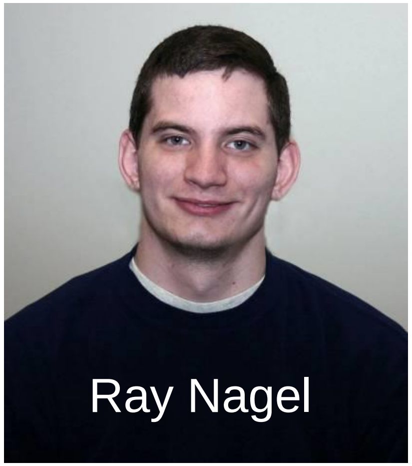
Order from Chaos

The design and interpretation of high-throughput crystallization screens to guide optimization

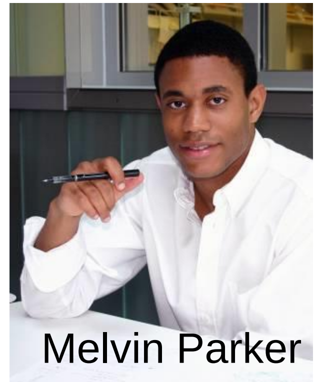
Edward Snell

Acknowledgements



Ray Nagel for programming support

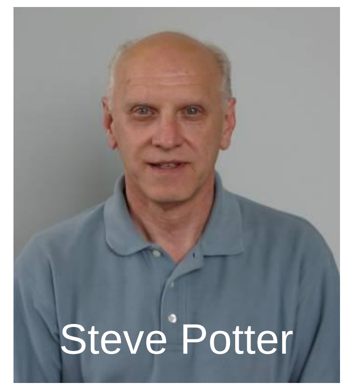
Joe Luft for discussions on the 1536 screen and analysis of the results



Melvin for the initial programming during his summer student project.

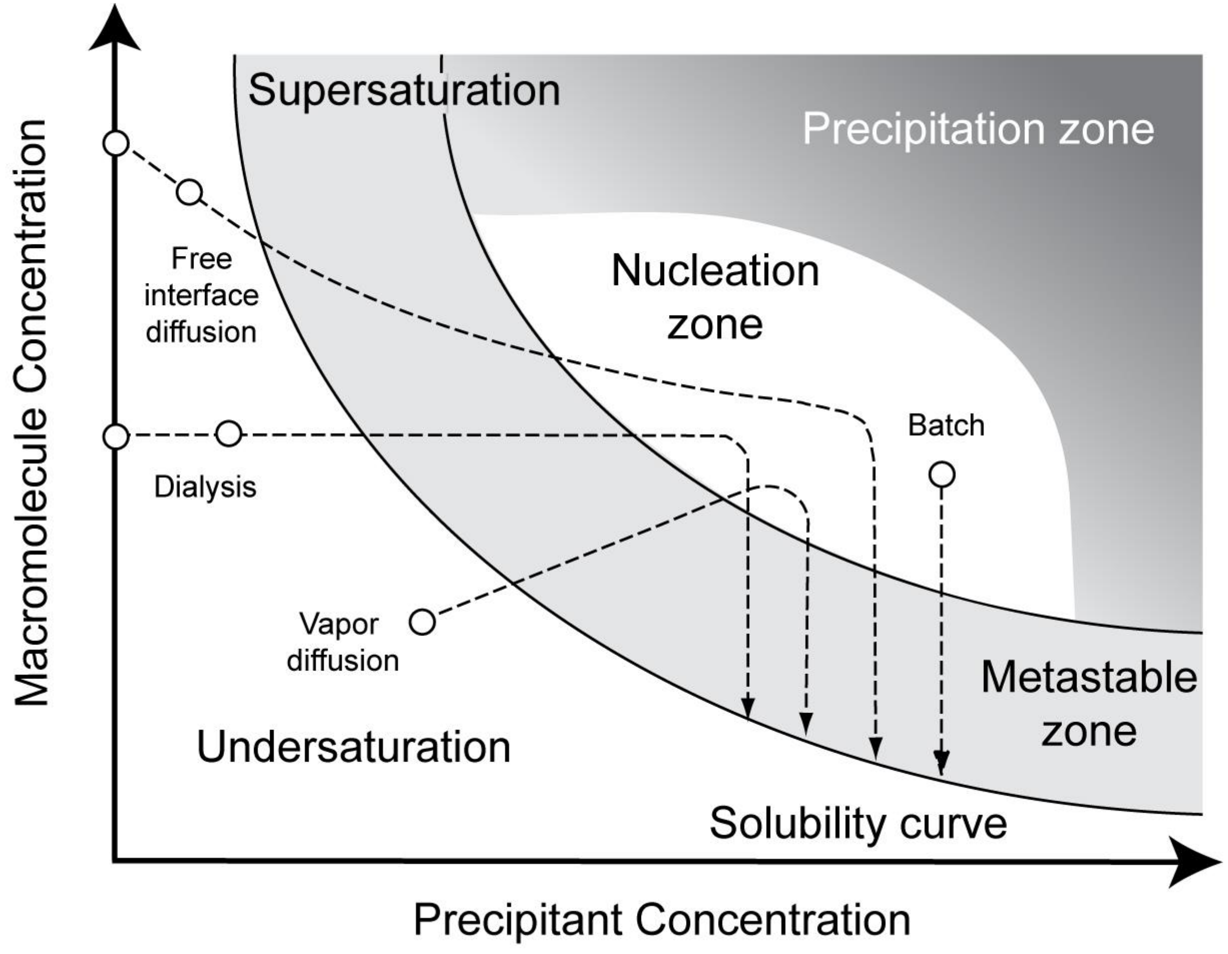
Ann, Miriem, Jen and Elizabeth for useful discussions, helping with the design of the chemical space, scoring images and testing the initial versions of the program.

Steve for providing multiple macromolecule data for testing and George for supporting the research.

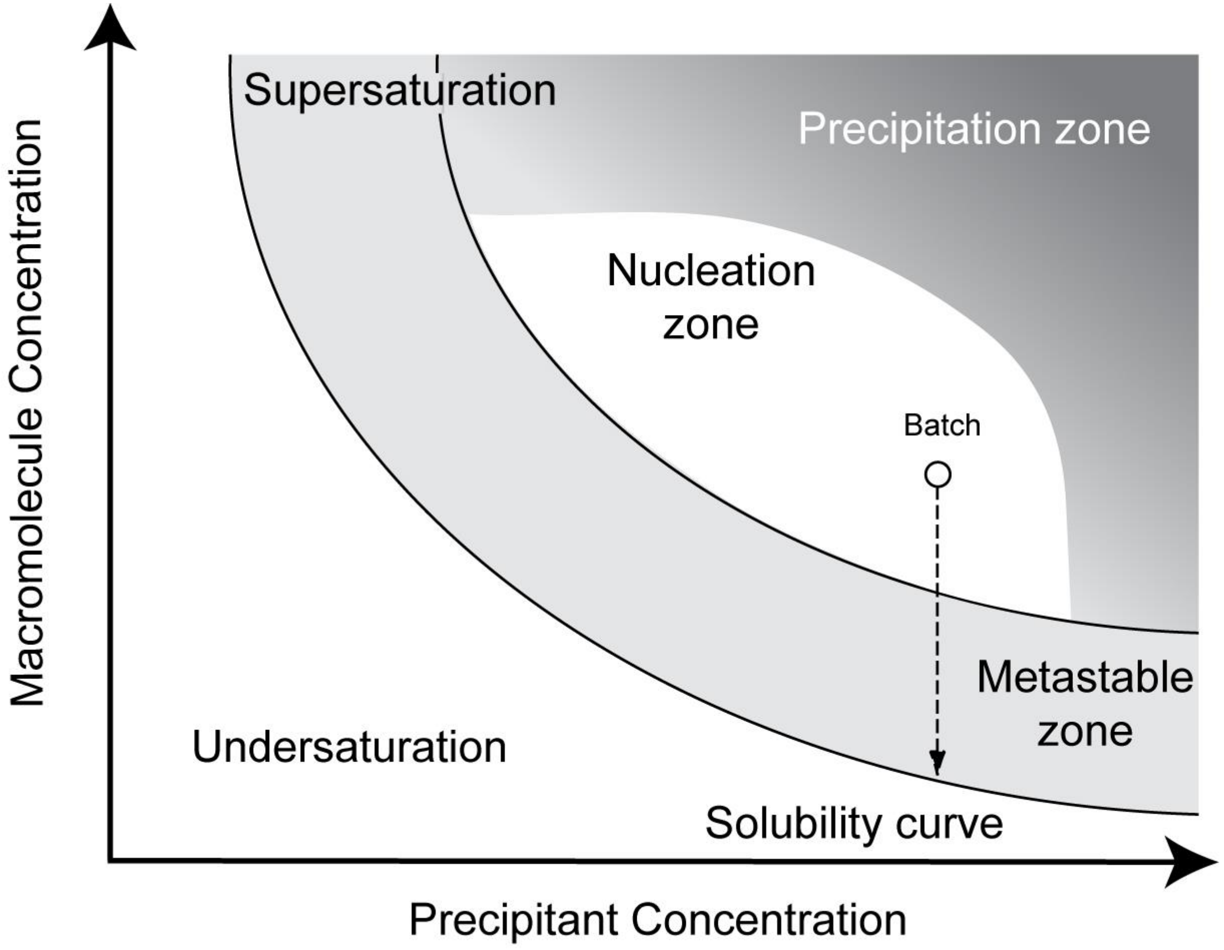


Not to forget the rest of the High-Throughput Lab, Tina, Angela and Ellie for putting up with me. Thanks also to Dean Myles, Hugh O'Neal and Flora Meilleur for samples.

