

PRINCIPAL INVESTIGATOR COVER LETTER AND TERMS AGREEMENT



Professor Evgeny Katz, Milton Kerker Chair in Chemistry

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Re: Biofuel Cells Based on Microbial Enzymes and Nanocomposite Materials for Powering Microelectronic Devices

I, Principal Investigator (PI) Prof. **Evgeny Katz**, hereby acknowledge that I have submitted a proposal to 2022 *U.S.-Ukraine Alternative Energy Research Competition RFP*. I will be collaborating with Prof. **Mykhailo Gonchar** of Institute of Cell Biology, NAS of Ukraine.

If awarded, I undertake this research in good faith and will uphold my portion of the collaborative work as proposed in the submission.

I attest that the information contained in this proposal is truthful and that it has been prepared with the full knowledge and consent of Ms. Shannon Robinson, leadership representative of Clarkson University.

I affirm that I have read and understand CRDF Global's policies and standards outlined within the 2022 *U.S.-Ukraine Alternative Energy Research Competition RFP*, including CRDF Global's Plagiarism Policy¹. I agree to adhere to CRDF Global's Plagiarism Policy, and understand that CRDF Global will not provide funding to an application in which plagiarism exists. If plagiarism is detected, penalties may be imposed up to and including my exclusion from this funding opportunity and barring my participation in future funding opportunities.

A handwritten signature in black ink, appearing to read "E Katz".

Principal Investigator Signature: professor Evgeny Katz

February 02, 2022
Date

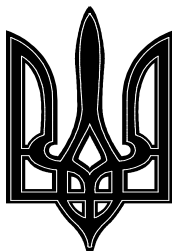
A handwritten signature in black ink, appearing to read "Shannon Robinson".

Institution Leadership Representative Signature:
Shannon Robinson
Associate Vice President for Research & Technology Transfer
/ Executive Director of Institutional Planning

February 03, 2022
Date

¹ [For more information, please see CRDF Global's Plagiarism Policy](#)

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Re: Biofuel Cells Based on Microbial Enzymes and Nanocomposite Materials for Powering Microelectronic Devices

I, Principal Investigator (PI) Prof. **Mykhailo Gonchar**, hereby acknowledge that I have submitted a proposal to *2022 U.S.-Ukraine Alternative Energy Research Competition RFP*. I will be collaborating with Prof. **Evgeny Katz** from Clarkson University (Potsdam, NY, USA).

If awarded, I undertake this research in good faith and will uphold my portion of the collaborative work as proposed in the submission.

I attest that the information contained in this proposal is truthful and that it has been prepared with the full knowledge and consent of Prof. **Andriy Sibirny**, leadership representative of Institute of Cell Biology, NAS of Ukraine.

I affirm that I have read and understand CRDF Global's policies and standards outlined within the *2022 U.S.-Ukraine Alternative Energy Research Competition RFP*, including CRDF Global's Plagiarism Policy². I agree to adhere to CRDF Global's Plagiarism Policy, and understand that CRDF Global will not provide funding to an application in which plagiarism exists. If plagiarism is detected, penalties may be imposed up to and including my exclusion from this funding opportunity and barring my participation in future funding opportunities.

Head of Department of Analytical Biotechnology,

Prof., Dr., DrSci

Mykhailo Gonchar

February 01, 2021

Principal Investigator

Signature

Date

Director of Institute of Cell Biology, NAS of Ukraine,

Prof., Dr., DrSci



Andriy Sibirny

February 01, 2021

Institution Leadership Representative Signature

Date

²For more information, please see CRDF Global Plagiarism Policy

CRDF GLOBAL COVER SHEET

GENERAL PROJECT INFORMATION			
Project Title (not to exceed 25 words)	Biofuel Cells Based on Microbial Enzymes and Nanocomposite Materials for Powering Microelectronic Devices		
Amount Requested (excludes cost-shares)	Total	U.S. Sub-Team	Ukrainian Sub-Team
	\$80,000	\$30,000	\$50,000
Research Categorization ³	Research Area	Sub-Research Area	Research Focus
	1. Natural Sciences	1.6 Biological Sciences; 1.4 Chemical Sciences.	1.6.4 Biochemistry and Molecular Biology; 1.4.5 Electrochemistry.
Research Involves use of Human/Animal Subjects	None	Project Duration	12

UKRANIAN SUB-TEAM					
INSTITUTION INFORMATION					
Institute Name	Institute of Cell Biology, NAS of Ukraine	Institution Type	Research Institute		
Mailing Address	14/16 Drahomanov str.				
	Lviv	79005	Ukraine		
PRINCIPAL INVESTIGATOR INFORMATION					
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Residency Status	Legal Citizen				
INSTITUTION LEADERSHIP REPRESENTATIVE INFORMATION					
Name	Sibirny	Andriy	Andriyovych	Position/Title	Director, DrSci, Pofessor
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Total number of Ukrainian sub-team members, including Ukrainian PI, graduate students, secondary collaborators					8

³ Please reference the CRDF Global Research Areas document found here:
http://www.crdfglobal.org/sites/default/files/crdf-global-research-areas_updated-june-2015.pdf

U.S. SUB-TEAM					
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Highest Degree	PhD		Electrochemistry	1983	
U.S. Residency Status	Legal Citizen		Gender	Male	
INSTITUTION LEADERSHIP REPRESENTATIVE INFORMATION					
Name	Robinson	Shannon	N/A	Title	Associate VP for Research & Technology Transfer
E-mail	srs@clarkson.edu		Telephone #	1-315-268-6475	
Have you received a grant under a previous CRDF Global program or been a participant in a CRDF Global-funded workshop?				No	
If yes, please list program and grant number or workshop title in the following text box			N/A		
Total number of U.S. team members, including PI, students, and secondary collaborators					3

⁴ If you do not know your congressional district, please click on this [link](#) and search for your representative using your Institution's zip code.

PROJECT ABSTRACT
Should not exceed 350 words

Biofuel cells (BFCs) are the novel bioelectrochemical devices that use living microorganisms or their enzymes to generate electricity via enzymatic oxidation of organic substrates, including industrial waste products. The aim of the project is a development of new enzymatic BFCs based on microbial enzymes coupled with catalytic nanocomposite materials. Cheap technological byproducts (alcohols, formaldehyde, phenols) or waste compounds found in environment will be used as the “fuel” for the power production.

The scientific and technical objectives of the project are defined as follows: 1) cultivation of yeast and fungal cells; isolation and purification of alcohol oxidase and laccase; 2) synthesis and characterization of nanocomposite materials with mediator or enzyme-like activity; 3) construction, characterization of laboratory prototypes of BFCs, based on purified oxidoreductases and nanocomposites, and their testing for the powering of microelectronic chips.

The novelty of the project is based on the idea of combining the microbial oxidoreductases with the mediator systems coupled with nanocomposite materials. The obtained nanocomposites will be used for various aims: as electron transfer mediators, matrices for immobilization of biocatalysts, and nanozymes (such as artificial peroxidases) for biocathode construction.

The innovation of the project is based on preparation of a small size (millimeter size) biofuel cell for powering microelectronic chips for various sensors and to provide self-powered operation by extracting power from the environmental sources in remote locations (“install-and-forget”). The sensing will be followed by wireless information transfer, also powered by the installed biofuel cell. The systems will find future applications for environmental monitoring and homeland security. The advantages of a biocatalytic system over lithium batteries will be particularly pronounced upon miniaturization of the power supply.

This project is based on already established collaboration between scientists of the Institute of Cell Biology, NAS of Ukraine and the Department of Chemistry and Biomolecular Science of Clarkson University in Potsdam (NY, USA). The scientist from Ukrainian team is already performing research at Clarkson University, USA.

PROJECT NARRATIVE

Should not exceed ten (10) pages. Text should be Arial font size 10 within 1-inch margins

The search for new renewable energy technologies and approaches for the utilization of technogenic by-products (often toxic) are the main problems of modern science. In this regard, the development of biofuel cells (BFCs) based on microbial enzymes capable of utilizing the by-products as substrates with the simultaneous generation of electricity can help to solve these two actual problems – cheap renewable energetics and environmental protection [Beretta *et al.*, 2019; Das *et al.*, 2014].

The aim of the project is a development of new BFCs based on purified microbial enzymes, immobilized onto electroconductive nanocomposite materials. As substrates of enzymatic reactions and primary energy sources, it is planned to use low-cost and often toxic technogenic by-products for which there is a problem of their utilization, in particular: methanol and formaldehyde – for the yeast alcohol oxidase (AO) and phenol derivatives – for the fungal laccase. As the sources of the enzymes, there will be used mutant yeast strains and classically selected fungal producers, constructed/selected at the Department of Analytical Biotechnology (DAP), Institute of Cell Biology, NAS of Ukraine. The mentioned above enzymes have useful biotechnological characteristics (high thermostability, high affinity for target substrates) and are easily isolated by simple and cheap procedures, developed at DAP.

The novelty and innovative power of the project are based on idea of combining the yeast and fungal enzymes with the new mediator systems (based on synthetic dyes, ferrocene, cyanoferrate derivatives, etc.), and nanocomposite materials coupled with mediator systems. The obtained nanocomposites will be used for various aims: as electron transfer mediators, as matrices for immobilization of biocatalysts and as nanozymes (such as artificial peroxidases) for biocathode construction. The important novelty is the functional integration of the BFCs producing electric power and microelectronic devices (sensor chips) consuming the produced power.

