OMB Number: 4040-0010
Expiration Date: 12/31/2022

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R) **Use Current Standard SF 424 Form**

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

○ Pre-application ● Application ○ Changed/Corrected Application

4.a. Federal Identifier
[redacted]

b. Agency Routing Number

2. DATE SUBMITTED
2021-06-15

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: [redacted]

Legal Name*: [redacted]
Department: [redacted]
Division: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*:
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: First Name*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix: [redacted]
Position/Title: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*:
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

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*
Orbitrap Exploris 480 Mass Spectrometer for Biomedical Research

12. PROPOSED PROJECT
Start Date* 02/01/2022
Ending Date* 01/31/2023

13. CONGRESSIONAL DISTRICTS OF APPLICANT
[redacted]

Use Current PAR Opportunity Number

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr First Name*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix:

Position/Title: [redacted]

Organization Name*: [redacted]

Department: [redacted]

Division: [redacted]

Street1*: [redacted]

Street2:

City*: [redacted]

County: [redacted]

State*: [redacted]

Province:

Country*: [redacted]

ZIP / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$881,950.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$881,950.00
d. Estimated Program Income*	$0.00

16.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

17. 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.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix: [redacted]

Position/Title*: [redacted]

Organization Name*: [redacted]

Department: [redacted]

Division:

Street1*: [redacted]

Street2:

City*: [redacted]

County: [redacted]

State*: [redacted]

Province:

Country*: [redacted]

ZIP / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative*

[redacted]

Date Signed*

06/15/2021

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter1029115345.pdf

424 R&R and PHS-398 Specific
Table Of Contents

SF 424 R&R Cover Page ... 1
Table of Contents ... 3
Performance Sites ... 4
Research & Related Other Project Information ... 5
Project Summary/Abstract(Description) ... 6
Project Narrative ... 7
Bibliography & References Cited ... 8
Equipment ... 9
Other Attachments ... 18
Instrumentation_Plan1029115372 ... 18
Letters_of_Support1029115347 ... 64
Research & Related Senior/Key Person ... 67
PHS398 Cover Page Supplement ... 149

OMB Number: 4040-0010
Expiration Date: 12/31/2022

Project/Performance Site Location(s)

Project/Performance Site Primary Location

○ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: [redacted]
Duns Number: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: [redacted]

Additional Location(s) File Name:

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: — 1 — 2 — 3 — 4 — 5 — 6 — 7 — 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

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 - positive or negative - 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. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	[redacted]
8. Project Narrative*	[redacted]
9. Bibliography & References Cited	[redacted]
10.Facilities & Other Resources	
11.Equipment	[redacted]
12. Other Attachments	[redacted]

Funding Opportunity Number: PAR-21-126. Received Date: 2021-06-15T17:17:18.000-04:00

Abstract

This grant application requests funds to purchase a Thermo Scientific Orbitrap Exploris 480 and UltiMate 3000 RSLCnano HPLC, and FAIMS (high field asymmetric ion mobility spectrometry) interface for shared use in the [redacted] The requested system will provide enhanced capabilities and expanded capacity for protein identification and relative quantification as well as valuable information for structure determination of proteins and protein complexes. Project summaries for 14 investigators are presented, with major focus on cancer and infectious disease (HIV, TB and SARS-CoV-2). The global proteomics experiments proposed by the user group will be conducted by data-independent acquisition mass spectrometry (DIA-MS). Use of this technique in the MSL over the past three years has demonstrated its extraordinary capabilities for comprehensive protein identification with greater accuracy for relative quantification, in an efficient and remarkedly reproducible manner. DIA-MS is being used in the MSL for numerous studies requiring discovery proteomics [including analysis of proteins from cells, tissues, secretomes, alveolar lining fluid (ALF)] and for interactomics. Cross-linking-MS is a new service that will be offered by the MSL; it will be an important partner for protein structure analyses conducted by cryo-electron microscopy in the new [redacted] In 2020, the user group of the [redacted] submitting samples for protein/peptide analysis consisted of 27 individual investigators in 17 departments at [redacted] in addition to 11 researchers from nine different outside institutions. Submissions to the [redacted] for protein identification/relative quantification dramatically increased when investigators became aware of the incredible power of DIA-MS. This resulted in a progressively growing backlog of DIA-MS samples. The level of interest in DIA-MS is evidenced by the support letters written for grant applications by [redacted]: >70% of the 35 letters written in 2020 discussed DIA-MS experiments (in most cases as the only MS-based technique being planned). DIA-MS analyses are currently being conducted on a Thermo Scientific Orbitrap Fusion Lumos mass spectrometer, yielding previously unprecedented numbers (>5,000) of protein identifications and relative quantifications for single injections of cell lysate samples. Results acquired in the Thermo "demo" lab showed that the requested Exploris outperformed the Lumos for DIA-MS analysis of aliquots of the same samples (6,665 vs 5,248 proteins). Acquisition of the requested Exploris is essential to handle the rapidly-expanding submissions and increasing backlog for DIA-MS in the MSL as well as to provide new capabilities for protein/protein complex structure analysis via cross-linking-MS. The analytical support that will be provided by the Exploris 480 and FAIMS interface will significantly advance the productivity of a large number of NIH-funded investigators studying important and diverse problems in biomedical research.

Project Narrative

Acquisition of the requested Exploris 480 mass spectrometer and accessories will advance the research efforts of a large number of investigators who study a wide range of biomedically-relevant problems. The projects described in the proposal focus on cancer and infectious disease [HIV, TB and SARS-CoV-2 (COVID-19)]. The Exploris 480 has extraordinary capabilities for efficient and accurate determination of protein identities and relative quantities (>6,000 proteins identified in a single analysis), providing critically-important information about biological processes as a way to understand mechanisms of disease development and progression, contributing to the discovery of new, more effective drug treatments.

Bibliography and References Cited



Equipment

The instrumentation requested from Thermo Scientific includes: Orbitrap Exploris 480 mass spectrometer, UltiMate 3000 RSLCnano HPLC, nanospray ion source, high field asymmetric ion mobility spectrometry (FAIMS) interface, nitrogen generator, Powervar uninterrupted power supply, and installation materials, as detailed below and in the attached quote. [redacted] An Omni Lab Solutions instrument table is also being requested (quote attached). List prices are shown for each line item and the discounts applied to the totals.

Item	Price
Instrument	
Orbitrap Exploris 480	$892,767.00
Accessories	
UltiMate 3000 RSLCnano HPLC and components	$100,570.33
Nanospray Flex ion source	$20,903.80
FAIMS Pro interface	$95,000
Nitrogen generator	$15,919.94
Powervar uninterrupted power source[2]	$12,346.78
Additional items needed for installation	$12,319.95
Onsite training, three days	$4,500.00
Subtotal	$1,154,327.80
Discounts and trade-in	-$283,431.56
Shipping	$5,400.00
Thermo Scientific subtotal	*$876,296.24*
Omni Lab Solutions instrument table	$5,665.00
Discount	-$566.50
Shipping	$555.00
Omni Lab Solutions subtotal	*$5,653.50*
Total	**$881,949.74**

[1]HPLC, autosampler, solvent rack, valve
[2]Powervar and distribution module

Contact PD/PI: [redacted]

Sales Quotation

Quote Number	Version	Quotation Date	Page
[redacted]	1.00	May 25, 2021	1
Lead Time	**Payment Terms**	**Freight Terms**	**Expiration Date**
[redacted]	NET 30 DAYS UPON INVOICE DATE	Origin - Prepay And Add	[redacted]
Company Name		**Customer Name**	
[redacted]		[redacted]	
* See end pages of quote for PO Submission details			

Thermo Electron North America LLC
5225 Verona Road
Madison WI 53711

1400 Northpoint Parkway
West Palm Beach FL 33407

An Orbitrap LTQ Velos Pro (SN03031B), installed in 2010 at Sue Weintraub's lab at UTHSC-San Antonio, is traded in for exchange of quote credit. The $30K trade-in credit has been reflected in the final discount of the Exploris 480 quote (CPQ-00359528).

Customer Info:		**Sales Contact Info:**	
Customer Name:	[redacted]	Contact Name:	[redacted]
Company Name:	[redacted]	Phone:	
Address:	[redacted]	Email:	[redacted]
Phone:	[redacted]		
Email:	[redacted]		

[redacted]

Item	Description	Unit Price	Qty	Total Price
1.1	BRE725533 Orbitrap Exploris 480 Basic System w/ IC S/N: SN03031B The Thermo Scientific™ Orbitrap™ Exploris 480 with EASY-IC is a high-performance, small-footprint benchtop quadrupole Orbitrap hybrid system that combines a high capacity transfer tubecapillary, an electrodynamic ion funnel, the most advanced quadrupole technology, an ion routing multipole, and Orbitrap analyzer. The high-field Orbitrap mass analyzer features high-resolution accurate mass analysis, with and without HCD fragmentation. The instrument includes the Thermo Scientific™ EASY-IC™ Ion Source generates internal calibrant ions for real-time mass calibration of any mass spectrum	$892,767.00	1	$892,767.00
1.2	FMS02-10001 FAIMS Pro The Thermo Scientific™ FAIMS Pro High Field Asymmetric Waveform Ion Mobility Spectrometry Interface FAIMS Pro provides an additional mode of separation after sample ionization and prior to ion introduction into the MS, and is beneficial for bottom-up, top-down proteomics, and quantitative proteomic workflows. FAIMS Pro is based on novel cylindrical electrodes with a minimized analytical gap utilizing N2 (15 L/min-Standby, 31 L/min-Max) as the separation gas while maintaining separation capability. Users can easily maintain FAIMS Pro due to the uniquely designed tool-free one-way assembly. The device is optimized for flow rates from 100 nl/min to 25 uL/min and is only compatible with Thermo Scientific TNG mass spectrometers. The kit contains Main Control Box, Housing adapter with connected RF coil box with bundled cable, and one electrode assembly. The FAIMS Pro ships separately and will be installed in the field.	$95,000.00	1	$95,000.00
1.3	BRE0017135 Merkur CountKit NA	$404.19	1	$404.19

Contact PD/PI: [redacted]

Sales Quotation

Quote Number	Version	Quotation Date	Page
[redacted]	1.00	May 25, 2021	[redacted]
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Company Name		**Customer Name**	
[redacted]		[redacted]	

