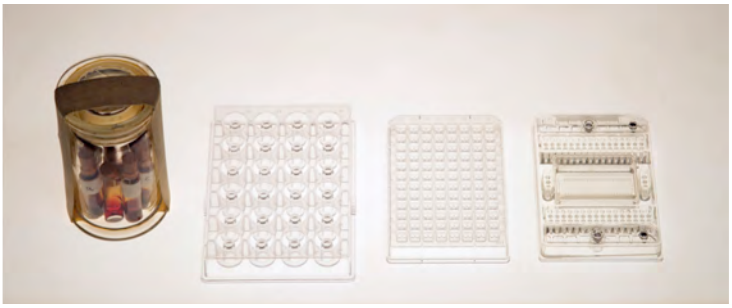
Protein Crystallisation

Fabrice GORREC



Introduction to Biophysical Techniques Lecture Series
5 March 2020

MRC Laboratory of Molecular Biology | STRUCTURAL STUDIES | PROTEIN & NUCLEIC ACID CHEMISTRY | UNIVERSITY OF CAMBRIDGE

1

What is briefly presented in this presentation

Generalities
X-ray crystallography, protein self-assembly, crystal properties, current context

Initial considerations (sample as the main variable)
Molecular Biology and Biophysics

Nucleation and growth
Solubility, supersaturation, nucleation and growth mechanisms

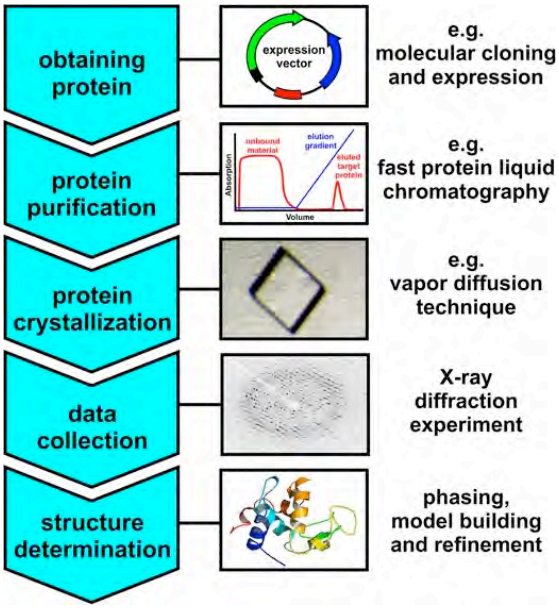
Initial screening in vapor diffusion nanoliter droplets
Technique, phase diagram, automation and miniaturisation, screens, strategy at the LMB

Assessments of experiments
Clear droplets, precipitate, microcrystals, other

Optimisation protocols
The 4-corner method, additive screening and random microseed matrix screening

2

Structure determination with X-ray crystallography



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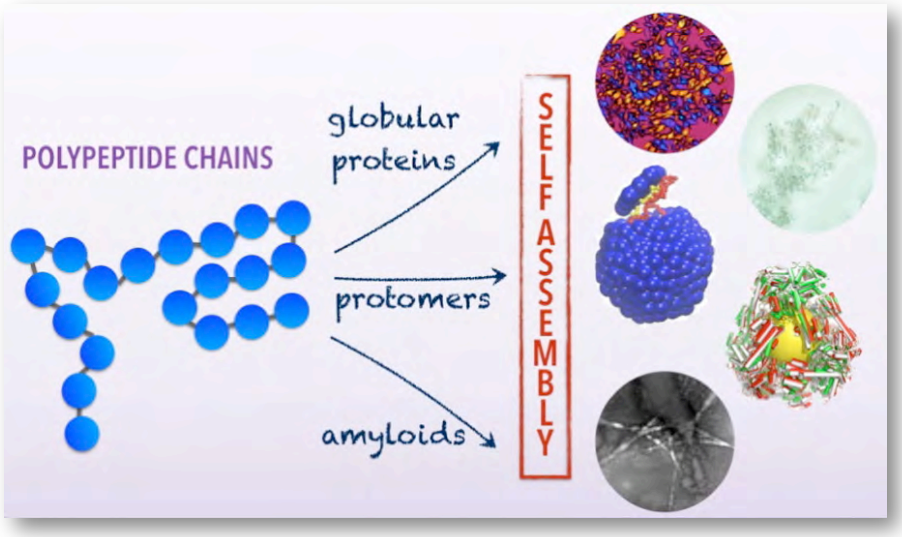
Bijelic & Rompel, ChemTexts (2018)

Physics of protein self-assembly



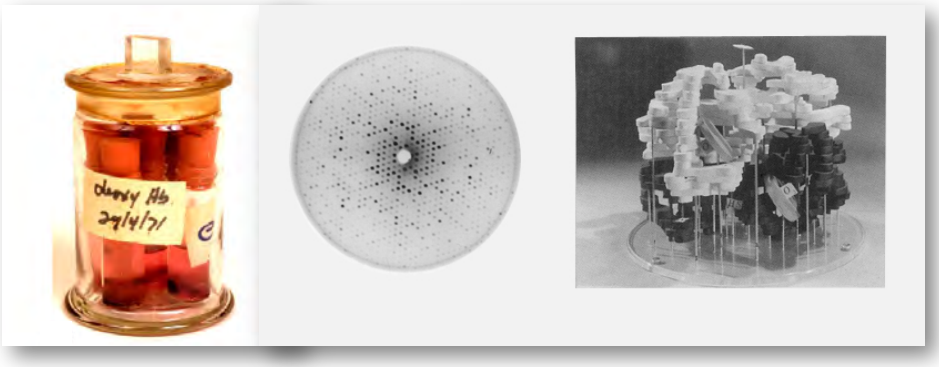
McManus et al., Curr Opin Colloid Interface Sci, 2016

4

While most physiological protein-protein interactions are well characterised, the nature of crystal packing contacts varies on a case by case basis and hence protein crystallisation is approached as a stochastic process.

