Chemoselective and laser cleavable probes for in situ protein lipoylation detection by laser desorption/ionization mass spectrometry

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EXPERIMENTAL SECTION

Chemicals

Dess-Martin periodinane, lipoic acid (LA) and (+)-sodium L-ascorbate were purchased from J&K Scientific. 5-hexyn-1-ol, azido-polyethylene glycol-thiol (N_3-PEG-SH, 5000 Da), Cyanine5-azide (Cy5-azide) and tris (4-methoxyphenyl) methanol were supplied by Aladdin reagent. Tetrachloroauric (III) acid trihydrate, sodium citrate tribasic dihydrate, triphenylmethyl mercaptan, tris (2-carboxyethyl) phosphine (TCEP) and tris [(1-benzyl-1H-1,2,3-triazol-4-yl) methyl] amine (TBTA) were provided by Sigma-Aldrich. N-ethylmaleimide (NEM) and chloroform-d for nuclear magnetic resonance (NMR) was purchased from Beijing Innochem co., LTD. The deionized water used was prepared by a Milli-Q water purification system from Millipore (Milford, MA, USA).

Cells

Human neuroblastoma (SK-N-SH) cells, human embryonic kidney 293 (HEK 293) cells and Michigan cancer foundation-7 (MCF-7) cells were obtained from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). The Dulbecco's modified eagle medium (DMEM) with penicillin and streptomycin was purchased from Jiangsu KeyGEN BioTECH Corp., Ltd., added 10% fetal bovine serum obtained from Zhejiang Tianhang Biotechnology Co. Ltd. Cells were cultured in a humidified incubator with 5% CO_2 at 37 °C.

Instruments

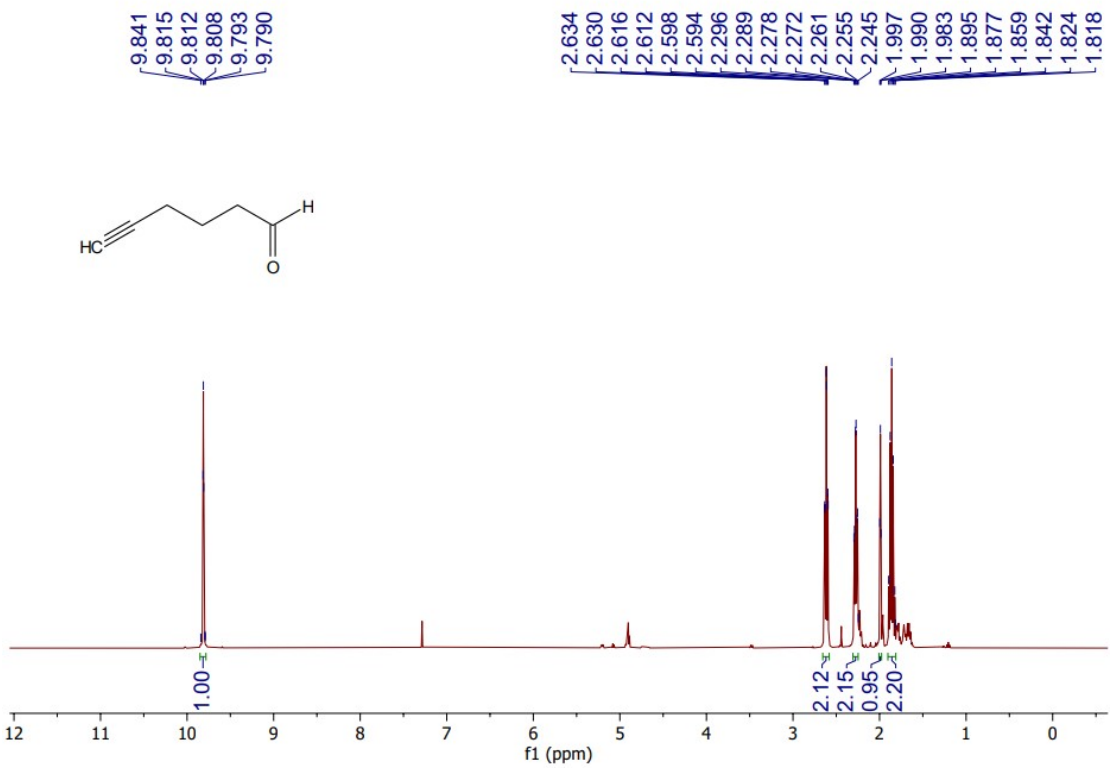
Laser desorption/ionization mass spectrometry (LDI-MS) analysis was performed in reflection positive mode using a Bruker Ultraflextreme time-of-flight mass spectrometer (Bruker Daltonics, Billerica, Germany) equipped with a 355 nm smart beam Nd:YAG pulsed laser, within a mass range of 0-1000 Da. Each mass spectrum was obtained with 200 laser shots at 1000 Hz, and the laser size and laser power energy were set to medium and 57% respectively.

