APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
11/16/2009	20101377
3. DATE RECEIVED BY STATE	**State Application Identifier**

1. * TYPE OF SUBMISSION

☐ Pre-application ☒ Application ☐ Changed/Corrected Application

4. Federal Identifier

5. APPLICANT INFORMATION

*** Organizational DUNS:** 804355790

* Legal Name: The Regents of the Univ. of Calif., U.C. San Diego

Department: Health Sciences SPO Division: School of Pharmacy

* Street1: 9500 Gilman Drive

Street2: MC 0742

* City: La Jolla County: San Diego

* State: CA: California Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 92093-0742

Person to be contacted on matters involving this application

Prefix: * First Name: Andrea Middle Name:

* Last Name: Rollins Suffix:

* Phone Number: (858)822-4109 Fax Number: (858)822-0834

Email: vchsgrants@ucsd.edu

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* 1956006144A1

7. * TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:

☒ New ☐ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY:

National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Real-Time Imaging of metabolic Communication

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*

N/A

13. PROPOSED PROJECT:

* Start Date: 07/01/2010 * Ending Date: 06/30/2014

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant: CA-053 b. * Project: CA-053

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. * First Name: Pieter Middle Name:

* Last Name: Dorrestein Suffix: Ph.D.

Position/Title: Assistant Professor

* Organization Name: The Regents of the Univ. of Calif., U.C. San Diego

Department: N/A Division: Sch of Pharmacy and Pharm Sci

* Street1: 9500 Gilman Drive

Street2: MC 0636

* City: La Jolla County: San Diego

* State: CA: California Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 92093-0636

* Phone Number: (858)534-6607 Fax Number: (858)822-6857

* Email: pdorrestein@ucsd.edu

OMB Number: 4040-0001
Expiration Date: 04/30/2008

16. ESTIMATED PROJECT FUNDING

a. * Total Estimated Project Funding	1,029,804.00
b. * Total Federal & Non-Federal Funds	1,029,804.00
c. * Estimated Program Income	0.00

17. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

18. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

☒ *** I agree**

**** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.***

19. Authorized Representative

Prefix: * First Name: Rachel Middle Name:

* Last Name: Cook Suffix:

* Position/Title: Grant Analyst

* Organization: The Regents of the Univ. of Calif., U.C. San Diego

Department: Health Sciences SPO Division: Sch of Pharmacy and Pharm Sci

* Street1: 9500 Gilman Drive

Street2: MC 0742

* City: La Jolla County: San Diego

* State: CA: California Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 92093-0742

* Phone Number: (858)822-4109 Fax Number: (858)822-0834

* Email: vchsgrants@ucsd.edu

*** Signature of Authorized Representative**: Rachel Cook

*** Date Signed**: 11/16/2009

20. Pre-application Add Attachment | Delete Attachment | View Attachment

21. Attach an additional list of Project Congressional Districts if needed.

Add Attachment | Delete Attachment | View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

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RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: The Regents of the Univ. of Calif., U.C. San Diego

* Street1: 9500 Gilman Drive

Street2: MC 0636

* City: La Jolla County: San Diego

* State: CA: California Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 92093-0636

Project/Performance Site Location 1

Organization Name:

* Street1:

Street2:

* City: County:

* State: Province:

* Country: USA: UNITED STATES * ZIP / Postal Code:

Additional Location(s) Add Attachment Delete Attachment View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Close Form | Print Page | About

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☐ Yes ☒ No

1.a If YES to Human Subjects

Is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Exemption Number: ☐1 ☐2 ☐3 ☐4 ☐5 ☐6

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? ☐ Yes ☒ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators? ☐ Yes ☒ No

5.b. If yes, identify countries:

5.c. Optional Explanation:

6. * Project Summary/Abstract 1235-ProjectsummaryNov05.pdf | Add Attachment | Delete Attachment | View Attachment

7. * Project Narrative 1239-Narrative.pdf | Add Attachment | Delete Attachment | View Attachment

8. Bibliography & References Cited 1238-Bibliography.pdf | Add Attachment | Delete Attachment | View Attachment

9. Facilities & Other Resources 1236-Facilityresources1.pdf | Add Attachment | Delete Attachment | View Attachment

10. Equipment 1237-Equipment1.pdf | Add Attachment | Delete Attachment | View Attachment

11. Other Attachments Add Attachments | Delete Attachments | View Attachments ☐

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Project summary: Metabolic exchange is a universal phenomenon. It is essential to every organism, from those as simple as bacteria to complex higher eukaryotes such as humans. While metabolic exchange enables cooperation and coordination between the ~70 trillion cells in an average human being, even unicellular organisms rely on metabolic exchange to adapt to environmental stress and form biofilms. Cellular communication allows stem cells to differentiate, cancer cells to proliferate, neurons to fire, bacteria to sense a quorum and pathogens to survive in human hosts. The chemical diversity of the molecules used for communication is extraordinary, and includes small ions such as calcium, small molecules such as secondary metabolites, fatty acids, peptides, but also carbohydrates, proteins and nucleic acids. Despite the universal nature of metabolic exchange, there are few methods that can characterize the communication between cells in a systematic and sensitive fashion, let alone real-time. In this proposal, our focus will be on the application and adaptation of desorption electrospray mass spectrometry to enable the real-time live cell detection and the characterization and visualization of metabolic exchange in important biological processes. We aim to accomplish this in both a spatial as well as temporal fashion. These tools will improve our understanding of secreted biomarkers, microbiome-human cell interactions and understanding the complexities of infectious disease that derive from the cooperation between different types of cells (e.g. *Bacilli* with macrophages, neutrophils or T-cells) and interkingdom communication. Ultimately it may drive the development of new therapeutic strategies or interventions based on paradigms involving inter-cellular metabolic communication in a system wide fashion.

This proposal aims to develop real-time monitoring of molecular entities involved in metabolic exchange of pathogen-immunological cell populations. Our ability to “visualize” metabolic exchange between different cell populations could lead to new therapeutic paradigms.

