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Controlled dispensing and mixing of pico- to nanoliter volumes using on-demand droplet-based microfluidics

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Abstract

We present an integrated droplet-on-demand microfluidic platform for dispensing, mixing, incubating, extracting and analyzing by mass spectrometry pico- to nanoliter-sized droplets. All of the functional components are successfully integrated for the first time into a monolithic microdevice. Droplet generation is accomplished using computer-controlled pneumatic valves. Controlled actuation of valves for different aqueous streams enables accurate dosing and rapid mixing of reagents within droplets in either the droplet generation area or in a region of widening channel cross-section. Following incubation, which takes place as droplets travel in the oil stream, the droplet contents are extracted to an aqueous channel for subsequent ionization at an integrated nanoelectrospray emitter. Using the integrated platform, rapid enzymatic digestions of a model protein were carried out in droplets and detected on-line by nanoelectrospray ionization mass spectrometry.

Keywords

Lab-on-a-chip; Droplet; Mixing; Valving; ESI-MS detection

1 Introduction

Droplet or plug-based microfluidic devices, in which femto-to-nanoliter sized aqueous volumes are encapsulated and dispersed in an immiscible oil phase, have emerged as attractive platforms for small volume analysis due to their ability to limit dilution caused by diffusion and Taylor dispersion while minimizing surface-related adsorptive losses (Roach et al. 2005). Droplet-based platforms have been employed to, e.g., encapsulate and sort single cells, effect polymerase chain reaction-based analyses with high throughput, study enzyme kinetics and protein crystallization, and synthesize small molecules and polymeric particles (Theberge et al. 2010; Yang et al. 2010; Teh et al. 2008; Huebner et al. 2008; Chiu and Lorenz 2009; Song et al. 2006; Chiu et al. 2009).

Currently, most microfluidic droplet generators are based on T-junction (Thorsen et al. 2001; Garstecki et al. 2006) or flow focusing (Anna et al. 2003; Ward et al. 2005) mechanisms, both of which create uniform droplets by utilizing the interface instability between oil and aqueous streams. Droplet volume and generation frequency depend on several factors, including the physical properties of the immiscible phases, flow rates, intersection geometry, etc. Droplet generation in these cases is spontaneous and droplet

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volume and generation frequency are interrelated. Also, droplet uniformity becomes difficult to achieve when applied to low frequency (e.g., <1 Hz) droplet generation (Kelly et al. 2009). For such low frequencies, on-demand droplet generation may be favorable as it ensures precise control and fine manipulation of individual droplets. Various approaches have been developed to generate droplets on demand (He et al. 2005a; He et al. 2005b; Park et al. 2011; Bransky et al. 2009; Galas et al. 2009; Zeng et al. 2009; Choi et al. 2010; Abate et al. 2009), the most promising of which in terms of facile control over both droplet size and generation frequency is pneumatic valving (Unger et al. 2000). For example, Galas et al. (2009) utilized a single pneumatic valve that was embedded in an active connector and assembled close to a T-junction to regulate the flow of the dispersed phase. Constant pressures were applied on the inlets of two immiscible liquids to drive the flow in the microchannel. Individual droplets were created by briefly opening the valve. The aqueous droplet size depended on the valve actuation time and frequency, as well as the pressure applied at the oil inlet. As such, the droplet volume, spacing and speed could be controlled accurately and independently. This device not only generated periodic sequences of identical droplets, but also enabled the production of non-periodic droplet trains with different droplet sizes or spacing. Lin and coworkers (Zeng et al. 2009) also reported a similar platform for pneumatic valve-assisted on-demand droplet generation. Negative pressure was applied at the outlet of the device to drive the flow of the two immiscible liquids through the microchannel. The dependence of droplet size on the valve actuation time and applied pressure was investigated. In addition, they utilized several aqueous flow channels, each with independently controlled microvalves, to generate arrays of droplets containing different compositions by alternately actuating the valves.

In addition to controlled droplet generation, combining droplets of different composition is of crucial importance in relation to the development of microreactors as it allows precise and reproducible mixing of reagents at well-defined points to initiate reactions (Theberge et al. 2010). Ismagilov and coworkers (Song et al. 2003) carried out the pioneering work of combining different reagents into droplets by flowing two reagent solutions in a microchannel as two separate laminar streams and using an inert center stream to prevent their contact prior to droplet formation. Droplets having a gradient in reagent concentrations were achieved by varying the relative flow rates of the three streams (Zheng et al. 2003). A subsequent winding channel was designed to accelerate mixing by chaotic advection (Song et al. 2003). Weitz and colleagues reported a robust picoinjector to add reagents to droplets in microfluidic systems (Abate et al. 2010). The picoinjector was controlled by an electric field to trigger the injection of a controlled volume of reagents into the droplets selectively. Niu et al. (2011) developed a microdroplet dilutor, in which a trapped sample droplet merged and mixed with a series of buffer droplets and then released a sequence of output droplets with gradient concentrations. Recently, Kennedy and coworkers utilized a series of reagent addition tees to add reagents into segmented samples for screening of enzyme inhibitors (Sun et al. 2012a). High-throughput, label-free screening was demonstrated, but carry-over resulting from the tee was 2-9% for one reagent addition and increased with more additions.

