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9102-136

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U.S. DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM

COVER PAGE

(THIS PAGE MUST BE THE FIRST PAGE OF THE APPLICATION)

BOX No. H-182-17 Bldg. 3714-H
FOLDER US/DOE Univ. Research Inst. Program 9102-136

1. Name of Institution: CASE WESTERN RESERVE UNIVERSITY
2. Department: BIOMEDICAL ENGINEERING
3. Street: 2040 Adelbert Road 4. City: Cleveland
5. County: Cuyahoga 6. State: OH 7. ZIP: 44106 8. Congressional District: 21st
9. Telephone: Area Code 216 Office: 368-3005 Home: [redacted]
10. Title of Application: ATOMIC FORCE MICROSCOPE FOR BIOMEDICAL RESEARCH
11. Area of Proposed Research (Select ONE) A. BIOMED./ENVIRONMENTAL

(A) Biomedical/Environmental
B. Chemical/Coal Science
C. Geosciences
D. Materials Research
E. Plant Science/Microbiology
F. Other

12. Research Subcategory (See Section II of DOE/URI FY 91 Announcement) LIFE SCIENCE STUDIES
13. Total DOE Funding for Research in Selected Area (During the last two fiscal years): $ 672,527
14. Estimated Purchase Price of Equipment: $ 140,200 15. Amount requested from DOE: $ 128,500

List all Federal agencies which are currently considering proposals from the institution involving the same or similar equipment.

16. Agency: N/A Agency Proposal Number:
Agency: Agency Proposal Number:

NOTE: The institution is responsible for immediately informing the URI program manager in writing if a proposal involving similar or related equipment is submitted to a federal agency prior to the announcement of DOE's URI awards.

List and federal agency which has provided funds to the institution during the past two years for the same or similar equipment.

Agency: N/A Amount of Funds: N/A

Please check one of the following:
☒ I authorize outside peer review of this proposal.
☐ I do not authorize peer review of this proposal.**

Signature of Principal Investigator: Roger D. Kamm Date: 11/28/90

Name and Title of Institutional Official (President or Designee)
James Kemp
Assistant Director
Signature:
Date: 12/4/90
Area Code/Telephone: (216) 368-4510

REPOSITORY Oak Ridge Operations
COLLECTION Records Holding Area
Documents 1944-1994

Is Applicant Delinquent on any Federal Debt? ☐ Yes (If, "Yes," attach an explanation) ☒ No

* Note – The application will be evaluated by reviewers in this field.
** Note – May prevent full consideration of this application.

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TABLE OF CONTENTS

ATOMIC FORCE MICROSCOPE FOR BIOMEDICAL RESEARCH

SUMMARY

This purpose of this application is to request funding to purchase an Atomic Force Microscope (AFM), that will enable major advances in ongoing energy-related biomedical research associated with characterizing the structure and function of biological macromolecules. The AFM is innovative new instrumentation that is expected to have a major impact on molecular-level biomedical research by providing direct, three-dimensional topographical images of biological macromolecules down to the nanometer level. This can be accomplished in air or in aqueous in situ environments, without any special requirement for high vacuum, conductive coatings, or for samples with highly ordered crystalline structures. AFM images can be obtained fast enough (few seconds/image) to observe many biological processes in real time and thus provide potential access to image molecule-molecule or molecule-cell interactions. The principal components of the AFM include: (i) the AFM "head," which includes the force detection assembly, and an optical system for the measurement of minute movements in the interactive probe tip; (ii) the sample stage mounting, which includes a piezoelectric tube scanner; and (iii) dedicated, computer-based, signal processing and data analysis systems, that converts the voltage signals into a three-dimensional image.

The proposed research thrust area will focus on DOE-related research in life sciences on characterizing the three-dimensional structure of perturbed biological macromolecules, and will encompass DNA, and plasma proteins involved in interfacial phenomena. Emphasis will be on imaging and identifying structural and/or functional alterations induced by exposure of the macromolecule to some foreign agent such as ionizing radiation, or artifical surfaces. Specifically, the proposed initial experiments will include (i) imaging the effects of radon

damage to DNA structure; (ii) direct observations of DNA lesions, such as single strand breaks and DNA-protein crosslinks after treatment with phthalocyanines and visible light or ionizing radiation; (iii) the characterization of radiation-induced damage to biomolecules such as enzymes encapsulated in microemulsions or gels; (iv) characterizing the structure and functional properties of plasma proteins, following attachment to artificial surfaces, and will include studies on fibronectin using a series of monoclonal antibodies that recognize specific epitopes of fibronectin; (v) similar experiments on von Willebrand factor and (vi) coagulation factor XII.

This new instrumentation, has wide potential applications in biomedical research (and potential users). The AFM equipment requested in this application is intended for shared use by faculty members and students of Case Western Reserve University is not currently available on our campus.

In recent years, an increasing number of graduate and medical students have been trained in the area of characterization of biological macromolecules (8 Ph.D degrees conferred in 1989). Furthermore, the University is committed to a major effort in biomedical research. This includes the construction (underway) of a new biomedical sciences building that will house over 80 new faculty members. Consequently, this provides the prospect of further increases in the number of graduate students who might benefit from the aquisition of this equipment.

U.S. DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM

BUDGET PAGE

ESTIMATED COSTS

Instrumentation	Requested of DOE	Institution's Cost Sharing *(1)*	Other Federal Funds *(2)*	TOTAL
A. **Purchase Price** *(3)*	$120,000	9,700	-0-	$129,700
Maintenance *(4)*	8,500	X X X X X	X X X X X	8,500
Subtotal:	$128,500	9,700	-0-	$138,200
B. **Other Allowable Costs**				
1. **Shipping/Handling**	X X X X X	-0-	-0-	-0-
2. **Building/Laboratory**	X X X X X	$2,000	-0-	$2,000
Subtotal:		$2,000	-0-	$2,000
C. **TOTAL**	$128,500	$11,700 *(5)*	-0-	$140,200

NOTES:

(1) Non-Federal funds only. (However, may be provided by a third party.)

(2) Estimate funds to be obtained from other Federal agencies for purchasing the instrument, etc.

(3) Only the purchase price of the instrumentation is eligible for DOE funding through this program.

(4) See discussion of eligible maintenance costs on page 11.

(5) Only those costs specified above are eligible as cost sharing. Installation, operation, maintenance, travel and training costs, or faculty and student salaries, etc. are ineligible as cost sharing. Review discussion of eligible and ineligible costs on pages 10 and 11.

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A. **Purchase Price (List components and unit prices.)**

Description/Vendor	Quantity	Total Estimated Unit Price	Requested of DOE	Institution's Cost Sharing	Total
The proposed vendor for components of the equipment is Digital Instruments (Santa Barbara, CA)					
1. Nanoscope II system with atomic force microscope	1	$89,000	$79,300	$9,700	$89,000
2. Upgrade to Nanoscope III (improved signal processing & data analysis revisions are scheduled for release by the Company during 1991)	1	10,000	10,000	-0-	10,000
3. Off-line data analysis system	1	15,000	15,000	-0-	15,000
4. 12 micron scanning head (AFM)	1	2,750	2,750	-0-	2,750
5. 12 micron scanning head (STM)	1	2,200	2,200	-0-	2,200
6. X-Y sample stage for AFM	1	3,000	3,000	-0-	3,000
7. Fluid cell	1	500	500	-0-	500
8. Video color printer	1	3,500	3,500	-0-	3,500
9. Cantilever probes (one wafer)	1	1,000	1,000	-0-	1,000
10. Calibration standards	1	250	250	-0-	250
11. Vibration isolation (table and pulley system)	1	2,500	2,500	-0-	2,500
12. MAINTENANCE (two-year service contract)	-	8,500	8,500	-0-	8,500
Subtotal:		$ 138,200	$ 128,500	$9,700	$ 138,200

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DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM
GRANT AND CONTRACT SUMMARY FORM

Institution Name: **Contract Date:**

	Current Grant/Contract/Subcontract No. (and contractor name if subcontract)	Principal Investigator	Title	From MO	From YR	To MO	To YR	Total Award Value	$ AMT Awarded for FY Period 1988 to 1990	DOE Technical Monitor/Location
1a.	DE-FG02-88ER60617	Bakale, George	"Ionization in Liquids	11	88	11	89	$85,000	$171,041	
1b.	DE-FG02-88ER60617	Bakale, George	"Ionization in Liquids	11	89	11	90	86,041		
1c.	DE-FG02-88ER60617	Bakale, George	"Ionization in Liquids	11	90	11	91			
2a.	DE-FG02-88ER60658	Evans, Helen H.	"Mutagenicity of Radon and Radon Daughters"	1	89	1	90	$206,975	$447,486	
2b.	DE-FG02-88ER60658	Evans, Helen H.	"Mutagenicity of Radon and Radon Daughters"	1	90	1	91	$240,511		
3.	DE-FG02-89ER60587	Goldthwait, David A.	"Repair of DNA treated with Irradiation and chemical carcinogens"	6	89	6	90	54,000	54,000	
									$672,527	

Provide information only on those research projects which are directly related to the selected principal research area and which were active during the period from October 1, 1988 to September 30, 1990. Do not include grants or contracts for the following: (1) workshops, (2) education/training projects or other non-research projects, (3) facilities, (4) instrumentation, (5) those projects completed before October 1, 1988.

See discussion on page 8 for additional requirements relating to subcontracts.

RELATED FEDERAL AGENCY GRANTS AND CONTRACTS

Agency/Agency Contact	Principal Investigator	Grant/Contract No.	Title	Grant/Contract Dates From MO	From YR	To MO	To YR	Total Award Value (TDC)
1a. NIH-NCI	Evans, Helen H.	CA-15901-15	Mutants and Altered Radioresponse of Cells and Tumors	12	85	11	90	$ 818,113
1b. NIH-NCI	Evans, Helen H.	CA-15901-15	Mutants and Altered Radioresponse of Cells and Tumors	12	90	11	93	630,132
2. NIH-NCI	Oleinick, Nancy L.	CA-48737-01	Phthalocyanine Photodynamic Therapy: Mechanistic Studies	7	90	6	93	1,771,386
3. NIH-NCI	Oleinick, Nancy L.	CA-15378-18	Radiation Induced Modifications of Protein Synthesis	12	84	11	90	-
				12	90	11	91	233,784
4. NIH-NHLBI	Anderson, James M.	HL-33849	Biomedical Polymers: Cell Activation and Interleukin 1	4	85	3	88	-
				4	88	3	93	474,550
5. NIH-NCI	Culp, Lloyd A	CA-27755	Fibronectin: Proteoglycan Binding in Adhesion Sites	4	81	3	89	-
				4	89	3	94	514,210
6. NIH-NHLBI	Marchant, Roger E.	HL-40047	New Biomedical Interface Materials	3	88	2	93	330,000

Only provide information on research projects which are directly related to the selected principal research area.

6. ATOMIC FORCE MICROSCOPE FOR BIOMEDICAL RESEARCH

PROPOSED RESEARCH THRUST AREA:

Characterization of the Structure and Function of Perturbed Biological Macromolecules

A. OVERVIEW

This application is to request funding to purchase an Atomic Force Microscope (AFM) that will enable major advances in ongoing energy-related biomedical research associated with characterizing the structure and function of biological macromolecules. The designated research area of life sciences will focus on identifying the structural and/or functional alterations induced by exposure of the macromolecule to some foreign agent such as ionizing radiation and artificial surfaces. In this application, we shall demonstrate that the proposed equipment will have a profound impact on the designated research area and will provide a major stimulus for future growth in this energy-related research, through the initiation new collaborative research, by providing new opportunities for acheiving innovative and ambitious research goals, and by training increasing numbers of present and future graduate students in a growing area of national research effort. Many of the federally funded projects cited in this application have received continuous financial support for many years, some for almost two decades, and is clear testament to the high standard of biomedical research that has been performed by the faculty at our University in the past, as well as providing evidence for a promising future. Furthermore, the University has recognised this need for advanced biomedical research. This commitment includes the construction (in progress) of a new biomedical sciences building that will house over 80 new faculty members.

