SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
ANALYTICAL CHEMISTRY
INORGANIC CHEMISTRY

Received
31 July 2014

Accepted
4 November 2014

Published
20 November 2014

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A Ruthenium(II) Complex Supported by Trithiacyclononane and Aromatic Diimine Ligand as Luminescent Switch-On Probe for Biomolecule Detection and Protein Staining

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A new ruthenium(II) complex has been developed for detection of biomolecules. This complex is highly selective for histidine over other amino acids and has been applied to protein staining in an SDS-PAGE gel.

Luminescent transition metal complexes constitute an important class of compounds that have found increasing applications in inorganic photochemistry[1–9], phosphorescent materials for optoelectronics[10,11] and luminescent sensing applications[2,3,12–16]. In particular, ruthenium(II)-diimine complexes such as $[Ru(bpy)_2(dppz)]^{2+}$ (bpy = 2,2′-bipyridine; dppz = dihydro[3,2-*a*:2′,3′-*c*]phenazine), have received particular attention due to their "molecular light switch effect" when bound to DNA[17–20]. Over the last two decades, the topic of luminescent transition metal complex–nucleic acid interactions has been firmly established as an important field of research[21–32].

By comparison, reports on the development of luminescent transition metal complexes for protein detection and protein staining have been considerably scarcer. SYPRO Ruby dye, a ruthenium(II) complex of undisclosed formulation, is commercially available as a reagent for protein gel staining[33,34]. However, its unknown composition hinders investigation and optimization of the staining protocol. We have previously developed cyclometallated iridium(III) complexes bearing 2-phenylpyridine (ppy) ligands for protein detection and staining[35]. As part of our continuing efforts, cyclometallated platinum(II) complexes with 2-phenyl-1,10-phenanthroline ligands have also been reported for protein gel staining and cellular imaging[36]. On the other hand, we have recently reported the photophysics of a series of ruthenium(II) and osmium(II) complexes with the general formula $[M^{II}(N^{\wedge}N)(X)_3(L)]^{n+}$ where $(X)_3$ are facial or meridional tridentate ligands, and N^N are bpy-like aromatic diimines[8]. Envisioning that the $[M^{II}(N^{\wedge}N)(X)_3]^{n+}$ core could serve as a luminescent switch-on/off probe when it binds with biomolecules, we designed a water-soluble ruthenium(II) complex based on the $[Ru^{II}(N^{\wedge}N)(X)_3]^{n+}$ core as a reagent for biomolecule detection and protein staining. We report herein a new ruthenium(II) complex bearing 1,4,7-trithiacyclononane ([9]aneS3) and 4,4′-dimethoxycarbonyl-2,2′-bipyridine (dcmb) (complex **1′**, Fig. 1). Complex **1′** is highly selective for histidine over other natural amino acids, and also functions as a switch-on luminescent probe for protein in solution and for protein staining in a polyacrylamide gel.

Results

Synthesis. Complex **1** was synthesized by refluxing $[Ru([9]aneS3)(dmso)Cl_2]$ with dcmb in methanol under argon for 90 min; recrystallization by slow diffusion of diethyl ether into an acetonitrile solution of **1** gave the acetonitrile-ligated complex $[Ru(dcmb)([9]aneS3)(CH_3CN)]^{2+}$ (**1′**) in 72% yield. The structures of the complexes were confirmed by ^1H and ^{13}C NMR spectroscopy and mass spectrometry. These complexes were sufficiently

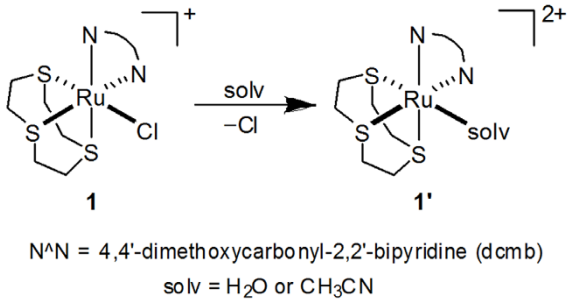

Figure 1 | Chemical structure of ruthenium(II) complexes 1′, formed from the *in situ* hydrolysis of complex 1.

stable to be handled in air under ambient conditions in solution and solid forms. For example, complex **1′** was stable in aqueous solution for 72 h at room temperature, as revealed by UV-Vis spectroscopy. The absorption spectrum of **1′** in acetonitrile displays two absorption peaks [λ/nm (ε_{max}/dm^3 mol^{-1} cm^{-1})]: 367 nm (6870) and 493 nm (7120) (Fig. S2†). Excitation of **1′** (31 μM) at 450 nm in acetonitrile resulted in an emission with λ_{max} at 692 nm, a quantum yield of 2.334×10^{-3}, and a lifetime of 0.272 μs. This emission was assigned as the triplet $d_\pi(Ru^{II}) \rightarrow \pi^*(N^{\frown}N)$ charge-transfer in nature based on comparison with analogous complexes reported previously[7,37–47]. The replacement of the bpy ligand with dcmb has been previously reported to red-shift the emission maximum of ruthenium(II) complexes[48]. The long-wavelength emission of **1′** is especially attractive for luminescent sensing applications, as interference from endogenous fluorophores (autofluorescence) can be reduced.

