National Science Foundation
Small Business Innovation Research Program

PROJECT SUMMARY

NSF AWARD

NAME OF FIRM

Protein Solutions, Inc.

ADDRESS

390 Wakara Way, Room 63, Salt Lake City, Utah 84108

PRINCIPAL INVESTIGATORS (NAME AND TITLE)

Suzanne Winters, Ph.D., V.P. for Research & Development

TITLE OF PROJECT

LUNULA COLONY™: Bioluminescence for Science Education

TOPIC TITLE	TOPIC NUMBER
Education & Human Resources	26

TECHNICAL ABSTRACT (LIMIT TO 200 WORDS)

We propose to develop unique hands on, integrated science education which will motivate children (and their parents!) to observe, experience LUNULA COLONY™ consists of transparent flasks and bags containing cultur bioluminescent marine microalgae, pyrocystis lunula. This photosynthetic is unusual in that it is hardy, robust, nontoxic, bioluminescent, and to range of conditions and environments (including classrooms and homes!). bioluminescence (light production) is an intense blue color and occurs u or shaking. The LUNULA COLONY™ product will include all needed material to set up, maintain, and observe bioluminescence and to enhance the obse interests. The product is analogous to existing ANT FARM and Sea MONKEY commonly sold in toy and science stores, museum gift shops, and via direc but will be far more exciting, more desirable, and more useful for scienc purposes.

We propose to develop a range of bioluminescence educational materia LUNULA COLONY™ for various age and grade groups (Grades 1-3, 4-6, and 7-9 materials will supplement and expand existing state and district core cur an emphasis on inquiry and discovery-based learning. The materials and e will be student question and curiosity driven.

Emphasis is placed on means for teacher and science curriculum coord education, testing, and evaluation.

KEY WORDS TO IDENTIFY RESEARCH OR TECHNOLOGY (8 MAXIMUM)

Bioluminescence, Science Education, Toys, Children's Products, Biotechnol

POTENTIAL COMMERCIAL APPLICATIONS OF THE RESEARCH

Science Education, Children's Toys and Novelties, School Supplies, Teache

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D. OPPORTUNITY IDENTIFICATION AND SIGNIFICANCE*

Protein Solutions, Inc. (PSI) intends to develop, manufacture, and market a new line of educational, innovative products, which use the phenomenon of bioluminescence [1-4] to motivate, entertain, and educate children. This proposal specifically addresses LUNULA COLONY™, a culture of phytoplankton which bioluminesce.

This project will develop hands-on integrated science educational materials based on the phenomenon of bioluminescence (1). The problems of our current science curricula are outlined in *Project 2061: Science for All Americans* (6). This report concludes that the problems in much of current science education include the "learning of answers more than exploration of questions, memory at the expense of critical thought, ... reading in lieu of doing"(6). PSI intends to address this critical need by developing materials, "toys", discovery aids, and new curricula using the excitement of bioluminescence.

Nearly everyone who discovers and observes bioluminescence is impressed and motivated to see and learn more. In these times where children have their senses constantly stimulated to near exhaustion, bioluminescence is a relatively unknown, unexperienced phenomenon which can readily compete for a student's attention and interest (4). It provides an opportunity to discover something totally new, eliminating or minimizing any preconceived notions or attitudes, which have been demonstrated to inhibit comprehension and retention.

The materials will aid in the observation, discovery and learning of basic concepts in various science fields (Figure 1). Teachers and their students will develop new and expanded understanding of science concepts in a completely new domain - bioluminescence. The concepts and understanding derived from bioluminescence are general and applicable to the full range of scientific and technical subjects (Figure 1). The emphasis will be on inquiry, discovery, conceptual learning, and the nature of the scientific endeavor.

We believe that by using bioluminescence we can develop exciting inter- and multi-disciplinary materials to encourage inquiry, thereby motivating children in the discovery of science as a fascinating and challenging endeavor.

A major problem with science education in the United States is that elementary school teachers, jr. high teachers, and even high school teachers either have a strong and fundamental fear and anxiety about science, or their science skills are in one of the classical disciplines, which may make them unable or ineffective at teaching science as an integrated subject, or even to relate their discipline to other disciplines and to students' personal experiences.

PSI's objectives are to develop courses, materials, laboratory experiences, and a variety of inquiry and discovery -based products with which to educate an entirely new generation of teachers, with which to provide in-service and other education activities for existing teachers, and with which to educate students. LUNULA COLONY™ provides an excellent introduction to the interplay of biology, chemistry, physics, and environmental science (Figure 1).

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* References are in Section P.

Figure 1: Bioluminescent organisms and their observation are shown as the center of an integrated science "wheel". Each of the classical specialties or disciplines are indicated with selected subject examples. These subjects and topics can all be directly observed and experimentally studied via bioluminescence.

LUNULA COLONY™ is a transparent container of bioluminescent marine micro-organisms which produce a brilliant blue light upon mechanical stimulation. Two different marine microalgae have been identified as candidates for this product, Pyrocystis lunula and Pyrocystis noctiluca (9). These are barely visible with the naked eye. With a small magnifying lens (provided with the product) they can be discovered and observed. With the use of a low power microscope, internal structures, cell division, and other features can be observed.

These organisms are dinoflagellates and are hardy and robust. They can be maintained for up to 6 months in totally closed containers and for 1-2 years with minimal care and "feeding" (adding supplemented sea water).

