OMB No. 0925-0195
Expiration Date 1/31/95

Department of Health and Human Services
Public Health Service

Small Business Research Program
PHASE I GRANT APPLICATION

Follow instructions carefully.

Leave blank – for PHS use only.

Type	Activity	Number
Review Group		Formerly
Council Board *(Month, year)*		Date Received

TLE OF APPLICATION *(Do not exceed 56 typewriter spaces)*

irect Reading, Quantitative Bioluminescent Biosensors

LICITATION NO. SBIR 94-3

3. PRINCIPAL INVESTIGATOR

☒ New Investigator

AME *(Last, first, middle)*

heer, Robert

3b. SOCIAL SECURITY NO.

253-35-1321

OSITION TITLE

- Research Scientist

3d. MAILING ADDRESS *(Street, city, state, zip code)*

390 Wakara Way, Room 31
Salt Lake City, Utah 84108

ELEPHONE AND FAX *(Area code, number, and extension)*

801 585-5361
801 585-5361 (call first)

MAN SUBJECTS

a. ☐ NO ☐ YES — If "Yes," exemption no. of — IRB approval date — 4b. Assurance of compliance no.

5. VERTEBRATE ANIMALS

5a. ☒ NO ☐ YES — If "Yes," IACUC approval date — 5b. Animal welfare assurance no.

TES OF PROJECT PERIOD

11/1/94 *Through:* 4/30/95

7. COSTS REQUESTED

7a. Direct Costs: $ 72,188

7b. Total Costs: $ 97,454

FORMANCE SITES *(Organizations and addresses)*

otein Solutions, Inc.
) Wakara Way, Room 31
lt Lake City, Utah 84108

9. APPLICANT ORGANIZATION *(Name, address, and congressional district)*

Protein Solutions, Inc.
390 Wakara Way, Room 31
Salt Lake City, Utah 84108
Cong. Dist. #

10. ENTITY IDENTIFICATION NUMBER

Federal Tax #: 87-0451813

11. SMALL BUSINESS CERTIFICATION

☒ Small Business Concern ☐ Women-owned
☐ Socially and Economically Disadvantaged

OTICE OF PROPRIETARY INFORMATION: The information identified by iks (*) on pages _____ application constitutes trade secrets or information that is commercial ncial and confidential or privileged. It is furnished to the Government in ence with the understanding that such information shall be used or sed only for evaluation of this application, provided that, if a grant is ed as a result of or in connection with the submission of this application, ivernment shall have the right to use or disclose the information herein extent provided by law. This restriction does not limit the governments o use the information if it is obtained without restriction from another .

14. NAME OF CORPORATE OFFICIAL

J.D. Andrade
TELEPHONE: 801 585-5361
FAX: 801 585-5361 (call first)
TITLE: President
ADDRESS: Protein Solutions, Inc.
390 Wakara Way, Room 31
Salt Lake City, Utah 84108

SCLOSURE PERMISSION STATEMENT: If this application does not result ıward, is the Government permitted to disclose the title only of your ed project, and the name, address, and telephone number of the ste official of your firm, to organizations that may be interested in ting you for further information or possible investment?

YES ☐ NO

BITNET/INTERNET ADDRESS:

JDANDRAD@CC.UTAH.EDU

NCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that the nts herein are true, complete and accurate to the best of my knowledge. I am that any false, fictitious, or fraudulent statements or claims may subject me to , civil, or administrative penalties. I agree to accept responsibility for the scientific t of the project and to provide the required progress reports if a grant is awarded ult of this application.

SIGNATURE OF PERSON NAMED IN 3a *(In ink. "Per" signature not accepted.)*

Robert J. Scheer

R. Scheer

DATE: 4/13/94

LICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the nts herein are true, complete and accurate to the best of my knowledge, and he obligation to comply with Public Health Service terms and conditions if a grant ded as a result of this application. I am aware that any false, fictitious, or nt statements or claims may subject me to criminal, civil, or administrative s.

SIGNATURE OF PERSON NAMED IN 14 *(In ink. "Per" signature not accepted.)*

J.D. Andrade

DATE: 4/12/94

6-1 (Rev. 1/92)

PRINCIPAL INVESTIGATOR:

ABSTRACT OF RESEARCH PLAN

NAME, ADDRESS, AND TELEPHONE NUMBER OF APPLICANT ORGANIZATION

Protein Solutions, Inc. (PSI)
390 Wakara Way, Room 31
Salt Lake City, Utah 84108
(801) 585-5361

YEAR FIRM FOUNDED	NO. OF EMPLOYEES *(include all affiliates)*
1988	5

TITLE OF APPLICATION

Direct Reading, Quantitative Bioluminscent Biosensors

KEY PROFESSIONAL PERSONNEL ENGAGED ON PROJECT

NAME	POSITION TITLE	ORGANIZATION
R. Scheer	Principle Investigator	Protein Solutions, Inc. (PSI)
J. Andrade	Co-Investigator	PSI
To be appointed	Post Doctoral Fellow	PSI
D. Min	Graduate Student	PSI
V. Hlady	Consultant	University of Utah
J. Janatova	Consultant	University of Utah

ABSTRACT OF RESEARCH PLAN: State the application's long-term objectives and specific aims, making reference to the health-relatedness of the project, describe concisely the methodology for achieving these goals, and discuss the potential of the research for technological innovation and commercial application. Avoid summaries of past accomplishments and the use of the first person.

The abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. Since abstracts of funded applications may be published by the Federal Government, do not include proprietary information. DO NOT EXCEED 200 WORDS.

This project is an outgrowth of two different R & D efforts leading to two product lines originally targeted to science education. We now find that these two unique and very different technologies can be combined to produce what one might call analytical chemistry without instruments. The basic idea is to build the entire instrument into the sampling device.

Readout and detection utilizes light produced by bioluminescence. Specificity is provided by the use of enzymes. In this project we focus on direct analysis of NADH* using bacterial luciferase coupled with other enzyme-mediated biochemical processes which either produce or consume NADH and/or FMN*.

Light intensity is a complex function of enzyme, substrate, and NADH concentrations and is a dynamic kinetic process. Light intensity is notoriously difficult to quantitate using the human eye. The sensor is therefore designed so as to produce light in a particular area of a two dimensional sample slide. It is the position of the light, rather than its intensity, which is related to substrate concentration. Man can directly detect the spatial position of a light source in a reproducible and quantitative manner.

The sensors utilize stable bacterial luciferase films coupled to a capillarity-based sample distribution system. The result will be specific, quantitative, rapid, direct reading, inexpensive and disposable analytical devices. Analytes suitable for initial development include simple alcohols, sugars, and amino acids.

(A)

* NADH = nicotine adenine dinucleotide
FMN = flavin mononucleotide

Provide key words (8 maximum) to identify the research or technology.

Biosensor, Bioluminscence, Luciferase, NADH, Enzyme, luminscence

Provide a brief summary of the potential commercial applications of the research.

Clinical measurements, home-care, education, biotechnology, personal diagnosis, bedside monitoring, patient monitoring.

Using continuation pages if necessary, describe the specific functions of the personnel and consultants. Read the instructions and justify all costs requested.

Dr. Robert Scheer, Principle Investigator, will be half time on this project for its six month duration.

