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Your University Education

Opportunities and Responsibilities

Twentieth Annual Honors Convocation Lecture
University of Alabama at Birmingham
May 21, 1995

by Joseph D. Andrade
Professor of Bioengineering and Materials Science & Engineering
Co-Director, Center for Integrated Science Education
University of Utah

Thank you. Hello. ***Congratulations!*** You are here to receive honors and you will soon receive a degree. You are here to celebrate -- to enjoy your success. This is an *honors* convocation. I presume that means you are all intelligent, motivated, hard working, and have achieved some level of personal, individual excellence.

Many of you will continue your studies -- graduate school -- professional schools. Some of you will more fully enter the "real world." Some of you already know it -- though your own backgrounds, jobs, community service, student teaching or other means.

I've brought three "friends" to help me today. Although their statements may be a bit out of context, their words help make my point -- in an enjoyable and identifiable way.

Here's the first one:

> I believe the children are our future.
> Teach them well and let them lead the way;
> Show them all the beauty they possess inside.
> Give them a sense of pride.
> To make it easier,
> Let the children's laughter
> Remind us how we used to be.

"The Greatest Love of All"
The *Whitney Houston Album*

Whitney Houston -- my first "helper" today, is talking about *you.* You were the children -- now -- you are now educated adults. The future is now -- and is your responsibility. Your past is prologue. Whitney is also singing about your kids -- born and unborn -- and the futures they will have and make, and the kids of your neighbors and of the homeless.

The real world is different from the University. As the University of Alabama at Birmingham is mainly an urban school, many of you know that -- some of you don't -- and it may be a rude or unpleasant introduction.

Your university experience has, I hope, provided you with societal and historical perspective, with social and communication skills, with critical thinking skills, and with problem-solving skills. I hope it has provided you with self confidence and with sensitivity to the needs, qualities, potential, and aspirations of others -- of all components of society. And I hope it has provided you with a sense of ethics and values.

That real world is far from perfect. It has many problems and needs which we'd all prefer not to think about -- especially today: drugs, gangs, rape, crime, violence, racism, disease, dementia, AIDS, health care, poverty, homelessness, unemployment, debt, unwanted pregnancy, irresponsibility, and discrimination.

My "friend," Anne Murray, expresses it better than I can :

> I come home this evening
> Thinking the news will be the same
> Somebody takes a hostage --
> Somebody steals a plane.
> How I want to hear the anchorman talk about a county fair,
> And how we cleaned up the air,
> How everybody learned to care.
> Oh, tell me nobody was assassinated in the whole durn world today
> And in the streets of Ireland, all the children had to do was play
> And everybody loves everybody in the Ole USA
> We sure could use a little good news today!

"A Little Good News"
A Little Good News Album
©1983 Capitol Records, 4XT 12301.

You are a member of society, as is each of your parents, your professors, bosses, friends, colleagues. You will soon be conferred with a degree -- attesting to your special skills, education, perspective, and training. You must use them -- not only in your job or advanced studies, but in the conduct of your society and communities.

Direct some of your time and energy to help enhance the societies and communities in which you exist. That includes: the planet (its lands, ocean, air, and water -- the environment and the biosphere), the nation, the state, and of course your city and local community.

That does not mean only serving on a local, high-rent district school board or community council while perhaps ignoring the homeless, the unemployed, the disadvantaged, as you drive by their areas in a secure, comfortable, very *well-locked* expensive automobile.

You *must* be involved. Do not listen to those who say you can ignore these societal issues and concerns in order to focus on your graduate work, on medical school, or on your first key job. Sure you have to focus and build your careers, but please don't use such focus as an excuse for personal irresponsibility. Members of communities have responsibilities which can neither be ignored nor delegated. Those who advise you otherwise are themselves a key part of the problem -- the problem of personal irresponsibility which pervades our weakening democracy.

As an educated member of society *you* must insist on ethical and responsible behavior -- by the press and the media, by your teachers and professors, by your family and friends, by politicians, by business men, by lawyers -- by everyone. Do not let any of them lie, cheat, distort, mislead, even misquote -- you must be honest and critical with yourself and with them.

Part of your University education dealt with the development of critical thinking skills -- being able to separate fact from fiction, reality from fantasy -- to detect lies, distortions, fraud, and misrepresentation. Excuse my Western frankness, but I call it "crap detection." Your degree certifies you as an educated crap detector -- and believe me, your communities need you to utilize that skill.

If you are aware of lies, fraud, dishonesty -- and do not address it -- then you are an accomplice. Ignorance may be bliss -- but not for you. Your days of innocence and ignorance are over. Now, if you don't do something, it's not because you're ignorant or unaware -- it's because you're lazy and irresponsible.

Walter Lippmann, 75 years ago, said "There can be no liberty for a community which lacks the means by which to detect lies." You are the lie detectors -- detect and confront them. Neil Postman said that intelligence is mainly the capacity to grasp the truth of things.

You must have the guts, the self confidence, the societal responsibility to grasp the truth and to point out and expose non-truth.

You must also be supportive, positive, and uplifting. You must be leaders, visionaries, planners of your communities -- perhaps not immediately -- but in the very near future.

These are not chores and duties. They are challenges and opportunities.

I once heard a child, dying of cancer, say her major goal in life was "to leave the world better than I found it." Your goal can be no less. You do have the time and resources to improve and enhance the world.

Let me share a favorite quote from a not well known author:

> What does a man need -- really need? A few pounds of food each day, heat and shelter, six feet to lie down in -- and some form of working activity that will yield a sense of accomplishment. That's all -- in the material sense. And we know it. But we are brainwashed by our economic system until we end up in a tomb beneath a pyramid of time payments, mortgages, preposterous gadgetry, playthings that divert our attention from the sheer idiocy of the charade.
>
> The years thunder by. The dreams of youth grow dim where they lie caked in dust on the shelves of patience. Before we know it, the tomb is sealed.
>
> Sterling Hayden, *Wanderer*, Bantam Books, 1963, p. 23.

You cannot predict where the future will find you. You cannot predict how long you will live or how satisfying that life will be. but you can ask yourself -- right now -- openly and honestly -- What is really important? How can I make this world a better place?

Although life will be unpredictable, challenging, and exciting, the secret of a satisfying life is indeed in helping to make the world a better place. In the words of my third and final "helper" this afternoon, a fellow named James Taylor -- Enjoy that Ride!

Since we're only here for a little while
We might as well show some style
Give us a smile, now...
Isn't it a lovely ride?
Sliding down and gliding down, oh
Try not to try too hard
It's just a lovely ride.
Now the thing about Time is that Time isn't really real.
It's all on your point of view
How does it feel for you?
Einstein said he could never understand it all
Planets spinning through space
The smile upon your face.
Welcome to the human race.
Isn't that a lovely ride? Oh, yeah,
Sliding down and gliding down,
Try not to try too hard.
It's just a lovely ride!

from "The Secret o' Life"
James Taylor (Live)

Columbia C2T 47056, CT 57305

Thank you, and good luck.

APPLYING BIOLUMINESCENCE TO GENERAL SCIENCE EDUCATION: SCIENCE WITHOUT WALLS TELECOURSE

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Introduction

Bioluminescence is a nearly ideal subject with which to experience the scientific process and critical science concepts and themes.

We have developed bioluminescent dinoflagellate cultures which enable upper elementary and junior high teachers and students (1) to readily experience bioluminescence, closed ecosystems, circadian rhythms, protozoa and optics. Much of the experience is conducted in the dark. Science in the Dark has been an effective way to reduce science anxieties and fears and to encourage teachers to develop a fresh, positive and instructive attitude towards hands-on science in their classrooms.

These materials have now been included in a television based distance learning course: *Science Without Walls: Science in Your World* (2). We utilize bioluminescence as an effective way of imparting the scientific experience and method to television viewers throughout Utah.

Science Without Walls Telecourse

We have previously reported (1) our experience with a ten hour hands on inservice course for teachers titled Integrated Science Concepts and Themes. This course extensively utilized bioluminescence, particularly the dinoflagellate Pyrocystis lunula, as a unique experimental tool with which to develop scientific observation skills and provide the opportunity to formulate many different and specific scientific hypotheses. With such observational skills and hypotheses in hand, the students can move forward to design, conduct, and analyze simple experiments using only the Pyrocystis lunula cultures.

Bioluminescence readily connects to practically all of the basic concepts and themes developed in Project 2061 (3) (Figure 1) and used in Science Without Walls (2), (Figure 2). The course connects science with the arts and with the humanities and relies heavily on integrated science concepts and themes, philosophies which came out of the American Association for the Advancement of Science Project 2061 Report: *Science for All Americans*. (3) (Figure 1).

Figure 1. Basic Concepts & Themes, Traditional Disciplines, and Bioluminescence

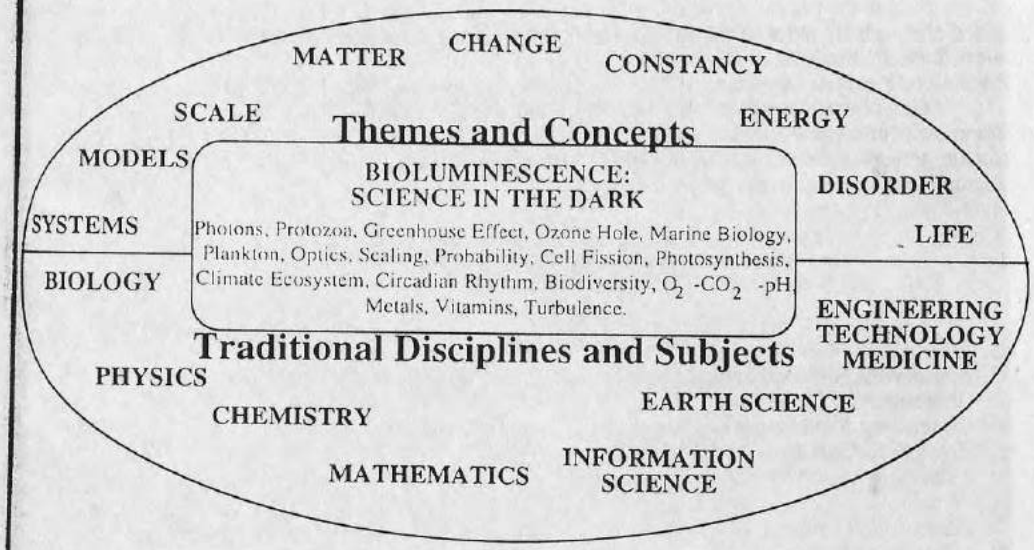
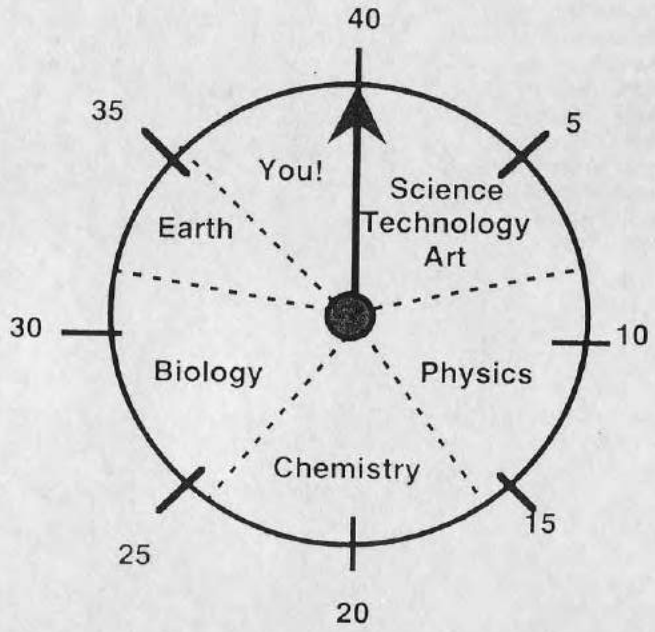


Figure 2. General Design of the Science Without Walls Telecourse



In order to minimize the teaching of science as a virtual subject rather than as a real subject, the course also involves a unique Lableless Lab, basically a science kit which all students are required to obtain and use to conduct weekly, semi-quantitative experiments. (4). This course took about 15 months to write and produce, and was launched October 1, 1996 as a telecourse for the University of Utah's Division of Continuing Education, aired through their local KULC Channel 9, a special television channel for Continuing Education/Distance Learning courses.

The course consists of forty half hour programs covering all of science in an integrated coherent fashion. (Table 1) It is designed primarily for non-science majors and for the general public and has as one of its major goals the empowerment of citizens and residents to become involved in public and political issues which may have a science or technological component.

Table 1. The forty half hour programs/topics in Science Without Walls: Bioluminescence is used mainly in Program 32 and parts of 27 and 28.

1: The World of Science-The World of Art
2: Observing And Perceiving: The Senses
3: Patterns And Numbers
4: Extending Your Senses
5: Integrated Concepts And Themes: Systems and Models
6: Scale
7: Constancy, Change, & Matter
8: Energy, Disorder & Life
9: Physicists In The Wild
10: Inertia, Gravity, & Senator Garn
11: Energy, Efficiency, Entropy
12: Interstate Physics
13: Action At A Distance
14: From Magnets To Electricity
15: From Electrons To Light
16: From Newton To Quanta
17: Chemists In the Wild
18 Your Personal Periodic Table
19: From Atoms to Molecules
20: From Metals To Water
21: From Water To Solutions
22: Molecular Alchemy
23: Very Personal Chemistry
24: Guns And Bombs
25: Biologists In The Wild
26: What is Life?--Diversity And Extinction
27: What Is Life?--The Very Early Days
28: What Is Life?--From Bacteria to You
29: Energy In: Fuel & Light
30: Energy Out: Biomass and Work
31: Information In: The Senses
32: Information Out: Language
33: Your Brain And Consciousness
34: Is There Intelligent Life on Earth?
35: Planetary Medicine: Gaia
36: Your Stuff: Cars And Transportation
37: Luck And Risk: Personal Statistics
38: Medicine & Health--Yours
39: Creativity--Yours
40: Where Do We Go From Here?

The Future

We are developing programs (going beyond Program 38) where we discuss health care, encouraging the student to be interested not only in clean and healthy living styles, but also in functioning as their physician's assistant, to help serve as eyes and ears, as an information gathering source, to aid health care practitioners in their efforts in diagnosing and treating the student's ailments.

Acknowledgments

Most of our work on the development of bioluminescence for science education has been funded by Protein Solutions, Inc., Salt Lake City, UT, USA. Our courses for teachers on Integrated Science Concepts and Themes based on bioluminescence were funded by the U.S. Department of Education, Eisenhower Grant Funds, administered by the Utah State Board of Regents.

Bioluminescence footage was provided by Edi Widder, James Morin, and Protein Solutions, Inc., Salt Lake City. The overall telecourse project was funded by the State of Utah Higher Education Technology Initiative and by the University of Utah, including its Center for Integrated Science Education.

We thank all of those who provided help, assistance, advice, footage, visuals, ideas, and support.

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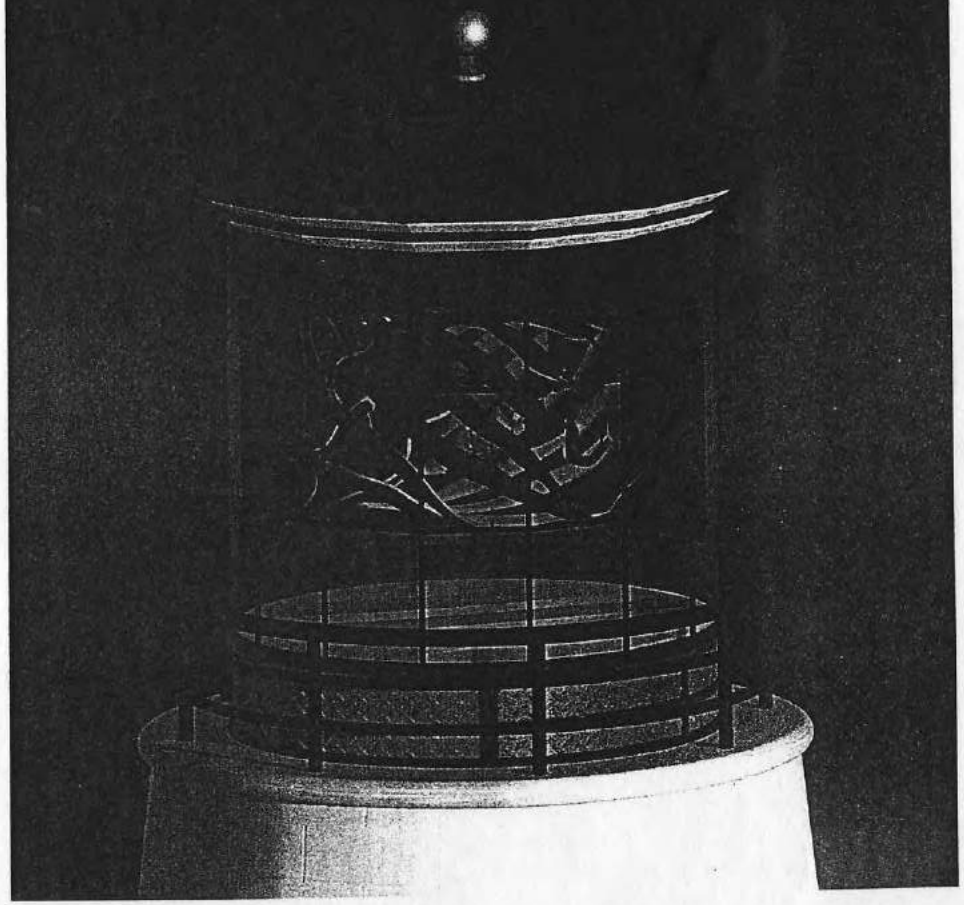
BIOLUMINESCENCE and CHEMILUMINESCENCE

Molecular Reporting with Photons



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DIRECT READING BIOSENSORS: ANALYTICAL CHEMISTRY WITHOUT INSTRUMENTS

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Introduction

Health care cost concerns in many nations are driving a growing interest in point of care-based technologies for screening, diagnosis, and even treatment.[1,4,5] Innovations and enhanced technologies in meter-less chemical analysis devices, employing immobilized and dry reagents, make it possible for individual patients to monitor their own glucose, cholesterol, pregnancy hormone, and other parameters.

There is growing need for devices which can use non-invasively derived samples, particularly urine and saliva. There is an evolving trend in encouraging and empowering consumers and potential patients with greater education, awareness, and responsibility for their own health care.[1] This has lead to a recent proliferation in home medical, self diagnosis, computer packages. These products attest to the growing interest in a the public becoming more involved in assuming more responsibility for their own education and health care.

There is also a growing trend throughout the world in interactive, hands-on science and technology centers/museums, in which human health and physiology are a very major and a very popular component. These centers provide a means for the typical citizen to not only monitor physical parameters related to their physiology, but chemical ones as well. Over the next 10-20 years, such experience is expected to lead to a segment of the population that is more interested and involved in their own health care, and insistent on the availability of materials and technologies which will permit such involvement.

