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Tetrahedral DNA Nanostructure-Corbelled Click Chemistry-based Large-scale assembly of Nanozyme for ratiometric fluorescence Assay of DNA Methyltransferase Activity

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Table S1. Sequence information for the nucleic acids used in this study.

Oligonucleotide Sequence(5'-3')	
DNA_1	biotin-TTTTTTTTTTGGACGGAACAGCTTTGATCGCGAACC
DNA_2	HS-TTTTTTTTTTCTTCGGTTCGCGATCAAAGCTGTTCCG
Tetra-7A	GAGCGTTAGCCACACACACAGTCTTTTTTTT TGAGGT-alkyne
Tetra-7B	NH_2-C6-TTAGGCGAGTGTGGCAGAGGTGT
Tetra-7C	NH_2-C6-CGCCTAAACAAGTGGAGACTGTG
Tetra-7D	NH_2-C6-AACGCTCACCACTTGAACACCTC
Tetra-13A	ACACTACGTCAGAACAGCTTGCATCACTGGTCACCAGAGTATTTTTTTTTTGAGGT-alkyne
Tetra-13B	NH_2-C6-ACGAGCGAGTTGATGTGATGCAAGCTGAATGCGAGGGTCCT
Tetra-13C	NH_2-C6-TCAACTCGCTCGTAACTACACTGTGCAATACTCTGGTGACC
Tetra-13D	NH_2-C6-TCTGACGTAGTGTATGCACAGTGTAGTAAGGACCCTCGCAT
Tetra-17A	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTATTTTTTTTT TGAGGT-alkyne
Tetra-17B	NH_2-C6-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
Tetra-17C	NH_2-C6-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
Tetra-17D	NH_2-C6-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
Tetra-26A	GCCTGGAGATACATGCACATTACGGCTTTCCCTATTAGAAGGTCTCAGGTGCGCGTTTCGGTAAGTAG ACGGGACCAGTTCGCC TTTTTTTT TGAGGT-alkyne
Tetra-26B	NH_2-C6-CGCGCACCTGAGACCTTCTAATAGGGTTTGCGACAGTCGTTCAACTAGAATGCCCTTTGGGCTGTTCCGGGTGTGGCTCGTCGG
Tetra-26C	NH_2-C6-GGCCGAGGACTCCTGCTCCGCTGCGGTTTGGCGAACTGGTCCCGTCTACTTACCGTTTCCGACGAGCCACACCCGGAACAGCCC
Tetra-26D	NH_2-C6-GCCGTAATGTGCATGTATCTCCAGGCTTTCCGCAGCGGAGCAGGAGTCCTCGGCCTTTGGGCATTCTAGTTGAACGACTGTCGC
/DNA	Azide-AGTA GGTTGTATAGTT

