National Science Foundation
Small Business Innovation Research Program

PROJECT SUMMARY

NSF AWARD NO.
9161158

NAME OF FIRM
Protein Solutions, Inc.

ADDRESS
390 Wakara Way #65
Salt Lake City, UT 84108

PRINCIPAL INVESTIGATORS (NAME AND TITLE)
S. Winters, V.P. Research and Development

TITLE OF PROJECT
Bioluminescence Based Discovery Materials for Science Education

TOPIC TITLE
Education/Human Resources

TOPIC NUMBER
26

TECHNICAL ABSTRACT (LIMIT TO 200 WORDS)

This project proposes to develop hands on integrated science materials based on the phenomenon of bioluminescence. Specifically, we propose to develop the science and technology necessary to develop novel teaching aids and the curricula for use with these aids to enhance the exploration and discovery of basic concepts in various scientific fields in a totally integrated, inquiry and hands-on approach.

In Phase I of this project, we demonstrated the feasibility of maintaining bioluminescent organisms in the laboratory, stimulation of these organisms to glow "on command" and basic environmental requirements. During Phase II, we propose to evaluate a much expanded candidate list of bioluminescent organisms and to develop the technology necessary for organism reproduction in the laboratory. In addition, Phase II efforts will involve development of stimulation methods of these organisms appropriate for use as teaching aids. Emphasis will be on development of observation and hands on experiments appropriate for use in integrated curricula for grades K-12.

KEY WORDS TO IDENTIFY RESEARCH OR TECHNOLOGY (8 MAXIMUM)
Bioluminescence, Integrated Science, Discovery, Stimulation

POTENTIAL COMMERCIAL APPLICATIONS OF THE RESEARCH
Science Education Kits
Children's Toys and Novelties
Teacher Education Materials and Kits
Workshops and Inservices

Table of Contents

A.	Cover Page (NSF 1207A -- Phase II)	1
B.	Project Summary (NSF 91-20)	2
	Table of Contents	3
C.	Identification and Significance of the Opportunities	4
D.	Background	6
E.	Phase I Research	7
	1. Summary or Phase I Objectives	7
	2. Background	7
	3. Results	7
	4. Conclusion	9
F.	Phase II Research Plan	10
	1. Objectives	10
	2. Year 1: Studies of Individual Organisms	10
	a. Dinoflagellates	11
	b. Bacteria	11
	c. Fungi	11
	d. Annelids	11
	e. Millipedes	12
	f. Mollusca	12
	g. Cnidaria	12
	h. Ctenophora	12
	i. Crustacea	13
	j. Fish	13
	3. Methods:	13
	4. Year 2: Ecosystems	15
G.	Expected Results	16
H.	Summary	17
I.	References	17
J.	Personnel and Consultants	20
K.	Facilities and Equipment	21
L.	Current and Pending Support	21
M.	Commercial Potential -- Phase III	22
N.	Budget and Justification	22
Appendix A: Support letters and vitae		25

C. Identification and Significance of the Opportunity

Protein Solutions, Inc. (PSI) is researching and developing innovative educational products which use the phenomenon of bioluminescence (1-4, 6, 11, 12) to motivate, entertain, and educate children.

The problems of our current science curricula are outlined in *Project 2061: Science for All Americans* (5). 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"(5). PSI is addressing this critical need by developing materials, "toys", discovery aids, and new curricula supplements 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, minimizing 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 is 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, junior 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 their 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 and their parents. Bioluminescence provides an excellent introduction to the interplay of biology, chemistry, physics, and environmental science (Figure 1).

A major objective of this Phase II effort is to assess and develop cultures of bioluminescent organisms, both single species and multiple species, in small ecosystems. Cultures of single species provide the opportunity for relatively simple, novel teaching aids, science kits, and related tools for integrated science discovery. Potential commercial products based on single organisms include LIGHT CRAWLERS, studied in Phase I, LIGHT TUBES, bioluminescent marine worms in transparent tubes, also

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.

researched in Phase I, NIGHT-LIFE, PSI's bioluminescent plankton, and other novel and exciting ideas based on bioluminescent organisms.

Ecosystems are important education, research and development tools (41-43) for the eventual construction and application of closed life support systems such as Biosphere II. Initial ecosystem development efforts will 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 EcoSystems (BES). Such systems would be dramatic tools for the study of the interactions among organisms. BES would serve as dramatic demonstrations of ecology, ecosystems, and partially closed life support environments.

Another opportunity for application of this technology exists in the development of totally bioluminescent displays for public aquaria. The Monterey Bay Aquarium in California and the Oregon Coast Aquarium in Newport, Oregon, have outstanding exhibits of species which have bioluminescent relatives, but few museums or aquaria have developed exhibits with specific attention to bioluminescence(41). Consider the impact of a huge tank of bioluminescent organisms, each species communicating with others of its own kind and warding off predators of other species with brilliant flashes of blue, green or yellow light! The Mystic Marine Life Aquarium in Mystic, Conn., learning of our expertise in bioluminescence and science education, has already expressed significant interest in developing such an exhibit (27).

PSI's primary objective is education. We expect that products based on the research and technology proposed here will lead to enhanced science education. Successful development of this technology will provide a new, exciting introduction to the joy of discovery and exploration, and the impetus for developing questions and hypotheses. Educational reform to integrate the various divisions of science is now recognized as a critical step which must be taken if the U.S. is to compete with the advances in other developed nations (10). Bioluminescence based products provide excellent introductions to the interplay of biology, chemistry, physics, geology, and environmental sciences (Figure 1).

D. Background

Flashing fireflies on summer evenings, glowing ocean surf, and other forms of natural bioluminescence have always been a target of curiosity. Ancient scientists studied bioluminescence, but it was not until the 1670's the English chemist Robert Boyle described some of its fundamental properties. Although investigated for many years, scientists have been slow to exploit bioluminescence in the laboratory. Now scientists are using bioluminescence to study gene expression and developmental biology. Other applications in biology, medicine and agriculture are now being developed.

Bioluminescence is the light produced by certain plants and animals (1-4, 6). It is not only fascinating to observe, but can be packaged and discovered so as to entertain a child while teaching her/him basic principles of biology, chemistry and physics. Luminous species are widely scattered taxonomically with no clear cut pattern discernible(44). Many luminous shrimps are known but no luminous crab. Many luminous squid are known but only a single bioluminescent octopus. Bioluminescent centipedes and millipedes are not uncommon (38, 39). Almost half of the animal phyla contain bioluminescent forms (44). The majority of the luminous organisms are marine (3, 6, 16, 17, 44, 45).

Among crustaceans, bioluminescence is especially abundant in the copepoda, shrimps and ostracods. Luminous copepoda are widely distributed throughout the waters of the world. Some are surface dwellers, while others live in the deep sea (45).

To our knowledge, there are no other commercial efforts toward the development of stable laboratory cultures of bioluminescent organisms. Investigators involved in bioluminescent research generally do not maintain cultures of these organisms for more than a few months and rarely have attempted organism reproduction through complete life cycles. There are no known educational products based on the phenomenon. There are few exhibits in public museums or aquaria demonstrating bioluminescence (41). From our experience, there are few other phenomena which can excite, motivate and interest children and adults alike.