Facility resources:

The Dorrestein lab is associated with the Skaggs school of pharmacy and pharmaceutical sciences, the center for marine biotechnology and biomedicine and department of chemistry and biochemistry. Therefore the Dorrestein lab has two different lab spaces. The first lab space is our wet-lab that is located in the biomedical science building. This lab is equipped with three fume hoods, and is designed for 16 people. In addition, we have a smaller room, which is currently used for storage of supplies but in the future will be used to house a second mass spectrometer, a 37^{o}C room and three office spaces that can hold 12 students total. (currently there are 9 students) These rooms are all located in the biomedical science building. The LTQ-FTMS with nanospray and DESI sources and MALDI-TOF imaging systems are located in the Skaggs School of pharmacy and pharmaceutical sciences, which is just 300 yards away from the biomedical science building.

In Sum, these facilities are sufficient to carry out all the proposed work.

Equipment.

Dorrestein.
The Dorrestein labs is equipped with incubators, culturing rooms (mammalian and non mammalian cells), water baths, gel electrophoresis apparatus, PCR equipment, and power supplies, 2 Gel documentation systems for both UV and visible detection (Agarose and SDS-gel), small centrifuges, centrifuges for larger cultures with 4 and 10-liter capacity, shaker for culturing, cell culturing facilities, sonicators, 3HPLC's, lab computers (~10 in total), these are used for data analysis, research and documentation of our work, *an LTQ-FT-ICRMS equipped with an Advion nanospray robot a Prosilia DESI system and nanospray capabilities*, this instrument is set up for secured remote access so that we can monitor the progress and control the progress of this instrument via the internet, *a MALDI-TOF imaging system*, pressure bomb for making our own capillary columns for proteomic studies to be used in some of the micro mass spectrometric studies and a microscope. An Agilent ion trap instrument with nanoinfusion. In addition through the marine biotechnology and the Moores cancer center we have access to screens against a variety of cell lines and organisms. Finally, the pharmacy school has recently purchased two 600Mhz NMRs with cryoprobes, including a 1.7 mm probe for the characterization of natural products. Furthermore we have A CD instrument, a liquid handling system, autoclaves, dishwashers that are available in the School of pharmacy.

In short, we have all the equipment available to carry out the work outlined in this proposal.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. * First Name: Pieter Middle Name:

* Last Name: Dorrestein Suffix: Ph.D.

Position/Title: Assistant Professor Department: N/A

Organization Name: The Regents of the Univ. of Calif., U.C. San Diego Division: Sch of Pharmacy and Pharm Sci

* Street1: 9500 Gilman Drive

Street2: MC 0636

* City: La Jolla County: San Diego

* State: CA: California Province:

* Country: USA: UNITED STATES * Zip / Postal Code: 92093-0636

* Phone Number: (858)534-6607 Fax Number: (858)822-6857

* E-Mail: pdorrestein@ucsd.edu

Credential, e.g., agency login: pdorrestein

*** Project Role:** PD/PI **Other Project Role Category:**

***Attach Biographical Sketch** 1234-Nov152009Dorrestein2page Add Attachment Delete Attachment View Attachment

Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

PROFILE - Senior/Key Person 1

Prefix: * First Name: Middle Name:

* Last Name: Suffix:

Position/Title: Department:

Organization Name: Division:

* Street1:

Street2:

* City: County:

* State: Province:

* Country: USA: UNITED STATES * Zip / Postal Code:

* Phone Number: Fax Number:

* E-Mail:

Credential, e.g., agency login:

*** Project Role:** **Other Project Role Category:**

***Attach Biographical Sketch** Add Attachment Delete Attachment View Attachment

Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

ADDITIONAL SENIOR/KEY PERSON PROFILE(S)		Add Attachment	Delete Attachment	View Attachment
Additional Biographical Sketch(es) (Senior/Key Person)		Add Attachment	Delete Attachment	View Attachment
Additional Current and Pending Support(s)		Add Attachment	Delete Attachment	View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on the Sample Form. Follow this format for each person. **DO NOT EXCEED FOUR PAGES**.

NAME	POSITION TITLE
Pieter C. Dorrestein	Assistant Professor Skaggs School of Pharmacy and Pharmaceutical Sciences and Departments of Pharmacology, Chemistry and Biochemistry
eRA COMMONS USER NAME pdorrestein	

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(S)	FIELD OF STUDY
Northern Arizona University, Flagstaff, AZ	B.S.	1998	Chemistry
Cornell University, Ithaca, NY	M.S.	2001	Chemistry
Cornell University, Ithaca, NY	Ph.D.	2004	Chemical Biology
University of Illinois, Urbana-Champaign, IL	Postdoc.	2006	Bioanalytical Chem.

A. Positions and Honors

Positons and Employment

1999-2004 Research Assistant, Cornell University (08/1999 - 05/2004)
2004-2006 Postdoctoral NIH NRSA fellow, University of Illinois (6/2004 – 9/2006)
2006-present Assistant Professor, University of California, San Diego (9/2006-present)

Professional Memberships

2001-present American Chemical Society
2005-present American Society of Mass Spectrometry
2006-present Biomedical Science program
2007-present Center for Marine Biotechnology and Biomedicine
2008-present Center for Computational Mass Spectrometry

Honors (recent)

2008 Beckman young investigator.
2008 V-foundation scholar in cancer research.
2008 Lilly young investigator award in analytical chemistry.

B. peer-reviewed publications

10 out of 54 articles.