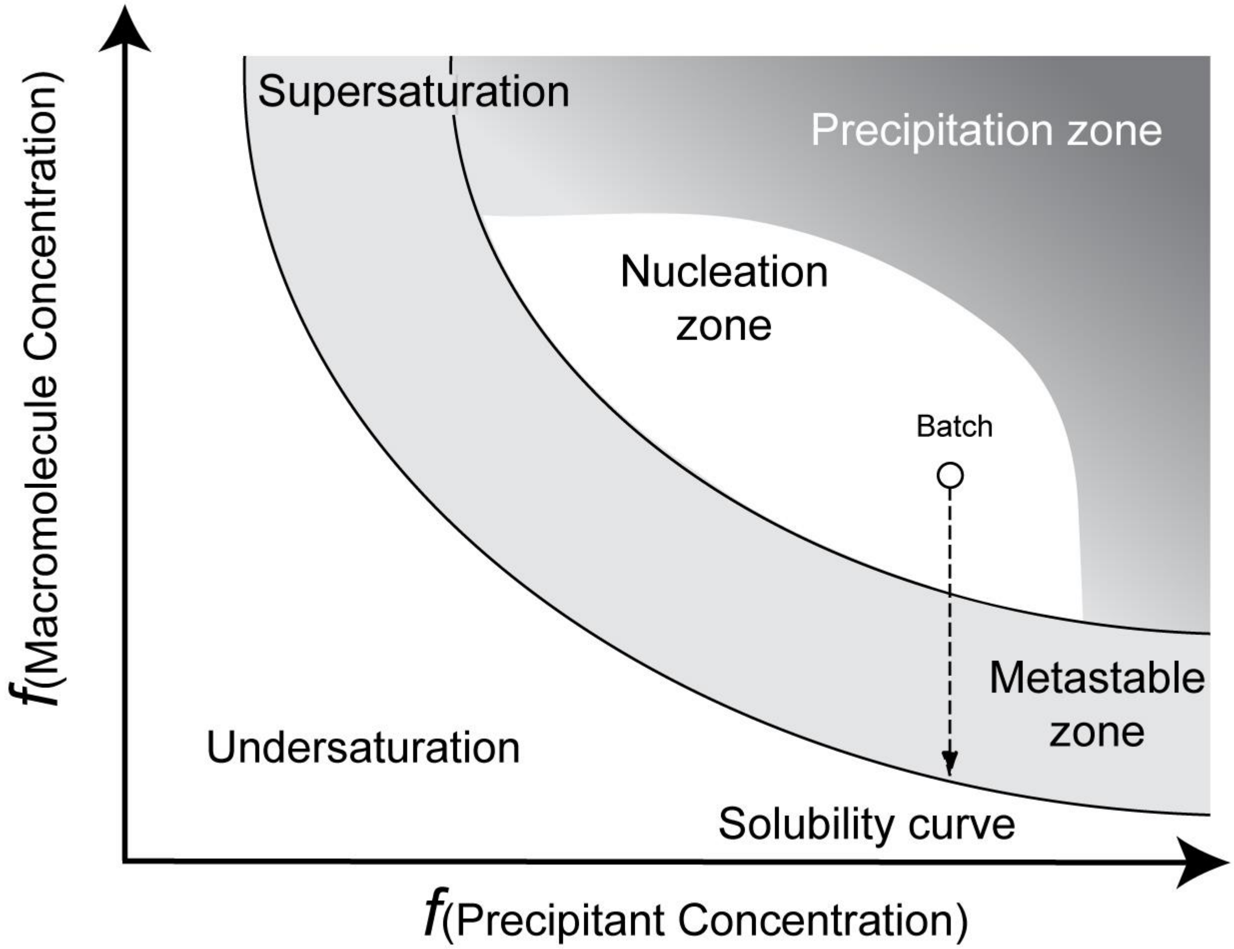
Simplified phase diagram for crystallization



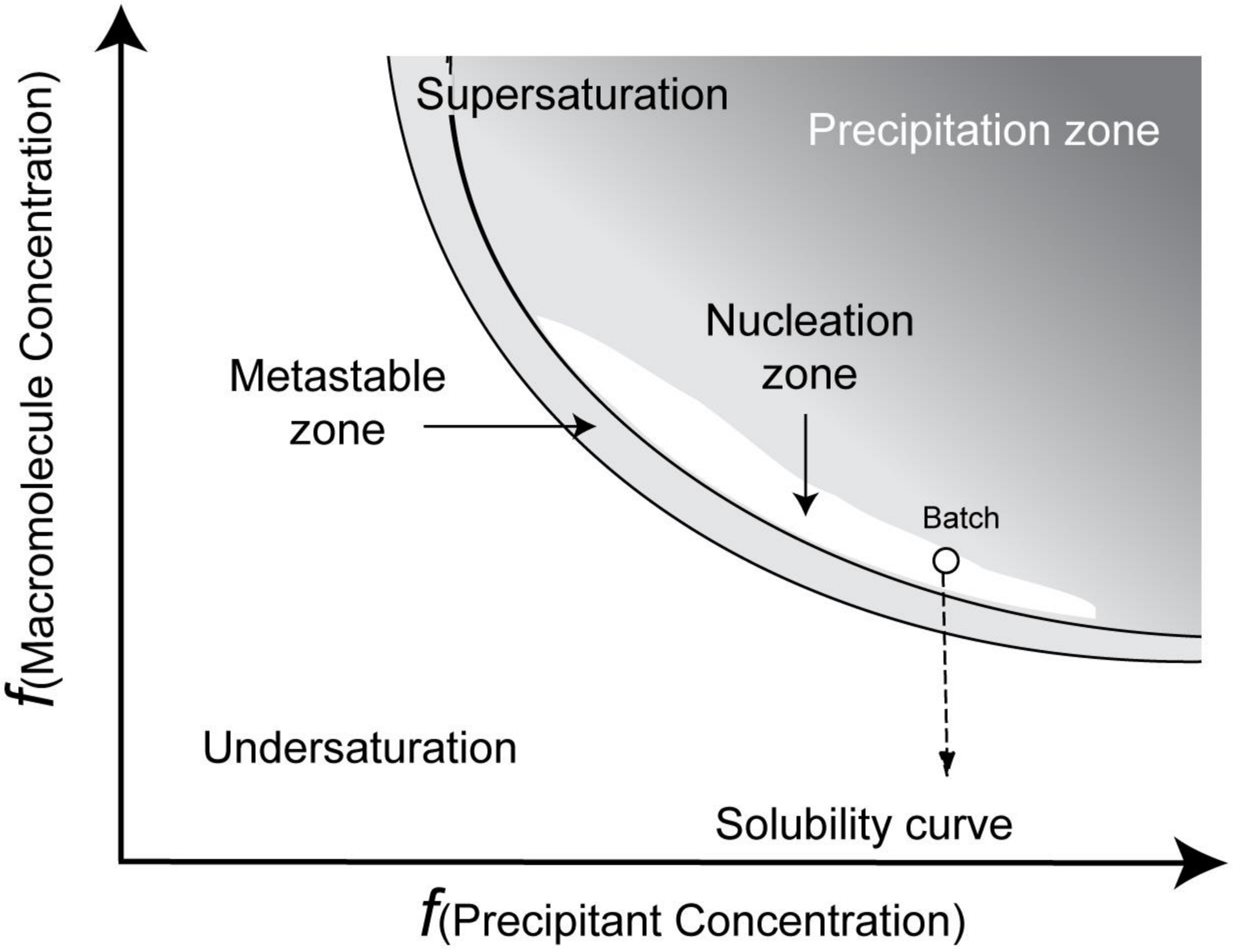
Even simpler phase diagram for crystallization



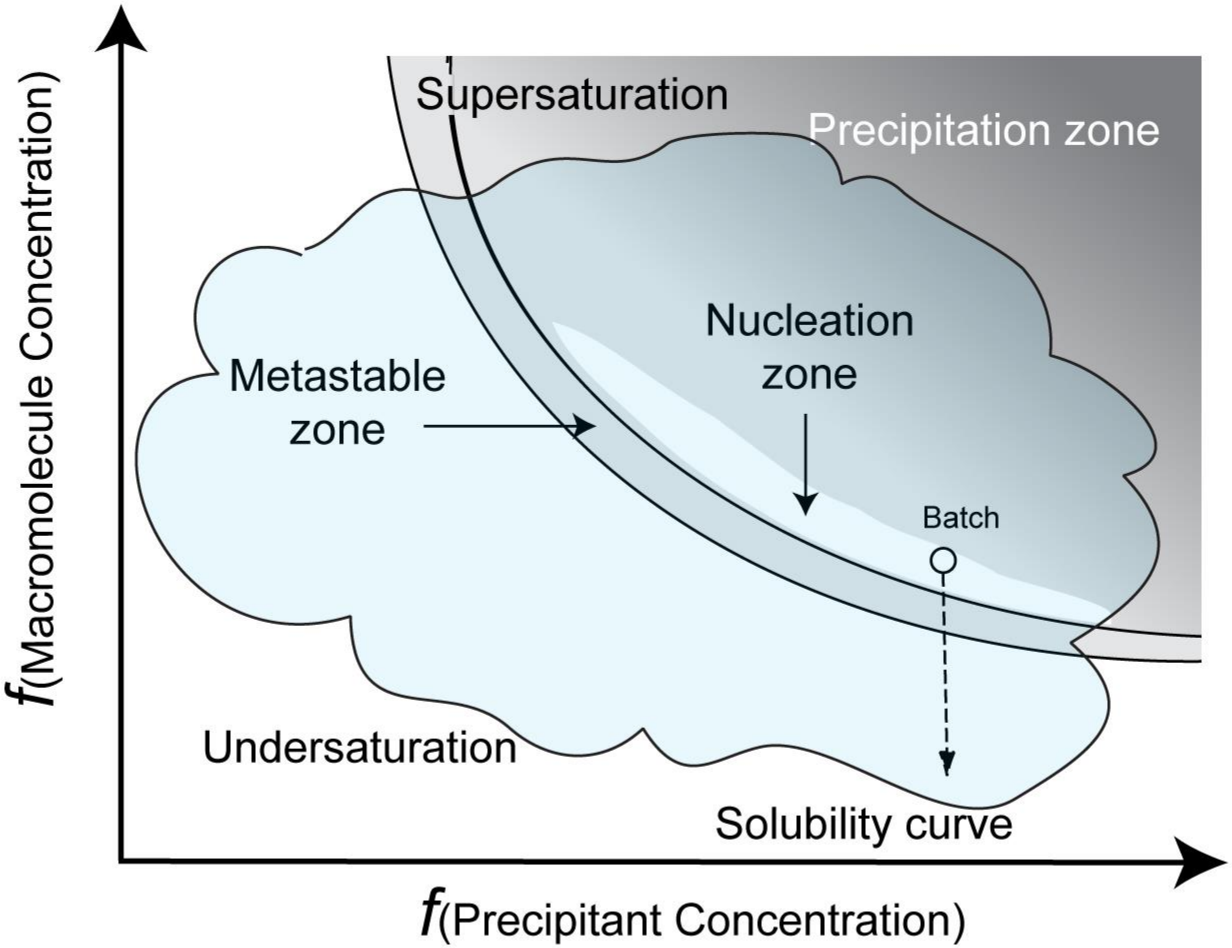
Start to throw some reality into the equation



