

American Chemical Society
DIVISION OF ANALYTICAL CHEMISTRY
ABSTRACTS

221st ACS National Meeting

San Diego, CA
April 01-05, 2001

C. L. Wilkins, C. Fenselau, Program Chairs

SUNDAY AFTERNOON

• **New Developments in Enantiomeric Analysis Through Advances in Chiral Selective Separation and Detection**

D. Bobbitt, Organizer; D. Bobbitt, Presiding Papers 1 - 7

SUNDAY EVENING

• **General Papers**

D. B. Chase, Organizer Papers 8 - 148

MONDAY MORNING

• **ACS Award in Analytical Chemistry**

P. Vouros, Presiding Papers 149 - 154

MONDAY AFTERNOON

• **ACS Award in Analytical Chemistry**

P. Vouros, Organizer; D. M. Desiderio, Presiding Papers 155 - 161

TUESDAY MORNING

• **Probing Intrinsic Reactivity Features by Mass Spectrometry**

G. Cooks, Organizer; G. Cooks, Presiding Papers 162 - 167

• **Near Field Spectroscopy: A Tool for Nanoanalysis**

S. Stranick, Organizer; S. J. Stranick, Presiding Papers 168 - 173

TUESDAY AFTERNOON

• **Probing Intrinsic Reactivity Features by Mass Spectrometry**

G. Cooks, Organizer Papers 174 - 179

• **Nanoelectrochemistry**

D. Feldheim, Organizer; D. Feldheim, Presiding Papers 180 - 185

WEDNESDAY MORNING

• **ACS Award in Chromatography**

G. Guiochon, Organizer; G. Guiochon, Presiding Papers 186 - 191

• **High Throughput Mass Spectrometry in Drug Discovery**

S. A. Hofstadler, Organizer; S. A. Hofstadler, Presiding Papers 192 - 197

WEDNESDAY AFTERNOON

• **ACS Award in Chromatography**

G. Guiochon, Organizer; K. -. Hupe, Presiding Papers 198 - 203

• **LC/MS of Biomolecules**

D. Jones, Organizer; D. Jones, Presiding Papers 204 - 207

THURSDAY MORNING

• **Recent Advances in Microfluidics**

I. Fritsch, Organizer; D. Cunningham, Organizer; I. Fritsch, Presiding

Papers 208 - 213

THURSDAY AFTERNOON

• **Recent Advances in Microfluidics**

I. Fritsch, Organizer; D. Cunningham, Organizer; D. D. Cunningham, Presiding

Papers 214 - 219

DIVISION OF ANALYTICAL CHEMISTRY

1. POLYMERIC SURFACTANTS: NEW REAGENTS FOR SEPARATING CHIRAL COMPOUNDS. *Isiah Manuel Warner, Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, Fax: 225-388-3971, isiah.warner@chem.lsu.edu*

Over the past several years, we have developed chiral polymeric surfactants as mobile phase additives for use as chiral separation media in capillary electrophoresis. The advantages of these polymeric surfactants over conventional micelles include 1) increased stability, 2) zero critical micelle concentration, 3) no deleterious monomer interactions, and 4) demonstrated selectivity in micellar electrokinetic chromatography (MEKC). We have recently shown that polymeric surfactants are broadly applicable to a variety of analyses, including enantiomeric separations. Our studies have shown that these polymers are usually more suitable than conventional micelles in MEKC. In this talk, I will focus on the use of polymeric surfactants as chiral separation reagents in MEKC. Some details will be given on our experiments which are designed to understand how chiral separations work in these polymers. The use of the results of these experiments to design and produce better chiral separation reagents will be discussed. In particular, the ability to fine-tune these reagents for particular separations will be demonstrated. The advantages of these reagents in comparison to regular micelles and cyclodextrins will also be discussed with an emphasis on the wide variety of possible applications. A comparison of the use of polymeric surfactants for separations will also be made directly to separations by use of conventional (unpolymerized) micelles. The advantages of combining CE with polymeric surfactants to achieve improved analytical measurements will be highlighted.

2. RECENT DEVELOPMENTS IN THE CE SEPARATION OF ENANTIOMERS USING SINGLE-ISOMER CHIRAL RESOLVING AGENTS. *Gyula Vigh, Dawn K. Maynard, Wenhong Zhu, Pavel Glukhovskiy, Brent M. Busby, Silvia Sanchez-Vindas, and Shulan Li, Department of Chemistry, Texas A&M University, College Station, TX 77842-3255, vigh@mail.chem.tamu.edu*

A family of pure, single-isomer hepta-sulfated - cyclodextrin and octa-sulfated -cyclodextrin derivatives were synthesized on the large scale using sequentially-applied protecting group chemistry. Each intermediate and final product was thoroughly characterized by HPLC, indirect UV-detection capillary electrophoresis, 2D 1H and 13C NMR spectroscopy, and MALDI-TOF-MS. When possible, single crystals of the pure products were grown to obtain the respective X-ray crystal structures. The new single-isomer resolving agents were used as chiral resolving agents for the CE separation of the enantiomers of acidic, basic, zwitterionic and neutral analytes in low pH and high pH background electrolytes according to the guidance of the charged resolving agent migration model (CHARM model).

The acetylated and methylated 6-sulfato cyclodextrins have sufficiently high solubilities in acidic, 100% methanol background electrolytes and were used for the separation of a large variety of weak bases, including very hydrophobic ones, thus extending the scope of CE enantiomer separations.

3. COMPARISON OF VIBRATIONAL CD, ELECTRONIC CD, AND OPTICAL ROTATION FOR ENANTIOMERIC PURITY DETERMINATION OF MIXTURES OF CHIRAL PHARMACEUTICAL MOLECULES. *Laurence A. Nafie¹, Teresa B. Freedman¹, Changning Guo¹, Rina K. Dukor², and Rekha D. Shah². (1) Department of Chemistry, Syracuse University, 1-014 CST, Syracuse, NY 13244-4100, Fax: 315-443-4070, LNAFIE@SYR.EDU, (2) BioTools, Inc, (3) R.W. Johnson Pharmaceutical Research Institute*

The production of chiral pharmaceutical molecules that are optically pure is becoming increasingly important. In the production of such molecules, techniques must be available for specifying the chiral purity of the samples, the absolute configuration of the active pharmaceutical molecule, and in some cases

the lowest energy conformations of the molecule in solution. Within the past several years, vibrational circular dichroism (VCD) has been shown to be an effective method of probing the optical purity and molecular stereochemistry of chiral pharmaceutical molecules. The instrumentation used to measure and calculate VCD will be described and examples of VCD spectra will be presented. We will demonstrate the use of VCD to measure optical purity in terms of percent enantiomeric excess, absolute configuration by comparison to *ab initio* calculations, and molecular conformation for a variety of pharmaceutical molecules. Following this introduction to current VCD technology will compare the capabilities of three principal optical techniques for determining enantiomeric excess in mixtures of chiral molecules of pharmaceutical interest. In particular, we have measured the VCD, electronic CD and optical rotation of several pairs of molecules that can be related as reactant and product in simple chemical reactions. This has allowed us to test the relative ability of these three chiroptical methods to follow the enantiomeric purity of mixtures of chiral molecules during a chemical reaction. We have obtained spectroscopic data for the pairs of chiral molecules 2-methyl butyric acid and 2-methyl butanol, camphor and borneol and 2-phenyl propionic acid and 2-phenyl propanol. We will discuss the relative strengths and weaknesses of VCD, CD and optical rotation under a variety of sample conditions for these pairs of chiral molecules. From these examples it will be shown that in many cases, unique advantages are available with VCD and that this technique will grow in importance for the analysis of chiral molecules as instrumentation and theoretical methods become more broadly used in the future.

4. ENANTIOSELECTIVE CHROMATOGRAPHY USING CARBON DIOXIDE-BASED MOBILE PHASES AND POLARIMETRIC DETECTION. *Fiona Geiser, Chiral Technologies, Inc, 730 Springdale Drive, P.O. Box 564, Exton, PA 19341, Fax: 610-594-2325, fgeiser@chiraltech.com, Rekha Shah, RW Johnson Pharmaceutical Research Institute, and Linda Betz, Department of Chemistry, Widener University*

In this presentation, we describe the use of supercritical fluid chromatography (SFC) instrumentation for the rapid development of analytical and preparative enantioselective chromatography methods. Model systems include a variety of analytes with acidic, basic, or nonelectrolyte functionality. Procedures will be described for enantioselective chromatography of (1*RS*, 2*RS*)-Tramadol hydrochloride (HCl), an analgesic commercialized as the +*trans* form of 2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol. Tramadol HCl enantiomers were preparatively chromatographed on CHIRALPAK AD chiral stationary phase (CSP) using a mobile phase of Carbon Dioxide-Methanol (90:10, v/v). Benefits of in-line polarimetric detection include direct confirmation of order enantiomer elution and rapid estimation of optimum enantioselective methods. Mobile phases containing CO₂ have been found to facilitate significant reduction of liquid mobile phases as well as direct chromatography of HCl salts and carboxylic acids without the use of basic and acidic additives, respectively. Essential factors for productive preparative separations include solute stability and solubility in the organic modifier, column injections exceeding 1.5 g of racemic mixture per kg chiral stationary phase, and short cycle times. Productivity values for most separations (purified product per vol-time) have been found to be sufficient for producing significant quantities of single enantiomers for use in drug discovery.

5. REAL-TIME ASSESSMENT OF ENANTIOMERIC PURITY IN PREPARATIVE-SCALE SEPARATIONS USING A COMBINED UV AND POLARIMETRIC DETECTOR RESPONSE RATIO. *Donald R. Bobbitt II¹, Sean W. Linder¹, and Gary W. Yanik². (1) Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR 72701, Fax: 501-575-4049, dbobbitt@uark.edu, (2) PDR-Chiral Inc*

The development of methodology appropriate for the rapid and precise assessment of enantiomeric purity is a critical need in both analytical and preparative

scale enantio-selective separations. In analytical-scale applications, this information is important for separation optimization. In preparative-scale situations, real-time assessment of enantiomeric purity is critical to decisions related to product purity, and/or the need for, and the type, of additional processing. The process is further complicated in preparative-scale applications by the fact that well-resolved enantiomeric distributions are not observed due to the severity of peak overlap and asymmetry. Recently, we have shown that laser-based polarimetric detection, in combination with ultraviolet detection, can be used to assess enantiomeric purity in real-time as an adjunct to the separation process. A mass-independent response function is obtained from the ratio of the normalized polarimetric signal relative to the normalized UV signal. This response ratio will be shown to be equivalent to the enantiomeric excess and independent of concentration and chromatographic resolution. This methodology is useful in defining optimum separation conditions, and for directing process-scale separations. The methodology will be evaluated as a function of enantiomeric excess, chromatographic resolution and peak asymmetry.

6. ANALYSIS AND FATE OF ENANTIOMERS OF PESTICIDES AND OTHER ENVIRONMENTAL POLLUTANTS. *Arthur W. Garrison, and J. Jackson Ellington, National Exposure Research Laboratory, U.S. Environmental Protection Agency, Ecosystems Research Division, 960 College Station Rd., Athens, GA 30605, Fax: 706 355 8202, garrison.wayne@epa.gov*

Up to 25% of pesticides and other environmental pollutants are chiral and exist as sets of mirror image isomers, or enantiomers, that usually differ in their microbiological transformation rates and toxicities. To provide for more accurate risk assessment of these chiral pollutants it is, therefore, important to determine the relative biotransformation rates of their enantiomers so as to predict their persistence and occurrences in the environment, and to estimate their relative toxicities. These studies require enantiomer separation techniques based on chiral chemistry. We have adapted and applied such separation techniques using high pressure liquid chromatography, gas chromatography and capillary electrophoresis. Examples will be shown of the separation of the enantiomers of organophosphorus, organochlorine, imidazolinone, phenoxyacid and other classes of pesticides, as well as of PCBs and other environmental pollutants. In addition, applications of these separation techniques to determine the environmental occurrences of enantiomers and the enantioselectivity of microbial transformation of chiral pollutants will be discussed.

7. UTILITY OF LASER-BASED POLARIMETRIC AND CIRCULAR DICHROISM HPLC DETECTION FOR PHARMACEUTICAL APPLICATIONS. *Thomas J. Edkins¹, Peter Meier², Lisa A. Roseman¹, and Rekha D. Shah¹.* (1) *New Product Research, The R.W. Johnson Pharmaceutical Research Institute, Welsh and McKean Roads, Spring House, PA 19477-0776, Fax: 215-540-4683,* (2) *New Product Development, The R.W. Johnson Pharmaceutical Research Institute c/o Cilag AG*

The commercialization of enantioselective HPLC detectors, such as those based on laser-based polarimetry and circular dichroism, has popularized their use among many chromatographers. When these detectors are used with an enantioselective separation, they produce a bimodal signal and thereby give confirmatory evidence that the separation has indeed occurred.

The production of this bimodal signal can be exploited for the benefit of drug development in many ways. First, it can be used to confirm/validate that an enantioselective separation has taken place. We will show data using these detectors in this mode for: 1) validation of an enantioselective HPLC separation method for an early-phase drug candidate, 2) confirmation of the presence of enantiomers in support of an enantioselective crystallization, and 3) utilization as a method development tool for screening enantioselective HPLC columns.

A second mode of use is quantification without separation. Once the enantiomeric purity is determined via an enantioselective separation, the detector is simply calibrated with varying proportions of each isomer. When combined with UV (or RI) detection, we can obtain weight % and %ee in one injection. We will show recent work with this technique, in support of monitoring the synthesis of some chemical intermediates, and compare those results to earlier results using this technique.

A third mode of use is known as quantification with partial separation. Useful analytical information can be obtained with these detectors even under poor or marginal separation conditions. This technique is particularly valuable when a

baseline enantioselective separation is not yet available or cannot be easily maintained, or when separation speed (but not resolution) is critical. We will discuss the important considerations when using this mode, such as: 1) the estimation of peak area overlap using simulated response models; 2) the use of residuals and back-calculations to examine calibration curves and 3) the utility of this mode as a semi-quantitative versus quantitative technique.

8. "UNIVERSAL" HPLC DETECTOR FOR COMBINATORIAL LIBRARY QUANTITATION IN DRUG DISCOVERY?. *Wenni Li, Mark Piznik, Kevin Bowman, and John Babiak, Analytical Chemistry, Pharmacopeia, Inc, CN 5350, Princeton, NJ 08543, Fax: 732-422-0156, weli@pharmacop.com*

A true 'universal' detector is capable of providing equivalent response to all compounds in a combinatorial library. Therefore, application of a 'universal' detector to the chromatographic analysis of combinatorial libraries, in theory, would allow the quantitation of the entire library compounds with a single external standard. Does such 'universal' HPLC detector exist? Refractive index (RI) detector and evaporative light scattering detector (ELSD) are commonly referred to as 'universal' HPLC detectors. Recently, Antek reported the use of a chemiluminescent nitrogen detector (CLND) as a means of quantitation of combinatorial library compounds. Which one is a true 'universal' detector among these three detectors? Does combinatorial library quantitation require only ONE 'universal' detector or MULTIPLE detectors? In searching for a true 'universal' HPLC detector, the basic theory, equivalent response, of each 'universal' detector was studied and evaluated. The advantages and disadvantages of RI, ELSD, and CLND will be discussed.

9. 2-D IR CORRELATION SPECTROSCOPY FOR A BIOLOGICAL MATRIX. *Aminiel Awichi, Giri Srikanthan, and Wei Zhao, Department of Chemistry, University of Arkansas, 2801 South University Ave., Little Rock, AR 72204, Fax: 501-569-8838, Wxzhao@ualr.edu*

2D infrared correlation spectroscopy has been used to examine individual components in a representative biological matrix containing glucose, bovine serum albumin and triacetin. Various perturbations including temperature and concentration have been used to generate 2D correlation spectra in the range of near IR and middle-IR. The features in the spectra characterizing individual components will be presented and discussed. WZ acknowledges the Financial Support from the Research Corporation.

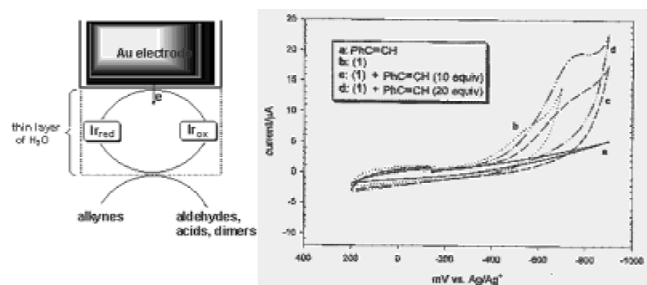
10. CLCOPERON/REPORTER GENE BASED SENSING SYSTEM FOR DIHYDROXYLATED (CHLORO-) BIPHENYLS. *Xiyun Guan¹, H-M. Lehmler², L. G. Bachas¹, and S. Daunert¹.* (1) *Department of Chemistry, University of Kentucky, Rose Street, Lexington, KY 40506, Fax: 859-323-1069, xguan1@pop.uky.edu,* (2) *Graduate Center for Toxicology, University of Kentucky*

Hydroxylated (chloro-)biphenyls (PCB-diols) are major metabolites of polychlorinated biphenyls (PCBs). It has been previously demonstrated that PCB-diols have estrogenic/anti-estrogenic effects. We are reporting here that PCB-diols can activate the chlorocatechol degradation pathway, a catabolic mechanism of further degradation of the major metabolite, chlorocatechol, in a large number of chlorinated compounds. This suggests a possible alternative way to degrade PCBs. With GC-MS, it is shown that PCB-diols are substrates for the ClcA enzyme. Given the importance of the PCB-diols, we also developed a sensing system for them using *Pseudomonas putida* bacteria harboring the plasmid pSMM50R-B', in which the reporter gene, *lacZ*, was fused to the *clcB* gene under the control of the ClcR, the regulatory protein. Both 3,4-biphenyldiol and 4-chloro-3', 4'-dihydroxy-biphenyl can be detected with this sensing system at concentrations as low as 1 μM using a 2-h induction period.

11. ACTIVATION OF TERMINAL ALKYNES BY ELECTROCHEMICAL REDUCTION OF [CP*IR(III)(TPPTS)(NCMe)₂](OTf)₂ IN THIN LAYER OF WATER ON GOLD ELECTRODE. *Woonsup Shin, Daesung Chong, and Chong Shik Chin, Department of Chemistry, Sogang University, 1 Shinsu-dong, Mapo-gu, Seoul 121-742, South Korea, Fax: 2-701-0967, shinws@ccs.sogang.ac.kr*

Thin aqueous layer containing [Cp*Ir^{III}(TPPTS)(NCMe)₂](OTf)₂ (1, Cp* = C₅Me₅⁻, TPPTS = P(*m*-C₆H₄SO₃Na)₃, OTf = ⁻SO₃CF₃) was formed on gold electrode and

series of terminal alkynes ($\text{HC}^{\text{R}}\text{CR}$; $\text{R}=\text{C}_6\text{H}_5$, $\text{C}_6\text{H}_4\text{CH}_3$, $\text{CH}_2\text{C}_6\text{H}_5$, C_6H_9) in $\text{ClCH}_2\text{CH}_2\text{Cl}$ were activated by electrochemical reduction of **1** through liquid/liquid interface. The electrochemical reduction current of **1** increases as increasing the concentration of terminal alkynes. The electrolysis of **1** in aqueous layer with terminal alkynes in organic layer produced hydrated and dimerized products of alkynes.



12. ADVANTAGES OF NONLINEAR LASER-INDUCED GRATING SPECTROSCOPIC METHOD OVER FLUORESCENCE METHODS FOR TRACE-CONCENTRATION ANALYSIS. *Mirna Lopez, Julia A. Schafer, and William G. Tong, Department of Chemistry, San Diego State University, San Diego, CA 92182, Fax: 619-594-2442, william.tong@sdsu.edu*

Multi-photon laser-induced grating spectroscopy is presented as a versatile and sensitive liquid-phase detection method with many advantages over other optical techniques including fluorescence-based methods. This nonlinear absorption-based method complements fluorescence detection methods well, and it yields comparable or better detection sensitivity levels. Advantages of laser wave-mixing spectroscopy include detection of both chromophores and fluorophores, detection of many biomolecules without the use of labels or tags, coherent signal beams that can be collected with 100% efficiency, and detection of positive signals against dark background. Taking advantage of quadratic signal dependence on absorption coefficient, the environment of a biomolecule can be studied more effectively.

13. AFM IMAGING OF 2-D DNA NANOARRAYS UNDER ATMOSPHERIC CONDITIONS. *Ava C. Dykes¹, Michael Norton¹, W. L. Manner², Nadrian Seeman³, and Furong Liu³. (1) Department of Chemistry, Marshall University, 400 Hal Greer Blvd., Huntington, WV 25755, Fax: 304-696-3243, caudill3@marshall.edu, (2) Microscopy and Surface Science Skill Center, Union Carbide Corporation Technical Center, (3) Department of Chemistry, New York University*

The utility of DNA as a building material for nanometer scale, molecular self-assembly has been demonstrated by the construction of polyhedra, knots, Borromean rings, devices and two-dimensional crystals of double crossover (DX), triple crossover and Holliday junction parallelograms. DX crystals have been characterized previously in solution by AFM. Because the use of a fluid cell is not always convenient, a sample preparation method for imaging in air has been developed. In this paper, we report the stringent sample preparation conditions and imaging parameters used to obtain high quality AFM images of DX crystals in air. Imaging DNA in air presents challenges not encountered in solution studies, chiefly salt deposition and hydration effects. Salt deposits are minimized by alternately rinsing and drying the samples. Imaging in intermittent mode with extremely low drive amplitude and using a low force constant cantilever diminishes sample-associated hydration layer interactions by minimizing contact with the tip.

14. ANALYSIS OF CORTICOSTERONE IN RAT PLASMA USING ELECTROSPRAY IONIZATION MASS SPECTROMETRY. *Padma Marwah, Ashok K. Marwah, and Henry A. Lardy, Enzyme Research Institute, Dept of Biochemistry, Wisconsin University at Madison, 1710 University Ave., Madison, WI 53705, pmarwah@facstaff.wisc.edu*

A simple, fast yet highly sensitive and specific method based on HPLC coupled to electrospray ionization mass spectrometry has been developed for the quantitation of corticosterone in rat plasma. After extraction of rat plasma (0.1

ml) with diethyl ether using 5-pregnen-3 β -ol-20-one-16 α -carbonitrile (SIGMA) as internal standard, HPLC was performed on a short C8 column (Zorbax-SB 4.6 mmx50 mm) using a methanol-water gradient (54% to 90% in 6 min). Detection was performed on single quadrupole mass spectrometer in selected ion monitoring mode ($m/z=369$ and 364). The detection limit of the assay was 9 femto mole (~ 3 pg) of corticosterone on column. In vitro data was subjected to curve fitting (cubic, $r^2=0.9999$). The recovery of corticosterone after extraction ranged from 84 to 92%. The coefficient of variation for intra assay and inter assay precision ranged from 1.3% to 9.0%. The assay is routinely used in our lab to examine corticosterone levels in stressed rats.

15. ANALYSIS OF LEAD ISOTOPE RATIOS IN STANDARD REFERENCE MATERIALS. *Kimberly A. Givler, and Richard D. Foust Jr., Department of Chemistry, Northern Arizona University, Flagstaff, AZ 86011-5698, Fax: 520-523-8111, kag8@dana.ucc.nau.edu*

Lead isotope ratios are commonly used as a means of identifying sources of lead pollution in the environment. However, though standard reference materials commonly have certified lead concentrations, ratios of lead isotopes in such materials are not known. We have determined ratios of Pb207/Pb206 and Pb208/Pb206 for ten plant, soil, sediment, and coal SRM's. Plant materials analyzed were National Bureau of Standards SRM's 1571 (Orchard leaves), 1573 (Tomato leaves), and 1575 (Pine needles). Coal materials tested were NIST SRM's 1632b (Buminous Coal), 1633a (Coal fly ash), and 1635 (Subbituminous Coal). Sediments and soils analyzed were NBS SRM 1646 (Estuarine sediment), and NIST SRM's 2709 (San Joaquin Soil), 2710 (Montana Soil), and 2711 (Montana Soil). Plant samples were prepared by microwave digestion with trace metal grade concentrated nitric acid. Coals, sediments, and soils were prepared by microwave digestion with the same nitric acid and 30% hydrogen peroxide. Method was verified by lead concentration determination by GFAAS. Isotope ratios were analyzed by ICP-MS.

16. ANALYTICAL UTILITY OF SOL-GEL TECHNOLOGY IN THE DETERMINATION OF ENVIRONMENTAL POLLUTANTS. *Nahada Allison, and Maurice O. Iwunze, Department of Chemistry, Morgan State University, Baltimore, MD 21251, miwunze@morgan.edu*

Chrysene of varied concentration was encapsulated in a sol-gel glass and characterized with fluorescence spectroscopic technique with a view to using it as a sensor for selected PAH's. Analytical sensitivity of this technique was observed to be about $2.26 \times 10^{10}\text{M}$. This value was compared with that obtained in a solution of ethyl acetate which was $5.80 \times 10^9\text{M}$, indicating about an order of magnitude lower that that obtained in the sol-gel glass. The limit of detection of both techniques was in the range below 0.1 ppb. These observations clearly show that the use of sol-gel glass as a sensor for PAH determination is a promising analytical technique. Its utility will be discussed with respect to environmental pollutant determination.

17. APERTURELESS NEAR-FIELD SCANNING IR MICROSCOPY OF ROUGH SURFACES. *Boris B. Akhremitchev, and Gilbert C. Walker, Department of Chemistry, University of Pittsburgh, 219 Parkman Ave, Pittsburgh, PA 15260, Fax: 412-383-9646, borbor@pitt.edu*

Infrared near field microscopy using an apertureless probe technique has been accomplished to study the surfaces of a cast copolymer film. Two basic models for the predicted signal and the experimental data are presented. The first model includes plane wave light scattering by a conductive sphere and an infinitely wide absorptive layer placed on a semi-infinite conductor. This model shows infrared signal dependence on the layer absorption and predicts topographic coupling. The experimental data also indicate that a significant component in the infrared contrast arises from the probe following the sample's topography, and a method to minimize the effect is demonstrated. The three dimensional finite difference time domain method was used to calculate scattering from an inhomogeneous surface. Both constant tip-sample gap and constant tip-substrate height analysis were made. Calculations for dielectric and Lorenzian materials and the implications for obtaining chemical contrast in near field imaging are discussed.

18. APPLICATION OF COVALENTLY BOUND POLYMER MULTILAYERS FOR EFFICIENT METAL ION SORPTION. *Jaycoda S. Major, and Gary J. Blanchard, Department of Chemistry, Michigan State University, East Lansing, MI 48824-1322, Fax: 517-353-1793, majorjay1@yahoo.com*

The potential utility of thin films in a variety of applications such as separation, second harmonic generation and sensors has been a subject of much research effort in recent years. In this work, a layer-by-layer deposition scheme is used to construct a multilayer assembly capable of metal ion sorption. We use an alternating co-polymer of 4-hydroxyphenylmaleimide and ethylvinylether-2-diisopropylphosphonate, where the hydroxyl functionality is reacted with adipoyl chloride resulting in covalent (ester) linkages. Subsequent to multilayer assembly, the phosphonate groups are deprotected/hydrolyzed using bromotrimethyl silane (BTMS), the deprotection of which renders these groups active for further reaction, in this case metal ion sequestration. XPS data for the multilayer assembly after exposure to Zr⁴⁺ show that ~13 atom% of the multilayer assembly is Zr⁴⁺, a number larger than that expected for saturation. Preliminary QCM data reveal that the kinetics of metal ion uptake is very fast. We characterize these multilayer systems using ellipsometry, QCM gravimetry, XPS, FTIR, UV-Visible, and ³¹P-NMR spectroscopies.

19. APPLICATION OF SCANNING ELECTROCHEMICAL MICROSCOPE FOR PROBING REDOX ACTIVITY OF LIVING CELLS. *Biao Liu, Department of Chemistry and Biochemistry, Queens College and Graduate Center of The City University of New York, Flushing, NY 11367, Fax: 718-997-5531, bliu@qc.edu, and Michael V. Mirkin, Queens College of CUNY, Flushing, NY 10016*

We describe here several SECM-based approaches to measuring the rates and investigating the pathway of transmembrane charge transfer. The differences were detected in the redox responses given by nontransformed human breast epithelial cells (MCF-10A), MCF-10A cells that were genetically engineered to overexpress PKCa (11a), and highly metastatic breast cancer cells (MDA-MB-231). The slower mediator regeneration rates observed for 11a and MDA-MB-231 cells may be related to a high level of PKCa expression, a common factor in these cell lines. This was further supported by the fact that a MDA-MB-231 cell treated with PKCa inhibitor (Go6976) showed higher mediator regeneration rate. one-dimensional current profiles and gray-scale constant-height images of cells were also obtained. The images of the mixture of MCF-10A cells and MDA-MB-231 cells suggest that metastatic cells can be detected in a large field of normal breast cells using the differences in their redox reactivities.

20. ARE WE NOT OBSESSED WITH LINEARITY?. *Ashok K. Marwah¹, Padma Marwah¹, and Henry A. Lardy². (1) Enzyme Research Institute, Dept. of Biochemistry, Wisconsin University at Madison, 1710 University Ave, Madison, WI 53705, Fax: 608-265-2904, amarwah@facstaff.wisc.edu, pmarwah@facstaff.wisc.edu, (2) enzyme research institute, dept of biochemistry, wisconsin university at madison*

Esthetically pleasing linear plots of data can not always be derived from complex measurements. This paper discusses the analysis of LC-MS data using curve estimation regression analysis and weighted linear regression analysis models. The reported data were generated by using calibration standards prepared with human plasma and rat plasma, liver and brain at concentrations ranging from 1 ng/ml to 1000 ng/ml. Various oxygenated derivatives of dehydroepiandrosterone were used for the purpose. Comparison of curve fitting and weighted linear regression analysis is discussed.

21. ASSEMBLY OF ALTERNATING POLYMERIZED MEDIATOR AND ENZYME MODIFIED ELECTRODE BY LAYER-BY-LAYER ADSORPTION. *Shin-ichiro Suye, Hideo Okada, Shu Nojima, and Mikio Sakakibara, Department of Applied Chemistry and Biotechnology, Fukui University, 3-9-1, Bunkyo, Fukui, 910-8507, Japan, Fax: +81-776-27-8747, suye@acbio.fukui-u.ac.jp*

Polymerized mediator, enzyme, and polyion layers on the surface of chemically modified surface of an electrode were assembled electrode by layer-by-layer adsorption. Polymerized mediator (PEI-Fc) was prepared by covalently immobilization of ferrocene carboxylic acid at the amino groups of poly(ethylenimine)

(PEI) using water soluble carbodiimide. PEI-Fc and glucose-6-phosphate dehydrogenase (G6PDH) were assembled in combination with alginate acid and PEI, respectively. The electrode responded linearly to G6P in the range of 0.04 to 2.0 mM. The effects of the deposition number and order of the enzyme and mediator layers on the performance characteristics of the biosensors will be presented. are described.

22. ASSOCIATION OF YERSINIA ENTEROCOLITICA PHOSPHOLIPASE A2 WITH PHOSPHOLIPID LANGMUIR MONOLAYERS. *Alexa Barnoski Serfis, Katherine Grant, and Sam Brancato, Department of Chemistry, Saint Louis University, 3501 Laclede Ave., Monsanto Hall 204, St. Louis, MO 63103, Fax: 314-977-2521, barnoski@slu.edu*

Phospholipases are capable of hydrolyzing phospholipid molecules, and one phospholipase of importance is PLA2 secreted from *Yersinia enterocolitica*. This bacterial pathogen secretes PLA2 upon contact with colonic epithelial cells, resulting in an extreme inflammatory response. PLA2 is believed to participate in the pathogenesis of *Y. enterocolitica* through destabilization of the cell membrane as lysophospholipids accumulate in the membrane vicinity. The role in pathogenesis has only begun to be studied, and our efforts involve the determination of PLA2 interactions with phospholipid monolayers using surface pressure and fluorescence microscopy techniques. DPPC monolayers were formed on a buffered aqueous subphase and compressed to various initial surface pressures. The protein was then injected beneath the monolayer, with subsequent increases in surface pressure occurring as the protein penetrated the monolayer. Surface pressure measurements and fluorescence microscopy images indicated protein interactions were most favorable when the monolayer was tightly packed and when calcium was present in the subphase.

23. CAPILLARY ELECTROPHORESIS COUPLED TO THE VOLTAMMETRIC DETECTION OF UNDERIVATIZED OLIGONUCLEOTIDES. *Sara Brazill, and Werner Kuhr, Department of Chemistry, University of California Riverside, Pierce Hall, Riverside, CA 92521, Fax: 909-787-4713, Brazill@citrus.ucr.edu*

Capillary electrophoresis (CE) is a powerful separation technique with advantages that include: speed, resolution and efficiency. In the work to be presented, native oligonucleotides are separated by CE and detected using Sinusoidal Voltammetry (SV) at a copper electrode under alkaline conditions. The use of high pH is necessary for detection at copper, and also minimizes possible secondary structure leading to greater separation efficiency of longer DNA fragments. SV is used as the electrochemical detection method and offers both selectivity and sensitivity. A copper microelectrode is positioned at the outlet of the capillary and a sine wave is used as the excitation waveform. Native oligonucleotides are detected at copper through electrocatalytic oxidation at high pH. The data collected is monitored in the frequency domain, where it is easier to discriminate between the background and the faradaic signal. The combination of CE with SV provides a highly efficient, sensitive method to separate native DNA fragments.

24. CHARACTERIZATION OF INTERMOLECULAR FORCES IN CAPILLARY ELECTROCHROMATOGRAPHY USING PARTITION COEFFICIENTS. *Loranelle L. Shultz-Lockyear, Aubrey E. Cavender, and Brian S. Dossey, Department of Chemistry, Fort Lewis College, 1000 Rim Drive, Durango, CO 81301, Fax: 970-247-7567, lockyear_l@fortlewis.edu*

A systematic study of capillary electrochromatography (CEC) and capillary electrophoresis (CE) has been performed to characterize differences in retention mechanisms. The capacity factor (k') for each of a set of polycyclic aromatic hydrocarbons (PAHs) has been determined and related to fundamental thermodynamic quantities to help describe retention on a molecular level. The capacity factor and linear solvation energy relationships are used to define specific interactions between the solute (analyte) and solvent (mobile or stationary phase). This information is particularly useful for determining the intermolecular forces in play during a CEC separation wherein a charged solute is both partitioning between phases and concurrently migrating in the applied electric field. Results of these experiments can be used to predict retention characteristics for new solutes and stationary phases.

