Form B: IGEM-HERC Full Proposal Cover Sheet

Idaho State Board of Education

PROPOSAL NUMBER: (to be assigned by HERC)	TOTAL AMOUNT REQUESTED: $187,100
Proposal Track (select one): • Initial Startup	

TITLE OF PROPOSED PROJECT: **Development of Methodologies, Instrumentation, and Assay Kits for Screening of Toxic Organic Pollutants in Environmental Sources**

SPECIFIC PROJECT FOCUS: This research proposal addresses the urgent need of accurate screening of toxic organic pollutants in environmental sources. Many of those chemicals have been classified by the United Nations Environmental Program as persistent organic pollutants (forever chemicals) and listed as a major threat to health. Among those, Polychlorinated Biphenyls (PCBs) and Per/Poly Fluorinated Alkyl Substances (PFAS) are considered as damaging the immune, reproductive, nervous, and endocrine systems, together with attributes like carcinogenic or genotoxic. Currently, Mass Spectrometry (MS) methods and instrumentation are employed as the dominant analytical techniques for detection and monitorization of organic pollutants. While these instruments may have the required sensitivity, they are very costly and time-consuming owing to the requirement of expensive, specialized equipment, highly specialized personnel, the need for sample transportation from sites to specialized labs, and laborious sample preparation. Therefore, the development of novel detection and monitorization systems that enable the rapid analysis of organic pollutants in environmental factors is urgently needed. While multiple such systems have been proposed, none of them came to utilization and/or commercialization. To answer this critical need, the academic/industrial team proposes to develop and optimize instrumentation and methodologies for accurate field testing of the presence of specific organic pollutants in environmental factors. We will employ the Kinetics Exclusion Assay (KinExA) technology and instrumentation developed and commercialized by Sapidyne Instruments in Boise, Idaho, for three decades. The proposed project aims at producing and testing assay kits to be used with the KinExA instruments for precise determination of organic pollutants in environmental sources at a fraction of the current cost while providing opportunities for portable, field measurements. In strong collaboration with Sapidyne Instruments, the Boise State University's multidisciplinary team will develop and test assay kits to be used with the KinExA technology for aptamer-based PCB and antibody-based PFAS detection and quantification in water samples. The attainment of these goals will reinforce Idaho's strength in production and commercialization of unique analytical instruments and technologies, substantially contribute to maintaining a healthier environment, and stimulate collaboration between Boise State University and the private sector in Idaho. The proposed research will also provide undergraduate and graduate students with multidisciplinary knowledge, skills, and research experience hence enhancing their readiness for high-end positions in Idaho and elsewhere.

PROJECT START DATE: 07/01/2024	PROJECT END DATE: 06/30/2025
NAME OF INSTITUTION: Boise State University	DEPARTMENT: Office of Sponsored Programs
ADDRESS: 1910 University Dr., Boise, ID 83725-1135	
E-MAIL ADDRESS: osp@boisestate.edu	PHONE NUMBER: 208-426-4420

NAME:	TITLE:	SIGNATURE:

PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR	Daniel Fologea	Professor	
CO-PRINCIPAL INVESTIGATOR	Denise Wingett	Professor	

NAME OF PARTNERING COMPANY: Sapidyne Instruments	COMPANY REPRESENTATIVE NAME: Elizabeth Hopkins, CEO

SIGNATURE:

Authorized Organizational Representative	NAME: Cara Greenlee Director, Office of Sponsored Programs	SIGNATURE: Cara Greenlee

1. Name of Primary Idaho public institution: Boise State University

2. Project Title: Development of Methodologies, Instrumentation, and Assay Kits for Screening of Toxic Organic Pollutants in Environmental Sources

3. Name and project-related credentials of Principal Investigator directing the project.

The proposed project will be directed by Dr. Daniel Fologea, who will act as the Principal Investigator. Dr. Fologea is a Professor of Physics and Biophysics with the Department of Physics and Biomolecular Sciences Graduate Program at Boise State University (BSU). Dr. Fologea joined BSU in 2011; he is an expert in the development of aptamer-based sensing systems, production, and characterization of liposomes for medical and bio-analytical applications, user of the KinExA technology, and collaborator with Sapidyne Instruments since 2012.

4. Name and project-related credentials of other key personnel directing the project

Dr. Denise Wingett, Professor of Biology with the Department of Biology and Director of the Biomolecular Sciences Graduate Program at BSU, will act as a Co-PI for the proposed project. Dr. Wingett joined BSU in 2003; she is an expert in immunology, with a special focus on antibody production and characterization.

Summary and Significance. This research proposal addresses the urgent, world-wide need of monitoring toxic organic pollutants in environmental sources. After decades of use and due to their inertness, bioaccumulation of organic pollutants in the environment led to the attainment of concentration levels that pose a real threat to the health of ecosystems and humans. Among those, Polychlorinated Biphenyls (PCBs) and Per/Poly Fluorinated Alkyl Substances (PFAS) are considered damaging to the immune, reproductive, nervous, and endocrine systems, with like carcinogenic and genotoxic attributes. Food and Drug Administration (FDA) mandates tolerances of 0.2-3.0 ppm PCBs for all foods and limits PCBs in paper food-packaging materials to 10 ppm[1]. Environmental Protection Agency (EPA) goal for drinking water's maximum contaminant level (MCL) is zero, and the enforceable MCL for PCBs in public water systems is 0.0005ppm[1]. With respect to PFAS, the EPA announced in 2021 the Agency's PFAS Strategic Roadmap; the second-year progress report (released in December 2023) includes specific strategies for i**nvesting in research, development, and innovation to increase understanding of PFAS methods, human health and environmental risks, and technologies.** In Idaho, the Department of Health and Welfare, the Department of Environmental Quality, and other local and federal partners are coordinating to assess the presence of PFAS in the environment[2]. The fifth round of the EPA's Unregulated Contaminant Monitoring Rule (UCMR5) fulfills a key commitment in EPA's PFAS Action Plan by including testing for all 29 PFAS that are within the scope of EPA Methods 533 and 537.1. In the EPA National Primary Drinking Water Regulation (NPDWR) proposed in the winter of 2023, the legally enforceable levels of Perfluorooctanoic acid (PFOA) and Perfluorooctanesulfonic acid (PFOS), two common PFAS compounds, are set to 4 ppt (4 ng/L), with proposed non-enforceable level goals of zero.[3]

Currently, Mass Spectrometry (MS) methods and instrumentation are employed as the dominant analytical techniques for detection and monitorization of organic pollutants[4-8]. While these instruments may have the required sensitivity, they are very costly and time-consuming owing to the requirement of expensive, specialized equipment, highly specialized personnel, the need for sample transportation from sites to specialized labs, and laborious sample preparation. Consequently, in many cases only highly suspicious samples undergo proper screening. Therefore, the development of novel detection and quantification systems that enable the rapid analysis of organic pollutants in environmental sources is urgently needed. While multiple such systems have been proposed[7-12], none of them have came to utilization and/or commercialization.

To answer this critical need, the academic/industrial team proposes to develop and optimize instrumentation and methodologies for accurate testing of the presence of specific organic pollutants in environmental samples. We will employ the Kinetics Exclusion Assay (KinExA) technology[13, 14] and instrumentation developed and commercialized by Sapidyne Instruments in Boise, Idaho. Since 1995, several hundreds of KinExA instruments have been sold and used around the world (including EPA, one of the first users) for analytical measurements in science, biomedical applications, toxicology, and pharmacology[15-21]. More recently, Sapidyne utilized the KinExA technology in the development of the portable SCOUT instrument (Figure 1), intended for field measurements.

Figure 1. The line of KinExA instruments produced by Sapidyne Instruments in Boise, ID. Left) The stand-alone KinExA 4000, equipped with autosampler. Center) and Right) The prototype SCOUT instrument for field use.

The KinExA instruments are basically microfluidic systems fitted with a high sensitivity detector capable of measuring the fluorescence signal resulted from the specific capture of the analyte of interest by a capture phase[17, 18]. The major difference between the stand-alone and portable instrument is the autosampler; this is not included in the portable instrument therefore the samples are manually introduced for measurements. Nonetheless, the capture phase, reagents, and automatic handling of the samples with the embedded microfluidic systems are identical for both instruments. The capturing phase needed for KinExA are small, microscopic-sized beads (a pack of ~15 µL beads) functionalized with biorecognition elements for specific binding to the analytes of interest. Currently, capture phases have been developed by Sapidyne and hundreds of scientists around the world for use with KinExA for other analytical purposes[15-21]; however, only one report shows successful development for the purpose of detection of polycyclic aromatic hydrocarbons in environmental sources[22]. To address this gap, the proposed project aims at expanding the instrument capabilities to precise determination of organic pollutants in environmental sources at a fraction of the current cost while providing opportunities for portable, field measurements. In this respect, we will fulfill this urgent need through the project detailed in this application.

