

Electrophoresis in 2005: 22nd Annual Meeting of the American Electrophoresis Society

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Electrophoresis is often considered to be the domain of biologists only. The common techniques were largely developed by biochemists and molecular biologists to help solve biological problems. Nevertheless, from the earliest days, chemical engineers have also been involved in studies of electrophoretic mechanisms, recognizing the value of electrophoresis as a high-resolution separations technology. Due to the mutual interests in electrophoretic separations, it was logical that when the American Electrophoresis Society (AES) was searching for a larger organization with which to hold its annual national meeting, the American Institute of Chemical Engineers (AIChE) arose as an appropriate choice. AIChE accepted AES's proposal and the two societies have held joint meetings for the past 5 years. AIChE's annual technical meeting is organized by dedicated staff and attended by an interested audience. AES, the much smaller of the two societies, holds its meetings as topical sessions within the AIChE annual conference. The first such joint meeting took place in 2001 and was considered a success. Since then, four more combined meetings followed; the fifth was held from October 31 through November 3, 2005, in Cincinnati (OH, USA). This latest meeting was the 22nd annual meeting of AES. There were 72 oral presentations and 14 posters on display. The lectern and audiences were shared between the two societies in areas of overlapping interests. Presentations and posters were made by

biologists and engineers alike, and the audiences were also composed of a mix of biologists and engineers. Sessions covered proteomics, microfluidics, bioinformatics, detection and electrokinetics. The meeting was jointly organized by Adrienne Minerick of Mississippi State University (MS, USA) and Victor M Ugaz of Texas A&M University (TX, USA). They are members of both AES and AIChE and both organizers are AES Council members. This review summarizes the meeting's oral presentations. Abstracts of the talks can be found by accessing the AIChE Conferences page directly through the AIChE web site [1] or by linking to that page through the AES web site [2]. Descriptions of the two societies can be found on their respective websites.

Plenary session

Drilling deeper into the proteome

The AES meeting opened with a session devoted to three plenary talks. Chong Ahn (University of Cincinnati, OH, USA) chaired the session, which was co-chaired by Adrienne Minerick. Mark P Molloy (Australian Proteome Analysis Facility, Ltd., NSW, Australia) spoke about recent schemes for deep proteome analyses by first removing high-abundance proteins. Early approaches towards drilling into the proteome utilized sample prefractionation schemes based on differential protein solubility in various solvents and on electrophoretic separation parameters (e.g., isoelectric point [pI]).

In an effort focused towards protein biomarkers in human biofluids, Molloy's group is developing an approach to remove at least the 100 most abundant proteins from human serum, thereby paving the way to detect less abundant proteins that may have diagnostic potential. This strategy, termed cyclic abundant protein immuno-depletion, utilizes suites of polyclonal immunoglobulin (IgY) antibodies raised in chickens, and involves successive rounds of immunization with fractionated serum and/or plasma. Antibodies obtained from the first immunization round are used to deplete the highest abundance proteins. The process is repeated with the depleted serum for a second immunization, then a third, and so on, through several cycles. Each IgY antiserum depletes samples of a different abundance class of proteins. A different approach for deeper proteomic drilling involves using certain commercially available matrix-assisted laser desorption/ionization (MALDI) capture targets that provide high sensitivity for protein identification. At the Australian facility, deeper drilling of the proteome augments a systems biology approach that uses bioinformatics to weave together data from disparate experimental approaches for a holistic view of biological activity.

Microfluidic oral diagnostics

Anup K Singh (Sandia National Laboratories, CA, USA) spoke about an automated handheld device for detection of biomarkers in saliva, which is being developed in his group. Analysis of oral fluids has great potential in clinical diagnosis of both oral and systemic diseases. Oral fluids, especially saliva, offer many advantages over other bodily fluids because they are easy to collect using noninvasive methods in both clinical and nonclinical settings. However, only a handful of devices currently exist for medical diagnostics based on the analysis of oral fluids. The described device performs rapid microfluidic chip-based immunoassays (<3 min) using saliva as a

sample with low sample volume requirements (10 μ l) and appreciable sensitivity (nM–pM). Immunoassays are performed in glass microchips with channels containing crosslinked polyacrylamide gels cast *in situ* by photopolymerization. The polymeric monoliths are cast in minutes and are easily tailored to obtain the desired pore size (10 nm–1 μ m) and surface properties. To perform microchip immunoassays, samples are mixed with fluorescently labeled antibodies prior to the separation of bound and unbound immune species by native polyacrylamide gel electrophoresis (PAGE), with detection by a laser-induced fluorescence (LIF) detector. Microchip immunoassays developed to date are compatible with complex samples such as saliva and blood serum, and were used to detect a wide range of analytes such as interleukin 6, tumor necrosis factor A, C-reactive protein and tetanus IgG. To improve the detection limit, an integrated chip-based preconcentrator is used that typically provides a 100-fold concentration of analytes in a few minutes. The immunoassay chip is being integrated with miniaturized high-voltage power supplies and a LIF detector as a portable device.

Funding opportunities at the National Institutes of Health

As might be expected by the title of her talk, *Funding opportunities in technology development at the National Institutes of Health* (NIH), the presentation by Brenda Korte (National Institute of Biomedical Imaging and Bioengineering [NIBIB], MD, USA) drew many academics. The mission of the NIBIB is to improve human health by leading the development and accelerating the application of biomedical technologies. The Institute is committed to integrating the physical and engineering sciences with the life sciences in order to advance basic research and medical care. With a focus on technology development in interdisciplinary areas, the institute is uniquely suited to enable and promote fundamental discoveries in a wide range of scientific areas such as sensors, lab-on-a-chip devices and general bioanalytical instrumentation. This talk addressed current

programs and funding opportunities in technology development both at the NIBIB and NIH. These programs cover a broad spectrum of efforts ranging from early-stage high-risk and/or high-impact projects to biomedical technology resource centers and large-scale bioengineering research partnerships that include early clinical testing.

