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Selective fluorogenic imaging of hepatocellular H_2S by a galactosyl azidonaphthalimide probe†

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We have developed a galactosyl azidonaphthalimide probe for the selective fluorogenic imaging of hepatocellular H_2S, an important gaseous transmitter produced in the liver.

Despite its toxicity, hydrogen sulfide (H_2S) has been identified as an important gaseous transmitter in many cellular signalling pathways.[1] Cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) are the two major enzymes responsible for the production of H_2S with L-(homo)cystein as the substrate. Because of the tissue-specific distribution of these enzymes, H_2S is predominantly generated in the liver.[2] However, this metabolic organ is vulnerable to damage from the aberrant expression of redox-active species including H_2S, leading to hepatic diseases such as liver cirrhosis.[3] As a result, selective detection of hepatocellular H_2S represents an important goal to aid not only cell biology research but also disease diagnosis.

Conventional techniques for H_2S detection depend on chromatographic and electrochemical sensors, which are not applicable for live cell imaging.[4] To address these issues, numerous fluorescence small-molecule probes for H_2S have been developed over recent years. The basis for the fluorogenic response (or fluorescence "OFF–ON") of the probes relies on H_2S-mediated reduction of azides,[5,6] copper sulfide precipitation[7,8] and nucleophilic addition strategies.[9–11] All these probe prototypes address sensitive and selective detection of the species, even in live cells and *in vivo*.[12–14] Nevertheless, although elegant H_2S probes with cellular trapping,[15] lysosome[16] and mitochondria targeting[17] abilities have been developed, fluorogenic probes that address hepatocyte-selective imaging of the species are rare.

Recently, we[18–21] and others[22–26] determined that the introduction of a glycosyl moiety (as a targeting agent) to a fluorescence probe can greatly enhance its selective internalisation by cells derived from a certain tissue. This could be ascribed to the selective, sugar-receptor-mediated endocytosis of the glycoprobes by the cells. Here we show that a galatosyl azidonaphthalimide probe has the ability to image H_2S selectively in hepatocytes among other cells, due to galactoside receptor-promoted endocytosis.

The desired **DT-Gal** was synthesised by a click-coupling of alkynyl naphthalimide **1** with azido galactoside **2**, followed by a sequential azidation and deacylation (Fig. 1a). The presence of the azide weakens the fluorescence of naphthalimide by an ICT process, and a subsequent reduction by H_2S produces aminonaphthalimide, enhancing the fluorescence.[14] **DT-OH** which is a known H_2S probe that lacks the galactosyl targeting agent was used as a control.[27]

With these probes in hand, we tested their fluorescence response to H_2S in an aqueous solution (PBS, pH 7.4). The presence of increasing H_2S caused a gradual fluorescence increase of both **DT-OH** (Fig. 1b) and **DT-Gal** (Fig. 1c) with the latter being more sensitive. This might be a result of the better water solubility of the latter glyco-probe. Among a range of anions (Fig. 1d) and amino acids (Fig. S1, ESI†), both probes showed good selectivity. Mass spectroscopic analysis indicated that the aminonaphthalimide derivative of **DT-Gal** was produced upon reaction with H_2S (Fig. S2, ESI†).

A kinetic investigation determined that **DT-Gal** showed rapid fluorogenic response to H_2S (Fig. 1e), and good linearity was observed by plotting the fluorescence intensity of the probe as a function of increasing H_2S (Fig. 1f). The limit of detection of **DT-Gal** for H_2S was determined to be 0.78 μM ($3\sigma_b/k$). Additionally, we observed that the probe functioned well over a wide pH range (4–12, Fig. 1g), suggesting its applicability for cellular analysis.

Subsequently, we tested the ability of the probes to image H_2S in live cells. We used the human hepatoma cell line (Hep-G2) with over-expressed asialoglycoprotein receptor (ASGPr) that

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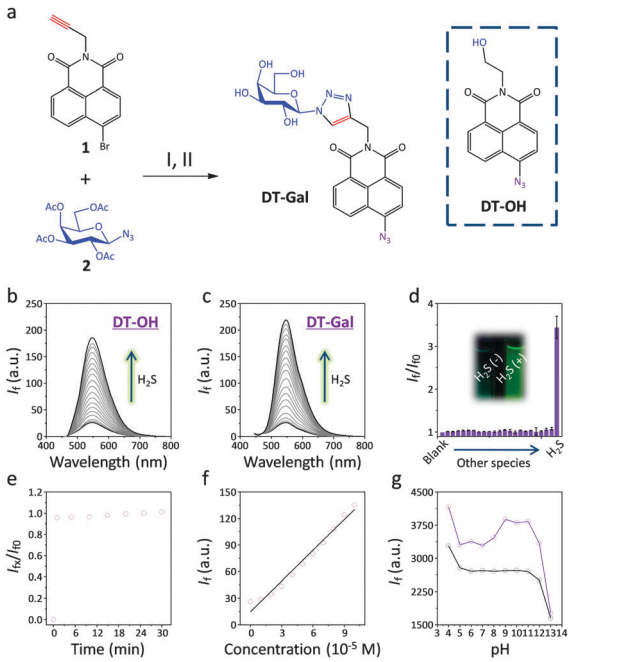

