A copper porphyrin for sensing H_2S in aqueous solution via a "coordinative-based" approach.

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Supporting Information Placeholder

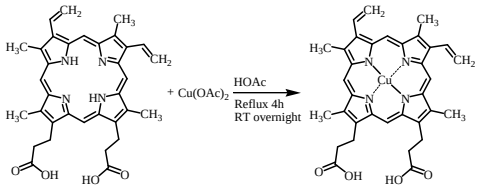
ABSTRACT: A 'turn on' fluorescence-based probe for sensing H_2S in water via a coordinative-based approach has been successfully devised. The probe can selectively detect H_2S in aqueous solutions over other anions, biothiols and common oxidants such as H_2O_2. ^{1}H NMR and ESI MS experiments provide evidences that the turn-on response in the presence of H_2S is due to binding of the target anion to the copper center.

For centuries, hydrogen sulfide (H_2S) has been viewed primarily as a noxious chemical species.[1-4] More recent studies have broadened its traditional view as a noxious chemical species and patently established H_2S as an essential physiological mediator and cellular signaling species,[1-8] but our understanding of H_2S chemistry and its far-ranging contributions to physiology and pathology is still mostly unknown.[9] The complex biological roles of H_2S and potential therapeutic implications constitute a challenging motivation for devising new ways to monitor its production, trafficking, and consumption in living systems. Recently, fuorescence-based systems for H_2S detection have been proposed as selective probes for biological applications.[9-20] These probes can detect H_2S in aqueous solution with high sensitivity and selectivity. Existing fluorescence-based probes implemented so far have been subdivided into four different categories (depending on the reaction mechanism by which analyte recognition occurs):[1;19] *i*) azide-to-amine reduction;[12;21-27] *ii*) nucleophilic addition;[28-31] *iii*) copper displacement;[32-36] *iv*) nitro-to-amine reduction.[37;38] All the sensors belonging to the above categories make use of organic molecules which change their fluorescence intensity when interacting with H_2S. Sensors belonging to category *iii* constitute an exception since in this case the molecule acting as the recognition element is a metal complex. In this case recognition builds on the displacement of the metal from the fluorophore's environment to generally produce fluorescence turn-on changes via H_2S-mediated precipitation of Mt-S.[1;32-34;39-41] Despite all the advantages of the above mentioned probes, some shortcomings typically involve slow response and poor water solubility. In our opinion a limitation of the fluorescent sensors developed so far is the irreversibility of the reaction by which H_2S recognition occurs, which renders the devices not reusable. With a coordinative-based approach one may, in principle, be able to remove H_2S from the metal center of the sensor and ensure a reversible H_2S binding process. This would be advantageous for practical sensing applications allowing reusability of the sensing device. It is well known that H_2S can bind to heme proteins, inducing different responses that in turn modulate its cytotoxic and cytoprotective activities.[42] Thus, the first system we devised as a fluorescent H_2S sensor by exploiting a coordination-based approach makes use of a heme protein. In particular, we employed myoglobin from horse skeletal muscle (Mb).[43] A limitation of our Mb monitoring system was the low amplitude for the fluorescence signals. Furthermore, by subsequent additions of H_2S, Mb(Fe^{3+}) was reduced to the ferrous form: the cuvette sample resulted in a mixture of Mb(Fe^{3+})-H_2S, Mb(Fe^{2+})-H_2S and Mb(Fe^{2+}). A similar reduction had been observed by Scheidt et al. for the hydrosulphide (HS^-) coordination in iron porphyrinates.[44]

More recently we reported that cobalt containing peptide deformylase (Co-PDF) can be implemented as a H_2S sensor by the same coordinative-based approach.[45] The Co-PDF system operates as a "turn-off" devise which is a limitation for real applications.

This time, to improve our H_2S sensors and overcome some of their limitations we focused on designing a simple copper porphyrin complex.

