ESC-497D, Guest Lecture

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Sensor Devices

- Sensors fall into two general categories:
  - **Physical** parameters of biomedical importance include pressure, volume, flow, electrical potential, and temperature, of which pressure, temperature, and flow are generally the most clinically significant, and lend themselves to the use of small *in vivo* sensors.
  - **Chemical** sensing generally involves the determination of the concentration of a chemical species in a volume of gas, liquid, or tissue. The species can vary in size from the H+ ion to a live pathogen and when the species is complex, an interaction with another biological entity is required to recognize it. When such an entity is employed, the sensor is considered a biosensor. It is, in general, necessary to distinguish this chemical from a number of similar interferents, which can be technologically challenging, but this is an area in which biosensors excel.

Physical Sensors

- Modern methods of measuring the temperature of a bulk material such as blood or tissue are based on temperature dependent electrical properties of matter. Devices include:
  - thermocouples,
  - resistance temperature detector (RTD) sensors,
  - thermistors,
  - silicon diodes, microcomputer-based applications
- The sensor must be placed into tissue or blood, so to maintain accuracy there should be little transfer of heat into or out of the body along the leads to the thermometer.

Chemical Sensors

- A variety of chemical sensors employing different transduction mechanism are currently employed fro in vivo and in vitro measurement of biological parameters.
- These are summarized in the table

TABLE 2 Transducers Used in Chemical Sensors

Transducer	Mode of measurement
Ion-selective electrode, gas-selective electrode, FET	Potentiometry—determination of surface concentration of charged species
Oxygen electrode, electrochemical electrode	Amperometry—monitoring available concentration of electrochemically active species
Low impedance electrodes for monitoring conductance, impedance, admittance	Monitoring changes in bulk or surface electrical properties caused by altered molecular concentrations
Optical waveguides with detection of absorption, fluorescence, phosphorescence, chemiluminescence, surface plasmon resonance	Photometry
Thermistors, RTD, calorimeters	Monitoring temperature change induced by chemical reaction
Piezoelectric crystal SAW, BAW, etc., with chemically selective coating	Change in sound absorption or phase induced by binding to device

Biosensors

- Biosensors are sensors that use biological molecules, tissues, organism or principles (Nature makes the best sensors)
- The development of biosensors is driven by increased need for biochemical information in the medical community, the knowledge that nature senses these chemical best, combined through emerging technologies to interface the biochemical with physical transducers.

TABLE 4 Biological Components of Biosensors[a]

Binding	Catalysis
Antibodies	Enzymes
Nucleic acids	Organelles
Receptor proteins	Tissue slices
Small molecules	Whole organisms
Ionophores	

USE OF BIOCHEMICALS FOR CHEMICAL DETECTION

Advantages and disadvantages of biosensors are summarized in the table.

Advantages for binding
- "Uniquely" high selectivity
- Possibility of raising antibodies to nearly all antigens
- Antibodies and biotin-avidin system allow selective attachment of markers and reporters of binding
- High binding constants possible
- Several possible detection modalities
- Ion flux through gated channels can provide gain

Advantages for catalysis
- For every biochemical there is an enzyme that can be used to detect its presence
- High selectivity possible with some enzymes
- Several possible detection modalities
- Enzymatic cascades can provide gain
- Universality of redox coupling and pH effects permit common transduction schemes

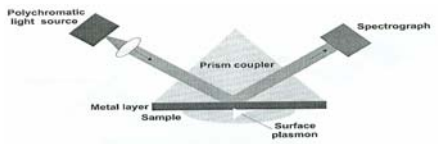
Disadvantages of biosensors
- Biomolecules generally have poor thermal and chemical stability compared with inorganic materials
- The function of the biological component usually dictates that they must have narrow operating ranges in temperature, pH, ionic strength
- Susceptibility to enzymatic degradation is universal
- Need for bacteriostatic techniques in their fabrication
- Time-dependent degradation of performance is guaranteed with the use of proteins
- Production and purification can be difficult and costly
- Immobilization can reduce apparent activity of enzymes or kill them outright
- Most live organisms need care and feeding

Optical Biosensors

- **Today**, the optical biosensor technologies include
  - fiber optic biosensors,
  - planar waveguides,
  - the displacement flow sensors,
  - sensors based on time-resolved fluorescence,
  - electrochemiluminescence,
  - surface plasmon resonance,
  - resonant mirrors,
  - interferometry.
- The science for ***future technology*** of biosensors development includes four different methods for producing new recognition elements
  - genetic engineering of proteins,
  - chemical synthesis,
  - combinatorial selection of nucleotide-based receptors,
  - molecular imprinting
- Two methods for immobilizing receptors on biosensors
  - sol gels
  - semi-synthetic membranes
- Two methods for producing very bright signals (PEBBLES and quantum dots), and soft lithography for surface patterning and microfluidics.

