Electronic Supplementary Material (ESI) for Analyst.

ALP-assisted chemical redox cycling amplification strategy for ultrasensitive fluorescent detection of DNA methylation

Hongding Zhang,[a]* Sifei Wu,[a] Zhenhua Xing,[b] Hai-Bo Wang[a]*

[a]*College of Chemistry and Chemical Engineering, Xinyang Key Laboratory of Functional Nanomaterials for Bioanalysis, Xinyang Normal University, Xinyang 464000, PR China*

[b]*Xinyang Branch, Henan Boiler and Pressure Vessel Inspection Technology Research Institute, Xinyang 464000, PR China.*

Table of contents: Experimental section, FigureS1-S3, Scehme S1, Table S1-S4.

* Corresponding author. Tel.: +86 376 6391172; Fax: +86 376 6391172.

E-mail address: zhanghongding0606@163.com (H.D. Zhang), wanghaibohn@163.com (H.-B. Wang).

Chemicals and Materials

Tris(2,2'-bipyridyl) ruthenium(II) chloride hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$) were provided from Sigma-Aldrich (USA). Tetraethyl orthosilicate (TEOS), 1-hexanol, chloroform, cyclohexane, and ammonia solution ($NH_3 \cdot H_2O$, 25%) were purchased from Aladdin Industrial Co., Ltd (Shanghai, China). Potassium permanganate ($KMnO_4$), 4-Morpholineethanesulfonic acid (MES), triton X-100, alkaline phosphatase (ALP), 2-phosphate-L-ascorbic acid trisodium salt (AAP), tris(2-carboxyethyl) phosphine (TCEP), streptavidin (SA), bovine serum albumin(BSA), glutaraldehyde and phosphate were obtained from Sangon Biotech Co., Ltd (Shanghai, China). 5mC-antibody (5mC Ab) was supplied from Abcam Trading CO., Ltd (Shanghai, China). Egg phosphatidycholine (PC-98T) and Cholesterol were bought from Shanghai Advanced Vehicie Technology (A. V. T.) Pharmaceutical Ltd (Shanghai, China). MaxsorpTM modified 96-well plates and dialysis bag (MW≤1000) used in this work were received from Thermo Fisher Scientific Inc (America). The DNA sequence (such as biotin modified capture DNA, unmethylated DNA (MLH1-C), Methylated DNA (MLH1-mC), random unmethylated DNA (Random-C), and random methylated DNA (Random-mC)) used in this work were synthesized from Sangon Biotech Co., Ltd (Shanghai, China), all of them were purified by HPLC and further confirmed by the mass spectrometry. The DNA sequences were shown in the **Table S1**. PBS buffer 1 (pH 7.4) was composed of 10 mM of NaCl and 10 mM of PBS. PBS buffer 2 (pH 7.4) was composed of 10 mM of NaCl, 10 mM of $MgCl_2$, and 10 mM of PBS.

Apparatus

A scanning electron microscope (SEM) of Hitachi S-4800 and a transmission electron microscopy (TEM) of Tecnai model G2 F20 were used to characterize the surface morphology of the nanomaterials. An X-ray photoelectron spectroscopy of Thermo Scientific K-ALPHA 0.5 EV was adopted to analysis the element composition of the nanomaterials. A ZetasizerNanoZS/Mastersizer 3000E instrument was employed to study the Zeta potential and the size of the nanomaterials. An atomic

force microscope (AFM) of Bruker Dimension Ion was used to record the AFM images of the obtained liposomes. A UV-vis spectrometer of Hitachi U-3900H was adopted to collect the UV-vis absorption spectra. A fluorescence spectrophotometer of Hitachi F-7000 was used to acquire the photoluminescence spectra. The excitation voltage was set as 500 v and both the emission and excitation slits were 5 nm.

