APPENDIX A - PROPOSAL COVER
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

SBIR 91-1 SOLICITATION PROPOSAL COVER

(Instructions on Reverse Side)

PROPOSAL NUMBER
(TO BE COMPLETED BY PROPOSER)

	4 DIGIT SUBTOPIC NUMBER	LAST 4 DIGITS OF FIRM PHONE NO.	CHANGE LETTER
91-1	12 06	1259	

ENTER PROPOSAL NUMBER ON APPENDICES B & C

PROJECT TITLE: Bioluminescent Closed EcoSystems (BCES)

16

FIRM NAME: Protein Solutions, Inc.

MAIL ADDRESS: 6009 Highland Drive

CITY: Salt Lake City STATE: UT ZIP CODE: 84121

AMOUNT REQUESTED: $ 49,386 (PHASE I) DURATION: 6 MONTHS (PHASE I)

OFFEROR CERTIFIES THAT:

	YES	NO
1. As defined in Section 2 of the Solicitation, this firm qualifies as a:		
1.1 Small business	X	
1.2 Minority and disadvantaged small business		X
1.3 Women-owned small business		X
NOTE: 1.2 and 1.3 are not eligibility requirements for SBIR and the offeror may decline to indicate status by stating "Decline" across boxes.		
2. A minimum of two-thirds of the research and/or analytical effort for this project will be carried out within the firm if an award is made.	X	
3. The primary employment of the principal investigator will be with this firm at the time of award and during the conduct of the research.	X	
4. Proposals of similar content have (indicate Yes) or have not (indicate No) been submitted to another agency and the details required by Section 5.10 of the Solication are included in the proposal.		X

ENDORSEMENTS

	Principal Investigator	Corporate/Business Official
Typed Name	J.D. Andrade	J.D. Andrade
Title	Chief Scientific Officer	President
Telephone No.	(801) 581-4379	(801) 277-1259

Signature of Principal Investigator: JDA Date 8/5/91

Signature of Corporate Business Official: JDA Date 8/5/91

PROPRIETARY NOTICE (IF APPLICABLE, SEE SECTIONS 5.4.1 & 5.5)

NOTICE: No proprietary information is included except in a Proprietary Addendum. The information (data) on pages __________ in the Proprietary Addendum section of this proposal constitute a trade secret and/or information that is commercial or financial and confidential or privileged. It is furnished to the Government in confidence with the understanding that it will not, without permission of the offeror, be used or disclosed other than for evaluation purposes; provided, however, in the event a contract is awarded on this proposal, the Government may obtain additional rights to use and disclose this information (data).

APPENDIX B - PROJECT SUMMARY

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

SBIR 91-1 SOLICITATION

(Instructions on Reverse Side)

PROPOSAL NUMBER
(TO BE COMPLETED BY PROPOSER)

	4 DIGIT SUBTOPIC NUMBER	LAST 4 DIGITS OF FIRM PHONE NO.	CHANGE LETTER
91-1	12 06	1259	

AMOUNT REQUESTED: $ 49,3

TITLE OF PROJECT

Bioluminescent Closed EcoSystems (BCES)

TECHNICAL ABSTRACT (LIMIT 200 WORDS)

We propose to assess the feasibility of developing materially closed ecosystems utilizing bioluminescent organisms. We anticipate that such an ecosystem would include bioluminescent dinoflagellates (phytoplankton), several zooplankton, marine worms, and small shrimp. Using the exis literature and experience with closed and partially closed small scale ecosystems, together with our experience with bioluminescent organisms, we will assess the feasibility of coupling these two areas to produce Bioluminescent materially Closed EcoSystems (BCES). Such systems would be a dramatic to for the study of the interactions among organisms. BCES would serve as a dramatic demonstrations of ecology, ecosystems, and materially closed life support environments.

BCES would have considerable application as teaching and education tools. In addition, the application of bioluminescent organisms in larger scale closed ecosystems could serve as sensors, probe and direct visual indicators of the state and health of components of the ecosystem. Studies involving BCES will provide information and experience relevant to NASA programs in Bioregenerative Life Sup Using Microorganisms.

It is important that teachers, students, and the general public be made aware of the close conne between NASA's interests and objectives on life support of man and space, and the parallel problems of maintenance and enhancement of a hospitable terrestrial environment for man. The development and availability of materially closed ecosystems, and particularly bioluminescent ones, as research and educa tools could significantly enhance the general public's awareness of the importance of NASA's objectives mission.

POTENTIAL COMMERCIAL APPLICATIONS

Model system for research and development;
Science education and educators;
Museum and science center exhibits;
Educational decoration for homes and offices.

KEY WORDS (LIMIT 8) Ecology, science education, model system, Food Webs, Ecosphere, ecosystem, bioluminescence, life support.

