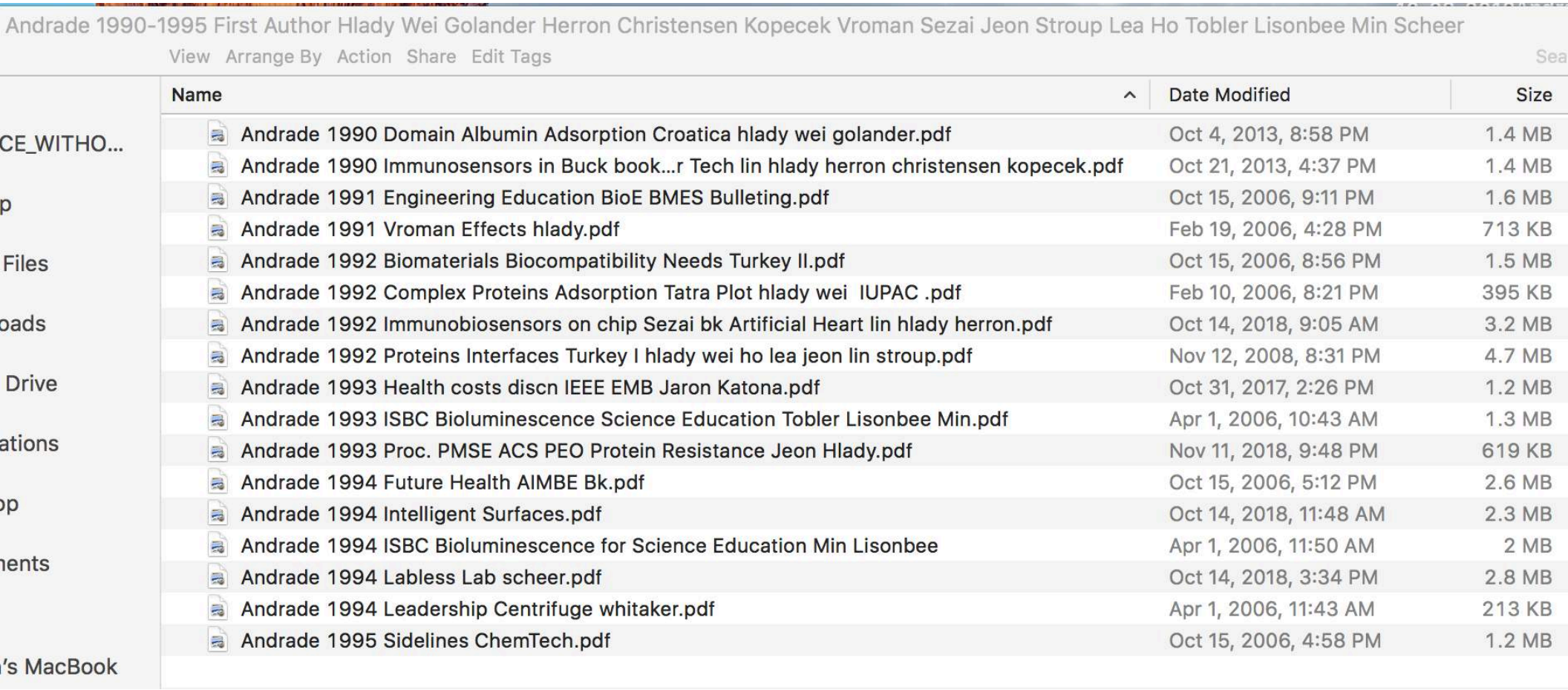

Andrade 1990-1995 First Author Hlady Wei Golander Herron Christensen Kopecek Vroman Sezai Jeon Stroup Lea Ho Tobler Lisonbee Min Scheer
View Arrange By Action Share Edit Tags
Name
Date Modified
Size
Andrade 1990 Domain Albumin Adsorption Croatica hlady wei golander.pdf Oct 4, 2013, 8:58 PM 1.4 MB
Andrade 1990 Immunosensors in Buck book...r Tech lin hlady herron christensen kopecek.pdf Oct 21, 2013, 4:37 PM 1.4 MB
Andrade 1991 Engineering Education BioE BMES Bulleting.pdf Oct 15, 2006, 9:11 PM 1.6 MB
Andrade 1991 Vroman Effects hlady.pdf Feb 19, 2006, 4:28 PM 713 KB
Andrade 1992 Biomaterials Biocompatibility Needs Turkey II.pdf Oct 15, 2006, 8:56 PM 1.5 MB
Andrade 1992 Complex Proteins Adsorption Tatra Plot hlady wei IUPAC .pdf Feb 10, 2006, 8:21 PM 395 KB
Andrade 1992 Immunobiosensors on chip Sezai bk Artificial Heart lin hlady herron.pdf Oct 14, 2018, 9:05 AM 3.2 MB
Andrade 1992 Proteins Interfaces Turkey I hlady wei ho lea jeon lin stroup.pdf Nov 12, 2008, 8:31 PM 4.7 MB
Andrade 1993 Health costs discn IEEE EMB Jaron Katona.pdf Oct 31, 2017, 2:26 PM 1.2 MB
Andrade 1993 ISBC Bioluminescence Science Education Tobler Lisonbee Min.pdf Apr 1, 2006, 10:43 AM 1.3 MB
Andrade 1993 Proc. PMSE ACS PEO Protein Resistance Jeon Hlady.pdf Nov 11, 2018, 9:48 PM 619 KB
Andrade 1994 Future Health AIMBE Bk.pdf Oct 15, 2006, 5:12 PM 2.6 MB
Andrade 1994 Intelligent Surfaces.pdf Oct 14, 2018, 11:48 AM 2.3 MB
Andrade 1994 ISBC Bioluminescence for Science Education Min Lisonbee Apr 1, 2006, 11:50 AM 2 MB
Andrade 1994 Labless Lab scheer.pdf Oct 14, 2018, 3:34 PM 2.8 MB
Andrade 1994 Leadership Centrifuge whitaker.pdf Apr 1, 2006, 11:43 AM 213 KB
Andrade 1995 Sidelines ChemTech.pdf Oct 15, 2006, 4:58 PM 1.2 MB

CROATICA CHEMICA ACTA CCACAA 63 (3) 527—538 (1990)

CCA-1956

YU ISSN 0011-1643
UDC 541.183
Conference Paper (Invited)

A Domain Approach to the Adsorption of Complex Proteins: Preliminary Analysis and Application to Albumin*

*Joseph D. Andrade,** Vladimir Hlady, Ai-Ping Wei and Carl-Gustav Gölander*

Department of Bioengineering, Center for Biopolymers at Interfaces, University of Utah, 2480 MEB, Salt Lake City, Utah 84112 USA

Received May 5, 1990

Albumin consists of three large domains with differences in electrostatic nature, charge-pH characteristics, and denaturability. The interfacial activity of albumin is due, at least in part, to the interfacial activity of its constituent domains. Consideration of the structure and interfacial activity of the various domains permits new and more precise hypotheses to be developed, with which new and better experiments can be designed. Such hypotheses allow one to evaluate and compare adsorption data, including kinetics and isotherms, adsorbed layer thickness, refractive index, lateral cohesion, multilayer formation, etc.

We feel strongly that each different protein is a unique molecular personality, which must be understood and considered if we are to more fully understand and apply the interfacial behavior of complex proteins.

INTRODUCTION

The adsorption of simple, model proteins at simple, model interfaces is qualitatively understood. We[1-3] and others[4] have hypothesized and shown that adsorption at short contact or residence times can be qualitatively predicted from, and correlated with, the surface chemistry of the protein globule, as deduced from x-ray crystallographic coordinates. Wei and others have demonstrated that adsorption at the air-water interface at long residence times correlates with the conformational stability of the model protein.[1] This correlation has also been suggested by Lyklema and Norde[5] and by others.[2,3,5]

Although much work remains before a reasonable theory is available for the prediction of model protein adsorption at model interfaces, we feel that it is now possible to cautiously and qualitatively approach the problem of the adsorption of complex proteins at model interfaces.

The objective of this paper is to outline an approach to the problem and to consider the adsorption of a »model« complex protein, albumin, at model interfaces. We expect to address other complex proteins in subsequent papers.

* Based on an invited lecture presented at the *8th »Ruđer Bošković« Institute's International Summer Conference on the Chemistry of Solid/Liquid Interfaces* Red Island, Rovinj, Croatia, Yugoslavia, June 22 — July 1, 1989.

52. S. Damodaran and K. B. Song, *Biochim Biophys Acta* **954** (1988) 253.
53. D. E. Graham and M. C. Phillips, *J. Colloid Interface Sci.* **70** (1979) 415, 427.
54. N. Watanabe, T. Shirakawa, M. Iwahashi, K. Ohbu, and T. Seimiya, *Coll. Polymer Sci.* **264** (1986) 903.
55. W. Norde, *Adv. Colloid Interface Sci.* **25** (1986) 267.

SAŽETAK

Tumačenje adsorpcije kompleksnih proteina teorijom domena: prethodna analiza i primjena na albumin

J. D. Andrade, V. Hlady, Ai-Ping Wei i C.-G. Gölander

Albumin se sastoji od tri različite domene, koje se razlikuju u strukturnom pogledu, karakterističnom odnosu naboja i pH, te prema sklonošću prema denaturaciji. Međupovršinska aktivnost albumina određena je, velikim dijelom, aktivnošću svake od te tri strukturne domene. Uzimajući u obzir međupovršinsku aktivnost svake strukturne jedinice, može se rastumačiti i sveukupna međupovršinska aktivnost albumina. Takav pristup analizi koji se oslanja na strukturne jedinice albumina, omogućuje razvijanje novih i preciznijih pretpostavki, pomoću kojih se mogu dizajnirati novi i bolji eksperimenti. Iste predpostavke dozvoljavaju novu ocjenu i usporedbu dosadašnjih eksperimentalnih podataka za kinetiku adsorpcije i adsorpcijske izoterme, debljinu adsorbiranog sloja i stvaranje višestrukih slojeva, indeks loma, lateralnu koheziju itd. Pristup opisan u radu zasniva se na hipotezi da svaki jednostavni protein ima jedinstvenu molekularnu osobenost, čije je poznavanje preduvjet za tumačenje i korištenje međupovršinske aktivnosti kompleksnih proteina.

EROM COMPLEXITY TO SIMPLICITY

Although the behavior of 'simple' proteins at 'simple' interfaces is qualitatively understood, the many plasma proteins responsible for blood coagulation and blood »compatibility« are not simple and their 3-D structures are not known. Likewise, many of the tear proteins implicated in contact lens interactions are also complex and structurally undefined.

Complex proteins can be made simpler by treating them as if they are constructed of functional and structural domains[6,7]. Structural domains are generally defined and identified as regions of the protein of relatively high packing density, which can be identified from the x-ray crystallographic coordinates using various algorithms.[8-10]

Rapid progress is being made in the prediction of structural domains from amino acid sequences.[11] Structural and functional domains can also be deduced from enzyme cleavage data[12] and from exon analysis of the DNA sequence.[13] These methods, coupled with careful analysis of the functions of the various domains, have allowed the development of schematics or »cartoons« of the functional structures of complex proteins, including plasma proteins.[13,14]

The identity and thermodynamic autonomy of structural domains can often be deduced from denaturation (unfolding)[15] and calorimetric studies.[16-18] The intrinsic stability of the protein is the free energy of folding and can be obtained from calorimetric or solution (urea or guanidinum chloride) denaturation studies.[1,15,16] Advances in the sensitivity of calorimetry and in the analysis of the scanning curves show, for many complex proteins, that the individual domains are calorimetrically independent and their individual thermal characteristics can be resolved.[17,18]

The »surface chemistry« of proteins can be obtained directly from x-ray crystal structures or from 2D NMR solution structures, when such data are available. By use of molecular computer graphics, one can readily discern the nature of the protein »surface«.[1-4] Such analysis leads to a much better appreciation of the surface chemical virtuosity of a protein. One rapidly begins to appreciate that different »faces« or regions on the protein surface can have very different surface and interface activities.

In the many cases where the three dimensional structure is not known, a simple analysis of the amino acid sequence is often helpful. Today most of the known sequences are organized in protein data banks which can be accessed via computer, using a modem and a telephone line, such as the Protein Identification Resource (PIR) in the USA,[24] and the Institute Pasteur in Europe.[26] Both of these databases contain annotated protein amino acid sequences. To use these data banks effectively, one also needs access to programs which can search the database, extract the raw information and process the data.[26,27] One initial question to ask is how homologous is the sequence in comparison to other known sequences. Homologous proteins may have similarities in main-chain folding and possibly in interfacial behavior. Aligning sequences is particularly useful when one of the homologous proteins has a known tertiary structure.

In addition to homology searches one can predict protein secondary structure from the amino acid sequence.[28] Four common areas of predictions are:

Typical secondary structures (helices, turns, coils);

Trans-membrane helices;

Antigenic sites;

Signal and target sequences.

As the overall accuracy of secondary structure prediction methods is only about 60%, they can only provide a starting point for further, more refined analysis by other methods.

As charged amino acids are generally on the surface of the protein, regions of unusually high electrostatic character, positive or negative, are often clues to particular electrostatic binding characteristics. Such an analysis readily identifies the heparin-binding plasma proteins,[19-21] as they all have domains or amino acid sequences rich in Lys and Arg.

Certain aspects of the surface chemistry can be derived from appropriate ligand-binding studies, especially using fluorescent probes. Probes are available which sense or »report« on many different microenvironments, including charge, potential, and hydrophobicity. Wei[1] recently showed that one such probe can be used to obtain an »apparent surface hydrophobicity« parameter for various model proteins. The surface hydrophobicity of the proteins correlates with their surface activity at the air/water interface.[1]

The interactions of proteins and their protease cleavage fragments with chromatographic surfaces provides clues as to interface characteristics and activities. Affinity chromatography data are especially valuable in identifying »specific« binding properties of proteins and fragments.

The interfacial behavior of a complex protein may be largely dominated by the interfacial activity of only one domain or even a sub-domain. For example, exposure of blood plasma to heparin-Sepharose materials results in the depletion of heparin-binding proteins, such as antithrombin III, with little depletion of the other plasma proteins.[19,20] Although this is an expected result, it suggests that the interfacial activity of the heparin-binding proteins — on a sulfonated surface — is dominated by their richly positive regions or domains.

We propose that the initial adsorption event is a function of the interfacial activity of the various domains or regions of the protein, and primarily on the particular surface chemistry of the domain or region. For many protein — solid surface systems one of the domains can be expected to »dominate« the interfacial activity of the protein, for example:

domains with large hydrophobic patches at hydrophobic surfaces;

anionic protein regions on cationic surfaces; and

cationic protein regions on anionic surfaces.

We further propose that the conformational accommodation (»denaturation«) of the protein at the interface will be a function of the stability of each of the individual protein domains — *i. e.* how »hard« or »soft« are each of the domains.[1,5,29] Domain hardness or softness can be qualitatively assessed by thermal or solution denaturation measurements.[1,15-18]

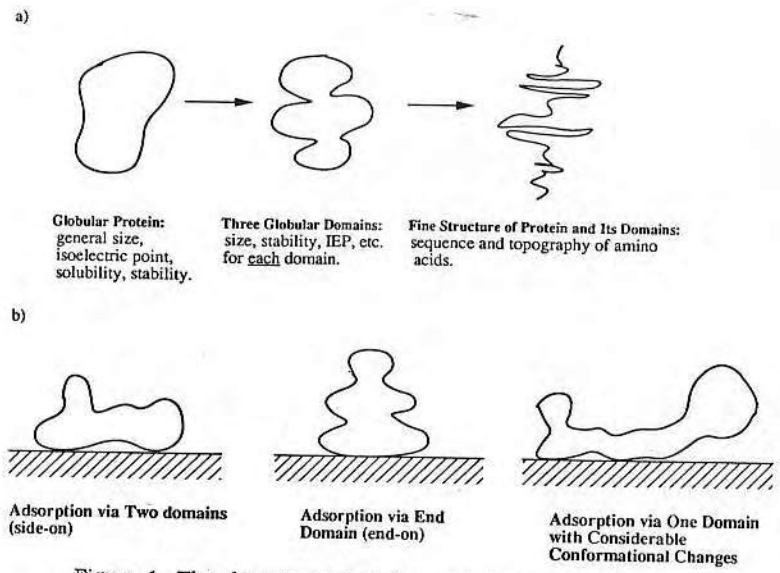


Figure 1. The domain concept for protein interfacial activity.
a) Left: the »classical« approach: proteins are colloidal particles with certain average particle characteristics; center: the domain approach: globular proteins, such as albumin, consist of structural and functional domains interconnected by flexible, hinge-like regions; right: each domain can be treated and modelled to determine its unique properties and characteristics.
b) Multi-domain proteins at a rigid interface; left: adsorption via one of the two domains; center: adsorption via one terminal domain (end on); right: adsorption via one dominant domain which conformationally alters and »spreads« on the surface with time.

Our simplistic model of a complex protein (Figure 1) is that it is a collection of simple domains, tied together via relatively flexible polypeptide segments. Clearly such a model is naive, simplistic, and unrealistic — but it is far better, in our opinion, than treating a protein as an undefined, unknown globule.

APPLICATION TO ALBUMIN

Albumin is a useful protein with which to begin our analysis because it:[34]
contains no carbohydrate;
consists of three fairly distinct domains;
has a high α-helix content;
has many —S—S— bonds;
has important ligand binding properties; and
the 3D x-ray structure is now available for human albumin (35).

Albumin is the major protein component of blood plasma and serum. Its collision rate with surfaces and interfaces is over 7 times greater than that

of any other plasma protein[30]. It is not surprising, therefore, that albumin adsorption dominates the plasma protein adsorption process at short contact times[30]. At longer times adsorbed albumin may be removed from the surface, as other proteins with higher interfacial activity may more strongly interact. For this and other reasons, a complex adsorption hierarchy is observed on most surfaces exposed to blood plasma[30,31]; this phenomenon has been called the »Vroman Effect«[32,33]. What one »sees« on an interface exposed to plasma (or to any other complex protein solution) depends on when one »looks«, that is, the surface composition is — in general — time dependent.

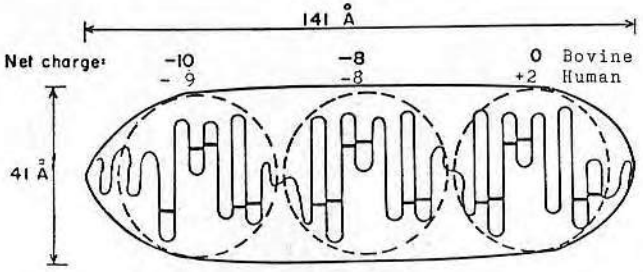


Figure 2. The »tennis ball« model of albumin, showing the three major domains and the disulfide-bonded alpha helical subdomains. Domain I is the N-terminal, domain III the C-terminal. Note the differences in overall charge in the various domains (from Ref 34).

Figure 2 (modified from Ref.[34]) shows the general size, shape, and amino acid sequence folding pattern for albumin. Although bovine and human albumin are similar in general folding properties, their net charge and charge distributions are different. The general shape can be viewed as three tennis balls (the large domains) in a can or cylinder.[34]

The large domains are normally called I, II, and III, going from the N terminal (left in Fig. 2) to the C terminal. The N terminal end binds Cu^{++} and Ni^{++}. The two high affinity fatty acid binding sites are in Domain III and in Domain II near the interface with Domain III (see Fig. 8 in Ref. 34). Other fatty acid sites are located in the center of Domains II and I. There are a variety of binding sites for other ligands.[34] The presence of fatty acid significantly enhances the conformational stability of albumin.

The overall denaturation temperature is increased from about 60° C (defatted) to 80° C (fatted) at pH 7.0[36], which must reflect an increase in stability in Domain III. Domains II and III tend to have higher individual denaturation temperatures than Domain I, probably due largely to their fatty acid binding[36].

Domain I has the highest net charge (—9 for human, while Domain III is +2 in human and zero in bovine albumin, according to Ref. 34. A simulated titration of bovine albumin reveals the estimated net charge on each of the three domains (Figure 3).

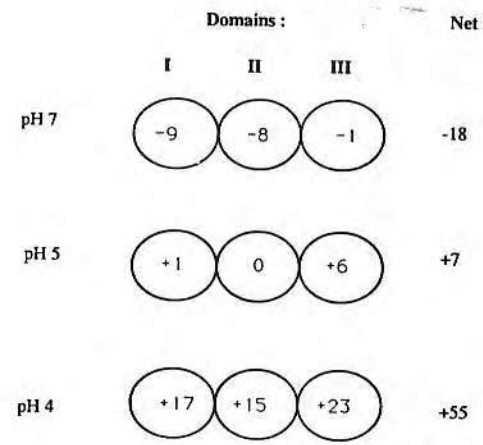


Figure 3. Simulated charge of the three domains in bovine albumin using following pK values*: $pK_{Arg} = 12.48$, $pK_{Lys} = 10.79$, $pK_{His} = 6.00$, $pK_{Asp} = 3.65$, $pK_{Glu} = 4.25$, $pK_{Tyr} = 10.13$, $pK_{Cys} = 8.3$, N-terminal $pK = 9.6$, C-terminal $pK = 2.34$. Newly created N-terminal and C-terminals of each domain were made untitratable by setting the pK equal to 1 or 13, respectively. Note the close agreement with the values reported in Ref. 34 (Figure 2). These results are also in qualitative with Norde's data on the titration of adsorbed and solution human albumin (55).

Albumin undergoes several pH sensitive transitions, especially the N — F transition in going from pH 7 to pH 4 or lower. Most of the expansion of the molecule at lower pH (the F form) is probably the result of expansion or unfolding of Domain III[37].

There is some evidence that the C-terminal (Domain III) may be looser or less compact that the N-terminal (Domain I)[40,41], however the N- terminal region may refold more rapidly than the C-terminal[40]. The evidence suggests that Domains I, II, and III fold and refold somewhat independently[40,41].

Alkyl chain surfactant binding occurs towards the C-terminal, leading to loss in α-helix content; »... the more stable N-terminal unfolds later...« (Ref. 41, p. 125). Although surfactant treatment leads to a loss in α-helix, it is believed »... that the large loops are the most stable against denaturation by surfactants.« (Ref. 41, p. 125).

Various peptic and tryptic fragments of albumin have been studied.[40,42] The peptic fragments have been exposed to surfactants and to urea and GdnCl denaturants. Urea denaturation is at about 6M, GdnCl denaturation at about 2M[42]. No dramatic differences were observed, although much of Domain III was not represented in these fragments. The thermal denaturation studies of albumin which are available[36] are on different fragments and therefore difficult to compare to the solution denaturation studies.

It is interesting to compare the Brown model of albumin[34,38] with the newly available 3D structure (see Fig. 3 in Ref. 35). Feng, *et al.* recently succeeded in obtaining high resolution scanning tunneling microscopy (STM)

images of individual human albumin molecules on a single crystal graphite surface[39]. Their images exhibit a remarkable similarity to the published 3-D image[35].

TABLE I

Preliminary hypotheses coupling human albumin structural properties to interfacial activity at pH ∽ 7.

Surface	
Positively Charged	Domains I and II are negatively charged and would be preferentially bound. As these domains are less stable than Domain III, one would expect the adsorbed albumin to be more denatured than on other surfaces.
Negatively Charged Surfaces	Domain III is weakly positive and would tend to adsorb; there is little denaturation due to the stability of Domain III when it contains bound fatty acid.
Hydrophobic Surfaces	The first loop in domain I is probably hydrophobic and would tend to bind. As this loop is less stable than other loops, a slow time dependent denaturation can be expected.

Based on an analysis of the available structures and images, Table I presents several very preliminary hypotheses regarding the surface activity of albumin.

ALBUMIN AT MODEL INTERFACES

There is a large literature on the adsorption of human and bovine albumin. Here we briefly review a number of key papers dealing with model surfaces and interfaces.

The Brown model of albumin is based on an association of the hydrophobic faces of the α-helices in the subdomains[34,38]. The amphiphilic nature of these helices suggest that an apolar interface could significanlty disturb the normal hydrophobic association of albumin.

Peptides and proteins often assume amphiphilic secondary structures at oil/water, lipid/water, and air/water interfaces. The apolar nature of the interface may induce an amphiphilic structure or may drive the adsorption and ordering of an existing helical structure[43,44]. The amphiphilicity of known helices can be viewed using the Edmundson »wheel« projection[45] and quantified using Eisenberg's hydrophobic moment analysis[46].

Krebs and Phillips have studied the α-helix contribution to the surface activity of proteins[47] as probed by surface pressure measurements.

There is a strong correlation between helix amphiphilicity and surface activity.

Albumin readily adsorbs at air/water and oil/water interfaces — the resulting decreases in surface and interfacial tensions can be easily monitored.[48-51] Although no work has yet been reported on the surface or interfacial tension behavior of individual albumin fragments, Damodaran and Song[52] have published a most interesting study on the surface activity of bovine serum albumin (BSA) »structural intermediates.« They fully reduced

As the individual albumin domains are roughly 40 × 40 Å in size, it is clear that only a small part of one of the domains can dominate the electrostatic adsorption process; note that at physiologic ionic strength the Debye length is less than 10 Å.

The presence of various counterions may significantly alter the electrostatic behavior, particularly multivalent anions (PO_4^{-3}, SO_4^{-2}) and cations (Ca^{++}, Mg^{++}, Al^{+++}, etc.). Ions may serve as bridging agents as well as serving to mask surface charged groups on the protein.[55]

It is also clear, however, with reference to Figure 4, that protein-protein lateral interactions can be very important. This is the normal explanation for the maximum in adsorbed amount at the isoelectric point, a behavior, which tends to be more pronounced on hydrophobic surfaces where the adsorptive interaction is predominantly hydrophobic. If we assume that the intrinsic surface hydrophobicity of each of the domains are comparable, then that domain exhibiting the least charge, and thus the least solubility, at a particular pH might tend to be preferentially adsorbed. At pH ~7, the least charge is on domain III, while at pH 5 it is domains I II. Thus as the pH decreases there may be a transition from »end on« to a partial »side on« orientation.

We must examine the actual position and distribution of charges and of hydrophobic residues in each domain and even in each subdomain. Such analyses are in progress.

We will not attempt here to review and analyze the voluminous literature on albumin at solid/liquid interfaces. A more complete analysis of the domain characteristics of albumin and of the albumin adsorption literature will be presented in a later, more complete paper. Our purpose here is merely to set the stage for a domain approach to protein adsorption. With a set of cartoons and hypotheses in mind (Table 1, Figure 4), we are now in a position to wade into the enormous literature on albumin adsorption. Please wish us luck!

Acknowlegements — We thank our colleagues and co-workers over the years for interesting discussions on these topics, particularly J-N Lin, J. Herron, and E. Brynda. This work was supported by the Center for Biopolymers at Interfaces — a University of Utah-industry consortium.

REFERENCES

1. A-P. Wei, *M. Sc. Thesis*, University of Utah, Sept. 1990; also A-P. Wei, J. N. Herron, and J. D. Andrade: in: D. J. A. Crommelin, (Ed.), *Biotherapie*, 1990, in press.
2. J. D. Andrade and V. Hlady, *Adv. Polymer Sci.* **79** (1986) 1.
3. D. Horsley, J. Herron, V. Hlady, and J. D. Andrade, in: T. S. Horbett and J. Brash, (Eds.), *Proteins at Interfaces, Amer. Chem. Soc. Symp. Series*, **343**, 1987, pp. 290.
4. C. S. Lee and G. Belfort, *Proc. Nat. Acad. Sci., USA*, **86** (1989) 8392.
5. H. Lyklema and W. Norde, this volume.
6. D. B. Wetlaufer, *Proc. Nat. Acad. Sci. USA* **70** (1973) 697.
7. G. E. Schulz and R. H. Schirmer, *Principles of Protein Structure*, Springer, 1979.
8. C. Chothia, *Ann. Rev. Biochem.* **53** (1984) 537.
9. M. H. Zehfus, *Proteins* **3** (1987) 90.
10. R. Unger, D. Harel, S. Wherland, and J. L. Sussman, *Proteins* **5** (1989) 355.

11. T. Kikuchi, G. Nemethy, and H. A. Scheraga, *J. Protein Chem.* **7** (1988) 427.
12. G. Weber and L. B. Young, *J. Biol. Chem.* **239** (1964) 1424.
13. C. C. F. Blake, K. Harlos, and S. K. Holland, *Cold Spring Harbor Symp. Quantitative Biol.* **52** (1987) 925.
14. L. Patthy, *Cell* **41** (1985) 657.
15. C. N. Pace, B. A. Shirley, and J. A. Thomson in: T. E. Creighton, (Ed.), *Protein Structure: A Practical Approach*, IRL Press, 1989.
16. P. L. Privalov, *Adv. Protein Chem.* **33** (1979) 167.
17. P. L. Privalov, *Adv. Protein Chem.* **35** (1982) 1.
18. P. L. Privalov, *Ann. Rev. Biophys. Chem.* **18** (1989) 47.
19. C-H. Ho, *MSc Thesis*, University of Utah, April, 1990.
20. C-H. Ho, V. Hlady, J. D. Andrade, and K. Caldwell, *J. Biomed. Materials Res.*, (1990) in press.
21. A. D. Cardin and H. J. R. Weintraub, *Arteriosclerosis*, **9** (1989) 21.
22. Molecular Probes, Inc., Eugene, Oregon.
23. A. Kato and S. Nakai, *Biochim. Biophys. Acta* **624** (1980) 13.
24. National Biocemical Research Foundation, Georgetown University Medical Center, 3900 Reservoir Rd., NW, Washington, DC 20007.
25. Inst. Pasteur, 25 Rue du So. Roux, 75724, Paris Cedex 15, France.
26. P. Argos in: T. Creighton, (Ed.), *Protein Structure: A Practical Approach*, IRL Press, Oxford, 1989, pp. 169—190.
27. A. M. Lesk, (Ed.), *Computational Molecular Biology*, Oxford University Press 1988.
28. P. T. Chou and G. D. Fasman, *Adv. Enzymology* **47** (1978) 45.
29. J. D. Andrade, V. Hlady, J. Herron, and A-P. Wei, *Croat. Chem. Acta* **60** (1987) 495.
30. J. D. Andrade and V. Hlady, *Ann. N. Y. Acad. Sci.* **516** (1987) 158.
31. L. Vroman, *Sem. Thromb. Hemost.* **13** (1987) 79.
32. J. L. Brash and P. Ten Hove, *Thromb. Haemostas.* **51** (1984) 326.
33. T. A. Horbett, *Thromb. Haemostas.* **51** (1984) 174.
34. T. Peters, Jr., *Adv. Protein Chem.* **37** (1985) 161.
35. D. C. Carter, X-M. He, S. H. Munson, P. D. Twigg, K. M. Gernert, M. B. Broom, and T. Y. Miller, *Science* **244** (1989) 1195.
36. E. Tiktopulu, *Molecular Biology* **19** (1985) 884.
37. M. Y. Khan, *Biochem. J.* **236** (1986) 307.
38. J. R. Brown, and P. Shockley in: P. C. Jost and O. H. Griffith, (Eds.), *Lipid-Protein Interactions, Vol. 1*, J. Wiley and Sons, New York, 1982, pp. 25—50.
39. L. Feng, C-Z. Hu, and J. D. Andrade, *J. Colloid Interface Sci.* **126** (1988) 650.
40. K. O. Johanson, D. B Wetlaufer, R. G. Reed and T. Peters, Jr., *J. Biol. Chem.* **256** (1981) 445.
41. K. Takeda, M. Shigeta, and K. Aaoki, *J. Colloid Interface Sci.* **117** (1987) 120.
41. K. Takeda, A. Wada, T. Nishimura, T. Ueki, and K. Aoki, *J. Colloid Interface Sci.* **133** (1989) 497.
43. E. T. Kaiser and I. J. Kezdy, *Proc. Nat. Acad. Sci. USA* **80** (1983) 1137.
44. W. F. DeGrado and J. D. Lear, *J. Am. Chem. Soc.* **107** (1985) 7685.
45. M. Schilfer and A. B. Edmunson, *Biophys. J.* **7** (1967) 121.
46. D. Eisenberg, R. M. Weiss, and T-C. Terwillinger, *Nature* **299** (1982) 371.
47. K. E. Krebs and M. C. Phillips, *FEBS Lett.* **175** (1984) 263.
48. E. Katona, A. W. Neumann, and M. A. Moscarello, *Biochim Biophys. Acta*, **534** (1978) 275.
49. M. Deyme, A. Baszken, J. E. Pruust, E. Perez, G. Albrech, and M. M. Boissonnade, *J. Biomed. Materials Res.* **21** (1987) 321.
50. R. D. Banall, J. A. D. Annis, and P. A. Arundel, *J. Biomed. Materials Res.* **12** (1978) 653.
51. D. R. Absolom, C. J. Van Oss, W. Zingg, and A. W. Neumann, *Biochim. Biophys. Acta* **670** (1981) 74.

6) Y.Ikariyama, H.Kunoh, M.Aizawa, Biochem.Biophys.Res.Commun. 128, 987-992 (1985).
7) M.Aizawa, Y.Ikariyama, K.Emoto, Proc. 2nd Intl Meeting Chemical Sensors, Bordeaux, 622-625 (1986)
8) M.Aizawa, Y.Ikariyama, M.Tanaka, H.Shinohara, Proc. ECS Symp. Chemical Sensors 362-369 (1987)
9) M.Aizawa, Y.Ikariyama, M.Tanaka, H.Shinohara, Tech.Digest 8th Sensor Symp. 7-12 (1989)
10) M.Aizawa, M.Tanaka, Y.Ikariyama, R.W.Murray, "Chemical Sensors and Microinstrumentation" (R.E.Murray, R.E.Dessy, W.R.Heineman, J.Janata, W.R.Seitz, eds.), Am.Chem.Soc., Washington,DC, 129-138 (1989)

Biosensor Technology ed. R P Buck, W E Hatfield, M Umana, E F Bowden Dekker, 1990

17

Immunosensors: Remaining Problems in the Development of Remote, Continuous, Multi-Channel Devices

J.D. Andrade, J-N. Lin, V. Hlady, J. Herron, D. Christensen, and J. Kopecek, Department of Bioengineering, University of Utah, Salt Lake City , Utah, 84112

1 GOALS AND OBJECTIVES

The goals and objectives of the Immunosensors Program at the University of Utah are to develop the science and engineering basis for optically based immunosensors. This includes the development of sensors for proteins and other antigens, the development of multi-channel sensors (including built-in calibration and reference channels), the development of sensors which are capable of remote on-line and continuous or semi-continuous function and the development of sensors which are biocompatible and stable. This chapter will serve as a 1989 progress report on these various goals and objectives.(1)

Figure 1 summarizes these goals. An animal or patient is shown, which could also represent a bioreactor or biochemical reactor. An extracorporeal loop is shown, for example, the extracorporeal blood pathway in hemodialysis or cardiopulmonary bypass during open-heart surgery. A connector is shown in that loop which contains a multi-channel immunosensor. Outputs of the various channels are appropriately referenced, ratioed, and otherwise processed to permit the quantitative analysis of the analytes of interest. The output is shown on a continuous or semi-continuous plot of analyte concentration as a function of time.

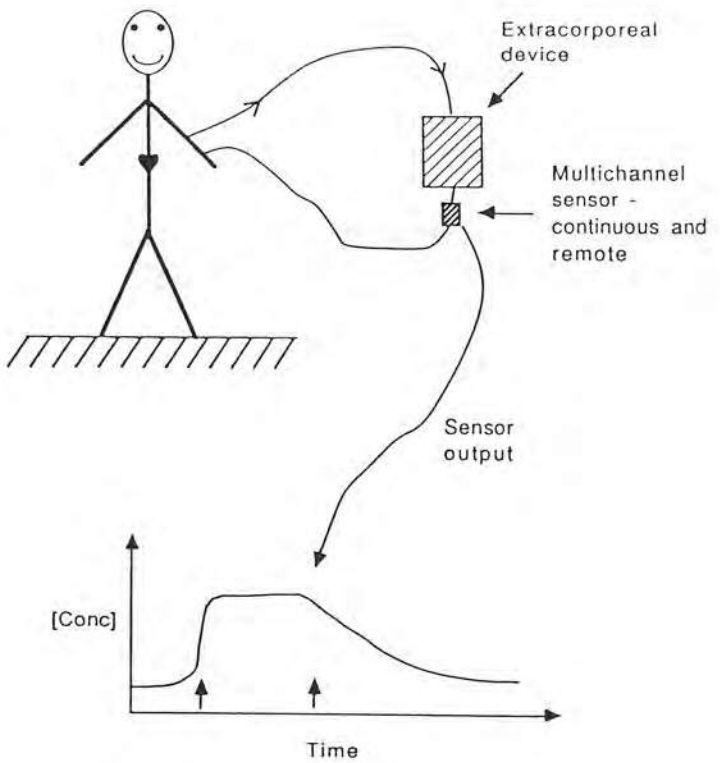


FIGURE 1
On line, continuous, remote sensing is the long-range goal of the Utah program. An array of such sensors can be applied in extracorporeal blood circuits for the monitoring and control of cardiovascular devices, in process control in the biotechnology and biochemical engineering industries, and for water monitoring in environmental engineering. Our focus is on immunoassay technologies for proteins, hormones, and drugs.

Application areas include:

1. Biochemical engineering and biotechnology, primarily for process control;
2. Medical diagnostics;
3. Extracorporeal device monitoring and control (Figure 1);
4. Implanted device monitoring and control;
5. Biomedical research, such as the monitoring of analyte concentrations in experimental animals and in cell cultures;
6. Water and waste-water monitoring of environmental pollutants and contaminants.

Our approach is based largely on immunoassay; thus, we are interested in applying these sensors to the assay of the following general classes of antigens/haptens:

1. Coagulation proteins
2. Complement proteins
3. Antibody levels
4. Lipoproteins
5. Enzymes
6. Enzyme inhibitors
7. Hormones
8. Growth factors and regulators
9. Drugs
10. Viruses and bacteria

Coagulation proteins are our current applications focus.

2 INTRODUCTION AND BACKGROUND

Most of our work is based on the use of antibody or antigen immobilized on a quartz or amorphous silica surface.(2) Silica is used because of its optical properties, specifically its high optical transmission, low fluorescence, and relatively high refractive index. The latter permits one to use total internal reflection optics and the interfacially bound evanescent wave as a means to excite fluorescence on the solution side of the solid liquid interface without exciting bulk fluorescence in the solution phase.(3)

This approach has been studied by a large number of groups for its possible immunosensor application.(4-8) In our case, we have used principally a flat quartz plate excited by a single total internal reflection geometry. This so called total internal reflection fluorescence (TIRF) method has been widely used by us and others for the fundamental study of protein adsorption and immobilization at solid liquid interfaces as well as for fluorescence immunosensor development.(1,3) We have also used silica

optical fibers and developed a sensing region in the fiber by the appropriate removal of cladding and coating (4), as have others.(4,5)

In Figure 2 a conventional silica core fiber is shown, but with the end stripped to remove the cladding and protective coating. The distal end of the fiber has been coated with a black rubber or epoxy to prevent light from propagating from the end of the fiber into the bulk solution. The stripped fiber surface is shown to contain immobilized Immunoglobulin and, after exposure to specifically bound antigen, the fluorescence intensity plot demon-

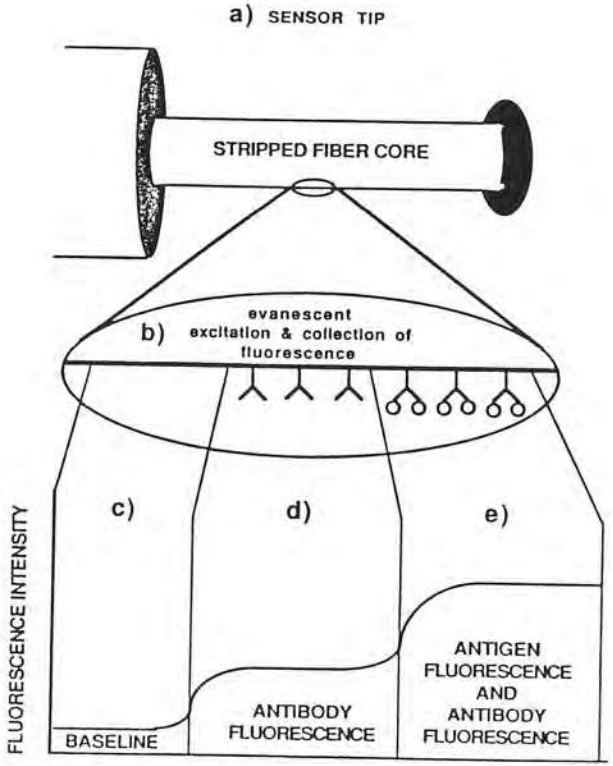


FIGURE 2
A typical optical fiber evanescent immunosensor design. From Reichert et al. (Ref. 16)

strates that the same fiber can be used to excite interfacial fluorescence as well as to collect a portion of the fluorescence generated. If the excitation wavelength is in the range of 280-290 nanometers, the intrinsic UV fluorescence of proteins is detected.(3) Thus, the immobilization of an unlabeled antibody can be directly probed and monitored.(6) In addition, if the antigen is fluorescent or if a fluorescently labeled antigen is added as a reagent to compete with unlabeled antigen for the antibody binding sites, then a conventional competitive fluoroimmunoassay is produced, and the visible fluorescence of the labeled antigen can be readily detected and measured.

More recently we have been working with multi-channel detection primarily through the use of a two-dimensional CCD (charge coupled device) camera.(7) We have demonstrated that various channels, ie., different spatially distinct regions of immobilized antibody, can be readily detected. Figure 3 demonstrates the optical geometry for such an experiment.

3 ANTIGEN-ANTIBODY BINDING

The sensitivity and specificity of an immunosensor is based on the sensitivity and specificity of the interaction of specific antibodies with a particular antigen.

Figure 4 presents the basic antibody antigen equilibrium equation and the plot based on the assumed reversibility of these interactions. The plot shows antigen binding as a function of antigen concentration. The dynamic range of an immunoassay or immunosensor is usually considered to be roughly from 10 to 90% saturation of the antibody used, or about two orders of magnitude in antigen concentration.(8) If one wishes to sense a much wider range of concentrations, then two or more antibody channels are required each with a different antibody with the appropriate binding constant.

In the case of a multi-epitope antigen it may be desirable to use an array of channels, each containing a different monoclonal antibody. In this way, the dynamic range could be enhanced

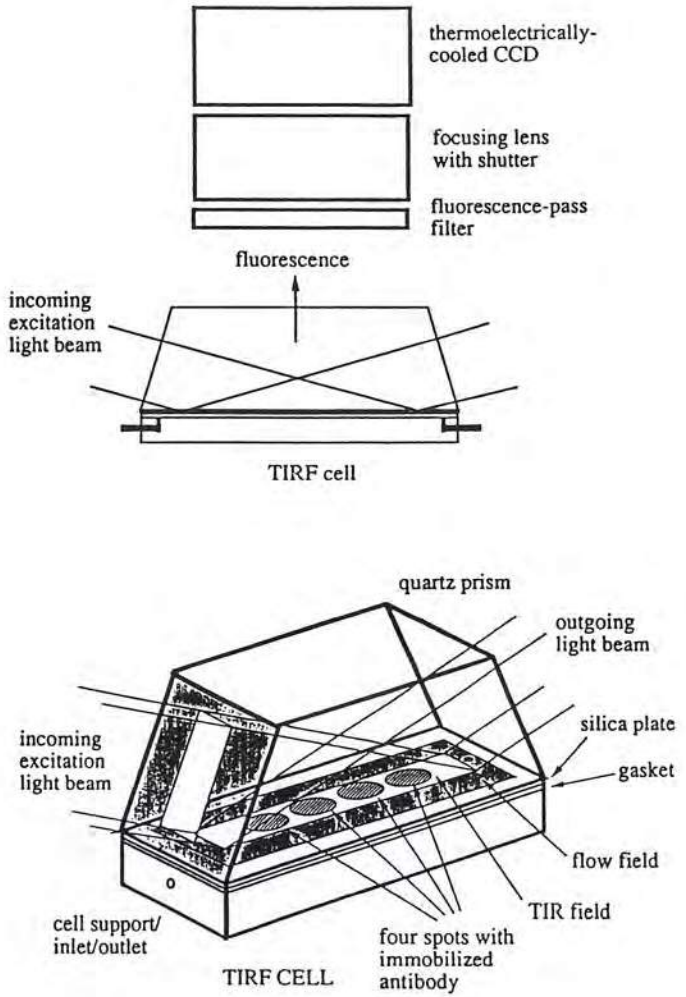


FIGURE 3
Use of the single reflection TIRF geometry for the development of multi-channnel immuno sensors. A cooled CCD is used as a position and wavelength sensitive detector. Four different immobilized Ab regions are detected. (From Ref. 7)

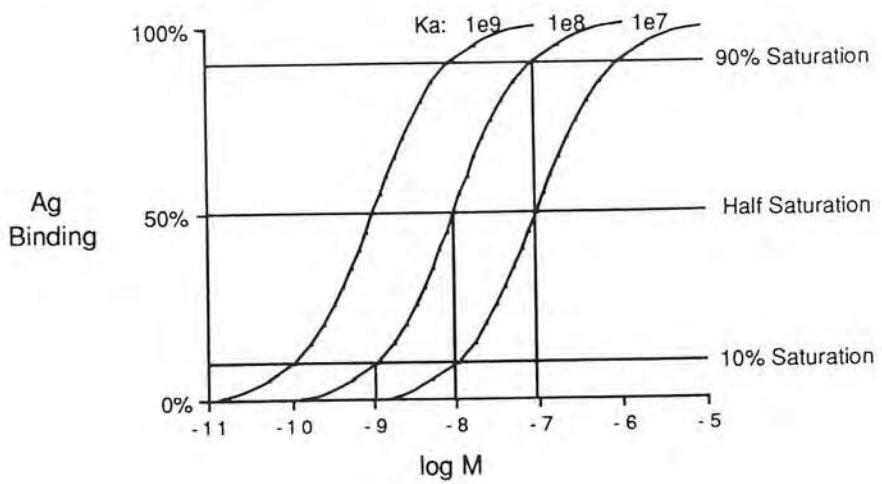


FIGURE 4
Bound Ag - Bulk Ag equilibrium curves, based on a reversible Ag-Ab interaction:

$$Ab + Ag \underset{K_2\ off}{\overset{K_1\ on}{\rightleftharpoons}} Ab\text{-}Ag; \qquad K_a = K_1/K_2 = \frac{[Ab\text{-}Ag]}{[Ab]\,[Ag]}$$

when $K_a \sim 1/[Ag]$, Ab sites are ~ 50% saturated.

Range of immunoassay is usually defined as:

$$\frac{0.1}{K_a} < [Ag] < \frac{10}{K_a},$$

i.e. 10 to 90% saturation results in two orders of magnitude range for [Ag].

and discrimination against nonspecific binding could be improved as well.

For much of our work, we have chosen to work with a model system of anti-fluoroscein monoclonal antibodies developed by Voss and coworkers at the University of Illinois.(9) The binding thermodynamics of this system have been studied and presented by Herron et al.(10) The overall association constant as a function

optical fibers and developed a sensing region in the fiber by the appropriate removal of cladding and coating (4), as have others.(4,5)

In Figure 2 a conventional silica core fiber is shown, but with the end stripped to remove the cladding and protective coating. The distal end of the fiber has been coated with a black rubber or epoxy to prevent light from propagating from the end of the fiber into the bulk solution. The stripped fiber surface is shown to contain immobilized Immunoglobulin and, after exposure to specifically bound antigen, the fluorescence intensity plot demon-

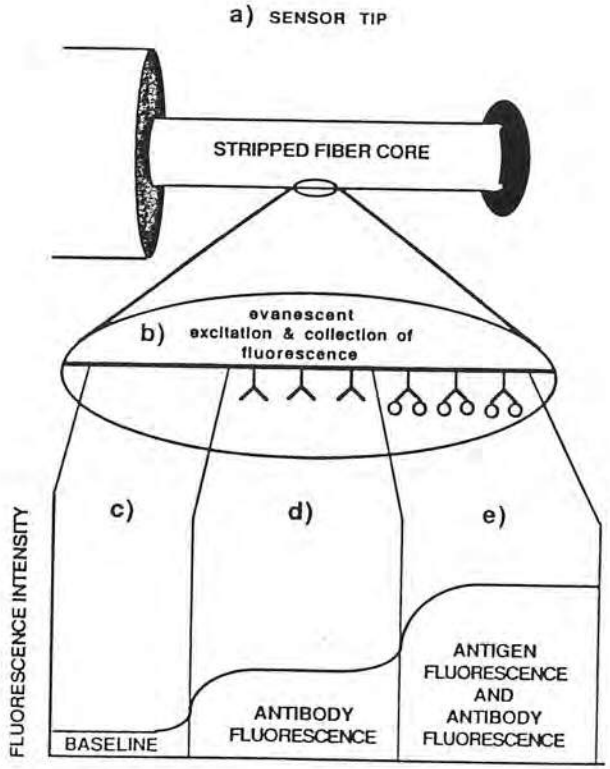


FIGURE 2
A typical optical fiber evanescent immunosensor design. From Reichert et al. (Ref. 16)

strates that the same fiber can be used to excite interfacial fluorescence as well as to collect a portion of the fluorescence generated. If the excitation wavelength is in the range of 280-290 nanometers, the intrinsic UV fluorescence of proteins is detected.(3) Thus, the immobilization of an unlabeled antibody can be directly probed and monitored.(6) In addition, if the antigen is fluorescent or if a fluorescently labeled antigen is added as a reagent to compete with unlabeled antigen for the antibody binding sites, then a conventional competitive fluoroimmunoassay is produced, and the visible fluorescence of the labeled antigen can be readily detected and measured.

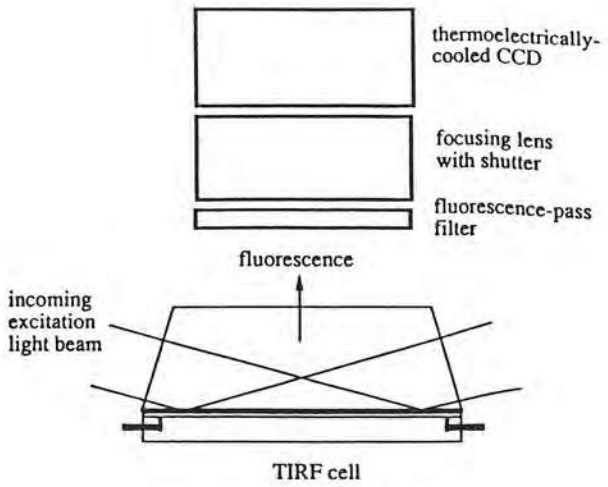
More recently we have been working with multi-channel detection primarily through the use of a two-dimensional CCD (charge coupled device) camera.(7) We have demonstrated that various channels, ie., different spatially distinct regions of immobilized antibody, can be readily detected. Figure 3 demonstrates the optical geometry for such an experiment.

3 ANTIGEN-ANTIBODY BINDING

The sensitivity and specificity of an immunosensor is based on the sensitivity and specificity of the interaction of specific antibodies with a particular antigen.

Figure 4 presents the basic antibody antigen equilibrium equation and the plot based on the assumed reversibility of these interactions. The plot shows antigen binding as a function of antigen concentration. The dynamic range of an immunoassay or immunosensor is usually considered to be roughly from 10 to 90% saturation of the antibody used, or about two orders of magnitude in antigen concentration.(8) If one wishes to sense a much wider range of concentrations, then two or more antibody channels are required each with a different antibody with the appropriate binding constant.

In the case of a multi-epitope antigen it may be desirable to use an array of channels, each containing a different monoclonal antibody. In this way, the dynamic range could be enhanced



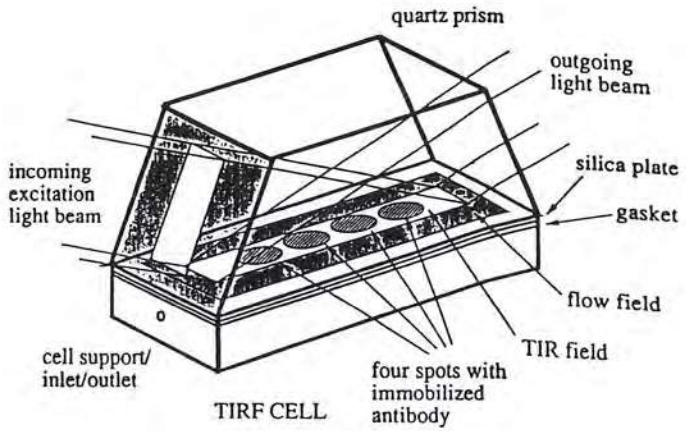


FIGURE 3
Use of the single reflection TIRF geometry for the development of multi-channnel immuno sensors. A cooled CCD is used as a position and wavelength sensitive detector. Four different immobilized Ab regions are detected. (From Ref. 7)

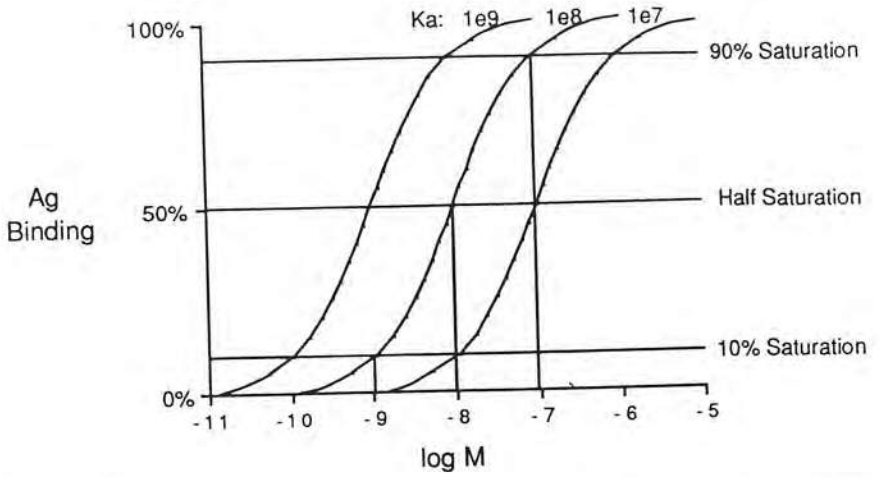


FIGURE 4
Bound Ag - Bulk Ag equilibrium curves, based on a reversible Ag-Ab interaction:

$$Ab + Ag \underset{K_2\ \text{off}}{\overset{K_1\ \text{on}}{\rightleftharpoons}} Ab\text{-}Ag; \qquad K_a = K_1/K_2 = \frac{[Ab\text{-}Ag]}{[Ab][Ag]}$$

when $K_a \sim 1/[Ag]$, Ab sites are ~ 50% saturated.