In-channel droplet fusion is another attractive approach to combine different reagents in individual droplets to initiate or terminate the confined reactions. The process of droplet merging introduces convective flows into the system, resulting in far more rapid mixing than relying on diffusion alone (Rhee and Burns 2008). Droplet fusion is readily achieved by bringing two or more surfactant-free aqueous droplets into contact. Both passive and active methods have been developed to control droplet fusion. For passive fusion devices, droplet coalescence is usually initiated by utilizing specially designed fusion elements in the channel. For example, Bremond et al. (2008) incorporated an expanded coalescence chamber in the channel network in which two droplets were brought into close proximity

and merged together before entering a narrow channel. Fidalgo et al. (2007) reported a method for droplet fusion based on a surface energy pattern inside a microfluidic channel where the segmented flow was disrupted and the droplets were trapped and fused together. However, this approach could potentially cause cross contamination between droplets from the patterned surface. Niu et al. (2008) developed a pillar-induced droplet merging device, in which rows of pillars were constructed in the channel network serving as passive fusion elements or chambers. The first trapped droplet in the pillar array was suspended and merged with succeeding droplets until the surface tension was overwhelmed by the hydraulic pressure.

Active fusion methods that can be controlled externally and selectively have also been developed using, e.g., electric fields (Priest et al. 2006; Link et al. 2006; Zagnoni and Cooper 2009; Niu et al. 2009a) and laser pulses (Baroud et al. 2007) to trigger coalescence. To perform active droplet fusion effectively, synchronization of droplets is a key factor because fusion efficiency relies on the droplets being in very close proximity (Thiam et al. 2009). Special designs are often employed to synchronize droplets in two parallel channels, which then merge into a single channel downstream to realize droplet coalescence (Zeng et al. 2009; Bremond et al. 2008; Frenz et al. 2008). However, such systems can potentially be disturbed by factors including flow rate and channel back pressure, which may reduce the fusion efficiency. Recently, Jambovane et al. (2011) used valve-based droplet generation for multiple reagents to perform controlled reactions and establish chemical gradients among arrays of droplets. Droplets were generated at a valve-controlled side channel and then different reagents were added to the droplets as they passed by similar side channels downstream, requiring careful timing of valve actuation. Shemesh et al. (2011) employed a piezoelectric actuator to control droplet generation and the synchronized droplets merged in a downstream chamber to form fused droplets with adjustable composition and volume.

In this work, we have employed pneumatic valves for on-demand droplet generation and present two strategies for combining reagents into droplets. In one approach, active reagent mixing is enabled by the synchronous actuation of pneumatic valves separating aqueous channels from the oil stream in a “double T” configuration. Independent computer-based operation of the two valves leads to a high degree of control over droplet dispensing and reagent dosing, enabling sequences of droplets containing different compositions to be easily created. Alternatively, the valves corresponding to different aqueous inputs can be actuated in a staggered fashion, causing the plugs to travel downstream separately. Fusion of the two plugs takes place spontaneously at a region of expanded channel cross-section. The latter approach results in more rapid mixing. Incubation takes place as droplets travel in the oil channel. This integrated microfluidic platform was used to combine myoglobin and pepsin from separate aqueous streams into droplets to perform rapid in-droplet digestions. The droplet contents were analyzed online by mass spectrometry (MS) using a previously developed droplet extraction interface and integrated nanoelectrospray ionization (nanoESI) source.

2 Experiments

2.1 Materials

Fluorinert FC-40, leucine enkephalin, methionine enkephalin, apomyoglobin, pepsin, glacial acetic acid, sodium carbonate, sodium bicarbonate, and hexamethyldisilazane (HMDS) were purchased from Sigma-Aldrich (St. Louis, MO). Fluorescein was obtained from Fluka (Buchs, Switzerland). HPLC-grade methanol was purchased from Fisher Scientific (Fair Lawn, NJ). Water was purified using a Barnstead Nanopure Infinity system (Dubuque, IA). ESI buffer solution was prepared by mixing water and methanol at the ratio of 9:1 (v/v), and adding 0.1% (v/v) acetic acid. Carbonate buffer (10 mM, pH 9.3) was filtered using 0.2 μ m

syringe filters (Pall Life Sciences, Ann Arbor, MI) before use. PDMS elastomer base and curing agent were purchased as Dow Corning Sylgard 184 from Ellsworth Adhesives (Germantown, WI).

