



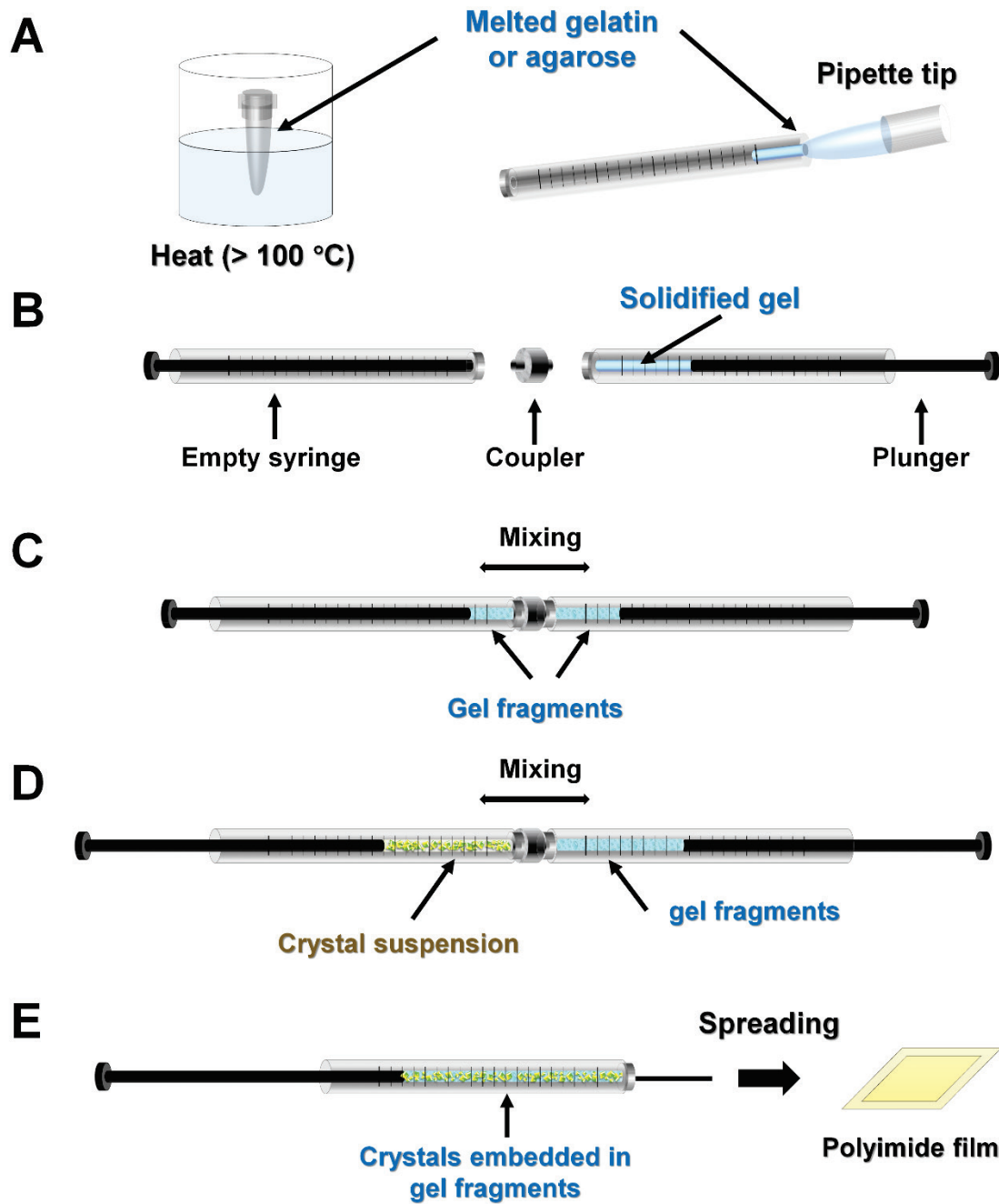
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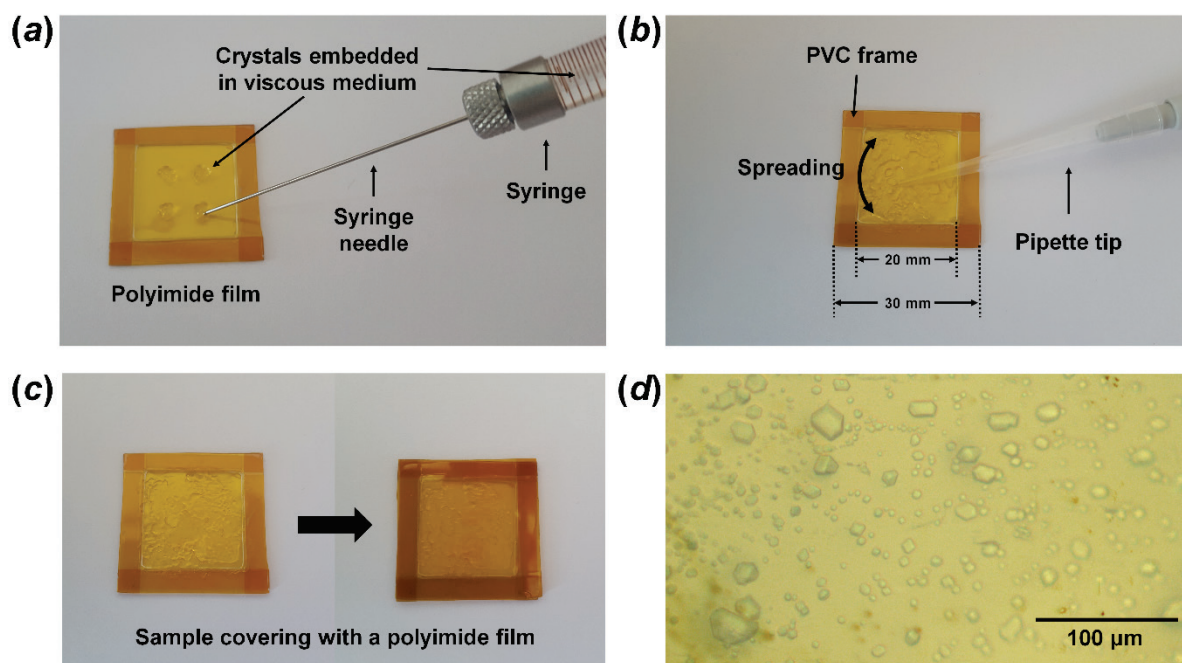
Supporting information for article:

