Automation Highlights from the Literature

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Laboratory Automation and High-Throughput Chemistry

High-Throughput Protein Purification and Quality Assessment for Crystallization

The ultimate goal of structural biology is to understand the structural basis of proteins in cellular processes. In structural biology, the most critical issue is the availability of high-quality samples. Structural biology-grade proteins must be generated in the quantity and quality suitable for structure determination using x-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy. The purification procedures must reproducibly yield homogeneous proteins or their derivatives containing marker atom(s) in milligram quantities. The choice of protein purification and handling procedures plays a critical role in obtaining high-quality protein samples. With structural genomics emphasizing a genome-based approach in understanding protein structure and function, a number of unique structures covering most of the protein folding space have been determined, and new technologies with high efficiency have been developed.

At the Midwest Center for Structural Genomics (MCSG) of Argonne National Laboratory, the authors, Kim et al., have developed semiautomated protocols for high-throughput parallel protein expression and purification. A protein, expressed as a fusion with a cleavable affinity tag, is purified in two consecutive immobilized metal affinity chromatography (IMAC) steps. The first step is an IMAC coupled with buffer exchange, or size exclusion chromatography (IMAC-I), followed by the cleavage of the affinity tag using the highly specific Tobacco Etch Virus (TEV) protease. The second step is IMAC and buffer exchange (IMAC-II) to remove the cleaved tag and tagged TEV protease. These protocols are implemented on multidimensional chromatography workstations and show that many proteins can be successfully produced in large scale. All methods and protocols used for purification, some developed by MCSG and others adopted and integrated into the MCSG purification pipeline and more recently the Center for Structural Genomics of Infectious Diseases (CSGID) purification pipeline, are discussed. (Kim, Y., et al., Methods 2011, 55, 12–28)

Comparison of Commercial Extraction Systems and PCR Assays for Quantification of Epstein-Barr Virus DNA Load in Whole Blood

The automation of DNA extraction and the use of commercial quantitative real-time PCR assays could help obtain more reliable results for the quantification of Epstein-Barr virus DNA loads (EBV VL). This study compares two automated extraction platforms and two commercial PCRs for measurement of EBV VL in 10 EBV specimens from Quality Control for Molecular Diagnostics (QCMD) and in 200 whole-blood (WB) specimens from transplant (n = 137) and nontransplant (n = 63) patients. The WB specimens are extracted using the QIAcube or MagNA Pure instrument; VL are quantified with the EBV R-gene quantification kit (Argene) or the artus EBV RG PCR kit (Qiagen) on the Rotor-Gene 6000 real-time analyzer, and the results are compared with those of a laboratory-developed PCR. DNA was extracted from the QCMD specimens by use of the QIAamp DNA minikit and is quantified by the three PCR assays. The extraction platforms and the PCR assays show good correlation (R > 0.9; p < 0.0001), but as much as 10% discordant results are observed, mostly for low viral loads (<3 log[10] copies/mL) and standard deviations that reach as high as 0.49 log(10) copy/mL. In WB, but not in QCMD samples, Argene PCR tends to give higher VL values than artus PCR or the laboratory-developed PCR (mean difference for the 200 WB VL, −0.42 or −0.36, respectively). In conclusion, the two automated extraction platforms and the two PCRs provide reliable and comparable VL results, but differences greater than 0.5 log(10) copy/mL remain between the

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Using the Zebrafish Photomotor Response for Psychotropic Drug Screening

Because psychotropic drugs affect behavior, we can use changes in behavior to discover psychotropic drugs. The original prototypes of most neuroactive medicines were discovered in humans, rodents, and other model organisms. Most of these discoveries were made by chance, but the process of behavior-based drug discovery can be made more systematic and efficient. Fully automated platforms for analyzing the behavior of embryonic zebrafish capture digital video recordings of animals in each individual well of a 96-well plate before, during, and after a series of stimuli. To systematically analyze the thousands of behavioral recordings obtained from a large-scale chemical screen, Kokel and Peterson transform these behavioral recordings into numerical barcodes, providing a concise and interpretable summary of the observed phenotypes in each well. Systems-level analysis of these behavioral phenotypes generates testable hypotheses about the molecular mechanisms of poorly understood drugs and behaviors. By combining the in vivo relevance of behavior-based phenotyping with the scale and automation of modern drug-screening technologies, systematic behavioral barcoding represents a means of discovering psychotropic drugs and provides a powerful, systematic approach for unraveling the complexities of vertebrate behavior. (Kokel, D.; Peterson, R. T., Methods Cell Biol. 2011, 105, 517–524)

