Biosample Preparation by Lab-on-a-Chip Devices

Synonyms

Sample pre-fractionation; Preparatory separation; Microfluidic devices; Nanofluidic device; BioMEMS

Definition

Sample preparation is usually defined as a series of cellular and molecular separation/fractionation steps required or recommended in order to obtain higher sensitivity and selectivity of downstream biosensing or bioanalysis.

Overview

Given the complexity of most biological samples, sample preparation has been, and will be, one of the critical challenges in bioanalysis [1]. A general flow of proteomic sample preparation steps with subsequent detection is shown in Fig. 1.

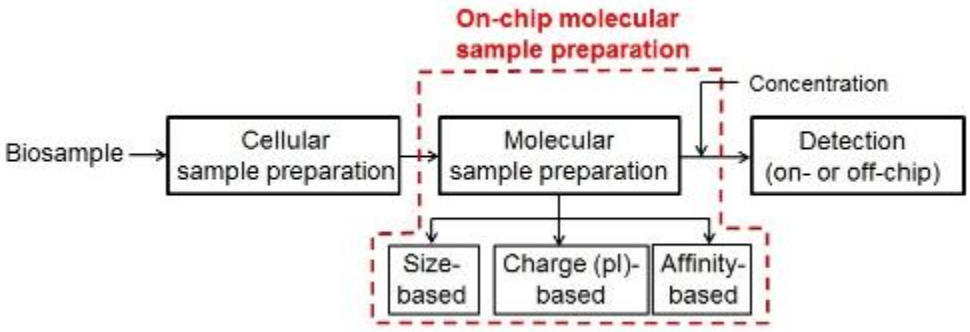

Fig. 1 General proteomic sample preparation flow

The main objective of this chapter is to review the on-chip molecular sample preparation methods. For an on-chip sample preparation for large biological objects, such as cells and particles, prior to the molecular sample preparation, there are several methods available such as dielectrophoresis, hydrophoretic filtration, and separation via magnetic forces, acoustic forces, and optical forces. These sample preparation methods are taking an increasingly important role in the on-chip sample preparation. However, a detailed description of these methods is beyond the scope of this review and the interested readers are referred to other review papers on this topic [2-5]. Typically, most biosensing involves detection of low-concentration target molecules over molecular backgrounds with much higher concentrations. In genomic biosensing, this problem is largely resolved by polymerase chain reaction (PCR), which can be used to increase the number of nucleotides with a specific sequence by several orders of magnitudes. However, in proteomic biosensing and bioanalysis, the issue of sample preparation still remains as a serious technical bottleneck, since there is no PCR equivalent for proteins and other biomolecules. For example, blood plasma or serum from any source is valuable for proteomics-based discovery of biomarkers for diseases or for discovery of novel drug targets. Detection of these proteins has potential diagnostic values; however, the major challenge is the complexity of common biomolecule samples. It is estimated that there are more than 10 000 protein species present in a serum sample. Moreover, most biomarker proteins are generally present at very low concentrations (< pg/ml), while others, such as albumin and immunoglobulins, are present in very large amounts (> mg/ml). This large concentration variation poses a formidable challenge to currently existing biomolecule detection techniques, most of which do not have low enough detection sensitivity and large enough dynamic range. It is expected, therefore, that the detection of low-abundance protein species or biomarkers would be possible only by better sample preparation and sorting. Conventionally, two dimensional (2D) protein gel electrophoresis, coupled with mass spectrometry (MS) has been the norm of proteomics research for decades, while multidimensional liquid chromatography coupled with MS is getting wider use due to ease of automation. Both techniques demonstrate similar separation peak capacity (up to ~3000) and dynamic range of detection (~10^4) when coupled with MS.

Microfluidic engineering has the potential to improve the proteomic sample preparation processes significantly, by the

automation and integration of many, laborious fractionation steps on a chip. While microfluidic devices for 2D protein separation [6] have already been demonstrated, the following technical issues still need to be addressed before the wider application of microfluidic 2D protein separation devices.

1. Preparatory separation should be able to process large sample volume. While small sample consumption is beneficial for some applications (such as single-cell analysis), most proteomic samples (blood, for example) are available in the volume larger than ~1 μL. Most microfluidic separation systems are based on miniaturized capillary electrophoresis, which is essentially an analytic (not preparatory) technique. Therefore, the overall sample throughput and the detection sensitivity in such separation are limited.
2. Any preparatory separation device should be designed with the downstream sensing in mind. Many capillary electrophoresis separations utilize polymeric sieving media or reagents like carrier ampholytes, which are detrimental to most downstream detection systems (such as MS) by causing huge background and nonspecific binding.
3. Since there is no PCR-like signal amplification process for proteins or peptides, there must be efficient sample pre-concentration steps in the overall process. The low-abundance molecules could be efficiently separated, but in order to cover several-order-of-magnitude concentration ranges, one needs to concentrate these purified dilute, low-abundance species into higher concentration.
4. Usually, more than one separation strategy would be needed to deal with the complexity of proteome. However, there is a need for developing separation techniques by pI (isoelectric point) or size (kD), rather than other properties such as hydrophobicity. One reason is that size-based separation would be an ideal method to eliminate most abundant proteins (albumin/globulin) from a given sample, which are typically larger than signaling molecules. Also, fractionation based on size (kD) or pI values will significantly reduce database searching time at the end of MS (or tandem MS)-based proteomic analysis.

The above issues come from the fact that technical requirements for preparatory separation are quite different from those of analytic separation. For the last decade, many microchip-based separation processes have been developed, demonstrating high resolution and speed. However, the requirement for preparatory separation/fractionation is different from that of analytical separation. Generally speaking, resolution is less stressed while sample throughput becomes more important. Accordingly, to meet the demand for high throughput, continuous-phase separation (free-flow electrophoresis, transverse electrophoresis) has been gaining increasing popularity. In this continuous-flow mode separation, molecules are fractionated continuously into different streams, based on different molecular properties (size, charge, electrophoretic mobility, etc.). As a result, the biomolecules are separated and flow to different output channels at the end of the main separation chamber, where the fractionated samples can be collected continuously, as shown in Fig. 2.

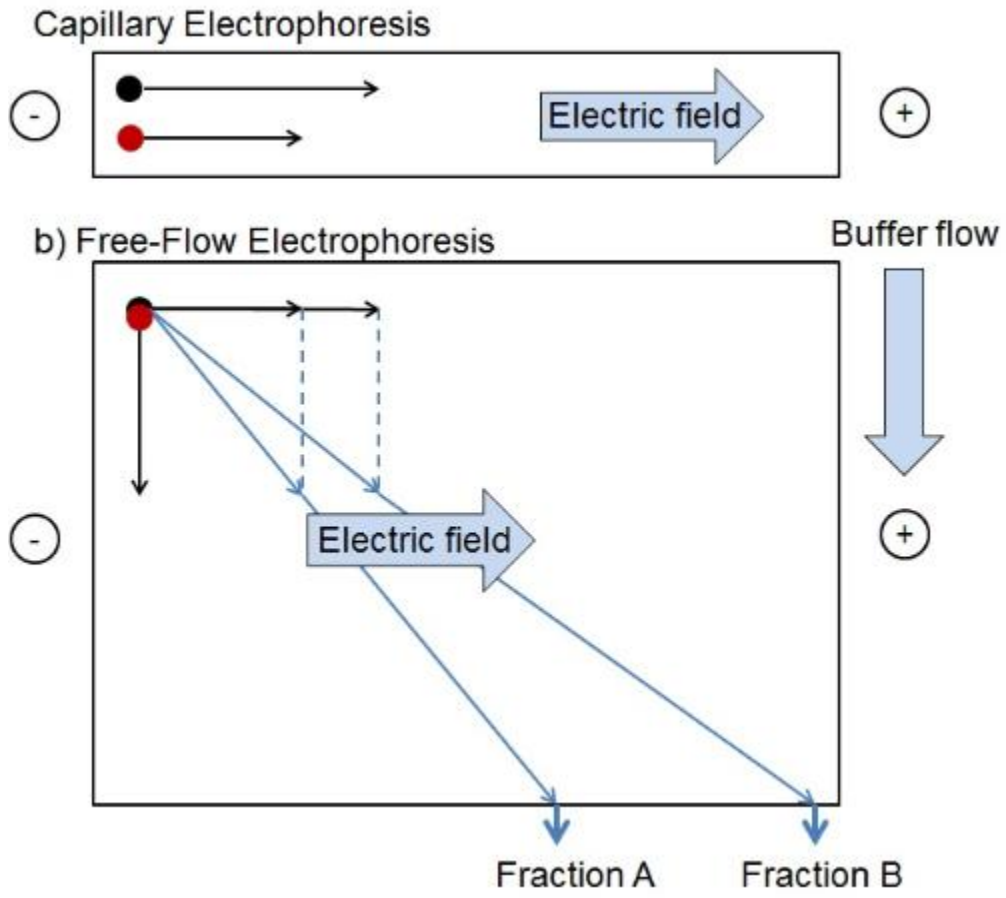

Fig. 2 Comparison between (a) batch-mode capillary electrophoresis and (b) continuous-mode free-flow electrophoresis

