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Coumarin-derived Fe^{3+}-selective fluorescent turn-off chemosensors: synthesis, properties, and applications in living cells†

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A novel coumarin-based fluorogenic probe bearing the tris unit (**DAT-1**) with high selectivity and suitable affinity toward Fe^{3+} over other cations tested was developed in biological systems. The sensing mechanism was studied by quantum calculations. The probe can be applied to the monitoring of Fe^{3+} in aqueous solution with a pH span of 3–8. In addition, biological imaging, membrane permeability and nontoxicity demonstrate that **DAT-1** could act as a turn-off fluorescent chemosensor for Fe^{3+} in living cells.

Introduction

The design and synthesis of high selectivity and sensitivity chemosensors to detect metal ions in the ecological environment and biology have attracted a great deal of attention.[1] A chemosensor shows a significant change in electrical, electronic, magnetic, or optical signal when it binds to a specific guest counterpart.[2] Current chemosensors have many advantages, including high sensitivity, low cost, easy detection, and suitability as a diagnostic tool for biological purposes.[3] As an essential trace element in biological systems, Fe^{3+} plays an important role in living organisms and metabolism. Many enzymatic reactions involving iron are related to oxygen metabolism, electron transfer and the mitochondrial respiratory chain.[4] Although iron is indispensable for the proper functioning of all living cells, it is disadvantageous when present in excess. Excessive Fe^{3+} in the human body has been found to be related to an increased incidence of certain cancers and the dysfunction of certain organs.[5] Moreover, hereditary hemochromatosis is characterized by excess iron that causes tissue damage and fibrosis with irreversible damage to various organs.[4a,6] Furthermore, iron homeostasis is an important factor involved in neuroinflammation and the progression of Alzheimer's disease.[4a,7] At the same time, iron deficiency can be as harmful as iron overload, or even more harmful. It is well known that anemia is due to iron deficiency. Therefore, a convenient and rapid method for detecting the concentration of Fe^{3+} in biological samples is of great interest in biological and environmental concerns. Recently, many fluorescent chemosensors for Fe(III)-selective detection were reported and have been used with some success in biological applications.[8] Nevertheless, a few of them have defects in actual practice such as cross-sensitivity toward other metal cations, long response times, poor water solubility, a narrow pH detection span, a low fluorescence quantum yield in aqueous media, and ligand cytotoxicity.[9]

Of late, coumarin-based chemosensors have been extensively used as fluorescent labeling reagents for their large molar extinction coefficient, relatively long excitation and emission wavelengths and high fluorescence quantum yield.[10] Here, we synthesized a novel coumarin-derived Fe(III)-selective chemosensor *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-7-hydroxycoumarin-3-carboxamide (**DAT-1**) which is highly selective and sensitive toward Fe^{3+} over other common metal ions such as Na^+, K^+, Ca^{2+}, Fe^{2+}, Mg^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Ag^+, Cu^{2+}, Zn^{2+}, Cr^{3+}, Cd^{2+}, Mn^{2+}, Hg^{2+} and Pb^{2+} in buffered aqueous solutions, and the sensing mechanism was studied by quantum calculations.

Experiment

Ethyl-7-hydroxy-3-carboxylate **2** (ref. 10) was synthesized according to the procedures reported in the literature. 2-Amino-2-(hydroxymethyl)-3-propanediol (tris) was purchased. **DAT-1** was prepared by the standard method with a minor modification.[11] Compound **2** (234 mg, 1 mmol) was treated with tris (121 mg, 1 mmol) in anhydrous ethanol (5 mL). The reaction was then refluxed for 8 h. The mixture was concentrated under vacuum and the crude product was purified by column chromatography (CH_2Cl_2 : CH_3OH = 10 : 1) to give **DAT-1** as a yellow solid (185 mg, yield: 60 %), mp. 239–241 °C. ^{1}HNMR (400 MHz, DMSO-d_6): δ = 11.09 (s, 1H), 8.96 (s, 1H), 8.81 (s, 1H), 7.81 (d,

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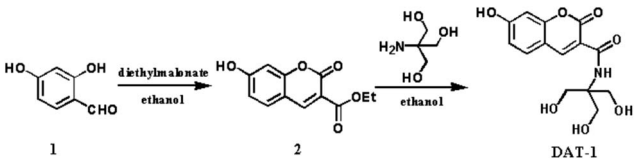

Scheme 1 Synthetic procedure of DAT-1.

