

necessary to perform proteolysis in order to facilitate identification of a protein of interest, as digest peptides form a characteristic “fingerprint” of the parent protein.

Cross References

- ▶ [Lab-on-Chip Devices for Protein Analysis](#)
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Proteomics

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Proteomics in Microfluidic Devices

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Definition

Proteomics is the study of the collection of proteins (the proteome) expressed in a given system. The field of proteomics can be divided into three subtypes:

- *profiling* – identification and quantification of the proteins in a sample and drawing comparisons between samples,
- *functional* – the study of the behavior of specific proteins, and
- *structural* – the study of protein folding and the complexes it forms.

Microfluidics is characterized by devices that contain channels or features with dimensions measured in microns, and are capable of transporting and analyzing sample volumes in the nanoliter range.

Overview

Proteomics is a field that first gained prominence in the mid 1990s. The sheer number of proteins as well as the number of variations that comprise the proteome presents a huge challenge for analysis. Serum protein concentrations can vary by several orders of magnitude, and often

a single disease will affect both high- and low-abundance proteins to varying degrees. In addition to basic protein structures and sequences, a vast array of post-translational modifications exists that significantly impacts the functionality, structure, and activity of the native proteins. The primary applications of microfluidics in proteomics have been in the profiling and functional subtypes – structural proteomics has not yet been extensively studied on the microscale.

Profiling proteomics is used to identify all of the proteins in a sample or to compare changes in protein expression between several samples. Due to the complexity of the proteome, accurate analysis requires a great deal of sample processing, including proteolytic digestion, followed by multidimensional separations and mass spectrometry. Separations and mass spectrometry are covered in other chapters of the *Encyclopedia* ▶ [lab-on-chip devices for protein analysis](#), ▶ [chromatographic chip devices](#), ▶ [interfaces between microfluidics and mass spectrometry](#), but chemical processing for profiling proteomics will be discussed here. Readers are encouraged to see Friere and Wheeler [1] for a comprehensive discussion of proteome profiling in microfluidics.

The study of functional proteomics on the microscale comprises an assortment of assays to identify functional groups on proteins and evaluate binding events to determine functional properties of proteins. Functional proteomics is exploited in *enzyme assays* and *immunoassays* to characterize the behavior of a protein within a biological system.

There are a number of ways in which enzymatic studies contribute to the understanding of the proteome. Enzymes are commonly used in proteomics to investigate analytes that are difficult to measure by conventional means. Quantitation of small molecules with enzymatic methods provides insight into the concentration and activity of the proteins associated with those molecules. A great variety of these enzymatic assays have been carried out in microfluidic devices. Another function of enzymatic assays is in kinetics; measurements of properties of enzymes such as the Michaelis–Menten constant (the concentration of substrate when the reaction rate is half the maximum rate) and the turnover number (the number of moles of substrate that are converted to product per catalytic site per unit time) are vital to understanding the mechanics of the proteome, and are used to characterize the effects of known drugs and discover new ones.

The immunoassay is another widely used technique that has been applied to a diverse set of proteomic applications, including the identification and quantization of protein markers for diagnosis of diseases where laboratory facilities are not readily available. Immunoassays can be

designed to detect very small amounts of antigens and to measure the concentrations quite accurately; in addition, further information about the disease markers, such as the existence of structural changes and posttranslational modifications, can be elucidated. Since immunoassays typically comprise a number of repetitive steps and require expensive reagents, they are a ripe target for adaptation to the microscale format, where sample sizes, reagent quantities, and analysis times are all greatly reduced.

Basic Methodology

The methods used in microscale proteomics applications can be divided into two categories, related to the features of microfluidic devices, and the nature of the analyses and assays themselves.

Features of Microfabricated Proteomic Devices

In proteomics applications, the most important features of microfluidic systems are related to delivery of fluid reagents, immobilization of proteins, mixing and reactions, and geometric microarrays.

Movement of Fluids

A critical decision for any microfluidic system is how to generate and control the flow of fluid. The most common methods rely on external pumps or electrokinetic flow. Conventional pumps are too large for pumping \sim nL volumes of solutions, requiring splitters to isolate a fraction of the pressure to apply to the device. Smaller pumps such as syringe pumps are used frequently, but can be difficult to interface with microchannels in a device. Another common option is using electroosmotic (or electrokinetic) flow (EOF) to move analytes through a chip. This method is advantageous because it doesn't require any pressure to be applied on the sometimes fragile chips, it can move fluids at fast rates (exceeding 33 mL/min), and electrodes can easily be integrated into the chip itself. The magnitude of EOF is a function of the density of charge on microchannel walls (or on the surface of packed media); thus, careful consideration must be made in determining the materials used to fabricate devices. If materials without adequate charge density are used (or if the native EOF is too weak or strong), there are a number of coatings that can be used to tune the direction and magnitude of EOF ([▶electroosmotic flow](#)). Electrokinetic effects can also be used to separate the constituents of solutions; however, this can be one of its biggest disadvantages. Any fluid pumped by this method will have its components at least partially separated from one another, creating an inherent bias in any injected sample plug, and flow rates will vary due to