This eliminates the need for careful sample loading and ensures higher throughput.
Typical biosample analysis could involve many (up to ~10) steps, which are currently of manual operation. Therefore, integration of different separation and fractionation steps is highly important, in order to build meaningful sample preparation microsystems. Two main challenges are buffer exchange and flow rate (volume) matching between each component. Some of the techniques that are very useful for analytical separation are not adequate for preparatory separation, because of specific buffer requirements or reagents to be used. Efficient buffer exchange methods in a microfluidic system have not been fully explored yet, as well as desalting of the sample (required for MS-based biosensing).

Basic Methodology

Filtration and Size-Based Separation

Gel filtration chromatography and gel electrophoresis are the two most commonly used techniques for separation of biologically relevant macromolecules (such as nucleic acids and proteins) based on size. Both techniques use gelatinous materials consisting of cross-linked three-dimensional pore networks, where the sieving interactions with the migrating biomolecules determine the separation efficiency. Depending on the relative size of the macromolecule compared with the gel mean pore size (e.g., the ratio of the radius of gyration R_g of the molecule to the gel mean pore size a), three basic separation mechanisms [7] have emerged to explain how flexible macromolecules migrate through a constraining gel medium - Ogston sieving (R_g / a < 1), entropic trapping (R_g /a ~1), and reptation (R_g / a > 1). In Ogston sieving, the macromolecule is smaller than the gel pores or constrictions, and the molecular sieving occurs because of steric

interactions of the macromolecules with the gel pore network. Since $R_g / a < 1$, the molecules move rather freely through the gel matrix, assuming their unperturbed conformations. Entropic trapping applies when $R_g / a \sim 1$, and the conformation of the flexible macromolecule must deform or fluctuate to pass through the gel medium's spatial constrains. At each point, the number of accessible conformations defines the molecule's local entropy. Entropy differences derived from the gel medium's spatial heterogeneity drive molecules to partition or localize preferentially in less constrictive spaces, where their enhanced conformational freedom raises entropy. Reptation can be envisioned as a long flexible macromolecule occupying multiple pores threading its way through the gel in a snake-like fashion, which is very similar to the reptation in a tube process proposed by deGennes for entangled synthetic polymers. In reptation, only the end segments can escape as the molecule undergoes random curvilinear motion along the tube axis.

Both gel filtration chromatography and gel electrophoresis represent the current standard for size-based separation of biomolecules in laboratories. However, poor separation resolution in gel filtration chromatography and difficult sample recovery with gel electrophoresis make neither method optimal in separating complex mixtures for downstream analysis. Various microchip-based separation systems have been developed by using liquid or solid polymeric materials as sieving media contained in microchannels, and such systems have demonstrated fast separation of various biomolecules (e.g., DNA, proteins and carbohydrates) with rather high resolution [8, 9]. However, the foreign sieving matrices pose intrinsic difficulties for the integration of automated multi-step bioanalysis microsystems. Furthermore, these microchip-based systems are limited to analytical separation of biomolecules, due to the difficulty of harvesting purified biomolecules for downstream analysis.

Recently, there has been great interest in switching from disordered porous gel media to patterned regular sieving structures, in the hope of achieving more efficient separation than gels in terms of separation speed and resolution [10]. While there are many different techniques to fabricate micrometer- or nanometer-sized sieving pores, it is sometimes more important to consider and understand the detailed molecular interaction with micro- or nanopore sieves before designing a molecular filter. Biomolecule interaction with molecular filters or sieves can be quite complicated, especially for the case of biopolymers such as long DNA. Intuitions based on filtration process of macroscopic objects can be quite misleading for the interaction between nanosized molecular sieves and nanometer-sized biomolecules in liquid environments.

Charge-Based (pI-Based) Separation

The charge density of biomolecules is related to their electrophoretic mobility; therefore electrophoresis in a free solution (capillary zone electrophoresis (CZE)) can be a method to separate biomolecules based on the charge density. The free solution electrophoretic mobility μ_e is a characteristic feature of each analyte, which is determined by the complicated balance among the electrical driving force on the analyte, electrical driving force on the counterions within the Debye layer on the analyte backbone and the frictional force from the surrounding fluid. Therefore, it is not a trivial matter to determine or calculate the electrophoretic mobility of a given protein/peptide a priori from the sequence information. Also, electrophoretic mobilities of many proteins among a given proteome can be similar. Therefore, the CZE is not an ideal technique for separating a very complex protein mixture for sample preparation purposes.

The most powerful variant for charge-based separation is isoelectric focusing (IEF). To perform this separation technique, a linear pH gradient has to be established first either in the gel or in the microchannel. Once the pH gradient has been established, the biomolecules such as proteins and peptides migrate to the position where the pH equals their specific isoelectric point (pI). At this specific position, the net charge of the molecules becomes zero and they stop migrating. Molecules with different pI values focus at different positions with the pH gradient, thus allowing an effective charge-based separation of the molecules. This is a powerful fractionation technique because pI of a given target protein can be easily and accurately estimated from amino acid sequences; therefore one can collect and analyze only the pI region of interest. This could significantly cut down database searching time after MS detection, which can be quite time-consuming.

Because of its utility, IEF has been employed in many different forms even at macroscopic scales. Miniaturization of IEF would have benefits of employing small potentials. In IEF, the separation resolution does not depend of the length of the channel. The focused peak width σ can be given as the following equation:

$$\sigma = \sqrt{\frac{D(dx/d(\mathrm{pH}))}{E(d\mu/d(\mathrm{pH}))}} \sim \frac{L}{\sqrt{V}} \quad (1)$$

Here, D is the diffusion constant of the protein, E (= V / L, L is the channel length) is the electric field and μ is the mobility of the protein. While dμ / d(pH) is an inherent property of the protein, dx / d(pH) (pH gradient in length) scales as ~L for a given pH range determined by the ampholyte used in the experiment. The separation resolution R_s is given as ~d / σ, where d is the separation distance in the microchannel between the two peaks of interest. Since d also scales as ~L, R_s is only proportional to $V^{1/2}$, independent of the length of the channel L. In other words, for a given applied potential, the separation resolution does not change as the channel length is decreased, because of increased field strength in the channel makes the focused peak narrower. However, the time it takes to achieve IEF in the channel is decreased, because of shorter channel length as well as higher electric field strength.

One important consideration for the miniaturization would be the ways to achieve a pH gradient. The best resolution can be achieved by immobiline gel, where ampholyte chemical groups are polymerized into a gel. The other method to obtain a pH gradient is to use carrier ampholytes in solution form. The ampholytes are small buffer molecules with a wide range of isoelectric points that form a pH gradient when an electric field is applied. Typically, the electric field is generated between a basic solution (catholyte) such as ammonium hydroxide at the cathode and an acidic solution (anolyte), e.g. phosphoric acid, at the anode. It is possible to establish a natural pH gradient, relying on reduction-oxidation process near the electrode [11]. However, such a natural pH gradient is not stable enough for practical applications. Instead, adding carrier is preferred, in order to obtain more stable pH gradient. This is true even when immobiline gel is used. More stable separation is achieved by adding carrier ampholytes to the gel solution. Therefore, the use of carrier ampholytes has been the method of choice for microfluidic IEF devices. This, however, can interfere with subsequent analysis such as MS.

