SUPPORTING INFORMATION FOR

Nanoliter-Scale Protein Crystallization and Screening with a Microfluidic Droplet Robot

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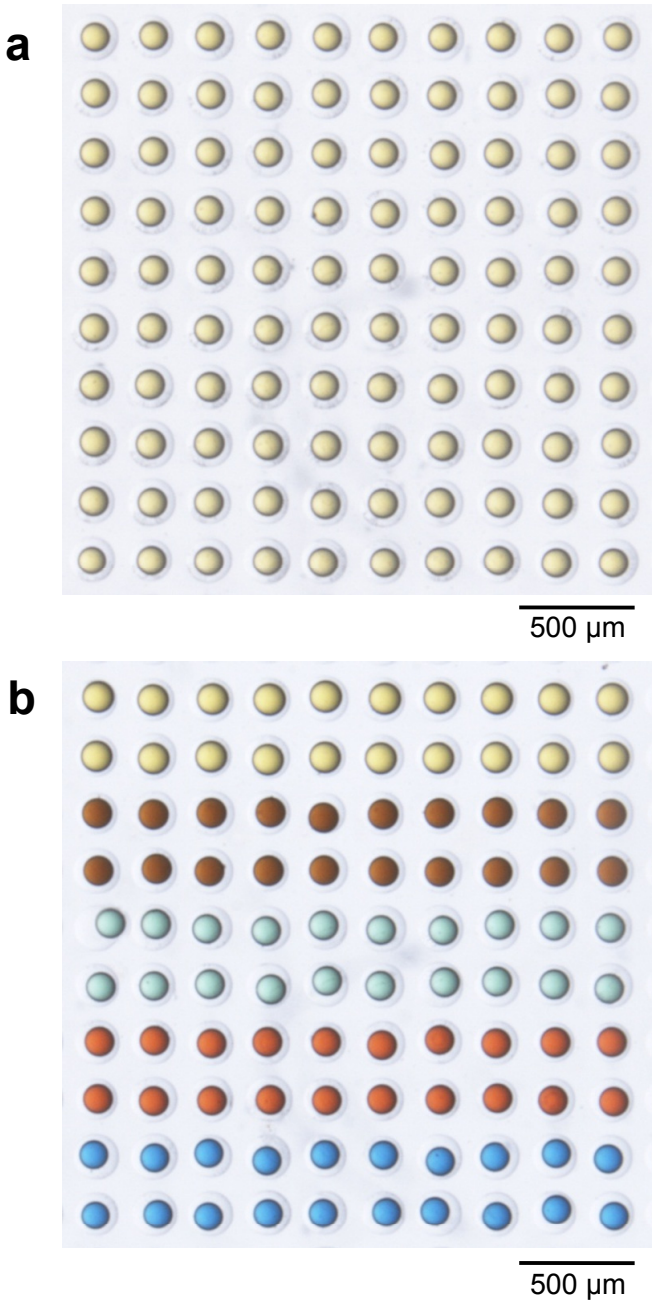

Figure S1. (a) Microscopic image showing 100, 1.98 nL droplets containing 5 mM sodium fluorescein and 30% PEG 6000 in 50 mM borate buffer. The relative standard deviation (RSD) of droplet diameter is 1.78% (n=100). (b) Microscopic image showing an array of droplets containing 30% PEG 6000 and five different dyes in 50 mM borate buffer. The RSDs of droplet diameters are in the range from 0.58% to 1.16% (n=20).

