What's in a drop? Correlating observations and outcomes to guide crystallization

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Only approximately 11% of the proteins we target for crystallography yield a crystallographic structure.

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On the need for an international effort to capture, share and use crystallization screening data

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When crystallization screening is conducted many outcomes are observed but typically the only trial recorded in the literature is the condition that yielded the crystal(s) used for subsequent diffraction studies. The initial hit that was optimized and the results of all the other trials are lost. These missing results contain information that would be useful for an improved general understanding of crystallization. This paper provides a report of a crystallization data exchange (XDX) workshop organized by several international large-scale crystallization screening laboratories to discuss how this information may be captured and utilized. A group that administers a significant fraction of the world's crystallization screening results was convened, together with chemical and structural data informaticians and computational scientists who specialize in creating and analysing large disparate data sets. T *Acta Cryst.* (2012). F68 crystallization ontology for the crystallization community was proposed. This paper (by the attendees of the workshop) provides the thoughts and rationale leading to this conclusion. This is brought to the attention of the wider audience of crystallographers so that they are aware of these early efforts and can contribute to the process going forward.

At least 99.8% of crystallization experiments produce an outcome other than crystallization.

In other words, “what’s not in a drop?”

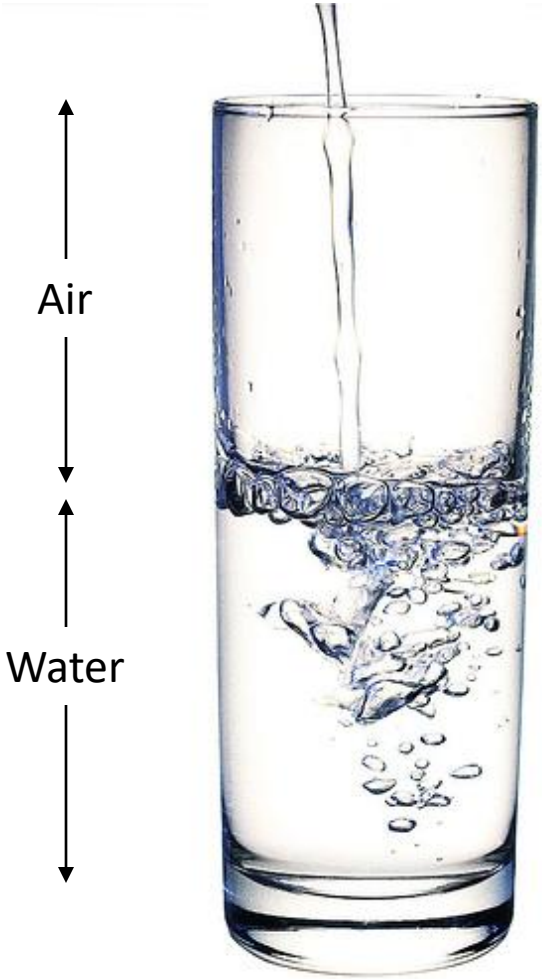
i.e. a crystal

Or simplifying further, “Good and Bad”

and bad can be good!

or ugly

Pessimists, Optimists, and Crystallographers



Consider a glass of water

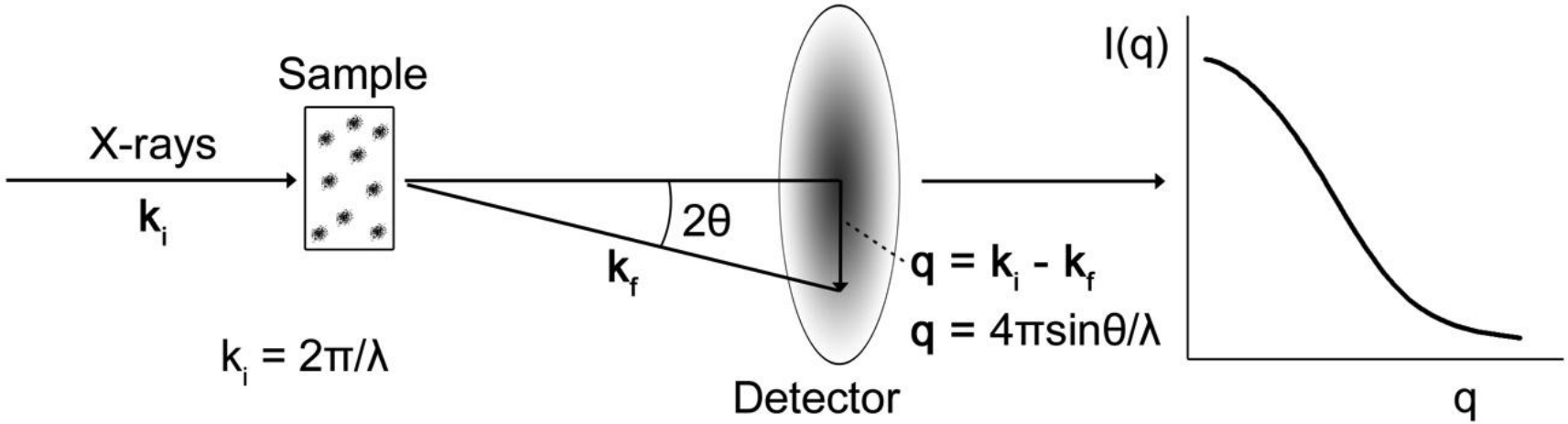
Pessimist
(the glass is half empty)

Optimist
(the glass is half full)

Crystallographer
(the glass is completely full)