E. Phase I Research

1. Summary of Phase I Objectives

The overall objective was to identify, select and maintain bioluminescent organisms appropriate for use in integrated science educational materials using bioluminescence as the discovery and observation vehicle. Specifically,

1. Identify, select and collect bioluminescent earthworm, marine worm, and other marine species;
2. Establish the feasibility of long term stable populations; and
3. Assess stimulation methods (chemical, acoustical, electrical and/or mechanical) for producing the bioluminescent response.

2. Background

PSI proposed the development of "Light Crawlers", an analog to the well known Ant Farm®, but using terrestrial bioluminescent earthworms. Several species of bioluminescent earthworms are found in many parts of the world, several in the United States (18). The use of bioluminescent earthworms enhances the excitement children develop upon exploration, motivating them for more in depth discovery and exploration.

Bioluminescence in earthworms has long been known; references to this property go back to 1670. Perhaps the best known case of luminescence in the Oligochaetae is that of *Microscolex phosphorous*, a worm whose original home is probably in South America but which has been carried to many parts of the world and has established itself in the Unites States (7). Greenish yellow light appears when the worms are stimulated. The light is given off by a luminous slime which usually exudes from the anal aperture and sometimes from the mouth. The dried slime can be made luminous again by the addition of water (7, 18).

3. Results

Due to severe drought conditions in areas where terrestrial earth worms are found, resulting in unsuccessful collecting attempts, we directed our activities to bioluminescent marine organisms. Two species of marine worms, *Pontodrillus bermudensis (Oligochaetae)* and *Chaetopterus variopedatus (Polychaetae)* and the sea pansy, *Renilla kollikeri (Anthazoa)* were acquired (7, 9, 18, 25). These organisms emit a brilliant luminescent glow in response to mechanical stimulation. The appropriateness of each of these species for use as a teaching aid in terms of the ease of maintenance and

reproduction, and methods of stimulation of the bioluminescence display were investigated.

Pontodrillus bermudensis is a marine littoral worm 5-7 mm long and only 2-3 mm in diameter, very pale pink in color and fast moving. *P. bermudensis* is available from marine specimen suppliers on the east and west coasts. Two collections of *P. bermudensis* were obtained: one from Gulf Marine Specimens (Panacea, FL.) and one collected by the principal investigator in Shell Harbor, FL. *P. bermudensis* is most commonly found in the intertidal zone in areas containing large amounts of turtle grass. Searches in similar locations but vegetated with *Spartina* had no worms. The worms seem to prefer coarse calcareous bottom material and are often found in groups rather than singly. The worms were shipped to the laboratory within the sand in which they were dug in Styrofoam containers. Upon opening, most of the worms were found in the bottom of the containers in the denser, wetter and more anaerobic portions of the sand. In addition, turtle grass was shipped with the worms.

Results of the light experiments indicate that *P. bermudensis* prefers to remain in darkness, as expected. Although reports from investigators familiar with *P. bermudensis* suggested that they tolerate dry conditions better that wet (8), all farms in which tide lines were simulated with gravel and sand levels behaved differently. Although burrows were observed leading to the surface of the sand above the water line, where turtle grass was available, 80% of all observations showed the worms completely submerged in sea water. The worms appeared healthy and quickly moved to the interior of the tank when the construction paper was lifted from the sides of the farm, exposing the worms to light.

Food preference experiments indicate that *P. bermudensis* prefers turtle grass to all other options offered and, in the absence of turtle grass, no distinctions could be found. It was unclear if the worms were eating any of the other alternative foods. Although burrows were observed to lead up to the food, changes in the amount of food on the surface were undetectable.

Both the stock farms and the experimental study farms of *P. burmudensis* remained viable for approximately 3 months. Stimulation experiments with *P. bermudensis* were difficult. Due to the low level of intensity and the difficulty in stimulating the bioluminescent response, *Pontodrillus bermudensis* was eliminated as a candidate for further study.

Chaetopterus variopedatus, a marine polychaete known as the parchment worm, is a white, tube dwelling worm consisting of three greatly dissimilar regions: a flattened anterior region of eleven segments, a middle region with wing-like projections and containing a cup organ and large fan, and a less differentiated posterior region (9, 14). Specimens were obtained from Pacific Biomarine Laboratories (Englewood, CA) and shipped in chilled polyethylene bags with ample salt water and sandy mud.

The *Chaetopterus* were obtained later in the Phase I study -- the tank was only in operation for approximately 4 weeks. Observations include the ability of the worm to perform "U" turns in the glass tubes.

A luminescence response to light tapping on the side of the glass tube has been observed. The blue-green glow was visible around the peristomial tentacles, the notopodia, cup organ and fans with moderately dark-adapted eyes. A holding chamber was built to confine the worm in order to photograph it with a highly sensitive video camera system. *Chaetopterus* has a free swimming larval stage which is also capable of

producing light. With the installation of the algal turf scrubber filtration system, we are now observing the larvae.

Specimens of the sea pansy, *Renilla kollikeri (Anthazoa)* (25, 26) were purchased from Pacific Biomarine Laboratories and shipped overnight with adequate sea water in chilled, Styrofoam containers. *Renilla* are relatively warm water organisms found on intertidal sand and mud flats extending down to 20 meters. It has a heart shaped disk or rachis approximately 5 cm in diameter which bears polyps. From the underside a fleshy purple peduncle extends downward to the substrate.

Movement of the animal is accomplished by wavelike motions of the rachis propelling the animal over the sand substrate. Animals were observed moving on a daily basis but tended not to travel too far from the other *Renilla* in the tank. Several *Renilla* were generally clustering around the airstone and air tubes inferring that the water circulation methods for delivering oxygen rich water to the bottom of the tank were ineffective. Since the change in water flow to accommodate bidirectional flow and removal of the reverse gravel filter, the *Renilla* were healthy and responsive.

Stimulation of *Renilla* by tactile means resulted in the expected and spectacular response. The bioluminescence is intense and may be observed with mildly dark adapted eyes. Luminescence is confined to the upper surface of the rachis. The polyps are immediately withdrawn in response to mechanical stimulation followed by contraction of the entire body. Following tactile stimulation, luminescent waves arise in the affected region and run over the animal. Individual waves may be discerned and succeed each other rapidly at first but soon decrease in intensity and frequency. Repeated stimulation of the autozooids results in a long lived glow. Herring has studied the nervous control and propagation of the light waves and found that the waves may cancel each other out (26).

An additional bioluminescent organism investigated is the marine dinoflagellate *Pyrocystis lunula*, a single celled phytoplankton approximately 250 microns long and commercially available from biological specimen suppliers (17, 33).

4. Conclusions

While the extended drought in the Southeast limited our pursuit of terrestrial worms, we have developed considerable expertise in the maintenance of several species of bioluminescent marine organisms.

The marine environment is very rich in bioluminescent organisms and permits the development of small scale ecosystems for science education and public display purposes.

