



Microseed it!

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Douglas Instruments Limited (near Oxford, UK):

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- Contact dispensing allows microseeding
- Almost no protein is wasted
- The same system can be used for small and large drops
 - 2-d grid
 - 7-d Central Composite etc
- Optimization

Feature chart

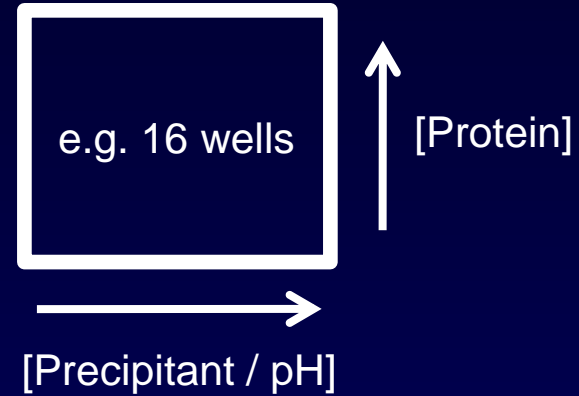


Feature	<u>OryxNano</u>	<u>Oryx4</u>	<u>Oryx8</u>
Vapor Diffusion Drops	✓	✓	✓
Vapor Diffusion Multiple drops per reservoir single Protein	✓	✓	✓
Vapor Diffusion Multiple drops per reservoir 2 Proteins	✓	✓	✓
Vapor Diffusion Multiple drops per reservoir 3 Proteins	✓		✓
Vapor Diffusion Microseed Matrix Screening	✓	✓	✓
Vapor Diffusion Additive Experiments		✓	✓
Microbatch with automatic oiling		✓	✓
Microbatch Additive experiments		✓	✓
Microbatch Microseed Matrix Screening		✓	✓
Optimization			✓
Quick and easy 2-D grid, 3 ingredients	✓	✓	✓
Quick and easy 2-D grid, 4 ingredients	✓		✓
7-D optimization grids			✓
Central Composite, multi-variate experimental designs (7-channel)			✓
Tools			
Microlytic Crystal Forma filling		✓	✓
Fluidigm filling		✓	✓

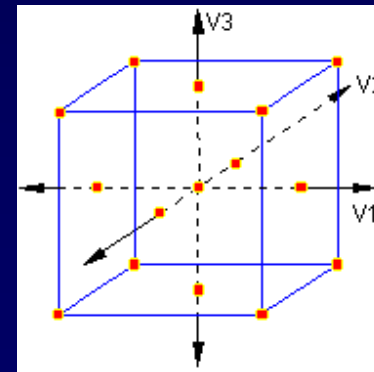


Experimental Design for Optimization

Quick and easy – 2d grid



More powerful – 7d multivariate designs



Xstep Optimization



XSTEP Optimization - [C:\Crystallization Experiments\Optimizations\ProteinX.xdb]

