(1) PSI-94-DOE — 1

U.S. DEPARTMENT OF ENERGY
BUSINESS INNOVATION RESEARCH
LICITATION NO. DOE/ER-0598

APPENDIX A

DOE USE ONLY

94-I

COVER PAGE

TIONS. "This submission is to be used only for DOE evaluation purposes. All government and non-Government personnel handling o ensure that the information contained herein is not duplicated, used, or disclosed in whole or in part for any purpose other than to ermission of the offeror (except that if a grant is awarded on the basis of this submission, the terms of the grant shall control disclosure shall not by itself be construed to impose any liability upon the Government or Government personnel for any disclosure or use of data

b
btopic (a-d)

nt Films Based on Photoproteins

tions, Inc. (PSI)

kara Way, Room 31

STATE: Utah ZIP: 84108

92

CERTIFICATIONS

The above organization certifies that:

It is a small business and meets the definition stated in Section 2.3.

A minimum of two-thirds of the funded research or analytical effort will be performed by the applicant organization (see Section 5.5).

It will comply with the provisions regarding: (1) lobbying, (2) debarment, suspension, and other responsibility matters, and (3) drug-free workplace requirements. (See Appendix E and Appendix F.) Inability to certify to any or all statements requires explanation.

It has provided the necessary information requested in Section 3.4.4 if it has received more than 15 Phase II SBIR awards in the preceding five fiscal years.

rements in Sec. 1.5)

s., Dr.

st

3128

Date: 2/11/94

Corporate/Business Certifying Official

TYPE NAME, Indicate Mr., Mrs., Ms., Dr.

Dr. Joseph D. Andrade

Title: President

Telephone No. (801) 581-4379

Signature of Corporate/Business Certifying Official

Date: 2/11/94

PROPRIETARY NOTICE (IF APPLICABLE, SEE SECTION 5.4)

ibmission, these data shall not be disclosed outside the Government and shall not be duplicated, used, or disclosed in whole or in warded to this applicant as a result of or in connection with the submission of these data, the Government shall have the right to nt provided in the funding agreement. This restriction does not limit the Government's right to use information contained in the data if riction. The data in this submission subject to this restriction are contained on pages:

17

CONTENTS

IDENTIFICATION AND SIGNIFICANCE OF THE OPPORTUNITY*

The major goal and objective is to produce intrinsically luminescent, biodegradable coatings and films which can be readily applied to almost any surface or substrate under typical environmental conditions, producing a uniform glow for periods ranging from hours to days and eventually even weeks. This technology would have a number of major applications:

- The uniform illumination of areas which are difficult to reach, and unusual shapes or geometries which are inaccessible to conventional light sources;
- Illumination in hazardous environments where conventional light sources may be undesirable;
- For a variety of sensor, sensing, and measurement functions.
- For motivational and educational purposes related to luminescence, photon generation and detection, and vision; and,
- For cosmetic or novelty purposes.

This technology has the potential to produce films which can provide continuous, low level, uniform illumination; it also has the potential to provide semi-directional photon emission and partial polarization. If these latter two objectives are indeed realized during Phase II, these bioluminescent films will be applicable to a much broader range of applications, including signage, display, emergency lighting, and specialty sensors.

* To save space, references which can be found in the publication lists in the Personnel section are referred to only by name.

U.S. DEPARTMENT OF ENERGY

APPENDIX B

SMALL BUSINESS INNOVATION RESEARCH PROGRAM
PHASE I - FY 1994-I
PROJECT SUMMARY

DOE USE ONLY

94-I

24	b
Topic No. (1-43)	Subtopic (a-d)

FIRM NAME, ADDRESS, TELEPHONE NUMBER: Protein Solutions, Inc. (PSI), 390 Wakara Way, Rm. 31, Research Park, Salt Lake City, Utah 84108, (801) 585-3128

TITLE OF PROJECT: Luminescent Films Based on Photoproteins

NAME AND TITLE OF PRINCIPAL INVESTIGATOR: Robert Scheer, Ph.D.

TECHNICAL ABSTRACT (Limit to space provided.):

Bioluminescence is an enzyme dependent chemical oxidation process which results in photon emission. Although it is a chemiluminescent reaction, the fact that an enzyme macromolecule is intimately involved leads to a set of unique characteristics and a wide range of potential applications. Bioluminescent organisms may produce short, intense, directional flashes of light (fireflies), as well as relatively intense continuous emission (flashlight fish).

