National Science Foundation
Small Business Innovation Research Program

PROJECT SUMMARY

NSF AWARD NO.

NAME OF FIRM
Protein Solutions, Inc.

ADDRESS
6009 Highland Drive, Salt Lake City, Utah 84121

PRINCIPAL INVESTIGATORS (NAME AND TITLE)
Suzanne Winters, Ph.D., V.P. for Research & Development

TITLE OF PROJECT
NIGHT COLONY: Science in the Dark

TOPIC TITLE	TOPIC NUMBER
Education & Human Resources	26

TECHNICAL ABSTRACT (LIMIT TO 200 WORDS)

We propose to develop unique science education products which will motivate children (and their parents!) to observe and experience scientific phenomena. The product, NIGHT COLONY, will consist of a transparent container with a culture of bioluminescent marine microalgae. The startling bioluminescence (light production) is an intense blue color and occurs upon mechanical stimulation. A light tap on the container results in light production. The NIGHT COLONY product will include all needed materials and accessories to set up, maintain, and observe bioluminescence and to enhance the observer's science interests. The product is analogous to existing ANT FARM and Sea MONKEY products commonly sold in toy and science stores, museum gift shops, and via direct marketing, but will be far more exciting, more desirable, and more useful for science education purposes.

The product will be available with a range of educational materials and modifies for various age and grade groups (Grades 1-3, 4-6, and 7-9). The materials will supplement and expand existing state and district core curricula, with an emphasis on inquiry and discovery-based learning. The materials and experiments will be student question and curiosity driven.

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

KEY WORDS TO IDENTIFY RESEARCH OR TECHNOLOGY (8 MAXIMUM)
Bioluminescence, Science Education, Toys, Children's Products, Biotechnology

POTENTIAL COMMERCIAL APPLICATIONS OF THE RESEARCH
Science Education, Children's Toys and Novelties

TABLE OF CONTENTS

I. IDENTIFICATION AND SIGNIFICANCE OF THE INNOVATION

Protein Solutions, Inc. (PSI) intends to develop, manufacture, and market a new line of educational, innovative products, NIGHT LIFE products, which use the phenomenon of bioluminescence [1-3] to motivate, entertain, and educate children. This proposal specifically addresses the first product of this line, NIGHT COLONY, a living "night light."

A new awareness is emerging that the 90's will be a decade of increased emphasis on education. Most states have already adopted programs to enhance education at all levels, in both public and higher education. The National Science Foundation's budget for science education has increased dramatically in recent years. This will result in a significantly increased demand for products which have an educational component, including gifts and novelties for children and more serious products purchased by parents and teachers to enhance the education of their children and their students. PSI will be addressing this interest with products that stimulate a child's natural curiosity and eagerness to learn.

PSI is focused on the development and application of bioluminescence as an educational and product development pool. This is a unique technology which has not been utilized in educational products or toys. Children (and their parents!) are fascinated by bioluminescence. It is a new, "magical", and very stimulating experience for them [4].

NIGHT COLONY, a transparent container of bioluminescent marine micro-organisms which produce a brilliant blue light upon mechanical stimulation, is the first NIGHT LIFE product. Two different marine microalgae have been identified as candidates for this product, Pyrocystis lunula and Pyrocystis noctiluca. 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 behaviors may be observed.

Successful development and manufacture of NIGHT COLONY will provide a new, exciting introduction to the joy of discovery and exploration, and the impetus for developing questions and curiosity.

We argue that there is a revolution developing in science education, particularly at the elementary and high school levels, in that science must be taught as an integrated discipline, rather than in the highly piecemeal fashion of biology, chemistry, physics, and mathematics, which is now the norm [5]. 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, curricula 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. NIGHT COLONY provides an excellent introduction to the interplay of biology, chemistry, physics, and environmental science (Figure 1).