The target copper complex (CuPPIX) was obtained in good yield by mixing an equimolar amount of protoporphyrin IX and $Cu(CH_3COO)_2$ in glacial acetic acid by following a literature procedure (see Scheme 1).[46]



Scheme 1

The stoichiometry was confirmed by ESI-MS where the major peak at 663.1 m/z units corresponds to the mononuclear $[(CuPPIX)K]^+$ species. Less intense peaks at 647.2 and 623.7 were visible and were attributed to the species $[(CuPPIX)Na]^+$ and $[CuPPIX]^+$. No evidence for binuclear species in solution was found. Because of the paramagnetism of the copper center, the ^{1}H NMR spectrum of CuPPIX showed featureless broad resonances.

CuPPIX was further characterized by UV-vis and fluorescence spectroscopy in MilliQ water solution (Figures S1 and S2 in the SI). The UV-vis spectrum of CuPPIX (which resembles those of similar metal porphyrin complexes[46;47]) exhibits the B-band (Soret band) at 385 nm with a shoulder at 343 nm. The Q-bands appeared collapsed into two transitions at 538 nm and 575 nm, due to an increase in symmetry of the molecule upon addition of the metal ion (fig S1 in the SI). The fluorescence spectrum displayed intense fluorescence originating from the first (S_1) excited singlet state (the Q state). The band with two peaks at 623 nm and 682 nm is mirror symmetric to the absorption spectrum in the Q region. The fluorescence originating from the second (S_2) excited singlet state (the B state) is generally quenched in copper porphyrins because of an efficient relaxation pathway ending on a triplet state. The fluorescence spectrum of CuPPIX is consistently weaker than that of the free porphyrin ligand (fig S2 in the SI) which is most likely due to

the coordination of the free ligand to the paramagnetic Cu^{2+} centre.[40]

H_2S binding to CuPPIX was firstly assessed via UV-vis spectroscopy. When KSH (a commonly employed H_2S donor) was added to a MilliQ water solution of CuPPIX a clear change of the initial spectrum was observed. Fig. 1 shows a typical UV-vis spectrum of a solution containing $2*10^{-5}$ M of CuPPIX in which the hyperchromic shift of the Soret band is particularly evident.

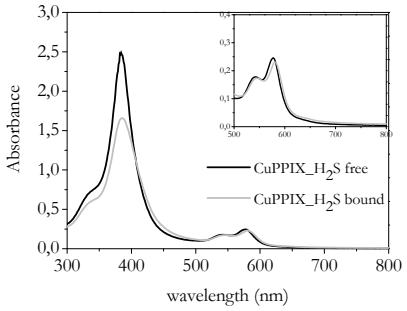


Figure 1. Electronic absorption spectra of CuPPIX free and upon addition of an excess of KSH (rt, MQ water, pH = 12.6). [CuPPIX] = $2*10^{-5}$ M; [KSH] = $2*10^{-4}$ M.

Then the system was studied via fluorescence spectroscopy. In the presence of KSH, a significant enhancement of the S_2 fluorescence emission was observed. Fig. 2 shows a typical fluorescence emission spectrum of a solution containing $2*10^{-5}$ M of CuPPIX when excited at 386 nm.

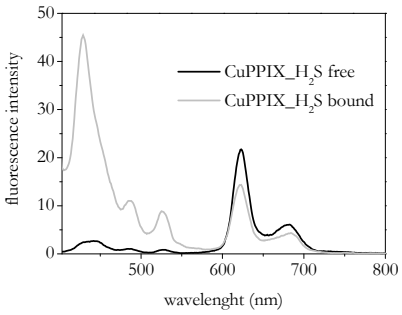


Figure 2. Emission spectra of CuPPIX free (λ_{ex} = 386 nm) and upon addition of an excess of KSH (rt, MQ water, pH = 12.6). [CuPPIX] = $2*10^{-5}$ M; [KSH] = $2*10^{-5}$ M.