J.D. Andrade is budgeted at one full month, but will spend considerably more time on the project. He is a Professor of Bioengineering and Materials Science at the University of Utah and has about 4.5 months out of 12 months to devote to other duties, including his activities at Protein Solutions, Inc.

Dr.s Scheer and Andrade will be assisted by a half time post doc who is a full time employee of Protein Solutions.

D. Min is a student under J. Andrade's direction at the University of Utah and will work essentially full time on this project during its 6 months duration. This is part of his Ph.D. work in the Department of Materials Science at the University of Utah. Protein Solutions, Inc. has a Technology Transfer Agreement with the University and the University is fully aware of this working relationship. Mr. Min's activities and progress towards his degree will in no way be hindered by this affiliation. PSI encourages full and timely publication.

Dr. Vladimir Hlady, Associate Professor of Bioengineering, and Dr. J. Janatova, Associate Research Professor of Bioengineering, are both involved as consultants; in Dr. Hlady's case, to aid with 2-D photon imaging of the sensor films and in Dr. Janatova's case, to assist and advise with protein purification characterization and related biochemistry topics. Their letters of collaboration are enclosed. Other expenses are for sample analyses by outside laboratories. These include surface analysis by local laboratories, as well as elemental and related chemical analyses.

Total Direct Costs are $72,188. No fixed fee is requested. PSI's Indirect Costs are 35% of Total Direct Costs. The detailed duties of each of the personnel are listed in the text of the proposal in Table 1.

RESOURCES AND ENVIRONMENT

1. FACILTIES: Describe the facilities to be used and briefly indicate their capacities, pertinent capabilities, relative proximity and extent of availability to the project. Include laboratory, clinical, animal, computer, and office facilities at the applicant organization, at any other performance site listed on the FACE PAGE, and at sites for field studies. Using continuation pages if necessary, include an explanation of any consortium arrangements with other organizations.

PSI has 1,200 square feet of research space at 390 Wakara Way, Room 31, SLC, Utah 84108. Laboratory, computer, and office facilities are adequate for the work proposed. In addition, PSI is a member of the Center for Biopolymers at Interfaces at the University of Utah, a State/University/Industry consortium, and as a member has access to specialized laboratories and equipment at the University. The equipment is available on a fee for service basis, which is budgeted in the Other Costs category.

2. MAJOR EQUIPMENT: List the most important equipment items already available for for this project, noting the location and pertinent capabilities of each.

No unusual or specialty equipment is required for this project. The ink jet printer, necessary for film formation and patterning, is already available. Various microscope and camera detection systems are present. The 2-D CCD camera for detailed imaging and calibration, is available through the University of Utah and our consultant, Dr. V. Hlady. Typical protein purification and characterization facilities will be set up but no special or expensive equipment is required. Dr. J. Janatova's lab is equipped with a full range of protein separation and characterization equipment, and her facilities will be utilized as necessary as part of the CBI membership and services.

Robert J. Scheer
390 Wakara Way, Room 31, Salt Lake City, UT 84112
Ph. (801)585-3128
e-mail (rob.scheer@m.cc.utah.edu)

EDUCATION

Ph.D. in Materials Science and Engineering, September 1993, University of Utah, Salt Lake City, UT. Dissertation emphasis: Mechanical, interfacial, and surface study of composite materials.

B.S. in Mechanical Engineering, 1989, Duke University, Durham, NC. GPA 3.76. Emphasis: Fracture mechanics and failure analysis of polymeric materials.

UNIVERSITY HONORS

National Science Foundation Fellow, University of Utah Graduate Research Fellow, Dean's List Duke University, Academic All American

Duke University Magna Cum Laude, Scholastic Societies: Tau Beta Pi and Pi Tau Sigma.

EXPERIENCE

Principle Investigator
Protein Solutions, Inc. Salt Lake City, UT. 1994 - present. Directed research for the design and implementation of novel science education materials.

Research Assistant
University of Utah, Salt Lake City, UT. 1989 - 1994. Tested mechanical properties of polymers and composites, studied surfaces and interfaces, tested adhesive bonds on the microscopic scale, and developed stress analyses related to materials testing.

Instructor/Tutor
University of Utah and Salt Lake Community College, Salt Lake City, UT. 1991 - present. Planned, instructed, and graded for undergraduate physical science classes. Served as tutor and teaching assistant.

Engineering Technician
Sandia National Laboratory, Albuquerque, NM. Summer, 1988 and Summer, 1989. Designed engineering experiments for failure analysis of ceramic materials, and extensively researched current experimental techniques for determining material fracture toughness. Designed engineering experiments for strength testing of brittle materials, and performed CAD. Interacted with diverse engineering disciplines on a major research project.

AFFILIATIONS: American Society for Mechanical Engineers, ASM International, The Minerals, Metals, and Materials Society, American Physical Society, The Center for Biopolymers at Interfaces

PUBLICATIONS:

Scheer, R.J. and J.A. Nairn. "Variational Mechanics Analysis of Stresses and Failure Analysis in Microdrop Debond Specimens." *Composites Engineering*, Vol. 2, No. 8, pp. 641-654, 1992.

Scheer, R.J. Ph.D. Dissertation, "An Energy Based Analysis of Fiber-Matrix Adhesion." University of Utah, 1993.

Andrade, J.D. and R.J. Scheer. "Applying 'Intelligent' Materials for Materials Education: The Lableess Lab™." *Proc., 2nd Annual Conference on Intelligent Materials*, Tech. Publ. Co., 1994, in press.

OTHER GRANT AND CONTRACT SUPPORT

The only federal grant or contract support is a Phase I SBIR from the National Science Foundation titled, "The Lableess Lab™: Polymer Materials." Dr. Robert Scheer is Principle Investigator of that grant which runs from February 15, 1994 through August 14, 1994. We fully anticipate submitting a Phase II application in support of this work.

A Department of Energy SBIR Phase I was submitted on February 15 titled, "Luminescent films Based on Photoproteins."

A National Science Foundation STTR grant, Phase I was submitted on March 15 titled, "Direct Reading, Quantitative Biosensors for ATP-dependent Processes." That grant is related to the present application in that it does utilize a similar sensor concept, but is implemented using firefly luciferase and ATP "consumase" enzyme, and is intended to analyze and diagnose ATP-dependent processes. There is no direct overlap between the two grants. Although they deal with similar sensor concepts, the different chemical and biochemical systems utilized are completely different. Dr. Scheer is also Principle Investigator in that proposed application.

PSI's other funding arises from stock purchases by its founders and by local investors. It also has some minimal sales income from its science education products.

There is no budgetary or commitment overlap among these various applications.

TABLE OF CONTENTS

Cover Page/Face Page 1
Abstract Page 2
Budget 3
Budget Justification 4
Other Grant and Contract Support 5
Table of Contents 5
BioSketches (Scheer, Andrade) 6
Significance 8
Background 8
Phase I Research Objectives/Specific Aims 16
Phase I Research Plan 18
References 22
Commercial Potential 23
Personnel 24
Consultant Letters (not part of page limitation)
Checklist

SIGNIFICANCE

There are two very special molecules that play unique and central roles in biology, adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide NAD and its phosphate form (NADPH). NADPH is a ubiquitous electron donor and ATP is generally recognized as one of the key energy currencies in biology. The two molecules act in a cyclic manner and can be regenerated or recharged. "They are the basic coupling agents of cellular metabolism." (14). A very large number of biochemical enzyme processes involve one of these two molecules.