LDI- Fourier transform ion cyclotron resonance (FTICR) MS analysis was performed with a Bruker 15 T SolariX FTICR mass spectrometer (Bruker Daltonics, Billerica, Germany) equipped with a 355 nm smart beam Nd:YAG pulsed laser.

^{1}H-NMR spectrum was recorded on Avance NEO 400 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). Transmission electron microscope (TEM) measurements were performed on a HT 7700 (Hitachi, Tokyo, Japan). Ultraviolet-visible (UV-vis) absorption spectra were acquired on a PerkinElmer Lambda 1050+ spectrophotometer (Perkin Elmer, MA, USA). Fluorescence imaging was conducted on an FV 1000-IX81 confocal laser scanning microscope (CLSM) (Olympus, Tokyo, Japan).

Synthesis and characterization of butyraldehyde probe (BAP)

The synthesis of BAP was according to the previous study of Chu Wang, et al.[1] ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.81 (s, 1H), 2.63 - 2.59 (m, 2H), 2.30 - 2.25 (m, 2H), 1.99 (t, J = 2.6 Hz, 1H), 1.86 (p, J = 7.1 Hz, 2H).



^{1}H NMR spectrum of compound butyraldehyde probe

Synthesis of gold nanoparticles (AuNPs)

100 μL of tetrachloroauric (III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$, 85 mg/mL) was added to 100 mL deionized water. After the water was brought to boil, 1 mL of 3% w/v sodium citrate tribasic dihydrate ($Na_3C_6H_5O_7$) was immediately added, and the color of the solution slowly changed from light purple to red. Based on this method, diameter of AuNPs was about 15 nm.[2, 3]

Synthesis of MTs/N_3@AuNPs

0.25 mM N_3-PEG-SH (5000 Da) and 25mM triphenylmethyl mercaptan (MTs) were added into the AuNPs solution and reacted for 12 h at 25℃. The obtained MTs/N_3@AuNPs were washed by water for at least three times and dispersed in deionized water for use. For reaction condition optimization, different concentrations of N_3-PEG-SH and MTs were evaluated to get the best formulation, and the above conditions were confirmed to be optimal.

In situ detection of protein lipoylation in cells

Cells were cultured in DMEM for 24 h after passage. After cells washed with phosphate-buffered saline (PBS), 4% paraformaldehyde (PFA) was used for cells fixed at 4℃ overnight, and then cells were washed with PBS and penetrated with 0.2% Triton-X100 for 10 min. To exclude the “side reactions” between BAP and cysteine, the cells were treated with 100 mM NEM 3 h for blocking protein thiol after washing with PBS. For experimental group, cells were washed with PBS and incubated with 20 mM BAP and 10 mM TCEP in an acidic buffer system (pH=3) for 12 h at 37℃. Two

control groups were set while one without BAP added and another with BAP added for 5 min to exclude the nonspecific adsorption of cells. Then the three groups of cells were treated the same as PBS was used for washing the cells. After that, 500 μL of reaction solution (0.2 mM TBTA, 0.2 mM TCEP, 1.0 mM $CuSO_4$, and 2.0 mM sodium ascorbate in MTs/N_3@AuNPs) was added for click reaction for 6 h. Finally, the entirely washed cells (1% Tween-20 and PBS) were scraped and directly spotted on the target plate for LDI-MS detection.

