SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
MONOSACCHARIDES
SENSORS
FLUORESENT PROBES

Received
26 November 2013

Accepted
5 February 2014

Published
3 March 2014

Correspondence and requests for materials should be addressed to X.-P.H. (xphe@ecust.edu.cn); J.L. (jli@mail.shcnc.ac.cn) or G.-R.C. (mrs_guorongchen@ecust.edu.cn)

Substitution Pattern Reverses the Fluorescence Response of Coumarin Glycoligands upon Coordination with Silver (I)

De-Tai Shi[1], Xiao-Li Wei[1,2], Yayun Sheng[1], Yi Zang[2], Xiao-Peng He[1], Juan Xie[3], Guixia Liu[1], Yun Tang[1], Jia Li[2] & Guo-Rong Chen[1]

[1]Key Laboratory for Advanced Materials & Institute of Fine Chemicals, and Shanghai Key Laboratory of New Drug Design, East China University of Science and Technology, 130 Meilong Rd., Shanghai 200237, PR China, [2]National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 189 Guo Shoujing Rd., Shanghai 201203, P. R. China, [3]PPSM, Institut d'Alembert, ENS de Cachan, CNRS UMR 8531, 61 Avenue du Pt Wilson, F-94235 Cachan, France.

Development of sugar-based fluorescence (FL) chemo-probes is of much interest since sugars are biocompatible, water-soluble and structurally rigid natural starting materials. We report here that fluorescent glycoligands with two triazolyl coumarin moieties installed onto the different positions of an identical glucosyl nucleus exert completely reversed optical response to a metal ion. C3,4-, C2,3- and C4,6-di-substituted coumarin glucosides synthesized by a click reaction similarly showed a selective FL variation in the presence of silver (I) among a range of metal cations in an aqueous solution. However, the variation was determined to be converse: the FL of the C3,4-ligand was quenched whereas that of the C2,3/C4,6-ligand tangibly enhanced. FL and NMR titrations suggested that this divergence was due to the distinct complexation modes of the conformationally constrained ligands with the ion. The optimal motifs of the ligand-ion complexation were predicted by a computational simulation. Finally, the C2,3-ligand was determined to be of low cytotoxicity and applicable in the FL imaging of silver ions internalized by live cells.

Fluorescent glycoligands (FGs)[1] are recently defined structural frameworks for the development of ion probes[10,40]. These compounds are structured by a sugar as the template upon which to install a diverse range of Lewis bases (as the metal chelation site) and fluorophores (as the optical reporter) by chemical modifications[5,8,10,28,29]. Employment of sugars, a cheap natural starting material, as the central platform lies on their structural diversity and rigidity[1–11], high biocompatibility[12,13] and good water solubility[14–17].

The copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition reaction (Cue-AAC)[18–20], a prototype of the click chemistry[21], has found wide applications in the construction of chemo-probes since the 1,4-disubstituted 1,2,3-triazole it forms represents a versatile ion coordination site[8,10,22–24,41]. We recently reported that Cue-AAC is also a promisingly suitable tool for fabrication of FGs[8,15,24–27]. By a twofold Cue-AAC between two azido fluorophores and a di-alkynyl glycoside, the resulting bis-triazolyl FGs exhibited distinct ion sensibilities in terms of the different fluorophores introduced[10,24–26]. Meanwhile, the epimeric identity of the glycosyl scaffold also impacts the ion coordination properties of the ligands[15,27–29].

Owing to its potential toxicity but wide-range utility, much attention has been paid to the monitoring of silver (I) ions. In recent years, some fluorescent probes for Ag^+ have been developed. However, due to the heavy metal effect of the ion, the majority of the probes suffered from FL quenching which is suboptimal for detection of biological samples[30]. Furthermore, some FL 'turn-on' probes reported are easily interfered with a competing metal or have limited solubility in aqueous media[31–34].

Here, we unravel an interesting discovery that by appending coumarins to different substitution positions of a glucoside via the Cue-AAC, the fluorescence (FL) change of the resulting FGs is totally converse upon coordination with silver (I). A 'turn-on' probe that shows excellent sensitivity and selectivity for silver (I) in both aqueous and buffer media was determined to be of low toxicity and of applicability in FL imaging of silver ions internalized by cells. The ligand-ion complexation modes and the mechanism of the reversed FL alternation were proposed.

Results

As shown in Fig. 1, we have determined in a previous study that a C3,4-disubstituted bis-triazolyl coumarin glucoside (**DT3**) showed selective and remarkable FL quenching upon coordination with Ag^+ in water[15]. The present study initiated with the interrogation of the metal-ion sensibility of two other structurally analogous coumarin glucosides, **DT1** and **DT2**, synthesized by a microwave-assisted two-fold Cue-AAC[35]. The only structural divergence is that two same triazolyl coumarin moieties were substituted on the different positions of the glucosyl platform to display these functional groups in diversely constrained manners[5,8,10,28,29].