In the area of therapeutic drugs and drugs of abuse, there are already major initiatives in most nations which have led to simple screening tests and devices for monitoring such drugs, or their metabolytes, in blood, urine, saliva, sweat, and hair.

We are embarked on projects to research and develop consumer friendly, dipstick-type devices applicable to non-invasively derived fluids for education, analytical, and potential diagnostic usage.

Rationale

There are two very special molecules that play unique and central roles in biology: adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH) and its phosphate form (NADPH), a ubiquitous electron donor. ATP is generally recognized as the energy currency in biology.[3] The two molecules are closely coupled in many biochemical processes and can be regenerated or recharged. "They are the basic coupling agents of cellular metabolism."[3] A very large number of biochemical enzyme processes involve one of these two molecules.

It is very fortuitous that biology has evolved two bioluminescent processes dependent on these two molecules: the firefly luciferase reaction, which acts on firefly luciferin in the presence of ATP to produce an oxidized product which chemiluminesces.[2,6] The bacterial luciferase reaction, which in the presence of alkyl aldehydes, and $FMNH_2$, produced by an NAD(P)H reaction, also produces an excited chemiluminescent product which chemiluminesces.[2] Both reactions produce photons with high efficiencies in the presence of oxygen. However, both the luciferases and luciferins involved are chemically different.[2]

There is a large body of literature on the development of biosensors for ATP and ATP-dependent processes and for NADPH and NADPH-dependent processes, using the firefly and bacterial luciferase enzymes, respectively. Such biosensors generally employ fiberoptic or other wave guided means of delivering the luminescence to a device which can accurately measure light intensities. Although one of the most portable and most sensitive photon detectors available to the scientist or physician is his or her own eye, it is notoriously difficult to calibrate for accurate measurements of even relative light intensity. The human two dimensional photon detection system, however, can reliably and accurately measure changes in spatial position.

We are using the human eye's spatial detection capabilities as the readout system, focusing on analysis of carbohydrates and other key molecules using ATP-dependent kinase-based, phosphorylation reactions.

Sensor Components

An ATP specific sensor, based on firefly luminescence requires several critical components and technologies.[8] The firefly luciferase enzyme is critical because it provides the specificity for ATP. The enzyme can be, and has been genetically engineered, and otherwise modified, to enhance its purification, its immobilization, and its stability.[8]

Luciferin is normally produced by synthetic means, is available from a wide range of sources, and is relatively expensive. Luciferase, luciferin, and even ATP require various stabilization and protection technologies and enhancements for a practical sensor.

Typical dry reagent technology-based sensors incorporat the critical reagents in or on various support materials, including cellulose and gels. Our work involves entrapping recombinant luciferase in low melting agaroses containing carbohydrate additives which facilitate their complete dehydration and later rehydration[8]. Such a sensor is specific and sensitive for ATP.

The major application of such ATP analysis is in hygiene monitoring, the detection of small quantities of bacteria or other cells, primarily in the food, dairy, and food processing industries.[6] The surface or device of interest is sampled with a swab, the collected cells transferred to a reagent cocktail, which releases intracellular ATP.

"Rapid hygiene monitoring" methods are growing rapidly due to the growing need in minimizing bacterial contamination in many industries. This is a generic biomass detection technology; it does not speciate, that is, it does not indicate whether the bacteria detected are indeed pathogenic. The method serves as a monitor for cleanliness and hygiene, that is, as a routine screening technique. A very wide range of products and methods, largely based on bacterial culture and defined media, are available for speciation analysis.

Our group is working on dry reagent, dipstick-based, highly sensitive sensors for ATP-based hygiene monitoring, using simple photographic film detection.

It is perhaps surprising that there has not been more interest in using the exquisite imaging photon detector, which practically all of us have, the human eye. The eye is so beautifully accommodating, adaptable, and auto-ranging, that it is a notoriously bad detector of photon intensity, the basic signal in practically all fluorescence and luminescence-based analytical devices. But the human eye is ideally suited for the detection of images or patterns.

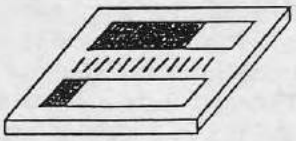
We have developed a set of technologies which allows ATP concentration to be measured by the spatial position of the bioluminescence, permitting a quantitative detector designed and optimized for human visual detection.

Analytes

Most analytes can be measured or monitored by a variety of methods. A good example is glucose.[4] There are at least 6 different ways to analyze glucose using biosensors.[4] One glucose analysis pathway is to react it with ATP in the presence of hexokinase, or even more specific enzymes, to produce glucose phosphate. The consumption of ATP due to the phosphorylation of glucose is a direct measure of glucose concentration, hence, a glucose sensor based on ATP-specific bioluminescence.

Admittedly, this is not new, but its implementation in a biosensor with the characteristics noted (Figure 1), coupled with direct visual detection, serves as a demonstration for the more widepread application of enzyme and substrate specific analysis based on ATP consumption or production. This method lends itself to the development of sensors for practically all mono-, di-, and poly-saccharides.

Such sensors will make it possible to enhance research and diagnosis in a wide range of problems and pathologies related to metabolism and bioenergetics, obviating the requirement for generally more expensive and time consuming standard analytical methods, often based on gas and liquid chromatography.



Dipstick-Dry Reagent Personal Sensors:

Characteristics:

Direct Reading
Disposable
Inexpensive
Ultra Sensitive
Quantitative
Wide Dynamic Range
Rapid
Stable

Figure 1. Sensor Characteristics

Conclusions

This is a report of work in progress. The sensing technologies and devices discussed are not yet commercially available - or available for extended testing. At this stage they are laboratory prototypes undergoing much more extensive test and evaluation

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3

Poly(ethylene oxide) and Protein Resistance

Principles, Problems, and Possibilities

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Poly(ethylene oxide) (PEO)-based protein-resistant surfaces function principally by a steric exclusion mechanism involving very high surface mobility and surface dynamics of the PEO chains. For such a surface to be effective, the dynamics and mobility of the chain must be maximized and, contradictorily, the underlying surface must be entirely covered by the PEO chains. Because of geometric constraints, these criteria are optimally met on highly curved surfaces; PEO probably cannot be used to make ideally flat surfaces as optimally protein-resistant as surfaces with low radii of curvature. A curved surface simply has more room for end-attached polymer chains than a flat surface.

SURFACES RESISTANT TO PROTEIN ADSORPTION AND CELL ADHESION are needed, particularly in the health care product and biotechnology industries. Although much has been done in the preparation, characterization, and even application of poly(ethylene oxide) (PEO) surfaces over the last 15 years, controversy in the field is considerable, and most of the key scientific questions are still open. A volume edited by J. M. Harris provides a concise, up-to-date, authoritative presentation of the field (*1*).

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ADVANCES IN CHEMISTRY SERIES 248

Hydrophilic Polymers
Performance with Environmental Acceptance

J. Edward Glass, EDITOR
North Dakota State University

Developed from a symposium sponsored by the Division of Polymeric Materials: Science and Engineering, Inc., at the 206th National Meeting of the American Chemical Society, Chicago, Illinois, August 22–27, 1993

American Chemical Society, Washington, DC 1996

Background

PEO and poly(ethylene glycol) (PEG) are used for a wide variety of interface engineering applications. Higher-molecular-weight PEO is widely used to stabilize aqueous colloids and dispersions, generally by means of physical adsorption followed by steric repulsion of the modified particles. Lower-molecular-weight PEG, roughly in the 1000 to 4000-Da range, is commonly used as a prepolymer in the synthesis of polyurethanes, epoxies, silicones, and other polymers. Low-molecular-weight PEG can also be readily coupled to hydrophobic chains to make a wide variety of nonionic surfactants that are widely used in the chemical industry, biochemistry, and the biotechnology industry (*1*).

Polymerized ethylene oxide is a somewhat anomalous molecule. It is both hydrophilic and hydrophobic, because it is soluble in both aqueous and nonpolar solvents. In solution it tends to be highly dynamic, and yet it can readily pack and form crystalline solids. Despite its dynamics and mobility, it can complex and aggregate, develop specific helical and near-helical conformations, and interact and complex with a variety of ionic and hydrogen-bonding structures. PEO, as a molecule alone and as part of other molecules, is generally nontoxic and is considered safe for a wide variety of cosmetic, food, and biomedical applications. PEO and its derivatives are readily available in a range of purities and molecular weights and are relatively inexpensive and easy to obtain. Here we focus on interface modification by PEO and PEO-based polymers and the optimization of the protein resistance of such surfaces.

The heterogeneity and dynamics characteristic of proteins are also characteristic of many solid surfaces, particularly those of synthetic polymers (*2*). Hydrophobic solid surfaces may be relatively homogeneous, as in poly(dimethylsiloxane), or very heterogeneous, as in semicrystalline polyethylene or block polyurethanes. All polymer surfaces are highly heterogeneous because of the sizes of polymer molecules, which are of the same order as the sizes of the individual protein molecules. Because of steric exclusion and a tendency to satisfy entropic concerns, polymer chains tend not to interpenetrate very effectively, and this failure further enhances the macromolecular granularity of polymeric surfaces. Polymers have a range of molecular dynamics and molecular relaxation processes, including the glass transition and side chain relaxations, that further contribute to the complexity of such interfaces. The time scales of relaxation processes for polymers are of the same order as those for proteins, which is not surprising, because both polymers and proteins are macromolecules. Although the terminology is different, the mechanisms and processes are basically the same (*3*).

The dynamics of both the protein and the polymer and the wide

repertoire of intermolecular interactions possible between protein and polymeric surfaces lead to the conclusion that most proteins adsorb on most interfaces (*4*). Proteins are polymeric surfactants and are not only adsorbed but are also generally conformationally altered as a result of interfacial activity (*2*). The major interest in PEO surfaces is therefore in finding a way to minimize or eliminate the tendency for protein adsorption. This problem can be almost completely avoided by developing protein-resistant surfaces or interface-resistant proteins.

Protein-Resistant Surfaces

Minimizing protein adsorption requires some knowledge and understanding of the structures of proteins (*5*) and their interfacial behavior (*2, 4, 6*).

Regardless of whether the underlying substrate is highly hydrophobic, highly ionic, or highly hydrogen bonding, the protein has regions on its surface that can indeed interact with the substrate (*5, 6*). The protein itself has loops, tails, helices, and sheets that can make their way through the PEO layer and interact with the substrate below. A variety of bridging, pinning, and related processes can then further complicate the problem (*7*).

The major interactions that drive the interfacial activity and adsorption of proteins are the water structure-driven hydrophobic effect, electrostatic interactions, and strong hydrogen-bonding interactions characterized by cooperative, multiple hydrogen bonds. A typical isolated hydrogen bond does not play much of a role in aqueous solutions, because that bond is largely satisfied by interactions with the 55 M water that is present. Isolated, random hydrogen bonds are generally unimportant. They become important when a multiplicity of such bonds, either acceptors or donors, occurs and the complementary component is on the other surface. Multiple hydrogen bonds that match up in space to form a cooperatively interacting structure consisting of 3 to 5 or more hydrogen-bonding units can be a strong, effective means of interfacial adhesion, as they are in biorecognition. Such matching requires multiple bonds *and* stereo complementarity.

That neutral, highly hydrophilic polymers tend to have minimal or very weak interactions with most aqueous proteins has been well known for over half a century. The development of the dextrans (Sephadex) and the agaroses (Sepharose) for protein chromatography and electrophoresis demonstrates that such matrices have relatively weak protein interactions. These interactions are weak because the matrices are generally nonionic, thereby minimizing electrostatic interactions, and highly water soluble and hydrophilic, thereby minimizing hydrophobic interactions. Although the gels and surfaces produced by such

polymers are extensively hydrogen bonded, they tend to be highly dynamic and random; therefore, cooperative hydrogen-bonding processes are not generally a problem. However, some proteins are indeed retained on such gels, often because of hydrogen-bonding interactions and in some cases because of residual charge or a hydrophobic character.

A neural, highly hydrophilic polymer that is also very dynamic at the surface has another mechanism by which to minimize protein interactions. By being neutral and hydrophilic, the polymer has already minimized enthalpic interactions, but by also being highly dynamic, the polymer has provided the interface with a high entropy. Any process that tends to decrease or minimize this interface entropy, such as by decreasing the dynamics or mobility of the polymer chains at the interface, will be unfavorable from a free-energy perspective. Adsorption on such a surface will therefore pay a high free-energy penalty, which must generally be paid in enthalpic currency. If no enthalpic interactions are available, such surfaces are said to be repulsive by an entropic, surface dynamics mechanism. This mechanism can also be related to steric exclusion and osmotic pressure. These processes have been extensively modeled and discussed by de Gennes (*8*) and are now being widely applied in the biomaterials/biotechnology community (*1*, *6*).

Figure 1 presents a schematic but comprehensive summary of PEO surfaces.

Figure 1a represents low-molecular-weight PEO (1000 to 4000 Da)

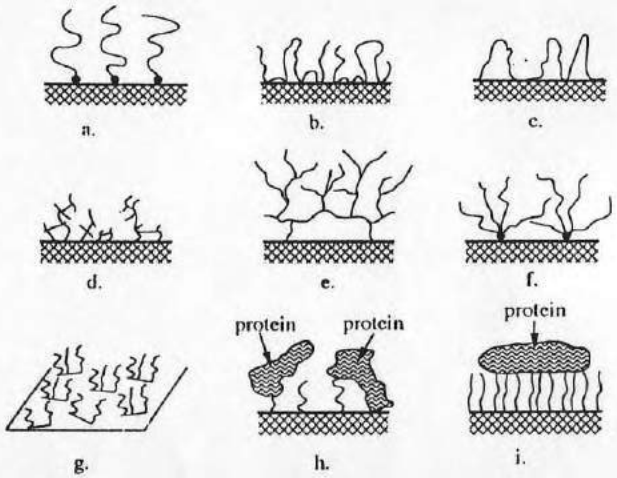

Figure 1. Some of the many structures and configurations that have been suggested for PEO and PEO-derived polymers attached to surfaces. See *text for details.*

tethered at one end to a particular surface. A wide variety of surface modification technologies and PEO derivatives are available for such surface modification. That the chains extend into solution as indicated is highly unlikely. In most studies, achieving a very high density of chains on the surface is difficult.

Figure 1b is the common illustration for high-molecular-weight PEO adsorbed onto particles or other surfaces. Here the very high molecular weight and the highly cooperative nature of polymer segmental adsorption lead to loops, tails, and trains that have been extensively characterized and modeled. The loops and tails provide a means of steric repulsion between two particles containing adsorbed PEO, although the dynamics of adsorption can clearly also lead to bridging and thus to colloidal aggregation rather than stabilization. Figure 1b also illustrates the adsorption of PEO block copolymer surfactants; an adsorbable block pins the molecule to the surface and the PEO block (loops or chains) extends into solution (*9*). Another variation is a graft copolymer, for example, with PEG chains on a hydrophobic backbone, which results in adsorption at a hydrophobic surface and PEG chains extending into solution (*10*).

Figure 1c represents a PEO chain bound to the surface, by both ends; that is, it forms a loop. This structure may occur in many types of block copolymers containing PEO block segments. It may also be part of many PEO surface modification reactions in which the PEO reagent is homobifunctional rather than heterobifunctional, as is required for the ideal situation in Figure 1a.

Figure 1d represents ethylene oxide attached to an activated surface and a PEO-like network growing out from the surface (*11*). It could also represent the plasma polymerization of ethylene oxide films (*12*). Such a film would be expected to be highly cross-linked and much less dynamic than the others indicated.

Figure 1e is an example of so-called surface amplification in which PEO is tethered to multifunctional entities such as carbohydrates or polysaccharides, which in turn are tethered to the surface (*13*). Although in principle this amplification leads to a much larger number of binding sites per unit area for the PEO chains, in practice the steric constraints imposed by the mobility and steric repulsion characteristics of PEO probably limit the number of binding sites to the same extent as in Figure 1a.

Figure 1f represents another version of surface amplification: the star polymer geometry. This polymer could be thought of as a sort of hybrid between those in Figure 1b and 1e in which a nucleus, often containing a multihydroxyl carbohydrate, is used to grow ethylene oxide chains from each reactive functional group, thereby producing a PEO star. The center or base of the star can then be appropriately

attached to a surface, or the entire process can be initiated from the surface (*11*). This structure is also reminiscent of the Tetronic family of polymeric surfactants, in which PEG chains extend from four poly(propylene oxide) chains attached to a tetrafunctional nucleus (*9*).

Figure 1g represents a block or graft copolymer designed for optimum adsorption (*10*) that is surface cross-linked between the chains or between the polymer blocks and the surface either by specific cross-linking reactions or by plasma reactions (*14*).

As Figure 1h shows, PEG chains are often used to provide a tether between a protein or other biomolecule and the surface (*15*). This approach is being widely applied in biosensors in which an antibody must function as if it were in solution and yet be tethered within several hundred angstroms of an interface to provide a means of transducing a binding event into a signal (*16*). The covalently coupled protein sitting on the end of a dynamic and mobile chain, however, has extensive mobility and dynamics of its own and will interact with the underlying substrate unless the surface is exceptionally well covered and passivated by PEO or some other means.

If we could prepare a maximally dense PEO surface, we might have a packed "crystal" of PEO that would then adsorb proteins, as Figure 1i shows. Such a surface would of course not be mobile or dynamic and would not sterically or entropically exclude or resist protein adsorption.

Although the reactions in Figures 1e and 1f have the advantage of leading to a very well covered surface and avoiding the potential problems of a bare substrate, there may well turn out to be little difference between the reactions in Figures 1a, 1e, 1f, and possibly even 1d because the excluded volumes of the chains themselves prevent a very high local concentration of PEO. If this excluded volume is decreased by solution "tricks" (*17*), then after equilibration in water, the final surface will probably be less mobile and less dynamic than is required for optimum protein resistance.

Another concern with Figure 1 is that we have assumed a particular surface structure that is homogeneous, that is, not patchy. We have little evidence to indicate that such homogeneity is indeed the case. Thus the problem is even more complex than is sketched in Figure 1.

Clearly, *the surface must be fully covered by PEO to minimize protein interaction* with the underlying surface. However, if the surface is "overcovered," as in Figure 1i, then the surface becomes adsorptive.

It is, therefore, not surprising that even crude, simplistic models of hypothetical spherical proteins interacting with ideal PEO brush surfaces suggest that protein resistance is a function of protein radius,

PEO molecular weight, and the number of PEO chains per unit area on the surface (*18*).

With all this complexity, one might ask, Why PEO? Why not consider other approaches to the passivation of surfaces with respect to protein adsorption? Protein-resistant surfaces tend to be neutral, thereby minimizing electrostatic interactions, and highly hydrophilic, thereby minimizing hydrophobic interactions (*2, 4*). Of all the neutral, hydrophilic, water-soluble/swellable polymers readily available, PEO appears to be the most mobile, the most dynamic, and the least interactive (*6, 9, 10, 18*).