The technology developed during this Phase I feasibility effort is highly encouraging and demonstrates the feasibility of using bioluminescence for integrated science education. The luminescent displays are awe inspiring and provide excellent opportunities for the development of science educational displays and demonstrations. We have received a great deal of interest from teachers and from national aquaria interested in our marine bioluminescence experience (27). With the national recognition of the current crisis in science education and the need for motivational materials, it is apparent that bioluminescent education products developed from this technology have an excellent future.

F. Phase II Research Plan

1. Objectives

We propose to develop integrated science education materials using bioluminescence as the "discovery" and observation vehicle. The technology required for the development of these materials requires research and development efforts to determine the appropriate species and selection of individuals for the highest degree of luminescence while minimizing difficulty in maintenance of the organism. We further propose to study means to stimulate the bioluminescence.

A major objective of this Phase II effort is to assess and develop cultures of single species and multiple species in partially closed ecosystems utilizing bioluminescent organisms. Cultures of single species provide the opportunity for relatively simple, novel teaching aids, science kits, and related tools for integrated science discovery.

The Phase II effort has the following objectives:

1. Identification and selection of bioluminescent organisms for use in single and multiple organism cultures based on size, ease of maintenance, food requirements and specific needs;
2. Optimization of culture media/substrate material for the establishment of long term stable cultures;
3. Development of stimulation methods (chemical, acoustical, electrical or mechanical for maximum intensity and sustained display;
4. Assess the feasibility of developing ecosystems of multiple organisms for long term survival

Although Phase I of this project focused mainly on bioluminescent annelids to demonstrate feasibility, we propose to expand the work to other classes of organisms which may be appropriate for use in science educational products. Our general objective is to synthesize and combine our existing experience with the knowledge available on bioluminescent organisms and small scale ecosystems to create new and innovative systems for the study of chemistry, physics, ecology, earth, and environmental sciences (Figure 1).

Note: PSI does not plan to routinely collect or to use organisms collected from the natural environment. Only organisms which we can culture, maintain, and reproduce in the laboratory will be considered for our science educational products and services.

2. Year 1: Studies of Individual Organisms

The science involved in the development of bioluminescent educational materials requires efforts in the culture, growth, and handling of the bioluminescent organisms -- in supplemented sea water for marine organisms and appropriate organic substrates for terrestrials -- and means to stimulate the bioluminescence by physical or chemical methods. Growth of the organisms to large densities requires optimization of culture conditions, including light cycles, composition of the medium, temperature, salinity, and nutrient requirements.

Initial efforts will involve identification and selection of appropriate species. There are bioluminescent species in nearly half of all major phyla (44). Some phyla have received more than their fair share of luminous species such as the Ctenophora. Many of

the Cnidaria are luminescent, whereas only one luminous species is known in the phylum Nemertinea. Chordate representatives are evidenced by a number of fish which either possess photophores themselves or light organs containing symbiotic luminous bacteria. Obviously, from such a large selection, only a few species can be investigated. We already have considerable experience with some luminous species. Those and other candidates are discussed below.

a. Dinoflagellates

Dinoflagellates are single celled protozoa. Luminous species are responsible for the " phosphorescence" of the ocean. The light emitted when the water is disturbed is characteristically emitted as a rapid flash which lasts only a fraction of a second (17). Individual cells, depending on the species, will flash repeatedly with repeated stimulation.

PSI has already invested about $60,000 in preliminary research on bioluminescent dinoflagellates for science education and as food sources for the marine organisms used in our Phase I studies. In these preliminary studies, we have demonstrated that *Pyrocystis lunula* can be cultured and maintained in non-laboratory environments (34) and that cultures can be maintained up to 6 months in totally sealed containers, with no oxygen or carbon dioxide exchange. We have demonstrated successful alteration of the light/dark circadian rhythms of these organisms. These materials are already being used by the University of Utah's Center for Integrated Science Education for pre-service and in-service teacher education. The dinoflagellate studies are the subject of a pending Phase I proposal submitted to NSF to further develop our understanding of these organisms (LUNULA COLONY Phase I SBIR -- submitted June, 1992).

b. Bacteria

Luminescent bacteria occur throughout the oceans: a one liter sample of sea water will contain some luminous bacteria, and may contain hundreds. They also occur as symbionts; some species are held within special light organs of certain fish and squid serving to provide luminescence for the host. Some of these same bacteria and others occur as intestinal and parasitic symbionts and may be important in the overall ecology of marine organisms in terms of cycling of nutrients and intestinal flora of fish (15, 16).

Luminous bacteria can readily be grown in the laboratory. Indeed, anyone having kept fish or meat in the refrigerator a bit too long, may have noticed a glow when searching for a late night snack.

PSI has experience in the culture of several bioluminescent vibrios.

c. Fungi

Numerous species of bioluminescent fungi are known. The commonly observed "Foxfire" is a North American species in which the fruiting body maintains a continuous low intensity glow. PSI has limited experience with fungi. We will consider the possible addition of luminous fungi to our "Light Crawler" earthworm farms for development of bioluminescent terrestrial ecosystems.

d. Annelids

Several species of bioluminescent earthworms are found in many parts of the world, several in the United States (18). PSI has gained extensive experience with

representatives from this phylum during our Phase I feasibility study. We expect to continue research and development efforts on marine species. The terrestrial species will be studied as soon as significant rainfall in the southeastern United States enables collection of specimens (38, 39).

e. Millipedes

Nearly all species of the genus *Motyxia*, i.e. *luminodesmus*, in the United States are bioluminescent. They are common in California, where the most studied species, *M-sequorae*, lives in moist humus, is 40 mm long, and glows greenish-white brightly over its entire body -- including appendages. The drought in California may make collection difficult. This organism may be ideal for a small terrestrial ecosystem product.

f. Mollusca

Latia neritoides, the New Zealand limpet, is the only fresh water bioluminescent species which spends its entire life cycle in the aquatic environment. *Latia* occupies a variety of habitats where hard surfaces are available, generally in fast flowing, stony bottomed streams (19). The most important investigations concerning the biology of *Latia* include those of Gray (20), Pelseneer (21), and Bowden (22), summarized recently (19). Cormier, Wampler and Hori (23) discussed the biochemistry of the luminescence reaction, which was originally described by Shimomura (24).

Moore has published results of laboratory maintenance of *Latia* and investigation of stimulation of the luminescent response (19). Feeding habits and preferences as well as mating has been reported. This organism may provide a relatively simple candidate for Phase II investigation due to its fresh water habitat. PSI's consultant, Greg Anderson, resides in New Zealand and is eager to assist in these efforts (see consultants' letters in Appendix).

g. Cnidaria

Renilla kollikeri of the Class Anthozoa, a sea pansy, exhibits a brilliant luminescent display in response to tactile stimulation (25, 26). A multiple series of luminous waves follows a single stimulus. In extreme cases, a frenzy of repetitive discharges may be induced from many different points in the nerve net, each initiating a separate wave of luminescence (26). An alternative response to strong stimuli is the production of a brief even glow, decaying over the period of approximately 30 seconds.

