

REPORT DOCUMENTATION PAGEForm Approved
OMB NO. 0704-0188**COPY**

Public Reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comment regarding this burden estimates or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188,) Washington, DC 20503.

1. AGENCY USE ONLY (Leave Blank)		2. REPORT DATE June 15, 2005	3. REPORT TYPE AND DATES COVERED Final Technical Report 5/1/99 – 10/31/04	
4. TITLE AND SUBTITLE Texas Consortium for the Development of Biological Sensors			5. FUNDING NUMBERS DAAD19-99-1-0207	
6. AUTHOR(S) Andrew Ellington, Ph.D., et. al.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Texas at Austin 2500 Speedway, MBB 3.424 Austin, TX 78712			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U. S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211			10. SPONSORING / MONITORING AGENCY REPORT NUMBER 39825.27-LS-mur	
11. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.				
12 a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited.			12 b. DISTRIBUTION CODE	
14. SUBJECT TERMS			15. NUMBER OF PAGES 40	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OR REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION ON THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL	

NSN 7540-01-280-5500

Standard Form 298 (Rev.2-89)
Prescribed by ANSI Std. Z39-18
298-102**GENERAL INSTRUCTIONS FOR COMPLETING SF 298**

The Report Documentation Page (RDP) is used for announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to **stay within the lines** to meet **optical scanning requirements**.

Block 1. Agency Use Only (Leave blank)

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least year.

Block 3. Type of Report and Dates Covered.

State whether report is interim, final, etc. If applicable enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, and volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s) project number(s), task number(s), and work unit number(s). Use the following labels:

C - Contract	PR - Project
G - Grant	TA - Task
PE - Program Element	WU - Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. (if known)

Block 11. Supplementary Notes. Enter information not included elsewhere such as; prepared in cooperation with....; Trans. of....; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement.

Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NORFORN, REL, ITAR).

DOD - See DoDD 4230.25, "Distribution Statements on Technical Documents."

DOE - See authorities.

NASA - See Handbook NHB 2200.2.

NTIS - Leave blank.

Block 12b. Distribution Code.

DOD - Leave Blank

DOE - Enter DOE distribution categories from the Standard Distribution for unclassified Scientific and Technical Reports

NASA - Leave Blank.

NTIS - Leave Blank.

Block 13. Abstract. Include a brief (*Maximum 200 words*) factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subject in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code (NTIS *only*).

Block 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (Unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

REPORT DOCUMENTATION PAGE (SF298)
(Continuation Sheet)

Category 1-a

Manuscripts submitted but not published

- Harvey, B.R., Shanafelt, A., Baburina, I., Hui, R., Vitone, S., Iverson, B.L., and Georgiou, G. (Submitted). Engineering of Recombinant Antibody Fragments to Methamphetamine by Anchored Periplasmic Expression (APEX). *J Immunol Methods*.
- Mabry, R., Rani, R., Geiger, R., Hubbard, R., Carrion, R., Brasky, K., Patterson, J., Georgiou, G., and Iverson, B.L. (Submitted). High Affinity Antibody Fragments Lacking Fc Regions Confer Protection Against Inhalation Anthrax. *Proc Natl Acad Sci USA*.
- Schmid, M.J., Manthiram, K., Willson, J.C., Bell, K.M., Ellington, A.D., and Willson, C.G.. (Submitted). Feature multiplexing a novel probe placement methodology for microarray devices. *Proc Natl Acad Sci USA*.
- Sooter, L.J., and Ellington, A.D. Automated selection of transcription factor binding sites. (2004). *JALA*. In Review.

Category 1-b

Papers published in peer-reviewed journals

- Chen G., Hayhurst, A., Thomas, J.G., Harvey, B.R., Iverson B.L. and Georgiou, G. (2001). Isolation of High Affinity Ligand-Binding Proteins by Periplasmic Expression with Cytometric Screening (PECS). *Nat Biotechnol*, **19**:537-42.
- Cho, E.J., Rajendran, M., and Ellington, A.D. (2004). "Aptamers as Emerging Probes for Macromolecular Sensing," *Topics for Fluorescence Spectroscopy*, J.R. Lakowicz, ed., Plenum Press, New York.
- Christodoulides, N., Tran, M., Floriano, P.N., Rodriguez, M., Goodey, A., Ali, M., Neikirk, D.P., and McDevitt, J.T. (2002). A microchip-based multianalyte assay system for the assessment of cardiac risk. *Anal Chem*, **74**(13):3030-36.
- Collett, J.R., Cho, E.J., Lee, J.F., Levy, M., Hood, A.J., Wan, C., and Ellington, A.D. (2005). Functional RNA microarrays for high-throughput screening of anti-protein aptamers. *Anal Chem*, **338**(1):113-23.
- Cox, J.C., Hayhurst, A., Hesselberth, J., Bayer, T.S., Georgiou, G., and Ellington, A.D. (2002). Automated selection of aptamers against protein targets translated *in vitro*: from gene to aptamer. *Nucleic Acids Res*, **30**(20):108-22
- Curey, T.E., Salazar, M.A., Oliveira, P., Javier, D.J., Dennis, P.J., Rao, P., and Shear, J.B. (2002). Enzyme-Based Sensor Arrays for Rapid Characterization of Complex Disaccharide Solutions. *Anal Biochem*, **303**:42-48.
- Faulkner, S.B., Syrett, H.S., Davidson, E.A., and Ellington, A.D. (2004). *In vitro* evolution through PCR-mediated mutagenesis and recombination. *PCR Technology Current Innovations, Second Edition*, Chp. **36**, 371-80.

- Flores, H., and Ellington, A.D. (2002). Increasing the thermal stability of an oligomeric protein, beta-glucuronidase. *J Mol Biol*, **315**(3):325-37.
- Goodey, A.P., Lavigne, J.J., Savoy, S.M., Rodriguez, M.D., Curey, T., Tsao, A., Simmons, G., Wright, J., Yoo, S.J., Sohn, Y.S., Anslyn, E.V., Shear, J.B., Neikirk, D.P., and McDevitt, J.T. (2001). Development of Multianalyte Sensor Arrays Composed of Chemically Derivatized Polymeric Microspheres Localized in Micromachined Cavities. *J Am Chem Soc*, **123**:2559-70.
- Griswold, K., Mahmood, N., Iverson, B.L., and Georgiou, G. (2003). Effects of Codon Usage on the Expression of *Fusarium solani* Cutinase in the *Escherichia coli* Cytoplasm. *Prot Expr Purif*, **27**:134-42.
- Harvey, B., Georgiou G., Hayhurst, A., Iverson, B.L. and Rogers, G. (2004). Anchored Periplasmic Expression (APEX): A Versatile Technology for the Isolation of High Affinity Antibodies from Combinatorial Libraries. *Proc Natl Acad Sci USA*, **101**:9193-98.
- Hayhurst, A. and Georgiou, G. (2001). High Throughput Antibody Isolation. *Curr Opin Chem Biol*, **5**:683-89.
- Hesselberth, J.R., Robertson, M.P., Knudsen, S.M., and Ellington, A.D. (2003). Simultaneous detection of diverse analytes with an aptazyme ligase array. *Anal Biochem*, **312**:106-12.
- Jeong, K.J., Kawarasaki, Y., Gam, J.S., Harvey, B.R., Iverson, B.L. and Georgiou, G. (2004). A Periplasmic Fluorescent Reporter Protein and Its Application in High-Throughput Membrane Protein Topology Analysis. *J Mol Biol*, **341**:901-09.
- Kaehr, B., Allen, R., Javier, D.J., Currie, J., and Shear, J.B. (2004). Guiding Neuronal Development with in situ Microfabrication. *Proc Natl Acad Sci USA*, **101**:16104-08.
- Kim, J.W. and Neikirk, D.P. (2003). Infrared wavelength selective micromachined microbolometers. Presented at *SPIE's First International Symposium on Microtechnologies for the New Millennium 2003*, Maspalomas, Gran Canaria (Canary Islands), Spain, May 19-21.
- Kirby, R., Cho, E.J., Gehrke, B., Bayer, T., Park, Y.S., Neikirk, D., McDevitt, J., and Ellington, A.D. (2004). Aptamer-based Sensor Arrays for the Detection and Quantitation of Proteins. *Anal Chem*, **76**(14):4066-75.
- Lee, J.F., Hesselberth, J.R., Meyers, L.A., and Ellington, A.D. (2004). Aptamer Database. *Nucleic Acids Res*, **32**:D95-D00. Database Issue.
- Levis, R., Weiss, R., Chen, G., Iverson, B.L., and Georgiou, G. (2001). Production of Correctly Folded Fab Antibody Fragment in the Cytoplasm of *Escherichia coli* *trxB* *gor* Mutants via the Co-expression of Molecular Chaperones. *Prot Expr Purif*, **23**:338-47.
- Maynard, J. A. and Georgiou, G. (2000). Antibody Engineering. *Annu Rev Biomed Eng*, **2**:339-76. [Note: Acknowledgement cites US Army rather than the MURI program specifically]
- Maynard, J.A., Chen, G., Georgiou, G., and Iverson, B.L. (2002). In vitro Scanning Saturation Mutagenesis. *Methods Mol Biol*, **182**:149-63.
- Maynard, J.A., Leppla, S.H., Bratt, K., Patterson, J.L., Iverson, B.L. and Georgiou, G. (2002). Protection Against Anthrax Toxin by Recombinant Antibody Fragments Correlates with Antigen Affinity. *Nat Biotechnol*, **20**:577-01.

- Mehnaaz, F.A., Kirby, R., Goodey, A., Rodriguez, M., Ellington, A.D., Neikirk, D., and McDevitt, J. (2003). DNA Hybridization and Discrimination of Single-Nucleotide Mismatches Using Chip-Based Microbead Arrays. *Anal Chem*, **75**(18):4732-39.
- Meiring, J.E., Schmid, M.J., Grayson, S.M., Rathsack, B.M., Johnson, D.M., Kirby, R., Kannappan, R., Manthiram, K., Hsia, B., Hogan, Z.L., Ellington, A.D., Pishko, M.V., and Willson, C.G. (2004). Hydrogel Biosensor Array Platform Indexed by Shape. *Chem Mater*, **16**(26):5574-80.
- Miao, W., Choi, J.P., and Bard, A.J. (2002). Electrogenerated Chemiluminescence 69: The Tris(2,2'-bipyridine)ruthenium(II), $(\text{Ru}(\text{bpy})_3^{2+})$ /Tri-*n*-propylamine (TPrA) System Revisited-A New Route Involving TPrA⁺ Cation Radicals. *J Am Chem Soc*, **124**:14478-85.
- Miao, W. and Bard, A.J. (2003). Electrogenerated Chemiluminescence. 72. Determination of Immobilized DNA and C-Reactive Protein on Au(111) Electrodes Using Tris(2,2'-bipyridyl)ruthenium(II) Labels. *Anal Chem*, **75**:825-34.
- Miao, W. and Bard, A.J. (2004). Electrogenerated Chemiluminescence. 77. DNA Hybridization Detection at High Amplification with $[\text{Ru}(\text{bpy})_3]^{2+}$ -Containing Microspheres. *Anal Chem*, **76**:5379-86.
- Miao, W. and Bard, A.J. (2004). Electrogenerated Chemiluminescence. 80. C-Reactive Protein Determination at High Amplification with $[\text{Ru}(\text{bpy})_3]^{2+}$ -Containing Microspheres. *Anal Chem*, **76**:7109-13.
- Park, B.H., Park, Y.S., Sohn, Y.S., and Neikirk, D.P. (2003). Fabrication of bead size sorting chip for chemical array sensor. Presented at *SPIE's First International Symposium on Microtechnologies for the New Millennium 2003*, Maspalomas, Gran Canaria (Canary Islands), Spain, May 19-21..
- Park, B.H., Sohn, Y.S., and Neikirk, D.P. (2003). Development of micro bead size selection chip for a chemical array sensor. *$\mu\text{TAS}2003$ / microTAS2003: The 7th International Conference on Miniaturized Chemical and BioChemical Analysis Systems*, Squaw Valley, California USA, October 5-9, pp. 631-34.
- Sohn, Y.S., Tsao, A., Anslyn, E.V., McDevitt, J.T., Shear, J.B., and Neikirk, D.P. (2000). Liquid Flow Through an Array-Based Chemical Sensing System. *Proc SPIE-Int Soc Opt Eng*, **4177**:212-19.
- Sohn, Y.S. (2001). MEMS Based Microfluidic Structure for Biological and Chemical Sensor Array. PhD dissertation in Department of Electrical and Computer Engineering. Austin: The University of Texas at Austin.
- Sohn, Y.S., Goodey, A.P., Anslyn, E.V., McDevitt, J., Shear, J.B., and Neikirk, D.P. (2001). Development of a Micromachined Fluidic Structure for a Biological and Chemical Sensor Array. Presented at the *Fifth International Conference on Miniaturized Chemical and Biochemical Analysis Systems*, Monterey, California, October 21 - 25.
- Sohn, Y.S., Goodey, A.P., Anslyn, E.V., McDevitt, J.T., Shear, J.B., and Neikirk, D.P. (2001). MEMS Based Microfluidic Structure for Biological and Chemical Sensor Array. Presented at *TEX MEMS III*, Dallas, TX, June 7, p. 23.
- Sohn, Y.S., Goodey, A.P., Anslyn, E.V., McDevitt, J.T., Shear, J.B., and Neikirk, D.P. (2004). A micromachined fluidic structure for capillary-based sample introduction into a microbead array chemical sensor. *Sensor Letters*, **2**(1):69-72.
- Sooter, L.J., Riedel, T., Davidson, E.A., Levy, M., Cox, J.C., and Ellington, A.D. (2001). Toward automated nucleic acid enzyme selection. *Biol Chem*, **382**(9):1327-34.
- Thompson, K.M., Syrett, H.A., Knudsen, S.M., and Ellington, A.D. (2002). Group I aptazymes as genetic regulatory switches. *BMC Biotechnol*, **2**:21.

Category 1-c

Papers Published in non-peer-reviewed journals or in conference proceedings

N/A

Category 1-d

Papers presented in meetings but not published in conference proceedings:

- a. Mabry, R., Maassen, C., Lee, S., Mohamed, N., Brasky, K., Pattersen, J., Spitalny, G., Casey, L., Iverson, B.L., and G. Georgiou, "Protection Against Anthrax Toxin by Recombinant Heteropolymers Targeting Protective Antigen". 2003 IBC Antibody Engineering Conference, San Diego, CA.
- b. Harvey, B.R., Mabry, G.R., Iverson, B.L. and G. Georgiou "Antibody Immunotherapies to BW Agents" AICHE Annual Meeting, San Francisco, CA (2003).
- c. Harvey, B.R., Iverson B.L. and G. Georgiou : "Flow Cytometric Selection of Antibodies Targeting Bacterial Toxins and Drugs of Abuse" DakoCytomation Life Sciences Users Meeting, Fort Collins, CO (September 2003).
- d. G. Georgiou "Directed Evolution and Engineering of Chaperone Specificity" 11th European Congress on Biotechnology, Basel, Switzerland (2003).
- e. Mabry, R., Maassen, C., Mohamed, N., Brasky, K., Pattersen, J.L., Spitalny, G., Casey, L., Iverson, B.L. and G. Georgiou "Protection Against Anthrax Toxin" 225th American Chemical Society National Meeting, New Orleans, LA (2003).
- f. Mabry, R., Maynard, J.A., Maassen, C., Leppla, S.H., Brasky, K., Patterson, J.L. Iverson, B.L. and G. Georgiou, "Protection Against Anthrax Toxin by Targeting Protective Antigen" Army Science Conference, Orlando, FL (2002).
- g. Hayhurst, A., Iverson, B.L. and Georgiou, G. "Multiplex analysis of single chain antibody fragment affinities using surface plasmon resonance" IBC 13th International Conference on Antibody Engineering, San Diego, (December, 2002).
- h. Harvey, B.R., Iverson, B.L., and Georgiou, G. "Anchored Periplasmic Expression (APEX) of Protein Libraries for Flow Cytometric Selection." 224th ACS National Meeting, Boston, MA (2002).
- i. Georgiou, G. "Development of Neutralizing Antibodies to Anthrax and other Biological Warfare Agents", AIMBE Annual meeting, Washington, D.C. (2002).
- j. Maynard, J., Braat-Maassen, L., Leppla, S.H., Brasky, K., Patterson, J., Iverson, B., and Georgiou G. "Engineering Antibody Therapeutics which Neutralize Bacterial Toxins" Department of Chemical Engineering, University of Texas at Austin, Austin, TX (2001).
- k. Georgiou, G. "Display-less Screening of Antibody and Enzyme Libraries using PECS (Periplasmic Expression & Cytometric Screening)" IBCs 12th Annual International Conference on Antibody Engineering, San Diego, CA (2001).
- l. Maynard, J., Braat-Maassen, K., Leppla, S. H., Brasky, K., Patterson, J., Iverson, B., and Georgiou, G. "Targeted Therapy against Anthrax: Prospects for Developing Recombinant Antibody Therapy" National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD (2001).

