SUPPORTING INFORMATION

Control and measurement of the phase behavior of aqueous solutions using microfluidics

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Contents

S1. Drawings and photolithographic masks of the Phase Chip

Drawings are produced using a computer aided design program, AutoCad (AutoDesk Inc.) as shown in Figure S1.1. The Phase Chip consists of two PDMS layers. The *flow layer* contains the flow channels (green), in which the microdrops are formed and transported, the wells (blue) where the drops are stored, and valves (dark green) which are used to direct the flow of the drops. The *control – reservoir layer* contains both the control channels, used to open and close the push-up valves[1], and the reservoir, used to control the chemical potential of the stored drops (orange). Four masks, one corresponding to each color, are used to build these layers. High resolution photo masks are fabricated on transparencies with a one or two day turn-around time after email submission of AutoCad files (CAD/Art Services Inc., OR).

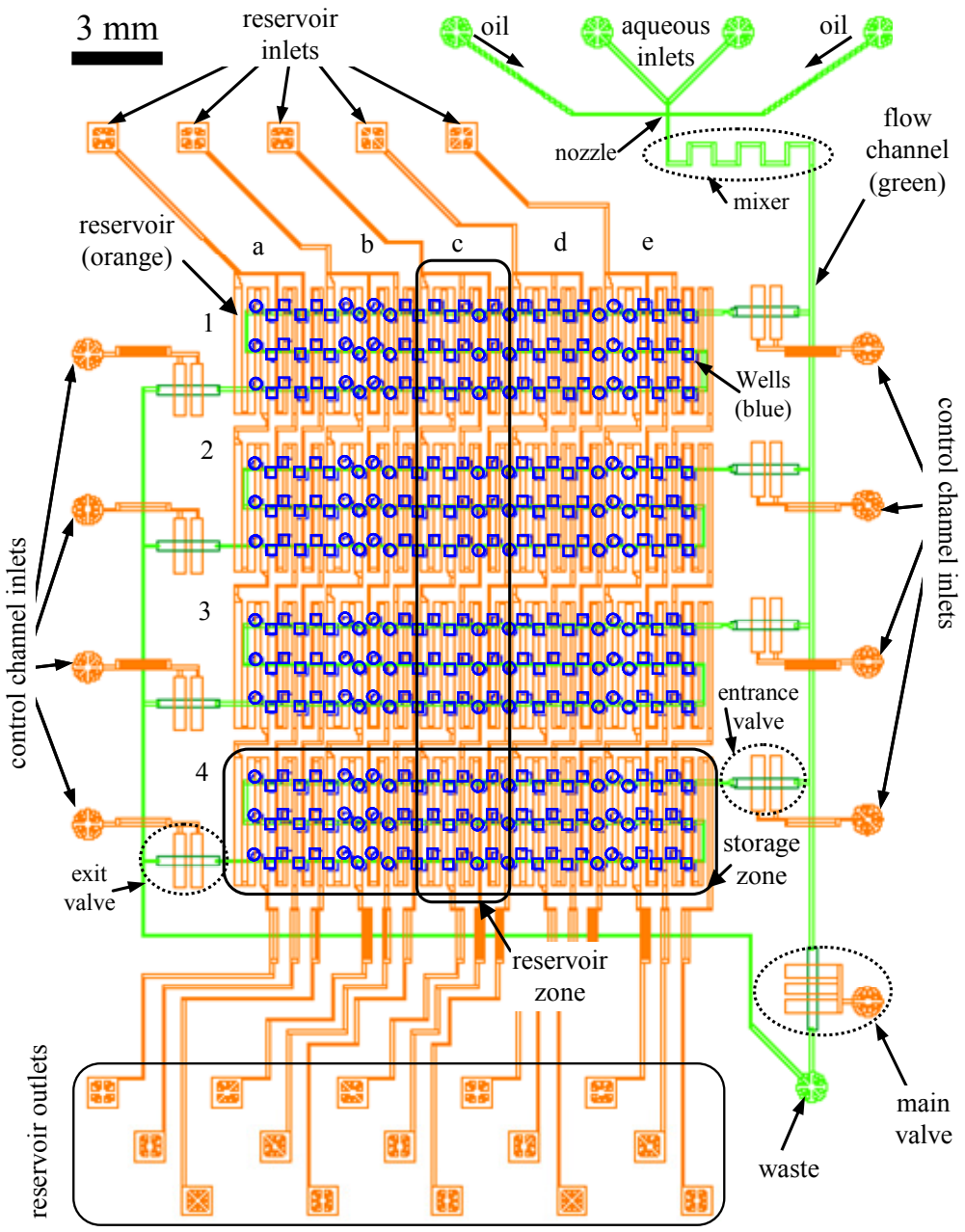

Figure S1.1. The Phase Chip, rendered here using AutoCAD, is a two-layer PDMS device. This drawing was transferred onto four masks made of plastic film. Three masks were used to construct the flow layer (flow channel – green, wells – blue, valves – dark green) and one mask was used for the control – reservoir layer (control channels and reservoir – orange). The chip contains four storage and five reservoir zones.

