Electronic Supplementary Material (ESI) for ChemComm.
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Supporting Information

Electrochemical detection of glutathione based on accelerated CRISPR/Cas12a trans-cleavage with MnO_2 nanosheets

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Experimental

Materials and instruments

Glutathione (GSH), manganese chloride ($MnCl_2$), sodium chloride (NaCl), tris (2-carboxyethyl)phosphine hydrochloride (TCEP), mercaptohexanol (MCH), hexaammineruthenium(III) chloride ($[Ru(NH_3)_6]^{3+}$), arginine, glutamine, glycine, histidine, lysine, leucine, serine, threonine, fructose, sucrose, folic acid, glucose, tetramethylammoniam hydroxide and hydrogen peroxide (H_2O_2) and ITO slides were ordered from Sigma (USA). Cas12a was purchased from New England Biolabs Ltd. (Beijing, China). A colorimetric GSH kit was acquired from Beyotime Biotechnology, Inc. (Shanghai, China). All other chemicals were of analytical grade and used without further purification. Solutions prepared in this work utilized ultrapure water, which was purified by a Millipore water purification system (18 MΩ·cm). DNA and RNA strands were synthesized and purified by Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences were shown in Table S1. Human serum samples were provided by Affiliated Hospital of Nantong University with written consent from participants (Nantong, China).

TEM image was taken by an FEI Tecnai G20 transmission electron microscope (FEI, USA). UV-vis absorption spectra were obtained by a NanoDrop OneC spectrophotometer (Thermo Scientific, USA). Electrochemical measurements were carried out on a CHI 660E workstation (CH Instruments, China). Polyacrylamide gel (PAGE) image was taken by a GelDoc XR^+ Imaging System (Bio-Rad, USA). Zeta potentials were recorded by a Zetasizer Nano ZS90 at 25°C (Malvern, UK).

Preparation of MnO_2 NSs

The two-dimensional nanosheets were synthesized according to a previous report.[1] Briefly, tetramethylammonium hydroxide with the concentration of 0.6 M was blended with 3 wt% H_2O_2.

20 mL of $MnCl_2$ with the concentration of 0.3 M was added in 40 mL of the above solution. The resulted solution was stirred overnight at room temperature. Next, the bulk MnO_2 NSs were centrifuged at 2000 rpm for 10 min, which were further washed with distilled water and methanol, respectively. Afterward, MnO_2 NSs were dried at 60 °C and resuspended with pure water. The NSs were ultrasonicated for 12 h before further use.

Preparation of ssDNA modified electrode

The substrate gold electrode was pretreated. First, it was incubated with piranha solution (98% H_2SO_4 : 30% H_2O_2 = 3 : 1) for 5 min (*Caution: the solution reacts violently with organic solvents*). After careful rinsing, the electrode was polished with P5000 sand paper and alumina slurry, respectively. The gold electrode was polished to a mirror-like surface, which was further ultrasonicated. Next, the electrode was scanned by cyclic voltammetry in 500 mM H_2SO_4 for electrochemical cleaning. After rinsing with pure water, it was incubated with 0.8 μM ssDNA for 8 h and then 1 mM MCH for 0.5 h.

GSH-mediated NSs digestion and Mn^{2+}-assisted CRISPR/Cas12a cleavage

Cas12a/crRNA complexes were assembled by incubating Cas12a and crRNA with the concentrations of 200 nM for 20 min. Standard GSH solutions with a series of concentrations were then prepared. After blending GSH with MnO_2 NSs with the concentration of 60 μg mL^{-1}, 100 μL of the solution was then mixed with 150 μL of Cas12a/crRNA and 50 μL of DNA duplex (dsDNA-1 and dsDNA-2) (300 nM). ssDNA modified electrode was incubated with the above solution for 15 min.

Electrochemical detection

Electrochemical measurements were performed on a CHI660D electrochemical analyzer (CH Instruments, China) with a traditional three-electrode system. The electrochemical properties of

the working electrode were studied by CC, CV, EIS, and SWV. The electrolyte for CV and EIS was 5 mM $[Fe(CN)_6]^{3-/4-}$ containing 1 M KNO_3. The parameters included 100 mV s^{-1} scanning rate, 0.21 V biasing potential, 5 mV amplitude, and frequency range from 0.1 to 100000 Hz. The electrolyte for CC was 10 mM Tris–HCl with 50 μM $[Ru(NH_3)_6]^{3+}$ (pH 7.4). The electrolyte for SWV was 20 mM Tris-HCl buffer (pH 7.5) containing 160 μM TCEP, 100 mM NaCl and 50 mM $MgCl_2$. The parameters included 60 mV s^{-1} scanning rate, and scan range from 0.1 to -0.7 V.