To assess whether the amount of the fluorescence enhancement of CuPPIX varies with the concentration of the analyte, the fluorescence intensity of the system was monitored after the addition of increasing amounts of KSH solution. Figure S3 in the SI shows that there is a clear dependence of the fluorescence intensity displayed by the CuPPIX on the KSH concentration.

To obtain an indication on the selectivity of the construct, the fluorescence intensity of CuPPIX in the presence of biologically relevant and potentially competing thiols [e.g., L-cysteine (L-cys) and glutathione (GSH)] or of a range of anions or of common oxidants was checked. Figure 3 shows the obtained results. For the species investigated we observed fluorescence trends completely different than that found with KSH suggesting a good selectivity of our probe in the experimental conditions tested.

In order to gain independent evidence for the binding of H_2S to CuPPIX we examined the reaction via NMR spectroscopy. At first we analyzed the ^1H NMR spectrum of complex CuPPIX in the presence of an excess H_2S. No significant change in the initial ^1H NMR spectrum was observed upon addition of H_2S to a D_2O solution of CuPPIX, excluding that the excess of anion can extract the metal from the complex causing the release of the porphyrin ligand. It is well known that metals can catalyze the oxidation of HS^- to polysulfides and also that copper easily undergoes redox chemistry. To exclude a possible oxidation of HS^- to HSSH[48] (see fig S4 in the SI), we tracked the reaction between the title complex and H_2S via quantitative NMR analysis. We followed a literature protocol for detecting and determining the possible presence of polysulfide species in solution which consists in the alkylation of the polysulfide ions with dimethyl sulfate.[49;50] The reaction was carried out in water with a complex/KSH molar ratio of 1/20 at room temperature under aerobic conditions. At the same time a control reaction (without complex) was also performed. No peaks ascribable to disulfides or polysulfides could be detected. Perfectly superimposable ^1H and ^{13}C NMR spectra for the two reactions were obtained (see fig S5 in the SI). This clearly indicates that HS^- is not oxidated to disulfides/polysulfides by the title complex. Then we studied the reaction between CuPPIX and H_2S by electrospray ionization mass spectrometry in aqueous solution. No peaks ascribable to the free ligand could be detected which confirms what we had already seen via NMR. The major peak at 679.24 m/z units is consistent with the mononuclear $[(CuPPIX)NaHS]^-$ species which suggests analyte binding to the copper complex. To the best of our knowledge, this is the first example for such a reaction. Typically, in the presence of H_2S, literature copper complexes undergo to a demetallation reaction with concomitant release of the organic ligand.[40] Coordination of organic bases to copper porphyrins had been already observed by Glazkov et al.[51;52]

Figure 3. Relative fluorescence emission at 430 nm (λex= 386 nm) of CuPPIX (20 μM) in the presence of KSH (20μM), Glutathione (GSH), Cysteine, NaCl, NaF, CH_3COOK, H_2O_2 (1.2 mM) in aqueous solution.

In conclusion CuPPIX can be successfully used as a 'turn on', sensitive and selective fluorescence probe for the detection of H_2S in aqueous environments *via* a coordinative-based approach. The probe can selectively and sensitively detect HS^- anions in water over other anions, biothiols and common oxidants such as H_2O_2. ^1H NMR and ESI MS experiments provide clear evidence that the turn-on response in the presence of H_2S is ascribed to binding of the target analyte to the copper center. We are currently investigating the mechanism by which the S_2 fluorescence in CuPPIX is

prompted by the coordination of the hydrogen sulfide to the copper center.

Experimental Section

Materials: All chemicals used for the synthetic work were obtained from Sigma-Aldrich or Strem Chemicals and were of reagent grade. They were used without further purification.