5

Structure of Haemoglobin (60's – 80's)



Stock *et al.*, Prog Biophys Mol Biol (2015)

6

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Current context

Advanced technology but …
Much more challenging nature of samples

>>> Low yield of useful crystals (“Bottleneck”)

>>> More ways and means required for crystallisation

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Yield of useful crystals

Sample name	MW (kDa)	Conc. (mg/mL)	conditions	hits
Prp8	220	20	1536	2
HIV-capsid domain	36	10	1632	20
SUN	25	15	1632	80
Hexameric HIV-1 capsid	144	27	1632	30
Spliceosomal RNA helicase	240	15	1632	2
mRNA export factor	73	18	1632	1
Autophagy protein	32	12	1728	2
GTPase-Effector comlex	50	10	1820	6
cmd1	16	7	1440	2
MMK	38	13	1440	3
U1 snRNP	120	4	480	2
Centriole protein	20	30	1536	5
Avg.	**85**	**15**	**1512**	**13**

(Gorrec, LMB data, 2015)

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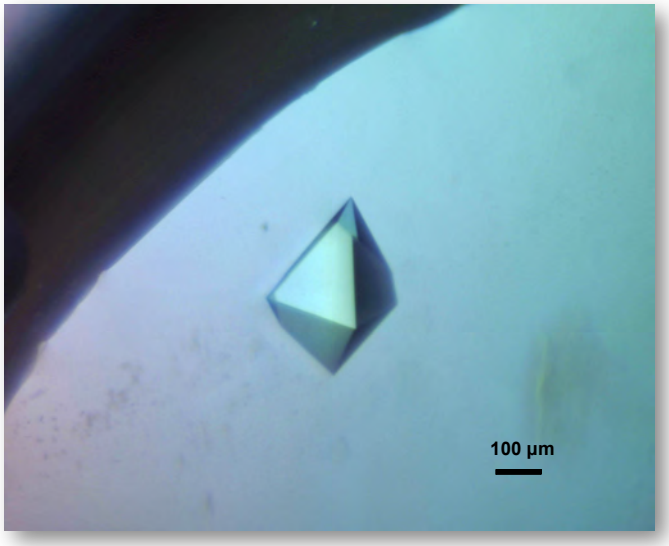
Protein crystals



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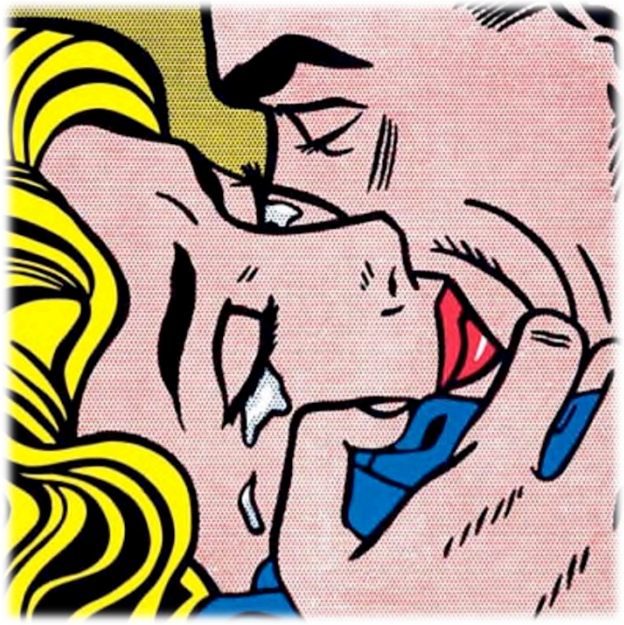

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"Unfavourable" properties

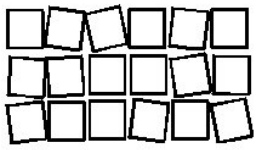
Made of 'flexible material'

Weak interactions and limited contact areas

High solvent content (30-95%)

Impurities

Poorly ordered (high mosaicity)



Highly exaggerated representation of crystal mosaicity

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Initial considerations

(sample as the main variable)

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Make use of…

Nature >>> Different expression systems

Cloning/Mutagenesis >>> More or less drastic alterations

Sequence >>> Comparison/Predictive tools

Literature >>> *e.g.* Stabilizing Partner/Ligand/Additive

Biophysics >>> 'pre-crystallisation' assays, *e.g.* Thermal Shift

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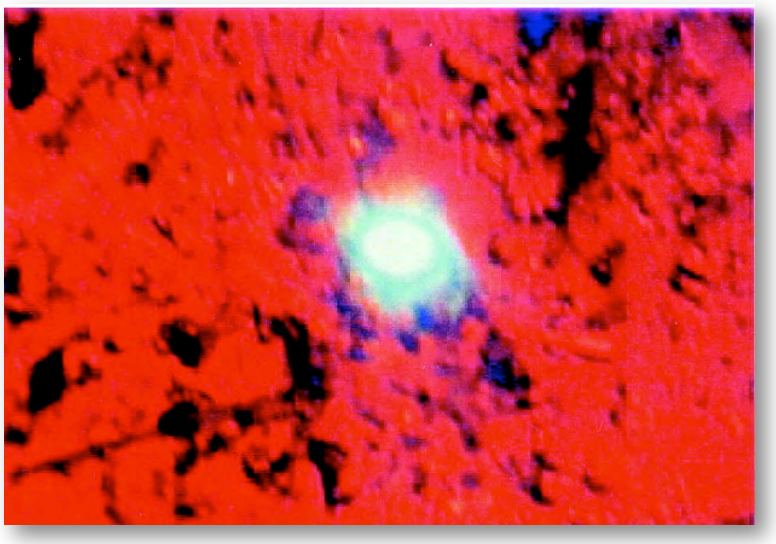
Inherited congenital cataract



Pande *et al.*, PNAS, 2001

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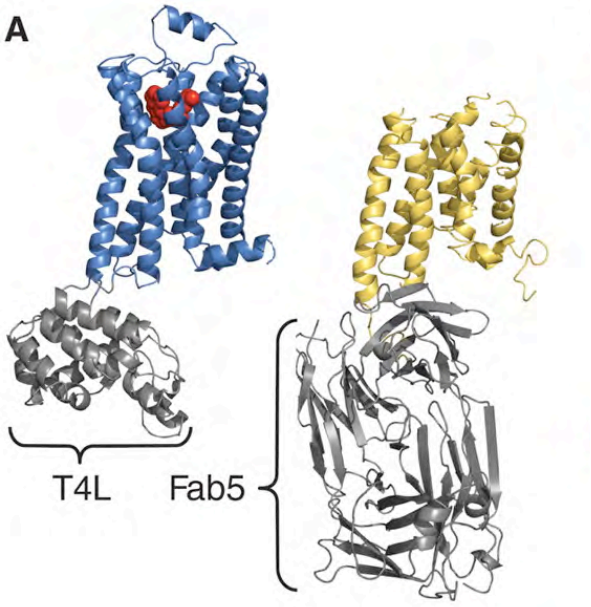
Protein fusion (and chaperone)



