Electrophoresis in 2004: The Annual Meeting of the American Electrophoresis Society

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Proteomics owes much to electrophoresis and vice versa. In fact, proteomics was built around 2D gel electrophoresis (2DE) with its maps of large protein ensembles. The notion that multiple proteins can be visualized and identified in parallel grew from looking at hundreds to thousands of proteins arrayed in a single gel entity. On the other hand, proteomics has spurred renewed interest in advances and improvements in electrophoretic methodologies. New gel-based methods and capillary and microchip electrophoresis technologies address the developing needs of proteomic analysis. Proteomic applications are a natural extension of genomic research, where electrophoresis also plays a vital role.

These concepts were in evidence at the 2004 Annual Meeting of the American Electrophoresis Society (AES). Annelise E Barron of Northwestern University organized the meeting. It was held at the Convention Center in Austin (TX, USA), from November 7 through November 11, 2004, as a series of topical sessions within the larger annual meeting of the American Institute of Chemical Engineers (AIChE). Abstracts of the talks can be found as topical session D by scrolling through the Conferences link on the AIChE website [101]. Two jointly sponsored bioinformatics sessions were sessions 10 (T2003) and 12 (T2004) of topical session 2.

The AES is dedicated to the theoretical and practical development of electrophoretic and electrokinetic technologies including those using gels, microfluidic chips and capillaries. The society was formed in 1973 to promote excellence in science and techniques, cross disciplines and facilitate collaboration and communication between members. No other US organization is more focused on the important field of electrophoresis. Information about the AES can be found on the group's website [102]. The association between AES and AIChE has been fruitful and synergistic, fostering an interdisciplinary approach toward the refinement of electrophoretic methods.

The AES/AIChE joint meeting consisted of 11 formal sessions extending over 4 days, a poster session and a workshop on 2DE. The themes of the formal sessions were proteomics, bioinformatics, microfluidics and electrokinetics. A total of 68 formal presentations were made at the meeting. These presentations as well as the workshop and poster session are summarized in this report.

Workshop

Practical aspects of 2DE

Prior to the start of the formal sessions, Nancy Kendrick (Kendrick Labs, WI, USA), the Immediate Past President of AES, organized another of her popular workshops on 2DE. Kendrick's workshops have been a highlight of previous meetings. Scott Rodkey (University of Texas-Houston Medical School, TX, USA) opened the workshop with an overview of the 2DE method and a discussion of the chemistry and uses of carrier ampholytes that continue to be important constituents of isoelectric focusing systems, even in the era of immobilized pH gradients. In recognition that the success of any 2DE run starts right at the beginning with sample preparation, Nancy Kendrick and Gary Smejkal (Proteome Systems, MA, USA) gave two separate talks about this ever-important topic. Every protein sample is different and has its own nuances, but the scientific community is constantly perfecting general methods of sample preparation. 2DE gel images are commonly analyzed by use of any of several software packages available for this purpose. The various software packages differ subtly in their algorithms and coding, but they all perform the requisite functions starting with spot identification and continuing through the tests for statistical significance of identified spots. Marcia Goldfarb (Anatek-EP, ME, USA), Paula Maia (Syngene, MD, USA) and Erich Gombocz (IO Informatics, CA, USA) highlighted the features of three different 2DE analysis packages. Frank Witzmann (University of Indiana, IN, USA) rounded off the presentations by describing his work on the use of 2DE methods in toxicology and by discussing some of the problems associated with bioinformatics when dealing with large data sets of gel images. A question and answer session with the speakers, chaired by David Garfin (AES), concluded the workshop.

Plenary session

The meeting included two well-attended plenary talks. Ronald Pethig (University of Wales, Bangor, UK) spoke about dielectrophoresis, one of the more intriguing recent developments in electrophoretic separations technology, and Walter Blackstock (University of Sheffield, UK) discussed the current status of proteomic research. The plenary session was organized by Chair Annelise E Barron (Northwestern University, IL, USA) and Vice Chair Kenneth F Reardon (Colorado State University, CO, USA).

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Microphysiometry systems based on microdielectrophoresis

In his talk, Pethig described the principles and applications of dielectrophoresis. The phenomenon has been known for many years, but has only recently been used with any regularity. Dielectrophoresis is defined as the motion imparted to uncharged particles as a result of polarization induced by nonuniform electric fields. With dielectrophoresis, alternating current (ac) fields are used and particle motion depends on a particle's polarization characteristics. Audio, radio and microwave frequencies have been used for dielectrophoresis. The dielectrophoretic force on a particle is a function of its (field-dependent) dipole moment and the presence of a gradient in the electric field (such as between asymmetric electrodes). The ac electric field induces a dipole moment in the particle, which, in the field gradient, experiences a force toward either the high-intensity field region or the low-intensity region depending on the polarization characteristics of the particle. Dielectrophresis can be scaled for the manipulation of nanosized particles such as colloidal particles, cells, bacteria, viruses and macromolecules, and lends itself to microfluidic technologies for electrode design (field gradient), flow pattern (separation) and detection (microscope). By means of control of the medium, the frequency of the ac field and the field gradient, particles can be forced to move in either direction, stand still or even rotate. This talk, along with others at the meeting, indicate that dielectrophoresis may move toward the forefront of electrophoretic separation methods in the not-too-distant future.