LUNULA COLONY™ provides a teaching aid which may be used in classrooms from kindergarten through grade 12. Consider the fascination of the traditional Ant Farm used in classrooms throughout the country and extrapolate to the increased fun in observing organisms which produce their own light!

PSI has tested this approach during the 1991-92 school year by offering three inservice courses in the Salt Lake City, Jordan, and Davis County school districts. There is strong interest in and support for adding the exploration of bioluminescence into their curricula.

Successful development and commercialization of LUNULA COLONY™ and other bioluminescence products will help increase the interest and enthusiasm of children through the process of discovery, thereby enhancing science education. .

E. BACKGROUND, APPROACH, AND BENEFITS

1.*Background and Proposed Research:*

Flashing fireflies on summer evenings, glowing ocean surf, and other forms of natural bioluminescence have always been a target of curiosity. Ancient scientists Aristotle and Pliny the Elder studied bioluminescence but it was not until the 1670's that English chemist Robert Boyle described some of its fundamental properties. Scientists are using bioluminescence to study gene expression and developmental biology. Other applications in biology, medicine and agriculture are underway, including the arena of clinical medicine and diagnostics [1-5, 7].

Bioluminescence is the light *produced by* certain plants and animals. It is not only fascinating to observe, but can be packaged and discovered in ways which entertain a child while teaching him/her basic principles of biology, chemistry and physics via an integrated, multi-disciplinary approach to science education (6).

The so-called phosphorescence of the sea is due to the bioluminescence of dinoflagellates. The light emitted when the water is disturbed is characteristically emitted as a rapid flash which lasts only a fraction of a second (5, 7). Individual cells, depending on the species, will flash repeatedly with repeated stimulation. These dinoflagellates will be used in LUNULA COLONY™ to produce light when agitated or mechanically stimulated (9).

We propose to develop integrated science educational materials using bioluminescence as "discovery" and observation vehicles. LUNULA COLONY™ will

consist of a transparent COLONY, an owner's manual, LUNULA NUTRIENTS™, a sampling pipet, a Petri dish, and blotter paper.

The science involved in the development of LUNULA COLONY™ requires research efforts in the culture, growth and handling of the bioluminescent microorganisms in supplemented sea water and means to stimulate the bioluminescence by physical and chemical methods. One major advantage over chemiluminescent products* is the ability to control the light emission, essentially to induce light on command. Another major educational advantage is that *bio*luminescence is the result of living organisms, thus allowing the integration of concepts of biology with those of chemistry and physics.

Growth of the organisms to the high densities needed for LUNULA COLONY™ requires optimization of the culture conditions and of the sea water composition (10, 11). PSI proposes to develop culture techniques for production of the microorganisms. Key parameters to be studied are temperature, salinity and nutrient requirements, including trace metals and vitamin supplements. Pyrocystis noctiluca and Pyrocystis lunula will be emphasized due to their high bioluminescence intensities, hardiness, and relatively large size (9, 11, 12).

The requirements for shipping and storage of the LUNULA COLONY™ will be studied and optimized. How long can they be stored in the dark? What are their temperature requirements? How can they be shipped?

PSI is uniquely positioned to enter the educational bioluminescence field. We have had extensive interaction with experts in the field, including those knowledgeable about dinoflagellates and other bioluminescent organisms. J.D. Andrade, President of PSI, has been involved in the field of bioluminescence for over 5 years (13). One of our staff has attended a workshop on marine phytoplankton culture and techniques at the Provasoli Guillard Center for the Culture of Marine Phytoplankton in Maine. We have performed enough preliminary research with these organisms to determine that they are hardly, tolerant of typical classroom and retail store environments, and have adequate shelf lives to consider their commercialization (11,14, 17A).

In addition, we have been actively working with educators and administrators from the Salt Lake City and Davis County school districts to introduce bioluminescence into an experimental science curriculum (15). We are also working with the Children's Museum of Utah to develop an interactive, hands-on bioluminescence exhibit (16). We sincerely believe that the development of this technology will result in an expanding, successful and profitable business venture which will stimulate children's fascination with science (17B). The State of Utah has provided a grant to the University of Utah's Center for Integrated Science Education to conduct seven inservice workshops: Light from Life: Using Bioluminescence for Integrated Science Education. These will be conducted September 1992 - May 1993.

2. *Innovativeness and Originality*:

Bioluminescence materials are not used in science education. Bioluminescence is a relatively unknown biological phenomenon which is rarely even mentioned in curriculum materials or textbooks. Yet it is incredibly interesting and stimulating.

* The Lite Stiks sold by American Cyanamid are based on chemiluminescence and do not involve living organisms.

Although it is possible to purchase some bioluminescent material from North Carolina Biological and related suppliers, the materials are not available in forms, quantities, or prices which make them useful in the elementary environment. Suitable printed teaching materials are not available.

Dinoflagellates are normally considered to be delicate organisms which are difficult to culture and maintain (8-10). They also have a reputation of being toxic (8, 9). There are thousands of different dinoflagellates - some produce toxins, many others do not. Many are non-bioluminescent - others are. Many are fragile - some are hardy. After thorough consideration of the scientific literature and discussions with many experts on dinoflagellates and bioluminescence, we have selected two very hardy, nontoxic, bioluminescent dinoflagellates, which can be maintained in totally sealed environments for up to 6 months (9, 11, 14). All they require is 8-10 hours/day of light and moderate temperatures. These results are so original and innovative that they are the basis of a current patent application (17A).

