STRUCTURAL
BIOLOGY

Volume 78 (2022)

Supporting information for article:

Data collection from crystals grown in microfluidic droplets

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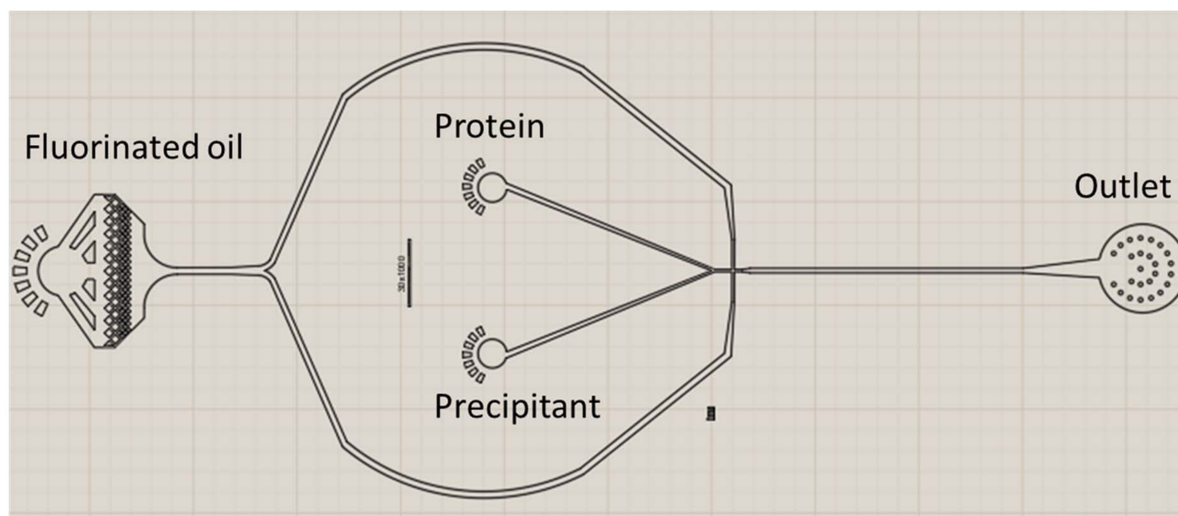


Figure S1 Schematic diagram of the in-house droplet generator chip. The chip was designed using AutoCAD and fabricated using soft lithography. In the PDMS chip the droplet forming channels are 30 µm wide and 100 µm deep. The fluorinated oil inlet has a filter to eliminate particles from the droplets. The inlets for the protein and precipitant have no filters and the features around the hole provide flexibility when a steel pin is connected. The inlets are connected with a steel pin and FEP tubing to a syringe pump. The outlet has small pads to ensure proper spacing between the bottom of the chip and the steel pin. In addition, they focus the droplet flow to the pin. The droplets are collected in an Eppendorf tube connected with a FEP tubing to the chip.