25. CHARACTERIZATION OF KINETIC BEHAVIOR OF FERROCENE-TAGGED DNA AT GOLD MICROELECTRODES USING SINUSOIDAL VOLTAMMETRY. *Sharin Bender, Sara A. Brazill, and Werner G. Kuhr, Department of Chemistry, University of California at Riverside, Pierce Hall, Riverside, CA 92521, sharin@citrus.ucr.edu*

Sinusoidal voltammetry (SV) is an electrochemical technique that is similar to continuous cyclic voltammetry (CV). SV utilizes a large amplitude sinusoid as the excitation waveform, as opposed to a triangular waveform exploited in CV. In contrast to CV, the frequency domain response of SV yields a linear background current that is primarily isolated in the fundamental frequency, while the faradaic current is non-linear and is observed in the higher harmonics. Analyzing the response in the higher harmonics has been shown to improve the signal-to-background ratio by up to two orders of magnitude. Since the frequency domain is dependent on the rate of electron transfer, this non-linear faradaic current response can be utilized to observe kinetic behavior. The electron transfer kinetics of immobilized ferrocene-tagged DNA on a gold electrode have been observed using the frequency response obtained from this technique in attempt to theoretically understand the kinetic behavior of these systems.

26. CHARACTERIZATION OF LEAD GLAZES AND LEAD LEACHING PROPERTIES OF GLAZED CERAMICS FROM THE SOLIS VALLEY, MEXICO USING INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY AND DIFFUSE REFLECTANCE FOURIER TRANSFORM IR SPECTROSCOPY: ARE WE WHAT WE EAT OUT OF? *Sara C. Tunstall, Rebecca Klein, Sachi DeCou, and Dula Amarasiriwardena, School of Natural Science, Hampshire College, West Street, Amherst, MA 01002, dula@hampshire.edu*

Lead poisoning is a major health concern in many parts of the world. Lead oxide used in ceramic glazes is an effective fluxing agent, which lowers the firing temperatures (~ 500 °C), yet provide clear and lustful ceramic product, therefore attractive to many pottery manufacturers. As a result, potters in Mexican villages continue to use lead-based glaze materials in ceramics so that they could manufacture more earthenware at a reduced cost. Pottery shards and liquid glazes were collected from Santa Maria de Conchesda in the Solis Valley, Mexico. Dried liquid glazes, post-fired glaze material were analyzed by DRIFT. Results of the DRIFT analysis demonstrate that PbO (1429 cm⁻¹) is the active form of lead found in liquid glazes and both materials contain silica band at 1025-1110 cm⁻¹ regions. Although much of the PbO remain in its original form but some reaction between silica and PbO occurs during the firing perhaps forming lead bisilicate. DRIFT spectroscopy can be used for the identification of chemical ingredients in the glaze materials pre and post firings. Pottery shards were leached in 0.02M citric acid (pH 2.05) for a minute and leached elemental concentrations including lead were analyzed using ICP-MS. The concentrations of lead in citric acid extracts of pottery ranged from 0.3 to 80.3 µg/ml while the control pottery sample fired with lead-free glaze had a lead concentrations of 0.1 µg/ml. Copper, iron, manganese and also leached out from a decorated pottery shard. Elemental distributions on glaze surfaces were identified by laser ablation (LA)-ICP-MS. Nitric acid extracts from soils, teeth, glaze materials and pottery shards were analyzed for lead isotope ratios (207/206Pb vs. 208/206Pb) by ICP-MS. Four clusters were identified by lead isotope ratio analysis. Two of the Solis deciduous teeth had lead isotope ratios similar to those of the Solis' soils while other two showed distinct lead isotope signatures comparable to lead isotope ratios found in the Santa Maria pottery. This study demonstrates applicability of lead isotope ratios for identifying the source origins, and indicates the potential health risk within the Solis Valley community owing to the lead leached from pottery.

Lead poisoning is a major health concern in many parts of the world. Lead oxide used in ceramic glazes is an effective fluxing agent, which lowers the firing temperatures (~ 500 °C), yet provide clear and lustful ceramic product, therefore attractive to many pottery manufacturers. As a result, potters in Mexican villages continue to use lead-based glaze materials in ceramics so that they could manufacture more earthenware at a reduced cost. Pottery shards and liquid glazes were collected from Santa Maria de Conchesda in the Solis Valley, Mexico. Dried liquid glazes, post-fired glaze material were analyzed by DRIFT. Results of the DRIFT analysis demonstrate that PbO (1429 cm⁻¹) is the active form of lead found in liquid glazes and both materials contain silica band at 1025-1110 cm⁻¹ regions. Although much of the PbO remain in its original form but some reaction between silica and PbO occurs during the firing perhaps forming lead bisilicate. DRIFT spectroscopy can be used for the identification of chemical ingredients in the glaze materials pre and post firings. Pottery shards were leached in 0.02M citric acid (pH 2.05) for a minute and leached elemental concentrations including lead were analyzed using ICP-MS. The concentrations of lead in citric acid extracts of pottery ranged from 0.3 to 80.3 µg/ml while the control pottery sample fired with lead-free glaze had a lead concentrations of 0.1 µg/ml. Copper, iron, manganese and also leached out from a decorated pottery shard. Elemental distributions on glaze surfaces were identified by laser ablation (LA)-ICP-MS. Nitric acid extracts from soils, teeth, glaze materials and pottery shards were analyzed for lead isotope ratios (207/206Pb vs. 208/206Pb) by ICP-MS. Four clusters were identified by lead isotope ratio analysis. Two of the Solis deciduous teeth had lead isotope ratios similar to those of the Solis' soils while other two showed distinct lead isotope signatures comparable to lead isotope ratios found in the Santa Maria pottery. This study demonstrates applicability of lead isotope ratios for identifying the source origins, and indicates the potential health risk within the Solis Valley community owing to the lead leached from pottery.

27. CHARACTERIZATION OF STATIONARY PHASES IN GC AND OPTIMIZATION OF CHROMATOGRAPHIC SEPARATIONS BY SOLVATION PARAMETER MODEL. *Qinglin Li, and Colin F. Poole, Department of Chemistry, Wayne State university, 180 Chemistry, 5101 Cass Ave., Detroit, MI 48202, Fax: 313-577-1377, qli@chem.wayne.edu*

The Solvation Parameter Model is used to characterize stationary phases for packed columns and capillary columns in gas chromatography. All phase characteristics are quantified by different sets of system constants. Selectivity differences as well as selectivity equivalence is identified with cavity formation

and dispersion interactions, n- and p-electron pair interactions, dipole-type interactions and hydrogen-bond interactions. Columns from different manufacturers are compared. Interfacial adsorption is also studied on packed columns. The model is used to predict solute retention properties in optimizing chromatographic separations.

28. CHARACTERIZATION OF THIN COLLOIDAL AU MULTILAYER FILMS AS OPTICALLY TRANSPARENT THIN LAYER ELECTRODES. *Andrea J. Osisek, and John N. Richardson, Department of Chemistry, Shippensburg University, 1871 Old Main Drive, Shippensburg, PA 17257-2299, ao8100@ark.ship.edu*

Colloidal Au multilayer films on glass substrates have recently been characterized as electrodes for investigating redox reactions in liquid solution. Here, we take advantage of the optical transparency of these films to develop and characterize novel optically transparent thin layer electrodes. Representative spectroelectrochemical data acquired using traditional light-absorbing redox probes such as o-tolidine and methylene blue will be presented. Possible advantages of these new electrodes include ease of fabrication and direct control over the electrode surface nanostructure.

29. CHEMILUMINESCENCE DETECTION IN CAPILLARY ELECTROPHORESIS USING AN ULTRA-FAST CO-CATALYZED PEROXYOXALATE CHEMILUMINESCENT REACTION AND ELECTROKINETIC REAGENT DELIVERY. *Chris Kuyper, Katie Denham, Jim Dickson, Jacqueline Murray, and Robert Milofsky, Chemistry, Fort Lewis College, 1000 Rim Drive, Durango, CO 81301, Fax: 970-247-7567, clkuyper@fortlewis.edu*

Despite the impressive limits of detection and inherent selectivity afforded by peroxyoxalate chemiluminescence (POCL) detection, efficient coupling of POCL to capillary electrophoresis (CE) remains limited by the relatively slow kinetics of the reactions that drive imidazole catalyzed chemiluminescence. Moreover, oxalate esters, used in POCL, are sparingly soluble in polar solvents and hydrolyze rapidly, presenting an additional challenge with respect to detection following aqueous phase separations. In this poster, a novel method for coupling an ultra-fast POCL reaction to CE is presented. Post separation electrokinetic delivery of the POCL reagent bis(2,4,6-trichlorophenyl)oxalate (TCPO) was accomplished using a commercially available micro tee. Electrokinetic addition of TCPO allowed for precise control of the ratio of TCPO to the chemiluminescence (CL) reagents 1,2,2,6,6-pentamethylpiperidine (PMP) and 1,2,4-triazole (triazole), spiked into the running buffer. This novel method for CL reagent delivery avoided the problems and costs associated with using pressure or mechanical pumps to deliver reagents post separation. Use of this dual-component system (PMP and triazole) resulted in intense CL with half-lives of less than 2 seconds. Optimum conditions for CE-POCL detection were investigated using stopped-flow kinetics. The detection limit for 3-aminofluoranthene, following separation by CE, was <0.95 nM.

30. CHIRAL HPLC ANALYSIS OF VASOCONSTRICTOR DEGRADATION IN LOCAL ANESTHETIC INJECTIONS. *Jeffrey R. Ammann¹, Fernandina Cancañón¹, Brian F. Paulus¹, and Geoffrey A. Thompson². (1) Chemistry Branch, US Army Dental Research Detachment, 310B B St. Building 1-H, Great Lakes, IL 60088, Fax: 847-688-7380, jeffrey.ammann@na.amedd.army.mil, (2) Dental Biomaterials Branch, US Army Dental Research Detachment*

Degradation of the vasoconstrictor component ([-]-epinephrine bitartrate) of amide-based local anesthetics renders the injection less efficacious. Although the anesthetic component is relatively stable to harsh environmental conditions, the vasoconstrictor is sensitive to temperature, light, and susceptible to oxidation. We present a method involving the in situ quantification of biologically active and inactive enantiomers of epinephrine via chiral HPLC separation and subsequent UV-Vis detection. The enantioselectivity of selected chiral stationary phases, ionic strength of mobile phase, pH and flow rates were investigated. Individual samples were exposed to above ambient temperatures (37, 45, and 60°C), sunlight, temperature cycling and freezing (-20°C). Injection aliquots (10 µL) were analyzed via chiral HPLC at predetermined times. Good selectivity was obtained using a Cyclobond I 2000 RSP stationary phase (250mm × 4.6mm, 5 µm particle size). The mobile phase was 100 mM phosphate buffer (pH=4) at a flow rate of 0.5 mL/min. The epinephrine enantiomers were detected at 280 nm.

31. CHIRAL SELECTIVE MOLECULAR SENSORS FOR AMINO ACIDS BY ELECTROCHEMICAL DETECTION. Yanxiu Zhou¹, Kalle Levon¹, and Tsutomu Nagaoka². (1) Department of Chemical Engineering, Chemistry and Material Science, Polytechnic University, 6 Metrotech Center, Brooklyn, NY 11201, Fax: 718-260-3125, yzhou@duke.poly.edu, (2) Department of Applied Chemistry, Yamaguchi University

Highly selective molecular sensors for enantio-recognition of amino acids were prepared based on the monolayer molecular imprinting technique coupled with supporting electrolyte-free, buffer-free potentiometry. These sensors exhibited recognition properties towards one optical isomer of racemic N-carbobenzoxy-L-aspartic acid (CBZ-Asp) without any pre-separation process. The enantiomeric selectivity coefficients of the sensors for the counter isomers were in the range of 0.004 – 0.009. These supramolecular sensors translated an enantioselective molecular recognition event into a potential change. These supramolecular sensors translated an enantioselective molecular recognition event into a potential change to detect optically active CBZ-Asp in a concentration range of 5.0–10⁻⁶ – 0.012 M. Mechanistic investigation revealed that cationic amino acid was inserted into the cavity imprinted in the octadecyltrichlorosilane (ODS) layer (physical interaction) after dissociation to the electrically neutral amino acid by releasing a proton (chemical interaction). The sensitivity of these sensors could not come from molecular imprinting technology itself rather from the transducer of sensor. Furthermore, a supporting electrolyte-free, buffer-free potentiometric method was proposed for electrochemical enantio-discrimination and used in this research.

32. CHLORIDE DETERMINATION IN HIGH IONIC STRENGTH SOLUTION OF AMMONIUM ACETATE USING NEGATIVE ION ELECTRON SPRAY IONIZATION HPLC/MS. Shijiang Liang¹, Margaret J. Kupferle¹, Souhail Al-Abed², and Karen Miller Korana¹. (1) Department of Civil and Environmental Engineering, University of Cincinnati, P.O. Box 210071, Cincinnati, OH 45221-0071, liang.mark@epamail.epa.gov, (2) National Risk Management Research Laboratory, U.S. EPA

A precise ion chromatography method has been developed for the determination of chloride in high ionic strength ammonium acetate solutions (10E-5 M to 5 M) using sodium carbonate/sodium bicarbonate as eluent. Negative ion electrospray ionization (ESI) mass spectrometry was used for quantitation without electronic suppression. The retention time for chloride was 5.6 min. Linearity was obtained up to a concentration level of 1000 mg/l chloride when bromide was used as an internal standard. A detection limit as low as 62.5 mg/l (100 ml injection, 1.5 ml/min flow rate, and 1:44 split ratio) was achieved.

33. COLUMN MAINTENANCE AND LIFETIME. Jon R. Fisher, Sheila J. Iuliano, and Kevin O'Donnell, Technical Service, TosoHaas, 156 Keystone Drive, Montgomeryville, PA 18936, Fax: 215-283-5035, rsjrf@rohmmaas.com

With the advent of high-throughput screening, HPLC column lifetime has become even more important. HPLC columns might be required to perform thousands of separations in very short time periods. This study evaluated the effect of column maintenance on the stability of a TSK-GEL reversed phase chromatography (RPC) column. The column, the TSKgel Super-ODS, was packed with two micron spherical silica with a C18 functionality. A mixture of sulfa antibiotics was separated on the RPC column using an isocratic elution. Column performance was assessed using resolution, efficiency, and peak retention. Factors such as column cleaning and the use of pre-column filters were found to have a profound impact on column lifetimes. With proper maintenance, the column provided reproducible results for greater than 5,000 injections.

34. CONFORMATIONAL ANALYSIS OF HPLC SEPARATED 5-SUBSTITUTED CIS- AND TRANS- 2-PHENYL-1,3,2-DITHIARSENANES. Paul C. Bossle, AMSSB-RRT-CA E3300, U.S. Army, Edgewood Chemical & Biological Center, 5183 Blackhawk Road, Aberdeen Proving Grounds, MD 21010-5424, Fax: 410-436-1846, Scott Malcom, Department of Chemistry, University of Delaware, George W. Wagner, Research and Technology Directorate, U.S. Army ECBC, and Fu-Lian Hsu, AMSSB-RRT-TC, U.S. Army, Edgewood Chemical & Biological Center

The cis- and trans- isomers of 5-substituted 2-phenyl-1,3,2-dithiarsenanes are the adducts of the Lewisite simulant, phenyl dichloroarsine and the 2-substituted 1,3-propanedithiols. The isomers were separated by RPLC and identified by PDA/MS. Conformational assignments are determined by ¹H and ¹³C NMR analysis.

35. CONTROL OF THE IMMOBILIZATION OF BIOMOLECULES AT THE NANOSCALE ON CELLULOSE NANOFIBERS AND CARBON NANOTUBES. Ramesh B. Iyer, Jiangling Liu, Stephen M. C. Ritchie, Vasillis Gavallas, Jianquan Wang, Jamie Hestekin, Dibakar Bhattacharyya, and Leonidas G. Bachas, Department of Chemistry, University of Kentucky, Rose Street, C/P bldg, Lexington, KY 40506-0055, Fax: 859-323-1069, rbiyer0@sac.uky.edu

Two aspects of enzyme immobilization that are critical to their performance are the orientation of the protein relative to the solid support and the choice of the support. This presentation will focus on the properties of cellulose nanofibers (30-60 nm diameter with high aspect ratio), and carbon nanotubes as immobilization matrices. Enzymes can be site-specifically immobilized onto these supports by protein spacer methods or binding domains. For example, subtilisin was site-specifically immobilized onto cellulose nanofibers by a protein spacer method leading to high catalytic efficiency. Cellulose nanofibers functionalized with polyamino acid were used for high capacity heavy metal ion capture. Enzyme immobilization on cellulose nanofibers by using the cellulose-binding domain will also be discussed.

36. CYSTEINE-FREE MUTANT OF AEQUORIN: APPLICATION IN THE DEVELOPMENT OF BIOLUMINESCENCE-BASED IMMUNOASSAY FOR DIGOXIN. Suresh Shrestha, Insook R. Paeng, Sapna K. Deo, and Sylvia Daunert, Department of Chemistry, University of Kentucky, Lexington, KY 40506, Fax: 859-323-1069

Aequorin is a photoprotein found in the jellyfish, *Aequorea victoria*. It has been used as a sensitive label in the detection of various biomolecules. A cysteine-free mutant of aequorin produced recombinantly in our laboratory has been shown to be more active and stable than native aequorin. In this study, we employed this mutant of aequorin as a label in the development of immunoassay for a model analyte, digoxin. Digoxin is a cardiac glycoside and is used in the treatment of congestive heart failure. This drug has a very narrow therapeutic range of 1.0-2.4 nmol/L requiring therapeutic drug monitoring. The derivative of digoxin was chemically conjugated to the mutant protein and used in the immunoassay performed in a sequential binding format. The assay provided a detection limit of 1 × 10⁻¹² M for digoxin, and it was used in the determination of digoxin in serum samples. Potential interferents of digoxin were also evaluated to determine the specificity of the assay.

37. DETECTION OF OPTICALLY TRANSPARENT OR WEAKLY ABSORBING LIQUID-PHASE ANALYTES USING SENSITIVE LASER WAVE-MIXING SPECTROSCOPY. Jim Knittle, and William G. Tong, Department of Chemistry, San Diego State University, San Diego, CA 92182, Fax: 619-594-2442, william.tong@sdsu.edu

Forward-scattering laser wave mixing is presented as a sensitive absorption-based multi-photon spectroscopic technique for trace-concentration analysis of liquid-phase analytes. In condensed-phase systems, the thermal properties of the analyte and the solvent have a significant influence on the sharpness of the wave-mixing gratings, and hence, the resulting signal intensity. Since laser wave mixing is an absorption-based method, it is effective in detecting both chromophores and fluorophores with detection sensitivity levels comparable to those of fluorescence-based methods. Many weakly absorbing and optically transparent

compounds can be detected directly or indirectly using this nonlinear laser method.

38. DETERMINATION OF HYDOXYL CONTENT BY FTIR. *Keri L. Angone, and Nancy L. Coster, DSM Desotech, 1122 St. Charles Street, Elgin, IL 60120, Fax: 847-468-7703*

The synthesis of urethane acrylates is dependent on the equivalence of NCO and OH present. Since the synthesis of polyols has some degree of variability, it is also important for the polydispersity of the oligomer to have a calculated hydroxyl number for each individual batch of polyol. A standard method of calculating the hydroxyl number is to dissolve the sample in dimethyl formamide and react with a pyromellitic dianhydride reagent. Following the reaction, the excess reagent is hydrolyzed and titrated with potassium hydroxide solution. This can be a timely and cumbersome test. The use of silicone based polyols can be particularly challenging since side reactions have been known to occur during the hydrolysis of excess reagent. Therefore, it would be beneficial to develop a method that would allow us to use the material in its original form. Utilizing materials with known hydroxyl values, a calibration curve was established using FTIR. This allows for an accurate and timely evaluation of incoming raw materials.

39. DETERMINATION OF LANGMUIR SORPTION DISTRIBUTIONS OF BASIC SOLUTES IN REVERSED-PHASE CHROMATOGRAPHY SYSTEMS TO CHARACTERIZE SECONDARY ELUTE-STATIONARY PHASE INTERACTIONS. *Brett J. Stanley, and Jolie R. Krance, Department of Chemistry, California State University San Bernardino, 5500 University Parkway, San Bernardino, CA 92407, Fax: 909-880-7066, bstanley@csusb.edu*

The elution of bases in reversed-phase HPLC often results in asymmetric peak shapes due to secondary interactions with the modified silica surfaces typically utilized. Column test procedures record the retention times and peak asymmetries of basic elutes to identify the importance of such interactions, and column manufacturers have developed base deactivated reversed-phase silicas to reduce them. To continue progress in this field, a procedure has been developed by which the total heterogeneity of the solute-surface interactions can be characterized *in situ*. The approach obtains sorption isotherms using the frontal analysis method, and models them with a Langmuir isotherm model modified to allow a continuous distribution of binding constants over a wide range of values. The distributions yield the number of adsorption sites secondary to the primary hydrophobic partitioning mechanism. The relative strength and the quantitative amount of adsorption at each of these sites are readily determined. Results for several basic solutes and commercial C₁₈ phases are given.

40. DETERMINATION OF NITRITE AND NITRATE IN NATURAL WATERS BY SURFACE-ENHANCED RAMAN SCATTERING. *Erika Z. Gannon¹, Brandy Neely², and Brian D. Gilbert¹. (1) Department of Chemistry and Physics, Coastal Carolina University, P.O. Box 261954, Conway, SC 29528, Fax: 843-349-2841, eureka2584@aol.com, bgilbert@coastal.edu, (2) Marion High School*

Raman signals of molecules adsorbed to roughened noble metal surfaces can be enhanced by several orders of magnitude (10^4 to 10^7 greater than normal Raman scattering). Under favorable conditions, such surface-enhanced Raman scattering (SERS) makes detection of femtomolar concentrations possible. We have used SERS to detect nitrite and nitrate in natural waters at the picomolar level. Detection limits for NO₂⁻ and NO₃⁻ are in the 10- 50 nM range by standard colorimetric methods. Samples containing nitrite and nitrate were prepared according to standard methods for colorimetric analysis. The resulting azo dye solutions were added to colloidal metals (Ag, Au or Ag/Au alloys), and their SERS spectra were collected at 1=633 nm. SERS signals were found to be linear over a wide range of nitrite and nitrate concentrations. We will discuss the reproducibility of the method and its use for detection of nitrite and nitrate in natural water samples.

41. DETERMINATION OF THE STANDARD HETEROGENEOUS ELECTRON-TRANSFER RATE CONSTANT FOR NITROBENZENE DERIVATIVES BY USE OF ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY. *Charoenkwan Kraiya, and Dennis H. Evans, Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19711*

The effect of methyl substitution on the standard electron-transfer rate constant of nitrobenzene derivatives was determined at mercury electrodes in acetonitrile by electrochemical impedance spectroscopy (EIS). The rate constant was determined for electron exchange between the nitrobenzenes and their anion radicals. Five derivatives (2-nitrotoluene, 2-nitro-m-xylene, nitromesitylene, nitroindurene and pentamethylnitrobenzene) were chosen in this study in order to show the influence of steric repulsion between the *ortho* methyl groups and the nitro group, which hinders conjugation. The rate constant for each compound was obtained from two different analyses. The first required knowledge of the reactant concentration (C*) and electrode area (A) and the measurement was made with the DC potential set to the standard potential. The second method did not require knowing C* and A and the measurements were made at a series of DC potentials. Comparison of the rate constants obtained from those two analyses will be presented.

42. DEUTERIUM NMR ANALYSIS OF GLYCOGEN METABOLISM IN LIVER CELL. *Liwei Cao¹, Ruoqing Yang², Dean Sherry³, Craig Malloy¹, and Christopher Newgard². (1) Mary Nell and Ralph B. ROgers Magnetic Resonance Center, UT Southwestern Medical Center at Dallas, 5801 Forest Park Rd., Dallas, TX 75390, Fax: 214-648-5881, lcao@mednet.swmed.edu, (2) Gifford Laboratories for Diabetes Research, UT Southwestern Medical Center at Dallas, (3) Mary Nell and Ralph B. Rogers Magnetic Resonance Center, UT Southwestern Medical Center at Dallas*

Adenovirus-mediated gene transfer has been applied to change hepatic glucose disposal and production to reverse the metabolic abnormalities in diabetes and obesity. To investigate the contribution to hepatic glycogen increases through direct and indirect pathways, we present a novel ²H NMR analysis to quantitate positional ²H-enrichment of glucose from hydrolysis of hepatic glycogen, following ingestion of ²H₂O (4%). Our data clearly showed direct evidence that hepatic overexpression of protein targeting to glycogen (PTG), a member of the family of glycogen targeting subunit (GTS), increases glycogen contents in hepatocytes by enhancing indirect pathway in the absence of medium glucose. Further studies show no significant differences for the NMR profiles by overexpression of different GTS in hepatocytes. This NMR analysis provides a crucial metabolic measurement that is difficult to achieve otherwise. Furthermore, the method is sufficiently sensitive only need 3-10 mg glucose sample.

43. DEVELOPING FLUORESCENT PROBES FOR CATECHOLS. *Douglas E. Stack, Nicole M. Burns, Anastacia L. Hill, and Clark B. Diffendaffer, Department of Chemistry, University of Nebraska at Omaha, 6001 Dodge Street, Omaha, NE 68182-0109, Fax: 402-554-3888, destack@mail.unomaha.edu*

Metabolites of estrogen react with DNA to form catechol estrogen-DNA (CE-DNA) adducts. The detection of these adducts has been conducted primarily by high performance liquid chromatography (HPLC) separation using electrochemical detection (ED). HPLC-ED provides good sensitivity, but selectivity can be an issue when analyzing complex biological mixtures. To improve both the sensitivity and selectivity of CE-DNA adduct detection, we have sought to develop fluorescent probes specific to the catechol moiety. The reaction of catechols with activated gem-dibromides, produced by bromination of malonate esters, leads to the formation of an acetal, spiro ring system incorporating the catechol oxygens. When this reaction is performed on malonate esters that contain fluorescent groups, the resulting acetal product can be detected using HPLC fluorescence detection. The use of malonate esters containing either fluorene or anthracene fluorophores was conducted. Structural identification of the catechol derivatives was also investigated using fluorescence line narrowing spectroscopy.

44. DEVELOPMENT AND APPLICATION OF A CU(II) SENSITIVE NAFION® BASED OPTODE. Peter Iles¹, Terry J. Sands², Terrence J. Cardwell³, Robert Catrell³, John Farrell², and Spas Kolev³. (1) Department of Chemistry, Salt Lake Community College, 4600 South Redwood Road, PO Box 30808, Salt Lake City, UT 84130-0808, Fax: 801-957-4821, ilespe@slcc.edu, (2) Department of Applied Chemistry, RMIT University, (3) Department of Chemistry, LaTrobe University

A Cu(II) sensitive flow-through optode was developed by the immobilization of 4-decyloxy-2-(2'-pyridylazo)-1-naphthol (DPAN) into a membrane of the perfluorosulfonated cation-exchange polymer Nafion. DPAN is a derivative of the well-known colorimetric reagent and metallochromic indicator 1-(2'-pyridylazo)-2-naphthol (PAN) and similarly to PAN it forms colored complexes with a variety of transitional metal ions. The optode membrane was prepared by curing a mixture of DPAN and a commercial solution of 5% Nafion in lower aliphatic alcohols and water onto a glass substrate. The optode was studied in a single-line flow system shown schematically. The injection of the samples was performed by advancing the multi-port selection valve to the port connected to the sample solution and aspirating the sample solution for a predetermined time interval. After each optode measurement, a HCl acid solution was passed through the measuring cell to facilitate the dissociation of the corresponding metal complexes and exchange the metal ions released in the membrane with protons from the solution. The carrier solution flowing through the system prior to injection was deionized water. The optode sensitivity to Cu(II) ions, which give a blue colored complex with DPAN, was found to exceed that to other frequently analyzed metals ions (i.e., Zn(II), Fe(II/III), Pb(II), Cd(II), Al(III), Hg(II), Mn(II), Bi(III)) by several orders of magnitude. The optode flow system was optimized with respect to membrane and solutions composition, flow rate and the operation of the multi-port selection valve. Under optimal conditions the detection range for Cu(II) ions was between 2×10^{-6} and 0.8 mol/L. The optode flow system was utilized for the accurate quantitative determination of copper in two bronze samples and Cu(II) ions in tap water. Very good agreement (within 3%) with atomic absorption spectrometry results in all three cases was observed.

45. DEVELOPMENT OF A LONG-TERM DATABASE TO DETERMINE NUTRIENT LEVELS IN LAKE GREENWOOD, SOUTH CAROLINA. Marshall L. Deanhardt, Science Division, Lander University, Greenwood, SC 29649, Fax: 864-388-8130, ldeanhar@lander.edu

Lake Greenwood has been plagued by problems with excessive algal growth. Excessive nutrients (in particular, phosphate) are believed to be responsible for the undesirable algal blooms. This paper describes the development of a database that tracks nutrient levels in water flowing into, circulating through, and out of the Lake. Three independent techniques were used to measure nutrient levels: capillary electrophoresis (CE), colorimetry (ascorbic acid method for ortho and total phosphate), and direct potentiometry (ion selective electrode for nitrate). Results reported in this paper will help identify excessive nutrients entering and exiting the Lake and may also identify the causes and possible methods of controlling algal blooms within the Lake.

46. DEVELOPMENT OF A REAGENTLESS BIOSENSING SYSTEM FOR GLUCOSE. Bethel V. Sharma¹, Lyndon L. E. Salins², and Sylvia Daunert². (1) Division of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536, bvshar@pop.uky.edu, (2) Department of Chemistry, University of Kentucky

The wild-type E.coli periplasmic d-glucose, d-galactose binding protein (GBP) has been employed in the design of a reagentless optical biosensing system for the detection of glucose. Labeling of the protein was accomplished by replacement of the calcium ion present in the single calcium-binding site of GBP with a lanthanide metal. Upon binding glucose, a conformational change in GBP based on its characteristic two domain hinge-like motion causes a change in the fluorescence intensity dependent upon the amount of glucose present in the sample. Calibration curves for glucose have been obtained using several different lanthanides as reporter elements. Results indicate that the system is nearly four times more responsive using terbium than when using europium in steady-state fluorescence measurements. The selectivity of the system against other sugars, as well as the potential applications in the development of a blood

glucose monitoring system in the treatment of diabetic patients will be discussed.

47. DEVELOPMENT OF A SENSING SYSTEM FOR cAMP BASED ON THE cAMP RECEPTOR PROTEIN. Sylvia Daunert, and Agatha Feltus, Department of Chemistry, University of Kentucky, College of Pharmacy, Lexington, KY 40506, ajfelt00@pop.uky.edu

CRP is a cAMP-binding protein from the bacterium Escherichia coli used in the regulation of transcription from various operons. The protein exists in two different conformations depending upon whether or not cAMP is bound. By taking advantage of this large conformational change upon cAMP binding, a sensing system for cAMP was developed, in which the signal from a fluorescently-labeled CRP is dependent upon the amount of cAMP present. In this system environmentally-sensitive thiol-reactive fluorophores are used to site-specifically label the binding protein at various sites chosen through examination of CRP's crystal structure. Using this approach, detection limits on the order of 10-14 M cAMP using the fluorophore acrylodan have been achieved. Optimization of this sensing system depends upon choosing the optimal fluorophore and location on the protein. We will also investigate the environment of the fluorophore using binding and lifetime studies. Applications of this sensing system for cAMP will also be discussed.

48. DEVELOPMENT OF AN ASSAY FOR 6-KETO PGF1 α EMPLOYING 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE: SENSING PROSTACYCLIN IN PHYSIOLOGICAL FLUIDS. Phillip M. Douglass¹, Sapna K. Deo², C. Mark Ensor², Marc Madou³, and Sylvia Daunert². (1) Department of Pharmaceutical Sciences, University of Kentucky, Rose Street, Lexington, KY 40536, pmdoug0@pop.uky.edu, (2) Department of Chemistry, University of Kentucky, (3) Department of Materials Science and Engineering, Ohio State University

Prostacyclin (PGI₂) has been employed in the therapy of several diseases, most notably pulmonary hypertension. The lack of an accurate and readily available assay for the measurement of PGI₂ has been a major challenge for clinicians in determining its proper dosing. Our strategy in the development of a sensing system for PGI₂ involves the use of the enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in the detection of 6-keto PGF1 α , the first metabolite in the metabolic pathway of PGI₂. A new plasmid has been prepared by inserting the gene encoding human 15-PGDH into a high expression vector. The plasmid was transformed in E. coli and the protein was expressed. Following purification, the enzyme was characterized with respect to its activity and kinetic properties. A potentiometric-based assay for 6-keto PGF1 α was then developed. Upon addition of increasing concentrations of 6-keto PGF1 α , in the presence of 15-PGDH and NAD⁺, we were able to construct calibration curves correlating electrochemical potential to 6-keto PGF1 α levels in solution. The selectivity of the assay system for 6-keto PGF1 α was evaluated against potential interfering compounds.

49. DEVELOPMENT OF AN ELECTRICALLY TUNABLE, COMPENSATED, OPEN CYLINDRICAL ION TRAP MASS SPECTROMETER. Ryan M. Danell, Ken L. Ray, and Gary L. Glish, Department of Chemistry, University of North Carolina at Chapel Hill, CB# 3290, Venable Hall, Chapel Hill, NC 27599, Fax: 919-962-2388, rdanell@email.unc.edu

Ion trap mass spectrometers have enjoyed increased use over the past years due to their high performance, relatively low cost, and several key technology advances which have extended their applicability. The use of higher order fields, in particular, has become a popular method to enhance ion trap performance. The compensated cylindrical ion trap is a unique design that allows higher order field components to be electrically, rather than mechanically tuned, possibly during an experiment. The geometry also provides potential for more efficient ion injection and detection, as well as convenient application of photodissociation techniques. A custom cylindrical ion trap system has been built and allows complete control over all operation parameters during any experimental step. The methodology and techniques used to construct this research grade instrument, as well as control software designed for it will be discussed. Data obtained from initial characterization experiments also will be presented.

50. DEVELOPMENT OF BIO-INSPIRED POLYMERS FOR EFFICIENT NITRIC OXIDE DELIVERY. *Karen M. Padden, and A. S. Borovik, Department of Chemistry, University of Kansas, Malott Hall, 1251 Wescoe Hall Dr, Lawrence, KS 66045-7582, Fax: 785-864-5396, kmpadden@eagle.cc.ukans.edu*

Template copolymerization of molecules into highly cross-linked network polymers has been used to create mono-dispersed immobilized sites in synthetic polymers for reversible binding of gaseous analytes. Polymerizable functional groups are arranged around template molecules or ions which upon copolymerization with cross-linking monomers, form immobilized sites. These sites contain microenvironments that have the same size and shape, as well as, functional group orientation as the template molecule. The template design will be based on biological systems found in nature. The polymeric materials will contain immobilized metal complexes, which are inspired by proteins that store and release nitric oxide. There are several key architectural features found in the active sites of these proteins that promote the efficient storage and release of NO. This poster will describe efforts to incorporate molecular components, analogous to structural features of protein active sites, into a polymer host to produce materials with tunable NO binding/release properties.