NAME AND ADDRESS OF OFFEROR

Protein Solutions, Inc.
6009 Highland Drive
Salt Lake City, Utah 84121

PRINCIPAL INVESTIGATOR

J.D. Andrade

Table of Contents

Part 1: Identification & Significance of the Invention

Introductory Paragraph

We propose to assess the feasibility of developing materially closed ecosystems utilizing bioluminescent organisms [1-5]. We anticipate that such an ecosystem would include bioluminescent dinoflagellates (phytoplankton), several zooplankton, marine worms, and small shrimp. Using the existing literature and experience with closed and partially closed small scale ecosystems, together with our experience with bioluminescent organisms, we will assess the feasibility of coupling these two areas to produce Bioluminescent materially Closed EcoSystems (BCES). Such systems would be dramatic tools for the study of the interactions among organisms. BCES would serve as dramatic demonstrations of ecology, ecosystems, and materially closed life support environments.

BCES would have considerable application as teaching and education tools. In addition, the application of bioluminescent organisms in larger scale closed ecosystems could serve as sensors, probes, and direct visual indicators of the state and health of components of the ecosystem. Studies involving BCES will provide information and experience relevant to NASA programs in Bioregenerative Life Support Using Microorganisms.

Background & Rationale

Closed ecosystems are important education, research, and development tools for the eventual construction and application of closed life support systems for man in space [6-27]. In addition, closed ecosystems are ideal models, for the study of environmental issues and ecology and for the education of the general population in these areas. Small stable, materially closed ecosystems have been developed and can serve as the basis for more definitive and extensive work.

It is important that teachers, students, and the general public be made aware of the close connection between NASA's interests and objectives on life support of man in space, and the parallel problems of the maintenance and enhancement of a hospitable terrestrial environment for man. The development and availability of materially closed ecosystems, and particularly bioluminescent ones, as research and education tools could significantly enhance the general public's awareness of the importance of NASA's objectives and mission.

Using the limited resources available in a Phase I SBIR, we propose to assess the feasibility and practicality of developing bioluminescent closed ecosystems (BCES). In Phase II we would proceed to actually develop and study such ecosystems, and in Phase III to proceed with their commercialization. Protein Solutions, Inc. has already made a major commitment to bioluminescence as a tool and motivator for science education at all levels. It has the vision, the motivation, and the resources to guarantee the commercial development and dissemination of bioluminescent closed ecosystems.

Although closed ecospheres are commercially available from two firms [21,22], and are used for display and education purposes, they tend to be relatively static, and students and audiences rapidly loose interest. Closed ecosystems containing bioluminescent organisms allow direct observation of many of the processes occurring in the ecosystem, including

- food consumption, grazing, predation;
- predator avoidance;

• effects of mechanical disturbance or agitation; and possibly
• mating displays and sexual behavior.

Bioluminescent closed ecosystem are self illuminating, essentially self sensing systems, and should therefore be intrinsically interesting and dramatic to observe and to study.

Those who have observed and experienced bioluminescence generally never forget it. Fireflies on a dark summer night, glow worms or millipedes on a dark path, bioluminescent algae in a dark sheltered bay or on the surface of the sea, or the light emanating from disturbed marine crustaceans in tide pools on a dark night [1-5]. In our present age of intense artificial lighting, video games, and environmental problems, most of the population has not observed bioluminescence. When they first experience it they are generally amazed and almost awestruck -- it seems to be magic.

Bioluminescence is a relatively common and yet largely unknown phenomenon. It has developed and evolved many times. At least seven different independent systems are known and reasonably well characterized. The major functions of bioluminescence are for sexual displays/mating and for predator aversion in the dark. Its only major commercial or economic application at present is as a label or detection aid in diagnostic and clinical chemistry laboratories.

We have been studying and working with bioluminescence for several years. When we first discovered and observed it, we, too, became fascinated and "hooked." We have used every opportunity to demonstrate and explain the phenomenon to colleagues, co-workers, friends, family, and children. We have painted ourselves with it for the benefit of infants and preschoolers on dark nights, and we have demonstrated it at numerous dinners, cocktail parties and meetings, for its novelty and conversation enhancement characteristics. We have demonstrated and presented it to preschoolers and to high schoolers, to college undergrads and to engineering graduate students, always with the same effect: an intense interest, awe, and curiosity and a genuine desire to learn more.

Our long range aim is simply to develop and produce integrated science teaching materials based on bioluminescence.

Part 2: Phase I Technical Objectives & Work Plans

General Objectives

We propose to assess the feasibility of developing materially closed ecosystems in the one to five gallon range, consisting primarily of bioluminescent organisms, ranging from bioluminescence phytoplankton (dinoflagellates) to small bioluminescent shrimp. The focus in this Phase I application will be on marine ecosystems and on relatively small marine organisms. We expect the Phase I feasibility study to lead to a Phase II experimental and development effort. We then expect to move on to the development of marine ecosystems using larger bioluminescent organisms, including fish. We also expect to explore the feasibility of a fresh water closed bioluminescent ecosystem in a subsequent SBIR application.