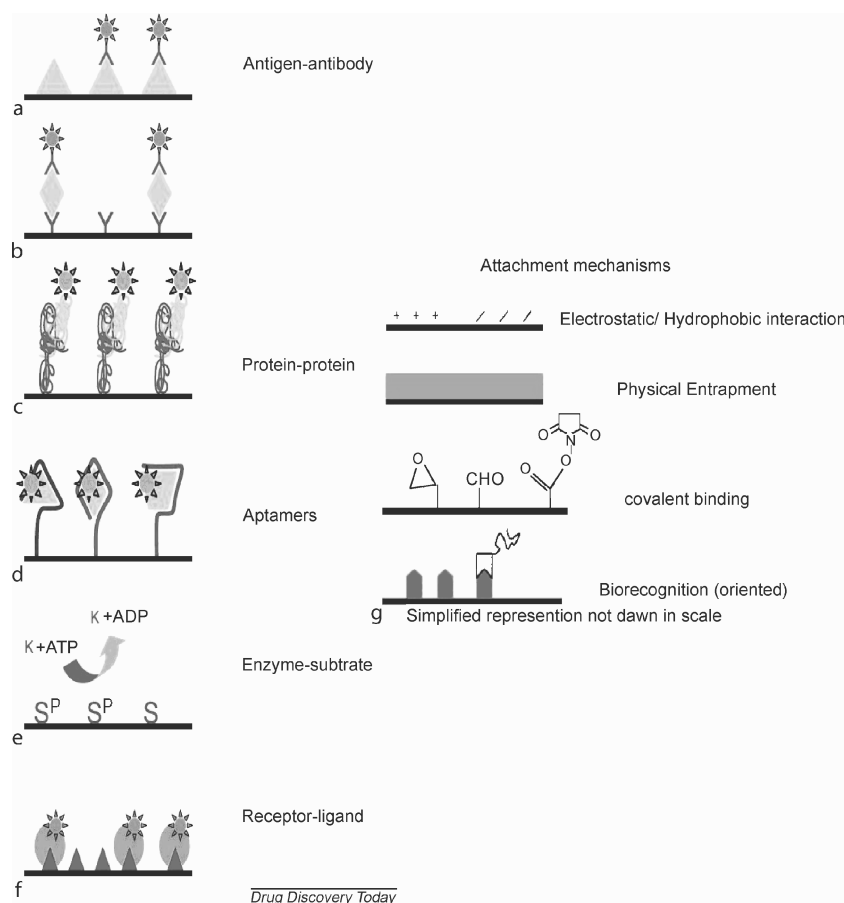
protein adsorption on the interior of the chip or with Joule heating in the device. A final disadvantage is that all solutions must contain buffer ions in a particular concentration range, which is not convenient for all applications.

There are several less common alternatives for controlling fluid flow in microchannels. [▶Digital microfluidics \(DMF\)](#) is an alternative fluid handling method that is beginning to be used in proteomic applications. In DMF, discrete droplets are manipulated on a surface (rather than as streams in enclosed channels) via electrowetting and/or dielectrophoretic forces generated when an electrical potential is sequentially applied to electrodes in an array. This technique can be used with a wide variety of different fluids and can be applied to some aspects of sample preparation and purification. Hydrodynamic pumping, in which flow is driven by siphoning from one fluid reservoir to another, is an option for driving flow that does not introduce a sampling bias. It can, however, be difficult to control; consistency and fine-tuning of flow rate are problematic. Centrifugal pressure flow is another attractive option for fluid movement in protein studies, as it is insensitive to pH, ionic strength, and other characteristics of the fluid that may need to be varied for different assays. Centrifugal pumping is also relatively unaffected by the deposition of analytes onto channel walls (unlike EOF, DMF, etc.); however, it typically relies on single-use valves and therefore flow can only be driven one-time, and in one direction.

Immobilized Proteins

There are two basic formats in which assays are incorporated in microfluidic devices: homogeneous, or solution-based, assays, and heterogeneous, or surface-based, assays. Both approaches significantly reduce sample and reagent consumption relative to their macroscale counterparts, but heterogeneous assays are emerging as the dominant method for proteomics, as they can be adapted to many different types of interactions (Fig. 1a–f) [2].

Heterogeneous assays require proteins or other substrates to be immobilized on surfaces, which allows for conservation and repeated use of the immobilized bed, as well as facilitating the analysis of a sample without need for a separate concentration or mixing step. Also, the binding event or interaction between analytes is not restricted by equilibrium conditions or adequate mixing/diffusion of reagents. Finally, immobilization makes it easier to incorporate more than one reaction-zone in a device, increasing the number of analytes that can be performed on a sample and taking greater advantage of the benefits of a microfluidic system. Several properties are required for useful surface-immobilized proteins, including: the sur-



Proteomics in Microfluidic Devices, Figure 1 (a)–(f) Several heterogeneous protein assay formats (reprinted from [2]). A variety of protein binding events can be studied, ranging from antibody/antigen to protein/protein to enzyme/substrate interactions. (g) Strategies for the immobilization of proteins on solid supports (reprinted from [4]) (► Color Plate 14)

face must have a high binding capacity for the capture protein, the immobilized protein must retain its biological activity, the protein must be spatially accessible, and there must be a low level of *nonspecific* interactions between proteins in solution and the coated surface.