PSI has successfully maintained cultures of *Renilla* for nearly 4 months as a result of the Phase I feasibility effort. The Phase II project will emphasize this fascinating organism. Cultures of *Renilla* in combination with luminous Ctenophores will be investigated.

h. Ctenophora (40)

Ctenophores are among the brightest of luminous animals. Like the *Renilla*, ctenophores produce a series of multiple flashes at each stimulus, each flash of about 300 milliseconds duration and the response propagated at up to 14 cm-1 (26). Marine experts at the Monterey Bay Aquarium in California have developed exhibits of these comb jellies, all of which are luminescent. Unfortunately, there is little discussion of the bioluminescence phenomenon in the Monterey exhibit.

Generally, maintenance of these fragile creatures requires a circular tank with unique flow dynamics for maintenance. The "planktonkreisel" (described below) has been used successfully for long term maintenance of ctenophores (28).

i. Crustacea

Among the eight subclasses of Crustacea, three contain species which are luminous: Ostracoda, Copepoda and Malacostraca. Some of the bioluminescent species contain photophores on the body while others secrete luminous material into the water (30, 35).

An immense amount of work has been carried out on ostracods (30), since certain species can be dried and preserved indefinitely in the dry state without any deterioration of the luminescent substance. Addition of water to dry powdered Cypridina (*Vargula hilgendorfi*) results in a bright blue luminescence lasting some time (Available from Carolina Biological Supply Co.).

Currently, we have no information regarding attempts to culture any of the copepoda in the laboratory except for the work of our consultant, Professor James Morin, at UCLA (15). Extensive literature reviews and discussion with investigators involved with these organisms will be done prior to serious consideration of *Cypridina* (*Vargula*) as a candidate species.

j. Fish

The anatomical and physiological expression of luminescence reaches its peak in the fishes (15, 16, 31, 45). Photophores are both more numerous and more diverse than in other organisms. The diversity extends not only to different species but also to the variety of luminous organs within a single species. All luminous fish are marine. Although many are deep sea fishes, numerous benthic and coastal luminous species are known. The Midshipman fish (*Porichytes sp.*) is a common fish found in the northern Gulf of Mexico (*P. porosissimus*) and on both the east and west coasts of the U.S. (*P. notatus* and *P. myriaster*). The life history of the Atlantic Midshipman fish has been described in detail (31). Generally, these fish are bottom dwellers preferring mud bottoms into which they burrow during daylight hours. They are tolerant to wide and rapid swings in temperature, salinity and sea bottom composition making this fish a promising candidate for further investigation.

Midshipman fish are commercially available and may be hatched in the laboratory from freshly collected eggs (31). To our knowledge however, no one has attempted to sustain these fish through a complete life cycle.

3. Methods

Task 1. Selection of appropriate species will be made following extensive literature investigations and discussion with experts on candidate species. PSI has identified and studied two non-toxic species of bioluminescent dinoflagellates, two species of marine annelid worms, and one species of Cnidaria. With further exploration of the literature and consultation with experts in the field, we will identify several other species that are suitable for maintenance and culture for science educational displays, tools and toys. These will be identified and final selections made based upon

availability, bioluminescent intensity and duration, ease of culture, ease of transport and long term viability.

Task 2 Development of appropriate culture vessels in the form of aquaria and terraria -- our Phase I effort has shown that traditional marine aquaria and filtration systems are not always appropriate, particularly for planktonic species which would be filtered from the tank using ordinary mechanical filtration systems (41). Lighting fixtures will be purchased or built optimized for the particular requirements of each selected species. For example, we learned during Phase I that *Renilla* remain considerably healthier and have brighter luminescent displays, if we used blue actinic lighting.

Special heating or cooling requirements of the individual organisms must be met by refrigeration or heating units appropriate for the particular application. Based on our current experience and following consultation with experts, improved facilities are needed for large dinoflagellate cultures (33).

A device known as the "planktokreisel" has shown much success for maintenance of organisms requiring superior water movement such as ctenophores (28). It consists of a round glass vessel, a central column facilitating a continuous rotating water current via a jet outlet and an inside sand filter. These will be built for maintenance of plankton, ctenophores and cnidaria.

Algal turf scrubbers have demonstrated an immense improvement for providing oxygen saturated water to the *Renilla* and *Chaetopterus* tanks. These are not commercially available and must be custom built for each tank (41).

Task 3 Optimization of culture media- For the marine organisms, several commercially available artificial sea waters are currently under investigation. Guillard F/2 Supplement provides manganese chloride, ferrous chloride, biotin, B12 vitamin and other needed ions and nutrients (33). We will evaluate various artificial sea waters with and without various supplements for each of the marine species studied (34). We will develop and test formulations for optimization of growth and bioluminescence emission. Additives to increase the viscosity of the medium will also be considered for purposes of forming a homogeneous suspension of the dinoflagellates and bacteria for use in teaching aids and science toys. The optimum media developed for each of the different organisms will be proprietary and potentially patentable.

Task 4 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 medium be left unattended (assuming adequate oxygen and carbon dioxide levels are maintained) without depletion of nutrients? Do the organisms degrade their own environment with metabolites or decay products not accommodated by the filtration systems?

Task 5 Life cycle investigation- For many of these organisms, complex life cycles may force their elimination as candidate species. However, with proper

planning of tank development, free swimming larval stages can be accommodated (32, 37). In the case of the bacteria and dinoflagellates, division rates must be assessed and the media optimized. Cell densities and division rates for these organisms will be measured by standard methods (33).

Following establishment of stable cultures of the selected organism, breeding in captivity will be a major emphasis of this effort (15, 30, 32). We will rely on the experience and advice of our outstanding consultants. The probability of survival will depend on the particular requirements (food, light, temperature, water currents) of each organism. We do not intend to market any products which could potentially disrupt or deplete natural populations.

Task 6 We will continue the development of supporting teaching and educational material which will be available as product accessories for inquisitive customers and for teachers and schools. Currently, in conjunction with the Center for Integrated Science Education at the University of Utah, we are providing materials for in-service training for teachers in school systems throughout Utah.

4. Year 2: Ecosystems (36, 37, 41-43)

During year 2, we propose to assess the feasibility of developing ecosystems utilizing combinations of some of the bioluminescent species investigated during the first year's efforts. Several different ecosystems may be envisioned. A marine example might include luminous bacteria, dinoflagellates (phytoplankton), several zooplankton, marine worms and small shrimp. An example of a terrestrial analogue is the Light Farm, containing luminous bacteria, worms and fungi. Even the addition of firefly larva (continuously luminous) may be possible. Using the existing literature and experience with partially closed small scale ecosystems, together with our experience with bioluminescent organisms, we will investigate the feasibility of coupling these two areas to produce Bioluminescent EcoSystems (BES). Such systems would be dramatic tools for the study of the interactions among organisms. BES would have considerable application as teaching and educational tools.

There is tremendous opportunity for application of this technology in the development of totally bioluminescent displays for public aquaria. The Monterey Bay Aquarium in California and the Oregon Coast Aquarium in Newport, Oregon have outstanding exhibits of species which have bioluminescent relatives but few museums or aquaria have developed exhibits specifically for bioluminescence. Consider the impact of a huge aquarium of bioluminescent organisms, each species communicating with others of its own kind and warding off predators of other species with brilliant flashes of blue, green or yellow light!

