BIOCHEMISTRY AND MOLECULAR BIOLOGY OF BIOSENSORS AND BIOPROBES

Organizers: Joseph D. Andrade, Daphne Kamely and James Lear
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Plenary Sessions Page

Poster Sessions

Biochemistry and Molecular Biology of Biosensors and Bioprobes

Keynote Address

CG 001 CHEMICAL RECOGNITION IN BIOLOGICAL SYSTEMS, Larry J Kricka, Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA 19104

Recognition of a chemical substance by a molecule or molecular complex is central to biological processes, eg immunity, histocompatibility. A wide range of recognition molecules have evolved which have exquisite specificity and these include transport proteins, lectins, DNA, receptors, and immunoglobulins. The immunoglobulin antibodies represent a versatile class of molecular recognition molecules and the perfection of monoclonal techniques has established antibodies as the most important type of recognition molecule for analytical and therapeutic applications. The mechanism of molecular recognition has been extensively studied and factors found to be important include shape , disposition of hydrophobic and hydrophilic groups, dehydration, and hydrogen bonding . Molecular recognition underlies many analytical methods and intact biological structures (crab antennules), cells, and individual molecules have been utilized to impart specificity to the analytical process. Various approaches have been explored for the production of new molecular recognition systems. The binding property of an enzyme can be modified by chemical attachment of ligands (hybrid enzymes). Alternatives to enzymes can be made by raising monoclonal antibodies to transition state intermediates in chemical reactions (catalytic antibodies). A recent goal has been the de novo synthesis of molecules with defined recognition properties. Selectivity for different anions and cations has been achieved using crown ethers. Cyclophane, cryptophane, and calixarene derivatives have been synthesized which recognize uncharged molecules. Binding molecules for peptides have been produced from antisense peptides (peptides coded by antisense DNA) and these molecules have affinity for the corresponding sense peptide.

Currently it is not possible to predict the composition and structure of a molecule required to produce a defined binding specificity for a complex substance. Much remains to be learned about the interplay of interactions which confer specific binding properties on a molecule, but the reward for elucidating these factors will be analytical methods with improved specificity and sensitivity.

Recognition, Transduction and Membranes

CG 002 BIOSENSORS BASED ON SOLVATED BILAYERS AT THE ELECTRODE SURFACE, C. Gitler*, H. Garty, Y. Shai, D. Bach, S. Steinberg[1], I. Rubinstein[1],A. Shenzer[2], M. Sheves[2], T. Bercovici, and I. Yuli
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The attachment of functional solvated lipid bilayers to the surface of gold electrodes is under study. The designed mechanism of attachment is based on the stabilization of the fluid bilayer with retention of bulk water between the bilayer and the electrode. It is modeled on the biological principles used to form a periplasmic space in bacteria and the oligosacharide-phosphoinositide attachment of proteins to membranes.

Amphipathic peptides have been synthesized capable of forming ion channels accross the lipid bilayers. The role of intramembrane proline on the channel properties has been critically examined. By means of attachment of side-chain groups that undergo Föester energy-transfer, the effect of transmembrane potential on the conformation of the polypeptides is under study. Polypeptides that induce gated electron channeling will be described.

The availability of a functional bilayer at the electrode surface allows the extension of biological sensory perception to the area of microelectronics.

CG 003 MULTIDIMENSIONAL INFORMATION PROCESSING OF FLUORESCENCE SIGNALS FROM SELECTIVE INTERACTIONS OF RECEPTORS AND ENZYMES AT LIPID MEMBRANES, Ulrich J. Krull, John Brennan, R. Stephen Brown and Dimitrios P. Nikolelis, Chemical Sensors Group, Department of Chemistry, Erindale Campus, University of Toronto, 3359 Mississauga Road North, Mississauga, ON Canada L5L 1C6

Research in my group has as its overall objective the development of biosensors that use chemically-selective membranes to measure the concentration of specific species in complex media. The fundamental idea is that a protein which can bind selectively to a specific organic or biochemical species, can be incorporated into an ordered lipid membrane assembly such that selective binding events lead to changes in the lipid structure (transduction) which can be measured quantitatively. The primary advantages of this method of detection are that it provides an intrinsic amplification of the original chemical signal, and that it is applicable to interactions of enzymes, antibodies, receptors and lectins, and therefore provides a sensitive generic strategy for sensor applications. The central problem to be solved is how the alteration of the structure of a lipid membrane that is caused by binding events of the protein can give rise to an analytical signal. My research group specializes in electrochemical methods and fluorescence spectroscopy to obtain a quantitative measure of binding.

This presentation will compare the microscopic physical structure of monolayer and bilayer lipid membranes to suggest a mechanism of ion permeability, and will show that chemically-selective materials can be deposited within domains to prepare lipid membranes that provide concurrent fluorescence and electrochemical signals. Fluorescence microscopy has been used to study the structure of lipid bilayers, and of lipid monolayers at an air-water interface. Structural information is derived from analysis of the spatial distribution of dye in membranes, and from spectral analysis (wavelength, average intensity, time-resolved intensity). Control of domain distribution within phospholipid membranes has been accomplished by use of edge active species and chemically-selective proteins. Fluorescence techniques have been used for studies of selective interactions of concanavalin A (lectin) with polysaccharide, of acetylcholinesterase (enzyme) with acetylcholine and of acetylcholine receptor with carbamylcholine (agonist) and α-bungarotoxin (toxin). Examples of significant research results include: determination that fluorescent lipid membranes can be used for quantitative analysis; that instrumental and chemical drift can be eliminated by using ratios of fluorescence intensities collected at different wavelengths; that time-resolved fluorescence intensities can be used to distinguish between the binding of agonist and antagonist to the same selective protein.

CG 004 RECONSTITUTION OF PROTEINS IN POLYMERIZED BILAYER ASSEMBLIES, David F. O'Brien, Department of Chemistry, The University of Arizona, Tucson, AZ 85721.

The recent availability of polymerizable lipids now allows for the reconstitution of membrane proteins in polymerized bilayer membranes. The enhanced chemical stability of the membranes from some polymerizable lipids opens the way to prepare robust bilayer membranes. Both the incorporation of the ATP synthetase and bacteriorhodopsin in monomeric diacetylenic lipid vesicles have been reported. In each case significant protein functionality was retained after photopolymerization of the membranes. Subsequent to these early studies we showed that purified delipidated bovine rhodopsin may be reconstituted by insertion into partially polymerized bilayer membranes. The sensitive nature of rhodopsin dictated the strategy employed to incorporate rhodopsin into polymerized bilayers. Rhodopsin is acutely sensitive to light, therefore the lipid bilayer was photopolymerized before incorporation of the rhodopsin. However, attempts to insert rhodopsin into 100% polymembranes were unsuccessful, therefore the membranes were prepared with domains of polymerized lipid and nonpolymerized lipid. The successful reconstitution of the rhodopsin was demonstrated by tests of the chemical photochemical, and enzymatic functionality of the membranes. Reconstitution of the enzymatic functionality requires the recombination of both the rod cell G protein and PDE in isotonic media with the rhodopsin membranes. The extent of binding of these surface associated enzymes was the same whether the membranes were polymerized or unpolymerized. Thus the biocompatibility of the PC membrane surface was not altered by the polymerization within the bilayer. Light stimulation of the rhodopsin results in activation of both peripheral proteins and a rapid hydrolysis of cyclic GMP in the normal manner found with natural rod cell membranes.

Biochemistry and Molecular Biology of Biosensors and Bioprobes

Interfaces and Immobilization

CG 005 ADSORPTION OF COMPLEX, MULTI-DOMAIN PROTEINS, J.D. Andrade, A-P Wei, V. Hlady, Department of Bioengineering, University of Utah, Salt Lake City, UT 84112

Common globular proteins range in complexity from small, single domain molecules (lysozyme, myoglobin, superoxide dismutase, for example) to very large, multi-domain structures (IgG, IgM, fibronectin, fibrinogen, etc.). Protein adsorption is dependent on the initial collision process followed by the conformational adaptation ("denaturation") of the protein to its new microenvironment. The initial collision and residence time is dependent on the surface chemistry of the protein and of its constitient domains. The conformational adaptation is dependent on the intrinsic stability (ΔG folding) of the protein and of its individual domains. The adsorption and separation behavior of proteins on charged and on hydrophobic surfaces will be rationalized and explained, in part, by a domain interfacial activity analysis.

This work is supported in part by the Center for Biopolymers at Interfaces, University of Utah.

CG 006 ADSORPTION OF MACROMOLECULES, POLYELECTROLYTES AND PROTEINS

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A subtle balance between energetic and entropic factors determines the adsorption of uncharged and charged macromolecules and proteins. Although these molecules differ in the respective adsorption behaviour at interfaces, there are still many similarities. Adsorption of uncharged and charged macromolecules is driven by the segmental adsorption energy, but opposed by the loss of conformational entropy and the entropy of mixing. Protein adsorption is largely determined by the internal stability of whole molecule. Rigid, "hard" proteins rely on energetic adsorption forces, like electrostatic interactions between protein and surface. Flexible, "soft" proteins, especially those containing several different domains, are also influenced by entropic contributions. By increasing the surface concentration of the macromolecules, interactions between neighboring adsorbed molecules start to play role in determining the adsorption. Different factors will influence the macromolecular adsorption processes in a different kinetic fashion which often gives rise to interesting effects, like: quasi-irreversible adsorption with no desorption upon dilution but with readily exchange between surface and solution molecules, "overshoot" of adsorption, preferential and/or competitive adsorption from macromolecular mixtures, and some others.

