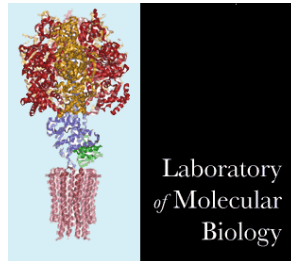


Innovative protein crystallization screens

Fabrice GORREC

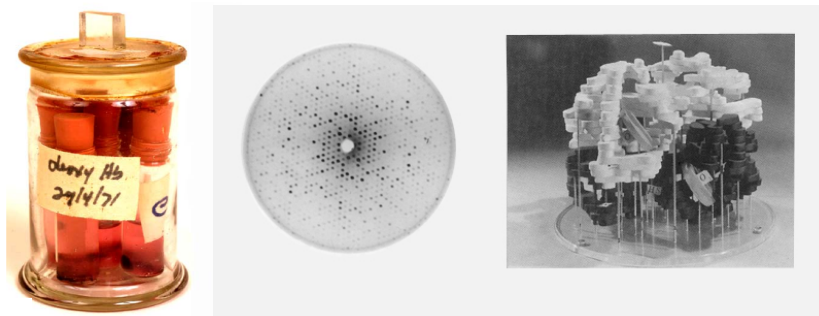


MRC-LMB

LMB: (more than) 50 years ago



Structure of hemoglobin (60's)



New LMB (2012)



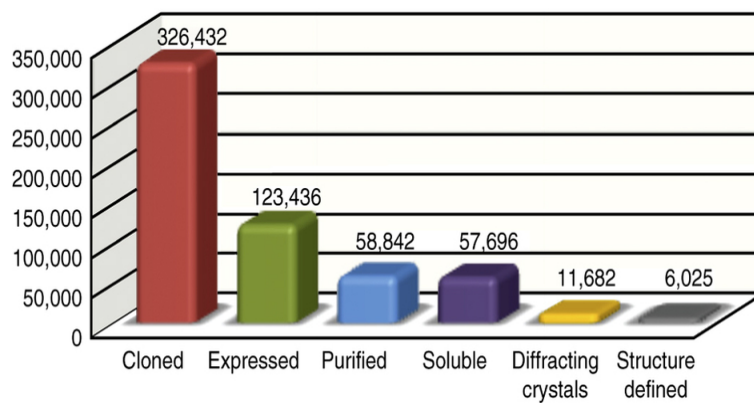
bicycle
therapeutics

HEPTARES
therapeutics



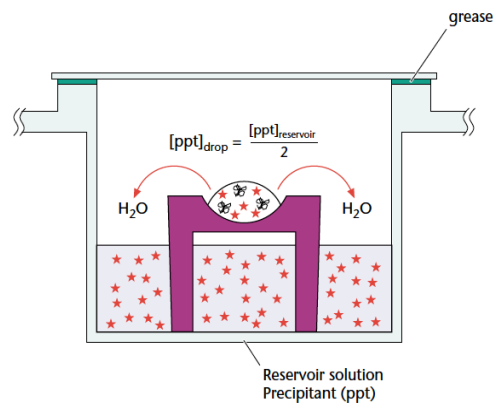
Protein crystallization

Bottleneck of protein crystallography



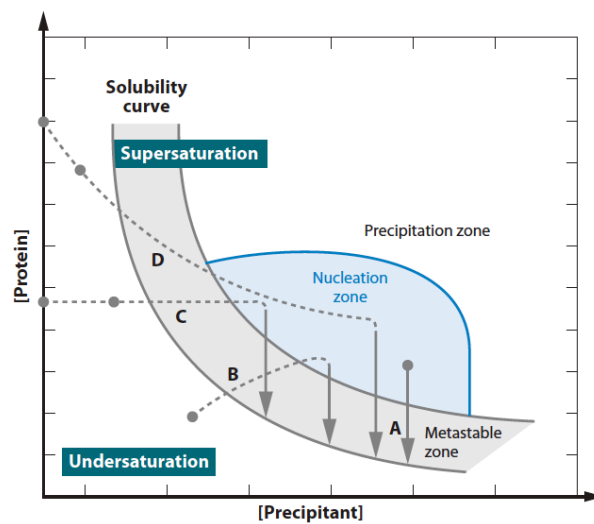
Statistics related to the structure determination process in various Structural Genomic Consortia (SGCs). From 'Porous nucleating agents for protein crystallization' Khurshid *et al.* (2014).

Vapor diffusion



From 'Crystallization of Proteins and Protein-Ligand Complexes', McPherson (2001).

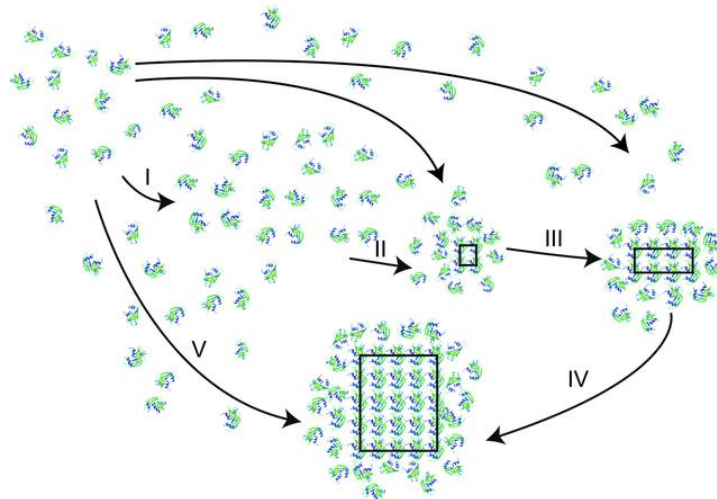
Supersaturation



Phase diagram based on two of the most commonly varied parameters: protein and precipitant concentrations. (A) Batch crystallization (B) Vapor diffusion. (C) Dialysis. (D) Free-interface diffusion. From 'Protein crystallization: from purified protein to diffraction-quality crystal' Chayen and Saridakis (2008).

Molten globule

Plausible nucleation pathways for macromolecular crystals



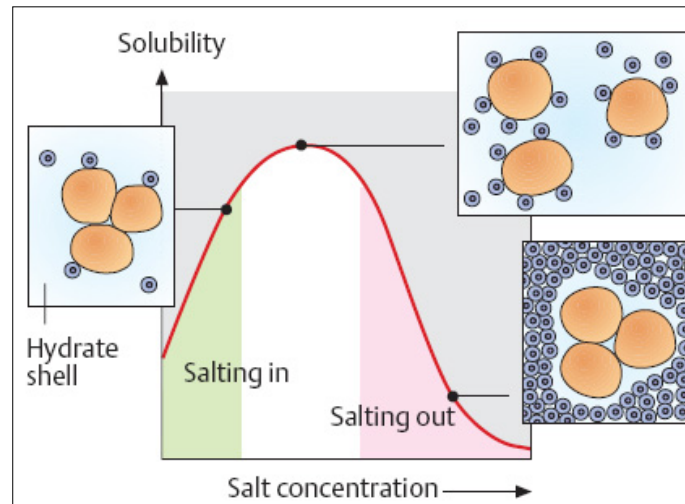
As the supersaturation is increased (I) association of molecules into larger disordered aggregates is promoted. (II) Cores of aggregates (III) critical nucleus (IV) crystal growth. From 'Mechanisms, kinetics, impurities and defects: consequences in macromolecular crystallization' McPherson and Kuznetsov (2014).

