Electronic Supplementary Information

Accurate and Sensitive Total Genomic DNA Methylation Analysis from Sub-nanogram Input with Embedded SERS Nanotags

Yuling Wang, [a,§,*] Eugeen J. H. Wee[a, §] and Matt Trau[a,b,*]

[a]Centre for Personalized Nanomedicine, Australian Institute for Bioengineering and Nanotechnolgy (AIBN), [b]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, 4072, Australia

[*]E-mail: y.wang27@uq.edu.au; m.trau@uq.edu.au

[§]Authors contributed equally.

Experimental Section:

Reagents. 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB), 4-mercaptobenzoic acid (MBA), $HAuCl_4$, sodium citrate dehydrate and sodium borohydride were purchased from Sigma/Aldrich/Fluka. Streptavidin (SA) was purchased from Invitrogen Life-Technology. Methyl-CpG binding domain 2 (MBD)/IgG1-Fc domain fusion protein and streptavidin coated magnetic beads were purchased from New England Biolabs (NEB). EDC and s-NHS were purchased from Sigma-Aldrich. 4-Mercapto-3-nitro benzoic acid (MNBA) was prepared by breaking the disulfide bond in DTNB with $NaBH_4$ as reducing agent before usage.

Preparation of SERS clusters. Gold nanoparticles (AuNPs) with the diameter of 60 nm were synthesized by citrate reduction of $HAuCl_4$.[1] Silica-coated AuNPs clusters were prepared according to the reported protocol.[2-4] Briefly, the as-prepared AuNPs were incubated with Raman reporter solution (MNBA and MBA) overnight, followed by the centrifuge to remove the excess Raman reporter molecules. PBS buffer were added into AuNPs solution to induce the part aggregation of AuNPs and form the clusters. Silica-coated clusters of AuNPs were then performed by using the hydrolysis of TEOS. Shell thickness was controlled by the amount of TEOS. Purified SERS clusters were obtained by using the Gradient Density Centrifuge as reported.[5]

Preparation of SERS nanotags. SA and MBD modified SERS nanotags are prepared according to our previous work.[6-7] Briefly, silica-coated SERS clusters were functionalized by carboxyl group, followed by the activation of the carboxyl group with EDC/s-NHS. SA was then incubated with the activated SERS clusters to form the SA functionalized SERS cluster. To bind MBD on SERS clusters, protein G were first conjugated on the carboxyl-activated SERS clusters, followed by the binding of MBD on protein G based on the strong affinity of Fc from MBD and protein G. Human IgG in 0.5%BSA was further used to block the surface to minimize the non-specific binding.

DNA preparation. WGA DNA was generated using the REPLI-g Ultra-Fast Mini kit (Qiagen, Australia) and purified using the DNeasy Blood and Tissue kit (Qiagen, Australia). An aliquot of WGA DNA was then treated with SssI methyl-transferase (NEB) overnight and purified to generate highly methylated genomic DNA (M-WGA). An aliquot of the SssI-treated DNA was used to evaluate the methylation conversion efficiency by digestion with the methylation-sensitive HpaII restriction enzyme (NEB). Only reactions with no detectable digestion via gel electrophoresis were used in downstream experiments. Genomic DNA from Jurkat cells representing before and after 5-aza-2′-deoxycytidine treatment were purchased from NEB. DNA from Tumor and normal biopsies were generously provided for by Darren Korbie (The University of Queensland, Australia) and used as is. Prior to the collection of biopsy samples, ethics approval was obtained from The University of Queensland Institutional Human Research Ethics Committee (Approval No. 2011001315) and informed consent was obtained from all subjects prior to sample collection. Methods pertaining to clinical samples were carried out in accordance with approved guidelines.

To generate biotinylated DNA fragments, 50 ng of gDNA was enzymatically digested with the endonucleases DpnII and MseI (7.5 units each, NEB) at 37 °C in a 20-μL reaction supplemented with the NEB Buffer 3.1 system to generate <1000 bpDNA fragments with 5′overhangs. After 30 min, the reaction was supplemented to a final volume of 25 μL with 5 units of Klenow fragment (3′→5′exo-) DNA polymerase (NEB, USA) and 5 μM of biotin-14-dUTP, dATP, dGTP and dCTP and incubated at 37 °C for another 30 min to fill in the overhangs and biotinylate the fragmented DNA. Biotinylated DNA fragments were finally purified using the Agencourt Ampure XP SPRI purification kit and re-suspended in water.