We already have considerable experience with certain bioluminescent organisms.

Our general objective is to synthesize and combine the experience which already exists with the knowledge available on bioluminescent organisms to create a new and

innovative system for the study of ecology and life support processes, Bioluminescent Closed EcoSystems (BCES).

Specific Questions:

1) What is the optimum number of different organisms which will be required to develop and maintain a stable closed ecosystem? This is of course a complex and open ended question. The systems developed by Hansen and Folsome [14-16], and commercialized by Ecosphere Associates, in Tucson, Arizona, probably contain hundreds of different organisms [21], although only several of the larger organisms, particularly the shrimp, are visible to the naked eye. The fresh water Ecolarium, available from Aquarium World in Wilmington, North Carolina, also has a large diversity of species, but appears to be even less characterized than the marine system [22].

We propose at this stage in our research and knowledge to assess the feasibility of systems ranging from three to six different species. The primary producer would be a bioluminescent phytoplankton, possibly pyrocystis lunula, a hearty, easily maintained, and brightly bioluminescent organism [33-38]. An omnivorous copepod, probably metridia lucens, or a similar bioluminescent organism, should readily feed on the dinoflagellates [41-44]. As small shrimps are the most complex organisms which have been maintained to date in totally closed marine ecosystems [14,21], we will survey and then select bioluminescent shrimp as the third and most complex organism, possibly the Antarctic Krill [53], or an organism similar in terms of feeding behavior and bioluminescence.

In addition, we will investigate an omnivorous dinoflagellate (noctiluca miliaris), a large organism which is very brightly bioluminescent [33,34,46], an ostracod (vargula hilgindorfi), often called the Japanese Sea Firefly, which has a particular bright and intense bioluminescence [49,55,57], and a marine shoreline worm (pontodrilis bermudensis), which is also brightly bioluminescent [50,51].

Ideally, the more complex six species ecosystem would be the most desirable, because one should be able to observe interactions among all of the different organisms via their bioluminescence responses.

In addition, the ecosystem would contain a full complement of the marine bacteria required for a balanced marine environment, including those normally found in a balanced marine aquarium [48,58].

2) Can bioluminescent dinoflagellates serve as primary produces for BCES? The answer to this question is probably yes, but there are definite food preferences among the various zooplankton [28-32], and this will have to be thoroughly examined.

We have maintained pyrocystis lunula and pyrosystis noctiluca, another bioluminescent phytoplankton, in culture in our labs without difficulty [38]. They grow and multiply readily in artificial sea water preparations such as Instant Ocean, supplemented with the normal microalgae trace metal mix known as Medium F/2 [35], which is commercially available.

The bioluminescent phytoplankton we have studied have no known toxin production, and should therefore be suitable food sources for the zooplankton. We will attempt to avoid diatom production (diatioms are *not* bioluminescent) and competition by minimizing the use of silicate producing containers and by eliminating silicates from all media [35,48].

with respect to their optimum laboratory culture conditions, including temperature, media composition, pH, dissolved oxygen and CO_2 requirements, etc.

For example, the optimum conditions for the growth of dinoflagellates [33-35] are quite different from those found in a balanced marine aquarium [48,58]. Nevertheless, dinoflagellates obviously grow in the open ocean, and so a compromised set of initial conditions will be required.

We may also have to seed the ecosystem with a sacrificial organism to maintain life until the system settles down and stabilizes. One possibility would be to seed the system with brine shrimp (artemia)eggs, which readily hatch when exposed to sea water, and produce the highly motile nauplii. The carnivorous and omnivorous zooplankton should use this as a food source during the break-in period of the ecosystem.

In order to insure the health and well-being of the phytoplankton population, upon which all of the other organisms depend, we may attempt to design the system with an elevated CO_2 level initially. This should guarantee that the phytoplankton thrive and will perhaps hold down slightly the growth of the other organisms during the break-in period.

7) What are the optimum physical and chemical conditions required for a BCES? We certainly have to address the issue of volumes, areas, and their appropriate ratios. In the materially closed ecosystems which have already been developed, they tend to be one volume of air phase to one volume of water phase, in some cases with a soil or sand phase included. They have also tended to be relatively small. Ecosphere Associates sells systems ranging from 1/4 to 2 liters and as large as 100 liters for museum and exhibition applications [21].

Our analysis will be focused on volumes in the 1 to 4 liter range, and up to 20 liters if we feel it necessary. Our analysis will be concentrated on a system containing about 40% by volume air, 40% by volume water, and 20% by volume solid phase, probably a carbonate sand or gravel, as used by Folsome and co-workers [14-16,24-27].