Our preliminary work on orientation and self assembly of firefly luciferase at air/water and lipid/water interfaces, and our related work on the long term stabilization of luciferase in gels, has provided the basis for the study of luciferase based films as unique luminescent materials.

We propose to study the firefly and bacterial luciferases and their respective substrates and co-factors at air/water, lipid/water, and solid/water interfaces. Monolayer films will also be transferred using Langmuir Blodget techniques onto the surface of transparent solid materials, surface modified so as to facilitate the transfer and maintain enzyme orientation. The luciferase films and assemblies will then be characterized with respect to their luminescence properties in terms of intensity, duration, directionality, and polarization, as well as stability of the film under various storage and use conditions.

The hope and expectation is that luciferase films will be deposited on many different kinds of surfaces and devices and readily activated to provide uniform light illumination in otherwise dark environments.

KEY WORDS: Photoproteins, Luciferases, Monolayers, Self Assembly, Biomolecular Materials.

ANTICIPATED RESULTS/POTENTIAL COMMERCIAL APPLICATIONS as described by the applicant. (Limit to space provided).

The goal is to produce films and paints which can coat a wide range of surfaces to provide uniform, low-level illumination in otherwise dark environments. We expect that such films will eventually be applicable using various spray or other remote deposition technologies. The availability of easily applied luminous coatings, which can produce light for periods ranging from hours to perhaps even weeks, leads to an enormous range of applications in industry, education, and consumer products.

(All information provided on this page is subject to release to the public.)

BACKGROUND

Protein Solutions, Inc. (PSI) was founded in early 1988 to develop and produce innovative science educational products. We now manufacture Night-Life: Science in the Dark™, a bioluminescence-based science kit designed for upper elementary students and their teachers. We also manufacture and distribute Galaxsea™, a bioluminescent product for the high school and adult market which introduces them to the wonders and beauty of bioluminescence.

Bioluminescence is an enzyme dependent chemical oxidation process which results in photon emission. Although it is a chemiluminescent reaction, the fact that an enzyme macromolecule is intimately involved leads to a set of unique characteristics and a wide range of potential applications. Bioluminescent organisms may produce short, intense, directional flashes of light (fireflies), as well as relatively intense continuous emission (flashlight fish).

The use of biologically generated reflectors, filters, wavelength shifters, and even optical guided wave devices has permitted biological light to be generated in unique colors, with some directionality, and even remotely from the point of generation. There is some evidence that the organelles (scintillons) and even the enzymes themselves may be highly ordered and self assembled, such ordering and self assembly may indeed provide some level of directional and polarized emission.

The photoproteins involved in these processes, the luciferase series of oxidative enzymes, are now readily available in inexpensive form, produced by recombinant means.

During the last 5 years, PSI has been deeply involved in all aspects of bioluminescence including several studies dealing with its application to biosensors. We have funded a study at the Center for Biopolymers at Interfaces at the University of Utah on the behavior of firefly luciferase, the photo protein/enzyme responsible for catalyzing bioluminescence in the North American firefly. We have studied its adsorption at solid/liquid interfaces (Hlady), air/water interfaces (Wang), and its denaturability or stability in solution (Wang). We have recently initiated a similar set of studies dealing with bacterial luciferase, the enzyme responsible for bioluminescence in various species of marine bacteria (Min). These studies, together with our commercial work on bioluminescent phytoplankton (the dinoflagellate *Pyrocystis Lunula*) have provided a deep and diverse background in practical bioluminescence.

J.D. Andrade, President and Chief Scientific Officer (CSO) of PSI, has studied proteins at interfaces for over 25 years (Andrade). Our consultant, V. Hlady, an Associate Professor of Bioengineering at the University of Utah, has also studied firefly luciferase interfacial behavior and has extensive experience with the study and monitoring of luminescence processes at interfaces (Hlady).

There has been very little published on the interfacial properties of photoproteins. Although there is a rich and varied literature on bioluminescence, and major international conferences nearly every year, there has been very little interest or activity in the interfacial or film forming potential of these unique photoproteins, nor on their application for anything other then sensors. Luciferases are extensively used as labels for a wide range of clinical diagnostic chemical tests. Since the firefly luciferase reaction is dependent on an adenosine triphosphate (ATP) co-factor, it has been extensively used in

aided through interaction with our consultant, Dr. Vladimir Hlady, who has extensive experience in interfacial and near field (evanescent) fluorescence and luminescence studies (Hlady).