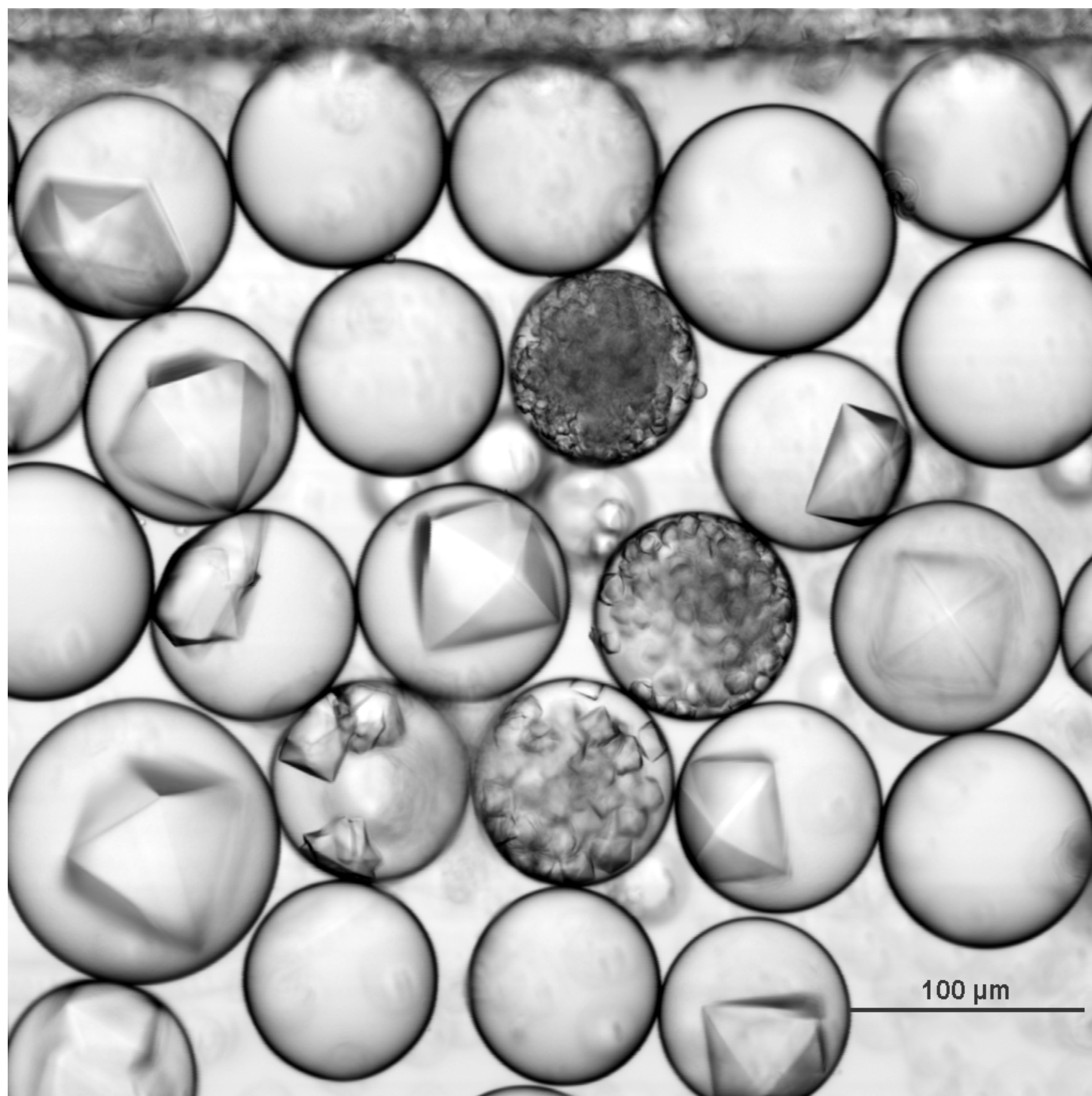


Figure S2 Protein crystallization in microfluidic droplets. Changes in protein concentration and flow ratios (protein, precipitant and oil) can control droplet occupancy and number of crystals in the droplet. Scale bar: 100 µm.