Although there are some aesthetic advantages to a sphere geometry, we will also consider bottle and cubic shaped volumes.

8) How can BCES be monitored non-invasively? The primary monitor and sensor of BCES activity will of course be the bioluminescence itself. As we attempt to identify and select the optimum species for a BCES, one of the factors will be the wavelength or color of bioluminescence. Although most of the dinoflagellates and zooplankton bioluminesce in the blue, there are some organisms that bioluminesce in the green, and even other colors. It may be possible to optimize organism selection with a respect to a variety of colors.

The intensity and time course of bioluminescence will also help to identify the species present and the particular stimulus which led to the bioluminescence [5]. Generally, the bioluminescence is stimulated mechanically, either by force gradients in the water produced by swimming, wakes, direct collisions, or by feeding and grazing. It can of course also be produced for sexual display purposes. This would of course be desirable to incorporate in the BCES, such as by including vargula hilgindorfi.

One of our objectives is to assess how a closed BCES can be non-invasively monitored [15]. The CO_2 concentration in the air phase can be monitored by infrared absorbance, and the various chlorophyll contents in the organisms by the absorbance and

3) Which zooplankton normally feed on dinoflagellates and can therefore be the intermediate part of the food chain? The copepods are the most dominant marine zooplankton and tend to be omnivorous, feeding on diatoms and dinoflagellates, as well as on other plankton [28,32]. Many copepods are brightly bioluminescent and indeed they and related zooplankton can be major sources of bioluminescence of the sea [39,40].

Metridia lucens and metridia longa are available on the East Coast, and are brightly bioluminescent [42,45]. Although omnivorous, they do prefer animal food over phytoplankton if they have their choice. Many copepods are readily maintained in laboratory culture and in synthetic sea water environments [48,43,44].

Noctiluca miliaris, classified normally as a dinoflagellate, is also readily cultured in the laboratory [33,34,46,48]. It has a specific gravity normally less than that of sea water and tends to float at the surface.

Vargula hilgindorfi, an ostracod crustacean, appears to be much more difficult to rear in laboratory culture, as it tends to eat its young (J. Morin, personal communication). If there is an adequate supply of food available from other organisms, it is possible that cypridina could be a stable component in a closed steady state ecosystem.

4) Which of the more complex and larger marine bioluminescent organisms will be suitable for the BCES? We have already suggested that small euphausiid shrimp may be the most suitable for this application. Shrimp are the most complex animals that have been successfully maintained in closed ecosystems -- for periods of 5 years or more [14,15,21]. Many of these organisms are bioluminescent [5]. They have complex photophors (light organs), in many instances these photophors can actually be rotated, probably to maintain an appropriate level of counter illumination for camouflage purposes. They can produce bioluminescence for long periods or times, more or less continuously, as well as produce shorter flashes. Some of the shrimp are so transparent that their internal structures are visible, and are therefore particularly interesting to observe and study [3,5,54,55].

Antarctic Krill are shrimp of this type [53], and are bioluminescent, although it is not clear if they can be maintained in the laboratory under the conditions required for the other organisms in the BCES.

We are not nearly as familiar with the shrimp organisms as we are with the others. A major effort of the project will be to learn what we can about bioluminescent shrimp and other higher order organisms in order to select those which would be most appropriate for a BCES.

We may also look at small squid, which have a rich variety of means of producing bioluminescence [5,52,57].

5) Which marine worm species may be most suitable for a BCES? The marine littoral earthworm pontodrilis bermudensis, an oligochaete, is readily collected and maintained in the laboratory in artificial sea water. It is not known whether it can be maintained in culture for long periods, nor if it will be suitable as part of a closed ecosystem [50,51].

6) What are the particular problems involved in multi-species cultures in closed ecosystems? This is a very difficult question,and has not been widely addressed in the literature, although there is some information available in the marine ecology and the marine aquaculture literature [47,48]. After thoroughly digesting that literature, our approach to the problem will begin by tabulating the various organisms which we have initially selected

fluorescence spectra of the various chlorophylls present (see also discussion under Budget Explanation).

9) How can the bioluminescence be externally stimulated? The main stimulant for bioluminescence is mechanical. Therefore, a slight tapping on the wall of the BCES will elicit dinoflagellate bioluminescence and probably that of most of the other organisms as well.

A rotation of the container so as to move the water surface will certainly result in bioluminescence. It may be necessary to design the container and the system so as to permit a periodic movement of of the water, such as a simple simulation of waves and tides. This would be done with a simple 12 hour rotation or movement cycle to possibly simulate daily tides [48]. This may be particularly important for the marine worm species.

