Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information for Article:

Plug-and-play polymer microfluidic chips for hydrated, room-temperature fixed-target serial crystallography.

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Section S1: Detailed protocol for the fabrication of cyclic olefin copolymer (COC) microfluidic chips.

A) Fabrication of a primary silicon mold

A primary silicon mold was fabricated by Ravata Solutions (Davis, CA, USA). Briefly, a 16-µm thick layer of a negative photoresist NR78-8000P (Futurrex Inc., Franklin, NJ, USA) was spun on a 150 mm silicon wafer (#590, University Wafer Inc., Boston, MA, USA) at 800 rpm for 40 seconds followed by a soft bake at 150 ºC for 1 minute. An EVG 620 contact aligner was used to expose the photoresist-coated wafer through a transparency photomask (CADArt Services, Brandon, OR, USA) at 260 mJ/cm^2 followed by a post-exposure bake at 110 ºC for 2.5 minutes. The wafer was developed in a TMAH-based developer for 1 minute. Plasmatherm ICP Deep Silicon Etcher was used to selectively etch 300-µm deep features in the silicon wafer. The photoresist was stripped using a DMSO-based stripper. The silicon mold surface was activated using a 30-minute UV-ozone treatment in a Jelight 42 UV-O Cleaner to generate surface hydroxyl groups. Vapor phase silanization was carried out in a vacuum desiccator with some modification of the protocol described by Bhushan et. al. [1] to deposit a passivating self-assembled layer of 1H,1H,2H,2H-perfluorodecyltrichlorosilane (FDTS, #SIH5841.0, Gelest Inc., Morrisville, PA, USA) on the surface of the silicon mold to allow for easy detachment of the secondary PDMS mold.

B) Fabrication of a secondary Sylgard 184 (PDMS) mold

The two-part Sylgard 184 silicone elastomer kit (PDMS) was mixed in a weight ratio of 5:1 monomer to curing agent and degassed in a vacuum desiccator for 30 minutes. Heavy-duty aluminum foil was used to create a walled reservoir around the silanized silicon mold. The degassed PDMS mixture was poured into the reservoir and placed in a pressure chamber at 30 psi for 5 minutes to displace or dissolve any air bubbles entrapped in the micropatterned features. The reservoir-mold was subsequently placed on a level surface in a laboratory oven at 120 ºC for 1 hour to cure the PDMS mixture. Once cooled, a scalpel was used to carefully cut and detach the cured-PMDS mold (~5-inch diameter) from the silicon primary. The smooth side of the PDMS secondary mold and a new 150-mm silicon wafer were oxygen-plasma treated for 60 s at 50 W, 0.79 Torr, 25 sccm O_2 using a Plasma Equipment Technical Services (PETS) RIE system and immediately brought into contact and placed on a hot plate at 120 ºC for 1 hour to bond the two surfaces together. The patterned PDMS mold surface was passivated with a self-assembled monolayer of FDTS as described above.

C) Hot-embossing through-holes in COC sheets

A 500-nm sacrificial layer of water-soluble poly vinyl alcohol (PVA, #363170, Millipore Sigma, St. Louis, MO, USA) was spin-coated onto a UVO-treated silicon wafer by dispensing 3-4 mL of 9 wt.% PVA solution in MilliQ water at 2000 rpm for 60 seconds. The wafer was softbaked at 100 ºC for 10 minutes. A 240-µm thick sheet of cyclic olefin copolymer (Europlex 0F304, Roehm America LLC, Sanford, ME, USA) was adhered to the PVA-coated wafer using an

intermediate “glue” layer of spin-coated 15 wt.% COC 8007 dissolved in sec-butylbenzene. The water-soluble PVA layer served a dual purpose as an adhesion promoting layer and sacrificial layer to allow controlled detachment of the COC sheet when required.