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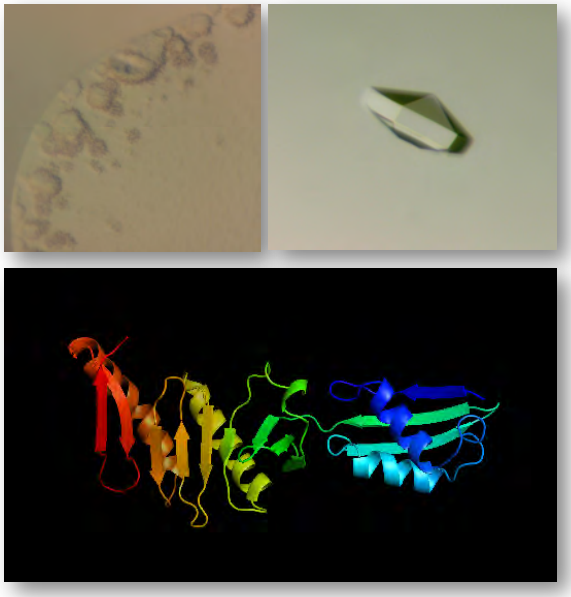
Rosenbaum *et al.* Science, 2007

Multiple constructs



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F. van den Ent *et al.*, Mol Microbiol, (2008)

Make use of…

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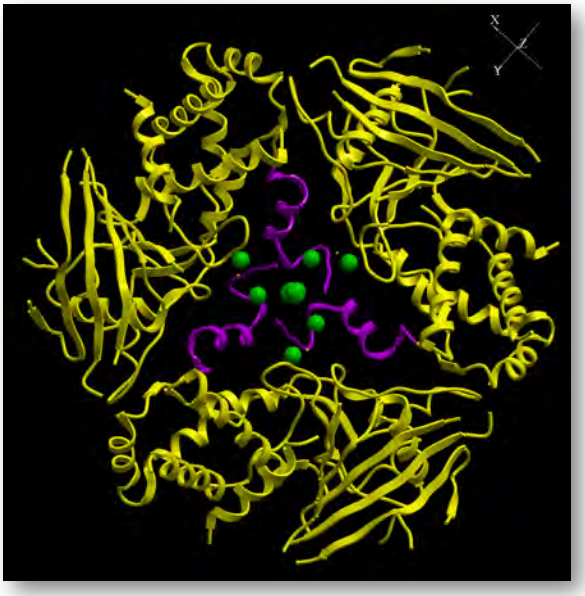
Integration of additive



Crystal structure of the Human Protein Tyrosine Phosphatase Receptor Type J (PDB ID: 2CFV). 20
With the permission of Alastair J. Barr (University of Westminster, London).
See also: Trakhanov & Quiocho (1995); McPherson & Cudney (2006); Zhang *et al.* (2011)

Make use of…

Nature >>> Different expression systems

Cloning/Mutagenesis >>> More or less drastic alterations

Sequence >>> Comparison/Predictive tools

Literature >>> *e.g.* Stabilizing Partner/Ligand/Additive

Biophysics >>> 'pre-crystallisation' assays, *e.g.* Thermal Shift

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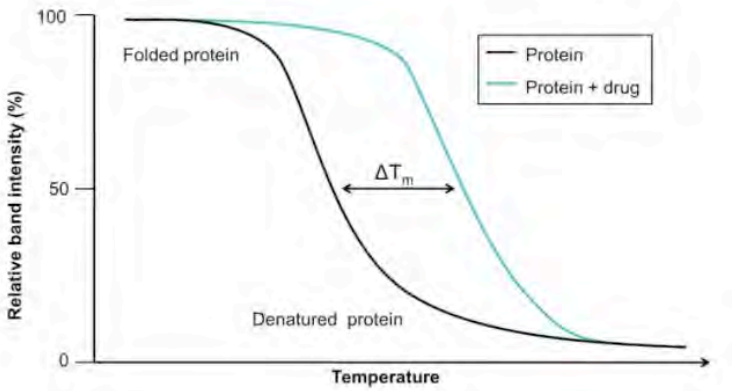
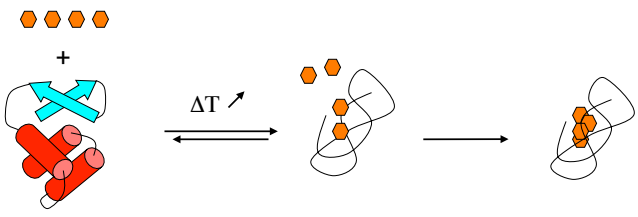
Thermal shift assays





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Limits of pre-crystallisation assays

Not always suitable

Relatively low concentrations of proteins and reagents tested

Single parameter investigated

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And also make use of…

"Common sense and Creativity at all time"

Meindert Lamers

Protein Expression, Crystallisation and Mutagenesis

LMB crystallography courses (2013)

https://www2.mrc-lmb.cam.ac.uk/research/scientific-training/crystallography-course-2013/

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Bottom line

MW

Aim high... but try lower

Purity

Single band on SDS-page

Concentration

2 mg/mL min. but 10 mg/mL is a better start

Solubility

Test early the effect of [Glycerol], [NaCl] and buffer-pH

Stability and Structural Homogeneity

Biophysics assays

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Nucleation and growth

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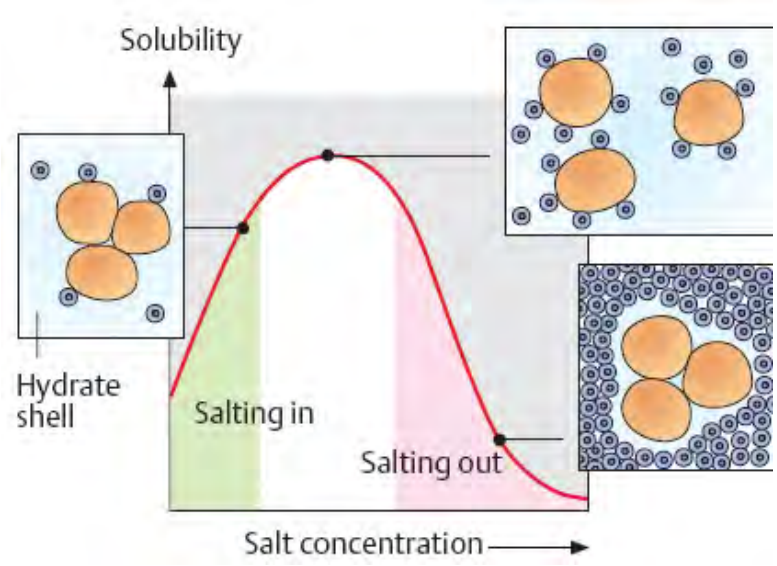
Hydration shell: Salting-out