5. Project Objectives(s) and total amount requested. The long-term goal of the proposal is to provide validated methodologies, instrumentation, and assay kits for rapid detection and quantification of toxic organic pollutants in environmental sources by employing the Kinetics Exclusion Assay (KinExA) technology developed and commercialized by Sapidyne Instruments in Boise, ID. To complete the long-term goal, this IGEM proposal will leverage the talents and expertise of research-active faculty from BSU and Sapidyne Instruments to achieve the following objectives:

Objective 1. Develop aptamer-based methodologies and assay kits for KinExA detection and measurement of PCBs in environmental samples.

Objective 2. Develop antibody-based methodologies and assay kits for KinExA detection of PFAS in environmental samples.

Objective 3. Lab testing of the assay kits for PCBs and PFAS with the standalone KinExA 4000 and the portable Scout instrument developed by Sapidyne Instruments.

To achieve the proposed objective, we request a total amount of $187,100.

6. Resource commitment. The proposed project is firmly aligned with the current strategic plan mapped by BSU, and it matches four out of the five established major goals. The project will contribute to: a) Improve Educational Access and Student Success by enhancing the comprehensive student experience, b) Innovation for Institutional Impact by expanding and implementing leading-edge innovations that integrates training, research, and creative activities, c) Advance Research and Creative Activity by fostering transformational practices and providing support, and d) Trailblaze Programs and Partnerships by partnering with industry organizations and fostering path breaking interdisciplinary activities to address workforce, research, education, and service.

Furthermore, BSU developed and adjusted academic programs prioritizing specialized education, training, and investments intended to keep pace with the current progress and demand in biosciences, bioengineering, and environmental sciences. In direct relationship to the proposed project, a Water Initiative project was initiated at Boise State University in the fall of 2023. The first meeting provided a broad view of Boise State's research, creative activity, education and outreach around water, networking with colleagues across campus, and gathering information on faculty working on water from a variety of perspectives. Within

this initiative, BSU hosted a Department of Environmental Quality Water Quality Workshop in February 2024, and two other workshops are planned this spring. In the same line, BSU co-organized with Sapidyne Instruments in the fall of 2023 an International Workshop focused on the analytical applications of the KinExA technology in drug discovery, industry, and environmental sciences.

The PI, Co-PI, and personnel involved in the proposed project have well established and equipped research labs to conduct the research tasks proposed in this project, together with full access to shared facilities. In addition, we will benefit from the committed support of Sapidyne Instruments in terms of equipment, assistance, personnel, and research tasks, as described later in this proposal and supporting letters. The location of the company in Boise and the strong and successful collaboration between BSU and Sapidyne with respect to preparation of capture phases, utilization of aptamers as bio-recognition elements, and expansion of the instrument's analytical capabilities will make it very difficult for anyone else to complete this project in a timely manner.

7. Specific project plan and timeline. The objective-specific tasks to be undertaken, the Technology Readiness Level (TRL, as defined by Department of Defense, DoD, and Department of Energy, DoE), timelines, and proposed lead are detailed next.

Objective 1. Develop aptamer-based methodologies and assay kits for detection and measurements of Polychlorinated Biphenyls (PCBs) in water-based solutions.

Summary. In this objective we will use aptamers as PCB-specific biorecognition elements, prepare capture phases using Polymethyl-methacrylate (PMMA) and glass microbeads by using non-covalent and covalent immobilization strategies, measure the affinity and specificity of aptamers for their target, and determine the analytical range of detection for each construct. The proposed tasks of this objective, detailed next, will provide advancement from the current TRL2 to TRL3. This objective will be led by PI Fologea, a recognized expert in utilizing the KinExA technology for aptamer-based detection of analytes.

Task 1.1 Non-covalent functionalization of PMMA and glass beads with PCB aptamers. This task employs indirect functionalization with aptamers by employing intermediate layers constructed by exploiting the strongest non-covalent interaction known in nature, biotin-streptavidin. This procedure was developed and reported for the first time for PMMA functionalization with aptamers by PI Fologea in 2020.[13] Through this established procedure, we will produce aptamer-based capture elements capable of interacting specifically with PCBs of interest (i.e., PCB72, and PCB77). We will utilize PMMA and glass beads with surface modified to create a strong absorption layer. Notably, the methodology for producing absorbance layers on glass beads was initially developed and brought to realization by the PI Fologea through a collaboration with Sapidyne Instruments during his sabbatical. This task will employ investigating at least seven different aptamers for PCBs; they will be selected based on their reported affinities and specificities[6, 11, 23-27], and custom-ordered from IDT-DNA technology. *This task is anticipated to be completed within the first two months of the project.*

Task 1.2. Covalent immobilization of aptamers on glass beads. This task employs a technology developed by the PI Fologea at BSU. The procedure consists of surface preparation, silanization, and covalent immobilization of DNA aptamers. The glass microspheres will be first functionalized with a heterobifunctional linker containing at one end a reactive silane group and at the opposite end a reactive N-Hydroxy-Succinimide (NHS); the two ends are connected through a 1k PEG molecule (NHS PEG Silane, Nanocs). To vary the density of NHS reactive moieties on the surface, the exposure to silanes will be performed for durations ranging from one to eight hours with one-hour increments. Each batch of beads will be further functionalized with aptamers against PCBs (the same aptamer sequences proposed for Task 1.1 but with an amino group added to the 3' end). We anticipate that the covalent immobilization of aptamers will lead to an unsurpassed stability of the capture phase (from months to years). *This task is anticipated to be completed within months 2-3 of the project.*

Task 1.3 Lab testing of the PCB assay kits testing with standard solutions. All the functionalized bead packs will be included in assay kits for PCB detection and quantification to be further tested in lab conditions by employing analytical standards. The analytical standards will be prepared from pure compounds and will cover a concentration range much larger than what is currently recommended for PCB measurements. Each PCB kit will be characterized with the stand-alone KinExA 4000 and portable SCOUT instrument for determination of affinity, dynamic range, and specificity (by employing mixtures of congener PCBs). *This task is anticipated to be completed within the first three months of the project.* This objective will result in

production of PCB assay kits containing functionalized beads and all the solutions and reagents needed to run the analyses of environmental samples proposed in Objective 3.

Preliminary work. The application of aptamer-based technologies for analyte detection by employing KinExA was first demonstrated by PI Fologea (Figure 2), hence providing strong evidence-based support for the successful completion of this objective.

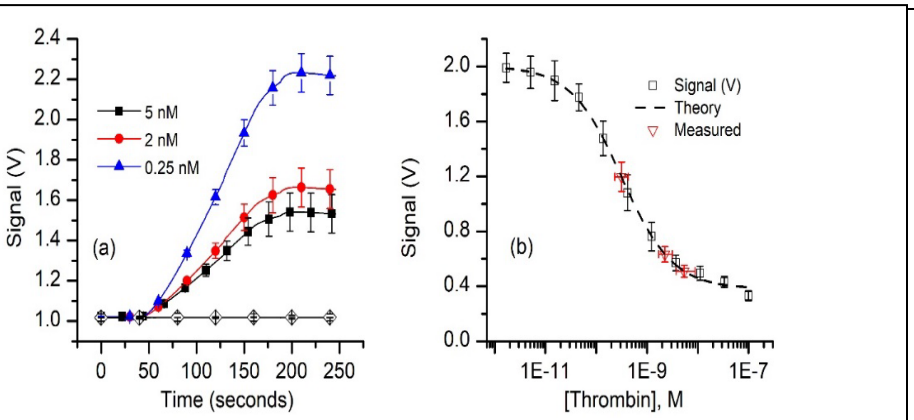

Figure 2. Example of successful development of aptamer-based technology and KinExA for successful measurement of analytes in complex biological samples. Published by PI Fologea in Sensors, *20*(12), 3442, 2020; ref.13

Objective 2. Develop antibody-based methodologies and assay kits for detection and measurement of PFAS in water-based solutions.

Summary. This objective is focused on full characterization of an anti-PFAS antibody produced by Sapidyne Instruments and development of assay kits for PFAS determination in water-based solutions. Besides the characterization of antibodies for affinity and specificity, we aim at identifying the sequence such that the antibodies may be produced in the future in a cost-effective manner. This objective will provide advancement from the current TRL2 to TRL3; the proposed research tasks will be led by Dr. Denise Wingett, an internationally recognized expert in immunology, in strong collaboration with Sapidyne Instruments and PI Fologea. Fulfillment of this objective implies extensive use of the shared Core Sequencing facility at BSU; a Material Technology Agreement (MTA) between BSU and Sapidyne Instruments is currently undergoing the approval process.

