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III: EXECUTIVE SUMMARY

The development of surfaces resistant to adsorption, adhesion, deposition or fouling by macromolecules and by particles is of considerable practical, scientific, and theoretical interest. Surfaces resistant to the adsorption of proteins and to the adhesion of cells are especially needed, particularly in the health care products and biotechnology industries. Most peptides and proteins are very surface active. Indeed, the structure of a protein in solution can often be viewed as an intramolecular micelle. 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).

The investigators in this proposal have all been working on various aspects of the PEO/protein problem for many years--although we have worked together and collaborated extensively on these studies, and a number of joint publications have resulted--this materials research group project will provide the means for a much more fully coordinated and integrated collaborative effort. By the sharing and co-supervision of graduate students, by directing and focusing our individual and collaborative efforts to the hypotheses, key problems and controversies in the proposal, and by regular meetings and seminars involving all participants, we expect to make rapid progress on this interesting and important problem.

We propose to address two general hypotheses/themes in this work:

1) 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.

2) Due to geometric constraints, the above criteria can be met only on highly curved surfaces; that is, that ideally flat surfaces cannot be made as optimally protein-resistant, based on PEO, as can surfaces with low radii of curvature.

We propose to address these hypotheses by application of theory and simulation as well as by experiment. The simulations and experiments will be addressed from three different perspectives:

1) The nature of PEO homopolymer and copolymers in aqueous solutions;
2) The behavior of these molecules at interfaces, including the air/water, lipid/water, and various solid/water interfaces; including their immobilization by adsorption, covalent coupling, and cross-linking.
3) The characterization of PEO-containing surfaces and the interaction of such surfaces, as well as the appropriate controls, with a set of model proteins whose three-dimensional structure, solution behavior and interfacial behavior are well characterized.

Our work will be aided by a distinguished group of international advisors: P. de Gennes (theory of polymers at interfaces and in solution), H. Ringsdorf (monolayers and

lipid/water interfaces), and T. Matsuda (PEO surface immobilization). Dr. Sang Il Jeon will also assist with several of the modeling studies.

We request a three-year project at about $270,000 per year, which will permit the eight investigators, the four advisors, and four shared/co-supervised students to optimally address these issues. We anticipate several additional students will also work in this program, supported by training grant and fellowship funds. We also expect that the funds now available to the individual investigators for their PEO/protein resistance studies will continue, and will also contribute to the activities in this program.

IV. RESULTS OF RESEARCH UNDER PRIOR NSF SUPPORT:

J.D. Andrade, PI

Title: "Protein Interactions with Gradient Surfaces"

Award: INT-87-19079, International Cooperative Research

This international cooperative research award, which ended July 30, 1992, facilitated the collaboration between Drs. Hai Bang Lee and Jin Ho Lee at the Korean Research Institute of Chemical Technology, and J.D. Andrade, V. Hlady, and Y.S. Lin at the University of Utah. Dr. Andrade and Mr. Lin visited the Korean group in May and June, 1992 respectively, to complete the joint work. Drs. Hai Bang Lee and Jin Ho Lee will visit the University of Utah in October, 1992 to help complete the final report. Dr. Sang Il Jeon, of Kangrung University (Kangrung, S. Korea) has also been a participant in the work.

The Utah group focused on the preparation of hydrophobic/hydrophilic gradient surfaces on silica and glass, using silane chemistries in a diffusion gradient, and focused on the study of protein interactions with these gradient surfaces. The Korea group focused on the preparation of gradients by corona discharge techniques, their characterization, and their study with respect to cell adhesion and invitro cell culture.

Both groups are continuing their studies and their collaboration. It is expected that the interaction will be a very long-term one.

Publications:

The joint work also stimulated an SBIR application, the ATP-Corona Pen, a portable device for monitoring bacterial populations on surfaces using bioluminescence and corona discharge treatment, submitted by Protein Solutions Inc., Salt Lake City, to NASA, July 1992.

J.D. Andrade and H.B. Lee, "Using Gradient Surfaces for Biomaterials Education", Abst., 3rd annual fall Biomed. Engrg. Soc. Meet., 1992.

Y.S. Lin, V. Hlady, and J. Janatova, "Adsorption of Complement Proteins on Hydrophobicity Gradient Surfaces", Biomaterials (1992) in press.

J.H. Lee, J.W. Park, and H.B. Lee, "Cell Adhesion...Plasma Treatment", Biomaterials, 12 (1991) 443.

V: JUSTIFICATION FOR MRG MODE OF SUPPORT:

"The MRG program provides support for collaborative multi-investigator efforts which addresses major problem areas in materials research...proposals should demonstrate that the group activity is integrated and coordinated" (from NSF Program Solicitation, NSF 92-93).

We feel that the group identified in this proposal is ideally suited for MRG support. J.D. Andrade and S.W. Kim have been close friends and collaborators since 1969 when they both started their academic careers at the University of Utah. Kim's primary appointment is in the Department of Pharmaceutics while Andrade's is in Materials Science and Bioengineering. They have shared common research interests in many areas, and have shared graduate students and co-authored papers.

Karin Caldwell joined the University of Utah in 1968 in the Department of Chemistry. She began collaborating with the investigators in Materials Science and Bioengineering in 1985. She directs the Center for Biopolymers at Interfaces (CBI), a University/Industry/State research consortium. She has stimulated a great deal of activity on the campus on the study of PEO, particularly using high surface area systems and chromatography/field flow fractionation (FFF) techniques.

J. Kopecek joined the faculty as professor of Bioengineering and Pharmaceutics in 1989, having spent several years prior to that time as visiting professor. He works closely with Kim and Andrade and co-directs (together with S. W. Kim) the Center for Controlled Chemical Delivery (CCCD).

James Herron joined the faculty of the Department of Pharmaceutics and Bioengineering as assistant professor in 1988, after serving for three years as a post-doc. in the Department of Biology, working in the area of antibody crystallography. A strong collaboration developed between Herron and the group in Engineering in 1984 in the area of optical immunosensor development. This initial collaboration has led to a wide range of collaborative projects and activities.

Dr. V. Hlady was appointed associate professor of Bioengineering in 1992. From 1990 through 1991 he served as associate research professor of Bioengineering, and had previously served as visiting associate professor. He has worked extensively on the study of protein interactions with surfaces, including PEO-gradient surfaces.

Dr. Jules Magda joined the University in 1988 as assistant professor of Chemical Engineering, and also accepted an appointment as assistant professor of Materials Science in 1991. The last several years he has been interacting with the other investigators in the group through the Center for Biopolymers at Interfaces.

Natalya Rapaport joined the faculty as Research Professor of Materials Science in 1991 after serving as the distinguished Clyde Professor, a visiting professorship in the College of Engineering, in 1990. Dr. Rapaport has developed a very strong collaboration with several of the investigators in this project and through the Center for Biopolymers at Interfaces.