Synthesis of CuPPIX: 0.5 g (0.89 mmol) of protoporphyrin IX in 300 ml of glacial acetic acid is boiled gently with about 1.5 g (8.9 mmol) of copper(II) acetate. The solution is left under stirring and at reflux for 4 hours and then overnight at ambient temperature. After conversion to the Cu(II)-protoporphyrin is complete, as indicated by the absence of free base absorption bands in a visual spectroscope, the solution is transferred to a separatory funnel with about 400 cc. of benzene. Water is added to the separatory funnel, and the resulting benzene layer is washed several times with water to completely remove the reaction solvent and inorganic salts. The resulting benzene solution is evaporated to dryness under vacuum to yield the purple-brown solid. Yield: 84%. C34H32CuN4O4: calcd. C 65.42, H 5.17, N 8.98; found C 65.52, H 5.22, N 8.78 MS (ESI acetonitrile): m/z (%) 663.1 (100) $[CuPPIX-K]^+$, 647.2 (80) $[CuPPIX-Na]^+$, 623.7 (50) $[CuPPIX]^+$.

Quantum yield, ΦF (H_2O, λexc = 386 nm): $9{,}5*10^{-4}$.

Absorbance and fluorescence measurements. Absorption spectra were recorded on a Cary-50 Spectrophotometer, using a 1 cm quartz cuvette (Hellma Benelux bv, Rijswijk, Netherlands) and a slit-width equivalent to a bandwidth of 5 nm. Fluorescence spectra were measured on a Cary Eclipse Spectrophotometer in a 10 × 10mm2 airtight quartz fluorescence cuvette (Hellma Benelux bv, Rijswijk, Netherlands) with an emission band-pass of 10 nm and an excitation band-pass of 5 nm. Both absorption and fluorescence measurements were performed in MilliQ water at room temperature. Fluorescence quantum yield (ΦF) value was measured in optically diluted solutions using phenol (ΦF = 0.014 in MilliQ water) as standard, according to the equation:

$$\Phi_F\, s = \Phi_F\, r\, (Is/Ir)(Ar/As)(\eta_F/\eta_F)^2$$

where indexes s and r denote the sample and reference, respectively. I stands for the integrated emission intensity, A is the absorbance at the excitation wavelength, and η is the refractive index of the solvent.