The aim of the proposed scientific project is a combination of fundamental science with applied biotechnology using advanced interdisciplinary approaches.

The scientific and technical objectives of the project are defined as follows:

1. Cultivation of the yeast and fungal cells; isolation and purification of oxidoreductases (alcohol oxidase and laccase) – promising biocatalysts for the construction of biofuel cells (BFCs);
2. Synthesis and characterization of nanocomposite materials with mediator or enzyme-like activity;
3. Construction of laboratory prototypes of enzymatic BFCs based on purified oxidoreductases and nanocomposites, and studying their operational parameters;
4. Construction of the small (mm²) BFCs compatible with the size of microelectronic chips and activation of the microelectronic sensors with the power produced by the BFCs.

This project is based on collaboration between scientists of the Department of Analytical Biotechnology of the Institute of Cell Biology, NAS of Ukraine (Lviv, UA) and the Department of Chemistry and Biomolecular Science of Clarkson University in Potsdam (NY, USA). Scientific partners (UA and US) are carrying out innovative scientific research in the complementary fields. Both teams are high-level professionals in various fields, including microbiology, enzymology, nanotechnology, biotechnology, analytical chemistry, and bioelectrochemistry. Notably, the teams have a long-term collaboration and Dr. Oleh Smutok (UA collaborator) is presently performing research at Clarkson University (US team) [<https://www.clarkson.edu/people/oleh-smutok>].

Biofuel cells (BFCs) are the novel bioelectrochemical devices that use living microorganisms or their enzymes to generate electricity through enzymatic oxidation of organic substrates [Gamella *et al.*, 2018]. This innovative technology has a number of potential applications: generation of renewable energy, especially for powering electronic mini- and microdevices [Bollela *et al.*, 2020], production of bio-hydrogen, wastewater treatment, water desalination, etc. [Logan *et al.*, 2019].

Notably, comparing to the fuel cells based on metal catalysts, the BFCs are capable of converting chemical energy into electricity using biological catalysts. This new technology offers several advantages over traditional batteries, including the use of renewable and clean catalysts, the selectivity of the reaction, fuel flexibility, and the ability to operate at milder temperature [Ganzales-Solino & Di Lorenzo, 2018]. Taken together, all these advantages lead to an economically viable process. While traditional fuel cells designated as “low temperature” operate at approximately 80 °C, biofuel cells can operate in the range of 20 to 40 °C and at physiological pH. These properties make BFCs an interesting alternative especially for devices that need to operate at milder temperatures and conditions [Katz (Ed.) 2014]. Moreover, the variety of reactions that can be catalyzed by enzymes enables the use of various fuel types. BFCs are highly promising due to combination of desirable technological features, such as efficiency, selectivity and biocompatibility of the enzymes and therefore, they could be used to power implantable medical devices [Neifar *et al.*, 2018].

The BFCs operational principle is based on the catalytic oxidation of organic substrates by a biocatalyst, which produces the electrons that are transferred to the anode, generating an electric current. A biocatalytic cathode performs O₂ reduction, thus, closing the electric circle. A decisive influence on the BFCs performance

is the efficiency of electron transfer from the substrate (“fuel”) through biocatalysts to the electrode surface (anode and cathode) [Gunawardena & Fernando, 2008; Sayed & Abdelkareem, 2017]. There are two main types of BFCs – microbial and enzymatic. Although microbial BFCs are characterized by a low power density (up to 0.56 mW/cm² - [Ren *et al.*, 2016], they exhibit high stability (up to several months); they are cheaper than enzymatic BFCs and capable of simultaneous utilization of different substrates. However, their widespread use is limited by a low current density caused by a slow electron transport through cell membranes to the anode. The enzymatic BFCs typically have a much higher current density, but capable only of partial oxidation of the fuel and have a limited shelf life (7-10 days) due to a low stability of the enzymes [Bullen *et al.*, 2006].

The main aim of the current project is a construction of new enzymatic BFCs on the basis of purified enzymes from the yeast and fungal sources, immobilized onto electroconductive nanocomposites. Low-cost and often toxic technogenic by-products, for which utilization is actually a problem, will be used as substrates of enzymatic reactions (energy sources). Therefore, the project intends to combine two scientific goals – cheap renewable energetics and environmental protection. The designed biofuel cell, after their characterization, will be tested for powering microelectronic sensor devices. For this purpose, we will specifically design small-size (a few mm²) biofuel cells compatible by their size with the electronic chips.

The BFCs, based on using purified alcohol oxidase (AO), isolated from the cells of thermotolerant methylotrophic yeast *Ogataea polymorpha*, will be used for generation of electrical energy due to oxidation of methanol or/and formaldehyde – the toxic technogenic pollutants of wood, paint and the other industries. A peculiarity of AO-overproducing cells of the mutant yeast strain of *O. polymorpha* C-105 (*gcr1 catX*) is an impairment of glucose catabolite repression and catalase deficiency [Gonchar *et al.*, 1998], as well as the thermostability of the enzymes (including AO). AO is a FAD-containing octameric protein with a wide substrate specificity (oxidation of primary alcohols and formaldehyde), which uses oxygen as an electron acceptor. The oxidation of methanol catalyzed by AO and electrochemical current generation looks as follows:

- 1) $\text{CH}_3\text{OH} + \text{AO-FAD} \rightarrow \text{AO-FADH}_2 + \text{CH}_2\text{O}$ (enzymatic reaction);
- 2) $\text{AO-FADH}_2 + 2 \text{ mediator}_{\text{ox}} \rightarrow \text{AO-FAD} + 2 \text{ mediator}_{\text{red}} + 2\text{H}^+$ (mediator reaction);
- 3) $2 \text{ mediator}_{\text{red}} \rightarrow 2 \text{ mediator}_{\text{ox}} + 2 \text{e}^-$ (electrochemical reaction).

In general, this enzyme-electrochemical scheme is typical for the other BFCs based on oxidases with a difference in the nature of their cofactor, substrate, enzymatic product or mediator type.

For the BFCs, based on purified laccase, phenol derivatives (a toxic waste product from the chemical and pharmacological industries) will be used as a “fuel” source. Laccase is a copper-containing enzyme with a wide range of substrate specificity and can use oxygen as an electron acceptor releasing water as a by-product [Tominaga *et al.*, 2014]. Laccase has a high redox potential (from 400 mV to 800 mV vs. Ag/AgCl reference electrode), making it a promising cathode biocatalyst [Mano *et al.*, 2018; Ghosh *et al.*, 2019]. The ability of laccase for direct electron transfer to electrode modified with gold nanoparticles, is the basis for the design of mediator-free enzymatic BFCs [Kavetsky *et al.*, 2019].

In the current project, electroconductive nanomaterials based on noble and transition metals or their hybrid forms [Holade *et al.*, 2014], as well as carbon materials (graphene derivatives [Koushanpour *et al.*, 2016], carbon nanotubes and carbonated cellulose fibers) will be used for increasing the current density at the electrode. To improve the efficiency of the electron transfer in the BFCs, we plan to use nanocomposites – synthesized nanomaterials, additionally modified by inorganic and organic electron-transfer mediators (synthetic dyes, cyanoferrate derivatives, etc.) [Zhao *et al.*, 2017]. Carbon-based materials, modified with noble and transition metals (electrodeposited Pt, Pt nanoparticles, hybrid metal nanoparticles), including peroxidase-like nanozymes, are planned to be used to improve the BFCs performance [Stasyuk *et al.* 2020]. The main operational parameters (current and power density, maximum operating time, etc.) for the constructed laboratory prototypes of enzymatic BFCs will be investigated. The application of nanocomposites with bound enzymes (AO or laccase) will allow us to significantly improve the catalysis rate, both of the anode and cathode reactions, as well as will increase the operating time of the constructed BFCs up to several months [Gervasio *et al.* 2020]. So, the expected specific power densities of constructed enzymatic BFCs will be in the range from 1.2 to 2.2 mW·cm⁻².

The realization of the current project will allow combining original ideas, the novel technological approaches and experimental skills of the UA-team (specialists in the field of microbiology, enzymology, analytical biotechnology, nanotechnology) and US-group (specialists in the field of bioelectrochemistry, analytical chemistry, nanochemistry). The useful properties of the isolated yeast and fungal enzymes as well as of bionanocomposites will allow the construction of new enzymatic BFCs with improved characteristics. The expected results of the project are beyond the current level of technology and therefore are innovative and transformative.

Milestones

Task 1. Cultivation of yeast and fungal cells; isolation and purification of oxidoreductases (alcohol oxidase and laccase) – promising biocatalysts for the construction of biofuel cells (BFCs)

1.1 Screening of mushroom fungi by their ability to overproduce extracellular laccase in liquid cultures.

Screening of more than 25 strains of the mushrooms belonging to the genera *Trametes* and *Phallus*, available in the collection of the Institute (UA), for the level of extracellular laccase activity in liquid cultures *in vitro* will be performed. The best enzyme's overproducers will be selected for further investigation.

1.2 Cultivation of mutant yeast cells and selected mushrooms, overproducing the target oxidoreductases, alcohol oxidase and laccase.

The optimal conditions for the cultivation of the producers will be determined, ensuring the maximum yield of the target enzymes (composition of the culture medium, intensity of aeration, temperature, time of cultivation, etc.). To maintain the highest activity of the enzymes inside the cells (AO) or in extracellular culture (laccase), the biomass (or extracellular culture) of the organisms will be frozen or lyophilized under optimal conditions.

1.3 Isolation, purification, enzymatic and electrochemical characterization of the enzymes: alcohol oxidase (AO) and laccase.