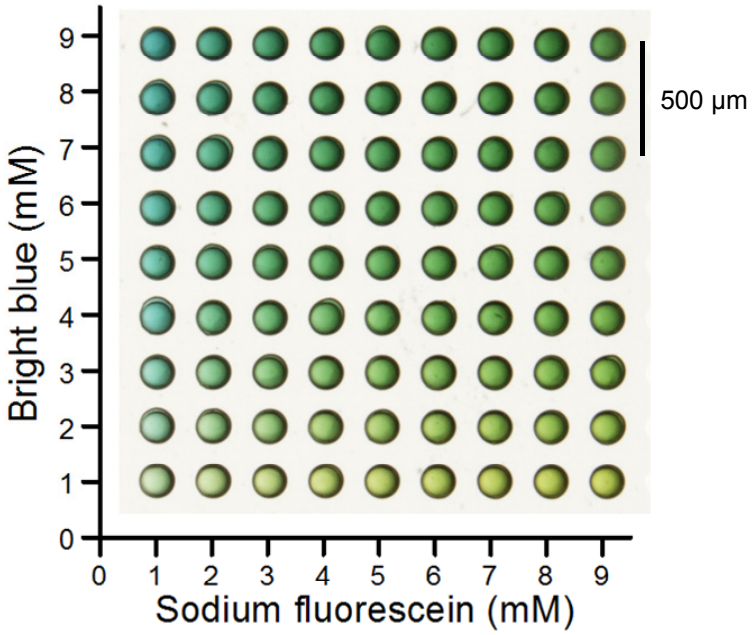

Figure S2. Generation of two-dimensional concentration gradients of two dyes (sodium fluorescein for yellow color and bright blue for blue color) in a droplet array with the droplet robot.

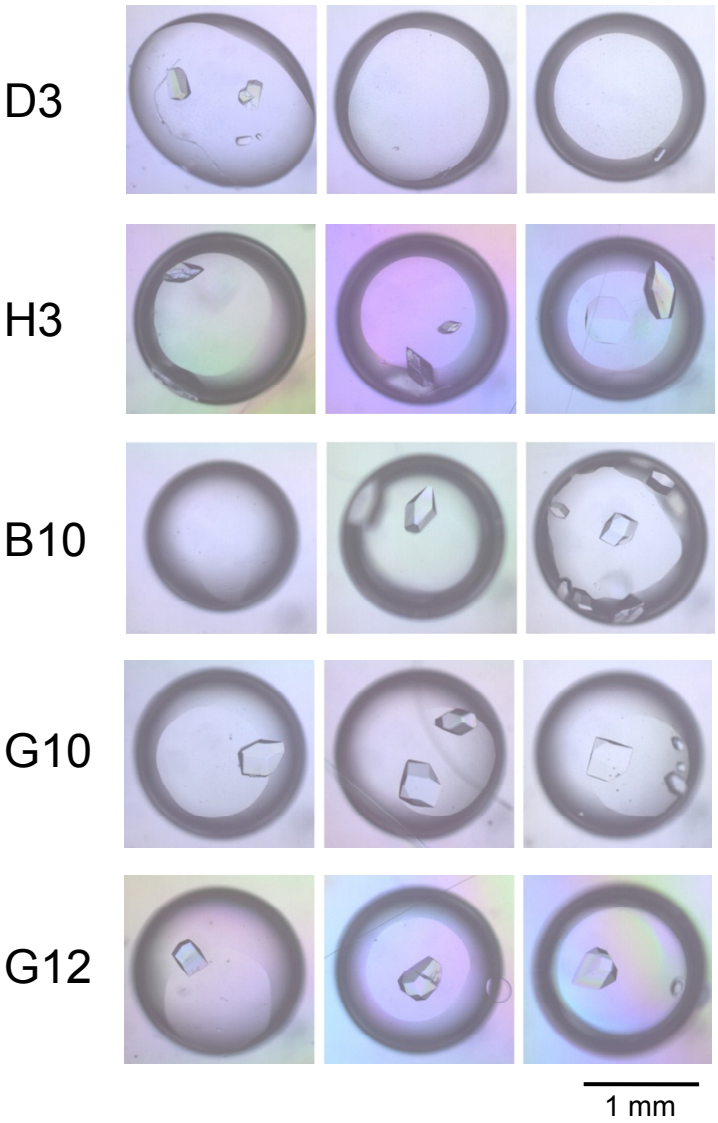

Figure S3. The scaling-up crystallization results for lysozyme with identified precipitants of D3, H3, B10, G10, and G12. The crystallization volume is 2 µL containing 1 µL protein and 1 µL precipitant solution. Protein: 50 mg/mL lysozyme in 0.1 M NaAc buffer (pH 4.6). Precipitant compositions: D3: 0.1 M HEPES pH 7.0, 30% v/v Jeffamine ED-2001® Reagent pH 7.0; H3: 0.2 M sodium malonate pH 7.0, 20% w/v polyethylene glycol 3350; B10: 0.8 M succinic acid pH 7.0; G10: 0.2 M magnesium chloride hexahydrate, 0.1 M Bis-Tris pH 5.5, 25% w/v polyethylene glycol 3350; G12: 0.2 M magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 25% w/v polyethylene glycol 3350. Crystallization volume: 2 µL containing 1 µL protein and 1 µL precipitant.