3. *Expected Results:*

The proposed R&D project has been planned and organized to test the feasibility of developing and maintaining viable dinoflagellate cultures for use in science education. The research proposed here is expected to lead to:

1. high density cultures of single and mixed populations of dinoflagellates which can be maintained for up to 6-12 months with little care or attention. We expect to identify specific feeding and CO2 requirements for their viability and their long term stability and function (10, 12, 14).

2. a polymeric membrane capable of providing the necessary gas requirements for these organisms which may be fashioned into shapes of interest to children. The gas needs of the dinoflagellates will be a function of temperature and of the light requirements and must be determined. Several gas permeable polymers are commercially available which may satisfy these requirements (14, 18). The PI has extensive experience with gas transfer membranes and in the design of products with the needed gas transfer requirements (see Section Q).

3. Printed materials to aid the students, their parents, and their teachers in enhancing their curiosity, experimentation, and concept development.

4. *Commercial Application*:

After feasibility is demonstrated by this Phase I project, we propose to move into Phase II to further develop and optimize LUNULA COLONY™ and then to move into production and commercial sales in Phase II. We anticipate no difficulty in obtaining funding for Phase III (full commercialization) of the project. We will develope a marketing plan which utilizes existing distributors and catalog companies which service the school and science kit/science gift markets (17B). We expect to use college undergraduates and teachers as part-time technical representatives.

Several novelty products are currently on the market which generate light through chemiluminescence, such as "light stiks" (see earlier footnote). There are also many "glow" products which involve phosphorescence. However, these do not significantly address the educational market nor attempt to stimulate or enhance scientific interest. These products lack the flexibility, versatility, efficiency, control, and interest level available with bioluminescence.

F. PHASE I RESEARCH OBJECTIVES

The project has the following specific objectives:

1. Develop methods to produce high densities of different dinoflagellates and their mixtures for optimum bioluminescence.

2. Develop the process to produce cultures which have high stability and which have a guaranteed product life of 6-12 months.

3. Optimize the culture medium.

4. Develop an optimum container/membrane with appropriate gas transfer requirements.

5. Establish shipping and storage conditions to maintain viable cultures.

6. Develop teaching and educational materials and product accessories to enhance customer interest in science education.

G. PHASE I RESEARCH PLAN

The program objectives will be reached by the performance of 14 specific tasks (keyed to Objectives 1-6 above):

Task 1-1. Selection of dinoflagellates with sufficient bioluminescence- There are many known species of bioluminescent dinoflagellates. PSI has identified and studied two non-toxic varieties, Pyrocystis noctiluca and Pyrocystis lunula (9-10, 12). With further exploration of the literature and consultation with experts in the field, we may find that other species are more suitable for this application. These will be identified and final selections made based on availability, bioluminescent intensity and duration, ease of culture, ease of transport, and long term viability.

Task 1-2. Improvement of incubators and lighting systems- Dinoflagellates maintain normal circadian rhythms and are sensitive to fluctuations in temperature and light. Following consultation and based on our current experience, improved incubators and lighting systems will be installed in our laboratory. Different organisms respond differently to light/temperature cycles (8-12).

Task 1-3. Development of culture/transfer techniques- The key references on culturing dinoflagellates emphasize laboratory conditions and small amounts (8, 9). We recently participated in a workshop sponsored by the Provasoli Guillard Center for Culture of Marine Phytoplankton in which the important criteria for successful maintenance of phytoplankton were discussed and practiced. Techniques developed so far will be optimized and recorded for each of the selected dinoflagellates. Our efforts will be focused on large quantities at high density and with a high bioluminescence.

Task 1-4. Identification of division rates- Each species of dinoflagellates has a particular division rate. The culture media has an influence on the rate of division. Cell densities and division rates will be measured by standard methods (19).

Task 1-5. Maximization of cell densities for maximum bioluminescent intensity- Currently, PSI has developed sufficient expertise to maintain moderate density cultures of Pyrocystis lunula (about 5,000 cells/ml). Experiments will be conducted to maximize the cell densities of individual species by altering light cycles, temperature and availability of CO2. These experiments will be explored simultaneously with efforts to optimize the artificial sea water (11).

Task 2-1. Influence of temperature/ light on long term stability- Using the optimum cell densities determined above for each species selected, experiments will be conducted to determine the influence of varying light cycles and temperatures for the purpose of long term stability and product life issues. These factors are critical in design of the product itself as well as in the development of packaging and shipping containers to maintain a viable, dense culture of dinoflagellates.

Task 2-2. Media rejuvenation experiments- Requirements for long term maintenance will necessarily address the issue of rejuvenation of the culture medium. Questions which must be answered include: How long can the culture be left unattended (assuming adequate CO2) without the depletion of nutrients? Do the organisms degrade their own environment with metabolites or decay products? Answers to these types of questions will be critical not only to culturing these organisms by PSI but also for the consumer. These issues will be addressed in the maintenance instructions to be packaged with the product.

Task 2-3. Mixed cultures- Experiments will be conducted to determine if mixed cultures, i.e. more than one species present in a single culture medium, will provide a more favorable product, either in terms of intensity or duration of light, or longer term stability of the culture. Since the different species of dinoflagellate respond differently to stimulation, it may be that a LUNULA COLONY™ consisting of a mixture of organisms will prove more optimal. Each of the selected organisms will be cultured in combination with the others and observations made as to light intensity, duration, and long-term viability of the culture and growth rates. The dinoflagellates are "plants" and should therefore coexist without problem.