It is very convenient that biology has evolved bioluminescence processes dependent on these two molecules: the firefly luciferase reaction, which acts on firefly luciferin in the presence of ATP to produce an oxidized product which chemiluminesces with a very high efficiency; and the bacterial luciferase reaction, which in the presence of alkyl aldehydes, such as decanal, together with NADPH, also produces an excited product which chemiluminesces also of relatively high efficiency. Both reactions require the presence of oxygen. The luciferases involved are different. The "luciferins" involved are different (1, 2, 20).

There is a large literature on the development of biosensors for ATP and ATP-dependent processes and for NADPH and NADPH-dependent processes, using the firefly and bacterial luciferase enzymes, respectively. Such biosensors generally involve fiberoptic or other waveguided means of delivering the luminescence to a device which can accurately measure light intensities (13,15-17, 23-28, 31). Although one of the most portable and most sensitive photon detectors available to the scientist or physician is his or her own eye, it is notoriously difficult to calibrate for accurate measurements of even relative light intensity. The human two dimensional photon detection system, however, can reliably and accurately measure changes in spatial position.

We propose to utilize this characteristic as the readout system for a quantitative, inexpensive, disposable, analytical device for the analysis of chemicals involved in NADH-dependent enzyme processes.

BACKGROUND

Protein Solutions, Inc. (PSI) was founded in early 1988 to develop and produce innovative science educational products and personal sensors. 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 (35).

Bioluminescence is an enzyme dependent chemical oxidation process which results in photon emission (1, 2). The photoproteins involved in these processes, the luciferase series of oxidative enzymes, are now readily available in inexpensive form, produced by recombinant means (10).

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, air/water interfaces, and its denaturability or stability in solution (36). We have recently initiated a similar set of studies dealing with bacterial luciferase,

the enzyme responsible for bioluminescence in various species of marine bacteria. These studies, together with our commercial work on bioluminescent phytoplankton (the dinoflagellate *Pyrocystis lunula*) have provided a deep and diverse background in practical bioluminescence.

Luciferases are extensively used as labels for a wide range of clinical diagnostic chemical tests (12, 18, 25). Since the firefly luciferase reaction is dependent on an adenosine triphosphate (ATP) co-factor, it has been extensively used in the development of biosensors for the measurement of ATP. Likewise, bacterial luciferase utilizes a ubiquitous chemical in bioenergetic pathways, and has therefore been widely applied for biosensing applications. 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 (31, 19, 13).

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 (8). 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 (3-8). 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 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 (3-6).

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. We are already proceeding to develop this technology for educational and commercial purposes. Mr. Dong Min is now performing similar studies with bacterial luciferases, suggesting similar results for bacterial luciferase.

PSI has been working closely with the Center for Biopolymers at Interfaces and the Dept. of Bioengineering at the University of Utah for the past five years. In addition, the Dept. of Bioengineering, particularly Dr.s V. Hlady and J. Janata, has a wealth of experience in several technologies which are important to the proposed work. They have extensive experience in fluoroimmunosensors (37), thin film and waveguide optics, protein and enzyme immobilization (34, 36), protein and enzyme stabilization, protein and enzyme purification and characterization, and, more recently, the extensive experience in bioluminescence of both intact organisms and purified luciferases (35, 36), as well as some experience with dry reagent analytical systems. All of these are important and help provide the basic science foundation for this project. More recent studies dealing with capillarity and spreading, again primarily directed to dry reagent systems, as well as to protein separation for science education purposes, are also important components.

This very extensive multi- and inter-disciplinary background is what makes this project possible. PSI's existing Technology Transfer agreement with the University of Utah includes the technologies and topics required for this application.

Technical Approach/Rationale

The goal is to develop miniature analytical biochemistry systems, specific for particular aqueous analytes which are quantitative, rapid, direct reading (by self luminescence), sensitive, with long shelf life, stable, disposable, and inexpensive.

In this project we are proposing to assess the feasibility of such sensors for NADH-dependent processes utilizing bacterial luciferase. Because there are literally hundreds, perhaps thousands, of enzymes which are involved with NADH consumption or ATP production, most of which are specific to another chemical substrate, for example, alcohols, sugars, amino acids, and bile acids, these "front end" enzymes permit the development of individual sensors or sensor channels for each of those substrates (28, 29, 13-17, 23, 24).

In this Phase I feasibility application we do *not* consider the front end enzymes. Rather, we will focus on the enabling technology to measure NADH concentration. A variety of sensors already exist for measuring ATP through the use of firefly luciferase (13, 15) and several for NADH using bacterial luciferase. However, these require sophisticated light detection and calibration systems, because they rely on light intensity.

The bacteria bioluminscent enzyme system consists of an NAD(P)H:FMN oxidoreductase (1,2,13,44) and a luciferase which emits light at 490 nm in the presence of FMN, NAD(P)H, a long-chain aliphatic aldehyde and molecular exygen. $FMNH_2$ is produced via the appropriate oxido-reductase:

$$NAD(P)H + FMN + H + \xrightarrow[\text{oxidoreductase}]{\text{NAD(P)H:FMN}} NAD(P)^+ + FMNH_2$$

The $FMNH_2$ and a long chain aldehyde (decanal) (RCHO) are then acted upon by bacterial luciferase:

$$FMNH_2 + RCHO + O_2 \xrightarrow{\text{luciferase}} FMN + RCOOH + H_2O + LIGHT$$

The quantum yield of this reaction is of the order of 10%. Various biochemicals and enzymes (E) can be analysed by coupling the luciferase and the oxidoreductase to a third reaction which produces NADH or NADPH:

$$XH_2 + NAD(P)^+ \xrightarrow{E} X + NAD(P)H + H^+$$

Either XH_2, the reduced substrate or the dehydrogenase (E) catalysing the oxidation of XH_2, can be measured.

