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Scanning Tunneling Microscopic studies
on Human fibrinogen deposited on highly
oriented pyrolytic graphite.* L. Feng, C-Z
Hu and J. D. Andrade, University of Utah--
Individual human fibrinogen molecules,
deposited from aqueous solution and dried on
highly oriented pyrolytic graphite, have been
studied by a scanning tunneling microscope
(STM). Since fibrinogen has a large height
(~100Å) but its electron density is much
smaller than that of the substrate, the STM
tip can not follow its contour, although the
tip senses its presence. Therefore fibrino-
gen molecules often show a slab shape with a
flat plateau rather than their typical 3-node
shape. They are apparently squeezed and
deformed by the STM tip. The shape and
radius of a tip may play an important role
for the interpretation of the results.
During the observation fibrinogen molecules
may be fairly mobile, as the molecule drifts
on the substrate under the influence of the
tip and the bias voltage. Such a dynamic
process implies that the tip is mechanically
in contact with fibrinogen and that the in-
teractions are weak between the substrate and
fibrinogen.

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# SURFACE ATOMIC STRUCTURES OF BIOMEDICAL CARBONS OBSERVED BY STM

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For decades, people have been puzzled as to why biomedical carbons show good biocompatibility; it is probable that surface structure and morphology are responsible for their properties[1,2]. One of the principal needs, therefore, is an appropriate method to examine the surface in detail. Scanning tunneling microscopy (STM) provides a chance of looking at an atomic level. Our recent STM studies on three carbon biomaterials reveal their surface morphology, atomic packing, nano-crystallite patches, and effects of the polishing process.

Three as-received carbons, glassy carbon (GC), silicon-alloyed low temperature isotropic carbon (LTIC), and ultra-low temperature isotropic carbon (ULTIC), were cleaned with pure water, acetone and dichloro-methane, sequentially, and air dried. GC and LTIC were also polished by hand with a 0.05 $\mu$m alumina slurry, sonicated in the above solutions, and air dried. A NanoScope II (Digital Inst.) with STM Head-A was used. All the samples were imaged with mechanically sharpened Ir/Pt tips at the bias voltage of 0.1V and the tunneling current of 1.0 nA, and all the images were obtained in air.

Among the three as-received samples GC shows the highest ordering structure with many discernible "quasi-crystalline" turbostratic patches on the order of 50Å. The apparently disordered patches are considered to be those with a non-horizontal orientation to the surface. Many misplaced atoms and line dislocations highlight the lack of long range ordering within a single "crystallite", although most atoms are arranged in a hexagonal manner. The distance between two adjacent atoms is usually larger than that in the graphite lattice, however.

Under an SEM the as-received LTIC shows a very rough and porous surface with lots of granules. On the nano-scale individual granules are actually quite smooth. Quasi-crystallite patches are also observed but atoms are far less orderly packed and the range of ordering is shorter; no distinguishable individual atoms were observed. Nonetheless, some areas with atomic arrays can still be seen, although not as frequently and noticeably as in the case of GC. On ULTIC, on the other hand, an atomic array does not appear at all while a number of apparently ordered areas can be barely seen. The difference in the intra-crystallite ordering is considered to be

dependent on the temperatures under which the carbon was formed.

After polishing the GC surface preserves its atomically ordered regions to some extent. Upon polishing the surface of LTIC becomes much smoother and non-porous whereas it loses all its ordered structures. It is virtually converted into an ULTIC-like surface.

Such a fundamental change of the LTIC surface structure must play a role in its surface energetics. It is the consensus in the carbon community that the edges and defects of a graphite crystallite have higher energy and activities towards a chemical reaction than its basal plane[1,3]. Thus polishing LTIC actually activates the surface by destroying the original somewhat crystalline structure and by creating more edges and defects, in addition to macroscopically smoothing the surface.

The present work indicates that the change in surface disordering and energetics may be more important than surface morphology. It is commonly believed that surface polishing improves biocompatibility of a carbon material, mainly by making it a smoother surface, such that species would not be trapped in surface pores[1]. Nevertheless, in the protein dimension the surface is quite flat. The surface with higher energy may be preferred when it interacts with proteins by keeping them in their native states. Or we can envision that such an activated surface tends to adsorb certain protein and/or non-protein species faster and more strongly, and then gets passivated more easily. It is this passivated surface that endows LTIC with good performance in a physiological environment. Meanwhile, the surface structures do not change much for graphite and GC after being polished. This might be the reason why they may be less suitable than LTIC or ULTIC as biocompatible materials[2].

In summary, this work studied the surfaces of biomedical carbons on an atomic scale and revealed that polishing changes the surface "crystalline" structure to a more "amorphous" structure.

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## THE BIOACTIVITY OF ADSORBED FIBRINOGEN: A DOMAIN ANALYSIS

APPROACH. L. Feng and J.D. Andrade, Department of Bioengineering and Center for Biopolymers at Interfaces, 2480 MEB, University of Utah, Salt Lake City, UT 84112

Over the years the role of adsorbed fibrinogen in platelet adhesion and activation has shifted dramatically. We now understand that complex proteins such as fibrinogen can interact with the surface by many different domains and in many different orientations, depending on the nature of the surface and on the competitive protein collisions present. We further understand that once the protein is adsorbed, depending on its adsorption free energy, its neighbors, and the rain of other colliding proteins, it may undergo time-dependant orientational and/or conformational alterations. All of these processes directly influence what portion of the protein molecule is exposed to other proteins, to platelets, and to other cells colliding with the now protein-coated surface. Depending on the particular orientation and conformation of adsorbed fibrinogen, various amino acid sequences and other features will be presented to the surrounding solution. We suggest that considerable insight into fibrinogen's interface-dependant properties can be attained by a careful consideration of the protein's unique structure. We initiate such an analysis using structural cartoons and icons based on a domain approach and using available information in the literature. Acknowledgments: This work has been partially supported by the Center for Biopolymers at Interfaces, University of Utah, and by NIH Grant HL 44538

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and $P_v$. From blood sampled at the minimum and control blood volumes, we determined the change in plasma density, $\Delta\rho_p$. For 10% hemorrhage with cyclic period of 2 min, $\Delta\rho_a$ over one cycle was $0.90 \pm 0.05$ g/l and $\Delta\rho_p$ was $0.07 \pm 0.02$ g/l. From $\Delta\rho_p$ we assessed the fluid restitution augmenting the blood volume as 6% of the shed volume. From the measured $\Delta\rho_a$ and $\Delta\rho_p$, we found that the volume shift offsets hypovolemia in the macrocirculation by 60% of the hemorrhaged volume. Because the density response and the volume shift were unaffected by cycle period, sympathetic ganglionic blockade via hexamethonium, and pentobarbital anesthesia, we concluded that neural and hormonal responses are not the principal mechanisms controlling the shift. A step hemorrhage in conscious and anesthetized rabbits revealed that the cardiac output reduction was unaffected by anesthesia, while the increase in total systemic resistance $R_{sys}$ observed in conscious rabbits was abolished. $P_a$ was well maintained in conscious rabbits during both the cyclic and steady state trials decreasing by $8 \pm 1$ mmHg for 10% hemorrhage, but fell markedly by $26 \pm 4$ mmHg after hexamethonium and anesthesia. $\Delta P_v$ equalled $2.3 \pm 0.3$ mmHg and was unaffected by hexamethonium, anesthesia or cycle period. Because of the altered responses of $R_{sys}$ and $P_a$ to hemorrhage, and the similarity in $\Delta V_s$ for conscious and anesthetized rabbits, we concluded the volume shift is not mainly controlled by autoregulation. Hypovolemia is known to cause a passive change in mean circulatory filling pressure resulting in a change in $P_v$. $\Delta P_v$ is the major determinant of the change in microvascular pressure due to the large compliance and low resistance of the veins. The linear correlations between $\Delta V$, $\Delta P_v$, $\Delta\rho_a$ and the volume shift support the conclusion that a reduction in microvascular pressure, due to $\Delta P_v$ induced by hemorrhage, results in the passive recoil of microvessel walls, shifting a volume of blood from the micro to the macrocirculation.

Amy J. LaForte

Dissertation Advisor: Dr. Jen-Shih Lee

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August 1992

## THREE-DIMENSIONAL RECONSTRUCTION AND HEMODYNAMICS OF CAPILLARY NETWORKS IN HYPERTENSIVE SKELETAL MUSCLE

An elevated total peripheral resistance is associated with essential hypertension. Structural adaptation of the microvascular network in skeletal muscle is one possible mechanism for this elevated peripheral resistance. Accurate topological and dimensional descriptions of microvascular networks can provide realistic hemodynamic network models which can be used to evaluate the structurally-based change in microvascular resistance. However, the complicated arrangement of the capillary network in thick skeletal muscle has hindered the description of its structure. Therefore, new computer software capable of reconstructing, visualizing, and analyzing complex vessel networks was developed, and three-dimensional reconstructions of arterio-venous capillary units in vasodilated gracilis muscle of 12–13 weeks old WKY and SHR rats were performed using 1 $\mu$m ink-filled histological sections. Geometrical capillary unit resistances were computed from Poiseuille's equation and the network conductance matrices, and this parameter was found to be statistically equal in WKY and SHR, indicating no structurally-based alterations. Heterogeneity of the geometrical unit resistances within each strain, however, indicated structure differences among units. The structure differences may be correlated to the spatial distribution of muscle fiber types in a capillary unit. A new method for fiber typing using plastic sections was established and flow simulations using a hemodynamic model were carried out. Blood flow was found to be higher around oxidative muscle fibers than around glycolytic muscle fibers. Fiber composition adjustments on the unit resistances, however, do not reduce the heterogeneity. It is shown that the pressure gradient along the arteriole pathway inside the capillary unit also plays an important role in determining the blood flow distribution. The flow simulation result actually implies lower functional capillary unit resistance in SHR rats. The spatial arrangements of capillary units within a muscle may affect the unit resistance, and an arrangement with more capillary units in parallel is likely to contain individual units with higher resistances. It is possible that the capillary unit interconnections with arterioles and venules, in addition to the pressure gradient and muscle fiber composition, are important determinants of the unit resistance.

Hsiang-Hsin Hsiung

Dissertation Advisor: Dr. Thomas C. Skalak

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May 1993

## BIOMEDICAL CARBON SURFACES AND THEIR INTERACTIONS WITH PLASMA PROTEINS

The nature of adsorbed plasma proteins on a blood contacting implant mediates the interaction between the surface and blood, and determines its blood compatibility. This work studies adsorption of plasma proteins on low temperature isotropic carbon (LTIC), a reputed blood compatible material, and tests hypotheses on carbon-protein interactions.

This work measures surface structures and properties of LTIC and other biomedical carbons using scanning electron microscopy, X-ray photoelectron spectroscopy, contact angle goniometry, and scanning tunneling microscopy. The carbons consist of different sizes of quasi crystallites and domains. Although containing a fairly large amount of oxygen, the LTIC surface shows hydrophobic characteristics. Polishing smoothes the surface of LTIC but also makes surface atomic packing more disorganized.

This work applies four major techniques to the investigation of protein adsorption: differential scanning calorimetry, AC impedance, radioisotopes, and two-dimensional electrophoresis. Albumin and fibrinogen are the principal proteins and LTIC is the substrate. Some small proteins and, silica and gold substrates are also used. Measured parameters of protein adsorption are: conformational changes, adsorption rates, isotherms, elutability and reversibility, and competitive adsorption. Factors affecting these parameters are discussed, such as substrates, surface charge, protein solution concentration and composition, ionic strength, and protein solution stability.

Major observations of protein adsorption on LTIC are as follows.

- The surface denatures all adsorbed proteins studied.
- The adsorption rate is high.
- The more negatively charged surface adsorbs more proteins.
- The surface is saturated by proteins with high surface concentrations.
- The adsorption is irreversible.
- The surface is not selective to adsorbed protein.
- Proteins with lower solution stability are more easily adsorbed.
- Albumin has a relatively low surface activity.
- Fibrinogen has high adsorptivity.
- Hydrophobic interactions are the principal interaction.

A four stage process is suggested for protein adsorption on LTIC when it contacts blood: diffusion, adsorption, denaturation, and modification. The nature of protein adsorption on LTIC favors the hypothesis that LTIC tenaciously adsorbs a layer of bland plasma proteins on its surface. This proteinaceous film "biolizes" the surface and renders it blood compatible. LTIC acquires such adsorption properties through its unique surface characteristics: hydrophobicity, relatively high surface energy, rigidity, and microdomain structure.