The Mystic Marine Life Aquarium in Mystic, Conn., learning of our expertise in bioluminescence and science education, has already expressed interest in developing such a display (27).

The project objectives will be further reached by the performance of the following specific Year 2 tasks:

Task 7 Identification of compatible organisms- Compatibility will depend not only on having compatible environmental conditions (media components, light, temperature), but also on predation and disease (37).

We propose to assess the feasibility of systems ranging from three to six different species. The primary food producer would be a bioluminescent phytoplankton, possibly *Pyrocystis lunula*, a hearty, easily maintained and brightly bioluminescent organism (17, 34). An omnivorous copepoda, probably *Metridia lucens*, or similar organism, should readily feed on the dinoflagellates (35). As small shrimp are already used in totally closed ecosystems (43, 46), it is likely that small bioluminescent shrimp will be suitable. The addition of the Midshipman fish may be possible since crustaceans are the primary food of these fish (31).

Task 8 Primary food production- We anticipate that dinoflagellates may be adequate as the primary food producer. However, given definite food preferences among the various zooplankton, this will have to be thoroughly examined. 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 and competition by minimizing the use of silicate producing containers and by eliminating silicates from all media.

G. Expected Results

The proposed R&D project has been planned to expand the feasibility demonstrated during the Phase I effort to other bioluminescent organisms for use as science discovery materials. Given our experience with maintaining bioluminescent organisms, and the experience of others (our consultants) in developing and maintaining specially long lived, stable ecosystems (15, 41), we anticipate considerable success.

A significant emphasis will be on the development of curriculum supplements for use with our bioluminescence products. Phase II will include discovery experiments which will be field tested in classrooms by expert teachers. PSI has been actively working with educators and administrators from Utah school districts to introduce bioluminescence into an experimental science curriculum. The reception has been enthusiastic.

If new science curricula are to be effectively implemented, research and development of student conceptions and misconceptions as well as the teachers' knowledge and skill must be assessed. PSI is actively working with the Center for Integrated Science Education at the University of Utah to develop these teaching aids in conjunction with expert educators and subject matter specialists to optimize the effectiveness of materials developed by PSI.

As research on bioluminescent organisms is not a well developed subdiscipline, we cannot anticipate every eventuality and problem. We certainly expect to be able to select, collect, grow, reproduce, and maintain the needed bioluminescent species.

Additionally, we anticipate developing interest from museums and aquaria in our development efforts. We expect to evolve a commercial activity in the area of large bioluminescent exhibitions.

H. Summary

Substantial evidence exists for the commercial feasibility of bioluminescent based science discovery products. There is national recognition of the current crisis in science education. Most states have already adopted programs to enhance science education at all levels. This will result in significantly increased demand for products which have an educational component. The trend in this revolution is for an integrated approach to science education, to connect or merge the different scientific subject via discipline bridging themes. Bioluminescent based science discovery materials provide unique vehicles for this integrated approach.

I. References

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

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

7. J.E. Wampler, "Earthworm Bioluminescence" in Bioluminescence and Chemiluminescence, M.A. Deluca and W.D. McElroy eds., Academic Press, New York (1981) pp 249-256.

8. G.E. Gates, India Mus., 27 (1925) 471.

9. M. Anctil, J.P. De Waele, M.J. Miron, and A.K. Pani, "Monoamines in the Nervous System of the tube worm Chaetopterus Variopedatus (Polychaeta): Biochemical Detector and Sero Toxin Immunoreactivity," Cell Tissue Res., 259 (1990) 81-92.

10. The National Commission on Excellence in Education, *A Nation at Risk*; Government Printing Office; Washington, DC (1983).

11. M. Root, "Glow-in-the-Dark Biotechnology," BioScience, 38, 11 (1988) 745-747.

12. A.K. Campbell, "Living Light: Chemiluminescence in the Research and Clinical Laboratory," TIBS, 11, March (1986) 104-108.

13. The New Encyclopedia Britannica: Macropaedia Vol. 2 15th Edition, Chicago (1974) 1028-1032.

14. J.A. Colin Nicol, "Studies on *Chaetopterus Variopedatus* I The Light Producing Glands," J. Marine Biol. Assoc., 30 (1952) 417-431.

15. J.G. Morin, Personal Communication and J.G. Morin, "Coastal Bioluminescence: Patterns and Functions," Bull. Marine Sci., 33 (1983) 787-817.

16. P.J. Herring, A.K. Campbell, M. Whitfield, and L. Maddock, Light and Life in the Sea, Cambridge University Press, 1990.

17. J.W. Hastings, "Bacterial and Dinoflagellate Luminescent Systems" in Bioluminescence in Action; P.J. Herring ed. Academic Press (1978) 129-170.

18. J.E. Wampler, "Earthworm Bioluminescence," in Bioluminescence and Chemiluminescence, Academic Press (1981) 249-258.

19-24. S.C. Moore, "Aspects of the Behavior, Morphology, Physiology and Ecology of the Luminescent Freshwater Pulmonate 'Limpet' Latia neritoides (Mollusca: gastropoda) Master's Thesis, Biological Sciences Depart. Univ. of Waikato (1987) and references cited therein.

25. P.A.V. Anderson and J.F. Case "Electrical Activity Associated with Luminescence and other Colonial Behavior in the Pennatulid Renilla kollikeri," Biol. Bull., 149 (1975) 80-95.

26. P.J. Herring, "Bioluminescence of Invertebrates other than Insects," in Bioluminescence in Action, P.J. Herring Ed., Academic Press (1978) 199-240.

27. Neil Overstrom, Exhibits Director, Mystic Marine Life Aquarium, Mystic, Conn., Phone (203) 536-9631.

28. W. Greve, "The 'Planktonkreisel,' a New Device for Culturing Zooplankton," Marine Biol., 1 (1968) 201-203.

29. P.J. Herring, "Bioluminescence in the Crustacea," J. Crustacea Biol., 5 (4) (1985) 557-573.

30. J.G. Morin and A.C. Cohen, "Bioluminescent Displays, Courtship and Reproduction in Ostracodes," in Crustacean Sexual Biology R.T. Baur and J.W. Martin Eds., Columbia University Press (1991) 1-16.

31. E.D. Lane "A Study of the Atlantic Midshipmen, Porichthys porosissimus, In the Vicinity of Port Aransas," in Contrib. in Marine Sci., 12 (1967) 1-53.

32. F-S Chia and M.E. Rice, eds., Settlement and Metamorphosis of Marine Invertebrate Larvae, Elsevier, 1978.

33. J.R. Stein, ed., Handbook of Physiological Methods: Culture Methods and Growth Measurements, Cambridge University Press, 1973.

34. 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.

35. S.M. Hag, "Nutritional Physiology of Metridia lucens and *M. longa* From the Gulf of Maine," Limnol. Ocean, 12 (1967) 40-57.

36. J.N. Kremer and S.W. Nixon, A Coastal Marine Ecosystem, Simulation and Analysis, Springer Verlag (1978).

37. M. Levandowsky, "Multi Species Cultures and Microcosms," in O. Kinne, ed., Marine Zoology, vol. 3, pt. 3, Wiley (1977) 1399-1458.