Task 3-1. Optimization of artificial sea water and supplements- Several commercially available sea waters are under investigation for use in LUNULA COLONY™ (11). Guillard F/2 Supplement® provides manganese chloride, ferrous chloride, biotin, B12 vitamin, and other needed ions and nutrients. We will evaluate various artificial sea waters with and without various supplements (11). We will develop and test formulations for optimization of growth and bioluminescence emission. Additives to increase the viscosity of the medium will also be explored for purposes of forming a homogeneous suspension of the organisms. An example of a material which may be considered is polyethylene glycol, a non-toxic, inert polymer. The optimum media will be proprietary and possibly patentable.

Task 3-2. Optimization of container configuration/materials- Most of PSI's work to date has used small volume laboratory flasks (125 ml to 2000 ml). We propose to utilize larger volumes and less expensive containers, including plastic bags and 5-30 gallon bucket/barrels (10). Container material properties to be evaluated include transparency, wetting characteristics, and the possible effect of low molecular weight impurities or additives. The effect of air bubbling and enriched CO_2 gas bubbling for increased gas transport will also be evaluated.

Task 4-1. Determination of CO2 requirements- Dinoflagellates use CO_2 and expire O_2 as part of their photosynthetic processes.* The rate per cell will determine the surface area and permeability of the materials used in the culture (18). Experiments will be performed to determine gas exchange requirements and limits. For example, is it possible to pressurize LUNULA COLONY™ with CO2 for shipping and storage without hurting the organisms? The CO_2, HCO_3, pH equilibria will be carefully studied. The respiration of the organisms will also be studied, including the feasibility of a totally sealed system (an Ecosphere or Biosphere) (20).

Task 4-2. Identification of appropriate gas transfer membranes-When CO2 requirements are established for particular cell densities, various materials will be evaluated for permeation rates of CO_2, O_2, and water.. Examples of materials which may be evaluated include polyethylene and ethylene-vinylacetate copolymers (18).

Task 5-1. Stress testing of cultures for limits of viability- Experiments will be conducted to determine limits of viability which will be critical in shipping and shelf life. Parameters which will be tested include temperature, partial and total darkness, mechanical agitation, and CO_2 storage capacity.

Task 6-1 We will continue the development of supporting teaching and educational materials which will be available as product accessories for inquisitive customers and for teachers and schools. This will be a major part of the Phase II effort. All of the previous tasks will help form the basis for laboratory experiments in the classroom.

It is emphasized that the over-all objectives of the tasks described above are to establish feasibility of growing dinoflagellates and designing and building a LUNULA COLONY™ product. The proposed project will be followed by more extensive Phase II studies and Phase III commercialization.

During Phase II, we expect to produce a science fair handbook and a variety of materials and other aids to assist teachers in using these cultures in their classrooms. A range of questions and experiments, which we propose to develop include:

- The effect of light intensity on bioluminescence and on growth rate.
- The effect of light duration and cycle on bioluminescence intensity and division rate.
- The effect of color, temperature, and light duration on bioluminescent intensity and division rate.
- Change in culture media with time,
- Correlation of pH changes with cell density and bioluminescence intensity.
- Response of the organisms to suboptimal environments leads to cyst formation, followed by regeneration of the cultures when the conditions improve.

The above are all topics which are more appropriate for Jr. High and High School Biology classes, but they are also the type of information which we need to fully understand the organisms and their potential for science education.

* They also respire during the dark cycle, consuming O_2 and producing CO_2. The photosynthesis/respiration ratio for these organisms is ~2 to 3 (12).

For the elementary classroom environment the major emphasis will be on such concepts as protozoa, photosynthesis, cell division, function of bioluminescence, role of light/dark cycles, effective temperatures, and the need for air, including possibly the role of oxygen and carbon dioxide.

The large size of these organisms, about 1/5 of a millimeter, makes it very easy to observe them in low power microscopes, or even through the use of a slide projector using a microslide projection device. Because of their approximate 7 day cell division time, and the high cell numbers in the cultures, students and teachers can observe cells in every stage of division.

The proposed schedule and time requirements for carrying out each of the identified tasks are summarized in Table 1.

No serious or insurmountable difficulties are anticipated. Areas of potential concern are listed below with appropriate discussion and possible solutions.

1. Algal contamination of culture- It may be possible, if careful handling and transfer techniques are not used, that other algae may contaminate the culture media. It may be necessary to suppress their growth by composition adjustment of the culture media. However, it is likely that the unique nutritional requirements of dinoflagellates will themselves act as inhibitors. The media used for reproduction and maintenance of dinoflagellates are inhibitory to many other microorganisms (10).

2. Safety testing- Because of the high safety expected of materials used by young children, safety and toxicity testing of the media and of the organisms will be carried out. No allergic or toxic indications have been reported for the dinoflagellates which we propose to employ (9). Acute toxicity studies of the present cultures are negative (no measurable toxicity) (21).

3. Cycle Maintenance- Dinoflagellates respond to light and dark cycles. The cultures and products will have their light-dark cycles adjusted to synchronize with the end user's needs and expectations.

