Supplementary Information for

"Supermolecule Assisted Imaging of Low-Molecular Weight Quaternary Ammonium Compounds by MALDI-MS of their Non-covalent Complexes with Cucurbit[7]uril"

Di Chen,[ab] Jun Han,*[ac] David Schibli,[a] Juncong Yang,[a] Zhenzhong Zhang,[b]

Christoph H. Borchers*[defg]

a. University of Victoria - Genome British Columbia Proteomics Centre, Victoria, BC V8Z 7X8, Canada.

b. School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou 450001, China.

c. Division of Medical Sciences, University of Victoria, Victoria, BC, V8P 5C2, Canada.

d. Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, V8P 5C2, Canada.

e. Gerald Bronfman Department of Oncology, Jewish General Hospital, McGill University, Montreal, Quebec, H3T 1E2, Canada.

f. Segal Cancer Proteomics Centre, Lady Davis Institute, Jewish General Hospital, McGill University, Montreal, Quebec, H3T 1E2, Canada.

g. Center for Computational and Data-Intensive Science and Engineering, Skolkovo Institute of Science and Technology, Moscow 121205, Russia.

Electronic Supplementary Information (ESI) available. See DOI: 10.1039/x0xx00000x

* To whom correspondence should be addressed. E-mail address: junhan@proteincentre.com and christoph.borchers@mcgill.ca

Chemicals and reagents

Rat brain specimens were purchased from Pel-Freez Biologicals (Rogers, Arkansas, USA). As described in the sample information sheet, the brain tissue specimens were flash-frozen by slow immersion in liquid nitrogen to avoid shattering after harvesting. The use of the animal specimens involved in current study was in accordance with current requirements of the Canadian Council on Animal Care and was approved by the Ethics Committee of the University of Victoria. MALDI matrix alpha-cyano-4-hydroxycinnamic acid (CHCA), LC-MS grade solvents including water (H_2O) and methanol (MeOH), analyte standards including choline chloride, phosphocholine chloride calcium salt tetrahydrate, cucurbit[6]uril hydrate (CB[6]) and cucurbit[7]uril hydrate (CB[7]) were all purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Trimethylvinylammonium bromide (neurine) was bought from Tokyo Chemical Industry (Tokyo, Japan). Stock solutions of all analyte standards were prepared in H_2O/MeOH (50/50, v/v) at the concentration of 10 mM. CB[7] was prepared in H_2O at the concentration of 10 mM. All these solutions are diluted to desired concentrations with proper solvents if needed.

Tissue Preparation and Sectioning

The frozen rat brain specimens were mounted in a Microm HM500 cryostat (Waldorf, Germany) and sectioned at −20 °C into 14-μm slices. For each specimen, two successive sections were collected. The one section was thaw-mounted onto conductive indium−tin oxide (ITO) coated glass slides (Bruker Daltonics, Leipzig, Germany) for MALDI-MSI analysis, and hematoxylin and eosin (H&E) staining was

performed for the other section to provide a general overview of the brain tissue's structure. After sectioning, the tissue sections were dried under vacuum for 30 min.

On-tissue inclusion and matrix coating for MALDI-MSI

On-tissue CB[7] inclusion and the matrix coating were performed by directly spraying several cycles of CB[7] solution and CHCA solution in turn (the two-step procedure) or the mixture solution of CB[7] and CHCA (single-step procedure) on the tissue section using a Bruker Daltonics ImagePrep sprayer (Bremen, Germany). Each spray cycle was composed of a 3 s spray step, a 60 s incubation step, and a 90 s drying step. For comparison, CB[7]-free tissue section was also performed and carried out for MALDI-MSI, in which no CB[7] was deposited.

MALDI-MS and MALDI-MSI analysis

All the experiments were performed on an Ultraflex II-TOF/TOF mass spectrometer equipped with a smartbeam™ solid state laser (Bruker Daltonics, Billerica, MA, USA) operated in positive-ion, reflectron mode. Mass spectrometer calibrations were conducted before every experiment performed and postrun calibrations would be carried out again to obtain accurate mass spectra measurements. CHCA and CB[7] related signals were used for internal calibration.