B. BACKGROUND AND EQUIPMENT DESCRIPTION

The AFM and its precursor, the scanning tunneling microscope (STM), are innovative and revolutionary new instruments capable of resolving surface detail down to the atomic level (1-4). The exciting potential of AFM is to offer direct, nondestructive, submolecular three-dimensional imaging of biological macromolecules down to the nanometer level. Imaging can be accomplished in controlled atmosphere, air or in aqueous in-situ environments. Thus, there is no special requirement for high vacuum environments, the need for high energy radiation sources, conductive coatings (or replicas) on samples (as for TEM and SEM), or samples possessing highly ordered crystalline structures. The images can be obtained fast enough (few seconds/image) to observe many biological processes in real time and thus provide potential access to image dynamic molecule-molecule or molecule-cell interactions. Our proposed initial experiments (Section 6E), describe sub-molecular imaging of structural and/or functional alterations induced by exposure of a biological macromolecule to some disruptive foreign agent such as radon and radon daughters. The effects of radon on DNA, for example, are usually determined by functional assays at the cellular level. In the proposed experiments, the effects will be directly observable at the molecular level. The results from other experiments will yield similar new insight into these molecular level events. It is anticipated that the fundamental information that will be obtained from the proposed experiments on this energy-related research area will have considerable impact not only on the research progress but on future experimental design and perspective.

Currently, there is no atomic force microscope available on our campus. Indeed, this is the case for most universities in the United States, although the precursor STM has been commercially available for about 3-4 years. The invention of the AFM was first described in 1986 by Binnig, Quate and Gerber (2), while much

of the subsequent developmental research has been carried out by P.K. Hansma's group at the University of California at Santa Barbara. Based on Hansma's prototype AFM, a commercial instrument was developed by Digital Instruments Inc. for use on their "Nanoscope" STM system. The AFM became commercially available in the spring of this year (1990). Consequently, publications describing applications of the AFM, particulary in the biological area are extremely few. However, following two publications in Science by Hansma and coworkers (4,5) the enormous potential impact of AFM for applications in the biomedical sciences became clear.

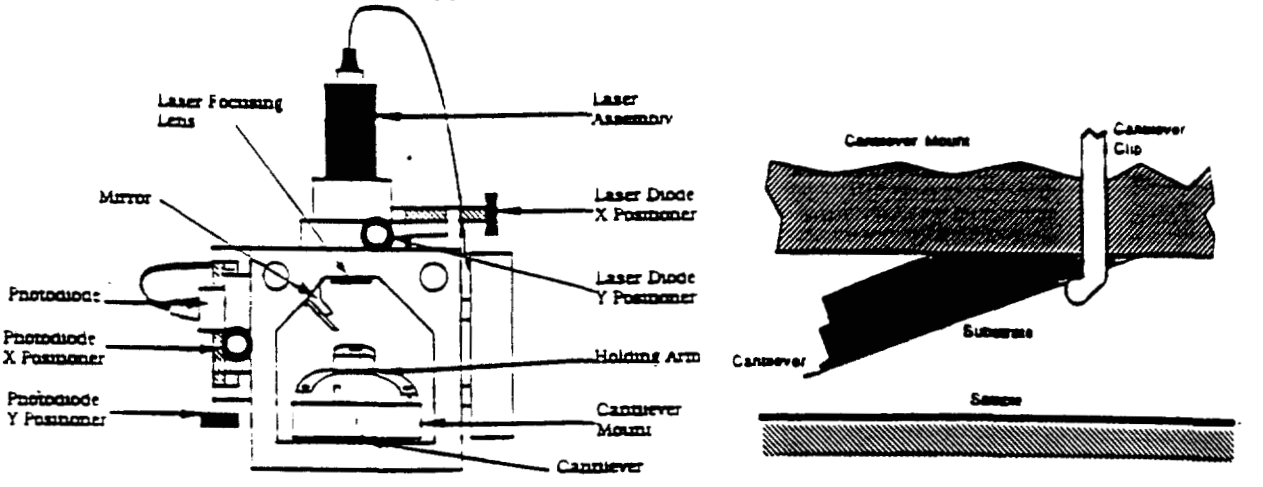


Figure 1. The diagram on the left illustrates the atomic force microscope head, and the cantilever arrangement is shown on the right.

The principal components of the AFM, which has an estimated cost of $138,200, include: (i) the AFM "head," (figure 1) which consists of the force detection assembly, and an optical system for the measurement of minute movements in the interactive probe tip; (ii) the sample stage mounting, which includes a piezoelectric tube scanner, and provides the precise x,y,z movement of the stage; (iii) and dedicated, computer-based, signal processing and data analysis systems, that coverts the voltage signals, into a three-dimensional image. In addition, the proposed Nanoscope II/III equipment incorporates STM capability as part of the system. The key component of the AFM is the force-sensing assembly, which measures variations in the interatomic (tip-sample) repulsive or attractive forces between a

flexible stylus and the surface of interest. AFM operates by scanning a surface with a sharp tip (ideally of atomic dimensions) mounted on a flexible cantilever. The tracking force between the probe tip and the biological molecule can be adjusted to extremely small values of 1 nanoNewton and less. The deflections are measured using a focused laser beam which is reflected off the gold-coated cantilever to a two-element photodiode detector. The optical detection allows distances of a fraction of an angstrom to be measured. The image processing system converts the voltage signal, as a function of probe position, into a three dimensional image. The dedicated software then allows manipulation of the scanning parameters and subsequent data analysis.

The AFM has been used to obtain atomic resolution of both conductors (6) and nonconductors (7), including native oxides on silicon (8), and molecular level images of insulating polmers (9). In 1988, molecular level images of DL-leucine crystals were reported that showed resolution and identification of individual methyl groups (10). Subsequently, Hansma and coworkers (11) reported on images obtained in air and under water of polyaniline on glass, as well as real-time in situ imaging of fibrinogen and thrombin activated polymerization of fibrin. Similar in-situ dynamic imaging of murine monoclonal immunoglobulin has been obtained (12). The ability of AFM to image whole cells (human lymphocytes) and surface features of cell membranes in which details were resolved down to 10nm, has been demonstrated recently (13). However, AFM images of delicate biological molecules or cell membranes with better than 10nm resolution has not been reported, yet.

In the past two years, the progress that has been made in using scanning tunneling microscopy for characterizing biological macromolecules has been remarkable, particularly in regard to the resolution of the images. This may be attributed to improvements in single processing, sample preparation methods, and

scanning conditions employed. For example, Driscoll and coworkers (14) recently reported on images obtained of uncoated duplex DNA in a vacuum environment, which showed the double helical structure, base pairs, and atomic scale substructure. This was achieved despite the system requirement to establish a nanoamp electron tunneling current. This requirement presents no experimental difficulties for conductive samples, but in the case of biological samples it can lead to no image (complete insulation), images which are not reproducible, and even the possibility of mis-interpreting artifact for submolecular detail. These comments are included to highlight two important considerations. The resolution and reproducibility of biological macromolecules images obtained by AFM is potentially much greater than that for STM, because AFM depends on measurement of interatomic forces and not on sample conductivity (i.e., need for coatings) or the ability to establish a tunneling current through an uncoated biological sample which will not conduct electrons. Secondly, experiments with STM have been in progress for several years, whereas the AFM is a much more recent development in which most published images at 10nm resolution have been obtained under aqueous conditions, where molecular mobility on the surface will be higher than for dry samples.

C. PRELIMINARY STUDIES

A series of preliminary experiments have been carried out by the Principle Investigator. AFM and STM experiments were performed during last summer (1990), in the laboratory of Dr. Joseph Andrade, Department of Bioengineering, University of Utah, Salt Lake City. The goal of the experiments was to evaluate the potential of AFM, and to a lesser extent STM, for obtaining molecular-level resolution of biological macromolecules. During these experiments, several different proteins were imaged in air, in aqueous buffer solutions and under dynamic flow conditions. The proteins evaluated included purified human von

Willebrand Factor (vWF), recombinant plasminogen activator inhibitor (PAI), coagulation factor XII, and some solid surfaces including mica which had been surface modified by chemical derivatization. Several examples of AFM and STM images are shown in figures 2-9

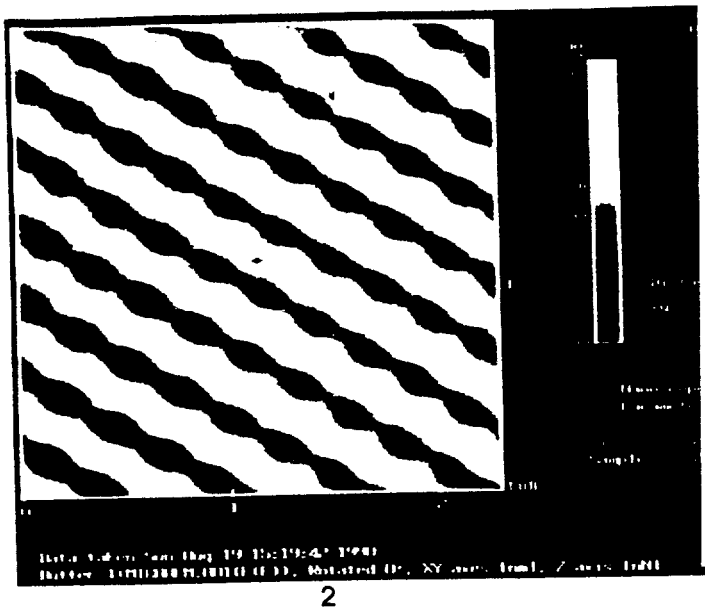
vWF is a large plasma glycoprotein that mediates adhesion of platelets to substrata such as subendothelium, and acts as a carrier molecule for coagulation protein factor VIII. VWF exists in the circulation as large disulfide-linked multimers, ranging in molecular weight from 0.5 to 20 million. VFW has been visualized previously by TEM as a flexible molecular filament (usually coiled) of varying lengths, typically 60 nm to over 1100 nm fully extended (15). The large size of vWF, and its role in thrombus formation, made it an attractive biological macromolecule for preliminary investigation.

The ability to obtain three dimensional images of biological macromolecules such as proteins by AFM, depends on the interactive forces between the two-dimensional solid surface and the protein, relative to the tracking force, which is of the order of 1 nN, applied to the protein by the cantilever tip. If the tracking force (which can be reduced during the experiment), is greater than the surface-protein adhesive force then the protein will be "moved" by the probe tip. This phenomenon was observed for vWF deposited on freshly-cleaved mica. Thus, vWF molecular aggregates 40 nm thick (width dimension) seen in a 1.3 x 1.3 micron image (obtained using a 12 micron scanning head), became 100 nm thick vWF molecular aggregates in the following image. This effect of protein "manipulation" was illustrated when the probed area was viewed at higher scan area (8 x 8 micron) and scan rate shown in figure 3(#5). It should be noted that the typical resolution obtained in these studies was similar to published images of fibrinogen/fibrin (11) and IgG molecules (12).