S2. Manufacturing Process

S2.1. Photolithography

As shown in Figure S2.1a, fabrication of the Phase Chip begins with drawing the microfluidic circuit and producing the masks as described in section S1. Each of the two PDMS layers are formed from molds constructed of photoresist spun-coated on silicon wafers. The flow layer mold is made from three applications of photoresist. Two of the applications consist of negative resist; the first for the flow channel and the second for the wells corresponding to the light green and blue masks, respectively. The third application consists of positive resist for the valves corresponding to the dark green mask.

With negative photoresist (SU8-2025, Microchem Inc.) the portion that is exposed to UV light becomes relatively insoluble to the developer (1-Methoxy-2-Propanol Acetate, Sigma-Aldrich) and remains on the wafer while the unexposed portion of the photoresist is dissolved by the developer. With positive photoresist (AZ-P4903, Clariant Inc.) the exposed portion of the photoresist is dissolved by the developer and the portion of the photoresist that is unexposed remains attached to the wafer.

Valves consist of three components; a portion of the flow channel with a rounded cross-section on top (dark green), the control channel with a rectangular section below that is oriented perpendicular to the flow channel (orange) and a thin membrane of PDMS separating the control and flow channels. In the closed state, high pressure is applied to the control channel displacing the thin membrane which seals the rounded flow channel. When the pressure is released the elasticity of the membrane opens the valve. Positive photoresist is used for the valves because it is a thermoplastic polymer which melts on heating. Upon melting, positive photoresist channels with rectangular cross section reshape into channels with semicircular cross-sections which improves the sealing of the valves [1].

The first step in constructing the molds is to clean the silicon wafers (3 inch diameter silicon wafer, Silicon Sense Inc.) by rinsing with methanol and iso-propyl alcohol and blow drying with nitrogen.

Figure S2.1 illustrates the photolithographic process for negative photoresist. After a spin-coated, exposed wafer is placed in the developer, the unexposed photoresist dissolves leaving the exposed, crosslinked photoresist attached to the silicon wafer (Figure S2.1b). This wafer, with the mask's pattern sculpted in bas relief out of photoresist, becomes the mold from which the PDMS layer is fabricated. In order to make three-dimensional structures, such as the storage wells, an additional photolithographic step is employed, in which a second layer of negative photoresist is spin-coated on top of the undeveloped, but exposed first layer, as illustrated in Figure S2.1c. The detailed process conditions for fabricating the molds for the two PDMS layers are described in Table S2.1a.