2.2 Design and fabrication

Figure 1a shows the design of an integrated microfluidic device, which includes two layers and three functional sections. The control layer contains two microchannels for valving (dashed line indicated in Figure 1b), and all other microchannels and microstructures are fabricated on the flow layer. The first functional section is the “double T” intersection indicated in Figure 1b, which is integrated with two valve control channels (dashed line) crossing over the side channels to regulate the introduction of dispersed aqueous solutions into the continuous oil flow channel. This section is responsible for on-demand droplet generation and active fusion. The width of all channels shown in Figure 1b is 100 μm . The lengths of the main and two side channels are 3 cm and 0.5 cm, respectively. The distance between the two side channels is 100 μm , and the intersectional area for valving is 100 $\mu\text{m} \times 100 \mu\text{m}$. In general, the nozzles of two side channels could be designed in different dimension to control droplet generation. The second functional device section is the stable oil-water interface used to extract the oil-encapsulated aqueous plugs to an aqueous stream. An array of cylindrical posts with 15 μm diameter and $\sim 3 \mu\text{m}$ gaps between adjacent posts separates the two streams (Kelly et al. 2009; Sun et al. 2012b). The oil channel is tapered prior to the extraction interface such that width of both microchannels separated by the interface is 50 μm . The third functional section is the integrated ESI emitter, which was created as reported previously (Kelly et al. 2009; Sun et al. 2010; Kelly et al. 2008), i.e., by cutting the device based on patterned guide lines to generate a tapered apex at the terminus of the aqueous channel. The emitter angle is 50° as defined by two symmetric guide lines (Sun et al. 2010).

To create the devices, two separate templates were produced on silicon substrates using three photomasks based on the pattern shown in Figure 1a. The template defining the valve control channels was formed by patterning SU-8 photoresist (Microchem, Newton, MA) with 25- μm -tall features of rectangular cross-section. The flow layer was patterned on another silicon substrate using two masks. The side channels for injection of dispersed aqueous solutions were first patterned with AZ P4620 photoresist (Capitol Scientific, Austin, TX) that was spin-coated at 2000 rpm for 30 s, exposed in a contact photomask aligner (4006, Neutronix-Quintel, Morgan Hill, CA) and developed using 1:3 AZ 400K developer (Capitol Scientific) in water. The patterned resist was thermally softened and reflowed (Unger et al. 2000) to a rounded cross-section ($\sim 10 \mu\text{m}$ high) at 150 °C for 30 min and then stabilized at high temperature (180 °C). Other microstructures on the flow layer were aligned with the rounded side channels and patterned with SU-8 photoresist to create 25- μm -tall channels with rectangular cross-sections. The silicon template defining the control layer was modified with HMDS using vapor deposition to assist in releasing the PDMS membrane from the patterned template. A 10:1 ratio (w/w) of PDMS base to curing agent was then mixed, degassed under vacuum, and poured onto the patterned flow layer template to a thickness of 1–2 mm. The mixed and degassed PDMS was also spin-coated on the surface-modified control layer template at 2000 rpm for 30 s to achieve a thickness of $\sim 50 \mu\text{m}$. Both substrates were then cured in an oven at 75 °C for 2 h. After removing the patterned PDMS from the flow layer template, several small through-holes were created at the ends of all flow channels by punching the substrate with a manually sharpened syringe needle (NE-301PL-C; Small Parts, Miramar, FL). The flow layer PDMS piece was then cleaned and treated with oxygen plasma (PX-250; March Plasma Systems, Concord, CA) for 30 s to activate the surface for bonding. It was immediately aligned on top of the control layer PDMS membrane (still on the silicon wafer) based on the alignment marks under a

stereomicroscope (SMZ-U, Nikon, Tokyo, Japan) and assembled together to enclose the flow channels. After placing in an oven at 75 °C for 2 h to form an irreversible bond, the PDMS assembly containing the flow and control layers was removed from the control layer silicon template, through-holes were punched at the end of all control channels, and the substrate was bonded to an unpatterned PDMS piece to enclose the control channels using oxygen plasma treatment. The assembled PDMS microdevice was cured in an oven at 120 °C for at least 48 h to recover its hydrophobicity (Sun et al. 2010). Before coupling with MS, an integrated ESI emitter was created as described previously (Kelly et al. 2008). Figure 1c shows a photomicrograph of the valve-integrated double-T intersection, where the nozzles of two side channels are 50 μm wide. The droplet extraction region with a stable oil-water interface (dark line between adjacent posts) is shown in Figure 1d. To render the interface more robust and reliable, the aqueous stream channel (top) was selectively modified to be hydrophilic by corona discharge while the oil phase flow channel (bottom) remained hydrophobic by protecting the surface with water (Sun et al. 2012b).