Towards a cell map

Blackstock shared his view that proteomics is trying to be too global and researchers should match expectations to reality. In other words, presently used techniques work best when applied to simple pathways. The field of proteomics can be divided into two disciplines: expression and functional proteomics. Expression proteomics, as the name implies, deals with changes in expression between differing biologic states. This was the original paradigm for proteomics and makes use of tools such as 2D gels, image analysis and cluster analysis. The pharmaceutical industry has lost interest in the 2D gel approach and considers it to be too low tech and too labor intensive. Furthermore, this approach really targets high-abundance proteins that make up as little as approximately 13% of a proteome, whereas proteins defined as being moderately abundant constitute approximately 41% of a proteome with the so-called low-abundance proteins making up the remaining 46%. Moderate- and low-abundance proteins are generally the most interesting. On the other hand, functional proteomics focuses on protein–protein complexes and their role in biologic processes. Assemblies of ten or more protein molecules carry out the major processes in cells. An aim of functional proteomics is the isolation of intact assemblies for direct investigation of the associated chemical pathways. Isolation procedures often include affinity methods, particularly immunoaffinity methods. One lesson to be remembered from this talk was that proteomics is still defining itself. Gel-based methods remain valuable in the discovery phase of a proteomic project, with other fractionation techniques applicable during more targeted research.

Proteomics

Frontiers in proteomics

This session, chaired by Thomas Kodadek (University of Texas Southwestern Medical Center, TX, USA) and George Georgiou (University of Texas, TX, USA), focused on novel technologies for the isolation of protein-binding reagents. Investigators in proteomics agree that high-affinity protein ligands such as antibodies are extremely valuable tools for the construction of protein-detecting microarrays and many other applications, but their production is currently too slow and expensive. In separate lectures, Thomas Kodadek and Shuwei Li (University of Texas Southwestern Medical Center, TX, USA) described efforts to create and screen combinatorial libraries of peptoids (substituted glycines) for high-affinity protein ligands. It was shown that lead compounds with low micromolar equilibrium dissociation constants are easily isolated from such libraries. A novel technique to rapidly mature these lead compounds into ligands with low nanomolar affinities was described and a new approach to the creation of peptoid and peptide microarrays using digital photolithography was also discussed. Ki Jun Jeong (University of Texas, TX, USA) described a different approach to the isolation of high-affinity ligands for proteins or small molecules. This is a two-hybrid-type platform that allows libraries of antibodies or other proteins to be displayed on the surfaces of bacterial cells and sorted for their ability to bind a fluorescent target molecule. Patrick Dougherty (University of California, CA, USA) described an adaptation of this approach to screening peptide libraries that resulted in the isolation of several high-affinity peptide–protein complexes. Mark J Dickman (University of Sheffield, UK) discussed the development of open-capillary affinity chromatography platforms for the rapid and sensitive analysis of protein–protein interactions. Taken together, these presentations nicely illustrated advances in technology dedicated to manipulating and analyzing the proteome in a high-throughput fashion.

New methods for proteome analysis

Kenneth F Reardon and Phillip C Wright (University of Sheffield, UK) cochaired this session in which 2DE and analysis of proteins derived from gels were the main topics of discussion. Christina Bondy (University of Wisconsin, WI, USA) described efforts aimed at understanding and improving the reproducibility of polyacrylamide gel electrophoresis and Marcia Goldfarb presented details of software for 2DE analysis. Michimasa Kishimoto (Osaka University, Japan) described a new algorithm for the analysis of the time courses of silver staining of 2DE gels. The approach described a statistical analysis of spots as they appear during silver development that allows spot overlaps to be accounted for and low-abundance proteins to be identified

