Molecular Devices

DOI: 10.1002/ange.201406002

Sequential Logic Operations with a Molecular Keypad Lock with Four Inputs and Dual Fluorescence Outputs**

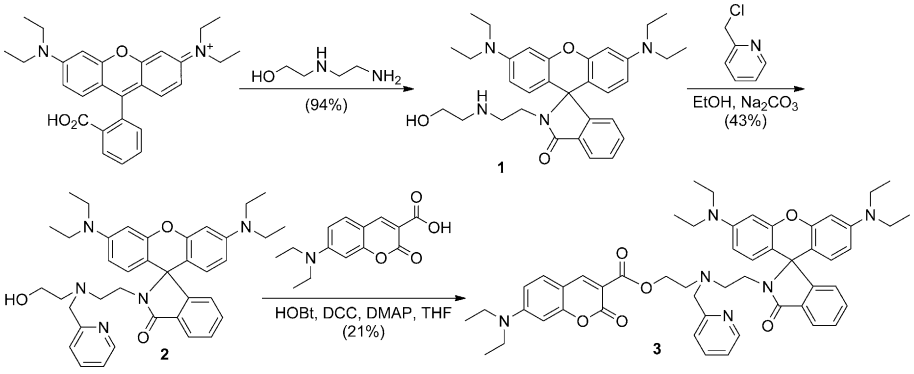
*Xiong-Jie Jiang and Dennis K. P. Ng**

Abstract: *A novel coumarin–rhodamine conjugate was prepared, and its metal binding properties were studied by UV/Vis and fluorescence spectroscopy. The conjugate serves as a ratiometric and highly selective fluorescent sensor for Hg^{2+} ions. Its metal-responsive spectral properties were utilized to construct a molecular keypad lock with four inputs and dual fluorescence outputs. The complexity of this molecular logic network can greatly enhance the security level of this device.*

Molecular computing has emerged as a promising alternative for information processing.[1] Compared with traditional silicon-based circuitries, molecular logics could result in smaller and more efficient devices. A vast number of molecular systems that can mimic the functions of individual logic gates have been reported.[2] Even for some complicated functional devices, such as adders/subtractors, encoders/decoders, and multiplexers/demultiplexers, the corresponding molecular mimics have been developed.[3] In contrast to these combinational logic systems, where the input history is of no consequence for the device function, sequential logic networks depend on both the input combination and the input sequence. Mimicking sequential logic operations is therefore more challenging, and its development is still in the infancy.[4]

As a sophisticated class of sequential logic systems, molecular keypad locks have received considerable attention. As they can only be unlocked by the correct input combination and sequence, that is, the password, they can serve as security devices for information protection at the molecular level. Shanzer et al. reported the first molecular keypad lock, which was based on a fluorescein–linker–pyrene assembly with an acidic iron chelator (ethylenediaminetetraacetate, EDTA), a base (NaOAc), and UV light as the inputs.[5] Since then, a number of related systems with various inputs and outputs have been sporadically reported.[6] Most of them only involve two inputs (excluding light) and a single output, which enables them to function as so-called crossword puzzles. Systems with three inputs[7] or dual outputs[8] have remained extremely rare. Apart from these molecular systems, a number of biomolecular keypad locks that are based on the use of enzymes,[9] DNA,[10] aptamers,[11] antibodies,[12] or bacterial toxins[13] have also been developed. Some of them work with three inputs, and one DNA-based system that can have five inputs has been described,[10f] but these systems only feature a single output channel. There has been an impetus to develop molecular keypad locks with a larger number of inputs and outputs as this can enhance their security level. Herein, we report a novel coumarin–rhodamine conjugate that can perform sequential logic operations and function as a molecular keypad lock with four inputs and dual fluorescence outputs. To the best of our knowledge, such complex molecular devices have not been reported thus far.

The coumarin–rhodamine conjugate (compound **3**) was prepared by typical condensation and substitution reactions using rhodamine B as a starting material (Scheme 1). This



Scheme 1. Synthesis of conjugate **3**. DCC = 1,3-dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine, HOBt = 1-hydroxybenzotriazole.

compound and the intermediate products **1** and **2** were fully characterized with various spectroscopic methods and elemental analysis (for details, see the Supporting Information).

