NSF 95-116
Multidisciplinary Research in
Optical Science & Engineering

Pre-Proposal

To: Director, MPS Office of Multidisciplinary Activities
The National Science Foundation, Room 1005
4201 Wilson Boulevard
Arlington, VA 22230

Title: Photons from Biology: The Bio-Photon Project

Abstract:

Photons are basic to biology. Biology has learned to efficiently collect photons and transduce and utilize such energy via photosynthesis. Biology also utilizes photons for sensing and imaging purposes via vision. What is not as well known is the fact that biology knows how to produce photons via bioluminescence. Bioluminescence is an enzyme-based chemiluminescence process utilizing specific substrates (luciferins), oxygen, and often one or more co-factors.

We propose to develop ordered arrays of photoproteins and utilize these ordered arrays as optical microcavities and as components of optical devices. We address the question: "Does biology utilize microcavity optics?" If so, can we learn from this? If not, can we couple micro-cavity optical principles to bio- and chemi-luminescent-based devices?

The second major question: "Can man apply biology's unique bio-reactors?" refers to the fact that many fish and marine organisms have developed unique photo-organs, living flashlights. We propose to "develop" photoorgans based on highly dense, possibly ordered, cultures of marine bioluminescent bacteria. A variety of fish, such as the flashlight fish, have bright, highly efficient photoorgans. The unique micro-bioreactors used for the maintenance of these highly dense cultures will be studied from an engineering or materials science perspective.

We ask a third major question, "Can we develop a selective or artificial symbiosis between photosynthetic and non-photosynthetic bioluminescent organisms in order to develop and produce nearly infinitely rechargeable luminescent devices?"

The Bio-Photon Project thus consists of three major components:

- Photoproteins in Ordered Arrays;
- Artificial Photoorgans; and
- Artificial Symbiosis.

Potential applications include instrument-less biosensors, intelligent fibers and fabrics, living optical materials, potential display applications, and regenerable lighting.

The project involves a distinguished international advisory board representing marine biology, molecular biology and biotechnology, materials science, electrical and optical engineering, bioengineering, surface and interface chemistry, physics, and ecology. The project includes a strong education component involving the Center for Integrated Science Education (CISE), high school teachers, and a local science education products company. Corporate collaboration is strong. Collaborations and technology transfer relationships are already in place.

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Submitted: _____ (date) *by* _____ (signature)
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Objectives/Specific Aims (See Table 1, Time Plan/Schedule - next page):

Figure 1 presents the subjects and questions addressed and the bio-photon devices we expect to develop. Figure 2 shows the general organization and management and includes the groups involved, their expertise, and their general areas of focus and responsibility.

Part I -- Photoproteins and Ordered Arrays:

Firefly luciferase: expression in *E. coli*, purification, characterization, adsorption, immobilization, orientation and ordering at interfaces, stability in the immobilized state, bioluminescent activity, characterization of ordered array bioluminescence with respect to directionality, intensity, and polarization. Engineered firefly luciferase: to enhance its orientation, immobilization, and ordering in arrays. Firefly luciferin and luciferin derivatives: sources, stability, and application.