more reliably than from a completely developed silver-stained gel. The algorithm was tested with gels run using proteins extracted from human liver cancer cells. Xunbao Duan (Massachusetts General Hospital, MA, USA) presented data on the depletion of albumin and immunoglobulin G from serum with antibodies and Protein A bound to agarose beads. Removal of the two high-abundance proteins made some previously unseen low-abundance proteins visible in 2DE gels. Next, Scott Rodkey described his process for the synthesis of high-resolution carrier ampholytes. Synthesis of carrier ampholytes involves coupling of polyamines with unsaturated compounds via Michael addition reactions. Purification of polyamine reagents, such as pentaethylenehexamine, prior to reaction with the organic acid component of carrier ampholytes provides accurate control of synthesis, eliminating lot-to-lot variability. Martin E Barrios-Llerena (University of Sheffield, UK) reported on studies of *Cyanobacterial* proteomes by both 2DE and mass spectrometry (MS) and multidimensional liquid chromatography (LC) tandem MS (MS/MS). In this study, the protein identification outcomes for sequenced and unsequenced species were compared. The 2DE-based analysis worked better for identifying new proteins than the chromatographic method when the genome of the organism was not sequenced. Conversely, multidimensional LC/MS/MS outperformed 2DE for protein identification with the sequenced organism. Thus, no single experimental technique was sufficient for a complete proteome analysis. Kelvin H Lee (Cornell University, NY, USA) presented work on the use of isobaric tags (stable isotope labels) in a shotgun proteomics approach for the study of translation in *Escherichia coli* and *Pseudomonas syringae*. These isobaric tags rely on MS/MS analysis for both the identification and quantification of protein expression. Nancy Kendrick closed the session with a provocative discussion on the effect of post-translational modifications (PTMs) on peptide mass fingerprinting (PMF). Experience with proteins from many different organisms demonstrates that the success of PMF in identifying proteins depends in part on the species from which the proteins were obtained. The data suggest that PTMs inhibit both in-gel staining and PMF (by matrix-assisted laser desorption/ionization; MALDI) and that the extent and types of PTMs influence protein identification.

Biomedical applications of proteomic technologies

Alex Kurosky and Larry Denner (both of The University of Texas Medical Branch, TX, USA) cochaired the session on Biomedical Applications of Proteomic Techniques. Xunbao Duan (Massachusetts General Hospital, MA, USA) presented results of a proteomic study on skeletal muscle wasting induced by burns and sepsis. A rat model system was used to mimic sepsis in burn patients. 2DE with PMF by MALDI time-of-flight (TOF) identified seven proteins in the pH 5–8 range that are differentially regulated following burn injury. In addition, the levels of several nonessential amino acids in the muscle were measured by high-performance liquid chromatography (HPLC). The results demonstrated that multiple therapeutic approaches (amino acid plus antioxidant supplementation and heat shock therapy) may be needed in the treatment of muscle wasting in patients with severe burn injury. Xin-Ming Li discussed proteomic studies being carried out at the Fox Chase Cancer Center (PA, USA) to identify protein expression patterns and signaling pathways that were altered in response to imatinib mesylate. Imatinib mesylate, a serine transferase inhibitor that is effective in the treatment of chronic myelogenous leukemia, is in clinical trials to determine its efficacy and safety in patients with ovarian or primary peritoneal carcinoma. Through use of 2DE gels with overlapping pH ranges from 4 to 11, more than 2000 protein spots from cancer cell cultures could be analyzed reproducibly. Expression levels of more than 900 proteins that changed between two- and 34-fold during imatinib mesylate treatment were targeted for study. John Wiktorowic (National Cancer Institute) presented his design for a capillary chip device in which protein separations mimicking 2DE can be accomplished. The device is designed for fluorescently labeled proteins. First, dimension electrophoresis is by nongel polymer sieving and the second dimension is isoelectric focusing. Thus, molecular weight fractionation is performed first followed by isoelectric point separation with the switch between the two dimensions carried out automatically. The advantage of running the focusing dimension as the second step is that proteins are held in place for extended periods, allowing for long signal collection times and thus high signal-to-noise ratios. Erin J Finehout (Cornell University, NY, USA) presented results of a proteomic study of the cerebrospinal fluid (CSF) from patients with Alzheimer's disease compared with CSF from normal patients and patients with other neurodegenerative disorders. By means of 2DE and PMF (MALDI-TOF/TOF), 82 different proteins were found. These proteins will be used to search for proteins that can form a basis for simple diagnostic methods. Frank Witzmann (Indiana University, IN, USA) presented a *tour de force* study of protein changes in a rat model of alcoholism in which 60 large-format 2D gels are used per run and also includes an LC/MS/MS shotgun approach. Rats susceptible to alcoholism were given access to ethanol and compared with an alcohol-naive control group. Proteins from five distinct brain regions were compared using sophisticated statistical techniques. The data suggests that ethanol produces significant changes in highly abundant proteins in three limbic regions involved in regulating alcohol drinking.