Enzymes, isolated from the mutant yeast cells and the culture medium of the selected fungi will be concentrated and/or purified using ion exchange and/or affinity chromatography. Purified preparations of AO and laccase will be investigated using different approaches: electrophoretic (to test their purity), spectrophotometric (to determine kinetic parameters – K_M and K_{cat}) and electrochemical (activity toward electron-transfer mediators, determination of redox potential).

Task 2. Synthesis and characterization of nanocomposite materials with mediator or enzyme-like activity

2.1. Synthesis of nanomaterials based on noble and transition metal nanoparticles by chemical reduction.

The nanosized materials of noble and transition metals or their hybrid forms (which will consist of 2-3 different noble or transition metals) will be obtained using chemical reduction from the corresponding ions. Citrate, ascorbate or sodium borohydride will be used as reducing agents in optimal ratios of reactants.

2.2. Carbon-based nanomaterial screening.

The different carbon materials (graphene derivatives, carbon nanotubes, carbonized lignocellulose fibers and their composites) will be characterized by cyclic voltammetry for testing their electrochemical performance in connection to the biocatalytic processes.

2.3. Synthesis of nanocomposite materials with high efficiency of electron transfer from biocatalyst to electrode and characterization of their electron-mediator activity.

To improve communication between the biocatalyst and the electrode, screening and additional modification of the synthesized nanomaterials by inorganic electron transfer mediators (synthetic dyes, electrically conductive films, ferrocene, cyanoferrate derivatives, etc.) will be carried out. The electrochemical characteristics of the obtained nanocomposite materials and their compatibility with BFC biocomponents will be investigated. For enzymatic BFCs, based on laccase, optimization of the methods for direct mediator-less transfer of electrons *via* the nanocomposites will be carried out.

2.4. Screening and synthesis of new nanocomposite materials possessing nanozyme (nanoperoxidase) activity.

In order to create the novel BFC cathodes, the screening and synthesis of new nanomaterials with nanozyme (peroxidase) activity will be performed. The best selected nanozymes will be tested for their ability to reduce hydrogen peroxide at the electrode. The catalytic activity of the nanozymes will be compared to the process catalyzed by the microperoxidase (MP-11). The best catalytic species will be used in the next experimental steps.

Task 3. Construction, characterization of laboratory prototypes of BFCs, based on purified oxidoreductases and nanocomposites.

3.1. Investigation of the possibility of using selected nanomaterials as an immobilization matrix for covalent or physical binding of biocatalysts on the electrode surface.

In order to ensure a high local concentration of the BFC biocatalyst and to increase its stability, screening for covalent and physical immobilization of the purified preparations of AO and laccase on the electrode surface will be carried out. The nanosized carriers will be functionalized by carboxyl and amino groups using appropriate reagents (ω -mercaptohexadecanoic acid, cysteamine – depending on chemical nature of nanomaterial) followed by covalent immobilization of the corresponding enzymes.

3.2. Screening of fixing polymer membranes of different nature.

Screening of various polymeric membranes (electrodeposited, photopolymeric, dialysis) will be carried out for their ability to reliably retain enzymes on the surface of the electrodes, a good electrical conductivity and a high permeability for enzymes' substrates.

3.3. Investigation of the basic operational parameters of enzyme-based BFCs.

The basic operational parameters will be investigated for the designed laboratory prototypes of enzymatic BFCs, based on AO and laccase: current density, power density and maximum operating time. The most effective BFC variants will be selected.

Task 4. Testing of the best selected BFCs for the powering of microelectronic chips.

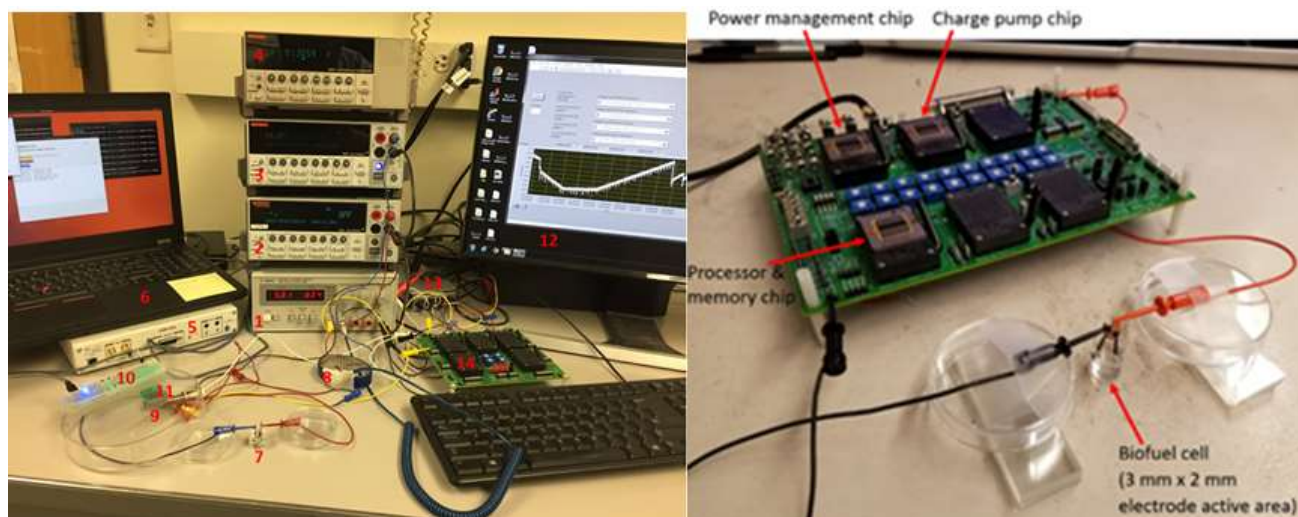


Fig. 1. (Left photo) The experimental setup for studying the microelectronic device operation and characterizing its different components. (Right photo) An example of the electronic microchip powered by a small size biofuel cell. A similar setup will be used in the proposed project when the new biofuel cell is integrated with a sensor microelectronic chip.

Figure 1 shows the setup used to test the different components individually and also, when configured as a complete system, autonomous system operating without external power. The shown setup includes the biofuel cell operating with an aqueous model solution. A sourcemeter (Keithely 2401 Sourcemeter) is used to characterize the sensor by measuring current consumption at a given voltage and can also accelerate tests by pre-charging the supercapacitor. Another sourcemeter is used to characterize the energy harvesting of the sensor by mimicking a biofuel cell. An electrometer (Keithely 6514 System Electrometer) is used to monitor 1.2 V from the 'Power Management' chip to confirm that the sensor works properly by providing voltage to the load circuits. To avoid impacting the low-power circuits, a high impedance electrometer is used. To monitor the voltage of the supercapacitor (V_{cap}), the node is connected to an analog buffer (Texas Instruments, OPA4354AIDR) powered by a power supply (Agilent E3630A) to avoid influence on this low-power circuit. Then, the voltage is converted to a digital format by an analog-to-digital converter module (National Instruments, NI-9229 & NI cDAQ-9171). The results are displayed on the monitor using a LABVIEW program. A laptop and a custom light programming module are used to generate an optical signal and program the sensor. A universal software radio peripheral (USRP) (Ettus, USRP X310), a custom antenna and the same laptop are used to wirelessly download the measured data from the sensor. After the biofuel cell and microelectronic sensor are characterized in terms of current, voltage and power, the biofuel cell will be connected to the microelectronic sensor (Figure 1, left) and the sensor will be operated autonomously powered only from the biofuel cell.

Autonomously operating microelectronic devices continuously powered by small biofuel cells will be beneficial for various environment-monitoring functions, including biosensors designated specifically for homeland security and military applications. Further miniaturization of the sensor device and connected biofuel cell would allow their operation as implantable biomedical devices. The microelectronic device can accommodate the biocatalytic electrodes directly in the structure of the device, thus further reducing the device size and eliminating unnecessary wiring connecting the biofuel cell and the electronic device.

Ukrainian and USA partners (UA and US) have all the necessary specialized equipment to carry out the planned research under the current project.

UA has the material and hardware support for the cultivation of the yeast and fungal cells (culture media, sterile boxes, autoclaves, thermostats, thermostatic shakers, disintegrators, centrifuges "Sorvall", "Eppendorf", lyophilizer "Christ alpha 1-2 LDplus"; the equipment for isolation, purification of enzymes and study of their

physicochemical and kinetic characteristics (chromatographic columns, chromatographic carriers, apparatus for vertical electrophoresis of proteins VE-2M Helicon, peristaltic pumps, fraction collectors, refrigerators, spectrophotometers "SHIMADZU UV-1650", fluorometer "Quantech filter"); equipment for synthesis and characterization of micro- and nanoparticles (atomic force microscope "Solver P47-PRO (NT-MDT)", scanning electron microscope "SEM-microanalyser REMMA-102-02", transmission electron microscope "REM-100", fluorescence microscope "Axio Lab. A1", optical microscopes); apparatus for conducting electrochemical studies (commercial electrodes of different configuration and different surface area values, electrochemical cells, potentiostats "CHI 1200A" and "PGstat16"; potentiostat/galvanostat MTech SPG-500L, enzymatic kits for methanol, formaldehyde and phenols assay to evaluate the fuel substrate consumption in BFCs.