51. DEVELOPMENT OF THE SCANNING ELECTROCHEMICAL MICROSCOPE (SECM) FOR STUDYING LIVE MAMMALIAN CELLS IN VITRO. *Johanna M. Liebetrau¹, John E. Baur¹, and Paul A. Garris², (1) Department of Chemistry, Illinois State University, Normal, IL 61790-4160, jmliebe@ilstu.edu, (2) Department of Biological Sciences, Illinois State University*

The scanning electrochemical microscope (SECM) is uniquely suited to advance biological investigations of live mammalian cells in vitro as a result of its capability to image morphology and chemical release from surfaces. Feasibility studies have been conducted using cultured PC12 cells, an immortal cell line widely accepted as a model system for studying neuron function. After screening for biocompatibility, five different redox mediators were selected for preliminary topographical imaging of PC12 cells. These results will be presented. In addition, studies were conducted to demonstrate the capability of the SECM to record induced morphological changes of the cells. These results demonstrate the utility of the SECM for investigating neuron differentiation, a subject of intense interest, and changes in cell volume in response to osmotic events. Of critical importance to the success of scanning electrochemical microscopy in biology is the development of probes with improved spatial and chemical resolution. Fabrication of carbon fiber microelectrodes that meet these objectives has been a central focus of this project.

52. DIFFERENTIAL PULSE VOLTAMMETRY FOR A FIRST-ORDER EC PROCESS. *Myung-Hoon Kim, Department of Science, Georgia Perimeter College, 2101 Womack Rd., Dunwoody, GA 30338-4497, Fax: 770-551-7097, mkim@gpc.peachnet.edu, Ronald L. Birke, Department of Chemistry, City College, City University of New York, and Myung-Zoon Czae, Department of Chemistry, Hanyang University*

Theoretical expressions for the differential pulse voltammetry (DPV) for a reversible electron transfer coupled with an irreversible follow-up first-order chemical reaction (EC) is derived. The current peaks as given by the current expressions are analyzed in terms of several parameters such as a ratio of anodic-to-cathodic peak currents, a separation in the peak-potentials, and a ratio of anodic-to-cathodic half-peak-widths in order to characterize the EC process and distinguish it from other types of electrode processes. The anodic peak is found to be more susceptible to the post kinetics than the cathodic peak. The new parameter of the peak-width ratio is much more sensitive to the post kinetics than the peak separation. The peak current ratio and the peak-width ratio have comparable sensitivities to the kinetics. Hence, the peak-width ratio is a better diagnostic criteria than the peak separation which has a poor sensitivity. This phenomenon is different from cyclic voltammetry (CV) in which the peak separation is as sensitive as the peak current ratio. The new criteria for EC with DPV is tested and successfully applied to several Co(III) complex systems, including coenzyme B12. The homogeneous rate constant for the follow-up step is estimated from the measurements of the experimental values of the parameters.

53. DIFFUSE REFLECTANCE FTIR SPECTROSCOPIC STUDY OF POLAR AND POLAR-EMBEDDED STATIONARY PHASES FOR NORMAL AND REVERSED-PHASE HPLC. *Kristina G. Proctor, Chad P. Gonzales, Cheryl A. Brown, and Loretta Sandoval, Department of Chemistry, University of Southern Colorado, 2200 N. Bonforte Boulevard, Pueblo, CO 81001, Fax: 719-549-2580, proctor@uscolo.edu*

Surface interactions of aminopropyl (-CH₂CH₂CH₂NH₂) and long chain alkylamide derivitized silicas (SiO₂) have been comparatively studied using diffuse reflectance FTIR spectroscopy (DRIFTS). In the case of the reversed-phase material, polar-embedded functionalities are strategically implemented to interact with unreacted surface silanol sites (SiOH). The surface concentrations of accessible, silanol sites (3740 cm⁻¹) on the unreacted and chemically modified surfaces have been quantified using a titration procedure. The presence and nature of hydrogen bonding interactions of amine and carbonyl constituents have been ascertained through respective infrared bands in the N-H and C=O stretching regions of the respective DRIFT spectra. Data and results will be presented with respect to the presence and proportion of ligand sites that experience interaction with surface silanol sites.

54. DNA MICROARRAY TECHNOLOGY IN THE HUMAN GENOME PROJECT. *Christie M. Sayes, and Steven A. Soper, Department of Chemistry, Louisiana State University, 232 Choppin Hall, Baton Rouge, LA 70803, christie.sayes@usa.net*

The human genome project is focused on completing the first draft of the entire complement of nucleotide bases (3x10⁹) comprising this genome. New tools are rapidly being developed to interrogate the DNA structure on a genome-wide scale and eventually possess the ability to determine the structure at the single nucleotide base level. DNA microarrays, which consist of high-density arrays of oligonucleotide probes that contain unique sequences, are one such tool that can interrogate the DNA structure. As the interest in microarrays increases, the likelihood of microarrays associated with genetic testing for diseases in individuals and populations also increases. Along these lines, various analytical methods have been employed to help in the success of microarray technology. In terms of readout modes, fluorescence spectroscopy is a common mode. This presentation discusses the use of fluorescence lifetime identification protocols for increasing the information content associated with reading DNA microarrays. Specifically, this presentation focuses on difficult algorithms used for processing lifetime data obtained using time-correlated single photon counting methods for monitoring hybridization events on solid substrate materials.

55. ELECTRIC FIELD DIRECTED SELF-ASSEMBLY OF NUCLEIC ACID PROBES AND PNA-DNA HYBRIDIZATION REACTIONS. *Lisa Henke, Max Planck Institute for Polymer Research, Ackermannweg 10, Mainz 55128, Germany, Fax: 011 49 6131 379 100, henke@mpip-mainz.mpg.de, and Wolfgang Knoll, Max-Planck-Institute for Polymer Research*

Molecular diagnostics of genetic material in the form of DNA biosensors provides a highly sensitive and specific route to detection of pathogens and elucidation of genetic variations among cells. It has recently been shown that electric fields may be utilized to control the transport of DNA to surfaces, as well enhance the hybridisation reaction kinetics and improve the stringency of nucleic acid interactions at an interface. Electric field strength thus provides an independent parameter to control hybridisation reactions in addition to salt concentration, pH, temperature and chaotropic agents. We have incorporated a multi-electrode array with individually addressable sensing chips and a SPM/SPFM detection format that provides a unique opportunity to study PNA-DNA hybridisation reactions as well as the self-assembly process of nucleic acids at the gold surface. The use of PNA probes incorporated with electric control should greatly enhance the specificity and speed of hybridisation reactions providing improved diagnostic systems.

56. ELECTROACTIVE POLYMER FILMS CONTACTED WITH SUPERCRITICAL CARBON DIOXIDE. *John Christopher Hutchison, Dongil Lee, Joseph M. DeSimone, and Royce W. Murray, Kenan Laboratories of Chemistry, University of North Carolina-Chapel Hill and NSF-STC, Chapel Hill, NC 27599-3290, hutchiso@email.unc.edu*

Current interest in supercritical carbon dioxide (sc-CO₂) as an environmentally friendly solvent highlights a need to develop analytical methods applicable to sc-CO₂ based systems. We are investigating the use of reporter films based on poly(ethyleneoxide) with included salts contacted with sc-CO₂ as analytical tools and to answer fundamental scientific questions of diffusion and electron transfer. Impedance spectroscopy and voltammetry experiments illustrating progress in this area will be described.

57. ELECTROCHEMICAL ANALYSIS OF THIAMIN PYROPHOSPHATE AND OTHER THIAMIN DERIVATIVES. *Jeffrey Allen Sutton, and Masangu Shabangi, Department of Chemistry, Southern Illinois University Edwardsville, 1918 Terrace Drive, Alton, IL 62002, nottusffej@hotmail.com*

Thiamin pyrophosphate is the metabolically active coenzyme of a class of enzymes that catalyze acyl group transfer reactions. Its deficiency in humans results in an ultimately fatal condition known as beriberi, characterized by neurological disturbances causing pain, paralysis and atrophy of the limbs. Currently, most commonly used techniques for assessing the status of thiamin in foods, pharmaceutical preparations and clinical applications are based on a process called the thiochrome procedure. Methods based on the thiochrome procedure are both costly, time consuming, and they pose difficulties in accurately determining various forms of thiamin. By electrochemically characterizing various forms and derivatives of thiamin, new approaches that minimize steps in sample treatments and show cost efficiency to thiamin determination in various media and samples can be accomplished. The results of the electrochemical investigation of thiamin pyrophosphate and other derivatives, along with the optimization of experimental conditions in terms of solvents, pH, and electrolytes will be presented.

58. ELECTROCHEMICAL AND SURFACE ANALYSIS STUDY OF COPPER CORROSION PROTECTION BY 1-PROPANETHIOL AND PROPYLTRIMETHOXYSILANE: A COMPARISON WITH 3-MERCAPTOPROPYLTRIMETHOXYSILANE. *Rolando J. Tremont, and Carlos R. Cabrera Sr., Department of Chemistry, University of Puerto Rico, P.O. Box 23346, San Juan, PR 00931, Fax: 787-756-8242, rtremont@rrpac.upr.clu.edu*

The chemisorption of alkanethiols over copper has been input to practice. Here, we present an alkanethiol and a silane that are very effective protectors of surfaces against corrosion. The 1-propanethiol (1-PT) and propyltrimethoxysilane (PTS) molecules were investigated as a copper corrosion inhibitors in 0.100 mol L⁻¹ KCl solution and compared to 3-mercaptopropyltrimethoxysilane (MPS). The corrosion inhibition was studied as a function of the 1-PT and PTS concentration in ethanol, between 1.0×10^{-7} mol L⁻¹ and 1.0×10^{-2} mol L⁻¹. Inhibition efficiency were calculated from Tafel plots done in 0.100 mol L⁻¹ KCl solution. The inhibitor efficiency improved concomitant with an increase on 1-PT and PTS concentration. Maximum efficiency was obtained at a 1-PT and PTS concentration of 1.0×10^{-3} mol L⁻¹ and 1.0×10^{-5} mol L⁻¹, respectively. The adsorption behavior of the 1-PT and PTS followed a Langmuir isothermal function. Polarization studies indicate that the 1-PT and PTS are an anodic as well as a cathodic inhibitor, in presence of dissolved oxygen. When the exposure time was varied in 0.100 mol L⁻¹ KCl solution, lost efficiency in the copper corrosion protection was observed in the three (MPS, PTS and 1-PT) compounds. However, the 1-PT compound maintained an excellent protection in the first 12 hours, afterward it had a significant loss in inhibition efficiency. Surface analysis studies with Auger electron spectroscopy (AES) and X-ray photoelectron spectroscopy (XPS) showed that the organic compounds modify the Cu surface. Scanning electron microscopy (SEM) analysis showed that the organic compounds protect the copper surface against the KCl solution corrosive attack, showing better behavior the 1-PT followed by MPS and PTS.

59. ELECTROCHEMICAL RESPONSE OF HEMOGLOBIN AT A SURFACTANT COVERED ELECTRODE. *Lyman H. Rickard, George J. Hager, and Joseph M. Pigga, Department of Chemistry, Millersville University, Millersville, PA 17551, Fax: 717-872-3985, Lyman.Rickard@millersville.edu*

Because many proteins function in biological systems to transfer electrons there is considerable interest in the electrochemical behavior of these proteins at electrode surfaces. Recent investigations have shown an increased electrochemical response for heme proteins at electrodes which have been covered by a surfactant film. Results of an enhanced electrochemical response of hemoglobin at a surfactant film electrode will be described. The dependence of the electrochemical response on time, experimental conditions and method of surfactant film preparation will also be described.

60. ELECTROCHEMISTRY IN SUPERCRITICAL CO₂. *Dongil Lee, John Christopher Hutchison, Joseph M. DeSimone, and Royce W. Murray, Kenan Laboratories of Chemistry, University of North Carolina Chapel Hill and NSF-STC, Chapel Hill, NC 27599-3290, dilee@email.unc.edu*

Supercritical CO₂ has recently received wide attention in the fields of cleaning, extraction and polymer synthesis. Applications of supercritical CO₂ prompt a need for measurement tools that support basic investigations of chemical reactions in supercritical CO₂ media. Supercritical CO₂ is a challenging medium for electrochemical investigations owing to its low dielectric constant and resulting high resistivity. We have studied several approaches to this problem, including the design and synthesis of new supporting electrolyte systems and cosolvents. Microelectrode voltammetry, diffusion rates, and ionic conductivity of these new media will be presented.

61. ELECTROCHEMISTRY OF MELANIN FILMS: DIRECT MEASUREMENT OF THE REVERSIBLE OXIDATION OF DHI-MELANIN AND ITS CU AND ZN ADDUCT. *Shirley Gidanian, and Patrick J. Farmer, Department of Chemistry, University of California, Irvine, Irvine, CA 92697-2025, Fax: 949-824-2210*

Synthetic melanoid films, formed by electrochemical deposition onto electrode surfaces by oxidative polymerization of 5,6-dihydroxyindole solution were used to directly measure the chromophore's redox reactivity by voltammetry and coulometry. Films on optically transparent electrodes allowed correlation of spectral changes with electrochemical potential. Spectroelectrochemical titrations under nitrogen yield a reversible reduction occurring at E_{1/2}=250 mV vs. NHE. At higher potentials, an irreversible bleaching becomes dominant which is accelerated in the presence of oxygen. Loss of absorbance due to this irreversible reaction is greater at higher wavelengths, which allows differentiation between the two reactions. Exposure of these samples to Cu²⁺ and Zn²⁺ leads to increased absorbance at 500 nm. We believe this effect is caused by interaction of hydroquinone functionalities of melanin with metal ions to make stable complexes. Our data suggest that coordination of Cu²⁺ with melanin is stronger than Zn²⁺, and E_{1/2} of these samples are also different.

62. ELECTROSPRAY MASS SPECTROMETRY STUDY OF SOLVOTHERMAL GROWTH OF SEMICONDUCTOR NANOCRYSTALS. *Orlando E. Raola, Department of Chemistry and Biochemistry, University of California Santa Barbara, Santa Barbara, CA 93106-1905, Fax: 805-893-4120, oraola@chem.ucsb.edu, and Geoffrey F. Strouse, Department of Chemistry, University of California Santa Barbara*

ESMS has been used to monitor the species involved in the solvothermal growth of CdSe and MoS₂ nanocrystals from cluster molecular precursors. The size of the resulting nanocrystals is calculated from their absorption spectra and they have a narrow size distribution (~5%) estimated by TEM. Samples of the growth solution were drawn at different temperatures, dispersed in dry acetonitrile to yield solutions approx. 10⁻⁴ mol L⁻¹ that were directly injected to a TurbolonSpray source. Positive and negative ions were analyzed at optimized orifice and ring voltages. The evidence for different growth paths can be inferred from the presence of various molecular and fragment ions in the mass spectra.

63. ENHANCED ATOMIC FORCE MICROSCOPY IMAGING OF DNA HYBRIDIZATION BY UTILIZING DNA-CAPPED GOLD NANOPARTICLES. *Shubo Han, Jianqiao Lin, and Feimeng Zhou, Chemistry and Biochemistry, California State University, Los Angeles, 5151 State University Drive, Los Angeles, CA 90032, Fax: 323-343-6490, hanshubo@hotmail.com, linjianqiao@netscape.net, fzhou@calstatela.edu*

A novel assay for selective determination of polynucleotides using AFM together with the formation of the probe-target-DNA/gold nanoparticle sandwich structure at gold surface is described. A 17mer probe was attached to the surface for subsequent hybridization with a polynucleotide target. The hybridization efficiency can only be estimated to be about 1.1% since certain surface features could not be resolved. The 30mer-capped gold nanoparticles, not only provides another dimension of selectivity, but also reoriented the previously formed probe-target hybrid in such a way that the strands of the target become tethered with respect to the surface. Due to the improvement in resolving the hybridized target molecules, an accurate determination of the hybridization efficiency (16.5%) was achieved. Quartz crystal microbalance (QCM) was also employed to quantify the hybridization and the results are compared with the findings of AFM.

64. ENZYME ELECTRODES FOR RAPID AMPEROMETRIC BLOOD GLUCOSE LEVEL DETERMINATION. *Mark F. Sistare, and Phyllis Palmer, Glucose monitoring systems, BD Technologies, 21 Davis Drive, Research Triangle Park, NC 27709, Fax: 919-313-6402, Mark_Sistare@bd.com*

Enzyme-modified electrodes for the rapid determination of blood glucose levels have been studied. These electrodes are sensitive to changes in glucose concentration over the range typically observed in clinical samples, 50 – 500 mg/dL, or 3 – 30 mM. Response times of less than ten seconds have been observed for these electrodes, in which glucose oxidase is either electrodeposited on the electrode surface or entrapped in an electrogenerated polymer matrix. For these non-mediated systems, in which H₂O₂ generated by the glucose oxidase is measured, the signal obtained in low-concentration solutions exceeds 1 µA/mm² and 150 nA/mm² for the electrodeposited and polymer-entrapped enzyme films, respectively. The effects of both electrodeposition conditions and the nature of the electrogenerated polymer on the sensitivity of these electrodes will be presented.

65. ESI- AND MALDI-MS OF ALUMINUM PEPTIDES AND PROTEINS. *Patrick A. Limbach, Jessica A. Ragas, Courtney Patrick, Melissa E. Griggs, and Melissa Bailey, Department of Chemistry, Louisiana State University, 232 Choppin Hall, Baton Rouge, LA 70803, Fax: 225-388-3458, plimbac@unix1.sncc.lsu.edu, jragas@cs.com*

Aluminum is toxic to the central nervous system (CNS). Impairment by aluminum of normally nontoxic substances, such as behaviorally active amines and peptides, might lead to the dysfunction of the CNS. As a necessary first step in understanding the role of aluminum in such diseases, methods must be developed that can characterize aluminum-binding compounds. In this work, we describe our use of electrospray ionization mass spectrometry (ESI-MS) and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) for the analysis of aluminum containing peptides and proteins. A description of optimal sample preparation methods for each technique will be presented, as well as a comparison of the merits of ESI-MS vs. MALDI-MS for locating the binding sites of aluminum in proteins.

66. ESTIMATION OF ESSENTIAL ELEMENTS IN FINGERNAIL SAMPLES IN NON-INDUSTRIALIZED PAKISTANI POPULATION. *Shahnaz Kazi, Rukhsana Kazi, Gul Kazi, Tasneem Kazi, and Syed Ali, Analytical Chemistry, NCEAC, University of Sindh, Jamshoro, Pakistan, kazishahnaz@hotmail.com, ghkazi@yahoo.com*

The concentration of four essential element viz. Iron(Fe) Zinc(Zn) Copper(Cu) and Manganese(Mn) were determined in finger nails of 375 human subjects by Atomic Absorption Spectrophotometry. All of these subjects belonged to non-industrial areas. Samples were washed by modified method described by S.Kazi and et al1. The sample solutions were prepared by wet acid digestion

method. Estimation was performed using Atomic Absorption Spectrophotometer of Hitachii Model 180-50. The purpose of the study was to evaluate concentration of essential elements in fingernails and the effects of various physiological parameters like age, sex. The mean and standard deviation values are presented for each element along with a summary of the effects of age, sex, and nutritional values on the concentration of each of these elements in nails. The results showed that the concentration of Zn, Fe and Mn is sex dependent. Comparative study with other reported data for population of other countries was also performed. All these data represent a significant contribution to existing information on nails of non-industrialized population of Pakistan.

67. ESTIMATION OF RECEPTOR-LIGAND INTERACTIONS BY THE USE OF A TWO-MARKER SYSTEM IN AFFINITY CAPILLARY ELECTROPHORESIS. *John Kaddis, Ying Zhang, and Frank A. Gomez, Chemistry and Biochemistry, California State University, Los Angeles, 5151 State University Drive, Los Angeles, CA 90032, Fax: 323-343-6490, MITSRAYIM@YAHOO.COM*

The study of receptor-ligand interactions by affinity capillary electrophoresis (ACE) requires an accurate form of analysis. Here, we examine the use of two non-interacting standards (markers) in the analysis of binding constant data in ACE studies. This concept is demonstrated using two model systems: carbonic anhydrase B (CAB, EC 4.2.1.1) and arylsulfonamides, and vancomycin (Van) from *Streptomyces orientalis* and the dipeptide N-acetyl-D-Ala-D-Ala. In this procedure a plug of receptor and non-interacting standards is injected, and analysis of the change in the relative migration time ratio, RMTR, of the receptor, relative to the non-interacting standards, as a function of the concentration of the ligand, yields a value for the binding constant. The findings described here demonstrate that data from ACE studies can best be analyzed using two non-interacting standards yielding values comparable to those estimated using other binding and ACE techniques.

68. EVALUATION OF ASSOCIATION CONSTANTS BETWEEN BASIC ENANTIOMERS AND SULPHATED CYCLODEXTRINS USING COUNTER-FLOW PARTIAL FILLING CE TECHNIQUE. *Angela H. Wu, and David C. Locke, Department of Chemistry and Biochemistry, Queens College and Graduate Center of the City University of New York, 65-30 Kissena Blvd., Flushing, New York, NY 11367, Fax: 718-997-5531, wxhqc@qcunix1.qc.edu*

Counter-flow partial filling capillary electrophoresis was used to determine association constants of various chiral amine pharmaceuticals with sulfated cyclodextrin (CD) as chiral selector. Various lengths of CD solution in background electrolyte (BGE) were vacuum-introduced into the detector end of the capillary. Analyte solution was injected into the other end. Enantioseparation was based on counter-flow of the CD and analyte zones. The analytes pass through the zone of BGE containing CD, which is moving towards the injection end, and interact with it transiently and differentially. The fused-silica capillary was coated with polyacrylamide and phosphate BGE with a low pH was chosen in order to minimize the interference by EOF. Because of the strong interactions between the enantiomers and the polycharged selector, concentrations as low as 0.1mM CD were needed to achieve the chiral separation. It was found that the apparent migration times change linearly with the partial-filling fraction. A new method to evaluate the apparent association constant between chiral compounds and charged cyclodextrin is proposed using this linearity. Association constants were compared for different brands of sulfated cyclodextrins.

69. EVALUATION OF CALCIUM AND MAGNESIUM IN HUMAN SCALP HAIR OF LOCAL POPULATION OF HYDERABAD SINDH. *Shahnaz Kazi¹, Rukhsana Patel¹, Gul Kazi², and Tasneem Kazi². (1) Department of Chemistry, Govt. K.B.M.S College, Hyderabad, B/99-462, Tilak-in-cline, Hyderabad 71000, Pakistan, kazishahnaz@yahoo.com, (2) Analytical Chemistry, NCEAC, University of Sindh*

Many elements appear in hair in concentrations related to those in blood, but the relationship for Calcium(Ca) and Magnesium(Mg) is complicated by disorders that probably affect their state in the blood. The Ca and Mg content in hair of 625 inhabitants living in different localities of Hyderabad Sindh was assayed by means of Atomic Absorption Spectrophotometer, by preparing sample solution by wet digestion procedure(S. Kazi and et al.1999). A comparison with the nutritional values, and biological and physiological factors like age

and sex at the same time reveal possible effect of these factors on the availability of Calcium and Magnesium to incorporation in the hair. The hair of female exhibited statistically significant higher values of Calcium and Magnesium in comparison to male's hair although case of external contamination, the inverse relation was found. Concentration of Ca and Mg is also strongly aged related too. The AAS of Hitachi model 180-50 was carefully calibrated for Ca and Mg estimation. Precautions were taken during sampling, washing, wet digestion with concentrated Nitric acid and Hydrogen per oxide and analysis.

70. EVALUATION OF METALLIC MOLECULAR BANDS IN HYBRID ROCKET PLUMES. Lize Wilcox¹, Kari Maxwell¹, and M. Keith Hudson². (1) Department of Chemistry, University of Arkansas at Little Rock, 2801 S. University, Little Rock, AR 72204, biff117@msn.com, (2) Graduate Institute of Technology, University of Arkansas at Little Rock

This study deals with the emission spectrum of a hybrid rocket plume when the fuel is doped with various salts. Interaction at high metal concentrations will be studied which give rise to molecular band emissions. The region of interest is in the ultraviolet-visible region (300-750nm). The most likely molecular band emissions are from the excited states of the metal oxides or metal hydroxides formed by these metals in the presence of the oxidizer feed in the hybrid rocket chamber. Initial studies have hinted at a linear relationship between concentration and intensity for some metals, yet show non-linearity for other. Metals such as calcium, barium, and copper show a linear relationship, yet manganese, magnesium, and strontium show a non-linearity relationship. Such spectra can be used as diagnostic tools when troubleshooting rocket engine performance and in formulating various pyrotechnics.

71. FLUORESCENCE-BASED GLUCOSE SENSORS. Peter Harms, Yordan Kostov, and Govind Rao, Department of Chemical and Biochemical Engineering, University of Maryland, Baltimore County, 1000 Hilltop Circle, ECS Room 100, Baltimore, MD 21250, harms@umbc.edu

Transdermal glucose sensing remains the Holy Grail of biosensors, and has driven many areas of research. A glucose sensor based on Concanavalin A and dextran with NIR fluorescent dyes was constructed. The sensor was optically interrogated through a layer of chicken skin, and the sensor response was characterized. A sensor based on boronic acid was also constructed that exhibits rapid and reversible changes in fluorescence lifetime with glucose. This sensor was characterized for sensitivity and specificity. The two methods were compared, highlighting the benefits and limitations of each system.

72. FRAGMENTATION OF MULTIPLY CHARGED PEPTIDES: EFFECTS OF PROLINE, ARGinine, ASPARTIC ACID, AND HISTIDINE. Justin C. Rose, and Elaine M. Marzluff, Department of Chemistry, Grinnell College, 1116 8th Ave, Grinnell, IA 50112, Fax: 515-269-4285, rosej@grinnell.edu

Understanding the fragmentation patterns of peptides and proteins in the gas phase is useful for determining the primary sequence of unknown peptides using mass spectrometry. The present study examines the effect of proline, arginine, aspartic acid and histidine in a peptide sequence on the gas phase dissociation patterns observed as a function of charge state. Peptides are introduced into the mass spectrometer using electrospray ionization and fragmentation is induced using low energy collision induced dissociation. For most peptides in low charge states, we observe primarily the loss of neutral molecules such as water and ammonia. At higher charge states proline containing peptides exhibit preferential cleavage adjacent to proline. In addition, selective cleavage adjacent to aspartic acid, and histidine is observed, and this fragmentation is more pronounced when these amino acids are next to proline. Mechanisms are proposed for these observed cleavage patterns.

73. FUNCTIONALIZED DENDRIMER MONOLAYER AS A PLATFORM FOR BIOSPECIFIC AFFINITY-SENSING. Hyun C. Yoon, Mi-Young Hong, Dong-Eun Lee, and Hak-Sung Kim, Department of Biological Sciences, Korea Adv. Inst. Sci. & Technol, 373-1, Kusung-dong, Yusung-ku, Taejon 305-701, South Korea, Fax: 82-42-869-2610, hcyoon@bioneer.kaist.ac.kr

For an affinity biosensing interface with high sensitivity and selectivity, construction of molecularly organized sensing surface representing high density of ligand

groups with adequate accessibility, fulfilling efficient affinity reaction and easy signal generation, is required. In this respect, functionalized dendrimer was employed for construction of monolayers on gold surfaces and used for affinity biosensing. As a model system, an electrochemical affinity biosensor based on avidin-biotin interaction was developed. A double-functionalized monolayer of ferrocenyl-tethered and biotinylated dendrimer was constructed on gold as an electrochemical affinity biosensing surface. And, a new approach to the development of a regenerable affinity sensing surface was attempted. The sensing surface was constructed by using dendrimers, whose surface chain-end groups have been modified with biotin or its analogue. The resulting monolayer acted as affinity recognition interfaces for avidin and anti-biotin antibody molecules, showing biospecific "on-off" reaction cycles when displacement and association steps were repeated.

74. GOLD COATED SiO₂ MODIFIED WITH SELF-ASSEMBLED MONOLAYERS AS A POTENTIAL STATIONARY PHASE FOR LIQUID CHROMATOGRAPHY. Yemilyn Ortiz¹, Jose M. Cintron², and Luis A. Colon². (1) Department of Chemistry, University of Puerto Rico at Cayey, Ave. Antonio R. Barcelo, Cayey, PR 00737, PR, yemilyn@centroweb.net, (2) Department of Chemistry, SUNY at Buffalo

High performance liquid chromatography (HPLC) has been used for many biological assays such as separations of proteins. Separation and quantification of proteins will allow monitoring changes in protein expression that are linked to particular conditions such as cancer, cell aging, and cell response to environmental stress. Typically in HPLC separations silica based supports are used to incorporate stationary phases. However, such silica based stationary phases have some limitations, particularly the pH range at which they can be utilized since they lack stability at high and low pHs. We are investigating self-assembled monolayers (SAMs) on gold-coated silica. This approach has the potential of circumventing the stability problems associated with silica. SAMs of (1-Mercaptoundec-11-yl)di(ethylene glycol) on gold-coated silica has been shown to eliminate irreversible adsorption of an enzyme, for example, on gold surfaces. We are investigating the use of SAM material as a potential stationary phase in HPLC for proteins separations. We synthesized the (1-Mercaptoundec-11-yl)di(ethylene glycol). We attached SAMs onto electroless gold coated silica. We evaluated stationary phase by microbore HPLC. The column was proven to be able to separate simple organics with good resolution. The preliminary results obtained for the protein mixtures demonstrates the potential use of the material for biological assays.

75. HARDWARE TOOL FOR QUANTITATION OF MENISCUS-BASED ARTIFACTS IN MICROPLATE READERS. Tom A. Beumer, Business Area Immunodiagnosics, Organon Teknika BV, PO BOX 84, Boxtel NL 5280AB, Netherlands, Fax: +31 411 654427, t.beumer@teknika.btl.akzonobel.nl, and Wim Carpay, Business Area Nucleic Acid Diagnostics, Organon Teknika BV

Microplate readers use a vertical light path for their photometric analysis. This inevitably makes the meniscus in the liquid-air interface part of the optical system. From a physical point of view this meniscus is a diverging lens, regardless of the direction of the light beam. This lens is the only optical 'component' that cannot be specified on forehand because the shape of the meniscus is determined by interactions between liquid and plate. In the practice of absorbance measurements, the meniscus may increase absorbance values especially in the lower range. Classically, during periodic instrument checkups, this artifact is analysed using a microplate filled with a detergent solution. Not only labour intensive but also highly unspecified and user dependent. We patented (WO 00/08440) and developed a 'solid-state' tool in which the liquid lenses have been replaced by glass or polymer lenses of appropriate refractive index, size and surface curvature to mimic the complete range of meniscus shapes that may occur in real applications. We present the optical analysis of the complete optical system as it was made to generate lens specifications. Readings of this calibration device showed a good correlation between the bias that is introduced by liquid meniscii and the results generated with this tool.

76. HIGH THROUGHPUT ANALYSES IN NANOLITER BEAKERS USING AIR SEGMENTATION IN CAPILLARY TUBES. *Dana M. Spence, and Mark Dittman, Department of Chemistry, Saint Louis University, 3501 Laclede, St. Louis, MO 63103, Fax: 314-977-2521, spenced@slu.edu*

Our group has recently demonstrated the ability to perform air-segmented continuous flow analyses in fused silica capillary tubing with inside diameters less than 75 micrometers. The work presented here will expand on previous work by showing the ability of such a system to perform high throughput analyses of nanoliter samples. Since each liquid segment is actually a discrete sample, reactions can be monitored and measured in each of the segments. Each segment is approximately 40 nL in volume. In addition, no physical debubbling or electronic bubble gating is required since chemiluminescence detection is employed; thus, there are no fluctuations due to passing air segments. A high pressure syringe pump is used to propel all reagents through the system. Since liquid segments are measured at a rate of approximately 1 Hz using a PMT, it should be possible to measure over 3,500 samples per hour.

77. HIGH THROUGHPUT IN VIVO PHARMACOKINETICS STUDIES AND IN VITRO ASSAYS BY USING BECKMAN LIQUID HANDLING SYSTEM. *Xinchun Tong, Junying Wang, Ida E. Ita, Song Zheng, James V. Pivnichny, and Patrick Griffin, Basic Chemistry, Merck & Co., Inc, PO Box 2000, RY800-B205, Rahway, NJ 07065, Fax: 732-594-9545*

Automation of plasma sample preparation for pharmacokinetic studies and in vitro liver microsome incubations has been achieved using Beckman Coulter Biomek liquid handling system. This Biomek system served as a robot to perform full automation plasma sample preparation tasks, which included serial dilution of standard solutions, pipetting plasma samples, addition of standard and internal standard solutions, and performing solid phase extraction (SPE) using the Waters OASIS 96-well plate. A modified vacuum system was installed to provide zero, low and high stages of vacuum to accommodate the needs at different processing steps. With this automation, extraction recovery has been significantly improved. The full automation of the entire sample preparation process requires approximately two and half-hours for a typical pharmacokinetic study (51 samples, 24 standards and 9 quality controls). The Biomek system has also been used to prepare in vitro liver microsome studies to evaluate the stability of potential drug candidates in several animal species. The overall improvement in throughput facilitates knowledge gathering for in-vivo and in-vitro correlation. Extensive validation has been made to ensure the accuracy and reliability of these two methods.

78. HPLC AND MS ANALYSIS OF ENZYMATICALLY-PRODUCED HALOGENATED AMINO ACIDS: IMPLICATIONS FOR BIOCHEMICAL PATHWAYS OF PHAGOCYTE-MEDIATED TISSUE INJURY. *George C. Yeh, Joseph P. Gaut, Jaeman Byun, and Jay W. Heinecke, Department of Medicine, Washington University School of Medicine, 660 S. Euclid Ave., Campus Box 8046, St. Louis, MO 63110, Fax: 314-362-0811, gccyeh@yahoo.com*

Peroxidase-derived oxidants have been proposed to play important roles in inflammation and host defense. In the presence of physiological levels of chloride and bromide, the heme enzymes myeloperoxidase and eosinophil peroxidase preferentially produce hypochlorous acid and hypobromous acid, respectively, which can correspondingly chlorinate and brominate biomolecules. Recent work has demonstrated that myeloperoxidase utilizes a transhalogenation mechanism to brominate nucleobases. This study uses reverse phase HPLC and ESI/MS/MS to investigate the relevance of transhalogenation to the halogenation of proteins and amino acids by myeloperoxidase and hypochlorous acid. Both hypochlorous acid and myeloperoxidase form N-acetyl-3-chloro-L-tyrosine and N-acetyl-3-bromo-L-tyrosine at physiological halide levels. Chloride enhances N-acetyl-L-tyrosine bromination over enzymatic incubation with bromide alone. Increasing levels of bromide suppress N-acetyl-L-tyrosine chlorination. Studies with taurine indicate that bromination proceeds via chloride oxidation, consistent with a transhalogenation scenario. These findings provide evidence that interhalogen species are viable biological oxidants and that transhalogenation is a physiologically relevant pathway.

79. HYPERBOLIC WAVE PROPAGATIVE PARTIAL DIFFERENTIAL EQUATION SOLUTION IN ELUTION CHROMATOGRAPHY. *Kal Renganathan Sharma, Chemical Engineering, Bharathidhasan University, Shanmugha College of Engineering, Vignana Vihar, Tirumalaisamudram, Thanjavur 613402, India, Fax: 66596, sharma@chem.sce.ac.in*

The hyperbolic adsorption wave propagative partial differential equations are written for elution gas chromatograph. The solution for the pulse feed is given by the method of Laplace transforms. Axial dispersion term is neglected and the transient terms are included. The solution is an exponential decay multiplied with the modified Bessels function of the zeroth order. The non-dimensionalized variables w and η are expressed as a product in the argument of the Bessels function. The step input is solved for by the method of separation of variables and the solution is represented by the Fourier series for the case when infinite propagation speed of mass can be assumed. When the finite speed of mass transport is accounted for the solution is obtained by the method of Frobenius and the solution represented by an infinite series that is convergent.