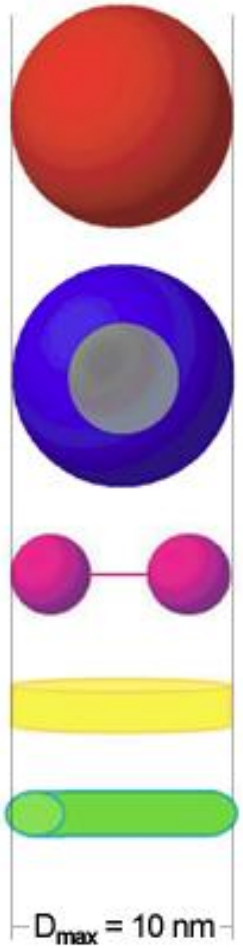
Fantasy

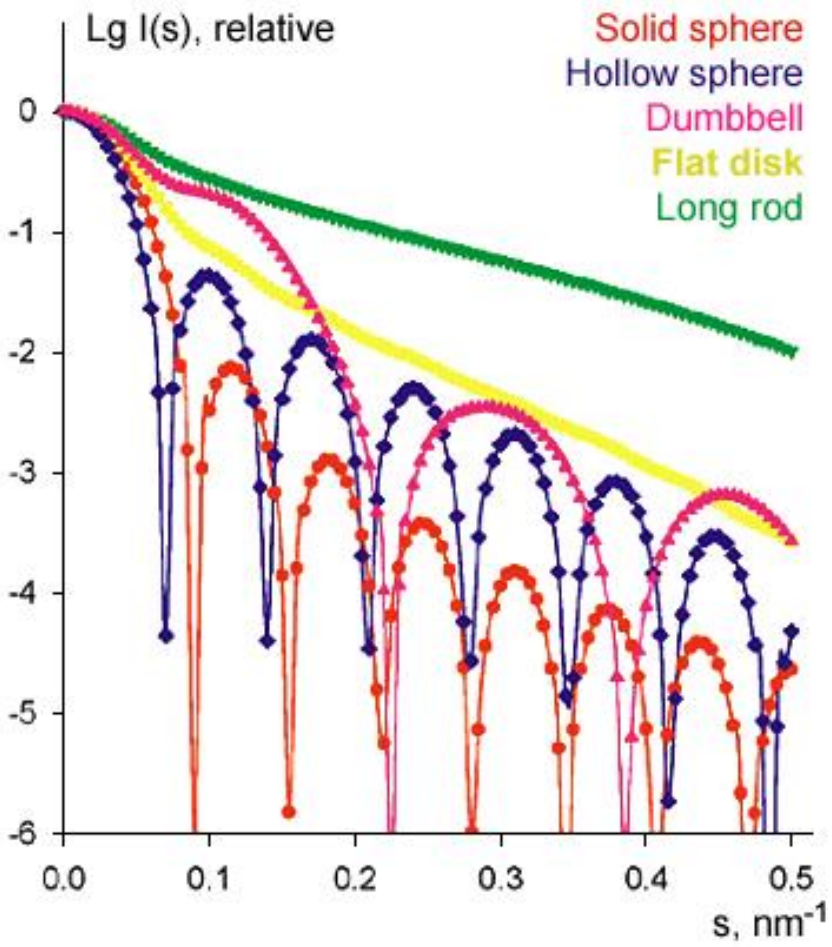
Before Crystallization Screeing



$$I(q) = \int 4\pi r^2 \cdot \bar{\rho}^2(r) \cdot \frac{\sin qr}{qr}\, dr$$

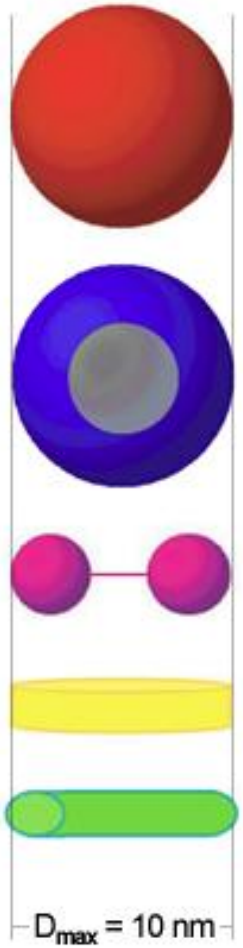
Data

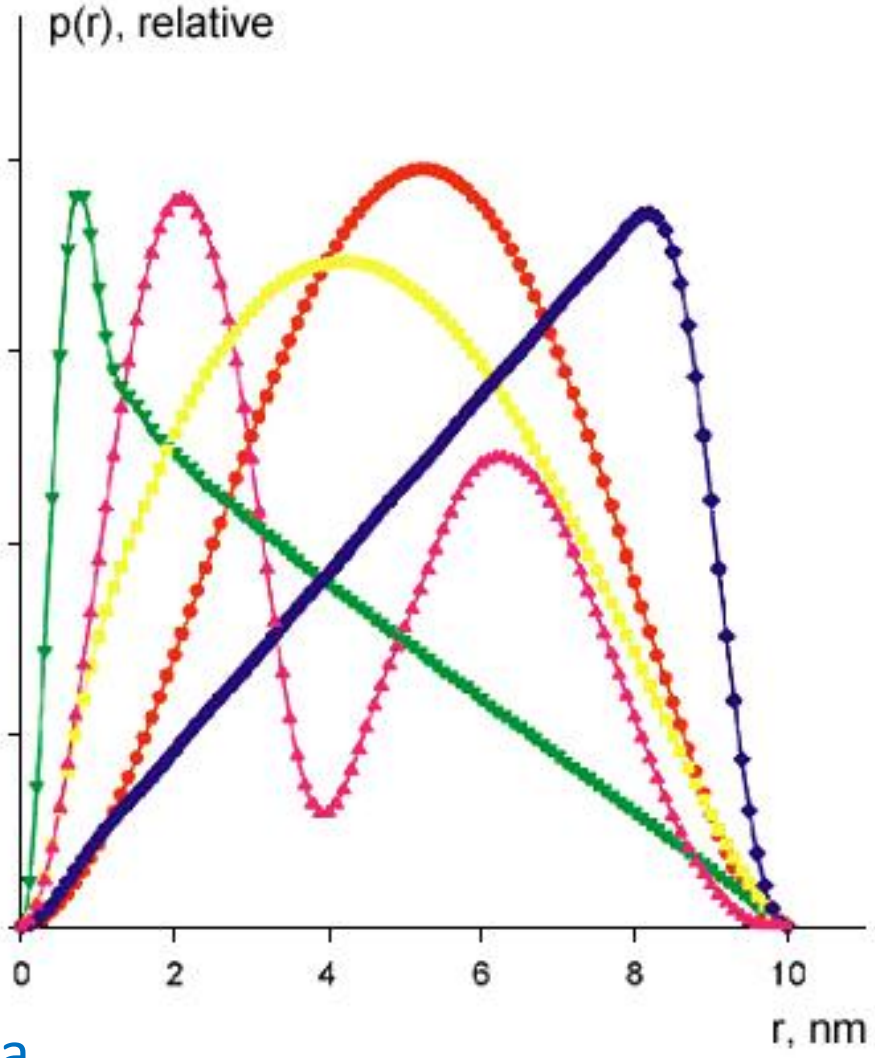




From: Small-angle scattering studies of biological macromolecules in solution, Svergun and Koch, Rep. Prog. Phys., 1735-1782 (2003)