These investigators have all been working on various aspects of polyethylene oxide science and engineering, on proteins and their behavior at interfaces, and on various applications of these topics to a range of industrial problems. They have all been involved with the Center for Biopolymers at Interfaces (CBI), a University/

Industry/State Consortium, with 25 members representing a wide diversity of health care, biotechnology, and scientific instrument companies. In addition Drs. Kim and Kopecek direct the Center for Controlled Chemical Delivery, which also works closely with industry on problems and developments in the drug delivery arena. This group, and most of the departments they represent, have a long track record of successful inter- and multi-disciplinary, collaborative research.

Figure 1 below shows you each of these individuals and attempts to show their collaborative activities related to PEO and to Proteins, as evidenced by joint publications and jointly supervised students. The availability of MRG funding will enable this group to become even more cohesive, interactive, and collaborative, and to direct their efforts and talents to the important topics proposed.

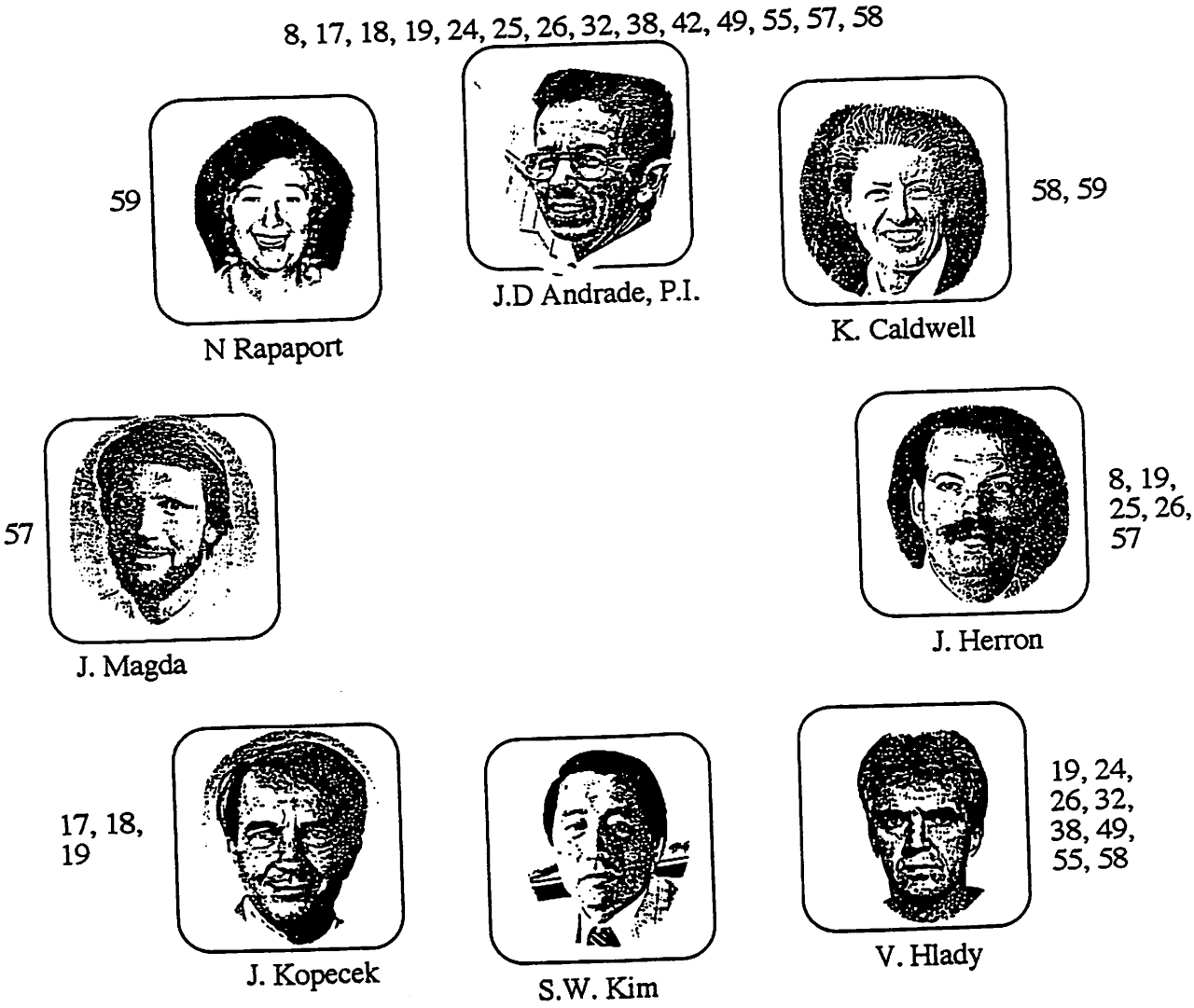

Figure 1: The eight MRG investigators, their joint publications, and jointly supervised students (References are on p29).

VI. RESEARCH PLANS:

A. Overview:

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 particle. 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 very widely used in the chemical industry, in biochemistry, and in the biotechnology industry (1-3).

Polymerized ethylene oxide is somewhat of an anomalous molecule (4-8). 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. In this proposal we focus on interface modification by PEO and PEO-based polymers (Figure 2) and the optimization of the protein resistance of such surfaces.

Although e and f in Figure 2 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" (7, 8), 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.

Let us now briefly consider the problem of what happens to these different surface geometries when presented with a protein solution, containing proteins highly interactive for the underlying substrate. Consider proteins of the order of 50,000 to 100,000 Daltons, but consisting of a set of interconnected, smaller domains in the range of 10,000 to 15,000 Daltons; this is the structural and functional building block concept, now well accepted in structural biochemistry (22-24).

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 (24-26). 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 (27) to further complicate the problem. Another concern with Figure 2 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 2.