File Edit View Experiment Tools Mark Execute Configuration Window Help

Not Connected

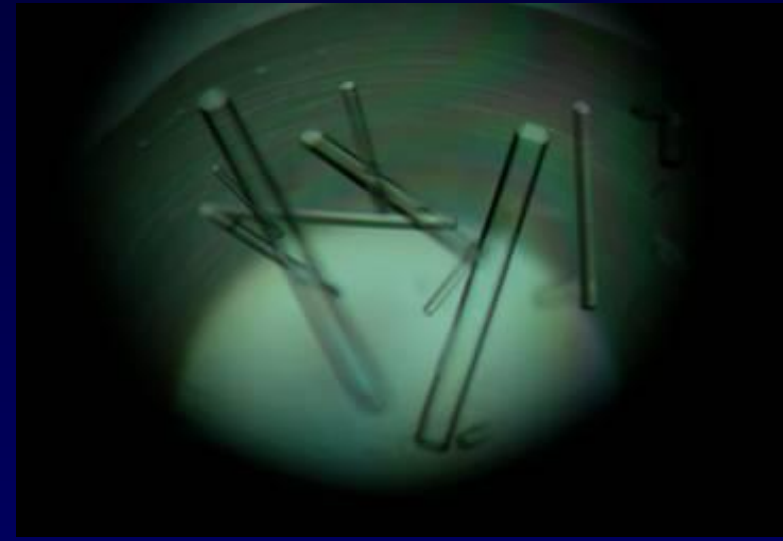
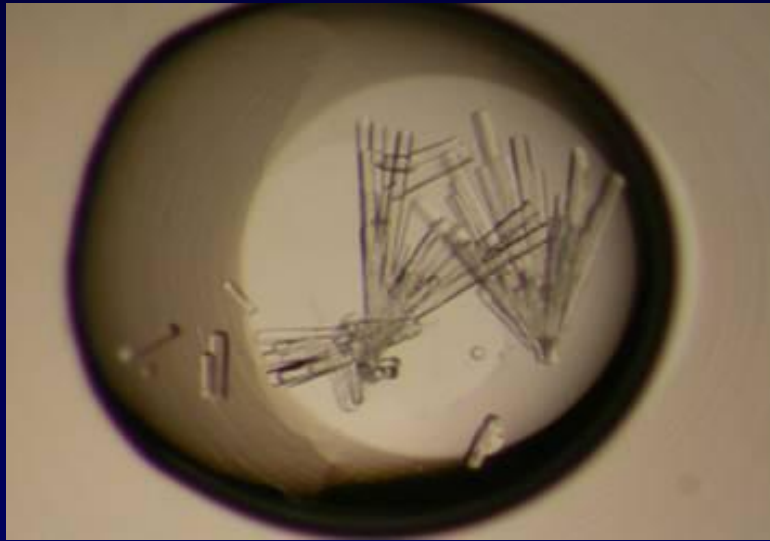
Small Cacodylate 2D screen 2

8 mg/ml ProteinX 50 % PEG 8000 2 M Na-formate 1 M cacodylate pH 6.5	1A 3.08 14.88 0.03 0.00	1B 3.08 14.88 0.03 0.10	1C 3.08 14.88 0.07 0.00	1D 3.08 14.88 0.07 0.10	1E 3.08 18.13 0.03 0.00	1F 3.08 18.13 0.03 0.00
8 mg/ml ProteinX 50 % PEG 8000 2 M Na-formate 1 M cacodylate pH 6.5	2A 3.08 18.13 0.07 0.00	2B 3.08 18.13 0.07 0.10	2C 4.60 14.88 0.03 0.00	2D 4.60 14.88 0.03 0.10	2E 4.60 14.88 0.07 0.00	2F 4.60 14.88 0.07 0.00
8 mg/ml ProteinX 50 % PEG 8000 2 M Na-formate 1 M cacodylate pH 6.5	3A 4.60 18.13 0.03 0.00	3B 4.38 17.25 0.03 0.09	3C 4.60 18.13 0.07 0.00	3D 4.28 16.88 0.07 0.09	3E 3.08 16.50 0.05 0.05	3F 3.08 14.88 0.05 0.05
8 mg/ml ProteinX 50 % PEG 8000 2 M Na-formate 1 M cacodylate pH 6.5	4A 3.84 16.50 0.03 0.05	4B 3.84 16.50 0.05 0.00	4C 3.84 16.50 0.05 0.10	4D 3.84 16.50 0.07 0.05	4E 3.84 18.13 0.05 0.05	4F 4.60 16.50 0.05 0.05
8 mg/ml ProteinX 50 % PEG 8000 2 M Na-formate 1 M cacodylate pH 6.5	5A 3.84 16.50 0.05 0.05	5B 3.84 16.50 0.05 0.05	5C 3.84 16.50 0.05 0.05	5D 0.00 0.00 0.00 0.00	5E 0.00 0.00 0.00 0.00	5F 0.00 0.00 0.00 0.00
8 mg/ml ProteinX 50 % PEG 8000 2 M Na-formate 1 M cacodylate pH 6.5	6A 0.00 0.00 0.00 0.00	6B 0.00 0.00 0.00 0.00	6C 0.00 0.00 0.00 0.00	6D 0.00 0.00 0.00 0.00	6E 0.00 0.00 0.00 0.00	6F 0.00 0.00 0.00 0.00
8 mg/ml ProteinX 50 % PEG 8000 2 M Na-formate 1 M cacodylate pH 6.5	7A 0.00 0.00 0.00 0.00	7B 0.00 0.00 0.00 0.00	7C 0.00 0.00 0.00 0.00	7D 0.00 0.00 0.00 0.00	7E 0.00 0.00 0.00 0.00	7F 0.00 0.00 0.00 0.00

