THE UNIVERSITY OF UTAH

COLLEGE OF ENGINEERING
OFFICE OF THE DEAN
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SALT LAKE CITY, UTAH 84112
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October 8, 1986

Dr. W. Thompson
Division of International Programs
U.S. Federal Republic of Germany Program
National Science Foundation
Washington, D.C. 20000

Dear Dr. Thompson:

Enclosed are two copies of the Final Report on our US-Federal Republic of Germany Cooperative Science Program, Grant Int 82-02351, which ended on August 31, 1986.

Thank you for this support, which has led to a very productive and long-lasting collaboration. Please let me know if you need further information.

Sincerely,

J.D. Andrade, Ph.D.
Dean

JDA/pk

encl.

cc: J. Vialpando
H. Ringsdorf

NATIONAL SCIENCE FOUNDATION
Washington, D.C. 20550

FINAL PROJECT REPORT
NSF FORM 98A

PLEASE READ INSTRUCTIONS ON REVERSE BEFORE COMPLETING

PART I—PROJECT IDENTIFICATION INFORMATION

1. Institution and Address	2. NSF Program	3. NSF Award Number
University of Utah Salt Lake City, UT 84112	U.S. Federal Republic of Germany Coop Sci.	Int 82-02351
	4. Award Period: From 9/1/82 To 8/31/86	5. Cumulative Award Amount: 12,400

6. Project Title

Protein Adsorption on Defined Polymeric Monolayers

PART II—SUMMARY OF COMPLETED PROJECT *(FOR PUBLIC USE)*

Protein adsorption on defined polymeric monolayers was studied via the Total Internal Reflection Fluorescence (TIRF) Technique at the University of Utah using polymeric monolayers prepared in the laboratory of H. Ringsdorf, Institute for Organic Chemistry, Gutenberg University, Mainz, Germany. Prof. Ringsdorf, 3 of his students (H. Schupp, B. Hupfer, and H. Bader) and a postdoctoral fellow (Paul Meller) participated in the work and all visited the Utah group at least once (using non-NSF funds). J. Andrade, D. Gregonis, S.W. Kim, and M. Reichert visited the Mainz group during the grant period. Langmuir-Blodgett monolayer techniques, particularly using polymerizable diacetylene derivatives, were introduced and transferred to the Utah group. Surface analysis and characterization (contact angle dynamics, streaming potential, and X-ray photoelectron spectroscopy) and protein adsorption methods (principally TIRF) were transferred to the Mainz group. The collaboration resulted in 4 publications to date, which are attached. Work continued through the last day of the grant and is ongoing using other funds. Prof. Ringsdorf will spend 6 weeks in Salt Lake City as a Visiting Professor in Spring, 1987. Drs Monty Reichert (Utah) and Paul Meller (Mainz) have submitted a joint grant. Continued exchanges and collaboration are assured.

PART III—TECHNICAL INFORMATION *(FOR PROGRAM MANAGEMENT USES)*

1. ITEM *(Check appropriate blocks)*	NONE	ATTACHED	PREVIOUSLY FURNISHED	TO BE FURNISHED SEPARATELY TO PROGRAM	
				Check (✓)	Approx. Date
a. Abstracts of Theses	✓				
b. Publication Citations		4			
c. Data on Scientific Collaborators					
d. Information on Inventions					
e. Technical Description of Project and Results					
f. Other *(specify)*					

2. Principal Investigator/Project Director Name *(Typed)*	3. Principal Investigator/Project Director Signature	4. Date
		10/2/86

NSF Form 98A, 12-85. Supersedes All Previous Editions

Form Approved OMB No. 3145-0058

A Proposal to the

National Science Foundation

U.S.-Germany Cooperative Science Program

Title: Protein Adsorption on Defined Polymeric Monolayers

from

J.D. Andrade, P.I., D.E. Gregonis, and R. Van Wagenen
Department of Materials Science and Engineering
and
Department of Bioengineering
University of Utah
Salt Lake City, Utah 84112

in collaboration with

Prof. Dr. Helmut Ringsdorf
Johannes Gutenberg-Universitat
Fachbereich Chemie
Institut fur Organische Chemi D-6500 Mainz
Johann-Joachim-Becher-Web 18-20 Postfach 3980
WEST GERMANY

J.D. Andrade, P.I.
Professor

R.H. Boyd, Chairman,
Department of Materials
Science and Engineering

Peter W. Barber, Chairman, Department of Bioengineering

Authorized Signature
University of Utah

From July 1, 1982 through June 30, 1984 (24 months)
Total Requested for Entire Period: $19,169

Mail To: Central Processing Section
ATTN: U.S.-Germany Cooperative Science Program
National Science Foundation
Washington, D.C. 20550

NOTICE OF RESEARCH PROJECT

SCIENCE INFORMATION EXCHANGE

Smithsonian Institution
NATIONAL SCIENCE FOUNDATION

PROJECT SUMMARY

SIE Project No.

