Supporting information for article:

3D-MiXD: 3D-printed X-ray compatible microfluidic devices for rapid, low-consumption serial synchrotron crystallography data collection in flow

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**Figure S1** Overview of the 3D-MiXD geometry showing the direction of printing as well as the different layer types referred to in the main text. The XZ plane is a cut through the median line of the device. The YZ plane is a cut through at the flow-focusing region, showing the inlet channels. The XY plane is a view from the bottom of the device. The device dimensions are 8.88 x 23.10 x 2.32 mm. Due to ongoing design updates and improvements we encourage interested readers to contact the corresponding authors for the latest 3D-CAD geometry files.
Figure S2 Radial integrations of the background (3D-MiXD and buffer) during data collection. 10 sequential images containing no visible hits were averaged and radially integrated at 3 different time points for both ADC and lysozyme (3 mm runs). No appreciable increase in background can be detected over the course of the data collection. No significant difference was detected in the background arising from either crystallization cocktail.
**Figure S3** Computational fluid dynamic simulation of the device running at 10:1 flow focusing ratio. Two simulations were performed, one in water and a second using the viscosity of 5% PEG 6000 solution in water and the experimentally measured density of the buffer. a) 3D rendering of the sample flow inside the device (from particle trajectories) showing the 3D hydrodynamic flow-focusing which places the sample in the center of the channel, away from the walls. YX and ZX orientations are shown. b) histograms of the sample distribution (flow lines) for both simulations along the Y axis (XY plane in a) and the Z axis (ZX plane in a). The sample stream width is 28 µm. c) Concentration plots of a ligand diffusing in from the side channels ($D = 6.7 \times 10^{-10}$ m$^2$ s$^{-1}$). The central YX and ZX planes are shown. The average ligand concentration experienced by the sample is above 50% of the maximum value at the beginning of the main channel (after the flow-focusing cross). This change in concentration within the short mixing cross is sufficient to provide the initial jump for reaction initiation. The actual rate of reaction and concentration of intermediate species present will be dependent on the kinetics of the system as well as the concentration of the ligand.
Figure S4  Computational fluid dynamic simulation of the device running at 5:1 flow focusing ratio for water and PEG (as per Figure S3). a) 3D rendering of the sample flow inside the device. YX and ZX projections. b) histograms of the sample distribution (flow lines) for both simulations along the Y axis (XY plane in a) and the Z axis (ZX plane in a). The sample stream width is 48 µm. c) Concentration plots of a small ligand diffusing in from the side channels (D = 6.7x10⁻¹⁰ m² s⁻¹, central YX and ZX planes). The average ligand concentration is above 50% of the maximum value 0.5 mm into the main channel (after the flow-focusing cross).
Figure S5 Microscopy image of the device running at 10:1 flow-focusing ratio in water. A dye was added to the fluid in the central channel (sample) to provide contrast. The flow-focused stream is ~26 μm.

Table S1 Comparison of algorithms for reflection integration within CrystFEL for 3 ADC datasets

All datasets were 100% complete across all resolution shells (with separate Fiedel pairs – Friedel’s law was held true for the final merging of the data shown in the manuscript).

| Reflection distribution | ADC 0p5mm 3 methods | ADC 0p5mm Xgandalf only |
|-------------------------|----------------------|-------------------------|
|                         | 288 0.7% Dirax (third method) | 46690 100% Xgandalf |
|                         | 42639 98.6% Mosflm (first method) | 46690 100% total |
|                         | 303 0.7% XDS (second method) | |
|                         | 41230 100% Total | |

| Merging Statistics | Merging Statistics |
|--------------------|--------------------|
| Resol. (Å)         | N. Reflections | Red. | SNR <I>/σ(I)> | Rsplit % | CC* | Resol. (Å) | N. Reflections | Red. | SNR <I>/σ(I)> | Rsplit % | CC* |
|--------------------|-----------------|------|---------------|----------|-----|------------|-----------------|------|---------------|----------|-----|
| 8.29               | 11579505        | 2610.9 | 26.80 | 3.65 | 1.00 | 8.29 | 11069799 | 26.80 | 3.65 | 1.00 | 27.72 | 3.50 | 1.00 |
| 3.81               | 7367285         | 1670.2 | 20.31 | 4.49 | 1.00 | 3.81 | 7029049 | 1593.5 | 21.30 | 4.39 | 1.00 | |
| 3.19               | 5398154         | 1227.1 | 12.17 | 7.62 | 1.00 | 3.19 | 5145385 | 1169.7 | 12.53 | 7.44 | 1.00 | |
| 2.84               | 5127519         | 1172.8 | 11.68 | 7.62 | 1.00 | 2.84 | 4888183 | 1118.1 | 11.34 | 7.44 | 1.00 | |
| 2.61               | 4846879         | 1100.8 | 18.42 | 7.62 | 0.99 | 2.61 | 4618198 | 1048.9 | 17.94 | 7.44 | 0.99 | |
| 2.44               | 4572471         | 1041.3 | 23.88 | 7.62 | 0.98 | 2.44 | 4354182 | 991.6 | 4.25 | 24.57 | 0.98 | |
| 2.31               | 4369797         | 991.6 | 27.27 | 7.62 | 0.97 | 2.31 | 4167095 | 945.6 | 3.57 | 29.57 | 0.97 | |
| 2.20               | 4152524         | 950.0 | 36.10 | 7.62 | 0.96 | 2.20 | 3961310 | 906.3 | 2.69 | 38.72 | 0.95 | |
| 2.11               | 4007327         | 916.8 | 48.01 | 7.62 | 0.94 | 2.11 | 3820364 | 874.0 | 1.94 | 54.17 | 0.92 | |
| 2.04               | 3927799         | 886.0 | 70.82 | 7.62 | 0.86 | 2.04 | 3743405 | 844.4 | 1.32 | 83.18 | 0.83 | |