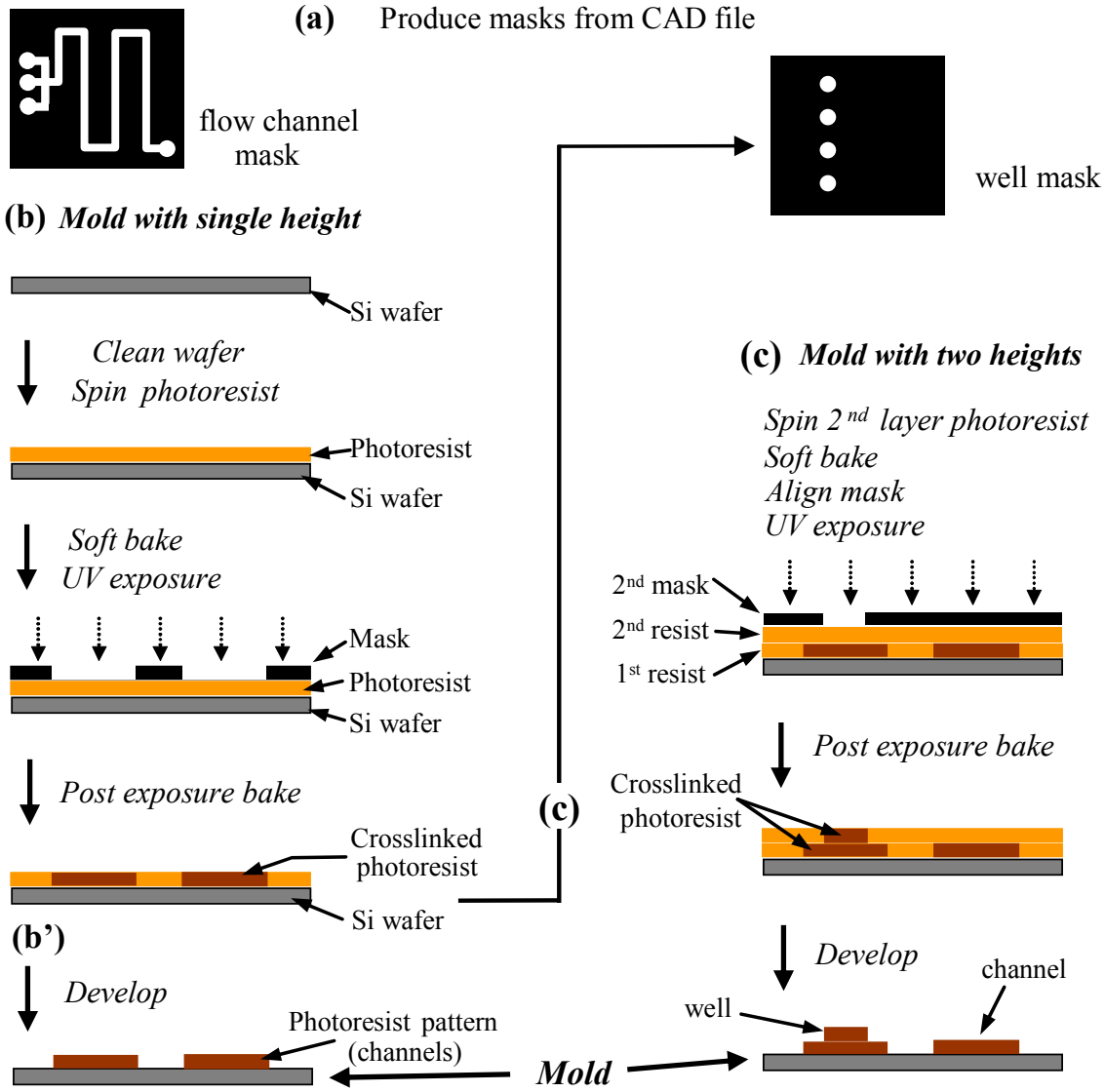

Figure S2.1 Photolithographic fabrication of PDMS molds using negative photoresist. **(a)** A microfluidic design is drawn using a CAD program and a mask is produced on a mylar sheet. **(b)** Photoresist is spun on a silicon wafer. The resist is exposed to UV light through the flow channel mask (green in Fig. S1.1) and baked, which crosslinks the resist. **(b')** For a mold with resist features of a single height (i.e. the control – reservoir layer) unexposed resist is removed by the developer. **(c)** Storage wells require molds with resist of two heights. Before development an additional photoresist layer is processed after aligning the well mask (blue in Fig. S1.1) over the flow channels.

S2.2. Soft lithography

Soft lithography [2], or the construction of microfluidic devices from poly(dimethyl siloxane) (PDMS), is illustrated in Figure S2.2. A commercially available liquid PDMS kit (Sylgard184, Dow Corning) consists of a pre-polymer and a crosslinker and is designed to be mixed at a weight ratio of 10:1, respectively. To build a single layer PDMS device the mold is placed in a Petri dish that is slightly larger than the wafer and the mixed, degassed liquid PDMS is poured over the mold to a thickness of 5 mm and then cured by baking at 65 ˚C for 25 minutes. The resulting transparent, flexible silicone rubber is peeled off the mold, leaving relief features from the mold imprinted into the PDMS slab. Injection holes are punched through the PDMS in order to insert tubing that transports fluids into the device. At this point the PDMS slab has channels that are open on one surface and needs to be sealed. For a single layer device the PDMS is directly bonded to glass by exposing both surfaces to oxygen plasma and then placing the two surfaces in contact as in Figure S2.2**a**.