4) Extensive bacterial luciferase work is planned during the first year of Phase II, followed by monolayer transfer and multi film formation studies in year II. The case for a rigid environment during photo emission is much stronger for bacterial luciferase than for firefly luciferase. However, we are not as experienced with bacterial luciferase as we are with the firefly protein. It will take some additional time through Phase I and the first part of Phase II to reach that level of experience and sophistication qith bacterial luciferase..

If the experiments indeed show that there is some evidence for polarization and/or directionality of photo emission from highly oriented and ordered firefly and/or bacterial luciferase films, then that will likely lead to a set of fairly basic studies with our University of Utah colleagues. These will be quite exciting studies dealing with a very fundamental basis of photon emission and chemiluminescence *(23). The practical development and commercialization of such behavior, however, will be the basis of the SBIR project.

EXPECTED RESULTS AND COMMERCIAL POTENTIAL

We expect to:

1) Significantly enhance our ability to produce purified firefly and bacterial luciferases in quantity; to stabilize these proteins in gels and other films; to apply these preparations in a range of spray, dip, and other coating processes -- to produce luminescent films and coatings on a variety of surfaces which will remain active for periods ranging from hours to days and, hopefully, weeks.

2) The basic research understanding and technology developed during this project should permit preparation of luminscent films involving oriented and ordered photoproteins with enhanced luminescent properties. We expect that these films will be not only much brighter, but far more stable and durable than their more random and heterogeneous gel-based counterparts.

Specific details of commercial potential and application are difficult to assess at this point because the technology to produce intrinsically luminescent films and coatings has never really existed before (excepting the old radium-based paints).

There is certainly immediate commercial potential in our existing product line of innovative science education materials; we envision a set of kits around luminescence.

Another product, designed mainly for the general consumer/hardware market, is Light Paint™ for the handy man or technician who must work in environments that are difficult to light or hazardous to light by conventional means. Imagine working under your car for example, and rather than trying to hold your flashlight between your teeth, you simply spray the underbelly of the car with a uniform, light emitting film -- completely biodegradable. You

the development of biosensors for the measurement of ATP. Likewise, bacterial luciferase utilizes FMNH2, also a ubiquitous chemical in bioenergetic pathways, and has therefore been widely applied for biosensing applications (1-3). Up until very recently such applications were frustrated by the relative instability of these enzymes and the difficulty in incorporating them in practical and reliable biosensors.

A year ago Protein Solutions, Inc. became interested in a new science education product based on another unique biological phenomena -- the ability of certain plants and animals to survive almost total desiccation for extended periods and to spring back to life when rehydrated, a phenomenon called anhydrobiosis (19). In developing our new science educational product, Resurrection™, we of course learned that most of these plants and animals depend on a unique disaccharide, trehalose, for their ability to withstand severe desiccation stress without denaturation of their proteins and enzymes or disruption of their cell membranes (14-19). Trehalose apparently serves two major functions. In high concentration it tends to prevent phase separation and crystallization, and it tends to substitute for water normally hydrogen bonded to proteins and cell membranes (14, 18) thereby stabilizing their structures when the last molecules of water are removed by desiccation or drought. Trehalose is now being widely applied to the stabilization of enzymes and antibodies and is being studied for the stabilization of air dried cells, tissues, and organisms (14-17).

Mr. C.Y. Wang, a Ph.D. student in the Department of Bioengineering at the University of Utah, has been working on the trehalose stabilization of firefly luciferase for the past year under a contract between PSI and the Center for Biopolymers at Interfaces. Mr. Wang has succeeded in stabilizing firefly luciferase, together with its substrate, luciferin, in agarose gels for extended periods. More importantly, such agarose/luciferase/luciferin gels can be completely dehydrated and desiccated, maintaining their clarity and transparency, and then rehydrated with full enzyme activity after extended periods. If such rehydrated gels are now exposed to ATP, they of course luminesce (Wang).

We are already proceeding to develop this technology for educational and commercial purposes. It serves as the basis however, for the much more ambitious and significant project described in this application.