Li Feng

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# AN ICON REPRESENTATION OF PROTEIN STRUCTURE AND PROPERTIES

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## Introduction

We have developed a set of icons (cartoons) to represent particular characteristics of protein structure and interfacial properties. The goal is to provide a direct graphical view of protein molecules, or their structural domains, so that they can be easily understood. An icon is intended to describe two aspects of a parameter: what it is and its magnitude. This paper presents fifteen icons and their physical significance. Their application to both small proteins and multi-domain proteins are exemplified.

An understanding of protein structure and properties, and their interrelationship is important in biology, biochemistry bioengineering, pharmaceuticals and food science [2]. Recently we proposed a graphical method, called "the Tatra plot", for examining protein structure and surface activity [3,4]. The Tatra plot consists of a multi-axis coordinate, providing a visual representation to facilitate the recognition among the parameters. In this paper, we develop a set of icon (cartoons) to describe proteins or their domains. These icons are directly visualized and easily understood, enabling viewers to comprehend their contents quickly and efficiently.

An icon shows the nature of a parameter and its magnitude, which can be a steady property (e.g., molecular weight), or a dynamic property (e.g., surface tension kinetics). Describing the later is more difficult than a steady property. The icon shows the degree of the change of a property by the magnitude of a scale (e.g., temperature), the amount of a quantity (e.g., denaturants), the size of an object (e.g., effective surface hydrophobicity), or the number or concentration of an entity (e.g., surface charge). A quantitative approximation is expressed as low, medium, or high. Thus a set of three icons were designed to describe a parameter, but only one of them was used for a given protein.

## Materials & Methods

In designing an icon we must balance two criteria: comprehensivity and simplicity. An icon should be easy to understand and provide semi-quantitative information. However, it has to remain a simple expression. Usually compromises are made between these two criteria for a specific icon, since enhancing comprehensivity often sacrifice simplicity, or vice versa. Since our goal is to represent the essence of the parameter in a cartoonish way, a picture always serves as the main body of an icon; the use of text is kept to a minimum.

Here most icons were derived for the parameters in the Tatra plot [3,4]. They are related to surface and interfacial behavior. The individual parameters and their descriptions are given in the full paper. The parameters are divided into five groups: molecular size, structural stability, hydrophobicity, hydrophilicity, and surface activity.

## Results

Figure 1 applied the icon approach to the example of lysozyme (LSZ), whose characteristics and Tatra plots are described elsewhere [3,4]. The paper shows the icon representation of lysozyme, myoglobin, superoxide dismutase, albumin, and fibrinogen -- including the individual domains of fibrinogen [8]. We can deduce, for example, that domains D and Aa C of Fibrinogen are more surface active than domain E.

## Conclusions

Protein structure and property parameters can be illustrated by the icon representation. It is a simple and efficient way to convey the many complex parameters involved in protein structure and properties.

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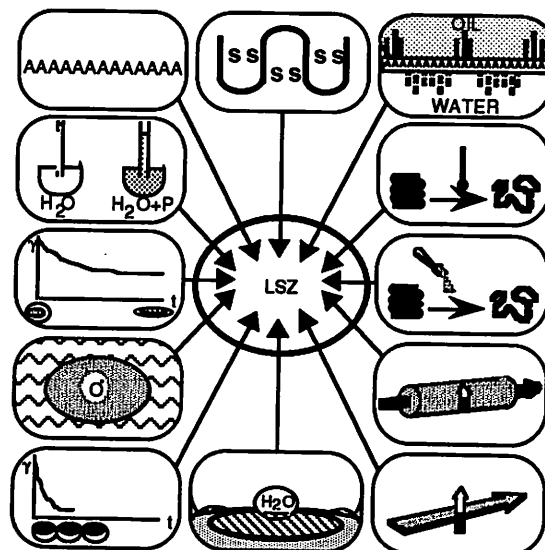[8] Feng, L; Andrade, J. D. in T. Horbett and J. Brash, Eds., *Proteins At Interfaces II*, ACS, 1995, in press.

Figure 1. Icon Representation of Lysozyme (LSZ)

# PROTEIN ADSORPTION ON LOW TEMPERATURE ISOTROPIC CARBON

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## Introduction

LTIC represents a large family of carbons that generally show some degrees of biocompatibility, including diamond-like carbon, pyrolytic graphite, carbon-carbon composites , carbon fibers, and even diamond, in addition to LTIC, ULTIC, and GC. LTIC is an interesting material because it possesses some properties that are not generally considered relevant to a "good" blood compatible material. How plasma proteins adsorb on a surface is a central issue in addressing the problem of thrombosis.

Carbon surfaces are thought to be conditioned by adsorbed proteins. The debate is focused on how the proteinaceous film passivates the surface. We consider two major hypotheses:

The weak interaction hypothesis states that the carbon surface is chemically inert and thus does not strongly interact with proteins. Micro-domains of carbon may also provide a "balanced" force to accommodate proteins. The adsorbed proteins preserve their native conformations at the blood-solid interface and the adsorption process should be reversible; adsorbed proteins are exchangeable.

The strong interaction hypothesis, suggests that the LTIC surface is coated with a thick film of bland plasma proteins. The proteins are held so tenaciously that they can not be easily removed. The surface becomes blood compatible through modification by this "bland" proteinaceous film, most likely albumin. According to this hypothesis, the conformation of the inner layer of adsorbed proteins should be changed to achieve a strong protein-surface interaction. This adsorption process should be irreversible; proteins are not exchangeable.

## Materials and Methods

We carried out two sets of experiments: LTIC surface analysis and protein adsorption. The details are in the literature on now in press.

## Results

Surface induced denaturation is a process of protein unfolding in the presence of the surface, implying a strong interaction between them. Usually a hydrophobic surface tends to denature adsorbed proteins due to hydrophobic interactions. From its hydrophobic nature we expected that LTIC would denature proteins. Our DSC study shows that LTIC denatures all the proteins studied in a number of buffers of different pH.

Both impedance and radioisotope-labeling techniques produced similar protein adsorption isotherms, indicating that LTIC has a strong tendency to adsorb proteins.

Competitive adsorption from an albumin/fibrinogen binary system confirms that there is no preferential adsorption of either albumin or fibrinogen on LTIC.

This work clearly demonstrates a very important attribute of the LTIC surface: it tenaciously and irreversibly adsorbs proteins. In fact, no effective way has been found to completely elute adsorbed proteins from LTIC.

When LTIC first contacts blood, plasma proteins begin to collide with the solid surface. The protein molecules that hit the surface are promptly retained by the surface. LTIC does not discriminate or select among the plasma proteins. Thus the composition of adsorbed proteins is similar to that of proteins that collide with the bare surface. Among the initially adsorbed proteins, albumin is the most abundant one (about 70% of the total). The adsorbed proteins are rapidly denatured by the LTIC surface through hydrophobic interactions. Globular plasma proteins are likely flattened on the surface, resulting in a dense and relatively impervious protein film that completely covers the LTIC surface. Little or no bare LTIC surface is exposed to blood after this adsorption process. The proteins that intimately reside on the surface are tenaciously held. The association is so strong that ordinary means of elution can not remove them. Thus the protecting film is stable and resistant to being displaced.

Since LTIC is actually covered, protected, and thus modified by the bland proteins (albumin and perhaps the albumin-IgG complex), this protein covered surface becomes blood compatible. The proteinaceous film prevents the surface from contacting potentially active plasma proteins (e.g., Factor XII) and platelets. The film does not activate them nor does it trigger the coagulation cascade.

Even though adsorbed albumin in some isolated areas will occasionally detach for some reason, the spots will be instantaneously replenished and patched by adsorption of circulating albumin so that the entire protecting film remains intact.

## Conclusion

What is unique for LTIC and perhaps other carbons is that they possess the following characteristics:

The protein protecting film on LTIC consists of mostly bland proteins, which is the result of its nonselective adsorption and the absence of displacement of adsorbed proteins. This property gives the surface a relatively permanent blood compatibility.

Strong interactions of LTIC with proteins may originate from its relatively high surface energy in addition to its hydrophobicity. The surface heterogeneity of LTIC is also important. Its function is not necessarily in preserving the native conformation of adsorbed proteins but rather in enhancing the adhesion between the surface and the adsorbed denatured proteins.

Chain motion in LTIC is severely restricted by the two dimensional network. Subsequently, rigid LTIC

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ADSORPTION OF THREE PLASMA PROTEINS ON THE SILICA SURFACE WITH WETTABILITY GRADIENT.   C.G. Gölander, V. Hlady, Y.S. Lin and J. D. Andrade, Center for Biopolymers at Interfaces, Department of Bioengineering, University of Utah, Salt Lake City, Utah, 84112

The adsorption of three plasma proteins on the modified silica surface was studied by total internal reflection fluorescence (TIRF) method. The surface was prepared by two-phase solution silanization using dichlorodimethylsilane (DDS) and showed a smooth gradient of wettability along one dimension. The adsorption of FITC-labeled proteins: albumin, immunoglobulin and fibrinogen was carried out from a single protein solution and from a mixture. The single protein adsorption experiment showed that the protein affinity for the surface remains unchanged along the wettability gradient; immunoglobulin > fibrinogen > albumin. The adsorbed amount was larger at the non-wetting side of the gradient. The adsorption from protein mixture showed that after 120 minutes of contact time the silica surface is largely depleted of adsorbed immmunoglobulin. The elutability of adsorbed protein was tested using a non-ionic surfactant solution.

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# BIOLOGICALLY COMPATIBLE SURFACES

D. Gough, J. D. Andrade, and R. VanWagenen
*University of Utah*

The often adverse interaction of living tissue with foreign materials is perhaps the most pressing problem currently delaying further advances in modern implant medicine. The interface between hydrophobic materials and the living system is commonly the site of many undesirable interactions such as platelet adhesion, blood clotting, protein adsorption, and denaturation, and in general, gross modification of the local biochemistry. Implant materials presently in use meet physical and mechanical demands but are generally dead, passive discontinuities in a dynamic living system.

Many approaches to the problem of compatibility have been pursued. One has been neo-intima formation, a technique commonly used in vascular surgery. A highly porous Teflon or Dacron prosthesis is implanted, around which a viable inner surface is allowed to form. Limitations have been bleeding, calcification, and adverse tissue growth. Another possibility for thrombo-resistance has been heparinization.[1] The anticoagulant may be adsorbed, attached to the surface by a quaternary ammonium salt complex, or even milled in with the polymer. Long term studies indicate that there is significant hemolysis resultant from these methods of heparinization.

One approach to the problem which has been largely overlooked is the hydrophilic surface. Since vascular lining is itself hydrophilic, it seems that a hydrophilic gel which could be bonded directly to a more rigid material would have a much more favorable interaction in the aqueous physiological media. Several such gels have been prepared and show promise.

Acrylic hydrogels, in which water content can be varied from 20% to 97%, have been investigated.[2] Biological properties of these hydrogels have been studied in many types of biological tissue, including blood,[3] and tend to be compatible.[4] These materials are quite stable in aqueous media and are not hydrolyzed but have very poor mechanical properties.

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2. O. Wichterle and D. Lim, *Nature* 185, 117 (1960).

3. M. F. Refojo, *J. Appl. Poly. Sci.* 9, 2425, 3161 (1965).

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severe nonlinearities present during the transient response phase and the nonstationarities in the steady state phase required the placement of stringent restraints and modifications on the adaptive schemes and their drug rate predictions. This was needed to produce the required stability and reliability necessary in clinically controlled variable management. The principal finding in the development of this process was that the restraints and modifications required on the adaptive controller produced control policies similar to much less complex controllers. This finding provided the impetus for further investigations, which resulted in the final controller's design.

The final controller design is a two-part device which employs a "wait-and-see" controller during the transient response phase and a "reset" controller in the steady state phase (WAS-I controller). It uses empirically derived parameter values and requires a montonic drug-controlled variable relationship. It produces symmetrical control policies, but will manage only single input-single output systems. Testing of the controller involved over 20 canines and numerous combinations of the aforementioned states, drugs, and variables. The results demonstrate consistency and reliability in producing clinically acceptable control. Therefore, the contribution of this thesis is a controller which satisfies, to the degree tested, all the goals set forth in the previously stated purpose.