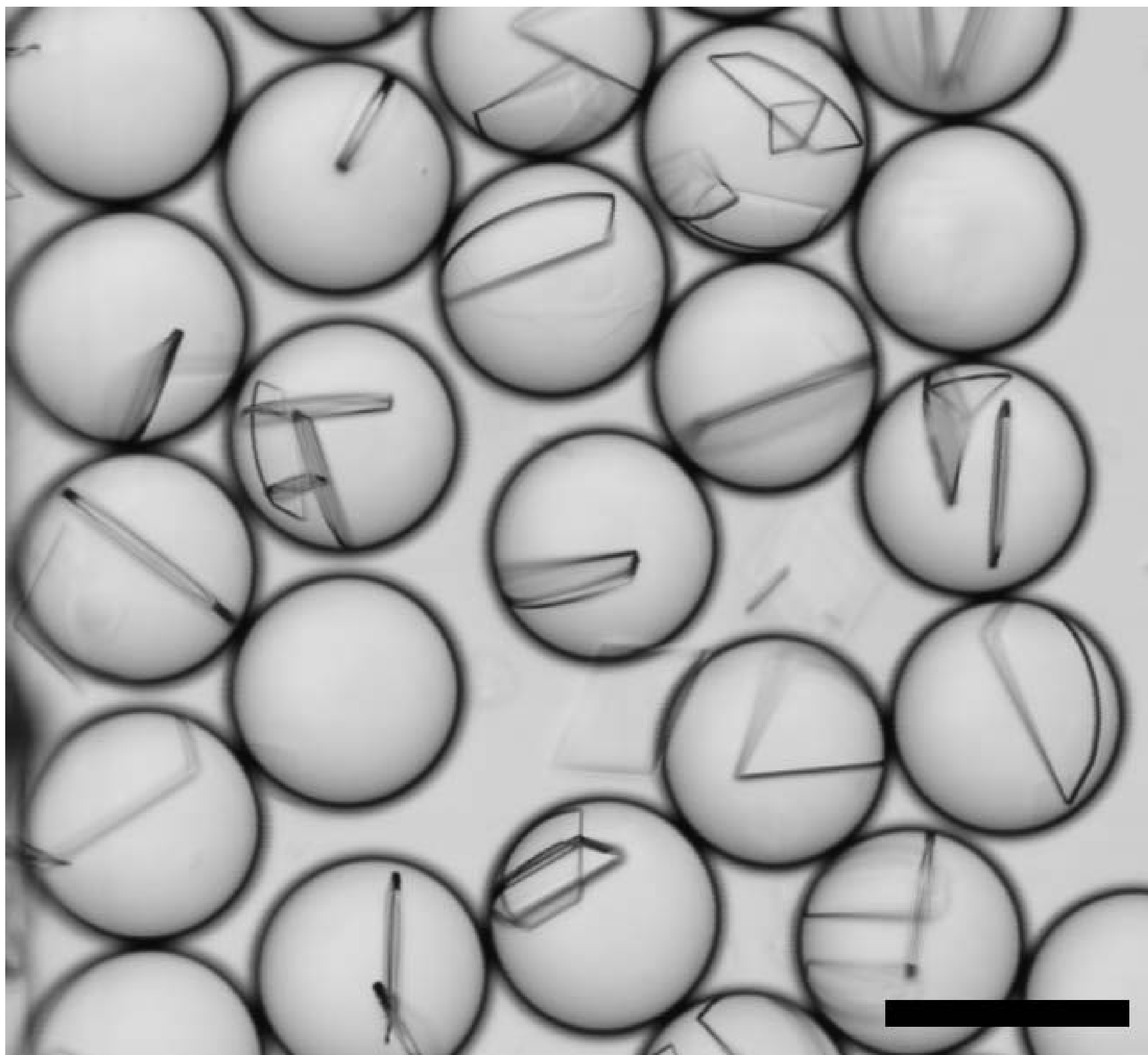


Figure S3 Different crystal forms in microfluidic droplets. Many crystal forms can be reproduced in the microfluidic droplet. The crystals of a beta-lactamase enzyme are shown. Scale bar: 100 μm.

