Supporting Information

Location of carbon-carbon double bonds in unsaturated lipids using microdroplet mass spectrometry

Kai Luo[1], Hao Chen[2], Richard N. Zare[1*]

[1]Department of Chemistry, Fudan University, Jiangwan Campus, Shanghai 200438, China

[2] Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ 07102, USA

*Correspondence should be addressed to Richard N. Zare; Email: rnz@fudan.edu.cn

Keywords: unsaturated compounds, fatty acids, microdroplets, chloroauric acid

1. Experimental

1.1 Variation of microdroplet reaction products with flight distance

In the IA-$HAuCl_4$ microdroplet mass spectrometry, some new species with *m/z* values of 199.1, 185.1, 129.0, and 115.0 appear with a relative abundance of 39%, 18%, 44%, and 8% of the IA signal in the mass spectrum, companied with the relative abundance of 18% and 8% for $[IA+O-H]^-$ and $[IA+2O-H]^-$ in the mass spectrum when the flight distance when the flight distance is increased from 10 to 20 mm (**Figure S1**). These new species with *m/z* of 185.1169 (theoretical mass = 185.1178, mass error = 4.86 ppm), 129.0548 (theoretical mass = 129.0552, mass error = 3.09 ppm), and 115.0388 (theoretical mass = 115.0395, mass error = 6.04 ppm) have an integer multiple difference of 14.0123 to the diagnostic ion of 199.1327 (theoretical mass = 199.1334, mass error = 3.51 ppm). Especially, there are two other species with an *m/z* of 145.0862 (theoretical mass = 145.0870, mass error = 5.51 ppm) and 231.1249 (theoretical mass = 231.1238, mass error = 4.75 ppm) when magnified 5 times in the mass spectrum. These are intermediates produced by ROS in the $HAuCl_4$ microdroplet reaction.

Interestingly, we noticed that the flight distance will affect the new species produced in the mass spectrum. The different flight distances from 5 mm to 40 mm were checked to further verify this behavior. The detailed results about the different reaction time (the microdroplet flight speed of 84 m/s)[1] are presented in **Figure S2**, which are all relative abundances compared to the IA signal. The mass spectrum revealed a diagnostic ion at 199.1 corresponding to $[IA+O-H]^-$ with a reaction time of 0.06 ms. New species at 199.1, 185.1, 129.1, and 115.1 were found in the mass spectrum, accompanied with $[IA+2O-H]^-$ for a reaction time of 0.12 ms. These results may indicate a different reaction mechanism reflected in the reaction time[2], and two different mechanisms are presented in **Figure S3.**

For the flight distance of 20 mm, some new species were found in the mass spectrum (**Figure S4**), having *m/z* values of 397.3 and 381.3. These correspond to species having an added oxygen atom $[NA+2O-H]^-$ and $[NA+O-H]^-$, the value of 287.2 and 173.1 as the intermediate species, 255.2, 241.2, 157.1 and 143.1 as the reaction species, respectively. All of these species with mass errors less than 4.3 ppm fit the rules of integer multiple difference of 14.0123 to the diagnostic ion of 255.2029. Compared with the result of IA, NA (24:1(15Z)) has the same ROS reaction mechanism (**Figure S5**) in the $HAuCl_4$ microdroplet reaction. The corresponding curve for the different species mediated by the reaction time are shown in **Figure S6**.

1.2 Possible mechanism analysis for the new microdroplet reaction of monounsaturated fatty acids

An analysis was made of the species appearing in the microdroplet mass spectrum obtained from unsaturated fatty acids using $HAuCl_4$ as the oxidizing agent. Some species fit the rule of $n(CH_2)$ with a single C=C bond, like IA and NA; and some with the rule of $n(CH_2)$-2H with two C=C bonds, like RA. The species losing CH_2 revealed that there is a new reaction for the $HAuCl_4$ as the oxidizing agent in the microdroplet reaction, which promotes generous amounts of ROS produced at the air-water interface. The possible mechanism indicated that there are different ROS species taking part in the oxidizing process accompanying the addition of one or two *O* atoms to the unsaturated fatty acids, For one *O* atom added to the unsaturated fatty acid $[M+O-H]^-$, it directly reflects OH attacking the C=C bond of unsaturated fatty acids to form epoxidation followed by breakage to yield diagnostic ions.[3] For the addition of 2*O* atoms $[M+2O-H]^-$, it reflected that superoxide radical O_2^- first attacks one hydrogen to form an allyl carbon that goes on to form the intermediate species with 2*O* atoms in the mass spectrum. All of this is mainly mediated by the flight distance between the tip of ESI source and the inlet of the mass spectrometer. The possible mechanism might involve the process of epoxidation of hydroxyl radical, superoxide radical oxidation, and 1,2,3-trioxolan rearrangement at the air-water interface of the microdroplet.[4-6] The final species was ultimately formed in the mass spectrum after the process of ring opening, Criegge ion formation, and isomerization.

