The effect of audible sound on protein crystallization

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Fig. S1. Crystal number with and without audible sound. Total number of crystals with and without audible sound. Crystallization conditions after mixing: 20 mg/ml lysozyme, 40 mg/ml NaCl (Error bar: standard deviation, n=30). It demonstrated that nucleation is correlative with frequency of audible sound.



Fig. S2. Examples of crystal morphology with (a-d) and without (a'-d') 1000 Hz audible sound. a and a': lysozyme; b and b': concanavalin A; c and c': glucose isomerase; d and d': proteinase K. The crystallization technique was sitting-drop method.

SI Supplementary Tables

Proteins	Abbreviate	Cata No.	Suppliers	Buffers					
Followings are proteins used at Northwestern Polytechnical University for sound effect study									
Lysozyme	Lys.	E05801	Seikagaku	100 mM Sodium acetate, pH4.					
Catalase	Cat.	C40	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Subtilisin AVII	Sub.	P5380	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Thaumatin	Thau.	T7638	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Insulin	Ins.	Z5500	Sigma-Aldrich	100 mM Ammonia					
Concanavalin A VI	Con.	L7647	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Ribonuclease A I	Rib. I	R4875	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Ribonuclease A III	Rib. III	R5125	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Ribonuclease A XII	Rib. XII	R5500	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
α-chymotrypsinogen AII	Chy.	C4879	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Proteinase K ^a	Prok.	P6556	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Cellulase	Cel.	C0615	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Papain	Pap.	P3125	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Lactalbumin	Lac.	K7015	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Glucose Isomerase	Glu.	HR7-100	Hampton Research	25 mM HEPES sodium, pH7.0					
Followings are proteins used at University of Hamburg for sound effect verification study									
Proteinase K	Prok.	105281	Merck Millipore	25 mM HEPES sodium, pH7.0					
Thaumatin	Thau.	T7638	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Lysozyme	Lys.	HR7-110	Hampton Research	50 mM Sodium acetate, pH4.6					
Lipase B	Lipb.	HR7-099	Hampton Research	25 mM HEPES sodium, pH7.0					
Ribonuclease A I	Rib. I	R4875	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Carbonic Anhydrase	Carb.	C3934	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
α-Glucosidase	α-Glu.	G0660	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					
Superoxide Dismutase	Sup.	82515	Sigma-Aldrich	25 mM HEPES sodium, pH7.0					

Table S1. Proteins and their buffers used in the investigation

a: The lot number of proteinase K utilized at 1000 Hz and 5000 Hz was 119K8602 and 039K8618, respectively.

Proteins	Screen kits	No sound		5000 Hz	
		Rep. 1	Rep.2	Rep.1	Rep.2
Proteinase K (20 mg/ml)	Classics	5	3	10	10
	Index HR	0	0	3	1
Thaumatin (20 mg/ml)	Classics	1	1	3	1
	Index HR	1	1	3	2
Lysozyme (20 mg/ml)	Classics	12	9	12	11
	JCSG	8	8	15	18
	Index HR	14	13	16	14
Lipase B (20 mg/ml)	Index HR	12	12	10	10
Ribonuclease A1 (24 mg/ml)	Index HR	0	0	1	1
Carbonic Anhydrase (14 mg/ml)	Index HR	1		1	
α -Glucosidase (9 mg/ml)	Index HR	0		3	
Superoxide Dismutase (4.5 mg/ml)	Index HR	8		9	

Table S2. Number of crystallization hits with and without sound irradiation inverification study in University of Hamburg

Note: in total 21 comparisons were conducted. Among these comparisons, 16 showed positive effect under sound irradiation, and 3 showed the same effect with and without sound irradiation. Only 2 showed reversed results. We sampled all comparisons and applied one-sample *t*- test to the results. This result showed that sound irradiation at 5000 Hz revealed a significant effect on the number of protein crystallization hits (P = 0.0264 < 0.05).

SI Supplementary Video

Video 1. DLS monitoring of the particle size evolution against time with and without sound irradiation.

This movie contains in-situ monitoring of the cluster size evolution in the crystallization solution against time with and without sound irradiation. It shows that the cluster size is more homogeneous when the sound irradiation is applied than that without sound irradiation. The result verified that sound irradiation indeed affects the solution status which may cause difference in protein crystallization.