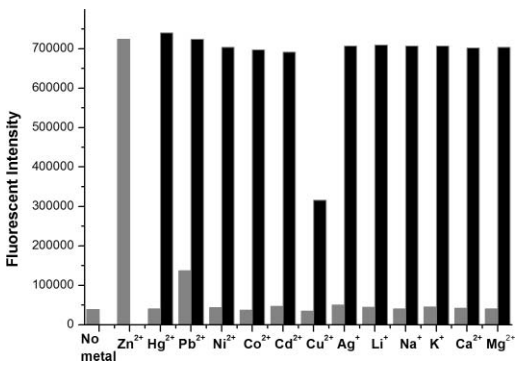


Fig. 7 Metal ion selectivity profiles of **SPQN** (10 μM) in the presence of various metal ions in buffer solution (50 mM, HEPES, 50% ethanol, pH = 7.4): (gray bars) fluorescence intensity at 650 nm in the presence of 1 equiv. of Zn^{2+}, Hg^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Li^{+}, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}; (black bars) fluorescence intensity in the presence of 1 equiv. of Zn^{2+} followed by 1 equiv. of Hg^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Li^{+}; 300 equiv. of Na^{+}, K^{+}; 100 equiv. of Ca^{2+}, Mg^{2+}.

As Zn^{2+} metal binding to the pendant chelating groups of **5**, the intramolecular charge transfer (ICT) from amide side group to the quinoline ring is enhanced and its fluorescent signal is increased (Fig. 8). It is noteworthy that upon excitation of the quinoline moiety of **SPQN** at 326 nm, the emissive peak at 401 nm overlaps significantly with the major absorption peak of the merocyanine moiety of **SPQN** (Fig. S7, ESI†). To examine whether fluorescence energy transfer (FRET) could occur between the two fluorophoric systems of **SPQN**, a fluorescence titration of **SPQN** against increasing concentrations of Zn^{2+} was conducted by exciting the quinoline moiety of **SPQN** at 326 nm. In contrast to the control compound **5**, as shown in Fig. 9, only the quenching of fluorescence signal of **SPQN** at 398 nm was observed. The red-shifted fluorescence signal centered at 488 nm was completely erased by the inner filter effect caused by the red color of the merocyanine moiety of **SPQN**.[9a,14] Concomitantly, the merocyanine moiety of **SPQN**, serving as the energy acceptor, is excited to give a new emissive peak at 650 nm. A clear FRET

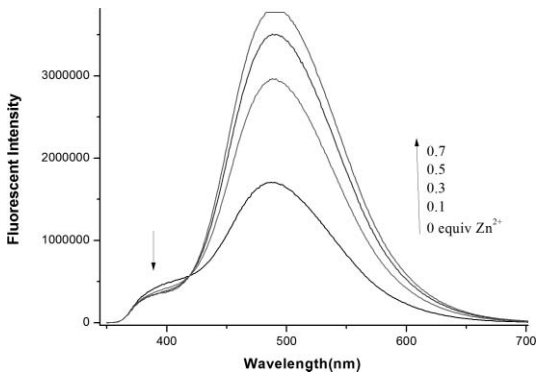


Fig. 8 Fluorescence spectra (λ_{ex} = 326 nm) of control compound **5** (10 μM) in buffer solution (50 mM, HEPES, 50% ethanol, pH = 7.4) in the presence of different concentrations of Zn^{2+}.

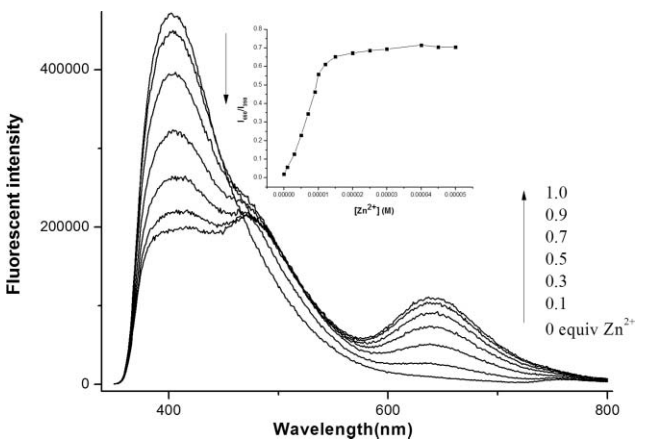
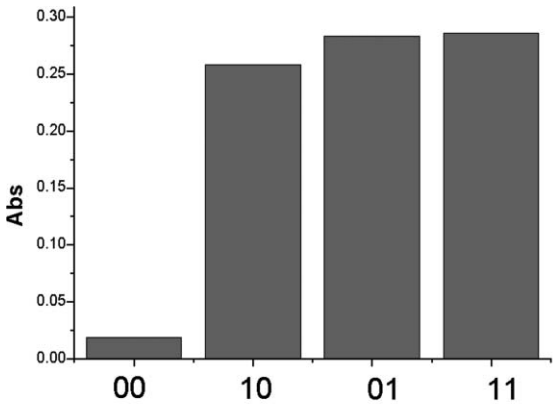