Task 2.1 Production and characterization of high affinity anti-PFAS IgY antibodies. Sapidyne Instruments succeeded in premiere to produce and isolate anti-PFAS antibodies from the egg yolk of immunized chickens, and the preliminary testing carried out by the company indicated a strong affinity for PFOA (see the preliminary results below). To achieve the highest sensitivity required for measuring extremely low level of pollutants, antibodies with highest attainable affinity are required. In this project, this will be achieved by longer than average immunization schedules of chicken, together with the testing of several immunogens (i.e., Human Serum Albumin, Human IgG1, Keyhole Limpet Hemocyanin, etc). The IgY antibodies will be weekly extracted from egg yolk, purified, and tested for affinity and specificity with the KinExA instrument by following the long-established procedures developed by Sapidyne Instruments for antibody characterization, kinetics, equilibrium, and concentration assays. *This task is anticipated to be completed within the first four months of the project.*

Task 2.2 Selection and characterization of optimal antibodies for Monoclonal Antibody Production. To develop hybridomas for monoclonal antibody development, splenocytes from the chickens producing optimal antibodies will be harvested and fused with a myeloma cell line (MuH1), and then incubated under avian cell culture conditions. Selection for successful fusion will take place specifically using hypoxanthine-aminopterin-thymidine medium (HAT) selection[28, 29]. Once sufficient fusion is achieved, cell colony titers will be screened for specific antibody production using Enzyme Linked Immunosorbent Assays (ELISA), Surface Plasmon Resonance (SPR), and Flow Cytometry. Positive titer colonies will be expanded for further proliferation and testing. Supernatant from the hybridomas will undergo a minimal affinity screening in a kinetic exclusion assay. The antibodies inhibited by the least amount of PFAS sample will be considered high affinity binders and move on to complete characterization by Kinetics Exclusion Assay and sequencing.

This task is anticipated to be completed within the first six months of the project.

Task 2.3 Utilize Illumina sequencing to identify the sequences of optimal antibody candidates. In order to provide an easy-to-make antibody, we will need to sequence the top antibody candidates for long term productions. The hybridoma that display the highest affinity will be chosen for sequencing. The sequencing service will be provided through the shared Core Sequencing facility at BSU, equipped with the Illumina NextSeq 1000 sequencer. The hybridomas that display the highest affinity (as determined in Task 2.2) will undergo DNA extraction, solubilization, and sequencing. These sequences will be selected for further used in plasmids and subsequent transfections for the purpose of long-time commercial antibody production. In this task, we will also utilize the training opportunity provided by the Core Sequencing so at least two students will achieve the knowledge and skills needed to conduct sequencing experiments with the high-end instrument. *This task is anticipated to be completed within months 3-9 of the project.*

Task 2.4 Lab testing of the PFAS assay kits with standard solutions. Each assay kit for PFAS detection and quantification will contain functionalized beads (prepared both with PMMA and glass beads) and all the solutions and reagents needed for further testing in lab conditions by employing analytical standards. The analytical standards will be made from pure compounds and will cover a concentration range starting from 0.01 pM and up to 1000 pM. Each developed kit will be characterized with the stand-alone KinExA 4000 and portable SCOUT instrument for precise determination of affinity, dynamic range, and specificity (by employing mixtures of similar PFAS). *This task is anticipated to be completed within months 6-12 of the project.* All the antibody-functionalized bead packs will be included in PFAS assay kits to be further tested in lab conditions by employing environmental water samples in Objective 3.

Preliminary results. Sapidyne Instruments already produced and provided a preliminary characterization of anti-PFOA antibodies by chicken immunization with two distinct immunogen bioconjugates. The results shown in Figure 3 provides evidence of strong binding of the produced antibodies to PFOA conjugates, with little nonspecific binding.

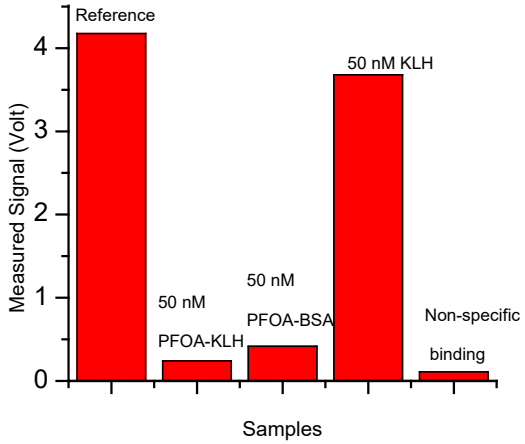

Figure 3. Preliminary data of strong and specific anti-PFOA binding to target analytes. The data indicates a strong decrease of the signal measured with the KinExA instrument in the presence of target analytes, indicative of strong binding. Courtesy of Sapidyne Instruments.

Objective 3 Lab characterization and optimization of the assay kits for field measurements

Summary. This objective is focused on thorough testing and optimization of the assay kits for field conditions. The proposed investigations include stability assays and software adjustments.

This objective pertains to the experimental proof of concept and lab validation of the proposed technology, hence advancing from the current TRL2 to TLR 3/ TLR4. The conjugated research efforts will be conducted together by BSU (PI Fologea and Co-PI Wingett) and Sapidyne Instruments.

Task 3.1 Lab measurements on environmental samples. This task encompasses measurements for PCBs and PFAS on water samples collected from local rivers, canals, and lakes. All the samples will be prepared by utilizing the currently recommended extraction method for each category of pollutant. The purified samples will be tested with the stand alone Kinexa 4000 instrument, the portable SCOUT instrument, and by Mass Spectrometry (MS) assays, which are available as shared facilities at BSU. If no pollutants will be detected by either method in any or all of the samples (indicative of “clean” samples), they will be spiked with the organic pollutants of interest and re-tested by both methods for data comparison. The stand-alone

KinExA instrument is also equipped with temperature control in the range 5-40 ^{0}C; all the samples will be tested within this temperature range to allow calculating the temperature correction coefficients to be used for field measurements. The temperature correction factors will be used to update the software analysis package and the validity of the corrections will be verified by performing the measurements with the portable SCOUT instrument inside a temperature-controlled environment. *This task is anticipated to be completed within months 6-12 of the project.*

Task 3.2 Stability analysis. The resulting assay kits will be tested for stability for the entire duration of the project (starting with the production date). We are planning weekly measurements for kits stored refrigerated and/or frozen (i.e., the antibody-based kits), or at room temperature (especially the aptamer-based kits). Based on our prior work with similar kits developed for KinExA, we anticipate the antibody-based kits to be functional for several months in the freezer, several weeks in the refrigerator, and several days at room temperature. However, we expect a much greater stability, of at least 9 months for the aptamer-based kits kept at room temperature; this is possible given the excellent stability of the aptamers (compared to antibodies) and the fact that the aptamer-based beads may be preserved dried. *This task will be initiated from the production of the first assay kit (anticipated to be completed within the first two months of the project) and will be finalized in the last month of the project.*

How will this project move along in the future? The collaboration between BSU and Sapidyne instruments will progress beyond the duration of the proposed project through federal grant applications (National Science Foundation - NSF, National Institute of Health - NIH, Department of Defense - DoD, Department of Energy - DoE) and external validation of the proposed technologies by third parties and federal agencies for advancement to TRL 7 (full scale, system prototype demonstration in a relevant environment) within three years from the successful completion of the proposed project. It is projected that Sapidyne Instruments will complete a system operated over the full range of expected conditions (TRL9) within five years from the successful completion of the proposed project.

8. Potential economic impact. Sapidyne Instruments started the production of the KinExA line of instruments in Boise, ID, in 1995. The excellent analytical capabilities of the instruments led to world-wide commercialization; several hundred instruments have been sold around the world. With its headquarters in Boise, Sapidyne has offices in Germany and distributors in Japan, Singapore, South Korea, and China. Besides commercializing instruments, Sapidyne achieves revenue through the instrument-loan program, consumable and accessories for their line of instruments, and analytical services for external customers. Therefore, to turn this project into a profitable one, we will leverage on Sapidyne's extensive experience in promoting, marketing, and commercializing their instruments and methodologies. We anticipate that the success of this project will lead to a tremendous expansion of an Idaho-located business, increased competitiveness of the state in a global market, development of novel partnerships for commercialization, and penetration of new markets.

The technology developed through this project is possible only in Idaho. We anticipate a positive economic impact **during the first year of the project** through new hires and job opportunities. A more significant impact on Idaho's economy is projected within one year from the finalization of the project. Given the world-wide interest in pollution with health-threatening organic compounds, we anticipate that the large dissemination of the results will lead to a great interest in this technology from USA and the world. Therefore, we expect an increased demand for the instruments produced by Sapidyne, which will also lead to opening of highly qualified jobs for manufacturing. It is also anticipated that the existing users of KinExA instruments will purchase the analytical kits to expand their analytical capabilities of their research groups and institutions; given the anticipated low cost of the kits, we do not see any barrier for entering the market for existing KinExA users. Since Sapidyne is the producer of these kits, more manufacturing jobs will be created for entry level scientists and technicians. A third source of potential revenue is represented by the analytical services offered by Sapidyne to numerous customers (water analysis for organic pollutants is not currently included in their portfolio of applications). The instrument's loan program offered by Sapidyne will provide opportunities for affordable assessments and monitorization of bioremediation efforts, which is an additional source of revenue. The completion and validation of the portable instrument is anticipated to significantly boost the sales not only as a cheaper alternative for a high-performance lab instrument but for direct field use. An important source of future revenue will be the anti-PFAS antibodies, which will be produced and commercialized by Sapidyne, and/or licensed to third parties.

