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A highly sensitive TTF-functionalised probe for the determination of physiological thiols and its application in tumor cells

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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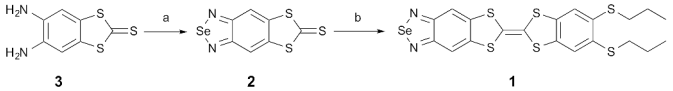
A tetrathiafulvalene (TTF)-fused piazselenole as a novel redox-active probe for highly sensitive determination of physiological thiols by electrochemical detection has been synthesised and successfully tested in intracellular non-protein thiol detection, reaching a detection limit of 10^{-10} M.

Physiological thiols such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) play vital roles in maintaining biological systems.[1] As a principal intracellular low-molecular-weight thiol, GSH has been proven to be effective at combating oxidative stress and maintaining redox homeostasis that is pivotal for cell growth and function.[2] Decreased GSH levels are associated with the pathogenesis of a number of diseases including cancer,[3] diabetes,[4] AIDS[5] and cataracts.[6] Therefore, a sensitive, selective and rapid detection of GSH is of significant importance to the early diagnosis and treatment of the diseases.

In the past few decades, various methods for detection of GSH and related thiols have been developed.[7,8] Among them, electrochemical and fluorescent assays have widely been studied and applied.[9] Fluorescent assays have become increasingly used in intracellular thiol imaging,[10] although conversely electrochemical detection methods have stimulated considerable interest because of the apparent advantages of simplicity, sensitivity, and low instrumental cost, *etc*.[11] In particular, electrochemical measurement of GSH in untreated samples is the most attractive path in terms of simplicity. So far, numerous direct and indirect electrochemical methods for the detection of thiols have been reported.[12] However, they often suffer from difficulties or drawbacks in terms of high overpotentials, toxicity, the need for complex electrode modifications and the lack of sufficient selectivity.[13] In response, we have introduced a new concept of using a piazselenole as an electrochemical probe for rapid, sensitive, and selective detection of cellular thiols.[14] Presumably, the strong nucleophilicity of sulfhydryl group surrounding the proteins to cleave a Se–N bond, might account for its high sensitivity and rapid response to GSH.

Inspired by this work, we set ourselves the synthetic task to fuse together tetrathiafulvalene (TTF) and piazselenole; each of these units has widely appeared in sensor and optoelectronic applications.[15,16] TTF, as a strong π-electron donor, is not only a main component of organic (super)conductors,[15] but also frequently used in the construction of a large variety of donor-acceptor (D–A) systems for photoinduced intramolecular charge-transfer (ICT) processes.[15a-d] In contrast, piazselenole, as an electron-deficient moiety, is often used in the construction of low band gap polymers.[16b,c] Moreover, it has been proven to be sensitive and reactive to mercapto groups and also exhibits encouraging biological activity.[14] The present study aims to further improve the sensitivity of the probe for the detection of GSH by direct fusion of the TTF moiety to the piazselenole core. To the best of our knowledge, this is the first case of electrochemical and fluorescent detection of GSH and application in a cancer cell using a probe containing both Se-N bonds and a TTF moiety.

As outlined in Scheme 1, target compound **1** was obtained by a phosphite-mediated cross-coupling reaction of 4,5-bis(propylthio)-1,3-dithiole-2-one with thione precursor **2**. The latter was prepared by a well-established reaction[17] of diamine **3** with SeO_2. Both of the new compounds **1** and **2** were purified by chromatographic separation, and have been fully characterised (ESI†).

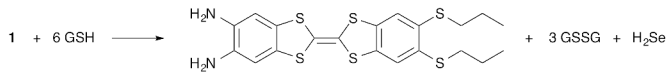


Scheme 1 Synthetic route to compound **1**. Reagents and conditions: a) SeO_2, cyclohexane; b) 4,5-bis-(propylthio)-1,3-dithiole-2-one, triethylphosphite/toluene, 120 °C.