10) How long can a BCES be sustained? This is a very difficult question. The existing commercial marine ecospheres tend to be stable for 5 years or more, which includes the shrimp population [14,21]. Although the numbers of shrimp are relatively small, they do go through normal life cycles of reproduction and death in the closed ecosystem. We see no reason why this cannot be accomplished in a BCES as well.

Our long range goal is to develop BCES which will be stable for multiple years.

Summary

It is important to emphasize that our major objective is to assess the feasibility of a BCES. This will be done primarily by extensive literature work and extensive discussion with experts. The actual experimental work to be done in this Phase I is relatively limited. Most of the experimental work will be reserved for the Phase II part of the project.

Part 3: Recent Developments

There has been considerable interest in the development of closed life-support systems for man in space [6-9,17]. Perhaps the most ambitious experiment along these lines is the Biosphere 2 Project in Arizona, in which a number of men and women are being housed and sustained in a completely materially closed ecosystem [9].

Our approach is obviously much more modest, and is directed mainly towards the development of an innovative and important tool for the study of closed ecosystems. In addition, this tool will enable the general population, students, and teachers to directly experience and observe a closed ecosystem community, leading to an enhanced awareness and understanding of ecosystems.

We are leaning very heavily on three sources of information:

one, the existing closed ecosystem literature [6-27];
two, the extensive literature on bioluminescent organisms [1-5, 55-57]; and
three, our own experience with the culture of bioluminescent organisms in the laboratory.

To date our work has focused on bioluminescent dinoflagellates and particularly pyrocystis lunula. We have large cultures of this organism in our laboratory ranging from 100 milliliters to 5 gallons, in a variety of artificial and natural supplemented sea water media. We have grown it under a wide range of light conditions; it has maintained itself in a completely closed environment for up to six months, and in environments open to air for

over a year. These cultures are known to have been maintained for as much as 30 years in laboratory environments.

We have interacted and communicated with a large number of experts on bioluminescent dinoflagellates and their culture, including

W.G. Hastings, Harvard University (Boston, Massachusetts) [5],
Robert Guillard, Provasoli-Guillard Center for the Culture of Marine Phytoplankton (W. Boothbay Harbor, Maine) [35],
Barbara Prezellin, University of California, Santa Barbara (California) [36],
Donald Anderson, Woods Hole Oceanographic Institute (Massachusetts) , and
Larry Brand, University of Miami, Oceanography School (Florida).

In addition to our ongoing work on bioluminescent dinoflagellates, we are in the process of developing a simple educational product titled NIGHT COLONY, which consists of a single culture of pyrocystis lunula for student experimentation and observation. This is also the basis of a current SBIR Phase I application to the National Science Foundation.

We have begun to investigate bioluminescent earthworms; that is the basis of another Phase I application to NSF called LIGHT CRAWLERS. Here we have relied very heavily upon our discussions with Dr. John Wampler (University of Georgia, Athens), an expert on bioluminescent worms [50,51]. We are beginning a worm culture and growth program in our facility.

We have established a number of small marine aquaria in order to obtain experience with a marine microcosm with multiple species.

We have had a particular interest in the vargula hilgendorfi organism because of its particularly bright bioluminescence, and also because the dried organism can produce a very bright bioluminescence from its ground up powder when wetted with water. This has been a very impressive demonstration of bioluminescence which we have used with school children, teachers, and potential investors. Dr. James Morin, at the University of California, Los Angeles, has kindly provided us with considerable information about the vargula species, which he has studied in Southern California and in the Caribbean.

We have also experimented briefly with bioluminescent fungi and bacteria, although we have no plans at present to incorporate these classes of organisms in the BCES.

Part 4: References and Bibliography

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   K.H. Nealson and C. Arnesan, "Marine Bioluminescence: About to See the Light," *Oceanus* 28(3)(1985)13.
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Part 5: Relation to Phase II

Why Phase II? This is such a complex problem involving so many disciplines that we feel that most of the Phase I effort needs to be a paper feasibility study. We simply must talk with experts in all areas of biology to learn of requirements for these various types and classes of organisms before we can rationally design a BCES.

The results of the Phase I project will be a set of recommendations with which to initiate the experimental work on BCES. It is that experimental work which would be the basis of the Phase II application. By that time Protein Solutions, Inc. expects to be well underway with its development of dinoflagellates and bioluminescent worms in individual cultures and will be in a position to consolidate that experience together with the information and experience gained during this phase I study.

The Phase II objectives will be to produce stable, readily observable, bioluminescent closed ecosystems for research and education purposes. It is expected that prototypes will be demonstrated and available at the conclusion of Phase II, and that in Phase III we will move into commercial production, marketing, and sales.

Possible NASA applications of these closed ecosystems include:

1) demonstration to the mass media and to the political community of the concept of materially closed ecosystems and how they can be developed to sustain man and other organisms in space environments.