Viscous medium-based crystal support in sample holder for fixed-target serial femtosecond crystallography

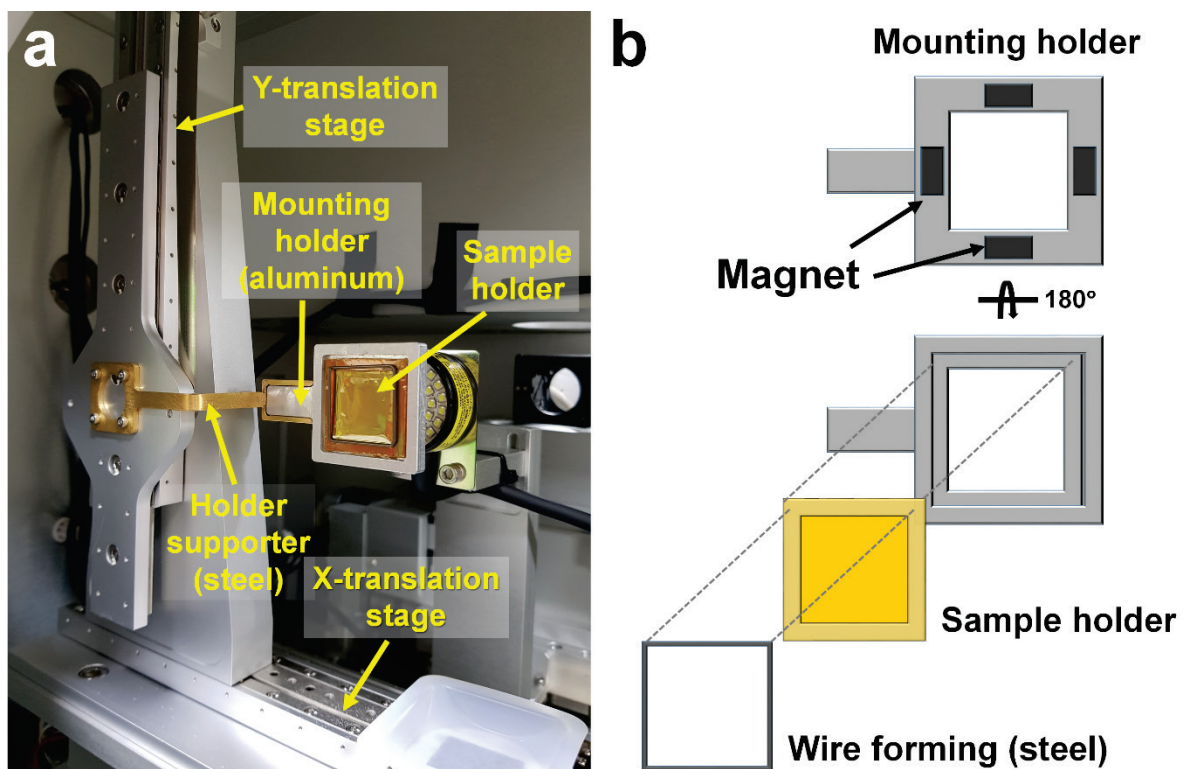
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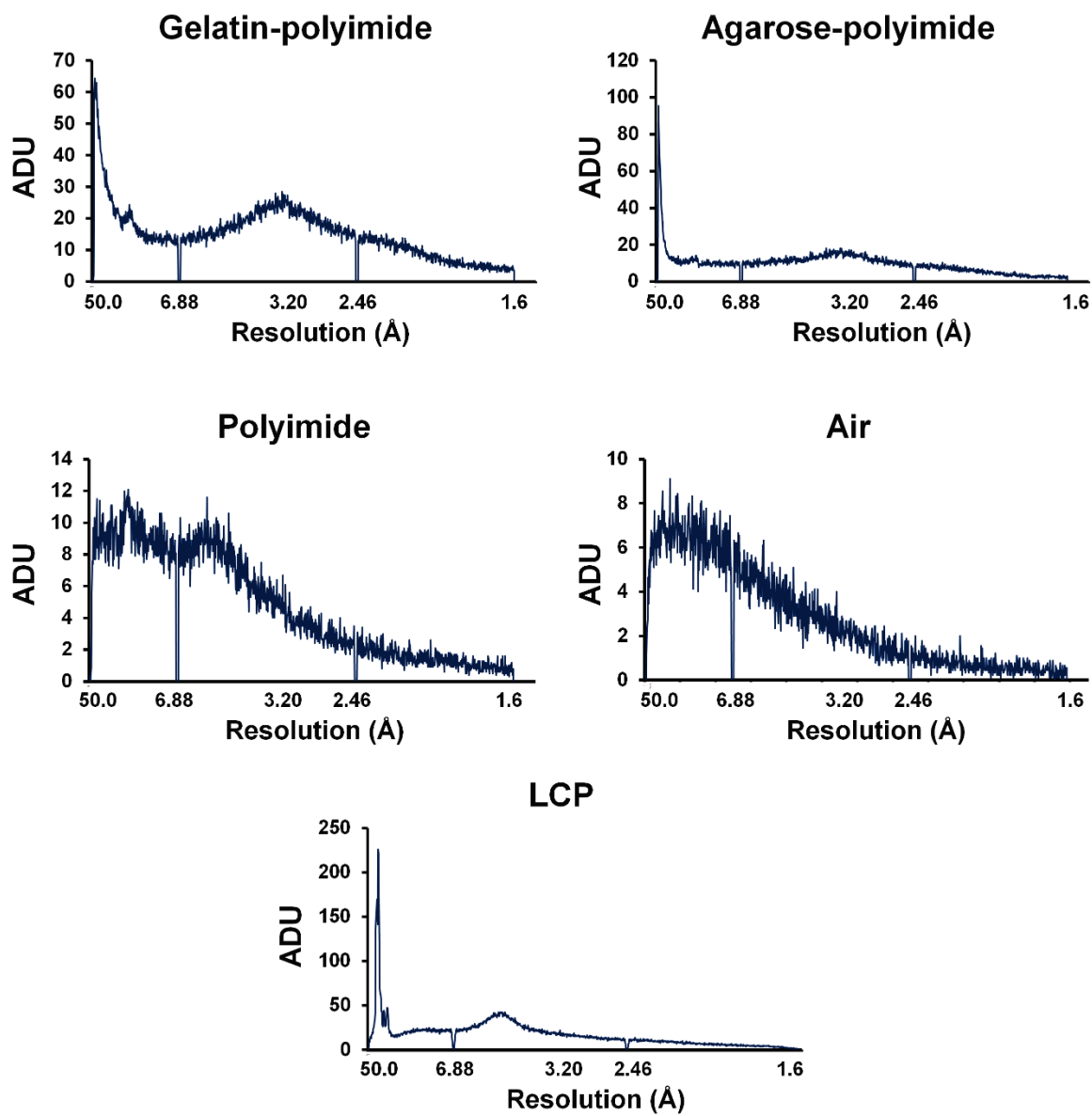
Supplementary Figure S1. Sample preparation for crystal embedding in gel fragment. (a) Gelatin or agarose powder are melted in hot water (> 100 °C) and transferred to a syringe using a pipette. (b) Melted gel is solidified in syringe at room temperature. The syringe is then connected to an empty syringe via a coupler. (c) The gel fragments are produced by moving the plunger back and forth in the dual-syringe setup. (d) The syringe containing the gel fragment is then connected to the syringe containing the crystal suspension and gently mixing. (e) Crystals embedded in gel fragments are spread on a polyimide film.