The academic partner is planning on contributing to the future profitability of this project by:

-large dissemination of the scientific data through presentation at regional, national, and international conferences focused on analytical methods and water quality issues.

-dissemination of data through publications of articles in high impact factor journals focused on toxicology, environmental sciences, and bioanalytical methods.

-work with Sapidyne Instruments for grant applications targeting federal agencies (NSF, NIH, DoD, DoE).

-present the experimental results at seminars and meetings organized at BSU and focused on water quality; as we indicated in the institutional commitment, BSU initiated the Water Initiative project and hosted the Department of Environmental Quality Water Quality workshop. Through this initiative, we will disseminate our findings to specialists in the field, analytical labs, and state representatives.

-identify and seek patent protection across all three Objectives and partner with Sapidyne on the commercialization of the technology, thereby generating licensing revenue for Boise State which can be reinvested for further development of additional assay kits.

-hiring of undergraduate and graduate students, providing them an opportunity for hands-on real-world research and development, thereby generating a pipeline of qualified workforce for academia, industry, national labs, and state and federal agencies.

9. Criteria for success/Metrics. The proposed research project addresses the "Human-Environment Interactions" FY2025 funding priority areas and it is in line with the Strategic Plan mapped by BSU. In addition, we anticipate significant contributions to the field, the institution, and the needs of the State of Idaho presented in the higher education research strategic plan. Consequently, the proposed metrics are intended to provide a measure of the successful completion of the project while considering the institution and state priorities. The next metrics will be considered and included in the HERC reports as they are achieved.

- report on the successful development status of assay kits for PCBs and PFAS
- produce Standard Operational Procedures and Quality Assurance protocols for each of the assay kits
- report number of new hires made by Boise State and Sapidyne Instruments. We anticipate hiring one graduate student (as a Research Technician) and at least four undergraduate students at BSU; Sapidyne Instruments anticipates hiring two staff/scientist individuals before the end of the project.
- indicate the number of sponsored project proposals resulted from partnerships between BSU and Sapidyne. We anticipate one NSF proposal to be sent for review during the duration of the project, and a DoD grant request to be completed no later than the summer of 2025.
-report the number of published articles, articles in preparation, and presentations made by participants at this project. We anticipate one publication, two manuscripts under preparation, and six scientific presentations to be made for the project's duration.
- provide evidence of student training by Sapidyne Instruments, synergetic activities, and inclusion of technologies developed by Sapidyne into teaching materials.
- provide evidence for seminars organized focused on water quality at BSU and confirmation of participation.

10. Brief description of anticipated development/challenges barriers and how you will deal with unanticipated research and development challenges. Given the expertise of the participants to this project and the support evidence presented by the preliminary results, we do not anticipate any challenges or barriers with the progress of the scientific tasks presented in this proposal. However, some technical barriers may occur due to technical limitations originating in the necessity to accurately measure very low levels of organic pollutants in water samples. For example, the aptamers proposed in Objective 1 to measure the concentrations of PCBs may not present sufficient sensitivity to detect the presence of organic pollutants at very low concentrations. In this case, we will utilize the traditional exclusion assay protocols, which will consider immobilization of complementary DNA on the beads (instead of aptamers) and detect the free aptamers in equilibrated mixtures of aptamers and PCBs. This will ensure a greater sensitivity of the method but would require an additional equilibration time (~30 minutes) before initiating the measurements.

A similar barrier may be encountered for the anti-PFAS antibodies (i.e., low sensitivity); in such a situation, we will continue with the immunization protocols for several weeks so the affinity of the antibodies will be improved. In the same line, a potential failure of the sequencing procedures will be mitigated by continual extraction of selected IgY antibodies from eggs. This will not limit the antibody production, but it will extend

the time needed for batch production and increase their cost. In this case, we will attempt to complete the sequencing by employing collaborative efforts in future grant applications.

11. Budget. The proposed budget ($187,100) and the categories are fully described in the budget form included with this application.

12. Budget justification

LINE ITEM REQUEST	JUSTIFICATION	TOTAL REQUEST
Personnel (salary and fringe)	See below	$94,900
Other Direct Costs	See below	$92,200
		$187,100

Personnel Costs. In terms of salary and fringe benefits, we request funds for:

-Salary summer for PI Fologea (5 weeks in summer 2024, and 5 weeks in summer of 2025). The PI will exclusively use the times for full dedication to the investigations pertaining to the proposed project. The total requested for this category is $36,900.

-Salary for a Research Technician. We will hire a self-supported graduate student as a Research Technician to work for the successful fulfillment of the project's objectives. The hourly appointment will be for the entire duration of the project, 20 hours/week, $23/hour. The total requested for this category is $25,200.

-Undergraduate students. We project to hire at least four undergraduate students to contribute to the investigations proposed in this project. We project ~20 hours/week/student during the summer months, and an average of ~10 hours/week/student for academic semesters. The undergraduate students will be paid $15/hour. It is anticipated that the students will work with PI Fologea and Co-PI Wingett. They will be selected from the departments of Physics, Biology, Chemistry, and Materials Science and Engineering. We request a total of $32,800 for undergraduate students.

Materials/Recharge Centers. The materials and supplies requested in the budget include:

a) High-cost categories. We do not anticipate any of the individual items to exceed $1000; however, the cumulated cost within the same category (indicated below) will exceed this amount.

-PCB aptamers and complementary DNA molecules (functionalized and/or labeled) will be custom ordered from IDT-DNA technology. We project on using at least seven distinct pairs aptamer-cDNA with various labels and conjugations, at an estimated cost of $2,700/pair. We will test for maximum efficiency seven pairs of aptamers and complementary DNA with biotin and covalent conjugation, fluorescent labels, and additional purification. The optimal pair to be identified in this project will have a projected price of $700/pair, which will be sufficient for more than one thousand samples to be analyzed. Budget requested for aptamers and complementary DNA molecules: $18,900.

-Mass Spectrometry kits for PCB and PFAS. We will purchase kits and reagents for mass spectrometry determination of organic pollutants and comparison with KinExA data. The MS kits (ThermoFisher) must ensure the highest purity and be conform with current EPA recommendations. The spectrometry measurements will be performed in shared facilities at BSU (see below), but the kits and consumable must be purchased separately to avoid any prior contamination. We request $7,200 for PCB kits and $7,800 for PFAS kits; total requested for spectrometry kits is $15,000.

-SPR chips. We request $3,500 for SPR chips to be utilized with the SPR instrument (Horiba) available in the PI's lab for alternative determination of affinities.

-Core Facilities. We are planning on using shared Core Facilities at BSU. The Core Sequencing facility will be responsible for the sequencing tasks proposed in Objective two, together with training of undergraduate and graduate students. We estimate this service to incur a cost of ~$10,400. We will also engage the shared HPLC-MS Core Facility at BSU for analytical services (as described in Objective 3) and the Flow Cytometry Core Facility for clone selection (Objective 2), at an estimated cost of $5,200. These costs are calculated by considering the discounted rates provided to BSU users but do not include the cost of materials and supplies.

b) Other materials and supplies:
-raw materials for bead preparation: PMMA and glass beads, BSA, biotin-BSA, Streptavidin, buffers, conjugation chemicals, silanes, glove-box accessories, inert gases (nitrogen/argon), and cleaning agents. These raw materials will be purchased from Fisher Scientific, Sigma, or other approved vendors.
-materials and supplies for preparation of ani-PFAS antibodies: chemicals, buffers, culture media, cell lines, supplies for flow cytometer, ELISA kits, sterile glassware and plasticware, supplies for cell culture, bioconjugation reagents.
-analytical standards of PCBs and PFAS will be purchased as high purity compounds and as mixtures from state approved vendors.
All the materials and supplies must be of highest purity. We request $39,200 for other materials.

13. Project management. This project will be executed by Boise State University in collaboration with Sapidyne Instruments. Contingent to project approval, hiring of undergraduate students, research technician, establishment of teams, and distribution of tasks will be initiated before the starting date. The planning for the one-year duration of the project is presented below.