Microfluidics

Microfluidic chips for biomedical analysis

This session was chaired by Adrienne R Minerick (Mississippi State University, MS, USA) and Rebecca A Zangmeister (National Institute of Standards and Technology). Paschalis Alexandridis (University of Buffalo, NY, USA) opened

the session with an investigation of the influence of dielectrophoretic forces on the transport of biologically infectious agents, such as the vesicular stomatitis virus, from dilute solutions onto arrays of microelectrodes decorated with polylysine. A complementary trapping approach, suitable for micron-sized bacteria species, was presented by Jayne Wu (University of Tennessee, TN, USA). Wu discussed the development of a new strategy of using long-range ac electroosmotic flow to collect and concentrate bacteria along the stagnation line of an electrode resulting in an increase in concentration by four orders of magnitude relative to the bulk. Gloria Thomas (Mississippi State University, MS, USA) described the immobilization of biomolecules by physical entrapment in polyacrylamide hydrogels in microfluidic channels as applied to the avian lymphoma/CD30 cancer model. The polymeric structures provide a conceptually simple alternative to extensive surface chemistry and (or) streptavidin and biotin linkages often used to immobilize biologic molecules in microfluidic systems. Michael Roper (University of Virginia, VA, USA) discussed the development of a multidimensional separation method on a microfluidic platform that enabled determination of the amount of C-reactive protein in the serum of patients who have undergone hip replacement surgery. Heather Pridemore (Tennessee Tech University, TN, USA) discussed microchannel geometry in relation to the behavior of the transport of macromolecules in nanostructured gel materials. Matthew Kerby (Brown University, RI, USA) spoke on the development of an integrated microfluidic device to measure binding of polymer microspheres targeted to a ligand-coated wall under controlled Hele Shaw flow theory shear conditions. Christa Hestekin (Northwestern University, IL, USA) presented successful detection results of microchip electrophoresis-based tandem single-stranded (ss) conformational polymorphism/heteroduplex analysis of single-base mutations in the p53 gene with separation times of under 5 min. Mark Burns (University of Michigan, MI, USA) closed the session with a presentation describing an advanced integrated microfluidic lab-on-a-chip device. The device (measuring 1 cm on a side) performs two biochemical reactions in series followed by electrophoresis, enabling it to perform genetic analysis, such as distinguishing between three influenza A subtype genomes, in a few minutes. The implementation of such microfluidic devices could significantly speed detection of disease outbreaks.

Microfluidic chips for proteome analysis

Development of microfluidic chips for proteome analysis is still in the nascent phase. Many designs are scaled-down versions of capillary systems. Thus, much effort is directed toward ways to improve resolution and (or) detection sensitivity. A session organized by Andrea Chow (Caliper Technologies Corp., CA, USA) and Steve Jacobson (Indiana University, IN, USA) dealt largely with resolution and sensitivity issues. Joshua I Molho (Caliper Life Sciences, CA, USA) described improved protein-sizing sensitivity in the Caliper system through integration of an isotachophoresis preconcentration step with sodium dodecyl sulfate (SDS) gel electrophoresis. The switch between isotachophoresis preconcentration and zone electrophoresis is accomplished in a single, integrated channel network using only control of pressures and voltages at the chip wells. Hamed Shadpour (Louisiana State University, LA, USA) showed a system that couples SDS capillary gel electrophoresis with micellar electrokinetic chromatography for 2D protein separations in poly(methyl methacrylate) (PMMA) microchips. Size discrimination in the SDS mode is accomplished by nongel polymer sieving. The separation mechanism in the micellar mode is based on differences in the distribution coefficient between the micellar and the nonmicellar (aqueous) phases. Detection is possible by laser-induced fluorescence, with runs taking just a few minutes. Brian Kirby presented work from Sandia National Laboratories (CA, USA) on integrating microfluidic HPLC and mass spectrometer sample injection. The device shown was fabricated in fused silica wafers. All flows in the device are controlled by high-pressure microvalves. Yanou Yang (Cornell University, NY, USA) described a simple polymer electrospray emitter source for coupling a polymer-based microchip to a mass spectrometer. This microchip was employed for analysis of methylphenidate extracted from human urine samples and also for solid-phase extraction and preconcentration prior to injection. Gloria Olivier (Brown University, RI, USA) presented a systematic study of the electrophoretic migration of SDS–protein complexes in dilute polymer solutions in microchips. Electrophoretic mobility was found to depend on the concentration of polymer and SDS, but apparently not on the molecular weight of the polymer. In analogy with gel electrophoresis, mobility was found to decrease logarithmically with the molecular weight of the protein and was independent of the electric field in the separation channel. The results suggested that the denatured protein molecules migrate as rigid rod-like molecules.

Genomic assays in microchannel electrophoresis systems

Cochairs for this session were Victor M Ugaz (Texas A&M University, TX, USA) and Annelise E Barron. The presentations explored ongoing efforts to develop microchannel electrophoresis systems capable of performing DNA separations in low-power, portable formats. Research in these areas is playing an important role in the drive to meet future societal needs for increased throughput of genomic analysis at greatly reduced cost (e.g., the US$1000 genome). Discussions dealt with device fabrication, advanced gel matrices, design of novel assays, fundamentals of DNA migration and the influence of operational parameters on separation performance. Steven Soper (Louisiana State University, LA, USA) described work in his group aimed at developing polymer-based microchips for high-efficiency DNA separations with applications in both DNA sequencing and molecular diagnostics. A family of microchannel designs was constructed in PMMA substrates, with detection