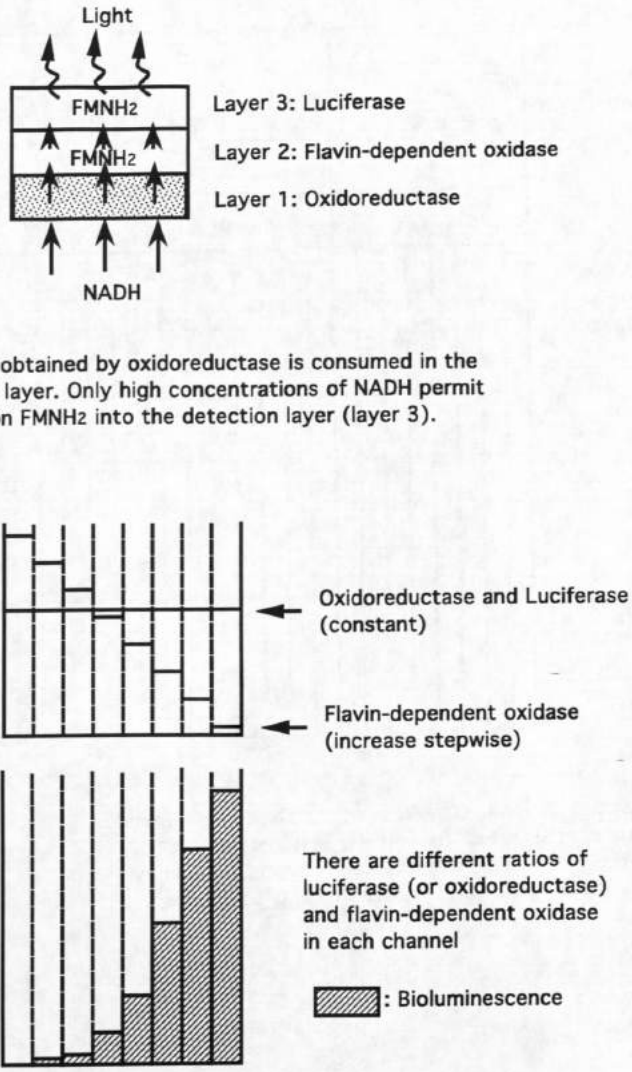
Our unique and novel approach is to produce a spatial distribution of the emitted light which is proportional to the analyte concentration. The human photon detector is therefore used primarily to assess *spatial position* rather than absolute light intensity. This will be achieved by carefully controlling the luciferase concentration in the sensing gel and by modulating, tuning, and "filtering" the $FMNH_2$ concentration through the use of a second $FMNH_2$ consuming enzyme, a Flavin-dependent oxidase. This oxidase is selected to have a very rapid turn over, and to serve as an concentration regulator to the sensor. The FMN consumase is deposited in different spatial regions of the one or two dimensional sensor at different concentrations. At the top of Figure 1 we show a layer of the oxidase immobilized in an agarose gel and containing its specific substrate, an aminoacid in the case of amino acid oxidase (29). The optimum oxidase to be used is one of the objectives of this study.

A sample containing NADH is distributed and diffuses into the oxido-reductase layer, producing $FMNH_2$ which is then regulated or filtered by the flavin-dpendent oxidase layer. The "filtered" $FMNH_2$ then diffuses into the luciferase gel producing light. The luciferase gel layer of course contains the optimum concentration of the decanal substrate. Photons are emitted which are then detected by the operator viewing from the top of the page. The middle panel shows that the device consists of eight channels, each with a different oxidase concentration. For a uniform input of $FMNH_2$ into the oxidase channels, a different $FMNH_2$ concentration is delivered to the uniform luciferase layer. This results in the light emission pattern shown on the bottom. Depending on the specific oxidase concentrations, the luciferase concentration, and the concentration of NADH, the light output will be a maximum where the oxidase concentration is minimum and will be a minimum where the oxidase concentration is maximum.

By appropriate design of these concentration ratios, and by appropriate selection of an oxidase with the appropriate turnover rate, which is matched to the delivery of $FMNH_2$ into the oxidase gel, a specific spatial illumination pattern is obtained. The human eye is very good at detecting changes in contrast and spatial position.

Figure 2 shows three NADH concentrations, eight oxidase channels, and two different luciferase concentrations. NADH and$FMNH_2$ concentration is schematically represented by the length of the arrows and is shown being delivered to the oxidase gel layer, resulting in a change in the $FMNH_2$ concentration delivered to the luciferase gel. That concentration is shown being delivered to a uniform luciferase gels of two *different* luciferase concentrations. The figure thus shows two different sensors, each exposed to three different NADH concentrations. There is obviously an increase in the light intensity as a function of increased NADH; more importantly is the fact that the spatial position, the inflection between high and low light output, changes as a function of NADH concentration. This means that a *spatial position is directly correlated with NADH level.* Spatial position can be measured by an operator viewing the device without special instruments and without great concern for dark adaptation or specific intensity values. There are of course significant kinetic issues which must be addressed -- such analyses are major parts of the Phase I objectives and research plan.

There are many possible design concepts for such a sensor. Figure 3 shows a dual sensor (two luciferase concentrations in a rectangular configuration); other designs will be considered during the Phase I study. We rely on dry reagent technologies and a capillarity-driven analyte sample delivery system (22, 38, 39). On the top of Figure 3 the sample is deposited over a rectangular area designed to accommodate a constant volume; the sample is distributed by wicking uniformly along the long dimension of the sample. In the right half of the rectangular device we assume the luciferase gel has a high concentration with a low concentration on the left half.



$FMNH_2$ obtained by oxidoreductase is consumed in the second layer. Only high concentrations of NADH permit diffusion $FMNH_2$ into the detection layer (layer 3).

Fig. 1 Basic principle of the "spatially-sensitive" NADH sensor using luciferase, oxidoreductase, and flavin-dependent oxidase.

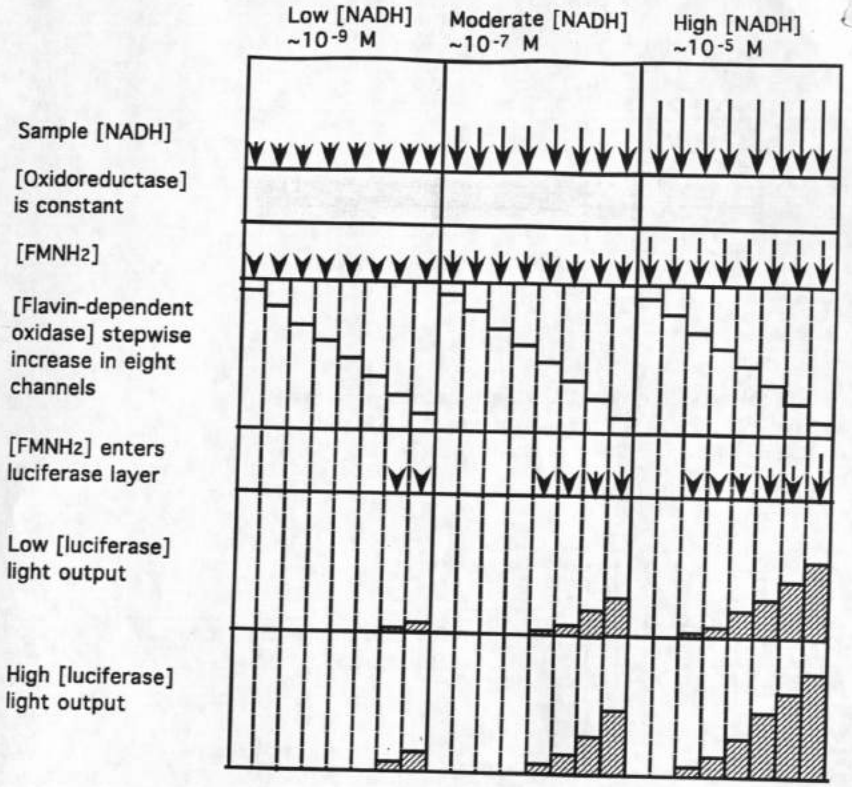


Fig. 2 Spatial detection of analyte concentration. The flavin-dependent oxidase serves as a spatial "filter" for $FMNH_2$. By tuning analyte concentration to the turnover rate of the analyte consuming enzymes, a spatial pattern is produced which is related to applied analyte concentration.