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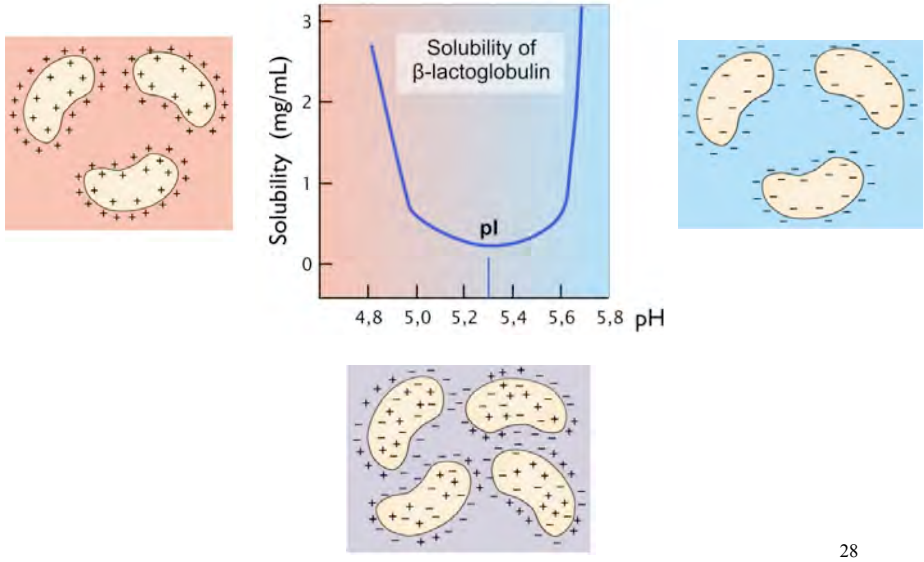
Density/landscape of charges



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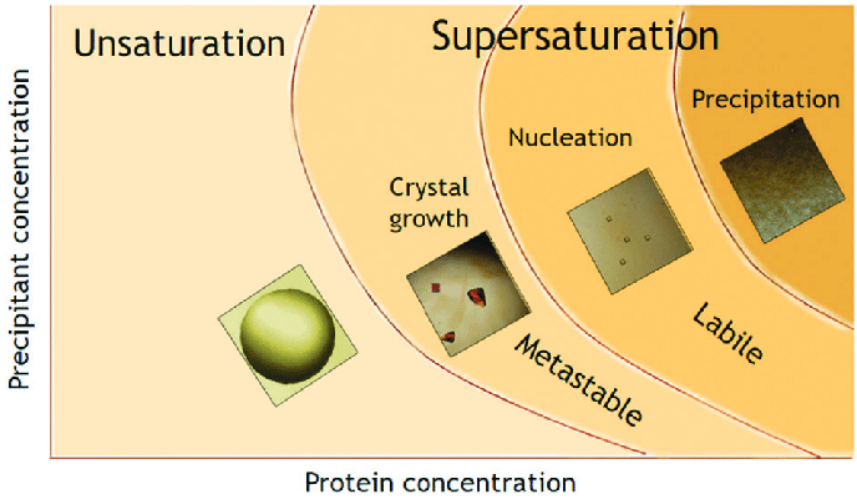
Supersaturation



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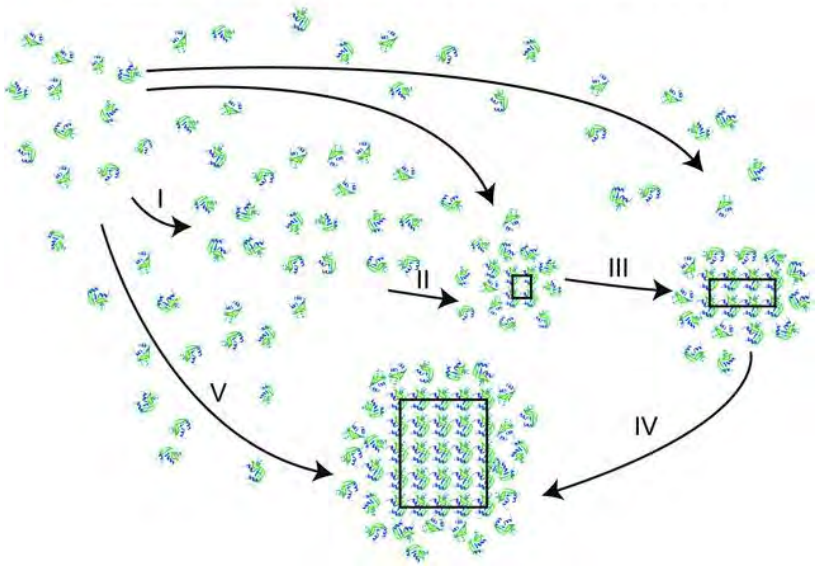
Nucleation: Molten Globule Theory



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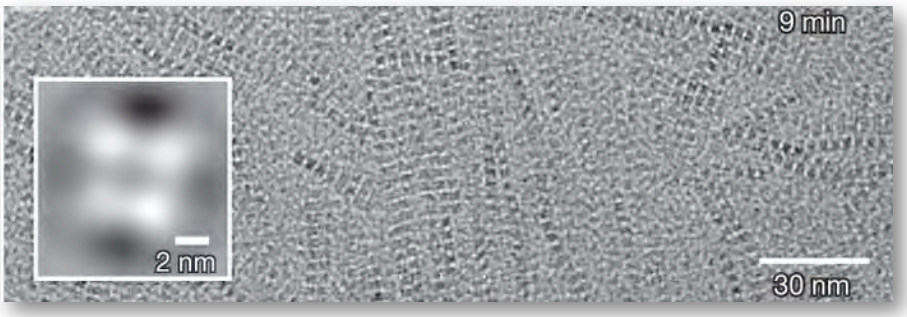
McPherson & Kuznetsov (2014)

Nucleation: (ordered) building blocks



Van Driessche *et al.* (2018)

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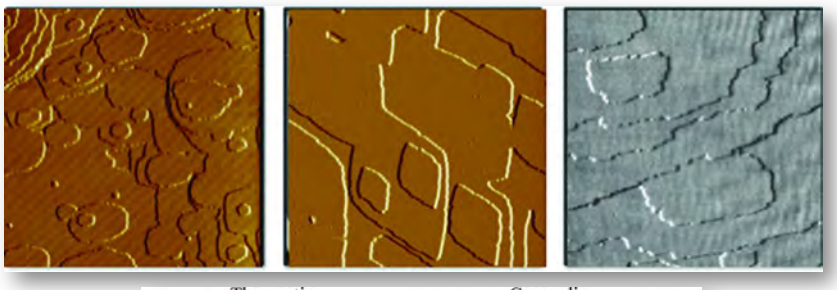
Growth