* See end pages of quote for PO Submission details

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Madison WI 53711

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Item	Description	Unit Price	Qty	Total Price
1.5	ES072 Nanospray Flex Ion Source Kit (NG)	$20,903.80	1	$20,903.80
1.7	00109-99-00046 VALVE,2 POS,6PRT,RHEODYN MXT715-004,RoHS	$5,808.23	1	$5,808.23
1.8	OPTON-30962 GEN N2, GENIUS XE35,120V,35L MIN,ROHS	$15,919.94	1	$15,919.94
1.9	C0012-01-00077 Powervar UPS SPII 6.0kVA, RoHS	$11,352.80	1	$11,352.80
1.10	C0202-99-00182 PV PDU 2xL6-20R,2x6-20R,4x5-15R, NOT EU The PDU module for the Powervar UPS systems acts as a “powerstrip” with numerous plug receptacles. The L14-30R (receptacle on UPS) mates with the L14-30P (Plug on PDU) 10’ long power cord. The unique thing about this plug/receptacle combination is that it contains two hot legs, a neutral and a ground, so it’s a four conductor set up (“14”), allowing both 115 and 230 VAC to be distributed. The “30” stands for the current rating of the device. These receptacles allow the user to plug in all the power cords that belong to their LCMS system in one place and keep them protected from power surges and from short power failures “brown-outs” of the mains power to the building. The PDU can supply both 125V and 230V depending on the plugs being used. As a rule, any outlet that is a Nema 5-XX will provide 125 V output, while any Nema 6-XX will provide 230V output. Each PDU can be matched up with any of the LSMS Powervar Security Plus II UPS systems. The differences in the 3 PDUs that are offered relate to the different receptacles that each of our MS systems require. This PDU works in conjunction with any LSMS Powervar UPS system. It is designed for use with the Exploris 120 & 240, ID-X, Fusion, Fortis, Quantis, and Endura MS systems. It contains the following receptacles: • 4 x 5-15R rated at 15A (120V) • 2 x 6-20R rated at 20A (230V) • 2 x L6-20R rated at 20A (230V)	$993.98	1	$993.98
1.4	BRE0021870 Cal Solution, Flexmix, 2x10mL	$1,133.00	1	$1,133.00
1.6	701-054849 Trade-In Service Deinstallation Labor	$0.00	1	$0.00
1.11	702-022409 3 DAYS LSMS ONSITE SPPRT (EDU&GOV ONLY)	$4,500.00	1	$4,500.00

Sales Quotation

Quote Number	Version	Quotation Date	Page
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[redacted]	NET 30 DAYS UPON INVOICE DATE	Origin - Prepay And Add	[redacted]
Company Name		**Customer Name**	
[redacted]		[redacted]	

* See end pages of quote for PO Submission details

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Madison WI 53711

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West Palm Beach FL 33407

Orbitrap Exploris 480 Basic System w/ IC - Technical Minimum Bundle Subtotal: $1,048,782.94
Orbitrap Exploris 480 Basic System w/ IC - Technical Minimum Bundle Discount: - $230,732.25
Orbitrap Exploris 480 Basic System w/ IC - Technical Minimum Bundle Total: $818,050.69

Item	Description	Unit Price	Qty	Total Price
2.1	5041.0010A NCS-3500RS NANO PROFLOW, NANO-CAP-SYSTEM Combines binary gradient nano pump, ternary gradient micro pump, and column compartment; best-in-class retention time precision with ProFlow technology, settable flows of 0–1500 nL/min at pressures up to 900 bar (nano-LC); 50–2500 µL/min at 620 bar (ternary pump), GDV 25 nL (nano pump) and 220 µL (ternary pump), resp.; column temp.: (ambient +7°C)-75 °C, 2 optional switching valves; contains UltiMate 3000 NCS-3500RS nano-LC ProFlow module and installation accessories (ship kit). NOTE: switching valves are not included and need separate order.	$61,854.03	1	$61,854.03
2.2	5826.0020 WPS-3000TPL RS AUTOSAMPLER Provides high precision and accuracy even at UHPLC pressures up to 1034 bar; supports even smallest sample amounts with full and partial loop filling options; Analytical scale pulled-loop sampler w/ sample thermostatting (4-45 °C), injection volume 0.02-20 µL (field-upgradable to 125 µL), 3 segments for vial racks/wellplates; contains WPS-3000TPL RS module and installation accessories (ship kit; includes 3 x 48 pos. sample racks).	$31,432.13	1	$31,432.13
2.3	5035.9245 SRD-3400 SOLVENT RACK, 4 DEGASS. LINES Solvent Rack with integrated analytical 4-channel vacuum degasser. Offers secure and functional positioning of the solvent reservoirs and for automatic degassing of 4 typical HPLC solvents. Supports analytical flows up to 12 mL/min per channel.	$4,483.17	1	$4,483.17
2.4	6041.0001B LOW-DISPERSION VALVE 2P-10P,C82,N60C,NCS	$2,801.00	1	$2,801.00
2.5	6000.1004 6-PIN MINI-DIN SIGNAL CABLE(5M) Signal Cable with 6-Pin Mini DIN Connector, 5 m	$51.34	1	$51.34
2.6	6720.0345 MS CONNECTION KIT RSLCNANO MS Connection Kit for RSLCnano	$1,282.00	1	$1,282.00
2.7	161089 CYTOCHROME C DIGEST, 1,6NMOL, LYOPHILIZE	$83.00	1	$83.00
2.8	6720.0310 NANO LC KIT,PRECONCENTRATION,NCS-3X00 nanoViper Application Kit for Preconcentration Nano LC on UltiMate 3000 RSLCnano Systems	$2,622.43	1	$2,622.43

Sales Quotation

Quote Number	Version	Quotation Date	Page
[redacted]	1.00	May 25, 2021	[redacted]
Lead Time	**Payment Terms**	**Freight Terms**	**Expiration Date**
[redacted]	NET 30 DAYS UPON INVOICE DATE	Origin - Prepay And Add	[redacted]
Company Name		**Customer Name**	
[redacted]		[redacted]	

* See end pages of quote for PO Submission details

Thermo Electron North America LLC
5225 Verona Road
Madison WI 53711

1400 Northpoint Parkway
West Palm Beach FL 33407

Item	Description	Unit Price	Qty	Total Price
2.9	6000.1001 POWER CORD USA-VERSION Power Cord (125V) 3xAWG18, 2.0 m	$0.01	3	$0.03

UltiMate 3000 RSLCnano Pre-concentration nano LC Subtotal: $104,609.13
UltiMate 3000 RSLCnano Pre-concentration nano LC Discount: - $22,699.32
UltiMate 3000 RSLCnano Pre-concentration nano LC Total: $81,909.81

Item	Description	Unit Price	Qty	Total Price
3.0	6041.5292 TBG,NANO COL TO MS,IDXODXL 20X280UMX1M Single Side Open Ended Fused Silica Capillary to Connect Column to MS incl. 10-32 nanoViper Fitting, ID 20 µm, OD 280 µm, Length 1 m	$245.09	1	$245.09
4.0	6041.5293 TBG,NANO COL TO MS,IDXODXL 20X360UMX1M Single Side Open Ended Fused Silica Capillary to Connect Column to MS incl. 10-32 nanoViper Fitting, ID 20 µm, OD 360 µm, Length 1 m	$245.09	1	$245.09
5.0	6041.5294 TBG,CAP COL TO MS,IDXODXL 50X280UMX1M Single Side Open Ended Fused Silica Capillary to Connect Column to MS incl. 10-32 nanoViper Fitting, ID 50 µm, OD 280 µm, Length 1 m	$222.13	1	$222.13
6.0	6041.5295 TBG,CAP COL TO MS,IDXODXL 50X360UMX1M Single Side Open Ended Fused Silica Capillary to Connect Column to MS incl. 10-32 nanoViper Fitting, ID 50 µm, OD 360 µm, Length 1 m	$223.42	1	$223.42

Additional Items Subtotal $935.73
Additional Items Total $935.73

Quote Subtotal: $1,154,327.80
Promotions / Discounts: - $283,431.56

Contact PD/PI: [redacted]

Sales Quotation

Quote Number	Version	Quotation Date	Page
[redacted]	1.00	May 25, 2021	[redacted]
Lead Time	**Payment Terms**	**Freight Terms**	**Expiration Date**
[redacted]	NET 30 DAYS UPON INVOICE DATE	Origin - Prepay And Add	[redacted]
Company Name		**Customer Name**	
[redacted]		[redacted]	

* See end pages of quote for PO Submission details

Thermo Electron North America LLC
5225 Verona Road
Madison WI 53711

1400 Northpoint Parkway
West Palm Beach FL 33407

Quote total less discounts:	**$870,896.24**
Shipping:	**$5,400.00**
Quotation Totals:	**$876,296.24**

Trade In Instrument	Age	Serial Number
LTQ Velos Pro STD	10 years	SN03031B

The discount above includes a one-time special allowance for the customer's existing system. Please note - This transaction requires the transfer of title for the existing system from the customer to Thermo Electron North America LLC. In the event that the customer opts to retain title to the existing system, the discount will not be available.

Sales tax exemption/resale certificates or direct pay permits must be provided with the order documents, if applicable. If valid sales tax exemption documentation is not provided, buyer shall pay all applicable federal, state and local taxes in addition to the price stated on this quotation.

Competitive monthly payment options are available. For more information, please contact financialservices@thermofisher.com or call (800) 986-9731 ext. 7

Contact PD/PI: [redacted]

Purchase Order - Submission Details

Quote Number	Version	Quotation Date	Page
[redacted]	1.00	May 25, 2021	[redacted]
Lead Time	**Payment Terms**		**Expiration Date**
[redacted]	NET 30 DAYS UPON INVOICE DATE		[redacted]
Inco Terms 1	**Inco Terms 2**	**Shipping Method**	
Origin - Prepay And Add		Fed Ex Ground	
See below			

Thermo Electron North America LLC
5225 Verona Road
Madison WI 53711

1400 Northpoint Parkway
West Palm Beach FL 33407

To place your order and expedite shipment, please 1) sign and date the last page of this document; and 2) fax or e-mail it along with your Purchase Order to one of the addresses below.

Please note-all Purchase Orders must show the vendor name of Thermo Electron North America LLC

Thermo Electron North America LLC
5225 Verona Road
Madison WI 53711

or

Thermo Electron North America LLC
1400 Northpoint Parkway
West Palm Beach FL 33407

Complete System Orders:
Fax: 412-200-6542
e-mail: usmadorderprocessing@thermofisher.com

Parts or Service Orders:
Fax: 877-680-2565
e-mail: USMAD.ServiceOE.PO@thermofisher.com

Notes:

- Items marked with an asterisk (*) on the face of the quotation are non-Thermo Electron North America LLC products.
- Prices, warranty, installation and service on the items quoted herein are available only in the United States and may not be otherwise assigned.
- Tax exemption certificates or direct pay permits must be provided with the order documents, if applicable. If tax exemption documentation is not provided, buyer shall pay federal, state and local taxes in addition to the price stated on this quotation.
- Buyer shall not export or re-export technical data or products supplied by Thermo Electron North America LLC in violation of applicable export regulation. Buyer who exports products purchased hereunder assumes all responsibility for obtaining required export documentation, authorization, and payment of all applicable fees.
- All prices are quoted in USD.

