SYMPOSIUM O

Chemical and Biological Sensors–Materials and Devices

April 2 – 4, 2002

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* Invited paper

SESSION O1: MICROFLUIDICS AND SENSING SYSTEMS
Chair: Paul S. Cremer
Tuesday Morning, April 2, 2002
City (Argent)

8:30 AM *O1.1
PLASTIC MICROFLUIDIC DEVICES: ELECTROKINETIC MANIPULATIONS, LIFE SCIENCE APPLICATIONS AND PRODUCTION TECHNOLOGIES. Hilary Lackritz, La Shaun Berrien, ACLARA BioSciences, Microtechnology and Materials, Mountain View, CA.

Rapid developments in microfluidic devices over the past 20 years have provided a wealth of ideas, information, and results on the construction, functioning, and applications of emerging 'lab-on-a-chip' technology. We present highlights of this technology and its evolution, focusing on plastic microfluidic devices that utilize electrokinetics to manipulate samples and reagents. Examples from ACLARA's product line will be used to highlight technical points. Key considerations in the selection of polymeric materials of manufacture are outlined and methods of production are overviewed. A brief summary of common detection methods is included, along with a summary of recent applications of direct- and alternating-current fluidic motivation, including electrophoresis, electroosmosis, and dielectrophoresis. Examples show how this technology is poised to enable a versatile analytical laboratory-on-a-chip, with remarkable implications for the scale and economy of high-throughput chemical manipulations in the modern laboratory.

9:00 AM *O1.2
POLYMERIC LAMINATE TECHNOLOGY FOR A RAPID DIFFUSION IMMUNOASSAY. Paul Yager, Hugh Chang, Anson Hatch, Kenneth Hawkins, University of Washington, Department of Bioengineering, Seattle, WA.

The performance of quantitative immunoassays is largely restricted to centralized laboratories because of the need for long assay times, complex and expensive equipment, and highly trained technicians. If a wider range of the 700 million immunoassays performed annually (in the US alone) could be run more inexpensively, more frequently, and at the point of care, the health of millions of patients could be improved, and we would have an important new tool in the detection of chemical and biological warfare agents. To make the assay technology accessible, it must also be inexpensive. We have been developing microfluidic devices and processes that allow chemical analyses to be performed both rapidly and in a format that lends itself to inexpensive mass production. These rely on the low Reynolds number conditions present in microchannels, and the interdiffusion of molecules between flowing laminae. In the T-sensor, the interdiffusion of an analyte present in one stream with some form of indicator pumped into the device in another stream produces a change in the distribution of the indicator optical properties at the interface between the two streams. A microfluidic diffusion immunoassay (DIA) has just been demonstrated that provides biochemical processes and a common analytical platform that are well suited to such miniaturized and simplified instrumentation. In this T-sensor based assay, the transport of antigens perpendicular to flow is affected by binding to soluble antibodies. This assay has been demonstrated in the format of competition immunoassay using fluorescence imaging detection. DIA can be used for monitoring drugs, hormones, and other larger analytes. The use of polymeric laminates for the formation of the microfluidic devices is central to the inexpensive implementation of the technology. The assay presents specific materials challenges, not all of which have yet been fully addressed.

9:30 AM *O1.3
PLASTIC MICROCHANNEL DEVICES FOR MULTIPLEXED BIOCHEMICAL REACTIONS, ANALYSIS, AND DETECTION. Alexander P. Sassi, ACLARA BioSciences, Inc., Mountain View, CA.

In the push towards greater throughput in pharmaceutical compound screening, genotyping, and gene expression studies, systems are needed which can sense (detect) low analyte concentrations rapidly and with low cost-per-analysis. Multiplexing can accomplish higher throughput: by sensing multiple analytes within a single device and/or by forming arrays of miniaturized sensors on a single device. Often the sensing device requires samples that are somehow prepared, and therefore, sample preparation steps must keep pace with the sensing device for the overall crude sample-to-answer process to become faster. ACLARA BioSciences has been developing replicated, plastic microchannel devices to address high throughput needs in analyte sensing and sample preparation. This talk will focus on two examples of ACLARA's plastic LabCard^{TM} technology, one for microchannel electrophoresis and one for DNA amplification. ACLARA's microchannel LabCard devices are arrays of microfluidic patterns in plastic. Each pattern can be used to separate multiple analytes by electrophoresis. These microchannel devices have been used separate DNA fragments, eTag^{TM} reporters and enzymatic reaction products significantly faster than in conventional electrophoresis devices. These types of separations are used widely in genotyping, gene expression, and candidate drug screening applications. Amplification of DNA by the polymerase chain reaction (PCR) is a sample preparation step often performed before analyzing the DNA by hybridization, mass spectrometry, or electrophoresis. ACLARA's Plurex^{TM} device is an array of 96 spiral channels in the standard SBS 96-well card footprint. Each channel uses spatially addressable multiplexed biochemical amplification (SAMBA) to amplify 50 DNA fragments simultaneously in 10 defined reaction zones, a throughput of 4800 amplicons per device. When designing standard tube-based PCR to amplify multiple DNA fragments for large-scale studies, significant assay design is required to eliminate cross-reactivities. By isolating amplification zones spatially, the time and cost of multiplexed PCR assay design can be reduced.

10:30 AM *O1.4
T. Michalske, Sandia National Laboratories, Albuquerque, NM.

ABSTRACT NOT AVAILABLE

11:00 AM *O1.5
POLYMERIC MATERIALS AND FABRICATION METHODS FOR CHEMICAL SENSING. Ralph G. Nuzzo, University of Illinois, Department of Chemistry, Urbana, IL.

I will discuss our recent progress in the areas of materials synthesis and microfabrication, highlighting as examples systems that develop new capabilities for chemical sensing. The utility of Soft-Lithography and a recently developed Decal Lithothgraphy method as fabrication methods for chip-based sensors will be explored. Several prototype sensor designs that integrate chemical separations and non-spectroscopic detection methods will be discussed.

11:30 AM O1.6
DETECTION OF DNA HYBRIDIZATION IN MICROCHAMBERS FABRICATED WITH UV-CROSSLINKED HYDROGEL. Gi Hun Seong, Richard M. Crooks, Texas A&M Univ, Dept of Chemistry, College Station, TX.

There is currently great interest in combining the functional components that are necessary for performing complex chemical and biochemical analysis into small, integrated units. Much of the current research activity in this field is concentrated in DNA analysis devices. One way to analyze multiple DNA targets, such as for gene expression, is with arrays of DNA probes contained within microfluidic devices. In this talk, the fabrication of microfluidic devices in poly(dimethylsiloxane) (PDMS) was performed by standard photolithography techniques and UV-crosslinked hydrogels (poly(ethylene glycol)diacrylates) (PEG-DA) were used to spatially define microchambers. PEG-DA could be crosslinked into hydrogels by introducing terminal acrylate functional groups that could take part in photopolymerization reactions. Also, for strong hydrogel attachment onto walls in microchannels, the surface of PDMS and glass in microchannels were modified with 3-(trichlorosilyl)prophyl methacrylate (TPM) to create a reactive surface onto which the hydrogel was covalently affixed during photopolymerization. Single-strand DNA (ssDNA) probes labeled with biotin were immobilized onto microbeads coated with streptavidin and microbeads coated with biotinylated ssDNA probes was packed simply by injection of microbead solutions through an inlet hole. Here, three oligonucleotides were designed as probes and four oligonucleotides as targets. The hybridization of fluorescein labeled ssDNA targets to complementary probes was observed by fluorescence microscopy but no binding was observed to noncomplementary probes. The specific DNA target was isolated by treatment with alkali (O.1 N NaOH) solution without mixing with other DNA targets hybridized in nearby microchambers. Also, we investigated the reuse of microbeads. However, after 3 cycles of hybridization/denaturation, the signal-to-noise ratio was significantly decreased. The signal decrease is believed to be a result of the deterioration of binding between streptavidin and microbeads.

SESSION O2: SENSOR ARRAYS AND DEVICES
Chair: Michael J. Sailor
Tuesday Afternoon, April 2, 2002
City (Argent)

1:30 PM *O2.1
CROSS-REACTIVE OPTICAL SENSOR ARRAYS FOR SMARTER SENSING. David R. Walt, Shannon Stitzel, Keith Albert, Dept of Chemistry, Tufts University, Medford, MA.