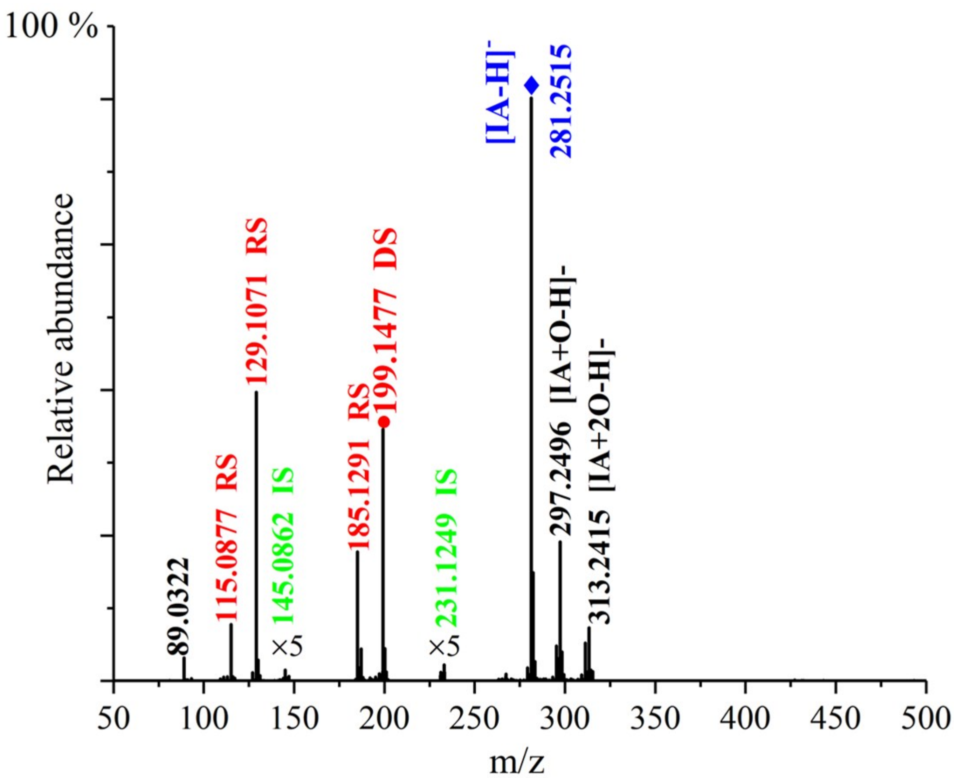

Figure S1. Elucidation the location of C=C in IA (18:1(11Z)). Mass spectrum of IA + $HAuCl_4$ obtained in microdroplet mass spectrometry with a flight distance of 20 mm.