Possible further developments in automatic controllers of physiological variables is presented. Also discussed are possible problem areas in, requirements for, and uses of clinical applications of automated controllers in the intensive care system.                    Order No. 75-11,645, 305 pages.


A FUNCTIONAL ANALYSIS OF AN ENZYME–MEMBRANE ELECTRODE

GOUGH, David Arthur, Ph.D.
University of Utah, 1974

Chairman: Joseph D. Andrade

The analytical use of biochemical-specific electrodes in health-related applications is increasing. A semi-empirical model of one type of sensor, the enzyme-membrane electrode, or enzyme electrode is presented. The model, based on the theory of the oxygen cathode and enzyme-membrane kinetics, is used to describe a glucose-specific sensor in which enzyme glucose oxidase is immobilized as a membrane onto a Clark oxygen electrode.

As a background, essential literature on the theory of the oxygen electrode and on immobilized enzymes is briefly reviewed. A study of solution-phase kinetics of glucose oxidase is reported, and the observed kinetic constants are used to model the behavior of glucose oxidase immobilized as a membrane.

A semi-empirical model of the enzyme electrode is presented. The analysis is rigorously accurate when the cathode radius is small, the substrate concentrations are lower than the respective Michaelis constants, and the catalytic ability of the membrane is small compared to the rate of substrate transport through the media. The treatment describes steady state and pre-steady state concentration profiles of electrodes in various types of physiological media and allows prediction of response to various substrate concentrations and measuring conditions. The three cases considered in steady state and pre-steady state are, an enzyme electrode (1) in substrate-evolving media, where there is no diffusion to the probe, (2) in moving media, or media where a boundary later is otherwise formed, such that substrate must diffuse across the boundary layer to the probe, and (3) in stationary, homogeneous media, where substrate must diffuse to the electrode. The equations predict different electrode response in each case and relate response to construction variables and measuring conditions.

An empirical term relating substrate concentration to electrode response was evaluated for many representative electrode designs. The steady state results suggest that electrode response may be optimized by the proper combination of construction variables. With a relatively small cathode, increasing the amount of catalyst in the enzyme membrane permits detection of lower glucose concentrations, although the detectable range remains approximately constant. Similar effects are noticed when temperature is increased or the pH optimum is approached. With larger cathodes the range of detectable glucose is noticeably shortened and more enzyme activity is required to effectively reduce the transport of uncatalyzed oxygen across the membrane. In all cases a characteristic thickness and activity of the enzyme membrane seems to be necessary for function. Substantially increasing the thickness of the enzyme membrane or the total membrane thickness may reduce the steady state response to glucose.

The pre-steady state response of the electrode after an instantaneous concentration change was taken as the most practical method of experimentally confirming the model. The time-dependent response was observed for a number of electrodes of different constructions and compared to computer simulations. It was found that electrodes with small cathodes, having low activity enzyme membranes, responded predictably to glucose changes in low concentration ranges when stirred. Electrodes or experimental conditions that deviated from the assumptions of the derivation were less predictable.

The analysis is useful to understand the function of enzyme electrodes. It predicts the optimization of improved analytical sensors and is useful for simulation of the response of certain electrodes.                    Order No. 75-8698, 144 pages.

EFFECT OF LOW OXYGEN TENSION ON CALCIUM WASHOUT DYNAMICS FROM GUINEA PIG LEFT ATRIUM

HUANG, Han-Min, D. Sc.
Washington University, 1974

Chairman: Professor G. R. Little

Guinea pig left atria were incubated with Sr-85 (a substitute for Ca-47) and EDTA–Co-60 (extracellular label) and then subjected to continuous outflow with isotopically inactive Krebs at constant temperature 25°C and flow rate 1 ml/sec. The Krebs solution for incubation and washout were bubbled with either 95% $O_2$ or 10% $O_2$. Radioactivity counts in the tissue were recorded using a Gamma-probe to $\mu$LINC digital computer system for real time two channel data collections.

Extracellular and intracellular washout curves were separated from raw data by time transformation and functional subtraction and analyzed by SAAM computer program to calculate the mean washout time constants. It was found that mean extracellular washout time constant at 1 CPS was increased from 55±2.5 to 164±29 seconds, and mean intracellular washout time constant at 1 CPS was increased from 214±19 to 581±70 seconds in going from 95% $O_2$ to 10% $O_2$.

Furthermore, compartmental analysis was used to describe the kinetics of Sr-85 in terms of transfer rate constants. A three-compartment model in series consisting of (1) extracellular (2) intracellular and (3) protein-bound spaces was found to fit the data. Transfer rate constant from extracellular space to the exterior was found to decrease about 2 to 1, and transfer rate constant from extracellular to intracellular space was found to increase three-fold in going from 95% oxygen to 10% oxygen, while the other transfer rate constants were not significantly changed.

**7090**

NITRENDIPINE MAY STIMULATE A CALCIUM PUMP IN VASCULAR MUSCLE.
Kent Hermsmeyer and Ronald Mason*. The Cardiovascular
Center, University of Iowa, Iowa City, IA 52242

Nitrendipine is a new dihydropyridine calcium ($Ca^{++}$)
antagonist proposed to block contraction by blocking entry of
$Ca^{++}$ into vascular muscle cells. Recording of membrane
potential ($E_m$) and contraction stimulated by norepinephrine
(NE) in the rat caudal artery showed that nitrendipine (10 nM
to 1 μM) rapidly induced hyperpolarization and relaxation.
In NE concentrations up to 1 μM, 10 nM nitrendipine hyper-
polarized by 2-3 mV accompanied by larger relaxation than the
voltage-tension relationship would predict. In other experi-
ments, NE was added to caudal arteries in either 0 $K^+$ solu-
tion or 0 $Ca^{++}$ solution. 0 $K^+$ solution completely abolished
the action of nitrendipine on $E_m$ and relaxation. Contrac-
tions to NE were larger in 0 $K^+$ solution than in control
solution, and were not blocked by up to 1 μM nitrendipine. In
solutions with no added $Ca^{++}$ (22 μM $Ca^{++}$ by $Ca^{++}$ ion elec-
trode), nitrendipine more strongly blocked NE contractions
(from 30% in control to 60% in low $Ca^{++}$). Enhanced action of
nitrendipine in low $Ca^{++}$ and its blockade in 0 $K^+$ suggest
that nitrendipine acts on $K^+$-sensitive $Ca^{++}$ transport impor-
tant for relaxation of vascular muscle. This hypothesized
stimulation of a $Ca^{++}$ pump by $Ca^{++}$ antagonists might be dif-
ficult to differentiate from block of $Ca^{++}$ entry in ion flux
experiments (same net result). Further $E_m$ experiments will
be needed to test the $Ca^{++}$ pump hypothesis.

**7091**

NIMODIPINE, A NEW C.    SEPARATE VASCULAR BEDS.
ACTIVATED ATPase OF                                Univ. of
Ronald A. Janis. M
1956, New Haven, CT U                              dulla for con-
The effects of nin _____ 1483 _____        400 sites
nitrophenyl)-3,5-pyridi                      apid changes
methylethyl ester), a                        in calcula-
triphosphatase (ATPase) ac.                  iliac arter-
deoxycholate-treated rat ao$_{Na^+, K^+}$-   bane. Medul-
ed ATPase was measured as th. and            gical sec-
activity, measured in the pres Box           efined
and $Mg^{-}$-ATPase, measured in th.         cts of the
cations. All assays were carrie.             a dorsal
$MgATP$, 0.2 mM EDTA, 32 mM Tris (p           and celiac
The ATPase activities (in μmol $Pi/hr$        to

| | Total-ATPase | $Mg$-. |
|---|---|---|
| Control | 189 ± 10 | 169 |
| Nimodipine (1.5μM) | 207 ± 13 | 162 ± |

(Mean ± S.E. for duplicate measuremer.
each of which contained aortic membran.
Nimodipine was found to stimulate this enzy.
microsomes from rat vas deferens. For both
smooth muscle the concentration of nimodipi.
half-maximal stimulation of $Na^+, K^+$-activated A.
than 15 nM. Verapamil at high concentrations
stimulated $Na^+, K^+$-activated ATPase. The relations
the effects of these calcium entry blockers on smo.
tone and on ATPase activities has yet to be defined.

**7092**

AGONIST-RELEASABLE INTRACELLULAR $Ca^{2+}$ POOL MAY MODULATE THE
CONTRACTILE RESPONSE TO $Ca^{2+}$ INFLUX. Rodger D. Loutzenhiser*
and C. van Breemen. Department of Pharmacology, University of
Miami, Miami, FL 33101

Several agonists induce the release of an intracellularly
bound Ca pool in arterial smooth muscle. This releasable Ca
pool refills only in the presence of extracellular Ca. Refil-
ling of the release pool of isolated rabbit aorta was deter-
mined by first depleting the pool and then measuring the con-
tractile response to agonists under Ca-free conditions, after
reloading for various times in Ca containing buffer. Lanthanum
which blocks both stimulated Ca influx and the nonspecific Ca
leak, prevented refilling, while D-600, which blocks only
stimulated Ca influx did not, suggesting that the pool refills
via the Ca leak pathway. However, the rate of refilling and
size of the pool were increased by KCl depolarization, indi-
cating that the Ca in this pool may also enter via the stimu-
lated Ca influx (ie. D-600 sensitive) pathway. $^{45}Ca$ uptake
measurements demonstrated that the extent of refilling was
only dependent upon the amount of Ca which entered the cell
and not the entry pathway. The rate of tension development in
response to KCl was slowed when the release pool had been de-
pleted and accelerated in the presence of an agonist capable
of releasing this pool. These data indicate that the agonist-
sensitive pool refills using Ca which has just entered the
cell. Furthermore, an agonist-induced reduction in the seque-
stration capacity of this pool may facilitate the entry of Ca
into the myoplasm during agonist-stimulation of Ca influx.

**7093**

THE LOSS OF NE STIMULATED Ca UPTAKE DURING WASHING OF RABBIT
AORTA IN ICE-COLD La-CONTAINING AND ZERO Ca MEDIA. Philip
Aaronson*, Cornelis van Breemen, and Pauline Zera*. University
of Miami, Department of Pharmacology, Univ. of Miami, Miami,
FL 33101

The quantitation of cellular Ca in smooth muscle tissues
generally involves washing tissues labeled with $^{45}Ca$ in warm
or cold media containing La or EGTA (ethyleneglycol-bis-(β-
aminoethyl ether)N,N' tetraacetic acid) in order to remove
extracellular bound label. Cellular Ca is then estimated by
extrapolating the monoexponential slow efflux component. In
order to investigate whether stimulated Ca influx can be ac-
curately measured using this technique, rabbit aorta rings
were labeled for 15 min in the presence or absence of $10^{-5}$
norepinephrine (NE) and then allowed to wash out into ice cold
Ca free media containing 2 mM EGTA or 10 mM La. Comparison of
washout curves from control and stimulated tissues indicated
that NE induced a loss of Ca from a rapidly exchanging tissue
pool, as well as an uptake of Ca into a more slowly exchanging
pool. During washing, the stimulated Ca uptake was progres-
sively lost. The half time for this loss was approximately
12 min in the La containing medium and 24 min in the EGTA con-
taining medium. These results suggest that NE induces a re-
lease of extracellular Ca, and further indicate that extended
washing of tissues in cold media may lead to an underestimate
of NE-stimulated Ca uptake, especially in La-containing media.
(Supported by NIH grants HL 07188 and HL 27559)

*Ab-Van W-4*

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BIOCHEMICAL TRANSDUCERS (7094–7095)

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**7094**

POTENTIAL SENSOR APPLICATIONS OF TOTAL INTERNAL REFLECTION
FLUORESCENCE (TIRF) SPECTROSCOPY. Richard A. Van Wagenen* and
Joseph D. Andrade, Department of Bioengineering, Univ. of
Utah, Salt Lake City, Utah 84112

Conventional immunoassay technology requires the use of a
labeled antigen (Ag) or antibody (Ab) and a process for sep-
arating the complex (bound fraction) from the unbound label
(free fraction). Total internal reflection (TIR) [1] at an
interface containing immobilized Ab or Ag permits direct mon-
itoring of fluorescently-labeled (extrinsic fluorescence) Ag*
or Ab* (the * denotes the molecule is fluorescently
labeled) due to the formation of the surface-bound Ab-Ag* or
Ag-Ab* complex [2-3]. Many of the Ag and all of the Ab of in-
terest are intrinsically fluorescent in the ultraviolet due
to tyrosine and/or tryptophan amino acid residues. We have
employed this intrinsic fluorescence property in the total
internal reflection mode to monitor the adsorption of proteins
at solid-liquid interfaces [4]. Preliminary studies sug-
gest that total internal reflection intrinsic fluorescence
(TIRIF) can be used as a direct assay of Ag-Ab interactions
and thus as a reagent-less, label-less immunoassay probe.