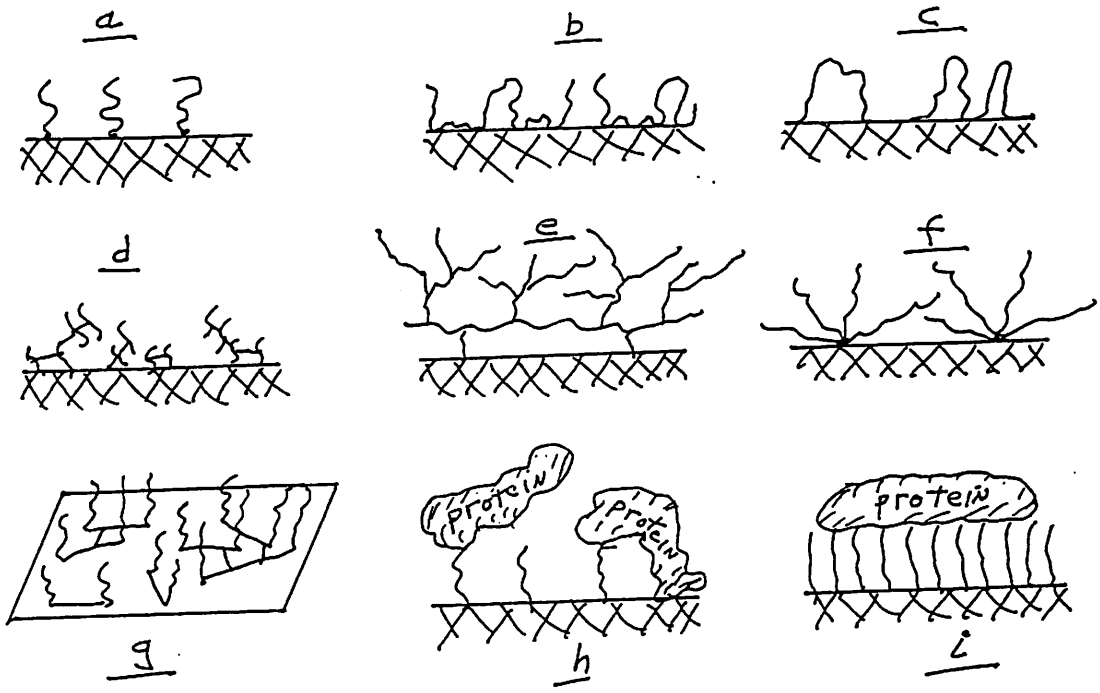

Figure 2: Some of the many structures and configurations which have been suggested for PEO or PEO-derived polymers attached to surfaces.

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 (9-10). 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 on to 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 (11, 12). 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 (13, 14). 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 (17). 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 (18).

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 (15). 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. 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 (16). This is also reminiscent of the tetronic family of polymeric surfactants, where h PEG chains extend from four PPO chains attached to a tetrafunctional nucleus (17).

g) represents the case of a block or graft copolymer designed for optimum adsorption (18), which is then surface cross-linked between the chains (20, 21) or between the polymer blocks and the surface (21) by either specific cross-linking reactions or via plasma reactions (21).

h) PEG chains are often used as a means to provide a tether between a protein, or other biomolecule, and the surface (7). 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 (19). 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, therefore, 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 Figure 2i 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 (28, 29).

Figure 3 shifts our focus away from the interface and, for better understanding, to the behavior of PEO molecules in solution. There is considerable activity and controversy regarding the solution behavior of PEO. The behavior of a single , reasonably high molecular weight chain of PEO in aqueous solution is not well understood.

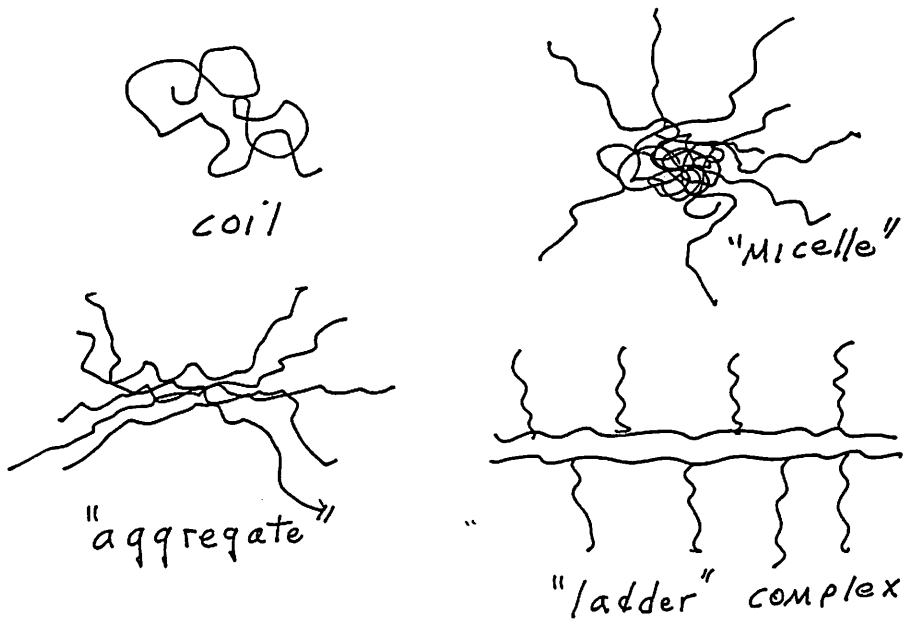

Figure 3: Some PEO solution structures

a) indicates a more or less random coil, which would be the case under theta solvent conditions. Although such conditions can be approximated for PEO in aqueous solutions, there has been little study of the behavior of PEO in solutions of biochemical and physiologic interest. The behavior of PEO in solution is known to be a strong function of temperature, ionic strength, specific ions present, and other factors (4-8). Under some conditions the coil will be somewhat collapsed and one can perhaps think of the molecule as an intramolecular micelle; other conditions would produce a more extended conformation. When PEO is connected to other entities, as in block co-polymers or surfactants, then a variety of other structures can evolve, including more or less typical micelle structures (b), macromolecular aggregates (c), various macromolecular complexes (d), and other structures (3).

When such complex molecules as PEO and its copolymers (Figure 3) are induced to reside at an interface, the mere presence of the interface and the interactions with the solid substrate perturbs the equilibria noted in Figure 3, so that the real world will be some hybrid between Figure 2 and Figure 3, and will of course depend on the specific solution conditions present.

With all this complexity, one might ask <u>why PEO?</u> Why not consider other approaches to the passivation of surfaces with respect to protein adsorption?

Water soluble polymers and water swellable gels, so called hydrogels, have been studied and used for decades to enhance the biocompatibility, blood compatibility, and protein resistance of materials (30, 31). Nearly all proteins have hydrophobic residues and hydrophobic patches on their surfaces and of course in their interior, have various positive and negatively charged groups dispersed on their surfaces (22-26), and generally tend to be capable of significant conformational change and adaptation at interfaces. Nearly all surfaces which are significantly hydrophobic or have positive or negative charge on their surfaces tend to bind or adsorb protein. Thus protein-resistant surfaces

tend to be neutral, to minimize electrostatic interactions, and highly hydrophilic, to minimize hydrophobic interactions. Of all the neutral, hydrophilic, water soluble/swellable polymers readily available (31-35). PEO appears to be the most mobile, the most dynamic, and the least interactive. PolyHEMA is actually quite hydrophobic and adsorbs proteins. PVP and PAAM have very strong dipole moments and H-bonding characteristics which sometimes lead to protein complexation. The polysaccharides have rigid rings with multiple hydrogen-bonding groups. Their helical structure often leads to interactions and to local junction point formation (such as in cellulose and agarose). It is in fact these interactions that give polysaccharide gels their unique chemical properties coupled with high water absorbancy.

Albumin is a protein which has been used for surface passivation. Its disadvantage is instability to interface protease activity as well as possible interactions with other proteins. The "neutral" phospholipids, such as phosphatidyl choline, are somewhat dynamic in the plane of the lipid film, and, although minimally interactive with proteins (36, 37), can also bind and complex with many types of proteins and protein domains (38). Of all of these polymers and surfaces, polyethylene oxide appears to be the least interactive.

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 (39). 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" (40). Nevertheless, of all the polymers we know, it appears to have the highest potential for the development of truly protein-resistant surfaces (41-43).

Over the years the investigators in this grant have worked with most of the polymers noted above and have concluded that PEO has the greatest potential for the development of practical protein-resistant surfaces, and the greatest interest in terms of the <u>science</u> of protein resistance.