Traditional condition

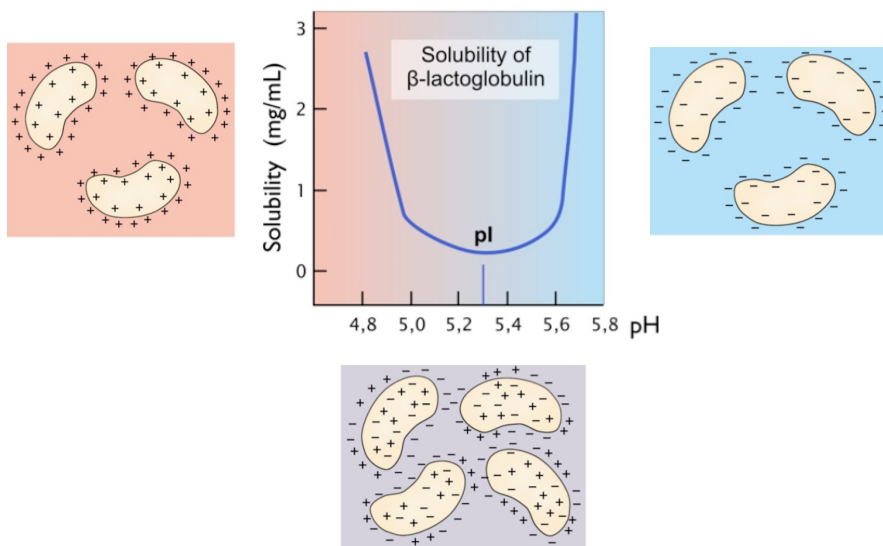
Precipitant +/- Buffer +/- Additive

All the components can alter the numerous parameters associated to the protein (solubility/stability/other) and the experiments (Thermodynamics and Kinetics).

Salting-out

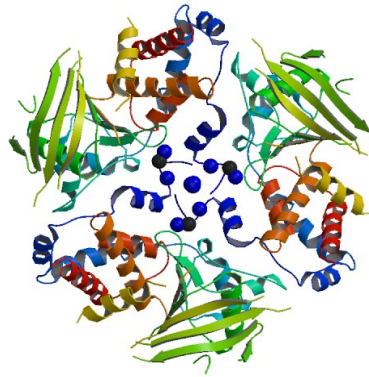


pH and pI



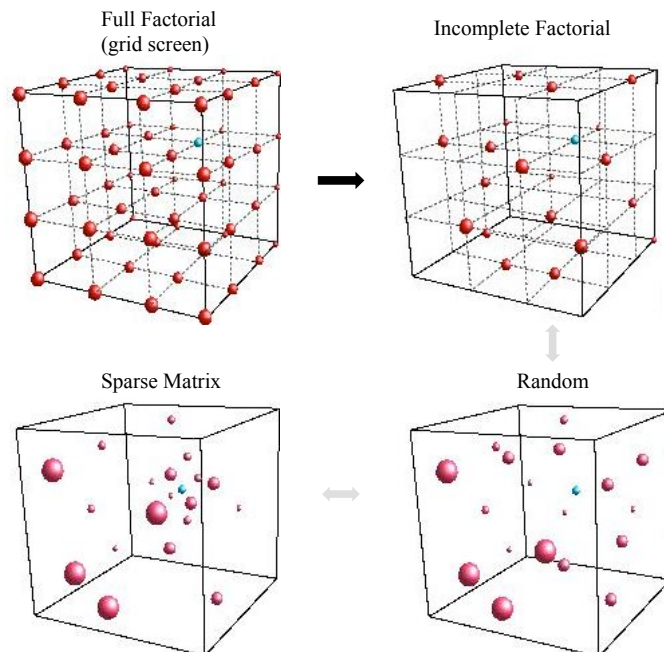
Additives

- Tuning effects of precipitants/buffers
- Stabilizing the protein
- Alter conformation/oligomeric state
- Packing bridges (cross-linking)
- Experimental phasing



Crystal structure of the Human Protein Tyrosine Phosphatase Receptor Type J (PTPNJ, PDB ID: 2CFV). with the permission of Alastair J. Barr (University of Westminster, London).

Sampling (screens)

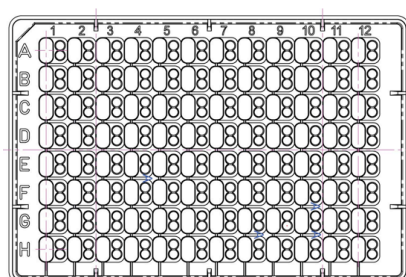
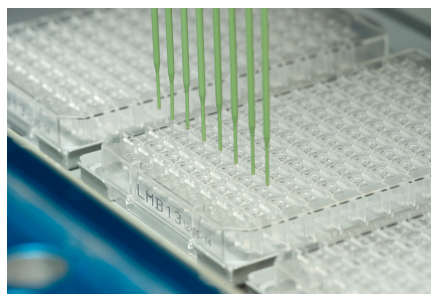
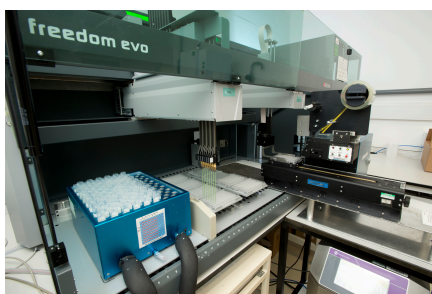


