Microseed it!

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Half the funding was provided by Opticryst, *a consortium of European institutions and companies.*
Submitted to Crystal Growth and Design, January 2011

Random microseeding: a theoretical and practical exploration of seed stability and seeding techniques for successful protein crystallization

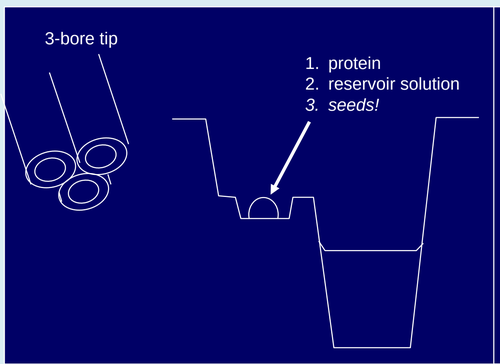
Synopsis. Random Microseed Matrix-Screening, which comprises automatic seeding into random screens, is a recent method that often gives spectacular results. We created a sensitive, reproducible and quantitative assay for crystal seeds, and evaluated several variations of the method. We make recommendations here for avoiding salt crystals, stabilizing seed stocks, crystallizing protein complexes, and preparing unconventional seed stocks and nucleants.

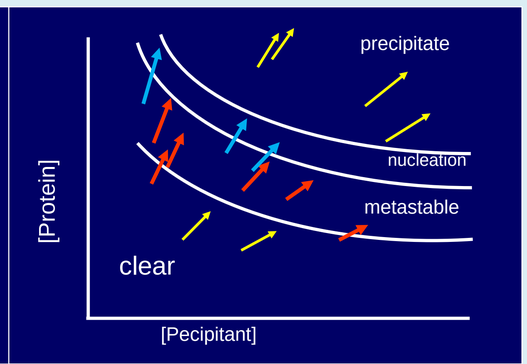
Background

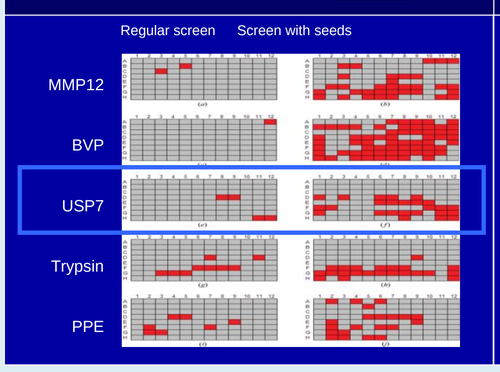
D'Arcy et al. Acta Cryst. (2007). D63. 'An automated microseed matrix-screening method for protein crystallization'

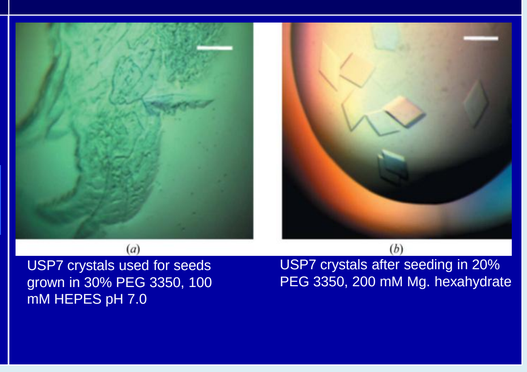
1. *Added seed crystals to a* random screen
2. *Suspended crushed crystals in the reservoir solution that gave the hits used ("hit solution")*
3. *Automated!*

This gave: (1) more hits (2) better crystals. Crystallization is more likely to take place in the metastable zone (red arrows).