H1*	AGTAGGTTGTATAGTTCAAAGTAACTATACAACCTACTACCTCA-SH
H2*	SH-ACTTTGAACTATACAACCTACTTGAGGTAGTAGGTTGTATAGTT

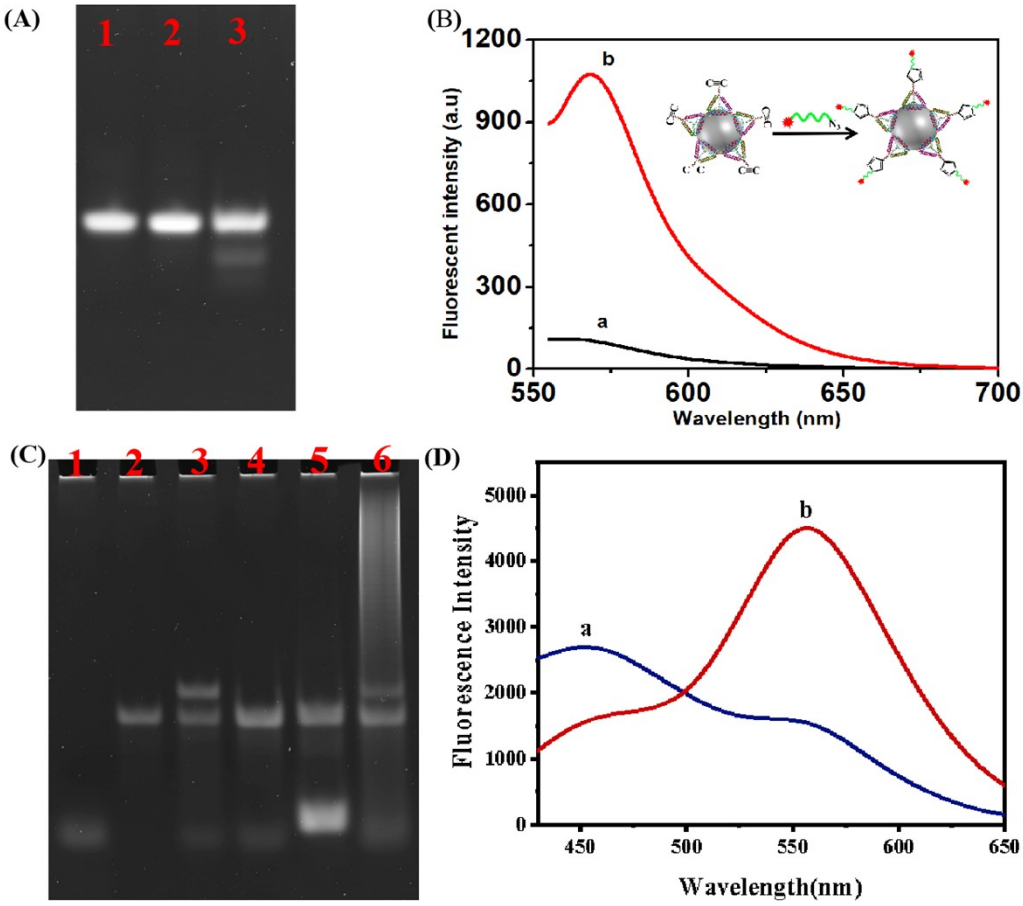

Figure S1. **(A)** Gel electrophoresis analysis of Dam MTase: (Lane **1**) 0.5 μM dsDNA + 10 U/mL Dam MTase; (Lane **2**) 0.5 μM dsDNA + 20 U/mL Dpn I; (Lane **3**) 0.5 μM dsDNA + 10 U/mL Dam MTase + 20 U/mL Dpn I. **(B)** Fluorescence response of Alk-DTN-MBs under different conditions after "click" chemistry reaction: Cy3 labeled azide-*l*DNA + Cu^+ + Alk-DTN-MBs (a), Cy3 labeled azide-*l*DNA + Alk-DTN-MBs (b). **(C)** Gel electrophoresis analysis of the "click" chemistry reaction-mediated hybridization chain reaction: (Lane **1**) azide-*l*DNA; (Lane **2**) alkyne-tetra-A; (Lane **3**) azide-*l*DNA + alkyne-tetra-A + Cu^+; (Lane **4**) azide-*l*DNA + alkyne-tetra-A; (Lane **5**) azide-*l*DNA + alkyne-tetra-A + H1 + H2; (Lane **6**) azide-*l*DNA + alkyne-tetra-A + Cu^+ + H1 + H2. **(D)** The feasibility of the sensor for Dam MTase assay under different conditions: (a) in the presence of Dam MTase (10 U/mL); (b) in the absence of Dam MTase.

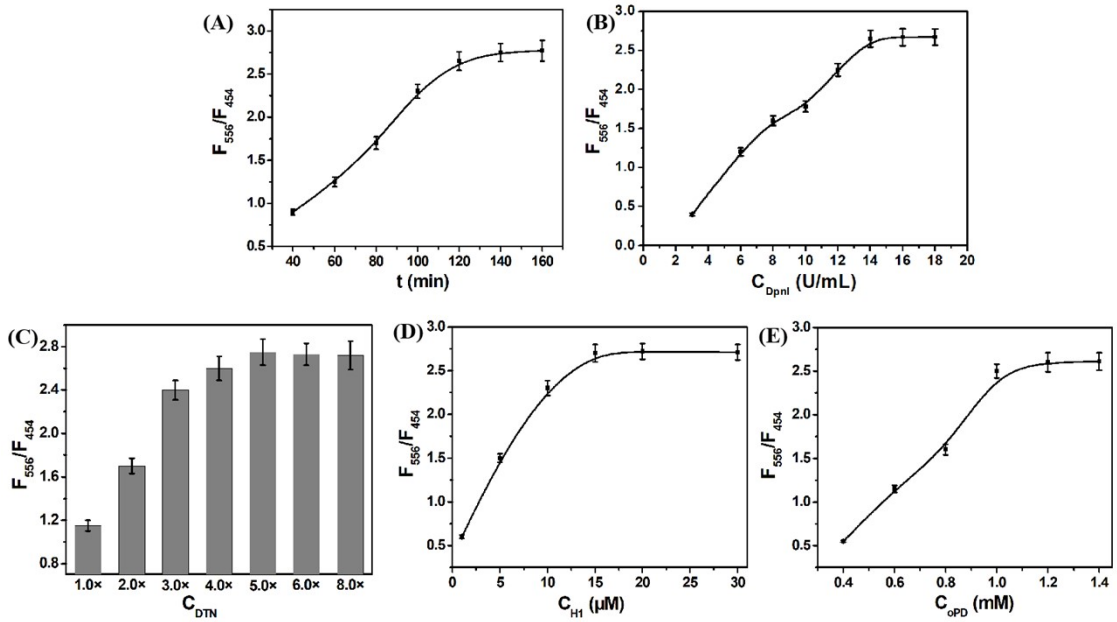

Figure S2. Effects of **(A)** methylation time, **(B)** DpnI concentration, **(C)** DTN probe concentration, **(D)** H1 (or H2) probe concentration, and **(E)** oPD concentration. The Dam concentration is 10 U/mL. Error bars represent the standard deviation from three independent experiments.

