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Supporting information for article:

High-viscosity sample injection device for serial femtosecond crystallography at atmospheric pressure High-viscosity sample injection device for serial femtosecond crystallography at atmospheric pressure

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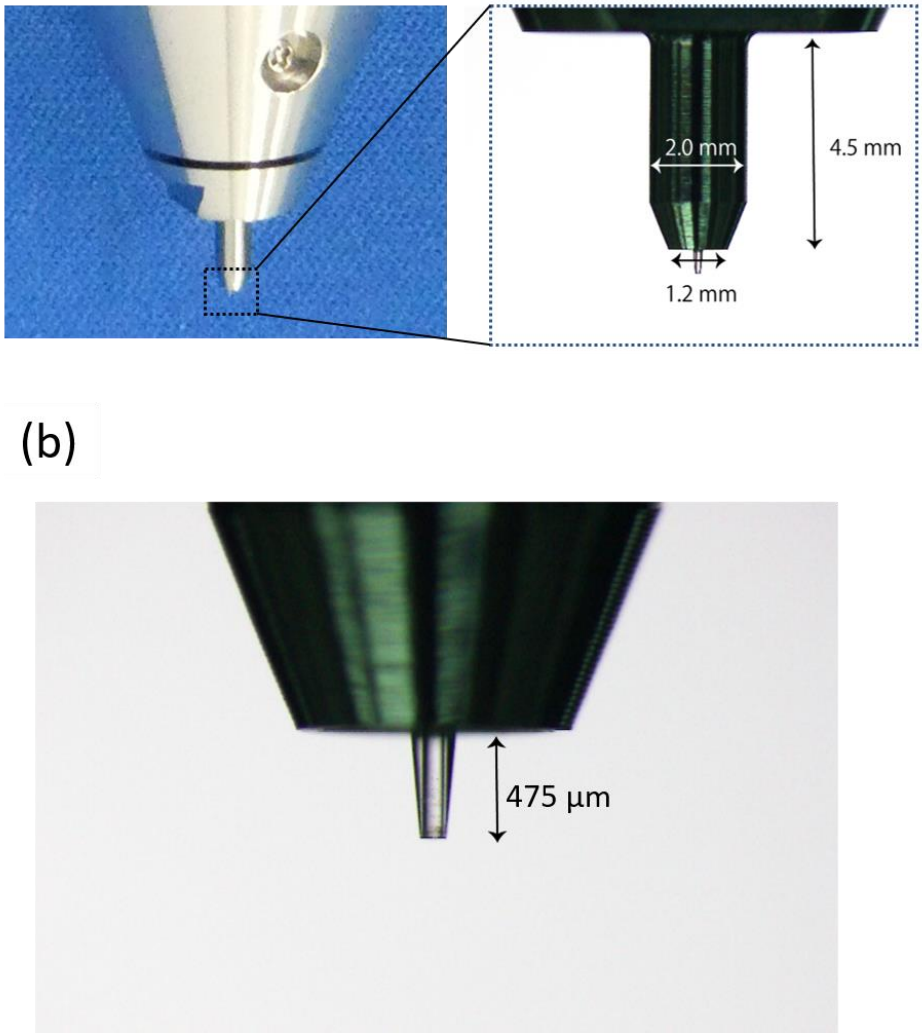

Figure S1 Images of the injector nozzle. (a) Magnified view of an outer metallic nozzle for a sheath gas of the HVE injector. The OD of the outer nozzle is 1.2 mm. (b) Magnified view of the capillary nozzle tip. The capillary is sharpened to a small taper angle (<10°) and protrudes from the outer nozzle by 475 µm.