Binding analysis. We first examined the binding of complex **1′** to the amino acid histidine. Encouragingly, we found that the emission intensity of complex **1′** in 5% CH_3CN/95% H_2O solution at λ_{max} = 630 nm was enhanced by up to 6.9-fold at [His]/[**1′**] = 48 (Fig. 2). As a control, we tested the response of complex **1′** to the amino acid glycine. The addition of glycine gave less than 0.2-fold increase in the emission intensity of **1′** even up to [Gly]/[**1′**] = 78 (Fig. S4†). The addition of groups of all the amino acids (each at 2.4 mM or *ca.* 50-times excess of the metal complex) also did not give a significant luminescent response (Fig. S5†). To investigate the binding property of complex **1′**, electrospray ionization positive-ion mass spectrometry (ESI-MS) was adopted for the binding between complex **1′** and histidine. No covalent attachment peak was observed for complex **1′** upon incubation with histidine for 5 h at 20°C (Fig. S6†). Compared with the complex $[Ir(ppy)_2(solv)_2]^+$ (where solv = H_2O or CH_3CN) we previously reported as a covalently binder to histidine, there was an additional peak center at *m/z* 656.1 which corresponds to the covalent attachment[23] indicating that complex **1′** may not covalently bind to histidine. These results demonstrate that complex **1′** might have another interaction mode and high selectivity towards histidine over other natural amino acids, as only the addition of histidine could give a significant luminescent response.

Luminescence response. To study the luminescence response of **1′** towards proteins, we chose the common protein standard bovine albumin serum (BSA) as the test analyte. We observed that the emission intensity of **1′** at λ_{max} = 630 nm was greatly enhanced upon addition of BSA (Fig. 3). An 18-fold increase in the emission intensity of **1′** was registered at [BSA]/[**1′**] = 1.2. We hypothesize that the binding of **1′** to the histidine residues of the protein protects the aromatic diimine moiety from the aqueous environment, thereby suppressing non-radiative decay of the excited state and promoting ^{3}CT emission.

Absorption titration. An absorption titration experiment was performed to further investigate the binding of **1′** to BSA. Isosbestic points were observed at 437 nm and 513 nm (Fig. S7†). Using the Scatchard equation, the binding constant *K* at 20 °C was determined to be 1.70×10^5 mol^{-1} dm^3 *D* (Fig. S7 Inset†)[49].

Gel electrophoresis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS) is one of the most important techniques in biochemistry and molecular biology to detect and quantitate protein levels. Conventional protein-staining methods include colloidal-silver staining, Coomassie Brilliant Blue staining and Ponceau S staining. However, most involve time-consuming procedures and multiple reagents. The popular and commercially available Coomassie Brilliant Blue (CBB)[50] stain requires a long destaining time for optimal performance. We were thus interested to see if we could apply ruthenium(II) complex **1′** to the staining of protein bands in an SDS-PAGE gel.

Fig. 4 shows an emissive image of a gel containing BSA after staining with **1′** (2.6 mg/20 mL) for 30 min. The lowest quantity of the protein mixture detected after staining with **1′** was 0.625 μg of protein (Fig. 4, left). The sensitivity of this system is at least comparable to Coomassie Brilliant Blue staining (Fig. 4, right). Note that in Fig. 4, complex **1′** was applied to the gel for only 30 min and required no destaining step, whereas Coomassie Brilliant Blue was applied for 60 min and was destained over a period of one to two days. This result demonstrates the simplicity and convenience of this protein staining protein utilizing ruthenium(II) complex **1′**. Whereas the SYPRO Ruby dye staining solution contains 7% acetic acid, which is a mild irritant, the method described here utilises only 5% acetonitrile, a relatively more benign reagent. We also observed that an increase in the staining time enhanced the sensitivity of the system (Fig. S8).

Complex **1′** is postulated to bind histidine residues. Consequently, **1′** should be able to readily detect histidine-rich or histidine-tagged proteins in a protein gel (Fig. 5). Gratifyingly, we observed that **1′** was able to detect 6×His-tagged NF-κB p50 within 30 min, whereas the sensitivity to NF-κB p50 without histidine-tag is relatively reduced (Fig. S9). This property may be useful for the analysis of His-tagged proteins during purification, for example during nickel-column chromatography.