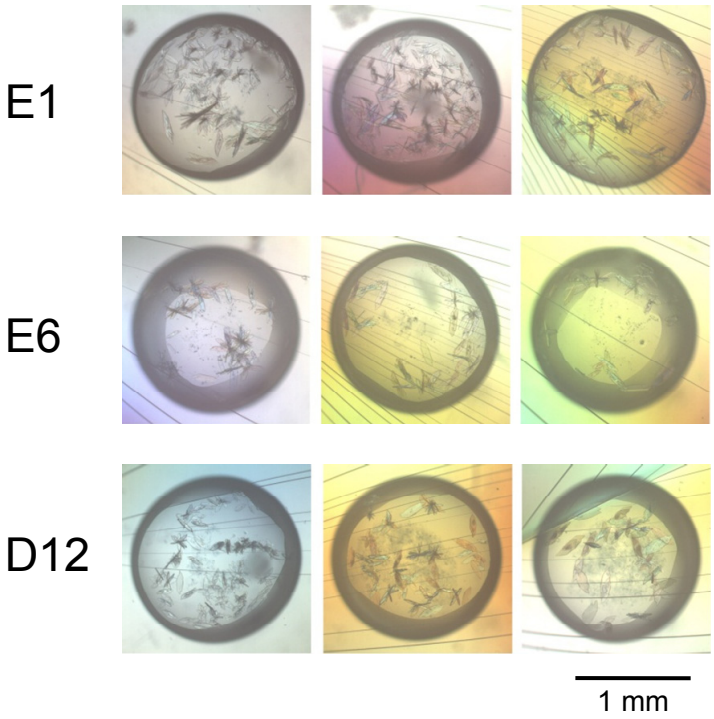

Figure S4. The scaling-up crystallization results for xylanase with identified precipitants of E1, E6 and D12. The crystallization volume is 2 µL containing 1 µL protein and 1 µL precipitant solution. Protein: 36 mg/mL xylanase in 0.18 M sodium/potassium phosphate buffer (pH 7) containing 43% glycerol (w/v). Precipitant compositions: E1: 0.2 M calcium chloride dehydrate, 0.1 M BIS-TRIS pH 6.5, 45% v/v (+/-)-2-methyl-2,4-pentanediol; E6: 0.05 M calcium chloride dehydrate, 0.1 M BIS-TRIS pH 6.5, 30% v/v polyethylene glycol monomethyl ether 550; D12: 0.2 M calcium chloride dehydrate, 0.1 M BIS-TRIS pH 5.5, 45% v/v (+/-)-2-methyl-2,4-pentanediol. Crystallization volume: 2 µL containing 1 µL protein and 1 µL precipitant.