DNA Methylation Assay: For the whole genome DNA methylation analysis, a previously establish protocol for highly stringent MBD assay (Ref.13, Wee et al, clinical epigenetics,

2015) was adopted with minor changes. Briefly, biotin-labeled gDNA fragments were first mixed with SERS-MBD nanotags and incubated on ice for 15 min. Since the binding of MBD on gDNA was sensitive to the salt concentration, 1.25x MBD buffer (NEB) was used for the binding of SERS-MBD nanotags on gDNA in the presence of 1 ng of fragmented salmon sperm DNA as blocking agent. Afterwards, streptavidin-magnetic beads and SERS-SA nanotags with the concentration of 0.1 nM were added for the incubation of magnetic beads, SERS-SA with gDNA for 10 min. The samples were then washed by 1.25x MBD buffer solution three times under magnet to remove the free SERS-MBD and SERS-SA nanotags. After washing, 60 μL PBS buffer was added to redisperse the sample for SERS test.

Instruments. Silica-coated gold nanoparticles with Raman reporters were characterized by TEM (JEOL-2100, 200keV). SERS spectra were recorded with a portable IM-52 Raman Microscopy. The 785 nm line was used for excitation of Raman scattering. SERS spectra were obtained at 1 sec integration with laser power (70 mW) by using a quartz cuvette. All the SERS spectra used are the raw data collected from the Raman microscope.

References:

1. G. Frens, *Nature. Phys. Sci.* **1973**, *241*, 20–22
2. B. Küstner, M. Gellner, M. Schütz, F. Schöppler, A. Marx, P. Ströbel, P. Adam, C. Schmuck, S. Schlücker, *Angew.Chem.Int.Ed.* **2009**, *48*,1950-1953.
3. M. Salehi, L. Schneider, P. Ströbel, A. Marx, J. Packeisen, S. Schlücker, *Nanoscale*. **2014**, *6*, 2361-2367.
4. Y.M.Lai, S. S. Schlücker, *RSC Adv.* **2015,** *5,* 13762-13767.
5. D. Steinigeweg, M. Schütz, M. Salehi, S. Schlücker, *Small*, **2011**, *7*, 2443-2448.
6. Y.Wang, R.Vaidyanathan, M.J. Shiddiky, M.Trau, *ACS Nano*, **2015**, *9*, 6354-6362.
7. Y. Wang, S. Rauf, Y.S. Grewal, L.J. Spadafora, M.J. Shiddiky, G.A. Cangelosi, S. Schlücker, M. Trau, *Anal Chem.* **2014**, *86*, 9930-9937.