Discussion

In conclusion, we have developed a new ruthenium(II) complex supported by trithiacyclononane and an aromatic diimine as a versatile luminescent switch-on probe for amino acid and protein detection and for protein staining. This complex can be used to selectively sense histidine over all other natural amino acids, and is postulated to bind protein through coordination to histidine residues. There are a wide range of alternate stains such as the traditional methods: Coomassie Brilliant Blue, colloidal-silver stain and SYPRO. However, the Coomassie Brilliant Blue requires a long destaining time for optimal performance, colloidal-silver stain involves multiple steps and reagents, as a result the process is relatively time consuming. The untreated silver stain sample is incompatible for mass spectrometry whereas the undisclosed SYPRO Ruby dye limits the investigation and optimization of the staining protocol.

Recently, some metal complex protein staining dyes have been reported via covalent and non-covalent binding which were reviewed by Lo and co-workers[51]. Generally, luminescent transition metal complexes derivatized with the reactive functional groups have been designed as covalent protein labels. Whereas, the noncovalent dyes generally equip with hydrophobic ligands for non-specific binding or append with a biological substrate that shows specific binding to its receptor protein.

In this study, complex **1′** shows the selectivity towards histidine. However, unlike our previous report iridium(III) complex shows

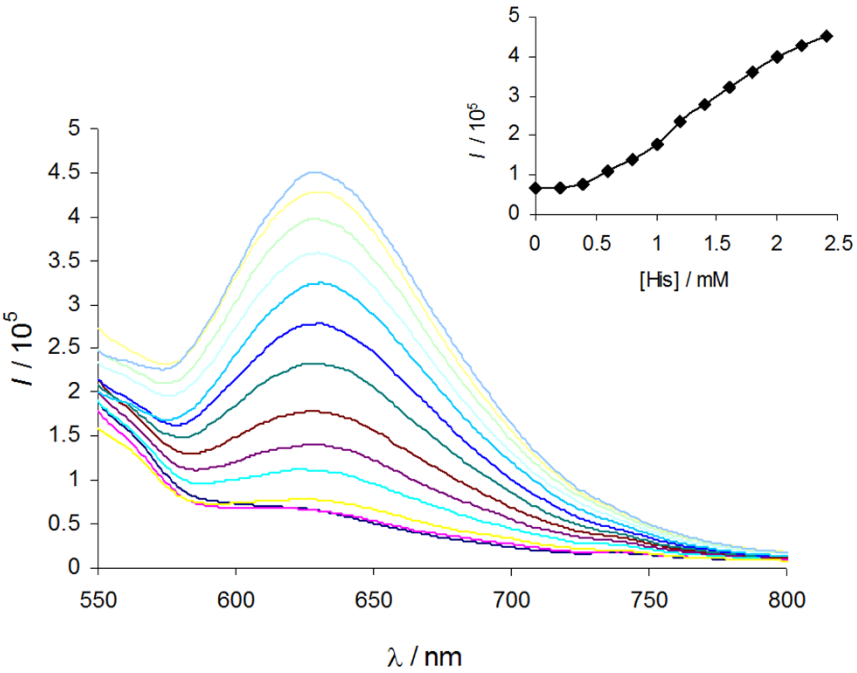

Figure 2 | Phosphorescence emission spectra of 1′ (50 μM) in 5% CH_3CN/95% H_2O with increasing concentration of [His]/[1′] (0–48) at 20°C. Inset: phosphorescence emission intensity at 630 nm *vs.* His concentration.

that it covalently and selectively binds to histidine, complex **1′** might display another type of interaction mode since the ESI-MS doesn't show the covalent attachment peak upon addition of histidine. Therefore, it indicates that the interaction mode may not be covalent binding and it is rare to see the noncovalent protein staining dye display the selectivity. We have applied this complex to SDS-PAGE protein gel staining to detect BSA and His-tagged proteins. Importantly, staining can be complete in less than 30 min and no destaining step is required, with a sensitivity comparable to the highly popular, but time-consuming Coomassie Brilliant Blue dye. This complex also possess a favourable emission maximum approaching the red region, which is attractive for sensing applications as the effects of autofluorescence from endogenous fluorophores can be avoided. Potentially, these complexes could be developed into probes for protein levels in cellular imaging. As the $[d_\pi(Ru^{II}) + L] \rightarrow \pi^*(N^{\wedge}N)$ ^3CT emission of these ruthenium(II)