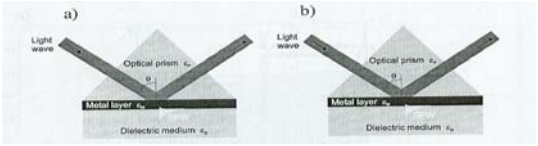
Surface Plasmon Resonance

- Surface plasmon resonance (SPR) biosensors use surface plasma waves (SPW) to probe biomolecular interactions occurring at the surface of a sensor. Plasma waves behave like nearly free electron plasmas.
- History:
  - Wood (1092): observed the spectra of continous light source using a relection diffraction grating, noticed narrowdark bands in the spectrum of diffracted light
  - Fano (1941) grating is due to the SPW
  - Otto (1968): SPW may be excited using attenuated total reflection.
  - Nylander et al 1982, detection of gas
  - Biacore,2001, biosensor technology



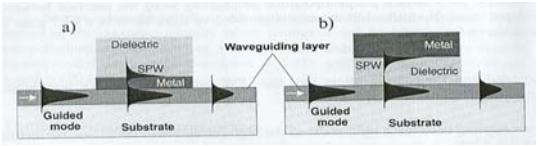
SPR: Surface Wave

- The coupling between a light wave in the high refractive index dielectric medium and an SPW at the metal - low-refractive index dielectric interface can be established by the total internal reflection method. A light wave passes through a high refractive index prism and is totally reflected at the prism base generating an evanescent wave penetrating a metal film in the Kretschmann configuration (Figure a) or a dielectric layer in the Otto configuration (Figure b). This evanescent wave propagates along the interface with the propagation constant, which can be adjusted to match that of the SPW by controlling the angle of incidence. Thus, the matching condition can be fulfilled allowing the evanescent wave to be coupled into the SPW.



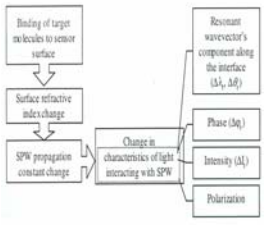
SPR: Waveguides

- Light propagates in a waveguide in the form of guided modes. The electromagnetic field of a guided mode is concentrated in the wave guiding layer. A fraction of the optical energy propagates in the form of an evanescent wave in the low-refractive index medium surrounding the waveguiding layer. In the section of the waveguide containing an SPW-active metal film, this evanescent wave can excite an SPW at the outer (in Figure a) or inner (Figure b) surface of the metal film.



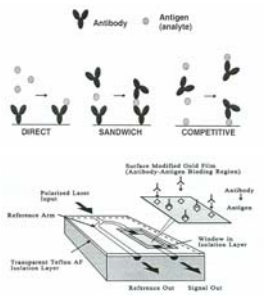
SPR-Bionsensors

- In principle, any phenomenon which gives rise to a change in the refractive index at the surface of the SPW -active metal film can be observed and quantified by means of an SPR sensor.
- SPR biosensors are SPR sensing devices that incorporate biomolecules, which recognize and are able to interact with selected analytes. These biomolecular recognition elements are immobilized on the SPR sensor surface. When a solution containing analyte molecules is brought into contact with the SPR sensor, analyte molecules in solution bind to the recognition elements on the sensor surface, producing an increase in the refractive index at the sensor surface. This change produces a change in the propagation constant of the SPW and is eventually measured by measuring a change in one of the characteristics of the light wave interacting with the SPW



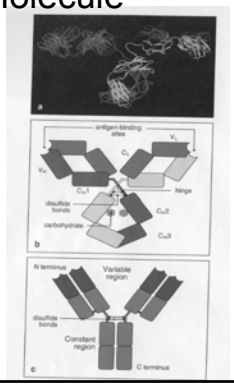
SPR-Biosensors

- SPR biosensors have been demonstrated for detection of various analytes. Various detection formats have been employed in SPR biosensing in order to optimize performance of SPR biosensors for specific applications. The main detection formats include direct, sandwich and competitive assays (Figure). In the direct detection format, a sample containing target molecules is brought into contact with the sensor surface coated with respective biomolecular recognition elements (e.g., antibodies). Binding of analyte molecules to antibodies produces an increase in the refractive index at the sensor surface. The SPR sensor instrument translates this change into a change in sensor response. A sandwich assay consists of two steps. In the first step, the analyte molecules bind to antibodies immobilized on the sensor surface as in the direct detection format. In the second step, the sensor is incubated with a solution containing secondary antibodies, which bind to the previously captured analyte, enhancing the specific sensor response.

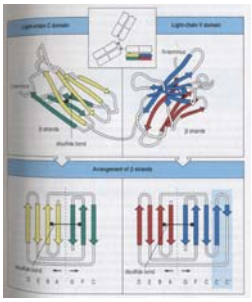


Antibody Molecule

- Structure of an antibody molecules is shown in the figure.
  - Panel a illustrates a ribbon diagram based on the X-ray crystallographic structure of an IgG antibody, showing the course of the backbones of the polypeptide chains. Three globular regions form a Y. The two antigen-binding sites are at the tips of the arms, which are tethered to the trunk of the Y by a flexible hinge region.
  - A schematic representation of the structure is given in panel b, illustrating the four-chain composition and the separate domains comprising each chain.
  - Panel c shows a simplified schematic representation of an antibody molecule.



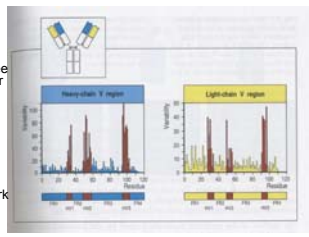
Similar domains of immunoglobulin



- The structure of immunoglobin constant and variable domains are shown in the figure.
- The upper panel shown schematically the folding pattern of the constant (C) and variable (V) domains of an immunoglobulin light chain. Each domain is a barrel-shaped structure in which strands of polypeptide chain (β strands) running in opposite directions (antiparallel) pack together to form two β sheets, which are held together by a disulfide bond.
- The characteristic four-strand plus three strand (C-region type domain) or four-strand plus five strand (V-region type domain) arrangements are typical immunoglobulin superfamily domain building blocks, found in a whole range of other proteins as well as antibodies and T-cell receptors.