Antibodies

CG 007 GENERAL ASPECTS OF ANTIBODY STRUCTURES AND THEIR RELATIONS TO LIGAND BINDING, Allen B. Edmundson*, James N Herron +, Luke Guddat* and Edward W. Voss,Jr.#, Harrington Cancer Center*, Amarillo, TX 79106, Dept. of Pharmaceutics +, Univ. of Utah, Salt Lake City, UT 84108, and Dept. of Microbiology#, Univ. of Illinois, Urbana-Champaign, IL 61081. Three-dimensional structures determined by X-ray analyses will be used to illustrate various shapes, sizes and complementary surfaces available for ligand binding in the following immunoglobulin fragments: (1) the native (unliganded) BV04-01 murine Fab which binds single-stranded DNA; (2) the complex of the BV04-01 Fab with a trinucleotide of deoxythymidylate; (3) the murine 4-4-20 Fab-fluorescein complex crystallized in 2-methyl-2, 4-pentanediol; (4) and (5) two forms of the 4-4-20 Fab-fluorescein complexes crystallized in polyethylene glycol; (6) and (7) two forms of the human (Mcg) Bence-Jones dimer crystallized in ammonium sulfate and deionized water. The Bence-Jones dimer and the parental IgG1 immunoglobulin containing the same light chain were mapped for peptide binding by the methods of Mario Geysen and his colleagues. Ligand-protein complexes were prepared both by diffusion of selected peptides into preexisting crystals of the Bence-Jones dimer and by cocrystallization in ammonium sulfate. Representative crystal structures of these complexes will be described. Peptide binding fell into two principal categories: (1) cases in which there were no major conformational changes in the protein; and (2) examples in which there were conformational adjustments in both the protein and peptide. Significant conformational changes also accompanied the binding of a trinucleotide by the BV04-01 Fab. The native and liganded forms of this protein crystallized in different space groups. Rigid body shifts were detected in the domain associations of the liganded form. In particular the VH domain was rotated about 8° about its long axis and swiveled about 6° away from the VL domain, with consequent expansion of the binding site for DNA. The overall structure of the third hypervariable loop of the VH domain was also different in the liganded form, and local conformational changes were accentuated in and around the chief contact residue (tryptophan 107) for DNA. This work was supported by Grant CA 19616, awarded by the National Cancer Institute, Dept. of Health and Human Services.

CG 008 THERMODYNAMICS AND MOLECULAR MECHANISMS OF Ag-Ab BINDING AND REGULATION, James N. Herron, Department of Pharmaceutics, University of Utah, Salt Lake City, UT 84112.

The antigen-antibody interaction is a paradox. While a particular antibody is very selective for its antigen, antibodies with specificities for almost any biomolecule can be elicited. Furthermore, a wide spectrum of antibodies with different affinities can be generated to a single antigen. These properties make antibodies ideal recognition elements in immunoassays and biosensors. Both the selectivity and diversity of the antigen-antibody interaction results from the unique three-dimensional structure of the immunoglobulin molecule, which consists of several homologous domains of highly conserved amino acid sequence, and six short regions (ca. 10 amino acids) of highly variable amino acid sequence. These variable regions are called complentarity-determining regions (CDR) and form the antigen binding site. Another paradox is the very nature of high affinity binding. The hydrophobic effect is the major thermodynamic driving force of antigen binding, but the selectivity of the active site probably resides in very specific interactions between active site residues and the antigen. Furthermore, the antigen binding event is often associated with a significant loss of vibrational and configurational entropy in both the antigen and antibody molecules, which partially offsets the favorable contribution to binding from the hydrophobic effect. Thus, the dynamic behavior of an antibody molecule is an important part of its function. Our laboratory has investigated these issues by correlating physico-chemical data obtained for antigen binding in solution with three-dimensional structures of antigen-antibody complexes. We are currently working with several different monoclonal antibodies which bind haptens in both crystal and solution. Of particular interest is a series of monoclonal antibodies which bind fluorescein, a fluorescent hapten. These antibodies are ideal for studying the molecular mechanisms of antigen binding because they exhibit a wide range of binding constants (10^6 to 10^{10} M^{-1}), and a variety of experimental techniques are available for measuring their kinetics, affinities, thermodynamics and dynamic behavior. Recently, we have determined the three-dimensional structure of the antigen binding fragment of a high affinity anti-fluorescein antibody and have used this information to examine the structural basis of its behavior in solution. Finally, because these antibodies are so well-characterized, they are finding increasing use as "calibration standards" in biosensors and and in other biotechnological applications.

CG 009 ANTIBODIES AT INTERFACES, Jinn-Nan Lin, Department of Bioengineering, University of Utah, Salt Lake City, UT 84112.

There has been considerable interest in the use of optical fibers or waveguides for remote spectroscopic sensing of biomolecules. We and others have previously demonstrated the detection of antibody-antigen binding at silica/liquid interface via total internal reflection fluorescence (TIRF) spectroscopy. The objective of the Immunosensors Program at the University of Utah is to develop remote, on-line, semi-continuous, and multichannel sensors for the detection of proteins and other antigens. To develop such a sensor, interfacial chemistry plays an important role. Immobilization of antibody on optical surfaces and reversibility of surface bound antibody-antigen complexes are the two important issues which will be discussed. The reversibility of an immune complex on a surface is closely related to the physical states of the immobilized antibody, thus these two issues are not completely separable. (1) Immobilization of antibodies. Reviewing the current literature on covalent immobilization of biomolecules on solid surfaces, one is normally overwhelmed by the large number of coupling chemistries. Many different functional groups have been derivatized on various types of solid surfaces to match functional groups on biomolecules. However, the effects of the adsorption properties of solid supports and biomolecules on their surface activity have not been discussed anywhere so far. We have recently demonstrated that the adsorption properties of the antibodies play an important role in their covalent immobilization on certain types of solid supports. Surface activities of native and partially denatured antibodies on silica and hydrogel surfaces were determined. It was found that the surface activity of the denatured antibody is higher than that of the native antibody on silica surfaces, indicating a surface indued phenomenon. (2) Antibody-antigen reversibility. Antibody-antigen binding in solution has been thought a reversible process. However, it has been observed that most antibody-antigen binding on solid surfaces is virtually irreversible. Several hypotheses have been suggested to explain this interesting phenomenon: 1) change in solution microenvironment at interfaces, 2) nonspecific adsorption, 3) antibody/antibody lateral interactions, 4) conformational change of immobilized antibody, 5) multivalent binding, and 6) diffusion and mass transport effects. The results of several key experiments will be presented and all hypotheses will be discussed.

Enzyme Recognition

CG 010 ORGANIC PHASE ENZYME ELECTRODES, Geoffrey F. Hall and Anthony P.F. Turner, Biotechnolgy Centre, Cranfield Institute of Technology, Cranfield, Bedfordshire MK43 0AL, U.K.

Enzymic reactions can be carried out in monophasic organic solvents. The use of enzymes in organic solvents can give rise to a number of advantageous properties compared to an aqueous reaction medium (1). These property changes are described and the reasons why they might give rise to enzyme electrodes with desirable properties are discussed. Two enzyme electrodes that function in chloroform and in chloroform/hexane are described. Firstly, an enzyme electrode using tyrosinase (EC 1.14.18.1) is presented that operates in chloroform and will detect a range of phenols at micromolar concentrations (2). Tyrosinase oxidises phenols to quinones which can be reduced at a graphite electrode to give a current proportional to the initial phenol concentration. An application of the sensor is demonstrated by detecting p-cresol concentrated from an aqueous sample into chloroform or butyl acetate. Secondly, cholesterol oxidase (EC 1.1.3.6) is used in conjunction with an oxygen electrode to create an enzyme electrode that can determine cholesterol in 50/50 chloroform/hexane. The use of the sensor for simple cholesterol measurements in butter and margerine is presented. Finally, a technique is described that can be used for the study of bioelectrochemical reactions in organic solvents. A bi-enzymic reaction is carried out inside swollen reverse micelles formed by the non-ionic detergent Triton X-100 in butyl acetate. The enzymes used are alcohol dehydrogenase (EC 1.1.1.1) and diaphorase (EC 1.8.1.4). The alcohol dehydrogenase catalyses the conversion of ethanol to acetaldehyde with the concomitant reduction of its cofactor nicotinamide adenine dinucleotide (NAD+) to NADH. Diaphorase reoxidises the NADH and can utilise a number of electron acceptors. It is demonstrated that in this system these may be water-soluble or solvent-soluble. This work represents preliminary studies in an area that is attracting increasing research interest.

(1) Dordick, J.S., Enzyme Microb. Technol., 11 (1989), 194-211.
(2) Hall, G.F., Best, D.J. and Turner, A.P.F., Anal. Chim. Acta, 213 (1988), 113-119.