2004

1. Dorrestein, P.C., Yeh, E., Garneau-Tsodikova, S., Kelleher, N.L., Walsh, C.T., Dichlorination of a Pyrrolyl-S-Carrier protein by FADH2-Dependent Halogenase PltA During Pyoluteorin Biosynthesis. **Proc. Natl. Acad. Sci. USA.** (2005) 102(39), 13843-13848. PMID: 16162666
   In this paper I was able to show that the flavin dependent halogenation, a reaction important for the function of many natural products, used a protein linked substrate. Furthermore we demonstrated with C13 and N15 depleted material that one halogenase was responsible for halogenating two positions on the substrate.
2. Dorrestein, P.C., Zhai, H., McLafferty F.W., and Begley, T.P. The Biosynthesis of the Thiazole Phosphate Moiety of Thiamin (Vitamin B1): The Sulfur Transfer Mediated by the Sulfur Carrier Protein ThiS. **Chemistry and Biology.** (2004) 11(10), 1373-81. PMID: 15489164
   A post-translationally modified thiocaxbocylated ubiquitin-like protein transfers its sulfur to the substrate that is covalently linked to a second protein thiazole synthase. Furthermore during the catalytic rearrangement of the sugar substrate one of its hydroxyl groups ends up on the C-terminal end of the ubiquitin protein.
3. Dorrestein, P.C., Blackhall, J., Straight, P.D., Fischbach, M.A., Garneau-Tsodikova, S., Edwards, D.J., McLaughlin, S., Lin, M., Gerwick, W.H., Kolter, R., Walsh, C.T., Kelleher, N.L. Activity Screening of

Carrier Domains within Nonribosomal Peptide Synthetases Using Complex Substrate Mixtures and Large Molecule Mass Spectrometry. **Biochemistry** (2006) 45(6), 1537-1546. PMID: 16460000
In this paper I demonstrated that high-resolution mass spectrometry can be used to screen for the substrate of non-ribosomal peptide synthetases, proteins involved in the biosynthesis of many of our therapeutic agents (e.g. vancomycin and penicillin.)

4. Dorrestein, P.C., Van Lanen, S. G., Li, W. Zhao, C., Deng, Z., Shen, B., Kelleher, N.L., The Bifunctional Glyceryl Transferase/Phosphatase OzmB Belonging to the HAD Superfamily That Diverts 1,3-Bisphosphoglycerate into Polyketide Biosynthesis. **Journal of the American Chemical Society** (2006) 128 (32), 10386–10387. PMID: 16895402
In this paper we demonstrated that bis-phosphoglycerate, a primary metabolite, is diverted into polyketide biosynthesis via the formation of a covalent intermediate and phosphatase activity by a bifunctional enzyme.
5. Dorrestein P.C., Bumpus S. B., Calderone C. T., Garneau-Tsodikova S., Aron Z. D., Straight P.D., Kolter R., Walsh C. T. and Kelleher N. L. Facile Detection of Acyl- and Peptidyl- intermediates on Thiotemplate Carrier Domains via Phosphopantetheinyl Elimination Reactions During Tandem Mass Spectrometry. **Biochemistry** (2006) 45 (6), 1537 -1546. PMID: 17042494
This paper describes the use of a gas phase rearrangement for the analysis of phospho-pantetheinylated proteins. This assay is becoming the assay of choice for many of the top labs that are investigating these type of systems. Just in the last year, 4 PNAS, 4 Science and 1 Nature article have used this assay, highlighting the utility of this assay.
6. Calderone, C.T., Kowtoniuk, W.E., Kelleher, N.L., Walsh, C.T., Dorrestein, P.C. Convergence of isoprene and polyketide biosynthetic machinery: isoprenyl-S-carrier proteins in the pksX pathway of *B. subtilis*. **Proc. Natl. Acad. Sci. USA.** (2006) 103, 8977-8982. PMID: 16757561
In this paper we show that an HMG-CoA reductase machinery, normally involved in the biosynthesis of a cholesterol precursor, is utilized in converting a polyene into a methylated polyene.
7. Simmons, T. L., Coates R.C., Clark B. R., Engene N., Gonzalez D., Esquenazi, E., Dorrestein P. C., Gerwick W. H. Biosynthetic Origin of Natural Products Isolated from Marine Microorganism-Invertebrate Assemblages. **Proc. Natl. Acad. Sci. USA.** (2008) 25,105, 4587-94. PMID: 18250337
In this article we demonstrated the proof-of-principle that imaging mass spectrometry can be used to look at natural products in a spatial fashion from microorganisms.
8. Esquenazi, E., Coates, C., Simmons, L., Gonzalez, D., Gerwick, W.H., Dorrestein, P.C. Visualizing the spatial distribution of secondary metabolites produced by marine cyanobacteria and sponges via MALDI-TOF imaging. **Molecular BioSystems.** (2008) 4, 562-570. (cover article) highlighted in analytical chemistry and chemical biology. PMID: 18493654
In this article we demonstrated that imaging mass spectrometry can be used to peer into the heterogenous chemical microenviroments of complex marine assemblages.
9. Ng,J., Bandeira, N.,Liu, W.T. Ghassemian, M., Simmons, T. L., Gerwick, W.H., Linington, R., Dorrestein P.C., Pevzner, P., Dereplication and De Novo Sequencing of Nonribosomal Peptides. **Nature Methods**, (2009) 6(8),596-9. PMID: 19597502
This article describes three different developments to structurally characterize complex peptides by mass spectrometry. 1) It describes the development of databse searches against a in silico generated library. 2) It describes a method for de novo sequencing cyclic peptides with non-canonical amino acids. 3) the paper describes a novel mass taggin approach for non-canonical amino acids.
10. Yang, Y.L., Xu, Y., Straight, P., Dorrestein, P.C., Translating metabolic exchange with imaging mass spectrometry. **Nature Chemical Biology** (2009, accepted, online Nov 08, 2009). No PMID available yet. *This paper describes the observation of multiplexed metabolic exchange by imaging mass spectrometry between organisms and demonstrated that microorganisms not only secrete natural products to kill other organisms but they also secrete them to silence the defensive arsenal of a second species. This approach enables us to uncover the hidden world of metabolic exchange as if we had molecular eyes.*

C. Research Support

Beckman foundation (PI) 09/01/08-09/01/11. R01GM086283-01 (PI) 09/01/08-08/30/11. NIH R01 GM085128-01 (subcontract) 08/01/08-07/31/13. V-foundation (PI) 10/30/08-10/30/10. Hearst Foundation (PI).

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
* First Name: Pieter
Middle Name:
* Last Name: Dorrestein
Suffix: Ph.D.

* New Investigator? ☒ No ☐ Yes

Degrees: Ph.D.

