Idaho Incubation Fund Program

Progress Report Form

Proposal No.	**IF18-005**
Name:	**Daniel Fologea (PI), Denise Wingett (co-PI)**
Name of Institution:	**Boise State University**
Project Title:	**Engineered Advancements in Measuring Molecular Interactions in Support of Local Bio-industry**
Reporting Period:	**July 1, 2017 to January 1, 2018**

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1. Summary of project accomplishments for the period just completed and plans for the coming reporting period

The major objective of this project is the development and testing of novel technologies pertaining to the reconstitution of both artificial and natural lipid membranes supported by solid supports for integration with the KinExA instrument produce by Sapidyne, a Boise company which is the sole developer and producer of the instrument. The achievement of this objective will ensure a significant advantage and marketability over competing instruments by providing novel capabilities of investigating the functionality of biological systems in health and disease, diagnostics, and drug development.

The major milestones / timeline for the period just completed, as described in the approved proposal, are: i) Screen and select student applicants / at award notification; ii) Coat glass beads with artificial membranes and test their interactions with the KinExA instrument / 2 months; iii) Coat glass beads with cell membranes, and iv) Prepare patent / 3-12 months.

The major accomplishments in relation to the milestones of the project are:

Milestone i): **Screen and select student applicants.** Four undergraduate students and one graduate student have been selected to participate on this project. Detailed information of the students is provided in the "Faculty and student participation in the project" section.

Milestone ii): **Coat glass beads with artificial membranes and test their interactions with the KinExA instrument.** For this task, we produced artificial membrane systems around large glass beads (~50 μm diameter) to be used in the KinExA instrument. The procedure consisted of mixing the glass beads with lipid mixtures in organic solvents, followed by forced evaporation of the organic phase under vacuum for 48 hours. The lipid mixture consisted of 10 mg Asolectine, 4 mg Cholesterol, and 0.6 mg Ganglioside GM1 (which is the target lipid for Cholera Toxin subunit B – CTB, for further studies of binding with KinExA), dissolved in 200 μL chloroform. After hydration of the lipids in a physiological buffer, the formed multilayers have been thinned to bilayers by sonication and tested for CTB binding with the KinExA instrument. To assess the binding, we used a FITC-labelled CTB that presents green excitation under blue illumination. Major results: After bead preparation, their ability to bind the FITC-CTB was assessed by fluorescence microscopy. The bare glass beads showed the absence of any non-specific binding of the FITC-CTB, while the GM1 containing beads provided a strong fluorescent signal, indicative of binding. In the same illumination conditions, the beads prepared with lipids and no GM1 showed no interaction, as indicated by the absence of green fluorescence. Next, we proceeded with introducing the beads in the flow cell of the KinExA instrument and tested the binding/unbinding of FITC-CTB. Our results clearly indicate that the functionalized beads are capable of binding the fluorescent ligand, therefore proving the feasibility of our approach for quantifying protein-membrane interactions by using artificial systems.

Milestone iii): **Coat glass beads with cell membranes**. For this task, we used Jurkat cells (lymphocyte) and sheep red blood cell (RBC) as precursors for the supported membranes. The cells have been mixed with the glass beads and subjected to sonication in a water-bath sonicator for four minutes. The membrane breaking-reforming process enabled reconstitution of the membranes around the glass beads, which have been imaged by fluorescence microscopy in the