Initial screening

1,920 conditions

LMB plate	kit	supplier	tubes	basic description
LMB01	Crystal Screen 1	Hampton Research	48	Sparse Matrix (pH 4.6-8.5)
	Crystal Screen 2	Hampton Research	48	Stochastic sampling (pH 4.6-9.0)
LMB02	Wizard 1	Rigaku	48	Stochastic sampling (pH 4.5-10.5)
	Wizard 2	Rigaku	48	Stochastic sampling (pH 4.5-10.5)
LMB03	Grid Screen Ammonium Sulfate	Hampton Research	24	Grid screen, conc. $\text{AmS} = 0.8\text{--}3.2\text{ M}$ and buffers pH 4.0-9.0
	Grid Screen PEG/LiCl	Hampton Research	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	24	Grid screen, conc. NaKPO4 + 0.8-1.8 M at pH 5.0-8.2
	Grid Screen Sodium Chloride	Hampton Research	24	Grid screen, conc. NaCl = 1.0-4.0 M and buffers pH 4.0-9.0
LMB04	Grid Screen PEG 6000	Hampton Research	24	Grid screen, conc. PEG 6000 + 1.30 %w/v and buffers pH 4.0-9.0
	Grid Screen MPD	Hampton Research	24	Grid screen conc. MPD = 10-65 %w/v and buffers pH 4.0-9.0
LMB05	MemFac	Hampton Research	48	Sparse Matrix for membrane proteins (pH 4.6-8.5)
	PEG-Ion	Hampton Research	48	Grid screen, conc. PEG 3350 + 20% w/v and various salts at 0.2 M (no buffers)
LMB06	Natrix	Hampton Research	48	Incomplete factorial (pH 5.6-8.5)
	Crystal Screen Lite	Hampton Research	48	Crystal Screen 1 with one-half of the original precipitant concentrations
LMB07	Custom Screen Lite	Molecular Dimensions	48	Crystal Screen 2 with one-half of the original precipitant concentrations
	Wizard Crys 1	Rigaku	48	Stochastic sampling with conditions cryoprotected using low MW PEGs (pH 4.5-9.4)
LMB08	Wizard Crys 2	Rigaku	48	Stochastic sampling with conditions cryoprotected using low MW PEGs (pH 4.5-10.1)
	JB51	JenaBioScience	24	Incomplete factorial based on various PEGs (pH 4.6-9.0)
	JB52	JenaBioScience	24	Incomplete factorial based on PEG 4000 (pH 4.6-8.5)
	JB53	JenaBioScience	24	Incomplete factorial based on PEG 4000 (pH 4.6-8.5)
LMB09	JB54	JenaBioScience	24	Incomplete factorial based on medium MW PEGs (pH 6.5-8.5)
	JB55	JenaBioScience	24	Incomplete factorial based on heavy MW PEGs (pH 6.5-9.5)
	JB56	JenaBioScience	24	Incomplete factorial based on AmS (pH 4.6-8.5)
	JB57	JenaBioScience	24	Incomplete factorial based on MPD (pH 4.6-8.5)
LMB10	JB58	JenaBioScience	24	Incomplete factorial based on MPD and ethanol (pH 4.6-8.5)
	JB59	JenaBioScience	24	Incomplete factorial based on common salts and 2-propanol (pH 4.6-8.5)
	JB510	JenaBioScience	24	Incomplete factorial based on common salts (pH 4.6-8.5)
LMB11	Clear Strategy Screen 1 pH 4.5	Molecular Dimensions	24	Grid screen with various PEGs
	Clear Strategy Screen 1 pH 5.5	Molecular Dimensions	24	Grid screen with various PEGs
	Clear Strategy Screen 1 pH 6.5	Molecular Dimensions	24	Grid screen with various PEGs
	Clear Strategy Screen 1 pH 7.5	Molecular Dimensions	24	Grid screen with various PEGs
LMB12	Clear Strategy Screen 2 pH 4.5	Molecular Dimensions	24	Grid screen with various PEGs
	Clear Strategy Screen 2 pH 5.5	Molecular Dimensions	24	Stochastic sampling
LMB13	Clear Strategy Screen 2 pH 6.5	Molecular Dimensions	24	Stochastic sampling
	Clear Strategy Screen 2 pH 7.5	Molecular Dimensions	24	Stochastic sampling
	Clear Strategy Screen 2 pH 8.5	Molecular Dimensions	24	Stochastic sampling
	Clear Strategy Screen 2 pH 9.5	Molecular Dimensions	24	Stochastic sampling
LMB14	Index	Hampton Research	96	Mix of Sparse Matrix and Grid screens (pH 3.0-9.0)
LMB15	SaltRX	Hampton Research	96	Grid screen including 22 unique salts versus salt concentration and pH (3.1-9.0)
LMB16	MemStart	Molecular Dimensions	48	Sparse Matrix for membrane proteins (pH 4.0-10.0)
LMB17	MemSys	Molecular Dimensions	48	Grid screen for membrane proteins (mostly PEGs, pH 3.5-9.5)
LMB18	ICSG+ sulfate	Qiagen	96	Sparse Matrix (pH 4.0-10.0)
LMB19	Morphosol screen	Molecular Dimensions	96	Grid screen including mixes of additives and cryoprotected conditions (pH 6.5-8.5)
LMB20	PI minimal screen	JenaBioScience	96	Incomplete Factorial (pH 4.0-9.5)
LMB21	PI-PEG screen	JenaBioScience	96	Incomplete factorial for membrane proteins (pH 4.8-8.8)
LMB22	Morphous II screen	Molecular Dimensions	96	Grid screen including mixes of additives (heavy atoms) and cryoprotected conditions (pH 6.5-8.5)

Technology development



MRC Laboratory of Molecular Biology

Select LMB screen name and plate well number

LMB screen name: Well:

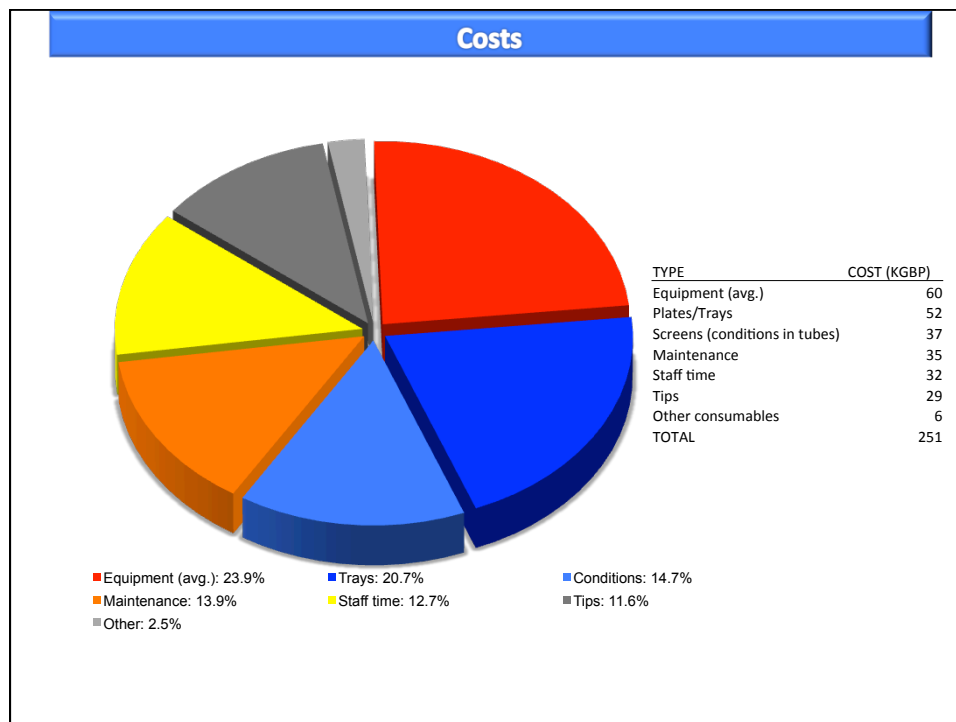
Commercial name	Supplier	Tube number
Crystal Screen 1	Hampton	5