We feel a very major factor is the way in which the hydrophilic polymer chains interact with water. Indeed it is perturbation and the structure of water, particularly around apolar solutes and surfaces, which is the driving force for the hydrophobic interaction. Thus any polymer which perturbs the surrounding water structure is likely to induce at least a small level of hydrophobic interaction with the appropriate regions on a protein surface. 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. The particular properties of PEO which we feel are important in its protein resistance have been summarized (4, 6, 17, 28, 41-43). 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. 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. 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.

Thanks to the assistance and tutoring provided by Professor de Gennes (see section on Advisors), we performed a theoretical analysis of the interaction of a hypothetical spherical protein with an ideal PEO surface, deducing an estimate of the role of molecular weight, distance between chains, PEO "hydrophobicity", and protein particle size on the steric exclusion behavior (28, 29).

Direct measurements of the steric repulsion between PEO surfaces (45) and between a surface of PEO and one of protein (46) are now available, thanks to the surfaces forces apparatus (47). 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 (55). Prime and Whitesides (44) 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 (48, 51-54). 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 (29, 49). 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 (16, 41, 50).

B. Specific Projects:

We propose to address these issues via the specific hypotheses presented in the Executive Summary, using the molecular structures and geometries noted in Figure 4, the various experimental techniques and apparatus in Figure 5, and the interface geometries in Figure 6. Please refer to the captions of those figures for details.

The systems noted in Figure 4 will be characterized in solution (using solution rheology--Magda, ESR--Rapaport, light scattering--Magda, Kopecek, and at interfaces (using chromatography and FFF--Caldwell), and at air-water interfaces (dynamic surface tension--Magda and monolayer trough--Andrade).

The array of techniques noted in Figure 5, suitable to different geometries and conditions, will be applied to the specific set of studies indicated below. There are clearly many other techniques which would provide very important and even vital information for this study, particularly infrared spectroscopy and NMR spectroscopy (60, 61). Although the facilities and expertise in NMR and IR are available on our campus, they were not included because the present group of investigators does not have strong expertise with those methods, and has not yet involved the experts in those areas on our own campus. During the course of the first year of the project, we will endeavor to gets these individuals involved, particularly Dr. K. Knutsen, Department of Pharmaceutics, an expert in IR, who has an interest in PEO-containing liposomes and Dr. David Grant in the Department of Chemistry, an expert in the application of NMR spectroscopy, including its use for the determination of water proton relaxation times. Indeed our group had experience and activity in this area many years ago (60, 61), and we fully expect to refurbish and modernize that interest and activity in the first year or two of the grant, and fully incorporate these needed experimental methods.

Given the severe page constraints of an MRG proposal, i.e. the technical section is limited to a maximum of 13 pages, we very briefly summarize only some of the proposed experiments, by investigator, and with reference to the summary figures already presented:

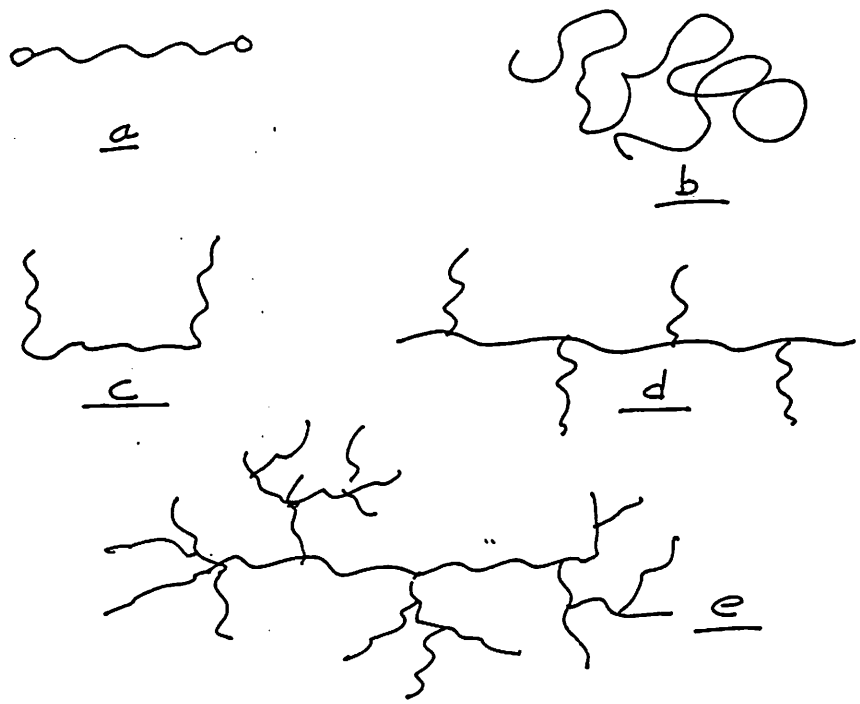

Figure 4: The various types of PEO molecules for use in this MRG project.

a) Low molecular weight PEG derivatized with various functional groups, including homobifunctional and heterobifunctional molecules (9, 10). These reagents are used to modify surfaces in order to prepare the model surfaces for subsequent protein-resistance studies. Molecular weights will be in the range of 500 to 5,000 Daltons. The purchase, characterization, and/or preparation will be under Kopecek's supervision.

b) Higher molecular weight PEO (10^5-10^6 Dalton) will be examined, particularly with respect to physical adsorption (Hlady).

c) PEO containing multi-block polymers, particularly the block co-polymer surfactants known as Pluronics, Synperonics, and Tetronics (17), will continue to be studied using polypropylene oxide and polybutylene oxide center blocks (Caldwell).

d) PEO graft co-polymers will continue to be studied, primarily based on methacrylate monomer co-polymerization (Andrade, Kopecek)(18).

e) Branched or fractal-like systems permitting multi-point attachment of PEO chains, so called surface amplification methodology, will also be studied (Kim).