ADC 1 mm 3 methods
ADC 1 mm Xgandalf only

| ADC 1 mm 3 methods | ADC 1 mm Xgandalf only |
|--------------------|-------------------------|
| 1199 1.3% Dirax (third method) | 120312 100% Xgandalf |
| Reflection distribution | 89455 | 97.5% | Mosflm (first method) | 120312 | 100% | total |
|-------------------------|-------|-------|-----------------------|--------|------|-------|
| 1111                    | 1.2%  |       | XDS (second method)   |        |      |       |
| 91765                   | 100%  |       | total                 |        |      |       |

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### Reflection distribution

| Resolution (Å) | N. Reflections | Red. | SNR <I/σ(I)> | Rsplit % | CC*  |
|----------------|----------------|------|--------------|----------|------|
| 8.69           | 13843570       | 3613.6 | 28.31 | 3.29 | 1.00 |
| 4.00           | 8742916        | 2294.1 | 22.71 | 4.03 | 1.00 |
| 3.35           | 6607718        | 1739.3 | 13.09 | 7.34 | 1.00 |
| 2.99           | 5939939        | 1559.9 | 7.99  | 12.43 | 0.99 |
| 2.74           | 5638430        | 1484.2 | 5.48  | 18.08 | 0.99 |
| 2.57           | 5320550        | 1405.3 | 3.83  | 27.07 | 0.98 |
| 2.43           | 5091175        | 1343.0 | 3.05  | 35.97 | 0.96 |
| 2.31           | 4839523        | 1286.1 | 2.64  | 40.46 | 0.95 |
| 2.22           | 4714302        | 1240.0 | 2.08  | 52.43 | 0.91 |
| 2.14           | 4569935        | 1203.2 | 1.87  | 56.10 | 0.97 |

### ADC 1.5 mm 3 methods

| Reflection distribution | 1016 | 1.4% | Dirax (third method) | 103146 | 100% | Xgandalf |
|-------------------------|------|------|----------------------|--------|------|----------|
| 72062                   | 97.3%|      | Mosflm (first method) |        |      | Xgandalf |
| 952                     | 1.3% |      | XDS (second method)  |        |      | total    |
| 74030                   | 100% |      | total                |        |      |          |

### Merging Statistics

| Resolution (Å) | N. Reflections | Red. | SNR <I/σ(I)> | Rsplit % | CC*  |
|----------------|----------------|------|--------------|----------|------|
| 9.09           | 10014355       | 2987.6 | 24.77 | 3.61 | 1.00 |
| 4.19           | 6400323        | 1934.2 | 21.41 | 4.26 | 1.00 |
| 3.51           | 5190343        | 1573.8 | 13.49 | 6.79 | 1.00 |
| 3.13           | 4118355        | 1245.3 | 8.11  | 12.04 | 0.99 |
| 2.87           | 4102229        | 1240.8 | 5.53  | 18.66 | 0.99 |
| 2.69           | 3879545        | 1184.2 | 3.94  | 26.78 | 0.98 |
| 2.54           | 3753535        | 1133.3 | 2.91  | 36.88 | 0.96 |
| 2.42           | 3597471        | 1090.1 | 2.44  | 44.24 | 0.94 |
| 2.32           | 3456045        | 1051.4 | 2.11  | 52.10 | 0.91 |
| 2.24           | 3369296        | 1015.5 | 1.73  | 63.93 | 0.89 |

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| 4.19           | 6400323        | 1934.2 | 21.41 | 4.26 | 1.00 |
| 3.51           | 5190343        | 1573.8 | 13.49 | 6.79 | 1.00 |
| 3.13           | 4118355        | 1245.3 | 8.11  | 12.04 | 0.99 |
| 2.87           | 4102229        | 1240.8 | 5.53  | 18.66 | 0.99 |
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| 2.32           | 3456045        | 1051.4 | 2.11  | 52.10 | 0.91 |
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| 952                     | 1.3% |      | XDS (second method)  |        |      | total    |
| 74030                   | 100% |      | total                |        |      |          |

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