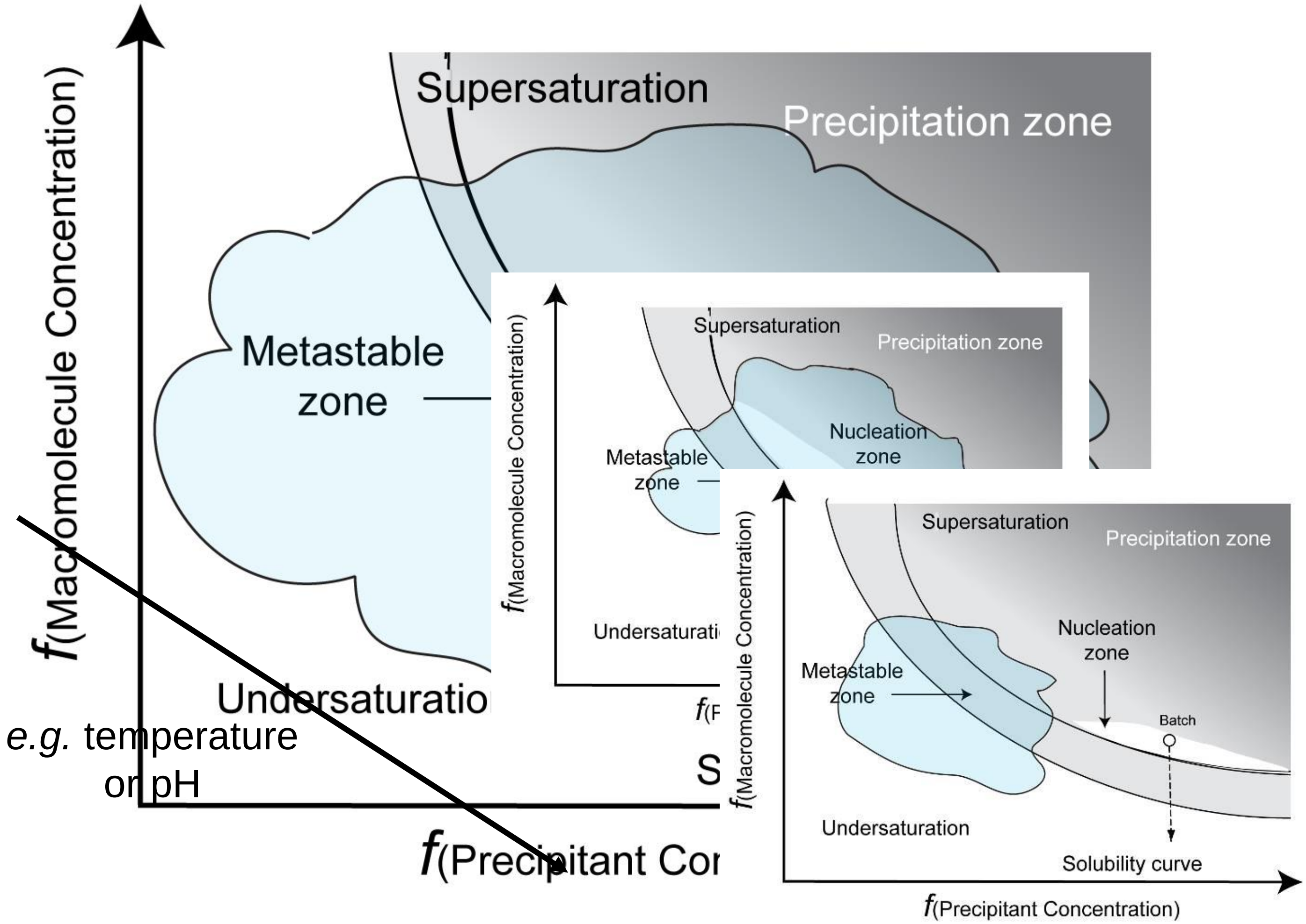
And reduce the chances of crystallization a little



Add the experimental space we sample



And the fact that it's not just two dimensions



Lets introduce a typical crystallographer …

Wile E. Coyote (Genius)

Overconfidentii Vulgaris

(Cristali Coltivatore Optimista)

And the crystal of interest …

Road Runner
(Beep beep)

Disappearialis Quickius

(Cristallio Perfetto)

And how the rules of the crystallographer relate to crystallography …

1. **Road Runner cannot harm the Coyote except by going "Beep! Beep!"**
2. **No outside force can harm the Coyote - only his own ineptitude or the failure of Acme products.**
3. **The Coyote could stop anytime - If he was not a fanatic.**
4. **No dialogue ever, except <u>"Beep! Beep!"</u>**
5. **Road Runner must stay on the road - for no other reason than that he's a roadrunner.**
6. **All action must be confined to the natural environment of the two characters -- the southwest American desert.**
7. **All tools, weapons, or mechanical conveniences must be obtained from the Acme Corporation.**
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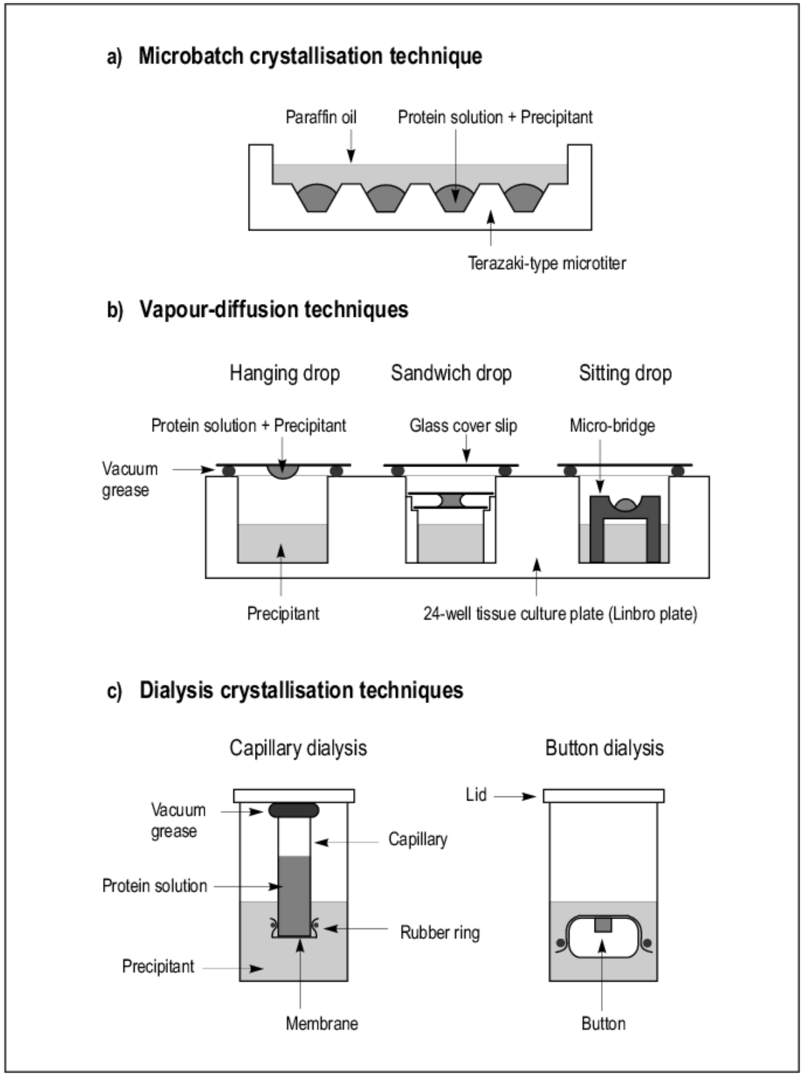
Crystallizing Macromolecules



Many different methods but they all have things in common:

- They are designed to traverse the crystallization phase diagram.
- They use many different kinds of solutions to sample crystallization space at many points.

Catching Road Runners

Growing Crystals

HAMPTON RESEARCH

Molecular Dimensions Limited

Jena Bioscience

QIAGEN

Crystallization is complex

How do we grow crystals?