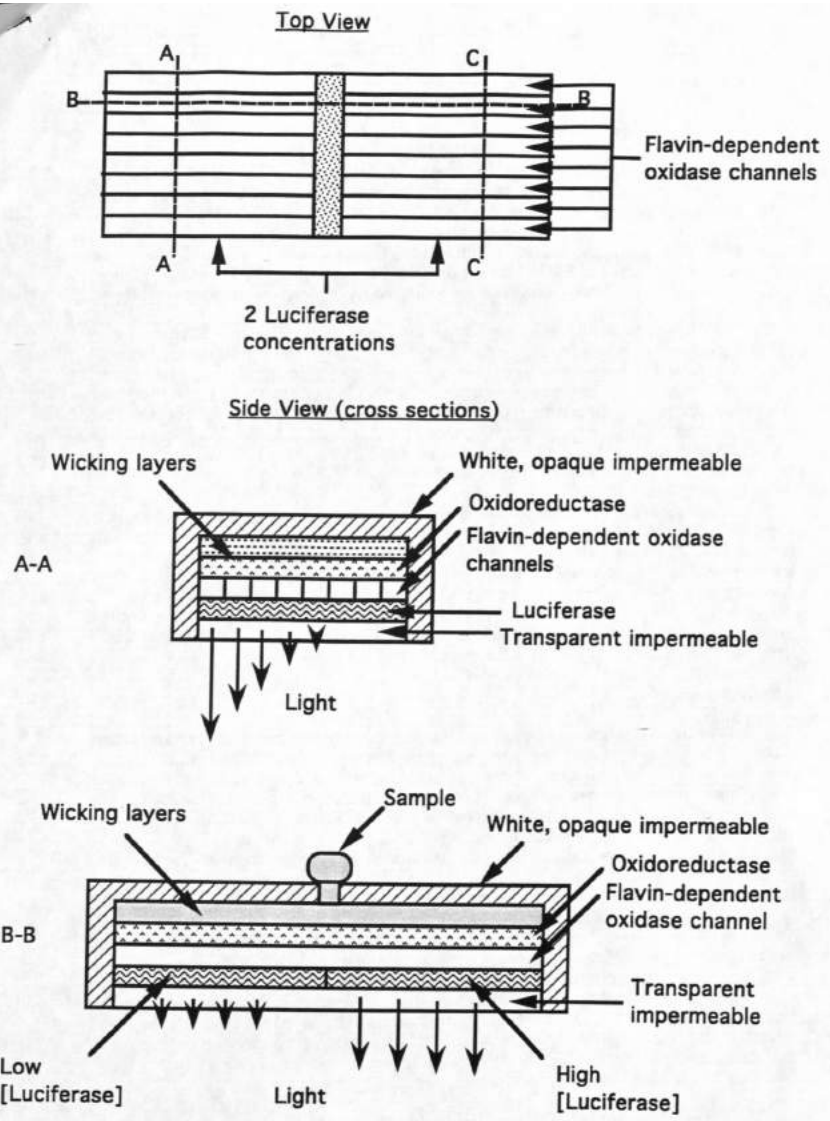


Fig. 3 Preliminary design concept for the spatially-sensitive NADH sensor involving dry reagent technologies and a capillary-based analyte (sample) delivery mechanism.

In the other dimension of the device we show the eight oxidase channels. As one goes from the center of the device to the right or left in the top view, we have to understand that the NADH is being delivered by wicking and that the amount delivered will be a function of distance from the center. The side views are AA and BB sections. AA is a section through the eight oxidase gels in the left hand portion of the device. Here you see the wicking layer which has distributed the sample to this part of the device. The NADH will then move into the various channels where it will be acted upon, resulting in different concentrations of $FMNH_2$ being delivered to the uniform luciferase gel. The different intensities of photon emission are indicated by the length of the photon arrows.

The BB cross section is a longitudinal cross section; we have not attempted to show the kinetics of wicking. Depending on the feasibility studies to be done in Phase I, we may require a timing step prior to luminescence observation. It may also be possible to design the device so the timing step is not critical. Going back to the top view, looking from the bottom, imagine a pattern of light emission, varying with time, to the right and to the left, and varying in spatial position along the AA and CC axes. The intensities and spatial positions along AA and CC will be different because these two sections of the device involve different luciferase concentrations.

A number of other variables which must be optimized include the actual sample size to be delivered, the rate of wicking, the diffusion of the appropriate substrates into the two gels, and the specific time or times when the luminescence should be observed by the user. Although one could argue that there are many variables and therefore considerable complexity in the system, it is that very complexity which allows us to design and develop a device in which one can simply *see* the analyte concentration by virtue of an optical luminescent pattern, visually detected by the operator without the need for other instruments. That is the potential and the challenge.

Anticipated Benefits:

Patterns and images are much easier to detect, to remember, and to process than numbers. Man is exquisitely constructed to deal with visual patterns and pattern recognition. The design, development, and application of analytical devices which are self luminescing and generate a specific pattern which can be directly correlated or related to analyte concentration, has enormous potential benefits. In addition to greatly simplifying routine measurements in a range of analytical, environmental, biotechnological, and medical/diagnostic laboratories, such technology has the potential for being applied widely in schools, education, and in the home for a variety of monitoring, diagnostic, and other measurement purposes.

Looking at health care alone, a one trillion dollar per year industry in the United States 2, 12, 32), chemical/clinical diagnostics accounting for perhaps 5-10% of that, and with the growing role of analytical chemistry in the biotechnological and environmental areas, it has been estimated that the biosensor industry is in the range of one to ten *billion* plus dollars per year (32). With the growing interest in home-based clinical chemistry measurement and a growing movement towards home care and personal care, it is clear that the opportunity for simple, inexpensive, disposable, and specific analytical biochemistry devices should grow dramatically (32).

It is important to note that our spatially sensitive NADH sensing system is also adaptable to the firefly luciferase ATP system and to a variety of chemiluminescent systems (12, 21). It is also important to note that, with the exception of the firefly and bioluminescent marine bacteria, most of the 30 or so other different, unique

bioluminescent systems in nature have yet to be extensively applied to analytical chemistry (1), with the possible exception of calcium monitoring based on aequorin and related photoproteins. It has been estimated that there are at least 30 and may indeed be several hundred different, unique, bioluminescent systems involving different luciferases, different luciferins and different co-factors (1, 2, 20). That all remains to be explored and applied.

PHASE I RESEARCH OBJECTIVES

The Phase I research objectives are summarized in Table 1 which is divided up into three general categories: Fundamental Data (Obj.1-4); Device Design and Prototype (Obj. 5-7); Testing and Evaluation (Obj. 8-9); and Preliminary Work for Phase II (Obj. 10-11). Responsibilities are indicated in the far right column. The eleven tasks are briefly explained here and described in more detail in the next section.

Table 1. Summary of Phase I Research Objectives.

Task	**PSI**
P.I.	**(Scheer)**
Fundamental Data:	
1. Protein Preparation/Characterization	Min/Janatova
2. Luciferase gels/oxidase/trehalose/ agarose/starch	Min/postdoc
3. Gel spreading/preparation/stability	Scheer
4. Capillarity -- sample delivery and transport	postdoc
Device Design and Proto-type:	
5. Simulation of capillarity, gel diffusion and enzyme reaction rates	Scheer/postdoc Andrade
6. Gel/Enzyme Patterning -- Printing	postdoc/Hlady
7. Device Design and Fabrication	Scheer/postdoc.
Testing and Evaluation:	
8. Optical Imaging	Scheer/Hlady
9. Device Testing	Scheer/postdoc.
Preliminary Work for Phase II:	
10. "Front End" Enzymes -- Recommendation	Andrade/postdoc
11. Firefly System -- Comparison	Andrade/postdoc

1. *Protein Preparation and Characterization.* We are experienced with the production of firefly luciferase by recombinant means from E. coli (10). The bacterial luciferase source is cultures of marine, bioluminescent bacteria, followed by extraction and purification of the bacterial luciferase (42,43). In addition the oxidases will also be purified and characterized. At the present time this is likely to be an amino acid oxidase, although a number of other possibilities will be examined. This part of the project will prepare the proteins in suitable quantities for the other components.