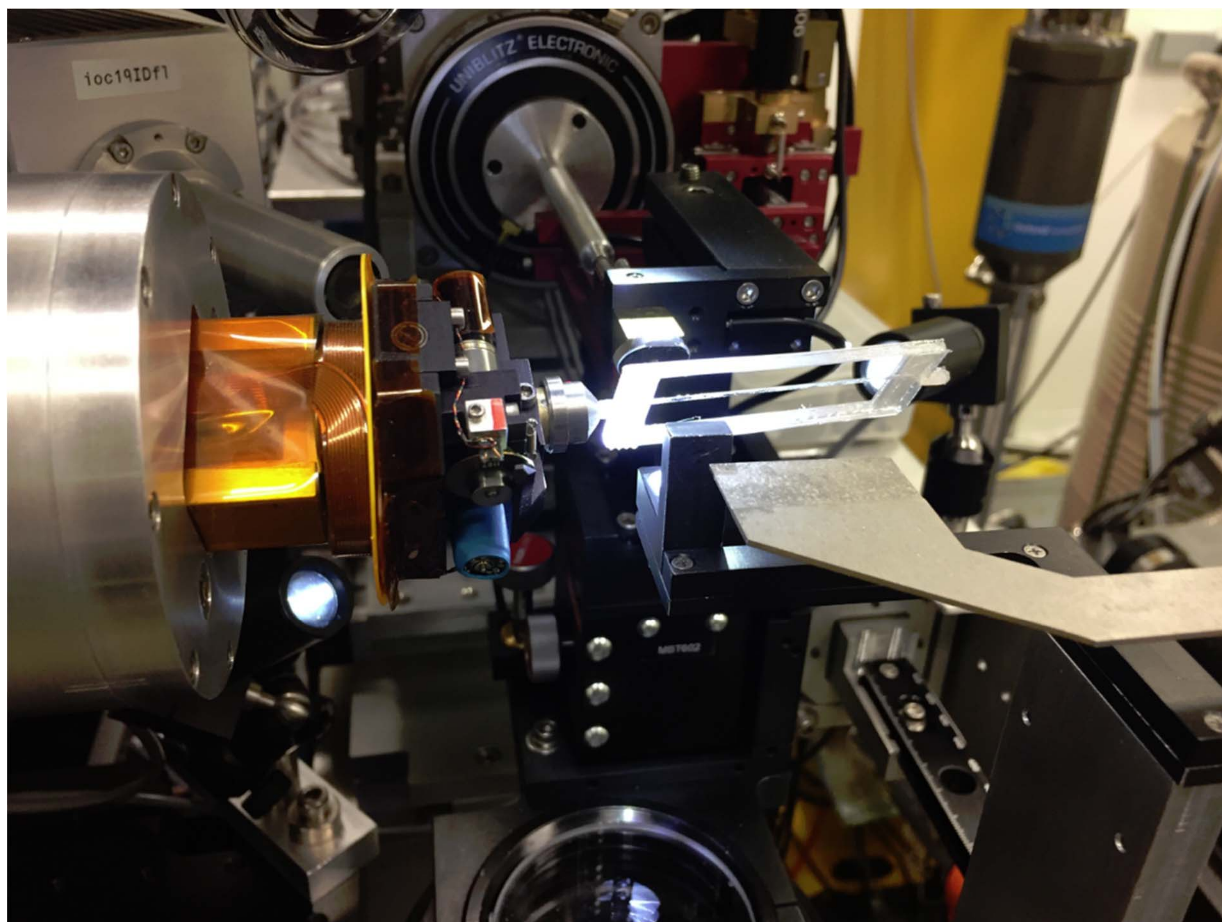
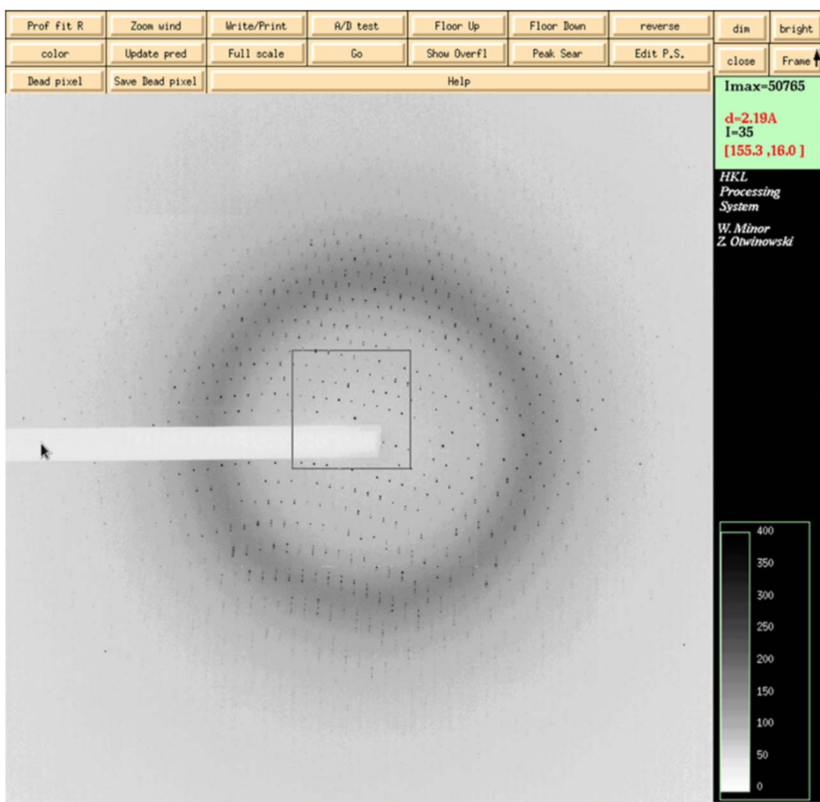
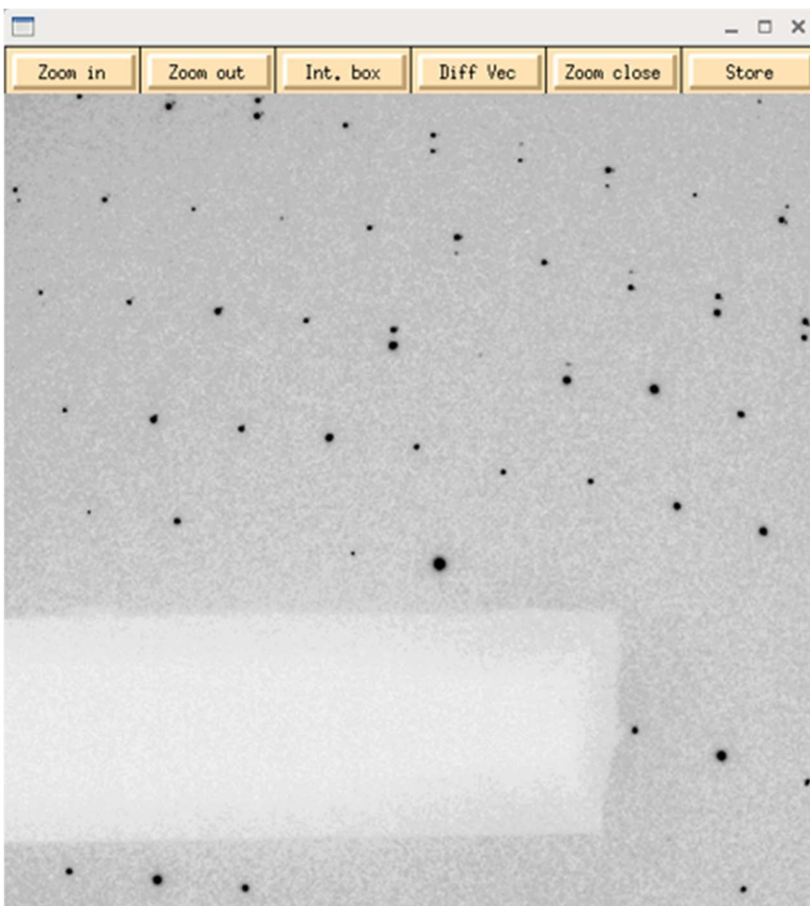


Figure S4 Data collection at the 19-ID from capillary mounted into a 3D-printed holder. Data collection was performed at 100 K or 295 K.