In contrast, AFM images of vWF obtained after rinsing, drying, and re-imaging showed individual vWF molecules adsorbed on the mica, that were not perturbed by the tracking force applied by the cantilever tip, shown in figures 4 and 5. From scan areas of 200 x 200 nm to 1 x 1 micron (using the 12 micron head), individual vWF molecules were observed. As in previous TEM studies (15), VWF molecules observed by AFM covered a wide range of dimensions. The largest "individual molecules" observed were up to 800 nm in the longest dimension, but molecular sizes in the order of 100 nm to 400 nm were most common with apparent measurements of 2-4 nm in the 'z' direction. These preliminary experiments demonstrate that three dimensional molecular level images of protein molecules such vWF can be obtained by using AFM (16, see also ref. 17, published Nov. 1990) as well as with STM (examples shown in figures 6 and 7).

Figure captions. Figure 2. AFM image which shows atomic-scale resolution (2.3nm x 2.3nm full scale) for cleaved mica imaged under water. Figure 3. vWf (0.4 mg/mL) deposited on mica and imaged in air, shows the effect of the probe tip on the vWF, full scales range from 1368nm (#1,2) up to 8663nm (#5). Figures 4 & 5 show individual vWF molecules after rinsing and drying the previous samples, full scales range from 380nm up to 1 micron. Figure 6 shows a vWF molecule (image has been low-pass filtered and expanded to a scale of 60nm) obtained from STM experiments. Figure 7a shows an unfiltered STM image (52nm full scale) of recPAI, the structure of this molecule is not known, but is believed to be similar to alpha-1 proteinase inhibitor which the has 3 antiparallel beta sheets, shown in 7b, while very similar to the STM image, the measured x and y dimensions for PAI are larger by about a factor of 1.6. Figure 8 demonstrates imaging under water, in which molecules of coagulation factor XII (25u/mL) are shown. We were able also to prepare and image mica with a modified surface using chemisorbed organic monolayers, although preliminary experiments did not reveal atomic-level surface structure.

* "Use or disclosure of the application data in the entire section entitled "Preliminary Studies" are subject to restrictions.



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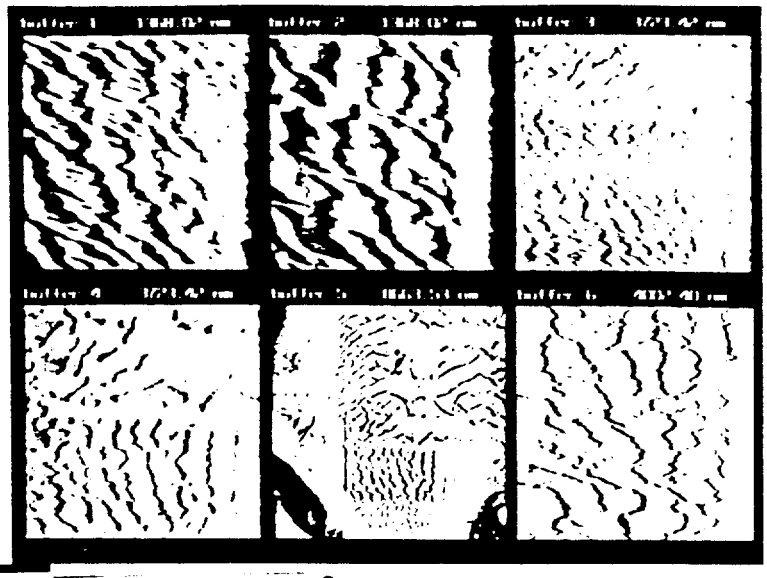

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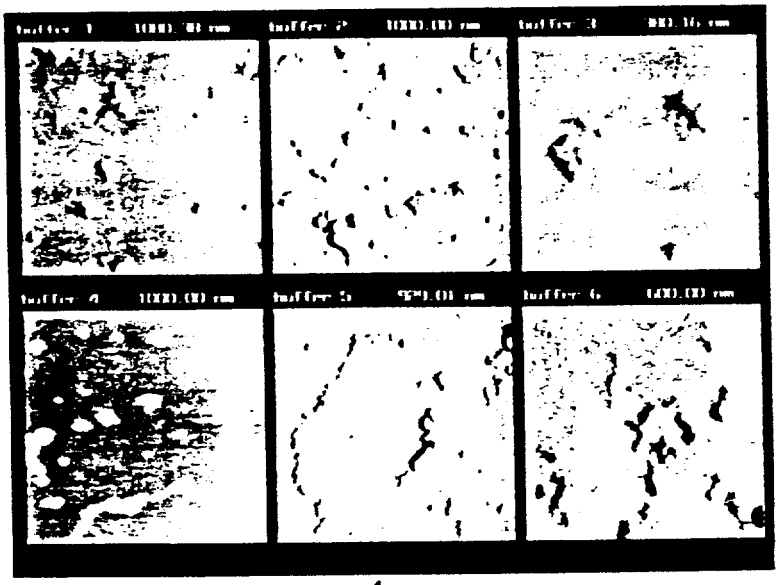

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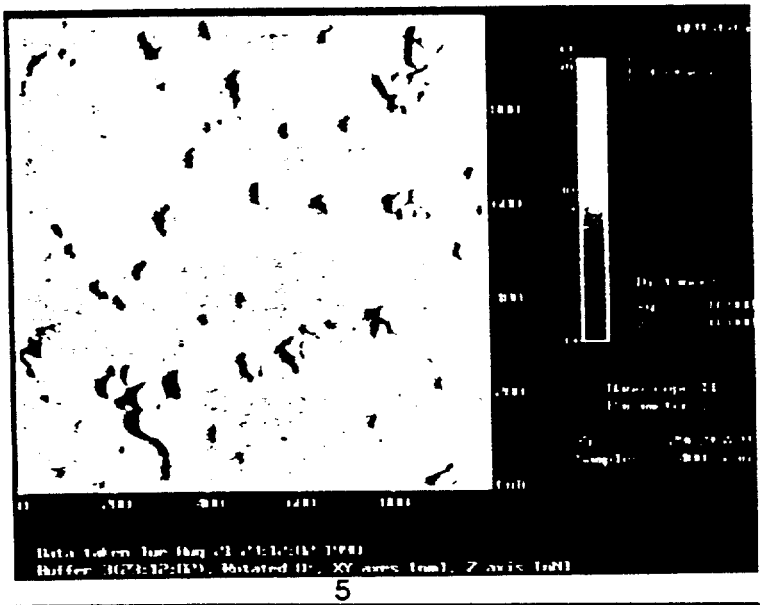

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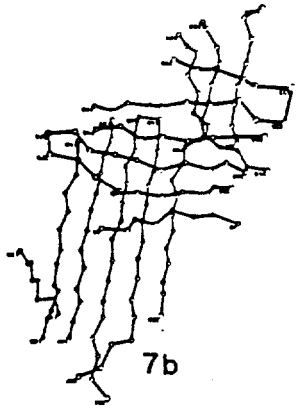

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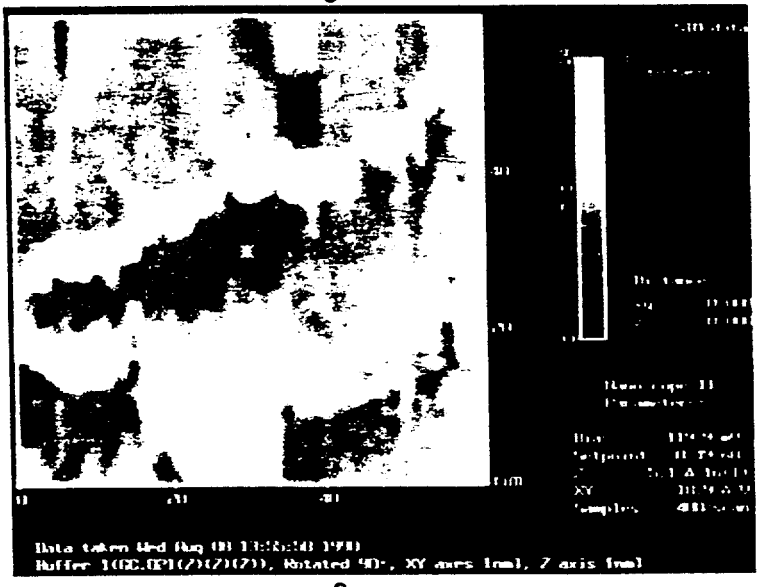

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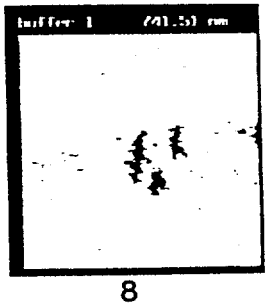

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D. PRINCIPAL RESEARCH AREAS/PROJECTS

The proposed research thrust area will focus on DOE-related research on characterizing the three-dimensional structure of perturbed biological macromolecules, and will encompass DNA and specific plasma proteins involved in interfacial phenomena. Emphasis will be on imaging and identifying structural and/or functional alterations induced by exposure of the macromolecule to some foreign agent such as radon. The requested equipment provides the possibilty of direct imaging of biological molecules and interactive events that could be alluded to only through indirect measurement and detection. Specifically, the proposed initial experiments will include (i) molecular-level characterization of the effects of radon/radon daughter induced damage to DNA; (ii) characterization of radiation-induced damage to biomolecules such as enzymes encapsulated in microemulsions or gels, that will elucidate the role that electron-related processes play in effecting radiobiological damage; (iii) direct observations of DNA lesions, such as single strand breaks and DNA-protein crosslinks after treatment with ionizing radiation or phthalocyanines and visible light; (iv) characterizing the effect of artificial surfaces on the binding and functional alterations to plasma proteins and enzymes which include fibronectin, which will be studied with a series of monoclonal antibodies that recognize specific epitopes of fibronectin; (v) similar experiments on von Willebrand factor and the effect on platelet binding (vi) and coagulation factor XII.

1. DOE FUNDED RESEARCH PROJECTS

(i). "Mutagenicity of Radon and Radon Daughters;" Funding: $598,385, for the period 4/88-12/90; Anticipated funding: $730,000 for the period 1/91-12/93, $730,000; P.I., Helen Evans.

This DOE funded project has been renewed recently for a further three years. Three graduate students have received training from this program. Currently, one graduate student (Thom Deahl) is assigned to this project.

The objective of this research project is to investigate the dose-response relationship of the lethal and mutagenic effects of the exposure of cells to radon and its decay products. The nature of the mutagenic lesions will be studied as a function of dose rate and of the repair capabilities. During the previous three years of research, our results have demonstrated that (i) The mutagenicity of alpha radiation from radon and radon daughters is similar to that of low LET (linear energy transfer) radiation at equitoxic radiation doses; (ii) The majority of $TK^{-/-}$ mutants induced by radon and radon daughters harbor multilocus lesions; (iii) Almost all of the $TK^{-/-}$ mutants of strain LY-R16 have lost the entire active tk allele; (iv) The percentage of mutants with deletions extending from the tk gene to the gk gene was greater than the percentage induced by X radiation in the repair-efficient strain LY-R16; (v) The percentage of mutants with multilocus deletions is reduced by repair after exposure to X radiation, but not after exposure to radon and radon daughters; and (vi) The radon daughters attached to cells or incorporated into cells contributes significantly to the total dose.