Proteomics

Electrophoresis-based approaches (Part 1)

Advances in technology and approach continue to allow deeper exploration of gene expression patterns and illumination of the interplay among the many factors governing cellular response. Specifically, proteomics is advanced by technologies with the capability of providing quantitative information with ever improving levels of sensitivity and throughput. This session focused on the development of novel techniques to address the limitations in current technology, including ultrasensitive protein detection, high-throughput antibody creation and optimization, the development of protein-detecting microarrays, and other related technologies. It was chaired by Ray Grant and co-chaired by Feng Wang (both with Procter & Gamble, OH, USA).

The session opened with a talk by Hsin-Yao Tang (The Wistar Institute, PA, USA) on microscale isoelectric focusing (IEF) to enhance detection of low-abundance proteins in human serum. Microscale IEF is based on immobilized pH gradient technology. Samples usually contain 300–400 μ g of protein in approximately 650 μ l of solution. This group incorporated microscale-IEF pre-fractionation into a novel 4D separation strategy to reduce sample complexity and allow detection of lower abundance proteins. The strategy consists of three orthogonal protein separations: major protein immunodepletion, microscale-IEF and 1D sodium dodecyl sulphate (SDS) PAGE. The result is a 2D array of pixels or gel slices that is equivalent to a low-resolution 2D gel, with each pixel in the array containing a group of proteins with known pI and molecular-weight ranges. Each slice is then digested with

trypsin followed by nanocapillary reversed-phase tryptic peptide separation prior to tandem mass spectrometry (MS/MS) analysis. When human serum was analyzed, more than 2700 proteins, spanning up to 9 orders of magnitude, were identified using Human Proteome Organization criteria for high-confidence assignments. Of interest is that a substantial number of low-abundance proteins (<100 ng/ml to pg/ml range) were identified.

Hongwei Xie (University of Minnesota, MN, USA) described the use of free flow (FF) IEF for preparative fractionation of complex peptide mixtures prior to MS-based proteomic analysis. In this strategy, peptides are fractionated by FF-IEF in the first separation dimension, followed by capillary liquid chromatography, MS/MS and sequence database searching. Using their approach, the group resolved and fractionated a complex mixture of peptides from chromatin-associated proteins in *Saccharomyces cerevisiae*, using peptide pIs as an additional constraint in protein identification.

John E Wiktorowicz (University of Texas Medical Branch, TX, USA) discussed the device he is developing: a liquid-based capillary/chip system that is an analog of 2D gel electrophoresis (2DE). Proteins are labeled to saturation with a covalent fluorescent dye prior to separation. This 2D device is said to be rapid, accurate and sensitive, and to permit real-time monitoring and quantitative recovery of proteins for post-separation processing and identification.

John F Hassard (DeltaDOT Ltd, UK) spoke about an instrument for real-time imaging of unlabeled biomolecules as they traverse an electrophoresis gel. The technique, label-free intrinsic imaging, utilizes dynamic multipixel detection and advanced signal processing algorithms to enhance the signal-to-noise ratio in unlabeled systems. The analysis of the very large data set is achieved by data acquisition from each molecule as it crosses each pixel at a specific time point. This allows a space–time correlation technique, termed vertexing, to be applied. Vertexing enables very high signal-to-noise ratios that exceed traditional single-detector

absorption imaging techniques. DNA sequencing has been achieved on standard kit-based cycle sequencing that can read lengths of 600 bp. Standard protein markers and *Escherichia coli* lysates were successfully imaged in less than 10 min. The technique was also applied to separations of bacterial species.

Jim Kraly (University of Washington, WA, USA) presented a 2D capillary system employing sieving in the first dimension, coupled to micellar electrokinetic chromatography in the second dimension. Proteins are fluorescently labeled prior to separation and are detected by LIF inside a sheath flow cuvette using a fiber-coupled single photon counting module. The 2D capillary electrophoresis (CE) system requires only nanoliters of sample, has limits of detection in the zeptomole range, and analysis time is less than 1 h.

Paul Todd (Space Hardware Optimization Technology, Inc., IN, USA) demonstrated his multistage electrophoresis device. With this instrument, a starting sample is placed in a cuvette in a sliding plate. The sample cuvette then becomes the lower half of a shear cell by sliding under an inverted, buffer-filled cavity in an upper plate. A vertical electric field is applied to the shear cell, and separands are collected in the upper half of the shear cell when the plates slide with respect to each other. This process is repeated until all desired separands are collected in up to 20 fractions on the basis of electrophoretic mobility. Applications of this technology to protein analysis include: pH-dependent isolation of proteins with specific pIs; concentration of proteins by sequential volume reduction; and debulking of protein solutions to reveal low-abundance constituents.

Electrophoresis-based approaches (Part 2)

Electrophoresis technology continues to be a vital tool for quantitative proteomic analysis. This session focused on the development of electrophoretic technologies and their application to proteomics. Presentations described advances in gel-free protein separations, protein staining, methods for analyzing membrane proteins, and mass spectrometric methods

for resolving mixtures of peptides. The session chair was Feng Wang and the co-chair was Ray Grant.

In the opening presentation, Bradley B Jarrold (Procter & Gamble) described a method for enriching the concentration of low-abundance skeletal muscle proteins prior to 2DE. In the case of muscle tissue, highly abundant structural proteins such as actin, myosin and tropomyosin compromise the detection and analysis of more biologically relevant proteins. The protocol described exploits high-pH extraction to reduce or remove abundant structural proteins from skeletal muscle crude membrane preparations in a manner suitable for 2DE. Muscle tissue is powdered and lysed in Tris base, then partitioned into a supernatant and pellet. Treatment of the pellet with high-pH conditions releases structural proteins from the membrane. MS identification demonstrates that the majority of protein spots reduced or removed by high-pH treatment were contractile or contractile-related proteins. Removal of these proteins enabled successful detection and identification of minor proteins. Removal of structural proteins also results in significant improvement of gel quality and the ability to load enough material for the detection of lower abundance protein classes.

Ivan C Gerling (University of Tennessee Health Science Center, TN, USA) described an evaluation of sequential staining of proteins in the same gel for detecting the post-translational modifications of phosphorylation and glycosylation. The net conclusion of these studies validates the sequential use of commercially available fluorescent stains for phosphorylation, glycosylation and total protein for identification of post-translationally modified proteins in 2D gels.