What are the disadvantages to PEO? The long-term stability of PEO on a surface is somewhat questionable; that is, it may be susceptible to local oxidation processes. The fact that PEO may weakly complex with proteins, particularly charged proteins, as it does with certain types of charged polymers, is also of some concern (*19*). PEO also has a tendency to form weak complexes with certain ions, particularly potassium. In fact, PEG has been called a "poor man's crown ether" (*20, 21*). Nevertheless, of all the polymers we know, PEO appears to have the highest potential for the development of truly protein-resistant surfaces (*1, 6, 22*).

A very major factor in this potential is the way in which the hydrophilic polymer chains interact with water. Although PEO solutions do not behave as ideal solutes and certainly do perturb the structure of water somewhat, they are apparently the least perturbing of all of the common neutral hydrophilic polymers. Although the nonbonding oxygen orbitals in PEO provide hydrogen-bonding capacity and indeed are largely responsible for the solubility of the molecule, this hydrogen bonding requirement is easily satisfied by water without significant perturbation of the structure of water (*22, 23*). A lack of significant perturbation in the structure and the fact that the ethyl moieties in the PEO chain are largely accommodated by the water structure minimize hydrophobic interactions. These two facts suggest that PEO indeed has minimal interactions with other solutes in aqueous solutions. In addition, the PEO chain is highly mobile and dynamic, thereby creating an entropic "insurance" that can more than compensate for any weak attractions that may be present (*18*). The end result is a weak or sometimes quite strong repulsive interaction between proteins and many types of PEO surfaces that results in very low protein adsorption. This interaction is what we define as protein resistance.

Direct measurements of the steric repulsion between PEO surfaces (*24, 25*) and between a PEO surface and a protein surface (*26*) are now available, thanks to the surface forces apparatus (*25, 26*). Direct measurement of steric exclusion and the imaging of surfaces via steric exclusion were accomplished in our group by atomic force microscopy

(*24*). Prime and Whitesides (*27*) recently presented a study of the adsorption of four different proteins on oligo(ethylene oxide) self-assembled monolayers with various oligo(ethylene oxide) surface concentrations. The protein resistance was roughly proportional to increasing surface coverage and increasing oligo(ethylene oxide) molecular weight.

Conclusions and Summary

The ideas presented in this discussion are not without controversy and criticism. Many studies in the literature argue that PEO surfaces are not particularly biocompatible. Other studies argue that if a PEO surface is resistant to one protein, it may not be very resistant to another protein (*6*, *18*). Is there a specificity to PEO's protein resistance? Others argue that PEO surfaces may not be stable and in time may be degraded or otherwise deteriorated and thereby lose their passivity or protein resistance (*11–13*).

PEO-based protein-resistant surfaces function principally by a steric exclusion mechanism involving very high surface mobility and surface dynamics of the PEO chains. For such a surface to be effective, the dynamics and mobility of the chain must be maximized and, contradictorily, the underlying surface must be entirely covered by the PEO chains. Because of geometric constraints, these criteria are optimally met on highly curved surfaces; ideally flat surfaces probably cannot be made as optimally protein-resistant with PEO as surfaces with low radii of curvature. A curved surface simply has more room for end-attached polymer chains than a flat surface.

Acknowledgments

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INTERFACIAL PHENOMENA AND BIOPRODUCTS

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2

Proteins at Interfaces: Principles, Problems, and Potential

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I. INTRODUCTION

The principles of protein adsorption have been presented in a number of monographs, review papers, and conference proceedings [1–10].

We will discuss protein adsorption by referring to the complexity axis concept (Fig. 1). The complexity of the protein is represented by one qualitative axis, ranging from relatively simple globular proteins (insulin, myoglobin, lysozyme) to very complex multidomain proteins (lipoproteins, fibronectin, and fibrinogen). Multicomponent protein solutions (blood plasma and tears) are also represented [9]. The surface or interface on which these proteins may act is also considered in terms of complexity, represented on the horizontal axis.

One usually thinks of the air/water interface as the simplest interface, followed in complexity by model lipid/water interfaces and liquid/liquid interfaces. One can then consider more complex polymer/water interfaces and, finally, complex solid surfaces, such as block copolyurethanes. As protein and interfacial complexity increase, the complexity of the interfacial interactions also increases. We will discuss these interactions in terms of the complexity, heterogeneity, and dynamics of the proteins and the surfaces.

II. PROTEINS AT SIMPLE INTERFACES

Figure 2 summarizes much of what we think we know about protein adsorption [6]. Consider a kinetic model for the adsorption of a single protein onto a model surface. The arrival of protein at the interface is assumed to be driven solely by diffusion processes, dependent on bulk concentration and diffusion coefficient, producing a collision rate. The surface chemistry of the protein and the surface

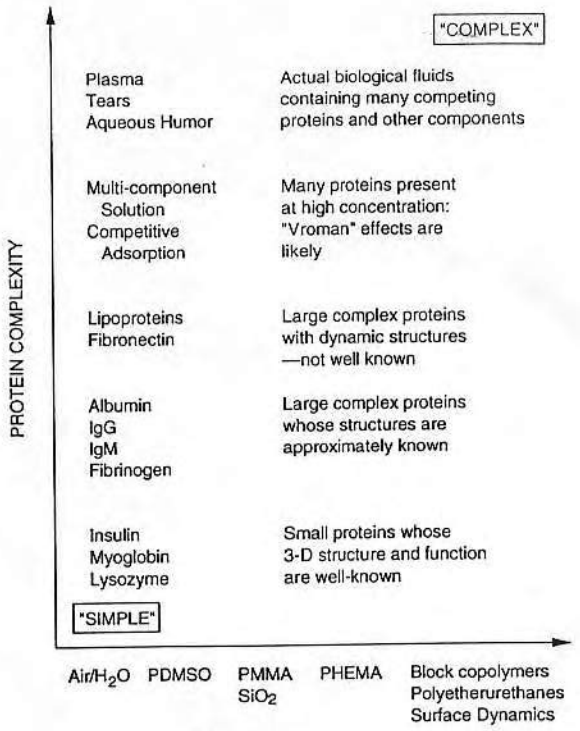

Figure 1 A protein adsorption complexity matrix. The lower left corner represents simple proteins at simple interfaces; the upper right represents complex proteins at complex interfaces. Protein complexity increases on the vertical axis, and surface or interface complexity increases on the horizontal axis. (See text for details; based on Refs. 9 and 71.)

determine the residence time due to the initial interaction energy. The dynamics or denaturability of the protein itself, together with its residence time, probably controls the surface denaturability of the protein. We assume that the protein denatures with time at the interface, represented by a rate constant. With increasing residence time, denaturation reaches a maximum. With increasing denaturation, the interaction energy in the adsorbed state is increased, and the probability for desorption, or the rate of desorption, is decreased. This is all illustrated in Figure 2 [4–6].

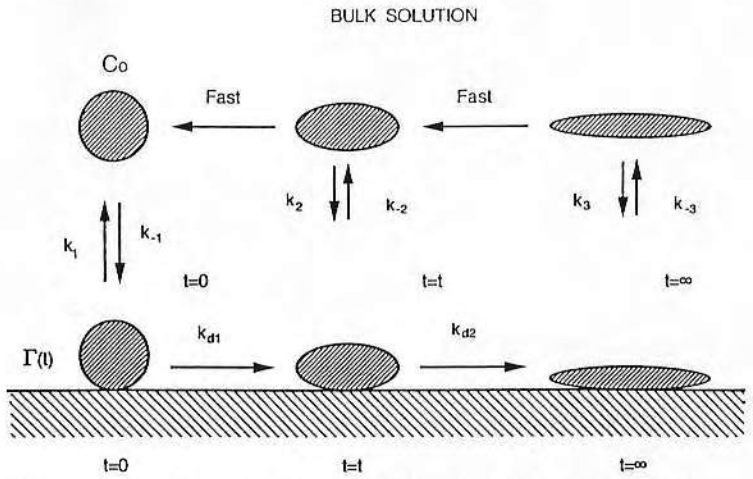

Figure 2 A general kinetic model for protein adsorption (based on Refs. 9 and 71).

The reality of the process is that proteins are not homogeneous particles. Not all collisions are equally effective in adsorption, and different protein surfaces, or faces, result in differing interaction energies with the protein, and, therefore, differing tendencies for surface denaturation [6,8–10].

Figure 3 presents a set of hypothetical proteins that we use for discussion and educational purposes: *anisotropin*, *domainin*, and *cooperatin*. Each can be considered a model system, helping us to illustrate, discover, and understand certain characteristics of real protein interfacial behavior. Each is described in the extensive caption to Figure 3. Although these model proteins "exist" in only two dimensions, they will be useful to illustrate many of the key concepts.

Anisotropin has four very different nonequivalent faces or surfaces; each can interact at the interface by a different mechanism. Molecular graphical images of the three-dimensional structure of lysozyme, myoglobin, and ribonuclease show such protein surface heterogeneity [20]. Thus, anisotropin is a crude model for such small, globular proteins. *Domainin* represents an ultrasimplified multidomain protein, such as activated fibrinogen. We have given generic characteristics to each of the major domains. In reality, *each* of the domains would be anisotropin-like, with its own array of heterogeneity and different faces. Thus, the heterogeneity of *anisotropin* is multiplied many times over in *domainin* by virtue of the different domains in the protein and the potential for multidomain interactions at the interface.

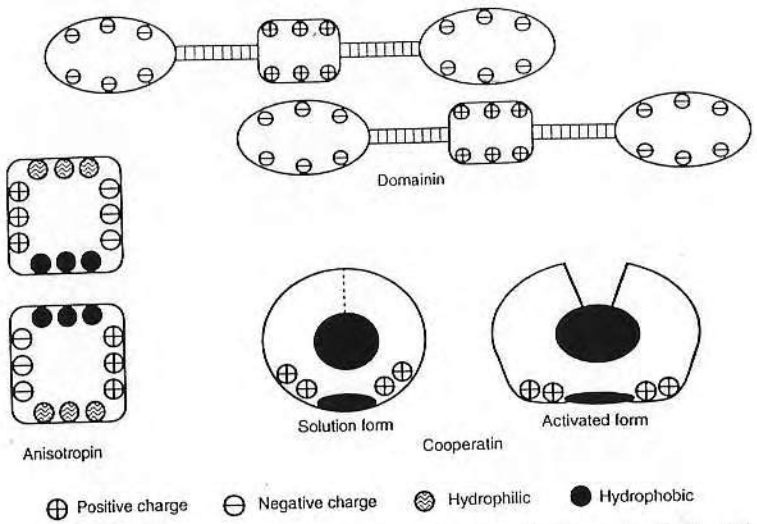

Figure 3 Three hypothetical model proteins used to help discover and understand the principles of protein interfacial behavior. At the far left, *Anisotropin* is a two-dimensional model that represents, in highly exaggerated form, some of the characteristics of small model proteins commonly used for basic protein adsorption studies, such as lysozyme, ribonuclease, and others. Anisotropin is shown as a small square with each of the faces or edges of the square representing different concentrations of amino acid residues. One face is highly negatively charged, another highly positively charged, another is hydrophilic but neutral, and the fourth represents a hydrophobic face or patch. All of these features are seen to various extents in proteins whose three-dimensional structures are readily available. Sometimes the patches are small, sometimes larger. Two anisotropin molecules are illustrated because you may want to play with them. For example, given the nature of anisotropin, it is likely that it would exist as a dimer in solution with the hydrophobic faces of each monomer in contact and with an orientation leading to electrostatic stabilization.

The reader is urged to photocopy the illustration, cut out the various proteins, and manipulate and play with the cutouts to experience these two-dimensional interactions.

At the top is *Domainin*. Domainin, a more complex multidomain protein represents, in exaggerated and schematic form, activated fibrinogen, for example. One might think of domainin as having three different domains, each one anisotropin-like, but very different in their individual characteristics. Note that domainin may associate laterally.

Cooperatin illustrates interface-induced conformational changes. Two forms of cooperatin are shown: the folded or compact solution form and the interfacially denatured, open, active form (refer to the text for more details). Cooperatin, an example of a more dynamic molecule, is more easily denaturable at an interface and introduces a different level of complexity to the problem of proteins at interfaces.

Anisotropin represents the simplest semirealistic case, domainin represents a second level of complexity, and cooperatin represents a still greater level of complexity. Now imagine these same concepts in three dimensions and one begins to appreciate the true complexity of protein interfacial processes!

Cooperatin is a dynamic, nonrigid model protein, still maintaining the concept of heterogeneity or semispecific regions or patches. With *cooperatin* we allow for the possibility of conformational change due to a change in the local microenvironment or local thermodynamics. Examples may include proteins in the contact activation pathway of coagulation or interfacially induced complement activation. We call such a conformational change "denaturation," to be discussed later.

Anisotropin has four faces: hydrophobic, positively charged, negatively charged, and neutral hydrophilic. Although all collisions are equally probable, only those collisions, that result in interaction energies in the range of kT, provide the residence times necessary for subsequent interfacial processes. Protein adsorption on neutral hydrophilic surfaces, for example, tends to be relatively weak, whereas adsorption of proteins on hydrophobic surfaces tends to be very strong and often partially irreversible. Adsorption on charged surfaces tends to be a strong function of the charge character of the protein, the pH of the medium, and the ionic strength [4].

To predict the initial contact or the orientation of adsorbed protein which would lead to the maximum interaction, we need to know something about the external surface chemistry of the proteins themselves. This is a simple problem for proteins whose three-dimensional structures are well known, such as insulin, myoglobin, and lysozyme [12–14]. In these cases the X-ray crystallographic coordinates of the protein are readily available for display on a computer screen. One can easily visualize the different faces or surfaces of the protein with respect to their hydrophobic charge and neutral hydrophilic character and readily formulate hypotheses as to their possible surface interaction [9–14].

Although significant attempts have been made to calculate the interaction energies between proteins and model surfaces in different orientations [13,14], the major problem with these simulations is incorporating the extremely important roles of water and the hydrophobic interaction. Fortunately, one can also gain insight into the mechanism and nature of the adsorption process via an intuitive, common sense approach which considers collision rates and multiple faces (Fig. 4).

In the case of electrostatic interactions, the analysis is more straightforward as done by Ho and Hlady for a number of model proteins [14] using the Delphi electrostatic field simulation package available through Biosym, Inc. [15]. A similar analysis has been applied to ribonuclease interaction with mica surfaces [16]. These analyses show that local electrostatic patches on the protein will interact with complementary charges on the surface or interface. They also show that the overall charge on the protein, or its isoelectric point, is virtually meaningless with respect to protein interfacial behavior at normal physiologic

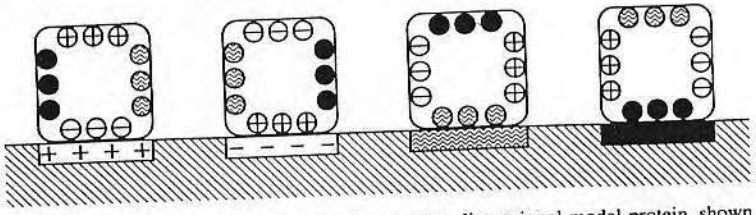

Figure 4 A schematic view of anisotropin, our two-dimensional model protein, shown interacting with surfaces of comparable character. In the case of a neutral hydrophilic polymer surface, one expects weak or little adsorption; in the case of a hydrophobic polymer one expects strong adsorption via the orientation shown. In the case of charged surfaces, one expects moderate or variable adsorption, depending on the electrostatic nature of the interaction, which is a function of the ionic strength and pH of the solution, charge density, and charge location (based on Ref. 9).

ionic strength conditions. Most proteins are larger than 20 to 30 Å, but the electrical double layer (Debye) length in physiologic solutions is only 8 to 10 Å. Therefore, the interaction of a protein with a surface is dictated largely by its collision orientation and the nature of the charged species on the particular face that happens to collide with the surface [10,12,14,16]. The nature of the charge on the other end of the protein molecule is not particularly important for a first approximation. That is why the *domainin* model (Fig. 3) is quite important. Depending on the nature of the surface or interface, it is only the domain with the opposite charge density that is likely to be "recognized," based only on electrostatic interactions.

At close distances (smaller than the Debye length) any charges on the surface will "feel" the charges on the nearest protein face. The ensuing force will enhance or retard protein approach. However, when the distance between the interacting charges becomes very small, both sets of charges will have to desolvate (lose their water or hydration). This process is energetically very costly, and, even for unlike charges, the energy difference may favor hydrated charges.

Therefore, when one speaks about the strongest interaction between charges, one has to add a caveat about the distance between such interactions. At contact, much of the interactive strength is lost due to dehydration of charges; if there are no other interactive components the protein may not stick. Perhaps it will glide or diffuse on its own surface hydration layer until if finds a spot that can provide energy for dehydrating charges (probably by hydrophobic area dehydration). Thus "simple" electrostatic arguments are not so simple!

Another complication to such analyses is the protein's ability to bind with itself, forming dimers, tetramers, hexamers, or other oligomers. This is best illustrated in the case of insulin whose interfacial behavior has been extensively studied [17,18]. The dimerization and oligomerization of insulin in solution is quite well known. Current studies of the adsorption of insulin are considering the "face" approach and the roles of dimer and hexamer structures [18].

Another complication is that a protein, normally a monomer in solution, may be induced to dimerize in the adsorbed state. This could be considered as a specific manifestation of lateral interactions in adsorbed protein layers.

The reader should make a number of enlarged overhead transparencies of Figure 3. Cut several *anisotropin* "molecules," out of those transparencies three or four *domainin* molecules, and at least one *cooperatin* molecule. It might be helpful to take some transparency pens and color code the three "proteins." We like to use green for neutral but hydrophilic character, black or grey for hydrophobic or apolar character, red for carboxyl or other negative charge character, and blue for amino or other positive charge character. This is consistent with the standard CPK color coding often used in molecular graphical simulations [11].

Now play with your two *anisotropin* molecules and you will see that one can easily form a hydrophobic dimer, which is electrostatically stabilized at the top and bottom. A purely electrostatic dimer is also possible. Now, you may wish to make some transparencies or drawings similar to the surface or interface in Figure 4. In addition to the orientations and interactions shown in Figure 4, produce some composite, heterogeneous, or patchy solid surfaces, that is, solid surfaces with both positive and negative charge and with hydrophobic character. You will see that the different patches or regions on the surfaces can adsorb *anisotropin* in different orientations, some leading to lateral interactions that may help to stabilize dimers or even more complex associations of surface-bound proteins.

