Supplementary Data

#	File name	File Type	Description
0	Supplementary Data.pdf	Pdf	Word document containing captions for 7 videos, tables, and process conditions.
1	Movie S1 Silicon Wafer.mpg	MPEG	MPEG video showing photoresist master fabrication.
2	Movie S2 PDMS Master.mpg	MPEG	MPEG video showing fabrication of the PDMS mold for microfluidics.
3	MovieS3 COC patterned chip.mpg	MPEG	MPEG video showing patterning of microfluidic features in COC.
4	Movie S4 COC Cover.mpg	MPEG	MPEG video showing fabrication of the COC lid.
5	Movie S5 Sealing the chip.mpg	MPEG	MPEG video showing the sealing of the chip.
6	Movie S6 Loading the Chip.mpg	MPEG	MPEG video showing loading of the protein solution into the store-then-create chip.
7	Movie S7 Zero waste.mpg	MPEG	MPEG video showing zero waste storage in the store-then-create chip.
8	Store Then Create.dwg	Autocad	Autocad file of the design of the Store-Then-Create chip.
9	Al Mold Layer 1.SLDPRT	Solidworks	Solidworks file of the first layer of the aluminium mold for the lid.
10	Al Mold Layer 2.SLDPRT	Solidworks	Solidworks file of the decond layer of aluminium mold for the lid.
11	Cover.SLDPRT	Solidworks	Solidworks file of the lid, which is produced by the combined two-layer aluminium mold.
12	Mold for Ferrule.SLDPRT	Solidworks	Solidworks file of the plexiglas mold used for making the ferrule.

Rapid Prototyping of Cyclic Olefin Copolymer (COC) Microfluidic Devices

Movies S1-S5 provide detailed instructions for each of the manufacturing steps. **Movies S6-S7** illustrate operation of the protein crystallization device.

Movie S1: Photoresist master fabrication.

The video shows spin coating the photoresist (SU8-3010, Microchem Inc.). SU8 photoresist processing was performed according to the manufacturers specification. In brief, after spinning the resist on a silicon wafer (Silicon Sense, Inc.) it is pre-exposure baked at 95 ºC for 20 min on a hotplate and subsequently placed in the mask aligner. After covering the wafer with a photomask, the photoresist is exposed to 360 nm UV light. Then a post-exposure bake is performed at 95 ºC for 5 min. The wafer is then allowed to cooled down to room temperature by placing it on a sheet of cleanroom paper. The last step is PGMEA (2-methoxy-1-methylethylacetate) development to remove uncured photoresist. Multiheight wafer processing is performed as previously described[1].

Movie S2: Microfluidic PDMS mold fabrication.

A CNC milled plexiglass frame is placed around the rim of raised photoresist. PDMS (Dow Corning, Sylgard 184) is mixed (10:1 ratio by mass) in a mixer (AR-250, Thinky, Inc) and then poured into the Plexiglas frame. The mold is then placed in vacuum for 10 minutes to remove small bubbles trapped in the microfluidic features. A layer of Mylar sheet (DuPont-50 micron) is placed on top of the liquid PDMS, which eases demolding. A glass slide is placed on the Mylar sheet and clamped so that it can be placed securely in the oven for baking at 70ºC for at least 2 hours.

Movie S3: Microfluidic feature patterning in COC.

A CNC milled aluminum frame is placed on top of a Kapton sheet (5 MIL Kapton film, American Durafilm, Inc). The PDMS mold is inserted into the aluminum frame. COC pellets (Grade 8007, TOPAS) are poured on the PDMS mold. Another piece of Kapton sheet is placed on the COC pellets. Heat and pressure are varied according to **Table S1**.

Table S1. Temperature, pressure and time settings of thermo-press to replicate the microfluidic PDMS mold in COC.

Step start time [min]	Step duration [min]	Temperature [ºC]	Pressure [psi] (MPa)
0	8	170	0
8	6	150	200 (1.38)
14	10	25	200 (1.38)

In the first step, before applying any pressure, the heating plates of the thermopress are lowered onto the assembly, to heat the pellets, frames and molds for about 8 min until the COC pellets have softened. In a second step, pressure is applied to press the molten COC into the PDMS master. Softening the pellets first prevents damage to the PDMS master by hard COC pellets.