Isotachophoresis (ITP) utilizes two different buffer systems, one as the leading electrolyte and the other as the trailing electrolyte. The leading electrolyte has a higher mobility than that of the analytes while the trailing electrolyte has a lower mobility. When an electric field is applied, the ions of the leading electrolyte migrate fastest and those of the trailing electrolyte slowest. Then, the ions of the analytes spread into the gradient of the electrical strength set by the mobilities of the terminating electrolytes. However, for practical applications, it is often difficult to find the appropriate terminating electrolytes with the required mobilities. The other limiting factor for this separation technique is that the ions have to be of the same polarity in order to be separated.

Counter-flow gradient focusing such as electric field gradient focusing (EFGF) and temperature gradient focusing (TGF) is another focusing method that is based on a balance between electrophoretic motion of an analyte and combined hydrodynamic and electroosmotic counter flow in the opposite direction to that of the electrophoretic migration. Along the gradient generated in the channel, either of electric or temperature field, there is a point where the sum of both velocities becomes zero and the analyte becomes focused. Like IEF and ITP, this method offers an advantage to separate and concentrate analytes at the same time.

Affinity-Based Separation

The affinity-based sample preparation method on microchips is another powerful tool for separation of biomolecules. Conventional affinity-based separation methods such as liquid chromatography or affinity chromatography have been successfully implemented in microfluidic chip format. The way the chromatography works in the microchannel is essentially the same as in the conventional method. In capillary electrochromatography (CEC), which combines the separation power of both liquid chromatography and capillary electrophoresis, the analyte is forced through a column of the stationary phase by electroosmosis instead of pressure. After this step, the gradient elution with a varying solvent composition, for instance 5% to 50% methanol, flows across the column and separates the analyte mixture depending on how well it mobilizes the analyte. The more hydrophobic component will elute first if the methanol content is high. However, when the methanol concentration is low, the hydrophilic analyte will elute more readily. The affinity chromatography is based on the specific interaction between an immobilized ligand and the target protein to be separated.

The last two variants of affinity-based separation are micellar electrokinetic chromatography (MEKC) and

electrochromatography (EC). MEKC uses surfactants which form micelles as pseudo-stationary phase in the microchannel. During electrophoresis, the analytes partition into the micelles depending on their hydrophobicity. Through the interaction with the micelles, the retention time of the molecules can be increased. In this way, even neural molecules can be separated. Electrochromatography utilizes an electrokinetic flow instead of pressure-driven flow to bring the mobile phase through a packed bed consisting of silica with a large negative surface charge. This induces an electro-osmotic flow (EOF) which drives the separation. A plug-like velocity profile brings a higher efficiency than HPLC.

Signal Amplification and Preconcentration

Several research groups have reported ways to preconcentrate samples in "Lab-on-a-Chip" devices. While most on-chip preconcentration approaches evolve from conventional capillary electrophoresis and chromatographic column techniques, these preconcentration techniques play an increasingly important role in chip-based system. The basic preconcentration strategies applied on microfluidic devices can be classified into three large categories: electrokinetic preconcentration, chromatographic preconcentration and membrane preconcentration.

Electrokinetic Preconcentration

Field-amplified stacking (FAS) (Fig. 3a) is a technique with a long history, first introduced by Mikkers et al. in the late 1970s [12].

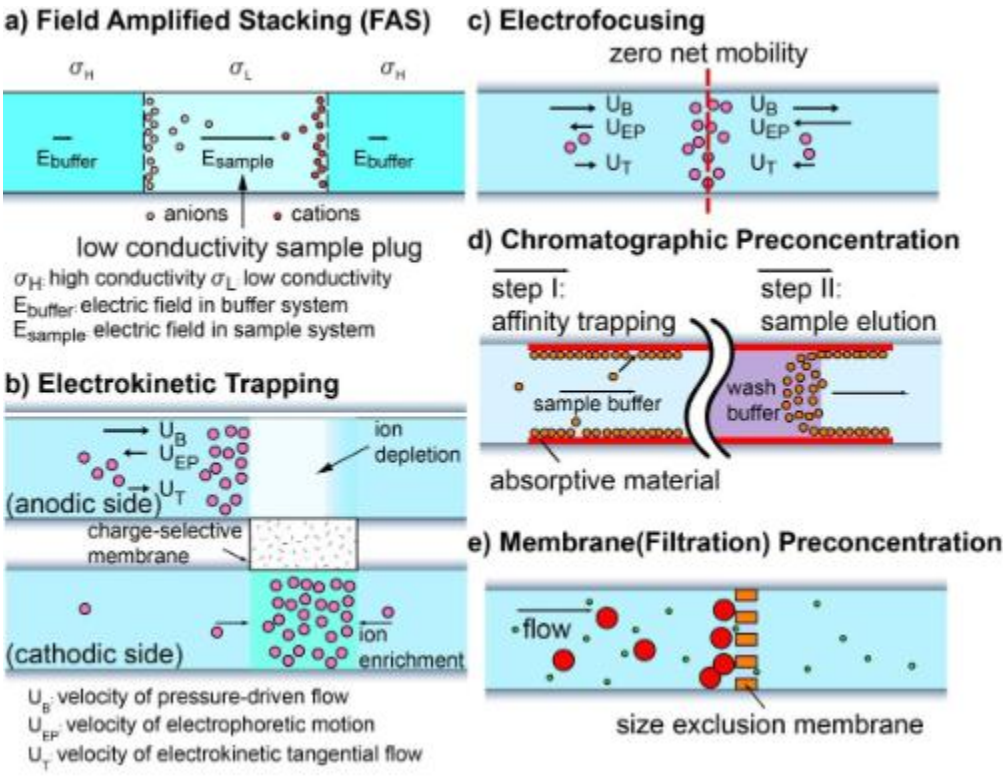

Fig. 3 Various types of preconcentration strategies. (a) Field amplified stacking, (b) electrofocusing (various types), (c) electrokinetic trapping (both anodic and cathodic side), (d) chromatographic preconcentration and (e) membrane preconcentration

The mechanism relies on manipulating buffer concentration to achieve local field amplification. The relation between the electrical field (E) and buffer concentration (C) can be defined by the relative conductive (γ) as:

$$\gamma = \frac{c_L}{c_H} = \frac{\sigma_L}{\sigma_H} = \frac{E_{\text{buffer}}}{E_{\text{sample}}} \quad (2)$$

As a result, when we introduce low conductivity (σ_L) sample plug into capillaries or microfluidic channels with high conductivity running buffer, most of the potential drop will be applied onto the injected sample. This locally amplified field will therefore drive/stack sample to the ends of the plug by electrophoretic force. With a given plug size, one can increase the enhancement factor by increasing the relative conductivity. Even though FAS is one of the simplest preconcentration schemes to implement on microchips, the band broadening at the stacking boundary caused by hydrodynamic mixing from both flow injection and mismatched EOF makes it difficult to have highly focused peak.

Transient isotachophoresis and micellar electrokinetic sweeping can be viewed as extensions of the stacking concept of FAS. In the case of isotachophoresis, with the understanding of a sample's electrophoretic mobilities, the sample plug is sandwiched by leading electrolyte (LE) and terminating electrolyte (TE) instead of the same high conductivity buffer. Based on the order of descending mobilities, the constituents will separate into distinct zones between high mobility LE and low mobility TE (relative to sample constituents) upon the application of the separation voltage. Moreover, once the steady-state gradient is achieved, the boundaries between samples can be maintained by a self-focusing mechanism which alleviates the dispersion problem from which FAS suffers. Both FAS and isotachophoresis use buffer manipulation to achieve local field enhancement that helps sample stacking. Micellar electrokinetic sweeping, on the other hand, works on changing the electrophoretic mobilities of samples by labeling them with micelle compounds (surfactants). Once the correct compound is chosen, one can use a small plug of fast moving micelle compounds to sweep samples rapidly out of the sample zone by hydrophobic interaction. The enhancement factor of micellar electrokinetic sweeping can be further improved by coupling with FAS, however, it is still limited by analytes' affinity to the micellar compounds.