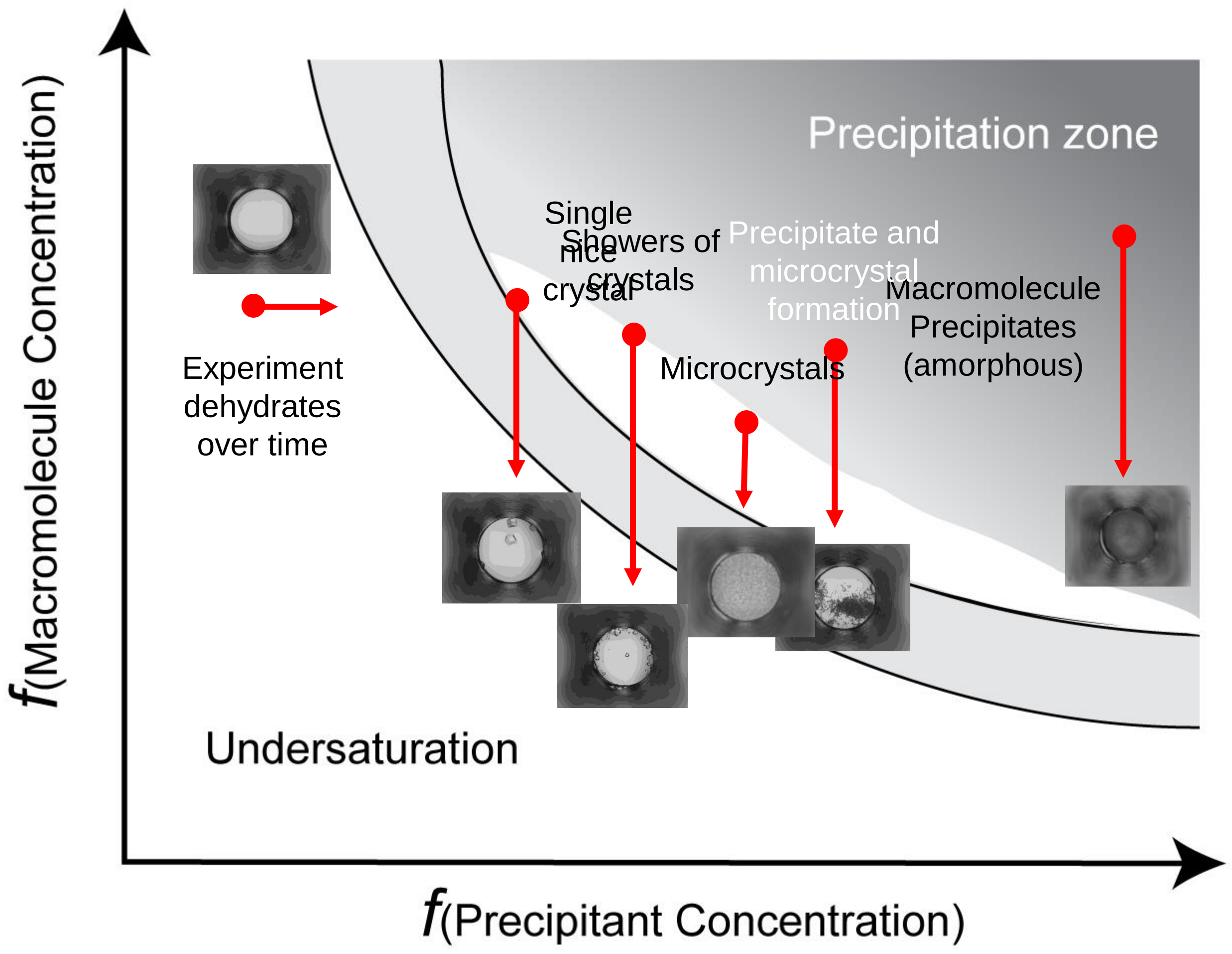
- Multiple guess?
- Intelligent design?

Set up many small scale experiments in conditions likely to be favorable for crystallization

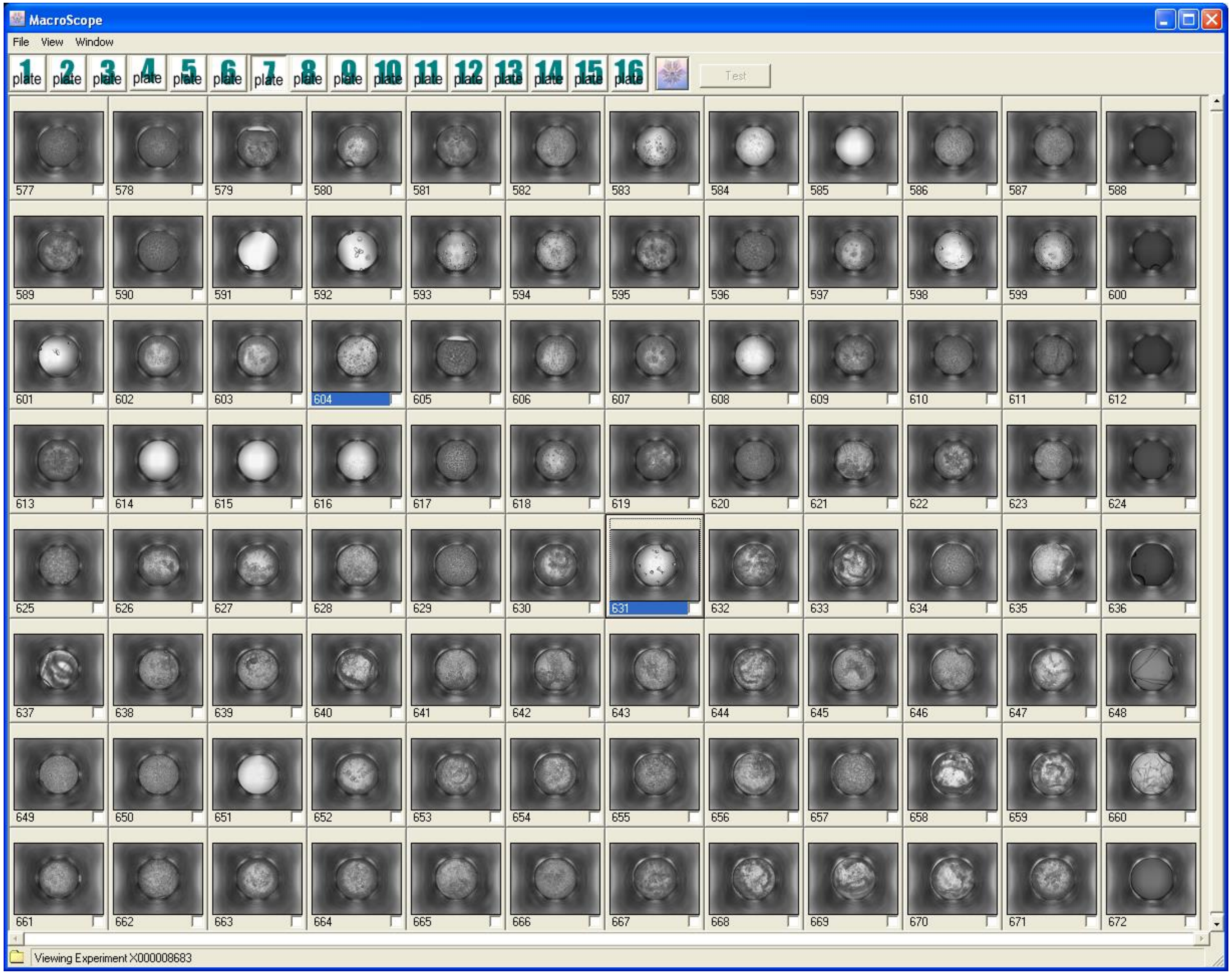
- Limited by amount of sample, time and effort.
- How many conditions is optimum? Divergent views (we'll return to this later)

Lets do the experiment

What results can we expect to see?



What do we actually see?



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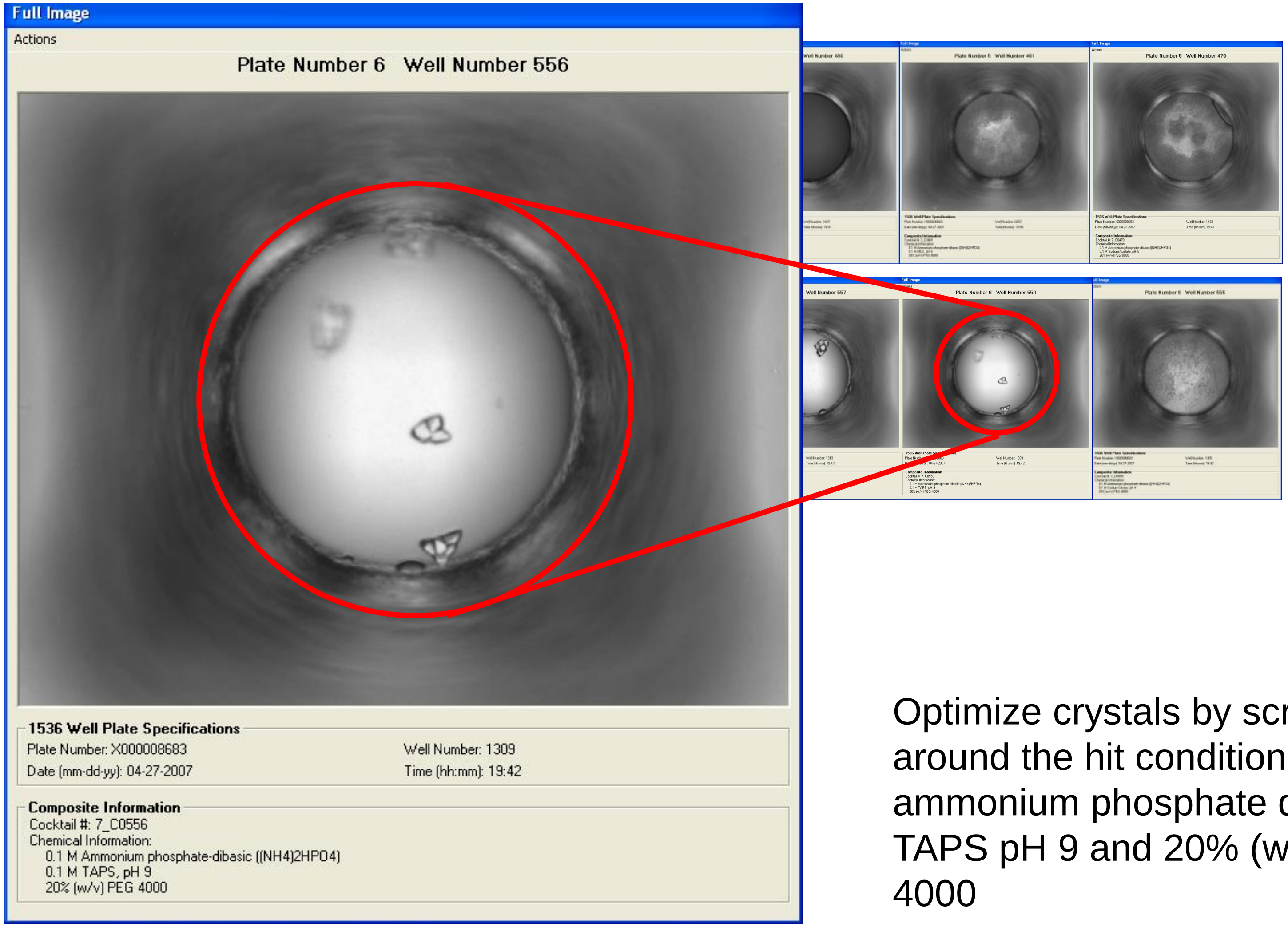