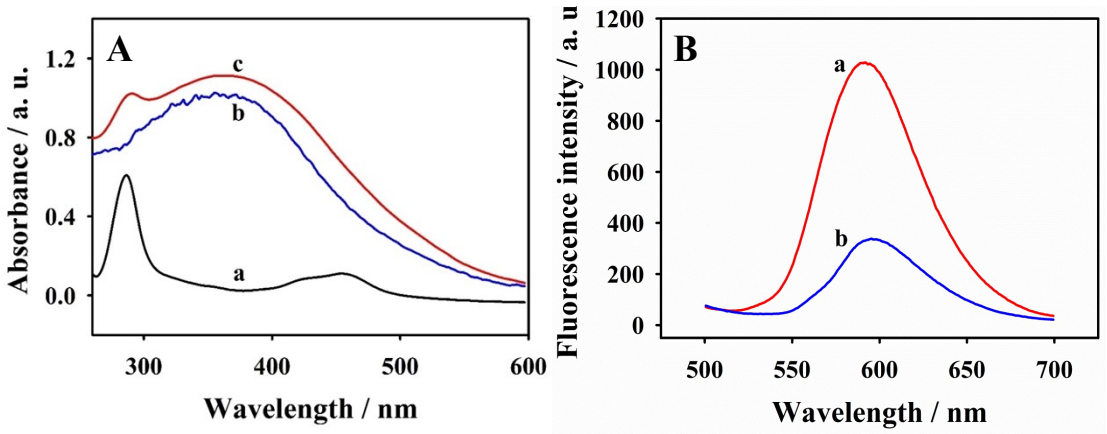


Fig. S1 (A) UV-vis spectra of Ru@SiO_2 nanoparticles (curve a), MnO_2 nanosheets (curve b) and Ru@SiO_2@MnO_2 nanocomposites (curve c). (B) Fluorescence spectra of Ru@SiO_2 nanoparticles (curve a) and Ru@SiO_2@MnO_2 nanocomposites (curve b).

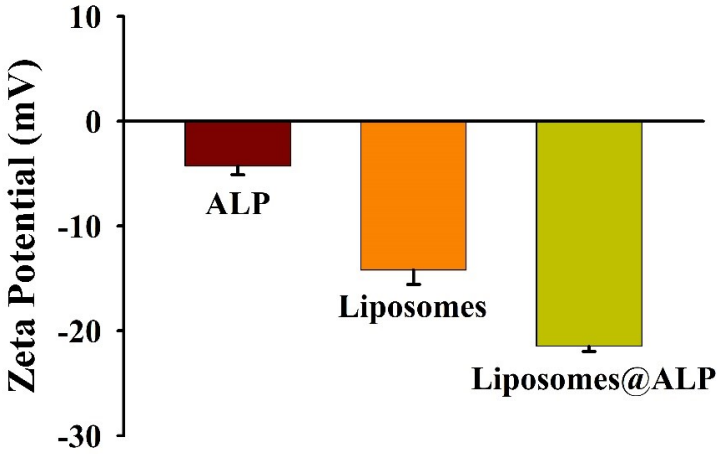
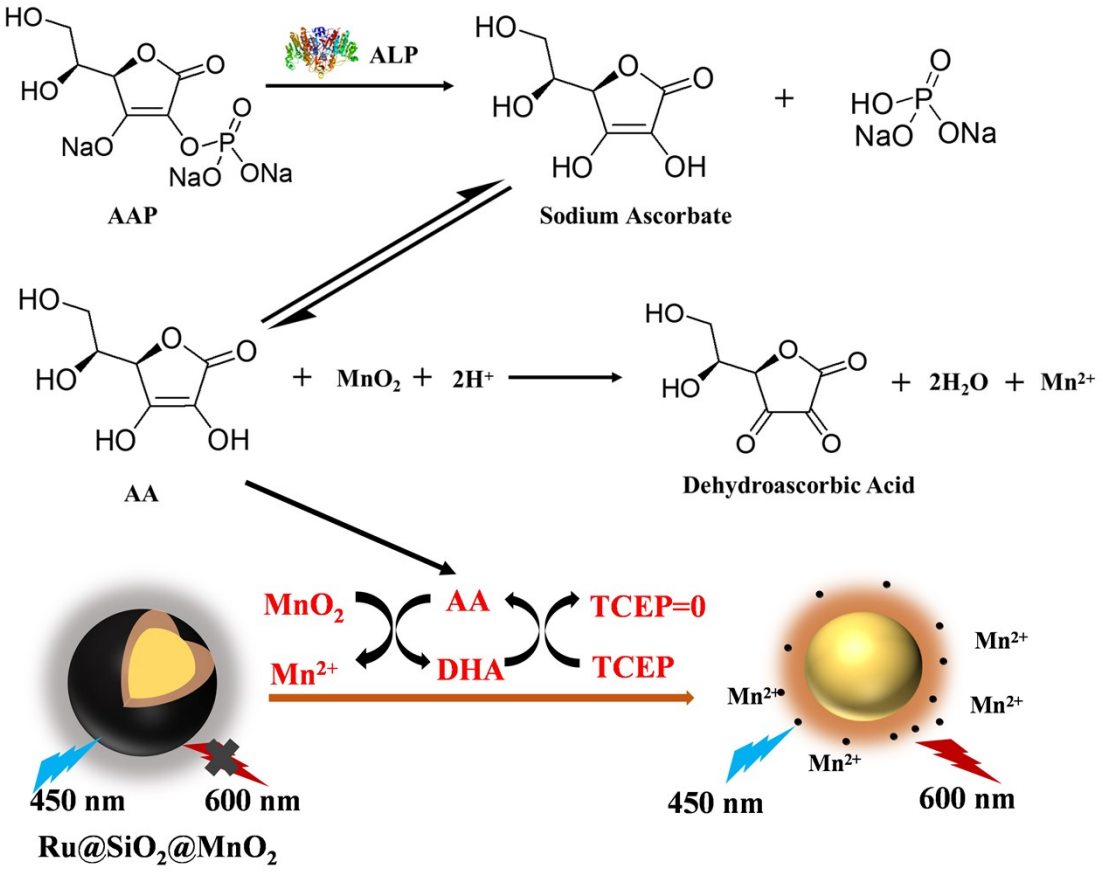