- m. Maynard, J., Braat-Maassen, K., Leppla, S.H., Brasky, K., Patterson, J., Iverson, B., and Georgiou, G. "Engineering Antibody Therapeutics" American Institute of Chemical Engineers National Meeting, Reno, NV (2001).
- n. Maynard, J., Hayhurst, A., Happe, S., Harvey, B., Fitza, M., Iverson, B., Georgiou, G. "Combinatorial antibody libraries in biological recognition" Controlled Release Society, San Diego, CA (2001).
- o. Maynard, J., Merkel, T., Keith, J., Iverson, B. and Georgiou, G. "Engineering Antibody Therapeutics against Pertussis Toxin" National Institutes of Health, Bethesda, MD (2001).
- p. Maynard, J., Leppla, S.H., Iverson, B., and Georgiou, G. "Engineering Antibody Therapeutics which Neutralize Anthrax Toxin" 4th International Conference on Anthrax, American Society for Microbiology, Bethesda, MD (2001).
- q. Maynard, J., Leppla, S.H., Iverson, B., and Georgiou, G. "Engineering Therapeutic Antibodies" 2nd Cambridge Health Tech Conference on Recombinant Therapeutics (2001).
- r. Grayson, Scott M.; Schmid, Matthew J.; Meiring, Jason, E.; Desai, Vijay; U, Daniel, S.; Manthiram, Kalpana; Ellington, Andrew D.; Willson, C. Grant. "Self-assembled Shape-encoded Hydrogel Sensor Array." 17th Polymer Networks Conference, Bethesda, MD; August 15-19, 2004.
- s. Grayson, Scott M.; Schmid, Matthew J.; Meiring, Jason, E.; Desai, Vijay; U, Daniel, S.; Manthiram, Kalpana; Ellington, Andrew D.; Willson, C. Grant. "Self-assembled of Shape-encoded Hydrogel Sensors." AIChE 2004 Annual Meeting, Austin, TX; November 7-12, 2004.
- t. Matthew J. Schmid, Jason E. Meiring, Romy Kirby, Kalpana Manthiram, Scott Grayson, Andrew D. Ellington, and C. Grant Willson. "Functionalizing Hydrogel Based Biosensors with DNA Oligomers for Single Nucleotide Polymorphism Detection." ACS Fall 2003, New York, NY
- u. Jason E. Meiring, Matthew J. Schmid, Benjamin M. Rathsack, David M. Johnson, Romy Kirby, Ramakrishnan Kannappan, Kalpana Manthiram, Ryan J. Russel, Michael V. Pishko, Andrew D. Ellington, and C. Grant Willson. "Hydrogel Biosensor Arrays Indexed Through Shape Recognition." ACS Fall 2003, New York, NY
- v. Jason E. Meiring, Matthew J. Schmid, Scott M. Grayson, Romy Kirby, Kalpana Manthiram, Benjamin I. Hsia1, Andrew D Ellington, and C. Grant Willson. "Development of a shape-encoded self-assembled hydrogel biosensor array." ACS Spring 2004, Los Angeles, CA.

Category 2

Scientific Personnel Supported by this Project and honors/awards/degree received (during interim period):

Postdocotral Candidates:

Seth Cockrum
 Marc Rodriquez
 Litao Yang
 Wei Niu

Robert Mabry
Min Jeong Seo
Christian Cobaugh
Yoon Park
Y. W. Kim
David James Javier
Zack Hogan
James Willson
Jennifer Stots
Chris Pruet
Charles Sung
Ben Rathsack
Jason Meiring
Matthew Schmid
Dave Johnson
Ali Mehnaaz
Barrett Harvey

Masters Recipients

Heather Sims
Sarah Faulkner

Ph.D. Recipients

Jennifer A. Maynard, Ph.D. Chemical Engineering (2002). Current Position: Asst Professor Dept of Chemical Engineering, U. Minnesota
Barrett Harvey, Ph.D. Molecular Biology (2004). Current Position: Senior Scientist, Merck
Joseph Manimala, Ph.D. in Biochemistry (2004). Current Position: Scientist, NIH
Letha Sooter, Ph.D. Biochemistry (2004). Current Position: Postdoctoral Fellow, MIT
Young-Soo Sohn, Ph.D. completed in 2004
Byunghwa Park, Ph.D. completed in 2004
Scott Grayson, Ph.D. completed in 2004

Postdoctoral Fellows

Wujian Miao
Sanghamitra Mohanty
Zhenlin Zhong
Jeff Cook

Category 3

Report of Inventions:

The MURI grant co-sponsored the invention of several technologies that have been licensed to major pharmaceutical companies and inventions that have been licensed to major pharmaceutical companies, shown below in parenthesis

“Isolation of Binding Proteins with High Affinity to Ligands” Chen, G., Hayhurst, A., Thomas, J., Iverson, B.L. and G. Georgiou, U.S patent application #09/699,023. [E.L. Lilly, Bioren]

"Combinatorial Protein Library Screening by Anchored Periplasmic Expression," Harvey, B.R., Georgiou, G. and B. L. Iverson, U.S. patent application #60/396,058. [E.L. Lilly, Bioren]

"Recombinant Therapeutic Antibodies for the Treatment of Anthrax," Maynard, J.A., B.L. Iverson and G. Georgiou, U.S. patent application #10/288,269. [Elusys Therapeutics].

"High affinity Antibodies to the Anthrax Toxin Isolated by Anchored Periplasmic Expression," Harvey, B.R., Georgiou, G. and B. L. Iverson, U.S. patent application #60/396,058. [Elusys Therapeutics]

"An APEx 2-Hybrid System Technology for Protein:protein Interaction Discovery" Harvey, B.R., Iverson, B.L., Jung, K.J. and G. Georgiou US Application pending [E. L. Lilly]

"Methods for the Tethering of Proteins on the Periplasmic Membrane of *E.coli*" Harvey, B.R., Iverson, B.L., Jung, K.J. and G. Georgiou US Application pending [E.L.Lily]

"Development of spotted cell Microarray"

"A Computerized Pattern Recognition and Part Identification System." Meiring, Jason E.; Michaelson, Timothy B.; Willson, C. Grant *United States Patent* application, disclosure made to UT on Feb 24, 2003

"Sensory Array with multiplexed features for the detection of analytes." Matthew J. Schmid, C. Grant Willson *United States Patent* application, disclosure made to UT on April 25, 2003

"Method and System for the Analysis of Saliva Using a Sensor Array". US patent application PATENT #5119-00555, December 2003

"Portable Instrument for Microarray Analysis". UT disclosure, December 2003

"On-Chip Combination of Chemical and Cellular Panels for Analysis of Fluid Samples". UT disclosure, December 2003

"A Microchip-based system for HIV Diagnostic". UT disclosure, December 2003

"Customized testing ensembles for complex fluid analysis using portable integrated microfluidics/detecting units". UT disclosure, December 2003

U.S. Patent Application No. 09/661,658: "Allosterically Regulated Ribozymes", 2000. Inventors: Ellington, Andrew D., Robertson, Michael P., Thompson, Kristin, Hesselberth, Jay, Cox, J. Colin, Riedel, Tim. E., Davidson, Eric A., and Sooter, Letha. (Archemix)

U.S. Patent Application No. 09/666,870: "Method and Apparatus for Identifying Allosterically Regulated Ribozymes", 2000. Inventors: Ellington, Andrew D., Robertson, Michael P., Cox, J. Colin, Riedel, Timothy E., and Davidson, Eric A. (Archemix)

U.S. Patent Application No. 09/883,119: "Regulatable, Catalytically Active Nucleic Acids", 2002. Inventors: Ellington, Andrew, D., Hesselberth, Jay, Thompson, Kristin, Robertson, Michael P., Sooter, Letha, Davidson, Eric A., Cox, J. Colin, Riedel, Timothy E., Wilson, Charles, Cload, Sharon T., and Keefe, Anthony D. (Archemix)

PCT International Patent Application No. PCT/US01/19119: "Allosterically Regulated Ribozymes", 2001. Inventors: Ellington, Andrew D., Robertson, Michael P., Thompson, Kristin, Hesselberth, Jay, Cox, J. Colin, Riedel, Tim. E., Davidson, Eric A., and Sooter, Letha. (Archemix)

PCT International Patent Application No. PCT/US02/30548: "Regulatable, Catalytically Active Nucleic Acids", 2003. Inventors: Ellington, Andrew, D., Hesselberth, Jay, Thompson, Kristin, Robertson, Michael P., Sooter, Letha, Davidson, Eric A., Cox, J. Colin, Riedel, Timothy E., Wilson, Charles, Cload, Sharon T., and Keefe, Anthony D. (Archemix)

Australian Patent Application No. AU 6691701: "Allosterically Regulated Ribozymes", 2003. Inventors: Ellington, Andrew D., Robertson, Michael P., Thompson, Kristin, Hesselberth, Jay, Cox, J. Colin, Riedel, Tim. E., Davidson, Eric A., and Sooter, Letha. (Archemix)

Australian Patent Application No. AU 6848101: "Regulatable, Catalytically Active Nucleic Acids", 2002. Inventors: Ellington, Andrew, D., Hesselberth, Jay, Thompson, Kristin, Robertson, Michael P., Sooter, Letha, Davidson, Eric A., Cox, J. Colin, Riedel, Timothy E., Wilson, Charles, Cload, Sharon T., and Keefe, Anthony D. (Archemix)

Canadian Patent Application No. CA 2412664: "Regulatable, Catalytically Active Nucleic Acids", 2002. Inventors: Ellington, Andrew, D., Hesselberth, Jay, Thompson, Kristin, Robertson, Michael P., Sooter, Letha, Davidson, Eric A., Cox, J. Colin, Riedel, Timothy E., Wilson, Charles, Cload, Sharon T., and Keefe, Anthony D. (Archemix)

European Patent Application No. EP 1364009: "Regulatable, Catalytically Active Nucleic Acids", 2002. Inventors: Ellington, Andrew, D., Hesselberth, Jay, Thompson, Kristin, Robertson, Michael P., Sooter, Letha, Davidson, Eric A., Cox, J. Colin, Riedel, Timothy E., Wilson, Charles, Cload, Sharon T., and Keefe, Anthony D. (Archemix)

Japan Patent Application No. 2004515219: "Regulatable, Catalytically Active Nucleic Acids", 2002. Inventors: Ellington, Andrew, D., Hesselberth, Jay, Thompson, Kristin, Robertson, Michael P., Sooter, Letha, Davidson, Eric A., Cox, J. Colin, Riedel, Timothy E., Wilson, Charles, Cload, Sharon T., and Keefe, Anthony D. (Archemix)

RECEPTOR GROUP

Ellington

Statement of problem(s):

- (1) There is a general bottleneck in the development of biosensors for sensor platforms. We undertook to determine whether high-throughput, automated methods could be applied to the development of aptamer and other nucleic acid biosensors.
- (2) Biosensors typically require immobilization or other complex handling in order to signal the presence of threat agents. For example, PCR frequently requires the extraction of DNA or RNA, sandwich assays require wash steps, and so forth. We were interested in identifying reagents that would react in solution to directly yield signals, or that could be readily mounted on sensor platforms without the need for multiple processing steps.

Statement of solution:

- (1) Under the ARO-MURI, we acquired robotic workstations and developed specialized hardware, software, and protocols for the high-throughput selection and arraying of aptamers. We have also developed bioinformatics methods and tools that can be used to handle the large amounts of data that these selections generate. More detail is provided in points (1) – (4), below.
- (2) We furthered the development of allosteric nucleic acid enzymes: aptazymes. Aptazymes are unique reagents that can recognize small molecule or protein analytes and convert non-covalent recognition into a change in covalent bond state. These changes in covalent bond state can in turn be directly coupled to optical or other sensor readouts. During the execution of the ARO-MURI we developed a number of aptazymes and adapted them to a number of sensor platforms. More detail is provided in (5) – (7), below.

Summary of most important results (relevant publications cited):

- (1) We acquired a number of high end robotic workstations, and developed specialized hardware, software, and protocols for automated nucleic acid selections (Sooter et al., 2001; Cox et al., 2002). We can now carry out up to eight selections in parallel and complete six selection cycles in a single day. This is at least a hundred fold increase in throughput over manual methods. These technical innovations have already proven to be of military relevance, in that a biosensor we have selected against the protective antigen (PA) of *B. anthracis* is in field trials.
- (2) Adaptation of nucleic acid sensor elements to the electronic tongue (ET; Kirby et al., 2004). During the execution of the MURI proposal, we worked with John McDevitt and the electronic tongue group on adapting functional nucleic acid sensor elements (hybridization probes, aptamers, and aptazymes) to the electronic tongue (ET). The bead-based nucleic acid sensor elements proved were readily adapted to and functioned well in the context of the ET.
- (3) We also developed simple methods for printing nucleic acid chips that can be used with COTS technology that is typically applied to gene expression microarrays (Collett et al., 2005). Using these technologies, hundreds of aptamer spots can be printed and protein targets can be detected in the pg / ml range, which is well within the concentration range of many cellular proteins in a lysate.

- (4) We have generated software tools that can be used to archive and analyze the large amounts of data that are derived from selection experiments (Lee et al., 2004). In particular, we created and continue to maintain the Aptamer Database (www.aptamer.icmb.utexas.edu), the only complete compendium of aptamer sequences. This was especially important, as aptamers are not catalogued in GenBank. We have also developed tools to identify short and compare short motifs in natural and unnatural sequence libraries.
- (5) Optimization of an aptazyme ligase. We had previously selected an aptazyme ligase (L1) from a random sequence population. During the course of the award, we carried out additional selections to optimize the speed of this ligase, reduce its size, and better understand critical sequences, structures, and reaction mechanisms.
- (6) Adaptation of the aptazyme ligase to novel analytes. The initial development efforts with the L1 ligase were against small analytes, such as ATP and theophylline, and involved appending aptamer domains to the ligase, leading to ligand-gated conformational changes. However, when the same approaches were tried with protein analytes there was little success. We reasoned that binding of larger protein analytes led to steric hindrance of ligand-gated conformational changes. We therefore randomized the catalytic core and allosteric binding site of the L1 ligase, and selected for aptazymes that were dependent upon protein targets. We obtained ligases that were up to 100,000-fold activated in the presence of their cognate protein analytes, and that could discriminate against non-cognate analytes based on both sequence and tertiary structures. These protein-dependent aptazymes were new and unique molecular reagents that continue to be studied and used throughout the sensor community. Aptazymes were also adapted to function in cells (Thompson et al., 2002).
- (7) Generation of an aptazyme ligase array. In order to show how aptazyme reagents could be used for array analyses, we were able to react and immobilize aptazymes in parallel, such that small organic, oligonucleotide, peptide, and protein analytes were identified in parallel. This was the first (and to our knowledge remains one of the only) examples of how a single type of molecule could be used for the recognition of a widely divergent set of analytes (Hesselberth et al., 2003).
- (8) Generation of novel enzyme reporters. In order to enhance the signals that could be garnered from aptazyme and other biosensors, we wished to generate robust enzyme reporters that would operate in a variety of environments. To this end, we used directed evolution and shuffling methods (Faulkner et al., 2004) to generate variants of the well-known reporter enzyme beta-glucuronidase that were resistant to low pH, to modification by aldehydes, and to thermal denaturation (Flores and Ellington, 2002). We have subsequently recombined these various mutations to make GUS variants that are extremely robust to a wide variety of conditions.
- (9) Establishment of a biosensor community at the University of Texas at Austin. One of the goals of the ARO-MURI from its inception was to strengthen interactions between a wide array of researchers on campus. As a result, the Ellington lab continues to collaborate with the Shear lab, the Willson lab, the Georgiou lab, the Anslyn lab, and the McDevitt lab. In particular, Shear and Ellington have recently developed technologies for the photopolymerization of protein architectures that may be useful for probing neural networks, and (in collaboration with David Shine at Baylor) have pitched these results to DARPA for spinal cord repair and regeneration. Similarly, the Ellington lab continues to work with the Willson lab on the seminal development of MUFFINS technologies for biosensing and multiplexing. Neither of these interactions would have come about without the MURI award.
- (10) Engendering corporate transitions. The biosensor work carried out under the ARO-MURI has transitioned to the private sector. In particular, automated selection experiments are carried out by Archemix (Boston, MA), a company started by Dr. Ellington for aptamer therapeutics. Dr. Ellington

currently works with Echo Technical, an Austin-based company, on the development of a miniaturized biosensor suite (the BRIEFCASE) that can be used for assaying biothreat agents in the field. Finally, based on experiences that were garnered through the ARO-MURI and interactions with military personnel and agencies, Dr. Ellington continues to perform research of relevance to the DoD through SBIR and STTR grants with a variety of companies. Essentially, the high-throughput methods touted in (1), above, have now allowed the Ellington lab itself to act as a high-throughput supplier of aptamer biosensors to the private sector.

Georgiou / Iverson

Statement of problem(s):

Our work has focused on the development of high throughput technologies for the generation of ultra-high affinity antibodies for diagnostic and therapeutic applications. Specific emphasis was placed on the development of antibodies for the detection of the protective antigen protein (PA) of *Bacillus anthracis*.

