APPLICATION
Valid until Sept. 82

NATO GRANTS
for international collaboration in research

NATO Scientific Affairs Division, B-1110 Brussels, Belgium

1 Project Title *(max. 10 words)*

Protein Adsorption onto Model Organic Surfaces

2 Principal Investigators *(Give names and affiliations of one senior scientist in each research group; indicate by an * the Project Co-ordinator and give his/her official address, telephone and telex numbers)*

*J.D. Andrade, Department of Bioengineering, College of Engineering, University of Utah, Salt Lake City, Utah 84112 U.S.A.
Telephone: (801) 581-8509 TELEX: TWX 910 925-5283 UNI UTAH SLC

Professor H. Ringsdorf, Johannes Gutenberg-Universitat, Fachbereich Chemie, Institut fur Organische Chemi D-6500 Mainz, WEST GERMANY

3 Project Identification

(a) Keywords *(max. 15)*

adsorption, protein, polymer, monolayers, tryptophan, fluorescence, Raman Spectroscopy

(b) Subject Code

42 (+ 12 *If interdisciplinary*)

4 Timing of the Project

(a) Starting Date : 9/1/82

(b) Estimated Duration : 2 years

5 Support requested *(for first year only)*

In US $

(a) (b) For Visits Abroad *(Give names, destination and duration of visits)*	Travel	Living
J.D. Andrade or co-worker from Salt Lake City To Mainz, Germany. 4 week visit	1,200	1,500
H. Ringsdorf or co-workers from Mainz to Salt Lake City Utah, U.S.A. 4 week visit	1,200	1,500
Sub-totals	(a) 2,400	(b) 3,000
(c) Other Expenditure *(Specify)* Page charges and reprints of joint papers		(c) 600
Total (a) + (b) + (c)		6,000

March 1981

6 Research Plan *(no more than 2 additional pages may be used)*

Describe the current state-of-the-art, outline objectives and methods of investigation, and give relevant references.

Protein adsorption at polymer aqueous solution interfaces is a problem important in physical biochemistry, polymer surface science and surface science in general. Although there has been considerable work on protein adsorption at solid-liquid interfaces [1], it is only 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 [2], which enables information on the conformational state and orientation of the adsorbed protein to be deduced.

H. Ringsdorf and his group at the Gutenberg University in 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 [3]. Such polymeric monolayer and multilayer films, because of their 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 in producing polymerizable monomers which are analogs to cell membrane phospholipid structures [3]. 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 [4], X-ray photoelectron spectroscopy, and total internal reflection fluorescence spectroscopy [2], based on the published methods of Watkins and Robertson [5]. 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 [2]. 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 [2]. As the total internal reflection method samples approximately 1,000 angstroms 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 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 diffration-derived structure of appropriate reference proteins, such as lysozyme and myoglobin.

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 studied by total internal reflection UV fluorescence [2].

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, human serum albumin monomer (mercaptalbumin monomer) and 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 [7] and gamma globulin [2]. 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 chaacterization , 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.

References

1. F. MacRitchie, Adv. Prot. Chem., 32 (1978) 283.

2. 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, (1982) in press.

3. L. Gros, H. Ringsdorf, and H. Schupp, Angew Chem. Int. Ed. 20, (1981) 305, and references cited therein.

4. 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.

5. R.W. Watkins and C.R. Robertson, J. Biomed. Materials Res., 11, (1977) 915.

6. R.E. Crandall, J. Janatova, and J.D. Andrade, Prep. Biochem., 11, (1981) 111.

7. R. Stoker, M.S. Thesis, University of Utah, August 1981.

7 International Co-operation

Describe the roles to be played by each research team indicating relevant work done, state the importance of this co-operation for the project, and justify visits to be made.

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. It is expected that the first trip would involve a member of the Mainz group coming 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. The second trip in the first year would be someone from the Salt Lake group to 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.

8 Participants

(a) Provide below a list of scientists, postdoctoral fellows and research students working on the project

(b) Attach a short curriculum vitae for each Principal Investigator (using the forms provided)

Name	Discipline	Highest Degree	Affiliation	% of time to be spent on project
Andrade, J.D.	Bioengineering/ Materials Science	Ph.D.	University of Utah Salt Lake City, UT U.S.A	20%
Van Wagenen, R.	Bioengineering	Ph.D.	University of Utah Salt Lake City, UT U.S.A.	75%
Gregonis, D.E.	Polymer Chemist	Ph.D.	University of Utah Salt Lake City, UT U.S.A.	20%
Ringsdorf, H.	Organic-Polymer	Ph.D.	Gutenberg, Universitat D-6500 Mainz, W. GERMANY	20%
Bader, H.	Organic chemistry	M.S.	Gutenberg, Universitat D-6500 Mainz, W GERMANY	50%