Hot embossing was performed with a EVG501 semi-automated wafer bonding system. The PDMS secondary mold was placed on the bottom platen with the COC/PVA coated Si wafer resting on top of the mold features. The temperature was first ramped up to 120 ºC and then 12 kN of force was applied for 15 min under high vacuum (7.5E-6 Torr). The assembly was allowed to cool below the glass transition temperature (Tg = 78ºC) of COC under pressure before raising the piston and demoulding the embossed film. The residual 20-30 µm COC layer was etched using oxygen plasma treatment in the PETS RIE instrument (500 W, 330 mTorr, 25 sccm O_2, 30 minutes). The wafer was immersed in a water bath to dissolve the PVA layer and release the 200-micron thick COC supports with through-holes.

D) Laser cutting

A double-sided pressure sensitive adhesive layer (3M F9460PC) was attached to 0.5- or 1-mm sheets of poly methyl methacrylate (PMMA, Clarex optical grade cast acrylic, Astra Products, Copiague, NY, USA) and laser cut using a Trotec Speedy 400 CO_2 laser cutter to make rigid frames with inlet and outlet ports. Similarly, a 25-µm or 48-µm spacer film (AR 92734 and AR 92712 respectively, Adhesives Research Inc., Glen Rock, PA, USA) was laser cut to make the sample flow layer.

E) Solvent bonding and chip assembly

COC thin films of desired thicknesses 2-5 µm were spin-coated onto a UV-ozone treated silicon wafer and softbaked as described above. Hot-embossed COC supports were placed on a stack of filter papers soaked with a solution of 35:65 vol.% cyclohexane: acetone for 30-60 seconds and dried with a nitrogen gun to turn the surfaces “tacky” as described by Keller et al. [2]. The solvent treated side was brought into contact with the spin-coated film (on a silicon wafer) and manually pressed to bond the two together. The PMMA frames (with adhesive) were adhered to the respective top and bottom side COC supports by aligning inlet/outlet features in the two layers. The edges of the assembly were scored with a scalpel and released gently by immersion in a water bath. The COC thin film has very low adhesion to the silicon wafer and a PVA sacrificial layer was not found necessary for detachment. The COC thin film surface was hydrophilized using atmospheric plasma treatment for 3 minutes on the high setting in a Harrick PDC-32G Basic Plasma Cleaner before being using in the chip assembly. The top and bottom sides of the chip were bonded using the pressure-sensitive adhesive flow layer by aligning the micropatterned windows to create an enclosed microfluidic chip. A contact dwell time of 24-72 hours was required to ensure strong adhesion between all the layers before using the chip.

Section S2: COC grade 8007 spin curves.

10-20 wt.% COC pellets (Grade 8007, T_g = 78 ºC; #24750, Polysciences Inc., Warrington, PA, USA) were dissolved in sec-butylbenzene (b.p. 174 ºC; TCI Chemicals, Portland, OR, USA) overnight on a hot plate at 150 ºC. A Millex-SV 5.0 µm syringe filter was used to filter the solution prior to spin-coating. 3-5 mL of solution was dispensed on a 5-inch UV-ozone treated silicon wafer and spun at a speed of 1000-4000 rpm. The wafers were softbaked at 100 ºC for 10 minutes prior to use.