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   235.
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   Cooper, et al. (eds.), Morphology, Structure and Inter. of
   Biomaterials, ACS Adv. Chem. Series, in press.

**7095**

DESIGN FACTORS IN ENZYME ELECTRODES FOR GLUCOSE. David A.
Gough* and John K. Leypoldt* (SPON: B.W. Zweifach). Univ. of
California, San Diego, La Jolla, CA. 92093

We are developing a potentially implantable glucose sensor
for possible use in diabetes. The sensor is based on the
"enzyme electrode" principle in which a membrane containing
immobilized glucose oxidase is mounted over an oxygen sensing
electrode. Glucose and ambient oxygen diffuse into the mem-
brane and react enzymatically, with the unconsumed oxygen
being detected at the electrode and indicating the glucose
concentration. Progress in the development of this sensor
has previously been hindered by the lack of a detailed under-
standing of the processes in the membrane. We have developed
a model of mass transport and reaction within the membrane,
taking into account such features as the concentration bound-
ary layer in the solution adjacent to the membrane, substrate
partitioning, nonlinear two-substrate reaction kinetics, and
enzyme inactivation. The model predicts the sensor response
to glucose concentration under various conditions of oxygen
concentration, enzyme loading, internal and external mass
transfer resistances, and is therefore useful in sensor de-
sign. We have tested this model with a novel, membrane-
covered rotated disc electrode that we have developed which
allows control of the external mass transfer resistance and
independent evaluation of the internal mass transfer and the
reaction kinetics. (Supported by the Juvenile Diabetes Fdn.
and NIH Grant AM 27541).

# POLY(ETHYLENE GLYCOL) SURFACES TO MINIMIZE PROTEIN ADSORPTION

D.E. Gregonis, D.E. Buerger, R.A. Van Wagenen, S.K. Hunter, and J.D. Andrade

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

Proteins adsorb to almost all surfaces during the first few minutes of blood exposure, and the amount, type, conformation, and orientation of these bound proteins primarily determines the overall blood compatibility response of the material. To attempt to clarify these protein interactions, it was felt necessary to learn how to minimize or eliminate this irreversible protein adsorption process. Surfaces which minimize protein adsorption are not only of interest for blood-materials interactions but other applications as gel permeation chromatography, contact lens polymers, coatings to minimize biofouling, separation membranes, and surfaces for immunoassay detection methods.

In this work, protein adsorption is measured using total internal reflectance intrinsic fluorescence (TIRIF) techniques [2] using the inherent fluorescence of tryptophan containing proteins. This method eliminates artifacts sometimes found when using various labelling procedures. Excitation light at 280 nm is totally reflected to a quartz-aqueous buffer interface. Due to the nature of the total reflectance process, the photoenergy exponentially decays into the aqueous buffer. The 280 nm energy is adsorbed by tryptophan in solution and re-emits the light at 330-350 nm. This signal intensity provides a measure of protein concentration at or near the interface. The aqueous buffer is in a thermostated flow cell in which the surface can be challenged with various protein and buffer rinse regimes.

We have found that covalent binding of poly (ethylene glycol) (PEG) molecules to the quartz interface essentially eliminates irreversible protein binding in physiological buffer solutions. To accomplish this bonding, the quartz is treated with vapors of $\gamma$-aminopropyl triethoxysilane (APS) to introduce amino groups at the surface. The terminal hydroxyl groups of PEG are reacted with phosgene to form bis-$\alpha$,$\omega$-chloroformate PEG derivatives. This intermediate reacts with the amino functionalized glass to covalently bond the PEG via a urethane linkage. All these surface reactions are characterized by advancing-receding aqueous contact angles and X-ray photoelectron spectroscopy (ESCA).

The protocol for protein adsorption-desorption at the quartz and modified quartz surfaces use the following solutions: 1) Human immunoglobulin (IgG) (100 mg/100 ml) followed by phosphate buffered saline (PBS), pH 7.4 rinse; 2) Human albumin (4 g/ 100 ml) followed by PBS rinse; 3) Human serum followed by PBS rinse. The fluorescent counts are normalized to a zero background intensity of the PBS solution and a 10,000 counts fluorescence intensity of 0.10 mg/ml tryptophan monomer calibration solution. The fluorescence counts are now measured after the protein are allowed to adsorb to the surface followed by a thorough rinse of PBS in low shear, laminar flow conditions. These fluorescence signal intensities are shown in the table below.

| Surface | Adsorbed IgG Fluorescence | Adsorbed Albumin Fluorescence | Adsorbed Serum Protein Fluorescence |
|---|---|---|---|
| Clean quartz | 25,330 ± 1,400 | -- | 36,900 ± 360 |
| APS-quartz | 31,840 | 30,090 | 128,110 |
| PEG-MW 750 | 5,390 | 5,430 | 12,500 |
| PEG-MW 1,900 | 580 ± 820 | 410 ± 700 | 9,730 ± 1,210 |
| PEG-MW 3,400 | 590 | 790 | 3,630 |
| PEG-MW 5,000 | 440 ± 500 | 320 ± 480 | 2,050 ± 260 |
| PEG-MW 14,000 | 390 | 390 | 1,610 |

From this table, it is shown that PEB effectively minimizes irreversible protein adsorption, and the higher the molecular weight of the bound PEG, the more effective it is for this purpose. Studies are in progress to optimize this PEG binding reaction at surfaces and to determine its hydrolytic stability. Preliminary blood studies of these surfaces have also been performed.

2. R.A. Van Wagenen, S. Rockhold, and J.D. Andrade, "Probing Protein Adsorption: Total Internal Relection Intrinsic Fluorescence," in S.L. Cooper and N.A. Peppas, (eds.), Advances in Chemistry Series, 199, 353-370 (1982).

Second World Congress on Biomaterials
10th Annual Meeting of the Society for Biomaterials
Washington, D.C., April 27-May 1, 1984

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Ab-Haub-1

# LTI CARBON SURFACES

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## Background

LTI carbon is one of the very few synthetic materials generally recognized as suitable for long term blood contact applications (1). Although a large number of hypotheses have been formulated with respect to the blood tolerability of materials, a general theory or mechanism is not yet available. It is known that in certain situations the local hemodynamics can play a predominant role (2) and in most cases the solid-blood interfacial properties can play a predominant role (2,3). It is assumed that understanding the plasma protein adsorption process onto solids used for blood-contact applications will lead a better understanding of solid-blood interactions (1-3).

A number of preliminary studies of plasma protein adsorption onto LTI carbon surfaces are available (4-7). Radioiodinated -($I^{125}$)-proteins have been utilized by Kim, et al. (4) to measure adsorption of individual proteins and protein mixtures on LTI carbon. His results indicate the carbon very rapidly adsorbs albumin. This is consistent with Kim's model of blood interactions via a platelet-adhesion mechanism (8). Microcalorimetric and electrophoretic mobility studies of proteins onto LTI carbon have been done by Nyilas, et al. (5). The extension of the adsorbed layers have been measured directly using ellipsometry by Fenstermaker et al. at NBS (6,7).

Characterization of surface properties of LTI carbons has been rather limited. Baier, utilizing contact angles and infrared spectroscopy (9, 10),and Schoen, utilizing scanning electron microscopy (11), have studied such surfaces before and after blood exposure. Epstein et al. have utilized electrochemical methods to study the LTI carbon-aqueous solution interface (12, 13).

Extensive measurements have been made on the bulk, composition and structure of LTI carbons and experiments with modified surfaces have been reported (15). Direct measurement of the composition of LTI carbon surfaces by modern surface analytical tools however are not available. Here we report Auger and X-ray photoelectron spectroscopy studies of such surfaces. It is expected that such data, coupled with electro-chemical and protein adsorption data, may aid in understanding why LTI carbon is relatively blood compatible.

## Materials and Methods

Unalloyed LTI carbon samples were prepared in the conventional manner (14). For the photoelectron studies, three lots of samples (A, B, C) from the same coating run were prepared. Specimens from a different coating run (Lot D) were used for Auger studies.

Lot A was placed in clean glass vials immediately after coating and examined without any subsequent handling or treatment. Lot B was ultrasonically cleaned in isopropyl alcohol, air dried, and stored in glass vials prior to study. Lots C and D were ground and polished in several steps and contacted metal oxides, silicon carbide, diamond, water, detergent, isopropyl alcohol, and ethyl alcohol. The final cleaning was done in ethyl alcohol. The samples of Lot C were packaged in a soft blue foam and are representative of the surface which is normally delivered to medical device manufacturers for further processing. Lots A and B have very rough porous surfaces. Lot C and D are highly polished yet still shows some porosity and microscratches at 100X magnification.

X-ray photoelectron spectroscopy was done on a Hewlett-Packard 5950B ESCA utilizing monochromatic $A\ell K\alpha_{1,2}$ radiation. The samples were mounted in air, inserted into the spectrometer and analyzed in a $10^{-9}$ torr vacuum at ambient temperature utilizing 800 watts of x-ray power (at the x-ray anode, not at the sample). The instrument resolution was normally 0.8 eV measured as the full width at half maximum of the C-1s line from graphite. All spectra are charge referenced to the C-1s line at 284.0 eV. Wide scans (0 to 600 eV) were performed for surface elemental analyses as well as detailed 20 eV scans of the C-1s (275 to 295 eV) and O-1s (520 to 540 eV) regions. The spectra were not resolution "enhanced" or curve resolved.

Auger electron spectroscopy was done on a Physical Electronics Industries Auger microprobe Model 541. Surface analyses were carried out at $10^{-9}$ torr. Depth profiling was performed using the in-situ ion beam sputtering gun at $10^{-5}$ torr. Scan speeds used were on the order of 2-5 eV per second. The primary beam voltage was generally maintained at 3KV while the modulating voltage was 3V at low energies (<600 eV) and 6V for higher energies.

Please note that the ESCA and Auger studies were not obtained on identical lots. Auger studies were performed at the General Atomic Co. in Oct. 1976, the ESCA studies were done at the University of Utah in February, 1977.

## Results and Conclusions

Lot A had a highly porous surface with carbon particulate matter on the surface. The surface composition as determined by ESCA is given in Table 1. Note the low oxygen content, which appears to be part of a carbon-oxygen bond. No other elements are evident on the surface.

Lot B had finer carbon particulates on the surface but otherwise is identical to A, including the ESCA results (Table 1).

Ab-Hlady-1

**A.** DIVISION OF

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Time Required for Presentation _____

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63rd ACS Coll. Surface Sci Symp.
ACS Seattle June 19-23, 1989

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THE DISTRIBUTION OF BINDING SITES ALONG THE WETTABILITY GRADIENT ON THE MODIFIED SILICA SURFACE. V. Hlady, Y.S. Lin and J. D. Andrade, Center for Biopolymers at Interfaces, Department of Bioengineering, University of Utah, Salt Lake City, Utah, 84112

The surfaces with some gradient-like varying characteristics are finding an increased use in protein adsorption studies. One of such surfaces is the silica surface with the wettability gradient which can be prepared by two-phase solution silanization using dichlorodimethylsilane (DDS). Although the contact angle of such surface changes smoothly from approx. 0 to 88 degree, the adsorption/desorption experiments done with some proteins show unexpected results at the middle portion of the gradient. Several methods of fluorescence spectroscopy were used to analyse the degree of heterogeneity of the binding sites distribution along the wettability gradient. The spectroscopic mapping of the gradient was achieved employing the total internal reflection fluorescence geometry combined with the CCD detection of the fluorescence emission of adsorbed fluorescent polarity probes.