One can examine the three-dimensional structure of those real proteins for which such information is available. In an early study, we showed that the adsorption of hen and human lysozyme on neutral, apolar, and charged surfaces could be qualitatively understood by considering the external surface chemistry of the two different lysozyme molecules [11]. From the major faces, knowing the collision rate, and assuming random collisions, one can begin to rationalize the kinetics of adsorption and estimate the initial interaction energies, at least at the instant of collision. Unfortunately, the problem becomes more complex from that moment on. We then expanded our matrix of model proteins and studied their behavior at air/water interfaces by dynamic surface tension techniques [9,12,19,20,111]. Our goal was to correlate the three-dimensional and surface structure of the protein in solution, its initial adsorption at air/water interfaces (determined by dynamic surface tension methods), its stability or denaturability in solution, and its tendency to denature upon long-term contact at the air/water

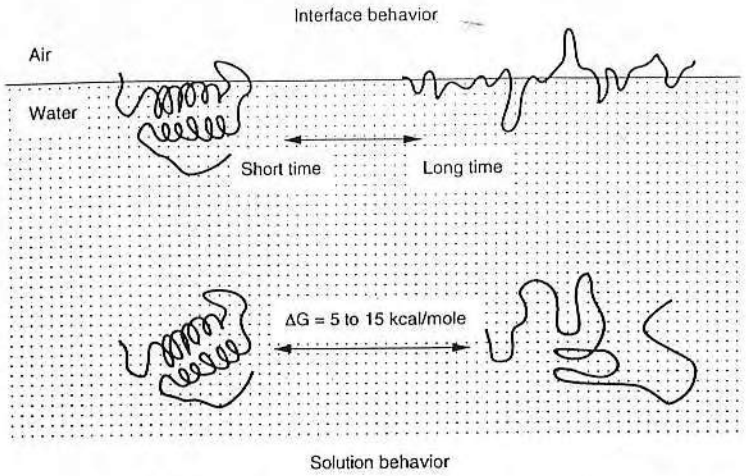

Figure 5 The relationships between protein structure in solution, protein solution denaturability, and protein behavior at the model air/water interface (see text for discussion; based on Ref. 9).

interface (again using dynamic surface tension). Figure 5 illustrates the objectives of this study, that is, the correlation of surface properties in solution, solution denaturability, and behavior at the air/water interface. Denaturability was assessed by calorimetry and by urea and guanidinium chloride perturbation, deduced by changes in fluorescence. The surface chemical nature of the protein was assessed by examining its external surface chemistry using molecular graphics and by fluorescent probe titration or hydrophobic chromatography [12,20,21]. A relative, effective surface hydrophobicity (ESH) parameter was then deduced [12].

After considering a wide range of parameters and, particularly, the adsorption principles illustrated in Figures 1–4, we selected twelve variables and qualitatively began to examine the correlations between them [12,20]. The variables were plotted on radial axes with the axes arranged and scaled to emphasize and even exaggerate correlations among the various parameters (Fig. 6). We call this multiparameter radial plot a "Tatra Plot." Others call it a spider or star plot [22,23].

Extensive protein Tatra Plots and the details of the multiparameter correlation have been presented elsewhere [9,12]. The important conclusion is that, as a first approximation, one can correlate and rationalize the air/water interfacial behavior. For small, globular, model proteins, one can qualitatively correlate and even predict their time-dependent interfacial behavior through their external surface chemistry (the initial collision event), their solution stability (their tendency to undergo interfacially induced denaturation or conformational change), and their total or overall nonpolar amino acid character, coupled with

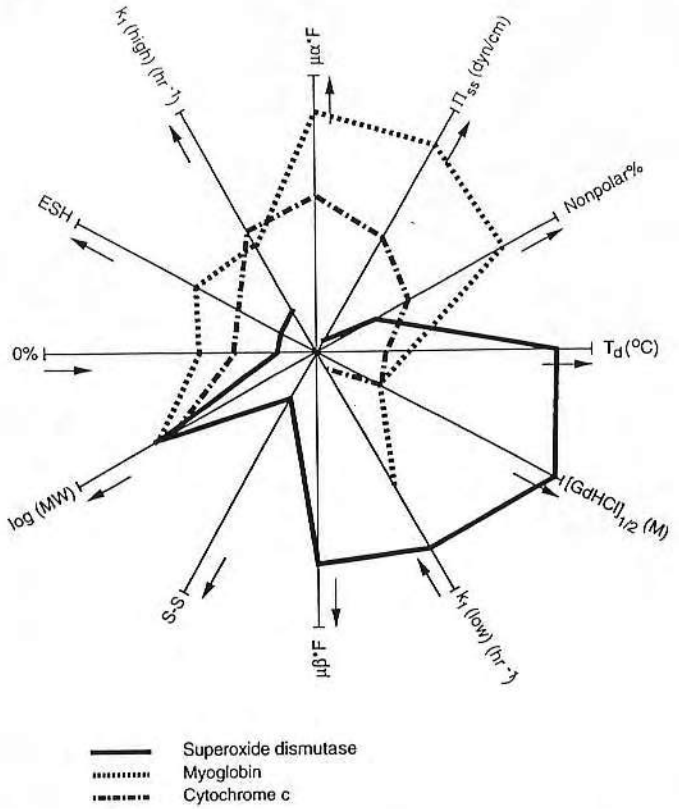

Figure 6 The Tatra Plot—a radial axis, multiparameter correlation involving the bulk and surface characteristics of model proteins and their behavior at the air–water interface (dynamic surface tension) at various dissolved protein concentrations. Three different model proteins are presented: superoxide dismutase (hydrophilic, nondenaturing, and surface inactive); cytochrome C (moderately hydrophobic, moderately denaturable, moderate surface activity); and myoglobin (only moderately hydrophobic but easily denatured and quite surface active) (see Refs. 9 and 12 for details).

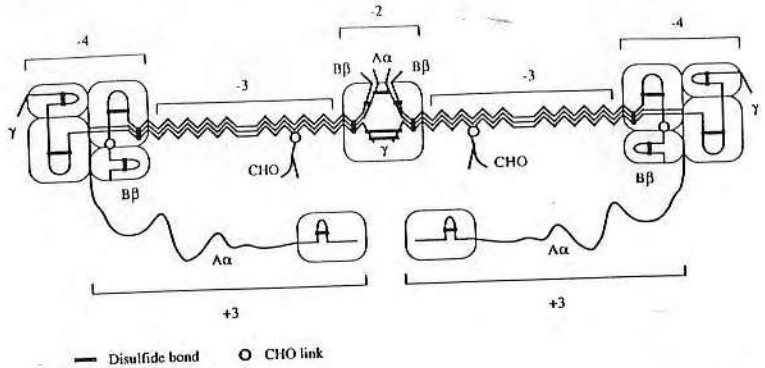

Figure 7 A schematic two-dimensional representation of the structure and properties of human fibrinogen. Note the different fixed charge and thermal denaturation properties of the various domains. Fibrinogen has a strong similarity to *domainin* (see Ref. 83 for details).

details of their structure and the number of disulfide bonds (the protein's ability to conformationally denature fully at the interface and expose its hydrophobic interior to the air phase). This work was recently extended by Tripp who included a set of additional proteins and used a better measure of dynamic surface tension characteristics [19,111].

This multiparameter, multiprotein correlation is now awaiting a modeler/theoretician who can produce a more quantitative and predictive model and theory from the simple, qualitative parameterization/correlation. We would be eager to collaborate with that individual.

And now *domainin*: We have been stimulated and fascinated over the years by the growing appreciation in the protein biochemical community of the role and the importance of structural and functional domains. One of the best examples is the important plasma protein fibrinogen (Fig. 7) to which *domainin* bears a striking resemblance.

Although each protein is a unique and distinct molecular machine and molecular personality, proteins can be considered as constructed of a multiplicity of smaller domain subunits [24,39]. For example, in the case of coagulation proteins, functional and structural domains include heparin-binding domains, growth factor domains, kringle sequences, carboxy-glutamic acid-rich, calcium-binding domains, and others [38]. High-sensitivity calorimetry studies of fibrinogen and its protease-derived fragments suggest 12 domains in the fibrinogen molecule, with denaturation temperatures of 45, 55, 90, and 100°C [27]. Fibronectin is another example. It has at least 20 calorimetrically identified

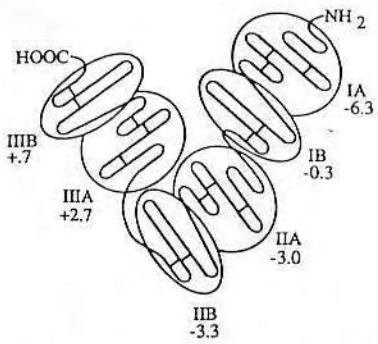

Figure 8 A schematic model of the domain nature of human serum albumin, based on the published three-dimensional structure [33] and utilizing homological modeling software [15, 32].

domains [28], and it is likely that its complex adsorption behavior will be partially understood through a domain analysis.

The optimistic view is perhaps best described by Chothia [29]:

> The apparently complex structure of proteins is, in fact, governed by a set of relatively simple principles. Individual proteins arise from particular combinations of and variations on these principles. An analogous situation is found in linguistics, where a set of simple grammatical rules governs the generation of different, and sometimes complex, sentences.

Others have suggested a protein structural linguistics [30].

We have attempted to apply some of these concepts to the analysis of the interfacial behavior of albumin [31,32]. Albumin is the simplest of the multidomain proteins for initiating this analysis. It is a major component of blood plasma; it has no bound carbohydrate; it consists of three, roughly 20-kilodalton domains; it is high in disulfide cross-link content [34]. It has a high degree of α-helicity and is somewhat myoglobin-like; it binds a variety of ligands, including fatty acids and calcium. The crystal structure for human albumin is now available [33].

With the three-domain model of albumin (Fig. 8) we have done a very preliminary electrostatic analysis. A computerized *simulated* titration of the three domains as a function of pH, and a simple analysis of the possible electrostatic behavior of those domains was done [31,32]. From these analyses, a set of hypotheses has been generated for analyzing albumin adsorption data [31,32].

Let us now become familiar with our various *domainin* molecules. *Domainin* molecules can be arranged end to end into a weakly electrostatically

bonded *domainin* "polymer." *Domainin* can also be associated side to side, that is, laterally, forming a two-dimensional, electrostatically associated polymeric sheet. Fibrinogen has some similarity to *domainin*. Its highly negatively charged fibrinopeptides A and B are removed by exposure to thrombin. This gives the C-terminal ends of the α- and β-chains of fibrinogen a high positive charge, providing us with a real world example of *domainin*. It has been suggested that this activated fibrinogen associates weakly, electrostatically, side to side and end to end in the early stages of the fibrin clot and network development [35].

Another good example of the domain approach to rationalizing protein interfacial behavior is the study done by Ho et al., dealing with the adsorption of plasma proteins on heparin-containing particles [36]. The heparin-containing particles could explain the adsorption properties of the complex mixture of plasma proteins by an amino acid sequence analysis which focused on regions of the sequence with high positive charge, assuming that the interaction with heparin chains was primarily electrostatic. This analysis rationalized much of the behavior of vitronectin, antithrombin, lipoproteins, and other heparin-binding proteins on hydrophilic, negatively charged, solid surfaces. Therefore, it is not necessary that one have a three-dimensional crystal structure of the entire protein to begin considering the details of its interfacial orientation and interactions.

It is clear that a domain approach to protein adsorption and immobilization helps greatly to simplify the apparent complexity of the process. In fact, we have been quite successful in applying these concepts to a variety of problems involving the covalent immobilization of antibodies for biosensor and related applications [37].

And now *Cooperatin*: both *anisotropin* and *domainin* (Fig. 3) were considered relatively rigid proteins. *Cooperatin* is much less rigid, and the transparency model for *cooperatin* is slightly more complicated. That is why there are two drawings for *cooperatin* at the base of Figure 3. On the right is the expanded, or activated version of *cooperatin*. Make a transparency of this figure and cut out the expanded *cooperatin*. Fold the edge of the cooperatin transparency adjacent to the left side of the "active site" so that the active site is hidden and your two-dimensional, folded *cooperatin* now looks like the solution form of cooperatin in Figure 3.

It is clear that you can expand and contract *cooperatin* at will, exposing and covering the "active site." You will note that the "bottom" of the *cooperatin* molecule, using the orientation in Figure 3, has a number of surface patches, or faces, not unlike *anisotropin*. In this case, we have shown some positively charged domains and some hydrophobic patches. Now, if you assume that *cooperatin* collides with those faces in approaching a heterogeneous or patchy surface, you may get the sequence of steps shown in Figure 9. The hydrophobic association at t=0 leads to an increased residence time for *cooperatin* at the interface. As it jostles, wiggles, or vibrates, pinned down by its hydrophobic

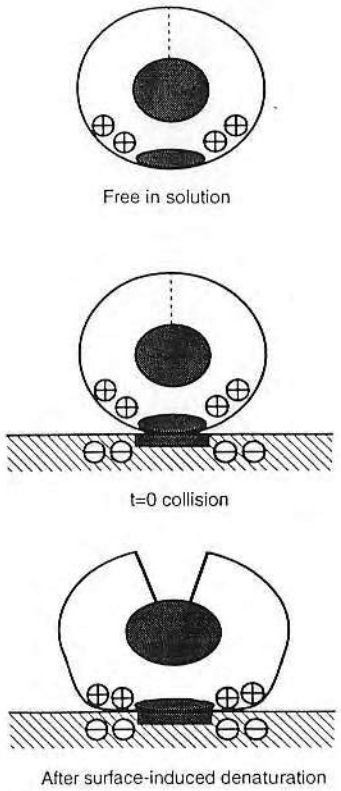

Figure 9 Our model protein *cooperatin* (from Fig. 3) is shown free in solution, at the instant of contact with a heterogeneous surface, and after conformational accommodation (denaturation?) and "activation" by the adsorption process. The protein develops enzymatic activity due to the adsorption process.

attachment feet, it quickly "discovers" another kT or two of electrostatic interaction available. Depending on the conformational stability of *cooperatin*, the actual magnitude of that increased adsorption free energy may lead to conformational change, thereby maximizing its interaction at the interface (bottom of Fig. 9). This can only happen if the protein literally changes its three-dimensional structure, resulting in the interfacially activated form. Thus *cooperatin*, with no enzymatic activity normally, has been interfacially activated to expose its putative or hidden enzyme active site. *Cooperatin* is a proenzyme or zymogen.

Given the electrostatic interaction/dehydration argument presented earlier, the actual adsorption mechanism could be an initial (long-range) electrostatic interaction, followed by hydrophobic dehydration at the solid/liquid interface. In either case, there may be sufficient adsorption free energy to facilitate a conformational change and the activation of cooperatin.

Cooperatin may be a model for a number of the proteins/enzymes in the contact activation system of coagulation and the complement system of molecular immunology [38]. That is why in our earlier model protein study that led to the Tatra plots we were so concerned with the "softness" or "hardness," that is, the conformational stability, of those single-domain model globular proteins [8,9] also discussed by Norde [112]. That is also the reason why the thermal denaturation temperature and the denaturant concentrations required to unfold these model proteins significantly were included as axes in the Tatra Plot. Another axis relates to the amphilicity of α-helices, because of their tendency to interact with and order at hydrophobic surfaces [45].

This discussion is beginning to suggest that so called "nonspecific" interactions of proteins may indeed be "semispecific." Given knowledge of the three-dimensional structure and/or the domain nature of proteins, one should be able to design surfaces with the appropriate complementarity to insure that the proteins deposit or adsorb in particular orientations, producing two-dimensional ordering or desired patterns, thereby permitting interface-designed assembly for specific applications. This has been demonstrated in a series of elegant studies by the groups of Ringsdorf and Grainger [113,114].

We must now consider the heterogeneity and/or domain nature of the solid surfaces themselves.

III. SURFACES AND INTERFACES

The heterogeneity and dynamics characteristic of proteins are also characteristic of many solid surfaces, particularly synthetic polymers [40].

Referring to the complexity axis paradigm in Figure 1, we consider the air/water interface among the simplest interfaces at which to consider protein adsorption. Although that interface is considered homogeneous at the level of the flickering cluster of water (10 Å), water molecules are constantly leaving and entering the interface from the water and air sides. A protein colliding at such an interface immediately feels the incredible energetic discontinuity and tends to be immediately bound, or at least retained, for a period of time much longer than the actual collision event.

Proteins are hydrophilic and soluble; thus, they tend to stay in solution, that is, to be initially rejected from the interface, just as hydrophilic, small ions are rejected from air/water interfaces [41].

However, most proteins also have hydrophobic patches on their surface. Even the most highly hydrophilic proteins can have substantial hydrophobic

character exposed [11]. As seen with *anisotropin* (Fig. 3), if that hydrophobic face or patch is oriented towards the air/water interface during the collision event, then the protein is likely to be adsorbed by a hydrophobic interaction process. The air side of the air/water interface is among the most hydrophobic of "materials." The interfacial free energy is about 73 ergs/cm^2 [46]. There is nothing on the other side of the interface for the water molecules to bind to. The number of gas molecules on the air side is negligible with respect to the molecular concentration on the water side of the interface. Thus water is even more highly self-structured at an air surface that it is at a classical apolar hydrophobic surface, leading to a very strong hydrophobic interaction potential. This is presumably the major driving source for adsorption at the air/water interface.

There are other factors, however. Ions are indeed rejected from the air/water interface; in fact, this is the basis of membrane desalination processes. Therefore, the local ionic strength and pH microenvironment are also different, which may contribute to the protein adsorption mechanism. Nevertheless, it is reasonable to say that the air/water interface is a highly hydrophobic interface; proteins that collide with an orientation that exposes a hydrophobic patch or face are likely to be retained or adsorbed, at least for a short period of time.

Hydrophobic solid surfaces are similar in many respects to the air/water interface, but an increased level of complexity is introduced. Hydrophobic solid surfaces may be relatively homogeneous (polydimethylsiloxane) or very heterogeneous (semicrystalline polyethylene). All polymer surfaces are highly heterogeneous in the size of polymer molecules, which are similar in size to individual protein molecules. Due to steric exclusion and the tendency to satisfy entropic concerns, polymer chains do not interpenetrate very effectively, further enhancing the macromolecular granularity of polymeric materials.

Polymers have a range of molecular dynamics and molecular relaxation processes, including the glass transition and side chain (beta) relaxations [40], which further contribute to the complexity of such interfaces. The dynamics of polymers and their role in polymer surface properties have been discussed by us and others and related conceptually and qualitatively to protein interfacial processes [1,47].

It is important to point out the time scale of relaxation processes for polymers is of the same order as that for proteins—not surprising as both are macromolecules. Although the terminology is very different, the mechanisms and processes are basically the same. This has been discussed by Chan and Dill in a recent review [42].

As one moves along the solid surface complexity axis of Figure 1, microcrystalline and semicrystalline materials must be considered. Low temperature isotropic (LTI) pyrolitic carbon is a good example [43,44]. This extensively used material, which has a reputation for blood compatibility in heart valve disk and related applications, is often thought of as a pure, homogeneous, rigid material. However, it is now known, and actually has been known ever since the

material was introduced into medical practice, that it is highly heterogeneous, with a variety of random microcrystallites and amorphous boundaries. The heterogeneity of LTI carbon, in crystallite size and crystallite boundary dimensions, is again of the same order as small globular proteins.

Synthetic polymers can have a wide range of crystalline characteristics, although the single crystal lamella, the basic structural unit of polymer crystals, is roughly 100 Å thick, again in the same range as that of proteins. In these semicrystalline systems, low molecular weight polymers and impurities are rejected from the growing crystal front, thus the surface often consists of low molecular weight or other noncrystalline material.

Adsorption experiments on polymer surfaces cannot be interpreted without knowing the nature of the solid surface in the specific environment in which it is exposed to protein, that is, in equilibrium with the water, pH, ions, and other components of the protein solution itself [1,46].