ASSOCIATED CONTENT

UV-vis, fluorescence and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) Lin, V. S.; Chang, C. J. *Curr.Opin.Chem Biol* **2012**, *16*, 595-601.
(2) Reiffenstein, R. J.; Hulbert, W. C.; Roth, S. H. *Annu.Rev Pharmacol.Toxicol.* **1992**, *32*, 109-134.
(3) Smith, R. P. *Can.Med.Assoc.J.* **1978**, *118*, 775-776.
(4) Smith, R. P. *American Scientist* **2010**, *98*, 6-9.
(5) Chen, C. Q.; Xin, H.; Zhu, Y. Z. *Acta Pharmacol.Sin.* **2007**, *28*, 1709-1716.
(6) Li, L.; Moore, P. K. *Trends Pharmacol.Sci.* **2008**, *29*, 84-90.
(7) Li, L.; Moore, P. K. *Biochem.Soc.Trans.* **2007**, *35*, 1138-1141.
(8) Wang, R. *Physiol Rev* **2012**, *92*, 791-896.
(9) Liu, J.; Sun, Y. Q.; Zhang, J.; Yang, T.; Cao, J.; Zhang, L.; Guo, W. *Chemistry* **2013**, *19*, 4717-4722.
(10) Wang, X.; Sun, J.; Zhang, W.; Ma, X.; Lv, J.; Tang, B. *Chem.Sci.* **2013**, *4*, 2551-2556.
(11) Zhou, G.; Wang, H.; Ma, Y.; Chen, X. *Tetrahedron* **2013**, *69*, 867-870.
(12) Chen, S.; Chen, Z. J.; Ren, W.; Ai, H. W. *J.Am.Chem Soc.* **2012**, *134*, 9589-9592.
(13) Lin, V. S.; Lippert, A. R.; Chang, C. J. *Proc.Natl.Acad.Sci.* **2013**, *110*, 7131-7135.
(14) Chen, T.; Zheng, Y.; Xu, Z.; Zhao, M.; Xu, Y.; Cui, J. *Tetrahedron Letters* **2013**, *54*, 2980-2982.
(15) Mao, G. J.; Wei, T. T.; Wang, X. X.; Huan, S. Y.; Lu, D. Q.; Zhang, J.; Zhang, X. B.; Tan, W.; Shen, G. L.; Yu, R. Q. *Anal.Chem* **2013**, *85*, 7875-7881.
(16) Quek, Y. L.; Tan, C. H.; Bian, J.; Huang, D. *Inorg.Chem* **2011**, *50*, 7379-7381.
(17) Tropiano, M.; Faulkner, S. *Chem Commun.* **2014**, *50*, 4696-4698.
(18) Takashima, I.; Kinoshita, M.; Kawagoe, R.; Nakagawa, S.; Sugimoto, M.; Hamachi, I.; Ojida, A. *Chemistry* **2014**, *20*, 2184-2192.
(19) Kumar, N.; Bhalla, V.; Kumar, M. *Coord. Chem. Rev.* **2013**, *257*, 2335-2347.
(20) Lippert, A. R. *J.Inorg.Biochem.* **2014**, *133*, 136-142.
(21) Lippert, A. R.; New, E. J.; Chang, C. J. *J.Am.Chem Soc.* **2011**, *133*, 10078-10080.
(22) Peng, H.; Cheng, Y.; Dai, C.; King, A. L.; Predmore, B. L.; Lefer, D. J.; Wang, B. *Angew.Chem Int.Ed Engl.* **2011**, *50*, 9672-9675.
(23) Yu, F.; Li, P.; Song, P.; Wang, B.; Zhao, J.; Han, K. *Chem Commun.* **2012**, *48*, 2852-2854.
(24) Das, S. K.; Lim, C. S.; Yang, S. Y.; Han, J. H.; Cho, B. R. *Chem Commun.* **2012**, *48*, 8395-8397.
(25) Montoya, L. A.; Pluth, M. D. *Chem Commun.* **2012**, *48*, 4767-4769.
(26) Zheng, K.; Lin, W.; Tan, L. *Org.Biomol.Chem* **2012**, *10*, 9683-9688.
(27) Wu, Z.; Li, Z.; Yang, L.; Han, J.; Han, S. *Chem Commun.* **2012**, *48*, 10120-10122.
(28) Qian, Y.; Karpus, J.; Kabil, O.; Zhang, S. Y.; Zhu, H. L.; Banerjee, R.; Zhao, J.; He, C. *Nat.Commun.* **2011**, *2*, 495.
(29) Liu, C.; Pan, J.; Li, S.; Zhao, Y.; Wu, L. Y.; Berkman, C. E.; Whorton, A. R.; Xian, M. *Angew.Chem Int.Ed Engl.* **2011**, *50*, 10327-10329.
(30) Liu, C.; Peng, B.; Li, S.; Park, C. M.; Whorton, A. R.; Xian, M. *Org.Lett.* **2012**, *14*, 2184-2187.
(31) Zhao, Y.; Zhu, X.; Kan, H.; Wang, W.; Zhu, B.; Du, B.; Zhang, X. *Analyst* **2012**, *137*, 5576-5580.
(32) Sasakura, K.; Hanaoka, K.; Shibuya, N.; Mikami, Y.; Kimura, Y.; Komatsu, T.; Ueno, T.; Terai, T.; Kimura, H.; Nagano, T. *J.Am.Chem Soc.* **2011**, *133*, 18003-18005.
(33) Choi, M. G.; Cha, S.; Lee, H.; Jeon, H. L.; Chang, S. K. *Chem Commun.* **2009**, 7390-7392.
(34) Hou, F.; Huang, L.; Xi, P.; Cheng, J.; Zhao, X.; Xie, G.; Shi, Y.; Cheng, F.; Yao, X.; Bai, D.; Zeng, Z. *Inorg.Chem* **2012**, *51*, 2454-2460.
(35) Wang, M. Q.; Li, K.; Hou, J. T.; Wu, M. Y.; Huang, Z.; Yu, X. Q. *J.Org.Chem* **2012**, *77*, 8350-8354.
(36) Cao, X.; Lin, W.; He, L. *Org.Lett.* **2011**, *13*, 4716-4719.
(37) Wu, M. Y.; Li, K.; Hou, J. T.; Huang, Z.; Yu, X. Q. *Org.Biomol.Chem* **2012**, *10*, 8342-8347.
(38) Xuan, W.; Pan, R.; Cao, Y.; Liu, K.; Wang, W. *Chem Commun.* **2012**, *48*, 10669-10671.
(39) Zhang, D.; Jin, W. *Spectrochim.Acta A Mol.Biomol.Spectrosc.* **2012**, *90*, 35-39.
(40) Santos-Figueroa, L. E.; de la Torre, C.; El Sayed, S.; Sancenon, F.; Martinez-Manez, R.; Costero, A. M.; Gil, S.; Parra, M. *Europ. J. Inorg. Chem.* **2014**, *2014*, 41-45.
(41) Ye, Z.; An, X.; Song, B.; Zhang, W.; Dai, Z.; Yuan, J. *Dalton Trans.* **2014**, *43*, 13055-13060.
(42) Pietri, R.; Lewis, A.; Leon, R. G.; Casabona, G.; Kiger, L.; Yeh, S. R.; Fernandez-Alberti, S.; Marden, M. C.; Cadilla, C. L.; Lopez-Garriga, J. *Biochemistry* **2009**, *48*, 4881-4894.
(43) Strianese, M.; De, M. F.; Pellecchia, C.; Ruggiero, G.; D'Auria, S. *Protein Pept.Lett.* **2011**, *18*, 282-286.
(44) Pavlik, J. W.; Noll, B. C.; Oliver, A. G.; Schulz, C. E.; Scheidt, W. R. *Inorg.Chem.* **2010**, *49*, 1017-1026.
(45) Strianese, M.; Palm, G. J.; Milione, S.; Kuhl, O.; Hinrichs, W.; Pellecchia, C. *Inorg.Chem.* **2012**, *51*, 11220-11222.