Fig. 9 Fluorescence spectra (λ_{ex} = 326 nm) of **SPQN** (10 μM) in buffer solution (50 mM, HEPES, 50% ethanol, pH = 7.4) in the presence of different concentrations of Zn^{2+}. Inset: dependence of the ratio of fluorescence intensities at 650 and 398 nm wavelengths of the probe on the Zn^{2+}.

process from the quinoline moiety to the merocyanine moiety of **SPQN** took place.

The FRET process was saturated when 1 equiv. of Zn^{2+} was added to the probe. The red-shifted emissive peak of the quinoline moiety of **SPQN** emerged when more than 1 equiv. of Zn^{2+} was introduced (Fig S8, ESI†). On the basis of the efficient FRET observed in **SPQN**, a ratiometric detection of Zn^{2+} is observed as shown in the inset of Fig. 9. When as little as 0.1 equiv. of Zn^{2+} was added to the probe, an enhancement of 3.0-fold was observed for I_{650}/I_{398}.

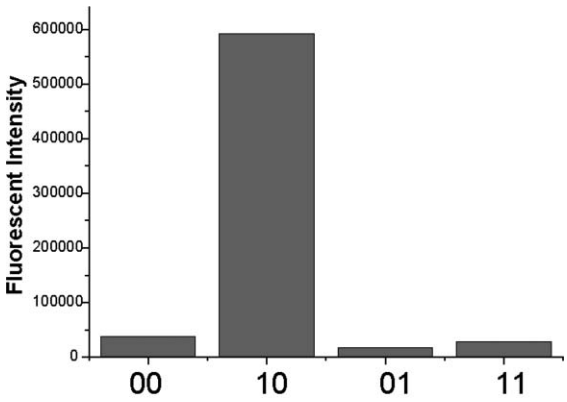
Spiropyran derivatives have been used to construct molecular logic circuits of high complexity.[15] The optical output signals of **SPQN** in response to metal ions binding can be used to design **OR** and **INHIBIT** (**INH**) logic gates. The **OR** gate is one of the basic logic gates that implement logical disjunction, which results in a high output if one or both inputs to the gate are high.[16] The absorbance of **SPQN** (10 μM) at 520 nm (Output 1) can be enhanced by Cu^{2+} or Zn^{2+} or both, enabling the **OR** logic function when 1 equiv. of Cu^{2+} and 1 equiv. of Zn^{2+} are taken as inputs (Fig. 10).



Input		Output	
Zn^{2+}	Cu^{2+}	OR (Absorbance)	
0	0	Low (0.018)	0
1	0	High (0.258)	1
0	1	High (0.283)	1
1	1	High (0.286)	1

Fig. 10 Absorbance output of **SPQN** (10 μM) at 520 nm in the presence of chemical inputs, Zn^{2+} (10 μM) and Cu^{2+} (10 μM), and the logic table of the **OR** gate.

On the other hand, the fluorescence output of **SPQN** at 650 nm (Output 2) was utilized to fabricate a two-input **INH** logic gate.[17] The output signal of an **INH** gate is inhibited by one of the active inputs. As shown in Fig. 11, a basic two-input **INHIBIT** action can be obtained for **SPQN** (10 μM) with Zn^{2+} (10 μM) and Cu^{2+} (20 μM) as inputs. Fluorescence emission enhancement at 650 nm of **SPQN** is observed only in the presence of 1 equiv. of Zn^{2+} and the absence of Cu^{2+}, displaying the output as "1". Under other circumstances the fluorescence of **SPQN** is quenched, leading to output "0".



Input		Output	
Zn^{2+}	Cu^{2+}	INH (Fluorescence)	
0	0	Low (3.78)	0
1	0	High (59.3)	1
0	1	Low (1.69)	0
1	1	Low (2.82)	0

Fig. 11 Fluorescence output of **SPQN** (10 μM) at 650 nm ($\lambda_{ex} = 520$ nm) in the presence of chemical inputs, Zn^{2+} (10 μM) and Cu^{2+} (20 μM), and the logic table of the **INH** gate.