Our general plan for future research consists of three specific aims: (1) To continue the study of the effect of dose rate on the cytotoxic and mutagenic response of the L5178Y cells and on the nature of the mutagenic lesion; (2) To study the variation of the cytotoxic and mutagenic response of L5178Y cells as a function of cell cycle stage; and (3) To study the molecular nature of radon/radon

daughter-induced deletions and rearrangements in the hprt and tk genes in human cells, with regard to the size of the lesion and the nucleotide sequence of the break-points. Our long-term goal is to determine the nucleotide sequence surrounding the break points in autosomal genes, since the presence of a homologous chromosome may influence the gene rearrangements which result from radiation injury.

Proposed AFM Experiments

Characterization of the radon/radon daughter-induced damage, such as identification of deletion size and strand breaks is a major goal of the planned research. An instrument capable of molecular-level imaging would have consderable impact on this specific goal of the project. The AFM, requested in this application, would initiate new experiments designed to exploit the instument's molecular-level resolution. The following initial experiments are envisaged: To employ AFM (and STM) to study DNA and chromosome breakage, following irradiation of cells or DNA in vitro. The effects of exposure to X-radiation and alpha particles from radon and radon daughters could then be compared directly. Initially, these experiments will be performed using the larger 12 micron microscope scanning head, which will provide reproducible images, proceeding from the large 12-15 micron field down to the nanometer scale (the ultimate resolution depends on the scanning head size and the image data pixel density, approx. 400 x 400, which is to be increased will be to 526 x 526). This approach will permit statistical calculations and comparisons of gross molecular dimensions for a large number of DNA molecules irradiated at a given dose rate (e.g., 40 cGy/hr). Parallel experiments will be performed on cDNA isolated from mutants containing deletions and/or rearrangements, and derived from the irradiation experiments on human $TK^{+/-}$ lymphoblast TK6 cells. In subsequent AFM experiments, exposure dose rates will be varied from 1 cGy/hr to approximately 40 cGy/hr. The irradiated DNA and control

samples will then be imaged with the 0.7 micron scanning head, with the objective of documenting specific alterations in helical structure, base pairs, and ultimately close to atomic-scale substructure. In addition to providing potential new information at the molecular and sub-molecular level, these studies will have impact on our overall project objective of determining the effect of dose rate on mutagenic response.

In this DOE Project, there are numerous additional potential applications in which both AFM and STM could be utilized. For example, one effect of ionizing radiation is the induction of crosslinks between DNA and nuclear proteins. Visualization and quantification of DNA-protein crosslinks (DPC) could be obtained through the application of AFM, and the effects of X radiation and alpha particles could be characterized, quantified and compared. Current calculations of radiation-induced crosslinked DNA yields are based on four indirect methods such as filter binding(18), which are complicated by the simultaneous induction of single strand breaks (SSB) along with DPC. There is a significant need in this project area for the AFM technique, so that differences in DPC and SSB in irradiated DNA can be distinguished and quantified. Similarly, certain repair enzymes and/or DNA binding proteins complex with damaged DNA and these complexes could be visualized directly and compared, following exposure to ionizing radiation under different conditions. A further extension of this work includes imaging the repair enzyme/ damaged DNA complex formation under aqueous real-time conditions. Clearly, there is considerable scope and potential impact from applications of the AFM system to this DOE project area.

(ii). "Ionization of Liquids;" $171,041 (FYs '88/90); P.I. George Bakale.

This DOE funded project project is currently at the beginning of the first year of a three-year renewal. Four students and two Post-doctoral fellows have received training from this program.

The rationale of the "Ionization in Liquids" project is that the physico-chemical principles deduced from studies of electron-chemical interactions can be applied to the primary ionization process and therby used to elucidate the roles that electron-related processes play in effecting radiobiological damage. Inferred in this rationale is that cellular electron-transfer processes can be disrupted by either an ionizing event or an electrophilic or electrophobic chemical and that this disruption can be manifested in a deleterious biological effect. In order to learn how this disruption occurs, the transport and reaction properties of excess electrons are studied in model liquids and biomimetic systems, in which attempts are made to simulate the cellular microenvironment over the time regime at which electron transfer occurs in biological systems. The excess electrons serve in these interdisciplinary studies as physico-chemical probes of the electron-accepting potentials of chemicals that are strongly correlated with the chemicals' biological activities. The primary experimental method that we use in our studies is pulse conductivity, which we have applied for two decades to study charge-carriers having mobilities that vary over a millionfold range in systems that extend from the liquid rare gases to reversed micells having molecular weights that exceed 10^6. The applicability of our model system to testing chemical carcinogens and "non carcinogens" has been described recently in detail (19). The k_e (electron attachment rate constant) test developed in our laboratory, using the pulse conductivity technique, has provided a means of obtaining an unequivocal measure of electron-carcinogen interaction, and provides a link between chemical physics and carcinogenesis.

Proposed AFM Experiments

The AFM that is requested in this application would complement the "Ionization in Liquids" project by providing a means by which radiation-induced damage to biomimetic systems could be delineated at the molecular level. Characterization of

this damage in such a model system would permit extension of the study to molecular characterization of radiation-induced damage in more complex biological systems, an example of which is the damage induced by radon and its daughters to mouse lympoma cells (see previous project description). The capabilities of this new instrumentation raises intriguing possibilities of *in situ* observations of biological damage induced by a single ionizing event via "before" and "after" data collections. Recently published reports (e.g.14) have indicated that the STM is capable of angstrom-level measurement of biological macromolecules. The AFM has similar potential resolution, and greater applicablity to biological systems. Ultimately, in the case of the single ionizing event mentioned above, the AFM might allow visualization of a single radon atom juxtaposed to a biomolecular target. The description of the proposed study that follows would serve as a firm basis from which this molecular radiobiological goal could be achieved.

Characterization of radiation-induced damage to biological macromolecules such as enzymes, encapsulated in microemulsions or gels is the primary objective of the proposed initial experiments with the AFM. The initial studies would be conducted using the Biochemical Oncology Division's x-ray, cobalt-60 or cesium-137 irradiation sources to irradiate the system found most amneable to AFM analysis. The same system would then be exposed to radon and the damage induced by this high LET (linear energy transfer) radiation would be compared to that observed in the lower LET x- and gamma irradiations. The biomimetic system proposed to be characterized by AFM is the most thoroughly characterized reversed-micelle system, *viz.* Aerosol OT (AOT, sodium di-2-ethylhexylsulfosuccinate)/H_2O/isooctane (20). Our earlier (21,22) and current (19) pulse conductivity studies of AOT/H_2O/isooctane system have significantly contributed to a better understanding of the kinetics and dynamics of electron attachment to water pools encapsulated in AOT, as well as providing new insight into electron-micelle and micelle-micelle

interactions. In this system, the micelle radius r_m can be adjusted from 1.5 to 10 nm by changing the molar H_2O/AOT ratio from <1 to >50 respectively. For the initial in situ AFM experiments, we shall use micelle radii in the range 5 to 10 nm, which is within the current resolving power for AFM imaging in liquid systems. The amphiphilic wall of AOT that encapsulates the pool of water serves as a pseudo-membrane, which we could now visualize and study through application of the AFM. In addition, a variety of enzymes can be encapsulated in water pools and have been shown to retain their biologic catalytic activity (23); these would also be studied by AFM. Further, the AOT/H_2O/isooctane microemulsion can be converted to a gel (24), which may serve as an alternative matrix in which to conduct these AFM studies.

2. OTHER RESEARCH PROJECTS IN THE DESIGNATED THRUST AREA.

The projects outlined in this section are funded by NIH agencies. Three of the projects are concerned with studies on radiation or chemical-induced alterations to DNA, and the second three projects are concerned with the effects of solid surface-induced structural and functional alterations to plasma protein macromolecules.

(i). "Mutants and Altered Radioresponse of Cells and Tumors;" NIH (NCI); Funding for the period: 12/85-11/90 = $818,113; for 12/90-11/93, $630,132 is anticipated; P.I., Helen H. Evans.

This project has received financial support from NIH (NCI) for the past fifteen years. The objectives of this project are to (1) investigate the induction of mutations by low doses of ionizing radiation in cultured mammalian cells and how the dose-response relationship is affected by dose rate, the type and chromosomal location of the target gene, and repair capabilities of the treated cells and (2) to study the molecular mechanisms responsible for the sensitivity of L5178Y strain LY-S to ionizing radiation in comparison to closely related radiation-resistant

strains. Large multilocus deletions and rearrangements may be frequently involved in carcinogenesis. The mechanism of the induction of large jDNA lesions will be investigated by assessing the role of (1) unrepaired DNA double-strand breaks (2) the cell-cycle stage of the exposed cells; (3) post-irradiation DNA degradation; (4) fragile chromosome sites; (5) mitotic recombination.

Proposed AFM studies

The potential applications and impact of the AFM towards achieving the project goals are similar to those I have described for my DOE funded project.

(ii) "Radiation Induced Modifications in Protein Synthesis;" Funding (annual direct costs): for the period 12/89-11/90 = $233,784, with a similar funding level for FY 90/91; P.I., Nancy L. Oleinick.

This project has received continuous financial support from the NIH (NCI) for the past eighteen years. The long-term objective of this research project is the detailed characterization of ionizing radiation-induced DNA damage and processing, as modulated by chromotin structure. DNA -protein crosslinks (DPC) have been selected for study. The proposed research will test the hypothesis that irradiation of mammalian cells create DPC by stabilizing pre-existing associations between chromosomal loops and the nuclear matrix. A primary goal of this research is to acheive a greater understanding of the molecular details of radiation damage in mammalian cells, and provide a conceptual framework and methods for evaluating the importance of a DNA lesion common to many cancer chemotherapeutic agents. As described in the DOE project of Helen Evans, the requested AFM equipment would have a major impact in describing molecular details of DPC and for a technique that would provide precise sizing of DPC. In addition, the availability of AFM and STM to provide sensitive measurements of the mutant DNA molecules would permit detailed studies at lower, more relevant doses of radiation.

(iii) "Phthalocyanine Photodynamic Therapy: Mechanistic Studies;" Funding (annual direct costs): $590,462; period of support: 7/90-6/93; P.I., Nancy L. Oleinick.

This is a new Program Project concerned with the elucidation of the mechanism(s) of photosensitization with phthalocyanines at the molecular level, cellular and tissue levels and development of new phthalocyanines with improved photodynamic and biodistribution properties. Phthalocyanines have great promise as sensitizers for photodynamic therapy (PDT) of cancer. We have shown in previous studies that certain tertiary and quaternary amines linked to the metal can improve the cellular uptake and photosensitizing properties of these compounds. The proposed research will test the hypothesis that amine moieties linked to appropriate metals within the phthalocyanine ring can provide highly efficient photosensitization.

As part of this research, processes invloved in PDT cytotoxicity will be investigated. This is the area in which the AFM would have greatest applicability and potential impact. This research would include studies on the involvement of DNA degradation in post treatment interactions of PDT with ionizing radiation-induced damage will be explored as will the potential of two-photon photochemical processes

(iv) "Fibronectin: Proteoglycan Binding in Adhesion Sites;" NIH (NCI); annual direct costs for FY '89/90: $85,888; period of support: 4/81-3/89, 4/89-3/94; P.I., Lloyd Culp.

This project is concerned with characterizing the structure and function of the biological macromolecule fibronectin and its response (i.e., alteration in structural and/or functional properties) to the presence of artificial surfaces. Ten graduate students have been trained on this project with four graduate students currently involved in working on the structure and function of fibronectins.