Nunc_HLA | 5 Channel | Additive | Concentration Mode | Drop: 2.12µl | Oct 19 2001 @ 23:51 | Starting point based on Additive Design

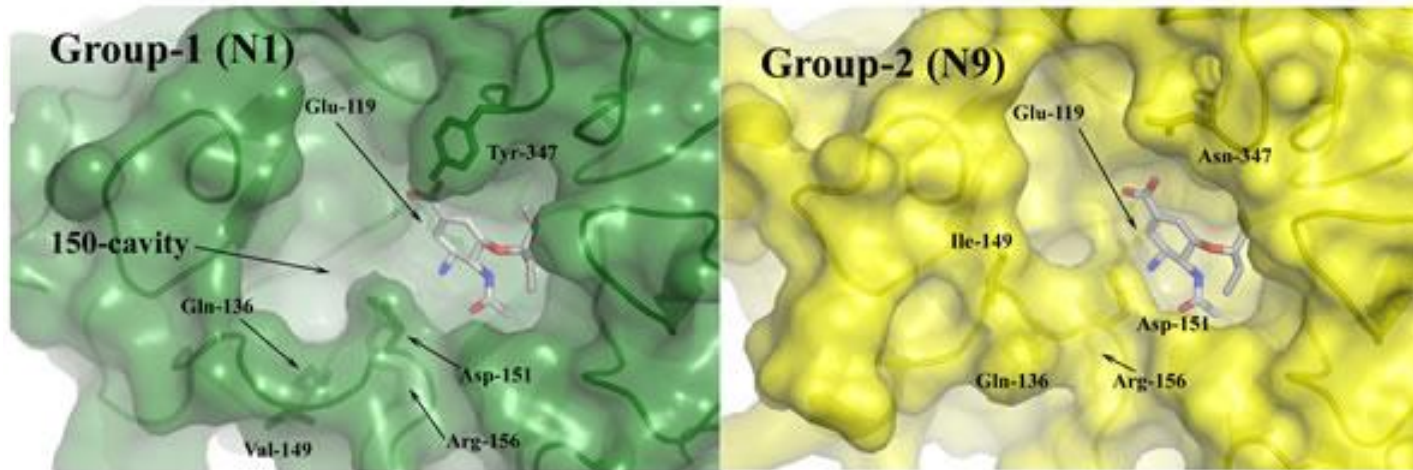
Microbatch-under-oil

NTD N-tropic MLV- capsid protein



G. B. Mortuza, L. F. Haire, A. Stevens, S. J. Smerdon, J. P. Stoye & I. A. Taylor. *Nature* (2004) 431 481-485.

Structure of N1 neuraminidase from H5N1 avian flu virus



The active site of the N1 neuraminidase enzyme (left) contains an additional cavity missing from some other forms.

Russell R. J., *et al. Nature*, vol 443 Sept 7, 2006 pp45-49

Structure solved with Oryx8 with around 500 μg of protein

random Microseed Matrix-Screening (rMMS)



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

1. Add seed crystals to a random screen
2. Suspend crushed crystals in the reservoir solution that gave the hits used ("hit solution")
3. Automate!