Optimize crystals by screening around the hit conditions, *i.e.* 0.1 M ammonium phosphate dibasic, 0.1 TAPS pH 9 and 20% (w/v) PEG 4000

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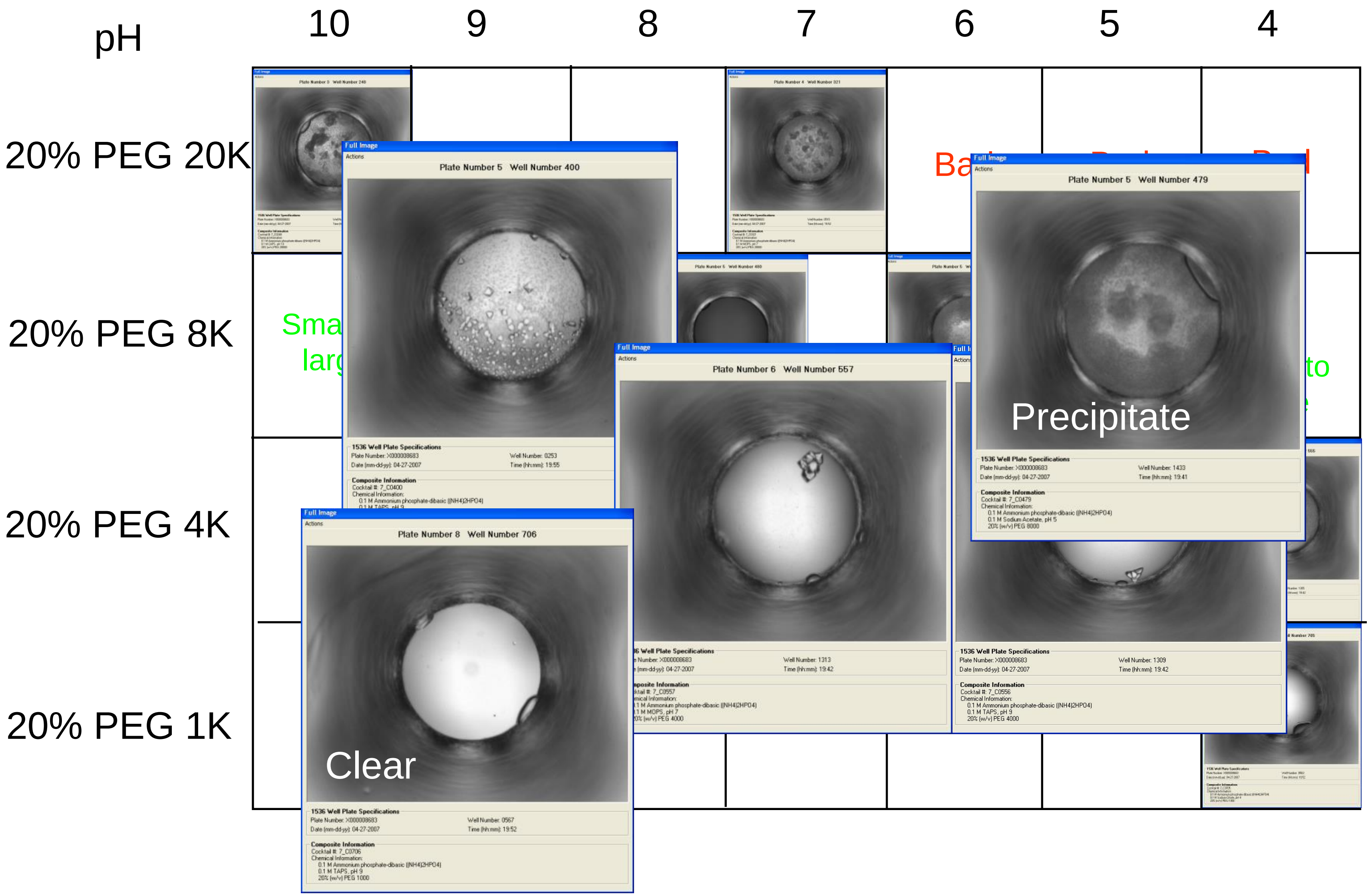
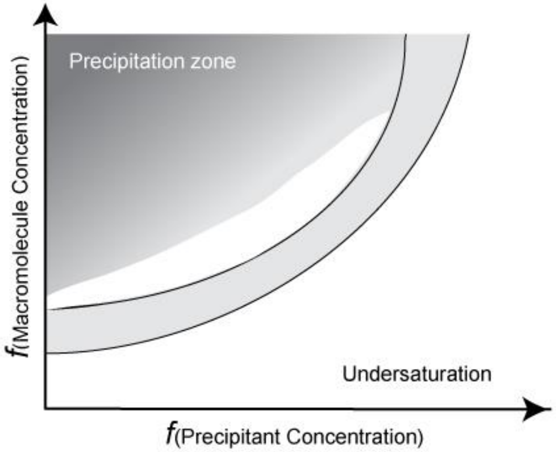
If we plot the results in chemical space the road becomes clear

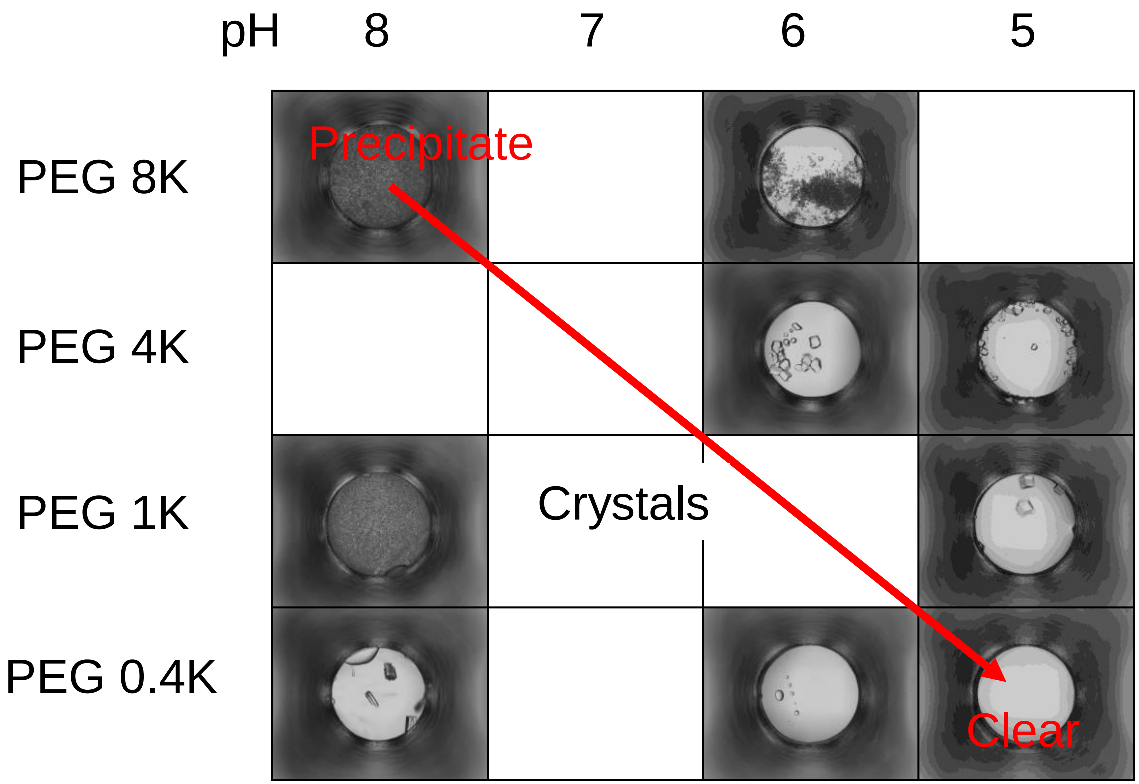


Chemical space provides a vector for optimization

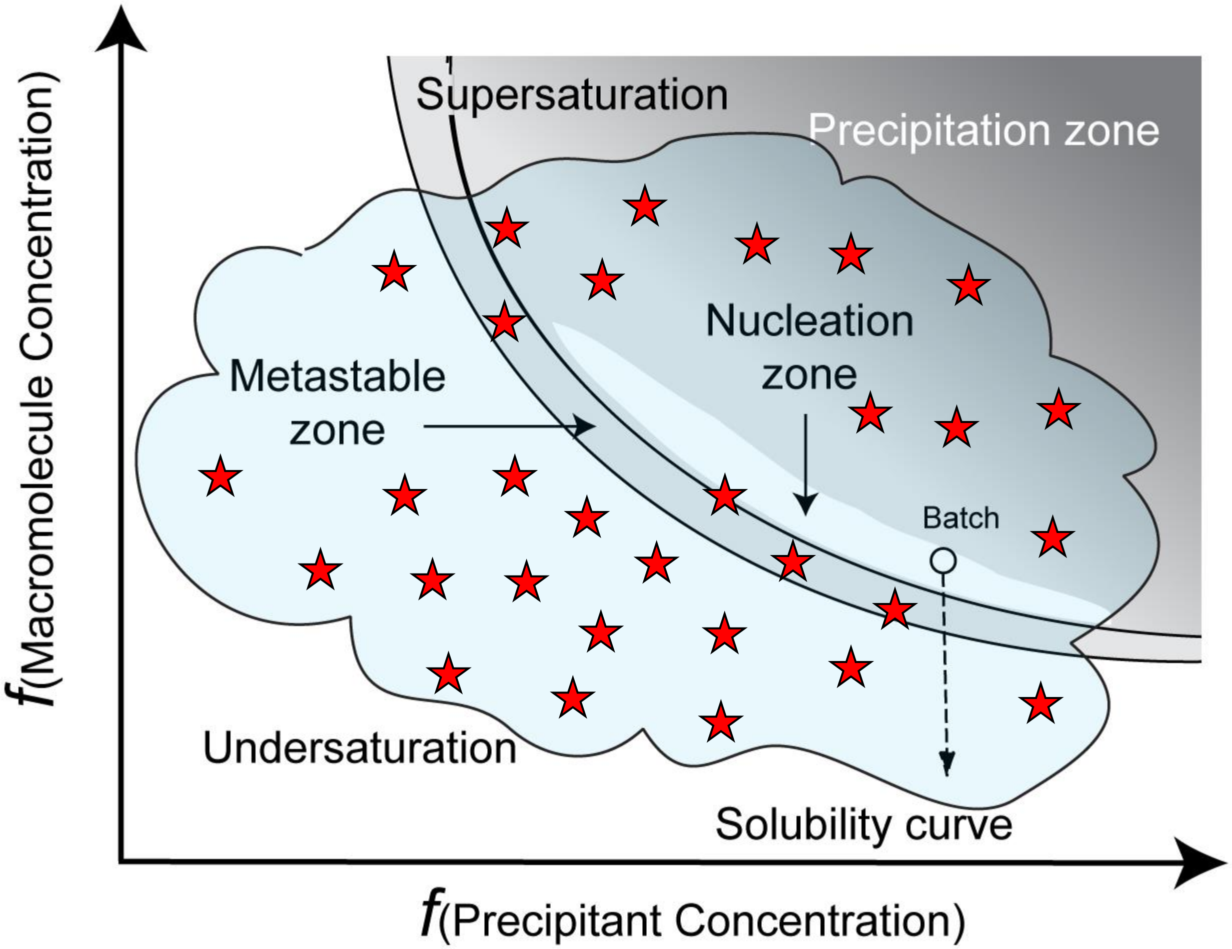
In this case the path from precipitate through crystals to clear is obvious. The phase diagram is reversed. Also clear are the number of chemical conditions that have not been sampled.



