Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Disulfide Bound-Molecular Beacon as a Fluorescent Probe for the Detection of Reduced Glutathione and its Application in Cells

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Chemicals. The molecular beacon (see Table S1) designed in this work were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Reduced glutathione (GSH), cysteine (Cys), glucose, BSA, and *N*-ethylmaleimide (NEM) were purchased from Sigma-Aldrich. The stock solutions of 0.05 M GSH was prepared by dissolving the powder in water. Double-distilled deionized water was used throughout the experiments. All the chemicals employed were of analytical reagent grade and were used without further purification.

Table S1. DNA Sequences

MB	5'-FAM-GCT GGA CAG AGT ATATA TCA ATT TTT TTT AGT CCA GC-TAMRA-3'
SSMB （6 base pairs in the stem region）	5'-FAM-GCT GGA AG AGT AT-S-S- ATA TCA ATT TTT TTT A T CCA GC-TAMRA-3'
SSMB （7 base pairs in the stem region）	5'-FAM-GCT GGA CAG AGT AT-S-S-ATA TCA ATT TTT TTT AGT CCA GC -TAMRA-3'
SSMB （8 base pairs in the stem region）	5'-FAM-GGCT GGA CAG AGT AT-S-S- ATA TCA ATT TTT TTT AGT CCA GCC-TAMRA-3'
SSMB （9 base pairs in the stem region）	5'-FAM-GCGCT GGA CAG AGT AT-S-S-ATA TCA ATT TTT TTT AGT CCA GCG C-TAMRA-3'
T-Hg^{2+}-T MB	5'-FAM-CCT CCA AAA GGT GG-DABCYL-3'

Apparatus. Fluorescence emission spectra were recorded on a Hitachi F-4600 fluorescence spectrofluorometer (Japan) with quartz micro-cuvette (Generay BiotechCo., LTD, Shanghai). Fluorescence images of cells were obtained with a Leica DMI600B CS inverted microscope equipped with an Andor iXon X3 EMCCD camera. Pipettors were purchased from Thermo Scientific.

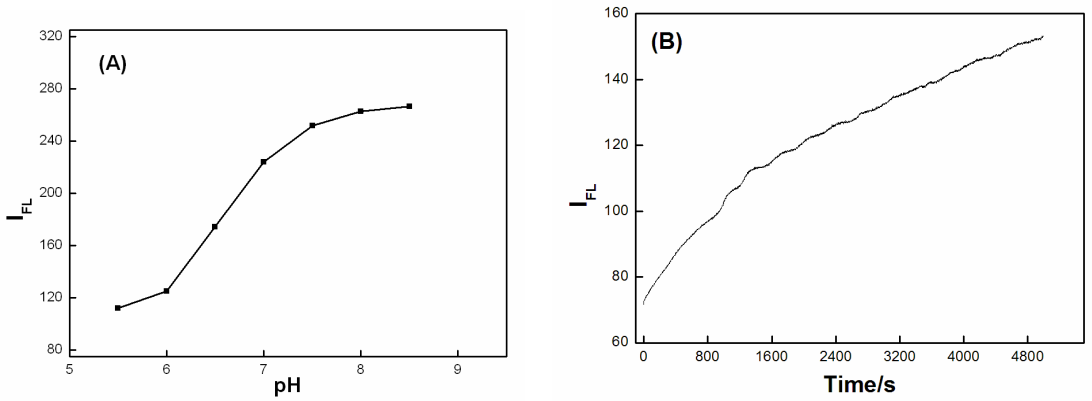

Fig. S1 (A) Fluorescence intensity-pH profiles for SSMB in the presence of 6.0×10^{-8} M GSH. (B) Time-dependent changes in the fluorescence of SSMB with 8.0×10^{-9} M GSH.

The Optimization of Molecular Beacons. SSMB with a stem that is too short can be relatively unstable and result in open molecular beacons even in the absence of target, leading to a low

signal : noise ratio (the fluorescence intensity in the presence of GSH vs blank control assay). Further, SSMB with a long stem can be too stable and might not open effectively to target, resulting in low signal : noise ratio as well. [S1] With proper design of the number of base pairs comprising the stem of molecular beacons, the improved signal : noise ratio should be achieved which enables SSMB to function for GSH analysis platform with very high sensitivity. Fig. S2 shows the fluorescence intensity changes upon analyzing GSH at 5.0×10^{-8} M by different SSMB that include 6, 7, 8 or 9 base pairs in the stem region, respectively. (see Table 1 for the sequences). We found that SSMB with 7 based pairs in the stem region yields the satisfactory performance.