Fig. 1 (a) Synthesis of **DT-Gal** and the structure of **DT-OH**. Reagents and conditions: (I) $CuSO_4{\cdot}H_2O$, Na ascorbate in H_2O–CH_2Cl_2. (II) NaN_3 in DMF, and then Et_3N. Fluorescence titration of (b) **DT-OH** (10 μM) and (c) **DT-Gal** (10 μM) in the presence of increasing H_2S (0–200 μM). (d) Fluorescence change of **DT-Gal** (10 μM) in the absence (blank) and presence of, from left to right, F^-, Cl^-, Br^-, I^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}, HSO_4^-, $S_2O_3^-$, PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, Phe, Ser, Thr, Pro, Val, His, Gly, Cys, GSH and H_2S (100 μM), where I_f and I_{fo} are the fluorescence intensity of the probe in the presence and absence of analyte, respectively (inset: photographed fluorescence change of **DT-Gal** in the presence of H_2S). (e) Plotting the fluorescence change of **DT-Gal** (10 μM) with H_2S (200 μM) as a function of time, where I_{fx} and I_{f0} are the fluorescence intensity of the probe with H_2S at different time points and that of the probe alone, respectively. (f) Plotting the I_f of **DT-Gal** (10 μM) in the presence of increasing H_2S. (g) Plotting the I_f of **DT-Gal** (10 μM) without H_2S (black) and with H_2S (200 μM) (blue) at different pH. All fluorescence spectra were recorded in PBS (0.05 M, pH 7.4 or varied pH as indicated) with excitation at 426 nm.

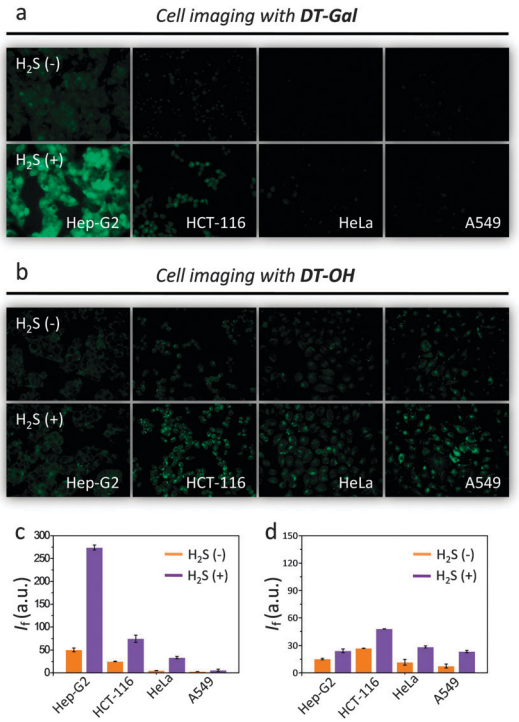

Fig. 2 Cell imaging (a for **DT-Gal** and b for **DT-OH**) and quantification of the fluorescence intensity (I_f) (c for **DT-Gal** and d for **DT-OH**) in the absence (–) and presence (+) of H_2S (Hep-G2: human liver cancer; HCT-116: human colon cancer; HeLa: human cervix cancer; A549: human lung cancer).

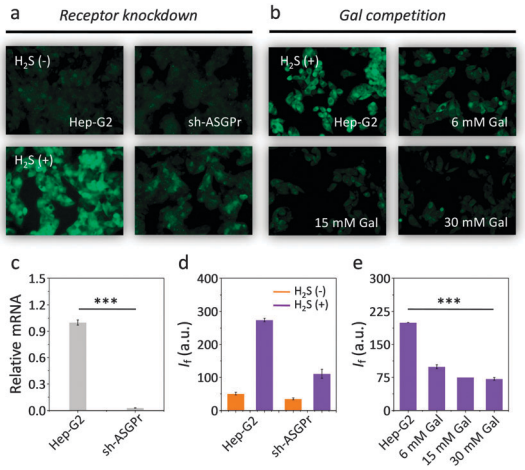

Fig. 3 Cell imaging (a) and fluorescence quantification (d) of **DT-Gal** for Hep-G2 and sh-ASGPr with reduced ASGPr expression. Cell imaging (b) and fluorescence quantification (e) of **DT-Gal** with Hep-G2 in the absence and presence of increasing competing free galactose. (c) ASGPr mRNA level of Hep-G2 and sh-ASGPr measured by PCR (***$P < 0.001$).