Fibronectin is involved in mediating the extracellular matrix adhesion responses of a variety of animal cell types, including fibroblasts (mouse 3T3 cells and human papillary and reticular skin fibroblasts) which play critical roles in wound healing processes in the human and animals and neuroblastoma tumor cells (both rodent and and human) which provide a model of peripheral neuron interaction with matrices. Adhesion responses of fibroblasts (mouse Balb/c 3T3 cells) and human Platt neuroblastoma cells have been examined with plasma fibronectin (pFN) adsorbed on glass surfaces derivatized with "self-assembling" organic monolayers, that consist of a alkyl chain with a terminal functional group. Six different chemical endgroups interfacing with the bound pFN were prepared in order to examine surface-induced regulation of pFN function (25). The six interfacing groups (in order of increasing polarity) included: methyl, olefin, bromo, cyano, diol, and carboxyl. Using an ELISA assay with polyclonal antibodies to human pFN, we have tested the binding parameters of pFN on these derivatized surfaces (26). All surfaces bind pFN to virtually the same saturable level, indicating that any differences in cell responses cannot be due to differences in the amounts of pFN bound. Furthermore, cell attachment (3T3 or Platt neuroblastoma cells) was virtually identical when pFN-adsorbed substrata were assayed. However, after initial cellular attachment, cellular responses such as generated microfilament stress fibers showed chemical end-group specific patterns. These studies indicated that the conformation of pFN on these surfaces differed significantly such that the multiple cell surface receptors required for these responses were altered by interaction with substratum-bound pFNs. This was confirmed when stress fiber organization was tested for inhibition by including a small synthetic peptide in the medium of cells— Gly-Arg-Gly-Asp-Ser-Pro which contains the critical RGDS sequence recognized by the alpha-5-beta-1 member of the integrin family of receptors. On glass and hydrophobic surfaces, stress fibers failed to form, while

on bromo- surfaces stress fibers were unperturbed. These and other studies with neuroblastoma cells have confirmed that the conformation of pFNs on artificial surfaces can be altered in such ways that cell surface receptor functions are subsequently changed in physiological responses. Furthermore, these functional alterations occur in cell type-specific ways (i.e., fibroblasts display different patterns to neuronal cells) and appears independent of the underlying support material (e.g., glass or metals) (27).

Proposed AFM Studies

Our preliminary studies raise several questions regarding the conformation of fibronectins on various "inert" artificial surfaces (often referred to as "biomaterials") and the subsequent functions of these molecules for mediating the specialized functions of differentiated cell types at many sites in the body. AFM offers an ideal, and needed, technique to examine in submolecular detail a variety of hypotheses on the structure and function of these complex adhesion-mediating glycoproteins. Currently, there is no direct methodology that could be used to examine our hypotheses. Specifically, AFM will be used to examine (initially on freshly-cleaved mica) sub-monolayer and full monolayer densities of pFN. The relative homogeneity and heterogeneity of the conformations of neighboring pFN molecules will be examined. These experiments then will be extended to include pFN interactions with our model organic surfaces. Possible alignment of FN molecules on some derivatized substrata may permit ordered arrays of cell surface receptor interaction, thereby regulating specific cell responses; this possibility can now be investigated by exploiting AFM methodologies, which has to date not even been considered in the literature.

As demonstrated in our previous studies, proteolytic fragments of fibronectins harboring specific binding domains have taught us a great deal about the permutation of multiple receptors required for obtaining optimal responses from

various cell types. For example, neuronal cells require a different array of receptors and recognize different cell-binding domains on fibronectins than do fibroblasts, chondroblasts, or muscle cells. Proteolytic fragments of FNs can now be adsorbed to derivatized surfaces and evaluated for cell response and, in parallel, for conformational properties by AFM, and by reactivity with a family of monoclonal antibodies recognizing specific epitopes which have been mapped by conventional biochemical and immunological means. These monoclonal antibodies can also be used to "map" the availability of specific epitopes on a population of adsorbed FN molecules on these various substrata. Finally, our laboratory is generating cDNAs against various regions of fibronectin mRNA and generating synthetic "adherons" with various permutations (natural and rearranged) of the binding domains of these molecules. In parallel with the testing of responses of various cell types to derivatized surfaces adsorbed with "adheron" proteins, "adheron" protein structure and conformation will be examined by AFM.

(v) "Biomedical Polymers: Cell Activation and Interleukin 1;" annual direct costs for FY '89/90: $95,000 period of support: 4/85-3/88, 4/88-3/93; P.I., James M. Anderson.

Efforts in our laboratory are currently directed toward developing a fundamental understanding of human blood protein and cellular interactions with biomaterials. Application of the atomic force microscope technique to our studies offers the unique opportunity to characterize the structure and possible function of biological macromolecules which are present at solid interfaces in materials utilized in implants, prostheses, artificial organs and biomaterials. For the past 20 years, graduate students from the Departments of Pathology, Biomedical Engineering, and Macromolecular Science, have carried out the major portion of research in my laboratory. At the present time I have four Ph.D.

candidates working in this area. If the AFM were made available, I would anticipate utilization by two or more students from my laboratory.

Current experimental evidence points toward protein adsorption as being the initial event when solid materials come into contact with blood and tissues of the human body. To date, an understanding of this protein adsorption phenomenon has not been forthcoming, as it relates to the actual in vivo environment where blood and protein containing fluids come into contact with these materials. Recently, we have published (28,29) on two complementary immunological assays, surface radioimmunoassay and immunogold scanning electron microscopy which have enabled us to identify significant reactive proteins at surfaces of materials in contact with human blood. These significant proteins include coagulation factor XII (Hageman factor), the protein responsible for initiation of the intrinsic coagulation system, high molecular weight kininogen, and prekallikrein, have been identified on human blood-contacting artificial surfaces, even though these proteins are present in blood in microgram per mL concentrations. Examples of our silver-enhanced gold labeling technique are shown in figure 9 (28).

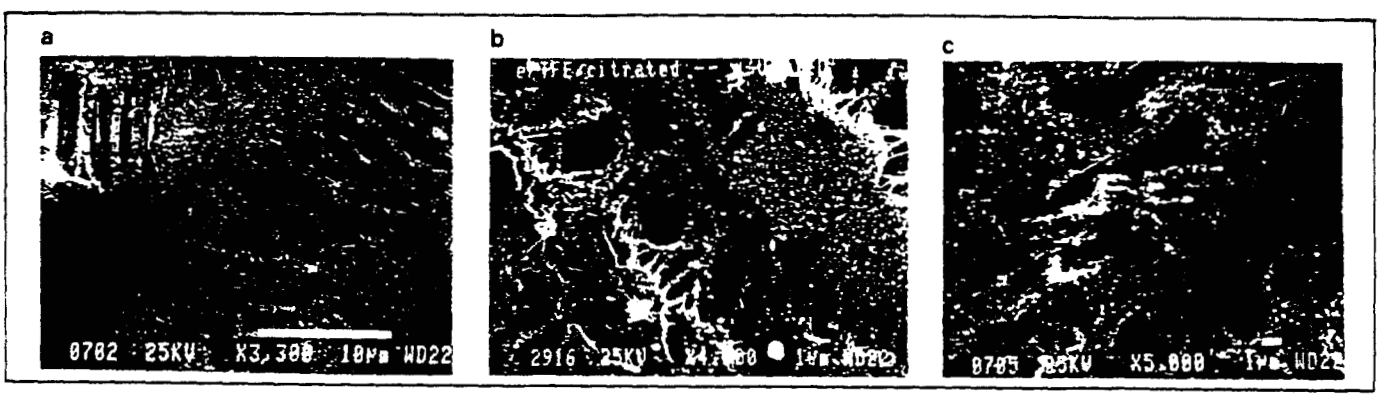

Fig. *· Surface adsorbed proteins from human blood. Visualization of silver coated immunogold-protein A conjugated beads and SEM. from in vitro recirculation system, 5 min. and stained for a) Hageman factor, X3300. b) factor VIII/vWF, X4000. and c) fibrinogen. X4000.*

Proposed AFM Studies

Together with Dr. Oscar D. Ratnoff (discoverer of FXII, Hageman factor), we shall undertake a fundamental investigation of factor XII adsorption with solid surfaces and its activation. Factor XII is unique in that it requires adsorption

to a surface for its activation and subsequent initiation of intrinsic coagulation. The application of atomic force microscopy will enable us to develop an understanding of the conformational and configurational interactions which occur as a first step in thrombosis formation on biomaterials. A fundamental mechanistic understanding of this process is desperately needed, inorder to address major clinical

The use of AFM in these studies to determine the conformational and configurational states of adsorbed proteins will provide important information that will be correlated with techniques currently available in our laboratory for evaluating protein and cellular activation on surfaces. For example, our current imaging methodology, discussed previously, utilizes rabbit anti-human polyclonal antibodies. Quantification is achieved by gold-bead counting using SEM visualization. Utilization of the AFM and STM technique would not only provide a direct visualization of the adsorbed proteins, but enable quantification of antibody binding to the different proteins in our gold-labelled preparations. This will graetly enhance our ability to quantify the different adsorbed proteins. The intrinsic interaction which occurs between surfaces of different chemistries and morphologies and their ability to adsorb proteins in different states will be determined. Furthermore, as these proteins interact with cells, it may be possible to elucidate protein structural requirements for cellular adhesion, activation and release of toxic agents such as superoxide anion.

(vi) (a) "New Biomedical Interface Materials;" NIH (NHLBI); annual direct costs (FY 89/90), $65,000; period of support: 3/88-2/93; P.I., Roger E. Marchant and (b) "Molecular level Approach to Blood-Materials Interaction" NIH (NHLBI); annual direct costs $79,505, period of support: 4/90-3/91; Co-P.I. Roger E. Marchant.

Our research efforts are concerned with the design, preparation and surface characterization on new bioactive material interfaces, prepared by novel

modification techniques, that will reduce thrombosis formation on blood-contacting artificial surfaces. A major effort on this, and related projects, is the study of plasma protein molecules that are associated with thrombus formation at the material-blood interface. Currently, three graduate (one Ph.D. candidate and two M.S.) students are involved in this project area, two of whom would utilize the AFM/STM imaging techniques in the proposed experiments. As part of their research, students in my laboratory gain experience in several surface specific characterization techniques and imaging methodologies, and have excellent theoretical and practical backgrounds to benefit from, and exploit, the AFM equipment requested in this application. The proposed studies, outlined and described below, build upon the results of the preliminary AFM and STM experiments.

Proposed AFM Studies

The interactions of plasma protein molecules at solid interfaces will be investigated at the sub-molecular, molecular and cellular (gel filtered platelets) levels, using AFM. The overall goal is to elucidate and determine the mechanism(s) of protein binding and conformational changes that occur on different artificial surfaces and to examine the hypothesis that alterations in the structural and functional properties of a bound protein macromolecule are determined by specific protein-surface interactions. The fundamental issues to be addressed include (i) the effect of surface chemical structure on protein and platelet binding; (ii) the orientation of the adsorbing protein and the subsequent conformational alterations in protein structure; (iii) the effect of nearest neighbor interactions on the organization of the adsorbed macromolecules and (iv) the alteration in functional properties of the adsorbed protein (purified vWF), in the presence or absence of a competing protein (i.e., fibrinogen) or a non-platelet binding protein (i.e., albumin), as shown by vWF

binding to receptor glycoproteins (GP1b and GPIIb/IIIa) on the platelet membrane. Direct evidence to address these issues would be possible using the AFM. Furthermore, through the application and exploitation of AFM/STM, quantitative imaging of protein binding and conformational changes can be accomplished for the first time. The proposed studies will involve the adsorption of vWF (at submonolayer and monolayer surface densities) on mica and gold (for initial parallel STM studies), and surface modified mica and gold. vWF-surface interactions will be studied under static conditions in air, under aqeous media and then under dynamic flow conditions that provide reproducible and calculable shear forces at the interface. The effect of surface chemistry on vWF binding will be investigated by engineering a series of organic surfaces, controlled with respect to functionality, surface energy and charge. The effect of monoclonal antibodies to one or both of the GPIIb/IIIa and GPIb binding domains which are present on each vWF subunit will be used to examine vWF-platelet interactions under the various experimental conditions.