US has a high-tech modern equipment for the design and operation of BFCs: Electrochemical equipment: Potentiostat/Galvanostat (Autolab, ECO Chemie) PGSTAT12 (two sets) – with additional modules (FRA2 impedance measurements module; BIPOt bipotentiostat module; ADC750 fast sampling module for fast chronoamperometric measurements; SCAN-GEN fast potential scanning module for ultra-fast cyclic voltammetry; ECD module for low current measurements on nanoelectrodes; FI20 filter and integrator module for chronocoulometric measurements; Rotating disk-ring electrode setup); Surface plasmon resonance instrument (Autolab, ECO Chemie) SPRINGLE connected with the potentiostat/galvanostat for in situ electrochemical-SPR measurements; Quartz crystal microbalance (QCM) (Autolab, ECO Chemie) connected with the potentiostat/galvanostat for in situ electrochemical-QCM measurements; Optical Contact Angle and Surface Tension Meter (KSV Instrument Ltd) Modular CAM 200 connected with the potentiostat/galvanostat for in situ electrochemical-contact angle measurements; Orion 3-Star pH Meter; Keithley 236 Source-Measure Unit; ELISA instrument; SpectraMax i3x Microplate Reader (Molecular Devices, LLC., CA, USA). Optical/biochemical equipment: Shimadzu Spectrophotometer UV-2401PC; Barnstead Nanopure Diamond Lab Water System; Refrigerated Universal Centrifuge (Labnet) Z300K; Clinical and Educational Incubator (Barnstead); Excellence XS Analytical Balance (Mettler). Available shared equipment (University facilities): AFM, STM, SAXS and WAXS techniques, HPC, SLS, DLS, particle size analyzers, high resolution SEM, TEM, NMR, Perkin Elmer LS 50B Fluorescence spectrometer, and mass spectrometer for proteomic analysis, Leica TCS SP5 II Laser Scanning Confocal Microscope, dynamic light scattering instrument (Malvern Zetasizer Nano), etc. US team has a great experience in developing small BFCs for powering electronic mini- and microdevices (Fig. 1).

The list of project authors/participants:

US-Team:

Prof. Evgeny Katz;
Dr. Oleh Smutok;
MS Daniel Massana (PhD student)
MS Paulina Wells (PhD student)
MS Ronaldo Badenhorst (PhD student)

UA-Team:

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PhD Olha Demkiv;
PhD Andriy Zakalskiy;
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MS Oksana Zakalska;
MS ANNA MOROZ (PhD student from October 2022)
BS Daryna Mruga (graduate student).

REFERENCES CITED

This section must only include bibliographic citations and not be used to provide parenthetical information outside of the Project Narrative

- Beretta G., Daghighi M., Espinoza Tofalos A., Franzetti A., Mastorgio A.F., Saponaro S., Sezena E. (2019) Progress Towards Bioelectrochemical Remediation of Hexavalent Chromium. *Water*, 11, 2336. <https://doi.org/10.3390/w11112336>
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STUDENT/POST-DOCTORAL RESEARCHER MENTORING PLAN
This should not be used to circumvent the Project Narrative page limit

Ukrainian Sub-Team Participant

A young researcher Bohdan Pshoniuk graduated with a Master Degree from the Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies in 2020. We plan to expand his level of knowledge and experience in related fields, namely, Molecular Biology and Analytical Biotechnology. We encouraged Bohdan to attend lectures at the Ivan Franko National University of Lviv and the Institute of Cell Biology, NAS of Ukraine for additional training courses: Genetic Engineering, Bioinformatics, Molecular Biology, and a special training course on Microbial Protein Production. Attending by the young researcher new disciplines will ensure his personal and professional growth. He will receive a thorough knowledge in modern protein bioengineering: selection of protein overproducers, immobilization of the enzymes to create bioreactors, acquaintance with modern fundamentals of protein engineering, approaches to obtaining artificial, semi-synthetic proteins.

Publications and presentations are expected to result from the work supported by the grant. These will be prepared under the direction of Prof. Gonchar M. and in collaboration with researchers from his team in the Institute of Cell Biology. Bohdan Pshoniuk will receive guidance and training in the preparation of manuscripts for scientific journals and presentations at conferences. Bohdan Pshoniuk will be encouraged to travel to attend scientific meetings and conferences, and to present his research in the form of seminars or poster presentations. It will promote his social activity and formation of high efficiency and a responsible attitude to the educational and scientific process. Bohdan will receive appropriate social and psychological counseling and support from the Department staff if necessary.

Technology Transfer activities will include regular contact with researchers in the Institute of Cell Biology. Young researcher Bohdan Pshoniuk will be given an opportunity to become familiar with the university-industry relationship including applicable confidentiality requirements and preparation of invention disclosure applications.

All this will help Bohdan in participation in research projects of the Department of Analytical biotechnology, Institute of Cell Biology, and will be a prerequisite for continuing his studies as a PhD student for his future career.

STUDENT/POST-DOCTORAL RESEARCHER MENTORING PLAN
This should not be used to circumvent the Project Narrative page limit

US Sub-Team Participants

As a group, we have been very successful in the mentorship of junior researchers, as indicated by their successful placement. Dr. Marcos Pita, the first postdoc in Dr. Katz's group at Clarkson University, has received a tenure-track position in Spain, after being rated No.1 on the national competition list, later he received tenure and promotion to the next academic level. Another postdoc, Dr. Vera Bocharova, received the prestigious Wigner scholarship in Oak Ridge National Laboratory; she is presently a head of a research group. Dr. Jan Halámek received a tenure position of the Director of the Forensic Science Institute at the Texas Tech University. Dr. Roberto Luz returned back to Brazil where he received a tenure-track professor position. The most recent postdoc, Dr. Maria Gamella, returned back to her home country (Spain) where she received an academic position. A few more postdocs found jobs in industry in the USA. Dr. Nataliia Guz is in the process of getting an academic position after 2-year maternity leave. In the last five years, seven Ph.D. graduates from Dr. Katz's group received postdoc positions in universities and government labs, or industry jobs.

Our mentorship of junior researchers has included research guidance, and emphasizing independent creativity balanced in the framework of a group effort. In addition, all our students and postdocs receive support and advisement in preparing their CVs, web sites, job searches, career stages, job market environment and networking. This is accomplished in group and individual discussions. Presentations are scheduled in seminar/interview formats and then critiqued. We even discuss interview behavior and dress code.

Junior researchers are also exposed to our network of colleagues and seminar visitors, as well as attend and present at conferences. The former research networking has included joint work and co-authorship with leading researchers and their groups locally and at other institutions: most recently, with Prof. Joseph Wang (UCSD; <http://joewang.ucsd.edu/>), Prof. Sergiy Minko (University of Georgia; <http://nsmmlab.com/>), Prof. M.J. Schöning (Aachen University of Applied Sciences, Germany; <https://www.fh-aachen.de/menschen/schoening/>), Prof. D.M. Kolpashchikov (University of Central Florida; <https://sciences.ucf.edu/chemistry/people/kolpashchikov-dmitry/>), and many others (<https://webspace.clarkson.edu/~ekatz/Collaborators.html>). Generally, our publication record is in top journals, and high citation of articles (https://webspace.clarkson.edu/~ekatz/publications_most_cited.htm) has facilitated career development of graduate students and postdocs. Particularly, numerous highlights on our research on implantable bioelectronics and biosensors, including highlights in *Nature*, *Science*, *Scientific American*, *New Scientist*, *New York Times*, as well as in various magazines, radio and TV programs have encouraged students and postdoctoral scholars.



Dr. Nataliia Guz (from the Katz group) received an award for the best poster presented at the conference: "Soft Magnetic Materials and Systems",

Postdoctoral researchers and graduate students of Dr. Katz have received numerous awards (including those from ACS, ECS, NYS and others) for research and presentation quality.

Specifically, with postdoctoral researchers, we have also encouraged development of communication and management skills by mentoring graduate and undergraduate students, and sharing leadership roles in the team, as well as preparing presentations, publications, and occasionally helping write grant proposals.

KEY PARTICIPANT INFORMATION FORM
Complete ONE for each participant involved

TEAM MEMBER INFORMATION				Ukrainian Sub-Team Participant	
Last Name (surname)	Gonchar	First Name (Given)	Mykhailo	Middle	Vasylyovych
Current Position	Head of Department of Analytical Biotechnology		Classification on Project		Researcher/Engineer
Institute Name	Institute of Cell Biology, NAS of Ukraine				
Complete Mailing Address	14/16 Drahomanov str.		Lviv	79005	Ukraine
E-mail Address	mykhailo1952@gmail.com		Telephone #		+380322612144
Highest Degree/ Year Awarded	DrSc		Biochemistry		2001
Gender	Male				
Description of project role (responsibilities, expertise, level of effort on project):					
Principal investigator, supervision of the UA-team research, preparation of scientific reports and international publications. Representation of the results on the international conferences and forums.					

TEAM MEMBER INFORMATION				Ukrainian Sub-Team Participant	
Last Name (surname)	Gayda	First Name (Given)	Galina	Middle	Zufarivna
Current Position	Senior Researcher		Classification on Project		Researcher/Engineer
Institute Name	Institute of Cell Biology, NAS of Ukraine				
Complete Mailing Address	14/16 Drahomanov str.		Lviv	79005	Ukraine
E-mail Address	galina.gayda@gmail.com		Telephone #		+380322612144
Highest Degree/ Year Awarded	Dr		Bioorganic chemistry		1983
Gender	Female				
Description of project role (responsibilities, expertise, level of effort on project):					
Coordination and realization of experiments focused on microbiology (screening the fungal producers of laccase) and enzymology (isolation, purification and enzymatic characterization of alcohol oxidase, laccase). Biofunctionalization of the nanocarriers by purified enzymes for the construction of biocatalysts for biofuel cells. Screening of new nanocomposite materials possessing nanozyme (nanoperoxidase) activity. Participation in preparing scientific reports and international publications.					