Conclusions

To extend the metal ion sensing ability of spiropyran-based materials, by appending a multi-ligating moiety onto a spiropyran scaffold, we have developed a "naked-eye" and fluorescence turn-on chemosensor **SPQN** for Zn^{2+}. Selective detection of Zn^{2+} has been realized in aqueous solutions with outstanding sensitivity. All biologically relevant metal ions and toxic heavy metals such as Cd^{2+}, Pb^{2+} and Hg^{2+} did not interfere with the zinc ion detection. Since the fluorescence signal output of the probe is in the near IR region (*i.e.* 650 nm) where the autofluorescence of biological samples is minimal, its potential use for *in vivo* detection of Zn^{2+} is implicated. The binding mode of sensor and zinc was established by the combined UV-vis, fluorescence and NMR method. In addition, when **SPQN** interacts with Zn^{2+}, a fairly efficient FRET took place between the quinoline (donor) and the merocyanine (acceptor) moieties of **SPQN**. In essence, **SPQN** has shown its ability for sensing Zn^{2+} in aqueous solutions through three different channels: turning the colorless sensor to red, giving an enhanced fluorescence signal at 650 nm and delivering a ratiometric FRET output at I_{650}/I_{398}. In addition, the color and fluorescence responses of **SPQN** upon metal chelation were employed for developing truth tables for **OR** and **INHIBIT** logic gates.

Experimental

1 General methods

The melting point was determined with a MEL-TEMPII melting point apparatus (uncorrected). ^{1}H NMR and ^{13}C NMR spectra

were recorded on a VARIAN INOVA or Bruka Advance-III 400 spectrometer (at 400 and 100 MHz, respectively) in $CDCl_3$. Low resolution mass spectra were recorded on a Finnigan MAT SSQ-710 mass spectrometer while high resolution mass spectra were obtained on a Bruker Autoflex mass spectrometer (MALDI-TOF) or electrospray ionization high-resolution mass spectra on an API Qstar Pulsari mass spectrometer. Fluorescent emission spectra and UV-vis spectra were collected on a PTI luminescence lifetime spectrometer and a Cary UV-100 spectrometer, respectively. Unless specified, all fine chemicals were used as received.

2 Synthesis of the precursors of SPQN and SPQN

2.1 Synthesis of 2-bromo-*N*-(quinolin-8-yl)acetamide (3). 8-Aminoquinoline (0.5 g, 3.46 mmol) was dissolved in dry dichloromethane (20 ml) and triethylamine (0.35 g, 3.46 mmol) was added into this solution. After the mixture was stirred at 0 °C for 10 min, bromoacetyl bromide (0.84 g, 4.16 mmol) was introduced dropwise to the stirred solution over a period of 20 min. The reaction mixture was then stirred at room temperature for 3 h. Subsequently the solvent was removed, the crude product was purified by column chromatography on SiO_2, using petroleum ether–ethyl acetate (6 : 1) as the eluant to afford **3** as white solids (0.88 g, 96% yield) Mp: >300 °C, ^{1}H NMR (400 MHz, $CDCl_3$): 10.72 (1 H, s), 8.86 (1 H, dd, J = 4.0, 1.6 Hz), 8.75 (1 H, dd, J = 5.6, 3.6 Hz), 8.18 (1 H, dd, J = 8.0, 3.6 Hz), 7.57 (2 H, m), 7.49 (1 H, dd, J = 8.4, 4.0 Hz), 4.14 (2 H, s); ^{13}C NMR (100 MHz, $CDCl_3$): 164.0, 148.6, 138.7, 136.3, 133.8, 127.9, 127.2, 122.5, 121.8, 116.6, 29.7. HRMS (ESI): m/z calcd for $C_{11}H_{10}N_2OBr$ $[M + 1]^+$ 264.9976, found, 264.9957.