The metal-ion binding properties of compounds **1**–**3** were first examined in CH_3CN with fluorescence spectroscopy. Compound **1** showed a very weak fluorescence, which was not affected by the presence of metal ions, including Na^+, K^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, or Pb^{2+} ions. In contrast, compound **2** exhibited a highly selective fluorescence en-

[*] Dr. X.-J. Jiang, Prof. D. K. P. Ng
Department of Chemistry
The Chinese University of Hong Kong
Shatin, N.T., Hong Kong (China)
E-mail: dkpn@cuhk.edu.hk

[**] We thank The Chinese University of Hong Kong for offering a Postdoctoral Fellowship to X.J.J.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201406002.

hancement (at 580 nm) towards Hg^{2+} ions (Supporting Information, Figure S1). It is likely that the additional pyridyl group in **2** imparts a multidentate environment that can strongly bind Hg^{2+} ions, which can subsequently trigger the conversion of the non-fluorescent spirocyclic form of rhodamine into the fluorescent open-ring structure. Hence, the selectivity towards Hg^{2+} ions may be attributed to the preferential binding of these ions and their unique properties for promoting this ring-opening process.[14] A Job's plot of the fluorescence data revealed a 1:1 binding stoichiometry between **2** and Hg^{2+} ions. The binding constant K was determined to be $(1.2 \pm 0.1) \times 10^5\,\text{M}^{-1}$ by non-linear fitting of the titration curve (Figure S2).[15]

The fluorescence spectrum of **3** displayed a strong band at 460 nm, which is due to the coumarin moiety (Figure 1). Upon addition of Na^+, K^+, or Ca^{2+} ions, the spectrum showed

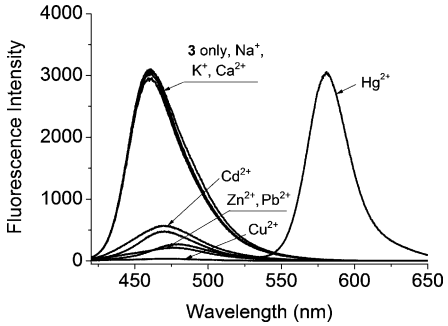


Figure 1. Changes in the fluorescence spectrum of **3** (10 μM) in CH_3CN upon addition of 10 equivalents of various metal ions ($\lambda_{ex} = 365$ nm).

negligible changes. However, the addition of Cu^{2+}, Zn^{2+}, Cd^{2+}, or Pb^{2+} ions largely quenched the fluorescence. Interestingly, the addition of Hg^{2+} ions not only quenched the fluorescence at 460 nm, but also induced a strong emission band at 580 nm, which could be assigned to the open-ring form of rhodamine. The effects on the absorption spectrum of the conjugate are shown in Figure S3. Whereas Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} ions caused a bathochromic shift of 18–32 nm and a slight enhancement in the intensity of the absorption band of coumarin, Hg^{2+} ions gave an additional band at 560 nm, which is due to the rhodamine moiety. These colorimetric and fluorescence changes could be easily seen with the naked eye (Figure S4). Apart from this advantage and the high selectivity, the ratiometric fluorescence response could greatly enhance the sensitivity of this probe, enabling it to be superior to the conventional intensity-based turn-on/turn-off fluorescent Hg^{2+} sensors.[16]

Figure 2a shows the changes in the fluorescence spectrum of **3** upon titration with Hg^{2+} ions in CH_3CN. The emission band at 460 nm decreased gradually, and a new emission band at 580 nm emerged almost concomitantly. A closer examination of the profiles (Figure 2b) revealed that the decrease in fluorescence intensity at 460 nm ceased after one equivalent of Hg^{2+} ions had been added, whereas the new band at 580 nm appeared after approximately 0.6 equivalents of Hg^{2+} ions

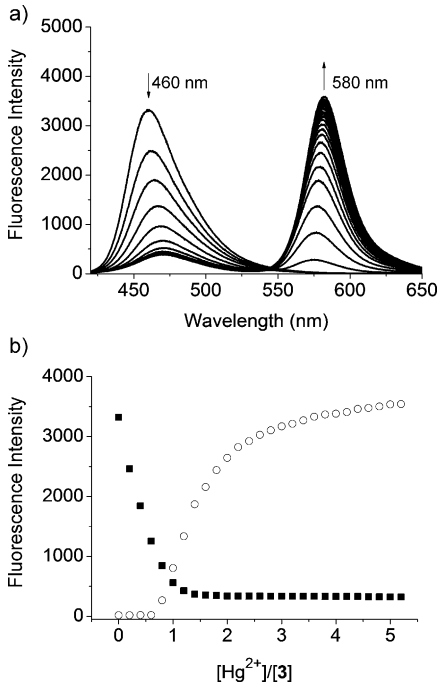


Figure 2. a) Changes in the fluorescence spectrum of **3** (10 μM) upon titration with Hg^{2+} ions in CH_3CN ($\lambda_{ex} = 365$ nm). b) Changes in the fluorescence intensities at 460 nm (■) and 580 nm (○) as a function of the $[Hg^{2+}]/[\mathbf{3}]$ ratio.