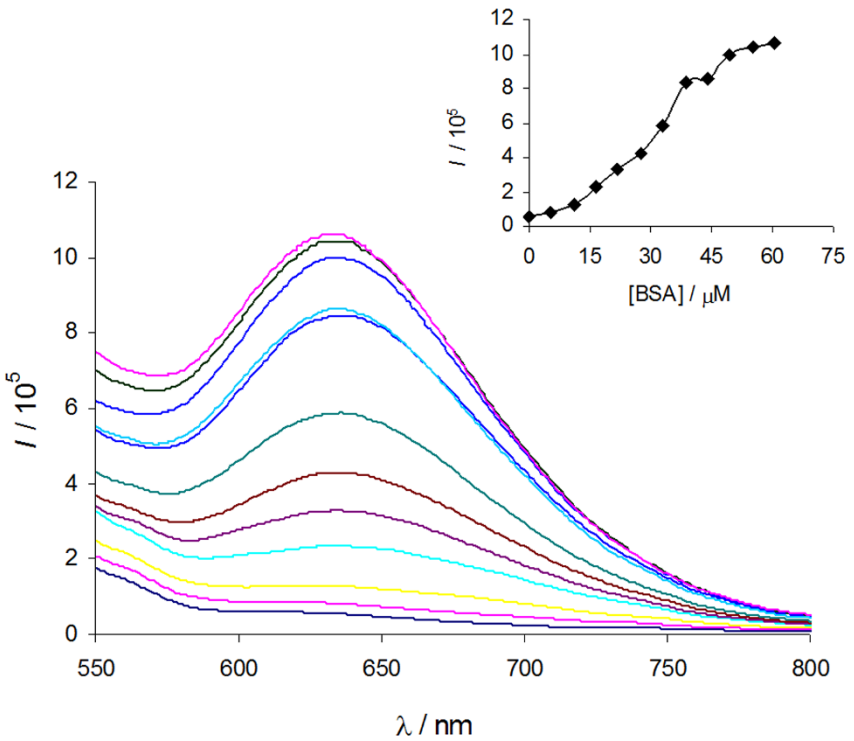

Figure 3 | Phosphorescence emission spectra of 1′ (50 μM) in H_2O with increasing concentration of [BSA]/[1′] (0–1.2) at 20°C. Inset: phosphorescence emission intensity at 630 nm *vs.* BSA concentration.

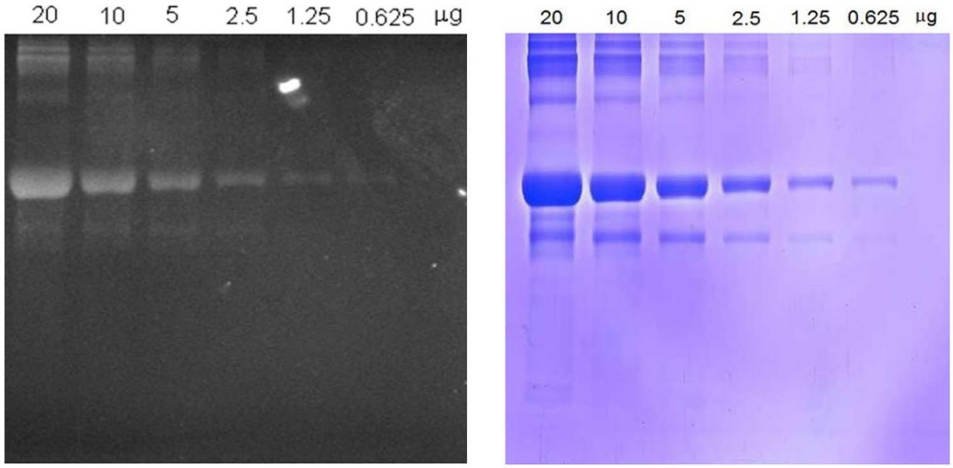

Figure 4 | Left: Emissive SDS-PAGE analysis of BSA with complex 1′ (2.6 mg/20 mL) as the detecting agent (staining time: 30 min); Right: Control SDS-PAGE analysis of BSA with Comassie Brilliant Blue staining (staining: 60 min; destaining: 24–48 h).

complexes is sensitive to the auxiliary ligands, we envisage that further optimization of the emission maximum, sensitivity and selectivity of the system is possible.

Methods

General Procedures. All chemicals and solvents (AR grade) for syntheses were used as received. Bovine serum albumin (BSA, product no. A8531) was purchased from Sigma Chemical Co. Ltd. and used without further purification. Broad-range molecular weight protein standards (Catalog no. 161-0317) and all chemicals for SDS-PAGE were purchased from Bio-Rad. UV-Visible spectra were recorded on Cary UV-300 (for UV-Visible absorption titration) and a Shimadzu UV-1700 spectrophotometer. Emission spectra were recorded on PTI QM-4/2005 Spectrophotometer. ^{1}H NMR spectra were recorded on Bruker 400 DRX FT-NMR spectrometers. Peak positions were calibrated with solvent residue peaks as internal standard. Electrospray mass spectrometry was performed on a PE-SCIEX API 3000 triple quadrupole mass spectrometer.

