

# Single cell mass spectroscopy data collected to investigate metabolomics affected by cell-cell interactions in 2020 and 2021

**Website:** <https://www.bco-dmo.org/dataset/890877>

**Data Type:** experimental

**Version:** 1

**Version Date:** 2023-03-01

## Project

» [Collaborative Research: Creatine Cycling in Marine Bacterial and Phytoplankton Assemblages](#) (Creatine Cycling)

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## Abstract

Single-cell mass spectrometry (SCMS) was integrated with fluorescence microscopy to investigate metabolomics affected by cell-cell interactions in 2020 and 2021. These data were used to create a table in the publication of the results by Chen et al. (2022).

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## Coverage

**Temporal Extent:** 2020 - 2021

## Methods & Sampling

Cell-cell interaction systems: the device utilized for the indirect co-culture system was Corning Transwell inserts (Corning Incorporated Life Science, Tewsbury, MA, USA) in the format of a 6-well plate with a permeable membrane (pore size: 0.4  $\mu\text{m}$ ). Gridded glass coverslips (ibidi USA Incorporation, Fitchburg, WI, USA) were used for cell attachment in the direct co-culture system.

The materials used to fabricate the single-probe include dual-bore quartz tubing (O.D. 500  $\mu\text{m}$ , I.D. 127  $\mu\text{m}$ , Friedrich & Dimmock, Inc., Millville, NJ, USA) and fused silica capillary (O.D. 105  $\mu\text{m}$ , I.D. 40  $\mu\text{m}$ , Polymicro Technologies, Phoenix, AZ, USA). The Single-probe was fabricated following our published protocols. Briefly, three major components (i.e., a Nano-ESI emitter, a dual-bore quartz tip, and a fused silica capillary) were integrated to prepare a Single-probe. Dual-bore quartz needles were produced by pulling the dual-bore quartz tubing using a laser micropipette puller (Sutter P-2000, Sutter Instrument, Novato, CA). The nano-ESI emitters were pulled from the fused silica capillaries using a butane micro torch. A Single-probe was fabricated by embedding a fused silica capillary and a nano-ESI emitter into those two channels of a dual-bore quartz needle.

The Single-probe was coupled to a Thermo LTQ Orbitrap XL mass spectrometer for SCMS analysis. The sampling solvent (acetonitrile supplemented with 1% formic acid) was used for the SCMS experiment at a flow rate of ~0.05 µl/min.

#### Instruments

The Thermo LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Waltham, MA, United States) was used. The SCMS experiment parameters included a mass range of m/z 200-1500 for positive ion mode and m/z 50-900 for negative ion mode, mass resolution 60,000, ionization voltage 4.5 kV, 1 microscan, and 100 ms max injection time.

## Data Processing Description

### Data processing

Single cell data was exported from the mass spec .raw files into Excel Sheets and submitted to BCO-DMO. Each individual table was concatenated into a cohesive table (see BCO-DMO data manager processing notes).

Metabolites data from single cells were searched against three online metabolomics databases (Metlin, HMDB, and GNPS) for tentative labeling. Tandem MS (MS2) analyses were performed for molecular structure verifications.

### BCO-DMO Data Manager Processing Notes:

- \* All Sheets from Excel files "Cell-cell interaction project\_neg.xlsx" and "Cell-cell interaction project\_pos.xlsx" were exported as a csv file per sheet. These sheets were bundled together with a sheet inventory providing experimental metadata to and made available as a supplemental file "single\_cell\_csvs.zip."
- \* Each cell table (csv) was imported into the BCO-DMO data system totaling 199 tables. These were concatenated into a cohesive table with additional columns to for metadata contained in the original excel filename, and sheet name.
- \* Additional Columns "Ion\_mode" "Cell\_Line" "Cell\_Number" and "Description" were added to the combined data table using a lookup table provided by the data submitter that matched information about each Sheet position in the Excel file and which Cell line and cell number it corresponded to.
- \* sheet inventory and combined data table were reviewed by the original data submitter to ensure the provided metadata was represented accurately in the published data.