had been added, and its intensity increased continuously with the $[Hg^{2+}]/[\mathbf{3}]$ ratio. The Job's plots that were constructed by monitoring these two bands (Figure S5) suggest a 1:1 binding between the coumarin moiety and the Hg^{2+} ions, followed by a further 1:1 complexation between the rhodamine part and Hg^{2+} ions, which results in ring opening of the rhodamine moiety (Figure S6). By non-linear fitting of the titration curves for these two bands, the corresponding binding constants K_1 and K_2 were determined to be $(3.7 \pm 0.6) \times 10^7\,\text{M}^{-1}$ and $(2.0 \pm 0.1) \times 10^6\,\text{M}^{-1}$, respectively.[15] These values are larger than the binding constant of **2**, which confirms the presence of cooperative effects that are due to the coumarin unit. Similarly, upon addition of Hg^{2+} ions, the coumarin absorption band shifted bathochromically to approximately 436 nm, and a new absorption band at 560 nm, which is due to the open-ring structure of rhodamine, appeared (Figure S7). Their titration profiles were very similar to those of the fluorescence counterparts and were also in accord with a stepwise complexation process.

The binding behavior of **3** with other metal ions, including Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} ions, was also investigated in a similar fashion. As shown in Figure S8–S11, all of these metal ions formed a 1:1 complex with **3**, and the emission band that is due to the rhodamine moiety was not observed. The corresponding binding constants are summarized in Table S1, which also includes the values for Hg^{2+} ions for comparison. Cu^{2+} ions exhibited the strongest complexation with **3** with a binding constant of $(1.6 \pm 0.3) \times 10^8\,\text{M}^{-1}$, which is about four times larger than the binding constant for Hg^{2+} ions.

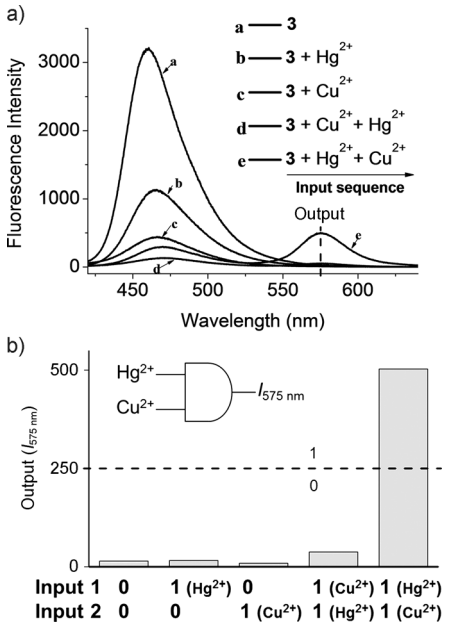


Figure 3. a) Fluorescence spectra of **3** (10 μM) in CH_3CN under different input conditions; $[Hg^{2+}]=5$ μM, $[Cu^{2+}]=10$ μM ($\lambda_{ex}=365$ nm). b) Outputs in the fluorescence channel at 575 nm in response to two sequential inputs.

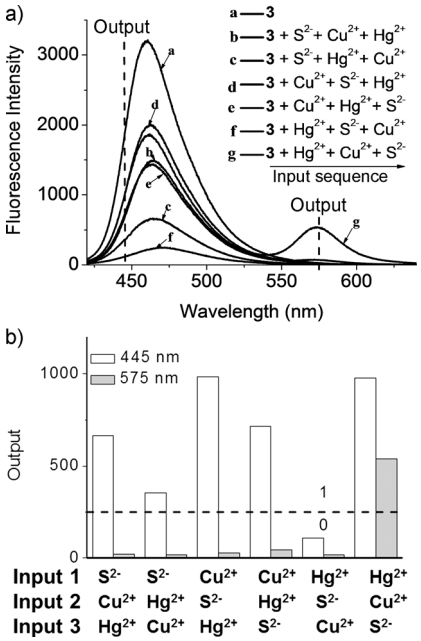


Figure 4. a) Fluorescence spectra of **3** (10 μM) in CH_3CN under different input conditions; $[Hg^{2+}]=5$ μM, $[Cu^{2+}]=10$ μM, $[S^{2-}]=10$ μM ($\lambda_{ex}=365$ nm). b) Outputs in the dual fluorescence channels at 445 and 575 nm in response to three sequential inputs.