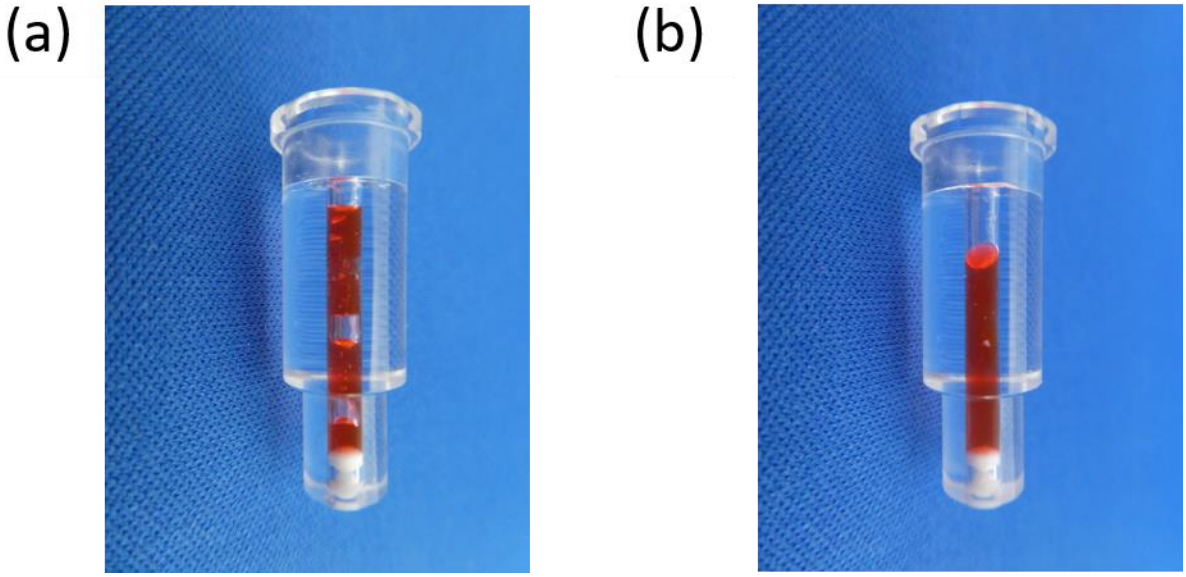

Figure S2 Sample reservoir before (a) and after (b) centrifugation. Grease colored with red ink was loaded into a 200-μL sample cartridge. The sample was centrifuged at 8000 ×*g* for 20 s. The bottom side of the cartridge was covered with a flexible film (Parafilm) to prevent samples leaking.

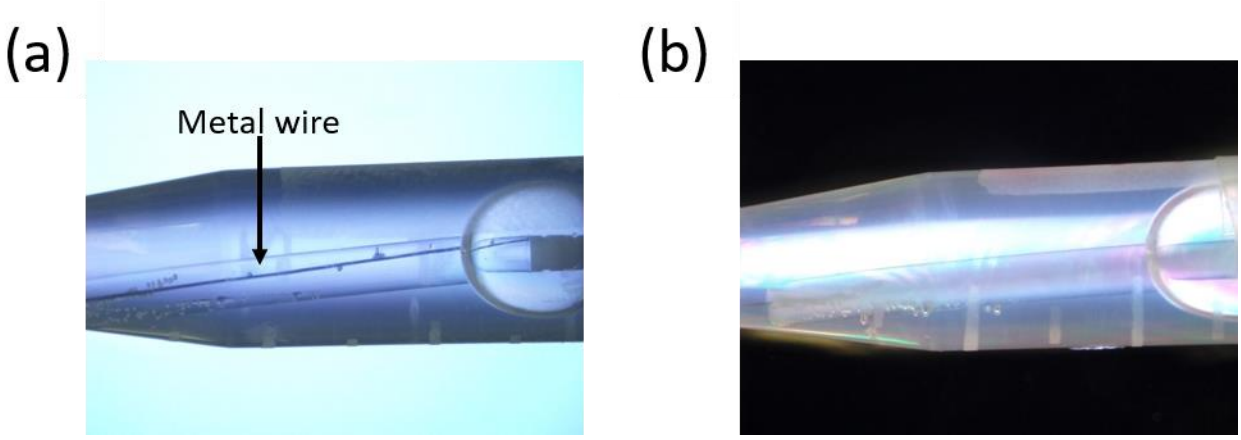

Figure S3 LCP crystallization using a metal wire. Before (a) and after (b) the appearance of crystals