McPherson & Kuznetsov (2014)

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Initial screening in vapor diffusion nanoliter droplets

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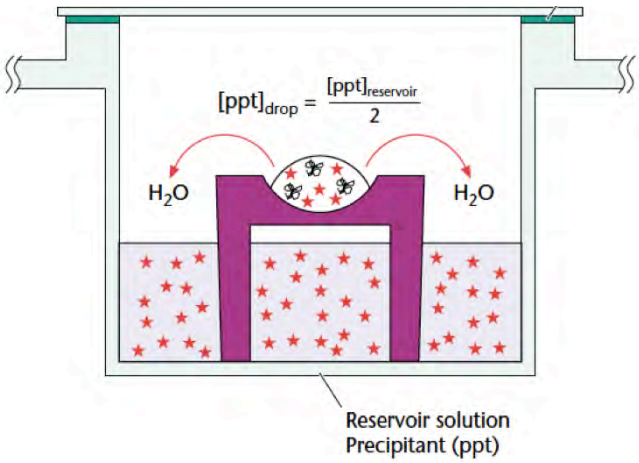
Vapour diffusion experiments



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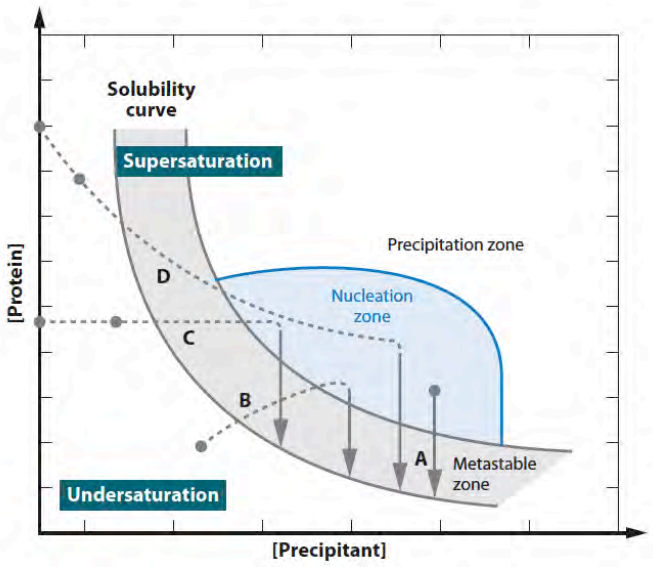
McPherson (2001)

Phase diagram (B: vapor diffusion)



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Chayen & Saridakis (2008)

Crystal screening kits

Crystal Screen — Reagent Formulation

Tube Number	Salt	Tube Number	Buffer †	Tube Number	Precipitant
1.	0.02 M Calcium Chloride dihydrate	1.	0.1 M Sodium Acetate trihydrate pH 4.6	1.	30% v/v 2-Methyl-2,4-pentanediol
2.	None	2.	None	2.	0.4 M Potassium Sodium Tartrate tetrahydrate
3.	None	3.	None	3.	0.4 M mono-Ammonium dihydrogen Phosphate
4.	None	4.	0.1 M Tris Hydrochloride pH 8.5	4.	2.0 M Ammonium Sulfate
5.	0.2 M tri-Sodium Citrate dihydrate	5.	0.1 M HEPES - Na pH 7.5	5.	30% v/v 2-Methyl-2,4-pentanediol
6.	0.2 M Magnesium Chloride hexahydrate	6.	0.1 M Tris Hydrochloride pH 8.5	6.	30% w/v Polyethylene Glycol 4000
7.	None	7.	0.1 M Sodium Cacodylate pH 6.5	7.	1.4 M Sodium Acetate trihydrate
8.	0.2 M tri-Sodium Citrate dihydrate	8.	0.1 M Sodium Cacodylate pH 6.5	8.	30% v/v iso-Propanol
9.	0.2 M Ammonium Acetate	9.	0.1 M tri-Sodium Citrate dihydrate pH 5.6	9.	30% w/v Polyethylene Glycol 4000
10.	0.2 M Ammonium Acetate	10.	0.1 M Sodium Acetate trihydrate pH 4.6	10.	30% w/v Polyethylene Glycol 4000
11.	None	11.	0.1 M tri-Sodium Citrate dihydrate pH 5.6	11.	1.0 M mono-Ammonium dihydrogen Phosphate
12.	0.2 M Magnesium Chloride hexahydrate	12.	0.1 M HEPES - Na pH 7.5	12.	30% v/v iso-Propanol
13.	0.2 M tri-Sodium Citrate dihydrate	13.	0.1 M Tris Hydrochloride pH 8.5	13.	30% v/v Polyethylene Glycol 400
14.	0.2 M Calcium Chloride dihydrate	14.	0.1 M HEPES - Na pH 7.5	14.	28% v/v Polyethylene Glycol 400
15.	0.2 M Ammonium Sulfate	15.	0.1 M Sodium Cacodylate pH 6.5	15.	30% w/v Polyethylene Glycol 8000
16.	None				M Lithium Sulfate monohydrate
17.	0.2 M Lithium Sulfate monohydrate				Polyethylene Glycol 4000
18.	0.2 M Magnesium Acetate tetrahyd				Polyethylene Glycol 8000
19.	0.2 M Ammonium Acetate				v/v iso-Propanol
20.	0.2 M Ammonium Sulfate				w/v Polyethylene Glycol 4000
21.	0.2 M Magnesium Acetate tetrahyd				v/v 2-Methyl-2,4-pentanediol
22.	0.2 M Sodium Acetate trihydrate				w/v Polyethylene Glycol 4000
23.	0.2 M Magnesium chloride hexahyd				v/v Polyethylene Glycol 400
24.	0.2 M Calcium Chloride dihydrate				v/v iso-Propanol
25.	None				M Sodium Acetate trihydrate
26.	0.2 M Ammonium Acetate				% v/v 2-Methyl-2,4-pentanediol

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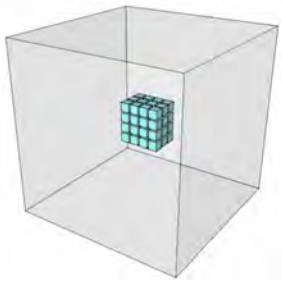
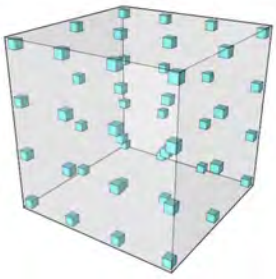
Typical kit in 15ml falcon tubes.