Biochemistry and Molecular Biology of Biosensors and Bioprobes

CG 011 AMPEROMETRIC BIOSENSORS BASED ON ELECTRICAL WIRING OF REDOX ENZYMES, Adam Heller, Department of Chemical Engineering, The University of Texas at Austin, Austin, TX 78712-1262. The redox centers of oxidoreductases are usually electrically insulated by protein or glycoprotein layers. As a result, most oxidoreductases cannot be directly electrooxidized or electroreduced on metal electrodes. If, however, the insulating protein or glycoprotein is modified by incorporating in it molecular electron relays, it is possible to transfer electrons between a redox center of an enzyme and a relay and between a relay and the metal electrode. An effective way electrically connecting redox centers of enzymes to electrodes is through large redox macromolecules that are electrostatically and/or covalently bound to the enzymes. For example graphite-adsorbed redox polycations of 150,000 - 300,000 daltons, with a dense array of poly(vinyl-pyridine) complexes of $[Os(bipyridine)Cl]^{1+/2+}$ centers will electrostatically bind to glucose oxidase, a polyanion at pH 7. Amperometric glucose sensors based on such redox-polymer-wired enzymes have response times of less than a quarter of a second and are simple to make. In structures with multiple layers of wired enzymes, interconnected by 3-dimensional networks of redox polymers, glucose diffusion limited current densities as high as $0.5mAcm^{-2}$ have been reached, and the signal/noise ratio is better than $10^3/1$.

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Molecular Biology of Ion Channels and Receptors

CG 012 CHARGE CARRIERS AND ION CHANNELS IN DEPOSITED AND SUSPENDED LIPID MEMBRANES

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Development of receptor- or ion channel-based biosensors has been impeded in part by the fragility of the lipid membrane. Attempts to improve the lifetime of the membrane have included the use of polymerizable lipids, deposition of the membrane onto small-pore substrates, and the transfer of films onto solid supports using a variety of adsorption or transfer techniques. The approach taken for this work is to develop techniques for measuring the function of ionophores and ion channels deposited onto conducting electrodes. Phospholipid films containing the ionophore valinomycin have been transferred onto platinum electrodes using the Langmuir-Blodgett technique. The a.c. impedance of these films has been characterized at low frequency as a function of potassium concentration. It was found that the change in conductance of the film increased as a function of the potassium ion concentration in the medium. Results were obtained for both high and low sodium background concentrations. Attempts are underway to extend these techniques to the analysis of a transmembrane ion channel, the voltage-dependent anion channel (VDAC), purified from the mitochondria outer-membrane of yeast; preliminary results will be discussed. Long-term stability of the films will also be addressed.

CG 013 The Glutamate and Nicotinic Receptor Gene Families

Heinemann, S., Boulter,J., Connolly, J.,Deneris, E., Duvoisin,R., Hartley, M., Hermans-Borgmeyer, I., Hollmann, M., O' Shea-Greenfield, A., Papke, R. and Rogers, S. Molecular Neurobiology Laboratory, The Salk Institute P.O. Box 85800 San Diego, California 92138.

We have used a molecular genetic approach to identify a superfamily of genes that code for the glutamate and nicotinic receptors which are the major excitatory receptors in the mammalian brain. Previous work identified seven genes that code for subunits of nicotinic acetylcholine receptors expressed in the mammalian brain. We have named the genes alpha2, alpha3, alpha4, alpha5, beta2, beta3, and beta4; Duvoisin, R. M., E. Deneris, J. Patrick and S. Heinemann, Neuron. 3 487-496, 1989. In our terminology the genes coding for the muscle nicotinic acetylcholine receptor are called alpha1, beta1, gamma, delta and epsilon. Thus, the nicotinic acetylcholine receptor family is encoded by twelve genes in the mammalian genome. We have shown that five of the brain or neuronal genes code for subunits that can form at least six different functional nicotinic acetylcholine receptors when expressed in Xenopus oocytes. We have studied the pharmacology of these receptor subtypes and demonstrated that they have different susceptibilities to agonists and toxins. The existence of multiple nicotinic receptor subtypes and the fact that they are expressed throughout the brain suggests that the nicotinic acetylcholine receptor is a major excitatory system in the mammalian brain. One can now speculate that the many diverse effects of nicotine, including addiction may be due to nicotine stimulation of this major excitatory receptor system.

The glutamate receptor system is thought to be involved in the first steps of learning and memory acquisition and is perhaps the most important excitatory receptor system in the mammalian brain. We have used an expression cloning approach to identify a family of glutamate receptor genes. One gene called GluR K1 codes for a functional glutamate receptor of the kainate subtype. The primary structure and the physiology of the GluR K1 glutamate receptor indicates that it is a member of the ligand-gated channel family, Hollmann,M., O' Shea-Greenfield, A., Rogers, S.W. and Heinemann, S., Cloning by functional expression of a member of the glutamate receptor family: The primary structure of a rat brain kainate receptor; Submitted 1989.

CG 014 DESIGN OF PEPTIDE MEMBRANE CHANNELS AND STRATEGIES TO USE ION BINDING IN FIBEROPTIC CATION SENSORS.

F. C. Szoka, Jr.*, J. N. Roe#, A. S. Verkman and R. A. Parente*, Departments of Pharmacy*, Bioengineering# and Medicine, University of California, San Francisco, CA 94143.

The possibilities for detection and signal amplification using ligand specific ion channels are a major rationale for their use in biosensors. Advances in the understanding of channels and peptide membrane interactions now permit the design of synthetic ion or environmentally triggered channels. GALA is a 30 amino acid peptide that undergoes a random coil to an amphipathic α helix transition as the pH is reduced from 8 to 5. In its low pH conformation GALA is able to form channels in phosphatidylcholine bilayers at lipid/peptide ratios of 20,000/1. The GALA sequence, W-E-A-A-L-A-E-A-L-A-E-A-L-A-E-H-L-A-E-A-L-A-E-A-L-E-A-L-A-A, was chosen to ensure a transbilayer dimension. Amino acid position in the sequence was based upon hydrophobic moment calculations and adjusted using computer graphics. The E residues were aligned so that the high charge density at neutral pH destabilized the helix. At low pH GALA assembles into a transmembrane oligomer through which molecules of up to 1000 daltons can permeate. Strategies to couple ion transport or binding to optical detectors based upon fluorescence energy transfer have been used to construct of a potassium sensitive fiberoptrode. The sensor was fabricated by coating the distal end of a single optical fiber in a PVC solution containing a hydrophobic dye 7-(n-decyl)-2-methyl-4-(3',5'-dichlorophen-4'-one)indonaphthl-1-ol (MEDPIN), a plasticizer, the ionophore valinomycin and the fluorescent dye $DiIC_{18}$. The binding of K^+ to valinomycin results in the formation of a ternary complex among K^+, valinomycin and the MEDPIN. In the ternary complex the MEDPIN is deprotonated and undergoes a 150 nm Stokes shift in its absorbance spectra. The K^+ induced change in MEDPIN absorbance was detected from the fluorescence quenching of $DiIC_{18}$. The sensor exhibited a 57% decrease in fluorescence with increasing K^+ concentration (0-5mM) at pH 7.5 and was not effected by Na^+. The response time to a 1.3 mM increase in K^+ was 10 s. In a similar fashion one can create sodium or other cation selective detectors. This demonstrates effective coupling of ion binding to the generation of an optical signal.

Polynucleotides and Other Approaches

CG 015 DNA AT INTERFACES

Karin D. Caldwell, Center for Biopolymers at Interfaces, Department of Bioengineering, University of Utah, Salt Lake City, UT 84112

The separation and recognition of DNA, whether in single or double stranded form, is generally mediated by the presence of an interface. Although, at present, most separations involve electrophoresis in an inert gel network whose sole purpose is spatial confinement of the sample, several processes exist which owe their selectivity to the affinity between the DNA and a particular surface composition. Recognition, in turn, is often performed by means of ligand binding, in the case of double stranded DNA, or hybridization with a complementary probe, in the case of single stranded DNA. In either case, the recognition step follows the attachment of the sample to a blotting membrane or other surface which will permit its detection. Despite the common use of such surface mediated detection, exemplified by the Southern blotting technique, few systematic studies exist which aim at optimizing the surface for binding of the sample in a conformation with the highest affinity for the probe.

The presentation will discuss existing techniques for examining the conformation of immobilized or adsorbed DNA. Environmental effects such as solvent composition and surface chemistry will be shown to have a major effect on the hybridization reaction. Attempts at lowering the detection limits of surface bound DNA will also be addressed.

CG 016 PEPTIDES AS SPECIFIC RECOGNITION DEVICES. H. Mario Geysen T.J.Mason and S.J. Rodda, Coselco Mimotopes Pty Ltd, (wholly-owned subsidiary of the Commonwealth Serum Laboratories), Cnr Duerdin & Martin Streets, Clayton, Victoria 3052, Australia. Identification of the features of an antigen which determine recognition by antibodies would be of great value to the understanding of protein-protein interactions in general, and to the design of peptides which specifically bind antibodies. Recent advances in methods for the simultaneous synthesis of large numbers of peptides have made possible the systematic assessment of the effects of amino acid substitutions on recognition between peptide epitopes and the corresponding antibody. This form of analysis identifies the allowed substitution pattern for each residue. A total of 103 epitopes within 63 well-defined antigenic peptides homologous with the relevant antigen sequences were identified, and the contribution of each amino acid residue to the antibody binding activity of each epitope was investigated(1). For each residue in the epitope, complete sets of peptide analogues containing single amino acid replacements were used to determine the alternative amino acids for which antibody binding activity was retained. The data are summarized in a replaceability matrix which indicates relationships between amino acids in terms of recognition by antibodies. In addition, the average number of residues with limited replaceability in continuous epitopes, and the frequency with which each amino acid is found in those epitopes were determined. Finally, the potential for cross-reactivity between different peptides and a given antibody will be discussed in the context of their use as diagnostic reagents.