Range of immunoassay is usually defined as:

$$\frac{0.1}{K_a} < [Ag] < \frac{10}{K_a},$$

i.e. 10 to 90% saturation results in two orders of magnitude range for [Ag].

and discrimination against nonspecific binding could be improved as well.

For much of our work, we have chosen to work with a model system of anti-fluoroscein monoclonal antibodies developed by Voss and coworkers at the University of Illinois.(9) The binding thermodynamics of this system have been studied and presented by Herron et al.(10) The overall association constant as a function

of temperature for three different anti-fluoroscein monoclonals is given in Figure 5. This is bulk solution data and demonstrates that the overall association constant can vary by up to two orders of magnitude over a temperature range of some 40 to 50 degrees centigrade. It was this behavior that led to the concept of the thermal regulation of antigen antibody binding which our group is now studying.(8)

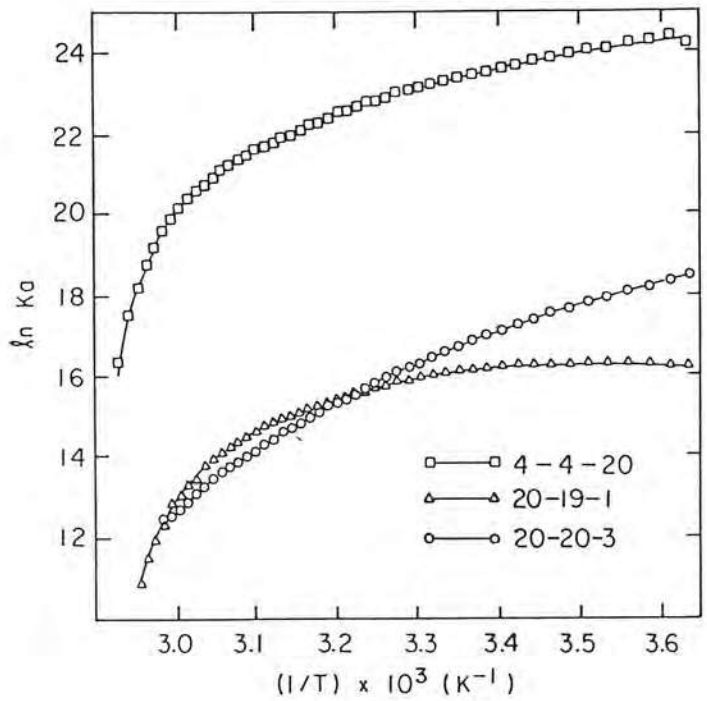


FIGURE 5
Association constants, Ka, as a function of reciprocal temperature for 3 antifluorescyl monoclonal antibodies. (from Ref. 10)

Table 1 presents the binding kinetics for five of the anti-fluoroscein monoclonals, ranging in overall association constant from 5 X 10^7 to 3 X 10^{10} moles $^{-1}$. The table shows that the association rate constant is relatively constant (of the order of 5 X 10^6) while the dissociation constant or its reciprocal, the dissociation lifetime, changes by a factor of 500. It is clear, therefore, that the overall association constant of an antigen-antibody complex can be governed almost entirely by the dissociation rate constant. The dissociation lifetime is the response time of an immunosensor as it attempts to respond to changes in circulating analyte concentration. Therefore, for the development of a truly reversible, continuous immunosensor, the dissociation lifetime issue has to be addressed and considered.

TABLE 1
Ag-Ab binding data for 5 antifluorescyl monoclonal antibodies. The "sensitivity" of a sensor is best indicated by Ka, the overall affinity or association constant. The sensitivity of 50% Ab saturation is shown at the far right. The response time is given by the dissociation lifetime, which is the reciprocal of the off-rate constant (from Ref. 10).

Clone	Association Rate	X	Dissociation Lifetime	=	Affinity Ka	[Ag] at 50% Saturation
	$M^{-1}\ s^{-1}$		s		M^{-1}	(M)
4-4-20	6.28×10^6		5376		3.38×10^{10}	3.10^{-11}
20-19-2	1.28×10^6		454		5.81×10^8	2.10^{-9}
20-20-3	1.08×10^7		38		4.10×10^8	2.10^{-9}
6-10-6	5.33×10^6		14		7.46×10^7	1.10^{-8}
20-4-4	4.67×10^6		11		5.14×10^7	2.10^{-8}

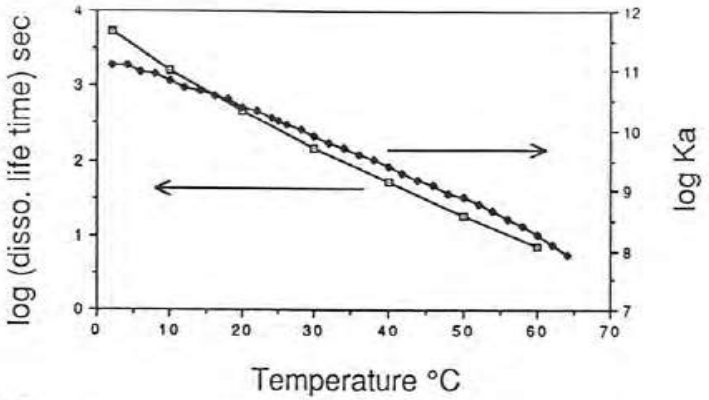


FIGURE 6
The dissociation lifetime (on the left) for the 4-4-20 antifluorescyl monoclonal antibody as a function of temperatures. Log Ka data is given on the right axis. Note that Ka decreases an order of magnitude with a 20° increase in temperature. (from Ref. 10)

Figure 6 presents the dissociation lifetime data for one of the antibodies, the 4-4-20 clone, as a function of temperature. Here one can readily see that the response time of a sensor can be improved by an order of magnitude with a 20 degree change in local temperature.

This discussion of antigen/antibody binding, however, has used data taken in bulk solution. Under those conditions, antigen/antibody interactions are reversible. However, the situation may change significantly when one of the components in the binding reaction is immobilized at a solid liquid interface. We will next discuss the question of antibody immobilization followed by the issue of the reversibility of antibody/antigen interactions at interfaces.

4 ANTIBODY IMMOBILIZATION

A wide range of immobilization methods have been developed and applied. We have experimented with: (11)

- surface modification by the use of silane chemistries;

- the use of cross-linking and coupling agents, including glutaraldehyde;
- the use of a precursor film of protein (such as bovine serum albumin);
- the use of proteins which may help to at least partially orient the immobilized antibodies, such as protein A.

In our case the reactions are constrained by the fact that we need to immobilize at an interface which is optically compatible with the total internal reflection/evanescent wave optical separation method previously described. In addition to the detailed immobilization chemistry, it is important to consider the collision and adsorption of the antibody to the interface, a precursor step to the covalent chemistry employed in the immobilization process. (12) Thus, it is important to understand the collision, orientation and adsorption of antibody at the solid/liquid interface.

An up-to-date, although qualitative, picture of protein adsorption is presented in Figure 7. The protein must first approach and collide with the surface, given by an overall adsorption rate constant. It may also desorb from the surface, given by the desorption rate constant. When these two processes are equal, then we say we have an equilibrium amount of protein adsorbed on the surface. However, Langmuir adsorption does not generally apply to the adsorption of macromolecules because a variety of other processes and interactions are present. One of the most interesting is the fact that the protein may conformationally change and the degree of that change may be a function of residence time at the surface. This is indicated by the time dependent conformational change depicted in Figure 7.

Another problem is that antibodies, in addition to being large macromolecules, have different, distinct domains. The domains may have their own particular interface activity characteristics. This is indicated at the bottom right of Figure 7 which shows a two-domain protein interacting with a surface by one of its domains.

If there is more than one type of protein present in the solution, then there are multi-component, competitive adsorption and immobilization processes which also occur. This is illustrated at the bottom left of Figure 7.

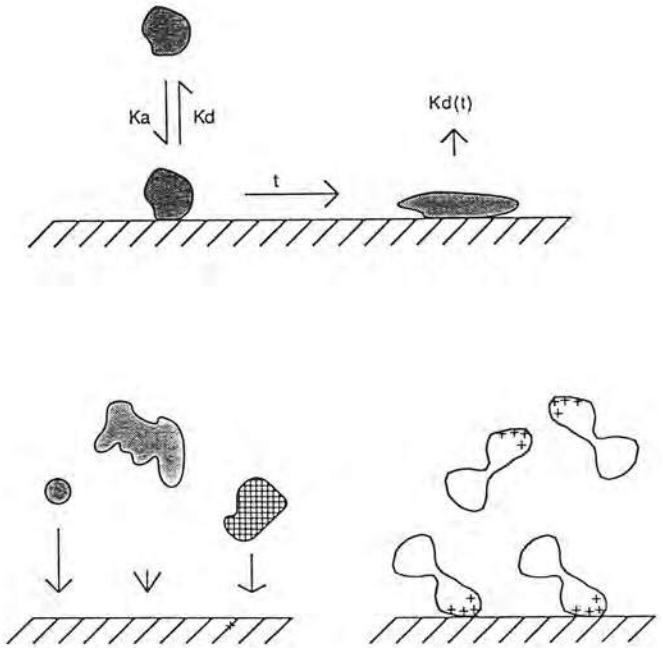


FIGURE 7
Top: Adsorption from a single protein solution can be described in terms of adsorption (Ka) and desorption (Kd) rate constants at initial contact ($t=0^{\circ}$). With increasing residence time, t, at the surface the adsorbed protein may conformationally change or denature, generally resulting in a greatly decreased desorption rate, represented by Kd(t).

Bottom Left: Adsorption from a multi-component protein solution can be treated in terms of the concentrations and diffusion coefficients of each component--ie. in terms of the collision rate of each component--and a measure of the affinity of each component for the interface and the denaturability of each component. (17,18)

Bottom Right: The adsorption of complex, multi-domain proteins may be dominated or controlled by the interface activity of one key domain, such as the positively charged binding domains in the heparin-binding plasma proteins. (19,20)

Immunoglobulins are highly complex proteins with a variety of distinct functional and structural domains. There are hinge regions and switch regions, the function of which might depend on the local micro-environment and mode or mechanism of adsorption and immobilization. These regions, in turn, may affect the binding constant of the active site in ways which are not fully understood at present. Intentionally denaturing IgG prior to its adsorption at a solid/liquid interface (12) can result in a significant increase in the surface antigen binding capacity. Lin, et al. demonstrated that it is possible to engineer the immobilization of antibodies to optimize their properties at the interface by control of their structure in solution.(12) This work is continuing with a detailed investigation of the solution, denaturation and structural characteristics of model antibodies in the hopes of optimizing means to adsorb and then immobilize these molecules with enhanced antigen binding characteristics.

5. ANTIGEN/ANTIBODY REVERSIBILITY

A true immunosensor requires that the sensor respond to changes in the circulating analyte concentration. Most immuno "sensors" developed to date are not true sensors but rather are detectors. They are designed to measure the analyte concentration and then either are discarded or regenerated by some process.

Our goal is to develop immunosensors which are inherently reversible and can continuously sense circulating analyte concentration. The problem is illustrated briefly in Figure 8. The bottom half basically demonstrates immobilized antibody interacting with its antigen and shows that although the on-rates in the bulk solution and interface case may be similar, the off-rate constant is much smaller in the case of immobilized antibody, meaning that the dissociation lifetime is much longer, which mean then that the response of the sensor is very slow. The question is why?

This issue has been addressed briefly by several authors (15) and more completely by Lin et al. in a recent paper which

presents six general hypothesis for the observation of partial irreversibility in immobilized antibody systems.(2)

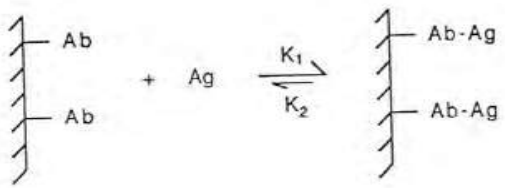
1. Diffusion and mass transport effects. In many systems, particularly highly porous particulate systems, these effects can be very pronounced and indeed dominate the system, so that the irreversibility is only apparent. When proper account is taken of concentration, diffusion, and mass transport effects, the system may be behaving reversibly or similarly to its behavior in bulk solution.

Solution:

$$Ab + Ag \underset{K_2}{\overset{K_1}{\rightleftharpoons}} Ab\text{-}Ag$$

$$Ka = \frac{K_1}{K_2} \sim 10^6 - 10^{12} \quad M^{-1}$$

Surface:



$$(Ka)_{surface} >> (Ka)_{solution}$$

$$(K_2)_{surface} << (K_2)_{solution}$$

FIGURE 8
Bulk solution Ab-Ag equilibria (left) are not generally observed when Ab or Ag is immobilized at an interface (right)--see Ref. 3.

2. The immobilized antibody is in a different conformation than the antibody in solution, thus influencing the nature of the antibody/antigen interaction. This is very difficult to directly measure, other than through on and off rate constants, because the conformational change of the antibody need not necessarily be a gross or very major change. Protein conformational changes are normally detected by changes in the circular dichroic spectra, infrared or Raman spectra, or by tryptophan fluorescence emission. All of these are relatively global measures of protein structural conformation. There could be a significant alteration in the Fab antigen binding site without a global conformational change. Such a local conformational change would not be detected by common techniques.
3. Antibody/antibody lateral interactions. The concentration of antibody at an interface is generally significantly higher than the concentrations used in bulk solution studies. Antibodies are known to readily aggregate, particularly under elevated concentration conditions. It may well be that two dimensional aggregation, perhaps due to lateral interactions at an interface, may significantly affect the antigen binding characteristics, which could result in a slow off-rate.
4. Change in solution micro-environment. It is well known that a change in local solution environment can change the nature of antigen/antibody interactions. For example, in affinity-chromatography columns immobilized antigen is normally released through the use of eluents which change the local solution environment and therefore the nature of the antigen/antibody binding. Although this is not fully understood, it is well known that low pH, eluents containing chaotropic salts, and eluents containing substances which minimize hydrophobic interactions are particularly effective in the removal of bound antigen from immobilized antibody columns.

5. Non-specific adsorption. The antigen may bind to the antibody through its particular epitope interacting with the Fab region of the antibody, but it may also bind by non-specific mechanisms to perhaps other parts of the immobilized molecule and to regions of the surface which are not covered with antibody. Such non-specific binding is a particular problem in the case of protein antigens, which are large macromolecules and can undergo non-specific interactions by a variety of mechanisms. This was illustrated nicely by Lin et al. (2) and also by Schram et al.(15)
6. Multi-valent binding. This is particularly important for larger antigens. If the antigen can be bound by more than one binding site, then it is a cooperative binding process. If there are two or more distinct and perhaps even independent binding events or binding sites holding down the antigen, then even if the antibody-antigen bond is released, the non-specific bond to the surface at that instant in time is maintained. This increases the probability that the antigen antibody bond will reform or reassociate. That makes it almost impossible to ever completely desorb or remove a bound antigen. This is exactly the mechanism believed to be responsible for the irreversibility of physical adsorption processes of proteins and other macromolecules. The multiplicity of binding sites means that the probability that all of the binding sites would be released at the same time is zero; thus, desorption does not occur. This would be expected to be observed in the case of large antigens, but not necessarily in the case of a very small antigens or haptens.

It is clear that all six of the above hypotheses and mechanisms are operable, some more so than others, in a particular experimental situation. It will be very difficult to isolate and sort out the individual components responsible for the irreversibilty of antigen/antibody interactions at interfaces. Lin et al. have summarized the variables affecting the behavior of immobilized antibody/antigen interactions.(2)

6. OTHER ISSUES

A truly remote fluoro-immunosensor will require some means to deliver fluorescently labelled antigen, as the basic principle of such a sensor is that a fluorescently labeled antigen competes with unlabeled circulating antigen for the finite number of antibody binding sites. We have previously described a number of approaches to solve this problem. Those studies are ongoing. Dr. J. Kopecek and co-workers are continuing the development of controlled delivery methods for fluorescent antigens.(21)

Earlier it was noted that it might be possible to regulate the antigen/antibody binding constant by changes in temperature or even by other means. We have performed a number of studies along these lines. However, it is necessary to immobilize antibody by means that permit the reversibility of the antibody/antigen interaction, before one can expect to regulate the nature of the binding constant. Our studies on binding constant regulation are continuing, based on the assumption that we will indeed develop methods to solve the "irreversibility" problem described above.

Non-specific binding is, of course, a problem which must be minimized. Although certain aspects of the problem can be overcome by the use of appropriate reference channels, it is clearly desirable to minimize the problem as much as possible. In our case, this is done through the use of polyethylene oxide as a "protein-repulsive" coating to minimize protein adsorption. This has been discussed extensively elsewhere.(22) We are also developing methods to immobilize antibody through the use of polyethylene oxide tethers or spacers in the hopes of minimizing non-specific adsorption to the spacer or to the chemical linkage itself.

Finally, there may be another problem with the development of truly remote sensors based on immobilized antibodies, particu-

larly in blood and related environments. Blood is known to contain a variety of protease pro-enzymes, which can become activated under appropriate conditions to produce proteolytic activity. If protease activity is indeed generated locally at the surface of an immunosensor, perhaps due to non-specific binding of coagulation or complement proteins, and if such proteases attack the immobilized antibody, then the sensor surface will degenerate with time and will eventually cease to function. That is one of the reasons why it is so important to build multi-channel devices with the appropriate calibration and reference channels to account for decrease or change in immobilized antibody concentration or activity. It is also why the non-specific adsorption problem is so important to eliminate and not simply to reference or calibrate out, as noted earlier. If we minimize the non-specific adsorption of all proteins then we also minimize the possibility of interfacially induced protease activation. Again, our approach to this problem is through the polyethylene oxide surface approach. Studies on the stability of immobilized antibodies are only now being initiated in our laboratory.

7. CONCLUSIONS

The development of truly remote, continuous, high sensitivity and specific immunosensors, capable of functioning remotely in blood or in other biological environments, is still in its early stages. There are a number of important technologies which must be developed. As the work progresses, one can envision the development and eventual production of multichannel biosensors based on microintegrated devices incorporating biochemical, optical and even electronic functions.

This paper has briefly reviewed the general concept and at least one approach to each of the major problems.

ACKNOWLEDGEMENTS

We acknowledge discussions and assistance from many colleagues and coworkers, including: A.Edmundson, M.Block, D.Gregonis, J.Ives, M.Reichert I.Skurnik, P.Suci, D.Tirrell, R.VanWagenen and E.Voss. The early phases of the work were funded by the Office of Naval Research, Biomolecular Engineering Program. Application to coagulation proteins was funded via NHLBI grant HL37046. Biocompatibility and reagent delivery aspects are supported in part by the Center for Biopolymers at Interfaces and by the National Science Foundation (Grant ECE86-02107). Studies dealing with Ag-Ab binding constant regulation are funded by the U.S. Army Research Office. The authors thank Ms. Carol Felis for skillful and personable preparation of the manuscript.

REFERENCES

1. Andrade, J.A., Herron, J., Lin, J-N., Yen, H., Kopecek, J., Kopeckova, P. (1988). On-Line Sensors for Coagulation Proteins: Concept and Progress Report, Biomaterials, 9: 76.
2. Lin, J-N., Herron, J., Hlady, V., and Andrade, J.D. (1989). Dissociation of Ab-Ag Complexes on Solid Surfaces: An Overview, Biosensors: in press.
3. Hlady, V., Reinecke, D.R., and Andrade, J.D. (1986). Fluorescence of Adsorbed Protein Layers. I., J. Coll. Interface Sci. 111, 555.
4. Block M.J., and Hirschfeld, T.B. (1987) US Patents 4 558 014 (1984), 4 447 546 (1984), 4 582 809 (1986), 4 654 532 (1987).
5. Place, J.F., Sutherland, R.M., and Dahne, C. (1985). Biosensors 1: 321.
6. Andrade, J.D., and Van Wagenen, R.A. (1983). Process for Conducting fluorescence Immunoassays Without Added Labels and Employing Attenuated Internal Reflection, US Patent 4 368 047.
7. Hlady, V., Lin, J-N., and Andrade, J.D. (1989). Spatially Resolved Detection of Ab-Ag Reactions at S/L Interfaces Using Total Internal Reflection Excited Ag Fluorescence and CCD Detection, Biosensors, in press.

8. Andrade, J.D., Lin, J-N., Herron, J., Reichert, M., and Kopecek J. (1986). Fiber Optic Immunodetectors: Sensors or Dosimeters, Proceedings of SPIE 718: 280.

9. Kranz, D.M., Herron, J.N., Voss, E.W. (1982). Mechanisms of Ligand Binding by Monoclonal Anti-fluorescyl Antibodies, J. Biol. Chem. 257: 6987

10. Herron, J.N., Kranz, D.M., Jameson, D.M., Voss, E.M. (1986). Thermodynamic Properties of Ligand Binding by Monoclonal Anti-fluorescyl Antibodies, Biochem. 25: 4602.

11. Lin, J-N., Herron, J., Andrade, J.D., and Brizgys, M. (1988). Solid Phase Immobilization of Antibodies for Optical Biosensor Application, IEEE Trans. Bio-Med Eng. 35: 466.

12. Lin, J-N., Chang, I-N, Herron, J., Andrade, J.D. (1989). Influence of Adsorption of Native and Modified Ab on Their Activity, J. Immunologic. Methods, in press.

13. Andrade, J.D. and Hlady, V. (1986). Protein Adsorption and Materials Biocompatibility, Adv. Poly. Sci., 79: 1.

14. Norde, W. (1986) Proteins at Solid Interfaces, Adv. Colloid Interface Sci. 25:, 267.

15. Schramm, W., Yang, T., and Midgley, A.R. (1987). Surface Modification with Protein A for Uniform Binding of Monoclonal Antibodies, Clin. Chem., 33: 1338.

16. Reichert, W.M., Bruckner, C.J., Joseph, J. (1987). L-B Films and Black Lipid Membranes in Biospecific Surface-Selective Sensors, Thin Solid Films, 152: 345.

17. Andrade, J.D. and Hlady, V. (1987). Plasma Protein Adsorption - The Big Twelve, Ann New York Academy Science 516: 151.

18. Andrade, J.D. and Hlady, V. (1987). Protein Adsorption -- The Denaturation Correlation, Croatica Chemica Acta 60: 495.

19. Andrade, J.D., Hlady, V., and Wei, A-P. (1990). A Structural and Functional Domain Approach to Protein Adsorption, Croatica Chemica Acta, submitted.

20. Ho, C-H. (1990). Adsorption of Plasma Proteins Detected by 2-D Gel Electrophoresis, MSc Thesis, in preparation, University of Utah.

21. Yen, H-R., Kopecek, J., Andrade, J.D. (1989). Optically Controlled Ligand Delivery, 1, Makromol. Chem. 190: 69.

22. Lee, J-H., Kopecek, J., Kopeckova, P., and Andrade, J.D. (1989). Surface Properties of PEO-Containing Polymeric Surfactants: Protein-Resistant Surfaces, J. Biomedical Materials Res. 23: 351.

Bioengineering: A Model for Engineering Education

JOSEPH D. ANDRADE

Chairman, Department of Bioengineering
University of Utah
Salt Lake City, UT 84112

Introduction

There is a growing dissatisfaction in the engineering and industrial communities with the present nature of engineering education. A variety of studies and reports have recommended significant modifications of engineering curricula. Bioengineering can – and perhaps should – serve as a model, a paradigm, for all of engineering education. Bioengineers – with their broad perspectives in science and technology – can and should assist in the curriculum modification and development at all levels of the education system.

Engineering Education Today

"The primary task has always been to make practical use of converting scientific theory into useful application . . . to provide for mankind's material needs and well-being . . . The engineer must have a fertile imagination, must be creative . . . accept new ideas" *(1)*.

This implies that engineering students and engineers must know science and they must have some appreciation and knowledge of man's "material needs and well being." The problem with engineering education today is that it is focused almost entirely on science and technology rather than on mankind's needs. Most curricula are heavily loaded with basic and applied science courses and with the methods and techniques "required" to apply scientific information to practical ends. There is a heavy emphasis on mathematics, computer simulation, computer aided design and analysis and on the basic and applied sciences relevant to the engineering discipline being studied.

Very few engineers in society know much about engineering fields different from their own. Most students receive little introduction to general engineering and spend most of their time, after learning the basic science and mathematical principles, on highly specialized courses in their particular engineering discipline. Although most engineers today work in teams, they have difficulty communicating and interacting with one another.

A very important problem deals with mankind's "material needs and well being." Engineering educators and their students generally leave it to non-engineers to decide what mankind's material needs and well being should be. Engineers are not interested in societal or social problems. They have difficulty in dealing with issues and problems that are not analytical or quantitative. They have difficulty in communicating and interacting with non-engineers. They are perceived by the general public as not interested in important social issues, more or less irresponsible or aresponsible with respect to societal problems and concerns, and generally ignorant, or at best apathetic, about such issues. They tend to take a dim view of encouraging students to partake in liberal or general education courses. They oppose any significant modification in curricula, in particular any that would decrease the emphasis on highly specialized engineering courses. These are some of the fundamental problems with engineering education today.

The National Academy of Engineering and the National Research Council recently issued a report, *Engineering Education and Practice in the United States (2)*. They made 23 major recommendations on the subject of engineering education and practice, including:

> "the curricula must be expanded to include greater exposure to a variety of non technical subjects . . ."
>
> "improve the communication skills of engineers, as well as their ability to understand and adapt to changing conditions . . ."
>
> ". . . require restructuring of the standard four year curriculum by various means. The committee recommends that extensive disciplinary specialization be postponed to the graduate level."
>
> "American engineers must become more sensitive to cultural and regional differences . . . and need to appreciate the financial, political, and security forces at play internationally. The non technical components of engineering education ought to include exposure to these aspects of contemporary engineering."

This report was issued in 1985. Although there has been considerable discussion and concern with its recommendations, it has not yet had a dramatic impact on engineering education.

Engineers, when they do speak up on social and political issues, tend to criticize social, economic, and political leaders for their lack of scientific and technical background and for their inability to deal with complex problems. And those same engineers who are doing the criticizing generally fail to become involved in the solutions to these complex problems. It is simply easier to criticize than to act.

It is beginning to be said widely that "we increasingly need effective leaders in business, politics, and education who possess

sound training in science and engineering, and who also have personal skills, aesthetic experience, and a knowledge of history and culture" *(3)*. And yet there is little or nothing in most engineering curricula to encourage and develop the leadership talents of scientists and engineers.

Samuel Florman, a civil engineer who has written and spoken widely on these issues, has expressed it very well: "Something is very wrong with a profession whose young people, starting at age eighteen, turn their backs on the general culture and immerse themselves totally in technical studies . . . If engineers were to become more liberally educated, the profession as a whole would improve and become more worthy of esteem" *(4)*. And yet it is quite common knowledge in engineering colleges and departments that "most faculty and students have viewed non engineering courses as a bothersome waste of time" *(4)*.

Edward Wenk, of the University of Washington College of Engineering: "Higher education should be distinguished by breadth, rather than specialization, and should be problem centered, rather than discipline centered. Graduates should be versatile, capable of self discovery and self expression, able to distinguish truth from propaganda and hyperbole, eager to participate in governance, able to make discussions, prepared for lifelong learning and willing to assume a balance between self interest and social interest, between getting and giving" *(5)*.

A number of national engineering leaders are also now beginning to speak out on these issues. In particular, Dr. George Bugliarello, president of the Polytechnic University of New York and a bioengineer, has argued that "Engineering can best carry out its social purpose when it is involved in the formulation of the response to a social need, rather than just being called on to provide a quick technological fix." He continues, "engineering as a force of society can and should intervene in correcting a social purpose it perceives as detrimental...the dominance of a purely technical role of engineering, particularly in our country, can be attributed primarily to the sociological characteristics of engineering, and to the inadequacy of engineering education in preparing for broad society leadership. The rigorous professionalism of engineering has been achieved in our country at the expense of preparation for broader leadership roles" *(6)*.

Karl Pister, former dean of the College of Engineering at U.C. Berkeley: "We must not continue to isolate the education and experience of engineers from the environment in which engineering is practiced. We must respect the principle that the significance of something lies primarily not in itself, but in its relation to other things." He also stated, "it is a bit ironic to deplore the problem of communication between engineers and non engineers when there is a serious problem of communication among engineers of differing disciplines" *(7)*.

What does all of this have to do with bioengineering?

What is Bioengineering?

Bioengineering is a complex inter- and multi-discipline. To some it means biomedical engineering, the application of engineering principles and methods to the practice of medicine and the improvement of health care. To others it means biotechnology and biochemical engineering and the use of biological principles and processes for the production of chemicals, materials, and drugs. To others it means agricultural engineering, or a modified and modernized version of agricultural engineering. There is a very small, but growing, group that sees biology as just as critical and important to *all* of engineering as are chemistry, physics, and mathematics today *(8-10)*. To those of you reading these pages, however, it primarily means biomedical engineering.

Is there anything unique or special about biomedical engineering that can serve as a paradigm or model for the necessary changes in engineering education? I think so. Most biomedical engineering programs require a strong background in human or mammalian physiology, and most require at least some background in organic chemistry and biochemistry. Most curricula also include some introduction to medicine, medical problems, and pathology. Although one can argue that much of this background may not be in great depth, it does provide an appreciation and awareness of the multivariate and complex nature of living systems, and particularly of the human organism. Thus of all engineering disciplines, bioengineers are the most prepared to address "mankind's material needs and well being", which was part of our earlier definition of engineering. The issue is one of awareness and perspective in order to design and build an object or device that will help enhance mankind's "well being." One cannot be ignorant in the nature of man and mankind, and of the perturbations and perhaps problems that our devices can produce and cause. This is the fundamental argument for the broadening of an engineering education, and an argument for including a biological and life sciences component in *all* of engineering education. How can you produce goods and devices for man if you know nothing about man himself or herself?

In addition to its multidisciplinary nature, bioengineering is inherently interdisciplinary. A bioengineer is not simply someone who knows some medicine and physiology, and perhaps some electrical engineering. If that were all that were required, then one would be far better off in getting an electrical engineer and a physician together to solve the problem. Such a collaboration does not work unless the physician is committed to learning some electrical engineering, or the electrical engineer is committed to learning some medicine. The problem is not simply one of multidisciplines, it is one of interdisciplines.

J. Prausnitz, Professor of Chemical Engineering at Berkeley: "Scholars in the history of knowledge have shown convincingly that in any discipline significant growth inevitably occurs at the periphery, at the interface that separates one discipline from another. When communication between disciplines declines, creation of new knowledge is impaired." Thus biophysics came out of the junction between biology and physics, biochemistry between biology and chemistry, materials science is an interdiscipline involving chemistry, physics and engineering, and now is even beginning to involve biology. The best and most effective bioengineers are not multidisciplinarians; they are interdisciplinarians. They look at the problems or opportunities in one area from the perspective of another area.

Every established discipline carries baggage — a set of

implicit assumptions and understandings about what is known, what is possible. A team of individual disciplinarians brings those constraints and boundary conditions through its members to each problem that the team addresses. Expert A tells Expert B that we can do this and we cannot do that. Expert B doesn't challenge that; after all, who is he to challenge Expert A's analysis and decision?

The interdisciplinarian, on the other hand, "diffuses" along the interface between the two disciplines and is either ignorant of the implicit assumptions and boundary conditions, which the experts accept, or is so confident that he ignores them. In a sense that's what bioengineers are. They think they know enough physiology, and they think they know enough engineering, to attack these interdisciplinary problems, and they are generally successful. If they knew far more engineering, or far more physiology, or were specialists in a particular medical area, they might know that what they were trying to do would not be possible for various "reasons" – reasons often inherent to and limited to that particular discipline. Basically, an interdisciplinarian has little respect for disciplines. Bioengineers have enough background to feel comfortable with the terminology, the problems, and some of the basic science, and they have the self confidence and the motivation to learn what they need to know to solve the problem and complete the project.

There is a major problem with bioengineering education, however, and it is the same problem that faces all of engineering. Although we have been very successful in merging the life sciences and the medical sciences with engineering, and have been successful developing new devices and methods for application to medicine and health care, we, like our engineering brothers in the more classical disciplines, have not really considered whether "mankind's material needs and well being" are being improved by our activities.

There is growing concern in this country with the costs of health care, with maintaining life, and extending life under conditions where the patient's quality of life is very poor. Although undergraduate engineering education usually includes a token course in economics and in cost-benefit analysis, graduate engineering education, including graduate bioengineering education, has generally ignored such issues. Those days are over.

In our own department we are now teaching a course titled Bioengineering and the Costs of Health Care to introduce our students to the problem. The solutions are not analytical, they are not quantitative, they are not simple; they are highly societal and political in nature – topics with which bioengineers, as well as all other engineers, generally feel uncomfortable.

Another attribute of bioengineering education is communication. Bioengineers often function as bridges between disciplines in order to help facilitate communication between medical specialists, life science specialists, and engineering specialists.

So What?

Bioengineers tend to work with people. They have some appreciation of mankind's social and material needs. They tend to have broader interests than engineers in the more classical disciplines. They tend to work in multi- and inter-disciplinary teams and often function as catalysts and communicators in such teams. They are often hired by industry to help lead and manage product development teams. They thus have a perspective and background that can be very useful to their colleagues in academia and industry. They can speak out and offer their assistance in the modification and enhancement of engineering education. Bioengineering can serve as a model or paradigm for engineering education in general.

Bioengineering students can become involved as teaching assistants in introductory and more general engineering courses, using their background and experience to provide a multi- and inter-disciplinary perspective which might otherwise be lacking in such courses.

Bioengineering educators can involve their more classical colleagues not only in research, but particularly in teaching activities, and offer their own services as lecturers and participants in key engineering courses.

Although changing the perceptions and assumptions of professionals is extremely difficult, we cannot wait for the present generation of relatively conservative engineering educators to retire before engineering education is substantially changed. Although education is an excruciatingly slow process, we should all become involved in that process.

The sooner we modify and enhance engineering education, the sooner we can expect engineering graduates to improve the nation's economic competitiveness; more importantly, the sooner we can expect them to begin to utilize rational, logical, and quantitative skills in leadership positions in all areas of society.

How Should We Change Engineering Curricula?

One possibility is to minimize the overspecialization within single, well defined disciplines. Some of the departments in my own College of Engineering list and teach nearly 100 different courses in their specific disciplines!

Many schools have found considerable success with a more project-oriented curriculum. After obtaining an appropriate grounding in basic science and the major courses in the particular discipline, students go on to participate in projects, often team projects, in which they learn what they need as they go along, and much of that learning is done independently. This has been called "just in time learning" or "just in time education", analogous to "just in time manufacturing" processes. Such an experience prepares students for real world, problem-solving activities.

Nobody really remembers much of their highly specific engineering courses five years into their career. In fact, it is highly probable that some of the methods and even principles that are being taught in these courses will be obsolete, or at least inappropriate, five years from now.

Education should emphasize lifelong learning and should provide students with an awareness and respect for all parts of the library, not just their own narrow discipline. A *projects orientation* also facilitates the inclusion of such topics as environmental impact, cost-benefit analysis, benefit-risk analysis, and ethics and

societal responsibilities. Historical and cultural perspectives can also be easily woven in.

There have been pleas from major engineering educators to unjam the curriculum *(11)*. It could be significantly unjammed by just eliminating the highly specific courses and replacing them with a projects-oriented approach to learning. The problem is that most engineering educators are uncomfortable with project courses because many of them have not had relevant project experience. Many of the present generation of engineering professors feel uncomfortable on any subject outside of their own particular course specialty. This of course argues for some creative leadership at the department and dean level, including workshops, conferences, and industry experiences for those faculty who most desperately require them.

We must encourage students (and faculty!) to take courses in the humanities and liberal arts. We must convince them that such courses are just as important – even *more* important – than specific engineering courses *(12, 13)*. They must learn to write, to speak, to communicate. They must learn that mankind's material needs and weil being involve far more than "material" things. They must assume some leadership in society.

Change does not happen spontaneously. The only thing we can count on happening without our intervention is the increase of entropy and the aging process. Positive change requires planning, leadership, and motivation. Take the time to become involved!

Acknowledgements

I thank my colleagues at the University of Utah who have been very involved in enhancing engineering education, especially Ken Horch, Doug Christensen, Richard Normann, John Wood, Magdy Iskander, Eric Stroup, and Robert Huefner.

REFERENCES

1. Beakley, B.C., D.L. Evans, J.B. Keats, *Engineering: An Introduction to a Creative Profession.* 5th ed., MacMillan Publ. Co., New York, 1986, p. 3.
2. –, *Engineering Education and Practice in the United States.* National Academy Press, 1985, Executive Summary, pp. 11-23.
3. Mark, H. and L. Carver, Educating engineers for leadership. *Engrg. Educ.*: 104, Nov. 1988.
4. Florman, S.C., The civilized engineer. *Issues in Science and Technology*: 82, Summer 1989.
5. Wenk Jr., E., Portents for reform in engineering curricula. *Engrg. Educ.*: 99, Nov. 1988.
6. Bugliarello, G., *Engineering and Its Social Function,* presented at National Academy of Engineering Annual Meeting, Oct. 3, 1990.
7. Pister, K.S., *Engineering the 21st Century*, speech on April 3, 1990.
8. Pilkington, T.C., F.M. Long, R. Plonsey, J.G. Webster and W. Welkowitz, Status and trends in biomedical engineering education. *IEEE Eng. Med. Biol. Mag.*: 9, Sept. 1989.
9. Johnson, A.T. and D.C. Davis, Biological engineering: A discipline whose time has come. *Engrg. Educ.*: 15, Jan/Feb. 1990.
10. Bugliarello, G., Bioengineering: Merging the artifact and its maker. *Mech. Eng.*: 46, Sept. 1989.
11. VanValkenburg, M., Unjamming our curricula. *Engrg. Educ.*: 456, April 1989; More on unjamming. *Engrg. Educ.*: 52, Jan/Feb. 1990.
12. Opsuta, M., Eleven strong votes for non-engineering electives. *Graduating Engineer*: 45, Nov. 1983.
13. Johnston Jr., S.S., S. Shaman and R. Zemsky, *Unfinished Design - The Humanities & Social Sciences in Undergraduate Engineering Education.* Assoc. of Amer. Colleges, Washington, DC, 1988.

Biomedical Engineering Applications in the Life Sciences Programs for Space Station Freedom

DANIEL W. BARINEAU

KRUG Life Sciences, Inc.
1290 Hercules, Suite 120
Houston, TX 77058

The approaching construction of Space Station Freedom (SSF) has presented the engineering community with a wide range of challenges to overcome. From a biomedical engineering (BME) standpoint, problems must be addressed concerning various equipment design parameters (weight, mass, volume, power, run time, amount of automation, data generation, etc.) as well as the condition of the equipment operators (astronauts). SSF currently has three different programs to fill the requirements for basic human research in microgravity, in-flight operational monitoring and countermeasures, and on-orbit acute clinical care. These are respectively the Space Biology Initiative (SBI), Biomedical Monitoring and Countermeasures program (BMAC), and the Crew Health Care System (CHeCS).

In the SSF scenario, the three life sciences programs follow a prescribed pattern in function and organization, with SBI being the research program, BMAC being the operationally designed

J. Biomater. Sci. Polymer Edn, Vol. 2, No. 3, pp. 161–172 (1991)

Vroman effects, techniques, and philosophies

J. D. ANDRADE* and V. HLADY

Department of Bioengineering and Center for Biopolymers at Interfaces, University of Utah, 2480 MEB, Salt Lake City, UT 84112, USA

Received 25 June 1990; accepted 20 August 1990

Abstract—Leo Vroman's work on blood–materials interaction over the years has motivated and influenced much of our work in this field. Here we show how most of our studies on proteins at interfaces can be traced to Vroman's ideas presented in *Blood* over 25 years ago. Specifically, we briefly discuss simple proteins at simple interfaces, complex interfaces, complex proteins at interfaces, multi-parameter phenomena, and scientific communication and education.

Key words: Vroman effects; blood; protein adsorption; competitive adsorption; gradient surfaces; denaturation.

INTRODUCTION

In 1967 a little red book entitled *Blood* was published by the American Museum of Natural History [1]. Although designed as a popular science book, it proved to be the most complete and thorough treatment of blood and blood proteins at interfaces then available. The hypotheses, methods, and general approach to the problem of contact activation in blood compatibility presented in that book have served as the basis for the research careers of many investigators, and indeed of the field in general. There are a number of reasons for the book's major influence and popularity: (1) it is exceptionally well written, it is a delight to read, and, through the entertaining and effective use of metaphor and analogy, it helps to present very difficult concepts in very understandable ways; (2) Vroman, in the book, and indeed throughout his career, tends to take a more global, panoramic view of a phenomenon than do most scientists. He tends to be much more interested in seeing and deducing the nature of the forest rather than experimenting upon and describing each individual tree and branch; (3) a variety of methods and techniques are presented in the book and have been employed by Leo Vroman during his research career to detect, illuminate, and generally understand the behavior and interactions of proteins at interfaces.

One of us (J.D.A.) literally stumbled across this book in 1967 in the children's section of a small Denver library. Leo Vroman kindly agreed to advise Paul Predecki and Joe Andrade at the University of Denver in a study dealing with blood-compatible materials. He first suggested that albumin might be a particularly compatible protein to attempt to bind and immobilize at material surfaces, which led to the formation of the albumin hypothesis, which was later extensively studied by a number of research groups worldwide [2].

*To whom correspondence should be addressed.

[illegible] present the current [illegible] investigators. The book is a delight to read. Leo's expert use of words, of images, of metaphor have made the book a science classic. Here follow a number of quotations from the book:

> The vast, untidy wilds in the world seem to withdraw almost eagerly from advancing man. The forests drain themselves of beasts and flatten into meadows, the meadows are turned into suburbs, and the suburbs are swallowed by our cities. The only thriving wildlife still barely touched by the mind is the hot, confusing, and poorly lit world within himself. I am a nature lover; but maybe for lack of larger scenery, I have learned to enjoy the tabletop-size scenery inside me, and inside you of course. The strangely shaped glands, and bones, the transparent lungs, the madly dense tangle of cells in the brain hold worlds within worlds; and, through it all and always, streams the blood. (p. ix)

> Unlike the machine gun, the syringe is an awesome weapon to hold, because it forces the attacker so close to his victim, who will then look at him and think: What do you think you are doing? It is easier to kill a distant crowd with a missile than to prick a child with a pin. (pp. x–xi)

> . . . if something alive is normally invisible, it will become quite abnormal as soon as it is made visible. If you want to see an earthworm as it normally lives, you must look at it only when it is gone. (p. xi)

> I knew my rabbits. I made it a point to look deeply into the eyes of each new arrival. Into its long, hot ears I would softly discuss the weather, so that when the time came for me to design little tests for my little theories, I would think long and hard in order to be sure there was no way but through an animal's death. And then, like all biologists I am sure, I tried to do as many quick little tests as possible in the short twilight of life remaining within the organs, like a man making light for reading a map by setting it on fire. (p. 17)

> When I was a little boy, I loved our piano and I hated playing it. One of the few really good things I could do with it was to undamp its strings with the pedal, meanwhile open the top lid, stick my head inside, and shout. All through its eternal darkness I could then hear the echo of my colorless yell breaking into a brilliant spectrum of pure notes, as each string vibrated in resonance with its own pitch that it had recognized in the mixture of my voice. The energy it needed for this resonance could have come from only one source: my own sound. It must have absorbed that particular bit of energy. (p. 56)

> . . . globular protein molecules have their hydrophobic amino acids hidden inside them to keep them dry. I now believe that those which can

The book did more than present the current status of the field of contact activation. It basically presented a philosophy, a way of looking at the world, and a way of doing science which tended to stimulate and encourage a generation of investigators. The book is a delight to read. Leo's expert use of words, of images, of metaphor have made the book a science classic. Here follow a number of quotations from the book:

> The last jungles in the world seem to withdraw almost eagerly from advancing man. The forests drain themselves of beasts and flatten into meadows, the meadows are turned into suburbs, and the suburbs are swallowed by our cities. The only thriving wildlife still barely touched by the mind is the hot, confusing, and poorly lit world within himself. I am a nature lover; but maybe for lack of larger scenery, I have learned to enjoy the tabletop-size scenery inside me, and inside you of course. The strangely shaped glands, and bones, the transparent lungs, the madly dense tangle of cells in the brain hold worlds within worlds; and, through it all and always, streams the blood. (p. ix)

> Unlike the machine gun, the syringe is an awesome weapon to hold, because it forces the attacker so close to his victim, who will then look at him and think: What do you think you are doing? It is easier to kill a distant crowd with a missile than to prick a child with a pin. (pp. x–xi)

> . . . if something alive is normally invisible, it will become quite abnormal as soon as it is made visible. If you want to see an earthworm as it normally lives, you must look at it only when it is gone. (p. xi)

> I knew my rabbits. I made it a point to look deeply into the eyes of each new arrival. Into its long, hot ears I would softly discuss the weather, so that when the time came for me to design little tests for my little theories, I would think long and hard in order to be sure there was no way but through an animal's death. And then, like all biologists I am sure, I tried to do as many quick little tests as possible in the short twilight of life remaining within the organs, like a man making light for reading a map by setting it on fire. (p. 17)

> When I was a little boy, I loved our piano and I hated playing it. One of the few really good things I could do with it was to undamp its strings with the pedal, meanwhile open the top lid, stick my head inside, and shout. All through its eternal darkness I could then hear the echo of my colorless yell breaking into a brilliant spectrum of pure notes, as each string vibrated in resonance with its own pitch that it had recognized in the mixture of my voice. The energy it needed for this resonance could have come from only one source: my own sound. It must have absorbed that particular bit of energy. (p. 56)

> . . . globular protein molecules have their hydrophobic amino acids hidden inside them to keep them dry. I now believe that those which can

> open easily will do so when they see a hydrophobic surface, and will turn themselves inside out to paste themselves with their fatty hearts onto that surface. (p. 63)

> We may have grown like that, onto specific spots of rock with electric charges on their surface just right to form patterns from the molecules of protein they adsorbed, forming lacey films tied together with hydrogen and hydrophobic bonds, mutating, but growing their way out of one disaster into another, and meanwhile trapping chaos in our meshes and creating order from it, locking in fats and phospholipids, and blowing micelles up to bubbles that adsorbed more protein and held specific enzymes safely inside them. One very special bubble, a deceptively simple one, is the red blood cell . . . (p. 100)

> It is bad enough that we cannot see small enough; it is worse that we cannot see briefly enough. If we could see one million times one millions times faster, we could see that the water is made up of always forming and breaking clusters, but our slow senses only give us an unchanging, smudged image, a statistical, dead average for our slow, long-living mind. One minute in a uniformly moist, white fog to us may be a year long series of floods interrupted by many dry spells to a tiny, fast, and briefly living bug. (p. 137–138)

> I think the most obvious property, the very nature even of life, seems to be its dedication to making simple matter more complicated, more and more structured, thereby opposing the physical laws that want lifeless matter to become less and less shaped, less organized, more chaotic. (pp. 157–158)

> . . . simplification means giving more structure, not less. A chair will look like a very complex structure if you force yourself to forget its purpose. (p. 158)

A careful reading of the book leads us to conclude that 25 years ago Leo Vroman asked the critical questions and set the directions which have governed the last quarter century of work on blood compatibility and biointerfacial chemistry. With the benefit of 25 years of experience and hindsight, the reader can find references in the book to:

(1) the complex, competitive, adsorption hierarachy, which came to be known later as the Vroman effect;
(2) the concepts of hydrophobicity and hydrophilicity of surfaces and interfaces, including the question of the hydrophilicity of the endothelium;
(3) the role of polymer surface dynamics in biointerfacial processes, including the unique structure and properties of lipid bilayers and cell membranes;
(4) the importance of protein structure and composition, including the important effect which a single amino acid replacement can have on the behavior and properties of a protein;
(5) the importance in looking at complex biological phenomena as a whole, as an image, rather than in attempting to overly dissect it into an infinity of

individually meaningless variables — he emphasized the need to look for correlations and relationships between sets of variables rather than to overly focus on or quantitate one or two individual parameters; and

(6) the importance of communicating our scientific philosophies, theories, and results in a manner which can be readily assimilated and understood.

SIMPLE PROTEINS AT SIMPLE INTERFACES

It was clear to Leo Vroman that the composition and structure of a protein was responsible for its interfacial behavior, and that its conformational stability influences its behaviour at interfaces, particularly at the nonpolar air–water interface, where it could distort, denature, and expose its hydrophobic heart to the surface.

Many years later, long after techniques and methods became readily available to present the three-dimensional structure and chemistry of a protein molecule on a computer screen, we initiated a study of human and hen lysosyme adsorption at solid–liquid interfaces [3] and, later, at air–water interfaces. That study has since been expanded to include other model proteins whose three-dimensional structures are known. Their adsorption at the air–water interface was studied by dynamic surface tension methods. The surface nature and structure of each of the proteins were examined by displaying the three-dimensional crystal coordinates on a computer screen and studying the various 'faces' or surfaces of the individual molecules. The surface hydrophobicity of each of the proteins was further explored through the use of fluorescent probe titration to develop an 'effective surface hydrophobicity' parameter. Finally, each of the proteins was studied in solution via urea and guanidinium chloride perturbation of their solution structure, thereby evolving a free energy of folding, from which an estimate could be derived of the stability of each of the proteins employed. These various parameters — effective surface hydrophobicity, intrinsic stability, and interfacial behavior from the dynamic surface tension technique — are correlated.

These studies have led to the following hypotheses: (1) the initial collision or contact event between a protein and an interface is influenced, and even controlled, by the external surface chemistry of the individual protein molecule. Although proteins collide with the interface in a variety of different orientations, many, if not most, of those collisions will be ineffective. Only those collisions in which the protein presents a face or surface which leads to a significant adsorption free energy will have the appropriate residence time to result in adsorption. (2) *After* the initial collision event, the protein may conformationally adapt to its new environment. Those proteins which are the least stable will have the greatest probability of conformationally adapting or denaturing at the interface. Figure 1 [4] qualitatively presents four limiting cases for adsorption at the air–water interface: proteins which have a high degree of surface hydrophobicity and are either hard, i.e. stable, or soft, i.e. unstable, in solution; and proteins which have very little surface hydrophobicity and are hard or soft in solution. This leads to four limiting classes of adsorption behavior at the air–water interface, and probably at hydrophobic interfaces in general. Proteins with high surface hydrophobicity result in a large decrease in surface tension, even at very short times. Soft or unstable proteins denature at the surface, thereby exposing their nonpolar interior and resulting in a further decrease in surface tension with time. (See ref. [4] for further explanation.)