2) NASA's goals in improving science education in the United States can be enhanced by the availability of such closed ecosystems in classrooms, science centers and museums, and even in homes throughout the country. Protein Solutions, Inc. is interested in developing various educational modules which expand the closed ecosystem concept to NASA's interests in manned space exploration and habitation.

Part 6: 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.

Another market is 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 $2B/year.

Yet 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. A joint proposal to NSF's Informal Science Education Program will be submitted in February, 1992.

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

It is expected that PSI's initial bioluminescence products will retail in the price range from roughly $15 to $100, with significant volume discounts for teachers and school districts. A bioluminescent closed ecosystem is expected to sell for $150 and up, depending on size and complexity.

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.

Initial test marketing of all of these products will be in local specialty shops. 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.

Part 7: The Company: Protein Solutions, Inc. (PSI)

PSI is a biotechnology firm developing unique engineering and consumer products based on biology and biotechnology. PSI's goal is to develop a family of science education materials, using proteins as key components. PSI is now developing products for education, toy, and pet markets. PSI expects to develop strategic partnerships with larger, more established firms with marketing expertise in its product areas.

PSI was founded in January 1988 by Joe Andrade, Peter Gerity, and James McRea. Initial funding for PSI has been provided by its founders via personal stock purchases. The company has contracted with the University of Utah for fundamental research and development studies in bioluminescence. The company is privately held. The major stockholders are the three directors and the University of Utah Research Foundation.

PSI's officers and directors feel that the '90s will be a decade of increased emphasis on education and that there will be a growing demand for products in these areas.

PSI has chosen to focus on *bioluminescence* as the first of its core technologies, and to develop a set of products for the educational, consumer products, and novelty markets.

A wide range of animals and certain plants have the ability to produce light. Light produced by natural organisms is called bioluminescence. It is not fluorescence. It is not phosphorescence. It does not need any external source of light or energy. The product is light -- natural, colorful -- without bulbs, batteries, wires, or other light sources -- unencumbered, portable, simple.

PSI can produce light on demand -- for a wide variety of unique, innovative products. Our materials are obtained entirely from biological sources; they are nontoxic and biodegradable.

PSI will perform research and development in house, advance the core technology, develop product prototypes, and test market. After product feasibility and marketing potential has been conclusively demonstrated, PSI will manufacture and distribute the products, probably in cooperation with larger firms with the appropriate sales and marketing expertise.

PSI is beginning with bioluminescence; the company then expects to move on to other critical research and development arenas, in each case identifying and developing a set of products and markets which grow significantly with time in overall complexity, cost, market size and revenue potential.

By developing and maintaining the *core technologies and key personnel within PSI*, the company has the opportunity to combine, synthesize, and employ the varied technologies into new, unique, and much larger market arenas. By this strategy PSI expects to have a very significant growth rate and to develop one or more key new product areas every 2 to 3 years.

PSI's products will be completely unique, and will have little or no direct competition. Many of the technologies are patentable. The products will be targeted to existing markets and to an existing customer base.

PSI is closely involved with the University of Utah, is a member of the University Center for Biopolymers at Interfaces, an industrial consortion through which PSI has funded fundamental research dealing with the bioluminescent enzyme, firefly luciferase. Through this Center PSI has access to a variety of analytical and research facilities at the University on a fee for service basis (please see letter on next page).

PSI is also a participant in the University's newly formed Center for Integrated Science Education (CISE), a group of faculty interested in producing courses, curricula, and experimental kits and materials to permit science to be taught in a fully integrated basis, rather than in the disciplinary approach so common today. CISE is working closely with the various school districts and the State of Utah Department of Education. The Center is also working very closely with the Graduate School of Education, in the development of courses and other mechanisms by which elementary teacher candidates can obtain a strong grasp of scientific concepts and principles and thereby encourage their students to learn science by concept, by discovery, and by experiment. PSI's bioluminescent products and materials are already being utilized by CISE.

Protein Solutions, Inc.'s laboratories are located in the University of Utah Research Park, 390 Wakara Way. It has 1,125 square feet of space of mixed office and laboratory character, which will be increased by early next year to 2,500 square feet, primarily additional laboratory space. It has a three year lease on that space which goes through July of 1994. Existing facilities are adequate for the preliminary experiments required for this Phase I application and for the more extensive experiments planned for Phase II.