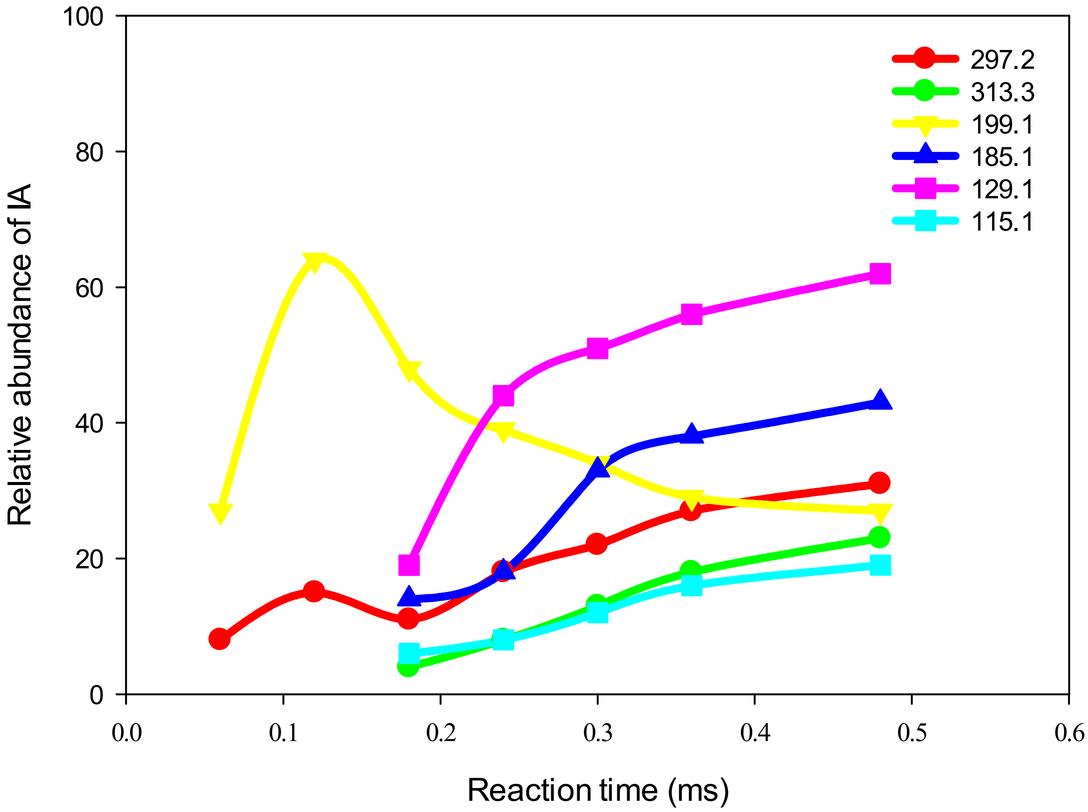

Figure S2. The relative abundance curves for the different species of 313.2, 297.2,199.1, 185.1, 129.1, and 115.1 corresponding to the different reaction time from 0.06 ms to 0.48 ms in the IA + $HAuCl_4$ microdroplet mass spectrum.

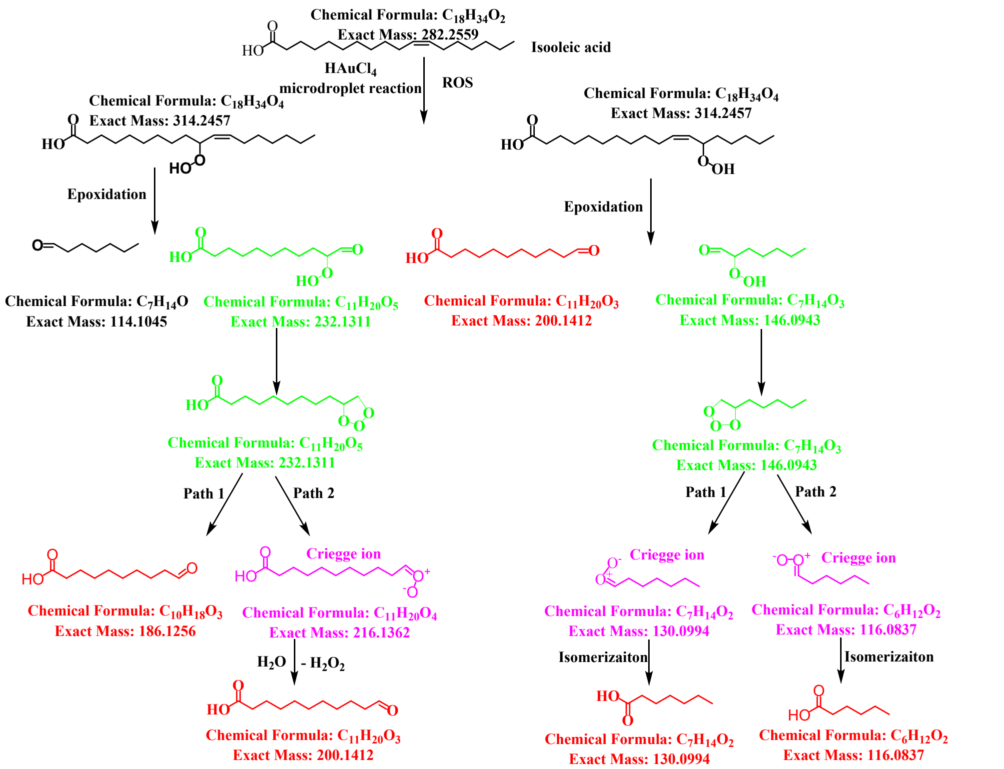

Figure S3. Possible ROS reaction mechanism for the species of IA with HAuCl4 as the oxidizing agent in microdroplet mass spectrometry.