9 Referees *(Suggest three referees, in countries other than participants', and provide their full addresses)*

(a) Professor Y. Feijen, Polymer Division, Department of Chemical Technology, P.O. Box 217, Twente University of Technology, Enschede, THE NETHERLANDS

(b) Dr. Adam Baszkin, Laboratoire de Physico-Chimie des Surfaces, 45, Rue Des St-Peres, 75006 Paris, FRANCE

(c) Professor Pat Hendra, Department of Chemistry, University of Southampton, Southampton, 509 5NH England, UNITED KINGDOM

10 Support estimated necessary for continuation $ 6,000
(Comment if substantially different from Item 5)

11 Other Support

(a) Can you provide a rough estimate of the total cost of this project ? yes
If yes, how much is it ? $25,000

(b) What are the other sources of support for this project ?

U.S. NIH Grant HL18519
H. Ringsdorf has research support from German government and University sources.

12 Have any of the Principal Investigators been supported in the past by a NATO Research Grant ?
If so, please specify the grant number and confirm that it has been formally closed.

Yes, Professor H. Ringsdorf was a participant in Grant 1077 which was recently closed (A. Gomes will confirm closure of Grant 1077).

13 3-16-82
Date

Signature of Project Co-ordinator

curriculum vitae

Andrade, J.D.
Name

Affiliation and official address : Department of Bioengineering
University of Utah
Salt Lake City, Utah 84112 U.S.A.

Date and place of birth : July 13, 1941
Hayward, California, U.S.A.

Nationality : U.S.A.

Education *(degrees, dates, universities)*

B.S., 1965 San Jose State University (California)
Ph.D., 1969 University of Denver (Colorado)

Career/Employment *(employers, positions and dates)*

1969-Present: Assistant, Associate, and Professor of Materials Science, Bioengineering, and Pharmaceutics, University of Utah.

Specialization *(specify)*

(i) main field Polymer Surface Chemistry

(ii) other fields Biomedical Engineering

(iii) current research interests Protein and Macromolecule Adsorption

Honours, Awards, Fellowships, Membership of Professional Societies

Distinguished Research Award, 1981, University of Utah
Ebert Prize, J. Pharmaceutical Sciences 1978

Publications *(list selected publications on page 2)*

- Number of papers in refereed journals : 52
- Number of communications to scientific meetings : 25
- Books 1 and 1 in preparation

Recent selected publications *(additional pages should not be attached and reprints should not be enclosed)*

D.L. Coleman, D.E. Gregonis, and J.D. Andrade, "Blood-Materials Interaction: The Minimum Interfacial Free Energy and the Optimum Polar/Apolar Ratio Hypotheses," J. Biomedical Materials Research, in press.

B. Hupfer, H. Schupp, R. Van Wagenen, J.D. Andrade, and H. Ringsdorf, "Surface Analysis of Functionalized Oriented Polymer Mono- and Multilayers," Colloid and Polymer Science, in press.

J. Chen and J.D. Andrade, "Effect of Reductive Methylation in the Adsorption of Hen Lysozyme - Note," J. Colloid Interface Science, in press.

J.D. Andrade, D.E. Gregonis, and L.M. Smith, "Biomedical Aspects of Polymer Surfaces - Polymer-Water Interface Dynamics," in K.L. Mittal, ed., Physiocochemical Aspects of Polymer Surfaces, Plenum, 1982, in press.

L. Smith, C. Doyle, D.E. Gregonis, and J.D. Andrade, "Surface Oxidation of Cis-trans Polybutadiene," J. Appl. Polymer Sci., in press.

B. Hupfer, H. Schupp, J.D. Andrade, and H. Ringsdorf, "Photoelectron Mean Free Paths in Polydiacetylene Mono- and Multilayers," J. Electron Spectroscopy, 23, 103 (1981); preliminary communication.

G.K. Iwamoto, R. Van wagenen, and J.D. Andrade, "Insulin Adsorption Using Intrinsic Tyrosine Interfacial Fluorescence," J. Colloid Interface Sci., in press; Note.

R. Van Wagenen, S. Rockhold, and J.D. Andrade, "Probing Protein Adsorption. II. Total Internal Reflection Intrinsic Fluorescence," in S.L. Cooper, N. Peppas, et al., eds., Morphology, Structure, and Interactions of Biomaterials, ACS Adv. Chem. Series, (1981) in press.

R.N. King, J.D. Andrade, S.M. Ma, D.E. Gregonis, and L. Brostrom, "Interfacial Tensions at Acrylic Hydrogel-Water Interfaces," J. Coll. Interface Sci., in press (1982).

R.E. Crandall, J. Janatova, and J.D. Andrade, "Effects of Radioiodination and Fluorescent Labeling on Albumin," Prep. Biochem., 11, (1981) 111.