Polyacrylamide gel electrophoresis

Electrophoresis was carried out in the Tris-boric acid buffer solution (90 mM, 1 mM EDTA, pH 8.0) at 100 V for 60 min. The collected gel was stained with 4S Red Plus solution. After careful rinsing, the gel was imaged under UV light by a Gel DocTM XR+ Imaging System (Bio-Rad, USA).

ITO slide modification and fluorescence observation

Bare ITO glass slide was cleaned with the solution (NH_4OH : H_2O_2 : H_2O = 1 : 1 : 5) for 20 min at 80 °C. After careful rinsing and then dried with nitrogen atmosphere, the glass slide was incubated in 1% APTES prepared in 95% for 1 h and then 10% GA for 2 h. It was rinsed and further incubated with aminated ssDNA-2 for 2 h at room temperature in a humid chamber. The DNA modified ITO glass slide was observed using a Leica TCS SP5 microscope (Leica Microsystems, Germany). To demonstrate the interaction between MnO_2 NSs and ssDNA-2, the DNA modified ITO glass slide was incubated with MnO_2 NSs (60 μg mL^{-1}) for 15 min before fluorescence observation.

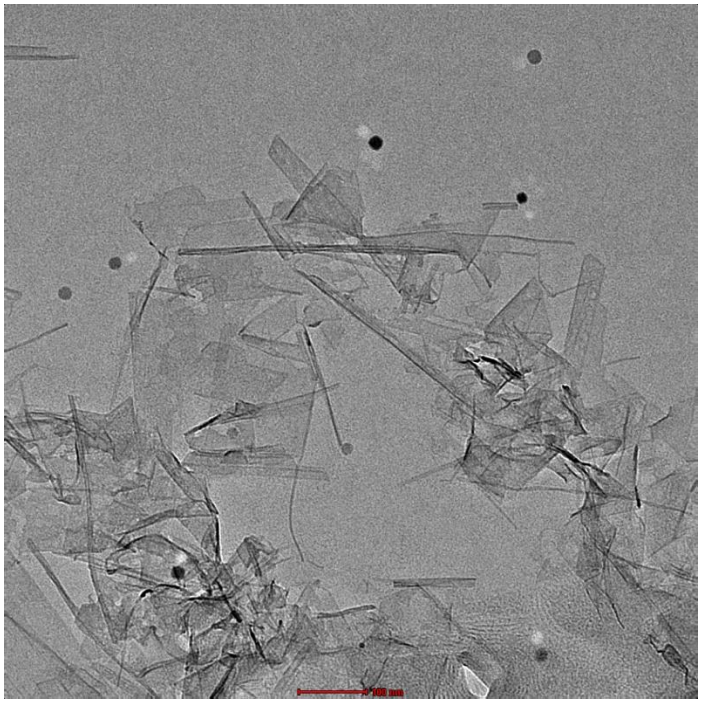

Figure S1. TEM image of MnO_2 nanosheets.

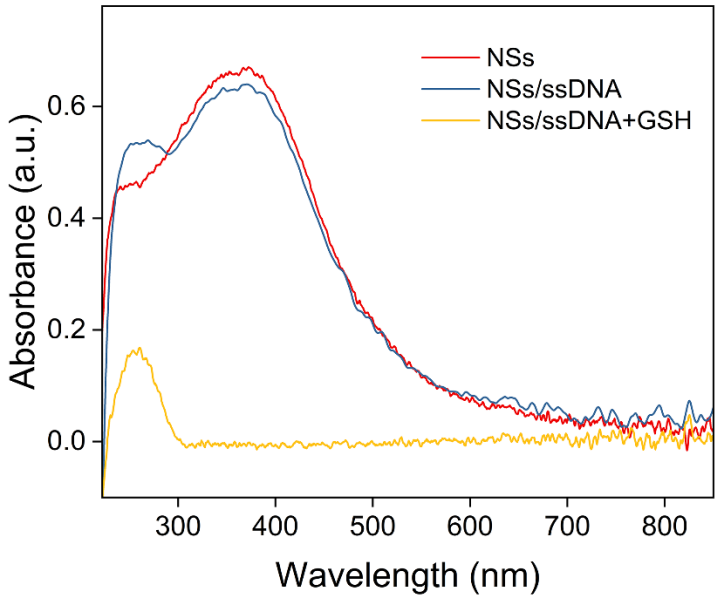

Figure S2. UV–vis absorption spectra of MnO_2 nanosheets after interaction with ssDNA in the absence and presence of GSH.