USP7 crystals used for seeds grown in 30% PEG 3350, 100 mM HEPES pH 7.0

USP7 crystals after seeding in 20% PEG 3350, 200 mM Mg. hexahydrate

Methods

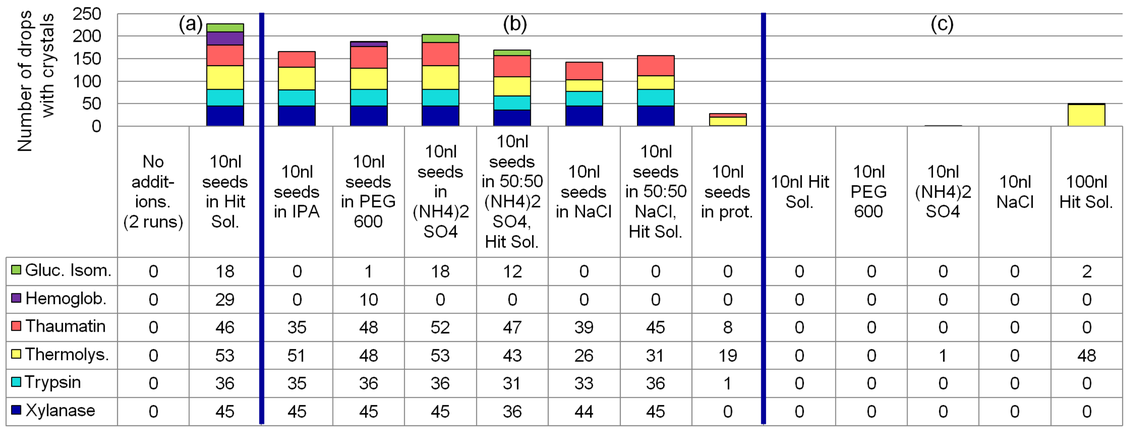
Proteins used

Protein	Source	Concentration
Glucose Isomerase	Hampton Research	33 mg/ml
Hemoglobin	Sigma Aldrich	60 mg/ml
Thaumatin	Sigma Aldrich	30 mg/ml
Thermolysin	Sigma Aldrich	15 mg/ml
Trypsin	Sigma Aldrich	30 mg/ml
Xylanase	Macro Crystal	36 mg/ml

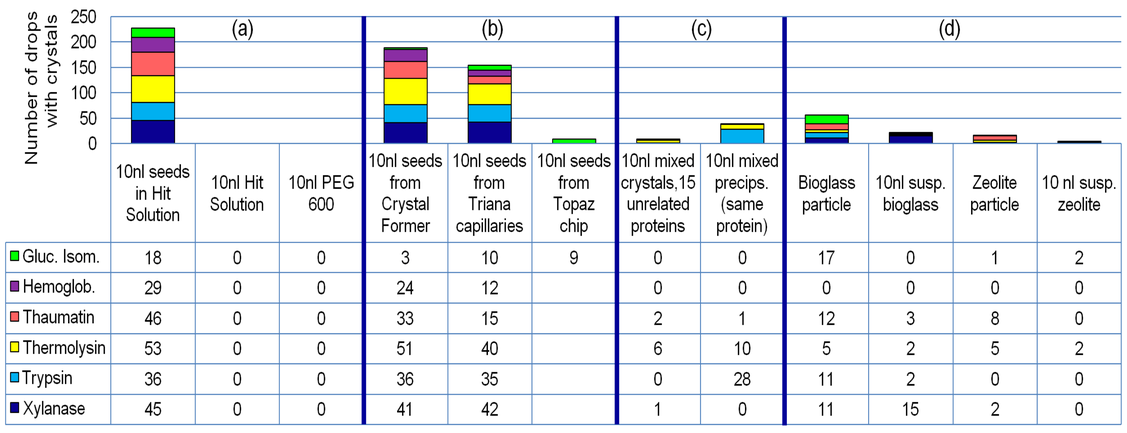
"Receptive Conditions" *(below) were identified where:*
(1) crystals don't grow without seeds in four drops, but
(2) crystals grow in at least three out of four drops with seeds.