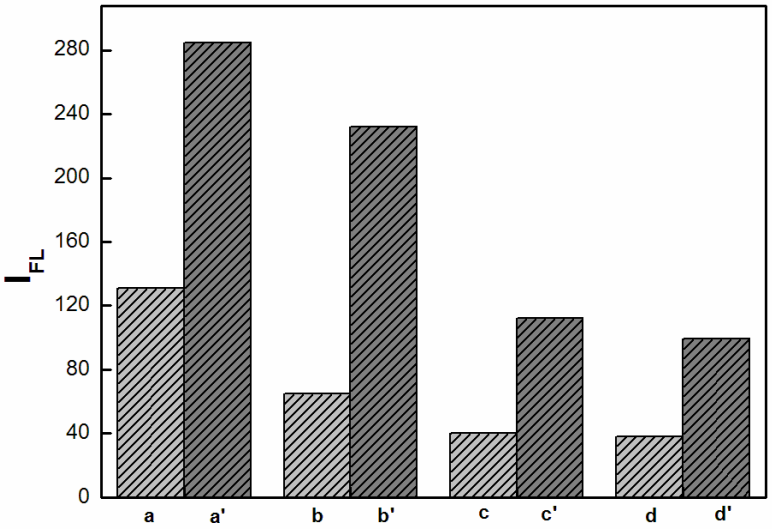

Fig. S2 Fluorescence intensities in the absence of GSH (5.0×10^{-8} M) (light gray bars) or in the presence of target nucleic acid (gray bars), respectively, using various lengths of the SSMB stem: (a) and (a') 6 bp of the stem region; (b) and (b') 7 bp of the stem region; (c) and (c') 8 bp of the stem region; (d) and (d') 9 bp of the stem region respectively.

Optimization of Reaction Temperature. The melting temperature of a MB is considerably higher than that of the corresponding MB formed from two separate DNA molecules with identical sequences. [S2] The analysis process is based on the delicate stabilities of the duplexes in SSMB and the temperature at which GSH analysis was activated had to be optimized in order to increase the sensitivity. According the results of temperature variations at 20 °C, 25 °C, 30 °C and 35 °C, we selected 25 °C for the subsequent experiments according the high signal : noise ratio guidelines.

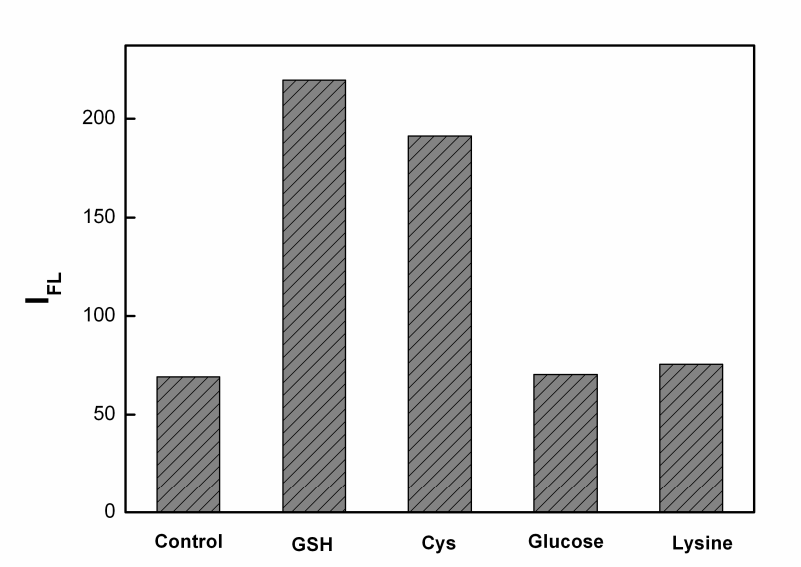

Fig. S3 Fluorescence emission intensity at 4.0×10^{-8} M GSH, Cys, Glucose, Lysine and control experiment.