Interactions of plasma proteins with solid surfaces and the perturbations to structural and functional properties that are caused by the surface are important phenomena that affect bioadhesion events in many physiologic and pathologic processes including thrombus formation on artificial surfaces. The proposed research using AFM will provide new fundamental information at the molecular and sub-molecular levels that has not been attainable previously. Secondly, platelet adhesion to surface-bound plasma proteins such as vWF or fibrinogen, is a critical event in thrombus formation, especially at interfaces characterized by high wall shear rates. Defining platelet surface receptors and adhesive molecules involved in thrombosis is important to an understanding of fundamental molecular mechanisms of the process and to the design of therapeutic strategies and modified biomaterials.

6b. FACULTY INVESTIGATORS

Each of the participating faculty members has well-established expertise and experience in their respective biomedical research area as demonstrated by their resumes. This experience is reflected in the quality of their publications and by the long periods of federal support the investigators have received for individual research grants. Furthermore, if this application is approved, it is anticipated that, in addition to the ovvious benefits of the equipment, the new collaborations that have been established during the course of preparing this application will further simulate energy-related research in life sciences. However, the requested AFM equipment is innovative and until recently (spring 1990) was not commercially available. The Principal Investigator is the only faculty member at C.W.R.U. with previous experience with the requested AFM equipment This experience was obtained during the summer of 1990 in the laboratory of Dr. Joseph Andrade, Department of Bioengineering at the University of Utah. Dr. Andrade received the initial prototype instrument from Dr. Paul Hansma's group (University of California at Santa Barbara) and in June 1990 one of the first AFMs to be delivered from Digital Instuments.

6C.LIFE SPAN, OPERATION AND MAINTENANCE

There is no atomic force microscope on campus of our University. The proposed equipment will be housed in a 750 sq. ft. laboratory located in the Wickended Building (room 515), Department of Biomedical Engineering, which is under Dr. Marchant's supervision. This laboratory contains a Digilab FTS-40 Fourier Transform Infrared Spectrometer, and a class 100 clean hood and laboratory benches for sample preparation. It should be emphasized that the AFM is predominantly electronic intrumentation; consequently, the equipment is expected to be "robust" and durable, with a life time greater than ten years. The system is "upgradable" such that new improvements can be incorporated at the appropriate time. Digital

Instruments will provide service and warranty, including any costs associated with parts and labor, for the first year after the date of purchase. The maintenance cost for the second year will be provided by Digital Instruments at an estimated cost of $8,500 (i.e., <7% of the total equipment cost). This has been included as part of the proposed Budget on page 5. Maintenance costs for years 3-5 will be covered by user fees. A "Cost Center" entitled "Biomolecule Characterization Laboratory" and will also include the FT-IR (8 collaborators cuuently use this equipment). The Cost Center will generate approximately $80,000/yr. (no Institutional overhead), to be used to hire and pay for a full-time technician in year 2, day-to-day supplies, and future maintenance and system upgrades. The Cost Center system works exceptionally-well for many laboratories on our campus with expensive equipment.

Dr. Roger Marchant will be responsible for the day to day management of the instrument and will provide the technical expertise and will fulfill the initial training needs and supervision of faculty and student users. After appropriate training, Mr. Xiang Wang will be given the responsiblity for day to day operations and training users. Mr. Wang is a research assistant in the Department of Biomedical Engineering under Dr. Marchant. He has extensive training in signal processing and computer programming. He is partially supported by the Department for the day to day maintenance and operation of the Department's computers, which are located in the room adjacent to the proposed AFM laboratory. In addition he is responsible for the user log for our FT-IR equipment, which enables estimate of monitoring.

6D INSTITUTIONAL COST SHARING

The University will contribute $11,700 towards the cost of the equipment. $9,700 directly for the equipment and $2,000 to cover modifications to the laboratory.

7. Estimated Cost and Budget

Cost of the Equipment components is shown on page 6 of this application. Shipping and installation costs will be covered by the vendor, Digital Instruments. Maintenance costs have been addressed in section 6c. There are no special requirements for installing the atomic force microscope. However, in order to achieve high resolution images, it is important to provide additional vibration isolation. This is best accomplished by placing the AFM scanning head on a supporting mount suspended by a pulley-bungee cord system. Installation of the mountings for the pulley system will be the only laboratory renovation required and the costs will be covered by the University.

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Attachment 1

FACULTY PARTICIPANTS AND RESUMES

1. Dr. Roger E. Marchant, Assistant Professor of Biomedical Engineering and Macromolecular Science.

2. Dr. Helen H. Evans, Professor of Radiology, Biochemistry, & Environmental Health Sciences.

3. Dr. George Bakale, Senior Research Associate, Department of Radiology

4. Dr. Nancy L. Oleinick, Professor of Radiology, Biochemistry, & Environmental Health Sciences.

5. Dr. Lloyd A. Culp, Professor of Molecular Biology and Microbiology

6. Dr. James M. Anderson, Professor of Pathology, Biomedical Engineering and Macromolecular Science.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel, consultants, and collaborators listed on page 4
Photocopy this page for each person

NAME	POSITION TITLE	BIRTHDATE (Mo., Day, Yr.)
Roger E. Marchant	Assistant Professor	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Research and Professional Experience

Nominated by Case Western Reserve University for a 1990 PEW Scholars award in Biomedical Sciences.

Member of NIH SBIR special study section, Surg. & Bioeng. (AHR-B1), 1989, 1990.

Chairman, NIH SBIR special study section, Surg. & Bioeng. (AHR-B1), 1990.

Member of NIH-NIDDK SBIR Ad Hoc Review Committee, 1990

Program Chairman and Organizer, Cleveland Symposium on Advances in Biomaterials Science and Engineering, Cleveland, Ohio, March 1989 & October 1990.

Assistant Professor of Macromolecular Science, 1988-.

Assistant Professor of Biomedical Engineering, 1988-.

Associate Editor, Journal of Biomedical Materials Research, January 1987-.

Senior Research Associate, Department of Macromolecular Science, Case Western Reserve University, 1984-88.

The Award for Outstanding Research (Ph.D. category), 2nd World Congress on Biomaterials, Society for Biomaterials, Washington, D.C., April 1984.

Research Chemist, Central Research Centre, Dunlop Tyre Co., Birmingham, England, 1969-74

Selected Publications (1987-90)

Observations of Von Willebrand Factor Deposited on Mica and Imaged by Atomic Force Microscopy, R.E. Marchant, A.S. Lea, J.D. Andrade, P. Brockenstedt and D. Ginsberg, Trans. Soc. Biomater., (accepted).

A Hydrophilic Plasma-polymerized Film Composite with Potential Application as an Interface for Biomaterials, R.E. Marchant, S.D. Johnson, B.H. Schneider, M.P. Agger and J.M. Anderson, J. Biomed. Mater. Res., 24, 1521, (1990).

In Vivo Leukocyte Interactions on Pellethane Surfaces, M.R. Brunstedt, J.M. Anderson, K.L. Spilizewski, R.E. Marchant, and A. Hiltner, Biomaterials, 11, 370, (1990).

Immobilization of Glucose Oxidase onto Plasma Polymerized Films for an Oxygen Sensor, M. Danilich and R.E. Marchant, IUPAC, 33, (1990).

The Effect of Albumin Coating on the In Vitro Blood Compatibility of Dacron Arterial Prostheses, K. Kottke-Marchant, J.M. Anderson, Y. Umemura and R.E. Marchant, Biomaterials, 10, 147-155 (1989).

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	POSITION TITLE	BIRTHDATE (Mo., Day, Yr.)
Helen H. Evans	Professor of Radiology, Biochemistry, & Environmental Health Sciences	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Research Assistant, Department of Radiology & Biochemistry Western Reserve University	1953-1956
Senior Instructor, Department of Biochemistry, Western Reserve University	1956-1957
Assistant Professor of Biochemistry, Western Reserve University	1957-1964
Assistant Professor of Radiology, Western Reserve University	1961-1964
Special Postdoctoral Fellow (NIH), Division of Microbiology, Scripps Clinic and Research Foundation, LaJolla, CA	1965-1966
Associate Professor of Biochemistry and Radiology, Case Western Reserve University	1964-1976
Visiting Professor, McArdle Laboratory for Cancer Research University of Wisconsin	1973-1974
Professor of Biochemistry & Radiology, Case Western Reserve	1976-Present
Professor of Environmental Health Sciences, Case Western Reserve	1985-Present
Professor of Oncology, Case Western Reserve University	1987-Present

SERVICE: Radiation Study Section, NIH, 1973-1977
Department of Energy Committee of Principal Scientists for the Radon Program, 1988-Present

SELECTED PUBLICATIONS

Evans, H.H., Ricanati, M., and Horng, M.F., "Deficiency in DNA Double-Strand Break Repair in Mouse Lymphoma Strain L5178Y-S", Proc. Natl. Acad. Sci., USA 84, 7562-7566, 1987.

Evans, H.H., Rerko, R.M., Mencl, J., Clay, M.E., Antunez, A.R., and Oleinick, N.L., "Cytotoxic and Mutagenic Effects of the Photodynamic Action of Chloroaluminum Phthalocyanine and Visible Light in L5178Y Cells", Photochem. Photobiol., 49, 43-47, 1989.

DeMarini, D.M., Brockman, H.E., de Serres, F.J., Evans, H.H., Stankowski, L.F., Jr., and Hsie, A.W., "Specific-Locus Mutations Induced in Eukaryotes (Especially Mammalian Cells) by Radiaton and Chemicals: A Perspective", Mutation Research, 220, 11-29, 1989.

Evans, H.H., Nielsen, M., Mencl, J., Horng, M.F., and Ricanati, M., "The Effect of Dose Rate on X Radiation-Induced Mutant Frequency and the Nature of DNA Lesions in Mouse Lymphoma L5178Y Cells", Radiat. Res. 122, 316-325, 1990.

Evans, H.H., "Ionizing Radiation Mutagenesis in Mammalian Cells", in UCLA Symposium on Molecular and Cellular Biology: Ionizing Radiation Damage to DNA: Molecular Aspects", Ed. S.S. Wallace and R.B. Painter, Wiley-Liss, New York, pp. 211-219, 1990.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	POSITION TITLE	BIRTHDATE (Mo., Day, Yr.)
George Bakale	Senior Research Associate	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

PROFESSIONAL EXPERIENCE

1962-63 Research Assistant, Union Carbide Company, Technical Service Laboratory, Tarrytown, NY
1963-67 Graduate Research Assistant, Chemistry Department, CWRU
1967-68 Postdoctoral Research Associate, Division of Radiation Biology, CWRU
1968-71 Research Fellow, Mellon Institute of Science, Carnegie-Mellon Univ., Pittsburgh, PA
1972-74 Guest Scientist, Hahn-Meitner-Institut fur Kernforschung, West Berlin, Germany
1975-6/85 Assistant Professor of Radiology, CWRU
1985-6/90 Associate Professor of Radiology, CWRU
7/90- Senior Research Associate, Radiology Department, CWRU
1975-90 Visiting Scientist, Hahn-Meitner-Institut, West Berlin, Germany; 11 visits of one to five months duration
8-10/83 Visiting Scientist, Interuniversity Reactor Instituut, Delft, The Netherlands

Publications, selected presentations and invited lectures, 1988-present

Bakale, G., "Theoretical Implications of the k_e Carcinogen-Screening Test", in Chemical Carcinogens, P. Politzer and F.J. Martin, eds. (Elsevier, Amsterdam, 1988), pp. 322-344.