Pair distribution function

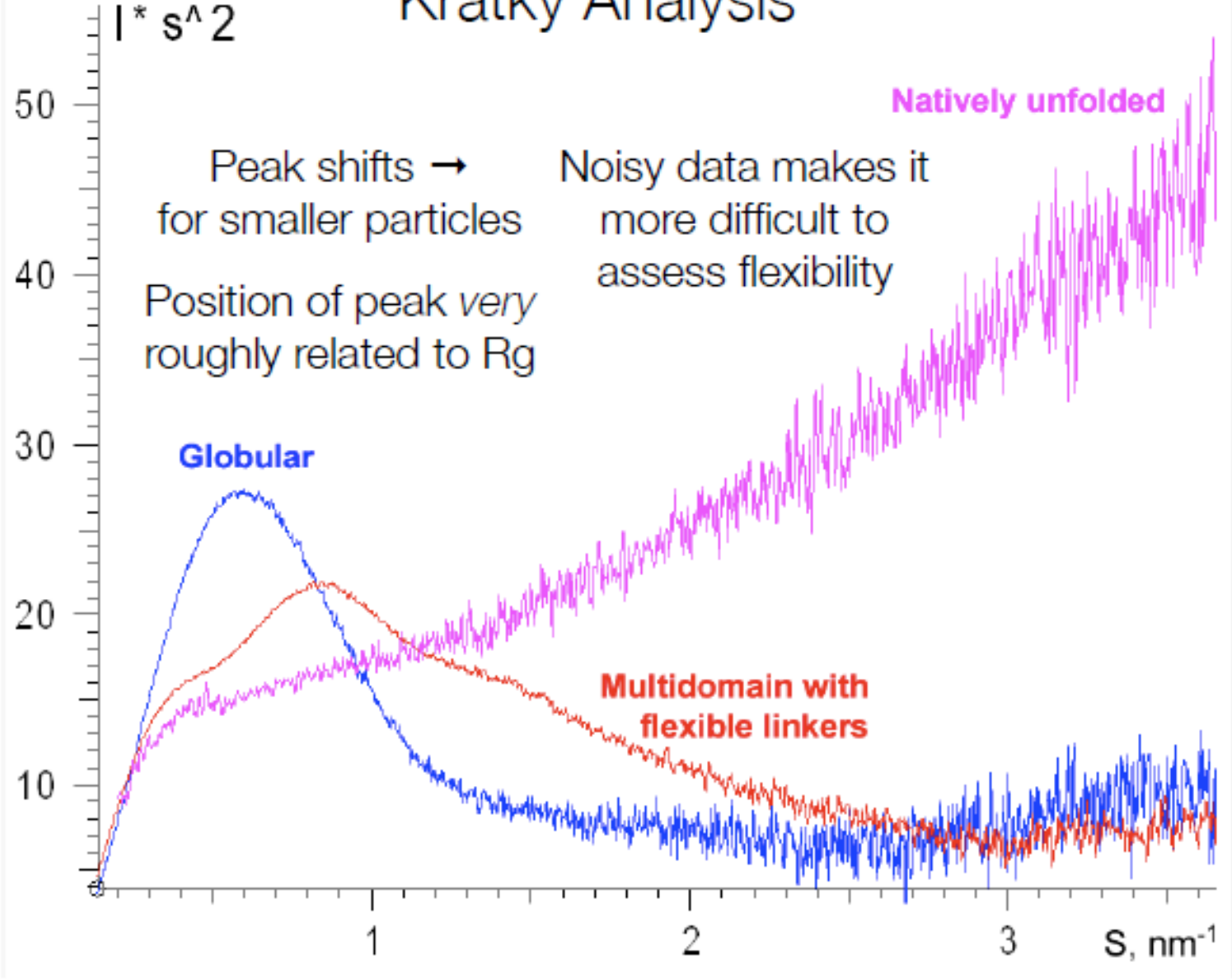




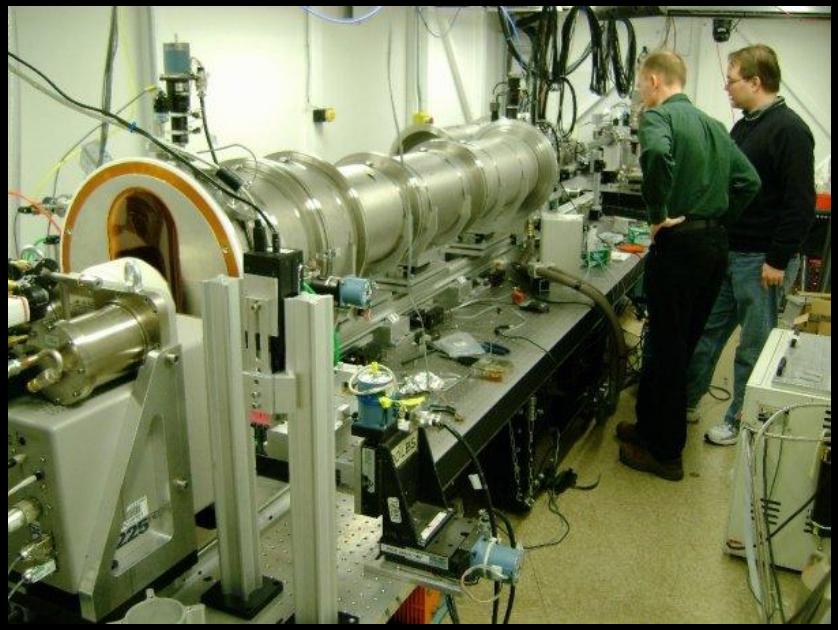
Fourier transform of data.

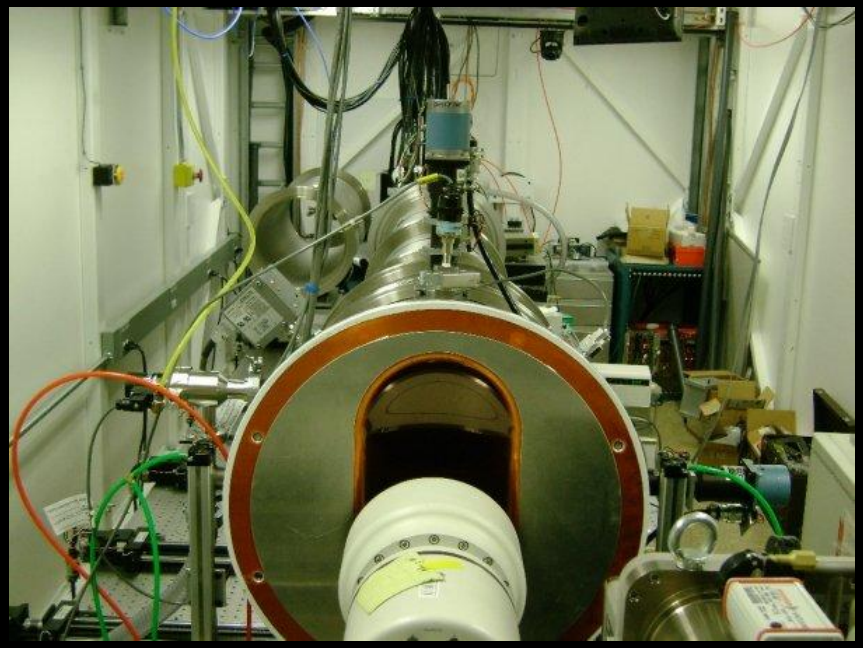
From: Small-angle scattering studies of biological macromolecules in solution, Svergun and Koch, Rep. Prog. Phys., 1735-1782 (2003)

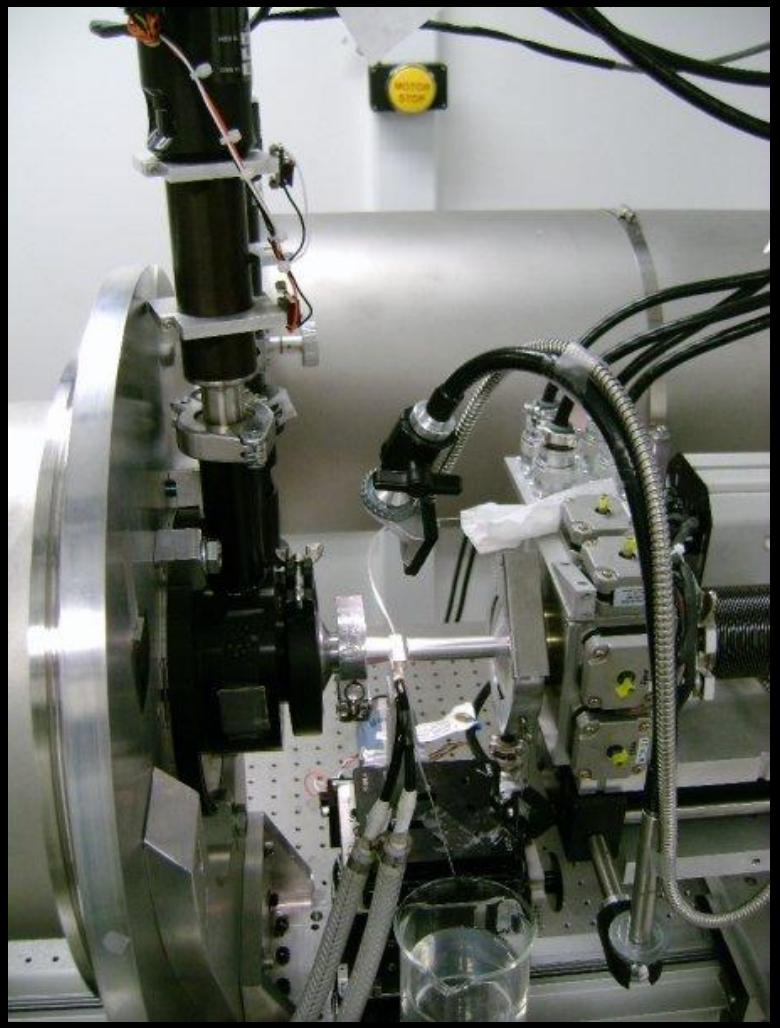
Kratky Analysis



http://www.saxier.org/forum/viewtopic.php?t=337







Beamline 4-2 SSRL

Time per concentration series – approximately 10 to 15 minutes. In high-throughput mode 24 samples in 3 to 4 hours.