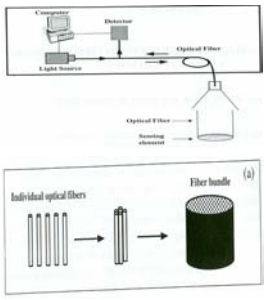
Hypervariability in V domains

- A variability plot derived from comparison of the amino acid sequences of several dozen heavy-chain and light-chain V domains is plotted in the figure.
- At each aminoacid position the degree of variability is the ratio of the number of different amino acids seen in all of the sequences together to frequency of the most common amino acid. Three hypervariable regions (HV1, HV2, and HV3) are indicated in red and are also known as the **complementarity-determining regions, CDR1, CDR2, CDR3**. They are flanked by less variable framework regions (FR1, FR2, FR3 and FR4, shown in blue or yellow)



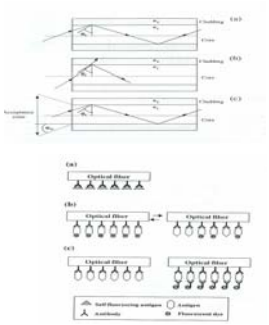
Fiber Optic Biosensors

- Optrode-basedfiber optic biosensors (bio-optrodes) are analytical devices incorporating optical fibers and biological recognition molecules. Optical fibers are small and flexible "wires" made out of glass or plastic that can transmit light signals, with minimal loss, over long distances. The light signals are generated by a sensing layer, which is usually composed of biorecognition molecules and dyes, coupled to the fiber end. Light is transmitted through the optical fibers to the sensing layer where different optical phenomena such as absorption or luminescence are used to measure the interactions between the analyte and the sensing layer.



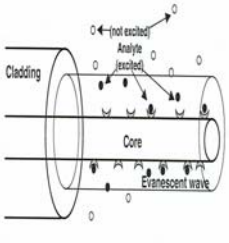
Fiber Optic Biosensors

- Bio-optrodes can be used for remote analytical applications including clinical, environmental, and industrial process monitoring. In the last decade, due to the rapidly growing use of fiber optics for telecommunication applications, new fiber optic technologies have been developed resulting in high-quality and inexpensive optical fibers that can be used for bio-optrode applications. Recent advancements in bio-optrode technologies include the development of nanoscale bio-optrodes, enabling measurements inside single living cells, and the development of multi-analyte and reagentless bio-optrodes.



Evanescent Wave Fiber Optic Biosensors

- When light is launched down a waveguide placed in contact with a lower refractive index material, if conditions are present to allow total internal reflection of this light, an electromagnetic component of the light extends out from the surface of the waveguide into the lower index medium. This electromagnetic field, the evanescent wave, has a limited penetration depth and can be used to specifically excite fluorophores bound to, or in close proximity to, the waveguide surface. Evanescent wave fiber optic biosensors have been developed utilizing the limited penetration depth of the evanescent wave to detect a variety of analytes. These sensors are able measure optical events at the fiber's surface with relatively little interference from the bulk solution. The ability of these instruments to detect analytes rapidly and specifically, even in the presence of complex sample matrices, has been demonstrated both under laboratory conditions and in the field.

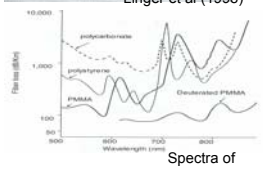


Evanescent Wave Fiber Optic Biosensors

- Optical fibers possess a number of related physical characteristics that can be used to distinguish them, such as radius, reflective index, etc.
- Monomode fibers, such as those used in telecommunication industry, typically have a core size of 5-10 μm and propogate only a single mode at any given wavelength
- Multimode fibers posses the advantages of good light transmission over short and medium distances with a wide variety of optical components
- Plastic fibers can be injected molded to fit the user's and instrument's requirements. Furthermore, dopants can be added to change the refractive index over a wide range. The chief problem with the plastic fibers is the limited spectra range for which they can be used. (due to high attenuation in –CH absorption bands)

ANALYTE, 2000

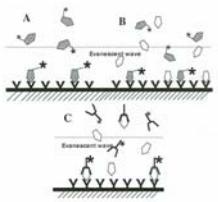
Linger et al (1998)



Spectra of various plastics

Evanescent Wave Fiber Optic Biosensors

- Formats for affinity assays:
- A. Direct binding assay. A fluorescent analyte or nonspecifically stained analyte binds to a recognition molecule immobilized on the fiber probe.
- B. Competitive immunoassay. Labeled and unlabeled analyte compete for binding to the immobilized recognition biomolecule.
- C. Sandwich immunoassay. A fluorescent complex forms when the immobilized recognition molecule binds to the analyte and the fluorophore-labeled tracer binds to the analyte at the surface of the optical fiber.



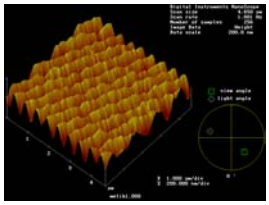
Evanescent Wave Fiber Optic Biosensors

Table 1. Evanescent fiber optic biosensors for detection in complex matrices.

