# Mass spectrometry analysis of single mammalian cells obtained using the redesigned T-probe from laboratory experiments performed in 2017 and 2018

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**Data Type**: experimental
**Version**: 1
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**Project**
» Collaborative Research: Creatine Cycling in Marine Bacterial and Phytoplankton Assemblages (Creatine Cycling)


| Contributors | Affiliation | Role |
|---|---|---|
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**Abstract**
Mass spectrometry analysis of single mammalian cells obtained using the redesigned T-probe from laboratory experiments performed in 2017 and 2018. These results were published in Zhu et al. (2019).

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## Coverage

**Temporal Extent**: 2017-05 - 2018-01

### Methods & Sampling

The following are excerpts from Zhu et al., 2019. Please refer to this publication for more details.

Methodology

During the SCMS analysis, the redesigned T-probe was coupled to the in-home developed SCMS platform employed in our previous SCMS studies. Briefly, this platform includes an XYZ-translational stage system, two digital microscopes, and a Thermo LTQ Orbitrap XL mass spectrometer. Cells in both control and drug treatment groups were used for the SCMS experiments (detailed sample preparation procedures are described in the Supporting Information). Irinotecan is a common anticancer drug for the treatment of colon cancer that inhibits the function of Topoisomerase I, leading to DNA damage and cell apoptosis. This drug compound was selected to treat live HCT-116 colorectal cells in our experiments to demonstrate the change of cellular metabolites upon the treatment of anticancer agent. Specifically, cells were first treated using 18 mM irinotecan for 45 min, and then rinsed and detached using trypsinization. Afterwards, a droplet of cell suspension solution was placed onto a glass slide, which was attached to the XYZ-stage system controlled by a LabView software package (incremental step size = 0.1 mm). Using two digital microscopes as the visual guide, the sampling

probe tip initially located above the sample plate was submerged into the solution containing cells by lifting the Z-stage. Upon selecting a target cell, the sampling probe can precisely draw the target cell with visual guidance. The XYZ-stage was then immediately lowered down to free the sampling probe tip from the culture medium and stop the suction of culture medium. Due to the complex composition of the cell culture medium that may affect the detection sensitivity, caution should be taken to minimize its amount withdrawn during cell sampling. This is particularly important for future analysis of patient cells suspended in complex biological fluids such as blood, urine, saliva, and cerebrospinal fluid (CSF). After a single cell was withdrawn, the solvent provided through the solvent-providing capillary (flowrate = 0.5 mL/min) mixed with the cell at the T-junction, and cell lysis rapidly occurred inside the nano-ESI emitter. In our SCMS analysis, an ionization voltage (~4 kV) was applied to the conductive union and transmitted throughout the solution inside the solvent-providing capillary and the nano-ESI emitter to ionize the cell lysis for MS analysis.

**Instrument**

Thermo LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Waltham, MA, United States). Mass analyze parameters were as follows: mass resolution 60,000, +4 kV ionization voltage at positive ion mode (0.05–0.07 μA of ion current), 1 microscan, 100 ms max injection time, and automatic gain control on.

**Location of experiments:** University of Oklahoma, Norman, OK 73019
**Cell line:** HCT-116 (human colon cancer cell line)

**Data Processing Description**

Processed using Geena 2 online software (Romano et al., 2016).

LabView version used: “LabView 2011, Service Pack 1, Version 11.0.1f2, 32-bit. National Instruments Corporation (2011).”

BCO-DMO data manager processing notes:
* Loaded Sheet 1 "Genna 2 data" from file "Redesigned T probe data_BCODMO.xlsx" into the BCO-DMO data system.
* Unpivoted data. Transformed from many intensity columns with multiple header rows (Date,Cell Group,Cell Number) to one Intensity column and added columns for Date, Cell_Group, and Cell_Number. First column named "mass_to_charge."
* Date (month and year, e.g. Aug_2017) format converted to ISO 8601 format yyyy-mm (e.g. (2017-08)
* metadata included in the data file extracted to metadata.

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## Data Files

| File | |
|---|---|
| **mass_spec_metabolites.csv** | (Comma Separated Values (.csv), 282.83 KB) MD5:6a6a2e0797181756279e00310a0d0ff9 |
| Primary data file for dataset ID 851838 | |

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## Related Publications

Liu, R., Pan, N., Zhu, Y., & Yang, Z. (2018). T-Probe: An Integrated Microscale Device for Online In Situ Single Cell Analysis and Metabolic Profiling Using Mass Spectrometry. Analytical Chemistry, 90(18), 11078–11085. doi:10.1021/acs.analchem.8b02927
*Methods*

National Instruments Corporation (2011). LabView 2011, Service Pack 1, Version 11.0.1f2, 32-bit. Available from https://www.ni.com/pdf/manuals/lv2011SP1.html
*Software*