While FAS and its related techniques are well-established, their merit for sample preparation device is limited since these techniques usually require special buffer arrangements or reagents in the system. Electrofocusing (Fig. 3b) is another class of techniques used to separate and concentrate analytes by manipulating both hydraulic and electrophoretic driving forces in the microchannel. Electrofocusing can occur whenever the net molecular velocity profile is converging, as shown in Fig. 3b, by either controlling the flow and/or electric field of the two zones. Such a condition can be obtained by changing channel geometry, electric field of each zone or temperature of each zone. Electrofocusing can be achieved in a continuous fashion (collection of molecules until enough concentration is reached), and does not require any special buffers or ionic strength arrangements. However, the collection would be critically dependent on the specific electrophoretic mobility of the target.

Another novel electrokinetic preconcentration technique is electrokinetic trapping (Fig. 3c), utilizing unique electrokinetic properties of perm-selective membranes. As a perm-selective membrane, one can use traditional membranes (such as Nafion®), nanochannels or charged polymer monoliths. When a current is applied through such a perm-selective membrane/nanochannel, concentration polarization of ions can occur (even at moderate buffer concentrations), causing the ion concentration of anodic side to decrease (ion depletion) and that of cathodic side to increase (ion enrichment). Both phenomena can be (and have been) utilized for concentrating biomolecules. In the cathodic side, biomolecules and ions can be enriched due to the ion enrichment process. In the anodic side, ion depletion region can be used in a similar manner as a stacking boundary, although the physical mechanism in this case is different from FAS. Unlike FAS, both cations and anions are collected at the same boundary. This is because both ionic species are repelled by concentration polarization process from the ion-selective membrane/nanochannel. The advantage of electrokinetic trapping is that the preconcentration can be less sensitive to specific molecular electrokinetic properties (such as electrophoretic mobility), therefore providing a generic way for various types of molecules. However, concentration polarization and related phenomena are generally poorly understood, and the linearity and stability of the trapping is sometimes an issue.

Chromatographic Preconcentration

Chromatographic preconcentration (Fig. 3d) is also called solid-phase extraction. It usually involves two steps. First, analytes are retained by affinity binding force onto an appropriate stationary subject. Then, with the application of elution buffer, the analytes can be eluted into a more concentrated form. Non-microfluidic examples of this strategy are widely used, such as commercially available trap column for mass spectrometry and SELDI (surface-enhanced laser desorption and ionization)-MS. Affinity reagents and bead systems are well-developed for trapping proteins and peptides and commercially available, which makes its implementation to microfluidic format rather straightforward. Also, chromatographic preconcentration techniques can also desalt biosamples, which is another important benefit especially for MS. One of the drawbacks of chromatographic preconcentrations, however, is that the preconcentration can be biased

(hydrophobic vs. hydrophilic), and the washing (elution) step could potentially re-dilute the concentrated plugs. In addition, chromatographic preconcentration can be limited by the number of binding sites (binding surface area) in the systems.

Membrane Preconcentration

In membrane preconcentration (Fig. 3e), gel or porous membranes are used to concentrate molecules bigger than the size of the pores. By adjusting the pore size, one can allow the passage of buffer ions and small molecules but exclude larger molecules of interest. With the formation of nanofilters or nanoporous membranes within the microfluidic systems, this strategy can be implemented easily. Membrane (filtration)-based preconcentration will not have any chemical bias (mainly dependent on the size of the molecule), but continuous membrane filtration could generate eventual clogging of the system, which is one of the main problems in this technique.

Key Research Findings

Filtration and Size-Based Separation

For size-separation of biomolecules, it is imperative to have a molecular sieving structure incorporated in the system. Formation of polyacrylamide gel in a microchannel is one viable option, but solid-state, artificial sieving systems are much preferred due to their mechanical and chemical stability. Artificial nanosized molecular sieving systems can be fabricated using a variety of techniques [13-18], that can be subdivided into lithographic methods, e.g., standard photolithography and e-beam lithography, and non-lithographic methods such as colloidal templating, anodized alumina pore fabrication, packed nanospheres, and superparamagnetic particle arraying.

Regular arrays of micrometer- or nanometer-sized pillars have been fabricated by different groups with either photolithography (with pillar diameter and spacing down to1μm) or e-beam lithography (with pillar diameter and spacing down to 100nm) on silicon substrates. The advantages of such microlithographically fabricated devices include the precise control over the sieving matrix geometry and the design flexibility. By applying two alternating electric fields of different directions and different magnitudes, Huang et al. recently devised a DNA prism device that can continuously separate long DNA molecules with high speed [19]. In this design, the longer DNA molecules only follow the strong electric field component while the shorter ones migrate in the direction of the sum electric field vector. However, their application was largely limited to rather large biomolecules (long DNA).

The concept of Brownian ratchets has been applied to construct asymmetric obstacle courses that provide a spatially asymmetric steric potential for biomolecule separation [20, 21]. The basic idea is to use such asymmetric obstacles to rectify the Brownian motion laterally and thereby to deflect diffusing biomolecules based on their sizes. So far, the Brownian ratchet systems have been successfully demonstrated for long DNA and phospholipids [15,16], even though the separation resolution reported so far was not ideal.

More recently, a microfabricated nanofilter array system was developed, which can be used for separating various biomolecules such DNA molecules and proteins [22-24]. The unique feature of this class of molecular filters is that only standard photolithography is needed to create ~10nm sized molecular filters. Using the same system, one can employ different sieving mechanism. For long DNA molecules, entropic trapping mechanism is used, while small DNA and proteins can be separated by Ogston sieving mechanism. These devices demonstrated speed and resolution comparable to or better than the conventional techniques (i. e. pulsed field gel electrophoresis for long DNA, and capillary gel electrophoresis for proteins). One important advantage of a nanofilter array system is that the separation efficiency could be further improved by increasing the nanofilter density, by using advanced high-resolution (photo or e-beam) lithography techniques.

In terms of sample preparation, continuous-flow separation is also highly desirable for micro-/nanofluidic devices because of the low sample throughput. We choose two recent developments here that we believe represent the current advance of this particular exciting area: the Tango device (or the bump array) (by Huang et al. [25]) that separates long DNA molecules by asymmetric bifurcation of laminar flow [25], and the anisotropic nanofilter array (ANA) (by Fu et al.) that separates DNA and proteins based on the different sieving characteristics along two orthogonal directions within the ANA structure [23]. The Tango device employed arrays of micrometer-sized pillars and spacing, with each pillar column slightly shifted with respect to the previous one in a direction perpendicular to the flow direction. Longer DNA molecules are displaced as they flow through the pillar array while shorter DNA molecules remain in the feeding streamlines (i. e. the deterministic lateral displacement), leading to efficient separation. It is believed that as the gap size of pillar array is

reduced using nanofabrication, the Tango device can be used to fractionate biologically relevant molecules. The design of the ANA consists of a 2D periodic nanofilter array, and the designed structural anisotropy in the ANA causes different sized biomolecules to follow radically different trajectories leading to separation. So far, continuous-flow Ogston sieving-based separation of short DNA and proteins as well as entropic trapping-based separation of long DNA molecules have been demonstrated with the ANA structure. The design of the structural anisotropy is the key for the continuous-flow biomolecule separation, and it can be applied to any sieving mechanism across the nanofluidic sieving structure along the orthogonal direction (size-, charge- or hydrophobicity-based) that can lead to differential transport across the nanofilters. Highly efficient, continuous-flow molecular separation would be possible as long as one can create a separation system that is anisotropic in nature.

However, there is a limitation in terms of the maximal achievable sample throughput rate because of the planar nanochannel with a nanometer-scale cross-sectional area for separation. The maximum flow rate achievable with the planar nanochannel device was only 1 nL/h. To further increase the flow rate, Pan et al. built a massively parallel vertical nanoarray [26]. To decrease the gap size of photolithographically patterned microchannels with a gap size of ~1 μm, a thermal oxidation process was applied on Si with a volume expansion of about a factor of 2.3. By controlling the oxidation time, the gap size of the vertical nanochannels could be controlled down to below 100 nm and the nanochannels could be built with a uniform gap size of 55nm and an aspect ratio (depth-to-width ratio) as high as 400 [26]. Using the massively parallel high-aspect ratio nanochannels, the sample processing rate could be increased by 1000-fold up to 1 mL/h. Another strategy to increase the sample throughput is to implement an out-of-the-plane design by sandwiching a nanoporous membrane layer between two device layers so the membrane is facing perpendicular to the flow direction. In this way, the surface area for filtration can be increased substantially. One of these so-called 3D nanofiltration device has been demonstrated in [27]. The membrane used was 4 μm thick polysilicon diaphragm, consisting of an array of 10 nm wide and 45 μm long slit pores with a 2 μm offset. It was used for hemodialysis and hemofiltration.