Synthesis. [([9]aneS3(dcmb)Ru(Cl)](PF$_6$), complex 1. [Ru([9]aneS3)(dmso)Cl$_2$] (0.1 g, 0.2 mmol) was added to a solution of 4,4′-dimethoxycarbonyl-2,2′-bipyridine dcmb (0.4 mmol) in methanol (20 mL) and the reaction mixture was refluxed under argon for 90 min. Upon cooling to room temperature, the resultant mixture was filtered and the filtrate was added to a saturated NH_4PF_6 solution to afford a red precipitate. The precipitate was washed with diethyl ether and dried under vacuum. The solid was recrystallized by slow diffusion of Et_2O into an acetonitrile solution to give deep red crystals. Yield: 0.098 g, 72%. ^{1}H NMR (400 MHz, CD_3CN): δ 2.45–2.78, 2.84–2.95, 2.98–3.10 (m, 12H, [9]aneS3); 4.03 (s, 6H, $COOCH_3$), 8.02 (dd, 2H, J = 5.6, 1.6 Hz, dcmb); 8.94 (s, 2H, dcmb); 9.22 (d, 2H, J = 6 Hz, dcmb). ESI-MS: m/z 589 [M$^+$].

Absorption Titration. UV-Visible spectra were recorded on Cary UV300. A solution of the **1′** (23 µM) was prepared in H_2O. Aliquots of a millimolar BSA stock solution were added. Absorption spectra were recorded in the 320–600 nm range, after equilibrium at 25°C for 20 min. The intrinsic binding constant K was determined from the plot if $D/\Delta\varepsilon_{ap}$ vs D:

$$\frac{D}{\Delta\varepsilon_{ap}} = \frac{D}{\Delta\varepsilon} + \frac{1}{\Delta\varepsilon K}$$

where D is the concentration of BSA, $\Delta\varepsilon_{ap} = |\varepsilon_a - \varepsilon_F|$ and $\Delta\varepsilon = |\varepsilon_B - \varepsilon_F|$. The apparent extinction coefficient, ε_a, is obtained by calculation A_{obs}/[metal complex]. ε_B and ε_F correspond to the extinction coefficient of BSA-metal complex adduct and the extinction coefficient of free metal complex, respectively.

Emission Titration. Emission measurements were carried out by using PTI QM-4/2005 Spectrophotometer. All measurements were made with 15 nm entrance and exit slit. A stock solution of the Ru(II) complex (50 µM) was prepared in acetonitrile. Aliquots of a millimolar BSA stock solution or various natural amino acids were added. Emission spectra were recorded in the 500–850 nm range, after equilibration at 25°C for 20 min.

Sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). SDS-PAGE analysis was conducted according to Bio-Rad Mini-Protean® 3 cell Instruction Manual.

Protein detection. After electrophoresis, the gels were stained with **1′** at room temperature. **1′** (2–3 mg) was first dissolved in 1 mL acetonitrile followed by addition of H_2O (19 mL). Approximately 20 mL of staining solution was used for a typical mini-gel (8 cm × 7 cm × 1.5 mm). The gel was placed into water for 10 min and then transferred into the staining solution. The container was covered with aluminum foil to protect the dye from light. The gel was gently agitated for 30 min at room temperature using an orbital shaker (50 RPM). After staining, the gel was viewed using a 300 nm UV transilluminator directly. Detection and imaging of the gels were conducted using AlphaImager™ 2200 imaging system. For the control gel, Coomassie Brilliant Blue stain was used to visualize the protein sample. The gels were