Activities and Tasks	Months												Lead
	1	2	3	4	5	6	7	8	9	10	11	12	
Project planning/teams/task distribution													PI, Co-PI, SI
Materials and supplies purchase													PI/Co-PI
Task 1.1 PCB aptamer functionalization													PI
Task 1.2 Covalent Immobilization													PI
Task 1.3 Lab testing													PI, SI
Task 2.1 Antibody production													Co-PI, SI
Task 2.2 Antibody selection													Co-PI, SI
Task 2.3 Sequencing													Co-PI
Task 2.4 PFAS lab testing													SI,Co-PI, PI
Task 3.1 Environmental sample testing													SI, PI
Task 3.2 Stability testing													PI/SI
Alternative approaches													PI, Co-PI, SI
Reports, grant writing, submission													PI, Co-PI, SI

PI: Dr. D. Fologea; Co-PI: Dr. D. Wingett; SI: Sapidyne Instruments

14. Additional institutional and other sector support

-The PI and the Co-PI have research included in their institutional workload. The PI has a nine-month appointment, with 40% research efforts during the academic year. The PI's participation at this project during the semesters will be considered part of research efforts. It is anticipated that at least 80% of the PI's research activities to be dedicated to this project. The Co-PI has a 12-month appointment; their research efforts dedicated to this project will be fully considered as part of their research workload.
- The PI and the Co-PI have dedicated laboratories and facilities to complete the proposed research tasks (as described in the equipment list). The institution will host the instruments (which will be loaned by Sapidyne free of charge) in the PI's lab for the entire duration of the project. The institution will also ensure that all the purchases will be carried out by obeying the current state and institutional requirements. In addition, the PI and Co-PI will work close with the Environmental Health and Safety office for proper disposal of the chemical waste resulted from this project, in accordance with current regulations.

Sapidyne Instruments will provide continual support for this project through:

-free loan of a KinExA 4000 instrument for the entire duration of the project. This instrument will be provided free of charge and installed in the PI's lab at BSU. The estimated cost for one-year loan (BSU would benefit from a 20% discount) is $59,400.
-free, full maintenance and service of the instrument for the entire project duration. The estimated cost (including 20% BSU discount) is $8,800.
-free training to undergraduate and graduate students involved in this project; this will be in addition to the training that will be provided by the PI on the loaned instrument. The estimated cost (BSU discounted) is $2,200.
-free access for testing of the assay kits with the prototype SCOUT instrument ($2,200).
-engage the CEO and three other scientists from Sapidyne, together with the instruments available in their R&D lab (12 KinExA 4000, and one SCOUT instrument) in the research tasks described in Objective 2 and

3 (including antibody production). This commitment will imply funds exclusively from Sapidyne Instruments. Estimated contribution: $66,000.
-dissemination of scientific data at large scientific venues for promoting the KinExA technology and its applications ($18,000).
-active collaboration with the BSU partner for grant applications to NSF, NIH, DoD, DoE and other federal agencies.
-promoting and marketing the technology and instruments through their partners, distributors, and media.

15. Future funding. Concurrent with this project and after its successful finalization, we will seek:
-NSF funding for the development of high affinity aptamers for PCBs and PFAS. This will enable production of very stable capture beads, compatible with fully portable measurements of organic pollutants in environmental waters by employing the SCOUT instrument produced by Sapidyne. The request for funding is projected to be completed by BSU in collaboration with Sapidyne in the spring of 2025.

-NIH funding for studies on bioaccumulation of organic pollutants into fat tissues and their health impact.
-DoD funding for the expansion of the analytical capabilities in soil samples. Together with the analytical methodologies resulted from this current project, we intend to contribute to the bio-remedial efforts currently conducted by DoD at hot-spot military sites. The request for funding will be completed in the summer of 2025 by Sapidyne in collaboration with BSU.
Beyond the duration of this project, we will also seek federal funding from:
-Department of Energy, for investigations of PCB pollution levels resulted from the use of transformer oils.
-Department of Agriculture, for detection and quantification of PCBs and PFAS accumulation in food (fruits, vegetables, fish, etc.).

References:

1.CDC/ATSDR. *PCB Standards and Regulations*. 2024; https://www.atsdr.cdc.gov/csem/polychlorinated-biphenyls/standards.html. 2.DEQ, I. *PFAS and Idaho Drinking Water*. 2024; Available from: https://www.deq.idaho.gov/water-quality/drinking-water/pfas-and-idaho-drinking-water/. 3. EPA.2024; Available from: https://www.epa.gov/sdwa/and-polyfluoroalkyl-substances-pfas. 4. Huset, C.A. and K. M. Barry, Methods X, 2018.DOI: 10.1016/j.mex.2018.06.017. 5.Guo, W., B. Pan, et al., International Journal of Environmental Research and Public Health, 2019. 6. Verdian, A., E. Fooladi, et al., Talanta, 2019. DOI:10.1016/j.talanta.2019.04.059. 7. Rodriguez, K.L., J.-H. Hwang, et al., Micromachines, 2020. 8. Gavrilaș, S., C.Ș. Ursachi, et al., Sensors, 2022. 9. Clark, R.B. and J.E. Dick, ACS Sensors, 2020.DOI: 10.1021/acssensors.0c01894. 10. Da Silva, B.F., A. Ahmadireskety, et al., Methods X, 2020.DOI: 10.1016/j.mex.2020.101111. 11. Yuan, X., Z. Jiang, et al., ACS Omega, 2020.DOI: 10.1021/acsomega.0c02846. 12. Langenbach, B. and M. Wilson, International Journal of Environmental Research and Public Health, 2021. 13. Smith, M.H. and D. Fologea, Sensors, 2020.DOI: 10.3390/s20123442. 14. Herrera-Gutierrez, J., S.J. Burden, et al., RNA, 2023.DOI: 10.1261/rna.079651.123. 15. Blake, R.C., X. Li, et al., Biochemistry, 2007.DOI: 10.1021/bi062164j. 16. Bromage, E.S., T. Lackie, et al., Biosensors and Bioelectronics, 2007.DOI: 10.1016/j.bios.2006.10.001. 17. Darling, R.J. and P.-A. Brault, ASSAY and Drug Development Technologies, 2004.DOI: 10.1089/adt.2004.2.647. 18. Darwish, I.A., T.A. Wani, et al., Talanta, 2013.DOI: /10.1016/j.talanta.2013.03.034. 19. Glass, T.R., N. Ohmura, et al., Analytical Chemistry, 2007.DOI: 10.1021/ac061288z. 20. Latesh, L., C. Sheila, et al., Journal of Biomolecular Screening, 2014.DOI: 10.1177/1087057114560123. 21. Li, X., S.L. Kaattari, et al., Sensing and Bio-Sensing Research, 2016.DOI: 10.1016/j.sbsr.2016.02.003. 22. Li, X., S.L. Kaattari, et al., Sensing and Bio-Sensing Research, 2016.DOI: 10.1016/j.sbsr.2016.02.003. 23. Chen, M., N. Gan, et al., Microchimica Acta, 2016.DOI: 10.1007/s00604-015-1695-1. 24. Cheng, R., S. Liu, et al., Journal of Hazardous Materials, 2018.DOI: 10.1016/j.jhazmat.2017.07.057. 25. Fu, C., Y. Wang, et al., Analytical Chemistry, 2015.DOI: 10.1021/acs.analchem.5b02508. 26. Liu, Y., Q. Zhou, et al., Analyst, 2013.DOI: 10.1039/C3AN00818E. 27. Yuan, L., Q. Fu, et al., RSC Advances, 2021.DOI: 10.1039/D0RA10285G. 28. Fishman, J.B. and E.A. Berg, Cold Spring Harbor Protocols, 2018.DOI: 10.1101/pdb.prot099150. 29. Pereira, E.P.V., M.F. van Tilburg, et al., International Immunopharmacology, 2019.DOI: 10.1016/j.intimp.2019.05.015.

FORM D: IGEM-HERC Full Proposal Budget Sheet

Track (select one): *Initial Startup*
PI First & Last Name: Daniel Fologea

Project Title: Development of Methodologies, Instrumentation, and Assay Kits for Screening of Toxic Organic Pollutants in Environmental Sources
Milestone description (if applicabl *Enter milestone number and/or brief description here*

Insert more rows in each section, as needed. *Copy/paste cell formulas, as needed.* *See cell notes for additional information.*
Do not remove or hide rows. *Shaded areas have preset formulas.*

Personnel

Name	FTE (opt)	Months	Base Salary	Salary Request	Fringe Rate	Other Ben Rat	Fringe Reques	Total
Daniel Fologea (PI) - Summer		2.5	$98,786.27	$27,440.63	0.3444		$9,450.55	**$36,900.00**
Undergraduate Assistant - AY		2.07692308	$31,200.00	$5,400.00	0.04		$216.00	**$5,600.00**
Undergraduate Assistant - AY		2.07692308	$31,200.00	$5,400.00	0.04		$216.00	**$5,600.00**
Undergraduate Assistant - AY		2.07692308	$31,200.00	$5,400.00	0.04		$216.00	**$5,600.00**
Undergraduate Assistant - AY		2.07692308	$31,200.00	$5,400.00	0.04		$216.00	**$5,600.00**
Undergraduate Assistant - Summer		0.92307692	$31,200.00	$2,400.00	0.1		$240.00	**$2,600.00**
Undergraduate Assistant - Summer		0.92307692	$31,200.00	$2,400.00	0.1		$240.00	**$2,600.00**
Undergraduate Assistant - Summer		0.92307692	$31,200.00	$2,400.00	0.1		$240.00	**$2,600.00**
Undergraduate Assistant - Summer		0.92307692	$31,200.00	$2,400.00	0.1		$240.00	**$2,600.00**
Research Technician - AY		4.61538462	$47,840.00	$18,400.00	0.04		$736.00	**$19,100.00**
Research Technician - Summer		1.38461538	$47,840.00	$5,520.00	0.1		$552.00	**$6,100.00**
								$94,900.00