In our design, the heights of the PDMS master or mold (we use these two terms interchangeably) and aluminum frame are equal. This is because we desire to produce sheets of featured COC that are less than 150 microns thick. The aluminum frame has drainage channels to relieve the pressure on the molten COC and to allow excess COC to flow out of the mold. Ultimately, because the thermopress plates rest on the aluminum frame, the actual pressure that is applied by the thermopress is not important. The COC sheet formed in this process assumes an irregular shape when it flows beyond the PDMS mold. Excess COC is trimmed off with a scissor. Thin marks on the COC, which form at the interface between the PDMS master and aluminum frame, serve as guidelines for cutting. To fabricate thicker COC chips, simply adjust the relative heights of PDMS master and its aluminum frame to yield the desired COC chip height (**Figure S1**). We did not explore whether the COC chips could be made thinner by using higher temperatures to lower the viscosity.

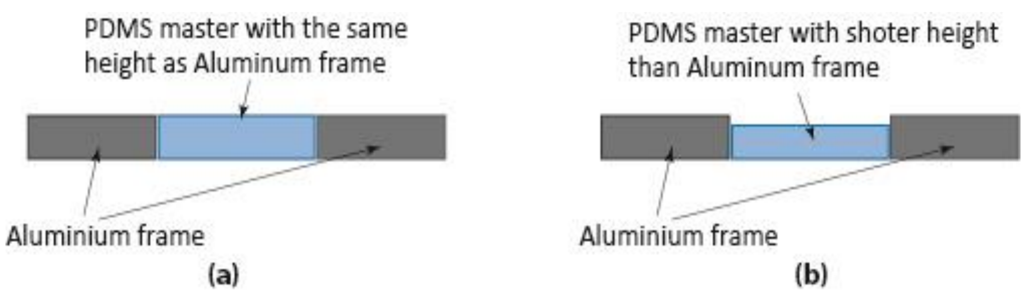

Fig. S1. **(a)** Using a PDMS master and aluminium frame with the same height results in thin COC chips (~100 μm). **(b)** Thicker COC chips can be made by reducing the height of PDMS master.

Movie S4: COC lid fabrication.

The 2-layer aluminum mold is placed inside the aluminum frame on a piece of Kapton sheet. A thin layer of Kapton tape (1 Mil Polyimide Kapton® with adhesive backing) is taped onto the aluminum mold to yield a clear surface finish on the final COC lid. COC pellets are poured on the aluminum mold, inside the aluminum frame. A small piece of Kapton sheet (4 Mil) is placed on top of the pellets to obtain a clear surface finish on the other side of the COC lid and to facilitate demolding. An aluminum slab (29.5 x 39.5 x 5 mm) is placed on top of the Kapton sheet. Pressure and heat is applied based on **Table S2**.

Table S2. Temperature, pressure and time settings of thermo-press to mold the COC lid/manifold.

Step start time [min]	Step duration [min]	Temperature [ºC]	Pressure [psi] (MPa)
0	8	170	0
8	6	150	200 (1.38)
14	10	25	200 (1.38)

Demolding is performed with a small benchtop press (Grizzly Arbor Press - 1/2 Ton) to separate the mold and frames. A knife is used to separate the two mold layers from the lid. Edges of the COC cover are cleaned with a file and holes are drilled through the fluidic access ports (Cameron Micro drill press, Model 214-A-1). Drilling is done with the lid submerged in water in a petri dish to reduce heat. Otherwise the low temperature COC could melt, gum up the drill bit and distort the hole. Solidworks files (Al Mold Layer 1.SLDPRT & Al Mold Layer 2.SLDPRT & Cover.SLDPRT) are included.

Movie S5: Sealing the chip.

The COC lid is soaked in a mixture of Ethanol-Decalin, 85%-15% by weight for 5 min. We used a plate shaker (OMEGA SKR-14) to mix the fluids. After soaking, the COC lid is rinsed with ethanol and air dried. The COC lid is mated with the PDMS stability form with the flat surface on top. The COC with the microfluidic features is placed on the COC lid while channels are aligned with holes on the COC lid. Since all the pieces are made by casting, they all have the same dimensions so aligning the holes is not difficult. A PDMS slab is placed on top of the patterned COC to make the pressure uniform. Similarly to other processing steps, two pieces of Kapton sheets are placed on the bottom and top of the assembly before turning on the thermopress. For sealing, only a comparably small amount of heat and pressure is applied to avoid unwanted distortions (**Table S3**). For our set-up applying 43 psi (0.3 MPa) (approximately 80 lbs on a 3 x 4cm chip) constituted the optimal pressure to ensure a strong bond, while preventing deformation of microfluidic features.