Dark red single crystals of compound **1** were obtained by the solvent evaporation method. X-ray diffraction reveals that this compound crystallises as solvated compound $\mathbf{1}\cdot(C_6H_{14})_{0.5}$ in the monoclinic system, *P*2(1)/*c*. The bond lengths in the TTF moiety are in the range expected for neutral TTF derivatives,[18] as illustrated in an ORTEP drawing of the π-conjugated molecule (ESI†). In the crystal packing, the molecules are prone to show head to head dimerisation via intermolecular Se-N interactions with short contacts of 2.8273(30) Å.

As expected, the probe **1** undergoes one reversible reduction wave at -1.26 V corresponding to the reduction of the piazselenole moiety, and two reversible oxidation waves at 0.70 V and 1.06 V (*vs* Ag/AgCl), for the formation of the TTF radical and dication species (ESI†). Moreover, its optical absorption spectrum in THF shows a broad and intense band peaking around 498 nm, ascribed to an ICT from the TTF to the piazselenole units. Remarkably, the corresponding CT fluorescence is also observed at 680 nm. It can therefore be deduced that the optical HOMO-LUMO gap of only 2.08 eV is in good agreement with the electrochemical gap of 1.96 eV (ESI†).

An investigation on the electrochemical behaviour of **1** on a Au electrode in Britton-Robinson buffer solution (BR buffer, pH 2.0) (ESI†) indicates that **1** is an electroactive species with sensitive electrochemical signals. A cathodic peak at -0.247 V is attributed to an irreversible 6-electron electrochemical reduction of **1** on the electrode surface.[14] Compared to that of piazselenole, the reduction potential of **1** is negatively shifted by 141 mV due to the electron-donating effect of the TTF core. Upon the addition of GSH from 0.5 nM to 5.0 nM to the solution of probe **1**, the peak current decreases sequentially. This observation can be accounted for by the consumption of the electroactive probe **1** with GSH as shown in Scheme 2. The strong nucleophilicity of sulfhydryl in GSH causes the Se-N bond cleavage, whereby the selenium heterocycle breaks down, yielding a product containing the TTF-fused *o*-phenylene-diamine (OPD) moiety. This also holds true for a decrease in the intensity of the CT fluorescence upon the addition of GSH (ESI†).



Scheme 2 Reaction of probe **1** with GSH.

The peak current decrease (ΔI_P) of probe **1** recorded by differential pulse voltammetry was used for GSH detection (Fig. 1). A linear relationship between the ΔI_P of probe **1** and GSH concentrations was plotted with concentrations of GSH in the range of 1.0×10^{-10}-1.0×10^{-9} M. The linear regression equation was obtained as: ΔI_P (10^{-7} A) = -0.376 + 0.306 × C_{GSH} (10^{-10} M) (γ = 0.9901) with the detection limit of 1.0×10^{-10} M (3σ, n = 11). Similarly, there is a linearity between fluorescence intensity change (ΔF) and the common logarithm of concentrations of GSH in the range of 1.0×10^{-8} - 2.0×10^{-5} M, and the regression equation was obtained as: ΔF = 141.7396 + 14.8171 × lgC_{GSH} with a linear coefficient of 0.9938. All these results indicate that **1** acts as a redox and fluorescent probe to detect GSH qualitatively and quantitatively.

The various relevant bioanalytes including metal ions, bioamines, reactive oxygen species, and biological antioxidants were employed for control experiments to evaluate possible interference effects on monitoring GSH. As illustrated in Fig. 2, various detected metal ions yielded no or only negligible interference with GSH detection. As for the bioanalytes including Vc (1.5 μM) and dopamine (2.5 μM), although their concentrations are much higher than that of GSH (5.0 nM), hence 300-fold and 500-fold GSH concentration, respectively, the resulting ΔI_P values are far below the one caused by GSH. Similar to our previously reported results, probe **1** can also selectively respond to GSH compared with other analytes.[14] The reactivity of probe **1** with non-protein thiols was further evaluated (ESI†), suggesting that probe **1** shows the best response to GSH among non-protein thiols such as cysteine (Cys), dithiothreitol (DTT), thioglycolic acid (TA) and 6-mercapto-1-hexanol (MCH).