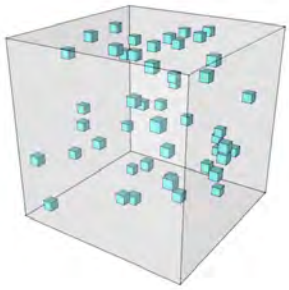
Screen formulation



Grid screen



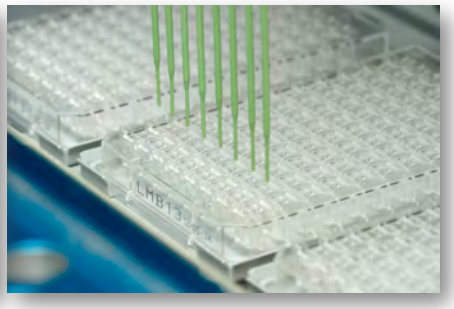
Incomplete factorial



Sparse matrix

created by
LMB Visual Aids
www.visaids.net

Automation and miniaturisation



https://search.mrc-lmb.cam.ac.uk/screens2/search.html

LMB screen name: LMB 01
Well: A01 Find

Commercial name	Supplier	Tube number
Crystal Screen 1	Hampton	5

Component Name	Conc	Unit	pH
MPD	30	% v/v	
sodium citrate tribasic dihydrate	0.2	M	
HEPES sodium salt	0.1	M	7.5

Select screen name and/or component name and/or key word (screen, chemical)

Commercial screen name: none
Component name: MPD
Key word(s): HEPES Find

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Main strategy at LMB: large initial screen

2,112 initial conditions in 22 pre-filled plates (‘LMB plates’)

Vapor-diffusion droplets (initial screen: 100 nL Protein+ 100 nL Condition)

Plate name	kit	supplier	CN*	tubes	basic description
LMB01	Crystal Screen 1	Hampton Research	HR2-110	48	Sparse Matrix (pH 4.6-8.5)
	Crystal Screen 2	Hampton Research	HR2-112	48	Stochastic sampling (pH 4.6-9.0)
LMB02	Wizard 1	Rigaku	1009530	48	Stochastic sampling (pH 4.5-10.5)
	Wizard 2	Rigaku	1009531	48	Stochastic sampling (pH 4.5-10.5)
LMB03	Grid Screen Ammonium Sulfate	Hampton Research	HR2-211	24	Grid screen, conc. AmS = 0.8-3.2 M and buffers pH 4.0-9.0
	Grid Screen PEG/LiCl	Hampton Research	HR2-217	24	Grid screen, conc. PEG 6000 = 0-30 %w/v, conc. LiCl = 1.0 M and buffers pH 4.0-9.0
	Quick Screen	Hampton Research	HR2-221	24	Grid screen, conc. NaKPO4 = 0.8-1.6 M at pH 5.0-8.2
	Grid Screen Sodium Chloride	Hampton Research	HR2-219	24	Grid screen, conc. NaCl = 1.0-4.0 M and buffers pH 4.0-9.0
LMB04	Grid Screen PEG 6000	Hampton Research	HR2-213	24	Grid screen, conc. PEG 6000 = 5-30 %w/v and buffers pH 4.0-9.0
	Grid Screen MPD	Hampton Research	HR2-215	24	Grid screen conc. MPD = 10-65 %w/v and buffers pH 4.0-9.0
	MemFac	Hampton Research	HR2-114	48	Sparse Matrix for membrane proteins (pH 4.6-8.5)
		Hampton			Grid screen, conc. PEG 3350 = 20% w/v and

Gorrec F. & Löwe, *JoVE*. 2018

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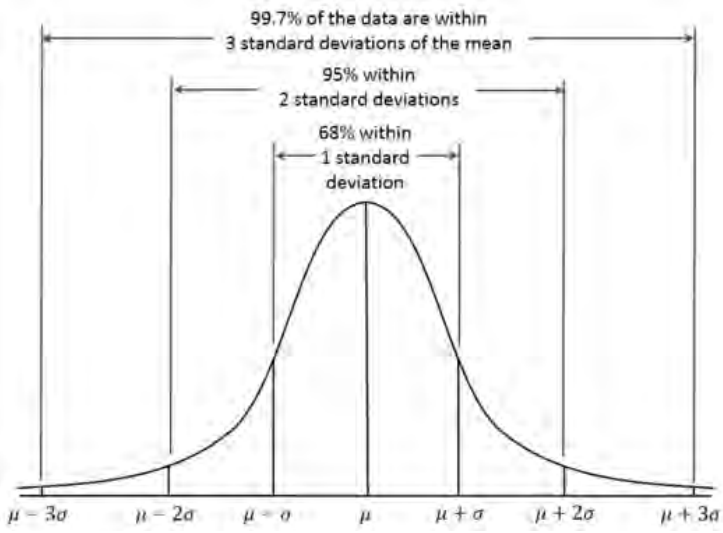
Normal distribution



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Pros and cons of our main strategy

Pros: Increase chances of exclusive hits

Reduce requirements of other steps

Cons: Sample consumption (16 μl x 22 plates = 352 μl)

Time consumption (assessing experiments)

Plate name	kit	supplier	CN*	tubes	basic description
LMB01	Crystal Screen 1	Hampton Research	HR2-110	48	Sparse Matrix (pH 4.6-8.5)
	Crystal Screen 2	Hampton Research	HR2-112	48	Stochastic sampling (pH 4.6-9.0)
LMB02	Wizard 1	Rigaku	1009530	48	Stochastic sampling (pH 4.5-10.5)
	Wizard 2	Rigaku	1009531	48	Stochastic sampling (pH 4.5-10.5)
LMB03	Grid Screen Ammonium Sulfate	Hampton Research	HR2-211	24	Grid screen, conc. AmS = 0.8-3.2 M and buffers pH 4.0-9.0
	Grid Screen PEG/LiCl	Hampton Research	HR2-217	24	Grid screen, conc. PEG 6000 = 0-30 %w/v, conc. LiCl = 1.0 M and buffers pH 4.0-9.0
	Quick Screen	Hampton Research	HR2-221	24	Grid screen, conc. NaKPO4 = 0.8-1.6 M at pH 5.0-8.2
	Grid Screen Sodium Chloride	Hampton Research	HR2-219	24	Grid screen, conc. NaCl = 1.0-4.0 M and buffers pH 4.0-9.0
LMB04	Grid Screen PEG 6000	Hampton Research	HR2-213	24	Grid screen, conc. PEG 6000 = 5-30 %w/v and buffers pH 4.0-9.0
	Grid Screen MPD	Hampton Research	HR2-215	24	Grid screen conc. MPD = 10-65 %w/v and buffers pH 4.0-9.0
	MemFac	Hampton Research	HR2-114	48	Sparse Matrix for membrane proteins (pH 4.6-8.5)
LMB05	PEG/Ion	Hampton	HR2-126	48	Grid screen, conc. PEG 3350 = 20% w/v and