38. S.P. Hopkin and H.J. Reed, The Biology of Millipedes, Oxford U. Press, 1992.

39. N.B. Causey and D.L. Tiemann, "...Bioluminescent Millipedes...", Proc. Amer. Phil. Soc., 113 (1969) 14-33.

40. M-L Hernandez-Nicaise, "Ctenophora," in Microscopic Anatomy of the Invertebrates, vol. 2, Wiley-Liss (1991) 359.

41a. The most common bioluminescence exhibit in public aquaria are flashlight fish, which are expensive to collect and difficult to maintain.

41b. W.H. Adey and K. Loveland, Dynamic Aquaria, Academic Press, 1991.

42. W. Adey, "Ecosystem Encounters," Science Teacher, Sept. 1992, p. 22.\

43. C.E. Fulsome and J.A. Hanson, "Emergence of Material Closed System Ecology," in N. Polunin, ed., Ecosystem Theory and Application, Wiley (1986) 209.

44. J.W. Hastings, "Biological Diversity, ...Bioluminescent Systems," J. Mol. Evol., 19 (1983) 309.

45. T. Goto, "Bioluminescence of Marine Organisms," in P.J. Scheuer, ed., Marine Natural Products, 3 (1980) Academic Press, p 179.

46. "Ecosphere," Ecosphere Associates, Tuscon, Arizona (available from Carolina Biological Supply Co.).

J. Personnel and Consultants

Suzanne Winters, Ph.D., Vice President for Research and Development, will serve as Project Manager and Principle investigator. Dr. Winters has worked in technology development for the past 5 years with CardioPulmonics, Inc. as Director of Membrane Technology. She joined Protein Solutions, Inc. in November, 1991. Suzanne served as P.I. of the Phase I project. Her brief vita is in the Appendix.

Joseph D. Andrade, Ph.D., President and founder of Protein Solutions, Inc., has been studying bioluminescence for the past five years, primarily for science motivation and education. He is internationally recognized for his inter and multi-disciplinary approaches to science and engineering. He is Director of the Center for Integrated Science Education. He has taught high school general science, chemistry and biology and has assisted in elementary school science instruction on a regular basis for several years. Dr. Andrade has been working on biomaterials and biotechnology problems for the past 25 years. His vast experience with the nature an behavior of proteins is directly applicable to the complex proteins involved in bioluminescence.

Mr. Andras Pungor is a Research Associate in the Department of Bioengineering. Mr. Pungor has a Master's Degree in Electrical Physics from Hungary, and has had considerable experience as a Physics instructor in his home country. He is a member of the technical staff of the Department of Bioengineering and the Surface Analysis Laboratory at the University of Utah. The laboratory does a variety of contract analytical and development work for industry. He has unique and special skills in the development of scientific devices and apparatus involving electronics, optics, and mechanical components. His time and supplies will be handled on a fee for service basis and billed via the Surface Analysis Laboratory at the University of Utah.

John Wampler, Ph.D., is professor of Biochemistry at the University of Georgia in Athens. He is internationally recognized for his work on bioluminescent earthworms and related organisms (7,18). Dr. Wampler provided considerable advice and assistance during the Phase I project, instructing Dr. Winters, the P.I., on the topic of bioluminescent earthworms, their physiology, collection, and study.

Dr. James Case, V.P. for Research at the University of California, Santa Barbara, and with the Marine Biotechnology Center at UCSB, is internationally recognized for his work on bioluminescence in marine organisms. Dr. Case has provided considerable advice and input during the Phase I, particularly with respect to the biology, collection, and maintenance of the sea pansy *Renilla.* He also is an expert on the mid-shipman fish. PSI staff have visited his laboratory several times (see Appendix).

Walter Adey is one of the Directors of the National Aquarium in Washington DC, part of the Smithsonian Institution. Dr. Adey, together with Dr. Lovelock, authored the recent excellent and important book *Dynamic Aquaria* in which the principles of the development and construction of marine ecosystems are carefully developed for the first time.

Dr. James Morin is Professor of Biology at UCLA in Los Angeles, and is also widely known for his work on bioluminescence, particularly in marine organisms. He and his co-workers have discovered and identified many new bioluminescent *Vargula*

species in the Gulf of Mexico. PSI staff have visited Dr. Morin, and he has aided us extensively in the Phase I project.

Dr. Vladmir Hlady is Associate Professor of Bioengineering at the University of Utah, an expert on optics and the measurement of luminescence and fluorescence. His optical spectroscopy and engineering laboratory and his consulting purposes will also be utilized in the Phase II (see Appendix).

Gregory Anderson is President of McBain laboratories in Minnesota, with a Division in New Zealand. Mr. Anderson has been actively involved as a design engineer of flow and reactor systems for over 14 years. He has been instrumental in PSI's investigation of the New Zealand limpet (see Appendix).

Dr. J.W. Hastings, Professor of Biology at Harvard, is one of the world's experts on dinoflagellate and bacterial bioluminescence. Dr. Hastings serves on PSI's scientific advisory board, and also will provide considerable advice and input to the project, as will

Mr. William Kelley, another member of PSI's scientific advisory board. Mr. Kelley was one of the founders of Aquarium Systems, a corporation that sells artificial sea water and related materials for hobbyists and exhibition aquaria. Mr. Kelley has 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 for PSI's existing bioluminescent plankton product development.

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 (see Appendix). PSI is also a key corporate participant in the University's Center for Integrated Science Education (CISE). 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 evaluation studies. The laboratory space (1500 ft^2) 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 at the University of Utah (see Appendix).

L. Current and Pending Support

PSI has invested $80,000 (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.

PSI submitted a Phase I application titled: LUNULA COLONY: Bioluminescence for Science Education, to the National Science Foundation on June 15, 1992. A Phase I SBIR was submitted to NASA on July 15, titled The ATP Corona Pen. A Phase I to the Department of Agriculture was submitted on 8/26/92, titled "Aquaculture of Marine Phytoplankton in Enclosed Environments."

There is no overlap between this Phase II proposal and the Phase I applications.

M. Commercial Potential

Throughout the Proposal we have discussed the commercial potential of the research and the technology derived from it. The markets for PSI's bioluminescence educational products are children, their parents, and their teachers. With a growing recognition that our educational system must increase its emphasis on science, mathematics, and technology, PSI expects an increased demand for products which have a significant educational component.

PSI is already test-marketing a science education kit for the 8-14 age group titled "NIGHT-LIFE™: Science in the Dark!," which uses its bioluminescent phytoplankton. PSI expects to launch an adult novelty science product in early 1993 called GALAXSEA™, Bioluminescent Plankton. The company is already working with catalogers, distributors, and a variety of retail outlets. It has conducted an extensive market survey of the science education materials market, including science and technology museum gift shops, aquarium shops, pet and fish stores, retail chain outlets such as The Nature Company and Worlds of Wonder, and of course the entire public and private education distribution system. The national public education market K-12 for science, texts, and materials is over 2 billion dollars per year.