Purchase Order - Submission Details

Quote Number	Version	Quotation Date	Page
[redacted]	1.00	May 25, 2021	[redacted]
Lead Time	**Payment Terms**		**Expiration Date**
[redacted]	NET 30 DAYS UPON INVOICE DATE		[redacted]
Inco Terms 1	**Inco Terms 2**	**Shipping Method**	
Origin - Prepay And Add		Fed Ex Ground	
See below			

Thermo Electron North America LLC
5225 Verona Road
Madison WI 53711

1400 Northpoint Parkway
West Palm Beach FL 33407

TERMS AND CONDITIONS OF SALE FOR PRODUCTS AND SERVICES

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OMNI LAB SOLUTIONS

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Attention:	[redacted]	Quote Number:	[redacted]
Company:	[redacted]	Quoted By:	[redacted]
Project Description:	Mass Spec Bench for Thermo Exploris System	Date:	[redacted]

Quote Valid	Terms	FOB	Approved Quote
Thru Nov 7, 2021	Net 30	Factory	Must Accompany P.O.

Qty	Part #	Description	Unit Price	Total
1	OL2IT48E-FSF	Enclosed Instrument Table 48W x 33D x 37H with Chemical Resistant Phenolic Work Surface and Flush Steel Frame (47-3/4W x 30D x 30-1/4H) on Heavy Duty Casters	$2,310.00	$2,310.00
1	OL2NS-S-R	Noise Suppression Module for Single Vacuum Pump 22W x 28-1/2D x 27H with SONEX Paneling and Two (2) 117CFM Fans with Hose Grommets in Rear and Vented Door Hinged Right	$1,942.00	$1,942.00
1	OL2SOS-S	Slide-Out Pump Maintenance Shelf for Above Noise Suppression Module, Single	$193.00	$193.00
1	OL2ODP-XL	Oil Drip Pan with Mounted Shock Absorbers, Large	$290.00	$290.00
1	OL2DB21	Drawer Bank 20-3/4W x 22D x 27H with Three (3) Full Extension Drawers and One (1) Pull-Out Writing Shelf	$825.00	$825.00
1	OL2E	Mounted 120V - 8 Outlet Industrial Surge Suppressor, Metal Housing with 12ft Cord (Rated at 3840 Joules/97,000 Amps)	$105.00	$105.00
			Subtotal	**$5,665.00**

Product Total	$5,665.00
10% Education Discount Applied	-$566.50
7.75% CA Sales Tax	N/A
FedEx LTL Freight (Crated/Assembled, Loading Dock Required)	$555.00
Total	**$5,653.50**

Contents of Instrumentation Plan

Introduction
Background
Justification of Need
Description of Instrument Requested
Orbitrap Exploris 480 mass spectrometer
Accessories and Additional Items
Inventory of Mass Spectrometers at [redacted] and Accessible Institutions
Need for Requested Instrument
Accessible User Time (AUT) for the Requested Instrument
Technical Expertise
[redacted]
Technical Staff
[redacted]
Research Projects (in decreasing %AUT order; grouped by analysis requirements)
Discovery proteomics (DIA-MS protein identification/relative quantification)
[redacted]
Cross-linking MS/protein structure (FAIMS + DDA-MS) and discovery proteomics (DIA-MS)
[redacted]
User Summary Table
Organization/Management Plan
Location
Personnel Responsibilities
Instrument Management
Scheduling
Attracting New Users to the Mass Spectrometry Laboratory
Data Backup
Quality Assurance
Citing Use of the Instrument
Advisory Committee
Biohazard Precautions
Financial Plan
Institutional Commitment
Overall Benefit

Background

This grant application requests funds to purchase a Thermo Scientific Orbitrap Exploris 480 mass spectrometer, UltiMate 3000 RSLCnano HPLC, and FAIMS (high field asymmetric ion mobility spectrometry) interface for shared use in the [redacted] The requested system will provide enhanced capabilities and expanded capacity for protein identification and relative quantification as well as crucially-important information for protein and protein complex structure determination for a large number of NIH-funded investigators studying diverse biomedical research disciplines. The [redacted] was established in 1979 under the direction of [redacted] At that time, the single instrument in the laboratory was a Hewlett-Packard model 5982 quadrupole GC/MS. In [redacted] was awarded a Shared Instrumentation Grant (SIG) for purchase of a Finnigan MAT 212 double focusing magnetic sector mass spectrometer, configured for probe and capillary GC sample introduction (EI and CI) as well as fast-atom bombardment (FAB). With this early "soft" ionization technique, it became possible to conduct unique experiments on minute quantities of platelet-activating factor, a highly potent naturally-occurring phospholipid (1, 2) in addition to the analysis of synthetic peptides. Since that time, the resources in the laboratory continued to expand through the acquisition of a variety of additional instruments for protein and small molecule analysis, facilitating the progression of the [redacted] to conduct increasingly complex and comprehensive proteomics experiments. Current HPLC-electrospray ionization (ESI) mass spectrometers in the [redacted] are:

Thermo Scientific Orbitrap Velos Pro (2010, SIG)

Thermo Scientific Q Exactive in the laboratory (2012, SIG)

Agilent 6224 ESI-TOF MS (2013, SIG)

Thermo Scientific Orbitrap Fusion Lumos/ETD (2016, [redacted]

The Q Exactive is used for small molecule/targeted metabolomics experiments. As discussed below, the Orbitrap Velos Pro will reach the end of service support in October 2021. It is planned that if this proposal is successful, the Orbitrap will be traded in as part of the purchase of the requested instrument.

Data processing in the [redacted] is accomplished using a combination of Mascot/Mascot Distiller (Matrix Science) Scaffold 5 and Scaffold LFQ (both from Proteome Software) for data-dependent acquisition MS (DDA-MS), and Scaffold DIA (Proteome Software) for data-independent acquisition MS (DIA-MS). With this powerful complement of instruments and software, the [redacted] is able to provide comprehensive services related to protein analysis/characterization to a wide range of investigators in addition to (limited) analysis of "small molecules."

In 2020, the user group of the [redacted] submitting samples for protein/peptide analysis consisted of 27 individual investigators in 17 departments at [redacted] in addition to 11 researchers from nine different outside institutions. Except for the ESI-TOF, the mass spectrometers in the laboratory are not made available as "open-access" instruments, but rather, they are only operated by [redacted] personnel. This arrangement has undoubtedly contributed to the outstanding performance and service history of all [redacted] mass spectrometers. Extensive one-on-one training in experimental design, data analysis and results interpretation is provided to all users.

Protein identification/relative quantification has been a frequently-requested service in the [redacted] most often for analysis of cell lysates, secretomes and pull-down experiments. Prior to installation of the Lumos, the majority of experiments were accomplished with a label-free method in which proteins are separated by 1-D SDS PAGE and then the entire gel lane is divided into slices ("GeLCMS"), followed by reduction/alkylation, digestion, MS analysis, Mascot database searching and post-processing in Scaffold. The Mascot files are either viewed individually in Scaffold to examine results for each slice or else they are combined using the "MudPIT" option to assess the sample as a whole. In most cases, relative quantification was based on spectral counts. Use of an initial 1-D gel separation has been particularly important for proteomics studies of bacteriophage with long-time collaborator [redacted] because it also permits localization of sites of proteolysis of head and inner body proteins (3) which would not be possible if the lysates were directly digested. This strategy was also used for a large infection study of a model *Salmonella* bacteriophage SPN3US (4) that was the first big experiment conducted on the Lumos.

With the addition of the Lumos to the [redacted] it became realistically possible to transition to a more powerful method for protein identification/relative quantification—either a reporter ion-based method or data-independent acquisition mass spectrometry (DIA-MS). TMT/iTRAQ experiments had not been conducted in the MSL in the past due to limitations of the Orbitrap, and although in widespread use in many laboratories, there are a number of concerns about reporter ion-based methods that lowered enthusiasm for its use. These include: the expense of the reagents; the need for verification of efficiency of derivatization; pre-fractionation that is usually required at the peptide level; loss of accuracy of quantitative results due to precursor co-isolation and ratio compression; one quantitative "read" per peptide (with dynamic exclusion), and, very importantly, limitations of experimental design/experimental group comparison. New MS scan strategies have been introduced that improve quantitative accuracy, but this comes at the expense of numbers of proteins identified/quantified. In view of the issues associated with reporter ion-based methods, DIA-MS seemed like a much more effective approach for the MSL. In preparation for establishing DIA-MS in the [redacted] sufficient sample quantities of a representative four-group experiment were prepared by [redacted] The samples were: breast cancer cells (MCF-7/TamR), cells plus drug 1 (ERX11; ER coregulator binding modulator-11, developed by [redacted] and colleagues), cells plus drug 2 (palbociclib), cells plus the combination of drug 1 and drug 2. Preliminary analysis using GeLCMS (12 slices) was conducted with 100-µg aliquots of one biological replicate of each group. The complete experiment took 17 days: sample preparation, four days; MS analysis, seven days; Mascot database searching, five days; Scaffold processing, less than one day. There were 6,025 proteins identified (two peptide minimum, <1% protein FDR), with multiple apparent differences in relative quantity indicated by spectral counting. These samples were then used to establish initial DIA-MS scan parameters. Analysis of biological triplicates of the four experimental groups resulted in the identification and relative quantification of >4,400 proteins. [redacted] Since then, substantial efforts have been made to optimize sample preparation, MS analysis and data processing. Key elements for the success of the workflow include: S-Traps (Protifi) for highly-reproducible tryptic digestion/sample cleanup; protein (EZ Q; Thermo Fisher) and peptide (Pierce Quantitative Fluorometric Peptide Assay) quantification; Scaffold DIA (Proteome Software, a complete system that starts with Thermo "raw" files and permits statistical assessment of the data, in-depth visual inspection of the results and generation of Excel files for post-processing and pathway analysis); and predicted spectral libraries produced by Prosit (6). Analysis of cell lysates and tissue samples on the Lumos in the [redacted] now regularly yields >5,000 proteins identified and quantified (two peptide minimum, 1% protein FDR) using single injections of each sample (plus a three-injection empirically-corrected chromatogram library). Markedly larger numbers of proteins can be identified/quantified when multiple gas-phase fractions are used (see below).

Justification of Need

Description of Instrument Requested

Mass spectrometer

The main instrument requested is a Thermo Scientific Orbitrap Exploris 480 mass spectrometer. It was decided to limit consideration for this proposal to Thermo mass spectrometers for many compelling reasons, including the long history of successful use of Thermo instruments in the [redacted] familiarity with instrument operating systems, similarity in protocols for calibration and tuning with existing [redacted] mass spectrometers, generation of the same data file types as the other proteomics instruments in the lab and the company's consistently outstanding and prompt service support. The next decision was the model of mass spectrometer. Since there is already a Tribrid in the lab (a Lumos) with capabilities for multiple sophisticated scan functions, an Orbitrap Eclipse is not needed. A Q Exactive Plus was also not considered a good option because it is based on a >10-year-old design and does not have the requisite ability for efficient DIA-MS. This left choosing between the other Orbitrap series proteomics instruments—the Exploris 240 and 480.