Ubiquitin, 40% PEG, 0.1M zinc acetate

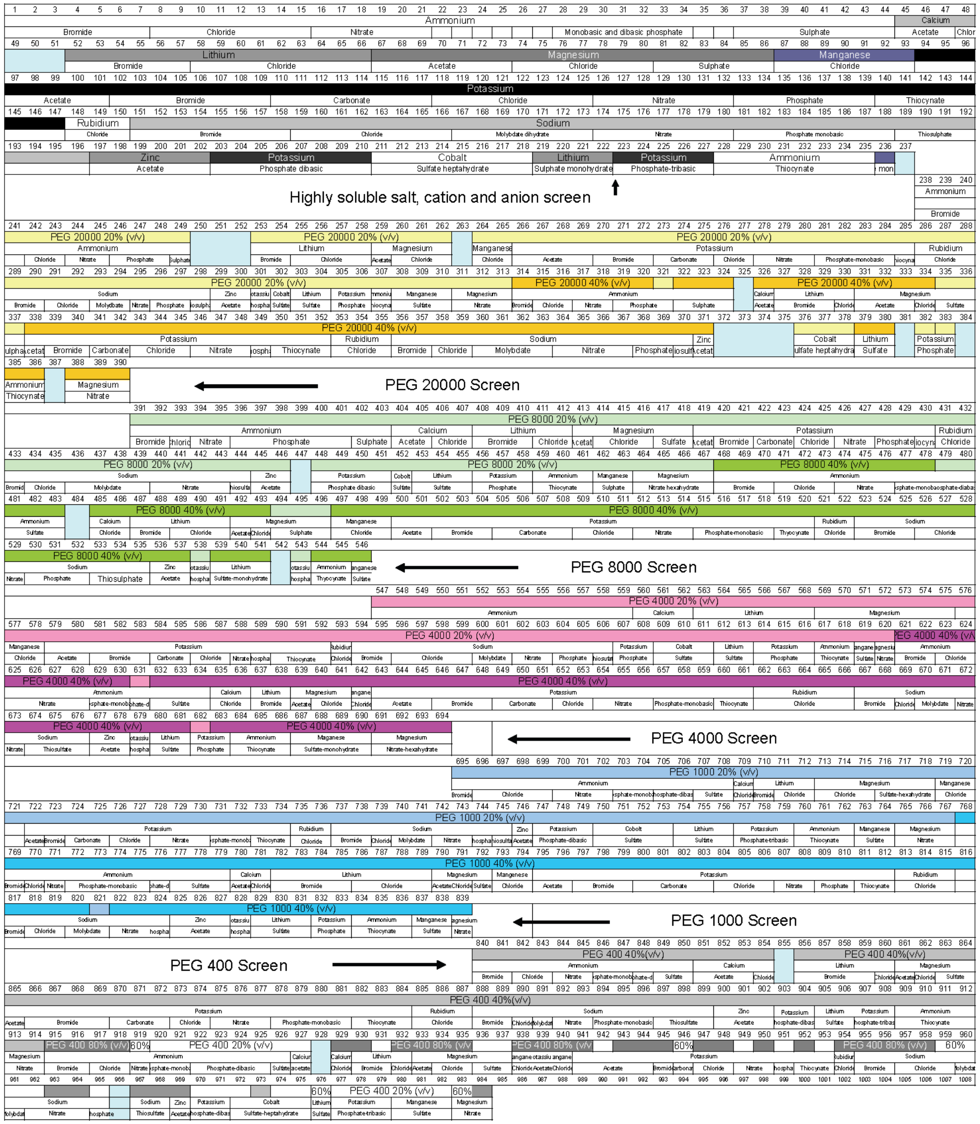


It also illustrates the space we do <u>not</u> sample



We only sample discrete points within the sampling space

The HWI crystallization cocktail screen.



The 1536 diverse chemical cocktails (Luft et al., 2003). The 984 in-house conditions comprise a incomplete factorial sampling of 36 salts, eight buffers, and 5 different PEGs.

The remainder of 1536 cocktails are comprised of commercial screens available from Hampton Research. Specifically, in order of use; the Natrix Screen, Quick Screen, Nucleic Acid Screen, Sodium Malonate Grid, PEG/Ion, PEG 6000 Grid, Ammonium Sulfate Grid, Sodium Chloride Grid, HT Screen, Index and the SaltRx screen.

The Commercial Screens in the HWI crystallization cocktails

The commercial screens incorporate several distinct mechanisms of sampling the crystallization space. Examples are shown here.

The original Hampton Research 1+2 sample a set of conditions known to produce crystals in the past with the predominant variable being pH. Although described as a sparse matrix the number of samples is small and the distribution in chemical space wide therefore it is difficult to relate results from one condition to results from other conditions. This is the primary reason that crystallization today is target focused.

Salt RX				
Magnesium				
Formate dihydrate	0.4M	B11	B12	C1
	0.7M		C2	
Sulfate hydrate	1.0M	G1	G2	G3
	1.8M	G4	G5	G6
Lithium				
Sulfate monohydrate	0.8M	F7	F8	F9
	1.0M		F11	
	1.5M	F10		F12
Potassium				
Sodium tartrate	0.6M		H1	H3
	1.2M		H2	H4
Thiocynate	0.5M	H5	H6	H7
DL-Malic acid				
	1.2M		C9	
	2.2M		C10	
Succinic acid				
	0.5M		E11	
	1.0M		E12	
Tacsimate				
	35%		H11	
	60%		H12	
		pH		
		5	6.9	8.2
Sodium	1.0M	E1	E2	E3

The SaltRx screen samples 22 crystallization salts with varying concentration and pH. A true sparse matrix where conditions can be related in terms of chemical space.

None						
A2						
A3						
C6						
C7						
C8						
C9						
D6		D11		C1	C5	C12
D7				C4	C11	D12
D8				D9	D2	
				D10	D3	
					D5	
E1		E9	F2	F8	G4	H3
E2		E10	F3	F9	G5	H4
E3		E11	F4	F10	G6	H5
E4		E12	F5	F11	G7	H6
E5		F1	F6	F12	G8	H7
E6			F7	G1	G9	H8
E7				G2	G10	H9
E8				G3	G11	
G4					G12	
					H1	

A number of Grid screens are incorporated, in this case Sodium Chloride. These provide a fine sampling of a small subset of individual conditions and serve to indicate the sensitivity (or lack of it) to small changes in precipitant conditions.

Sodium Chloride						
Conc	pH					
(M)	4	5	6	7	8	9
1	A1	A2	A3	A4	A5	A6
2	B1	B2	B3	B4	B5	B6
3	C1	C2	C3	C4	C5	C6
4	D1	D2	D3	D4	D5	D6

A special case – The Hampton Research Index Screen

Hampton Research Index Screen

Note, the HT screen is not a convential screen as such. It is designed to sample a range of reagents and provide an indication of the appropiate chemical area and variables that w ould be appropiate for crystallization and should be used in this manner.

pH	Ammonium Sulfate 2.0M	Sodium chloride 3.0M	Magnesium formate dihydrate 0.3M	Magnesium formate dihydrate 0.5M
3.5	A1	A7		
4.5	A2	A8		
5.5	A3	A9	B1	
6.5	A3	A10		B2
7.5	A5	A11	B3	
8.5	A6	A12		B4

Classic salt versus pH

Hits here indicate that a variation of salt concentration and pH in a grid screen has a strong potential for crystallization

pH	Sodium phosphate
5.6	B5
6.9	B6
8.2	B7

Neutralized organic acids (ph 7.0)
B9
B10
B11
B12
C1
C2
C3
C4
C5

pH	High supersaturation salt and low polymer
5.5	C8
6.5	C6
8.5	C7
7	C9
	C10
	C11
	C12

pH	Low ionic strength systems
3.5	D4
4.5	D5
5.5	D6
6.5	D7
	D10
	D11
7	D2
	D3
7.5	D8
8.5	D9

pH	Non-volatile organics
5.5	D12
	E2
6.5	E1
	E3
	E6
	E9
	E10
7.5	E4
	E7
	E8
	E11
8.5	E5
	E12

PEGs and Salts as a function of pH

	3.35K					10K	3.35K
pH	Ammonium sulfate	Sodium chloride	Lithium sulfate monohydrate	Ammonium acetate	Magnesium Chloride hexahydrate	Ammonium acetate	Mixed chloridehydrates
5.5	F6	F10	G2	G6	G10	F5	
6.5	F7	F11	G3	G7	G11		
7.5	F8	F12	G4	G8	G12		F4
8.5	F9	G1	G5	G9	H1		

PEG 3350 and salts

%	Potassium sodium tartrate tetrahydrate	Sodium malonate pH 7.0	Ammonium citrate tribasic pH 7.0	Succinic acid pH 7.0	Sodium formate	DL-Malic acid pH 7.0	Magbesium formate dihydrate	Zinc acetate dihydrate	Sodium citrate tribasic dihydrate	Potassium thiocyanate	Potassium bromide
15				H5			H8				
20	H2	H3	H4		H6	H7		H9	H10		
25											
30										H11	H12

Coarse test for chemical conditions likely to produce crystallization

Sherlock and Watson.