In contrast to the lithographically patterned nanostructures, as described above, non-lithographically patterned nanostructures are an interesting alternative approach for fabricating nanoporous sieving matrices for molecular filtration. A clear advantage of this approach is that it doesn't require sophisticated nanofabrication facilities and time-consuming and expensive fabrication processes. Based on the self-assembly process inside the microchannels driven by evaporation, solid-state sieving matrices out of polymer or silica colloids and magnetic bead columns have been developed for nanofluidic size-based separation. As for the colloids, ~ 1.5μm monodisperse plain polystyrene (PS) microspheres and 900nm silicon beads were used. An electrophoretic separation of a low DNA mass ladder was demonstrated using a 900nm silica bead matrix at 15 V/cm. Using a matrix of 330nm silica spheres, a mixture of four proteins with 20-205 kDa was also separated successfully. The resolution was 2.64 between the 20.1- and 116-kDa proteins and 3.92 between the 116- and 205-kDa proteins, respectively [28]. With the magnetic bead (average bead size 570nm) columns, it was possible to separate λ-phage, 2λ-DNA, and bacteriophage T4 DNA [29]. Baba group used a similar approach to build a solid sieving matrix out of core-shell type nanospheres [18]. DNA fragments up to 15 kbp were analyzed within 100 s under a pressure of 2.5 kPa for 1s.

Charge-Based Separation

IEF-based sample fractionation devices have been developed and commercialized, but miniaturizing them into a microfluidic format is being studied actively. An advantage to scale down the IEF-based separation is that the Joule heating can be minimized due to the large surface to volume ratio, therefore, higher electric field than in macroscale separation devices can be used which increases the separation efficiency. Especially, there have been tremendous efforts to increase the throughput of charge-based separation by operating the microfluidic device in a continuous mode (see Fig. 2b). The critical component of a 2D continuous flow separation chip is a membrane between the separation chamber and the electrode reservoirs with sufficient hydrodynamic resistance to pressure while allowing efficient coupling of the electrical field into the separation process. Several solutions have been proposed such as small, large microchannels, UV cured acrylamide, nitrocellulose, or agarose gel or a glass wall [30].

The Yager group from the University of Washington fabricated a multi-stacked Mylar continuous-flow separation device with palladium electrodes in direct contact with the solution. Since the electrodes were in direct contact with the solution, only a small voltage, 2.3V, could be applied over a 1.27mm wide channel [11]. Because of the small distance, however, it generated sufficient electric field, ~18V/cm, for the separation of a binary mixture of proteins. The same group used the electrolysis of the buffer, H^+ at the anode and OH^- at the cathode, to generate a natural pH gradient across the microchannel without using any carrier ampholytes [11]. This low-voltage approach, however, requires a significant

amount of time for separation at around 4 minutes. To reduce the separation time, Zhang et al. developed a high-voltage μ-FFE (free flow electrophoresis) device by isolating the separation channel from the electrode reservoirs using narrow (4μm wide) microchannels [31]. Their FFE microchip with 10μm deep channels in a polydimethylsiloxane (PDMS) substrate allowed an electric field up to 270V/cm and they could successfully separate two fluorescent dyes, rhodamine 110 and fluorescein, as well as two amino acids in 2s. However, the high voltage induced a strong EOF which deteriorated the separation result. The μ-FFE device developed by Kohlheyer incorporates two new improvements compared to the previous ones [32, 33]. First, it is a multifunctional μ-FFE device which can perform free-flow zone electrophoresis and free-flow IEF. Depending on the separation method to be used, the only parameter to be changed is the width of the sample by hydrodynamic focusing. This can be performed easily on the run by changing the flow rates in the two side channels. Second, they implemented a polymerized acrylamide as the salt bridge between the main separation channel and the electrode reservoirs. This proved to be more effective in suppressing the EOF than the microchannel as the salt bridge.

Song et al. developed a simple fabrication method to create a salt bridge for free-flow zone electrophoresis in PDMS chips by surface printing a thin hydrophobic layer on a glass substrate. Since the surface-printed hydrophobic layer prevents plasma bonding between the PDMS chip and the substrate, an electrical junction gap can be created for free-flow zone electrophoresis. With this device, they demonstrated a separation of positive and negative peptides and proteins at a given pH in standard buffer systems and validated the sorting result with LC/MS. Furthermore, they coupled two sorting steps via off-chip titration and isolated peptides within specific pI ranges from sample mixtures, where the pI range was simply set by the pH values of the buffer solutions. This free-flow zone electrophoresis sorting device, with its simplicity of fabrication, and a sorting resolution of 0.5 pH unit, can potentially be a high-throughput sample fractionation tool for targeted proteomics using LC/MS [34]. An electrostatic induction through a glass wall membrane enabled 50% coupling of the applied electric field for free-flow electrophoresis without any cross flow between the separation channel and the reservoirs [35]. Isotachophoresis (ITP) has been also realized in microchip format for the free-flow electrophoresis [36]. A sample mixture of fluorescein, eosin G and acetylsalicylic was separated in less than a minute. Song et al. developed a novel ampholyte- and gel-free pI-based continuous-flow sorting technique [37]. Their method differs from previous approaches in that this continuous sorting process involves no external power supply and no special ampholytes. Instead, they utilized the diffusion potential generated by the diffusion of different ionic species in situ at the laminar flow junction.

Free-flow electrophoresis has also been successfully applied to separate subcellular particles such as organelles (mitochondria) [38]. This is facilitated by the fact that these particles contain many different proteins and amphoretic molecules. The sorting in microscale devices offers obvious advantages compared to the conventional ones. Less heat is generated by using only ~2V compared to 2000V of conventional devices and this causes less damage of organelles. The result shows that free-flow electrophoresis can be applied to various organelles, even for organelles that are larger and do not have uniform pI values.

There have been several attempts to combine IEF and capillary gel electrophoresis (CGE) on a single chip [39-41]. Resembling 2D gel electrophoresis, the electrokinetically focused proteins were transferred into orthogonal microchannels directly for subsequent capillary gel electrophoresis. Wang et al. developed a two dimensional separation chip by integrating an active PDMS micro valve [42]. A 2D integration of micelle electrokinetic chromatography (MEKC) in the first dimension and capillary zone electrophoresis (CZE) in the second dimension has been shown in [6]. A 2D chip combining SDS (sodium dodecyl sulfate) μ-CGE in the first dimension and MEKC in the second dimension has been built in PMMA [43].

Affinity-Based Separation

While the technology for affinity-based separation is very well developed for capillary-based separation, chromatography in microchips has not been as popular as electrophoresis, mainly due to the difficulty in packing microchannels with beads. In particular, if the channels are not straight but are curved into serpentine configurations, it is quite a challenge to achieve a uniform packing of the channels. One of the promising techniques is the sol-gel technology since it doesn't require any frits to pack particles inside the microchannel [44]. To deposit a solid phase structure out of reversed-phase stationary phase particles (5 μm, C4) on the substrate, a micropatterning technique was applied using a reversibly bonded PDMS sheet. Its proof of concept was demonstrated with a mixture of thiourea and the peptides (Trp-Ala, Leu-Trp

and Trp-Trp) under isocratic chromatographic conditions. Instead of packing affinity beads, creating a polymer monolith [45, 46] is much preferred, with controlled, relatively uniform pore sizes, variety of chemical groups one can incorporate into the system, and covalent linkage to the channel wall (therefore no need of frits to hold the monolith stationary).
Often, fittings for microfluidic systems cannot handle the high pressure required for chromatographic separation, and electrokinetic flow can be used in lieu of pressure-driven flow. Using the microchip chromatography, driven either by elecroosmosis or pressure, a separation of low-molecular-weight neutral and basic compounds and acidic proteins has been demonstrated. He et al. demonstrated CEC of peptides in a microfabricated system [47]. They mimicked the packed bed by etching an array of support structures into a quartz substrate, the so-called collocate monolith support structures. These columns were prepared with polystyrene sulfate for stationary phase. A mixture of tryptic peptides from ovalbumin was separated in the CEC isocratically as opposed to the gradient elution mode.
In capillary-based separations, technologies based on microbeads with specific chemistry are well-developed and mature, and utilizing such bead systems in microfluidic channels would be of tremendous value. Oleschuk et al. developed a design that allows exchange of packing materials [48]. This can be utilized for solid-phase extraction (SPE) and CEC. Using CEC, a separation of BODIPY and fluorescein could be achieved with a mobile-phase composition of 30% acetonitrile / 70% aqueous 50mMammonium acetate. The BODIPY is hydrophobic and has a higher affinity for the column than the fluorescein, causing slower elution of BODIPY. In addition to affinity-based separations, microchannels with packed beads could provide other functionalities for microfluidic sample preparation systems, such as peptide digestions [49, 50], removal of majority proteins and extraction of DNA from cells [51]. It was shown that peptide digestion reaction can be significantly expedited (~10min) compared with solution-phase digestion process.