There are several notable advantages of an Exploris 480 compared to a 240, particularly related to ion optics and scan rate. There is an ion funnel on the 480 rather than the S-lens on the 240. When combined with the larger ion transfer tube orifice on the 480, substantially more ions enter into a 480 than a 240, resulting in enhanced ion transmission. This translates into a four-fold improvement in detection limits for the 480 [manufacturer's specifications: 50 fg reserpine on column with S/N 100:1 for the 480 versus 200 fg reserpine on column with S/N 100:1 for the 240]. In addition, the scan rate on the 480 is twice as fast as on a 240 (40 Hz

versus 20 Hz). As such, many more MS2 spectra can be acquired per scan event on a 480. The advantages in ion transmission and scan rate lead to enhanced performance for DDA-MS and DIA-MS, both of which are important for the user group of the requested instrument.

Comparison of these performance characteristics of the Exploris 480 and 240 was convincing that the 480 is the instrument of choice for DIA-MS in the MSL, because the combination of the substantially brighter ion source and markedly faster scan speed of a 480 will yield more protein identifications and relative quantification with greater quantitative accuracy than a 240. Because the 480 can functionally scan twice as fast as a 240, 50-msec ion inject times are needed on a 240 to accumulate the same ion counts as in 25 msec on a 480. When a longer ion inject time is used, there are fewer points across an HPLC peak, which introduces more error in quantitative measurements. Another important consideration is the windowing scheme used for DIA-MS. On the Lumos in the [redacted], 12-m/z windows across a range of 390 – 1010 were initially used for single injection analyses. Subsequent optimization studies showed that 8-m/z windows yielded more identifications and a higher percentage of proteins quantified, as shown in **Table 1** for analysis of one sample of control ovarian cancer cells

Table 1. Comparison of DIA-MS scan strategies for analysis of ovarian cancer cell proteins: Control and KDM1a knockout

	4 m/z x2GPF[1]			8 m/z x1GPF[2]			12 m/z x1GPF[3]		
# Proteins	C	KO-1	KO-2	C	KO-1	KO-2	C	KO-1	KO-2
Total ID	5289	5289	5289	4910	4910	4910	4673	4673	4673
# Quantified									
Not 0	5240	5242	5240	4623	4619	4624	4032	4030	4030
>2	4052	4054	4053	3115	3113	3108	2315	2312	2312
=2	800	806	802	800	801	809	786	786	788
=1	388	382	385	708	705	707	931	932	930
=0	49	47	49	287	291	286	641	643	643

[1]4 m/z staggered windows, 400 - 700 m/z and 700 - 1000 m/z
[2]8 m/z staggered windows, 390 – 1010 m/z
[3]12 m/z staggered windows, 390 – 1010 m/z

and two biological replicates of the same cells with KDM1a knocked out [data processing with Scaffold DIA (Proteome Software)]. In addition to identifying and quantifying a smaller number of proteins, a drawback of wider windows is there is a greater chance for interference from co-eluting peptides, decreasing the number of interference-free fragment ions used for quantification. With fewer interference-free fragment ions, quantitative accuracy suffers.

In the course of the Lumos DIA-MS optimization studies, it was surprising to see the striking benefit in terms of identification and relative quantification when two gas-phase fractions (GPF; 400 - 700 m/z and 700 - 1000 m/z) are used for data acquisition instead of a single injection of 390 – 1010 m/z (**Table 1**). As can be seen in the table, over 99% of identified proteins were quantified (5,240 of the 5,289 identified proteins) with 4 m/z x 2 GPF, which was substantially better than when single range 8-m/z or 12-m/z windows were used. An Exploris 480 scans slightly faster than a Lumos, so similar if not better results can be anticipated. The ability to use this scan strategy is yet another asset of an Exploris 480, because a 240 does not scan fast enough for a 4 m/z x 2-GPF windowing scheme. Choice of the scan strategy for an experiment depends to a large extent on the goals of the study, the numbers of samples/experimental groups, the peptide quantities for injection, the investigator's budget, and available instrument time. If an Exploris 480 were added to the MSL, there would be sufficient capacity to permit use of 4 m/z windowing schemes whenever needed for projects that would benefit from even deeper proteome coverage with enhanced quantitative.

As a final step in determining if the Exploris 480 is the best choice for the [redacted] parallel samples of the same ovarian cancer cells and KDM1a knockouts that were used to generate the data in **Table 1** were prepared (three biological replicates of each group). One set was analyzed on the Lumos in the MSL and the other on an Exploris 480 in the New Jersey Thermo demo lab. [Note that at that time, the New Jersey Thermo demo chemists had not as yet conducted DIA-MS experiments on an Exploris 480; [redacted] provided the windowing scheme and scan parameters to use for the analyses and conducted all of the data processing.] Both sets were frozen after digestion prior to analysis. As can be seen in **Table 2**, there were substantially more protein identifications and a higher proportion of relative quantifications by the Exploris 480 than the Lumos, analyzing aliquots of the same samples and using the same windowing schemes (8 m/z, single injections) and scan parameters. These results clearly demonstrate the outstanding DIA-MS capabilities of the Exploris 480.

Table 2. DIA-MS analysis of ovarian cancer cell proteins: Control and KDM1a knockout

	# Proteins					
Lumos (MSL)	C1	C2	C3	KO1	KO2	KO3
Total ID	5258	5258	5258	5258	5258	5258
# Quantified						
Not 0	5072	5076	5080	5076	5079	5074
>2	3595	3587	3589	3587	3592	3585
=2	846	853	856	845	848	852
=1	631	636	635	644	639	637
=0	186	182	178	182	179	184
Exploris (Thermo)						
Total ID	6665	6665	6665	6665	6665	6665
#Quantified						
Not 0	6605	6604	6605	6605	6602	6603
>2	5318	5321	5318	5318	5324	5313
=2	839	835	838	841	831	836
=1	448	448	449	446	447	454
=0	60	61	60	60	63	62

Accessories and Additional Items (item number of Thermo Scientific quote in parentheses)

HPLC (item 2.1). A new UltiMate 3000 RSLCnano HPLC is being requested in this proposal. An UltiMate 3000 RSLCnano HPLC is currently being used with the Orbitrap Velos Pro. This HPLC could be directly transferred to the Exploris when the Orbitrap is traded in. However, despite the fact that the existing UltiMate is in excellent operating condition and has been maintained under warranty or service contract since installation, it would be close to 12 years old at the time of Exploris installation. Moreover, the new version of the UltiMate 3000 RSLCnano HPLC is fitted with a ProFlow flow meter instead of the “Classic” flow meter on older systems. The ProFlow provides substantially better retention time reproducibility than the Classic, which would be a major benefit for DIA-MS. Other notable advantages include: flow meter equilibration in half the time required for the “Classic” flow meter; and no requirement for solvent calibration after mobile phase refresh or change. Taken together, there is strong justification for purchase of a new HPLC for use with the requested Exploris.

Nanoelectrospray ion source (item 1.5). This request includes a Nanospray Flex ion source. All of the proteomics analyses conducted in the MSL use nanoflow HPLC (usually 400 nl/min) to separate peptides prior to introduction into the mass spectrometer. The other proteomics mass spectrometers in the laboratory are fitted with New Objective PicoView electrospray interfaces which provide excellent reliability and versatility. The PicoView interface that is currently in use on the Orbitrap Velos Pro cannot be adapted for an Exploris 480. In addition, the FAIMS Pro interface that is also being requested (see below) can connect directly with the Flex ion source in contrast to a PicoView interface where there is interference from the cables. Since outstanding results

were obtained from the samples analyzed in the Thermo "demo" lab using the Thermo Flex source, this is the model selected for purchase with the requested Exploris 480.

FAIMS Pro interface (item 1.2). A high field asymmetric waveform ion mobility (FAIMS) interface is requested to provide the capability to filter electrosprayed ions on the basis of their charge state.

Nitrogen generator (item 1.8). A nitrogen supply is needed for operation of the FAIMS Pro interface. Using a nitrogen Dewar as the FAIMS gas supply is not practical due to the gas volume needed for interface operation.

Uninterruptible power supply/power conditioner (items 1.9 and 1.10). The requested Powervar is an uninterruptible power supply and power conditioner. It is needed to assure the proper quality of power supplied to the Exploris and HPLC and to provide power during a switchover to emergency power during an outage. The "PV PDU" module is the bank of receptacles for connecting the mass spectrometer and accessories to the Powervar.

Training (item 1.11). The Tune software and methods editor are the same on an Exploris 480 as on a Lumos, so the staff of the MSL will not need instruction on basic instrument operation. With the new vacuum manifold design of the Exploris 480, the staff members will be able to clean the ion optics as needed rather than wait for a service engineer. Training for this critical activity and for operation and optimization of the FAIMS Pro interface cannot be realistically accomplished in the brief spare time that might be available during installation. Three days is the minimum time offered for Thermo training.

Other Thermo Items. (a) The additional items to be ordered are needed for instrument installation by the service engineer, including calibration solutions, assorted tubing, cables and power cords. (b) There will not be a charge for deinstallation of the Orbitrap Velos Pro (item 1.6) so that it can be traded in as part of the Exploris 480 purchase.

Omni Lab Solutions. An Omni Lab Solutions instrument table with a chemical-resistant work surface, drawer bank and noise suppression module for the mechanical vacuum pump is being requested to house the Exploris. Omni desks are in use with the all of the mass spectrometers in the MSL, and a table similar to the one requested supports the Q Exactive. These units have proven to be versatile, sturdy and durable for many years.

Inventory of Mass Spectrometers at [redacted] and Accessible Institutions

[redacted] The [redacted] is the only protein analysis/proteomics mass spectrometry laboratory at [redacted]. [redacted] provides lipidomics analyses on collaboration or fee-for-service basis. The mass spectrometers in [redacted] laboratory are: (all from Thermo Scientific): two TSQ Altis, a TSQ Quantiva and a Q Exactive. None of his instruments is used for proteomics. Several laboratories in the Departments of Pathology, Pharmacology and Psychiatry have single or triple quadrupole LC-MS systems that are used for targeted drug analysis. [redacted] has two Thermo Scientific Q Exactive HF X mass spectrometers. One is used for imaging MS and the other for analysis of small molecules/metabolites. [redacted] also has a Bruker GC-triple quadrupole for targeted metabolomics.

[redacted] The Proteomics and Protein Biomarkers Core at [redacted] has the following instrumentation: Thermo Scientific Orbitrap Elite, Bruker microTOFMS, Bruker Ultraflextreme MALDI-TOF/TOF (imaging). The main proteomics service provided at [redacted] is based on TMT-labeling. Numerous [redacted] investigators submit samples to the [redacted] for varied MS support, including DIA-MS.

[redacted] There was previously a state-of-the-art mass spectrometry facility at [redacted], under the direction of [redacted] whose research focus is metabolic disease (using MS-based proteomics and metabolomics). [redacted] provided MS service for several [redacted] investigators; others at the institute either used the [redacted] or sent samples elsewhere. Not long after [redacted] in leadership roles in 2017 and the institute's emphasis shifted to virology and infectious disease, [redacted] moved [redacted]. In the absence of other MS expertise, it was decided to close the mass spectrometry facility at [redacted], sell the instruments, and use the [redacted] instead. Many experiments have already been conducted for [redacted]

[redacted] researchers in the past three years and, as can be seen in the project descriptions for [redacted] many are ongoing, with more planned for the future.

