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Rationally designed fluorescence 'turn-on' sensor for Cu^{2+} †

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A rationally designed, coumarin-based fluorescent sensor imino-coumarin (IC) displays high selectivity for Cu^{2+} over a variety of competing metal ions in aqueous solution with a significant fluorescence increase. DFT/TDDFT calculations support that the fluorescence '*turn-on*' of IC originates from blocking the electron transfer of the nitrogen lone pair upon complexation with Cu^{2+}. IC was successfully applied to microscopic imaging for detection of Cu^{2+} in LLC-MK2 cells (*in vitro*) and several living organs (*in vivo*).

Copper is the third element in abundance among the essential heavy metals (next to iron and zinc) in the human body and plays an important role in various physiological processes.[1] Abnormal uptake of certain levels of Cu^{2+} by animals is known to cause Wilson's disease, gastrointestinal disease, hypoglycemia, dyslexia, and infant liver damage.[2] Thus, detecting trace amounts of Cu^{2+} is important not only for environmental applications, but also for toxicity determinations in living organs. One of the tools for detecting Cu^{2+} is to utilize a fluorescent chemosensor. Cu^{2+} complexation is well known to induce intrinsic fluorescence quenching,[1,3] and most artificial fluorescent chemosensors give rise to quenching of signals upon Cu^{2+} binding.[1] Only a few examples were reported to be fluorescence turn-on sensors toward Cu^{2+}.[4] Most of these studies have been conducted in organic media due to poor solubility of the chemosensors in aqueous solution. For practical applications, fluorescence sensors which 'turn-on' in the presence of the analyte are superior to those which 'turn-off'. Also, sensing in organic media has intrinsic difficulties for applications in environmental and biological systems.[4a,b] Therefore, a fluorescence 'turn-on' chemosensor with high selectivity towards Cu^{2+} that functions in aqueous solution is highly desirable.

To date, a variety of signaling mechanisms have been proposed and utilized for optical detection of different species, including photo-induced electron/energy transfer (PET),[5] metal–ligand charge transfer (MLCT),[6] intramolecular charge transfer (ICT),[7] excimer/exciplex formation,[8] and excited-state intra-/intermolecular proton transfer (ESIPT).[9] Recently, Wang *et al.* raised a new signal mechanism, C=N isomerisation, for the design of fluorescent sensors.[10] We further developed this mechanism as fluorescence enhancement by orbital control (FEOC) for Cu^{2+}.[11] To design a new fluorescent "turn-on" Cu^{2+} sensor, herein, we intend to adapt the recent strategy FEOC.[11] The basic architecture of the FEOC sensor is comprised of a fluorophore, the sp^2 hybridized nitrogen lone pair (–C=N–), and a chelator site.[10] Based on such a logical strategy, we designed and synthesized the imino-coumarin **IC** (Fig. 1). **IC** exhibits a distinct fluorescence increase in the presence of Cu^{2+}, while competing metal ions do not induce fluorescence changes. The fluorescence behaviour was originated from the blocking of the electron transfer from the nitrogen lone pair to the fluorophore upon complexation with Cu^{2+} as revealed by DFT/TDDFT calculations. **IC** was successfully applied to microscopic imaging for detection of Cu^{2+} in LLC-MK2 cells (*in vitro*) and several living organs (*in vivo*).

IC was synthesized by refluxing 7-diethylamino-coumarin-3-aldehyde[10] and 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol in absolute ethanol to give an 85% yield of the chemosensor. The molecular structure of **IC** was verified by ^{1}H- and ^{13}C-NMR spectroscopy (Fig. S12 and S13, ESI†, respectively), FAB-MS, and X-ray crystal structure analysis (Fig. 1).‡

IC shows a characteristic UV-Vis absorption band at 450 nm in 80% aqueous MeCN (v/v). In the presence of Cu^{2+}, the absorption at 450 nm decreased and a small irregular band appeared at longer wavelength (Fig. S1, ESI†). Fluorescence titration spectra of Cu^{2+} for 3.0 μM **IC** in 80% aqueous MeCN are shown in Fig. 2. **IC** is a weak fluorescent molecule with a maximum emission at 515 nm. Upon gradual addition of Cu^{2+}, fluorescence was remarkably enhanced with a red shift to 525 nm. Job's plot analysis of the fluorescence spectra (Fig. S2, ESI†) showed a maximum at 0.5 mole fraction of Cu^{2+},

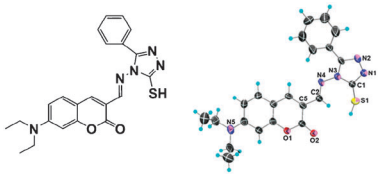

Fig. 1 Structure of a new chemosensor **IC** and crystal structure of **IC** with displacement atomic ellipsoids drawn at the 30% probability level.