FL spectroscopy was used to primarily test the FL change of the FGs in the presence of a range of metal cations. The FL intensity of both **DT1** (Fig. 2a) and **DT2** (Fig. 2b) enhanced evidently only in the presence of Ag^+ in MeOH in a concentration-dependent manner. The quantum yields of **DT1** and **DT2** in MeOH were determined to be 0.06 and 0.12, respectively (reference compound: 9,10-diphenylanthracene). A red-shifted shoulder was observed while the ion solution (pre-dissolved in water) was added to the probe solution (Fig. 2c for **DT1** and Fig. 2d for **DT2**), which might be caused by the presence of water that increases the polarity of the system. To test this, the FL of the more sensitive **DT1** alone was measured in a series of premixed aqueous solvents (MeOH/H_2O). As shown in Fig. S2, increasing the water content of the system resulted in gradual decrease of the original emission peak (λ_{max} = 425 nm) and increase of the red-shifted peak (λ_{max} = 475 nm); the new peak was found to predominate in a highly aqueous medium (H_2O/MeOH = 4 : 1, V/V).

With this aqueous medium we further measured the Ag^+-sensing property of **DT1**. To our delight, results showed that the FL enhancement of the FG is similarly dependent on Ag^+ concentration with a satisfactory linear range from 0 to 20 μM (Fig. S3b). The limit of detection of the probe was determined to be 1.7 μM ($3\sigma/k$), and the probe also showed excellent metal cation selectivity in this 80% aqueous solution (Fig. 2f). Further addition of increasing I^- to the **DT1**-Ag^+ complex recovered gradually the FL of the ligand, suggesting that the complexation is reversible (Fig. S3c and Fig. S3d). Additionally, co-existence of a series of competing ions did not impact the sensitivity of the probe for Ag^+ (Fig. S3e).

Interestingly, we have determined previously that, in the presence of the same ion (Ag^+), the FL of the C3,4-disubstituted coumarin FG, **DT3** (Fig. 1), quenched sharply in water[15]. Since the only structural distinction between **DT1**/**DT2** and **DT3** lies in the substitution pattern of the bis-triazolyl coumarin moieties upon the glucosyl platform, we deduced that they probably adopt different complexation modes with the ion leading to the reversed FL variations observed.

To elaborate this intriguing observation, a series of additional spectroscopic analyses were performed. First, by measuring the response of **DT1** and **DT3** towards Ag^+ in a uniform solvent system (10 μM each in H_2O/MeOH = 4 : 1, V/V), we substantiated that their FL indeed changed conversely (Fig. 3a and Fig. 3b). The dissociation constants of **DT1** and **DT3** with Ag^+ in this system were measured to be 2.2×10^{-4} M^{-1} and 5.2×10^{-5} M^{-1}, respectively. The quantum yields of **DT1** and **DT3** in water (reference compound: 9,10-diphenylanthracene) were determined to be 0.04 and 0.40, respectively. After addition of 100 μM of Ag^+, the quantum yield of **DT1** increased (0.10) while that of **DT3** decreased (0.23). A Job plot analysis suggested that the C2,3-substituted **DT1** forms a 2 : 1 complex with Ag^+ (Fig. 3c), whereas complexation of the C3,4-substituted **DT3** follows a 1 : 1 stoichiometry (Fig. 3d).

Next, we resorted to ^{1}H NMR titration of the two FGs for gaining a better understanding of the coordination modes. We used **DT1′** and **DT3′** (Fig. S5) which are the protected forms of **DT1** and **DT3**, respectively, because of their better spectral resolution. Since DMSO-d_6 was used as the deuterated solvent, we preliminarily confirmed that the trend in FL increase and decrease of **DT1′** and **DT3′** in the presence of Ag^+ in DMSO (Fig. S4) accords with those of **DT1** and **DT3** in H_2O/MeOH, respectively.

Fig. S5 displays the partial (aromatic part) spectral change of both glycoligands in the presence of increasing Ag^+. From the spectra we noted that: 1) Complexation of **DT1′**-Ag^+ and **DT2′**-Ag^+ follows a 2 : 1 and 1 : 1 stoichiometry, respectively, which is in agreement with the result yielded by the Job plot analysis; 2) All aromatic protons (triazole-*H* and coumarin-*H*) of **DT1′** shifted, whereas only the triazole protons and two pairs of coumarin protons adjacent to the triazole of **DT3′** shifted. The second observation suggests that while both triazole and coumarin moieties of **DT1′** participated in the ion coordination, probably only the *N*-atoms of triazole groups of **DT3′** chelated a silver ion.

A quantum chemical calculation was further conducted to predict the binding motif of **DT1** and **DT3** with silver (I). After optimizations, it was simulated that, of the three possible binding motifs

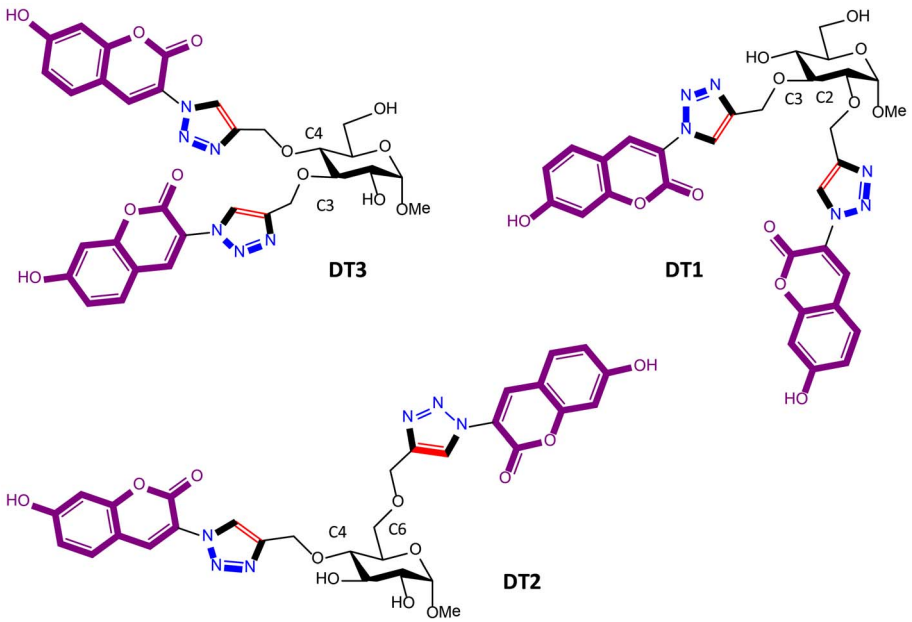

Figure 1 | Structure of the bis-triazolyl coumarin glucosides DT1, DT2 and DT3.