On the basis of these metal-dependent spectral properties, a sequential AND logic gate could be constructed using Hg^{2+} (0.5 equiv) and Cu^{2+} (1 equiv) ions as the inputs and either the fluorescence intensity or the absorbance of the rhodamine moiety as the output. As shown in Figure 3, only the addition of Hg^{2+} ions followed by the addition of Cu^{2+} ions gave a noticeable fluorescence at 575 nm. It is likely that upon addition of 0.5 equivalents of Hg^{2+} ions, they were bound by **3** in a 1:1 stoichiometry, but the amount was not sufficient to cause ring opening of the rhodamine moiety. Further addition of 1 equivalent of Cu^{2+} ions displaced the Hg^{2+} ions as a result of their stronger binding to **3**. The displaced Hg^{2+} ions then induced rhodamine ring opening, which resulted in fluorescence emission at 575 nm. When the order of addition was reversed, the rhodamine ring could not be opened as the binding to Hg^{2+} ions was not strong enough to displace the Cu^{2+} ions from the binding sites. This proposed mechanism is shown in Figure S12. A similar logic gate could also be constructed by using the absorbance of rhodamine (at 556 nm) as the output (Figure S13).

This system was then extended by introducing S^{2-} ions (1 equiv) as the third input and one additional fluorescence channel at 445 nm as the second output. It was believed that the S^{2-} ions could preferentially form complexes with the metal ions, although the resulting metal sulfides could not be observed because of their low concentration (in μM). The results are shown in Figure 4. By defining Output 3 as "1" only when both Output 1 and Output 2 are "1", an advanced logic gate could be constructed with this system, and the corresponding truth table is shown in Table S2. It can be seen that only in the presence of all three inputs in the correct sequence, that is, Hg^{2+}, Cu^{2+}, and S^{2-}, strong fluorescence could be seen at both 445 and 575 nm, which then turned on Output 3. None of the other conditions could achieve the same result. These sequential logic operations could then be adopted to construct a molecular keypad lock with three inputs and dual fluorescence outputs. As shown in Figure S14, out of the six possible combinations, only the password "HCS" ($H=Hg^{2+}$, $C=Cu^{2+}$, $S=S^{2-}$) could turn on both channels and unlock the device. With the same three-input system, the absorbance of rhodamine (at 556 nm) could also be used as the output to obtain a similar sequential logic gate (Scheme S15).

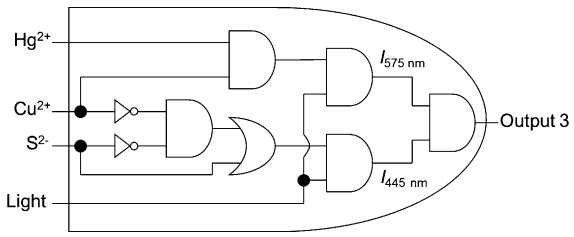
Taking light (at 365 nm) as the fourth input, an even more advanced molecular keypad lock could be constructed. The corresponding truth table and a schematic representation of the integrated logic gates are shown in Table 1. Only in the presence of all four inputs in the correct sequence, that is, Hg^{2+}, Cu^{2+}, S^{2-}, and light, strong fluorescence could be observed at both 445 and 575 nm, which eventually turned on Output 3. A total of four inputs results in 24 possible input combinations, but only the password "HCSL" (L = light) could light up both fluorescence channels and unlock the keypad lock (Figure S16).

In conclusion, a novel ratiometric and highly selective fluorescent sensor for Hg^{2+} ions that is based on a coumarin–rhodamine conjugate has been prepared. This chemosensor can function as an advanced molecular keypad lock with four inputs (Hg^{2+}, Cu^{2+}, S^{2-}, and light) and dual fluorescence outputs, which can greatly enhance its security level.

Received: June 7, 2014
Published online: July 30, 2014

Keywords: coumarin · fluorescent sensors · keypad locks · rhodamine · sequential logic

Table 1: Truth table for combinatorial logic operations with four inputs based on compound **3**.