NSF Award No.

FOR NSF USE ONLY

Directorate/Division	Program or Section	Proposal No.	F.Y.

NAME OF INSTITUTION (INCLUDE BRANCH/CAMPUS AND SCHOOL OR DIVISION)

College of Engineering
University of Utah

ADDRESS (INCLUDE DEPARTMENT)

Department of Materials Science and Engineering
University of Utah
Salt Lake City, Utah 84112

PRINCIPAL INVESTIGATOR(S)

J.D. Andrade

TITLE OF PROJECT

Protein Adsorption on Defined Polymeric Monolayers

TECHNICAL ABSTRACT (LIMIT TO 22 PICA OR 18 ELITE TYPEWRITTEN LINES)

We propose to thoroughly surface characterize the highly oriented model polymer films prepared by Ringsdorf and his group in their laboratories and in our laboratories by a series of techniques which have now become routine in our laboratory. We further propose to study these oriented, characterized model films with respect to their protein adsorption characteristics using a totally new technique: total internal reflection intrinsic UV fluorescence (TIRF) spectroscopy, which permits the online real time measurement of adsorption at a well characterized, low surface area surface using unlabeled, native proteins. This is done by exciting the intrinsic UV fluorescence of the tryptophan residues common to the proteins of interest. In addition to the kinetic adsorption and desorption information, this technique provides spectroscopic information, i.e., the UV fluorescence emission spectrum, which provides some information on the conformational state of the adsorbed protein.

1. Proposal Folder
2. Program Suspense
3. Division of Grants & Contracts
4. Science Information Exchange
5. Principal Investigator
6. Off. of Govt. & Pub.Progs

NSF FORM 4 (8-80)

Abstract

The interfacial properties of polymer-water interfaces are important in many applications, including adhesion, chromatography, medicine, and protective films. Dr. H. Ringsdorf of the Gutenburg University in Mainz, West Germany, is internationally recognized for his work in the general area of synthetic polymer chemistry and particularly for oriented polymer systems. Such oriented systems are highly useful as model systems for the study of polymer surface properties and for the preparation of membrane models and analogs. Dr. Ringsdorf is submitting a proposal to the West German officials for a joint collaborative program. This is the companion U.S. proposal.

We propose to thoroughly surface characterize the highly oriented model polymer films prepared by Ringsdorf and his group in their laboratories and in our laboratories by a series of techniques which have now become routine in our laboratory. We further propose to study these oriented, characterized model films with respect to their protein adsorption characteristics using a totally new technique: total internal reflection intrinsic UV fluorescence (TIRF) spectroscopy, which permits the online real time measurement of adsorption at a well characterized, low surface area surface using unlabeled, native proteins. This is done by exciting the intrinsic UV fluorescence of the tryptophan residues common to the proteins of interest. In addition to the kinetic adsorption and desorption information, this technique provides spectroscopic information, i.e., the UV fluorescence emission spectrum, which provides some information on the conformational state of the adsorbed protein.

The research work is funded by two ongoing NIH grants. Funds requested in this application are only for the foreign travel to permit this international collaboration. Two trips per year from Salt Lake City to Frankfurt-Mainz, Germany are requested for the two year period of the grant: one each year for J.D. Andrade the P.I., the other for D.E. Gregonis and R. Van Wagenen (one trip each). We request the program be funded for two years beginning approximately July 1, 1982.

1. Background

Protein adsorption at polymer aqueous solution interfaces is a problem important in many fields of interest in physical biochemistry, polymer surface science and surface science in general. Although there has been some considerable work done on protein adsorption at solid-liquid interfaces, it is only very recently that work has been done on extensively and well characterized surfaces of controlled surface properties. Also, it has only been recently that methods have been developed for the study of the adsorption of well characterized unlabeled proteins by interface spectroscopic means [1], which enables information on the conformational state and orientation of the adsorbed protein to be deduced.