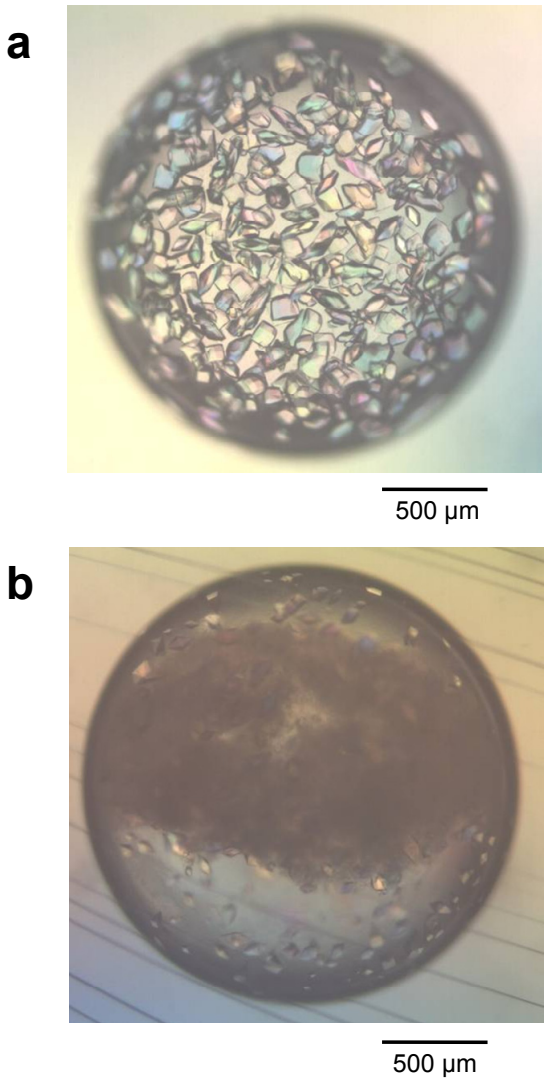

Figure S5. Large-volume crystallization of (a) lysozyme and (b) thaumatin with microbatch method. The crystallization volume is 4 μL containing 2 μL protein and 2 μL precipitant. Crystallization conditions: (a) protein, 50 mg/mL lysozyme in 0.1 M NaAc buffer (pH 4.6); precipitant, 10%(w/v) NaCl in 0.1M NaAc (pH 4.6); protein/precipitant mixing ratio, 1:1. (b) protein, 30 mg/mL thaumatin in 0.1 M ADA buffer (pH 6.5); precipitant, 2M sodium / potassium tartrate in 0.1M HEPES (pH 7.5); protein/precipitant mixing ratio, 1:1.