Vertebrate olfaction and gustation, noted for their sensitivity and selectivity, provide intriguing models for creating cross-reactive sensor arrays. In contrast to sensors made with selective receptor chemistries, such arrays employ pattern recognition algorithms that are first trained to recognize sensor response patterns corresponding to particular analytes or mixtures. To mimic these biological sensing systems, a sensor array was developed comprising thousands of bead sensors, randomly dispersed across an etched optical fiber array. These bead sensors are impregnated with solvatochromic dyes, which alter their fluorescence emission spectra in response to micro-environmental changes due to the analyte's polarity. Each sensor type is cross-reactive and has unique fluorescence response patterns to different analytes. The sensor array is small, sensitive and reproducible and has rapid response and recovery times. The critical aspect of such arrays is their ability to retain an 'odor memory' over time. The ability to train the arrays to recognize complex aspects of various odors will be described. Possible applications range from pollution monitoring, medical diagnosis, food quality control, and land-mine detection.

2:00 PM *O2.2
SPR IMAGING MEASUREMENTS OF DNA, PEPTIDE AND PROTEIN MICROARRAYS. Emily A. Smith, Greta J. Hurtt, Terry T. Goodrich, Hye Jin Lee, and Robert M. Corn, Department of Chemistry, University of Wisconsin, Madison, WI.

The identification and application of bioaffinity interactions in a large scale array format has become an indispensable tool for modern biological research. For example, bioaffinity interactions such as DNA-DNA and antigen-antibody interactions are regularly employed to ascertain the presence of a particular DNA sequence in a sample or to identify various microbial and viral species. The technique of surface plasmon resonance (SPR) imaging is emerging as a powerful tool for the study of bioaffinity interactions in a surface array format. SPR imaging is a label free detection method that monitors the presence of a biopolymer on a chemically modified gold surface by the change in the local index of refraction that occurs upon adsorption. The specific binding interactions of DNA, RNA, peptides, proteins, antibodies, and enzymes can be characterized through the use of SPR imaging measurements of highly reproducible biomolecule arrays on gold surfaces. This talk will highlight our recent developments in the implementation of near infrared SPR imaging for the detection of biomolecules onto DNA, peptide and protein microarrays for the study of DNA-protein interactions, the direct detection of ribosomal and messenger RNA, and the study of protein-protein interactions (proteomics). A significant portion of this research is focused on the fabrication of well-characterized, robust and bioactive arrays of biomolecules on gold surfaces. Novel methodologies that employ microfluidics in order to fabricate the arrays and reduce the sampled target volume will be presented.

3:00 PM O2.3
THIN FILM MICROARRAYS WITH IMMOBILIZED DNA FOR HYBRIDIZATION ANALYSIS. J.P. Conde[1], V. Chu[2], R. Cabeca[2], F. Fixe[3], A. Faber[3], D. Goncalves[3], G.N.M. Ferreira[3,4] and D.M.F. Prazeres[3]. [1]Department of Materials Engineering, Instituto Superior Técnico, Lisbon, PORTUGAL. [2]INESC Microsystems and Nanotechnologies, Lisbon, Portugal. [3]Center for Biological and Chemical Engineering, Instituto Superior Técnico, Lisbon, PORTUGAL. [4]Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Faro, PORTUGAL.

Biochips, particularly those based on DNA (DNA chips), are powerful devices that integrate the specificity and selectivity of biological molecules with electronic control and parallel processing of information. This combination will potentially increase the speed and reliability of biological analysis. Examples of current applications of DNA chips include genomic analysis to screen and identify single nucleotide polymorphisms (SNPs) or to sequence gene fragments, pathogen identification, and gene expression profiling.
Thin film technology is used to manufacture the devices in such products as displays (thin film transistors), scanners and imagers (photodetectors). Integrating thin-film technology into biological sensors such as DNA chips, enzyme electrodes and microarrays can increase the functionality of these devices by enabling on-chip control, data acquisition and analysis.
The objective of this work is to develop a DNA-chip capable of positioning the DNA probes in the appropriate site or pixel (i.e. addressing), and detecting on-chip hybridization using thin-film materials on plastic substrates. To achieve this objective, we have developed a procedure to immobilize DNA probes on a microarray patterned on a flexible plastic substrate. This procedure, which will be described in quantitative detail, involves the chemical activation of the film surface, the introduction of amine functionality via a step of silanization, the coupling of an adequate crosslinker and finally the immobilization of the DNA probe. The response of different thin-film materials and plastic substrates to the immobilization procedure will be discussed. After immobilization, it is important to confirm that the DNA probes in the patterned pixels keep their ability to hybridize with their complementary strands. This test is achieved by marking the target DNA with a fluorescent molecule and allowing it to hybridize with the immobilized probes, and will be described in detail. A prototype chip is demonstrated using an array of functionalized thin-film SiO_2 on a polyimide substrate.

3:15 PM O2.4
A MICROMAGNETIC GENE CHIP: MAGNETIC LABELING AND DETECTION OF DNA. M.M. Miller[a], M.A. Piani[b], J.C. Rife[a], C.R. Tamanaha[c] and L.J. Whitman[a]. [a]Naval Research Laboratory, Washington, DC. [b]NOVA Research, Inc., Alexandria, VA. [c]Geo-Centers, Inc., Fort Washington, MD.

The Bead Array Counter (BARC) biosensor system uniquely combines a DNA microarray, magnetic microbeads, giant magnetoresistive (GMR) magnetic field sensors, and microfluidics to detect and identify biological molecules.[1,2] Our initial focus is on the detection of biological warfare agents. The core of the sensor is a microchip containing an array of 64 GMR sensors. Distinct single stranded DNA capture probes are immobilized above each sensor. Complementary DNA in a sample is allowed to hybridize on the chip, and is then labeled with magnetic microbeads that are detected by the GMR sensors. The overall system sensitivity is a convolution of the chemical and instrumental sensitivities. The chemical sensitivity is determined by the effectiveness of the hybridization and labeling assay, and the instrumental sensitivity by the micromagnetics of the bead-sensor interaction. We have been able to achieve a chemical sensitivity of 0.1 fM, and our current sensors can detect as few as ten 2.8 μm-diameter Dynabeads (covering only 0.2% of a sensor). We have demonstrated an overall system sensitivity per sensor of 10 fM for a 30 μl sample with a total assay time of $\sim$30 min. We are now exploring the use of peptide nucleic acid (PNA) capture probes to enhance the chemical sensitivity, and working to develop high-magnetization microbeads to increase the instrumental sensitivity. The interdisciplinary challenges to making a complete, integrated sensor system based on this technology will be discussed.
[1]Edelstein *et al.*, Biosensors & Bioelectronics **14**, 805 (2000).
[2]M.M. Miller *et al.*, J. Mag. and Mag. Mat. **225**, 138 (2001).

3:30 PM *O2.5
PROTEIN AND OLIGONUCLEOTIDE NANOARRAYS GENERATED BY DIP PEN NANOLITHOGRAPHY. Chad A. Mirkin, K.B. Lee, and S.J. Park, Northwestern University, Chemistry Department and Institute for Nanotechnology, Evanston, IL.

Methods based on Dip-Pen Nanolithography for generating combinatorial arrays of oligonucleotides and proteins will be described. The use of such arrays for generating complex inorganic materials and biological sensors will be discussed.

4:00 PM O2.6
CL-ION SENSING USING ELECTROLYTE-SOLUTION-GATE DIAMOND FETS. Kwang-Soup Song, Toshikatsu Sakai, Hirofumi Kanazawa, Yuta Araki,Hitoshi Umezawa, Minoru Tachiki and Hiroshi Kawarada, School of Science and Engineering, Waseda University, Tokyo, JAPAN, CREST, Japan Science and Technology Corporation (JST), JAPAN.

The ion sensitive field effect transistors (FETs) of hydrogen-terminated polycrystalline diamond have been introduced for the first time. Diamond has many advantages for electrochemical applications such as wide potential window (3.0$\sim$3.5V), chemical stability, biocompatibility, etc. The as-grown CVD diamond surface was terminated by hydrogen to obtain the surface p-type conductivity. The diamond surface channel was exposed directly into the electrolyte solutions with pH range 1-13. The Vds-Ids show perfect pinch-off and saturated current-voltage characteristics. The threshold voltages of the diamond FET are insensitive to pH values in electrolyte solutions. But in hydrochloric acid (HCl) solutions, potassium chloride (KCl) solutions, and sodium chloride (NaCl) solutions, the threshold voltages of the diamond FET are shifted according to the density of Cl-ions with 30 mV / decade. By iodine ions and bromine ions, the threshold voltages are also affected largely. These results indicate that the H-terminated diamond surface has a high sensitivity to halogen ions. Moreover, it is expected that the H-terminated diamond surfaces can be used for biosensor with the immobilization of enzyme on the diamond surface.