Equipment

Item Description	Units	Unit Cost	Total
	1		**$0.00**
	1		**$0.00**
	1		**$0.00**
	1		**$0.00**
	1		**$0.00**
	1		**$0.00**
			$0.00

Travel

Tentative Date(s)	# persons	Total days	Transit cost/ person	Lodging/ day	Meal per diem	Total
						$0.00
						$0.00
						$0.00
						$0.00
						$0.00
						$0.00
						$0.00

Participant Support

Description	# persons	Cost/ Stipend	Total
			$0.00
			$0.00
			$0.00
			$0.00
			$0.00
			$0.00

Other Direct Costs

Item	Units	Cost	Total
Materials/ Supplies	1	$39,200.00	**$39,200.00**
Publication Charges	1		**$0.00**
Consultants (add consultant travel here)	1		**$0.00**
Computer Services	1		**$0.00**
Subcontract 1	1		**$0.00**
Subcontracts 2			**$0.00**
Other - PCB aptamers and complementary DNA molecules, price per pair	7	$2,700.00	**$18,900.00**
Other - PCB kits, spectrometry; includes columns, EPA-approved reagents, tubing, consumables, injectors	1	$7,200.00	**$7,200.00**
Other - PFAS kits, spectrometry; includes columns, EPA-approved reagents, tubing, consumables, injectors	1	$7,800.00	**$7,800.00**
Other - SPR Chips	1	$3,500.00	**$3,500.00**

Other - Recharge Center Services	1	$15,600.00	**$15,600.00**
			$92,200.00
		TOTAL DIRECT COST REQUEST	**$187,100.00**

Appendix A: Facilities and Equipment

Project Title: Development of Methodologies, Instrumentation, and Assay Kits for Screening of Toxic Organic Pollutants in Environmental Sources

PI Fologea Lab, Boise State University, Room MPCB 309

Research Laboratory: The PI's membrane biophysics lab at Boise State University has ca. 1,200 sq ft; an additional shared room of similar size is available and has direct access from the main research lab. All the equipment described in the PI's list is available in the research lab. The research lab is BSL2-designated, has water (including a reverse osmosis water purification system – 18 Mohm), gas, power, high speed internet connections, and three fume hoods. For enhanced safety and security, individual lab access is provided through ID card. When needed, all the personnel may use multiple shared facilities and equipment available at our institution for sample preparation, material characterization, and imaging.

Equipment available for equilibrium, kinetics, and concentration measurements:

- KinExA 4000 Instrument with embedded autosampler, Sapidyne Instruments; this instrument will be loaned for free from Sapidyne Instruments for this project
- Temperature Controlled Box (2 ^{0}C – 50 ^{0}C); holds the KinExA 4000 or any other instrument no larger than 3' x 2' x 3'
- Surface Plasmon Resonance (SPR) with imaging, Horiba Scientific
- Glove box
- Dynamic Light Scattering (Malvern) for size and zeta potential analyses
- Carry UV-VIS-NIR spectrophotometer (Varian)
- Portable VIS spectrophotometer
- Fluoromax 4 fluorometer (Horiba)
- Nikon TS-500 fluorescence microscope (Nikon)
- Olympus IX-71 epifluorescence/TIRF microscope (Olympus) with high resolution color camera (Olympus) and single photon camera (Acton), stereo microscopes, wide field microscopes
- pH meters with microelectrodes, cytometer, portable spectrometer with optical fiber (400-800 nm)
- Portable KinExA instrument (SCOUT prototype, Sapidyne Instruments), available at any time to the PI for measurements at Sapidyne Instruments
- High-energy sonicators (bath and tip) for sample dispersion
- Dialyzers (cassette, bag, chamber, and electro-dialysis)
- Centrifuges (three low-speed bench centrifuges, and two high-speed, refrigerated).

Other equipment available in the PI's lab: three complete electrophysiology systems (amplifier plus digitizer) for characterization of transport through planar lipid membranes and membrane-ligand interactions (Molecular Devices), BSL2 cabinet, CO_2 incubator, pH meters, liposome extruders systems (Avanti Polar Lipids, complete, with custom temperature controller), portable fluorometer, Quartz Crystal Microbalance for monitorization of surface interactions

Co-PI Denise Wingett's Laboratory, Boise State University, Science Building Room 218

- High resolution optical fluorescent microscope (Leica DM2500 B)
- Immunomagnetic selection equipment for cell isolation (Stemcell RoboSep 20000)
- Nuaire tissue culture hoods and incubators (two each) for mammalian cell culture
- BioTech Synergy MX plate reader (fluorescence, luminescence, absorbance)
- NanoDrop LITE (Thermo Scientific) spectrophotomer
- Sony FACS SH800S (fluorescent activated cell sorter) for cell isolation
- BD InFlux FACS (fluorescent activated cell sorter) for cell isolation
- Coulter Epics XL 3-color flow cytometer for cell characterization
- BD FacsCaliber 4-color flow cytometer for cell characterization
- Savant Concentrator/Vacuum System
- BioRad Thermocycler C1000X Touch with dual blocks
- Jouan refrigerated table top centrifuge
- Hermle refrigerated microfuge
- Molecular biology supplies (e.g. electrophoresis rigs, pipettors, etc.)
- 96-well plate harvester for proliferation assays
- Incubators and water baths
- Full complement of molecular biology and cell culture supplies
- Refrigerated centrifuges and microfuges
- Temperature controlled incubator/shaker
- Microbiological culturing supplies
- Various -80^{o}C freezers, refrigerators, and liquid nitrogen storage
- Microbalances

Additional equipment available in the Department of Biological Sciences

- An additional 3000 sq. ft. of lab space has shared equipment including a walk-in warm-room, walk-in cold-room, autoclave facilities, and a core area which houses shared high-speed centrifuges, spectrophotometers, scintillation counters, Kodak gel documentation systems, additional -80^{o}C freezers and liquid nitrogen storage. A water purification system as well as two large autoclaves are located on the same floor laboratory.

Other facilities available for this project at Boise State University:

- **The Flow Cytometry Core Facility** (FCCF) at Boise State University is overseen by Dr. Wingett and also located in her laboratory. This recharge center was created to aid researchers and graduate students in using flow cytometry and fluorescent activated cell sorting in their research. The FCCF is equipped with a state of the art Sony SH800S cell sorter with three lasers and six detectors. This instrument is capable of two-way sorting into various tube volumes, as well as sorting into plates. The SH800S is highly automated and features automatic compensation to account for spillover between detectors. The instrument is microfluidic based using a chip to create droplets encapsulating a sample.

There are three different chip sizes to account for various size cells or particles. The shared instrumentation facility also houses a four-laser BD INFLUX cell sorter. This instrument supports four-way sorting, plate sorting for single cell isolation, 9-color analysis, and can operate at up to 200,000 events per second. The BD INFLUX is equipped with a small particle detector allowing particle detection near 200 nm.

- **The Boise State Sequencing Core** provides Next Generation Sequencing (NGS) solution to research. Besides sequencing, the core offers training, DNA and RNA extraction, library preparation, sequencing, qPCR, and bioinformatics support. The Sequencing Core is a part of the Genetics & Infectious Disease Lab on Boise State University's campus. The core has an Illumina NextSeq 1000 which is capable of generating between 10-360 GB of data which corresponds to 100M to 1.2B reads, depending on r sequencing needs. Read lengths range from 50-600 base pairs.
- **Bruker Daltonics maXis Quadrupole Time-of-Flight (Q-TOF) Mass Spectrometer.** The Bruker Q-TOF Mass Spectrometer is a hybrid tandem mass spectrometer with outstanding performance including fast acquisition rate (up to 30 Hz for small molecules, up to 5Hz dynamic for peptides), high resolution (50,000 Full Sensitivity and Resolution), high-resolution EIC (0.5 – 1mDa on typical LC peaks), and excellent sensitivity (1 pg Reserpine >100:1 S/N RMS). This mass spectrometer is coupled with a Dionex Ultimate 3000 HPLC system and an innovative Captive electrospray source.
- **Thermo Scientific Velos Pro Dual-Pressure Linear Ion Trap (LIT) Mass Spectrometer.** The Themo Scientific Velos Pro Linear Ion Trap Mass Spectrometer offers Trap-HCD (Higher-Energy Collisional Dissociation) combined with CID (Collision-Induced Dissociation), and PQD (Pulsed-Q Dissociation) to enhance coverage and sensitivity of proteomic analysis.
- **Thermo Scientific Trace GC Ultra with ITQ 900 ion trap mass spectrometer.** The Trace GC Ultra supports a maximum temperature of 450 °C, utilizes a split-splitless injector, as well as an AS 3000 autosampler with a 105-position sample tray. The GC is coupled with the ITQ 900 ion trap mass spectrometer with a 10 – 900 AMU mass range, electron impact or chemical ionization capabilities, full scan or MS(n) scan modes, and a direct insertion probe. Data acquisition and analysis is performed using Xcalibur software.
- **Thermo Scientific Trace 1300 GC with ISQ 7000 single quadrupole mass spectrometer.** The Trace 1300 gas chromatograph enables instant-connect injector and detector modules for increased flexibility. It supports a temperature range of 3-450 °C with a 4-minute cool-down time. It utilizes a split-splitless injector and an AS 1310 autosampler with a 155-position sample tray. The GC coupled with the ISQ 7000 single quadrupole mass spectrometer with a 1.2 – 1100 AMU mass range, and is capable of full scan and SIM scanning modes.
- **Thermo Scientific Trace GC Ultra with flame ionization detector (FID).** This Thermo Scientific Trace GC Ultra gas chromatograph utilizes a flame ionization detector (FID) for sensitive, reliable, and universal detection. This instrument is also equipped with a split-splitless injector, as well as an AS 1310 autosampler with a 155-position sample tray. Data acquisition and analysis is performed using Xcalibur software.
- **Agilent 1100 Series high-performance liquid chromatograph**. The Agilent 1100 Series high-performance liquid chromatograph is equipped with a quaternary gradient elution pump, solvent degasser, column heating up to 80 °C, and a 100-position autosampler. The system is capable of up to a 10 ml/min flow rate and 400 bar of pressure. The UV-Vis photodiode array detector enables a wavelength range from 190 – 950 nm, capable of monitoring up to five wavelengths simultaneously as well as full spectrum monitoring. Fluorescence detection and MS coupling are also available.

