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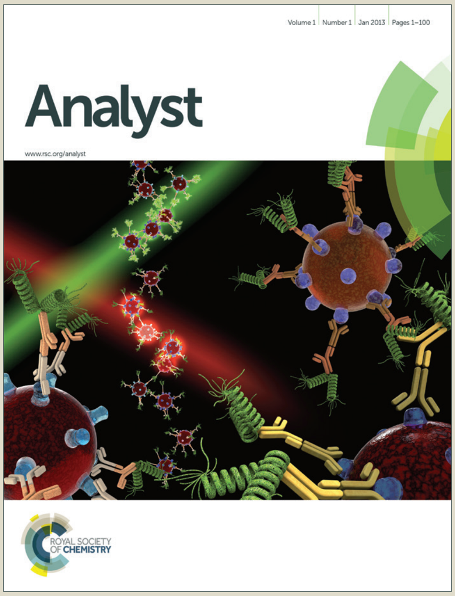

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Reaction and Detection Click in High-voltage Assisted Laser Desorption Ionization Mass Spectrometry

Ran Qiu, Hai Luo*

Beijing National Laboratory for Molecular Sciences, College of Chemistry
and Molecular Engineering, Peking University, Beijing, 100871, China.
E-mail: hluo@pku.edu.cn Tel: +86-10-62751499 Fax: +86-10-62751708

Running Title: Reaction and Detection Click in HALDI-MS

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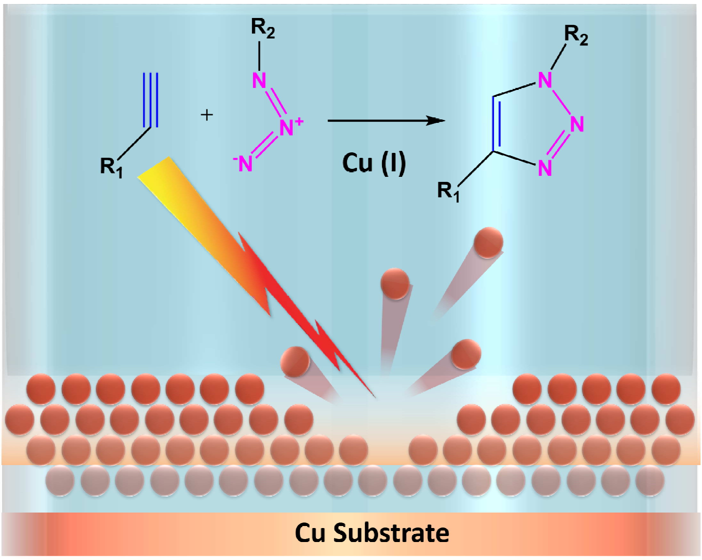
Click by Laser@Cu in HALDI-MS: Click reaction (copper-catalyzed azide-alkyne cycloaddtion) catalyzed by "naked" copper ions (without ligand) *in situ* generated from copper substrate by laser ablation in high-voltage assisted laser desorption/ionization (HALDI) ion source is demonstrated.



Ambient ion sources [1] of mass spectrometry (MS) have been treated as a special form of reaction vessels. Several recent papers [2] demonstrated that chemistry in ambient ion sources could be much different from that in traditional conditions, due to the unique chemical/physical environment within. Significant reaction rate enhancement was shown in a wide range of reactions done in electro-spray ionization (ESI) based ambient ion sources, which was attributed to the chemical effects induced by desolvation.[2] Chemically inert covalent bonds (C-H and C-C) were activated in ambient environment by the high electric field in desorption electro-spray ionization (DESI) [3] and paper-spray ion source,[4] respectively. Heterogeneous birch reduction was observed in low-temperature plasma (LTP) ion source.[5] However, the novel chemistry induced by laser in several laser desorption (LD) based ambient ion sources has seldom been studied. Laser ablation on metal substrate can generate metal ions (plasmas), clusters and nano-materials.[6] Our previous work has shown that in the laser desorption spray post-ionization-MS (LDSPI-MS) analysis of proteins on copper substrate, the laser could ablate the copper substrate to *in situ* generate “naked” copper ions (without ligand), with which proteins could form corresponding copper (I)-protein complexes.[7] In this paper, we will demonstrate that these “naked” copper ions can successfully catalyze click reaction[8] (copper-catalyzed azide-alkyne cycloaddition) in ambient environment. The novel way to *in situ* generate and apply this new catalyst may largely expand the application ranges of ambient mass spectrometry and be conducive to the future development of organometallic catalysis.