The Phase Chip is a two layered PDMS device and therefore is fabricated from two molds. The *flow* mold contains the flow channel, the wells and the valves. The flow layer is formed by pouring a 5 mm thick layer of PDMS on top of the flow mold. The *control / reservoir* mold contains the valve control channels and the reservoir. The control / reservoir layer is formed by spin coating a 40 μm thick layer of PDMS over the control / reservoir mold. The weight ratio of pre-polymer and crosslinker for the thick flow layer is 5:1 so the crosslinker is in excess, and 20:1 for the thin control / reservoir layer so the pre-polymer is in excess. Each PDMS layer is partially cured until the surfaces have just stopped being tacky as determined by touching with a bare finger. After this partial curing, the thick flow layer is removed from the flow mold and aligned with the reservoir / control layer, which is still attached to its mold. Alignment is done by hand using ~ 2X magnification on a stereo zoom microscope. After alignment, the two layer assembly is baked again at 85 ˚C for 30 minutes to bond the layers. While baking, crosslinkers from the partially cured flow layer, where they are in excess, diffuse into the control / reservoir layer and pre-polymer, which is in excess in the control / reservoir layer diffuses into the flow layer [3]. The pre-polymer and crosslinker react, which produces a crosslinked elastomer spanning the interface, bonding the layers together. Next, the two-layer PDMS slab is peeled off the control / reservoir layer mold. Injection holes to insert tubing that transports fluids into the device are punched through the slab using a 25 gauge Luer stub (McMaster, Inc.) and debris is removed by blowing the holes with dry nitrogen gas. The control channels and reservoir are open on one surface. In order to close the open face, the PDMS slab and a glass substrate are exposed to a oxygen plasma then placed one on the other forming a very tight and irreversible seal [4]. The detailed conditions for fabricating the mold and processing the PDMS are described in Table S2.1.