SESSION O3: SENSING WITH NANOPARTICLES
Chair: Richard M. Crooks
Wednesday Morning, April 3, 2002
City (Argent)

8:00 AM O3.1
A HIGHLY SENSITIVE AND SELECTIVE SURFACE-ENHANCED NANOBIOSENSOR. Amanda J. Haes and Richard P. Van Duyne, Northwestern University, Department of Chemistry, Evanston, IL.

Nanosphere lithography (NSL) derived triangular Ag nanoparticles were used to create an extremely sensitive and specific optical biological and chemical nanosensor. NSL generated nanoparticles are ideal for sensor studies for the following reasons: 1) the nanoparticles are confined to a surface at a fixed interparticle spacing, 2) the nanoparticles are homogenous in size and shape, and 3) the dielectric environment surrounding the particles is easily controlled. Using simple UV-vis spectroscopy, the model system, biotinylated surface-confined Ag nanoparticles were used to detect streptavidin down to one picomolar concentrations. This response can be amplified by exposing the streptavidin-coated surface with biotinylated Au colloids. The system was vigorously tested for nonspecific binding interactions and was found to display virtually no adverse results. The extremely sensitive and selective response of the Ag nanoparticle sensor indicates an exciting use for biological and chemical sensing.

8:15 AM O3.2
MASSIVELY PARALLEL ELECTRICAL DETECTION OF DNA USING OLIGONUCLEOTIDE-MODIFIED Au NANOPARTICLE MATERIALS. So-Jung Park, Zhi Li, T. Andrew Taton, Rongchao Jin, Chad A. Mirkin, Northwestern University, Department of Chemistry and Institute for Nanotechnology, Evanston, IL.

Herein, we present a new array based DNA detection method utilizing oligonucleotide functionalized Au nanoparticles as a circuit element and DC conductivity changes associated with target-probe binding events to quantify the amount of target DNA in solution. The detection method takes advantage of a selective binding event between a capture oligonucleotide strand located between two electrodes and a target oligonucleotide in solution. The target oligonucleotide has contiguous recognition elements, which are complementary to the capture strand and a nanoparticle probe. Therefore, when the device with the pair of electrodes is immersed in a solution containing the appropriate probe and target, particles fill the gap. Exposing the active component of the device to a solution of Ag(I) and hydroquinone (photographic developing solution) closes the circuit and dramatically increases conductance across the electrode gap due to the nanoparticle-initiated silver reduction. Significantly, we have found that oligonucleotide-modified nanoparticles exhibit unusually sharp denaturation properties over salt concentration gradients, which has been exploited to achieve selectivity without a thermal stringency wash, a significant step towards a hand-held detection system for DNA. The detection system is amenable to massive multiplexing, exhibits higher selectivity than fluorophore and previous nanoparticle-based approaches, and at present is 10 times more sensitive than analogous fluorophore probe-based detection with a conventional fluorescence microscope. This work shows how nanoscopic materials can provide major advantages over conventional molecule-based approaches to detection.

8:30 AM *O3.3
CHEMICAL SENSING WITH METAL NANOWIRES. Fred Favier, Erich Walter, Mike Zach, Stacey Rogers, Erik Menke, Reginald Penner, Department of Chemistry, University of California, Irvine, CA.

Arrays of metal nanowires (metal NWAs) can be employed to detect molecules in a contacting gas phase. Detection is based on a change in the electrical conductivity of the array induced by the adsorption, or absorption, of the target molecule by the nanowires.
Arrays of mesoscopic palladium wires, prepared by electrodeposition, form the basis for hydrogen sensors and hydrogen-actuated switches that can exhibit a response time as fast as 20 ms. These devices were constructed by electrodepositing palladium mesowires on a highly oriented pyrolytic graphite surface, and then transferring these mesowires to a cyanoacrylate film supported on a glass slide. The application of silver contacts to the ends of 10 to 100 mesowires - arrayed electrically in parallel - produced sensors and switches that exhibited a high conductivity state in the presence of hydrogen, and a low conductivity state in the absence of hydrogen. After an initial exposure to hydrogen, 15 to 50 nanoscopic gaps are formed in each mesowire. These nanoscopic gaps or "break junctions" close in the presence of hydrogen gas and reopen in its absence as hydrogen is reversibly occluded by the palladium grains in each wire, and the palladium lattice expands and contracts by several percent. The change in resistance for sensors and switches was related to the hydrogen concentration over a range from 1% to 10%.
The challenge now is to prepare NWA sensors that function in liquids, and which possess a selectivity for a variety of different molecules. Progress in this direction will be summarized in this presentation.

9:00 AM O3.4
ALLOYED NANOWIRES FOR TUNABLE BIOMECHANICAL SENSOR APPLICATIONS. S. Gemming, Institut für Physik, Technische Unversität, Chemnitz, GERMANY.

Biosensors such as arrays of DNA strands have a high potential to monitor even chemical reactions with a rather low reaction enthalpy. For the tuning and contacting of such DNA arrays gold and gilded silver clusters or rods have been employed successfully. In order to gain a better understanding of the silver-gold alloying on the nanoscale, three alloyed two-shell nanowires have been investigated by first-principles density-functional calculations, using the plane-wave band-structure code ABINIT [1]. As discovered previously for pure Ag and Au nanowires [2], all investigated alloyed structures are stable local minima of the cohesive energy and the generalized string tension. Compared with the pure bulk phases the wires are metastable. The dense-packing effects at the pure gold wire surface, which lead to the stabilization of chiral structures [2] with specific diameters, also prevails for silver contents less than roughly 50%. Beyond 50% of Ag the wires become less stable with increasing wire diameter. However, this reordering of the wire stability does not depend linearly on the Ag : Ag ratio. An "island of stability" occurs for Ag contents of 10% to 30%, with thinner wires favoring the higher Ag content. Thus, a gold-coated Ag chain would be the best, structurally stable, candidate for a DNA microcontact.
[1] The ABINIT code is a common project of the Universite Catholique de Louvain, Corning Incorporated, and other contributors (URL http://www.pcpm.ucl.ac.be/abinit).
[2] E.Tosatti, S.Prestipino, S.Köstlmeier, A.dal Corso, F.di Tolla, *Science 291* (2001) 288.

9:15 AM O3.5
SEMICONDUCTOR NANOWIRES FOR NANOTECHNOLOGIES: FROM FUNDAMENTAL CHEMISTRY AND PHYSICS TO NANOELECTRONICS AND CHEMICAL AND BIOLOGICAL SENSORS. Yi Cui, Charles M. Lieber, Harvard Univ, Dept of Chemistry and Chemical Biology, Cambridge, MA.

Semiconductor nanowires represent critical building blocks for nanotechnologies, such as nanoelectronics and nanosensors, since their properties can be precisely defined. Herein we present results addressing key aspects of the synthesis, electronic properties and device applications of semiconductor nanowires with an emphasis on silicon-based materials. We show that during synthesis, the properties of silicon nanowires, such as diameter, length, dopant type (n and p-type) and doping concentration could be precisely controlled. The n-type and p-type nanowires also have been assembled into other key functional nanoelectronic devices including field effect transistors, p-n junctions, complementary inverters and bipolar transistors. The key parameters of the devices will be discussed. The large sensitivity of precisely doped nanowires to gate-voltage in solid state devices also suggests that molecular gating could be utilized to create ultra-sensitive chemical and biological sensors. Therefore, we describe a nanowire based sensor that enables label-free, highly-sensitive real-time electrical detection of a wide range of chemical and biological species. The coexistence of NH_2 and SiOH group on nanowire surface results in linear pH-dependence of NW conductance over large dynamic pH range and these NWs could thus function as very good nanoscale pH-sensors. Biotin-modified surface is used to detect streptavidin down to at least picomolar range. In addition, we show the concentration dependent detection of antigen-antibody type of binding. Lastly, detection of a metabolic indicator Ca^{2+} is demonstrated. These results imply that NWs have the great potential for super high integration, enabling simultaneous multispecies detection and revolutionizing genomics and proteomics.

9:30 AM *O3.6
HIGHLY MULTIPLEXED BIOASSAYS USING SELF-ENCODED METAL NANOPARTICLES. Michael J. Natan, Chief Technical Officer, SurroMed, Inc., Mountain View, CA.

