Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supporting Information

Surface Enhanced Raman Scattering Based Sensitive Detection of Histone Demethylase Activity using Formaldehyde-Selective Reactive Probe

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EXPERIMENTAL SECTION

Reagents and Materials. Lysine specific demethylase 1 (LSD1) and its substrate peptide, histone H3 dimethyl lysine-4 peptide (Item No. 700403), LSD assay buffer (Item No. 700401) were purchased from Cayman Chemical Company (Ann Arbor, MI, U.S.A.). The diMe H3K4 substrate peptide was Ala-Arg-Thr-Lys(Me_2)-Gln-Thr-Ala -Arg-Lys-Ser-Thr-Gly-Gly-Lys-Ala-Pro-Arg-Lys-Gln-Leu-Ala-NH_2, where Lys(Me_2) denoted dimethylated lysine residue that could be demethylated by LSD1. Another histone H3 dimethyl lysine-9 peptide (diMe H3K9), which was chosen to validate the substrate specificity of LSD1, was synthesized and purified (>95% purity) by A' Peptide Co. Ltd. (Shanghai, China). The short hydrophilic peptide used for stabilizing AuNPs was Cys-Ala-Leu-Asn-Asn, which was synthesized and purified (>95% purity) by A' Peptide Co. Ltd.. The rationale for the peptide is that the thiol group facilitates a covalent anchoring on AuNPs surface, the hydrophobic side chains in position 2 and 3 promotes the self-assembly of the peptide, uncharged and hydrophilic amino acid in positions 4 and 5 and the negatively charged C-terminal improve the stability of the nanoparticles. Purpald

(4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole), 2-PCPA (trans-2-phenylcyclopropyl-amine) and formaldehyde (37% w/w) were obtained from Sigma Aldrich Corporation (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd (Beijing, China). Solutions were prepared using ultrapure water, which was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) and had an electric resistance >18.3 MΩ.

Preparation of Peptide-stabilized AuNPs. Citrate-capped AuNPs (~30 nm) were prepared by citrate reduction of $HAuCl_4$ according to a documented protocol,[S1] as briefly described as follows: 2.5 mL 1% (w/w) sodium citrate was added rapidly under vigorous stirring in 200 mL boiling solution of 0.01% (w/w) $HAuCl_4$. Within several minutes, the solution color changed from yellowish to wine red. The solution was heated under reflux for another 20 min to ensure complete reduction, and then slowly cooled to room temperature and stored at 4 ºC before use. The concentration of these AuNPs was determined to be ~0.3 nM based on an extinction coefficient of 3.0×10^9 M^{-1} cm^{-1} at 530 nm for 30 nm AuNPs using a UV/visible absorption spectrometer (UV 2450, Shimadzu, Kyoto, Japan).

It is noted that AuNPs with different diameters have unequal SERS enhancement effects, and maximized enhancement is achieved with AuNPs of 60-80 nm.[S2] However, AuNPs of the size always require complicated synthesis and exhibit decreased stability. In our assay, AuNPs of 30 nm can be prepared easily with high stability and desirable monodispersity.

The as-prepared AuNPs solution was concentrated to 1 nM via centrifugation at 10000 rpm for 10 min followed by re-suspension in ultrapure water. A 48 μL aliquot of peptide solution (3.75 μM) was added slowly into 600 μL AuNPs solution to prepare the peptide-stabilized AuNPs. The pH of the mixture was maintained ~7.0 by adding 0.1 M NaOH during the labeling process. After 8 h, the mixture was concentrated via centrifugation at 10000 rpm for 10 min followed by re-suspension of the sediment in 1 mL ultrapure water. This step was repeated three times and the peptide-stabilized AuNPs were re-dispersed in 300 μL ultrapure water and stored at 4 ºC until use. The

final concentration of peptide-stabilized AuNPs was ~2 nM, assuming no significant loss of AuNPs during the preparation process.