It is now well accepted that many surfaces, particularly those of hydrophilic synthetic polymers, oxidized metals, and most ceramics, hydrate and develop gel-like layers at the interface [46]. Many polymer systems restructure their surface regions, driven by interfacial free energy minimization, to present interfaces to the protein aqueous solution very different than presented to air or vacuum, the typical environment of most surface characterization techniques. We and others have discussed these problems and concerns repeatedly [40,48].

As one moves further along the polymer complexity axis, we consider block copolymer systems, which are designed to separate into phases due to incompatible macromolecular blocks. The biomedical polyether urethanes are the best examples [48,64,65]. Depending on the relative hydrophobicity and hydrophilicity of the "hard" and "soft" segment blocks and the relative composition and molecular weight of each segment, one can have a broad range of solid structures with widely differing mechanical and physical properties. The property differences are controlled by chemical nature, block size, and degree of phase separation.

Most commonly hard segment blocks are dispersed through a less hydrophilic, somewhat elastic, soft segment matrix. Optimum mechanical properties correlate with relative block sizes in the 100 Å range.

It is clear that a small globular protein approaching such a surface has many "choices" to make. A shower of identical protein molecules colliding with such a heterogeneous block copolymer surface creates widely diverse interactions. Consider *anisotropin*. There are a variety of orientations by which it may approach the surface. It may collide in the middle of a hard segment block, it may collide in the middle of a soft segment region, it may collide at the interface between the hard and soft segment, or it may collide and be somewhat retained in one position, and then find itself diffusing along the surface until it reaches another point or site at which the interaction energy is stronger or more

optimal. Time is the critical variable in all of these processes. A relatively "soft" protein may immediately begin to adapt conformationally (denature) upon collision. With a harder protein, that process may be greatly slowed down; conformational adaption may not begin to occur until after it has "sampled" various regions of the surfaces by translational, two-dimensional diffusion. Either diffusing or stationary on the surface, it may suddenly find itself in collision with one of its solution brothers or laterally kicked or otherwise perturbed by a sister diffusing laterally on the surface.

Even if the surface were ideally homogeneous, it is obvious that there is a great heterogeneity in the orientation of the rain of proteins colliding with that surface. This heterogeneity leads to a wide range of adsorbed states, some of which may readily desorb, and others which are oriented so that they have a high adsorption free energy with the surface and are essentially bound.

Most polymer surfaces, however, are also dynamic. The very presence of the protein on the surface exposes the polymer molecule to a new microenvironment, with a new local microthermodynamic system. The polymer chain suddenly finds itself in a new chemical environment, that is, *under* the protein rather than underneath an aqueous solution; the polymer, through its own relaxation processes and internal dynamics, begins to accommodate in both enthalpic and entropic terms (Fig. 10).

The suggestion is that *both* the protein and the polymer are accommodating to each other with some of the same relaxation processes and time scales, some rapid and some very slow [42].

All of this is not much different from so-called "specific" reactions in biochemistry. Although the old biochemical specificity paradigm has utilized a rigid lock and key model, there is now general appreciation that both the ligand and its receptor are dynamic; the interaction occurs in a highly cooperative fashion. Because the nature of the bonding partners changes during the bonding event, the final structural or conformational state differs from the initial state. This results in interesting hysteretic properties and great differences between so-called "on rates" and "off rates" in many processes, including antigen–antibody interactions and certain classes of enzyme–substrate reactions.

Given this analogy with "specific biochemistry," the range and repertoire of dynamic variables involved with protein interactions on dynamic surfaces leads to the possibility of a *statistical specificity*, which we will discuss later.

There is a way to avoid the problem partially by designing and preparing a surface which minimizes all protein interaction and indeed even repels proteins from the interface.

IV. PROTEIN-RESISTANT SURFACES

It is possible to avoid the protein adsorption problem almost completely by developing protein-resistant surfaces or interface-resistant proteins. The major

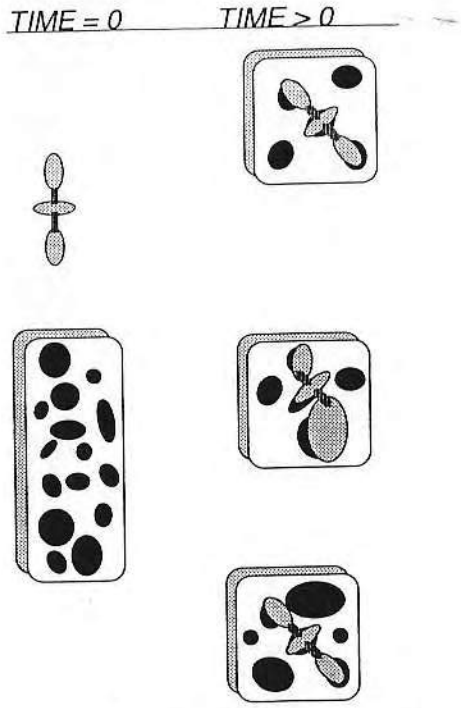

Figure 10 Speculations on domain-based "statistical specificity," assuming a multidomain protein (top, left) interacting with a multidomain polymer (bottom, left) whose domains are roughly comparable in size to those of the protein (e.g., certain polyether urethanes). Top: domain matching and interaction complementarity; middle: protein denatures or adapts to try to match polymer; bottom: polymer surface adapts to adjust to a "less-adaptable" ("hard") protein. The important variables include protein and polymer domain sizes and surface chemistries, protein domain denaturation temperatures, and polymer domain glass transition temperatures.

interactions which drive the interfacial activity and adsorption of proteins are the water-structure-driven hydrophobic effect, electrostatic interactions, or strong hydrogen bonding interactions characterized by cooperative, multihydrogen bonds. A typical isolated hydrogen bond does not play much of a role in aqueous solutions, because that bond is largely satisfied by interactions with the

55-molar concentration of water which is present. Isolated, random hydrogen bonds are generally unimportant. They become important when there are many such bonds, either acceptors or donors, with the complementary component on the other surface. If those multiple hydrogen bonds can match up to form a cooperative interaction consisting of three to five or more hydrogen bonding units, then this can be a strong, effective means of interfacial adhesion, just as in biorecognition. But it requires multiple bonds *and* stereo complementarity.

For more than half a century, it has been well-known that neutral, highly hydrophilic polymers have minimal or very weak interactions with most aqueous proteins. The development of the dextrans (Sephadex) and the agaroses (Sepharose) for protein chromatography and electrophoresis demonstrate that such matrices have relatively weak protein interactions. Because they are generally nonionic, they minimize electrostatic interactions and because they are highly water soluble and hydrophilic, they minimize hydrophobic interactions. Although they are extensively hydrogen bonded, the gels and the surfaces produced by such polymers tend to be highly dynamic and random; therefore, cooperative hydrogen bonding processes are not generally a problem. However, some proteins are retained on such gels, often attributed to hydrogen bonding interactions and, in some cases, to residual charge or hydrophobic character.

A neutral, highly hydrophilic polymer that is also very dynamic at the surface has another mechanism for minimizing protein interactions. Being neutral and hydrophilic, it has already minimized enthalpic interactions. Being highly dynamic, the interface has high entropy. Any process which tends to decrease or minimize this interfacial entropy, for example, by decreasing the dynamics or mobility of the polymer chains at the interface, will have unfavorable free energy. Adsorption on such a surface will, therefore, pay a high free energy penalty, which must generally be paid for in enthalpy. If there are no enthalpic interactions available, such surfaces are said to be repulsive by an entropic, surface dynamics mechanism. This can also be related to steric exclusion and osmotic pressure. These processes have been extensively modeled and discussed by deGennes [49] and are now being widely applied in the biomaterials/biotechnology community [50,51].

The polymer with optimum protein repulsive characteristics is polyethylene oxide (PEO), or, in low molecular weight form, polyethylene glycol (PEG) [53]. Many workers have succeeded in producing PEO-rich surfaces which reduce or inhibit protein adsorption to varying degrees. We will not discuss this subject in detail here; a current review is available [50]. The protein resistance characteristics of PEO surfaces can be a strong function of PEO molecular weight, the distance between grafted chains, the molecular weight of the protein itself, salt type and concentration, temperature, and the nature and types of proteins in the mixture, that is, competitive protein adsorption processes [50–53].

Polymeric surfactants, particularly triblock and other multiblock surfactants utilizing PEO, have enabled a number of groups to develop very effective and relatively simple processes to produce PEO-rich surfaces for biomedical and biotechnological applications [51,52].

One can also modify the protein directly through reactive PEGs [53,54]. This has been called "PEG-elating" a protein. For several decades, Enzon Corp. has been developing these techniques and processes for enzyme and protein replacement therapies [54].

Some protein preparations are now commonly stabilized with PEO-PPO-PEO surfactants. They bind to the insulin dimer or hexamer, for example, thereby minimizing its interfacial adsorption and aggregation [55]. The same concepts and additives are now being applied in biotechnology to minimize the surface activity of interfacially sensitive proteins during downstream processing operations.

Immunosensors and other biosensors with greatly reduced nonspecific binding are being prepared by immobilizing antibodies through neutral, hydrophilic PEO tethers and by otherwise coating and covering the underlying surface with PEO, dextran, or other protein-resistant hydrophilic polymers [56,57]. The minimization of nonspecific binding can enhance the sensitivity and specificity of immunosensors and other specific recognition-based diagnostic chemistry methods [57].

V. COMPETITIVE PROTEIN ADSORPTION (LEO VROMAN'S EFFECTS)

The interest in the blood compatibility of medical devices and the tear compatibility of contact lenses has strongly driven attempts to understand the complexity of plasma protein and tear protein interactions with materials. Although it is one thing to look at a single, purified protein solution and consider its complex interaction with a range of interfaces, it is more difficult to look at a complex mixture of proteins, all dynamically interacting with the interface and with each other, over a broad time span.

The brief discussion here will focus on plasma proteins, but the same principles and concepts apply to all multiprotein mixtures. There are many reviews summarizing the many proteins in blood plasma, including their structure, concentration, and function [58]. Although there have been a number of pioneering studies which have attempted to directly measure the competitive adsorption of large numbers of proteins from plasma [59–61], only in the last several years have a number of techniques become available which permit such studies without Herculean efforts or commitments.

Normally one studies competitive protein adsorption by radiolabeling one protein and then studying its adsorption from a mixture of proteins [62]. A separate set of experiments is required to study the adsorption of a second protein

from the same mixture of proteins. This approach has been applied by a number of investigators, using primarily radioiodine or fluorescence labels. In some cases, specific antibody methods were used to identify specific proteins in the adsorbed state [63,64].

In recent years, very high resolution, ultrasensitive, one- and two-dimensional gel electrophoresis and high performance liquid chromatography have enabled a number of groups to study the adsorption of complex plasma protein mixtures as a function of time, without the need for labeling [36,44,61,65,67].

Although these techniques are not nearly as quantitative as those employing radio labels, they do offer the advantages of

Looking at a very large number of proteins at the same time
Avoiding labels and potential label-associated artifacts
Saving an enormous amount of time and money

The benefit of these techniques is that they allow investigators to screen semiquantitatively and to look for general trends, allowing the formulation of more specific hypotheses which can then be quantitatively assessed by more classical means.

We will review two recent such studies from our group which provide a perspective on the enormous potential of these multidimensional, semiquantitative methods [44,65].

A. Case 1: Strong Interactions—LTI Carbon [44,68]

Feng recently completed a Ph.D. thesis dealing with plasma/protein interactions with low temperature isotropic carbon (LTIC), a material with a domain-mosaic structure [44]. LTIC represents a large family of carbons with good biocompatibility despite their structural differences. In ancient times charcoal and lampblack were used as tattooing materials due to their inertness to tissue [43]. Feng asked, "Do carbons have something in common that grant them all the property of biocompatibility"? LTIC is a "mystical" material because it possesses some properties not generally considered appropriate for a "good" blood-compatible material. Table 1 summarizes how LTIC defies those "general rules."

Despite its wide application and acceptance as an important biomaterial, there are few systematic studies of LTIC's blood compatibility based on plasma protein adsorption. Feng consolidated the proposed hypotheses into two major ones:

The weak interaction hypothesis—the surface does *not* strongly interact with proteins

The strong interaction hypothesis—the surface becomes blood-compatible through modification by a "bland" proteinaceous film, that is, the passivation can be attributed to a first layer of adsorbed proteins inactive to later adsorbed proteins

Table 1 Common Accepted Indicators or Correlators of Blood Compatibility and the Properties of LTI Carbon

Blood compatible material should be:	Blood compatible LTIC actually is:
Hydrophilic	Hydrophobic
Soft (low modulus)	Hard (high modulus)
Low surface energy	Relatively high surface energy
Nonconductive	Electrically conductive
Negative resting potential vs. NHE	Positive resting potential vs. NHE

Source: Ref. 44.

Judged from the activities of plasma proteins after adsorption on artificial surfaces [66], the bland protein is most likely albumin. According to this hypothesis, the conformation of the inner layer of absorbed proteins is altered at the interface to achieve a strong protein–surface interaction. The adsorption process is irreversible, and the firmly adsorbed proteins are not exchangeable.

The weak interaction hypothesis suggests that the carbon does not strongly interact with proteins. Any proteins which may be adsorbed preserve their native conformation and are not structurally altered or biochemically activated.

Feng applied a wide range of techniques to study the competitive adsorption of plasma proteins onto LTIC substrates, including AC impedance [69], labeled proteins [70], differential scanning calorimetry [71], and semiquantitative two-dimensional gel electrophoresis [68]. We review his conclusions very briefly here. The details are in the thesis and in papers [44,68–71].

One might think that LTIC carbon is an ideal model surface. Indeed that is one of the reasons why it was initially chosen for Feng's study. However, upon closer examination, one finds that it is a heterogeneous surface composed of small crystallites varying in size from 20 to 100 Å, with a variety of crystallite orientations exposed to the surface. Polishing of the carbon results in smoothing of the surface, but also smears the atomic structure, increases oxidation (probably primarily carbon atoms on the edges of the graphitic like planes), and causes other changes [72]. Carbon is also moderately hydrophobic, as measured by advancing contact angles (60–80° with water), and yet has a fairly high oxygen content, as measured by X-ray photoelectron spectroscopy. It is, however, a highly rigid surface, as well as a conductive material.

Feng's major conclusions were:

1. LTIC is characterized by a microporous, oxidized, hydrophobic, and domain-mosaic structure. Although the oxygen content remains constant, polishing reduces the porosity and the heterogeneity of the surface.
2. LTIC denatures albumin, fibrinogen and the four small model proteins studied. Hydrophobic and possibly charge transfer interactions are thought to be the major driving forces for surface denaturation on LTIC.

3. The adsorption rate on LTIC is high, reaching 50% of the plateau value in about 10 s, and about 80% in 2–3 min for albumin with solution concentration higher than 0.50 mg/ml.
4. Saturated (plateau) values of the adsorption isotherms require a high bulk concentration in the case of albumin and fibrinogen and result in a high surface plateau concentration. Multilayer adsorption is suspected.
5. The adsorption process is highly irreversible on LTIC. Displacement of adsorbed proteins is almost negligible for surface concentration below the plateau value.
6. Adsorption of both albumin and fibrinogen on LTIC is only moderately suppressed in the presence of other plasma proteins.
7. Neither fibrinogen nor albumin is preferentially adsorbed on the LTIC surface from the binary system. Their surface concentrations are linear functions of their mass compositions in the mixture.

These results suggest that LTIC surfaces have high affinity for *all* proteins. This may be the most valuable property of LTIC and perhaps other carbons. Such a strong interaction between the surface and proteins has two important consequences for biocompatibility: forming a "bland" protein-protecting film and perhaps disabling adsorbed "harmful" proteins.

The protein film formed from blood on LTIC consists of mostly bland proteins, a result of the predominant concentration of albumin, the nonselective adsorbing property of LTIC, and the absence of displacement of adsorbed proteins. Albumin is certainly the first protein to arrive at surface [73] because of its high concentration, but it has a relatively low adsorption propensity compared with other plasma proteins. If carbon can adsorb and retain the albumin, the surface will be protected and become biocompatible according to the albumin-passivation hypothesis [74,75]. This protein film must be stable to give the surface relatively permanent blood compatibility. Many solid surfaces have low affinity for albumin and do not retain the adsorbed albumin.

Adsorbed proteins that are denatured generally lose their biochemical functions. For example, by significantly altering the tertiary and secondary structure of fibrinogen, the LTIC surface would cripple its capability to bind to platelets.

We suggest that the LTIC surface possesses four features that are responsible for its high adsorption of proteins:

High surface energy and high dispersion forces
Modest hydrophobicity
Charge-transfer capability
Rigidity

The *high surface energy* (50 dynes/cm^2) [74] of LTIC is very important. The pure hydrophobic interaction may be treated as a "passive interaction"

because its driving force is from the surrounding water; a high surface energy provides a "positive" component for protein adhesion. Consequently, LTIC may have a greater intrinsic capability for adsorbing and retaining plasma proteins than lower surface energy polymers.

A surface with *modest hydrophobicity* may be an ideal substrate for proteins. Despite its hydrophobicity, the LTIC surface contains a fairly large amount of oxygen. Proteins often show maximum affinity to surfaces of intermediate polarity [77]. It is likely that the oxygen content is clustered at the defect areas, the boundaries between the crystalline domains [72]. The balance of the polar and nonpolar components and the nano separation of hydrophobicity should strengthen protein–surface interactions by providing proper sites for different peptide segments.

Charged transfer (or electron donor–acceptor) interactions attract molecules via π–σ or π–π interactions. Aromatic amino acids (tryptophan, tyrosine, and phenylalanine) strongly interact with aromatic groups [78] and with atoms with nonbonding lone electron pairs [79]. Composed of fused imperfect aromatic rings, LTIC is an excellent candidate for such interactions. Oxidation may produce different surface regions with different interactions. For instance, the quinone (>C=O)-like region is likely to be an electron acceptor while the phenol (>C–OH)-like group should behave as an electron donor [80]. The charge-transfer interaction not only partially explains why LTIC tenaciously adsorbs proteins, but it also provides a mechanism explaining why LTIC strongly denatures proteins: strong interactions with 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 charge-transfer interaction because both interactions can result from the very same surface regions or groups [78].

The *rigid surface* provides a solid ground upon which proteins can anchor. Surface dynamics, as in many organic polymers, may weaken the association of the surface and the adsorbed protein. LTIC is a rigid material. Its atoms are commonly bonded to three (sp^2 hybrid) or four (sp^3 hybrid) neighboring carbon atoms, forming an imperfect two-dimensional network. Chain segment motion is severely restricted, and the surface is hard and firm.

In summary, carbon is covered by plasma proteins that are strongly adsorbed and strongly denatured. The strong, tenacious, self-healing, proteinaceous film appears bland to subsequent plasma protein collisions, thereby minimizing interfacially activated processes.