recognises galactosides[18–21] as well as cancer cells without the receptor expression such as human colon cancer HCT116, cervix cancer HeLa and lung cancer A549.[18] We observed that, the addition of **DT-Gal** to the cell lines pre-treated with H_2S led to fluorescence enhancement for Hep-G2, but only a slight fluorescence increase for other cells (Fig. 2a and c). In sharp contrast, co-incubation of **DT-OH** with the cells led to slight, *unselective* fluorescence enhancement (Fig. 2b and d). These results suggest that the presence of the galactosyl warhead facilitates the selective internalisation of the H_2S probe probably by ASGPr-mediated endocytosis.[18–21] Notably, the assumed endocytosis led to much stronger fluorescence generation of **DT-Gal** than the **DT-OH** without a targeting group.

To corroborate that the imaging was a result of selective ASGPr–galactose interaction, we used a Hep-G2 cell line with a reduced ASGPr expression level (sh-ASGPr)[21] to incubate with **DT-Gal**. We observed that addition of the galactosyl probe to sh-ASGPr resulted in a much weaker fluorescence compared to Hep-G2 (Fig. 3a and d). This correlates with the distinct ASGPr expression level of the two cell lines (Fig. 3c). Moreover, pre-incubation of increasing free galactose with Hep-G2 inhibited the fluorescence enhancement of **DT-Gal** in a concentration-dependent manner (Fig. 3b and e). These data support the assumption that the strong and selective fluorogenic signal produced by **DT-Gal** in raw Hep-G2 cells is facilitated by ASGPr-mediated endocytosis.

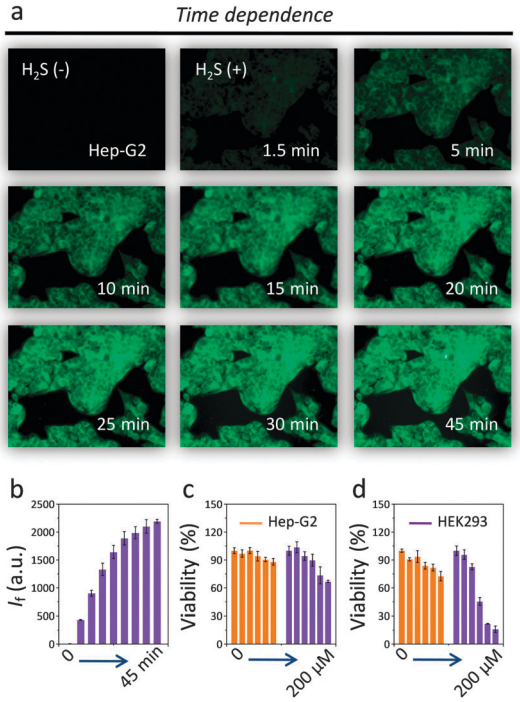

Fig. 4 Cell imaging (a) and fluorescence quantification (b) of **DT-Gal** for Hep-G2 with increasing incubation time. Cytotoxicity of (c) **DT-Gal** (0–200 μM) and (d) **DT-OH** (0–200 μM) for Hep-G2 and HEK293.

We also tested the kinetics of **DT-Gal** for H_2S imaging in the hepatocellular cell line. We observed a time-dependent fluorescence enhancement of the imaging experiments and equilibrium was achieved in about 30 min (Fig. 4a and b). Note that this cellular kinetics is slower than that in solution probably because of the complexity of the cellular environment. Interestingly, **DT-Gal** (Fig. 4c) was much less toxic towards Hep-G2 as well as a human kidney cell line (HEK293) than **DT-OH** (Fig. 4d). This suggests that, in addition to its targeting ability, the presence of the galactosyl moiety might also mitigate the intrinsic cytotoxicity of naphthalimide-based compounds.[28]

We note that a similar azidonaphthalimide probe for H_2S has been previously reported.[29] However, unlike our **DT-Gal** probe, that probe does not contain a cellular targeting unit, which is vital for target-specific imaging *in vivo*. Since azidonaphthalimide can be reduced to the fluorescent amino derivative within cells,[25] we carried out a time-dependent imaging assay with Hep-G2 and just **DT-Gal** (Fig. S3, ESI†). While the fluorescence increased slightly, the co-existence of H_2S results in significantly enhanced fluorescence. This clearly demonstrates that the fluorescence enhancements observed here are due to H_2S recognition within the cells.

In conclusion, a galactosyl azidonaphthalimide based fluorogenic probe for hepatocellular-selective imaging of H_2S was developed, which sets a basis for the target-specific imaging of H_2S in the liver, the main organ that produces this gaseous transmitter.