The effect of peptide concentration in preparation of peptide-stabilized AuNPs at a fixed AuNPs concentration (1 nM) was investigated to improve the signal-to-background ratio. A series of peptide solutions (3.75 μM) with different volumes were mixed with 600 μL AuNPs solution, making final concentration of peptide 150 nM, 300 nM, 600 nM, and 1200 nM, respectively. According to the above step, a batch of peptide-decorated AuNPs was prepared. Then we utilized these peptide-stabilized AuNPs for the assay of 500 nM LSD1. It is found that peptide-decorated AuNPs were unstable in the assay medium at peptide concentration smaller than 300 nM, while higher peptide concentration decreased the SERS signal intensity. Note that other stabilizing agents such as PEG, polymer coating, or silica shell coating, have also been used for decorating AuNPs in SERS detection. However, the short peptide capping agent has the advantages such as low cost, ease for preparation, and high SERS enhancement ratio. Besides, we tried simply using citrate-capped AuNPs in our assay. In that case, the obtained SERS signals, though gave substantially stronger enhancement, showed very poor reproducibility due to the uncontrolled property of the electrolyte-induced aggregation processes. This poor reproducibility disabled us to quantitative detection of histone demethylases.

Activity Analysis of Lysine Specific Demethylase 1 (LSD1). 10 μL LSD1 samples with different concentrations were added into 10 μL HEPES buffer (50mM, pH 7.5) containing 20 μM diMe H3K4 peptide substrate, making LSD1 final concentrations ranging from 0 nM to 500 nM. The mixture was incubated at 37 ºC for 30 min to allow complete demethylation of the substrate peptide by LSD1. A 4 μL aliquot of freshly prepared Purpald solution (20 mM, dissolved in 0.2 M NaOH) was added into the mixture and incubated for 10 min at room temperature. Then 6 μL peptide-stabilized AuNPs solution was added in the mixture. After incubating for 5 min, the resulting solution was dripped on a silicon wafer and subjected to SERS measurements. The Raman spectra were recorded using a confocal Raman system Laboram 010 (Horiba JobinYvon, France) with 632 nm HeNe laser. The

SERS spectra were acquired using a laser beam exposure time of 10 s and an accumulation of 1 scan. We also performed control experiments with an equal volume of the diMe H3K9 peptide or ummethylated H3K4 peptide in place of diMe H3K4. Further control experiment was performed by incubating the diMe H3K4 peptide in the presence of 500 nM LSD1 and its inhibitor, 2-PCPA (50 μM) at 37 ºC for 30 min, followed by the addition of Purpald and peptide-stabilized AuNPs solution.

MALDI-TOF Mass Spectrometry Characterization. The demethylation reaction was also characterized using MALDI-TOF mass spectrometry. The matrix was prepared by dissolving α-cyano-4-hydroxycinnamic acid (CCA) in the acetonitrile and water mixture (1:1 volume ratio) containing 0.1% (v/v) trifluoroacetic acid, making CCA final concentration 10 mg mL^{-1}. The sample solution (0.5 μL) was mixed with 0.5 μL matrix solution, dripped on the spot in a polished steel sample holder and air-dried at room temperature. The mass spectrum was acquired in deflection mode on a Proflex III mass spectrometer (Bruker, USA) equipped with a nitrogen laser of 337 nm with 3 ns duration pulse. The acceleration voltage was set to 20 KV. Laser power was set as 30%. The spectrum was an average of 100 laser shots.

References

[S1] K. C. Grabar, R. G. Freeman, M. B. Hommer, M. J. Natan, *Anal. Chem.*, 1995, **67**, 735-743.

[S2] L. Guerrini, D. Graham, *Chem. Soc. Rev.*, 2012, **41**, 7085-7107.

frequency (cm^{-1})	assignments
708	S-C-N stretching vibration
833	ring breathing
1095	N-N stretching vibration
1212	ring vibration
1282	ring stretching vibration
1385	ring stretching vibration
1463	N=N stretching vibration

Tab. S1 Frequencies and assignments for Raman bands of the Purpald-formaldehyde adduct.