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:
 Component name:
 Key word(s):





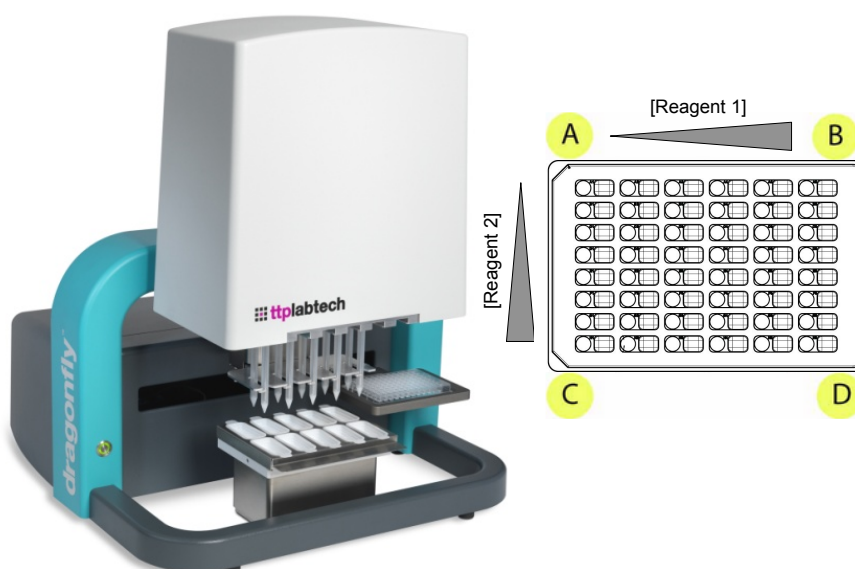
Yield

Sample name	MW (kDa)	Conditions	Hits
Prp8	220	1536	2
HIV-capsid domain	36	1632	20
SUN	25	1632	80
Hexameric HIV-1 capsid	144	1632	30
Spliceosomal RNA helicase	240	1632	2
Mex67:Mtr2	73	1632	1
Atg13	32	1728	2
Arl1-BIG1(DCB domain)	50	1820	6
cmd1	16	1440	2
MMK	38	1440	3
Avg.	79	1466	13

Yields of quality diffraction crystals observed at the LMB during the crystallization of 10 optimized samples (2012). Despite a relatively large number of initial conditions employed (avg. 1512), useful crystals are observed in a small number of experiments (avg. 13 “hits” which means a yield < 1%).

Other steps

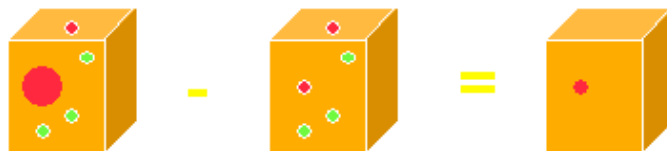
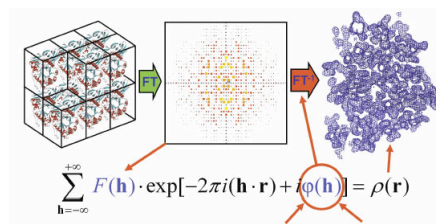
Four corners optimization



Flash-cooling



Isomorphous replacement



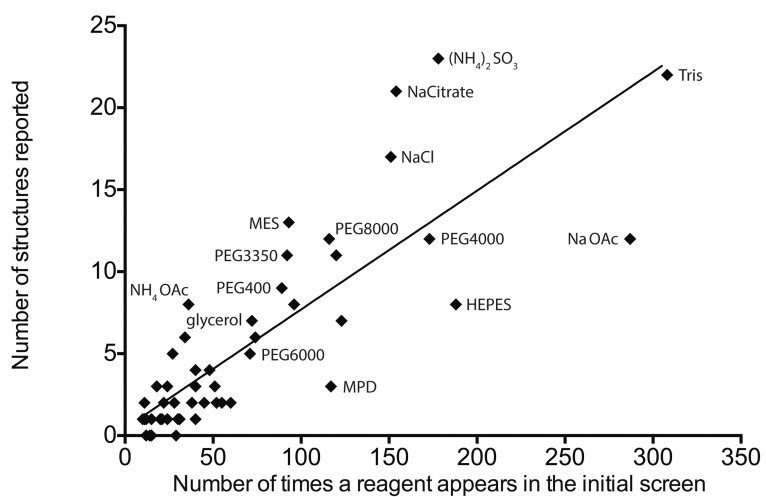
<http://www.ruppweb.org/Xray/Phasing/Phasingt.html>

‘There are so many dimensions associated with the parameter space to be explored that it is problematic or impossible to perform an analysis that has any statistical significance’.

Richard E. Bellman

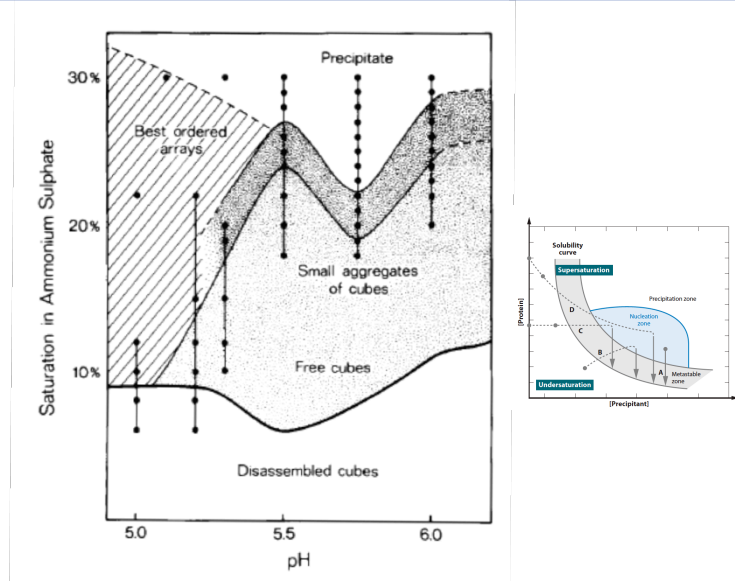


Under-sampling



Statistics related to published conditions between 2002-2009. From 'The current approach to initial crystallization screening of proteins is under-sampled' Gorrec (2013)

Complex behavior of the samples



Phase diagram showing the relationship between Clathrin trimers and trimer precipitates, monomeric cubes, and various cube aggregates. From the article 'Clathrin cubes: an extreme variant of the normal cage' Sorger *et al.* (1983).