Data acquisition and processing were performed with flexControl 3.0, flexAnalysis 3.0 and flexImaging 2.1 (Bruker, Bremen, Germany). All mass spectra were acquired over the mass range of *m/z* 50-2000. For direct MALDI-MS analysis, profiling data was acquired by accumulating 500 laser shots per spot. For MALDI-MSI analysis, profiling data was acquired by accumulating 20 laser shots per pixel

with the minimum raster step size of 200 μm. The signal of the target analyte was identified by comparing the experimental *m/z* value of CB[7]-analyte noncovalent complex with theoretical value within an allowable mass error of ±15 ppm.

Histological Staining

H&E staining was performed according to previously reported procedures[1, 2] to obtain histological optical images.

Table. S1 Measured and calculated *m/z* signals and their relative abundance of the QACs, CB[7] and QAC-CB[7] complexes in the positive-ion mode.

Complexes	Measured *m/z* and relative abundance	Calculated *m/z* and relative abundance	Mass Error (ppm)
Neurine	86.0979	86.0964	17.4
Choline	104.1082	104.1069	12.57
Phosphocholine	184.0752	184.0733	10.3
	1163.352, 100.0%	1163.350, 100.0%	1.7
CB[7]-H^+	1164.357, 60.1%	1164.353, 56.8%	3.4
	1165.352, 19.8%	1165.355, 18.7%	-2.6
	1185.340, 100.0%	1185.332, 100.0%	6.7
CB[7]-Na^+	1186.338, 58.9%	1186.335, 56.8%	3.1
	1187.339, 17.6%	1187.337, 18.7%	2.2
	1201.301, 100.0%	1201.306, 100.0%	-4.2
CB[7]-K^+	1202.310, 58.8%	1202.308, 56.8%	0.9
	1203.303, 23.2%	1203.309, 25.9%	-5.0
	1248.439, 100.0%	1248.444, 100.0%	3.9
Neurine-CB[7]	1249.442, 62.7%	1249.441, 64.4%	-0.7
	1250.444, 22.2%	1250.440, 21.7%	-3.6
	1266.450, 100.0%	1266.455, 100.0%	4.1
Choline-CB[7]	1267.452, 62.8%	1267.451, 65.1%	-0.8
	1268.455, 22.5%	1268.450, 24.8%	-3.8
	1346.416, 100.00%	1346.256, 100%	0.8
Phosphocholine-CB[7]	1347.419, 62.9%	1347.258, 60.9%	-0.1
	1348.421, 23.2%	1348.259, 21.9%	-0.8