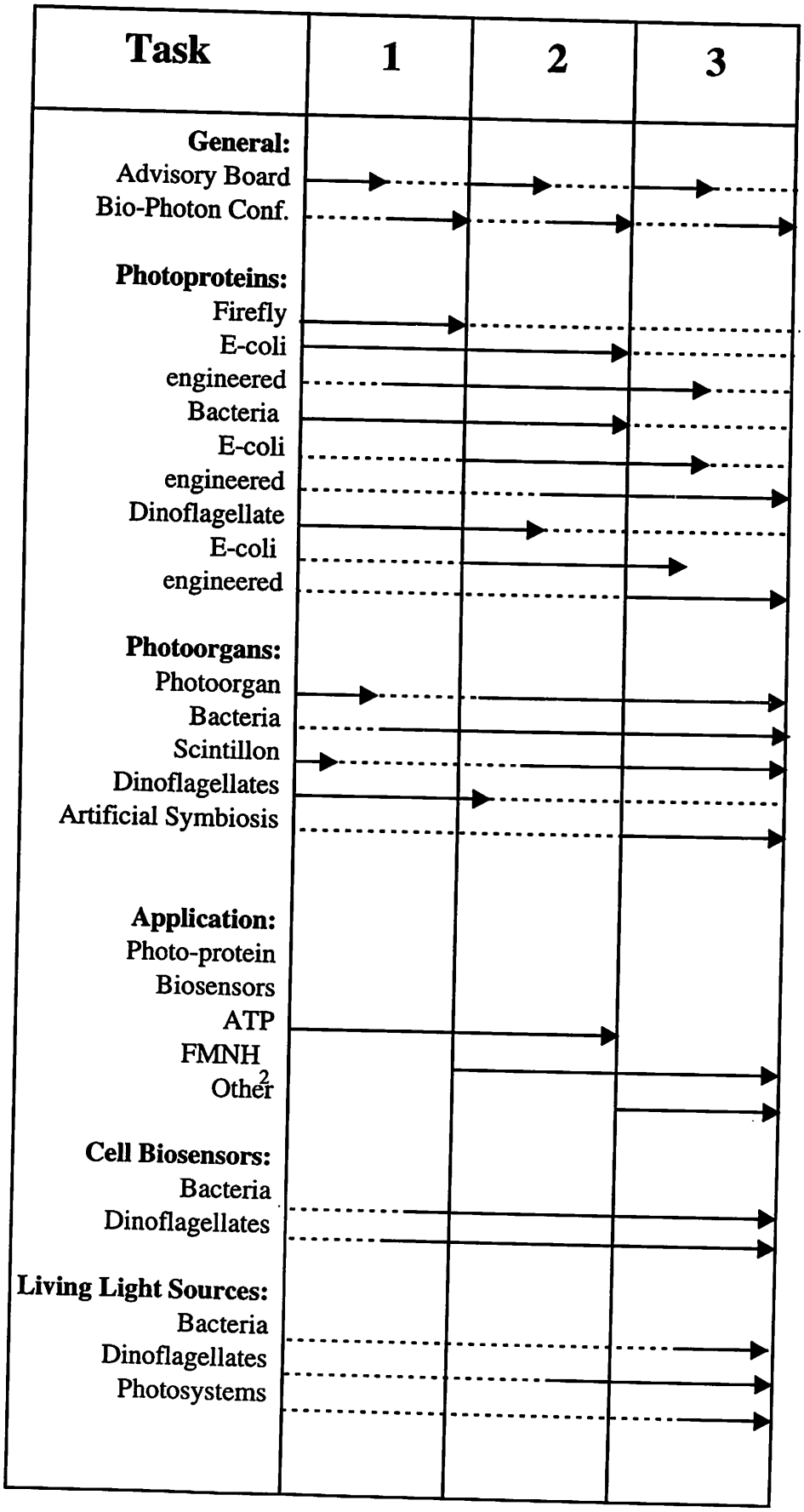
Bacteria luciferase: culture of marine bacteria under a variety of culture conditions, bioluminescence expression, extraction, purification, and characterization of bacterial luciferase. Expression in *E. coli*, purification, characterization, adsorption immobilization, orientation and ordering at interfaces, stability in the immobilized state; bioluminescent activity, characterization of ordered array bioluminescence with respect to directionality, intensity, and polarization. Expression of bacterial luciferases in *E. coli* to enhance purification, orientation, and ordering.

Dinoflagellate luciferases: culture of three different species of dinoflagellate under a variety of culture conditions, the study of bioluminescence intensities and characteristics, preliminary study of scintillon morphology and characteristics, extraction, purification, characterization and application of luciferase and luciferin derived from these organisms. Expression in *E. coli*, purification, characterization, adsorption, immobilization, orientation and ordering at interfaces, stability in the immobilized state, bioluminescent activity, characterization of ordered array bioluminescence with respect to directionality, intensity, and polarization. Expression of engineered dinoflagellate luciferase to enhance orientation, immobilization, and ordering. Extraction, purification, and characterization of Luciferin-Binding Protein (LBP); expression in *E. coli.*

Part II -- Artificial Photoorgans:

Photoorgans: Careful evaluation and analysis of the properties and nature of bacteria photoorgans in marine species. Analysis of bioprocess engineering aspects of bacterial photoorgans. Development of means to produce high bacterial density cultures, including means to orient and even "order" the bacteria.