B. Case II: Statistical "Specificity"—Polyether Urethanes

Tingey studied a series of polyurethane microbeads of varying soft and hard segment compositions [65]. The study clearly showed the dynamic characteristics of polyurethane surfaces, their ability to reorient in aqueous environments,

Table 2 Plasma Proteins Evaluated by the Two-Dimensional Electrophoresis Solute Depletion Technique

	Depletion of plasma proteins[a]		
	Relative	Depletion, %	
Plasma protein	amount[b]	Carbon[c]	Silica[d]
Albumin	100	15	21
α1 Acid glycoprotein	6	53	31
α1 Antichymotrypsin	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
αB 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 balance.
[c]25 mg in 1.7 ml solution.
[d]330 mg in 1.7 solution.
Source: Ref. 65.

and the role of the bulk chemical composition and bulk morphology in the surface chemistry. He followed the interactions of some 16 different plasma proteins using the two-dimensional gel electrophoresis technique (Table 2). He also correlated the protein depletion behavior with platelet adhesion, but only the protein results will be briefly discussed here.

Although one cannot draw definitive conclusions from his study because of the semiquantitative nature of gel electrophoresis and the relatively limited compositional range of the polyurethane particles evaluated, a variety of interesting correlations were observed and protein-specific hypotheses formulated. Although most of the proteins behaved similarly on the surfaces, with protein adsorption increasing with block size, a number of more protein-specific correlations were observed.

Haptoglobin (Hp) and antithrombin III (AT3) were depleted very little on most of the urethanes, whereas fibrinogen, apolipoprotein A-1, and hemopexin were heavily depleted, indicating that the proteins do not adsorb in amounts based solely on their plasma concentrations. The individual proteins apparently are able to differentiate the differences at the polyurethane interfaces. There were also some significant differences between two series of polyurethanes: the low, hard segment and high, hard segment series.

It is interesting that most of the proteins which exhibited some differences, or "specificity," among the materials have not been extensively studied for their adsorption characteristics. As a result of these observations, there is now considerable interest in the role of hemopexin, a plasma transport protein which complexes heme. It has some structural and compositional homologies with the more well-known plasma protein, vitronectin [80]. Vitronectin is difficult to study by this technique because apparently it does not stain efficiently in these two-dimensional gel preparations. The apparent compositional similarities between vitronectin and hemopexin [80,81] and the well-known propensity of vitronectin to influence blood compatibility suggest that the adsorption behavior of both proteins is worth studying in greater detail. The adsorption of apolipoprotein A-4 and apolipoprotein A-1 indicates that low-density lipoproteins adsorb as intact units and that more attention should be given to the role of the interfacial activity of lipoproteins.

There was a strong correlation between the depletion or adsorption of specific proteins and the hard segment domain size, with maximum depletion ocurring on those samples with hard segment domains in the range of 100 Å. There was also a strong correlation with soft segment block molecular weight, with maximum depletion correlating with a relatively low molecular weight of 1,000 for the tetramethylene oxide materials.

Others, particularly Takahara, have observed strong correlations between platelet adhesion and the morphology and composition of polyurethanes [82]. It has been suggested that phase purity may be one of several important parameters determining platelet interactions with polyurethanes. Okano [106], Gibbons [107], and Sakurai [108] have also shown correlations between micromorphology and protein adsorption.

The general hypothesis is that there may be some matching or "specificity" between the surface domain characteristics of polyurethanes and the major structural and functional domains of some of the plasma proteins (Fig. 10). Perhaps it would be instructive to look at the domain structure of fibrinogen [83], apolipoprotein A1, hemopexin, antithrombin 3, and complement C3.

Tingey also utilized the "Tatra-Plot," discussed in the early part of this chapter, to search for correlations between a number of the key variables. These plots showed that phase purity, theoretical hard segment domain size, and hard segment surface composition all correlate strongly with total protein adsorption and with platelet adhesion. The correlations were different in the two major

compositional systems studied, suggesting that different mechanisms of protein adsorption and platelet adhesion may be involved in the two polyurethane series.

The conclusion we draw from this extensive study, whose data are still being digested and interpreted, is that surface compositional and surface property differences, which may be considered quite subtle by classical interfacial characterization, can result in significant differences in protein adsorption. Indeed, in the interaction with a complex protein mixture such as dilute plasma, there is some specificity in the interaction between certain plasma proteins and certain polyurethane compositions. These interactions correlate with the microphase separation properties of the polyurethanes. This has led to the domain interaction or *domain specificity hypothesis* that, although expressed earlier for cell interactions with multi phase surfaces [84], also hold for protein interactions *if* the phase dimensions are in the range of proteins or of domains within proteins.

VI. THE DOMAIN MODEL OF FIBRINOGEN

The insight provided by a domain analysis of human albumin discussed earlier, together with our other data and experience indicating that protein structural domains may play important roles in their interfacial activity, led us to examine the structural nature of fibrinogen to relate it to its potential interfacial properties. This was recently accomplished by Feng (Fig. 7) leading to a number of specific hypotheses with which to evaluate the large volume of fibrinogen adsorption data in the literature [83].

This approach can be extended to most other proteins of interest. Although ideally one needs to know the three-dimensional crystal structure of the protein, considerable insight into mechanisms of interfacial behavior can be derived from consideration of a good cartoon model. Knowledge of the interfacial activity of vitronectin, laminin, fibronectin, fibrinogen, and albumin can be enhanced by the structural information available [85]. Modern sequence analysis and structural homological tools make such a task practical and efficient today.

VII. CONTINUING PROBLEMS, NEW HYPOTHESES

Although there is much that we know qualitatively about protein interfacial processes, there are many important topics which must be qualitatively understood before we can assemble a comprehensive model and theory.

A. Chemical Reactivity

There is an implicit assumption throughout most of the protein adsorption field that only secondary interactions are involved in the adsorption process, that is,

that primary, covalent chemical changes do not occur. Such an assumption is probably invalid, even for the simplest model systems [86]. It is well known that pH and ionic strength within 20 Å of an interface are dependent on the nature of the solid surface, as well as on the properties of the bulk solution. Interfacial properties change during the course of adsorption or other interfacial modification. That means that a protein colliding with such an interface, or already deposited on the surface, is in an ionic environment very different from its counterpart in the bulk. A protein on a hydrophobic surface, for example, finds itself in the midst of an enormous dielectric constant gradient, ranging from as low as 2 or 4 on the hydrophobic surface to a value in excess of 80 in the aqueous ionic solution. This, of course, leads to changes in the ionization of ionizable amino acid residues, changes in electrostatic interactions, both intra- and extramolecularly, and, of course, dramatic changes in the nature of solvent-based hydrophobic interactions [87]. These changes can be as great as those in enzyme-active sites, which are well known for their potential to produce covalent bond changes in specific susceptible molecules.

In addition we have already established that adsorption processes can induce enzymatic activity in proenzymes (zymogens) by virtue of conformational changes during adsorption. Depending on the orientation and mobility of such adsorbed enzymes, they in turn may act on other colliding proteins, or even on their adsorbed neighbors, to elicit chemical changes and even chain cleavage, changing the chemistry of the bulk system as well as the interface. There is evidence of such processes in the literature [88,89], although most studies today do not consider such artifacts.

It is now also well known that the high concentration of proteins at the interface leads to a chemical potential very different from the more dilute solution phase. Such macromolecular *crowding effects* can lead to a set of interactions involving aggregation, assembly, or other effects [90–92].

B. Surface Diffusion and Surface Mobility [93–95]

Molecules in the adsorbed state may have considerable two-dimensional mobility, dependent on the strength of their adsorption, the number and mobility of their neighbors, and other factors. Although molecules are confined to an interface by adsorption and/or solubility considerations, they can diffuse along the interface. Adsorbed molecules, even proteins, are not necessarily fixed or static. They may have considerable interfacial mobility. Polymer molecules may actually translate by two-dimensional random diffusion. Because there are regions or patches of the surface with less affinity than other, two-dimensional diffusion may be far from random, and may even be somewhat directional in nature. Adsorbed molecules may have considerable dynamics—various domains may be unadsorbed. There may be particular loops or segments of the polyamino acid chains that are still flexing into solution, unperturbed by the adsorbed state

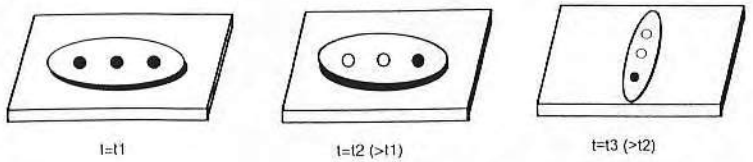

Figure 11 A hypothetical mechanism for surface (lateral) diffusion/translation. Each individual attachment site ("foot") is potentially reversible. ● represents a bound site; O represents an unbound site on the protein. As one or more sites are statistically released (desorbed), the protein may rotate or pirouette around the remaining attachment site until pinned down again by multiple (cooperative) sites (see Ref. 93).

of the rest of the molecule. These motions, and this lateral mobility, may contribute to the exchange process, to the kinetics of adsorption, and to the nature and shape of adsorption isotherms. A protein may be adsorbed strongly by one domain with the rest of the molecule largely unadsorbed. The adsorbed protein may rotate or pirouette on the surface [93] (Fig. 11). Depending on the asymmetry of the molecule and the nature of its adsorbed state, the adsorbed protein may indeed sweep out a much larger area than expected from the size and shape of the molecule in solution. One can even envision the protein statistically "walking" along the surface, due to the reversible and statistical nature of the on and off rates and the fact that different sites or domains on the molecules are involved in the adsorption process.

These effects are more pronounced at surfaces and interfaces which are themselves dynamic, such as lipid bilayers, synthetic elastomers, and related materials with considerable mobility at room and body temperature. The importance and role of membrane protein diffusion is becoming increasingly recognized [94,109]. It is likely that similar behavior will be recognized as important in protein adsorption.

C. Intermolecular Interaction Coupling

We normally treat intermolecular forces and interactions as separable, summable processes. We like to calculate van der Waal's interactions, electrostatic interactions, steric exclusion interactions, and even hydrophobic interactions without really considering the effect of any one interaction on another [96,97]. We are beginning to realize that this is far too simplistic, although convenient. This problem is addressed to some extent in the voluminous literature on the direct measurement of interaction forces between surfaces using the molecular forces apparatus; it is best illustrated and expressed by the recent work of Urry and co-workers, who show that the nature of the electrostatic

interaction in polyamino acids is greatly influenced and perturbed by nearby hydrophobic activity, and vice versa [87,98].

It has become common to take a model protein of known three-dimensional structure and calculate its interaction energy with a model surface, using modern computer molecular graphical software and algorithms [99,100]. However, the potential functions used in the software for such calculations are empirically derived from a very large data base [15], utilizing environments very different from that at a solid surface. Furthermore, the potential functions required are likely to be very different for the parts of the protein immediately adjacent to the solid, compared with those portions of the protein more in equilibrium with the bulk solution phase. This may be related to the concept of the Z-dimension gradient, now being considered by Matsuda [101]. Not only is there a gradient in dielectric constant, noted earlier, but there is a gradient in protein mobility as one moves outward from the surface to the bulk phase. This means that gradients exist in steric exclusion, intramolecular dynamics, ion exchange, and, of course, electron and ion conduction.

The models for adsorbed proteins which we apply to interpret various experimental techniques, such as double layer capacitance, ellipsometry, and evanescent wave spectroscopies, assume a homogeneous protein layer, a homogeneous refractive index, a constant dielectric constant, and constant density. It is now very clear that those assumptions must be challenged.

D. Statistical Specificities

We have established that most practical solid surfaces are not homogeneous. We know that protein "surfaces" are also far from homogeneous. Domain heterogeneity and interdomain interactions, coupled with specific functional group complementarity, lead to the concept and the potential of *statistical specificity* (Fig. 10) [86]. That is, *there is no such thing as a nonspecific interaction, nor is there any such thing as a purely specific interaction*, although biochemistry has come very close. There is a continuous spectrum of interactions ranging from the relatively nonspecific to the highly specific. Statistics and probability apply throughout the entire interaction spectrum. We are now calling the very large region between ideal nonspecific processes and the highly heterogeneous specific processes the regime of *statistical specificity* [86]. This is a well-known field in modern synthetic organic chemistry, dealing with the development of synthetic approaches to molecular recognition and artificial enzymes.

E. Cell Reprocessing

We have ignored cells in this discussion, choosing to focus exclusively on noncellular, nonliving solutions and systems. It is clear, however, that as one moves into the more complex environment of cellular systems and intact organisms, the level of complexity and uncertainty increases dramatically. In addition

to their own array of interfacial activities and processes, cells can modify their environment by secreting solutes, including ions and macromolecules, and can generally reprocess any particular interface to their preconceived liking. One must, therefore, be unusually cautious in extending any so-called "principles" of protein adsorption to these more complex environments. Fortunately there is increasing activity in monitoring the nature of and changes at an interface directly beneath a cell attached to a solid surface. Such studies are likely to have enormous impact on the understanding of the interactions between tissues and materials, including bioadhesion, biomineralization, implant fixation and integration, wound healing, biodegradation, and related topics.

VIII. A PLEA FOR THEORY

We need theoreticians who are not afraid of complexity, and who can deal with the multivariate and multidisciplinary aspects of these complex problems.

The study of proteins at interfaces has been, almost exclusively, an experimental, empirical field. There is an enormous quantity of data available, most of it minimally interpreted, and most of it largely uncorrelated and unused for developing broader pictures which can lead to a more comprehensive understanding of the field. We need individuals who feel comfortable with complex multicomponent systems, who feel comfortable with the structure and nature of complex proteins and other macromolecules, who feel comfortable with surface and interfacial chemistry and physics, and who feel comfortable with the thermodynamics and kinetics of biochemical solutions. They must also be particularly well versed in the mathematical techniques of complexity and uncertainty. We need one or more individuals who can do for proteins at interfaces what Pierre deGennes, for example, has done for polymer science and for certain aspects of synthetic macromolecules at interfaces. Indeed, deGennes, himself, may be starting to address this problem [102]. Others who have addressed components of this overall problem include Dill [103], Sevastianov [110], Talbot [104], and Willems [105]. We urge them to continue!

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COMMENTARY

by J.D. Andrade

Science Without Walls: Science In Your World

How should one teach nonscience majors science? In the modern university, nontechnical majors are, almost by definition, majors in the fine arts, the humanities, or the social sciences. Graduates from nonscience/nontechnical programs will not find work in laboratories, nor will they wear white lab coats or be involved with technical apparatuses, manipulations, or calculations. Their interaction with science will be in their everyday world. They should experience science in their university courses in a manner and environment that are indeed relevant to *their* everyday world—which is not necessarily the world of science or engineering faculty.

"Science Without Walls: Science in Your World," a video-intensive telecourse, is designed as an integrated, coherent, interrelated science experience for undergraduates not intending to major in science or engineering. No such course or project has previously been attempted, to our knowledge, although the book by James Trefil and Robert M. Hazen, *The Sciences: An Integrated Approach* (New York, John Wiley & Sons, 1995; 2d ed. 1998), has similar objectives.

The content was organized into 40 half-hour programs in six general sections or units: Science and Art; Physics; Chemistry; Biology; Earth; and You! To get to the wider student- and general-population audience, the course was developed for television and is now regularly broadcast on Utah's statewide educational TV channel. It uses video segments to illustrate and demonstrate processes and phenomena. The objective from the very beginning was that, wherever possible, video clips would be on the screen rather than a professor's talking head.

The design and content of the course were based on a number of pedagogical strategies. Students learn best and most effectively when the content is practical and directly relevant to their everyday needs and lives. To *experience* science, one has to *do* science. Science cannot be learned or appreciated in a spectator role. Most laboratories and researchers' technical jargon reinforce students' preconceptions that science is different from and unrelated to their interests and their world.

We minimize the use of formal laboratories, emphasizing kitchens, bathrooms, garages, and the natural outdoor world. Scientists are treated as informal, friendly, fallible, and human—and they don't wear white coats! Homework and personal laboratory experiments emphasize involvement with local museums and related institutions. Assignments also involve interaction with public and other agencies and sources as well as direct communication with local, accessible professionals, such as pharmacists and physicians.

The Lableless Lab for "Science Without Walls" is a small science kit of generally available materials that the students use to conduct the experiments and observations associated with each of the 40 programs. There always has been considerable concern in offering science or other experience-based courses via television with the argument that students cannot gain the hands-on experience normally required in the laboratory components of on-campus courses. This is certainly true, but everyday materials and living situations can be far more relevant and meaningful than a formal or standard laboratory.

The normal high school sequence for the teaching of the sciences—biology to chemistry to physics—is inappropriate and illogical. We use the sequence of *first* physics, *then* chemistry, and *then* biology. This is because physics provides the fundamental rules and laws of the natural world, upon which both biology and chemistry are dependent. Chemistry provides the understanding of the elements, the molecules, and the materials of the natural world, upon which biology is dependent. Biology, although a unique science, is dependent on the rules and understanding derived from both physics and chemistry.

The various sciences are historically treated as distinct and separate in high school and even in junior high, divorced from the students' everyday world. Science must be viewed and experienced in the context of the nonsciences for nonscience students to accept and understand the relevance of science to their everyday lives. Nonscience students are interested generally in the fine arts, the humanities, or the social sciences; thus, science must be made relevant to these disciplines and areas of study. There is particular emphasis in "Science Without Walls" on the connections and similarities between the sciences and the arts.

Students need heroes and role models. They need people and individuals with whom they can identify and whom they can emulate. We have made extensive use of individual personalities.

A unique aspect of the course is an emphasis on music. Each of the programs concludes with music tied to the content of that particular program. The pedagogical rationale here is that most students are interested in music, particularly various forms of popular music. If they can begin to see and experience the connections between science topics and the music to which they listen everyday, they will start to appreciate science and its connections to their everyday lives.

"Science Without Walls" shows that students must be responsible. University telecourses tend to attract older students with a myriad of commitments and responsibilities. The course is targeted to adults, with the goal of empowering them to act as concerned, literate, educated members of a democratic society. The course gives them the background and motivation to become appropriately involved with such issues.

The major objective of "Science Without Walls" is to provide minimum scientific literacy for the general population, including university undergraduates. The goal is not to make scientists out of them or to teach them to solve physics or chemistry problems, but to get them to understand the basic concepts and themes that underlie our natural world and to provide them with the background and confidence to take additional science courses and to become involved in the scientific and technological issues important to their nation, state, community, and family. ●

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CHEMICAL SENSORS IN THE HOME: Discussion Paper

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Many medical conditions require the measurement of one or more biochemicals in order to facilitate diagnosis, manage a disease or condition, or monitor a treatment. Physicians and other health care providers regularly order a range of chemical tests, generally performed on blood or urine. The samples are generally obtained by the health care provider and are sent to centralized analytical laboratories, which perform the analyses and report the results. Historically, the techniques and procedures involved require skilled personnel and specialized instrumentation.