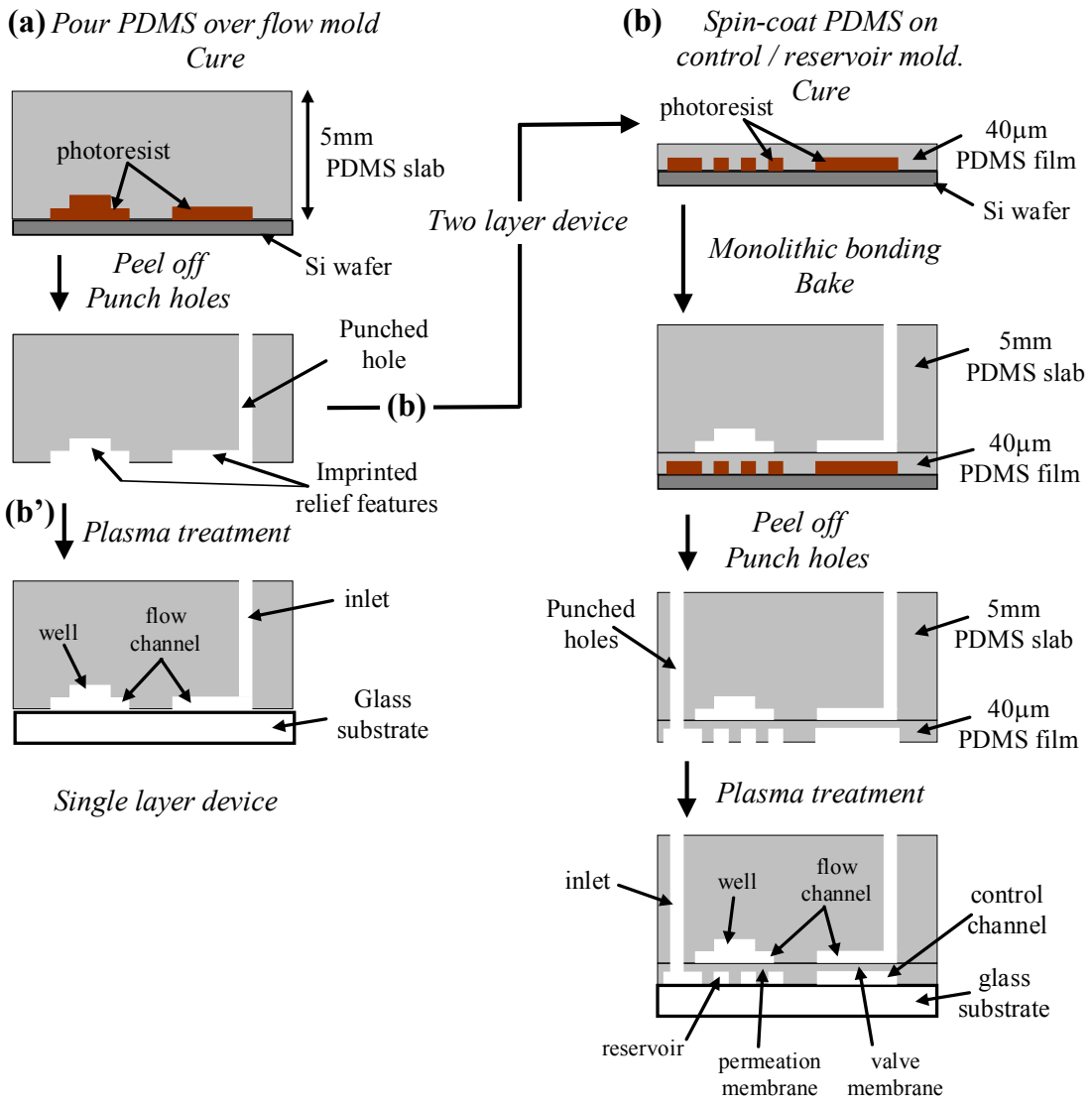

Figure S2.2 Microfluidic device fabrication using soft lithography. **(a)** Liquid PDMS is poured onto the flow mold and then cured at 65 ˚C. The soft elastomer is peeled off, leaving patterns embossed in the PDMS. Access holes are punched through the PDMS. **(b')** If the device is a single layer of PDMS it is bonded to a glass substrate by an oxygen plasma treatment. **(b)** If the device is composed of two PDMS layers, then the thick flow layer is aligned on the thin control / reservoir (multi layer soft lithography), cured in an oven to bond the two layers, removed from the control / reservoir mold, additional access holes are punched and finally the device is bonded to a glass substrate.

(a)

Pre-treatment			Squirt wafer with methanol, isopropyl alcohol (IPA) Dry with compressed N_2
Flow Layer: Channel	1st PR[1] Coat Negative resist	PR: SU8-2025 thickness: 30µm	Spin: 0rpm → 800rpm / 5sec → 2800rpm / 60sec Ramp rate: 100rpm/s 300rpm/s Soft bake: 65ºC / 2min → 95ºC / 5min
	1st Exposure	Mask Id: Flow	Exposure: 700 mJ, 365 nm Post exposure bake: 65ºC / 2min → 95ºC / 4min
Flow Layer: Well	2nd PR Coat Negative resist	PR: SU8-2007 thickness: 7µm	Spin: 0rpm → 800rpm / 5sec → 2800rpm / 60sec Ramp rate: 100rpm/s 300rpm/s Soft bake: 65ºC / 1min → 95ºC / 2min
	2nd Exposure	Mask Id: Well	Exposure time: 10sec Post exposure bake: 65ºC / 1min → 95ºC / 2min
	Develop		Developer: 1-Methoxy-2-Propanol Acetate Rinse with IPA
Flow Layer: Valve	3rd PR Coat Positive resist	PR: AZ-P4903 thickness: 40µm	HMDS[2] treatment. Spin: 0rpm → 500rpm / 3sec → 1500rpm / 60sec → 500rpm / 0sec Ramp rate: 100rpm/s 100rpm/s 100rpm/s Wait 10min Soft bake: 65ºC / 3min → 105ºC / 5min
	3rd Exposure	Mask Id: Valve	Exposure time: 50sec
	Develop		AZ400k[3] diluted 4 times with DI water Rinse with water
	Reshape positive resist		Bake: 70ºC → 110ºC / 10min
Control / Reservoir Layer	PR coating on second wafer Negative resist	PR: SU8-2025 thickness: 30µm	Spin: 0rpm → 800rpm / 5sec → 2800rpm / 60sec Ramp rate: 100rpm/s 300rpm/s Soft bake: 65ºC / 2min → 95ºC / 5min
	1st Exposure	Mask Id: Control	Exposure time: 15sec Post exposure bake: 65ºC / 2min → 95ºC / 4min

PR[1]: Photoresist; HMDS[2]: hexamethyldisilazane; AZ400k[3]: the developer for AZ-P4903.