2.3 Operation and detection

Figure 2 shows a schematic of the setup for operation of the pneumatic valve-integrated droplet-on-demand platform with nanoESI-MS detection. The computer-operated solenoid-based valve controller and the associated software were described previously (Sun et al. 2011a). The valve control channels in the PDMS device were filled with water to avoid introduction of bubbles into the flow channels. The continuous oil (Fluorinert FC-40) flow in the main channel was controlled by a syringe pump (PHD 2000; Harvard Apparatus, Holliston, MA) through a fused-silica capillary (75 μm i.d., 360 μm o.d.; Polymicro Technologies, Phoenix, AZ). One end of the capillary was connected to a 50 μL syringe (Hamilton, Reno, NV) and the other end was inserted into a ~ 2 mm long section of Tygon tubing (TGY-101-5C; Small Parts, Miramar, FL), which was in turn inserted into the through-hole of the oil flow channel on the microchip. The dispersed aqueous solutions were stored in sealed vials with a nitrogen gas inlet to pressurize the liquid, and an outlet to allow liquid to be transferred into the microchannels through a capillary. Before operation, the transfer lines and microchannels were purged of any air bubbles. The pneumatic valves were actuated to disperse aqueous solutions into the oil flow channel to generate droplets.

The microfluidic device was coupled with a linear ion trap mass spectrometer (LTQ, Thermo Fisher, Waltham, MA, USA) to analyze the droplet contents. The microchip emitter was positioned 3 mm in front of the MS inlet capillary, which was heated to 120 °C. The ESI buffer solution was infused into the aqueous channel from a syringe via a capillary transfer line. A syringe pump controlled the infusion rate. The electrospray potential of ~ 2.5 kV from a high voltage power supply was applied to the syringe needle. A fluorescence detection system was also employed for monitoring droplet generation and extraction. The UV light generated by a mercury lamp (Olympus, Tokyo, Japan) was passed into an inverted optical microscope (IX71, Olympus), and the fluorescence was collected with a digital camera (CoolPix 995Nikon).

3 Results and discussion

3.1 On-demand droplet generation

A single side channel and its corresponding valve (Figure 1b) were used to characterize on-demand droplet generation while the other valve was kept closed to block the unused side channel. Figure 3a shows the pneumatic valve-controlled generation of an individual droplet. The valve is initially closed and an aqueous fluorescein solution is confined in the side channel. When the valve is briefly opened, the aqueous solution is driven into the oil channel by a moderate constant pressure (8–10 psi) and a small aqueous droplet having a

volume of ~500 pL is produced and then flushed downstream by the continuous oil flow. Compared with conventional microfluidic droplet generation techniques based on a T-junction or flow focusing, which depend on the spontaneous breakup of the aqueous flow stream to produce droplets, our valve-integrated system can generate droplets with precise control over droplet volume, generation frequency, and velocity. Droplet velocity is determined by the syringe pump driving the oil stream, while the droplet generation rate is controlled by the valve actuation frequency, which is defined in the software. Droplet spacing is determined by the interval between valve openings and the oil flow velocity. Droplet volume depends on several parameters including the valve actuation time, the pressure of the aqueous solution, the oil phase flow rate, and the valve control pressure (Sun et al. 2011a). While the pneumatic valve can be actuated at frequencies as high as 100 Hz, we limited our studies to lower frequencies to prevent droplets from inadvertently coalescing in the oil channel and for compatibility with MS detection. Figure 3b shows the fluorescence detection of multiple droplets generated at 2.2 Hz with relative standard deviation (RSD) of peak width 3.5%. Figure 3c shows a series of droplets formed by increasing valve actuation times, and the linear relationship between droplet volume and valve actuation time is shown in Figure 3d.

3.2 Valve-controlled droplet merging and mixing

Reagent combination and mixing play key roles in initiating reactions within droplets. The efficient and active droplet fusion method that we developed here relies on two pneumatic valves integrated at the double-T intersection (Figure 1b). Simultaneous opening of two valves results in the creation of an individual aqueous plug containing both reagents. The process of droplet creation and mixing is shown in Figure 4a using fluorescein solutions of two different concentrations as a model system. Upon actuation, the oil between the two side channels is quickly displaced, and the two aqueous streams collide and combine. No sample cross contamination was observed because of the applied pressures, the rapid valve actuation and the offset between the two side channels. The two liquids mixed together by a combination of diffusion and convection (Rhee and Burns 2008). The mixing process was further investigated using Figure 4b, which shows the fluorescence detection of three droplets at different positions in the oil channel. Peaks 1 and 3 contained dilute and concentrated fluorescein, respectively, and Peak 2 was a combined plug containing both fluorescein concentrations. The peak intensities were normalized to Peak 3. Scan A was performed just downstream of the double-T intersection, which was the early stage of the mixing process. The relative intensity of peak 1 (dilute fluorescein droplet) is 0.63. Peak 2 has a shape of a right-angled triangle on the top, which is consistent with the gradient fluorescence distribution in the fused droplet shown in the inset image. The relative intensity of the leading edge (0.65) is similar to peak 1, and the relative intensity of the trailing edge (0.95) is close to peak 3. When the fused droplet moved downstream, the liquids mixed more thoroughly. For instance, Scan B shows the droplets moving ~1.5 s after generation and fusion. A small triangle shape is still on the summit of peak 2. But the intensity of the leading edge increased to 0.75, which approaches that of the trailing (0.91). The fused droplet shown in Scan C (~3.75 s) mixed completely because the fluorescence distribution across the peak was uniform, as confirmed by the similar intensity across the peak (0.84 to 0.87).