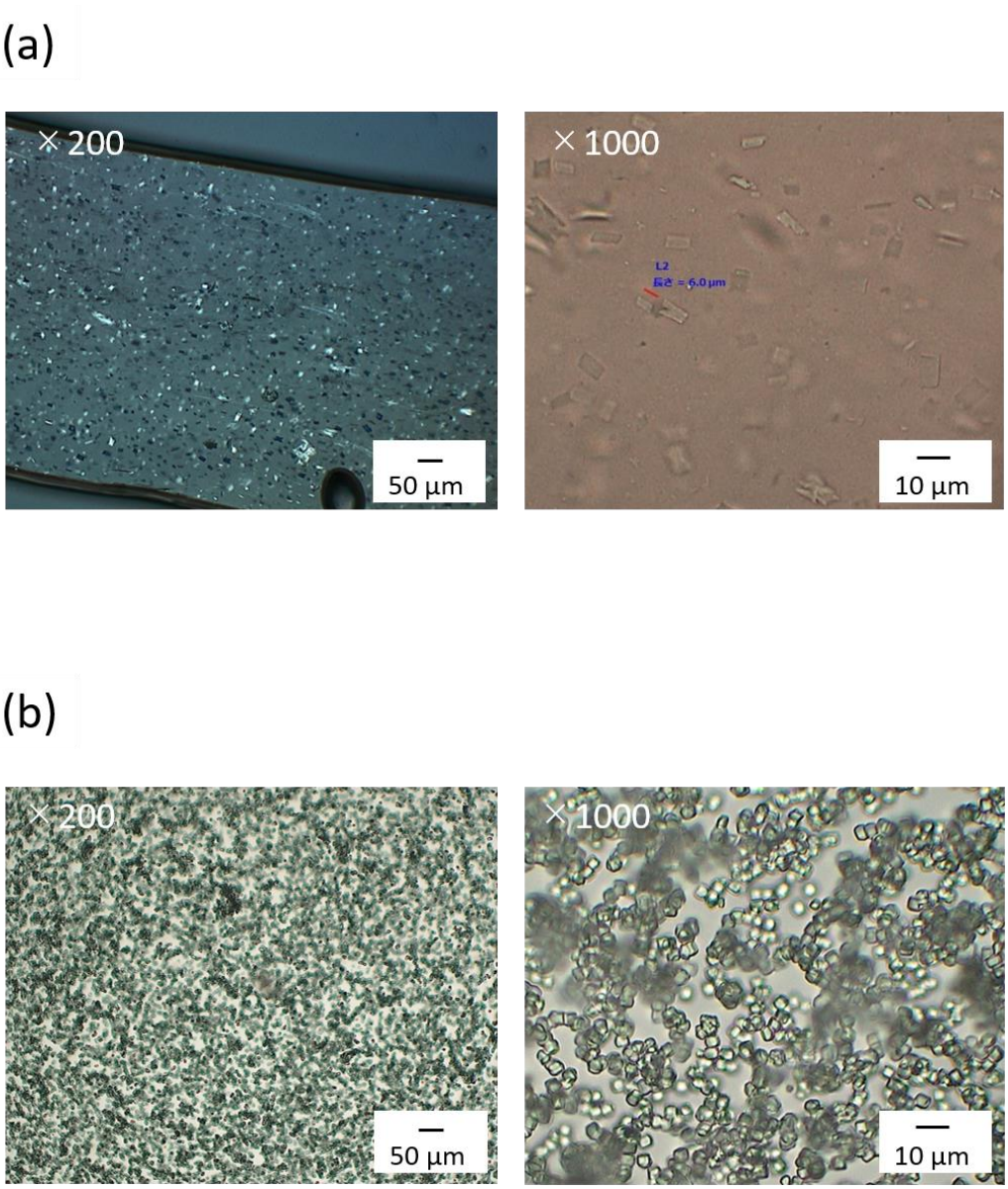

Figure S4 (a) $A_{2A}AR$ crystals in LCP used for data collection. The crystal density was estimated to be approximately one-tenth of the density of the lysozyme crystals. (b) Lysozyme crystals (~5 µm in size) used for data collection. The crystal density of 2.3×10^8 crystals/mL was determined by counting the crystals in a cell-counting plate.

