



To  
Dr. Bing Wu  
Review Editor  
JoVE

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**Resubmission of our manuscript entitled “Analysis of complex molecules and their reactions on surfaces by means of cluster-induced desorption/ionization mass spectrometry”**

Dear Dr. Wu,

Thank you and the reviewers for the overall very positive response and helpful comments on our manuscript entitled “Analysis of complex molecules and their reactions on surfaces by means of cluster-induced desorption/ionization mass spectrometry”.

We addressed all issues raised by the reviewers as listed in detail below (point by point). We also addressed all editorial comments. All changes are highlighted in cyan in the resubmitted manuscript. With these changes, we hope that the manuscript is now suitable for further processing and publication in *JoVE*.

Yours sincerely

Michael Dürr (on behalf of all co-authors)

P.S.: We also updated the ppt-file with material available for production of the video article.

## Response to the reviewers' reports / list of changes:

Reviewer #1:

**Comment:** Page 2, Line 117-121: The explanations of these sentences seem to be ambiguous. How low is the SO<sub>2</sub> cluster induced desorption? The authors should describe these points clearly.

Answer: We now explicitly give the missing information.

**Changes:** Starting line 124, we write:

"With the current set-up, typical desorption rates of cluster-induced desorption of biomolecules are of the order  $10^{-3}$  nm/s. A high depth resolution in the range of 1 to 2 nm has been observed for mixed lipid/peptide samples<sup>17</sup>."

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**Comment:** Page 8, Line 334-341: In Fig. 5(E), the simulated curves of the "Sim k<sub>1</sub>" and "Sim k<sub>1</sub>, k<sub>2</sub>, k<sub>3</sub>" are shown, but the explanations for the rate constants "k" are not enough. For example, "k" is not described both in the text and the figure caption. The authors should carefully explain "k" in the text or figure caption. In addition, what is the reason why at least three rate constants are needed, or provide evidence that the two rate constants are not enough?

Answer: We explain k<sub>i</sub> and the reason why three different values are necessary.

**Changes:** Starting line 330, we write:

"Inspection of the experimental results (symbols) reveals three different regimes: a fast increase of  $\bar{d}$  operative for  $t < 50$  s, an intermediate regime for  $50 \text{ s} < t < 200$  s, and a slow but almost continuous increase for  $t > 200$  s. The experimental results were simulated by means of Monte Carlo simulations; pseudo-first-order reaction kinetics with reaction constants  $k_i$  were assumed for the H/D exchange at the functional groups of the molecules investigated<sup>22</sup>. A good agreement between simulations and experimental results in all three regimes was only obtained when at least three different rate constants  $k_i$  for the H/D exchange in angiotensin II molecules were applied."

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**Comment:** Page 11, Line 441-442: The explanations of these sentences seem to be ambiguous. How do you use trifluoro acid (TFA)? Is TFA added to the SO<sub>2</sub> cluster? Or is it mixed with the sample as a matrix?

Answer: We clarified this statement.

**Changes:** Starting line 440, we write:

"The samples then may be treated by ionizing agents such as trifluoro acid, e.g., by exposure of the sample to the vapor pressure of the ionizing agent."

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**Comment:** Figure 6 (B): What is the difference between open red circles and filled red circles? The authors should better explain it in the figure caption.

Answer: We added the information in the figure caption of Fig. 6.

**Changes:** Starting line 418, we write:

“...as obtained from two independent sets of data (filled and open symbols).”

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Reviewer #2:

**Comment:** Authors claimed that the protonation is due to sulfuric acid forming from SO<sub>2</sub> and H<sub>2</sub>O. How are the desorption and ionization take place? They have do more efforts to give a clear mechanism. Negative mechanism is also missed.

Answer: We explain in more detail and more precisely the respective mechanisms.

**Changes:** Starting line 90, we write about the desorption:

“When the clusters impact on the sample surface, they first provide the energy necessary for desorption. In addition, due to the high dipole moment of SO<sub>2</sub>, they also serve as a transient matrix in which the desorbing molecule is dissolved during the desorption process<sup>11,18,19</sup> (Figs. 1 and 2).”