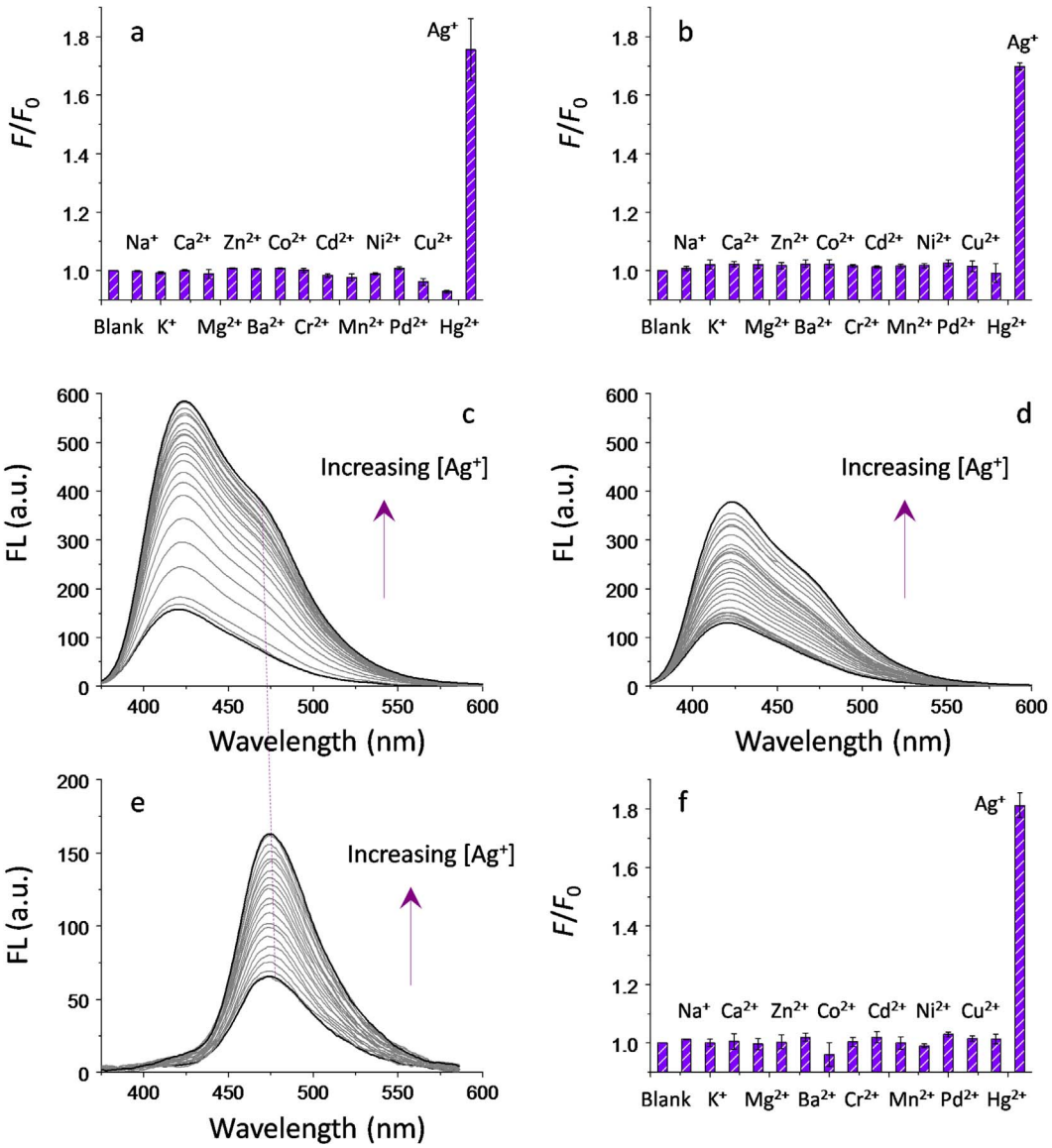

Figure 2 | FL change of 10 μM of (a) **DT1** and (b) **DT2** in the absence and presence of various metal cations (100 μM) in MeOH. FL titration of 10 μM of (c) **DT1** and (d) **DT2** in the presence of increasing Ag^+ (0 to 55 μM for **DT1** and 0 to 50 μM for **DT2**) in MeOH. (e) FL titration of 10 μM of **DT1** in the presence of increasing Ag^+ (0 to 84 μM) in H_2O/MeOH = 4 : 1 (V/V). (f) FL change of 10 μM of **DT1** in the absence and presence of various metal cations (100 μM) in H_2O/MeOH = 4 : 1 (V/V). For all FL spectra, λ_{ex} = 350 nm; for the original FL spectra of (a), (b) and (f) in the absence or presence of the cations, see Fig. S1a, Fig. S1b and Fig. S3a, respectively.