(46) Dorough, G. D.; Miller, J. R.; Huennekens, F. M. *J.Am.Chem.Soc.* **1951**, *73*, 4315-4320.
(47) Oliver I.T.; Rawlinson W.A. *Biochemical Journal* **1955**, *61*, 641-646.
(48) Hartle, M. D.; Sommer, S. K.; Dietrich, S. R.; Pluth, M. D. *Inorg.Chem* **2014**, *53*, 7800-7802.
(49) Filpponen, I.; Guerra, A.; Hai, A.; Lucia, L. A.; Argyropoulos, D. S. *Ind.Eng.Chem.Res.* **2006**, *45*, 7388-7392.
(50) Argyropoulos, D. S.; Hou, Y.; Ganesaratnam, R.; Harpp, D. N.; Koda, K. Holzforschung, 2005, 59, 124-131.
(51) Glazkov, Y.; Vrublevskii, A. I.; Kruglik, E. K.; Kachura, T. F. *J. Appl. Spectrosc.* **1981**, *35*, 1254-1259.
(52) Vrublevskii, A. I.; Glazkov, Y.; Kachura, T. F. *J. Appl. Spectrosc.* **1984**, *41*, 1166-1170.

SYNOPSIS TOC.

We devised a new, fast, simple and cost-effective probe for monitoring hydrogen sulphide. Proof-of-principle results that a copper porphyrin complex can be successfully implemented as a turn-on sensing system for H_2S via a coordination-based approach are reported.