Figure 5 describes a passive droplet fusion for more rapid mixing at a region of increasing channel width (Figure 5a). By staggering droplet generation for the two reagents by 33 ms, a pair of droplets containing different dyes that were separated by a small (~100 μm) plug of oil was produced (Figure 5b). As the plugs moved downstream to the widening region, the first droplet slowed down, resulting in coalescence with the incoming droplet, which is depicted in Figure 5c in detail. The liquid mixing was observed clearly and it took

approximately 0.5 s to mix thoroughly in this case. Optimizing the geometry of the mixing region and reducing droplet size should enable significant further reduction in mixing time.

The linear dependence of droplet volume on valve actuation time, and the independent valving for the two aqueous streams, provides a high degree of control over merged droplet composition and size (i.e., volume can be held approximately constant even as composition changes). Figure 6 shows arrays of 6 droplets containing different ratios of two dyes by controlling the operation of two valves. The series of droplets is repeated four times to demonstrate the excellent reproducibility of the technique (average of 2.3% RSD in peak height for the 5 most intense peaks). Such control should be useful in forming droplets with gradient compositions for rapidly optimizing reactions and for studying reaction kinetics.

3.3 In-droplet protein digestion and on-line MS detection

Using this platform to initiate reactions, apomyoglobin (1 $\mu\text{g}/\mu\text{L}$) and the protease pepsin (1 $\mu\text{g}/\mu\text{L}$) from separate aqueous streams were merged into droplets to perform in-droplet protein digestion with online nanoESI-MS detection. Pepsin was selected over trypsin due to its compatibility with the acidic conditions used for ESI and because pepsin itself is not observed in the mass spectrum due to a paucity of basic amino acid residues (Loo et al. 1992). The reaction was not complete, as the droplets had just ~ 18 s to incubate as they traveled to the extraction region of the device. However, a significant number of identifiable peptides were produced during that brief time due to the rapid mixing and high concentration of protease. Following extraction from the oil stream into an aqueous ESI buffer stream, the contents were rapidly transferred to an integrated electrospray emitter. MS detection was performed as described previously (Sun et al. 2011b). Figure 7a shows the MS detection of the droplets. The averaged mass spectrum from one of the peaks is shown in Figure 7b. While intact apomyoglobin is still evident in the spectrum (see Figure 7c for reference), indicating incomplete digestion, a number of identifiable peptide fragments are present as well. These peptides are labeled in Figure 7b with m/z values and the corresponding identifications are listed in Table 1. Simply increasing the incubation time by incorporating a longer oil channel should dramatically improve digestion efficiency. Further optimization of the enzymatic digestions is ongoing and will be published as a separate study, but the initial results illustrate the utility of the platform for performing rapid in-droplet digestions and analyzing the contents by nanoESI-MS.

4 Conclusions

An integrated PDMS microfluidic device has been developed to generate droplets on demand, control droplets fusion, and detect the droplet compositions using nanoESI-MS. PDMS membrane-based pneumatic valves were assembled at a T-junction or double T intersection to precisely regulate the introduction of the dispersed liquids into the continuous oil phase channel. Aqueous droplets were produced on demand by actuating the valves under computer control. Accurate synchronization of droplets could be realized by controlling the operation of two valves carefully. The droplets could be actively merged and rapidly mixed in the generation area, or passively fused together at a region of increasing channel cross-section. Using this microfluidic platform, rapid in-droplet protein digestions were performed and finally analyzed by MS following the droplet extraction. With further optimization, the high degree of control over droplet composition, rapid mixing and compatibility with MS analysis should lead to novel applications, including high-rate, label-free kinetic studies. Additional applications will be made possible when the platform is combined on-line with liquid chromatography (Niu et al. 2009b). In that case, eluent from a nanoLC separation can be selectively combined with reagents into droplets prior to MS analysis for, e.g., combined top-down and bottom-up proteomic analyses for improved

protein identification and characterization of post-translational modifications (Wu et al. 2009).

