

Supporting Information

**Reaction and Detection Click in High-voltage Assisted Laser
Desorption Ionization Mass Spectrometry**

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Contents

1 Experimental Details.....	2
1.1 Chemicals and reagents.....	2
1.2 Off-line click reactions	2
1.3 ESI-MS/MS	3
1.4 HALDI-MS/MS.....	3
1.5 HPLC-UV	4
1.6 NMR	4
2 Results and Discussion	5
2.1 Other similar examples of click reactions successfully done in HALDI source	5
2.2 Control experiments on the bulk copper plate	7
2.3 Factors influential to the yield of the in-source reaction.....	8
2.4 In-source click reactions on Fe and Si surface, respectively.....	10
2.5 Compound characterization data.....	12
3 References	12

1 Experimental Details

1.1 Chemicals and Reagents

HPLC-grade methanol and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA, USA). HPLC-grade dichloromethane was purchased from Kemiou Chemical Reagent Company (Tianjin, China). AP-grade chloroform was purchased from Beijing Chemical Works (Beijing, China). Pure water was purchased from Wahaha Company (Hangzhou, Zhejiang, China). 4-ethynyl-N,N-dimethylaniline, phenylacetylene, benzyl azide solution (0.5 M in dichloromethane), 1-(azidomethyl)-4-chlorobenzene solution (0.5 M in dichloromethane), Copper (I) acetate (CuOAc) were obtained from Sigma Aldrich (St. Louis, MO, USA), which were of AP-grade or higher. Ethylenediaminetetraacetic acid (EDTA) was obtained from Sinopharm Chemical Reagent Beijing Co. LTD (Beijing, China). All chemicals were used as received without further purification.

The gold and the silicon sample plates, respectively, were obtained from department of microelectronics in Peking University (Beijing, China). The gold plate was fabricated by sputtering a layer of gold (1600 Å) on a silicon substrate. These two kinds of plates (Si and Au) were ultrasonicated in 1:1 methanol/water before use. The iron and copper plates, respectively, were purchased from Alfa Aesar. Before use, these two kinds of plates (Fe and Cu) were first polished with abrasive paper (grit 240–360) and then ultra-sonicated in 1:1 methanol/water for several minutes.

1.2 Off-line click reactions

These syntheses were done according to what Shao *et al.* had described earlier.¹ Briefly, 0.5 M azide and alkyne, respectively, were dissolved in dichloromethane solution, and then 10 % eq. copper (I) acetate (CuOAc) was added as catalyst. The reaction was kept going on for 48 h at room temperature, and the coupling product was then precipitated spontaneously. The precipitate was dissolved in chloroform and washed by saturated EDTA aqueous solution to remove

remaining CuOAc catalyst. The washed organic layer was then put under reduced pressure to remove solvent. After that, the product was re-dissolved in 1:1 MeOH/water (*ca.* 10^{-5} M) and analyzed by ESI-MS/MS to get its characteristic fragments. By comparing the unique fragmentation patterns of the off-line reaction products to those of the in-source reaction products (via HALDI-MS/MS), one could identify the in-source reaction products were the corresponding 1,2,3-triazoles.

1.3 ESI-MS/MS

ESI-MS/MS was done using a Thermo Finnigan LCQ Advantage MAX ion trap mass spectrometer (San Jose, CA, USA). The main experimental parameters used were as follows: spray voltage 5.5 kV, capillary voltage 4.0 V, tube lens offset -50.0V, heated capillary temperature 250°C, and nebulizing gas (N₂) pressure 0.4 mPa. In MS/MS mode, the isolation width was set at 5 Th. All mass spectra were acquired in positive mode with 3 microscans and were recorded by the instrument software (Xcalibur version 1.4 SR1).

1.4 HALDI-MS/MS

The HALDI source has previously been described in detail.² The sample plate mounted to an XYZ translation stage was placed below the extended ion transfer tube, and a high voltage of typical 5.5 kV was applied to the sample target. Irradiation from a pulsed Nd:YAG laser (Lai Yin Opto-Electronics Technology, Beijing, China) was focused onto the sample droplet by a set of lens mounted on a linear translation stage for easy alignment. The incident angle of the laser beam onto the sample target was fixed at 45°. The laser was operated at a frequency of 20 Hz with a wavelength of 1064 nm. The typical laser energy was *ca.* 2.2 mJ/pulse with a pulse width of 10 ns. The horizontal distance (*d*) and vertical distance (*l*) between the mass spectrometer inlet and the illuminated spot were 3 mm and 10 mm, respectively.