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix:
* First Name: Andrea
Middle Name:
* Last Name: Rollins
Suffix:

* Phone Number: (858)822-4109
Fax Number: (858)822-0834
Email: vchsgrants@ucsd.edu

* Title: HSSPPO Director

* Street1: 9500 Gilman Drive
Street2: MC 0742
* City: La Jolla
County: San Diego
* State: CA: California
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 92093-0742

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4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/registry/index.asp. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Tracking Number:GRANT10470360 Funding Opportunity Number:RFA-GM-10-009 Received Date:2009-11-16T13:58:59-04:00

PHS 398 Modular Budget, Periods 1 and 2

OMB Number: 0925-0001

Budget Period: 1

Start Date: 07/01/2010 End Date: 06/30/2011

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	175,000.00
Consortium F&A	
* Total Direct Costs	175,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	On campus rate, modified total direct costs	54.5	154,561.00	84,236.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Region IX, 415-437-7820

Indirect Cost Rate Agreement Date: 05/28/2004

Total Indirect Costs: 84,236.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 259,236.00

Budget Period: 2

Start Date: 07/01/2011 End Date: 06/30/2012

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	175,000.00
Consortium F&A	
* Total Direct Costs	175,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	On campus rate, modified total direct costs	54.5	152,517.00	83,122.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Region IX, 415-437-7820

Indirect Cost Rate Agreement Date: 05/28/2004

Total Indirect Costs: 83,122.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 258,122.00

PHS 398 Modular Budget, Periods 3 and 4

Budget Period: 3

Start Date: 07/01/2012 End Date: 06/30/2013

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	175,000.00
Consortium F&A	
* Total Direct Costs	175,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	On campus rate, modified total direct costs	54.5	150,269.00	81,897.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Region IX, 415-437-7820

Indirect Cost Rate Agreement Date: 05/28/2004

Total Indirect Costs: 81,897.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 256,897.00

Budget Period: 4

Start Date: 07/01/2013 End Date: 06/30/2014

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	175,000.00
Consortium F&A	
* Total Direct Costs	175,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	On campus rate, modified total direct costs	54.5	147,796.00	80,549.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Region IX, 415-437-7820

Indirect Cost Rate Agreement Date: 05/28/2004

Total Indirect Costs: 80,549.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 255,549.00

PHS 398 Modular Budget, Periods 5 and Cumulative

Budget Period: 5

Start Date: | End Date:

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	
Consortium F&A	
* Total Direct Costs	

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.				
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number)

Indirect Cost Rate Agreement Date | Total Indirect Costs

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($)

Cumulative Budget Information

1. Total Costs, Entire Project Period

*Section A, Total Direct Cost less Consortium F&A for Entire Project Period	$	700,000.00
Section A, Total Consortium F&A for Entire Project Period	$	
*Section A, Total Direct Costs for Entire Project Period	$	700,000.00
*Section B, Total Indirect Costs for Entire Project Period	$	329,804.00
*Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period	$	1,029,804.00

2. Budget Justifications

Personnel Justification	1241-Budgetjustification1115.p	Add Attachment	Delete Attachment	View Attachment
Consortium Justification		Add Attachment	Delete Attachment	View Attachment
Additional Narrative Justification		Add Attachment	Delete Attachment	View Attachment

Budget justification.

Dr. Pieter Dorrestein, PhD. PI ***0.6 calendar month***

Pieter C. Dorrestein is an assistant professor at the Skaggs School of Pharmacy and Pharmaceutical Sciences and the departments of Pharmacology, Chemistry and Biochemistry. In addition, he is a member of the Moores Cancer Center and the Center for Marine Biotechnology and Biomedicine at the Scripps Institution of Oceanography. Dorrestein currently has a 6.4T Thermo LTQ-FT-ICR-MS with nanospray and DESI ion sources and MALDI-TOF system for use in his research program. Dr. Dorrestein's research involves the use of novel mass spectrometric approaches and proteomics to solve problems related to the biosynthesis of therapeutics of natural origin, his laboratory develops of novel mass spectrometry based methods that enable the characterization of naturally occurring therapeutics, his laboratory also identifies the targets of natural products that are in the therapeutic development pipeline and his laboratory investigates the functional roles of novel post-translational modifications as a result of therapeutic stimulation.

TBN. Post-doctoral fellow ***12 calendar months***

A post-doctoral fellow in Dorrestein lab. This post-doc will get familiar with the methodologies to analyze the samples. A major aspect of this post-doc will be the dereplication to know molecules and/or structural elucidation of the most interesting ions that are observed in these images.

Jeramie Watrous, Graduate student ***6 calendar months***

The development of real time mass spectrometry methods to monitor metabolic exchange is Jeramie's thesis project. He was trained as a chemist and worked for Watson company after graduation. Here he was responsible for the analytical support for NDI filings. Since his arrival to my laboratory a year ago he has been a creative force. For his 4-week rotation project I asked him to define the mycobacterial pupylome and succeeded, this paper just got accepted. Further more he is a co-author on an imaging review that will be featured on the front cover of Natural Product Reports. He is now becoming familiar with DESI and the first images are incredible and will submit a paper on this shorly, however the step will be real time imaging, the subject of this proposal. This will take funds and time to get established. Should this grant get funded, Jeramie can devote 100% of his time to this effort.

Jane Yang, Graduate student ***3 calendar months***

Jane's project is on the quantitative analysis of metabolic exchange and the development of novel mass spectrometric approaches to define the ions we observe. 50% of Jane's time will be devoted to the efforts outline in this grant.

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OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page and PHS398 Checklist. The responses provided on these pages, regarding the type of application being submitted, are repeated for your reference, as you attach the appropriate sections of the research plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)		Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1242-Aims.pdf	Add Attachment	Delete Attachment	View Attachment
3. Background and Significance		Add Attachment	Delete Attachment	View Attachment
4. Preliminary Studies / Progress Report		Add Attachment	Delete Attachment	View Attachment
5. Research Design and Methods	1243-Researchdesignandmetho	Add Attachment	Delete Attachment	View Attachment
6. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
7. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

Attachments 8-11 apply only when you have answered "yes" to the question "are human subjects involved" on the R&R Other Project Information Form. In this case, attachments 8-11 may be required, and you are encouraged to consult the Application guide instructions and/or the specific Funding Opportunity Announcement to determine which sections must be submitted with this application.

8. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
10. Targeted/Planned Enrollment		Add Attachment	Delete Attachment	View Attachment
11. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

12. Vertebrate Animals		Add Attachment	Delete Attachment	View Attachment
13. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
14. Multiple PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
15. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
16. Letters of Support		Add Attachment	Delete Attachment	View Attachment
17. Resource Sharing Plan(s)		Add Attachment	Delete Attachment	View Attachment

18. Appendix Add Attachments | Remove Attachments | View Attachments

Title: Real-Time Imaging of Metabolic Communication.

Aim: *In this proposal we aim to develop of a mass spectrometry based approach to observe and characterize multiplexed metabolic exchange in real-time.* While processes in metabolic exchange have tremendous pharmacological importance, there is currently no technology capable of observing them in real-time. Metabolic exchange defined as secreting molecules or recognizing secreted molecules that mediate a message from one cell population to the next, is a universal phenomena to all life. Examples of metabolic exchange are the release of chemokines and cytokines upon pathogen invasion, the release of virulence factors, the secretion of antibiotics or quorum sensing by microbes. The molecules involved in these messages are very diverse ranging from small molecules such as hydrogen peroxide, nitric oxide, calcium, potassium ions but also lipids, natural products and polynucleotides, peptides and proteins. Our aim is to develop mass spectrometry based approaches to capture this enormous diversity with cells grown in culture. *Our ability to visualize metabolic exchange in real-time will largely depends on creative sample preparation and the use of modern ionization strategies such as desorption electrospray ionization (DESI) combined with imaging methodologies.* Preliminary results have already demonstrated that imaging mass spectrometry can capture both the molecular diversity as well as the spatial distribution of metabolic exchange. The key feature of this proposal is to extend these applications to real-time and demonstrate its applicability to investigate the metabolic exchange involved in microbe-immune responses. These developments would lead to the first tools that enable the direct observation of the multiplexed nature of metabolic exchange in real-time. Progress for the EUREKA proposal will be monitored via the following set of 3 sub aims.

Sub aim a. Design a DESI-stage to support the cell cultures for real-time imaging mass spectrometry. Our current DESI system is not able to handle Petri-dishes. In the first few months we will adapt our current DESI system to accommodate Petri-dishes. Should this not be sufficient for the observation of molecules involved in metabolic exchange one solution may be the modification of the target plate to contain a large number of permeable grids ~100 µm spacings to which the growth media and cells can be added to retain the spatial information during the DESI process.

Sub aim b. Develop semi-rigid media for macrophage growth (e.g. RAW or THP1 cells) and bacterial growth. One of the challenges in this proposal is to develop universal culturing conditions to prevent the washing effect often seen with DESI. There are three approaches that will be investigated. First, we will optimize the conditions to grow macrophages, much like some tumor cell lines, in an agar media. Second, there are a variety of cell support matrices (e.g. agar based, cellular support matrices) that can be used for solid support of the macrophages, we will assess if these matrices be amenable to DESI. Finally, collagen coating or tack coating of surfaces may enable the cells to partially activate and adhere to different surfaces and we will evaluate if this support enables DESI without washing away the individual cells. Once macrophage growth conditions have been developed that are DESI tolerant, the system will be evaluated to support growth of microbes on the same or slightly adjusted media support. We expect that this last step will develop without too much trouble.

Sub aim c. Identify exchange factors involved in metabolic exchange between a *Bacillus sp.* and macrophages and *Pseudomonas sp.* and macrophages. Using the methodologies developed in sub aim a and b, the interactions of these organisms will be monitored in a real-time fashion. We picked these two organisms as they are known to metabolically interact with the immune system.[1,2] The most interesting candidates that appear to regulate the morphology of the macrophages or chemokine/ cytokine production will be subjected to isolation and dereplication to known molecular entities or their structures will be determined *de novo*, if unknown.

1. The Challenge: Metabolic exchange, including communication is a universal phenomenon. It is essential to every organism, from those as simple as bacteria to complex higher eukaryotes such as humans. While metabolic communication enables cooperation and coordination between the ~70 trillion cells in an average human being, even unicellular organisms rely on metabolic communication to adapt to environmental stress and form biofilms. Cellular communication allows stem cells to differentiate, cancer cells to proliferate, neurons to fire, bacteria to sense a quorum and pathogens to survive in human hosts. The chemical diversity of the molecules used for communication is extraordinary, and includes small ions such as calcium, small molecules such as secondary metabolites, fatty acids, peptides, but also carbohydrates, proteins and nucleic acids. Despite the universal nature of metabolic exchange, there are few methods that can characterize the communication between cells in a systematic and sensitive fashion. Instead, many traditional approaches involve characterizing intracellular response to single factors, which fails to capture the dependences and synergy from the convergence of multiple factors on a cell. Therefore the challenge of this proposal is the development of systematic tools that can capture the multiplexed nature of the metabolic response from complex interactions, such as an immunological stimulation by microbes, in both a spatial fashion and real-time.

2. Potential Impact: In this proposal, our focus will be on the application and adaptation of desorption electrospray mass spectrometry to enable the real-time live cell detection and the characterization and visualization of metabolic exchange in important biological processes. We aim to accomplish this in both a spatial as well as temporal fashion. These tools will improve our understanding of secreted biomarkers, microbiome-human cell interactions and understanding the complexities of infectious disease that derive from the cooperation between different types of cells (e.g. *Bacilli* with macrophages, neutrophils or T-cells) and interkingdom communication. Ultimately it may drive the development of new therapeutic strategies or interventions based on paradigms involving inter-cellular metabolic communication in a system wide fashion.