A route to highly multiplexed bioassays using segmented metal nanoparticles has recently been described. This approach is based an encoding pattern that exploits the differential optical reflectivity of adjacent segments within individual particles. One advantage to the approach is obvious: because the particles are identified using differential reflectivity, the entire fluorescence emission region can be used to quantitate bound materials. Another advantage is the very large number of uniquely identifiable particles. This presentation will focus on recent advances in the synthesis of the striped particles, different approaches to particle readout, and demonstration of multiplexed analyses of proteins, oligonucleotides, and low MW species.

10:30 AM *O3.7
SUPPORTED POLYMERS AND MOLECULAR ASSEMBLIES ON NANOPARTICLES AND THEIR USE IN BIOSENSING. R.M. Jones,

L. Lu, D. McBranch and D. Whitten, QTL Biosystems, Santa Fe, NM and Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ.

Superquenching of fluorescent polyelectrolytes - both conjugated polymers and dye pendant J-aggregate polylysines - has been demonstrated for aqueous and mixed aqueous-organic solutions by oppositely charged electron transfer and energy transfer small molecule quenchers. Enhanced superquenching has been obtained by using the same fluorescent polyelectrolytes in several supported formats such as silica or polymeric microspheres or clay nanoparticles in aqueous suspensions. Smaller oligomers and even monomers (or mixtures of monomers) may be assembled on microspheres or nanoparticles to afford self-assembled "polymers" that also exhibit superquenching. Recent investigations have provided a quantitative picture of the factors which may control or limit superquenching in solution and in surface-assembled complexes. The polymers and supported assemblies can be used in biosensing and bioassays for a number of molecules ranging from small molecules to proteins. The talk will both the photophysics of these assemblies and the use of the nanoparticle supported polymers in several formats for biosensing applications.

11:00 AM O3.8
TARGETED NANOMACHINES: BIOLOGICAL SCREENING WITH OPTICALLY ENCODED MICRON-SIZED NANOPOROUS SILICON PARTICLES. Frederique Cunin, Thomas A. Schmedake, Jamie R. Link, Michael J. Sailor, University of California - San Diego, Dept of Chemistry and Biochemistry, La Jolla, CA.

A novel method for encoding micron-sized nanoporous silicon particles has been developed based on the interference reflection spectrum. Films of nanoporous silicon are optically encoded by changing the electrochemical conditions during porosification. Modulation of the current density used in the etch results in a modulation of porosity in the films. Optical structures such as Rugate filters or Bragg stacks can be generated in this fashion. The Rugate filters display sharps lines (∼15 nm FWHM) in their optical reflectivity spectrum, and the wavelength of the feature can be tuned over the entire visible spectrum and out to the near IR by appropriate choice of etch conditions. Ultrasonic treatment of the film in an aqueous or nonaqueous solvant generates micron-sized encoded particles. The use of these particles in fluorescence-based and other biological assays will be described.

11:15 AM O3.9
RED-EMITTING BIOLOGICAL SENSORS SYNTHESIZED BY A REVERSE MICELLE TECHNIQUE. V.J. Leppert, G.D. McCool, F.T. Quinlan, P. Stroeve, S.H. Risbud, UC Davis, Dept. of Chem. Eng. and Mat. Sci., Davis, CA; J. Feng, I.M. Kennedy, UC Davis, Dept. of Mech. and Aero. Eng., Davis, CA; B.D. Hammock, UC Davis, Dept. of Entomology, Davis, CA; K.S. Lam, UC Davis Medical Center, Div. of Hemat. and Oncol., Sacramento, CA.

Visible wavelength absorbing and red-emitting materials are of interest for application as biosensors due to the potential for reduced background fluorescence from biological materials at longer wavelengths. PbSe nanocrystals have been synthesized by a reverse micelle technique and subsequently capped with mercaptopropionic acid for functionalization with biological molecules. The nanocrystals, characterized by high-resolution transmission electron microscopy, are 6 nm in diameter on average, well below the Bohr exciton radius; and selected area electron diffraction confirms the cubic phase of PbSe. Fluorescence measurements show a 10 nm FWHM peak at 640 nm using an excitation wavelength of 540 nm. The large Bohr exciton radius in PbSe results in strong quantum confinement effects at relatively large particle sizes, allowing the emission wavelength to be easily varied by changing the particle size, in turn opening up the possibility for signal multiplexing in the red portion of the electromagnetic spectrum.

11:30 AM O3.10
EXTERNAL CONTROL OF DNA HYBRIDIZATION AND ENZYME ACTIVITY VIA COVALENTLY ATTACHED NANOCRYSTAL ANTENNAS. Kim Hamad-Schifferli, Christine Ko, Joseph Jacobson, Media Lab; Jian Ping Shi, Shuguang Zhang, Center for Biomedical Engineering, MIT, Cambridge, MA.

Metal nanocrystals can be used as antennas for controlling the activity of biological systems. The authors present results in which the activity of DNA and proteins are controlled by covalently linked 1.4nm diameter Au nanocrystals. The nanocrystals are inductively heated, which is accomplished by an alternating external magnetic field (frequency ∼1GHz) that induces eddy currents in the nanocrystals. As a result, the nanocrystals transfer heat to the biomolecule to which they are attached. Induction heating of nanocrystals linked to DNA oligonucleotides in solution has been shown to dehybridize the DNA in a manner that is localized and reversible. Induction heating of antisense oligos appended with a nanocrystal is shown to turn off translation arrest, permitting translation. In addition, nanocrystals have also been attached to the enzyme Ribonuclease S, allowing specific and reversible control of the hydrolysis of RNA.

SESSION O4: SENSING WITH MONOLAYERS AND BILAYERS
Chair: Robert M. Corn
Wednesday Afternoon, April 3, 2002
City (Argent)

1:30 PM *O4.1
STOCHASTIC SENSING WITH ENGINEERED PORE-FORMING PROTEINS. Hagan Bayley, The Texas A&M University, System Health Science Center, Dept. of Medical Biochemistry & Genetics, College Station, TX.

Sensor elements are being made in our laboratory by engineering transmembrane protein pores. Analyte molecules modulate the ionic current driven through the engineered pores by a transmembrane potential. Stochastic sensing, which uses currents from single pores, is an especially attractive prospect. This approach yields both the concentration and identity of an analyte, the latter from its distinctive current signature. Further, several analytes can be detected simultaneously with a single sensor element. In one example of stochastic sensing, the bacterial pore-forming protein staphylococcal alpha-hemolysin has been altered to permit the detection of divalent metal cations by using mutagenesis to place a cation-binding site within the conductive pathway (1, 2). In a second example, the hemolysin pore has been modified with cyclodextrins, which act as non-covalent molecular adapters, to allow the detection of a variety of small organic molecules (3, 4). Finally, ligands have been covalently attached to the hemolysin pore, either directly or through polymer tethers, permitting the detection of proteins and DNA (5, 6).
References:
1. O. Braha, L.-Q. Gu, L. Zhou, X. Lu, S. Cheley and H. Bayley, (2000) Nature Biotechnology 17, 1005-1007.
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3. L.-Q Gu, O. Braha, S. Conlan, S. Cheley and H. Bayley, (1999) Nature 398, 686-690.
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2:00 PM O4.2
DEVELOPMENT AND CHARACTERIZATION OF NICKEL-BINDING MSCL MUTANTS. Sharon E. Jones, Cojean Wang, Lawrence L. Brott, Rajesh R. Naik, Paul Blount*, Morley O. Stone, US Air Force Research Laboratory, Materials & Manufacturing Directorate, Wright-Patterson Air Force Base, OH. *Department of Physiology, University of Texas, Southwestern Medical Center, Dallas, TX.

The Mechanosensitive channel of Large conductance (MscL), a homopentameric membrane-bound channel protein, allows bacteria to rapidly adapt to changing environmental conditions such as osmolarity. The MscL channel opens in response to increased membrane tension. The opening of the channel allows for efflux of cytoplasmic constituents into the extracellular environment. We hypothesize that an MscL channel with a nickel atom co-ordinately bound in the pore could be used to generate a solid-state relay device that can be thermally or osmotically activated. In order to test this hypothesis, we are generating a series of individual point mutants (R13H, G30H, K31H, S34H, S35H) and a combined mutant (G30H, K31H, S34H, and S35H). These mutations are positioned on the inner face of the MscL pentameric structure, in and near the channel pore. Homopentameric units of these mutants may be able to bind nickel. Nickel binding will be measured through a fluorophore efflux assay. Our goal is to use the nickel-binding MscL mutants in the development of a cell-free polymeric solid-state relay system.