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References

- Abate AR, Hung T, Mary P, Agresti JJ, Weitz DA. High throughput injection with microfluidics using picoinjectors. *Proc Natl Acad Sci U S A*. 2010; 107:19163–19166. [PubMed: 20962271]
- Abate AR, Romanowsky MB, Agresti JJ, Weitz DA. Valve-based flow focusing for drop formation. *Appl Phys Lett*. 2009; 94:023503.
- Anna SL, Bontoux N, Stone HA. Formation of dispersions using “flow focusing” in microchannels. *Appl Phys Lett*. 2003; 82:364–366.
- Baroud CN, de Saint Vincent MR, P. DJ. An optical toolbox for total control of droplet microfluidics. *Lab Chip*. 2007; 7:1029–1033. [PubMed: 17653345]
- Bransky A, Korin N, Khoury M, Levenberg S. A microfluidic droplet generator based on a piezoelectric actuator. *Lab Chip*. 2009; 9:516–520. [PubMed: 19190786]
- Bremond N, Thiam AR, Bibette J. Decompressing emulsion droplets favors coalescence. *Phys Rev Lett*. 2008; 100:024501. [PubMed: 18232876]
- Chiu DT, Lorenz RM. Chemistry and Biology in Femtoliter and Picoliter Volume Droplets. *Accounts Chem Res*. 2009; 42:649–658.
- Chiu DT, Lorenz RM, Jeffries GDM. Droplets for ultrasmall-volume analysis. *Anal Chem*. 2009; 81:5111–5118. [PubMed: 19507850]
- Choi J-H, Lee S-K, Lim J-M, Yang S-M, Yi G-R. Designed pneumatic valve actuators for controlled droplet breakup and generation. *Lab Chip*. 2010; 10:456–461. [PubMed: 20126685]
- Fidalgo LM, Abell C, Huck WTS. Surface-induced droplet fusion in microfluidic devices. *Lab Chip*. 2007; 7:984–986. [PubMed: 17653339]
- Frenz L, Blouwolff J, Griffiths AD, Baret JC. Microfluidic production of droplet pairs. *Langmuir*. 2008; 24:12073–12076. [PubMed: 18823095]
- Galas JC, Bartolo D, Studer V. Active connectors for microfluidic drops on demand. *New Journal of Physics*. 2009; 11:075027.
- Garstecki P, Fuerstman MJ, Stone HA, Whitesides GM. Formation of droplets and bubbles in a microfluidic T-junction-scaling and mechanism of break-up. *Lab Chip*. 2006; 6:437–446. [PubMed: 16511628]
- He M, Edgar JS, Jeffries GDM, Lorenz RM, Shelby JP, Chiu DT. Selective encapsulation of single cells and subcellular organelles into picoliter- and femtoliter-volume droplets. *Anal Chem*. 2005a; 77:1539–1544. [PubMed: 15762555]
- He M, Kuo JS, Chiu DT. Electro-generation of single femtoliter- and picoliter-volume aqueous droplets in microfluidic systems. *Appl Phys Lett*. 2005b; 87:031916.
- Huebner A, Sharma S, Srisa-Art M, Hollfelder F, Edel JB, deMello AJ. Microdroplets: a sea of applications? *Lab Chip*. 2008; 8:1244–1254. [PubMed: 18651063]
- Jambovan S, Kim DJ, Duin EC, Kim S-K, Hong JW. Creation of stepwise concentration gradient in picoliter droplets for parallel reactions of matrix metalloproteinase II and IX. *Anal Chem*. 2011; 83:3358–3364. [PubMed: 21456571]
- Kelly RT, Page JS, Marginean I, Tang K, Smith RD. Dilution-Free Analysis from Picoliter Droplets by Nano-Electrospray Ionization Mass Spectrometry. *Angew Chem Int Ed*. 2009; 48:6832–6835.