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Notes and references

1 P. Kamoun, *Amino Acids*, 2004, **26**, 243.
2 J. Yan, F. Teng, W. Chen, Y. Ji and Z. Gu, *Exp. Ther. Med.*, 2012, **4**, 832.
3 S. Fiorucci, E. Antonelli, A. Mencarelli, S. Orlandi, B. Renga, G. Rizzo, E. Distrutti, B. Shah and A. Morelli, *Hepatology*, 2005, **42**, 539.
4 A. Tangerman, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2009, **28**, 3366.
5 A. R. Lippert, E. J. New and C. J. Chang, *J. Am. Chem. Soc.*, 2011, **133**, 10078.
6 H. Peng, Y. Cheng, C. Dai, A. L. King, B. L. Predmore, D. J. Lefer and B. Wang, *Angew. Chem., Int. Ed.*, 2011, **50**, 9672.
7 K. Sasakura, K. Hanaoka, N. Shibuya, Y. Mikami, Y. Kimura, T. Komatsu, T. Ueno, T. Terai, H. Kimura and T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 18003.
8 F. Hou, L. Huang, P. Xi, J. Cheng, X. Zhao, G. Xie, Y. Shi, F. Cheng, X. Yao, D. Bai and Z. Zeng, *Inorg. Chem.*, 2012, **51**, 2454.
9 Z. Xu, L. Xu, J. Zhou, Y. Xu, W. Zhu and X. Qian, *Chem. Commun.*, 2012, **48**, 10871.
10 X. Cao, W. Lin, K. Zheng and L. He, *Chem. Commun.*, 2012, **48**, 10529.
11 Y. Chen, C. Zhu, Z. Yang, J. Chen, Y. He, Y. Jiao, W. He, L. Qiu, J. Cen and Z. Guo, *Angew. Chem., Int. Ed.*, 2013, **52**, 1688.
12 W. Xuan, C. Sheng, Y. Cao, W. He and W. Wang, *Angew. Chem., Int. Ed.*, 2012, **51**, 2282.
13 V. S. Lin and C. J. Chang, *Curr. Opin. Chem. Biol.*, 2012, **16**, 595.
14 F. Yu, X. Han and L. Chen, *Chem. Commun.*, 2014, **50**, 12234.
15 V. S. Lin, A. R. Lippert and C. J. Chang, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 7131.
16 T. Liu, Z. Xu, D. R. Spring and J. Cui, *Org. Lett.*, 2013, **15**, 2301.
17 S. K. Bae, C. H. Heo, D. J. Choi, D. Sen, E. H. Joe, B. R. Cho and H. M. Kim, *J. Am. Chem. Soc.*, 2013, **135**, 9915.
18 K.-B. Li, Y. Zang, H. Wang, J. Li, G.-R. Chen, T. D. James, X.-P. He and H. Tian, *Chem. Commun.*, 2014, **50**, 11735.
19 X.-P. He, R.-H. Li, S. Maisonneuve, Y. Ruan, G.-R. Chen and J. Xie, *Chem. Commun.*, 2014, **50**, 14141.
20 H.-L. Zhang, Y. Zang, J. Xie, J. Li, G.-R. Chen, X.-P. He and H. Tian, *Sci. Rep.*, 2014, **4**, 5513.
21 H.-L. Zhang, X.-L. Wei, Y. Zang, J.-Y. Cao, S. Liu, X.-P. He, Q. Chen, Y.-T. Long, J. Li, G.-R. Chen and K. Chen, *Adv. Mater.*, 2013, **25**, 4097.
22 K. EI-Boubbou, D. C. Zhu, C. Vasileiou, B. Borhan, D. Prosperi, W. Li and X. Huang, *J. Am. Chem. Soc.*, 2010, **132**, 4490.
23 M. H. Lee, J. H. Han, P.-S. Kwon, S. Bhuniya, J. Y. Kim, J. L. Sessler, C. Kang and J. S. Kim, *J. Am. Chem. Soc.*, 2012, **134**, 1316.
24 K. Y. Zhang, K. K.-S. Tso, M.-W. Louie, H.-W. Liu and K. K.-W. Lo, *Organometallics*, 2013, **32**, 5098.
25 M. Sawa, T.-L. Hsu, T. Itoh, M. Sugiyama, S. R. Hanson, P. K. Vogt and C.-H. Wong, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 12371.
26 D. Wu, S. Cheung, R. Daly, H. Burke, E. M. Scanlan and D. F. O'Shea, *Eur. J. Org. Chem.*, 2014, 6841.
27 C. Yu, X. Li, F. Zeng, F. Zheng and S. Wu, *Chem. Commun.*, 2013, **49**, 403.
28 X. Li, Y. Lin, Q. Wang, Y. Yuan, H. Zhang and X. Qian, *Eur. J. Med. Chem.*, 2011, **46**, 1274.
29 L. A. Montoya and M. D. Pluth, *Chem. Commun.*, 2012, **48**, 4767.