1	Glucose Isomerase	JCSG+	2-2	2 M (NH4)2SO4, 0.2 M NaCl, 0.1 M Na MES, PH 6.5
2	**Glucose Isomerase**	**JCSG+**	**2-43**	**25%(w/v) PEG 3350, 0.2 M (NH4)2SO4, 0.1 M bis-tris**
3	Hemoglobin	JCSG+	2-25	30%(w/v) Jeffamine ED-2001, 0.1 M Na HEPES, PH 7.0
4	**Hemoglobin**	**JCSG+**	**2-33**	**30%(w/v) PEG 2000 MME, K thiocyanate**
5	Hemoglobin	JCSG+	2-34	30%(w/v) PEG 2000 MME, K bromide
6	Hemoglobin	JCSG+	2-44	25%(w/v) PEG 3350, 0.2 M NaCl, 0.1 M bis-tris, PH 5.5
7	Thaumatin	Structure screen 1	7	30%(w/v) PEG 4K, 0.2 M ammonium acetate, 0.1M Na citrate, PH 5.6
8	Thaumatin	Structure screen 1	9	20%(v/v) IPA, 20%(w/v) PEG 4K, 0.1 M Na citrate, PH 5.6
9	Thaumatin	Structure screen 1	14	30%(w/v) PEG 8K, 0.2 M (NH4)2SO4, 0.1 M Na cacodylate, PH 6.5
10	Thaumatin	Structure screen 1	15	20%(w/v) PEG 8K, 0.2M magnesium acetate, 0.1 M Na cacodylate, PH6.5
11	Thaumatin	Structure screen 1	32	2 M (NH4)2SO4, 0.1 M tris, PH 8.5
12	**Thaumatin**	**Jena Bioscience Membrane screen3**	**D5**	**1.5 M Li2SO4, 0.1 M Na HEPES, PH 7.5**
13	Thermolysin	JCSG+ (2:1 water)	1-2	20%(w/v) PEG 3K, 0.1 M Na citrate, PH 5.5
14	Thermolysin	JCSG+ (2:1 water)	1-21	20%(w/v) PEG 6k, 0.1 M citric acid, PH 5.0
15	Thermolysin	JCSG+ (2:1 water)	2-18	10%(v/v) MPD, 0.1 M bicine, PH 9.0
16	**Thermolysin**	**JCSG+ (2:1 water)**	**2-19**	**0.8 M succinic acid, PH 7.0**
17	Thermolysin	JCSG+ (2:1 water)	2-21	2.4 M Na malonate, PH 7.0
18	Thermolysin	JCSG+ (2:1 water)	2-22	0.5%(w/v) Jeffamine ED-2001, 1.1 M Na malonate, 0.1 M Na HEPES, PH 7.0
19	**Trypsin**	**Jena Bioscience Membrane screen3**	**D3**	**1.5 M NaCl, 0.1M Na acetate, PH 4.6**
20	Trypsin	Jena Bioscience Membrane screen3	D3	1.5 M NaCl, 0.1M Na acetate, PH 4.6
21	Trypsin	Jena Bioscience Membrane screen3	D6	2 M NaCl, 0.1 M Na citrate
22	Trypsin	Jena Bioscience Membrane screen3	D6	2 M NaCl, 0.1 M Na citrate
23	Xylanase	Structure screen 1	32	2 M (NH4)2SO4, 0.1 M tris, PH 8.5
24	Xylanase	Structure screen 1	37	30%(w/v) PEG 4K, 0.2 M Na acetate, 0.1 M tris, PH 8.5
25	**Xylanase**	**Structure screen 1**	**45**	**4 M Na formate**
26	Xylanase	Jena Bioscience Membrane screen3	B5	3.5 M (NH4)2SO4, 0.25M NaCl, 50mM Na/K phosphate, PH 7.5
27	Xylanase	Jena Bioscience Membrane screen3	D4	1.5 M K phosphate, PH 7.0

Results



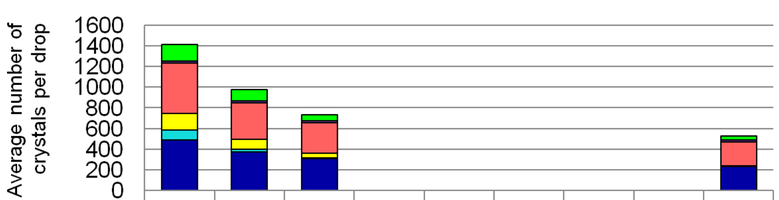
	No addit-ions. (2 runs)	10nl seeds in Hit Sol.	10nl seeds in IPA	10nl seeds in PEG 600	10nl seeds in (NH4)2 SO4	10nl seeds in 50:50 (NH4)2 SO4, Hit Sol.	10nl seeds in NaCl	10nl seeds in 50:50 NaCl, Hit Sol.	10nl seeds in prot.	10nl Hit Sol.	10nl PEG 600	10nl (NH4)2 SO4	10nl NaCl	100nl Hit Sol.
Gluc. Isom.	0	18	0	1	18	12	0	0	0	0	0	0	0	2
Hemoglob.	0	29	0	10	0	0	0	0	0	0	0	0	0	0
Thaumatin	0	46	35	48	52	47	39	45	8	0	0	0	0	0
Thermolys.	0	53	51	48	53	43	26	31	19	0	0	1	0	48
Trypsin	0	36	35	36	36	31	33	36	1	0	0	0	0	0
Xylanase	0	45	45	45	45	36	44	45	0	0	0	0	0	0