Detlef Schumann (University of Cincinnati) extolled the virtues of mini-gels for optimizing sample extraction conditions and the conditions for running and staining 2D gels. The idea is that mini-gels offer valuable information on the compatibility of sample preparation methods with 2DE protocols, with the advantage that they require significantly less sample and time than high-resolution large gels.

Nancy Kendrick (Kendrick Laboratories, WI, USA) described the method used in her laboratory for analysis of phosphorylated proteins. She advocated the use of carrier ampholyte-based 2DE methods, as they facilitate the use of SDS, as compared with immobilized pH gradient methods.

Phillip C Wright (University of Sheffield, UK) showed comparisons of the proteomes of closely related *Cyanobacteria*. Using a combination of multidimensional chromatography, 2DE, image analysis, spot matching and electrospray ionization (ESI) MS/MS, together with protein-protein BLAST analysis, functional phylogenetic relationships were developed between these cyanobacteria to act as a guide for future biotechnological exploitation.

Kunal Aggarwal (Cornell University, NY, USA) described the use of an isobaric tagging shotgun approach to simultaneously study protein expression of *E. coli* at four different biological states. Isobaric tags are isotopically distinguishable adducts that are attached to proteins prior to MS. The four commercially available tags were used to label each of the four biological states being studied. Initially, the tags all have molecular masses of 145 Da. During ionization in the mass spectrometer, the tags lose balance segments of 31, 30 or 29 Da, allowing for the identification of each different reporter tag on the peptides. In this study, 780 unique proteins, representing all the major functional groups, were identified. These include some low-abundance proteins and transcription factors. Protein quantitations using either isobaric tags or 2DE were compared, and both these methods were observed to yield reasonably similar protein expression ratios. However, the isobaric tagging method demonstrated lower coefficients of variation in the quantitation of proteins that were poorly stained on 2DE gels. Not all the proteins detected using 2DE were identified using the isobaric tagging shotgun approach, demonstrating that the two methods are complementary.

Advances in proteomic analysis: biomedical applications

Proteomics offers approaches for the global investigation of the molecular basis of disease, drug action and development.

The goal of these studies is to ultimately provide a basis for early disease detection and, eventually, rational design of pharmacological intervention. This session explored novel applications of electrophoretic and mass spectrometric technologies to analyze proteomes for ultimate clinical benefit. It was chaired by Charles S Henry (Colorado State University, CO, USA) and co-chaired by Aaron Timperman (West Virginia University, VA, USA).

Aaron Timperman began the session with a discussion of a microfluidic system for 2D separations of intact proteins with online ESI-MS analysis. Critical components of this system are microfluidic channels that are effectively terminated by nanocapillary membranes. The membranes are used as analyte concentrators, and have been used to achieve concentration factors in excess of 300-fold. Small 1-nm diameter ions can be concentrated in front of membranes with 10-nm diameter capillaries, but the mechanisms of this electrokinetic concentration process are not fully understood. Additionally, Timperman's group is developing polyethylene glycol (PEG)-terminated self-assembled monolayers for minimizing nonspecific adsorption on the surfaces of the glass microfluidic channels. The PEG monolayers are effective in preventing protein adsorption, and concomitantly reduce electro-osmotic flow. The electro-osmotic flow on PEG-coated channels is reduced by 90% compared with the native glass surface, and the separation efficiencies achieved with these coatings are comparable to other commercially available permanent coatings.

Michael A Freitas (The Ohio State University, OH, USA) talked about post-translational modifications to core histones. These modifications play a critical role in gene activities such as chromatin remodeling, regulation of gene transcription, DNA recombination and DNA repair. Localization of these modifications is an important first step in the characterization of their molecular function. There is mounting evidence that multiple modifications work in a synergistic fashion and give rise to a histone

code that regulates many of these activities. Recently, the Freitas group identified 20 novel modification sites by high mass accuracy Fourier transform ion cyclotron resonance MS/MS on the four core histones purified from bovine thymus, and have developed quantitative proteomic strategies to screen for modification changes in transformed cell lines and human patients with acute myeloid or chronic lymphocytic leukemia.

Anne MacLachlan (Cincinnati Children's Hospital, OH, USA) stressed that it is important to understand the sources and magnitude of both technical (experimental) and biological (naturally occurring) variability for assessing the statistical significance of differential expression protein patterns and for the accurate interpretation of the experimental results. The number of biological replicates considered for analysis should be large enough to obtain reasonable estimates for biological variability and, therefore, enable a valid interpretation of observed differences in sample populations. This group automated the collection of reproducible protein profiling data from large sample sets MALDI time-of-flight (TOF) MS. Their protocol has been applied to the analysis of proteins that are differentially expressed between challenged and controlled samples of biological fluids.

Bin Cao (National University of Singapore, Singapore) described proteomic studies on the growth pattern of *Pseudomonas putida* in the presence of two growth substrates (phenol and sodium glutamate) and a nongrowth substrate (4-chlorophenol). The organism grows in two exponential phases. Cells preferentially utilize phenol in the first growth phase and glutamate in the second. 2DE analysis provided protein profiles during cell growth under various culture conditions. The presence of 4-chlorophenol inhibits the degradation of phenol and sodium glutamate to different extents due to differences in its toxicity to different sets of proteins.

Xunbao Dun (Massachusetts General Hospital, MA, USA) described proteomic studies of hepatic responses to cutaneous burns in mice. Acute-phase

proteins were upregulated and antioxidant enzymes were downregulated. These responses are consistent with known metabolic phenomena. In addition, expression of some antiapoptotic chaperone proteins and protein synthesis-related proteins are likely responses to burn-induced apoptosis and oxidative stress and meet the needs for protein synthesis in the regenerating liver. The proteomic data offer a fairly comprehensive overview of altered molecular events in liver, and provide the basis for identifying new avenues and therapeutic targets for improving clinical outcomes following severe burn injury and trauma.

Daisy-Malloy Hamburg (University of Cincinnati) demonstrated a protocol that can be used to identify proteins from bacterial strains for which no DNA or protein sequence exists. A particular set of proteins are characterized using MALDI-TOF-MS. The resultant molecular weights are then compared with DNA or protein sequences from reference strains, allowing for straightforward identification of proteins from any nonreference strains. The method was validated with ribosomal proteins from various strains of *Thermus thermophilus*, for which sequence information was available for a few, but not all, strains.