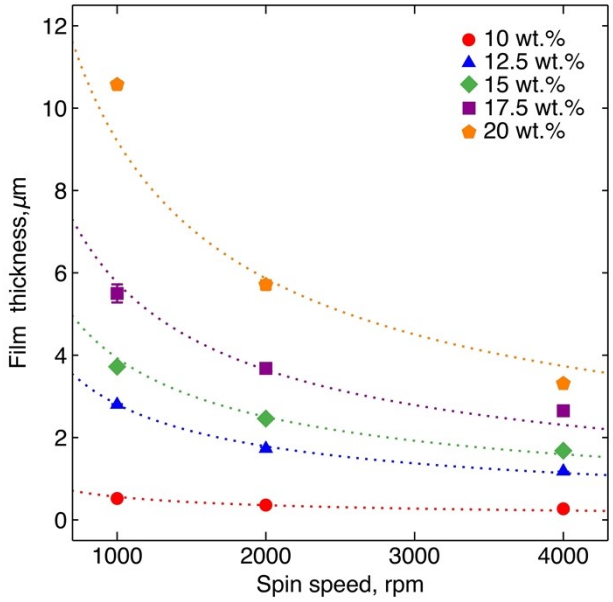

Figure S1: Spin curves showing COC film thickness as a function of spin speeds for different concentrations of COC 8007 dissolved in sec-butylbenzene. The dotted lines are fitted curves capturing the inverse relationship between dry film thickness and spin speed using equation $T = \frac{G}{\omega^{\alpha}}$, where T is the film thickness, ω is the spin speed and G and α are fitted parameters. An α value of 0.65 accurately captured the trends for all concentrations except for the solution with the highest viscosity (20

Section S3: Contact angle measurements of oxygen-plasma treated COC films.

A Ramé-Hart goniometer was used to measure the advancing and receding contact angles for COC thin films attached to both a silicon wafer and a COC support frame. After measuring the native surface contact angle, the films were atmospheric-plasma treated for 3 minutes using a Harrick PDC-32G Basic Plasma Cleaner to render them hydrophilic. Weekly measurements were taken on samples stored under ambient conditions over a period of four weeks. The average and standard deviation values reported are from n=5 independent measurements. The receding contact angles after plasma treatment (not shown) were consistently less than 10-20° over the duration of the study.

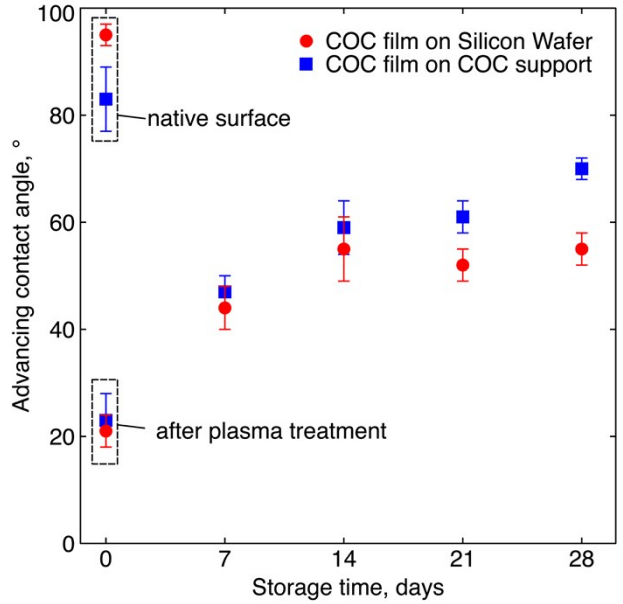

Figure S2: Contact angle measurements for COC 8007 thin films show a large decrease in the advancing contact angle from ~ $83-95°$ for the native COC surface to ~ $22°$ after atmospheric plasma treatment. Upon storage in ambient conditions, reorganization of surface groups at the COC-air interface results in a fast recovery to ~$47°$ and ~$59°$ after 1 and 2 weeks respectively, beyond which the increase is significantly slower.

Section S4: Sample loading into the microfluidic chip.

Hydrophilic surface treatment of the COC films prior to chip assembly enabled facile loading of aqueous solutions into the chip using a standard 10-µL micropipette. Assembled chips could be stored under ambient conditions for up to 2-4 weeks prior to use while still retaining sufficient wetting properties. While filling the chip with crystallization solution, it was important to ensure that the micropipette tip created a good seal when placed at the inlet to ensure the solution filled the flow/spacer layer instead of pooling at the inlet. A quick, one-step dispense was used to fill the chip in order to maintain the solution "front" such that the entire chip was filled without entrapping any air bubbles at the center where the spacer was unsupported.

