

## **Bio-Chemo-Opto-Mechanical (BioCOM) Sensors for Real-Time Characterization for D&D Applications (82799)**

### **Research Objective**

Basic and applied research is being conducted to develop simple to use chemical and biological sensor chips utilizing bio-chemo-mechanics for real-time, in-situ, detection of technetium, mercury, uranium, copper, and lead for deactivation and decommissioning applications. The bio-chemo-opto-mechanical (Bio-COM) chip involves properly fashioned arrays of micromachined silicon cantilevers containing embedded deformable diffraction gratings functionalized with chemically selective coatings. Adsorption of specific molecules on the cantilever array leads to bending, which changes the diffraction of light from the array. The bio-chemo-opto-mechanical (BioCOM) chips will be designed to contain an array of pixels, with each pixel containing an array of microcantilever springs in which one surface is derivatized with either an antibody coating or a self-assembled monolayer (SAM) coating for detecting Hg(II), Hg(0), Cu(II), Pb(II), U(VI), or  $\text{TcO}_4^-$ . The BioCOM sensor platform also offers the advantage of simultaneous measurement of many analytes using a single chip. The readout mechanism can be a reflected laser beam, producing a diffraction pattern, or in an ideal case the diffraction of daylight resulting in a change of color. In the latter case the proposed sensors would not require any external power, external or on-board electronics, or fluorescent dyes and associated optics, which will keep its fabrication and operation costs low while making it simple to use for real-time environmental monitoring.

### **Research Progress and Implications**

This report summarizes the work in the first 33 months (June, 2004) of a three year project. Despite its high sensitivity, the cantilever platform offers no intrinsic chemical selectivity. One surface of the silicon microcantilever can be functionalized so that a given molecular species will be preferentially bound to that surface upon its exposure to an analyte stream. Therefore, detection sensitivity is vastly enhanced by applying an appropriate coating on one cantilever surface. Such a coating can, in principle, provide selectivity as well. In this program, advantage is being taken of this fact to create sensors for contaminant metal ion species, in several cases based on antibody antigen interactions in solution. Many different self-assembled monolayers (SAM) have been and continue to be under development. Once the SAMs are tested and optimized, we will apply them on one surface of the deformable diffraction cantilever array for determination of analytes.

Our collaborator Diane Blake at Tulane has been developing antibodies for U, Pb, and other heavy metals. A derivative of 1,10 phenanthroline that binds to  $\text{UO}_2^{2+}$  with nanomolar affinity was found to be a very effective immunogen for the generation of antibodies directed toward chelated complexes of hexavalent uranium. The synthesis of 5-isothiocyanato-1,10-phenanthroline-2,9-dicarboxylic acid and its use in the generation and functional characterization of a group of monoclonal antibodies that recognize the most soluble and toxic form of uranium, the hexavalent uranyl ion ( $\text{UO}_2^{2+}$ ), has been accomplished. Three different monoclonal antibodies (8A11, 10A3, and 12F6) that recognize the 1:1 complex between  $\text{UO}_2^{2+}$  and 2,9-dicarboxy-1,10-phenanthroline (DCP) were produced by the injection of BALB/c mice with DCP- $\text{UO}_2^{2+}$  covalently coupled to a carrier protein. Equilibrium dissociation constants for the binding of DCP- to anti  $\text{UO}_2^{2+}$  bodies 8A11, 10A3, and 12F6 were 5.5, 2.4, and 0.9 nM, respectively. All three antibodies bound the metal-free DCP with roughly 1000-fold lower

affinity. Binding studies conducted with structurally related chelators and 21 metal ions demonstrated that each of these three antibodies was highly specific for the soluble DCP-  $\text{UO}_2^{2+}$  complex. The three antibodies identified possess sufficient affinity and specificity to support the development of immunoassays for hexavalent uranium in environmental and clinical samples. An attempt was made to attach 5-isothiocyanato-1,10-phenanthroline-2,9-dicarboxylic acid to a silicon cantilever which had been treated with trimethoxysilylpropylamine. The antibody does not appear to recognize the DCP-  $\text{UO}_2^{2+}$  species when it is bound to a surface.

Studies were conducted on a monoclonal antibody directed against Pb(II) complexed with a protein conjugate of diethylenetriaminepentaacetic acid (DTPA). Binding curves obtained with aminobenzyl-DTPA or its complexes with Ca(II), Sr(II), and Ba(II) were highly sigmoidal, characterized by Hill coefficients of 2.3 to 6.5. Binding curves obtained with the Pb(II) and In(III) complexes of aminobenzyl-DTPA were hyperbolic, but in each case the apparent affinity of the antibody for the chelator-metal complex was higher in the presence of excess chelator than it was in the presence of excess metal ion. In the presence of excess chelator, the equilibrium dissociation constant for the binding of aminobenzyl-DTPA-Pb(II) to the antibody was  $9.5 \times 10^{-10}$  M. Binding curves obtained with the Hg(II) and Cd(II) complexes of aminobenzyl-DTPA were biphasic, indicative of negative cooperativity. Further binding studies demonstrated that aminobenzyl-DTPA-Hg(II) opposed the binding of additional chelator-metal complexes to the antibody more strongly than did aminobenzyl-DTPA-Cd(II). The Fab fragment differed from the intact antibody only in that the apparent affinity of the Fab was generally lower for a given chelator-metal complex. These data are interpreted in terms of a model where: (i) aminobenzyl-DTPA and its complexes bind both to the antigen binding site and to multiple charged sites on the surface of the compact immunoglobulin; and (ii) the bound, highly charged ligands interact in a complicated fashion through the apolar core of the folded antibody.

