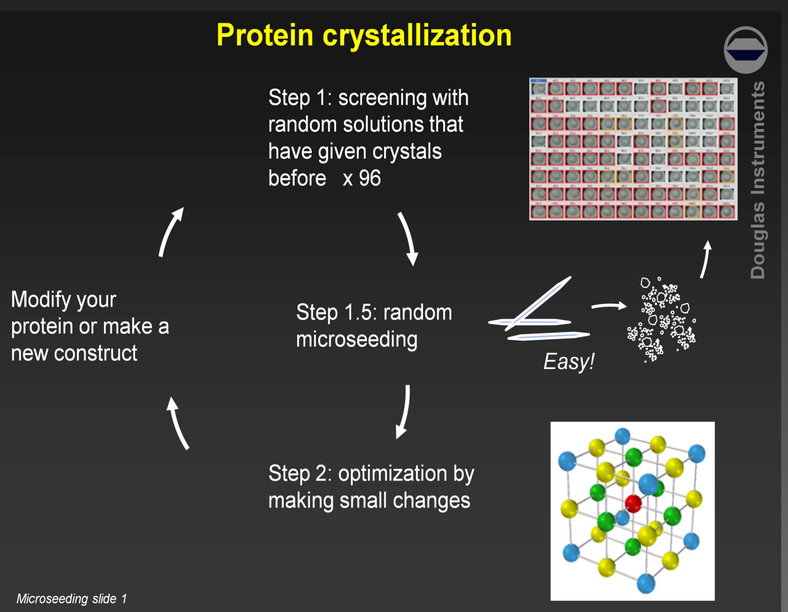
Understanding “random” MMS microseeding – how new strategies can improve productivity

(A review of published work including our own)

Patrick Shaw Stewart, Stefan Kolek, Richard Briggs, Peter Baldock, Douglas Instruments Limited, near Oxford, UK:

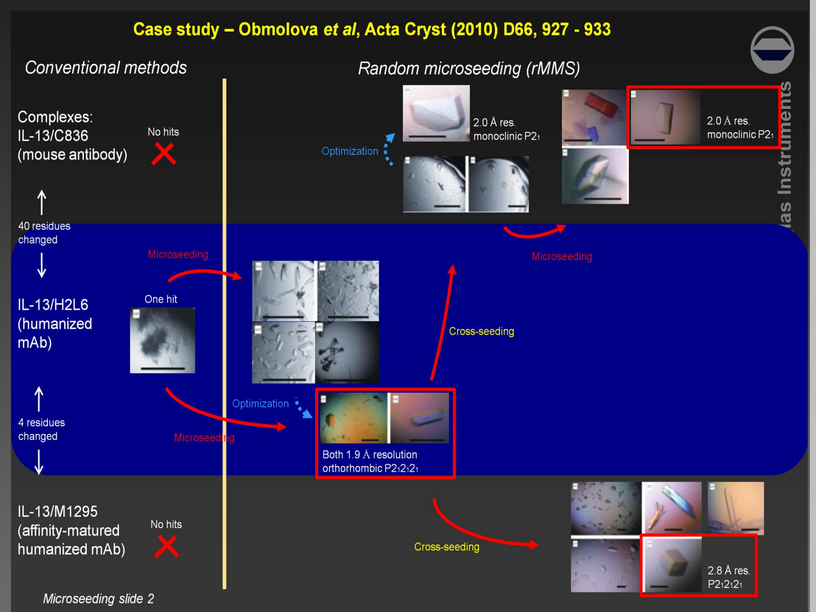
Introduction to “random” microseeding

Protein crystallization

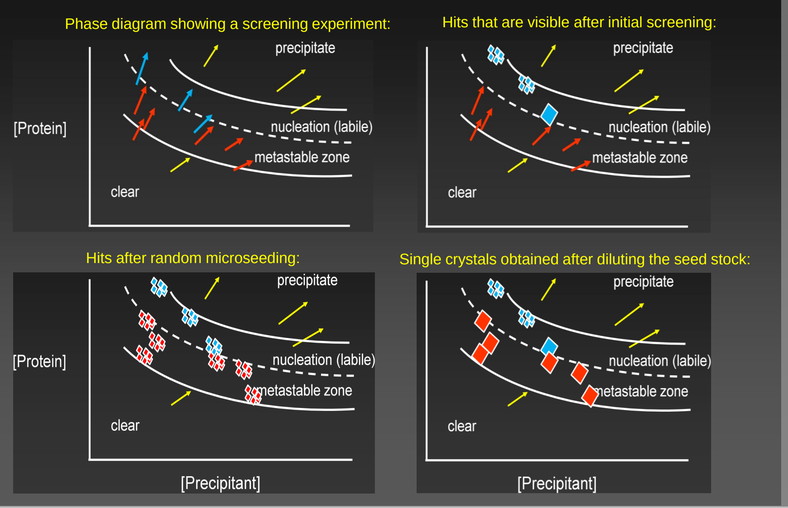


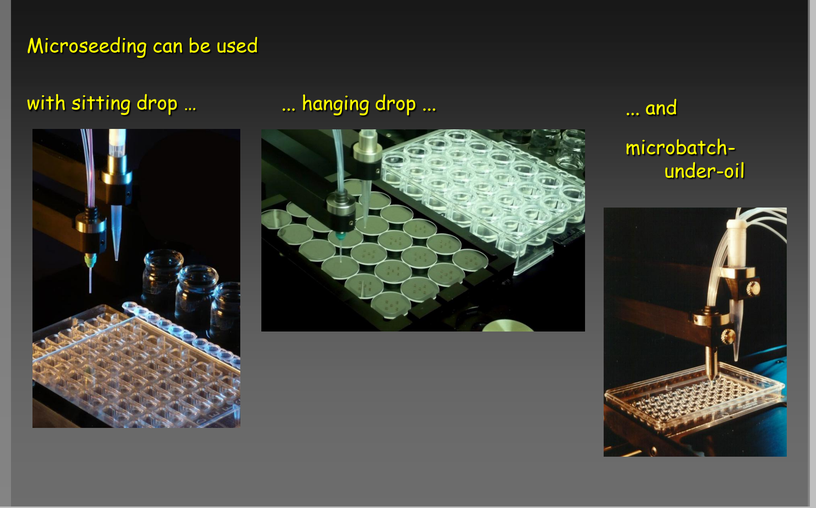
Microseeding slide 1

Case study – Obmolova *et al*, Acta Cryst (2010) D66, 927 - 933



Microseeding slide 2

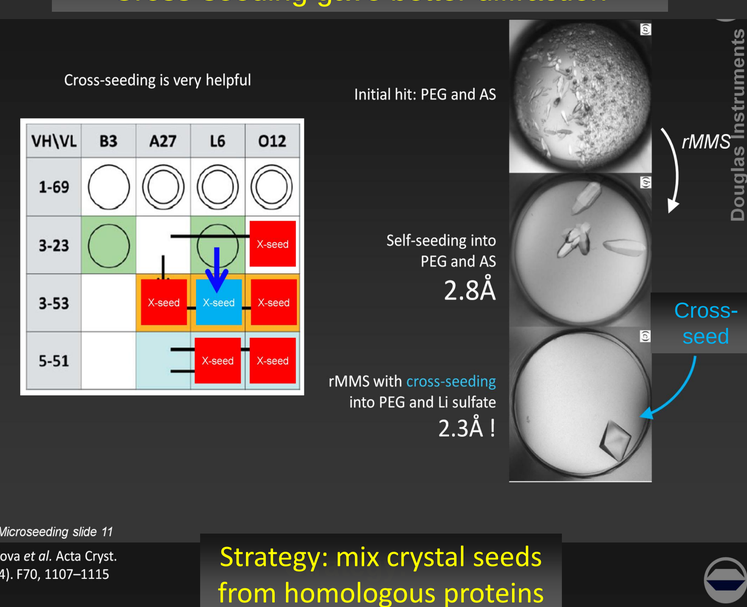




CASE STUDY

Obmolova, Galina, et al. "Protein crystallization with microseed matrix screening: application to human germline antibody Fabs." *Acta Cryst. F* 70.8 (2014): 1107-1115.