J = 8.5 Hz, 1H), 6.89 (dd, J = 8.5, 1.7 Hz, 1H), 6.82 (d, J = 1.6 Hz, 1H), 4.86 (s, 3H), 3.65 (s, 6H). ^{13}CNMR (400 MHz, DMSO-d_6): δ 169.08, 166.84, 166.39, 161.52, 153.23, 137.19, 119.62, 118.82, 116.19, 106.96, 67.44, 65.30, 45.27, 45.06, 44.86, 44.65, 44.44, 44.23, 44.02. HRMS (ESI): m/z, calcd for $(M - H)^-$ 308.0770; found 308.0798 (Scheme 1).

Results and discussion

The **DAT-1** chemosensor contain a coumarin group used as the fluorescent signal unit and a 2-amino-2-(hydroxymethyl)-1,3-propanediol (tris) acting as a possible recognition unit. Since coumarin derivatives exhibit several advantages such as large Stokes shifts, good photostability and high fluorescence quantum yields (the quantum yield of the system was calculated to be 0.75), they could be used as efficient fluorophores. And the tris which includes three hydroxyl groups and an amide group is expected to serve as a potential binding group.[11] Furthermore, the tris group can improve the water solubility of chemosensors due to its hydrophilic properties.

The structure of **DAT-1** was confirmed by ^{1}H NMR, ^{13}C NMR, FT-IR, ESI-MS and X-ray analysis (Fig. S7–S10†). The single crystal of **DAT-1** was obtained under vapor diffusion of CH_3OH into a solution of water and characterized using X-ray crystallography (Fig. 1).

Fig. 2a shows the change in the fluorescence spectra of **DAT-1** upon the addition of Fe^{3+} in the Na_2HPO_4–citric acid buffer solution at pH 4.8. Upon the addition of an increasing amount of Fe^{3+}, the emission intensity of **DAT-1** at 448 nm gradually decreased. The fluorescence emission intensity were sensitively and proportionately decreased with an increasing concentration of Fe^{3+} as noted from the relationship between the fluorescence emission intensity at 350 nm and Fe^{3+} concentration (Fig. 2b). Therefore, the **DAT-1** could be used for

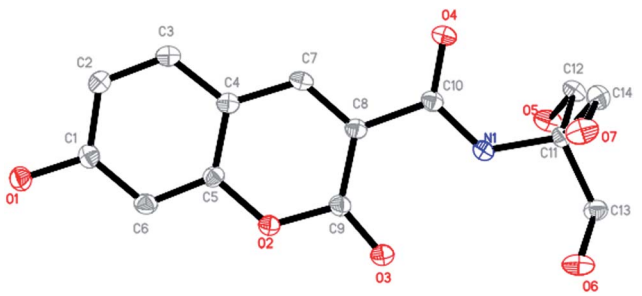

Fig. 1 View of the structure of DAT-1 with displacement atomic ellipsoids drawn at the 30% probability level. All hydrogen atoms are omitted for clarity.