Dimiter N Petsev (University of New Mexico, NM, USA) talked about a microchip for separating charged proteins by means of the nascent technique of electric field-gradient focusing. The device operates by independent manipulation of the electrophoretic and electro-osmotic forces and velocities in the channel. Electro-osmosis is kept constant along the channel, but the opposing electrophoretic force is made to vary from one region to another. The net force on a given protein molecule becomes zero at a unique point, while all other solutes are swept away. Similar to IEF, this method concentrates separated molecules into spatially distinct bands. The separation of a binary mixture of bovine serum albumin and phycoerythrin demonstrated that the technique is feasible.

Carla MR Lacerda (Colorado State University) explored the response of mixed bacterial cultures, considered a

meta-organism or community, to cadmium stress. Comparison of 2D gels from cadmium-exposed and control cultures revealed that the community reacts by changing its protein profile, with both increased and decreased expression of substantial numbers of proteins that change with time.

Focus on bioinformatics

Ruth VanBogelen (Pfizer, MI, USA) chaired and Philip Andrews (University of Michigan, MI, USA) co-chaired a session concerning bioinformatics in proteomics. Since proteomics technologies generate dauntingly large amounts of heterogeneous data types, they are highly dependent on, and integrated with, informatics. This session covered laboratory information management systems, image analysis systems and databases needed for generation and maintenance of proteomics data.

Gordon A Anderson (Pacific Northwest National Laboratory, WA, USA) spoke about the informatics challenges of high-throughput proteomics. The complex multistage analyses of proteomics studies require tracking of experimental conditions and sample pedigrees. Conducting proteomics research at any significant level of throughput using MS requires automated information management, as the volume of data is too large and the processing rates required are too rapid to be managed manually. In order to manage this large volume of data and metadata, an information storage and management system was developed. This system performs the required data management tasks in addition to automation of the data process pipeline. It converts the data produced by multiple disparate mass spectrometers into information about the proteins that are present in biological samples. It has been in operation for over 3 years in support of proteomics research, and has successfully managed information relating to thousands of experiments.

Romesh Stanislaus (Medical School of South Carolina, SC, USA) described AGML Central, an open-source public proteomic infrastructure for disseminating 2DE proteomics data. It is based on

the annotated gel-markup language (AGML) an Extensible Markup Language (XML) standard developed for the annotation of 2DE data. AGML Central infrastructure consists of an input layer with a growing collection of converters from proprietary formats. The conversion layer creates AGML documents with the input information and data stored in a database for future reference. An analysis layer consists of many analytical and search tools from an ever-growing list of Matlab toolboxes, visualization tools and tools for searching the database.

Andreas PM Weber (Michigan State University, MI, USA) presented results of bioinformatics and proteomics approaches towards identifying chloroplast envelope membrane transporters. The availability of fully sequenced plant genomes, and the progress in proteomics techniques and bioinformatics are allowing for the identification of previously unknown plastid envelope membrane transporters.

Carol Giometti (Argonne National Laboratory, IL, USA) described GelBank, a public website designed to provide access to proteome analysis results as they are validated and published [3]. GelBank includes the complete genome sequences of approximately 130 microbes, along with numerous tools that provide the capability to search the sequence databases for specific protein functions and amino acid sequences. This site also includes web applications pertinent to 2DE analysis, such as titration curves for collections of proteins and 2DE pattern animations. The database is currently populated with protein identifications from the Argonne Microbial Proteomics studies, and will accept data input from outside users interested in sharing and comparing results from proteome experiments.

Joel S Bader (Johns Hopkins University, MD, USA) reported on protein-protein interactions in the human proteome identified by use of a high-throughput, two-hybrid screen. The raw network contains over 30,000 unique interacting pairs. Bioinformatics analysis identified a high-confidence core network, evolutionary comparisons to

model organism networks and estimates of the underlying complexity of human protein-protein interactions.

Dexter R Pratt (Genstruct, Inc., MA, USA) described the development and application of a very large-scale causal, computable model of biology, which has been used to identify molecular cause-and-effect hypotheses consistent with data from proteomic experiments. Large-scale causal analysis is a powerful new systems-based approach for the interpretation of molecular state measurements in drug discovery. Automated causal analysis can be used to define upstream networks of molecular events, which could result in experimentally observed protein changes. It can be used to identify possible causal pathways linking initial experimental perturbations to observed protein or phenotypic changes.

Ramon M Feliciano (Ingenuity Systems, Inc., CA, USA) described a systems biology application for computational analysis of high-throughput experimental datasets. The company markets the world's largest curated database of biological networks created from millions of individually modeled relationships between proteins, genes, complexes, cells, tissues, drugs and diseases. Network models of biomolecular pathways are computationally generated using this database and sets of significantly expressed genes from proteomics and microarray datasets. These models are further annotated with functional and physical relationships such as gene regulations, protein-protein interactions, post-translational modifications and drug-target interactions.

Lawrence I Grossman (Wayne State University, MI, USA) addressed the question of which genes might have been involved in the adaptive evolution responsible for the emerging phenotypes of extended life spans and the enlarged brains relative to their body size, particularly neocortex, of humans and other anthropoid primates as compared with other organisms. The available comparative genomic data was examined using bioinformatic and evolutionary genetic techniques. In particular, the evolution of the genes involved in aerobic energy

metabolism correlates strongly with the increased demand for aerobic energy in the expanded anthropoid primate cortex. Furthermore, the evolutionary rate of mitochondrial genomes among select vertebrates compared with the encephalization quotient, a measure that normalizes brain-to-body size, demonstrates that the mitochondrial-encoded genes that are involved in accepting electrons from cytochrome c have accelerated rates of change that result in a reduction of electrostatic charge in primates.

BioMEMS & microfluidic technology: cell & biomolecule analysis

This session, chaired by Steven A Soper (Louisiana State University, LA, USA) and co-chaired by Patrick Limbach (University of Cincinnati), addressed microfluidic technology being developed to probe chemical and biochemical responses at the cellular and subcellular levels. The ability to study processes at the single-cell level promises to provide a host of information with benefits in the area of therapeutics and drug discovery.