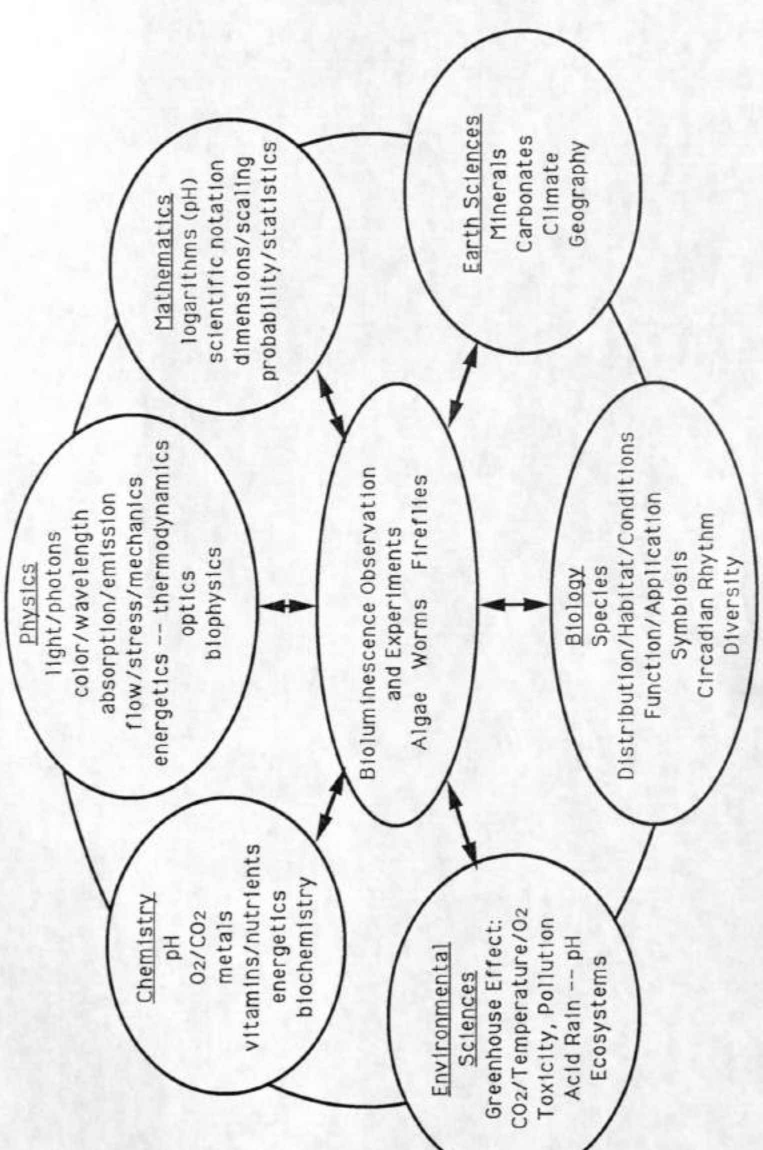

Figure 1: Bioluminescent organisms and their observation are shown as the center of an integrated science "wheel". Each of the classical specialties or disciplines are indicated with selected subject examples. These subjects and topics can all be directly observed and experimentally studied via bioluminescence.

NIGHT 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 had very positive responses from the Salt Lake City, Jordan, and Davis County school districts, and from the State Office of Education; there is strong interest in adding the exploration of bioluminescence into their curricula.

Thus, successful development and commercialization of NIGHT COLONY and other NIGHT LIFE products will help increase the interest and enthusiasm of children through the process of discovery, thereby enhancing science education. .

II. BACKGROUND, APPROACH, AND ANTICIPATED RESULTS

Background:

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 [1-4, 6].

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 child's attention and interest.

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 [5].

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 occurs upon stimulation and lasts only a fraction of a second [1-3] but individual cells, depending on the species, will flash repeatedly with repeated stimulation. These dinoflagellates will be used in NIGHT COLONY to produce light when agitated or mechanically stimulated [7, 8].

Approach:

We propose to develop integrated science educational toys and materials using bioluminescence as "discovery" and observation vehicles. NIGHT COLONY will consist of a transparent container of "sea pets", an owner's manual, food packets, a sampling pipet, and a NIGHT SLIDE for observation.

The science involved in the development of NIGHT 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 NIGHT COLONY requires optimization of the culture conditions and of the sea water composition.

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 light intensities, hardiness, and relatively large size [9].

The requirements for shipping and storage of the NIGHT COLONY will be studied and optimized. For example, how much carbon dioxide must be stored in the NIGHT COLONY to maintain an adequate shelf life of the organisms? How long can they be stored in the dark? Can they be shipped and stored completely sealed?

PSI is uniquely positioned to enter the educational bioluminescence toy market. We have had extensive interaction with experts in the field, including those knowledgeable about dinoflagellates and other bioluminescent organisms [10]. J.D. Andrade, President of PSI, has been involved in the field of bioluminescence for over 3 years [11]. 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.

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 [12]. We are also working with the Children's Museum of Utah to develop an interactive, hands-on bioluminescence exhibit [13]. 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 and educational toys.