Cross-seeding gave better diffraction



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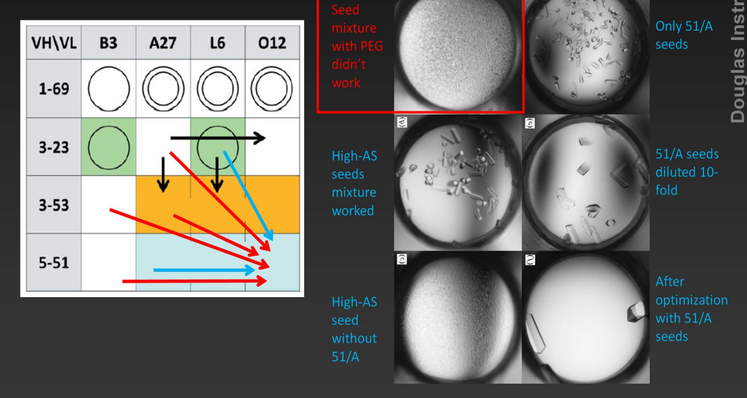
Obolova *et al.* Acta Cryst. (2014). F70, 1107–1115

Strategy: mix crystal seeds from homologous proteins



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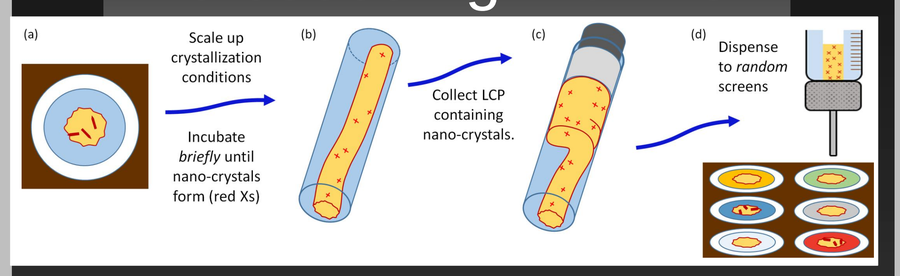
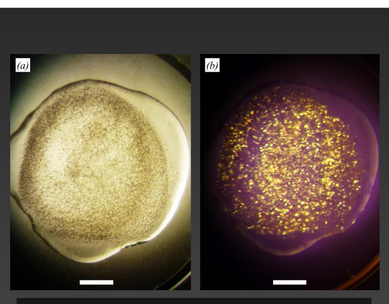
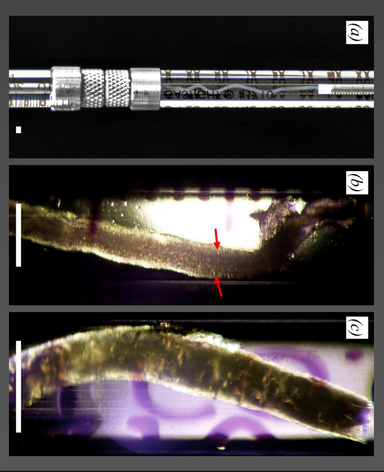
Microseeding with LCP



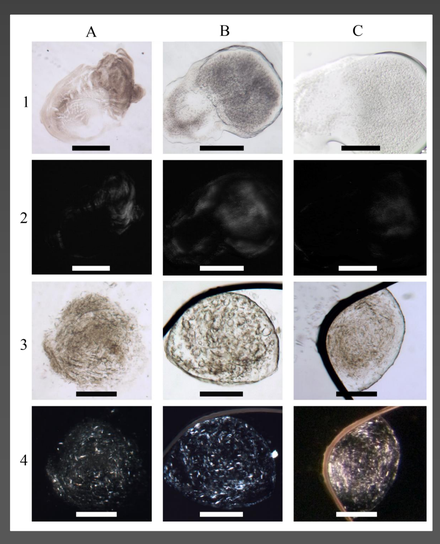


The seed conditions: OmpF in LCP with 1.1 M KSCN, 2.1 M LiNO3, 0.1 NaOAc pH 4.6

Screen	Cond	Main precipitant	Without seeding Count	Comment	With seeding Count	Comment
JCSG+	17	40% MPD	0		1	Small crystals
JCSG+	18	40% ethanol	0		1	Small crystals
JCSG+	22	50% PEG 200	0		1	Very small crystals
JCSG+	30	40% PEG 300	0		1	Very small crystals
JCSG+	43	40% PEG 400	0		1	Very small crystals
JCSG+	53	40% MPD	0		1	Small crystals
JCSG+	64	20% Jeffamine	1	Very small crystals	0	
JCSG+	66	10% MPD	0		1	Very small crystals
Memb 1	5	48% PEG 400	0		1	Very small crystals
Total			1		8	



Top: the appearance of the LCP in the syringe.
Middle : LCP with small crystals, ready to use.
Bottom: crystals have grown too large to be used.