H. Ringsdorf and his group and Mainz, have developed means to produce biochemical analog monomers which can be made to form monolayers at the air-solution interface. These monomer monolayers can be transferred by classical Langmuir-Blodgett procedures to suitable solid substrates. The transferred monolayers can then be polymerized to provide a polymeric monolayer of defined orientation and physical properties [2]. Such polymeric monolayer and multi-layered films, because of the defined orientation,structure, and chemical and physical character are ideal model substrates with which to study the adsorption of biological macromolecules, particularly proteins and enzymes. In addition, the Ringsdorf group has succeeded recently in producing polymerizable monomers which are analogs to cell membrane phospholipid structures [2]. Thus, the polymer film formed can in many respects be considered to be a polymeric analog of a lipid bilayer structure. The study of adsorption of proteins on such surfaces, including such multilayers impregnated with appropriate proteins, may provide important information on the interaction of proteins with model cell membrane analogs, including information which should be useful in considerations of protein-membrane receptor interactions.

J.D. Andrade's group in Salt Lake City have applied modern surface analytical methods to the characterization of polymer surfaces for study of protein adsorption processes. These techniques include interface energetics, obtained by contact angle methods directly at polymer-aqueous solution interfaces [3], X-ray photoelectron spectroscopy, and total internal reflection fluorescence spectroscopy [1], based on the published methods of Watkins and Robertson [4]. The total internal reflection fluorescence technique allows one to study protein adsorption at the solid-liquid interface in situ in real time using only the intrinsic tryptophan fluorescence of tryptophan containing proteins as the spectroscopic probe [1]. Studies performed with the adsorption of unlabeled native albumin and gamma globulins onto hydrophilic quartz and polydimethyl siloxane-coated hydrophobic substrates have clearly documented the feasibility of the technique for the study of protein adsorption in situ. Recent work with this technique has clearly established that the fluorescence emission spectrum of the adsorbed protein film can also be obtained dynamically during the course of adsorption [1, 5]. As the total internal reflection method samples approximately 1000 angstrons in the bulk solution, not only does one obtain information on the adsorbed protein but one obtains the bulk protein signal simultaneously. Thus by appropriate computer subtraction techniques and by reference to the fluorescence emission spectrum of the bulk protein in solution, one can deduce directly and unambigously the fluorescence emission of the adsorbed material. By studying the desorption kinetics and the spectroscopic properties of the irreversibly bound film, one obtains spectroscopic information on the irreversibly bound component of the adsorbed protein as well as the reversibly bound component. All of this can be

done dynamically, kinetically, with resolution times of the order of one second, as a function of temperature, pressure, flow rate, protein concentration and, of course, the nature and type of solid surface. In addition, this group is developing a model for protein adsorption by direct consideration of hydrophobic, electrostatic (ionic), and charge transfer interactions of both the well characterized solid surface and of the well characterized protein. The latter is obtained by using the three dimensional X-ray diffraction-derived structure of appropriate reference proteins, such as lysozyme and myoglobin.

2. Proposed Work/Research Plan

In this program we propose to prepare surfaces of mono and multi polymeric layers produced by monomers which are analogs of lipids in cell membranes and related monomers. This will be accomplished by the Ringsdorf group using clean, well characterized glass and/or quartz substrates provided by the Salt Lake group. This work will be done by a member of the Mainz group coming to Salt Lake City and working on a suitable Langmuir trough in Salt Lake City. The surfaces will then be surface characterized using techniques previously described by Andrade and his group in the Salt Lake City laboratories. The samples will then be mounted in the total internal reflection apparatus and studied by total internal reflection UV fluorescence [1].

Model proteins whose 3-D structures are well defined and which are presently being studied by Andrade's group on other substrates will be utilized initially. These include myoglobin and lysozyme. Two plasma proteins are also selected. One is human serum albumin monomer (mercaptalbumin monomer), the other is human gamma globulin. Both are presently being studied on conventional surfaces. The Salt Lake group has considerable experience in the purification and characterization of bovine mercaptalbumin monomer [6] and is presently involved in purification and characterization programs dealing with fibronectin [5] and gamma globulin [1]. In addition, there is experience in the group in the handling and the characterization of all of the above mentioned proteins. The protein characterization will include gel electrophoresis, circular dichroism, Raman spectroscopy, and fluorescence spectroscopy.

The major objectives of this program are to determine the protein adsorption characteristics of cell membrane model analogs prepared by the use of polymerizable monomers which form monolayers at liquid-air interfaces. These oriented, well structured, well characterized monolayers and multilayers of differing surface and related properties are expected to be very useful model systems for the study of membrane-protein interactions. It is expected that these results combined with the studies in the literature utilizing actual cell membrane interactions and with the existing studies on protein adsorption at conventional surfaces, will enable us to make some deductions on expected receptor geometries on cell membranes for the selected proteins and on the selection and design of surfaces for optimizing and/or minimizing the interaction of selected proteins. This latter topic is, of course, useful in the handling, purification and storage of proteins and for developing and designing methods for protein separation and purification. In addition, the information obtained will probably be useful in assisting the design of site directed molecules for targeted drug delivery. Both of the key collaborators and their research groups are very excited at this collaboration and look forward to a productive and stimulating collaboration.