Summary of most important results:

- Invented a pair high throughput screening methodologies (Periplasmic Expression and Cytometric Screening (**PECS**) and Anchored Periplasmic Display (**APEX**)) for the efficient isolation of very high affinity antibodies from large, combinatorial libraries expressed in *E.coli* bacteria
- Isolated antibody fragments that neutralize the Protective Antigen (PA) component of the *Bacillus anthracis* exotoxin with picomolar affinity
- Demonstrated that the high affinity anti-PA antibodies confer protection against challenge with holotoxin in the rat model
- Showed that antibody fragments conjugated to polyethylene glycol (PEG) for extending their half life in serum confer protection towards very heavy challenge with inhalation anthrax. The antibody fragments were able to confer protection even though they lacked an Fc region and therefore were unable to recruit immune cells or complement. These results demonstrated that neutralization of a key virulence factor, in this case the PA protein is sufficient to mediate clearance of pathogenic bacteria.
- Developed methods for the efficient production of antibody fragments in bacteria engineered to have a cytoplasm that allows the formation of disulfide bonds, thus allowing oxidative protein folding to take place.
- Jointly with the Ellington lab we developed methods for the parallelization of antibody and aptamer isolation.

Anslyn

For the analysis of individual analytes in a complex mixture, the use of a unique, highly selective receptors is typically required for each analyte to be detected. An alternative approach is to use devices that rely on a series of chemosensors where analysis of complex mixtures arises from patterns produced by the combined response of all the chemosensors in the array. This approach has been particularly successful for vapor phase analysis.

Patterns can also be created that are diagnostic for single analytes. We previously postulated that combinatorial receptors biased towards a class of analytes would be effective in an array setting for the analysis

of structurally similar analytes in solution. Using in part funding from MURI, we demonstrated the first instance of solution-based sensing utilizing a combinatorial library of receptors that can differentiate between nucleotide phosphates.

The receptors consist of a rationally designed core with a binding cleft possessing guanidinium groups. These guanidinium groups impart an affinity for nucleotide triphosphates, and are appended with tripeptides to incur differential binding properties. A previously reported screening of this same 4913 member library led to the identification of individual solution phase sensors that are highly selective for adenosine 5'-triphosphate (ATP) over guanosine 5'-triphosphate (GTP). The objective of the year of funding was to determine whether the patterns generated by a library of receptors in an array can discriminate between structurally similar compounds like ATP and GTP.

Thirty beads from the library were randomly chosen, given an index number, and placed in a micromachined chip-based array platform that has been previously reported. The sample is introduced over the array and passes around and through the beads to exit the bottom of the wells. Red, green, and blue (RGB) transmitted light intensity values were recorded for each bead in the array over the period of the assay via a charge-coupled device (CCD).

The signaling protocol used with the array platform was an indicator-displacement assay similar to those exploited in many of our single analyte sensing schemes. To impart color to the library members an anionic chromophore, fluorescein, was introduced into the array containing different members from the combinatorial

library of receptors. The cationic receptors associate with the indicator, bringing about a distinct orange color to each bead. Blank beads show no orange color indicating little indicator uptake. Upon exposure to solutions of nucleotide phosphates, the analyte displaces the indicator, fluorescein, at different rates and each bead loses color.

The RGB intensity values for the thirty library beads in the array are recorded over time after a 2 mL injection of a 20 mM sample of ATP, GTP, or adenosine 5'-monophosphate (AMP) in 25 mM HEPES buffer (pH 7.5). Three trials were performed for ATP, GTP, and AMP for a total of nine trials, and absorbance values were calculated by taking the negative log of the ratio of the blue channel intensity over the red channel intensity for each bead. Figure 1A shows a representative normalized absorbance trace for two of the beads in the array after an injection of GTP. Figure 1B shows a trace of the same two beads after an injection of AMP.

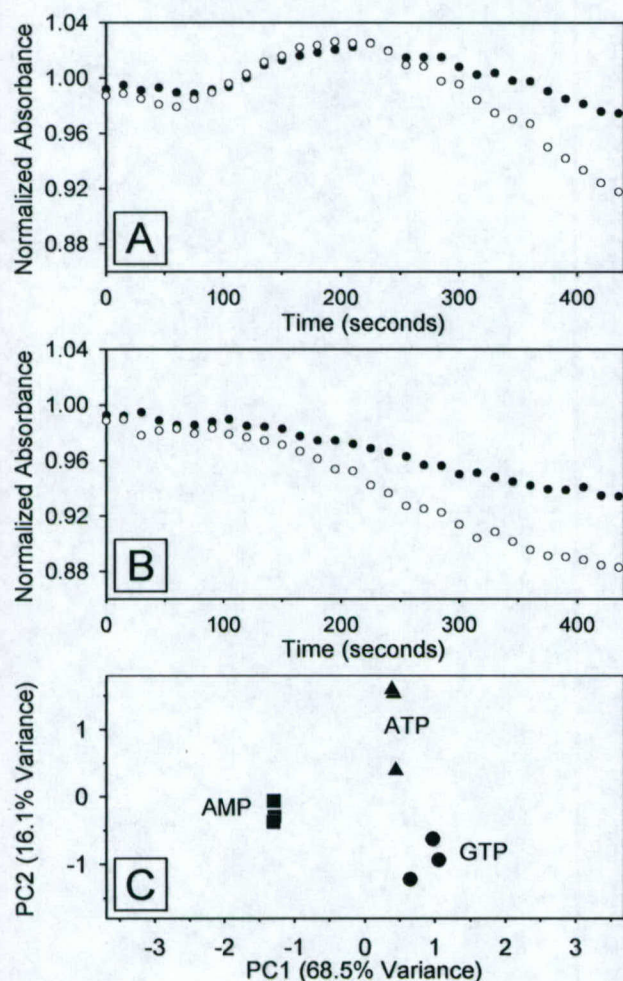


Figure 1. Absorbance values over time and principal component plot. To aid slope comparison, the plots were shifted so that the initial 30 data points prior to sample injection (not shown) had an absorbance of 1.00. (A) 20 mM GTP sample in 25 mM HEPES buffered aqueous solution (pH 7.5) while eluting with 25 mM HEPES buffer (pH 7.5) Bead 23 and Bead 28. (B) 20 mM AMP sample in 25 mM HEPES buffered aqueous solution (pH 7.5) while eluting with 25 mM HEPES buffer (pH 7.5) Bead 23 and Bead 28. (C) PCA score plot of the three trials for each

The slope of the absorbance values from 210-435 seconds was calculated for each bead in each sample and used for comparison. Figure(s) 1A and 1B reveal that each chemosensor responds differently to various nucleotide phosphate samples. The slope of bead 28 differs by 40% between the AMP and GTP trials. Whereas, the slope value for bead 23 in each plot differs by only 26%. Although, these slopes are easily differentiated by qualitative visual inspection, the rates of displacement for several trials can be compared more quantitatively using pattern recognition algorithms.

Principal component analysis⁸ (PCA) was utilized to reduce the dimensionality of the data set. For each of the nine trials, a slope value was recorded for each of the thirty beads. To identify patterns in the data, a principal component (PC) axis is calculated to lie along the line of maximum variance in the original data. Subsequent PC axes lie along lines describing diminishing levels of variance. The coordinates of the sample relative to the PC axes are termed scores and can be used as an indicator of correlation between analytes. Proximity in space on a score plot directly correlates to similarities in indicator displacement rates. Figure 1C shows a two dimensional score plot for the first two principal components (PC1 and PC2) with clustering of the AMP, GTP and ATP samples with one possible ATP outlier. PCA demonstrates that the array of library sensors can differentiate between structurally similar molecules such as AMP, GTP and ATP.

Factor loading values can be used to evaluate the individual sensors in the array. The values correspond to the cosine of the angle between a principal component axis and the original variable axis. Therefore, similar loading values correspond to similar sensor responses. Furthermore, loading values approaching 1 or -1

indicate that the individual sensor played a significant role in the formation of a particular component axis. Nine beads were chosen to be sequenced based upon their factor loading values and the sequencing results are shown in Table 1. Although some beads have nearly identical factor loading values, the sequences do not reveal any particular homology. Since 86% of the beads in the array had a factor loading value with an absolute magnitude greater than 0.7 on PC1, it is thought that the combination of these differential receptors is necessary for analyte discrimination.

Tripeptide Sequence	Factor Loading (PC1)	Factor Loading (PC2)	Bead Index Number
Asp-Asn-Ser	-0.98	0.14	28
Phe-Trp-Phe	-0.88	-0.16	23
Thr-Thr-Ser	-0.88	0.39	33
Val-Asn-Tyr	-0.84	-0.20	24
Val-Pro-Ala	0.01	0.21	14
Ala-Met-Thr	0.17	-0.91	34
Val-Gly-Ile	0.74	-0.46	6
Ser-His-Tyr	0.75	-0.47	25
Thr-Thr-Ile	0.89	-0.36	2

Table 1. Sequencing results and factor loading values for the first two principal components (PC1 and PC2)

In conclusion, we found that the use of a combinatorial library of sensors and indicator-displacement assays in the context of a chip-based array allows for the differentiation of structurally similar analytes in aqueous solutions. The coupling of supramolecular chemistry principles with pattern recognition lead to this new protocol for sensing.

PLATFORM GROUP

McDevitt

- Adapted the 'electronic tongue' to antibody biosensors
- Developed methods to allow the 'electronic tongue' to function as an enhanced DNA chip
- Developed sensor array for the detection of aflatoxins
- Adapted taste chip technology for rapid screening and detection of bacillus spores and bacteria (military, homeland defense)- Testing of the technology for possible use in US Postal System (homeland defense)
- The taste chip system has been interfaced, tested, automated (with software control of detection and fluidics system) with bioaerosol collection equipment (military, homeland defense)
- Development and testing of the following types of instruments: a) hand-held taste chip reader (pH, electrolyte detection), b) shoe box taste chip reader (protein, antibody detection), c) smart trigger for bioaerosol collection and detection units (bacillus spore, bacteria detection).
- Developed CD4-lymphocyte (T-helper) cell counting prototype for monitoring progress of HIV infection in resource poor settings

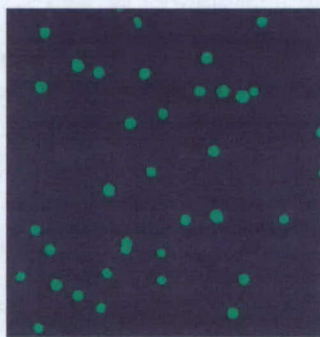
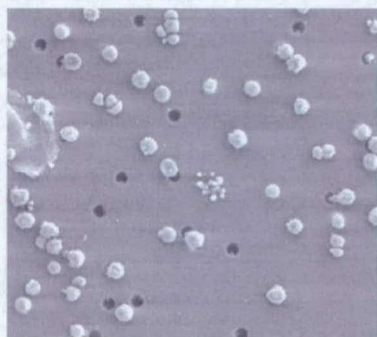
Overview: Recent work from the McDevitt laboratory has led to the development of a powerful new "electronic taste chip" technology. By mimicking the chemical features of the human taste bud, the chip has the capacity to analyze rapidly the chemical and biochemical content of complex fluids such as human blood and saliva, environmental samples, and bioaerosol specimens. This technology is extremely versatile, making it suitable for the measurement of electrolytes, protein antigens, antibodies, whole bacteria and DNA/RNA. While these chips exhibit impressive analytical and diagnostic capabilities as compared with gold standards (such as pH meters for acidity, ELISA for protein analysis, FDA approved automated instruments for cardiac risk factors and planar DNA chips for nucleotide detection), their compact design and low cost also allows for their use in numerous civilian and military applications which require autonomous operation. Moreover, because molecular detection is confined to a miniaturized chamber etched into a silicon chip, multiple tests can be performed simultaneously. Testing requires a single drop of fluid and disposable cartridges, customized for specific applications can be created using highly parallel chip fabrication and solid-state bead synthetic procedures. This electronic taste chip technology can be used to identify and quantify analytes in the solution-phase via colorimetric and fluorescence changes to receptor and indicator molecules that are covalently attached to the polymer microspheres. Sensor arrays are created by placing individual microspheres into micromachined cavities in small silicon chips. The optical response of each microsphere is monitored in real-time using a charged coupled device (CCD), allowing for near-real-time analysis of complex fluids. Most recently, microbead arrays have been fashioned specifically for the detection of chemical weapons precursors and degradation products as well as for the identification of bacterial spores from the bacillus family.

The McDevitt group has focused on the design and optimization of the instrumentation that services the taste chip platform. High quality designs for flow cells have been developed and tested in a variety of real-world application areas (human medicine, environmental samples, bioaerosols). The development of highly effective algorithms that can be used to translate the array color information to analyte identity and concentration has been completed. Furthermore, in collaboration with the Ellington group, work has now been completed that allows for the efficient adaptation of the taste chip approach for the rapid and direct detection of DNA oligonucleotides. This work documents a number of significant advances in the following areas: (1) An elegant approach has been described which affords the simple incorporation of DNA capture probes into porous microbeads. (2) This biotin-avidin docking methodology allows for use of much longer capture probes than is possible with current generation gene chips. (3) Unusually high sequence specificity is afforded by the unique microenvironment within the porous beads. It is likely that the bead arrays will show greater discrimination

against non-cognate sequences than conventional DNA chips. (4) Since each bead serves as its own micro-reactor, multiplexed assays can be readily accommodated using the structure. (5) The inclusion of micro-fluidic channels and micro-drains at the bottom of the wells that hold the beads facilitates the rapid delivery of analytes and thus the rapid completion of assays. (6) The 3-dimensionality of the bead along with a chromatography-like sequestration capability greatly increases the signaling capacity for the bead systems relative to flat chip arrays. The large signaling capacity may in turn afford the capacity for rapid DNA analyses to be completed using simple hand-held reader units.

Sensor Array Research Highlights:

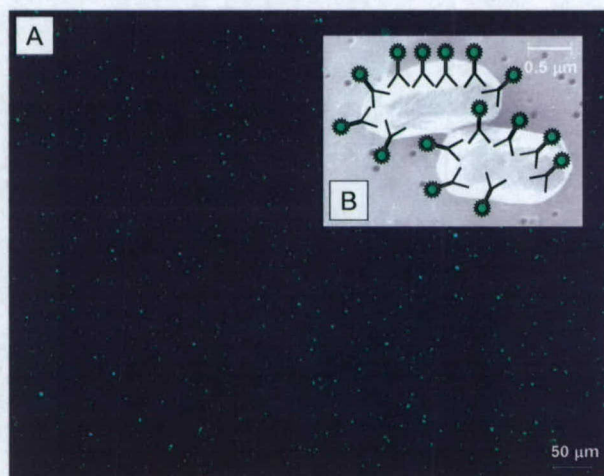
1) HIV Microchip Program for Resource Poor Settings: This program combined the expertise of three groups in an effort to accelerate the development, testing and deployment of a powerful chip-based technology suitable for the early detection and monitoring of important emerging infectious diseases. Here, Dr. Bruce Walker, who serves as the Director of the Division of AIDS for Harvard Medical School along with his collaborator, Dr. William Rodriguez, the Director of International Programs for the Division of AIDS and a specialist in infectious diseases, provided the medical expertise for the effort. At the University of Texas at Austin, the laboratories of Dr. John McDevitt and Dr. Dean Neikirk have become recognized leaders in the field of lab-on-a-chip devices, and have begun development of microchip devices that can diagnose illnesses ranging



from cardiac diseases to anthrax to HIV. This joint UT-Harvard-MGH program targets the rapid development and deployment of the microchip-based technology in point-of-care diagnostics for the entire range of emerging infectious diseases. Key to the advances in this area are efforts which serve to miniaturize diagnostic systems whereby new nano-materials and new nano-device concepts are combined so as to provide complete diagnostic systems that

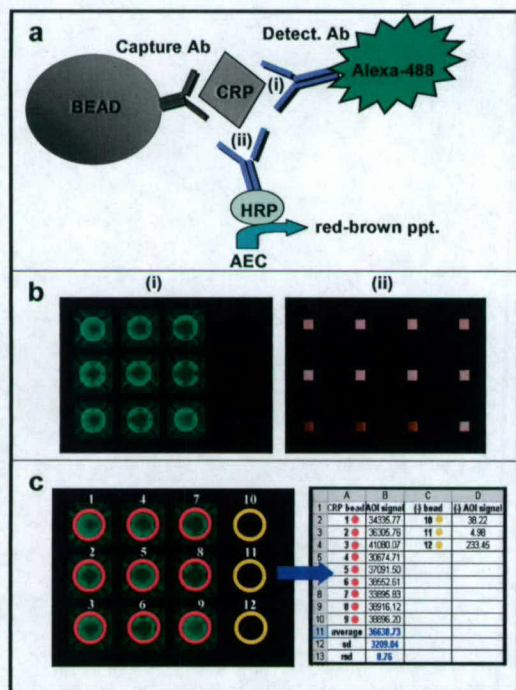
can operate at the point-of-care with reduced cost.