Enables two important things – eat and sleep!

High throughput protocol

Up to 12 different PCR strips.

3-7 different concentrations per sample.

For high-throughput studies, 2 samples per strip, 24 samples in total

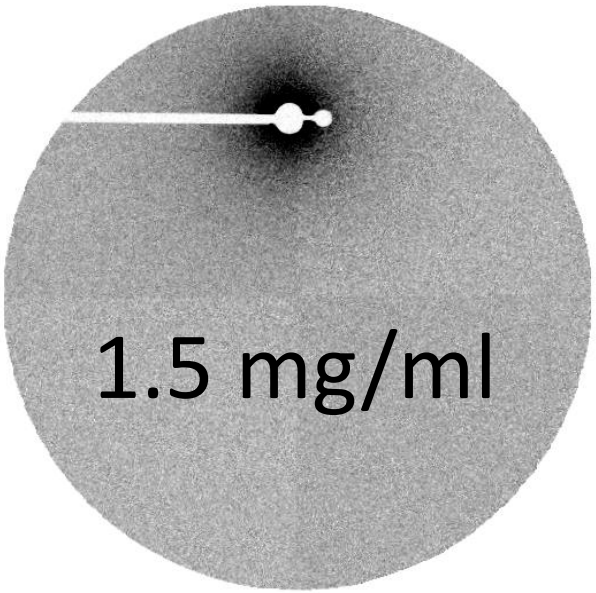
Start with buffer then lowest concentration first. End with buffer

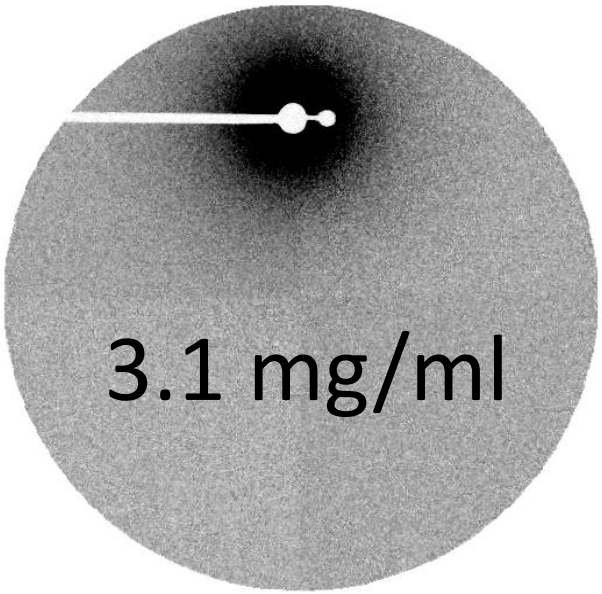
8 exposures, 1-2s each dependent on sample molecular weight, buffer and concentration.

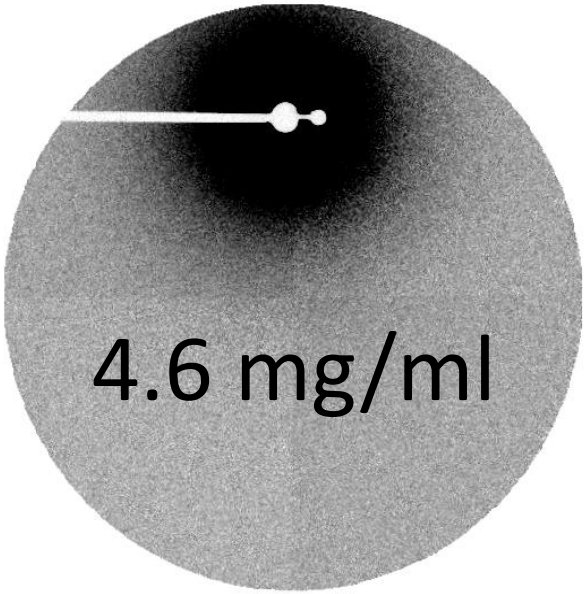
Oscillate sample to minimize radiation damage

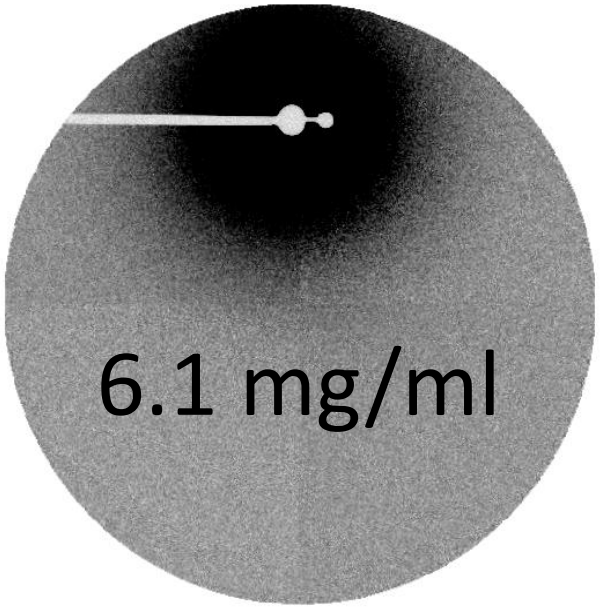
Repeat the buffer.