PSI is also working closely with the Children's Museum of Utah and the Mystic Marine Life Aquarium in its development and eventual marketing of marine exhibits utilizing bioluminescence.

PSI is also entering into an agreement with a local firm, Sea-Base, to greatly scale up the production of marine bioluminescent organisms for its science education products.

These follow on funding agreements and commitments, and an overall commercialization plan will be submitted soon. The Phase III commercialization plan will discuss product development and improvement, product testing, test-marketing, marketing, patent procurement, publicity, and public relations. PSI's Phase III follow-on funding commitments will include a mix of stock stales, advance orders, and ongoing sales revenues, as well as contract research activities.

N. Budget and Justification

Suzanne Winters is Project Manager and Principal Investigator. She will manage all activities relating to organism evaluation and selection, acquisition, maintenance and reproduction attempts. She will direct design engineering of the environmental chambers and related water handling systems. She will have responsibility for all data management activities for the project.

J.D. Andrade will act as liaison between laboratory studies and educational personnel for review of appropriateness of materials for educational curricula development.

A to be appointed post doctoral fellow will be responsible for overseeing daily activities in the laboratories. This individual will also be intimately involved in environmental chamber design and construction and computer interface software development for computer-controlled water handling systems.

John Tobler, laboratory technician, will work directly under the post doctoral's supervision. He has primary responsibility for sea water composition and analysis.

An undergraduate student (part time) will be employed for routine organism maintenance.

Equipment: Permanent equipment necessary for carrying out this research includes: gas and electrolyte analyzer with appropriate electrodes ($10,000) and a personal computer data station ($3,000) which will act as a driver for all water handling systems and aquaria. All tanks, lighting systems and water handling equipment require custom designing and will be purchased under equipment charges. Estimated costs are 6 x $1,000/tank system ($6,000).

Consultants: See section J and Appendix. Consultants will be compensated at $400/day plus travel expenses. We have budgeted 4 consultant days/year for 2 years.

Supplies: routine laboratory glassware $2,000; artificial sea salts and supplements $1,200; chemistry test kits $500; aquaria supplies (tubing, gravel, food, etc.) $4,000; gas and electrolyte electrodes and cartridges $3,000; construction materials $2,000; miscellaneous $1,000.

Other: Analytical services $3,000 for microbiological identification and toxicity testing, and for materials analysis.

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.

UNIVERSITY OF CALIFORNIA, SANTA BARBARA

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO — SANTA BARBARA • SANTA CRUZ

OFFICE OF RESEARCH DEVELOPMENT — SANTA BARBARA, CALIFORNIA 93106

19 October 92

Dr. Suzanne Winters
V.P. Research and Development
Protein Solutions, Inc.
390 Wakara Way, Rm 65
Salt Lake City, UT 84108 FAX 801 585 5361

Dear Dr. Winters,

I will be most interested in acting as a consultant on your Phase II SBIR project, "Bioluminescence-Based Discovery Materials Science Education." I understand this work will include reviewing the project and providing input.

I am delighted that you were able to use Renilla in your initial work since it is an organism which we have used extensively in university level instruction and holds great promise as a vehicle for experimentation by lower school students on the fundamentals of nervous system function using only the simplest apparatus. Our local marine resources can undoubtedly provide you with other examples.

Of course you understand that this proposed consulting agreement must be in conformity with University of California policy.

Best wishes,

James F. Case
Associate Vice Chancellor for Research
Professor of Neurobiology

JAMES F. CASE

August, 1990

Present Appointment: Professor of Neurobiology;
Associate Vice-Chancellor for Research
University of California
Santa Barbara, CA 93106
(805) 961-2913,-3639,-4188

Personal Data: Born 27 October, 1926, Bristow, OK
Married, 5 children

Education:

1945-48 University of Kansas, no degree
1948-51 Johns Hopkins University, PhD in Biology
Thesis: Adrenal-Pituitary Axis in Embryonic Life

Professional Experience:

1946-48 Collector, Division of Mammals, Univ. Kansas Museum of Natural History
Teaching Assistant: Intro. Biol. U.K.
1949 Teaching Assistant: Intro. Biol. J.H.U.
1949 Student: Summer courses, Marine Biological Laboratory, Woods Hole, MA. Invertebrate Embryology
1949 Investigator, Mt. Desert Island Laboratory, Salisbury Cove, ME. Tissue culture
1950 Jr. Instructor: Graduate Biology, J.H.U.
1950 Instructor, Children's School of Science, Woods Hole, MA. (summer)
1951 Physiologist, U.S. Department of Agriculture, Agricultural Research Center, Beltsville, MD. Avian endocrinology
1951-55 Military Service: 2d/Lt to Capt; attended Company Officer's Course, Brooke Army Medical Center; assigned to Chemical Corps with duty at Army Chemical Center, MD, Physiology Div. Research in insect neurophysiology.
1956 Physiologist (GS-11) Physiology Division, Army Chemical Center (insect neurophysiology research)
1957 Postdoctoral student, Nerve-Muscle Training Program, Marine Biological Laboratory. Director: Steven Kuffler.
1957-62 Assistant to Associate Professor, Department of Zoology, University of Iowa, Iowa City
1958 Summer Investigator, Marine Biological Lab.
1959-60 Summer Instructor and Investigator, Invertebrate Zoology, Marine Biological Lab, Woods Hole, MA
1962- Associate Professor III to Professor VIII, Department of Biological Sciences, U. C. Santa Barbara
1964 Research: Eniwetok, Marshall Isls. 2 mos.
1965- Oceanographic Cruises: approximately 30
1968-71 Instructor and Instructor-in-Charge, Invertebrate Zoology and, later, Experimental Invertebrate Zoology, Marine Biological Lab. (summers)
1969 Member, Alpha Helix New Guinea Biological Expedition, 2d Leg: Madang and N. New Guinea

Coast, KarKar Isl., N. G. Highlands (bioluminescence)
1972-82 Field studies of fireflies: New Guinea, Thailand, Malaysia - 5 occasions of up to 1 month
1975 Chief Scientist, Alpha Helix, S.E. Asian Biolum. Expedition, 1st leg: Amboina and Banda Islands
1980 Senior Queen's Fellow in Marine Science, Dept. Neurobiology R.S.B.S. Australian National University and Lizard Is. Res. Station
1980-84 Chairman, Dept. Biological Sciences, UCSB
1986-88 Associate Vice Chancellor for Research (Act'g), UCSB
1987 Chief Scientist, ONR Biowatt Cruise 5, Sargasso Sea (bioluminescence)
1988- Associate Vice Chancellor for Research, UCSB
1990 Alboran Sea Bioluminescence Program
1990-91 MLML N. Atlantic Bioluminescence Component: HIDEX and MOORDEX deployments

Other Professional Activities:

