Ratiometric fluorescent assay for DNA methylation based on alkaline phosphatase triggered *in-situ* fluorogenic reaction

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Table S1 The list of 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-mC (MLH1)	CCA ATA GGA AGA G(mC)G GAC AG
MLH1-C	CCA ATA GGA AGA GCG GAC AG
Random-mC	TGG AGT CCT GCA (mC)GA GAC TAG
Random-C	TGG AGT CCT GCA CGA GAC TAG

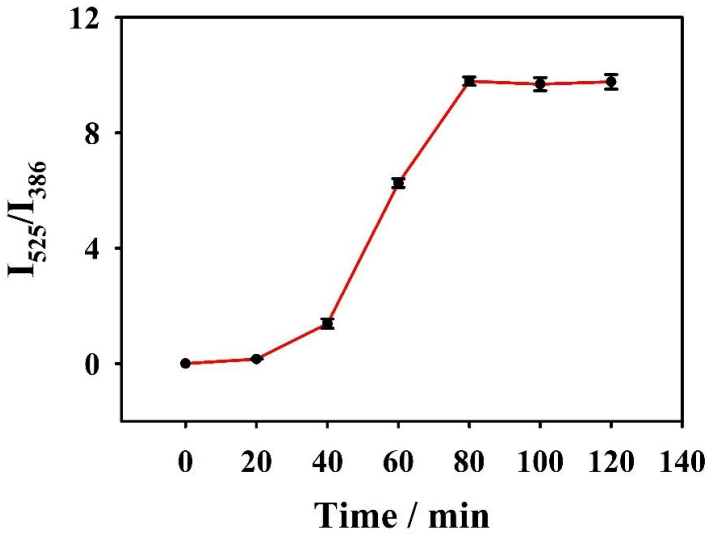

Fig. S1 The effect of reaction time on the in-situ fluorogenic reaction between AA and 2,3-DAN. The error bars stand for three parallel experiments.

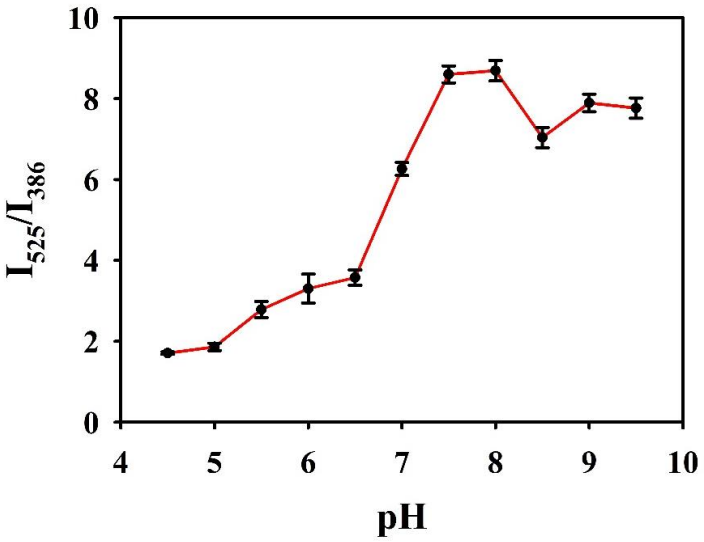

Fig. S2 The effect of pH on the in-situ fluorogenic reaction between AA and 2,3-DAN. The error bars stand for three parallel experiments.

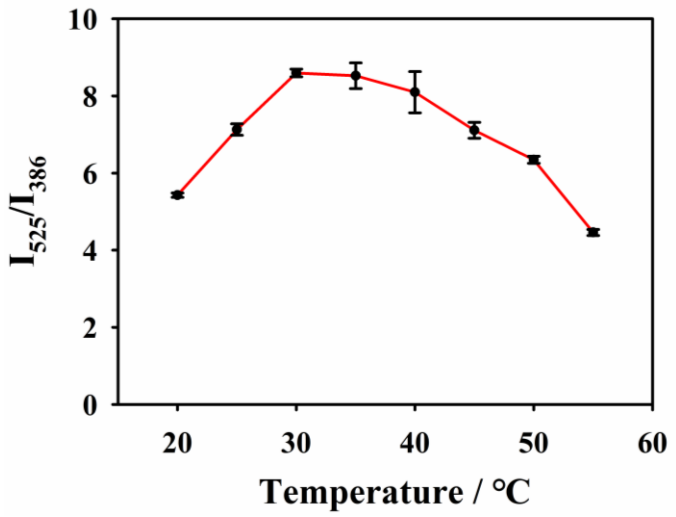

Fig. S3 The effect of temperature on the in-situ fluorogenic reaction between AA and 2,3-DAN. The error bars stand for three parallel experiments.