Firefly luciferase is a 60,000 Dalton labile protein with a good deal of surface hydrophobicity, as well as negative electrostatic character (Wang). Its two binding sites for ATP and luciferin are in very close proximity. Given what we already know about its behavior at polar and non polar solid surfaces (Hlady), its behavior at the air/water interface, and its interaction with various lipids and surfactants, we propose that it can be highly oriented to produce monolayers and films with unique luminescent properties, in contrast to the random and statistical distribution and orientation likely to be the case in our agarose gels.

PHASE I -- TECHNICAL OBJECTIVES

1) Production and Purification of Firefly Luciferase:

2) Luciferase Immobilization in Gels;

3) Development of a Spray Process for Gel Coatings;

eliminate balancing flashlights, stringing cumbersome electric lights, and the potential for sparking and fire hazards.

PHASE I -- WORK PLAN AND PROJECT DESCRIPTION

1) Production and Purification of Firefly Luciferase:

Although much of our work with firefly luciferase has utilized firefly-derived material, we have initiated studies on the cultivation of E-coli using an appropriate plasmid which expresses firefly luciferase. This recombinant firefly luciferase is then extracted from the E-coli and subjected to a series of purification steps (21, Wang). That purification is at present somewhat cumbersome, involving a number of steps. Our knowledge of the interfacial behavior of the protein (Andrade, Wang) should enable us to develop a simplified purification protocol with a much greater yield and activity. Crude preparations are appropriate, however, for the more general film forming studies described below. Highly purified preparations are required for the interfacial orientation and ordering/self assembly studies, also described below.

The protocol for firefly luciferase expression in E-coli and for purification are readily available and will not be repeated here (Wang, 21).

2) Immobilization of Firefly Luciferase in Low Gelling Temperature Agarose:

Our experience on the stabilization of firefly luciferase in gels has been limited to a specific kind of agarose. There is a report from a Russian group (24) that starch is effective for bacterial luciferase; we have some very preliminary work suggesting that other gels may work under certain conditions (Wang). This study will involve a range of hydrophilic gel forming materials, including polyacrylamide, polyvinylpyollidone, polyvinyl alcholol, as well as several agaroses, starches, and celluloses.

The goal is to softly immobilize the enzyme in the gel, generally in the presence of its luciferin substrate, and allow ATP to diffuse into the gel from an external source. This is the most effective method for biosensing applications. In the case of a luminescing film, we also want to incorporate the ATP in the gel itself. Obviously if both reagents are in the gel together with the enzyme, then in the presence of oxygen they are rapidly consumed by the chemiluminescent reaction. The way to prevent this is to eliminate oxygen or one of the key reagents, neither of which is practical for commercial applications. The other way to prevent it is to completely desiccate the gel so that the various components are almost totally immobile. The degree of immobility will of course be a function of residual water content, the particular characteristics of the macromolecular components of the gel itself, that is its glass transition temperature (7, Andrade), the degree of residual moisture, humidity in the storage environment, and a variety of other factors.

The highly desiccated gel will be characterized with respect to background glow under various relative humidity conditions for extended periods of time. This will be done using a fiber optic photometer and a time and position based sampling system, which will enable a single spectrometer/photometer to monitor a wide range of samples continuously.

At higher relative humidity, where the diffusion of luciferin and ATP are increased and therefore the overall luminescence is greater, then a simple TV camera will be used to monitor an array of different gels under identical relative humidity conditions.

4) Study the orientation and packing of semi-pure and pure firefly luciferase at buffer/water interfaces by dynamic surface tension and Langmuir Blodget pressure-area (11-13, Wang) techniques;

   Study the role of phospholipids in the orientation, stabilization, and activity of firefly luciferase at air/water and lipid/water interfaces (20, Wang);

   Study the role of trehalose in the sub phase as a function of buffer type, pH, and ionic strength on the behavior of luciferase monolayers;

5) Begin work on the transfer of luciferase monolayers to solid supports by conventional monolayer transfer methods (11) (this objective would only be partially satisfied during Phase I and would be a key part of the first year, Phase II studies); characterize luinescent propoerties of these layers;

6) In preparation for the Phase II studies involving bacterial luciferase, develop the means and expertise to produce and prepare bacterial luciferase in quantity (Min); and,

   Perform preliminary work on the behavior of bacterial luciferase at air/water interfaces, (the more extensive monolayer and interfacial work on bacterial luciferase would be accomplished in the first year of Phase II).