Entry	Input 1[a] (Hg^{2+})	Input 2[a] (Cu^{2+})	Input 3[a] (S^{2-})	Input 4[a] (light)[b]	Output 1 ($I_{445\ nm}$)[c]	Output 2 ($I_{575\ nm}$)[c]	Output 3
1	0	0	0	0	0 (0)	0 (0)	0
2	1	0	0	0	0 (0)	0 (0)	0
3	0	1	0	0	0 (0)	0 (0)	0
4	0	0	1	0	0 (0)	0 (0)	0
5	0	0	0	1	1 (1712)	0 (24)	0
6	1	1	0	0	0 (0)	0 (0)	0
7	1	0	1	0	0 (0)	0 (0)	0
8	1	0	0	1	1 (518)	0 (16)	0
9	0	1	1	0	0 (0)	0 (0)	0
10	0	1	0	1	0 (220)	0 (11)	0
11	0	0	1	1	1 (3018)	0 (36)	0
12	1	1	1	0	0 (0)	0 (0)	0
13	1	1	0	1	0 (101)	1 (515)	0
14	1	0	1	1	1 (1780)	0 (24)	0
15	0	1	1	1	0 (1670)	0 (30)	0
16	1	1	1	1	1 (978)	1 (541)	1

[a] Input *i* as the i^{th} input. [b] At 365 nm. [c] Threshold value of $I = 250$.

[1] a) A. R. Pease, J. F. Stoddart, *Struct. Bonding (Berlin)* **2001**, *99*, 189 – 236; b) A. P. de Silva, S. Uchiyama, *Nat. Nanotechnol.* **2007**, *2*, 399 – 410; c) M. Amelia, L. Zou, A. Credi, *Coord. Chem. Rev.* **2010**, *254*, 2267 – 2280; d) H. Tian, *Angew. Chem.* **2010**, *122*, 4818 – 4820; *Angew. Chem. Int. Ed.* **2010**, *49*, 4710 – 4712; e) Y. Wu, Y. Xie, Q. Zhang, H. Tian, W. Zhu, A. D. Q. Li, *Angew. Chem.* **2014**, *126*, 2122 – 2126; *Angew. Chem. Int. Ed.* **2014**, *53*, 2090 – 2094.

[2] a) A. P. de Silva, N. D. McClenaghan, *Chem. Eur. J.* **2004**, *10*, 574 – 586; b) D. C. Magri, T. P. Vance, A. P. de Silva, *Inorg. Chim. Acta* **2007**, *360*, 751 – 764.

[3] a) D. Margulies, G. Melman, A. Shanzer, *Nat. Mater.* **2005**, *4*, 768 – 771; b) D. Margulies, G. Melman, A. Shanzer, *J. Am. Chem. Soc.* **2006**, *128*, 4865 – 4871; c) U. Pischel, *Angew. Chem.* **2007**, *119*, 4100 – 4115; *Angew. Chem. Int. Ed.* **2007**, *46*, 4026 – 4040; d) J. Andréasson, U. Pischel, *Chem. Soc. Rev.* **2010**, *39*, 174 – 188; e) A. P. de Silva, *Chem. Asian J.* **2011**, *6*, 750 – 766.

[4] a) G. de Ruiter, E. Tartakovsky, N. Oded, M. E. van der Boom, *Angew. Chem.* **2010**, *122*, 173 – 176; *Angew. Chem. Int. Ed.* **2010**, *49*, 169 – 172; b) U. Pischel, *Angew. Chem.* **2010**, *122*, 1396 – 1398; *Angew. Chem. Int. Ed.* **2010**, *49*, 1356 – 1358; c) G. de Ruiter, L. Motiei, J. Choudhury, N. Oded, M. E. van der Boom, *Angew. Chem.* **2010**, *122*, 4890 – 4893; *Angew. Chem. Int. Ed.* **2010**, *49*, 4780 – 4783; d) M. Kumar, N. Kumar, V. Bhalla, *Chem. Commun.* **2013**, *49*, 877 – 879.

[5] D. Margulies, C. E. Felder, G. Melman, A. Shanzer, *J. Am. Chem. Soc.* **2007**, *129*, 347 – 354.