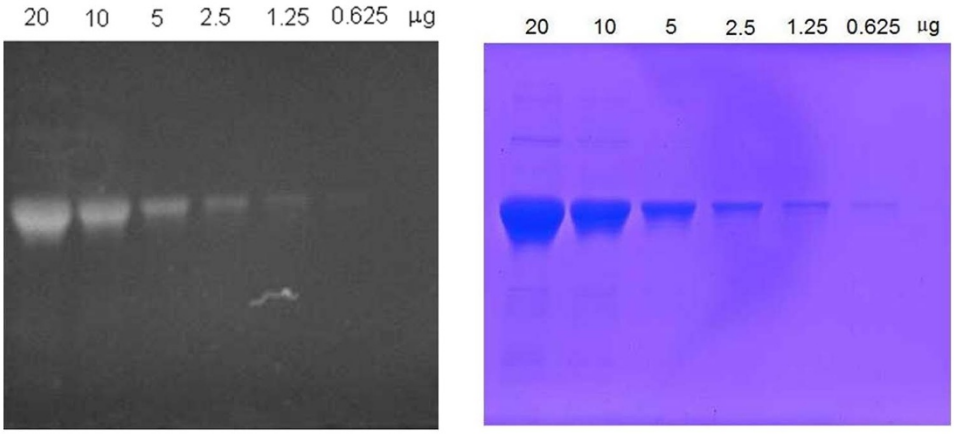

Figure 5 | Left: Emissive SDS-PAGE analysis of 6×His-tagged NF-κB p50 with complex 1′ (2.3 mg/20 mL) as the detecting agent (staining time: 30 min); Right: Control SDS-PAGE analysis of BSA with Comassie Brilliant Blue staining (staining: 60 min; destaining: 24–48 h).

stained for 1 h followed by destaining in deionised H_2O for 24–48 h to reduce the background staining.