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# PROTEIN ADSORPTION STUDIES USING SURFACES WITH A HYDROPHOBICITY GRADIENT

V. Hlady*, Y-S. Lin, W. Norde[1], C-G. Gölander[2] and J.D. Andrade,
Center for Biopolymers at Interfaces, University of Utah, Salt Lake City, UT
84112, USA, [1]Agricultural University, Wageningen, The Netherlands,
[2]Institute for Surface Chemistry, Box 5607, S-11486 Stockholm, Sweden,

## INTRODUCTION

A convenient way to systematically study how surface chemistry affects protein adsorption and protein surface-solution exchange is to utilize surfaces with gradient-like varying characteristics (1). We have reported on the adsorption of three plasma proteins from the single solutions and from the protein mixtures onto the wettability gradient surface (2). In this presentation we report on the experimental total internal reflection fluorescence adsorption study which enabled us to follow protein adsorption and desorption kinetics simultaneously along the wettability gradient dimension with < 1 sec resolution.

## EXPERIMENTAL

The instrument used to follow simultaneously protein adsorption kinetics along one linear dimension of the gradient surface is based on combination of total internal reflection fluorescence and computerized fluorescence detection using thermoelectrically-cooled charge-coupled device (CCD) camera. The schematics of the TIRF-CCD instrument is shown in Fig 1. The evanescently excited fluorescence is imaged on the slit of monochromator and dispersed on the face of CCD array in such way that its spatial information is fully conserved. With combination of "binning" of the signal across the CCD wavelength dimension and fluorescence "flat-fielding", a single profile of fluorescent emission from the gradient surface can be obtained in a fraction of a second.

## SELECTED RESULTS

Initial adsorption and desorption kinetics of several different proteins has been followed from the single protein solutions, or from the solution mixtures. Fig. 2 shows the three-dimensional plot of adsorption-desorption of immunoglobulin G (0.01 mg/ml, PBS buffer, pH 7.4, I = 0.19) onto wettability gradient surface from flowing solution. Several interesting features are evident from Fig 2: (a) the adsorbed amount of IgG is very low at hydrophilic side of the gradient surface and it increases at wetting transition region of the gradient, (b) at the hydrophobic side of the gradient the IgG adsorption shows an "overshoot". The 11 minutes desorption with PBS buffer smooths out the "overshoot" effect and final adsorption values reflect only the wettability profile of the gradient surface.

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2. C-G. Gölander, Y-S. Lin, V. Hlady and J.D. Andrade, Colloids and Surfaces, in press, 1989.
*On leave of absence from "Ruder Boskovic" Institute, POBox 1016, 41000 Zagreb, Yugoslavia.
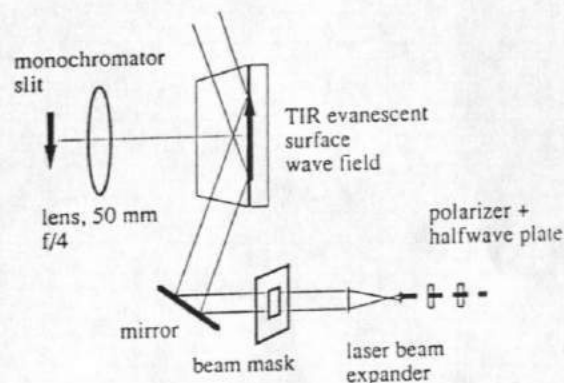
Figure 1. Schematics of total internal reflection fluorescence (TIRF) technique applied to study of protein adsorption on gradient surfaces
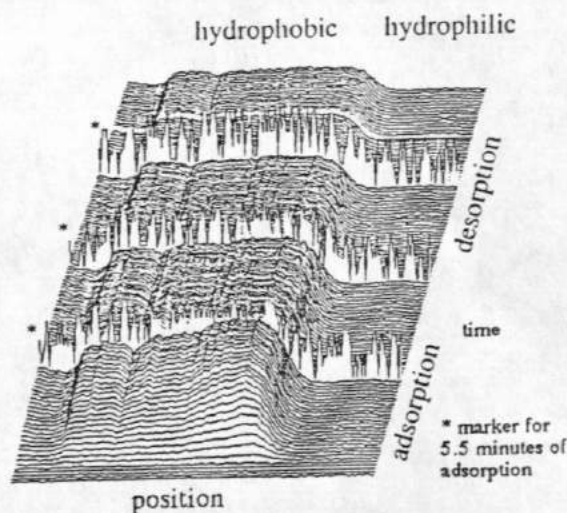


Figure 2. Time-resolved kinetics of IgG adsorption/desorption onto DDS gradient surface from flowing solution (0.01 mg/ml IgG, PBS, pH 7.4, I = 0.19). Fluorescence intensity profiles were taken every second, each tenth profile is actually shown. Markers represent 5.5 minutes. Desorption starts after 11 minutes of adsorption.

SPATIALLY-RESOLVED TOTAL INTERNAL REFLECTION FLUORESCENCE
METHOD APPLIED TO PROTEIN ADSORPTION KINETICS FROM COMPLEX
SOLUTION MIXTURES

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In the development of blood- and tissue compatible materials, there is a need
for increasing understanding of the detailed relationship between the nature of
the surface and its blood response. Any foreign material in contact with blood
initially responds by adsorbing plasma proteins. The structure and composition
of adsorbed protein layer is dynamic: proteins adsorb, desorb and exchange
with other proteins from blood. Even before the steady state composition of
adsorbed layer is reached, blood proteins replace each other in a well-defined
order; for example: albumin-IgG-fibrinogen-HMW kininogen. These phenomena
are commonly called the Vroman effect.

A convenient way to systematically study how surface chemistry affects
adsorption and protein exchange is to utilize surface compositional gradients.
We used wettability gradient surfaces and coupled them with total internal
reflection fluorescence and fluorescent labeling methods to permit the study of
the Vroman effect in the time range from seconds to minutes. The three most
abundant plasma proteins were studied: albumin, IgG and fibrinogen. Their
adsorption was studied from the single protein solution and from the protein
mixture solution. In each experiment only one of the three proteins was labeled
with fluorescein isothiocyanate and its adsorption/desorption kinetics was
determined from its surface fluorescence. Adsorption of fluorescein-labeled IgG
showed a profound influence on the wettability of the surface. At low protein
concentrations ranging from 1/1000 to 1/100 of respective plasma
concentrations the transient adsorption of IgG was restricted only to the
wettability transition region of the gradient surface and could not be observed at
either hydrophobic nor hydrophilic side. We found that both albumin and
fibrinogen are capable of exchanging with adsorbed IgG. Furthermore, we found
that the efficiency of the IgG-albumin and IgG-fibrinogen exchange process was
dependent on the chemistry of the surface.

*On leave of absence from "R. Boskovic" Institute, Zagreb, Yugoslavia.

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# 4th INTERNATIONAL CONFERENCE ON POLYMERS IN MEDICINE

## BIOMEDICAL AND PHARMACEUTICAL APPLICATIONS
Riva del Garda ITALY
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## ABSTRACTS

# UNIVERSITY OF UTAH
## Direct Imaging of Protein-Surface Interactions by SFM
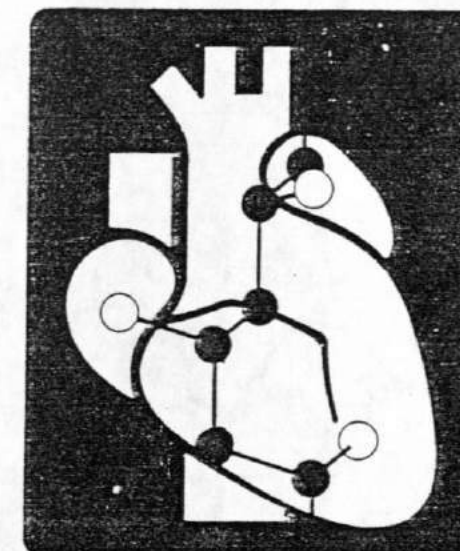
Within seconds after initial contact of blood with an artificial surface the over 200 different blood proteins begin to collide with and competitively adsorb onto the interface. Although there has been much progress in understanding and controlling protein interfacial activity, it is desirable to be able to observe protein-surface and protein-protein interactions directly with resolution approaching molecular protein dimensions. The advent of new scanning force microscopy (SFM) has encouraged us to design and perform experiments by which the dynamics of protein adsorption are observed directly, on a molecular scale, *in situ* and under physiologically relevant conditions.[1]

In the scanning probe microscope, one scans the probe mounted on a weak cantilever in the very close proximity of the sample. The probe react to the forces originating from the sample by deflecting the cantilever away or towards the sample. Scanning the probe over the sample by following the constant force, creates images which reflect the topographical features of the sample. The scanning can be easily performed in any liquid transparent medium.

We have performed a number of studies aimed at understanding limitations of the SFM methods and its applicability for direct imaging of processes involving blood proteins, platelets and surfaces of polymeric biomaterials. While studying the process of adsorption of immunoglobulins onto the atomically flat mica surfaces we observed the SFM-probe induced aggregation of IgG and denaturation of individual molecules of IgM.[2] In order to prevent the excessive loads experienced by the protein molecules on the sample we have modified the surfaces of the SFM probes by chemically grafting short chains of PEO. The presence of the PEO chains on the SFM probe introduces a new imaging mechanism based on steric repulsion. In this imaging mechanism any compression of PEO chains by the approaching sample will transduce the repulsive forces onto the cantilever deflection at the probe-sample distances where the ubiquitous van der Waals forces are negligible.

Currently, our efforts are focused towards:

- direct observation of protein adsorption from dilute plasma,
- imaging of soft polymer surfaces by a force modulation imaging method, and
- high resolution imaging of surface adhered platelets under solution and *in situ*.

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# Solution depletion and two dimensional gel electrophoresis - a method for the study of the competitive adsorption of plasma proteins

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## Introduction

When biomaterials are exposed to human plasma, there is often a problem of compatibility, because many plasma proteins are highly surface active. The recent recognition of the Vroman Effect has shown that plasma protein adsorption is a complex competitive adsorption process.

We have developed an experimental method for studying the competitive adsorption of plasma proteins by a solution depletion method. Two dimensional polyacrylamide gel electrophoresis (2-D PAGE) enables the detection and the identification of all plasma proteins at the same time. With the help of image analysis software, silver stained gels can be analysed and quantitated. By this method, we are able to study the competitive adsorption of plasma proteins.

In order to develop and verify this method, we have selected a well characterized model system for our initial studies. Plasma proteins usually contain different structural domains; each domain may adsorb differently on different surfaces. As heparin binding domains are present in many plasma proteins, we have chosen for our initial study the family of plasma proteins with heparin-binding domains. They are among the more important proteins in plasma with respect to coagulation and their specific binding functions and sequences are relatively well defined. These heparin binding proteins include : antithrombin III (ATIII), fibronectin (FN), vitronectin (VN), complement factor C3 (C3), histidine-rich glycoprotein (HRG), plasminogen (PMG), heparin cofactor II (HC II), and apolipoproteins (Apo-AI, Apo-AII, Apo-AIV, Apo-B, Apo-CI, Apo-CII, Apo-CIII, Apo-E).

## Materials and Methods

Human plasma is our protein sample. High surface area particles containing immobilized heparin made for affinity chromatography are used as the adsorbing bed. A solution depletion method was used to study protein - heparin interactions. Two different experiments were applied : (1) For time dependent adsorption measurements, the concentration of plasma solution and amount of heparin Sepharose are fixed while adsorption time is varied; (2) For the adsorption isotherm experiments, the concentration of the plasma solution and adsorption time are fixed while the amount of heparin-Sepharose is varied.

High resolution semi-quantitative 2-D PAGE is the analytical method applied. In the first dimension the proteins are separated according to their isoelectric point and in the second dimension they are separated by molecular weight.

After separation, high-sensitivity silver staining is employed for detection. Using a charge coupled device (CCD) camera (Photometrics LTD., Tucson, Arizona) interfaced with a Macintosh II to photograph the gel. The resulting image of individual protein optical densities is quantified by public domain analysis software - Image (National Institutes of Health).

## Result and discussion

Three important heparin binding plasma proteins were chosen for our study : ATIII, C3, and Apo-Al.

The time dependent adsorption results for ATIII and Apo-Al are shown in Figure 1. Figure 2 displays the isotherm.
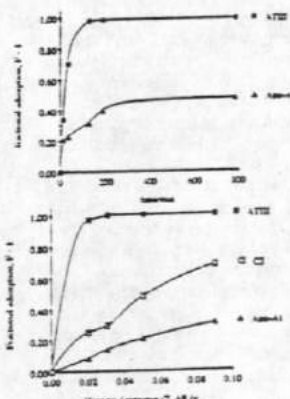


Fig. 1 ATIII and ApoAI time dependent adsorption. This plot shows the fraction of protein bound or depleted at different time intervals.