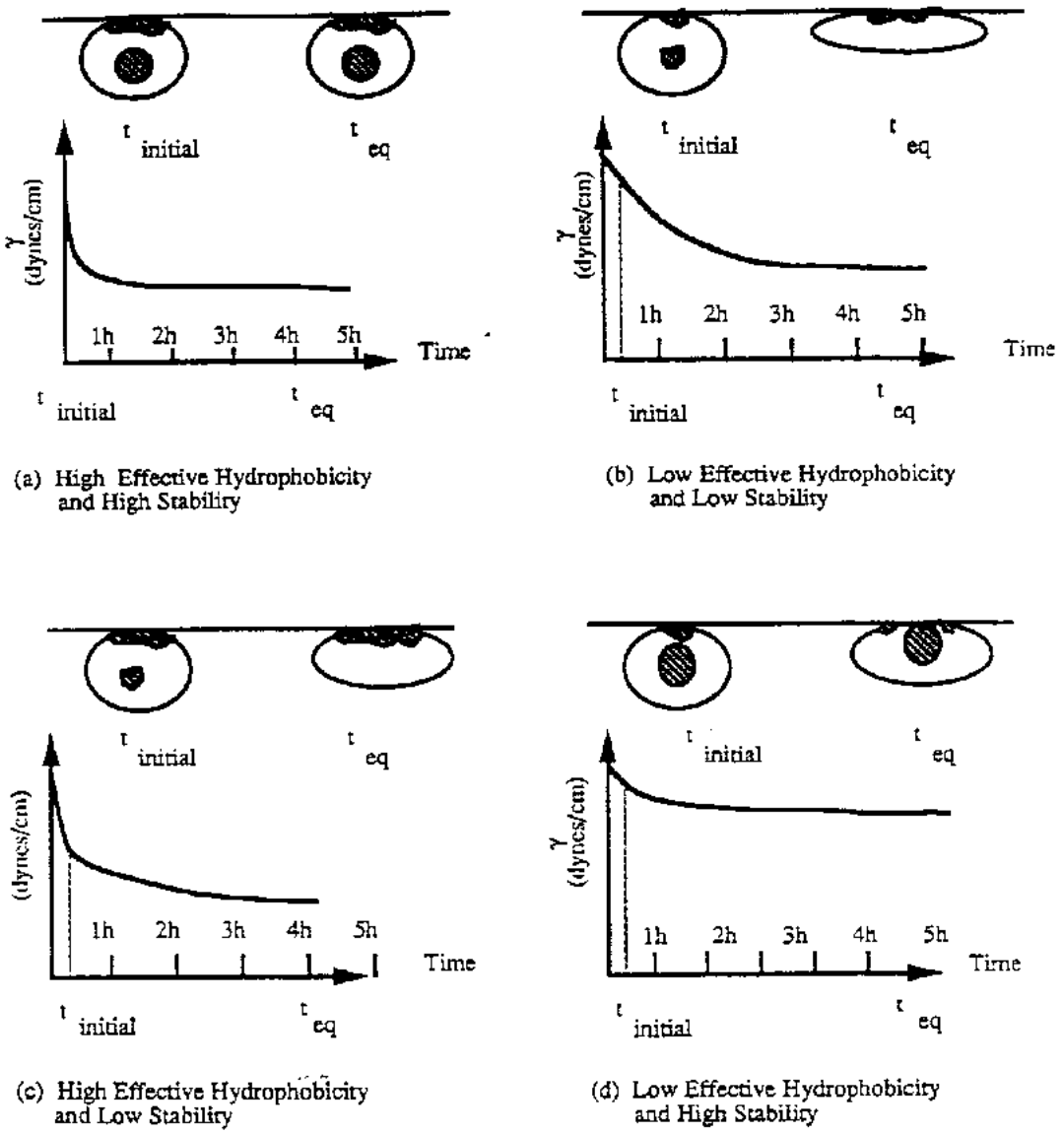


Figure 1. Conceptual illustration of the role of stability and effective hydrophobicity in the reduction of surface tension of small model proteins in high bulk concentrations at the air–water interface (taken from ref. [4]) (see text for discussion).

These studies were done using model, small, single-domain proteins in the hope of serving as a basis for application to larger, more complex multi-domain proteins. Such studies are now in progress [5].

COMPLEX INTERFACES

Classical surface chemistry assumes that its solid surfaces are homogeneous, rigid, and immobile. *Blood* clearly suggests that Leo Vroman appreciated and understood why most biological interfaces are not rigid or immobile. He also treated the topic in his scientific papers:

> . . . blood flows over the soft bed of endothelial membrane molecules that bend with the slightest atom wind too softly for protein molecules to adhere. (*Thromb. Res.* **1**, 437 (1972))

> . . . the apparent simplicity of the interactions of plasma and endothelial surfaces is maintained by the complexity of the substrate. The complexity of the interaction of plasma and polymer surface is caused by the simplicity of the substrate. *Ann. N.Y. Acad. Sci.* **516**, 300 (1987))

Although we feel that the latter quotation has one or two decades to go before it is fully appreciated, the biomaterial/biocompatability community has responded to the need for an appreciation of the surface properties and surface nature of the materials placed in contact with blood and blood proteins.

In *Blood*, Vroman clearly talked about the surface characteristics of different adsorbants and chromatographic supports used for the purification of different classes of plasma proteins. He was also clearly tuned to the dynamics of the endothelium and of cell membranes in general. It was his demonstration that the endothelium was hydrophilic which led one of us, many years ago, to begin to formulate the interfacial free energy hypothesis, which provided a rationale for the study and application of hydrogels as blood-contacting materials [6].

The dynamics of surfaces have really formed the basis of the current large interest in polyethylene oxide surfaces as protein-resistant, or protein-repulsive, materials [7]. One could even say, with the current interest and activity in block copolyurethanes, particularly the parts dealing with their dynamics and restructuring in various environments [8] can at least be qualitatively traced to the interface dynamic concepts suggested in *Blood*.

COMPETITIVE ADSORPTION AND DESORPTION — THE VROMAN EFFECTS

The development of specific antibodies as probes and detectors for adsorbed proteins by Vroman and co-workers in the 1960s and 1970s confirmed his earlier observations and suspicions that blood protein adsorption involved a hierarchical series of collision, adsorption, and exchange processes. These observations and concepts were confirmed by Horbett [9], Brash and Ten Hove [10], and others, and came to be known as 'the Vroman effect' [9,10]. The effect turned out to be a series of effects which are now widely studied and the subject of this symposium. One of those effects relates to competitive collision, adsorption, and exchange processes dependent on concentration, diffusion coefficient, and adsorption affinity. This Vroman effect is now reasonably well modeled, assuming rigid protein molecules which do not denature. The problem of incorporating the denaturability of a protein, and particularly of a complex multi-domain protein, into such multicomponent kinetic adsorption models has yet to be effectively formulated and presented.

Our interest in the Vroman effect led to our application of a powerful, albeit somewhat cumbersome and time-consuming, analytical method known as 'two-dimensional gel electrophoresis'. In *Blood*, Vroman clearly presented the concept of electrophoresis as a means for protein separation and even identification. In the chapter which he cleverly called 'How We Play with Proteins', he briefly presented chromatography and electrophoresis: 'But all never goes well with plasma. It contains so many proteins that it would demand an impossibly sharp image to reveal all its components.' (p. 52). Twenty-five years later, protein separation techniques have

indeed advanced and developed to the point where the individual components of plasma can be separated, detected, and even quantified. Instruments and techniques for high resolution two-dimensional gel electrophoresis became readily available several years ago. We chose to apply the method to the study of the competitive adsorption of plasma proteins using high surface area particles and a solution depletion methodology. Until recently, our group had focused primarily on flat surfaces of low surface area and on the use of radio-labeled and fluorescently labeled proteins for our studies. But it was clear to us that the ability to see 50–100 *different* plasma proteins, competitively interacting in real time without labels or other potential artifacts, provided a seductive and powerful opportunity with which to study the Vroman effect. With the kind assistance and tutelage of the Andersons, pioneers in the development of two-dimensional gel electrophoresis [11], and with the help of Dennis Reader and Jess Edwards at the National Institute for Standards and Technology, we have shown that this technique is indeed immensely powerful [12]. Although at this stage in its development it is only semi-quantitative, it does provide a multi-parameter, multi-protein assessment of the Vroman effect. The work to date has focused on heparin-binding proteins interacting with heparin–Sepharose particles and, more recently, on plasma proteins interacting with a set of polyether urethanes [8]. The major advantage of, and problem with, the technique is the immense volume of data generated. Since 50–100 different proteins can be detected, it is virtually impossible to look at all of the proteins in every experiment. Although all of the data are recorded and available, we have found it necessary to limit our attention and analysis to only certain subsets of proteins, such as the heparin-binding proteins [12] or those proteins which appear to be unusually depleted and, therefore, active for the particular surfaces being studied. For example, in the case of the polyurethanes, preliminary data suggest that three major proteins have particular affinities for polyether urethanes [8]: fibrinogen, lipoproteins, and hemopexin, a protein with a strong homology with vitronectin, but which has been little studied with respect to protein adsorption.

COMPLEX PROTEINS AT INTERFACES

Now that methods are available for studying the competitive adsorption of complex plasma proteins, we have become interested in attempting to develop mechanisms and hypotheses for the interfacial activity of the individual plasma proteins. Since each plasma protein is a unique molecular personality, and generally has an unknown three-dimensional structure, it becomes necessary to use approximation techniques to begin to assess the interfacial activity of the individual proteins. Building on our limited success with model proteins at air–water interfaces, discussed earlier, and on the rapid development over the last 10 years of the domain concept of complex globular protein structures [13], we have begun to examine key plasma proteins in terms of their individual domain building blocks and the potential interfacial activity of each individual building block. The analysis for albumin utilizes the Brown model of the structure of albumin [14], coupled with an analysis of the low resolution X-ray crystal structure which became available in 1989 [15]. Since the three major domains of albumin vary significantly in terms of charge characteristics as a function of the pH, it is possible to develop electrostatic models for the interaction of albumin at various charged and uncharged surfaces as a function of the pH.

Such models suggest hypotheses related to orientation and thickness of adsorbed layers [5], which can be readily tested using existing literature data or through newly designed experiments. The amphilicity of the helical structure of albumin also suggests strong interactions of hydrophobic interfaces. One could even imagine that adsorption at hydrophobic interfaces would not necessarily require a significant change or decrease in alpha helical content because of the amphiphilic nature of the helices and the tendency of the hydrophobic interface to partially stabilize amphiphilic helices. Theses concepts and approaches have already been presented in a preliminary way [5]. The availability of a refined structure for albumin [16] now permits a detailed analysis of the charge distribution and hydrophobic amino acid distribution of the molecule, and should allow the formulation of specific and testable hypotheses. An analysis of fibrinogen from a domain point of view is now in process. Although the three-dimensional structure of fibrinogen is not available, domain-based models are known, and are sufficient for at least a preliminary assessment of interfacial activity. A comparable situation is the case for IgG: its general domain structure and characteristics are well known.

MULTI-PARAMETER PHENOMENA

Vroman clearly understood and predicted the complex multi-parameter nature of plasma protein interfacial processes. In the 25 year since *Blood* was written, it has become possible to observe directly a number of the key variables simultaneously and in real time using optical and spectroscopy techniques which have become available in recent years.

About 10 years ago, we chose to apply total internal reflection fluorescence (TIRF) spectroscopy to study the kinetics of adsorption processes at low surface area flat interfaces. Fluorescence is a useful technique because it also provides information on the micro-environment of the fluor, which can be interpreted in terms of its local conformation and structure.

Vroman is a master at developing simple means for the observation of complex phenomena. A technique which stimulated a great deal of thinking and activity in the interface community was the elegant convex lens on slide method, which was developed to study surface to volume ratio effects in plasma protein adsorption [14]. The establishment of a surface to volume ratio gradient, and the ability to observe the effects of such a continuous gradient on protein adsorption, was, we think, what really led to the verification and acceptance of Vroman's concepts on competitive protein adsorption and exchange. We think that this development helped lead the biomaterials community to consider other possible gradients and multi-parameter experiments. The introduction by Elwing *et al.* of surface compositional gradients [17] thus received an enthusiastic acceptance by many parts of the biointerface community.

Our own group adopted Elwing's methods and coupled them with total internal reflection fluorescence and fluorescent labeling methods to permit the study of the Vroman effect in the time range from seconds to minutes using surfaces with continuous surface property gradients. This work has led to the direct visualization of adsorption and exchange processes, now commonly known as the Vroman effect (Fig. 2).

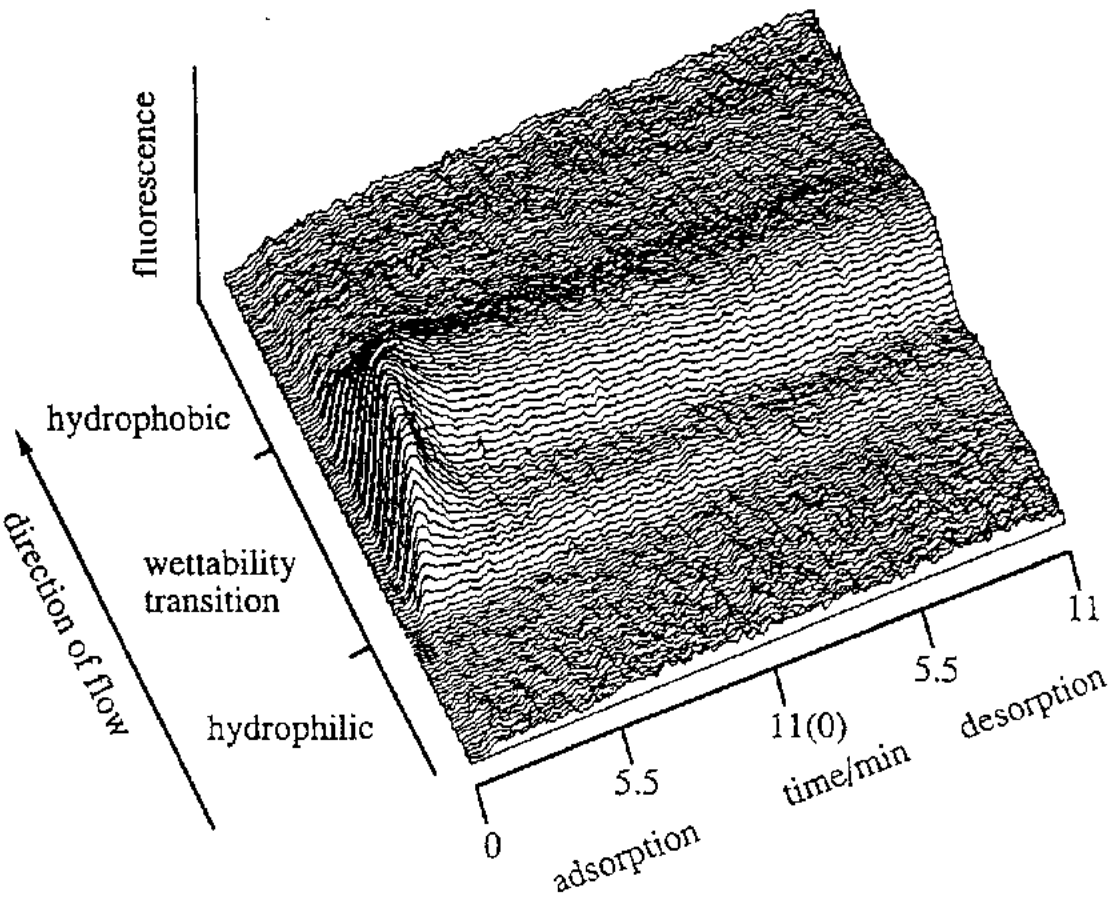


Figure 2. Three-dimensional presentation of fluorescein-labeled-IgG adsorption/desorption kinetics onto a wettability gradient surface as measured by spatially resolved TIRF. The phosphate buffer solution contained IgG ($c_{(IgG)} = 0.013$ mg/ml) and two other unlabeled proteins: albumin and fibrinogen ($c_{(Alb)} = 0.041$ mg/ml, $c_{(Fgn)} = 0.003$ mg/ml). The adsorbed amount of IgG is proportional to the fluorescence signal. Adsorption of the labeled IgG is thus detected in the presence of the unlabeled albumin and fibrinogen — all three proteins are, of course, competing for the limited number of binding sites. Note that the fluorescence intensity in the center of the gradient (the wettability transition region) first increases and then decreases, suggesting that IgG adsorbs but is then replaced by albumin and/or fibrinogen. This directly demonstrates the protein/surface/time dependence of competitive protein adsorption, i.e. the Vroman effect (taken from ref. [18]).

DISCUSSION

In the past 25 years it has become possible to directly measure and present the phenomena, the trends, and the images which have been in Leo Vroman's mind for over 25 years. Although he was able to translate those images into sketches and cartoons, the images and cartoons were treated by many as somewhat fanciful, and perhaps even as fantasy. It is really only now, with 25 years of hindsight behind us, and with techniques and equipment which permit those images to be derived from actual experiments rather than mental constructs, that his ideas have become acceptable to the general scientific community.

It is now safe to say that protein adsorption is, at least qualitatively, reasonably well understood. In our opinion, the next big hurdle is a chemical one. Most of our models, theories, and interpretations assume that protein molecules are stable with respect to their primary chemical bonds. Although we tolerate secondary bonding changes, i.e. denaturation, we generally assume, implicitly, that changes in covalent

bonds are not occurring. We can no longer tolerate such simplification and such luxury. We all know that there are innumerable proteases and preproteases present in plasma, many of which are activated by interfacial processes. Some of Leo Vroman's initial and early experiments with the ellipsometer dealt with protease effects at interfaces. Protease-dependent enzyme cascade systems of blood coagulation and protease-dependent systems of complement activation are well known, and yet we generally ignore the protease effects and changes which are complicating our ultra-simplified protein adsorption experiments and their interpretations. John Brash, Leo Vroman, and others have clearly shown that protease-dependent cleavage and fragmentation of proteins does indeed occur. Given that we can identify, or at least predict, the nature of the protease activity which may be present, and knowing the amino acid sequence and structure of the proteins with which we are dealing, we can predict the cleavage profile for virtually all of the proteins of interest. The task now is to couple that information with our knowledge of denaturation and stability at interfaces and to begin to evolve a comprehensive and global picture of plasma proteins at interfaces.

SCIENTIFIC COMMUNICATION AND EDUCATION

Part of the major success of *Blood* was its very effective use of analogy and metaphor. This is clearly shown in most of Leo Vroman's writings in both the literary and the scientific literature. For example:

> Introducing plasma to a relatively artificial surface the way we do is, on a small scale, like using a pocketknife to section tissue, and then peering at the knife for an image of what we cut. The simpler the knife, the harder it is, and the sharper the false image of nature it creates. (*Thromb. Res.* **1**, 437 (1972))

> Facing a hail of miscellaneous eggs, we cannot expect to come away clean. Unless they are hard-boiled ones, we are most likely to become coated rapidly with a relatively thin film of matter from the most numerous and most fragile eggs. Similarly, no interfaces may exist that, facing blood plasma, can escape being coated with the most abundant and fragile plasma proteins. (*J. Biomed. Mater. Res.* **3**, 43 (1969))

> . . . Wherever the well trained scientist aims he must dive into detail to crash with his brain impaled on his pinpoint specialty. (*Thromb. Diath. Haemorrh. Suppl.* **42**, 167 (1970))

Leo felt strongly that his experiments and results must be relevant to and relatable to the general public:

> Whenever I become so lost into the beauty of smaller and smaller things that no one will tunnel behind me, I must stop and turn myself around, and come back to the world. The day I come home unable to explain to a child what I did, that day is lost, and that child has receded from me.

In this day and age when we are all very concerned with the scientific literacy of the general population, and indeed even with the scientific population, it is imperative that all of us attempt to effectively communicate the joys, rewards, and achievements of science to our acquaintances, our children, our grandchildren, and to the general community at large. It is only if all scientists and technical people do their part in communicating that we can expect the community at large to appreciate and support our work, and, even more importantly, to be able to make intelligent judgements and decisions regarding scientific and technological issues. Leo's artistic and literary skills, coupled with his exquisite scientific intuition and perspective, have been very effective. Perhaps the greatest honor that we can bestow upon Leo Vroman is our own commitment to attempt to emulate him in some limited ways.

The following quotation from one of his artistic colleagues may be appropriate:

> So how do you go about teaching them something new? By mixing what they know with what they don't know. Then when they see in their fog they think, 'Ah, I know that,' and then it's just one more step to, 'Ah, I know the whole thing,' and their mind thrusts forward into the unknown and they begin to recognize what they didn't know before, and they increase their powers of understanding. (Picasso)

HAPPY BIRTHDAY, LEO!

Acknowledgements

Our work has been supported by the Center for Biopolymers at Interfaces, University of Utah; the Ruder Boskovic Institute, Zagreb, Yugoslavia; and the National Heart, Lung, and Blood Institute. We thank our many colleagues and co-workers over the years for their contributions, insight, and discussions; and we particularly thank Leo Vroman for his leadership, guidance, perspective, and stimulation over the past 25 years.

REFERENCES

1. L. Vroman. *Blood*, Natural History Press, (1967).
2. J. D. Andrade, D. L. Coleman, S. W. Kim and D. J. Lentz, in: *Artificial Liver Support*, pp. 84–93, R. Williams and I. M. Murray-Lyan (Eds), Pitman Medical (1975).
3. D. Horsley, J. Herron, V. Hlady and J. D. Andrade, in: *Proteins at Interfaces*, p. 290, T. S. Horbett and J. Brash (Eds), Am. Chem. Soc. Symp. Series 343 (1987).
4. A.-P. Wei. M.Sc. Thesis, University of Utah (1990); also A.-P. Wei, J. N. Herron and J. D. Andrade, in: *Biotherapie*, D. J. A. Cromlin (Ed.), (in press).
5. J. D. Andrade, V. Hlady, A.-P. Wei and C. G. Golander. *Croat. Chem. Acta* 63, 527 (1990).
6. J. D. Andrade. *Med. Inst.* 7, 110 (1973).
7. J. H. Lee, J. Kopecek and J. D. Andrade. *J. Biomed. Mater. Res.* 23, 351 (1989).
8. K. G. Tingey, K. Sibrell, J. M. Lambert, D. D. Soloman, K. D. Caldwell and J. D. Andrade. *Trans. Soc. Biomater.* 13, 136 (1990).
9. T. A. Horbett. *Thromb. Haemostas.* 51, 174 (1984).
10. J. L. Brash and P. Ten Hove. *Thromb. Haemostas.* 52, 161 (1985).
11. N. L. Anderson, P. R. Tracy and N. G. Anderson, in: *The Plasma Proteins*, 2nd edn, Vol. 4, pp. 221–271, F. Putman (Ed.), Plenum Press, New York (1985).
12. C.-H. Ho, V. Hlady, J. D. Andrade and K. Caldwell, *J. Biomed. Mater. Res.* (in press).
13. G. E. Schulz and R. H. Schirmer, *Principles of Protein Structure*, Springer, New York (1979).
14. L. Vroman and A. L. Adams. *J. Colloid Interface Sci.* 111, 391–397 (1986).

15. D. C. Carter, X.-M. He, S. H. Munson, P. D. Twigg, K. M. Gernert, M. B. Broom and T. Y. Miller. *Science* **244**, 1195 (1989).
16. D. C. Carter and X.-M. He. *Science* **249**, 302 (1990).
17. H. Elwing, S. Welin, A. Askendal, U. Nilsson and I. Lundstrom. *J. Colloid Interface Sci.* **119**, 203 (1987).
18. V. Hlady. *Appl. Spectrosc.* **45**, in press (1991).
19. J. R. Brown and P. Shockley, in: *Lipid*–Protein Interactions, Vol. 1, pp. 25–50, P. C. Jost and O. H. Griffith (Eds), John Wiley, New York (1982).

Clinical Materials **11** (1992) 19–23

Needs, Problems, and Opportunities in Biomaterials and Biocompatibility

J. D. Andrade

Department of Bioengineering, Center for Biopolymers at Interfaces, University of Utah, Salt Lake City, Utah, USA

Abstract: There are four topics related to biomaterials and biocompatibility which I feel are key problems, are often unrecognized, and are therefore rich opportunities for work in the near future: (i) the covalent instability of proteins, (ii) the concept of statistical specificity and statistical heterogeneity, (iii) the issue of solid surface dynamics and surface relaxation, and (iv) the growing concern with the costs of health care and of medical research. Each is briefly discussed in this paper.

THE COVALENT INSTABILITY OF PROTEINS

It is normally assumed in the treatments of protein adsorption that proteins are stable with respect to their covalent bonds (see Andrade *et al.* and Norde in this volume). It is of course well known to protein chemists that proteins are not particularly stable, but this instability has been largely ignored by the biomaterials community. In addition to problems with sulfhydryl and disulfide chemistries and the general oxidation of proteins, there is the perhaps even more ubiquitous and important issue of protease effects at interfaces. The contact activation system of blood coagulation and the complement system largely consist of pro-enzymes, which are activated to have various protease activities. These proteases act on subsequent proteins leading to a cascade behavior, resulting in coagulation or complement activation. In the case of contact activation it is interaction of selected proteins at an interface that leads to protease activity and subsequent activation steps. Thus it is reasonable to expect that adsorbed proteins, particularly in studies involving complex protein mixtures such as plasma or tears, are likely to be modified by protease components of the mixture or by pro-enzymes which have in turn been activated by interfacial processes.

It is particularly surprising, for example, that in electrophoretic studies of adsorbed proteins, which have been eluted from surfaces by surfactant or other means, the electrophoresis pattern shows a high concentration of material in the 10–20 kilodalton range. This is exactly what one expects from nonspecific protease cleavage of complex proteins. In addition, adsorbed proteins, due to conformational alterations imposed by the adsorption process, may experience very different protease susceptibilities from the same proteins in their native conformation in solutions. Thus, not only are proteins likely to be covalently altered at interfaces, but the nature of that covalent alteration may be very different from that of the same protein exposed to the same enzyme in solution. The good news, however, is that control studies of adsorbed proteins exposed to protease enzymes, followed by subsequent elution and analysis, should provide unique information as to the conformational changes in the adsorbed state (Fig. 1).

Surface oxidation and disulfide/sulfhydryl changes may be particularly pronounced with protein studies at air/water interfaces. Those interested in this area should look to protein food and foam literature where such effects have been considered in some detail. Biologically modified material surfaces will of course also be suscep-

Clinical Materials 0267–6605/92/$05.00

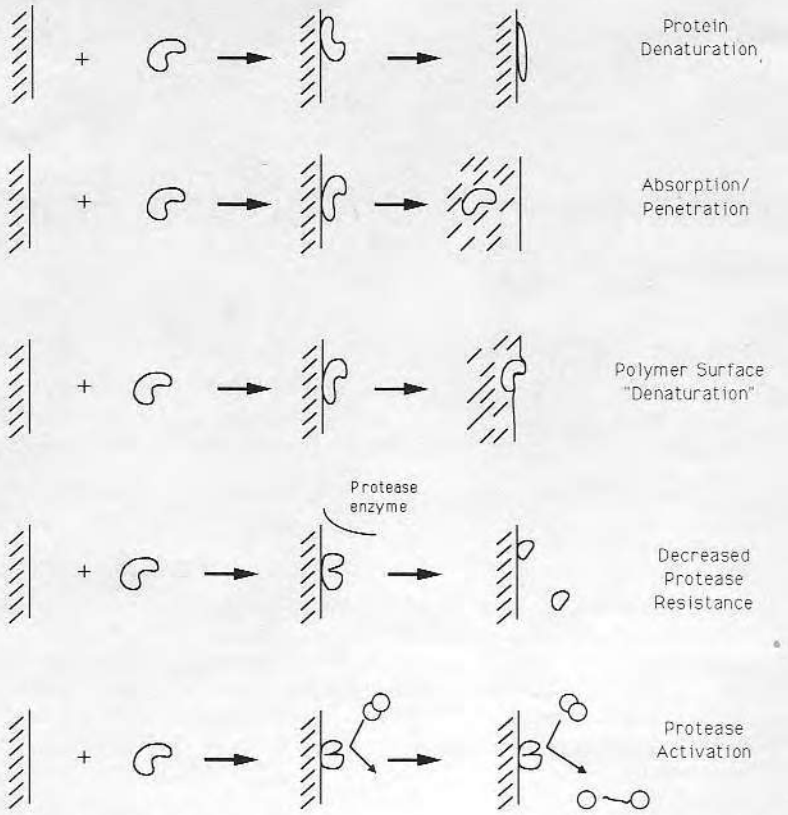


Fig. 1. A schematic view of some protein interfacial processes. At the top is the now commonly accepted phenomenon of protein adsorption, followed by conformational change (denaturation) in the adsorbed state. Below that is illustrated the phenomena of protein absorption, or penetration, into certain polymer systems. This is of concern in the case of very high water content, loosely crosslinked systems interacting with very small proteins, such as certain hydrogel contact lens material interacting with lysozyme and other small proteins in the tears. The center panel shows the phenomena of polymer surface relaxation or denaturation, in response to an adsorbed protein. If the polymer surface is particularly soft it may relax, flow, or otherwise adapt to the presence of the adsorbed protein. We may say that the polymer surface now 'denatures' in response to the new protein microenvironment. This, of course, could be a precursor to the absorption or penetration step illustrated just above. The bottom two panels refer to the chemical changes that we have been discussing. First a protein in solution that is normally resistant to certain enzyme or protease attack may, as a result of conformational changes imposed by the adsorption process, be susceptible to protease attack in the adsorbed state. Thus an adsorbed protein may show decreased protease resistance. This of course can be used in a positive way by using various protease enzymes as probes of adsorbed proteins, and then examining the digest by electrophoresis and other techniques. The bottom panel attempts to illustrate the activation of a pro-enzyme to an enzyme as a result of conformational changes imposed by the adsorption process. The adsorbed enzyme is now protease active and can act on other proteins colliding with the surface, again to produce protein fragments. This can also be turned around and used as a probe of the adsorption process by simply attempting to measure the enzyme activity of adsorbed proteins.

tible to protein covalent chemical changes and problems.

SOLID SURFACE DYNAMICS AND RELAXATION

It is now generally accepted and understood that polymer surfaces are dynamic and relax or change in response to a change in environmental conditions, particularly if the polymer is amphilic in nature, that is it contains both polar and nonpolar groups or components.[1] In equilibrium with air or vacuum, the nonpolar components tend to dominate the interface, thereby minimizing the surface energy. In an aqueous environment, the polar components tend to dominate, thereby minimizing the interfacial free energy. The relaxation process can be rapid or slow, depending on the intrinsic dynamics of the polymer involved. Although this has not been thoroughly studied, it is generally accepted that the surface relaxation times will be of the same order as the bulk relaxation times. Some of the bulk relaxation mechanisms that have been discussed include the glass transition temperature and the beta relaxation, which occurs in the vicinity of room temperature in most methacrylate systems.

The subject of polymer dynamics and relaxation is well treated in all basic polymer science and polymer materials textbooks. Although it is gen-

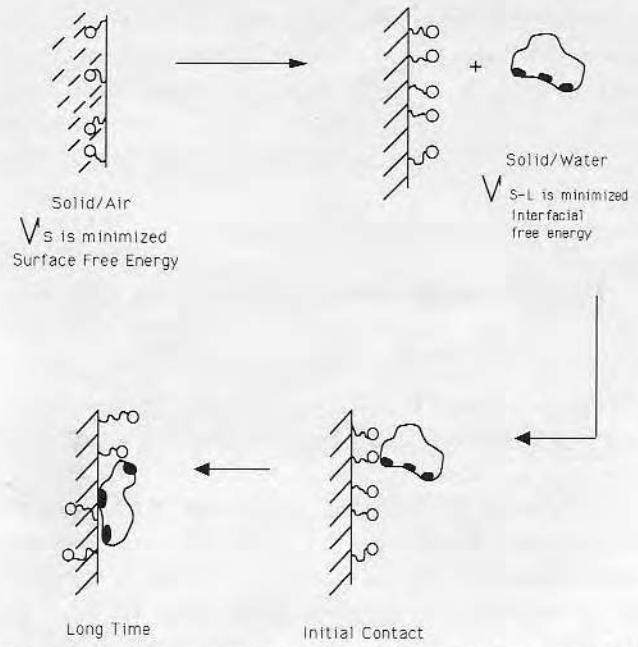


Fig. 2. Some schematic illustration of consequences of solid surface dynamics and relaxation phenomena (see text for discussion). Γ_s is the solid surface energy; Γ_{s-L} is the interfacial free energy.

erally understood that the same polymer exhibits a different surface under water than it does in air,[1] what is not generally understood is that it exhibits still a different surface when in contact with protein. This is illustrated in a schematic way in Fig. 2. In the upper right we show a polymer with hydrophilic side chains or side groups at the air interface. The side groups tend to be buried inside the polymer, thereby minimizing its surface free energy. When exposed to water, the controlling thermodynamic parameter is the interfacial free energy; thus the polar groups tend to migrate to the interface, thereby minimizing the interfacial free energy. This is the part that is well understood and has been reasonably well studied.

If a protein now contacts a surface, then of course the principles of protein adsorption apply, and the initial contact of protein is a function of the surface chemistry of the polymer, equilibrated in water, and the surface chemistry of the protein molecule. This has already been discussed (see Andrade *et al.* in this volume). However, once the protein has adsorbed and makes contact, then of course the protein has its own interface and its own dynamics, and begins to conformationally adapt and denature in response to its interfacial environment. For example, in the bottom left illustration in Fig. 2 we show the dark hydrophobic patches on the protein surface. These hydrophobic patches now provide an environment in many ways similar to the air environment illustrated in the upper left. The polymer now senses this more hydrophobic environment and begins to adapt its surface locally to that environment. Thus, we may have regions of the polymer surface in which the polar chains are oriented outwards and other regions where they are oriented inwards. Thus the adhesion mechanism may be considerably different at various contact or residence times. The initial adhesion in the early stages of adsorption, as indicated in the bottom center, may be very different from the long-term adhesion in later stages of adsorption, indicated at the bottom left.

The hypothesis, therefore, is that the surface denatures or conformationally adapts in response to the adsorbed or adsorbing proteins. This, of course, has not been well studied. There is probably some data in the chromatography literature, but there is very little in the biomaterials literature.

STATISTICAL SPECIFICITY

Although most polymer and protein adsorption is considered to be nonspecific in nature and most biochemical processes are considered to be highly specific, it is clear that these are merely limits. Biochemistry is indeed primarily based on functional group matching, steric complementarity, steric adjustment, and cooperativity. This leads to association constants that are quite high and interactions that are highly specific.

Nonspecific binding or adsorption of proteins and polymers generally occurs with low binding energies per segment. The large number of segments or contact point leads to very high binding energies but relatively low specificity.

Figure 3 attempts to illustrate how a family of more or less random synthetic polymers can contain individuals that can combine with high specificity to biomolecules. This merely has to do with the statistical placement of groups involved in the binding process. This can be called the 'Jacqueline and Marcel effect', in honor of the Jozefowicz's and their coworkers who have demonstrated such behavior with modified polystyrene systems.[2]

Let us assume that the biomolecule, perhaps heparin which binds through its anionic groups to cationic groups, requires a certain minimal number and appropriate placement of sites for effective binding. Using affinity chromatography with an immobilized heparin column, one could clearly select for those components of the polymer mixture

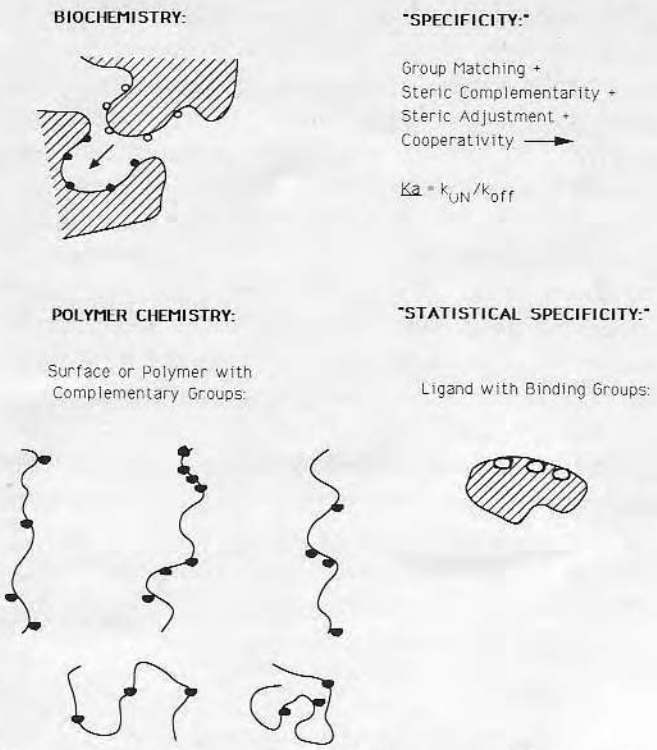


Fig. 3. Schematic illustration of the concepts of biochemical and statistical specificity. The upper panel demonstrates the typical view of biochemical specificity, which is based on functional group complementarity and matching, steric complementarity, steric adjustment, and cooperativity. The statistical specificity approach is shown on the lower panel. Here we see five different synthetic polymer molecules which have been statistically or randomly derivitized, shown by the circles. The functional groups are shown at different concentrations and at different regions of the molecule. The polymer molecule is itself shown in a number of different conformations. One must now imagine how such a statistical distribution of conformations and arrangement of functional groups will interact with a particular ligand, illustrated at the far right. Simply make a transparency of that ligand and play with it on this particular figure and you will see how certain orientations and combinations should lead to specific and strong binding, whereas most of the orientations and interactions will lead to weak and relatively nonspecific binding.

which indeed do bind to heparin with a particular binding constant or binding strength. That subset of the population could then be said to 'specifically' interact with heparin, whereas those polymers whose statistical placement of cationic groups is different would not bind or would bind much less. They would then be said to not bind or to bind nonspecifically.

Clearly the random nature of synthetic polymers would lead to a binding constant distribution function and one could define high binding, low binding, specific binding, etc., with respect to the nature of the distribution function. This approach helps explain the specificity observed with the sulfonated polystyrenes and related polymers developed by the Jozefowicz's and their coworkers,[2] and should be readily modeled using standard statistical synthetic polymer concepts. This concept is well developed in biochemistry and indeed has been used to model the specificity and cross-reactivity of antibodies.[3]

ETHICS AND THE COSTS OF HEALTH CARE

The costs of health care appear to be rising in most parts of the developed world, from a low about 6% of gross national product in Britain to about 12% in the United States. Most of Europe is somewhere in between. There are major driving forces, particularly in the United States, to reduce the costs of health care. This is already starting to lead to changes in research and development funding priorities. One can expect that in the near future all research proposals in the biomaterials and bioengineering areas will require a justification in terms of their potential impact on decreasing, or at least evidence that their successful implementation will not increase, the costs of health care; that is proposals will have to contain a cost-benefit analysis.

We should begin asking the question: 'How can biomaterials and bioengineering research and development be used to decrease the costs of the more expensive procedures?' We can also expect that there will be an increased emphasis on prevention and cures rather than on device-related treatments.

The increasing concern with animal experimentation will also lead to an increased emphasis on modeling, simulation, hypothesis formulation, and general full and effective use of existing information before proceeding with animal experimentation. There are many instances in modern biomaterials research where the investigators are not fully aware of relevant work which preceded their interest or activity in the area; that is 'many people do not do their homework'. This is of course difficult in our field because of its broad and interdisciplinary nature, but it is essential that we make effective use of all existing information. Ignorance of the literature will be no excuse.

There has also been the tendency to approach problems in a 'shotgun' fashion, that is to simply try a wide variety of approaches and mix up a large number of variables, rather than to think through the problem. Such an approach is no longer tolerable and, indeed, from a cost-benefit analysis

point of view can be considered completely ineffective and possibly even unethical. At least qualitative modeling and simulation must be done in order to formulate testable hypotheses and thereby design efficient experiments.

There is also a tendency in many quarters to argue that one cannot begin to appreciate the literature outside one's own speciality and that therefore there is a right, or even an obligation, to ignore it. This is, and will become increasingly, intolerable. If the investigator chooses not to make the commitment to learn the appropriate literature, collaborators must be found in those allied fields who will, by working together, make full and effective use of the existing information and of the resources available.

The increasing concern with bioethics, with the minimization of animal and human experimentation, and the drive to reduce the cost of research and development and of health care in general, will make it essential that research and development proposals in biomaterials and bioengineering consider these points. There is certainly going to be increased emphasis on modeling and simulation, on hypothesis formulation, on in-vitro testing, and on the increased use of cell culture and simple organisms in lieu of more complex animal and human experimentation (see Harmand in this volume).

ACKNOWLEDGMENTS

I thank Marcel and Jacqueline Josefowicz for stimulating discussions on the concept of statistical specificity and for the opportunity to spend one month in their laboratory in the Spring of 1988, in which this topic, among others, was discussed. I also thank E. Piskin and the EUROBIOMAT initiative for the opportunity to participate in the Conference.

REFERENCES

1. Andrade J. D. (ed.), *Polymer Surface Dynamics*. Plenum Press, New York, 1988.
2. Jozefowicz, J. & Jozefowicz, M. *J. Biomat. Sci., Polym. Ed.*, **1** (1990) 147.
3. Inman, J. K., Barnett, A. L., Highet, P. F., *J. Cell. Biochem.* (*Suppl.*), **35** (4) (1987).

Pure & Appl. Chem., Vol. 64, No. 11, pp. 1777–1781, 1992.
Printed in Great Britain.

Adsorption of complex proteins at interfaces

J.D. Andrade, V. Hlady, A.P. Wei

Department of Bioengineering, 2480 MEB, University of Utah, Salt Lake City, Utah 84112, USA

Abstract - We have previously shown that the adsorption of model proteins at model interfaces can be quantitatively understood via a careful consideration of the proteins' three dimensional structure and its stability. Using computer molecular graphics, dynamic surface tension, fluorescence probes and labels, and solution denaturation data, we can relate the chemical and structural properties of proteins to their interface behavior. We have developed a novel means to present these data and correlations in a simple radial plot (the "Tatra" plot). We are now extending these approaches to complex multi-domain proteins. Albumin consists of three large domains with differences in electrostatic nature, charge-pH characteristics, and denaturability. The interfacial activity of albumin is due, at least in part, to the interfacial activity of its constituent domains. Consideration of the structure and interfacial activity of the various domains permits new and more precise hypotheses to be developed, with which new and better experiments can be designed. Such hypotheses allow one to evaluate and compare adsorption data, including kinetics and isotherms, adsorbed layer thickness, refractive index, multilayer formation, etc. We feel strongly that each different protein is a unique molecular personality, which must be understood and considered if we are to more fully understand and apply the interfacial behavior of complex proteins. Expanded treatments of these topics are available in References 1-4.

PRINCIPLES OF PROTEIN ADSORPTION

The principles of protein adsorption have been presented in a number of monographs, review papers, and conference proceedings [1-8].

The arrival of protein at the interface is assumed to be driven solely by diffusion processes, which are dependent on bulk concentration and diffusion coefficient. That results in a collision frequency [9]. The particular surface chemistry of the protein and of the surface dictates the residence time due to the initial interaction energy. The surface dynamics, or denaturability, of the protein itself, together with the residence time, probably controls the surface denaturability of the protein. The protein tends to denature with time at the interface. 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.

The reality of the process of course is that proteins are not homogeneous particles. Not all collisions are equally effective in adsorption, and different protein "surfaces," or faces, result in different interaction energies with the protein, and therefore different tendencies for surface denaturation. We attempt to illustrate this in Figure 1, in which the protein is shown as having four "faces:" a hydrophobic face, a positively charged face, a negatively charged face, and a neutral hydrophilic face. Although all collisions are equally probable, it is only those collisions which result in interaction energies in the range of kT which will provide the residence times necessary for subsequent interfacial processes to occur. 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 [8].

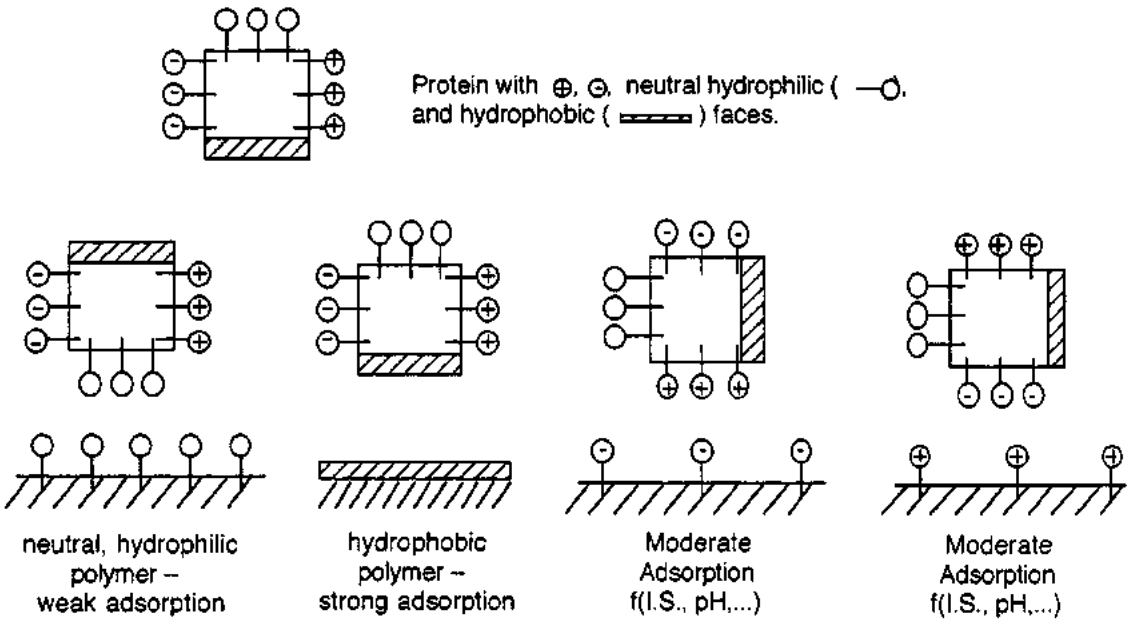


Figure 1: A schematic view of four-sided "protein," with one face being hydrophobic, one face being negatively charged, one face positively charged, and one face of neutral hydrophilic character, shown interacting with surfaces of comparable character. In the case of a neutral hydrophilic polymer surface, one would expect weak or little adsorption, whereas in the case of a hydrophobic polymer one would expect strong adsorption via the orientation shown. In the case of charged surfaces, one would expect moderate or variable adsorption, depending on the electrostatic nature of the interaction, a function of the ionic strength and pH of the solution, charge density, charge location, etc. (from Ref. 1).

In order to predict the initial contact event, 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. In these cases the X-ray crystallographic coordinates of the protein are readily available, and can be displayed on a computer screen. One can very 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 [10,11].

We have studied a matrix of model proteins at air/water interfaces by dynamic surface tension techniques [13]. 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 interface (again using dynamic surface tension). Denaturability was assessed by calorimetry and by urea and guanidinium chloride perturbation deduced by fluorescence changes. The surface chemical nature of the protein was assessed by examination of its external surface chemistry using molecular graphics and by the use of fluorescent probe titration. A relative, effective surface hydrophobicity (ESH) parameter was then deduced [11,12].

After consideration of a wide range of parameters, we selected twelve variables and began to qualitatively examine the correlations between them using radial axes with the axes arranged and scaled so as to emphasize and even exaggerate correlations among the various parameters. We call this multi-parameter radial plot a "Tatra Plot" [11].

Figure 2 presents the Tatra Plot for superoxide dismutase, a highly stable protein whose surface is extremely hydrophylic and thus exhibits little surface activity at the air/water interface. The upper left quadrant depicts protein surface hydrophobicity. The lower left quadrant depicts the stability of the protein. The upper right reflects surface activity. We are still experimenting with the variables, their placement, and their scaling, and the Tatra Plot is far from being completed or optimized at this time.

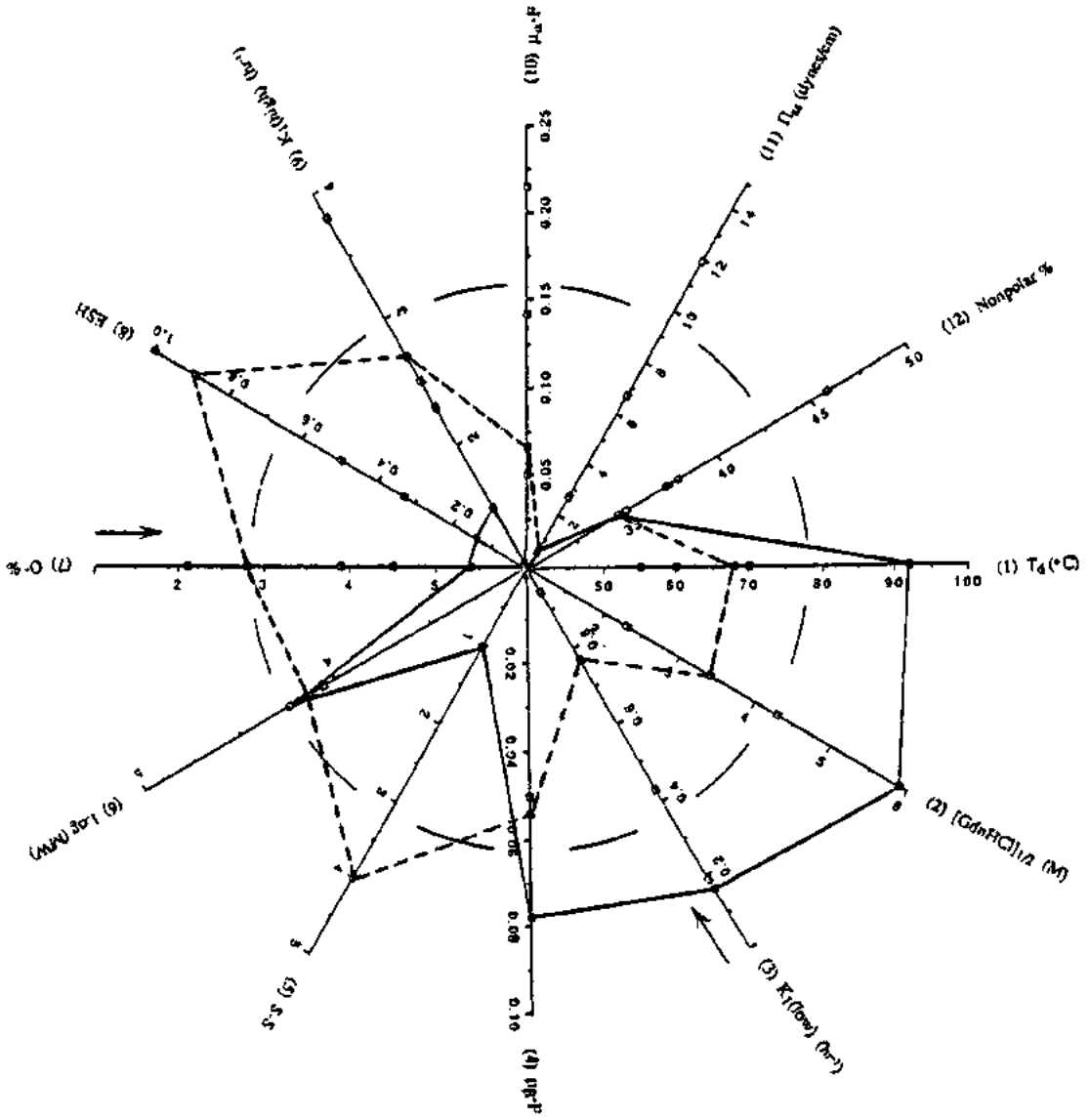


Figure 2: The Tatra Plot, Tatra parameters, and the data for superoxide dismutase and ribonuclease (dotted lines). Axes 1-5 are stability or stability related parameters. Axes 7-9 are parameters of effective surface hydrophobicity or related to it. Axes 10-12 are steady state surface activity. The directions for all axes, except for 3 and 7, are increasingly outward. It is clear that superoxide dismutase is a very stable, hydrophilic and relatively non-surface active protein (from Reference 11).

Figure 2 also gives comparable data for ribonuclease. It is less stable than superoxide dismutase, as indicated by the partial collapse of the points in the lower right quadrant; ribonuclease has slightly less surface hydrophobicity and less surface stability.

The proteins studied to date fall into three different categories in terms of their structure, function, and surface activity relationships [11].

Given the apparent success of the multi-variate Tatra Plot approach to correlating the behavior of model proteins at air/water interfaces, we are now extending the approach to the solid/liquid interface [1]. We have taken a limited set of parameters and used them to develop a preliminary Tatra Plot representation for model protein adsorption on polystyrene lattices, using the data of Norde and co-workers [8,13].

In the air/water interface case, hydrophobic interactions are believed to dominate the adsorption behavior and this is clearly reflected in the strong correlation with the hydrophobic surface character of the protein. In the case of charged surfaces electrostatics plays a major role, and this is strongly reflected in the shape of these preliminary Tatra Plots. Hydrophobic interactions also play a major role

We are still in the process of optimizing the Tatra Plot representation for solid/liquid interfaces [14].

COMPLEX PROTEINS

What do these concepts and results have to do with the more practical problem of the behavior of complex plasma and tear proteins at biomaterials surfaces? We feel strongly that the way to understand the behavior of a complex protein is to look at its various structural domain building blocks. In the last 10 years it has become evident in structural biochemistry that, 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. 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. Fibrinogen is an excellent example. High sensitivity calorimetry studies of fibrinogen and of its protease derived fragments suggest 12 domains in the fibrinogen molecule with denaturation temperatures of 45°, 55°, 90°, and 100° C [15]. We are only now beginning t o analyze fibrinogen in terms of its domain structure, with the hope of beginning to understand its behavior at solid/liquid interfaces. Fibronectin is another example. It has at least 20 calorimetrically identified domains [16], and it is likely that its complex adsorption behavior will be partially understood through a domain approach.

The optimistic view is perhaps best described by Chothia [17]: "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 govern the generation of different , and sometimes complex, sentences." Others have suggested a protein structural linguistics [18].

We have attempted to apply some of these concepts to the analysis of the interfacial behavior of albumin [19]. Albumin is perhaps the simplest of the multi-domain proteins with which to initiate 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 alphahelix content, high in disulfide cross-link content. It has a high degree of alpha-helicity and is somewhat myogiobin-like; it binds a variety of ligands, including fatty acids and calcium. The crystal structure, refined to the four angstrom level, for human albumin is now available [20].