The circadian rhythms which these organisms exhibit provide an excellent entree to a discussion of biological rhythms and cycles. The light/dark cycles are readily reprogrammed. We have constructed a variety of very simple units using a small fluorescent light and an inexpensive timer, contained within a black cardboard box. The teacher or other user can set the timer for whatever day/night cycle is desired. In this way, students and teachers can readily program the organisms, so that the bioluminescence is observable during any desired part of the day.

The elementary, Jr. high, and high school classrooms which have already utilized these cultures have simply set up a little activity corner for those students or group of students performing the experiments and observations.

H. RELATED RESEARCH

PSI has already invested about $60,000 in preliminary research on bioluminescent dinoflagellates for science education. In these preliminary studies, we have demonstrated that pyrocystis lunula can be cultured and maintained in nonlaboratory environments, including homes, classrooms, and offices (11).

TABLE I. PROPOSED SCHEDULE FOR R&D WORK

Specific R&D Task to be performed	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Task 1-1.	|---|					
Task 1-2.		|-----|				
Task 1-3.	|----------	----------	----------	----------	---|	
Task 1-4.			|----------	----------	---|	
Task 1-5.			|----------	----------	---|	
Task 2-1.				|----------	----------	---|
Task 2-2.						|---|
Task 2-3.			|----------	----------	---|	
Task 3-1.		|----------	----------	----------	---|	
Task 3-2.					|---|	
Task 4-1.			|----------	----------	---|	
Task 4-2.					|---|	
Task 5-1.				|----------	----------	---|
Task 6-1	|----------	----------	----------	----------	----------	---|

We have found that no special lighting conditions or sources are required - normal room lighting and normal room temperatures are satisfactory. We have found that no special containers are required, only that they be transparent and clean.

We have found that the cultures can be maintained for up to 6 months in totally sealed containers, with no oxygen or CO_2 exchange (14, 17A).

We have found that the organisms exhibit no measurable acute toxicity and appear to be completely safe (21).

We have found that their light/dark cycle/circadian rhythm can be very easily altered and programmed.

We have found that they can be shipped by first class mail across the country with little difficulty, as long as they are delivered and opened within 3 days.

We have found that students and teachers respond very positively and enthusiastically to these cultures (15).

Although these are all very promising results, considerable, additional research is required to get beyond the gee whiz stage of application of these novel cultures. Most of our preliminary results are very qualitative in nature. As these materials begin to be used in classroom environments, in science fairs, and science projects, it is important that we much more thoroughly understand their behavior on a solid scientific footing.

That information is not available in the technical literature. It must be generated. With such information in hand (the subject of this Phase I proposal), we will then be in a much stronger position to develop the teaching materials to enable the cultures and organisms to be most effectively used in the classroom and in other science education environments.

I. SENIOR PERSONNEL AND ADVISORS

Suzanne Winters, Ph.D., Vice President for Research and Development, Protein Solutions, has been with the company for approximately 1 year. She has a strong background and experience in the area of medical devices, and in particular in the development of materials and systems for the transfer of oxygen and carbon dioxide. She has a Bachelors Degree in Zoology, and a strong and driving interest in biology and in integrated science education. She is the PI of PSI's current Phase I grant, LIGHT CRAWLERS™: Bioluminescence Based Discoveries for Science Education, which involves the development and application of bioluminescent earthworms and marine worms for science education purposes. Her vita is in Section Q.

J.D. Andrade, Ph.D., President and Chief Scientific Officer of Protein Solutions, has been working on bioluminescence for the past 5 years and on bioluminescent dinoflagellates for the past 2 years. He has supervised all of the work to date in the area of bioluminescent dinoflagellates. He has conducted 3 inservices during the past 8 months and has distributed pyrocystis lunula cultures to at least 50 different teachers, friends, co-workers, and advisors for their input and critique. Joe taught high school general science, chemistry, and biology and has assisted in elementary school science instruction on a regular basis for a 3 year period. He has a strong interest in integrated science education. Dr. Andrade has been working on biomaterials and biotechnology problems for the past 25 years. He is an accomplished scientist with 5 books and over

100 peer-reviewed publications. Joe is also Director of the University of Utah's Center for Integrated Science Education (CISE).

Advisors:

Trish Stoddart, Ph.D. is Assistant Professor of Educational Studies in the Graduate School of Education at the University of Utah. Dr. Stoddart's major research area is the assessment of teacher knowledge, and the means by which incorrect concepts can be relearned. She sees bioluminescence as an ideal tool with which to study teacher pre-conceptions and with which to motivate teachers to restructure their concepts. She will function as an advisor to the project.

Barbara Andrade is a first and second grade teacher with twelve years of teaching experience. She also serves as secretary of Protein Solutions, Inc. She has been involved with PSI's efforts in the use of bioluminescence for science education for several years. She advises in the development and formulation of the bioluminescence concepts into practical and effective science education tools.

Vladimir Hlady, Ph.D. is Associate Research Professor of Bioengineering at the University of Utah. He is an expert on optics, particularly the measurement of luminescence and fluorescence at solid/liquid interfaces. His optical spectroscopy and engineering laboratory is a resource for more detailed light intensity and light duration studies. Dr. Hlady will serve as an informal advisor.

Dr. J.W. Hastings, Professor of Biology at Harvard University is one of the world's experts on dinoflagellate bioluminescence. Dr. Hastings serves on PSI's Scientific Advisory Board, and provides considerable advice.