Comparison Between SSMB and MB for Analysis GSH. GSH, SSMB and MB solution were all prepared with 0.02 M Tris-HCl buffer (pH 7.5). 25 μL of 2.5×10^{-6} M SSMB or MB was placed in a 0.5 mL eppendorf tube (EP tube), followed by adding 25 μL 5.0×10^{-8} M of GSH, and the mixture was incubated at 25 °C for fluorescence detection. The results obtained are presented in Fig. S4. As can be seen, the fluorescence enhancement for GSH/SSMB is obvious. By contrast, the fluorescence intensity of GSH/MB is as much as its blank assay. These indicate that the cleavage based on thiol-disulfide exchange should give rise to fluorescence enhancement.

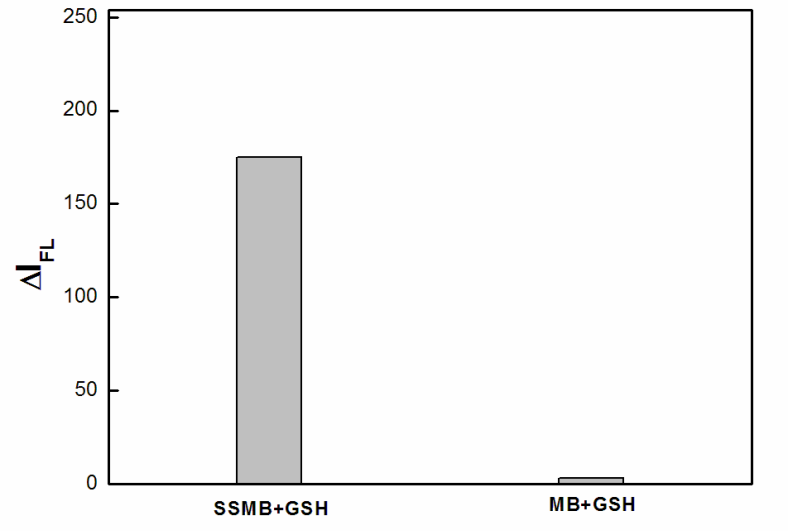

Fig. S4 The fluorescence analysis 5.0×10^{-8} M of GSH using SSMB or MB.

Detection of GSH in Buffer. Different concentrations of GSH and SSMB solution were all prepared with 0.02 M Tris-HCl buffer (pH 7.5). 25 μL of 2.5×10^{-6} M SSMB was placed in a 0.5 mL eppendorf tube (EP tube), followed by adding 25 μL different concentrations of GSH, and the

mixture was incubated at 25 °C for fluorescence detection. Fluorescence assays were performed on a F-4600 reader. Samples were excited at 495 nm.

Cell Culture. Cells were cultured at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. K562 cells (leukemia cell) were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 units/mL penicillin and 50 μg/mL streptomycin.

Detection of Intracellular Thiols. The cell density was counted with a hemocytometer. A 1.0 mL of 1.6×10^{-6} suspended K562 cells was centrifuged at 3500 rad min^{-1} for 5 min in culture medium, washed twice with ice-cold PBS buffer (pH 7.5). After that, they were suspended again in PBS buffer with the same volume as they had been grown and stepwise diluted to different concentrations with PBS buffer, making 25 μL of K562 cell suspensions contain 80, 200, 500, 800, 1000, 5000, 8000, 10000 cells in 0.5 mL EP tube, respectively. K562 Cell homogenates were obtained by disrupted cell suspensions 25 μL for 30 min in ultrasonic disintegrater. During sonic disruption, the temperature was maintained below 4 °C with ice bath. The homogenates were immediately added into 25 μL of 2.5×10^{-6} M SSMB incubating at 25 °C for fluorescence detection.