2) Systems for Sampling and Detection of Bio-aerosols: In October 2001 anthrax spores from two contaminated letters were released into the occupied environment of the U.S. Postal Service Brentwood facility in Washington, DC. Two postal workers died from exposure to the spores and 20 others became ill. The Brentwood facility has been closed since that time. This incident reveals the vulnerability of the mail system to



pathogenic bioaerosols; however, it suggests that if mail machines were equipped with detectors, not only would contamination of the postal workers and facilities be precluded, but also potential recipients of contaminated mail would be protected. Recent work from the McDevitt group at The University of Texas at Austin as well as the McFarland group at Texas A&M University has led to the development of a powerful bio-aerosol collection and detection system that is suitable for the rapid and sensitive detection of anthrax spores in postal settings. The relevant apparatus has been designed for the collection of aerosols from a critical region of the machines, and that aerosol is transported to an aerosol-to-hydrosol transfer stage, where the particulate matter is deposited into a small flow rate of

liquid (about 15 drops/min). The hydrosol then flows onto a detector system developed at UT wherein a filter collects particles, exposes them to a “visualization cocktail” and then automatically detects the bio-signatures therein via optical methods. Blind tests have been completed demonstrating the efficacy of the new system using a non-hazardous stimulant for anthrax spores, bacillus subtilus. Response times below 15 minutes and spore detection thresholds less than 1000 CFU are obtained.



3) Novel Microchip-based Multi-Analyte Assay System for Assessment of Cardiac Risk: The development of a novel chip-based multi-analyte detection system with a cardiac theme is reported. This work follows the initial reports of “electronic taste chips” whereby multiple solution phase analytes such as acids, bases, metal cations and biological cofactors were detected and quantitated. The newly fashioned “cardiac chip” exploits a geometry that allows for isolation and entrapment of single polymeric spheres in micromachined pits while providing to each bead the rapid introduction of a series of reagents / washes through microfluidic structures. The combination of these miniaturized components fosters the completion of complex assays with short analysis times using small sample volumes. Optical signals derived from single beads are used to complete immunological tests that yield outstanding assay characteristics. The power and utility of this new methodology is demonstrated here for the simultaneous detection of the cardiac risk factors, C-reactive protein and interleukin-6, in human serum samples. This demonstration represents the first important step toward the development of a useful cardiac chip that targets numerous risk factors concurrently

and one that can be customized readily for specific clinical settings.

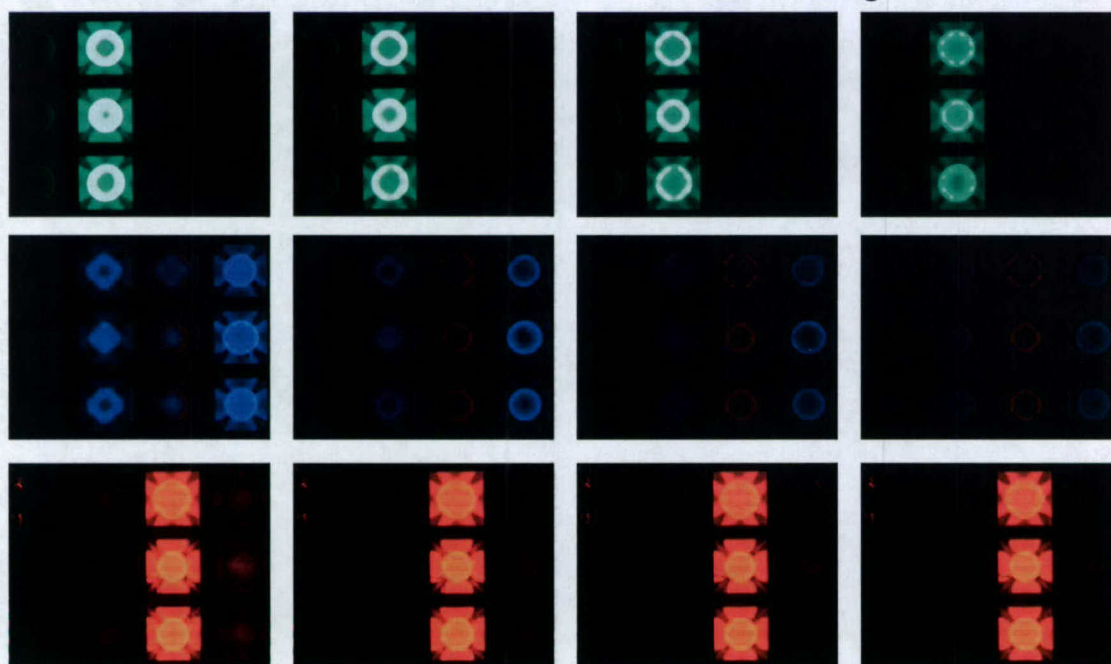
4) Development of Multi-analyte Sensor Arrays Composed of Chemically Derivatized Polymeric Microspheres Localized in Micromachined Cavities: The development of a chip-based sensor array composed of individually addressable polystyrene-polyethylene glycol and agarose microspheres has been demonstrated. The microspheres are selectively arranged in micromachined cavities localized on silicon wafers. These cavities are created with an anisotropic etch and serve as miniaturized reaction vessels and analysis chambers. A single drop of fluid provides sufficient analysis media to complete ~100 assays in these microetch pits. The cavities possess pyramidal pit shapes with trans-wafer openings that allows for both fluid flow through the microreactors/analysis chambers as well optical access to the chemically sensitive microspheres. Identification and quantitation of analytes occurs via colorimetric and fluorescence changes to receptor and indicator molecules that are covalently attached to termination sites on the polymeric microspheres. Spectral data is extracted from the array efficiently using a charge-coupled device (CCD) allowing for the near-real-time digital analysis of complex fluids. The power and utility of this new microbead array detection methodology is demonstrated here for the analysis of complex fluids containing a variety of important classes of analytes including acids, bases, metal cations, sugars and antibody reagents.

5) Multi-shell Microspheres with Integrated Chromatographic and Detection Layers for Use in Array Sensors: The initial demonstration of an array-based sensor composed of integrated chromatographic and detection elements has been completed. The new concepts described here allow for the batch production of multi-component sensing ensembles whose chromatographic and detection functions can be chemically tailored to a given application by exchanging either of the integrated elements. Moreover, this work establishes a

method for the facile generation of a collection of low-selectivity sensors based on a single detection scheme. Such complementary, overlapping elements are the building blocks of cross-reactive sensor arrays. The extension of this concept to include various other N, S, O, P-based ligands, chelators, crowns and cryptands should further enhance the scope and discriminatory capabilities of the outer shells, allowing for the creation of additional interesting "coordination chemistry" theme chips. Furthermore, with the appropriate components, this integrated technique should be readily applicable to other classes of analytes. Future efforts may exploit analogous components drawn from various branches of chromatography including molecular exclusion, ion exchange, and affinity chromatography. Toxic metal cations such as those derived from lead, mercury and cadmium as well as various radioactive lanthanides can be detected readily with this approach.

6) DNA Hybridization and Discrimination of Single-Nucleotide Mismatches Using Chip-Based

Microbead Arrays: The development of a chip-based sensor array composed of individually addressable agarose microbeads has been demonstrated for the rapid detection of DNA oligonucleotides. Here, a "plug and play" approach allows for the simple incorporation of various biotinylated DNA capture probes into the bead-microreactors which are derivatized in each case with avidin docking sites. The DNA-capture probe containing



microbeads are selectively arranged in micromachined cavities localized on silicon wafers. The micro-cavities possess trans-wafer openings, which allow for both fluid flow through the

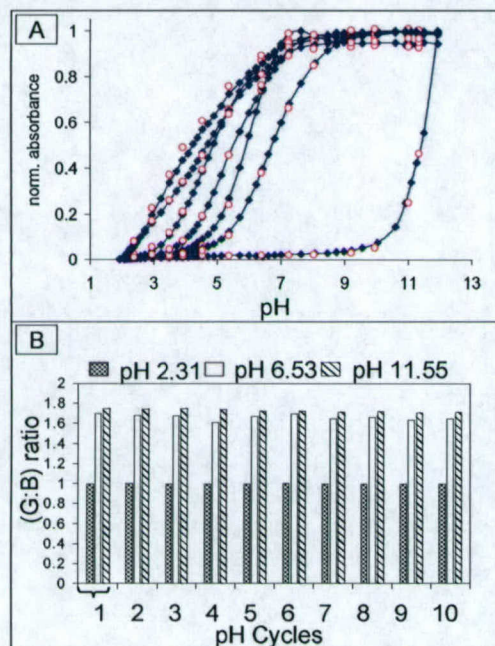
microreactors/analysis chambers as well as optical access to the chemically sensitive microbeads. Collectively, these features allow for the identification and quantitation of target DNA analytes to occur in near-real-time using fluorescence changes that accompany the target sample. The unique 3-dimensional microenvironment within the agarose bead as well as the microfluidics capabilities of the chip structure afford a fully integrated package that fosters rapid analyses of solutions containing complex mixtures of DNA oligomers. These analyses can be completed at room temperature through the use of appropriate hybridization buffers. For applications requiring analysis of $\leq 10^2$ DNA sequences, the hybridization times and point mutation selectivity factors exhibited by this lab-on-a-chip bead-array method exceed the operational characteristics in many respects for the commonly utilized planar DNA chip technologies. The utility of this DNA detection microbead array methodology was demonstrated for the analysis of fluids containing a variety of similar 18-base oligonucleotides. Hybridization times on the order of minutes with point mutation selectivity factors greater than 3,800 and limit of detection values of $\sim 10^{-14}$ M are obtained readily with this microbead array system. This work was recently published in the journal of Analytical Chemistry, acknowledging support from the MURI program.

7) pH Chip: One of the initial goals of this program has been development and the refinement of the Total Analysis System for use as a reliable and powerful analytical instrument. Likewise, before attempting complex multi-analyte detection, it was essential to fully characterize the sensor in a conceptually simple single analyte environment. With this in mind, a series of pH indicating dyes were immobilized onto the beads with the assistance of the Anslyn group. In order to ensure a broad analytical range, the dye selection was made so that a large range of pKa values were covered. Although numerous dye candidates were studied in this context, a total

of six strategic dyes were selected for more careful analysis in the context of a customized chip that was optimized for pH detection. These six dyes, along with appropriate blank beads, were used to explore the accuracy and reproducibility of the array for measurement of this standard analyte.

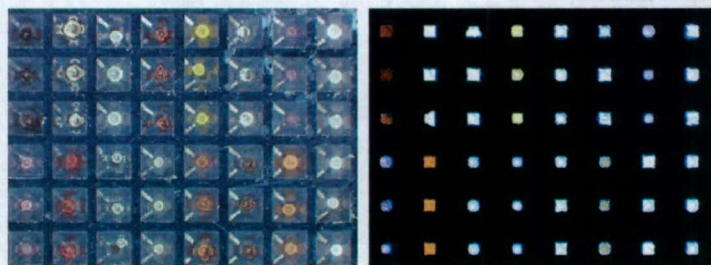
Careful analysis of the optical response curves reveals that pH changes as low as 0.01 can be sensed by the array. Furthermore, while many of the beads lead to accurate measurement over a range of 2 to 3 pH units, the combination of the six beads affords an impressive dynamic range that spans 10 decades of concentration. Beads selected from the same batch of indicator dyes yield very similar results. Collectively, these results suggest that the sensor array technology can yield impressive analytical data comparable to that achieved by commercially available pH meters.

With the new instrumentation, reproducibility, reversibility, concentration thresholds and response times have by now been measured for both static and flow-based experiments. Analysis of the

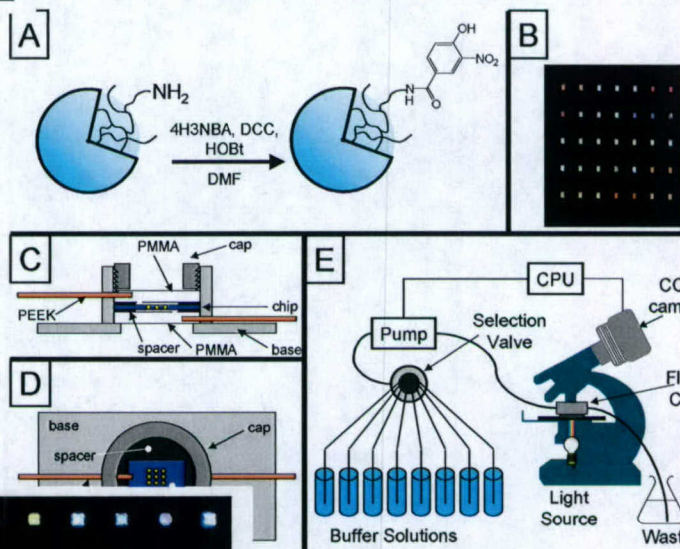


properties yields a bead to only ~2%. These results suggest that the initial few selected beads can be expected optical signatures prepared from the same

8) Assays Developed for electrolyte determination: The table



progress for selected cases.



individual beads optical response bead variance of reproducibility training set of a used to define the recorded for beads batch.

electrolyte below summarizes the bead types that are included within 42-element

array used for electrolyte determination. All of the assays described (except for the FRET systems) were completed in water fluids. Studies designed to explore any interference effects and detection thresholds were also performed. Sample limitations, usage limitations and quantitative factors are now in

A 6 by 7 array is shown here using top illumination before wetting and the same system with transmitted light after wetting. The 42-element array is composed of the indicator derivatized beads as summarized in Table 1. Table 1 also lists the bead locations and known analytes.

Assays in Development by McDevitt group
August 17, 2000

Table 1. Summary of bead types that are included within 42-element array used for electrolyte determination.

Indicator Molecule(s)	Bead Matrix	Array Position (row, column)	Detection Method(s)	Analyte Class(es)	Known Analytes	pH Range
g-Cresolphthalein complexone	PS-PEG	(1,6)(3,6)	Abs.	pH, metal cations	H ⁺ , Ba ²⁺ , Ca ²⁺ , Mg ²⁺ , Mo ⁶⁺	~10-12
Xylenol Orange	PS-PEG	(4,2)(6,2)	Abs.	pH, metal cations	H ⁺ , Al ³⁺ , Hg ²⁺ , La ³⁺ , Ni ²⁺ , Pb ²⁺ , Y ³⁺ , (~35 total)	~6-8
Methyl Thymol Blue	PS-PEG	(4,4)(6,4)	Abs.	pH, metal cations	H ⁺ , Al ³⁺ , Ca ²⁺ , Cu ²⁺ , Fe ²⁺ , Mn ²⁺ , Pb ²⁺ , Y ³⁺ (~25 total)	~9-11
Thymolphthalexon	PS-PEG	(4,3)(6,3)	Abs.	pH, metal cations	H ⁺ , Ba ²⁺ , Ca ²⁺ , Mg ²⁺ , Sm ³⁺ , Yb ³⁺	~8-10
Chrome Azurol S	PS-PEG	(1,7)(3,7)	Abs.	pH, metal cations	H ⁺ , Al ³⁺ , Cu ²⁺ , Fe ²⁺ , Mg ²⁺ (~20 total)	~11
4-hydroxy-3-nitro-phenylacetic acid	PS-PEG	(1,5)(3,5)	Abs.	pH	H ⁺	~5-8
Alizarin complexone	PS-PEG	(1,4)(3,4)	Abs.	pH, metal cations	H ⁺ , La ³⁺ , Ce ³⁺ , Zr ⁴⁺	~2-12
Ferrocene carboxylic acid	PS-PEG	(4,6)(6,6)	Abs.	Redox	H ⁺ , Ascorbate, TCNQ	
Eosin B	PS-PEG	(4,5)(6,5)	Abs.	pH	H ⁺	~2-6
Zincon	PS-PEG	(4,1)(6,1)	Abs.	pH, metal cations	H ⁺ , Cu ²⁺ , Ga ³⁺ , Ni ²⁺ , Zn ²⁺	~8
Fluorescein	PS-PEG	(4,7)(6,7)	Abs., Fluor.	pH, solvent	H ⁺ , protic, aprotic solvents	~5-7
Quinoxaline	PS-PEG	(1,1)(3,1)	Abs.	Anions	F ⁻ , Cl ⁻ , Br ⁻	
Coumarin/Fluorescein	PS-PEG	(1,3)(3,3)	FRET	Solvents	EtOH, MeOH, CH ₃ CN, CH ₂ Cl ₂ , DMF, Toluene	
Protein Combinatorial Library	PS-PEG	(1,2)(3,2)	Abs., Fluor.	Anions	ATP, GTP, AMP	

9) Development of Pattern Recognition Methods: As described above, The McDevitt group has developed a number of theme chips for customized applications. The results in all of these areas were quite promising. Likewise, by incorporating automated optical and mathematical analysis algorithms into our procedures, we have increased the utility of our system. Efforts were also devoted in this task to compare a number of popular algorithms. Attempts were made to select methods that worked well with our micro-bead array, as well as with the membrane-based approach. Methods were selected based on their capacity to complete analyses in a rapid and efficient manner while exhibiting the most accurate characteristics.

10) Analytical Models: For this task, efforts were devoted towards the development of a basic understanding of the factors that dictate the response characteristics of the individual beads. Here attempts were made to develop an analytical model that would allow for a fitting of the experimental curves to theoretical ones based on the interplay of variables such as flow rate, diffusion constants, analyte concentration, equilibrium binding constants, extinction coefficients and path lengths. This analytical model allowed for the more accurate interpretation of data as well as helped in the design of more effective bead-based analysis systems.