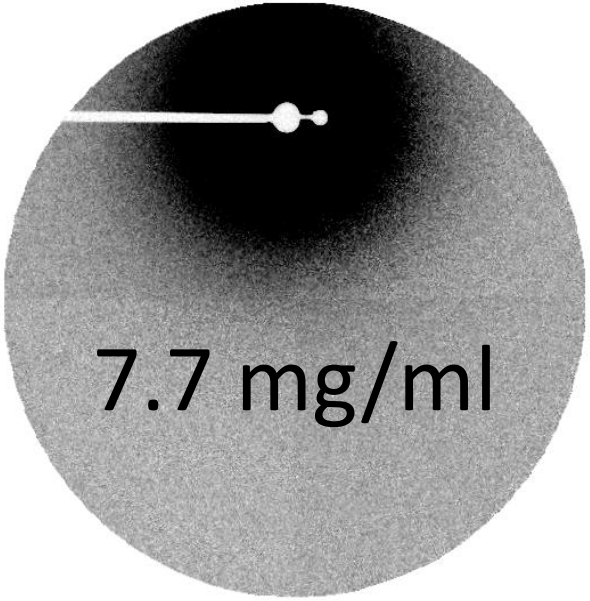
Load next sample


1.5 mg/ml

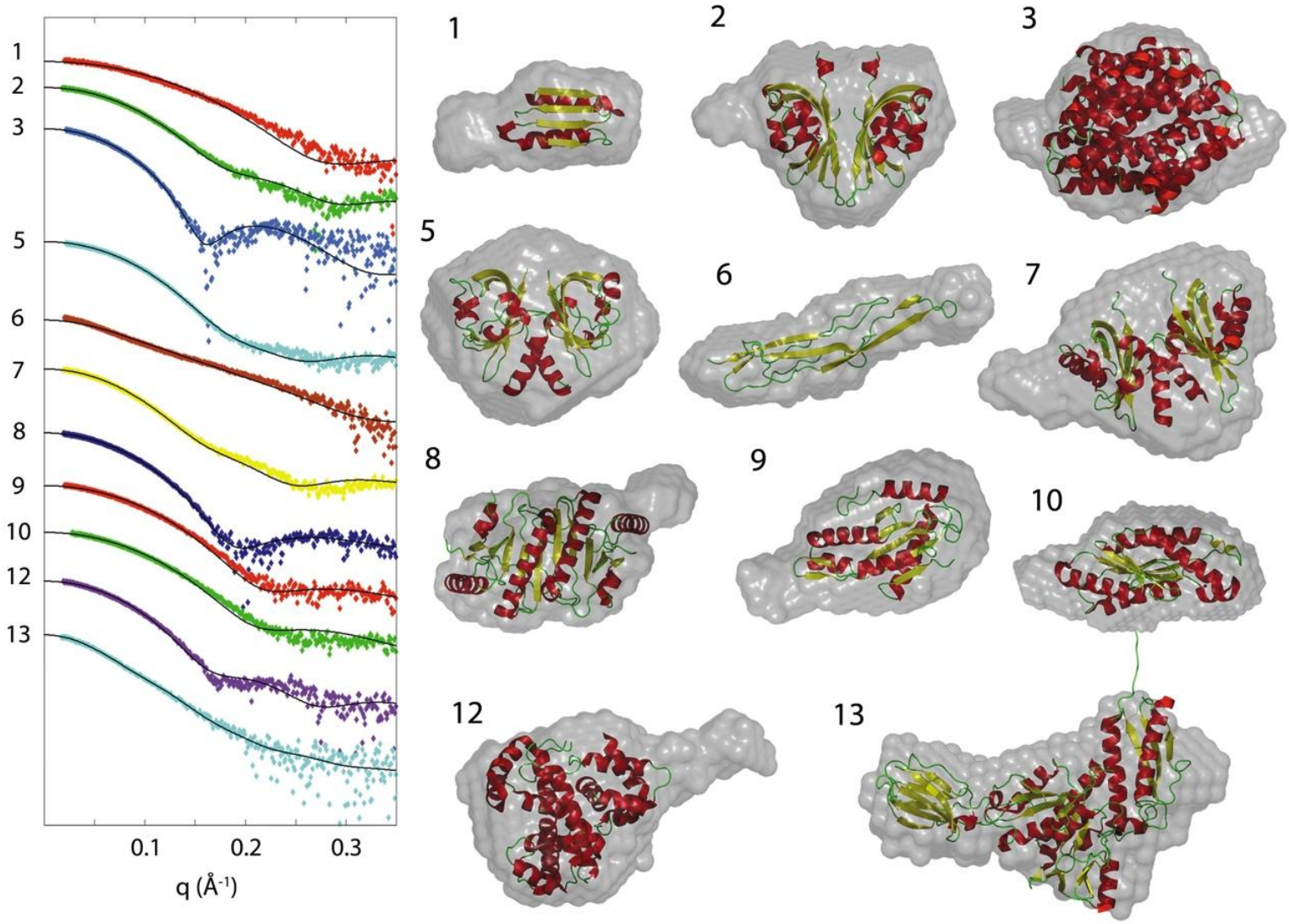

3.1 mg/ml


4.6 mg/ml

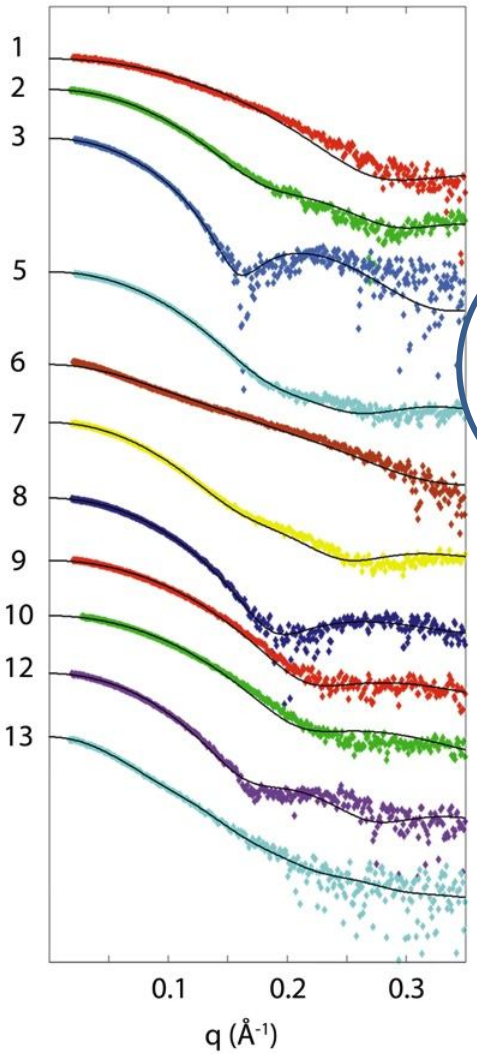

6.1 mg/ml

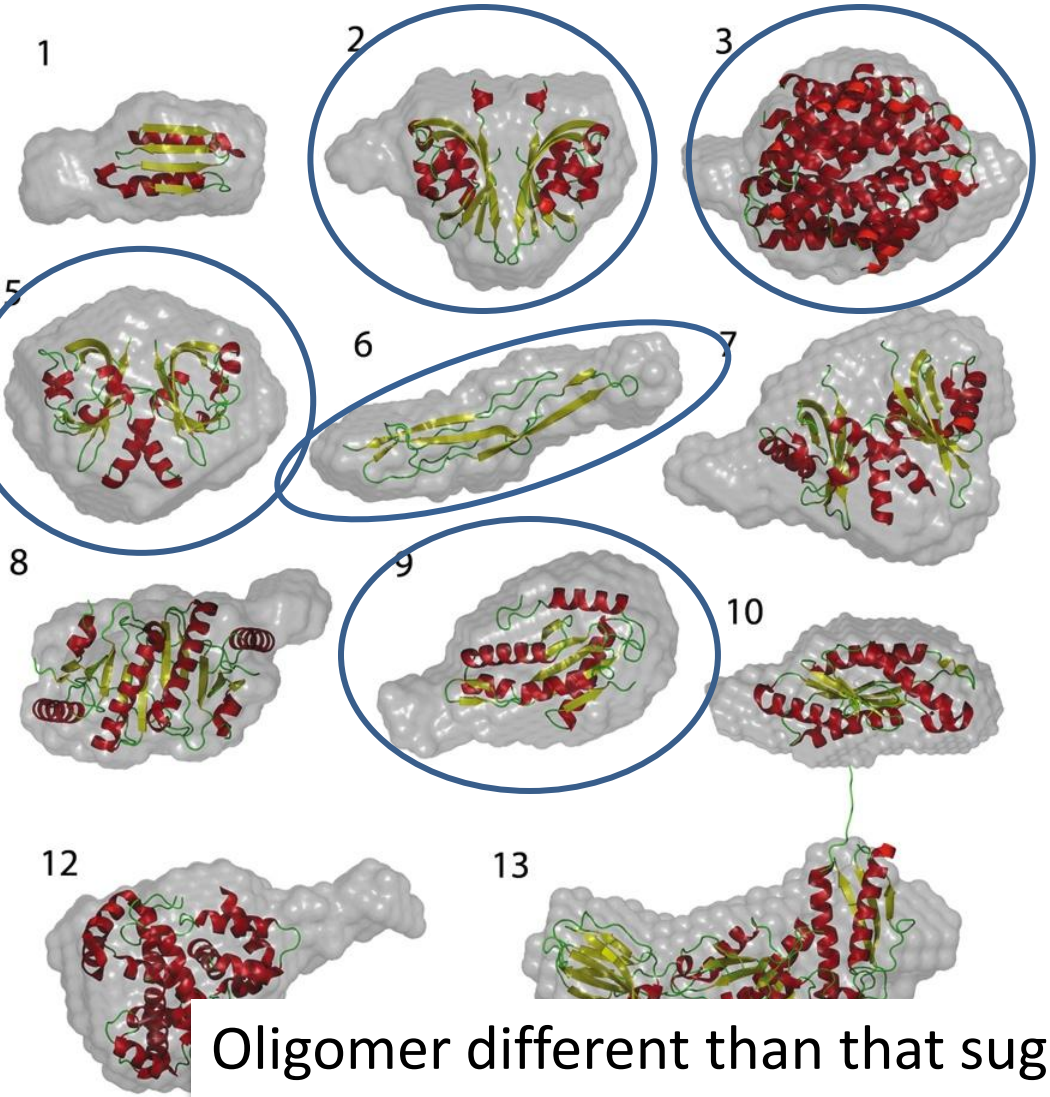

7.7 mg/ml

Comparing X-ray structures

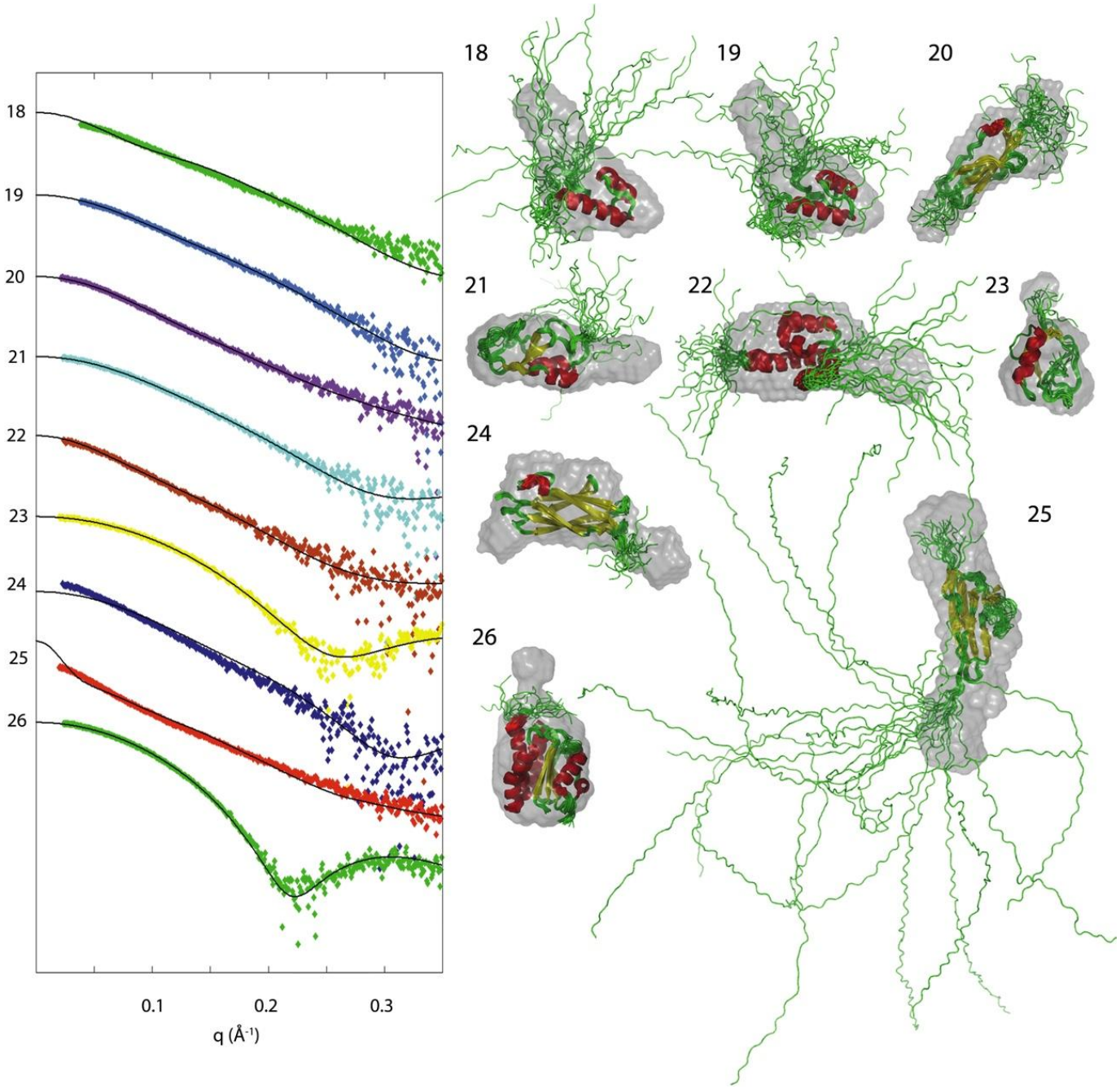


Comparing X-ray structures



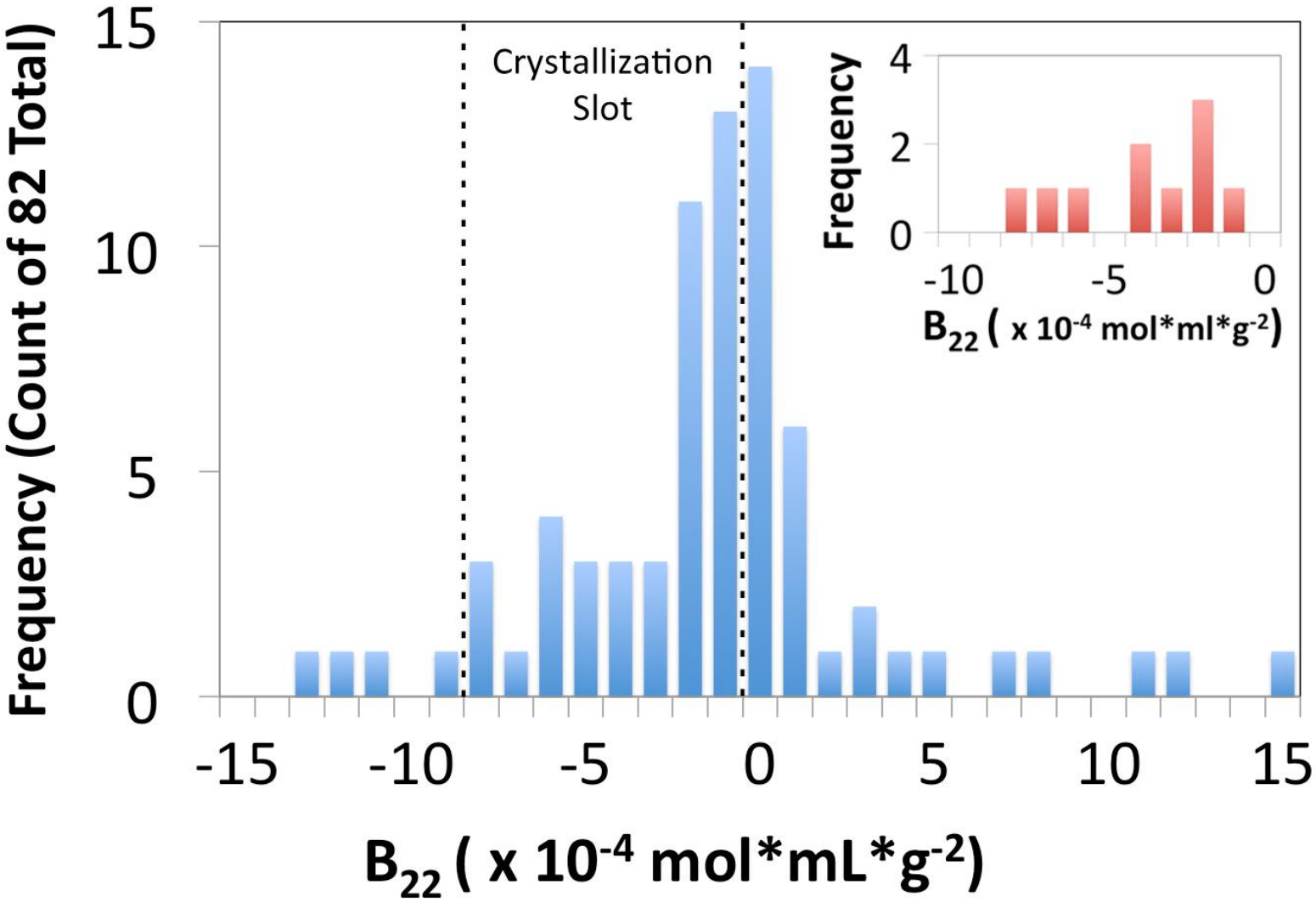


Oligomer different than that suggested by asymmetric unit of PDB



Comparing NMR structures

20 lowest energy Conformations shown


Frequency (Count of 82 Total)
15
10
5
0
Crystallization
Slot
-15
-10
-5
0
5
10
15
B22 (x 10-4 mol*mL*g-2)
Frequency
4
2
0
-10
-5
0
B22 (x 10-4 mol*ml*g-2)

High-throughput