CLSM imaging of protein lipoylation in cells

Cells were treated the same as described in section "In situ detection of protein lipoylation in cells" until BAP incubated. Two control groups were also set. After cells washed with PBS, reaction solution (0.05 mM Cy5-azide, 0.2 mM TBTA, 0.2 mM TCEP, 1.0 mM $CuSO_4$, and 2.0 mM sodium ascorbate) was added for click reaction for 1 h. Finally, the entirely washed cells were used for confocal imaging.

LA inhibition experiment

20 mM LA and 20 mM TCEP were mixed and reacted for 30 minutes. Next, 20 mM BAP was added and reacted at 37°C for 12 hours. The pre-incubated BAP was then used to incubate with cells as the BAP-MTs/N_3@AuNPs strategy described in section "In situ detection of protein lipoylation in cells".

MS imaging of tissues

Fresh frozen tissues were cut to 10 μm slice with serial sections under -20 ℃ using a Leica CM1950 cryostat (Leica Microsystems GmbH, Wetzlar, Germany) and thaw mounted onto conductive indium tin oxide (ITO) glass. One specimen was used for hematoxylin and eosin (HE) staining and the other serial sections were used for MSI. The spatial resolution was set at 100 and 200 μm for mass spectrometry imaging (MSI).

In order to exclude some of the chemical species that negatively affect analysis of proteins and peptides including salts and lipids, tissue washing procedure was adopted.[4] The standard procedure in our laboratory calls for a 30 s wash in 70% ethanol, a 30 s wash in anhydrous ethanol, a 30 s wash in Carnoy's (10% glacial acetic acid, 60% ethanol, 30% chloroform) solution, a 30 s wash in deionized water containing 0.2 % trifluoroacetic acid (TFA), and a final 5 min wash in deionized water, followed by drying under ambient conditions.[5]

100 mM NEM was added to the surface of tissues and incubated for 3h. After washing with PBS, 20 mM BAP and 10 mM TCEP in an acidic buffer system (pH=3) was added to the surface of tissues and incubated for 12 h at 37℃. After washing with PBS, reaction solution (0.2 mM TBTA, 0.2 mM TCEP, 1.0 mM $CuSO_4$, and 2.0 mM sodium ascorbate in MTs/N_3@AuNPs) was added for click reaction for 6 h. Finally, the entirely washed tissue sections were placed into a vacuum desiccator and preparing for LDI-MSI analysis.

Human glioma tissue was obtained during surgery. All experiments were performed in accordance with the Measures for the Ethical Review of Biomedical Research Involving Human Subjects issued by the National Health and Family Planning Commission of The People's Republic of China, and approved by the Medical Ethics Committee of Beijing Tiantan Hospital Affiliated to Capital Medical University.

Informed consents were obtained from human participants of this study.

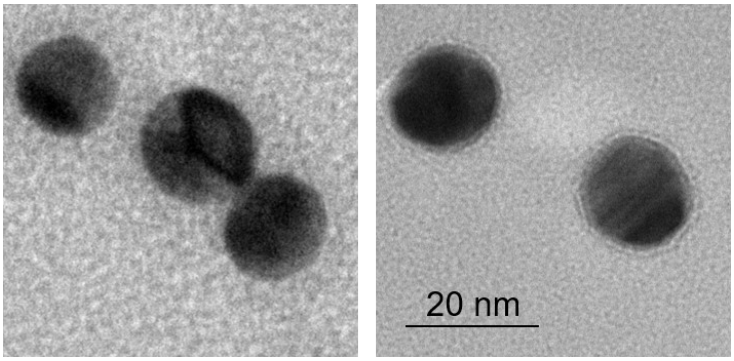


Figure S1. TEM images of bare AuNPs (left) and MTs/N_3@AuNPs (right).