In microfabricated devices, protein immobilization is most often carried out on glass or silicon supports, using a wide variety of surface chemistries [3]. The most common methods for attachment of proteins to surfaces are depicted in Fig. 1g [4], ranging from simple (e.g., adsorption of proteins via electrostatic or hydrophobic interactions with the surface, entrapment in immobilized polymers, etc.) to more sophisticated techniques, relying on covalent bonding and biological recognition (e.g., biotin/streptavidin). In general, the most robust immobilized protein beds are formed by covalent attachment. One popular method requires two steps: a first layer is formed by silane chemistry such that the surface presents a reactive group (e.g.,

epoxy, amino, thiol, etc.), and a second layer (e.g., the protein to be immobilized or a linker molecule) is covalently attached via the immobilized reactive groups. Other strategies include covalent attachment to functionalized polymer coatings on the interior of the device or linkage to the free end of a self-assembled monolayer (SAM) on a gold surface. Most of these methods result in incomplete coating of the surface, and some reactive groups are left vulnerable to interaction with target proteins during analysis; this phenomenon is called *non-specific interaction*, and can negatively impact assay performance. A number of strategies exist for blocking surfaces to prevent non-specific binding of analytes, most of which involve coatings of polymers (e.g., polyethylene glycol) or proteins (e.g., albumin or casein) that will not interact with the sample. However, even with these precautions, interactions between analytes and the surface of the device are very difficult to eliminate.

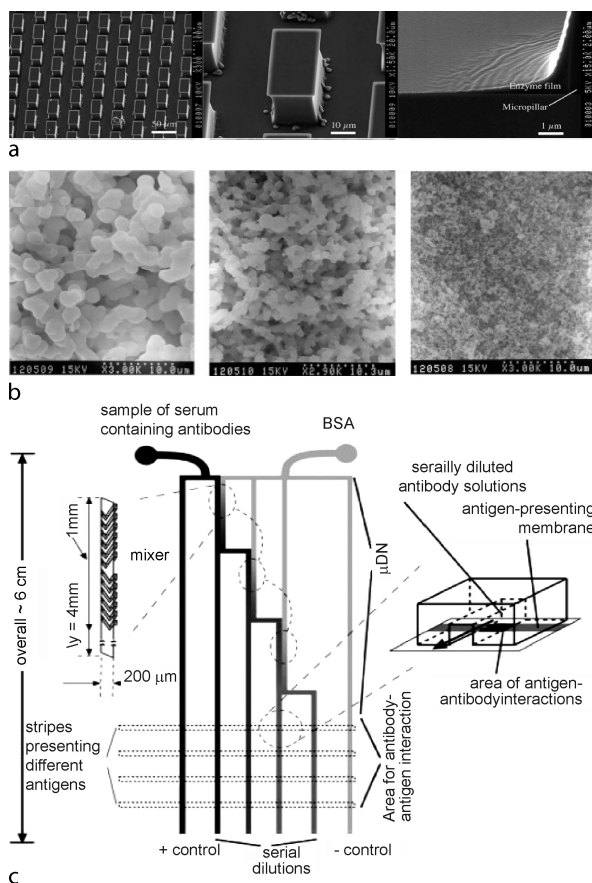
Mixers and Microreactors

In contrast to heterogeneous assays, in homogeneous assays, solutions of an enzyme and a substrate or an *antibody* and antigen are combined in a channel to evaluate the enzyme kinetics or properties of inhibitors, or measure the amount of displaced antigen, which requires thorough mixing. In low Reynold's Number microfluidic channels, turbulent mixing is not possible, and diffusive mixing dominates. Diffusive mixing is a reasonable technique for small molecules; however, enzymes and other large molecules diffuse much too slowly for adequate mixing in many proteomic applications. A number of passive mixing schemes in microfluidic systems have been developed – for example, a network of interconnecting channels of varying lengths and widths is capable of mixing solutions being pumped by electroosmotic flow, while patterned surface grooves enable mixing in a pressure-flow device.

One solution to the challenges of mixing proteins in microfluidic systems is to immobilize proteins in *microreactors* [5]. These systems typically consist of chambers of enzymes immobilized on beads, micropillars [6], or porous polymer monoliths [7] (Fig. 2a–b). Such systems have large surface area-to-volume ratios, which minimizes diffusion time for reactions with solution-phase reagents. Microreactors can be used either for the conversion of an analyte to another form that is more easily detected, or for direct studies of the properties of enzymes and substrates. One of the most common uses is for the digestion of proteins for proteome profiling, but such systems can also be used for the removal of amino acid residues from peptides or proteins or for enzyme kinetic studies.