2:15 PM *O4.3
MACROMOLECULAR INTERACTIONS WITH SUPPORTED PHOSPHOLIPID BILAYERS. Anne Feng Xie and Steve Granick, Department of MS&E, University of Illinois, Urbana, IL.

A picture emerges of significant adsorption-induced structural and dynamical heterogeneity in zwitterionic phospholipid bilayers (DMPC, dimyristoylphospholipid choline) when polyelectrolytes (primarily PMA, polymethacrylic acid) and DNA are allowed to adsorb to sparse surface coverage, at temperatures both above and below the fluid-gel

phase transition temperature. The structural evidence for heterogeneity comes from in situ FTIR measurements in the mode of attenuated total reflection; the dynamical evidence, from FCS (fluorescence correlation spectroscopy) of labelled lipids. Experiments are underway seeking to visualize this heterogeneity.

3:15 PM *O4.4
THE MOLECULAR ORIGIN OF SOLVATION FORCES.
Michael Grunze, Tomohiro Hayashi, Alexander Pertsin, University of Heidelberg, Dept of Physical Chemistry, Inst of Applied Physical Chemistry, GERMANY.

Nonspecific interactions between biomolecules and solid surfaces are a general problem in biosensors. One of the forces leading to non specific interactions are solvation forces, which are believed to correlate with the water affinity of a surface. Here we discuss our grand canonical Monte Carlo simulations to study the behavior of water confined between structureless model walls and self-assembled alkanethiol monolayers (SAMs). The affinity of the confining surfaces for water is assessed in terms of hydration pressure and an analysis of the water structure using various distribution functions. Primary attention is given to large surface-to-surface separations (40 and more), where the oscillations of the hydration pressure and water density have practically decayed. Despite fairly short-ranged potentials used in describing the surface-water interaction, the hydration pressure remains, at these separations, quite perceptible, as do the deviations of the mid-point water density from that of bulk water. It is found that a high surface-water binding energy, as well as the ability of the surface to form multiple hydrogen bonds with a water molecule, is not sufficient for the surface to be hydrophilic. Surface-induced water structuring, such as orientational ordering, may strongly impair the water affinity of a surface by perturbing the natural hydrogen bonding network characteristic of bulk water. We discuss these results in the context of non-specific protein adsorbing and protein resistant surfaces.

3:45 PM *O4.5
SENSORS BASED ON SURFACE PLASMON RESONANCE: FUNDAMENTAL ASPECTS. Charles T. Campbell, L.S. Jung, J. Shumaker-Parry, H. Zaeri, M.H. Gelb, P.S. Stayton, K. Nelson, R.A. Aebersold, Chemistry Department, University of Washington, Seattle, WA.

The adsorption of molecules from liquid solutions onto solid surfaces can be monitored with high sensitivity and fast time response using surface plasmon resonance (SPR). Simple methods convert the signal into adsorbate concentrations. Such measurement of adsorption / desorption kinetics and equilibrium coverages allows not only chemical sensing, but also monitoring of the build-up of the sensing film itself. The sticking probability (the rate of adsorption per molecule-surface collision) directly expresses the difficulty encountered by a molecule in scaling the barrier to adsorption. A method extending this concept to adsorption in liquid solutions is applied to transient measurements of alkylthiol adsorption onto gold from ethanol. Results show a fixed transition state stabilization per methylene. Applications of gold-thin-film SPR sensors in quantifying biological interactions will be described also. A gold surface containing a few biotin headgroups in a self assembled alkylthiolate monolayer of mainly oligo(ethylene glycol) (OEG) headgroups selectively adsorbs the protein streptavidin with a structure that depends on the biotin / OEG ratio. The free biotin sites in the resulting streptavidin monolayer have been used as strong linker sites for further attachment of intact, biotinylated lipid vesicles and biotinylated, double-stranded oligonucleotides to the surface. These complex biological films then provide a surface template that can be used to probe the kinetics and equilibrium binding constants for: (1) peripheral membrane proteins binding to vesicle walls, and (2) the binding of DNA-binding proteins to select oligonucleotide sequences. By detecting the reflected light at a high-contrast angle with spatial resolution using a simple CCD camera, these SPR measurements can be performed quite simply in a microscopy mode to probe the distribution of species across the surface in real time. This is being applied to develop a high-throughput method for the study of the interactions of DNA binding proteins with immobilized DNA arrays.

SESSION O5: SENSING WITH BILAYERS, CELLS, AND POLYMERS

Chair: Sylvia Daunert
Thursday Morning, April 4, 2002
City (Argent)

8:00 AM O5.1
DIRECT OBSERVATION OF CHOLERA TOXIN AGGREGATION AND CHOLERA-GM_1 BINDING AT LOW CONCENTRATIONS. Daniel E. Hooks, Basil I. Swanson, Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM.

Los Alamos National Laboratory is currently developing a simple, compact sensor for the rapid detection of multiple biological pathogens (protein toxins, viruses, and bacteria). Our sensor system targets specific proteins that are markers for pathogens and is based on species selective thin films that mimic receptors on cell membrane surfaces, the target of pathogens. Our initial efforts have been focused on the development of a single-channel, hand-held, battery operated instrument for sub-nanomolar detection of cholera and ricin toxins. Because the signal transduction relies on proximity-based fluorescence changes of the reporting molecules in the lipid bilayer, the nature of target aggregation is a primary concern. Previous AFM studies of bilayer-bound cholera toxin (CT) and cholera toxin B (CTB) subunit demonstrated that CTB was prone to aggregation while CT was not. This result suggests that CTB may not be a reliable substitute for CT during instrument development. However, all of these experiments were conducted at very high concentrations (10-20 mole percent) of both reporting molecule and target agent. Such high concentrations do not accurately reflect the physiological conditions mimicked in our sensor. We have performed AFM imaging of low concentrations of cholera toxin and cholera toxin B subunit bound to GM_1 receptors in a gel-phase bilayer. Experiments were conducted by injecting toxin into the AFM liquid cell with a fluid POPC bilayer containing GM1 receptors supported on a hydrophilic substrate at room temperature. The system was then cooled, inducing the bilayer into the gel phase, which facilitated imaging of the bound molecules at low concentration. The aggregation properties of the CT and CTB molecules will be compared.

8:15 AM O5.2
EFFICIENT IN-COUPLING OF REPORTER EMISSION FROM A PHOSPHOLIPID BILAYER INTO A PLANAR OPTICAL WAVEGUIDE. Robert W. Springer, Daniel E. Hooks, Karen M. Grace, W. Kevin Grace, Roy M. Goeller, Basil I. Swanson, Los Alamos National Laboratory, Los Alamos, NM.

Los Alamos National Laboratory is currently developing a simple, compact sensor for the rapid detection of biomolecules that are signatures for pathogens (protein toxin, viral or bacterial). Our sensor system is based on phospholipid bilayer films decorated with recognition molecules that mimic natural receptors on cell membrane surfaces, the target of pathogens. The binding event between the receptors and the target biomolecules results in receptor aggregation and the accompanying proximity-based fluorescence changes of reporter dyes that are attached to the receptors. The bio-active membrane architecture is directly coupled to a planar optical waveguide. The design of this waveguide, which must also act as a bilayer support, is critical to the operation of the instrument. High-index, low-loss materials that also support a stable bilayer and allow grating-coupled guiding are needed. The design and preparation of two-layer films, including a high-index amorphous guiding material (e.g., alumina, zirconia, and titania) and a thin bilayer-compatible material (silica), and an integrated in-coupling grating will be presented. Approaches to integration of this waveguide structure into an optical biosensor device will be discussed.

8:30 AM *O5.3
INVESTIGATION OF SURFACE INTERACTIONS FOR SHEAR ACOUSTIC WAVE DEVICE IMMUNOSENSORS. R.W. Cernosek[1], C.A. Bailey[1], B.A. Chin[1] and V. Vodyanoy[2], Auburn University; [1]Materials Research and Education Center; [2]Inst. for Biological Detection Systems, Dept. of Anatomy, Physiology, & Pharmacology, Auburn, AL.