Anticipated Results:

The proposed R&D project has been planned and organized to test the feasibility of developing and maintaining a viable dinoflagellate culture for use as a science educational toy. The research proposed here is expected to lead to:

1. High density cultures of single and mixed populations of dinoflagellates which may be maintained viable for up to six months with little care or attention. We expect to identify specific feeding and CO2 requirements for their viability and long term stability and function.

2. A polymeric membrane capable of providing the necessary gas requirements for these organisms which may be fashioned into a shape attractive 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. The PI has extensive experience with gas transfer membranes and in the design of products with the needed gas transfer requirements.

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

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

After feasibility is demonstrated by this Phase I project, we propose to move into Phase II to further develop and optimize NIGHT COLONY and to move into production and commercial sales. We anticipate no difficulty in obtaining funding for the follow-on Phase II (optimization and test marketing) and Phase III (full commercialization) of the project.

Several novelty products are currently on the market which generate light through chemiluminescence, such as "light stiks" (see footnote on page 5). These 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 and control available with bioluminescence. To our knowledge, no other product is available or under development which can compete with NIGHT LIFE products.

III. PROGRAM OBJECTIVES

The project has the following specific objectives:

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

B. Develop the process to produce cultures which have high stability and which guarantees a product life of three to six months.

C. Optimize the culture medium.

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

E. Establish shipping and storage conditions to maintain a viable culture of the dinoflagellates.

F. Develop the teaching and educational materials and product accessories to enhance customer interest and science education.

IV. PROGRAM PLAN

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

Task A-1. Selection of dinoflagellates with sufficient bioluminescence- There are several known species of bioluminescent dinoflagellates. PSI has identified and studied two non-toxic varieties, Pyrocystis noctiluca, and Pyrocystis lunula [19]. 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 A-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.

Task A-3. Development of culture/transfer techniques- There are several references on culturing dinoflagellates emphasizing laboratory conditions and small amounts [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 A-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 and will be explored in efforts on Task C-1 below. Cell densities and division rates will be measured by standard methods [18].

Task A-5. Maximization of cell densities for maximum bioluminescent intensity- Currently, PSI has developed sufficient expertise to maintain moderate density cultures of the Pyrocystis strains (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 again be explored simultaneously with efforts to optimize the artificial sea water (Task C-1).

Task B-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 B-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 B-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 NIGHT 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 C-1. Optimization of artificial sea water and supplements- Several commercially available sea waters are under investigation for use in the NIGHT COLONY. Guillard F/2 Supplement® provides manganese chloride, ferrous chloride, biotin, B12 vitamin, and other needed ions and nutrients [9]0. PSI is now evaluating various artificial sea water with and without various supplements [14]. We will do testing to develop 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 C-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 bag and 5-10 gallon bucket/barrel cultures [9]. Container material properties to be evaluated include transparency, wetting characteristics, and the possible effect of low molecular weight impurities or polymer additives. The effect of air bubbling and enriched CO_2 gas bubbling for increased gas transport will also be evaluated.

Task D-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. Experiments will be performed to determine gas exchange requirements and limits. For example, is it possible to pressurize NIGHT 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)[16].

Task D-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 silicones and porous polypropylenes.

Task E-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 F-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.

It is emphasized that the over-all objectives of the tasks described above, including testing to be carried out, is to establish feasibility of growing dinoflagellates and designing and building a NIGHT COLONY product. The proposed project will be followed by Phase II and Phase III studies

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

---

* 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 [15].

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 A-1.	├──┤					
Task A-2.	├──────┤					
Task A-3.	├──────────────────────┤					
Task A-4.			├──────────────┤			
Task A-5.			├──────────────────┤			
Task B-1.			├──────────────────┤			
Task B-2.					├──────┤	
Task B-3.		├──────────────────┤				
Task C-1.		├──────────────────┤				
Task C-2.					├──────┤	
Task D-1.		├──────────────────┤				
Task D-2.					├──────┤	
Task E-1.				├──────────┤		
Task F-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, if this is determined to be a problem, 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.

2. Safety testing- Because of the high safety expected of toys and related products, safety and toxicity testing of the media and of the organisms will be carried out. Nelson Laboratories, located in Salt Lake City, is a contract laboratory for toxicity testing and will be used to test product safety. No allergic or toxic indications have been reported for the dinoflagellates which we propose to employ [19].

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.

V. REFERENCES

1. *National Geographic Magazine* has 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. Hughe, "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. *Project 2061*, Science for All Americans, Summary Report.

6. 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.