Microarrays

Biological microarrays are two-dimensional arrangements of immobilized spots of molecules on a surface. When a microarray is exposed to a solution containing unknown analytes bearing an appropriate label, analytes binding to particular spots on the surface can be rapidly identified. DNA microarrays were introduced in the late 1990s, and have become a widely used tool for high-throughput genomic analysis; however the development of similar tools for proteome analysis has been hampered by the diverse properties of proteins. For example, all DNA molecules inherently possess specific capture properties through complementary base-pairing; proteins do not have such a universal property. Additionally, protein capture *ligands* tend to be more delicate in structure and function than the relatively robust DNA molecule. Finally, small amounts of DNA can be detected quite easily using amplification techniques such as the polymerase chain reaction (PCR); no such amplification technique exists for proteins.



Proteomics in Microfluidic Devices, Figure 2 (a) An enzymatic microreactor containing micropillars that have been coated with trypsin for protein digestion. Reprinted from [6]. (b) Porous monolith microreactors of various pore sizes. Pore size is determined by the ratio of monomer to porogen (solvent) during polymerization. Reprinted from [7]. (c) A microdilution network (μ DN) for the quantitation of multiple antigens. The device uses a series of T-intersections to sequentially dilute a serum sample. Herringbone patterned areas are used as mixers in the dilution channels. Several antibodies have been deposited on a membrane using a microfluidic network, and the membrane is incorporated in the bottom of the μ DN device. Reprinted from [12]

Despite these disadvantages, the promise of multiplexed detection of analytes has made protein microarrays a popular research topic in microfluidic implementations of proteomic analyses.

The immobilized spots used in protein microarrays may contain protein molecules, or smaller molecules such as inhibitors or ligands with which a protein in solution may interact. Very small spots are advantageous in that higher detection throughput can be achieved with minimal amounts of printed material and sample. Spots are typically deposited either by contact methods, such as printing arrays that use needles to deposit droplets directly on the

surface, or by non-contact methods, including the use of capillaries or ink jet dispensers to deposit droplets with nanoliter-picoliter volumes.

An alternative to microarrays is the use of arrays of multiple parallel channels. Typically, such structures are used to carry out a number of duplicate assays on one or more samples, to perform a serial dilution of a single sample for quantitation of a given protein, or to detect multiple targets in a single sample. In the latter case, an array of channels may be used in conjunction with a printed array of spots or bands – the channel network is used for fluid delivery to the printed array (Fig. 2c).

Analyses and Assays

Microfluidic devices are used in a variety of profiling and functional proteomics applications; the goal in much of this work is to reduce sample and reagent consumption and increase throughput. The primary application in profiling proteomics is in the preparation of protein samples for separations and mass spectrometry, while the major functional proteomics tools are enzymatic and immunological assays.

Sample Preparation for Profiling Proteomics

The identification of unknown proteins often requires that they be digested (or divided) into a group of constitutive peptides, which are interrogated by mass spectrometry. Comparing the masses of the peptides to genomic databases enables absolute identification by means of peptide mass fingerprinting (PMF). Proteins are digested by exposure to proteolytic enzymes (e.g., trypsin), or other lytic reagents (e.g., cyanogen bromide, CNBr); this is often part of a multistep process requiring 12–18 h, including mixing the sample with buffer and denaturant, mixing and incubating the sample with reducing agent to reduce disulfide bonds, mixing and incubating the sample with alkylating agent to prevent disulfide bonds from reforming, and finally, mixing and incubating with lytic reagent. This process is extremely tedious and time-consuming, making it an attractive application for microfluidics.

Several device configurations have been used for proteomic processing in microfluidic devices, including open channels, immobilized beads, and other solid phase media. Open-channel devices for tryptic digestion and reduction of disulfides in proteins are capable of achieving complete tryptic digestion in timeframes ranging from 15 min to 5 s. Immobilized beads increase the surface-to-volume ratio further; for example, a bed of trypsin-modified beads immobilized in a microfluidic chamber can be used very effectively for *proteolysis* of relatively large volumes of

flowing sample. An alternative method for increasing the surface area-to-volume ratio in microfluidic devices is to use trypsin-modified monoliths, formed from polymer plugs cured in situ, or from membranes sandwiched between channels. Trypsin and other enzymes can also be copolymerized within hydrogel microstructures – the hydrogel is a biocompatible matrix that reduces the likelihood of damage to the enzyme during and after immobilization.

Microfluidics is promising for developing tools with integrated chemical processing of proteomic analytes, by virtue of fast reaction kinetics. Sample purification and concentration can be carried out on the microscale by methods such as solid phase extraction on hydrophobic surfaces ► [lab-on-chip devices for sample extraction](#) or affinity chromatography ► [chromatographic chip devices](#). Immunoassays on magnetic beads have been used not just for detection of antigens (described below), but also as a purification method for the isolation of a target for mass spectrometry. However, to our knowledge, there have been no reports of implementing a fully integrated process, including stepwise reduction, alkylation, and digestion. Microfluidic tools for chemical processing will likely not be widely adopted until this critical benchmark is achieved.