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## Data Files

File	
<b>Cell-cell interactions</b>	
filename: cell_cell_interactions.csv	(Comma Separated Values (.csv), 517.69 MB) MD5:68af5c134857c6a821a7e4c5cc4d218e
Cell-cell interaction mass to charge, intensity, and sample metadata. See "Parameters" section for more details of the data columns.	
This is the main data table for this dataset and combines all single-cell subtables contained in "single_cell_csvs.zip."	

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## Supplemental Files

## File

### Single-cell csv files (data in alternate format)

filename: single\_cell\_csvs.zip

(ZIP Archive (ZIP), 28.34 MB)  
MD5:350d8dabecbbb24637723d9da4a6de18

This file bundle contains one csv file per cell in the experiment. These files were concatenated together to form the main data table in this dataset. This file bundle includes a "sheet\_inventory.csv" which provides metadata for each csv file (cell-cell interaction cell line information).

Each csv file contains:

- \* m.z: This is the m/z (mass/charge) for ions detected in single cell
- \* Intensity: The intensity of ions at m/z
- \* Relative: relative intensity normalized by highest peak intensity (100%)

Each file contains six header lines as output from the instrument-specific file format. These header lines contain information about where each table was extracted from in the instrument .raw file.

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## Related Publications

Chen, X., Peng, Z., & Yang, Z. (2022). Metabolomics studies of cell-cell interactions using single cell mass spectrometry combined with fluorescence microscopy. *Chemical Science*, 13(22), 6687–6695. <https://doi.org/10.1039/d2sc02298b> <https://doi.org/10.1039/D2SC02298B>

*Results*

Liu, R., Zhang, G., Sun, M., Pan, X., & Yang, Z. (2019). Integrating a generalized data analysis workflow with the Single-probe mass spectrometry experiment for single cell metabolomics. *Analytica Chimica Acta*, 1064, 71–79. doi:[10.1016/j.aca.2019.03.006](https://doi.org/10.1016/j.aca.2019.03.006)

*Methods*

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](https://doi.org/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](https://doi.org/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](https://doi.org/10.3791/53911)

*Methods*

Smith, C. A., Maille, G. O., Want, E. J., Qin, C., Trauger, S. A., Brandon, T. R., ... Siuzdak, G. (2005). METLIN. *Therapeutic Drug Monitoring*, 27(6), 747–751. doi:[10.1097/01.ftd.0000179845.53213.39](https://doi.org/10.1097/01.ftd.0000179845.53213.39)

*Methods*

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](https://doi.org/10.1002/cpbi.11)

*Methods*

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## Parameters

Parameter	Description	Units
Ion_Mode	Ion mode (pos=positive ion mode, neg=negative ion mode)	unitless
Cell_Line	The specific cell line that was used for studying cell-cell interactions in co-culture systems.	unitless
Cell_Number	Cell number for each Cell_Line and Ion mode.	unitless
Description	Cell with or without co-culture in cell-cell interaction experiments. (e.g. "Control cell line" or "Cell-cell interaction cell line (with resistance)").	unitless
Mass_to_Charge	Mass to charge ratio (m/z). This is the m/z for ions detected in single cell	unitless
Intensity	The intensity of ions at m/z	unitless
Relative	relative intensity normalized by highest peak intensity (100%)	unitless
path_name	Path name of the same data in an alternate format. This path name is the corresponding csv file containing the same m.z, Intensity, Relative data columns within the file bundle "single_cell_csvs.zip" which contains a csv file per cell. See processing notes section for more information.	unitless

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## Instruments

<b>Dataset-specific Instrument Name</b>	Thermo LTQ Orbitrap XL
<b>Generic Instrument Name</b>	Mass Spectrometer
<b>Dataset-specific Description</b>	Thermo LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Waltham, MA, United States)
<b>Generic Instrument Description</b>	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  $^{15}\text{N}$  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
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1634630</a>

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