A



B

Figure S5 Diffraction images of BcGH2SBD crystals collected at 100 K from flat glass capillary (A, B).

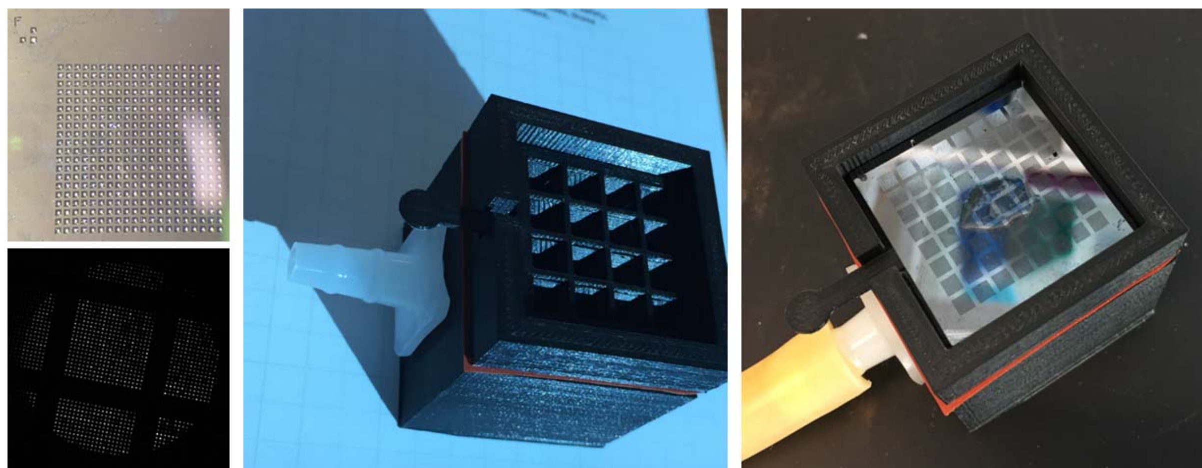
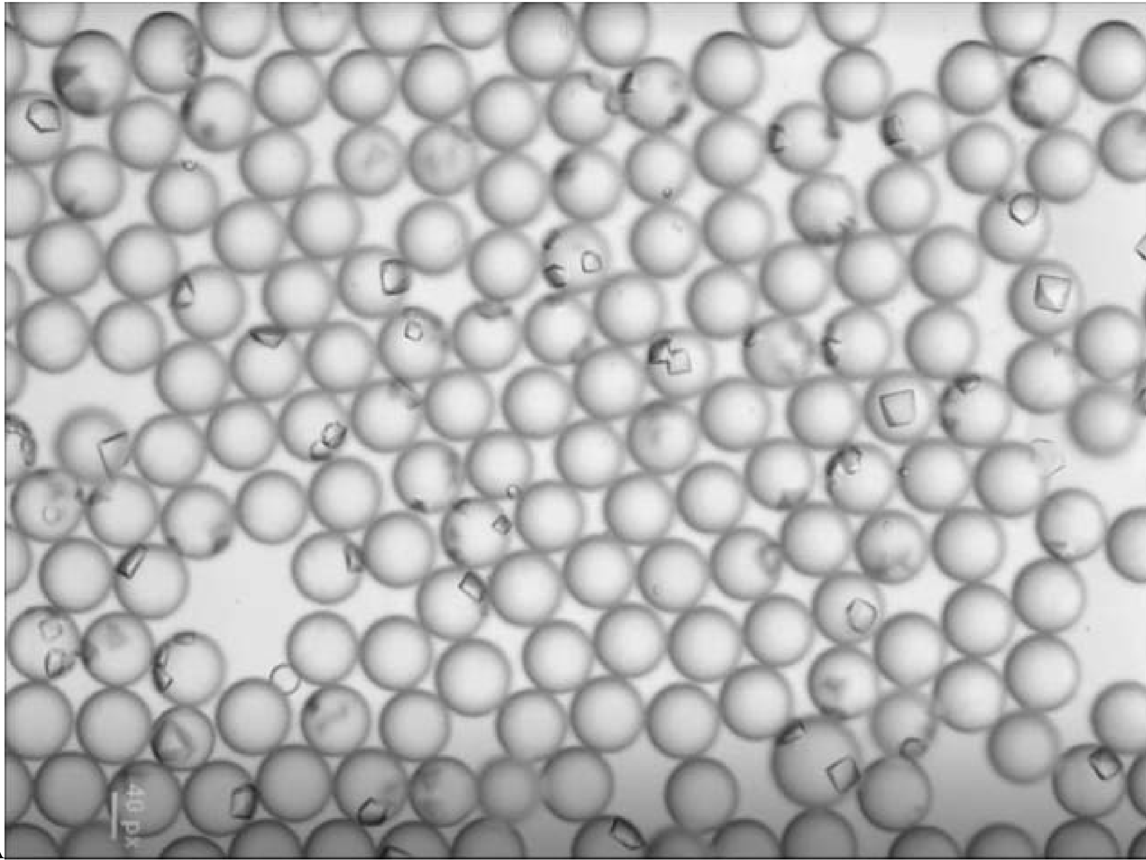
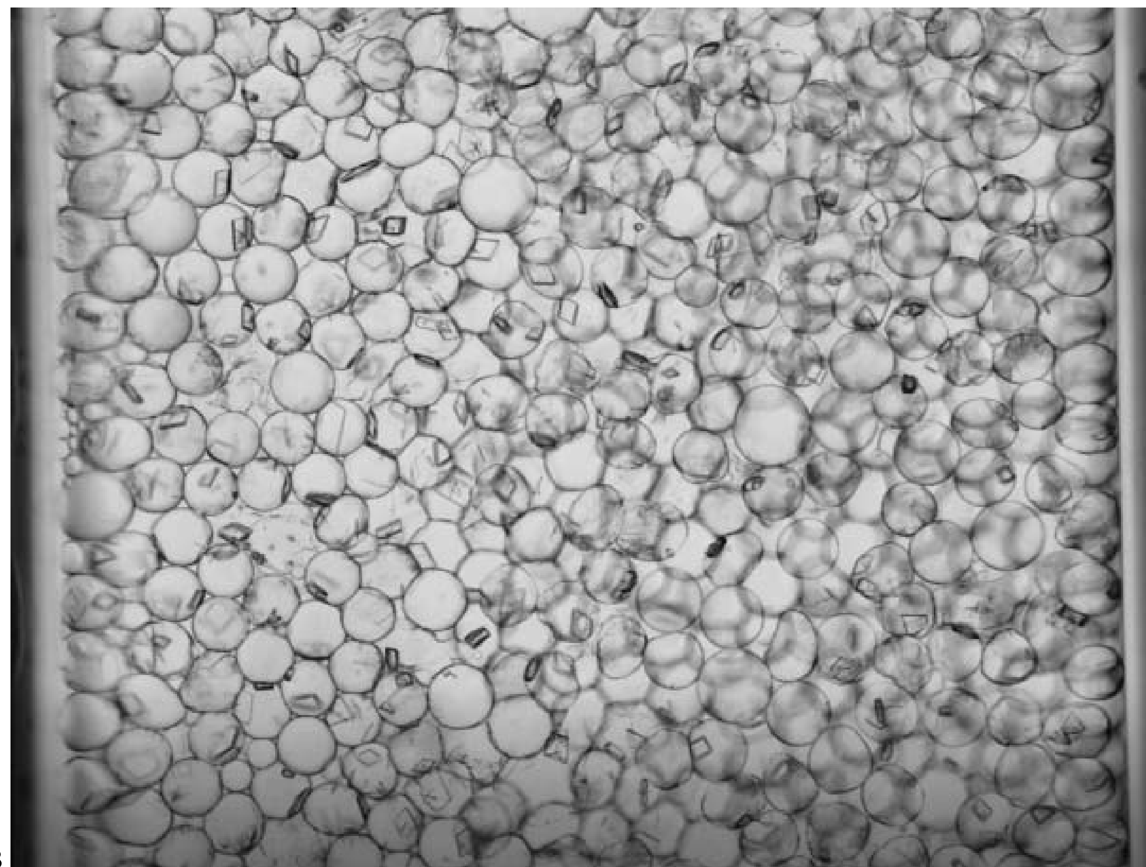