80. IDENTIFICATION OF OVERLAPPING NEAR IR BANDS OF THE ANOMERS OF GLUCOSE USING 2-D IR CORRELATION SPECTROSCOPY. *Giri Srikanth, Aminiel Awichi, and Wei Zhao, Department of Chemistry, University of Arkansas, 2801 South University Ave., Little Rock, AR 72204, Fax: 501-569-8838*

Near-infrared spectroscopy is a useful tool to determine glucose in biological matrices. The identification is based on three broad features located in the range of 4000-5000 cm^{-1} which may be related to the C-H combination bands. Because alpha anomer and beta anomer of glucose are in equilibrium in the solution, the observed broad features may come from the overlapping of vibrational modes of the anomers. To reveal the origin of the near IR features, we have conducted near and middle-IR experiments to measure the spectral changes of individual pure anomers based on the mutarotation. For constructing 2D IR correlation spectra, different spectra have been recorded in a periodical time after the anomers are dissolved into water. We have identified a hidden feature at 4350 cm^{-1} for alpha anomer and a hidden feature at 4270 cm^{-1} for beta anomer. The assignment of these two bands has been discussed. WZ acknowledges the Financial Support from the Research Corporation.

81. IMMOBILIZATION OF PHOSPHORYLATED PROTEINS ON ALUMINA NANOPARTICLES: TOWARD NANOSIZE BIOSENSORS. *Ju Li, Yuzhong Wang, Jianquan Wang, Melanie Harvey, David A. Atwood, and Leonidas G. Bachas, Department of Chemistry, University of Kentucky, Rose street, Lexington, KY 40506, Fax: 859-323-1069, jli4@pop.uky.edu*

The goal of this research is to understand the parameters that control the function of enzymes upon their site-selective immobilization on nanoparticles in order to design nanosize biosensors. Alumina particles with a 25(\pm 5) nm diameter can be obtained from tetrametallic $\text{Al}(\text{R}_2\text{Al}(\mu\text{-O}i\text{Pr})_2)_3$ ($\text{R}=\text{Me, Et, iBu}$) precursors. The enzyme pepsin was used as a model protein to demonstrate the advantages of site-specific immobilization with nanoparticulate materials. Pepsin is a protease consisting of a single polypeptide with only one phosphoserine group. Comparison of the properties of immobilized pepsin on different forms of alumina will be presented. To explore the nature of bonding between a phosphorylated protein/peptide and alumina nanoparticles, we reacted phosphoserine with LAIOH, the only known soluble molecule possessing a terminal Al-OH unit (where L is a tetradentate Salen-based ligand). The reaction product was characterized by NMR, EI-MS, IR, elemental analysis and X-ray crystallography.

82. IN SITU ANALYSIS OF VOLATILES OBTAINED FROM CATALYTIC CRACKING OF POLYETHYLENE. *Nathan D. Hesse, Rong Lin, Edouard Bonnet, Jesse Cooper III, and Robert L. White, Department of Chemistry and Biochemistry, University of Oklahoma, 620 Parrington Oval, Norman, OK 73019, Fax: 405-325-6111, natehesse@ou.edu*

The effects of solid acid catalyst pore size and acidity on polyethylene catalytic cracking are examined by comparing temperature-dependent volatile product slate changes when the polymer is cracked by HZSM-5, HY, and MCM-41. Volatile product distributions depend on catalyst acidity and pore size. With HZSM-5, paraffins are detected initially and olefins are produced at somewhat

higher temperatures. Aromatics are formed at temperatures 30–40°C higher than those required for olefin production. Small olefins (C3–C5) are the most abundant products when HZSM-5 and MCM-41 catalysts are employed. In contrast, cracking with HY produces primarily paraffin volatile products (C4–C8). HY pores are large enough and acid sites are strong enough to promote disproportionation reactions, which lead to formation of volatile paraffins. In comparison, the smaller HZSM-5 pores inhibit residue formation and facilitate production of small alkyl aromatics. Volatile product variations can be rationalized by considering the combined effects of catalyst acidity and pore size on carbenium ion reaction pathways.

83.

IN VITRO ASSAY FOR TRANSGENIC COTTON PLANTS. *Bao-Hong Zhang, Hong-Mei Wang, and Fang Liu, Key Laboratory of Cotton Genetic Improvement of the Ministry of Agriculture, Cotton Research Institute, Chinese Academy of Agricultural Sciences, Anyang 455112, China, zbh68@yahoo.com*

2,4-D resistant plants of transgenic cotton (*Gossypium hirsutum* L.) were produced using *Agrobacterium tumefaciens* containing a plasmid carrying the neomycin phosphotransferase II (npt II) and 2,4-dichlorophenoxyacetic acid (2,4-D) monooxygenase (tfd A) genes. An in vitro assay was performed to determine the sensitivity of seed germination, and the growth of seedlings of transgenic and non-transgenic cotton to various concentrations of kanamycin and 2,4-D. The results indicated that kanamycin caused the cotyledons of non-transgenic plants to turn white, but transgenic plants grew normally. Seed germination and seedling growth of non-transgenic plants were strongly inhibited by 2,4-D, but only slightly for transgenic plants. Transgenic plants and non-transgenic plants can be clearly distinguished by the use of 2 mg/L 2,4-D in seed germination medium. There was a high correlation between the response of seed germination and the growth of seedlings to kanamycin or 2,4-D, based on the germination ratio, albino ratio, dry weight or fresh weight. On this basis, we developed a rapid method for identifying transgenic plants, which has been verified in the field. These findings will allow identification of cotton transformants at an early stage of plant development, saving time and improving cultivars containing the 2,4-D resistance trait.

84.

INCREASED SELECTIVITY OF ELECTROCHEMICAL MEASUREMENTS USING COMPOSITE MODIFIED ELECTRODES. *Shelley D. Minteer, and Sam Brancato, Department of Chemistry, Saint Louis University, 221 North Grand Blvd., St. Louis, MO 63103, Fax: 314-977-2521, minteers@slu.edu*

Composite modified electrodes have been developed using silane coated glass beads and Nafion. Silanes with various functional groups were covalently attached to the glass beads in order to exclude certain redox species on the basis of size, structure and hydrophobicity. The four silanes employed in this study were 3-aminopropyltrimethoxysilane, 3-chloropropyl-trimethoxysilane, 2-phenylethyl-trimethoxysilane, and 3-methacryloxypropyl-trimethoxysilane. The redox species Ru(bpy)₃²⁺, Fe³⁺, hydroquinone, and methyl viologen were chosen as analytes due to their electrochemical properties and to illustrate the range of functionality of the modified electrodes. Cyclic voltammetry measurements were performed with both the composite modified and Nafion electrodes, and the KD1/2 term was determined. The KD1/2 term was then used to calculate the ratio of electrochemical flux of the composite compared to a Nafion modified electrode. The results support that the silane coated glass bead/ Nafion composites have differing selectivities for redox species depending on the functional groups on the silane.

85.

INDUCED ORIENTATIONAL ANISOTROPY DYNAMICS AND INVERSE RAMAN SPECTROSCOPY OF RHODAMINE 640. *John Delacruz, and Gary J. Blanchard, Department of Chemistry, Michigan State University, 326 Chemistry, Michigan State University, East Lansing, MI 48824, Fax: 517-353-1793, delacruz@photon.cem.msu.edu*

We report on the rotational diffusion behavior of Rhodamine 640 (R640) in a series of n-alcohols, methanol to n-decanol. The purpose of this work is to elucidate the intermolecular interactions present in these systems. Solvent viscosity and chain length play an important role in the reorientation dynamics. These data, in conjunction with inverse Raman scattering measurements of R640 in the same solvents allows us to develop a fundamental understanding of

both intramolecular and intermolecular coupling of the electronic and vibrational coordinates of the probe molecule. By comparing the reorientation and inverse Raman information, we expect to gain new insight into the solvent-induced perturbation to the intramolecular relaxation dynamics of the chromophore.

86.

INTEGRATION OF GENETICALLY ENGINEERED SENSING ELEMENTS WITH A COMPACT DISC MICROFLUIDIC PLATFORM. *Brett R. Wenner¹, Phillip M. Douglass², Marc J. Madou³, and Sylvia Daunert¹. (1) Department of Chemistry, University of Kentucky, Chemistry/Physics Bldg, Lexington, KY 40506, (2) Department of Pharmaceutical Sciences, University of Kentucky, (3) Department of Materials Science and Engineering, Ohio State University*

The coupling of genetically engineered sensing elements with a compact disc-based microfluidic platform has proven to be a novel analytical tool for analyte detection. The phosphate binding protein (PBP) and calmodulin (CaM) are examples of such engineered proteins which have previously been employed as sensing elements for the detection of inorganic phosphate and phenothiazines, respectively. These assays have been coupled with a compact disc platform which utilizes centrifugal force to drive the liquid reagents throughout the microfluidic structures. In this way, the reagents can be released and mixed with samples containing the analyte of interest. The use of fluorescence-based detection provides a highly sensitive means to assay in microscale applications. Beyond the fundamental issue of analyte detection, lies the concern of long-term storage of reagents. The versatility of this microfluidic platform has been further enhanced by investigating the feasibility of dry storing reagents on the disk for later use.

87.

INTERROGATING INTERACTIONS OF 7-AZATRYPTOPHAN WITH MICELLES. *Lee Kelepouris, and Gary J. Blanchard, Department of Chemistry, Michigan State University, E. Lansing, MI 48824, Fax: 517-353-1793, kelepour@photon.cem.msu.edu*

To better understand the fundamental optical response of a fluorescent probe in heterogeneous systems we have investigated the nonnatural amino acid 7-azatryptophan. The fluorescence properties of this probe are monitored in aqueous micellar systems. Azatryptophan is studied in free form and as a dipeptide in aqueous solutions of anionic, cationic, and neutral surfactants above their critical micelle concentration. The various forms of this probe report on the molecular environment though changes in fluorescence lifetime and reorientation dynamics. The fluorescence response of azatryptophan is discussed in terms of its environment, specifically changes mediated by the adjacent amino acid in a dipeptide and interactions with the micellar system.

88.

INVESTIGATING DIFFUSIONLESS ELECTRON TRANSFER OF ADSORBED CYTOCHROME C THROUGH AN ATTENUATED TOTAL REFLECTANCE SPECTROELECTROCHEMICAL GEOMETRY. *Walter J. Doherty III, Neal R. Armstrong, and S. Scott Saavedra, Department of Chemistry, University of Arizona, 1306 E. University, Tucson, AZ 85721-0041, Fax: 520-621-8407, wjd@u.arizona.edu*

Cytochrome c is a small, heme-bearing protein that shuttles electrons in the respiratory chain of mitochondria. The redox-active heme is bound in a hydrophobic pocket flanked with a high density of positive charge. Electron transfer between cyt-c and its complimentary oxidase and reductase is therefore strongly governed by electrostatic interactions and may be orientationally dependant. The spontaneous adsorption of cyt-c to indium tin oxide (ITO) thin films facilitates spectroelectrochemical probing of cyt-c at the electrode surface. Using an attenuated total reflectance (ATR) geometry one can monitor both the spectral and electrochemical changes as a function of applied potential and incident polarization for a monolayer of adsorbed cyt-c. These capabilities allow one to obtain information about both adsorption orientation and electron transfer kinetics.

89. INVESTIGATING HYDROLYTIC POLYMERIZATION OF ZIRCONIUM USING THE FLUORESCENT PROBE 1-PYRENECARBOXYLIC ACID. *J. J. Tulock, and G. J. Blanchard, Chemistry, Michigan State University, E. Lansing, MI 48824, Fax: 517-353-1793, tulock@photon.cem.msu.edu*

Considerable interest has been focused on the hydrolytic polymerization of Zr in aqueous solution. Key issues in this area are characterizing the structure, size and mechanism for growth of hydrous Zr polymers. Much of what is currently known about the aqueous chemistry of Zr has come from the study of Zr solutions and their crystalline products as a function of pH, temperature, and degree of aging. Apart from X-ray scattering measurements, many experimental strategies employed to date provide only a limited in-situ perspective concerning growth and aggregation of Zr polymers. We present a novel approach to study the structure and growth of hydrous Zr polymers using the fluorescent probe molecule 1-pyrenecarboxylic acid (PCA) where the probe is introduced, as a trace impurity (~ ppm), into Zr solutions of various ages. The fluorescence lifetime and rotational diffusion dynamics of PCA are studied as a function of solution age and we discuss these data as they relate to polymerization and aggregation phenomena of Zr in aqueous solutions.

90. INVESTIGATION OF MODEL CONJUGATED ORGANIC/ORGANIC INTERFACES BY SURFACE RAMAN SPECTROSCOPY. *Adam M. Hawkrigde, Domenic J. Tiani, and Jeanne E. Pemberton, Department of Chemistry, University of Arizona, 1306 E. University Blvd, Tucson, AZ 85721, Fax: 520-621-8248, hawkrigd@email.arizona.edu*

The facilitation and optimization of charge transport across conducting polymer interfaces is crucial for the realization of commercial organic light-emitting diodes (OLED). To date, little information is known at the molecular level concerning the parameters that influence charge transport across these interfaces. A first attempt to model the complex hetero-organic interfaces that exist in such devices is presented here using a novel emersion approach developed in this laboratory. Emersion involves the introduction and controlled removal of a solid substrate from a liquid. Upon removal of an organic substrate from an organic liquid, the molecular orientation of the organic-organic interface established in solution is retained. Specifically, a conjugated polymer surface was modeled using self-assembled monolayers (SAMs) of phenyl-terminated alkanethiols on silver. These substrates were exposed to several simple conjugated organic solvents including benzene, toluene, pyridine, and pyrrole. The nature of the chemical interactions between these molecules and the phenyl-terminated SAMs was determined by surface Raman spectroscopy on ultrathin (< 5 nm) emersed layers of these organics. Thicknesses of the emersed layers were determined via manual-null ellipsometry.

91. INVESTIGATION OF NEAR-IR FLUORESCENT DYES FOR USE AS CELL STAINS IN CYTOTOXICITY STUDIES. *Richard J. Williams¹, LaVentrice Taylor², and Duane Hill². (1) Department of Chemistry, Morgan State University, 1700 East Cold Spring Lane, Baltimore, MD 21251, Fax: 410-319-3778, rwillia6@morgan.edu, (2) Department of Biology, Morgan State University*

The use of chromophores from the ultraviolet (UV) and visible regions as stains and dyes is a very effective tool in several bio-analytical techniques. While conventional UV-visible spectroscopy techniques can provide good sensitivity and specificity, they are often limited in sensitivity by interference from the background spectral properties of non-targeted components in the UV-visible region. Bio-analytical techniques that incorporate fluorescent near-infrared (near-IR) dyes offer several advantages as an alternative to techniques that utilize chromophores in the uv-visible region. Near-IR dyes typically exhibit strong molar absorptivity coefficients and good quantum yields. In this study, several commercially near-IR dyes are compared to calcein, a standard visible fluorophore, as possible stains and labels for use in cell toxicity monitoring techniques. Comparisons of detection sensitivity and cell loading ability are reported along with cell viability. Also, an interpretation of the outcome of selected cells being exposed to known toxins is presented.

92. KINETIC AND OPTIMAL MOISTURE STUDIES OF METHANOTROPH-ENRICHED WHOLE SOIL SAMPLES OVERLYING COAL BED METHANE SEEPS. *Christian David Adams¹, Kevin W. Mandernack², Christopher H. Mills², and Robert E. Milofsky¹. (1) Department of Chemistry, Fort Lewis College, 1000 Rim Drive, Durango, CO 81301, cdadams@fortlewis.edu, (2) Department of Chemistry and Geochemistry, Colorado School of Mines*

Thermogenic coal bed methane seeps overlying the Fruitland formation in the Southwest USA have stimulated areas of methanotroph-enriched soils. This enrichment creates an ideal environment for studying methane oxidation by methanotrophic bacteria. Soil samples were collected from two sites overlying active methane seeps. Activity was measured as consumption of methane, monitored with a gas chromatograph. It was shown that linear decreases in methane concentration were due to microbial activity and not other soil adsorption processes. These methanotroph-enriched soils were also investigated to determine optimal moisture content for methane uptake and the enzymatic kinetic parameters K_m and V_{max} . Optimal moisture levels were observed to change with location and season. Initial kinetic data show a correlation between methane consumption and location.

93. LOW-COST OXYGEN SENSING USING UNFILTERED FLUORESCENCE. *Yordan V. Kostov, Peter Harms, and Govind Rao, Department of Chemical and Biochemical Engineering, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250, Fax: 410-706-2623, kostov@umbi.umd.edu*

Oxygen fluorescent sensors use filters or monochromators for wavelength separation. This strongly diminishes the light intensities, imposing the need for sophisticated photodetectors. Furthermore, this significantly increases the cost of the sensor. Filtering works well only when the sample under test exhibits no fluorescence. If it does, sensor's dynamic range significantly decreases (in some cases - down to 0). Optical shielding gives satisfactory results; however, it severely affects sensor's response time. Here we present a method and a device for filterless oxygen sensing. The approach allows for accurate oxygen detection in very high background (1 mM fluorescein) without the use of any optical filters or optical shielding of the sensor from the background. The ambient light is not affecting the sensor's operation, too. The device was built using only conventional electronics. The method allows for use of standard semiconductor technologies and has the potential for development of ultra-low-cost, high-reliability long-lasting oxygen sensors.

94. MASS SPECTROMETRIC CHARACTERIZATION OF EPOXYISOPROSTANE PHOSPHOLIPIDS THAT ACTIVATE ENDOTHELIAL CELLS TO PRODUCE INTERLEUKIN-8. *Ganesamoorthy Subbanagounder¹, Jason W. Wong¹, Hans Lee¹, Kym F. Faull², Alan M. Fogelman¹, and Judith A. Berliner¹. (1) Department of Medicine/Cardiology, University of California, Los Angeles, 47-123 CHS, UCLA School of Medicine, Los Angeles, CA 90024, Fax: 310-206-9133, gsubbanagounder@mednet.ucla.edu, (2) Department of Psychiatry & Biobehavioral Sciences and the Neuropsychiatric Institute, University of California, Los Angeles*

We have previously reported that 1-palmitoyl-2-epoxyisoprostane-3-phosphatidylcholine (PEIPC) found in oxidized 1-palmitoyl-2-arachidonoyl-3-phosphatidylcholine (Ox-PAPC) and lesions activated endothelial cells (EC) to bind monocytes. In the present study we identify the active components of Ox-PAPC that are responsible for the production of interleukin-8 (IL-8) in EC. Employing LC/MS methods to isolate various components from Ox-PAPC, we found that molecules with m/z 828.5 and 810.5 accounted for the majority of the activity to produce IL-8 in EC. Tandem MS analyses suggest that these molecules are isomeric epoxyisoprostane phospholipids. Chemical derivatization studies demonstrate the importance of epoxide and carbonyl groups for biological activity. Quantitative MS analysis showed that the levels of m/z 828.5 were increased by 2 to 6 fold when EC were exposed to IL-1 β (10 ng/ml) for 24 h compared to control cells. These studies identify specific oxidized phospholipids act as potent activators of endothelial cells to produce inflammatory chemokines important in atherogenesis.

95. MEDIUM-THROUGHPUT PKA SCREENING OF PHARMACEUTICALS BY PRESSURE-ASSISTED CAPILLARY ELECTROPHORESIS. *Zhongjiang Jia, Tore Ramstad, and Min Zhong, Pharmaceutics/Pharmacia Corp, Kalamazoo, 7000 Portage Road, Kalamazoo, MI 49001, zhongjiang.jia@am.pnu.com*

A fast screening method for the determination of the dissociation constants (pKa) of acidic, basic, and multivalent compounds was developed by using pressure-assisted capillary electrophoresis (PACE). External air pressure was applied to shorten the analysis time. The separation efficiency decreases as air pressure increases. However, it was found that air pressure does not affect the measurement of electrophoretic mobility and pKa significantly when it is less than 2 psi. The method was evaluated in terms of accuracy, precision, and ruggedness by using a set of 48 compounds with literature pKa values ranging from 2 to 10. The difference between the measured pKa values and literature values is less than 0.2 units. The throughput is approximately 20 compounds per day with a 12-point measurement ranging from pH 2.5 to 11. It was demonstrated that this method is applicable for pKa screening of pharmaceuticals with diverse chemical structures.

96. MESOPOROUS SILICA BASED FLUORESCENCE SENSORS. *Victor S-Y. Lin, Jianguo Huang, and Cheng-Yu Lai, Department of Chemistry, Iowa State University, 1710 Gilman Hall, Iowa State University, Ames, IA 50011, Fax: 515-294-0105, vsylin@iastate.edu*

The recent discovery of flexible synthetic pathways to structurally well-defined, periodic, mesoporous, high surface area silicas with tunable pore diameters and narrow pore size distributions, has led to extensive research efforts in exploring the potential applications of these materials in chromatography, catalysis, and sensor design. Selective functionalization of the inner pore surface of these mesoporous materials with a variety of molecular moieties provides an excellent microenvironment for developing synthetic "enzyme or antibody active sites" for highly selective molecular recognition centers. We have recently developed a simple procedure for the syntheses of multi-functionalized mesoporous materials via an interfacial electrostatic interaction directed co-condensation reaction. Utilizing these derivatized mesoporous silicas, a series of fluorescence sensory systems with multi-functional group selectivity have been designed and fabricated to detect biogenic catecholamines, DNA's, and aminoglycosyl antibacterial agents.

97. MOBILE PHASE VOLUME AND VELOCITY IN PCSFC-MS: HIGH-THROUGHPUT ANALYTICAL METHOD FOR COMBINATORIAL LIBRARIES. *Benjamin G. Kinney, Geoffrey E. Barker, and Jonathan Krakover, Analytical Chemistry, Ontogen Corporation, 2325 Camino Vida Roble, Carlsbad, CA 92009, Fax: 760-930-0955, ben.kinney@ontogen.com*

The production of large numbers of compounds in combinatorial chemistry has driven a need for a fast, robust, informative, and easily automated technique. To date, the primary limitations of pcSFC-MS has been its long analysis time, difficulty in maintaining repeatability, and its lack of application to a wide variety of compounds. Very few attempts have been made to use SFC as a high-throughput chromatographic method in a drug discovery setting. High throughput pcSFC-MS has many advantages over high-throughput LC-MS including higher linear velocity limits as a consequence of reduced back-pressure and higher solvation rates which in turn reduces mobile phase throughput time. SFC can be much more cost effective over HPLC because when CO₂ is used as the primary mobile phase one can reduce solvent costs, and greatly reduce waste disposal costs. The need for better information during targeted library development has created a need to balance high throughput analysis and high quality separations with the costs of implementing them. This requires a more in-depth examination of the physical parameters involved in the analysis.

In this work we investigate the effect of linear velocity and mobile phase volume on pcSFC chromatographic performance and throughput. We have focused not only on the speed of analysis, but also on the quality of the separation needed to allow full pcSFC-MS characterization of a mixture of compounds. We specifically address peak retention as a percent of the gradient time, peak width, peak capacity, system pressure, and peak separation of two closely eluting peaks. We also verify the equivalence of linear velocity changes

and robustness of the system of by comparing component elution time as a fraction of gradient time.

98. MOLECULAR MOTIONS OF LONG DNA CONFINED IN SMALL SPACE. *Masanori Ueda, Noritada Kaji, and Yoshinobu Baba, Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Tokushima, CREST, 1-78 Shomachi, Tokushima 770-8505, Japan, Fax: +81-88-633-9507, uedam@fc.ph.tokushima-u.ac.jp*

We studied electrophoretic-behaviors of a long DNA molecule confined in a small space. DNA was stained with fluorescence dye, and incubated in linear polymer solutions or gel systems etc. The solution including DNA was placed on a miniaturized electrophoretic cell on a fluorescence microscope. We applied AC electric fields (sinusoidal and pulsed fields) to the solution, observing the molecular motions of the individual DNA. DNA shows several types of motion, depending on the external field's frequency, voltage, and the obstacle's concentration. Especially, under the electric field around 10 Hz and 200 V/cm, DNA is stretched out almost fully because of the entanglement between DNA and obstacles. This stretching is observed with a wide range of DNA sizes (48.5kbp - 1Mbp) and gel concentrations (agarose, 0.5% - 2%). We will talk about the possible application of the DNA stretching to biological methods.

99. MONITORING OXIDATION IN VEGETABLE OILS BY NEAR-IR SPECTROSCOPY. *Randy L. Wehling, Gulgun Yildiz, and Susan L. Cuppett, Department of Food Science and Technology, University of Nebraska, 143 Filley Hall, Lincoln, NE 68583-0919, Fax: 402-472-1693, rwehling1@unl.edu*

Near-infrared (NIR) spectroscopy has been used successfully in our laboratory to monitor oxidation levels in vegetable oils. Models have been developed to measure peroxide values in both soy and corn oil, using Partial Least Squares (PLS) regression and Multiple Linear Regression (MLR), from NIR transmission spectra. Peroxide values can be successfully measured in both corn and soy oil using a single model. The most successful model was based on PLS regression of first derivative spectra. When this model was applied to validation sample sets containing equal numbers of corn and soy oil samples, with peroxide values ranging from 0-20 meq/kg, a correlation coefficient of 0.99 between titration and NIR values was obtained, with a standard error of prediction (SEP) equal to 0.72 meq/kg. For both types of oil, changes occurred in the 2068 nm region of the NIR spectra as oxidation levels increased. These changes appear to be associated with the formation of hydroperoxides during oxidation of the oils.

100. MULTIPLEXED IMMOBILIZATION OF BIOMOLECULES ONTO A GOLD BAND ELECTRODE ARRAY. *C. Brandon Davis, and Werner G. Kuhr, Department of Chemistry, University of California Riverside, Pierce Hall, UC Riverside, Riverside, CA 92507, Fax: 909-787-4713, davischr@citrus.ucr.edu*

In the work presented here it is demonstrated that biotin-LC-hydrazide may be quickly and efficiently deposited on the surface of gold by application of 3 consecutive potential scans, simplifying derivitization of gold with biomolecules. Electrodeposition of biotin-LC-hydrazide allows a fast one step procedure that completely covers the gold surface. The subsequent attachment of avidin to the biotinylated surface forms the molecular sandwich necessary to immobilize any biotinylated molecule to the gold. Utilization of the electrodeposition of biotin and biotin/avidin technology has the advantage that it allows for an array of gold band electrodes to be multiplexed with several distinct sequences of DNA probes because the gold electrodes are individually addressable. On the gold band electrode array we demonstrate the addressability of the electrodeposition by derivitizing electrodes 1 and 3 with a HIV DNA probe and electrode 2 and 4 with a TB DNA probe. Upon exposure with one of the fluorescently tagged DNA targets two of the electrodes specific for the target sequence was hybridized. The fluorescently labeled DNA targets are then detected using a fluorescence microscope equipped with a CCD and the appropriate excitation and emission filters. This will allow for an inexpensive and easily fabricated biosensor device for the precise recognition and subsequent detection of a specific complementary DNA target for diagnosis and genetic screening.

101. NANOLITER-RANGE ELECTROCHEMICAL PLATFORM SUITABLE FOR THE PRODUCTION OF SMALL-VOLUME SENSORS. *J. Christopher Ball*¹, *Janet K. Lumpp*², *Sylvia Daunert*¹, and *Leonidas G. Bachas*¹. (1) Department of Chemistry, University of Kentucky, Lexington, KY 40506-0055, Fax: 859-323-1069, jcball0@pop.uky.edu, (2) Department of Electrical Engineering, University of Kentucky

An electrochemical-based platform for small volume (nanoliter) analysis has been developed. The platform consists of electrochemical cells with embedded electrodes fabricated by using screen printing and laser micromachining. The fabrication process has been investigated to determine the protocols for creating electrochemical nanovials with features that can be predicted. Cyclic voltammetry and chronoamperometry are used to characterize the electrochemical properties of the resulting nanocells, along with SEM to visualize the profile of the structure. These nanoliter-range analysis systems can serve as the basis for the further fabrication of small volume sensors. An example of this is the incorporation of enzymes to produce nanoliter amperometric biosensors. These biosensors can be produced either by immobilizing an enzyme in the working electrode carbon thick-film matrix or immobilization within an electropolymerized conducting polymer. To demonstrate this use of the platform, the enzyme glucose oxidase (GOx) has been used.

102. NMR OF CADMIUM BOUND PHYTOCHELATINS. *Dallas L. Rabenstein*, and *Stephen M. Spain*, Department of Chemistry, University of California, Riverside, Riverside, CA 92521, dlrab@mail.ucr.edu, nuclearmr@hotmail.com

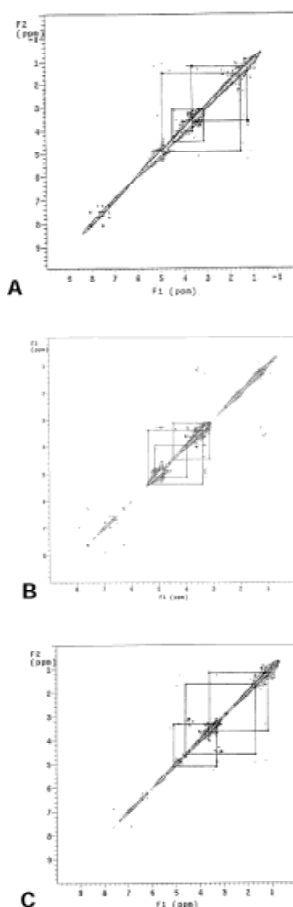
Phytochelatins are cysteine-rich polypeptides produced by plants grown in heavy metal contaminated environments as a mechanism for heavy metal detoxification by chelation of the metal ion. They consist of repetitions of the dipeptide γ -Glu-Cys and a C-terminal glycine (γ -Glu-Cys)_nGly, where n ranges from 2 to 11 (PC2-PC11). We report here on studies of the binding of PC2, PC3, and PC4 with cadmium using high-resolution ¹H NMR. Complexes were formed by titration of the phytochelatins with Cd²⁺ and evaluating the one-dimensional ¹H and ¹³C spectra obtained. Structure and binding information were obtained through TOCSY and ROESY spectra on the stoichiometric bound complexes in combination with molecular modeling techniques.

103. NMR STUDIES FOR THE QUALITY CONTROL OF HERBAL EXTRACTS. *Mohammed Kamil*¹, *Alappat F. Jayaraj*¹, *Faizan Ahmed*¹, *Conjeevaram J. Gunasekar*¹, *Samuel T. Stephen*¹, *Mohammed Osman E. Sheikh*¹, and *Aref Shehi*². (1) Department of Pharmacognostic Sciences, Zayed Complex for Herbal Research & Traditional Medicine (ZCHRTM), Ministry of Health, Abu Dhabi, P.O. Box 29300, United Arab Emirates, Fax: 971 2 5826919, drkamil@emirates.net.ae, jrjaj@emirates.net.ae, (2) Director, ZCHRTM

NMR technique is normally applied to structural determination and conformational analyses of pure compounds. It has rarely been applied to herbal extracts and assay because of its complexity [1]. An attempt has been made to use NMR as an extension of quality control parameter for differentiating between three herbal alcoholic extracts of *Zizyphus spinachristi*, *Teucrium stocksianum* and *Caralluma arabica* which are being generally used for the treatment of diabetes mellitus. ¹H and COSY spectra of the above three extracts were recorded on a Varian Gemini 400 MHz NMR spectrometer. All the spectra were recorded in deuterated methanol. Chemical shift, multiplicity and the coupling constants of the characteristic signals for the three extracts are given in the table:

<i>Zizyphus spinachristi</i>	<i>Teucrium stocksianum</i>	<i>Caralluma arabica</i>
1.17(s), 1.28(s), 6.62(s), 7.23(t,7.6), 7.52(m)	1.17(t,7.3), 1.28(s), 6.77(t,2.3), 6.95 (d,8.7), 7.89 (d,8.8)	1.17(t,7.0), 1.28(s), 6.2 (d,2.1), 6.4(m), 7.84(d,2.1)

The multiplicity of the signal at 1.17 ppm (which is a singlet in the case of *Zizyphus*) differentiate *Zizyphus* from the rest of the samples, besides the singlet at 1.28 which are common for all the three samples. COSY spectra of the above three extracts are shown in the figure. The characteristic correlation pattern of the proton signals differentiate *Teucrium* from the *Caralluma* sample.



COSY spectra (showing the correlation) of the absolute alcohol extracts of (A) *Zizyphus spinachristi*, (B) *Teucrium stocksianum* and (C) *Caralluma arabica*

Correlation patterns are being used for the confirmation of natural products in a mixture by comparing the correlation maps of the suspected component and the mixture.

104. NOVEL WAY OF DETERMINING PEAK PURITY BY HPLC-HPLC. *Chan Y. Ko*, and *Arthur P. Micheal*, New Product Research/Analytical Development, The RWJ Pharmaceutical Research Institute, Welsh & McKean Roads, Spring House, PA 19477-0776, Fax: 215-540-4683

The development of analytical HPLC methods for pharmaceutical actives requires that the purity of the main peak be evaluated to assure that there are no co-eluting impurities. Traditional means for assessing chromatographic peak purity include: (1) a comparison of UV absorption spectra obtained at several points during the elution profile of the component of interest (using the peak purity algorithm associated with the diode array detector), and (2) comparison of mass spectra obtained during the elution profile. We have been evaluating the alternate technique HPLC-HPLC to determine peak purity. To use this method, a sample is chromatographed on a capillary HPLC system (using a 150 × 1.0 mm, i.d. HPLC column). The peak of interest in the chromatogram is then diverted through a valve to another HPLC system equipped with a traditional size column (150 × 4.6 mm, i.d.), generally using a different mode of separation. The flow rate in the capillary system is on the order of 50 fL/min, and the transfer of the chromatographic peak into the larger column does not cause deleterious band spreading. Since a different mode of separation is employed on the second HPLC system, the probability of separating a potential impurity co-eluting with the major component is greatly increased. Now, the traditional means for assessing chromatographic peak purity could be applied by overlaying UV spectra captured throughout the main peak (using the peak purity program associated with the diode array detector) and HPLC-MS.

105.

OBSERVATION OF SIZE-SPECIFIC QUENCHING CHARACTERISTICS OF ENCAPSULATED MOLECULES. *Niya Diggs, and Maurice O. Iwunze, Department of Chemistry, Morgan State University, Baltimore, MD 21251, Fax: 410-319-3778, miwunze@morgan.edu*

Steady-state fluorescence quenching techniques, with $K_3Fe(CN)_6$ as quencher, was used to characterize various sized biomolecules, encapsulated in sol-gel glass. The observed quenching profile is sensitive to the size of the biomolecule. When smaller sized biomolecules show both dynamic and static quenching profiles, the larger molecules exhibit size-exclusion phenomenon. The observed quenching rate constant and the accessibility factors for these molecules will be used to characterize the efficacy of sol-gel technology for encapsulated biosensors.

106.