Another member of PSI's Scientific Advisory Board is Mr. William Kelley. Mr. Kelley was one of the founders of Aquarium Systems, a corporation that sells artificial seawater and related materials for hobbyists and exhibition aquaria. Mr. Kelley has also been involved in the design and development of exhibition aquaria nationally, including the Mystic Marine Life Aquarium in Mystic, Connecticut. He has retired in Salt Lake City and has been of immense help in the area of culture media for dinoflagellates.

Other dinoflagellate experts with whom Dr. Andrade has already consulted over the last 2 years and whom have offered their advice include Dr. Barbara Prezelin, Department of Biological Sciences, University of California, Santa Barbara; Dr. L. Brand, University of Miami, School of Oceanography, Miami; Dr. James Morin, Professor of Biology, UCLA, Los Angeles; Dr. Robert Guillard, the National Phytoplankton Culture Facility, West Boothbay Harbor, Maine.

J. CONSULTANTS AND SUBCONTRACTS - NONE

K. FACILITIES AND EQUIPMENT

The work on this project will be carried out by PSI, Inc. in its laboratories located in the University Research Park, 390 Wakara Way, Salt Lake City, 84108. PSI is a member of the Center for Biopolymers at Interfaces at the University of Utah, one of the State's Centers of Excellence (Section Q). PSI is a key corporate participant in the University's Center for Integrated Science Education (CISE) (~~Section Q~~). PSI has a Technology Transfer agreement with the University of Utah Research Foundation. PSI's laboratories are equipped to perform the necessary biological, chemical, engineering, and

(see next page)

evaluation studies. The laboratory space (1500 ft²) includes a culture room, a general biology lab, and normal office and instrument room space. Sophisticated equipment which may be required may be used by our team ~~on a time-sharing cooperative basis~~ at the University of Utah (see ~~Section Q~~). next page

(A)

L. CURRENT AND PENDING SUPPORT

PSI has a current NSF Phase I award, which expires September 30, 1992: LIGHT CRAWLERS: Bioluminescence-Based Discoveries for Science Education. That project does not overlap with this proposal.

(B)

PSI has invested $60,000 (provided by its founders and major stock holders) in the initial studies and product development. PSI expects to continue funding the project from stock-derived funds. PSI is now discussing equity investments by a number of local investors and investment groups.

LUNULA COLONY: Bioluminescence

PSI ~~is~~ submitting ~~an additional~~ Phase I application titled: ~~The Lableas Lab: Polymer Materials~~. ~~There is no overlap between that companion proposal and this one.~~ for Science Education, to the National Science Foundation on June 15, 1992

~~The PI has no other support commitment at this time.~~

A second SBIR/ARO proposal submitted to NASA on July 15, titled The ATP Corona Pen.

M. COMMERCIAL POTENTIAL

The initial market for PSI's bioluminescence products are children, their parents and their teachers. With the recognition that our educational system must increase its emphasis on science and mathematics, a dramatically increased demand for products which have a significant educational component is expected during the 90's (17B).

Although PSI has not had the resources to do a complete market analysis, it is clear that the market is large. For example there have been over ten million Ant Farms sold by its original inventor and developer, Milton Levine. There at least two other manufacturers of ant farms who have a significant fraction of the market. The estimated sales volume for these ant farms and accessories over the years is $25 million.

A key market are the public school districts and teachers. It is estimated that $5B/year is spent in K-12 public education for books and materials. The part spent for science-related books and materials is over $2B/year (17B).

Another market is museum and science center gift shops. PSI is working with The Children's Museum of Utah and the University of Utah to erect a display dedicated to bioluminescence.

PSI's competitors are manufacturers of scientific educational toys. However, no other bioluminescent toys or science kits are currently on the market. In fact a number of science products/science gift distributors have already learned of PSI's bioluminescent science materials activities and have made inquiry as to the availability of these products for distribution and marketing.

It is expected that PSI's bioluminescence products will retail in the price range from roughly $5 to $100, with significant discounts for teachers and school districts.

In summary, the science educational market is already very large and is likely to be expanding in the next decade, based on the recognition of need for a renewed emphasis in science education (17B). The ability to produce bright light in various patterns and under various degrees of stimulation will attract potential buyers to PSI's bioluminescent products. That attraction and novelty, coupled with the educational potential of the product, should guarantee a strong and loyal clientele.

It is PSI's intention to develop and manufacture LUNULA COLONY™ in Salt Lake City as an educational product based on bioluminescence. LIGHT CRAWLERS, a bioluminescent worm farm, analogous to the Ant Farm, is the subject of a current SBIR Phase I project. A more sophisticated product, NIGHT LAB, a complete laboratory with accessories for more in-depth exploration of the phenomenon of bioluminescence, will also be developed.

N. EQUIVALENT PROPOSALS

No similar proposal has been funded, is pending, or is about to be submitted by Protein Solutions, Inc. to the National Science Foundation or any other agency.