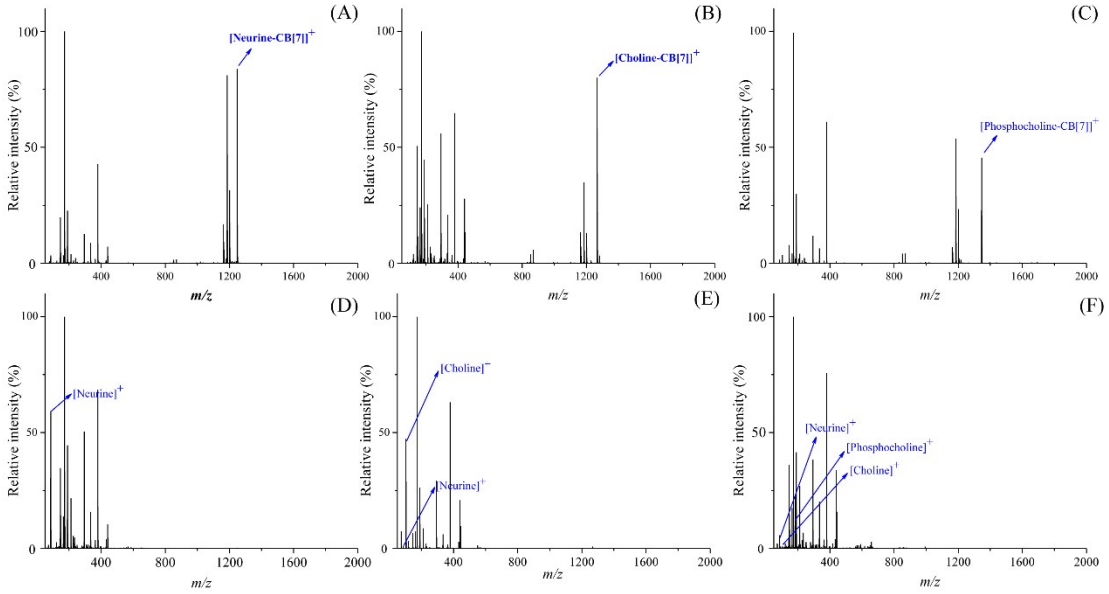

Fig. S1. Mass spectra of QACs or QAC-CB[7] complexes. (A) Neurine-CB[7] complex, (B) choline-CB[7] complex, (C) phosphocholine-CB[7] complex, (D) neurine, ((E) neurine and choline, and (F) neurine, choline, and phosphocholine.

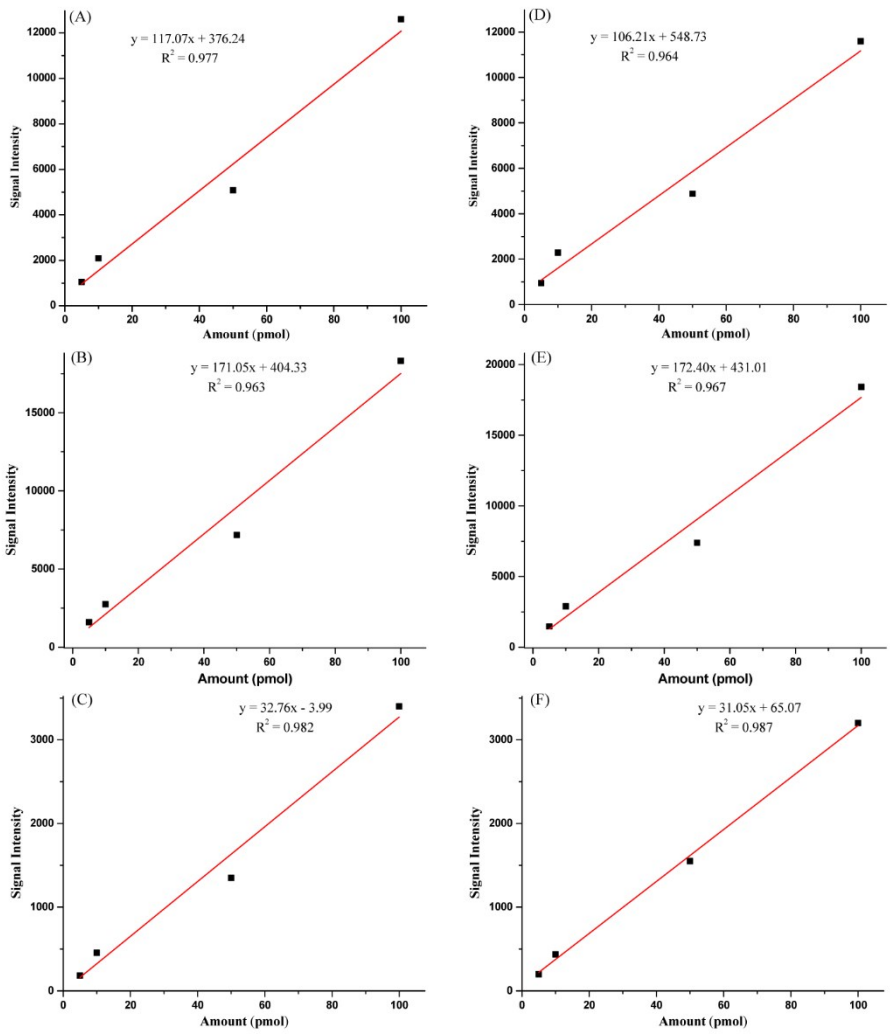

Fig. S2 Linearity curves for MALDI-MS detection of (A) neurine, (B) choline, (C) phosphocholine, (D) CB[7]-neurine, (E) CB[7]-choline and (F) CB[7]-phosphocholine.