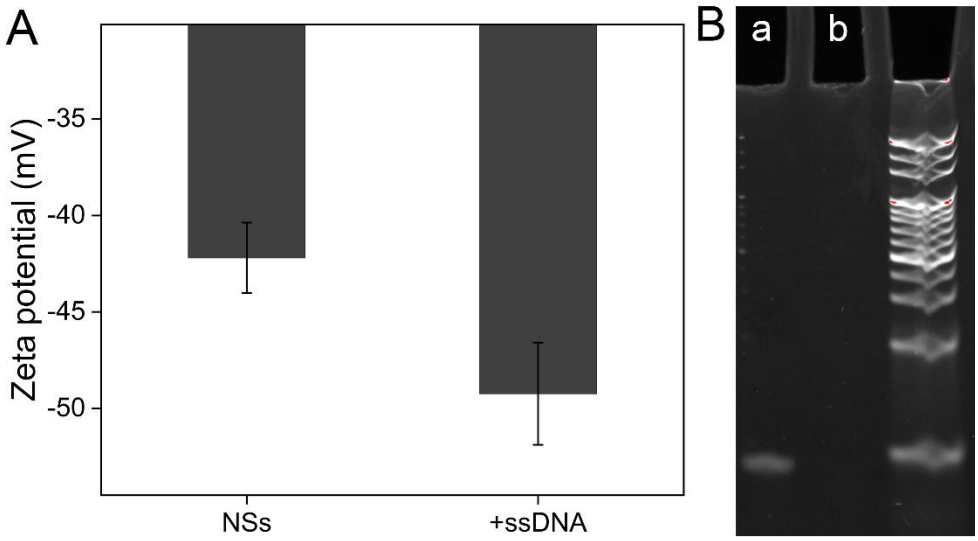

Figure S3. (A) Zeta potentials of MnO_2 nanosheets after ssDNA incubation. (B) PAGE image of ssDNA (a) before and (b) after CRISPR/Cas12a trans-cleavage.

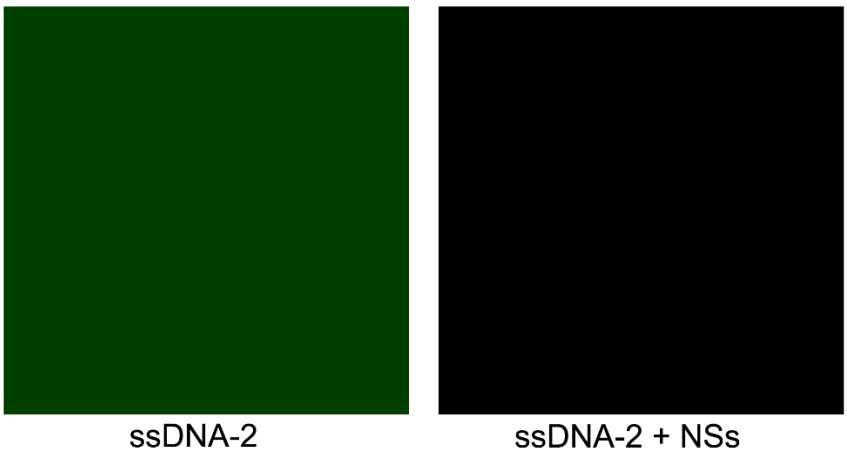

Figure S4. Fluorescent images of ssDNA-2 modified ITO slide before and after incubation with MnO_2 NSs.

Table S1. DNA and RNA sequences used in this work.

Name	Sequence (5' to 3')
dsDNA-1	TCATAGTTAGCGTAACGATCTAAAGTTTTGTCGTC
dsDNA-2	GACGACAAAACTTTAGATCGTTACGCTAACTATGA
crRNA	UAAUUUCUACUAAGUGUAGAUGAUCGUUACGCUAACUAUGA
ssDNA	SH-$(CH_2)_6$-TTTTACGATCGACTTCCG-MB
ssDNA-2	NH_2-$(CH_2)_6$-TTTTACGATCGACTTCCG-FAM

Table S2. Comparison of the analytical performances of recent GSH assays.