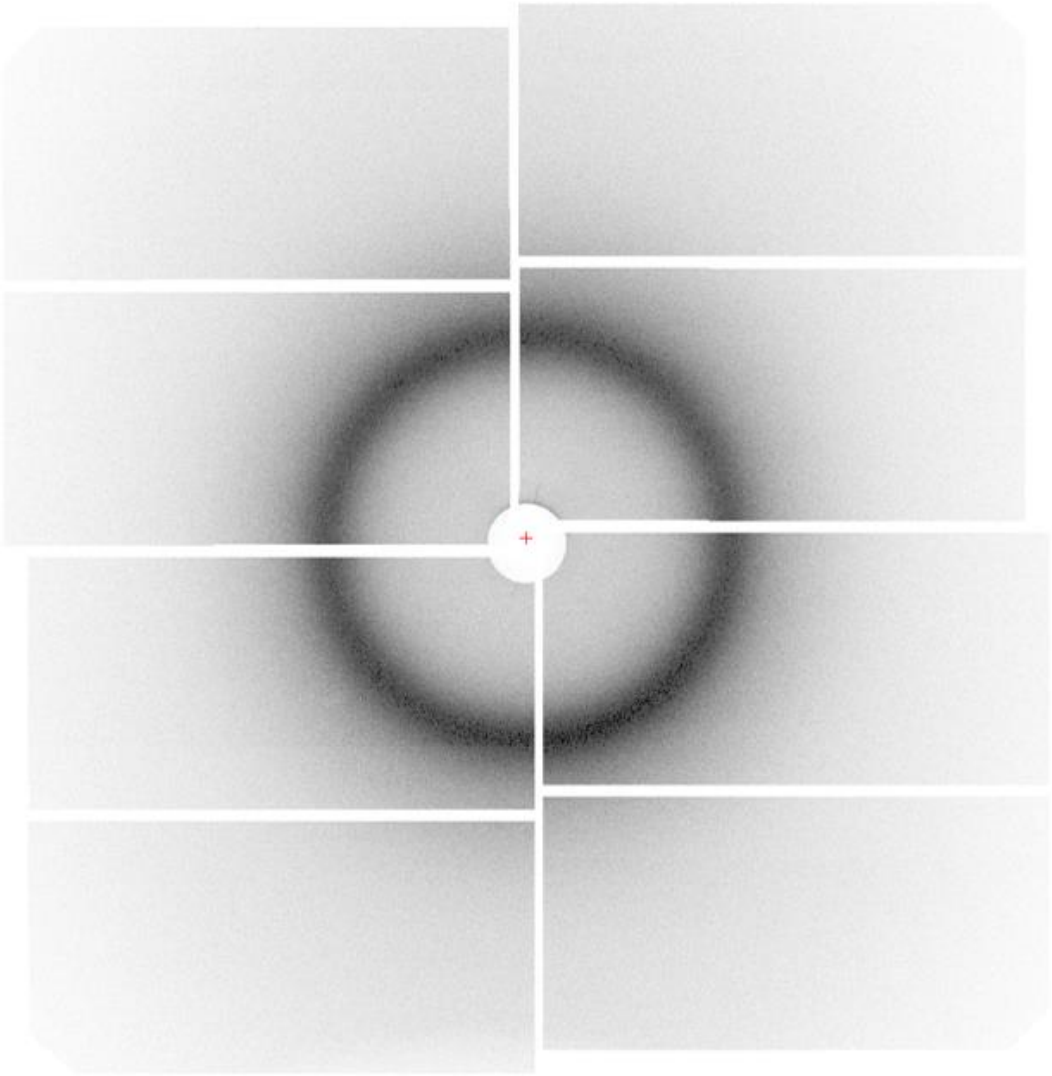

Figure S5 Representative image not counted as a hit for the $A_{2A}AR$ crystals. The image shows weak scattering patterns from the lipids.

Sample	Matrix/Sample flow rate (µl/min)	Resolution (Å)	Indexed images	PDB-ID	CXIDB	References
Human orexin-2 receptor	LCP/0.42	2.3	18,359	5WS3	74	(Suno *et al*., 2018)
Nitric oxide reductase	Hydroxyethyl cellulose/1.25	2.0 2.0 2.1 2.1	34,811 35,309 108,597 109,519	5Y5I 5Y5J 5Y5K 5Y5L	63	(Tosha *et al*., 2017)
Influenza M2 channel	LCP/0.42	1.4 1.45 1.4	34,113 29,173 63,249	5JOO 5UM1 5TTC	-	(Thomaston *et al*., 2017)
Cytochrome c oxidase	LCP/0.48	2.3	8,211	5NDC	-	(Andersson *et al*., 2017)
Lysozyme	Hydroxyethyl cellulose/0.43 or 0.75	1.8 1.45	29,593 40,787	5WR9 5WRA	50	(Sugahara *et al*., 2017)
Lysozyme	Nuclear grade grease/0.42	2.0	19,271	5WRB	47	(Sugahara *et al*., 2017)
Thaumatin	Hydroxyethyl cellulose/0.47	1.55	43,350	5WR8	49	(Sugahara *et al*., 2017)
Proteinase K	Hydroxyethyl cellulose/0.47	1.50	47,503 (native), 30,930 (derivative)	5WRC	45, 48	(Sugahara *et al*., 2017)
Photosystem II	Nuclear grade grease/5.6 or 2.8	2.35 2.35 2.50 2.50	64,985 51,482 54,956 63,711	5WS5 5WS6 5GTH 5GTI	-	(Suga *et al*., 2017)
Bacteriorhodopsin	LCP/2.5	2.00 2.10	243,639 17,724 18,937 20,509	5B6V 5B6W 5H2H 5H2I	53	(Nango *et al*., 2016)

			20,888 20,587 19,826 14,803 15,777 16,851 19,175 15,371 15,589 13,648	5H2J 5B6X 5H2K 5H2L 5H2M 5B6Y 5H2N 5H2O 5H2P 5B6Z		
Bacteriorhodopsin (Bicelle)	Synthetic grease super lube/0.25 or 0.95	2.10	23,347	5B34	43	(Nakane, Hanashima, *et al.*, 2016)

Table S1 Successful SFX results using the HVC injector at SACLA.

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Movie S1 Close-up view of the sample stream from the HVC injector at beanline 3 of SACLA. Microcrystals of A_{2A}AR complexed with ZM241385 embedded in LCP were extruded from the nozzle of 75-µm ID. The sample stream was continuously supplied without curling up although the stream was chopped by the XFEL beam.