John F Hassard demonstrated how his company's label-free imaging technique (as described in Proteomics, Part 1) can be applied to the isolation of proteins. A system of bifurcated channels in a plastic microfluidic chip was designed. The branches in the chip have embedded exclusion electrodes, with specific biomolecules in the mixture being separated into an isolation channel.

Sirimon O-Charoen (University of Michigan) described a numerical simulation study of flow and diffusion in microfluidic channels used for oligonucleotide synthesis. This study suggested a washing method for reducing the solvent amount needed. Also, the effects of flow rate, diffusion coefficient and feeding characteristics to the fluid replacement result were studied. The feeding styles included continuous and pulsed, with various pulsing sizes.

Adrienne Minerick described a dielectrophoretic device for separating red blood cells by blood type (A, B, AB or O). In dielectrophoresis, nonhomogenous alternating current fields probe the interior characteristics of cells. This differs from

linear electrokinetic tools, which separate or manipulate objects based on surface charge and radius. Genetically or geometrically similar cells have similar, but still distinct, resonant frequencies in the MHz range. In the dielectrophoretic field, the blood cells align into pearl chains and aggregates in either regions of high field intensity or regions of low field intensity. These behaviors are quantified as a function of field frequency, field intensity and length of time in storage. This work could help develop new dielectrophoresis-based microdevices for rapid, point-of-care blood typing methods. It could also serve as a baseline from which abnormalities in blood, due to disease or physiological imbalances, can be measured.

Kyung Eun Sun (University of Michigan) also made use of dielectrophoresis, in this case, to stretch single DNA molecules. The surface properties and the electrode characteristics of the dielectrophoretic device affect the molecular stretching. For example, the hydrophilicity of the surface between the electrodes affected the degree to which single DNA molecules could be reliably stretched, with low-contact angle surfaces (hydrophilic) between the electrodes decreasing the efficiency of stretching. Surfaces treated with high silane concentrations performed better, presumably due to a decrease in nonspecific adsorption of DNA. The shape and dimensions of the electrodes also affected the efficiency of stretching. Annealing of gold electrodes was necessary to remove most of the field points formed by the methods of construction, and enabled controlled stretching to be obtained.

Hsiang-Yu Wang (Purdue University, IN, USA) studied on-chip electrical lysis of biological cells. On-chip electrical lysis of biological cells is of interest due to the possibility of recovering the contents of cells without introducing lysing agents that may interfere with subsequent biological assays. In this study, green fluorescent protein (GFP)-expressing *E. coli* cells were lysed continuously while flowing through a microfluidic device. The direct current field was intensified in a defined section of the microfluidic channel by

altering the channel geometry. Local lysis field strengths of 1500 V/cm were required for the lysis of 95–100% of GFP-expressing *E. coli*, as followed by plate counts and fluorescence spectroscopy. This is a considerably lower overall voltage than with previous devices.

Thomas N Chiesl (Northwestern University, IL, USA) presented studies on the effect of polymer architecture on the non-gel, polymer sieving of DNA molecules undergoing CE. With as little as 0.13% mol dihexylacrylamide, the physically crosslinked polymer, poly(acrylamide-co-dihexylacrylamide) yields remarkably improved electrophoretic DNA separations when compared with a linear polyacrylamide. Physically crosslinked systems offer significant advantages over both linear polymers and covalently linked crosslinked gels, due to separation performance (or speed) and because the physical crosslinks can be broken (reversibly) with applied shear and loaded into microchannels.

Matthew B Kerby (Brown University, RI, USA) described a continuous-flow microfluidic reactor developed to study the kinetics of a linear *in vitro* transcription reaction of mRNA. In this reactor, cDNA templates were either covalently attached to the channel or in packed beds.

Biomedical & diagnostic applications

Medical diagnostic kits encompass a wide variety of portable analytical devices used to monitor and screen for medical conditions. They are rapidly being developed for use on a single test basis and show promise as indispensable tools for clinical research, medical laboratories and at-home self testing. Research in the areas of sample introduction, preparation, electrokinetic transport of biofluids, development of quantitative detection sensors, and the incorporation of genomic and proteomic biomarkers are needed to further the advancement of biomedical microdevices. This session concerned biomedical microdevices for analyses on either the cellular or subcellular level. The chair of this session was Joseph J Biernacki (Tennessee Technological University, TN, USA) and the co-chair was Adrienne R Minerick.

Bruce Gale (University of Utah, UT, USA) described work on the use of field-flow fractionation for handling and separating nanoparticles in microdevices. Nanoassembled sensing arrays that can be combined with a variety of other microfluidic components and optical detection components, such as those using fluorescence or absorbance, and electrical detection systems using conductivity or amperometry, were also discussed. Paul JA Kennis (University of Illinois) described the fabrication of a microfluidic chip device for the combinatorial synthesis of a library of small molecules and the subsequent screening of the library using integrated detection, with all fluid steps performed at the nanoliter level.

Christa N Hestekin (Northwestern University) demonstrated a device for the tandem single-strand conformational polymorphism and heteroduplex analysis on a microchip electrophoresis platform. The two analytical procedures are excellent, complementary, electrophoretic methods for genetic mutation detection. Single-base mutations in the p53 gene exons 5–9 were detected by the chip electrophoresis device, with separation times of less than 10 min. p53 is important in the pathogenesis of a variety of human cancers.

Prasanna K Thwar (University of Michigan) described work aimed at the development of assay methods for single cells. This group is developing an emulsion droplet-based technique to probe a single cell over long periods of time. This technique is based on encapsulating a single cell in a water-in-oil emulsion droplet, and actively manipulating it using dielectrophoresis.

Wyatt N Vreeland (National Institute of Standards and Technology, MD, USA) described the development of a prototype polymer-based microfluidic system for the rapid, multiplexed analysis of forensic short tandem-repeat DNA samples. The microfluidic system is an eight-channel device fabricated in commercially available plastic. The device can also be employed as a single-use device.