ON-COLUMN LIGAND SYNTHESIS COUPLED TO PARTIAL-FILLING AFFINITY CAPILLARY ELECTROPHORESIS. *Ying Zhang, Cynthia Kodama, Cecilia Zurita, and Frank Gomez, Chemistry and Biochemistry, California State University, Los Angeles, 5151 State University Drive, Los Angeles, CA 90032, Fax: 323-343-6490, zhangwen22@yahoo.com, ckodama@calstatela.edu, zuritalopez@hotmail.com*

This paper describes a two-step procedure whereby on-column ligand synthesis and partial-filling affinity capillary electrophoresis (PFACE) are coupled to each other to determine the binding of 9-fluorenylmethyl carbamate (Fmoc) D-Ala-D-Ala species to vancomycin (Van) from *Streptomyces orientalis*. In this technique three separate plugs of sample are injected onto the capillary column and electrophoresed. The initial sample plug contains a D-Ala-D-Ala terminus peptide and two non-interacting standards. Plug two contains a solution of Fmoc-amino acid N-hydroxysuccinamide (NHS) ester. The third sample plug contains an increasing concentration of Van partially-filled onto the capillary column. Upon electrophoresis the initial D-Ala-D-Ala peptide reacts with the Fmoc-amino acid NHS ester yielding the Fmoc-amino acid D-Ala-D-Ala peptide. Continued electrophoresis results in the overlap of the plug of Van and Fmoc-amino acid D-Ala-D-Ala peptide and non-interacting markers. Analysis of the change in the relative migration time ratio, RMTR, of the Fmoc-amino acid D-Ala-D-Ala peptide relative to the non-interacting standards, as a function of the concentration of Van, yields a value for the binding constant.

107.

PAMAM-DYE/SOL-GEL COMPOSITES AS CHEMICAL SENSOR MATERIALS. *S. Scott Saavedra, and Muditha D. Senarath-Yapa, Department of Chemistry, University of Arizona, 1306 E. University, Tucson, AZ 85721-0041, Fax: 520-621-8407, saavedra@u.arizona.edu*

We are investigating analytical applications of dye modified dendrimers encapsulated in sol-gel materials. A fourth generation PAMAM dendrimer is an attractive choice for a macromolecular carrier of indicator dyes, due to its inherently globular shape and its 64 surface amine groups. An amine reactive sensor molecule for an analyte can be attached to the PAMAM surface through an isothiocyanate coupling reaction, which quantitatively eliminates dye leaching from hydrated, porous sol-gel monoliths. The microenvironment of the dye on the PAMAM surface can be altered by co-derivatization of the surface amines with other moieties (e.g. fatty acids). Recent results using this strategy to "tune" sensor response will be presented.

108.

PHOTOIONIZATION INTERFACE FOR LC/MS. *Karl A. Hanold¹, Matthew D. Evans¹, Steven M. Fischer², Patricia H. Cormia², and Jack A. Syage¹. (1) Syagen Technology, Inc, 1411 Warner Ave, Tustin, CA 92780, Fax: 714-258-4404, khanold@syagen.com, (2) Agilent Technologies, Inc*

In this work we describe the performance of low pressure and atmospheric pressure photoionization sources (LPPI and APPI) for sampling liquid flows. The APPI source was interfaced to an Agilent 1100 series LC/MS. Our previous photoionization mass spectrometry (PI MS) instruments were developed for high-throughput air and liquid screening of chemical weapons, semiconductor process compounds, and combinatorial chemistry drug samples. Photoionization (PI) directly ionizes compounds to molecular ion M⁺. Nonpolars that are not easily ionized by electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) can be ionized by PI. APPI is an important complement to ESI

and APCI by expanding the range and classes of compounds that can be analyzed. APPI is a potentially important ion source for combinatorial chemistry. Detection limits of 3 pg (reserpine) have been measured, which is comparable to APCI. APPI achieves significantly better sensitivity than APCI at flow rates below 200 microL/min, making it a useful source for capillary LC and CE. The APPI source is linear up to 10 ng injected quantity (highest level tested so far). This work will also discuss the role of dopants to improve sensitivity for select classes of compounds.

109.

PHOTOIONIZATION MASS SPECTROMETRY FOR HIGH-THROUGHPUT PHARMACEUTICAL ANALYSIS. *Jack A. Syage, Matthew D. Evans, and Karl A. Hanold, Syagen Technology, Inc, 1411 Warner Ave, Tustin, CA 92780, Fax: 714-258-4404, jsyage@syagen.com*

Photoionization mass spectrometry (PI MS) is emerging as an important tool for high-throughput pharmaceutical drug analysis. PI MS meets the requirements for many applications where electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) underperform. The PI source uses a gas discharge cell to generate a choice of wavelengths ranging from about 8 to 12 eV. A 10 eV source is commonly used because nearly all molecules of analytical interest have ionization potentials below 10 eV, yet all air constituents and many common solvents (e.g., H₂O, MeOH, CH₃CN, halogenated solvents) have ionization potentials above 10 eV and are not ionized. In this talk we describe the principles of PI, compare the properties of PI to ESI and APCI, and discuss the specific benefits for pharmaceutical drug analysis. This work focuses on atmospheric pressure PI (APPI) using nebulized liquid flows and electrospray flows. PI vs. ESI mass spectra of combinatorial library samples show simplified molecular ion signal with minimal extraneous signal due to salt ionization and complexation and no Na⁺ adduct complexation. A Leap Mini Pal autosampler has been integrated with the APPI source. Results are presented for the APPI source operating with a quadrupole ion trap, time-of-flight (QitTof) system and with a commercial quadrupole system. Results will be shown demonstrating large dynamic range and sensitivity comparable to APCI.

110.

PHOTOMODIFIED ANTIBODY DETECTION OF GLUTAMINE SYNTHETASE. *Manjula Nakka, and Boyd Haley, Department of Chemistry, University of Kentucky, Chemistry- Physics building, Rose Street, Lexington, KY 40506, Fax: 859-257-3040*

Glutamine Synthetase (GS) is located primarily in the astrocytes of brain tissue and lysis of these cells would release GS into the cerebrospinal fluid(CSF). Astrogliaosis occurs during many neurological disease states including Alzheimers disease and amyotrophic lateral sclerosis. Therefore, the detection of GS in CSF could be an important aid in the diagnosis of many neurological diseases. However, GS is reported to be present in CSF at low pg/mL levels in these diseases and would be difficult to detect with standard immunodetection methods. Therefore, a method of photomodification of GS antibody, to generate an efficacious radioimmunoassay that easily detected GS in a 0.5 mL CSF sample via a single antibody procedure was accomplished. A ³²P label is attached to the antibody by a photolabeling technique. Using lumbar CSF samples GS was easily detected with phosphorimaging using this procedure. The highest level detected was over 40 times that detected in the samples with the lowest level. This indicates that GS levels vary greatly from patient to patient and validates this technique as a sensitive procedure to quantify GS in CSF.

111.

PHOTOPHYSICAL INVESTIGATION OF POLYAMINES CONTAINING NAPHTHYL DERIVATIVES. *Siddharth Pandey¹, Kristin A. Fletcher¹, Ashley E. Hendricks¹, Michael W. Fennie², and Mary C. O'Sullivan². (1) Department of Chemistry, New Mexico Institute of Mining and Technology, 801 Leroy Pl, Socorro, NM 87801, Fax: 505-835-5364, pandey@nmt.edu, fletcher@nmt.edu, (2) Department of Chemistry, Canisius College*

Trypanosomatidae parasites are responsible for many human and animal diseases including African Sleeping Sickness, Chagas' disease, and Nagana cattle disease. Since current treatment of trypanosome infections is difficult and often ineffective in controlling the chronic phases of these diseases, more effective anti-trypanosomal drugs are urgently needed. A class of amine-related compounds that contain hydrophobic groups shows promise. However,

conformational information regarding their interaction with the protein has not been investigated. Toward this end, we have investigated the fundamental photophysical properties of spermidine or spermine derivatives with varying number of N-substituted 2-naphthylmethyl groups dissolved in buffer solutions of varying pH. Electronic absorbance and steady-state emission/excitation data indicate presence of molecular aggregates. These aggregates either involve naphthyl moieties or are formed as a consequence of electron transfer from amine nitrogen(s) to the naphthyl moiety (moieties). We further investigated the aggregation phenomenon in these compounds by lowering the pH of the solutions.

112.

POROUS SILICON VAPOR SENSOR BASED ON LASER INTERFEROMETRY. *Jun Gao, Ting Gao, and Michael J. Sailor, Department of Chemistry and Biochemistry, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0358, Fax: 858-534-5383, jgao@ucsd.edu*

Certain porous silicon (PS) films exhibit well-resolved Fabry-Pérot fringes in their optical reflection spectra due to thin-film interference. The fringes shift to higher wavelengths when the PS film is exposed to vapors from organic solvents, as a result of an increase in the average refractive index of the PS silicon layer. If a single-wavelength laser is used as the light source, the shift of the Fabry-Pérot fringes upon analyte adsorption results in a change in the reflected light intensity, which correlates with the concentration of the analyte (ethanol) in an air stream. Based on this principle, a PS vapor sensor has been demonstrated. With the optimization of PS thickness and porosity, a detection limit of 250 ppb and a dynamic range of nearly five orders of magnitude have been achieved. Experimental results also suggest that capillary condensation is responsible for the vapor adsorption and the high sensitivity in these PS vapor sensors.

113.

POROUS SILICON VAPOR SENSOR WITH ENHANCED SELECTIVITY BY SURFACE MODIFICATION. *Ting Gao, Jun Gao, and Michael Sailor, Department of Chemistry and Biochemistry, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0358, tgao@ucsd.edu*

Porous silicon (PS) thin films that display Fabry-Pérot fringes are chemically modified to generate hydrophobic or hydrophilic materials. The chemical modification reactions involve thermal oxidation, oxidation with ozone, and electrochemical grafting of hydrocarbons. The films are then used as components in a laser interferometer that acts as a sensitive vapor sensor. Surface modification affects the sensitivity and specificity of the sensors. Thus a PS sensor with a hydrophilic surface has greater sensitivity to hydrophilic solvents such as ethanol, and the hydrophobic surface responds preferentially to molecules such as hexane.

114.

POTENTIAL DEPENDENT ORIENTATION OF SOLVENT MOLECULES ON PLATINUM (111) ELECTRODE SURFACE STUDIED BY SUM FREQUENCY GENERATION. *Steve Baldelli¹, Gabor A. Somorjai¹, Philip N. Ross², and Yuen-Ron Shen³. (1) Department of Chemistry, University of California, Berkeley, CA 94720, sbqz@zplink.net, (2) Material Sciences, Lawrence Berkeley National Laboratory, (3) Department of Physics/Materials Science Division, U of CA/Lawrence Berkeley National Laboratory*

Sum Frequency Generation, SFG, vibrational spectroscopy is used to investigate the orientation of acetonitrile (CH₃CN), dimethylsulfoxide (DMSO), propylene carbonate, nitromethane (CH₃NO₂) and chloroform at the Pt(111) electrode. These organic molecules are studied as a function of electrode potential to determine how this influences the adsorbate structure at the interface. The SFG results indicate acetonitrile is oriented by the electric field with the C-C bond perpendicular to the surface. The orientation is predominately with the CH₃ group toward the metal between 200-600 mV and with the CN group toward the metal above 800 mV, (vs. NHE, Normal Hydrogen Electrode). The orientation is reversible between 0-1400 mV. The other molecules show a potential dependant spectrum that is related to the reorientation of molecules at the Pt(111) surface. The alignment of molecular dipole along the surface normal is in response to the surface charge at the electrode and is reversible within a given potential window. The potential where reorientation of molecules at the surface occurs, as determined SFG, is related to the dielectric constant of the solvent.

115.

PROBING THE REDOX PROPERTIES OF MEDIUM-CHAIN ACYL-COA DEHYDROGENASE WITH DIENOYL-COA LIGANDS. *Avery W. Stephens¹, Lian Luo¹, Marian T. Stankovich¹, Alasdair F. Bell², and Peter J. Tonge². (1) Department of Chemistry, University of Minnesota, 207 Pleasant Street S.E., Minneapolis, MN 55455, Fax: 612-626-7541, step0117@tc.umn.edu, (2) Department of Chemistry, SUNY at Stony Brook*

Medium-chain acyl-CoA dehydrogenase (MCAD) is a flavoprotein capable of oxidizing fatty acyl thioesters to their corresponding fatty enoyl thioesters. This enzyme utilizes the flavin adenine dinucleotide (FAD) cofactor as the redox center in the active site, allowing the oxidation state(s) of the enzyme to be observed using UV-visible spectroscopy. The energetics of the oxidation mechanism is measured using a three-electrode spectroelectrochemical cell. 2,4-Dienoyl-CoAs are product-like ligands which bind to MCAD and induce responses that are similar to naturally occurring products, enoyl-CoAs. One of these dienoyls, hexadienoyl-CoA, produces responses that include (1) pKa shifts of the catalytic base, (2) redox potential shifts of the flavin cofactor, and (3) polarization of the pi-bond conjugation within the ligand. The dienoyl-CoAs are detectable above 300 nm which allows the MCAD/dienoyl-CoA interactions to be investigated using UV-Vis spectroscopy, electrochemistry and Raman spectroscopy. (Grant support: GM20434 to AWS, GM29344 to MTS, MCB9604254 and DAAG559710083 to PJT).

116.

QUALITATIVE AND QUANTITATIVE ANALYSES OF CORDYCEPS SINENSIS BY USING REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. *King Wah Ma, and Foo Tim Chau, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong, Hong Kong, Fax: 852-23649932, kwma@fg702-6.abct.polyu.edu.hk*

Cordyceps sinensis, is probably the most unusual energizer ever to become popular. It is a peculiar fungus that grows on certain adult caterpillars native to the high mountains of China. The fruiting bodies of the fungus have long been used in that country to enhance endurance and speed recovery from exhaustion. Recently, *Cordyceps* have been studied extensively. The fruit body and attached mycelium of *Cordyceps* have been the herb of choice in China to treat "lung" and "kidney" asthenia syndromes. It has also been used to modulate the immune system and as an adjuvant in cancer therapy. In this work, three main classes of active components in *Cordyceps sinensis*, nucleosides, ergosterol and cordycepic acid were extracted from the commercial products and determined by reversed-phase High Performance Liquid Chromatography (RP-HPLC). Both the isocratic and gradient elution methods were applied successfully to separate and identify the active components in *Cordyceps*. The concentrations of the active components were then compared between the commercial products. New standardization procedures were developed for quality control of *Cordyceps* products. The method as developed in this work can also be applied to other Chinese medicines.

117.

QUANTIFICATION OF THE PAH (POLYCYCLIC AROMATIC HYDROCARBONS) METABOLITES AS A BIOMARKER FOR PAH EXPOSURE. *Chris Smith, Wenlin Huang, Charisse Walcott, Vince Maggio, James Grainger, and Don Patterson, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway, F17, Atlanta, GA 30341, wfh7@cdc.gov*

Polycyclic Aromatic hydrocarbons (PAH), generated from a spectrum of incomplete organic combustion, dietary and occupational sources, have been categorized as an external factor associated with the development of cancer in humans. In this study, an analytical method was developed to measure PAH metabolites using GC/MS. Human urine samples (with ¹³C labeled PAHs added as internal standards) are hydrolyzed by an enzyme (β-glucuronidase/Arylsulfatase), extracted by SPME (Solid Phase Micro Extraction) fibers, then derivitized by MSTFA (N-Methyl-N-(trimethylsilyl)-trifluoroacetamide)). Separation is done using a DB-5MS capillary column, and the quantitative analysis is performed on a high resolution Mass Spectrometer. The SPME method is faster, less susceptible to contamination and requires less solvent than traditional liquid-liquid extraction and solid phase extraction techniques. The GC/MS method developed here allows 18 mono-hydroxy PAHs to be analyzed in a single run of 20 minutes. The results from the analyses of approximately 500 human urine samples will be discussed.

118.

QUANTIFICATION OF VOLATILE ORGANIC COMPOUNDS IN SOIL-VAPOR EXTRACTOR HEADSPACE BY SOLID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY. *Kayla A. Hamersky, and Diana S. Aga, Department of Chemistry, University of Nebraska at Kearney, 1107 W. 26th St., Stout Hall 313, Kearney, NE 68845, Fax: 308-865-8399, hamerskyk@unk.edu*

A cost-effective and accurate quantification method for perchloroethylene (PCE) in the headspace of soil-vapor extractors was developed using a solid-phase microextraction (SPME) apparatus, comprised of a Carboxen/Polydimethylsiloxane fiber. The analytes were concentrated by exposing the fiber to the contents of a collection bag for five minutes, allowing the analytes to partition into the solid phase. The fiber was inserted directly into the injection port (heated at 250°C) of a gas chromatograph with mass spectrometer detector (GC/MS) to thermally desorb the analytes into a 30-m VOCOL fused silica capillary column with a 0.25mm ID and a 1.50µm film. Quantification of PCE was achieved using selected ion monitoring of the base peaks of the analyte and internal standards (1.0ppm chlorobenzene-d5, 1,4-difluorobenzene, and bromochloromethane) to construct a calibration curve. This approach increased the specificity and signal-to-noise ratio of the method, which resulted in a detection limit of 0.50ppm.

119.

QUANTITATIVE ANALYSIS OF SYNTHETIC GALACTOSYL CERAMIDE ANALOGS BINDING TO HIV-1 GP120 USING TOTAL INTERNAL FLUORESCENCE MICROSCOPY. *Yingmei Gu, Rachel Y. LaBell, David F. O'Brien, Jacquelyn Gervay-Hague, and S. Scott Saavedra, Department of Chemistry, University of Arizona, Tucson, AZ 85721, Fax: 520-621-8407, yingmeig@u.arizona.edu*

Galactosyl Ceramide (GalCer) is an alternative cell receptor for HIV-1 infection in CD4 negative cells. The successful design and synthesis of analogues mimicking GalCer may lead to development of agents that can block HIV-1 infection. In this study, several GalCer analogues have been synthesized. The binding of these synthetic GalCer analogues to gp120 was measured by total internal fluorescence microscopy, on planar supported bilayer membranes bearing the analogues. The surface coverage of gp120 bound to the membrane was also determined, using in situ internal calibration. The effects of membrane phase behavior and length of the spacer group linking the Gal to the membrane surface on binding affinity and surface coverage will be discussed.

120.

QUANTITATIVE DETECTION OF PROSTACYCLIN THROUGH A SENSITIVE, BIOLUMINESCENT IMMUNOASSAY. *Urvee A. Desai¹, Sapna K. Deo¹, Michael D. Poon², and Sylvia Daunert³. (1) Department of Chemistry, University of Kentucky, Chemistry-Physics Building, CP 205, Rose Street, Lexington, KY 40506, Fax: 859-323-1069, uadesa0@sac.uky.edu, (2) The Zena and Michael A. Wiener Cardiovascular Institute, Mount Sinai School of Medicine, (3) Departments of Chemistry and Pharmaceutical Sciences, University of Kentucky*

This work describes a solid-phase assay for 6-keto-prostaglandin $F_{1\alpha}$, the stable hydrolysis product of prostacyclin (prostaglandin I_2). Prostacyclin is a potent inhibitor of platelet aggregation and a powerful vasodilator with anti-thrombotic properties and it is, therefore, employed as a therapeutic drug for pulmonary hypertension. The immunoassay for 6-keto-prostaglandin $F_{1\alpha}$ was developed using the bioluminescent protein, aequorin. A conjugate was constructed between the carboxyl group of 6-keto-Prostaglandin $F_{1\alpha}$ and lysine residues of aequorin by conventional chemical conjugation methods. The binding properties of 6-keto-Prostaglandin $F_{1\alpha}$ towards its antibody and the bioluminescent properties of aequorin were retained in the conjugate, which was then used to generate a dose response curve for the analyte. Real sample analysis was also carried out using this assay strategy.

121.

RAPID OPEN ACCESS CHANNEL ELECTROPHORESIS/MALDI-FTMS OF PEPTIDES AND OLIGOSACCHARIDES. *Jun Liu, Michele Stone, Ben Garcia, Ken Tseng, and Carlito Lebrilla, Department of Chemistry, University of California, Davis, One Shields Avenue, Davis, CA 95616, Fax: 530-752-8995, juliu@ucdavis.edu, lebrilla@chem.ucdavis.edu*

Electrophoretic separation of biological mixtures in open microchannels in combination with MALDI-MS is introduced. Separation is achieved with a Rapid

Open Access Channel Electrophoresis (ROACHE) device that has been designed and fabricated on a glass microchip. A solution of MALDI matrix and the run-buffer is applied to the chip prior to separation. After the separation, the solvent is evaporated under vacuum, and crystals are formed in the channel. The microchip is placed into a specially designed ionization source of an external source Fourier transform mass spectrometer (FTMS). The samples are ready for MALDI-MS analysis without further treatment. Separation of simple mixtures of peptides and oligosaccharide-peptide mixtures are shown.

122.

RATIONAL DESIGN OF A FUSION PROTEIN FOR THE DETECTION OF CALCIUM AND CALMODULIN ANTAGONISTS. *Emre Dikici, Sapna Deo, and Sylvia Daunert, Department of Chemistry, University of Kentucky, 205 Chemistry Physics Building, Lexington, KY 40506, Fax: 859-232-1069, edikc0@pop.uky.edu*

A fusion protein between calmodulin and enhanced green fluorescent protein (EGFP) was developed in order to monitor the conformational changes induced by Ca^{+2} binding. In the presence of Ca^{+2} , calmodulin undergoes a conformational change and allows for interactions with calmodulin-binding proteins, peptides, and drugs such as trifluoropiperazine and phenothiazine. Upon binding to the analyte, the calmodulin conformational changes lead to a change in the fluorescence of EGFP that can be correlated to the concentration of the corresponding analyte. This method can be used as a sensor for Ca^{+2} and calmodulin-binding proteins. It also can be used for high throughput screening of calmodulin antagonists. Finally, since neither calmodulin nor EGFP is toxic to the cell, this fusion protein can be used for *in vivo* sensing of calmodulin agonists/antagonists in whole cell detection systems.

123.

RECOVERY AND DEGRADATION OF COMPLEXED CYANIDE. *Shyam Shukla, Chemistry Department, Lamar University, Beaumont, TX 77710, Fax: 409-880-8270, shuklass@hal.lamar.edu, Alka Shukla, Chemistry Department, Southeast College, 6815 Rustic, Houston, TX 77270, shukla_1998@yahoo.com, John L. Margrave, Department of Chemistry, Rice University, Jose Parga, Metallurgy Department, Institute Technology, and Jan Miller, Department of Metallurgy and Materials Science, University of Utah*

This paper is a result of consortium effort and it can be divided in two parts. In the first part, we will examine the use of titania for photomicroelectrochemical degradation of complexed cyanide. We will discuss the effects of supplementary oxidants on titania to destroy cyanide. In the second part, we will discuss recovery/destruction of complexed cyanide. In this regard, the air-sparged hydrocyclone (ASH) has been used as a reactor for the treatment of cyanide solutions in two ways: first for cyanide recovery by acidulation using the Mexican modification of the Mills-Crowe process and second for cyanide destruction by oxidation with the use of chlorine dioxide (ClO_2). In both cases excellent performance can be achieved using the high capacity ASH technology.

124.

REPORTER GENE TECHNOLOGY IN THE DESIGN OF A BIOSENSING SYSTEM FOR ARSENITE. *Jessika Feliciano, Yue Liu, and Sylvia Daunert, Department of Chemistry, University of Kentucky, Rose Street, Chem-Phys Bldg, Lexington, KY 40506-0055, Fax: 859-323-1069, jsfeli0@pop.uky.edu*

A sensitive and selective optical sensing system for arsenite based on genetically engineered bacteria will be presented. The basis of this system is the *ars* operon, which confers the ability to certain bacteria to survive in environments contaminated with antimonite, arsenite, and arsenate. Using the specificity of the *ars* operon for antimonite and arsenite, a highly selective whole-cell sensing system has been developed using *cobA* as the reporter gene and *E. coli* cell line AW10, in which the chromosomal *ars* operon is deleted, as the host. The response for arsenite and antimonite was measured on samples that mimic the composition of a real sample from the contaminated waters of Bangladesh. The selectivity of the system was demonstrated by evaluating several oxoanions and soft metals as possible interferences. The potential applications of this reagentless biosensing system in environmental samples will be discussed.

125. SENSITIVE SUB-DOPPLER NONLINEAR SPECTROSCOPIC METHOD FOR SIMPLIFIED ISOTOPE-RATIO MEASUREMENT. *Helen R. Kemp, Ron D. Briggs, Julia A. Schafer, and William G. Tong, Department of Chemistry, San Diego State University, San Diego, CA 92182, Fax: 619-594-2442, william.tong@sdsu.edu*

Sub-Doppler degenerate four-wave mixing spectroscopy is presented as a simple method for isotope-ratio measurement without the use of expensive mass analyzers. It offers excellent sensitivity at isotope resolution using inexpensive low-power lasers. The use of counter propagating input laser beams yields sub-Doppler spectral resolution that is suitable for hyperfine analysis and isotope-ratio measurement. It offers important advantages over other popular isotope-capable techniques. Unlike mass spectrometry, this nonlinear technique offers unambiguous isotope ratio information since it is based on unique hyperfine structures.

126. SEPARATION OF PROPOXYPHENE ENANTIOMERS USING CAPILLARY ELECTROPHORESIS. *Thomas C. Werner, Tania Magoon, and Keiko Ota, Department of Chemistry, Union College, Union Street, Schenectady, NY 12308, Fax: 518-388-6795, wernert@union.edu, magoon@union.edu*

We are collaborating with the New York State Forensic Investigation Center in Albany, NY to develop an efficient protocol for the separation of propoxyphene enantiomers using capillary electrophoresis (CE). Currently, the Forensic Center employs a crystallization method that is not only subjective but provides no hard copy of results and requires at least 1 mg of sample. Lurie et al. have reported the separation of d- and l-propoxyphene in 20 minutes by CE using a mixture of methanol, neutral and ionic cyclodextrins (CD) as additives. We have been able to achieve baseline separation (R1.5) using either heptakis (tri-2,3,6-O-methyl)-beta-CD or polymers of beta-CD and gamma-CD formed by reaction of the CD monomers with epichlorohydrin. Typical conditions include a pH 8.23, 25 mM borate buffer containing 300 mM of CAPS and 30 mM of the CD additive. Separation of the propoxyphene isomers occurs in about five minutes. This method is being adapted for use by the Forensic Center.

127. SEPARATION OF TAXANES BY REVERSED PHASE LIQUID CHROMATOGRAPHY ON FLUORINATED AND HYDROCARBONACEOUS STATIONARY PHASES. *Ralf Dolfinger, Department of Chemistry, Queens College and Graduate Center of the City University of New York, 65-301 Kissena Blvd., Flushing, NY 11367, rado_69@hotmail.com, and David C. Locke, Department of Chemistry, Queens College of the City University of New York*

Fluorinated stationary phases have been used successfully in the past to separate paclitaxel and related taxane compounds using reversed phase liquid chromatography. The advantage of fluorinated phases compared to octyl or octadecyl phases was believed to be based on the particular chemical selectivity exhibited by the fluorinated surface of the stationary phase. The poster will show a comparison of the separation of fifteen taxane compounds on a regular octyl phase and several fluorinated phases. Detailed studies of the effect of mobile phase composition and temperature on the separation will be presented. The data will conclude the primary reason for the better performance of fluorinated phases in the separation of taxanes.

128. SPATIAL RESOLUTION INCREASE OF QUASI-DISTRIBUTED FLUORESCENT SENSOR ARRAYS ON OPTICAL FIBERS. *Barry J. Prince, Alan W. Schwabacher, and Peter Geissinger, Department of Chemistry, University of Wisconsin-Milwaukee, P.O. Box 413, Milwaukee, WI 53201-0413, Fax: 414-229-5530, baz@uwm.edu, geissing@uwm.edu*

Pulsed laser readout of quasi-distributed fiber-optic sensor arrays allows for the determination of the location of a sensing event along the fiber. When using fluorescent sensors, however, the spatial resolution of such arrays is limited by the fluorescence lifetimes. We report here a technique utilizing two optical fibers: one to deliver an excitation pulse to the sensor regions, and the other to collect sensor fluorescence and deliver it the detector. The coupling between the fibers is purely evanescent. We demonstrate that this scheme reduces the minimum spacing of adjacent sensors by at least two orders of magnitude. Moreover, the parameters of each fiber may be adjusted independently for

optimum signals. The sensor regions can be prepared on one fiber and exposed to the experimental conditions whilst completely separated from the detection apparatus. A separate contribution presents a novel combinatorial chemistry method for the efficient preparation of large linear sensors arrays.

129. STRATEGIES FOR THE SCREENING AND DETECTION OF TRIAZINE HERBICIDE CONTENT IN ENVIRONMENTAL SAMPLES USING ANTIBODY COUPLED CAPILLARY ELECTROPHORESIS. *Charlene D. Crawley, Monica K. Little, and Litticia Clay, Department of Chemistry, Virginia Commonwealth University, 1001 West Main Street, Richmond, VA 23284, Fax: 804-828-8599, cdcrawle@saturn.vcu.edu, Katrice17@vcu.org*

Triazine herbicides are among the most common class of pesticides used to kill weeds on non-crop land, and its widespread usage has led to the contamination of drinking water. Capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) have been used to measure triazine content. The use of monoclonal and polyclonal antibodies to selectively isolate and analyze environmental contaminants can drastically improve the sensitivity and resolution of the CE assay. Anti-atrazine monoclonal antibodies have been used in the on-line pre-concentration and analysis of the atrazine content in well water samples. An evaluation of the atrazine responses using glutaraldehyde-entrapped monoclonal antibodies on fused silica capillaries will be compared with responses obtained for antibody adsorbed to C8 hydrophobically modified capillaries. In addition, the electropherograms of two monoclonal antibodies to atrazine obtained using capillary isoelectric focusing (cIEF), and capillary sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) will be correlated with their CE and ELISA performances.

130. STRUCTURAL ANOMALIES THAT AFFECT THE BINDING AND ACTIVITY OF POLYMERASES. *Hellen F. Thomson, and Stephen Winkle, Department of Chemistry, Florida International University, 11200 SW 8th St., Miami, FL 33199, Fax: 305-348-3772*

DNA serves as a target molecule for several types of enzymes and may assume a wide variety of structural motifs depending upon the local sequence. Previous results from our lab suggest the presence of alternate structures can affect polymerase activity with respect to end-labeling and amplification. We have examined the effects of the (CG)₃ segment and cruciform structures found in the bacteriophage phiX174, on DNA polymerase I binding and activity. Gel mobility shift assays suggest that DNA polymerase I may bind to the DNA in the absence of cognate binding sequences. Restriction enzyme digests show altered activities suggesting that binding is in the vicinities of the (CG)₃ segment and the palindromic region. PCR assays indicate the alternate conformations alter the polymerase activity, generating anomalous products. The data presented suggests that the complex secondary structures may alter the inherent activity of polymerases, including locating binding sites and amplification of DNA fragments.

131. STRUCTURE-FUNCTION RELATIONSHIPS OF OCTADECYLSILANE STATIONARY PHASES BY RAMAN SPECTROSCOPY. *Christopher J. Orendorff, Michael W. Ducey Jr., and Jeanne E. Pemberton, Department of Chemistry, University of Arizona, 1306 E. University Blvd, Tucson, AZ 85721, Fax: 520-621-8248, chriso@u.arizona.edu*

Raman spectroscopy is used to examine the effects of temperature, surface coverage, nature of alkylsilane precursor (octadecyltrichlorosilane, methyl octadecyldichlorosilane, or dimethyloctadecylchlorosilane), and method of preparation (surface or solution polymerization) on alkylsilane conformational order in a series of high-density octadecylsilane stationary phases* in the presence of solvent. Conformational order is assessed using empirical spectral indicators. Conformational order of the alkyl chains is observed to be dependent on solvent, generally increasing in polar solvents and decreasing in nonpolar solvents. Raman spectroscopy is sensitive to subtle changes in the solvated conformational order of the alkyl chains as a function of temperature, surface coverage, nature of the alkylsilane precursor and preparation method. These results are used to propose solvent-stationary phase molecular interactions.

* The authors gratefully acknowledge Dr. Lane C. Sander of the National

Institute of Standards and Technology for the kind gift of the high-density stationary phase materials.

132.

STUDIES ON ELECTROCHEMICAL REACTIONS OF METALLOTHIONEIN ADSORBATES AT MERCURY FILM ELECTRODES. *Fayi Song, Alejandro L. Briseno, and Feimeng Zhou, Department of Chemistry and Biochemistry, California State University, Los Angeles, 5151 State University Drive, Los Angeles, CA 90032, Fax: 603-754-5471, songfayi@yahoo.com, abrisen2@earthlink.net, fzhou@calstatela.edu*

Rabbit liver metallothionein (MT) molecules were adsorbed onto thin mercury films preformed onto glassy carbon electrodes and subsequently transferred into MT-free phosphate buffers. The redox behavior of the surface-confined MT was studied by cyclic voltammetry and differential pulse voltammetry, while the amount of MT adsorption was quantified by a flow-injection quartz crystal microbalance. Two reversible redox waves were observed. The overall voltammetric characteristics were found to be remarkably analogous to that of adsorbed cysteine and cystine molecules. The two redox waves were found to be transformable by holding the potential at a negative potential (e.g., -1.2 V) to reduce the MT adsorbate or by applying a more positive potential (e.g., at -0.1 V) to oxidize the adsorbed MT molecules. Based on these voltammetric studies, we postulate that there exist two types of surface orientations among the adsorbed MT molecules.

133.

STUDY OF β -LACTAMASE INHIBITORS USING AN EGFP FUSION PROTEIN. *Libby G. Puckett, Jennifer C. Lewis, Sylvia Daunert, and Leonidas G. Bachas, Department of Chemistry, University of Kentucky, Rose Street, Lexington, KY 40506-0055, Fax: 606-323-1069, lpuck0@pop.uky.edu*

Bacterial resistance to β -lactam antibiotics occurs through the production of β -lactamases. β -Lactamase is an enzyme that catalyzes the cleavage of the β -lactam ring of antibiotics such as penicillin and ampicillin. To study this hydrolysis, a fusion protein was constructed between enhanced green fluorescent protein (EGFP) and β -lactamase. When the β -lactam ring is cleaved, there is a decrease in the local pH. Subsequently, the fluorescence of EGFP decreases which can be correlated to the amount of antibiotic present. Other hydrolytic enzymes can also be conjugated to EGFP. This EGFP/ β -lactamase fusion protein has also been used to study two common β -lactamase inhibitors - clavulanic acid and sulbactam sodium. Both are irreversible competitive inhibitors of β -lactamase that are commonly combined with β -lactam antibiotics in order to prevent bacterial resistance. Upon addition of the inhibitor, β -lactamase is unable to hydrolyze the β -lactam ring of penicillins, and the fluorescence of EGFP is unaltered.

134.