presence of lipophilic dyes. Both products showed that the cell membranes have been reconstituted around the glass beads, as observed from the fluorescence yielded upon exposure to appropriate excitation wavelengths. To test the binding with the KinExA instrument, we proceeded by using the RBC-coated glass beads and FITC-antibody capable of binding specifically components of the RBCs. Although the testing of the binding with the KinExA instrument showed specific binding, we encounter problems with repeatability when using the same batch of functionalized glass beads. The analysis of the pressure curves indicated an unusual change in the pressure of the system during the flow of the buffer over the beads. After consultation with Sapidyne, we concluded that the reconstituted membranes were not stable enough and portions of the membranes were shedding while exposed to the fluid flow. This was also observed from microscopy imaging experiments, which indicated an incomplete coating of the membrane, detrimental to the supported membrane stability. To eliminate this major roadblock, we proceeded with an alternative approach for functionalization of intact cell membranes. In this approach, we aimed the binding of the cell membranes directly onto the surface of the beads by using a strong linker such as a biotin-streptavidin system (the strongest non-covalent bond in nature). Our first attempt to functionalize the beads with streptavidin failed since the proteins were not absorbed on the surface of either PMMA or glass beads. Therefore, we decided to functionalize both the beads and cell membranes with biotin, followed by cross-linking with streptavidin. In this respect, we used biotinylated BSA protein, which is very strongly absorbed on the surface of PMMA beads, that are largely used as a solid phase for KinExA experiments. The biotinylated beads have been tested by fluorescence microscopy with FITC-streptavidin, showing an excellent binding between the beads and target proteins. The next major step was the functionalization of the cell membranes with biotin. To achieve this objective, we used a biotinylated lipophilic linker, FSL-biotin. This linker is capable of self-inserting into any lipid membranes in an orientation-specific manner, hence exposing the biotin to the extracellular environment. After cell membrane biotinylation, we cross-linked the beads and the cell membranes in the presence of streptavidin. Both microscopy imaging and binding experiments performed with the KinExA instrument indicate an excellent stability of the functionalized beads, therefore demonstrating the superiority of this approach for studying ligand-cell membrane interactions. With these findings, we are progressing with the analysis of stability, which was proposed to be finalized within the last six months of the project.

1.1 Plans for the coming reporting period

In accordance to the milestones and timeline presented in the proposal, our plans for the upcoming reporting period are:

-finalize the investigations on the stability of artificial and natural membranes reconstituted on beads and establish standard operational procedures to produce and characterize supported membranes for using them with the KinExA instrument.

-quantify antibody affinity for cell surface antigens with KinExA, and quantify the affinity of CTB to artificial membranes containing variable amounts of GM1.

-disseminate the scientific results through publications and presentations.

-submit patent application to the Office of Technology Transfer at Boise State.

-update the documentation of the available KinExA procedures by including full descriptions of the novel technologies.

2. Summary of budget expenditures for the period just completed

The initial budget was amended **with prior approval** as follows:

-the graduate student fee remission cost ($11,898) was distributed between undergraduate student salaries ($5,949), and OE (materials and supplies) $5,711. $238 has been added to the total fringe benefits. This re-distribution was required because the two graduate students working on this project received the student fee remission from other sources.

-prior ISBOE approval has been obtained to purchase a biosafety cabinet ($6,960.66) needed for cell culturing in the PI lab, which is used in conjunction with the CO_2 incubator. The total equipment budget did not change because of this acquisition.

Below it is the summary of the budget expenditures for the reported period:

Salaries

PI summer. Budgeted: $7,867; Spent: $7,867; Burn rate: 100%

Undergraduate students. Budgeted: $11,349; Spent: $3,229; Burn rate: 28.5%

OE

Materials and supplies, Computers, Red Laser upgrade, Recharge center. Budgeted: $37,511; Spent: $14,608.85; Burn rate: 38.9%

Capital equipment. Budgeted: $14,800; Spent: $10,747.64; Burn rate:72.6%

Note: Capital equipment purchased for this reporting period: Biosafety cabinet ($6,960.66), CO_2 incubator ($3,786.69)