Starting line 99, we write about the ionization:

“For biomolecules, ionization typically takes place via uptake or abstraction of a proton at a basic or acidic functional group, respectively<sup>11</sup>. If water molecules are present in the sample, SO<sub>2</sub> molecules from the cluster can react with these water molecules forming sulfuric acid<sup>18</sup>. The latter can act as an efficient proton source which further promotes the ionization process in case of ionization via proton uptake (positive ion mode)<sup>13,18</sup>.”

In line 385, we added in the figure caption of figure 3:

“The positive ion signal is more intense due to the influence of the SO<sub>2</sub> clusters<sup>18</sup>.”

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**Comment:** Schematic representation for the method is required to be easy for following.

Answer: We improved the schematic representation of our method and set-up in Fig. 1. We added information in the figure caption of Fig. 1.

**Changes:** Starting line 359, we write:

“Cluster-induced desorption/ionization is performed in a high-vacuum vessel. A beam of SO<sub>2</sub> clusters (yellow dots) is produced via supersonic expansion of a SO<sub>2</sub>/He gas mixture from a pulsed nozzle. During cluster-surface impact, surface molecules are desorbed and ionized. Molecular ions (red/orange dots) are transferred via a biased grid, a dual ion funnel inlet, and octopolar ion guides into the ion trap for mass spectrometry.”

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**Comment:** *Molecular dynamics simulations of cluster-induced desorption is not working.*

Answer: The animation of the MD simulations works in the ppt-file we provided in addition to the main manuscript. It does not work when the file has been converted, e.g., to pdf. It will for sure work in the professionally produced video article in JoVE.

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**Comment:** *Authors discussed SIMS, desorption electrospray ionization (DESI) in introduction. They have to include also other methods such as MALDI-MS (TrAC Trends in Analytical Chemistry 2017, 89, 68-98), SALDI-MS (Microchimica Acta 2018, 185 (3), 200), matrix assisted ionization vacuum (MAIV) (Anal. Chem., 85 (2013), pp. 2005-2009), Anal. Chem., 86 (2014), pp. 6208-6213).*

Answer: We compare DINEC with MALDI and related techniques in the introduction of our article. We added two references.

**Changes:** Starting line 108, we write:

“DINEC can be applied to any sort of solid sample which is compatible with high-vacuum conditions. No special sample preparation is required, in particular no matrix has to be applied prior to DINEC-MS measurements, in contrast to matrix-assisted laser desorption/ionization (MALDI) mass spectrometry and related techniques<sup>20,21</sup>.”

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**Comment:** *Positive mode and negative mode should used instead of cation and anions in Figure 3.*

Answer: We changed the figure and figure caption accordingly.

**Changes:** Starting line 379, we write:

“Mass spectra (top panel: positive ion mode, bottom panel: negative ion mode) as obtained after cluster-induced desorption/ionization from an angiotensin II sample.”

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**Comment:** *Language should be revised carefully. Several sentences are very long for 3-5 lines. Authors should make the sentence shorter.*

Answer: We shortened sentences longer than 3 lines.

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**Comment:** *Minor Concerns: There are several typos.*

Answer: We carefully proofread the article.

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Editorial comments:

**Comment:** *Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."*

Answer: If applicable, we introduced the respective sentence in the figure captions. The submission is accompanied by the files Copyright\_Fig2.pdf and Copyright\_Figs5+6.pdf in which the respective sentences are labelled yellow.

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**Comment:** *Unfortunately, there are a few sections of the manuscript that show significant overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Please rewrite lines 62-94, 322-326, 335-338.*

Answer: We rewrote the respective lines (62-96, 314-321, and 329-333 in the revised manuscript).

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**Comment:** *Please use superscript arabic numerals to cite references in text. Please remove the brackets before and after the reference number.*

Answer: We changed the style to cite references in the text.

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**Comment:** *Step 2.2.1: Please write this step in the imperative tense.*

Answer: This step is now written in imperative tense.

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***Comment:*** *Please do not abbreviate journal titles for references.*

Answer: Full journal titles are now given.