Table 1. Time Plan
Years



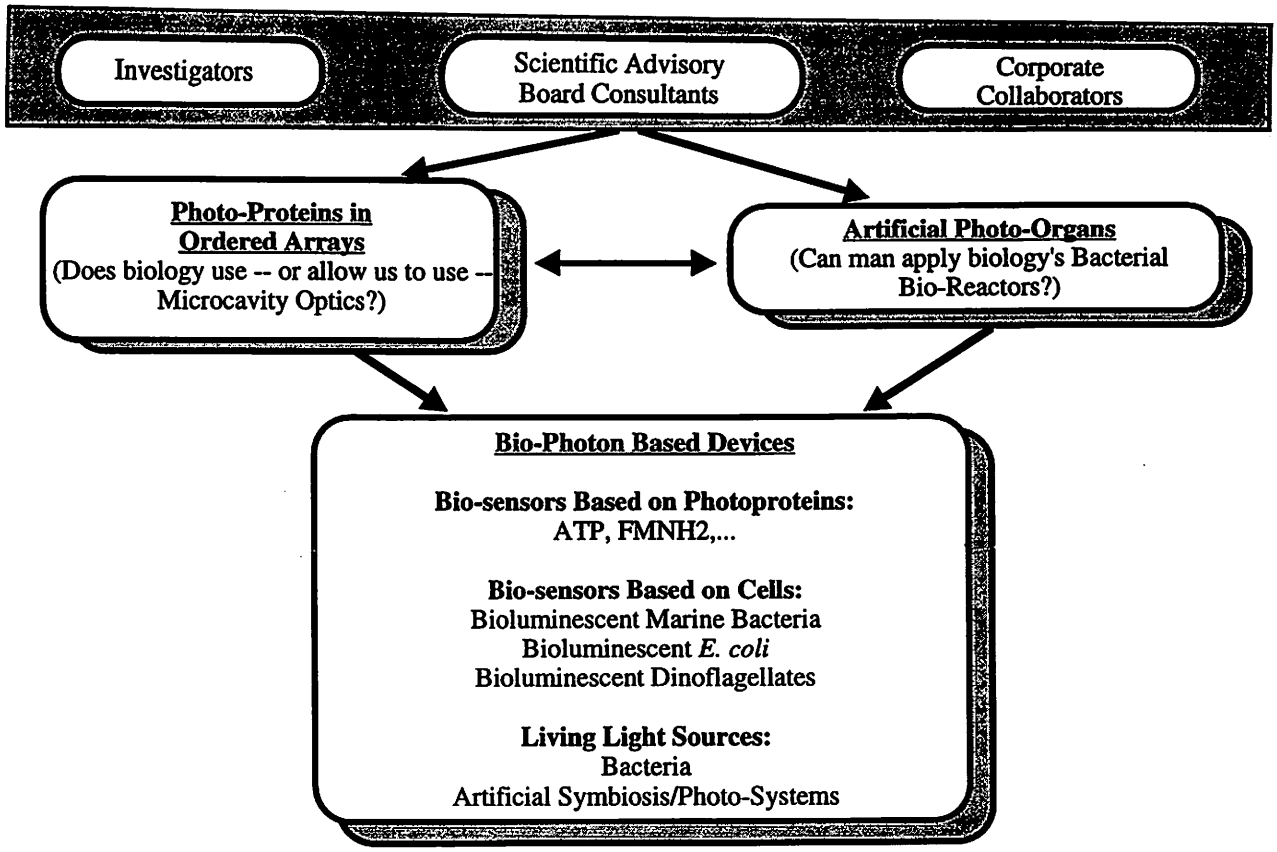
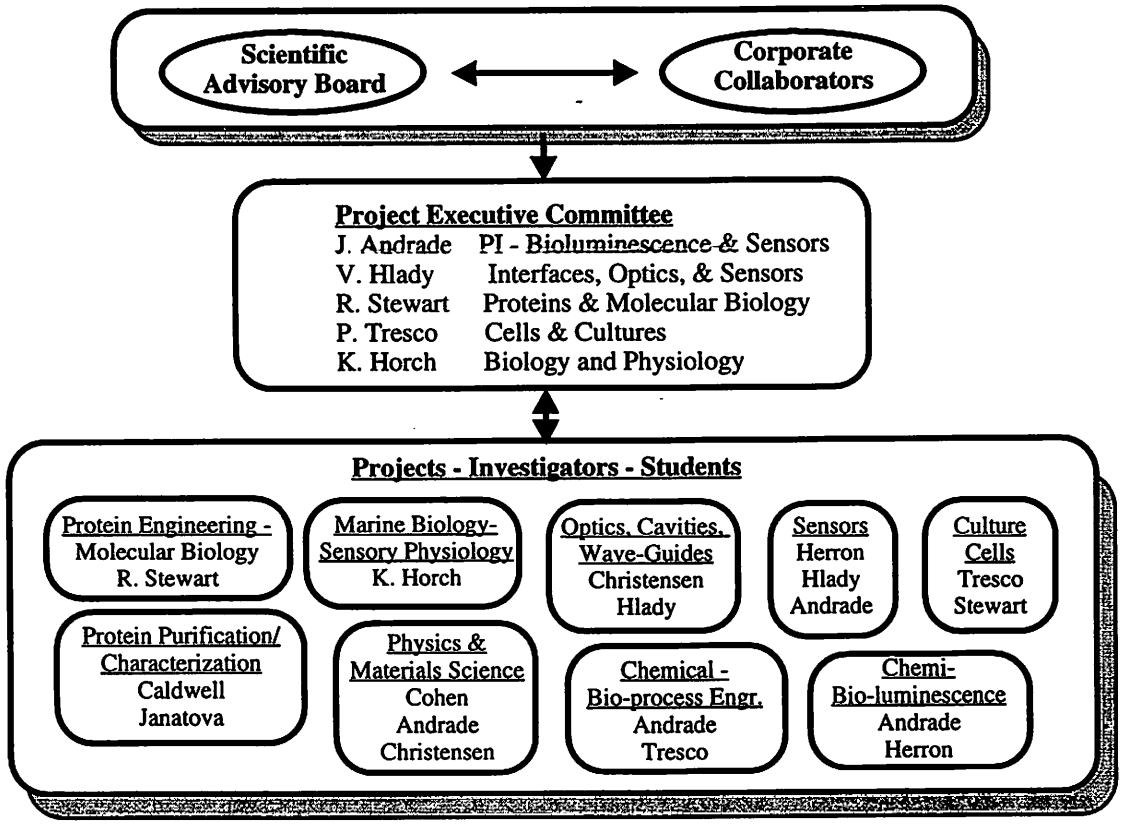