Need for Requested Instrument

The requested Exploris 480 and FAIMS interface will have a major impact on biomedical research at [redacted] by providing enhanced performance and substantially increased capacity for discovery proteomics using DIA-MS along with exciting new capabilities for protein structure analysis when paired with cryo-electron microscopy (cryo-EM). Submissions to the [redacted] for comprehensive protein identification/relative quantification have been exhibiting a consistent increase as more faculty members become aware of the power of DIA-MS, and current users of DIA-MS plan new, larger experiments. Interest in the technology is clearly evidenced in the support letters [redacted] has written for grant applications. In 2020, 35 letters were written for 20 [redacted] nvestigators in nine departments, 25 of them (>70%) proposed DIA-MS experiments. In addition, 15 letters were provided for eight investigators at six outside institutions—all requesting DIA-MS analyses. This trend has continued in 2021 (as of June 7): 19 letters for 12 [redacted] investigators in eight departments, 12 proposing DIA-MS; four letters for three outside investigators, three with DIA-MS. Of the support letters in 2020, 72% were for NIH grant applications. Before the [redacted] had to limit operations due to COVID-19, there was at least a one-month backlog of samples that had been already submitted for DIA-MS. The [redacted] esumed full operation in June 2020, with staff members working split shifts to permit safe sample processing and utilization of laboratory equipment. Although productivity has been high with the current arrangement, there is now a two-month backlog for DIA-MS analyses. The current heavy load for the Lumos is going to increase further in the near future as three new faculty members begin to submit samples to the MSL (see project descriptions of [redacted] The projects of all three of these researchers will greatly benefit from the capabilities of the requested Exploris 480 in terms of global proteomics, and two will also conduct numerous cross-linking mass spectrometry (XL-MS) experiments.

In the past, the Orbitrap Velos Pro in the [redacted] was regularly operated at near capacity for a wide variety of protein analyses, although demand has declined slightly in the last year as a number of experiments transitioned to DIA-MS on the Lumos. As indicated above, the Orbitrap will reach its end of service support in October 2021. Thermo has agreed to sell a six-month "Essential" contract that will end in April 2022. Coverage under that contract will be on a best effort basis, subject to commercially-available resources. After that time, parts, on-site service and telephone technical support will be billed at Thermo's established rate schedule. For example, a preventative maintenance visit will cost approximately $14,000 (including consumables). Labor for a two-day repair visit will be at least $10,000, with parts ranging from a few thousand dollars for boards and power supplies to $40,000 for a new lens assembly. Although the Orbitrap has had an excellent operating history over its long period of use, there is no way to anticipate when a major repair might be needed and whether the necessary parts will be available to complete the repair. In view of the lower performance capabilities of the Orbitrap—in particular its inability for DIA-MS—it will soon not be cost-effective to keep the Orbitrap running to handle the many straightforward analyses that are regularly conducted on it. This will result in an even greater overload for the Lumos based on just the existing sample queues for both instruments.

Protein structure has been a major focus for at least two decades in the [redacted] with highly-regarded experts and instrumentation for NMR spectroscopy and X-ray crystallography, comprising an [redacted]. The [redacted] core has taken a major leap forward with recruitment of two additional structural biology faculty members, [redacted] and with the installation of a new Glacios cryo-electron microscope and state-of-the-art direct detector (Falcon 4) and energy filter (Selectris) in early 2022. This will be the only such configuration of its kind in the entire U.S. and will add transformative capabilities to the institution's research infrastructure by facilitating rapid, high resolution data collection for structure determination using single particle analysis and eventually, cryo-electron tomography (ET). The facility will propel [redacted] to become a national leader in identifying innovative solutions to health problems, such as metabolic syndromes and cancer, that are particularly prevalent in the [redacted]. Many of the protein samples that will be subjected to cryo-EM analysis include very large multi-protein complexes and molecular machines that are highly dynamic—essential to eukaryotic cell biology, and implicated in myriad human pathologies.

[redacted] have already been collaborating with [redacted] for MS analyses, most notably cross-linking experiments that provided extremely valuable information that was needed to validate cryo-EM structures (see [redacted] project description). Cross-linking-mass spectrometry (XL-MS) is an essential part of these experiments, as illustrated in [redacted] project description. For example, XL-MS has been successfully used to resolve contacts in cryo-EM models when resolution is limiting (> 5 Å), providing essential data for docking, as well as model-based and de novo building (7, 8). This strategy has been applied by many groups to conformationally dynamic targets—including those generated recombinantly, and those isolated from native sources with higher levels of sample heterogeneity and unknown binding partners. Because such challenging systems are increasingly being studied by cryo-EM, there is an increasing need for XL-MS to assign ambiguous regions.

While the XL-MS data for [redacted] were extremely informative, there were only two, highly-purified proteins that were cross-linked, greatly simplifying interpretation of the results. But, as discussed above, that is not representative of many XL-MS studies where the number and size of proteins that are cross-linked are much larger, and many of the informative ions are of low relative abundance in a very complex mixture (7-9). The requested FAIMS interface will be a tremendous facilitator of these analyses, because cross-linked tryptic peptides are typically of 3+ or higher charge state, and with FAIMS, the lower charge state ions can be filtered out, thereby enabling enrichment of the ions that are most informative for specific localization of cross-links. In addition, the outstanding detection limits of the Exploris 480 will significantly reduce the amount of material required for successful cross-link identification, which is crucial given the difficulty associated with reconstituting and purifying the large complexes and proteins that will be studied by cryo-EM. As documented in an [redacted] "FAIMS is highly beneficial for XL-MS studies by expanding the proteome coverage of cross-links while improving the efficiency and confidence of cross-link identification." With the expertise of [redacted] to guide them, there are multiple [redacted] faculty who are already lined up for cryo-EM structure analysis of proteins and protein complexes, many of whom have samples (grids) that are ready for imaging. Having regular access to high-performance mass spectrometry capabilities for XL-MS is essential for their planned studies of multi-component and dynamic systems, and with the FAIMS interface, it will be possible to more accurately resolve cross-links in rigid as well as more mobile regions.

The requested Exploris 480 and accessories are, therefore, critically important for the [redacted] in three main ways. First, the larger number of identified and quantified proteins afforded by DIA-MS on the Exploris 480 will yield a greater depth of coverage for global, discovery proteomics and interactomics studies, markedly enhancing pathway analysis of the DIA-MS data. Second, in conjunction with a FAIMS interface, it will bring new capabilities to the core for structural biology analyses of proteins and complexes based on cross-linking-MS, which will be an essential partner for the new cryo-EM facility that will be brought online in spring 2022. Third. it will add crucially-needed capacity for high-performance mass spectrometry instrument time, thereby reducing sample backlog to a more reasonable and manageable level.

Accessible User Time (AUT) for the Requested Instrument

An instrument like the Exploris 480 can operate 24x7 since there is a refrigerated autosampler on the HPLC and samples can be queued for unattended analysis. Accessible user time (AUT) was calculated based on 8,766 instrument operating hours per year (24 hours/day x 365.25 days/year) minus 1,234 hours for the following: weekly calibration; preventative maintenance; new method development; new protocol implementation; HPLC and mass spectrometer repairs. This results in a total AUT rounded to 7,500 hours per year. The user group listed in this proposal for the requested instrument is projected to require 84.5% of the total AUT; 5% AUT is anticipated for investigators who are not in the listed user group and will have a small number of protein complexes for cryo-EM analysis, leaving 10.5% available for new users. The projected AUT per user is shown in **Table 3** after the users' project descriptions.

Technical Expertise

[redacted] will be responsible for overall supervision of the requested Exploris 480, FAIMS interface and accompanying HPLC system. [redacted] Biology and Director of the [redacted] She has been actively engaged in biomedical gas chromatography and mass spectrometry since [redacted] n the mid-to-late 1990's, as technology facilitated analysis of much larger molecules, [redacted] interests expanded to include the characterization of proteins by mass spectrometry. She is a co-author on [redacted] scientific papers published in peer-reviewed journals in addition to [redacted] chapters. [redacted] previously served on the [redacted]

There are two full-time staff members in the [redacted] oth have extensive expertise in biomedical mass spectrometry and have exceptional skills in sample preparation and MS analysis. Their careful attention to detail contributes to the high quality of results reported by the [redacted] as well as the reliable performance of the Core's instruments, with minimal down-time other than routine maintenance. They are both highly capable of performing all activities in the laboratory.

Research Projects

The projects of 14 users of the requested Exploris 480 are described below, grouped by types of analyses that are needed. The first group proposes to use the Exploris 480 for discovery proteomics analysis conducted by DIA-MS. The users in the second group need cross-linking MS for protein/protein complex structure determination (predominantly in conjunction with cryo-electron microscopy that will be conducted in the new [redacted] as well as DIA-MS for protein identification/relative quantification. The users are listed in decreasing percent AUT order within each group; details about selected grants are provided. The projected AUT is not different enough among the users to warrant division into major and minor categories. [Their biosketches are provided together in alphabetical order to facilitate examination.] Figures and tables are numbered separately for each project description. [redacted] The DIA-MS analyses that are needed for the projects will be especially impacted by availability of the Exploris 480 either due to limitations in sample quantity or the need for more comprehensive protein identification/relative quantification. The FAIMS interface will facilitate structure/cross-linking analysis, which is a new service for the [redacted] All users of the [redacted] will benefit from the increased capacity and decreased turnaround time for analyses.