Figure S6 Testing the loading of the silicon chip with droplet emulsion at the 19-ID.

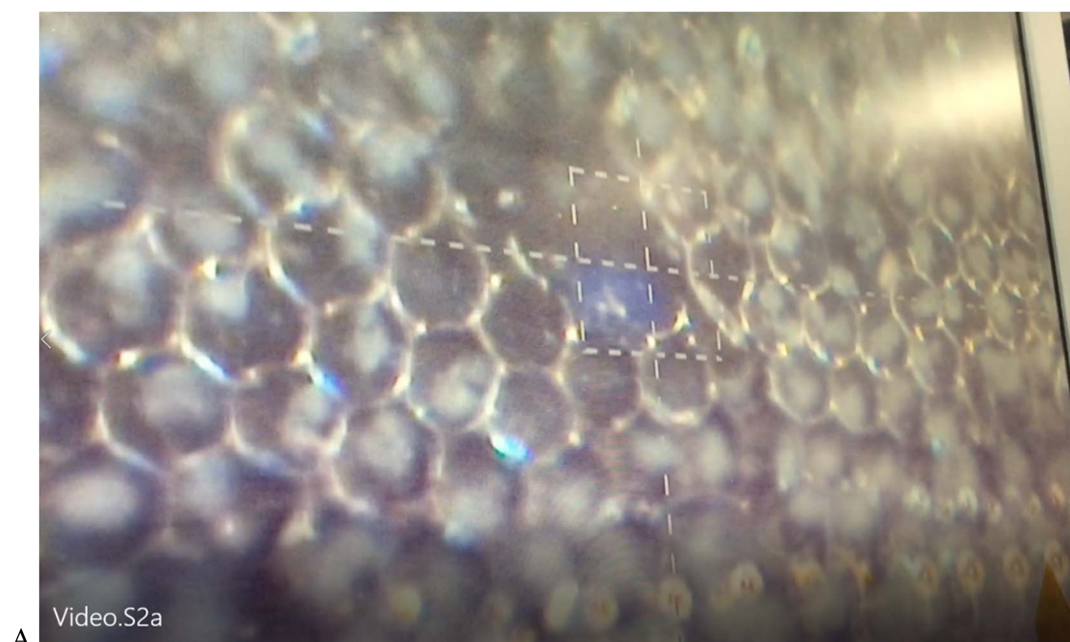


A

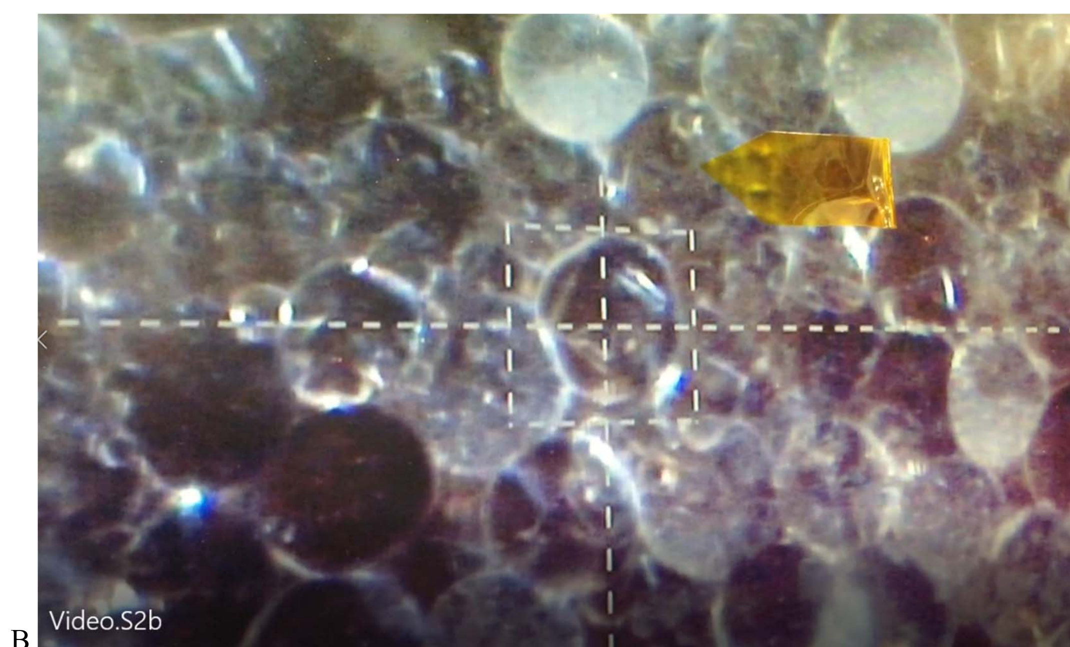


B

Video S1. The microfluidic droplets tightly packed into flat glass capillaries can be monitored for crystal growth under the confocal microscope. Sample videos are shown for BvSIAE (A) and a beta-lactamase enzyme (B).



A



B

Video S2. Comparison of fluorinated oils for room temperature data collection at the beamline. The droplets generated using FluoSurf oil (Dolomite Microfluidics, Royston, UK) even with 5% surfactant quickly coalesced when exposed to the x-ray dose used for generic data collection from crystals (A). When using droplet generation oil for probes used in digital PCR (Bio-Rad, Hercules, CA, USA), the droplets remained stable for the duration of a short < 30 s data collection session (B).

Table S1 Macromolecule production information for BcGH2SBD (accession No. EEF92278.1).