Signal Amplification and Preconcentration

Given the importance of sample preconcentration in many biosensing applications, several sample preconcentration techniques, including field-amplified sample stacking (FAS), isotachophoresis (ITP), electrokinetic trapping, micellar electrokinetic sweeping, chromatographic preconcentration, electrofocusing (various types) and membrane filtration preconcentration have been developed. Each technique has its own advantages and disadvantages.Since protein samples are often dilute and the amount of sample injected into the microfluidic device is limited, an online protein concentration prior to electrophoretic separation steps is also required.

Field-Amplified Stacking and Other Related Techniques

FAS can be implemented on microchips in a very similar manner as capillary electrophoresis. However, the requirement of low ionic strength sample buffer for FAS puts limitation on its use as general preconcentration technique. Variations of the technique, such as transient isotachophoresis and micellar electrokinetic sweeping, have been more successfully used. Jung et al. reported on-chip transient isotachophoresis by introducing TE and LE into a T-junction simultaneously to achieve fast sample loading, preconcentration and separation [52]. By combining ITP with CE on a microfluidic chip, they demonstrated millionfold concentration increase of 100 fM Alexa Fluor 488 by a factor of 2 x 106 within 2 min. and an LOD of 100 fM. Micellar electrokinetic sweeping, pioneered by the Terabe group, combines field-amplified stacking with affinity concentration using micelles [53], and provides very high concentration factors. These techniques were originally developed for capillary electrophoresis; therefore they are well suited for enhancing the sensitivity of microchip-based separation and detection. However, they require special arrangements of buffers with different ionic concentrations, which makes the coupling to the downstream biosensing challenging, limiting their use as sample preparation devices. For example, micellar electrokinetic sweeping relies on a detergent additive (sodium dodecyl sulfate), which has a negative impact on the downstream analysis.

Electrofocusing and Electrokinetic Trapping

Newer techniques such as electrofocusing and electrokinetic trapping could be ideal alternatives for proteomic sample preconcentration. One of the benefits electrofocusing offers is that the collection can be run continuously, and therefore, concentration can be arbitrarily increased (with a limitation imposed by crystallization and other technical issues). Electrofocusing can continuously collect molecules by applying two different (electrophoretic and hydraulic, typically) driving forces in the opposite direction in a microchannel or capillary to trap molecules. Various types of electrofocusing [54-59], which differ in the method to generate a gradient in electrophoretic mobility (temperature [60, 61], electric field

[58, 59], etc.), have been demonstrated in microfluidic and capillary systems. Depending on the focusing time, these techniques can achieve typically up to~105-fold concentration enhancement. An detailed review on different electrofocusing methods can be found in [62].

Electrokinetic trapping techniques [63-74] have been recently demonstrated as an efficient way of concentrating protein samples. Different membrane materials can be used, such as polymer monolith (Singh and coworkers), Nafion® (Swerdlow and coworkers), nanochannels in silicon and nanofluidic Nafion membrane (Han and coworkers), porous silica membrane (Ramsey and coworkers) and even nanochannels created by reversible bonded PDMS on a glass substrate (Hasselbrink and coworkers). A detailed review on the nanochannel- and Nafion-membrane-based electrokinetic concentrators with regard to fabrication and applications can be found in [75]. These techniques demonstrate impressive concentration factors (up to ~10^6) as well as the flexibility to be coupled to downstream analysis. These techniques are dependent on the ion depletion and concentration polarization, which as a generic process are quite common to most nanoporous membrane systems. Therefore, there is no specific buffer requirement, as long as the ionic strength is moderate (~10mM or less). An interesting observation from the electrokinetic trapping-based concentration was that the concentration enhancement factor seemed to be dependent on the initial sample concentration. For an initial concentration of 33fM of GFP, an increase of 10^7 times within 40 min. was obtained while the enhancement factor for 33 pMol was only 7 x 10^4 times [76]. A similar dependence of the concentration enhancement factor was observed by Kim et al. with the PDMS concentrator chip [72]. Shackman et al. calculated that the highest concentration enhancement factor reported corresponds to a velocity of 8 cm/s for a focused plug width of 20 μm [62]. This velocity would require electric field strength of over 40 000 V/cm. Since the voltage applied was only 30V, the most plausible explanation according to them was that the ion depletion zone was acting probably as a highly efficient pump and transported the molecules against the stacking zone at the speed mentioned above. Electroosmosis of the second kind was cited as a potential explanation for this phenomenon [62]. However, the reason for the dependency of the concentration speed on the initial sample concentration is still unknown and needs further investigations.

Chromatographic Preconcentration/Membrane Preconcentration

Several groups [77, 78] have demonstrated affinity-based molecular preconcentration systems in a microfluidic format. Affinity chemical groups can be directly coated to the surface of the glass microchannel, although techniques using microbeads (Harrison and coworkers) and polymer monoliths (Frechet and coworkers) provide better functionality, larger binding surface area and flexibility in fabrication and integration. The concentration factor in these systems is eventually determined by the surface binding area. For efficient capturing from larger sample volume (~1μL or more), a larger microchannel is required.

Membrane preconcentration [79-82] is a microscale version of membrane filtration and dialysis, which is well established. Typically gel or other polymeric materials (Singh and coworkers) as well as nanofluidic channels/solid membranes (Ramsey and coworkers) are used as a molecular filter. Hatch et al. demonstrated an integration of a size-exclusion membrane (MW cutoff ~ 10 kDa) with a cross-linked polyacrylimde inside a single glass microchip to perform a preconcentration with a subsequent separation. This device allowed a fast concentration over 1000-folds within less than 5 min., enabling a detection of less than 50fM with 30 min. of preconcentration [81]. For the size-exclusion membrane, a degassed solution of 22% acrylamide/bisacrylamide/ (15.7:1) with 0.2 w/v photoinitiator was used. The sieving matrix was photopolymerized after filling the channel with 0.2% (w/v) photoinitiator in 1 x Tris/glycine/SDS buffer. Through this on-chip integration of concentration and separation, an improved efficiency and resolution have been achieved. As this examples shows, an advantage of this technique is that one can combine preconcentration with filtration/separation in a single step. A disadvantage of this technique would be that it becomes progressively more difficult for smaller proteins and peptides, and it is also limited by the ambiguity of the molecular weight cut-off of the nanoporous filter membrane materials. Even in the case of regular nanofilters, molecular size filtration of biomolecules can be quite complicated due to conformation changes of biomolecules.

Future Directions for Research

The importance of sample preparation in bioanalysis is expected to draw more attention in the future, since this is currently one of the major bottlenecks in biosensing. For preparatory separation, continuous-flow fractionation is much

preferred over elution-type separation, due to the flexibility of integration and higher sample processing rates. More and more fractionation techniques would be made into a continuous-flow format, possibly by adopting anisotropic sieving system designs. Since typical sample preparation could easily involve many (up to ~10) different separation/reaction/preconcentration processes, integration of these individual steps on a single device would be highly desirable for practical application. Recent developments in sample preconcentration devices have the potential to enhance any new and existing biosensors in terms of sensitivity and selectivity, if properly integrated with preparatory separation steps.