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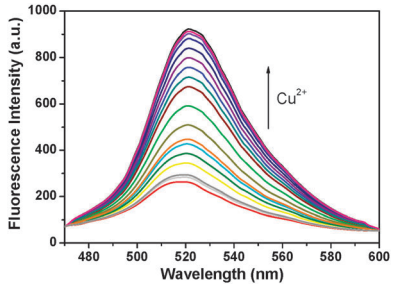

Fig. 2 Fluorescence titration spectra of **IC** (3.0 μM) in 80% aqueous MeCN (phosphate buffer, pH = 7.2) upon addition of $CuCl_2$ (0–20.0 μM) with excitation at 460 nm.

indicating the formation of a 1 : 1 adduct between **IC** and Cu^{2+}. A peak at *m*/*z* 482.1674 corresponding to the adduct $(\mathbf{IC}–H + Cu)^+$ in the MALDI-TOF mass spectrum (Fig. S3, ESI†) provides further evidence for a 1 : 1 complex between **IC** and Cu^{2+}. Based upon a 1 : 1 stoichiometry, the association constant of **IC** with Cu^{2+} was calculated to be 3.34×10^4 M^{-1} from the fluorescence titration data.[12]

To assess the pH dependence of the fluorescent response of **IC** to Cu^{2+}, fluorescence changes of **IC** (3.0 μM) in the absence and presence of 25 equivalents of Cu^{2+} in 80% aqueous MeCN were determined (Fig. S4, ESI†). In the absence of Cu^{2+}, **IC** gave only slight fluorescence variation as the pH of buffer solution varies from 1 to 13. On the other hand, in the presence of Cu^{2+}, the fluorescence intensity at 515 nm exhibited marked increase in the region of pH 4–11 with a good plateau between pH 5 and 8. Thus, the optimal pH range for detection of Cu^{2+} is pH 5–8. Hereafter, the discussion will be done for the spectral data performed in phosphate buffer solution (pH = 7.2) in 80% MeCN.

Fig. 3 displays the fluorescence changes of **IC** in the presence of various metal cations. The very weak fluorescence of **IC** was only marginally increased upon addition of a mixture including the four alkali metal cations, the four alkaline earth metal cations, Cd^{2+}, Co^{2+}, Fe^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}. However, when Cu^{2+} was added, the fluorescence was remarkably enhanced, indicating that **IC** is an excellent selective fluorescent "turn-on" sensor for Cu^{2+}.

The crystal structure (bare structure, BS) seems to be inadequate for metal ion coordination, thus, we calculated another structure (coordination structure, CS) as well as the crystal structure by DFT calculations with 6-31G* basis sets using a suite of Gaussian 03 programs.[13] In gas-phase, BS was

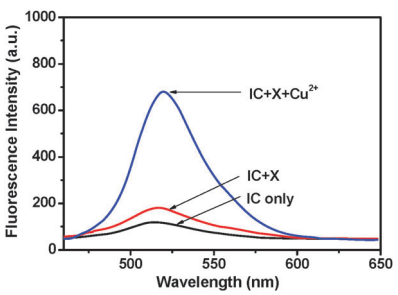

Fig. 3 Fluorescence spectra of **IC** (3.0 μM) (phosphate buffer, pH = 7.2) in 80% aqueous MeCN in the presence of Cu^{2+} (20 μM) and a metal ion mixture of the four alkali metal cations, the four alkaline earth metal cations, Cd^{2+}, Co^{2+}, Fe^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} (100 μM in each) with excitation at 460 nm.

more stable than CS by 4.41 kcal mol^{-1}, however, in PCM calculation considering the solvent effect, the energy difference was only 0.88 kcal mol^{-1} (Fig. S5, ESI†).[14] The gas-phase structure is not always the same as the solid or liquid one, as previously reported.[15] In addition, the bare structure may be changed when coordinated as observed in our recent study.[11] Furthermore, for **IC**, CS can bind Cu^{2+} more tightly than BS because it is better organized. Therefore, we adopted CS to study the fluorescence behavior of **IC** and **IC**–Cu^{2+} complex. The optimized structures of **IC** and **IC**–Cu^{2+} are shown in Fig. 4. For **IC**–Cu^{2+}, various geometries were tested, and reported in ESI.† According to calculated results and our recent study,[16] in **IC**–Cu^{2+} complex, two water molecules are believed to be involved as shown in Fig. 4. **IC** has a little structural change from **IC** upon Cu^{2+} binding; the dihedral angle C=N–N–C is 44.3° in **IC** and 41.3° in **IC**–Cu^{2+}.