Typically, 200 μ L 5 mM **1** and 5 mM **2** (The chemical structure of **1** and **2** could be seen in

Scheme 1) reaction solution (v/v, 1:1 MeOH/H₂O) was deposited on the substrate. Both the biased high voltage (typically of 5.5 kV) and the laser should be turned on to get corresponding MS and tandem MS signals.

1.5 HPLC-UV

An Agilent eclipse XDB-C18 column (particle size 3.5 μ m, 2.1 x 150 mm) was used on an Agilent 1100 series high performance liquid chromatography (HPLC) equipment (Agilent Technology, CA, USA) with a UV detector. The retention time (1.56 min) of the product **3** (see Scheme 1) was measured by the corresponding click reaction product synthesized according to section 1.2, the chemical structure of which was confirmed by NMR data (see section 2.6 for details). 65 % acetonitrile and 35 % water were used as the eluent (with a flow rate of 2 mL/min), and the UV absorption was detected at 260 nm. The injection volume of each sample was 10 μ L. Each detection was done for at least three times. The HPLC-UV detection of the in-source reaction solution was done in less than 1 min after the laser was off. Considering the retention time of **3** in column was 1.56 min, the reaction still went on for less than 2.56 min (less than 1 min for transference from the HALDI ion source to the HPLC inlet and 1.56 min for HPLC-UV analysis).

1.6 NMR

¹H-NMR data was measured by an AVANCE III Bruker-500 MHz NMR spectrometer (Bruker, Switzerland). Chemical shifts were measured relative to residual solvent peaks as internal standards set to δ 7.26 (CDCl₃).

2 Results and Discussion

2.1 Other Similar Examples of Click Reactions Successfully Done in HALDI Source

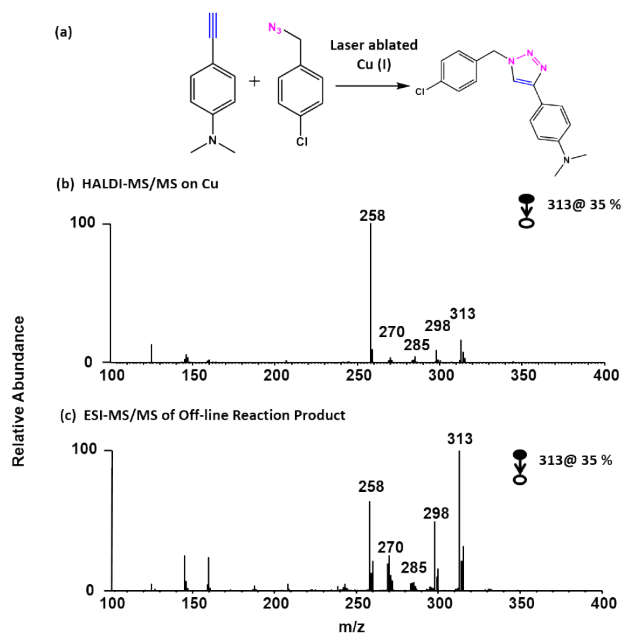


Fig. S1 (a) Click reaction of 4-ethynyl-*N,N*-dimethylaniline and 1-(azidomethyl)-4-chlorobenzene was successfully done in HALDI source. (b) 200 μ L 5 mM 4-ethynyl-*N,N*-dimethylaniline and 5 mM 1-(azidomethyl)-4-chlorobenzene (in v/v 1:1 MeOH/H₂O), respectively, was deposited on copper substrate. HALDI-MS/MS experiment was done upon it and the characteristic fragments were clearly shown here (when the reaction went on for 5 min). (c) Off-line click reaction of these two reactants was done according to section 1.2. ESI-MS/MS analysis of the product showed identical fragments compared with (b), confirming the product in (b) was the same as that in (c). This result showed the success of the in-source click reaction.