High-voltage assisted laser desorption/ionization (HALDI)[9] is a new LD based ambient ionization technique, which combines desorption and ionization into one step, thus reducing the dilution effect in the LDSPI process. The HALDI-MS/MS experiments were done as shown in scheme 1. 200 μL reaction solution (5 mM **1** and 5 mM **2**, respectively. Sol.: v/v, 1:1 MeOH/H_2O) was deposited on the copper substrate, on which high voltage of 5.5 kV was biased. Once the laser was on, charged microdroplets were desorbed and then underwent subsequent desolvation and ionization processes similar to that of ESI, generating ions of the analyte(s). By comparing the characteristic MS/MS fragments, one could unambiguously identify the reaction product.

Fig. 1 (b) shows the HALDI-MS/MS spectrum of the product *in situ* formed in the in-source reaction when the laser irradiation was on for 5 min. By comparing the characteristic fragmentation pathways in fig. 1 (b) to that of the off-line click reaction product synthesized according to Shao *et al.*,[10] whose ESI-MS/MS spectrum is shown in fig. 1 (a), we can confirm that the product of the click reaction is successfully detected (identical fragmentation pathways indicated the same product). More similar examples of the in-source click reaction are shown in fig. S1-S2 in the supporting information.

There are three possible factors that may have affected the in-source click reaction: the copper substrate, the laser and the biased high voltage. It is likely that the bulk copper substrate might be catalytically active in this experiment, because Sharpless and coworkers have used copper turnings to catalyze this reaction.[11] However, control experiments shown in fig. S3 clearly demonstrated that the copper substrate used here had extremely low catalytic ability without laser irradiation during the time under study (5 min). The influence of laser energy and biased high voltage, respectively, on the amount of the product is shown in fig. S4-S5, which demonstrates that the amount of the product is closely related to the laser energy and insensitive to the biased high voltage. Based on these results, it is clear that the laser plays an important role here in the success of this catalytic reaction.

Since laser ablation was indispensable to the success of the in-source click reaction, there were two possibilities: 1) laser irradiation alone induced this reaction, or 2) the reaction was catalyzed by laser ablated metal ions. Some authors claimed that this reaction could proceed slowly in high temperature.[12] To confirm this reaction was not accomplished by heating effect of the laser but by *in situ* generated copper ions, we performed control experiments on gold substrate. Clearly shown in fig. 1(c), there are no characteristic fragments of **3** in the MS/MS spectrum of *m/z* 279 if the HALDI substrate is gold. By comparing the different results on Cu and Au substrate, one can come to the conclusion that the laser alone does not induce this reaction and the copper substrate is indispensable to yield the product **3**. Considering the previous experimental data in our lab[7] and the widely accepted [13] and newly re-affirmed [14] mechanism of click reaction, we propose that it is the laser ablated Cu (I) ions that have catalyzed this reaction. HALDI-MS/MS spectra corresponding to the same reaction on Fe and Si substrates, respectively, also without the target product due to the lack of *in situ* generated copper catalyst, are shown in fig. S6-S7. These two examples again confirm that it is the laser ablated copper catalysts that really "click" this reaction.

A closer look at the extracted ion chromatogram (EIC) of the HALDI-MS/MS spectra further testifies our conclusion ("click" by "naked" Cu(I) ions). Fig. 1 (d) shows the extracted ion chromatogram (EIC) of m/z 224 in the afore-mentioned HALDI-MS/MS experiment on copper substrate. The m/z 224 is the most abundant MS/MS fragment of product **3**, so it can be assumed that the continuous increment of the signal intensity of this peak in fig. 1 (d) indicates that more and more products are forming in the reaction solution. While the extracted ion chromatogram (EIC) of m/z 224 in the afore-mentioned HALDI-MS/MS experiment on gold substrate is shown in fig. 1 (e). Random appearance of m/z 224 and its signal intensity of noise level (NL: 8.29×10^2), suggest that almost no product is formed under this experimental condition (similar results on Fe and Si substrate are shown in fig. S6-S7). These results are consistent with our proposal that it is the laser ablated Cu (I) ions that have catalyzed this in-source click reaction.

Ambient mass spectrometry has evolved into the science and technology of ions in the open air. In this paper, the first example of click reaction catalyzed by cuprous ion *in situ* generated by laser ablation in ambient environment is demonstrated. As a new way to generate new molecules *in situ*, the presented method has an influence not only in analytical chemistry, but also offer a potentially useful strategy to synthetic chemistry, *viz.* adding "naked" metal ions to initiate reactions. When using LD based ambient ionization MS to do analysis of target molecules, on the other hand, to eliminate the interfering signals of the corresponding reaction products formed *in situ*, the choice of proper substrate (chemically inert with regard to the analyte) is needed.