1. Szacilowski, K., Macyk, W., Drzewiecka-Matuszek, A., Brindell, M. & Stochel, G. bioinorganic photochemistry: frontiers and mechanisms. *Chem. Rev.* **105**, 2647–2694 (2005).
2. Zhao, Q., Huang, C. & Li, F. Phosphorescent heavy-metal complexes for bioimaging. *Chem. Soc. Rev.* **40**, 2508–2524 (2011).
3. Zhao, Q. *et al.* A highly selective and multisignaling optical−electrochemical sensor for Hg^{2+} based on a phosphorescent iridium(III) complex. *Organometallics* **26**, 2077–2081 (2007).
4. You, Y. *et al.* Phosphorescent sensor for robust quantification of copper(II) ion. *J. Am. Chem. Soc.* **133**, 11488–11491 (2011).
5. Chen, H. *et al.* Selective phosphorescence chemosensor for homocysteine based on an iridium(III) complex. *Inorg. Chem.* **46**, 11075–11081 (2007).
6. Xiong, L. *et al.* Phosphorescence imaging of homocysteine and cysteine in living cells based on a cationic iridium(III) complex. *Inorg. Chem.* **49**, 6402–6408 (2010).
7. Wu, Y. *et al.* Ratiometric phosphorescence imaging of Hg(II) in living cells based on a neutral iridium(III) complex. *Inorg. Chem.* **50**, 7412–7420 (2011).
8. Sauvage, J. P. *et al.* Ruthenium(II) and osmium(II) bis (terpyridine) complexes in covalently-linked multicomponent systems: synthesis, electrochemical behavior, absorption spectra, and photochemical and photophysical properties. *Chem. Rev.* **94**, 993–1019 (1994).
9. Kalyanasundaram, K. Photophysics, photochemistry and solar energy conversion with tris(bipyridyl)ruthenium(II) and its analogues. *Coord. Chem. Rev.* **46**, 159–244 (1982).
10. Chan, C.-W., Cheng, L.-K. & Che, C.-M. Luminescent donor-acceptor platinum(II) complexes. *Coord. Chem. Rev.* **132**, 87–97 (1994).
11. Hissler, M. *et al.* Platinum diimine complexes: towards a molecular photochemical device. *Coord. Chem. Rev.* **208**, 115–137 (2000).
12. Yang, Y., Zhao, Q., Feng, W. & Li, F. Luminescent chemodosimeters for bioimaging. *Chem. Rev.* **113**, 192–270 (2012).
13. Zhao, Q., Li, F. & Huang, C. Phosphorescent chemosensors based on heavy-metal complexes. *Chem. Soc. Rev.* **39**, 3007–3030 (2010).
14. Lo, K. K.-W. *et al.* Luminescent transition metal complex biotin conjugates. *Coord. Chem. Rev.* **250**, 1724–1736 (2006).
15. Balzani, V., Sabbatini, N. & Scandola, F. "Second-sphere" photochemistry and photophysics of coordination compounds. *Chem. Rev.* **86**, 319–337 (1986).
16. De Silva, A. P. *et al.* Signaling recognition events with fluorescent sensors and switches. *Chem. Rev.* **97**, 1515–1566 (1997).
17. McConnell, A. J., Song, H. & Barton, J. K. Luminescence of $[Ru(bpy)_2(dppz)]^{2+}$ bound to RNA mismatches. *Inorg. Chem.* **52**, 10131–10136 (2013).
18. Lim, M. H., Song, H., Olmon, E. D., Dervan, E. E. & Barton, J. K. Sensitivity of $Ru(bpy)_2dppz^{2+}$ luminescence to DNA defects. *Inorg. Chem.* **48**, 5392–5397 (2009).
19. Olson, E. J. C. *et al.* First observation of the key intermediate in the "light-switch" mechanism of $[Ru(phen)_2dppz]^{2+}$. *J. Am. Chem. Soc.* **119**, 11458–11467 (1997).
20. Friedman, A. E., Chambron, J. C., Sauvage, J. P., Turro, N. J. & Barton, J. K. A molecular light switch for DNA: $Ru(bpy)_2(dppz)^{2+}$. *J. Am. Chem. Soc.* **112**, 4960–4962 (1990).
21. Ma, D.-L., Chan, D. S.-H., Leung, C.-H. Group 9 organometallic compounds for therapeutic and bioanalytical applications. *Acc. Chem. Res.* DOI: 10.1021/ar500310z (2014).
22. Leung, C.-H. *et al.* Luminescent detection of DNA-binding proteins. *Nucleic Acids Res.* **40**, 941-955 (2012).
23. Ma, D.-L., Chan, D. S.-H., Yang, H., He, H.-Z. & Leung, C.-H. Luminescent G-quadruplex probes. *Curr. Pharm. Des.* **18**, 2058-2075 (2012).
24. Ma, D.-L. *et al.* Label-free sensing of pH and silver nanoparticles using an "OR" logic gate. *Anal. Chim. Acta* **733**, 78-83 (2012).
25. Lin, S., He, B., Chan, D. S.-H., Chan, P. W.-H., Leung, C.-H. & Ma, D.-L. A G-quadruplex-based platform for the detection of Hg2+ ions using a luminescent iridium(III) complex. *RSC Adv.* **4**, 54826-54831 (2014).
26. Leung, K.-H. *et al.* A highly selective G-quadruplex-based luminescent switch-on probe for the detection of nanomolar strontium(II) ions in sea water. *RSC Adv.* **2**, 8273-8276 (2012).
27. He, H.-Z. *et al.* Label-free detection of sub-nanomolar lead(II) ions in aqueous solution using a metal-based luminescent switch-on probe. *Biosens. Bioelectron.* **41**, 871-874 (2013).
28. Ma, D.-L., He, H.-Z., Chan, D. S.-H. & Leung, C.-H. Simple DNA-based logic gates responding to biomolecules and metal ions. *Chem. Sci.* **4**, 3366-3380 (2013).
29. Ma, D.-L., He, H.-Z., Leung, K.-H., Chan, D. S.-H. & Leung, C.-H. Bioactive luminescent transition-metal complexes for biomedical applications. *Angew. Chem. Int. Ed.* **52**, 7666-7682 (2013).
30. Leung, K.-H. *et al.* A G-quadruplex-selective luminescent switch-on probe for the detection of sub-nanomolar human neutrophil elastase. *RSC Adv.* **3**, 1656-1659 (2013).
31. Leung, K.-H. *et al.* Detection of base excision repair enzyme activity using a luminescent G-quadruplex selective switch-on probe. *Chem. Commun.* **49**, 5630-5632 (2013).
32. He, H.-Z. *et al.* Label-free luminescence switch-on detection of T4 polynucleotide kinase activity using a G-quadruplex-selective probe. *Chem. Commun.* **50**, 5313-5315 (2014).
33. Diwu, Z., Haugland, R. P. & Patton, W. F. Background-free, high sensitivity staining of proteins in one-and two-dimensional sodium dodecyl sulfate-polyacrylamide gels using a luminescent ruthenium complex. *Electrophoresis* **21**, 2509–2521 (2000).
34. Haugland, R. P. *Handbook of fluorescent probes and research products.* [Haugland, R. P. (9)] (Molecular Probes, Inc., Eugene, Oregon, USA, 2002).
35. Ma, D.-L. *et al.* A highly selective luminescent switch-on probe for histidine/histidine-rich proteins and its application in protein staining. *Angew. Chem. Int. Ed.* **120**, 3795–3799 (2008).
36. Wu, P. *et al.* Cyclometalated platinum(II) complexes as highly sensitive luminescent switch-on probes for practical application in protein staining and cell imaging. *Chem. Eur. J.* **15**, 3652–3656 (2009).
37. Wong, C.-Y., Lai, L.-M., Lam, C.-Y. & Zhu, N. Ruthenium carbene and allenylidene complexes supported by the tertiary amine−aromatic diimine ligand set: structural, spectroscopic, and theoretical studies. *Organometallics* **27**, 5806–5814 (2008).
38. Wong, C.-Y., Lai, L.-M., Leung, H.-F. & Wong, S.-H. Ruthenium(II) isocyanide complexes supported by triazacyclononane/trithiacyclononane and aromatic diimine: structural, spectroscopic, and theoretical studies. *Organometallics* **28**, 3537–3545 (2009).
39. Wong, C.-Y., Lai, L.-M. & Pat, P.-K. Ruthenium acetylide complexes supported by trithiacyclononane and aromatic diimine: structural, spectroscopic, and theoretical studies. *Organometallics* **28**, 5656–5660 (2009).
40. Wong, C.-Y., Lai, L.-M., Chan, S.-C. & Tai, L.-H. Photophysical and theoretical studies of ruthenium(II)−acetylide and −cyanide complexes with aromatic diimine and trithiacyclononane. *Organometallics* **29**, 6259–6266 (2010).
41. Wong, C.-Y., Lai, L.-M., Pat, P.-K. & Chung, L.-H. Osmium complexes containing N-heterocyclic carbene-based C, N, C-pincer ligands. *Organometallics* **29**, 2533–2539 (2010).
42. Chan, S.-C., Pat, P.-K., Lau, T.-C. & Wong, C.-Y. Facile direct insertion of nitrosonium ion (NO^+) into a ruthenium−aryl bond. *Organometallics* **30**, 1311–1314 (2011).
43. Chan, S.-C., Cheung, H.-Y. & Wong, C.-Y. Ruthenium complexes containing 2-(2-nitrosoaryl) pyridine: structural, spectroscopic, and theoretical studies. *Inorg. Chem.* **50**, 11636–11643 (2011).
44. Chung, L.-H., Chan, S.-C., Lee, W.-C. & Wong, C.-Y. Emissive osmium(II) complexes supported by N-heterocyclic carbene-based C^C^C-pincer ligands and aromatic diimines. *Inorg. Chem.* **51**, 8693–8703 (2012).
45. Chan, S. C., England, J., Lee, W. C., Wieghardt, K. & Wong, C. Y. Noninnocent behavior of nitrosoarene–pyridine hybrid ligands: ruthenium complexes bearing a 2-(2-nitrosoaryl) pyridine monoanion radical. *ChemPlusChem* **78**, 214–217 (2013).
46. Chung, L.-H. *et al.* Ruthenium(II) and osmium(II) complexes bearing bipyridine and the N-heterocyclic carbene-based C^N^C pincer ligand: an experimental and density functional theory study. *Inorg. Chem.* **52**, 9885–9896 (2013).
47. Chung, L.-H., Yeung, C.-F., Ma, D.-L., Leung, C.-H. & Wong, C.-Y. Metal–indolizine zwitterion complexes as a new class of organometallic material: a spectroscopic and theoretical investigation. *Organometallics* **33**, 3443–3452 (2014).
48. Xia, H. *et al.* Ruthenium(II) complexes with the mixed ligands 2,2′-bipyridine and 4,4′-dialkylester-2,2′-bipyridine as pure red dopants for a single-layer electrophosphorescent device. *J. Phys. Chem. B* **110**, 18718–18723 (2006).
49. Goodman, M. S., Hamilton, A. D. & Weiss, J. Self-assembling, chromogenic receptors for the recognition of dicarboxylic acids. *J. Am. Chem. Soc.* **117**, 8447–8455 (1995).
50. Neuhoff, V., Stamm, R. & Eibl, H. Clear background and highly sensitive protein staining with Coomassie Blue dyes in polyacrylamide gels: a systematic analysis. *Electrophoresis* **6**, 427–448 (1985).
51. Lo, K. K.-W., Choi, A. W.-T. & Law, W. H.-T. Applications of luminescent inorganic and. organometallic transition metal complexes as biomolecular and cellular probes. *Dalton. Trans.* **41**, 6021–6047 (2012).