R.A. Van Wagenen, D.L. Coleman, R.N. King, P. Triolo, L. Brostrom, L.M. Smith, and J.D. Andrade, "Streaming Potential Investigations: Polymer Thin Films," J. Colloid Interface Sci., 84, (1981) 155.

J.D. Andrade, "Surface Analysis of Materials for Medical Devices and Diagnostic Products," Med. Devices and Diag. Ind., 2 (1980).

R.A. Van wagenen, B.J. Zdasiuk, and J.D. Andrade, "Total Internal Reflection Fluorescence Studies of Albumin Adsorption onto Quartz," ACS Organic Coatings Preprints, 21, (1980) 749.

J. Janatova, R.E. Crandall, and J.D. Andrade, "An Analysis of the Heterogeneity of Albumin," Prep. Biochem., 10 (1980) 405.

J.D. Andrade, R.N. King, D.E. Gregonis, and D.L. Coleman, "Surface Characterization of Poly(hydroxyethyl Methacrylate) and Related Polymers. I. Contact Angle Methods in Water," J. Poly. Sci. Symp., 66, (1979) 313.

curriculum vitae

Ringsdorf, H.

Name

Affiliation and official address : Institut fur Organische Chemie
Johannes Gutenberg-Universitat
D-6500 Mainz, den
Johann-Joachim-Becher-Weg 18-20

Date and place of birth : WEST GERMANY **Nationality :**

July 30, 1929: GieBen, Germany WEST GERMANY

Education *(degrees, dates, universities)*

1952	Uni. of Frankfurt
1953	Uni. of Darmstadt
1955-59	Uni. of Freiburg

Career/Employment *(employers, positions and dates)*

1960-62	Research Associate, Polytechnic Institute of Brooklyn
1963-71	Assistant and Associate Professor, University of Marburg
1971-Present	Professor of Organic and Polymer Chemistry, University of Mainz
1979-Present	Member, Academy of Science, Mainz

Specialization *(specify)*

(i) main field Polymer Chemistry

(ii) other fields Pharmacologically Active Polymers

(iii) current research interests
Polyreactions in Ordered Systems
Polymeric Liquid Crystals
Polymers as Model Membranes

Honours, Awards, Fellowships, Membership of Professional Societies

MANY

Publications *(list selected publications on page 2)*

- Number of papers in refereed journals : 128
- Number of communications to scientific meetings : MANY
- Books 0

Recent selected publications *(additional pages should not be attached and reprints should not be enclosed)*

B. Hupfer and H. Ringsdorf, "Polymeric Monolayers and Liposomes as Models for Biomembranes and Cells," ACS-Symposium Series, 175 (1981).

H. Ringsdorf and A. Schneller, "Synthesis, Structure, and Properties of Liquid Crystalline Polymers," The British Polymer Journal, 13, (June 1981).

H. Ringsdorf, "Incorporation of ATP-Synthetase into Long-Term Stable Liposomes of a Polymerizable Synthetic Sulfolipid," FEBS-Letters, 132, 2, (1981) 313-318.

L. Gros, H. Schupp, and H. ringsdorf, "Polymere Antitumormittel auf Molekularer und Zellularer Basis?" Angew. Chem., 93, (1981) 311-332.

H. -H.Hub, B. Hupfer, H. Koch, and H. Ringsdorf, "Polymerization of Lipid and Lysolipid Like Diacetylenes in Monolayers and Liposomes," J. Macromol. Sci, - Chem., A15, (1981) 701-715.

H. Ringsdorf and H. Schupp, "Polymerization of Substituted Butadienes at the Gas-Water Interface," J. Macromol. Sci. - Chem., A15 (1981) 1015-1026.

A. Akimoto, K. Dorn, L. Gros, H. Ringsdorf, and H. Schupp, "Polymere Modell-membranen," Angew. Chem., 93 (1981) 108-109.

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H. Hupfer, H. Ringsdorf, and H. Schupp, "Polyreactions in Oriented Systems, 21 Polymeric Phospholipid Monolayers," Makromol. Chem., 182 (1981) 247-253.

D. Day, H.H. Hub, H. ringsdorf, "Polymerization of Mono- and Bi-Functional Diacetylene Derivatives in Monolayers at the Gas-Water Interface, Isr. J. Chem., 18 (1979) 325-329.

T. Hirano, W. Klesse, and H. Ringsdorf, "Polymeric Derivatives of Activated CP as Drug Delivery Systems in Antitumor Chemotherapy," Makromol. Chem., 180 (1979) 1125-1131.

D.R. Day and H. Ringsdorf, "The Monolayer Polymerization of 10,12-Nonacosadiynoic Acid Studied by a Spectroscopic Technique," Makrolmol. Chem., 180 (1979) 1059-1063.

V. Hofmann, H. Ringsdorf, A. Seganowa, W.-H. Wager, "Pharmakologisch-Aktive Polymere, 19," Makromol. Chem., 180, (1979) 837-841.