Fig. 2 ATIII, C3, and ApoAI adsorption isotherms. This plot shows the fraction of protein bound or depleted after adsorption on varing quantities of heparin Sepharose.

From the slope of the initial portion of the isotherm curve (Figure 2), the apparent binding constant, Ka, for the three important heparin binding proteins ATIII, C3 and Apo-Al can be calculated as presented is Table 1. The binding affinity for heparin is shown to increase in the order : ATIII > C3 > Apo-Al.

| Protein | Conc. in plasma | Apparent Ka |
|---------|-----------------|-------------|
| ATIII | $7.0 \times 10^{-6}$ M | $1.3 \times 10^{7}$ M$^{-1}$ |
| C3 | $6.5 \times 10^{-6}$ M | $0.8 \times 10^{5}$ M$^{-1}$ |
| Apo-Al | $3.6 \times 10^{-5}$ M | $0.2 \times 10^{5}$ M$^{-1}$ |

## Conclusions

-A subset of heparin binding plasma proteins have been mapped and identified on 2-D gels; other high concentration heparin binding proteins are now being mapped.

-A quantitative 2-D PAGE has been successfully used as a method to study the adsorption of plasma proteins.

-Preliminary results on three important heparin binding proteins (ATIII, C3, Apo-Al) suggest that the method is useful for the study of competitive protein adsorption. Kinetic as well as isotherm data are obtained.

-Binding constants of three important heparin binding proteins, ATIII, C3, and Apo-Al, are in the range of $10^{5}$ to $10^{7}$ M$^{-1}$

-Studies using other high surface area materials are now in progress.

## Acknowledgements

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MONDAY

The as received Lot C shows a much higher oxygen concentration and substantial quantities of silicon (Table 1). Other elements present in the surface include traces of sulphur, phosphorous, chlorine and possibly aluminum.

Lot C prewashed in methanol shows no evidence of silicon. The carbon/oxygen ratio is ca. 10:1. Traces of chlorine were present on the surface. The fact that the silicon lines were readily removed by a brief ultrasonic methanol wash suggests a surface contaminant as the source of silicon, perhaps a silicone release agent. The only likely source of such a contaminant is the blue foam in which the samples were packaged. ESCA examination of the foam revealed relatively high concentrations of silicon on the surface as well as nitrogen and chlorine suggesting a polyurethane foam with a silicone anti-stick or release agent and traces of possible NaCl from handling. Thus we tentatively identify the silicon on the as received Lot C as silicone material transferred from the foam packing material. Studies of methanol-cleaned Lot C material (Silicon-free) contacted with the foam confirmed that the silicon (silicone) on the foam readily transfers to the carbon.

Auger analysis of Lot D confirm the ESCA results obtained on C. Oxygen was found on the surface at a level on the order of 10 atomic percent. A trace amount (< 1 percent) of sorbed nitrogen was also generally detected. Depth profiling showed that both the oxygen and nitrogen are found only on the surface. At a depth of approximately 30-50 Å into the carbon, the oxygen signal had dropped by a factor of 10; nitrogen was no longer detected. Since Lot D was not packaged for shipment, these specimens did not contact the polyurethane foam.

Table I

Surface Elemental Ratios for Various LTI Carbon Samples

C:O:N:Si Ratio (Normalized to 100 Carbon Atoms)

| LTI Lot Code | Ratio |
|---|---|
| A | 100:1.8:0:0 |
| A | 100:1.2:0:0 |
| B | 100:1.4:0:0 |
| C | 100:8.5:0:1.9 |
| C | 100:11.0:1.0:1.5 |
| C "Methanol Cleaned" | 100:8.2:0:0 |
| Foam Packing Material | 100:39.3:2.8:12.3 |
| D | 100:10: <1:0 |
| D (50Å) | 100:1:0:0 |

These initial results from direct measurements of the composition of LTI surfaces confirm previous conclusions (15). The history of a biomaterial surface is important. Variability in test surfaces can be unintentionally produced by very subtle means. The fact that the impurities found on the LTI carbon surfaces could be readily removed shows that the mat-erial is inert but leaves open the question in the case of less inert materials. Furthermore, the role of tightly bound surface impurities in activating the clotting mechanisms should be investigated. Similar studies on alloyed LTI carbons will be performed in the future.

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We have produced three dimensional gels of hydroxyethyl methacrylate (HEMA) on a number of polymer substrates by $Co^{60}$ radiation-induced grafting and also by several chemical techniques. The resulting surfaces have significantly increased blood compatibility and reduced blood platelet depletion in studies performed by our group. We have also produced such gels on an activated carbon substrate. The system has been shown to be an efficient adsorber of undesirable molecular wastes in blood while showing minimal blood damage in many studies done in our lab on sheep.

Another method of hydrophilic gel formation is protein insolubilization. This has been used as a standard biochemical technique,[5] but has only recently been applied to the biocompatibility problem. Crosslinked gelatin and some modified collagen materials have been shown to be biocompatible;[6] an adsorbed protein layer on hydrophobic surfaces has been shown to be biocompatible[7] and produced virtually no platelet adhesion or clotting.

We have produced protein gels with controlled thickness of up to 15 micro $gm/cm^2$ covalently bonded to various substrates by several mechanisms. Cyanogen bromide has been used to activate cellulosic substrates to which albumin has been covalently linked through a well-known cross-linking agent, glutaraldehyde. Certain reinforced dialysis membranes with covalently linked protein by this method have been shown to retain diffusion characteristics.

A technique using $\gamma$-aminopropyltriethoxysilane has been used successfully to bind albumin to hydroxylated substrates such as polydimethyl siloxane, soda lime glass and $TaO_2$. The surface reacts with antialbumin, indicating that its antigenicity has not been drastically altered. Hydrophilicity as measured by contact angle can be controlled and biocompatibility has been increased. Cross-linked proteins are more resistant to proteolytic enzyme attack and appear to be stable up to 30 days in buffered saline at room temperature.

The rationale of the hydrophilic interface suggests reasons for observed compatibility. A neutral gel interface having a high water content should not adsorb compounds from aqueous solutions, should have minimal force asymmetry due to water cohesion, and therefore should not be recognized as a true interface.

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acts as a solid ground for proteins to anchor, fortifying their association with the surface.

Strong interactions between carbon and aromatic groups that are often buried inside the protein help produce protein unfolding (denaturation). The hydrophobic interaction between LTIC and proteins may be partly due to this charge-transfer interaction.

Our information indicates that there is a very strong interaction between the LTIC surface and the adsorbed protein. Thus the strong interaction hypothesis is considered more realistic and merits further investigation.

In summary, since LTIC is covered by plasma proteins, its surface properties are the result of this proteinaceous film. The function of the carbon substrate is to dictate the composition, structure, and stability of the proteinaceous layer.

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2

# COMPETITIVE PROTEIN ADSORPTION ON SOLID SURFACES STUDIED BY TWO DIMENSIONAL ELECTROPHORESIS

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## INTRODUCTION

The composition of adsorbed plasma proteins on a blood contacting material plays a crucial role in the blood compatibility of the material. To study such materials, it is important to know the surface composition, a result of competitive adsorption of plasma protein from blood. However, successful measurements of each individual adsorbed protein from plasma by conventional means are tedious and time-consuming because there are hundreds of different proteins in plasma. Using depletion methods, we studied the composition adsorbed proteins on two solid surfaces by two dimensional polyacryamide gel electrophoresis (2D PAGE), a technique that can simultaneously detect many proteins from a mixture qualitatively and semi-quantitatively [1,2].

## METHODS

Two-D PAGE separates a mixture of proteins first by their isoelectric points and then by the sizes of their molecular chains. Separated protein chains were revealed by silver stain and quantitated by computer densitometry. Two kinds of solid powders were used for depletion: silica and carbon (low temperature isotropic), a coating material for heart valves. The adsorbents were incubated with PBS diluted plasma. We ran 2D PAGE on both control and depleted samples so that the depletion percentage could be estimated for each detectable plasma protein.

## RESULTS

Depletion of plasma proteins[a]

| Plasma protein | Relative amount[b] | Depletion, % carbon[c] | Depletion, % Silica[d] |
|---|---|---|---|
| albumin | 100 | 15 | 21 |
| α1 acid glycoprotein | 6 | 53 | 31 |
| α1 antichymotrypson | 8 | 45 | 46 |
| antithrombin III | 9 | 29 | 31 |
| apolipoprotein A I | 21 | 29 | 91 |
| apolipoprotein A II | 4 | 50 | 98 |
| apolipoprotein A IV | 2 | 48 | 100 |
| apolipoprotein C III | 1 | 100 | 100 |
| apolipoprotein E | 3 | 100 | 98 |
| C4 | 1 | 81 | 100 |
| fibrinogen | 12 | 99 | 90 |
| Gc globulin | 5 | 56 | 34 |
| α B glycoprotein | 3 | 43 | 31 |
| G4 glycoprotein | 3 | 64 | 100 |
| α2 HS glycoprotein | 8 | 100 | 92 |
| haptoglobin | 64 | 36 | 29 |
| hemopexin | 22 | 59 | 12 |
| immunoglobulin | 44 | 46 | 30 |
| α1 macroglobulin | 4 | 97 | 100 |
| transferrin | 37 | 27 | 17 |

a) incubation in 1/30 diluted plasma overnight.
b) from control samples, showing the relative abundance.
c) 25 mg in 1.7 ml solution.
d) 330 mg in 1.7 ml solution.

## DISCUSSION

The patterns of plasma protein depletion are surprisingly similar on carbon and silica, which are very different in surface structures and properties. It implies that the protein depletion may involve multi-layer adsorption. In general, proteins with low plasma concentrations are more prone to depletion than the more abundant ones in term of depletion percentage. The first category includes apolipoprotein A II, apolipoprotein E, G4 glycoprotein, and α1 macroglobulin. The second one consists of albumin, haptoglobin, immunoglobulin, and transferrin. From this general trend, we consider that the depletion properties are partially determined by their biological functions.

It is probably more instructive to study the proteins with moderate plasma concentrations. Their depletion percentage is either high (fibrinogen or α2 HS glycoprotein), or low (α1 acid glycoprotein). There seems to be no correlation between their depletion properties and their molecular parameters, such as molecular weight, net charge, or carbohydrate content. However, analysis of their solution properties indicates that the degree of depletion for a particular protein is determined by its solubility more than by the type of surface. An important conclusion is that when interpreting the adsorption properties of a protein, one should consider its solution properties.

A few proteins show opposite depletion properties towards carbon and silica, such as hemopexin and apolipoprotein A I. Such surface dependent depletion is worth further investigation, particularly the relationship between their molecular structures and their adsorption properties.

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## ACKNOWLEDGEMENT

## S.Hjertén[*]

### The design of an agarose bed for high-performance hydrophobic-interaction chromatography

In hydrophobic-interaction chromatography (HIC) as well as in ion-exchange chromatography and many other types of chromatography it is often desirable that the separations are based only on interactions with the ligands, i.e., the matrix itself should be inert. Gel matrices of agarose used in conventional low pressure chromatography often fulfil this requirement. However, to be useful for HPLC the agarose beads must be rigid enough to withstand pressures up to at least 50 bar in order to permit the high flow rates typical for HPLC. This rigidity can be accomplished by crosslinking the agarose chains. Unfortunately, most crosslinking methods make the agarose beads relatively hydrophobic, which - as stated above - is less desirable. We will describe a new crosslinking method which does not have this disadvantage. Attachment of non-polar ligands to the crosslinked agarose beads by a recently introduced method gives an amphiphilic bed with good chromatographic properties: no other interactions than those based on hydrophobic bonds with the ligand have been observed; the interaction is not time-dependent, i.e., the appearance of a chromatogram is not affected by the residence time of the proteins on the column; high resolution can be obtained not only by gradient- but also by isocratic elution. The necessary conditions for high resolution upon isocratic fractionation of macromolecules by HIC and other chromatographic separation methods will be presented.

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## E. Hochuli and H. Döbeli

### α-Alkyl nitrilotriacetic acid adsorbents, a new type of metal chelating gel selective for proteins with neighbouring histidine residues

Porath et al. [1] demonstrated that immobilized cations as $Cu^{2+}$ or $Zn^{2+}$ are very useful for the recovery of proteins. The chelating ligands iminodiacetic acid (IDA) and tris (carboxymethyl) ethylene diamine (TED) have been bound covalently to oxirane-activated agarose and the resulting gels charged with metal ions. These affinity resins have since been used successfully for the purification of many proteins.