To get:

(1) more hits

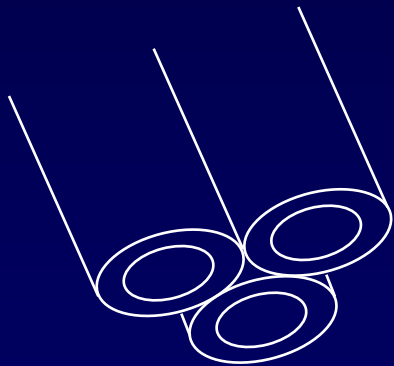
(2) better crystals



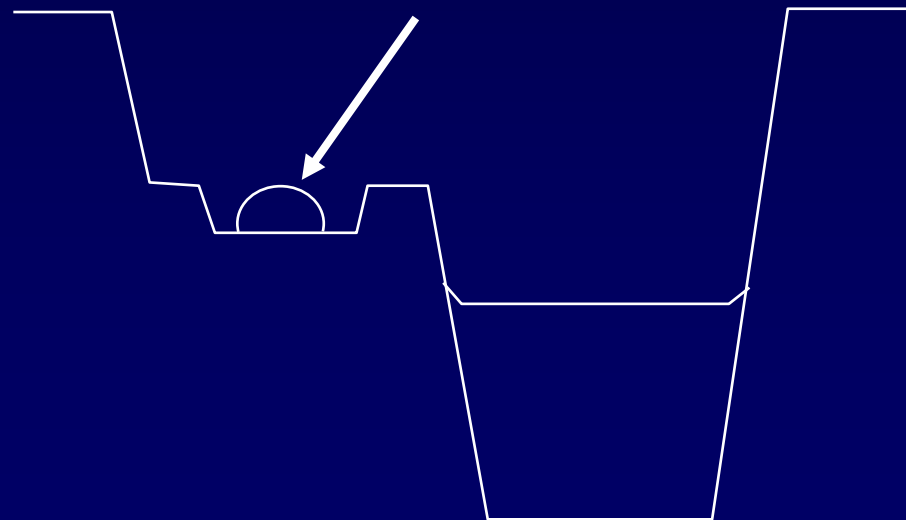
Microseeding in *screening* experiments

Allan D'Arcy
Novartis, Basle
2006 'Matrix-seeding script'

3-bore tip



1. protein
2. reservoir solution

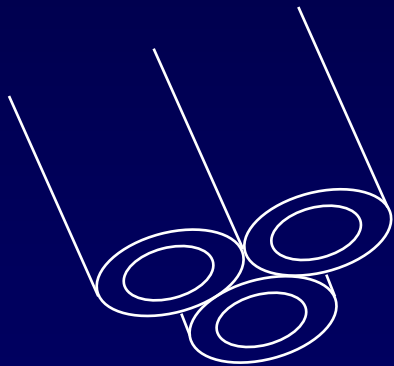




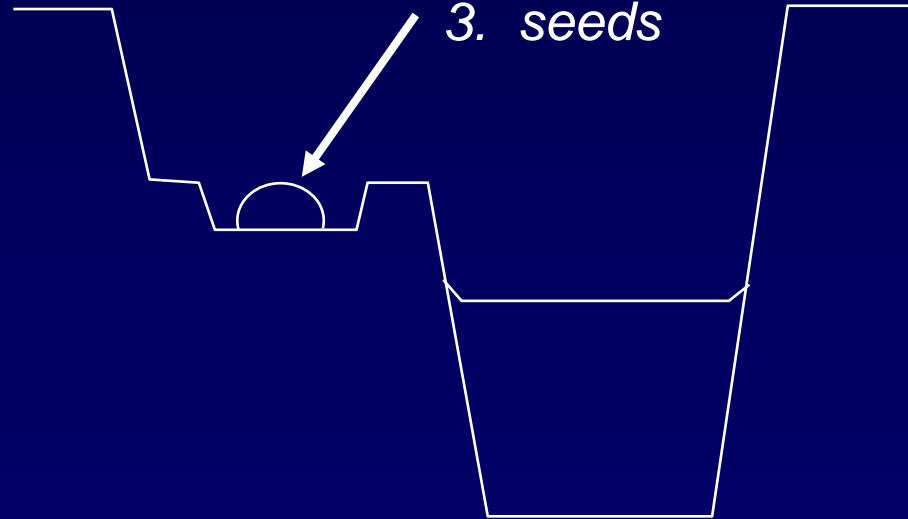
Microseeding in *screening* experiments

Allan D'Arcy
Novartis, Basle
2006 'Matrix-seeding script'