Discovery proteomics (DIA-MS protein identification/relative quantification)

[redacted] **Ph.D. (8.0% AUT)**
Professor, Department of Obstetrics and Gynecology

Therapeutic agents targeting nuclear receptor signaling in distinct molecular subtypes of breast cancer
NCI R01CA[redacted] [redacted] 12/15/17 – 11/30/22

FoXM1 inhibition: a novel therapeutic avenue to treat breast cancers
NCI 1R01CA[redacted] [redacted] 12/15/19 – 11/30/24

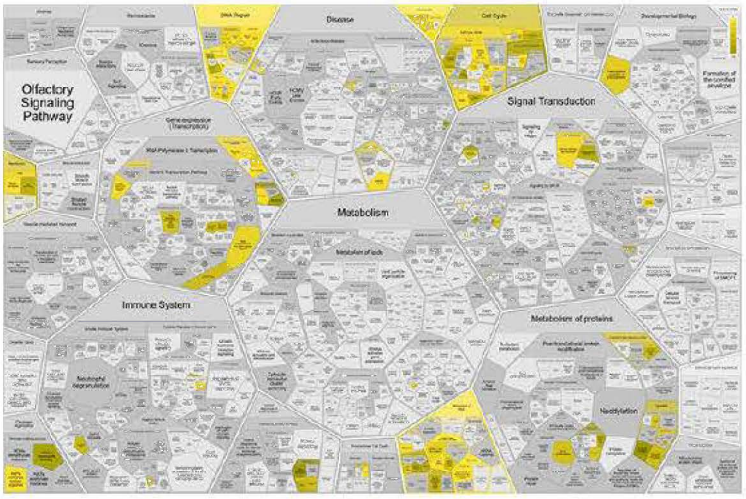
Targeted inhibition of leukemia inhibitory factor (LIF)/ LIFR axis for the treatment of Triple Negative Breast Cancer
DOD BC[redacted] [redacted] 02/01/18 – 01/31/22

Development of potent inhibitors of proto-oncogene PELP1 for treating advanced breast cancer
VA: [redacted] [redacted] 10/01/19 – 09/30/23

The [redacted] lab has made foundational contributions in elucidating the molecular biology of estrogen signaling in cancer. Importantly, [redacted] lab research has identified a new targetable site on the estrogen receptor, and this has led to the development of a new class of inhibitors (ERXs) for treating advanced breast cancer. These efforts have resulted in a new patent and RO1 funding from the National Cancer Institute. As part of this ongoing project, the group has been examining the efficacy of ERXs in enhancing the utility of currently approved therapies such as CDK inhibitors. The combination of ERX-11 and the CDK4/6 inhibitor palbociclib has been found to be synergistic in decreasing the proliferation of both endocrine therapy-sensitive and endocrine therapy-resistant BCa cells, in vitro, in xenograft models in vivo, in xenograft-derived explants ex vivo, and in primary patient-derived explants ex vivo. Importantly, the combination caused xenograft tumor regression in vivo. Unbiased global DIA-MS studies demonstrated decreases in the relative levels of numerous proliferation markers with combination therapy (1). Pathway analysis results of proteins exhibiting a larger decrease in relative quantity after treatment with the drug combination compared to treatment with palbociclib alone are shown in **Figure 1** on the following page.

Of all [redacted] lab numerous scientific discoveries, the identification, molecular cloning, and functional characterization of PELP1, a novel ER co-regulator, is one of the most significant to highlight. Specifically, Dr. [redacted] laboratory was the first to identify PELP1 as a proto-oncogene in the genesis of breast and ovarian tumors and as a novel tumor biomarker. Importantly, [redacted] and his trainees have generated several in vitro and in vivo transgenic mouse models and have also developed a small molecule inhibitor with high specificity and efficacy. [redacted] is firmly committed to the translation of his mechanistic research findings and is currently conducting preclinical studies on the PELP1 inhibitor for eventual applications in the clinic. He is also involved in the development of additional inhibitors of PELP1 (patent filed by [redacted]) and has secured

a [redacted] to support these drug discovery endeavors. DIA-MS studies are instrumental in elucidating the pathways modulated by PELP1 oncogene and also in understanding the specificity of PELP1 inhibitors currently in development.



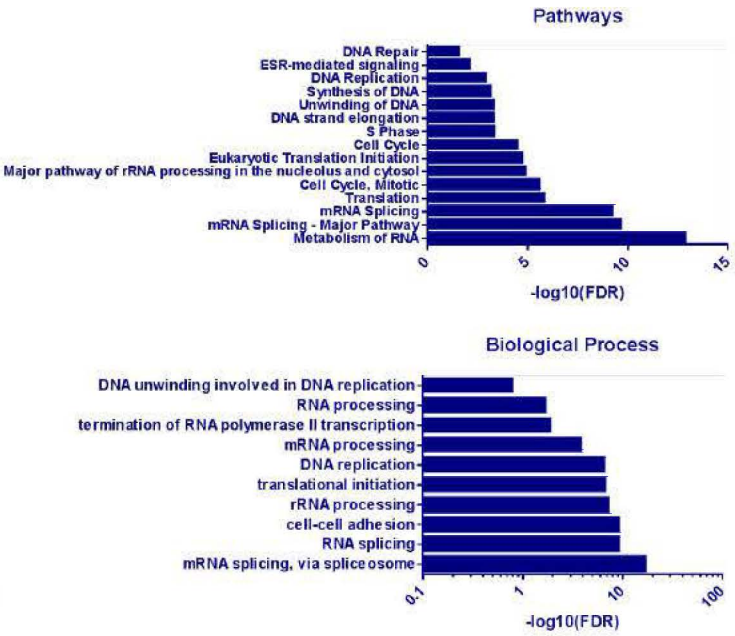


Figure 1. Proteins exhibiting a larger decrease in relative quantity after treatment with the drug combination (E+ERX-11+palbociclib) compared to E+palbociclib were uploaded into Reactome (https://reactome.org/). Pathways with over-representation are indicated in yellow. The corresponding pathways and biological processes for the top ten hits are shown to the right.

Another area of focus of the [redacted] lab is to exploit the tumor suppressor function of a second estrogen receptor, ERβ, in cancer prevention (2). Studies conducted using the MSL were of key importance in identification of unique proteins bound to ERβ, including several that function as regulators of mTOR, immunomodulatory, and apoptosis pathways [redacted] In a collaborative project with [redacted] group is developing novel ERβ agonists for the treatment of glioblastomas. Further, in collaboration with a [redacted] group has developed a new compound (EC359) that targets LIF/LIFR signaling in the tumor micro environment; these ongoing investigations are funded by a DOD Breakthrough Award. Currently, Dr. Vadlamudi's team is completing preclinical studies on these compounds in preparation for future clinical trials (3).

[redacted] group is extensively using DIA-MS analyses to understand the mechanisms of newly discovered drugs, and they anticipate six to seven DIA-MS experiments per year in the future as part of their funded NIH, DOD and VA grants. The larger numbers of proteins identified/quantified on the Exploris 480 compared to the Lumos will provide a greater sampling of protein differences among experimental groups, thereby increasing the input for subsequent pathway analysis and the likelihood of detecting significant pathway over-representation. A new direction of [redacted] research is characterization of the tumor microenvironment altered by small molecule inhibitors that target leukemia inhibitor factor receptor (LIFR). A part of these studies will require proteomic analysis of sorted single cell types from tumors, anticipating approximately 10,000 – 50,000 cells per sample. There will be sufficient protein to process via micro S Traps and analyze by DIA-MS. The enhanced performance of the Exploris compared to the Lumos will be particularly important to be able to identify and quantify proteins across a wider dynamic range, yielding data for many more lower abundance proteins.

References

1. [redacted]

2. [redacted]

[redacted], **Ph.D. (7.7% AUT)**
[redacted]

Exploring the impact of inflammaging on immune function during *Mycobacterium tuberculosis* infection
NIH/NIA P41 AG[redacted] 03/15/16 – 02/28/22
[redacted] PI in Project 1, PI Core B, Consultant in Project 4)

The Rv2623-Rv1747 interaction: regulation of the *in vivo* fate of *M. tuberculosis*
NIH/NIAID R01 A[redacted] 01/16/20 – 12/31/24

A major focus of the [redacted] laboratory is to define the impact of the lung alveolar environment in determining why the elderly, as well as diabetics and people living with HIV (PLWH) are more susceptible to acute and/or chronic respiratory infections than adults and healthy individuals, respectively (1-12). In particular, the group employs proteomics, lipidomics and systems biology approaches to study human alveolar lining fluid (ALF). ALF is the first environment that a lower-track respiratory pathogen encounters before infecting alveolar host cells and causing disease. The group hypothesizes that the initial interaction of a pathogen and ALF determines the pathway of infection in the lungs, along with subsequent clearance or establishment of the infection and the development of disease. As acute models of respiratory infection, they use coronaviruses (SARS-CoV-1 and SARS-CoV-2), influenza A/B, and for chronic respiratory infection, they use *Mycobacterium tuberculosis*, the etiological agent of tuberculosis (TB).

The mucosal lining of the interior of the alveolar space is composed of two layers: the surfactant lipid layer, mainly containing phospholipids [predominantly dipalmitoylphosphatidylcholine (DPPC) at 80%], and an aqueous hypophase, the ALF. The functions of ALF range from providing the alveoli with the necessary surface tension that allows gas exchange to occur, to innate defense of the lung through myriad soluble components dedicated to the degradation and elimination of pathogens that might reach the lower airways throughout an individual's life. Understanding how ALF components change with aging and disease, both in abundance and functionality, will yield insight into how pathogens interact with the human host. More specifically, these studies provide information about this first, heavily understudied, interaction of pathogens before they even encounter any host immune cells. In addition, studying ALF composition in various human cohorts (young, elderly, diabetics, PLWH) is important in order to define how ALF phenotypical and functional differences can be used to predict susceptibility to respiratory infections.

There are four major directions in the [redacted] lab:

i) Identify which components of ALF induce pathogens to replicate faster inside host cells, driving disease (2). These efforts are directed at defining the ALF-pathogen interactome, determining how pathogens react to the lung environment at the proteome and transcriptome levels, before encountering and infecting host cells (*NIH/NHLBI R01 pending*). ii) Define which ALF components are dysregulated and dysfunctional in the elderly population (*NIH/NIA P01 funded*), iii) in PLWH (*NIH/NIAID R01 pending*), and iv) in diabetics (*NIH/NIAID R01 pending*), driving their susceptibility to respiratory infections. Efforts

also focus on determining if replenishment of components to dysfunctional ALF improves resistance to such infections. To achieve these goals the group employs several *in vitro* models using primary human cells and bronchoalveolar lavage samples (human ALF), as well as *in vivo* models, rodents and non-human primates.

In previous preliminary studies, lipid (9, 10) and proteomic (9) assessments of adult and elderly human ALF were conducted by LC-MS/MS [data acquired at [redacted], before the group moved to [redacted]]. A total of 1,154 proteins was identified, with adults having 349 (30.2%) unique proteins, elderly 96 (8.3%), and 709 (61.4%) shared across both groups. Differences in relative abundance of proteins in the elderly ALF relative to adult were calculated based on the total MS2 TIC [total ion current; for representative plots see Supplemental material in (9)]. In general, elderly ALF contained higher levels of pro-oxidative/pro-inflammatory proteins and lower levels of anti-oxidative/anti-inflammatory proteins.

After interruptions related to the onset of the COVID-19 pandemic, the [redacted] was able to re-start operations in June 2020, and the first DIA-MS experiment conducted was for [redacted] group—analysis of the ALF composition of 21 healthy subjects—human adults (age 18 – 55) and elders (age 60 – 75). There were 1,794 proteins identified and quantified (minimum of two peptides, 1% protein-level FDR). Comparison of the two age groups revealed significant differences in 473 proteins ($p < 0.05$). Of these proteins, 315 exhibited a two-fold or greater difference in relative abundance [$\log_2$(fold change) of ±1]. Pathway analysis indicated decreased cellular function and increased cellular death mechanisms, as well as highly dysregulated immune functions in the elder group. In addition, there was altered expression of regulatory transcription factors that could explain the susceptibility phenotype observed in the elderly population. As an example, they found that the serum response factor (SRF) was downregulated in ALF from elders. SRF is hypothesized to control the homeostatic remodeling of the alveolar environment to keep lungs healthy. An enrichment map of the results generated in Cytoscape is shown in **Figure 1**.