accomplished by interfacing the chips with a near-infrared (IR) laser scanning microscope. Using a 4% linear polyacrylamide matrix, this system enabled separation of ligase detection reaction products in approximately a tenth the time of conventional capillary gel electrophoresis. The effects of surface treatments to control electroosmotic flow in order to optimize performance in DNA sequencing applications were also discussed. These systems show great promise as polymer-based multichannel chips for high-throughput DNA sequencing. In the following talk, cochair Annelise Barron expanded on the high-throughput sequencing theme by exploring a series of thermoreversible sieving matrices developed in her research group for use in high-performance DNA electrophoresis applications. Two classes of materials based on N-substituted acrylamides were investigated. Linear copolymers of *N*,*N*-diethylacrylamide and *N*,*N*-dimethylacrylamide incorporating a tailored lower critical solution temperature type volume phase transition, and linear copolymers of *N*-ethoxyethylacrylamide and *N*-methoxyethylacylamide exhibited interesting re-entrant-type volume-phase transition behavior. Read lengths of approximately 600 bases were demonstrated using both matrices. The dramatic thermally induced reduction in viscosity achievable in these systems makes them ideally suited as replaceable matrices for use in both automated DNA sequencers and electrophoresis microchips. Rebecca Zangmeister continued to focus on sieving gels by describing a novel method for immobilizing ssDNA probe molecules within polyacrylamide hydrogel plugs. A photopolymerization process allows these gel plugs to be precisely positioned within plastic microfluidic channel networks. Hybridization assays can then be easily performed by electrophoretically transporting fluorescently labeled ssDNA targets through the hydrogel plugs where they subsequently bind with the complementary immobilized ssDNA probes. Reproducibility, stringency and hybridization efficiency of the assay were explored. Rongsheng Lin (University of Michigan, MI, USA) described a microchip device incorporating arrays of individually addressable electrodes with the capability of extracting a migrating band from an electrophoresis gel. Using a specially designed side channel, any band of interest can be extracted from the middle of the gel rather than at the end and collected for subsequent analysis. The influences of various parameters, including channel geometry and microelectrode design, were explored, both experimentally and with simulations, to optimize the electrical field in the vicinity of the extraction region so that dispersion and leakage of neighboring DNA bands could be minimized. On a more fundamental note, Nancy Stellwagen (University of Iowa, IA, USA) presented research exploring the free solution mobility of a series of ssDNA oligomers measured by capillary electrophoresis in potassium diethylmalonate buffer. Although the measured mobilities exhibited strong sequence dependence, a correlation was found with the calculated free energy of melting of the most stable hairpin or homodimer form of each oligomer. This suggests that ss oligomers in free solution exist as an equilibrium mixture of hairpins, homodimers and/or random coils in rapid exchange, giving rise to an average mobility reflecting the relative proportion of each conformation in the mixture. The results of these studies are important in order to accurately interpret the results of oligomer separations. Shengnian Wang (The Ohio State University, OH, USA) presented an investigation of the conformation of double-stranded DNA during electrokinetic transport through various nanofluidic channel geometries. Nanochannels incorporating converging and diverging flow paths were fabricated using electron beam lithography techniques, and both steady-state and transient dynamics of DNA conformation were investigated. These studies are relevant to the design of optimized nanochannel geometries for use in a variety of microchip-based DNA analysis applications. Finally, Zheng Chen (University of Michigan, MI, USA) presented work exploring novel strategies to circumvent problems associated with ion depletion due to the limited buffer reservoir volumes available in many electrophoresis microchips. Often, these ion depletion problems cause mobility shifts that compromise separation performance. These issues were investigated by interfacing an electrophoresis microchip with a syringe pump to continuously deliver fresh running buffer into the separation channel during electrophoresis. The influence of operational parameters such as buffer flow rate and location of the on-chip electrodes within the buffer flow path were studied. The results of these studies may lead to enhancement of microdevice separation performance.

Electrophoretic biomolecule analysis on microfluidic platforms

Although microfluidic systems in their current forms are not capable of the resolution necessary to be truly useful during the discovery phase of proteomic studies, they have great potential when it comes to targeted research, quality control procedures and diagnostics. Thus, the session cochaired by Steven Soper (Louisiana State University, LA, USA) and Don Devoe (University of Maryland, MD, USA) on electrophoretic analysis of biomolecules on microfluidic platforms was timely and informative. Charles S Henry (Colorado State University, CO, USA) described a microchip device capable of detecting carbohydrates, amines and sulfur-containing compounds simultaneously. The device is a capillary electrophoresis/electrochemistry microchip that uses pulsed amperometric detection, the only method that can simultaneously detect the three categories of biomolecules without derivatization. The examples shown for this device were the screening for markers of renal function and the screening for carbohydrates in various biologic samples. Ying-Xin Wang (University of Maryland, MD, USA) demonstrated a microfluidic system combining capillary electrophoresis for separations of protein mixtures followed by electrospray deposition onto MALDI target plates, with offline MALDI-MS analysis of the deposited samples. This approach avoids inefficiencies associated with the differing and incompatible time