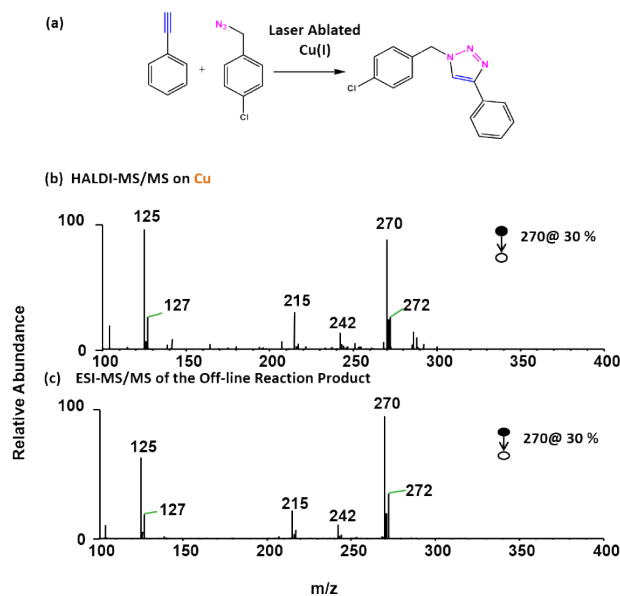


Fig. S2 (a) Click reaction of phenylacetylene and 1-(azidomethyl)-4-chlorobenzene was successfully done in HALDI source. (b) 200 μ L 5 mM phenylacetylene and 5 mM 1-(azidomethyl)-4-chlorobenzene (in v/v 1:1 MeOH/H₂O), respectively, was deposited on copper substrate. HALDI-MS/MS experiment was done upon it and the characteristic fragments were clearly shown here (when the reaction went on for 5 min). (c) Off-line click reaction of these two reactants was done according to section 1.2. ESI-MS/MS analysis of the product showed identical fragments compared with (b), confirming the product in (b) was the same as that in (c). This result showed the success of the in-source click reaction.