- Over 800 different proteins (all also screened for crystallization).
- 129 with X-ray structures
- 71 with NMR structures
- 32 with both X-ray and NMR
- Average 28 kDa, largest 2.1 Mda, smallest 3.8 kDa

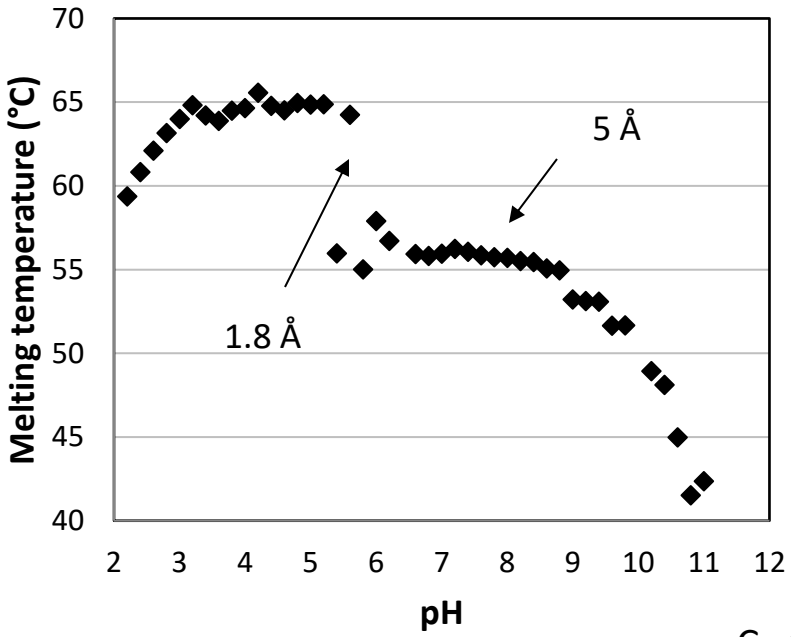
High-Throughput SAXS

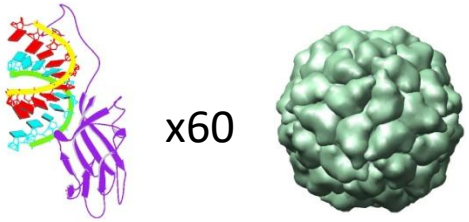
Some Examples

Over 80% of to 800 proteins we have screened with SAXS are globular and well folded

Plug for thermofluor

Satellite tobacco mosaic virus





Satellite tobacco mosaic virus (STMV) can undergo at least two physical transitions that significantly alter its mechanical and structural characteristics. At high pH the 17-nm STMV particles expand radially by about 5 Å to yield particles having diameters of about 18 nm...