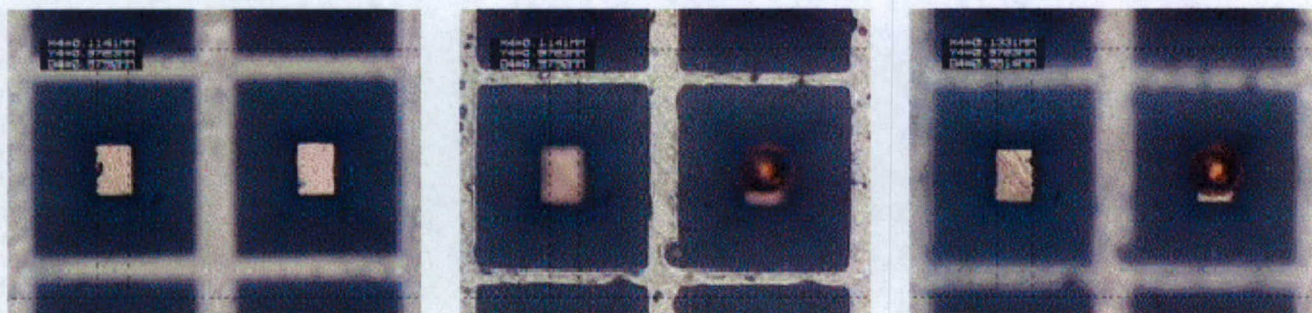
Neikirk

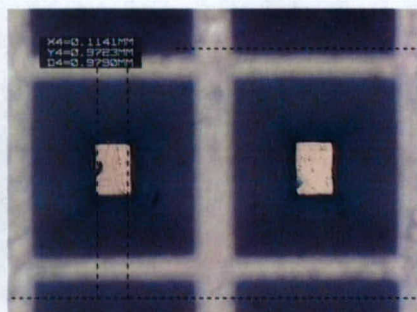
Major technical accomplishments in the last year:

- Development of a molded passive fluid introduction system driven only by capillary force.
- Demonstration of improved size-selective assembly method for microbead arrays.
- Development of a bi-layer check-valve suitable for use with in bead arrays.
- Development of a process for integrating electrodes into bead storage wells for use in localized array-based electrochemistry.

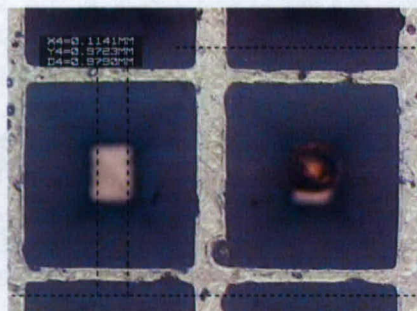
Our primary work under this program was the development of various micromachined platforms to support bead-based detection. We have fabricated two different sized "micro-well" arrays suitable for both large (approximately 200 micron) and small (approximately 50 micron) diameter beads. Under partial support from this grant (co-supported by the NIH) we have completed fabrication and testing of a surface tension driven sample introduction system. This structure allows the introduction of a sample in an aqueous solution without requiring any external active pump.

We completed work on the development of an injection molding processes to produce bead storage wells. For this process a silicon "master" is first made, which is then used to produce a mold for the production of final chips. Shown in Figure 1 are several photomicrographs showing molded chip, both empty and loaded with a bead. Surface finish and dimensional control are not as good as with a directly micromachined silicon chip, but should be adequate for bead confinement. The obvious advantage of such molded chips would be the extremely low cost fabrication.

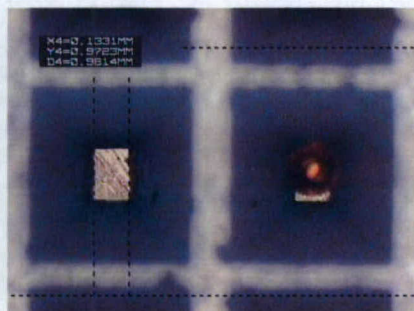




(a)



(b)

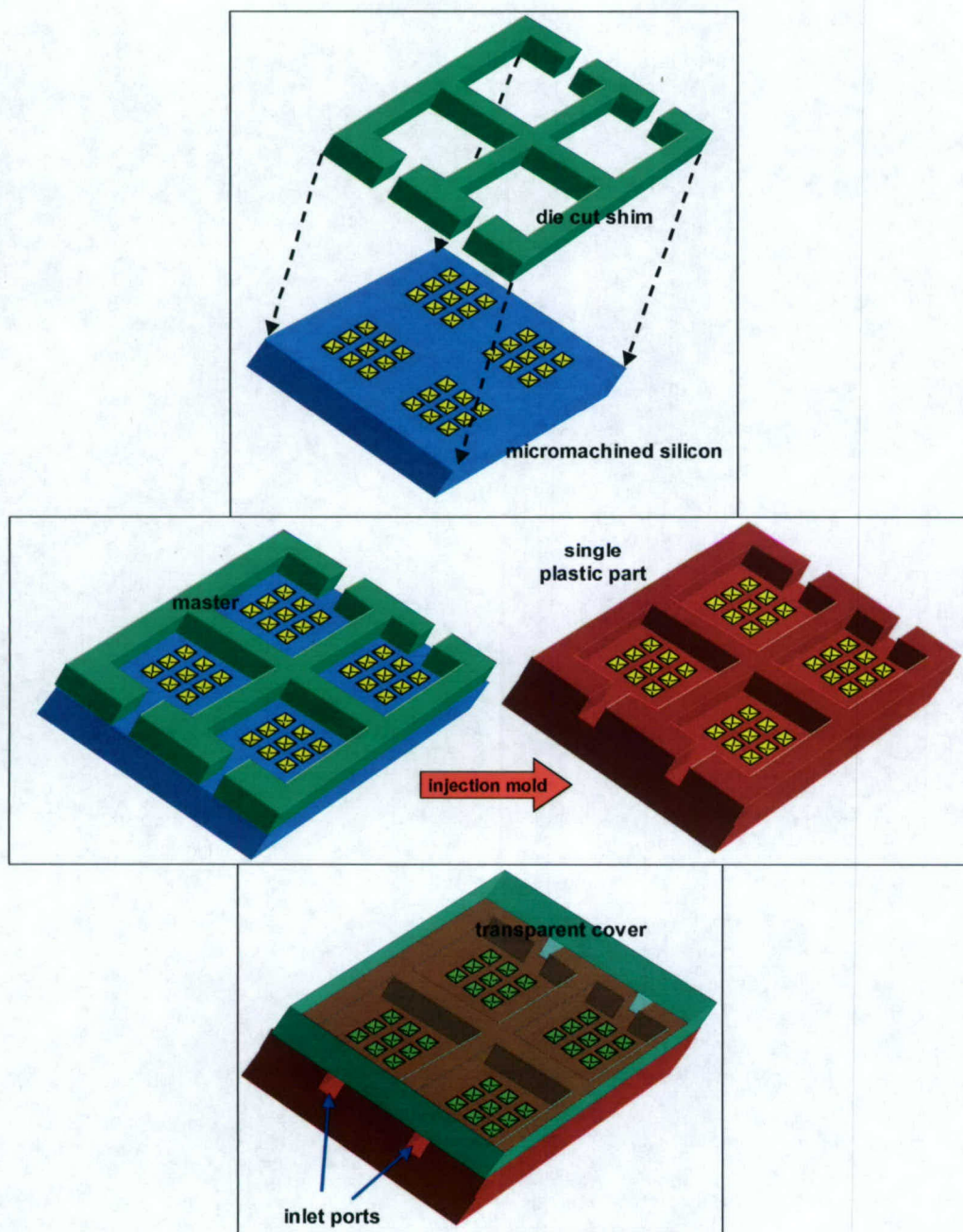


(c)

Figure 1: Photomicrographs of molded chips for bead confinement; (a) before bead insertion; (b) and (c): after bead insertion; (b) and (c) are identical, with a change in focal plane to show both the top of the chip (b), and the bottom (c).

Although this process cannot produce integrated cover fingers, it can be used to fabricate some structures that are difficult to make using only silicon processing. One example would make use of molding to fabricate “multi-sector” chips with integrated fluid flow paths, as illustrated in figure 2. In some cases there may be a need for multi-analyte sensing in which the reagents required for each analyte (or set of analytes) are mutually incompatible. In such a case, one of the easiest solutions is to fabricate a chip that has separate analyses chambers, isolated from one another. It can be difficult to directly fabricate leak-tight “walls” between the sectors of the chip using only silicon micromachining. However, a master can be made easily by bonding two separate pieces together, as shown in Figure 2. When this master is used to form a mold, the final injection molded part is one monolithic part, hence providing the leak free separations between the sectors of the chip.

The master for this device can actually be made quite simply by bonding a raised “wall” pattern around four micromachined chips.



We have now also fabricated molded chips that includes an integrated capillary-driven fluid introduction system. A master is first made by bonding a raised “wall” pattern around micromachined chips, then bonding two separate pieces together and forming a mold. The figures below show the basic sequence for master fabrication and the resulting molded chips. We have demonstrated that capillary forces are sufficient to draw liquid samples into the bead storage wells.



We have also demonstrated effective methods of bead size sorting for both agarose and PS-PEG microbeads using a micromachined chip that contains integrated top and bottom “cover layers” that act as size-selective sieves. In experiments, bead populations of $95 \pm 5 \mu\text{m}$, $105 \pm 5 \mu\text{m}$, $115 \pm 5 \mu\text{m}$, $135 \pm 5 \mu\text{m}$, $165 \pm 5 \mu\text{m}$, and $200 \pm 5 \mu\text{m}$ have now been self-assembled into an array. With these size-selected beads, different chemistries can be encoded as a function of bead size, allowing size to be used to distinguish different sensing sites within an array. By combining this with chemistries encoded as a function of bead size, we should be able to create a self-assembling multi-analyte array. To accomplish this, the bottom and top openings of a storage well must be controlled independently. In our latest chip, both the top and bottom window sizes are fabricated using etched thin film windows, independent of wafer thickness. Figure 1 illustrates how this can be done. Since in (100) silicon anisotropic etching will undercut openings in a mask layer until (111) bounding planes are reached, a mask layout as illustrated will produce a pyramidal storage well of size dictated by the location of the outer corners of the mask opening and the thickness of the wafer. The central opening in the cover layer acts as a filter to prevent beads larger than the opening from entering the storage well. Using a double sided aligner a simple square pattern is etched in the dielectric on the back of the wafer, aligned to the pattern etch in the top. This bottom opening will act as the filter that captures only beads large than its size. After anisotropically etching the silicon, both the top and bottom mask layers will remain, producing the integrated “sieves” necessary for the capture of specifically sized beads. Each well then accepts only beads whose diameters are smaller than the top window but larger than the bottom opening. Figure 1b shows a photomicrograph of one such storage well.

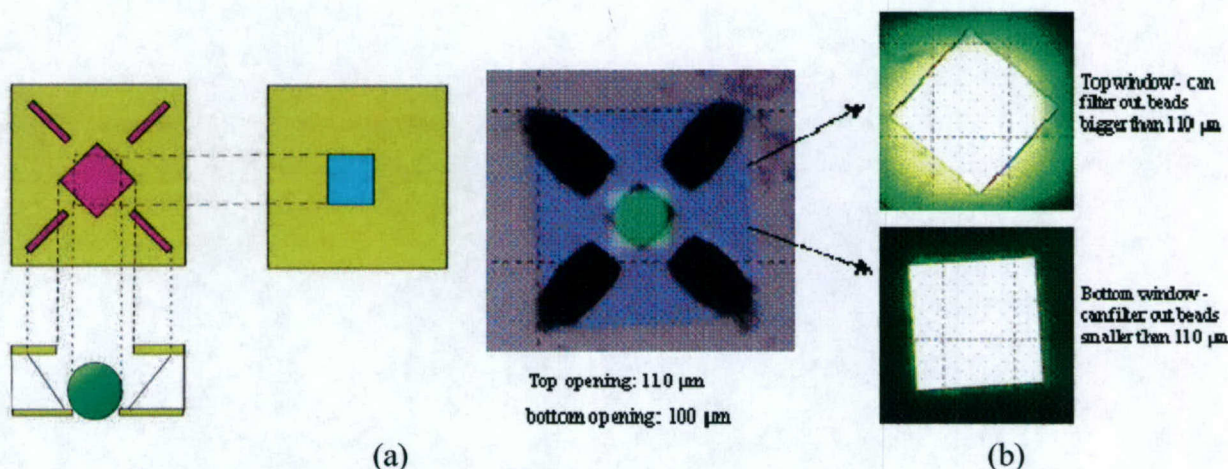


Figure 1: Double sided storage well with size selective capability.

Figure 2 shows the array we have tested. Both glass and agarose beads have been used to test the self-assembly of the array. Beads were transported in a water solution using a syringe. In this experiment, a drop of liquid was

placed on the surface of the chip, each drop containing beads of a different size. The liquid carrying the beads is drawn through the wells using a slight vacuum. The top cover layer windows prevent bigger size beads from entering an inappropriate well while the bottom window allows smaller size beads to be pulled completely through. Only beads of appropriate size enter and are retained in a particular location in the array. Once a bead occupies its pre-set location, the bead in the cavity blocks flow and the well does not capture more beads. We can also use a wash step to remove beads sitting on top surface of the chip; beads captured in the wells do not wash away. Figure 3 shows a photomicrograph of an array that was filled using small glass and large agarose beads.

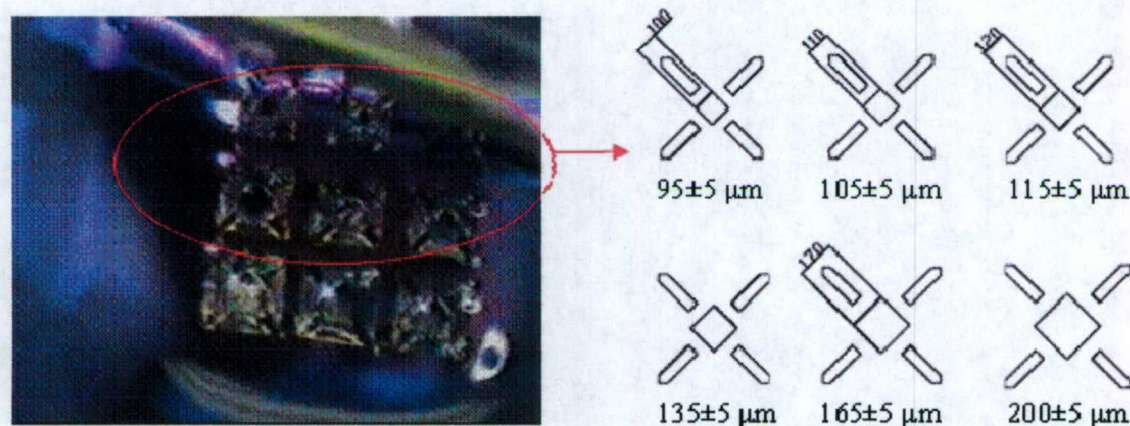


Figure 2: Photomicrograph of a storage well array with top and bottom size selective membranes.

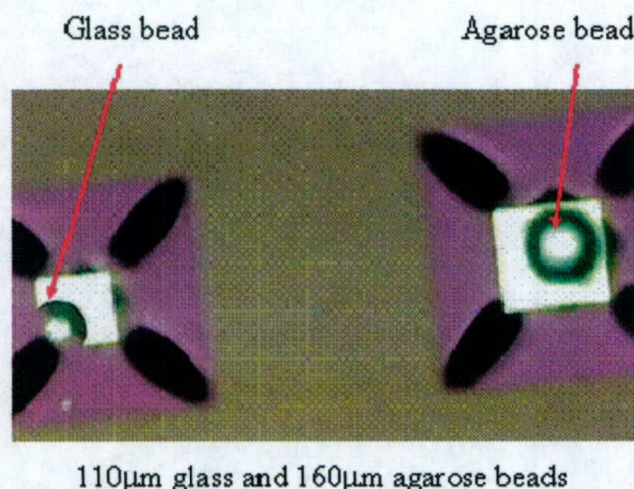


Figure 3: Photomicrograph of two storage wells that have self-selected beads of the desired size..

We have also begun the fabrication of a check valve structure compatible with our bead arrays. This is a critical microfluidic component, since any fluid flow system must avoid back-flow. This structure is also allows reduction of diffusion transport, one of the limiting factors in cross-talk between elements in an array that uses displacement assays. Figure 1 shows the general process flow used to make a storage well cover layer with the asymmetric characteristics required for a check valve. First, a silicon nitride layer is deposited on a silicon wafer and patterned. The material used as the structural layer is silicon enriched silicon nitride. The ratio of Si to N is targeted to be 4 to 1, and the film is deposited at 835 °C. Then, a sacrificial layer of poly-silicon is deposited at about 600 °C and patterned over the markings in the initial nitride layer. Afterwards, another layer of silicon nitride is placed on top of the sacrificial layer, and is patterned with a narrower opening. Finally, the

wafer is submerged in KOH at about 65 °C. Since KOH isotropically etches poly-silicon and anisotropically etches silicon, the poly layer etch enables the separation of the two nitride layers, while the initial opening still controls the size of the pyramidal cavity in the silicon substrate. Figure 2 shows SEMs of a fabricated device. Figure 2a shows an overall view of the device, while Figure 2b is a detail view of the two nitride layers. In the near future, testing of the device will be performed.

