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RPPR Final Report
as of 30-Jan-2019

Agency Code:

Proposal Number: 70421LSREP

Agreement Number: W911NF-17-1-0518

INVESTIGATOR(S):

Name: II Robert Blake
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Organization: **Xavier University of Louisiana**

Address: 1 Drexel Drive, New Orleans, LA 701251098

Country: USA

DUNS Number: 020857876

EIN: 720635884

Report Date: 30-Nov-2018

Date Received: 05-Dec-2018

Final Report for Period Beginning 01-Sep-2017 and Ending 31-Aug-2018

Title: New Instrumentation and Means to Study the Mechanisms and Pathways of Energy Generation in Microorganisms

Begin Performance Period: 01-Sep-2017

End Performance Period: 31-Aug-2018

Report Term: 0-Other

Submitted By: II Robert Blake

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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 2

STEM Participants: 4

Major Goals: The principal practical goal was to purchase the customized equipment/instruments necessary to construct and assemble a new and novel integrating cavity absorption meter (ICAM) to conduct accurate spectroscopic, fluorescence, electrochemical and other concomitant measurements on respiratory electron transfer reactions in intact bacteria. Purchase, delivery and installation were completed in April, 2018. The new instrument joins and complements existing spectrophotometers and fluorimeters on the third floor of the Pharmacy Addition at Xavier. All of these instruments are dedicated to studying the mechanisms and pathways for energy generation in microorganisms. The new ICAM is now in use.

Accomplishments: The types of new measurements that are possible with this new instrument and their expected outcomes are briefly summarized in Table I, below. With the acquisition of this instrument, the PI is now in the process of testing five new hypotheses that were originally generated from his recently-terminated DoD-funded project that would not be possible without access to this new customized instrument:

(i) the initial sequential reduction of redox biomolecules in the aerobic iron respiratory chain will be observed if the intact cells and their soluble reductant are rapidly mixed on the time scale of 1 to 2 milliseconds;

(ii) rapid, virtually instantaneous in situ measurements of oxygen concentration are possible using luminescent dyes whose excited state lifetimes are affected by dissolved oxygen;

(iii) photochemical action spectra conducted in the presence of inhibiting carbon monoxide will reveal the absorbance spectrum of the CO-binding terminal oxidase in each different iron respiratory chain;

(iv) absorbance measurements following the flash photolysis of CO from heme groups in the terminal oxidase will enable us to study the kinetics of oxygen binding to and reactivity with the terminal oxidase in the aerobic respiratory chain; and

(v) respiration-dependent changes in NAD(P)H concentrations within the cell may be quantified if in situ fluorescence measurements are conducted in intact cells during aerobic respiration on soluble iron.

Table I. Types and benefits of new measurements to be conducted on biological suspensions that scatter light.

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as of 30-Jan-2019

Measurement/Capacity Type	Results and Outcomes
Absorbance	Monitor the oxidation-reduction status of colored prosthetic groups in the presence of different concentrations of electron donors and acceptors
Fluorescence	i. Monitor energy metabolism-dependent changes in the concentration of NAD(P)H ii. Monitor energy metabolism-dependent changes in cellular pH using cell-permeant, pH-sensitive dyes iii. Monitor energy metabolism-dependent changes in membrane potential using potential-sensitive dyes
Luminescence lifetime	Monitor the energy metabolism-dependent concentration of molecular oxygen using dyes whose luminescent lifetimes are sensitive to oxygen
Oxygen microelectrode	Monitor the energy metabolism-dependent concentration of molecular oxygen using an oxygen microelectrode
Redox potential microelectrode	Conduct spectropotentiometric titrations of redox-active prosthetic groups either in vitro or in vivo
Ion-selective microelectrodes	Correlate metabolism-dependent changes in the concentrations of selected analytes with concomitant spectrophotometric or fluorimetric changes
Rapid-mixing capability	Extend the initial temporal range of the spectroscopic observations listed above to less than 100 milliseconds
Photochemical action spectra	Identify the absorbance properties of the terminal oxidases that are inhibited by carbon monoxide (CO)
Flash photolysis capability	Monitor the rapid reactions (microsecond) with oxygen when CO is dissociated from terminal oxidases by light absorption

In addition, the educational and research activities of STEM undergraduate students at Xavier University have benefited and will continue to benefit from the acquisition of this research instrument in that numerous student support programs (including the BUILD, COE, RISE, MARC programs) are always looking for active laboratories in which to place students for a productive research experience. The PI's laboratory currently hosts five undergraduate minority students, all of whom are active and collecting publishable data using the current equipment. The acquisition of this new capability has only increased the opportunities for students to have a productive experience in this laboratory.