SIGNIFICANCE AND PHASE II OBJECTIVES

The Phase I will provide a firm basis and foundation for the more challenging and comprehensive Phase II studies. Based on the results and experience gained in Phase I, and on our knowledge at present, we expect the following Phase II objectives:

1) The successful transfer of firefly luciferase monolayers onto solid supports resulting in oriented, ordered, and active monolayer and multilayer luciferase films.

2) Utilizing the trehalose stabilization/desiccation technology, we expect to incorporate luciferin and ATP between the luciferase layers with little consumption of those reagents, i.e., light emission, during the fabrication process. Such dry films, kept desiccated and dry should be stable indefinitely. The films will be reactivated in the presence of water. Reactivation will be studied as a function of relative humidity, as well as by various water/spray processes.

3) The luminescent characteristics of the as prepared, oriented, ordered films will be studied with respect to the directionality of photon emission, and the possible polarization of that emission. This is based on the fact that photon emission event likely occurs while the excited state molecule remains attached to the enzyme. The enzyme itself, being oriented, ordered, and largely immobile, and the relatively strong binding between the excited state intermediate and the enzyme, suggest the possibility of a partially polarized and possibly even partially directional photon emission. This is of course a controversial hypothesis (23). The consequences of such possible behavior are so significant, however, that they merit study. The study of the potential directional and polarization characteristics of the luminescence will be greatly

The goal of these studies, to be completed in the first 2-3 months of Phase I, is simply to determine an optimum gel immobilization process.

We expect that trehalose is essential for the stabilization of the protein, and that trehalose will also be most effective in stabilizing the protein in polysaccharide gels, i.e., agaroses, celluloses, dextrans, etc., and that the synthetic polymer gel matrices will be less effective. But that hypothesis remains to be tested.

3) Development of aSpray Process for Gel Coatings

Some years ago we utilized ink jet printing for multi-channel biosensor applications -- proteins could be deposited in various patterns on pape substrates. This technology was later modified to produce protein patterns on rigid planar substrates. The process is to spray the luciferase/luciferin/trehalose/agarose solution, followed by a drying step, followed by thin coating and spraying of the ATP component, including trehalose and additional agarose. In this manner the luciferase and ATP are physically separated; the low mobility and diffusion, due to partial hydration under normal relative humidity conditions, should not lead to significant luminescence. Full rehydration of the film would rapidly cause mobility of the ATP into the luciferase/luciferin layer, followed by bioluminescence.

Such a process would be further optimized during Phase II with the possible spraying of a third component which could serve as a barrier between the two layers or an outer layer which could serve as a permeability barrier, if required.

The major goal of this part of the project is to prepare films of variable thicknesses, luminescence intensities, and patterns such films will be homogeneous with respect to the orientation and the photo emission properties of the luciferase molecules, thus allowing a comparison with the behavior of the oriented films prepared by monolayer techniques.

4) Air/Water Interface Studies

We will conduct a more comprehensive set of studies on the pressure area properties of firefly luciferase at air/water and lipid/water interfaces as a function of trehalose concentration, lipid type, and pH (Wang).

Building on our experience to date with firefly luciferase at air/water and lipid/water interfaces, using a large, Lauda, monolayer trough (Wang), we will conduct a set of such studies using a small, miniature trough and a highly sensitive, CCD camera capable of nearly single photon detection (Hlady). We will directly monitor the low level luminescence from the single monolayer of luciferase as a function of pressure/area conditions. This experiment will be conducted in Dr. V. Hlady's (consultant) optical spectroscopy laboratory in the Center for Biopolymers at Interfaces.

Given sufficient sensitivity, we will also look at the polarization state of the light as a function of pressure/area conditions. The new trough will constructed so that the subphase can be altered at specific pressure/area conditions by introducing luciferin/ATP solutions. Similar experiments will be conducted using preformed lipid monolayers, followed by adsorption of luciferase from the subphase at the lipid/water interface. These studies will test the hypothesis that luciferase interacts at the pure air/water interface by hydrophobic means, and orients its major hydrophobic face or patch towards the air surface, similarly to the behavior of lysozyme at air/water interfaces (Andrade, Hlady,

however, and would prefer to stay with that system unless there is a good reason for moving to E-coli.

Purified bacterial luciferase is far less expensive than firefly luciferase. We expect to initiate gel studies and interfacial activity studies very early in Phase I using commercial material. By the end of Phase I we will be in a good position to do extensive monolayer studies of bacterial luciferase in Phase II.