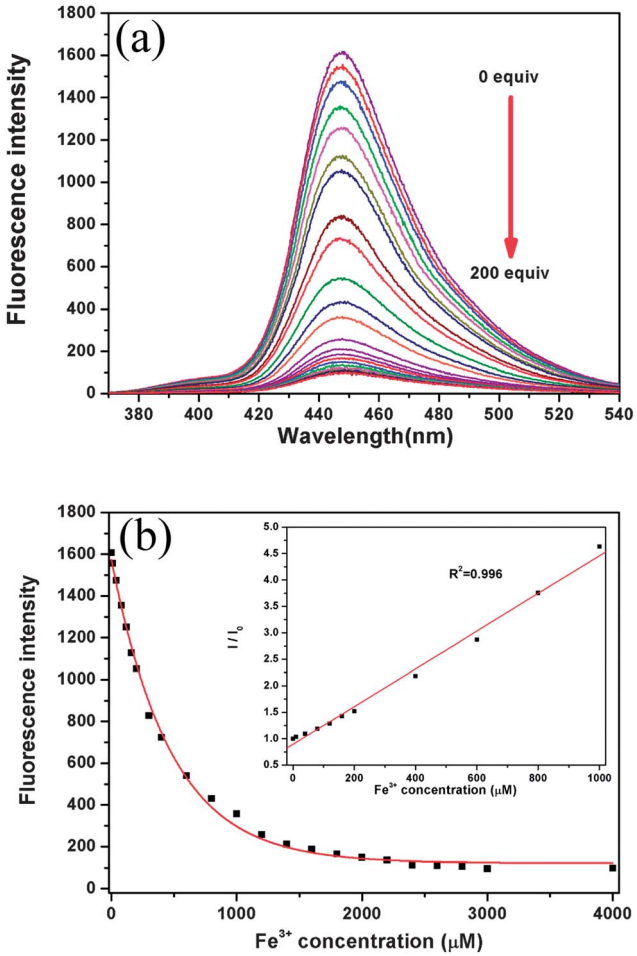

Fig. 2 (a) Fluorescence titration spectra (λ_{ex} = 350 nm) of DAT-1 (20 μM) with the addition of Fe^{3+} (0–200 equiv.) in the 0.2 M Na_2HPO_4–citric acid buffer. (b) Titration data points and the nonlinear least-squares fitting curve. Insert: the Stern–Volmer plot of fluorescence quenching of the DAT-1 by Fe^{3+}. R^2 = 0.996.

the development of an efficient method for Fe^{3+} ion detection. The linear relation of the obtained experimental data for Fe^{3+} detection could be best described as the Stern–Volmer equation. The Stern–Volmer relationship with an R^2 of 0.996 is given in the following equation (Fig. 2b, inset):

$$I_0/I = 1 + K_{SV}[Q]$$

where I and I_0 are the fluorescence emission intensities of the **DAT-1** in the presence and absence of Fe^{3+} respectively. Q is the Fe^{3+} concentration. K_{SV} is the Stern–Volmer quenching constant and is found to be 3.57 × 10^3 M^{-1}. The linear range of Fe^{3+} concentration was from 1 × 10^{-5} M to 1 × 10^{-3} M and the detection limit of Fe^{3+} was 3 × 10^{-7} M. It can be found that the presented method has wider detection range and higher sensitivity than many of fluorescent chemosensors for Fe^{3+}.

There was a remarkable selectivity of **DAT-1** for Fe^{3+} over various other metal ions in the competition experiments, which turned out to be applicable in environmental technology (Fig. 3). The fluorescence intensity of the chemosensor **DAT-1**

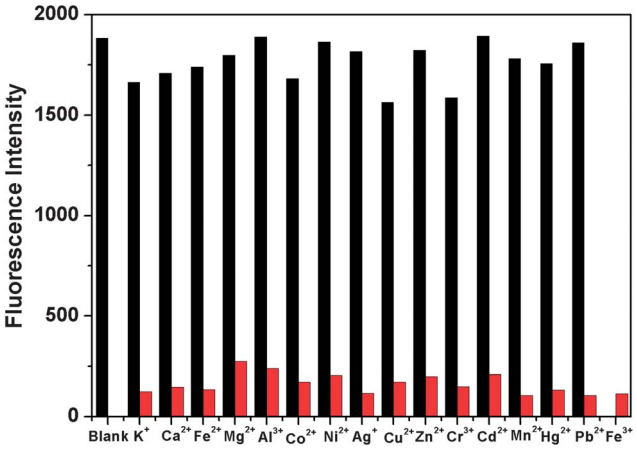

Fig. 3 Metal ions selectivity of **DAT-1** (20 μM) in the Na_2HPO_4–citric acid buffer solution. The black bars represent the fluorescence emission intensity of **DAT-1** and 100 equiv. of metal ions. The red bars represent the fluorescence response of **DAT-1** to 100 equiv. of Fe^{3+} containing 100 equiv. of other metal ions. $\lambda_{ex} = 350$ nm, $\lambda_{em} = 448$ nm.