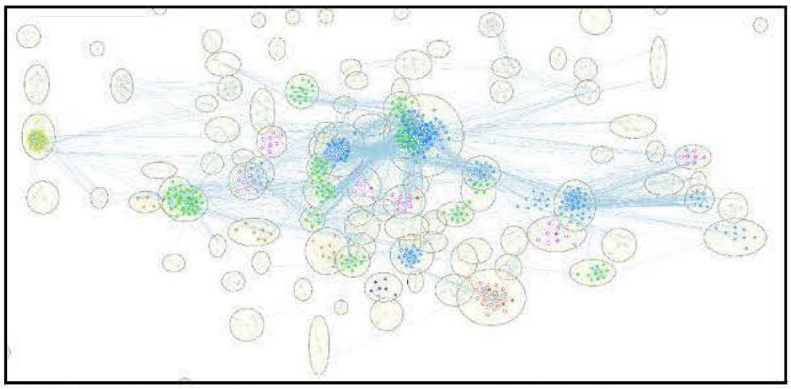


Figure 1. Map of pathway enrichment of the lung ALF proteome in healthy elder humans (60-75 years old) compared to adults (18-55) showing clusters of pathways involved in: cellular processes and homeostasis (**blue**), immune responses (**green**), lung alveolar environment remodeling and homeostasis (**violet**), oxidative stress (**orange**), regulatory processes (**purple**), intracellular killing mechanisms (**red**), and lipid metabolism and recognition (**yellow**).

Additional DIA-MS analyses are needed for the group to substantially expand their subject pool for discovery proteomics in order to more specifically associate subject characteristics with ALF protein composition. Due to the limited quantities available of human ALF, these analyses will significantly benefit from the outstanding performance of the Exploris 480 for DIA-MS. This study, comparing adult *vs.* elderly ALF, will involve at least 480 samples in a three-year period.

Another important branch of research in the [redacted] lab is a study of how *M. tuberculosis* adapts to the different environments that are encountered during infection and how the composition of these environments determines the way that *M. tuberculosis* behaves during infection *in vitro* and *in vivo*. They further are investigating how the composition of the *M. tuberculosis* cell wall manipulates the host, dictating latency infection or active TB disease. The group has identified several *M. tuberculosis* genes involved in infection progression within the host and generated 24 *M. tuberculosis* mutants, each of which lacks one of these genes. They plan to use DIA-MS to comprehensively characterize the proteomes of these *M. tuberculosis* cell wall mutants to investigate their correlation to TB pathogenesis. These analyses will significantly benefit from the enhanced depth of coverage for DIA-MS of the requested Exploris 480. This study will require multiple analysis sets over a period of at least four years.

The group's discovery research is based on delineating the regulatory pathways that control oxidative stress and inflammation in the lungs as we age. To identify these pathways it is critical that they identify the maximum number of proteins possible, which will allow them to narrow down transcription regulators that dictate the lung's

health. In their initial DIA-MS studies (discussed above), the group identified >470 proteins that were differentially abundant in the elderly population. With the availability of the requested Exploris 480, it is anticipated that they will be able to identify many more lower abundance proteins that will substantially improve the definition of the pathways involved in the increased susceptibility to respiratory infections with age.

References

Accessible User Time (AUT) for the Requested Instrument (repeated from above)
An instrument like the Exploris 480 can operate 24x7 since there is a refrigerated autosampler on the HPLC and samples can be queued for unattended analysis. Accessible user time (AUT) was calculated based on 8,766 instrument operating hours per year (24 hours/day x 365.25 days/year) minus 1,234 hours for the following: weekly calibration; preventative maintenance; new method development; new protocol implementation; HPLC and mass spectrometer repairs. This results in a total AUT rounded to 7,500 hours per year. The user group listed in this proposal for the requested instrument is projected to require 84.5% of the total AUT; 5% AUT is anticipated for investigators who are not in the listed user group and will have a small number of protein complexes for cryo-EM analysis, leaving 10.5% available for new users. The projected AUT per user is shown in **Table 3** below.

Table 3. Grant Funding of Major Users

User Name	Grant Number[1]	Project Title (Period)	Hrs (%AUT)
Discovery proteomics (DIA-MS protein identification/relative quantification)[2]			
[redacted]	R01CA[redacted]	[redacted]	340 (4.5%)
[redacted]	R01CA[redacted]	[redacted]	
[redacted]	R01CA[redacted]	[redacted]	
[redacted]	R01A[redacted]	[redacted]	340 (4.5%0
[redacted]	RO1AR[redacted]	[redacted]	360 (4.8%)
[redacted]	RO1AR[redacted]	[redacted]	
[redacted]	RO1EY[redacted]	[redacted]	
[redacted]	R01CA[redacted]	[redacted]	550 (7.3%)
[redacted]	R01A[redacted]	[redacted]	500 (6.7%)
[redacted]	R21A[redacted]	[redacted]	
[redacted]	R01CA[redacted]	[redacted]	480 (6.4%)
[redacted]	R01CA[redacted]	[redacted]	
[redacted]	R01CA[redacted]	[redacted]	460 (6.1%)
[redacted]	R01A[redacted]	[redacted]	575 (7.7%)
[redacted]	P01AG[redacted]	[redacted]	340 (4.5%)

User Name	Grant Number[1]	Project Title (Period)	Hrs (%AUT)
[redacted]	R01CA[redacted]	[redacted]	600 (8.0%)
[redacted]	R01CA[redacted]	[redacted]	
Discovery proteomics (DIA-MS) and cross-linking MS (DDA-MS). These users require the FAIMS interface[2]			
[redacted]	CA[redacted]	[redacted]	360 (4.8%)
[redacted]	R01GM[redacted]	[redacted]	
[redacted]	R01CA[redacted]	[redacted]	
[redacted]	R01GM[redacted]	[redacted]	400 (5.3%)
[redacted]	R01GM[redacted]	[redacted]	
[redacted]	R35CA[redacted]	[redacted]	460 (6.1%)
[redacted]	[redacted]	[redacted]	575 (7.7%)
Subtotal user group with project descriptions			6,340 (84.5%)
Additional users for cross-linking MS (DDA-MS) for structure in conjunction with cryo-EM			375 (5%)
New users			785 (10.5%)
Total			7,500 (100%)

[1] NIH funding; total grant support is provided in each investigator's biosketch; all listed users have NIH funding.

[2] The FAIMS interface will be needed for users who will submit samples for cross-linking-MS analysis. The other accessories will be needed by all users.

Organizational/Management Plan

Location. The requested Exploris 480 and accessories will be installed in the [redacted] which occupies space (1,034 sq. ft. total) in the Department of Biochemistry and Structural Biology in the Medical Building on the [redacted] The main laboratory is 713 sq. ft. plus an adjacent room for staff offices and data processing. It houses four mass spectrometers (all Thermo Scientific): Orbitrap Fusion Lumos Tribrid/ETD/ (RSLC NANO HPLC); LTQ-Orbitrap Velos Pro (RSLC NANO HPLC); Q Exactive (Ultimate 3000 HPLC system with diode array detector); ISQ GC-quadrupole MS. There is an Agilent model 6224 ESI-TOF-MS in a nearby room where [redacted] gel electrophoresis is conducted. [redacted]

Personnel Responsibilities. There are two full-time [redacted] staff members, [redacted] (see biosketches). [redacted] is responsible for overseeing day-to-day operations of the [redacted] His work assignments parallel those of [redacted] in terms of meeting with investigators and their laboratory staff members to finalize details for sample submission/analysis, scheduling analyses, sample receipt and logging into the institutional core service request system (iLab) and local database, preparing samples, performing analyses, reporting results, conducting necessary instrument calibration and QA/QC testing. [redacted] takes care of most of the routine maintenance in the core. He is also responsible for training users in operation of the ESI-TOF-MS, the only mass spectrometer in the [redacted] that is available for user access. Requests for instrument time on the ESI-TOF-MS are made through iLab; [redacted] performs calibration and HPLC set-up prior to scheduled use. On request, [redacted] provides text and prepares figures and graphs for grant applications, publications and presentations. [redacted] will share responsibility for use of the Exploris 480.

Instrument Management. There are no plans for the Exploris to be an "open-access" instrument, as has been the policy for the Orbitrap and Lumos and all previous proteomics mass spectrometers in the [redacted]. There are numerous reasons for this policy—mainly associated with the extremely-complex nature of a high-performance mass spectrometer and the multitude of potential ways that serious problems could be caused by an inexperienced operator. Direct user access of an instrument like an Exploris 480 is also not efficient. The laboratory staff members and [redacted] constantly monitor the progress of ongoing analyses (in person and remotely) and can not only intervene if necessary, but also they can be ready to start the next sample set as soon as a previous one is finished. [For large sample sets, blocks of vials are stored frozen and only placed in the autosampler as a group near the time for their analysis in order to maximize sample quality.] What is more important for users than hands-on instrument operation is training in experimental design, data analysis and interpretation. For all but the most routine analyses, extensive discussions are held with [redacted] before sample submission to be sure that the goals of the experiment are mutually understood and the plans for implementation are as optimal as possible. The user then communicates with [redacted] about sample submission. After MS analysis and data processing are complete, results are transmitted to the users in the form of a Scaffold file which can be examined and manipulated via a free Scaffold Viewer. Core users receive instruction from [redacted] in use of the Viewer. For all discovery proteomics and interactomics studies, before sending the file(s), [redacted] meets with the users (via Zoom) to show the results and help with interpretation. At that time, data are exported into Excel as needed, and initial pathway analysis is conducted using Reactome. In addition, students who submit samples are given more in-depth information about the underlying mass spectrometry, rationale for choice of MS method and additional details about the data processing workflow.

Scheduling. Service requests are submitted on-line through the [redacted] Mass Spectrometry Laboratory site in iLab. Users select the type of analysis and the number of samples. An e-mail notification is sent to [redacted] staff members who revise the request, if needed, to include any additional services that will be required; an e-mail is then sent to the user for financial approval. Users also fill out a sample submission form with contact information and details about the samples. Print and electronic copies of the sample submission form and any gel images associated with the project are maintained, along with a local Microsoft Access database of samples received, analyses conducted in the [redacted] and results files sent to the users. This arrangement provides essential continuity and back-up of project records over time. Unless prior arrangements have been made to accommodate deadlines for grant applications and/or manuscripts and presentations, projects are initiated in the order received; in-house users take precedence over investigators from outside institutions. [redacted] members have priority over other [redacted] users for samples submitted at the same time. Billing for [redacted] service is handled in the

office of the Director of Institutional Cores after a service request has been marked as "completed" in iLab by [redacted] staff and the charges reviewed by [redacted].

Attracting New Users to the Mass Spectrometry Laboratory. There are several avenues for attracting new users for the [redacted]. 1) In addition to the iLab site, the [redacted] maintains a website on the [redacted] domain [redacted] that will be updated to include the new instrument if this proposal is funded. 2) [redacted] periodically gives oral presentations at the [redacted] meetings, retreats and symposia to apprise Cancer Center members about core services. 3) As a Cancer Center Shared Resource, an [redacted] poster is displayed at Cancer Center symposia and annual retreats. 4) [redacted] has participated in the past in various [redacted] department meetings to inform faculty members of Core services; and will continue to do this in the future. 5) [redacted] will be a presenter at institutional core workshops that are planned for the near future. 6) If this proposal is funded, an announcement will be distributed campus-wide by the Vice President for Research, along with information about the enhanced services that will become available.