We have taken the three domain model of albumin and done a very preliminary analysis from an electrostatic point of view. 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 at various interfaces, allowed us to begin to formulate a number of hypotheses regarding the possible interfacial activity of albumin. These hypotheses now allow us to probe into the voluminous albumin adsorption literature and try to begin to make sense of that enormous data base. The analysis is continuing.

It is clear that a domain approach to protein adsorption and immobilization helps to greatly 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 [21].

SUMMARY

We feel that the adsorption of simple proteins at simple interfaces is qualitatively understood. This understanding is being extended to the behavior of complex proteins, even at complex solid interfaces, by the careful consideration of domains, domain properties, patchiness of the surface, and domain-patch interactions. In fact, this can be used to develop the concept of statistical specificity of surface interactions [2]. The various domains of complex proteins and the various domains and patches on complex surfaces each have their own surface activity and "denaturability" which must be characterized and incorporated in the analysis.

We must also note that surfaces which greatly decrease adsorption and may even be resistant to protein interactions are becoming available. Models and simulations, as well as experimental results of protein interactions with PEO surfaces, suggest that such surfaces do indeed work, and can be further optimized and enhanced for biomaterials and related applications [22-24].

We must also note that the new technique of scanning force microscopy shows potential not only in the direct imaging of proteins and complex biomaterial surfaces, but, perhaps even more importantly, in the manipulation, processing, and fabrication of protein interfaces [25,26].

Acknowledgement

We thank J.N. Herron and Kap Lim for our interactions and collaboration in the area of molecular graphics and model protein studies, Willem Norde for access to the data of model protein interactions with polystyrene lattices [13], and the Center for Biopolymers at Interfaces for partial support of the work. JDA thanks J. and P. Kopecek for a wonderful trip to Czechoslovakia's High Tatra Mountains, where the Tatra Plot approach was initially formulated.

REFERENCES

1. J.D. Andrade, V. Hlady, A-P Wei, C-H Ho, A.S. Lea, S.I. Jeon, Y.S. Lin, E. Stroup, in E. Piskin, ed., *Biologically Modified Biomaterial Surfaces*, Elsevier (1991) in press..
2. J.D. Andrade, in E. Piskin, ed., *Biologically Modified Biomaterial Surfaces*, Elsevier (1991) in press..
3. V. Hlady, J.D. Andrade, C-H Ho, L. Feng, and K. Tingey, *J. Clin. Mat.* (1991) in press.
4. J.D. Andrade and V. Hlady, *J. Biomat. Science-Polymer Ed. 2,* 161 (1991).
5. J.D. Andrade and V. Hlady, *Adv. Polymer Sci. 79,* 1(1986).
6. J.D. Andrade, ed., *Protein Adsorption*, Plenum Press, (1985).
7. T.S. Horbett and J. Brash, eds., *Proteins at Interfaces*, Amer. Chem. Soc. Symp. Series 343, (1987).
8. W. Norde in E. Piskin, ed., *Biologically Modified Biomaterial Surfaces*, Elsevier (1991) in press.
9. J.D. Andrade and V. Hlady, *Ann. N.Y. Acad. Sci. 516*, 158 (1987).
10. D. Horsley, J. Herron, V. Hlady, and J.D. Andrade, in *Proteins at Interfaces*, T.S. Horbett and J. Brash (Eds), *Am.Chem. Soc. Symp. Series 343*, 290 (1987).
11. A-P Wei, M.Sc. Thesis, University of Utah (1990).
12. A.P. Wei, J.N. Herron and J.D. Andrade in D.J.A. Crommelin and H. Schellekens, eds., *From Clone to Clinic* , 305-313 (1990).
13. T.Arai and W. Norde, *Colloids and Surface 51,* 1-15 (1990).
14. V. Hlady, C.H. Ho, J.D. Andrade, and W. Norde, in preparation (1991).
15. P.L. Privalov and L.V. Medved., *J. Mol. Biol. 159* , 665 (1982).
16. L.V. Tatunashvili, V.V. Filimonov, P.L. Privalov, *J. Mol. Biol.211,* 161-169 (1990).
17. C. Chothia, *Ann. Rev. Biochem 53,* 537 (1984).
18. M.H. Zehfus, *Proteins 3,* 90 (1987).
19. J.D. Andrade, V. Hlady, A-P Wei, C-G Golander, *Croatica Chem. Acta. 63*, 527 (1990).
20. D.C. Carter, X-M He, S.H. Munson, P.D. Twigg, K.M. Gernert, M.B. Brown, T.Y. Miller, *Science 244*, 1195 (1989).
21. J-N Lin, I-N Chang, J.D. Andrade, J.N. Herron, and D.A. Christensen, *J. Chromatography 542,* 41-54 (1990).
22. J.H. Lee, J. Kopecek, and J.D. Andrade, *J. Biomed. Mater. Res. 23,* 351 (1989).
23. S.I. Jeon, J.H. Lee, J.D. Andrade, and P.G. deGennes, *J.Colloid Interface Science 142* , 159 (1991).
24. J.M. Harris, ed., *Biomedical Applications of Polyethylene Glycol Chemistry* (1991) in press.
25. J.N. Lin, B. Drake, A.S. Lea, P.K. Hansma, and J.D. Andrade, *Langmuir 6*, 509 (1990).
26. A.S. Lea, A. Pungor, V. Hlady, J.D. Andrade, J.N. Herron, and E.W. Voss, Jr., *Langmuir* (1991) in press.

10. Immuno-Biosensors: The Clinical Chemistry and Coagulation Laboratory on a Chip

Joseph D. Andrade, Jinn-Nan Lin, Vladimir Hlady, and James Herron

INTRODUCTION AND BACKGROUND

The 21st century is only 9 years away. Many of our preliminary ideas and experiments today will be commercial products by the beginning of the 21st century. Japan has a national program in Bioelectronics and Biochips [1]. Some of the first medical products using bioelectronics/biochip concepts will be biosensors [2–5]. Biosensors have the potential of replacing and enhancing many of the tests and functions now provided by clinical chemistry and coagulation laboratories. Virtually any clinical chemistry test can, in principle, by transferred to a chip. A chip here means any small, integrated device with high reliability. The integrated miniature clinical chemistry laboratory could include micro electron-mechanical, optical, and biochemical systems, or MEMOBS for short.

There are two fundamentally different objectives and directions in the miniature clinical chemistry field: 1) the development of simple, easy to use methods and devices for the quantitative and specific assay of clinical chemistries in individual blood, urine, or tear samples; this approach is often called a single or "one-shot" measurement device; and 2) the development of remote biosensors suitable for continuous monitoring and perhaps for feedback control of artificial organs and/or drug delivery devices [5].

The "one-shot" device is analogous to the present day clinical laboratory, where a patient sample is measured for various analytes of interest. The remote, continuous device—a true sensor—is analogous to an on-line pH or pO_2 electrode, which can respond continuously—and remotely—to a change in analyte concentration.

Just as the development of immunoassays revolutionized clinical chemistry, the development of immunosensors will revolutionize immunoassay. The increasing sensitivity of multi-monoclonal antibody based immunoassay makes it possible to perform highly sensitive measurements with small sample volumes. It is possible to do a number of different immunoassays

Department of Bioengineering 2480 MEB, University of Utah, Salt Lake City, USA

ARTIFICIAL HEART

The Development of Biomation in the 21st Century

Editor

YUKIYASU SEZAI, MD, FACS

Chairman and Professor,
The Second Department of Surgery,
School of Medicine,
Nihon University, Tokyo,
Japan

Assistant Editor

J. PATRICK BARRON

SARDER ABDUN NAYEEM, MBBS

W.B. SAUNDERS COMPANY

with a small volume of serum or plasma. The techniques and methods being developed to produce one-shot "sensors" for single analytes can be readily combined and multiplexed to provide many different analyte sensors or channels on a single device. One can envision, for example, a liver function chip—or a thyroid function chip—which would measure all of the biochemistries needed for a clinical chemistry assessment of liver or thyroid function. One can even imagine 100 or more channels—the only real limitation is the total sample volume required and the individual channel or analyte sensitivities needed.

A problem is how to display and utilize multiple channels of biochemical information [6]. Specialized clinical chemistries are expensive, so they are generally used to confirm diagnosis. The availability of many different clinical chemistries will allow chemistry-assisted diagnosis, as well as the confirmation of diagnosis. Multi-channel analyte date will also permit physicians to more completely and quantitatively assess the effect of their treatments and therapies.

The development of one-shot, miniature automated biochemical measurement systems is relatively straight forward. What is required is a sufficiently long range and appropriately funded effort.

A more difficult problem is the development and practical application of truly remote and continuous biosensors—that has been the goal of our group [5] (Figure 10-1).

An animal or patient is shown, which could also represent a bioreactor or biochemical reactor (Figure 10-1). An extracorporeal loop is shown, for example, the extracorporeal blood pathway in hemodialysis or cardiopulmonary bypass during open-heart surgery. A connector is shown in that loop which contains a multi-channel immunosensor. Outputs of the various channels are appropriately reference, ratioed, and otherwise processed to permit the quantitative analysis of the analytes of interest. The output is shown on a continuous or semi-continuous plot of analyte concentration as a function of time.

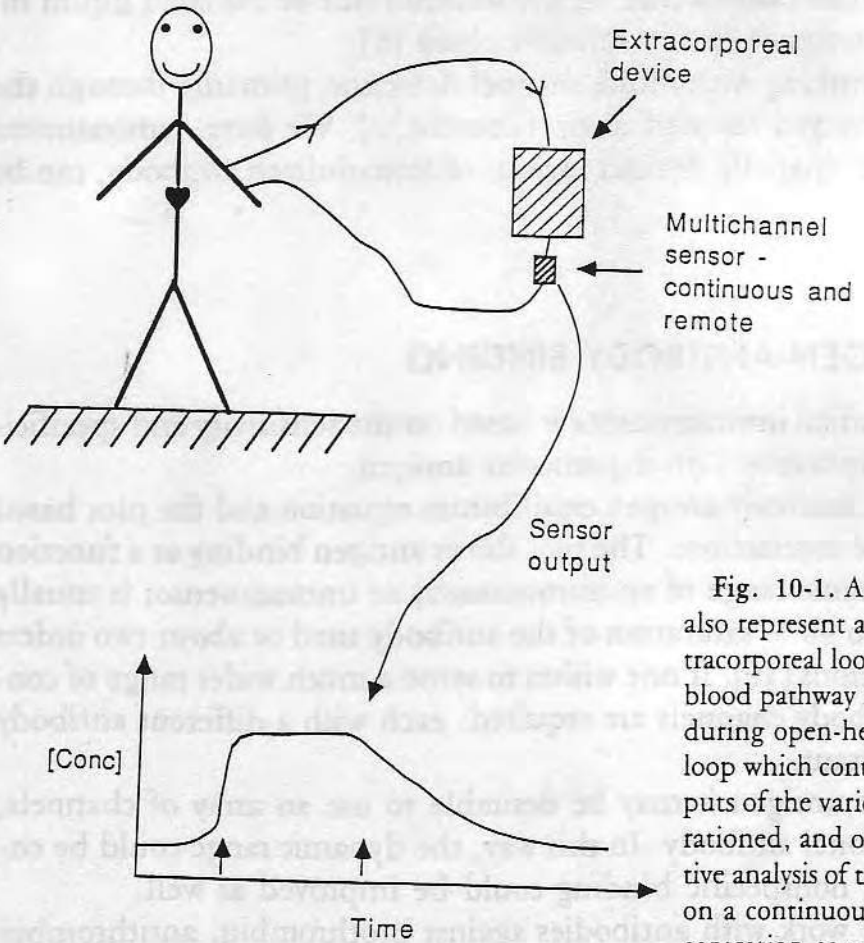


Fig. 10-1. An animal or patient is shown, which could also represent a bioreactor or biochemical reactor. An extracorporeal loop is shown, for example the extracorporeal blood pathway in hemodialysis or cardiopulmonary bypass during open-heart surgery. A connector is shown in that loop which contains a multi-channel immunosensor. Outputs of the various channels are appropriately referenced, rationed, and otherwise processed to permit the quantitative analysis of the analytes of interest. The output is shown on a continuous or semi-continuous plot of analyte concentration as a function of time.

Application areas include:

1. Biochemical engineering and biotechnology, primarily for process control;
2. Medical diagnostics;
3. Extracorporeal device monitoring and control (Figure 10-1);
4. Implanted device monitoring and control;
5. Biomedical research, such as the monitoring of analyte concentrations in experimental animals and in cell cultures;
6. Water and waste-water monitoring of environmental pollutants and contaminants.

Our approach is based largely on immunoassay; thus, we are interested in applying these sensors to the assay of the following general classes of antigens/haptens:

1. Coagulation proteins
2. Complement proteins
3. Antibody levels
4. Lipoproteins
5. Enzymes
6. Enzyme inhibitors
7. Hormones
8. Growth factors and regulators
9. Drugs
10. Viruses and bacteria

Coagulation proteins are our current applications focus.

Most of our work is based on the use of antibody or antigen immobilized on a quartz or amorphous silica surface [7]. Silica is used because of its optical properties, specifically its high refractive index, which permits one to use total internal reflection optics. The interfacially bound evanescent wave is the means to excite fluorescence on the solution side of the solid liquid interface without exciting bulk fluorescence in the solution phase [8].

More recently we have been working with multi-channel detection primarily through the use of a two-dimensional CCD (charged coupled device) camera [9]. We have demonstrated that various channels, i.e., different spatially distinct regions of immobilized antibody, can be readily detected.

ANTIGEN-ANTIBODY BINDING

The sensitivity and specificity of an immunosensor is based on the sensitivity and specificity of the interaction of specific antibodies with a particular antigen.

Figure 10-2 presents the basic antibody antigen equilibrium equation and the plot based on the assumed reversibility of these interactions. The plot shows antigen binding as a function of antigen concentration. The dynamic range of an immunoassay or immunosensor is usually considered to be roughly from 10 to 90 % saturation of the antibody used or about two orders of magnitude in antigen concentrations [10]. If one wishes to sense a much wider range of concentrations, then two or more antibody channels are required, each with a different antibody with the appropriate binding constant.

In the case of a multi-epitope antigen it may be desirable to use an array of channels, each containing a different monoclonal antibody. In this way, the dynamic range could be enhanced and discrimination against nonspecific binding could be improved as well.

Although we have done some work with antibodies against prothrombin, antithrombin III, and albumin, most of our work has been with a model system of anti-fluoroscein monoclonal

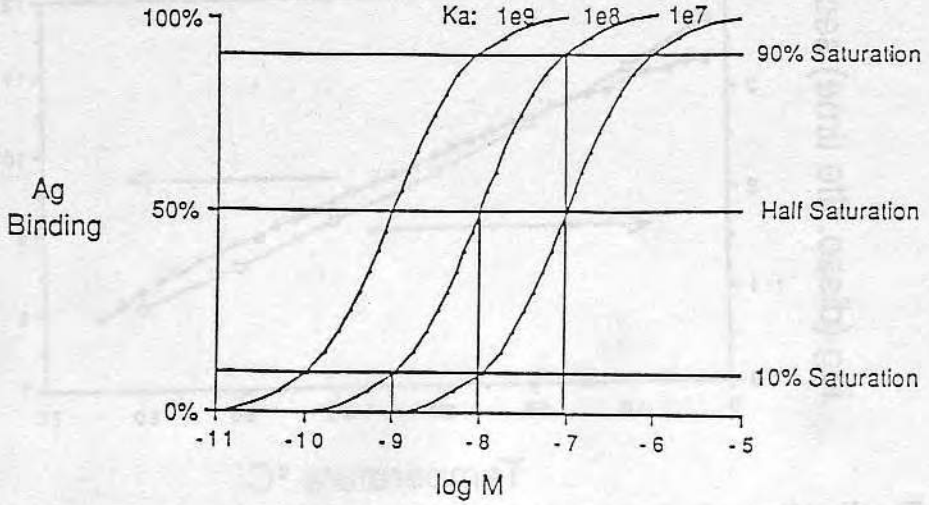


Fig. 10-2. Bound Ag—Bulk Ag equilibrium curves, based on reversible Ag-Ab interaction:

$$Ab + Ag \underset{K_2\ off}{\overset{on\ K_1}{\rightleftharpoons}} Ab - Ag; \qquad Ka = K_1/K_2 = \frac{[Ab - Ag]}{[Ab]\ [Ag]}$$

when $K_a \sim 1/[Ag]$, Ab sites are $\sim$ 50 % saturated. Range of immunoassay is usually defined as:

$$\frac{0.1}{K_a} < [Ag] < \frac{10}{K_a},$$

i.e. 10 to 90 % saturation results in two orders of magnitude range for [Ag].

antibodies developed by Voss and coworkers at the University of Illinois [11]. The binding thermodynamics of this system have been studied and presented by Herron et al. [12]. The overall association constant as a function of temperature for three different anti-fluoroscein monoclonals is well known [12]. The overall association constant can vary by up to two orders of magnitude over a temperature range of some 40 to 50 degrees centigrade. It was this behavior that led to the concept of the thermal regulation of antigen antibody binding which our group has been studying [10].

The binding kinetics for five of the anti-fluoroscein monoclonals have been determined [12]. The overall association constants range from 5×10^7 to 3×10^{10} moles $^{-1}$. Although the association rate constant is relatively constant (of the order of 5×10^6), the dissociation constant or its reciprocal, the dissociation lifetime, varies by a factor of 500. Therefore, the overall association constant of an antigen-antibody complex can be governed almost entirely by the dissociation rate constant. The dissociation lifetime is the response time of an immunosensor as it attempts to respond to changes in circulating analyte concentration. Therefore, for the development of a truly reverssible, continuous immunosensor, the dissociation life time issue has to be addressed and considered [8, 10].

Figure 10-3 presents the dissociation lifetime data for one of the antibodies, the 4-4-20 clone, as a function of temperature. Here one can readily see that the response time of a sensor can be improved by an order of magnitude with a 20 degree change in local temperature.

This discussion of antigen/antibody binding, however, has used data taken in bulk solution. Under those conditions antigen/antibody interactions are reversible. However, the situa-

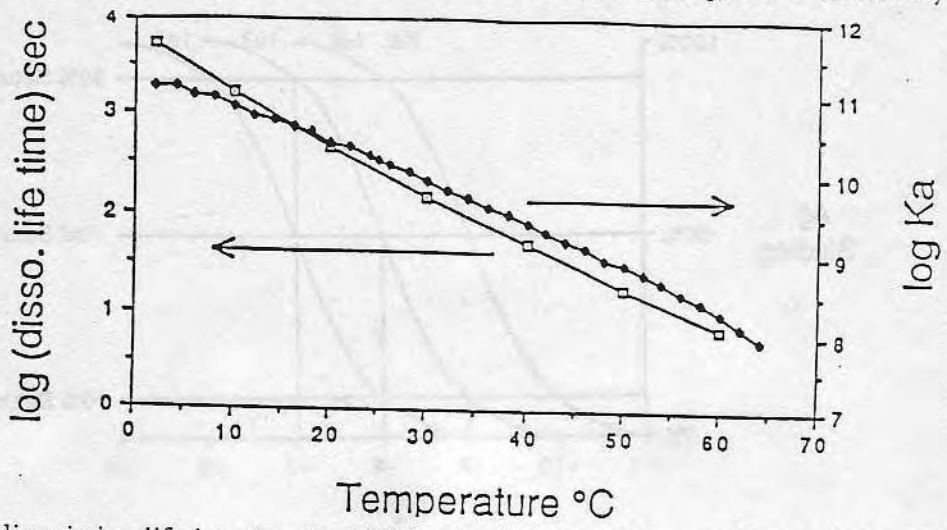


Fig. 10-3. The dissociation lifetime (on the left) for the 4-4-20 antifluorescyl monoclonal antibody as a function of temperatures. Log K_a data is given on the right axis. Note that K_a decreases an order of magnitude with a 20° increase in temperature (from Ref. 12).

tion may change significantly when one of the components in the binding reaction is immobilized at a solid liquid interface. We will next discuss the question of antibody immobilization followed by the issue of the reversibility of antibody/antigen interactions at interfaces.

ANTIBODY IMMOBILIZATION

A wide range of immobilization methods has been developed and applied [5]. In our case the reactions are constrained by the fact that we need to immobilize at an interface which is optically compatible with the total internal reflection/evanescent wave optical separation method previously described [8, 9]. In addition to the detailed immobilization chemistry, it is important to consider the collision and adsorption of the antibody to the interface, a precursor step to the covalent chemistry employed in the immobilization process [13].

Immunoglobulins are highly complex proteins with a variety of distinct functional and structural domains. There are hinge regions and switch regions, the function of which might depend on the local micro-environment and mode or mechanism of adsorption and immobilization. These regions, in turn,may affect the binding constant of the active site in ways which are not fully understood at present. Intentionally denaturing IgG prior to its adsorption at a solid/liquid interface [13] can result in a significant increase in the surface antigen binding capacity. Lin, et al. demonstrated that it is possible to engineer the immobilization of antibodies to optimize their properties at the interface by control of their structure in solution [13]. This work is continuing with a detailed investigation of the solution, denaturation, and structural characteristics of model antibodies in the hopes of optimizing means to adsorb and then immobilize these molecules with enhanced antigen binding characteristics.

ANTIGEN/ANTIBODY REVERSIBILITY

A true immunosensor requires that the sensor respond to changes in the circulating analyte concentration. Most "sensors" developed to date are not true sensors but rather are detec-

Solution:

$$Ab + Ag \underset{K_2}{\overset{K_1}{\rightleftharpoons}} Ab\text{-}Ag$$

$$Ka = \frac{K_1}{K_2} \sim 10^6 - 10^{12}$$

Surface:

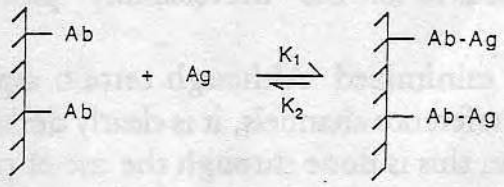


Fig. 10-4. Bulk solution Ab-Ag equilibria are not generally observed when Ab or Ag is immobilized at an interface (see Ref. 7).

$$(Ka)_{surface} >> (Ka)_{solution}$$

$$(K_2)_{surface} << (K_2)_{solution}$$

tors. They are designed to measure the analyte concentration and then either are discarded or regenerated by some process.

Our goal is to develop immunosensors which are inherently reversible and can continuously sense circulating analyte concentration. The problem is illustrated briefly in Figure 10-4. The bottom half basically demonstrates immobilized antibody interacting with its antigen and shows that although the on-rates in the bulk solution and interface case may be similar, the off-rate constant is much smaller in the case of immobilized antibody, meaning that the dissociation lifetime is much longer, which means then that the response of the sensor is very slow. The question is why?

This issue has been addressed briefly by others [14] and more completely by Lin, et al. in a recent paper which presents six possible reasons for the observation of partial irreversibility in immobilized antibody systems [7]:

1. Diffusion and *mass transport* effects.
2. The *immobilized antibody* is in a different *conformation* than the antibody in solution, thus influencing the nature of the antibody/antigen interaction.
3. Antibody/*antibody lateral interactions.*
4. Change in *solution micro-environment.*
5. *Non-specific adsorption.*
6. *Multi-valent binding.*

All six of the above hypotheses and mechanisms are operable, some more so than others, in a particular experimental situation. It will be very difficult to isolate and sort out the individual components responsible for the irreversibility of antigen/antibody interactions at interfaces.

OTHER ISSUES

A truly remote fluoro-immunosensor will require some means to deliver fluorescently la-

beled antigen, as the basic principle of such a sensor is that a fluorescently labeled antigen competes with unlabeled circulating antigen for the finite number of antibody binding sites. We have previously described a number of approaches to solve this problem. Those studies are ongoing [15].

Earlier it was noted that it might be possible to regulate the antigen/antibody binding constant by changes in temperature or even by other means. We have performed a number of studies along these lines. However, it is necessary to immobilize antibody by means that permit the reversibility of the antibody/antigen interaction before one can expect to regulate the nature of the binding constant. Our studies on binding constant regulation are continuing, based on the assumption that we will indeed develop methods to sole the "irreversibility" problem described above.

Non-specific binding is a problem which must be minimized. Although certain aspects of the problem can be overcome by the use of appropriate reference channels, it is clearly desirable to minimize the problem as much as possible. In our case, this is done through the use of polyethylene oxide as a "protein-repulsive" coating to minimize protein adsorption. This has been discussed extensively elsewhere [16]. We are also developing methods to immobilize antibody through the use of polyethylene oxide tethers or spacers in the hopes of minimizing non-specific adsorption to the spacer or to the chemical linkage itself.

Finally, there may be another problem with the development of truly remote sensors based on immobilized antibodies, particularly in blood and related environments. Blood is known to contain a variety of protease pro-enzymes, which can become activated under appropriate conditions to produce proteolytic activity. If protease activity is generated locally at the surface of an immunosensor, perhaps due to non-specific binding of coagulation or complement proteins, and if such proteases attack the immobilized antibody, then the sensor surface will degenerate with time and will eventually cease to function. That is one of the reasons why it is so important to build multi-channel devices with the appropriate calbration and reference channels to account for decrease or change in immobilized antibody concentration or activity. It is also why the non-specific adsorption problem is so important to eliminate and not simply to reference or calibrate out as noted earlier. If we minimize the non-specific adsorption of all proteins then we also minimize the possibility of interfacially induced protease activation. Again, our approach to this problem is through the polyethylene oxide surface approach [16]. Studies on the stability of immobilized antibodies are only now being initiated in our laboratory.

CONCLUSIONS

The development of truly remote, continuous, high sensitivity and specific immunosensors, capable of functioning remotely in blood or in other biological environments,is still in its early stages. There are a number of important technologies which must be developed. As the work progresses, one can envision the development and eventual production of multichannel biosensors based on microintegrated devices incorporating biochemical, optical and even electronic functions.

ACKNOWLEDGMENTS

We acknowledge discussions and assistance from many colleagues and coworkers, including; J. Kopecek, D. Christense, K. Caldwell, A. Edmundson, M. Block, D. Gregonis, J. Ives,

M. Reichert, I. Skurnik, P. Suci, D. Tirrell, R. Van Wagenen and E. Voss. The early phases of the work were funded by the Office of Naval Research, Biomolecure Engineering Program. Application to coagulation proteins was funded via NHLBI grant HL37046. Biocompatibility and reagent delivery aspects are supported in part by the Center for Biopolymers at Interfaces and by the National Science Foundation (Grant ECE86-02107). Studies dealing with Ag-Ab binding constant regulation are funded by the U.S. Army Research Office. The authors thank Ms. Jamie Healey for skillful and personable preparation of the manuscript. An expanded version of this paper is in press [5].

REFERENCES

1. Aizawa M, ed: 2nd International Symposium on Bioelectronic and Molecular Electronic Devices, R&D Association for Future Electronic Device, Dec. 12–14, 1988, Fujiyoshida, Japan.
2. Turner APF, et al, eds: Biosensors, Oxford University Press, 1987
3. Wise D: Biosensors, CRC Press, 1989
4. Buck RB, et al, eds: Biosensors, Dekker Publ. Co., 1990, in press.
5. Andrade JD, Lin J-N, Hlady V, Aerron J, Christensen, D, Kopecek J: Immunosensors; Remaining Problems..., Chapter in Ref. 4.
6. Vogt W: Trends in Anal. Chem. 3, 166, 1984
7. Lin J-N, Herron J, Hlady V, Andrade JD: Dissociation of Ab-Ag Complexes on Solid Surfaces: An Overview, Biosensors. in press, 1990
8. Hlady V, Reinecke DR, Andrade JD: Fluorescence of Adsorbed Protein Layers. I, J Coli Interface Sci 111, 555, 1986
9. Hlady V, Lin J-N, Andrade JD: Spatially Resolved Detection of Ab-Ag reactions at S/L Interfces Using Total Internal Reflection Excited Ag Fluorescence and CCD Detection, Biosensors, in press, 1990
10. Andrade JD, Lin J-N, Herron J, Reichert M, Kopecek J: Fiber Optic Immunodetectors: Sensors or Dosimeters?, Proceedings of SPIE 718: 280, 1986
11. Kranz DM, Herron JN, Voss EW: Mechanisms of Ligand Binding by Monoclonal Anti-fluorescyl Antibodies. J Biol Chem 257: 6987, 1982
12. Herron JN, Kranz DM, Jameson DM, Voss EM: Thermodynamic Properties of Ligand Binding by Monoclonal Anti-fluorescyl Antibodies Biochem 25: 4602, 1986
13. Lin J-N, Chang I-N, Herron J, Andrade JD: Influence of Adsorption of Native and Modified Ab on Their Activity. J Immunologic Methods 125: 67, 1989
14. Schramm W, Yang T, Midgley AR: Surface Modification with Protein A for Uniform Binding of Monoclonal Antibodies. Clin Chem 33: 1338, 1987
15. Yen H-R, Kopecek J, Andrade JD: Optically Controlled Ligand Delivery. I. Makromol Chem 190: 69, 1989
16. Lee J-H, Kopecek J, Kpeckova P, Andrade JD: Surface Properties of PEO-Containing Polymeric Surfactants: protein-Resistant Surfaces. J Biomedical Materials Res 23: 351, 1989

BOOK REVIEW

Artificial Heart: The Development of Biomation in the 21st Century

Editor: Yukiyasu Sezai, M.D. FACS.
Published by W. B. Saunders Company, Harcourt Brace Jovanovich, Inc. Philadelphia.
 $120.00

by Elaine Duncan

The attractive cover of this book beckons the die-hard artificial heart "groupie". "Buy Me!", it cries. A book this pretty must have the very latest information on artificial hearts, right? Well, not exactly.

"As part of the events marking the centenary of Nihon University, an international symposium was held on The Artificial Heart-Biomation in the 21st Century." This book is a compendium of the papers by the participants in the symposium. It strives to include a medical anthropological point of view and the role and contribution of the artificial heart to the humanity, as well as ethical aspects of the technological revolution of "biomation." This goal was intriguing and unique.

There are sixty-four chapters in the book, contributed by world-wide authors from universities, companies and consortiums from around the globe. For this reason alone it is shocking to see how many photographs in the book appear to be Jarvik-clones (such as the Thomas 60). Artificial heart "success" is repeatedly measured by survival times of animals in days.

(Continued from page 12)

Some of the chapters make interesting reading still today. One "find" was the article by Dr. Joseph D. Andrade et. al. about the "lab on a chip", titled, " Immuno-Biosensors, The Clinical Chemistry and Coagulation Laboratory on a Chip." Clearly one of the most difficult challenges to the long-term maintenance of a patient on a permanent assist or replacement device is to adequately understand the biological fluctuations induced by the mechanical replacement. Responses to drug therapies may not be the same for an assisted-patient. The exact conditions of the patient and his or her response to device parameter changes and drugs must be appreciated almost simultaneously, but no current laboratory methods can provide that. The technology proposed in this article will also have merit independent of the success or failure of the artificial heart technology.

For $120, this book could be an addition to a library or a nice "parting gift", but don't buy it for your personal collection unless you just like to collect handsome books.

The chapter by the Pierce group at Pennsylvania State University presented their original work on the electrical heart assist device, but at the time of this book, only sketches of human implant locations could be provided. The steam artificial heart from Russia certainly seemed interesting, but the technical discussion was scanty.

This is a continuing difficulty with books generated by symposia. By the time the hard-cover is published, the information is dated. Worse, the papers cannot be adequately critiqued and so the authors may produce any information that they choose.

One of the most ironic statements in the book was the conclusion offered in "The Total Artificial Heart — A subjective view from Berlin", by Dr. Klaus Affeld. He stated, " After many years of development of the artificial heart, a light is finally visible at the end of the tunnel, the reasons for this is that the basics of heart replacement and heart assists are understood and that the tools for the design of these devices are developed." *Oh really?*

The book has merit, don't misunderstand. In fact, someone went to a great deal of work to get these authors to return their manuscripts and photographs. This book is a lasting tribute to the extremely hard work of all of these scientists and engineers. What seems amazing, reading through chapter after chapter is that with all of this work, worldwide, why is there yet no total artificial heart on the market in the U.S.?

Simple answers are hard to come by, but it seems that eventually one program will succeed. When it does, this book will have collector's value, as well as merit as a general reference text.

The least interesting chapters deal with the "clinical experience" of use of the hearts in different countries. The statistics are numbing and have little appeal to the technologist. There is little or no discussion of the biomaterials testing conducted for these various devices and there is only meager emphasis on design qualification. The promised ethical and anthropological assessment of the artificial heart was not delivered.

(Continued on page 17)

Clinical Materials 11 (1992) 67–84

ANDR-43

Proteins at Interfaces: Principles, Multivariate Aspects, Protein Resistant Surfaces, and Direct Imaging and Manipulation of Adsorbed Proteins

J. D. Andrade, V. Hlady, A-P. Wei, C-H. Ho, A. S. Lea, S. I. Jeon, Y. S. Lin & E. Stroup

Department of Bioengineering, Center for Biopolymers at Interfaces, University of Utah, Salt Lake City, Utah, USA

Abstract. The principles of protein adsorption are briefly reviewed with emphasis on model proteins at model interfaces. Using a data set for protein behavior at the air/water interface, a multi-variate, multi-axes treatment of protein adsorption behavior is developed: the Tatra plot. By the careful placement and scaling of radial axes, representing 12 protein, surface, and interface parameters, one can begin to deduce various correlations between these parameters. The correlations are then used to formulate hypotheses with which to design additional experiments. The treatment is extended to the solid/liquid interface using data available in the literature for model proteins on model solid surfaces. We then present brief discussion of the extension of the technique to more complex surfaces, including means to parameterize solid/liquid interfacial properties, before proceeding to more complex proteins based on a structural domain approach to protein structure and function. A preliminary analysis employing albumin is briefly presented. We move on to protein resistant surfaces based on polyethylene oxide and present a rationale for the properties and behavior of such interfaces, including a preliminary theoretical model which may be useful for the design and optimization of protein resistant surfaces. Finally, we briefly present some preliminary atomic force microscopy studies of immunoglobulins on mica surfaces, demonstrating not only direct imaging of proteins at interfaces in an aqueous environment but, perhaps even more importantly, their manipulation, processing, and ordering.

PART I: PRINCIPLES OF PROTEIN ADSORPTION

The principles of protein adsorption have been presented in a number of monographs, review papers, and conference proceedings.[1–3] The chapter by Norde in this volume, and related papers by him and his co-workers, should also be consulted.

We like to begin a discussion of protein adsorption by reference to the complexity axis concept (Fig. 1), wherein the complexity of protein is represented as one qualitative axis. This axis ranges from relatively simple proteins, such as insulin, myoglobin, or lysozyme, to very complex proteins, such as lipoproteins and other multi-domain plasma proteins, for example fibronectin and fibrinogen. Very complex multi-component protein solutions, such as blood plasma and tears,[4] are also represented.

The surface or interface on which these proteins are acting is also considered in terms of complexity and represented on the horizontal axis.[4] One normally thinks of the air/water interface as perhaps being the simplest, possibly followed by model lipid/water interfaces or liquid/liquid interfaces. One can then consider polymer/water interfaces and finally very complex solid surfaces, such as the block co-polyurethanes. As protein and interface complexity increase, the type and number of interfacial interactions increase.

Clinical Materials 0267–6605/92/$05.00

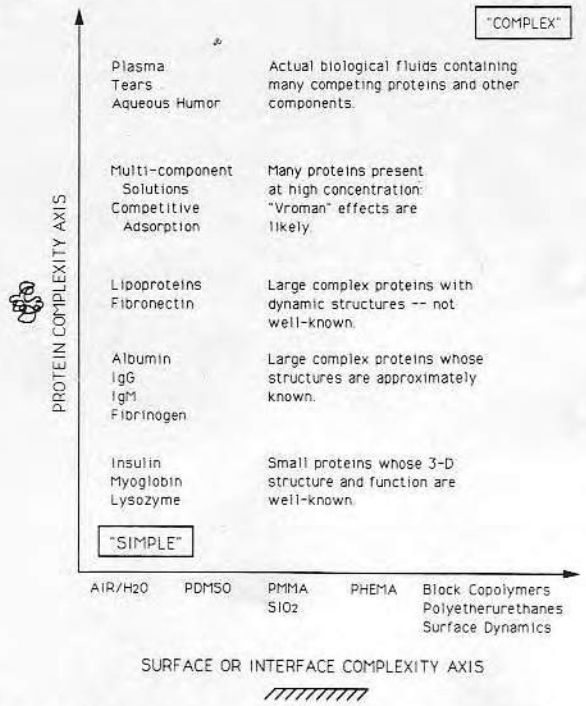


Fig. 1. A protein adsorption and complexity matrix. The lower left corner represents simple proteins at simple interfaces, whereas the upper right represents complex proteins at complex interfaces. Protein complexity is shown increasing on the vertical axes, whereas surface or interface complexity is shown as increasing on the horizontal axes. (See text for details from Ref. 4.)

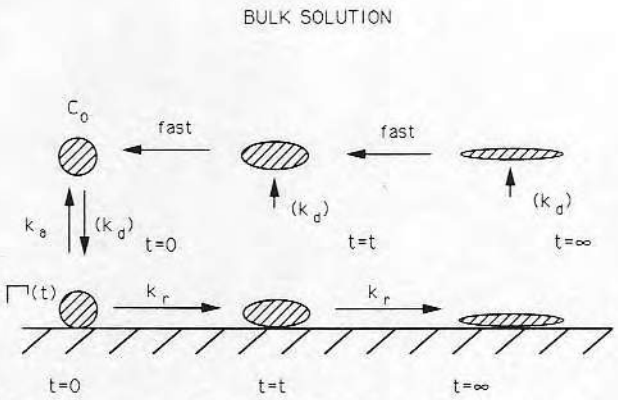


Fig. 2. A general kinetic model for protein adsorption. (From Ref. 4.)

We must consider the dynamics of the process and the ability of the protein and the surface to undergo conformational change. In the case of the protein this is called denaturation.[5] In the case of the solid surface this is often called surface dynamics[6] (see also the chapter by Andrade in this volume).

We begin our discussion by considering the lower left-hand corner of the complexity axis, i.e. simple proteins at simple interfaces, and we will focus on the air/water interface. Figure 2 summarizes much of what we think we know about protein adsorption.[4] Here we show a kinetic model for 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, which are dependent on bulk concentration and diffusion coefficient. That results in a collision frequency.[4] The particular surface chemistry of the protein and the surface dictates the residence time due to the initial interaction energy. The surface dynamics, or denaturability, of the protein itself, together with the residence time, probably controls the surface denaturability of the protein. We assume that the protein denatures with time at the interface; in this simple model system that is 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 Fig. 2.

The reality of the process of course is that proteins are not homogeneous particles. Not all collisions are equally effective in adsorption, and different protein surfaces, or faces, result in different interaction energies with the protein, and therefore different tendencies for surface denaturation. We attempt to illustrate this in Fig. 3, in which the protein is shown as having four faces: a hydrophobic face, a positively charged face, a negatively charged face, and a neutral hydrophilic face. Although all collisions are equally probable, it is only those collisions that result in interaction energies in the range of kT that will provide the residence times necessary for subsequent interfacial processes to occur. 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 a charged surface tends to be a strong function of the charge character of the protein, the pH of the medium, and the ionic strength (see Norde in this volume).

In order to predict the initial contact event or the orientation of adsorbed protein that 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. In these cases the X-ray crystallographic

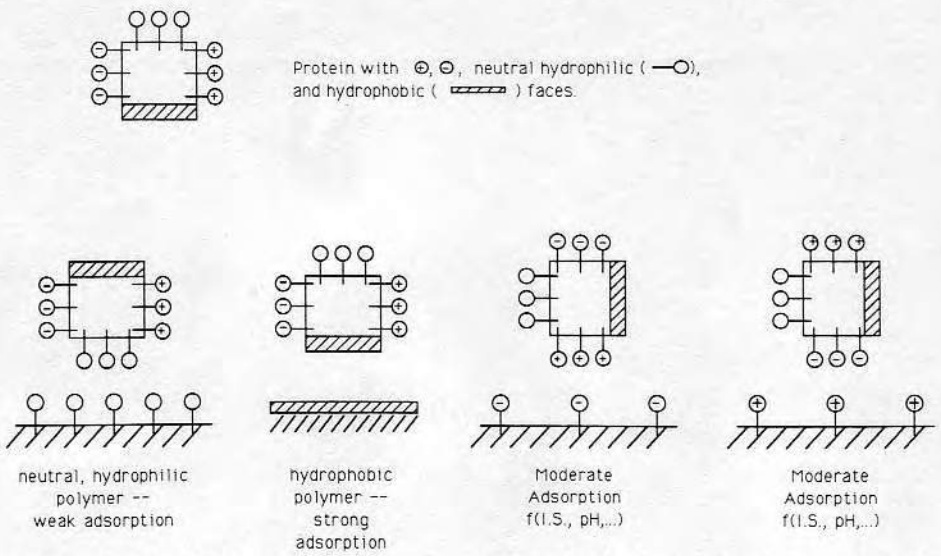


Fig. 3. A schematic view of a four-sided protein, with one face being hydrophobic, one face being negatively charged, one face positively charged, and one face of neutral hydrophilic character, shown interacting with surfaces of comparable character. In the case of a neutral hydrophilic polymer surface, one would expect weak or little adsorption, whereas in the case of a hydrophobic polymer one would expect strong adsorption via the orientation shown. In the case of charged surfaces, one would expect moderate or variable adsorption, depending on the electrostatic nature of the interaction, a function of the ionic strength and pH of the solution, charge density, charge location, etc.

Table 1. The set of model proteins chosen for the basic study of model proteins at the air/water interface. (From refs 9 and 13)

Proteins (abbreviations)	*Species*	*Strands of β-sheets*[a]	*Number of α-helices*[a]	*Number of amino acids*[a]	*Molecular weight*[a]	*Dimensions* (Å)	*Number of S—S bonds*	*pI*	*Nonpolar residues*[a] (%)
Cytochrome-c (CYTC)	Tuna heart	0 (0%)	5 (52%)	103	11353	25 × 25 × 37	0	10	38
Myoglobin (MYG)	Sperm whale	0 (0%)	8 (79%)	153	17183	44 × 44 × 25	0	7·8	46
Superoxide dismutase (SOD)	Bovine liver	8 (36%)	0 (0%)	151	15534	36 × 40 × 38	1	4·6	35
Lysozyme (LYZ)	Hen egg	5 (16%)	4 (27%)	129	14296	45 × 30 × 30	4	10·7	37
Ribonuclease-A (RNase)	Bovine pancreas	3 (37%)	3 (27%)	124	13673	38 × 28 × 22	4	9·6	35

[a] Calculations were based on data obtained from the Protein Data Bank, Brookhaven National Laboratory, Upton, New York, 1973. The percentage of α-helices and β-sheets are given in parentheses.

coordinates of the protein are readily available[7] and can be displayed on a computer screen. One can very easily visualize the different faces or surfaces of the protein with respect to their hydrophobic, charge, and neutral hydrophilic characters and readily formulate hypotheses as to their possible surface interaction.[8,9]

In an early study we showed that the adsorption of hen and human lysozyme on neutral, apolar, and charged surfaces could be at least qualitatively understood by consideration of the external surface chemistry of the two different lysozyme molecules. We could also rationalize the difference in adsorption behavior of human and hen lysozyme by this process.[8,10]

We then expanded our matrix of model proteins (Table 1)[9] and studied their behavior at air/water interfaces by dynamic surface tension techniques.[11] 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 denaturate upon long term contact at the air/water

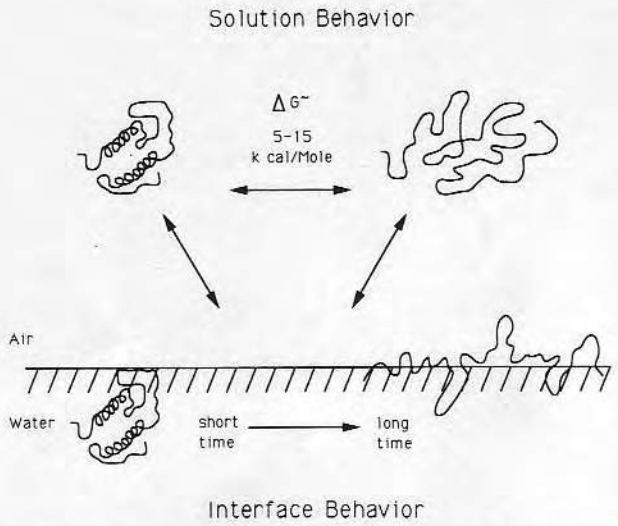


Fig. 4. The relationships between protein structure in solution, protein solution denaturability, and protein behavior at the model air/water interface. (See text for discussion.)

interface (again using dynamic surface tension). Figure 4 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 fluorescence changes. The surface chemical nature of the protein was assessed by examination of its external surface chemistry using molecular graphics and by the use of fluorescent probe titration.[12,13] A relative effective surface hydrophobicity (ESH) parameter was then deduced.[9]

After consideration of a wide range of parameters, and particularly the adsorption principles illustrated in Figs 2 and 3, we selected 12 variables and began to qualitatively examine the correlations between them[9,13] (Table 2). The variables were plotted on radial axes with the axes arranged and scaled so as to emphasize and even exaggerate correlations among the various parameters. We call this multi-parameter radial plot a 'Tatra plot'; it is described in detail in Ref. 9.

Figure 5 presents the Tatra plot for superoxide dismutase, a highly stable protein whose surface is extremely hydrophilic and thus exhibits little surface activity at the air/water interface. The upper left quadrant depicts protein surface hydrophobicity. The lower left quadrant depicts the stability of the protein. The upper right reflects surface activity. We are still experimenting with the variables, their placement, and their scaling, and the Tatra plot is far from being completed or optimized at this time.

Figure 6 gives the comparable data for lysozome and ribonuclease. The lysozome data are clearly pulled into the upper left quadrant, indicating its high surface hydrophobicity. It is much less stable than superoxide dismutase, as indicated by the partial collapse of the points in the lower right quadrant. Compare this with ribonuclease, which has slightly less surface hydrophobicity and less surface stability.

Figure 7 shows the data for myoglobin and cytochrome C—two proteins with similar Tatra patterns. In this case the surface hydrophobicity is lower than in Fig. 6 and the stability is much lower. These are more denaturable proteins. This ease in denaturability is reflected in the increased surface activity (the upper right quadrant), with myoglobin being more surface active than cytochrome.

It is clear that proteins studied fall into three different categories (Figs 5, 6, and 7) in terms of their structure, function, and surface activity relationships (for further details see Ref. 9). Given the apparent success of the multi-variate Tatra plot approach to correlating the behavior of model proteins at air/water interfaces, we are now extending the approach to the solid/liquid interface.

Table 3 presents a listing of solid surface properties that are believed to be important for protein adsorption, and a suggested parameterization of each of these properties. Much additional work is needed before many of these parameters can be appropriately characterized and utilized in the understanding and prediction of protein adsorption.

Figure 8 presents those adsorption parameters which might be useful for multi-variate correlation, based on protein adsorption kinetics and isotherm results. We have taken a limited set of parameters in Fig. 8 and Table 3, and used them to develop a preliminary Tatra plot representation for model protein adsorption on polystyrene lattices, using the data of Norde and co-workers (see Refs 14 and 15 and the chapter by Norde in this volume). The variables selected for this initial use of the Tatra plot for solid/liquid interface problems are listed in Table 4.

In the air/water interface case, hydrophobic interactions are believed to dominate the adsorption behavior, and this is clearly reflected in the strong correlation with the hydrophobic surface character of the protein. In the case of Figs 9 and 10, we utilized data obtained at two different solid surfaces

Table 2. The 12 parameters selected for expression in the Tatra plot in figs 5 to 7. (from ref. 9)

Number	*Label*	*Parameters*	*Value range*	*Physical significance*
1	Td (°C)	Temperature of thermal denaturation	$40 \rightarrow 100^{a}$ (°C)	Thermal stability
2	$[GdnHCl]_{1/2}$ (M)	GdnHCl concentration of 50% denaturation	$1 \rightarrow 6$ (M)	GdnHCl stability
3	K_1(low) (h^{-1})	Rate constant of surface tension kinetics at low bulk concentration (0·01 mg/ml)	$0 \leftarrow 1$ (h^{-1})	K_1 values were found to be limited by the process of surface-induced denaturation
4	$F\mu_\beta$	Average β-moment multiplied by fraction of β-sheets in molecule	$0 \rightarrow 0{\cdot}1$	A measure of both the quantity and amphilicity of β-sheets in structure
5	S—S	Number of disulfide bonds	$0 \rightarrow 5$	More S—S bonds would make a protein more stable, although the contrary may not be true
6	log (MW)	The logarithm of molecular weight	$3 \rightarrow 5$	Size of protein
7	O^-%	Percent accessible area of negatively charged oxygen atoms	$1 \leftarrow 6$	Inversely correlated with protein effective hydrophobicity
8	ESH	Effective surface hydrophobicity measured by *cis*-PnA binding and hydrophobic interaction chromatography (HIC)	$0 \rightarrow 1$	A measure of hydrophobicity of proteins in their native states
9	K_1(high) (h^{-1})	Rate constant of surface tension kinetics at low bulk concentration (1 mg/ml)	$1 \rightarrow 4$ (h^{-1})	K_1 values were found to be dependent on the hydrophobicity of proteins in their native states
10	$F\mu_\alpha$	Average α-moment multiplied by fraction of α-helices in molecule	$0 \rightarrow 0{\cdot}25$	A measure of both the quantity and amphilicity of α-helices in structure
11	Π_{ss} (dynes/cm)	Steady-state surface pressure values at bulk concentration of 0·05 mg/ml	$0 \rightarrow 15$ (dynes/cm)	Air/water surface activity of proteins at equilibrium
12	Nonpolar %	Percent of nonpolar residues in sequence, calculated on a molar basis	$30 \rightarrow 50$ (%)	Correlated to steady-state surface pressure values

[a] The direction of the arrow represents the outward direction in the plots.

that vary significantly in charge. Therefore electrostatics plays a major role; this is strongly reflected in the shape of these preliminary Tatra plots. Electrostatics are represented by the vertical axes: the upper vertical axis reflects the charge on the protein and the lower vertical axis is the charge on the surface. A simple calculation of the charge density shows that the charges on the polystyrene surface are of the order of 25 Å apart, and the intervening space on the surface is largely hydrophobic in nature. It is therefore clear that hydrophobic interactions must also be playing a major role; indeed, both lattices are relatively hydrophobic, as characterized by advancing contact angle measurements.[14] The similarity in behavior between ribonuclease and lysozyme is again evident on the positively charged polystyrene (PS). Adsorption on the negative PS, however, is very different for the two proteins (see the lower quadrant axes). The data for myoglobin in Fig. 10 show that, although its protein parameters are very different, certain aspects of the adsorption behavior are similar. For example, Mb on negative PS is similar to Lyson negative PS. Figure 11 presents lactalbumin, a negatively charged protein. Lactalbumin on the positive PS behaves in a similar way to Mb.

We are still in the process of optimizing the Tatra plot representation for solid/liquid interfaces, and continuing to attempt to interpret the data of Figs 9 to 11.

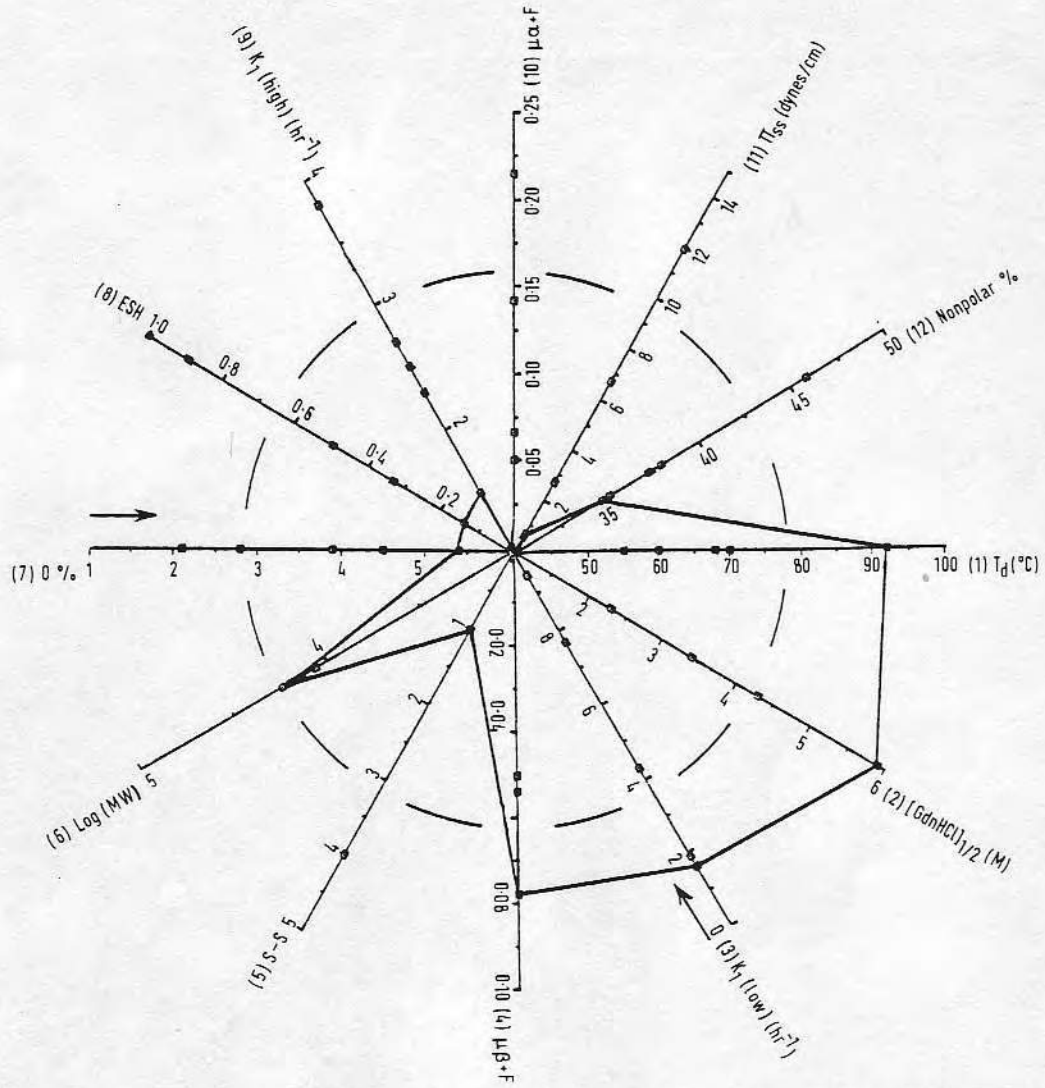


Fig. 5. The Tatra plot, Tatra parameters, and the data for superoxide dismutase. Axes 1–5 are stability related parameters. Axes 7–9 are parameters of effective surface hydrophobicity or related to it. Axes 10–12 are steady-state surface activity. The directions for all axes, except for 3 and 7, are increasingly outward. It is clear that superoxide dismutase is a very stable, hydrophilic, and relatively non-surface-active protein. (From Ref. 9.)