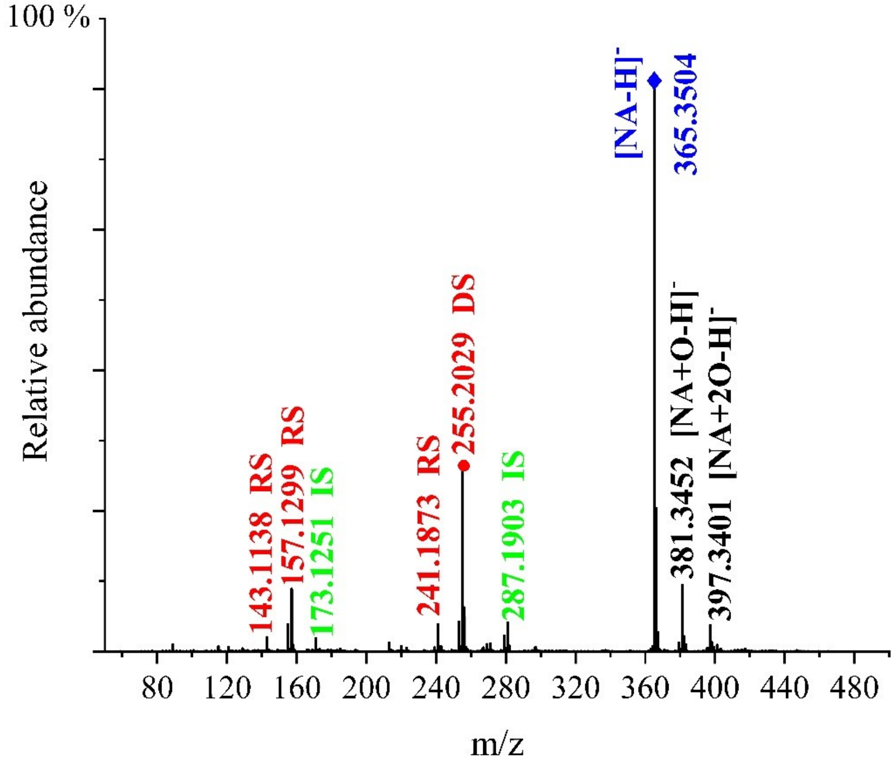

Figure S4. Elucidation the location of C=C in NA (24:1(15Z)). Mass spectrum of NA-$HAuCl_4$ obtained in microdroplet mass spectrometry with a flight distance of 20 mm.