When chelated on the tridentate IDA resin, $Cu^{2+}$ and $Zn^{2+}$ ions leave only one coordination site free for interactions with biopolymers, whereas $Ni^{++}$ ions do three. However, $Ni^{++}$ ions are not bound tightly enough and releasing of metal ions is observed during chromatography. The pentadentate TED resin binds metal ions with coor-

dination number six very strongly. but again only one coordination site remains for biopolymer interactions.

The new α-alkyl nitrilotriacetic acid (NTA) adsorbent, synthesized from lysine and bromoacetic acid. is a quadridentate chelating resin.

$$Agarose\text{-}OCH_2CH(OH)CH_2NH(CH_2)_4\text{-}CH(COOH)N(CH_2COO^-)_2Ni^{2+}$$

This stationary ligand occupies four positions in the metal sphere of $Ni^{2+}$, remaining still two for protein interactions.

The new NTA resin was tested on the specificity for many proteins and peptides having neighbouring histidine residues. Thus lactate dehydrogenase isoenzymes, the pig muscle enzyme having three, the heart enzyme no His on the surface, were separated. In an other experiment the model peptide His-His-Leu-Gly-Gly-Ala-Lys-Gln-Ala-Gly-Asp-Val having two His in position 1 and 2 is eluted from the chromatography column after the peptide Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Ser having three His in position 6, 9 and 13.

From many such specificity experiments it was concluded, that the new metal chelating resin charged with $Ni^{2+}$ ions is specific for a His-His affinity tail. With this knowledge a "genetic designed" purification procedure [2]. where the His-His affinity tail together with a specific cleavage site is fused by molecular biological methods to a protein of interest, was developed.

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## D. Horseley, A. Wei, J. Herron, J.D. Andrade and V. Hlady

### Simulation of protein Adsorption: Computer Graphics, Surface Tension and Solution Denaturation Studies of Lysozyme

Total internal reflection intrinsic fluorescence (TIRIF) spectroscopy and molecular graphics have been applied to study the adsorption behavior of two lysozymes (EC 3.2.1.17) on a set of three model surfaces. A recently devised TIRIF quantitation scheme was used to determine adsorption isotherms of both hen egg-white lysozyme (HEWL) and human milk lysozyme on three model surfaces. This preliminary study suggests that the adsorption of the two lysozymes are significantly different. Molecular graphics was used to rationalize the adsorption results from TIRIF in terms of proteins' surface hydrophobic/hydrophilic character [1].

*[handwritten: Biol. Chem. 368 (7) 1987 p.748-9 Oberammergau 8-17→21-1987]*

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These data are being correlated against solution denaturation and air/water surface tension data.

We suggest that data on the solution denaturation of proteins may be important in estimating protein lability and stability and, together with information on the surface tension and interfacial tension behavior of proteins, will help develop hypotheses and correlations with the actual solid/liquid interface behavior [2].

We suggest means to model and simulate the adsorption of simple proteins at model interfaces.

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## Robert Huber[*]

### Flexibility and Rigidity in Protein-Protein Protein-Pigment and Protein-Ligand Interaction

Proteins may be rigid or flexible to various degrees as required for optimal function. Flexibility at the level of amino acid side chains occurs universally and is important for binding and catalysis. Flexibility of large parts of a protein which rearrange or move are particularly interesting [1]. We differentiate between certain categories of large-scale flexibility although the boundaries between them are diffuse: Flexibility of peptide segments, domain motions and order-disorder transitions of spatially contiguous regions. The domains may be flexibly linked to allow rather unrestricted motion or the motion may be constrained to certain modes. The polypeptide segments linking the domains show characteristic structural features. The various categories of flexibility will be illustrated with the following examples: a) Small protein proteinase inhibitors which are rather rigid molecules and provide binding surfaces complementary to their cognate proteases but also show limited segmental flexibility and adaptation [2]. b) Large plasma inhibitors which exhibit large conformational changes upon interaction with proteases probably for regulatory purposes. [3] c) Pancreatic serine proteases which employ a disorder-order transition of their activation domain as a means to regulate enzymic activity. [4] d) Immunoglobulins in which rather unrestricted and also hinged domain motions occur in different parts of the molecule probably to allow binding to antigens in different arrangements. [5] e) Citrate synthase, which adopts open and closed forms by a hinged domain motion to bind substrates and release products and to perform the catalytic condensation reaction respectively. [6] f) Riboflavin synthase, a bifunctional multienzyme complex in which two enzymes (α and β) catalyse two consecutive enzymic reactions. The β-subunits form a shell in which the α-subunits are enclosed. Diffusional motion of the catalytic intermediates is therefore restricted. In

addition segmental rearrangement occurs in the assembly of the β-subunit. [7] In contrast rigidity is the dominant impression provided by the recent structures of the light harvesting complexes [8] and the reaction centres [9] involved in the photosynthetic light reactions. These are large protein pigment complexes in which the proteins serve as matrices to hold the pigments in the appropriate conformation and relative arrangement. Since motion would contribute to desactivation of the photo-excited states of the pigments and diminish the efficiency of light energy and electron transfer a functional role for reduced flexibility is easy to rationalize for these proteins.

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## J. R. Hunter, R. Z. Guzman, R. G. Carbonell, and P. K. Kilpatrick

*[handwritten: wrote]*

### Adsorption of Proteins at Gas-Liquid Interfaces

Proteins adsorb at gas-liquid interfaces in multiple layers, as evidenced by the radiotracer and ellipsometric experiments of Graham and Phillips (1). We have developed a kinetic model of protein adsorption isotherms and adsorption kinetics which incorporates multilayer adsorption, a dependence of activation energy of adsorption and desorption on surface concentration, and mass transfer effects in the bulk solution. The model successfully correlates experimental data of adsorption isotherms and adsorption rates for the three proteins β-casein, bovine serum albumin, and lysozyme. (EC 3.2.1.22).

*Abst: HSeang-1: Multi-Element analysis - 1995*

**A. TITLE OF PAPER**

Protocol Development For Quantitative Multi-Element Analysis Of Biological Samples By Inductively Coupled Plasma-Mass Spectrometry

**B. AUTHORS**
underline name of speaker

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Joseph D. Andrade
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**D. POSTER/ORAL**
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☒ Oral 5 Minutes required for presentation.
Symposium _____

**E. SPECIAL EQUIPMENT** _____

**F. ABSTRACT** (200 words or equivalent). Title of paper should be ALL CAPS; authors listed by first name, middle initial, last name; indicate full address with zip code. Single space with black carbon ribbon.

PROTOCOL DEVELOPMENT FOR QUANTITATIVE MULTI-ELEMENT ANALYSIS OF BIOLOGICAL SAMPLES BY INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY, Chiung-Sheng Hsiung, Joseph D. Andrade. K. Owen Ash, ARUP, 500 Chipeta Way, Salt Lake City, UT 84108

During the last decade, inductively coupled plasma-mass spectrometry (ICP-MS) has emerged as a promising and versatile means for trace element analysis in biological specimens. Our goal is to determine conditions for simultaneous analysis of multiple elements in biological specimens. High salt content in biological samples results in matrix induced signal suppression, polyatomic interference and changes in ion stabilities and detection limits. Using a Perkin-Elmer SCIEX ELAN 5000 ICP-MS, we investigated the feasibility of chemical modification with ethanol while varying two instrument parameters:

- Nebulizer gas flow rate----15 flow rate intervals of 0.025 liters/minute.
- Radio frequency power----1.0 to 1.4 Kilowatts at 100 Watt intervals.

Ion signal variations were assessed for more than 39 elements spiked in water. Optimized conditions for gas flow and radio frequency power produced patterns which were mass dependent. We confirmed reported findings that ethanol increases sensitivity for some element, while lowering gas flow requirements and increasing power requirements. These findings provide a base for continued development of simultaneous multi-elements analysis in biological specimens.

**G.** Mail the original and 2 copies directly to the symposium chair.

# OPTIMIZATION OF ICP-MS FOR QUANTITATIVE MULTI-ELEMENT ANALYSIS OF BIOLOGICAL SPECIMENS.

Chiung-Sheng Hsiung, Joseph D. Andrade, Department of Materials Science & Engineering, University of Utah, Salt Lake City, UT 84112, and K. Owen Ash, ARUP, Chipeta Way, Salt Lake City, UT 84108

The simultaneous determination of elements in biological specimens is increasingly crucial to the operation of a clinical chemistry laboratory. To accomplish this goal demands a versatile and reliable technique. Since the first launch of commercial ICP-MS instrumentation in 1984, this hybrid technique has emerged as a promising method of rapid multi-element analysis with superior detection limits. Yet, in a number of applications of biological specimens, the quadrupole-type ICP-MS suffers both spectroscopic and non-spectroscopic interferences. There are a number of strategies for reducing interferences and enhancing signals. Addition of an organic solvent to the analyzed samples is an easy, low-cost, and effective method.

Using a Perkin-Elmer Sciex Elan 5000 ICP-MS, equipped with pneumatic nebulizer, platinum sampler and skimmer cones, and argon plasma, we evaluated the feasibility of ethanol chemical modification using a systematic optimization procedure. Moreover, we also investigated the direct multi-element analysis of biological samples while varying two instrument parameters, nebulizer flow rate and rf power.

Maximum optimum signals, for a wide range of elements, were a function of atomic mass. The higher the mass of the element, the lower the rf power for maximum intensity. At a particular power, the optimum nebulizer flow rate for each element shifted regularly as the atomic mass changed. However, the power patterns and the nebulizer flow-rate patterns permitted an optimization of ICP-MS parameters for simultaneous analysis of multiple elements.

The power patterns, comparing optimum signals and interferences at different powers (1.0 kW-1.4 kW), suggested that the medium power range may be the better candidate for multi-element analysis. By analyzing the patterns of signals at a range of varied nebulizer flows, the nebulizer flow rate can be optimized using a multiple element standard covering wide atomic mass. We confirmed that ethanol chemical modification increased the optimized power and decreased the optimized nebulizer flow rate. The ICP-MS parameters should be optimized individually under ethanol conditions other than those optimized for non-ethanol samples. Otherwise, the effects of ethanol chemical modification may be obscured due to improper instrument operating parameters. Analyses of analytes with low ionization efficiency or heavy interferences were improved by ethanol due to signal enhancements and reduction of polyatomic overlap and matrix effects. These investigations provide further support for simultaneous multi-element analysis of biological specimens by ICP-MS.

THE STRUCTURE AND PHYSICAL PROPERTIES OF

PYROLYZED POLYIMIDE

by

Chen-Ze Hu

A dissertation submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Material Sciences and Engineering

The University of Utah

December 1986

# ABSTRACT

Polyimide was pyrolyzed in an argon atmosphere at various temperatures and times, and thermally converted to amorphous carbon films. The irreversible evolution of polyimide under progressive heat treatment is characterized by three successive structural changes: pyrolysis, carbonization and graphitization.

The polyimide starts to dissociate at pyrolysis temperatures above 500°C. At temperatures higher than 650°C most functional groups of polyimide decompose to evolve $CO$, $CO_2$, $H_2$ and $N_2$ gases which are released from the sample. The polyimide then gradually becomes more carbon-rich. It is believed that at pyrolysis temperatures higher than 650°C the polyimide starts to form heterocyclic structures with some oxygen and nitrogen incorporated into the heterocyclic carbon rings. The structure rearranges or recombines to form larger and denser heterocyclic networks at temperature higher than 700°C, causing an increase of electrical conductivity. This conclusion is supported by the ESCA and conductivity data. Raman, ESCA valence band and X-ray data indicate that the polyimide at pyrolysis temperatures less than 1000°C is amorphous carbon and no long term periodic structure can be detected. But at pyrolysis temperatures higher than 2000°C, the polyimide is converted to microcrystalline graphite.