Fig. S2 Zeta potentials of ALP, bare liposomes and ALP-encapsulated liposomes (Liposomes@ALP). The error bars represent standard deviation of three paralleled experiments.



Scheme S1 The reaction principles of the ALP-assisted chemical redox cycling reactions.

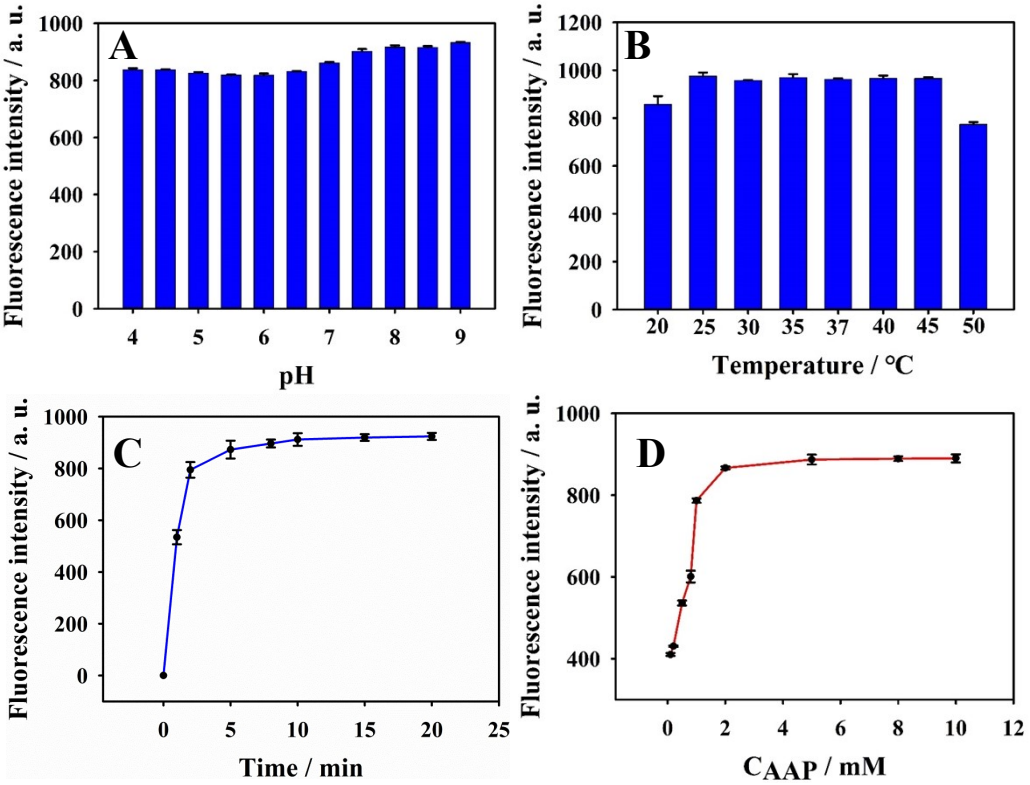


Fig. S3 Optimization of the reaction conditions of ALP-assisted chemical redox cycling dual signal amplification system. (A) The effect of reaction pH values. (B) The effect of reaction temperature. (C) The effect of incubation times. (D) The effect of AAP concentrations.

Table S1 DNA sequence used in this work.

Note	Sequence (5'-3')
Biotin capture DNA	CTG TCC GCT CTT CCT ATT GGT TTT TTT TTT-Biotin
MLH1-C	CCA ATA GGA AGA GCG GAC AG
MLH1-mC	CCA ATA GGA AGA G(mC)G GAC AG
Random-mC	TGG AGT CCT GCA (mC)GA GAC TAG
Random-C	TGG AGT CCT GCA CGA GAC TAG

Table S2 Comparisons of different fluorescent assays for the detection of ALP.