scales of capillary separation and electrospray ionization (ESI)/MS and provides the advantages of MALDI, such as sample archiving. Thomas N Chiesl (Northwestern University, IL, USA) presented further developments in ongoing studies of hydrophobically modified polyacrylamide matrices that selectively remove proteins and lipids from cellular lysates while allowing the rapid passage of DNA for subsequent analysis. The matrices selectively remove proteins and lipids from DNA during microchip and capillary electrophoresis. The inclusion of hydrophobic subunits into a polymer matrix for electrophoresis can lead to substantial and irreversible protein and lipid adsorption, due to hydrophobic interactions. DNA molecules, which are highly charged and hydrophilic, pass rapidly through the matrices by electrophoresis without being adsorbed, and are separated according to DNA size. The matrices are a family of water-soluble block copolymers of acrylamide and N-alkyl acrylamides. The polymer composition reported includes dihexyl- or octylacrylamide (C6-6 and C8, respectively) hydrophobic monomers. A second family of related copolymers consisted of combinations of hydrophilic acrylamide units and hydrophobic alkyl acrylamide subunits crosslinked via bisacrylamide. Li Zhu (Louisiana State University, LA, USA) described a microchip DNA sequencing device that scans in the near-IR (680 and 780 nm) and that has both dual-color and time-resolved fluorescence capabilities for highly multiplexed genomic assays from microchips. This microscope consists of two pulsed diode lasers that are coupled to the microscope head using optical fibers. The multichannel (16 independent channels) PMMA-based microchip was designed for DNA sequencing applications. The separation channels are configured in a radial design with a crossed-T injector and a common anodic reservoir. The electrophoretic separation of standard M13 sequencing ladders in the microchip with linear polyacrylamide as the polymer-sieving matrix was shown, as were results on sequencing DNA templates using both steady-state (color) and time-resolved (lifetime) discrimination techniques. Victor Ugaz studied DNA electrophoresis in photopolymerized, crosslinked polyacrylamide gels in microfluidic flow networks. The study presented was designed to obtain detailed understanding of mobility, diffusion and dispersion phenomena in the gel. The microdevice format allowed 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. Key factors governing separation performance were identified and the results were compared with biased reptation theory to extract information about the gel structure and predict achievable resolution. The crosslinked polyacrylamide gels offered good separation resolution at relatively low electric field strengths (10–20 V/cm), making them well suited for use in portable microdevice-based applications. Yue Kuo (Texas A&M University, TX, USA) reported a novel protein identification microsystem that uses an amorphous silicon thin-film transistor to drive a new type of microchannel electrophoresis device. This is a low-voltage microchannel device that can simultaneously separate and identify proteins. Proteins are identified by measuring the current versus time curve, which is then translated into mobilities. This *in situ* method requires no stains or optical scanning procedures. The solid-state transistor functions as a current regulator that suppresses current perturbations during operation, which can affect signal discrimination.

Bioinformatics

As used in this article, bioinformatics refers to the full spectrum of activities involved in the interpretation of biologic data, and includes the collection, analysis and storage of biologic information.

Bioinformatics & functional genomics III: focus on transcriptomics

Functional genomic approaches seek to identify gene markers for disease states. Christina Chan (Michigan State University, MI, USA) and Enoch Huang (Pfizer, MA, USA) organized a session on bioinformatics approaches to studies of transcriptomes. The work reported in this session made use of microarrays rather than electrophoretic techniques, but nonetheless represents an important step toward the proteomics of several systems. R Michael Raab (Massachusetts Institute of Technology, MA, USA) described transcriptional studies on hepatic insulin resistance in mice. The work focused on discovering new genes involved in hepatic function. Genes were sought that contribute to the underlying regulatory mechanisms that go awry during the insulin-resistant state, when hepatic glucose production is not repressed despite elevated levels of blood glucose and insulin. DNA microarrays containing 17,000 unique gene probes were used to monitor hepatic gene transcription under normal, insulin-resistant and fasting states in C57 mice. From these data, approximately 40 different genes that are highly discriminatory of the treatment groups were identified. The genes were studied with RNA interference gene silencing to examine their functions. Arul Jayaraman (Texas A&M University, TX, USA) spoke about transcriptional profiling of cytokine interactions in hepatic inflammation. DNA microarrays were employed to study two inflammatory mediators produced in the liver after injury, interleukin (IL)-1b and IL-6. The data suggest that IL-1b has a more pronounced effect on hepatocyte gene expression than IL-6 and plays a dominant role in initiating inflammatory response following systemic injury. Microarrays were also the tool of choice in the work presented by Kunal Aggarwal (Cornell University, NY, USA) on the relationship between mRNA and protein expression profiles in *E. coli*. The procedure consisted of measuring mRNA synthesis in response to induction of expression of a key ribosomal protein. Changes in the expression of mRNAs corresponded to different 50S and 30S ribosomal subunit proteins. A preliminary proteomic study of ribosomal proteins was also conducted that indicates an apparent lack of correlation between mRNA and protein levels. Laura R Jarboe (University of California, CA, USA)

