Electronic Supplementary Information (ESI) for:

Microarray-based resonance light scattering assay for detecting DNA methylation and human DNA methyltransferase simultaneously with high sensitivity

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Experimental methods

1. Reagents and materials

Restriction endonuclease HpaII, human DNA (cytosine-5) methyltransferase (Dnmt1) and corresponding buffers (CutSmart buffer and Dnmt1 reaction buffer) were obtained from New England Biolabs (NEB, UK). Synthetic oligonucleotides (as shown in Table S1) were purchased from Sangon Ltd. (Shanghai, China). Hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 3H_2O$), silver enhancer solution and methoxypolyethylene glycol amine (PEG-NH_2, MW 750) were purchased from Sigma-Aldrich Chemical Co. (USA). Sodium dodecyl sulfonate (SDS) was purchased from Aladdin Industrial Co. (Shanghai, China). Other chemicals were analytical grade. Aldehyde 3-D glass slides were obtained from CapitalBio Ltd. (Beijing, China). Milli-Q water (18.2 MΩ.cm) was used throughout the experiment.

Table S1. The oligonucleotides used in experiment[a]

Name	Sequence
p16-p	5'-NH_2-T_{10}-GGCACCAG**CCGG**AAGCAGC**ATACCCATACCCATA**-3'
p16-2-p	5'-NH_2-T_{10}-TTCAGGAGCGCGGGGTTCAC**ATACCCATACCCATA**-3'
p18-p	5'-NH_2-T_{10}-CCCTCCTG**CCGG**CTAGGCG**ATACCCATACCCATA**-3'
p21-p	5'-NH_2-T_{10}-CCCTCC**CCGG**GGCCCAG**ATACCCATACCCATA**-3'
p27-p	5'-NH_2-T_{10}-GCTAGGAG**CCGG**GGTGGCG**ATACCCATACCCATA**-3'
p16-m	5'-GCTGCTT**CMGG**CTGGTGCC-3'
p16-u	5'-GCTGCTT**CCGG**CTGGTGCC-3'
p16-2-m	5'-GTGAACCCCGMGCTCCTGAA-3'
p16-2-u	5'-GTGAACCCCGCGCTCCTGAA-3'
p18-m	5'-CGCCTAG**CMGG**CAGGAGGG-3'
p18-u	5'-CGCCTAG**CCGG**CAGGAGGG-3'
p21-m	5'-CTGGGCC**CMGG**GGAGGG-3'
p21-u	5'-CTGGGCC**CCGG**GGAGGG-3'
p27-m	5'-CGCCACC**CMGG**CTCCTAGC-3'
p27-u	5'-CGCCACC**CCGG**CTCCTAGC-3'
label	5'-SH-T_{10}-TATGGGTATGGGTAT-3'

[a] Red section is the recognition motif of HpaII. M indicates methylated cytosine. The underline sections are complementary sequences to label DNA on GNP probes. DNAs, p16-2-p, p16-2-m and

p16-2-u are control DNAs which do not contain HpaII recognition motif.

2. Preparation of DNA-GNP probe

The citrate stabilized 13 nm GNPs were synthesized by classical Turkevich–Frens method.[s1, s2] DNA-modified GNP (DNA-GNP) probes were prepared by a previously reported procedure.[s3] Generally, the GNP solution (8 nM, 300 μL) was incubated with 15 μL label oligonucleotide in aqueous solution overnight, then diluted with equal volume of PBS buffer (10 mM PB, 0.2 M NaCl, pH 7.5). After further incubation for another 10 h, the solution was evaporated to 100 μL by a vacuum concentrator (Eppendorf AG Co., Germany). Excess oligonucleotides were removed by repeated centrifugation (9000 rpm, 3 times). Finally, the DNA-GNP probes were dispersed in probe reaction buffer (0.67×SSC, 0.1% (w/v) SDS) and stored at 4 °C for further use.

3. Microarray fabrication

The alkylamine-modified oligonucleotides (probe DNAs: p16-p, p18-p, p21-p and p27-p) were dissolved in spotting buffer (3×SSC, 1.5 M betaine, 0.005% (w/v) SDS) at the concentration of 30 μM and spotted onto the aldehyde 3-D glass slide by a SmartArrayer 96 system (Capitalbio Ltd., Beijing, China). After an overnight incubation under 75% humidity at 37 °C, the slide was rinsed orderly with 500 mL washing buffer (1×SSC, 0.01% (w/v) SDS) and 500 mL water. After dried by centrifugation (480 g for 1 min), the microarray was separated into 12 independent subarrays by PTFE grid (Capitalbio Ltd., Beijing, China). Subsequently, the slide was blocked with 4 mg/mL PEG-NH_2 (in PBS buffer (50 mM PB, 0.15 M NaCl, pH 7.5)) at 30 °C for 1 h to inactivate remaining aldehyde groups. Then, the slide was washed with 30 mL PBS buffer for 3 min (3 times) and 30 mL Milli-Q water for 3 min (3 times), respectively. Finally, the slide was dried by centrifugation.