3-bore tip



1. protein
2. reservoir solution
3. seeds



Microseeding in screening experiments

Allan D'Arcy, Novartis, Basle. 2006 'Matrix-seeding script'



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The image shows two software windows. The background window is 'XYZV PlateLoader' with a 12x8 grid of wells. The foreground window is 'WaspRun - VD One protein seeding.xpp' with a 'Wells' tab selected. It displays configuration for 'Droplet One'.

Parameter	Value
Execute this drop	<input checked="" type="checkbox"/>
Protein Vol [ul]	0.15
Reservoir Vol [ul]	0.08
Seeding Vol [ul]	0.02
Calculated Protein [%]	60.00
Calculated Reservoir [%]	32.00
Calculated Seeding [%]	8.00
Final Drop Volume Incl. Seeding	0.25

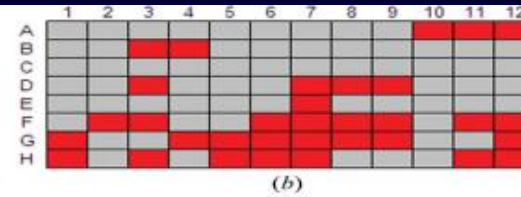
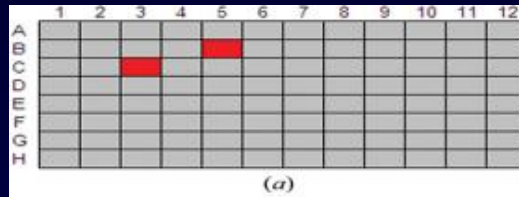
Microseeding in screening experiments



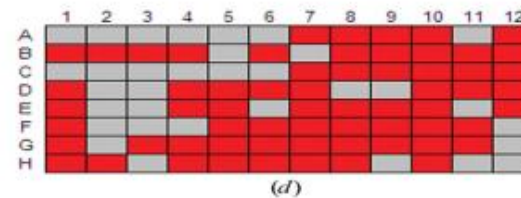
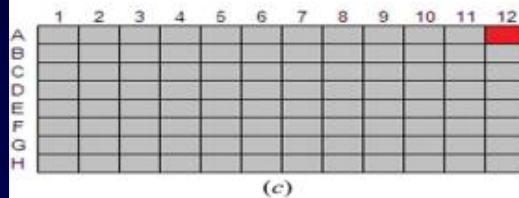
Regular screen

Screen with seeds

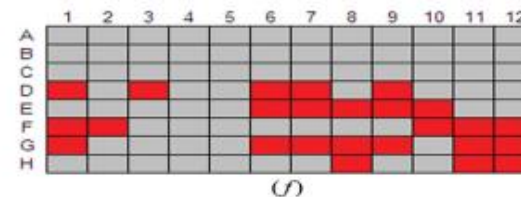
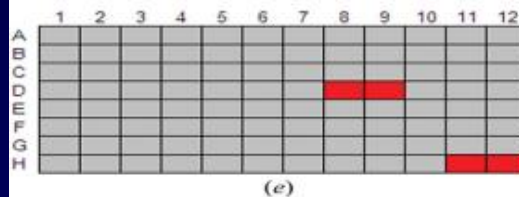
MMP12