Table S3. Temperature, pressure and time settings of thermal-press for sealing

Step start time [min]	Step duration [min]	Temperature [ºC]	Pressure [psi] (MPa)
0	8	65	43 (0.3)
8	10	25	43 (0.3)

Movie S6: Loading of the protein crystallization "store-then-create chip".
After surface treatment to render the chip fluorophilic, the chip is first primed by injecting a mixture of 78 vol% FC-43 fluorinated oil with 12 vol% surfactant (1H,1H,2H,2H-Perfluoro-1-octanol) using syringe pumps. Oil displaces the air and fills all the features (channels, wells and capillary valves). Air bubbles trapped in the storage wells dissolve into the oil, which is pressurized by flow driven by the syringe pumps. Once the oil is thoroughly degassed the sample is injected. In this movie we use red food dye to enhance the contrast between the oil and aqueous phases. The aqueous fluid passes through the channels and wells, but not through the capillary valves located on the well exit. Switching between oil and protein is done by a 2 position, 6 port valve (MV303, LabSmith) (**Figure S2**). When the desired amount of sample is loaded from the injection loop, the valve is switched back to the initial position to inject oil for the second time. Oil pushes the aqueous sample solution into the remaining unfilled wells and then purges the aqueous solution from the channels, leaving the sample isolated in the wells. Note that this method has zero dead volume. All sample injected into the chip will be stored into the chip's wells, as illustrated in **Movie S6**.

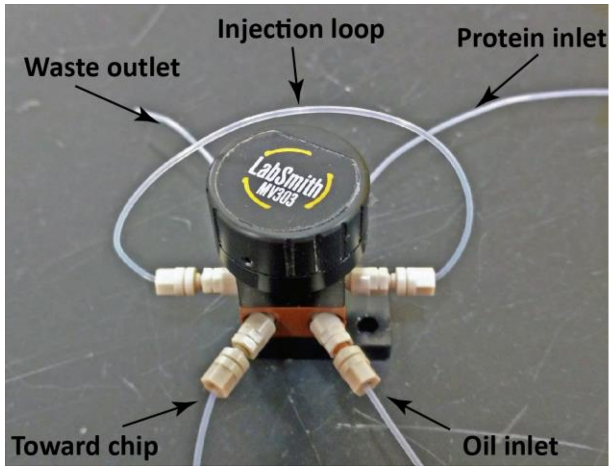

Fig. S2. A manual injection loop consisting of a LabSmith-MV303, 2 position – 6 port valve with 1/32 tubing was used for loss free sample loading.

Movie S7: Zero waste storage.

The protein crystallization store-then-create chip is designed to have zero waste for protein, which in this movie is replaced with red food dye. 100% of the sample is loaded into wells, with none of the sample residing in the channels or being expelled from the chip. In the beginning of the movie the chip is prefilled with FC-43 oil. A predefined volume of food dye (0.7 µL) is injected. No food dye is seen exiting the outlet.

Making ferrules.

First we CNC machined a Plexiglas mold for PDMS ferrules. 0.8 mm needles are placed in the holes. PDMS (Dow Corning, Sylgard 184) is mixed at a 10:1 ratio by mass in a mixer (AR-250, Thinky, Inc) and then poured into the Plexiglas mold. The assembly is baked in an oven at 70ºC for two hours. A Solidworks file for the Plexiglas mold (Mold for Ferrule.SLDPRT) is included.