After blocking step, the ssDNA microarrays were hybridized with suitable target DNAs in 25 μL hybridization buffer (4 ×SSC, 0.1% (w/v) SDS) at 55 °C for 2 h. Then, the slide was subjected to a series of rinses: (1) 30 mL hybridization buffer at

55 °C for 5 min (3 times), (2) 30 mL washing buffer at 50 °C for 5 min (3 times), and (3) 30 mL Milli-Q water for 3 min (3 times), respectively. After dried by centrifugation, the dsDNA microarrays were employed to analyze methylation level of DNA and detect Dnmt1.

4. **Methylated DNA measurement**

For DNA methylation analysis, 100 nM target DNAs containing serial proportions (0%, 0.001%, 0.01%, 0.1%, 1%, 10% and 100%) of methylated DNAs (p16-m, p18-m, p21-m and p27-m) and corresponding unmethylated DNAs (p16-u, p18-u, p21-u and p27-u) were hybridized with ssDNA microarrays. Then, the dsDNA microarrays were incubated with 250 U/mL HpaII in 1×CutSmart Buffer (50 mM KAc, 20 mM Tris-Ac, 10 mM $Mg(Ac)_2$ and 100 μg/mL BSA, pH 7.9 @ 25 °C) at 37 °C for 4 h, followed by rinsing with 30 mL washing buffer at 37 °C for 5 min (3 times) and 30 mL Milli-Q water for 3 min (3 times), respectively.

5. Methyltransferase measurement

A range of concentrations (0.04, 0.2, 1, 5, 25 and 100 U/mL) of Dnmt1 were prepared in 1×Dnmt1 Reaction Buffer (50 mM Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol and 5 % glycerol, pH 7.8 @ 25 °C) supplemented with 100 μg/mL BSA and 160 μM S-adenosylmethionine (SAM). After incubation with hemimethylated and unmethylated dsDNAs on microarray at 37 °C for 1 h, the slide was rinsed with 30 mL washing buffer at 37 °C for 5 min (3 times) and 30 mL Milli-Q water for 3 min (3 times). Then, microarray was treated with 250 U/mL HpaII as previous description.

6. DNA-GNP probe labeling

After HpaII digestion, microarrays were incubated with 3.5 nM DNA-GNP probes in probe reaction buffer (25 μL each subarray) at 37 °C for 1 h and then washed with hybridization buffer, washing buffer and water as previously described. Afterwards, 1 mL silver enhance solution (solution A ($AgNO_3$) and solution B (hydroquinone) were

mixed at the volume ratio of 1:1) were applied to the slide for 8 min, followed by washing with 30 mL water (3 times) and drying by centrifugation.

7. Data acquisition and processing

RLS images were acquired by ArrayIt SpotWare Colorimetric Microarray Scanner (Telechem. International Inc., USA) according to the manufacturer's preset parameters. RLS signal intensity was collected by ImageJ software and the background signal originating from the slide was subtracted prior to evaluation. The mean value and standard deviation of the RLS intensity were determined from 6 spot replicates per sample.