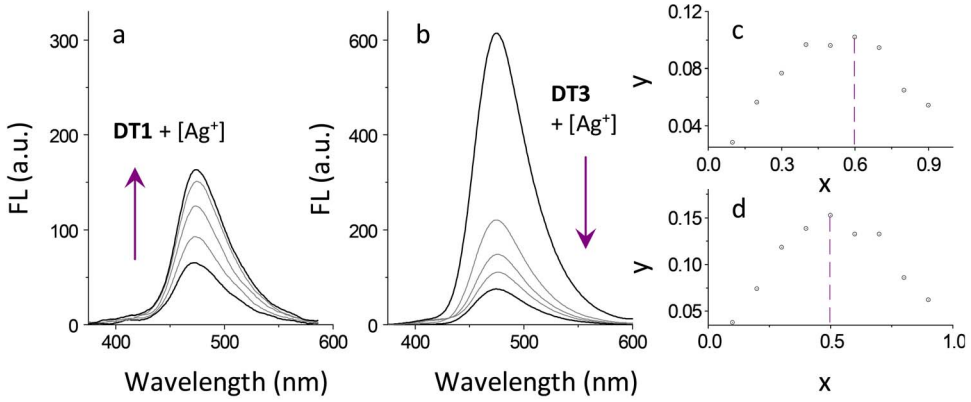

Figure 3 | FL titration of 10 μM of (a) **DT1** and (b) **DT3** in the presence of increasing Ag^+ (0 to 84 μM) in H_2O/MeOH = 4 : 1 (V/V). Job plot of (c) **DT1** and (d) **DT3** in complexation with Ag^+ (y = $F - F_0(1 - x)$, where F_0 is the original FL intensity of probe and F that upon addition of Ag^+). For all FL spectra, λ_{ex} = 350 nm.

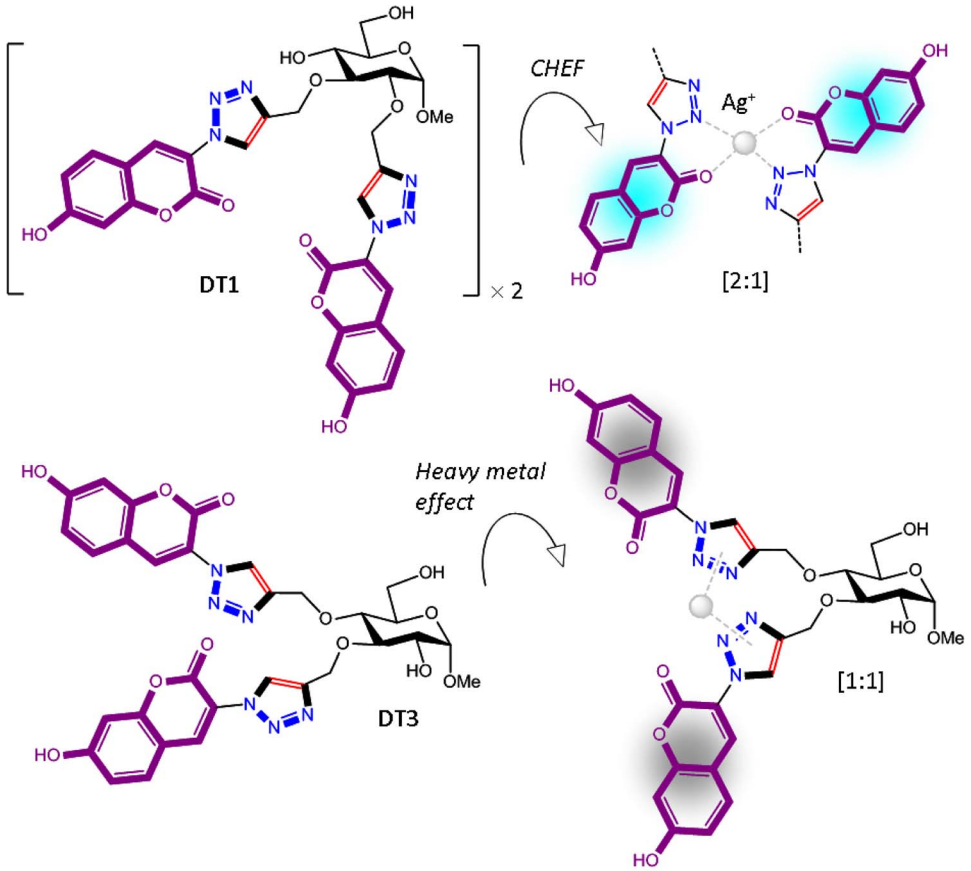

Figure 4 | The computationally simulated optimal complexation mode of DT1 (upper, the distance between triazole-*N2* and coumarin-carbonyl-*O1* and Ag^+ is 2.4 Å and 2.5 Å, respectively) and DT3 (lower) with a silver ion (the green balls stand for carbon atoms, blue for nitrogen, red for oxygen, white for hydrogen and grey for silver).