RELATED R & D

This was discussed earlier in the section on Background. Also refer to pucliation lists of Andrade, Hlady, Wang, and Min.

REFERENCES (refer also to publication lists in PERSONNEL)

1. K-D Gundermann and F. McCapra, *Chemiluminescence in Organic Chem.*, Springer-Verlag, 1990.
2. A. Campbell, *Chemiluminescence.*
3. Special issue, "Bioluminescece in the Sea," *Naval Research Reviews* **45** (1993) (#2).
4. J. Lee, "Bioluminescence: Biochemistry for Fun and Profit," in D.P. Valenzeno, ed., *Photobiological Techniques*, Plenum, 1991, pp. 297-321.
5. D. Phillips, ed., *Polymer Photophysics*, Chapman & Hall, 1985.
6. R.C. Ropp, *Luminescence and the Solid State*, Elsevier, 1991.
7. G.A. George, "Luminescence in the Solid State," in L. Zlatkevich, *Luminescence Techniques...*, Dekker, 1989, pp. 1-50.
8. J.B. Birks, *Photophysics of Aromatic Molecules*, Wiley, 1970.
9. J.E. Guillet, *Polymer Photophysics...*, Cambridge U. Press, 1984.
10. J.L. Houben and N. Rosato, "Light Migration and Energy Utilization in Biological Systems," in G. Montagnoli and B.F. Erlanger, eds., *Molecular Models of Photoresponsiveness*, Plenum, 1983, pp. 15-38.
11. H. Kuhn and D. Mobios, "Systems of Monomolecular Layers," *Angew. Chem. Int. Ed.* **10** (1971) 620-637.
12. H. Kuhn, "...Evergy Transfer in Molecular Systems," *J. Chem. Phys.* **53** (1970) 101-108.
13. J.D. Swalen, "Optical Properties of L-B Films," *J. Molec. Elect.* **2** (1986) 155-181.
14. T. Arakawa, S.J. Prestelski, W.C. Kenney, and J.F. Carpenter, "Factors Affecting Short-Term and Long-Term Stabilities of Proteins," *Adv. Drug Delivery Reviews* (1993), in Press.

Min). The interfacially oriented protein can then be "assembled" by increasing the pressure and decreasing the area per molecule.

It is also possible to observe the monolayer as a function of pressure/area conditions in a fluorescent set up using the intrinsic fluorescence of luciferin.

We fully expect to see some evidence of ordering and even 2 dimensional domain formation in these monolayers at the air/water interface, similar to what Ringsdorf, Grainger, and others have observed with other proteins at such interfaces (25). Almost all such studies in the past have utilized fluorescent approaches. This will, to our knowledge, be the first such study utilizing the intrinsic luminescence of the film.

5) Monolayer Transfer Study

These will only be done in a very preliminary way in Phase I.

Using our expertise with gradient surfaces, i.e., surfaces in which there is a continuous gradient in wettability along one major dimension of the sample (Hlady), we will be able to quickly screen the deposition characteristics of luciferase monolayers under various constant pressure conditions.

We fully expect that luciferase, transferred to a solid surface directly from the air/water interface, will be denatured and largely inactive (Wang). Since firefly luciferase is believed by many to be a lipoprotein and to require at least a partial lipid environment for its stability and activity (Wang, 20), the same transfer studies will be done with lipid/luciferase monolayers and on solid surfaces to which lipid has been previously transferred, thereby providing a lipidic environment for the luciferase layer.

Such experiments will also be done utilizing trehalose in the subphase, as it is expected to help stabilize both the lipid and the luciferase components of these complex layers.

Clearly there is much we need to know about the air/water interfacial behavior of these systems before we can begin to specify the parameters for the monolayer transfer component of the project. Most of that work will indeed be done in Phase II.

If we are successful in Phase I in producing mono and multilayers of luciferase on solid surfaces, then of course some preliminary characterization of these systems will be performed using the CCD camera system to determine the homogeneity, intensity, and possible polarization state of the luminescence. Again, we expect that most of this work would be during Phase II.

6) Preliminary Studies With Bacterial Luciferase

Tasks 1-4, described above, will be repeated for bacterial luciferase. In this case, at least at present, we are not using recombinant bacterial luciferase, but rather are using bioluminescent marine bacteria (2,3) from which we extract the bacterial luciferase. We expect to enhance the yield of this process to provide ample, inexpensive quantities of purified bacterial luciferase (Min).