TABLE 1. (a) Photolithographic conditions

(b)

Flow Layer: Thick PDMS slab	Pour mixed 5:1 ratio PDMS over flow layer master contained in a Petri dish to a 5 mm thickness. Bake at 65ºC for 25min in an oven. Cut out cured PDMS and punch access holes with a 20 gauge Luer stub.
Control / Reservoir Layer: Thin PDMS film	Spin coat 20:1 ratio PDMS on the control master. Spin: 0rpm → 800rpm / 5sec → 2000rpm / 60sec Bake on a hot plate at 85ºC for 5min.
Assemble thick and thin layers.	Align the detached thick slab onto thin film PDMS, which is still attached to the wafer using a 2X binocular with epi-illumination. Bond layers by placing on a hot plate at 85ºC for 30min. Peel PDMS off of wafer and punch access holes with a Luer stub. Bond two-layer PDMS on a glass substrate with an oxygen plasma treatment (100W for 9 sec. @ 300 mT O_2).

TABLE 1(b) Multilayer soft lithography

S3 Surface Treatment of the Flow Channel

PDMS is a widely used material to fabricate microfluidic devices for chemical applications involving aqueous solutions. PDMS is easy to handle, optically transparent and has a low manufacturing cost. However, PDMS has several recognized problems limiting practical use, such as its incompatibility with organic solvents [5], which swell the device, distorting micron size features [6] and the adsorption of biological molecules, such as DNA and proteins to the PDMS surface [7].

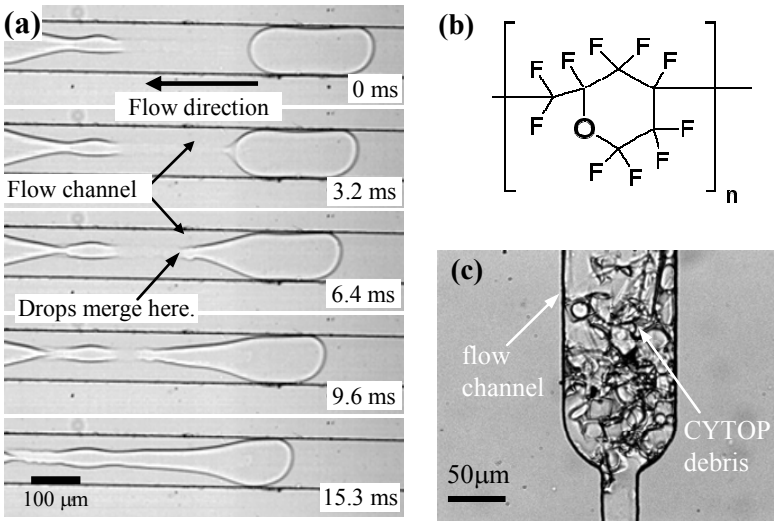

Figure S3.1 (a) The sequence of photographs show a protein drop flowing down a channel. Upstream (to the right) the aqueous drop is enveloped by oil and is not in contact with the PDMS. As the drop flows downstream it begins to wet the PDMS and later merges with another drop further downstream. The protein solution is 40 mg/ml of lysozyme (Seikugaku) in a buffer of 0.1 M sodium acetate, pH 4.5. The oil is FC-43 (3M) with an surfactant added (5%wt of Tridecafluoro octanol, Sigma-Aldrich). **(b)** The molecular structure of CYTOP perfluoro-polymer. It is coated on the surface of the channel as described in the text. After CYTOP treatment the fluorinated oil preferentially wets the PDMS surface, preventing the protein solution and water from adhering to the surfaces of the PDMS channel. **(c)** Excess CYTOP clogs the channel. If the concentration of CYTOP solution exceeds 0.5 %(w/w), the channel becomes blocked with CYTOP.

S3.1. Failure of the Phase Chip due to Adhesion of Aqueous Drops

Often aqueous drops in fluorocarbon oil will flow freely during the first minutes of operation, but with time we found that drops stuck to the surface of PDMS channels, as shown in Figure S3.1a. This problem was exasperated for aqueous drops containing proteins when drop adhesion occurred after only a few minutes of operation. Once a drop adhered to the surface, the following drop would merge with the stuck one and subsequently break free. Even drops of pure water began to stick to the surface of PDMS channels after several hours of continuous operation.

The cause of drop adhesion is not well understood. It could occur due to the adsorption of solutes in the drops, or as a result of a contaminated surface of the channels, since the sticking does not occur throughout the device. Figure S3.1a shows a mobile drop merging with a stuck drop in the flow channel. Normally, flushing of the channel with oil or with water fails to resolve the problem.