Acknowledgments

This work is supported by Hong Kong Baptist University (FRG2/13-14/008), Centre for Cancer and Inflammation Research, School of Chinese Medicine (CCIR-SCM, HKBU), the Health and Medical Research Fund (HMRF/13121482), the Research Grants Council (HKBU/201811, HKBU/204612 and HKBU/201913), the French National Research Agency/Research Grants Council Joint Research Scheme (A-HKBU201/12), the State Key Laboratory of Environmental and Biological Analysis and Strategic Development Fund of HKBU (SKLP-14-15-P001), the Science and Technology Development Fund, Macao SAR (103/2012/A3) and the University of Macau (MYRG091(Y2-L2)-ICMS12-LCH, MYRG121(Y2-L2)-ICMS12-LCH and MRG023/LCH/2013/ICMS).

Author contributions

L.H.C., D.S.H.C. and S.L. carried out all the experiments, performed the data analysis; D.L.M., C.H.L and C.Y.W. designed the experiments, analyzed the results and wrote 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: Wong, C.-Y. *et al*. A Ruthenium(II) Complex Supported by Trithiacyclononane and Aromatic Diimine Ligand as Luminescent Switch-On Probe for Biomolecule Detection and Protein Staining. *Sci. Rep.* **4**, 7136; DOI:10.1038/srep07136 (2014).