Chemical and biological detectors utilizing acoustic wave devices as the transduction platform often are categorized simply as mass detectors. The measured shift in acoustic device operating frequency is assumed directly proportional to the sorbed or bound surface mass, which for thickness shear mode resonators is described by the Sauerbrey equation [1]. Under ideal sensor conditions, such assumptions are valid. However, recent measurements using bacterial immunosensors developed at Auburn University [2] indicate responses are not due to mass alone and other surface phenomena must be considered. Bacteria being large viscoelastic organisms exhibit a mechanical compliance that leads to power dissipation at the driving frequency of the acoustic wave device. If dissipation in this surface layer is large enough, the frequency shift exceeds that of the mass contribution. Under these conditions, it is necessary to measure the oscillation magnitude as well as the resonant frequency to characterize the interaction. Models exist for shear acoustic wave devices that allow extraction of surface material parameters from the measured responses and conversely for prediction of sensor response under known or special conditions [3]. Application of these models to immunosensors leads to some interesting effects. For shear acoustic wave devices (thickness shear mode resonators, acoustic plate mode

devices, and shear-horizontal surface acoustic wave devices), penetration of the acoustic wave into the contacting medium is shallow - a boundary layer interaction only. This means target organisms suspended in solution (but not yet bound to the surface receptors) are not 'seen' by the acoustic sensor; a natural background rejection mechanism observed in recent testing [4]. Additionally, models predict that resonant interaction between bound organisms and the driving surface oscillation is possible, although such a phenomenon has yet to be observed under experimental conditions.

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9:00 AM *O5.4
HIGH SENSITIVITY DETECTION OF BACTERIAL ENDOSPORES VIA TB PHOTOLUMINESCENCE ENHANCEMENT. Nicholas F. Fell, Jr., Paul M. Pellegrino, and James B. Gillespie, U.S. Army Research Laboratory, Adelphi, MD.

Detecting bacterial endospores is a critical challenge to bioanalytical chemistry, since a number of serious diseases and health problems are caused by members of the spore-forming genera *Bacillus* and *Clostridium*. We have developed a highly sensitive method for their detection and have demonstrated detection limits of approximately 1000 CFU/ml. Our method is based on the presence of a marker compound in bacterial endospores, dipicolinic acid (dpa). When complexed with Tb and excited in the UV, the dpa enhances the photoluminescence emission of Tb by several orders of magnitude. We have investigated the potential for interference from other biological materials and chemicals and found that nothing other than bacterial endospores will give us a positive response to this test. Our investigation also showed that the presence of phosphate or organophosphate ions will reduce the observed signals. We have been able to overcome this problem through the addition of $AlCl_3$. The results of our interference studies and phosphate studies will be presented. Since only 10% or less of the dpa is released when the endospores are suspended in aqueous buffer, we have also examined methods for enhancing the release of dpa. Our results from both mechanical and chemical methods to enhance dpa release will be presented. The best we have achieved is a 20-fold increase in dpa release from *B. globigii* endospores in 2 minutes through the addition of dodecylamine and heating to 80°C. Our most recent efforts have been focused on developing and constructing a compact prototype of a sensor using our technique. We have obtained a compact spectrometer and quadrupled Nd:YAG laser for use in this prototype. Our initial examination of this system, shows sensitivity matching or exceeding that of our laboratory scale system, approximately 1000 CFU/mL. The system design and our characterization of its performance will be discussed.

9:30 AM O5.5
CELLS IN MICROPATTERNED HYDROGELS: APPLICATIONS IN BIOSENSING. Won-Gun Koh, Michael Pishko, The Pennsylvania State University, Department of Chemical Engineering, University Park, PA.

Here we will discuss the development of arrays of mammalian cells of differing phenotype integrated with microfluidics and microsensors for applications such as drug screening and used to monitor cellular effects of multiple chemical and biological candidates. To fabricate these arrays, we immobilized either single or small groups of cells in 3-dimensional poly(ethylene glycol) hydrogel microstructures fabricated on plastic or glass surfaces. These microstructures were created using either photolithography or printed using microarray robots. The resulting hydrogel microstructures were fabricated to dimensions as small as 10 microns in diameter with aspect ratios as high as 1.4. The gels were highly swollen with water to permit mass transfer of nutrients and potential analytes to the cells, and cell adhesion molecules were immobilized in the gel to allow cell attachment and spreading. Cell viability was confirmed using fluorescent assays and ESEM used to verified complete cell encapsulation. The specific and non-specific response of these cells to target molecules was monitored using optical or electrochemical detectors and analyzed to quantify the effect of these agents on the different phenotypes present in the array.

9:45 AM O5.6
IMMOBILIZATION OF LIVING CELLS IN SELF-ASSEMBLED NANOSTRUCTURES. Helen K. Baca, Jeb. H. Flemming, Zack Shaw, Department of Chemical and Nuclear Engineering, University of New Mexico, Albuquerque, NM; Darren Dunphy, Susan Brozik, Kim Butler, Sarany Singer, Tamara Hartenberger, Sandia National Laboratories, Albuquerque, NM; Maggie Werner-Washburne, Department of Biology, University of New Mexico, Albuquerque, NM; C. Jeffrey Brinker, Department of Chemical and Nuclear Engineering/Center for Micro-Engineered Materials, University of New Mexico and Sandia National Laboratories, Albuquerque, NM.

The ability of living cells to respond to an external stimulus by incorporating recognition, signaling and response into one small package makes them attractive candidates for use in environmental sensor applications. Cell-based biosensing systems can be genetically engineered by fusing relevant promoter sequences to the coding region of the green fluorescent protein (GFP) gene. Expression of the reporter gene produces a measurable fluorescent signal. Encapsulating engineered cells in a porous, host matrix would allow the cells to be protected and contained while retaining accessibility to the environment if the average pore diameter is smaller than the encapsulated species. Silica thin films formed by evaporation-induced self-assembly (EISA), with resultant uni-modal pore sizes and long range order, are promising immobilization materials for use in cell-based biosensors. EISA uses amphiphilic templates to direct condensation of a silica matrix that may be patterned by micro-molding, stamping or ink-jet printing. Biocompatible templates for the self-assembly process are necessary to ensure both viability of living material and uniform porosity of the surrounding structure. We report the synthesis of structured, patterned silica thin films using a series of biocompatible phospholipid templates. The type of mesophase obtained depends on the molar ratio of template to silica, while pore size can be adjusted by choice of template and condensation conditions. Biocompatibility of the phospholipids is assessed by using a two-color fluorescent probe protocol to measure viability of Saccharomyces cerevisiae after exposure to lipid concentrations necessary for EISA. Yeast cells that have been genetically modified to respond to the glucose/galactose nutrient shift have been immobilized in a porous, phospholipid-templated silica matrix and serve as a model system for optimization of signal transduction and detection.

10:30 AM *O5.7
BIOSENSING AND MICROANALYTICAL METHODS BASED ON GENETIC ENGINEERING STRATEGIES. Sylvia Daunert, Department of Chemistry, University of Kentucky, Lexington, KY.

The design of instruments and techniques capable of detection and quantification of small amounts of biomolecules is essential in gaining further insight into biological processes and in diagnostics. In that respect, miniaturization of analytical instrumentation has played an important role in the analysis of small volume samples. In addition, micromachining techniques have provided invaluable tools that allow for the design and fabrication of microstructures that can be used in microanalysis. In order to be able to detect the target molecules in small volumes, it is necessary to prepare bioreagents that provide enough sensitivity for the detection of the molecule. In our laboratory, we design new assays for biomolecules that are based on the bioluminescence generated by genetically engineered aequorin, a photoprotein isolated form the jellyfish Aequorea victoria. We also use other recombinant DNA methods to rationally design new biosensors. For this, binding proteins are engineered to introduce a unique cysteine that serves as the site of attachment for a fluorescent probe. Binding of the analyte to the binding protein causes a conformational change that alters the microenvironment of the attached flurophore. The change in fluorescence can then be related to the concentration of the analyte, constituting the basis for the development of the sensing systems. Examples of the integration of our biosensing systems on a centrifugal microfluidics CD platform will be presented.

11:00 AM *O5.8
AMPLIFYING POLYMERS FOR ULTRASENSITIVE SENSORS. Timothy M. Swager, Department of Chemistry and Center for MS&E, Massachusetts Institute of Technology, Cambridge, MA.

This presentation will describe the design of electronic polymers that have the ability to undergo self-amplified responses. Optimal energy and charge transport properties are key to the amplifying ability of these materials. Design principles have been developed that can be used to improve the mobility and lifetime of excitons will be presented. To elicit a selective sensor response different molecular recognition principles have been integrated into the polymers. Effective implementation of recognition elements requires effective transduction events that are compatible with the amplifying ability of the polymers. Designs based upon energy transfer, quenching, and excimer formation will be presented for the detection of DNA, Proteins, Explosives, and Ions.

11:30 AM O5.9
SIGNAL GENERATION FROM SWITCHABLE POLYDIACETYLENE FLUORESCENCE. Mary A. Reppy, Analytical Biological Services Inc., Wilmington, DE.