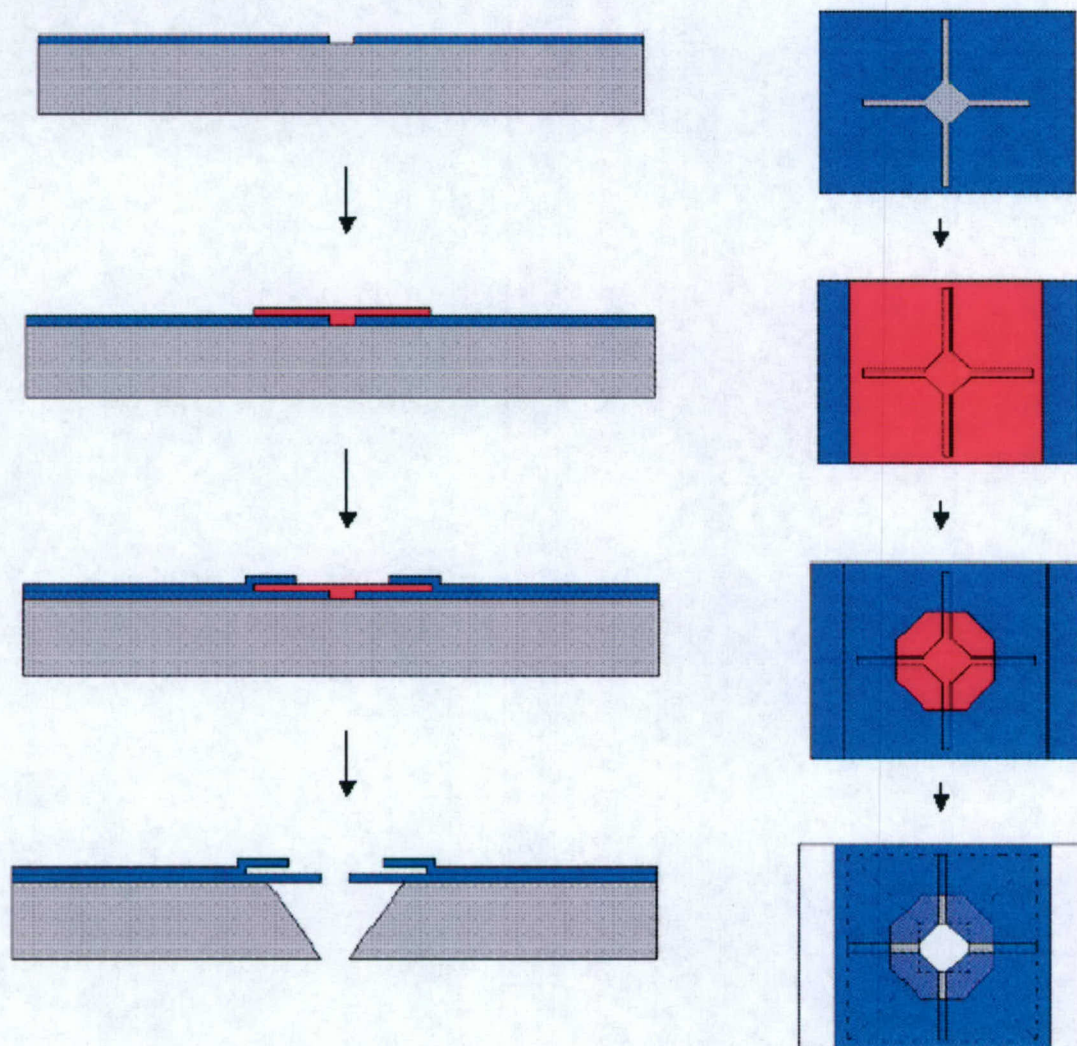
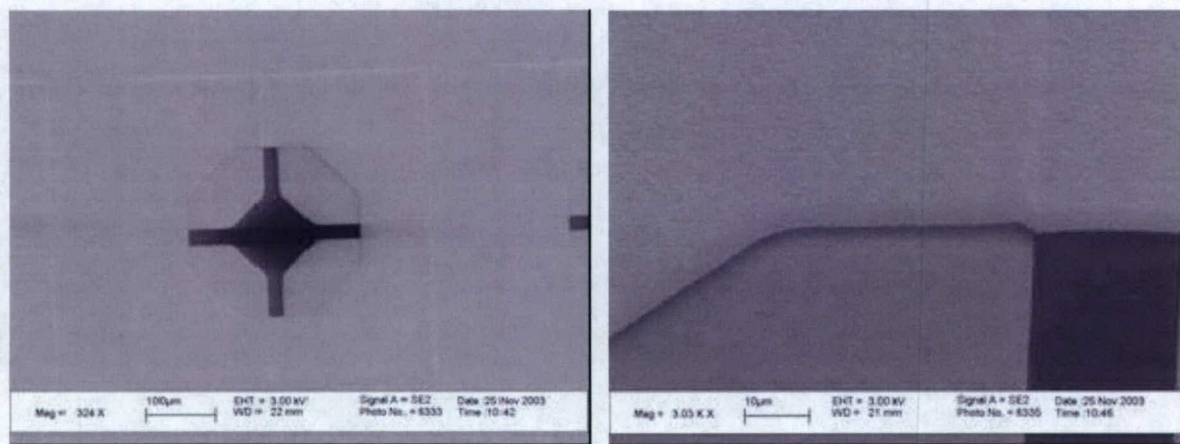


Figure 1: Diagram of the general process flow for a check valve that can be integrated with our bead storage arrays.



(a) (b)

Figure 2: SEM of the fabricated device.

Much of the chemistry used in our bead-based arrays is sensitive to environmental condition such as pH change and Ca^{2+} concentration. Hence, we have now begun the design and fabrication of a storage well array that could have the ability to control such environmental conditions separately in each well using integrated electrodes. Figure 1 shows a schematic illustration of such a chip. Using a modification of our integrated cover layer process, it should be possible to form conductive traces in the dielectric layers on top of our storage wells. The exposed end of this wiring will serve as the control electrode in a given storage well. It would also be possible to integrate address and sensing electronics into the silicon substrate if necessary. Figure 2 below shows a photomicrograph of our first prototype. Continued research is required to find optimum wiring and electrode materials, and to insure their process compatibility with bead storage well fabrication.

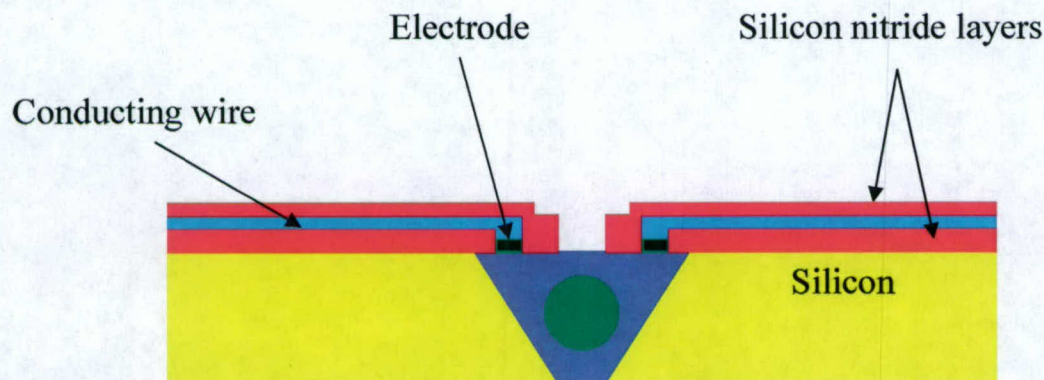


Figure 1. Cross-sectional view of the microdevice for local electrochemical control.

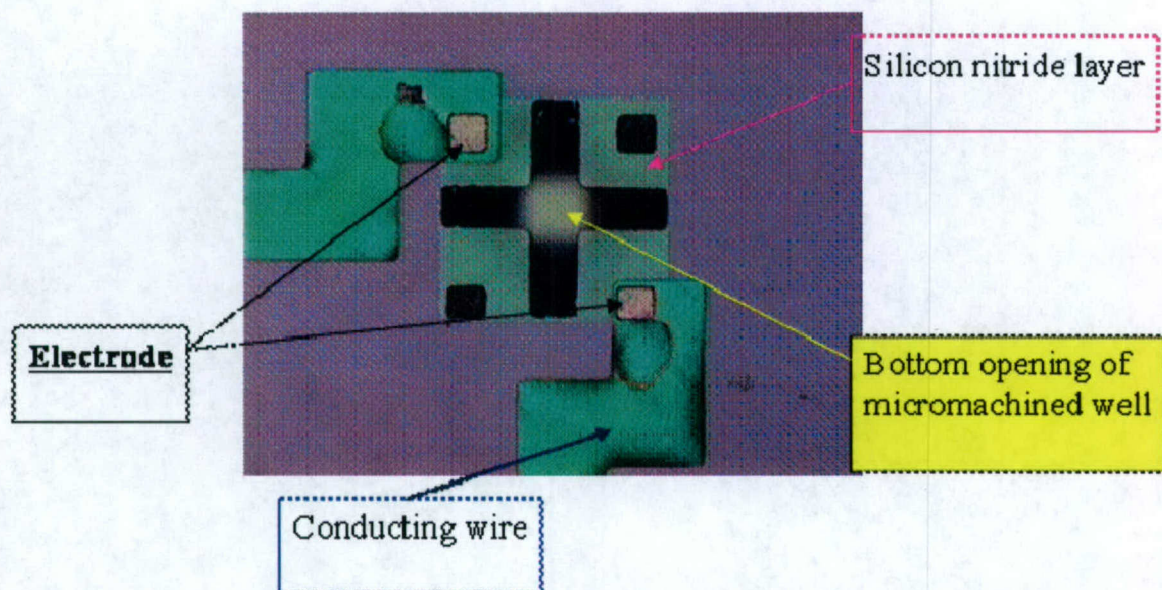


Figure 2: Photomicrograph of the top view of a prototype of the microdevice for local electrochemical control.

Wilson

Statement of problem studied:

The Willson Research Group has investigated methods of deploying the novel molecular recognition elements developed by collaborating research groups in the MURI program. This effort has focused on a lithographically fabricated multi-analyte array platform comprised of hydrogel materials. Depending on the physical scale of the sensing elements, they can either be covalently attached to the hydrogel matrix (e.g. oligonucleotides probes, and aptamers), or they are merely physically encapsulated, an important strategy for preserving the biological activity of larger and more complex sensing elements (e.g. antibodies, proteins, cells). The photolithographic techniques used to construct the arrays are amenable to mass production, and, therefore, promise to be a low cost alternative to traditional biosensor platforms.

Summary of important results:

The MUFFINS Platform

A multi-analyte sensor array platform has been developed which consists of analyte specific features that are indexed by shape, which are referred to as **M**esoscale **U**naddressed **F**unctionalized **F**eatures **I**ndexed by **S**hape (MUFFINS). The array features are mass produced in batches via contact photolithography from poly(ethylene glycol)diacrylate hydrogel pre-polymer containing biologically active sensing elements. Sensor features have been successfully fabricated as small as 50 microns in diameter. Because the each feature type is indexed by shape, they can be efficiently assembled into randomly-ordered arrays using high-throughput and inexpensive self-assembly processes. Due to parallel nature of both fabrication and assembly, the MUFFINS platform promises to be a cost-effective approach towards high-density, multi-analyte sensing (see Figure 1).

MUFFINS Platform

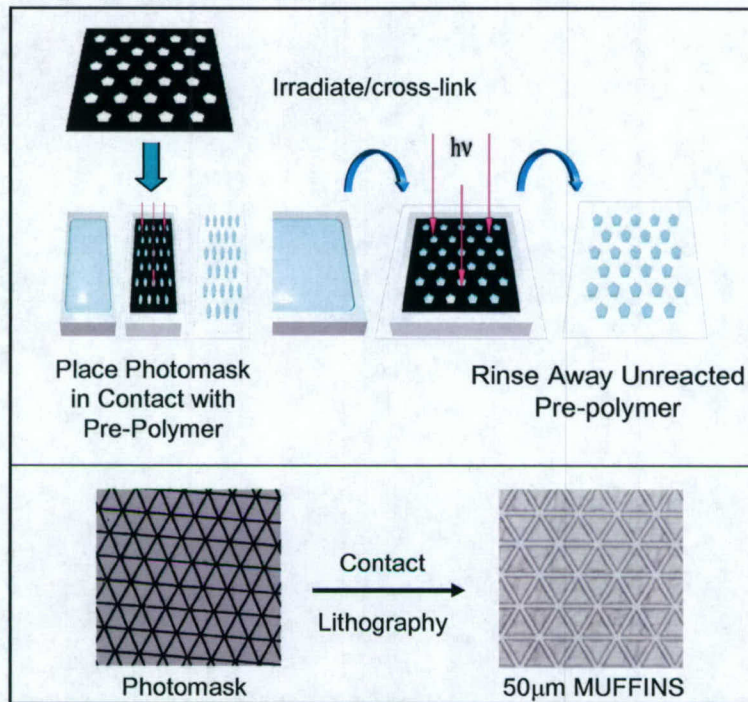


Figure 1: Schematic of contact photolithography process used to fabricate MUFFINS features and bright-field micrograph showing 50µm MUFFINS.

Shape Encoding/Recognition

A central tenet to the MUFFINS platform is rapid, accurate, pattern recognition software that can unambiguously determine the response and identity of each MUFFIN. The existing method of array assembly requires that MUFFINS be identified, regardless of position, rotation, or even inversion. Although commercially available software (such as optical character recognition) exhibit accuracy with specifically oriented characters, the systems fail when characters are rotated or inverted. Therefore, custom pattern recognition software has been developed, along with a MUFFINS shaping methodology called DOMINOES (Dots in an Ordered Matrix with INtegrated Orientation Enhancement System). In the DOMINOES systems, arrays of dots uniquely identify each type of MUFFINS feature. The dot arrays are similar in principle to barcodes. A 5x5 array of dots can be made quite compact and can encode over 33 million unique combinations. Three larger dots are placed on the side of the array to assist the software in determining rotation and inversion. The program takes the micrograph output of a detection array, scans it for dot patterns, and produces a composite image indicating which sensor features are producing a detection response. Trials of this software have shown fast and accurate recognition of over 98% of the array features. A picture illustrating the dot patterns and decoding methodology/process is shown in Figure 2.

Shape Encoding/Recognition of MUFFINS

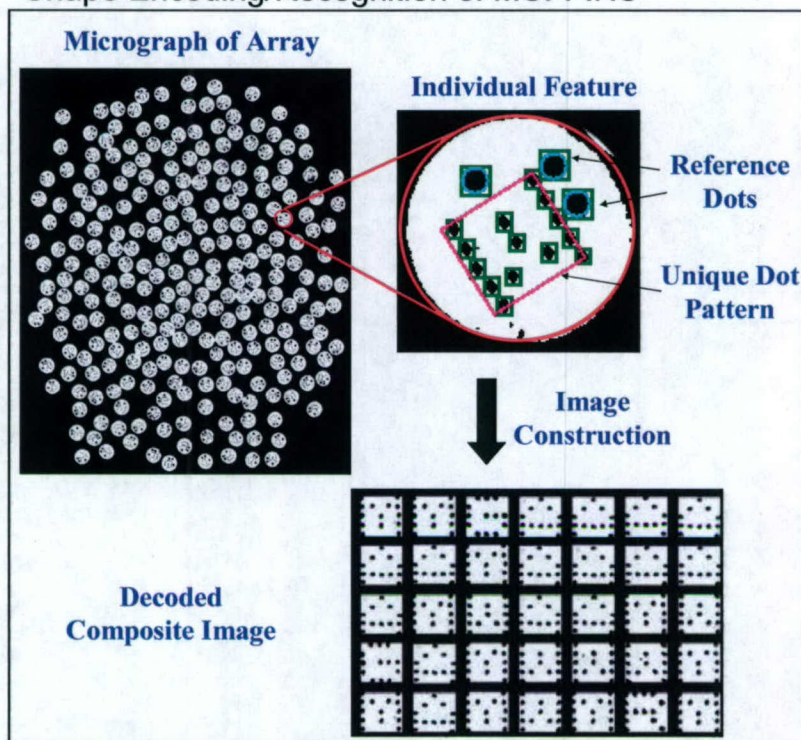


Figure 2: Diagram illustrating the dot patterns used to identify each type of MUFFINS feature.

Array Assembly

In order to make the MUFFINS sensing platform viable, a technique had to be developed to efficiently assemble the individual sensors into a dense but readable array. The individual cylindrical sensors can be set at the air-water interface of a water filled Petri dish, where the hydrostatic forces in between each individual sensor will lead to a randomly ordered aggregate. Balancing this force against that of mechanical agitation allows a sufficiently reversible assembly to enable dense hexagonal-phase packing. If the assembly process is carried out on a layer of hydrogel pre-polymer rather than water, polymerization of this liquid enables the array of sensors to be immobilized on the surface of a durable polymer film. Sensing with these arrays can be carried out by soaking the film in the appropriate DNA hybridization solution, and then rinsing away the unbound DNA with a series of washes (see Figure 3.) Each film can be reused multiple times.