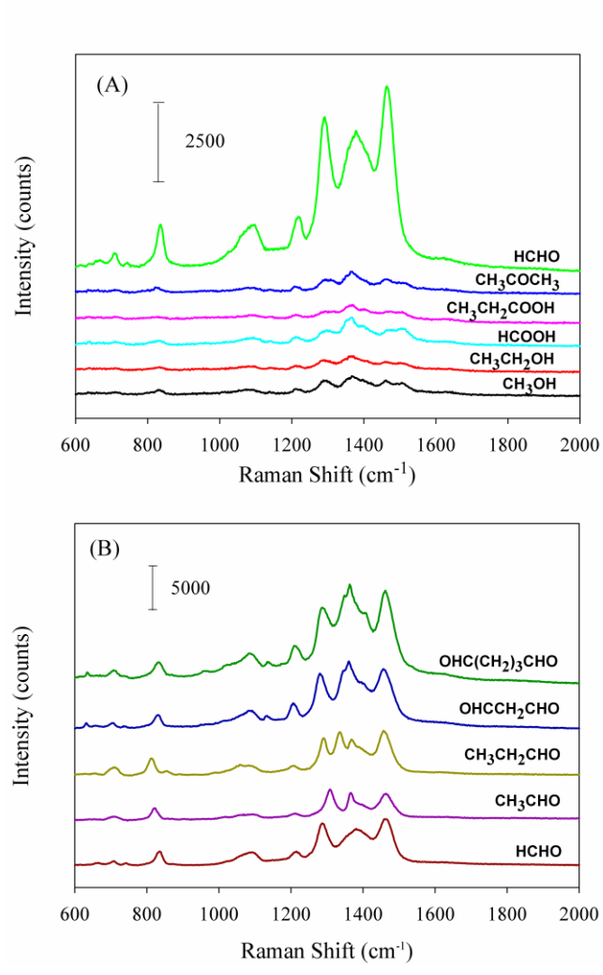

Fig. S1 Selectivity of Purpald toward other aldehydes and common alcohols, carboxylic acids and acetone. (A) SERS signals demonstrating the selectivity for formaldehyde over other common alcohols, carboxylic acids and acetone: methanol (black); ethanol (red); formic acid (cyan); acetic acid (pink); acetone (blue); formaldehyde (green). Formaldehyde has been used at 2 μM, others have been used at 5 mM. Each spectrum is offset by 800 counts. (B) SERS signals demonstrating Purpald also reacts with a wide varity of aldehydes: formaldehyde (dark red); acetaldehyde (dark pink); propanal (dark yellow); malondialdehyde (dark blue); glutaraldehyde (dark green). Formaldehyde and other aldehydes have been used at 2 μM. Each spectrum is offset by 5000 counts.

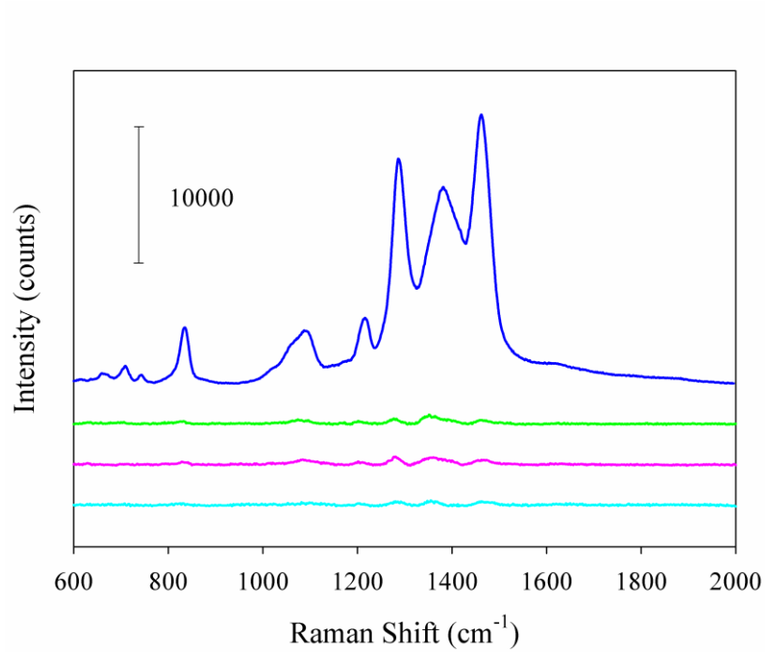

Fig. S2 Average SERS spectra across four repetitive assays obtained with different samples. Hela cell extracts (5-fold diluted, cyan), RPMI 1640 medium supplemented with 10% FBS (10-fold diluted, pink), glucose (10 mM, green), formaldehyde (8 μM, blue). Each spectrum is offset by 3000 counts.