Enzymatic Assays

Traditionally, enzymatic assays are performed in microtitre plates (plastic trays containing arrays of isolated wells), where an enzyme and its substrate are mixed in an individual well, and the substrate turnover is measured to determine the activity of the enzyme. Microfabricated devices that can perform enzymatic studies without interference between individual elements are highly desirable, as the macroscale method consumes a great deal of enzyme. There are three basic applications of enzymatic assays in proteomic studies:

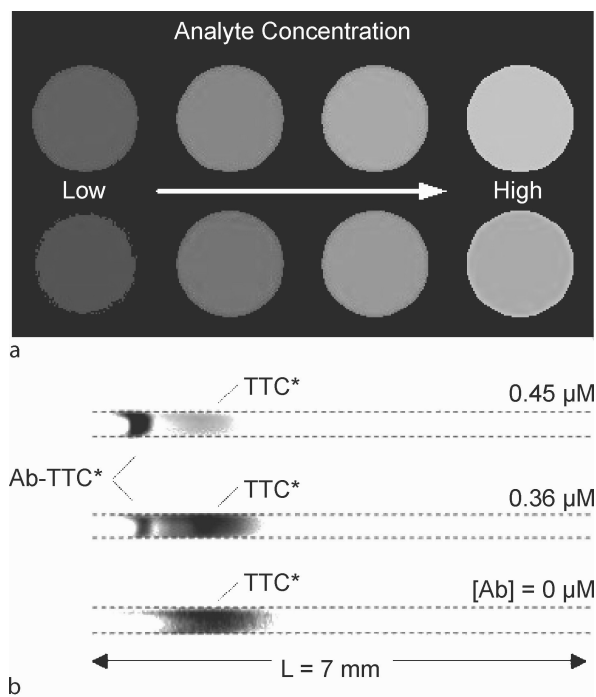
1. small molecule detection or sensing via an enzymatic reaction,
2. studies of enzyme–substrate, protein–ligand, and protein–protein interactions, and
3. kinetic studies.

The first type of enzyme application in microfluidics is chemical sensing. Sensors can be constructed in cases where an enzyme turns over a particular small-molecule substrate to produce a product quantifiable by fluorescence, chemiluminescence, absorbance spectroscopy, or electrochemical detectors. In cases where the substrate is not detectable itself, an enzymatic product can often be coupled to another enzyme that produces a detectable product. For example, there are a wide variety of small

molecules (such as nutrients, amino acids, and sugars) that can be coupled to the chemiluminescent reaction of luminol and peroxide in the presence of horseradish peroxidase. These enzyme-substrate assays were the first to be adapted to microfluidic devices – a great number of small-molecule sensors have been developed based on microfluidic channels with electroosmotic or hydrodynamic flow, and pre-loaded microfluidic cartridges containing nanoliter volumes of reagents have shown great promise as a replacement for 96-well plates for high-throughput screening [8].

The second type of enzyme application in microfluidics is protein interaction studies. In order to study interactions between enzymes and substrates, peptides, or other proteins, an alternative to microtitre plates is the microarray. These arrays are very often used in conjunction with microfluidic sample and reagent delivery systems; alternatively, microchannels can be used to deposit spots in an array. By immobilizing substrates or inhibitors on the array, it is possible to screen large numbers of molecules for their relative abilities to bind a particular enzyme. This is typically accomplished by tagging the enzyme with a fluorescent molecule and using the intensity of the array spots to determine the amount of enzyme bound (Fig. 3a). This is a promising approach for the identification of protein drug targets, or for the evaluation of compound libraries in drug discovery. Peptide fragments of proteins of interest have great potential as capture ligands, as they are easy to synthesize, stable, and able to mimic proteins. However, small molecules such as peptides are difficult to immobilize on a surface such that they are still accessible to the sample. Peptide fragments are also not as specific as complete proteins; for some applications, it is preferable to immobilize an entire protein to characterize its interactions with other proteins or enzymes of interest. Sample delivery to the elements of an array via microfluidics enables hundreds of different analyses to be performed on a single sample while reducing the size of that sample from microliter volumes to nanoliters.

A third type of enzyme application in microfluidics is kinetic studies, which may be carried out in either homogeneous or heterogeneous format. In homogeneous assays, adequate mixing of the enzyme, substrate, and any inhibitors that are being studied is crucial – this is done either via the merging of reagent streams at a T- or Y-shaped junction between channels or by means of a microfabricated mixer to ensure complete homogenization. A microfluidic mixer is advantageous – it ensures complete mixing of the enzyme and substrate and a smaller quantity of enzyme is consumed. Electrophoretically-mediated microanalysis, or EMMA, uses electrokinetic forces as the pumping method, which has the added ben-