Keywords

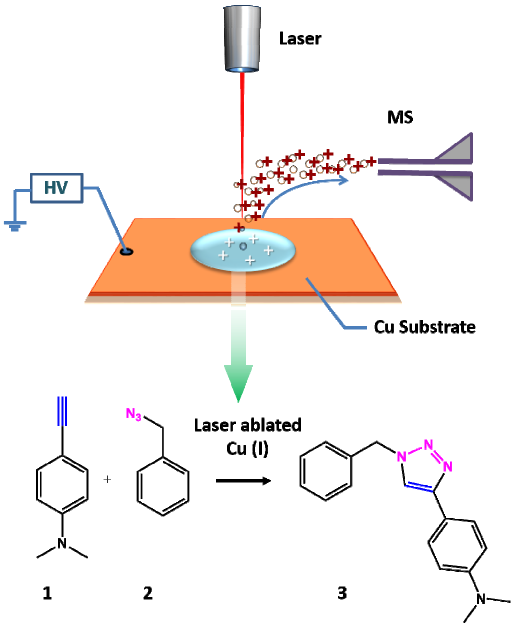
ambient ionization, click chemistry, mass spectrometry, laser ablation.

Acknowledgements

We thank Yanlong Jiang, Xinxin Ren, Chengsen Zhang for discussions and help on the work. We acknowledge the financial support from the National Natural Science Foundation of China (Nos.

20727002 and 21075005).

Electronic Supplementary Information available: [experimental details, other similar examples of click reactions, control experimental results, fig. S1-S7]. See DOI: 10.1039/c000000x/



Scheme 1 Click reaction matches with product identification by laser ablation in HALDI-MS

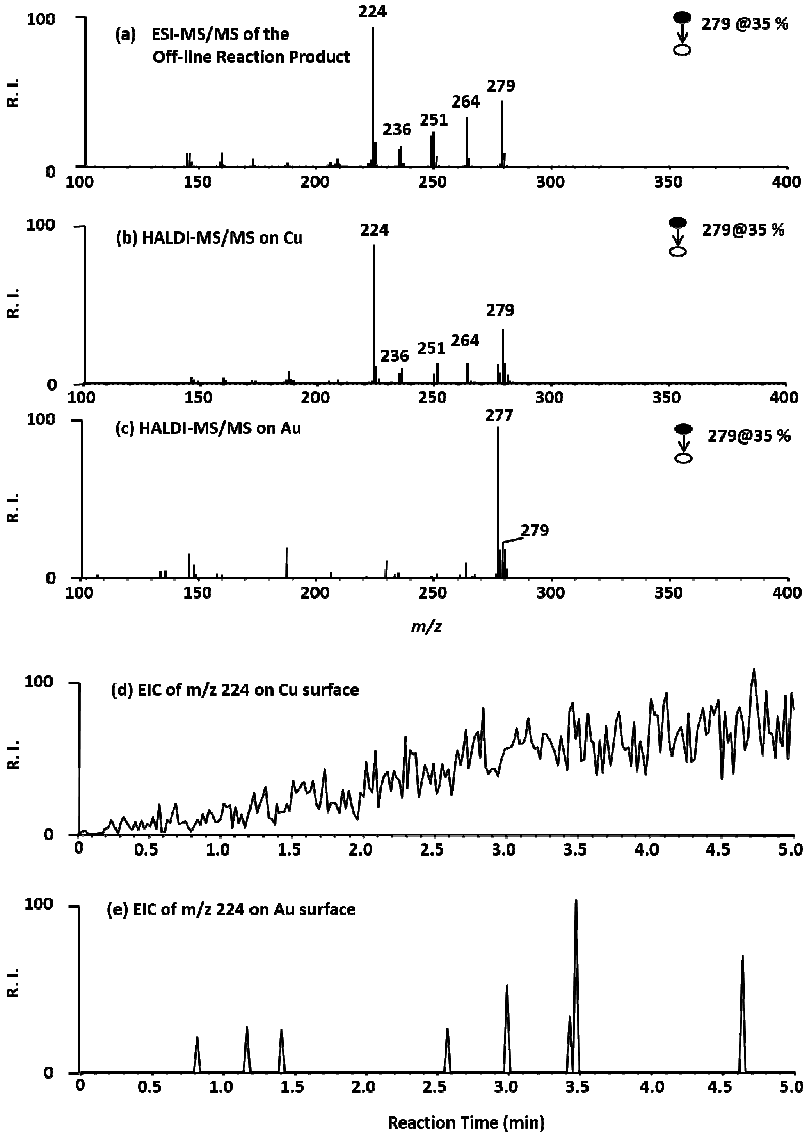


Fig.1 (a) ESI-MS/MS spectrum of the off-line product **3** of the click reaction shown in **Scheme 1**. The product was synthesized according to ref. 10. HALDI-MS/MS spectra of the in-source reaction solution on (b) Cu surface and (c) Au surface. Extracted ion chromatogram (EIC) of the most abundant fragment m/z 224 of **3** in the HALDI-MS/MS experiment on (d) Cu surface and (e) Au surface.

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