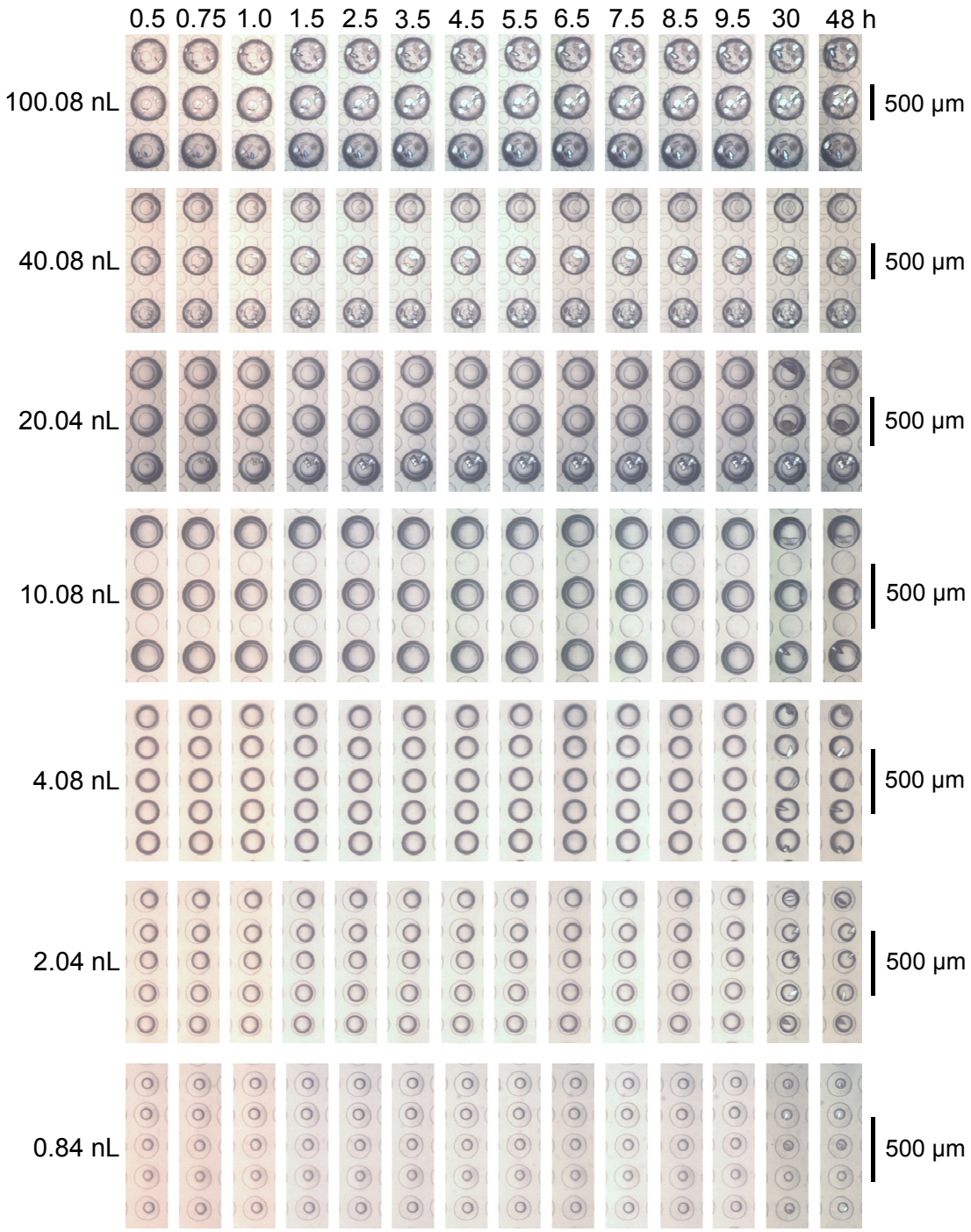

Figure S6. The effect of droplet volume on protein crystallization. Time-lapse microscopic images of droplets with different volumes from 0.84 nL to 100.08 nL. Crystallization conditions: protein, 50 mg/mL lysozyme in 0.1 M NaAc buffer (pH 4.6); precipitant, 10% (w/v) NaCl in 0.1M NaAc (pH 4.6); protein/precipitant mixing ratio , 1:1.

Table S1. The compositions of protein, buffer, and precipitant solutions in a 2D droplet array for studying protein phase behavior.

Droplet position (x, y)	Protein (nL)	Buffer (nL)	Precipitant (nL)
0, 0	0.84	6.72	0.84
0, 1	1.26	6.30	0.84
0, 2	1.68	5.88	0.84
0, 3	2.10	5.46	0.84
0, 4	2.52	5.04	0.84
0, 5	2.94	4.62	0.84
0, 6	3.36	4.20	0.84
0, 7	3.78	3.78	0.84
0, 8	4.20	3.36	0.84
1, 0	0.84	6.30	1.26
1, 1	1.26	5.88	1.26
1, 2	1.68	5.46	1.26
1, 3	2.10	5.04	1.26
1, 4	2.52	4.62	1.26
1, 5	2.94	4.20	1.26
1, 6	3.36	3.78	1.26
1, 7	3.78	3.36	1.26
1, 8	4.20	2.94	1.26
2, 0	0.84	5.88	1.68
2, 1	1.26	5.46	1.68
2, 2	1.68	5.04	1.68
2, 3	2.10	4.62	1.68
2, 4	2.52	4.20	1.68
2, 5	2.94	3.78	1.68

2, 6	3.36	3.36	1.68
2, 7	3.78	2.94	1.68
2, 8	4.20	2.52	1.68
3, 0	0.84	5.46	2.10
3, 1	1.26	5.04	2.10
3, 2	1.68	4.62	2.10
3, 3	2.10	4.20	2.10
3, 4	2.52	3.78	2.10
3, 5	2.94	3.36	2.10
3, 6	3.36	2.94	2.10
3, 7	3.78	2.52	2.10
3, 8	4.20	2.10	2.10
4, 0	0.84	5.04	2.52
4, 1	1.26	4.62	2.52
4, 2	1.68	4.20	2.52
4, 3	2.10	3.78	2.52
4, 4	2.52	3.36	2.52
4, 5	2.94	2.94	2.52
4, 6	3.36	2.52	2.52
4, 7	3.78	2.10	2.52
4, 8	4.20	1.68	2.52
5, 0	0.84	4.62	2.94
5, 1	1.26	4.20	2.94
5, 2	1.68	3.78	2.94
5, 3	2.10	3.36	2.94
5, 4	2.52	2.94	2.94
5, 5	2.94	2.52	2.94

5, 6	3.36	2.10	2.94
5, 7	3.78	1.68	2.94
5, 8	4.20	1.26	2.94
6, 0	0.84	4.20	3.36
6, 1	1.26	3.78	3.36
6, 2	1.68	3.36	3.36
6, 3	2.10	2.94	3.36
6, 4	2.52	2.52	3.36
6, 5	2.94	2.10	3.36
6, 6	3.36	1.68	3.36
6, 7	3.78	1.26	3.36
6, 8	4.20	0.84	3.36
7, 0	0.84	3.78	3.78
7, 1	1.26	3.36	3.78
7, 2	1.68	2.94	3.78
7, 3	2.10	2.52	3.78
7, 4	2.52	2.10	3.78
7, 5	2.94	1.68	3.78
7, 6	3.36	1.26	3.78
7, 7	3.78	0.84	3.78
7, 8	4.20	0.42	3.78
8, 0	0.84	3.36	4.20
8, 1	1.26	2.94	4.20
8, 2	1.68	2.52	4.20
8, 3	2.10	2.10	4.20
8, 4	2.52	1.68	4.20
8, 5	2.94	1.26	4.20

8, 6	3.36	0.84	4.20
8, 7	3.78	0.42	4.20
8, 8	4.20	0.00	4.20