2. *Proteins Immobilized in Agarose and/or Starch Gels Containing Trehalose.* Trehalose is a stabilizer for both the gel and the protein, permitting it to be totally

dehydrated and stored, and then rehydrated at a later date to full enzyme activity. Optimum conditions for the preparation of gels with the various enzyme concentrations desired will be explored. This is technology developed at the University of Utah and is already under transfer to PSI for firefly luciferase applications.

3. *Gel Spreading, Preparation, and Stability.* The gel solution (sol) has to be applied and spread on suitable supports in a uniform and homogeneous manner. At present this is done by a dipping or casting process. PSI will also develop technology of preparing thin gel layers by various printing processes (see Task 6 below).

4. *Capillarity: Sample, Delivery and Transport.* The sample containing the analyte will be deposited on a suitable device and spread across the appropriate portions of the gel by a capillarity and rehydration process. This technology is already applied in existing dry reagent chemistry diagnostic kits (38, 45, 46). There is experience at the University in this area, largely on thin layer chromatographic processes involving aqueous media for protein separation. That work will be initially done at the University and then transferred to PSI.

5. *Simulation of Capillarity Gel Diffusion and Enzyme Reaction Rates.* The selection of optimal conditions will be greatly aided by a theoretical model and simulation which includes capillarity-based analyte delivery, the diffusion of analyte through the gel, and its interaction with enzymes. The various turnover numbers will be simulated. Existing enzyme kinetic models, diffusion models and capillarity models will be used.

6. *Gel Enzyme Patterning and Printing.* J. Andrade experimented with ink jet printing for the deposition of proteins for biosensor applications some five years ago. Screen printing is now quite common in the biosensor community. We are confident that we can "ink jet" print and spray print protein/agarose and/or protein/starch solutions under conditions which will form the patterns desired for the multi-channel sensor.

7. *Device Design.* Based on the results of Tasks 1-6 above, and continued extensive discussion, the design for a prototype device will be finalized within the first four months of the project. The first decision is whether to use a rectangular device with spreading and analyte delivery in two directions (as in Figure 3), or to use a circular, radial multi-directional device. The decision will rest in large part on the results of Task five.

8. *The Prototype Devices Will Be Tested and Evaluated Through Bioluminescence Intensity, Spatial Distribution, and Ability to detect and determine NADH Concentration.* This will be done at the University using the optical imaging laboratory at the Center for Biopolymers at Interfaces with the assistance of Dr. V. Hlady, Consultant, as well as visually with a number of disinterested volunteers. CCD camera patterns and visually determined patterns will be compared to see if any particular training or experience is required for disinterested, unaided observers.

9. *Device Testing.* Task 8 will be extended to a range of NADH concentrations of normal analytical significance and interest. In addition, short term storage, dehydration, stability, and reproducibility experiments will be performed. This will be done in the second quarter in preparation for a thorough reanalysis of Tasks 1-9, development of an optimized device design, and development plan for the Phase II proposal.

10. *Preliminary Work Will Assess the Advisability of Incorporating Other Enzymes for the "Consumase" Portion of the Sensor, as Well as a Range of NADH/FNH$_2$-Dependent Enzymes to Extend the Analysis to Other Substrates.* The so-called "front end" enzymes which would be incorporated in a *fourth gel layer* on the front end of the sensor would thus make the sensor sensitive for other enzyme substrates. This will be an important component of the Phase II application.

11. *A Preliminary Analysis of the Firefly Luciferase System and a Comparison of its Advantages and Disadvantages with the Bacterial Luciferase System.* At this point there is no intention of doing extensive studies with the firefly system or making it an important part of the Phase II project, but nevertheless, given its importance and its complementarity to bacterial luciferase, it is important that such analysis be performed.

Basically, Tasks 1-4, the fundamental work which is the underpenning of the project, will be completed largely in the two months. Tasks 5-7 in months 3 and 4 Tasks 8-9 in month 5, and Tasks 9-11 in month 6.

PHASE I RESEARCH PLAN

1) Protein Preparation and Characterization.

Although bacterial firefly luciferase has been expressed in E-coli, we will use primarily material derived from marine bioluminscent bacteria. The luciferase and oxido reductase are readily extracted and purified from these sources (2, 45, 46).

There are many flavin-dependent oxidases -- our initial work will be with one of the aminoacid oxidases. We will quickly but thoroughly assess the field of flavin-dependent oxidases with respect to Vmax, availability, stability, and suitability for this application.

Our preliminary analysis suggests that a very major difference in V-max is required between the two oxidases (flavin-dependent and luciferase) to obtain the spatial sensitivity and localization of luminescence which is critical to this unique sensor concept. Commercial and reference preparations of amino acid oxidases are available for comparison and standardization purposes. (e.g. Sigma Chemical).

The proteins will be characterized by high pressure liquid chromatography and by non-denaturing and denaturing polyacrylamide gel electrophoresis. The initial protein purification and characterization work will be accomplished via the advice and assistance of our consultant, Dr. J. Janatova.

2) Enzyme/Trehalose Gel.

Our experience on the stabilization of luciferases in gels has been limited to low melting agaroses. There is a report from a Russian group (11) that starch is effective for bacterial luciferase; we have some very preliminary work suggesting that other gels may work under certain conditions. 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.

We expect that trehalose is the most effective additive for stabilizing the protein in polysaccharide gels, i.e., agaroses, celluloses, dextrans, etc. Although we have extensive experience with the stabilization of luciferases in trehalose/agarose gels, we

have little experience with other oxidase enzymes at this point. Trehalose has, however, been used by others for the stabilization of a wide variety of enzymes (6). Therefore, we anticipate no problem with the preparation of the oxidase/trehalose/agarose gel. The gels will be prepared with different enzyme/trehalose ratios. The enzyme activity can be directly measured by delivering reference amounts of $FMNH_2$ and then detecting the resulting $FMNH_2$ diffusion into a luciferase gel, essentially the basis of our biosensor concept. Analogous studies will be performed for the oxido reductase gels (Figure 2).

We will also experiment with dual enzyme gels, that is, trehalose/agarose gels containing both enzymes. Although this was not mentioned in the earlier schematic drawings, oxido-reductase, oxidase, and also oxidase/luciferase gels may indeed have some advantages.

Gels will be dried at different rates, subjected to storage under different temperature conditions, rehydrated at different rates, and evaluated for enzyme activity. We will also do a number of studies in which the rehydration is coupled with NADH analyte delivery, thereby evaluating the kinetics of hydration, enzyme activity recovery, and luminescence signal generation.