From the TDDFT calculations, λ_{max} of **IC** and **IC**–Cu^{2+} were calculated to be 386 and 416 nm, respectively, which are in good agreement with the experiment. In **IC**, the contributions of HOMO-2 → LUMO, HOMO-1 → LUMO and HOMO → LUMO transitions were 2.6, 94.0 and 3.4%, respectively. Due to the importance of the lone pair electrons of the nitrogen atom,[11] we carefully analyzed its contribution to the relevant orbitals. It was found that the nitrogen lone pair electron belongs to HOMO-2 in **IC**. Thus, HOMO-2 → LUMO transition (2.6%) is responsible for the fluorescence quenching. On the other hand, in **IC**–Cu^{2+}, the nitrogen atom has strong interaction with Cu^{2+} d-orbital to produce bonding type orbitals that block the PET process from the nitrogen lone pair electron to the coumarin, hence block the fluorescence quenching (Fig. S7–S10, ESI†). The possibility of the restricted C=N isomerization process of **IC** on coordination to Cu^{2+} could also contribute to the fluorescence enhancement along with the PET process.[10]

Biosensor molecules that can selectively monitor guest species in living systems have attracted a great attention.[17] In this context, we carried out an *in vitro* biological test utilizing **IC**. To determine the cell permeability of **IC**, kidney cells of a monkey (LLC-MK2) were incubated with **IC** (3.0 μM). As shown in Fig. 5, **IC** displays a non-fluorescent image in the absence of Cu^{2+}, whereas a strong confocal image with green fluorescence upon addition of Cu^{2+}. Thus, **IC** has potential applicability for *in vitro* qualitative detection of Cu^{2+}.

In addition to the cell line *in vitro* study, the contrast enhancing effect of **IC** with Cu^{2+} was further evaluated in ICR (Institute for Cancer Research) mice, *in vivo*. Necropsy was performed on a mouse after 3 days of oral feeding with Cu^{2+}. Lung, heart, liver, muscle, kidneys, and spleen were extracted, washed with phosphate buffered saline, and

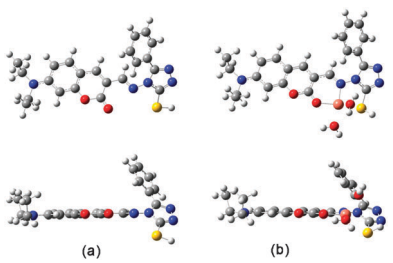

Fig. 4 Calculated structures of (a) **IC** and (b) **IC**–Cu^{2+}.

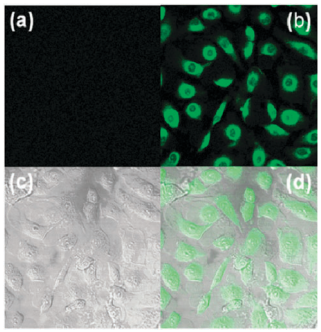

Fig. 5 Confocal fluorescence images of **IC** with Cu^{2+} in LLC-MK2 cells (excitation at 458 nm, Zeiss LSM 510 META confocal microscope, ×20 objective lens) in D-MEM (Dulbeccos-Modified Eagles Medium). Fluorescence image of (a) LLC-MK2 cells with **IC** (3.0 μM) (phosphate buffer, pH = 7.2) in 80% aqueous MeCN and (b) after addition of Cu^{2+}. (c) Bright-field transmission image of LLC-MK2 cells (50.0 μM). (d) An overlay image of (b) and (c).

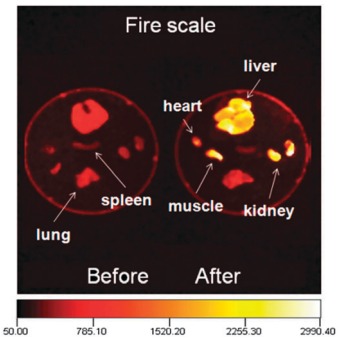

Fig. 6 Fire scale images of mouse organs with **IC** (20.0 μM) (phosphate buffer, pH = 7.2) in 80% aqueous MeCN and after addition of Cu^{2+} (80.0 μM).

subjected to imaging. The images reveal that Cu^{2+} accumulated only in the liver and kidney of the fed mouse as seen in Fig. 6, which is consistent with a previous report that Cu^{2+} preferentially accumulates in these organs.[8a] Thus, **IC** has a capability to sense Cu^{2+}-accumulation in specific organs.