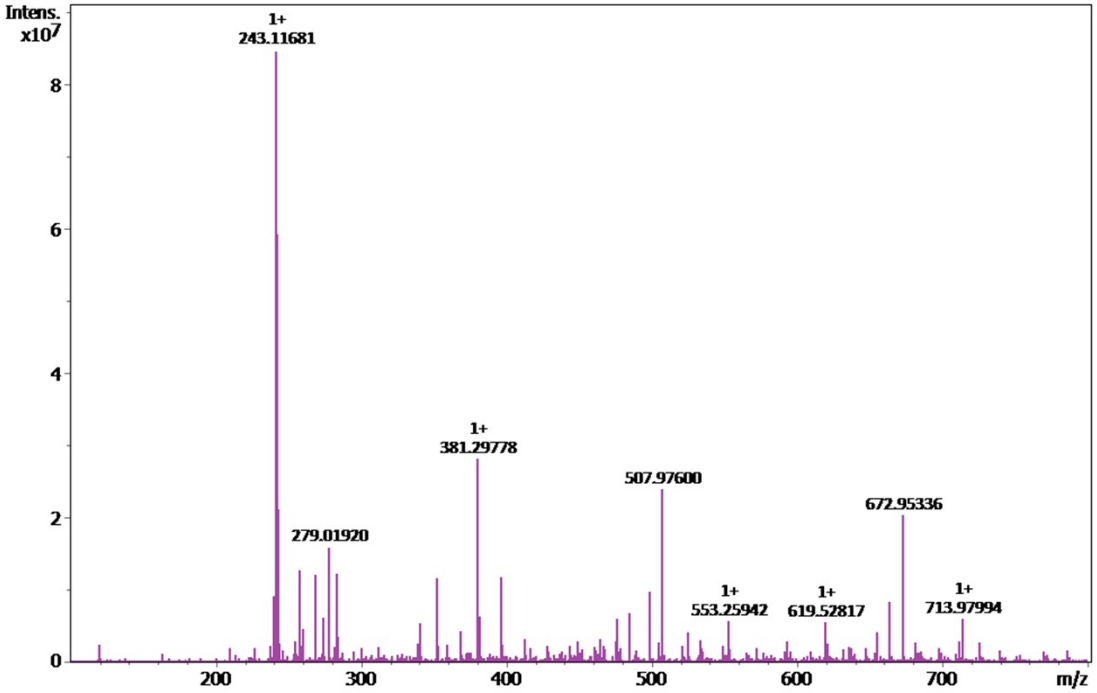


Figure S2. Accurate mass measurement of MTs/N_3@AuNPs using LDI-FTICR MS. *m/z* 243.1168 represented the triphenylmethyl cation released from MTs. Increasing the laser energy can obtain the signal of Au, but it will cause mass drift.