Technique	Strategy	Detection range (M)	LOD (M)	Ref
Fluorescence	double-stranded DNA templated silver nanoparticles	0 to 9×10^{-2}	3.7×10^{-4}	2
MALDI-MS	α-cyano-4-hydroxycinnamic acid as a reactive matrix	-	2×10^{-4}	3
Colorimetry	FeO_x/Pt/PEDOT	0 to 10^{-4}	10^{-5}	4
Visual sensor	thiol−ene click chemistry and the capillary action principle	10^{-5} to 10^{-3}	2×10^{-6}	5
Fluorescence	disulfide linkage rupture between FRET donor acceptor	10^{-6} to 10^{-5}	8×10^{-7}	6
Fluorescence	near-infrared fluorescent probe based on red shift	0 to 1.6×10^{-5}	2.52×10^{-7}	7
Colorimetry	copper aspartate nanofibers with multiple enzyme-like activities	1 to 5×10^{-5}	2.5×10^{-7}	8
Fluorescence	Mn-doped ZnS quantum dots and cellulose nanofibrils	2×10^{-7} to 2×10^{-5}	5.7×10^{-8}	9
Colorimetry	apigenin catalyzed AuNRs etching	5×10^{-8} to 2×10^{-5}	3.4×10^{-8}	10
CL	cobalt oxyhydroxide nanoflakes with oxidase-mimicking activity	10^{-8} to 10^{-6}	6.4×10^{-9}	11
DPV	glutathione peroxidase, graphene oxide and nafion	3×10^{-9} to 3.7×10^{-4}	1.5×10^{-9}	12
ECL	$AgGaS_2$ quantum dots	10^{-11} to 10^{-6}	8.03×10^{-12}	13
SWV	accelerated CRISPR-Cas12a trans-cleavage	10^{-11} to 10^{-5}	3.5×10^{-12}	this work

Table S3. Detection of GSH in serum samples before and after spiking.

Sample	Spiked (μM)	Detected (μM)	Elman method (μM)	RSD (%)	Recovery (%)
1	1	1.923	1.893	2.351	102.328
	5	5.837	5.924	3.293	92.794
2	1	1.965	2.041	1.535	98.469
	5	6.014	5.984	1.093	103.469
3	1	2.123	2.039	2.305	108.712
	5	6.102	6.084	1.532	106.680

References

1. K. Kai, Y. Yoshida, H. Kageyama, G. Saito, T. Ishigaki, Y. Furukawa and J. Kawamata, *J. Am. Chem. Soc.*, 2008, **130**, 15938-15943.
2. W. H. Bao and J. Ai, *Part. Part. Syst. Charact.*, 2023, **40**, 2300041.
3. L. M. Guo, C. S. Xiao, J. R. Li, X. Lin, N. Zhao, X. Y. Wang, L. J. Dong and X. H. Guo, *J. Am. Soc. Mass Spectrom.*, 2021, **32**, 2837-2841.
4. T. Chen, B. C. Li, B. C. Wang, X. Y. Luo, Z. Y. Fan, H. Zhao, J. Yang, B. Y. Ren, Y. P. Cai and R. F. Dong, *Mater. Today Nano*, 2023, **24**, 100413.
5. H. Wang, Z. Liu, J. Xiao, C. X. Li, J. L. Wang, X. J. Xiao, H. W. Huang, B. Shrestha, L. Tang, K. Q. Deng and H. Zhou, *Anal. Chem.*, 2021, **93**, 7292-7299.
6. Y. B. Zhang, S. Xia, S. L. Wan, T. E. Steenwinkel, T. Vohs, R. L. Luck, T. Werner and H. Y. Liu, *ChemBioChem*, 2021, **22**, 2282-2291.
7. K. P. Wang, G. Nie, S. Q. Ran, H. L. Wang, X. Q. Liu, Z. M. Zheng and Y. Zhang, *Dyes Pigm.*, 2020, **172**, 107837.
8. L. L. Lei, D. H. Song, L. H. Fan, B. Liu, M. Z. He, X. H. Sun, W. J. Xu, K. Tao, H. Huang and Y. X. Li, *Microchim. Acta*, 2022, **189**, 61.
9. X. W. Li, J. K. Qin and Y. C. Hu, *Microchem. J.*, 2023, **191**, 108763.
10. H. Khani, S. Abbasi, M. Tavakkoli Yaraki and M. B. Gholivand, *Microchem. J.*, 2022, **173**, 107006.
11. X. J. Yang, R. S. Li, C. M. Li, Y. F. Li and C. Z. Huang, *Talanta*, 2020, **215**, 120928.
12. S. Cheraghi, M. A. Taher, H. Karimi-Maleh, F. Karimi, M. Shabani-Nooshabadi, M. Alizadeh, A. Al-Othman, N. Erk, P. K. Yegya Raman and C. Karaman, *Chemosphere*, 2022, **287**, 132187.
13. J. Z. Zhang, X. Liu, H. X. Liu, J. Z. Wang, Y. W. Zhang and W. B. Zhao, *Anal. Chim. Acta*, 2022, **1226**, 340251.