Pi

Pi sampling



1, A, M	2, B, N	3, C, O	4, D, P	5, E, Q	6, F, R	7, G, S	8, H, T	9, I, U	10, J, V	11, K, W	12, L, X
1, H, O	2, I, P	3, J, Q	4, K, R	5, L, S	6, M, T	7, N, U	8, O, V	9, P, W	10, Q, X	11, R, Y	12, S, Z
1, C, Q	2, D, R	3, E, S	4, F, T	5, G, U	6, H, V	7, I, W	8, J, X	9, K, Y	10, L, Z	11, A, O	12, B, P
1, J, S	2, K, T	3, L, U	4, A, V	5, B, W	6, C, X	7, D, Y	8, E, Z	9, F, O	10, G, P	11, H, Q	12, I, R
1, E, U	2, F, V	3, G, W	4, H, X	5, I, M	6, J, N	7, K, O	8, L, P	9, A, Q	10, B, R	11, C, S	12, D, T
1, L, W	2, A, X	3, B, M	4, C, N	5, D, O	6, E, P	7, F, Q	8, G, R	9, H, S	10, I, T	11, J, U	12, K, V
1, G, M	2, H, N	3, I, O	4, J, P	5, K, Q	6, L, R	7, A, S	8, B, T	9, C, U	10, D, V	11, E, W	12, F, X
1, B, O	2, C, P	3, D, Q	4, E, R	5, F, S	6, G, T	7, H, U	8, I, V	9, J, W	10, K, X	11, L, Y	12, A, N

Δ (1-12)

'Pi sampling: a methodical and flexible approach to initial macromolecular crystallization screening'
Gorrec *et al.* (2011).

Pi sampler

Component Set 1

ID	Name	Stock Concentration	Screen Concentration	Concentration Unit	Δ Sort
1	acetate pH 4.8	1.0	0.05	M	4.8
2	acetate pH 5.2	1.0	0.05	M	5.2
3	MES pH 5.6	1.0	0.05	M	5.6
4	MES pH 6.0	1.0	0.05	M	6.0
5	ADA pH 6.4	1.0	0.05	M	6.4
6	ADA pH 6.8	1.0	0.05	M	6.8
7	HEPES pH 7.1	1.0	0.05	M	7.1
8	HEPES pH 7.3	1.0	0.05	M	7.3
9	Tris pH 7.6	1.0	0.05	M	7.6
10	Tris pH 8.0	1.0	0.05	M	8.0
11	Bicine pH 8.4	1.0	0.05	M	8.4
12	Bicine pH 8.8	1.0	0.05	M	8.8

Component Set 2

ID	Name	Stock Concentration	Max Screen Concentration	Min Screen Concentration	Concentration Unit	Δ Sort
A	PEG 200	90.0	22.5	0.0	% v/v	200.0
B	PEG 300	80.0	20.0	0.0	% v/v	300.0
C	PEG MME 350	80.0	20.0	0.0	% v/v	350.0
D	PEG 400	80.0	20.0	0.0	% v/v	400.0
E	PEG MME 550	80.0	20.0	0.0	% v/v	550.0
F	PEG 600	80.0	20.0	0.0	% v/v	600.0
G	PEG 1000	70.0	17.5	0.0	% w/v	1000.0
H	PEG 1500	70.0	17.5	0.0	% w/v	1500.0
I	PEG 2000	60.0	15.0	0.0	% w/v	2000.0
J	PEG MME 2000	60.0	15.0	0.0	% w/v	2000.0
K	PEG 3000	60.0	15.0	0.0	% w/v	3000.0
L	PEG 4000	60.0	15.0	0.0	% w/v	4000.0

Component Set 3

ID	Name	Stock Concentration	Max Screen Concentration	Min Screen Concentration	Concentration Unit	Δ Sort
M	PEG MME 2000	60.0	30.0	0.0	% w/v	

<http://pisampler.mrc-lmb.cam.ac.uk/>

Colin Palmer

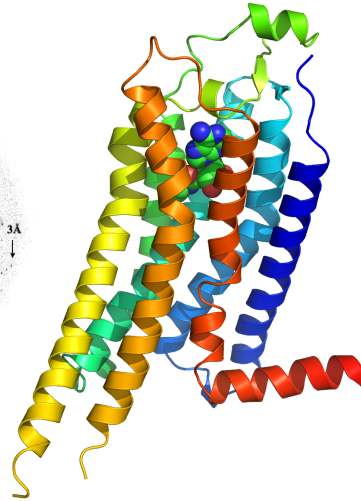
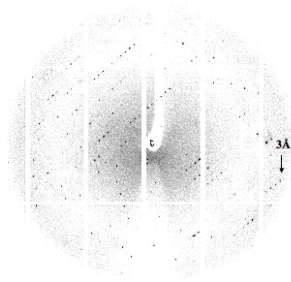


There are originally 12^3 or 1,728 possible combinations. The Pi Sampler, a web-based application, generates 96 combinations that correspond to conditions that are distant in property by using a main characteristic, and different concentrations, of the stock solutions

Formulations

Well	First component	Concentration	Second component	Concentration	Third component	Concentration
A1	acetate pH 4.8	0.050 M	PEG 200	22.500 % v/v	PEG MME 2000	0.000 % w/v
A2	acetate pH 5.2	0.050 M	PEG 300	20.000 % v/v	PEG 3000	0.000 % w/v
A3	MES pH 5.6	0.050 M	PEG MME 350	20.000 % v/v	PEG 4000	0.000 % w/v
A4	MES pH 6.0	0.050 M	PEG 400	20.000 % v/v	PEG 200	0.000 % w/v
A5	ADA pH 6.4	0.050 M	PEG MME 550	20.000 % v/v	PEG 300	0.000 % w/v
A6	ADA pH 6.8	0.050 M	PEG 600	20.000 % v/v	PEG MME 350	0.000 % w/v
A7	HEPES pH 7.1	0.050 M	PEG 1000	17.500 % w/v	PEG 400	0.000 % w/v
A8	HEPES pH 7.3	0.050 M	PEG 1500	17.500 % w/v	PEG MME 550	0.000 % w/v
A9	Tris pH 7.6	0.050 M	PEG 2000	15.000 % w/v	PEG 600	0.000 % w/v
A10	Tris pH 8.0	0.050 M	PEG MME 2000	15.000 % w/v	PEG 1000	0.000 % w/v
A11	Bicine pH 8.4	0.050 M	PEG 3000	15.000 % w/v	PEG 1500	0.000 % w/v
A12	Bicine pH 8.8	0.050 M	PEG 4000	15.000 % w/v	PEG 2000	0.000 % w/v
B1	acetate pH 4.8	0.050 M	PEG 1500	15.000 % w/v	PEG 4000	3.571 % w/v
B2	acetate pH 5.2	0.050 M	PEG 2000	12.857 % w/v	PEG 200	6.429 % w/v
B3	MES pH 5.6	0.050 M	PEG MME 2000	12.857 % w/v	PEG 300	5.714 % w/v
B4	MES pH 6.0	0.050 M	PEG 3000	12.857 % w/v	PEG MME 350	5.714 % w/v
B5	ADA pH 6.4	0.050 M	PEG 4000	12.857 % w/v	PEG 600	5.714 % w/v
B6	ADA pH 6.8	0.050 M	PEG 200	19.286 % v/v	PEG MME 550	5.714 % w/v
B7	HEPES pH 7.1	0.050 M	PEG 300	17.143 % v/v	PEG 600	5.714 % w/v
B8	HEPES pH 7.3	0.050 M	PEG MME 350	17.143 % v/v	PEG 1000	5.000 % w/v
B9	Tris pH 7.6	0.050 M	PEG 400	17.143 % v/v	PEG 1500	5.000 % w/v
B10	Tris pH 8.0	0.050 M	PEG MME 550	17.143 % v/v	PEG 2000	4.286 % w/v

Pi-PEG screen



'Agonist-bound adenosine A(2A) receptor structures reveal common features of GPCR activation' Lebon *et al.* (2011).