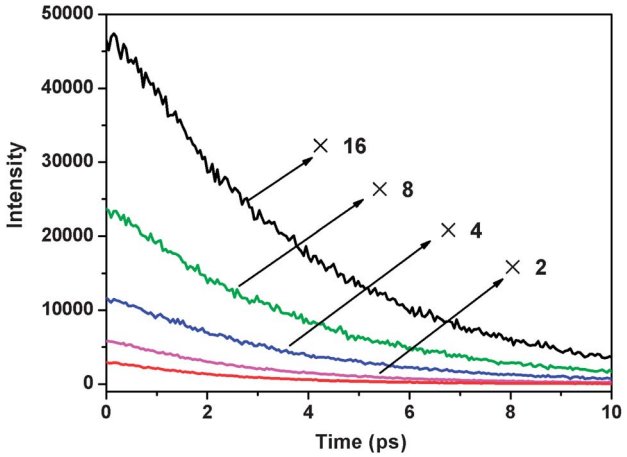

Fig. 6 Time-resolved fluorescence of **DAT-1** with an excitation at 405 nm in the absence (black) and in the presence of 1 equiv. (green), 5 equiv. (blue), 25 equiv. (magenta) and 50 equiv. (red) of Fe^{3+} ions.

was substantially diminished by the addition of Fe^{3+}, whereas other cations such as K^+, Ca^{2+}, Fe^{2+}, Mg^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Ag^+, Cu^{2+}, Zn^{2+}, Cr^{3+}, Cd^{2+}, Mn^{2+}, Hg^{2+} and Pb^{2+} did not cause any significant changes in the fluorescence emission intensity, even at a concentration of 100 equiv. of guest counterparts. Even with highly concentrated Na^+, K^+, Ca^+, and Mg^{2+} (~5.0 mM) under physiological conditions,[12] there were no fluorescence changes in **DAT-1.** The large decrease in fluorescence intensity could be applied to the detection of iron cations by the naked eye (Fig. 4). When the chemosensor (20 μM) was excited at 350 nm in the presence of various cations (100 equiv.) in Na_2HPO_4–citric acid buffer (pH 4.8), hardly any fluorescence response of Fe^{3+} was selectively observed (Fig. 4).

It is well-known that heavy metal ions such as Fe^{3+}, Cu^{2+}, Cd^{2+}, Hg^{2+} and Pb^{2+} tend to quench the luminescence through electron- and/or energy-transfer processes.[13] Because of the

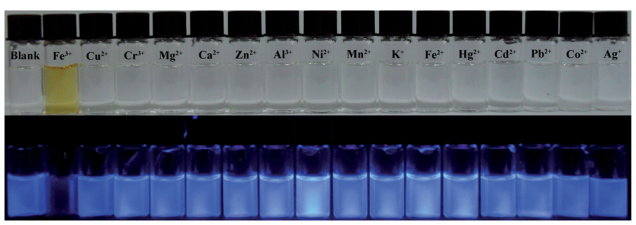

Fig. 4 Fluorescent (bottom) and color (top) responses of **DAT-1** (20 μM) in Na_2HPO_4–citric acid buffer solutions (0.2 M) upon the addition of (100 equiv.) metal ions in water.

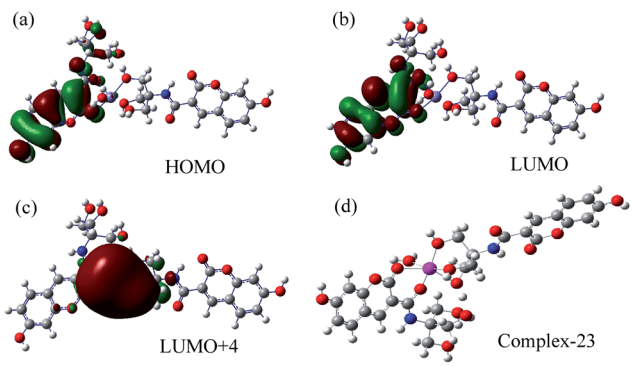

Fig. 5 The calculated frontier molecular orbitals of complex-23 (a–c) and the best structure of complex-23 (d).