TEAM MEMBER INFORMATION				Ukrainian Sub-Team Participant	
Last Name (surname)	Zakalskiy	First Name (Given)	Andriy	Middle	Yevstakhovych
Current Position	Researcher		Classification on Project		Researcher/Engineer
Institute Name	Institute of Cell Biology, NAS of Ukraine				
Complete Mailing Address	14/16 Drahomanov str.		Lviv	79005	Ukraine
E-mail Address	andriy.zakalskiy@yahoo.com		Telephone #		+380322612144
Highest Degree/ Year Awarded	Dr		Biochemistry		1985
Gender	Male				
Description of project role (responsibilities, expertise, level of effort on project):					
Cultivation of mutant yeast cells and selected fungi, overproducing the target oxidoreductases. Participation in construction of laboratory prototypes of microbial biofuel cells and studying their functional characteristics.					

TEAM MEMBER INFORMATION				Ukrainian Sub-Team Participant	
Last Name (surname)	Soldatkin	First Name (Given)	Oleksandr	Middle	Oleksiyovych
Current Position	Leading Researcher		Classification on Project		Researcher/Engineer
Institute Name	Institute of Molecular Biology and Genetics, NAS of Ukraine				
Complete Mailing Address	150, Acad. Zabolotnyi str.		Kyiv	03143	Ukraine
E-mail Address	alex_sold@yahoo.com		Telephone #		+380322612144
Highest Degree/ Year Awarded	DrSc		Biotechnology		2019
Gender	Male				
Description of project role (responsibilities, expertise, level of effort on project):					
Screening and modification of the synthesized nanomaterials by electron transfer mediators (synthetic dyes, electroconductive films, ferrocene, cyanoferrate derivatives, etc.). Screening of various polymeric membranes (electrodeposited, photopolymeric, dialysis) with an ability to reliably retain enzymes on the surface of the electrodes, possessing a good electrical conductivity and a high permeability for enzymes' substrates. Participation in the design of new enzymatic biofuel cells and studying their operational parameters.					

TEAM MEMBER INFORMATION				Ukrainian Sub-Team Participant	
Last Name (surname)	Stasyuk	First Name (Given)	Nataliya	Middle	Yevgenivna
Current Position	Researcher	Classification on Project		Researcher/Engineer	
Institute Name	Institute of Cell Biology, NAS of Ukraine				
Complete Mailing Address	14/16 Drahomanov str.	Lviv	79005	Ukraine	
E-mail Address	stasuk_natalia@ukr.net		Telephone #	+380322612144	
Highest Degree/ Year Awarded	Dr	Physical Chemistry		2013	
Gender	Female				
Description of project role (responsibilities, expertise, level of effort on project):					
Synthesis of nanocomposite materials with a high efficiency of electron transfer from biocatalyst to electrode and characterization of their electron mediator activity. Structural and morphological characterization of the obtained nanomaterials. Testing the possibility of using selected nanomaterials as an immobilization matrix for covalent or physical binding of biocatalysts on the electrode surface. Participation in the design of laboratory prototypes of enzymatic and hybrid biofuel cells and studying their operational parameters.					

TEAM MEMBER INFORMATION				Ukrainian Sub-Team Participant	
Last Name (surname)	Demkiv	First Name (Given)	Olha	Middle	Mykhailyvna
Current Position	Researcher	Classification on Project		Researcher/Engineer	
Institute Name	Institute of Cell Biology, NAS of Ukraine				
Complete Mailing Address	14/16 Drahomanov str.	Lviv	79005	Ukraine	
E-mail Address	demkiv@yahoo.com		Telephone #	+380322612144	
Highest Degree/ Year Awarded	Dr	Microbiology		2013	
Gender	Female				
Description of project role (responsibilities, expertise, level of effort on project):					
Screening of strains of the mushrooms for their synthesis of extracellular laccase in liquid cultures. Isolation, purification, enzymatic and electrochemical characterization of the laccase from the best enzyme producers. Synthesis of nanocomposite materials with a high efficiency of electron transfer from biocatalyst to electrode and characterization of their electron mediator activity. Participation in construction of laboratory prototypes of microbial biofuel cells and studying their functional characteristics.					

TEAM MEMBER INFORMATION				Ukrainian Sub-Team Participant	
Last Name (surname)	Zakalska	First Name (Given)	Oksana	Middle	Myroslavivna
Current Position	Engineer	Classification on Project		Technical/Scientific Support	
Institute Name	Institute of Cell Biology, NAS of Ukraine				
Complete Mailing Address	14/16 Drahomanov str.	Lviv	79005	Ukraine	
E-mail Address	zakalska@yahoo.com	Telephone #		+380322612144	
Highest Degree/ Year Awarded	MS	Microbiology		1986	
Gender	Male				
Description of project role (responsibilities, expertise, level of effort on project):					
Technical support in screening, cultivation, and stabilization of mutant and selected fungi - overproducers of laccas. Participation in the synthesis of nanocomposite materials for application in the construction of biofuel cells.					

TEAM MEMBER INFORMATION				Ukrainian Sub-Team Participant	
Last Name (surname)	Moroz	First Name (Given)	Anna	Middle	Andriyivna
Current Position	Engineer	Classification on Project		Researcher/Engineer	
Institute Name	Institute of Cell Biology, NAS of Ukraine				
Complete Mailing Address	14/16 Drahomanov str.	Lviv	79005	Ukraine	
E-mail Address	morozanna1998@gmail.com	Telephone #		+380322612144	
Highest Degree/ Year Awarded	MS	Microbiology		2021	
Gender	Female				
Description of project role (responsibilities, expertise, level of effort on project):					
Technical support in screening, cultivation, and stabilization of mutant and selected fungi - overproducers of laccas. Participation in the synthesis of nanocomposite materials for application in the construction of biofuel cells.					

TEAM MEMBER INFORMATION				Ukrainian Sub-Team Participant	
Last Name (surname)	Mruga	First Name (Given)	Daryna	Middle	Middle
Current Position	Engineer-Researcher	Classification on Project		Student	
Institute Name	Institute of Molecular Biology and Genetics, NAS of Ukraine				

Complete Mailing Address	150, Acad. Zabolotnyi str.	Kyiv	03143	Ukraine
E-mail Address	darynamruga@gmail.com	Telephone #		+380322612144
Highest Degree/ Year Awarded	Bc	Nanotechnology in Biology		2020
Gender	Female			
Description of project role (responsibilities, expertise, level of effort on project):				
Isolation, purification, stabilization of the enzymes.				

TEAM MEMBER INFORMATION				US Sub-Team Participant	
Last Name (surname)	Katz	First Name (Given)	Evgeny	Middle	N/A
Current Position	Full Professor		Classification on Project		Researcher/Engineer
Institute Name	Clarkson University				
Complete Mailing Address	Science Center, Department of Chemistry & Biomolecular Science, 8 Clarkson Ave		Potsdam, NY	13699	USA
E-mail Address	ekatz@clarkson.edu		Telephone #		1-315-268-4421
Highest Degree/ Year Awarded	PhD		Electrochemistry		1983
Gender	Male				
Description of project role (responsibilities, expertise, level of effort on project):					
Project management, supervision of the US-team research, preparation of scientific reports and international publications. Representation of the results on the international conferences and forums.					

TEAM MEMBER INFORMATION				US Sub-Team Participant	
Last Name (surname)	Smutok	First Name (Given)	Oleh	Middle	N/A
Current Position	Research Associate		Classification on Project		Researcher/Engineer
Institute Name	Clarkson University				
Complete Mailing Address	Science Center, Department of Chemistry & Biomolecular Science, 8 Clarkson Ave		Potsdam, NY	13699	USA
E-mail Address	osmutok@clarkson.edu		Telephone #		1-315-268-2389
Highest Degree/ Year Awarded	DrSc		Microbiology		2019
Gender	Male				
Description of project role (responsibilities, expertise, level of effort on project):					
Planning, coordination, and realization of experiments related with electrochemistry (construction and characterization of microbial, enzymatic biofuel cells on the base of microbial cells, oxidoreductases, and nanocomposite materials). Preparation of scientific reports and international publications.					

TEAM MEMBER INFORMATION				US Sub-Team Participant	
Last Name (surname)	Daniel	First Name (Given)	Massana	Middle	N/A
Current Position	PhD student		Classification on Project		Student
Institute Name	Clarkson University				
Complete Mailing Address	Science Center, Department of Chemistry & Biomolecular Science, 8 Clarkson Ave.		Potsdam, NY	13699	USA
E-mail Address	masim@clarkson.edu		Telephone #		1-347-524-3426
Highest Degree/ Year Awarded	BA		Chemistry		2017
Gender	Male				
Description of project role (responsibilities, expertise, level of effort on project):					
Performing electrochemical experiments on modified electrodes.					

TEAM MEMBER INFORMATION				US Sub-Team Participant	
Last Name (surname)	Paulina	First Name (Given)	Wells	Middle	N/A
Current Position	PhD student		Classification on Project		Student
Institute Name	Clarkson University				
Complete Mailing Address	Science Center, Department of Chemistry & Biomolecular Science, 8 Clarkson Ave.		Potsdam, NY	13699	USA
E-mail Address	kulpapk@clarkson.edu		Telephone #		1-347-524-3426
Highest Degree/ Year Awarded	MS		Biotechnology		2018
Gender	Female				
Description of project role (responsibilities, expertise, level of effort on project):					
Optimization of bioelectrodes architecture and procedure of its formation.					