MORPHEUS

Examples of PDB ligands

Ligand(s)	PDB ID	Structure Hits
1,2,3-HEPTANETRIOL	HTO	33
1,2-ETHANEDIOL (Ethylene glycol)	EDO	951
1,2-PROPANEDIOL (enantiomers R and S)	PGO, PGR	39
1,3-PROPANEDIOL	PDO	7
1,4-BUTANEDIOL	BU1	10
1,6-HEXANEDIOL	HEZ	18
2-(N-MORPHOLINO)-ETHANESULFONIC ACID (MES)	MES	297
2-[BIS-(2-HYDROXY-ETHYL)-AMINO]-2-HYDROXYMETHYL-PROPANE-1,3-DIOL (Bis-Tris)	BTB	26
2-AMINO-2-HYDROXYMETHYL-PROPANE-1,3-DIOL (Tris)	TRS	313
2-METHYL-2,4-PENTANEDIOL (MPD, enantiomers R and S)	MPD, MRD	446
3[N-MORPHOLINO]PROPANE SULFONIC ACID (MOPS)	MPO	20
4-(2-HYDROXYETHYL)-1-PIPERAZINE ETHANESULFONIC ACID (HEPES)	EPE	184
ACETATIC ACID, ACETATE, ACETYL	ACY, ACT, ACE	1780
ALANINE (enantiomers L and D)	ALA, DAL	32
AMINO, AMMONIA, AMMONIUM	NH2, NH3, NH4	554
BICINE	BCN	11
BROMIDE	BR	105
CACODYLIC ACID, CACODYLATE	CAD, CAC	121
CALCIUM	CA	3775
CHLORIDE	CL	2613
CITRIC ACID, CITRATE	CIT, FLC	353
D-GALACTOSE (anomers α and β)	GAL, GLA	82
D-GLUCOSE (anomers α and β)	GLC, BGC	230
DI(HYDROXYETHYL)ETHER (Di-ethyleneglycol)	PEG	168
D-MANNOSE (anomers α and β)	MAN, BMA	162
D-XYLOPYRANOSE (anomers α and β)	XYL, XYP	40
FLUORIDE	F	16

Stock solutions

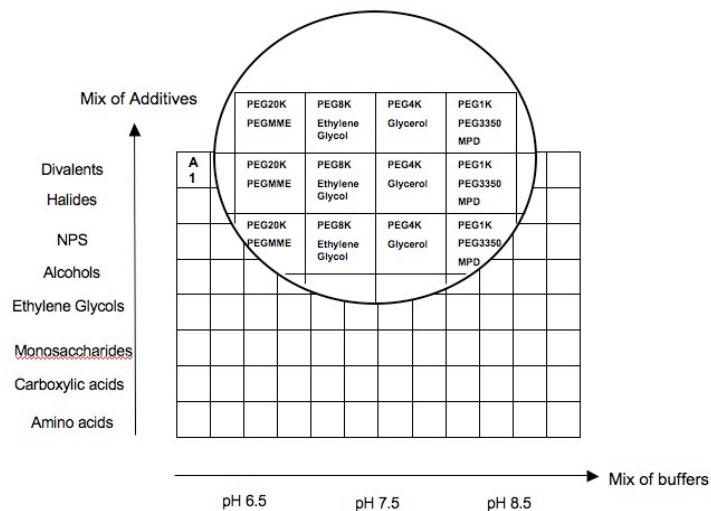
stock name	conc.	composition
Monosaccharides (Row F)	1.2M	0.2M D-Glucose; 0.2M D-Mannose; 0.2M D-Galactose; 0.2M L-Fucose; 0.2M D-Xylitose; 0.2M N-Acetyl-D-Glucosamine

stock name	conc.	composition
P550MME_P20K	60.0%	40%v/v PEGMME 550; 20%w/v PEG 20K
EDO_P8K	60.0%	40%v/v Ethylene glycol; 20%w/v PEG 8K
GOL_P4K	60.0%	40%v/v Glycerol; 20%w/v PEG 4K
MPD_P1K_P3350	75.0%	25%v/v MPD (racemic); 25%w/v PEG 1K; 25%w/v PEG 3350

pH	1 M MES (ml)	1 M Imidazol (ml)
6.1	36.0	14.0
6.3	33.5	16.5
6.5	30.6	19.4
6.7	27.5	22.5
6.9	25.0	25.0

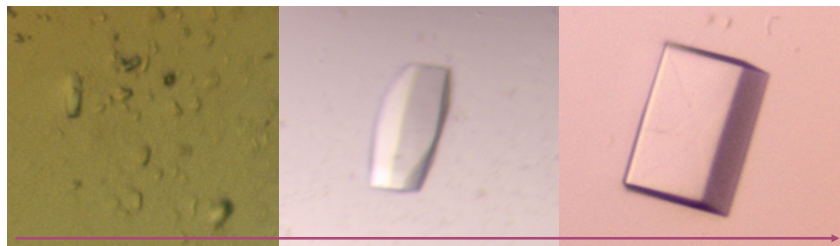
0.5 stock precipitants + 0.1 stock additives + 0.1 buffer-system + 0.3 water

MORPHEUS sampling

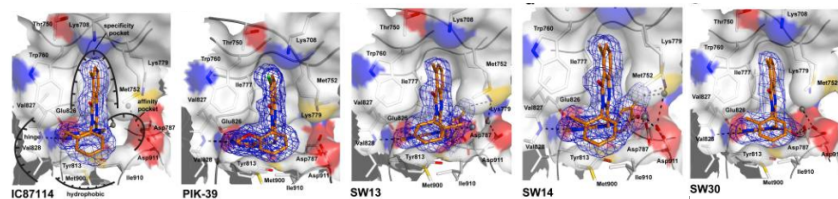


‘The MORPHEUS protein crystallization screen’ Gorrec (2009).

Case study



Optimization: crystal of the Catalytic subunit of the class IA PI(3)K p110δ. The original hit was obtained exclusively in Morpheus.



Amongst the compounds that are highly selective for the Kinase, only those interacting with the affinity pocket of the active site are potent. ‘The p110δ structure: mechanisms for selectivity and potency of new PI(3)K inhibitors’ Berndt *et al.* (2010).