Table S2. Determination of Dam MTase in the spiked human serum samples using the developed method

Samples	Added (U/mL)	Found (U/mL)	RSDs (%, n=3)	Recovery (%)
1	0.1	0.0972	3.2	97.2
	0.1	0.1025	3.7	102.5
	0.1	0.0983	3.6	98.3
2	1.0	1.032	4.2	103.2
	1.0	0.969	3.9	96.9
	1.0	1.027	4.5	102.7
3	10.0	10.39	2.5	103.9
	10.0	10.15	3.5	101.5
	10.0	9.96	4.0	99.6

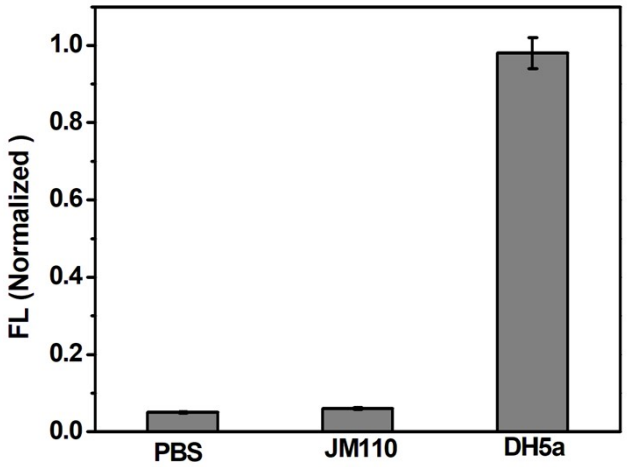

Figure S3. The proposed strategy-based detection of Dam MTase in complex biological sample: PBS buffer, JM110 and DH5α E. coli cell lysates.

Table S3. Comparison with other published methods for Dam MTase activity

Analytical method	Strategy	Linear range (U mL^{-1})	Detection limit (U mL^{-1})	Ref.
SERS	Au nanocube enhanced SERS biosensor coupled with strand displacement amplification (SDA)	10^{-4} − 0.5	8.65×10^{-5}	1
SERS	Nanoholes array (NHA) with the combination of Hybridization Chain Reaction (HCR)	0.002 − 200	2×10^{-4}	2
ICP-MS/cascade	Strand displacement amplification (SDA) and multicomponent nucleic acid enzyme (MNAzyme)	0.001–0.2	1.51×10^{-4}	3
Electrochemistry	AuNPs/ERGO hybrids and Hybridization Chain Reaction (HCR) strategy	0.02–10	0.0073	4
Electrochemistry	Porous organic polymer inorganic nanocomposite(Cu_2O@$FePPO_{PBADE}$)	0.005 − 100	0.0014	5
Ratiometric ECL	Polyaniline and anti-fouling peptidemodified electrode	0.05 − 100	0.02	6
Fluorescence	Terminal deoxynucleotidyl transferase induced activators to unlock the collateral cleavage activities (trans-cleavage) of CRISPR/Cpf 1 (TdT-IU-CRISPR/ Cpf 1)	1.59×10^{-3}– 3.18×10^{-1}	1.26×10^{-3}	7
Fluorescence	Single molecule fluorescence correlation spectroscopy (FCS) and Polystyrene polymer dots	0.025 − 3	0.025	8
Fluorescence	Isothermal autocatalytic hybridization reaction (AHR) circuit	0-1.0	0.011	9
Fluorescence	Cascade invasive reactions	0.1 − 10	0.002	10
Fluorescence	Entropy-driven reaction and toehold-initiated rolling circle amplification (TIRCA)	0.1 − 40	0.06	11
Fluorescence	Dissipative DNA networks	0.001 − 0.2	0.113	12
Fluorescence	HpaII-assisted and linear amplification-enhanced exponential amplification strategy	0.125 − 8	0.034	13
Single-molecule detection	purinic/apyrimidinic endonuclease 1 (APE1)-mediated cascade signal amplification platform	1.0×10^{-4}- 15	6.72×10^{-5}	14
Fluorescence	Tetrahedral DNA Nanostructure-Braced click chemistry based Large-scale assembly of Nanozyme	0.0025-10	0.001	This work

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