Appendix B: Biographical Sketches

PI: Daniel Fologea

Co-PI: Denise Wingett

IDENTIFYING INFORMATION:

NAME: Fologea, Daniel

POSITION TITLE: Professor

PRIMARY ORGANIZATION AND LOCATION: Boise State University, Boise, Idaho, United States

Professional Preparation:

ORGANIZATION AND LOCATION	DEGREE (if applicable)	RECEIPT DATE	FIELD OF STUDY
University of Bucharest, Romania, Bucharest, Not Applicable, N/A, Romania	PHD	04/2002	Physics/Biophysics
University of Bucharest, Bucharest, Not Applicable, N/A, Romania	BS	06/1993	Physics/Biophysics

Appointments and Positions

2011 - present	Professor, Boise State University, Department of Physics, Boise, Idaho, United States
2010 - 2011	Research Assistant Professor, University of Arkansas, Department of Biological Sciences, Fayetteville, Arkansas, United States
2007 - 2010	Research Associate, University of Arkansas, Department of Biological Sciences, Fayetteville, Arkansas, United States
2004 - 2007	Research Associate, University of Arkansas, Department of Physics, Fayetteville, Arkansas, United States
2003 - 2004	Scientist, University of Bucharest, Department of Genetics, Bucharest, Not Applicable, N/A, Romania
2000 - 2004	Chair, National Institute of Physics and Nuclear Engineering, Department of Life and Environmental Physics, Bucharest, Not Applicable, N/A, Romania
1993 - 2000	Scientist, National Institute of Physics and Nuclear Engineering, Bucharest, Not Applicable, N/A, Romania

Products

Products Most Closely Related to the Proposed Project

1. Herrera-Gutierrez J, Burden SJ, Kobernat SE, Shults NH, Smith M, Fologea D, Hayden EJ. Double-stemmed and split structural variants of fluorescent RNA Mango aptamers. RNA. 2023 Sep;29(9):1355-1364. PubMed Central PMCID: PMC10573287.
2. Stolarz AJ, Chhetri BP, Borrelli MJ, Jenkins SV, Jamshidi-Parsian A, Phillips JH, Fologea D, Gandy J, Griffin RJ. Liposome Formulation for Tumor-Targeted Drug Delivery Using Radiation Therapy. Int J Mol Sci. 2022 Oct 2;23(19) PubMed Central PMCID: PMC9569741.
3. Abatchev G, Bogard A, Hutchinson Z, Ward J, Fologea D. Rapid Production and Purification of Dye-Loaded Liposomes by Electrodialysis-Driven Depletion. Membranes (Basel). 2021 May 31;11(6) PubMed Central PMCID: PMC8228697.
4. Ayllon M, Abatchev G, Bogard A, Whiting R, Hobdey SE, Fologea D. Liposomes Prevent In Vitro Hemolysis Induced by Streptolysin O and Lysenin. Membranes (Basel). 2021 May 18;11(5) PubMed Central PMCID: PMC8157566.

5. Smith MH, Fologea D. Kinetic Exclusion Assay of Biomolecules by Aptamer Capture. Sensors (Basel). 2020 Jun 18;20(12) PubMed Central PMCID: PMC7348807.

Other Significant Products, Whether or Not Related to the Proposed Project

1. Bogard A, Abatchev G, Hutchinson Z, Ward J, Finn PW, McKinney F, Fologea D. Lysenin Channels as Sensors for Ions and Molecules. Sensors (Basel). 2020 Oct 27;20(21) PubMed Central PMCID: PMC7663491.
2. Whiting R, Stanton S, Kucheriava M, Smith AR, Pitts M, Robertson D, Kammer J, Li Z, Fologea D. Hypo-Osmotic Stress and Pore-Forming Toxins Adjust the Lipid Order in Sheep Red Blood Cell Membranes. Membranes (Basel). 2023 Jun 25;13(7) PubMed Central PMCID: PMC10385129.
3. Whiting R, Finn PW, Bogard A, McKinney F, Pankratz D, Smith AR, Gardner EA, Fologea D. Experimental Investigations on the Conductance of Lipid Membranes under Differential Hydrostatic Pressure. Membranes (Basel). 2022 Apr 29;12(5) PubMed Central PMCID: PMC9144669.
4. Bogard A, Finn PW, McKinney F, Flacau IM, Smith AR, Whiting R, Fologea D. The Ionic Selectivity of Lysenin Channels in Open and Sub-Conducting States. Membranes (Basel). 2021 Nov 19;11(11) PubMed Central PMCID: PMC8622276.
5. Shrestha N, Bryant SL, Thomas C, Richtsmeier D, Pu X, Tinker J, Fologea D. Stochastic sensing of Angiotensin II with lysenin channels. Sci Rep. 2017 May 26;7(1):2448. PubMed Central PMCID: PMC5446423.

Synergistic Activities

1. Mentor and supervisor of over 150 undergraduate and 12 graduate students from Physics, Engineering, Chemistry, Math and Biology at Boise State University since 2012. All undergraduates who have performed research in the PI's lab and graduated continued their education at either medical or graduate schools. The retention of undergraduate students who have performed research in the PI's lab is 100%. The undergraduate students co-authored 22 presentations at national and regional conferences. The PI was awarded the BSU-COAS Faculty Excellence Award in 2018, and the BSU-Undergraduate mentor of the year award in 2023.
2. Development of new curricular materials for "Introduction to Biophysics" (undergraduate) and "Molecular Biophysics" (graduate) classes and labs at Boise State University. The evaluation provided by students for those classes ranked the PI as the highest-rated teacher in the Department of Physics and Biomolecular Sciences Graduate Program.
3. Outreach activities for high school students: site-on visits, involvement of high school students in summer-research, development of a free tutoring program for high school students. The PI was awarded the Distinguished Mentor Award by the Rotary Club Boise for mentoring high school students in 2013.
4. Sole author or first author of three patents related to medical uses of liposomes. All three patents are currently licensed to biomedical companies.
5. Collaborator with Sapidyne Instruments (Boise, ID) since 2012. The collaboration resulted in improved technologies for preparation of capture phases by using glass beads, two PhD theses focused on application of the KinExA technology, a successful grant application, and training of

more than 12 undergraduate students. Four of the trained students have current scientist positions at Sapidyne.

Certification:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to domestic and foreign appointments and positions. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729-3733 and 3802.