S3.2 Rendering PDMS fluorophillic

PDMS surfaces coated with CYTOP (Asahi Glass Company) become fluorophilic. The continuous fluorinated oil phase wets the CYTOP treated solid surface, which reduces the adhesion of aqueous drops to PDMS. CYTOP-107M is a perfluoro-polymer, consisting of a 7 %(w/w) solution of poly (1,1,2,4,4,5,5,6,7,7,-decafluoro-3-oxa-1,6-heptadiene) (molecular weight 60,000 ~ 70,000) dissolved in CT-solv100 (perfluorotributylamine). The molecular structure of CYTOP is shown in Figure S3.1b. The

glass transition temperature of CYTOP is 108 ˚C, and the boiling point of the solvent is 100 ˚C. A CYTOP film doesn't interrupt the visual clarity of PDMS because the transmittance is more than 95 % for a 200 μm thick film in the frequency range of 400 nm ~ 700 nm. CYTOP must contain a coupling agent as according to the manufacturer the substrate doesn't require pre-treatment for direct adhesion onto metal, silicon, silicon oxide, and glass. Since the PDMS is a silicon-based organic material [4, 8], CYTOP can directly adhere to the surface of the PDMS and has been used to passivate the surface of the PDMS [7].

S3.3 Process Conditions for CYTOP Coating

The following multi-step process is used to deposit CYTOP on PDMS channels. CYTOP-107M solution is supplied as a 7 % (w/v) solution which we dilute to 0.25 %(w/v).

Step 1. Coating the flow layer with CYTOP.

The Phase Chip consists of a number of channels connected in parallel and series. CYTOP is injected into the Phase Chip with a syringe pump typically running at 30 μl /hr. The CYTOP solution flows down the path of least resistance, which leaves a number of channels filled with air. PDMS is permeable to air, which allows dead-end filling. In order to purge the air trapped in the device a counter pressure of 5-20 PSI was applied to the waste outlet.

CYTOP coating was done on a hot plate at 75 ˚C for 30 minutes. The following considerations may help to adjust CYTOP coating for other PMDS devices. CYTOP solvent is soluble in PDMS, but CYTOP is not. Thus there is a permeation flux of solution from the channel into the PDMS, which deposits CYTOP on the PDMS surface. It was reasoned that the permeation flux would increase with temperature since the boiling point of CYTOP-107M is 100 ˚C. Thus, the coverage of PDMS by CYTOP is assumed to increase with temperature, time, and CYTOP concentration. However, increasing the CYTOP concentration above 0.5% (w/v) leads to clogging of the channels as shown in Figure S3.2c.

Step 2. Removing CYTOP.

It is necessary to remove any extra CYTOP solution from the flow layer. Otherwise, as the CYTOP solution dries a thick film accumulates on the channel surface and can clog the flow channel. In order to remove the CYTOP solution, air is blown into the channel. The air pressure is increased up to 12 psi, but not higher as delamination of the multi-layer device is a possibility. To remove the CYTOP the valves are used to force the air through one section of the chip at a time. This removal process is also done on the hot plate at a temperature of 75 ˚C.

Step 3. Curing CYTOP.

The air dried CYTOP coated device is baked at two different temperatures. First, the device is baked for 2 hours at 110 ˚C to remove residual solvent. Second, the manufacturer recommends heating CYTOP above the glass temperature to form a smooth, durable fluorinated film. The glass transition of CYTOP is 108 ˚C and the 2nd bake is done at 210 ˚C for another 2 hours. The resulting CYTOP treated device performs better than the untreated device. In contrast to what is seen in Fig S3.1a, no adhesion of drops was observed, even after 48 hours of continuous production of aqueous drops in fluorinated oil (FC-43, 3M).

S4. Movies

Figure S4.1. Drop formation. Figure caption for movie: DropFormation.avi.

The Phase Chip is a poly(dimethylsiloxane) (PDMS) device which utilizes hydrodynamic focusing to produce drops of protein solution inside a continuous oil stream. This movie shows the nozzle, illustrated in Figure 1b. Channels are 100 microns wide and the drops are about 1 nl. This movie is not real time; drops are formed at 50 hz. The oil is introduced in channels on the top and bottom of the frame. The protein solution is premixed and enters from the right side of the frame. All fluids are driven by syringe pumps. The movie is an .avi file made with QuickTime.

Figure S4.2. Drop storage. Figure caption for movie: DropStorage.avi.