Analyte	Sample matrix	Reference
Cocaine, benzoylecgonine, and cocaethylene	Urine	Nath et al., 1999
Cocaine	Coca leaf extract	Topporada et al., 1997
TNT	River water, bilge water	Shriver-Lake et al., 1995a
TNT, RDX	Groundwater	Shriver-Lake et al., 1997
Bacillus globigii	Air samples	van Bergen et al., 2000 Ligler et al., 1998 Anderson et al., 1999
Ricin	River water, urine	Ogert et al., 1993
Yersinia pestis F1 antigen	river water blood, plasma, serum	Narang et al., 1997b Cao et al., 1995 Anderson et al., 1996
D-Dimer	plasma whole blood	Rowe et al., 1998 Rowe-Taitt, unpublished
Staphylococcal enterotoxin B	Clay, topsoil, pollen, smoke extracts	King et al., 1999
Staphylococcal enterotoxin B	Serum, urine, ham extract	Templeman et al., 1996
Specific antibody	Serum	Nath et al., 1997 Anderson et al., 1998
Giardia	Fecal extracts; pond, river and sea water	Anderson and Rowe-Taitt, 2001
Burkholderia cepacia	Ground water	Pease et al., 1995
Lipopolysaccharide (LPS)	20% plasma	James et al., 1996
Hormones, cytokines	plasma	Erb et al., 2001b
E. coli O157:H7	ground beef extract	DeMarco et al., 1999; DeMarco and Lim, 2002a,
E. coli O157:H7	unpasteurized apple juice	DeMarco and Lim, 2002b

Planer Waveguides

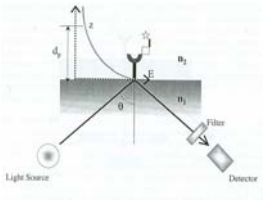
- Total internal reflection fluorescence (TIRF) is the process whereby fluorophores that are either attached to or in close proximity to the surface of a waveguide are selectively excited via an evanescent wave.The use of a planar waveguide allows the immobilization of multiple capture biomolecules and the possibility therefore of multianalyte detection on a single substrate. Planar waveguide TIRF has been used in the measurement of a variety of analytes including hormones, toxins, bacteria and viruses, leading to applications in areas such as environmental monitoring, clinical diagnostics and military defense. Analytes have been measured both in buffer and in complex matrices, such as whole blood, nasal secretions and soil suspensions. Detection limits both in buffer and complex matrices have been comparable. The continued development and miniaturization of the sensor instrumentation has led to systems that are fully automated and portable and would be highly competitive with current techniques upon transition to the commercial market.



Surface AFM scan of Ta_2O_5 (Demirel, unpublished data)

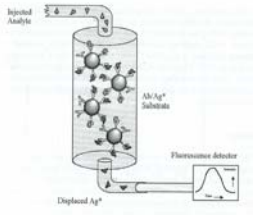
Planar Waiveguides Biosensors

- There are three techniques which can be grouped under the principle of reflectance:
  - attenuated total reflectance (ATR) which monitors alterations in the IR, visible and UV -regions;
  - surface plasmon resonance (SPR) which measures variations in refractive index;
  - total internal reflection fluorescence (TIRF) which monitors changes in fluorescence
- Fluorescence-based biosensors, which use planar substrates as waveguides, fall under the last category. The basic arrangement of the TIRF system is shown in Figure.



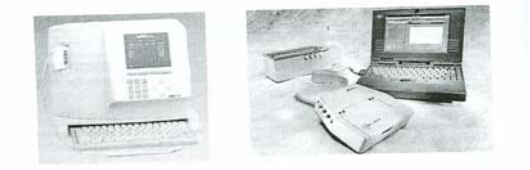
Flow Immunosensors

- The flow immunosensor combines the selectivity and sensitivity of traditional immunoassays with a non-equilibrium displacement reaction that allows rapid analysis of small-molecular weight compounds within minutes. Working assays have been developed for a wide range of molecules including explosives, drugs of abuse and environmental contaminants. Accurate determinations of analyte concentrations can be made on site, thus providing immediate feedback to field managers and law enforcement personnel. Side-by-side comparison of these measurements with laboratory instruments (HPLC, GC/MS) has demonstrated the accuracy and precision of the method. Commercial versions of the flow immunosensor have been engineered that integrate fluidics, electronics and computer control into a portable instrument. More recently, advanced laboratory prototypes of the biosensor have been fabricated to improve low end detection, extend the applications to underwater sensing, enhance field ruggedness and assist in the manufacturing process.



Flow Immunosensors

- Applications
  - Aviation security: TNT sensors
  - Drug detection: Patient levels of therapeutic drug
  - Environmental monitoring: pesticide, TNT
- Commercial prototypes developed based on flow immunosensor technology. Shown on the left is IMPACT test system, and right is the FAST 2000 system.