Part 8: Personnel

Joseph D. Andrade, Ph.D. Professor, Department of Bioengineering and Department of Materials Science, University of Utah, and President of Protein Solutions, Inc., has been studying bioluminescence for over 3 years, primarily for science motivation and education. 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)

Joe is best known for his work on biomaterials and biocompatibility, including the behavior of proteins at interfaces. He has also worked extensively in the area of biosensors, particularly fluorescence based immunosensors. In recent years he has become very interested and involved in the issue of integrated science education, the use of bioluminescence as a motivational tool and a vehicle for integrated science education and research. He is also involved in using general biological and ecological principles and concepts as a means to teach science in an integrated, comprehensive fashion. He feels strongly that bioluminescence is an ideal tool for such purposes (see Figure, page 18).

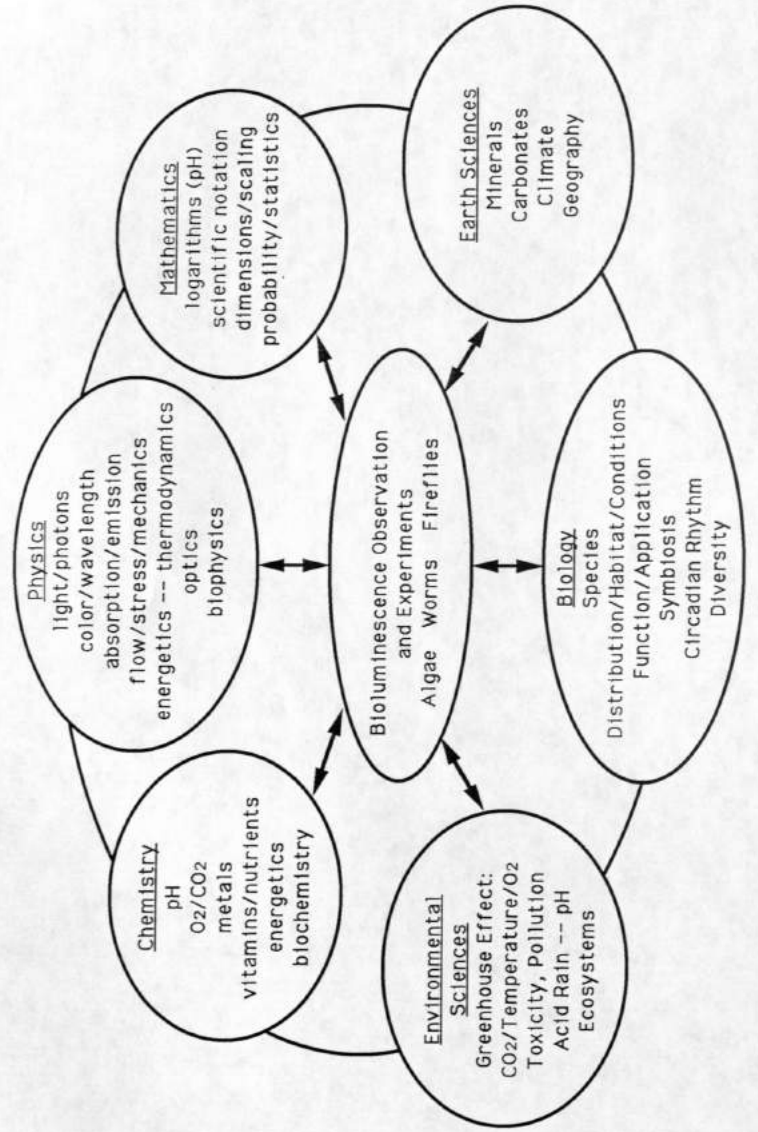

Figure: Bioluminescent organisms and their observation are shown as the center of an integrated science "wheel". Each of the classical specialties or disciplines are indicated with selected subject examples. These subjects and topics can all be directly observed and experimentally studied via bioluminescence.

Over the past 25 years of his scientific and engineering career he has authored over 100 peer reviewed papers, has edited five books, and holds five U.S. patents. His management experience in the University includes the Chairmanship of the Department of Bioengineering (two three year terms), and Dean of the College of Engineering (four years, 1983-1987). His industrial experience includes being one of the co-founders of a Salt Lake City corporation which is now part of Ohmeda, a division of BOC (British Oxygen Corporation). Ohmeda employs about 70 people in Salt Lake City and is a successful and growing corporation. He founded Protein Solutions, Inc. three years ago.

During the course of this Phase I project Joe will be employed less than 50% time with the University of Utah (see letter on page 23), and will be devoting a major portion of his time and activities to Protein Solutions, Inc.'s bioluminescence grants, contracts, and its related activities.

Part 9: Subcontracts and Consultants

No subcontracts. One consultant -- please refer to Budget Explanation page.

Part 10: Related Proposals and Awards

PSI recently (June 17, 1991) submitted two Phase I SBIR Applications to the National Science Foundation, Topic 26: Education and Human Resources:

NIGHT COLONY: Science in the Dark, and

LIGHT CRAWLERS: Bioluminescnece-Based Discoveries for Science Education.