Data Backup. Mass spectrometry "raw" files are acquired on each instrument computer and then transferred to a data processing workstation in the [redacted] (8 TB on two sets of mirrored drives for DDA-MS data and short-term backup; 10 TB on a separate workstation for DIA-MS files). For DDA-MS analyses, peak picking with Mascot Distiller is conducted on one of two local workstations, and searching with Mascot is accomplished on a virtual server in the new [redacted] data center. The files are then transferred back to the [redacted] for Scaffold post-processing on one of the workstations. Scaffold DIA on a separate workstation in the [redacted] is used for the complete DIA-MS processing workflow. Raw files are archived and backed up on a remote server in the [redacted] data center.

Quality Assurance. Mass spectrometers are calibrated weekly according to the manufacturer's instructions and are tuned according to the manufacturer's schedule. Annual preventative maintenance is conducted by a manufacturer's field service engineer (part of the maintenance agreement). A commercial quantitative peptide standard (Sigma MS Qual/Quant QC Mix) is analyzed at least twice before and after all analysis sets to document chromatography and mass spectrometry performance. The outputs of the analyses of the standards are visually inspected for chromatographic peak shape, retention time, intensity and quality of precursor and tandem mass spectra; data in selected files are searched with Mascot and assembled in Scaffold 5. Criteria have been established for determining acceptability. Final processing of all users' datasets (except for the ESI-TOF/MS) is conducted in Scaffold 5 for DDA data and Scaffold DIA for DIA-MS data. The Scaffold files permit direct, visual examination of the protein/peptide assignments and the tandem mass spectra.

Citing Use of the Instrument. There is a prominent statement on the [redacted] sample submission form on which users agree to acknowledge the services of the core and cite appropriate grants. In essentially all cases where data from the MSL are included in a manuscript or presentation, [redacted] is asked to provide input for content and formatting. At that time she gives the users the necessary text for the Acknowledgements section.

Advisory Committee. The Mass Spectrometry Laboratory Advisory Committee members are: [redacted] The committee meets once or twice a year unless unusual issues arise. The role of the committee is to recommend guidelines for laboratory operation (including sample scheduling policies and fees), to help plan future directions, and to be available for problem solving, if necessary. There is also an Institutional Core Committee (ICC) under the auspices of the office of the Vice President for Research, [redacted]. The committee is composed of "senior leaders from the University's schools, main research centers, scientific base, financial, administrative and regulatory units. It "provides advice to the Vice President for Research to ensure the Core Labs optimize their service to the University's scientists" and "ensures strategic representation of the University's schools, main research centers, scientific base, financial, administrative and regulatory units, and the [redacted] Core Laboratory Network in the management of core

laboratories." A report is submitted each year with details about [redacted] activities (scientific and financial); the minutes of the [redacted] Advisory Committee meeting(s) are included. The ICC annually evaluates the operation of each institutional core. Institutional funding for the cores is managed through [redacted] serves as a liaison between the cores and the office of the Vice President for Research and is an advocate for the needs of the cores and their staff.

Biohazard Precautions. Samples with any associated biohazard potential are processed in the submitting investigator's laboratory with appropriate precautions up to a stage in the workflow where the samples can be safely handled in the [redacted]. The [redacted] staff members and [redacted] must complete an annual Biosafety training course and, are, therefore, aware of necessary safe practices related to biohazards.

Financial Plan. Operation of the [redacted] is supported jointly by institutional funds, chargebacks, and the [redacted] A user fee schedule is in place for the [redacted], with the basis for charges depending on the type of analysis (detailed in iLab). Rates for analyses are determined by taking into consideration the numbers of samples analyzed in the previous year as well as costs for staff salaries, maintenance agreements and consumables relative to income from chargebacks. The rate for a given service is scaled according to the instrument time required for the analysis. In addition to analysis-specific instrument time, costs include fixed charges, such as sample preparation (e.g., Barocycler homogenization, 1-D SDS-PAGE, S-Trap digestion) and protein/peptide quantitative analysis. Rates are reviewed annually and adjusted as appropriate. The rates for all MS analyses are higher for outside academic institutions [redacted] and [redacted] for non-academic submissions. [redacted] members receive a 10% discount from the in-house rates, in accordance with institutional policies. The projected five-year budget for the requested Exploris 480 is shown in **Table 4** below.

Table 4. Estimated Annual Income and Expense for the Exploris 480

	Year 1	Year 2	Year 3	Year 4	Year 5
Income					
Chargebacks[1]	$90,000	$99,000	$108,900	$119,790	$131,769
Cancer Center salary support[2]	$6,300	$6,489	$6,684	$6,884	$7,091
VPR salary support[3]	$4,635	$4,774	$4,917	$5,065	$5,217
Income total	$100,935	$110,263	$120,501	$131,739	$144,076
Expense					
Salaries[2]	$60,300	$62,109	$63,972	$65,891	$67,868
Maintenance contract[4]	$0	$55,620	$57,289	$59,007	$60,777
Chemicals and supplies[5]	$15,000	$15,450	$15,914	$16,391	$16,883
Software licensing fees[6]	$6,500	$6,695	$6,896	$7,103	$7,316
Server and computer storage	$8,000	$8,240	$8,487	$8,742	$9,004
Expense total	$81,800	$139,874	$144,070	$148,392	$152,844
Net[7]	$19,135	($29,611)	($23,569)	($16,653)	($8,768)

[1]Estimated based on historical data in the [redacted] for DIA-MS and DDA-MS analyses, increased by 10% per year for anticipated increased usage; annual rate adjustment not applied

[2]Proportioned between Exploris 480 (45%), Lumos (45%) and Q Exactive (10%); increased by 3% per year

[3]For Core Director; 10% of salary, proportioned between Exploris 480 (45%), Lumos (45%) and Q Exactive (10%); increased by 3% per year

[4]Exploris 480, UltiMate 3000 RSLCnano HPLC and FAIMS Pro interface; increased by 3% per year

[5]Solvents, reagents, gasses, consumables, HPLC columns; increased by 3% per year

[6]Matrix Science, Proteome Software, TeamViewer, ShareFile; proportioned between Exploris 480 (50%) and Lumos (50%); increased by 3% per year

[7]Any net loss to be covered by the Office of the Vice President for Research

Values for expenses are based on knowledge gained during the long operating history of the [redacted] The price of the service contract for the Exploris 480 and accessories was provided by Thermo. It can be realistically anticipated that total chargeback income for the [redacted] will increase dramatically if this proposal is funded and an Exploris 480 is available to provide new capabilities for protein structure analysis along with critically-needed additional capacity for DIA-MS. Since continued support has been promised by the VPR and the [redacted] and MSL income is expected to increase commensurate with increased usage, the combined funds will be sufficient to cover service contracts and operating expenses for the Exploris 480 and accessories after the warranty period. It is indicated in the letter from [redacted], that institutional funds are available to cover repairs of core equipment in case of "catastrophic failure." These funds will not be needed for the Exploris and and accessories, because service contracts will be maintained for the duration of their operating lifetimes (as they have been for all other major instruments in the [redacted]).

Institutional Commitment. [redacted] provided continuing, strong institutional support for the [redacted] since the establishment of the core in [redacted] There have been service contracts maintained on all of the major instruments throughout the more the 40 years of the core, and full or partial support has been provided for the salary of at least one staff member for the last 15 years. [redacted] As documented in the letter from [redacted] the institution will support the requested instrument through continued funding for [redacted] operations [redacted]. Additional support is provided by the [redacted] 10% of the salaries of [redacted] and the [redacted] staff (see letter from [redacted]). As discussed above, since service contracts will be maintained for the Exploris 480 and accessories. there will not be a need to request support for repair of the Exploris 480 from the funds for "catastrophic failure" that is made available by the VPR. [These funds have been invaluable for purchase of items not covered by the Thermo service contracts, such as replacement batteries for the UPS/power conditioner for the Lumos.] Taken together, it can be projected that more than sufficient funds will be available from the combination of [redacted] chargebacks plus institutional and [redacted] support commitments to cover service contracts for the Exploris and accessories after the end of their warranties.

Overall Benefit. Acquisition of the requested Exploris 480 mass spectrometer, associated HPLC, and FAIMS interface will advance the research efforts of a large number of NIH-funded investigators at [redacted] and outside institutions by providing enhanced results for comprehensive proteomics analysis using DIA-MS (larger numbers of proteins identified and quantified across a wider dynamic range of sample quantities) as well as new avenues for obtaining protein structure information from cross-linking-MS experiments through use of FAIMS. The cross-linking-MS data will be of particular importance to investigators who will use the new [redacted] cryo-electron microscopy facility that will open in [redacted], many of whom already have their first set of grids prepared and are anxiously awaiting local cryo-EM access. The demand for cross-linking-MS is anticipated to increase substantially as colleagues learn of the cryo-EM results. The availability of comprehensive protein identification and relative quantification has already made a major impact on current users of the [redacted] by providing valuable insight into individual proteins and pathways that are altered by disease or drug treatment. The depth of coverage for identification and dynamic range for relative quantification afforded by DIA-MS for global/discovery proteomics and interactomics have expanded these investigations beyond transcriptomics to obtain a substantially more complete view of underlying biological mechanisms. As could be expected, the requests for DIA-MS in the [redacted] have continued to increase as more researchers at [redacted] become aware of this powerful technique. As a consequence, the backlog of samples has concomitantly increased, slowing down turnaround times to levels that impede progress. Based on the "demo" data obtained on the requested instrument, addition of the Exploris 480 to the [redacted] will not only result in DIA-MS data that are superior to those obtained on the Lumos, but it will provide the means to support a larger number of investigators in a timely manner. Availability of increased capacity for DIA-MS and new strategies for protein structure analysis will undoubtedly stimulate investigators (current and new users of the [redacted]) to plan experiments in the future that may take their research endeavors into exciting novel directions. Thus, acquisition of the Exploris 480 has the potential to positively impact a large number of NIH-funded investigations covering the full array of disciplines in biomedical research.

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr First Name*: [redacted] Middle Name [redacted] Last Name*: [redacted] Suffix:

Position/Title*: [redacted]
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

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

Degree Type: PhD Degree Year: [redacted]

Attach Biographical Sketch*: File Name: [redacted]

Attach Current & Pending Support: File Name: [redacted]

Funding Opportunity Number: PAR-21-126 . Received Date: 2021-06-15T17:17:18.000-04:00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 02/28/2023

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ○ Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

○ Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*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)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

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://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? ○ Yes ● No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

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

*Previously Reported: ○ Yes ○ No

6. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Funding Opportunity Number: PAR-21-126 . Received Date: 2021-06-15T17:17:18.000-04:00