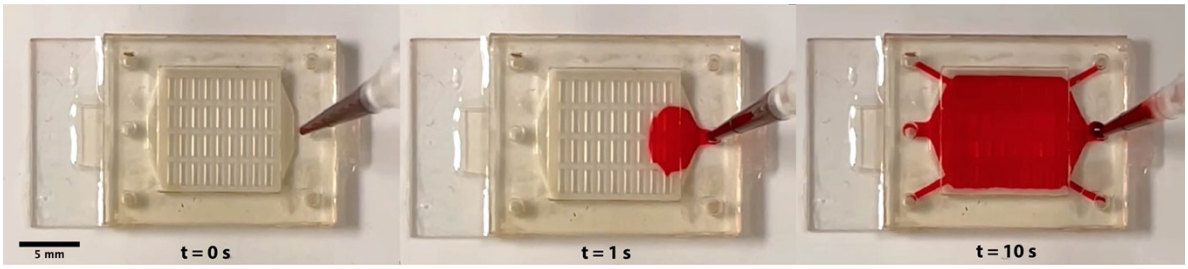

Figure S3: Snapshots of the COC microfluidic chip being loaded with ~8-10 µL of red dye solution over a 10-second period. The corner vents in the spacer layer (48 µm) allowed the wide flow chamber to fill uniformly with minimal bubble entrapment.

Section S5: Microscopy images of all protein crystals used in this work, grown on-chip.

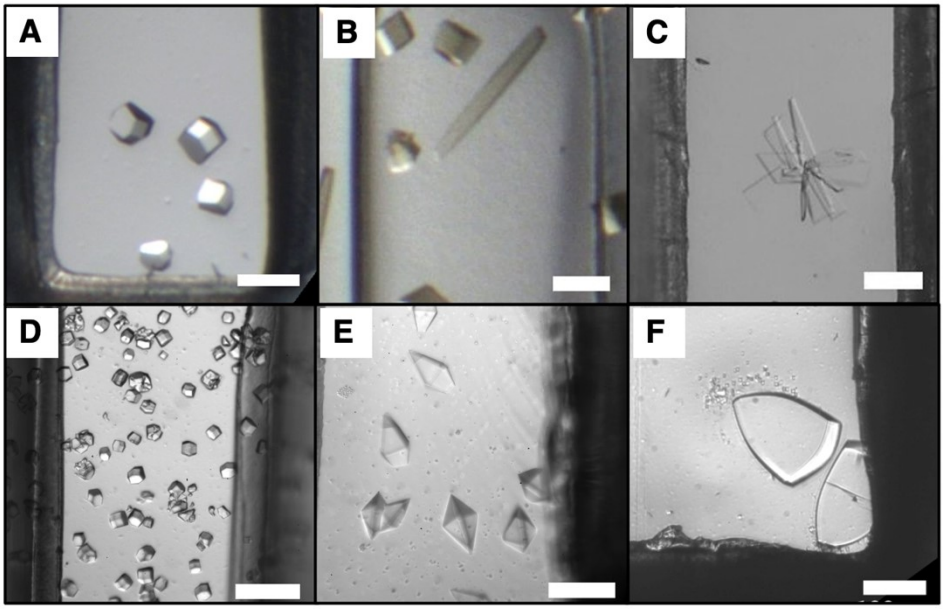

Figure S4: Optical microscopy images of all protein crystals used in this work, grown on-chip using micro-batch or vapor diffusion crystallization using conditions described in Table 1. (A) Lysozyme "aged", (B) catalase, (C) NSP5, (D) lysozyme "fresh", (E) thaumatin, (F) concanavalin-A. The scale bar is 200 µm.

Section S6: A comparison of merging statistics between fully hydrated and partially hydrated (excess buffer removed) catalase crystals.

A comparison of merging statistics between fully hydrated catalase crystals and ones with surrounding buffer removed did not show any significant changes in the unit cell parameters (~0.1 Å contraction). Slight improvements in the statistics for the second dataset can be explained by the fact that more crystals were included in the analysis.

Table S1: A comparison of merging statistics between fully hydrated catalase crystals and crystals with surrounding buffer removed.