Proteomics in Microfluidic Devices, Figure 3 Detection of binding of a protein analyte in a heterogeneous (a) or homogeneous (b) assay. In (a), the fluorescence intensity of spots on an array reflects the amount of the target protein in the sample solution. Different analytes are tagged with molecules that fluoresce at different wavelengths to simplify identification. In (b), the tetanus toxin-C-fragment (TTC) is fluorescently labeled and separated from the antibody-TTC complex in an electrophoretic channel for quantification. Reprinted from [11] (► Color Plate 14)

efit of separating product from substrate as the analysis proceeds. EMMA was first developed in capillary electrophoresis systems, but was readily adapted to microchannel setups [9]. Since a chip disperses Joule heat much more efficiently than a capillary does, the analysis can be run at higher potentials, increasing the speed of measurement. In most cases, a modified substrate is used that is converted to a fluorescent, chemiluminescent, or colored product by the enzyme, and optical methods are used to calculate factors such as the turnover number and Michaelis constant. Kinetic assays have also been carried out in centrifugal flow microfluidic devices [10], which are applicable for a wide variety of biological samples, including whole blood, plasma, and urine, that are incompatible with electroosmotic flow systems. Centrifugal devices are also easily adapted to perform multiple analyses on a single plastic device, reducing the costs of manufacturing. However, fresh enzyme is required for each determination, making enzyme recovery difficult. Heterogeneous enzyme microreactors eliminate the need for recovery

steps and dead time during the measurement. Microfluidic devices containing immobilized enzymes enable the study of kinetics with excellent enzyme recovery and decreased reagent consumption, increasing the overall efficiency of the method and greatly decreasing waste.

Immunoassays

Like enzymatic assays, immunoassays are conventionally implemented in microtitre wells, but they are even more easily adapted to the microscale. Homogeneous immunoassays are typically employed for the study of small molecules, while heterogeneous immunoassays are preferred for large-molecule detection.

A homogeneous assay is carried out by combining an antigenic sample and an antibody by means of electrokinetic flow in a microchannel. After the antigen binds the antibody, the complex can be separated from the remaining free antibody via electrophoresis to determine the amount of antigen present in the sample (Fig. 3b) [11]. On a microfluidic chip, this analysis can easily be multiplexed – a different assay can be performed in different channels on the same sample.

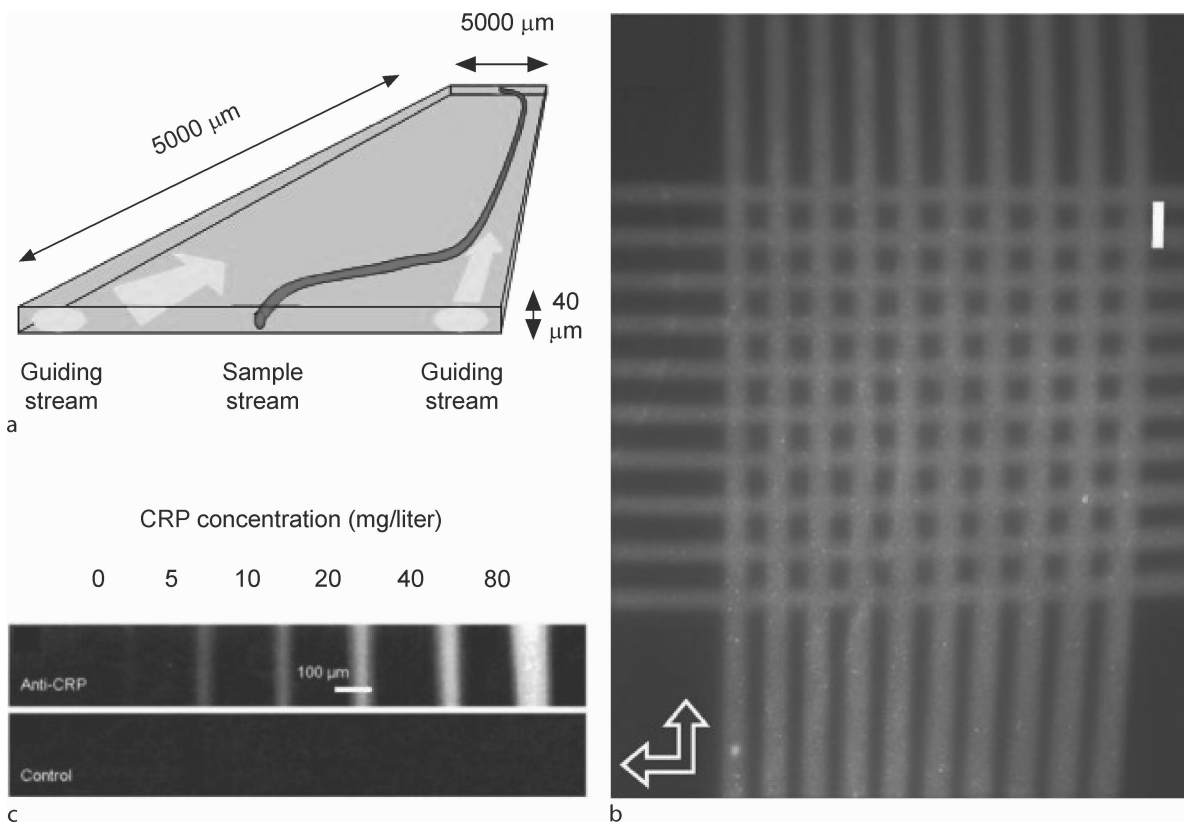
There are two common forms of heterogeneous assays used to detect and quantify disease markers: the direct-labeling method, in which the proteins in a sample are labeled with a detectable tag and isolated from a complex sample by means of a bed of immobilized antibodies, and the indirect-labeling method, in which two antibodies are used for each marker. Sandwich-type immunoassays, such as the enzyme-linked immunosorbent assay (ELISA), require two antibodies: an immobilized antibody used to capture a molecule of interest – a protein, a virus, or a small molecule (e.g., a hormone, pharmaceutical, or environmental pollutant) – and a second antibody in solution that is conjugated to a detectable tag. Immunoassays have also been carried out with detection via surface plasmon resonance microscopy, atomic force microscopy, and electrochemical sensors, eliminating the need for a detectable label. These assays are very useful because the antibody-antigen binding that must occur at each step is highly specific and thus has built-in redundancy – they are very sensitive and capable of quantitation of changes in concentration with a dynamic range of up to 2–3 orders of magnitude. As for enzymatic assays, microfluidic channels can be used either to deposit the first antibody onto a surface for immobilization or to direct the sample over the immobilized antibody (concentrating the antigen on the surface in the process) and any subsequent reagents – in some cases, microfluidic systems have been used for both purposes [12]. While basic immunoassays are well-suited for determining the pres-