(1) Cognitive Features of Continuous Antigenic Determinants, H.Mario Geysen, T.J. Mason & S.J. Rodda. (1988) J.Mol.Rec. Vol. 1, No. 1, 32-41.

CG 017 MICROORGANISMS AS BIOSENSOR DEVICES VIA MICROCALORIMETRY, Rex Lovrien and Irwin Boe, Biochemistry Department, Univ. of Minnesota, St. Paul, MN 55108

Microbial calorimetric analysis (MCA) uses bacteria adapted to specific analytes (carbon compounds) to metabolically combust such compounds to produce heat in proportion to the compound. Bacterial K_m values are about 0.01 to 1 μM for many analytes after adaption, which roughly sets the range of concentrations they sense. When adapted, bacteria usually 'burn' the target compound in 3-6 minutes (25°) if there is an excess of cells. From 30-80 Kcal of aerobic heat/mole of carbon atoms is usually gotten, depending on metabolic path (300 Kcal/mole glucose). Seebeck effect calorimeters are loaded with 1-2 ml. of cells (2 to 5 x 10^9 mg. bacteria) and 0.2-2 ml. of sample; 2-100 nanomoles of analyte. A number of features and advantages may be cited: One may create many "analytic reagents", adapted to the compound of interest, from common bacteria, E. coli, Pseudomonas, etc. Bacteria may be stored (frozen). MCA is applicable to analytes which lack chromogens, prochromogens, analytes difficult to tag with isotopes or to make volatile. The general method is simple and rugged, much because of the ability of whole cells to protect critical enzymes and supply them with cofactors. Numbers of such enzymes and transport particles are fragile and unstable outside the cells, so that even if they can be isolated, electrodes and related devices requiring isolated prooxidative enzymes sometimes are short lived. Because MCA needs only a rough excess of oxygen (air) MCA tolerates large fluctuations in background oxygen concentration in contrast to oxygen respirometry technique. The general method uses essentially the same systems, bacterial conversion/biodegradation, which occur on a large scale in soil, sewage, silage, intestinal fermentation.

A companion method, stripping, also uses adapted bacteria to remove interfering compounds from samples. Bacteria adapted to unwanted phenols, sugars, etc. nearly quantitatively remove them from samples to be analyzed. Applied to MCA, heats of microbial combustion of stripped samples are more assignable to sought for analytes, rather than to a sum of analyte + interfering compound heats. Microbial stripping has been underemployed for analysis in general.

Refs.: A.C.S. Symp. Series 399, N. Lewis, M. Paice, eds., Chap. 39 (1989); App. Environ. Microbiol. 53, 2935 (1987); Biotech. Bioeng., I. Boe and R. Lovrien, In press (1989).

Banquet Address

CG 018 A NEW GENERATION OF BIOSENSORS?, Paul Hansma, UC Santa Barbara, CA 93107.

Scanning probe microscopes such as the scanning tunneling microscope (STM), the atomic force microscope (AFM) and the scanning ion conductance microscope (SICM) may prove to be useful in a new generation of biosensors. These biosensors could consist of an array of different molecular binding sites. This array would be imaged by the scanning probe microscope, which could detect the presence or absence of a molecule bound to one of the sites. Such a biosensor could combine high sensitivity with the ability to sense many different molecules in each image of the array. This talk will focus on progress toward such a device. Scanning probe microscopes have already imaged individual amino acid molecules, DNA, proteins and other biological molecules. It has even been possible to image biological processes. For example, in this talk a video cassette tape will show the polymerization of individual fibrin molecules into a fibrin net. This ability to image individual molecules undergoing processes such as polymerization or binding to an active site may form the basis of a new generation of biosensors.

Processing and Scale-Up

CG 019 DNA OLIGONUCLEOTIDES: DIAGNOSTIC AND THERAPEUTIC APPLICATIONS, Lyle J. Arnold Jr., Genta Inc., 10955 John J. Hopkins Dr., San Diego, CA 92121

Advances in the synthesis and design of oligonucleotides now make DNA oligonucleotides widely useful in diagnostic assays. These advances include both heterogeneous and homogeneous non-isotopic assays which are simple to carry out and are as sensitive as isotopic assays. Chemiluminescent acridinium esters have allowed the development of assays with sensitivities superior to those of ^{125}I. Using acridinium esters, the first practical homogeneous DNA probe assay was developed which is about a million times more sensitive than homogeneous immunodiagnostic assays[1]. Synthesis scale-up to supply the chemiluminescent oligonucleotides for these assays was easily achieved using conventional phosphoramidite DNA synthesis methods. In contrast, the successful development of oligonucleotide therapeutics is highly dependent on the large scale synthesis of unique oligonucleotide constructions. Large scale synthesis is even more challenging because normal diesters for which DNA synthesis methods have been principally developed show minimal application as therapeutic agents, due in part to their rapid degradation by cellular enzymes. Consequently, the therapeutics area demands that analogs of normal oligonucleotides be synthesized on a large scale. Analogs that appear to be particularly promising as therapeutic agents are the methylphosphonate oligonucleotides wherein a methyl group replaces an oxygen of the normal diester backbone. Early work on the synthesis of these analogs using phosphoramidite chemistry made it possible to synthesize only milligram quantities of material. We have modified this chemistry to allow the synthesis of gram amounts of methylphosphonate oligonucleotides by taking into account the instability of the phosphite intermediates. In addition, other methods of synthesis are under consideration for the production of yet larger quantities of material. The chemistries underlying these diagnostic and therapeutic technologies as well as process scale up methods will be discussed.

[1]Clin. Chem., 35,1588-1599 (1989)

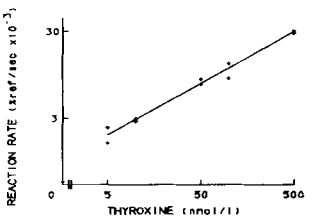
CG 020 **OPTICAL SURFACE TECHNIQUES.** E A H Hall, C Duschl, M J Liley. University of Cambridge, Institute of Biotechnology, Downing Street, Cambridge, CB2 3EF, UK.

The high attenation of reflection behaviour from dielectic multilayers has been exploited to study the adsorption/desorption of ultra-thin organic layers. The technique, which requires a non-absorbing dielectric (eg SiO_2) with dielectric constant $\epsilon_1 = \epsilon_{1,r}$, deposited to a $\epsilon_0 = \epsilon_{0,r} - i\epsilon_{0,i}$, exhibiting a highly reflecting surface (eg silicon), displays a degree of sensitivity **suitable for application in sensor devices.** A layer deposited at the SiO_2-sample interface modulates the reflection emanating from the lower surfaces. Where this layer is chosen as an analyte sensitive interface, the modulation is related to the analyte interaction. Multiple dielectic layer sandwiches can be devised, suitable for producing many different types of analyte recognition interface, with a degree of sensitivity which is frequently comparable with ellipsometry. The technique, known as **interference enhanced reflectivity** (IER) is reported for the first time for use in diagnostics.

By contrast, the optical surface technique of surface plasmon resonance has already been demonstrated as a diagnostic tool, and its merits and limitations assessed. These two techniques will be reviewed and contrasted particularly for application in the development of DNA probes and immunoassay sensors.

CG 021 A BIOSENSOR BASED ON SURFACE PLASMON RESONANCE - PRINCIPLES, PERFORMANCE AND APPLICATIONS, Denise Pollard-Knight and Stephen A. Charles, Amersham International plc, Pollards Wood Research Laboratories, Nightingales Lane, Little Chalfont, Buckinghamshire, England, HP8 4SP.

Surface plasmon resonance (SPR) has been used to detect changes in refractive index occuring when an antigen in solution binds to an antibody immobilised at a silver surface 60nm thick[1]. Typically, changes in refractive index of approximately 10^{-5} can be detected within 100nm of the surface. In theory, any molecular binding pair can be substituted for the antigen/antibody, e.g. DNA/DNA, enzyme/substrate, DNA/DNA binding protein and hormone/hormone receptor. The roles of the solution partner and the surface partner are often interchangeable. We have extended both the original assay work and the applications of SPR to include some of these alternative molecular binding pairs. This has necessitated the production of stable thin silver films and studies on the characteristics of the silver surface. In particular, methods for immobilisation of functional antibodies, enzymes and nucleic acids to the silver surface have been determined and quantified. Utilising instrumentation capable of scanning 6° angle of incidence in 3 milliseconds, we have demonstrated the application of SPR to, a) immunoassay of proteins in the 10^{-9}M range in less than one minute; b) immunoassay of haptens by antibody displacement in the 10^{-9}M range in less than one minute; c) detection of DNA/DNA hybridisation and displacement. Our SPR procedure is rapid, requires no labels (i.e. fluorophores, radiochemicals and enzymes) and involves simple protocols. The sensitivity is limited by the association/dissociation constant of the molecular binding pair, the refractive index change, the instrumental limit and non-specific binding. The sensitivity may be considerably enhanced by the use of refractive index probes or an enzyme label which produces an insoluble product at the surface from a soluble substrate.