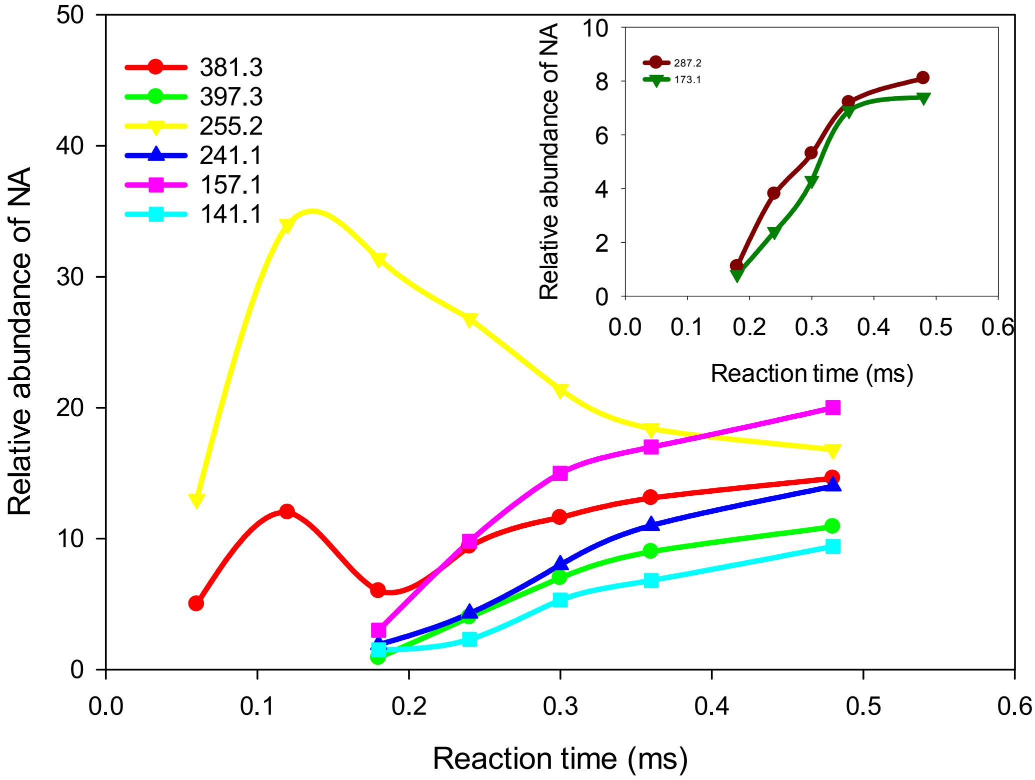

Figure S5. Relative abundance curves for the different species of 397.3, 381.3, 255.2, 241.1, 157.1, and 141.1 corresponding to different reaction times from 0.06 ms to 0.48 ms in the NA + $HAuCl_4$ microdroplet mass spectrometry. The insert is the relative abundance curves for the intermediate species of 287.2 and 173.1 with different reaction times.

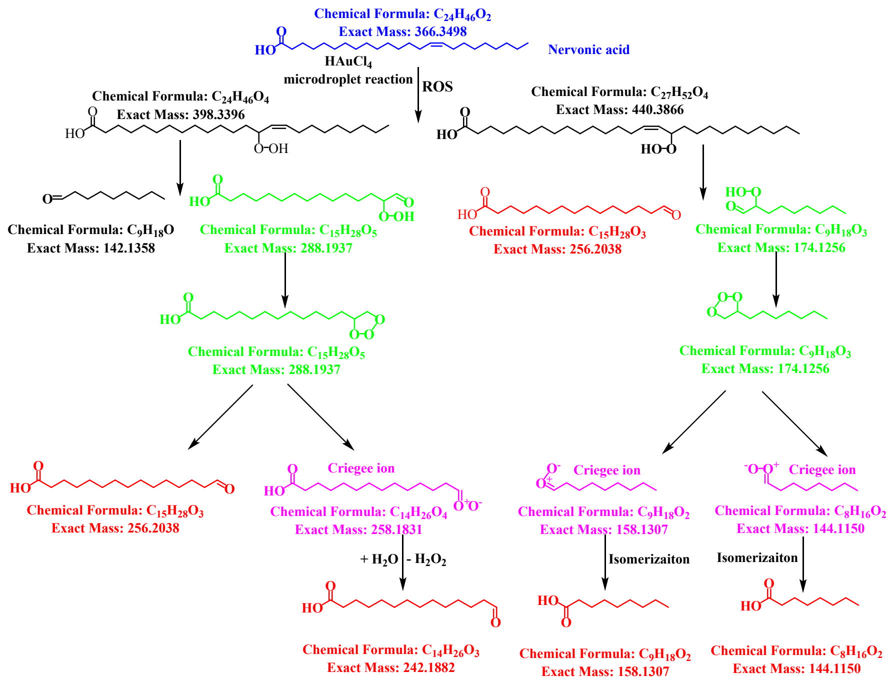

Figure S6. Possible ROS reaction mechanism for the species of NA with $HAuCl_4$ as the oxidizing agent in microdroplet mass spectrometry

References

1. J. K. Lee, S. Kim, H.G. Nam and R. N. Zare, *Proc. Nat. Acad. Sci. U.S.A.*, 2015, **112**, 3898-3903.

2. S. Enami, and A.J. Colussi, *Phys. Chem. Chem. Phys.*, 2017, **19**, 17044-17051.

3. Y. H. Lai, S. Sathyamoorthi, R. M. Bain and R. N. Zare, *J. Am. Soc. Mass Spectrom.*, 2018, **29**, 1036-1043.

4. C. Zhu, M. Kumar, J. Zhong, L. Li, J. S. Francisco, and X. C. Zeng, *J. Am. Chem. Soc.*, 2016, **138**, 11164-11169.

5. J. Qiu, S. Ishizuka, K. Tonokura, and S. Enami, *J. Phys. Chem. A*, 2018, **122**, 6303-6310.

6. F. Tang, T. Ohto, T. Hasegawa, W.J. Xie, L. Xu, M. Bonn, and Y. Nagata, *J. Chem. Theory Comput.*, 2018, **14**, 357-364.