- Kelly RT, Tang K, Irimia D, Toner M, Smith RD. Elastomeric microchip electrospray emitter for stable cone-jet mode operation in the nanoflow regime. *Anal Chem*. 2008; 80:3824–3831. [PubMed: 18419138]
- Link DR, Grasland-Mongrain E, Duri A, Sarrazin F, Cheng Z, Cristobal G, Marquez M, Weitz DA. Electric control of droplets in microfluidic devices. *Angew Chem Int Ed*. 2006; 45:2556–2560.
- Loo JA, Loo RRO, Light KJ, Edmonds CG, Smith RD. Multiply charged negative ions by electrospray ionization of polypeptides and proteins. *Anal Chem*. 1992; 64:81–88. [PubMed: 1310574]
- Niu X, Gielen F, deMello AJ, Edel JB. Electro-coalescence of digitally controlled droplets. *Anal Chem*. 2009a; 81:7321–7325. [PubMed: 19715363]
- Niu X, Gielen F, Edel JB, deMello AJ. A microdroplet dilutor for high-throughput screening. *Nature Chemistry*. 2011; 3:437–442.
- Niu X, Gulati S, Edel JB, deMello AJ. Pillar-induced droplet merging in microfluidic circuits. *Lab Chip*. 2008; 8:1837–1841. [PubMed: 18941682]
- Niu X, Zhang B, Marszalek RT, Ces O, Edel JB, Klug DR, deMello AJ. Droplet based compartmentalization of chemically separated components in two dimensional separations. *Chem Commun*. 2009b; 41:6159–6161.
- Park S-Y, Wu T-H, Chen Y, Teitell MA, Chiou P-Y. High-speed droplet generation on demand driven by pulse laser-induced cavitation. *Lab Chip*. 2011; 11:1010–1012. [PubMed: 21290045]
- Priest C, Herminghaus S, Seemann R. Controlled electrocoalescence in microfluidics: targeting a single lamella. *Appl Phys Lett*. 2006; 89:134101.
- Rhee M, Burns MA. Drop mixing in a microchannel for lab on a chip platforms. *Langmuir*. 2008; 24:590–601. [PubMed: 18069861]
- Roach LS, Song H, Ismagilov RF. Controlling nonspecific protein adsorption in a plug-based microfluidic system by controlling interfacial chemistry using fluorosurfactants. *Anal Chem*. 2005; 77:785–796. [PubMed: 15679345]
- Shemesh J, Nir A, Bransky A, Levenberg S. Coalescence-assisted generation of single nanoliter droplets with predefined composition. *Lab Chip*. 2011; 11:3225–3230. [PubMed: 21826345]
- Song H, Chen DL, Ismagilov RF. Reactions in droplets in microfluidic channels. *Angew Chem Int Ed*. 2006; 45:7336–7356.
- Song H, Tice JD, Ismagilov RF. A microfluidic system for controlling reaction networks in time. *Angew Chem Int Ed*. 2003; 42:768–772.
- Sun S, Slaney TR, Kennedy RT. Label free screening of enzyme inhibitors at femtomole scale using segmented flow electrospray ionization mass spectrometry. *Anal Chem*. 2012a; 84:5794–5800. [PubMed: 22656268]
- Sun X, Kelly RT, Danielson WF, Agrawal N, Tang K, Smith RD. Hydrodynamic injection with pneumatic valving for microchip electrophoresis with total analyte utilization. *Electrophoresis*. 2011a; 32:1610–1618. [PubMed: 21520147]
- Sun X, Kelly RT, Tang K, Smith RD. Ultrasensitive nanoelectrospray ionization-mass spectrometry using poly(dimethylsiloxane) microchips with monolithically integrated emitters. *Analyst*. 2010; 135:2296–2302. [PubMed: 20617264]
- Sun X, Kelly RT, Tang K, Smith RD. Membrane-based emitter for coupling microfluidics with ultrasensitive nanoelectrospray ionization-mass spectrometry. *Anal Chem*. 2011b; 83:5797–5803. [PubMed: 21657269]
- Sun X, Tang K, Smith RD, Kelly RT. Robust extraction interface for coupling droplet-based and continuous flow microfluidics. *Advances in microfluidics. In Tech*. 2012b:155–170.
- Teh S-Y, Lin R, Hung L-H, Lee AP. Droplet microfluidics. *Lab Chip*. 2008; 8:198–220. [PubMed: 18231657]
- Theberge AB, Courtois F, Schaerli Y, Fischlechner M, Abell C, Hollfelder F, Huck WTS. Microdroplets in microfluidics: an evolving platform for discoveries in chemistry and biology. *Angew Chem Int Ed*. 2010; 49:5846–5868.
- Thiam AR, Bremond N, Bibette J. Breaking of an emulsion under an ac electric field. *Phys Rev Lett*. 2009; 102:188304. [PubMed: 19518918]

- Thorsen T, Roberts RW, Arnold FH, Quake SR. Dynamic pattern formation in a vesicle-generating microfluidic device. *Phys Rev Lett*. 2001; 86:4162–4166.
- Unger MA, Chou H-P, Thorsen T, Scherer A, Quake SR. Monolithic microfabricated valves and pumps by multilayer soft lithography. *Science*. 2000; 288:113–116. [PubMed: 10753110]
- Ward T, Faivre M, Abkarian M, Stone HA. Microfluidic flow focusing: drop size and scaling in pressure versus flow rate driven pumping. *Electrophoresis*. 2005; 26:3716–3724. [PubMed: 16196106]
- Wu S, Lourette NM, Toli N, Zhao R, Robinson EW, Tolmachev AV, Smith RD, Pasa-Toli L. An integrated top-down and bottom-up strategy for broadly characterizing protein isoform and modifications. *J Proteome Res*. 2009; 8:1347–1357. [PubMed: 19206473]
- Yang C-G, Xu Z-R, Wang J-H. Manipulation of droplets in microfluidic systems. *Trends Anal Chem*. 2010; 29:141–157.
- Zagnoni M, Cooper JM. On-chip electrocoalescence of microdroplets as a function of voltage, frequency and droplet size. *Lab Chip*. 2009; 9:2652–2658. [PubMed: 19704980]
- Zeng S, Li B, Su X, Qin J, Lin B. Microvalve-actuated precise control of individual droplets in microfluidic devices. *Lab Chip*. 2009; 9:1340–1343. [PubMed: 19417898]
- Zheng B, Roach LS, Ismagilov RF. Screening of protein crystallization conditions on a microfluidic chip using nanoliter size droplets. *J Am Chem Soc*. 2003; 125:11170–11171. [PubMed: 16220918]

Figure 1.