AIBS Speakers Bureau
Commission on Undergraduate Education in Biological Sciences
Program Chairman, Division Chairman, Comparative Physiology Division of American Society of Zoologists
Editorial Boards: Biological Bulletin, J. Experimental Zool.
Editorial Reviewer: Science, Nature, J. Exptl. Biol., J. Exptl. Zool., Marine Biol., Biol. Bulletin, J. Compar. Physiology, National Geographic, Deep Sea Research.
Proposal Reviewer: NIH, NSF, Nat. Geogr. Soc., NATO
Trustee (classes of 1969 and 73 - 4yr terms) and Member Executive Committee, Marine Biological Laboratory (elected by members MBL Corporation and Trustees respectively)
Alpha Helix National Advisory Board (NSF)
Nominating Committee of AAAS
External Examiner-Physiology, University of Malayia
Member, BIDS Committee, Johns Hopkins Applied Physics Laboratory (Naval interests in bioluminescence)
Member Exec. Ctty., Ocean Optics and Bioluminescence Committee (BIOWATT), Office of Naval Research
Member, Bioluminescence Committee, DUMAND Project
Member, UCSB/ARCO Science and Technology Panel (dealing with problems arising from oil production near campus)
Member, U.C. Systemwide Ctty on Research
Member, U.C. Systemwide Ctty on Technology Transfer
Member Exec. Ctty., Marine Light/Mixed Layer Committee, Office of Naval Research (bioluminescence)
Member, DOD Interagency Bioluminescence Working Group
Invited Participant, Symposium on Tactical Oceanography, Office of Oceanographer of the Navy.
Initiatives Review Panel, Naval Research Laboratory, 1991

Consultancies: Dynamics Technology, Inc., Torrance Cal.
TASC, Inc., Rosslyn, Va.
Johns Hopkins University Applied Physics Lab

JAMES F. CASE

August, 1990

Present Appointment: Professor of Neurobiology;
Associate Vice-Chancellor for Research
University of California
Santa Barbara, CA 93106
(805) 961-2913,-3639,-4188

Personal Data: Born 27 October, 1926, Bristow, OK
Married, 5 children

Education:

1945-48	University of Kansas, no degree
1948-51	Johns Hopkins University, PhD in Biology Thesis: Adrenal-Pituitary Axis in Embryonic Life

Professional Experience:

1946-48	Collector, Division of Mammals, Univ. Kansas Museum of Natural History Teaching Assistant: Intro. Biol. U.K.
1949	Teaching Assistant: Intro. Biol. J.H.U.
1949	Student: Summer courses, Marine Biological Laboratory, Woods Hole, MA. Invertebrate Embryology
1949	Investigator, Mt. Desert Island Laboratory, Salisbury Cove, ME. Tissue culture
1950	Jr. Instructor: Graduate Biology, J.H.U.
1950	Instructor, Children's School of Science, Woods Hole, MA. (summer)
1951	Physiologist, U.S. Department of Agriculture, Agricultural Research Center, Beltsville, MD. Avian endocrinology
1951-55	Military Service: 2d/Lt to Capt; attended Company Officer's Course, Brooke Army Medical Center; assigned to Chemical Corps with duty at Army Chemical Center, MD, Physiology Div. Research in insect neurophysiology.
1956	Physiologist (GS-11) Physiology Division, Army Chemical Center (insect neurophysiology research)
1957	Postdoctoral student, Nerve-Muscle Training Program, Marine Biological Laboratory. Director: Steven Kuffler.
1957-62	Assistant to Associate Professor, Department of Zoology, University of Iowa, Iowa City
1958	Summer Investigator, Marine Biological Lab.
1959-60	Summer Instructor and Investigator, Invertebrate Zoology, Marine Biological Lab, Woods Hole, MA
1962-	Associate Professor III to Professor VIII, Department of Biological Sciences, U. C. Santa Barbara
1964	Research: Eniwetok, Marshall Isls. 2 mos.
1965-	Oceanographic Cruises: approximately 30
1968-71	Instructor and Instructor-in-Charge, Invertebrate Zoology and, later, Experimental Invertebrate Zoology, Marine Biological Lab. (summers)
1969	Member, Alpha Helix New Guinea Biological Expedition, 2d Leg: Madang and N. New Guinea Coast, KarKar Isl., N. G. Highlands (bioluminescence)
1972-82	Field studies of fireflies: New Guinea, Thailand, Malaysia - 5 occasions of up to 1 month
1975	Chief Scientist, Alpha Helix, S.E. Asian Biolum. Expedition, 1st leg: Amboina and Banda Islands
1980	Senior Queen's Fellow in Marine Science, Dept. Neurobiology R.S.B.S. Australian National University and Lizard Is. Res. Station
1980-84	Chairman, Dept. Biological Sciences, UCSB
1986-88	Associate Vice Chancellor for Research (Act'g), UCSB
1987	Chief Scientist, ONR Biowatt Cruise 5, Sargasso Sea (bioluminescence)
1988-1990	Associate Vice Chancellor for Research, UCSB Alboran Sea Bioluminescence Program
1990-91	MLML N. Atlantic Bioluminescence Component: HIDEX and MOORDEX deployments

Other Professional Activities:

AIBS Speakers Bureau
Commission on Undergraduate Education in Biological Sciences
Program Chairman, Division Chairman, Comparative Physiology Division of American Society of Zoologists
Editorial Boards: Biological Bulletin, J. Experimental Zool.
Editorial Reviewer: Science, Nature, J. Exptl. Biol., J. Exptl. Zool., Marine Biol., Biol. Bulletin, J. Compar. Physiology, National Geographic, Deep Sea Research.
Proposal Reviewer: NIH, NSF, Nat. Geogr. Soc., NATO
Trustee (classes of 1969 and 73 - 4yr terms) and Member Executive Committee, Marine Biological Laboratory (elected by members MBL Corporation and Trustees respectively)
Alpha Helix National Advisory Board (NSF)
Nominating Committee of AAAS
External Examiner-Physiology, University of Malayia
Member, BIDS Committee, Johns Hopkins Applied Physics Laboratory (Naval interests in bioluminescence)
Member Exec. Ctty., Ocean Optics and Bioluminescence Committee (BIOWATT), Office of Naval Research
Member, Bioluminescence Committee, DUMAND Project
Member, UCSB/ARCO Science and Technology Panel (dealing with problems arising from oil production near campus)
Member, U.C. Systemwide Ctty on Research
Member, U.C. Systemwide Ctty on Technology Transfer
Member Exec. Ctty., Marine Light/Mixed Layer Committee, Office of Naval Research (bioluminescence)
Member, DOD Interagency Bioluminescence Working Group
Invited Participant, Symposium on Tactical Oceanography, Office of Oceanographer of the Navy.
Initiatives Review Panel, Naval Research Laboratory, 1991

Consultancies: Dynamics Technology, Inc., Torrance Cal.
TASC, Inc., Rosslyn, Va.
Johns Hopkins University Applied Physics Lab

mcbain laboratories, inc.

September 22, 1992

Dr. Suzanne Winters
Protein Solution Inc.
390 Wakara Way
Salt Lake City, Utah
84108

Dear Dr. Winters;

I am writing to confirm my desire to work with your company on the proposed Latia Neritoides project.

As you know, I have a personal interest in this organism also. I am familiar with its habitat in New Zealand, and now that I am spending nearly half my time in that country, it would be quite feasible for me to assist you there.

Please keep me informed of your progress. I look forward to working with you on this project!

Sincerely,

Greg Anderson