Alex Berndt

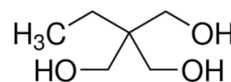
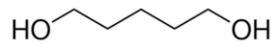
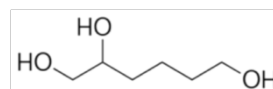
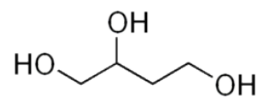
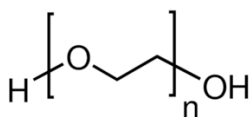
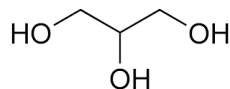


Roger Williams



MORPHEUS II

Polyols



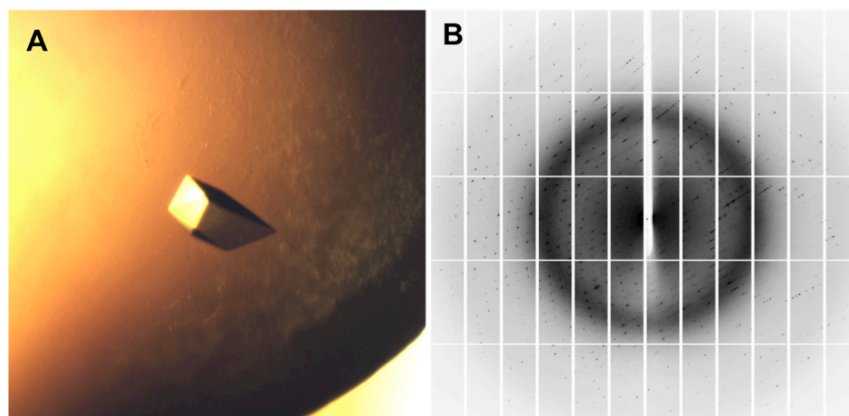
Chemical name	MW	Conc.	Unit
1,2-Ethandiol (ethylene glycol)	62.07	80	% v/v
1,2-Propanediol	76.09	80	% v/v
1,2,3-Propanetriol (Glycerol)	92.09	80	% w/v
1,2,6-Hexanetriol	134.17	80	% w/v
1,5-Pentanediol	104.15	80	% w/v
1,2,4-Butanetriol	106.12	80	% w/v
1,4-Butanediol	90.12	80	% w/v
PEG 200	200.00	80	% v/v
PEG 300	300.00	80	% v/v
1,2-Butanediol	90.12	80	% v/v
1,2-Hexanediol	118.17	80	% v/v
1,3-Propanediol	76.09	80	% v/v
1,6-Hexanediol	118.17	80	% w/v
2-Methyl-2,4-pentanediol (MPD)	118.17	80	% v/v
1,2-Pentanediol	104.15	80	% v/v
meso-Erythritol	122.12	80	% w/v
1,1,1-Tris(hydroxymethyl)propane	134.17	80	% w/v
2-Deoxy-D-ribose	134.17	80	% w/v
3-Methyl-1,3,5-pentanetriol	134.17	80	% w/v
Dianhydro-D-glucitol	146.14	80	% w/v
1-O-Methyl-2-deoxy-D-ribose	148.16	80	% w/v
D-(-)-Lyxose	150.13	80	% w/v
Adonitol	152.15	60	% w/v
L-(-)-Araabitol	152.15	60	% w/v
Xylitol	152.15	40	% w/v
2-Deoxy-D-galactose	164.16	50	% w/v
2-Deoxy-D-glucose	164.16	35	% w/v
L-(-)-Fructose	164.16	60	% w/v
D-(-)-Fructose	180.16	80	% w/v
L-(-)-Glucose	180.15	70	% w/v
myo-inositol	180.15	10	% w/v
D-(-)-Tagatose	180.15	80	% w/v
L-Rhamnose monohydrate	182.17	25	% w/v
Methyl-alpha-D-glucopyranoside	194.18	60	% w/v
Tetraethylene glycol	194.23	80	% v/v
Pentaethylene glycol	238.28	80	% v/v
Glycerol propoxylate (1PO/OH)	266.00	80	% w/v
PEE (3/4 EO/OH)	270.00	80	% w/v
Hexaethylene glycol	282.33	80	% w/v
D-(+)-Mellibiose	342.30	60	% w/v
Lactulose	342.30	50	% w/v
Sucrose (D-+)-Saccharose	342.30	60	% w/v
maltilol	344.31	80	% w/v
D-Lactitol monohydrate	362.33	50	% w/v
D-(+)-Trehalose dihydrate	378.33	50	% w/v
PEG 400	400.00	80	% v/v
PEP (5/4 PO/OH)	426.00	80	% w/v
Maltotriose	504.44	60	% w/v
PEG 600	600.00	80	% v/v
PEG 1500	1500.00	70	% w/v
PEG 6000	6000.00	50	% w/v

Heavy-atoms

Row	Mix name	Chemicals
A	LiNaK	0.3 M lithium sulfate, 0.3 M sodium sulfate, 0.3 M potassium sulfate
B	Divalent cations II	10 mM manganese chloride, 10 mM cobalt chloride, 10 mM nickel chloride, 10 mM zinc chloride
C	Alkalis	10 mM rubidium chloride, 10 mM strontium acetate, 10 mM cesium acetate, 10 mM barium acetate
D	Oxometalates	5 mM sodium chromate, 5 mM sodium molybdate, 5 mM sodium tungstate, 5 mM sodium orthovanadate
E	Lanthanides	5 mM yttrium (III) chloride hexahydrate, 5 mM erbium (iii) chloride hexahydrate, 5 mM terbium (III) chloride hexahydrate, 5 mM ytterbium (III) chloride hexahydrate
F	Monosaccharides II	0.2 M xylitol, 0.2 M D-(-)-fructose, 0.2 M D-sorbitol, 0.2 M myo-inositol, 0.2 M L-rhamnose monohydrate
G	Amino-acids II	0.2 M DL-arginine-HCl, 0.2 M DL-threonine, 0.2 M DL-histidine-HCl-H ₂ O, 0.2 M DL-5-hydroxylysine-HCl, 0.2 M trans-4-hydroxy-L-proline
H	Polyamines	0.1 M spermine-4(HCl), 0.1 M spermidine-3(HCl), 0.1 M 1,4-diaminobutane-2(HCl), 0.1 M DL-ornithine-HCl

‘The MORPHEUS II protein crystallization screen’ Gorrec (2015).

Case study



Quality crystal of *T. thermophilus* MreB used to determine the corresponding structure. Significant anomalous signal was induced with the presence of lanthanides in the crystal (The X-ray beam was tuned to the absorption edge of erbium : 1.2715 Å).