In the last several decades analytical and clinical chemistry has developed to the point where many useful analytical measurements can be made using relatively simple and inexpensive instrumentation and often by unskilled personnel. Some of these technologies have now become over the counter, readily available, kits and instruments for home use. The most common of these is quantitative glucose measurement, used regularly by millions of Diabetics throughout the world. Using a microlancet to generate a small (50-100 microliter) blood droplet, the patient transfers the blood sample to a dip stick device, which serves to collect the sample, performs needed separation steps, and delivers the sample to one or more analytical zones in which a specific chemical reaction is carried out, resulting in a signal which is read by a small, inexpensive analytical instrument. In the case of diabetes, glucose specific dipsticks are used and small hand held reflectance colorimeters, commonly called Glucometers, are employed with acquisition costs in the range of $30-100 or more.

The individual Dipsticks cost in the range of 50 cents to $2.00, depending on the manufacturer and quantity considerations. In addition to the enzyme based colorimetric assays used for glucose, immunoassays can also be employed, the most common of which is the over the counter pregnancy test. The current over the counter cholesterol test is an enzyme based colorimetric system.

The major Diabetes Control and Complications Trial (DCCT) recently documented the enhanced health benefits of tight glycemic control for diabetes. Regular monitoring of glucose and regulation of insulin intake leads to much more effective management of the disease and the minimization of the chronic complications which are so burdensome to both the patient and to the health care system.

The diabetes community is leading and driving major research and development activities to further improve the measurement and monitoring of glucose and of other metabolites important to diabetes, with an emphasis on sampling methods which do not involve the trauma and discomfort of blood sampling. There is a move towards the use of interstitial fluid as the analytical sample and even to the development of truly non-invasive methods of analysis. Considerable research and development is now being focused upon minimally invasive approaches for obtaining samples of interstitial fluids

"Home Care Technologies for the 21st Century", workshop co-sponsored by NSF and FDA, April 7-9, 1999, Rockville, MD (www.hctr.be.cua.edu).

Antimicrobial/ Anti-Infective Materials

Principles, Applications and Devices

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Toward Dollar Devices for Measuring Metabolic Biochemistry

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INTRODUCTION

LIFE BEGAN ON this planet roughly four billion years ago. Those very early, very primitive life forms developed around a phosphate-based biochemistry. Molecules involving phosphates became the basis of the energy currency of all known life forms on the planet. Similar phosphate-containing molecules became the basis of RNA and DNA, the information storage macromolecules that permitted those life forms to replicate [1].

Life as we know it involves four general classes of low molecular biochemicals: carbohydrates, amino acids, lipids, and nucleotides. The synthesis and degradation of those chemicals, the anabolic and catabolic processes that are the basis of all biochemistry, are dependent on the two key molecules of energetics: ATP (adenosine triphosphate) and NADH (nicotinamide adenine dinucleotide). These molecules are the fundamental basis of bioenergetics and, therefore, of all life as we know it, ranging from the most primitive, simple bacteria to the most recent primates [1,2].

The application of ATP- or NADH-specific bioluminescence reactions to the monitoring and measurement of various analytes is certainly not new [3,4]. The major application of bioluminescence for analytical purposes has been in bacteria hygiene monitoring [5]. This application is perhaps the most relevant for this volume. It is extensively developed, and the reagents, instruments, and methods are available from numerous

sources. Basically, one samples a surface or solution with suspected bacteria contamination and induces those bacteria generally through appropriate surfactant/detergent treatments to release their intracellular ATP, which is then, almost instantaneously, detected by the firefly luciferase reaction. "Hygiene monitoring" by this method is well known and widely applied. The main problem and limitation is that this particular approach does not lend itself to bacterial speciation, and it is also somewhat difficult to distinguish bacterial from non-bacterial cells by this method. Our emphasis is on the use of bioluminescence for the measurement of specific analytes. Thus, we will not discuss generic ATP-based hygiene monitoring any further.

Biochemical reactions involve enzymes and a small set of coenzymes, generally derived from vitamin precursors. The analysis and monitoring of the biochemistry of any life form depends on the ability to measure, specifically and quantitatively, the low molecular weight metabolites and the enzymatic reactions that facilitate that biochemistry. It is interesting and quite surprising that there are no simple, inexpensive means by which to measure and monitor the key metabolites of living systems [2]. Generally, their measurement requires analytical instruments and techniques: kilodollar spectrometers or electroanalytical instruments; tens to even hundreds of kilodollar high-performance liquid chromatographs or spectrophotometers; and nearly megadollar mass spectrometers. The only significant exception is the glucose dipstick and its companion glycometer, a small, relatively high-performance colorimeter that permits the quantitative measurement of glucose in a small drop of blood, used by hundreds of thousands of diabetics throughout the advanced world. It is the high incidence of diabetes in a relatively affluent part of the world that has encouraged many companies to invest millions of dollars in the development and manufacture of simple, inexpensive, high-performance analytical instruments, focused almost exclusively on glucose.

Although there is considerable interest in the monitoring of specific carbohydrates, amino acids, and other "nutrients" important to the biochemical process and biotechnology industries, the instruments required generally cost several thousand dollars or more, and each analyte of interest requires a special sensor, probe, electrode, etc., generally costing several hundreds of dollars.

Our goal is to provide means for the simple, quantitative, direct analysis of carbohydrates, amino acids, vitamins, and other low molecular weight molecules of interest to metabolism, metabolic abnormalities, nutrition, sports and physical performance, and related areas, including the biotechnology and bioprocess industries.

Our approach is based on a relatively well known but little used curiosity of biology: bioluminescence [3]. The bioluminescence in fireflies is based on an enzyme-catalyzed oxidation reaction utilizing ATP as a highly specific co-reactant. The bioluminescence of marine bacteria is closely coupled to an NADH-dependent enzyme reaction. Thus, mother nature has literally handed to us two unique, different, ultrasensitive, and highly specific reactions for the measurement and monitoring of ATP and/or NADH. The readout is photons, green and yellow in the case of the firefly reaction and blue in the case of the bacteria process. The reactions are highly sensitive to and quantitative for ATP or NADH over a five or more order of magnitude concentration range [4]. Because all of biochemistry depends on ATP or NADH, practically all biochemical reactions can be monitored via bioluminescence.

There is a large body of literature on the development of biosensors for ATP and ATP-dependent processes and for NADH and NADH-dependent processes, using the firefly and bacterial luciferase enzymes, respectively [5–9]. Such biosensors generally employ fiberoptic or other wave-guided means of delivering the luminescence to a device that can accurately measure light intensities [6,7].

For many of the analytes of interest, the bioluminescence is intense enough that the unaided eye can serve as the analytical instrument. In most other cases, a relatively inexpensive luminometer will suffice. For very low concentrations of analytes, in the pico to subpicomolar range, a more sophisticated photon counting luminometer is required.

We are now in the process of developing "dollar devices" for the analysis of galactose [10], phenylalanine [11], homocysteine [12], and lactate [13]. Others will follow.

A "SIMPLER" APPROACH TO BIOLUMINESCENCE-BASED ANALYSIS

Although bioluminescence analysis is well known and has been used regularly in research, analytical laboratories, and clinical laboratories [3,4], it has not been widely applied outside of those specialty areas for several reasons:

1. The exquisite sensitivity for very low ATP concentrations has encouraged the application of the technique to those problems where such sensitivity is indeed needed. Thus, it has acquired the reputation of an ultra-sensitive technique and has not been seriously considered for the measurement of analytes in the micromolar to millimolar range.

2. The luciferases and other reagents involved have developed a reputation of being somewhat labile, unstable, and perhaps difficult to utilize.
3. The nature of the bioluminescence reaction, and in particular its complex kinetics, made it necessary to develop rapid mixing techniques and to utilize an instrument capable of sensing a flash or short pulse of light. Application of trace concentrations required, in addition, a highly sensitive, and therefore relatively expensive, luminometer. Thus, the technique evolved a reputation for requiring an expensive instrument and a precise and somewhat sophisticated analysis protocol.
4. The widespread application of the firefly luciferase reaction to the monitoring of very low concentrations of ATP released from bacterial and other cells in hygiene-monitoring applications [5] led to a mysterious or "black magic" reputation because of the "cocktails"—the surfactants, detergents, and other agents required to disrupt cell membranes—needed to release the ATP. Those same reagents, of course, denatured and inactivated the luciferase involved; thus, these processes always involved a delicate balance and a careful optimization, and were often difficult to carry out in a reliable and reproducible manner.

About five years ago, we became convinced that ATP-based firefly luminescence and NADH-based bacterial bioluminescence could serve as a highly specific and sensitive means of monitoring metabolism. We began to develop an ATP detection platform that would obviate or minimize the problems noted above. This platform has been under development for the past several years. We are now in the process of developing an NADH detection platform. Our approach is based on the following considerations:

1. The biotechnology community knows how to express, produce, and purify proteins via simple organism cultures and processes. Indeed, recombinant firefly and bacterial luciferases have been known for several decades now, and recombinant firefly luciferase is commercially available.
2. The biotechnology and protein pharmaceutical industries have learned how to formulate, passivate, store, and reconstitute proteins and enzymes with considerable retention of activity [8]. We addressed the instability of firefly luciferase [9] using our experience, understanding, and control of the denaturation of proteins at interfaces and in solution [14].
3. A reaction that actually produces photons has many advantages. One does not have the problems associated with color perception, as in the case of reflectance colorimetry. One does not require a light source, as in the case of fluorescence spectroscopy. One does not require electrodes and their tendencies to become contaminated or to participate in side reactions, as in the case of much of analytical electrochemistry.

Practically all scientists, laboratory technicians, and even patients come equipped with two ultra-sensitive, portable, reliable, and inexpensive photon detectors: their own eyes. We realized, however, that most bioluminescence analysis is dependent on the measurement of an intensity [4], although the total number of photons, the integral of the intensity-time curve, can also be used. We know that the human eye is incapable of integrating photons. The eye is also a highly variable and, therefore, unreliable detector of photon intensity. The human eye's incredible ability to accommodate, to adjust its sensitivity to photon flux, makes it very difficult to calibrate and use as an analytical or quantitative measure of photon flux. Also, the human eye's exquisite photon sensitivity is really only manifested under dark adaptation conditions, which require 20 to 30 minutes of accommodation time for maximal sensitivity [15].

We appreciated, however, that the human eye is unsurpassed as an imaging device—as a position sensitive detector—with sophisticated and sensitive means to perceive changes or differences in relative photon yields as a sensitive function of position. We, therefore, undertook a means to transform the quantitative analytical signal from one relying on relative intensity to one relying on relative spatial position. Our current work involves both approaches, the more or less conventional intensity-based approach and a spatial or position approach.

THE ATP PLATFORM

The intensity approach is quite straight forward and has been used for decades, generally for specialized high-sensitivity analysis in clinical and research laboratories [3,4]. Reagents and even kits are available from many manufacturers, for example, Sigma Chemical Company.

Our group's growing interest in patient empowerment and cost-reducing healthcare technologies [16] led to a consideration of means by which a sensor could be produced that could be read without the need for an instrument, i.e., by the patient's own eyes. The problem, of course, was the human eye's astonishing ability to accommodate to changes in intensity, thereby making it a very non-objective intensity detector. The trick was to display the signal in space.

Although we cannot present the details of this approach here, the technology can best be appreciated using the metaphor of a glowing thermometer, where the length of the glow is proportional to ATP concentration. As long as the intensity of glow is sufficient to be recognized above the ambient light background, the observer can observe it and determine the position at which the glow ends. Adjacent to the glowing "thermometer" is a calibrated scale that reads out directly in ATP concentration. Thus, it is not the intensity of the luminescence that counts, but its position in space. The human eye is exquisitely designed to see positions in space, i.e., patterns, and has a variety of contrast enhancement mechanisms with which to facilitate such spatial discrimination.

We, therefore, have two different ways of measuring ATP. One is by measuring the absolute intensity of the glow, which can be made proportional to substrate concentration. This is the standard approach. It generally requires an analytical instrument, a type of luminometer that can objectively measure intensity. The second means, the spatial position of the glow, can be detected by an imaging device, such as a diode array, a CCD, or the human eye. These two different approaches can be coupled in the same sensor/device for increased reliability and sensitivity.

The same approach can apply to the measurement of NADH using a bacterial oxidoreductase/luciferase enzyme reaction. This, thus, leads to a generic ATP sensor and a generic NADH sensor. With these generic quantitative sensors in hand, one can then go forward to produce sensors specific for analytes which can be coupled to ATP or NADH.

SUBSTRATE SPECIFIC SENSORS

The simplest substrate-specific sensor is one in which the enzyme reaction produces ATP:

$$\text{substrate} + \text{ADP} \xrightarrow{E} \text{product} + \text{ATP}$$

A good example is the transformation of phosphoenolpyruvate (PEP) to pyruvate. The ATP sensor then measures the ATP produced, which directly correlates with substrate concentration.

Another typical reaction involves the consumption of ATP:

$$\text{substrate} + \text{ATP} \xrightarrow{E} \text{product} + \text{ADP}$$

In this case, the ATP sensor measures the decrease in ATP concentration; thus, light intensity correlates inversely with substrate concentration. A good example of the phosphorylation of galactose (Gal):

$$\text{Gal} + \text{ATP} \xrightarrow{\text{Galactokinase}} \text{Gal-1-P} + \text{ADP}$$

The galactokinase reaction can be carried out for some time, and the resulting ATP concentration then can be measured by the firefly luciferase reaction. This is a sequential or series sensor. The two enzyme reactions can also be used at the same time (a homogeneous or parallel sensor), with both enzymes competing for ATP. Because galactokinase is a much faster enzyme than luciferase, the simpler homogeneous approach is appropriate for a Gal sensor (Figure 1). Although the results are not linear, a simple function allows the integrated intensity to relate to Gal concentration.

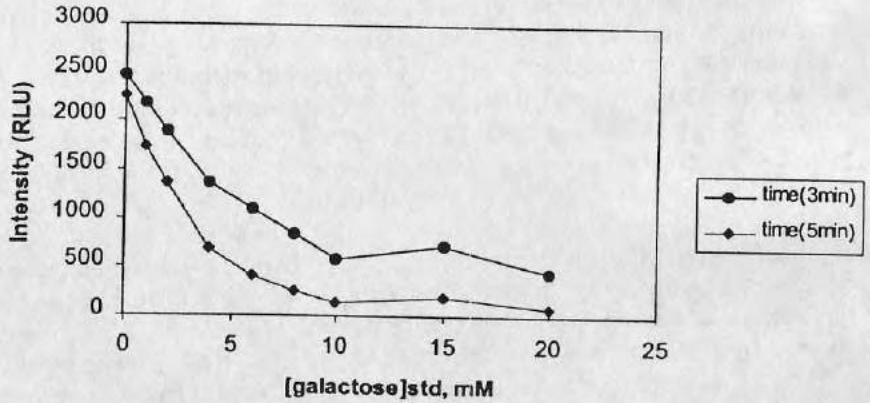

Figure 1. A preliminary galactose assay based on ATP depletion. Calibration curves for two different sampling times, 3 mins and 5 mins. Galactose standards were prepared in 0.025M gly-gly buffer, pH = 7.8. Assay conditions: [ATP] = 10 μM, [luciferin] = 25 μM, and [Mg^{++}] = 2.5 mM, [galactokinase] = 3.8 × 10^{-9} M (M.W. = 58,000), [luciferase] = 7.3 × 10^{-9} M (M.W. = 62,000), and [BSA] = 0.25 mg/mL. ATP, luciferase, galactokinase were mixed in 12 × 50 mm polypropylene test tube; galactose standards were added immediately after the addition of luciferin and Mg^{++}. Bioluminescence was recorded with a Turner Designs TD 20/20 luminometer for five minutes. The data points are the average of two experiments.

The "problem" is that the galactose sensor needs to operate in a fairly high galactose concentration range to be useful for the diagnosis and management of galactosemia, an inborn error of metabolism that afflicts one in every 50,000 newborns [10]. The ATP luciferase assay is already largely saturated in that ATP concentration range, so the sensor has to employ various design modifications that allow the ATP sensor to be responsive to the ATP concentration range encountered in the galactose/ATP reactions.

Perhaps the best example of a sensor utilizing the NADH detection platform is lactic acid or lactate, a key product of anaerobic glycolysis [13].

A lactate sensor was chosen as a model system, because lactate is important in clinical analysis, food analysis, and sports medicine [2]. Lactate monitoring using bacterial bioluminescence has many advantages, including simplicity and speed. The governing reactions are:

$$\text{lactate} + \text{NAD}^+ \xleftrightarrow{\text{LDH}} \text{pyruvate} + \text{NADH} + \text{H}^+ \quad (1)$$

$$\text{NADH} + \text{FMN} + \text{H}^+ \xrightarrow{\text{OR}} \text{NAD}^+ + \text{FMNH}_2 \quad (2)$$

$$\text{FMNH}_2 + \text{RCHO} + \text{O}_2 \xrightarrow{\text{LF}} \text{FMN} + \text{RCOOH}_2 + \text{H}_2\text{O} + \text{light} \quad (3)$$

where NAD is ß-nicotinamide adenine dinucleotide, LDH is lactate dehydrogenase, FMN is flavin mononucleotide, OR is NADH:FMN oxidoreductase, $FMNH_2$ is reduced flavin mononucleotide, RCHO is decanal, and LF is bacterial luciferase. NADH formation is catalyzed by LDH. Light is emitted after the serial reactions by OR and LF. The light intensity is proportional to the rate of NADH formed, which is proportional to lactate concentration in the solution. The inhibition effects of lactate, NAD, and pyruvate on the bioluminescent reactions were not critical. The optimum conditions for a lactate sensor based on bacterial bioluminescence (1 mmol/L lactate) were 10 U LDH, 75 mU OR, and 0.26 mU LF at pH 7.6.

We have discussed the optimal conditions for lactate analysis by bacterial bioluminescence and the interference of reactants and products on the reactions [13,17].

The NADH-based sensors are more complicated as both a luciferase (bacterial) and an oxidoreductase (FMN/NADH) are required. $FMNH_2$ is unstable and must be generated locally so that it can be acted upon by luciferase. The kinetics of the enzyme and other reactions suggest that the two enzymes must be in intimate contact with a high luciferase/oxidoreductase ratio [17].

The bacterial bioluminescence system can be applied as an enzyme sensor for biochemicals related to reduced nicotinamide adenine dinucleotide (NADH). A key problem is completeness of the conversion of

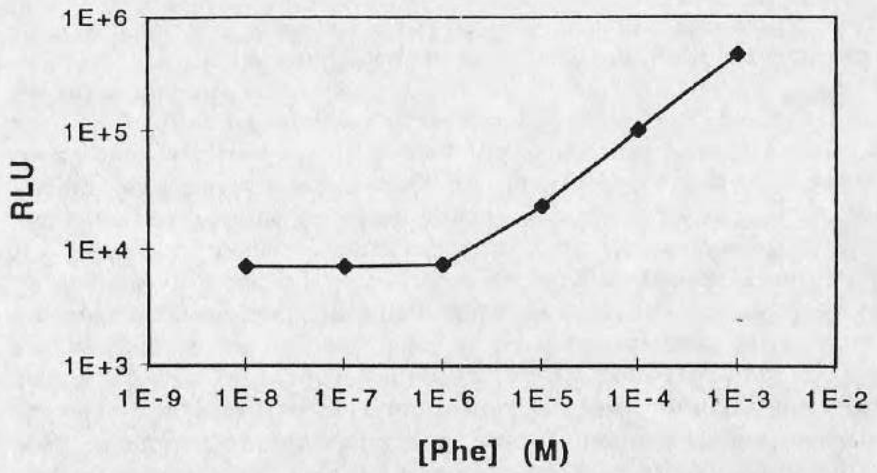

Figure 2. *A preliminary phenylalanine assay based on NADH production. Assays were started by adding the Phe solution to the cuvette containing all necessary reagents, including 0.1 mM NAD, 1 µM FMN, 0.1 m% dodecanal, 0.04 U PheDH, 0.1 nmol OR, and 0.1 nmol LF in 50 mM Phosphate buffer (pH 8.0). The total assay volume was 250 µL. Bioluminescence was measured with a TD-20/20, Turner Designs luminometer at room temperature using the integrated relative light unit (RLU) at 5 min. Both axes are log scale [17].*

reactants to products, because the efficiency of conversion affects the sensitivity. This problem is especially significant in multiple enzyme systems, because each enzyme may have different optimal conditions. The selection of the optimal pH value, the effect of reactants and products on the enzyme activities, and the enzyme concentrations and their ratios are important in the optimization of enzyme sensors [13,17].