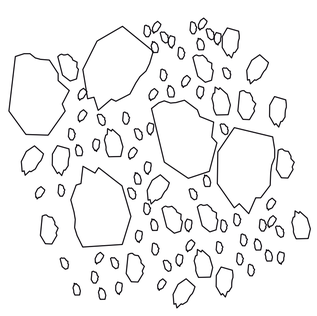
	10nl seeds in Hit Solution	10nl Hit Solution	10nl PEG 600	10nl seeds from Crystal Former	10nl seeds from Triana capillaries	10nl seeds from Topaz chip	10nl mixed crystals,15 unrelated proteins	10nl mixed precips. (same protein)	Bioglass particle	10nl susp. bioglass	Zeolite particle	10 nl susp. zeolite
Gluc. Isom.	18	0	0	3	10	9	0	0	17	0	1	2
Hemoglob.	29	0	0	24	12		0	0	0	0	0	0
Thaumatin	46	0	0	33	15		2	1	12	3	8	0
Thermolysin	53	0	0	51	40		6	10	5	2	5	2
Trypsin	36	0	0	36	35		0	28	11	2	0	0
Xylanase	45	0	0	41	42		1	0	11	15	2	0

Protein	Crystals in Hit Sol.	Crystals in Isopropanol	Crystals in PEG 600	Crystals in Amm.sul.	Crystals in NaCl	Crystals in protein stock
Gluc. Isom.	**OK**	Cracked	Shattered	**Cracked**	Dissolved	Dissolved
Hemoglobin	**OK**	Cracked	**OK**	Dissolved	Dissolved	Dissolved
Thaumatin	**OK**	**Cracked**	**OK**	**OK**	**OK**	**Grew**
Thermolysin	**OK**	**OK**	**Shattered**	**OK**	**Dissolved**	**Grew**
Trypsin	**OK**	**OK**	**Dissolved**	**OK**	**OK**	Dissolved
Xylanase	**OK**	**OK**	**Cracked**	**OK**	**OK**	Dissolved

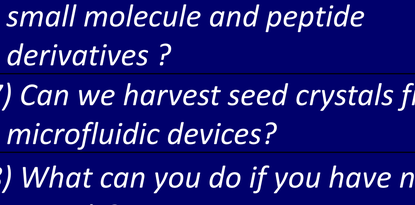
To investigate seed stability, seed stock in "hit solution" was incubated at room temp. for 0, 3, 6 and 24 hours



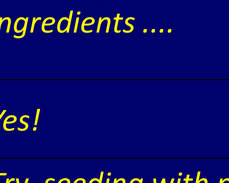
	0 hrs	3 hrs	6 hrs	9 hrs	12 hrs	15 hrs	18 hrs	21 hrs	24 hrs
Gluc. isom.	166	113	63						36
Hemoglob.	19	18	19						20
Thaumatin	489	357	293						228
Thermolysin	158	96	46						11
Trypsin	95	23	6.0						0.1
Xylanase	490	372	309						229

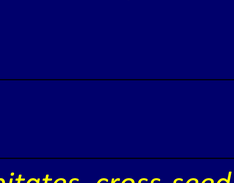


Seeds from microfluidic devices



Precipitates, cross-seeding, heterogenous nucleants,





Conclusions

Our questions:	*Take-home practical suggestions:*
(1) How can we get as many hits as possible?	*Stick to the 'hit solution' for suspending seed crystals for routine rMMS*
(2) How stable are the seed stocks?	*Not completely stable so use your seed stock quickly, then freeze. Or cross-link.*
(3) Is "preseeding" the protein stock helpful?	*Not so good! (Better than nothing. It's free!)*
(4) How can we avoid salt crystals?	*Suspend the seed crystals in PEG or NaCl (test by incubation)*
(5) How can we get more diverse crystals?	*Remove ingredients that you suspect may be interacting with your protein from the seed stock*
(6) How can we stabilize protein complexes, including heavy atom, small molecule and peptide derivatives ?	*Avoid high salt in your seed stock; remove ingredients ....*
(7) Can we harvest seed crystals from microfluidic devices?	*Yes!*
(8) What can you do if you have no crystals?	*Try seeding with precipitates, cross-seeding with mixed crystals, or bioglasss*