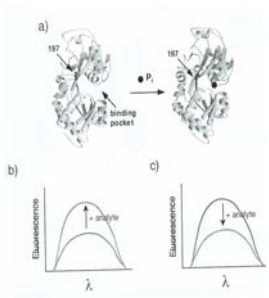


Genetic Engineering of Signaling Molecules

- In order to expand the capabilities of biosensors, there is a need to develop new signaling molecules. We will focus on molecules, produced through genetic engineering, that combine the recognition element with a signaling element (such as a fluorophore) in an effort to optimize the signal caused by the binding of the analyte to the recognition element. These systems, while not necessarily originally developed for an optical fiber, can be immobilized at the tip of the fiber either through chemical attachment or entrapment behind a membrane.
- Three different systems will be examined:
  - fluorophore-labeled binding proteins,
  - FRET-based systems,
  - bacteria-based sensors.
- These systems use optical signaling methods to reveal the binding event, taking advantage of molecular biological techniques to optimize the signal. The advantages and disadvantages of each system will be discussed, as well as the current state of the art of these biosensors.

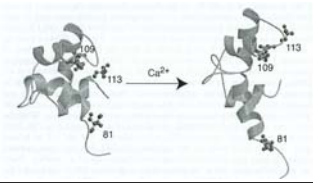
Fluorophore-label binding proteins

- Schematic of a fluorescently labeled protein sensing system. The protein is labeled with an environmentally-sensitive fluorophore such that the binding of the analyte changes the conformation of the protein, altering the solvation of the fluorophore. a) In this example, amino acid 197 of phosphate binding protein (PBP) is located near the binding pocket and will undergo a change in environment as PBP closes around its ligand, phosphate. This can result in either b) an increase or c) a decrease in fluorescence upon ligand binding. In some cases, the emission wavelength of the fluorophore can also change.



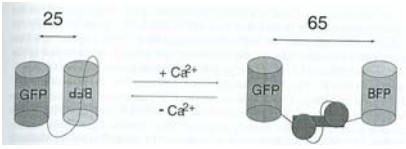
Fluorophore-label binding proteins

- Alterations in the C-terminal hydrophobic pocket of calmodulin upon calcium binding. Residue 109 is closer to the pocket than either residue 81 or 113. Labeling mutant calmodulins gives the most change with an MDCC-CaM109 conjugate. Labeling at 81 or 113 does not give as much fluorescence change upon calcium binding, presumably because the two residues are further from the hydrophobic pocket than amino acid 109. Adapted from Schauer-Vukasinovic et al. (1997).



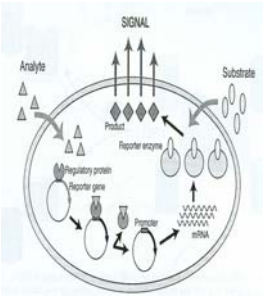
FRET-based systems

- FRET -based sensing system for Ca2+ based on a *BFP/GFP* pair bridged with a MLCK CaM binding site. The FRET donor BFP is separated from the acceptor GFP by a CaM recognition sequence from myosin light chain kinase (MLCK). CaM can only bind this sequence in the presence of Ca2+, increasing the distance between the fluorophores and decreasing the amount of FRET. The system, therefore, responds to the amount of Ca2+ present. Adapted from Miyawaki et al. (1997)



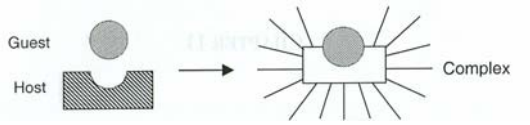
Bacteria-based systems

- Schematic of a bacteria-based sensing system. The bacteria are transformed with a plasmid containing the reporter gene under the control of an analyte-sensitive promoter. In the presence of the analyte, the regulatory protein is released from the promoter region, allowing transcription of the reporter gene. The mRNA is then translated into protein, which can be assayed. The amount of protein produced is proportional to the amount of analyte present, although there is amplification at each step so that there are many more proteins present than reporter genes. Sometimes it is necessary to also place the gene for the regulatory protein on the plasmid as well as the reporter gene, as the native levels of reporter protein within the bacteria are insufficient for proper regulation of transcription.



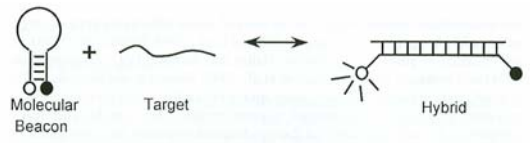
Artificial Receptors for Chemosensors

- Chemosensors are molecules of abiotic origin that signal the presence of matter which can be used to measure the concentrations of analytes in solution. They consist of artificial receptors tailored to reversibly bind the analyte with sufficient affinity and selectivity, a chromophore or fluorophore, and a mechanism for communicating between binding and optical signaling.



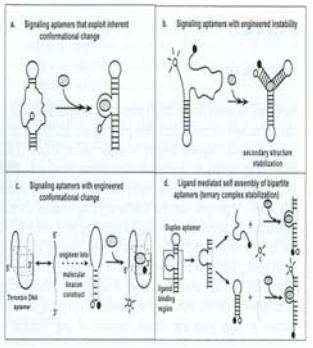
Nucleic Acids for Reagentless Biosensors

- Aptamers are single stranded nucleic acids (RNA, ssDNA, modified RNA or ssDNA), capable of binding tightly and specifically to their targets. They are isolated from combinatorial oligonucleotide libraries by a process known as *in vitro* selection. *In vitro* selection mimics the process of natural evolution in that a pool of nucleic acids are sieved for a desired functional property, such as the ability to bind to a target or catalyze a reaction. Once functional species have been isolated, they are preferentially amplified via conventional molecular biology techniques, such as reverse transcription, polymerase chain reaction, and *in vitro* transcription. Over multiple rounds of selection and amplification, quite large populations (> 10^{13} different sequences) can be sieved and those few, "fittest" nucleic acid species isolated