The lactate NADH system also works well as a homogeneous reaction system because the LDH enzyme is generally so much faster than the bacterial luciferase. A typical set of results for phenylalanine was given in Figure 2.

Galactose/galactokinase is a model for an ATP-specific analyte sensor, and lactate/lactate dehydrogenase is the model system for a NADH/bacterial luciferase-based sensor.

The sensors can be designed to measure enzyme activity rather than substrate concentration. For the example of galactokinase activity, we would apply the sample of interest to a sensor in which galactose and

ATP were already present. If there was indeed any consumption of ATP in the short term, it would suggest that galactokinase or other ATPase is present. Given the appropriate controls and sample preprocessing, one could then determine the galactokinase activity in the sample. If the sensor includes the appropriate enzyme, it is designed to be specific for substrate, but if it includes appropriate substrate, it can be made to be specific for enzyme activity.

MULTI-ANALYTE PANELS

Metabolism is an extremely complex chemical reaction network [18–20]. Although most of the individual reactions and the substrates, products, and enzymes involved are fairly well known, we are only beginning to appreciate the interdependencies of these various reactions and the overall complexity of the system, even for very simple organisms. Although the field of metabolic "engineering" is now developing [18–20], using the most sophisticated chemical engineering and biochemistry-based methods and insights, we are a long way from developing even a primitive understanding of the system.

It is, therefore, clear that just as "no man is an island," no analyte can be considered individually. To effectively monitor, understand, and possibly regulate a particular segment or part of metabolism, one needs to look at a set of analytes, including not only low molecular weight substrates and products, but also measures of specific enzyme activities. There is growing interest in disease-specific panels. This approach is already widely used in clinical medicine and clinical chemistry [2].

SAMPLES

The sensors we have described are not designed for on-line or continuous monitoring [6,7] Rather, they are designed for discrete samples, measured using a simple disposable device. Both the device and the sample are discarded. In industrial biochemical processes and in many biotechnological processes, the analyte sample is simply the culture or the perfusion medium being used.

For medical and clinical purposes, the sample of choice is generally blood, usually derived from a simple lancet-based fingertip, earlobe, or heal prick. Modern micro-lancets are almost painless and can readily generate a 100 to 200 microliter droplet, adequate for the devices described, even for a multi-channel device or panel.

It would be far more desirable to use a truly non-invasively derive sample, such as urine or sweat. There are many analytes in urine tha are appropriate for monitoring. Urinalysis is a highly developed are [2]. Indeed, a variety of semi-quantitative, multi-channel urine dip sticks are readily available. Occasionally, metabolites are excreted i urine, which may reflect the concentration of a blood analyte. Urin does have the problem that it can be dilute or concentrated, dependin on the time the sample was obtained and the nature of the individual' water intake. This problem can be partiallly alleviated, however, by th routine monitoring of creatinine and by the normalizing of urine ana lyte values to creatinine concentration. This is how most normal val ues for urine analytes are reported [2].

One reason why urine is not used for wider range of analytes is because data are not available for the correlation of analyte concentrations in urine and in blood. Blood is usually the sample of choice because it is essentially an instantaneous reflection of metabolic biochemistry, whereas urine, sweat, saliva, and other non-invasively derived fluids are not in equilibrium with blood or plasma. There are many analytes in sweat, and sweat may be particularly useful in the sports or physical performance arena.

OTHER TECHNOLOGIES

There are many other technologies useful for biochemical measurement and metabolic monitoring. Much of metabolism utilizes redox enzyme reactions, most of which couple to or through NADH. One can directly measure the resulting redox current or utilize that current to produce another product that can be readily monitored, either electrochemically or through some other means. For those analytes for which antibodies can be readily generated, highly sensitive and specific immunoassays can be used. This is the approach used in over-the-counter pregnancy tests. Immunoassays are generally unavailable and impractical or inapplicable to the monitoring of very low molecular weight metabolites. It is impractical to generate antibodies against simple carbohydrates and amino acids because such antibodies are simply undesirable biochemically and biologically. Although various tricks may be used to help generate such antibodies, generally immunoassay is not an appropriate technology for very low molecular weight metabolite analysis.

There are highly sophisticated separation methods for analysis, such as the use of mass spectrometry and gas/liquid chromatography

for carbohydrate and/or amino acid analysis. These techniques are clearly of a great multichannel nature. Once the separation conditions are worked out for the individual molecules, the methods are highly specific and sensitive, permitting complete profiling of literally hundreds and even thousands of analytes. However, such instruments are expensive, often difficult to use, and inappropriate for the typical small laboratory or home environment.

CONCLUSIONS

We have described means for the specific and sensitive measurement of common low molecular weight biochemicals using a simple, robust, specific, and highly sensitive set of bioluminescence-based technologies. Common analytes in the millimolar to micromolar range can be detected and measured using a disposable analytical device that can be directly read by the operator or patient. The devices have the appearance of a glowing "thermometer," with the length of the glow either directly or inversely proportional to the concentration of the analyte of interest. In the micromolar to nanomolar range, the same approach utilizes a simple luminometer, somewhat analogous in application and cost to the present generation of sophisticated glycometers for the measurement of blood glucose. Analyte concentrations in the nanomolar to picomolar or below range require a more sensitive analytical instrument, expected to cost in the range of $1,000 to $2,000.

Our goal is to design and produce disposable analytical devices in the dollar range. We also expect to develop and produce multi-channel, multianalyte devices appropriate to the monitoring and management of various metabolic diseases, sports and physical performance assessment, and nutrition assessment.

ACKNOWLEDEGMENTS

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for glucose analysis. Such fluid can be collected from the skin epidermal layer, which is devoid of blood vessels or nerves. The process is therefore painless and bloodless.

A problem with minimally invasive approaches to sampling is that the volume collected is often one microliter or less and thus considerably smaller than a typical blood glucose sample, generally 30 microliters or more. This presents a considerable challenge for current analytical methods of detection, which in the case of glucose is primarily electrochemical or reflectance colorimetry. There are many groups developing means for interstitial fluid collection and analysis (www.jdfcure.com and www.diabetes.org). It is likely that these efforts will be successful and that truly, minimally invasive, painless means for acquiring samples for biochemical analysis will become available in the very near future.

The problem of sensitivity is also being addressed by a number of groups, including our own. We use a bioluminescence approach to the analysis of glucose and other metabolites in blood, urine and interstitial fluids. Bioluminescence has an advantage over existing approaches to such measurements in that it is generally at least 100 times more sensitive. Bioluminescence is light produced by biological compounds undergoing specific, enzyme catalyzed chemical reactions. The most well known example of bioluminescence is the firefly, however, other organisms employ similar reactions to produce light, e.g., there are also bioluminescent bacteria, fish, and fungi. All bioluminescent reactions employ an enzyme, called luciferase, that makes the reaction possible, e.g. there is a firefly luciferase, a bacterial luciferase, etc. Practically all of biochemistry is linked to two very unique and ubiquitous molecules - ATP and NADH. ATP and NADH represent the "energy currency" of metabolism and are both linked via other enzymes to firefly luciferase bioluminescence and bacterial luciferase bioluminescence, respectively. In our approach, the bioluminescence is detected with an opto-electronic device, often a CCD camera. The amount of bioluminescence measured can be related to the specific biochemical of interest in the sample.

These technologies permit the development of sensors for a many other diseases. Of particular need are the many inborn errors of metabolism, exemplified by Phenylketonuria (PKU) and Galactosemia. PKU is an inherited enzyme deficiency disease which results in the accumulation of Phenylalanine to toxic levels. The disease can normally be managed by careful dietary control and by regularly measuring phenylalanine concentration in blood. Phenylalanine is an essential amino acid, so the patient and his health care provider must walk a fine line between providing adequate phenylalanine for growth and renewal and yet keep the circulating blood concentrations of this essential amino acid in ranges which are non-toxic and non-damaging. This requires frequent blood monitoring, preferably weekly. Given the relatively low incidence of that disease (one in ten thousand), the fact that the current analytical methods require a large volume of blood for analysis (of the order of 200 to 500 microliter), and that there are no home based sampling or analytical methods available, most PKU patients are not optimally monitored or managed.

The situation with Galactosemics is even more difficult. Although Galactosemia is a more complicated disease and is not as easily managed, it is important for Galactosemics to minimize their Galactose intake. The major source of Galactose is lactose in milk and diary products; Galactose is also common in a wide variety of fruits and vegetables. Galactose and Galactose-1-phosphate should be monitored regularly.. But as Galactosemia is even more of an "orphan disease" (incidence one in 50,000), the technologies and resources for convenient monitoring are simply unavailable.

Although industry has responded to the analytical and instrumentation challenges required for the appropriate monitoring and management of diabetes, that is only because it is a relatively high incidence disease. The numbers are such that it is cost effective to develop and market products for diabetes. When a disease has an incidence of one in ten thousand or significantly lower, it becomes extremely difficult to justify any such development or expenditure by a commercial entity. Hence we call such diseases "orphan diseases", as we are now familiar with the term "orphan drug".

It is clear that new and different incentives will need to be developed in order to meet the health care needs of these small segments of the population. The good news is that Diabetes is basically driving the entire analytical biochemistry field with respect to technologies which can be utilized at home.

A major application of home based over the counter biochemical measurement devices is likely to be in the nutrition and food supplement communities. The ability to monitor amino acids levels, vitamin levels, and a range of other nutrients and food supplements is of interest to these major segments of the population. It would be of interest for sports and physical performance enthusiasts, for example, to learn whether or not creatine supplements do indeed influence the circulating levels of this important bioenergetic chemical. Consumers taking mega doses of Vitamin C would learn that such dosages do not need necessarily lead to an increase in the circulating levels of Vitamin C, thus, perhaps, prompting them to modify their behavior or at least minimize their expenditures and intake.

Those empowered consumers and patients who are interested in their circulating blood or urine levels of various analytes can now, via the worldwide web, order such tests from centralized analytical laboratories and obtain such information privately, if they so choose. As the technology continues to miniaturize, as it continues to become more reliable and inexpensive and easy to apply, and as it becomes possible to make such measurements painlessly, using minimally or non-invasively derived fluids, there is likely to be growing interest in the application of home based analytical sensors. There is even talk of incorporating the analytical unit as a PC card or using existing CD drives directly as measurement devices.

The data derived from such measurements can thus easily be incorporated into PC based personal medical advisor software and thereby facilitate personal diagnosis and disease management. The present generation of instruments for the monitoring of glucose already includes the capability for recording and storing the data and for

downloading it on a regular basis to the health care provider. Such capabilities will of course be available in home based analytical chemistry devices for orphan and other metabolic diseases.

The key questions are:
What should be measured?,
How should the data be interpreted and utilized?
How can one develop incentives to facilitate the development of the technologies and instruments required for currently unmet health care needs?
How can these new technologies be incorporated in evolving tele-connected living environments?

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Sung Wan Kim at the University of Utah--the first 15 Years

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In 1969 a young University of Utah Ph.D. graduate in Physical Chemistry accepted a postdoctoral fellowship in Engineering working in the new area of biomaterials and biocompatibility. The young Dr. Kim very rapidly applied his strong physical chemistry skills and intuition -- and his innate creativity -- to that new field. The rest -- as the phrase goes -- is history.

Thirty one years have come and gone since Sung Wan Kim made the voyage from basic Physical Chemistry to Engineering, resulting in an incredibly innovative, creative, and productive career which shows no signs of slowing down.

Shortly after beginning his work in biomaterials and blood compatibility, the Department of Pharmaceutics recognized his skills, and he accepted a position as Assistant Professor of Pharmaceutics. He developed interests and activities in drug delivery. His papers on drug delivery began to appear in the mid to late 70's, initially on permeation in hydrogels.

S. W. Kim was one of the first to recognize the importance of saccharide residues in interfacial recognition and interaction, initiated by his work on the role of adsorbed glycoproteins on blood compatibility.

The heparin work began in the early 80's, as did that the insulin work. Dr. Kim thus developed two parallel, productive, interacting research areas:

blood and biocompatibility;
controlled drug delivery;

both have expanded and continued.

Very early in his career, Sung Wan became involved with the development and application of relevant animal models for testing and evaluation. He worked closely with the Utah artificial heart group on a wide range of blood interaction, shunt, and artificial heart studies.

Already in the mid 80's his papers used the terms bioactive surfaces and bioactive materials. Today these are popular and growing areas of biomaterials science, some 15 years after his pioneering work.

The Okano years began in the mid 80's (the Feijen years began even earlier!) -- resulting in the extensive work on environmentally responsive polymers, the field later called intelligent or smart materials.

The Feijen and Okano collaborations have continued today and on into tomorrow -- attesting to the importance and the strength of international collaboration in Sung Wan's work.

I will not continue the story, because the past 15 years are well known to most of you.

I have had the pleasure and good fortune to work and interact with Sung Wan Kim in that first decade of our careers. It was an exciting time -- working in a new and rapidly evolving area -- learning completely new things in new fields.

Sung Wan Kim has been -- and continues to be -- incredibly productive, creative, and well-funded. In addition to his many international and national awards, the University of Utah has recently awarded him the rank of Distinguished Professor ---the highest rank and award at that institution.

Congratulations, Sung Wan, and thank you for the past 31 years.

PROCEEDINGS

INTERNATIONAL SYMPOSIUM ON BIOMATERIALS AND DRUG DELIVERY SYSTEMS

In Conjunction with

SECOND ASIAN INTERNATIONAL SYMPOSIUM
ON POLYMERIC BIOMATERIALS SCIENCE

In Honor of Professor Sung Wan Kim's 60th Birthday

August 20(Sun) – 22(Tue), 2000
Shilla Cheju Hotel, Cheju Island, Korea

The Korean Society for Biomaterials
The Polymer Society of Korea
The Korean Pharmaceutical Society

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Beyond the Mono-Parameter Paradigm--Looking at the Whole Elephant

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KIM'S ELEPHANT

In the early 80's, during an interview for a local newspaper, Sung Wan Kim told the reporter:

> "A blind man trying to identify an elephant by examining only the trunk faces a nearly impossible task."

TOO MANY TRUNKS –NO ELEPHANTS!

Today Science--and most NIH study sections--insist on single hypothesis, single parameter proposals. To look at the whole elephant is considered to be "too ambitious", "lacking a specific hypothesis", "a fishing expedition", or other negative descriptor. How can one understand the elephant by examining only its toe, or its ear, or its trunk? By the time we formulate enough single parameter hypotheses and experiments, the elephant--and surely the investigator--would likely be dead.

There are many examples where our dependence on single parameter hypotheses can get us into trouble. In fact, the general public is now skeptical of much scientific and medical research because of the contradictory output from overly simplistic, mono parameter studies.

Albert Einstein said: "Science should be as simple as possible, but not simpler".

Here are some examples where it has been perhaps too simple:
(a set of newspaper clippings on medical and health single parameter studies). These are primarily chemical, biochemical, examples

A BIOCHEMICAL ELEPHANT – METABOLISM

The most well known and yet least understood biochemical elephant is metabolism. We have all seen and used biochemical maps and metabolic pathway charts. Such a map makes it crystal clear that biochemical reactions do not exist or operate in isolation. Every reaction is obviously dependent on many other reactions through the principles of reaction kinetics and equilibria. And yet we continue to look for "magic" single chemical parameter disease correlations. We study them and present those results to the general

public in the form of lifestyle recommendations., never warning them that this is such a tiny part of the biochemical elephant that it may well be irrelevant to the overall system.

MONOPARAMETER BRAINWASHING

We have indeed learned much from our simplistic single parameter approaches -- often called the reductionist method. That enormous knowledge base can now be used to understand the systems we have been reducing and dissecting for many decades.

The problem is that study sections, proposal reviewers, and most of the scientific system are now programmed, hard-wired, to only appreciate and understand reductionist science. We have all been doing single parameter reductionism for so long that it's all we know. The hard wired scientific establishment -- and perhaps most of us -- have great difficulty in appreciating that we now have the information, tools and skills to deal directly with multiparameter complex systems. Fortunately, the National Science Foundation has recognized this potential and this need with its new initiatives on biocomplexity.

THE 4M LAB – APPROACHING THE COMPLEXITY OF THE METABOLOME

We are beginning an initiative on biochemical complexity--to begin the modeling and simulation of major segments of metabolism, coupled with the means to directly measure many different metabolite concentrations simultaneously, thereby providing the multiparameter chemical data required for the development and utilization of models of complex biochemical networks. This work of course uses the fundamental principles of chemical reaction kinetics and reaction equilibria which Sung Wan Kim, and his famous Ph.D. supervisor, Henry Eyring, so extensively studied and developed.

Our new Laboratory for the Modeling, Measurement, and Management of the Metabolome (the 4M Lab) includes the following projects:

- the mathematical modeling of complex biochemical networks (MathWare);
- devices to collect and distribute small volumes of physiologic samples (ChipWare);
- specific enzymatic means to sensitively measure up to 50 different analytes/metabolites (ChemWare); , and
- methods to present multiparameter biochemical information in an easily visualized form (InfoWare) to permit an appreciation and understanding of complete biochemical elephants.

FROM COMPLEXITY TO SIMPLICITY—BACK TO COMPLEXITY

Although our work on complex systems is focused initially on metabolism, the mathematical modeling and multiparameter measurement and presentation approaches should have application to all areas of science. Modern scientific and analytical tools permit the monitoring and measurement of many different parameters simultaneously. Modern mathematical and data analysis tools permit us to deal with multiparameter data sets in a highly efficient and effective manner. It is no longer necessary to design and

conduct experiments using only monoparameter hypotheses. Indeed, given today's tools and skills, it is incredibly inefficient and misleading to do so. Nevertheless, we are all simple hypothesis, monoparameter trained and therefore hard wired. It will be difficult to overcome those decades of monoparameter brainwashing. Let us try. As Sung Wan suggested some 20 years ago, it is time to appreciate the whole elephant.

POSTERS

The three posters on ChipWare, ChemWare, and InfoWare present our current approach.

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