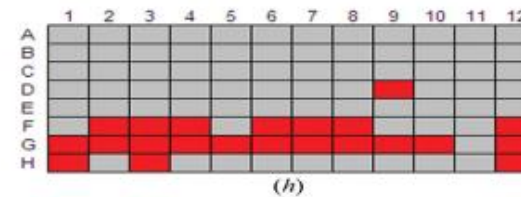
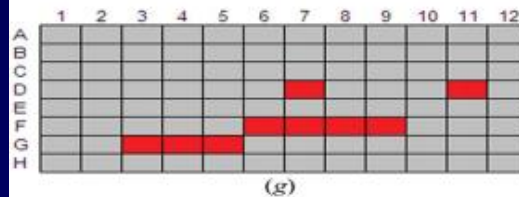
BVP



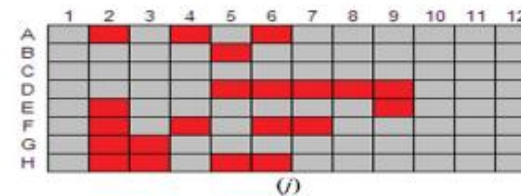
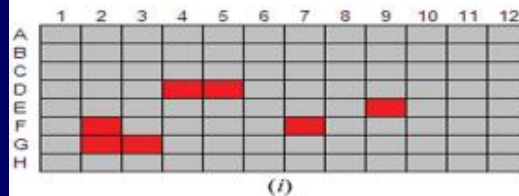
USP7