If this is not successful (economical), then we will use the recombinant E-coli approach, which is well known and well developed (3). We have an interest in marine bacterial bioluminescence for our other science educational studies and products,

15. J.F. Carpenter and J.H. Crowe, "Modes of Stabilization of a Protein by Organic Solutes During Desiccation," *Cryobiology* **25** (1988) 459.

16. J.H. Crowe, L.M. Crowe, D. Chapman, "Preservation of Membranes in Anhydrobiotic Organisms: The Role of Trehalose," *Science* **223** (1984) 701.

17. C. Colaco, et al., "Extraordinary Stability of Enzymes Dried in Trehalose," *BioTech.* **10** (1992) 1007.

18. B.P. Gaber, et al., "Interaction of Trehalose..." Molecular Modeling," in A.C. Leopold, ed., *Membranes, Metabolism, and Dry Organisms*, Cornell Univ. Press, pp. 231.

19. J.H. Crowe, et al., "Anhydrobiosis," *Ann. Rev. Physiol.* **54** (1992) 579.

20. A.F. Dukhovich, N.N. Ugarova, and I.V. Berezin, "Firefly Luciferase as a Complex of Protein with Lipids," *Dokl. Acad. Nauk SSSR* **289** (1986) 231-233.

21. K. Wood, Ph.D. Dissertation, Department of Chemistry, University of California, San Diego, 1989.

22. R.P. Feynman, QED, Princeton Science Lib., 1985.

23. The details of individual photon emission are not well understood (see refs. 4-13, 22). Although there has been much work on the absorption, fluorescence, and energy transfer properties of molecular assemblies and monolayers, to our knowledge therehave been no such studies utilizing photoproteins. We are wll aware of near- and far-field electromagnetics, evanescent waves, and the role oflocal dielectric properties on fluorescence and luminescence (Hlady, Andrade). Nevertheless, we will not be at all surprised if we find some "surprises" in the luminescent behavior of ordered luciferase films.

24. V. Kratysuk, Abstract, *Conference on Bioluminescence*, Maui, Dec., 1993.

25a. S.A. Darst, M. Ahlers, P.H. Meller, E.W. Kubakel, R. Blankenburg, H.O. Ribi, H. Ringsdorf, and R.D. Kornberg, "Two-dimensional Crystals of Streptavidin on Biotinylated Lipdi Layers and Their Interactions With Biotinylated Macromolecules," *Biophys. J.* **59** (1991) 387-396.

25b. D.W. Grainger, K.M. Maloney, X. Huang, M. Ahlers, M.A. Reichert, H. Ringsdorf, C. Salesse, J.N. Herron, V. Hlady, and K. Lim, "Binding, Interactions and Organization of Proteins with Lipid Model Membranes," in J.D. Gomez Fernandez, D. Chapman, I. Packer, eds., *Progress in Membrane Biotechnology*, Birkhauser Verlag, Zurich, 1991, pp. 64-82.

FACILITIES

PSI has 1,000 square feet of research space at 390 Wakara Way in the University of Utah Research Park. Most of the work will be done by Dr.s Scheer and Andrade and Mr.s Wang, Min, and a budgeted technician, in that space.

Funds are budgeted for the construction of a small monolayer trough which is already designed and which already exists at the University of Utah. It will be a simple

matter to duplicate this trough for the extensive studies in this Phase I project. It will require only two to three weeks to construct the trough; in those first several weeks of the project we will use the facilities available to us at the University of Utah through our membership in the Center for Biopolymers at Interfaces. These include a large Lauda conventional monolayer trough, as well as a small trough suitable for use on a microscope stage and for the optical monitoring experiments described above.

We have also budgeted the lease of a fiber optic spectrometer/fluorometer. We have budgeted a six month lease at 10% of purchase price/month. We expect to request the remaining funds for purchase of the instrument in the Phase II budget. 75% of the lease funds can be applied to the purchase price.

The other facilities in our lab are adequate for routine work. Any special, additional equipment or facilities which we might require are available through our affiliation with the University of Utah.

CONSULTANTS

Dr. Vladimir Hlady is budgeted at about one hour/week.

Dr. Hlady is an expert in interfacial fluorescence and interfacial luminescence. He also has personal experience with the interfacial behavior of luciferase and is an expert on protein behavior at interfaces. He and Dr. Andrade have worked very closely over the last ten years. He is also deeply committed to chemi- and bioluminescent based technologies. See biographical sketch.