Table 1 Fluorescence decay time constants of 1 in the presence of Fe^{3+} ions

Fe^{3+} (equiv.)	τ (ns)	Fe^{3+} (equiv.)	τ (ns)
0	3.68	25	2.76
1	3.60	50	2.30
5	3.42		

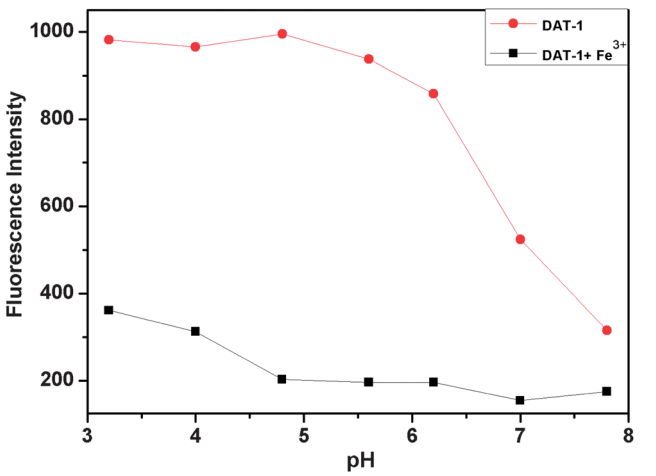

Fig. 7 Variation of fluorescence spectra of **DAT-1** (20 μM) in aqueous solution (Na_2HPO_4–citric acid buffer) (0.2 M) with and without Fe^{3+} (100 equiv.) ion as a function of pH. Fluorescence intensity at 448 nm.

specific structure, the recognition unit of our probe could complex with iron ions selectively. Thus the photoinduced electron transfer (PET) effect on N atom causes fluorescence quenching of coumarin. The fact that our **DAT-1** chemosensor can not complex with other ions besides iron ions is attributed to the specific recognition of the recognition unit to iron ions.[13e] Therefore, our probe cannot be quenched by other ions without PET effect. To prove the mechanism of fluorescence quenching, we carried out theoretical calculations (Fig. 5; details in Fig. S3†). From the single crystal structure of **DAT-1**, there are many coordination sites, but it is difficult to determine the structure of coordination between **DAT-1** and Fe^{3+}. We therefore designed 22 coordinations, considering all possible options except water as ligand. We found, however, that complex-23, with water as ligand, was the optimum structure compared with other complexes. This result agrees with the experimental data reported by other authors.[11] Thus we can assume that complex-23 was the best structure in this system. The electron distributions of HOMO, LUMO, and LUMO+4 of complex-23 were calculated by B3LYP/6-31G(d, p) and are shown in Fig. 5. The electronic distribution on LUMO+4 for complex-23 was extended to Fe, indicating the electron transfer from HOMO to LUMO+4. Thus the electrons transfer from **DAT-1** to Fe^{3+}, making the fluorescence quenching behavior possible. Indeed, it has been demonstrated in the literature that fluorescence quenching of the ligand may occur by the excitation energy transfer from the ligand to the metal d orbital and/or ligand to metal charge transfer (LMCT).[9,14]

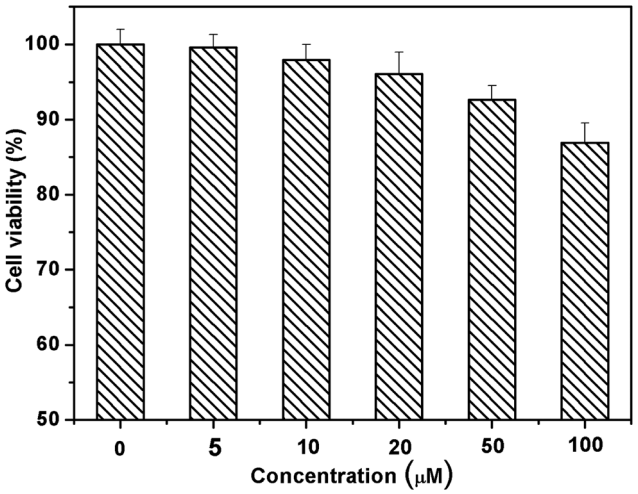

Fig. 8 Percentage of MCF-7 cell viability remaining after cell treatment with **DAT-1** (untreated cells were considered to have 100% survival). Cell viability was assayed by the MTT method (values: mean ± standard deviation).[15]