"We approached the case, you remember, with an absolutely blank mind, which is always an advantage. We had formed no theories. We were simply there to observe and to draw inferences from our observations"

Sherlock Holmes to Dr. Watson

I never get your limits, Watson. There are unexplored possibilities about you.

Sherlock Holmes on Dr. Watson.

Two pieces of related software under development;

- Sherlock to look at the individual 'crime', *i.e.* examine results from a single macromolecule
- Watson to tell the complete story, *i.e.* look at trends from many experiments.

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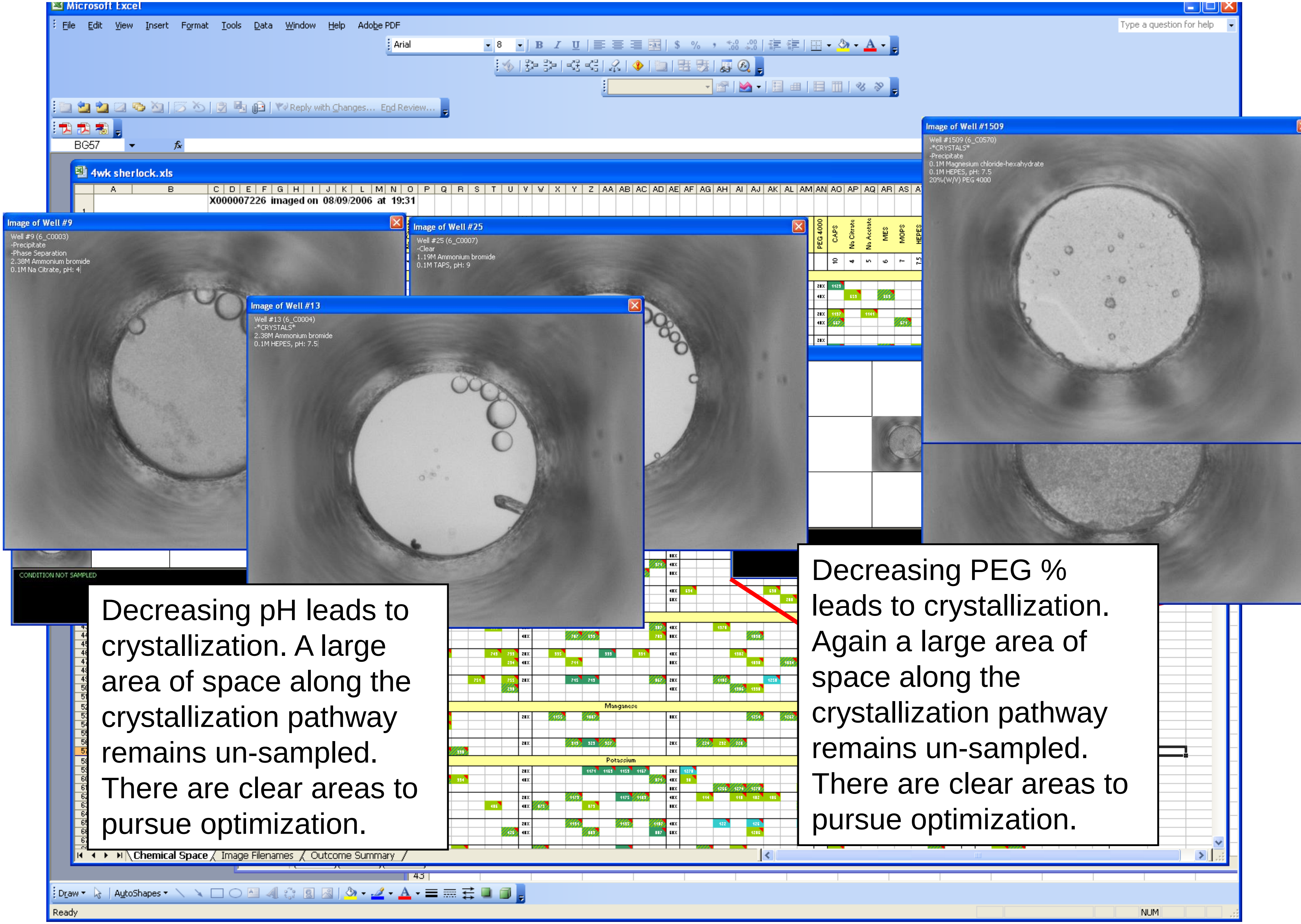
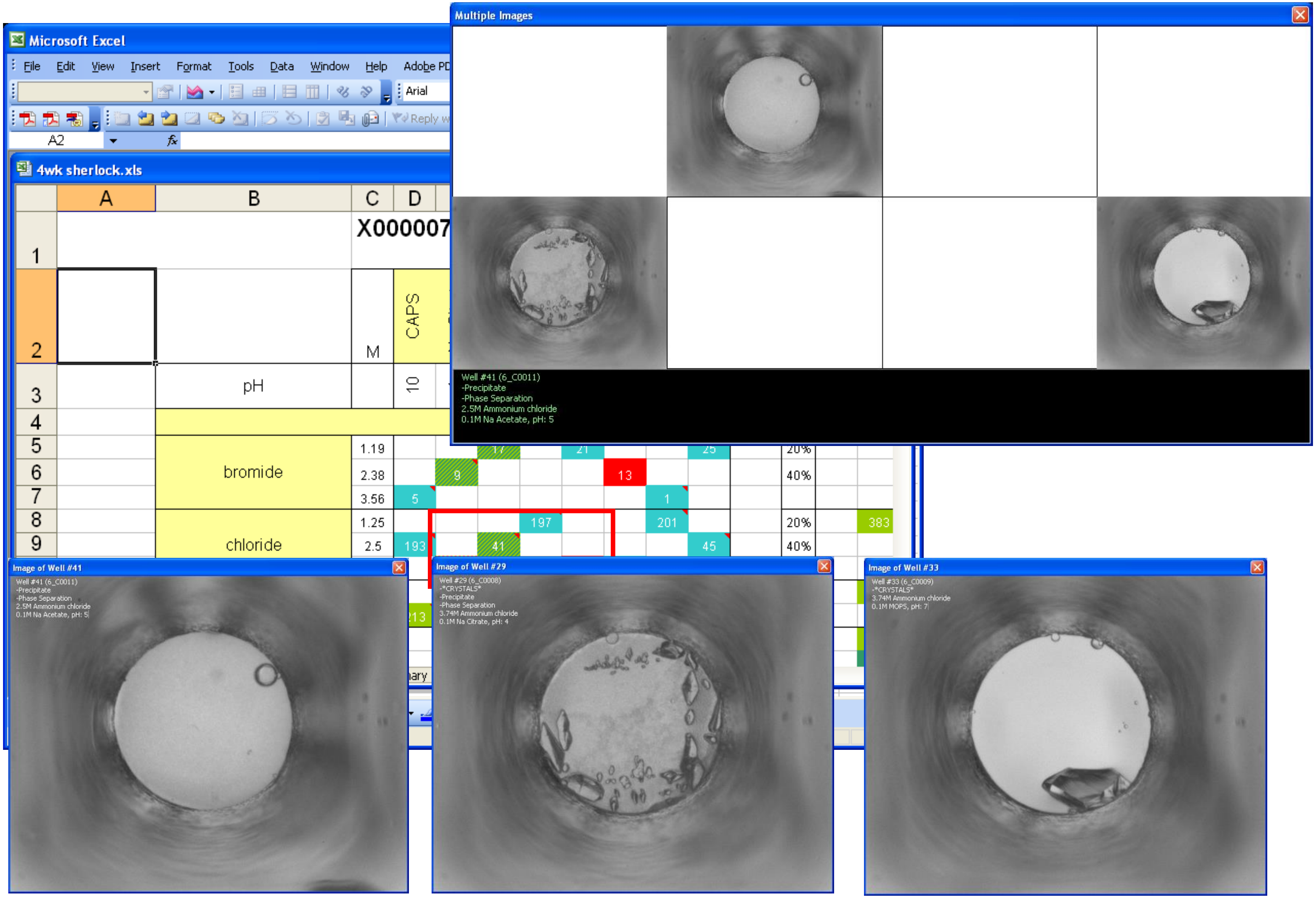


Decreasing pH leads to crystallization. A large area of space along the crystallization pathway remains un-sampled. There are clear areas to pursue optimization.

Decreasing PEG % leads to crystallization. Again a large area of space along the crystallization pathway remains un-sampled. There are clear areas to pursue optimization.