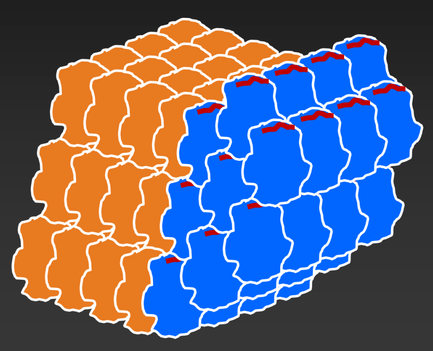


Results: new crystallization conditions found.
Top: no seed stock added
Bottom: with seed stock

Crystallizing complexes – cross-seed with crystals of one component

Cross-seeding

You don't have to match the unit cell, only one of the structural planes of the crystals



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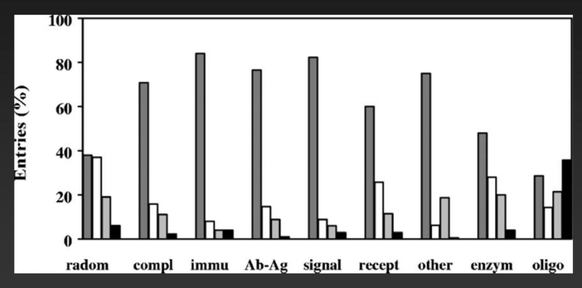
Crystallizing complexes

Radaev and Sun. Crystallization of protein-protein complexes. J. Appl. Cryst. (2002). 35, 674-676



- PEG / (NH4)2SO4 / other salts / organic solvents (including 2-propanol, MPD, ethanol)

Random uncomplexed samples, all *protein-protein complexes* included in this survey, immune complexes, antibody-antigen complexes, signal transduction complexes, receptor and ligand complexes, miscellaneous protein-protein complexes, enzyme related complexes, *oligomeric protein complexes*

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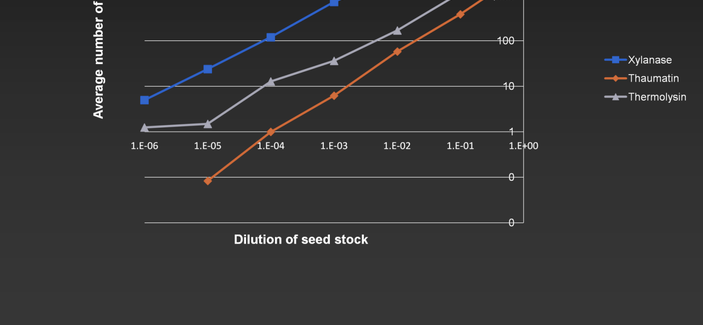
Focusing on “receptive” conditions



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Appearance of uncrushed crystals after incubation for one day

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

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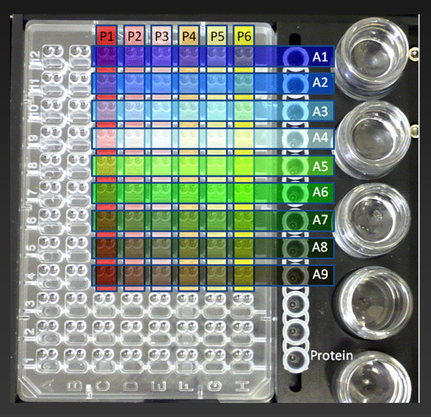
Experimental design for finding the right dilution of the seed stock

- If solutions to be optimized are arranged in columns up to 8 hits can be optimized in one experiment/plate
- 10^{-3} to 10^{-6} dilutions were required for 3 model proteins

New “combinatorial” experimental design



Microseeding:

A1: 100% seed stock
A2: 25% seed stock
A3: 6.3% seed stock
A4: 1.6% seed stock
A5: 0.4% seed stock
A6: 0.1% seed stock
A7: 0.02% seed stock
A8: 0.006% seed stock
A9: 0% seed stock (control)

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New “combinatorial” experimental design

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- “Additive Scatter” script can be used with 2-d grids
- Seed stocks vary orthogonally
- Up to 5 seed dilutions per plate
- Any size block can be filled