TEAM MEMBER INFORMATION				US Sub-Team Participant	
Last Name (surname)	Ronaldo	First Name (Given)	Badenhorst	Middle	N/A
Current Position	PhD student		Classification on Project		Student
Institute Name	Clarkson University				
Complete Mailing Address	Science Center, Department of Chemistry & Biomolecular Science, 8 Clarkson Ave.		Potsdam, NY	13699	USA
E-mail Address	badenhrw@clarkson.edu		Telephone #		1-347-524-3426
Highest Degree/ Year Awarded	MS		Chemistry		2019
Gender	Male				
Description of project role (responsibilities, expertise, level of effort on project):					
Synthesis and characterization of binding linkers for covalent immobilization of biomolecules on the surface of different type electrodes.					

CRDF GLOBAL BUDGET NARRATIVE FORM

Describe and justify the expenses included in each budget line item. If a category doesn't apply to your budget, please insert N/A for "not applicable" in the space provided.

Institution Name:	Institute of Cell Biology, NAS of Ukraine	Applicant type:	Primary Institution
Individual Financial Support (IFS)			
Describe the level of effort projected for the PI and other team participants. Provide justification for pay rate and any fringe benefits included.			
<p>Gonchar Mykhailo Vasylyovych will work as a Principal Investigator/Project Director for this project and will commit 12 calendar months and dedicate 48 % of his working time during this period;</p> <p>Gayda Galina Zufarivna will work as a Researcher for this project and will commit 12 calendar months and dedicate 45 % of her working time during this period;</p> <p>Zakalskiy Andriy Yevstakhovych will work as a Researcher for this project and will commit 12 calendar months and dedicate 30 % of his working time during this period;</p> <p>Stasyuk Nataliya Yevgenivna will work as a Researcher for this project and will commit 12 calendar months and dedicate 48.5% of her working time during this period;</p> <p>Demkiv Olha Mykhailyvna will work as a Researcher for this project and will commit 12 calendar months and dedicate 48.5% of her working time during this period;</p> <p>Soldatkin Oleksandr Oleksiyovych will work as a Researcher for this project and will commit 12 calendar months and dedicate 48.5% of her working time during this period;</p> <p>Zakalska Oksana Myroslavivna will work as a technical/scientific assistant for this project and will commit 12 calendar months and dedicate 20 % of her working time during this period</p> <p>Moroz Anna Andriyivna will work as a graduate research assistant for this project and will commit 12 calendar months and dedicate 17.2 % of his working time during this period.</p> <p>Mruga Daryna will work as a graduate research assistant for this project and will commit 12 calendar months and dedicate 17.2 % of his working time during this period.</p>			
Equipment, Supplies and Services (ESS)			
Justify the purpose and cost rationale of each ESS line item included in the budget. General or non-descript line items such as "supplies" or "services" are not acceptable. Please itemize.			
<p>We request an amount of \$2,276 for the General Supplies and Expendable Materials.</p> <p>Planar "DropSens" electrodes (4 mm gold - \$ 250.0, and 4 mm platinum - \$ 250.0), glass carbon electrode (\$ 231.0), gold screen printed electrode (\$ 425.0), platinum screen printed electrode (\$ 450.0), copper working electrode (\$ 200.0), glass electrochemical cells (\$ 250.0) and Amicon centrifugal filter unit (\$ 220.0) will be used for construction of model biofuel cells. The price of each position of supplies and materials is calculated as the producer's catalog price including the costs for transportation to Institute.</p>			
Travel			
Explain the need for travel - how the travel will benefit the project's aims - and your calculations of travel costs for domestic and foreign travel. Break down by airfare, hotel, per diem, etc.			
<p>We request \$ 17494.0 for foreign travel from Lviv to the Clarkson University (Potsdam, NY, USA). DrSc Gonchar M., Dr. Stasyuk N., and Dr. Demkiv O. are suggested to attend U.S. PI laboratory for collaborative work. The financial calculation is based on \$850 Amount airfare per person, \$151 Amount International lodging/Per Diem rates per person for 31 nights, \$160 Amount visa fee per person and \$60 Amount medical insurance per person based on the U.S. Government allowances in effect at the time of travel.</p> <p>We request \$ 259.0 for domestic travel as Conference Registration Fees \$ 200 and Other Travel Expenses \$59.0 for attendance at the International Scientific Conference (Ukraine).</p>			
Indirect Costs (IDCs)			
Justify indirect costs % of the total sub-team direct expenses requested. Indicate if a NICRA or other institutional IDC certification is applicable.			
Indirect costs (10 %) will be used for the cover of communal payments, communication items (internet, telephone and mail), office and accounting expenses.			
Cost Share (optional)			
If applicable, describe the cost share that is being undertaken. Describe what items the cost-share will be applied to.			
Not applicable			

CRDF GLOBAL BUDGET NARRATIVE FORM

Describe and justify the expenses included in each budget line item. If a category doesn't apply to your budget, please insert N/A for "not applicable" in the space provided.

Institution Name:	Clarkson University	Applicant type:	Primary Institution
Individual Financial Support (IFS)			
Describe the level of effort projected for the PI and other team participants. Provide justification for pay rate and any fringe benefits included.			
<p>Funding is requested for one Postdoctoral Research Associate to devote approximately 10% effort toward this project.</p> <p>Funding is also requested for one part-time graduate student who will also devote approximately 20% effort toward this project.</p> <p>Fringe benefits are charged on the Research Associate's salary at the anticipated rate of 29.1%.</p>			
Equipment, Supplies and Services (ESS)			
Justify the purpose and cost rationale of each ESS line item included in the budget. General or non-descript line items such as "supplies" or "services" are not acceptable. Please itemize.			
\$1,050 is requested for the purchase of the lab consumables required for the completion of the proposed project.			
Travel			
Not applicable			
Not applicable			
Indirect Costs (IDCs)			
Justify indirect costs % of the total sub-team direct expenses requested. Indicate if a NICRA or other institutional IDC certification is applicable.			
<p>Indirect Costs are calculated in accordance with Clarkson University's Indirect Cost Rate Agreement with the Department of Health and Human Services. Currently, Clarkson is using a rate of 53% of modified total direct costs (MTDC), per DHHS Agreement dated 03/11/20. If any rate other than the appropriate negotiated rate is applied to a project due to published program restrictions or sponsor policy, all unrecovered indirect costs are designated as cost sharing by Clarkson University. Clarkson's threshold for equipment is \$5,000 as allowed by the Uniform Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards (UG 2 CFR § 200).</p>			
Cost Share (optional)			
If applicable, describe the cost share that is being undertaken. Describe what items the cost-share will be applied to.			
Not applicable			

CRDF Global PROJECT MILESTONE PLAN (TEMPLATE/ SAMPLE)

Copy template to complete. Information should match the proposal Project Narrative and Project Budget

Reporting Period (Complete for each quarterly segment applicable to project duration.)			Responsible Team	
First Quarterly Reporting Period			Mark all that apply	
Milestone:	Description:	Associated Deliverable(s):	U.S. Sub-Team	Ukrainian Sub-Team
Cultivation of yeast and fungal cells; isolation and purification of oxidoreductases (alcohol oxidase and laccase) – promising biocatalysts for the construction of biofuel cells (BFCs)	Over 25 strains of fungi will be screened for their ability to produce laccase, and prospective producers of this enzyme will be selected. For cells of mutant strains of the yeast and selected fungi (overproducers of laccase), the cultivation conditions will be optimized. Enzymes, isolated from the mutant yeast cells and the culture medium of the selected fungi will be concentrated and/or purified using ion exchange and/or affinity chromatography. Purified preparations of AO and laccase will be investigated using different approaches: electrophoretic spectrophotometric and electrochemical.	Scientific report	\$3.750	\$8.500
Total Amount Requested for this Reporting Period:		\$12,250	\$3.750	\$8.500
Second Quarterly Reporting Period			Mark all that apply	
Milestone:	Description:	Associated Deliverable(s)	U.S. Sub-Team	Ukrainian Sub-Team
Synthesis and characterization of nanocomposite materials with mediator or enzyme-like activity	The methods of synthesis and functionalization of metal nanoparticles will be optimized and more than 10 variants of mono- and bimetallic nanoparticles hybrids (based on noble and/or transition metals) will be obtained. Among newly synthesized nanomaterials, artificial peroxidases (nanoperoxidases) will be selected. Conductive carbon materials – derivatives of graphene, carbon nanotubes and carbonated cellulose fibers will be tested and characterized. For the obtained nanomaterials, their physicochemical, catalytic and electrochemical characteristics will be investigated. Screening and additional modification of the synthesized nanomaterials by inorganic electron transfer mediators will be carried out and the electrochemical characteristics of the obtained nanocomposite materials	Scientific report	\$3.750	\$12.300