To elaborate upon the mechanism of fluorescence quenching which we speculate takes place by the energy and/or charge transfer model, fluorescence lifetime testing was conducted. The fluorescence decay behavior in the presence of Fe^{3+} is shown in Fig. 6, and the exponential fit results are summarized in Table 1. In the absence of Fe^{3+}, the fluorescence decays single exponentially by a 3.68 ns time constant, which is the lifetime of the S1 state of free **DAT-1**. The time constant (τ) of the lifetime decay component drops off when Fe^{3+} is added.

For biological applications of the chemosensor, the sensing should operate in a wide range of pH. Fig. 7 shows that in aqueous solution the suitable pH range for Fe^{3+} determination is pH 3–8 where the "on–off" fluorescence can be operated by the iron ion binding. Consequently, our present Fe^{3+}-selective receptor would be an ideal chemosensor for monitoring Fe^{3+}.

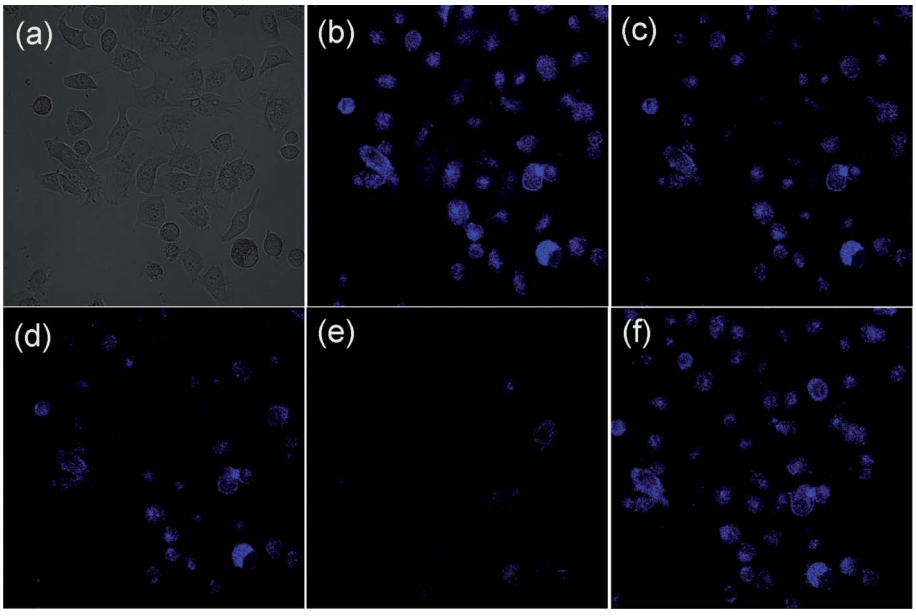

Fig. 9 Confocal fluorescence images of Fe^{3+} in MCF-7 cells (Olympus FV1000, 40× objective lens). (a) Bright-field transmission image of MCF-7 cells. (b) Fluorescence image of MCF-7 cells incubated with **DAT-1** (20 μM). Further incubated with addition of various concentrations of $FeCl_3$ [(c) 25, (d) 50, and (e) 100 equiv., respectively]. (f) Return of intracellular Fe^{3+} to the resting level was achieved by addition of EDTA (2 mM).