Protein	**Catalase (in solution)**	**Catalase (solution removed)**
Resolution range $(\dot{A})$	71.01 – 2.46 (2.50 - 2.46)	79.30 – 2.28 (2.32 – 2.28)
Unit cell dimensions	$a = b = 141.88\ \dot{A}$, $c = 103.523\ \dot{A}$, $\alpha = \beta = 90°$, $\gamma = 120°$	$a = b = 141.751\ \dot{A}$, $c = 103.461\ \dot{A}$, $\alpha = \beta = 90°$, $\gamma = 120°$
Space group	$P3_221$	$P3_221$
Data processing statistics		
Total reflections	203188 (8841)	790087 (38202)
Unique reflections	43769 (2111)	55195 (2684)
Multiplicity	4.6 (4.2)	14.3 (14.2)
Completeness $(\%)$	98.9 (96.3)	100.0 (98.6)
Mean $I/\sigma(I)$	7.2 (0.8)	9.0 (0.8)
R_{merge}	0.218 (2.168)	0.192 (2.387)
R_{meas}	0.244 (2.453)	0.199 (2.476)
R_{pim}	0.106 (1.119)	0.052 (0.651)
$CC_{1/2}$	0.949 (0.212)	0.997 (0.584)
Wilson B-factor	41.36	44.16

Section S7: A comparison between structures obtained in this work and the respective PDB references used as templates for phasing via molecular replacement.

PyMOL (*align* command) was used to perform secondary structure-based alignment of the structure solved in this work and the respective PDB reference used for phasing via molecular replacement. The alignment returned a root mean squared deviation (RMSD) value that quantitates the average deviation (in Angstroms) of each atom in a structure from the corresponding atom in the reference structure.

Table S2: Root mean square deviation (RMSD, Å) between structures obtained in this work and the respective PDB references used as templates for phasing via molecular replacement.

Structure (this work)	PDB reference	RMSD, Å
Lysozyme (fresh)	1VED	0.249
Lysozyme (aged)	1VED	0.238
Thaumatin	1RQW	0.118
Catalase	8CAT	0.313
Concanavalin-A	1SCR	0.121
NSP5	6CR3	0.416

Section S8: Effects of excess mother liquor removal/exclusion on background scatter intensity profiles.

On-chip crystal growth offers several opportunities to reduce the mother liquor derived background scattering of a chip-based measurement. Under certain conditions where growth of relatively large single crystals is favorable, the spacer layer thickness may limit crystal size, leading to crystal growth that spans the chip fluid layer and excludes excess mother liquor from the beam path as demonstrated for concanvalin-A (**Figure S5**). Interactions with the enclosing films may also allow for physical removal of the mother liquor while leaving crystals in place, reducing mother liquor derived background scattering as demonstrated for bovine liver catalase (**Figure S6**)