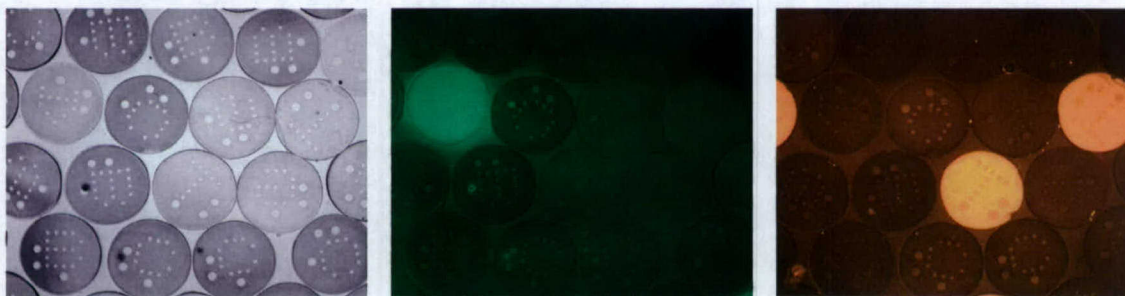


Figure 3: Images of reusable hydrogel sensor array (left) brightfield image of 1 X 1.2 cm window of array (center) sensing of fluorescein labeled DNA sample "A" and (right) sensing of TAMRA labeled DNA sample "T."

Feature Multiplexing

A novel probe indexing methodology, called feature-multiplexing, has been developed, which dramatically increases the information density of microarray devices. This methodology breaks from the

concept of indexing each probe onto an exclusive feature; rather, the probes are indexed such that each is placed within a unique set of multiplexed features. Using this technique a given number (n) of features can deploy $(2^n - 2)$ probes. The efficacy of this technique was demonstrated in a single-nucleotide-polymorphism (SNP) detection array using the shape-indexed, hydrogel-based MUFFINS-platform. The SNP-detection array was designed to screen for the presence of twenty-nine cancer causing missense SNPs found within the human p53 gene. The twenty-nine oligonucleotide probes corresponding to these SNPs were indexed into just five multiplexed-features, constituting an approximate six-fold increase in information density over traditional indexing methods.

Organizing the probes within the array is the key to increasing the density of the array beyond the customary limit of one probe per feature. To assign unique loading patterns for the probes used in this study, each was given a unique number (e.g. 1-29) and binary encryption code corresponding to that number (e.g. probe 21 = 10101). This unique code defined the loading pattern (the expression for probe 21, "10101", indicates that probe 21 is to be placed in features 1, 3 & 5). This process is illustrated in Figure 4.

Binary Encryption of Probes

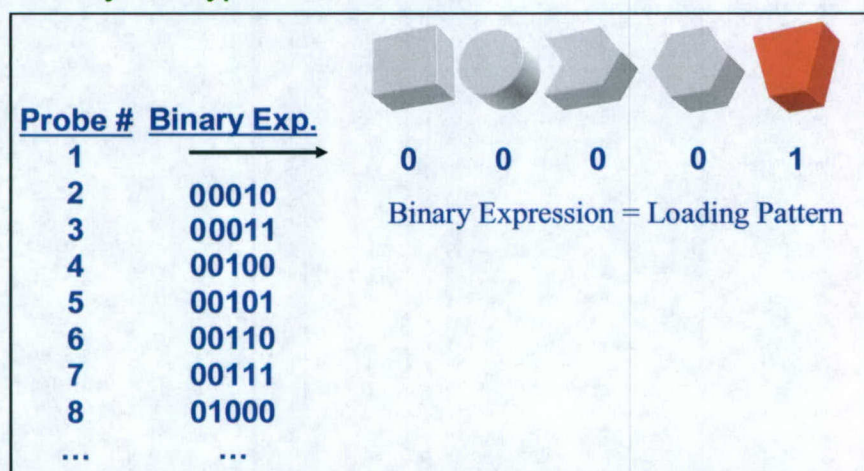


Figure 4 A graphic representation of the binary encryption process. Probe #1 is loaded into the trapezoid shaped feature.

The benefit to using this binary-number encryption strategy is that it simplifies the process of decoding the output of the array. The output pattern can be easily converted into a binary-expression, whereby each feature producing a recognition signal counts as a "1", and all other features count as a "0". The binary-expression identifies the number of the probe that corresponds to the positive detection response. Figure 5 shows micrographs of the p53 SNP detection array detecting target #21 and subsequent binary decoding process.

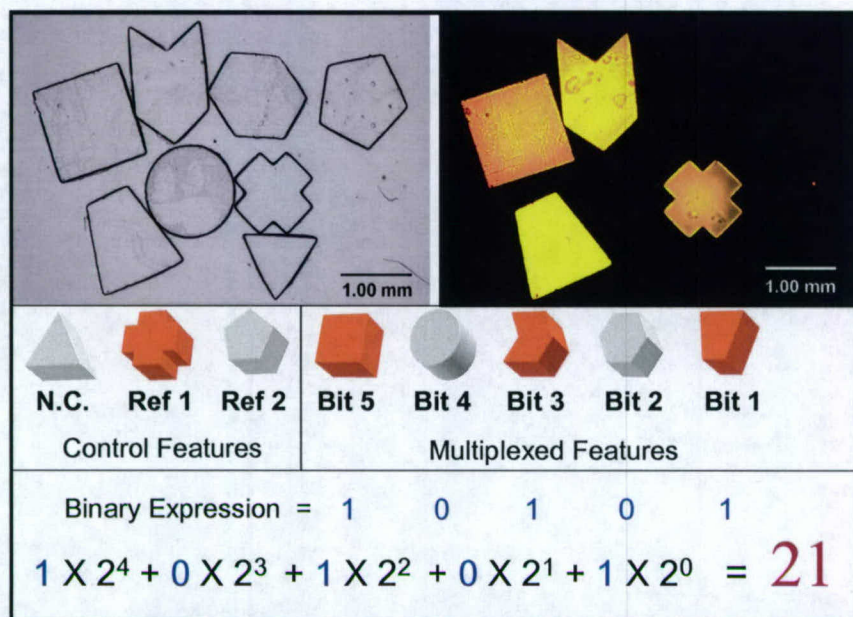


Figure 5: Bright-field and fluorescence micrographs of the detection response produced by the p53 SNP detection array; shown with corresponding binary decoding calculation. The target analyte corresponds to probe #21.

Probe Design Software

A computational method of designing oligonucleotide probes for DNA detection arrays has been developed. The program automates the probe selection process and optimizes the arrays signal-to-noise characteristics. It uses the Nearest Neighbor (NN) model to predict the perfect-match (PM) binding affinity of prospective probe sequences and selects ones that most closely match a pre-defined value. This normalizes the signal strength of the probes. The program also predicts the mismatch (MM) base pair interactions, thereby providing an estimate of the cross-hybridizational noise expected from the array. This data is then used to construct dissociation curves for the PM (signal) and MM (noise) interactions for each probe in the probe-set, which in turn can be used to forecast the signal-to-noise characteristics of the array as a function of various hybridization conditions (i.e. temperature, target concentration, and ionic strength). These capabilities enable the user to optimize both the probes as well as the assay conditions for their DNA detection array. The binding/dissociation characteristics for a sample probe-set are shown in Figure 6. The predicted signal strengths are plotted in green, the noise in blue, and the corresponding worst case signal-to-noise ratio in brown.

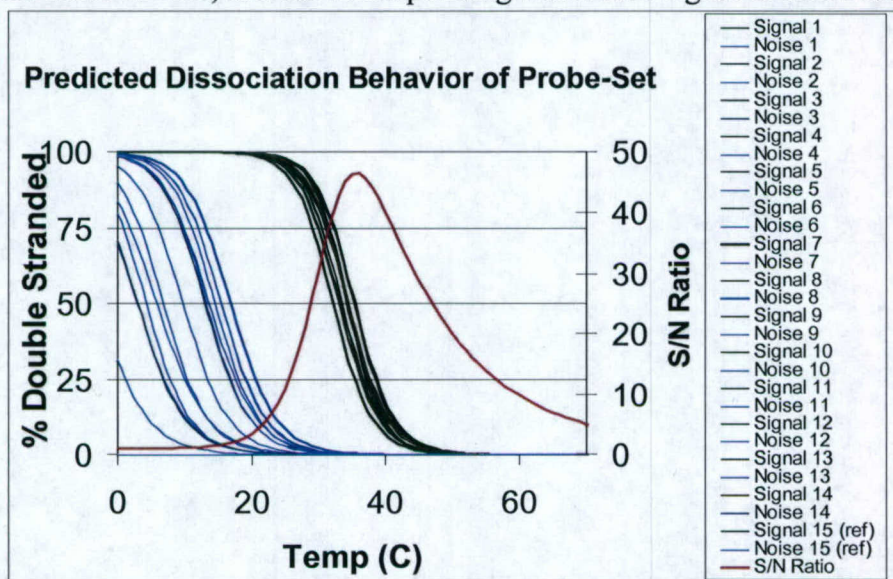


Figure 6: The predicted dissociation/binding characteristics for a sample probe-set plotted as a function of temperature.

SENSOR GROUP

Shear

Statement of the problem studied:

The Shear group has focused on developing and optimizing enzyme-based sensing strategies for array sensors. In particular, we have addressed a number of factors that have traditionally limited enzyme-based assays, including limited dynamic ranges, limited types of analytes that can be simultaneously quantified, and challenges in adapting sensor arrays to permit multiple sensing modalities to be used in parallel.

Summary of the most important results:

We have developed multiple strategies for exploiting the parallel nature of sensor arrays to extend the dynamic range typically achieved for enzyme-based sensing. Strategies examined to date include: (i) Use of multiple enzyme isoforms (e.g., lactate dehydrogenase, LDH, from bacteria versus LDH from mammalian sources); (ii) Use of different ratios of enzymes in multi-enzyme sequences (e.g., glucose oxidase coupled to HRP); (iii) Measurement of different intermediate products in multi-enzyme sequences (e.g., separate sensors based on glucose dehydrogenase or galactose dehydrogenase after enzymatic degradation of lactose); and (iv) Use of different quantities of a reactive "sink" to extend the working range of an enzymatic sensor (e.g., alcohol dehydrogenase as a sink together with an alcohol-oxidase reporting scheme). The variety of approaches now feasible for creating multiple sensor types for a given analyte has substantially extended the versatility and utility of this approach for array sensing, providing increases in dynamic range of more than an order of magnitude.

We have identified conditions in which diverse groups of enzymes that rely on different reporting systems can be implemented within a single "taste-chip" sensor array. In this work, we have collaborated with the Anslyn group to characterize mixtures containing numerous analytes, including lactate, malate, glutamate, ethanol, lactose, galactose, and glucose. Analysis across these broad classes of small molecules was accomplished using combinations of oxidases and dehydrogenases, with the former coupled to horseradish peroxidase (HRP) to generate quinoneimine dye and the latter employing a synthetic hydride-transfer reagent to catalyze formation of a tetrazolium dye from NADH. We have found that these sensing strategies can be used together with minimal interferences, good reproducibility, and sensitivity sufficient to detect a variety of analytes in samples such as serum and beverages.

We also have pursued a "bead-less" strategy for characterizing various analyte types with improved stability and sensor density, and in environments that are not easily amenable to interrogation using more conventional sensor platforms. Here, multiphoton excitation of a photosensitizer is used to elicit cross-linking of a protein directly onto a coverslip or other planar substrate. The fact that these structures can be made rapidly, where desired, and with precise 3D architectures makes this an attractive approach for fabrication; moreover, the very high concentrations of protein that are incorporated into structures offer potential for yielding high densities of immobilized enzymes, antibodies, and other receptor types. We have evaluated structures comprised of various proteins, and have demonstrated that cross-linked avidin retains strong and highly specific binding capacity for biotin, a finding that opens the possibility for creating a diverse range of optical- and electronic-based sensors for use within cellular environments.

Bard

Statement of problem studied:

On the basis of coreactant electrogenerated chemiluminescence (ECL) technology, in which tris(2,2'-bipyridyl)ruthenium(II) ($\text{Ru}(\text{bpy})_3^{2+}$) was used as an ECL precursor and tri-*n*-propylamine (TPrA) as a coreactant, two different approaches capable of detection of DNA hybridization and determination of C-reactive protein (CRP) at high amplification with $[\text{Ru}(\text{bpy})_3]^{2+}$ -containing microspheres were developed. In the first approach, polystyrene microspheres/beads (PSB) were used as the carrier of the ECL labels. p-ssDNA was attached to the surface of magnetic beads (MB) and hybridized with t-ssDNA with immobilized PSB containing a large number of water insoluble $\text{Ru}(\text{bpy})_3^{2+}$ species. With this approach, a large amplification factor of $\text{Ru}(\text{bpy})_3^{2+}$ species for each t-ssDNA can be achieved, when each PSB is attached to a limited number of t-ssDNA. The newly formed p-ssDNA-MB $\leftrightarrow$ t-ssDNA-PSB/ $\text{Ru}(\text{bpy})_3^{2+}$ conjugates were magnetically separated from the reaction media and dissolved in MeCN containing TPrA. ECL was produced upon the anodic potential scanning from 0 to 3.0 V vs. Ag/Ag^+ , and the integrated ECL intensity was found to be linearly proportional to the t-ssDNA concentration in a range of 1.0 fM to 10 nM under optimized conditions. In the second approach, biotinylated anti-CRP species were attached to the surface of streptavidin-coated magnetic beads (MB) and avidin-coated polystyrene microspheres/beads (PSB) entrapping a large number of electrogenerated chemiluminescence (ECL) labels ($\sim 10^9$ $\text{Ru}(\text{bpy})_3[\text{B}(\text{C}_6\text{F}_5)_4]_2/\text{bead}$) to form anti-CRP $\leftrightarrow$ MB and $\text{Ru}(\text{II})\text{cPSB}/\text{Avidin}\leftrightarrow$ anti-CRP conjugates, respectively. Sandwich type $\text{Ru}(\text{II})\text{cPSB}/\text{Avidin}\leftrightarrow$ anti-CRP < CRP > anti-CRP $\leftrightarrow$ MB aggregates were formed when $\text{Ru}(\text{II})\text{cPSB}/\text{Avidin}\leftrightarrow$ anti-CRP was mixed with anti-CRP $\leftrightarrow$ MB conjugates in the presence of analyte CRP. The newly formed aggregates were magnetically separated from the reaction media and dissolved in MeCN containing tri-*n*-propylamine (TPrA) as an ECL coreactant. ECL was carried out with a potential scan from 0 to 2.8 V vs. Ag/Ag^+ , and the ECL intensity was found to be proportional to the analyte CRP concentration over the range of 0.010-10 $\mu\text{g}/\text{mL}$. The CRP concentration of an unknown human plasma specimen was measured by the standard addition method based on this technique. Elimination of the nonspecific adsorption of the CRP system with several different blocking agents was also studied and 2.0% bovine serum albumin (BSA) was found to be best.

Summary of results:

1. Exploration of two different approaches of DNA/protein immobilization, separation and detection.

(a) DNA hybridization detection at high amplification with $\text{Ru}(\text{bpy})_3^{2+}$ -containing microspheres. Figure 1 schematically shows the general principle of this technique. A known ssDNA (probe ssDNA) is first attached to the surface of a magnetic bead, then the complementary ssDNA (t-ssDNA) coated polystyrene bead loaded with a huge number of ECL labels hybridizes with the probe ssDNA. The t-ssDNA-contained $\text{Ru}(\text{bpy})_3^{2+}$ /polystyrene beads are magnetically separated and transferred into an acetonitrile solution, in which polystyrene beads dissolve and the ECL label is released. This is followed by an ECL detection of the released ECL label mixed with a coreactant such as TPrA solution at an electrode.

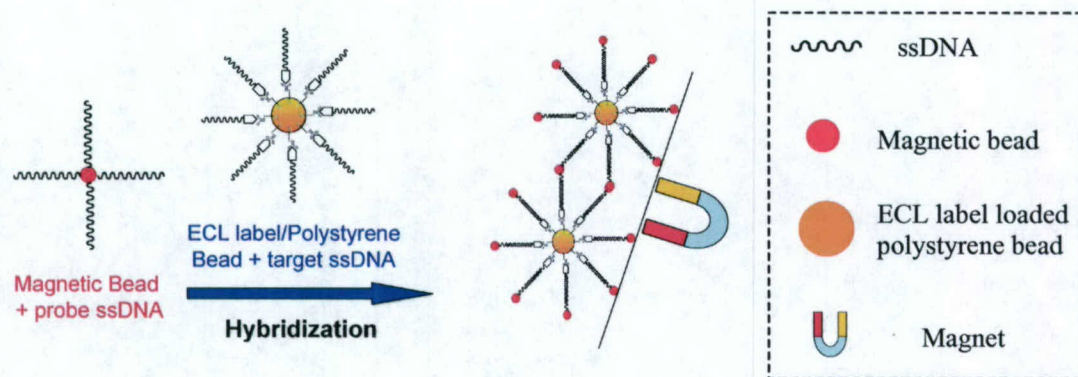


Figure 1. Schematic diagram of DNA hybridization a polystyrene bead as the ECL label carrier and a magnetic bead for the separation of analyte-contained ECL label/polystyrene beads.