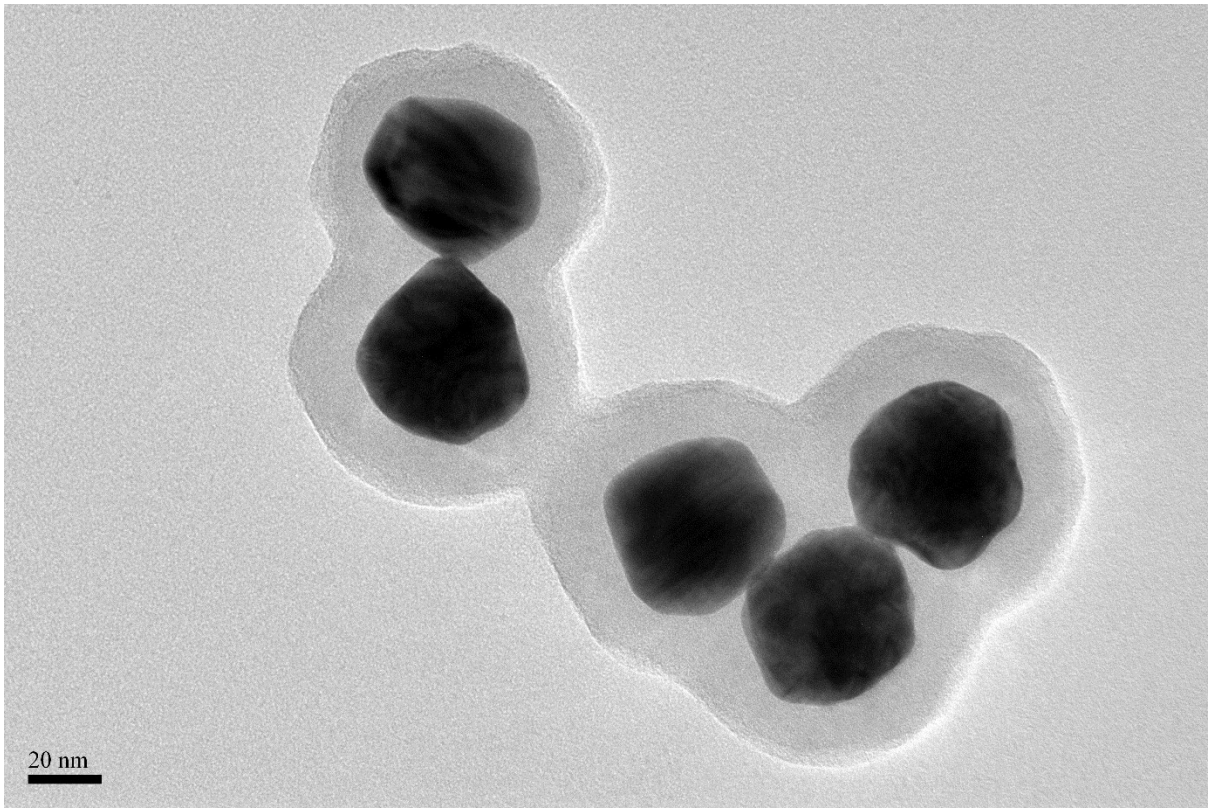

Fig.S1. Typical HR-TEM image of silica-coated SERS nanoparticles.

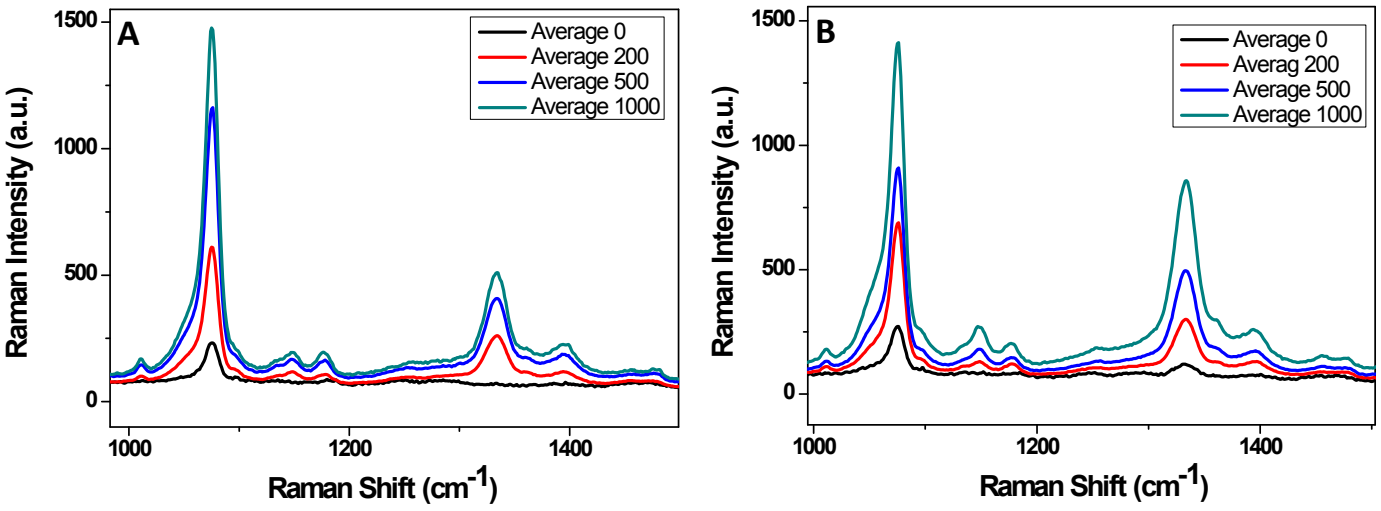

Fig. S2. SERS spectra for patient samples with different amount of input DNA from 0 pg to 1000 pg. (a) Normal tissue and (b) tumor tisssue.