Timothy Leong (Johns Hopkins University) described the parallel self assembly of 200- μm scale perforated, metallic

containers, with arbitrarily patterned faces, made from 2D photolithographically patterned precursors. After self assembly, the containers were filled with hydrogels, biological constituents or spherical beads that could be released by dissolution or agitation. The containers can be tracked in capillaries and microfluidic channels as a result of a radio-frequency shielding effect. Yuan Wen (The Ohio State University) described the design and fabrication of a microfluidic device for tissue engineering using multiple layers of poly(dimethylsiloxane) through photolithography and replica molding. Each layer served as part of a network of microfluidic channels for medium flow, serial drug dilution, mixers and cell culture chambers with the same dimensions as the wells of a 384-well plate. Tissue engineering scaffolds were placed in the chambers for 3D cell cultures on the chip. The microfluidic bio-reactor array was designed to test the effects of six different concentrations of a drug, with controls on two different types of cells in a perfusion 3D culture without interference. The numbers of cell types and drugs for the test can be easily expanded with similar designs.

Bioanalytical capillary electrophoresis & microdevice technology

Rebecca Zangmeister (National Institute of Standards and Technology) and Gloria Thomas (Mississippi State University) chaired the session devoted to the development and improvement of CE and electrophoretic microdevice platforms. The emphasis was on integrated sample preparation, gel formulations, microchannel surface functionalization, secondary separation mechanisms and microdevice design.

David Ross (National Institute of Standards and Technology) opened the session with a talk on the nascent technique of scanning temperature-gradient focusing of proteins. Temperature-gradient focusing is a counter-flow gradient-focusing method that shows promise as a separation tool, but which suffers from low peak capacity (i.e., the number of peaks that can be resolved). Briefly, temperature-gradient focusing

works by balancing the electrophoretic motion of an analyte against the bulk flow of buffer through a microchannel. The method is implemented by scanning the bulk flow rate over time, which sequentially focuses different ranges of analytes. The scanning is accomplished by controlling the pressure applied to the microchannel. Focused analyte peaks are detected as they pass a fixed detection point near the end of the gradient zone, giving a signal–applied pressure plot that is similar to a conventional chromatogram. The advantages of this method over previous implementations are that it gives an increased peak capacity, separations are repeatable and quantitative, and it is compatible with single-point LIF and ultraviolet absorbance detection typically used with CE. In addition, detection limits can be adjusted as needed by changing the scan rate to keep analytes in the focusing window for differing times.

Shane T Grosser (Carnegie Mellon University, PA, USA) described sequence-specific separation of target DNA by micellar electrokinetic chromatography. This is accomplished by attaching hydrophobic probes to the target DNA sequence. Probes consist of peptide nucleic acids appended to alkane tails, forming peptide nucleic acid amphiphiles. The uncharged probes bind DNA oligomers with high sequence specificity, tagging them for separation by either their hindered electrophoretic mobility or their partitioning to surfactant micelles in the running buffer. In the absence of micelles, probe attachment to short DNA oligomers is sufficient to alter the electrophoretic mobility, providing good resolving power between target and nontarget DNA.

Jennifer Lin (Northwestern University) described a method for the CE separation of DNA molecules by means of friction-inducing end labels. The technique provides an alternative and potentially very useful technique for separating DNA fragments according to size, in the absence of a sieving matrix, through the creation of novel bioconjugate moieties. Since no sieving matrix is necessary, the difficulties associated with loading viscous polymer solutions into narrow

microchannels are eliminated. In this technique, each DNA molecule in a sample is covalently modified with a unique frictional modifier or drag-tag that serves to modify the electrophoretic mobility of the DNA in a size-dependent fashion. In addition to DNA sequencing, the method was demonstrated for highly multiplexed mutation detection by single-base extension. Differently sized polypeptoid drag-tags are conjugated to primers specific to target loci. An optimized thermal cycling protocol is used to generate the products, and the differently sized drag-tags allow easy separation of each of the products in free solution in capillaries. Rapid separations were possible in glass microchips, with excellent resolution of the products achieved in less than 30 s.

Victor M Ugaz described the use of microfabricated gel electrophoresis devices incorporating on-chip arrays of electrodes, heaters and temperature sensors to measure diffusion and dispersion of single- and double-stranded DNA fragments in crosslinked polyacrylamide gels. The goal is to quantitatively determine the achievable level of separation resolution directly from measurements of fundamental physical properties associated with the gel matrix. The microdevice format allows a complete set of diffusion and dispersion data to be collected in approximately 1 h, as opposed to experiment times lasting several days using conventional sequencing equipment.

April L Dupre (University of Cincinnati) talked about the development of polymeric microchips for use in binding studies. This group has been investigating various surface-modification chemistries for poly(methylmethacrylate) to increase the electro-osmotic force to levels that are compatible with bioanalytical assays and electrospray MS. They have been examining the use of single-lane microchips as platforms to determine binding constants obtained from various protein–ligand systems.

Christopher P Fredlake (Northwestern University) described work on the development of optimal polymeric separation matrices and wall coatings for

DNA sequencing on microfluidic chips. Dynamically adsorbed polymeric wall coatings are preferred, and acrylamide-based polymers, other than linear polyacrylamide, show potential as high-performance DNA sequencing matrices. Modified acrylamide-based separation matrices, combined with hydrophilic channel-coating acrylamide polymers, achieved DNA sequencing read lengths longer than 540 bases, at 98.5% accuracy, with a total separation time of approximately 8 min, and achieved detection of the 540-base DNA fragment at 4.5 min, in comparison to reported read lengths of greater than 400 bases requiring 18–30 min.

Swomitra Mohanty (University of Wisconsin, WI, USA) described a way to extract DNA from a microchip for subsequent analysis. This is performed by integrating a removable capillary insert as part of the microfluidic platform for easy manipulation at these small volumes. The sample manipulation scheme presented makes products that are analyzed using microchips that are simple to interface with the macro world as well as the micro world. The device integrates a 2D micro-gel electrophoresis device with a removable capillary insert. A microscale device separates DNA based on size and nucleic acid sequence. Separation in one direction is based on the size of denatured DNA, while separation in the other direction is based on renatured secondary structure. The denaturing and renaturing environments are achieved by localized cooling and heating on-chip. One channel is heated locally using a resistive heat pad to create a denaturing environment, the second channel is cooled locally using a chemical cooler to create a renaturing environment for the second separation to occur. The removable capillary insert serves as part of the separation path during the separation process, but is a removable component. The electric field is used not only to perform separations, but also to move biological material into the insert. After migrating into the capillary, the biological material is then removed by the user to be used in post-separation analysis.