Since mouse urine is deeply related to fundamental metabolic processes,[18] using **IC** we also tried to detect Cu^{2+} in urine collected from a Cu^{2+}-fed mouse and a control mouse. Fig. S11 (ESI†) shows the fluorescence spectra of **IC** exposed to collected urine of the two mice, monitored at wavelengths of 460–700 nm upon excitation at 450 nm. The fluorescence intensity was strong at 531 nm when **IC** was added to the urine which was collected from a Cu^{2+}-fed mouse. On the other hand, urine from the control mouse gave only weak fluorescence, as in the case of a Cu^{2+}-fed mouse without **IC**. These results suggest that **IC** could be developed as a signaling system for Cu^{2+} in biological samples and encourages the possible application for Cu^{2+} detection in human urine.

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Notes and References

‡ Single crystal data for **IC**: $C_{22}H_{21}N_5O_2S$, M_w = 419.50, plate orange crystal, size: 0.30 × 0.15 × 0.12 mm, monoclinic, space group $P2_1/c$, a = 9.57100(10) Å, b = 15.5340(8) Å, c = 16.3360(6) Å, V = 2057.69(13) Å^3, T = 293(2) K, Z = 4, D = 1.354 Mg m^{-3}, ρ = 0.187 mm^{-1}, $F(000)$ = 880; 11 777 reflections measured, of which 4187 were unique (R_{int} = 0.0419). 273 refined parameters, final R_1 = 0.0599 for reflections with $I > 2\sigma(I)$, wR_2 = 0.1448 (all data), GOF = 1.222. Final largest diffraction peak and hole: 0.547 and −0.511 e Å^{-3}. CCDC 641149.

1 (*a*) D. Y. Sasaki, D. R. Shnek, D. W. Pack and F. H. Arnold, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 905; (*b*) R. Krämer, *Angew. Chem., Int. Ed.*, 1998, **37**, 772; (*c*) A. Torrado, G. K. Walkup and B. Imperiali, *J. Am. Chem. Soc.*, 1998, **120**, 609; (*d*) P. Grandini, F. Mancin, P. Tecilla, P. Scrimin and U. Tonellato, *Angew. Chem., Int. Ed.*, 1999, **38**, 3061.

2 (*a*) A. K. Jain, V. K. Gupta, L. P. Singh and J. R. Raisoni, *Talanta*, 2005, **661**, 355; (*b*) A. Mokhir, A. Kiel, D.-P. Herten and R. Kraemer, *Inorg. Chem.*, 2005, **44**, 5661; (*c*) J. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2007, **129**, 9838.

3 (*a*) Q. Wu and E. V. Anslyn, *J. Am. Chem. Soc.*, 2004, **126**, 14682; (*b*) J. V. Mello and N. S. Finney, *J. Am. Chem. Soc.*, 2005, **127**, 10124; (*c*) E. W. Miller, A. E. Albers, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2005, **127**, 16652; (*d*) Z. Xu, X. Qian and J. Cui, *Org. Lett.*, 2005, **7**, 3029; (*e*) K. Komatsu, Y. Urano, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2007, **129**, 13447; (*f*) Y.-Q. Weng, F. Yue, Y.-R. Zhong and B.-H. Ye, *Inorg. Chem.*, 2007, **46**, 7749; (*g*) Z. Xu, X. Chen, H. N. Kim and J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 127.

4 (*a*) P. Ghosh, P. K. Bharadwaj, S. Mandal and S. Ghosh, *J. Am. Chem. Soc.*, 1996, **118**, 1553; (*b*) M. B. Inuoe, F. Medrano, M. Inoue, A. Raitsimring and Q. Fernando, *Inorg. Chem.*, 1997, **36**, 2335; (*c*) K. Rurack, M. Kollmannsberger, U. Resch-Genger and J. Daub, *J. Am. Chem. Soc.*, 2000, **122**, 968; (*d*) Z. Xu, Y. Xiao, X. Qian, J. Cui and D. Cui, *Org. Lett.*, 2005, **7**, 889; (*e*) M. Royzen, Z. Dai and J. W. Canary, *J. Am. Chem. Soc.*, 2005, **127**, 1612.