Device design. (a) Overview of the droplet-on-demand microfluidic platform integrated with two valve-controlled side channels, a droplet extraction interface and a nanoESI emitter. (b) Schematic depiction of the droplet generation and mixing portion of the device. (c) Photograph of the double-T intersection. (d) Photograph of the droplet extraction region of the device. Water and oil fill the top and bottom channels, respectively, and the interface for the two liquids can be seen between the circular posts.

Figure 2.
Schematic of the experimental setup for droplet generation, mixing, and MS detection.

Figure 3.

(a) Micrograph sequences depicting pneumatic valve-controlled generation of an individual fluorescein droplet. The width of the oil flow channel was 100 μm , the oil flow rate was 0.5 $\mu\text{L}/\text{min}$, and the sample injection pressure was 8 psi. Valve actuation time and pressure were 33 ms and 25 psi, respectively. (b) Fluorescence detection of aqueous fluorescein droplets generated at 2.2 Hz. (c) A series of fluorescein droplets with increased volumes generated by sequentially increasing the valve actuation time ($t = 33$ ms). (d) Plot of the droplet volumes vs. different valve actuation times.

Figure 4.

(a) Micrograph sequences depicting the active droplet fusion of a pair of droplets containing different concentrations of fluorescein. The oil flow rate was $0.4 \mu\text{L}/\text{min}$ and the sample injection pressure was 10 psi. Valve actuation time and pressure were 33 ms and 40 psi, respectively, and the plugs were produced at a frequency of 0.45 Hz and had a volume of 2 nL. (b) Relative fluorescent intensity of the fused droplets (peak 2) recorded at different positions: A) ~ 0.5 mm, B) 4 mm, and C) 10 mm downstream from the intersection. Peaks 1 and 3 are droplets containing diluted and concentrated fluorescein samples, respectively.

Figure 5.

(a) Photography of the channel widening section with indicated dimension. (b) A pair of droplets, containing yellow and green dyes, respectively, moving in the narrow channel (100 μm wide). (c) Micrograph sequences depicting the passive droplet fusion at the channel widening section.

Figure 6. Relative intensity of an array of 6 droplets containing yellow (1) and green (6) food dyes with different volume ratios: 4:1 (2), 2:1 (3), 1:1 (4) and 1:2 (5) (yellow/green).

Figure 7.

(a) MS detection of the fused droplets mixing $1 \mu\text{g}/\mu\text{L}$ apomyoglobin with $1 \mu\text{g}/\mu\text{L}$ pepsin in water containing 0.1% formic acid (pH ~ 3). The flow rates of oil and ESI buffer streams were $0.1 \mu\text{L}/\text{min}$ and $0.4 \mu\text{L}/\text{min}$, respectively. (b) and (c) MS spectra of the fused droplet and $1 \mu\text{g}/\mu\text{L}$ apomyoglobin, respectively.

Table 1

Sequence of apomyoglobin and identification of peptide fragments from in-droplet digested apomyoglobin shown in Figure 7b.

GLSDGEWQQVLNVWGKVEADIAGHGQEVLRIRLFTGHPETLEKFDKFKHLKTEAEMKASEDLKKHGT
VVLTAALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHSHKHPGDFGADAQGAMTKA
LELFRNDIAAKYKELGFQG (Apomyoglobin sequence)

<i>m/z</i>	Mass	<i>z</i>	Position	Sequence
620.05	1856.0	3+	138-153	FRNDIAAKYKELGFQG
690.08	4133.9	6+	70-106	TALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEF
827.81		5+		
665.77	4653.4	7+	30-69	IRLFTGHPETLEKFDKFKHLKTEAEMKASEDLKKHGTVV
776.60		6+		
931.54		5+		
682.12	4767.5	7+	110-153	AIIHVLHSHKHPGDFGADAQGAMTKALE LELFRNDIAAKYKELGFQG
795.60		6+		
954.53		5+		
727.21	5082.8	7+	107-153	ISDAIIHVLHSHKHPGDFGADAQGAMTK ALELFRNDIAAKYKELGFQG
848.27		6+		
1017.56		5+		
1046.27	3133.6	3+	1-29	GLSDGEWQQVLNVWGKVEADIAGHGQEVLR