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

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

9. 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. 7.

10. J.W. Hasting, Harvard University, Boston, Massachusetts.
D. Anderson, Woods Hole, Mass: D. Brand, Univ. of Miami; B. Prezelin, Univ. of California Santa Barbara; R. Guillard, Boothbay Harbor, Maine.

11. 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 year (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 in 1990. Science curriculum specialists in the State Office of Education, several local school districts, and local educators and students were all excited. It was decided that there was sufficient interest and commitment among all involved to prepare a proposal to NSF in order to develop integrated science discovery materials based on bioluminescence.

12. State Office of Education; Bruce Griffin and LaMar Allred: Davis School District: David Steele and LaMont Jensen: Salt Lake School District; Ken Powell.

13. Richard Morris, The Children's Museum of Utah.

14. John Tobler and J. Andrade, "Culture of Bioluminescent Dinoflagellates in Non-Traditional Media," Abstract, utah Academy of Arts and Sciences Meeting, Salt Lake City, May 10, 1991.

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

16. 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.

17. *Toy Industry Fact Book: 1990-91*, Toy Manufacturers of America, 1991.

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

19. Some dinoflagellates produce toxins which can accumulate in certain shellfish. The organisms selected for NIGHT COLONY do not produce toxins [7,8].

VI. KEY PERSONNEL

Suzanne Winters, Ph.D., Vice President, Product Development, will serve as Project Manager. Dr. Winters has worked in technology development for the past 4 years with CardioPulmonics, Inc. as Director of Membrane Technology, where she was responsible for materials development of a gas transfer membrane. She recently joined Protein Solutions, Inc. where she is Vice President for Product Development and is working on bioluminescence-

based technologies. She holds a Bachelors Degree in Zoology and and Ph.D. in Pharmaceutics from the University of Utah. Her vita is in the Appendix.

Joseph D. Andrade, Ph.D. Chairman, Department of Bioengineering, University of Utah, and President of Protein Solutions, Inc., has been studying bioluminescence for over 3 years, primarily for science motivation and education. He 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. Joe is also Director of the University of Utah's Center for Integrated Science Education (CISE). His vita is in the Appendix.

PSI is a major corporate participant in CISE and has access to CISE faculty and staff, including education professionals (see Appendix). CISE's major goal is to teach science in fully integrated, highly inter- and multi-disciplinary ways. CISE involves faculty from the University of Utah's Colleges of Education, Science, and Engineering.

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 will directly advise and assist in the development and formulation of the bioluminescence concept into a practical and effective science education tool.

Rene Stofflett, Ph.D. is Assistant Professor of Education at Northern Illinois University. She was formerly a graduate student working under Dr. T. Stoddart. Dr. Stofflett was also involved in the discussions on the development and use of bioluminescence in elementary education. She is eager to apply the materials and products developed by PSI to her research and education activities in Northern Illinois.

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.

VII. FACILITIES

Most of the work on this project will be carried out by PSI, Inc. in its laboratories located at Northgate Business Center, 825 No. 300 West, Suite 145 in Salt Lake City and affiliated laboratories at the University of Utah. PSI is a member of the Center for Biopolymers at Interfaces at the University of Utah, one of the State's Centers of Excellence (Appendix). PSI is a key corporate participant in the University's Center for Integrated Science Education (CISE) (Appendix). PSI has a Technology Transfer agreement with the University of Utah Research Foundation. Our laboratories are equipped to perform the

necessary biological, chemical, engineering, and evaluation studies. Sophisticated equipment which may be required may be used by our team on a time-sharing cooperative basis at the University of Utah (Appendix).

VIII. CURRENT AND PENDING SUPPORT & FOLLOW ON FUNDING

PSI has no current outside support. A second proposal has been submitted to the National Science Foundation SBIR program titled LIGHT CRAWLERS: Bioluminescence-Based Discoveries for Science Education. That proposal does not overlap with this project.

PSI anticipates submitting an SBIR to NASA on August 7, dealing with closed system ecologies (ecospheres) [16].

In addition to the anticipated Federal SBIR funds, PSI has already 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 at the same level. PSI is now discussing equity investments by a number of local investors and investment groups.

PSI recently submitted a similar proposal to the State of Utah's Small Business Innovation Program (SBIP). That proposal was not funded on the grounds that an education materials industry is not part of the State's economic development plans!

IX. 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 the emphasis on science and mathematics, a dramatically increased demand for products which have a significant educational component will be seen during the 90's.

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.