STUDY OF STRUCTURE-FUNCTION RELATIONSHIPS FOR NOVEL MONOCLONAL ANTIBODIES USING CAPILLARY ELECTROPHORESIS. *Charlene D. Crawley, and Monica K. Little, Department of Chemistry, Virginia Commonwealth University, 1001 West Main Street, Richmond, VA 23284-2006, Fax: 804-828-8599*

Triazine herbicides are among the most common classes of herbicides used to kill weeds on non-crop land, and its widespread usage in urban areas has led to the contamination of drinking water. Capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC) have been used to measure triazine content. However, the sensitivity and resolution of the CE assay can be improved by the use of antibodies specific for the target analyte. Anti-atrazine monoclonal and commercial polyclonal antibodies have been used in the on-line pre-concentration and analysis of the atrazine content in well water samples. Previously, work has been done using glutaraldehyde to polymerize monoclonal antibodies in order to bind the antibody to the surface of fused silica capillaries. Results obtained using this mode of immobilization of the antibody, along with data generated from the adsorption of antibodies to C8 modified capillaries will be compared for similarities in their responses. These results will also be compared with those obtained by ELISA. Finally, a comparison of the isoelectric points (pI) of two monoclonal antibodies for identical pesticide antigens using capillary isoelectric focusing (cIEF) will be discussed.

135.

SUM FREQUENCY GENERATION SPECTROSCOPIC STUDY OF SURFACE SPECIES. *Peilin Chen¹, K.Y. Kung¹, Y. R. Shen², and Gabor A. Somorjai¹. (1) Department of Chemistry, University of California, Berkeley, 1 Cyclotron Rd., Bldg 66/427, Berkeley, CA 94720, Fax: 510-486-4995, pchen@mailman.lbl.gov, (2) Department of Physics, University of California*

Surface vibrational spectroscopy using infrared-visible sum frequency generation (SFG) was applied to the study of CO poisoning effect in ethylene hydrogenation reaction on the Pt(111) surface at various temperatures. In the presence of 3 Torr of CO, 10 Torr of ethylene and 100 Torr of hydrogen at room temperature, only CO was observed on platinum surface. Under such conditions, no product (ethane) was observed in the gas phase. When the temperature of platinum was increased to 150 C, which is above the CO desorption temperature, CO resonance peak decreased, and the reaction product, ethane, was detected by GC. However, the reactivity was three orders of magnitude lower than the same reaction without CO. Our result indicates that CO poisons the ethylene hydrogenation reaction by preventing ethylene from absorbing on surface.

136.

SURFACE COORDINATION OF A TRIRUTHENIUM CLUSTER ON PLATINUM NANOPARTICLES FOR METHANOL OXIDATION CATALYSTS. *Ramonita Diaz Ayala¹, Esteban R. Fachini¹, Carlos R. Cabrera¹, Emerilis Casado Rivera¹, and Sharon Files-Emperador². (1) Department of Chemistry, University of Puerto Rico, Facundo Bueso Bldg. - FB-B1, San Juan, PR 00931, ramonita_diaz@hotmail.com, (2) Department of Biology, University of Puerto Rico*

An inorganic surface modification was used to prepare the Pt:Ru/Vulcan catalysts by coordination of a triruthenium cluster on Pt nanoparticles, which provided a surface with catalytic activity for use in direct methanol fuel cells. The proposed method gave Ru atoms on Pt particles rather than usual Pt:Ru alloys. The adsorptive process followed a Langmuir isotherm, and the amount of deposited Ru could be controlled changing the adsorption conditions. The catalyst powders were characterized by X-ray fluorescence, TEM, XPS, XRD and electrochemical studies. The peak potential for methanol oxidation reached a minimum for some concentration around 0.5 mM. From chronoamperometric experiments, the better performance was reached for the catalyst prepared with solutions of Ru cluster at around 0.4 mM. The comparison between the synthesized and commercial catalysts, in the same experimental conditions, showed similar performance. The best catalyst presented a Ru:Pt ratio of 0.07, as given by XPS analysis.

137.

SURFACE PLASMON RESONANCE SPECTROSCOPY AND METAL FILM RESISTANCE CHANGES DURING Pb UNDERPOTENTIAL DEPOSITION AT Au AND Ag. *Roger Terrill, Mondona Zangeneh, and Edward Sambriski, Department of Chemistry, San Jose State University, One Washington Square, San Jose, CA 95192-0101, Fax: 408-924-4945, rterrill@jupiter.sjsu.edu*

Concurrent measurements of the shift in wavelength of the plasma resonance (SPR) and the increase in the resistance of thin Ag and Au films were made during the electrodeposition of single monolayers of Pb. Substantial responses were recorded using both metrics. The SPR data were interpreted to be in response to a uniform Pb layer that was increasing in thickness. Using this interpretation, the SPR shift is a linear function of Pb coverage and the data were used in this way to determine the coverage dependence of the resistance increase incurred by Pb ion deposition.

138.

SURFACE SECOND HARMONIC GENERATION STUDY OF LAYER ORDER IN SELF-ASSEMBLED MULTILAYERS. *Stephen B. Bakiomoh, and Gary J. Blanchard, Department of Chemistry, Michigan State University, East Lansing, MI 48824, Fax: 517-353-1793, bakiomoh@photon.cem.msu.edu*

The ability to control the structure and composition of organic thin films makes them attractive systems for tailoring specific surface properties and for the study of interfacial phenomena such as friction or lubrication, wetting and adhesion, electron transfer, and corrosion protection. Thus through the careful design, synthesis, and utilization of specific adsorbates, layered interfaces have found applications in the electronics and microelectronics industries. Controlling the molecular structure of interfaces is now within the domain of routine

chemical preparation however, for all the intended applications, there is a fundamental question of the extent of organization within the material. We report on the use of surface second harmonic generation to probe microscopic organization in aliphatic multilayer assemblies. We synthesize oriented multilayer assemblies where the increase in the net macroscopic dipole moment results in increase of the second harmonic intensity as a function of the number of layers. These assemblies were also used to study the $\chi^{(2)}$ nonlinear optical response of the inorganic interlayer of layered assemblies. The data reveal an overall decrease in order with increasing interface thickness.

139.

SURFACE-ENHANCED RAMAN SPECTROSCOPY-BASED SPECTROELECTROCHEMISTRY AS AN IN SITU VIBRATIONAL PROBE OF SERS-ACTIVE SITES ON AG SURFACES. *Vanessa B. Oklejas, and Joel M. Harris, Department of Chemistry, University of Utah, 315 South 1400 East Rm. #2020, Salt Lake City, UT 84112-0850, Fax: 801-581-8433, vanessa@chemistry.utah.edu*

SERS-based spectroelectrochemistry is used to characterize the potential dependence and chemical heterogeneity of SERS-active adsorption sites found on both electrochemically-roughened and highly-polished polycrystalline Ag electrodes. The nitrile stretching vibrational mode of adsorbed thiocyanate is used as an in-situ spectroscopic probe: the potential dependence of band position, shape, and scattering intensity of this mode are measured in order to investigate the chemical nature of SERS-active sites. Results obtained from thiocyanate on electrochemically-roughened Ag surfaces indicate that there is a diverse population of SERS-active adsorption sites onto which thiocyanate adsorbs. The local electronic character of these sites results in large vibrational bandwidths and significant SERS activity that are sensitive to applied potential. With the application of sufficiently large negative potential, many of these sites anneal and disappear which results in irreversible loss of SERS intensity. Results obtained from thiocyanate adsorbed onto highly-polished Ag show a discrete population of adsorption sites which exhibit a single nitrile vibrational frequency that tunes with applied potential. The potential dependence exhibited by scattering intensity of the nitrile stretching frequency is also measured using different incident excitation wavelengths to determine the charge-transfer contribution to the observed Raman scattering enhancement.

140.

TIP-ENHANCED RAMAN SPECTROSCOPY AS A NEW ANALYTICAL TOOL FOR NANOMATERIALS. *Yung-Doug Suh, Raoul M. Stoeckle, Volker Deckert, and Renato Zenobi, Department of Chemistry, ETH Zurich, Universitaetstr. 16, Zurich CH-8092, Switzerland, Fax: ++41-1-632-1292, suh@org.chem.ethz.ch*

Near-field Raman spectroscopy with aperture probes suffers from several difficulties stemming from the intrinsically low Raman scattering cross section. Long acquisition times in comparison with near-field fluorescence are required to obtain reasonable signal at each point. Therefore brighter near-field aperture probes with a high transmission coefficient for a given aperture diameter, along with a long-term stability of the scanner are desirable. In spite of these difficulties, 70-nm scale Raman mapping has been reported.

In an effort to push the limits for nanometer scale Raman spectroscopy, we combined apertureless scanning near-field optical microscopy (SNOM) and surface enhanced Raman spectroscopy (SERS). A sharp noble metal tip was brought into close proximity of a sample using a force feedback. Two different types of noble metal tips are used. One is an electrochemically etched gold tip attached to a tuning fork; the other is a silver-coated atomic force microscope (AFM) tip. The tip is illuminated by a focused laser beam from below through an oil immersion objective lens of an inverted optical microscope. Scattered light is collected by the same objective lens and then fed into a spectrograph coupled to a liquid nitrogen-cooled CCD camera to record Raman spectra.

Strong enhancement of the Raman signal from the sample was observed both for a gold tip and a silver-coated AFM tip, but only when they are in feedback. This localized enhancement under the tip enabled us to chemically identify features in the corresponding AFM image. Several examples of this tip-enhanced Raman spectroscopy, including molecular thin films of C₆₀, brilliant cresyl blue (BCB), carbon nanotubes, and an air bacteria will be presented.

141.

TOXICITY IN NAILS: A STUDY OF CD, CR, PB AND NI IN FINGER NAILS BY AAS. *Shahnaz Kazi, G. H. Kazi, and T. G. Kazi, NCEAC, University of Sindh, Jamshoro, Sindh, Pakistan, kazishahnaz@yahoo.com*

Human Beings by their own technology have introduced into his environment a number of toxic metals, at an unprecedented and constantly increasing rate. The pollution of a living environment had been a global concern of the present time. The present paper reviews the study of four toxic metals namely Lead Cadmium, Chromium and Nickel in fingernails of twenty different families of Sindh, Pakistan by Atomic Absorption Spectrophotometry (AAS). A total of 275 nail samples were examined. Samples were digested with concentrated Nitric acid and Hydrogen per oxide. The geometric mean and Standard deviation were calculated for each element along with a summary of the effects of age, sex, and nutritional values on the concentration of each of these elements in nails. In the analysis of trace and toxic elements in biological materials there is need not only to determine the element in the whole sample but also to relate the obtained analytical data with relevant biological parameters. The results showed that the concentration of Cadmium, Chromium, and Lead is sex dependent. The data represent a significant contribution to existing information on toxicity in nails of non-industrialized population of two major cities of Pakistan.

142.

TRACE METAL ASSESSMENT IN THE TATNUCK BROOK WATERSHED, WORCESTER MA, USA. *Anne M. Falke, Scott Kaplan, Nicole Arpin, Victoria V. Dune, and Kristina Ostlund, Department of Chemistry, Worcester State College, 486 Chandler St., Worcester, MA 01602, afalke@worcester.edu, skaplan@charter.net, narpin@worcester.edu, vdune@worcester.edu*

Trace metal assessment in fresh water streams and related areas streams has been very limited. From Spring 1998 through Fall of 1999 water samples were collected at various locations along the Tatnuck Brook Watershed, located in Worcester County, MA and evaluated for Copper, Cadmium, Nickel and Silver. Samples were collected on a weekly basis from various locations along the watershed. The water samples were preconcentrated with cobalt-ammonium pyrrolidine dithiocarbamate chelation. Concentrations of metals were determined by graphite furnace atomic absorption spectroscopy (AAS). Despite the fact that particular attention was paid sampling above and below the Old Coes Knife Factory, which was known to deposit tailings into the brook to build up a dam, no elevated metal concentrations were found downstream from the factory. Our hypothesis is that metals adsorbed to sediments in the brook. We will collect sediment cores at various locations above and below the factory site. Sediments will be digested in aqua regia and analyzed by graphite furnace AAS. Background data will be obtained from a site at the headwaters for the brook.

143.

ULTRATHIN SOL-GEL COMPOSITE SILICA-FERROCENE FILMS FABRICATED ON METAL SUBSTRATES. *Joseph W. Robertson, Jeffrey W. Anthis, and Jeanne E. Pemberton, Department of Chemistry, University of Arizona, 1306 E. University Blvd, Tucson, AZ 85721, Fax: 520-621-8248, robertso@u.arizona.edu*

A novel sol-gel approach has been developed for the fabrication of redox-active ultrathin silica-based oxides deposited onto metal substrates. The fabrication approach relies on the use of an ultra-dilute sol-gel solution modified to contain the redox species 1,1'-bistriethoxysilylferrocene deposited onto self-assembled monolayers (SAM) of (3-mercaptopropyl) trimethoxysilane on Ag or Au to produce electrochemically active hybrid sol-gel films. Raman spectroscopy has been used to study the hydrolysis and condensation reactions of 1,1'-bistriethoxysilylferrocene and tetramethoxysilane in order to better optimize the film formation conditions. The final ferrocene-modified silica films have been examined with a variety of optical spectroscopies including ellipsometry and infrared spectroscopy, the scanning probe microscopies AFM and STM, and electrochemical methods including cyclic voltammetry and ac impedance. These results will be presented, and their implications in terms of the electrical characteristics of these films will be discussed.

144.

USE OF 1-ALKYL-3-METHYLIMIDAZOLIUM BASED IONIC LIQUIDS FOR THE ELECTROPHORETIC SEPARATION OF POLYPHENOLS FOUND IN GRAPE SEED EXTRACTS. *Enrique G. Yanes, Samuel R. Gratz, and Apryll M. Stalcup, Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221-0172, Fax: 513-556-9239, yanosse@email.uc.edu*

Ionic substances with melting points at or close to room temperature are referred to as ionic liquids. Interest in ionic liquids for their potential in different chemical processes is increasing because they are environmentally benign and good solvents for a wide range of both organic and inorganic materials. In this study, a capillary electrophoretic method for resolving phenolic compounds found in grape seed extracts is reported. The method is simple and reproducible which uses 1-alkyl-3-methylimidazolium based ionic liquids as the running electrolytes. The separation mechanism seems to involve association between the imidazolium cations and the polyphenols. The role of the alkyl substituents on the imidazolium cations was investigated and will be discussed.

145.

USE OF ONLY PULSED ARGON IN THE QUADRUPOLE ION TRAP MASS SPECTROMETER TO IMPROVE TRAPPING AND MS/MS EFFICIENCY. *Allison S. Danell, and Gary L. Glish, Department of Chemistry, University of North Carolina at Chapel Hill, CB# 3290, Venable Hall, Chapel Hill, NC 27599, allison_danell@unc.edu*

The addition of a static pressure of heavy gases to the static pressure of He buffer gas in the quadrupole ion trap has been established as an effective method for increasing MS/MS efficiency. However, accompanying decreases in resolution and trapping efficiency have been observed. By pulsing a heavy gas into the ion trap, it can be removed before detection of the ions, and the resolution does not degrade. We have investigated the use of only pulsed Ar in the absence of any other added buffer gas in the ion trap. Ar was pulsed into the ion trap during specific stages of an experiment to quantify its effects on trapping and MS/MS efficiency. At several Ar pressures, increased trapping efficiency for peptide ions over a range of m/z values was observed. MS/MS efficiencies for these peptide ions also were higher than for those dissociated in a mixture of pulsed Ar and a static pressure of He buffer gas.

146.

USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY TO EXPLORE THE ROLE OF OXIDANTS IN THE PATHOPHYSIOLOGY OF DISEASE. *Joseph P. Gaut, George C. Yeh, Jaeman Byun, Hung Duy Tran, and Jay W. Heinecke, Department of Medicine, Washington University School of Medicine, 660 S. Euclid Ave., Campus Box 8046, St. Louis, MO 63110, Fax: 314-362-0811, gccyeh@yahoo.com*

Phagocyte oxidants are thought to be major mediators of our innate host defenses. Under pathological conditions, these oxidants might also contribute to host injury. In vitro, phagocyte peroxidases catalyze the formation of hypohalous acids, tyrosyl radical, and reactive nitrogen intermediates. These reactive species form characteristic end products with tyrosine. Hypochlorous acid forms 3-chlorotyrosine, hypobromous acid generates 3-bromotyrosine, tyrosyl radical forms dityrosine, and reactive nitrogen species generate 3-nitrotyrosine. Quantification of these end products from pathological samples would provide insight into the pathophysiological role of their oxidant precursors. Previous studies have utilized MS to quantify these molecules from biological material. However, their harsh derivatization conditions raise the potential for artifact generation. We have developed novel GC-MS methods that avoid such conditions and account for artifact generation during sample processing. We explored the utility of this technique to detect and quantify various biomarkers of oxidation from a variety of biological samples.

147.

USING RECEPTOR CONFORMATIONAL CHANGE TO DETECT LOW MOLECULAR WEIGHT ANALYTES BY SURFACE PLASMON RESONANCE. *Jason E. Gestwicki, Helen V. Hsieh, and J. Bruce Pitner, BD Technologies, 21 Davis Dr, Research Triangle Park, NC 27709, Fax: 919-313-6400, Bruce_Pitner@bd.com*

Small molecules in solution are typically difficult to detect directly using commercially available SPR (surface plasmon resonance) instruments. The low molecular weight of these small ligands (in these examples, < 360 mw) is not sufficient to cause a detectable change in refractive index on binding to

surface-bound receptors (e.g., antibodies). Some receptors, however, undergo extensive changes in tertiary structure upon binding ligands. Here we present data suggesting conformational changes in surface-bound receptors such as periplasmic binding proteins, calmodulin, and tissue transglutaminase can be detected by SPR. Further, this response can be used to monitor specific binding of ligands such as carbohydrates and metal cations even when a ligand of this molecular weight would not be detectable using traditional SPR methods. The observed concentration-dependent responses matched favorably with the known dissociation constants for these interactions. This approach has potential applications for developing biosensors and for screening small molecule drug libraries.

148.

WHOLE CELL-BASED SENSING SYSTEMS FOR METAL IONS. *Ranjit S. Shetty, Sridhar Ramanathan, Yue Liu, Janet Wolford, Puja Shah, and Sylvia Daunert, Department of Chemistry, University of Kentucky, Chemistry-Physics Bldg, Lexington, KY 40506, rssheto@sac.uky.edu*

Certain microorganisms can survive in environments polluted with toxic metal ions thanks to suitable resistance machinery that get triggered in the presence of these ions. On the other hand, some metal ions are critical for the growth and metabolic functions for the organisms at certain levels. Genetic determinants evolved in these organisms not only ensure that the cells are provided with the nutritional levels of the metal ions but also prevent their accumulation above toxic levels. The expression of these resistance genes is tightly regulated by regulatory proteins and depends on the availability of the specific metal ion in the cell. By exploiting the specific regulation and induction properties of the genes and regulatory protein with respect to specific metal ions, biosensing systems for detecting these metal ions have been developed. Whole cell-based sensing systems for cadmium, copper, arsenite and antimonite employing β -galactosidase and GFP reporters will be presented.

149.

ADVENTURES IN THE MASS SPECTROMETRY OF RNA. *James McCloskey, Department of Medicinal Chemistry, University of Utah, 30 S 2000 E RM 311A, Salt Lake City, UT 84112-5820, Fax: 801-581-7457, james.mccloskey@m.cc.utah.edu*

Mass spectrometry is emerging as a powerful technique for mapping of natural modifications in RNA, which are formed enzymatically following the transcription of DNA to RNA. From the standpoint of analytical chemistry the substantial challenge includes analysis of 200-400 component mixtures of oligonucleotides, recognition of modified nucleoside residues from the nearly 100 now known—or structure determination at the picomole level of new nucleosides—plus exact sequence placement of the modified residues in RNAs of molecular mass of up to 1M Da. The great importance of RNA in biology derives from the direct roles of transfer RNA and the ribosomal RNAs in translation of the genetic code and catalysis of protein synthesis. These functions of RNA are in turn fine-tuned through regional control of structure by strategically placed modified nucleosides. One of the most fascinating examples is the RNA from thermophiles, organisms which grow optimally between 70°C and 106°C. The RNA of these organisms contain numerous unexplored modifications, some of which appear to provide significant thermostability.

150.

INTEGRATION OF MASS SPECTROMETRIC AND BIOINFORMATICS TOOLS FOR STUDIES OF PROTEIN MACHINES. *A. L. Burlingame, Mass Spectrometry Facility, University of California, San Francisco, University of California, 513 Parnassus Ave., San Francisco, CA 94143-0446, Fax: 415-476-0688, alb@itsa.ucsf.edu*

Recently, spectacular developments have occurred in the mass spectrometry-based characterization of complex protein mixtures that have the potential to reveal not only the molecular players involved in maintaining cell homeostasis but also the dynamic changes in their covalent structure that are critical determinants of protein function and interaction. This methodology will reveal the expressed protein composition as well as the structural details of the physiologically active forms of proteins. In a post-genomic era that seeks to understand the function of proteins at the cellular and organismal level, a variety of mass spectrometric technologies will play essential roles. Progress on current

research involving problems of biological significance will be discussed. Supported by NIH NCRR Grant 01614.

151.

AUTOMATED IDENTIFICATION OF PEPTIDES AND PROTEINS AT THE ATTOMOLE LEVEL IN COMPLEX MIXTURES BY MASS SPECTROMETRY.

Donald F. Hunt, *Departments of Chemistry and Pathology, University of Virginia, Charlottesville, VA 22901, Fax: 804-296-3159, dfh@virginia.edu*

Presented here is novel methodology for the rapid, automated identification of proteins present at the attomole level in complex mixtures. Prior fractionation of the mixture by gel electrophoresis is not required and characterization of post translational modifications on the target proteins is facilitated in many cases. Representative examples will be presented from the analysis of (a) protein protein interactions (b) proteins involved in nuclear transport (c) proteins that allow a plant to synthesize its own fungicide, (d) proteins involved in signal transduction cascades (e) antigens presented by class I and II MHC molecules, and (f) proteins expressed uniquely by two different cell populations.

152.

LOW RESOLUTION PROTEIN STRUCTURES BY MASS SPECTROMETRY.

Bradford W. Gibson¹, *Malin Young*², *Birgit Schilling*¹, *Ning Tang*¹, *Christopher Collins*¹, *R. Kip Guy*³, *Andrew D. Leavitt*⁴, *Gavin Dollinger*⁵, and *Irwin D. Kuntz*¹. (1) *Department of Pharmaceutical Chemistry, University of California, San Francisco, 513 Parnassus Ave., School of Pharmacy, San Francisco, CA 94143-0446, Fax: 415-476-0688, gibson@socrates.ucsf.edu*, (2) *Sandia National Laboratory*, (3) *Department of Pharmaceutical Chemistry and Department of Cellular and Molecular Pharmacology, University of California at San Francisco*, (4) *Laboratory Medicine, University of California, San Francisco*, (5) *Small Molecule Drug Discovery, Chiron*

We have recently reported on a general method that identifies the fold family of a protein using a combination of chemical crosslinking, mass spectrometry and computational techniques with fibroblast growth factor as a model system (Young et al., 2000, PNAS 97:5802). We are now expanding this methodology to include new protein targets and to improve the efficiency of protein crosslinking and our ability to rapidly identify peptides obtained after proteolysis that contain the distance constraint information. For example, the orientation of the three domains of HIV-1 integrase is currently being determined in the presence and absence of DNA to obtain a better understanding of this enzyme's function in the integration of viral and host DNA. Moreover, we have developed new bifunctional reagents that will enable us to define the distances between crosslinked amino acids with higher precision that should allow us to apply this strategy for low-resolution protein structure determination.

153.

EMERGING ROLES OF MASS SPECTROMETRY IN PROTEIN ANALYSIS.

Steven A. Carr, *Department of Physical and Structural Chemistry, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, PA 19406, Fax: 610-270-6608, Steven_A_Carr@sbphrd.com*

From initial isolation to large-scale production, MS (often in combination with high resolution electrophoretic or chromatographic techniques) has emerged as the single most powerful tool for protein analysis. The unique capabilities of MS for structural characterization of posttranslational modifications kept interest and use of this technology alive in the biochemistry community during the fairly "dark-ages" of protein MS (pre-1980). Today, the ever increasing reliance on MS is the result of continuing improvement in the performance and degree of automation of the hardware and software, and the ability to easily generate data with high information content at exquisite sensitivity. This talk will emphasize the contributions that MS makes in the analysis of posttranslational modifications, and the potential for proteome-wide analyses. Use of MS to elucidate protein pathways for validation of drug targets, and to define structures of natural ligands of "orphan" receptors will also be described.

154.

FOUR DECADES OF STRUCTURE DETERMINATION BY MS: FROM ALKALOIDS TO HEPARIN.

Klaus Biemann, *Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, kbiemann@mit.edu*

In the late 1950's we demonstrated the potential and power of mass spectrometry (MS) for the determination of the structure of complex natural products.

The work on indole alkaloids quickly captured the attention of the organic chemists.

Mass spectrometric sequencing of small peptides more slowly developed into a new method for the elucidation of the primary structure of proteins. First we used gas chromatography in conjunction with MS and computer interpretation of the data, later fast atom bombardment ionization, four-sector tandem MS, and finally matrix-assisted laser desorption ionization MS. Throughout, specific enzymatic or chemical cleavage was an important part of the strategy.

More recently, we discovered that basic peptides form stable complexes with highly acidic molecules upon laser ionization. This led us to the development of a novel method for the sequencing of fragments of heparin, a linear highly sulfated polysaccharide of great biological importance.

155.

NOVEL TOOLS FOR THE FIELD OF PROTEOMICS.

Stephen A. Martin¹, *Marvin Vestal*², *Peter Juhasz*¹, *Brian Williamson*¹, *Jason Marchese*¹, *Armin Graber*¹, and *Dale Patterson*¹. (1) *Proteomics Research Center, Applied Biosystems, 500 Old Connecticut Path, Framingham, MA 01701, Fax: 508-383-7893, martinsa@appliedbiosystems.com*, (2) *R&D, Applied Biosystems*

Proteomics encompasses a broad range of technologies aimed at determining the identity and quantity of expressed protein in cells, their three-dimensional structure and interaction partners. Intense focus has been placed on the development of a series of processes that will enable researchers to complete these studies with appropriate sensitivity and throughput to leverage the corresponding genomics information. In the subset of proteomics focused on protein identification and quantification we have been investigating a series of novel technologies that may accelerate the overall work flow in Proteomics. Two key areas include isotope coded affinity tags (ICAT) and matrix assisted laser desorption ionization tandem time of flight (MALDI TOF/TOF). The ICAT reagent enables rapid relative quantification of protein expression without the requirement for 2D-gel electrophoresis. MALDI TOF/TOF couples a high throughput mode of ionization with tandem mass spectrometry. This combination provides both molecular mass and primary sequence information with MALDI.

156.

EVOLUTION OF MODERN MASS SPECTRAL APPROACHES TO CARBOHYDRATE AND GLYCOCONJUGATE ANALYSIS.

Catherine E. Costello, *Departments of Biochemistry and Biophysics, Boston University School of Medicine, Mass Spectrometry Resource, 715 Albany Street, R-806, Boston, MA 02118-2526, Fax: 617-638-6491, cecmsms@bu.edu*, and *Joseph Zaia*, *Department of Biochemistry, Boston University School of Medicine*

Carbohydrates and their conjugates present analytical challenges that exceed those of other biopolymers, because of their branched structures and their frequent occurrence as mixtures of closely related components that vary in the specificity and level of biological activity. Progress in organic mass spectrometry has usually been marked by the first application of each new technique to peptide and protein chemistry, yet the study of carbohydrates has followed closely. This focus occurred in the Biemann laboratory, with the result that the glycoproteins had early access to new technical developments, and strategies for carbohydrate and glycoconjugate analysis surfaced directly in the wake of protein methods. Emerging appreciation of the importance of carbohydrates and the increasing sophistication of mass spectrometer systems are presently stimulating a wealth of new approaches for glycobiology. These methods and the contribution of the MIT laboratory to their evolution will be highlighted in this presentation.

157.

ANALYSIS OF IN VIVO-FORMED DNA ADDUCTS BY MICROCAPILLARY LC-MS/MS.

Paul Vouros¹, *Robert J. Turesky*², *John R. Soglia*¹, and *Eric T. Gangl*¹. (1) *Chemistry, Northeastern University, Boston, MA 01742, p.vouros@nnet.neu.edu*, (2) *Nestle, Ltd, Lausanne, Switzerland*

It is well recognized that exposure to a wide variety of environmental chemicals—e. g., polyaromatic hydrocarbons and heterocyclic aromatic amines, compounds found in cigarette smoke and protein-containing foods—may lead to DNA damage via the formation of covalent compounds which are commonly referred to as DNA adducts. If not repaired before the onset of DNA replication, these DNA lesions may lead to mutation and, ultimately, the growth of cancer. DNA adducts can thus serve as useful molecular markers for assessing human

health risk. However, their detection and characterization is complicated by their presence in minute quantities in *in vivo* systems—typically a few modifications in one billion normal bases. This presentation will discuss methodology based on the use of micro-capillary HPLC coupled to tandem mass spectrometry for the *in vivo* analysis of DNA adducts of selected heterocyclic aromatic amines. Data from dose response studies in rats will be presented, demonstrating limits of detection and quantification of the adducts in the low part per billion range.

158.

MOLECULAR-ISOTOPIC STUDIES OF GEOMICROBIAL PROCESSES. *John M. Hayes, Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Mail Stop 8, Woods Hole, MA 02543, Fax: 508-457-2183, jhayes@whoi.edu*

Structural and isotopic analyses of organic compounds from sedimentary deposits can provide information about organisms and pathways of carbon flow in ancient ecosystems. In turn, this biological and geochemical information can help to constrain estimates of physical conditions such as temperature, abundances of nutrients, and concentrations of dissolved CO₂ and O₂. Two illustrative examples will be discussed: (1) estimation of concentrations of dissolved CO₂ and of rates of growth of haptophyte algae based on isotopic compositions of C₃₇ alkenones and (2) recognition of the organisms involved in the anaerobic oxidation of CH₄ based on the isotopic compositions of specific microbial lipids.

159.

BIOSYNTHESIS OF 5-OXO-ETE AND FORMATION OF A NOVEL BIOLOGICALLY ACTIVE METABOLITE, FOG7. *Robert C. Murphy, Rebecca C. Bowers, John Hevko, and Simona Zarini, Division of Cell Biology, National Jewish Medical and Research Center, 1400 Jackson St, Denver, CO 80206, Fax: 303-398-1694, murphyr@njc.org*

Lipid mediators of cellular activation include a family of metabolites of arachidonic acid formed through action of the enzyme 5-lipoxygenase. Leukotrienes and the less well studied products derived from 5-hydroperoxy eicosatetraenoic acid (5-oxo-ETE and 5-HETE) have profound effects on cells such as human eosinophils and neutrophils. We have investigated the biosynthesis of 5-oxo-ETE using mass spectrometry to follow a competitive reaction of deuterium labeled 5-HETE and unlabeled 5-HpETE as potential biosynthetic precursors in the peritoneal macrophage. Experiments carried out studying the metabolism of 5-oxo-ETE by the peritoneal macrophage led to the identification of an abundant metabolite corresponding to the addition of glutathione to the conjugated dieneone. The position of attack of the thiolate anion for glutathione on this unsaturated ketone was elucidated using an ion trap mass spectrometer and MS³. This metabolite was further investigated for biological activity and found to be highly potent for chemotaxis of human eosinophils and neutrophils. Additional metabolites of 5-oxo-ETE were identified and corresponded to cytochrome P-450 ω -oxidation and ω -6 reduced products. Insight into the biosynthesis and disposition of biologically active 5-oxo-ETE was made possible by using electrospray tandem mass spectrometry that permitted experiments to be carried out with relatively small quantities of isolated cells.

160.

ENVIRONMENTAL CHEMISTRY AND ELECTRON CAPTURE MASS SPECTROMETRY. *Ronald A. Hites, School of Public and Environmental Affairs, Indiana University, SPEA 410 H, Bloomington, IA 47405, Fax: 812-855-1076, HitesR@Indiana.edu*

Many toxic environmental contaminants are highly chlorinated; the polychlorinated biphenyls (PCBs) and dioxins are two examples. The analysis of these compounds in the environment frequently centers on gas chromatographic mass spectrometry, often using electron capture, negative ionization (ECNI). This ionization method is both specific (selective) and sensitive. Because ECNI can easily achieve sensitivities of a few attomoles for selected compounds, it is remarkably well suited for tracing the environmental movement of pollutants. This lecture will present examples of the application of ECNI to various problems of environmental science: Dioxins move from combustion sources to environmental sinks (such as soil, vegetation, and human tissue), and we have used ECNI to understand this process. The identification of spurious compounds in human tissue is of obvious importance, and ECNI has been an essential tool

in this research. Our laboratory has identified several interesting by-products of chlordane (a common insecticide) in human tissue using this approach.

161.

USE OF MASS SPECTROMETRY TO STUDY HUMAN PROTEOMES. *Dominic M. Desiderio, Neurology and Biochemistry, University of Tennessee, Memphis, 847 Monroe Avenue, Room 117, Memphis, TN 38163, Fax: 901-448-7842, ddesiderio@utmem.edu*

Comparative proteomics is used to study basic molecular mechanisms in the human pituitary (<http://utmem.edu/proteomics>) and CNS, the cerebellum of a mouse lurcher brain, human cerebrospinal fluid, and the effects of opioid drugs on phosphorylation. Idiopathic low back pain (CSF) and human macroadenomas (pituitary) are studied. Proteomics is used to experimentally test the hypothesis that metabolic defects in neuropeptidergic and other metabolic systems contribute to those pathologies; these studies are based on our previous tandem mass spectrometry measurements of neuropeptides in human tissues and fluids.

162.

REACTIVITY OF PLATINUM: METHANE ACTIVATION AND CIS-PLATIN. *Peter B. Armentrout, Department of Chemistry, University of Utah, 315 S 1400 E Rm 2020, Salt Lake City, UT 84112, Fax: 801-581-8433, armentrout@chemistry.utah.edu*

Platinum is known as a useful catalyst and has proven to be one of the most reactive elements. Guided ion beam tandem mass spectrometry studies have been conducted to examine the intrinsic reactivity of platinum cations in the gas phase. This talk will focus on two of our studies. In the first, we have elucidated the potential energy surface for reaction of Pt⁺ with methane, a system previously studied by Schwarz et al. Bond energies for Pt⁺-H and Pt⁺-CH_x (x=0 - 4) are determined and insight into the reaction mechanism is obtained. Good agreement with previous work and theoretical studies is observed. In the second study, we have experimentally and theoretically characterized the sequential bond energies of [Pt(NH₃)_x(Cl)_y]⁺ complexes where x=0 - 4 and y=0 - 2, gas-phase analogues of the anti-tumor drug, cis-platin.

163.

ENZYME CHEMISTRY IN THE GAS PHASE: HIGHLY SELECTIVE REACTIONS OF BIOMOLECULES. *J. L. Beauchamp, Department of Chemistry, California Institute of Technology, Noyes Laboratory 127-72, Pasadena, CA 91125, Fax: 626-568-8641, jlbchamp@its.caltech.edu*

The last decade of the twentieth century witnessed rapid development in experimental methodology for isolating and manipulating complex biomolecules in the gas phase. The challenge for chemists in the new millennium is to develop gas phase chemistry that provides the same selectivity for chemical transformation of these molecules that is afforded by enzyme reactions in solution. Important goals include the development of methods for cleaving amide linkages adjacent to specific amino acids in peptides and phosphate diester linkages adjacent to specific bases in nucleic acids. A few such reactions, such as the use of salt bridge chemistry to cleave amide linkages adjacent to aspartic acid, have already been identified. A more general strategy involves development of reagents that bind specific functional groups in biopolymers, with appropriate functionality to then carry out highly selective reactions in the gas phase. Progress in achieving these goals will be discussed.