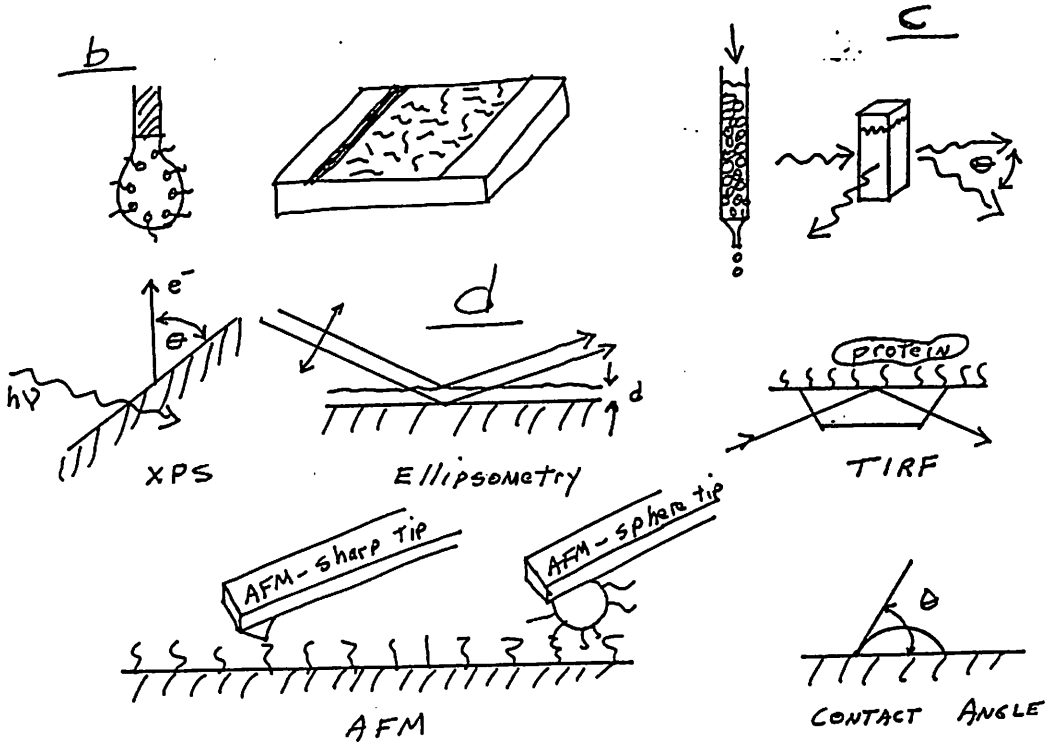

Figure 5: The experimental techniques and methods to be used in this MRG project.

a) Solution characterization methods and techniques (not shown) include solution rheology, particularly high resolution viscometry (Magda), molecular dynamics as probed by electron-spin resonance (Rapaport), molecular size and configuration as probed by inelastic light scattering (Kopecek), and fluorescence (Herron, Hlady).

b) Air/water interface studies: pendant drop dynamic surface tension (Magda) and monolayer trough and sub-monolayer studies (Andrade).

c) Solid/liquid interface techniques involving high surface-area particles: chromatography to determine weak interactions of PEO molecules to particle surfaces or of proteins to PEO-modified surfaces (Caldwell); surface dynamics of either spin-labeled PEO on the surface or spin-labeled protein interacting with the surface (Rapaport) (not shown); light scattering in particulate suspensions for particle size change upon adsorption (Kopecek, Caldwell); field flow fractionation to determine particle size change upon interaction (Caldwell) (not shown); fluorescence polarization and fluorescence energy transfer studies, primarily for protein interaction with modified particle surfaces (Herron, Hlady).

d) Flat surface techniques: X-ray photo electron spectroscopy as a routine characterization tool for determining surface concentration and, to some extent, layer thicknesses (Andrade); ellipsometry, mainly to determine layer thicknesses in the dry state (Andrade); internal reflection fluorescence spectroscopy (Hlady), mainly to determine protein interactions with modified surfaces; atomic force microscopy to determine probe/substrate interactions where one or both surfaces is modified by PEO (Hlady), contact angle--surface wettability (Andrade).

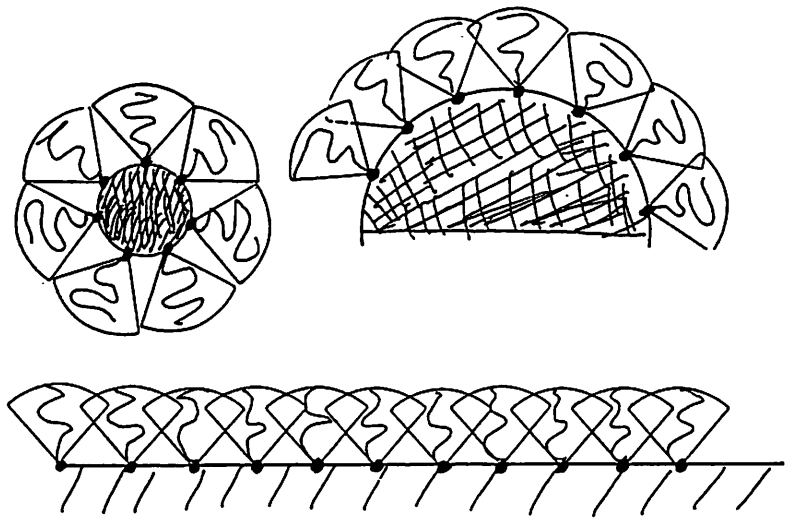

Figure 6: Our second major hypothesis relates to the role of radius of curvature of the substrate on the protein resistance characteristics of a PEO-treated surface. Using the same surface derivatization density, and assuming identical excluded volumes for each of the chains (incorrect because the excluded volume is a function of the radius of curvature), the general hypothesis can be visualized. There is simply more room near a curved surface than near a flat surface.

J. Kopecek: **Relationship between the structure of PEO containing graft copolymers and their solution properties**

Solution properties of copolymers will be characterized by GPC and by static and dynamic light scattering. Light scattering measurements will be performed with a Brookhaven Instruments goniometer, equipped with a He-Ne laser (vertically polarized, = 632.8 nm). Dynamic light scattering measurements will be performed using a standard laser light multiangle Brookhaven Instruments spectrometer.

The following characteristics of polymeric coils, micelles and aggregates can be evaluated:

1) Weight average molar mass
2) Second virial coefficient (a characteristics of the polymer-solvent interactions)
3) Radius of gyration of polymeric coils, micelles, or aggregates
4) Collective diffusion coefficient of scatterers
5) Hydrodynamic radius of polymeric coils, micelles or aggregates
6) Polydispersity index of micelles or aggregates
7) Average segmental density of micelles or aggregates
8) Association number of micelles or aggregates

In addition, our group will be involved in all covalent reaction and derivatization studies.

J. Magda **PEO-Containing Macromolecules in Solution and at the Air/Water Interface**

PEO chains adopt a "swollen random coil" configuration in water due to large osmotic pressure forces (62). These same osmotic forces swell PEO-containing macromolecules attached to solid surfaces, thereby creating an entropic barrier to protein deposition (28, 29).

The research program employs pendant drop tensiometry (63), and intrinsic viscosity measurements (64). Complementary solution measurements such as static light-scattering and electron spin resonance will be performed by Dr. Kopecek and Dr. Rapaport, respectively. Adsorption at the air/water interface will also be studied as a possible means of predicting adsorption of the same macromolecules at the surfaces of hydrophobic solids. Such an approach takes advantage of the accuracy and speed (< 10 minutes) of pendant drop tensiometry (57), and does not require radio-labelling of the adsorbing species.

Dynamic surface tensions are accurately measurable using pendant drop tensiometry with visual image digitization and temperature control up to 80 oC (57). Ubbelohde capillary viscometers immersed in temperature baths are used for intrinsic viscosity measurements.

The same hydrophobic moities which attract the amphiphile to the hydrophobic surface may also induce aggregation in aqueous solution (65). We will locate the CMC by measuring the surface tension as a function of polymer concentration for aqueous solution of PEO-containing amphiphiles (66). The advantage of this approach is the simplicity of pendant drop tensiometry, and its ability to access very low polymer concentrations (57). It may also be possible to locate the binodal of aqueous polymer solutions by measuring surface tensions as a function of temperature. Pure PEO homopolymer is known to precipitate from water at elevated temperatures; little is known about the phase diagram of the copolymers.