demonstrated the use of Network Component Analysis to reconstruct transcription factor activity from DNA microarray data. This type of analysis should further the understanding of the roles of transcription factors in both the regulation of target genes and interactions with other transcription factors. Jeremy S Edwards (University of Delaware, DE, USA) described a novel method being developed for analyzing thousands of genes on a single chip in order to develop a precise, cost-effective assay for whole-transcriptome analysis. This method is expected to augment other techniques that have limitations in terms of costs, quality and throughput. Correlations between various methods for estimating protein expression levels are only fair and were dependent on the methods themselves. Enrique T Munoz (Rice University, TX, USA) reported on an investigation into systematic biases in the estimation of gene expression rates from microarray data and from abundance within the Expressed Sequence Tag (EST) database. In particular, the investigation addressed whether the average protein length in *Caenorhabditis elegans* increases or decreases with expression level. It was suggested that a length bias exists primarily in the abundance-within-the-EST-database method, not being ameliorated by internal standards as in the microarray data. When this bias is eliminated, both microarray and abundance-within-the-EST-database give a monotonic decrease of spliced length with expression level and the correlation between the EST and microarray data improves. Joon Chong Yee (University of Minnesota, MN, USA) spoke about a large-scale project to functionally annotate expressed sequence tags from Chinese hamster ovary (CHO) cells. Over 14,000 transcripts were isolated and sequenced and a microarray for large-scale transcription profiling was constructed. A high degree of similarity was found with mouse ESTs. To refine the quality of annotation, CHO ESTs were translated into amino acid sequences, aligned with protein databases and mapped to mouse chromosome sites. In a parallel study, transcription in perturbed CHO and mouse hybridomas were compared. Orthologous genes in both cell types with similar expression profiles were identified by cluster analysis. The comparative approach against the better annotated mouse sequences generates novel insight into the function of CHO transcripts and should facilitate engineering CHO cells for better productivity and product quality.

Bioinformatics & functional genomics IV: focus on proteomics

The session was chaired by Kenneth Reardon and cochaired by Alfred Gaertner (Genencor, CA, USA). James R Swartz (Stanford University, CA, USA) spoke on cell-free preparations of protein libraries. Swartz showcased a technology to produce biocatalysts, polymers and proteins through cell free synthesis systems. This presentation was followed by a talk by A James Link (Tirrell Laboratory, CA, USA) on the use of azidohomoalanine for specific labeling of proteins, thereby enabling separation and tracking of localized protein expression activity (neurons in this case). Dacheng Ren (Cornell University, NY, USA) presented a proteomic study of the *P. syringae* Type III secretion system outlining a differential expression study using 2DE and MS/MS. Ren showed that quorum sensing plays a role in pathogenesis. Phillip C Wright presented work on quantitative proteomics of the archeon *Sulfolobus solfataricus* in which both 2DE and multidimensional LC were used along with ^{15}N metabolic labeling. Carla MR Lacerda (Colorado State University, CO, USA) highlighted a proteomic study that identified physiologic changes in an *E. coli* that had been metabolically engineered for enhanced trichloroethylene (TCE) degradation. Expression of a monooxygenase and exposure to TCE caused a variety of other proteins to be differentially expressed compared with the wild type. MP Nandakumar (University of Maryland, MD, USA) presented a proteome analysis of *E. coli* cultures under conditions that are relevant in large-scale bioprocesses. In addition, Nandakumar showed that deliberate deviations from steady state in feed batch fermentations could lead to higher protein expression. The session concluded with a clinical proteomics study by Patricia A Gonzales (University of Maryland, MD, USA) demonstrating a noninvasive technique to detect renal diseases. The goal was to study biomarkers in urine using antibody assays in microarray format. In summary, this session spanned a variety of topics including protein expression in cell-free, eukaryotic and prokaryotic systems, and subsequent characterization using proteomic technologies.