Thyroxine Immunoassay

[1]Nylander, C., Leidberg, B., Lind, T. (1982) Sens. Actuators, 3, 79-88.

CG 022 **PROTEIN RECONSTITUTION INTO PHOSPHOLIPID BILAYERS DEPOSITED ONTO PLATINUM ELECTRODES BY LANGMUIR-BLODGETT TECHNIQUES,**

Åke Sellström, Per-Åke Ohlsson, Göran Olofsson and Gertrud Puu, Department of NBC Defence, National Defence Research Establishment, S-901 82 Umeå, Sweden.

Advancing biosensor technology from the use of soluble proteins to biosensors based on membrane bound proteins imposes new problems concerning the immobilisation of these proteins. A biosensor operated with soluble proteins uses either biocatalysis or bioaffinity as its principle of detection, with membrane bound proteins, other reactions such as increased membrane permeability may be utilised.

We have used LB-technology to deposit phospholipid bilayers onto platinum electrodes. We will show that such "artificial cellmembrane" are stable enough to allow protein reconstitution of membrane bound proteins.

Basic requirements for the electrodes for a succesful transfer of a stable phospholipid bilayer will be discussed. Conditions for optimal "immobilisation" of membrane bound proteins will be given and the relative long-term (fourteen days) stability of the activity of a model protein, cytochrome oxidase, will be given.

Preliminary results aimed at evaluating the feasibility of using an increased membrane permeability as a biosensor signal are promising. Electrodes exposed to a NaCl or Na-citrate electrolyte, respond to an altered electrolyte concentration by a transient potential change. Also manipulations of the osmotic pressure of the electrolyte by sucrose will result in a transient potential change. The LB transferred phospholipid bilayer, accordingly, seems semipermable to small particles.

Commercialization: Needs and Opportunities

CG 023 CELLS ON SILICON: THE DETECTION OF CELL-AFFECTING AGENTS WITH THE LIGHT-ADDRESSABLE POTENTIOMETRIC SENSOR, John C. Owicki, Molecular Devices Corporation, 4700 Bohannon Drive, Menlo Park, CA 94025

Most devices that are called biosensors use a biological macromolecule with catalytic or binding specificity to modify the output of a physico-chemical sensor. A less-studied configuration, where living cells replace the macromolecules, is a biosensor in a narrower sense: the sensor directly transduces some strictly biological event. My talk will focus on an example of a cellular biosensor, the silicon microphysiometer [1]. Recently developed at Molecular Devices, this instrument uses a light-addressable potentiometric sensor [2] to detect perturbations in metabolic rates of cultured cells due to a variety of biological, chemical, and physical stimuli. Metabolic rate is identified with the rate of production of acidic metabolites such as lactic acid and carbon dioxide. Examples of applications, beyond metabolic studies in cell biology, include *in-vitro* toxicology, testing for chemo-therapeutic efficacy in oncology, and the discovery of new therapeutic drugs by the detection of the consequences of ligand-receptor interactions. The ability to do bioassays that are sensitive, reproducible, and convenient does have a price; the comparative complexity and fragility of cells pose interesting problems for the development of a robust sensor. Molecular Devices plans to introduce the silicon microphysiometer as a product in late 1990 or early 1991, and I will discuss some of the biological issues attendant to commercialization.

1. J.W. Parce, J.C. Owicki, K.M. Kercso, G.B. Sigal, H.G. Wada, V.C. Muir, L.C. Bousse, K.L. Ross, B.I. Sikic, and H.M. McConnell (1989) *Science*, scheduled for the 13 October issue.
2. D.G. Hafeman, J.W. Parce, and H.M. McConnell (1988) *Science*, **240**, 1182.

Novel Sensing and Transduction Methods; Interfacial Phenomena and Processes

CG 100 CAPACITIVE DEVICES INCORPORATING INTEGRAL MEMBRANE PROTEINS.
Nancy W. Downer, Jianguo Li, and H. Gilbert Smith, EG&G Mason Research Institute, 57 Union St., Worcester, MA 01608.
Integral membrane proteins form a significant subset of the biologically active molecules that will find applications in biosensors. We describe a model biosensor based on the light-transducing protein bacteriorhodopsin, bR, from bacterial purple membranes. A dialysis technique is employed to incorporate bR (or other integral membrane proteins) into membrane-like assemblies covalently bound at the surface of capacitive devices. Results with Ti/TiO_2 electrodes show a capacitance decrease of 600 nF/cm^2 after alkylsilanization with OTS and a further decrease of ≥100 nF/cm^2 after incorporation of protein/lipid from natural membrane by dialysis. Estimates of the layer thickness derived from these measurements are 30 Å and 50 Å for OTS and membrane, respectively. The same dialysis technique was used to form bR-containing membranes on the surface of a capacitive interdigitated array sensor. This model biosensor produced steady-state light-responses of several pF/cm^2 from incorporation of picomolar amounts of bR. These results indicate that the dialysis method used here can effectively couple membrane proteins to capacitive devices and that the resulting structures appear to be like single membrane layers. The studies will be extended to elucidate the molecular basis for the capacitance responses and to incorporate other membrane receptors that respond to chemical stimuli.

CG 101 APPROACHES TO THE DESIGN OF A Mb BASED BIO-SENSOR FOR CARBON MONOXOIDE.
Djordje Filipovic, Patrick S. Stayton, and Stephen G. Sligar, Departments of Biochemistry and Chemistry, University of Illinois, Urbana, Illinois, 61801, USA.
For the use of myoglobin as a CO-sensor at least two parts of the protein must be mutated. A sulfhydryl group for the coupling of the protein to an amphipathic carrier has to be introduced on the surface and the active site must be redesigned in order to improve the ratio of carbon monoxide and oxygen affinities (K_{CO}/K_{O2}).

Since the Fe-O_2-bond is normally bent whereas CO can also bind in the upright position, we try to discriminate between CO and O_2 by forcing upright ligation. In our group several residues in the active site of Mb have been mutated (Springer *et al.*, J. Biol. Chem. *264*, 3057-3060, 1989). The (K_{CO}/K_{O2}) value was altered up to 380-fold greater in these mutants but binding of dioxygen could not be completely prevented, because the ligand still was bound in a tilted position. Efforts to further discriminate between these two heme ligands by mutating other residues in the heme pocket of myoglobin as well as the incorporation of this protein sensor in 2D arrays will be presented.

CG 102 A SYSTEM FOR MOLECULAR INTERACTION ANALYSIS BASED ON SURFACE PLASMON RESONANCE; 2. IMMOBILIZATION OF BIOMOLECULES TO MODIFIED METAL SURFACE,
Bo Johnson, Gabriella Lindquist, Lena Stigh, Stefan Lofas and Jan Bergstrom, Pharmacia AB, Building F61-1, S-751 82 Uppsala, Sweden.
Methods for immobilization of biomolecules and other ligands to hydrogel modified metal surface have been developed for SPR-applications. These methods can be performed in a fully automated mode to give efficient covalent coupling via amino or thiol groups in a fast and reproducible way. Functionalized surfaces are obtained which can stand a wide range of conditions. Surface coverages of proteins from fractions of monolayers up to multilayers (50 ng/mm^2) can be obtained depending on the reaction parameters.

CG 103 A SYSTEM FOR MOLECULAR INTERACTION ANALYSIS BASED ON THE SURFACE PLASMON RESONANCE.
4. SPR APPLIED TO IMMUNOASSAY. Robert Karlsson, Mats Andersson, Åsa Frostell, Carin Gröningsson, Erik Herbai, Margareta Kjellgren and Inger Rönnberg. Pharmacia AB, Building F61-1, S-751 82, Sweden.
The SPR-technique makes it possible to detect protein interactions in real time without using labelled molecules. This is an attractive feature which clearly distinguishes SPR from the techniques like RIA and Elisa. We have used SPR for the development of sandwich and inhibition immunoassays. The sensitivity of the SPR-technique depends not only on the detection principle but in practice also on how the surface is modified and how the sample is brought in contact with the surface. In many cases flow cell design and flow rate determine the binding rate. In other cases ligand density can be of extreme importance for keeping unspecific binding to a minimum. The development of rapid immunoassay systems for Immunoglobuline E (IgE) down to the 10pM level and Thyroxine (T4) down to the nM level describe these effects.

CG 104 A SYSTEM FOR MOLECULAR INTERACTION ANALYSIS BASED ON SURFACE PLASMON RESONANCE.
3. CORRELATION OF THE SPR RESPONSE TO SURFACE CONCENTRATION. Mari Kullman Björn Persson, Håkan Roos and Esa Stenberg. Pharmacia AB, Building F61-1, S-751 82 Uppala, Sweden.
The system has been used for model studies of protein adsorption to a hydrogel immobilized to a metal film. The SPR response is related to the amount and the distribution of the adsorbed proteins within the hydrogel. The amount of proteins was determined by the use of radiolabelled proteins. Different proteins and labelling techniques were used and the radioactivity was measured in a surface scintillation counter. The SPR response was found to be linearily correlated to protein surface concentration in the range 2 - 50 ng/mm2. The protein distribution within the hydrogel will be discussed from a theoretical point of view.