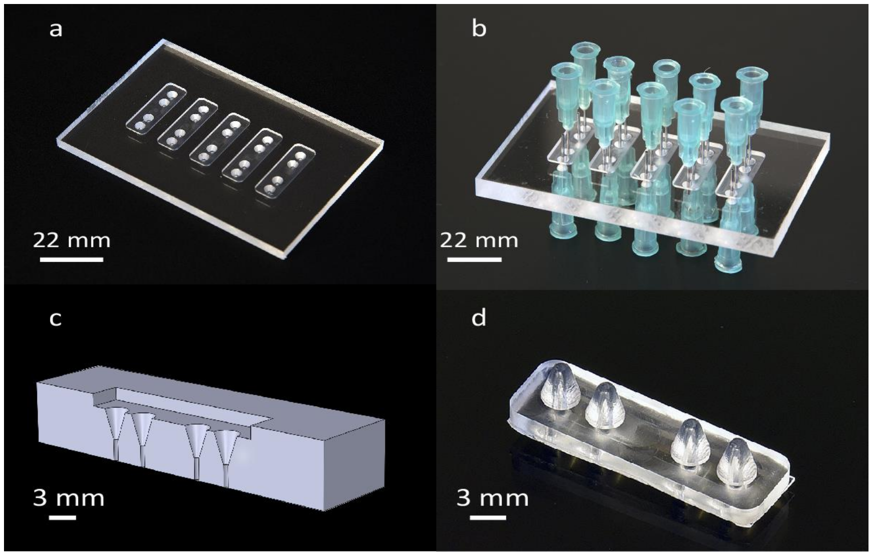

Fig. S3. a) CNC machined Plexiglas mold for casting PDMS ferrules. **b)** 0.8 mm needles are inserted in the holes so that the fluidic tubing feeds through the ferrule. **c)** Cross section of the Plexyglas mold, which is shown in (a). **d)** Actual PDMS ferule.

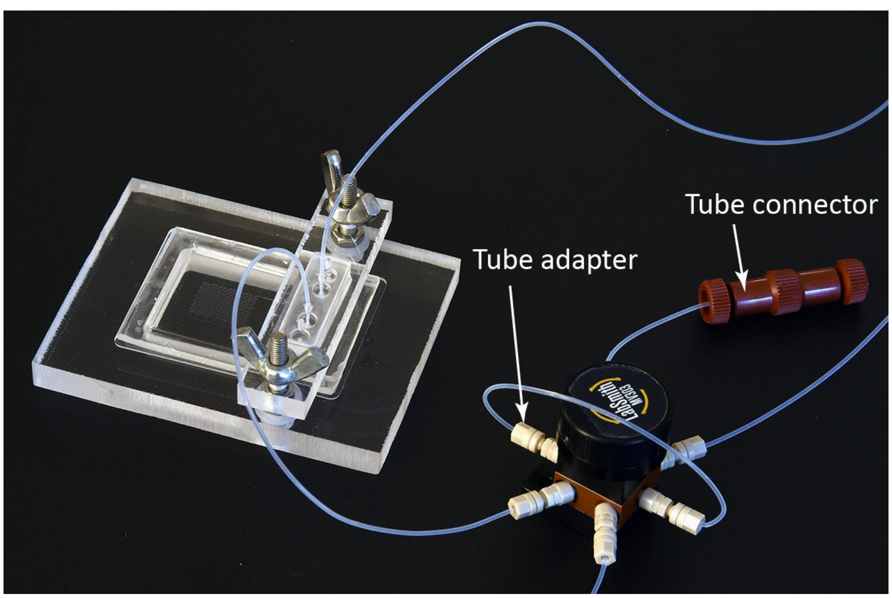

Fig. S4. COC chip and PDMS ferrule, mounted in CNC machined Plexiglas frame and connected to the 6-port, 2-position valve with injection loop. Only 2 of the 4 inlets into the chip are used in this chip design.

Plumping components:

Tube connector: IDEX, P-771

Tube adapter: LabSmith, C360-A132

Tubing: Cole Parmer, EW-06417-11

COC pellets specification

All the chips were made with TOPAS 8007x10[2, 3].

Table S4. Glass transition temperature and melting temperature of TOPAS 8007 COC.

Glass transition temperature [ºC]	Melting Temperature [ºC]
78	190-250

1. M. Heymann, S. Fraden and D. Kim, *J Microelectromech S*, 2014, **23**, 424-437.
2. *TOPAS® 8007X10 Date sheet* http://www.topas.com/sites/default/files/TDS_8007X10_english%20units_1.pdf.
3. TOPAS Brochure http://www.topas.com/sites/default/files/files/TOPAS_Brochure_E_2014_06(1).pdf.