(Fig. 4, the upper diagram and Fig. S6), the energy of a **DT3**-Ag^+ motif, where the two C3-triazolyl coumarin arms of two individual **DT1** molecules together chelate an ion (Fig. 4), was the lowest. By contrast, a 1 : 1 **DT3**-Ag^+ motif was simulated to be much less stable than the 2 : 1 counterpart (Fig. S7), which suggests that latter is optimal. The optimized motif of one molecule of **DT3** complexed with silver (I) illustrated that only the triazole groups of the ligand are involved in chelation (Fig. 4, the lower diagram).

Based on the above data, we proposed a plausible explanation with respect to the FL changes of the FGs in the presence of Ag^+ (Fig. 5). For **DT1**, two triazolyl coumarin arms that belong separately to two FG molecules coordinate with one silver ion through both the carbonyl groups of coumarin and nitrogen atoms of triazole, leading to a CHEF (chelation-enhanced fluorescence)-like mechanism. This is similar to a chelation mode recently described between a triazolyl coumarin-based chemo-probe and a heavy metal[36], as well as to some other simulated motifs including constrained glycoligands in complex with heavy metals[37,38]. In contrast, as only the triazole groups are in coordination with silver, the FL quenching of **DT3** could be possibly ascribed to a heavy metal effect[15,39].

To test the practicality of the FGs, a cell imaging assay was eventually performed with the 'turn-on' probe **DT1** (Fig. 6). We first tested the sensitivity of the probe for Ag^+ in HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] buffer (pH 7.3) that will be used for the cellular assay. As shown in Fig. 6a, the FL of **DT1** gradually enhanced with increasing Ag^+ in the buffer, corroborating its good water solubility and potential utility for FL imaging in live cells under physiological conditions. An MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] cell-viability assay (3-day incubation of the probe at varying concentrations with the cells) then revealed that the

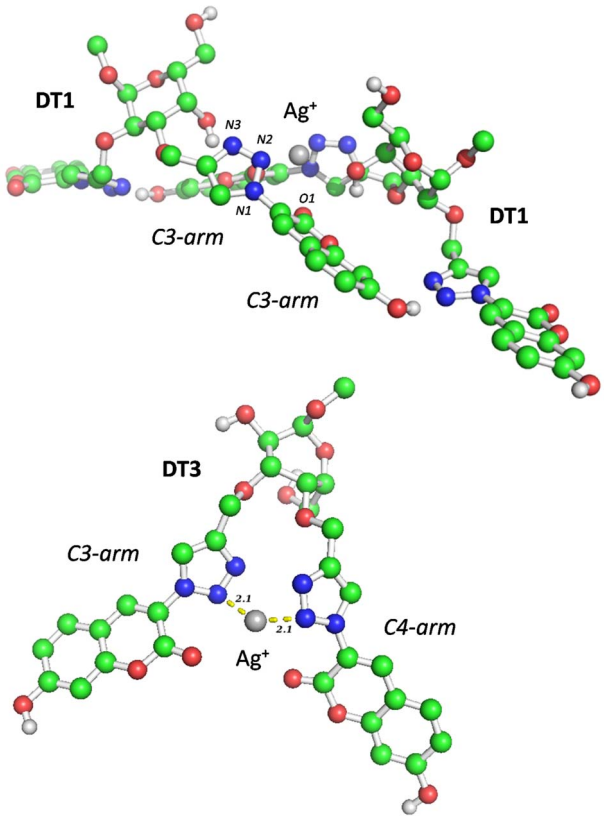

Figure 5 | The proposed coordination modes and mechanisms of the FL alternation of DT1 and DT3 in complexation with Ag^+.