CG 105 GENETIC ENGINEERING OF THIN FILM BIOSENSORS, Stephen Sligar and Paul Bohn, Departments of Biochemistry and Chemistry and The Beckman Institute for Advanced Science and Technology, University of Illinois, Urbana, IL 61801.
We utilize totally synthetic genes for two metalloproteins, sperm whale myoglobin and rat hepatic cytochrome, which are expressed to gram levels in *E. coli* (Proc. Nat. Acad. Sci. USA *83*, 9443 (1986) and *84*, 8961 (1987)). Through the specific modification of protein surfaces by recombinant DNA technology, we can provide unique attachment sites for aliphatic side-chains which can be positioned to order these cytochromes at an air-water interface. Transfer from the L-B trough to appropriate substrate allows detailed characterization by various thin-film optical techniques. Genetic engineering of protein active sites allows manipulation of macromolecular recognition events which are used to construct highly specific ligand affinities for use as biosensors. Our most recent results in this research endeavor will be presented.
Supported by the Biotechnology Research and Development Corporation and the Illinois Capital Development Board.

CG 106 A SYSTEM FOR MOLECULAR INTERACTION ANALYSIS BASED ON SURFACE PLASMON RESONANCE.

1. THE ANALYTICAL SYSTEM, Bengt Ivarsson, Ulf Jönsson, Csaba Urbaniczky, Esa Stenberg, Stefan Sjölander, Stefan Sowa, Ralph Stålberg, Henrik Östlin. Pharmacia AB, Building F61-1, S-751 82 Uppsala, Sweden.

The analytical system detects molecular interactions on a surface in real time without any labels such as radioisotopes or enzymes.
The basic features of this system are:

- **Detection of surface concentration by surface plasmon resonance (SPR), an optical technique whereby changes in optical properties in close contact with a surface can be followed in real time.**
- **The combination of optics, thin film deposition and surface chemistry to a detection surface.**
- **An integrated liquid handling system that optimizes the mass transport of molecules to the detection surface and thereby minimizing sample consumption.**

The basic features are put together in a sofware controlled instrument whereby completely automatic analysis of molecular interactions can be performed.

CG 107 ACETYLCHOLINE RECEPTORS IN LIPID BILAYERS ON A SILICONE WAFER SURFACE. Horst Vogel, Ecole Polytechnique Fédérale de Lausanne, Suisse

A mixed lipid/acetylcholine receptor (AchR) monolayer was formed at the air-water interphase of a Langmuir trough. Supported bilayers containing the AchR were prepared by transfering sequentially two monolayers from the air-water interphase onto the surface of a silicon wafer. A transparent indium-tin counter electrode was placed over a buffer layer covering the membrane. The complex impedance of the device was measured in the audio radio frequency range. At certain frequencies the impedance changed linearly with the logarithm of the acetylcholine concentration between 10^{-8} to 10^{-5}M. This effect is discussed in terms of activation and desensitizing of the AchR. The present observation shows the potential applicability of membrane protein receptors in planar supported membranes within a biosensor device.

CG 108 ELECTROCHEMICALLY DEPOSITED BIOFILMS IN BIOSENSORS. E A H Hall. J C Cooper, M K Deshpande. University of Cambridge, Institute of Biotechnology, Downing Street, Cambridge CB2 3EF, UK.

Electrochemically grown conducting polymers have been identified as good immobilisation matrices for biomolecules. Conducting polymers such as polypyrrole and polyaniline can be produced by electrochemical oxidation of the monomer in aqueous solution. Polymerisation in the presence of a biorecognition species, produces a hybrid network, with the species entrapped in the polymer matrix. Amperometric sensors have been designed which employ redox enzymes (eg glucose oxidase, polyphenol oxidase etc) or whole cells entrapped in a polypyrrole film to selectivly monitor a particular analyte. In these examples the polymer acts as an immobilisation matrix, and the assay is related to current density due to hydrogen peroxide or an artificial mediator.

By contrast, a polyaniline-redox enzyme model has been tested, where the specific analyte induced signal is related to a change in the conductivity of the polymer film. In this example the role of the polymer is greater than a simple immobilisation matrix, since it is the transducing agent for the assay.

Biochemistry and Molecular Biology of Biosensors and Bioprobes

Antibody- and Enzyme-Based Sensors

CG 200 CHEMILUMINESCENT CAPILLARY FIBER OPTIC IMMUNOSENSOR FOR VIRUS ANTIGEN DETERMINATION, Arenkov, P. Ya., Berezin, V.A., Department of Biophysics and Biochemistry, Dniepropetrovsk State University.

This immunosensor is based upon using identically made optrodes (pieces of optical fiber 60 mm in length) inserted into short glass or plastic capillaries (10 mm in length). Measuring optrodes had covalently immobilized monoclonal antibody to hemmaglutinin of influenza A/Tw virus on fragmentally stripped ends. Optrode with BSA was used as a control. Capillaries had the same dry antibody with peroxidase label on inner walls. Measuring was fulfilled by inserting one end of every optrode into a special binding of a photomultiplier and immersing the capillary end into a solution of nonvirulent influenza A/Tw virus. After following washing the capillary end pulled into an enhance chemiluminescent solution. The signal from photon counter was treated by a personal computer and out-put on printer. The measuring concentration range was 1-1000 ng/ml of the antigen, incubating time was 1 min. The sensitivity was 5 ng/ml. The measuring time was 0,3-4 min.

CG 201 A STUDY OF THE FRAGMENTATION BEHAVIOUR OF IgG MONOCLONAL ANTIBODIES OF SUB–CLASSES 1, 2a AND 2b, V.L.B. Braach–Maksvytis, +P. Anderson–Stewart, +R. Johanson, +T. Senese and +M. Svejkar, CSIRO PO Box 52 North Ryde, N.S.W. 2113 Australia and +Bioclone Australia Pty Ltd, 54c Fitzroy St, Marrickville N.S.W. 2204 Australia.

Antibodies have been used as receptor molecules in liposomal drug–delivery systems and biosensors. In particular $F(ab')_2$ and Fab' fragments have been used to reduce the size of the molecule and to avoid possible complement activation by the Fc fragment. Fab' fragments offer the further advantage of possessing a sulfhydryl group located well away from the binding site which can be used to covalently attach and orient the Fab' fragment onto the liposome or biosensor membrane.

Nine monoclonal antibodies raised against protein hormones were digested with pepsin to produce the $F(ab')_2$ fragments. In order to increase the accessibility of the antibody hinge region to the enzyme, it was necessary to treat the antibodies to an acid incubation before proceeding with the pepsin digestion. All IgG_1 and IgG_2a antibodies produced $F(ab')_2$ fragments to some extent while the IgG_2b antibody was further fragmented.

The highest yield of Fab' fragments was produced when freshly prepared $F(ab')_2$ fragments were immediately reduced with dithiothreitol, otherwise complete reduction to the light and heavy chains resulted. There was no difference in Fab' fragmentation behaviour between antibodies of the different sub–classes, hence individual fragmentation conditions were tailored to suit individual monoclonal antibodies.

CG 202 ANTIBODY-BASED SENSOR FOR POLYNUCLEAR AROMATIC COMPOUNDS: A REVIEW OF OUR EXPERIENCE, Guy D. Griffin, Michael J. Sepaniak, Bruce J. Tromberg, Jean-Pierre Alarie, and Tuan Vo-Dinh. Health and Safety Research Division., Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, Tennessee 37831, and Chemistry Department, University of Tennessee, Knoxville, TN 37996.

Microsensors to detect trace amounts of benzo(a)pyrene (BP) or certain of its metabolites have been developed by generating antibodies to BP (or the metabolite), and immobilizing these antibodies at the distal (sensing) end of an optical fiber. Advantage is taken of the fluorescence of BP as the basis of the analytical method, using laser (Argon ion) light excitation. Methods used to prepare an anti-BP specific antibody will be described, as well as studies designed to test the specificity of the antibody. Several techniques/configurations to immobilize the antibody to the optical fiber and to provide a reaction chamber for the antigen-antibody reaction will be discussed. The response characteristics of the completed device and experiments designed to test the regenerability of the sensor will be discussed. The sensor has been found capable of detection of femtomole amounts of BP in minute (5 μL) volumes. The device has been applied to detection of BP metabolites adducted to DNA. Further applications of the general principle to different toxic chemicals will be discussed.

CG 203 A STUDY OF SURFACE PROCESSES IMPORTANT TO THE OPERATION OF NADH-BASED AMPEROMETRIC BIOSENSORS, C. Korzeniewski, V. L. Berger, Department of Chemistry, The University of Michigan, Ann Arbor, MI 48109.

Electrochemical biosensors combine the specificity of biological recognition with the high sensitivity of electrochemical detection. One class of biosensors relies upon electrochemical oxidation of the enzyme cofactor nicotinamide adenine dinucleotide (NADH, reduced form), and is specific for substrates of dehydrogenase enzymes. These devices are constructed by immobilizing the enzyme and oxidized form of the cofactor (NAD^+) at the surface of a metal electrode. Enzymatic oxidation of substrate by NAD^+ to form NADH followed by electrochemical oxidation of NADH forms the basis of sensor operation. The short term performance of NADH-based biosensors is often limited by electrode fouling from the oxidation of NADH, and the high overpotential necessary for this reaction can lead to interferences from electroactive species in the sample. Electrochemical studies suggest decomposition of radical intermediates and the primary acid rearrangement product of NADH as possible surface poisons. We are using surface infrared spectroscopy and nmr spectroscopy to investigate the importance of these reaction pathways. Results of studies at bare metal and chemically modified electrodes will be presented.