Microsoft Excel
File Edit View Insert Format Tools Data Window Help Adobe PD
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X000007
CAPS
M
pH
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bromide
chloride
1.19
2.38
3.56
1.25
2.5
20%
40%
20%
40%
9
13
5
1
197
201
383
193
41
45
Multiple Images
Well #41 (6_C0011)
-Precipitate
-Phase Separation
2.5M Ammonium chloride
0.1M Na Acetate, pH: 5
Image of Well #41
Well #41 (6_C0011)
-Precipitate
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2.5M Ammonium chloride
0.1M Na Acetate, pH: 5
Image of Well #29
Well #29 (6_C0008)
-*CRYSTALS*
-Precipitate
-Phase Separation
3.74M Ammonium chloride
0.1M Na Citrate, pH: 4
Image of Well #33
Well #33 (6_C0009)
-*CRYSTALS*
3.74M Ammonium chloride
0.1M MOPS, pH: 7

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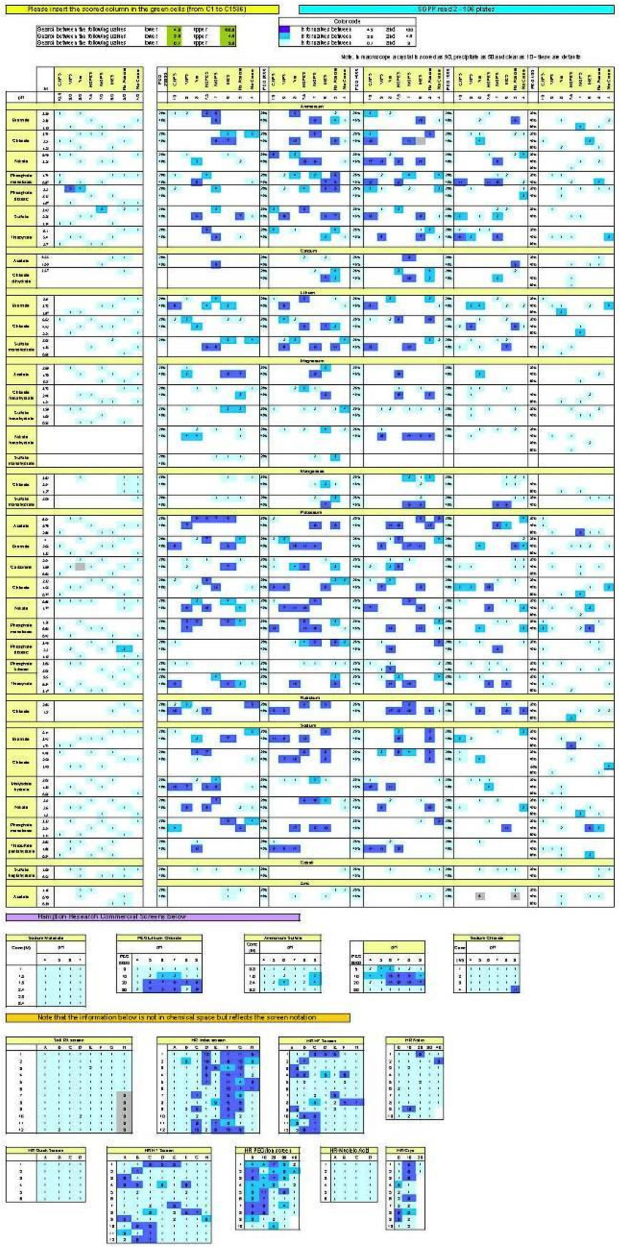
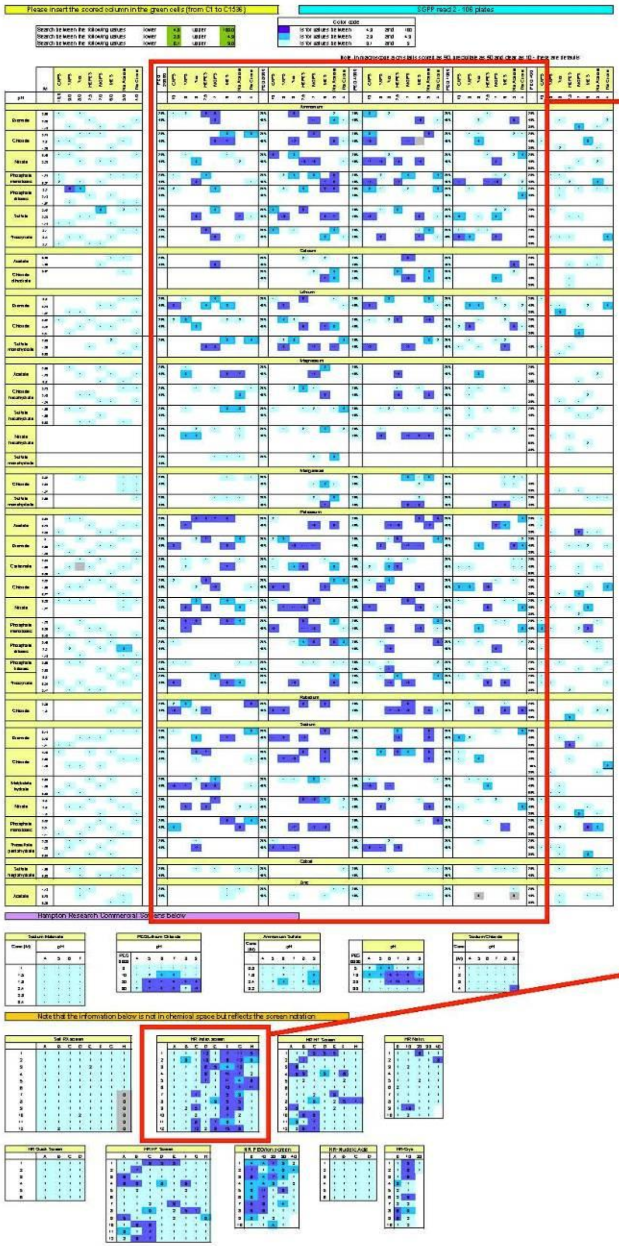


Watson looks at the complete picture rather than an single 1536 screen of one macromolecule.

In this case crystallization results for 106 macromolecues are shown several weeks into their growth. Only those samples showing crystals are tabulated here..

Dark blue indicates 5 or more crystal hits in the 106 conditions, medium blue is 3-4 hits and light blue is 1-2. Grey shows other conditions sampled (only 3 conditions) while white spaces are un-sampled regions of the incomplete factorial.



PEG 20K to 1K component of the screen

The index screen is working, it shows that PEG is a good chemical area to promote crystallization

Hampton Index Screen

Sherlock and Watson – Current Status

- Sherlock is currently being tested in the High-Throughput laboratory. The aim is to release it to external users as a beta version in the near future.
- There are several possible representations of chemical space available, only one was shown here.
- Currently it requires manual scoring of images. Developments in automated image analysis look very promising and there is near certainty that we can automatically score clear and precipitate images leaving a much smaller number of images to visually examine. Other research is underway to automatically score these as well.

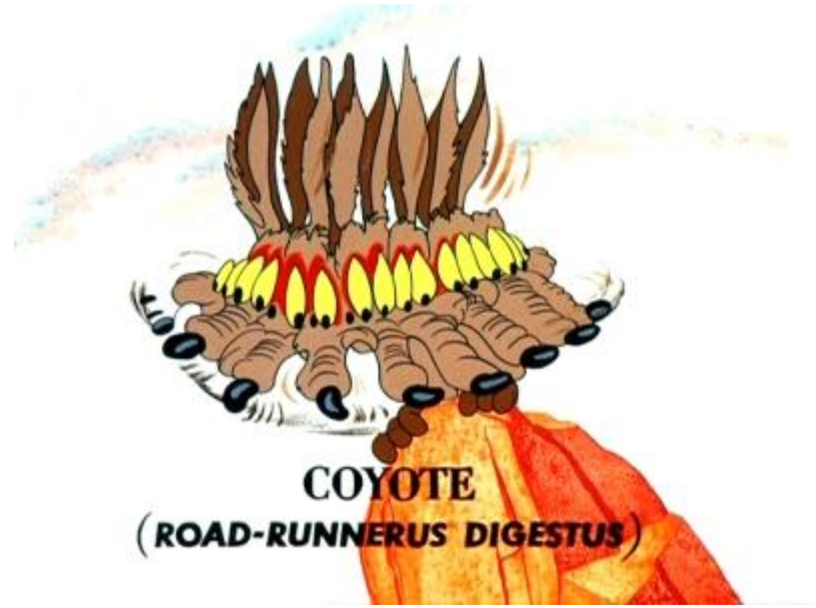
- Watson is under development and at present is only being used by a limited number of testers to analyze the performance of the HWI cocktails and commercial screens used in the laboratory.

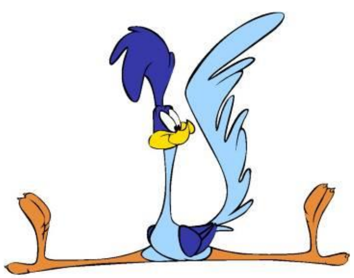
Future work

- To automatically flag patterns that may indicate potential regions for further exploration if a crystallization hit does not occur. For example, two results showing clear and precipitate separated by a long un-sampled chemically sensitive pathway.
- To produce separate programs for other screens.
- To incorporate time or temperature resolved data, predict the best optimization strategies or aid the interpretation of current optimization techniques such as Drop Volume Ratio/Temperature (DVR/T) Luft et al., 2007.

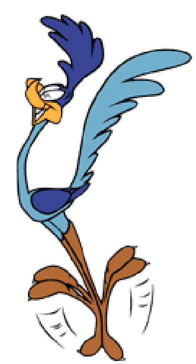
How many samples?

In using chemical space mapping to analyze a number of samples it has become clear that 1536 is a good number of experiments to try. It enables a wide range of chemical space to be investigated with sufficient detail to identify common regions for crystallization together with diversely separated regions where different crystal forms may result





It is important to investigate not a single hit but as many hits as you have sample. Visual observation only indicates a crystal, not that it diffracts well or even if it is a macromolecular crystal rather than salt or PEG. Spreading the effort among many hits is better than focusing exclusively on one.



Summary

- No experiment should be considered in isolation.
- In crystallization screening when you have a sparse matrix, incomplete factorial or any other designed sampling of chemical space the results build up a picture of the crystallization landscape.
- An experiment with no crystallization hits that which generates both precipitate and clear conditions is promising when those conditions are separated by an un-sampled chemically sensible direction.
- You should know what crystallization conditions you examined but more importantly how those relate to those that were not sampled.
- Optimize as many samples as you can.
- Check with X-rays as soon as possible.
- The axis of crystallization space have a complex relationship with those in chemical space. We have a limited understanding of those relationships and hopefully Watson will reveal a better story from the >9000 cases we currently have.
- There are many more variables to explore!

Questions?