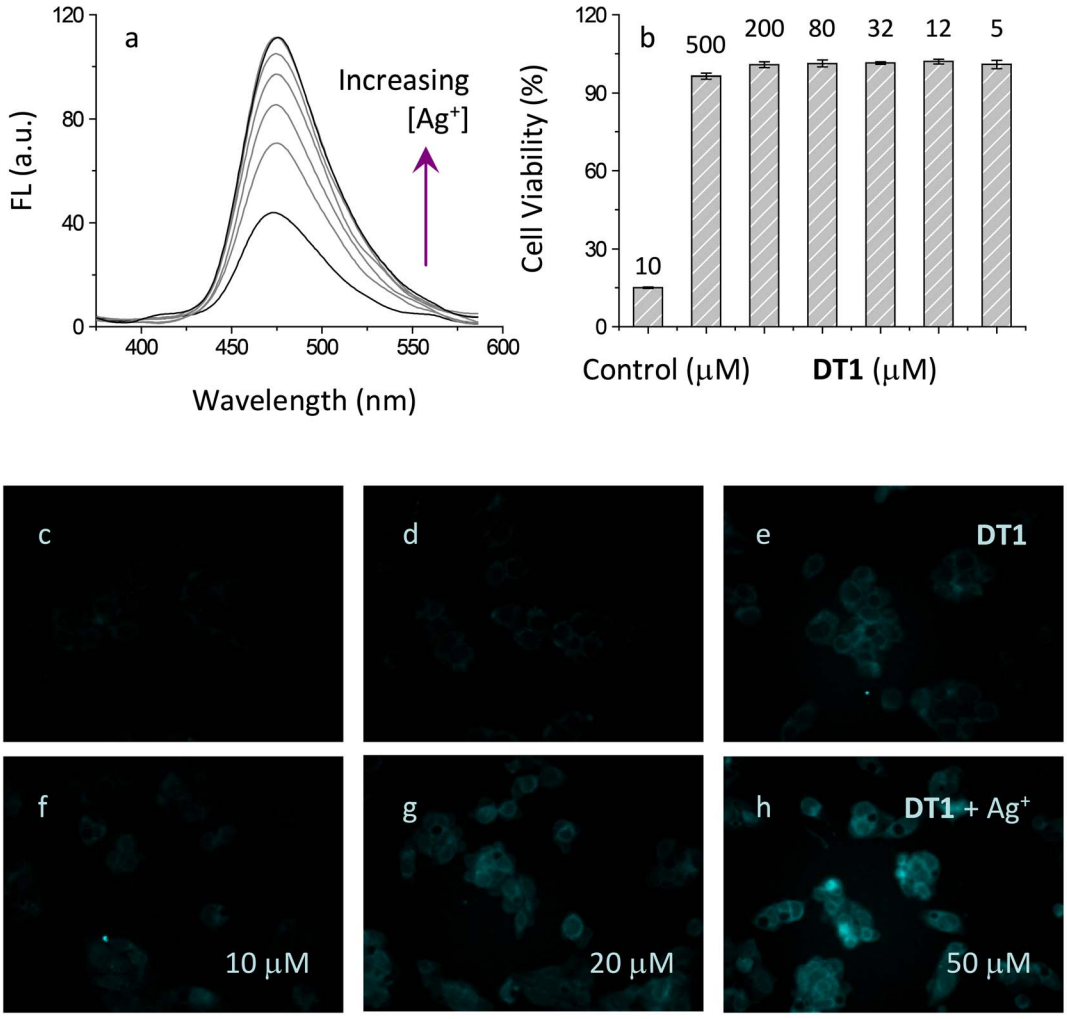

Figure 6 | (a) FL titration of 10 μM of **DT1** in the absence (the bottom curve) and presence of increasing Ag^+ (0–42 μM) in HEPES buffer (95% HEPES mixed with 5% DMSO, pH 7.3). (b) Cell viability of Hep-G2 in the presence of a control compound (doxorubicin) and **DT1** with increasing concentrations. FL images of Hep-G2 cells in the presence of (c) 10 μM, (d) 20 μM and (e) 50 μM of **DT1**. FL images of Hep-G2 cells preincubated with 20 mM of Ag^+ in the presence of (f) 10 μM, (g) 20 μM and (h) 50 μM of **DT1**.

probe was not toxic to Hep-G2 cells (human hepatoma) even at a relatively high concentration (500 μM, 50-fold the concentration used for sensing the ion in solution, Fig. 6b).

Next, by incubation of the probe alone with Hep-G2 cells at three different concentrations, only very weak FL was recorded (Fig. 6c, Fig. 6d and Fig. 6e). However, incubation of the cells with Ag^+ prior to loading of the probe led to emergence of clearly intensified FL. This implies that **DT1** could probably chelate silver ions internalized by the cells, thereby producing the enhanced FL (Fig. 6f, Fig. 6g and Fig. 6h). These results together support the promise of the coumarin-based FGs in monitoring silver ions in live cells.

Discussion

We unravelled with this research an unprecedented discovery that different substitution patterns of triazolyl coumarins upon an identical glucosyl platform could produce FGs with totally reversed optical response to a same heavy metal ion in an aqueous solution. By a series of analyses we determined that this interesting divergence was probably caused by the distinct coordination mode of the conformationally constrained glycoligands with the ion. A 'turn-on' C2,3-substituted FG that exhibited good water solubility and low cytotoxicity has proved suitable for imaging Ag^+ in live cells. This study thereby paves the way for the design and development of sugar-based fluorescent chemo-probes with tuneable FL owing to the conformational constraint of fluorophore-receptor moieties modified on a rigid glycosyl platform.

Methods

General. All chemicals and regents are of high commercially available grade, and were used as received. ^{1}H NMR spectra were recorded on a Bruker AM-400 spectrometer using tetramethyl silane (TMS) as the internal standard ($\delta = 0$). LC-MS was performed on a Waters ACQUITY UPLC™ system with a Quattro Mirco MS (triple quadrupole MS).

Fluorescence spectroscopy. The fluorescence measurements were carried out on a Varian Cary Eclipse Fluorescence spectrophotometer by using a path length of 10 mm with excitation at 350 nm by scanning the emission spectra between 360 nm and 600 nm. The bandwidth for both excitation and emission spectra was 5 nm. All cations tested are perchlorate salts prepared in a stock solution of 10 mM in H_2O, and were diluted to the indicated concentrations for testing.

MTS cell viability assay. Hep-G2 cells were plated overnight on 96-well plates at 5000 cells per well in growth medium. After seeding, cells were maintained in growth media treated at increasing concentrations (5.12 μM, 12.8 μM, 32 μM, 80 μM, 200 μM and 500 μM) of **DT1** (dissolved in DMSO, final concentration) for 72 h. 20 μL of MTS (Promega Corp) solution (2 mg/mL) was added to each well for 2 h at 37°C, and then the absorbance was measured on a SpectraMax 340 microplate reader (Molecular Devices, USA) at 490 nm with a reference at 690 nm. The optical density of the result in MTS assay was directly proportional to the number of viable cells. Each experiment was done in triplicate.

Cell imaging. Hep-G2 cells were cultured in DMEM supplemented with 10% FBS. Cells (1.5×10^4/well) were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. After pretreatment with 20 mM $AgNO_3$ in 50 mM HEPES for 30 min, the cells were incubated with the probe in 50 mM HEPES at different concentrations for another 30 min. Then the cells on the microplate were rinsed in warm HEPES and fixed by 4% paraformaldehyde in HEPES for 15 min at room temperature. After rinsing in HEPES three times (5 min each time), the fluorescence was eventually detected and photographed with an Operetta high content imaging system (Perkinelmer, US).