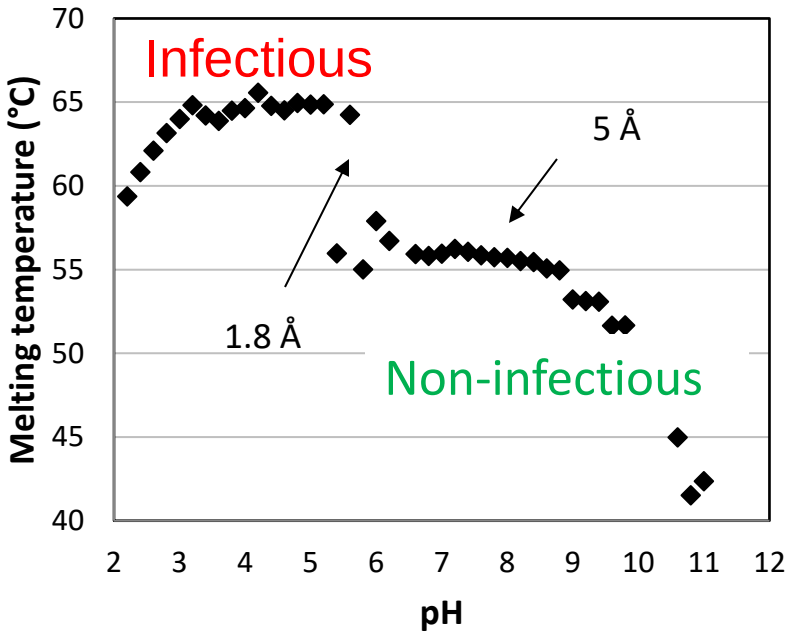
…While the native 17-nm particles crystallize as orthorhombic or monoclinic crystals which diffract to high resolution (1.8 Å), the enlarged 18-nm particles crystallize in a cubic form which diffracts to no better than 5 Å.

Kuznetsov, Larson, Day, Greenwood, and McPherson. Virology 284, 223-234 (2001).

Currently no data in the literature supports the prediction of crystallization conditions from T_m values. only the identification of ligands that stabilize macromolecules to improve crystallization outcomes

Higher melting temperature does not indicate better diffraction.