Design Optimization of Liquid-Phase Flow Patterns for Microfabricated Lung on a Chip

Microreactors experience significant deviations from plug flow due to the no-slip boundary condition at the walls of the chamber. The development of stagnation zones leads to widening of the residence time distribution at the outlet of the reactor. A hybrid design optimization process that combines modeling and experiments has been used to minimize the width of the residence time distribution in a microreactor. The process is used to optimize the design of a microfluidic system for an in vitro model of the lung alveolus. Circular chambers to accommodate commercial membrane–supported cell constructs are a particularly challenging geometry in which to achieve a uniform residence time distribution. Iterative computational fluid dynamics (CFD) simulations are performed to optimize the microfluidic structures for two different types of chambers. The residence time distributions of the optimized chambers are significantly narrower than those of nonoptimized chambers, indicating that the final chambers better approximate plug flow. Qualitative and quantitative visualization experiments with dye indicators demonstrate that the CFD results accurately predicted the residence time distributions within the bioreactors. The results demonstrate that such a hybrid optimization process can be used to design microreactors that approximate plug flow for in vitro tissue engineered systems. This technique has broad application for optimization of microfluidic body-on-a-chip systems for drug and toxin studies. (Long, C., et al., Ann Biomed. Eng., 2012, 40(6), 1255–1267.)

Microfluidic Chip Technology and Micro Reactor Technology

An Integrated Microfluidic Device for Rapid Cell Lysis and DNA Purification of Epithelial Cell Samples

In this article, Ha et al., from Hanyang University, Korea, describe the design and fabrication of a microfluidic device for cell lysis and DNA purification and the results of device tests using a real sample of buccal cells. Cell lysis is thermally executed for 2 min at 80 °C in a serpentine type microreactor (20 µL) using an Au microheater with a microsensor. The DNA is then mixed with other residual products and purified by a new filtration process involving micropillars and 50 to 80 µm microbeads. The entire process of sample loading, cell lysis, DNA purification, and sample extraction is successfully completed in the microchip within 5 min. Sample preparation within the microchip is verified by performing an SY158 gene PCR analysis and gel electrophoresis on the products obtained from the chip. The new purification method enhances DNA purity from 0.93 to 1.62 after purification. (Ha, S. M., et al., J. Nanosci. Nanotechnol. 2011, 11, 4250–4253)

Neuroscience Goes on a Chip

Advances in microelectronics, microfluidics, polymers, and microfabrication have enabled the creation of disposable labs-on-a-chip (LOCs) as the new tools for neuroscience research. The LOCs have been applied for a wide range of neurobiology studies, including cellular and molecular biochemical experimentations, morphological observations, and electrophysiological investigations. The integration of miniaturized components leads to analytical instrumentations with unprecedented automation, speed of analysis, and flexibility. These features make LOCs capable enough to replace their bulky and expensive bench-top counterparts. LOCs can be useful for genomic, proteomic, epigenomic, peptidomic, connectomic, and electrophysiological studies and also as effective tools for reductionist neuroscientists. Moreover, they can be applied at higher-level studies such as developmental neurobiology and behavioral investigations. This work provides an in-depth review of LOC platforms for neuroscience research. First, Soe et al.,
from the Center for Intelligent Systems Research, Deakin University, Australia, review the essential bench-top neuroscience instrumentation as per their functions and features. In addition, they present LOC counterparts for those bench-top instrumentations, and they offer perspectives on persistent challenges and our perception of opportunities based on LOC instrumentations in neuroscience research. (Soe, A. K. et al., Biosens. Bioelectron. 2012, 35(1), 1–13.)