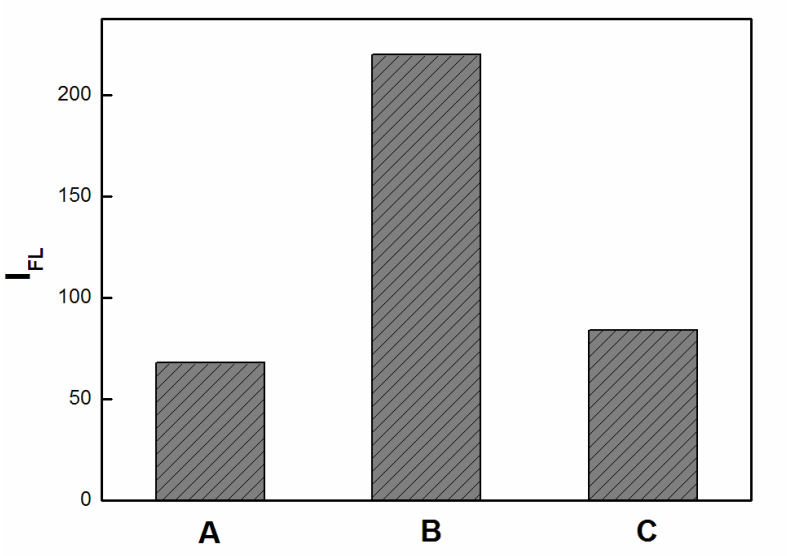

Fig. S5 Fluorescence produced by K562 cells homogenates with SSMB with (B) and without (C) a 30 min preincubation with NEM. (A) Control experiment: fluorescence produced with no K562 cells.

Table S2. Recovery of GSH Assay at Different Concentrations Spiked into 25 μL Cell Homogenates[a]

No.	Sample (M)	Spiked (M)	Found (M)	Recovery (%)
1	2.875×10^{-8}	1.5×10^{-8}	4.485×10^{-8}	107%
2	2.467×10^{-8}	1.0×10^{-8}	3.483×10^{-8}	102.6%

[a] Each sample was repeated for three times and averaged to obtain the recovery values.

Comparative Study between the Present Proposals (SSMB) and Literature Detections (T-Hg^{2+}-T MB) [S3]

T-Hg^{2+}-T MB detection of GSH was performed as followed processes according literature reported[S3] with a little modification. A 100 nM T-Hg^{2+}-T MB was incubated with 100 nM Hg^{2+} solution in 2.5 mL of 10 mM 3-(N-morpholino) propanesulfonic acid buffer containing 0.05 M $NaNO_3$ (pH 7.4) for 1 h to form the T-Hg^{2+}-T MB. Then, freshly prepared sample was added and incubated at 52 °C for 30 min. Table S3 and Fig. S6 show the comparison results between the present method and the literature method, indicating good agreement.

Table S3 GSH concentration spiked in cell homogenate samples[a]

Sample	The present method (10^{-8} M)	Literature method (10^{-8} M)	Relative deviation (%)
1	0.81	0.828	2.1
2	2.132	2.167	1.6
3	5.324	5.046	–5.5
4	10.329	9.858	–4.8
5	2.491	2.701	7.8

[a]Each value is the average of three measurements.

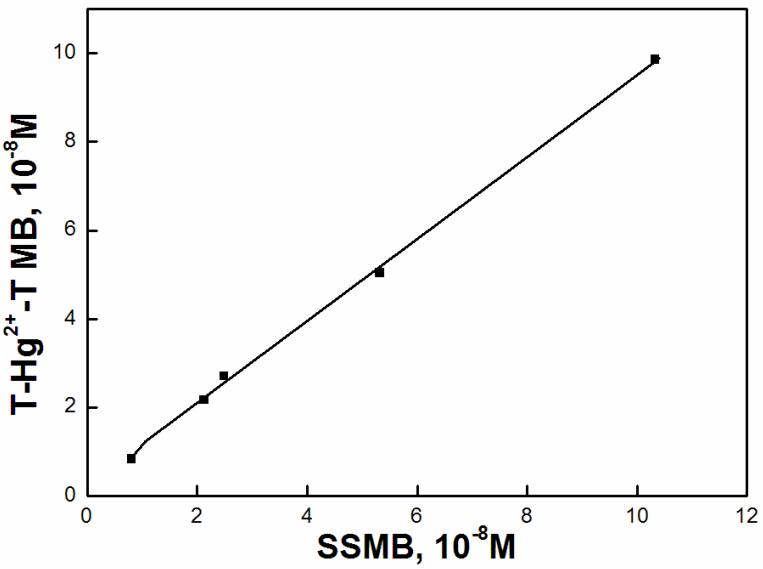

Fig. S6. The correlation of results for the spiked GSH in K562 cells by SSMB (x axis) and T-Hg^{2+}-T MB (y axis) with correlation coefficients of 0.999.

References for Supplementary Information

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S2. S. Tyagi and F. R. Kramer, *Nat. Biotechnol.*, 1996, **14**, 303.

S3. H. Xu and M. Hepel, *Anal. Chem.*, 2011, **83**, 813.