**Supplementary**

Table S1. Comparison of different serial crystallography experiments in different aspects

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Experiment | Protein model | Sample delivery setup | Data collection rate (Hz) | Indexable rate (%) | No. of indexable frames collected per hour | Total measuring time | Protein consumption | Light Source |
| This study | Lysozyme / proteinase K | microfluidic rotating-target | 2 | 2.27 / 2.08 | 137 / 91 | 18 h / 25 h | 4.32 mg / 1.88 mg | SR |
| Nam et al. (2020) 1 | Lysozyme | Viscous media | 10 | 10.99 - 33.00 | 3977 - 11889 | 7.6 min - 57.6min | 0.038 - 0.288 mg | SR |
| Monteiro et al (2020) 2 | Lysozyme | 3D-MiXD | 200 | 2.72 - 8.71 | 19554 - 62680 | ~ 50 min | 0.626 mg | SR |
| Doak et al. (2018) 3 | Lysozyme | Sheet-on-sheet sandwich | 30 | 73 | 60000 | 14 min | 0.112 mg | XFEL |
| Grünbein et al. (2018) 4 | ConA and ConB | GDVN | 30\* | 7.53\*\* | 8376 | 12 h | 50 g\*\*\* | XFEL |
| Beyerlein et al (2017) 5 | Lysozyme-CTO | Polyimide tape drive | 25 | 24.12 -26.97 | 16936 - 17410 | ~ 8.4 h | ~ 39.53 mg | SR |
| Owen et al. (2017) 6 | Myoglobin | Fixed target | 23 | 15 - 33 | 15462 - 36548 | 7 - 10 min | 2.5 - 4.8 mg | SR |
| Martin-Garcia et al. (2017) 7 | Lysozyme | LCP | 10 | 5.11 | 1554 | 12 h | 0.2 mg | SR |
| Roedig et al. (2017) 8 | BEV2 / CPV18 | Fixed-target | 10.79 / 33.61 | 3.99 / 87.97 | ~ 2112 / 71738 | ~ 10min / 14min | ~ 4 μg | XFEL |
| Stellato et al. (2014) 9 | Lysozyme | Capillary | 25 | 2.68 | 2367 | 17 h | 250 mg | SR |
| Boutet et al. (2012) 10 | Lysozyme | Liquid jet | 120 | 0.53 - 0.83 | 2299 - 3602 | ~ 3.4 h- 4.6h | ---- | XFEL |

\* Data-acquisition rate calculated from total collected images and total measuring time

\*\* Overall indexable rate of ConA and ConB

\*\*\* Proteins were extracted from 50 g of jack bean meal



Figure S1. Drawing information for processing PMMA and double-sided tape. Both PMMA and double-sided tape are circle shape with a diameter of 20 mm, which contains a groove with a width of 1 mm (inner diameter is 15 mm, outer diameter is 17 mm). Inside this groove, another groove with a width of approximately 2 mm (inner diameter is 4.48 mm, outer diameter is 8.96 mm) is machined to reduce the weight of the entire device. The semi-circular in the middle is machined to fit the size and shape of the motor axle.



Figure S2. The boundary size of the microfluidic rotating-target device.

**References**

1. K. H. Nam, Int J Mol Sci, 2020, 21, 3332.

2. D. C. F. Monteiro, D. von Stetten, C. Stohrer, M. Sans, A. R. Pearson, G. Santoni, P. van der Linden and M. Trebbin, Iucrj, 2020, 7, 207-219.

3. R. B. Doak, G. N. Kovacs, A. Gorel, L. Foucar, T. R. M. Barends, M. L. Grunbein, M. Hilpert, M. Kloos, C. M. Roome, R. L. Shoeman, M. Stricker, K. Tono, D. You, K. Ueda, D. A. Sherrell, R. L. Owen and I. Schlichting, Acta Crystallogr D, 2018, 74, 1000-1007.

4. M. L. Grunbein, J. Bielecki, A. Gorel, M. Stricker, R. Bean, M. Cammarata, K. Dorner, L. Frohlich, E. Hartmann, S. Hauf, M. Hilpert, Y. Kim, M. Kloos, R. Letrun, M. Messerschmidt, G. Mills, G. N. Kovacs, M. Ramilli, C. M. Roome, T. Sato, M. Scholz, M. Sliwa, J. Sztuk-Dambietz, M. Weik, B. Weinhausen, N. Al-Qudami, D. Boukhelef, S. Brockhauser, W. Ehsan, M. Emons, S. Esenov, H. Fangohr, A. Kaukher, T. Kluyver, M. Lederer, L. Maia, M. Manetti, T. Michelat, A. Munnich, F. Pallas, G. Palmer, G. Previtali, N. Raab, A. Silenzi, J. Szuba, S. Venkatesan, K. Wrona, J. Zhu, R. B. Doak, R. L. Shoeman, L. Foucar, J. P. Colletier, A. P. Mancuso, T. R. M. Barends, C. A. Stan and I. Schlichting, Nat Commun, 2018, 9, 3487.