The required substrates must also be included in the gel or appropriately delivered -- the particular amino acid for the amino acid oxidase gel and decanal (or other long chain aldehyde) for the luciferase gel layer. Aldehyde competes with $FMNH_2$ for luciferase (43). For optiimal bioluminescence, it is desireable to permit $FMNH_2$ binding followed by decanal. We are investigating several approaches to such staggered substrate delivery, including encapsulating the decanal in very small liposome or gel particles which swell and/or degrade upon hydration, releasing the decanal. Our group has experience in this area stemming from activities on controlled delivery of reagents/drugs for drug delivery and biosensor applications.

3) Gel Spreading.

We will improve the process of preparing thin gel films by a controlled dipping process followed by rapid cooling to induce gelation. We will also experiment with a conventional horizontal casting process. Surface treated Mylar films are commercially available, designed for optimum adhesion to thin agarose coatings, and are widely used for thin layer electrophoresis applications (FMC Corp.). We have considerable experience with these films already. They appear to be in almost ideal support for our enzyme films and perhaps for the prototype devices.

4) Capillarity: Sample Delivery and Transport.

Most existing dry film tabs used for clinical diagnostic applications use a wicking/capillarity means of delivering the analyte to the sample area and volume (38, 39, 45, 46). We have performed studies on the spreading of analyte containing solution, on glass fiber filters, and on protein sample delivery via capillarity on cellulose supports used for thin layer chromatography. We have also studied films prepared from ion exchange particles and size exclusion particles, used in special areas of chromatography, and applied them in thin film form, studied liquid transport and distribution in such films, and utilized them for protein separation. In fact, Protein Solutions, Inc. has another project to develop a simple, 2-dimensional *Protein Card* for the separation of tear proteins, urine peptides, and possibly saliva proteins for educational applications.

PSI has substantial experience and a strong commitment to capillarity-based sample delivery and separation processes. J. Andrade is a surface chemist with 25 years

of teaching and research experience and will personally supervise the capillarity-based experimental and simulation studies.

5) Simulation of Capillarity, Diffusion, and Enzyme Reactions.

The modeling and simulation studies will be broken into three parts during the first half of Phase I and then combined into a comprehensive, more complete model in the second half of Phase I. The parts include:

Capillarity. The parameters required for these simulations are either available in the literature or can be readily determined experimentally using various dyes, coupled with automatic time-dependent imaging of the fluid profile. Indeed this has already been done for a number of other studies.

Diffusion of NADH and $FMNH_2$ in the partially hydrated and fully hydrated gels will be estimated based on data in the literature for molecular diffusion through such gels (30). Because of the wide application of these agaroses for protein and enzyme encapsulation/immobilization, there is a substantial database which already exists and which we can adapt for our analyses.

Enzyme reaction rates will be determined experimentally for our particular preparations and will be used in standard enzyme kinetic models employing single enzyme/dual substrate and dual enzyme/single substrate competitive analysis.

The problem of course becomes more complex when we attempt to couple and combine the three individual phenomena to a complete simulation of our proposed device. Nevertheless, we are confident that the modeling exercise will prove to be very helpful in enhancing our understanding of the various phenomena and their coupling, and in the design of experiments with which to determine the optimum characteristics and specifications of our prototype devices.

6) Gel Patterning and Printing.

Some years ago we utilized ink jet printing for multi-channel biosensor applications -- proteins could be deposited in various patterns on paper substrates. This technology was later modified to produce protein patterns on rigid planar substrates. The process is to spray the enzyme/trehalose/agarose solution, followed by a drying step. The process is repeated, under spatial control, for the various enzyme gel layers required.

Given our earlier experience with protein patterning using moderately viscous protein solutions, we anticipate no serious problem with this part of the project. We expect that we will have to develop a local heating system for the cartridge and tubing on the ink jet to prevent the agarose solution from gelling prior to contact with the support which is being "printed." Technical staff responsible for this work have already had extensive experience using ink jet printing and related patterning techniques for chemical and biosensor development.

7) Device Design and Fabrication.

The understanding and experience derived from Tasks 1-6 will permit us to proceed to optimize our device design and fabricate prototypes. We anticipate no serious problems here. We will also perform a number of assessments of user preference. That is we will simulate the area and spatial distribution of luminescence and the expected photon flux of luminescence intensity for a variety of observers, aimed at assessing the

optimum pattern in spatial distribution to permit the observer to derive the most information regarding substrate concentration from light spatial position. Based on these perception studies and the data from Tasks 1-6, a set of prototype devices will be constructed.

8) Optical Imaging.

The prototype devices will then be exposed to appropriate concentrations of NADH and luminescence imaged with a sensitive CCD camera, capable of near single photon detection, as a function of time. We will thus be able to follow the development of the capillarity, diffusion, and enzyme reaction in real time on prototype devices, and on the various components of those prototype devices. The sensitive, time dependent, two dimensional optical imaging will allow us to really characterize these prototypes, and to quantitatively determine the photon yield as a function of spatial position and time. This work will be performed at the University of Utah in the Optical Imaging Laboratory via our consultant, Dr. V. Hlady.

Our preliminary calculations and suggestions suggest that the photon flux will be such that it can be detected by a normal dark adapted eye. During the Phase II program we expect to further enhance and optimize the device, and to design a packaging and inexpensive viewing port, which would enable the observation to be made with the non adapted eye. The results from Task 8 will be used to further enhance and improve the device, and for the construction of the second generation of prototypes for further testing.

9) Device Testing.

The second generation prototypes will then be subjected to extensive testing using a range of analyte concentrations, sample volumes, reading times, and degree of dark adaptation of the viewer. We will then be in a position to objectively assess practical feasibility and, if feasibility is determined, as expected, to proceed with the more definitive and extensive Phase II research and development activities.

10) Front End Enzymes.

A preliminary assessment of NADH-dependent enzymes for the front end of the generic sensor will be made in preparation for the Phase II effort. Again, there is an extensive literature and experience with such coupled enzyme systems for specific biosensor applications (13, 28). We will simply apply that knowledge and experience to our very unique sensor prototypes to develop enhanced and optimum prototypes for an array of different analytes during Phase II.

11) The Firefly System.

The firefly luciferase system has been used in conventional biosensors for a wide range of ATP-dependent enzymes. We feel there is considerable information and experience here, which will aid us in the further research and development of our bacterial luciferase dependent system.

References:

1. Special issue, "Bioluminescence in the Sea," *Naval Research Reviews* **45** (1993) (#2).
2. J. Lee, "Bioluminescence: Biochemistry for Fun and Profit," in D.P. Valenzeno, ed., *Photobiological Techniques*, Plenum, 1991, pp. 297-321.
3. 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.
4. J.F. Carpenter and J.H. Crowe, "Modes of Stabilization of a Protein by Organic Solutes During Desiccation," *Cryobiology* **25** (1988) 459.
5. J.H. Crowe, L.M. Crowe, D. Chapman, "Preservation of Membranes in Anhydrobiotic Organisms: The Role of Trehalose," *Science* **223** (1984) 701.
6. C. Colaco, et al., "Extraordinary Stability of Enzymes Dried in Trehalose," *BioTech.* **10** (1992) 1007.
7. 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.
8. J.H. Crowe, et al., "Anhydrobiosis," *Ann. Rev. Physiol.* **54** (1992) 579.
9. 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.
10. K. Wood, Ph.D. Dissertation, Department of Chemistry, University of California, San Diego, 1989.
11. V. Kratysuk, Abstract, *Conference on Bioluminescence*, Maui, Dec., 1993.
12. L.J. Kricka, et al., *Anal. Applic. of Bioluminescence and Chemiluminescence*, Acad. Press., 1989.
13. A. Roda, et al., "Coupled Reactions for the Determination of Analytes and Enzymes Based on ... Luminescence," *J. Biolum. Chemilum.* **4** (1989) 423-435.
14. F.H. Harold, *The Vital Force: Bioenergetics*, W.H. Freeman, Co., 1986.
15. L.J. Blum, et al., "Design of Luminescence Photobiosensors," *J. Biolum. Chemilum.* **4** (1989) 543-550.
16. P.R. Coulet, "Luciferase-based Sensors," in Proc. Second World Congress on Biosensors, *Biosensors 92*, Elsevier Adv. Tech., pp. 2-9.
17. P.J. Worsfold and A. Nabi, "Bioluminescent Assays...," *Anal. Chim.Acta* **179** (1986) 307.
18. T.O. Baldwin, et al., "...Immunoassay...Using Bacterial Luciferase," *Meth.Enzym.* **133** (1986) 248-264.
19. N.N. Ugarova and O.V. Lebedeva, "Immobilized Bacterial Luciferase..." *Appl Biochem Biotech* **15** (1987) 35-51.
20. J.W. Hastings, "Bioluminescence in Bacteria and Dinoflagellates," in *Light Emission by Plants and Bacteria*, Acad. Press, 1986, pp. 363-398.
21. I. Bronstein and A. Sparks, "...Enzyme Immunoassays With Chemilum...," in R.M. Nakamura, et al., eds., *Immunochemical Assays and Biosensor Technology*, Amer. Soc. Microbiol., 1992. pp. 229-250.
22. R.F. Zuk, et al., "Enzyme Immunochromatography," *Clin. Chem.* **31** (1985) 1144-1150.
23. U. Wollenberger, et al., "Biosensor for ADP...," *Anal. Lett.* **20** (1987) 657-668.
24. U. Wollenberger, et al., "Enhancing Biosensor Performance Using Multienzyme Systems," *TIBTech* **11** (1993) 255-262.
25. D. Griffiths and G. Hall, "Biosensors -- What Real Progress is Being Made?," *TIBTech* **11** (1993) 122-130.
26. A. Nabi and P.J. Worsfold, "...Assays...Using Immobilized Bacterial Luciferase," *Analytical Letters*, **22** (1989) 1861-1871.
27. S.M. Gautier, et al., "Bioluminescence-Based Fibre-Optic Sensor With Entrapped Co-Reactant...," *Analytica Chimica Acta* **243** (1991) 149-156.

28. A.Nabi and P.J. Worsfold, "Determination of Ethanol and Alcohol Dehydrogenase Using Co-immobilised Bacterial Luciferase and Oxidoreductase," *Analyst* **111** (1986) 531-533.
29. J.W. Thorner and H. Paulus, "Catalytic and Allosteric Properties of Glycerol Kinase from *E. coli*," *J. Biological Chem.* **248** (1973) 3922-3932.
30. For exameple, B.H. Schneider, et al., "Microminiature Enzyme Sensors for Glucose and Lactate Based on Oxygen Electrodes," *Sensors and Actuators*, **B1** (1991) 565-570.
31. N.N. Ugarova, et al., "Bioluminescent Microassay of Various Metabolites Using Bacterial Luciferase Co-immobilized with Multienzyme Systems," *Analytical Biochemistry* **173** (1988) 221-227.
32. J.D. Andrade, ed., *Medical and Biological Engineering in the Future of Health Care*, U. of Utah Press, 1994, see chapters by I. Karube and J. Andrade.
33. Andrade, J.D. and R.J. Scheer, "Applying 'Intelligent Materials for Materials Education: The Lableess Lab™," *Proc., 2nd Annual Conference on Intelligent Materials*, Tech. Publ. Co., 1994, in press.
34. J.D. Andrade, et al., "Proteins at Interfaces: Principles, Problems, and Potential," chapter in *Interfacial Behavior of Bioproducts*, J. Brash and P. Wojciechowski, eds., Dekkar, 1994, in press.
35. J.D. Andrade, et al., "Using Bioluminescence for Integrated Science Education," in A.A. Szalay, et al., eds., *Bioluminescence and Chemiluminescence: Status Report*, Wiley, 1993, pp. 69-73.
36. C.Y. Wang and J.D. Andrade, Ibid., pp. 99-103.
37. J.D. Andrade, et al., "Immuno-Biosensors: The Clinical Chemistry and Coagulation Laboratory on a Chip," in Y. Sezai, ed., *Artificial Heart: Biomation in the 21st Century*, Saunders, 1992, pp. 89.
38. O. Sonntag, *Dry Chemistry*, Elsevier, 1993.
39. K. Okuda, ed., *Automation and New Techniques in the Clinical Lab*, Blackwell, 1990.
40. A. Nabi and P.J. Worsfold, "Determination of Ethanol and Alcohol Dehydrogenase Using Co-immobilised Bacterial Luciferase and Oxidoreductase," *Analyst* **111** (1986) 531-533.
41. S.M. Gautier, et al., "Fibre-optic Biosensor Based on Luminescence and Immobilized Enzymes: Microdetermination of Sorbitol, Ethanol and Oxaloacetate," *J. Biolum. and Chemilum.* **5** (1990) 57-63.
42. T.F. Holzman and T.O. Baldwin, "Isolation of Bacterial Luciferases by Affinity Chromatography," *Biochemistry* **21** (1982) 6194-6201.
43. T.H. Holzman and T.O. Baldwin, "Reversible Inhibition of the Bacterial Luciferase Catalyzed Bioluminescence Reaction by Aldehyde Substrate" *Biochemistry* **22** (1983) 2838-2846.
44. R.H. Abeles, et al., *Biochemistry*, Jane & Bartlett, Publ., 1992; especially Chapter 16: Biological Oxidation: NAD^+ and FAD.
45. E. Diebold, et al., "Chemistry on a Stick," *Chemtech* (August, 1991) 462-467.
46. B. Walter, "Fundamentals of Dry Reagent Chemistries: The Role of Enzymes" in C.H. Suelter, ed., *Bioanalytical Applications of Enzymes* (1992) pp. 35.

COMMERCIAL POTENTIAL

Please refer to the later part of Background section relating to anticipated benefits. We have had considerable experience with major diagnostic and sensor companies. We expect considerable interest in this technology. PSI expects to enter into an appropriate agreement for commercial funding of the development as Phase III. PSI will itself fund and market these sensors for educational and home use.

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 (33). 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 was presented earlier.

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 (32, 37). About 5 years ago he became quite interested in bioluminescence and particularly in the firefly and bacteria luminescence systems. He will provide much of the interfacial biochemistry, bioluminescence, and biosensor expertise required. Joe is three quarter time Professor of Materials Science and Bioengineering at the University of Utah. His abbreviated vita was also presented.

Mr. Dong Min is a graduate student under Joe Andrade's direction, working on bacterial luciferases. He will be responsible for the luciferase studies. Refer to discussion in Budget Justification section.

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 other specialized studies which require University equipment and access can 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.

CONSULTANTS AND SUB-CONTRACTS

See Budget Justification for consultants Dr. V. Hlady and Dr. J. Janatova. Space limitations do not allow their biosketches to be attached. Their letters of collaboration follow.