**Microfluidic Single-Cell Analysis: From Promise to Practice**

Methods for single-cell analysis are critical to revealing cell-to-cell variability in biological systems, especially in cases in which relevant minority cell populations can be obscured by population-averaged measurements. To date, however, cell studies have been limited by the cost and throughput required to examine large numbers of cells and the difficulties associated with analyzing small amounts of starting material. Microfluidic approaches are well suited to resolving these issues by providing increased sensitivity, economy of scale, and automation. After many years of development, microfluidic systems are now finding traction in a variety of single-cell analytics including gene expression measurements, protein analysis, signaling response, and growth dynamics. With newly developed tools now being applied in fields ranging from human haplotyping and drug discovery to stem cell and cancer research, the long-heralded promise of microfluidic single-cell analysis is now finally being realized. (Lecault, V., et al. Curr. Opin. Chem. Biol. 2012).

**High-Throughput Analytics**

**Microchip Capillary Gel Electrophoresis of Multiply PEGylated High Molecular Mass (Glyco) Proteins**

PEGylation is the most successful approach to date to prolong the in vivo survival of recombinant proteins. The conjugation of the polymer to glycoproteins results in challenging analysis and furthermore requires a wide variety of analytical tools for determination of the extent of PEGylation. Authors Seyfried et al. from Vienna University of Technology, Austria, present MCGE with a noncommercial high-molecular-weight protein assay for the determination of the PEGylation degree with a focus on multiply PEGylation. To show the potential of the modified MCGE system high mass (e.g., coagulation factor VIII), the authors analyze PEGylated glycoproteins. For VWF, the influence of glycans and of the hydrodynamic radius on migration time and molecular weight determination are shown. The modified MCGE assay system turns out to be a powerful tool for rapid determination of the degree of PEGylation, demonstrating conjugate quality or reaction control of PEGylated proteins. This is the main advantage over time-consuming conventional SDS-PAGE. Furthermore, electrophoretic separation, staining, destaining, and fluorescence detection in one step combined with automated data analysis show that the MCGE system is a promising technique for high-throughput monitoring. The MCGE system can be used for rapid structure confirmation (“MCGE fingerprinting”) of multiply PEGylated glycoproteins beyond the 230-kDa molecular mass range. (Seyfried, B. K., et al., Biotechnol. J. 2012, 7, 635–641)

**GenomicTools: A Computational Platform for Developing High-Throughput Analytics in Genomics**

Recent advances in sequencing technology have resulted in the dramatic increase of sequencing data, which in turn requires efficient management of computational resources, such as computing time, memory requirements, and prototyping of computational pipelines. Tsirigos et al., from IBM T.J. Watson Research Center of New York, present GenomicTools, a flexible computational platform, comprising both a command-line set of tools and a C++ API, for the analysis and manipulation of high-throughput sequencing data such as DNA-seq, RNA-seq, ChIP-seq, and MethyIC-seq. GenomicTools implements a variety of mathematical operations between sets of genomic regions, thereby enabling the prototyping of computational pipelines that can address a wide spectrum of tasks, ranging from preprocessing and quality control to meta-analyses. In addition, the GenomicTools platform is designed to analyze large data sets of any size by minimizing memory requirements. In practical applications, where comparable, GenomicTools outperforms existing tools in terms of both time and memory usage. The GenomicTools platform (version 2.0.0) was implemented in C++. The source code, documentation, user manual, example data sets, and scripts are available online at http://code.google.com/p/ibm-cbc-genomic-tools. (Tsirigos, A., et al., Bioinformatics 2012, 28, 282–283)