[6] For examples, see: a) Z. Guo, W. Zhu, L. Shen, H. Tian, *Angew. Chem.* **2007**, *119*, 5645 – 5649; *Angew. Chem. Int. Ed.* **2007**, *46*, 5549 – 5553; b) M. Suresh, A. Ghosh, A. Das, *Chem. Commun.* **2008**, 3906 – 3908; c) W. Sun, C. Zhou, C.-H. Xu, C.-J. Fang, C. Zhang, Z.-X. Li, C.-H. Yan, *Chem. Eur. J.* **2008**, *14*, 6342 – 6351; d) J. Andréasson, S. D. Straight, T. A. Moore, A. L. Moore, D. Gust, *Chem. Eur. J.* **2009**, *15*, 3936 – 3939; e) W. Jiang, M. Han, H.-Y. Zhang, Z.-J. Zhang, Y. Liu, *Chem. Eur. J.* **2009**, *15*, 9938 – 9945; f) M. Kumar, A. Dhir, V. Bhalla, *Org. Lett.* **2009**, *11*, 2567 – 2570; g) R. Pandey, P. Kumar, A. K. Singh, M. Shahid, P.-z. Li, S. K. Singh, Q. Xu, A. Misra, D. S. Pandey, *Inorg. Chem.* **2011**, *50*, 3189 – 3197; h) Q. Zou, X. Li, J. Zhang, J. Zhou, B. Sun, H. Tian, *Chem. Commun.* **2012**, *48*, 2095 – 2097; i) S. Chen, Z. Guo, S. Zhu, W. Shi, W. Zhu, *ACS Appl. Mater. Interfaces* **2013**, *5*, 5623 – 5629; j) B. Rout, P. Milko, M. A. Iron, L. Motiei, D. Margulies, *J. Am. Chem. Soc.* **2013**, *135*, 15330 – 15333.

[7] Kumar et al. reported a thiacalix[4]arene-based molecular keypad lock with Hg^{2+}, K^{+}, and F^{-} ions as the inputs; see: M. Kumar, R. Kumar, V. Bhalla, *Chem. Commun.* **2009**, 7384 – 7386.

[8] Dhir et al. reported a melamine – pyrene conjugate, which formed gold microparticles upon sequential addition of Au^{3+} ions and ascorbic acid. The particle size distribution and fluorescence behavior were used as the dual output channels; see: M. Devi, A. Dhir, C. P. Pradeep, *Dalton Trans.* **2013**, *42*, 7514 – 7518.

[9] a) G. Strack, M. Ornatska, M. Pita, E. Katz, *J. Am. Chem. Soc.* **2008**, *130*, 4234 – 4235; b) J. Halámek, T. K. Tam, G. Strack, V. Bocharova, M. Pita, E. Katz, *Chem. Commun.* **2010**, *46*, 2405 – 2407; c) M. Zhou, X. Zheng, J. Wang, S. Dong, *Chem. Eur. J.* **2010**, *16*, 7719 – 7724.

[10] a) F. Pu, Z. Liu, X. Yang, J. Ren, X. Qu, *Chem. Commun.* **2011**, *47*, 6024 – 6026; b) Z. Huang, Y. Tao, F. Pu, J. Ren, X. Qu, *Chem. Eur. J.* **2012**, *18*, 6663 – 6669; c) W. Hong, Y. Du, T. Wang, J. Liu, Y. Liu, J. Wang, E. Wang, *Chem. Eur. J.* **2012**, *18*, 14939 – 14942; d) F. Pu, Z. Liu, J. Ren, X. Qu, *Chem. Commun.* **2013**, *49*, 2305 – 2307; e) Z. Zhou, Y. Liu, S. Dong, *Chem. Commun.* **2013**, *49*, 3107 – 3109; f) J. Zhu, X. Yang, L. Zhang, L. Zhang, B. Lou, S. Dong, E. Wang, *Chem. Commun.* **2013**, *49*, 5459 – 5461.

[11] Y. Liu, J. Ren, Y. Qin, J. Li, J. Liu, E. Wang, *Chem. Commun.* **2012**, *48*, 802 – 804.

[12] J. Halámek, T. K. Tam, S. Chinnapareddy, V. Bocharova, E. Katz, *J. Phys. Chem. Lett.* **2010**, *1*, 973 – 977.

[13] K. Zhu, J. Shen, R. Dietrich, A. Didier, X. Jiang, E. Märtlbauer, *Chem. Commun.* **2014**, *50*, 676 – 678.

[14] a) S.-K. Ko, Y.-K. Yang, J. Tae, I. Shin, *J. Am. Chem. Soc.* **2006**, *128*, 14150 – 14155; b) W. Lin, X. Cao, Y. Ding, L. Yuan, L. Long, *Chem. Commun.* **2010**, *46*, 3529 – 3531.

[15] J. Bourson, J. Pouget, B. Valeur, *J. Phys. Chem.* **1993**, *97*, 4552 – 4557.

[16] E. M. Nolan, S. J. Lippard, *Chem. Rev.* **2008**, *108*, 3443 – 3480.