	and their compatibility with the BFCs biocomponents will be investigated.			
Total Amount Requested for this Reporting Period:		\$ 16,050	\$ 3.750	\$ 12.300
Third Quarterly Reporting Period			Mark all that apply	
Milestone:	Description:	Associated Deliverable(s)	U.S. Sub-Team	Ukrainian Sub-Team
Construction, characterization of laboratory prototypes of BFCs, based on purified oxidoreductases and nanocomposites	New laboratory prototypes of enzymatic BFCs based on purified preparations of AO and laccase as well as nanoperoxidases and electron-mediated nanocomposites will be obtained and characterized. For BFCs, the possibility of creating the third-generation mediator-free enzyme bioelectrodes based on laccase in combination with nanomaterials will be investigated.		\$3.750	\$4.226
Analysis of Experimental Data	Both teams will conduct analysis on data collected by two institutions to combine them for the next researching stages	Scientific report	-	\$1.500
Total Amount Requested for this Reporting Period:		\$ 13,450	\$ 3.750	\$ 5.726
Fourth Quarterly Reporting Period			Mark all that apply	
Milestone:	Description:	Associated Deliverable(s)	U.S. Sub-Team	Ukrainian Sub-Team
Testing of the best selected BFCs for the powering of microelectronic chips	The optimized BFCs will be connected to the microelectronic sensor which be operated autonomously powered only from the BFC. The operational time of the microelectronic device powered with BFC will be analyzed in continuous mode.	Scientific report	\$3.750	\$4.225
Travel to US Institution	Members of the Ukrainian team will visit their partner institution in the USA. The main objective is face-to-face interaction in providing joint experiments and future publications	Trip Report Travel documents (copies of boarding passes, visa, other receipts) Photos	-	\$17.494
Completion of manuscript to be submitted for publication and final CRDF-report	The teams will work to prepare a final manuscript to be submitted for publication in the peer-reviewed journal and Final CRDF-report	Copy of the manuscript Final CRDF-report	-	\$1.755
Total Amount Requested for this Reporting Period:		\$ 30,250	\$ 3.750	\$ 23.474

PI OTHER SOURCES OF SUPPORT FORM

PI Name	Mykhailo Gonchar		
If no other sources of support, check "None." Otherwise, complete table below for each source (duplicate as needed).			<input type="checkbox"/> "None"
Project/Proposal Title	Development of biosensors and biofuel cells based on redox enzymes	Location of Research	Ukraine/Lithuania
Support	<input checked="" type="checkbox"/> Current <input type="checkbox"/> Pending Submission Planned in Near Future		
Source of Support	Ministry of Education and Science of Ukraine	Level of Effort (%)	10 %
Award Amount	\$ 8,000	Period Covered	03/20 – 12/22

PI OTHER SOURCES OF SUPPORT FORM

PI Name	Evgeny Katz		
If no other sources of support, check "None." Otherwise, complete table below for each source (duplicate as needed).			<input type="checkbox"/> "None"
Project/Proposal Title	HFSP Collaborative Research Grant	Location of Research	Clarkson University
Support	<input checked="" type="checkbox"/> Current <input type="checkbox"/> Pending Submission Planned in Near Future		
Source of Support	Human Frontier Science Program	Level of Effort (%)	0%
Award Amount	\$420,000	Period Covered	10/18 – 09/21
Project/Proposal Title	EAGER: NSF-BSF: Quorum Biosensing using Magnetic Field-Activated Molecular Machines	Location of Research	Clarkson University
Support	<input checked="" type="checkbox"/> Current <input type="checkbox"/> Pending Submission Planned in Near Future		
Source of Support	National Science Foundation	Level of Effort (%)	0%
Award Amount	\$150,000	Period Covered	08/19 – 01/21
Project/Proposal Title	Wearable-implantable Sensors for Continuous Monitoring of Biomarkers in Animals and Humans	Location of Research	Clarkson University
Support	<input type="checkbox"/> Current <input checked="" type="checkbox"/> Pending <input type="checkbox"/> Pending Submission Planned in Near Future		
Source of Support	Queensland University of Technology (DOD-prime)	Level of Effort (%)	0%
Award Amount	\$200,000	Period Covered	09/20 – 02/22

PREVIOUS CRDF GLOBAL AWARD FORM

Individual forms should not exceed one page.

CRDF Global Award Number:	UKB2-9044-LV-10		
Title of Previous Project:	Production of trial series and preparation of technical documentation for bioanalytical kit for enzymatic analysis of L-lactate in clinical diagnostics and beverage industries		
Start Date	05/2011	End Date	05/2012
Please briefly describe the previous research project. Be sure to provide specific information regarding results and objectives. Were all objectives of the research plan achieved? If not, what prevented you from doing so? Please list scientific publications and conference reports that were published as a result of CRDF Global award.			
<p>As a result of this CRDF-project, the optimization of a process of L-lactate cytochrome c-oxidoreductase (flavocytochrome b_2, FC b_2) production and its isolation have been performed. It was obtained preparation with 70 units of enzyme activity that allows produce 3 000 "Lactatest" kits. Optimization of composition parameters of the "Lactatest" (concentration of enzyme, chromogen, and buffer), storage form of the kit's components and optimization of reaction conditions were done.</p> <p>The manual (instruction) for using the proposed kit and description of laboratory technology to produce "Lactatest" were prepared according to the standards of Ukraine.</p> <p>The trial series of the kit "Lactatest" was prepared and its testing on the real samples of food products (dairy products, juices, ketchups etc.) as well as human liquids was done. The analysis of L-lactate by the use of "Lactatest" in sweat samples obtained from the trained athletes was also performed. The correlation level for obtained results with the reference methods was done.</p> <p>Several international publications with a high impact factor were published and acknowledgment of CRDF has been indicated.</p> <p><u>The list of publications with affiliated CRDF-grant # UKB2-9044-LV-10:</u></p> <ol style="list-style-type: none"> 1. Smutok O., Broda D., Smutok H., Dmytruk K., Gonchar M. (2011) Chromate reducing activity of the <i>Hansenula polymorpha</i> recombinant cells overproducing flavocytochrome b_2. Chemosphere, 83(4), 449-454 (IF - 3.613). https://doi.org/10.1016/j.chemosphere.2010.12.078; 2. Smutok O., Gayda G., Dmytruk K., Klepach H., Nisnevich M., Sibirny A., Puchalski C., Gonchar M., Sibirny V. Amperometric Biosensors for Lactate, Alcohols, and Glycerol Assays in Clinical Diagnostics / in epy "Biosensors - Emerging Materials and Applications", (2011), ISBN 978-953-307-328-6, pp. 401-446. 			
How will the work accomplished during this project contribute to the proposed research? Please address specific project results (data, models, methods) that the proposed project will further develop and/or build upon.			
<p>Due to the realization of the previous CRDF-project, a closer relationship between the scientific group of the Institute of Cell Biology, NAS of Ukraine and the company "Engineering Laboratory Ltd." was established. On the base of acquired experience, it will be easier to introduce our innovations into the practice, especially, in the fields of amperometric biosensors' and biofuel cell construction.</p>			

PREVIOUS CRDF GLOBAL AWARD FORM

Individual forms should not exceed one page.

CRDF Global Award Number:	UKB1-9048-KV-10		
Title of Previous Project:	Development of technology for production of recombinant human arginase as anticancer enzyme and element of biosensor for arginine		
Start Date	05/2011	End Date	05/2012
Please briefly describe the previous research project. Be sure to provide specific information regarding results and objectives. Were all objectives of the research plan achieved? If not, what prevented you from doing so? Please list scientific publications and conference reports that were published as a result of CRDF Global award.			
<p>The objective of the previous CRDF-project was to develop an innovative technology for expression of recombinant human arginase in yeast host and its purification to homogenous state and to make this product commercially available. This enzyme is currently not commercially available but is of great interest for anticancer enzymotherapy (medicine) and as an element of novel biosensor for arginine (analytical biotechnology).</p> <p>As a result of the project, we optimized the purification protocol for recombinant human arginase expressed in two species of yeasts, bakers' yeast <i>Saccharomyces cerevisiae</i> and methylotrophic yeast <i>Hansenula polymorpha</i> to make it cost-efficient. We achieved production of the purified preparations of the recombinant arginase sufficient for the use in bioanalytical devices and for further manipulations to be used in animal studies and clinical trials (in PEGylated form).</p> <p>Obtained in the frame of previous CRDF-project enzyme was used also for the other scientific investigations. As a result, some international publications with a high impact factor were published and acknowledgment of CRDF has been indicated.</p> <p><u>The list of publications with affiliated CRDF-grant # UKB1-9048-KV-10:</u></p> <ol style="list-style-type: none"> 1. Stasyuk N., Smutok O., Gayda G., Gonchar M., Koval'chuk. Y. (2011) A new bi-enzyme potentiometric sensor for arginine analysis based on recombinant human arginase i and commercial urease. Journal of Materials Science and Engineering: A, 1, 819-827; 2. Stasyuk N., Smutok O., Gayda G., Koval'chuk. Y., Vus B., Gonchar M. (2012) Bi-enzyme L-arginine-selective amperometric biosensor based on ammonium-sensing polyaniline-modified electrode. Biosens. & Bioelectron., 37(1), 46-52 (IF - 5.63) https://doi.org/10.1016/j.bios.2012.04.031 <p>Zakalskiy A., Zakalska O., Rzhepetskiy Y., Potocka N., Stasyk O., Horak D., Gonchar M. (2012) Overexpression of (His)(6)-tagged human arginase I in <i>Saccharomyces cerevisiae</i> and enzyme purification using metal affinity chromatography. Protein Expr Purif., 81, 63-68. (IF – 1.64) DOI:10.1016/j.pep.2011.09.001</p>			
How will the work accomplished during this project contribute to the proposed research? Please address specific project results (data, models, methods) that the proposed project will further develop and/or build upon.			
<p>The methods developed during this project will be valuable for the proposed project, namely, for isolation of the enzymes from cell extracts and their purification using ion exchange and affinity chromatography. There will be also used methods for stabilization of the enzymes and their immobilization on the electrodes, proposed in the earlier project.</p>			

CRDF INSTITUTIONAL DATA FORM (UKRAINE INSTIUTION)

Institution Name	Institute of Cell Biology		
Institutional Website	https://www.cellbiol.lviv.ua/		
Type of Organization	<input type="checkbox"/> International Organization <input type="checkbox"/> Government <input type="checkbox"/> Corporation <input checked="" type="checkbox"/> University		
Small Business (US Orgs Only)	<input type="checkbox"/> Small Business <input type="checkbox"/> WOSB <input type="checkbox"/> SDB <input type="checkbox"/> HUB-Zone <input type="checkbox"/> VOSB <input type="checkbox"/> SDVOSB <input type="checkbox"/> N/A		
<u>DUNS Number</u>	[565754314]	TIN/EIN (US Tax Liability Only)	[TIN/EIN]

Organizations must have a DUNS number to receive federal funding. For help applying for a DUNS number and more guidance on completing this form, please [click here](#).