Electrokinetics

Electrokinetics & microfluidics

Electrokinetics is the keystone concept of electrophoresis. The motions of colloids and other particles in both direct current and ac electric fields are studied in this discipline. James C Baygents (The University of Arizona, AZ, USA) and Darrell Velegol (Pennsylvania State University, PA, USA) organized a session on electrokinetics and microfluidics. Jeffrey A Fagan (Carnegie Mellon University, PA, USA) presented studies aimed at understanding the motion of colloidal particles in alternating electric fields near electrodes. This information can be of particular interest for a variety of applications, including biosensors and optical devices. Using total internal reflection microscopy, the motions of single particles were explored in three different electrolytes, nitric acid, sodium bicarbonate and potassium hydroxide, over 3 decades in frequency from 10 Hz to 10 kHz. The normal motion of a particle is dependent on the electrolyte and both the frequency and magnitude of the electric field. The results suggested that the driving mechanism for net particle motion is different for frequencies below and above several hundred Hertz. Matthew Preston (Carnegie Mellon University, PA, USA) discussed a similar topic, the mathematics of high-frequency colloidal electrokinetics. The numeric procedure used is appropriate for the solution of the standard equations of electrokinetics that are accurate over a frequency range from 1 Hz to over 100 GHz. It is applicable to a greater variety of nonaqueous as well as

aqueous colloidal systems and a wider frequency range than was previously available. Kellie M Smith (Mississippi State University, MS, USA) described studies of the dielectrophoresis of red blood cells. Red blood cells (erythrocytes) are ideal candidates for dielectrophoretic characterization as they have negatively charged membranes and highly conductive interiors. Due to the polarizability of cells, each species is susceptible to certain ac frequencies, known as resonant frequencies. The dielectrophoretic response of red blood cells included the formation of chains of cells along field lines and movement of the cells away from a pointed electrode and toward a flat electrode. Both of these phenomena are frequency dependent; the chains are longer at higher frequencies. The relationship between frequency and chain length as well the resonant frequencies determined for each blood type were quantified with the aim of developing dielectrophoretic microdevices for determining the blood type of single drops of blood. Jason G Kralj (Massachusetts Institute of Technology, MA, USA) showcased a microfluidic device that continuously separates microparticles based on size using dielectrophoresis. The purpose of the device is production of particles with narrow size distributions; particle size being a factor for micro- and nanoparticles in applications such as immunoassays, optical materials and catalysis. Separation of a mixture of particles with two different sizes was demonstrated. Rajiv Bharadwaj (Stanford University, CA, USA) talked about mathematical modeling of field-amplified sample stacking and showed experimental validation and simulation tools supporting the model. Jeffrey D Carbeck (Princeton University, NJ, USA) revisited the question of extracting physical information about proteins from electrophoretic mobilities (the ratio of net charge to coefficient of hydrodynamic drag). Protein charge ladders with measurable charge and hydrodynamic drag were used to determine differences in proton equilibria between native and denatured states of proteins. It was concluded that protein charge and the concentration of carbohydrate solutes contribute to the stability of proteins.

Poster session

As in previous years the poster session and reception was one of the highlights of the meeting, both in technical content and its ability to provide fertile ground for networking. The attendees were from diverse scientific backgrounds. Many posters were presented by graduate students who showcased their work fresh out of the laboratory, thus ensuring that the latest research trends were shown and discussed. The session was cochaired by Pedro Arce (Tennessee Tech University, TN, USA), Alfred Gaertner (Genencor, CA, USA) and Jasmine Gray (GE Healthcare, NJ, USA). This year, the room arrangements allowed for the posters to be displayed for almost the entire time of the meeting, giving ample time to review the posters in detail. Overall, 23 posters were shown spanning a range of subjects including microfluidics, electroosmosis, DNA analysis, proteomics, metabolic studies, analysis software and laboratory information management systems.

The poster reception was accompanied by complimentary appetizers and refreshments and included a 'Best Poster' contest. The posters were evaluated by a Judging Committee assembled by Pedro Arce consisting of Sharon Sauer (Rose-Hulman Institute of Technology, IN, USA), Adrienne Minerick and David Garfin. The prizes were distributed as follows: honorable mentions went to Shawn Llopis (Louisiana State University, LA, USA), Mario Oyander (Universidad Católica del Norte, Antofagasta, Chile) and Robert Meagher (Northwestern University, NY, USA) for their work on microdevices for DNA sequencing, modeling of copper electrokinesis and end-labeled free solution electrophoresis for DNA analysis, respectively. Second place went to Faisal Shaike (Texas A&M University, TX, USA), whose poster described a microfluidic device and technique to concentrate and analyze minute amounts of DNA, thereby eliminating laborious sample preparation steps. First prize was won by Christa Hestekin. Hestekins' work outlined microchip electrophoresis as a means for analyzing ss conformational polymorphisms and heteroduplex analysis to detect genetic mutations. Winners received certificates and monetary awards from AES. All in all, there were lively discussions on the research presented coupled with the opportunity to meet old and new friends.

As can be seen from the summaries above, the meeting provided something for just about everyone interested in the use of electrophoresis and related techniques in genomics and proteomics. The next joint meeting of AES and AIChE will be held in Cincinnati (OH, USA) from October 30 through November 4, 2005.

Websites

101 American Institute of Chemical Engineers
www.aiche.org
(Viewed March 2005)

102 American Electrophoresis Society
www.aesociety.org
(Viewed March 2005)

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