Finally, Ramos cells (a B-cell lymphoma cell line) were applied to evaluate the capability of this method in biological samples. As depicted in Fig. 3, the non-protein extracts of Ramos cells showed a significant decrease in peak current of probe **1** without preincubation with 1.0 mM thiol-blocking reagent *N*-ethylmaleimide (NEM) (gray bar). After non-protein thiols in Ramos cell extracts were consumed by pretreating with NEM (black bar), the ΔI_P values were significantly reduced. These experimental results clearly demonstrate that probe **1** shows sensitive response toward non-protein thiols in the extracts of Ramos cells.

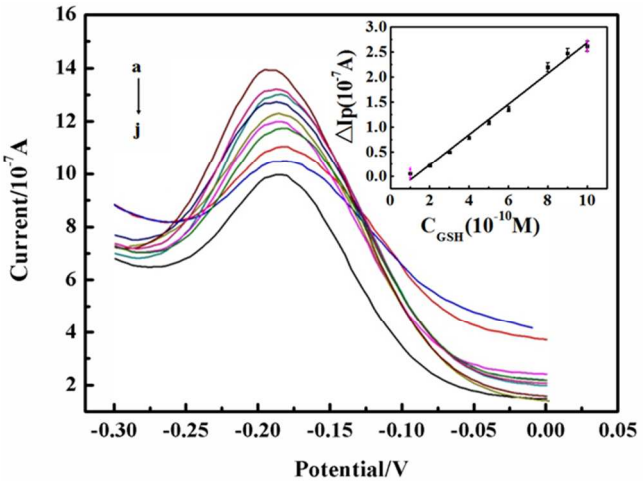


Fig. 1 Differential pulse voltammograms of probe **1** (25.2 μM) toward different concentrations of GSH (a → j: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 0.9, 1.0 nM) after incubation at 25 °C for 20 min in 0.2 M BR (pH 2.0).

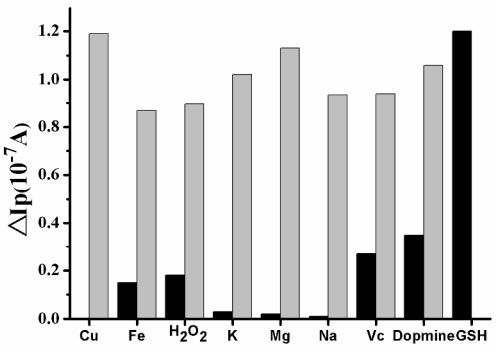


Fig. 2 Electrochemical responses of probe **1** (12.5 μM) to various bioanalytes in BR. Black bars represent values for analytes: Fe^{3+}, Mg^{2+}, Cu^{2+}, Na^{+}, K^{+}, dopamine, H_2O_2 (2.5 μM); Vc (1.5 μM); GSH (5.0 nM). Gray bars represent the values for the subsequent addition of 5.0 nM GSH to each of the analytes.

In conclusion, a novel electrochemical and fluorescent TTF-based probe for the detection of GSH was designed and synthesized. For the first time, a TTF-fused D-A system has been successfully applied for rapid, sensitive and selective detection of GSH with a detection limit of 10^{-10} M, by virtue of the strong nucleophilicity of sulfhydryl to cleave Se-N bonds. The application in the intracellular non-protein thiol detection demonstrates the promising potential of small-molecule probe-based electrochemical detection technology in clinical diagnostics.

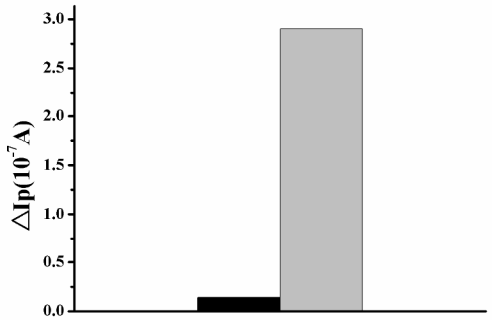


Fig. 3 Electrochemical responses of probe **1** (25.2 μM) toward the non-protein extracts of Ramos cells in BR. The gray bar represents no addition of NEM and the black bar represents the addition of 1.0 mM NEM.