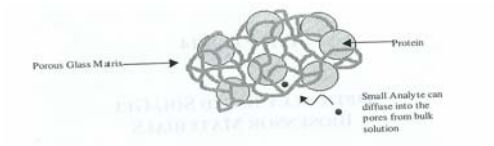
Nucleic Acids for Reagentless Biosensors

- Strategies for signaling aptamers. (a) Exploitation of small conformational changes. (b) Exploitation of larger secondary structural conformational changes. (c) Exploitation of tertiary structural conformational changes.(d) Exploitation of quarternary structural conformational changes. In the case of (a), the conformational change may be inherent to the aptamer. In (b) - (d), the conformational changes have been engineered into the aptamer by altering its secondary, tertiary, and / or quarternary structure.



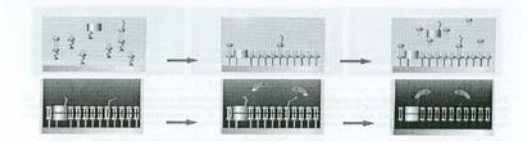
SOL-GEL Based Biosensors

- The sol-gel process is a chemical technique for synthesizing a silicate matrix around a biomolecule that can function as the recognition and signaling element for a sensor. Within the past decade, biologically doped sol-gel glasses have surfaced as having great potential in optical biosensor applications. The materials are transparent in the UV and visible spectra allowing for transmission of optical signals. The glass is porous such that small analyte molecules can diffuse through the matrix and reach the large biomolecule that is physically trapped. Biological molecules including heme proteins, enzymes, and antibodies can remain active within the porous sol-gel glass. The flexibility of the method has allowed the encapsulation of a wide range of biomolecules and cells, resulting in sensor materials able to detect small molecules in both gases and in liquids.



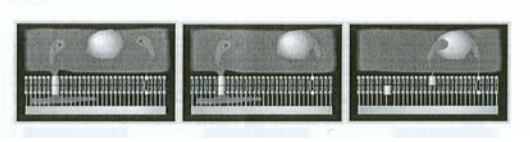
Membrane based biosensors

- A fresh gold surface is exposed to an ethanol solution of the tethering species (lipid bilayer) for 10 minutes. This produces the inner and part of the outer leaflet of the bilayer membrane. Following an alcohol rinse, a second ethanol solution brings the mobile elements. Rinsing with water spontaneously forms a lipid bilayer structure. Some of the lipids span the membrane, whilst the remainder are mobile within the two-dimensional plane of the membrane. Antibody fragments are then added in the aqueous solution and tethered using a streptavidin-biotin attachment (not shown). For large analyte detection involving a "sandwich assay" configuration, assembly is by adding an equimolar mixture of the two antibody fragments.



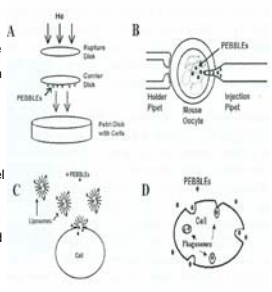
Membrane based biosensors

- The binding of analyte to the antibody fragments causes the conformation of gramicidin A to shift from conductive dimers to non-conductive monomers. This causes a loss of conduction of ions across the membrane. A competitive assay has also been devised in which the analyte causes the population of channel dimers to increase.



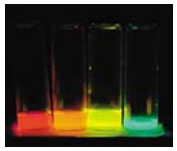
PEBBLE nanosensors

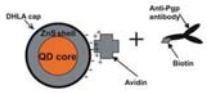
- PEBBLE nanosensors (Probes Encapsulated By Biologically Localized Embedding) are submicron-sized optical sensors designed specifically for minimally invasive analyte monitoring in viable, single cells with applications for real time analysis of drug, toxin, and environmental effects on cell function. PEBBLE nanosensors is a general term that describes a family of matrices and nano-fabrication techniques used to miniaturize many existing optical sensing technologies. The main classes of PEBBLE nano-sensors are based on matrices of cross-linked polyacrylamide, cross-linked decyl methacrylate, and sol-gel silica. These matrices have been used to fabricate sensors for H+, Ca2+, ~, Na+, Mi+, Zn2+, cr, N02-, O2, NO, and glucose that range from 20 nm to 600 nm in size. A host of delivery techniques have been used successfully to deliver PEBBLE nanosensors into mouse oocytes, rat alveolar macrophages, rat C6-glioma, and human neuroblastoma cells.



Quantum Dots in Biosensor

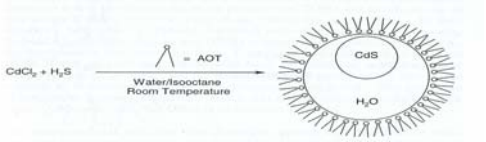
- We begin with a basic introduction to quantum dots, including their synthesis and some characteristic physical properties, then follow with a review of bio-related work involving semiconductor nanocrystals published todate. Work involving preparation and use of QD-protein conjugates in cellular imaging, quantitative immunoassays, and in early-stage energy transfer applications is reviewed, as well as uses of QD-DNA conjugates as nanoscale building blocks.