PART II: COMPLEX PROTEINS

What do these concepts and results have to do with the more practical problem of the behavior of complex plasma and tear proteins at biomaterials surfaces? We feel strongly that the way to understand the behavior of a complex protein is to look at its various structural domain building blocks. In the last 10 years it has become evident in structural biochemistry that, although each protein is a unique and distinct molecular machine and has molecular personality, proteins can be considered as constructed of a multiplicity of smaller domain subunits. 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. Fibrinogen is an excellent example. High sensitivity calorimetry studies of fibrinogen and of its protease derived fragments suggest 12 domains in the fibrinogen molecule with denaturation temperatures of 45, 55, 90, and 100 °C. We are only now beginning to analyze fibrinogen in terms of its domain structure, with the hope of beginning to understand its behavior at solid/liquid interfaces.[16,17] Fibronectin is another example. It has at least 20 calorimetrically identified domains,[18] and it is likely that its complex adsorption behavior will be partially understood through a domain approach.

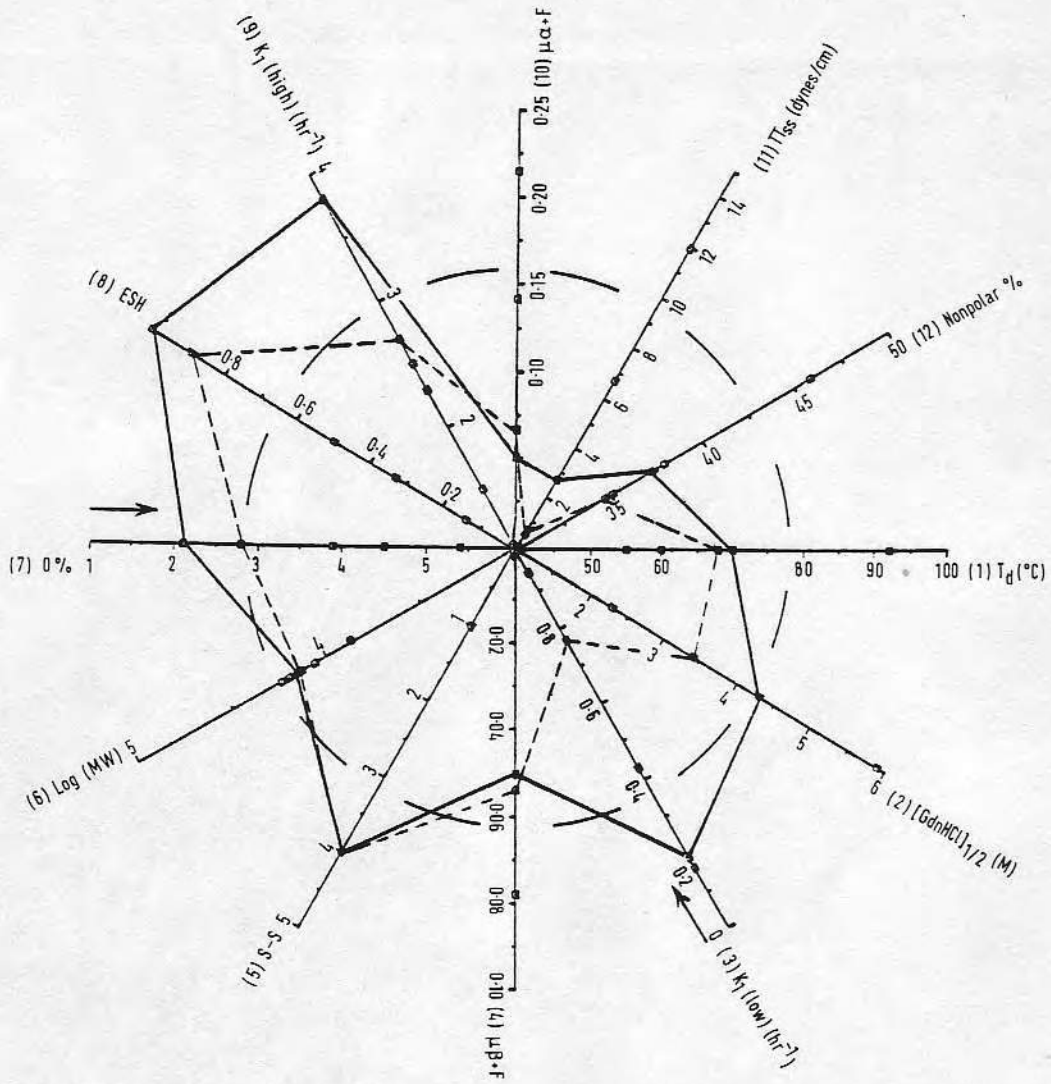


Fig. 6. The comparable Tatra plot data for ribonuclease and lysozyme (solid line). Here one sees the close similarity and behavior of these two proteins, although the surface tension decays for lysozyme in both the high and low solution concentrations are higher than for ribonuclease.

The optimistic view is perhaps best described by Chothia:[19]

> 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 govern the generation of different, and sometimes complex, sentences.

Others have suggested a protein structural linguistics.[20]

We have attempted to apply some of these concepts to the analysis of the interfacial behavior of albumin.[21] Albumin is perhaps the simplest of the multi-domain proteins with which to initiate this analysis. It is a major component of blood plasma; it has no carbohydrate; it consists of three, roughly 20 kilodalton domains; it is high in alpha-helix content and high in disulfide cross-link content; it has a high degree of alpha-helicity and is somewhat myoglobin-like; it binds a variety of ligands, including fatty acids and calcium. The crystal structure, refined to the 4 Å level, for human albumin is now available.[22,23]

We have taken the three domain model of albumin (Fig. 12) and done a very preliminary analysis from an electrostatic point of view. A computerized simulated titration of the three domains as a function of pH and a simple analysis of the possible electrostatic behavior of those

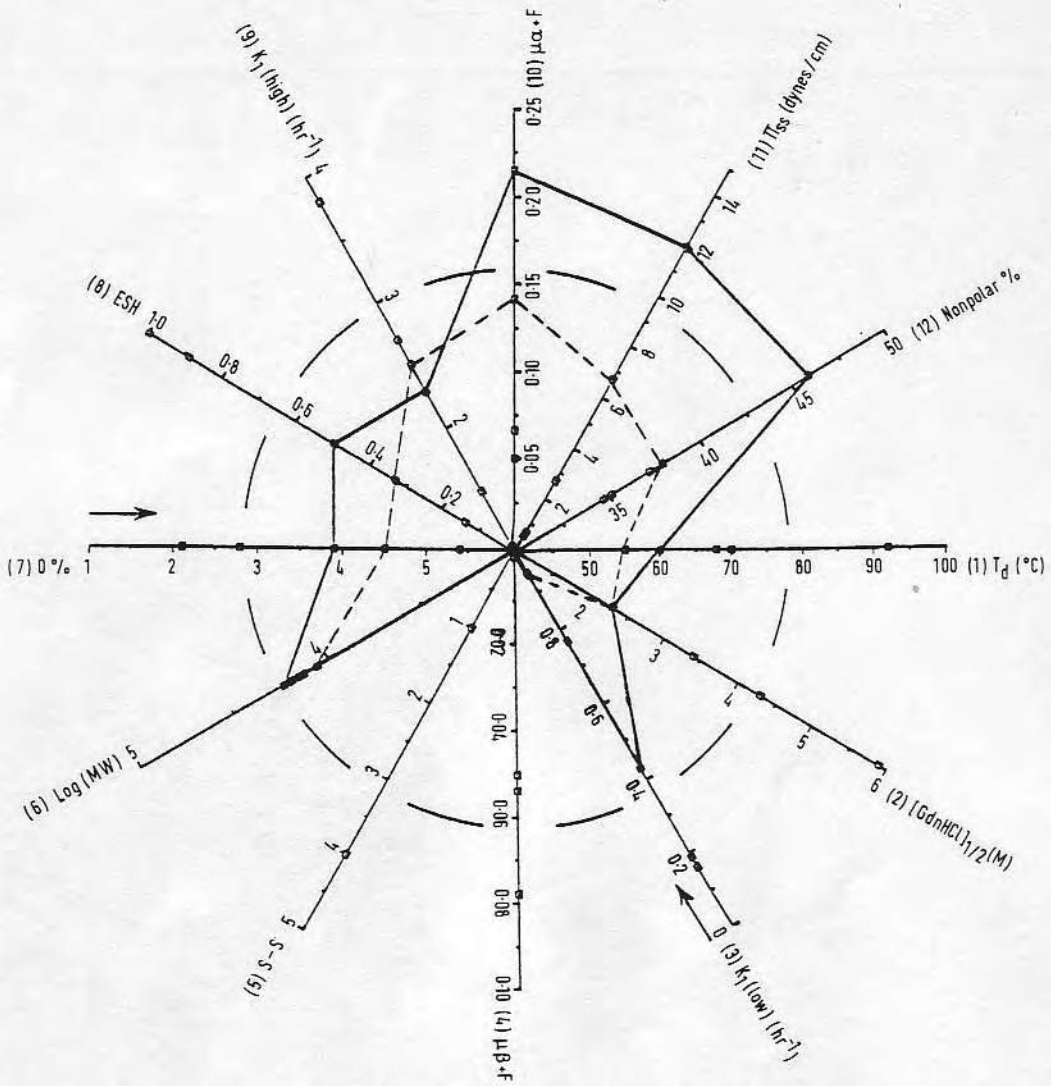


Fig. 7. The comparable Tatra plot data for cytochrome C and myoglobin (solid line). Again, the very general behavior is similar, but quite different from that observed in Figs 5 and 6. Myoglobin shows a much higher surface activity, presumably due to its higher nonpolar amino acid content, and perhaps its alpha-helix content and amphilicity. (See Ref. 9 for details.)

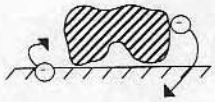
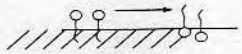
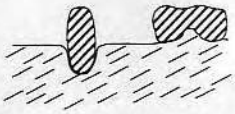
domains at various interfaces allowed us to begin to formulate a number of hypotheses regarding the possible interfacial activity of albumin (Table 5). These hypotheses now allow us to probe into the voluminous albumin adsorption literature and try to begin to make sense of that enormous data base. This has been done in a preliminary way in Ref. 21. The analysis is continuing.

It is clear that a domain approach to protein adsorption and immobilization helps to greatly 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 biosensors and related applications.[24]

PART III: PROTEIN RESISTANT SURFACES

There is now considerable interest in the development of protein resistant surfaces for biomaterials and medical device applications. This builds on a wealth of experience over the decades in the area of colloid science and chromatography, in which minimal interacting surfaces have been extensively developed, modeled, and applied, The fundamental rationale for protein resistant surfaces for biocompatibility purposes is that proteins that cannot be adsorbed cannot be interfacially activated. Most bioincompatiblity is believed to result from protein adsorption processes which change the structure and biochemical properties of proteins

Table 3. Solid surface properties important in protein adsorption. Although the first four parameters can be readily quantitated and presented for many surfaces, the bottom four are often difficult to characterize, and even more difficult to appropriately parameterize

Hydrophilicity	
Hydrophobicity	
Interfacial free energy	
Surface charge density	
Charge-transfer properties? Acid–base character	
Surface dynamics? Polymer transition temperatures	
Surface heterogeneity surface domains, ex. polyetherurethanes	
Surface topography	

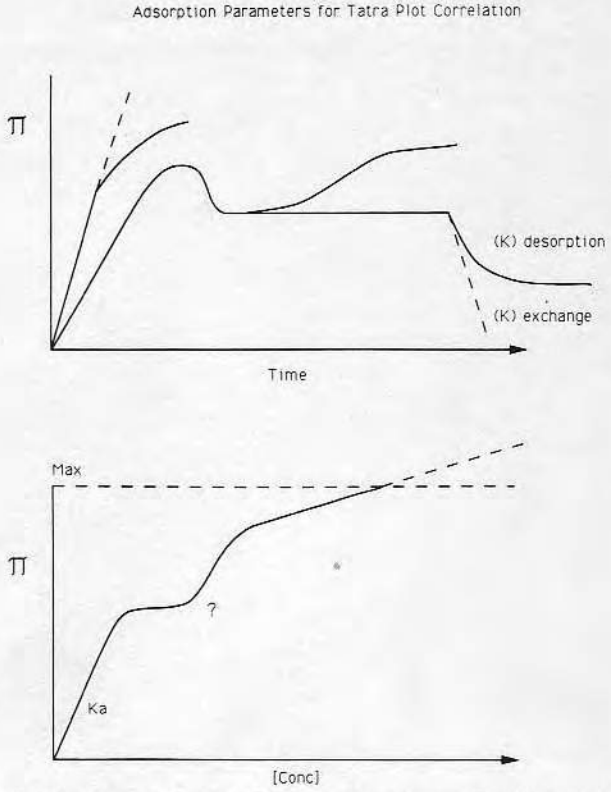


Fig. 8. Schematic and general drawing of protein adsorption and desorption kinetics and protein adsorption isotherms. A number of adsorption parameters are suggested for the preliminary Tatra plot analysis. (From Ref. 15.)

in a deleterious way. Surfaces that minimize adsorption would be expected to minimize such processes.[1-3,25]

Our own work and approach to the problem is summarized in Fig. 13 and ranges from the development of means to produce practical polyethylene oxide (PEO) surfaces on common biomaterials (utilizing the adsorption of a PEO containing polymeric surfactant[26,27]) to the use of covalently immobilized PEO as a tether for the immobilization of antibodies and other proteins.[28,29] We are also developing gradient surfaces as model systems for studying and optimizing protein resistant surfaces.[30] Modeling and simulation of such surfaces is also under way.[31]

Earlier in this paper we discussed adsorption from a protein perspective. Although we considered the properties of solid surface, we considered them primarily in terms of classical hydrophobic and electrostatic interactions. We now wish to consider them from the point of view of dynamics, particularly the dynamics of a surface that has little potential for intermolecular interaction with aqueous solutes.

Polyethylene oxide appears to be a nearly ideal polymer from such a perspective (Table 6).[26,32] It is a neutral molecule, and its weak hydrogen bonding characteristics can be easily satisfied by surrounding water molecules. Its low refractive index suggests that van der Waals interactions will be relatively low. Its stereochemical structure suggests that it tends to fit into the tetrahedral water lattice with minimal perturbation of water structure. It is perturbation of water structure, and particularly its enhanced structuring in the presence of hydrophobic solutes, that leads to the hydrophobic interaction. Thus solutes that minimally perturb water structure are expected to have little or no tendency to develop and promote hydrophobic interactions.

PEO is infinitely soluble in water and has exceptionally low chain rigidity. It is a dynamic and mobil molecule in solution. It fits almost all of the criteria for a surface that has minimal interactions of any kind with protein and for a surface that

Table 4. A very preliminary set of parameters for a Tatra plot relating to protein adsorption at solid/liquid interfaces. (From ref. 15)

$\Delta pH = PI - pH$	Measure of net protein charge; range from -5 to 5
$\mu_\alpha F$	Average α-moment multiplied by fraction of α-helices in molecule; range from 0 to 0·25
T_d (°C)	Denaturate temperature based on pH of buffer solution; range from 0 to 100 (°C)
Log (ionic strength)	Measure of screening of electrostatic interactions between proteins and surface; range from -4 to 0
Cos θ	Cosine of water contact angle, hydrophobicity of surface; range from $-0{\cdot}25$ to 1·0
σ	Surface charge; range from -3 to 3 ($\mu C/cm^2$)
$\left(\frac{\Gamma_{exp}}{\Gamma_{calc}}\right)_{max}$	Normalized surface coverage is related to lateral interactions in adsorbed protein layer; range from 0 to 0·8
$\Delta H^{mol}_{\Gamma \to 0}$	Molar enthalpy of adsorption at low surface coverages; range from -1500 to 1000 (kJ/mol)
$\Delta\zeta_{\Gamma \to max}$	Defined as: $\zeta_{(protein+surface)} - \zeta_{(only\ surface)}$; range from -50 to 50 (mV)
ESH	Effective surface hydrophobicity measured by *cis*-PnA binding and hydrophobic interaction chromatography (HIC); range from 0 to 1·0

would be of intrinsically high mobility and rapid dynamics, and is therefore optimum for steric exclusion.[31]

Some of these concepts were incorporated in a theoretical model, developed in collaboration with deGennes, in which the interaction of a hypothetical protein with an assumed PEO surface of known properties was simulated.[31] The basic model and its components are given in Fig. 14. The model suggests that a surface designed for optimal protein resistance should have the highest possible concentration of immobilized PEO and a maximum PEO molecular weight. This is of course difficult to practically accomplish because the steric exclusion characteristics of PEO make it difficult to achieve high surface concentration, at least from aqueous solutions. Therefore, tricks are clearly required in order to produce surfaces with a large surface concentration of PEO chains. The model also shows that if one assumes there is a weak hydrophobic interaction between PEO and protein, there is a balance, or an optimum, between the number of PEO chains per unit area and the size of the protein. For small proteins, one requires the highest surface concentration of PEO, preferably an interchain separation of 10 Å or so, whereas for very large proteins, the optimum may be in the range of 15–20 Å interchain distances.

This conclusion derives from a model involving a large number of assumptions, and is yet to be experimentally tested. Given this model, however, it is not particularly surprising to us that differences in protein adsorption and protein resistance are observed for different proteins on the same PEO surface. It is highly possible that a surface may be resistant to fibrinogen, and even IgG, while being adsorptive towards albumin, which some of our own preliminary data suggests.[30]

Another major problem is the patchiness of modified surfaces. In order to obtain a thorough characterization and understanding of gradient surfaces, we are now developing methods to characterize the patchiness of surfaces. In fact, it is indeed the patchiness, or the domain characteristics of multi-domain materials such as polyurethanes, that is probably responsible for their peculiar protein adsorption properties,[33] because their domain sizes are comparable to the sizes of protein domains. Thus patchiness of the same order with PEO surfaces, that is in the range of 25–200 Å, will certainly lead to adsorption results that are difficult to interpret. We are presently involved in a set of studies dealing with PEO gradient surfaces, well characterized with respect to surface concentrations and patchiness, in an attempt to partially resolve these complex issues.

PART IV: DIRECT IMAGING AND MANIPULATION OF ADSORBED PROTEINS[34–42]

The development of underwater atomic force microscopy (AFM) by Hansma and co-workers[39] and the ready availability of stable, reliable, commercial atomic force microscopes has made it possible for us and other groups to begin to apply AFM to the study of proteins at interfaces. After seeing the spectacular results of Hansma and co-workers on the adsorption of thrombin activated fibrinogen on mica,[39] we initiated a study in Hansma's laboratory to directly observe IgG adsorption.[35] This study, and the earlier fibrinogen study of Hansma, clearly documented the fact that one could directly observe the protein adsorption process under water, in real time, by atomic force microscopy.

We then proceeded to begin to develop the

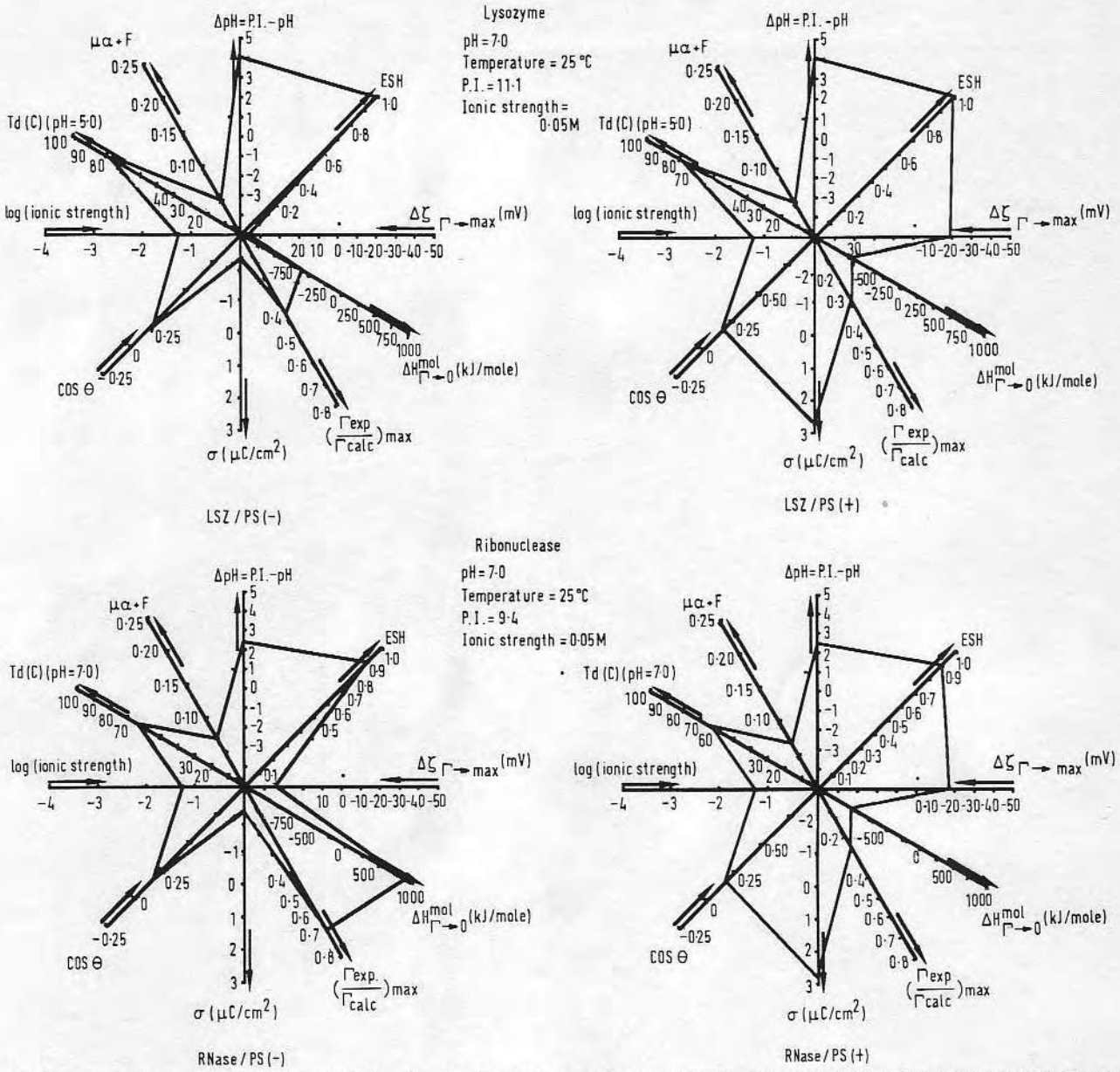
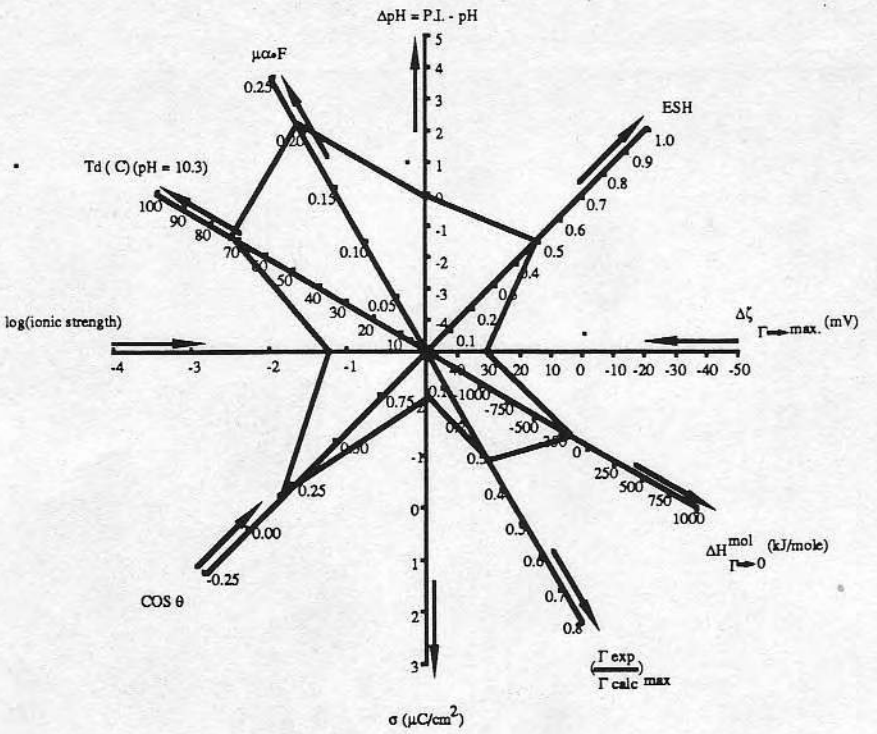


Fig. 9. A preliminary representation of a Tatra plot for ribonuclease and lysozyme adsorbed on charged polystyrene matrices. (From Ref. 15; adsorption data from Ref. 14.) Again there are similarities and differences in the results for ribonuclease and lysozyme. (See text for discussion.)

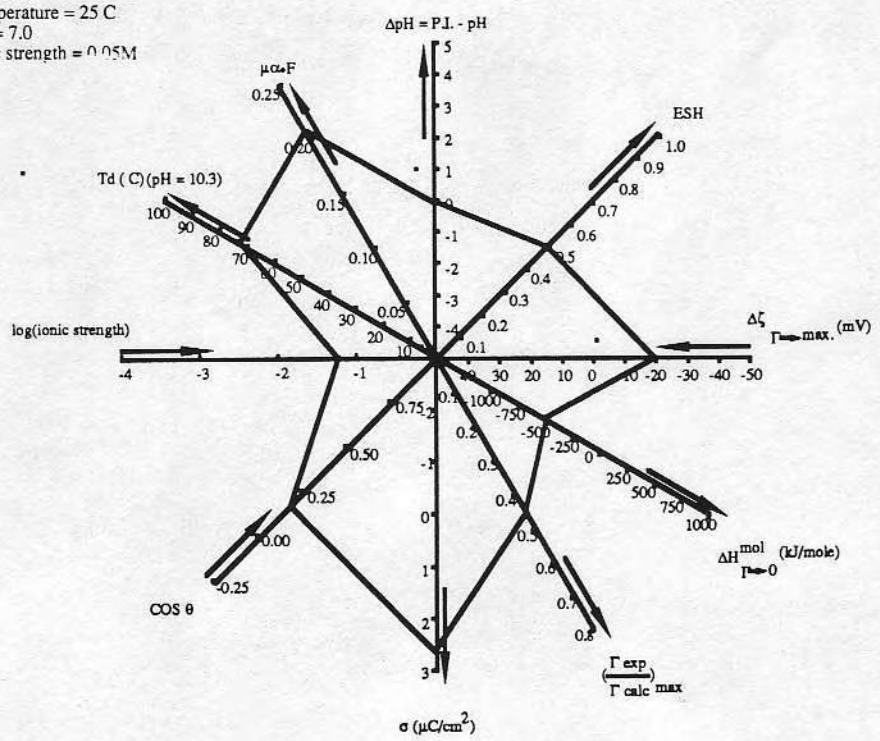
technique by careful consideration of possible artifacts and perturbations, which we tend to call 'protein sweeping with micro-brooms'. Although proteins at solid surfaces can be readily observed by AFM, it is clear that the forces of interaction between the micro cantilever tip and the surface result in significant perturbation of the protein films.[36] The sensitivity of present-day instruments requires that significant interaction forces be present between the tip and the surface. These forces are in the nanonewton range which, given the contact area between the tip and the surface, can even induce covalent bond rupture. Clearly, extensive deformation of the surface is possible when a force in the nanonewton range is applied through a rigid micro cantilever tip to the surface.[36–38]

Figure 15 presents a schematic showing how proteins can be swept along the surface when the sample is scanned with respect to the stationary rigid tip. The magnitude of this effect can be controlled by applying greater or lesser forces between the cantilever and the surface, by controlling the scanning rates, and by programming the scanning area.



Myoglobin
pH = 7.0
Temperature = 25 C
P.I. = 7.0
Ionic strength = 0.05M

MGB/PS(-)



MGB/PS(+)

Fig. 10. The comparable data for myoglobin, which has a very different set of protein properties, yet the adsorption behavior is comparable to that of ribonuclease.

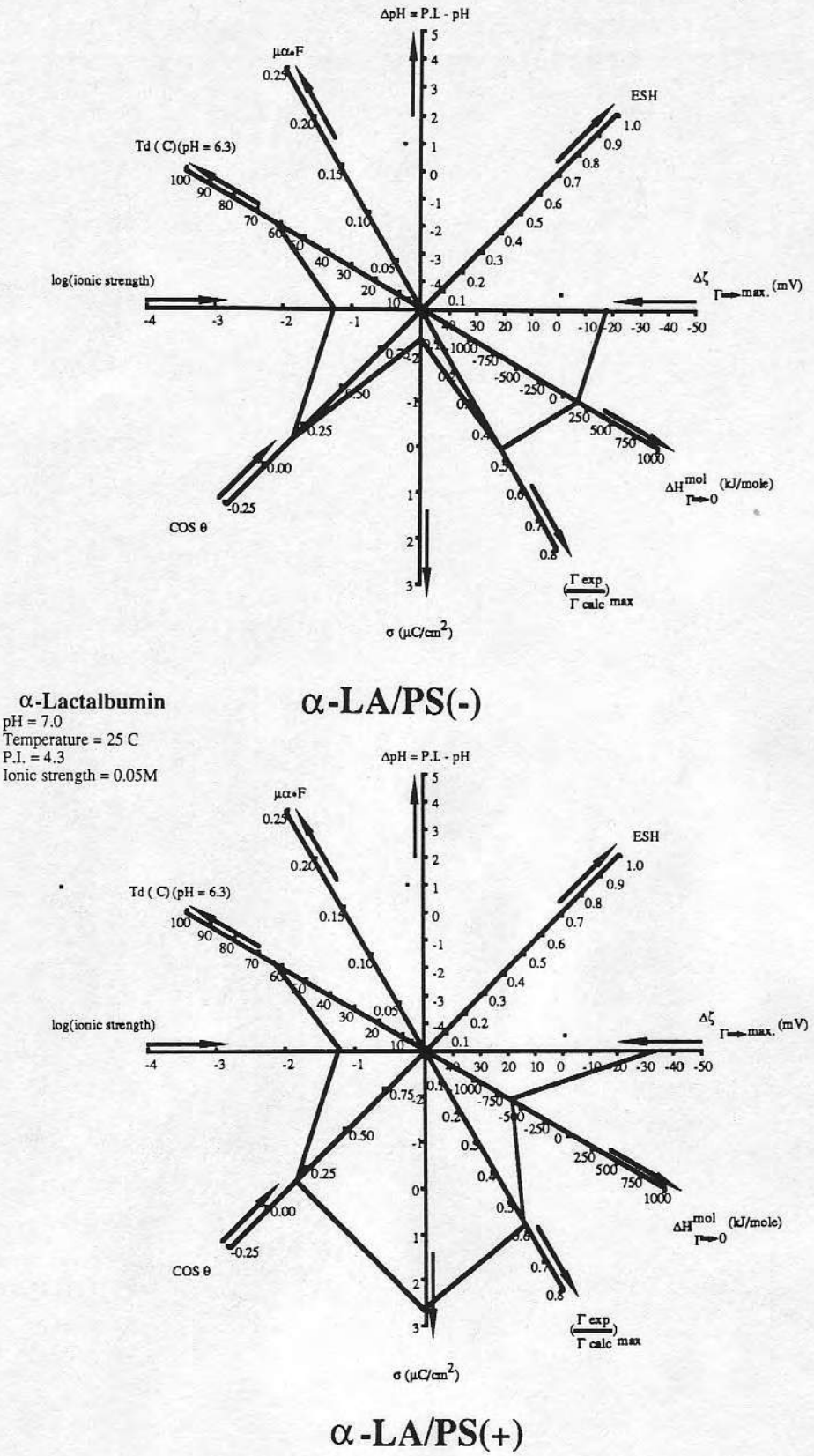


Fig. 11. The preliminary Tatra plot data for lactalbumin, a negatively charged protein with a low alpha-helix content. Now the pattern is very definitely different, and the adsorption slope is much different from that for the other proteins, probably representing the strong electrostatic nature of the interaction in this case.

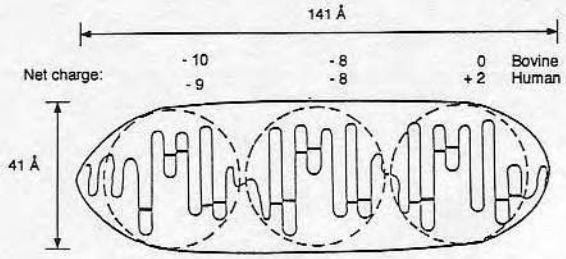


Fig. 12. The 'tennis ball' model of albumin, showing the three major domains and the disulfide-bonded alpha helical subdomains. Domain I is the N-terminal, Domain III the C-terminal. Note the differences in overall charge in the various domains. (From Refs 22 and 23.)

Table 5. Preliminary hypotheses coupling human albumin structural properties to interfacial activity at pH ~ 7. (From ref. 23)

Surface	
Positively charged	Domains I and II are negatively charged and would be preferentially bound. As these domains are less stable than domain III, one would expect the adsorbed albumin to be more denatured than on other surfaces
Negatively charged surfaces	Domain III is weakly positive and would tend to adsorb. There is little denaturation due to the stability of domain III when it contains bound fatty acid
Hydrophobic surfaces	The first loop in domain I is probably hydrophobic and would tend to bind. As this loop is less stable than other loops, a slow time-dependent denaturation can be expected

Table 6. Some of the properties of polyethylene oxide, which may be important in its surface modification and protein resistant characteristics. (From refs 27–32)

Why poly(oxyethylene)?

- It is a neutral macromolecule.
- It has a low refractive index (low VdW interactions).
- It has high water solvency and low rigidity.
- It has high mobility/dynamics.

$(C—C—O)_n$

POE = polyoxyethylene
PEO = polyethylene oxide
PEG = polyethylene glycol

Figure 16 is the result of an adsorbed layer of fibrinogen which was then scanned over particular areas so as to produce a 'U' pattern representing, for public relation purposes, the University of

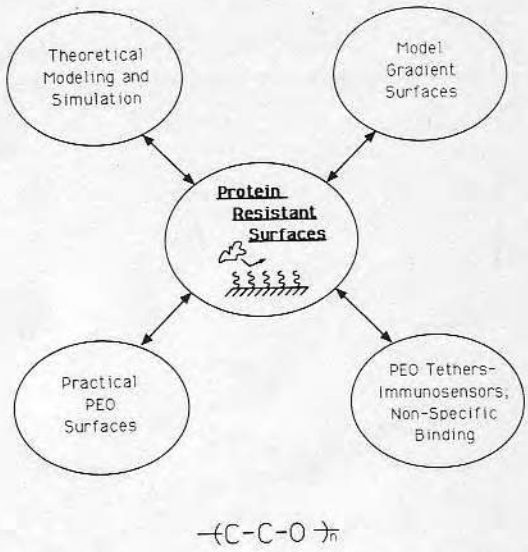


Fig. 13. Various approaches to protein resistant surfaces that we have been studying. Upper left, we have attempted to theoretically model grafted PEO using the deGennes scaling and brush model approach.[31] Lower left, we have been studying commercial and especially synthesized PEO-containing polymeric surfactants as the means for the practical and rapid treatment of biomaterials and devices.[26,27] Lower right, we have been employing immobilized PEO as a tether or spacer, with which to immobilize antibodies and other biomolecules.[28,29] Upper right, surfaces that contain a gradient in PEO concentration along one dimension of a material are now being developed and studied experimentally. These model surfaces should enable a rapid assessment of the parameters and characteristics required for optimum protein resistant surfaces.[30]

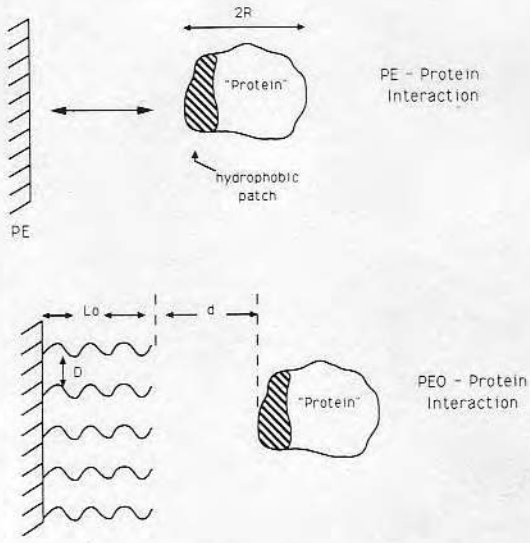


Fig. 14. The schematic model used for the preliminary theoretical treatment of protein interactions with PEO containing brush surfaces. (Refer to Ref. 31 for details.)

Utah. We are now in the process of attempting to apply this unique ability to sweep proteins to the design of experiments, and perhaps even to the

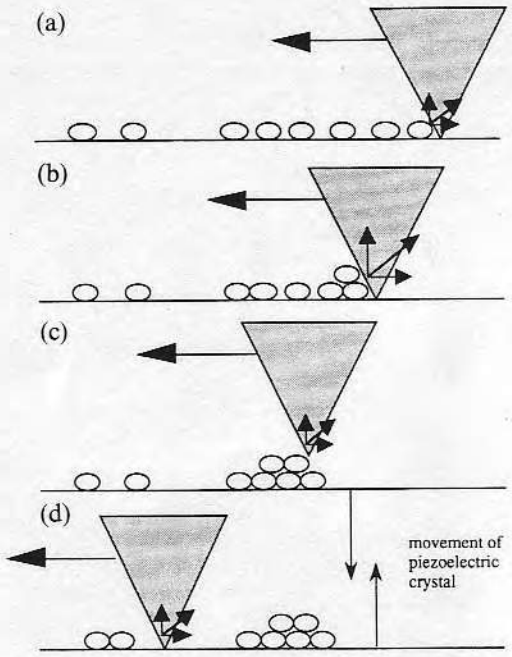


Fig. 15. The schematic illustration of the ability of an atomic force microscope to sweep adsorbed proteins over a surface using the contact force mode: (a) Tip moving in the fast scanning direction begins sweeping the proteins across the surface, provided the vertical force exerted on the tip by the protein is small. (b) As the protein begins piling up, the interaction of the aggregate with the surface increases, producing a larger vertical force exerted on the cantilever. (c) When the vertical force becomes sufficiently large to cause cantilever deflection, the feedback system retracts the piezoelectric crystal, as indicated by the downward arrow, to maintain constant force. (d) The piezoelectric crystal advances, as indicated by the upward arrow, when the vertical force is diminished and the sweeping process begins again. (From Ref. 36.)

development of devices involving preassembled protein arrays. We are also hopeful that we can apply this method to analysis of multi-domain polymer surfaces, such as polyetherurethanes, in the hopes of being able to directly image and characterize the morphology of such surfaces under water. This is done by utilizing a force modulation method and model systems involving rigid and soft polymer blends.[38]

It is likely that the sensitivity of AFM instruments will be improved substantially so that much smaller forces will be needed for the imaging process. The results to date, however, suggest one can indeed image proteins at surfaces, albeit at this time with relatively low resolution because of forces involved and the softness and lack of rigidity of proteins, especially in aqueous environments. It is clear that much progress is being made in overcoming some of these problems.[42] It is also clear, however, that these problems and potential artifacts can be important tools and developments in their own right, as we have demonstrated with the manipulation and processing of fibrinogen in Fig. 16. Indeed, the ability to move proteins around on surfaces into predesigned patterns and arrays may well be the most important biological application of scanning force microscopy.

SUMMARY

We have attempted to summarize most of what we think we know about protein adsorption in Fig. 17

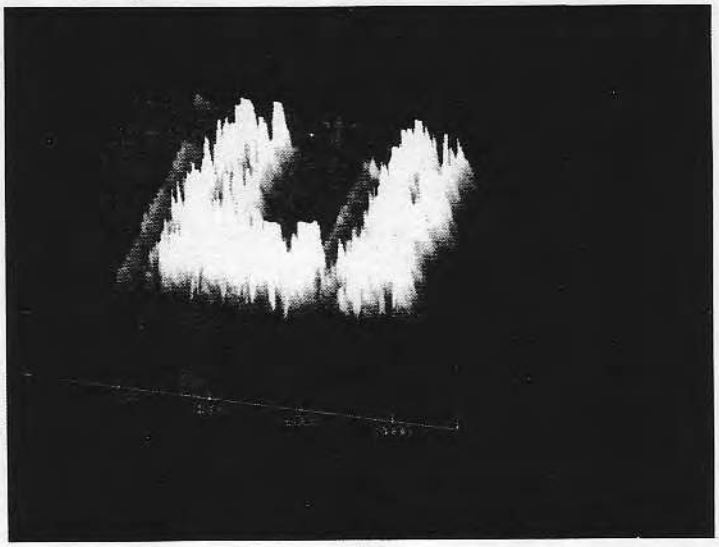


Fig. 16. The results of a programmed protein sweeping experiment using adsorbed fibrinogen to produce a block letter U, clearly demonstrating the ability of AFM and its scanning process to sweep and manipulate proteins on surfaces. (From Ref. 36.)

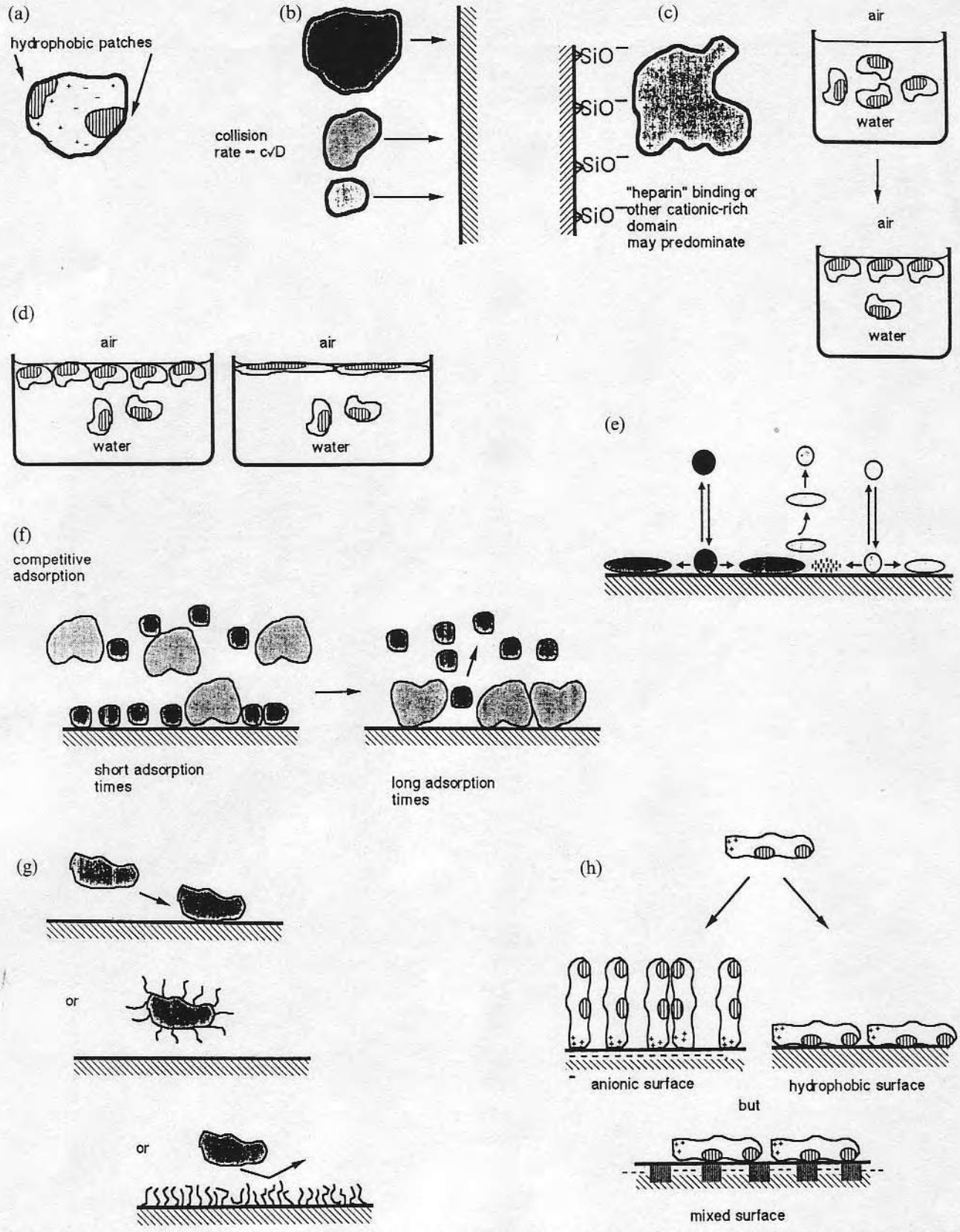


Fig. 17. A concise schematic summary of many of the principles of protein adsorption: (a) Each protein has its own distinctive and individual surface chemistry produced by the outer shell of amino acids and carbohydrate which interface with the liquid medium. (b), (c) Although protein molecules collide with the interface in many different possible orientations, one specific orientation will

(modified from Ref. 43). Figure 17 and its comprehensive caption serves as a graphical summary of most of the current principles of protein adsorption and protein interface engineering.

We feel that the adsorption of the simple proteins at simple interfaces is qualitatively understood. This understanding is being extended to the behavior of complex proteins, even at complex solid interfaces, by the careful consideration of domains, domain properties, patchiness of the surface, and domain–patch interactions. In fact, this can be used to develop the concept of statistical specificity of surface interactions (see Andrade in this volume). The various domains of complex proteins and the various domains and patches on complex surfaces each have their own surface activity and denaturability which must be characterized and incorporated in the analysis.

Surfaces that greatly decrease adsorption and may even be resistant to protein interactions are becoming available. Models and simulations, as well as experimental results of protein interactions with PEO surfaces, suggest that such surfaces do indeed work, and can be further optimized and enhanced for biomaterials and related applications.

Finally, the new technique of scanning force microscopy shows potential not only in the direct imaging of proteins and complex biomaterial surfaces but, perhaps even more importantly, in the manipulation, processing, and fabrication of protein interfaces.

ACKNOWLEDGMENTS

We thank J. N. Herron and Kap Lim for our interactions and collaboration in the area of molecular graphics and model protein studies, Willem Norde for access to the data of model protein interactions with polystyrene lattices,[14] and the Center for Biopolymers at Interfaces for partial support of the work. J.D.A. thanks J. and P. Kopecek for a wonderful trip to Czechoslovakia's High Tatra Mountains, where the Tatra plot approach was initially formulated.

REFERENCES

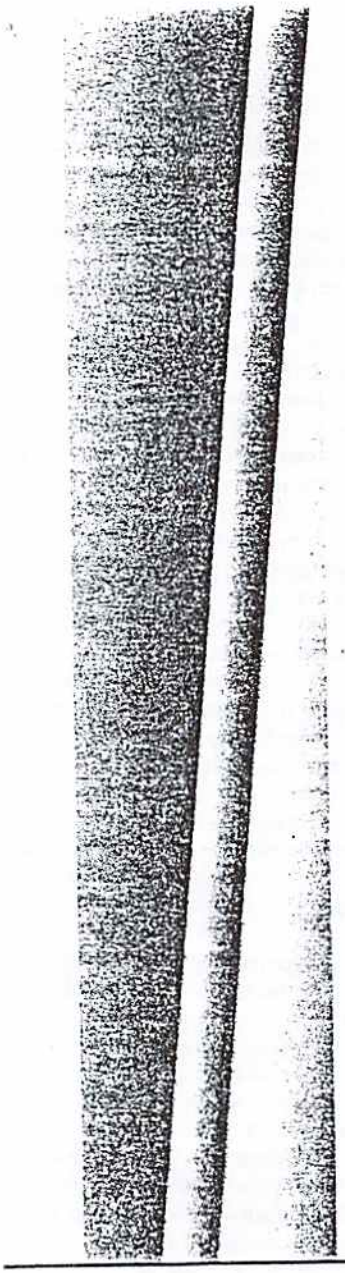
1. Andrade, J. D. & Hlady, V., *Adv. Polymer Sci.*, **79** (1986) 1.
2. Andrade, J. D. (ed.), *Protein Adsorption*. Plenum Press, New York, 1985.
3. Horbett, T. S. & Brash, J. (eds), *Proteins at Interfaces*, American Chemical Society Symposium Series 343, 1987.
4. Andrade, J. D. & Hlady, V., *Ann. N.Y. Acad. Sci.*, **516** (1987) 158.
5. Andrade, J. D., Hlady, V., Herron, J. & Wei, A-P., *Croat. Chem. Acta*, **60** (1987) 495.
6. Andrade, J. D. (ed.), *Polymer Surface Dynamics*. Plenum Press, New York, 1988.
7. *Protein Data Bank*, Brookhaven National Laboratory, Brookhaven, New York.
8. Horsley, D., Herron, J., Hlady, V. & Andrade, J. D., In *Proteins at Interfaces*, ed. T. S. Horbett & J. Brash, American Chemical Society Symposium Series 343, 1987, p. 290.
9. Wei, A-P., MSc thesis, University of Utah, 1990.
10. Horsley, D., Herron, J., Hlady, V. & Andrade, J. D., *Langmuir*, **7** (1991) 218–22.
11. Tornberg, E., Granfeldt, Y. & Hakansson, C., *J. Sci. Food Agic.*, **33** (1982) 904.
12. Nakai, S. & Li-Chan, E., *Hydrophobic Interactions in Food Systems*. CRC Press, Boca Raton, Florida, 1988.
13. Wei, A. P., Herron, J. N. & Andrade J. D., In *From Clone to Clinic*, ed. D. J. A. Crommelin & H. Schellekens. Kluwer Publishers, Amsterdam, 1990, pp. 305–13.
14. Arai, T. & Norde, W., *Colloids and Surface*, **51** (1990) 1–15.
15. Hlady, V., Ho, C. H., Andrade, J. D. & Norde, W. (in preparation).
16. Furie, B. & Furie, B. C., *Cell*, **53** (1988) 505.
17. Privalov, P. L. & Medved, L. V., *J. Mol. Biol.*, **159** (1982) 665.
18. Tatunashvili, L. V., Filimonov, V. V. & Privalov, P. L., *J. Mol. Biol.*, **211** (1990) 161–9.

---

contd.

probably result in the most stable adsorption, with hydrophobic surface patches oriented towards hydrophobic adsorption, with hydrophobic surface patches oriented towards hydrophobic interfaces, anionic patches oriented toward cationic surfaces, etc. (c). Domain and mosaic surfaces can be expected to have rich and complex interactions with the domains and mosaics on the surface of the protein. (d) If the collision rate is very high and the interface is populated with protein very quickly, the proteins may not show extensive time-dependent denaturation processes, although there may be some adjustments in packing, ordering, and lateral interactions. (d), (e) If the surface is not highly populated, the adsorbed protein may denature and/or spread at the interface, altering its confirmation and orientation to optimally adjust to its new microenvironment. Such events may result in the expulsion of less optimally oriented or bound proteins from the interface, as the more optimally oriented or bound protein spreads at the interface. The tendency for the protein to denaturate at the interface is related to its intrinsic stability, including the number of disulfide bonds. (e, f) The presence of two or more different proteins in the solution will result in competitive adsorption processes; the protein that can most optimally bind and accommodate at the interface tends to displace its less optimally bound neighbors. Thus, a complex hierarchy of adsorbed protein types and amounts can develop with time, related to solution concentration, size, collision rates, interface affinity, and denaturation tendencies. This behavior is now called the 'Vroman effect'. (g) It is possible to control and regulate protein adsorption, in part, by modifying the surface or interface or by modifying the protein, such as with steric exclusion modifiers. (h) Materials and interfaces with microheterogeneities of the same size as the structural domains or building blocks in proteins probably have a particularly rich and complex set of adsorption properties.