The PI has a record of 35 years of directing and maintaining externally-funded research projects that exploit spectroscopic methods to investigate biological phenomena. This new capability is providing new opportunities to conduct biochemical measurements that have not been possible heretofore.

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Training Opportunities: Four undergraduate students and a Postdoctoral Research Associate were trained on this new instrument during the reporting period.

Results Dissemination: Nothing to Report

Honors and Awards: The PI was recently awarded a 3-year grant from the National Science Foundation that will provide funds for the routine maintenance of this new, novel instrument after the end of DoD support.

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)

Participant: Richard Painter

Person Months Worked: 3.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Participant Type: Undergraduate Student

Participant: Bria Landry

Person Months Worked: 6.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Participant Type: Undergraduate Student

Participant: Brionna King

Person Months Worked: 4.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Participant Type: Undergraduate Student

Participant: Pham Nghi

Person Months Worked: 12.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Participant Type: Undergraduate Student

Participant: Olivia Griswold

Person Months Worked: 12.00

Funding Support:

Project Contribution:

RPPR Final Report
as of 30-Jan-2019

International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

FINAL PROGRESS REPORT

Title: New Instrumentation and Means to Study the Mechanisms and Pathways for Energy Generation in Microorganisms

Principal Investigator: Robert Blake II

We solicited and received price quotation #TWDQ2165-01 for \$261,936 from On Line Instrument Systems, Inc., to purchase the customized equipment/instruments necessary to construct and assemble a new and novel integrating cavity absorption meter (ICAM) to conduct accurate spectroscopic, fluorescence, electrochemical and other concomitant measurements on respiratory electron transfer reactions in intact bacteria. A copy of the formal quotation is attached below. Because On Line Instrument Systems, Inc., is the only vendor that constructs and sells this novel, unique type of instrument, only one formal price quotation was solicited.

Purchase, delivery and installation were completed in April, 2018. The new instrument joins and complements existing spectrophotometers and fluorimeters on the third floor of the Pharmacy Addition at Xavier. All of these instruments are dedicated to studying the mechanisms and pathways for energy generation in microorganisms. The new ICAM is now in use. The PI has recently acquired a 3-year grant from the National Science Foundation that will provide funds for the routine maintenance of the ICAM after the end of DoD support.

The types of new measurements and their expected outcomes are briefly summarized in Table I, below. With the acquisition of this instrument, the PI proposes to test five new hypotheses that were originally generated from his recently-terminated DoD-funded project that would not be possible without access to this new customized instrument:

(i) the initial sequential reduction of redox biomolecules in the aerobic iron respiratory chain will be observed if the intact cells and their soluble reductant are rapidly mixed on the time scale of 1 to 2 milliseconds;

(ii) rapid, virtually instantaneous *in situ* measurements of oxygen concentration are possible using luminescent dyes whose excited state lifetimes are affected by dissolved oxygen;

(iii) photochemical action spectra conducted in the presence of inhibiting carbon monoxide will reveal the absorbance spectrum of the CO-binding terminal oxidase in each different iron respiratory chain;

(iv) absorbance measurements following the flash photolysis of CO from heme groups in the terminal oxidase will enable us to study the kinetics of oxygen binding to and reactivity with the terminal oxidase in the aerobic respiratory chain; and

(v) respiration-dependent changes in NAD(P)H concentrations within the cell may be quantified if *in situ* fluorescence measurements are conducted in intact cells during aerobic respiration on soluble iron.

Table I. Types and benefits of new measurements to be conducted on biological suspensions that scatter light

Measurement/Capacity Type	Results and Outcomes
Absorbance	Monitor the oxidation-reduction status of colored prosthetic groups in the presence of different concentrations of electron donors and acceptors
Fluorescence	<ul style="list-style-type: none"> i. Monitor energy metabolism-dependent changes in the concentration of NAD(p)H ii. Monitor energy metabolism-dependent changes in cellular pH using cell-permeant, pH-sensitive dyes iii. Monitor energy metabolism-dependent changes in membrane potential using potential-sensitive dyes
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In addition, the educational and research activities of STEM undergraduate students at Xavier University have benefited and will continue to benefit from the acquisition of this research instrument in that numerous student support programs (including the BUILD, COE, RISE, MARC programs) are always looking for active laboratories in which to place students for a productive research experience. The PI's laboratory currently hosts five undergraduate minority

students, all of whom are active and collecting publishable data using the current equipment. The acquisition of this new capability has only increased the opportunities for students to have a productive experience in this laboratory.