3. Faculty and student participation in the project

The PI (Dr. Daniel Fologea, Physics) and the co-PI (Dr. Denise Wingett, Biology) fully participated in the developments related to this project for the reported period. Dr. Rebecca Hermann provided continuous assistance and technical expertise with cell culture initiation, maintenance, and assessment, including proper student training. All the participants underwent CITI training for biosafety and work with mammalian cell cultures. Together, the PI and the co-PI selected four undergraduate students to work on this project: Colleen Poulton and Jessika Dagostino (Biology), Lizzie Leung (Health Sciences), and Andy Bogard (Physics). The students have been hired as research assistants for this project, and worked an average of 10 hours/week (the stipend has been paid from the budgeted funds). Also, a BMOL graduate student, Mark Smith, was involved full time in the research work of this project. Mark benefited from a research assistantship from the BMOL graduate program but this research is a major component of his dissertation research. In addition, another graduate student (Marcelo Ayllon, a Hispanic graduate student) became fully involved in this project and decided to use the novel technology

for his dissertation research focused on quantifying the interactions between cholera toxin and artificial lipid membranes, with the goal of screening drug inhibitors and producing decoy targets for in vivo application. Three out of four undergraduate students are women, one is a native American, and one graduate student is Hispanic, therefore the workforce dedicated to this project has a great diversity index.

4. Progress with patents and copyrights

Preliminary discussions about patenting the technology of producing supported membranes for integration with the KinExA technology have been initiated with the Technology Officer at Boise State University when the award was announced. Our first disclosure draft included formation of supported membranes by using glass beads and sonication for both artificial and natural cell membranes. However, our new findings with regards to the excellent stability of either artificial or natural cell membranes supported by PMMA beads and crosslinked via biotin-avidin requires a major update of the disclosure for patenting. This work is currently underway, and it is within the timeline proposed for this task (3-12 months).

5. Technology licenses signed and start-up businesses created

The proposed technology is intended to be included into and offered with the line of KinExA instruments produced and commercialized by the partner company, Sapidyne instruments. Sapidyne is a well-established company, which operates worldwide from its headquarters in Boise and is the sole developer, manufacturer and supplier of the patented KinExA family of scientific instruments. The company has well established commercialization and marketing paths in place. Boise State University is seeking IP for the newly developed technology, which will be licensed by Sapidyne. The company will use their marketing and commercialization strategies for adoption of the newly developed technologies for their current and new customers.

6. Status of private part/industry partnerships

This project provided opportunities for developing an outstanding partnership with the industry partner, Sapidyne Instruments from Boise, which is the producer of the KinExA Instrument. Sapidyne loaned free of charge a KinExA3200 instrument and the Autosampler as kind-in contribution for the duration of this project, which have been set by the company in the PI's lab at Boise State University. They also provided multiple supplies for this project, and on-site training for the students and faculty involved in this project. Numerous meetings have been set up for discussing the progress with this project, the roadblocks, and for troubleshooting. Also, Sapidyne provided assistance with beads sorting, binding procedures, and donated multiple items required for cell cultures to the participant faculty. The company is extremely pleased with the progress of our investigations, especially with the proposed strategy of creating supported cell membranes by direct attachment of the cells to the PMMA beads. Once our scientific results are validated, the research team will disseminate the scientific results through presentations and publications, while Sapidyne will expose and promote the novel technologies together with the instrument at market fairs and scientific meetings at local, regional, national, and international venues. This technology will create a great advantage for the company over competitive instruments, which is expected to result in a significantly increased share market.

7. Other information and conclusions

All the milestones stated in the proposal for the reported period have been met or exceeded. A significant progress is reported ahead of time with regards to the stability of the supported membranes, which is a crucial achievement for the proposed development. Two abstracts with student first authors and related to the scientific findings have been accepted for presentation at the prestigious Biophysical Society meeting in February 2018 (San Francisco, CA), which will provide an excellent opportunity to present the new technology to more than 7,000 participants. All the undergraduate and graduate students participating at this project are included as co-authors of the presentations. The newly developed methods raised a sustained interest from several scientists at Boise State University, which are planning to use it for quantitative measurements to be included in several federal grant proposals. A manuscript that includes our findings is currently under preparation, and an updated disclosure discussed with the Technology Officer at Boise State University.