Bakale, G., "Electron Attachment and Ion Mobility in Hydrocarbons and Related Systems", Proceedings of the "International Workshop on Liquid State Electronics", November 7-10, 1988, West Berlin.

Bakale, G., "Detection of Mutagens and Carcinogens by Physicochemical Techniques", Hazard Assessment of Chemicals-Current Developments, Vol. 6, J. Saxena, ed., (Hemisphere Publishing, Washington, D.C., 1989), pp.85-124.

Mencl, J., Jostes, R., Cross, F., Gies, R., Bakale, G., Rao, P.S., Rerko, R., Evans, H., "Radon-Induced Mutation at an Autosomal Locus", 37th Annual Meeting of the Radiation Research Society, Seattle, WA, March 18-23, 1989.

Bakale, G. and McCreary, R.D., "Electrophilicity of Nongenotoxic Carcinogens and Genotoxic Noncarcinogens as Measured by the k_e Test", invited lecture presented at the Fifth International Conference on Environmental Mutagens, Cleveland, OH, July 10-15, 1989 and published in Mutation and the Environment M.L. Mendelsohn, ed. (Wiley-Liss, New York, 1990), pp. 355-365.

Bakale, G. and McCreary, R.D., "Response of the k_e Test to NCI/NTP-Screened Chemicals. I. Nongenotoxic Carcinogens and Genotoxic Noncarcinogens", Carcinogenesis, 11, in press.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	POSITION TITLE	BIRTHDATE (Mo., Day, Yr.)
Nancy L. Oleinick	Professor	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

PROFESSIONAL EXPERIENCE:

1967-69 Post-Doctoral Fellowship Awarded by NIAID, USPHS
1968-69 Instructor, Department of Biochemistry, School of Medicine, CWRU
1969-76 Assistant Professor, Departments of Radiology & Biochemistry, CWRU
1971-76 Assistant Professor, School of Dentistry, CWRU
1978-78 Visiting Professor, Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel
1979-82 & 1985-86 Associate Editor, Radiation Research
1976-85 Associate Professor, Department of Radiology (Division of Radiation Biology) and Department of Biochemistry (Assoc. Member) School of Medicine, and the School of Dentistry, CWRU
1983-85 Associate Professor, Department of Epidemiology and Community Health (Department of Environmental Health Sciences), CWRU
1984-88 Radiation Study Section, NIH
1985- Professor, Departments of Radiology, Environmental Health Sciences, and Biochemistry (School of Medicine), and Oral Biology (School of of Dentistry), CWRU
1986- Director, Division of Biochemical Oncology, Department of Radiology, and Radiation Biochemistry Program, Cancer Research Center
1986- Editorial Board, International Journal of Radiation Biology
1987- Member, CIRRPC Task Force on Neutron Radiobiology
1987- Professor, CWRU Cancer Research Center

SELECTED PUBLICATIONS

Ramakrishnan N, Oleinick NL, Clay ME, Horng MF, Antunez AR, Evans HH. DNA Lesions and DNA Degradation in Mouse Lymphoma L5178Y Cells After Photodynamic Treatment Sensitized by Chloroaluminum Phthalocyanine. Photochem. Photobiol. 50:373-378 (1989).

Chiu SM, Friedman LR, Oleinick NL. Formation and Repair of DNA-Protein Cross-Links in Newly Replicated DNA, Radiat. Res. 120:545-551 (1989).

Chiu SM, Friedman LR, Oleinick NL. The Fate of DNA-Protein Cross-Links Formed in Gamma-Irradiated Metaphase Cells. Int. J. Radiat. Biol. 58:235-247, 1990.

Ramakrishnan N, Clay ME, Friedman LR, Antunez AR, Oleinick NL. Post-Treament Interactions of Photodynamic and Radiation-Induced Cytotoxic Lesions. Photochem. Photobiol. 52:555-559, 1990.

Oleinick NL, Chiu SM, Friedman LR, Xue LY, and Ramakrishnan N. DNA-Protein Crosslinks: Windows into Chromatin Damage and Repair, in Ionizing Radiation Damage to DNA: Molecular Aspects (ed. by S. Wallace and R. Painter), Wiley-Liss, New York p. 59-67 (1990).

Oleinick NL Ionizing Radiation Damage to DNA: Molecular Aspects Radiat. Res., in press, 1990.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE	BIRTHDATE (Mo., Day, Yr.)
Culp, Lloyd A.	Professor of Molecular Biology and Microbiology	[redacted]

EDUCATION: Begin with college and include postdoctoral training.

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position. List in chronological order, previous employment, experience and honors. Include present membership on any Federal Government public advisory committee. List in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professor of Oncology/General Medical Sciences, CWRU School of Medicine (1987-present)
Professor of Molecular Biology and Microbiology, CWRU School of Medicine (1983-present)
Associate Professor of Microbiology, CWRU School of Medicine (1977-1983)
Assistant Professor of Microbiology, CWRU School of Medicine (1972-1977)
Harry A. Pinney Scholar in Cancer Research at Case Western Reserve University (1973-1975)
USPHS Career Development Awardee CA70709 (1974-1979)
Member of NIH Cellular Physiology Study Section (1978-1982)
Member of NIH Special Study Section (SSS-5) (1989-present)
Ad Hoc Reviewer for: American Cancer Society, National Science Foundation, Canadian Medical Research Council, U.S.-Israel Binational Science Foundation, American Heart Association, and many programs of the NIH

Recent Representative Publications:

Radinsky, R., Flickinger, K.S., Kosir, M.A., Zardi, and Culp, L.A. Adhesion of Kirsten *ras*$^+$ tumor-progressing and Ki-*ras*$^-$ revertant 3T3 cells on fibronectin proteolytic fragments. Cancer Res., 50:4388-4400 (1990).

Hershberger, R.P. andCulp, L.A. Cell type-specific expression of alternatively spliced human fibronectin IIICS mRNAs. Mol. Cell. Biol., 10:662-671 (1990).

Lewandowska, K., Balza, E., Zardi, L., and Culp, L.A. Requirement for two different cell-binding domains in fibronectin for neurite extension of neuronal derivative cells. J. Cell Science, 95:75-83(1990).

Lewandowska, K., Balachander, N., Sukenik, C.N. and Culp, L.A. Modulation of fibronectin adhesive functions for fibroblasts and neural cells by chemically-derivatized substrata. J. Cellular Physiol. 141:334-345 (1989).

Culp, L.A., Mugnai, G.M., Lewandowska, K., Vallen, E.A., Kosir, M.A. and Houmiel, K.L. Heparan sulfate proteoglycans of *ras*-transformed 3T3 or neuroblastoma cells: differing functions in adhesion on fibronectin. In *Structure and Activities of Heparin and Related Polysaccharides*, Ed. by F.A. Ofosu, I. Danishefsky and J. Hirsh. Annals of New York Academy of Sciences 556:194-216 (1989).

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	POSITION TITLE	BIRTHDATE (Mo., Day, Yr.)
James M. Anderson, M.D., Ph.D.	Professor of Pathology, Macromolecular Science & Biomed. Eng.	[redacted]

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]			

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Appointments and Experience:

Professor of Pathology, Macromolecular Science and Biomedical Engineering, 1984-
President, Society for Biomaterials, USA, 1984-85
Editor-in-Chief, Journal of Biomedical Materials Research, 1988-
Assistant Editor, Journal of Biomedical Materials Research, 1979-1987
Program Chairman, Second World Congress on Biomaterials, Washington, D.C., April 27-May 1, 1984
Participant, National Veterans Administration Cooperative Study of the Comparative Efficacy of Vascular Bypass Graft Materials in Lower Extremity Revascularization, Evaluation of Retrieved Vascular Grafts, 1982-
Member, Advisory Board, CRC Critical Reviews in Biocompatibility, 1983-
Member, Subcommittee of Blood-Materials Interactions, International Committee on Thrombosis and Haemostasis
Co-Chairman, International Symposium on Recent Advances in Drug Delivery Systems, Salt Lake City, Utah, 1983, 1985, 1987, 1989
Panel Member, NIH Consensus Development Conference on Clinical Applications of Biomaterials, Bethesda, MD, November 1-3, 1982
Associate Professor of Pathology, 1980-84
Associate Professor of Macromolecular Science, 1981-84
Associate Professor of Biomedical Engineering, 1982-84
Pathologist, Institute of Pathology, University Hospitals of Cleveland, 1979-
Assistant Professor of Pathology, 1976-79
Assistant Professor of Macromolecular Science, 1976-81
Senior Research Associate, Department of Macromolecular Science, 1970-76

Selected Publications

Analysis of protein adsorption on retrieved human vascular grafts using immunogold labelling with silver enhancement, N.P. Ziats, N.S. Topham, D.A. Pankowsky and J.M. Anderson, Cells & Materials, 1, 73-82 (1990).

Foreign-body giant cells and polyurethane biostability: in vivo correlation of cell adhesion and surface cracking, Q. Zhao, N. Topham, J.M. Anderson, A. Hiltner, G. Lodoen and C.R. Payet, J. Biomed. Mater. Res., 25 (1991).

Fibroblast stimulation by monocytes cultured on protein adsorbed biomedical polymers. I: Biomer® and polydimethylsiloxane, T.L. Bonfield, E. Colton and J.M. Anderson, J. Biomed. Mater. Res., 25 (1991).

Adsorption of Hageman factor (factor XII) and other human plasma proteins to biomedical polymers, N.P. Ziats, D.A. Pankowsky, B.P. Tierney, O.D. Ratnoff, and J.M. Anderson, J. Lab. Clin. Med., 116, 687-696 (1990).

Morphological characteristics of adsorbed human plasma proteins on vascular grafts and biomaterials, D.A. Pankowsky, N.P. Ziats, N.S. Topham, O.D. Ratnoff, and J.M. Anderson, J. Vasc. Surg., 11, 599-606 (1990).

DOE F 1600.5
(10-86)

OMB Contol No
1910-0400

U.S. Department of Energy

Assurance of Compliance

Nondiscrimination in Federally Assisted Programs

CASE WESTERN RESERVE UNIVERSITY (Hereinafter called the "Applicant") HEREBY AGREES to comply with Title VI of the Civil Rights Act of 1964 (Pub. L. 88-352), Section 16 of the Federal Energy Administration Act of 1974 (Pub. L. 93-275), Section 401 of the Energy Reorganization Act of 1974 (Pub. L. 93-438), Title IX of the Education Amendments of 1972, as amended, (Pub. L. 92-318, Pub. L. 93-568, and Pub. L. 94-482), Section 504 of the Rehabilitation Act of 1973 (Pub. L. 93-112), the Age Discrimination Act of 1975 (Pub. L. 94-135), Title VIII of the Civil Rights Act of 1968 (Pub. L. 90-284), the Department of Energy Organization Act of 1977 (Pub. L. 95-91), and the Energy Conservation and Production Act of 1976, as amended, (Pub. L. 94-385). In accordance with the above laws and regulations issued pursuant thereto, the Applicant agrees to assure that no person in the United States shall, on the ground of race, color, national origin, sex, age, or handicap, be excluded from participation in, be denied the benefits of, or be otherwise subjected to discrimination under any program or activity in which the Applicant receives Federal assistance from the Department of Energy.