Gorrec F. & Löwe, *JoVE*. 2018

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Assessments of experiments

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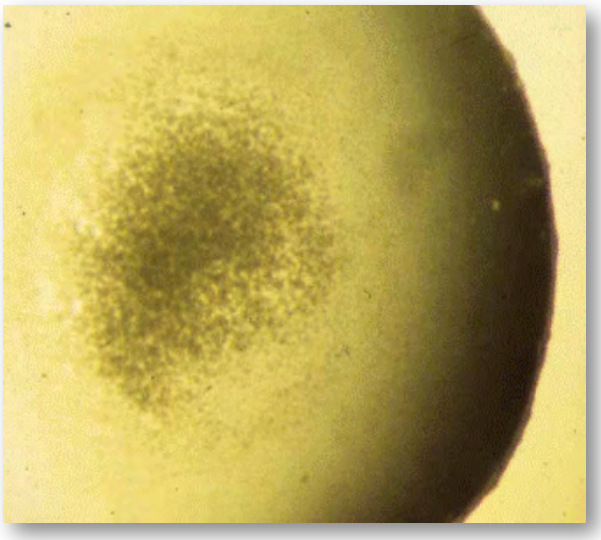
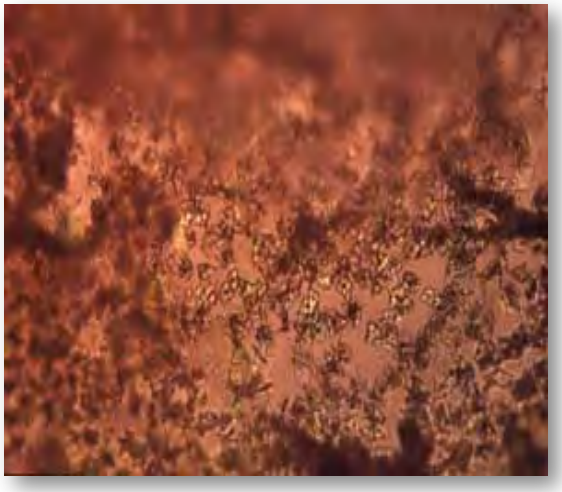
Clear droplets vs precipitate

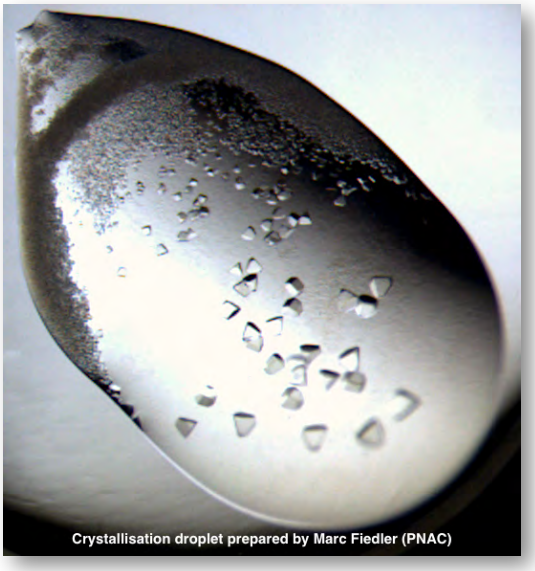


Microcrystals?



https://xray.teresebergfors.com

"Phase diagram droplet"



X-ray diffractometer at the LMB

Dom Bellini
Ext. 7839
07766155064
dbellini@mrc-lmb.cam.ac.uk
1S205

STRUCTURAL STUDIES

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Optimisation protocols

The 4-corner method

Additive screening

Random microseed matrix screening

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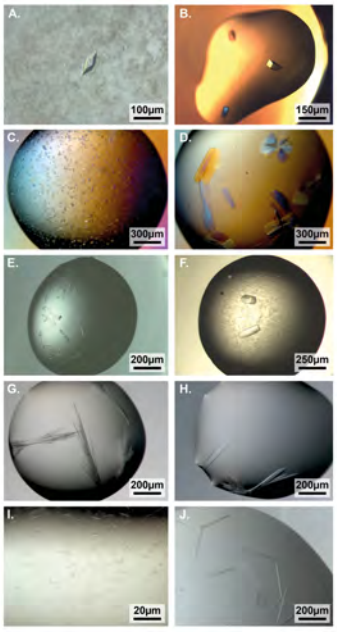
Examples of successful optimisations



A, B. OmpF and MreB resulting from initial screening with the fully automated systems, conditions: LMB07 well A4 and LMB20 well D12, size of droplets: 100 nL protein + 100 nL condition, work of Andrzej Szewczak (LMB).

C, **D**. Bar domain crystals before and after optimization of initial conditions with the 4-corner method, LMB02 B6, 1000 nL + 1000 nL, unpublished work of Leonardo Almeida-Souza (LMB).

E, **F**. Heavy chain of human dynein 1 N-terminal domain crystals before and after optimization of the initial conditions with the 4-corner method, LMB20 E6, 200 nL + 200 nL, then 500 nL + 500 nL, unpublished work of Edgar Morales-Ríos (LMB).

G, **H**. Complement factor D crystals before and after optimization of the condition with additive screening, initial condition from in-house custom screen, 200 nL + 200 nL, additive D6 from MORPHEUS additive screen (Molecular Dimensions), unpublished work of Matthias Bauer (LMB).

I, **J**. Viral envelope glycoprotein crystals before and after optimization of the condition with additive screening, LMB20 A2, 150 nL + 150 nL, then 200 nL + 200nL + 100 nL additive E5 from MORPHEUS additive screen, unpublished work of Yorgo Modis (University of Cambridge).