3. The Approach (3 pages): ***Exploring metabolic exchange with mass spectrometry.*** DESI-imaging mass spectrometry technologies will be adapted for the analysis of important biological processes involving real-time communication in a systematic fashion. These technologies will be indifferent to the cell type and growth surface, and will be performed on living cells. Our goals for the development of these mass spectrometry approaches are multifold but *one important criterion is that after the mass spectrometric analysis, the cells remain viable so that they can be subjected to tissue culturing, life cell imaging, or be used for other experiments such as expression studies with RT-PCR.* Currently, our laboratory is exploring communication in bacteria via different mass spectrometry means. For these studies, our laboratory has developed a MALDI-imaging based method that enables the visualization of the communication molecules of organisms in culture.[3-6] MALDI-TOF imaging, a mass spectrometry method fine-tuned by the lab of Caprioli, is an approach that enables control of the movement of the MALDI plate in the x-y direction, allowing the MALDI-laser to raster a sample.[7,8] The resulting collection of 100s to 1000s of spectra, collected as a grid, create a 2 dimensional mass spectrometry profile. Any ion observed in these spectra can be spatially visualized with a color and superimposed on an optical image of the sample. The MALDI-IMS approach for the visualization of the natural products involved in bacterial communication has now been adapted to cells grown on a thin layer of agar directly on top of the MALDI-target plate (Figure 1A).[9] However MALDI is a destructive method of ionization and therefore meaningful follow-up experiments are impossible. However ambient mass spectrometry approaches such as Desorption Electrospray Ionization (DESI), has proven itself to be a gentle method to ionize molecules.[10-12] In this proposal, we will adapt DESI to analyze secreted molecules. DESI is particularly well-suited to studies of metabolic exchange because it can be applied to most surfaces and it leaves cells intact. [11,12]

DESI ionizes molecules by spraying charged droplets onto a physical surface (Figure 1B). Upon impact, molecules present on the surface are ionized and then introduced into the atmospheric inlet of a mass spectrometer.[11] DESI-imaging is accomplished by moving a sample in an x-y direction under the DESI ionization source in a pre-defined fashion. The resulting mass spectra are binned at preset intervals to create a raster. Throughout the raster grid, different mass spectra are acquired and the ions of interest are displayed in color and

superimposed onto a picture of the sample. As the abundance of the molecular ion at a given position of the raster increases, so does the intensity of the color displayed, allowing for visualization of the distribution of ions.[13-15]

We are going to use DESI and DESI-imaging on an LTQ-FT-ICR-MS to analyze samples with limited sample preparation. How this will be accomplished is shown in Figure 1C. We plan on investigating communication within bacteria, in bacteria-host interactions and in human-cellular communication. For example we will culture two different cell types on biological Petri-dishes. We anticipate that additional cell support of the cells is needed so that the DESI-spray does not wash the cells away. The mass spectrometry on these samples will be carried out in an analogous fashion as was done with the MALDI-IMS except that we do not need to optimize the culture conditions to be amenable to mass spectrometry as DESI can be used to look at nearly any surface. These biological plates are then placed under a DESI-source that can be controlled in an x-y-direction. We will have to modify the DESI source to accommodate such plates and to keep them fixed in place during an imaging run. The required alterations to the DESI ion source will be performed at the UCSD machine shop. The resulting signals of interest will be represented with color (step 5, Figure 1C) and superimposed on a picture of the sample to enable the visualization of the communication molecules in a spatial fashion.

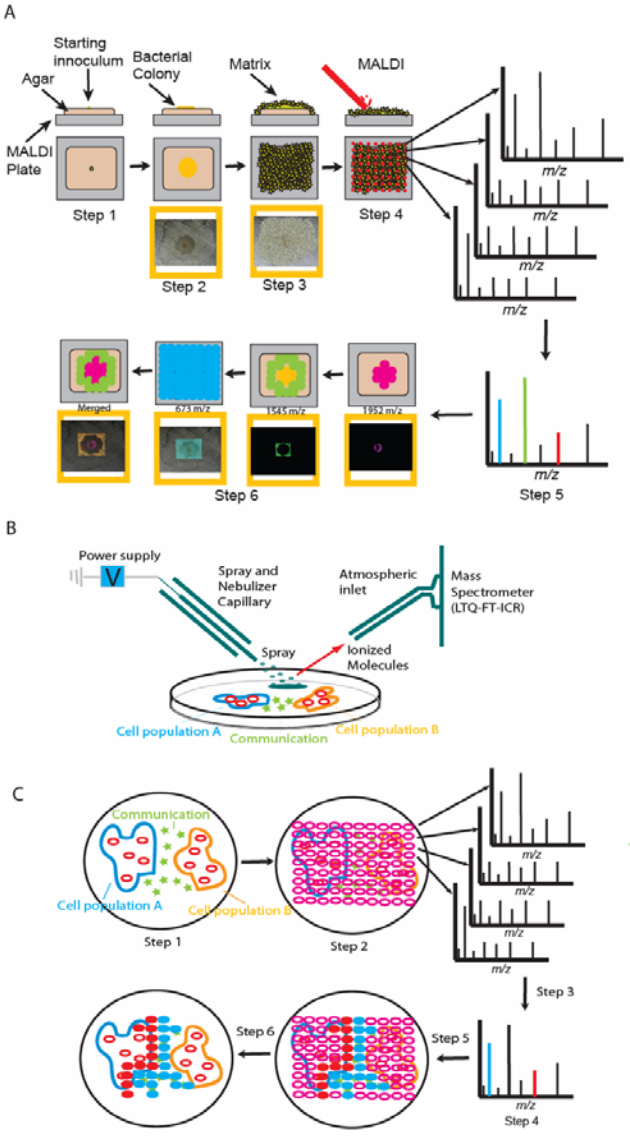

Figure 1. An overview of the thin layer MALDI imaging and DESI-imaging. A) Schematic of the thin layer agar MALDI-IMS approach. In step 1, a thin film of agar is deposited on top of the MALDI target plate. Step 2, a small inoculum of *Bacillus* was allowed to grow and then covered by Matrix and dried (step 3). In step 4, the sample is subjected to MALDI-imaging in predefined x-y movements and all the spectra are averaged. The different ions observed by MALDI-imaging are displayed with different colors and superimposed on a picture of the sample. B) Schematic of a DESI-imaging set-up for the visualization of metabolic communication. C) A schematic of how DESI-imaging may be applied to the observation of communication molecules. In step 1 we have a standard Petri-dish on which colonies of cells are grown (or a tissue slice on a standard glass slide). In step 2 DESI-imaging will be performed in a predefined raster. Following the collection of the mass spectra, ions are displayed with a color and superimposed onto a photograph of the sample that was analyzed (step 3-6).