Sensing, detection & integration in bioanalytical systems

This session dealt with developments in miniaturized sensing and analytical components for use in biomedical and genomic applications. Many advances have been made in the design of such devices. However, the ability to assemble and interface individual components in order to achieve a high level of integration in a complete working device continues to pose a host of challenges. In this session, aspects of integration, both among components at the microscale and between the microscale device and the macroscale external environment, were examined. The chair was Paul Takhistov (Rutgers University, NJ, USA) and the co-chair was Fred Batrell (Micronics, WA, USA).

Michael W Toepke (University of Illinois) spoke about the use of microfluidic devices for time-resolved spectroscopic studies of protein kinetics. Time-resolved spectroscopy can be achieved in microfluidic devices by activating a sample in a channel and using the length down the channel as time resolution. This talk covered the design, fabrication and operation of microfluidic chips that enable rapid mixing of samples while reducing sample dispersion. The device incorporates a custom calcium fluoride flow cell that is transparent in the infrared region. The results of time-resolved ultraviolet/visible and Fourier transform infrared spectroscopy studies of myoglobin and cytochrome oxidase obtained with these microfluidic chips were discussed.

Justin S Mecomber (University of Cincinnati) described surface modifications to plastic microchip devices. The group utilized nitrine chemistry to chemically modify the surface of various polymeric devices. Modifications enabled enzyme immobilization and improved electro-osmotic flow. The results demonstrate that photofunctionalization provides a simple, spatially controlled approach to microchip functionalization, and these chips facilitate the investigation of on-chip processing and subsequent mass spectral characterization of proteins.

Shramik Sengupta (University of Notre Dame, IN, USA) described a microfluidic device that enables bacterial samples to be loaded, incubated and subsequently exposed to multiple antagonists in parallel. The device enables detection of the presence of small numbers of bacteria (<1000 per ml) and establishment of the viability (or lack thereof) of a bacterial strain within 6 h using a frequency scan technique that allows resolution of the increases in bulk solution capacitance (caused by bacteria) from the surface capacitance and bulk resistances. The device could find use in basic, clinical, food and environmental microbiology.

Yali Gao (University of Toronto, ON, Canada) described an automatic electrokinetically controlled heterogeneous immunoassay with multiple analyte detection capability. Multiple antigens are immobilized on poly(dimethylsiloxane)-coated glass slides. Labeled second antibodies were used to detect antibodies in the test sample.

Andrei G Federov (Georgia Institute of Technology, GA, USA) presented a micromachined ultrasonic electrospray device consisting of three main components: a piezoelectric transducer to generate resonant ultrasonic waves within sample reservoir; a silicon wafer containing cavities that focus and amplify the ultrasonic waves; and a spacer. During operation, the high-pressure gradient close to the nozzle produces the ejection of a droplet stream. The device is interfaced with a TOF mass spectrometer. John F Hassard described a benchtop DNA sequencing instrument based on the company's label-free imaging technology (as described in Proteomics, Part 1).

Environmental & colloidal applications of electrokinetics

Pedro E Arce (Tennessee Technical University, TN, USA) chaired and Mario A Oyander (Universidad Catolica del Norte, Chile) co-chaired a session on electrokinetics. Electrokinetics involves the use of electrical fields and electrical forces (between surfaces and particles) to produce a motion of colloidal particles within a medium. This environment

could be a fluid, porous or fibrous medium. Notable applications include those related to environmental processes such as the decontamination of water or soil, the cleaning of water for drinking purposes, and the decontamination of industrial effluents. Electrostatic aspects of membrane-based separation processes and microfiltration in electrically enhanced processes are other examples of electrokinetic applications. Within this framework, a detailed analysis of particle-to-particle electrostatic forces, the experimental measurements of their magnitude and computer-based simulation approaches are relevant for the advance of processes and technology involving electrokinetics principles.

Minami Yoda (Georgia Institute of Technology) described a novel technique for measuring the zeta potentials near the walls of electrophoresis microchannels. Electro-osmosis, where ions in a conducting fluid are driven by an electric field, is commonly used in microfluidics to drive aqueous electrolyte solutions within the electric double layer next to the charged wall. This talk described nanoparticle image velocimetry with evanescent wave illumination, which can be used to measure velocities and, hence, changes in zeta potential within 250 nm of capillary walls. Comparison of analytical predictions and empirical measurements indicate that the Debye length underestimates the double-layer thickness by up to 1 order of magnitude over a 1000-fold variation in ionic strength.

Jeffrey A Fagan (Carnegie Mellon University) discussed the interesting phenomenon that application of an alternating electric field across a suspension of colloidal particles induces measurable, electrolyte-dependent forces on particles near the electrode, even at relatively high frequencies. The proposed mechanism is an electrohydrodynamic body force in the fluid due to a spatially inhomogeneous polarization of the electrode's double layer, influenced by the presence of the particles. In addition, charged particles in electrolytes with unequal ionic mobilities experience an additional force due to the interaction of a net electric field (from

the asymmetric polarization of the electrode under the particle) with the charge of the particle. This force increased with the magnitude of the electrode's equilibrium zeta potential and was directed towards the electrode for potassium hydroxide electrolyte.

Robert D Tilton (Carnegie Mellon University) described a way to control electro-osmotic fluid flows in gels by embedding negatively charged silica particles in the gels. Gels are a common matrix for biosensors, but hindered transport through the polymeric matrix slows down the response rate of such sensors, thereby limiting the applicability of gel-based sensors for processes with relatively rapid dynamics. These studies provide one mechanism to promote mass transfer in gels.