2.2 Synthesis of 2-azido-*N*-(quinolin-8-yl)acetamide (4). A mixture of **3** (0.8 g, 3.0 mmol) and sodium azide (0.4 g, 6 mmol) was dissolved in DMF (20 ml). The reaction mixture was stirred overnight at room temperature. Then water was added and the mixture was extracted repeatedly with ethyl acetate (3 × 30 ml). The combined organic layers were washed several times with water and dried by anhydrous sodium sulfate. After filtering and removal of the solvent *in vacuo*, the residue was purified by column chromatography on SiO_2, using petroleum ether–ethyl acetate (6 : 1) as the eluant to afford **4** as white solids (0.62 g, 90% yield). Mp: 69–71 °C, ^{1}H NMR (400 Hz, $CDCl_3$): 10.55 (1 H, s), 8.86 (1 H, dd, J = 4.4, 1.6 Hz), 8.76 (1 H, dd, J = 6.0, 1.6 Hz), 8.18 (1 H, dd, J = 8.0, 1.6 Hz), 7.56 (2 H, m), 7.49 (1 H, dd, J = 8.0, 4.4 Hz), 4.26 (2 H, s); ^{13}C (100 Hz, $CDCl_3$): 165.1, 148.7, 138.6, 136.4, 133.5, 128.0, 127.2, 122.5, 121.8, 116.8, 53.5. HRMS (ESI): m/z calcd for $C_{11}H_{10}N_5O$ $[M + 1]^+$ 228.0885, found, 228.0896.

2.3 Synthesis of 2-amino-*N*-(quinolin-8-yl)acetamide (5). To the solution of azide **4** (0.50 g, 2.2 mmol) in THF–water (6 : 1) (10 ml) was added triphenylphosphine (1.15 g, 4.4 mmol) at room temperature. The reaction mixture was then stirred at 85 °C for 24 h. The mixture was concentrated, and the residue was purified by column chromatography on SiO_2 using ethyl acetate–ethanol (5 : 1) as the eluant to afford **5** (0.32 g, 72% yield) as white solids. Mp: 108–110 °C; ^{1}H NMR (400 MHz, $CDCl_3$): 11.30 (1 H, s), 8.87 (1 H, dd, J = 4.4, 1.6 Hz), 8.84 (1 H, dd, J = 6.8, 2.0 Hz), 8.16 (1 H, dd, J = 8.4, 2.0 Hz), 7.56 (2 H, m), 7.46 (1 H, dd, J = 8.4, 4.4 Hz), 3.65 (2 H, s), 1.82(2 H, s); ^{13}C NMR (100 MHz, $CDCl_3$): 171.6, 148.6, 138.9, 136.3, 134.2, 128.1, 127.3, 121.8, 121.6, 116.5, 46.1. HRMS (MALDI-TOF): m/z calcd for $C_{11}H_{11}N_3O$ $[M^+]$ 201.0896, found, 201.0901.

2.4 Synthesis of (6-*tert*-butyl-1′,3′,3′-trimethylspiro[chromene-2,2′-indoline]-8-yl)methanol (7). To a solution of 5-*tert*-butyl-2-hydroxy-3-(hydroxymethyl)-benzaldehyde (3 g, 14.4 mmol) in ethanol (20 ml) was added 1,3,3-trimethyl-2-methyleneindoline (2.5 g, 14.4 mmol). The mixture was stirred at room temperature for about 12 h. Then the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel using petroleum ether–ethyl acetate (10 : 1) as the eluant to afford **7** as yellow oils (3.9 g, 75% yield), which could turn to red color after several days in the dark. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00–7.20 (m, 4 H), 6.81–6.87 (m, 2 H), 5.70 (d, 1 H, J = 10 Hz), 4.50 (m, 2 H), 2.67 (s, 1 H), 1.17–1.32 (m, 15 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.7, 147.8, 142.7, 136.7, 130.0, 127.7, 126.0, 125.9, 123.1, 121.5, 119.4, 118.0, 107.0, 104.0, 61.8, 51.3, 34.1, 31.5, 29.0, 25.7, 20.3. HRMS (MALDI-TOF): m/z calcd for $C_{24}H_{30}NO_2$ $[M + 1]^+$ 364.2271, found, 364.2244.