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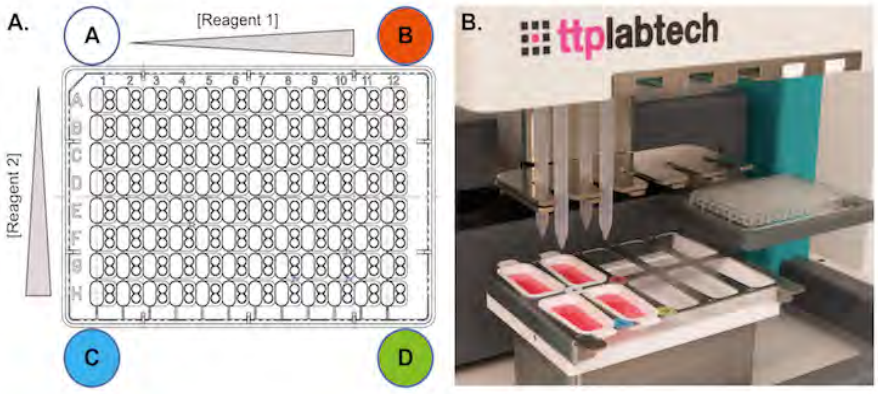
Gorrec & Löwe, JoVE (2018)

The 4-corner method



Excel applet to formulate screen and prepare the 4-corner solutions A, B, C and D:
(http://www2.mrc-lmb.cam.ac.uk/groups/JYL/WWWrobots/four-corners.html)

Stock *et al.*, Prog Biophys Mol Biol (2005)

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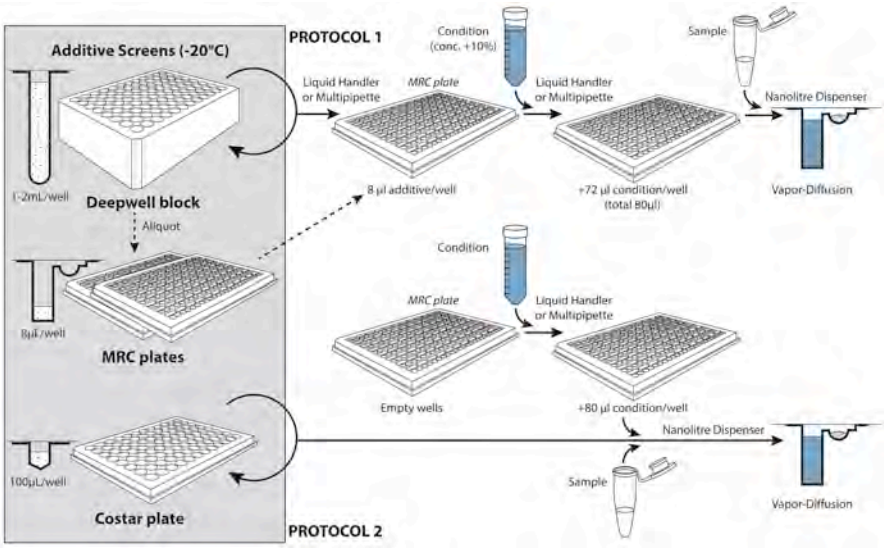
Additive screening



Gorrec & Löwe, JoVE (2018)

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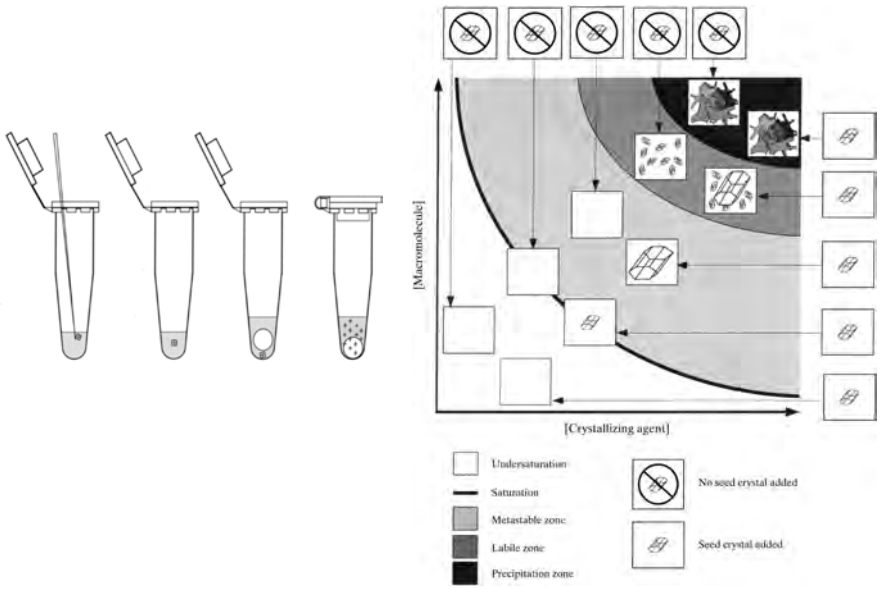
Random microseed matrix screening



Luft & DeTitta (2003)

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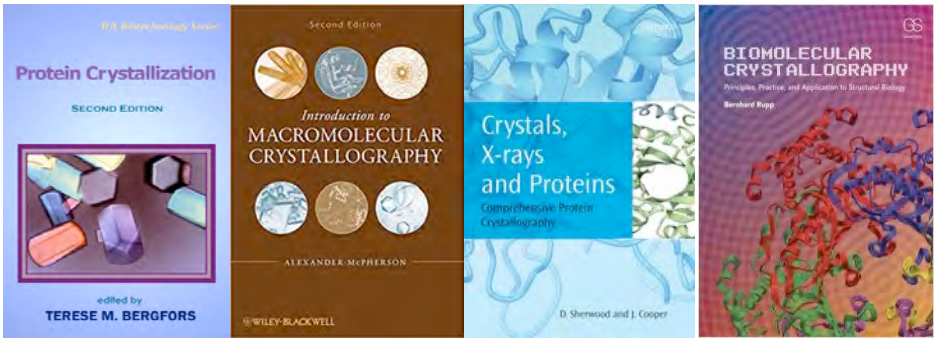
Books



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MRC Laboratory of Molecular Biology

STRUCTURAL STUDIES

Jan Löwe's group studies the bacterial cytoskeleton

PROTEIN & NUCLEIC ACID CHEMISTRY

Roger William's group studies phosphoinositide signalling

The End

'Merci pour votre attention', Fabrice (fgorrec@mrc-lmb.cam.ac.uk)

Talk will be made availble on my website:

https://www3.mrc-lmb.cam.ac.uk/sites/protein-crystallisation/