This work was supported by the National Natural Science Foundation of China (No. 21175077), the Taishan Scholar Program of Shandong Province and the Nature Science Foundation of Shandong Province (No. ZR2010BM025) as well as by the Swiss National Science Foundation (No. 200021-147143).

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details and crystallographic data for **1** CCDC 994184. For ESI and crystallographic data in CIF or other electronic format, see DOI: 10.1039/c000000x/

1 (a) K. S. Lee, T. K. Kim, J. H. Lee, H. J. Kim and J. I. Hong, *Chem. Commun.*, 2008, **46**, 6173; (b) R. Hong, G. Han, J. M. Fernández, B.-J. Kim, N. S. Forbes and V. M. Rotello, *J. Am. Chem. Soc.*, 2006, **128**, 1078.
2 (a) S. Pelletier and C. A. Lucy, *J. Chromatogr. A*, 2002, **972**, 221; (b) I. Rahman and W. MacNee, *Free Radic. Biol. Med.*, 2000, **28**, 1405.
3 A. Pastore, G. Federici, E. Bertini and F. Piemonte, *Clin. Chim. Acta*, 2003, **333**, 19.
4 K. Murakami, T. Kondo, Y. Ohtsuka, M. Shimada and Y. Kawakami, *Metabolism*, 1989, **38**, 753.
5 (a) R. Buhl, K. J. Holroyd, A. Mastrongeli, A. Cantin, H. A. Jaffe, F. B. Wells, C. Saltini and R. G. Crystal, *Lancet*, 1989, **2**, 1294; (b) D. M. Townsend, K. D. Tew and H. Tapiero, *Biomed. Pharmacother.*, 2003, **57**, 145.
6 W. A. Kleinman and J. P. Richie Jr, *J. Chromatogr. B*, 1995, **672**, 73.
7 (a) H. L. Li, J. L. Fan, J. Y. Wang, M. Z. Tian, J. J. Du, S. G. Sun, P. P. Sun and X. J. Peng, *Chem. Commun.*, 2009, 5904; (b) A. R. Ivanov, I. V. Nazimov and L. Baratova, *J. Chromatogr. A*, 2000, **895**, 157; (c) K. Amarnath, V. Amarnath, K. Amarnath, H. L. Valentine and W. M. Valentine, *Talanta*, 2003, **60**, 1229; (d) H. Jiang and H. X. Ju, *Anal. Chem.*, 2007, **79**, 6690.
8 (a) S. J. Wang, H. M. Ma, J. Li, X. Q. Chen, Z. J. Bao and S. N. Sun, *Talanta*, 2006, **70**, 518; (b) V. Hong, A. A. Kislukhin and M. G. Finn, *J. Am. Chem. Soc.*, 2009, **131**, 9986; (c) H. Y. Shiu, H. C. Chong, Y. C. Leung, M. K. Wong and C. M. Che, *Chem. Eur. J.*, 2010, **16**, 3308; (d) Y. B. Ruan, A. F. Li, J. S. Zhao, J. S. Shen and Y. B. Jiang, *Chem. Commun.*, 2010, 4938.
9 B. Tang, L. Yin, X. Wang, Z. Chen, L. Tong and K. Xu, *Chem. Commun.*, 2009, 5293.
10 B. Tang, Y. L. Xing, P. Li, N. Zhang, F. B. Yu and G. W. Yang, *J. Am. Chem. Soc.*, 2007, **129**, 11666.
11 J. Mauzeroll and A. J. Bard, *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 7862.