2.5 Synthesis of SPQN. To a dichloromethane solution (20 ml) of alcohol **7** (270 mg, 0.74 mmol) at 0 °C, was added 5 drops of $SOCl_2$. The mixture was stirred at room temperature for about 5 min. Saturated sodium bicarbonate (20 ml) was added to the reaction mixture immediately. Then the crude product **8** was extracted with dichloromethane (3 × 20 ml). The combined organic layers were dried, filtered and evaporated to dryness. To the acetonitrile solution (30 ml) of crude **8**, amide **5** (0.15 g, 0.74 mmol) and potassium carbonate (0.31 g, 2.22 mmol) were introduced successively. The reaction mixture was stirred at 85 °C for 3 h. Then water was added, and the mixture was extracted with ethyl acetate (3 × 20 ml) and dried by anhydrous sodium sulfate. After filtering and removal of the solvent *in vacuo*, the residue was purified by column chromatography on SiO_2 using petroleum ether–ethyl acetate (15 : 1–10 : 1) as the eluant to afford **SPQN** as yellow solids (0.146 g, 36% yield). Mp: 65–67 °C.^{1}H NMR (400 MHz, $CDCl_3$): 11.24 (1 H, s), 8.82 (1 H, dd, J = 7.2, 2.0 Hz), 8.78 (1 H, dd, J = 4.0, 1.6 Hz), 8.16 (1 H, dd, J = 8.0, 1.6 Hz), 7.56 (2 H, m), 7.46 (1 H, dd, J = 8.4, 7.2 Hz), 7.11(1 H, d, J = 2.4 Hz), 7.05-6.98 (3 H, m), 6.92 (1 H, d, J = 10.4 Hz), 6.76 (1 H, m), 6.43 (1 H, d, J = 7.6 Hz), 5.74 (1 H, d, J = 10 Hz), 3.85 (1 H, d, J = 13.6 Hz), 3.60 (1 H, d, J = 13.6 Hz), 3.23 (2 H, s), 2.68(3 H, s), 1.89 (1 H, s), 1.34 (1 H, s), 1.26 (9 H, s), 1.20 (3 H, s); ^{13}C NMR (400 MHz, $CDCl_3$): 170.7, 150.0, 148.5, 147.7,142.6, 139.0, 136.8, 136.2, 134.4, 130.2, 128.1, 127.6, 127.6, 127.3, 124.2, 122.9, 121.7, 121.5, 121.4, 119.3, 118.3, 118.2, 116.6, 106.8, 104.2, 52.6, 51.0, 49.6, 34.0, 31.5, 29.0, 25.7, 20.3. HRMS (MALDI-TOF) m/z calcd for $C_{35}H_{38}N_4O_2$ $[M^+]$ 546.2989, found 546.2979.

Acknowledgements

The work was supported by a grant from the Research Grant Council of Hong Kong (HKBU 200407).

Notes and references

1 For reviews, see: (*a*) *Fluorescent Chemosensors for Ion and Molecular Recognition*, ed. A. W. Czarnik, American Chemical Society, Washington, DC, 1993; (*b*) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, T. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem.*