Cross References

Electrokinetic Focusing
Electrokinetic Flow and Ion Transport in Nanochannels

References

1. Mariella, R., Sample preparation: the weak link in microfluidics-based biodetection. Biomedical Microdevices, 2008. 10(6): p. 777-784.
2. Gervais, L., N. de Rooij, and E. Delamarche, Microfluidic Chips for Point-of-Care Immunodiagnostics. Advanced Materials, 2011. 23(24): p. H151-H176.
3. Lim, Y.C., A.Z. Kouzani, and W. Duan, Lab-on-a-chip: a component view. Microsystem Technologies-Micro-and Nanosystems-Information Storage and Processing Systems, 2010. 16(12): p. 1995-2015.
4. Pamme, N., Continuous flow separations in microfluidic devices. Lab on a Chip, 2007. 7(12): p. 1644-1659.
5. Hou, H.W., et al., Microfluidic Devices for Blood Fractionation. Micromachines, 2011. 2(3): p. 319-343.
6. Rocklin, R.D., R.S. Ramsey, and J.M. Ramsey, A Microfabricated Fluidic Device for Performing Two-Dimensional Liquid-Phase Separations. Analytical Chemistry, 2000. 72(21): p. 5244-5249.
7. Viovy, J.L., Electrophoresis of DNA and other polyelectrolytes: Physical mechanisms. Reviews of Modern Physics, 2000. 72(3): p. 813-872.
8. Woolley, A.T. and R.A. Mathies, Ultra-High-Speed DNA Fragment Separations Using Microfabricated Capillary Array Electrophoresis Chips. Proceedings of the National Academy of Sciences of the United States of America, 1994. 91(24): p. 11348-11352.
9. Yao, S., et al., SDS capillary gel electrophoresis of proteins in microfabricated channels. Proceedings of the National Academy of Sciences of the United States of America, 1999. 96(10): p. 5372-5377.
10. Volkmuth, W.D. and R.H. Austin, DNA Electrophoresis in Microlithographic Arrays. Nature, 1992. 358(6387): p. 600-602.
11. Macounova, K., et al., Generation of natural pH gradients in microfluidic channels for use in isoelectric focusing. Analytical Chemistry, 2000. 72(16): p. 3745-3751.
12. Mikkers, F.E.P., F.M. Everaerts, and T.P.E.M. Verheggen, High-Performance Zone Electrophoresis. Journal of Chromatography, 1979. 169(Feb): p. 11-20.
13. Turner, S.W., et al., Monolithic nanofluid sieving structures for DNA manipulation. Journal of Vacuum Science & Technology B, 1998. 16(6): p. 3835-3840.
14. Han, J. and H.G. Craighead, Entropic trapping and sieving of long DNA molecules in a nanofluidic channel. Journal of Vacuum Science & Technology a-Vacuum Surfaces and Films, 1999. 17(4): p. 2142-2147.
15. Doyle, P.S., et al., Self-assembled magnetic matrices for DNA separation chips. Science, 2002. 295(5563): p. 2237-2237.
16. Sano, T., et al., Size-exclusion chromatography using self-organized nanopores in anodic porous alumina. Applied Physics Letters, 2003. 83(21): p. 4438-4440.
17. Baba, M., et al., DNA size separation using artificially nanostructured matrix. Applied Physics Letters, 2003. 83(7): p. 1468-1470.
18. Tabuchi, M., et al., Nanospheres for DNA separation chips. Nature Biotechnology, 2004. 22(3): p. 337-340.
19. Huang, L.R., et al., A DNA prism for high-speed continuous fractionation of large DNA molecules. Nature Biotechnology, 2002. 20(10): p. 1048-1051.

20. Chou, C.F., et al., Sorting by diffusion: An asymmetric obstacle course for continuous molecular separation. Proceedings of the National Academy of Sciences of the United States of America, 1999. 96(24): p. 13762-13765.
21. van Oudenaarden, A. and S.G. Boxer, Brownian ratchets: Molecular separations in lipid bilayers supported on patterned arrays. Science, 1999. 285(5430): p. 1046-1048.
22. Han, J. and H.G. Craighead, Separation of long DNA molecules in a microfabricated entropic trap array. Science, 2000. 288(5468): p. 1026-1029.
23. Fu, J.P., et al., A patterned anisotropic nanofluidic sieving structure for continuous-flow separation of DNA and proteins. Nature Nanotechnology, 2007. 2(2): p. 121-128.
24. Kaji, N., et al., Separation of long DNA molecules by quartz nanopillar chips under a direct current electric field. Analytical Chemistry, 2004. 76(1): p. 15-22.
25. Huang, L.R., et al., Continuous particle separation through deterministic lateral displacement. Science, 2004. 304(5673): p. 987-990.
26. Mao, P. and J. Han, Massively-parallel ultra-high-aspect-ratio nanochannels as mesoporous membranes. Lab on a Chip, 2009. 9(4): p. 586-591.
27. Fissell, W.H., et al., High-performance silicon nanopore hemofiltration membranes. Journal of Membrane Science, 2009. 326(1): p. 58-63.
28. Zeng, Y. and D.J. Harrison, Self-assembled colloidal arrays as three-dimensional nanofluidic sieves for separation of biomolecules on microchips. Analytical Chemistry, 2007. 79(6): p. 2289-2295.
29. Minc, N., et al., Quantitative microfluidic separation of DNA in self-assembled magnetic matrixes. Analytical Chemistry, 2004. 76(13): p. 3770-3776.
30. Pamme, N., Continuous flow separations in microfluidic devices. Lab on a Chip, 2007. 7: p. 1644-1659.
31. Zhang, C.X. and A. Manz, High-Speed Free-Flow Electrophoresis on Chip. Analytical Chemistry, 2003. 75: p. 5759-5766.
32. Kohlheyer, D., et al., Free-flow zone electrophoresis and isoelectric focusing using a microfabricated glass device with ion permeable membranes. Lab on a Chip, 2006. 6: p. 374-380.
33. Kohlheyer, D., et al., Microfluidic High-Resolution Free-flow Isoelectric Focusing. Analytical Chemistry, 2007. 79: p. 8190-8198.
34. Song, Y.A., et al., Free-Flow Zone Electrophoresis of Peptides and Proteins in PDMS Microchip for Narrow pI Range Sample Prefractionation Coupled with Mass Spectrometry. Analytical Chemistry, 2010. 82(6): p. 2317-2325.
35. Janasek, D., et al., Electrostatic induction of the electric field into free-flow electrophoresis devices. Lab on a Chip, 2006. 6(6): p. 710-713.
36. Janasek, D., et al., Isotachophoresis in free-flow using a miniaturized device. Analytical Chemistry, 2006. 78(11): p. 3815-3819.
37. Song, Y.-A., et al., Continuous-flow pI-based sorting of proteins and peptides in a microfluidic chip using diffusion potential. Analytical Chemistry, 2006. 78: p. 3528-3536.
38. Lu, H., et al., A microfabricated device for subcellular organelle sorting. Analytical Chemistry, 2004. 76(19): p. 5705-5712.
39. Herr, A.E., et al., On-Chip Coupling of Isoelectric Focusing and Free Solution Electrophoresis for Multidimensional Separations. Analytical Chemistry, 2003. 75(5): p. 1180-1187.
40. Li, Y., et al., Integration of Isoelectric Focusing with Parallel Sodium Dodecyl Sulfate Gel Electrophoresis for Multidimensional Protein Separations in a Plastic Microfludic Network. Analytical Chemistry, 2004. 76(3): p. 742-748.
41. Wu, D.P., J.H. Qin, and B.C. Lin, Electrophoretic separations on microfluidic chips. Journal of Chromatography A, 2008. 1184(1-2): p. 542-559.
42. Wang, Y.-C., M.H. Choi, and J. Han, Two-Dimensional Protein Separation with Advanced Sample and Buffer Isolation Using Microfluidic Valves. Analytical Chemistry, 2004. 76(15): p. 4426-4431.
43. Shadpour, H. and S.A. Soper, Two-Dimensional Electrophoretic Separation of Proteins Using Poly(methyl methacrylate) Microchips. Analytical Chemistry, 2006. 78(11): p. 3519-3527.
44. Jindal, R. and S.M. Cramer, On-chip electrochromatography using sol-gel immobilized stationary phase with UV absorbance detection. Journal of Chromatography A, 2004. 1044(1-2): p. 277-285.
45. Yu, C., F. Svec, and J.M.J. Frechet, Towards stationary phases for chromatography on a microchip: Molded porous polymer monoliths prepared in capillaries by photoinitiated in situ polymerization as separation media for electrochromatography. Electrophoresis, 2000. 21(1): p. 120-127.