Proof-of-concept that imaging mass spectrometry will enable us to visualize metabolic exchange. Many therapeutic agents in clinical use, such as penicillin, vancomycin or taxol, come from microbial sources, yet we know surprisingly little about the biological function of these natural products. However, it has become clear that bacteria communicate with natural products. Some natural products are required for virulence, quorum sensing, they may serve as defense mechanisms or they may be involved in other forms of bacterial communication.[16-18] The sporulation killing factor (skf) gene cluster was identified in 2003 and is involved in the cannibalistic behavior of *B. subtilis*.[19] The skf cluster is found in a domesticated strain called Py79. Cannibalistic behavior was dependent on Spo0A, a transcriptional regulator that is activated when spore formation is initiated. Spo0A activated cells up-regulate skfABCDEFGH resulting in the production of a secreted factor (skf) that lyses cells without Spo0A (Fig. 2)—lysis of sister cells provides nutrients for the survivors.

To date, the mature form of the killing factor remains undetected and uncharacterized even though *B. subtilis* is a model gram-positive organism. Obviously, the precise biological function of skf cannot be evaluated until the mature form is available. We therefore employed our MALDI-IMS approach and scanned a colony of Py79 adjacent to a colony containing a Spo0A deletion strain and observed many ions, including 2782 (Figure 2).[21] Fragmentation of the 2782 ion by TOF-TOF enabled us to obtain a sequence tag (Leu-Pro-His-Pro-Ala) that is also found in the gene product SkfA. To confirm the genetic source of the 2782 Da ion, a strain of Py79 with IPTG inducible expression of the skf gene cluster was subjected to MALDI-IMS (Figure 2c).This example demonstrates that IMS can be used to understand and discover molecular entities involved in metabolic exchange.

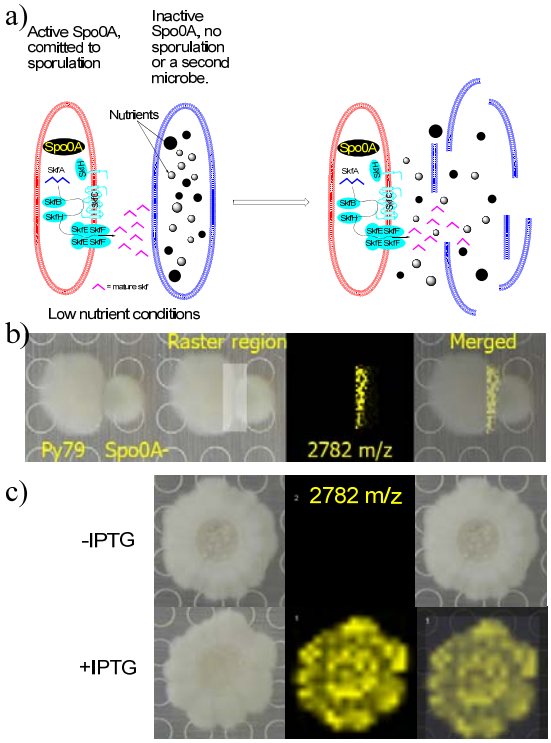

Figure 2. Identification of the cannibalistic peptide, skf, from *B. subtilis*. a) Schematic of cannibalistic behavior. b) MALDI-IMS of *B. subtilis* Py79 and the *Spo0A* deletion strain. The two strains were grown together. The image of the killing factor is shown in yellow. c) IPTG induction of the skf gene cluster in engineered Py79. The signal at 2782 is dependent on IPTG and is the same ion as that in panel b.

Potential advantages of the DESI-imaging approach. While the thin layer MALDI-IMS approach provided the first indication that imaging mass spectrometry can be used to observe communication molecules it is limited in its application due to its destructive nature. DESI-imaging on an LTQ-FT-ICR mass spectrometry instrument overcomes many of these limitations. Some of the limitations of MALDI-IMS are listed next. First, the sample is covered with matrix, introducing many low *m/z* background signals. Secondly, the sample needs to be thin enough to fit into the instrument and tolerate the passage of the acceleration voltage required for the time-of-flight detection. The requirement for the sample to be covered with matrix, the drying of the sample, and the thickness of the dried thin film agar impose severe limitations on the types of cells that can be analyzed. The adaptation of DESI-imaging does not require a matrix, thereby eliminating many high intensity background signals that obscure ions in the lower mass range (<1000 m/z), the region where many of the secondary metabolites, quorum sensing molecules, pheromones, hormones, peptides etc. involved in metabolic exchange are observed. With DESI-imaging, the sample does not need to be dried nor is there an acceleration voltage that needs to be applied to the target plate thus enabling DESI to be used with any media or surface. For example, all attempts to grow and visualize human cells in conjunction with *Pseudomonas aeruginosa* PAO1 to provide insight into the pathogen-host interactions by MALDI-imaging have failed. These experiments were unsuccessful because the human cells were unable to grow on the thin layer agar, although the PAO1 strain grew very well and was readily imaged. DESI-imaging also eliminates the severe consequences that desiccation has on cells being analyzed. An additional advantage is that DESI does not destroy the sample enabling experiments that are currently not possible. Thus it should be possible to do DESI-imaging on human cells, and localize a region of interest based on a signal that we believe is present and perhaps upregulated (e.g. the presence of secreted cytokines or small molecules, "the secretome", in the matrix). We anticipate that DESI should be gentle enough so that the cells should remain viable and they can be isolated for additional investigations such as RT-PCR or sub-culturing as it has been demonstrated that cells and surfaces remain intact after subjecting them to DESI.[21] Because MALDI is destructive, the same sample cannot be visualized in a temporal fashion. However with DESI-imaging it should be possible to analyze “the secretome” in real time, enabling one to monitor changes from the beginning of the co-culture to steady state, as the cells transform each other into a cooperative network.