Rev., 1997, **97**, 1515; (*c*) R. Martinez-Manez and F. Sancanon, *Chem. Rev.*, 2003, **103**, 4419; (*d*) T. Gunnlaugsson, M. Glynn, G. M. Tocci, P. E. Kruger and F. M. Pfeffer, *Coord. Chem. Rev.*, 2006, **250**, 3094.
2 Selected recent examples: (*a*) S. C. Burdette, G. K. Walkup, B. Spingler, R. Y. Tsien and S. J. Lippard, *J. Am. Chem. Soc.*, 2001, **123**, 7831; (*b*) P. Jiang, L. Chen, J. Lin, Q. Liu, J. Ding, X. Gao and Z. Guo, *Chem. Commun.*, 2002, 1424; (*c*) S-K. Ko, Y-K. Yang, J. Tae and I. Shin, *J. Am. Chem. Soc.*, 2006, **128**, 14150; (*d*) M. Taki, M. Desaki, A. Ojida, S. Lyoshi, T. Hirayma, I. Hamachi and Y. Yamamoto, *J. Am. Chem. Soc.*, 2008, **130**, 12564; (*e*) X. Zhang, Y. Xiao and X. Qian, *Angew. Chem., Int. Ed.*, 2008, **47**, 8025.
3 S. J. Lippard and J. M. Berg, *Principle of Bioinogranic Chemistry*, University Science Book, CA, 1994, pp. 10, 14, pp. 78–183.
4 (*a*) A. I. Bush, *Curr. Opin. Chem. Biol.*, 2000, **4**, 184; (*b*) M. P. Cuajungco and K. Y. Faget, *Brain Res. Rev.*, 2003, **41**, 44–56.
5 (*a*) Selected recent examples: R. Parkesh, T. C. Lee and T. Gunnlaugsson, *Org. Biomol. Chem.*, 2007, **5**, 310; (*b*) Y. Liu, N. Zhang, Y. Chen and L-H. Wang, *Org. Lett.*, 2007, **9**, 315; (*c*) H-H. Wang, Q. Gan, X-J. Wang, L. Xue, S-H. Liu and H. Jiang, *Org. Lett.*, 2007, **9**, 4995; (*d*) Y. Zhang, X. Guo, W. Si, L. Jia and X. Qian, *Org. Lett.*, 2008, **10**, 473; (*e*) L. Xue, C. Liu and H. Jiang, *Org. Lett.*, 2009, **11**, 1655; (*f*) J. Wang and C-S. Ha, *Tetrahedron*, 2009, **65**, 6959.
6 (*a*) S. Huang, R. Clark and L. Zhu, *Org. Lett.*, 2007, **9**, 4999; (*b*) S. C. Burdette, G. K. Walkup, B. Spingler, R. Y. Tsien and S. J. Lippard, *J. Am. Chem. Soc.*, 2001, **123**, 7831; (*c*) P. Jiang, L. Chen, J. Lin, Q. Liu, J. Ding, X. Gao and Z. Guo, *Chem. Commun.*, 2002, 1424.
7 (*a*) F. M. Raymo and S. Giordani, *J. Am. Chem. Soc.*, 2001, **123**, 4651; (*b*) S. Giordani and F. M. Raymo, *Org. Lett.*, 2003, **5**, 3559; (*c*) V. I. Minkin, *Chem. Rev.*, 2004, **104**, 2751.
8 (*a*) K. Kimura, T. Teranishi, M. Yokoyama, S. Yajima, S. Miyake, H. Sakamoto and M. Tanaka, *J. Chem. Soc., Perkin Trans. 2*, 1999, 199; (*b*) M. Tanaka, M. Nakamura, M. a. A. Salhin, T. Ikeda, K. Kamada, H. Ando, Y. Shibutani and K. Kimura, *J. Org. Chem.*, 2001, **66**, 1533; (*c*) S. A. Ahmed, M. Tanaka, H. Ando, K. Tawa and K. Kimura, *Tetrahedron*, 2004, **60**, 6029; (*d*) H. Sakamoto, H. Takagaki, M. Nakamura and K. Kimura,, *Anal. Chem.*, 2005, **77**, 1999; (*e*) K. Kimura, T. Uraumi, T. Teranishi, M. Yokoyama, H. Sakamoto, M. Okamoto, R. Arakawa, H. Moriguchi and Y. Miyaji, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2452.
9 (*a*) N. Shao, Y. Zhang, S. M. Cheung, R. H. Yang, W. H. Chan, T. Mo, K. Li and F. Liu, *Anal. Chem.*, 2005, **77**, 7294; (*b*) N. Shao, J. Y. Jin, H. Wang, Y. Zhang, R. H. Yang and W. H. Chan, *Anal. Chem.*, 2008, **80**, 3466.
10 (*a*) S. Aoki, D. Kagata, M. Shiro, K. Takeda and E. Kimura, *J. Am. Chem. Soc.*, 2004, **126**, 13377; (*b*) R. Badugu, J. R. Lakowicz and C. D. Geddes, *J. Am. Chem. Soc.*, 2005, **127**, 3635.
11 F. M. Raymo and S. Giordani, *J. Am. Chem. Soc.*, 2001, **123**, 4651.
12 Z. Xu, K-H. Baek, H. N. Kim, J. Cui, X. Qian, D. R. Spring, I. Shin and J. Yoon, *J. Am. Chem. Soc.*, 2010, **132**, 601.
13 K. C. Song, J. S. Kim, S. M. Park, K.-C. Chung, S. Ahn and S.-K. Chang, *Org. Lett.*, 2006, **8**, 3413.
14 (*a*) J. F. Holland, R. E. Teets, P. M. Kelly and A. Timnick, *Anal. Chem.*, 1977, **49**, 706; (*b*) R. Leese and E. L. Wehry, *Anal. Chem.*, 1978, **50**, 1193.
15 (*a*) F. M. Raymo and S. Giordani, *Org. Lett.*, 2001, **3**, 3475; (*b*) F. M. Raymo and S. Giordani, *J. Am. Chem. Soc.*, 2001, **123**, 4651.
16 P. Ghosh, P. K. Bharadwaj, S. Mandal and S. Ghosh, *J. Am. Chem. Soc.*, 1996, **118**, 1553.
17 (*a*) A. P. De Silva, I. M. Dixon, H. Q. N. Gunaratne, T. Gunnlaugsson, P. R. S. Maxwell and T. E. Rice, *J. Am. Chem. Soc.*, 1999, **121**, 1393; (*b*) T. Gunnlaugsson, D. A. Mac Donail and D. Parker, *Chem. Commun.*, 2000, 93.