CG 204 MEASUREMENT OF SPECIFIC ANTIBODIES IMMOBILIZATION ON SURFACES OF QUARTZ OPTICAL FIBER BIOSENSORS USING FLUORESCENCE AND ^{125}I LABELING METHODS, L.W. Jiang, A. Margaritis* and N.O. Petersen, Department of Chemical and Biochemical Engineering, The University of Western Ontario, London, Ontario, Canada, N6A 5B9.

Optical fiber biosensors are rapidly emerging as a new class of highly efficient and sensitive sensor systems with a wide range of industrial applications, which may include chemical and biochemical analytical laboratories, clinical-medical laboratories, on-line process monitoring and control including bioreactor control. One of the basic features of an optical fiber biosensor system is the immobilization of a biomolecule (enzyme, monoclonal antibodies, etc.) on the decladded surface of an optical fiber, such as quartz fiber, which can be used to interact with an analyte (substrate, antigen, etc.) present in a sample. Successful immobilization of the biomolecule on the surface of a quartz optical fiber is crucial to the sensitivity and stability of the biosensor system.

In this paper we present data on the covalent immobilization of protein A on the surface of quartz fibers with subsequent binding of specific antibodies to protein A. Fluorescence imaging was used to characterize the distribution of protein A, antibody and ligand binding to the quartz fiber surface. In addition, ^{125}I labeling of the components was used to make quantitative measurements of surface coverage by these components. Our data demonstrate that it is possible to obtain homogeneous surface coverage at near maximal surface densities.

CG 205 INTERACTION OF LIPOSOMES WITH SOLID PHASE ANTIBODIES, Anne Plant, Laurie Locascio-Brown, Steven Choquette, Richard Durst, Center for Analytical Chemistry, National Institute of Standards and Technology, Gaithersburg, MD 20899.

Antibodies are immobilized onto solid supports such as glass and silica for use in analytical devices and immunosensors. Liposomes (single bilayer phopholipid vesicles), are made immunoreactive by appropriate covalent and noncovalent modifications of their exterior membrane surface with derivatives of antigen or antibody molecules. Liposomes which are 0.2 μm in diameter each contain 2×10^5 highly absorbant and fluorescent molecules in their aqueous centers, and thus provide enhanced sensitivity compared to traditional immunoreagent tags. In addition, these structures have distinct advantages in certain immunoassay formats as a result of their multivalency. The affinity constant of liposomes for immobilized antibodies is several orders of magnitude higher than that of univalent analogs, and is dependent on the extent of derivatization of the liposomes. The increased affinity constant is a result of a decreased dissociation rate constant. We demonstrate the use of liposomes in an automated immunoassay system using flow injection analysis, and in a optical waveguide immunosensor. The implications for tailoring assay sensitivity and assay formats using liposomes will be discussed.

CG 206 DESIGN AND DEVELOPMENT OF A FLUORESCENCE-BASED FIBER OPTIC BIOSENSOR, B.R.Ratna*, Suresh.K.Bhatia+, Lynne Kondracki+, Frances S. Ligler, Lisa Shriver-Lake, Richard B.Thompson, Center for Bio/Molecular Science and Engineering, Code 6090, Naval Research Laboratory, Washington DC 20375-5000, *Department of Biochemistry, Georgetown University, Washington DC 20007, +Geo-Centers Inc., 10903 Indian Head Hwy., Ft. Washington, MD 20744.

A biosensor capable of detecting a wide range of substances via changes in the evanescent wave-excited fluorescence on the surface of an optical fiber is being developed at the Naval Research Laboratory. Novel chemistry for immobilizing functional antibodies on the surface of optical fibers at high densities has been developed. Initial immunoassays performed using radiolabelled antigens showed the nonspecific binding to be very low. A sensor testbed, in the evanescent wave configuration, has been built. The sensitivity of the device has been enhanced by incorporating novel components. Various factors influencing the efficiency of the device are being examined. For instance, efforts are being made to design an optical fiber in order to maximize the fluorescence detected. Issues such as reproducibility, calibration and quantitation are also being addressed.

CG 207 PROTEIN ENGINEERING OF SELF ASSEMBLY AND ELECTRONIC CENTER ORIENTATION IN TWO DIMENSIONS WITH THE REDOX ACTIVE CYTOCHROME B5, Patrick S. Stayton, Paul W. Bohn, Stephen G. Sligar, Departments of Biochemistry and Chemistry, University of Illinois, Urbana, IL, 61801.

The development of traditional electronic devices is based on the ability to manipulate materials by chemical and physical synthetic methods. Successful utilization of biological macromolecules in electronic devices requires similar synthetic control. Powerful advances in protein engineering techniques provide the means for modifying protein structure in ways applicable to bioelectronic devices. We report the results of such an approach using the structurally characterized, heme containing redox protein, cytochrome b_5. Unique cysteine residues have been genetically engineered on the cytochrome b_5 surface at two defined loci. These thiols have then been used as attachment sites to differentially orientate the prosthetic heme redox center both on a sulfhydral derivatized silicon oxide surface and on a Langmuir-Blodgett derived monolayer of a sulfhydral specific amphiphile. The integrity of cytochrome b_5 on these surfaces has been confirmed with resonance Raman spectroscopy and the orientation of the proteins determined by measuring the linear dichroism of the prosthetic heme group. These results demonstrate the ability to spread and orientate the electronic center of a redox proteins relative to an inert substrate using genetic engineering techniques.

CG 208 Regeneration of Immunosorbents: Factors Considered and Comparison of Adsorbed And Covalently Bound Antibody

G.C. Blanchard, C.G. Taylor, B.R. Busey and M.L. Williamson

1 Veterans Administration Medical Center, Boston, MA, USA 2 Transamerican Immunology, Inc., N. Quincy, MA, USA and 3 Department of Defense, US Army Chemical Research Development and Engineering Center (CRDEC), Aberdeen Proving Grounds, MD, USA.

The dissociation of ag-ab complexes in immunoanalyses would allow the reuse of adsorbed and covalently bound antisera and further promote the development of emerging biosensors and ELISA use in clinical settings for efficient and inexpensive detection and diagnosis of a broader range of analytes. A systematic approach to evaluating ag-ab dissociating solutions (ie. HCl, proprionic acid, ethylene glycol and SDS in urea) on commercial protein sorbent materials with diverse physical characteristics (ie. glass, membranes and plastic) was undertaken to determine the feasibility for reuse. Although subtle differences were evident in the reaction efficiencies, dissociation and reassociation, the reuse of all of the commercial immunosorbents in ELISA's proved to be highly feasible. The results are in agreement with findings by Hendry and Herrmann (1980) and Sutherland (1984), the later of whom alluded to multiple (upto 30) regenerations of antibody on glass. The model system provides a means for evaluating antibody operational stabilities on use with various denaturants or contaminants that may be present in analytical specimen samples. The experimental model and methods described lend themselves to useful alternatives for further probing the nature of non-covalent interactions at the antibody-surface interface and ag-ab combining site.

DNA Probes; Receptors and Organelles

CG 300 CONFORMATIONAL MAPPING OF THE MEMBRANE SPANNING DOMAINS OF THE BACTERIAL CHEMOSENSORY RECEPTOR TRG USING SITE-DIRECTED MUTAGENESIS AND CYSTEINE BIOCHEMISTRY. Gregory G. Burrows, David P. Dutton and Gerald L. Hazelbauer, Biochemistry/Biophysics program, Washington State University, Pullman, WA 99164-4660. The *Escherichia coli* chemosensory receptor Trg is an approximately 60 kDa polypeptide that spans the cytoplasmic membrane twice. These putative membrane spanning sequences (amino acid residues 17-46 and 199-222) link two separate domains of the receptor, one on the periplasmic side of the membrane that is responsible for ligand binding and a carboxy-terminal cytoplasmic domain that undergoes covalent modification in response to ligand binding. Circular Dichroic spectra of purified detergent-solubilized Trg indicate that the secondary structure of the protein is predominantly α-helix. The receptor is dimeric. Site-directed mutagenesis was used to create modified proteins with cysteine residues substituted at individual sites along the membrane spanning domains. Mutant strains expressing these proteins were behaviorally indistinguishable from wild-type. Partially purified mutant proteins were subjected to chemical modification by either [^{3}H]-N-ethyl maleimide or to the oxidizing catalyst Cu(II) (1,10-phenanthroline)$_3$. The extent of covalent modification by [^{3}H]-N-ethyl maleimide provides a means of examining the tertiary structure of the membrane spanning domains. Mutant proteins with cysteines exposed on the surface will be modified to a greater extent than those with cysteines buried within the structure. Cu(II) (1,10-phenanthroline) is used to catalyze disulfide formation between cysteine residues present on each mutant monomer, providing a measure of the proximity of these residues to each other in the dimeric structure. The mutant proteins show a range of covalent modification and cysteine oxidation products consistent with a model of the receptor as a dimer with four interacting membrane spanning α-helical domains. A discussion of the significance of this organization in terms of signal transduction is presented.