The PI has a record of 35 years of directing and maintaining externally-funded research projects that exploit spectroscopic methods to investigate biological phenomena. This new capability is providing new opportunities to conduct biochemical measurements that have not been possible heretofore.



QUOTATION

Prepared: 10/23/17 By: Travis Number: TWDQ2165-01

Robert Blake, Ph.D.

XAVIER UNIVERSITY OF LOUISIANA
 College of Pharmacy
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 New Orleans, Louisiana 70125

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Qty	Description	Ext. Price
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1 ■ OLIS CLARiTY 1000A

\$129,900.00

Study oxidation-reduction and other high speed processes of mitochondria, bacteria, and other organisms or biomolecules using this remarkable new RSM 1000 spectrophotometer enhanced with the OLIS CLARiTY Chamber. The "CLARiTY" chamber eliminates the affect of scatter and thus allows accurate absorbance (and fluorescence with optional enhancement) spectroscopy to be done on turbid sample suspensions.

The sample compartment is a dual beam CLARiTY Chamber. Within the "CLARiTY" chamber are two stirred quartz 'suspension presentation cavities,' (DSPC) each of which holds up to either 1mL, 4 mL, or 8 mL of sample. The DSPCs return effective pathlengths of 5, 20, or 30 centimeters, respectively. Ports are provided on four sides of the sample holders to allow entrance and exit of excitation and emission beams. Inlet ports through the top of the Chamber allow introduction of reactants into the sample (and reference) suspensions manually or with the OLIS Automatic Titrator (quoted separately). This integrating cavity system is calibrated once at the OLIS facility and does not require the frequent re-calibration required by integrating spheres.

The absorbance measurement beam is produced by a high stability 75W xenon arc lamp mounted to the patented DeSa Subtractive Double Grating Monochromator with Moving Intermediate Slit (aka, the RSM). The "Moving Intermediate Slit" is an OLIS StepDisk. The speed of the high precision stepping motore driven StepDisk is 100 scans per second. The StepDisk also has a static mode, which is employed for fixed wavelength with and without associated movement of the gratings (to duplicate a normal spectrophotometer's means of scanning).

The spectral range of the RSM is determined by the accessible 50 x 50 mm gratings. The default choice is a pair of gratings blazed at 500 nm with 400 lines/ mm which are useful from about 330-750 nm with a 231 nm span. These gratings can be exchanged in moments for others, which will provide different spectral ranges and resolutions.

All electronics and detection hardware are included. The provided computer hardware includes an OLIS verified and supported desktop PC with 3rd Gen. Intel Core i3-3240 3.4GHz, 4GB DDR3, 1TB HDD 7,200RPM, DVD-RW drive, 6 Total USB 2.0 Ports. Windows 7 Pro 64-bit (English version required), Keyboard & Mouse and 20" Class Monitor

All electronics and circuitry are housed in a free standing Master Control Box the approximate size and dimensions of the tower computer.

Software includes OLIS SpectralWorks for the OLIS CLARiTY 1000, a powerful, intelligently designed, and incredibly fast software package for controlling operation of the monochromator



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motors, signals from the detectors, as well as collecting, displaying, analyzing, and storing/retrieving data from the system. This software supports all available data collection rates of the hardware. Exclusive implementation of Matheson's SVD, Downhill Simplex, and Matrix Exponentiation algorithms are provided for instantaneously fast and precise 2D and 3D data fitting. Data storage/retrieval, including powerful import/export capabilities are provided for dealing with acquired data. Proprietary algorithms based on the published work by Fry and Javorfi are included for accurate pathlength correction along the entire spectral range of the data.

1 ■ Scanning Emission Module for the detection of fluorescence, OLIS CLARiTY 1000 version **\$19,400.00**

The first monochromator (the RSM) is fixed on an excitation wavelength, then second monochromator (after the sample) is fixed to a single emission wavelength or set to scan through numerous wavelengths to acquire an emission spectrum. A higher sensitivity Hamamatsu photon counting detector is included for success with low light level emission detection.

Presumes use of the rapid-scanning monochromator in its conventional scanning mode, not rapid-scanning. Module includes the OLIS single grating monochromator for UV/Vis scanning to be scanned in synchrony with the primary (RSM) monochromator on the OLIS CLARiTY 1000. In this way, the detector sees only the absorbance measurement wavelength, thus eliminating any inherent fluorescence from the absorbance reading. The monochromator thus functions as a continuously variable filter, taking the place of tens or hundreds of bandpass filters.

Software enhancement of the OLIS SpectralWorks software supports the synchronous and asynchronous scanning and acquisition from the new detector.