Figure 1: Bio-Photon Project -- Topics, Questions, and Applications.



The Bio-Photon Project:

Figure 2: Organization & Management

Bacteria: High density culture of marine bioluminescent bacteria. Development of means to pack and order marine bacteria in micro-bioreactors. Modification of culture medium and culture conditions to enhance ordering, orientation, and density of the bacteria. Modification of culture media and conditions to enhance total bioluminescent output. Optimization of all conditions to enhance stability and survivability of the artificial photoorgans.

Scintillons: Consideration of structure and function of dinoflagellate scintillons with respect to ordering, packaging, and self-assembly of artificial scintillons.

Artificial Symbiosis: Consideration of co-cultures of photosynthetic bacteria and bioluminescent bacteria, diatoms (photosynthetic) and bioluminescent bacteria, and of dinoflagellates (photosynthetic) and bioluminescent bacteria.

Applications -- Bio-Photon Based Devices:

Photoprotein-based biosensors: ATP based and $FMNH_2$ based biosensors using optimized ordered photoprotein arrays.

Cell-based biosensors: Use of high density bacteria and dinoflagellate cultures as means to detect toxins and other chemicals via bioluminescence inhibition or bioluminescence enhancement.

Living light sources. Use of high density artificial photoorgans as regenerable, long-lived light sources.

Anticipated Results/Relevance and Significance:

Ordered arrays of photoproteins will provide films and coatings of enhanced luminescence intensity, much as careful optimization of antibody immobilization has been important in enhancing immunobiosensor performance. There is some probability that properly oriented arrays of luciferases may provide some directional emission due to intrinsic microcavity behavior. This characteristic may be enhanced by co-immobilization and co-assembly with organic structures which provide appropriate dielectric properties.

Such arrays should provide some degree of polarization, although this is less likely for firefly and more likely for bacterial luciferase because of the expected nature of the activated state.

Given what little we know about bacterial bio "reactors" in photoorgans and lacking any evidence to the contrary, we feel that high density, viable marine bioluminescent bacterial cultures are possible. Indeed there is already industrial interest (Protein Solutions, Inc. -- confidential) in developing and marketing such devices as qualitative sensors.