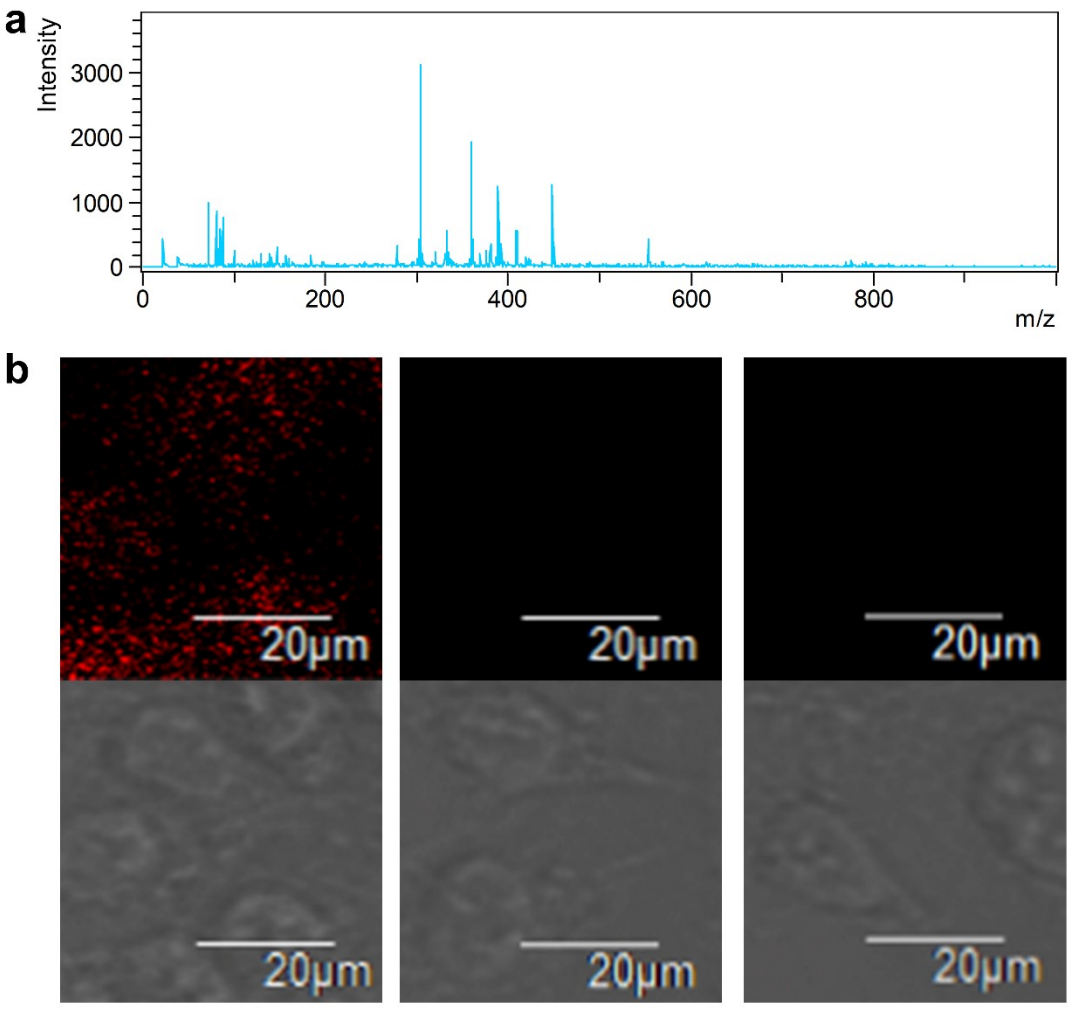


Figure S3. (a) MS spectrum of the BAP-MTs/N_3@AuNPs strategy for protein lipoylation analysis by LDI-MS with BAP incubated for 5 min. (b) CLSM imaging of protein lipoylation in cells labeled by Cy5-azide with BAP incubated for 12 h (left), 5 min (medium) and without BAP added (right).

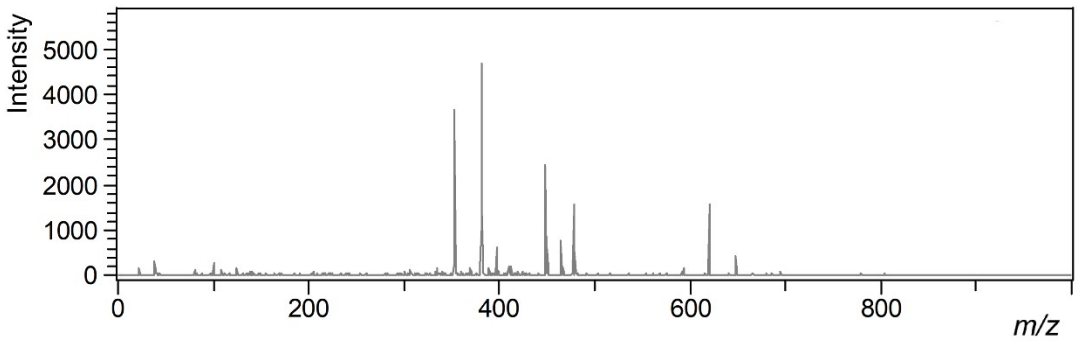


Figure S4. MS spectrum of the BAP-MTs/N_3@AuNPs strategy for protein lipoylation analysis by LDI-MS with BAP preincubated with LA.

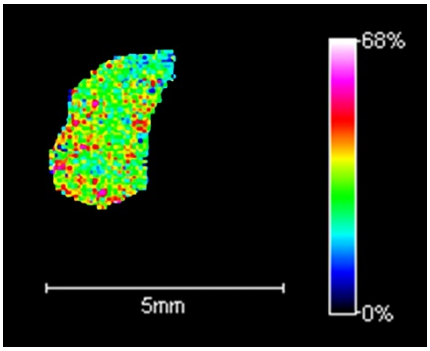


Figure S5. MS image of protein lipoylation in human brain glioma tissue.

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