Enabling the visualization of cellular events is important to provide a complete understanding of processes such as inflammatory responses to microbes. One of the challenging aspects of MALDI-imaging that is currently employed in the Dorrestein laboratory, is the structural identification of peptides or small molecules that are observed by imaging with tandem mass spectrometry. The interface of the DESI-source with an LTQ-FT-ICR-MS will overcome this problem because MS/MS is readily achieved in the linear trap portion of the instrument. Such an interface will enable us to obtain high-mass accuracy (~1 ppm) and high-resolution data (up to 500,000), enabling the generation of molecular formulas for all of the small molecular weight ions that are observed. In addition, tandem mass spectrometry is readily accomplished with CID in both low-resolution and high-resolution settings. This is important for the thorough characterization of unknown ions observed in the imaging runs. For all these reasons, real time DESI-imaging, if successful, will become a powerful enabling technology, especially compared to the current capabilities we have available to investigate multiplexed metabolic exchange.

4.0 The Appropriateness of EUREKA: The current proposal is appropriate for EUREKA as it will initiate the development of the tools necessary to study of molecules involved in metabolic exchange in a system wide or multiplexed fashion as opposed to individual entities. It is anticipated that the synergy between multiple factors involved in metabolic exchange can be captured using the approaches described in this proposal. Traditional NIH funding mechanisms do not fund the development of such tools especially when they are at such an early stage of the development and not targeted towards just one disease state. Nor do any of my current grants fund this type of work. The approaches outlined in this proposal, if successful, will have wide ranging implications. It may enable the understanding of multi-factorial communication in pathogen invasion and inflammatory processes but could ultimately be used in research beyond the scope of this proposal such as our understanding of our microbiome, stem cell differentiation or cancer proliferation. While we have shown that imaging mass spectrometry can capture metabolic exchange and be used to discover new molecular entities, extending this ability to live cells with the aim of capturing only the "secretome" in real-time would be a major advancement in the application of mass spectrometry to investigate organismal processes in a dynamic fashion. Typically, molecules involved in metabolic exchange have important biological and therapeutic functions (e.g. penicillin, vancomycin, rapamycin, cytokines, chemokines etc.) yet there are no tools available to truly study the synergistic production and effects of such molecules in a real-time and spatial fashion. As a part of this proposal we aim to expand IMS with these capabilities and demonstrate its effectiveness in the metabolic communication of microbe-microbe and microbe-immune cell (e.g. neutrophils, macrophages and T-cells) interactions.

5.0 The Likelihood of Success: The success of this proposal-the real-time detection of molecules involved in metabolic exchange- is not guaranteed. This is one of the reasons why this proposal is submitted as a EUREKA mechanism. It is clear from the MALDI imaging mass spectrometry in my laboratory that the search for important molecules involved in metabolic exchange is not "searching for a needle in a haystack" even though secretomes are complex mixtures of molecular entities. For example, using non real-time IMS approaches, we discovered the *Bacillus* sporulation killing factor, the sporulation delaying factor, established that >90% of all *Streptomyces* secrete a lantibiotic type peptide and established that microorganisms secrete antibiotic-type compounds to silence the defensive arsenal of surrounding organisms.[6] We are now adapting the MALDI based approaches to search for microbial molecules that regulate the inflammatory process. The present work is aimed at obtaining static pictures of metabolic exchange. However organisms are dynamic entities and therefore real-time monitoring using DESI would be an important development in our understanding of the functional roles of multi-factorial communication between cell populations. There are three aspects of this proposal that will require extensive development before it can be successful. 1) The development of the DESI parameters and cell growth conditions (e.g. matrix or agar embedded macrophages) so that cell populations and their metabolic output can be imaged without washing away. 2) The optimization of the DESI parameters enabling the detection of secreted molecules from a culture without killing the cells themselves enabling real-time analysis. 3) The adaptation of these approaches to the investigation of the interaction of multiple cell populations (e.g. *Bacilli*/ macrophage) in communication. Each one of the aspects will be an important milestone in the application of mass spectrometry to the investigation of cells in culture. Based on these milestones we anticipate that it will take 3-4 years to successfully develop a robust pipeline for real-time imaging of metabolic exchange.

6.0 Timeline and Milestones: The timeline and milestones are indicated in the table below.

In year one, our Prosilia DESI source and sample inlet will be adapted to hold culturing plates. Once the DESI can hold the plates, the culturing conditions for DESI analysis will be optimized. We anticipate that this is the most challenging aspect of the project. Next, the real-time DESI conditions will be developed. During this process we aim to observe the activation of virulence factors by *Pseudomonas*, the secretion of probiotic factors by *Bacilli*. Care will be taken to evaluate the effect that DESI has on the growing cells by monitoring morphology changes, and following SOS responders with fluorescent tags. Finally, we will perform transcriptional profiling or RT-PCR to verify that the response with DESI is identical or altered. Finally any interesting molecular candidates involved in the immune response (either by the macrophage or the Bacterial cell) we identify using the DESI approach will be structurally characterized. For this purpose mass spectrometry guided isolation will be performed using extractions, HPLC, gel filtration or other chromatographic methods as was done for the sporulation killing factor by *B. subtilis*. Many of these interactions are mediated by complex peptides and they will be analyzed with algorithms we have developed for this purpose in conjunction with the Pevzner lab. Below is a table that describes the timeline and progress of each of the steps.

Year											
1			2			3			4		
Design a DESI-stage											
	Optimize conditions for macrophage and bacterial growth for DESI										
				Real-time metabolic exchange between a *Bacillus sp.* and macrophages .							
					Structural characterization of factors involved in metabolic exchange.						
					Real-time metabolic exchange between *Pseudomonas sp.* and macrophages.						
						Structure characterization of factors involved in metabolic exchange.					

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PHS 398 Checklist

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

OMB Number. 0925-0001
Expiration Date: 9/30/2007

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. Assurances/Certifications (see instructions)

In agreeing to the assurances/certification section 18 on the SF424 (R&R) form, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the agency's application guide, when applicable. Descriptions of individual assurances/certifications are provided at: http://grants.nih.gov/grants/funding/424

If unable to certify compliance , where applicable, provide an explanation and attach below.

Explanation: Add Attachment | Delete Attachment | View Attachment