Osman Basaran (Purdue University) described pinch-off of fluid interfaces in the presence and absence of electric fields. Electric fields are being used or are being contemplated for use in applications as diverse as separations (extraction and distillation), chemical analysis (electrospray MS), manufacture of ceramic particles and polymer beads, and electrospinning of micro/nanofibers. What all of these applications have in common are the formation of finite time singularities and breakup of fluid interfaces. When a fluid interface breaks in the absence of an electric field, the type of singularity that arises is referred to as the pinch-off singularity. Such a singularity occurs when the radius of a thinning liquid filament tends to zero. In an electric field, the interface pinches off and also takes on a conical profile and a fine jet issues from the tip of the cone. Without a field, only the pinch-off occurs.

AT Conlisk (The Ohio State University) described a simulation of electro-osmotic transport in a three-component system in a nanochannel. For electro-osmosis in a channel with negatively charged walls, negatively charged species may move in an opposite direction to the direction of bulk fluid flow. Positive species are transported in the direction of fluid flow with significant decreases in transit time. The transport of negatively charged species in channels with

positively charged walls behaves similarly to the transport of positively charged species within negatively charged walls.

Mario A Oyander described a solution of the Poisson–Boltzmann equation under nonisothermal conditions. Prediction of electro-osmotic velocity profiles is a key factor in the study of electrokinetic processes, such as electro-assisted drug delivery, microelectrophoretic separations, soil remediation and material processing. The analysis is for a generalized case where temperature profiles developed across a rectangular batch cell cannot be neglected in the prediction of electrostatic potentials.

Ryan O O'Hara (Tennessee Technological University) spoke about the use of electrohydrodynamic methods for the uniform coating of metal wires. The study was of the motion of a cylindrical metal wire moving at constant speed within a coating solution inside an annular shell. The study considered Joule heating and, therefore, buoyancy flows, which were accounted for by using a heat-transfer model.

Advances in electrokinetics & electrophoresis

Electrokinetic techniques play leading roles in technologies ranging from nanoparticle characterization and directed electronics assembly, to micropumps and micromixers, to biosensors and DNA sequencing. A session organized by James C Baygents (University of Arizona, AZ, USA) and moderated by Paul Bowers (University of Arizona) was devoted to talks related to the development of new technologies in these areas, from both the fundamental and applied perspectives.

Ronald G Larson (University of Michigan) derived a model for the motion of long DNA chains entangled in a concentrated gel matrix in the presence of a strong electric field. The model is adapted from a tube-based slip-link approach, which was originally intended to model the rheology of entangled polymer fluids, and is suitable for solution by Brownian dynamics simulation. The model accounts for the constraining effect of the surrounding matrix, motion

due to the electric field and finite extensibility of the DNA chain. The model allows the investigation of the effect of molecular weight and field strength on the DNA drift velocity in a constant electric field, along with molecular stretching in an oscillating field. Chain-end motion through the matrix has a significant role in the DNA dynamics, particularly in oscillating fields.

Patrick Doyle (Massachusetts Institute of Technology, MA, USA) presented single-molecule studies of DNA molecules driven by an electric field through a microfabricated constriction. The electric field gradient that is generated is purely elongational in its effect on DNA, unlike with hydrodynamic flows that can lead to DNA chain tumbling and incomplete stretching.

Satish Kumar (University of Minnesota) used Brownian dynamics simulations to characterize the timescales involved in polymer electrophoresis through narrow constrictions. The polymer is modeled as a freely jointed bead–rod chain with a total charge distributed uniformly among the beads. The polymer is initially placed in a thick region, and an applied electric field drives it into the next thick region through the intervening narrow constriction. Upon reaching the entrance of the thin region, the polymer is entropically trapped. After the activation event, the polymer moves through the thin region and into the next thick region. The transit velocity of the polymer first increases with increasing chain length, and then decreases beyond a certain value of chain length. The work has relevance to the transport of polymers in microfluidic channels.

Jennifer S Lin applied drag-tags to DNA sequencing. Drag tags are genetically engineered protein polymers that are covalently attached to the primer molecules used to prime DNA synthesis. They modify the electrophoretic mobility of DNA molecules in a size-dependent fashion, a property that can be exploited in sequencing (see Bioanalytical CE and microdevice technology). Jeffrey A Fagan discussed observations of net interparticle motion above planar electrodes due to alternating electric fields. At frequencies

in the 100-Hz range, results suggest that electrode reactions are the root cause both of net lateral motions (including both aggregative or separating motions) and net vertical particle motion.

Xin Hu (The Ohio State University) presented work on microfluidic flow patterns in a single, fractal design, microfluidic network without surface charge patterning on the channel walls. The observed flow patterns of buffer solutions containing charged polystyrene microspheres and λ -DNA molecules is in strong agreement with simulation results.

James D Hoggard (Carnegie Mellon University) described a method for measuring the zeta potential of discs. Combining the hydrodynamic properties of a rotating disc, the solution of Laplace's equation for the potential and the electrokinetic boundary condition, an equation relating the zeta potential of the disc to the streaming potential is obtained. Theory predicts that the streaming potential is proportional to the rotation rate raised to the power of 3/2. Placing an electrode near the disc surface and a reference electrode at infinity enabled the streaming potential measurements. The experimental results agree well with the theory for silicon oxide discs in dilute potassium chloride solutions.

Posters

A wide variety of topics were on display in the 14 posters presented at this meeting. Topics ranged from mathematical solutions of the Poisson–Boltzmann equation for various systems to electrophoretic devices for analysis of chemical compounds, proteins and DNA, to new configurations for gel electrophoresis. Prizes were given to student contributors (first and second prizes and three honorable mentions). The first prize went to Marvi Matos of Carnegie Mellon University for studies on the incorporation of charged nanoparticles in gels to control electro-osmotic flow.

Once again, the synergy between biologists and chemical engineers engaged in electrophoresis produced a technical meeting of wide scope and relevance that was both practical and stimulating for attendees. Capsule summaries of all presentations

at the meeting are given in this review. Individual talks can be accessed in somewhat greater detail by visiting the abstracts on the AIChE website [1].

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Websites

- 1 American Institute of Chemical Engineers
www.aiche.org
- 2 American Electrophoresis Society
www.aesociety.org

- 3 Argonne National Laboratory Protein Mapping Group: GelBank
<http://GelBank.anl.gov>

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