Non-Profit/Charitable Status (Non-US Organizations Only)			
Is your organization registered as a charitable/non-profit entity?		<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Country of Registration	Ukraine	Registration #	1713044601024



Financial Controls & Audits		
Did your organization expend more than US \$750,000.00 in U.S. Government Federal Funding (Grants, Contracts, Subgrants, Subcontracts) in the previous fiscal year? If yes, please provide a copy of your single audit report, which is required under 2 CFR 200.	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No
Have you been audited in the past 3 years? If yes, please send a copy of the current report.	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No
Were there any material or significant findings in the audit report?	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No
Has your organization ever had a grant or contract terminated for cause?	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No
Does your organization utilize a financial manual to authorize expenses?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Does your organization utilize an accounting system to track expenses?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Does your organization have an ethics policy?	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No
Does your organization have a timekeeping system for labor such as timesheets?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Will your organization use an institutional bank account to receive funding?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Please identify the authorized institutional signatory for agreements: Sibirny A.A., Prof., Dr., DrSci. Director of Institute of Cell Biology, NAS of Ukraine		

Executive/Management Reporting Requirements	
CRDF Global may be required to publicly report the names and total compensation of the five most highly compensated individuals at the awardees' institution. If you meet any of the criteria below, you are exempt from this requirement. Please find and check any applicable exemption:	
1 In the previous tax year, institutional gross income from all sources was LESS than \$300,000.	<input type="checkbox"/> Exempt
2 The institution received LESS than 80 percent of its annual gross revenues in U.S. federal funding (Contracts, Grants, Subgrants, Subcontracts or Loans).	<input checked="" type="checkbox"/> Exempt
3 The institution received LESS than \$25,000,000 in annual gross revenues from U.S. federal funding sources (Contracts, Grants, Subgrants, Subcontracts or Loans).	<input checked="" type="checkbox"/> Exempt
4 Executive compensation is publicly reported under section 13(a) or 15(d) of the Security Exchange Act or section 6104 of the Internal Revenue Code.	<input type="checkbox"/> Exempt
I do not meet any of the exemptions above. I will provide the names and total compensation of the five most highly compensated executives. Click here for more information.	<input type="checkbox"/> <u>Not Exempt</u>

The information requested above must be provided in full and signed by an authorized institutional signatory, certifying that the information is true to the best of their knowledge. CRDF Global cannot proceed with an award to the institute without this information.

Past Performance				
Please list any applicable grants or contracts received from outside organizations. Successful completion is defined as zero suspensions or terminations for cause, audit findings or other discrepancies.				
Funding Source	Agreement Number	Total Funding	Successful Completion?	Type of Project
NATO	CBP.NUKR.SFP 984173	59.500 €	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Research Grant
EU-Poland	IPBU.03.01.00- 18-452/11-00	47.555 \$	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Research Grant
CRDF	UKB2-9044-LV- 10	9000 \$	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Research Grant
CRDF	UKB1-9048-KV- 10	9000 \$	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Research Grant

The information requested above must be provided in full and signed by an authorized institutional signatory, certifying that the information is true to the best of their knowledge. CRDF Global cannot proceed with an award to the institute without this information.

Signature _____ Andriy Sibirny, Prof., Dr., DrSci. _____ February 04, 2021 _____
 Name and Title _____ Date _____

Address Drahomanov Street, 14/16, Lviv 79005, Ukraine
 Phone +38 032 261-21-48
 E-Mail sibirny@cellbiol.lviv.ua

CRDF INSTITUTIONAL DATA FORM (U.S. INSTITUTION)

Institution Name	Clarkson University		
Institutional Website	https://www.clarkson.edu/		
Type of Organization	<input type="checkbox"/> International Organization <input type="checkbox"/> Government <input type="checkbox"/> Corporation <input checked="" type="checkbox"/> University		
Small Business (US Orgs Only)	<input type="checkbox"/> Small Business <input type="checkbox"/> WOSB <input type="checkbox"/> SDB <input type="checkbox"/> HUB-Zone <input type="checkbox"/> VOSB <input type="checkbox"/> SDVOSB <input checked="" type="checkbox"/> N/A		
DUNS Number	041590993	TIN/EIN (US Tax Liability Only)	15-0543659

Organizations must have a DUNS number to receive federal funding. For help applying for a DUNS number and more guidance on completing this form, please [click here](#).

Non-Profit/Charitable Status (Non-US Organizations Only)			
Is your organization registered as a charitable/non-profit entity?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No	
Country of Registration	USA	Registration #	15-0543659

Financial Controls & Audits		
Did your organization expend more than US \$750,000.00 in U.S. Government Federal Funding (Grants, Contracts, Subgrants, Subcontracts) in the previous fiscal year? If yes, please provide a copy of your single audit report, which is required under 2 CFR 200.	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Have you been audited in the past 3 years? If yes, please send a copy of the current report. (The report can be downloaded here: FY2019)	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Were there any material or significant findings in the audit report?	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No
Has your organization ever had a grant or contract terminated for cause?	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No
Does your organization utilize a financial manual to authorize expenses?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Does your organization utilize an accounting system to track expenses?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Does your organization have an ethics policy?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Does your organization have a timekeeping system for labor such as timesheets?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Will your organization use an institutional bank account to receive funding?	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Please identify the authorized institutional signatory for agreements: Shannon Robinson, Ed.D. Associate Vice Provost for Research & Technology Transfer Clarkson University 315-268-7766		

Executive/Management Reporting Requirements	
CRDF Global may be required to publicly report the names and total compensation of the five most highly compensated individuals at the awardees' institution. If you meet any of the criteria below, you are exempt from this requirement. Please find and check any applicable exemption:	
1 In the previous tax year, institutional gross income from all sources was LESS than \$300,000.	<input type="checkbox"/> Exempt
2 The institution received LESS than 80 percent of its annual gross revenues in U.S. federal funding (Contracts, Grants, Subgrants, Subcontracts or Loans).	<input checked="" type="checkbox"/> Exempt
3 The institution received LESS than \$25,000,000 in annual gross revenues from U.S. federal funding sources (Contracts, Grants, Subgrants, Subcontracts or Loans).	<input type="checkbox"/> Exempt
4 Executive compensation is publicly reported under section 13(a) or 15(d) of the Security Exchange Act or section 6104 of the Internal Revenue Code.	<input type="checkbox"/> Exempt
I do not meet any of the exemptions above. I will provide the names and total compensation of the five most highly compensated executives. Click here for more information.	<input type="checkbox"/> <u>Not Exempt</u>

The information requested above must be provided in full and signed by an authorized institutional signatory, certifying that the information is true to the best of their knowledge. CRDF Global cannot proceed with an award to the institute without this information.

Past Performance				
Please list any applicable grants or contracts received from outside organizations. Successful completion is defined as zero suspensions or terminations for cause, audit findings or other discrepancies.				
Funding Source	Agreement Number	Total Funding	Successful Completion?	Type of Project
National Science Foundation	032221	200,000 USD	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Research Grant
National Science Foundation	100099	330,000 USD	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Research Grant
National Science Foundation	032570	450,000 USD	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Research Grant

The information requested above must be provided in full and signed by an authorized institutional signatory, certifying that the information is true to the best of their knowledge. CRDF Global cannot proceed with an award to the institute without this information.



Signature

Shannon Robinson, Ed.D.

Associate Vice Provost for Research & Technology Transfer

February 03, 2021

Date

Address 8 Clarkson Avenue, CU Box 5630, Clarkson University, Potsdam, NY 13699-5630
 Phone 315-268-7766
 E-Mail srs@clarkson.edu

SUGGESTED REVIEWERS AND REVIEWERS NOT TO INCLUDE
(Optional)

Suggested Reviewers				
#	Name	Affiliation	Email	Brief Justification
1.	Sergiy Minko	University of Georgia, USA	sminko@uga.edu	Expert in advanced functional materials
2.	Michael J. Schöning	Aachen University of Applied Sciences, Germany	schoening@fh-aachen.de	Expert in modified electrodes and bioelectronics
3.	Antonio López	Instituto de Catálisis y Petroleoquímica, CSIC, Spain	alopez@icp.csic.es	Expert in bioelectrochemistry, bioelectronics and biofuel cells
4.	Sergey Shleev	Malmö University, Sweden	sergey.shleev@mah.se	Expert in bioelectrochemistry and biofuel cells
5.				