One of the Phase Chip's innovations is to exploit surface tension forces to guide each drop to a storage chamber, or well, illustrated in this movie and in Figure 1. The device is designed such that the channels flatten and elongate the drop. Wells, located to the side of the channel are deeper than the flow channel. A drop in a well can adopt shape that decreases its surface area and hence its surface energy. A drop that partially occupies both a channel and well will experience a gradient in surface energy, with the resulting force acting to drive and store the drop inside the well. As the wells exist as pockets on the sides of the channel, the enclosed, stored droplets are outside the flow stream and shielded from dislodgment by hydrodynamic forces.

Drops sequentially fill the wells, with the first drop going into the first well. Subsequent drops pass over all filled wells, entering the first empty well. To prevent coalescence of the drops during the loading process, surfactants must be added to the bulk, continuous phase. In this movie, the oil is hexadecane and the surfactant is Span80 (2% w/v)) and it was filmed at 2500 frames / sec.

Figure S4.3. Size matters. Figure caption for movie: LeapFrog.avi.

Care must be taken to match the volume of the drop to the wells. If the docked drop is too large it will protrude into the channel and the next drop will dislodge it. If the docked drop is too small, a second drop will share its well. In this movie, the drop is half the volume of the rectangular wells located upstream. Subsequent drops displace the stored drop, creating a cascade. Downstream there are cylindrical wells which match the drop size so that docked drops are not displaced by subsequent drops. Note that the cylindrical wells have small slots at twelve and six o'clock. These allow the oil to drain out of the slots as the aqueous drops enter the wells. Without the slots the drops are sometimes blocked from entering the wells because the oil they are displacing has no exit path. In this movie, the oil is hexadecane and the surfactant is Span80 (2% w/v) and it was filmed at 2500 frames / sec.

Figure S4.4. Drop storage without surfactant. Figure caption for movie: FastSlow.avi.

As in Figures 1 and S4.2, this storage method uses wells adjacent to, but connected to the flow channel. The drops flow by the wells when moving at high flow rates if the well is shallow in depth, comparable with the height of the flow channel. When the flow stops the drops spontaneously dock into the nearest well in a game of microfluidic musical chairs. In this method the first drop docks into the last well and the last drop docks into the first well. Without surfactant to stabilize the drop some mixing of drops occurs and typically the contents of about three or four adjacent drops are shared. In this movie a fluorinated oil (FC-43, 3M) mixed with a surfactant (12%w/w, Tridecafluoro octanol, Sigma-Aldrich) was used. The surfactant lowers surface tension making it easy to create drops and simultaneously slows coalescence of drops. Extraction of the drops from the wells is possible when the flow direction is reversed, making recovery of the droplets possible. The movie is recorded in real time.

Figure S4.5. Liquid – liquid phase transition. Figure caption for movie: LiquidLiquid.avi.

In Figure 5 the liquid – liquid phase boundary of a PEG / ammonium salt mixture is mapped. In Figures 5b, c and d three images are shown of a single drop as water flows out and the solutes concentrate. Two wells, similar to the well shown in Figures 5b,c and d, are shown in this 24 hour time lapse movie. The drop starts out in the single phase region (Figure 5b). As the drops shrink they take on a bluish tinge as the dye is concentrated. At a certain point there is a sudden flash as the liquid-liquid

phase boundary is crossed. Note that both drops cross the phase boundary simultaneously. As the drop further shrinks, the proportion of the dense liquid phase increases.

Figure S4.6. Two step protein crystallization pathway. Figure caption for movie: KineticEnhancement.avi

A time lapse movie of the second from the rightmost well in Figure 7 is shown in this timelapse movie of 138 hour duration. The reservoir is filled with 6M NaCl solution for 42 hours. The initially large drop shrinks thereby increasing both the lysozyme and PEG concentration. The protein / polymer (lysozyme / PEG) mixture precipitates indicating this condition is deep in the supersaturation region. After 42 hours the reservoir is replaced with a 2M NaCl solution. The drops swell, diluting the solute concentrations. The drops first became clear and then a single crystal grows at the oil/water interface.

Figure S4.7. Crystal annealing. Figure caption for movie: CrystallizeDissolveXylanase.avi.

A crystal of xylanase is alternately grown and dissolved by varying the contents of the reservoir. The xylanase (Hampton Research, HR7-104), was dialyzed against 0.4M potassium sodium tartrate tetrahydrate (Hampton Research, Crystal Screen HR2-110) and the initial protein concentration was 15.3 mg/ml. Xylanase does not crystallize under these conditions. When the reservoir contains high salt, water flows out of the protein solution, dehydrating the protein solution and the crystal grows. When the reservoir contains low salt, water flows into the protein solution and the crystal dissolves.

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