19. Chothia, C., *Ann. Rev. Biochem.*, **53** (1984) 537.
20. Zehfus, M. H., *Proteins*, **3** (1987) 90.
21. Andrade, J. D., Hlady, V., Wei, A-P. & Golander, C. G., *Croat. Chem. Acta*, **63** (1990) 527.
22. Andrade, J. D., Hlady, V., Wei, A-P. & Golander, C. G., *Croat. Chem. Acta*, **63** (1990) 527.
23. Peters Jr, T., *Adv. Protein Chem.*, **37** (1985) 161.
24. Chang, I-N. & Lin, J-N. (in preparation); also Chang, I-N., PhD thesis, University of Utah (in preparation).
25. Andrade, J. D., Nagaoka, S., Cooper, S., Okano, T. & Kim, S. W., *ASAIO*, **10** (1987) 75.
26. Lee, J. H., Kopecek, J. & Andrade, J. D., *J. Biomed. Mater. Res.*, **23** (1989) 351.
27. Lee, J-H., Kopeckova, P., Kopecek, J. & Andrade, J. D., *Biomaterials*, **11** (1990) 450.
28. Kopeckova, P., Kopecek, J. & Andrade, J. D., *New Polymeric Mater.*, **1** (1990) 289.
29. Lin, J-N., Chang, I-N., Andrade, J. D., Herron, J. N. & Christensen, D. A., *J. Chromatography*, **542** (1990) 41–54.
30. Lin, Y. S. & Hlady, V. (in preparation); also Lin, Y-S., PhD thesis, University of Utah (in preparation).
31. Jeon, S. I., Lee, J. H., Andrade, J. D. & deGennes, P. G., *J. Colloid Interface Sci.*, **142** (1991) 159.
32. Harris, J. M. (ed.), *Biomedical Applications of Polyethylene Glycol Chemistry*, Plenum Press, New York (in press).
33. Tingey, K., PhD thesis, University of Utah, 1991.
34. Feng, L., Andrade, J. D. & Hu, C-Z., *Scanning Microscopy*, **3** (1989) 399.
35. Lin, J. N., Drake, B., Lea, A. S., Hansma, P. K. & Andrade, J. D., *Langmuir*, **6** (1990) 509.
36. Lea, A. S., Pungor, A., Hlady, V., Andrade, J. D., Herron, J. N. & Voss Jr, E. W., *Langmuir*, **8**, (1992) 68.
37. Marchant, R. E., Lea, A. S., Andrade, J. D., Brockenstedt, P. & Ginsbert, D., *J. Colloid Interface Sci*, **148**, (1992) 261.
38. Stroup, E. W., Pungor, A., Lea, A. S., Hlady, V. & Andrade, J. D., Abstract, SPE Regional Technical Conference on Medical Plastics, Raleigh, N.C., October 1991.
39. Drake, B., Prater, C. B., Weisenhorn, A. L., Gould, S. A. C., Albrecht, T. R., Quate, C. F., Cannell, D. S., Hansma, H. G. & Hansma, P. K., *Science*, **243** (1989) 1586.
40. Weisenhorn, A. L., Drake, B., Prater, C. B., Gould, S. A. C., Hansma, P. K., Ohnesorge, F., Egger, M., Heyn, S-P. & Gaub, H. E., *Biophys. J.*, **58** (1990) 1251.
41. Wigren, R., Elwing, H., Erlandsson, R., Welin, S. & Lundstrom, I., *FEBS Letters*, **280** (1991) 225.
42. Andrade, J. D., Lea, A. S., Pungor, A., Hlady, V., Herron, J. N. & Voss Jr, E. W., Abstract, 7th International Conference on Surface and Colloid Science, July, 1991.
43. Andrade, J. D., Hlady, V., Herron, J. & Lin, J-N., 2nd International Symposium on Bioelectronic and Molecular Electronic Devices, R & D Association for Future Electronic Devices, Japan, 1988.



Improved Delivery and Reduced Costs Of Health Care Through Engineering

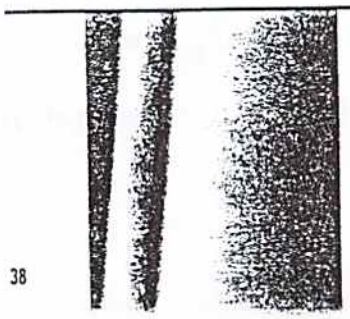
Discussion Meeting—April 23-24, 1992, Washington, DC

J.D. Andrade
Biomedical Engineering
University of Utah
Meeting Chairman

Meeting Organizers

Dov Jaron
Biological and Critical Systems
National Science Foundation

Peter Katona
Biomedical Engineering Programs
The Whitaker Foundation



Engineering can make major contributions to decreasing the costs of health care and to improving the quality and access of health care. This was the major conclusion of a two-day discussion meeting on the topic, involving 25 biomedical engineers, physicians, social workers, policy specialists, health administrators, physical therapists, nurses, economists, hospital administrators, research administrators and program managers from federal and private funding agencies.

The purpose of the meeting was to explore ways in which engineering research can contribute to the containment and reduction of health care costs and help to improve the cost effectiveness, quality and accessibility of the health care system.

Specific objectives included:

- Identification of areas for innovation and research.
- Examination of ways by which the engineering community can become a partner in addressing the health care problem.
- Discussion of mechanisms that would enhance cooperation between agencies.
- Consideration of restructuring of educational programs.
- Outline of plans for follow up activities.

Background

The health care system is undergoing fundamental changes. The rising costs of health care, the advancing age of our population, and the increased impact of medical technologies are important elements in the growing demand for improved care and for wider access.

Restoration of function and of individual independence are key criteria in health care. Much can be learned from regional, state, and particularly international comparisons and perspectives. The health care discussion in the United States often tends to be too parochial.

Enhanced communication and increased sharing of resources and information is desirable. Health care providers may have to be organized, networked, and managed so as to have broad access to expensive technology. There will be an increasing commitment to the development and use of more cost-effective technologies. Hospitals are expected to alter the way technology is acquired, managed, distributed and utilized. The demand to improve the productivity of every person involved in health care delivery will lead to increasing needs and demand for labor-saving technologies.

The health care delivery system is expected to continue to decrease its emphasis on tertiary care and accommodate to a rapidly increasing need for primary care services. Non-physicians are becoming responsible for primary services, including diagnosis and early treatment. Utilization of paraprofessionals in the health care system will continue to increase in hospitals, clinics, nursing homes, and private homes. There is a rise in the authority and responsibility of nurses and other nonphysician professionals. An increasing number of patients, especially the elderly, the disabled, and the chronically ill, can receive care in their homes, with clinical data transmitted from the home and monitored at a central location. Records, clinical information and on-line expert diagnosis will become available to remote sites.

Important demographic considerations include the rapid aging of our population and the fact that there will be fewer and fewer younger people to care for more and more older people. Older people are far more functional than they have been in the past and will be far more functional in the future than they are today. Not only the age demographics but the functionality of various age groups must be considered and effectively employed and utilized.

Engineering research can and must play a critical role in responding to these needs in

0739-5175/93/$3.00©1993

order to develop a more efficient, more cost-effective, and higher quality system. In many areas, engineers can and should assume a leading role.

Panel Recommendations: Areas for Innovation and Research

Quality and Production Engineering

The increasing emphasis on total quality management, continuous quality improvement, and information management will likely change the focus and direction of medical technology development. Such methods and concepts need to be studied and applied to the health care system. Measurements of quality need to be improved.

Personnel costs are a very major component of the total health care equation and all efforts must be made to increase health care worker productivity. Services and care can be enhanced significantly by very different assignment and distribution of staff and services, such as providing a pair of providers (nurse/physician) who perform all possible services to a set of patients, or by cross training, so those personnel in immediate contact with the patient perform the services required, rather then simply writing orders for others to perform services. Such diad models with permanent patient assignments lead to increased productivity, enhanced communications, and more effective care.

Potential major changes in the manpower makeup of hospitals are expected. A simple policy change with respect to Medicare payment for residents, for example, could lead to a significant change in the number of residents available in hospital environments. If that were to happen, there might be a need for increasing numbers of less trained health care professionals, which would produce a demand for more effective means of education, easier to operate and more intuitively understandable devices, and related significant changes.

It was noted that duplication of diagnostic services is a significant cost. Cost savings could be provided by better information handling and record keeping, and by increasing the bedside or near bedside availability of tests and related information.

Communication and Information Technologies

The development and availability of means for the collection, storage, correlation, and access of large quantities of medical information need to be studied and applied.

The integration of medical information and the use of such information for enhanced patient treatment, including application of expert systems for enhanced medical decision making, should be studied and developed.

Information and communications technologies and systems should be available to provide on-site information at the decision point. This information, together with available clinical practice guidelines and codification of diagnostic and therapeutic strategies, would permit more rapid and more effective on-site diagnosis.

There needs to be more effective clinical information transfer between the nurse, physician, pharmacist, and other health care professionals. New information systems should be clinically based and should support improved decision-making by physicians, nurses, and other clinicians. The use of networks or other information and communication technologies should make such information available to the attending health care professional.

There needs to be more enhanced information transfer between the home care paraprofessional and the nurse or tending physician. There needs to be better mechanisms to enhance and insure patient compliance.

There should be consideration of national or even international medical record systems, accessible via satellite and fiber optic communications networks. More effective and more remote noninvasive monitoring should be considered, including the opportunity for near continuous remote monitoring of at risk patients. Means to process and handle large volumes of information are needed, including neural networks, fuzzy logic, and expert systems.

The airline reservation system was given as an example of a real time, reactive system in which information is very readily stored, transferred, accessed and modified, internationally, in real time, with minimal cost. Why can there not be a health care equivalent?

Education

Modern technologies and methods must be applied to enhance education and training for all appropriate populations, including physicians, nurses, other health care professionals, patients, family, child patients, public officials, health policy professionals, the media, and the general public.

There is a need for education at all levels, including some introduction in the medical curriculum to the profession of engineering and the engineering approach to problems. NSF's strong interest in science and engineering education can and should apply to the entire problem of the costs of health care. National initiatives in science and technology education, from kindergarten through the university, can all involve various health and medical components. This may be a vehicle by which the population may be more optimally educated with respect to health care issues, including the minimization of unrealistic expectations.

Much of the education which physicians receive regarding medical technologies is the result of marketing initiatives from corporate representatives and other special interest groups. *Demarketing* must also be done—marketing information and marketing influences must be balanced with other, perhaps more objective, information.

Patient education and understanding is another very important priority. Technologies are needed to provide periodic or even constant reminders to encourage patients to resist temptation to reactivate bad habits or poor health practices.

The use of models and simulations should be increased in many areas. Modern education technologies and methods, especially modeling and stimulation, can identify target or most effective populations for activities related to medical compliance and behavior modification. Inexpensive monitoring and sensing can provide information and signals for enhancement of patient compliance.

Education technologies are helpful to develop realistic patient expectations and facilitate and improve patient feedback and provider-patient interaction. Education can lead to better informed patient decision making, including the ability and likelihood of patient concern for and understanding of quality of outcome and costs of procedures (i.e. of the "value" of the procedures).

More effective education and training can be accomplished using the skills and techniques of the game and entertainment

Engineering research must play a critical role in developing a more efficient, more cost-effective, and higher quality system

We need to consider how the components of health care expenditures might be favorably affected by future technology development

industry, particularly video, robotic, and virtual reality technologies.

The media often fuels unrealistic expectations. There are many examples of miracle medicine in the media. Health care professionals, biomedical engineers, and others must work with the media to make them more aware of the realities of medicine and health care.

The issue of risks, particularly as applied to health care, must be considered and communicated to the general public and to the media. The difference between voluntary and involuntary risk must be made clear. The role of risk awareness in influencing behavior is critical. The importance of patient choice and empowerment should be emphasized. Cost is unlikely to be brought under control without a general appreciation of the limits of technology and of the inability of devices to correct the normal wear and tear of the aging process. This includes the acceptance by patients of risks commensurate with the statistically demonstrated benefit. There must be reform of tort law which at the moment unrealistically presumes a uniform level of performance for "life-saving" devices.

Device Design and Manufacturing

There has been some tendency in medicine to introduce technologies into clinical practice because they are new or fashionable rather than being significantly more effective.

Technology which enhances the independence and mobility of disabled and geriatric populations is needed. Methods and mechanisms to reuse and recycle devices, such as equipment and patient aids, must be developed.

Many devices can be significantly improved—the man/machine interface in medical devices should be researched with the goal of producing more user-friendly devices. Many existing devices should be re-engineered to improve design, reliability, and utilization. Optimal design must be based on the point of view of the patient, the user, and the maintainer of such equipment. It would also consider the integration of various pieces of equipment. Other topics include reliability, longevity, reuse, resale, upgrading, abandonment, and patient independence.

New technologies may make it possible for a major reconsideration of medical device design and development. Cell and tissue engineering needs to be applied to a new generation of hybrid devices and even substitutes for devices. The developments in drug delivery technologies and minimally invasive devices can also be expected to lead to significant changes in practice.

Device design and development must also consider user and patient education and understanding.

Minimally Invasive Sensing

Home sensing, home monitoring, and home diagnosis should be further developed and expanded.

The wide range of developments and the rapidly increasing technology with respect to sensors and sensing has yet to be effectively applied in medicine. This is an area in which emphasis and application is expected.

Economic Analysis and Technology Planning

There needs to be research in the areas of hypothesis development and in the development of measures and indicators with respect to the effectiveness and costs of various technologies.

There is a need for research on incentives and deincentives regarding cost control and cost effectiveness. The whole issue of health care economics, cost, cost effectiveness, acquisition costs, utilization costs, real costs, total costs, expenditures, and other such terms, need to be well defined and made available to the general health care community, as well as to the biomedical engineering communities. There are difficulties in obtaining valid data with respect to life cycle costs, marginal costs, benefits, and effectiveness. These topics must be studied.

We need to develop formalized processes to consider the cost effectiveness of new technology—but with the following cautions:

- Recognizing that complete measures of cost effectiveness seldom exist.
- Allowing developers and evaluators to identify adjustments which, if made, could result in favorable cost effectiveness evaluations.
- Encouraging funding agents to take risks when cost effectiveness is not certain.
- Allowing second chances to show cost effectiveness.

We need to look at the components of health care expenditures; considering how each might be favorably affected by future technology development, and as a basis for global evaluation of the cost-effectiveness of a particular technology.

New and improved medical technologies and bioengineering research should be measured and compared against a set of criteria in order to assess their effectiveness, potential benefit, appropriateness, costs, and applicability in the modern health care environment. One set of criteria which could serve as a basis for discussion and research includes:

- cross training (applicability by a range of health care professionals);
- decreased costs;
- increased patient independence;
- decreased need for personnel time;
- increased quality as measured by outcomes;
- generation of data or related information important to hypothesis testing;
- increased access to targeted population or to the general population;
- more effective resource utilization;
- improved long term benefits;
- increased retirement age;
- minimization of health care inflation;
- compatibility with general societal objectives

Engineering Partnerships

Education

There must be close interaction between bioengineers and educators responsible for the education and training of physicians, nurses, and health care paraprofessionals. Social workers and other professionals involved with the care and counseling of the aged, handicapped, sick, and indigent must be a part of the engineering team—as they have insights and perspectives critical to successful health care outcomes.

The media are vital to communication with the general public. The media must be involved and educated—particularly with respect to cost/benefit/risk issues. The American Medical Writers Association and the National Science Writers Association are two groups with which bioengineers should communicate and interact.

Academic bioengineers must encourage the effective education of all undergraduates at their institutions. They cannot leave these topics and responsibilities to "others."

Health Care Providers

Engineers must develop collaborations and partnerships with hospital administrators and with health care providers. They must become aware of the problems and needs in a modern hospital, in modern clinics, and in physicians' office.

Although academic bioengineering programs are generally tied to research and teaching hospitals, they rarely have the opportunity to learn the activities and needs of primary and secondary care providers. On-site visits, internships, special projects, and related mechanisms are needed in primary care environments including doctors' offices, health maintenance and preferred provider organizations, clinics, etc.

Bioengineers should of course maintain their ties to tertiary care providers and to the high technology side of medicine, but with an increased interest and activity in decreasing the costs of care, while improving and simplifying the medical technologies involved.

Public Health

The greatest engineering contributions to the health and well being of our population in this century have come from involvement in the public health sector, particularly in water treatment, water purification, enhanced air quality, and related activities.

Engineers should seek increased interactions with the public health sector to address such problems as vaccination technologies, technologies to treat and relieve drug dependencies, and technologies and approaches to enhance the health and well-being in all sectors of the economy and of society.

Economists and Public Policy Professionals

Most programs dealing with health care economics and health care policy are not closely allied with engineering or bioengineering programs. In a large part this is due to the very wide gap in interest and skills of these two professional populations.

Engineers should begin to bridge that gap, should offer their services to health economics and public policy programs in their institutions and in their cities. Engineers should strive to play a much larger role in such public policy issues, as they can bring much needed technical and scientific skills to the discussion.

Deans, department chairs, and distinguished engineers should encourage their colleagues to become involved in such activities. The trend has been to discourage such involvement, and that trend must be reversed.

Federal Agencies

A large number of federal agencies are involved in health care funding and policy formulation. Many of these same agencies are involved in the funding of engineering and technical research and development through grant, contract, and small business programs. These agencies should begin to address the issue of the costs of health care and the role of engineering and technology in decreasing those costs.

Emphasis should be placed on initiatives and projects which have some likelihood of decreasing health care costs while maintaining or enhancing the quality of care and enhancing access to care.

The National Institutes of Health is completing a strategic planning process. It is likely that one of the outcomes of that planning process will be some coordinating body or committee within NIH to coordinate engineering and bioengineering activities throughout NIH. Bioengineering has been defined as a trans-NIH activity and it has been suggested that it be given an appropriate identity within the NIH mechanism. The Bioengineering Program in the National Science Foundation should interact with the appropriate entities in NIH and with other government agencies, including the Department of Defense, the Environmental Protection Agency, NASA, the Agency for Health Care Policy and Research (AHCPR), the Health Care Finance Administration (HCFA), and the Food and Drug Administration (FDA).

Recommended Follow-Up

The American Institute for Medical and Biological Engineering (AIMBE) held its annual meeting March 7-9, 1993 in Washington, DC. The theme of that meeting was "The Future of Health: The Role of Medical and Biological Engineering."

A workshop on "The Role of Engineering in Enhancing the Quality, Productivity, and Access of the Health Care System" should be held in the near future. The workshop should begin with a series of background and perspective lectures dealing with costs, demographics, technologies, and other issues.

There should be a general, wide-ranging, perspective and awareness lecture on each main topic, followed by a set of panel discussions which assess needs and problems which such technologies could address and solve. The panels should consist of physicians, nurses, engineers, health care professionals, paraprofessionals, patients, family members, and social workers experienced with various patient segments and populations.

A copy of the complete report, including a list of all participants and a set of comprehensive appendices, is available by request from the author.

Joseph Andrade is Professor of Bioengineering and of Materials Science at the University of Utah, Salt Lake City, where he has served as Chairman of the Department of Bioengineering and Dean of the College of Engineering. He directs the Center for Integrated Science Education at the university, and is program co-chair for the Utah Science Center project. He is Vice President for Public Policy of the American Institute for Medical and Biological Engineering, and chaired the March 1993 meeting, "The Future of Health: The Roles of Medical and Biological Engineering."

His major research area is proteins at interfaces and the application of proteins as biomolecular devices, and he involved in developing a number of unique bioluminescent materials and products for science education purposes.

He can be reached at the Department of Bioengineering, University of Utah, 2480 Merrill Engineering Building, Salt Lake City, UT 84112.

Bioluminescence and Chemiluminescence

Status Report

Proceedings of the VIIth International Symposium on Bioluminescence and Chemiluminescence, Banff, March 1993

Edited by

A.A. Szalay, L.J. Kricka and P. Stanley

1993

JOHN WILEY & SONS

Chichester · New York · Brisbane · Toronto · Singapore

USING BIOLUMINESCENCE FOR INTEGRATED SCIENCE EDUCATION

J.D. Andrade[1], J. Tobler[1], M. Lisonbee, D. Min

Center for Integrated Science Education, University of Utah, S.L.C., UT 84112
[1]Second Address, Protein Solutions, Inc. 390 Wakara Way, S.L.C., UT 84108

SUMMARY

Bioluminescence is a nearly ideal subject with which to experience the scientific process and critical science concepts and themes, particularly in the upper elementary and junior high school environment (4).

We have developed bioluminescent dinoflagellate cultures which enable teachers and students to readily experience bioluminescence, closed ecosystems, circadian rhythms, and a variety of principles of 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.

INTRODUCTION

A large number of elementary teachers readily admit to having fears and anxieties towards science and towards the teaching of science in their classrooms. Much of this fear stems from their own science experience in high school and college courses, where science historically is treated as a series of facts and laws which are to be memorized and minimally questioned. Frustrations, inadequacies, and anxieties which these teachers experienced in their own education stay with them during their teaching years, and become transmitted to their own students. Teachers cannot effectively present and develop the scientific process and experience for their students unless they feel comfortable in those processes and experiences themselves.

The teachers that are most in need of such experience are those which normally will not voluntarily sign up for inservice and related upgrade classes. We must go to them, their schools, and their districts in their locations.

Most teachers have never flunked bioluminescence because most have never seen it, particularly in the Western United States where fireflies are not common. Even those who have experienced fireflies are quite surprised and shocked to learn of the other sources of bioluminescence. As soon as the subject is raised, they are almost always intrigued, mystified, motivated, and otherwise curious and interested.

We decided to try to utilize bioluminescence as an effective way of imparting the scientific experience and method to elementary teachers in Utah. Some 200 teachers have experienced this course and are utilizing these concepts and activities in their classrooms.

INSERVICE COURSES

Almost all schools and school districts in the nation regularly have in service courses for their teachers. These generally provide continuing education for teachers to improve their teaching skills, their content knowledge, and improve their job rating and salary levels. In our case, the in service consists of 10 contact hours which are eligible for 1 quarter hour of university credit. The contact hours are also used in the district for advancement, promotion, lane change, and other compensation-related evaluations.

We usually begin the discussion with a brief overview of the major national education surveys, reports, and movements of the past decade, with particular reference to the Project 2061 report of the American Association for the Advancement of Science (1), and the report titled "Elementary Science Education in the Nineties (2)." We also refer heavily on the works of Howard Gardner of Harvard, particularly his Frames of Mind and his more recent book The Unschooled Mind: How Kids Learn and how Schools Should Teach (3).

We try to loosen the teachers up. We talk with them about the arrogance and the myopia of modern disciplinary intensive science. We share with them our conviction that the University is one of the worst places on Earth to learn science because we rarely teach it there. We teach specific disciplines. We rarely teach or emphasize the connections between those disciplines and the concepts and themes which are basic to all of those disciplines. We use the conclusions of these reports, i.e. that there are general science concepts and themes which pervade all of the fields (1,2). We work through those concepts and themes in a general and even superficial way, relying heavily on metaphor and analogy with everyday experience. For example, housework is a battle against entropy. We use concepts of balance and opposing forces and tendencies, sort of the Ying-Yang hypothesis, order and disorder, light and black, hot and cold. Our goal is to make them feel at ease, not to intimidate them in any way. We encourage them to ask questions and from those questions begin to develop hypotheses based on common sense and every day relevance and experience. We utilize as much as possible what we have around us, what they are wearing, what they are drinking or eating, what they are carrying, particular props or materials in the room. We also spend time getting acquainted, learning something about their backgrounds and their fears and anxieties. The goal is to get them to open up.

The classroom we select has no outside windows. The room can be made completely black. We talk with them about dark adaptation. We go from light to dark in a gradual way over 10-20 minutes, all the time talking about concepts and themes, encouraging and inducing them to ask questions, to open up. We begin the Science in the Dark experience with "sea fireflies" (Vargula hilgendorfi), a small crustacean which is almost legendary now in the bioluminescence community because of its ability to produce bright bioluminescence when the dry crustacean is ground to a powder and wetted.

We look at the crustacean, we conclude that it is dead and dry, and then we get someone to grind it up, always using a very white, large mortar and pestle. Another student (now it is nearly pitch black) sprays water into the powder. The brilliant glow in their, black, dark-adapted environment is dramatic and impressive. The oohs and aahs and let me touch it and see it go on for at least 5-10 minutes. The mortar is passed around. Everyone takes a turn. We grind a little more, we paint it on ourselves. By now the teachers are no longer squeamish and even the fishy smell of the Vargula does not bother them. Questions are being generated, hypotheses fly.

Why does it light up? Why does it need water? Does it stop when it is dry? What if I add more water? Why does it smell? Why is it blue? We simply encourage questions. We encourage the formulation of hypothesis, how would you test that? Let's try that again. We get into the scientific process. What is an experiment? What is an observation? What is a control? What is reliable, reproducible? We take the powder as far as is convenient. Now they grind up wintergreen LifeSavers, experiencing tribo-luminescence. More questions, more hypotheses. We usually pull out a chemiluminescent Lite Stik, and, in the dark, get one of the students to activate it. It is cold light, and that it is very different from normal light production. Many of them have experienced it. They start talking about two components. What is the noise inside? Why is it blue, green, or red, depending on the particular Lite Stik used. More hypotheses: why isn't the light hot, or is it? Warm hands, cold hands. How long does it last? Can you turn it off? Why does freezing stop it? By now we are off on a pretty wide-ranging discussion on light. How is light generated? What is light? How do light bulbs work? What is fluorescence? What is phosphorescence? That takes us into the structure of the atom and electrons in atoms. Electron atomic and molecular orbitals, excited states, photon generation and color, waves and wavelengths. We spend a good hour or more on the principles of optics and light generation. We pull out flashlights, we pull out pocket lasers, we simply take that discussion as far as is convenient and effective.

Sometimes these courses are given in three, 3-hour segments, sometimes in one three hour segment followed by a second 7-hour segment. They are given some general literature on bioluminescence, a set of questions, several additional sheets to read...a little hype about their kits, their bugs, their dinoflagellates. There is a lot of anticipation about constructing their own kit, their own ecosystem.

DINOS IN ACTION

We go into a dark environment in which we have an array of flasks containing bioluminescent dinoflagellates, pyrocystis lunula (6,7). The organism has been put on an alternate cycle so that it is approximately in the middle of its night cycle during this part of the course. They all hover around the collection of flasks, placed together in one dark, black box. We simply give them an enormous bang. The whole box lights up in a brilliant blue flash. Oohs and aahs, but much more intensive than in the first experience. They pull out the flasks, they begin to shake them. Again more questions. Why only when I shake them? Why don't they stay on? What is making this? Is this chemiluminescence? We caution them that this time the organism is alive. We give them another set of flasks. We tell them it is exactly the same thing. They shake them. They don't glow. There goes the hypothesis. We encourage them to formulate hypotheses and to discard hypotheses. Hypotheses are to be tested, not proven. We say they are plants, they need light. We start talking about light cycles, circadian rhythms, natural rhythms, jet lag. We put our dinos away and let them rest. Sometimes we utilize plastic bags containing dinos completely sealed in the bag, and we throw the bags around the classroom. They play catch with them. They notice that they light up in individual discreet points. They light up at the air/water interface. Some of them seem to be attached to the wall of the bag. They light up around bubbles. A lot of observation and primitive hypothesis formulation is going on. How do they live? How do we take care of them? What do they look like anyway?

We go to microscopes. We simply take a drop of the dense culture between an inverted petri dish, and look at them. They are large and moon shaped. What is all of that stuff inside? Here is one that has two nuclei, here is one that is more round.

What is going on? They all look different. The 7 day life cycle of pyrocystis lunula makes them an almost ideal protozoan with which to study all aspects of cell division and fission and of intracellular organelle structure and migration. Some of those who are involved in teaching biology and protozoa are absolutely fascinated. They think it is an ideal organism. Why haven't they heard about it before? How do they grow? What do they need? They are plants, photosynthesis, CO_2, respiration, oxygen production, little ecosystem. How do they live in the bags? How do they live in the flasks? How do they get air? How much light do they need?

We talk about sub-cultures, we talk about feeding them, we talk about seawater and supplements. We show a video we have put together on bioluminescence which simply demonstrates many of the things they have already experienced. We show segments of a BBC video, "Creatures of the Night Sea." We show segments of the David Attenborough, "Trials of Life" video "Communicating with Strangers," particularly the segment showing the flashing fireflies and deep sea creatures. What is the function of bioluminescence? Where is it found? Is the chemistry all the same? Is it different?

There is a real advantage to doing all this in the dark. They seem to be less intimidated; they can't see each other; nobody looks stupid, nobody knows or remembers who anybody is, so the questions and ideas just pour out of the darkness. We organize and formulate some of the questions into hypotheses, and encourage them to make their own experiments. They put together a kit that 10-15 students can work with.

CONCEPTS AND THEMES

We come back to integrated science concepts and themes. We are learning physics, optics, chemistry, biology, and environmental sciences. We talk about phytoplankton as one of the primary producers of the sea. We talk about the possible effect of the ozone hole (5). We talk about their role in minimizing the greenhouse effect. We talk about little ecosystems and big ecosystems.

A discussion of biospheres and ecospheres usually ensues, ranging from the tiny ecosystem represented by the pyrocystis lunula plastic bag or flask to back yards, to local regions, and to Biosphere 2 in Arizona. We talk about the problems and uncertainties with Biosphere 2, treating it as an experiment in the study of complex systems. We then move on to Biosphere 1, i.e. the Earth, and a variety of current issues such as the ozone hole, greenhouse effect, and global warming. We talk about phytoplankton as primary producers and as responsible for a significant fraction of the CO_2 consumption on the planet. We also discuss very recent studies dealing with the effect of increased UV on phytoplankton productivity and health in certain parts of the world (5).

NEEDLE A SCIENTIST

The inservice concludes with a plea to probe, particularly for those elementary teachers who have high science anxieties and concerns about their science background. We say "needle a scientist, needle a physicist, needle a chemist, needle a biologist"; ask them about the basic premises and foundations of their area of science. Throughout the inservice, we question. We look at basic premises, constantly asking why, and concluding that science is based on a small number of un-understood phenomena and building blocks. We encourage them to feel comfortable

about interacting with scientists, asking advice, ignoring the arrogance of many professional scientists, needling them, using them, appreciating them, and learning from them.

We always conclude with a discussion of current popular science writing with which they can probe deeper and enjoyably. We encourage them to use popular science writing in their classes as appropriate, particularly for those teachers involved in high school courses. We refer extensively to the books by Stephen J. Gould, John McPhee, Loren Eisely, Rachel Carson, E.O. Wilson, and many others.

ACKNOWLEDGEMENTS

Most of our work for the development of bioluminescence for science education has been funded by Protein Solutions, Inc., Salt Lake City, UT. We have recently obtained a grant to develop and conduct a series of Integrated Science Concepts and Themes inservices based on bioluminescence from the Department of Education Eisenhower Grant Funds administered by the Utah State Board of Regents.

REFERENCES:

1. FJ Rutherford, A Ahlgren, Science for All Americans (The Project 2061 Report), Oxford University Press, 1990.

2. Loucks-Horsley, et al., eds., Elementary School Science for the 90's, Andover, MA: The Network, 1991.

3. H Gardner, The Unschooled Mind: How Children Think and How Schools Should Teach, Basic Books, 1991.

4. M Gail Jones, "Bioluminescence," The Science Teacher 1993; Jan: 19.

5. RC Smith, et al., "Ozone Depletion:...Phytoplankton Biology...," Science 1992; 255: 952.

6. FJR Taylor, ed., The Biology of Dinoflagellates, Blackwell Sci., 1987.

7. DL Spector, ed., Dinoflagellates, Academic Press, 1984.

Ab/Andr. 23

Polyethylene Oxide and Protein Resistance: Principles, Problems, and Possibilities

J.D. Andrade, V. Hlady, and S-I Jeon*
Department of Bioengineering, 2480 MEB
University of Utah
Salt Lake City, UT 84112, USA
*Department of Chemistry
Kangreung University
Kangreung, Korea

Introduction:

Surfaces resistant to the adsorption of proteins and to the adhesion of cells are needed, particularly in the health care products and biotechnology industries. Although there has been a great deal of activity on the preparation, characterization, and even application of polyethylene oxide (PEO) surfaces over the last 15 years, there is considerable controversy in the field and most of the key scientific questions are still open. A volume edited by J. M. Harris, has just become available and provides a concise, up to date, authoritative presentation of the field (1).

Background:

Polyethylene oxide (PEO) and polyethylene glycol (PEG) have been 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 1,000 to 4,000 range, is commonly used as a pre-polymer in the synthesis of various polymers, including polyurethanes, epoxies, silicones, and other systems. Low molecular weight PEG can also be readily coupled to hydrophobic chains to make a wide variety of nonionic surfactants which are widely used in the chemical industry, in biochemistry, and in the biotechnology industry (1).

Polymerized ethylene oxide is somewhat of an anomalous molecule. It is both hydrophilic and hydrophobic, as it is soluble in aqueous and in non-polar solvents. In solution it tends to be highly dynamic, and yet it can readily pack and form crystalline solids. In spite of 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, and as part of other molecules, is generally non-toxic and 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 (see Figure 1, next column, and its long caption).

Although e and f in Figure 1 have the advantage that they lead to a very well-covered surface and avoid the potential problems of a bare substrate, it may well turn out that there is little difference between a, e, f, and possibly even d, due to the fact that the excluded volumes of the chains themselves prevent a very high local concentration of PEO. If this excluded volume is decreased by solution "tricks" (2), then it is likely that the final surface, after equilibration in water, will be less mobile and less dynamic than required for optimum protein resistance.

Regardless of whether the underlying substrate is highly hydrophobic, highly ionic, or highly hydrogen bonding, the protein has regions on its surface which can indeed interact with the substrate (3, 4). The protein itself has loops, tails, helices, and sheets, which can make their way statistically through the PEO layer, and interact with the substrate below. One can even envision a variety of bridging, pinning, and related processes to further complicate the problem (5). Another concern with Figure 1 is that we have assumed a particular surface structure that is homogeneous, i.e. not patchy, and there is of course little evidence to indicate that this is indeed the case. Thus the problem is even more complex than sketched in Figure 1.

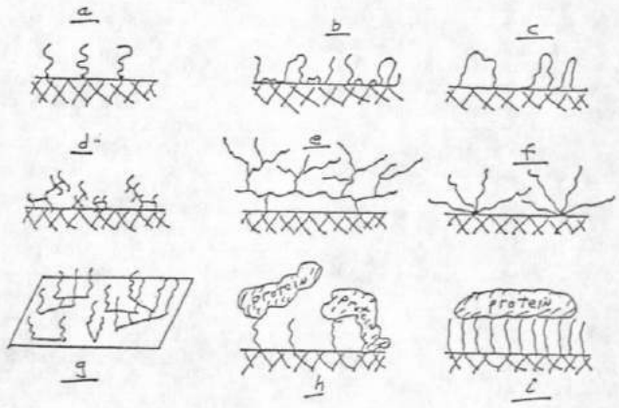


Figure 1: Some of the many structures and configurations which have been suggested for PEO or PEO-derived polymers attached to surfaces.

a) represents low molecular weight PEO (1,000 to 4,000 Daltons) tethered at one end to a particular surface. There are a wide variety of surface modification technologies and PEO derivatives available for such surface modification. It is highly unlikely that the chains extend into solution as indicated. In most studies it is difficult to get a very high density of chains on the surface.

b) is the common illustration for high molecular weight PEO adsorbed onto particles or other surfaces. Here the very high molecular weight, and highly cooperative nature of polymer segmental adsorption, leads to loops, tails, and trains which have been extensively characterized and modelled. The loops and tails provide a means of steric repulsion between two particles containing adsorbed PEO, although it is clear that the dynamics of adsorption can also lead to bridging and thus to colloidal aggregation rather than stabilization. This figure also illustrates the adsorption of PEO block copolymer surfactants, where an adsorbable block pins the molecule to the surface and the PEO block (loops or chains) extends into solution (6). Another variation is a graft copolymer, with PEG chains on a hydrophobic backbone, for example, resulting in adsorption at a hydrophobic surface with PEG chains extending into solution (6).

c) represents a PEO chain bound by both ends to the surface, that is a loop. This may be the structure in many types of block co-polymers containing PEO block segments. It may also be part of the situation in many PEO surface modification reactions, where the PEO reagent is homobifunctional rather than the heterobifunctional case required for the ideal situation in a.

d) is intended to represent the situation where ethylene oxide is attached to an activated surface and a PEO-like network is grown from the surface out. This could represent the plasma polymerization of ethylene oxide films (7). Such a film would be expected to be highly cross linked and much less dynamic than the others indicated.

e) is an example of so called surface amplification, where PEO is tethered to multi-functional entities, such as carbohydrates or polysaccharides which are in turn tethered to the surface (8). Although in principle this 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 this case to the same extent as in a.

f) represents another version of surface amplification--the star polymer geometry. One could think of this as sort of a hybrid between b and e 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 (9). This is also reminiscent of the Tetronic family of polymeric surfactants, where PEG chains extend from four PPO chains attached to a tetrafunctional nucleus (10).

g) represents the case of a block or graft copolymer designed for optimum adsorption (6), which is then surface cross-linked between the chains or between the polymer blocks and the surface by either specific cross-linking reactions or via plasma reactions (11).

Proc. PMSE (Polymeric Materials Science & Engineering), ACS, 69 (1993) 60-61

h) PEG chains are often used as a means to provide a tether between a protein, or other biomolecule, and the surface (6). This approach is being widely applied now 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 which provides a means of transducing a binding event into a signal (12). The covalently coupled protein, however, sitting on the end of a dynamic and mobile chain, will have 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 other means.

i) if we could prepare a maximally dense PEO surface, we might have a packed "crystal" of PEO which will then adsorb proteins, as shown. Such a surface would, of course, not be mobile or dynamic and would not sterically or entropically exclude or resist protein adsorption.

(end of caption)

It is clear that the surface must be fully covered by PEO to minimize protein interaction with the underlying surface. However, if the surface is "over" covered, as in Figure1i above, then the surface becomes adsorptive.

It is perhaps 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 (13).

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, to minimize electrostatic interactions, and highly hydrophilic, to minimize hydrophobic interactions (14). 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 (4).

What are the disadvantages to PEO? There is some question as to its long-term stability on a surface, that it may be susceptible to local oxidation processes, and there is some concern that it may weakly complex with proteins, particularly charged proteins, just as it does with certain types of charged polymers (15). It 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" (16). Nevertheless, of all the polymers we know, it appears to have the highest potential for the development of truly protein-resistant surfaces (1, 4, 17).

A very major factor is the way in which the hydrophilic polymer chains interact with water. PEO solutions, although they do not behave as ideal solutes and certainly do provide some perturbation of the structure of water, apparently are the least perturbing of all of the common neutral hydrophilic polymers. Although the non-bonding oxygen orbitals in PEO do provide hydrogen-bonding capacity, and indeed are largely responsible for the solubility of the molecule, this hydrogen bonding is easily satisfied by water, without significant perturbation in the structure of water (17, 18). 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, thus minimizes hydrophobic interactions. These two facts suggest that PEO indeed has minimal interactions in aqueous solutions with other solutes. In addition, the PEO chain is highly mobile and dynamic, thereby creating an entropic "insurance" which can more than compensate for any weak attractions which may be present (13). The end result is a weak and sometimes even quite strong repulsive interaction between proteins and many types of PEO surfaces, resulting in very low protein adsorption. This is what we define as protein resistance.

Direct measurements of the steric repulsion between PEO surfaces (19, 20) and between a surface of PEO and one of protein (21) are now available, thanks to the surfaces forces apparatus (20, 21). Direct measurement of steric exclusion and the imaging of surfaces via steric exclusion means has now been accomplished in our group by atomic force microscopy (19). Prime and Whitesides (22) recently presented a study of the adsorption of four different proteins on oligo (ethylene oxide) self-assembled monolayers of varying oligo (ethylene oxide) surface concentration. The protein resistance roughly scaled with increasing surface coverage and with increasing oligo (ethylene oxide) molecular weight.

This discussion is not without controversy and critique. There are many studies in the literature which argue that PEO surfaces are not particularly protein-resistant or biocompatible. There are other studies which argue that if a PEO surface is resistant to one protein, it may not be very resistant to another protein (4, 13). 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 (6).

Summary:

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, coupled with the contradictory requirement that the underlying surface must be entirely covered by the PEO chains. Due to geometric constraints, the above criteria should be optimally met on highly curved surfaces; ideally flat surfaces probably cannot be made as optimally protein-resistant, based on PEO, as can surfaces with low radii of curvature. There is simply more room for end-attached polymer chains near a curved surface than near a flat surface.

Acknowledgements:

Many parts of this work were supported by the Center for Biopolymers at Interfaces at the University of Utah and by NIH Grant HL-... S-I Jeon thanks KOSEF for a fellowship at the University of Utah.

References:

1. J. Milton Harris, ed., Poly(ethylene Glycol) Chemistry, Plenum, 1992
2. K.P. Antonsen and A.S. Hoffman, in Ref. 1; also K. Holmberg, et al., in Ref. 1.
3. C. Branden and J. Tooze, Intro to Protein Structure, Garland, 1991
4. J.D. Andrade, et al., Clinical Materials, 11 (1992).
5. H.E. Johnson and S. Granick, Science, 255 (1992) 966
6. J.H. Lee, et al., Biomaterials, 11 (1990) 455
7. G.P. Lopez, et al., J. Biomed. Materials Res., 26 (1992) 415
8. S.W. Kim, et al., personal communication
9. E.W. Merrill, Chapter in Ref. 1
10. J.H. Lee, et al., J. Biomed Materials Res., 23 (1989) 351
11. M-S Sheu, et al., Trans Soc for Biomaterials, 1992, p. 228
12. J.D. Andrade, et al., Chapter in R.B. Buck, et al., eds., Biosensor Technology, Dekker, 1990, p. 219
13. S.I. Jeon and J.D. Andrade, J Colloid Interface Sci., 142 (1991) 159
14. J.D. Andrade and V. Hlady, Adv Polymer Sci, 79 (1986) 1
15. E. Tsuchida and K. Abe, Adv Polymer Sci 45 (1982) 1
16. D. Balasubramanian and B. Chandani, J Chem Educ 60 (1983) 77
17. S. Nagaoka and Y. Mori, J Biomaterials Applic 2 (1987) 219 and citations therein.
18. G-C Golander, et al., Chapter in Ref. 1
19. A.S. Lea, Ph.D. Thesis, University of Utah, May, 1993.
20. P.F. Luckham and J. Klein, J Chem Soc Farad Trans, 86 (1990) 1363
21. M. Malmsten, et al., J Colloid Interface Sci 151 (1992) 579 and work in progress in the Claesson lab
22. K.L. Prime and G.M. Whitesides, J Amer Chem Soc., (1993) in press

CHAPTER 24

The Future of Health: The Roles of Medical and Biological Engineers

JOSEPH ANDRADE
University of Utah

"Members of communities have responsibilities. . . . They cannot leave these duties and responsibilities to others."

"Our community in general has simply chosen not to be involved. It is not only we who suffer the consequences of that choice, it also is our society that suffers."

"Engineers . . . know how to deal with systems, very complex systems, and they can help develop models and means to define and to address such complex problems."

"In some respects we must all do less, so we have the time to do what we do more compassionately, relevantly, and effectively. We need to teach less, but to teach it better; we need to educate in a more integrated and systems-like manner."

"We all hope and expect that a national health care plan will include legal liability reform and improvement of the federal regulatory structure."

"It is time engineers—and bioengineers in particular—began to lead."

In this book, we have been informed, educated, and enlightened by an array of provocative, informative, and well-written chapters. We have also been urged and encouraged to get more intimately, responsibly, and effectively involved—not only in the debate about health care costs and health plans but in the actual redirection of our own individual efforts and activities in reducing the costs, improving the quality, and significantly increasing access to health care in this country and around the world.

Let us consider six points:

- social responsibility,
- national values and needs,
- economics,
- quality, productivity, and efficiency,
- benefits and risks, and
- education and communication.

Social Responsibility

We are each members of a hierarchy of communities: our families, the institutions in which we work and contribute, our city, county, state, nation, and our lovely green and blue biosphere—the planet Earth. Except possibly at the biosphere level, we are members of each of these communities by choice. We could choose to be in a different state, in a different community, and even in a different family. Members of communities have responsibilities: they must be involved in the process of determining the community's values, its needs, and its governance. They should not leave these duties and responsibilities to others.

Unfortunately, the technical community, and engineering in particular, tends to relegate some of its social responsibilities to other members of the community. Often in one's upper elementary and junior high school years there begins to be a number of bifurcations: you are typified as a people person or as an analytical person; you are a life science person or you are a physical science person. Those splits may continue through high school, into college, and finally into our adult professional careers. The caricature of engineers and scientists is that they are not people people—they shun

Polymers Have "Intelligent" Surfaces: Polymer Surface Dynamics

J. D. ANDRADE

Departments of Materials Science and Bioengineering, Center for Biopolymers at Interfaces, 2480 MEB, University of Utah, Salt Lake City, UT 84112

ABSTRACT: The "intelligence" of synthetic macromolecules is currently a topic of great interest, popularity, and activity. The understanding of macromolecule mechanics and dynamics has reached the stage where unique and novel applications are being developed.

Here I present basic principles of polymer surface science, bulk polymer dynamics, and some selected areas in polymer surface dynamics.

I include a brief treatment of the complexity of highly dynamic, multi-domain, macromolecular surfaces and their interactions with highly dynamic, multi-domain "block copolymer" proteins which are important in biocompatibility and biotechnology.

BACKGROUND

POLYMER physicists and material scientists are well aware of the dynamics, the relaxations, and the multitude of conformational states which exist in most polymeric materials. Indeed, it is these very properties that are largely responsible for the unique set of physical and mechanical characteristics which have made polymer materials and the products derived from them so ubiquitous in every day life [1,2].

Biopolymers, and particularly proteins and polynucleic acids, have a history, long track record, and even expectation of "intelligence". Multiple conformational states, conformational flexibility, cooperativity, specific binding interactions, temperature and pH sensitivities, and a myriad of other properties are all common or expected with biopolymers [3], yet it is only recently that the same set of expectations has been extended to and attributed to synthetic macromolecules. This is largely because the two worlds have not overlapped in terms of the practitioners.

Fortunately that situation has changed dramatically in the last decade. The proliferation of programs and research groups in biomedical materials, drug delivery systems, medical devices, and biotechnology has produced hybrid scientists and engineers; their students and co-workers now expect synthetic macromolecules capable of far more than is normally reflected in polymer materials textbooks.

Our focus is on the "intelligence" of *surfaces and interfaces*, particularly those involving polymers. Surfaces and interfaces operate with the same set of thermodynamic and kinetic rules as do bulk phases. Indeed, the equations of surface thermodynamics are exactly the same as those of bulk thermodynamics with the exception that the interfacial work and interfacial free energy terms are included, rather than neglected as in the case of infinite bulk phase systems [4]. As soon as this is understood, then of course the "intelligence" of polymer surfaces becomes much less of a mystery. Surfaces and interfaces respond to their environments just as do bulk polymers. Surface and interface properties are time and temperature sensitive, as are bulk polymers [5].

The dynamics of polymer surfaces are well known in the adhesives and textile fiber communities. Indeed, there is a rich patent literature which goes back over 40 years [6]. However, because of the normal classical assumptions of surface chemistry and physics [4], i.e., that surfaces are homogeneous, flat, rigid, and immobile, the dynamic aspects of most polymer systems have been largely overlooked, ignored, and even unknown to the academic polymer science and surface science communities, until perhaps 20–30 years ago.

About 20 years ago, surface dynamics was rediscovered by the medical devices community, largely through studies on contact lenses [7]. Since then, there has been growing interest and application of polymer surface dynamics in medical and biomaterials problems [8].

INTERFACES

The major driving force for interfacial processes is their interfacial free energy (Figure 1) [9,47–49]. At the solid polymer/air or /vacuum interface this is often called the surface free energy, or even the solid surface tension. The interface orders or structures so as to *minimize* the surface free energy, thereby minimizing the overall energy of the system. The situation is quite different at a solid polymer/water interface. Here the very high surface tension of water itself, coupled with the characteristically low surface free energy of solid polymers, leads to the development

Associate Editor: G. Wallace.

1045-389X/94/05 0612-07 $6.00/0

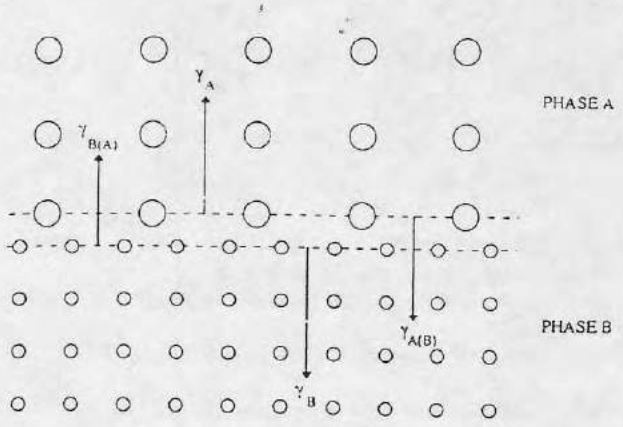


Figure 1. Hypothetical interface between two phases, A and B, and the surface tension and work of adhesion components (from References [9] and [52]).

of a high interfacial free energy or interfacial tension. This can be estimated; a now somewhat old and classical data set is shown in Figure 2 [9].

In Figure 1 we see a typical hypothetical interface between two phases, A and B. By γ_A and γ_B we refer to the surface free energy or surface tension of each of the individual phases. By $\gamma_{B(A)}$ we mean the interaction between Phase B and Phase A at the interface, and $\gamma_{A(B)}$, the interaction between Phase A and Phase B. These two parameters constitute the work of adhesion at the interface, W_{AB}. The interfacial free energy γ_{AB} is given by $\gamma_{AB} = \gamma_A + \gamma_B - W_{AB}$. It is this interfacial free energy that is the driving force for subsequent interface modification and interface restructuring processes.

Figure 2 shows what happens under ideal conditions at a solid/water interface. The vertical axis represents the interfacial free energy between the solid and water. The horizontal axis represents the surface free energy for the solid γ_A interfacing with water, γ_B. This is a somewhat academic and hypothetical plot so we allow the horizontal axis to go from zero to relatively high values. Let us take a typical

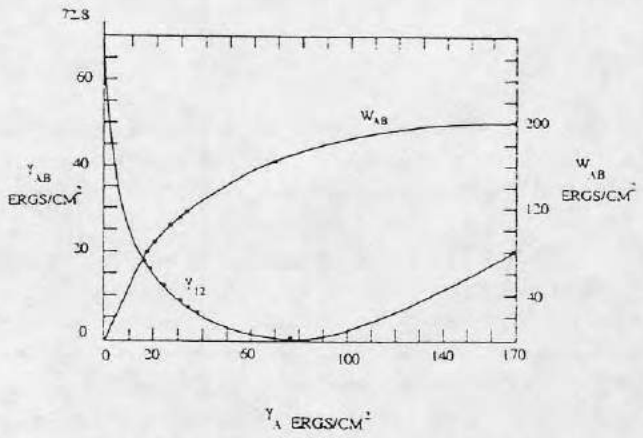


Figure 2. The interfacial free energy, γ_{AB}, and work of adhesion W_{AB}, for surfaces of different surface free energy, γ_A, in water (from Reference [9]).

hydrophobic polymer, such as polyethylene or polypropylene, which would have a surface free energy in the range of 25–30. The very high surface tension of water, 72.8 dynes/cm or ergs/cm^2, interacts largely by its own dispersion forces with the hydrophobic polymer, providing a work of adhesion component largely dispersive or apolar in nature. There is little or no polar interaction across the interface as there is no polarity; the dipoles in water really have nothing to interact with on the polyethylene surface, assuming a completely unoxidized hydrophobic polymer. Thus, the polar and hydrogen bonding interactions in water which are manifested at the interface are entirely uncompensated, and are therefore largely responsible for the residual interfacial free energy, γ_{AB}.

More highly polar polymers, for example an epoxy or nylon, would be in the range of perhaps 40–50 ergs/cm^2, have a somewhat lower interfacial free energy, because now there are mutual polar or dipolar interactions across the interface. The work of adhesion is also shown on the plot.

If one considers solids whose surface energies are much higher than that of water, then the interfacial free energy increases again, but this time most of that energy is coming from the uncompensated forces on the solid side of the interface. Gel-like solid materials of very high water content have a surface free energy or surface tension almost equal to that of water itself [12]. Such a material is shown at the position of about 72 ergs/cm^2 on the horizontal axis and would of course have a very low interfacial free energy against water. Because of such very low interfacial free energies, aqueous gel materials tend to have weak interfacial interactions, such as adsorption of proteins from aqueous solutions.

We have discussed these principles in a number of reviews and books over the years [4,5,8–12].