(b) CRP determination at high amplification with $\text{Ru}(\text{bpy})_3^{2+}$ -conatining microspheres. Figure 2 schematically displays the general principle of this technique. A magnetic bead (MB) immobilized with an antibody is mixed and reacts with an antibody-coated PSB pre-loaded with a large number of ECL labels in the presence of the antigen (analyte) species to form a sandwich-type MB < antigen > PSB aggregate that is separated magnetically from the reaction medium and transferred into an acetonitrile solution where the PSB is dissolved and the ECL label released. This is followed by an anodic ECL detection of the released ECL label using tri-*n*-propylamine (TPrA) as the coreactant, and the ECL signal produced within in a certain potential range is detected with a photomultiplier tube or CCD camera.

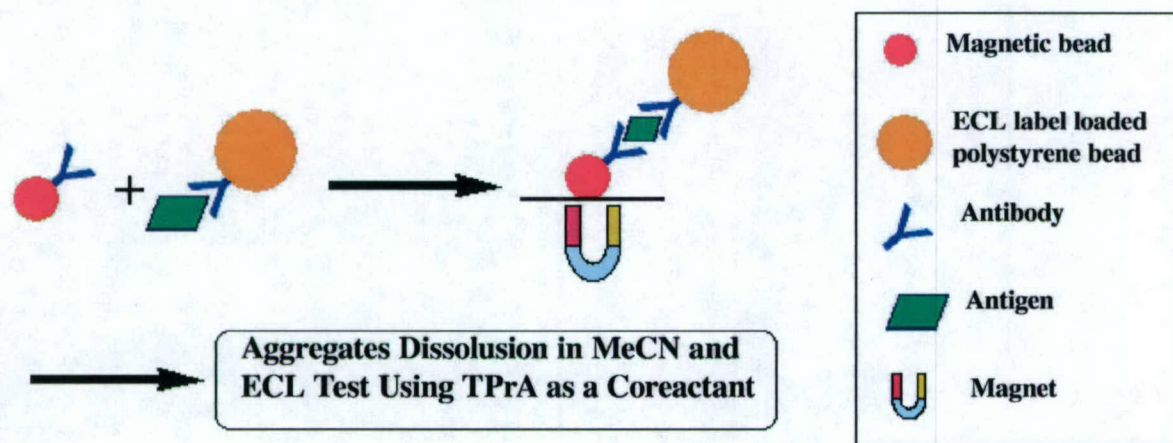


Figure 2. Schematic diagram showing the formation of a sandwich type aggregate between an antibody-coated MB and an antibody-coated PSB containing entrapped ECL labels in the presence of the antigen species, and the separation of the newly formed aggregate with a magnet as well as the subsequent dissolution and ECL detection in MeCN using TPrA as the coreactant.

2. Modification of t-ssDNA with ECL Tag. t-ssDNA was mixed directly with ECL tag in DMSO/MeCN with or without trace amounts of water to covalently attach ECL labels to the end of the ssDNA. This approach was more convenient than DNA labeling with $\text{Ru}(\text{bpy})_3^{2+}$ species carried out during the process of DNA synthesis, which requires the use of an automated DNA synthesizer.

3. Elimination of nonspecific adsorption for immobilized DNA determination. Nonspecific adsorption of the labeled species was significantly reduced by using a series of electrode treatments, such as blocking free –

COOH groups with ethanolamine, pinhole blocking with BSA, washing with EDTA/NaCl/Tris buffer and spraying with inert gases.

4. Selection and synthesis of $\text{Ru}(\text{bpy})_3^{2+}$ contained ECL labels that can diffuse and be maintained within polystyrene beads. Tris(2,2'-bipyridyl)ruthenium(II) tetrakis (pentafluorophenyl) borate ($\text{Ru}(\text{bpy})_3[\text{B}(\text{C}_6\text{F}_5)_4]_2$) was used as the ECL label for such a purpose. This complex (Figure 3) can be effectively loaded into polystyrene beads using a suitable organic solution and maintained entrapped within the beads during a series of modification of the beads in aqueous solutions. The complex was prepared by a metathesis reaction between $\text{Ru}(\text{bpy})_3\text{Cl}_2$ and $\text{Li}[\text{B}(\text{C}_6\text{F}_5)_4] \cdot n\text{Et}_2\text{O}$ ($n = 2-3$) in water.

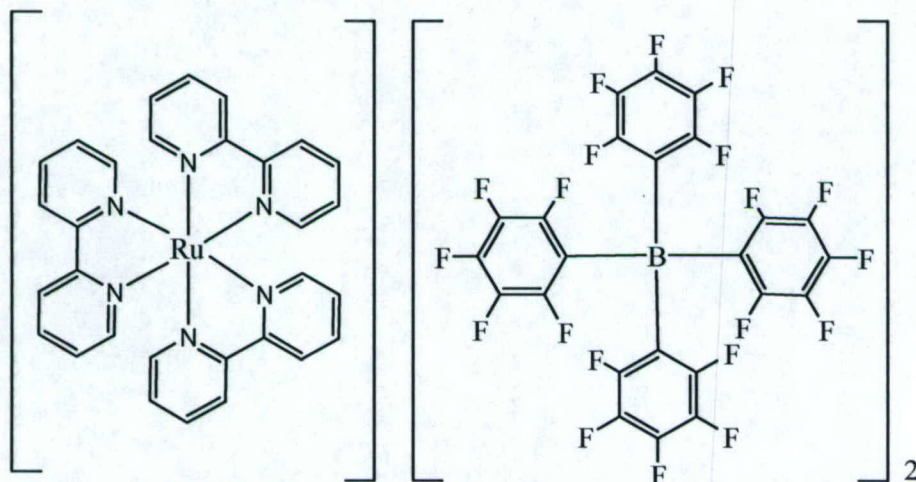


Figure 3. Chemical structure of Ruthenium(II) tris(2,2'-bipyridine) tetrakis(pentafluorophenyl) borate

5. Effective loading of the ECL labels into polystyrene beads. As shown in Figure 4, PSB modified with carboxyl groups are swelled in organic solvent/ECL label solution. The water-insoluble ECL labels diffuse into the polymer matrix, and are entrapped when the solvent is removed from the beads by vacuum evaporation or transfer to an aqueous phase. For $10\ \mu\text{m}$ $-\text{COOH}$ modified polystyrene beads, about 10^9 $\text{Ru}(\text{bpy})_3[\text{B}(\text{C}_6\text{F}_5)_4]_2$ molecules per bead has been found.

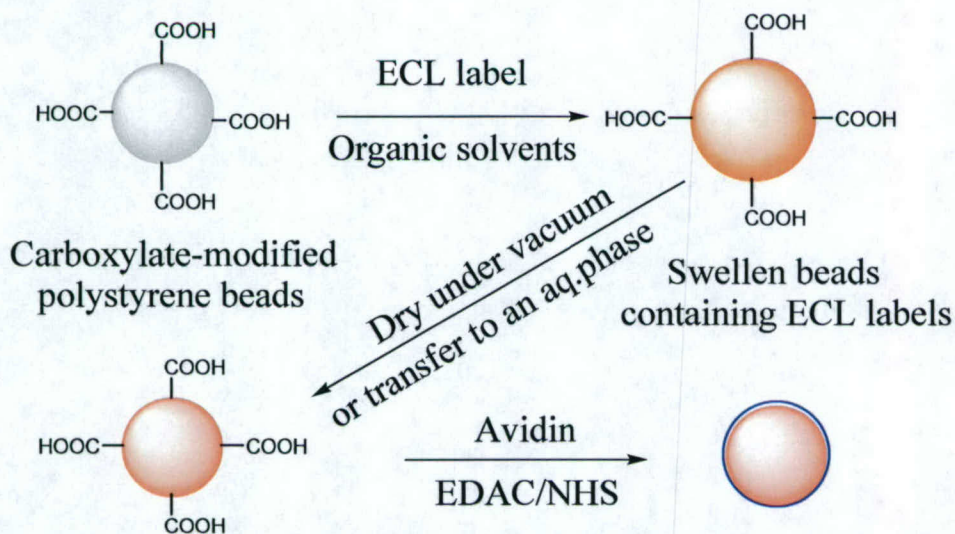


Figure 4. Internal loading of ECL labels into polystyrene beads.

6. The effective loading of the ECL labels into the polystyrene beads can be visually verified via their fluorescent images (Figure 5a) and the uniformity of the avidin layer on the surface of Ru(II)-PSB was suggested by the bright green fluorescent image of Ru(II)-PSB/avidin after the beads reacted with fluorescein biotin (Figure 5b).

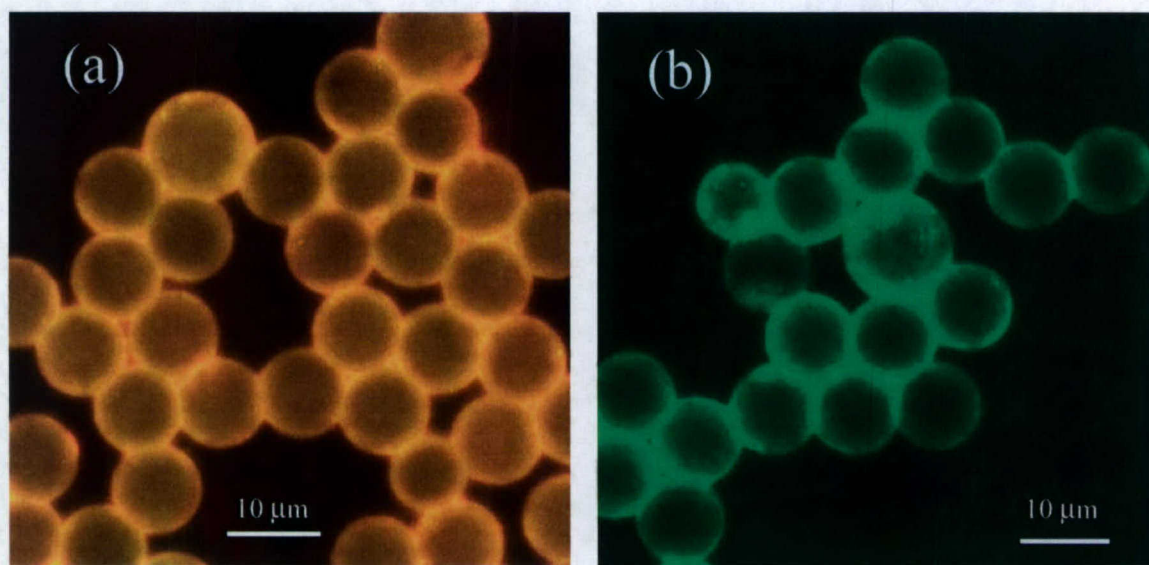


Figure 5. Fluorescent images of 10 μm diameter carboxylate polystyrene beads: (a) after entrapping of $\text{Ru}(\text{bpy})_3[\text{B}(\text{C}_6\text{F}_5)_4]_2$, and (b) after covalent binding of avidin onto the surface of $\text{Ru}(\text{bpy})_3[\text{B}(\text{C}_6\text{F}_5)_4]_2$ loaded beads. The exposure time was 30 s. The specimens were excited at $\lambda_{\text{ex}} \sim 490 \text{ nm}$.

7. DNA hybridization between p-ssDNA-MB and c-ssDNA-PSB/ $\text{Ru}(\text{bpy})_3^{2+}$, and magnetic separation are illustrated in Figure 6.

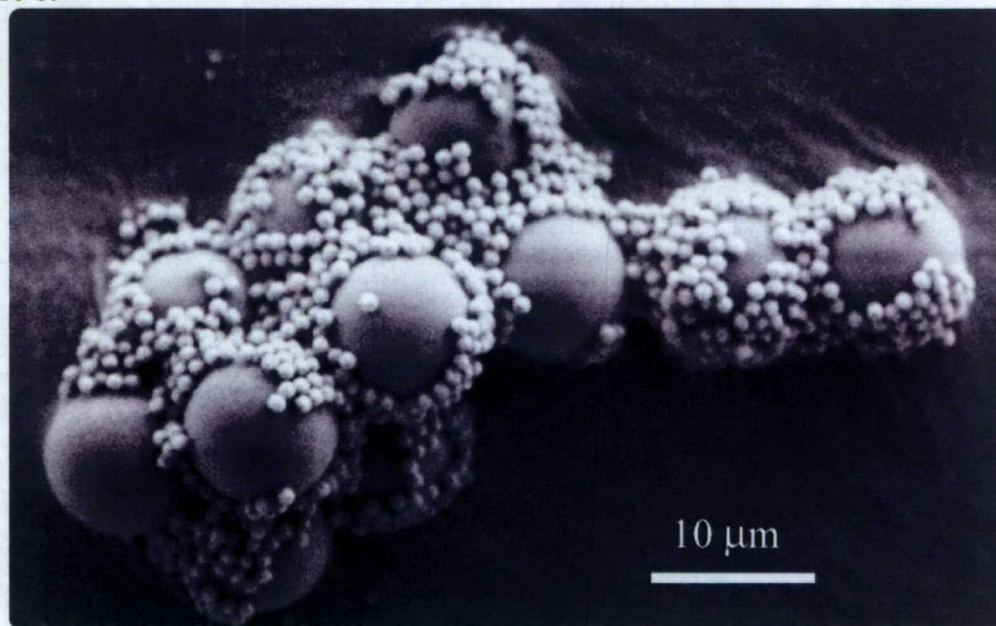


Figure 6. A SEM image obtained after the DNA hybridization between probe DNA-MB and complementary DNA-Ru(II)-PSB/Avidin.

7. Sandwich type $\text{Ru(II)}\text{-PSB/Avidin}\leftrightarrow\text{anti-CRP} < \text{CRP} > \text{anti-CRP}\leftrightarrow\text{MB}$ aggregates were formed when $\text{Ru(II)}\text{-PSB/Avidin}\leftrightarrow\text{anti-CRP}$ was mixed with $\text{anti-CRP}\leftrightarrow\text{MB}$ conjugates in the presence of analyte CRP. ECL detection of CRP was accomplished in 0.10 M TPrA-0.055 TFAA-0.10 M (TBA)BF₄ MeCN at a Pt electrode after the PSB < CRP > MB aggregates were dissolved in this solution. With this technique, the CRP detection limit is as low as 0.010 $\mu\text{g/mL}$, which is lower than those obtained from most of the presently available automated high-sensitivity CRP assay systems. CRP detection for human plasma was conducted with the traditional standard addition method to avoid possible sample matrix effects, and the CRP value obtained was consistent with the literature data. Nonspecific adsorption in CRP immunoassay was also studied using several different blocking agents, including 0.5% Triton X-100, 10% SuperBlock blocking buffer in PBS, 2.0% BSA, and combination of these. 2.0% BSA was found to be the best for the CRP system.

Category 5

Technology Transfer

Over the course of the execution of this grant, a number of companies have been started based in part on the technologies that have been described. In particular, the company Archemix (Boston, MA) was started in part based on aptamer and aptazyme array technologies.

Other technologies, such as the antibody display technologies that have been developed by the Georgiou / Iverson labs, and the electronic tongue technologies developed by McDevitt, Anslyn, Shear, and Neirkirk, have been productively licensed.

Finally, the research that has progressed under the auspices of this grant continues to be funded for development and commercialization. Some of the grants that have recently been awarded for further development of these technologies include:

- A. HSARPA SBIR with Echo Technical (HSB04.1.002); Contract number UTA04-312 (4/15/04 – 10/15/04)
- B. HSARPA SBIR with Accacia International; Solicitation FY04.1; Topic H-SB04. 1-002; (currently under negotiation)
- C. HSARPA SBIR with BioElectroSpec, Inc.; (HSARPA FY04.1); UTAA04-352
- D. HSARPA SBIR with Fluence (Topic No. H-SB04.1-002); UT OSP#300400205

MASTER COPY: PLEASE KEEP THIS "MEMORANDUM OF TRANSMITTAL" BLANK FOR REPRODUCTION PURPOSES. WHEN REPORTS ARE GENERATED UNDER THE ARO SPONSORSHIP, FORWARD A COMPLETED COPY OF THIS FORM WITH EACH REPORT SHIPMENT TO THE ARO. THIS WILL ASSURE PROPER IDENTIFICATION. NOT TO BE USED FOR INTERIM PROGRESS REPORTS; SEE PAGE 2 FOR INTERIM PROGRESS REPORT INSTRUCTIONS.

MEMORANDUM OF TRANSMITTAL

U.S. Army Research Office
ATTN: AMSRL-RO-BI (TR)
P.O. Box 12211
Research Triangle Park, NC 27709-2211

☐ Reprint (Orig + 2 copies)

☐ Technical Report (Orig + 2 copies)

☐ Manuscript (1 copy)

☒ Final Progress Report (Orig + 2 copies)

☐ Related Materials, Abstracts, Theses (1 copy)

CONTRACT/GRANT NUMBER: **DAAD19-99-1-0207**

REPORT TITLE: **Texas Consortium for the Development of Biological Sensors**

is forwarded for your information.

SUBMITTED FOR PUBLICATION TO (applicable only if report is manuscript):

Sincerely,

Andrew D. Ellington
University of Texas at Austin