PEO homopolymers can be conveniently and inexpensively characterized by intrinsic viscosity, for molecular weights as low as 1000 Daltons (67). However, intrinsic viscosity correlations (Mark-Houwink relations (64)) are lacking for PEO-containing copolymers. These can be developed from light scattering and intrinsic viscosity measurements on fractionated samples of known topology. The measurements also give information about chain flexibility and chain hydrodynamic volume (64). One can thus check the "universal calibration" hypothesis that GPC retention time is a universal function of hydrodynamic volume, regardless of copolymer architecture.

PEO surfactants are used commercially to protect materials from protein deposition (17, 56). The pendant drop apparatus will also be used to study the competitive adsorption of various proteins and various PEO amphiphiles at the air/water interface. This is accomplished by measuring the surface tension of an aqueous solution containing *two* different macromolecular species'. If one species is preferentially adsorbed to the interface, then the surface tension of the ternary solution will be similar to the surface tension of a binary solution containing only one of the amphiphiles (57).

N. Rapaport **PEO INTERACTION WITH BIOMATERIALS AND PROTEINS BY ELECTRON SPIN RESONANCE.**

The molecular conformation of PEO chains in aqueous solutions, the rapid movement of hydrated PEO chains attached to surfaces, and steric stabilization effects appear most important in the protein resistance of PEO surfaces.

Information on PEO interaction with surfaces and proteins can be produced by ESR using stable nitroxide probes extremely sensitive to the rotational and translational dynamics of their local microenvironment. The dynamics of spin-labeled PEO-chains attached to different surfaces can be correlated with their protein repulsion properties, thus elucidating the role of PEO chain mobility in surface passivity. Conformational changes of spin-labeled proteins in PEO-containing solutions and on PEO-coated and uncoated surfaces provides information on PEO interaction with proteins.

We introduced nitroxide spin labels into the ends of PEO-chains of a number of Pluronic copolymers that differed in the lengths of PPO and PEO blocks. Using spin-labelled Pluronics, we initiated an ESR investigation of the dynamics of PEO chains attached to the hydrophobic polystyrene latex surfaces (59).

We also spin-labeled albumin and cytochrome-C to follow their possible conformational changes in interaction with Pluronics in solution and Pluronic treated and untreated solid surfaces.

Our preliminary results reveal

- PEO chain motion on the latex surfaces is restricted in comparison with that in solution.
- PEO dynamics on PS latex particles depends on the PEO/PPO length ratio in the copolymer. PEO dynamics appears to correlate with protein repulsion.
- There is some evidence for PS particle size related differences in PEO dynamics, also well correlated with differences in protein uptake.
- The displacement of adsorbed surfactants from PS particles by plasma proteins can be monitored by ESR.

Non-ionic surfactants like Pluronic can form micelles in aqueous solutions; such micelles influence both the kinetics and thermodynamics of Pluronic adsorption on hydrophobic surfaces and the subsequent surface properties. There is little information on the correlation between the aggregation state of Pluronics and their surface properties. Only limited knowledge is available about the dependence of CMC on the length of PEO and PPO blocks and on their ratio in Pluronic copolymers.

We will monitor the aggregation state of Pluronic molecules in water by spin-probe method using the hydrophobic nitroxide 16-doxylstearic acid. The feasibility of this approach has been demonstrated (59), our preliminary results show that ESR spectra of 16-doxylstearic acid in Pluronic water solutions are very sensitive to the aggregation state of Pluronic in water.

16-doxylstearic acid is not spontaneously dissolved in water and gives a powder spectrum, represented by a broad singlet. Sonification of the probe in water leads to its dissolution; in this case the spectrum is characterized by a symmetrical triplet.

Immersion of 16-doxylstearic acid in a Pluronic solution has the same effect as sonification. The hydrophobic probe solubilization kinetics are monitorable as a function of concentration and type of Pluronic. The onset of Pluronic micelle formation (CMC) is

also clearly visible. Information about local concentrations of a spin probe inside Pluronic micelles can be produced from spectra recorded at 77 K.

The introduction of PS latex particles into the Pluronic micellar solution drustically changes the spectrum of a spin probe.

We propose to:

- determine CMC in water for a series of Pluronic copolymers depending on the lengths of PEO and PPO blocks and their ratio.
- investigate PEO chain dynamics on PS surfaces as a function of the aggregation state of Pluronic in a solution and as a function of particle radius.
- compare PEO chain mobility on the surface as a function of the length of PEO and PPO blocks and their ratio in a copolymer.
- compare the stability of surface coating as a function of the length of PEO and PPO blocks and their ratio.
- investigate the stability of Pluronic micelles and coatings in plasma protein solutions.
- extend investigations of the effect of substrate particle size on the mobility of PEO chains on PS surface.
- investigate the interaction of proteins (HSA, cytochrom C, fibrinogen, SOD) with PEO below and above CMC by monitoring possible protein conformational changes.

S.W. Kim **PEO Immobilization through surface amplification**

Our laboratory has grafted PEO onto polyurethane, styrene-co-aminostyrene, and HEMA-styrene surfaces. We have grafted PEO onto soluble polyurethane chains used as a nonthrombogenic surface. In addition, we have incorporated PEO as integral polymer segments of tri- and penta-block (PDMS-PEO-HEP, PDMS-PEO-HEP-PEO-PDMS) nonthrombogenic surfaces

All of these PEO coupled surfaces demonstrated decreased protein and cell (platelet) adhesion; especially with an optimal PEO MW of 3,500. Physical properties of PEO 3,500 including optimal hydration vs. chain extension, surface hydration, and surface mobility were discussed for the optimal chain length effect.

In all previous research, PEO was coupled directly to functional groups associated with the polymer surface. The type and availability of surface groups was critical for PEO coupling and its surface density. In order to increase the surface density of PEO, we propose to couple PEO through polyfunctional polymers (PFP) onto the surface. This procedure will greatly increase the surface density of PEO through "surface amplification".

Linear and branched PFP, such as polyvinyl alcohol, polyethyleneimine, and polyallylamine will be coupled onto a model surface, such as poly (styrene-co-p aminostyrene. This model surface has proven reliable in polymerization techniques to control the amount of surface functional groups. Immobilization of all the PFP will proceed.

Of primary interest is the optimum surface concentration of PEO to control protein and cell adhesion. Based on the determined amounts of PFP functional groups, varying molar ratios of PEO will be coupled to the PFP surfaces.

K. Caldwell and N. Rapaport **Protein Interaction with Adsorption Complexes between PEO containing Surfactants and Hydrophobic Latex Particles.**

The relative importance of the three factors thought to influence protein repulsion: surface concentration of PEO chains, the thickness of the ad-layer, and the mobility of the chins on the surface. We have reported the adsorption behavior of a group of Pluronics with comparable length of the PPo center block, but with varying lengths of the PEO blocks adsorbed to a series of polystyrene latex particles with diameters in the 70-500 nm range (68, 69).