**Automation Systems**

**Emerging Technologies in Mass Spectrometry Imaging**

Mass spectrometry imaging (MSI) as an analytical tool for biomolecular and biomedical research that targets accurate compound localization and identification. In terms of dedicated instrumentation, this translates into the demand for more detail in the image dimension (spatial resolution) and in the spectral dimension (mass resolution and accuracy), preferably combined in one instrument. At the same time, large-area biological tissue samples require fast acquisition schemes, instrument automation, and a robust data infrastructure. This review discusses the analytical capabilities of an “ideal” MSI instrument for biomolecular and
biomedical molecular imaging. The analytical attributes of such an ideal system are contrasted with technological and methodological challenges in MSI. In particular, innovative instrumentation for high-spatial-resolution imaging in combination with high sample throughput is discussed. Detector technology that targets various shortcomings of conventional imaging detector systems is highlighted. The benefits of accurate mass analysis, high mass resolving power, additional separation strategies, and multimodal three-dimensional data reconstruction algorithms are discussed to provide an insight in the current technological advances and the potential of MSI for biomedical research. This article is part of a special issue titled Mass Spectrometry Imaging (Jungmann, J. H., Heeren, R. M., J. Proteomics 2012).

A Logic-Tated Nanorobot for Targeted Transport of Molecular Payloads

Douglas, Bachelet, and Church from Harvard Medical School describe an autonomous DNA nanorobot capable of transporting molecular payloads to cells; sensing cell surface inputs for conditional, triggered activation; and reconfiguring its structure for payload delivery. The device can be loaded with a variety of materials in a highly organized fashion and is controlled by an aptamer-encoded logic gate, enabling it to respond to a wide array of cues. Douglas et al. implement several different logical and gates and demonstrate their efficacy in selective regulation of nanorobot function. As a proof of principle, nanorobots loaded with combinations of antibody fragments are used in two different types of cell-signaling stimulation in tissue culture. This prototype could inspire new designs with different selectivities and biologically active payloads for cell-targeting tasks. (Douglas, M., et al., Science, 2012, 335, 831–834)

Advances in Immunosensors

Recent Trends in Antibody-Based Sensors

This review by Holford et al., from Cranfield University, United Kingdom, details recent advances in the fields of immunosensors and closely related immunoassays in the past decade, together with a discussion of possible future trends. Immunosensors can be classified by the way in which they transduce the signal produced on the formation of an antibody-antigen complex. Recent advancements in these methods of detection and transduction are discussed in detail, with particular focus on electrochemical, optical, piezoelectric, and magnetic-based sensors. The varying applications of these sensors are also discussed. Some of the most significant advances include development of immunosensors for the continuous monitoring of analytes, point-of-care devices with lower unit costs, automation, reusability, and ease of use. Immunosensor technology has advanced at a prolific rate since its conception and has grown into a diverse area of ongoing research. (Holford, T. R., et al., Biosens. Bioelectron. 2012, 34, 12–24)

In Situ Comparative Studies of Self-assembly Adsorption of Bovine Serum Albumin on Nano Films by Atomic Force Microscopy

The self-assembly adsorption of function protein on crystal surfaces, as a common phenomenon, broadly takes place in many applications of biosensors, biocapsules, and bioMEMS/bioNEMS. To systematically investigate the different adsorption characteristics of the same function protein on two different crystal surfaces under the identical environment, a hybrid surface composing silica and discontinuous gold nano film (GNF) is fabricated by physical vapor deposition (PVD) and ultrasonic cleaning method, in which the dynamic process of the self-assembly adsorption of bovine serum albumin (BSA) is in situ observed by an atomic force microscope (AFM). The variations on the junction area of the two different surfaces are studied in the aqueous solution before and after injecting BSA with 0.05 mg/mL concentration. It is found that silica, compared with the same hydrophilic GNF, takes on a fairly weak adsorption force. The results indicate that the adsorption strength of BSA on the hydrophilic crystal surface is determined not only by hydrophilic property but also by other interaction forces, such as Van der Waals, and so on. Moreover, observation under the contact mode of AFM, BSA adsorbed on GNF has a great tendency to form a ridgelike topography. These results may be helpful in the application of immunosensors and other areas. (Ye, X., et al., J. Nanosci Nanotechnol. 2011, 11, 10765–10769)