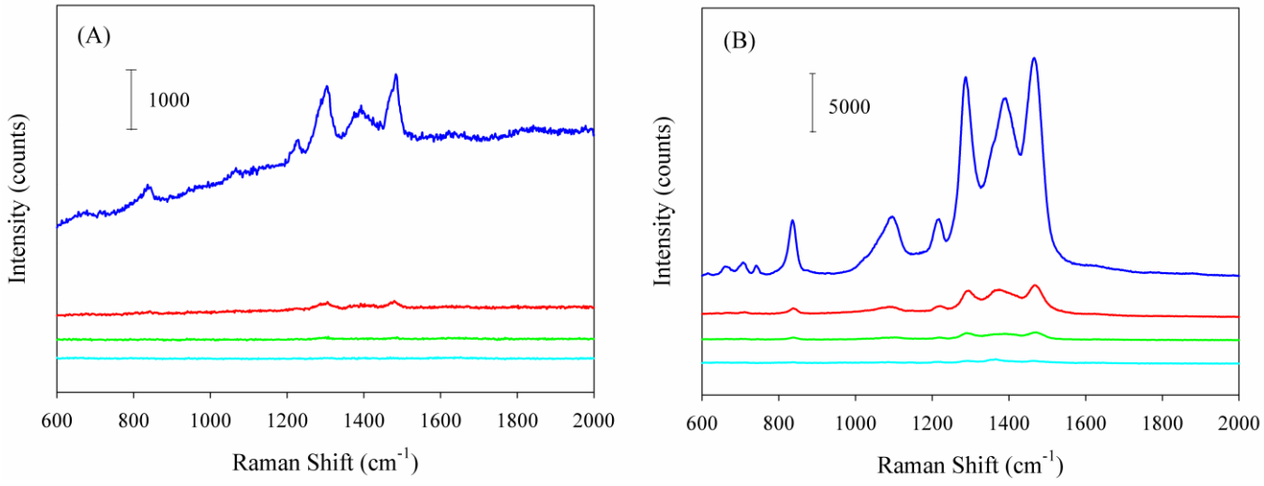

Fig. S3 Average Raman spectra across four repetitive assays for Pupald in responses to formaldehyde of different concentrations. 0 nM (cyan), 100 nM (green), 500 nM (red), and 8 μM (blue). (A) Resonance Raman spectra in the absence of AuNPs. Each spectrum is offset by 300 counts. (B) SERS spectra in the presence of AuNPs. Each spectrum is offset by 2000 counts.

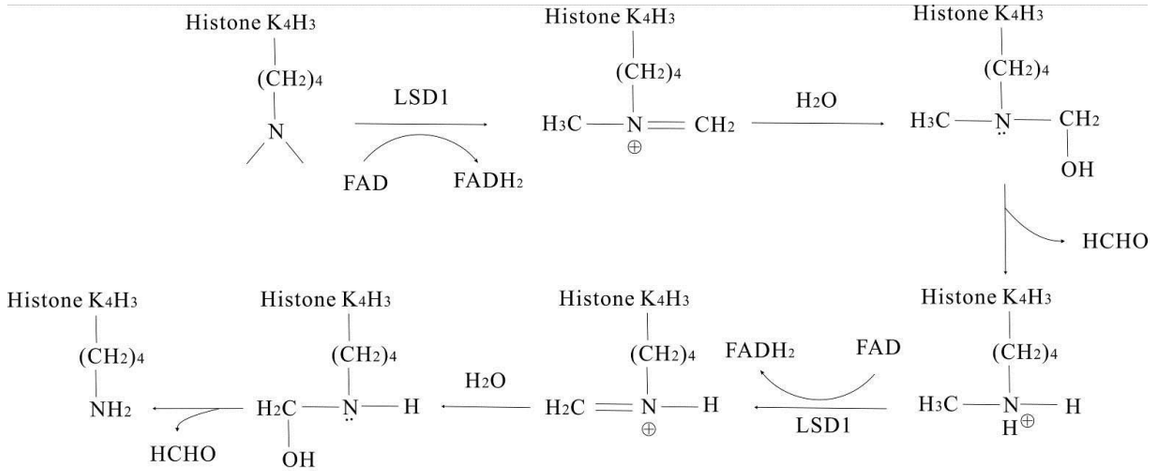

Fig. S4 The demethylation reaction process of diMe H3K4 substrate peptide catalyzed by LSD1. LSD1 catalyzes an amine oxidation of the protonated nitrogen using FAD as a co-factor, creating an iminium ion which spontaneously hydrolyzes to release a formaldehyde molecule and results in mono-methylated lysine. Then the mono-methylated lysine can also undergo the same reaction to become unmethylated.