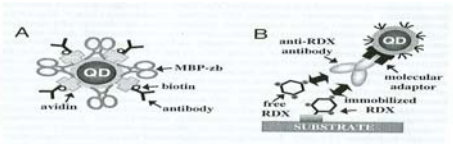
Quantum Dots in Biosensor

- Nano-engineered materials can be considered excellent candidates for a variety of advanced technological applications due to their extremely interesting optical and electronic properties, such as: (Bruchez et al. 1998; Chan and Nie 1998; Pathak et al. 2001)
  - Semiconductor Q-dots possess narrower emission spectra than conventional organic dye molecules.
  - Because of the broad absorption spectra of semiconductor Q-dots, there is great flexibility in the selection of the excitation wavelength.
  - The emission spectra of semiconductor Q-dots are size tunable. By varying the size and/or composition of the material, semiconductor Q-dots can be prepared with emission spectra at any visible wavelength.



Quantum Dots in Biosensor

- QD-antibody complexes for use in bioassays have been formed using adaptor proteins as bridges to link QDs with antibodies. Either naturally occurring protein bridges (e.g., avidin) or engineered recombinant protein bridges can be used in this capacity. In practice, mixed-surface QD conjugates have been made with both the antibody-binding adaptor protein and an engineered maltose binding protein derivative (MBP-zb) bound to their surface (Figure A). The mixed recognition elements on the particles allow separation of QD-antibody complexes from unbound antibody using affinity chromatography. After saturation of antibody binding sites with IgG (or biotinylated IgG when using the avidin bridge) and purification on a cross-linked amylose column to remove excess unbound IgG, various QD-antibody conjugates have been demonstrated to bind antigen directly adsorbed to microtiter plates. Sandwich- or competitive type assays were then performed in model systems for analysis of staphylococcal enterotoxin B (SEB) and for detecting low levels of the explosives TNT and RDX dissolved in water (Figure B).

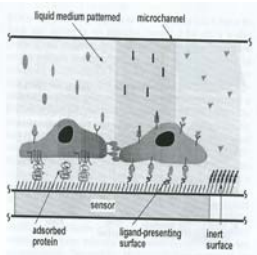


Soft Lithography and Microfluidic

- Optical biosensors necessarily involve an interface between synthetic materials and biological systems.
- Soft lithography is a set of techniques that includes 1) methods of fabricating microstructures in polymers, especially elastomers, 2) uses of these methods in combination with organic surface chemistry to generate micron-scale patterns on synthetic surfaces, and 3) uses of micro fluidic systems to pattern the composition of the fluid medium adjacent to a surface. These techniques allow the immobilization of biomolecules and cells at surfaces with micron-scale resolution, and for the control of the subsequent interaction of these species with liquid media. These techniques are compatible both with optical and electronic materials and with biological systems.
- We will focus on the use of soft lithography to fabricate micro fluidic systems and to position and manipulate living cells on surfaces.

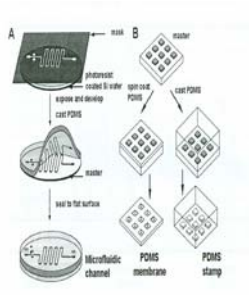
Soft Lithography and Microfluidic

- Controlling the interactions of a cell with its environment using soft lithography. Most of the environmental features sensed by the cell can be patterned using soft lithography or devices fabricated using soft lithography: the surface on which proteins adsorb or that presents ligands (patterning by microcontact printing); the identity of neighboring cells (membrane-based patterning or patterning using three-dimensional microfluidic systems); the composition of the extracellular medium (laminar flow patterning in microchannels).



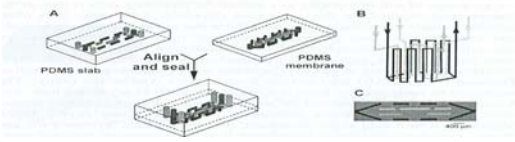
Soft Lithography and Microfluidic

- Schematic diagrams of the soft lithographic approach of fabricating microfluidic channels (A), membranes, and stamps (B). A) A transparency prepared on a high resolution printer (5000 dpi) is used as a photomask. Epoxy photoresist is spun onto a silicon wafer, exposed, and developed to create a master structure. Many (> 100) negative copies of the structure on the master can be formed by molding the structure into poly(dimethylsiloxane) (PDMS), an elastomeric polymer. To form a closed channel, the PDMS mold is sealed to a flat surface either covalently by oxidizing the surfaces in a low temperature plasma or non-covalently by applying pressure. B) Fabrication of a membrane (left) and a stamp (right) from a master. To form a membrane, PDMS is spun onto the master in a thin layer such that the features on the master create holes that tranverse the entire thickness of the layer. On a stamp, the negative of the features on the master are molded in bas-relief on one surface