International cooperation is assured. Andrade and his group would provide the surface characterization, the protein characterization, and the protein adsorption work as well as the analysis of the data and the modeling of the phenomena. Ringsdorf and his group would provide the organic chemistry expertise dealing with the

preparation of the polymerizable model monomers and the monolayer expertise in actually forming the mono and multi films and the polymerization of those films. The program is requested for two years with two trips per year. A member of the Mainz group will come to Salt Lake City, mainly for the purpose of bringing and providing the monomers and providing the appropriate polymer substrates. This individual would then be instructed in the methods of characterizing those polymer surfaces and in the protein adsorption instrumentation and methodology. It is expected that much of that technology and methods would be transferred via this individual back to Mainz. In the first year members of the Salt Lake group will go to Mainz mainly for the purposes of learning the synthetic methods and monolayer forming methods in the Mainz group, again to be able to transfer that methodology to Salt Lake City. The same general arrangement would occur in the second year, but at a much higher level of expertise. It is expected at the end of the two year collaboration, both groups would have become proficient in techniques which are now limited to only one of the groups and that both groups would then have the resources by which to continue the collaboration into the future.

A summary of the sample flow and planned work is given in the figure (next page).

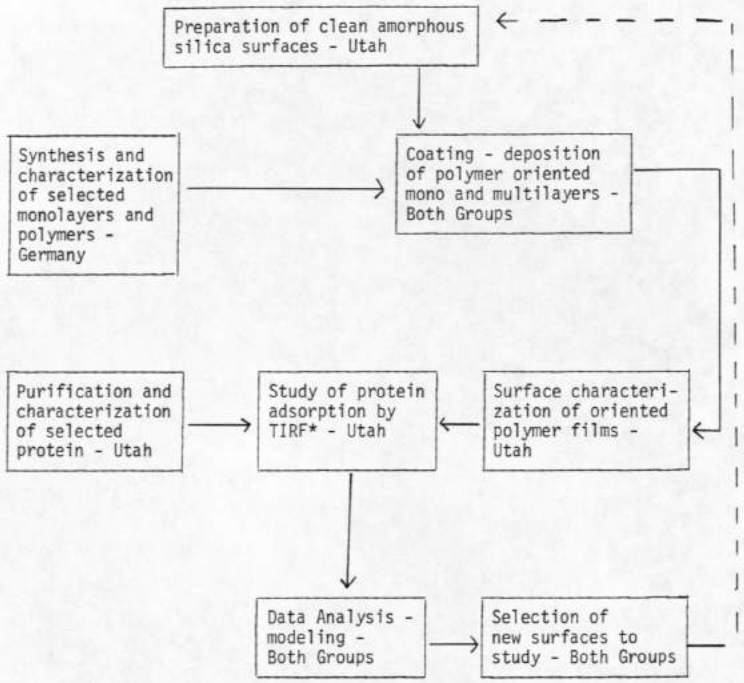
This international collaboration has already begun in a limited way. Two of H. Ringsdorf's students, H. Schupp and B. Hupfer, spent two months in J.D. Andrade's lab in Salt Lake City in April and May 1980. This short collaboration resulted in two publications [7,8], which confirmed that diacetylene monomers with lipid side chains could indeed by polymerized and transferred to solid surfaces. Surface characterization using contact angle methods, X-ray photoelectron spectroscopy, and interface electrical double layer potentials by the streaming potential technique [9], confirmed the expected surface and interfacial chemistry, compositions and properties of these materials. A preliminary study on four of these compositions was completed studying the adsorption of gamma globulins by the total internal reflection intrinsic UV fluorescence technique, demonstrating that the films were indeed suitable for the UV TIRF methodology. In addition, a study was completed of mono and multilayer films to determine the surface sensitivity of the X-ray photoelectron spectroscopy method by measuring photoelectron mean free paths in diacetylene mono and multilayers [7]. H. Ringsdorf has visited the lab in Salt Lake City once and J.D. Andrade has visited the Mainz lab several times in the past three years.

The preliminary work which has been done to date serves as the basis for the more extensive international collaboration requested in this proposal.