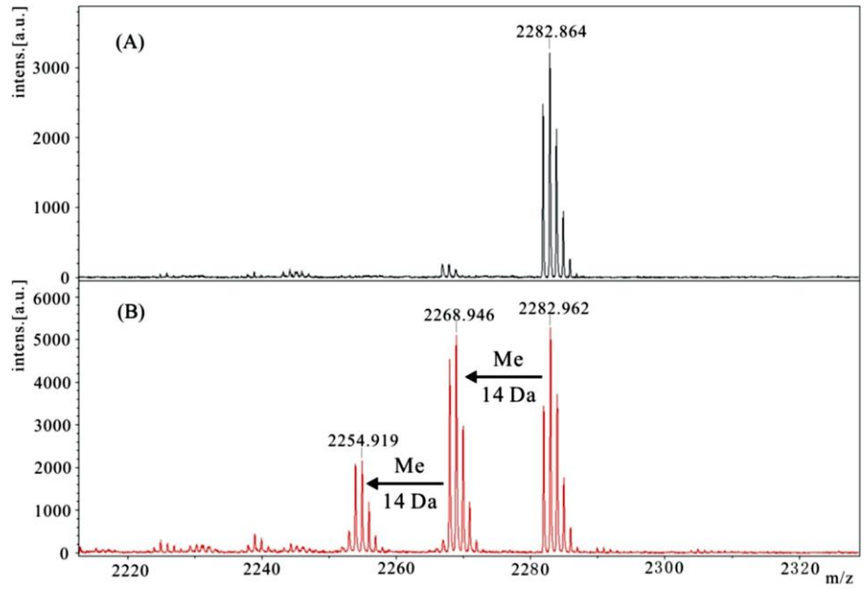

Fig. S5 MALDI-TOF mass spectrometry assay of the diMe H3K4 peptide in the absence (A) and presence (B) of 500 nM LSD1.

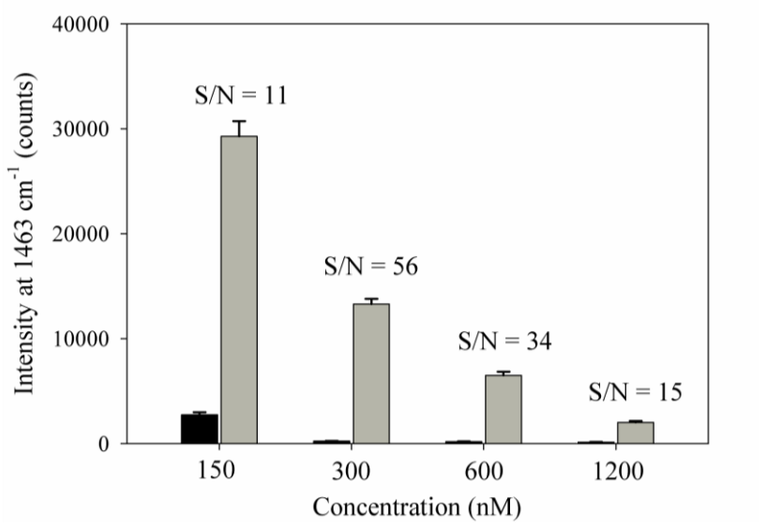

Fig. S6 Optimization of peptide concentration in preparation of peptide-stabilized AuNPs. Plot of intensities at 1463 cm^{-1} of SERS signals in the presence (gray) and absence (black) of 500 nM LSD1 versus concentrations of peptide. The error bars are standard derivations across four repetitive assays. The optimized peptide concentration was achieved at 300 nM.