2.2 Control Experiments on the Bulk Copper Plate

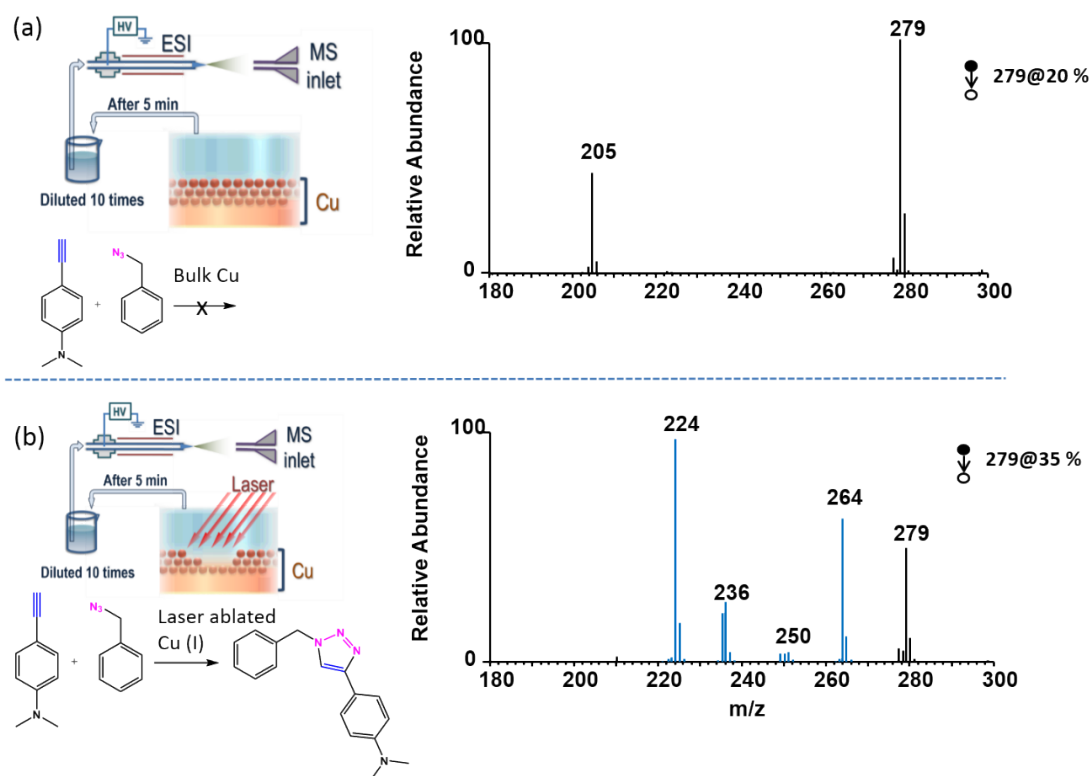


Fig. S3 Control experiments showing the bulk copper plate is not catalytically active, if not irradiated by the laser. (a) 200 μ L reaction solution of 5 mM **1** and 5 mM **2** (respectively, in v/v 1:1 MeOH/H₂O) was deposited on a copper plate for 5 min, then diluted ten times by v/v 1:1 MeOH/H₂O, and analyzed by ESI-MS/MS. No characteristic fragments (as shown in Fig. 1(a) in the paper) could be seen in the spectrum. The m/z 279 in the background might be attributed to remaining plasticizer (dibutyl phthalate) in ambient environment, as described by Li *et al.*³ previously. (b) the same solution as in (a) was deposited on a copper plate, with laser irradiation on for 5 min, then diluted ten times by v/v 1:1 MeOH/H₂O, and analyzed by ESI-MS/MS. Characteristic fragments (in light blue) of **3** were clearly seen. These experiments show bulk copper substrate used here has extremely low catalytic activity compared with the laser ablated Cu (I) ions.

2.3 Factors Influential to the Yield of the In-source Reaction

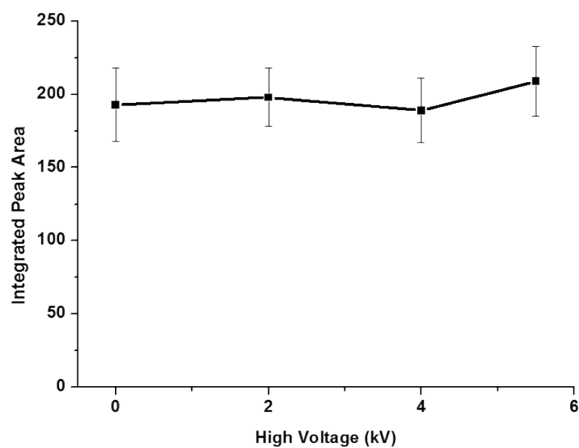


Fig. S4 The influence of biased voltage on copper plate on the amount of **3** generated from the in-source click reaction detected by HPLC-UV (see section 1.5 for details). All the experiments were done in HALDI ion source similar to what has been described in section 1.4, with laser energy of 2.2 mJ/pulse and reaction time of 5 min. The data shows the biased high voltage has no significant influence on the reaction.

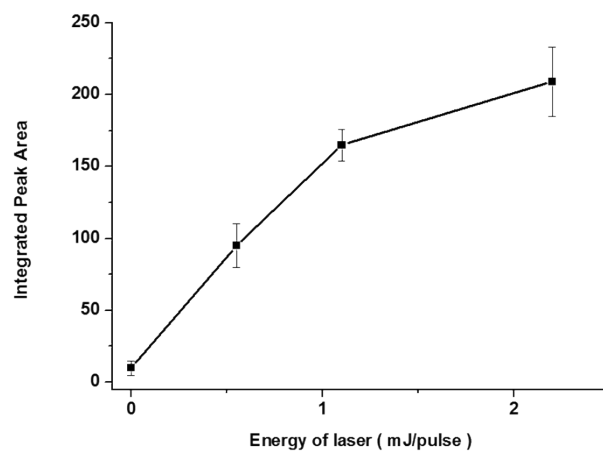


Fig. S5 The influence of laser energy on the amount of the product 3 generated from the in-source click reaction on copper substrate detected by HPLC-UV (see section 1.5 for details). All the experiments were done in HALDI ion source similar to what has been described in section 1.4, with biased voltage of 5.5 kV and reaction time of 5 min. The data shows the laser energy has a significant influence on the yield of the reaction.