3. Personnel (See also attached CV's)

The personnel involved include, in addition to Professors Andrade and Ringsdorf, the following members of the Salt Lake City group:

Dr. D.E. Gregonis is a polymer chemist who has worked together with J.D. Andrade on model systems for preparation of surfaces of defined characteristics for the study of protein adsorption and related biological phenomena. It is proposed that Dr. Gregonis spend some time with the Ringsdorf group in Mainz to learn some of the chemistry of phospholipid-analog polymers and related compounds suitable for preparing polymeric mono and multilayers. His curriculum vita is appended.



*TIRF = total internal reflection intrinsic fluorescence spectroscopy [1].

Figure 1. Sample flow Diagram.

Dr. R. Van Wagenen is a materials scientist with considerable experience in the general areas of interface electrical potentials and the use of the reflection fluorescence technique for the study of protein adsorption. The objective for his visit to Mainz, Germany would be to assist the Mainz group in setting up these two techniques in their laboratories, as well as assisting in the preparation of substrates and samples which will be further characterized by Dr. Van Wagenen and his colleagues in Salt Lake City upon his return.

Both Andrade and Ringsdorf intend to visit the respective laboratories at least once a year during this collaborative program.

Most of the experimental work of the Salt Lake City group will be funded as part of two ongoing NIH grants, HL18519 on Protein Adsorption, J.D. Andrade, P.I.; and HL26469, Blood-Materials Interactions of Model Polymers, D.E. Gregonis, P.I.

4. Travel

Two trips per year for each of the two years of the program are requested. One trip each year will be made by J.D. Andrade to plan that year's studies with H. Ringsdorf and his staff. During the first year D.E. Gregonis will travel to Mainz to learn some of the organic and polymer chemistry methods and techniques of the Ringsdorf group. This will be done early in the first year so that he will have a good understanding of the nature of the compounds to be studied later. Towards the middle of the second year and after a considerable amount of work has been done on the surface characterization and protein adsorption properties of these materials, R. Van Wagenen will travel to Mainz to assist the Mainz group in the setting up of the total internal reflection fluorescence technique.

In a companion proposal submitted to the German authorities, Ringsdorf will plan on making one visit each year to the Salt Lake City laboratory to assist in data analysis and further experiment planning. In addition, one or two of his people will visit each year, for a two to three month stay, to assist in polymer film preparation and characterization and to do the protein adsorption studies.

The synthetic organic and polymer chemistry strengths of the Ringsdorf group in Mainz coupled with the surface characterization and protein adsorption strengths of the Salt Lake group make this a logical, stimulating, and highly productive collaboration.

5. Budget - Two Year Period

Personnel

None - funding provided in existing grants --

Supplies

None - funding provided in existing grants --

Travel - Foreign

SLC - Frankfurt*

two in 1982 (estimated) at 1,200 each	$2,400
two in 1983 (estimated) at 1,400 each	$2,800

Per diem for U.S. participants while in Frankfurt-Mainz, West Germany. Estimate average of 20 days/trip, two trips/year, four trips total.
Present per diem rate = $94/day $7,520

Other

Publication costs/reprints	$ 500
Total Direct Costs (both years)	$13,220
Indirect Costs (both years)	$ 5,949
Total Costs (both years)	$19,169

*Salt Lake-Frankfurt air fares range from $984 to over $1,400 depending on carrier, season, and other variables.

6. References

1. R. Van Wagenen, S. Rockhold, and J.D. Andrade, Morphology, Structure, and Interactions of Biomaterials, S.L. Cooper, N. Peppas, et al., eds., ACS Adv. Chem. Series, (1981) in press.
2. L. Gros, H. Ringsdorf, and H. Schupp, Angew Chem. Int. Ed. 20, (1981) 305, and references cited therein.
3. D.E. Gregonis, R. Hsu, D.E. Buerger, L.M. Smith, and J.D. Andrade, in R.B. Seymour and G.S. Stahl, eds., Solvent-Property Relationships in Polymers, Pergamon, (1982), in press.
4. R.W. Watkins and C.R. Robertson, J. Biomed. Materials Res., 11, (1977) 915.
5. R. Stoker, M.S. Thesis, University of Utah, August 1981.
6. R.E. Crandall, J. Janatova, and J.D. Andrade, Prep. Biochem., 11, (1981) 111.
7. B. Hupfer, H. Schupp, J.D. Andrade, and H. Ringsdorf, J. Electron Spectroscopy, 23 (1981) 103; preliminary communication.
8. B. Hupfer, H. Schupp, R. Van Wagenen, J.D. Andrade, and H. Ringsdorf, submitted to Colloid and Polymer Sci.
9. R.A. Van Wagenen and J.D. Andrade, J. Coll. Interface Sci., 76 (1980) 305.