Holly and Refojo, studying the wettability of hydrogel soft contact lenses [7], showed that polyhydroxyethylmethacrylate (PHEMA)-based lenses appeared to behave as hydrophobic surfaces in air, and as much more hydrophilic surfaces under water. A general understanding from their studies, and many others on similar systems over the years, is that polyHEMA is a good example of a hydrophobic hydrogel [13]. HEMA is an amphiphilic monomer with weak surfactant properties [14], and polyHEMA is an amphiphilic polymer. When highly plasticized by water under equilibrium swelling conditions, the hydrogel interface is highly mobile and can rearrange or restructure to minimize its interfacial free energy. In air or vacuum this is accomplished by orienting the more apolar segments of the mer units towards the air or vacuum side of the interface, thereby minimizing the surface free energy and maximizing the surface hydrophobicity. Under water, however, it is the polar, more hydrophilic side chains that tend to orient towards the water phase, thereby functioning as weak surfactants and minimizing the interfacial tension. These effects are easily seen in dynamic contact angle experiments [15]. When a water droplet is advanced along a polyHEMA

surface, the advancing angle is quite hydrophobic whereas if that same water droplet is receded, the receding angle is low, suggesting a highly hydrophilic surface.

Such contact angle hysteresis experiments are well known and are one of the standard methods of characterizing the surfaces [4,30]. The polymer simply responds to its environment.

A now classical study by Pennings and Bosman [19] demonstrated the dynamic restructuring of polymers cast against a high energy gold substrate, and then allowed to restructure or reorient in air under different time and temperature conditions. From such a study they determined that the activation energy for surface restructuring was approximately equal to that of the beta relaxation for that particular polymer.

Mechanical and dielectric relaxation studies demonstrate dramatically the time/temperature dependency of polymer dynamics [1,2,20]. It may be reasonable to suggest that high molecular weight synthetic polymer systems are rarely, if ever, at equilibrium. What you see depends on when and how long you look. This is certainly true for polymer properties and is perhaps even more true for surface properties.

These effects are now well understood, but they have not yet made it fully into surface chemistry and polymer science textbooks, although they are becoming increasingly recognized in the chromatography field [51]. One finds the phenomena are being regularly rediscovered in the literature [21]. These effects have been known for a very long time in certain aspects of the polymer industry and in particular the textile community [6]. Reference [6] refers to a 1971 patent assigned to the 3M Corporation describing a set of copolymers which respond reversibly to changes in their environment, i.e., "intelligent" surfaces.

If the polymer surface contains ionizable groups which differ significantly in their polarity in going from the non-ionized to the ionized state, then a simple change in pH can dramatically change the polarity and wettability of the surface. This has perhaps been most elegantly demonstrated in a set of recent studies by Whitesides and his group utilizing essentially contact angle titrations [22]. Polymer surfaces containing azobenzene or other components which can undergo a trans to cis photoisomerization, with a significant increase in dipole moment and therefore polarity, also demonstrate significant wetting changes upon photoisomerization [23].

BULK POLYMER DYNAMICS

Polymers are dynamic materials [1,2]. Polymer chains and solid polymers exhibit a variety of motions. The ends, loops, and segments of the chains can experience a variety of relaxation processes which are time and temperature dependent. The most well known and quoted of these dynamic processes is the microBrownian motion characteristic of polymers held above their glass transition temperature, T_g, the temperature at which there exists motion of main chain segments involving perhaps 50–100 carbon/carbon bonds [16]. The glass transition temperature of polymethyl methacrylate (PMMA), a rigid polymer, is about 130°C, whereas for polydimethyl siloxane (PDMSO), a highly dynamic mobile polymer, it is $-120°$ to $-130°$C. The microBrownian motion of PDMSO is present at room temperature, whereas the microBrownian motion of PMMA is not activated until the temperature exceeds about 130°C.

However, even rigid polymer surfaces can be dynamic with respect to interfacial properties and processes. The glass transition temperature argument alone would suggest that PMMA would not restructure under water because the polymer molecules do not have sufficient mobility or dynamics to respond to the high interfacial tension at room temperature. But this is not so. The T_g refers only to microBrownian motion-activation of major segments or parts of the polymer chain. Many polymer systems have side chains; side chain rotation requires much lower activation energies. In the case of PMMA, the side chain rotation, i.e., the beta relaxation, is activated in the range of 20°–30°C; this is sufficient to provide at least some statistical amphilicity at polymer/water interfaces. Indeed, PMMA and other polymethacrylates can be spread as monolayers and characterized by pressure/area isotherms using Langmuir trough techniques [17]. As the number of carbon atoms in the side chain increases, they provide plasticization of the system, thereby decreasing its T_g and providing for a more dynamic and mobile polymer [18].

GRADIENT AND HETEROGENOUS SURFACES

A quarter of a century ago J. J. Bikerman in a wonderful monograph considered the problem of water droplets on heterogeneous surfaces [24].

Surfaces with a continuous range of surface character along one or more linear dimensions, gradient surfaces, have become popular in recent years for the study of the effect of a solid surface parameter on interfacial processes. They have perhaps been most widely employed in studies related to biomaterials and biocompatibility, particularly protein adsorption. Pioneered by Hans Elwing and co-workers, they have been utilized by Elwing and a number of other groups and have provided important, fundamental information on the role of surface properties on protein adsorption [25,26].

Very recently alkyl thiol monolayers have been used as model, highly ordered surfaces for a variety of applications [27]. These surfaces are so ordered and so ideal that they exhibit very low contact angle hysteresis. It was shown by Zisman many years ago [28] that only highly ordered, homogeneous, and packed rigid surfaces exhibit minimal contact angle hysteresis [4,28,29]. Virtually all other surfaces demonstrate significant hysteresis.

Whitesides and co-workers have prepared gradient surfaces using thioalkyl reagents. Although such surfaces vary in wettability along the linear dimension, at any particular

point on the surface the contact angle hysteresis is very low. Such modern, ideal surfaces allowed Whitesides to show what Bikerman had speculated upon decades earlier [24], that is, a water drop could be made to move along such a surface "searching" for a position which minimizes the system's total energy. This tendency can be made great enough that the water drop can even be induced to move up hill [30], an elegant demonstration of the importance and strength of interfacial energetics, and a reasonably high level of interfacial "intelligence".

Polymer blends, diblock polymers, and triblock polymers generally involve two or more distinct phases of different cohesive energy density and different surface character. Such materials are also interfacially dynamic on an even greater spatial dimension than the individual polymer chain processes discussed earlier. Assuming that at least one of the phases is highly mobile and above its glass transition temperature then, given sufficient equilibration time, the low energy phase tends to dominate in air, whereas the high energy phase tends to dominate under water. Admittedly this behavior is constrained in diblock and triblock systems by block size and stoichiometry, but nevertheless, the tendency is there. Systems with a polysiloxane continuous phase, for example, are almost totally dominated by the silicone component in air.

PROTEINS AND BIOCOMPATIBILITY

Proteins are complex polyamides with a wide range of amphiphilic and dynamic characteristics. Chan and Dill recently reviewed the motions and time scales for both proteins and synthetic linear polymers [31] and, although the nomenclature and the semantics are different in the two worlds, the processes and the principles are similar. The tendency for protein molecules to adsorb and then conformationally adapt (denature) at polymer/aqueous interfaces has been an area of major study for the past 30 years [32,33], largely driven by the need to enhance and improve the blood and/or tear compatibility of various medical devices and diagnostic products.

A number of "magic" biomaterials have been studied by our group from the point of view of interface dynamics and restructuring. Here we discuss some recent work on polyether urethanes, because they represent multi-phase polymer systems with the "intelligence" required to restructure their interfaces, depending on the environment.

Tingey [34] and others [35] have extensively characterized the surface properties of biomedical polyether urethanes both in and out of water. Briefly, there is a tendency for the low energy phase to dominate in air and the more high energy phase to dominate in water, although in many biomedical polyether urethanes the surface free energies of the soft segment and hard segment phases are roughly comparable, so this tendency is not particularly dramatic. It is far more dramatic in multiphase polymer systems where the two phases differ greatly in their respective surface free energies. Nevertheless, even in biomedical polyether urethanes, the tendency is there. In addition, and perhaps even more importantly, the phase dimensions in these systems are in the range of 100 angstroms or so, a particularly important scale for plasma and tear protein interaction.

PROTEINS AND INTERFACES

Proteins are highly anisotropic, dynamic, and conformationally unstable polymers and thus exhibit a wide range of interactions at interfaces. The ability of the same protein to interact at different interfaces by hydrophobic, polar, and electrostatic means, and by combinations of such forces, is now well known and has been extensively discussed [10,11,36]. The ability of such proteins to conformationally adapt, i.e., denature, at interfaces is also well known and qualitatively understood [36].

Such understanding, however, is most complete only for relatively small proteins of single domain character whose 3-dimensional structures are known. These have served as model proteins for the study of interfacial behavior and have greatly advanced our understanding of such processes [37]. However, most proteins in blood, tears, and other body fluids are not small, single domain, "model" proteins. Rather, they are of high molecular weight, ranging from perhaps 40,000 to several million, the crystal structures are generally unavailable, and they consist of a wide range and number of domains [38,39]. Most proteins of interest are analogous to block copolymers, in which different regions of the same molecule can have very different chemical and physical properties.

A good example is fibrinogen, one of the more concentrated proteins in blood plasma, whose function is to provide a polymer network and much of the mechanical strength of a blood clot. Fibrinogen has 12 thermodynamically autonomous domains [40,41] with a total molecular weight of about 350,000, involving 6 polyamino acid chains. One might think of this as a star polymer with 6 arms on the star, but the arms associate and self-assemble into 6 times 2 independent domains. These domains have different dynamics, i.e., thermal stabilities, they have different electrostatic characteristics, and they have different apolar characteristics. Thus the domains *each* have their own unique set of surface and interfacial properties, which means that the protein can interact with different interfaces by different domains, providing a wide range of possible orientations and interfacial ordering or self-assembly processes [11,41].

DOMAIN-BASED STATISTICAL SPECIFICITY

Both Feng [42] and Tingey [34] studied the adsorption of some 16 different proteins from dilute blood plasma onto rigid carbon surfaces and soft polyurethane surfaces, respectively. Feng found that adsorption largely correlated

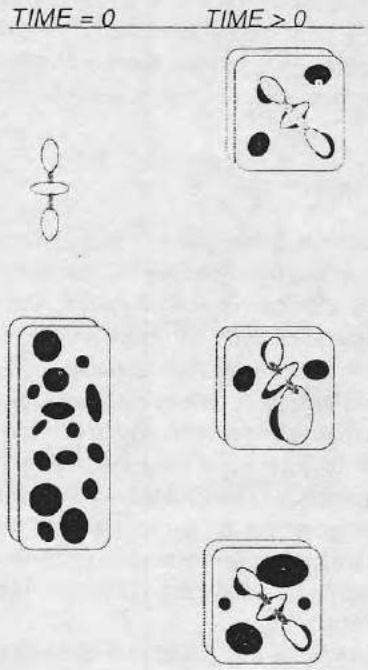


Figure 3. Speculations on domain-based "statistical specificity", assuming a multi-domain protein (top, left) interacting with a multi-domain polymer whose domains are roughly comparable in size to those of the protein (e.g., certain polyether urethanes). Top: domain matching and interaction complementarity; middle: protein denatures or adapts to try to match polymer; and bottom: polymer surface adapts to adjust to a "less-adaptable" ("hard") protein. The variables likely to be important include protein and polymer domain sizes and surface chemistries, protein domain denaturation temperatures, and polymer domain glass transition temperatures (from Reference [11]).

with the concentration of the protein in solution and that all proteins adsorbed irreversibly, with, to a first approximation, adsorption being independent of the particular properties of the individual proteins. The only real correlation observed was with the solubility of the respective proteins, as expected [11,42]. Tingey, on the other hand, using polyurethanes, found several proteins were highly depleted/adsorbed on certain polyurethanes, but not on others. There was a crude correlation with polyurethane domain size.

This had led to what we call the "multi-domain interaction hypothesis": a multi-domain protein interacting with a multi-domain polymer surface, where the protein and polymer domains are roughly of comparable dimensions, leads to a number of possible scenarios. If there is some matching or complementarity between the domains on the solid polymer, and the domains on the protein, then, in time, the adsorbed protein will orient to optimize such domain interaction matching (Figure 3, top). If the dimensional matching is not optimum and the protein is relatively "soft", i.e., easily denaturable [37,43], or at least the critical domains involved are easily denaturable, then the protein may conformationally adapt to the more rigid polymer surface. If, on the other hand, the protein is relatively "hard", i.e., its domains have high thermal denaturation temperatures, and it is interacting with a soft, more conformationally adaptable polymer, then the polymer surface may conformationally adapt to its new protein environment.

This domain matching may be the basis of "specificity" behavior in some systems. This is of course a speculative

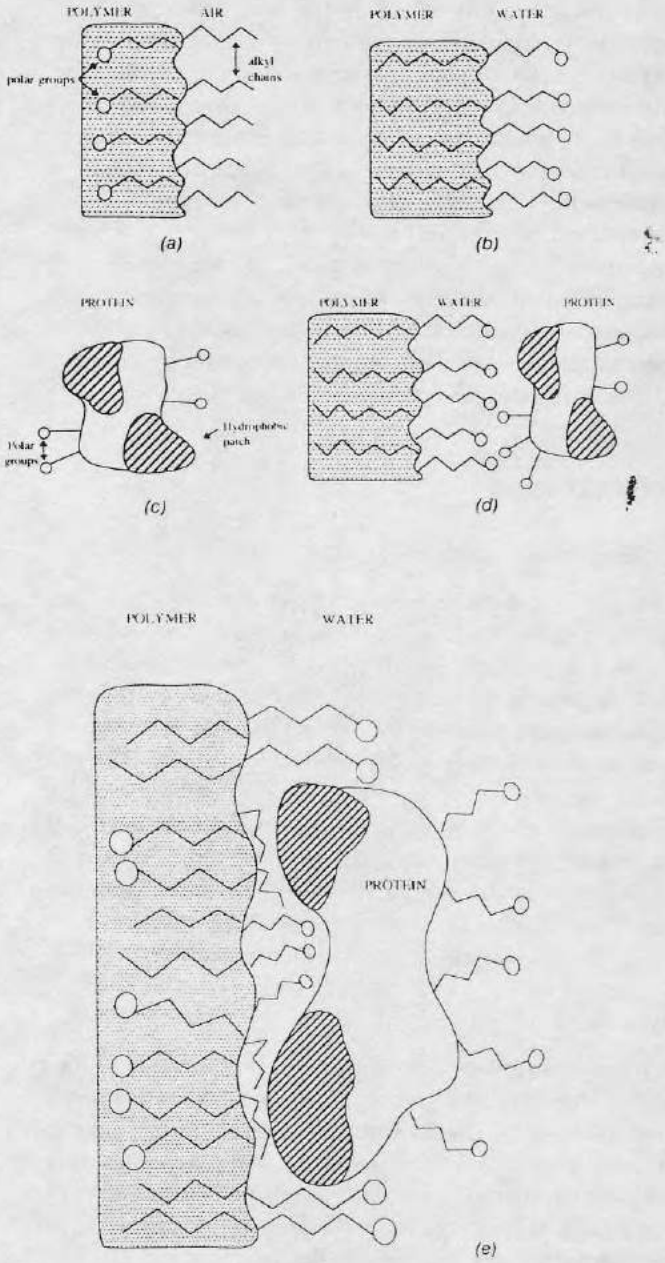


Figure 4. A schematic view of a dynamic polymer surface in different environments: (a) in air; (b) in water; (c) a protein molecule in water exhibiting its equilibrium "surface"; (d) the protein approaching a water-equilibrated surface; and (e) the polymer-adsorbed protein system with the protein shown interacting mainly via hydrophobic interactions. The polymer surface has restructured to hydrophobically interact with the protein and, where appropriate, interact with the aqueous phase by hydrophilic means (from Reference [53]).

hypothesis which will be difficult to test because of the difficulty in characterizing polymer surfaces and adsorbed protein films in aqueous environments with high spatial resolution and with conformational and orientational information.

Figure 4 shows a dynamic copolymer in air, in which the more hydrophobic side chains dominate the surface. We now understand the same polymer in water will rearrange its surface, given that it has sufficient mobility and time to do so, to express more of its hydrophilic and less of its hydrophobic character. But the story is not over if the polymer is in an aqueous solution which contains solutes or colloidal particles which can adsorb and modify the interface. In the final panel in Figure 4 we see that same water equilibrated polymer surface is now sitting *underneath* a protein with a multiplicity of domains and hydrophobic/hydrophilic character. Just as the protein attempts to accommodate to its new microenvironment, the polymer, too, is restructuring in response to *its* new microenvironment. They are both intelligent in this respect. Their mutual intelligences are complicated by the cooperativity and dependence of the various processes on each other.

CONCLUSIONS

Heterogeneity

Polymer surfaces are never homogeneous—there are simply different degrees of heterogeneity. The polymer chains are generally oriented parallel to the free surface [44]. Depending on the process and conditions for the particular polymer material, the orientation may be extensive, such as in a fiber or highly extruded material. Polymer chains are finite—there is a granularity or heterogeneity on the level of the size of individual polymer molecules due to the lack of interpenetration of these molecules. There may be microBrownian motion due to glass transitions and related relaxations. There may be side chain rotations. Proteins, too, are heterogeneous, as indicated earlier.

Dynamics

When dealing with macromolecules, everything is a function of time. As described by Chan and Dill [31], overall molecular conformations may require seconds to minutes, or even longer, to achieve. Routine adsorption equilibria may range from seconds to days. Synthetic polymer adsorption equilibria may require weeks or even months, whereas side chain motions may be in the microsecond to picosecond domain.

Cooperativity

Although not discussed in this paper, the interfacial phenomena considered tend to involve multiple cooperative interactions and many low energy binding sites. The sites may have different dimensions and different interaction energies. Although I only briefly discussed side chain rotations and large domain interactions, there is clearly a whole spectrum of motions and dynamics between these two limits.

Statistical "Specificity"

The heterogeneity of these systems, their dynamics, and the fact that the interactions are highly cooperative in nature lead to "specificity". Specificity is well known, and even expected, in biochemical systems. Enzymes interact with their substrates, and antibodies with their antigens, by a number of small functional groups or side chains, perhaps as many as five or six, arranged stereochemically to provide complementary interactions, yet each of these interactions, individually, are of low energy. The fact that such multi-site interactions are cooperative in nature leads to very high binding energies when complementarity, i.e., stereo specific matching, is optimum, producing exquisite recognition capabilities (specificity).

I have tried to show in this paper that some of the same behavior can be expected at polymer surfaces—there is a *statistical specificity.* This has, of course, been recognized by many other groups, and I have previously called this the "Marcel and Jacqueline Effect" because of the extensive work that Marcel and Jacqueline Josefovich have done in this area [45].

"Intelligence"

When one has heterogeneity, cooperativity, and dynamics, and then puts such a system in a different environment, it of course responds in a time dependent, complex manner to that new environment. That responsiveness, or accommodation, is often called "intelligence". It involves many levels of complexity which we are only beginning to appreciate. Such complexity provides the opportunity to design and develop novel materials and devices.

ACKNOWLEDGEMENTS

I acknowledge discussions and interactions, involving many hikes and innumerable glasses of wine, over the years with many friends, colleagues, and co-workers, but in particular, K. Caldwell, H. Ringsdorf, P. G. deGennes, P. Dryden, H. Elwing, L. Feng, V. Hlady, Y. I. Jeon, J. Kopecek, J. H. Lee, J. N. Lin, K. Tingey, A. P. Wei, and J. Zhang.

REFERENCES

1. Billmeyer, F. W., Jr. 1984. *Textbook of Polymer Science, 3rd Ed.* Wiley.
2. Rodriguez, F. 1982. *Principles of Polymer Systems, 2nd Ed.* McGraw Hill.

3. Creighton, T. 1992. *Proteins, 2nd Ed.* Cambridge: University Press.
4. Andrade, J. D., L. M. Smith and D. E. Gregonis. "Contact Angle and Interface Energetics", in [5], pp. 249-291.
5. Andrade, J. D., D. E. Gregonis and L. M. Smith. 1985. "Polymer Surface Dynamics", in *Surface & Interfacial Aspects of Biomedical Polymers, V. 1*, J. D. Andrade, ed., Plenum Press, pp. 15-41.
6. Sherman, P. O. and S. Smith. "Block and Graft Copolymers . . ." U.S. patent 3,574,791, April 13, 1971.
7. Holly, F. J. and M. F. Refojo. 1975. "Wettability of Hydrogels", *J. Biomed. Materials Res.*, 9:315-326.
8. Andrade, J. D., ed. 1988. *Polymer Surface Dynamics*. New York: Plenum Press.
9. Andrade, J. D. 1973. "Interfacial Phenomena and Biomaterials", *Med. Inst.*, 7:110-120; see also *Current Contents*, 45:24, 1985.
10. Andrade, J. D. and V. Hlady. 1980. "Protein Adsorption and Materials Compatibility", *Adv. Polymer Sci.*, 79:1-63.
11. Andrade, J. D., V. Hlady, L. Feng and K. Tingey. 1994. "Proteins at Interfaces: Principles, Problems, and Potential", in *Interfacial Behavior of Bioproducts*, J. L. Brash and P. W. Wojciechowski, eds., Dekker, in press.
12. Andrade, J. D., ed. 1976. "Hydrogels for Medical and Related Application", *ACS Symp. Series 31*, Amer. Chem. Soc.
13. Gregonis, D. E., D. I. Coleman, R. Hsu and D. Buerger. 1983. "Hydrophobic Hydrogels: Physical Characteristics and Blood Interactions", *Organic Coatings & Appl. Polymer Sci. Prep.*, 48:643-646.
14. Hattori, S., R. N. King and J. D. Andrade. 1978. "Surface Tension Measurements on Methacrylate Monomer Solutions, Selected Solvents, and Gel Extracts", *J. Bioengineering*, 2:113-118.
15. Gregonis, D. E., R. Hsu, D. E. Buerger, L. M. Smith and J. D. Andrade. 1982. "Wettability of Polymers and Hydrogels as Determined by Wilhelmy Plate Technique", in *Macromolecular Solutions*, R. B. Seymour and G. A. Stahl, eds., Pergamon Press, 120-133.
16. North, A. M. 1974. *Molecular Behavior and the Development of Polymeric Materials*, A. Ledwith and A. M. North, eds., London: Chapmand and Hall, pp. 358-403.
17. Gaines, G. L., Jr. 1991. "Monolayers of Polymers", *Langmuir*, 7:834-839.
18. Gregonis, D. E. and J. D. Andrade. "Model Polymers . . .", in [5], pp. 43-76.
19. Pennings, J. F. M. and B. Bosman. 1979. "Relaxation of the Surface Energy of Solid Polymers", *Colloid Polym. Sci.*, 257:720-724.
20. Hedvig, P. 1977. *Dielectric Spectroscopy of Polymers*. New York: Wiley.
21. Yasuda, T., M. Miyama and H. Yasuda. 1992. "Dynamics of the Surface Configuration Change of Polymers . . .", *Langmuir*, 8:1425.
22. Ferguson, G. S. and G. M. Whitesides. 1992. "Thermal Reconstruction of the Functionalized Interface of Polyethylene . . .", in *Modern Approaches to Wettability*, M. E. Schader and G. Loeb, eds., Plenum Press, pp. 143-177.
23. Hayasha, S., H. Sato and S. Sugawara. 1986. "Photo-Induced Wettability Change in Films Containing Spiropyran Microcrystals", *Polymer Journal*, pp. 227-235.
24. Bikerman, J. J. 1970. *Physical Surfaces*, Academic Press.
25. Elwing, H. and C. G. Golander. 1990. "Gradients in Chemical Composition", *Adv. Colloid Interface Sci.*, 32:317-339.
26. Hlady, V. 1988. "Hydrophobicity Gradient on Silica Surfaces", *Colloids and Surfaces*, 33:185-190.
27. Ulman, A. 1991. *Ultrathin Organic Films*, Academic Press.
28. Zisman, W. A. 1964. "Relation of the Equilibrium Contact Angle to Liquid and Solid Constitution", in *Contact Angle, Wettability, and Adhesion, Adv. Chem. Ser.*, F. M. Fowkes, ed., 43:1-51.
29. Johnson, R. E. and R. Dettre. 1969. "Wettability and Contact Angles", *Surface Colloid Sci.*, 2:83-153.
30. Chaudhury, M. K. and G. M. Whitesides. 1992. "How to Make Water Run Uphill", *Science*, 256:1539.
31. Chan, H. S. and K. A. Dill. 1993. "The Protein Folding Problem", *Physics Today*, February, pp. 24-32.
32. Andrade, J. D., ed. 1985. *Protein Adsorption*, New York: Plenum Press.
33. Horbett, T. S. and J. Brash, eds. 1987. "Proteins at Interfaces", *Amer. Chem. Soc. Symp.*, 343.
34. Tingey, K. 1994. "Protein and Platelet Interactions with Polyether Urethanes", Ph.D. thesis, University of Utah, Salt Lake City.
35. Takahara, A., N.-J. Jo and T. Kajiyama. 1989. "Influence of Aqueous Environment on Surface Molecular Mobility and Surface Microphase Separated Structure of Segmented Poly(Ether Urethanes) and Segmented Poly(Ether Urethane Ureas)", in *Progress in Biomedical Polymers*, E. G. Gebelein and R. Dunn, eds., New York: Plenum Press.
36. Andrade, J. D., V. Hlady, A.-P. Wei, C.-H. Ho, A. S. Lea, S. I. Jeon, Y. S. Lin and E. Stroup. 1992. "Proteins at Interfaces", in *Biologically Modified Polymeric Biomaterial Surfaces*, E. Piskin, ed., London: Elsevier Applied Science, 1992; reprinted from *Clinical Materials*, 11:1-4, 1992.
37. Wei, A. P., J. N. Herron and J. D. Andrade. 1990. "Role of Protein Structure in Surface Tension Kinetics", in *From Clone to Clinic*, D. J. A. Crommelin and H. Schellekens, eds., Amsterdam: Kluwer Publishers, pp. 305-313.
38. Patthy, L. 1993. "Modular Design of Proteases of Coagulation, Fibrinolysis, and Complement Activation", in *Methods in Enzymology*, Academic Press, p. 222.
39. Zehfus, M. H. 1987. "Continuous Compact Protein Domains", *Proteins*, 3:90-110.
40. Privalov, P. L. and L. V. Medved. 1982. "Domains in the Fibrinogen Molecule", *J. Mol. Biol.*, 159:665-683.
41. Feng, L. and J. D. Andrade. 1994. "Fibrinogen: Its Structure and Surface Properties", submitted to *Protein Engineering*.
42. Feng, L. and J. D. Andrade. 1994. "Protein Adsorption on Low Temperature Isotropic Carbon: III. Isotherms, Competitivity, Desorption, and Exchange of Human Albumin and Fibrinogen", *Biomaterials*, in press.
43. Norde, W. and A. C. I. Anusiem. 1992. "Adsorption, Desorption, and Re-Adsorption of Proteins on Solid Surfaces", *Colloids and Surfaces*, 66:73-80.
44. Stamm, M. 1992. ". . . Polymer Interdiffusion at Interfaces", in *Physics of Polymer Surfaces and Interfaces*, F. C. Sanchez, ed., Butterworth-Heinemann, p. 189.
45. Andrade, J. D. 1992. "Needs, Problems, and Opportunities in Biomaterials and Biocompatibility", in *Biologically Modified Polymeric Biomaterial Surfaces*, E. Piskin, ed., London: Elsevier Applied Science; reprinted from *Clinical Materials*, 11:1-4, 1992.
46. Rabe, T. E. and R. D. Tilton. 1993. "Surface Diffusion of Adsorbed Proteins in the Vicinity of the Substrate Glass Transition Temperature", *J. Colloid Interface Sci.*, 159:243-245.
47. Lawson, D. F. 1987. "Corona Discharge Activation and Reconstruction of Elastomer Surfaces", *Rubber Chem. Tech.*, 60:102-110.
48. Gagnon, D. R. and T. J. McCarthy. 1984. "Polymer Surface Reconstruction . . .", *J. Appl. Polymer Sci.*, 29:4335-4340.
49. Lee, S. H. and E. Ruckenstein. 1987. "Surface Restructuring of Polymers", *J. Colloid Interface Sci.*, 120:529-536.
50. Carre, A., S. Moll, J. Schultz, M. E. R. Shanahan. 1986. "Interpretation of Contact Angle Hystereses", in *Adhesion II*, K. W. Allen, ed., Elsevier Appl. Sci., pp. 82-96.
51. Hanson, M., K. K. Unger, J. Schmid, K. Albert and E. Bayer. 1993. "Chain Mobility of Polymeric Reversed Phase . . .", *Anal. Chem.*, 65:2249-2253.
52. Fowkes, F. M. 1964. "Attractive Forces at Interfaces", *Ind. Eng. Chem.*, 56:40-50.
53. Andrade, J. D. 1986. "Contact Angle Analysis of Biomedical Polymers: From Air to Water to Electrolytes", in *Polymers in Medicine II*, E. Chiellini, ed., Plenum Press, pp. 24-40.

reporters; they have disdain for politicians; and they tend not to serve on school boards, city councils, or state legislatures.

This book and the conference from which it came have argued that we must *all* be involved. Individuals with engineering and technical backgrounds can and should make significant contributions to a range of social and national problems. Their technical and analytical training and background can provide vital input to practically all socioeconomic issues and controversies, whether it be the role of technology in health care, the environment, and the biosphere, or in regard to weather and natural catastrophes. We must *insist* that these important issues and problems be addressed by our various communities and social systems. We must help generate the political will and the leadership to address these important societal concerns.

Our training and background provide a perspective as well as a set of analytical and critical tools. That perspective is often absent or is incompletely and ineffectively presented in sociological debates and deliberations. It is not because people do not want to listen; it is not because we are ostracized or kept from being involved; it is because our community in general has simply chosen not to be involved. It is not only we who suffer the consequences of that choice, it also is our society that suffers.

Those days are now over. We are seeing the development of a new ethic, a new sense of social responsibility in the scientific and technical communities. The old perception—and excuse—of the "two cultures" is weakening. We can look forward to an increasing level of involvement by the scientific and technical communities in the full spectrum of societal problems, and in particular the nature of health and of health care.

National Values and Needs

Engineers rarely attempt to solve problems that don't exist. They like to have well-defined, well-presented problems. The problem with the health care problem, as Senator Durenberger so forcefully put it, is that the problem has not been adequately defined. The problem is *not* just that we spend 14 percent of our GNP on health care; the problem is *not* just that there are nearly 40 million uninsured; The problem is *not* just that we have over 2,000 individual insurance companies, each with their own forms and bureaucracies—the problem is more than that. Samuel Thier said it very concisely: "What the system should be doing is providing the

proper balance of screening, prevention, diagnosis, treatment, and rehabilitation." Senator Durenberger went on to say: "What are the real health needs of the people? Is the medical market system with its dysfunctional manner depriving us of the resources we need to meet those real needs? Do we have to change our values to help solve this problem?" What do we really mean by "health care"? Have we defined health, health care, and health policy properly? They are defined very differently in other countries. These are difficult and not particularly analytical questions, and they are the kinds of questions that engineers don't like to address. Engineers and physical scientists chose their professions because they like well-posed problems; they become very uncomfortable if they must deal with such ill-posed questions and problems. Nevertheless, they are *our* problems and, as socially responsible adults and citizens of our communities, *we* must deal with them.

The state of Oregon has been involved in discussions and planning with respect to the general health and well-being of its population for the last five years or so.[1] After many years of public discussion and debate involving all sectors of the Oregon population, the state evolved the Oregon Health Care Plan, which defined and identified *their values and needs* with respect to health and quality of life. The plan addressed the issues of access, quality, costs, and economic constraints, and it formulated a standard benefits package for health and health care, with a major emphasis on prevention, healthful living styles, and early diagnosis. There was a deemphasis on expensive, heroic, and halfway technologies and procedures. The Oregon Plan, and the dialogue leading to it, has generated a considerable amount of criticism, discussion, and debate. It nevertheless serves as a model for what we as a nation and what we in other states and regional communities can do.

Scientists and engineers know that problems need to be at least partially defined before they can be effectively addressed and eventually solved. We really do need to decide if health care is a right, and/or how much health care is a right, and how much are we willing to spend on health care? What do we do about individuals who insist on living unhealthy life-styles? Do we wish to maintain the choice and independence so characteristic of the present system? Susan Bartlett Foote said it very concisely: "Choice has a cost"—and generally a significant cost. Do we want to empower patients to have a significant financial stake and intellectual stake in their health and well-being and in the selection of treatments or

nontreatments of their health-related problems? These primarily are all values questions—societal questions that need to be addressed in appropriate forums in order that the problem can be defined.

Economics

What are the costs of health care? Dr. Thier argued that perhaps we need a new calculus, to consider *all* of the costs and *all* of the benefits related to health and well-being. The costs of *your* health and well-being began at the moment of your conception, and they escalated from there. The cost of prenatal care can be allocated both to the mother as well as to the fetus, as can the costs of birth itself. Can we develop a calculus for the total costs and benefits of a human life? If my average life span is seventy years, what will it cost my parents, me, and my community from my conception to my death and burial? We have rarely, if ever, looked at the problem in such an inclusive manner.

Missing a vaccination early in life can lead to significant problems and inordinate costs later in life. We can't simply focus on the incremental cost of that vaccination, or on the incremental cost of the health-problem episodes resulting from a lack of the vaccination. They are all related. The integral stretches from conception to burial. It is an integral which includes an enormous amount of virtually unpredictable statistics and probabilities. Some of us know how to do integrals. Some of us know how to deal with noisy data. Some of us know how to trade quantity in data for quality in application. We can help address those complex problems.

Is the cost of hand-gun control, or gun control in general, a health care cost? Are the costs of drug prevention programs health care costs? And the costs of drug treatment programs—are they health care costs? Is the cost of a summer job for an inner-city teenager, who is likely to acquire both guns and drugs, a health care cost? And what are the health care costs to society at large if that gun-toting, drug-selling, unemployed teenager sells and distributes those drugs and uses that gun? These are all parts of the calculus—parts of this incredibly complex integral. We must now integrate, and not only over the life of an individual and over all of the individuals in society; we must also integrate and consider all of the cross-terms as well. The integral must include all of society. Engineers, at least certain kinds of them, know how to deal with systems, very complex systems, and they can help develop models and means to define and to address such complex problems.

We all know from personal experience that the scientific and technical communities and the medical communities respond to economic incentives. Incentives do indeed matter, and it is today's incentives which drive tomorrow's outcomes. If we can simply identify and define what we want tomorrow, we can implement incentives today which should help drive that outcome. That is again why it is so important to define the problem, so that we can put in place programs and incentives with which to address and to solve it.

Quality, Productivity, and Efficiency

We have seen that quality in the health care system can be significantly enhanced and improved. Improvement in quality and overall enhancement in productivity and efficiency also will lead to lower costs. Productivity and efficiency enhancement does not just mean minimizing labor costs. In fact, it may mean just the opposite. It may well mean taking a little more time to get an appropriate medical history, taking a little time to make the patient aware of his/her medical problem and to educate the patient about the trade-offs of different possible treatments or no treatment as well as to involve the patient in self-diagnosis and self-monitoring. These actions will increase labor costs in the time spent by physician, nurse, or health care provider. But such "increases" in labor costs might indeed be excellent investments in enhanced quality, productivity, and efficiency in the sense of achieving better health and a better health outcome over the longer term. Patients certainly do not want to talk to machines, and they do not want to talk to health care providers who function like machines. They want to talk to informed, involved, compassionate people who can listen as well as pronounce and prescribe.

Hunter and Foote both addressed the issue that there are far too many specialists in this nation, and that the ratio of specialists to primary-care providers is completely out of balance. Incentives are evolving to change that, but they could evolve more rapidly. The same situation is perhaps true in biomedical engineering. There is far more interest in graduate projects that tend to push the scientific and technology envelope, driven of course by incentives from the funding agencies, than there is on projects which may help to solve and meet a current health care need but that may involve more pedestrian or less exciting technologies.

Benefits and Risks

The scientific and technical community tends to have some appreciation of probability and statistics. Those with even the weakest introduction to the life sciences know that organisms eventually die, and that they die from myriad causes. Most of us know that the Gaussian distribution, or normal, curve is more or less endemic throughout biology. Yet, although these principles tend to be part of the education of some college and university students, they are not a part of the education of the general public nor of their lawyers. Unfortunately, these concepts are also not well known among many physicians and medical providers. When they are, they often are not transmitted effectively to their patients.

Keller (Chapter 2) discussed the issue of benefit and risk. Clearly there are more safe as well as less safe activities and procedures. Clearly there is malpractice. Clearly there are poorly designed and manufactured devices; and clearly we need effective regulation with respect to safety and efficacy. But just as clearly there are statistics and there are probabilities. It is no one's fault if an earthquake levels your town or your home. It may be *your* fault for choosing a home on an earthquake fault, if that fact was indeed known to you. Is it really society's fault if you live on a 500-year floodplain? Is it someone's fault if one out of 10,000 medical implants leads to a negative or even catastrophic outcome? Is it fair or right to insist that that device have a one in 1,000,000 failure rate, increasing its development, testing, and manufacturing costs by orders of magnitude to produce such reliability? Is it fair to make the other 999,999 individuals who receive that implant pay the exceptionally high cost required for that level of safety?

Lawmakers and lawyers must come to understand statistics and probability. You and I and our colleagues must educate them. We must see to it that these concepts are incorporated in all professional courses of study—in all majors, on all campuses, in all schools.

Education and Communication

Health care and the costs of health care are important to every single individual in society. We are all involved and we are all part of the problem. Every single group, every component of society, will have to change its own behavior patterns in order to effectively and successfully solve this problem.[2] In some respects, we must all

do less, in order that we have the time to do what we do more compassionately, relevantly, and effectively. We must encourage people, ourselves included, to get off of treadmills leading to nowhere, in order that we will have the time to reflect, to identify the problem, and to solve it. All groups must become far better informed and involved in the entire health care area. They must select their physician collaborators more carefully. They must design their experiments—particularly animal and human experiments—far more carefully and efficiently. They must attempt to develop the *societal impact statement*, described by Keller, regarding the future applications and societal impact of new medical procedures or technologies. They must consider and perhaps overcome the technological imperative, also described by Keller, and realize that most ideas and inventions never come to fruition or are never applied, perhaps for good reasons.

Lawyers and judges must be educated—many can be. Letters to the editor, radio and television interviews, discussions with reporters on local cases and controversies—these all help educate the media, the general public, and the law profession.[3] Simple, everyday, elementary-school-level examples of statistics, benefit versus risk, probability, and related topics can have significant impacts, particularly if the entire technical community begins to assume its education and communication responsibilities.

The media often fuels unrealistic expectations; there are many examples of miracle medicines in the media. Health care professionals, biomedical engineers, and others must work with the media to make them more aware of the realities of medicine and health care.

The issue of risks, particularly as applied to health care, must be considered and communicated to the general public and to the media. The difference between voluntary and involuntary risk must be made clear. The role of risk awareness in influencing behavior is critical. The importance of patient choice and empowerment should be emphasized. Cost is unlikely to be brought under control without a general appreciation of the limits of technology and of the inability of devices to correct the natural wear and tear of the aging process. This includes the acceptance by patients of risks commensurate with the statistically demonstrated benefit.

Politicians and elected officials certainly respond to letters and letters to the editor. If they were to receive even a handful of

inquiries or statements informing them of risk-benefit-cost issues, they and their staffers would respond. Dr. Healy said, "This puts an obligation on us, each in our own way, to tell the public what we are doing and how it is done. Keep in mind, after all, that the public perception of what we do is not always exact—and when that is so, it is our problem."

Scientists and engineers generally have poor communication skills. Such skills are not fostered in our curricula, and there are not many incentives for developing those skills. There is a general attitude, sort of an arrogant pedestal syndrome, that we don't want to lower ourselves to the level which would be required for dialogue with the general public or the media. Those with communication skills generally do not have the scientific and technical skills, so the information that is getting to the general public, with the exception of that provided by a few good science writers and reporters, is usually incomplete at best and completely erroneous or misrepresented at worst. You and I have to correct that. We have to challenge those reporters, those writers, those lawyers. We have to inform them, and the community influenced by them, that their scientific and technical facts either are correct or incorrect, that their analysis of a technical or semi-technical issue either is appropriate or inappropriate, that their consideration of risk is reasonable or unreasonable given the data and understanding at hand. Not to do so is socially irresponsible—it leads to the system and the problems we have, and the blame becomes ours for being so uninvolved.

Every major report on engineering education, medical education, undergraduate education in general, and public education over the last decade or so has said that we need to teach less, but teach it better, and that we need to educate in a more integrated and systems-like manner. We need to produce graduates and professionals who are good listeners, effective communicators, and responsible citizens. Although I have yet to see a significant response in the higher education sector to these reports, the public education sector is changing rapidly. There are major movements and activities throughout the nation to enhance education at the elementary, junior high, and high school levels, particularly in science and technology education, and that includes mathematics, statistics, and related topics. I am optimistic. As these students move into the college and university environments, as they challenge their professors and fellow students, as they move into the job market and the economy and interact with their co-workers and fellow citizens, I think they will help realize the fulfillment of the hope for a more responsible and involved electorate.

Prognosis and Predictions

Regarding the field of biomedical engineering in particular, George Bugliarello said: "The time has come to look at bioengineering not any longer as a specialized effort tucked into a corner of engineering or medicine but as a mainstream field involved in our efforts to maintain and enhance our species and our planet. But we must first deal with the health care question. That is perhaps the highest immediate priority of the biomedical engineering community." Specifically, we must deal with the following topics and problems.

1. *Health Care and Its Costs.* We must *each* examine the health care, health costs, and health technology fields objectively and decide where our individual talents and interests can be most effectively utilized. Although this book is a significant contribution to such study, it is not enough. There are a number of other excellent sources which should be consulted.[4] More importantly, we must stay current and involved in the ongoing dialogue and analysis of these complex problems.[5] We must encourage our students to learn something about economics, the health care calculus, benefits and risks, and what activities and efforts are needed and could really make an impact.

2. *The Health Care Products Industry.* There is no question that the technological imperative described by Keller and the funding incentives for new technological development have significantly changed. It is therefore unlikely that small incremental improvements in medical devices or technology will prove to be very cost-effective in the new calculus, unless they are accompanied by significant reductions in cost. New inventions and new technologies are likely to be of significant interest only if they represent major leaps or advances in either quality or cost-reduction. Incremental changes will probably be of decreasing interest.

3. *Information.* This volume has made a strong case for enhanced information and communications technologies, and for a computer-based patient medical record that is transportable around the country and even around the world.[6] It is likely that this initiative will

grow and will be implemented. It is also likely that medical-equipment manufacturers will have to design their equipment and their software, not only to comply but to lead in such efforts if they expect to be commercially successful.

4. *More Diagnostic Information.* The availability of rapid, high-speed, transparent information and communication networks, and the ability with them to analyze and cross-correlate large amounts of data from large populations, will drive an interest in having even more information, because the analysis of the information in such large databases will likely lead to new correlations, hypotheses, and insights into disease, treatment, and overall health and well-being. This in turn will drive an interest in even *more* information; so it is likely that there will be an interest in developing large data sets, even for normal patients, as reference and control values. My guess is that early in the twenty-first century when one orders any clinical chemistry, say from a blood or urine sample, hundreds of chemicals will be analyzed at the same time. It will be as easy to do one hundred clinical chemistry determinations on a sample of blood as it is to do one, six, twelve, or eighteen. There will of course be no additional charge for this extra information; it will simply be there because it is expected. Those companies who are used to making large amounts of money on single-test kits are likely to have a very rude awakening when kits and instruments that analyze 50, 100, or even 1,000 chemicals will be available at almost the same price.

5. *Academic Research.* Much academic research in medical institutions is funded by internal funds, often clinical funds "contributed" by academic clinicians. Other academic research is sometimes funded from monies attributed to indirect costs but then utilized internally by the institutions for other purposes. As our national health care plan evolves, it is likely that there will be significant constraints, if not outright elimination, of cost shifting and of the use of clinical incomes for other than clinical purposes. It is also likely that the use of indirect costs for other costs also may be curtailed.

There will be a significant shake-out, particularly in research hospitals and in teaching hospitals' research activities. This will lead to increased pressure on NIH and other funding agencies. The need for health-outcomes research will continue to increase, including the appropriate testing and evaluation of medical devices and

cost-benefit-risk studies and analyses. There will be a growing expectation that these studies should be at least in part funded by federal sources. That will make the finite research pie even more difficult to allocate.

6. *Regulation.* We all hope and expect that a national health care plan will include legal liability reform and an improvement of the federal regulatory structure. This will only happen if the scientific, engineering, and medical communities do everything they can to educate the public on such issues as benefits, risks, and costs. If that indeed happens, then there may well be a move to incorporate such concepts in the regulatory structure.

7. *Bio-Based Engineering.* Finally, there is some perceptible motion towards what George Bugliarello called "biomimesis," or what some of us have called bio-based engineering. Bioengineers trained in physiology, cell and tissue engineering, or biotechnology represent Bugliarello's biomimesis, but they are only the tiniest tip of the iceberg.

There is a wealth of biodiversity, much of it going back billions of years and involving millions if not billions of species. Engineers generally ignore it. Back in junior high and high school, you may have either loved to learn to dissect frogs, or you loved to learn about magnets or chemicals. The former sends you on a pathway to the life sciences and perhaps into medicine; the latter sends you on a pathway through the physical sciences and sometimes into engineering. The two pathways rarely meet or intersect in college or university environments or in professional life. Bioengineering and its sister disciplines, biophysics and biochemistry, are exceptions, although even in these cases there is a strong human (or at least mammalian) emphasis. Practically all of the rest of biology is totally ignored. However, the growing interest in bio-based engineering and biomimesis is expected to grow rapidly, leading to totally new solutions to a variety of industrial, environmental, medical, and related problems.

Summary

Senator Durenberger told a little story in a speech in Portland, Oregon, some years ago. He was challenged by an irate constituent as to why he had voted for a particular piece of legislation. His reply: "Madam, sometimes leaders have to lead." It is time engineers—and bioengineers, in particular—began to lead.

Acknowledgments

These topics and opinions have been discussed and formulated over the past several years with many colleagues, co-workers, and other friends. I particularly thank those curious, dynamic, and creative students who have participated in a set of nontraditional courses over the past few years: Critical Science Communications, Bioengineering and the Costs of Health Care, Using Bioengineering for Science Education, and Integrated Science Concepts and Themes. Very special thanks are extended to Dov Jaron and Peter Katona for encouraging the discussion on these important topics.

References

1. See J. Kitzhaber, "A Healthier Approach to Health Care," *Issues in Science and Technology* (Winter 1990–91): 59–65; and H.D. Klevit, A.C. Bates, T. Castanares, E.P. Kirk, P.R. Sipes-Metzler, and R. Wopat, "Prioritization of Health Care Services," *Archives in Internal Medicine* 151 (1991): 912–16; and E.M. Reingold, "Oregon's Value Judgment," *Time* (Nov. 25, 1991): 37.

2. W.L. Lanier and M.A. Warner, "New Frontiers in Anesthesia Research: Assessing the Impact of Practice Patterns on Outcome, Health Care Delivery, and Cost," *Anesthesiology* 78 (1993): 1001–4.

3. J. Marc Adam, "Countering the Growing Skepticism over Medical Technology," *Medical Devices and Diagnostic Industry* (June 1992): 28–34.

4. K.B. Ekelman, ed., *New Medical Devices: Invention, Development, and Use* (Washington, D.C.: National Academy Press, 1988).

S.B. Foote, *Managing the Medical Arms Race: Innovation and Public Policy in the Medical Device Industry* (Berkeley: University of California Press, 1992).

J.D. Bronzino, V.H. Smith, and M.L. Wade, *Medical Technology and Society: An Interdisciplinary Perspective* (Cambridge, MA: MIT Press, 1990).

A.C. Gelijns and E.A. Hahn, eds., *The Changing Economics of Medical Technology* (Washington, D.C.: National Academy Press, 1991).

A.C. Gelijns, ed., *Technology and Health Care in an Era of Limits* (Washington, D.C.: National Academy Press, 1992).

R.S. Dick and E.B. Steen, eds., *The Computer-Based Patient Record: An Essential Technology for Health Care* (Washington, D.C.: National Academy Press, 1991).

D. Feeny, G. Guyatt, P. Tugwell, *Health Care Technology: Effectiveness, Efficiency, and Public Policy* (Halifax, Nova Scotia: Institute for Research on Public Policy, 1986).

V.H. Smith and J.D. Bronzino, "Measuring the Costs of Health Care Technologies," *IEEE-Engineering in Medicine and Biology Magazine* (June 1993): 34–37.

J.D. Andrade, D. Jaron, and P. Katona, "Improved Delivery and Reduced Costs of Health Care Through Engineering," *IEEE-Engineering in Medicine and Biology Magazine* (June 1993): 38–41.

W.L. Lanier and M.A. Warner, "New Frontiers in Anesthesia Research: Assessing the Impact of Practice Patterns on Outcome, Health Care Delivery, and Cost," *Anesthesiology* 78 (1993): 1001–4.

J. Marc Adam, "Countering the Growing Skepticism over Medical Technology," *Medical Devices and Diagnostic Industry* (June 1992): 28–34.

5. An excellent set of sources is the *New York Times, Science, New England Journal of Medicine,* and *Journal of the American Medical Association.*

6. R.S. Dick and E.B. Steen, eds. *The Computer-Based Patient Record: An Essential Technology for Health Care* (Washington, D.C.: National Academy Press, 1991).

USING NOVEL BIOLOGICAL PHENOMENA TO ENHANCE INTEGRATED SCIENCE EDUCATION: BIOLUMINESCENCE

J.D. Andrade, M. Lisonbee, D. Min

Center for Integrated Science Education, 2480 MEB
University of Utah, S.L.C., UT 84112, USA

SUMMARY

A major shift in science education is now being implemented. There is growing interest in hands on, discovery-based science education. There is also interest in treating science as a coherent, integrated field of inquiry which can deal with complex subjects (1-3).

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 (2) 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.

INTRODUCTION

A large number of elementary teachers readily admit to having fears and anxieties towards science and towards the teaching of science in their classrooms (2). Much of this fear stems from their own science experience in high school and college courses, where science was treated as a series of facts and laws to be memorized and minimally questioned. The frustrations, inadequacies, and anxieties which these teachers experienced in their own education stay with them during their teaching years, and become transmitted to their own students. Teachers cannot effectively present and develop the scientific process and experience for their students unless they feel comfortable in those processes and experiences themselves.

The teachers that are most in need of such experience are those which normally will not voluntarily sign up for inservice and related upgrade classes. We must go to them, their schools, their locations.

A. K. Campbell, et al., eds.,
Bioluminescence and Chemiluminescence,
Wiley, 1994.

Andr-52

Applying "Intelligent" Materials for Materials Education: The Labless Lab™

J. D. ANDRADE AND R. SCHEER

ABSTRACT

A very large number of science and engineering courses taught in colleges and universities today do not involve laboratories. Although good instructors incorporate class demonstrations, hands on homework, and various teaching aids, including computer simulations, the fact is that students in such courses often accept key concepts and experimental results without discovering them for themselves. The only partial solution to this problem has been increasing use of class demonstrations and computer simulations.

We feel strongly that many complex concepts can be observed and assimilated through experimentation with properly designed materials. We propose the development of materials and specimens designed specifically for education purposes.

"Intelligent" and "communicative" materials are ideal for this purpose. Specimens which respond in an observable fasion to new environments and situations provided by the students/experimenter provide a far more effective materials science and engineering experience than readouts and data generated by complex and expensive machines, particularly in an introductory course. Modern materials can be designed to literally communicate with the observer. Although some such materials can be obtained from commercial and research sources and are suitable for experiencing and learning certain materials phenomena and behavior, there has been no concerted effort to develop materials specifically for education application.

We are embarked on a project to develop a series of Labless Labs™ utilizing various degrees and levels of intelligence in materials. It is expected that such Labless Labs™ would be complementary to textbooks and computer simulations and be used to provide a reality for students in courses and other learning situations where access to a laboratory is non-existent or limited.

J.D. Andrade, University of Utah, 2480 MEB, Salt Lake City, Utah 84112
R. Scheer, Protein Solutions, Inc., 390 Wakara Way, Rm. 31, Salt Lake City, Utah 84108

BACKGROUND AND RATIONALE

Undergraduate students in many science and engineering courses in the United States have little or no laboratory experience in such courses. The labless science and engineering course has become a very common feature in higher education. Although outstanding instructors attempt to overcome this deficiency with the use of classroom demonstrations, discovery based homework assignments, class projects, and computer simulations, many instructors may not have the time or inclination to utilize these tools, particularly in relatively large lecture environments. Labs are also not generally available for correspondence, distance learning, or TV/video courses. We feel there is a need for small, inexpensive, completely self contained laboratories which can supplement existing textbooks.

The several hundred undergraduate engineering programs in the country nearly all teach a course in materials science, most with a significant polymer component, and many of them teach a separate course in polymer materials. In addition, advanced high school chemistry or physics courses often include a significant polymer materials component. There are on the order of 40,000 introductory materials science texts sold annually in the U.S. and Canada (1-2). The National Science Teachers Association has a high school supplement text which is very popular with high school teachers throughout the country.

Although many of these texts come with instructions for experiments and demonstrations, in reality these are rarely performed due to the difficulty in obtaining the materials in a timely and inexpensive fashion.