FFF allows us to accurately determine the adsorption of surfactant per particle and the surface concentration (69), verified independently by isotope labelling methods. The thickness of the adlayer is determined by flow FFF and by dynamic light scattering (photon correlation spectroscopy, PCS), after careful fractionation of the adsorption complex to remove any aggregates formed. The dynamics of the PEO chains has been followed by ESR after substituteion of proxyIradicals for their terminal hydroxyls.

Briefly, our results from these studies can be summarized as (68, 69):

- For a given surfactant, both surface concentrations and adlayer thicknesses are strong functions of particle size, such that small particles take up fewer polymer molecules per unit area. this gives each PEO chain greater freedom of movement, manifested in thinner adlayers (for the smallest particles of 60 nm diameter, commensurate with the size of the PEO chain in free solution) and shorter ESR correlation times (close to the values seen for dissolved polymer).

- The size of the hydrophobic center block, rather than of the PEO flanking blocks, determines the surface concentration in adsorption to hydrophobic particles of a given diameter. Thus, triblocks of a given center block size adsorb with comparable surface concentration, despite large variations in the size of the flanking blocks. The longer the PEO chain, the greater the dynamics.

- The increase in dynamics is directly correlated with a decrease in protein uptake, as seen from exposures of these different adsorption complexes to 0.5 mg/mL of fibrinogen.

The significant suppression of fibrinogen adsorption to particles coated with pluronic F108 (17, 59, 66) prompted a closer look at adsorption complexes involving this surfactant, specifically as concerns their interactions with other proteins:

- Isotope labelled surfactants show no tendency to leak from their adsorption complexes with a 272 nm PS latex during week-long suspension times in a) PBS; b) a solution of human serum albumin (8mg/mL in PBS; and c) a solution of human IgG (5mg/mL) in PBS.

- However, there is a clear leakage of F108 when the coated particles are suspended in whole plasma, likely due to a displacement reaction, as it is paralleled by a significant protein uptake by the coated particles.

We propose to:

1. *Determine whether the protein uptake from plasma is due to lipoproteins,* which competitively displace the F108 surfactant from its complex with latex particles of different size.

2. Examine the mobility of the PEO chains before and after exposure to whole plasma. this will resolve whether an initial protection by the dynamic PEO chains is replaced in time by a similarly protective protein coat.

3. Determine whether or not the protein uptake from plasma changes the particles' adsorption of fibrinogen adn fibronectin.

4. Explore experimentally whether there exists a relationship between the size of PEO chains on a surface and the size of protein maximally repelled by these chains. Such a relationship has been predicted from theoretical considerations (29, 44).

J. Herron **Molecular Simulations of Protein Adsorption to PEO Surface**

Advances in computer technology have led to the development of computer programs which can simulate and display the structure and dynamics of macromolecules (70, 71). These programs come in two generic varieties — molecular graphics and molecular mechanics programs. Molecular graphics programs are used to display and manipulate molecules on a high resolution computer graphics system, while molecular mechanics programs are used to simulate molecular structure.

We have simulated the chain dynamics of polyethylene and polyethylene oxide (8, 72) and their interaction with model proteins (72). The current hypothesis is that the biocompatibility and protein resistant nature of PEO is due in part to its high degree of segmental flexibility. This hypothesis was tested by computing the dynamics trajectories *in vacuo,* for both PE and PEO over a period of 60 picoseconds. The initial conformation of the PEO molecule was an extended helical chain. Within the first 10 picoseconds, the extended conformation disappeared and was replaced by a more compact random coil structure, which persisted through the duration of the simulation. In contrast, PE retained its extended initial conformation throughout the entire simulation. When water was added to the simulations, PEO exhibited a lower degree of segmental flexibility, but was still considerably more flexible than PE.

One major concern in simulating the dynamic behavior of polymers, is how the program deals with hydrophobic interactions. Although the Discover Program does not explicitly include a hydrophobic term in its potential energy functions, BIOSYM (San Diego, CA) contends that the hydrophobic effect is implicitly included in the force field parameters, because many of them were derived empirically. Although the Discover force field appears to handle hydrophobic interactions implicitly, it would be desirable to use a force field which explicitly includes an energy term for solvation free energy. Recently, several researcher have included such a term in their simulations of macromolecules in solution and also on polymeric interfaces (73-75). We propose to repeat our simulations of PE and PEO chains *in vacuo*, but this time will include an explicit term for solvation free energy in our calculations. These results will be compared to our previous results obtained *in vacuo* without an explicit solvation term and also to our previous simulations in water.

The specific aim of this comparison is to see whether an explicit energy term adequately emulates the influence of solvent, or if simulations of a fully-solvated ensemble are required. This issue is of practical importance because the computations *in vacuo* are a lot less expensive than computations with full solvent. If including a

solvation free energy term in the force field proves successful, we plan to incorporate it into our simulations of model proteins on PE and PEO surfaces.

Molecular models for synthetic polymers such as polyethylene and polyethylene oxide are built using the Insight II program (Biosym Technologies, Inc.) on a Silicon Graphics 4D/70GT workstation. Molecular models for proteins (lysozyme, ribonuclease, myglobin, superoxide dismutase and cytochrome-c) are obtained from the Protein Data Bank (Brookhaven National Laboratory). Energy minimization and molecular dynamics simulations are performed using the Discover program (Biosym Technologies, Inc.) on either a Silicon Graphics 4D/220-S minisupercomputer or an IBM 3090-600J supercomputer. Proteins are docked with polymer surfaces using the ROT program developed at the Center for Biopolymers at Interfaces by Kap Lim. This program computes the non-bonded forces between the protein and the surface as the protein is moved towards the surfaces in small distance increments.

V. Hlady **MEASUREMENTS OF THE EFFECT OF SURFACE CURVATURE ON THE STERIC REPULSION FORCES BETWEEN PEG-COATED SURFACES**

Although PEG chains can be attached to surfaces in different ways, the chemistry of attachments seem to matter less than physics. Even with the same chemistry of attachment, the protective nature of PEG depends on the nature of the supporting surface and other factors.

The effectivenss of the surface PEG to repel solution species is a delicate balance of a number of parameters. We postulate that the most decisive role is played by the way PEG segments distribute themseves in the layers adjacent to the surface. The PEG segment density distribution will depend on:

- PEG segment-surface interactions (note that any preferential adsorption of segment will "flatten" the PEG chains onto the surface) (see Figure 2),
- the curvature of the surface (highly curved surfaces will cause a "dilution" of the PEG segments in the layer further away from the surface) (see Figure 6),
- the attachemnt of PEG chains (terminally grafted vs multi-point attachment),

The distribution of PEG segments in the layers adjacent to the surface will determine the forces which dominate the protective action of surface PEG. Steric repulsion forces bewteen PEG-coated mica surfaces have been confirmed and measured using the surface force apparatus (SFA) (76). Although the range of forces accessible by SFA method are rather large, there are some serious limitations of SFA method:

- the force measurement are limited to two surfaces of (coated)-mica,
- no surface imaging capability exist (typically, only a single point measurement is performed), and
- radius of curvature of the mica surface is rather large (1-2 sm) and for all practical purposes the forces measured describe interactions between flat surfaces.