164.

STRUCTURES AND ENERGETICS OF BIOPOLYMERS IN THE GAS PHASE. *Michael T. Bowers, Department of Chemistry, University of California, Santa Barbara, CA 93106, Fax: 805-893-8703, bowers@chem.ucsb.edu*

One of the important current problems in bioanalytical chemistry is developing mass spectrometric methods for accurate and rapid genome analysis. One of the factors that makes this difficult is the fact that polynucleotides tend to fragment during the MALDI process and the bigger they are the greater the problem. It is of interest, then, to try to understand the important fragmentation mechanisms so steps can be taken to prevent them from occurring. In this talk we will present structural data from ion mobility measurements on tetranucleotides along with theoretical calculations that are useful in testing fragmentation mechanisms in the literature. The question of zwitterion formation will be addressed as they play central and controversial roles in the suggested

fragmentation mechanisms. If time permits structures of di- and trinucleotides with transition metal ions attached will also be presented.

165. DIPOLE SUPPORTED STATES OF NEGATIVE IONS: ROTATIONS AND LIFETIMES. Joel M. Karty, David A. Walthall, and John I. Brauman, Department of Chemistry, Stanford University, Stanford, CA 94305-5080, brauman@stanford.edu

Negative ions whose neutral cores have a sufficiently large dipole moment are capable of supporting electronic states that can be characterized semiclassically as an electron bound in the field of a dipole. The ability to observe these states is related to their lifetimes, which in turn depends on their rotational motions. We describe a model that allows us to predict when such states can be observed.

166. ROLE OF WATER ON THE STRUCTURE OF CATIONIZED AMINO ACIDS. Evan R. Williams, Department of Chemistry, University of California, Berkeley, Berkeley, CA 94720, Fax: 510-642-8369, williams@cchem.berkeley.edu

For over a century, it has been recognized that amino acids exist as zwitterion in aqueous solution. In the absence of any solvent or cations, the most stable structure for all of the amino acids in the ground state is the neutral form. This clearly shows that water stabilizes the zwitterion forms of all amino acids. In the gas phase, the presence of a nearby charge can stabilize the zwitterion form of some amino acids even without solvent. For example, there is strong computational and experimental evidence that protonated dimers of arginine form a salt bridge in which one arginine molecule is in its zwitterionic form (the guanidine side chain protonated and the C-terminus deprotonated). Similarly, arginine bound to K⁺, Rb⁺, and Cs⁺ is in its zwitterionic form, whereas arginine bound to Li⁺ is not. By comparison, both experiment and theory indicate that glycine bound to the alkali metal ions forms a charge-solvated structure in which glycine is in its neutral form. Our goal is to determine how many water molecules are required to turn amino acids, such as glycine, into zwitterions in the presence of a charge, i.e., how many water molecules are required to go from a charge-solvated to a salt-bridge structure. Hydrated ions are formed using electrospray ionization and their dissociation kinetics investigated using blackbody infrared radiation dissociation in a Fourier-transform mass spectrometer. From the kinetic data, information about the structure of the ion is inferred. These structures are compared to those obtained using the highest levels of theory. From these results, it appears that as few as six water molecules are sufficient to make the solution-phase structure of valine the most stable form, i.e., cationized valine is zwitterionic with six water molecules. These and other biomolecule hydration studies will be reported.

167. STUDIES OF THE INTRINSIC REACTIVITIES OF MACRO-IONS VIA QUADRUPOLE ION TRAP MASS SPECTROMETRY. S. A. McLuckey¹, J. M. Wells¹, G. E. Reid¹, P. A. Chrisman¹, B. J. Engel¹, K. A. Newton¹, P. Pan¹, J. Wu¹, D. E. Goeringer², K. G. Asano², J. L. Stephenson Jr.², and D. E. Butcher³. (1) Department of Chemistry, Purdue University, 1393 Brown Laboratory, West Lafayette, IN 47907-1393, Fax: 765-494-0239, mcluckey@purdue.edu, (2) Chemical and Analytical Sciences Division, Oak Ridge National Laboratory, (3) Department of Chemistry and Physics, Western Carolina University

The reactivities of de-solvated ions derived from macro-molecules have both fundamental and applied implications. Studies of the intrinsic behavior of large de-solvated and partially solvated ions in the gas-phase may provide new insights into the detailed role of solvent in the condensed-phase reactivity of macro-ions. From an applied standpoint, the chemical reactivity of macro-ions plays a major role in determining the utility of mass spectrometry in macro-molecule analyses. We have been studying, in particular, the unimolecular dissociation behavior of peptide and protein ions under thermal and near-thermal dissociation conditions in a quadrupole ion trap. The observed fragmentation behavior is determined by the complex interplay of a number of experimental and fundamental variables. Experimental variables affect the ion internal energies and can affect the relative contributions of various isomeric conformations. Fundamental variables include peptide/protein structure (i.e., primary, secondary, tertiary) and charge state. This talk will present results that bear on the development the ion trap as a tool for studying high mass ions and recent

results obtained in studying the charge state fragmentation behavior of various model proteins.

168. NEAR-FIELD RAMAN AND IR ABSORPTION SPECTROSCOPY FOR MOLECULAR IMAGING. Y. Inouye, N. Hayazawa, Z. Sekkat, and S. Kawata, Department of Applied Physics, Osaka University, 2-1 Yamada-oka, Suita, Osaka 565-0871, Japan, Fax: ++81-6-6879-7330, ya-inouye@ap.eng.osaka-u.ac.jp

We have observed near-field Raman scattering of molecules using a metallic tip which we firstly proposed as a near-field scanning optical microscope probe. A silver-coated cantilever was used for locally enhancing electric field and Raman scattering. Stokes-shift lines of Rhodamine 6G and Crystal Violet molecules were obtained and assigned to each vibration mode. The enhancement factor of 40 and 40 nm spatial resolution corresponding of the radius of the metallic tip were achieved in the near-field Raman scattering detection. We observed intensity-enhancement of specific Stokes-shift lines, emergence of new peaks, and shift of specific Stokes-shift lines which might be induced by chemical interaction between the metallic tip and molecules as well as intensity-enhancement of Stokes-shift lines induced by locally enhanced field at the metallic tip. We will also show near-field IR absorption images using an apertured cantilever with an optically parametric amplifier and differential frequency generator.

169. SINGLE MOLECULES IN THE NEAR FIELD. Niek F. van Hulst, Maria F. Garcia-Parajo, Joost-Anne Veerman, and Henkjan Gersen, Applied Optics group, Dept Appl.Phys, MESA+ Research Institute, University of Twente, POBox 217, 7500AE Enschede, Netherlands, Fax: 31-53-4891105, n.f.vanhulst@tn.utwente.nl

With optical research at the nanometer scale (nano-optics) one enters the domain of sub-wavelength optics, evanescent and local fields. Using a nano-optical source individual nanoparticles or molecules can be excited. Alternatively sub-wavelength optical fields can be probed using a single emitter. We have studied the photodynamics of individual molecules at room temperature. Real-time single molecular singlet - triplet quantum jumping has been observed. Both triplet and fluorescence lifetime fluctuate due to the temporal and spatial heterogeneity of the host material. We have applied single molecular detection to study the photodynamical behavior of individual macromolecules, proteins and quantum dots. Moreover the characteristic local field distribution of the nano-source allows full 3D-orientation determination of the single molecular dipole moment.

170. NEAR FIELD IR MICROSCOPY OF NANOSTRUCTURED SURFACES. Gilbert C. Walker, Department of Chemistry, University of Pittsburgh, G-12 Chevron Science Center, Pittsburgh, PA 15260, Fax: 412-383-9646, gilbertw@vm2.cis.pitt.edu

We have recently developed an apertureless scanning near field infrared spectrometer with <100nm spatial resolution. We report the application of this tool for the analysis of nanostructured polymer surfaces. Calculations of the near field infrared signals using analytical and discretized models for light propagation that reveal the roles of topography and absorbances due to chemical composition will also be presented.

171. SCANNING NEAR-FIELD RAMAN MICROSCOPY WITH AND WITHOUT APERTURES. David N. Batchelder¹, D. Alastair Smith¹, François Demangeot², Jing Jing Wang¹, and Fiona C. Thorley¹. (1) Department of Physics and Astronomy, University of Leeds, Leeds LS2 9JT, United Kingdom, Fax: 44-113-2333900, d.n.batchelder@leeds.ac.uk, (2) Laboratoire de Physique des Solides, Université Paul Sabatier

Scanning near-field Raman microscopy (Raman SNOM) simultaneously provides sample topography and molecular identification with sub-micron spatial resolution. We have developed two types of Raman SNOM. In the first, the source is a metal-coated, tapered fibre optic with an aperture of about 100 nm diameter that is scanned in close proximity to the sample. This instrument has thus far been used at visible and ultraviolet wavelengths to study defects in strong Raman scatterers such as silicon and diamond. The second uses a metal-coated tip in a commercial atomic force microscope to scatter light from the near field of the laser-illuminated sample. This apertureless SNOM is

currently being used to study polydiacetylene nanoparticles in which there is strong resonance enhancement of the Raman scattering. Both confocal and direct imaging Raman microscopy, with detection in the far field, have proved invaluable in this study.

172.

ELECTRIC FIELD GRADIENT EFFECTS IN NSOM-RAMAN SPECTROSCOPY. *Eric J. Ayars¹, Hans D. Hallen², and Michael A. Paesler².* (1) Department of Physics, Walla Walla College, 204 S. College Avenue, College Place, WA 99324, Fax: 509-527-2867, ayarer@wwc.edu, (2) Department of Physics, North Carolina State University

Raman spectra of materials subject to strong electric field gradients, such as those present near a metal surface, can show significantly altered selection rules. We describe a new mechanism by which the field gradients can produce Raman-like lines. We develop a theoretical model for this "Gradient-Field Raman" effect, discuss selection rules, and compare to other mechanisms that produce Raman-like lines in the presence of strong field gradients. This mechanism can explain the origin and intensity of some Raman modes observed in Near-field Scanning Optical Microscopy (NSOM-Raman) and can also explain some Raman modes observed in SERS.

173.

CHEMICAL IMAGING WITH SCANNING NEAR-FIELD IR MICROSCOPY AND SPECTROSCOPY. *Stephan J. Stranick¹, Bruce Chase², and Chris A. Michaels¹.* (1) CSTL, NIST, 100 Bureau Drive, Gaithersburg, MD 20899, Fax: 301-926-6689, stephan.stranick@nist.gov, (2) Dupont, Experimental Station Research

The development of a scanning near-field microscope that utilizes infrared absorption as the optical contrast mechanism will be described. This instrument couples the nanoscale spatial resolution of a scanning probe microscope with the chemical specificity of vibrational spectroscopy. This combination allows the in situ mapping of chemical functional groups with subwavelength spatial resolution. Key elements of this infrared microscope include: a broadly tunable infrared light source producing ultrafast pulses with a FWHM bandwidth of 150 cm⁻¹, an infrared array-based spectrometer which allows parallel detection of the entire pulse bandwidth, and a single mode fluoride glass fiber probe which supports transmission from 2200 to 4500 cm⁻¹. Measurements exploring the coupling between the near-field infrared absorption magnitude and changes in tip-sample separation due to sample topography will be discussed. Images of thin film polymer blends and nanocomposites acquired in the C-H stretching region will be used to benchmark the chemical imaging capabilities of this microscope, focusing particularly on the absorption sensitivity of the spectrometer.

174.

UNBOTTLEABLE MOLECULES AS GENERATED AND PROBED BY ELECTRON-TRANSFER MASS SPECTROMETRY. *Helmut Schwarz, Department of Chemistry, Technical University of Berlin, Strasse des 17. Juni 135, D-10623 Berlin, Germany, Fax: ++49 30 314 21102*

Over the last decade, neutralization-reionization mass spectrometry has emerged as a powerful method for the generation and structural characterization of small, neutral molecules that are not accessible in the condensed phase and which are believed to play a central role in many fields of chemistry. Systems that will be discussed in detail include the celebrated water oxide and ethylenedione. In addition, emphasis will be given on the generation and theoretical description of *thermochemically stable, multiply charged diatomic molecules* by charge stripping mass spectrometry. This area is of topical interest in chemistry and physics. Of particular importance are small, *positively charged* molecules which exhibit positive proton affinities, e.g. LaO⁺ which has a proton affinity similar to that of CH₄.

175.

COMBINING ELECTROSPRAY IONIZATION WITH A FAST FLOW TECHNIQUE IN THE STUDY OF THE REACTIVITY OF PROTONATED BIOMOLECULES. *Chava Lifshitz, Department of Physical Chemistry, The Hebrew University of Jerusalem, Givat Ram, 91904 Jerusalem, Israel, Fax: 972-2-6522472, chavalu@vms.huji.ac.il*

Protonated biomolecules are introduced via an ESI source into a fast flow reactor. Collisionally stabilized protonated complexes of betaine/ammonia and diglycine/ammonia but not of bradykinin/ammonia were observed. Reaction with

ND3 leads to H/D exchange of the labile hydrogens of betaine and of diglycine. The ammonium ion stabilizes a betaine zwitterion in the gas phase forming a salt bridge structure as has been verified by DFT calculations. DFT computational results are in agreement with proposed mechanisms for H/D exchange between ND3 and protonated peptides. Exchange of the amide hydrogen takes place via a tautomerized peptide structure having a partial salt bridge character. On a time scale of several milliseconds doubly protonated bradykinin undergoes three and des-Arg9 bradykinin- six hydrogen exchanges. The additional H/D exchanges observed in the case of doubly-protonated des-Arg9 bradykinin are made possible by complexation of its less compact structure via hydrogen bonded intermediates that promote H/D exchange of amide hydrogens.

176.

PROBING INTRINSIC KINETICS OF REACTIONS WITH BARE-METAL, LIGATED-METAL, AND BIO-METALLIC IONS USING ICP/SIFT MASS SPECTROMETRY. *Diethard K. Bohme, Department of Chemistry, Centre for Research in Mass Spectrometry, Centre for Research in Earth and Space Science, York University, 4100 Keele St., Toronto, ON M3J 1P3, Canada, Fax: 416-736-5246, dkbohme@yorku.ca*

Metal ions exhibit a wide range in reactivity in a wide range of chemical and biochemical environments. Understanding this chemical diversity requires knowledge of the intrinsic reaction kinetics of bare metal-ion reactivity and their modulation by metal-ion ligation in these environments. This talk will survey results obtained recently for the kinetics of bare and ligated metal-ion reactions using a novel Inductively-Coupled Plasma/ Selected-Ion Flow Tube (ICP/SIFT) tandem mass spectrometer. Topics will include state-specific N-atom and O-atom transfer reactions with bare-metal ions, observed periodicities in bare metal-ion reactivity, spin-selective ring cleavage, spin-crossing effects in sequential metal-ion ligation, ligand-assisted reactions of metal ions, carbonaceous surface effects on metal-ion reactivity, and intrinsic ligation kinetics of bio-organometallic ion mimics. Neutral reactants chosen for these reactivity studies include nitrous oxide, carbon dioxide, oxygen, ammonia, cyanides and benzene.

177.

CHEMISTRY OF GAS-PHASE INCLUSION COMPLEXES. *Carlito B. Lebrilla, Seonghee Ahn, and Gabriela Grigorean, Department of Chemistry, University of California, Davis, One Shields Ave, Davis, CA 95616, Fax: 530-752-8995, cblebrilla@ucdavis.edu*

Complexes of oligosaccharide hosts and chiral guests are produced in the gas phase with a Fourier transform mass spectrometry. The host-guest complexes are produced by electrospray ionization and trapped in the analyzer where they are allowed to undergo guest exchange with a gaseous alkyl amine. The reactions are highly sensitive to the chirality of the guest compound. In this presentation, the nature of the enantioselectivity will be discussed as well as the analytical application of the guest exchange reaction, particularly with amino acids and pharmaceutical compounds. A number of hosts including derivatized and native α -, β -, and γ -cyclodextrin and their linear analogs will be used to illustrate that chiral selectivity depends complementary fit between the size of the host and the guest. The "three point interaction" is used to understand the enantioselectivity. We will show that the combination of a two-point attraction and a one-point repulsion provide optimal enantioselectivity.

178.

METAL ION-COORDINATED RADICALS: A NEW CLASS OF DISTONIC RADICAL CATIONS. *Chrys Wesdemiotis, Jianglin Wu, Jody M. Talley, and Michael J. Polce, Department of Chemistry, The University of Akron, 190 E. Buchtel Commons, Akron, OH 44325-3601, Fax: 330-972-7370, wesdemiotis@uakron.edu*

The lithium and sodium ion complexes of the polyglycol radicals (R[•]) HOCH₂CH₂O[•], HOCH₂CH₂OCH₂[•], and HOCH₂CH₂OCH₂CH₂[•] are produced in the gas-phase and the intrinsic chemistry of these novel distonic radical cations is investigated by tandem mass spectrometry. The unimolecular chemistry of [R[•] + X]⁺ is dominated by radical site reactions. The metal ion does not directly participate in these processes, but it promotes or impedes specific channels by preventing bond rotations in R[•], allowing for the formation of intermediary metal ion-bound heterodimer complexes, and influencing the dissociation energetics. The ion-molecule reactions of [R[•] + X]⁺ strongly depend on the reagent used.

Dimethyl ether adds to the charge site, triggering radical-site cleavages within R[•]; 1-butene is added to the radical site to yield new distonic ions that decompose consecutively at the new radical center; and methyl vinyl ether undergoes both these reactions. Overall, the charge as well as the type of radical site affect the ion-molecule reaction outcome.

179.

TOOLS FOR PROBING INTRINSIC REACTIVITY FEATURES: ENTROPY

DETERMINATIONS BY THE KINETIC METHOD. *Robert G. Cooks, Jeffrey Denault, and Taufika Islam, Department of Chemistry, Purdue University, 1393 BRWN, West Lafayette, IN 47907, Fax: 765-494-9421, cooks@purdue.edu*

Mass spectrometry provides many tools for probing intrinsic reactivity features. These include means to generate isolated or solvent-number controlled ions for study and means to react them in controlled environments for set times.

Structural tools based on dissociation products typically employ multiple stages of mass analysis. Dynamical measurements such as energy partitioning in the course of metastable ion decay help characterize potential energy surfaces.

Isotope effects, isotopic substitution and quantum chemical calculations all add to knowledge of intrinsic reactivity.

The importance of the measurement of thermochemical properties by mass spectrometry is indicated by the variety of methods that have been used and properties that have been measured. This talk presents a new formulation of the kinetic method of making thermochemical measurements. Like other measurements based on the kinetic method, the competitive dissociations of mass-selected cluster ions are measured. The approach is an alternative to the existing methods of Fenselau and coworkers and Wesdemiotis and coworkers of measuring entropy changes in the course of a reaction. In the cases tested it gives results in agreement with the earlier measurements.

180.

CHEMICALLY DOPING SINGLE MOLECULES TO ACHIEVE HIGH CONDUCTIVITY.

Clifford P. Kubiak, and Bala Sundari T. Kasibhatla, Department of Chemistry and Biochemistry, U of CA, 9500 Gilman Drive, DEPT 0358, La Jolla, CA 92093-0358, Fax: 858-534-5383, ckubiak@ucsd.edu

The preparation and electrical properties of self-assembled monolayers of organic molecules and their charge transfer complexes with electron acceptor "dopants" will be described. STM studies show that SAMs of organic thiols on gold exhibit high resistivities and behave as insulators. The conductance spectroscopy of organic "molecular wires" on Au(111) will be summarized to show how electrical conductance depends on (i) electronic structure of the molecule, (ii) length of the molecule, and (iii) nature of the end-groups that attach to gold. These studies show that the conductance of organic molecules is strongly dependent on even minor changes in the molecule, and that the Fermi energy of gold lies within the HOMO/LUMO gap of most simple organic molecules. The synthesis and characterization of SAMs of pentamethyl benzylthiol (PMBT), tetramethyl xylidithiol (TMXYL) and their 1:1 charge-transfer complexes with the electron acceptor, tetracyanoethylene (TCNE) will then be described. We will show that "doping" the thiol monolayers with TCNE triggers (i) a change in the structure of the monolayer and (ii) its electrical behavior shifts from insulating to conducting. Removal of the TCNE "dopant" with a stronger electron donor (TTF) affords monolayers of TMXYL which remain in the flattened orientation, with phenyl rings parallel to the Au(111) surface. STM studies show that the flat TMXYL monolayers are highly resistive. Together, these studies demonstrate that the higher conductance of the "doped" PMBT-TMXYL monolayers relative to the "undoped" TMXYL monolayers in either their vertical or horizontal orientation arises from the electronic structure of the surface confined charge transfer complexes and not from a structural change associated with the TMXYL phenyl rings.

181.

SIZE-SELECTIVE ELECTROCHEMICAL GROWTH OF METAL AND

SEMICONDUCTOR NANOWIRES. *Reginald M. Penner, Chemistry, UCI, Irvine, CA 92697-2025, Fax: 949 824 3168, rmpenner@uci.edu*

There are few methods for preparing metallic nanowires that are also long and dimensionally uniform. These structures may have applications ranging from interconnects to sensors. We have recently described (Science, Dec., 2000), a method for synthesizing molybdenum nanowires ranging in diameter from 15 nm to 1.0 μm with lengths of up to 500 μm (0.5 mm). Our involves the

electrodeposition of MoO₂ nanowires at step edges on a graphite surface followed by reduction in H₂ gas at 500C. Nanowires of molybdenum metal are thereby obtained. These nanowires are extremely uniform in diameter, and the mean nanowire diameter is proportional to the square root of the deposition time.

In this presentation, we shall touch on the following issues:

- What is the detailed mechanism of nanowire growth?
- Can nanowires composed of other materials be synthesized using this approach?
- How may these nanowires be manipulated?
- What are their mechanical and electrical properties?
- Can nanowires such as these be employed to make devices?

182.

CONSTRUCTION OF A PLATFORM FOR MOLECULAR ELECTRONICS.

Christopher Gorman, Department of Chemistry, North Carolina State University, Box 8204, Raleigh, NC 27695-8204, Fax: 919-515-8920, chris_gorman@ncsu.edu

Redox active macromolecules, self-assembled monolayers and scanned probe lithography have been used in our group to construct prototype molecular electronics devices. The role of the self-assembled monolayer foundation in constructing chemically well-defined architectures will be elucidated. The use of redox-active groups as nonlinear "device elements" will be illustrated.

183.

ELECTROACTIVE SAMs AND MULTILAYERS: NONLINEAR BEHAVIORS IN POTENTIAL MOLECULAR ELECTRONICS SYSTEMS.

Richard Lloyd Carroll, and Christopher Gorman, Department of Chemistry, North Carolina State University, Box 8204, Raleigh, NC 27695-8204, Fax: 919-515-8920, chris_gorman@ncsu.edu

Before the use of molecules in electronics systems is fully realized, the useful I/V behaviors of such systems must be understood. To this end, we have studied several classes of electroactive self-assembled films, both mono- and multi-layered. It is found that these types of films display strong Negative Differential Resistance (NDR) at room temperature. We have explored the potential of tuning the NDR by modifying the composition of the film, and molecular structure-property relationships for monolayer-based electronic behaviors will be presented.

184.

ELECTROCHEMISTRY IN NANOSTRUCTURED INORGANIC MATERIALS.

Mary Elizabeth Williams, and Joseph T. Hupp, Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL IL60208-3113, mbw@chem.nwu.edu

Abstract text not available.

185.

NANOBARCODES: ELECTROCHEMICAL SYNTHESIS OF NANOPARTICULATE TAGS.

Michael J. Natan, Surromed, Inc, 1060 East Meadow, Palo Alto, CA 94303, Fax: 650-855-9079, mnatan@surromed.com

This presentation will describe a series of nanoparticulate tags, comprising striped, cylindrical metal nanoparticles. I will discuss electrochemical synthesis of these novel materials, focusing on issues of reproducibility, scalability, and multiplexing. Aspects of optical and other forms of readout/interrogation will be shown, and recent applications in genomics, proteomics, and mass spectrometry will be highlighted.

186.

STATE OF THE ART IN HYPHENATION OF CAPILLARY SEPARATIONS METHOD WITH MS AND NMR.

Ernst Bayer, Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18, Tübingen D 72076, Germany, Fax: 4970-7129-5246, ernst.bayer@uni-tuebingen.de

In the last years fast progress of on-line coupling of capillary HPLC (CHPLC), capillary electrochromatography (CEC) and capillary gel electrophoresis (CGE) to MS and NMR has been achieved. An instrumentation has been designed which can alternatively employed for CHPLC, CEC and capillary electrophoresis (CE) and the hybrid pressurized electrochromatography (pCEC). A specially developed

frit-less CEC capillary avoids the problems encountered with the frits and non-compatible conjunctions between filled separation capillaries and open tubular transfer capillaries. This results in faster analysis in pCEC-NMR coupling. For coupling of capillary separation methods with electrospray MS (ES-MS) the most recent success was achieved by coupling of CGE with entangled polymers to ES-MS for the analysis of synthetic oligonucleotides (OD) as they are used in antisense therapy and primers for PCR. With the development of the new ionization method coordination lonspray in mass spectrometry (CIS-MS) the analysis of less polar analytes like vitamins, steroids, lipids and sugars is possible. Also new fragmentation patterns in MS-MS allow better structure elucidations. pCEC-CIS-MS is a very powerful new analytical method.

187.

DNA ANALYSIS BY CAPILLARY ELECTROPHORESIS. *Barry L. Karger, Barnett Institute, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, Fax: 617-373-2855, bakarger@lynx.neu.edu*

HPCE has become the method of separation for a variety of assays in the genomics field. Of particular note is that this technology has been used to sequence the human genome and the genomics of a variety of other organisms. Additionally, the method provides outstanding capability for mutational analysis. In this talk, we will present recent advances from our lab, detailing a variety of DNA separations using replaceable polymer matrices. The conditions necessary to achieve ultra-long DNA sequencing per run will be described. In particular, the use of high molecular weight linear polyacrylamide with dilute concentrations and elevated column temperatures will be shown to be necessary ingredients for long read lengths. Base-calling software, which promotes peak recognition and resolutions down to 0.25, will also be presented. In the mutation analysis area, we will demonstrate the power of constant denaturant capillary electrophoresis (CDCE). This approach, by providing very high resolution between mutants, permits determination of mutants at low frequencies in populations. Through the use of a fraction collector with multiple capillary arrays, the discovery of new mutants can be achieved. We will conclude the talk with an assessment of future directions of CE on DNA analysis.

188.

CAN CAPILLARY ELECTROPHORETIC TECHNIQUES COMPETE WITH HPLC IN RESPECT OF REPEATABILITY AND REPRODUCIBILITY. *Heinz Engelhardt, and Thomas Faller, Institute of Instrumental and Environmental Analysis, University of the Saarland, Postfach 151150, Saarbrücken D66129, Germany, Fax: 49681-302-2963, iaua@rz.uni-sb.de*

Capillary Electrophoresis (CE) has the potential of high efficiency and short analysis time. Despite these advantages its application in pharmaceutical and chemical industry for the analysis of small molecules is still hampered by reduced reproducibility and repeatability in qualitative and quantitative analysis compared with HPLC. It will be shown that the repeatability of the migration times in consecutive CE runs can be improved by proper choice of rinse steps in between each run. However no standard procedure can be applied. The rinse steps have to be optimized additionally for different separation systems, as very similar buffer systems can lead to great differences concerning the rinsing procedures. The poor reproducibility in quantitative analysis stems in part from older types of integration software, derived from GC or LC. With newer software taking in account the more or less triangular peak shapes in CE better reproducibility can be achieved. The main disadvantage of CE, however, are the usual low detection limits forcing the instruments to work at high noise levels. It can be shown that when the signal to noise ratio exceeds 35, quantitation accuracy approaches values standard in HPLC. In many cases these values can only be reached when for injection stacking techniques are applied. However, these problems originate from Lambert-Beer's law and the short optical path length in liquid capillary techniques. Only improved and more sensitive detectors like CE/MS coupling will help to overcome these problems in CE and CEC.

189.

RADIOCARBON ANALYSIS BY GAS CHROMATOGRAPHY AND ACCELERATOR MASS SPECTROMETRY OF ENVIRONMENTAL TRACE CHEMICALS. *Hartmut Frank, Environmental Chemistry & Ecotoxicology, University of Bayreuth, Bayreuth D-95440, Germany, Fax: 49-921-552334, Hartmut.Frank@uni-bayreuth.de*

The sources of environmental chemicals are often uncertain, especially when it comes to the question as to their anthropogenic or natural origin. This is

typically the case for environmental chloroorganic chemicals such as polychlorinated dibenzo-dioxins, airborne chlorocarbons, the various haloacetates or chloro- and nitrophenols. Chloroacetates may arise from photodegradation of the volatile chlorinated C2-hydrocarbons 1,1,1-trichloroethane, trichloroethene, and tetrachloroethane but natural formation has also been proposed. In order to differentiate between biological and industrial sources, several approaches may be employed, such as back-trajectory calculations for airborne pollutants, budget and inventory assessments, or isotope-ratio determinations. A particularly powerful method for differentiation of industrial chemicals, i.e. from a petrochemical "old" carbon source, and those of biological origin, i.e. from an atmospheric carbon source, are isotope ratio determinations, in particular the determination of the abundance of the carbon isotope ^{14}C . Methods for quantifying biogenic contributions by radiocarbon analysis using two-dimensional preparative capillary gas chromatography and accelerator mass spectrometry (2D-PCGC/AMS) are discussed. Particular efforts must be directed towards the reduction of the absolute mass required for $^{12}\text{C}/^{13}\text{C}/^{14}\text{C}$ -isotope ratio determination. Presently, amounts in the range of about 100 mg carbon are needed but upon appropriate optimization of sample preparation, this can be reduced to about 1 mg. Development of a gas ionization source for AMS is under way to enable the on-line coupling of GC-AMS, in order to further increase the sensitivity and to allow to work with quantities in the range of 100 ng carbon. Large-volume rain water samples are collected and processed for isolation of chloroacetates with 2D-PCGC on two columns with different polarities. ^{14}C -Containing chloroacetate standards are synthesized and AMS-analyzed together with standards containing "old" carbon, to calibrate for any contaminations. The combined evidence indicate that most brominated haloacetates are of natural origin, chloroacetates are predominantly anthropogenic, while environmental fluoroacetates seem to arise from both sources.

190.

ELECTROSMOTIC VS. PRESSURE-DRIVEN FLOWS IN FIXED BEDS: FROM INTERSTITIAL FLOW HETEROGENEITY TO INTRAPARTICLE AND FILM MASS TRANSFER. *Ulrich Tallarek, Institut für Verfahrenstechnik, Lehrstuhl für Chemische Verfahrenstechnik, Otto-von-Guericke Universität, Universitätsplatz 2, Magdeburg D 39106, Germany, Ulrich.Tallarek@Water.MF.WAU.NL*

The ultimate gain in performance of an electrokinetically-driven mobile phase as compared to pressure-driven flows through packed capillaries attracts a tremendous attention in capillary electrochromatography (CEC) because it is still unclear what the limiting contributions of the different physical mechanisms to this improvement can be. It includes a careful consideration of aspects like the column wall effects, column-to-particle diameter ratio (f), interstitial flow heterogeneity in general (i.e., on a macroscopic, but also on the pore level), the intraparticle transport characteristics as well as film mass transfer resistance.

Using a recently developed capillary-NMR device that allows the use of a non-invasive NMR approach for direct motion-encoding of the fluid molecules, we demonstrate that for charged particles packed into a charged capillary, wall effects due to a mismatch of zeta-potentials at the capillary surface (z_w) and the particles surface (z_p) may cause severe flow heterogeneity with a long-time tail of velocity correlation in the mobile phase. This effect strongly depends on the excess zeta-potential $z_{ex}=z_w - z_p$ and on f , not on external porosity heterogeneity (as in capillary HPLC).

We further demonstrate that the electroosmotic flow offers a superior stagnant mobile phase (intraparticle) and film mass transfer characteristics due to the existence of thin double layers on the internal and external surface of the porous particles. The intraparticle tortuosity factor is seen as one of the most important parameters in achieving a substantial electroosmotic perfusive solute transport. It seems that film mass transfer resistance is practically negligible in CEC over a wide range of conditions.

191.

FROM LC-NMR TO LC-NMR/MS UNDER FULL AUTOMATION. *Manfred Spraul, Ulrich Braumann, Martin Hofmann, and Markus Godejohann, HR-NMR applications and NMR hyphenation, Bruker Analytik GmbH, Silberstreifen, Rheinstetten D-76287, Germany, Fax: 49-721-5161-297, manfred.spraul@bruker.de*

LC-NMR has been established as a standard analytical tool in the last years. Due to the relatively low sensitivity of NMR, in most cases other analytical tools are needed to locate LC-peaks and to transfer them to the NMR for longer term

measurement. Up to now, UV has mostly been used to perform this task. However there are some drawbacks to this, as UV cannot be used quantitatively, several compounds have no UV absorbance and UV is not very structure specific. Other detectors possible like ECD or refractive index have similar problems. Therefore mass spectrometry is the method of choice to get more structure specificity, when selecting LC-peaks, that are worth to be investigated by NMR. At first, the development of LC-NMR towards a fully automatic technique is explained, then the hardware to perform peak selection and transfer into the NMR under MS selection criteria is introduced. It is shown, that system internal loop collection provides the best results for lowest concentration NMR measurement. The criteria and their connections are discussed, that allow to select peaks for the NMR based on UV, MS or other analogue detectors. On the MS side an ion trap mass spectrometer is interfaced to the LC-NMR system. This is primarily achieved by the use of a flow splitter, that divides the flow by 95% to the NMR and 5% to the MS. A dilutor system is added as well to allow dosing of dilution solvents or ionisation helping agents. The function of a delay loop is explained, that can change the timing of transfer to the MS. Application examples from natural product research demonstrate the power of combining NMR and MS information to get ideas about structure and purity of peaks investigated. An outlook to further developments is also presented.

192.

USE OF AFFINITY SELECTION MASS SPECTROMETRY TO SCREEN ORGANIC COMPOUNDS OF DIVERSE STRUCTURES FOR DRUG LEADS. *Houjun Yang¹, Xueheng Cheng¹, Abba Bakhomou², Philip Hajduk³, Isabella Lico⁴, Martin Voorbach¹, Peter Dandliker⁵, Mark Schurdak¹, Lan Gao¹, Alex Buko⁶, Laura Miesbauer⁶, Robert Schmitt², Yvonne Martin⁴, Bruce Beutel⁵, and David Burns¹.* (1) 4PN, Abbott Laboratories, 200 Abbott Park Road, Abbott Park, IL 60064, Fax: 847-935-7929, houjun.yang@abbott.com, (2) 4PT, Abbott Laboratories, (3) 47G, Abbott Laboratories, (4) 47E, Abbott Laboratories, (5) 47P, Abbott Laboratories, (6) 418, Abbott Laboratories

Biological screening is an important early step in modern pharmaceutical discovery. New screening technologies are constantly being evaluated for improved throughput, efficiency and compatibility with new targets. Affinity selection techniques have several important advantages over conventional activity assays, including minimal assay development and high throughput. Mass spectrometry (MS) is ideally suited for the identification of hit compounds from affinity selection screening. The use of MS has the added advantages of simultaneous hit detection and identification as well as low compound consumption due to high sensitivity of MS. We have developed an affinity selection mass spectrometry (AS/MS) technique that works for pools of organic compounds selected from our collection initially geared for high throughput screening. These pools of compounds, unlike combinatorial library, are composed of diverse structures mostly synthesized using traditional medicinal chemistry. These compounds are discrete, do not share the common core structures and represent the structural diversity of the compound collection.