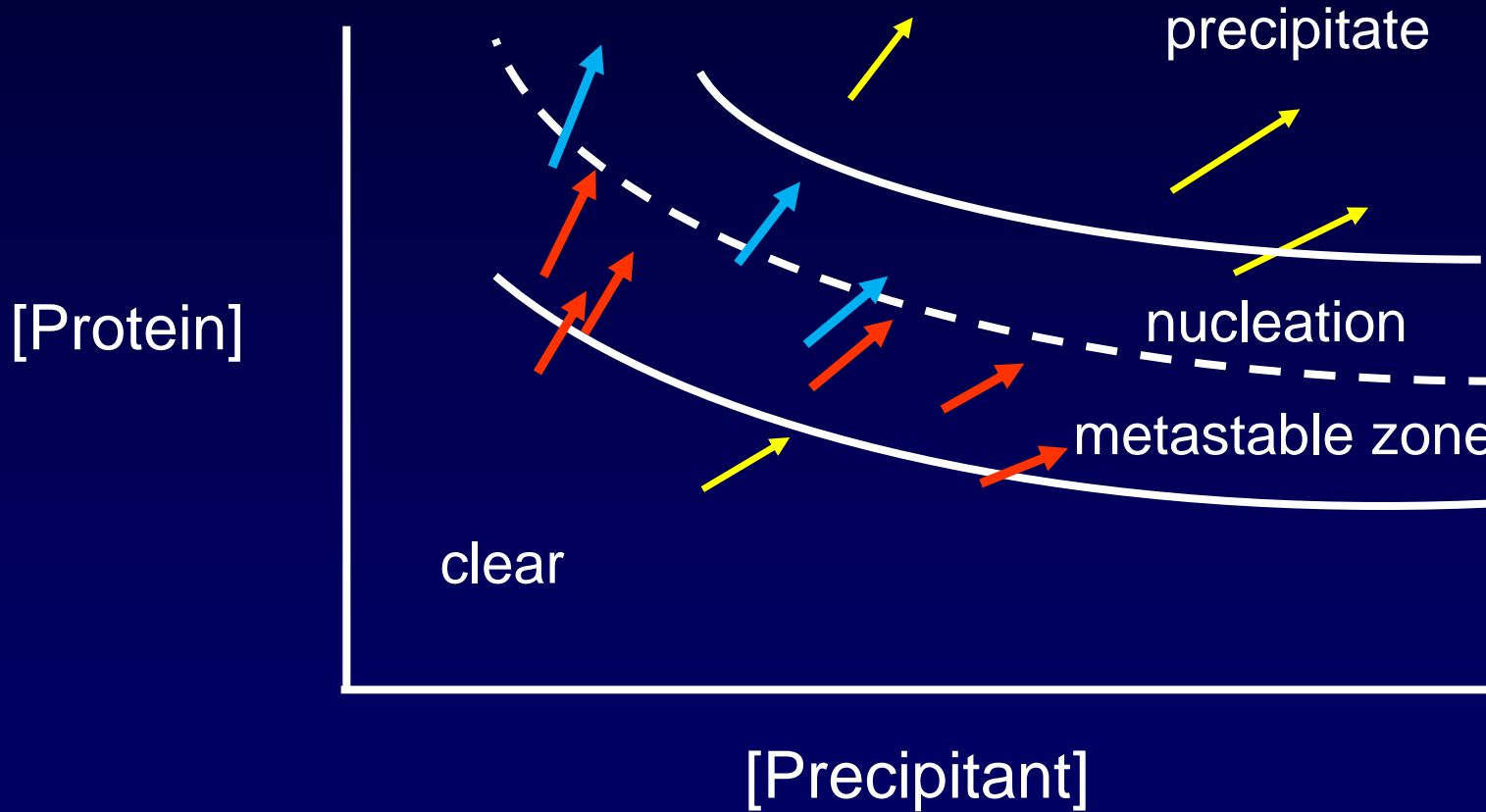
Trypsin



PPE

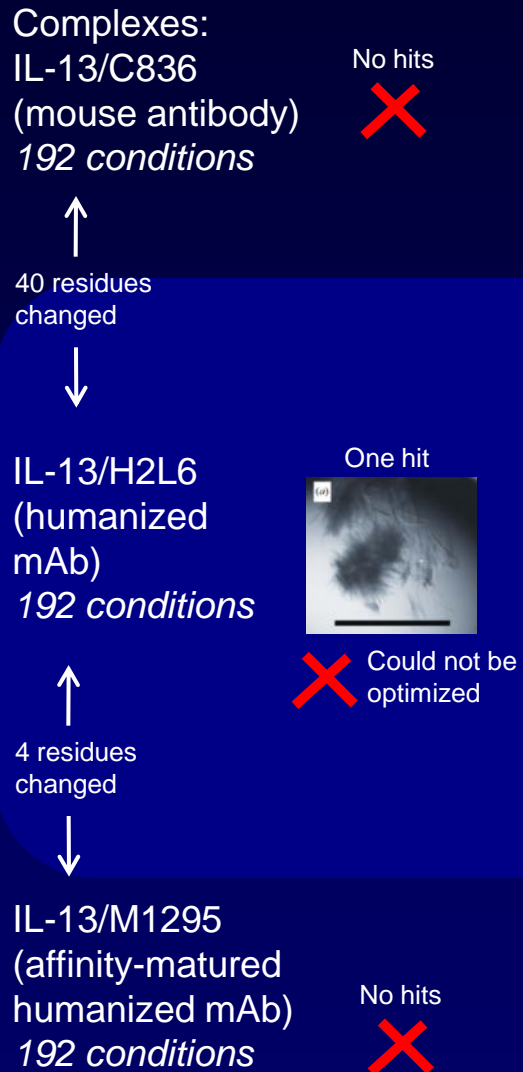


Phase diagram of a protein





Conventional methods





Conventional methods

Random microseeding (rMMS)

Complexes:
IL-13/C836
(mouse antibody)

No hits
X

↑
40 residues changed

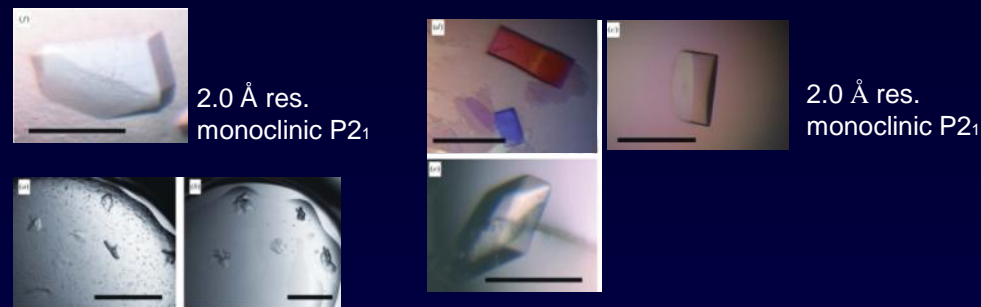
↓
IL-13/H2L6
(humanized mAb)