Two forms of polyimide were used in this work: Kapton, a fully cured polyimide film, and PI-2525, an uncured polyimide solution. The Kapton film was placed between two quartz plates during pyrolysis; the resulting polyimide is flat, has uniform conductivity, has high chemical resistance, and shows better mechanical strength than Kapton pyrolyzed in free standing conditions. The PI-2525 was spin-cast on different substrates and cured at 200°C. The pyrolyzed PI-2525 polyimide was supported by the substrate.
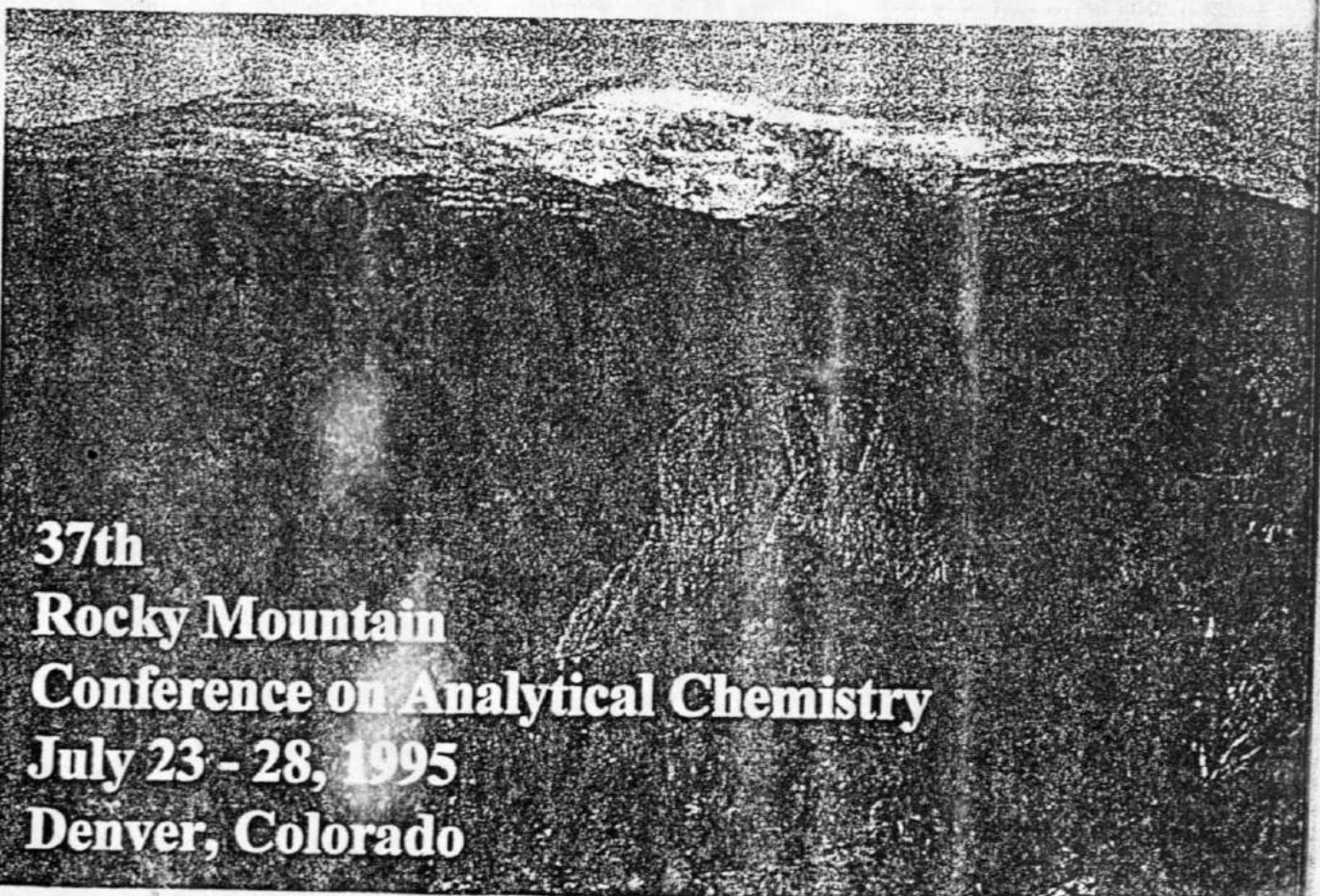
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photo by Jeffery Cornell

**WX5  Waveguide evanescent streak excitation of surface deposited dye monolayer fluorescence**

J. T. IVES, W. M. REICHERT, J. D. ANDRADE, and P. A. SUCI, U. Utah, Bioengineering Department, Salt Lake City, UT 84112.

The fluorescence emission from cyanine dye–arachidic acid monolayers Langmuir-Blodgett deposited onto 1–3 $\mu$m poly(styrene) waveguides was investigated. 488-nm light from an argon-ion laser is coupled into the waveguide as a guided mode using high-index LaSF$_5$ prisms. The evanescent field at the waveguide surface excites the dye fluorescence (emission maximum = 505 nm) which appears superimposed on the Raman spectrum of the poly(styrene) film. The sample size and excitation volume at the polymer surface was varied by increasing the monolayer coverage and through careful mode selection, respectively. Independent variation of these two parameters on the same waveguide provided a method to investigate the evanescent field intensity and effective depth of penetration. Emission spectra demonstrate an intensity increase proportional to the number of monolayers and the concentration of dye molecules. Emission variations due to mode selection are dependent on the number of monolayers and the interfacial amplitude and evanescent decay of the particular mode. We are analyzing the results with multilayer theory calculations and waveguide energy measurements using the Stokes and anti-Stokes Raman peaks of the poly(styrene) waveguide. This method is being developed for biomedical investigations of protein adsorption onto polymer surfaces.　　　　(12 min)

**WX6  Calculation of the number of guided transverse surface acoustic modes for integrated acoustooptical devices**

G. GOLAN, G. GRIFFEL, S. RUSCHIN, A. SEIDMAN, and N. CROITORU, Tel Aviv U., Faculty of Engineering, Ramat Aviv 69978, Israel.

The analogy between the electromagnetic wave equation and the wave equation for the propagation of acoustic waves is used to drive the cutoff lines and the number of guided transverse surface acoustic (Love) modes in stratified piezoelectric media. The combination of these two kinds of waves occurs in integrated acoustooptic devices where the upper layer is made by diffusion of metal atoms into a dielectric substrate. The efficiency of these types of device depends strongly on the overlapping integral of the light mode and the acoustic mode which propagate in the upper layer. In many applications it is important to design the structure parameters in such a way that only a single mode of each kind exists. The present theory describes simple-design tools which enable direct calculation of the proper thicknesses, indices of refraction, the piezoelectric constants of the structure, and the rf operating frequency range. A detailed computation is given for the cases of a two-layer and a buried layer structure.　　(12 min)

**WX7  Surface acoustic wave interaction with polarized light**

S. W. LI and E. BOURKOFF, Johns Hopkins U., Electrical Engineering & Computer Science Department, Baltimore, MD 21218.

It is of primary importance for acoustooptic signal processors to have high efficiency and wide bandwidth and, hence, enhanced dynamic range. In those devices employing the side entry technique, an unguided optical beam enters a substrate at grazing incidence. It is then totally internally reflected from the top surface on which a surface acoustic wave is propagating. As it propagates laterally through the substrate just beneath the surface, the optical beam is Bragg diffracted by the surface acoustic wave. Taking into account this particular interaction geometry, the Guassian nature of the optical beam and the decay of the surface acoustic wave, the interaction efficiency and incidence angular bandwidth for both $p$- and $s$-polarized light are calculated. The theoretical results are compared to experimental observation, which shows that for a YZ–LiNbO$_3$ substrate, $p$-polarized light has a higher diffraction efficiency over a wider incidence angular bandwidth than $s$-polarized light, a fact that can be used advantageously to enhance the dynamic range in these acoustooptic devices.　　　　(12 min)

**WX8  Coupled-mode theory of parallel waveguides**

AMOS HARDY and WILLIAM STREIFER, U. New Mexico, Electrical Engineering Department, Albuquerque, NM 87131.

A new coupled-mode formulation for parallel dielectric waveguides is described. The derivation includes radiation modes in addition to the individual guided modes and the coupling equations are obtained by requiring that the additional field be orthogonal to the guided modes. The results apply to any guided modes (TE, TM, or hybrid) in waveguides of arbitrary cross section, dissimilar index, and nonidentical shape. Additional index perturbations not included within the waveguides are encompassed by the theory. Propagation constants and mode patterns for the coupled modes computed according to this theory are shown to agree very well with numerical solutions for the system modes when the latter can be determined. Moreover, the new results are more accurate than those obtained from prior coupled-mode formulations. It is shown that even for lossless guides the coupling coefficient from waveguide $b$ to $a$ and from $a$ to $b$, described by $\kappa_{ab}$ and $\kappa_{ba}$, respectively, are not related by their complex conjugates if the guides are not identical. This fact does not violate energy conservation or reciprocity when the full expressions for the power flow are used. (12 min)

Wednesday　　　　　　　　**AFTERNOON**
16 October 1985　　　　　　　　**WY**
MONROE WEST

**4:00 PM  Surface Physics**

**Robert W. Boyd**, Presider

**WY1  Instabilities during laser melting of semiconductors**

J. LUSCOMBE, J. PRESTON, J. E. SIPE and H. M. VAN DRIEL, U. Toronto, Department of Physics, Toronto M5S 1A7, Canada.

We have studied, experimentally and theoretically, the liquid–solid morphologies[1,2] induced by the interaction of plane-polarized cw 10.6-$\mu$m laser radiation with silicon on a sapphire substrate. The experiments were performed at normal incidence, with silicon thicknesses ranging from 0.5 to 2 $\mu$m. Near the melt threshold, the large difference in optical properties of liquid and solid semiconductor drives an instability breaking the translational symmetry of the surface. The initial morphology of the nonuniform melting consists of lamellae structures, e.g., microscopic solid islands in a liquid background, which then evolve into a steady-state periodic arrangement of liquid and solid with increasing laser intensity. The structures have been studied using real-time microscopy and optical diffraction techniques. A key phenomenon in the evolution and maintenance of these surface structures is interference shielding, whereby existing liquid regions electromagnetically shield surrounding regions of solid, hindering further local melting. There is a strong competition in this process between the orientation of induced structure and the polarization of the incident field. A simple theory which exploits an analogy with the Ising model of disorder—order phase transitions is discussed.　　(12 min)

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**WY2  Paper withdrawn**

**WY3  Measurement of the optical damage threshold of wire grid polarizers at 10.6 $\mu$m**

M. MOHEBI, A. SAID, and M. J. SOILEAU, North Texas State U., Center for Applied Quantum Electronics, Department of Physics, Denton, TX 76203.

We report the results of a study of the laser-induced damage threshold of wire grid polarizers for pulsed laser radiation at 10.6 $\mu$m. The polarizers were wire grids of copper or silver deposited on ZnSe substrates and overcoated with an antireflection coating. The measurements were performed using linearly polarized, normal incident, 130-ns pulses from a hybrid CO$_2$ TEA laser. The laser was operated in the TEM$_{00}$ spatial mode and single-longitudinal mode with a pulse width of 130-ns (FWHM). The beam size at the sample was 0.36 mm (FW1/e$^2$M). Each site was irradiated only once (one on one measurement). A damage threshold of 0.8 J/cm$^2$ (6 MW/cm$^2$) was measured for the copper wire grid when the wires were parallel to the incident electric field (closed or low transmission condition). The damage threshold for the sample with silver wires was 2 J/cm$^2$ (15 MW/cm$^2$) for the same orientation. The damage threshold of both samples increased by more than a factor of 2 when the wires were oriented perpendicular to the incident electric field (the open or high transmission condition). Here we have defined the damage threshold as the lowest incident fluence (or irradiance) for which damage occurred.　　　　(12 min)

**WY4  Determination of loss mechanism in long-range surface plasmon modes**

RICHARD A. BOOMAN and DROR SARID, U. Arizona, Optical Sciences Center, Tucson, AZ 85721.

Long-range surface plasmon (LRSP) modes have been observed on very thin metal films sandwiched between two dielectrics[1] that can be excited using frustrated total internal reflection.[2] The intensity profile of the light in the reflected beam contains a structure caused by interference between the light reflected from the prism base and the light being reradiated by the mode. It is possible to determine the loss coefficient of the LRSP mode from this intensity profile, and if there is an interference zero in this profile, loss coefficients caused by dissipation and reradiation can also be determined.[3] A computer-controlled system that locates the plasmon resonance angle and also measures the reflectance vs angle and the intensity profile from a charge-coupled-device array was used for the data acquisition. We have measured the decay constants of several thin silver