His biosketch follows.

PERSONNEL

Dr. Robert Scheer, Principle Investigator, received his Ph.D. in Materials Science and Engineering in 1993 and has been working for PSI. He is Principle Investigator of PSI's Lableess Lab™ in Polymer Materials, an SBIR Phase I from the National Science Foundation. Rob's background is in polymers, polymer structure and morphology, and the modeling and testing of polymeric materials. Although he has not had an extensive biochemistry or biomaterials background, he is certainly learning fast. His abbreviated vita is appended to this section.

Dr. Joseph Andrade is President, founder, and Chief Scientific Officer of PSI. Joe has worked extensively with proteins, enzymes and antibodies for the past 25 years, particularly with their behavior at surfaces and interfaces. About 5 years ago he became quite interested in bioluminescence and particularly in the firefly and bacteria luminescence systems. He will provide the interfacial biochemistry and bioluminescence expertise required. Although Joe is three quarter time Professor of Materials Science and Bioengineering at the University of Utah, he spends a quarter of his time with Protein Solutions, Inc. and will be a major participant in this project. His abbreviated vita is also attached.

Mr. C.Y. Wang is a graduate student working under Joe Andrade's supervision at the University of Utah. Mr. Wang has worked on the firefly luciferase system for nearly four years and will be completing his Ph.D. studies on this system in late 1994 or early 1995. He will thus be available during a major part of the Phase I award to conduct some of the firefly luciferase-based studies and to transfer his rich, comprehensive expertise on that system to the others involved in the project. There is a distinct possibility that Mr. Wang would elect to stay on in a post-doctoral capacity on the Phase II project.

Mr. Dong Min is also a graduate student under Joe Andrade's direction, working on bacterial luciferases. He is following in Mr. Wang's footsteps and basically doing a similar series of studies, but with the bacterial luciferase rather than the firefly system. He will work closely with Mr. Wang to learn the techniques and methodologies, which he can apply to the bacterial problem, as well as being responsible for the luciferase studies when Mr. Wang completes his studies.

Both students will be supported directly through PSI. The University of Utah and Protein Solutions, Inc. have a Technology Transfer agreement and a full potential Conflict of Interests/Disclosure Policy. PSI also has a research contract with the University of Utah through which the more specialized studies which require University equipment and access will be conducted.

PSI is committed to an active disclosure and publication policy and will in no way inhibit or delay the students or faculty members involved in the publication of their discoveries and results in the general scientific literature. Mr. Min will be full time on this project and would continue as a full time student supported by and working at PSI during the Phase II portion of the project. The timing is such that he would be completing his Ph.D. studies at about the end of the tow year Phase II period.

The technician on the project will likely be *Mr. Andras Pungor*, who has worked extensively with Andrade, Hlady, and Scheer over the past several years on a number of projects. Mr. Pungor is an experienced electro optical mechanical engineer who has designed and built spectrophotometers and fluorometers in his native Hungary and has designed, built, and utilized a variety of electrochemical equipment, other analytical equipment, and scanning force microscopy equipment at the University of Utah.

OTHER PROPOSALS

PSI has no other pending proposals on this topic. It is currently working on a Phase I SBIR from the National Science Foundation: The Lableess Lab™, Polymer Materials.

Proposals are, however, being prepared for the NSF STTR Program in Chemical Instrumentation (tentative title: Bioluminescence-Based Multi-Enzyme Sensors), submission date, March 15, and possibly for the Department of Energy STTR Program, submission date, April 11, Topic 6, Biotechnology Resources and Applications. This latter one would likely be on the culture of marine bacteria.

There is no overlap between these Phase I applications and the work proposed in this Phase I proposal.

BUDGET JUSTIFICATION

We are budgeting the purchase of a fiber optic spectrometer/luminometer with appropriate sensitivity and versatility to be useful for a wide range of the studies proposed, for both the Phase I and Phase II programs. The expense of such an instrument is such that it will be necessary to lease it for the Phase I Program and then complete the purchase with Phase II funds; that is reflected in the budget.

The other major item in the budget is the construction of the small Langmuir trough discussed in the section on work plan.

"Testing Services" are also included for instrument time charges associated with the use of equipment in the Center for Biopolymers at Interfaces, as described earlier.