Market assessments performed by Toy Manufacturers of America estimates sales of Activity Toys, which include educational toys, for 1989 to be $1.4 billion. The segment of this market specifically addressing educational and scientific toys was $45 million in 1989[17].

PSI's key market will be the public school districts and teachers. It is estimated that $5B/year is spent in K-12 public education for books and materials. We estimate the part spent for science-related books and materials to be $1-2B/year.

Another market will be 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. A joint proposal to NSF's Informal Science Education Program will be submitted in August, 1991.

PSI's competitors would be manufacturers of scientific educational toys. However, no other bioluminescent toys or science kits are currently on the market.

It is expected that PSI"s bioluminescence products will retail in the price range from roughly $15 to $100, with significant volume discounts for teachers and school districts.

In summary, the educational toy 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. 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 NIGHT COLONY in Salt Lake City as the first of a line of educational toys based on bioluminescence. NIGHT COLONY falls into PSI's division of liquid products. Follow-on products in the dry category involve technology consisting of a powder containing the protein and the chemicals required for efficient bioluminescence, derived from a marine crustacean. When the powder is wet with water, the enzyme is reactivated and a brilliant bioluminescence results. This dry technology lends itself to the development of products such as paper, inks and paint. The market for these types of toys was over $200 million in 1989. LIGHT CRAWLERS, a bioluminescent earthworm farm, analogous to the ant farm, is the subject of a parallel NSF SBIR application. A more sophisticated teaching toy, NIGHT LAB, a complete laboratory with accessories for more in-depth exploration of the phenomenon of bioluminescence, will also be developed.

Initial test marketing of all of these products will be in local specialty shops such as Gregory's Toys and Adventures. Managers of these businesses have expressed significant and enthusiastic interest in these products. Direct marketing via the publications of the National Science Teachers Association and related groups will be used at a later date.

X. 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.

The University of Utah's Center for Integrated Science Education (CISE) recently (May 15, 1991) submitted a proposal to NSF's Materials Development Program. The proposal, NIGHT LIFE: Integrated Science Education Materials Based on Bioluminescence, focused on curricular development and curriculum integration. There is a slight overlap (estimated at 20% of this SBIR effort) in the area of bioluminescent dinoflagellates.

Suzanne Winters Ph. D.

V.P., Research & Development
Protein Solutions, Inc.
6009 Highland Drive
Salt Lake City, Utah 84121

2890 Live Oak Circle
Salt Lake City, UT 84117
(801) 272-4528

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 shumt 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.

GREGORY'S
TOYS & ADVENTURES

March 15, 1991

Joseph D. Andrade, President
Protein Solutions, Inc.
6009 Highland Drive
Salt Lake City, Utah 84121

Dear Joe:

I was pleased to learn that Protein Solutions, Inc. is proceeding with its plans to develop a range of children's discovery products based on bioluminescence. I have reviewed your description of the proposed NIGHT-COLONY product and feel that it would be very popular with our customers.

As you know, Gregory's Toys and Adventures provides toys and related products for a broad audience. We also have a very significant educational component in our stores. As I am sure you are aware, the science area is one that continues to draw more interest from consumers as well as educators. It is a real growth category. Our clientele prefer products which are safe, sound, educational, and entertaining. Your proposed NIGHT-COLONY product meets all of these requirements.

We look forward to participating in the initial test marketing of NIGHT-COLONY, which I understand may be available as early as late in 1991, hopefully in time for the pre-Christmas season.

We will be happy to display and to feature your product in our local and out of state stores. We look forward to your continued progress.

Sincerely,

Greg A. Gohlinghorst

Greg A. Gohlinghorst
President, Gregory's Toys & Adventures

THE CHILDREN'S MUSEUM OF UTAH ™

15 March 1991

Dr. J. D. Andrade, President
Protein Solutions, Inc.
6009 Highland Drive
Salt Lake City, Utah 84121

Dear Joe,

I was pleased to review your NIGHT-COLONY Proposal to the State SBIP Program. The availability of the technology which will be generated as a result of this project will greatly help you and me in our efforts to develop an interactive bioluminescence-based science exhibit in The Children's Museum of Utah.

The availability of this exhibit will of course provide exposure for your product and product ideas. We would also expect to be able to sell and distribute the product via our giftshop mechanism.

I look forward to continuing to work with you in the development of a complete set of bioluminescence interactive exhibits at The Children's Museum of Utah.

Good luck.

Very truly yours,

Richard

Richard R. Morris
Excecutive Director

RRM:bw