Andrzej Szewczak



Jan Löwe



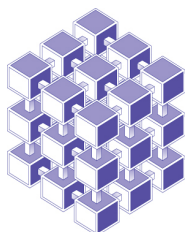
Commercialization

Conflicts of interest



Jena Bioscience

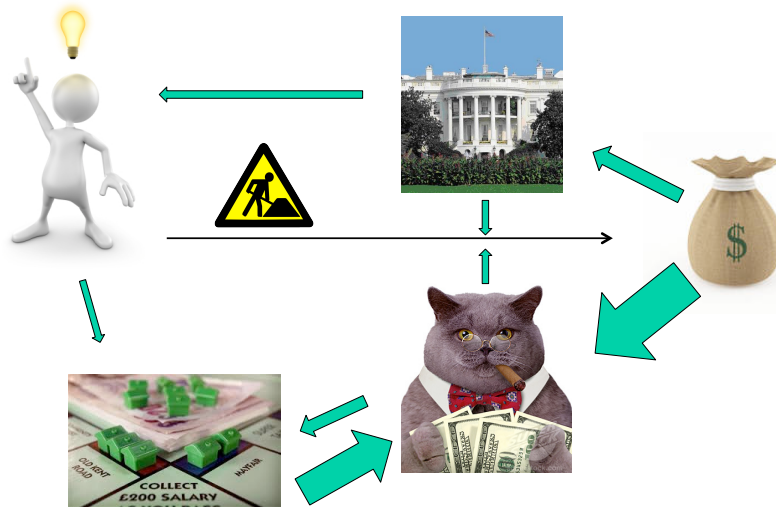
- Pi minimal screen
- Pi-PEG screen



Molecular
Dimensions

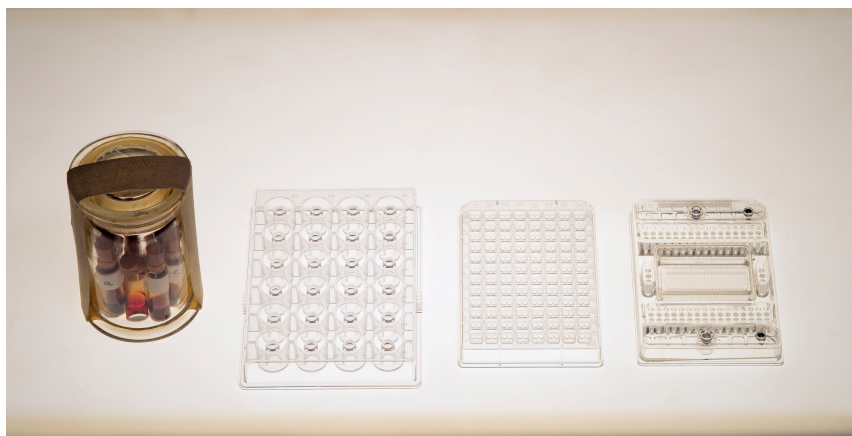
- **MORPHEUS**
- **MORPHEUS II**

The invention process in a Nutshell



Perspectives

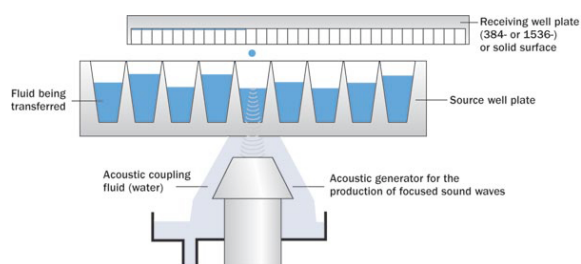
Miniaturization of assays



From left to right: Perutz's flask (80's, experiments in vials with few millilitres of sample), Chryschem tray (early 90's, microliter drops), MRC plate (early 2000's, nanoliter droplets) and the TOPAZ[®] integrated chip carrier (early 2000's, sub-nanoliter microchannels).

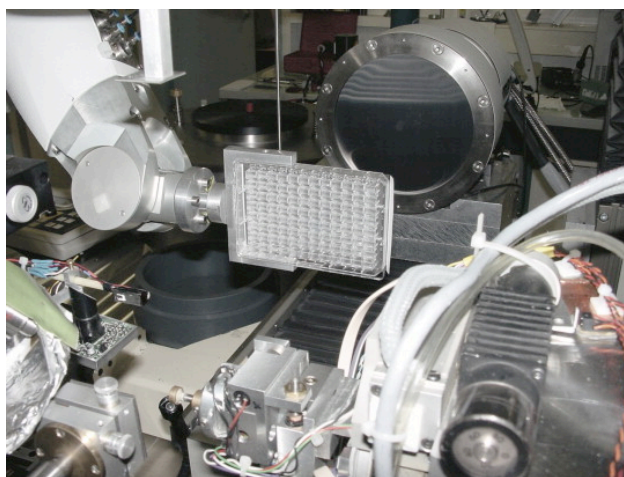
'Progress in macromolecular crystallography depends on further miniaturization of crystallization experiments' Gorrec (2014).

Acoustic technology



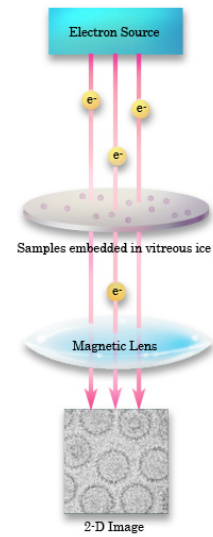
‘Nanolitre-scale crystallization using acoustic liquid-transfer technology’ Villaseñor *et al.* (2012).

X-ray diffraction in-situ



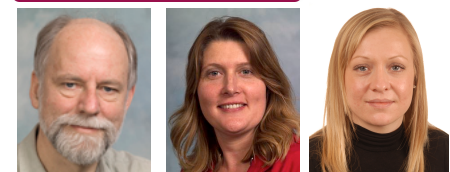
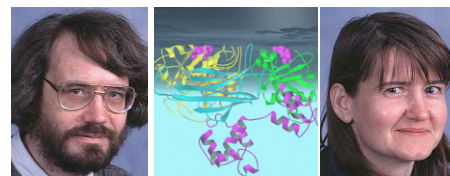
‘Automated analysis of vapor diffusion crystallization drops with an x-ray beam’ Jacquamet *et al.* (2004).

Cryo-EM

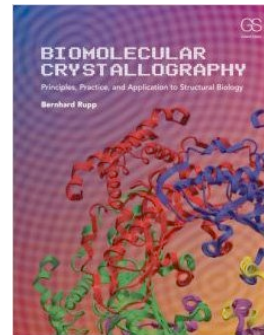
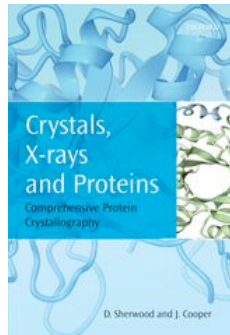
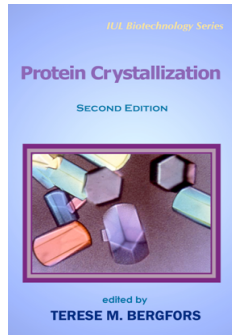


‘Overview and future of single particle electron cryomicroscopy’ Henderson (2015).

Acknowledgments



Books



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