Quantum chemical calculations. All the quantum chemical calculations were performed with Gaussian 09 software. The original geometries of all molecules were drawn using GaussView v5.08 program, which were further optimized by a density functional theory (DFT) with Becke's three-parameter hybrid exchange functional and the Lee-Yang-Parr correlation functional (B3LYP). In all calculations, a combined basis set was employed with LanL2DZ for silver and 6-31G(d) for other atoms.

1. Bellot, F., Hardré, R., Pelosi, G., Thérisod, M. & Policar, C. Superoxide dismutase-like activity of cobalt(II) complexes based on a sugar platform. *Chem. Commun.* **41**, 5414–5416 (2005).
2. Yuasa, R. H. *et al.* Hinge sugar as a movable component of an excimer fluorescence sensor. *Org. Lett.* **6**, 1489–1492 (2004).
3. Yuasa, H., Miyagawa, N., Nakatani, M., Izumi, M. & Hashimoto, H. A tongs-like fluorescence sensor for metal ions: perfect conformational switch of hinge sugar by a pyrene stacking. *Org. Biomol. Chem.* **2**, 3548–3556 (2004).
4. Qu, S., Lin, Z., Duan, C., Zhang, H. & Bai, Z. A sugar-quinoline fluorescent chemosensor for selective detection of Hg^{2+} ion in natural water. *Chem. Commun.* **42**, 4392–4394 (2006).
5. Charron, G. *et al.* Glycoligands tuning the magnetic anisotropy of Ni^{II} complexes. *Chem. Eur. J.* **13**, 2774–2782 (2007).
6. Xie, J., Ménand, M., Maisonneuve, S. & Métivier, R. Synthesis of bispyrenyl sugar-aza-crown ethers as new fluorescent molecular sensors for Cu(II). *J. Org. Chem.* **72**, 5980–5985 (2007).
7. Yang, Y.-K., Shim, S. & Tae, J. Rhodamine–sugar based turn-on fluorescent probe for the detection of cysteine and homocysteine in water. *Chem. Commun.* **46**, 7766–7768 (2010).
8. Garcia, L. *et al.* Sugars to control ligand shape in metal complexes: conformationally constrained glycoligands with a predetermination of stereochemistry and a structural control. *Inorg. Chem.* **49**, 7282–7288 (2010).
9. Mitra, A., Mittal, A. K. & Rao, C. P. Carbohydrate assisted fluorescence *turn-on* gluco–imino–anthracenylconjugate as a Hg(II) sensor in milk and blood serum milieu. *Chem. Commun.* **47**, 2565–2567 (2011).
10. Garcia, L. *et al.* Intrinsically fluorescent glycoligands to study metal selectivity. *Inorg. Chem.* **50**, 11353–11362 (2011).
11. Hsieh, Y.-C. *et al.* A sugar-aza-crown ether-based fluorescent sensor for Cu^{2+} and Hg^{2+} ions. *Carbohydr. Res.* **346**, 978–981 (2011).
12. Pujol, A. M. *et al.* Hepatocyte targeting and intracellular copper chelation by a thiol-containing glycocyclopeptide. *J. Am. Chem. Soc.* **133**, 286–296 (2011).
13. Lee, M. H. *et al.* Hepatocyte-targeting single galactose-appended naphthalimide: a tool for intracellular thiol imaging in vivo. *J. Am. Chem. Soc.* **134**, 1316–1322 (2012).
14. Singhal, N. K., Ramanujam, B., Mariappanadar, V. & Rao, C. P. Carbohydrate-based switch-on molecular sensor for Cu(II) in buffer: asorption and fluorescence study of the selective recognition of Cu(II) ions by galactosyl derivatives in HEPES buffer. *Org. Lett.* **8**, 3525–3527 (2006).
15. He, X.-P. *et al.* Creation of 3,4-bis-triazolocoumarian-sugar conjugates via flourogenic dual click chemistry and their quenching specificity with silver(I) in aqueous media. *Tetrahedron* **67**, 3343–3347 (2011).
16. Ma, X., Tan, Z., Wei, G., Wei, D. & Du, Y. Solvent controlled sugar–rhodamine fluorescence sensor for Cu^{2+} detection. *Analyst* **137**, 1436–1439 (2012).
17. Ke, B. *et al.* A fluorescent probe for rapid aqueous fluoride detection and cell imaging. *Chem. Commun.* **49**, 2494–2496 (2013).
18. Rostovtsev, V. V., Green, L. G., Fokin, V. V. & Sharpless, K. B. A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective ligation of azides and terminal alkynes. *Angew. Chem. Int. Ed.* **41**, 2596–2599 (2002).
19. Tornøe, C. W., Christensen, C. & Meldal, M. Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. *J. Org. Chem.* **67**, 3057–3064 (2002).
20. Wang, Q. & Hawker, C. Toward a few good reactions: Celebrating click chemistry s first decade. *Chem. Asian J.* **6**, 2568–2569 (2011).
21. Holb, H. C., Finn, M. G. & Sharpless, K. B. Click chemistry: diverse chemical function from a few good reactions. *Angew. Chem. Int. Ed.* **40**, 2004–2021 (2001).
22. Struthers, H., Mindt, T. L. & Schibli, R. Metal chelating systems synthesized using the copper(I) catalyzed azide-alkyne cycloaddition. *Dalton Trans.* **39**, 675–696 (2010).