We are currently designing a variant of the atomic force microscope by which local forces, including steric repulsion forces, can be directly and accurately measured in situ (55). The method is based on measuring the interaction force between a spherical probe (radia of curvature 50 nm - 5 μm) and the surface (radia of curvature ranging from 1 μm to infinity). The key elements of this new technique are the cantilevers with different spring constants onto which a spherical probe is attached and by which interaction forces of different magnitude can be examined. The probe and/or surface can be coated with any polymer including PEG. The measurements are not limited to a single point, in fact, we expect to be able to map a spatial distribution of forces as well as the surface topography using the same instrumentation.

We propose to:

1) complete development of the scanning force microscope that will be devoted to the measurement of steric repulsion forces and to the imaging of the spatial distribution of forces (estimated duration 1- 2 years).

2) measure the force-distance profiles using non-symetrical probe-surface combinations (i.e. spherical probe with attached PEG chains interacting with surfaces made of different materials but with no PEG attached). Variable parameters will be the radius of curvature of the probe, the surface density of PEG chains on the probe, the chemical nature of surface and the composition of solution.

The results will provide us with two kind of information:

a) the nature of steric interaction forces and
b) the adhesive PEG segment - surface interactions (if any).

Further extension of this experiment will be to deposit a layer of protein onto the surface and measure the interaction forces involved between the PEO-coated probe and protein-coated surface.

3) Measure the force-distance profiles for a probe-surface combination possesing a chemical symmetry (i.e. spherical probe and surface made of identical material with identical surface density of PEG chains on each). Variable parameters will be radius of curvature of the probe and the surface density of PEG chains. The experimental results will be compared with theoretical predictions, using both the molecular modeling simulation (this proposal, part directed by Herron) and self-consistent field theories (77). We expect to be able to obtain a picture of the distibution of PEG segments in the layers adjacent to the surface as a function of the surface curvature.

4) Image the spatial distribution of surface steric repulsion forces originating from the PEG chains attached to the flat surface, a feature of significant practical interest. We will have the capability of determining the spatial heterogeneity of the PEG coatings.

In summary we propose to terminally attach PEO chains to surfaces and to investigate the effects of distribution of PEG segments in the layers adjacent to the surface to the ensuing steric repulsion forces. To measure the forces we propose to develop a variant of scanning force microscopy which will enable us not only to measure local force-distance profiles between PEG-coated surfaces of different radii of curvature, but also to map the two-dimensional heterogeneity of polymer coatings

In summary, we have the techniques, facilities, and expertise to address the key hypotheses and themes noted in the Executive Summary.

Protein Adsorption expeiments will utilize our standard techniques (chromatography, TIRF, XPS, radio labelling) and a set of model proteins (lysozyme, mycglobin, superoxide dismutase, cytochrome c, and ribonuclease) with which we have had considerable experience (25, 26, 49, 57, 72). Please also refer to publications listed in Biosketches (Appendix A).

We would have liked to go into more detail, but 13 pages is a bit limiting for such an extensive project!

C. Resources and Facilities:

Adequate facilities and resources are available. The investigators all have access to equipment, space, and technical support in the Departments of Naterials Science, Chemical Engineering, Bioengineering, and Pharmaceutics, as well as the Center for

Biopolymers at Interfaces (CBI) and the Center for Controlled Chemical Delivery (CCCD). Caldwell directs CBI's particle characterization facility, Andrade directs the Surface Analysis Laboratory, Hlady directs the Interface Optics and Scanning Probe Microscopy Labs, Magda directs the Rheology Lab, and Kopecek directs the Synthesis Labs in both Pharmaceutics and Bioengineering. Please refer to Figure 5 for brief technique and facility descriptions.

D. Personnel and Organization:

This topic is covered in section "Justification for MRG Mode of Support" and Figure 1 in that section. The PI, Andrade, will coordinate and orchestrate the effort. By the sharing of students and staff and by regular meetings, seminars, and discussions, we can guarantee a fully interactive, collaborative project. See also Budget Justification and section on Education and Human Resources.

E: Advisors

Three distinguished, international colleagues and collaborators have been asked to serve as an advisory board for this project.

Pierre Gilles de Gennes, of the College de France in Paris, is internationally recognized for his innovative and creative work in a wide range of fields. Of relevance to this project is his work on scaling phenomena in polymer physics, modeling and simulation of polymer solutions, polymers at interfaces, and polymer gels and networks. PEO has generally been his favorite polymer, largely because of the properties which we described earlier: its mobility, dynamics, and solution behavior in both apolar as well as polar solvents. Professor de Gennes was a visiting professor at the University of Utah in the summer of 1985, where he, together with professor Brochard, gave a series of outstanding lectures dealing with polymers at interfaces. Those lectures were videotaped, and we still use them regularly for introducing our students and co-workers to these important topics and concepts. Pierre Gilles loves the Intermountain West and the Southwest, and he is also an avid skier. We anticipate having no trouble inducing him to return to Utah, particularly when the snow is of suitable quality. Dr. de Gennes was the winner of the Nobel prize in physics earlier this year.

Dr. Helmut Ringstorf is Professor of Organic Chemistry at the University of Mainz, Germany. Helmut Ringstorf is internationally recognized for his work on monolayers, polymerizable monolayers, liposomes, and generally on interfacial self-assembly. He, too, is somewhat of a "desert rat" and has been to Utah many times, serving as visiting professor, giving short courses, and giving a range of seminars and colloquia. He and his wife honeymooned in the Grand Canyon many, many years ago, and we have no trouble enticing them back to this part of the world, as their schedule and our resources permit.

Dr. Taki Matsuda is Director of Biomaterials and Bioengineering for the National Cardiovascular Research Center in Osaka, Japan. Dr. Matsuda is also internationally recognized for his work on biomaterials and biocompatibility. He has done extensive work on the immobilization of PEO, the use of PEO as a tether, and the development of patterned PEO surfaces based on photolithography and photochemical techniques. Dr. Matsuda has also been a frequent visitor to Utah, and is a close collaborator of Drs. Kim, Kopecek, and Andrade. He will be visiting professor at the University of Utah in October, 1992, and has given at least one seminar per year at Utah for about the past seven or eight years.

All three of these distinguished advisors travel widely and are often in the United States. We have not budgeted any international travel for their services, because we expect that they will be able to visit our project at least once per year during the course of one of their other trips to the United States. We have budgeted only domestic travel for them, a limited amount for local expenses while they are here, and an appropriate honorarium/consulting fee. We expect them to visit at least once per year for the three-year duration of this project. During their visits they will give lectures on their current work. They will also meet with the co-investigators and graduate students involved with this project. The meetings will be as a general group, and as various sub-groups on specific topics of mutual interest. We will specifically request their critique of our hypotheses, methods, and analyses. Where appropriate, we will urge them and encourage them to become involved with various aspects of the project. We expect that there will be collaborative as well as advisory activities resulting from this interaction.

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