P- REFERENCES/BIBIOGRAPHY/NOTES:

1. National Geographic Magazine has had many articles and photos of bioluminescence:

   P.A.Zahl, Nature's Night Lights, July, 1971, p.45,
   P.A.Zahl, Fishing in the Whirlpool, Nov, 1973, p.579.
   D.L. Teimann, Nature's Toy Train, The Railroad Worm, July, 1970, p.58.
   P.A. Zahl, "Fireflies", July, 1962, p.48

2. Several major encyclopedias include articles on bioluminescence:

   Encyclopedia Britannica
   McGraw Hill Encyclopedia of Science and Technology

3. Popular science articles include:
   K.H. Nealson and C. Arnesan, "Marine Bioluminescence: About to See the Light," Oceanus 28(3)(1985)13.
   P. Huyghe, "Wheels of Light, Sea of Fire," Oceans, Dec, 1987, p.21.
   M. Root, "Glow-in-the-dark Biotechnology," Biological Science 38 (11)(1988)745.
   A.K. Campbell, "Living Light," Trends in Biological Sci. 11 (1986)104.
   A.P. Neary and C.S.J. Walpole, "Bioluminescence-Chemical Light," Science Progress 70(1986)145.
   P.J. Herring, "How to Survive in the Dark: Bioluminescence in the Deep Sea," in M.S. Laverack, ed., Physiologic Adaptation of Marine Animals, Soc. of Experimental Biology of Great Britain, 39(1985)323-351.

4. There is a limited discussion of bioluminescence in science and nature books for children. The most complete is:

   A. and U. Silverstein, *Nature's Living Light*, Little, Brown, & Co., 1988.

5. F.A. Brown, Jr., "Bioluminescence", in *Comparative Animal Physiology*, (Cladd Prosser ed.), 3rd Edition (1973), pp. 951-966.

6. J. Rutherford, Project 2061: *Science for All Americans*, American Association for the Advancement of Science, Washington, DC, (1988) p. 14.

7. Although bioluminescence is largely unknown in the K-12 and college curricula, there is an extensive scientific literature:

   A.K. Campbell, Chemiluminescence, VCH Publ.,1988
   F.H. Johnson and Y. Haneda, Bioluminescence in Progress, Princeton Univ. Press, 1966.
   P.J. Herring, Bioluminescence in Action, Academic Press, 1978.
   P.J. Herring, A.K. Campbell, M. Whitfield, and L. Maddock, Light and Life in the Sea, Cambridge University Press, 1990.

   Much of the current scientific information is being published in the Journal of Bioluminescence and Chemiluminescence, John Wiley and Sons.

8. F.J.R. Taylor, *Biology of Dinoflagellates*, Blackwell Scientific Publ., 1987.

9. D.L. Spector, ed., *Dinoflagellates*, Academic Press, 1984.

10. G. Barnabe, ed., Aquaculture, E. Horwood, Publ, 1991.
A. Richmond, ed., *CRC Handbook of Microalgal Mass Culture*, CRC Press.
R.R.L. Guillard, "Culture of Phytoplankton..." in C.J. Berg, Jr., *Culture of Marine Invertebrates*, Hutchinson Ross Publ. Co., 1983; see also his Chapter in Ref. 9.

11. John Tobler and J. Andrade, "Culture of Bioluminescent Dinoflagellates in Non-Traditional Media," Abstract, Utah Academy of Arts and Sciences, Salt Lake City, May 1991.

12. B. Prezelin, "Photosynthetic Physiology of Dinoflagellates," Chapter in Ref. 8, pp. 174-223.

13. Our interest in bioluminescence began in 1985 when J. Andrade became interested in the subject and began doing some simple "discovery" experiments. Work began in earnest in the Fall of 1987. Protein Solution, Inc. (PSI) was established in early 1988 with the goal of developing bioluminescence for the children's education and toy markets. PSI has been funding bioluminescence work in Andrade's lab for nearly 3 years (about $60,000 total to date). It was already clear in 1987 that bioluminescence was a real attention getter and motivator of children and adults. Andrade's wife, Barbara, is a first grade teacher. Together they developed several demonstrations and experiments. The phenomena were presented to Dr. T. Stoddart and R. Stofflett in the Department of Educational Studies at the University of Utah in 1990. It was decided that there was sufficient interest and commitment among all involved to begin studies on integrated science discovery materials based on bioluminescence.

14. J. Tobler and J.D. Andrade, "Culture of Pyrocystis Lunula in Sealed Polyethylene Bags", Abstract, Utah Academy of Arts and Sciences, May, 1992.

15. State Office of Education: Bruce Griffen and LaMar Allred; Davis School District: David Steele and LaMont Jensen; Salt Lake School District: Kenneth Burton; Jordan District: Jean Woolam.

16. Richard Morris, The Children's Museum of Utah, Salt Lake City, Utah.

17A. J. D. Andrade and J. Tobler, Invention Disclosure: "Culture of Phytoplankton in Sealed Environments", June, 1992.

17B. M. McDonald, K. Thorimbert, and J. Andrade, "The Science Education Market", report to State of Utah Centers of Excellence Program, July, 1992; Center for Integrated Science Education.

18. K.D. Foote, "Highly Gas Permeable Polymer Membranes Applied to Algae Aquaculture", BSc. Thesis, Dept. of Materials Science, Univ. of Utah, Dec. 1991 (J.D. Andrade, Advisor).

19. J.R. Stein, ed., *Handbook of Phycological Methods: Culture Methods & Growth Measurements*, Cambridge Univ. Press, 1973.

20. C.E. Folsome and J.A. Hanson, "Emergence of Material-Closed-System Ecology", in N. Polunin, ed., Ecosystem Theory and Application, Wiley, 1986, pp. 269-288.