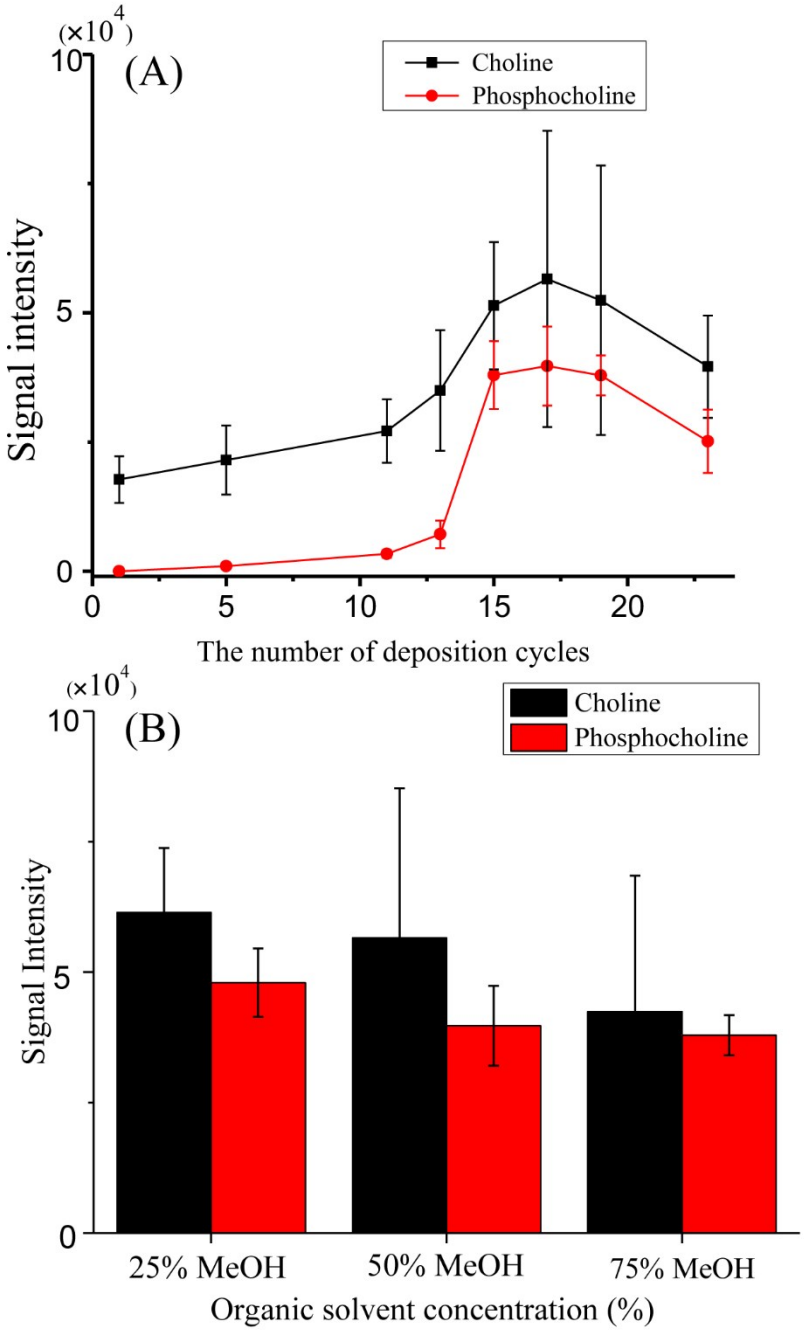

Fig. S3 Optimization of the on-tissue inclusion conditions. (A) The amount of CB[7] and (B) the organic solvent %s of CB[7] solution on ion intensities of choline and phosphocholine.