CG 301 CEREBRAL HORMONE-SENSITIVE CALMODULIN-INDEPENDENT ADENYLATE CYCLASE. PRIMARY STRUCTURE AND HYPOTHETICAL SPATIAL ORGANIZATION IN MEMBRANE. Albert N.Obukhov, Valery M.Lipkin, Nikolay V.Khramtsov. Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR

The adenylate cyclase system is an enzyme transmembrane complex, synthesizing cAMP in response to hormone binding to the cell. It consists of three components: receptor, regulatory GTP-binding proteins, and catalytic C-protein. Two forms of enzyme, calmodulin-sensitive and calmodulin-independent, are known to occur in the cerebral cortex. Affinity chromatography of bovine cerebral cortex membrane proteins, solubilized with Lubrol PX, on the monoclonal antibody immunosorbent yielded calmodulin-independent catalyst. The complete cDNA sequence (3486 bp), containing codes for the calmodulin-independent adenylate cyclase catalytic component, has been reconstituted. The polypeptide chain consists of 834 aa protein (Mr 91735) and 19 aa signal peptide. The computer-predicted enzyme spatial organization and behaviour in membrane are discussed.

CG 302 EXPRESSION OF ACTIVE STREPTAVIDIN IN *E. COLI* AND ITS APPLICATION TO NEW DETECTION SYSTEMS WITH STREPTAVIDIN-CONTAINING FUSION PROTEINS Takeshi Sano and Charles R. Cantor, Dept. of Molecular Biology, University of California, Berkeley, CA 94720. We have developed an expression system for the cloned streptavidin gene in *E. coli*. Although the streptavidin gene is extremely toxic to the host cells because of the strong biotin-binding affinity of the gene products, streptavidin could be expressed efficiently by using T7 RNA polymerase/T7 promotor expression system (Studier *et al.*, Methods Enzymol. In press) with a help of T7 lysozyme to reduce the basal level of the T7 RNA polymerase activity in the uninduced state. The expressed streptavidin reached more than 35 % of the total cell protein. The expressed streptavidin could be isolated easily by a simple purification procedure involving solubilization in 6 M guanidine·HCl, and the purified preparation showed almost full biotin-binding ability. We have also constructed several expression vectors for core streptavidin (truncated form), and they were also expressed efficiently by using the same T7 system. The core streptavidin should be free from aggregate formation and very stable against proteolysis. These properties of the recombinant core streptavidin would improve considerably the detection system by reducing non-specific binding. The establishment of an expression system for the cloned streptavidin gene will allow us to construct streptavidin-containing fusion proteins. By simply inserting the coding sequences of a target protein to the expression vector downstream of the cloned streptavidin gene, the target protein containing the streptavidin moiety could be expressed. The streptavidin-containing fusion proteins will offer a variety of applications for protein detection and isolation through its specific and strong binding-affinity for biotin. (Supported by NCI Grant CA39782)

Biochemistry and Molecular Biology of Biosensors and Bioprobes

CG 303 DEVELOPMENT OF A PCR ASSAY FOR DETECTION OF Toxoplasma gondii.
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Serology is currently the most widely used technique for the diagnosis of toxoplasmosis; however, it gives unreliable results with some patients (eg those with AIDS or being immunosuppressed). We have used the polymerase chain reaction (PCR) to amplify a toxoplasma-specific DNA sequence (that for the major surface antigen) and we have used this to examine a number of human and mouse tissues and body fluids for the presence of the parasite's DNA. The results indicate that the test is specific for toxoplasma and that it is capable of detecting different isolates of the parasite. The test has been used to detect toxoplasma in brain biopsies from AIDS patients, in the peripheral blood of transplant recipients, in the cord blood and placenta of congenitally-infected infants, in peritoneal fluids from mice infected with a virulent strain and in the brains of chronically-infected mice.

CG 304 STORAGE & GROWTH OF NEUROBLASTOMA CELLS IMMOBILIZED IN CALCIUM-ALGINATE BEADS. Michel Simonneau [1], Christian Tamponnet [2], Sylvie Boisseau [1], Pierre-Noël Lirsac [2], Jean-Noël Barbotin [3], Maurice Lievremont [2] & Chantal Poujeol [1]
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Calcium-alginate beads immobilization techniques were adapted to mammalian neuronal cells. In standard culture conditions (37°C; 5% CO_2), cell growth was observed inside beads. The number of cells increased 4 fold for 7 days of culture with pictures of cell division and differentiation which were visualized by electron-microscopy. Cell properties were maintained after short-term storage (2-3 days at 4°C): (i) properties of voltage-dependent ionic channels, specially Na^+ and K^+ channels, were tested by patch-clamp electrophysiological techniques, using both single channel and whole cell recordings (Leneveu & Simonneau, 1986; Valmier, Simonneau & Boisseau, 1989); (ii) expression of cell-adhesion membrane proteins was tested by immunohistochemistry using polyclonal antibodies against N-CAM molecules (Boisseau *et al.*, in preparation) (iii) morphological differentiation obtained by depletion of foetal calf serum in culture medium.

CG 305 A COMBINED SOLID PHASE EXTRACTION CHROMATOGRAPHY AND RADIOIMMUNOASSAY SYSTEM FOR SELECTIVE EXTRACTION, ELUTION AND QUANTITATION OF LND-796, A NOVEL CARDIOTONIC STEROID, AND ITS METABOLITES IN PLASMA, J.R. Skuster, E.J. Cabrera and R.M. Rydzik, Norwich Eaton Pharmaceuticals, Inc., Norwich, NY 13815 and G.D. Searle and Co., Skokie, IL 60077A
A highly specific and very sensitive assay was developed for measuring levels of LND-796, an aminosteroid cardiotonic, when closely related metabolites were also present in the samples. The methodology used an established radioimmunoassay (RIA) which could detect parent compound at the picogram/ml levels for quantitation. Because the RIA was strongly cross reactive with likely metabolites, a method was developed for separating them from parent compound. TLC was used as a tool for evaluating a series of reverse and normal phase systems for their extraction and elution properties. A chromatography system was developed which used cyanopropyl bonded Solid Phase Extraction (SPE) columns to extract and then selectively elute the compounds of interest. LND-796 and a marker metabolite were extracted from the aqueous phase using a reverse phase mode with nearly 100% efficiency. Other more polar metabolites were not bound to the SPE column. Using a normal phase elution system, the marker metabolite was selectively eluted, followed by LND-796. Solvents were removed by evaporation and the analyte concentrations determined using the RIA. Wide variations in the performance of cyanopropyl SPE columns were seen between those obtained from several manufacturers. The system allowed for the measurement of LND-796 at sub-nanogram/ml levels even when cross reactive metabolites were also present in the biological sample.

CG 306 USE OF VDAC IN A RECEPTOR-BASED BIOSENSOR MODEL SYSTEM, D.A. Stenger, T.L. Fare and E.L. Chang, Center for Bio/Molecular Science and Engineering, Code 6090, Naval Research Laboratory, Washington, D.C. 20375.

Voltage-dependent anion channel (VDAC) from baker's yeast is used for the development of a receptor-based biosensor model. The channel may be purified in sufficient quantities (1) and has a large conductance (4-5 nS) which can be modulated by application of a membrane potential (2). The electrical impedance of multi-channel bilayer lipid membranes (BLMs) is measured using a dual phase lock-in amplifier as a function of the applied d.c. offset voltage from 0.5 Hz to 5 KHz. This technique allows for a more detailed membrane characterization, is tolerant of background noise, and reduces electrode polarization. An increase of the in-phase (resistive) component occurs for offset values between 0 and 40 mV, corresponding to a 60% decrease in the VDAC conductance. Current efforts to make the impedance measurement on solid support VDAC-containing membranes formed by Langmuir-Blodgett film deposition will be discussed.

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CG 307 DNA PROBE CHROMATOGRAPHY FOR DETECTION OF SINGLE BASE SUBSTITUTIONS IN NUCLEIC ACIDS Akira Suyama, Masahiro Tagawa, Tatsuya Mitsuma and †Akiyoshi Wada, Department of BioEngineering, Nagaoka University of Technology, Nagaoka 940-21, Japan and †Department of Physics, The University of Tokyo, Tokyo 113, Japan

We have been developing the DNA probe chromatography in order to detect and separate nucleic acids according to base sequences. DNA probe columns used in this chromatography contain solid supports on which synthetic DNA oligomers of defined base sequences are immobilized at the 5'-terminals through hydrocarbon chain linkers. Nucleic acid samples injected into the columns are eluted by gradually elevating the column temperature. Samples are eluted at the temperature corresponding to base sequence homology to the sensor DNA probe. We have already reported results of applications of this method, which was proved to be powerful. However, the resolution of the method in sequence discrimination was not sufficient for detection of single base substitutions occurring in sample nucleic acids. In order to increase the resolution, we have recently improved the materials of the solid supports and immobilization procedures. As a result of this improvement, we have succeeded in complete separation of samples with single base substitutions occurring at the central part of the DNA probe sequence from intact ones within about one hour. Even for single base substitutions occurring at a quarter position from the DNA probe terminals, good separation is still obtained. The DNA probe chromatography with the detection ability of single base substitutions will definitely provide a powerful tool for various applications requiring nucleic acid separation according to base sequences including the diagnosis of genetic diseases. This study was performed in part through Special Coordination Funds of the Science and Technology Agency of the Japanese Government.