These applications involve single organism cultures and products based on dinoflagellates (Night Colony) and terrestrial earthworms (Light Crawlers). There is no overlap with this NASA SBIR application.

PSI also expects to submit a Phase I SBIR to the Department of Agriculture (August, 1991) in the area of sensors for aquaculture systems.

Part 11: Previous NASA SBIRs -- None.

Budget Explanation

Office and Library Supplies refers to normal paper, postage, computer disks, etc. Photocopying and inter-library loans are self-explanatory.

General lab supplies include containers, media, chemicals, pipets, and related materials for cell and organism culture and maintenance.

Consultant services are for Dr. John Shaffer, a former student of Clair Folsome, and one of the few individuals with considerable experience in totally closed ecosystems (see Ref.s 14, 16). Dr. Schaffer now resides in Huntington Bay, New York. His letter of interest and collaboration is on page 24.

Analytical services are for water and solution testing for analysis of container surfaces, and for monitoring of air gas concentrations and solution dissolved gas levels.

Information retrieval services are for computer based searching using the DIALOG information retrieval system.

Personnel (6 months, 26 weeks):

Person:	Hours/Wk:	Rate:	Fringe (~35%):	Total hourly cost:	Total Amount:
Andrade, P.I.	8	34.00	11.90	45.90	9,547
Tobler, Technician	40	7.40	2.59	9.99	10,389
Students (2)	20	7.00	--	7.00	7,280
Secretary/Library	15	8.00	--	8.00	3,120
				Total:	30,336

J. Andrade will be fully responsible for the project, will supervise the other personnel, and will do the library research, preliminary testing, and perform the feasibility study and analysis (see also page 23).

John Tobler, a biology technician, will aid in all parts of the study, and will be particularly involved in the experimental aspect.

Two graduate students, probably from the University of Utah, will participate and perform various parts of the analysis. One of the graduate students will be from the Bioengineering program and will focus on the non-invasive sensing feasibility study. He/she will examine methods for analyzing gas concentrations non-invasively, particularly infrared absorbance. Using the facilities and resources of the Center for Biopolymers at Interfaces on a fee for service basis the student will examine the feasibility of assessing the solution phase of the BCES using absorbance and fluorescence measurements. Not only can chlorphyl be assessed in this way, but since the various organisms have different types of luciferins, which are generally fluorescent, by the judicious use of luciferin and chlorophyl absorbance and fluorescence spectra, we expect to be able to obtain considerable analytical information on individual organism concentrations.

The second graduate student will probably come from the biology program and will work closely with J. Andrade in assessing the species diversity requirements of the project. A library assistant/secretary is also budgeted to assist with library work, reports, correspondence, etc.

Total personnel charges, including fringe benefits, are $30,336. No other direct costs are budgeted.

Small items of equipment, which are needed for this project, are either already available, or will be purchased from corporate funds. Travel necessary for the conduct of the project will also be covered from other corporate funds.

Overhead is 25% of total direct costs, or about $10,000. These funds are primarily for corporate administrative offices, space lease, and the services of an office manager.

Jonathon Shaffer
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n;(516)385-7176 or 385-7628.
FAX: 516-567-1025.

May 2 ,1991

Joe Andrade
College of Engineering
Univ. of Utah
Salt Lake City

Dear Dr. Andrade:

I'd be pleased to accept your offer to be listed as a consultant on your proposals regarding closed ecosystems.

Also I appreciate your consideration in passing my resume to your colleagues.

As a pioneer in CES I'm excited with their future applications. Ecological principles must be disseminated eventually to the general public on a level more relevant than the foxes versus the rabbits, or the beer can on the highway. CES are a very graphic (accurate) representation of the earth and many of its biogeochemical cycles.

I look forward to meet with you one day, good-luck in all endeavors. I will forward portions of my dissertation and publications shortly.

Sincerely,

Jon Shaffer

THE UNIVERSITY OF UTAH

August 1, 1991

To Whom It May Concern:

J.D. Andrade, Professor of Bioengineering and Materials Science and Engineering at the University of Utah, is employed 64% of full time by the University of Utah. In addition, Dr. Andrade is on sabbatical leave from September, 1991 through August, 1992, so his time is flexible. The activities he has proposed in this SBIR are consistent with his sabbatical leave goals.

As University of Utah policy is to pay only 80% of the state salary during a sabbatical, his full time percentage effort during the '91-'92 year is only 52%.

It is our understanding that his commitment and responsibilities with the University of Utah will be reduced to 50% or lower during the course of the SBIR project, to enable him to spend 50% or more time with Protein Solutions, Inc.

We are in support of his plans.

Sincerely,

R. Normann, Acting Chair
Department of Bioengineering

R. Boyd, Chair
Department of Materials Science and Engineering

D. Pershing, Dean
College of Engineering

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