Chemical and biological sensors require a material component that

acts as the transducer from the molecular level event of interest to a discernable output measurable in the macroscopic world. One such material is polydiacetylene, a conjugated polymer that can switch from a non-emitting to a fluorescent state in response to environmental changes. This attribute is harnessed to provide signal generation for bio-sensors and assays as a more sensitive alternative to the previously reported monitoring of polydiacetylene colorimetric shifts. For a given system the change in the emission is much greater than the change in the absorbance; both changes are usually irreversible. Polydiacetylene materials are suitable for a variety of sensing applications ranging from high-throughput screening of enzyme activity to environmental detection of micro-organisms. Liposomes and coatings were prepared from diacetylene surfactants with bio-interactive species such as antibodies or enzymatic substrates incorporated and the materials were polymerized to form polydiacetylene. Binding of the target to the antibodies or other ligands, or reaction of an enzyme with a substrate, led to the shortening of the conjugation length of the polymer backbone, which made the material fluorescent. The emission signal was significantly amplified by incorporation of fluorophores that accepted energy from the excited polymer backbones and fluoresced. This process increased the Stokes shift of the system for certain fluorophores, which led to lower backgrounds and increased sensitivity. Liposomes were prepared from a series of single and double-tailed diacetylene surfactants and their intrinsic fluorescence analyzed. The emission spectra and efficiency of energy transfer varied with the surfactant monomers as well as with the conditions and extent of polymerization. Model enzymatic assays and microbial sensors have been developed.

11:45 AM O5.10
STAR POLYMERS FOR MOLECULAR RECOGNITION.
Ebru Oral[1], Nicholas A. Peppas1, 2, NSF Program on Therapeutic and Diagnostic Devices. [1]School of Chemical Engineering. [2]Department of Biomedical Engineering, Purdue University, West Lafayette, IN.

Biological molecules such as enzymes and antibodies have exceptional recognition capabilities. These recognition capabilities are brought forth by a complex three- dimensional structure composed of small building blocks, all of which support the activity of a few sites. These sites actively select, bind and react with specific substrates. Star polymers are powerful candidates for the design of highly specific synthetic networks because of the presence of a large number of functional groups in a small volume. These functionalities can be derivatized with different groups to interact with different compounds or entire surfaces. We prepared methacrylated poly(ethylene glycol)(PEG) star polymers with 31 arms for use as molecularly imprinted polymers in microfabrication and biosensor applications. A 1 g sample of PEG star polymers with hydroxyl groups on chain ends were functionalized with methacrylate groups by reacting with 3 ml methacryloyl chloride for 16 hours at 40°C in tetrahydrafuran. The resulting mixture was filtered under vacuum and the yield of the reaction was 77%. The extent of methacrylation was observed by ^{1}H-NMR. Molecular imprinting was achieved by complexation and free-radical polymerization of methacrylated star polymers in the presence of glucose and cholesterol, which were used as template molecules. The crosslinking agents used were poly(ethylene glycol) dimethacrylate (PEGDMA) with the molecular weight of the PEG chain of 200, 600 and 1000. The polymers used as controls were prepared without the star polymers. Polymers were placed in a side-by-side diffusion cell to calculate the diffusion coefficients of glucose and structurally similar galactose and methylglucopyranoside through imprinted and non-imprinted gels. In an attempt to make the materials more specific, arms of the stars were further functionalized with histidine and arginine by methacrylating these amino acids and reacting them with the star polymers.

SESSION O6: SENSING WITH SILICON
Chair: Richard W. Cernosek
Thursday Afternoon, April 4, 2002
City (Argent)

1:30 PM *O6.1
SILICON OPTICAL BIOSENSORS: CONTROL OVER MULTIPLE LENGTH SCALES. Philippe M. Fauchet, Univ. of Rochester, Dept of Electrical and Computer Engineering and Center for Future Health, Rochester, NY; Benjamin L. Miller, Univ. of Rochester, Dept. of Chemistry and Center for Future Health, Rochester, NY.

We review the recent development of optical biosensors made of silicon. These biosensors are multilayer and microcavity light emitters made of porous silicon. The internal surface of the pores is functionalized to recognize harmful pathogens. We will show fast, sensitive, and selective detection of DNA segments, full DNA strands, proteins, and bacteria. The design of these biosensors relies on the control over three independent lenght scales corresponding to three different physical phenomena: quantum confinement (1-5 nm quantum dots), photonic bandgap (∼100 nm layers), and penetration into the pores (*10 nm to *100 nm).

2:00 PM O6.2
TOWARDS CONTINUOUS IN VIVO GLUTAMATE MONITORING WITH A SOL-GEL FIBER OPTIC BIOSENSOR USING PHOTOCHEMICAL ENZYME Co-FACTOR REGENERATION.
Jenna L. Rickus, UCLA, Neuroengineering Program, Los Angeles, CA; Pauline Chang, UCLA, Dept. of Chemical Engineering, Los Angeles, CA; Allan J. Tobin, UCLA, Brain Research Institute, Los Angeles, CA; Jeffrey I. Zink, UCLA, Dept. of Chemistry and Biochemistry, Los Angeles, CA; Bruce Dunn, UCLA, Dept. of MS&E, Los Angeles, CA.

Sol-gel encapsulation has recently surfaced as a successful approach to biomolecule immobilization. Proteins, including enzymes, are trapped in the pores of the sol-gel derived glass while retaining their spectroscopic properties and biological activity. The present paper covers our recent work in extending the unique capabilities of biomolecule-doped sol-gel materials to the detection of glutamate, the major excitatory neurotransmitter in the central nervous system. Previously we demonstrated the ability of glutamate dehydrogenase (GDH)-doped sol-gel materials to measure glutamate at varying concentrations. Currently, we are developing an in vivo fiber optic biosensor for glutamate along with methods to achieve continuous monitoring. Our goal is to monitor glutamate release in awake, behaving animals in real time with a temporal resolution of seconds to milliseconds and a spatial resolution of tens of micrometers. In our research to date we have encapsulated GDH in a silica sol-gel film on the tip of an optical fiber. GDH catalyzes the oxidative deamination of glutamate to α-ketoglutarate and the simultaneous reduction of NAD+ to NADH. To calculate the glutamate concentration, we observe the rate of change of NADH fluorescence as a function of time. The current sensors have a diameter of 200μm and can detect glutamate at physiologically relevant concentrations (μM - mM) within seconds. An important consideration for continuous in vivo monitoring is the incorporation of a self-sustaining NAD+ source. We have adopted a photochemical means of regenerating NAD+ from NADH, by irradiating thionine (3,7-diaminophenothiazin-5-ium) which we incorporate into the sol-gel sensor material. When excited with visible light (λabs $\approx$596 nm), thionine undergoes a reaction with NADH resulting in a non-fluorescent form of thionine and NAD+. We have characterized the kinetics of this reaction in the sol-gel matrix, and have shown that the reaction results in regenerated co-factor that is ! usable by GDH for the oxidation of glutamate.

2:15 PM O6.3
OPTICAL SENSING OF GASES USING POLYMER/POROUS SILICON BI-LAYER STRUCTURES. Ting Gao, Jun Gao, Michael J. Sailor, University of California, San Diego, Department of Chemistry and Biochemistry, La Jolla, CA.

Gas sensors consisting of porous silicon (PS) layers, thin polymer films, or a composite structure consisting of a polymer layer on top of a porous Si layer are studied and compared. All three types of structures exhibit well-resolved Fabry-Pérot fringes in their optical reflection spectra due to thin-film interference. For PS films, the fringes shift to higher wavelength upon exposure to chemical vapors due to the adsorption of the vapors in the micropores. Polymer thin films coated on flat silicon substrates also show well-resolved Fabry-Prot fringes that shift when the films are exposed to certain solvent vapors. In this case the spectral shifts are attributed to swelling of the films, while a non-solvent vapor will not cause significant swelling. For this reason the polymer-based vapor sensors offer better selectivity than bare nanocrystalline porous silicon films, although with lower sensitivity. Selective and sensitive vapor sensors can be obtained by using polymer-coated PS as the sensing medium, with the polymer layer functioning as a filter to provide selectivity. Time resolved results have been obtained from this bi-layer structure that demonstrate discrimination between the analytes methyl ethyl ketone, ethanol, proponol, and iso-butanol as analytes. In addition, the polymer-coated PS layers display greater stability in air and water than bare porous Si films.