The Exploratorium, an interactive, hands on science museum in San Francisco, and Klutz Press in Palo Alto, California, recently teamed up to produce a volume called The Explorabook, in which a range of hands on, discovery based experiments were incorporated into a small inexpensive book. The Explorabook is the largest selling children's science book in the United States today. It demonstrates the need for challenging scientific materials by the general public. That need is also present in higher education.

The recent Project 2061 report of the American Association for the Advancement of Science (AAAS) states (29):

> *"For teachers to be able to bring all students to the level of understanding and skill proposed in this report, they will need a new generation of books and other instructional tools...Textbooks and other teaching materials in current use are - to put it starkly - simply not up to the job."*

Although there has been major soul searching and even restructuring of education throughout the nation and a growing emphasis on hands-on, discovery, experiential activities, this renaissance has not yet significantly penetrated higher education. Science and engineering education in many institutions is still highly didactic in nature, relying on computer simulation and visualization to provide an "experimental" experience for students. There are two major problems with this approach :

1) Motivation -- students know that there is a difference between virtual reality and real reality;
2) Students otherwise excellently prepared for college and university work often lack experience in dealing with simple tools, in working with their hands, and in making simple common sense observations and deductions.

This is due to two major features of our society:

1) There is no need for major segments of the society to learn how to use simple tools or to do simple technical manipulations with their hands. We are a service oriented society -- everything is done for us. We have a whole generation of students, and even many of their parents, who lack simple tool utilization and basic observational skills.

   We have seen and taught university undergraduates with A- grade point averages who literally do not know the difference between a Phillips head and a slot head screwdriver, or what a crescent wrench is. We have the same problem in graduate school -- particularly with students from the Orient whose background is largely based on theory and problem-solving in the absence of hands-on or experimental experience.

2) The public education system in this country, up until very recently, has not emphasized experiential, common sense, hands-on approaches to situations and problems. As indicated earlier, that situation is of course changing and is changing very rapidly, but the college age generation that has been through that system needs help.

Another major need and opportunity is continuing education or distance learning. There is growing interest in making our educational system available to a much wider segment of society. Through the new national information highway and network there will be a proliferation of courses and even full degree programs available via our televisions and computer terminals. Although there is much that can be done in these arenas with excellent lecturers and presenters, with outstanding video segments, and with computer activities and virtual reality simulations, the bottom line is the same as discussed briefly above. Without actual hands-on, experiential, real reality experience, the topics and concepts presented will never be fully internalized -- will never become "common sense" for the individuals involved.

A Lableess Lab™ approach to such real reality will facilitate such internalization, such experience, and such learning.

POLYMERIC MATERIALS

Plastic and polymeric materials are ubiquitous in modern society. A significant fraction of all chemists and engineers work in polymer related industries or utilize polymers in scientific and engineering activities. Polymeric materials are unique because of their polymeric nature. Large molecular weight molecules behave, in general, very differently from low molecular weight molecules and molecular or atomic solids. Many of the rules of thumb learned in elementary physics and chemistry appear to not apply in the case of polymeric materials.

Most students come into polymer courses with various concepts and preconceptions which lead them to conclude that the behavior and properties of polymers are counter intuitive. It is therefore important that they fully discover and observe the properties and behavior of polymeric materials for themselves.

It is appropriate to begin our Lableess Lab™ effort with polymeric materials because they are readily available for a wide variety of applications and because they exhibit a range of phenomena which are very easy to observe, experience, and discover.

There is considerable interest in effective polymer education (3). The American Chemical Society Division of Chemical Education often includes polymer related articles in its Journal of Chemical Education (4, 5) and in its sessions at the American Chemical Society annual meetings. The ACS also has a Polymer Education Committee, as does the Society of Plastic Engineers (SPE). Polymer education is also of interest to the American

Institute of Chemical Engineers (AICHE) and the Materials Research Society (MRS). There is a Polymer Education Center at the University of Wisconsin, Steven's Point and a Polymer Education Newsletter (3). The Institute for Chemical Education at the Department of Chemistry at the University of Wisconsin, Madison, is also active in providing a variety of educational materials for discovery based chemistry and polymer education (6).

These activities are all helpful and indeed have greatly stimulated this project. However, the typical instructor, particularly in relatively large enrollment classes, often does not have the time or the inclination to assemble the materials, components, and equipment necessary to put together an effective discovery laboratory, particularly if the class is a lecture only course, which is typical for many introductory material science and polymer science courses.

"COMMUNICATIVE" MATERIALS

"Communicative" materials are those which alter their properties or characteristics when subjected to a perturbation by the observer. The material's response to the perturbation is easily and readily observed without recourse to any instruments or apparatus. The observer's own senses are sufficient. There are, of course, many examples from common, everyday practice: the change in color or optical density of a liquid crystal thermometer or a liquid crystal based computer screen; the change in hardness/softness and other mechanical properties of polymers due to changes in temperature; the polymerization or setting up of glues, adhesives, and caulks; the elasticity and extension of rubber bands; etc. Although these are all useful, there are many opportunities for enhancing the educational content of polymeric materials through the design and development of materials which can be used to direct the experience of key concepts and phenomena (Table 1).

Table 1 presents key concepts and topics in a typical introductory polymer science course, together with a set of possible Lableess Lab™ experiments.

Materials, polymers included, are developed for commercial application and for meeting some particular consumer or industrial need. We are developing materials specifically for science education. With these special materials the student can directly observe physical and chemical parameters and materials behavior. This is possible by utilizing gradient materials (20). We have been utilizing gradient surfaces for many years in our study of the surface properties and biocompatibility of medical polymers (7-9). We are now using these surfaces in biomaterials courses (10), because integration of materials and devices with surrounding tissues and the biocompatibility of materials and devices tends to be a strong function of the surface properties of the materials (surface free energy, polarity, hydrophobicity, charge density, roughness, and surface dynamics), which play important roles in various biocompatibility situations (27,28).

The gradient surface is one in which a distinct surface property is varied continuously from one end of the sample to the other (11). Exposure of a single surface to the appropriate biological environment permits a qualitative assessment of a wide range of functions and activities, thereby allowing general concepts to be discovered and deduced. For example, we now routinely prepare surfaces with a continuous spatial gradient in wetting, as indicated in Figure 1. Two such surfaces placed together with a small separation, allow the phenomenon of capillarity, as a function of wetting or contact angle, to be dramatically demonstrated. Such basic concepts of surface energy, hydrophobicity, and capillarity are directly observed and make a lasting and permanent impression. One does not need to graph or correlate the results of the experiment. One simply sees the correlations vividly (18).

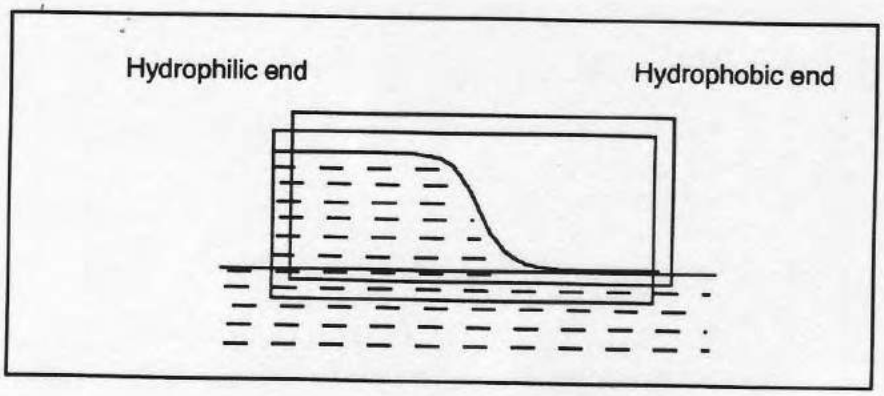


Figure 1. Capillary rise method for investigation of wettability gradients. Two transparent plates with wettability gradients are put together with a support that separates the plates. The lower edges of the parallel plates are then brought into contact with a trough filled with water. Water moves upward between the plates and the height of the liquid meniscus is determined by the wettability of the surface of the plates (from Ref. 11).

Gradients are used in particular areas of research. For example, biochemists commonly use gels with a gradient in cross link density, thereby developing a gradient in porosity. This is widely used in electrophoresis for protein analysis and separation (19). Polyelectrolyte gradient gels are used in electric fields to produce a gradient in pH, which allows the separation of macromolecules on the basis of net charge (19). Gradients in surface to volume ratio have been used to study concentration and mass transport dependent effects (12, 13).

Since so many of the properties of polymer materials are time and temperature dependent, it is almost mandatory to be able to produce different temperatures to permit such observations. A simple temperature gradient device used with gradient materials allows a wide range of relationships to be directly observed.

The thermalgradient device will provide a continuous temperature gradient from about room temperature to 60°C. A polymer film at a glass transition temperature of say 40°C placed on such a device will enable the student to directly view, feel, and experience the change in polymer properties with temperature above, below, and through the glass transition temperature.

We are using methacrylate homopolymers of varying alkyl chain length to demonstrate the effect of side chain length or internal plasticization on Tg and thus on the physical and mechanical properties (31). Using polymethyl methacrylate (Tg = 130C), polyoctyl methacrylate (Tg = -60), and intermediate alkyl chain lengths, we have a nearly ideal series of materials with which the students can directly experience Tg as a function of side chain character.

We are also developing polymers with a continuous gradient in composition so that composition variation and the consequent Tg variation are observable in a single sample. One end of the film is rigid (PMMA) and the other end is soft (POMA) -- this can be observed directly in the student's hand at body temperature.

The role of exogenous plasticisers, such as dioctyl phthalate in poly vinyl chloride, can be similarly experienced. PVC containing 35% DOP plasticizer is gradient extracted in methanol to provide a polymer film with a continuous range in plasticizer content from nearly zero to 35%.

In the case of an elastomer or a hydrogel network, a variation in crosslink density results in a directly observable change in behavior. Elongation under constant load varies inversely with crosslink density. In the case of a hydrogel, swelling and dimensional change varies inversely with crosslink density. In both cases, such materials are studied

as sets of individual samples which can be compared with one another, and studied as gradient samples in which the continuous range of characteristics can be observed in a single sample.

Temperature responsive polymers, initially poly isopropyl methacrylamide, which has been so widely applied as an "intelligent" polymer, are also being developed for these applications (20).

Polynorborene, a shape memory polymer, sold in Japan as a technical novelty, will also be used (32).

Other experiments and activities in the Lableess Lab™ include molecular weight separations using gel permeation chromatography, growth of spherulite's using appropriate polymers with low melting points, considerations of solubility and solubility parameter, changes in the ionization and redox behavior of polymers in response to electric field's and to different electrochemical environments, photo degradation, photo induced cross-linking, and certain aspects of polymerization.

The eventual goal is that all of the themes and concepts listed in Table 1 would be directly experienced.

Beside those polymer samples and the thermal gradient device, the Lableess Lab™ will include DC battery powered electrical experiments, a variety of optical experiments, and a small inexpensive device for force, stress, and strain experiments.

What does one do with these novel and unique polymer samples for science education? Table 1 presents a typical topic outline for an Introduction to Polymer Materials course. This represents Materials Science and Engineering 519, the required polymer course for Materials Science majors at the University of Utah (16). This is the first and last polymer materials course for those students who do not go on into the polymer science track.

The first column in Table 1 presents the general topic, followed by a column of commercial or conventional polymer examples, followed by a column of the special materials we are developing. The Table indicates how the new materials, as well as existing materials, are used by students to discover and observe the various concepts, properties, and behavior, covered during this class.

CONCLUSION/SUMMARY

A Lableess Lab™ for polymer materials is well on its way to becoming a real reality. Preliminary versions are being tested in a course at the University of Utah. A field test version keyed to several major textbooks in polymer science and engineering should be available by summer, 1995. Limited commercial distribution is expected in early 1996.

The goal is to develop a Lableess Lab™ in polymer materials which could be made available in classroom quantities with prices comparable to those of existing textbooks, i.e., in the $40-$60 range per unit. It is anticipated that there will be two such products, an introductory polymer materials version, and a more advanced version.

We are interested in learning of additional materials and phenomena which could be incorporated into the Lableess Lab™ in a very inexpensive manner. Lableess Labs™ in other appropriate science and engineering courses are also under development.

The Lableess Lab™ is a trademark of Protein Solutions, Inc., Salt Lake City, Utah.

ACKNOWLEDGMENTS

Our initial work in this area was funded by a small grant from the American Chemical Society's Division of Polymer Chemistry, Polymer Education Program. The application of the concept to a basic polymer materials science course at the University of Utah was funded by the University of Utah Teaching Committee. Most of the existing work on the

TABLE 1 MAJOR TOPICS IN A ONE QUARTER (10 WEEKS)
UNDERGRADUATE COURSE, INTRODUCTION TO POLYMER SCIENCE AND
POLYMERIC MATERIALS (MSE 519, UNIVERSITY OF UTAH) (16)

Topic	Key Concepts	Conventional Materials/Methods	Communicative Materials
Polymer Applications	Wide range of Properties and compositions	Examples from consumer products and engineering devices & machines	None
Macro molecules	High molecular weights. packing, entanglement	Individual examples	MW gradients comonomer gradients
Polymerization and copolymerization	Properties = f (composition, molecular weight)	Individual examples	MW gradients comonomer gradients
Morphology and Structure	Packing, Ordering, Melting	Individual examples, molecular simulation	Crystallization, annealing, ordering as f (T, t), comp. gradients
Block copolymers	Phase separation, incompatibility	Blends, diblock - triblock - copolymers	Blend gradient
Solubility and solutions	Solution Interactions and thermodynamics	Individual examples - MW, polarity, Temp.	Temp. gradient - LCST composition gradient
T - t Effects	T - t superposition; effect of properties on T and t	Specific examples	T gradient/comp. gradient
Cross-linking	Networks, elasticity, viscoelasticity	Individual examples	Cross link gradient; mechanical response
Additives	Plasticization	PVC - Plasticizer - individual samples	Plasticizer gradient, T gradient
Surface Properties	Wetting/surface modific.	Individual Examples	Wetting gradients, capillarity
Processing	Fiber formation, film molding	Thermoplastic, thermoset, T and processing	T gradient, cross link gradient composition gradient
Adhesives	Adhesion/surface properties	Hi and low energy polymers, contact angle, peel tests	Surface property gradient
Electrical fields	Conductivity, ionization, solubility	Mainly conductive polymers	Ionogenic polymers & biopolymers; electrically responsive polymers; T gradient; LCST; Elect. responsive
Biopolymers	structural proteins	Silk, collagen	Polypeptide gradients
Environmental issues	Cost, energy, solid waste, biodegradation	Specific examples, classes, stability	Composition/morphology gradients; stability

project is focused at Protein Solutions, Inc. in Salt Lake City, a science education products company, and funded by a small business innovation research (SBIR) grant from the National Science Foundation. J.D. Andrade acknowledges discussions on these topics with K. Caldwell, W. Callister, L. Feng, V. Hlady, and T. Matsuda.

REFERENCES

1. R.B. Deanin and R.R. Martin, "Survey of Polymer Education in U.S. Colleges and Universities", Preprints, Polymer Sci. and Engrg. (ACS), 45-56.

2. W. Callister, author of Materials Science & Engineering an Introduction, 2nd ed., Wiley, 1991, personal communication.

3. J. Droske, Polyed Information Center and Polymer Education Newsletter, Dept. of Chemistry, University of Wisconsin - SP, Stevens Point, WI 54481.

4. R.B. Seymour, "Recommended ACS Syllabus for Introductory Courses in Polymer Chemistry", J.Chem. Educ. 59 (1982) 652.

5. L.J.Mathias, "The Lab for Intro. Polymer Courses", J. Chem. Educ. 60 (1983) 990.

6. Institute for Chemical Education, Dept. of Chemistry, University of Wisconsin - Madison, 1101 University Ave., Madison, WI 53706-1396.

7. V. Hlady, "Hydrophobicity Gradient on Silica Surfaces", Colloids and Surfaces 33 (1988) 185-190.

8. C.G. Golander, "...Surfaces with a Hydrophobicity Gradient, Colloids and Surfaces 34 (1989).

9. J.H. Lee, "Wettability Gradient Surfaces Prepared by Corona Discharge", Trans. Soc. for Biomaterials, 17 (1991) 133.

10. J.D. Andrade and H.B. Lee, "Surface Property Gradients for Biomaterials Education", Abstracts, Fall Meeting of Biomed. Engrg. Soc. 3 (1992).

11. H. Elwing and C. G. Golander, "...Gradients in Chemical Composition", Adv. Colloid Interface Sci. 32 (1990) 317-339.

12. L. Vroman and A. Adams, "Proteins...in Narrow Spaces" (Lens on Slide Gradient Method) J. Colloid Interface Sci. 111 (1986).

13. H. Elwing, "Lens on Surface", J. Biomat. Sci. Polymer Ed. 3 (1991) 7-16.

14. F. Rodriguez, Principles of Polymer Systems 2nd ed., Hemisphere Publ. Co., 1984.

15. F. Billmeyer, Textbook of Polymer Science, 3rd ed., 1984, Wiley.

16. J. Andrade, Syllabus for MSE 519, Introduction to Polymeric Materials, Univ. of Utah.

17. M. McDonald, K. Thorimbert, and J. Andrade, "The Science Education Market", report to State of Utah Centers of Excellence Program, July, 1992; Center for Integrated Science Education, Univ. of Utah.

18. J.D. Andrade, "Polymers Have Intelligent Surfaces: Polymer Surface Dynamics," *J. Intelligent Materials Systems & Structures* (1994) in press.

19. C-H. Ho, V. Hlady, G. Nyquist, J.D. Andrade, K. Caldwell, "...Proteins...Studied by High-Resolution Two-Dimensional Gel Electrophoresis", J. Biomed. Materials Research 25, 423-441 (1991).

20. A.S. Hoffman, "Environmentally Sensitive Polymers and Hydrogels: "Smart" Biomaterials", MRS Bulletin, Sept. 1991, pp. 42-46.

21. Y. Osada, H. Okuzaki, and H. Hori, "Polymer Gel with Electrically Driven Motility", Nature 355 (1992) 242-3.

22. Y. Osada and M. Hasebe, "Electrically activated Mechanochemical Devices using Polyelectrolyte Gels", Chem. Letters (1985) 1280-1288.

23. T. Tanaka, "Gels", in C. Nicolin, ed., Structure and Dynamics..., Nijhoff Publ., 1987, pp. 237-257, see also Scientific American 244 (Jan. 1981) 124-138.

24. T. Tanaka, "Collapse of Gels in an Electric Field", Science 218 (1982) 467- 469.

25. G. Giannetti, et. al., "Effects of DC Electric Fields on Gels", in F.L. Carter, et. al., eds., Molecular Electronic Devices, Elsevier, 1988, pp. 369-380.

26. J. M. Guenet, Thermoreversible Gelation... Academic Press, 1992.

27. J.D. Andrade, ed., Hydrogels for Medical and Related Applications, ACS Symp. Series 31, 1976, American Chemical Society.

28. J.D. Andrade, ed., Surface and Interfacial Aspects of Biomedical Polymers, Vol. 1, Plenum, 1985, especially Chapters 2, 3.

29. J. Rutherford, Project 2061: *Science for All Americans*, American Association for the Advancement of Science, Washington, DC, (1988) p. 14.

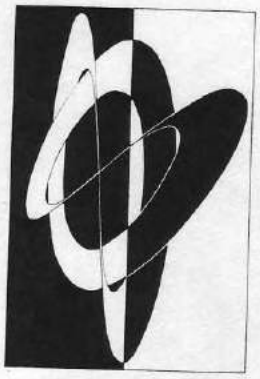
30. M. Goodwin, BSc. Thesis, University of Utah, Department of Materials Science and Engineering, Feb., 1994.

31. R. Larsen, BSc Thesis, University of Utah, Department of Materials Science and Engineering, March, 1994.

32. Ring and Stick product, Maruyoshi Co., Ltd., Japan.

PROCEEDINGS OF THE

SECOND INTERNATIONAL CONFERENCE ON INTELLIGENT MATERIALS



ICIM'94

Edited by

CRAIG A. ROGERS, General Chair
GORDON G. WALLACE, Technical Chair

June 5-8, 1994
Colonial Williamsburg, Virginia, U.S.A

Organized by
CENTER FOR INTELLIGENT MATERIAL SYSTEMS AND STRUCTURES
VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Sponsored by
ADVANCED RESEARCH PROJECTS AGENCY
NATIONAL CENTER FOR EARTHQUAKE ENGINEERING RESEARCH
NATIONAL SCIENCE FOUNDATION
SANDIA NATIONAL LABORATORIES
UNITED STATES ARMY RESEARCH OFFICE

TECHNOMIC PUBLISHING CO., INC.
LANCASTER · BASEL

Most teachers have never flunked bioluminescence. Many have never seen it, particularly in the Western United States where fireflies are not common. Even those who have experienced fireflies are quite surprised to learn of the other sources of bioluminescence. As soon as the subject is raised, they are almost always intrigued, mystified, motivated, and otherwise curious and interested.

We utilize bioluminescence as an effective way of imparting the scientific experience and method to elementary teachers in Utah. Nearly 400 teachers have now experienced this course and are utilizing these concepts and activities in their classrooms.

INSERVICE COURSES

We usually provide a ten hour course/workshop. We begin the discussion with a brief overview of the major national education surveys, reports, and movements of the past decade, with particular reference to the Project 2061 report of the American Association for the Advancement of Science (1), and the report titled "Elementary Science Education in the Nineties (2)." We also refer heavily on the works of Howard Gardner of Harvard, particularly his Frames of Mind and his more recent book The Unschooled Mind: How Kids Learn and how Schools Should Teach (3).

We try to loosen the teachers up. We talk with them about the arrogance and the myopia of modern discipline-based science. We share with them our conviction that the University is one of the worst places on Earth to learn science because we rarely teach it there. We teach specific disciplines. We rarely teach or emphasize the connections between those disciplines and the concepts and themes which are basic to all of those disciplines. We introduce them to the general science concepts and themes which pervade all scientific fields (Table 1) (1,2), relying heavily on metaphor and analogy with everyday experience. For example, housework is a battle against entropy. We use concepts of balance and opposing forces and tendencies, sort of the Yin-Yang hypothesis, order and disorder, white and black, hot and cold. Our goal is to make them feel at ease, not to intimidate them in any way. We encourage them to ask questions and from those questions begin to develop hypotheses based on common sense and every day relevance and experience. We utilize as much as possible what we have around us, what they are wearing, what they are drinking or eating, what they are carrying, particular props or materials in the room. We also spend time getting acquainted, learning something about their backgrounds and their fears and anxieties. The goal is to get them to open up.

SCIENCE IN THE DARK

The classroom we select has no outside windows. The room can be made completely black. We talk with them about dark adaptation. We go from light to dark in a gradual way over 10-20 minutes, all the time talking about concepts and themes, encouraging and inducing them to ask questions, to open up.

Table 1. General Themes and Concepts in Science, Mathematics, and Technology

EVOLUTION & DIVERSITY:	How does life change? Interdependence -- ecology -- ecosystems.
SYSTEMS:	The Universe, the Earth, your little toe, a bacteria? What to focus on.
SCALE:	Size, dimensions, measurement
STRUCTURE:	What is matter? How are things organized? What is their function? What is their shape?
CONSTANCY:	What is constant? How is it constant? Properties. "Constants".
CHANGE:	What is changing? How much? How fast? Why? Forces. Variables?
ENERGY:	What is it? Are there different kinds? How can we change it, use it?
DISORDER:	How are things disorganized? Why are most things statistical? What is entropy?
PREDICTION:	Models and theories -- experiments -- hypotheses -- the scientific process.
DECISIONS:	What and who is right? wrong? How can complex decisions be made? Who makes decisions?

We begin the Science in the Dark experience with "sea fireflies" (*Vargula hilgendorfi*), a small crustacean which is almost legendary now in the bioluminescence community because of its ability to produce bright bioluminescence when the dry crustacean is ground to a powder and wetted (available from Carolina Biological Supply Co.).

We look at the crustacean. We conclude that it is dead and dry. We get someone to grind it up, always using a very large white mortar and pestle. Another student (now it is nearly pitch black) sprays water into the powder. The brilliant glow in their, black, dark-adapted environment is dramatic and impressive. The oohs and aahs and "let me touch it and see it" go on for at least 5 minutes. The mortar is passed around. Everyone takes a turn. We grind a little more, we paint it on ourselves. By now the teachers are no longer squeamish and even the fishy smell of the Vargula does not bother them. Questions are being generated, hypotheses fly. "Why does it light up? Why does it need water? Does it stop when it is dry? What if I add more water? Why does it smell? Why is it blue? "

We simply encourage the questions. We encourage the formulation of hypothesis: how would you test that? "Let's try that again." We get into the scientific process. What is an experiment? What is an observation? What is a control? What is reliable, reproducible? We take the Vargula powder as far as is convenient.

Now they grind up wintergreen LifeSavers, experiencing triboluminescence (12). More questions, more hypotheses. We usually pull out a chemiluminescent Lite Stik, and, in the dark, get one of the students to activate it. It is cold light, and that is very different from normal light production (13). Many of them have experienced it. They start talking about two components. What is the noise inside? Why is it blue, green, or red, depending on the particular Lite Stik used. More hypotheses: why isn't the light hot, or is it? Warm hands, cold hands. How long does it last? Can you turn it off? Why does freezing stop it?

We are off on a pretty wide-ranging discussion on light. How is light generated? What is light? How do light bulbs work? What is fluorescence? What is phosphorescence? That takes us into the structure of the atom and electrons in atoms. Electron atomic and molecular orbitals, excited states, photon generation,color, waves and wavelengths. We spend a good hour or more on the principles of optics and light generation. We pull out flashlights, we pull out pocket lasers, we simply take that discussion as far as is convenient and effective.

Sometimes these courses are given in three, 3 hour segments, sometimes in one three hour segment followed by a second 7-hour segment. They are given some general literature on bioluminescence, a set of questions, several additional sheets to read...a little hype about their kits, their bugs, their dinoflagellates. There is a lot of anticipation about constructing their own kit, their own ecosystem.

DINOS IN ACTION

We go into a dark environment in which we have an array of flasks containing bioluminescent dinoflagellates, *Pyrocystis lunula* (6,7). The organism has been put on an alternate cycle so that it is approximately in the middle of its night cycle during this part of the course.

We package the dinos in sealed, transparent bags and -- in the dark -- have each of them catch a bag (8). The bag flashes blue as they catch it. Oohs and aahs, but much more intensive than in the first experience. They begin to shake them. Again more questions. "Why only when I shake them?" "Why don't they stay on?" "What is making this?" "Is this chemiluminescence?" We caution them that this time they are dealing with live organisms.

They notice that the bags light up in individual discreet points. They light up at the air/water interface. Some of them seem to be attached to the wall of the bag. They light up around bubbles. A lot of observation and primitive hypothesis formulation is going on. How do they live? How do we take care of them? What do they look like anyway?

We give them another set of bags. We tell them it is exactly the same thing. They shake them. They don't glow. There goes the hypothesis. We encourage them to formulate hypotheses and to discard hypotheses. Hypotheses are to be tested, not proven. We say they are plants, they need light. We start talking about light cycles, circadian rhythms, natural rhythms, jet lag. We put our dinos away and let them rest.

We go to microscopes. We simply take a drop of the dense culture between an inverted petri dish and look at them. They are large and moon shaped. What is all of that stuff inside? Here is one that has two nuclei, here is one that is more round. What is going on?

The long life cycle of *Pyrocystis lunula* makes them an almost ideal protozoan with which to study all aspects of cell division and fission and intracellular organelle structure and migration. Some of those who are involved in teaching biology and protozoa are absolutely fascinated. They think it is an ideal organism. Why haven't they heard about it before? How do they grow? What do they need? They are plants: photosynthesis, CO2, respiration, oxygen production, little ecosystem. "How do they live in the bags?" "How do they live in the flasks?" "How do they get air?" "How much light do they need?" We talk about sub-cultures, we talk about "feeding" them, we talk about seawater and supplements.

We show a video we have put together on bioluminescence which simply demonstrates many of the things they have already experienced. We show segments of a BBC video, "Creatures of the Night Sea." We show segments of the David Attenborough, "Trials of Life" video "Communicating with Strangers," particularly the segment showing the flashing fireflies and deep sea creatures (9).

What is the function of bioluminescence? Where is it found? Is the chemistry all the same? Is it different?

There is a real advantage to doing all this in the dark. They seem to be less intimidated; they can't see each other; nobody looks stupid, nobody knows or remembers who anybody is, so the questions and ideas just pour out of the darkness. We organize and formulate some of the questions into hypotheses, and encourage them to make their own experiments.

They put together a kit that 10-15 students can work with in their individual classrooms. The kit includes petri dishes, test tubes, pipets, media, and the *Pyrocystis lunula* colony (10).

.We give them a set of experiment sheets that they can utilize with their students, facilitating experiments on the role of light, on the health and stability of the cultures, their circadian rhythms, protozoan jet-lag, the role of turbulance and other means of excitation of the bioluminescence. There is even an experiment where they simply put the culture on an audio speaker cone and watch it luminesce in response to the beat and sounds in various audio tapes. This of course leads to discussions and experiments on the nature of sound, hearing, waves, and related topics. There is an experiment on light adaptation and dark adaptation, which gets into topics related to vision, light detection, photon generation and luminescence, and many other topics.

There are a set of microscope experiments which deal with observations of the dinoflagellates, their life cycle, and the nature of their light collection machinery. They evaluate the color of dense cultures, note that they are orangish or reddish, discuss the nature of chlorophyll and absorption and how these are different from green plants.

CONCEPTS AND THEMES

We come back to integrated science concepts and themes (Table 1). We are learning physics, optics, chemistry, biology, and environmental sciences. We talk about phytoplankton as one of the primary producers of the sea. We talk about the possible effect of the ozone hole (5). We talk about their role in minimizing the greenhouse effect. We talk about little ecosystems and big ecosystems.

A discussion of biospheres and ecospheres usually ensues, ranging from the tiny ecosystem represented by the *Pyrocystis lunula* plastic bag or flask to back yards, to local regions, and to Biosphere 2 in Arizona (11). We talk about the problems and uncertainties with Biosphere 2, treating it as an experiment in the study of complex systems. We then move on to Biosphere 1, i.e. the Earth, and a variety of current issues such as the ozone hole, greenhouse effect, and global warming. We talk about phytoplankton as primary producers and as responsible for a significant fraction of the CO2 consumption on the planet. We also discuss very recent studies dealing with the effect of increased UV on phytoplankton productivity and health in certain parts of the world (5).

NEEDLE A SCIENTIST

The course concludes with a plea to read and to probe, particularly for those elementary teachers who have high science anxieties and concerns about their science background. We say "needle a scientist, needle a physicist, needle a chemist, needle a biologist"; ask them about the basic premises and foundations of their area of science. Throughout the course, we question. We look at basic premises, constantly asking why, and concluding that science is based on a small number of un-understood phenomena and building blocks. We encourage them to feel comfortable about interacting with scientists, asking advice, ignoring the arrogance of many professional scientists, needling them, using them, appreciating them, and learning from them.

We always conclude with a discussion of current popular science writing with which they can probe deeper, enjoyably. We encourage them to use popular science writing in their classes as appropriate, particularly for those teachers involved in high school courses. We refer extensively to the books by Stephen J. Gould, John McPhee, Loren Eisely, Rachel Carson, E.O. Wilson, and many others.

THE FUTURE:

Bioluminescence is just the first step. It's designed to hook them, get them interested, get them stimulated, make them receptive; from then on, the sky's the limit.

Depending on the amount of time available and the specific interests and questions from the teachers, a variety of other topics are developed. We try to make them appreciate how important science and technical education is for the general public and not just for budding scientists and engineers.

We are developing programs where we will discuss health care and their role in educating students to be interested not only in clean and healthy living styles, but also in functioning as physician's assistants, that is to help serve as eyes and the ears, as information gathering source, to aid health care practitioners in their efforts in diagnosing and treating their ailments.

We are beginning work on a number of other unique, strange, uncommon biological phenomena for science education purposes. Stay tuned!

ACKNOWLEDGEMENTS:

Most of our work for 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 are funded by the the U.S. Department of Education, Eisenhower Grant Funds, administered by the Utah State Board of Regents.

REFERENCES:

1. FJ Rutherford , A Ahlgren, Science for All Americans (The Project 2061 Report), Oxford University Press, 1990.

2. Loucks-Horsley, et al., eds., Elementary School Science for the 90's, Andover, MA: The Network, 1991.

3. H Gardner, The Unschooled Mind: How Children Think and How Schools Should Teach, Basic Books, 1991.

4. M Gail Jones, "Bioluminescence," The Science Teacher 1993; Jan: 19.

5. RC Smith, et al., "Ozone Depletion:...Phytoplankton Biology...," Science 1992; 255: 952.

6. FJR Taylor, ed., The Biology of Dinoflagellates, Blackwell Sci., 1987.

7. DL Spector, ed., Dinoflagellates, Academic Press, 1984.

8. Available as the product Galaxsea™ from Protein Solutions, Inc., 350 West 800 North, Suite 218, SLC, UT 84103-1441, (801) 596-2675.

9. "Communicating With Strangers," available from Ambrose Video, 1290 Avenue of the Americas, Suite 2245, New York, NY 10104.

10. Available as the product Night-Life™ from Protein Solutions, Inc., 350 West 800 North, Suite 218, SLC, UT 84103-1441, (801) 596-2675.

11. A. Alling, M. Nelson, S. Silverstone, Life Under Glass, Biosphere Press, Tucson, 1993, (602) 825-6489.

12. W.L. Dills, Jr., "The Great Wintergreen Candy Experiment," *Science Scope*, Nov/Dec, 1992, 24-27.

13. A.K. Campbell, *Chemiluminescence* VCH Publ., 1988.

14. R. Tinnin and E. Buskey, "Bioluminescence: Living Light of the Sea," *Currents* **12** (3) (1994) 10-13.

"Leadership in the Academic Centrifuge"

by Joseph D. Andrade
Professor of Bioengineering and Materials Science
University of Utah
presented at the Whitaker Foundation Biomedical Engineering Research Conference
Snowbird, Utah, August 5, 1994

It is a pleasure to address this 1994 Whitaker Grantee Conference. You are all considered young or relatively new investigators in the early stages of your academic and research careers. The majority of you are in academic and research institutions and most of you are still several years away from tenure decisions. I would like to share with you some perspective on six short topics:

- The Academic Centrifuge,
- Education and Responsibility,
- Universities and Their Faculty,
- Communication With the Non-Technical Community,
- Leadership -- a Crisis in Higher Education; and,
- You -- Your Role and Involvement in the above five topics.

First, the Academic Centrifuge. Harold Shapiro, president of Princeton and former president of the University of Michigan, has expressed this most succinctly, "One challenge that bears critically on the future of education is the centrifugal force of academic specialization...centrifugal forces threaten to drive all the separate groups within the university community away from the core and to the periphery."

All of you are tubes or vials in an academic centrifuge. The nature of the tenure process in most universities, particularly the more prestigious research universities, with which most of you are affiliated, directs and insists that you focus practically all of your efforts in outstanding research performance and productivity, including, of course, research funding. In most traditional departments you are expected to be strong and solid in your specific discipline and particularly in a sub-discipline. Collaboration, particularly

with people outside of your department, and especially with individuals from widely different departments, is often treated with suspicion and grave concern. Most of you have already experienced this. Those of you who haven't probably will soon.

As bioengineers you are intrinsically inter- and multi-disciplinary. Your situation is perhaps a bit easier if you are in an interdisciplinary department. But even in "interdisciplinary" departments, the centrifugal forces are strong. You don't have time to be involved in other topics or in issues or concerns beyond your department, your college, and your university.

Your overwhelming commitment, drive, and direction is directly picked up by, and passed on, to your students. In the words of Frank Rhodes, president of Cornell, "What frustrates students...is the apparent disengagement of many of their teachers from any interest in the larger questions or even in the broader relationships and implication of the subjects they teach...We simply have not done well in linking...scholarship and professional practice to critical thinking about the fundamental issues of life."

Derek Bok, former president of Harvard, goes on to say, "There is much evidence to suggest an erosion of many forms of personal responsibility...at a time when these attitudes of trust and moral concern seem weakened and precarious, it is important to ask how they can be strengthened and what higher education can contribute to the process. "

Some of your students, particularly undergraduates, will expect you to at least have some interests in societal topics, in addition to your concerns with specific research projects and assignments. This all leads to a broader question as to the role and responsibility of universities in our society.

Donna Shalala, former president of the University of Wisconsin-Madison: "There are those who believe that universities can only reflect society. I am not among them. I believe that universities must be role models for society. We must have the courage to lead."

Vaclav Havel, president of the Czech Republic, has stated, "The only genuine backbone of all our actions...is responsibility." And yet, that is not discussed, at least not very much, in most professional programs.

There is growing concern with issues of ethics, but they tend to be focused on professional ethics, the number of authors on a paper, non-overlapping of grants, etc. There is little discussion of societal responsibilities.

Students must be made "to understand that to acquire special expertise is to acquire power and that it is dangerous to wield such power without learning to use it responsibly... No professional school should risk creating an impression that matters of moral and social responsibility are digressions, or sentimental irrelevancies, rather than integral parts of all sound analysis" -- again, Derek Bok, former president of Harvard.

Perhaps Albert Einstein said it best, "It is not enough that you should understand about applied science in order that your work may increase man's blessings. Concern for man himself and his fate must always form the chief interest of all technical endeavors. Never forget this in the midst of your diagrams and equations." Derek Bok continues that such concerns include "honesty, promise keeping, free expression, and helping others, for these are not only principles essential to civilized society, they are values on which all learning and discovery ultimately depend."

Part of this responsibility involves communication with the general public, including the press. We all have the responsibility to communicate what we do to others, not only for professional development and advancement, but because it is also in the best interest of society that it be informed and knowledgeable. Ultimately society is paying the research bill, and society has a right to know what it is purchasing. The people have a right to experience, or at least appreciate, scientific progress and scientific and technological achievements. This means being able to communicate not only with one's peers and colleagues, but also with the lay public and the lay press. Many of you have already experienced that it is not necessarily easy to communicate with the public, and particularly

with the press. What you say may be misinterpreted, distorted, or even outright misrepresented. After being burned you will want to avoid the press. But is essential that you do not succumb to such a temptation.

We have a scientifically illiterate society: this is a particular problem in our legal and our political systems. This is not surprising, because we as scientists and engineers have not generally been involved in that communication process. We have delegated it to others. The delegation clearly hasn't worked. We must all be personally and individually involved in the communication process whether we like it or not. It is possible to communicate what you do to the lay public. If Stephen Hawking can talk about advanced cosmological theories to the lay public, then surely you and I can communicate what we do in biomedical engineering, a subject which is generally highly relevant to the typical man or woman on the street.

In the words of Leo Vroman, a wonderful scientist well-known to some of you, "The day I have come home unable to explain to a child what I did, that day is lost and that child has receded from me."

It is, of course, necessary to make your comments relevant and to make extensive use of analogy and metaphor, and to otherwise relate what you are explaining and saying to the specific background and interest of your audience. Again, to paraphrase Albert Einstein: "Never forget this in the midst of your diagrams and equations."

Our final brief topic is leadership. Again, Donna Shalala: "If you asked Americans who the country's top ten leaders are they probably would not list a college president. It's really been relatively recently that the heads of the great universities were not significant leaders in this country."

Warren Bennis probably expresses this the best in his series of books on university unleadership. In *Why Leaders Can't Lead: The Unconscious Conspiracy Continues*, 1989: "In previous generations, at any given moment, there were a half dozen university heads who were known and respected throughout the world. James Conant, Robert Hutchins,

Clark Kerr, and their like did not merely run their universities but led a kind of constant national colloquy on the state of education in America. Their turf was not simply their university but all of education. I cannot remember the last time any university president addressed any problems beyond his or her own campus. Universities have changed, and so have university presidents."

"Today, almost no college or university president has spoken out significantly about Bosnia, Haiti, North Korea, health care, welfare reforms, the attack on the National Endowment for the Arts or dozens of other issues high on the national agenda." (W.H. Honan, *New York Time*, July 24, 1994).

I feel strongly that universities must provide much of the vision, the problem solving expertise, and even the conscience of society, and that responsibility should be expressed and endorsed by presidents or chancellors. Most of them, as have many of our national leaders, have a problem with, to quote George Bush, "The Vision Thing." In some cases presidents are actually told to lay low and not get their universities in trouble.

Society needs leaders. John W. Gardner's little book *On Leadership* expresses the reasons very succinctly. He also talks about the mechanisms of leader formation: "Leaders have always been generalists. Tomorrow's leaders will, very likely, have begun life as trained specialists, but to mature as leaders they must sooner or later climb out of the trenches of specialization and rise above the boundaries that separate the various segments of society." To use the Harold Shapiro academic centrifuge model, they have to climb out of their particular centrifuge tube and make their way more to the central core of the university -- where they can experience wider views and a larger perspective.

Buckminister Fuller, shortly after being introduced by a university president to give a commencement speech, is reported to have said, "What you fellows in the universities do is to make all the bright students into experts in something. That has some usefulness, but the trouble is it leaves the ones with mediocre minds and dunderheads to become the

generalists who must serve as the college presidents...and the Presidents of the United States. "

What does all this have to do with Whitaker Fellows and Assistant Professors? You were those bright students that Buckminister Fuller was referring to. Bright students who are now university professors, educating and training other bright students, practically all of whom go into appropriate tubes in the centrifuge. There is nothing wrong with this.

What I am suggesting and asking is that, if you have a natural tendency or a natural interest in more than one part or subject of the university, and indeed all of you do, otherwise you wouldn't be doing biomedical engineering, you consider (after you have tenure) climbing out of the centrifuge tube and getting involved in wider issues.

Don't let anyone tell you that service as a department chair, election to a school board, even service in your state capitol, detract from your career. What detracts or augments depend on what you want for your career -- and on the exercise of your responsibilities to your society.

I have a strong belief that the reason society is in such deep trouble today is that most of the people responsible for the various segments of that society were not effectively or appropriately educated in their undergraduate days. They generally do not have the vision, the background, the ethical convictions, nor the brains to deal with complex societal problems. Those of us in universities must be involved in assuring and guaranteeing that every student that goes through the system has an outstanding education and has some appreciation for social responsibility and leadership. That includes you -- and your students.

Good luck.

Don't stand on the sidelines

This is a call for the technical community to demonstrate a new sense of social responsibility, particularly in the ongoing health care debate.

Joseph Andrade

We are all members of a hierarchy of communities: our families, the institutions in which we work and contribute, our cities, counties, states, nations, and our lovely green and blue biosphere—the planet Earth. Except possibly at the biosphere level, we are members of each of these communities by choice. We could choose to live in a different state, in a different community, and even in a different family. Members of communities have responsibilities: They must be involved in the process of determining the community's values, its needs, and its governance. They should not leave these duties and responsibilities to others.

Unfortunately, the technical community, and the engineering community in particular, tends to relegate some of its social responsibilities to others. Often during the upper elementary and junior high school years a number of social bifurcations materialize: One is typified as a "people person" or as an analytical person; one is a life science person or a physical science person. These divisions may continue throughout our high school, college, and professional careers. The caricature of engineers and scientists is that they are not people people: They shun reporters; they have disdain for politicians; they tend not to serve on school boards, city councils, or state legislatures.

Individuals with engineering and technical backgrounds can and should make significant contributions toward solving a range of social and national problems. Their technical and analytical training and background can provide vital input into practically all socioeconomic issues and controversies, such as the role of technology in health care, the environment, and the biosphere as well as in weather and natural catastrophes. We must insist that these important issues and problems be addressed by our various communities and social systems. We must help generate the political will and the leadership to address these important societal concerns.

Our training and background provide a perspective as well as a set of analytical and critical tools. That perspective is often absent or is incompletely and ineffectively presented in sociological debates and deliberations—not because people do not want to listen and not because we are ostracized or kept from being involved but because our community in general simply has chosen not to be involved. It is not only we who suffer the consequences of that choice: Our society also suffers.

Those days are over. We see the development of a new ethic, a new sense of social responsibility in the scientific and technical communities. The old perception—and excuse—of the "two cultures" is weakening. We look forward to an increasing level of involvement by the scientific and technical communities in the full spectrum of societal problems, particularly in the nature of health and health care.

Defining the problem

Engineers rarely attempt to solve problems that don't exist. They like well-defined, well-presented problems. The health care problem has not been adequately defined. The problem is not only the 14% of our GNP spent on health care; it is not only the 40 million people in this country who are uninsured; it is not only the more than 2,000 individual insurance companies, each with its own forms and bureaucracy—the problem comprises all of these issues and yet is even more basic. What do we really mean by "health care"? Have we defined health, health care, and health policy properly? They are defined very differently in other countries. These questions are difficult; they are not particularly analytical, and they are the kinds of questions that engineers don't like to address. Engineers and physical scientists chose their respective professions because they like well-posed problems; they are very uncomfortable dealing with ill-posed questions and problems. Nevertheless, as socially responsible adult citizens, we must deal with the problems in our communities.

The state of Oregon has been involved in discussions and planning pertinent to the general health and well-being of its population for the past five years or so (*1–3*). After many years of public discussion and debate involving all sectors of the state's population, the Oregon Health Care Plan (Oregon Plan) evolved. This plan defined and identified values and needs with respect to health and quality of life; it addressed the issues of access, quality, costs, and economic constraints, and it formulated a standard benefits package for health and health care, with a major emphasis on prevention, healthful living styles, and

The author is with the University of Utah and Protein Solutions, Inc.

BRUCE VAN PATTER

early diagnosis. There was a de-emphasis on expensive, heroic, and halfway technologies and procedures. The Oregon Plan, and the dialogue preceding it, generated a considerable amount of criticism, discussion, and debate. It nevertheless serves as a model for what we as a nation and what we in other states and regional communities can do.

Scientists and engineers know that problems need to be at least partially defined before they can be effectively addressed and eventually solved. We really need to decide whether health care is a right, and/or how much health care is a right, and how much we are willing to spend on health care. What about individuals who insist on living unhealthy lifestyles? Do we wish to maintain the choice and independence characteristic of the present system? Do we want to empower patients to have a significant financial stake and intellectual stake in their health and well-being and in the selection of treatments or nontreatments of their health-related problems? These issues are primarily societal questions that must be addressed in appropriate forums to define the health care problem.

Calculating true costs

What are the true costs of health care? The costs of health and well-being begin at the moment of conception and escalate from there. Prenatal care costs can be allocated to both the mother and the fetus, as can the costs of birth itself. Can we develop a calculus for the total costs and benefits of a human life? If my average life span is 70 years, what will it cost my parents, me, and my community from my conception to my death and burial? We have rarely, if ever, looked at the problem in such an inclusive manner.

Missing a vaccination in childhood can lead to significant problems and inordinate costs later in life. We can't focus simply on the incremental cost of that vaccination or on the incremental cost of health problems resulting from not having had the vaccination; the issues are all related. The integral stretches from conception to burial, and it includes an enormous amount of virtually unpredictable statistics and probabilities. Some of us know how to do integrals. Some of us know how to deal with noisy data. Some of us know how to trade quantity in data for quality in application. We can help address those complex problems.

Is the cost of handgun control, or gun control in general, a health care cost? Are drug prevention and drug treatment programs health care costs? Is a summer job for an inner-city teenager, who is likely to acquire both guns and drugs, a health care cost? These items are all part of the calculus, this extremely complex integral. We must now integrate, over the life of one individual plus all individuals in society, considering all of the cross-terms. The integral must include all of society. Engineers, at least certain kinds of them, know how to deal with very complex systems, and they can help develop models and means to define and to address such complicated problems.

Benefits and risks

The scientific and technical community tends to have some appreciation of probability and statistics. People with even the weakest introduction to the life sciences know that organisms eventually die and that they die from myriad causes. Most of us know that the Gaussian distribution, or normal, curve is more or less endemic throughout biology. Yet although these principles tend to be part of the education of some college and university students, they are not a part of the education of the general public or of their lawyers. Unfortunately, these concepts are also not well known among many physicians and medical providers, and when they are, they

often are not communicated effectively to their patients.

Clearly there are more safe as well as less safe activities and procedures; there is malpractice; there are poorly designed and manufactured devices. We need effective regulation with respect to safety and efficacy. But, just as clearly, there are statistics and there are probabilities. No one is to blame if an earthquake levels your town or your home. It may be your error for having chosen a home on an earthquake fault, if that fact indeed was known to you. Is it really society's responsibility if you live on a 500-year flood plain? Is someone to be blamed if 1 out of 10,000 medical implants leads to a negative or even catastrophic outcome? Is it fair or right to insist that the device have a one-in-a-million failure rate, increasing its development, testing, and manufacturing costs by orders of magnitude to produce such reliability? Is it fair to make the other 999,999 individuals who receive that implant pay the exceptionally high cost required for an increased level of safety?

Everyone, but especially lawmakers and lawyers, must come to understand statistics and probability: You and I and our colleagues must educate them. We must ensure that these concepts are incorporated in all professional courses of study—in all majors, on all campuses, in all schools.

What we can do

Health care and its costs are important to every single individual in society. We are all involved and all part of the problem. Every single group, every component of society, will have to change its behavior patterns to effectively and successfully solve the health care problem (*4*). In some respects, we must all do less so that we have the time to accomplish those things more compassionately, relevantly, and effectively. We must encourage people, ourselves included, to get off treadmills leading to nowhere so that we will have the time to reflect, identify the problem, and solve it.

All groups must become far better informed and involved in the entire health care arena. They must select their physician collaborators more carefully. They must design their experiments—particularly animal and human experiments—far more carefully and efficiently. They must attempt to determine the societal impact (*5*) of new medical procedures or technologies. They must consider and perhaps overcome the technological imperative.

The issue of risks, particularly as applied to health care, must be considered and communicated to the general public and to the media. The difference between voluntary and involuntary risk must be made clear. The role of risk awareness in influencing behavior is critical. The importance of patient choice and empowerment should be emphasized. Cost will probably not be brought under control without a general appreciation of the limits of technology and of the inability of devices to correct the natural wear and tear of the aging process. This includes the acceptance by patients of risks commensurate with the statistically demonstrated benefit.

Contributing to the public view

The communication skills of scientists and engineers generally are not fostered in our curricula, and few incentives exist for developing those skills. Because people with communication skills often do not have scientific and technical skills, the information that reaches the general public (with the exception of that provided by a few good science writers and reporters) is usually incomplete at best and completely erroneous or misrepresented at worst. We must rectify this situation. We have to challenge those reporters, writers, and lawyers. We have to inform them, and the community they influence, that their scientific and technical facts are correct/incorrect, their analysis of a technical or semitechnical issue is appropriate/inappropriate, and their consideration of risk is reasonable/unreasonable given the data and understanding at hand. Not to do so is socially irresponsible; it leads to the system and the problems we have, and the blame becomes ours for being so uninvolved.

Every major report on engineering education, medical education, undergraduate education in general, and public education over the past decade or so has urged us to teach less, but teach better, and to educate in a more integrated and systems-like manner. We need to produce graduates and professionals who are good listeners, effective communicators, and responsible citizens.

Although I have yet to see a significant response to these reports in the higher education sector, the public education sector is changing rapidly. There are major movements and activities throughout the nation to enhance education at the elementary, junior high, and high school levels, particularly in science and technology education, which includes mathematics, statistics, and related topics. I am optimistic. As these students advance to college and university and challenge professors and fellow students and then join the job market and interact with co-workers and fellow citizens, I think they will help realize the fulfillment of the hope for a more responsible and involved electorate.

We need no less.

Acknowledgments

The topics and opinions presented here have been discussed and formulated over the past several years with many colleagues, co-workers, and friends. I particularly thank those curious, dynamic, and creative students who have participated in a set of nontraditional courses over the past few years: Critical Science Communications, Bioengineering and the Costs of Health Care, Using Bioengineering for Science Education, and Integrated Science Concepts and Themes. Very special thanks are extended to Dov Jaron and Peter Katona for encouraging discussion on these important topics.

References

(1) Kitzhaber, J. "A Healthier Approach to Health Care"; In *Issues Sci. Tech.* Winter 1990–91, pp. 59–65.

(2) Kievit, H. D.; Bates, A. C.; Castanares, T.; Kirk, E. P.; Sipes-Metzler, P. R.; Wopat, R. "Prioritization of Health Care Services"; In *Arch. Intern. Med.* **1991**, *151*, 912–16.

(3) Reingold, E. M. "Oregon's Value Judgment"; In *Time*, 25 November 1991, p. 37.

(4) Lanier, W. L.; Warner, M. A. "New Frontiers in Anesthesia Research: Assessing the Impact of Practice Patterns on Outcome, Health Care Delivery, and Cost"; In *Anesthesiology* **1993**, *78*, 1001–4.

(5) Keller, K. H. CHEMTECH **1995**, *25*(1), 7.

Adapted from "Medical and Biological Engineering in the Future of Health Care," edited by J. D. Andrade.

Joseph D. Andrade is a professor of bioengineering and materials science and engineering at the University of Utah (2480 Merrill Engineering Bldg.; Salt Lake City, UT 84112; 801-581-4379). Andrade founded and directs a small biotechnology company, Protein Solutions, Inc., which develops novel materials for science education. He served as vice president for public policy of AIMBE. (Send e-mail to <jandrad@cc.utah.edu>.)