Source organism	*Bacteroides cellulosilyticus* DSM 14838
DNA source	Genomic DNA
Forward primer	5′-TACTTCCAATCCAA TGCCACACTTCGGGAACAATCTTTCGATGAG-3′
Reverse primer	5′-TTATCCACTTCCAATGTTAAGTTATAGTAATACTCGCAACCGGAAGT-3′
Cloning vector	pMCSG68
Expression vector	pMCSG68
Expression host	*E. coli* BL21 (DE3) Gold
Complete amino acid sequence of the construct produced	SNATLREQSFDEAWLFHRGDIAEGEKQSLDDSQWRQINLPHDWSIEDIPGTNSPFTADAA TEVAGGFTVGGTGWYRKHFYIDAAEKGKCIAVSFDGIYMNADIWVNDRHVANHVYGYTAF ELDITDYVRFGAENLIAVRVKNEGMNCRWYTGSGIYRHTFLKITNPLHFETWGTFVTTPV ATADKAEVHVQSVLANTEKVTGKVILETRIVDKNNHTVARKEQLVTLDNKEKTEVGHALE VLAPQLWSIDNPYLYQVVNRLLQDDKVIDEEYISIGIRNIAFSAENGFQLNGKSMKLKGG CIHHDNGLLGAKAFDRAEERKIELLKAAGFNALRLSHNPPSIALLNACDRLGMLVIDEAF DMWRYGHYQYDYAQYFDKLWKEDLHSMVARDRNHPSVIMWSIGNEIKNKETAEIVDICRE LTGFVKTLDTTRPVTAGVNSIVDATDDFLAPLDVCGYNYCLNRYESDAKRHPDRIIYASE SYASQAYDYWKGVEDHSWVIGDFIWTAFDYIGEASIGWCGYPLDKRIFPWNHANCGDLNL SGERRPQSYLRETLWSDAPVSHIVVTPPVPSFPLNPDKADWSVWDFPDVVDHWNFPGYEG KKMTVSVYSNCEQVELFLNGESLGKQENTADKKNTLVWEVPYAHGILKAVSYNKGGEVGT ATLESAGKVEKIRLSADRTEIVADGNDLSYITLELVDSKGIRNQLAEELVAFSIEGDATI EGVGNANPMSIESFVANSRKTWRGSNLLVVRSGKSSGRIIVTAKVKALPVASITIT

The expression plasmid pAPC115077 contains residues 25-797 with a N-terminal ‘SNA” tag of the original protein sequence.

Table S2 Macromolecule production information for BvSIAE (accession No. ABR41743.1).

Source organism	Bacteroides vulgatus (strain ATCC 8482 / DSM 1447 / JCM 5826 / NBRC 14291 / NCTC 11154)
DNA source	Genomic DNA
Forward primer	5′-TACTTCCAATCCAATGCCGAACGCAAGTATTCTACTTTTTATGAGCAGA-3′
Reverse primer	5′-TTATCCACTTCCAATGTTAGTCAACATAAGGTTTTACAATATCACGCCA-3′
Cloning vector	pMCSG68
Expression vector	pMCSG68
Expression host	E. coli BL21 (DE3) Gold
Complete amino acid sequence of the construct produced	SNAERKYSTFYEQRATLFEELPVTSKDIIFLGNSITNGCEWAELFQNKNVKNRGISGDIC MGVYDRLDPIVKGKPAKIFLLIGINDVSRGTSADKIISEISMIVRKIKQESPKTKLYLQS VLPVNDCYGMFNGHTSRWQVVKQINDLLEPLAVKEGVAYIDLYSHFVEKETGKMNPVYTN DGLHLLGKGYLLWRDIVKPYVD

The expression plasmid pAPC113151 contains residues 21-219 with a N-terminal ‘SNA” tag of the original protein sequence.

Table S3 Primer pairs for the generation of mutants of BcGH2SBD.

The following primers were used in PIPE to introduce point mutations into the enzyme.

Mutation	Forward Primer Sequence	Reverse Primer Sequence
E426A	GGTAATGCTATCAAGAACAAAGAAACT GCTGAAATTGTGGATA	CTTGATAGCATTACCAATACTCCACA TGATAACACTAGGAT
E501A	GCTTCGGCTTCCTACGCATCCCAGGCTT ATGATTATT	GTAGGAAGCCGAAGCATAGATAATA CGGTCCGGAT
Y480F	TATAATTTTTGTTTAAACCGTTATGAATC GGATGCCAAA	TAAACAAAAATTATAACCACAAACAT CCAGAGGAGCC
E426Q	GGTAATCAGATCAAGAACAAAGAAACT GCTGAAATTGTGGATA	CTTGATCTGATTACCAATACTCCACA TGATAACACTAGGAT
E501Q	GCTTCGCAGTCCTACGCATCCCAGGCTT ATGATTATT	GTAGGACTGCGAAGCATAGATAATAC GGTCCGGAT

The numbering of the mutation sites refers to the original full-length protein.