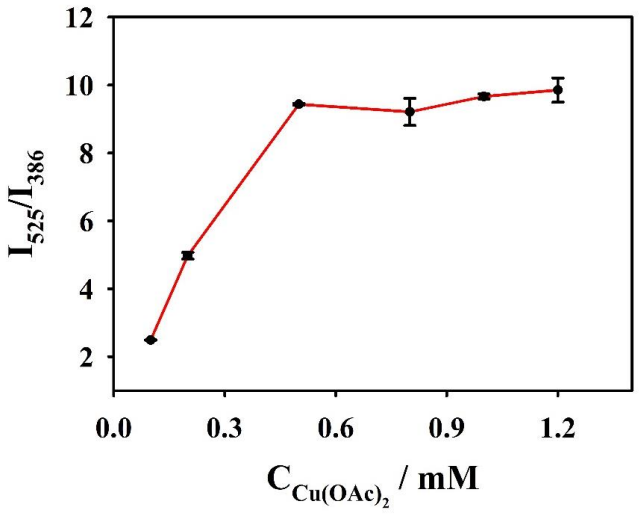

Fig. S4 The effect of $Cu(OAc)_2$ concentrations on the in-situ fluorogenic reaction between AA and 2,3-DAN. The error bars stand for three parallel experiments.

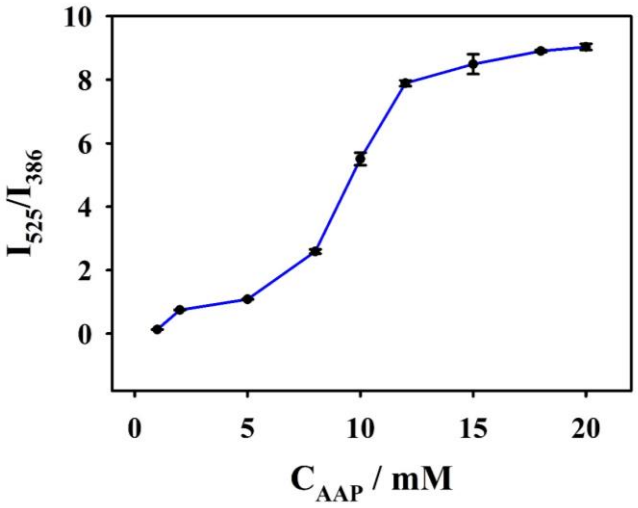

Fig. S5 The effect of AAP concentrations on the ALP-assisted in-situ fluorogenic reaction between AA and 2,3-DAN. The error bars stand for three parallel experiments.

Table S2 Comporisons of other fluorescent methods for ALP detection.

Fluorescent assays	Determination range ($mU \cdot mL^{-1}$)	Limit of detection ($mU \cdot mL^{-1}$)	References
DNAzyme-regulated CRISPR/Cas12a	0.05-10	0.04	1
Lysozyme-functionalized 5-methyl-2-thiour-acil gold/silver nanoclusters	0.5-10	0.0193	2
Glutathione-stabilized Cu nanoclusters	0.1-200	0.02	3
In situ fluorogenic reaction between dopamine and orcinol monohydrate	0-30	0.1	4
In situ fluorogenic reaction between terephthalic acid and ascorbic acid	0.05-20	0.016	5
ALP-triggered in situ fluorogenic reaction between ascorbic acid and o-phenylenediamine	0.1-30	0.06	6
ALP-triggered *in situ* fluorogenic reaction between AA and 2,3-DAN	1.0-0.1, 0.1-0.01	0.0036	This work

Table S3 Comparisons of other sensing assays towards DNA methylation detection.

Analytical methods	Linear range (M)	Detection of limit (M)	Reference
Rolling circle amplification	1×10^{-12} - 1×10^{-7}	1.42×10^{-13}	7
Oxidation damage base based amplification	1×10^{-13}-1×10^{-9}, 2×10^{-9}-1×10^{-7}	3.458×10^{-14}	8
Tannic acid-accelerated fenton chemical reaction amplification	2×10^{-14} -5×10^{-13}	1.4×10^{-15}	9
5mC antibody based electrochemical bioplatform	1.95×10^{-11} -9.5×10^{-8}	1.5×10^{-12}	10
5mC antibody based electrochemiluminescence assay	5×10^{-13} -5×10^{-8}	1×10^{-13}	11
ALP-triggered *in situ* fluorogenic reaction between AA and 2,3-DAN	2×10^{-13} -1×10^{-11}	8.2×10^{-14}	This work

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

	Sample	Added (pM)	Found (pM)	Recovery (%)	RSD(%)
Human serum	1	0.5	0.492	98.4	3.8
		1.0	1.053	105.3	4.2
		5.0	5.216	104.3	3.7
	2	0.5	0.503	100.6	2.9
		1.0	0.979	97.9	3.4
		5.0	5.104	102.1	2.5
	3	0.5	0.498	99.6	5.1
		1.0	1.035	103.5	3.3
		5.0	4.971	99.4	1.6

Reference:

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