ence of a given molecule in a sample and sometimes the quantity, they do not provide any other information about the target molecule. In recent work, immunoassays have been adapted to provide structural and post-translational modification information on marker proteins, however, these assays have not yet been widely applied to microfluidic devices and are still primarily performed in microtitre wells.

Key Research Findings

In the first report of on-chip proteolytic processing, Gottschlich et al. used open-channel devices for tryptic digestion and reduction of disulfides in proteins, which enabled complete processing in 15 min. More recently, Huang et al. [13] and Liu et al. [14] used surface adsorbed trypsin in channels to enable complete digestion in less than 5 s. In an alternative strategy, Yue et al. used a bed of trypsin-modified agarose beads immobilized in a weir in a glass microfluidic device to digest β -casein [15]. Peterson et al. used the related heterogeneous technique of forming methacrylate plugs cured in situ to increase the surface area-to-volume ratio to achieve tryptic digestion of myoglobin in ~ 11 s [16]. Sakai-Kato et al. developed similar methods using trypsin immobilized in a sol-gel matrix [17]. Gao et al. reported using a trypsin-modified polyvinylidene fluoride (PVDF) membrane in a PDMS microfluidic chip, enabling protein digestion in 3–10 min [18]. Each of these methods represents a vast improvement over conventional macroscale proteolysis, which typically requires hours-days. One promising approach for sample purification during processing is the use of digital microfluidics to remove hydrophilic impurities from proteomic samples [19].

Hadd et al. performed the first homogeneous assays to determine enzyme kinetics in a microfluidic chip – these assays relied on diffusional mixing of β -galactosidase and resorufin β -D-galactopyranoside (RBG) within a channel electrophoresis system [9]. This assay successfully characterized the kinetics of the enzyme, and reduced reagent consumption by four orders of magnitude over conventional assay methods. The same assay was later modified by Burke et al. by including a passive microfluidic mixer, resulting in a characterization of enzyme kinetics that more closely agreed with that obtained via conventional methods [20]. Puckett et al. developed a centrifugal microfluidic system in which homogeneous protein-ligand binding assays were performed to detect and characterize the binding interaction between phenothiazine antidepressants and calmodulin, a calcium-binding protein known to interact with this class of drugs [21]. This system utilized pre-measured aliquots of reagents,



Proteomics in Microfluidic Devices, Figure 4 (a) Use of hydrodynamic flow to create a microfluidic stream and direct it to specific areas of a larger channel. (b) The flow system is used to deposit biotinylated fluorescein in a grid pattern. The direction of flow during deposition is indicated by arrows. (c) Quantitation of C-reactive protein (CRP) in whole blood. Samples of blood were spiked with CRP and the microstream directed across a surface coated with anti-CRP before exposure with a Cy3-labeled secondary antibody. Reprinted from [24] under open access agreement with BioMed Central. (► [Color Plate 15](#))

increasing the speed and reproducibility of the analysis for high-throughput applications. In an effort to integrate multiple analyses on one device, Wang et al. incorporated two homogeneous assays into one microfluidic channel: an enzymatic assay for glucose and an immunoassay for insulin [22]. Electrokinetic effects were used for both fluid movement and separation of the components before detection. Through the use of pre- and post-column reactors and an electrophoretic separation of analytes, two independent measurements were made in the same space.