Given our limited experience with dinoflagellate cultures, admittedly pseudo-eukaryotic cells rather than prokaryotes (bacteria), we feel we can develop long lived dense bacterial cultures (primitive photoorgans), initially using a controlled nutrient delivery system.

Given our limited experience with co-culture of multi-dinoflagellate species -- and also the experience of closed ecosystems and mesocosms, we are confident that an artificial or selective symbiosis is indeed possible.

The real question is how practical and marketable such technologies might be. At least two of our corporate collaborators are betting on this -- and so are we -- although our experience base is too limited at this time to suggest specifics.

Budget:

We anticipate requesting a budget of approximately $300,000/year for 3 years -- a total of $900,000, which includes allowable indirect costs.

Personnel:

Please also refer to Figure 2, Organization and Management.

Joe Andrade, P.I., is Professor of Bioengineering and Materials Science at the University of Utah. He is former Chairman of the Department of Bioengineering and former Dean of the College of Engineering. Joe has worked on proteins at interfaces and biocompatability, biosensors and related areas for 25 plus years, has edited 6 books, and has about 125 peer reviewed technical papers. He is experienced in the management of research organizations and research groups, has produced some 45 Ph.D. and Masters students, consults for a number of industries and government agencies, and has been the founder or co-founder of two companies, including Protein Solutions, Inc., one of the industrial collaborators in this project. He has been working on luciferases and bioluminescence for the last 4 years. He has worked extensively in fluorescent-based biosensors using total internal reflection/fiber optics.

Vladimir Hlady, is a physical chemist/surface chemist/bioengineer who has worked extensively with biosensors, interfacial fluorescence, proteins at interfaces, and colloidal systems. He is particularly well recognized for his work on protein adsorption and total internal reflection fluorescence as applied to protein adsorption. His present work is focused on scanning force microscopy, protein patterning, and specific protein-ligand binding processes.

Russell Stewart, a new member of the Department of Bioengineering and its Bio-Based Engineering Program, is an expert on proteins involved in motility and transport and has a particular expertise in molecular biology and protein engineering. He is developing micro and nano engineering teaching laboratories together with Dr. Hlady. Dr. Stewart routinely uses protein engineering techniques and *E. coli* to produce proteins with novel engineered properties for his particular research applications. He will be largely responsible for the protein engineering/molecular biology parts of the project.

Ken Horch is a neuro-physiology, sensory systems bioengineer who, earlier in his career, was involved in marine biology, again, from a sensory and neural perspective. Ken is the closest thing we have to a marine biologist and will work closely with the Scientific Advisory Board consultants.

Doug Christensen is an expert on interfacial optics, wave guides, and the development of optically-based biosensors, he is also active in medical imaging and in the applications of ultra sound. Doug has worked in the area of optical microfabrication and in the modeling and analysis of optical systems, including optical microcavities.

James Herron is an antibody crystallographer with extensive experience in protein structure and function, including computer molecular graphics. He now heads up the fluoro-immunosensor activities and is an expert on fluorescence, fluorescence-energy transfer, and the use of proteins as specific recognition elements for biosensors.

Patrick Tresco is also part of the new Bio-Based Engineering Program -- he heads up the tissue engineering laboratories and has a particular interest in the development of devices based on cells in culture, on membranes and in fabric matrices, for potential medical application.

Karin Caldwell is Director of the Center for Biopolymers at Interfaces and an expert in chromatography and separations, as well as in proteins at interfaces. She, together with Jarmilla Janatova, a protein biochemist with extensive experience in protein purification and characterization, will be largely responsible for the protein extraction, purification, and characterization components of the project.

Rick Cohen has worked extensively in the area of luminescence materials and solid state physics and will function as a reality therapist to our thoughts, concepts, and models on the application of microcavity concepts to biochemistry and biology.

On campus advisors include Dr. Richard Normann, Chairman of the Department of Bioengineering and an expert on vision, Dr. Craig P. Taylor, Chairman and Professor of Physics and an expert on luminescence in amorphous systems.

Edward Pope is an expert on sol gel processing and on the immobilization of proteins and cells using sol-gel techniques. Although he is President of Matech, Inc., one of the industrial collaborators in this program, he also serves as adjunct professor of Materials Science.