2:30 PM O6.4
POROUS SILICATE FILMS TEMPLATED WITH BIO-COMPATIBLE PORE FORMING AGENTS. Darren R. Dunphy, Adam W. Cook, Kimberly Butler, Sanda National Laboratories, Materials Chemistry Department, Albuquerque, NM; Helen K. Baca, Zachary Shaw, University of New Mexico, Department of Chemical and Nuclear Engineering, Albuquerque, NM; C. Jeffrey Brinker, Sandia National Laboratories and University of New Mexico, Albuquerque, NM.

Entrapment of biomolecules inside a mesoporous silicate medium is an attractive technology for biosensor development; the silica

environment can increase biomolecular stability, while the well-connected pore structure allows facile mass transport through the hybrid biological/inorganic material. Standard silica templating agents (i.e. cationic, anionic, or block copolymer amphiphiles) have the potential to disrupt biomolecular structure, however, and are difficult to remove after material synthesis. For these reasons, small molecules, such as carbohydrates, carboxylic acids, or even peptides, are promising pore templating agents. As these compounds are biologically derived, they are generally very biocompatible, and are readily removed from templated silica using aqueous extraction. We will discuss the formation of thin silica films templated with these pore forming agents. Special emphasis will be given on comparing the resultant mesostructure in our thin films with that obtained in previous research where monolithic or powdered samples were synthesized. Also, requirements for efficient pore templating will be covered, along with the effect of film formation conditions on the final material porosity. Finally, the biocompatibility of mesoporous silicate film synthesis will be discussed for two cases; 1) entrapment of whole yeast cells and 2) entrapment of bovine serum albumin (BSA), a model protein. Biocompatibility will be assayed with either a fluorescent viability indicator (yeast), or intrinsic fluorescence (BSA), a measure of protein conformation.

3:15 PM O6.5
NITRIC OXIDE SENSORS OBTAINED THROUGH THE ENTRAPMENT OF IRON COMPLEXES IN SOL-GEL MATRIX.
Juliana C. Biazzotto, Carlos F.O. Graeff, DFM-FFCLRP-USP, Ribeirão Preto, BRAZIL; Roberto Mendonca Faria, IFSC-USP, São Carlos, BRAZIL.

Since nitric oxide (NO) was discovered as an important mediator of various physiological processes, several NO detection methods have been developed. Sol-gel process has been used for NO electrochemical sensors as well as biosensors. In this work we report the synthesis of the NO sensors, SGFeDETC and SGFeTFPP, consisting in the entrapment of the iron(III)-diethyldithiocarbamate (FeDETC) or iron(III)-tetra-pentafluorophenylporphyrin (FeTFPP), within a silica matrix by the sol-gel process. SGFeDETC was obtained by addition of the silica sol, composed of the tetraethyl orthosilicate (TEOS, 4.00 mL), ethanol (4.00 mL), concentrated HCl (15.0 μL) and sonicated for 10 min, to the FeDETC in dimethylformamide (ratio DETC:Fe, 2:1). For SGFeTFPP the silica sol comprising TEOS (4.00 mL), ethanol (4.00 mL), HCl 0,1 M (50.0 uL), water (310 uL) and Triton X100 (40.0 uL) after 10 min sonicated was added to a FeTFPP in dichloromethane. The resultant mixtures was maintained under stirring for 2 hours and allowed to stand at 30°C for aging. UV/Vis spectroscopy (Varian, Cary 50 spectrophotometer) and Electron Spin Resonance (ESR) (Varian E-4 X-Band spectrometer at room temperature) have been used to characterize the materials and sensors. UV/Vis spectra of the SGFeTFPP present a Soret band at 410 nm similar to that found in the solution. The binding of gaseous NO resulted in a red shift in the Soret absorption band (410 to 419 nm) of the FeTFPP in the matrix, as expected. In the case of SGFeDETC, after addition of sodium dithionite solution and bubbling NO gas, the ESR spectrum of the SGFeDETCNO exhibited a characteristic three-line ($g = 2.035$) similar to that found in solution. It was also observed that the iron nitrosyl diethyldithiocarbamate is more stable in the sol-gel than in solution. Studies towards NO quantification using both sol-gel iron complexes will also be presented. FAPESP and CNPq supported this work.

3:30 PM O6.6
GAS MOLECULAR RECOGNITION BY USING ORGANIC MONOLAYERS SELF-ASSEMBLED ONTO SILICON: FUNDAMENTAL AND TECHNOLOGICAL ISSUES.
Dario Narducci, Monica Bollani, Matteo Oldani, Istituto Nazionale per la Fisica della Materia and Dept. Materials Science, Univ. of Milano Bicocca, Milan, ITALY; Laura Raimondi, Dept. Organic and Industrial Chemistry, Univ. of Milano, Milan, ITALY.

Gas chemical sensors face hurdles that has limited their extensive development in both civil and industrial environments. Despite a widespread need, the large spectrum of chemicals for which a specific gas sensor need exists is extremely diversified, the market for each of them rarely exceeding a few hundreds units/year. In this way, chemical sensing appears to be a contradictory market niche. The realization of sensors requires technologies commonly available in the microelectronic industry only, as the making of sensor arrays and the integration of the device can be sustained only through an integrated production scheme. The production rate for each sensor is however extremely marginal with respect to microelectronic industry standards.
In this communication we will present a new technology overcoming most of these hurdles. It relies upon the use of a self-assembling method leading to modifications of Si surfaces through the formation of direct Si-C bonds. The technique, based upon room-temperature nucleophilic reaction between a halogenated Si surface and a suitable electrophilic precursor of the organic species, enabled monitoring of atmospheric pollutants down to the ppm range. We will present data showing how these sensors, operating at low temperatures (300-320 K) display a remarkable sensitivity toward gases such as CO, SOx, NOx, along with an enhanced selectivity. Comparisons with sensors based on metal oxides will be presented. The results obtained disclose remarkable opportunities of further technological implementation. Due to the infinite variety of organic fragments usable and to the possibility of designing ad hoc receptor molecules, sensor arrays can be devised dealing with complex organic mixtures, leading to flavor and perfume detection. At the same time, as gas sensor manufacturing require silicon surface functionalization to be carried out only after the making of the integrated board, specialized sensor arrays can be conceived also for quantitatively marginal applications.

3:45 PM O6.7
A SELF LOCKING TECHNIQUE WITH FAST RESPONSE AND HIGH SENSITIVITY FOR MICRO-CANTILEVER BASED SENSING OF ANALYTES.
Adosh Mehta, G. Muralidharan, Ali Passian, Suman Cherian, T.L. Ferrell, Thomas Thundat, Life Sciences Division, Oak Ridge National Lab, Oak Ridge, TN.

MEMS based microcantilevers have been employed as sensors in both liquid and ambient conditions. One scheme for detection is based upon monitoring the change in microcantilever resonant frequency as a function of the amount of adsorbed analyte. However, the sensitivity is limited by the accuracy of the frequency measurements, which is a function of the Q-factor of the vibrating element and the measurement bandwidth. In this paper, we present a feedback scheme for self-locking amplification of the small-amplitude thermal oscillations of the microcantilever. Using this approach, we demonstrate an improvement in the Q-factor by two to three orders of magnitude as compared to that of the undriven microcantilever. Use of this technique eliminates the need for lock-in detection and results in improved response times for sensor applications. We also present a theoretical model that predicts the allowed regimes of relevant parameters for which amplification is feasible. Experiments using the proposed feedback amplification technique show improved sensitivity for the detection of biological molecules in liquids, and for adsorbed vapors under ambient conditions.

4:00 PM O6.8
CHEMICAL SENSORS BASED ON PIEZORESISTIVE MICROCANTILEVERS.
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MEMS-based microcantilevers have been proposed for a variety of biological and chemical sensing applications. Measuring the magnitude of microcantilever deflection due to adsorption-induced bending, and following the variation in the resonant frequency of the microcantilevers due to the adsorbed mass are two techniques commonly employed for sensing analytes. Apart from possessing a high level of sensitivity to small changes in mass, microcantilevers are also very sensitive to small changes in temperature and hence the flow of heat. One way of achieving high sensitivity in thermal measurements is by using a bimaterial microcantilever and measuring its deflection as a result of thermal fluctuations. Commercially available piezoresistive microcantilevers are an example of bimaterial cantilevers and in this study, we propose the use of such cantilevers for sensing explosives. We show that sensing can be accomplished by following the differences in the thermal response of the cantilevers introduced by the presence of explosives adsorbed from the vapor phase onto the surface of the cantilever. We discuss the materials and design issues involved in determining the sensitivity of detection. In addition, we also show how selectivity can be achieved in this scheme of detection.