1 ■ For Rapid-scanning Fluorescence, an excitation source is added; both steady-state xenon arc and wavelength specific LEDs are included. **\$12,900.00**

1 ■ OLIS Rapid Sample Delivery System for Turbid Samples **\$28,900.00**

Our Useful, Simple, and Affordable stopped-flow for research grade stopped-flow work. The OLIS U.S.A. stopped-flow provides complete mixing, anaerobic performance, temperature regulation with use of water bath, and failsafe operation. Exclusive use of ceramic valves and fittings make this stopped-flow impervious to solvents and gas exchange. Unique 'interlock' safety system makes pre-mixing or other accidents impossible: the three valves, pneumatic actuator, and the stopped-flow syringe must be in the correct positions before the computer allows firing of the stopped-flow; the result is 100% security against inadvertent waste of reagents and/or damage to the stopped-flow and syringes. Tubing and mixer of the CLARiTY SF are selected to provide a high volume, high velocity flow from the two syringes to achieve a dead time of ~10 millisecond. First year warranty covers all issues; a modestly priced Warranty Renewable Package is available on an annual basis. Made in the USA*

1 ■ OLIS OxyDye Oxygen Measurement System **\$12,900.00**

This enhancement is suitable for use with in a wide range of spectrophotometers and fluorimeters and is not limited to use with OLIS equipment. The oxygen reading is



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synchronized to be non-interfering with other optical measurements on-going by the primary data analysis program.

The OxyDye method uses phosphorescence lifetime as a measure of oxygen concentration. A submicromolar concentration of dye (harmless to biological materials) is added to the given sample (dye purchased separately). A pulsed LED excites dye in the sample to luminescence and a PMT used as a photon counter detects the emitted light. The counts from the detector are collected by 100 digital counters on an FPGA card. The decay of the luminescence (its lifetime) varies from about 5 to 60 microseconds depending on the oxygen concentration. The LED excitation pulses can be repeated at rates to 5000 per second, so that only considerations of the S/N required in a particular experiment limits the response time of the system to the oxygen concentration. The acquired 100 points of the decay profile are read from the counters and are fitted to one exponential; the resulting decay constant (Tau) is converted to oxygen concentration using the Stern-Volmer equation. The measurement is unaffected by fluorescence and the intensity of the phosphorescence.

The OxyDye enhancement to an OLIS spectrophotometer includes the LED, fiber optics, photon counting module, supporting electronics, and dedicated data acquisition and handling software, accessed as a new icon.

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|----------|--|---------------------------|
| 1 | ■ Shutter Disk | <u>\$3,800.00</u> |
|
 | | |
| 1 | ■ Actinic Source for OLIS CLARiTY 1000 to induce photochemical events
A pulsed Xenon light source to be added to the emission monochromator.
This includes required mounting hardware for the emission monochromator and controlling software. | <u>\$14,900.00</u> |
|
 | | |
| 3 | ■ Additional DSPC
To expand the use of one Olis CLARiTY spectrophotometer beyond the limits of a given DSPC, a second one can be purchased to accommodate another volume or cell geometry.

Customer would like customized DSPCs to be equipped with removable caps for chimney to be air-tight (both with regards to material and an O-ring to seal the perimeter of the cap. Need to be equipped with at least 2-mm holes for 2 different microelectrodes.

For the purpose of the electrochemical measurement in the observation cell it needs to be possible to insert gold or platinum wires through the cap.

Volumes of DSPCs to be determined. | <u>\$7,500.00</u> |
|
 | | |
| 1 | ■ Peltier Temperature control capabilities for the CLARiTY DSPCs
Temperature control for CLARiTY measurements from -40°C to +110°C. This also includes the temperature controller TC1. | <u>\$8,500.00</u> |



On-Line Instrument Systems, Inc.
 130 Conway Drive, Suites A & B · Bogart, Georgia 30622 · www.olisweb.com
 Phone: (706) 353-6547 · Fax: (706) 353-1972 · E-mail: travis@olisweb.com

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- 1 ■ **Travel, On-Site Installation, System Verification, and Two Day User-Training Period by OLIS personnel (doctorate in biochemistry with expertise in spectroscopy).** \$5,100.00
 OLIS staff scientist will uncrate and setup the system, verify all system performance parameters, and train all laboratory staff in attendance on use and care of the OLIS equipment and software.

- 1 ■ **Taxation to be assessed @ 7%** \$17,136.00

SubTotal	<u>\$260,936.00</u>
Shipping Total	<u>\$1,000.00</u>
TOTAL	<u>\$261,936.00</u>

Deposit Amount* = \$130,968.00

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