Supplementary Figure S2. Preparation of viscous medium-based sample holder for FT-SFX. (a) The crystals embedded in gelatin were loaded onto the polyimide chip. (b) The sample was spread evenly using a pipette tip. (c) The polyimide chip was covered to prevent solution evaporation. (d) Microscope view of glucose isomerase embedded in gelatin on the sample holder.



Supplementary Figure S3. (a) The sample holder containing the crystals embedded in viscous medium enclosed by polyimide films was installed into the mounting holder on the translation stage. (b) Exploded assembly of sample holder and mounting holder.



Supplementary Figure S4. Intensity of background scatter in gelatin-polyimide, agarose-polyimide, polyimide film, air and LCP (60% (w/v) monoolein).

Supplementary Table 1. Data collection statistics of glucose isomerase and lysozyme

Glucose isomerase embedded in gelatin				
Resolution	Refractions	Completeness	Redundancy	Signal/Noise (SNR)
70.92-3.77	4920	100	722.1	6.61
3.77-2.99	4741	100	461.1	6.49
2.99-2.61	4728	100	392.5	5.80
2.61-2.38	4688	100	352.5	5.59
2.38-2.21	4680	100	324.9	5.63
2.21-2.07	4677	100	305.1	5.52
2.07-1.97	4633	100	289.4	5.13
1.97-1.89	4651	100	273.8	5.09
1.89-1.81	4636	100	190.6	3.02
1.81-1.75	4650	100	124.5	3.00
Lysozyme embedded in agarose				
Resolution	Refractions	Completeness	Redundancy	Signal/Noise (SNR)
79.37-3.88	1302	100	308.0	8.83
3.88-3.08	1198	100	204.2	9.21
3.08-2.69	1182	100	172.2	8.38
2.69-2.44	1164	100	153.9	8.22
2.44-2.27	1156	100	143.4	7.93
2.27-2.13	1151	100	133.7	7.17
2.13-2.03	1147	100	126.9	6.13
2.03-1.94	1159	100	121.0	5.10
1.94-1.86	1131	100	100.0	3.06
1.86-1.80	1138	100	63.5	2.06

Supplementary Table 2. Comparison of unit cell parameters for glucose isomerase and lysozyme crystals.

Sample	Glucose isomerase	Glucose isomerase	Glucose isomerase	lysozyme	lysozyme	lysozyme
Experiment	FT-SFX	FT-SFX	SMX	FT-SFX	FT-SFX	SMX
Sample delivery	gelatin polyimide film	nylon-mesh polyimide films	gelatin capillary	gelatin polyimide film	nylon-mesh polyimide films	gelatin capillary
X-ray source	PAL-XFEL	PAL-XFEL	PLS-II	PAL-XFEL	PAL-XFEL	PLS-II
Energy (ev)	9700	9700	12400	9700	9700	12400
Space group	I222	I222	I222	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2
Cell dimension						
a	92.63	93.05	94.52	79.98	78.22	79.74
b	98.23	99.00	100.17	79.98	78.22	79.74
c	101.61	101.92	103.57	38.22	37.76	38.56
Reference	this study	Lee et al. 2019	Nam 2020	this study	Lee et al. 2019	Nam 2020

References

- Lee, D., Baek, S., Park, J., Lee, K., Kim, J., Lee, S. J., Chung, W. K., Lee, J. L., Cho, Y. & Nam, K. H. (2019). *Sci Rep* 9, 6971.
- Nam, K. H. (2020). *J Appl Crystallogr* 53, 45-50.