Pan, N., Rao, W., Kothapalli, N. R., Liu, R., Burgett, A. W. G., & Yang, Z. (2014). The Single-Probe: A Miniaturized Multifunctional Device for Single Cell Mass Spectrometry Analysis. Analytical Chemistry, 86(19), 9376–9380. doi:10.1021/ac5029038
*Methods*

Pan, N., Rao, W., Standke, S. J., & Yang, Z. (2016). Using Dicationic Ion-Pairing Compounds To Enhance the Single Cell Mass Spectrometry Analysis Using the Single-Probe: A Microscale Sampling and Ionization Device. Analytical Chemistry, 88(13), 6812–6819. doi:10.1021/acs.analchem.6b01284
*Methods*

Rao, W., Pan, N., & Yang, Z. (2016). Applications of the Single-probe: Mass Spectrometry Imaging and Single Cell Analysis under Ambient Conditions. Journal of Visualized Experiments, (112). doi:10.3791/53911
*Methods*

Romano, P., Profumo, A., Rocco, M., Mangerini, R., Ferri, F., & Facchiano, A. (2016). Geena 2, improved automated analysis of MALDI/TOF mass spectra. BMC Bioinformatics, 17(S4). doi:10.1186/s12859-016-0911-2
*Software*

Xia, J., & Wishart, D. S. (2016). Using MetaboAnalyst 3.0 for Comprehensive Metabolomics Data Analysis. Current Protocols in Bioinformatics, 55(1), 14.10.1–14.10.91. doi:10.1002/cpbi.11
*Methods*

Zhu, Y., Liu, R., & Yang, Z. (2019). Redesigning the T-probe for mass spectrometry analysis of online lysis of non-adherent single cells. Analytica Chimica Acta, 1084, 53–59. doi:10.1016/j.aca.2019.07.059
*Results*

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| Mass_to_Charge | Mass to charge ratio (m/z) | dimensionless |
| Date | Date (month and year) in ISO 8601 format yyyy-mm | unitless |
| Cell_Group | Cell group describing treatment with or without anticancer drug Irinotecan. (Control = no drug treatment,Treated = with drug treatment) | unitless |
| Cell_Number | Cell Number | unitless |
| Intensity | Ion intensity (arbitrary unit) | unitless |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | Thermo LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Waltham, MA, United States) |
| **Generic Instrument Name** | Mass Spectrometer |
| **Dataset-specific Description** | Mass analyze parameters were as follows: mass resolution 60,000, +4 kV ionization voltage at positive ion mode (0.05–0.07 μA of ion current), 1 microscan, 100 ms max injection time, and automatic gain control on. |
| **Generic Instrument Description** | General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components. |

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## Project Information

**Collaborative Research: Creatine Cycling in Marine Bacterial and Phytoplankton Assemblages (Creatine Cycling)**

**Coverage**: Atlantic bight

*NSF Award Abstract:*
High rates of dissolved organic nitrogen (DON) production and utilization in aquatic systems are typically attributed to microbial activity. Though it is known that there is a tight coupling between the production and consumption of biologically available DON, the composition, dynamics, and ecological significance of this rapidly cycled DON pool are less well understood. This proposal focuses on a component of the DON pool, creatine, which is historically understood as a product of metazoan activity, but appears to be both produced by phytoplankton and consumed by marine bacteria. Creatine is present in seawater in measurable quantities, which led to the hypothesis that creatine may be a significant component of the marine DON cycle. DON cycling likely has a bearing on fundamental marine ecosystem processes with large implications for carbon and nitrogen turnover on a global scale. Broader impacts of this project will include outreach that focuses on connecting scientists with K-12 students through research experiences for teachers and lesson development in collaboration with the K20 Center for Educational and Community Renewal, a statewide education research and development center at the University of Oklahoma. The project will integrate the research with inquiry-based teaching of rural secondary science teachers through Authentic Research Experiences in oceanographic science and microbial ecology. The K20 network includes 96% of Oklahoma schools, providing a unique opportunity to impact STEM education in Oklahoma.

The results of this project will help develop a better understanding of DON cycling, the ecological context of creatine uptake activity, and identify both creatine-producing and consuming organisms in the marine environment. The importance of creatine cycling will be assessed via 15N tracer studies along the natural coastal-to-offshore productivity gradient observed in the North Atlantic. Tracer and molecular approaches will be used to investigate the importance of phytoplankton vs. bacteria in creatine uptake and, the taxonomic identities of creatine-utilizing bacteria will be interrogated via molecular, stable isotope probing (SIP), and RT-qPCR approaches.

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## Funding

| Funding Source | Award |
|---|---|
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1634630 |

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