The ability of biosensing molecules to selectively monitor guest species in living cells is of great importance for biological applications. First, we selected breast carcinoma cell lines (MCF-7) to investigate the toxicity of **DAT-1**. The toxicity data were obtained by performing 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assays as shown in Fig. 8. The cells were cultured in RPMI 1640 (Gibco) and supplemented with 10% fetal bovine serum (FBS; Gibco) at 37 °C in a humidified atmosphere of 5% CO_2. For all experiments, cells were harvested from subconfluent cultures by the use of trypsin and were resuspended in fresh complete medium before plating. *In vitro* cytotoxicity was assessed by using MTT reduction assays. In a typical procedure, about 2000 cells were plated in 96-well plates for 24 h to allow the cells to attach, and then incubated with **DAT-1** (20 μM) in 5% CO_2 at 37 °C. At the end of the incubation time, MTT solution (10 μL, diluted in culture medium to a final concentration of 1 mg mL^{-1}) was added, and then the mixture was incubated for another 4 h. Finally, the incubation solution was removed and dimethyl sulfoxide (DMSO, 150 μL) was added to each well. The absorbance of MTT was determined (λ = 490, 630 nm) by using a microplate reader (BioTek, ELX808). The cell viability obtained was expressed as a percentage relative to the control. The viability of untreated cells was assumed to be 100%. When the concentration of chemosensor **DAT-1** was 5 μM, more than 99% of the MCF-7 cells were alive. Even when the concentration of **DAT-1** increased to 100 μM, there were still about 87% of the MCF-7 cells alive. From the results of cytotoxicity experiments, the chemosensor **DAT-1** shows low toxicity even at a high concentration of 100 μM particles and an incubation time of 24 h, which means that **DAT-1** possesses high biocompatibility.

To determine the cell permeability of **DAT-1**, MCF-7 cells were incubated with **DAT-1** (20 μM) for 25–30 min at 37 °C, and washed with PBS to remove the remaining compound **DAT-1**. The results are shown in Fig. 9b. One can clearly observe significant confocal imaging changes of the medium upon addition of $FeCl_3$ for 20 min (25, 50, and 100 equiv., respectively) at 37 °C. MCF-7 cells incubated with **DAT-1** initially display a strong fluorescence image, but the fluorescence image immediately becomes faint in the presence of Fe^{3+} (Fig. 9c–e). Thus, the chemosensor **DAT-1** can be a suitable fluorescence chemosensing probe for Fe^{3+} detection in biological systems.

Conclusion

In summary, this study has reported the synthesis, properties, and cellular applications of a novel chemosensor **DAT-1** for Fe^{3+} sensing. This chemosensor exhibits high sensitivity and selectivity toward Fe^{3+} in aqueous solution. We simulated the mechanism of fluorescence quenching by quantum calculations. In terms of good optical properties, fine water solubility, excellent membrane permeability and non-toxicity, **DAT-1** could be one of the most important chemosensors for the detection of Fe(III) in living cells.

Acknowledgements

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Notes and references

1 (*a*) D. H. Vance and A. W. Czarnik, *J. Am. Chem. Soc.*, 1994, **116**, 9397–9398; (*b*) Z. C. Xu, J. Yoon and D. R. Spring, *Chem. Soc. Rev.*, 2010, **39**, 1996–2006; (*c*) J. S. Wu, W. M. Liu, J. C. Ge, H. Y. Zhang and P. F. Wang, *Chem. Soc. Rev.*, 2011, **40**, 3483–3495; (*d*) M. Baglan and S. Atilgan, *Chem. Commun.*, 2013, **49**, 5325–5327; (*e*) M. J. Peng, Y. Guo, X. F. Yang, L. Y. Wang and J. An, *Dyes Pigm.*, 2013, **98**, 327–332.

2 (*a*) T. W. Bell and N. M. Hext, *Chem. Soc. Rev.*, 2004, **33**, 589–598; (*b*) W. Wang, X. L. Wang, Q. B. Yang, X. L. Fei, M. D. Sun and Y. Song, *Chem. Commun.*, 2013, **49**, 4833–4835; (*c*) X. G. Li, Y. Z. Liao, M. R. Huang, V. Strong and R. B. Kaner, *Chem. Sci.*, 2013, **4**, 1970–1978; (*d*) Z. Yang, M. Y. She, B. Yin, J. H. Cui, Y. Z. Zhang, W. Sun, J. L. Li and Z. Shi, *J. Org. Chem.*, 2012, **77**, 1143–1147.

3 (*a*) C. Y. Lai, B. G. Trewyn, D. M. Jeftinija, K. Jeftinija, S. Xu, S. Jeftinija and V. S. Y. Lin, *J. Am. Chem. Soc.*, 2003, **125**, 4451–4459; (*b*) P. G. Sutariya, A. Pandya, A. Lodha and S. K. Menon, *Analyst*, 2013, **138**, 2531–2535; (*c*) G. Sivaraman, T. Anand and D. Chellappa, *Analyst*, 2012, **137**, 5881–5884; (*d*) X. J. Wu, Z. Li, L. Yang, J. H. Han and S. F. Han, *Chem. Sci.*, 2013, **4**, 460–467.