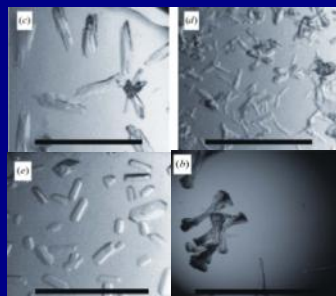
↑
4 residues changed

IL-13/M1295
(affinity-matured humanized mAb)

No hits
X



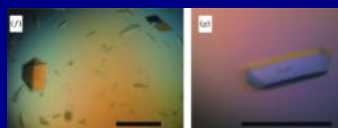
Microseeding



Microseeding

Cross-seeding

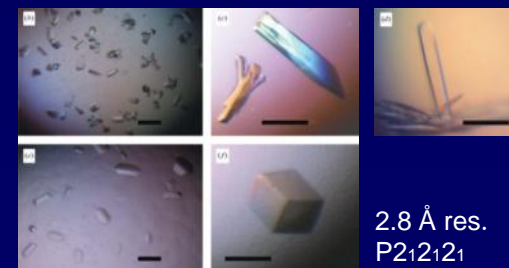
Optimization



Both 1.9 Å resolution
orthorhombic P2₁2₁2₁

Microseeding

Cross-seeding



2.8 Å res.
P2₁2₁2₁



Robotics for rMMS

Contact dispensing is very helpful

Preferably, you should *not* spin your seed stock

Sometimes you have very little seed stock e.g. only one crystal obtained



rMMS with membrane proteins

Crystals of membrane proteins are often unstable

Remember that the reservoir normally has no detergent!

Harvest several large drops *without dilution*

1.5 microlitres are enough!

MPL (Diamond Light Source) / Douglas Instruments: 2 of 5 projects worked very well

Thank you for listening!



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Scaling up



100 + 100 nl



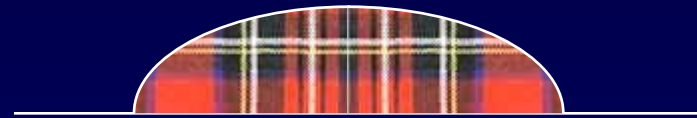
1 + 1 μ l



Scaling up



100 + 100 nl

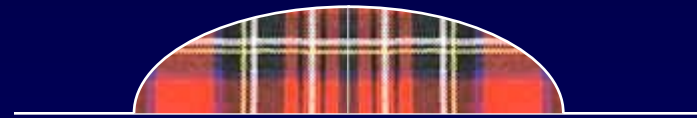


1 + 1 μ l

PRECIPITATION !!



Scaling up



High surface to volume ratio

- More protein is lost at the air/liquid interface
- Equilibration is faster

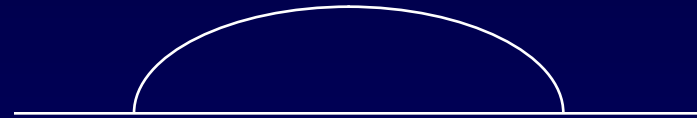
Low surface to volume ratio



Scaling up



Try 200 nl (protein) +
100 nl (reservoir solution)



Scales up to 1 + 1 μ l
(Heather Ringrose, Pfizer)



Scaling up



100 nl (protein) + 100 nl
(reservoir solution)

Equilibrates faster



Scales up to 0.5 + 1 μ l
(Heather Ringrose, Pfizer)

Increase the salt by 50 – 100%