2.4 In-source Click Reactions on Fe and Si Surface, respectively.

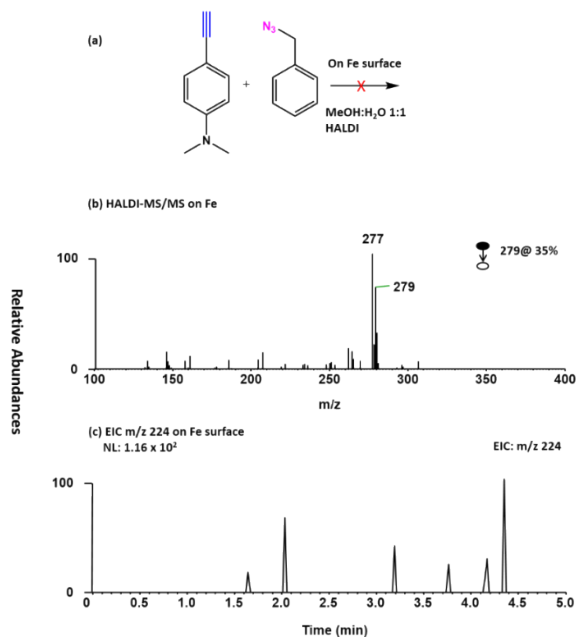


Fig. S6 HALDI-MS/MS experiment on Fe plate, to confirm the laser-irradiation alone was not the reason for the success of the in-source click reaction. (a) Reaction formula showing the reactants and the product, as well as the reaction conditions. (b) 200 μ L 5 mM1 and 5mM2 (respectively, in v/v 1:1 MeOH/H₂O solution) was deposited on an iron plate. HALDI-MS/MS experiment was done upon it, and no characteristic fragments (compared with fig. 1(a)) could be seen in the spectra (especially the most abundant one: m/z 224), showing no product was formed in the process. (c) EIC (Extracted ion chromatogram) in (b) of m/z 224, which was the most abundant fragment of **3**, was shown. The random appearance of m/z 224 and its signal intensity of noise level (NL: 1.16 x 10²) demonstrated that no product was formed.

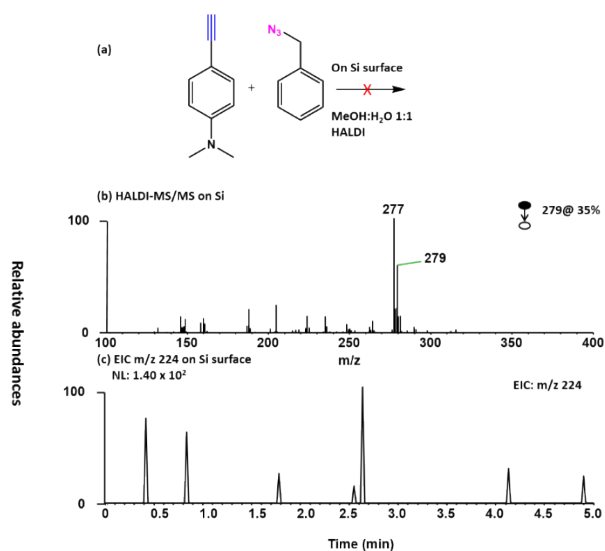
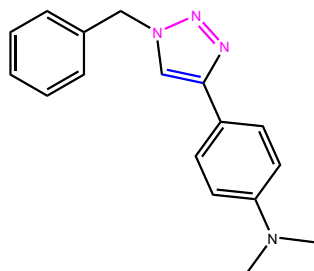


Fig. S7 HALDI-MS/MS experiment on Si plate, to confirm the laser-irradiation alone was not the reason for the success of the in-source click reaction. (a) Reaction formula showing the reactants, as well as the reaction conditions. (b) 200 μ L 5 mM1 and 5 mM2 (respectively, in v/v 1:1 MeOH/H₂O solution) was deposited on a silicon plate. HALDI-MS/MS experiment was done upon it, and no characteristic fragments (compared with fig. 1 (a)) could be seen in the spectra (especially the most abundant one: m/z 224), showing no product was formed in the process. (c) EIC (Extracted ion chromatogram) in (b) of m/z 224, which was the most abundant fragment of **3**, was shown. The random appearance of m/z 224 and its signal intensity of noise level (NL: 1.40 x 10²) demonstrated that no product was formed.

2.5 Compound characterization data



$^1\text{H-NMR}$ (in CDCl_3): $\delta_{\text{ppm}} = 2.97$ (s, 6H, $2\text{CH}_3\text{-N}$), 5.48 (s, 2H, CH_2), 6.73 (d, $J = 8.5$ Hz, 2H), $7.35\text{-}7.39$ (m, 5H), 7.52 (s, 1H, $\text{CH}_{\text{triazole}}$), 7.65 (d, $J = 8.5$ Hz, 2H), which is consistent with ref. 4.

3 References

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