Perhaps the greatest area of interest in microfluidics for proteomics applications is in multiplexed, heterogeneous assays. Kim et al. used microfluidic PDMS channels to pattern sol-gels on polyvinylacetate-coated glass slides under conditions mild enough to retain protein activity [23]. The use of a microfluidic channel system generated a well-defined, intricate pattern of immobilized protein that simplified data collection. Jiang et al. patterned antigens on a membrane via a microfluidic network, and

then positioned the membrane under a microdilution network to carry out serially-diluted immunoassays to detect multiple antigens on the patterned surface (Fig. 2c) [12]. This fluidic network enables the simultaneous quantitation of several molecules that vary widely in concentration – a major challenge in proteomics – in a single submicroliter sample. Brevig et al. developed a hydrodynamic microfluidic system that uses two streams of fluid to focus a sample and perform parallel immunoassays to quantitate levels of C-reactive protein, a well-known marker of inflammation (Fig. 4) [24]. As this method effectively creates a microscale stream within a larger chamber, it conserves sample but can also be carried out on whole blood without risk of clogging very small channels. In recent years, a great number of biochips have been made commercially available that use networks of microfluidic channels to perform analyses of over 100 different proteins using only 0.1 μL of whole blood [25]. These chips are capable of separating plasma from blood cells, and then directing the plasma sample across an ELISA array – the heterogeneous

array format and simplified sample prep are advantageous in terms of both speed of analysis and reduced sample consumption. This is very promising, as the cleanup of whole blood is a major bottleneck to the use of microfluidics in medical applications.

Future Directions for Research

The future of miniaturization in proteomic applications lies in achieving greater sophistication of devices. A variety of arrays have been used to investigate post-translational modifications such as phosphorylation and glycosylation, and it is only a matter of time before these are incorporated into microfluidic systems. Factors such as antibody synthesis currently limit the range of proteins that can be incorporated on an array, and devices that are capable of simultaneous quantitation of a protein and the study of its post-translational modifications, activity, etc., are not yet fully realized. Enzymatic microreactors are evolving in directions that will enable rapid screening of enzyme inhibitors for pharmaceutical purposes, industrial-scale synthesis, and environmental applications such as conversion of used grease to diesel fuel. Tools such as biochips mark the beginning of a shift from central laboratory to point-of-care testing, one of the hallmark aims of microfluidic technology. Together, these innovations will enable the development of a true total analytical system capable of greatly increasing the efficiency and throughput of any number of diagnostic, prognostic, and industrial processes.

Cross References

- ▶ Biosample Preparation Lab-on-a-Chip Devices
- ▶ Chromatographic Chip Devices
- ▶ Digital Microfluidics
- ▶ DNA Micro-arrays
- ▶ Interfaces between Microfluidics and Mass Spectrometry
- ▶ Lab-on-Chip Devices for Immunoassay
- ▶ Lab-on-Chip Devices for Protein Analysis
- ▶ Lab-on-Chip Devices for Sample Extraction
- ▶ Microfluidic Bioreactors
- ▶ Droplet Microreactors

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Proton Exchange Membrane Fuel Cells

► Microstructured Hydrogen Fuel Cells

PRT

► Resistance Temperature Detectors

Pump Head

Synonyms

Backpressure; Differential pressure; Head pressure

Definition

Pump head is the pressure rise from pump inlet to pump outlet. The *characteristic curve* of a pump relates pump head to flow rate. The pump head at which the flow rate is zero is called the *shut-off head* or *maximum head*.

Cross References

- Magnetic Pumps
- Membrane Actuation for Micropumps
- Peristaltic Pumps
- Positive Displacement Pump

Pyroelectric

► Pyroelectric Flow Sensors

Pyroelectric Flow Sensors

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Synonyms

Pyroelectric sensors; Pyroelectric; Thermal flow sensors

Definition

A *pyroelectric* is substance that generates an electrical charge during a temperature change. The *pyroelectric coefficient* is the measured change in the polarization with a variation in the temperature in a single-domain pyroelectric material. *Pyroelectric sensors*, in principle, work as thermal transducers. The pyroelectric sensing element converts a non-quantified thermal flux into an output measurable quantity like charge, voltage or current.

Overview

Pyroelectric Effect

The pyroelectric coefficient (p) of a material under constant stress and electric field is defined by the following expression:

$$p = \left(\frac{\partial P}{\partial T} \right)_{E, \sigma} \quad (1)$$

where P is the polarization, T the temperature, E the electric field and σ the elastic stress.

Pyroelectrics can be classified into two main categories:

- non-pyroelectric pyroelectrics, those whose polarization cannot be switched by an application of external electric field (such as some semiconductors and biological materials); and
- pyroelectric pyroelectrics, those whose polarization is obtained by poling and also can be switched by an electric field.

The pyroelectric effect of pyroelectric pyroelectrics usually exists below a certain transition temperature called the *Curie point*, T_c , in proper pyroelectrics and is more temperature dependent than that of the non-pyroelectric pyroelectrics.

Primary and Secondary Pyroelectric Effects

Thermodynamic analysis of the pyroelectric effect yields the expression

$$P_i^\sigma = P_i^\epsilon + d_{ijk}^T c_{jklm}^{T,E} \alpha_{lm}^\sigma \quad (2)$$

where P_i^σ is the total pyroelectric effect measured at constant stress, and P_i^ϵ , the pyroelectric effect at constant