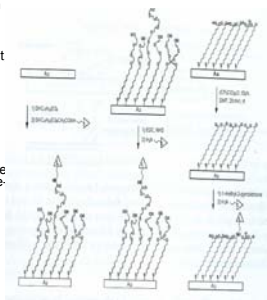
Soft Lithography and Microfluidic

- Three-dimensional microfluidic network for applying reagents to a surface in a discontinuous pattern, a technique called 3D MIMIC. A) Scheme for the fabrication of 3D-microfluidic stamp. The slab and the membrane are formed with different masters.The membrane contains segments of channel that will be in contact with the surface that is to be patterned. Vertical vias in the membrane connect the lower channels (in contact with surface) to the upper layer of channels in the slab. The aligned stack of the slab and the membrane are sealed (non-covalently) to the surface that is to be patterned. B) Schematic illustration of the fluid paths in the 3D network. C) Silicon oxide surface that has been etched with a network of channels such as in (A) and (B) in which different concentrations of etchant (hydrofluoric acid) were run through the three independent channels. Different shades of the etched regions correspond to different thicknesses of the oxide after etching



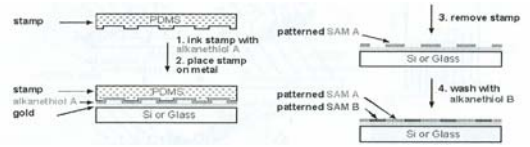
Soft Lithography and Microfluidic

- Generation of SAMs presenting specific ligands: A) Immersion of a gold substrate in a solution containing a mixture of an alkanethiol terminating in oligo(ethylene glycol) groups (OEG), and a second alkanethiol terminating in a ligand of interest generates a surface presenting the desired ligand (Roberts et al., 1998). B) A mixed SAM is formed by the immersion of a gold substrate in a solution containing a mixture of EG3OH-terminated and EG6-OCH2COOH-terminated alkanethiols. Activation of the carboxylic acid groups using N-hydroxysuccinimide (NHS) and 1ethyl-3-(dimethylaminopropy1)carbodiimide (EDC), followed by a reaction with an amine-terminated ligand, generates a surface presenting that ligand (Lahiri et al., 1999a, 1999b). C) A surface presenting interchain carboxylic acid anhydrides is generated by treating SAMs that present terminal carboxylic acid groups with trifluoroacetic anhydride. Reacting these activated SAMs with amine-terminated ligands results in a surface presenting a 1: 1 mixture of the desired ligand and carboxylic acid groups



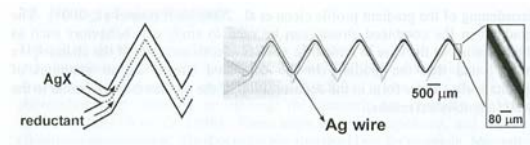
Soft Lithography and Microfluidic

- Procedure for patterning SAMs by micro contact printing: A stamp is inked with an alkanethiol and placed on a gold (or silver) surface; the pattern on the stamp is transferred to the gold by the formation of a SAM on the regions that contacted the substrate. The bare areas of the gold are exposed to a different alkanethiol to generate a surface patterned with a SAM that presents different chemical functionalities in different regions



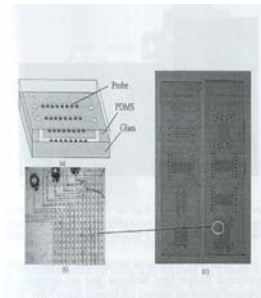
Soft Lithography and Microfluidic

- Demonstration of patterned surface chemistry achieved with laminar flowing streams in a microchannel. A silver wire deposited in a zigzag channel at the laminar flow interface between solutions containing the components of an electroless silver plating solution



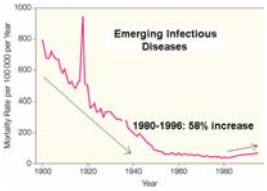
Soft Lithography and Microfluidic

- Schematic of a highly parallel biochannel array chip consisting of a PDMS microchannellayer and a micro array glass chip. (b) A close view of the high-density PDMS channel array running across gel pad based DNA array. The microchannels are 250 ~m wide and 50 ~m deep. (c) Micrograph showing two biochannel array chips, each has various channel array designs. The chip on the right-hand side consists of 52 channels in parallel (P. Grodinski et al, 2000)



Biodetection

- The increase in the rate of infectious diseases is one of the reasons for the scientific community to focus on biodetection. Simultaneous detection of several pathogens is a current trend in the area of biodetection. (Ligler et al. 2002; Taitt et al. 2002; Walt 2002) There are several methods in the literature for specific and sensitive detection of pathogens (Cox et al. 2000; Lan et al. 2000) such as liquid arrays (McBride et al. 2003) and array biosensors (Rowe et al. 1999a; Rowe et al. 1999b). Analytical methods for biodetection, such as liquid/gas chromatography and mass spectroscopy, require expensive equipment. Standard microbiological methods, such as culturing and microscopic examination are time consuming and labor intensive. Polymerase chain reaction (PCR) requires considerable sampling processing before analysis, and reagents are expensive. Antibody-based technologies are highly selective and specific. Several studies have been reported describing antibody-based sensors built on a variety of substrate platforms such as optical fibers and waveguides. (Baird and Myszka 2001; Baird et al. 2002; Holt et al. 2002; Kelly et al. 1999; Muegge and Richter 2002; Parikh et al. 2001; Steemers et al. 2000; Szurdoki et al. 2001)



Biodetection

- In additional to its scientific use, biodetection products fall into three major segments of nanobiotechnology: drug screening, bioimaging and sensor technologies. The market for nanobiotechnology has only existed for a few years, but is expected to grow rapidly at an annual rate of 28% worldwide. The current estimated worldwide market breakdown is: U.S. at 65%, Europe at 20%, Japan at 10%, and the rest of the world at 5%.