The key to microcantilever surface modification technology is to selectively modify one surface of a microcantilever with molecular recognition layers. General microcantilever modification methods using a multilayer film have been and continue to be under development. A microcantilever modified with a layer of acetyl cholinesterase (AChE) which responds to paraoxon has been a subject under investigation. AChE was immobilized on a microcantilever through a cross linker to a monolayer on gold surface of the microcantilever. The microcantilever underwent a maximum of 7 nm bending due to the inhibition of AChE by paraoxon that slightly changed the conformation of AChE.

### **Planned Activities**

The goal of this research program is to develop a sensor platform based on a complex microcantilever deformable diffraction gratings. These are often called flip-over bimaterial (FOB) beam to increase the deflection of the micromechanical cantilevers for chemical detection. The FOB has a special configuration such that a material layer is coated on the top and the bottom of the other material layer. The sensitivity is increased by incorporating multiple interconnections to FOB beams. Therefore, the beam deflection is converted into a linear displacement of a reflecting surface for interferometry. We have microfabricated these FOB sensors. The details of the fabrication process for FOB are described in publication by Lim et al.(2004).

Chemical selectivity is achieved by coating the arrays with selective coatings, designed by the principles of molecular recognition. The response of a given modified grating will depend

on the concentration of the analyte and the affinity of the analyte for the coating (e.g. interactions due to hydrogen bonding, complexation, and other molecular recognition bonding). The BioCOM chips are being designed to be arrays of pixels, each to be selective to an analyte of interest

DOE's needs have changed since this project was initiated, and an emphasis on subsurface and vadose zone issues will be the focus of future work. New approaches to sensors, particularly low power devices that can be placed in monitoring wells will continue to be a need. DOE's basic research focus for subsurface contamination is generally in four areas: (1) location and characterization of subsurface contaminants and characterization of the subsurface, (2) conceptual modeling, (3) containment and stabilization, and (4) monitoring and validation. The first and fourth of these goals benefit from new microcantilever sensor platforms under development in this project. Contaminants of concern across the general DOE complex broadly include: radionuclides, metals, and dense nonaqueous phase liquids (DNAPLs). More specifically, key chemicals by group are:

- Radionuclides: plutonium, strontium-90, cesium-137, isotopes of uranium, tritium, thorium, technecium-99, radium, and iodine-129
- Metals: lead, chromium VI, mercury, zinc, beryllium, arsenic, cadmium, and copper
- DNAPLs: carbon tetrachloride, trichloroethylene, dichloroethylene, tetrachloroethylene, chloroform, dichloromethane, and polychlorinated biphenyls

In the first group we have been working on  $\text{UO}_2^{2+}$  sensors as well as  $\text{TcO}_4^-$  sensors. Among the second group, we have developed selective coatings for a several of these metals, and work will continue in this area via the principles of molecular recognition. The latter group will be addressed with an array of organic coatings designed to recognize organic vapors by a combination of solubility and chemical affinity principles

Most of the selective coatings employed by us to date has depended on the preparation of alkane thiol reagents that form self assembled monolayers on a cantilever coated on one surface with gold. Work in this area will continue, but it will be supplemented with a new effort to develop the chemistry of silane reagents to selectively modify the surface of individual cantilevers in an array. We will emphasize photoactivation and photo-deprotection schemes in an effort to use a laser (or other tightly focused light beam) to select a single cantilever in an array for derivatization.

#### **Information Access – Publications in FY 04**

“Microcantilever sensors for physical, chemical, and biological detection” T. Thundat and A. Majumdar, Springer Verlag (2003) (in press)

X. Yan, Y. Lvov, T. Thundat, H. –F. Ji, “A General Method for Microcantilever Surface Modification by Multilayer for Biospecific Recognition”, Organic and Biomolecular Chemistry, 2003, 460-462

R.C. Blake II, J.B. Delehanty, M. Khosraviani, H. Yu, R.M. Jones, and D.A. Blake (2003) "Allosteric binding properties of a monoclonal antibody and its Fab fragment", Biochemistry, 42:497-598.

X. Yan, Y. Lvov, T. Thundat, H. -F. Ji, "A General Method for Microcantilever Surface Modification by Multilayer for Biospecific Recognition", *Organic and Biomolecular Chemistry*, 2003, 460-462

Yanjun Tang, Ji Fang, Xiaohe Xu, Hai-Feng Ji, Gilbert M. Brown, and Thomas Thundat, "Detection of Femtomolar Concentration of HF Using an SiO<sub>2</sub> Microcantilever," *Anal. Chem.*, **2004**, 76, 2478-2481

Thomas Thundat, Hai-Feng Ji and Gilbert M. Brown, "Environmental Monitoring Using Microcantilever Sensors," ACS Symposium Series, in press

R.C. Blake II and D.A. Blake Quantitative analysis of antibody-antigen interactions using immobilized ligands, *Progress in Monoclonal Antibody Research*, (F. Columbus, ed.) Nova Science Inc., Hauppauge, New York, 2004, in press.

X. Yan, Y. Tang, H.-F. Ji, Y. Lvov, T. Thundat "Detection of Organophosphate Using an AChE-Coated Microcantilever" *Instrument Science & Technology*. 2004, In press

R.C. Blake II, A.R. Pavlov, M. Khosraviani, H.E. Ensley, G.E. Keifer, H. Yu, X. Li, and D.A. Blake (2004)"Novel monoclonal antibodies with specificity for chelated uranium(VI): Isolation and binding properties", *Bioconjugate Chemistry*, submitted for publication.

S-H. Lim, J. Choi, R. Horowitz, and A. Majumdar, "Design and fabrication of a novel bi-morph micro-opto-mechanical sensor" (submitted).