4 (*a*) S. K. Sahoo, D. Sharma, R. K. Bera, G. Crisponi and J. F. Callan, *Chem. Soc. Rev.*, 2012, **41**, 7195–7227; (*b*) S. P. McCormick, M. Chakrabarti, A. L. Cockrell, J. Park, L. S. Lindahl and P. A. Lindahl, *Metallomics*, 2013, **5**, 232–241.

5 (*a*) B. J. Halliwell, *J. Neurochem.*, 1992, **59**, 1609–1623; (*b*) E. D. Weinberg, *Eur. J. Cancer Prev.*, 1996, **5**, 19–36; (*c*) D. Galaris, V. Skiada and A. Barbouti, *Cancer Lett.*, 2008, **266**, 21–29.

6 E. Beutler, *Blood Cells, Mol., Dis.*, 2007, **39**, 140–147.

7 W. Y. Ong and A. A. Farooqui, *J. Alzheimer's Dis.*, 2005, **8**, 183–200.

8 (*a*) L. Huang, F. P. Hou, J. Cheng, P. X. Xi, F. J. Chen, D. Bai and Z. Z. Zeng, *Org. Biomol. Chem.*, 2012, **10**, 9634–9638; (*b*) S. K. Sahoo, D. Sharma, R. K. Bera, G. Crisponi and J. F. Callan, *Chem. Soc. Rev.*, 2012, **41**, 7195–7227; (*c*) B. K. Kanungo, M. Baral, R. K. Bera and S. K. Sahoo, *Monatsh. Chem.*, 2010, **141**, 157–168.

9 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. H. Joo and J. S. Kim, *J. Am. Chem. Soc.*, 2009, **131**, 2008–2012.

10 K. Y. Chen, Y. Guo, Z. H. Lu, B. Q. Yang and Z. Shi, *Chin. J. Chem.*, 2010, **28**, 55–60.

11 J. N. Yao, W. Dou, W. W. Qin and W. S. Liu, *Inorg. Chem. Commun.*, 2009, **12**, 116–118.
12 T. D. Rae, P. J. Schmidt, R. A. Pufahl, V. C. Culotta and T. V. O'Halloran, *Science*, 1999, **284**, 805–808.
13 (*a*) J. L. Bricks, A. kovalchuk, C. Trieflinger, M. Nofz, M. Buschel, A. I. Tolmachev, J. Daub and K. Rurack, *J. Am. Chem. Soc.*, 2005, **127**, 13522–13529; (*b*) K. Kavallieratos, J. M. Rosenberg, W. Z. Chen and T. Ren, *J. Am. Chem. Soc.*, 2005, **127**, 6514–6515; (*c*) Y. Zheng, J. Orbulescu, X. Ji, F. M. Andreopoulos, S. M. Pham and R. M. Leblanc, *J. Am. Chem. Soc.*, 2003, **125**, 2680–2688; (*d*) D. T. Quang, H. S. Jung, J. H. Yoon, S. Y. Lee and J. S. Kim, *Bull. Korean Chem. Soc.*, 2007, **28**, 682; (*e*) T. E. Glass, *J. Am. Chem. Soc.*, 2000, **122**, 4522–4523.
14 L. S. Villata, E. Wolcan, M. R. Feliz and A. L. Capparelli, *J. Phys. Chem. A*, 1999, **103**, 5661–5666.
15 (*a*) Y. Cetin and L. B. Bullerman, *J. Agric. Food Chem.*, 2005, **53**, 6558–6563; (*b*) I. Roy, T. Y. Ohulchanskyy, H. E. Pudavar, E. J. Bergey, A. R. Oseroff, J. Morgan, T. J. Dougherty and P. N. Prasad, *J. Am. Chem. Soc.*, 2003, **125**, 7860–7865; (*c*) S. Andreescu, O. A. Sadik and D. W. McGee, *Chem. Res. Toxicol.*, 2005, **18**, 466–474.