Supporting figures

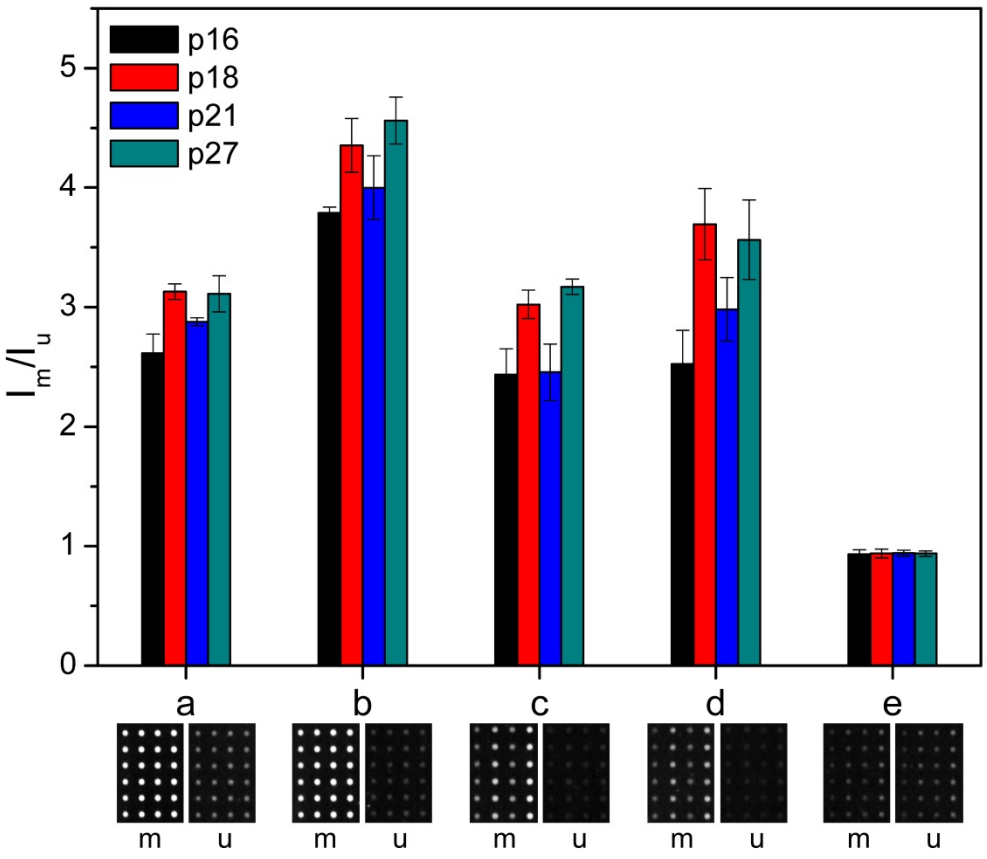

Fig. S1 The relative RLS intensities (I_m/I_u) as a function of concentrations of HpaII: (a) 500 U/mL, (b) 250 U/mL, (c) 125 U/mL, (d) 62 U/mL and (e) 0 U/mL. Letters m and u under the RLS images indicate hemimethylated and unmethylated substrates, respectively. The columns in RLS images (from left to right) contain p16, p18, p21 and p27, respectively.

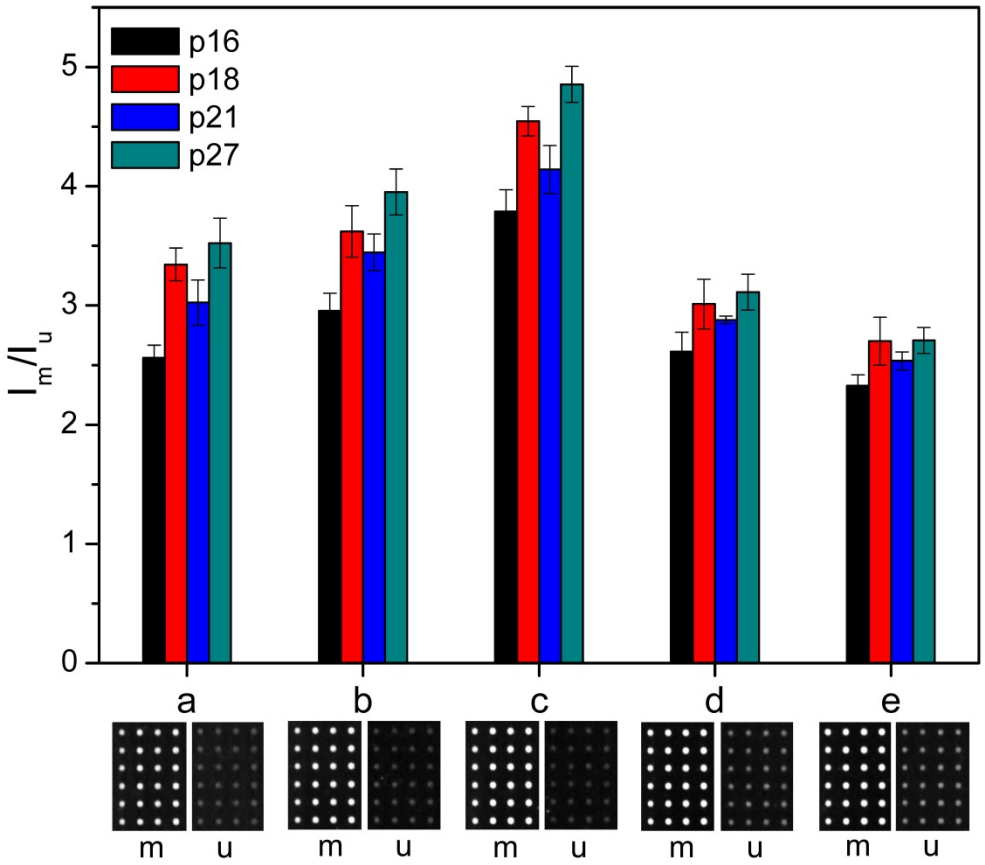

Fig. S2 The relative RLS intensities (I_m/I_u) as a function of concentrations of target DNAs: (a) 500 nM, (b) 200 nM, (c) 100 nM, (d) 50 nM and (e) 10 nM, respectively. Letters m and u under the RLS images indicate hemimethylated substrates and unmethylated substrates, respectively. The columns in RLS images (from left to right) contain p16, p18, p21 and p27, respectively.

Supplementary reference

s1. G. Frens, *Nature Phys. Sci.*, 1973, **241**, 20-22.

s2. J. Turkevich, P. C. Stevenson and J. Hillier, *Discuss. Faraday Soc.*, 1951, **11**, 55-75.

s3. A. G. Kanaras, Z. Wang, A. D. Bates, R. Cosstick and M. Brust, *Angew. Chem. Int. Ed.*, 2003, **115**, 201-204.