Fluorescent assays	Determination range ($mU \cdot mL^{-1}$)	Limit of detection ($mU \cdot mL^{-1}$)	References
The fluorogenic reaction between 2,3 –diaminonaphthalene and AA	0.1-60.0	0.08	1
Lysozyme-functionalized 5-methyl-2-thiour-acil gold/silver nanoclusters	0.5-10	0.0193	2
Sulfur quantum dots@ZIF-8 metal-rganic frameworks (SQDs@ZIF-8)	0.15-50	0.044	3
DNAzyme-regulated CRISPR/Cas12a	0.05-10	0.04	4
Enzyme-assisted signal amplification	0.01-8	0.0074	5
Glutathione-stabilized Cu nanoclusters	0.1-200	0.02	6
ALP-assisted redox cycling signal amplification	0.05-200	0.012	This work

Table S3 Comparison of various analytical methods towards DNA methylation detection.

Analytical methods	Linear range (M)	Detection of limit (M)	Reference
Oxidation damage base based amplification	1×10^{-13}-1×10^{-9}, 2×10^{-9}-1×10^{-7}	3.458×10^{-14}	7
Endonuclease-assisted PAM-free recombinase polymerase amplification	2×10^{-15} -2×10^{-11}	9.8×10^{-16}	8
Rolling circle amplification	1×10^{-12} - 1×10^{-7}	1.42×10^{-13}	9
GlaI cleavage assistant isothermal exponential amplification coupling with CRISPR/Cas12a	3×10^{-15}-4.8×10^{-14}, 4.8×10^{-14} -6×10^{-12}	1.25×10^{-15}	10
5mC antibody based electrochemiluminescence assay	5×10^{-13} -5×10^{-8}	1×10^{-13}	11
5mC antibody based electrochemical bioplatform	1.95×10^{-11} -9.5×10^{-8}	1.5×10^{-12}	12
ALP-assisted chemical redox cycling dual signal amplification	1×10^{-14} -8×10^{-13}, 8×10^{-13} -5×10^{-11}	2.9×10^{-15}	This work

Table S4 Detection of methylated DNA (MHL1) in human serum samples.

	Sample	Added (fM)	Found (fM)	Recovery (%)	RSD(%)
Human serum	1	20.00	20.18	105.4	5.7
		50.00	48.25	96.5	4.0
		100.00	89.40	89.4	2.1
	2	200.00	209.52	104.8	3.8
		500.00	482.76	96.6	4.9
		750.00	703.53	93.8	1.4

Reference:

1 J. Wen, Y. Hu, N. Li, D. Li, G. Zheng, Y. Zou, M. Zhang, and Li. Shui, *Anal. Chim. Acta*, 2022, **1230,** 340414.

2 M. Wang, X. Zhou, L. Cheng, M. Wang, and X. Su, *ACS Appl. Nano Mater.*, 2021, **4**, 9265-9273.

3 X.-X. Jiang, P. Li, M.-Y. Zhao, R.-C. Chen, Z.-G.. Wang, J.-X. Xie, and Y.-K. Lv, *Anal. Chim. Acta*, 2022, **1221**, 340103.

4 Y. Lai, M. Li, X. Liao, and L. Zou, *Anal. Chim. Acta*, 2022, **1233**, 340518.

5 X. Shang, Y. Yan, J. Li, X. Zhou, X. Xiang, R. Huang, X. Li, C. Ma, and X. Nie, *Spectrochim. Acta A*, 2023, **286**, 121939.

6 H. Zhang, B.-B. Tao, N.-N. Wu, L.-G. Chen, and H.-B. Wang, *Microchem. J.*, 2023, **193**, 109066.

7 Y. Zhang, C. Li, X. Zhang, Q. Xu, and C. Zhang, *Anal. Chem.*, 2020, **92**, 10223-10227.

8 S. Zhou, J. Dong, L. Deng, G. Wang, M. Yang, Y. Wang, D. Huo, and C. Hou, *ACS Sens.*, 2022, **7**, 3032-3040.

9 P. Yan, Y. Hao, Z. Shu, C. Gu, X. Zhou, X. Liu, and H. Xiang, *Mikrochim. Acta*, 2018, **185**, 299.

10 Q. Wu, X. Xiang, Y. Yuan, Y. Yu, M. Chen, J. Long, T. Xiang, and X. Yang, *Sens. Actuat B-Chem.*, 2023, **385**, 133675.

11 W. Wu, J. Wu, H. Huang, B. Qiao, C. Jiang, Y. Shi, C. Wang, H. Pei, Q. Xu, X. Wu, Q. Wu, and H. Ju, *Sens. Actuat. B-Chermical*, 2023, **375**, 132857.

12 E. Povedano, M. Gamella, R. M. Torrente-Rodríguez, V. Ruiz-Valdepeñas Montiel, A. Montero-Calle, G. Solís-Fernáez-García, M. Mendiola, D. Harddisson, J. Feliú, R. Barderas, J. M. Pingarrón, and S. Campuzano, *Anal. Chim. Acta*, 2021, **1182**, 338946.