21. White Eagle Toxicology Labs, Morristown, PA

Q. VITA AND LETTERS (next page)

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EDUCATION

1986 Ph.D. Pharmaceutics: University of Utah, Dissertation: "Immobilized Heparin via a Polyethylene Oxide Spacer for Protein and Platelet Compatibility", Joseph D. Andrade, advisor

1976 B.S. Zoology, Ohio Wesleyan University, Delaware, Ohio, cum laude

PROFESSIONAL EXPERIENCE

Feb, 1991 to present — Vice President, Product Development, Protein Solutions, Inc. Salt Lake City, Utah

- ◊ Submission of grant applications
- ◊ Research and development on bioluminescent educational products

October 1986 to Nov. 1990 — Director, Membranes Technology, CardioPulmonics, Salt Lake City, Utah

- ◊ Responsible for a group of 9 professionals plus technicians for R&D projects for start-up medical devices development company
- ◊ Supervised and assisted in the installation and set-up of a wet chemistry laboratory for small start-up research and development business
- ◊ Managed an $800K annual budget and approved all capital expenditures for installation and maintenance of two laboratories totaling $1.1 million;
- ◊ Submission of grant applications, proposals (success rate 75%) and patent applications
- ◊ Primary responsibility for wet and analytical chemistry labs and development of plasma polymerization coatings laboratory
- ◊ Coordination between research and development departments; significant responsibilities in marketing technology
- ◊ Development of a gas permeable, pharmacologically active membrane coating for a totally implantable artificial lung resulting in two patent applications
- ◊ Development of plasma etching techniques for chemical functionalization of silicone plastics and subsequent chemical modifications
- ◊ Provide an interface for technical and business development personnel

January 1986 to June 1986 — Symbion, Inc., Salt Lake City, Utah: Senior Materials Scientist

- ◊ Analysis and interpretation of retrieved Jarvik 7 artificial hearts for protein and cellular deposition and materials failures
- ◊ Preparation of reports to Food and Drug Administration on retrieved hearts for follow-up of original Jarvik-7 PMA
- ◊ Development of testing protocols for Jarvik 7-70 and supervision of animal experiments for PMA submission
- ◊ Development of a quantitative analysis system of animal data
- ◊ Functioned as the primary interface between development engineers and clean room manufacturing personnel for design revisions and production problem solving

February 1978 to December 1981 — Project Leader, Battelle Columbus Laboratories, Columbus, Ohio Biological Spectroscopy Facility

- ◊ Supervised and assisted ongoing molecular level study of the events occurring during whole blood contact with polymeric surfaces using Fourier Transform infrared spectroscopy for characterization and analysis
- ◊ Developed data compilation system and analysis for presentation to sponsors, biannually
- ◊ Designed and built a live animal shunt system and analytical cell for on-line, real-time data acquisition of blood protein adsorption phenomenon

September 1976 to February 1978 — Battelle Columbus Laboratories, Columbus, Ohio: Researcher, Analytical Chemistry Division

- ◊ Development of a fractionation and characterization scheme for Alaskan crude oil using gel permeation chromatography and gas chromatography / mass spectrometry for determination of toxicity and mutagenicity
- ◊ Frankford Arsenal, Philadelphia, PA; Set up an on-site analytical laboratory testing for trace explosive contaminants in conjunction with the U.S. Army

PUBLICATIONS

"Fourier Transform Infrared Spectroscopy of Protein Adsorption from Whole Blood: I. *Ex Vivo* Dog Studies", R.M. Gendreau, S. Winters, R.I. Leininger, D. Fink, and R.J. Jakobsen, Applied Spectroscopy 35, 353 (1981).

"Biological Applications of FT-IR or Bloody FT-IR", R. J. Jakobsen, S. Winters, and R. M. Gendreau, 1981 International Conference on Fourier Transform Infrared Spectroscopy, Proceedings, SPIE 289 469 (1981).

"Fourier Transform Infrared Spectroscopy of Protein Adsorption from Whole Blood: II. *Ex Vivo* Sheep Studies", S. Winters, R. M. Gendreau, R. I. Leininger, and R. J. Jakobsen, Applied Spectroscopy, 36, 404 (1982).

"Effects of Flow Rates and Solution Concentration on *In Situ* Protein Adsorption Behavior", R. J. Jakobsen, L.L. Brown, S. Winters, and R. M. Gendreau, Journal of Biomedical Materials Research, 17 199 (1983).

"Intermolecular Interactions in Collagen Self Assembly as Revealed By Fourier Transform Infrared Spectroscopy", R. J. Jakobsen, L. L. Brown, S. Winters, T. B. Hutson, D. J. Fink, and A. Veis, Science, 220, 1288 (1983).

IVOX: An Intracorporeal Device and Methodology for Temporary Augmentation of Blood Gas Transfer in Subjects with Acute, Potentially Reversible Respiratory Insufficiency", J.D. Mortensen, G.L. Berry, and S. Winters, Cardiac Chronicle (2) 1990.

"Night Colony: A Science Discovery Tool," Abstract, J.D. Andrade, J. Tobler, T. Stoddart, and S. Winters, Pacific Division, AAAS, Logan, Utah, June 23-27 (1991).

PATENTS

"Multifunctional Thrombo-Resistant Coatings and Methods of Manufacture", S. Winters, K.A. Solen, C.G. Sanders, G.L. Berry and J.D. Mortensen, submitted July 1987, pending.

"Gas Permeable Thrombo-Resistant Coatings and Methods of Manufacture", S. Winters, K.A. Solen, C.G. Sanders, G.L. Berry and J.D. Mortensen, submitted March 1990, pending.