193.

DETECTION OF OLIGONUCLEOTIDE: LIGAND COMPLEXES USING ELECTROSPRAY IONIZATION MASS SPECTROMETRY AS A COMPONENT OF HIGH THROUGHPUT SCREENING. *Jessica Robinson, and Michael Greig, High Throughput Discovery, Pfizer Global R&D - LaJolla, 3550 General Atomics Ct, San Diego, CA 92121, Fax: 858-455-3298, jessica.robinson@agouron.com*

In order for high throughput screening to yield valid inhibitors, secondary assays that rapidly provide an accurate answer regarding the specificity of interactions must be available. To meet this demand, we have developed an assay using electrospray ionization mass spectrometry (ESI-MS) to examine non-covalent interactions between small molecules and oligonucleotide substrates. This solution-based assay can be used to screen single and double stranded oligonucleotides simultaneously in the presence of individual or multiple compounds. The assay was validated using several known DNA intercalators and minor groove binders and has been applied to thousands of assay "hits." DOLCE-MS has enabled us to greatly reduced the number of potential lead candidates fulfilling the modern day pharmaceutical maxim of killing bad compounds early, thus saving time and money in lead development.

194.

ENZYME CHARACTERIZATION WITH MASS SPECTROMETRY AND HIGH THROUGHPUT SCREENING. *James E. Bruce¹, Xiaoting Tang¹, Carl Belke¹, Qian Huang¹, Yueming Li¹, Elizabeth Chen¹, Steven Gardell¹, Steven F. Brady², Victor M. Garsky², Mohinder Sardana¹, and Jules A. Shafer¹.* (1) Biological Chemistry Department, Merck Research Laboratory, P. O. Box 4, WP 26-104, West Point, PA 19486, Fax: 215-652-6913, James_bruce@merck.com, (2) Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486

Drug discovery efforts typically involve the identification of a suitable molecular target and subsequent development of a compound that can successfully alter the target molecule activity. Large chemical compound libraries, established through many years of research and development, can provide potential drug development leads for new targets since some compounds that are found active for one class of molecules, sometimes show activity with others as well. However, many cases arise where no known chemical structure is found suitable and in these cases, combinatorial synthesis is used to broadly vary structural parameters, in hopes that some region of the surveyed structural domain will provide insight allowing optimal lead compounds to be synthesized. Still however, such studies can fail to identify useful candidates if the varied properties are not accurately targeted. Mass spectrometry and high throughput screening are also beginning to play a role in the targeting optimal domains for combinatorial investigations through the elucidation of enzyme specificity. The results of these studies can then be used to increase understanding of the specific chemical behavior of the identified target molecule and thus, restrict synthetic efforts to structural features most likely to provide good inhibitors. This presentation will highlight the capabilities of mass spectrometry and high throughput screening for the characterization of enzymatic specificity and discuss the resulting impact on drug candidate identification.

195.

ITEMS ASSAY OF SULFOTRANSFERASE ENZYMES: HIGH THROUGH-PUT SCREENING OF POTENTIAL ENZYME INHIBITORS USING MASS SPECTROMETRY. *Julie A. Leary, Xue Ge, Dawn Verdugo, Carolyn Bertozzi, and Tammy Sirich, Chemistry Dept, University of California, Berkeley, CA 94720-1460, Fax: 510-642-9295, leary@socrates.berkeley.edu*

A technique has been developed to rapidly screen enzyme inhibitor candidates from complex combinatorial libraries. Libraries are screened using immobilized enzyme methods and ESI-FTICR mass spectrometry. After initial mass spectral identification of the library, it is subsequently incubated with the immobilized enzyme. The immobilized enzyme/inhibitor mixture is centrifuged and an aliquot of supernatant is again analyzed by ESI. Potential inhibitors are quickly identified by comparison of the spectra before and after incubation with the immobilized enzyme; i.e. relative abundance of inhibitors decrease or disappear after incubation due to binding with the enzyme. This method has been successfully demonstrated on the enzymes glutathione S-transferase and estrogen sulfotransferase; the latter library consisting of approximately 100 compounds. The immobilized enzyme can be recycled and reused for continuous screening of additional new libraries. IC-50's correlate very well with data generated using the mass spectrometry technique. The method allows for immobilization of approximately 30 pmol/well.

196.

RAPID IDENTIFICATION AND QUANTITATION OF DRUG METABOLITES USING STABLE ISOTOPES, ACCURATE MASS, AND AN ENHANCED CHEMILUMINESCENT NITROGEN DETECTOR. *Gavin Dollinger, Eric Taylor, Weiping Jia, James Chesko, and Barbara Hell, Small Molecule Drug Discovery, Chiron Corporation, 4560 Horton St., Emeryville, CA 94608, Fax: 510-923-8341, gavin_dollinger@cc.chiron.com*

Detailed metabolism studies of new drugs are typically performed using radiolabeled compounds. Because the methodology is both costly and time consuming, these crucial studies are not usually undertaken until late in the pre-clinical development process, when much time and expense has already been invested. We have developed an integrated methodology, using stable isotopes to identify metabolites, accurate mass spectrometry to determine their composition, MSn to elucidate their structure, and an enhanced chemiluminescent nitrogen detector to measure their concentration. The utility of this approach is demonstrated in two case studies of compounds in early develop-

ment. The speed and modest cost of our methodology renders metabolism studies feasible much earlier in the drug discovery process, where they can have a profound effect on compound selection, optimization and development.

197.

HIGH PERFORMANCE ESI-FTICR MASS SPECTROMETRY AS A HIGH THROUGHPUT SCREEN TO IDENTIFY RNA-BINDING LIGANDS. *Steven A.*

Hofstadler, Jared Drader, Kristin A. Sannes-Lowery, Eric E. Swayze, and Richard H Griffey, Ibis Therapeutics, A Division of Isis Pharmaceuticals, Inc, 2292 Faraday Avenue, Carlsbad, CA 92008, Fax: 760-603-2588, shofstadler@isisph.com

Fourier transform ion cyclotron resonance (FTICR) mass spectrometry (MS) is increasingly being used in the drug discovery arena as a tool to characterize macromolecules. In this work we describe the development of a parallel high-throughput screening (HTS) strategy to identify small molecules that bind RNA drug targets using FTICR as an alternative to classical high-throughput screening of combinatorial libraries. The MASS (Multitarget Affinity/Specificity Screening) assay takes advantage of the "intrinsic mass" label of each compound and target RNA by employing high resolution, high precision mass measurements. The ability to analyze complex mixtures allows complex mixtures to be screened in the presence of multiple RNA targets simultaneously. The identity of the small molecule(s) which bind, the RNA target to which it binds, the compound-specific binding affinity, and the location of the binding site on the RNA can be determined in one set of rapid experiments. In an automated format, this approach is capable of interrogating tens of thousands of molecular interactions in a single day.

198.

REPRODUCIBILITY OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COLUMNS: PACKED VS. MONOLITHIC COLUMNS. *Georges Guiochon, and*

Marianna Kele, Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, Fax: 865-974-2667, guiochon@novell.chem.utk.edu

The column-to-column and batch-to-batch reproducibility of several commercial brands of silica based C18 stationary phases for RPLC were studied. The data characterizing the retention, the hydrophobic and steric selectivity, the column efficiency, the peak symmetry and the hydrogen bonding capacity are reported for a group of thirty neutral, acidic, and basic compounds selected as probes. Careful attention was paid to minimize the systematic error contributions by adhering to a strict operational procedure. The column temperature and the eluent composition were carefully monitored. The reproducibility of series of conventional packed columns (Symmetry from Waters, Kromasil from Eka Chemicals, Vydac from The Separation Group, Luna from Phenomenex, and HyPURITY Elite from Hypersil) and of a monolithic column (Chromolith Performance RP-18e silica rod, from Merck) are compared. Data characterizing the performance of the newly developed monolithic type stationary phase are also presented. The special dual pore structure of this phase results in a permeability that is much higher than that of a packed column of comparable efficiency. This characteristic allows high-speed separations. The limitations of this phase are also discussed.

199.

ABOUT THE TOTAL PERFORMANCE OF MONOLITHIC HPLC-COLUMNS. *Karin*

Cabrera¹, H. Minakuchi², D. Lubda¹, and K. Nakanishi². (1) R&D Biochemistry and Separation, Merck, Frankfurter Strasse 250, Darmstadt D 64293, Germany, Karin.Cabrera@merck.de, (2) Division of Material Chemistry, Graduate School of Engineering, Kyoto University

Monolithic HPLC-columns are made of cylindrically shaped silica rods. Their unique feature is a homogeneous biporous structure with defined macro- and mesopores. Silica rod columns (ChromolithTM-columns) possess about 15% higher porosity as compared to particulate ones. Therefore, they show a much higher permeability and respectively much lower column back pressure. Consequently, several silica rod columns (from 10cm to 1.4m) can be coupled in series with no limitations in column backpressure. With a standard mixture of 6 alkylbenzenes plate numbers of N=108000 were obtained on a 1.4 m long silica rod column. We will demonstrate the usefulness of added separation efficiencies for the separation of structurally related compounds, as e.g. isomers. Furthermore, silica rod columns show very high separation efficiencies as high as those of 3.5 mm particulate silica materials if one compares the respective

van deemter curves. In combination with the low column backpressure they show extremely low values of separation impedance (Knox et al, Chromatographia 1977). The separation impedance (E) is a term which describes the total performance of columns considering the column back pressure and separation efficiency at the same time. Silica rod columns show E-values, which cannot be reached by particulate silica materials, even not by small ones. Therefore, the great advantage of silica rod columns is that they can be used at higher flow rates without losing the required resolution to separate the compounds. This will be demonstrated with applications of practical relevance.

200.

PREPARATIVE CHROMATOGRAPHY WITH CHIRAL STATIONARY PHASES. *Ernst*

Kuesters, Chemical & Analytical Development, Novartis Pharma AG, Bau 145, Lichstraße 35, Basel CH 4002, Switzerland, ernst.kuesters@pharma.Novartis.com

The preparation of enantiomerically pure drugs is a topic of increasing importance, and the number of methods using preparative chromatography on chiral stationary phases (CSP) is rapidly increasing.

In the first part a systematic approach for the method development for preparative chromatographic resolution of racemates is outlined. Difficulties encountered during the scale-up are mentioned and possible solutions discussed. The scope and limitations of this emerging technology will be illustrated with the separation of several racemates on different CSP. In addition, the potential of liquid chromatography in different modes is presented: namely, batch elution chromatography with repetitive injection, recycling chromatography and simulated moving bed (SMB) chromatography.

SMB chromatography allows the continuous counter-current separation of a feed into two streams of purified enantiomers. As a consequence the technology has emerged as a very promising tool offering high production rates. It is the scope of the second part to systematically describe the method development for a given separation problem. Based on experimentally determined adsorption isotherms the region of complete separation is derived and the optimization of possible SMB methods elucidated.

201.

CAPILLARY ELECTROCHROMATOGRAPHY: MASS SPECTROMETRY OF

ANTISENSE OLIGONUCLEOTIDES. *Hans J. Gaus, and L. L. Cummins, Carlsbad*

Research Center, Isis Pharmaceuticals, 2292 Farraday Avenue, Carlsbad, CA 92008, hgaus@isisph.com

Phosphorothioate deoxyoligonucleotides (SODN) represent the first generation of chemically modified antisense oligonucleotides used to alter gene expression by inhibiting transcription or translation of mRNA. So far, capillary gel electrophoresis (CGE), HPLC and electrospray ionization mass spectrometry (ESI-MS) are the most important analytical methods to characterize antisense oligonucleotides. We have explored micro-column separations including capillary electrochromatography (CEC) with on-line electrospray mass spectrometry to analyze SODNs. The effects of polarity and magnitude of applied voltage on efficiency and resolution have been studied. CEC-ESI-MS has been found to be a powerful method to analyze antisense oligonucleotides extracted from plasma and tissues or bulk drug material. Overall advantages are its versatility, efficiency, and sensitivity. CEC has been connected to several mass spectrometers from different manufacturers without modifying standard electrospray sources. The results obtained were compared to analytical methods generally applied for antisense oligonucleotide separations, such as CGE and HPLC-ESI-MS. The impact of results on future developments will be discussed.

202.

CAPILLARY ELECTROPHORETIC SEPARATIONS IN GENOMICS AND

PROTEOMICS: FROM CAPILLARIES TO CHIPS. *Aran Paulus, Genomics*

Institute, Novartis Research Foundation, 3115 Merryfield Row, Suite 200, San

Diego, CA 92121, Fax: 858-812-1570, aran.paulus@gnf.org

The changing environment in the pharmaceutical industry with a number of new approaches and technologies in drug discovery and development such as combinatorial libraries, high throughput screening, cell-based assays, genomics, proteomics and pharmacogenomics will have a lasting impact on the methodologies of the analytical sciences. An increasing number of compounds will be in lead finding and drug discovery programs and the number of targets is expected to increase through the completion of the Human Genome Project. At the same time, the industry is under pressure to reduce the overall development

time and costs. This puts a heavy burden on the analytics as in a regulated environment, drug efficacy, potency, safety and toxicity has to be demonstrated. As a consequence, a major task of analytical research for life science applications is the development of techniques that are capable of faster automated analysis through higher speed and multiplexing.

Capillary Electrophoresis (CE) is a powerful tool for the fast and efficient analysis of peptides, proteins and DNA samples. Examples of high throughput automated CE methods on 96-well plates for both the analysis and purification of oligonucleotides and PCR fragments will be discussed. CE on microfabricated devices offers even faster separations and higher throughput using multiple parallel lanes at similar costs than single lane operation. The manufacturing of microfabricated devices and applications for multiplexed analysis of genetic samples and enzyme assays will be presented. Finally, the potential of microfabricated devices for the integration of sample pretreatment steps such as purification and pre-concentration as well as for DNA samples amplification will allow a streamlined and fully automated sample-to-answer process.

203.

LC-MS, NEW METHODS ON BOTH SIDES OF THE HYPHEN. *Beate Behnke, Reinhard Doetzer, and Wolfgang Dreher, Agricultural Center Limburgerhof, Spectroscopy, BASF AG, APD/FS Li 444, Limburgerhof D-67114, Germany*

With the advent of atmospheric pressure ionization techniques, liquid chromatography in combination with mass spectrometry has become the technique of choice for analysis of biomolecules, drugs and pesticides. The quadrupole time-of-flight hybrid mass spectrometer (QqTOF) provides a new tool for structure elucidation of these molecules: by exact measurement the elemental composition of precursor and product ions is readily determined.

In order to fully exploit the sensitivity of the new hybrid QqTOF instrument the mass spectrometer is coupled to capillary and nano-LC. Compared to conventional LC, this approach leads to a substantial increase of mass sensitivity resulting in a significantly reduced sample consumption. Optimum separation efficiency combined with additional selectivity in the analysis of charged molecules is obtained by hyphenating the MS with electrochromatography.

204.

CHARACTERIZATION OF INTACT PROTEINS AND PROTEIN DIGESTS BY LC/MS AND LC/MS/MS. *Rong-Rong Zhu, Ling Santora, David Ouellette, Tsoyue Joanne Sun, and Kathleen Grant, BASF Bioresearch Corporation, 100 Research Dr, Worcester, MA 01605, zhur@basf-corp.com*

LC/MS/MS peptide mapping has been an essential tool for many protein chemists in the last decade. Characterization of modified proteins is an essential aspect of the development of novel biomedical agents. The common approach has involved analysis of protein digests, but we have been also using the LC/MS to characterize intact proteins and their modifications including biotinylation, phosphorylation and monoclonal antibody production processes. HPLC provides separation, desalting and concentration of proteins prior to MS analysis. Performance of Q-TOF and LCQ spectrometers will be compared for intact protein molecular weight measurements and peptide mapping of recombinant monoclonal antibodies. The wide m/z range and high resolution of the Q-TOF allow routine measurement of intact antibody molecular weight in the mass range of about 150 kDa. However, the LCQ is still a powerful tool for protein chemists due to its high sensitivity, ease of use, and data analysis of peptide maps.

205.

IN GOOD TASTE: LC/MS ANALYSIS OF COCOA BIOPOLYMERS. *Alyson E. Mitchell¹, J. F. Hammerstone², S. A. Lazarus², R. B. Rucker³, Roger S. Mercer⁴, and H. S. Schmitz².* (1) Department of Food Science and Technology, University of California, Davis, One Shields Avenue, Davis, CA 95616, aemitchell@ucdavis.edu, (2) Analytical and Applied Sciences Group, M&M/MARS, (3) Department of Nutrition, University of California, Davis, (4) Molecular Structure Facility, University of California, Davis

Procyanidins are a class of polyphenolic compounds found in foods commonly consumed in the diet such as wine, tea, and chocolate. Procyanidins exist as monomers and oligomeric units. LC/MS methodology was developed for the separation and quantification of oligomeric procyanidins found in cocoa. Procyanidins were separated, based on degree of polymerization and identified by electrospray ionization mass spectrometry with assisted ionization. Chromatograms show resolution of oligomeric procyanidin units through decamers.

ESI mass spectra indicate that multiply charged species form as the degree of polymerization increases, and that procyanidins form cluster ions in the gas phase, and easily undergo fragmentation. The addition of post-column ionization enhancers were screened for optimal ionization conditions.

206.

SEPARATION METHODS FOR LC/MS ANALYSIS OF PHYTOPLANKTON BIOMOLECULES. *A. Daniel Jones, Department of Chemistry, The Pennsylvania State University, 152 Davey Laboratory, University Park, PA 16802, Fax: 814-865-3314, djones@chem.psu.edu, and Janice L. Hironaka, Graduate Program in Plant Sciences, Oklahoma State University*

Phytoplankton play important roles in aquatic ecosystems but are often neglected as biochemical resources. Identification of some of these microalgae presents a significant challenge because of their diversity and their lack of distinguishing physical characteristics. Information-rich LC/MS approaches offer the prospect of rapid discrimination of different genera and species. HPLC/electrospray ionization MS analyses of proteins and signature peptides obtained from proteolysis has been explored using C18, C4, and cyano stationary phases interfaced to an orthogonal acceleration/time-of-flight mass spectrometer. LC/MS analyses of pigments based on atmospheric pressure chemical ionization (APCI) complement diode array detection. Responses of phytoplankton to elevated salinities often involve biosynthesis of protein-compatible solutes such as amino acids and polyols. These solutes have been surveyed using strong cation-exchange HPLC coupled to APCI mass spectrometry using a mobile phase gradient based on volatile acids and electrolytes.

207.

USE OF NARP HPLC AND CAPACITIVE ELECTROSPRAY FOR THE LC/MS ANALYSIS OF LIPID A AND OTHER HYDROPHOBIC BIOMOLECULES. *Murray Hackett¹, Eugene C. Yi¹, Sanghyun Park¹, Kerry Nugent², and Houle Wang².* (1) Department of Medicinal Chemistry, University of Washington, H-172D, Health Sciences Building, Seattle, WA 98195, mhackett@u.washington.edu, (2) Michrom BioResources

Non-aqueous reversed-phase (NARP) high performance liquid chromatography coupled with a capacitive electrospray ion source has been used for LC/MS analysis of complex mixtures of lipid A molecules, other membrane lipids and membrane bound proteins derived from Gram-negative pathogens. These analyses have been problematic with conventional reversed phase mobile phases. Analyses were carried out using a number of binary solvent systems evaluated in conjunction with C30, C18, C8 and C4 stationary phases. All experiments were carried out using an LCQ ion trap and a Michrom Magic HPLC inlet system optimized for either packed capillary (100 μm i.d.) or 1.0 mm stainless steel columns. One goal of the work was to separate lipid A isoforms in complex mixtures in their native form without chemical modification. The experimental ion source was either a cylindrical design or a parallel plate design optimized for flow rates in the range of 50 to 500 nL/min.

208.

AUTOMATED COLLECTION OF MICRO-VOLUMES OF WHOLE BLOOD FOR GLUCOSE MONITORING AND OTHER DIAGNOSTIC PRODUCT FLUIDICS SYSTEMS. *David D. Cunningham, Timothy P. Henning, Eric B. Shain, and Douglas F. Young, Dept. 9NL, Build. AP20-3, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6015, david.cunningham@abbott.com*

A variety of fluidic systems are incorporated into diagnostics products. Examples include the Abbott Vision system based on a unique, multichambered single-use test pack and two-dimensional centrifugation. The i-Stat point-of-care test cartridges are based on piercing pins and an air bladder that is mechanically actuated. Capillary forces control fluid flow in self-performing assays such as the home pregnancy test kits. Recently, the painless extraction of blood was accomplished by the application of vacuum and skin stretching around a lanced site on the forearm. Lancing the forearm gave an average of 0.8 μL of blood at ambient pressure and 3.1 μL at -5 psig using an extraction time of 30 sec., and increased by applying -5 psig for 30 seconds prior to lancing (7.8 μL) or by applying heat of 45 deg. C for 15 seconds (6.9 μL). Surprisingly when the skin was held in place by a mesh, the application of vacuum did not result in any increase. The geometry of the nosepiece was studied. Integrated devices were constructed incorporating a vacuum pump, lancing mechanism, and test strip.

Vacuum holds the skin in a fixed position. The skin is lanced through a hole in the strip and the strip automatically indexes over the lanced site. A mesh layer wicks the blood into the test strip where electrical contact is made to indicate the strip is filled.

209. MICROFLUIDIC DEVICES WITH INCREASED SAMPLE HANDLING CAPABILITIES.

Elisabeth Verpoorte, Laura Ceriotti, Antoine Daridon, Arash Dodge, Gian-Luca Lettieri, Jan Lichtenberg, Vincent Linder, Bruce Weiller, and Nico F. de Rooij, *Sensors, Actuators and Microsystems Laboratory, Institute of Microtechnology, University of Neuchâtel, Rue Jaquet-Droz 1, Neuchâtel CH-2000, Switzerland, Fax: +41 32-720-5711, sabeth.verpoorte@unine.ch*

There are a number of recent and noteworthy developments in the area of integrated chip-based chemistry, or lab-on-a-chip. Very important among these is the trend towards chips incorporating both multiple fluidic functions and non-fluidic elements for e.g. optical or electrochemical detection. Efforts are underway in our laboratory to develop multi-functional systems for fast chemical analysis in both the clinical and environmental domain. It is of great interest to gain a better understanding of flow properties in microchannels, to ultimately facilitate design of chips for different applications. In particular, microfluidic devices have been realized in our laboratory for heterogeneous immunoassay, sample preconcentration in the presence of an electric field (field amplified stacking), capillary electrochromatography using conventional particulate stationary phases, and micro flow injection analysis. This presentation will describe some recent developments in ongoing research projects focusing on these aspects of chip-based chemical analysis.

210. ADVANCES IN COMPUTER-AIDED DESIGN OF MICROFLUIDICS FOR APPLICATIONS IN BIOTECHNOLOGY.

John Harley, *Microcosm Technologies, 1820 Gateway Dr., Suite 330, San Mateo, CA 94404*

Advances in computer-aided design and simulation of microfluidics have enabled reliable simulation and modeling of physical processes in microsystems. Effects such as combinations of electrophoresis, electroosmosis, dielectrophoresis and pressure-driven flow can now be accurately modeled, providing valuable design insight that can guide the development of microfluidic devices. Combined with careful experimental studies, simulation can dramatically speed up the development of microfluidic systems. Specific examples of how simulation can and has been used to yield effective solutions to design problems specific to microfluidic systems for biotechnology applications will be presented. Examples of some of the different types of modeling approaches and the types of physics that can be modeled will be described. Examples of system level or reduced order modeling tools, which provide an even more dramatic speed up in the development process, will also be described.

211. HIGH THROUGHPUT SCREENING IN MICROCHIPS.

Andrea W. Chow, Sherri Biondi, Yevgeny Yurkovetski, Michael Spaid, Aileen Zhou, Anne R. Kopf-Sill, and J. Wallace Parce, *Caliper Technologies Corp, 605 Fairchild Dr, Mountain View, CA 94043, Fax: 650-623-0500, andrea.chow@calipertech.com*

For high throughput screening applications, we have developed a continuous flow microfluidic chip format that allows a few nanoliters containing fmoles of compounds to be brought onto our microchips sequentially at throughput rates of one sample per a few seconds. The current sipper chip format directly interfaces with compounds stored in a microtiter plate. We have developed many designs of microchannel networks in sipper chips for different classes of biochemical and cellular assays, including fluorogenic, non-fluorogenic (such as kinase), and cellular calcium flux. In these HTS applications, only nanoliters of reagents are consumed per compound tested. This feature can be especially enabling when reagents such as enzymes or substrates are difficult to synthesize or purify. After hits are detected in a primary screen, it is useful to develop a dose response curve on that compound. We have integrated automatic dilution of the compound over several orders of magnitude on-chip to measure dose response. To reduce compound usage defined by the microtiter plate formats, we are developing a LibraryCard™ format for high density, dry compound storage that is compatible with the sipper chips.

212. CHEMICAL CONTROL OF ELECTROSMOTIC FLOW IN POLYMER MICROFLUIDIC DEVICES.

Charles S. Henry, Yan Liu, J. Mark Bledsoe, and Chad D. Hopkins, *Department of Chemistry, Mississippi State University, 118 Hand Lab, Mississippi State, MS 39762, Fax: 662-325-1618, chenry@ra.msstate.edu*

Research in microfluidics has progressed rapidly in the last several years. Much of the research in microfluidics has focused on electroosmotic flow (EOF). EOF is attractive because it is simple to setup and operate. However its variation with small changes in pH, solution conditions, and surface chemistry give rise to a lack of reproducibility. Methods for stabilizing EOF with respect to pH using polyelectrolyte multilayer (PEM) coatings will be discussed. PEM coated channels show a 17% variation in EOF from pH 5-10 as compared to uncoated channels which show >40% variation over the same pH range. These coatings are attractive because they are easy to apply and stable for over 50 continuous runs. The simplicity of the coating process allows precision control of the surface chemistry within the microfluidic channel. The use of these coatings to generate new forms of field free flow will also be discussed.

213. PATTERNING FLOW AT THE MICROSCALE VIA CONFIGURABLE THERMOFLUIDIC ARRAYS.

Sandra M. Troian¹, Anton A. Darhuber¹, Jeffrey M. Davis¹, Walter W. Reisner², and Sigurd Wagner³. (1) *Dept. of Chemical Engineering, Princeton University, Princeton, NJ 08544, Fax: 609-258-0211, stroian@princeton.edu*, (2) *Department of Physics, Princeton University*, (3) *Department of Electrical Engineering, Princeton University*

Most microfluidic devices rely on mechanical or electrokinetic techniques for driving ultrasmall liquid volumes through an interior channel network. By contrast, we are exploring an open architecture design based on free surface flow. The driving mechanism relies on thermocapillary transport of liquid on a differentially heated substrate which is chemically patterned to confine flow along selected pathways. Flow pathways and reaction sites can be dialed in via an active matrix circuit which controls the resistive heating of individually addressable pixels. The multifunctional heating elements can route, flow and react liquid streams or droplets over large or small distances. In addition, the proposed technology is expected to be low voltage and low-current with no moving parts. In this talk, we discuss extensive in-house experimental and modeling efforts aimed at quantifying the parameters controlling flow throughput and mixing capability of such a device.

214. BIOANALYTICAL APPLICATIONS OF MICROCHIP CAPILLARY ELECTROPHORESIS WITH ELECTROCHEMICAL DETECTION.

Susan M. Lunte¹, R. Scott Martin¹, Andrew Gawron², Nathan Lacher², Barbara Fogarty³, and Fiona Reagan³. (1) *Department of Pharmaceutical Chemistry, University of Kansas, 2095 Constant Avenue, Lawrence, KS 66047, Fax: 785-864-5736, slunte@ukans.edu*, (2) *Department of Pharmaceutical Chemistry, University of Kansas*, (3) *Department of Chemistry, Limerick Institute of Technology*

Microchip-based analytical devices incorporating capillary electrophoresis are becoming increasingly popular for clinical assays, high throughput analyses, and DNA sequencing. Electrochemical (EC) detection has many advantages for use with microchip CE systems. Both the detector and the potentiostat can be miniaturized without a loss in sensitivity. It is also highly selective, and many biologically important analytes can be detected without derivatization. Chemically modified electrodes and multiple electrode systems can be utilized to increase selectivity for many types of compounds. Lastly, microelectrodes can be fabricated using the same procedures that are already used to construct microchips. In this paper, the design, fabrication, and evaluation of microchip CE-EC devices using both single and dual gold and carbon microelectrodes will be described. The potential of these devices for the analysis of biological samples will be demonstrated for the detection of neurotransmitters, amino acids, and peptides.

215. MICROFLUIDICS IN TRIAGE® LAB CHIPS: A NEW DIMENSION TO IMMUNOASSAYS. *Kenneth F. Buechler, Paul H. McPherson, Steve Lesefko, and Kevin Nakamura, Research and Development, Biosite, 11030 Roselle St., San Diego, CA 92121, Fax: 858-452-8613, kbuechler@biosite.com*

The Triage Lab Chip performs multiple, independent immunoassays in 15 min to measure targets in biological fluids. Blood or other biological fluid is added to the Lab Chip and a filter on the Lab Chip separates red blood cells from the plasma or other insoluble matter from the fluid. Surface architecture and relative hydrophobicity of microcapillaries control microfluidics in the Lab Chip. Precise microcapillaries (15µm to 250µm) are formed by the assembly of plastic lids and bases. The edges of the microcapillaries are made hydrophobic to prevent accelerated flow at capillary junctions. Hydrophobic surfaces are used to impede flow within microcapillaries to provide incubation of the sample and fluorescent antibodies. Structures within the microcapillaries allow fluid to move from high to low capillarity. The Lab Chip comprises a protein array microcapillary containing antibodies for capture of fluorescent antibody-target complexes. Target concentrations are determined from the fluorescence of the captured fluorescent antibody-target complexes at the surface of the Lab Chip. Fluorescence is measured in 2 min by a portable fluorometer called the Triage Meter. The Lab Chips currently on the market are the Triage Cardiac Panel and Triage BNP Test, which aid in the diagnosis of myocardial infarction and congestive heart failure, respectively. Details of the surface architecture, the microfluidics and performance of the Triage Lab Chips will be discussed.

216. EXPLORING A NEW APPROACH TO MICROFLUIDICS. *Ingrid Fritsch, Christopher S. Carter, and Zoraida P. Aguilar, Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR 72701, ifritsch@uark.edu*

Recent successes in high-throughput, small sample treatment, separations, and rapid chemical analysis have been made possible by advancements in microfluidics. Mechanical, electrokinetic, and centrifugal approaches are probably the most used methods, each with its set of advantages and disadvantages. We are exploring a new approach that involves magnetic fields and does not fall into previous microfluidics categories. With this new method, it may be possible to independently control several variables and forces to perform microfluidics on a greater variety of solutions, with a larger variety of device materials, while allowing reversal of flow without valves. The theory of how these variables and forces are expected to influence solution flow rate and direction in channels of different dimensions will be discussed. We will compare the predictions to our preliminary experimental results and describe advantages and disadvantages of using this new approach for development of a new generation of microfluidics devices.

217. BIOCHEMICAL EXPERIMENTATION ON MICROFABRICATED DEVICES. *J. Michael Ramsey¹, Stephen C. Jacobson¹, Christopher T. Culbertson¹, Roswitha S. Ramsey¹, Maxine McClain¹, and Robert S. Foote². (1) Oak Ridge National Laboratory, PO Box 2008, Oak Ridge, TN 37831-6142, Fax: 865-574-8363, ramseyjm@ornl.gov, (2) Chemical & Analytical Sciences Division, Oak Ridge National Laboratory*

The first condensed phase chemical separations were performed on a microfabricated device (microchip) nearly a decade ago. Although first viewed as a

curiosity, microchip separations have gained increasing interest over the past few years. Some attributes of this technology are the ability to perform moderately efficient separations 10 to 100 times faster than conventional technology while utilizing sample volumes that are 100 - 10,000 times smaller. In addition microchips promise to monolithically incorporate sample processing procedures, which allows automation of chemical and biochemical assays. We have been working on a number of microfabricated technologies that relate to the production of biochemical information. We have recently shown that microchip devices can be used for rapid, high-resolution separations of amino acids, peptides, and proteins. Moreover, integration of chemical processes such as proteolytic degradation and fluorescence derivitization have been incorporated. Cellular manipulations on microfabricated flow cytometry devices have also been demonstrated that include staining of cells with various dyes, incubation with antibodies, and cell lysis. The operation and performance characteristics of these devices will be discussed.

218. LABCD™: A PLATFORM FOR AUTOMATED LIFE SCIENCES RESEARCH. *Bruce L. Carvalho, Todd E. Arnold, David C. Duffy, Gregory J. Kellogg, and Norman F. Sheppard Jr., Tecan Boston, 200 Boston Avenue, Medford, MA 02155, Fax: 781-306-0837, bruce.carvalho@tecan.com*

The LABCD™ is a centrifugal microfluidics-based platform developed for integration and automation of fluid processing, detection, and analysis. In its simplest form, centrifugal force on a rotating disc is used to pump and valve fluids. We present two applications in which centrifugal microfluidics is integrated with detection and thermal regulation. A system that performs multiple (96) enzymatic assays simultaneously on microliter volumes using colorimetric and fluorescence detection has been developed, demonstrating the ability to perform highly parallel, miniaturized assays. A second application is the integration of raw sample processing with PCR. Samples are processed through cell lysis, sample conditioning, and mixture with PCR reagents and primers. The instrument that rotates the disc also employs co-rotating Peltier devices for lysis and thermal cycling. We present results showing amplification of specific targets from whole blood and bacterial samples.

219. 2-D ARRAY ASSEMBLY USING LIGHT AND INTERFACIAL PATTERNING. *Michael Seul, Sukanta Banerjee, Kairali Podual, Alice X. Li, and Chiu Chau, BioArray Solutions, LLC, 120 Centennial Avenue, Suite 105, Piscataway, NJ 08854, Fax: 732-457-8888, sukanta.banerjee@bioarrays.com*

Spatially modulated illumination as well as lithographic patterning of silicon electrodes have been used to create lateral impedance gradients which permit control of electric field-induced fluid flow as well as interfacial transport of colloidal particles. In the presence of these gradients, AC electric fields applied normal to the interface generate rectifying fluid flow and particle transport, directed from high-impedance regions to low-impedance regions of the electrode. The underlying electrohydrodynamic forces permit the rapid assembly of planar arrays of particles in designated regions of the electrode.