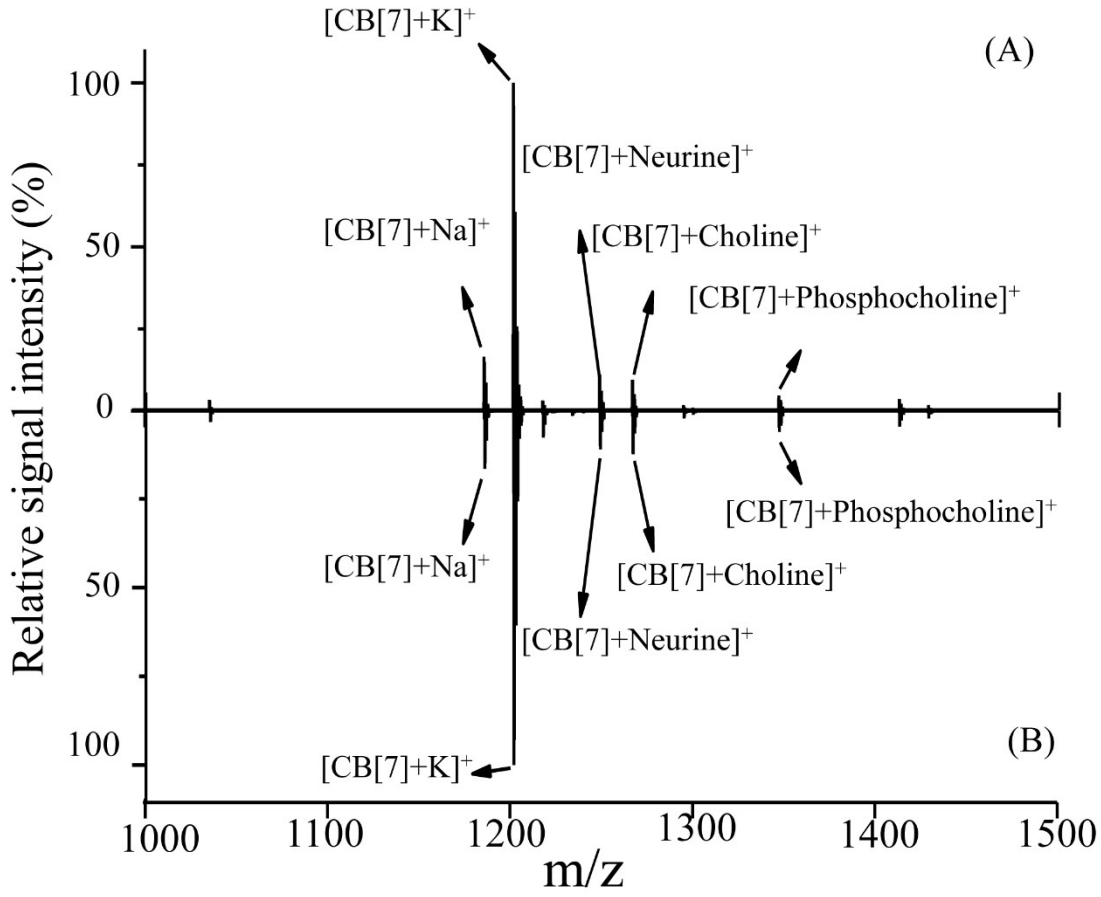

Fig. S4 Average mass spectrum obtained by conducting on-tissue inclusion by (A) two steps or (B) one step.

References

1. X. Wang, J. Han, A. Chou, J. Yang, J. Pan and C. H. Borchers, *Anal Chem*, 2013, **85**, 7566.
2. X. Wang, J. Han, J. Yang, J. Pan and C. H. Borchers, *Chem Sci*, 2015, **6**, 729.