5 (*a*) I. Aoki, T. Sakaki and S. Shinkai, *J. Chem. Soc., Chem. Commun.*, 1992, 730; (*b*) H.-F. Ji, G. M. Brown and R. Dabestani, *Chem. Commun.*, 1999, 609; (*c*) I. Leray, F. O'Reilly, J.-L. Habib Jiwan, J.-Ph. Soumillion and B. Valeur, *Chem. Commun.*, 1999, 795; (*d*) S. K. Kim, S. H. Lee, J. Y. Lee, R. A. Bartsch and J. S. Kim, *J. Am. Chem. Soc.*, 2004, **126**, 16499; (*e*) S. K. Kim, J. H. Bok, R. A. Bartsch, J. Y. Lee and J. S. Kim, *Org. Lett.*, 2005, **7**, 4839; (*f*) H. J. Kim and J. S. Kim, *Tetrahedron Lett.*, 2006, **47**, 7051.

6 (*a*) P. D. Beer, M. G. B. Drew, D. Hesek, M. Shade and F. Szemes, *Chem. Commun.*, 1996, 2161; (*b*) P. D. Beer, *Acc. Chem. Res.*, 1998, **31**, 71.

7 J. B. Wang, X. F. Qian and J. N. Cui, *J. Org. Chem.*, 2006, **71**, 4308.

8 (*a*) S. Nishizawa, Y. Kato and N. Teramae, *J. Am. Chem. Soc.*, 1999, **121**, 9463; (*b*) J.-S. Wu, J.-H. Zhou, P.-F. Wang, X.-H. Zhang and S.-K. Wu, *Org. Lett.*, 2005, **7**, 2133.

9 (*a*) A. Weller, *Z. Elektrochem.*, 1956, **60**, 1144; (*b*) A. Weller, *Prog. React. Kinet.*, 1961, **1**, 188.

10 J.-S. Wu, W.-M. Liu, X.-Q. Zhuang, F. Wang, P.-F. Wang, S.-L. Tao, X.-H. Zhang, S.-K. Wu and S.-T. Lee, *Org. Lett.*, 2007, **9**, 33.

11 H. S. Jung, K. C. Ko, J. H. Lee, S. H. Kim, S. Bhuniya, J. Y. Lee, Y. Kim, S. J. Kim and J. S. Kim, *Inorg. Chem.*, 2010, **49**, 8552.

12 (*a*) Association constants were calculated using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, United Kingdom; (*b*) K. A. Connors, *Binding Constants*, Wiley, New York, 1987.

13 M. J. Frisch, *et al.*, *Gaussian 03, Revision D.02*, Gaussian Inc., Pittsburgh, PA, 2006.

14 (*a*) N. V. Belkova, M. Besora, L. M. Epstein, A. Lledos, F. Maseras and E. S. Shubina, *J. Am. Chem. Soc.*, 2003, **125**, 7715; (*b*) J. Stare, A. Jezierska, G. Ambrozic, I. J. Kosir, J. Kidric, A. Koll, J. Mavri and D. Hadzi, *J. Am. Chem. Soc.*, 2004, **126**, 4437; (*c*) R. Fang, Z. Ke, Y. Shen, C. Zhao and D. L. Phillips, *J. Org. Chem.*, 2007, **72**, 5139.

15 S. J. Kim, M.-G. Jo, J. Y. Lee and B. H. Kim, *Org. Lett.*, 2004, **6**, 1963.

16 H. S. Jung, P. S. Kwon, J. W. Lee, J. I. Kim, C. S. Hong, J. W. Kim, S. H. Yan, J. Y. Lee, J. H. Lee, T. Joo and J. S. Kim, *J. Am. Chem. Soc.*, 2009, **131**, 2008.

17 (*a*) J. Thomas, D. B. Sherman, T. J. Amiss, S. A. Andaluz and J. B. Pitner, *Bioconjugate Chem.*, 2007, **18**, 1841; (*b*) S. V. Wegner, A. Okesli, P. Chen and C. He, *J. Am. Chem. Soc.*, 2007, **129**, 3474.

18 (*a*) M. Mahajna, G. B. Quistad and J. E. Casida, *Chem. Res. Toxicol.*, 1996, **9**, 1202; (*b*) S. Giri, J. R. Idle, C. Chen, T. M. Zabriskie, K. W. Krausz and F. J. Gonzalez, *Chem. Res. Toxicol.*, 2006, **19**, 818.