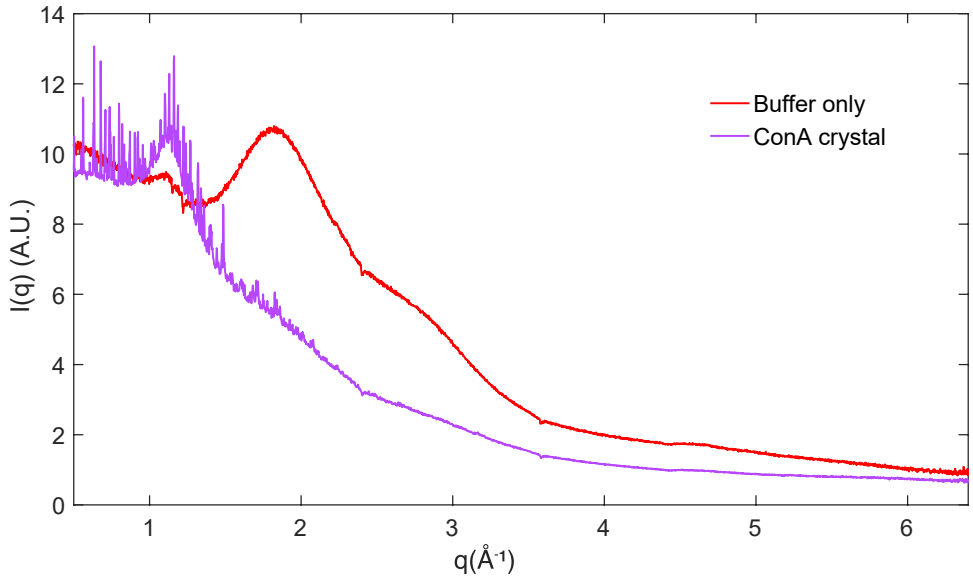

Figure S5: Radial averages of scattering from chip-spanning crystals of concanvalin-A (ConA, purple, average of three frames where the beam is perpendicular to the chip plane), compared to a buffer filled chip (red). Both are measured in chips with 3.7 µm COC enclosing films and 48 µm spacer layers. On-chip crystal growth in this case excludes the majority of buffer from the beam path, leading to a significantly diminished water solvent ring at ~q = 1.8 $Å^{-1}$.

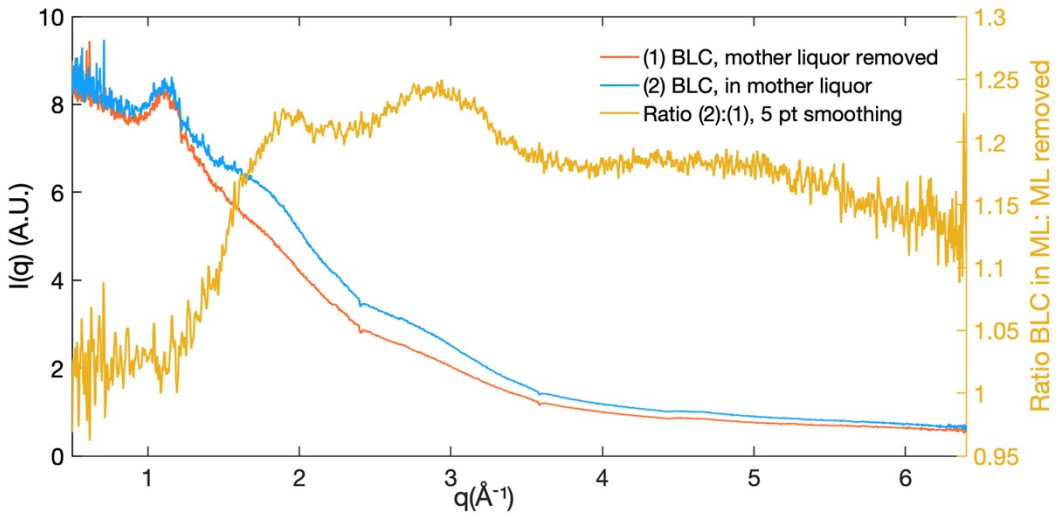

Figure S6: Radial averages of scattering from comparably sized bovine liver catalase (BLC) crystals in two hydration environments (average of three frames where the beam is perpendicular to the chip plane): one surrounded by mother liquor (blue) and one with mother liquor removed (orange). Mother liquor removal slightly decreases the contribution of the water solvent ring at ~q = 1.8 $Å^{-1}$. Both are measured in chips with 3.7 µm COC enclosing films and 48 µm spacer layers. The right axis shows the ratio of the radial intensity before mother liquor removal to the intensity after mother liquor removal with five-point smoothing (yellow).

References:

1 B. Bhushan, D. Hansford and K. K. Lee, *J. Vac. Sci. Technol. Vac. Surf. Films*, 2006, **24**, 1197–1202.

2 N. Keller, T. M. Nargang, M. Runck, F. Kotz, A. Striegel, K. Sachsenheimer, D. Klemm, K. Länge, M. Worgull, C. Richter, D. Helmer and B. E. Rapp, *Lab. Chip*, 2016, **16**, 1561–1564.