Certified by Fologea, Daniel in SciENcv on 2024-02-18 15:34:08

IDENTIFYING INFORMATION:

NAME: Wingett, Denise

POSITION TITLE: Director Biomolecular Sciences Graduate Programs

PRIMARY ORGANIZATION AND LOCATION: Boise State University, BOISE, Idaho, United States

Professional Preparation:

ORGANIZATION AND LOCATION	DEGREE (if applicable)	RECEIPT DATE	FIELD OF STUDY
Washington State University, Pullman, Washington, United States	PHD	12/1991	Biochemistry
Boise State University, Boise, Idaho, United States	BS	05/1986	Chemistry

Appointments and Positions

2012 - present	Director Biomolecular Sciences Graduate Programs, Boise State University, BOISE, Idaho, United States
2011 - present	Director, Flow Cytometry Core Facility, Boise State University, BOISE, Idaho, United States
2010 - present	Professor, Boise State University, BOISE, Idaho, United States
2010 - 2012	Chair, Department Biological Sciences, Boise State University, BOISE, Idaho, United States
2007 - 2010	Associate Professor, Boise State University, BOISE, Idaho, United States
2003 - 2007	Assistant Professor, Boise State University, BOISE, Idaho, United States
1998 - 2004	Medical Research Scientist, Boise VA Medical Center, BOISE, Idaho, United States
1994 - 1995	Assistant Medical Research Scientist, Boise VA Medical Center, BOISE, Idaho, United States
1994 - 1995	Research Assistant Professor, Portland VA Medical Center, Portland, Oregon, United States
1992 - 1994	Post-doctoral Fellow, Washington State University, Pullman, Washington, United States

Products

Products Most Closely Related to the Proposed Project

1. Eixenberger JE, Anders CB, Wada K, Reddy KM, Brown RJ, Moreno-Ramirez J, Weltner AE, Karthik C, Tenne DA, Fologea D, Wingett DG. Defect Engineering of ZnO Nanoparticles for Bioimaging Applications. ACS Appl Mater Interfaces. 2019 Jul 17;11(28):24933-24944. PubMed Central PMCID: PMC7010230.
2. Anders CB, Eixenberger JE, Franco NA, Hermann RJ, Rainey KD, Chess JJ, Punnoose A, Wingett DG. ZnO nanoparticle preparation route influences surface reactivity, dissolution and cytotoxicity. Environ Sci Nano. 2018 Feb 1;5(2):572-588. PubMed Central PMCID: PMC5823520.
3. Eixenberger JE, Anders CB, Hermann RJ, Brown RJ, Reddy KM, Punnoose A, Wingett DG.

Rapid Dissolution of ZnO Nanoparticles Induced by Biological Buffers Significantly Impacts Cytotoxicity. Chem Res Toxicol. 2017 Aug 21;30(8):1641-1651. PubMed Central PMCID: PMC5863281.

4. Rasmussen JW, Martinez E, Louka P, Wingett DG. Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert Opin Drug Deliv. 2010 Sep;7(9):1063-77. PubMed Central PMCID: PMC2924765.
5. Thurber A, Wingett DG, Rasmussen JW, Layne J, Johnson L, Tenne DA, Zhang J, Hanna CB, Punnoose A. Improving the selective cancer killing ability of ZnO nanoparticles using Fe doping. Nanotoxicology. 2012 Jun;6(4):440-52. PubMed PMID: 21635174.

Other Significant Products, Whether or Not Related to the Proposed Project

1. Anders CB, Chess JJ, Wingett DG, Punnoose A. Serum Proteins Enhance Dispersion Stability and Influence the Cytotoxicity and Dosimetry of ZnO Nanoparticles in Suspension and Adherent Cancer Cell Models. Nanoscale Res Lett. 2015 Dec;10(1):448. PubMed Central PMCID: PMC4648810.
2. Hanley C, Thurber A, Hanna C, Punnoose A, Zhang J, Wingett DG. The Influences of Cell Type and ZnO Nanoparticle Size on Immune Cell Cytotoxicity and Cytokine Induction. Nanoscale Res Lett. 2009 Sep 16;4(12):1409-20. PubMed Central PMCID: PMC2894345.
3. Olson RD, Headley MB, Hodzic A, Walsh GM, Wingett DG. In vitro and in vivo immunosuppressive activity of a novel anthracycline, 13-deoxy, 5-iminodoxorubicin. Int Immunopharmacol. 2007 Jun;7(6):734-43. PubMed Central PMCID: PMC2002547.
4. Matthies KM, Newman JL, Hodzic A, Wingett DG. Differential regulation of soluble and membrane CD40L proteins in T cells. Cell Immunol. 2006 May;241(1):47-58. PubMed PMID: 16963006.
5. Reddy KM, Feris K, Bell J, Wingett DG, Hanley C, Punnoose A. Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. Appl Phys Lett. 2007 May 24;90(213902):2139021-2139023. PubMed Central PMCID: PMC2153488.

Synergistic Activities

1. Idaho Innovation Award, Finalist, Early-Stage Innovation Category, 2010
2. Health Care Hero Honoree, Educator Category, Idaho Business Review, 2008
3. Wingett D, Punnoose A, Reddy KM. Preferential killing of cancer cells and activated human T-cells using the selective toxicity of zinc oxide nanoparticles. Patent No. US 8,187,638, May 29th, 2012.
4. Wang H, Punnoose A, Wingett D, Reddy KM, Feris K. Fluorescent particles comprising nanoscale ZnO layer and exhibiting cell-specific toxicity. Patent No. US 7,939,560B2, 2011.
5. Women of the Year, Idaho Business Review, 2008

Certification:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to domestic and foreign appointments and positions. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729-3733 and 3802.

Certified by Wingett, Denise in SciENcv on 2024-03-05 17:57:51

Appendix C: Senior Personnel

No services and contributions are expected from consultants, visiting professors, postdoctoral associates, and other senior personnel. The qualifications of the PI and Co-PI are described in the biosketches included in this application.

Appendix D: Other

Letter of Support – Sapidyne Instruments

Letter of Interest – City of Boise

Dear Members of the Idaho Global Entrepreneurial Mission Initiative IGEM-HERC,

I am writing to confirm the support provided by Sapidyne Instruments to the IGEM-HERC application submitted by Dr. Daniel Fologea and Dr. Denise Wingett of Boise State University (BSU).

Sapidyne Instruments is a technology and contract research organization headquartered in Boise, Idaho. Since 1995, our company developed, produced, and commercialized the line of KinExA instruments and technologies focused on determining intermolecular interactions through solution-phase affinity and kinetics measurements. Our instruments have been installed in government and research labs around the globe.

Sapidyne Instruments established long and fruitful collaborations with scientists from BSU, and we have hired and will continue to hire BSU graduates; in fact, I am a BSU graduate as well.

Sapidyne Instruments is looking forward to partnering with BSU for their proposal entitled "Development of Methodologies, Instrumentation, and Assay Kits for Screening of Toxic Organic Pollutants in Environmental Sources." Through this proposal, we seek to provide an answer to the critical need for fast and accurate quantification of organic pollutants such as Polychlorinated Biphenyls and Per/Poly Fluorinated Alkyl Substances in the environment. The proposed technology can be achieved only in Idaho from a public/private collaboration between BSU and Sapidyne Instruments.

For the successful completion of the proposed project, Sapidyne Instruments will engage in project management, multiple research tasks, dissemination activities, and marketing and commercialization efforts, as described in the proposal. However, as a partner to this proposal, **we will provide, at no cost to BSU, expertise and specialized services currently included in our commercial offer.** All the committed costs detailed in this letter will be supported by Sapidyne Instruments and considered in-kind contributions to this project. The provided services are listed below, and the estimated contribution includes a 20% discount from list price (which would be applied for BSU scientists):

-12-month rental of KinExA instrument to the PI's lab, $59,400

-maintenance and warranty coverage for the project's duration, $8,800

-training of undergraduate and graduate students (in addition o the training provided by the PI), $2,200

-full access to testing the developed assay kits with the portable SCOUT prototype, $2,200

In addition, we will engage four of our scientists and the CEO for contributing to the research tasks described in Objectives 2 and 3 (estimated cost: $66,000) and will commit $18,000 for dissemination of scientific data at large scientific venues for promoting the KinExA technology and its applications.

I look forward to working with Boise State University to accomplishing this much needed goal.

Sincerely,

Elizabeth Hopkins
President & CEO
Ehopkins@sapidyne.com

PUBLIC WORKS DEPARTMENT
MAYOR: Lauren McLean | DIRECTOR: Stephan Burgos

March 12, 2024

Idaho Global Entrepreneurial Mission Initiative
Idaho Department of Commerce
700 W. State St.
Boise, ID 83702

Subject: Letter of Interest for Development of Methodologies, Instrumentation, and Assay Kits for Screening of Toxic Organic Pollutants in Environmental Sources

Dear IGEM-HERC:

The City of Boise is sharing this letter to express our interest in the IGEM-HERC proposal titled "Development of Methodologies, Instrumentation, and Assay Kits for Screening of Toxic Organic Pollutants in Environmental Sources", sent by Dr. Daniel Fologea and Dr. Denise Wingett from Boise State University in collaboration with Sapidyne Instruments, a Boise-based company.

The City of Boise has a long-standing commitment to protecting water quality in the Boise River through effective treatment and renewal of wastewater at our Water Renewal Facilities. The pollutants this project seeks to monitor using innovative technologies are a growing concern for communities across our nation. We routinely monitor for pollutants at the Water Renewal Facilities and major industries in town. We anticipate that the successful realization of this project will substantially impact our efforts of identifying sources of pollutants and controlling or eliminating their contributions to our treatment system. This project will assist our ability to maintain a clean environment, all while contributing to a better health of people in our city and state.

Respectfully,

Austin Walkins
Source Control Manager