23. Shi, D.-T. *et al.* K. Bis-triazolyl indoleamines as unique "off–approach–on" chemosensors for copper and fluorine. *Analyst* **138**, 2808–2811 (2013).
24. Zhang, Y.-J. *et al.* Highly optically selective and electrochemically active chemosensor for copper (II) based on triazole-linked glucosyl anthraquinone. *Dyes Pigm.* **88**, 391–395 (2011).
25. Song, Z. *et al.* 'Click' to bidentate bis-triazolyl sugar derivatives with promising biological and optical features. *Tetrahedron Lett.* **52**, 894–898 (2011).
26. Tang, Y.-H. *et al.* Discovery of a sensitive Cu(II)-cyanide "off-on" sensor based on new *C*-glycosyl triazolyl bis-amino acid scaffold. *Org. Biomol. Chem.* **10**, 555–560 (2012).
27. He, X.-P., Xie, J., Chen, G. & Chen, K. Pyrene excimer-based bis-triazolyl pyranoglycoligands as specific mercury sensors. *Chin. J. Chem.* **30**, 2874–2878 (2012).
28. Cisnetti, F., Guillot, R., Thérisod, M., Desmadril, M. & Policar, C. Stereocontrol by a pair of epimeric sugar-derived ligands of the coordination sphere of copper(II) Complexes. *Inorg. Chem.* **47**, 2243–2245 (2008).
29. Cisnetti, F. *et al.* Metal complexation of a D-ribose-based ligand decoded by experimental and theoretical studies. *Eur. J. Inorg. Chem.* **2012**, 3308–3319 (2012).
30. Zhang, J. F., Zhou, Y., Yoon, J. & Kim, J. S. Recent progress in fluorescent and colorimetric chemosensors for detection of precious metal ions (silver, gold and platinum ions). *Chem. Soc. Rev.* **40**, 3416–3429 (2011).
31. Kumar, M., Kumar, R. & Bhalla, V. Optical chemosensor for Ag^+, Fe^{3+}, and cysteine: information processing at molecular level. *Org. Lett.* **13**, 366–369 (2011).
32. Zhang, B. *et al.* A highly selective ratiometric fluorescent chemosensor for Ag^+ based on arhodanineacetic acid–pyrene derivative. *New J. Chem.* **35**, 849–853 (2011).
33. Tsukamoto, K., Shinohara, Y., Iwasaki, S. & Maeda, H. A coumarin-based fluorescent probe for Hg^{2+} and Ag^+ with an *N′*-acetylthioureido group as a fluorescence switch. *Chem. Commun.* **47**, 5073–5075 (2011).
34. Lin, G. *et al.* An *ortho*-methylated fluorescent chemosensor based on pyrromethene for highly selective and sensitive detection of Ag^+ and Hg^{2+} ions. *Mater. Chem. Phys.* **141**, 591–595 (2013).
35. Xue, J.-L. *et al.* Construction of triazolyl bidentate glycoligands (TBGs) by grafting of 3-azidocoumarin to epimeric pyranoglycosides via a fluorogenic dual click reaction. *Carbohydr. Res.* **363**, 38–42 (2012).
36. Ho, I.-T. *et al.* Design and synthesis of triazolyl coumarins as Hg^{2+} selective fluorescent chemosensors. *Analyst* **137**, 5770–5776 (2012).
37. Yang, S.-T., Liao, D.-J., Chen, S.-J., Hu, C.-H. & Wu, A.-T. A fluorescence enhancement-based sensor for hydrogen sulfate ion. *Analyst* **137**, 1553–1555 (2012).
38. Chen, K. H. A pyrenyl-appended triazole-based ribose as a fluorescent sensor for Hg^{2+} ion. *Carbohydr. Res.* **345**, 2557–2561 (2010).
39. Ast, S. *et al.* Integration of the 1,2,3-triazole "click" motif as a potent signaling element in metal ion responsive fluorescent probes. *Chem. Eur. J.* **19**, 2990–3005 (2013).
40. Garcia, L. *et al.* An intrinsically fluorescent glycoligand for direct imaging of ligand trafficking in artificial and living cell systems. *New J. Chem.* **37**, 3030–3034 (2013).
41. Li, K.-B. *et al.* A per-acetyl glycosyl rhodamine as a novel fluorescent ratiometric probe for mercury (II). *Dyes Pigm.* **102**, 273–277 (2014).

Acknowledgments

We thank the 973 project (2013CB733700), the National Science Fund for Distinguished Young Scholars (81125023), the National Natural Science Foundation of China (21176076, 21202045 and 81173033), and the Key Project of Shanghai Science and Technology Commission (13NM1400900).

Author contributions

G.-R.C., J.X., J.L. and X.-P.H. discussed and conceived the idea. S.-D.T. synthesized the compounds and performed the optical tests; X.-L.W. performed the biological tests; Y.S. performed the calculation. Y.Z. supervised the biological tests; G.L. and T.Y. supervised the calculation. X.-P.H. wrote the paper. All authors commented on the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Shi, D.-T. *et al.* Substitution Pattern Reverses the Fluorescence Response of Coumarin Glycoligands upon Coordination with Silver (I). *Sci. Rep.* **4**, 4252; DOI:10.1038/srep04252 (2014).