5. K. R. Beyerlein, D. Dierksmeyer, V. Mariani, M. Kuhn, I. Sarrou, A. Ottaviano, S. Awel, J. Knoska, S. Fuglerud, O. Jonsson, S. Stern, M. O. Wiedorn, O. Yefanov, L. Adriano, R. Bean, A. Burkhardt, P. Fischer, M. Heymann, D. A. Horke, K. E. J. Jungnickel, E. Kovaleva, O. Lorbeer, M. Metz, J. Meyer, A. Morgan, K. Pande, S. Panneerselvam, C. Seuring, A. Tolstikova, J. Lieske, S. Aplin, M. Roessle, T. A. White, H. N. Chapman, A. Meents and D. Oberthuer, Iucrj, 2017, 4, 769-777.

6. R. L. Owen, D. Axford, D. A. Sherrell, A. L. Kuo, O. P. Ernst, E. C. Schulz, R. J. D. Miller and H. M. Mueller-Werkmeister, Acta Crystallogr D, 2017, 73, 373-378.

7. J. M. Martin-Garcia, C. E. Conrad, G. Nelson, N. Stander, N. A. Zatsepin, J. Zook, L. Zhu, J. Geiger, E. Chun, D. Kissick, M. C. Hilgart, C. Ogata, A. Ishchenko, N. Nagaratnam, S. Roy-Chowdhury, J. Coe, G. Subramanian, A. Schaffer, D. James, G. Ketwala, N. Venugopalan, S. L. Xu, S. Corcoran, D. Ferguson, U. Weierstall, J. C. H. Spence, V. Cherezov, P. Fromme, R. F. Fischetti and W. Liu, Iucrj, 2017, 4, 439-454.

8. P. Roedig, H. M. Ginn, T. Pakendorf, G. Sutton, K. Harlos, T. S. Walter, J. Meyer, P. Fischer, R. Duman, I. Vartiainen, B. Reime, M. Warmer, A. S. Brewster, I. D. Young, T. Michels-Clark, N. K. Sauter, A. Kotecha, J. Kelly, D. J. Rowlands, M. Sikorsky, S. Nelson, D. S. Damiani, R. Alonso-Mori, J. S. Ren, E. E. Fry, C. David, D. I. Stuart, A. Wagner and A. Meents, Nat Methods, 2017, 14, 805-810.

9. F. Stellato, D. Oberthur, M. N. Liang, R. Bean, C. Gati, O. Yefanov, A. Barty, A. Burkhardt, P. Fischer, L. Galli, R. A. Kirian, J. Meyer, S. Panneerselvam, C. H. Yoon, F. Chervinskii, E. Speller, T. A. White, C. Betzel, A. Meents and H. N. Chapman, Iucrj, 2014, 1, 204-212.

10. S. Boutet, L. Lomb, G. J. Williams, T. R. M. Barends, A. Aquila, R. B. Doak, U. Weierstall, D. P. DePonte, J. Steinbrener, R. L. Shoeman, M. Messerschmidt, A. Barty, T. A. White, S. Kassemeyer, R. A. Kirian, M. M. Seibert, P. A. Montanez, C. Kenney, R. Herbst, P. Hart, J. Pines, G. Haller, S. M. Gruner, H. T. Philipp, M. W. Tate, M. Hromalik, L. J. Koerner, N. van Bakel, J. Morse, W. Ghonsalves, D. Arnlund, M. J. Bogan, C. Caleman, R. Fromme, C. Y. Hampton, M. S. Hunter, L. C. Johansson, G. Katona, C. Kupitz, M. N. Liang, A. V. Martin, K. Nass, L. Redecke, F. Stellato, N. Timneanu, D. J. Wang, N. A. Zatsepin, D. Schafer, J. Defever, R. Neutze, P. Fromme, J. C. H. Spence, H. N. Chapman and I. Schlichting, Science, 2012, 337, 362-364.