46. Ericson, C., et al., Electroosmosis- and pressure-driven chromatography in chips using continuous beds. Analytical Chemistry, 2000. 72(1): p. 81-87.
47. He, B., J.Y. Ji, and F.E. Regnier, Capillary electrochromatography of peptides in a microfabricated system. Journal of Chromatography A, 1999. 853(1-2): p. 257-262.
48. Oleschuk, R.D., et al., Trapping of bead-based reagents within microfluidic systems: On-chip solid-phase extraction and electrochromatography. Analytical Chemistry, 2000. 72(3): p. 585-590.
49. Wang, C., et al., Integration of immobilized trypsin bead beds for protein digestion within a microfluidic chip incorporating capillary electrophoresis separations and an electrospray mass spectrometry interface. Rapid Communications in Mass Spectrometry, 2000. 14(15): p. 1377-1383.
50. Jin, L.J., et al., A microchip-based proteolytic digestion system driven by electroosmotic pumping. Lab on a Chip, 2003. 3(1): p. 11-18.
51. Tian, H.J., A.F.R. Huhmer, and J.P. Landers, Evaluation of silica resins for direct and efficient extraction of DNA from complex biological matrices in a miniaturized format. Analytical Biochemistry, 2000. 283(2): p. 175-191.
52. Jung, B., R. Bharadwaj, and J.G. Santiago, On-chip millionfold sample stacking using transient isotachophoresis. Analytical Chemistry, 2006. 78(7): p. 2319-2327.
53. Quirino, J.P. and S. Terabe, Exceeding 5000-Fold Concentration of Dilute Analytes in Micellar Electrokinetic Chromatography. Science, 1998. 282(5388): p. 465-468.
54. Hori, A., et al., Electroconcentration by Using Countercurrent Due to Pressurized Flow and Electrophoretic Mobility. Analytical Chemistry, 1993. 65(20): p. 2882-2886.
55. Huang, Z. and C.F. Ivory, Digitally controlled electrophoretic focusing. Analytical Chemistry, 1999. 71(8): p. 1628-1632.
56. Ivory, C.F. and Z. Huang, Dynamic field-gradient focusing of proteins. Abstracts of Papers of the American Chemical Society, 1999. 217: p. U876-U876.
57. Ross, D. and L.E. Locascio, Microfluidic temperature gradient focusing. Analytical Chemistry, 2002. 74(11): p. 2556-2564.
58. Humble, P.H., et al., Electric field gradient focusing of proteins based on shaped ionically conductive acrylic polymer. Analytical Chemistry, 2004. 76(19): p. 5641-5648.
59. Kelly, R.T., Y. Li, and A.T. Woolley, Phase-Changing Sacrificial Materials for Interfacing Microfluidics with Ion-Permeable Membranes To Create On-Chip Preconcentrators and Electric Field Gradient Focusing Microchips. Analytical Chemistry, 2006. 78(8): p. 2565-2570.
60. Munson, M.S., et al., Temperature gradient focusing with field-amplified continuous sample injection for dual-stage analyte enrichment and separation. Analytical Chemistry, 2007. 79(16): p. 6201-6207.
61. Shackman, J.G., M.S. Munson, and D. Ross, Temperature gradient focusing for microchannel separations. Analytical and Bioanalytical Chemistry, 2007. 387(1): p. 155-158.
62. Shackman, J.G. and D. Ross, Counter-flow gradient electrofocusing. Electrophoresis, 2007. 28(4): p. 556-571.
63. Vollmer, S., et al., Peptide enrichment by microfluidic electrocapture for online analysis by electrospray mass spectrometry. Analytical Biochemistry, 2008. 374(1): p. 154-162.
64. Astorga-Wells, J., et al., Formation of stable stacking zones in a flow stream for sample immobilization in microfluidic systems. Analytical Chemistry, 2007. 79(3): p. 1057-1063.
65. Vollmer, S., et al., Microfluidic electrocapture interfaced with electrospray mass spectrometry. International Journal of Mass Spectrometry, 2007. 259(1-3): p. 73-78.
66. Astorga-Wells, J., et al., Microfluidic electrocapture for separation of peptides. Analytical Chemistry, 2005. 77(22): p. 7131-7136.
67. Astorga-Wells, J., et al., Microfluidic systems and proteomics: Applications of the electrocapture technology to protein and peptide analysis. Analytical Biochemistry, 2005. 345(1): p. 10-17.
68. Astorga-Wells, J., T. Bergman, and H. Jornvall, Multistep microreactions with proteins using electrocapture technology. Analytical Chemistry, 2004. 76(9): p. 2425-2429.
69. Astorga-Wells, J. and H. Swerdlow, Fluidic preconcentrator device for capillary electrophoresis of proteins. Analytical Chemistry, 2003. 75(19): p. 5207-5212.
70. Astorga-Wells, J., H. Jornvall, and T. Bergman, A microfluidic electrocapture device in sample preparation for protein analysis by MALDI mass spectrometry. Analytical Chemistry, 2003. 75(19): p. 5213-5219.
71. Wang, Y.C., A.L. Stevens, and J.Y. Han, Million-fold preconcentration of proteins and peptides by nanofluidic filter.

Analytical Chemistry, 2005. 77(14): p. 4293-4299.
72. Kim, S.M., M.A. Burns, and E.F. Hasselbrink, Electrokinetic protein preconcentration using a simple glass/poly(dimethylsiloxane) microfluidic chip. Analytical Chemistry, 2006. 78(14): p. 4779-4785.
73. Foote, R.S., et al., Preconcentration of proteins on microfluidic devices using porous silica membranes. Analytical Chemistry, 2005. 77(1): p. 57-63.
74. Liu, V., Y.A. Song, and J.Y. Han, Capillary-valve-based fabrication of ion-selective membrane junction for electrokinetic sample preconcentration in PDMS chip. Lab on a Chip, 2010. 10(11): p. 1485-1490.
75. Kim, S.J., Y.A. Song, and J. Han, Nanofluidic concentration devices for biomolecules utilizing ion concentration polarization: theory, fabrication, and applications. Chemical Society Reviews, 2010. 39(3): p. 912-922.
76. Wang, Y.-C., A.L. Stevens, and J. Han, Million-fold Preconcentration of Proteins and Peptides by Nanofluidic Filter. Analytical Chemistry, 2005. 77(14): p. 4293-4299.
77. Li, J.J., et al., Integrated system for high-throughput protein identification using a microfabricated device coupled to capillary electrophoresis/nanoelectrospray mass spectrometry. Proteomics, 2001. 1(8): p. 975-986.
78. Yu, C., et al., Monolithic porous polymer for on-chip solid-phase extraction and preconcentration prepared by photoinitiated in situ polymerization within a microfluidic device. Analytical Chemistry, 2001. 73(21): p. 5088-5096.
79. Khandurina, J., et al., Microfabricated porous membrane structure for sample concentration and electrophoretic analysis. Analytical Chemistry, 1999. 71(9): p. 1815-1819.
80. Song, S., A.K. Singh, and B.J. Kirby, Electrophoretic concentration of proteins at laser-patterned nanoporous membranes in microchips. Analytical Chemistry, 2004. 76(15): p. 4589-4592.
81. Hatch, A.V., et al., Integrated Preconcentration SDS−PAGE of Proteins in Microchips Using Photopatterned Cross-Linked Polyacrylamide Gels. Analytical Chemistry, 2006. 78(14): p. 4976-4984.
82. Herr, A.E., et al., Microfluidic immunoassays as rapid saliva-based clinical diagnostics. Proceedings of the National Academy of Sciences, 2007. 104(13): p. 5268-5273.

Biosample Preparation by Lab-on-a-Chip Devices

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