12 (a) X. Hun, W. Sun, H. Zhu, F. Du, F. Liu, Y. Xu and Y. He, *Chem. Commun.*, 2013, **49**, 9603; (b) G. Wu, J. Zhou, X. Jiang, X. Guo and F. Gao, *Electrocatalysis*, 2013, **4**, 17; (c) T. J. O'Shea and S. M. Lunte, *Anal. Chem.*, 1993, **65**, 247; (d) M. Zhou, J. Ding, L. Guo and Q. Shang, *Anal. Chem.*, 2007, **79**, 5328; (e) V. Adam, J. Baloun, I. Fabrik, L. Trnkova and R. Kizek, *Sensors*, 2008, **8**, 2293.
13 (a) T. Inoue and J. R. Kirchhoff, *Anal. Chem.*, 2000, **72**, 5755; (b) T. Inoue and J. R. Kirchhoff, *Anal. Chem.*, 2002, **74**, 1349.
14 W. Wang, L. Li, S. Liu, C. Ma and S. Zhang, *J. Am. Chem. Soc.*, 2008, **130**, 10846.
15 (a) X. Guégano, A. L. Kanibolotsky, C. Blum, S. F. L. Mertens, S.-X. Liu, A. Neels, H. Hagemann, P. J. Skabara, S. Leutwyler, T. Wandlowski, A. Hauser and S. Decurtins, *Chem. Eur. J.*, 2009, **15**, 63; (b) H.-P. Jia, J. Ding, Y.-F. Ran, S.-X. Liu, C. Blum, I. Petkova, A. Hauser and S. Decurtins, *Chem. Asian J.*, 2011, **6**, 3312; (c) C. Jia, S.-X. Liu, C. Tanner, C. Leiggener, A. Neels, L. Sanguinet, E. Levillain, S. Leutwyler, A. Hauser and S. Decurtins, *Chem. Eur. J.*, 2007, **13**, 3804; (d) M. Bendikov, F. Wudl and D. F. Perepichka, *Chem. Rev.*, 2004, **104**, 4891; (e) J. L. Segura and N. Martin, *Angew. Chem. Int. Ed.*, 2001, **40**, 1372; (f) D. Canevet, M. Sallé, G. X. Zhang, D. Q. Zhang and D. B. Zhu, *Chem. Commun.*, 2009, 2245; (g) J. Liao, J. S. Agustsson, S. Wu, C. Schönenberger, M. Calame, Y. Leroux, M. Mayor, O. Jeannin, Y.-F. Ran, S.-X. Liu and S. Decurtins, *Nano Lett.*, 2010, **10**, 759.
16 (a) C. Saravanan, S. Easwaramoorthi, C.-Y. Hsiow, K. Wang, M. Hayashi and L. Wang, *Org. Lett.*, 2014, **16**, 354; (b) C. M. MacNeill, R. C. Coffin, D. L. Carroll and N. H. Levi-Polyachenko, *Macromol. Biosci.*, 2013, **13**, 28; (c) S. Das, P. B. Pati and S. S. Zade, *Macromolecules*, 2012, **45**, 5410; (d) A. Cihaner and F. Algi, *Adv. Funct. Mater.*, 2008, **18**, 3583.

17 (a) K. T. Post, R. D. Cody and R. J. Dennis, *J. Org. Chem.*, 1988, **46**, 4065; (b) M. Renson, Ins. Patai and Z. Rappoport (Eds), *The Organic Chemistry of Selenium and Tellurium Compounds, Vol. 1* (John Wiley & Sons, Chichester, 1986) chap. 13, p. 329.

18 (a) F. Pop, A. Amacher, N. Avarvari, J. Ding, L. M. Lawson Daku, A. Hauser, M. Koch, J. Hauser, S.-X. Liu and S. Decurtins, *Chem. Eur. J.*, 2013, **19**, 2504; (b) A. Amacher, H. Luo, Z. Liu, M. Bircher, M. Cascella, J. Hauser, S. Decurtins, D. Zhang and S.-X. Liu, *RSC Adv.*, 2014, **4**, 2873.

Table of Content

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