Applicability and Period of Obligation

In the case of any service, financial aid, covered employment, equipment, property, or structure provided, leased, or improved with Federal assistance extended to the Applicant by the Department of Energy, this assurance obligates the Applicant for the period during which Federal assistance is extended. In the case of any transfer of such service, financial aid, equipment, property, or structure, this assurance obligates the transferee for the period during which Federal assistance is extended. If any personal property is so provided, this assurance obligates the Applicant for the period during which it retains ownership or possession of the property. In all other cases, this assurance obligates the Applicant for the period during which the Federal assistance is extended to the Applicant by the Department of Energy.

Employment Practices

Where a primary objective of the Federal assistance is to provide employment or where the Applicant's employment practices affect the delivery of services in programs or activities resulting from Federal assistance extended by the Department, the Applicant agrees not to discriminate on the ground of race, color, national origin, sex, age, or handicap, in its employment practices. Such employment practices may include, but are not limited to, recruitment, recruitment advertising, hiring, layoff or termination, promotion, demotion, transfer, rates of pay, training and participation in upward mobility programs; or other forms of compensation and use of facilities.

Subrecipient Assurance

The Applicant shall require any individual, organization, or other entity with whom it subcontracts, subgrants, or subleases for the purpose of providing any service, financial aid, equipment, property, or structure to comply with laws cited above. To this end, the subrecipient shall be required to sign a written assurance form, however, the obligation of both recipient and subrecipient to ensure compliance is not relieved by the collection or submission of written assurance forms.

1063107

Data Collection and Access to Records

The Applicant agrees to compile and maintain information pertaining to programs or activities developed as a result of the Applicant's receipt of Federal assistance from the Department of Energy. Such information shall include, but is not limited to,the following: (1) the manner in which services are or will be provided and related data necessary for determining whether

any persons are or will be denied such services on the basis of prohibited discrimination; (2) the population eligible to be served by race, color, national origin, sex, age and handicap; (3) data regarding covered employment including use or planned use of bilingual public contact employees serving beneficiaries of the program where necessary to permit effective participation by beneficiaries unable to speak or understand English; (4) the location of existing or proposed facilities connected with the program and related information adequate for determining whether the location has or will have the effect of unnecesarily denying access to any person on the basis of prohibited discrimination; (5) the present or proposed membership by race, color, national origin, sex, age and handicap, in any planning or advisory body which is an integral part of the program; and (6) any additional written data determined by the Department of Energy to be relevant to its obligation to assure compliance by recipients with laws cited in the first paragraph of this assurance.

The Applicant agrees to submit requested data to the Department of Energy regarding programs and activities developed by the Applicant from the use of Federal assistance funds extended by the Department of Energy. Facilities of the Applicant (including the physical plants, buildings, or other structures) and all records, books, accounts, and other sources of information pertinent to the Applicant's compliance with the civil rights laws shall be made available for inspection during normal business hours on request of an officer or employee of the Department of Energy specifically authorized to make such inspections. Instructions in this regard will be provided by the Director, Office of Equal Opportunity, U.S. Department of Energy.

This assurance is given in consideration of and for the purpose of obtaining any and all Federal grants, loans, contracts (excluding procurement contracts), property, discounts or other Federal assistance extended after the date hereto, to the Applicants by the Department of Energy, including installment payments on account after such data of application for Federal assistance which are approved before such date. The Applicant recognizes and agrees that such Federal assistance will be extended in reliance upon the representations and agreements made in this assurance and the the United States shall have the right to seek judicial enforcement of this assurance. This assurance in binding on the Applicant, its successors, transferees, and assignees, as well as the person whose signature appears below and who is authorized to sign this assurance on behalf of the Applicant.

12/7/90
(Date)

ROGER MARCHANT, PROFESSOR
CASE WESTERN RESERVE UNIVERSITY
(Name of Applicant)

[redacted]

[redacted] OH 44106
(Address)

(James Kemp)
(Authorized Official)

( ) [redacted]
(Applicant's Telephone Number)

1063108

CERTIFICATION REGARDING DRUG-FREE WORKPLACE REQUIREMENTS

This certification is required by the Drug-Free Workplace Act of 1988 (Pub. L. 100-690, Title V, Subtitle D) and is implemented through additions to the Debarment and Suspension regulations, published in the Federal Register on January 31, 1989.

An organizational applicant certifies that it will provide a drug-free workplace by:

(a) Publishing a statement notifying employees that the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance is prohibited in the grantee's workplace and specifying the actions that will be taken against employees for violation of such prohibition;

(b) Establishing a drug-free awareness program to inform employees about–

(1) the dangers of drug abuse in the workplace;
(2) the grantee's policy of maintaining a drug-free workplace;
(3) any available drug counseling, rehabilitation, and employee assistance programs; and
(4) the penalties that may be imposed upon employees for drug abuse violations occurring in the workplace;

(c) Making it a requirement that each employee to be engaged in the performance of the grant be given a copy of the statement required by paragraph (a);

(d) Notifying the employee in the statement required by paragraph (a) that, as a condition of employment under the grant, the employee will–

(1) abide by the terms of the statement; and
(2) notify the employer of any criminal drug statute conviction for a violation occurring in the workplace not later than five days after such conviction;

(e) Notifying the agency within ten days after receiving notice under subparagraph (d)(2) from an employee or otherwise receiving actual notice of such conviction;

(f) Taking one of the following actions, within 30 days of receiving notice under subparagraph (d)(2), with respect to any employee who is so convicted–

(1) taking appropriate personnel action against such an employee, up to and including termination; or
(2) requiring such employee to participate satisfactorily in a drug abuse assistance or rehabilitation program approved for such purposes by a Federal, State, or local health, law enforcement, or other appropriate agency;

(g) Making a good faith effort to continue to maintain a drug-free workplace through implementation of paragraphs (a), (b), (c), (d), (e), and (f).

1063109

Place of Performance: The applicant shall insert in the space provided below the site(s) for the performance of work done in connection with the specific grant: (street address, city, county, state, zip code)

DEPT. OF BIOMEDICAL ENGINEERING
CASE WESTERN RESERVE UNIVERSITY
10900 Euclid Avenue
Cleveland, OH 44106

An applicant who is an individual certifies that, as a condition of the grant, he or she will not engage in the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance in conducting any activity with the grant.

This assurance is given in connection with any and all financial assistance from the Department of Energy after the date this form is signed. This includes payments after such date for financial assistance approved before such date. The applicant recognizes and agrees that any such assistance will be extended in reliance on the representations and agreements made in this assurance, and the United States shall have the right to seek judicial enforcement of this assurance. This assurance is binding on the applicant, its successors, transferees, and assignees, and on the authorized official (or individual applicant, as appropriate) whose signature appears below.

Organization Name Award Number

James Kemp, Assistant Director, Research Administration Name and Title of Authorized Representative

Signature Date

1063110

CERTIFICATION REGARDING DEBARMENT, SUSPENSION, AND OTHER RESPONSIBILITY MATTERS - PRIMARY COVERED TRANSACTIONS

1. The prospective primary participant certifies to the best of its knowledge and belief, that it and its principals:

a. Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency;

b. Have not within a three-year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State or local) transaction or contract under a public transaction; violation of Federal or State anti-trust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;

c. Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph 1.b. of this certification; and

d. Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State or local) terminated for cause or default.

2. Where the prospective primary participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

CASE WESTERN RESERVE UNIVERSITY
Organization Name

Award Number

James Kemp, Assistant Director, Research Administration
Name and Title of Authorized Representative

Signature

Date

10631111

(See Reverse)

Instructions for Certification

1. By signing and submitting this proposal, the prospective primary participant is providing the certification set out below.

2. The inability of a person to provide the certification required below will not necessarily result in denial of participation in this covered transaction. The prospective participant shall submit an explanation of why it cannot provide the certification set out below. The certification or explanation will be considered in connection with the department or agency's determination whether to enter into this transaction. However, failure of the prospective primary participant to furnish a certification or an explanation shall disqualify such person from participation in this transaction.

3. The certification in this clause is a material representation of fact upon which reliance was placed when the department or agency determined to enter into this transaction. If it is later determined that the prospective primary participant knowingly rendered an erroneous certification, in addition to other remedies available to the Federal Government, the department or agency may terminate this transaction for cause or default.

4. The prospective primary participant shall provide immediate written notice to the department or agency to whom this proposal is submitted if at any time the prospective primary participant learns that its certification was erroneous when submitted or has been erroneous by reason of changed circumstances.

5. The terms "covered transaction," "debarred," "suspended," "ineligible," "lower tier covered transaction," "participant," "person," "primary covered transaction," "principal," "proposal," and "voluntarily excluded," as used in this clause, have the meanings set out in the Definitions and Coverage sections of the rules implementing Executive Order 12549. You may contact the department or agency to which this proposal is being submitted for assistance in obtaining a copy of those regulations.

6. The prospective primary participant agrees by submitting this proposal that, should the proposed covered transaction be entered into, it shall not knowingly enter into any lower tier covered transaction with a person who is debarred, suspended, declared ineligible, or voluntarily excluded from participation in this covered transaction, unless authorized by the department or agency entering into this transaction.

7. The prospective primary participant further agrees by submitting this proposal that it will include the clause titled "Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transaction," provided by the department or agency entering into this covered transaction, without modification, in all lower tier covered transactions and in all solicitations for lower tier covered transactions.

8. A participant in a covered transaction may rely upon a certification of a prospective participant in a lower tier covered transaction that it is not debarred, suspended, ineligible, or voluntarily excluded from the covered transaction, unless it knows that the certification is erroneous. A participant may decide the method and frequency by which it determines the eligibility of its principals. Each participant may, but is not required to, check the Nonprocurement List (Telephone No. [financial assistance administrator]).

9. Nothing contained in the foregoing shall be construed to require establishment of a system of records in order to render in good faith the certification required by this clause. The knowledge and information of a participant is not required to exceed that which is normally possessed by a prudent person in the ordinary course of business dealings.

10. Except for transactions authorized under paragraph 6 of these instructions, if a participant in a covered transaction knowingly enters into a lower tier covered transaction with a person who is suspended, debarred, ineligible, or voluntarily excluded from participation in this transaction, in addition to other remedies available to the Federal Government, the department or agency may terminate this transaction for cause or default.

CERTIFICATION REGARDING LOBBYING

This certification is required by Section 319 of Public Law 101-121 and the OMB Governmentwide Guidance for New Restrictions on Lobbying; Interim Final Guidance, as published in the *Federal Register* on December 20, 1989.

NOTE: Based on OMB guidance dated March 23, 1990, and Civilian Agency Council Letter No. 90-04 dated April 4, 1990, this certification applies only to the instant transaction and not to all transactions.

Certification for Contracts, Grants, Loans, and Cooperative Agreements

The undersigned certifies, to the best of his or her knowledge and belief, that:

a. No Federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

b. If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

c. The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

CASE WESTERN RESERVE UNIVERSITY

Organization Name — Award Number

JAMES KEMP, ASSISTANT DIRECTOR, RESEARCH ADMINISTRATION

Name and Title of Authorized Representative

Signature — Date

10631-13