Interesting Aside

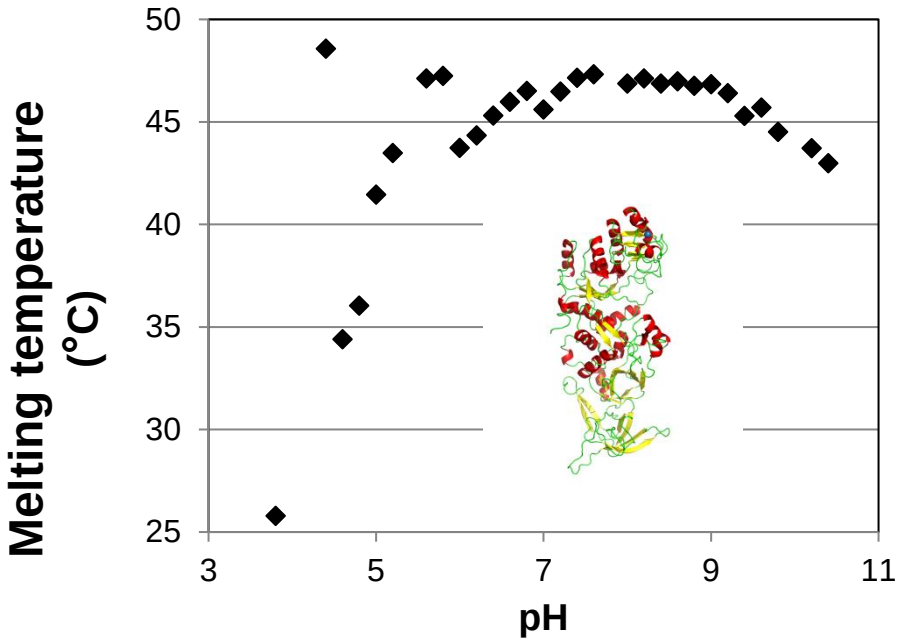


We know where to 'trap' virus particles to look at their dynamic mechanism – a whole new talk.

***"In the life-cycles of viruses, dramatic morphological changes in their capsid structure are needed** to allow them to carry out the diverse set of functions required **for replication**. All virus capsids must form readily, have structural integrity, and have the proper biological trigger in order to be infectious."* *Canady et al., Journal of Molecular Biology, 299 573-584 (2000)*

We have an assay to determine if a virus particle is functional and to develop lead drug candidates – i.e. mix a quantity of potential therapeutic compounds and look for a lack of shift in melting temperature across the pH range (or other conditions) of interest

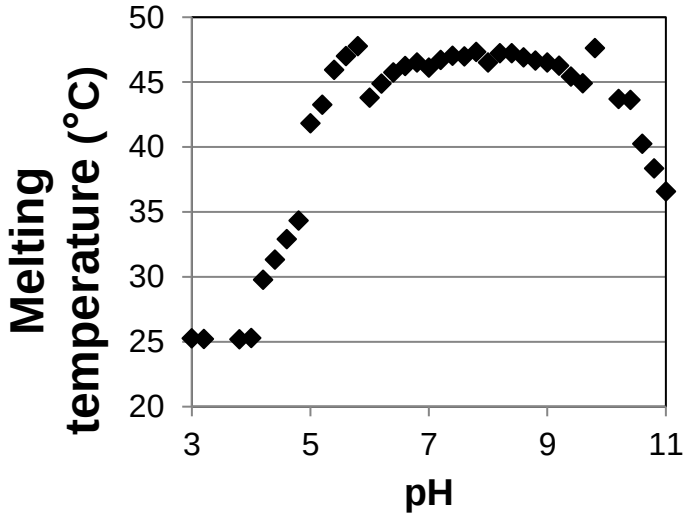
Gln-4

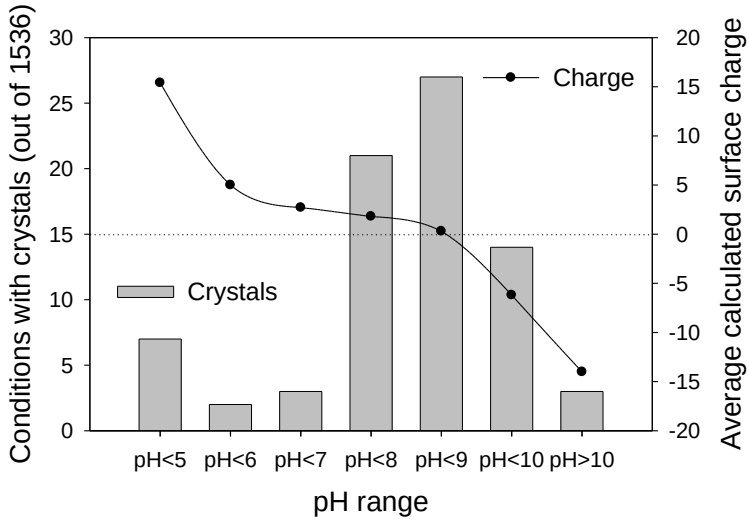


Preferred crystallization pH – 7

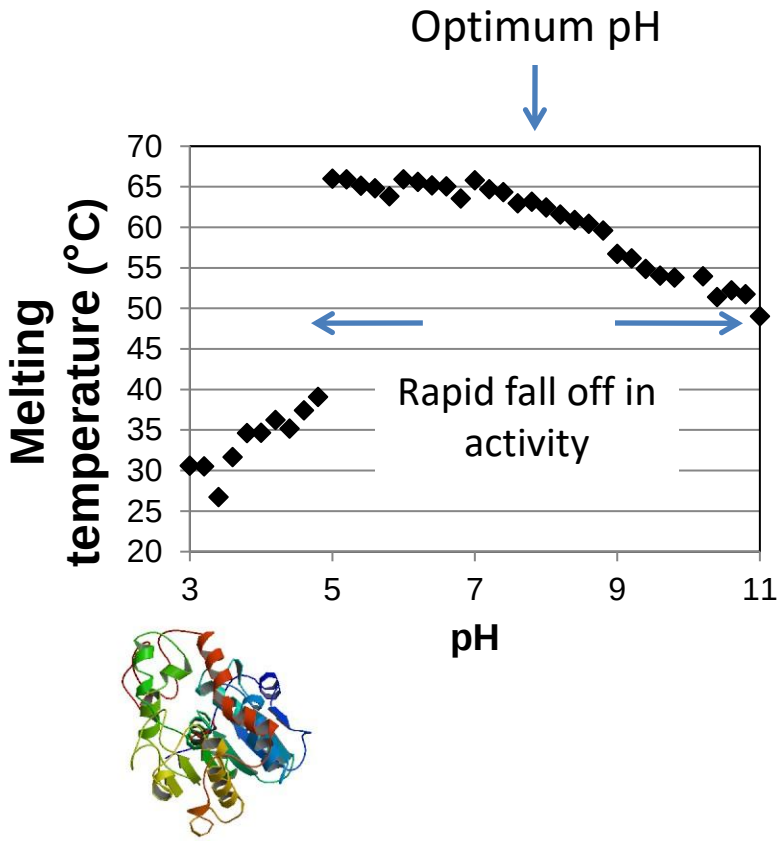
2.3A data collected on N-terminal arm

N-terminal arm





Lipase

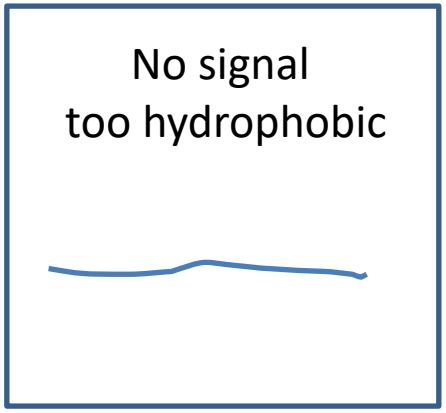


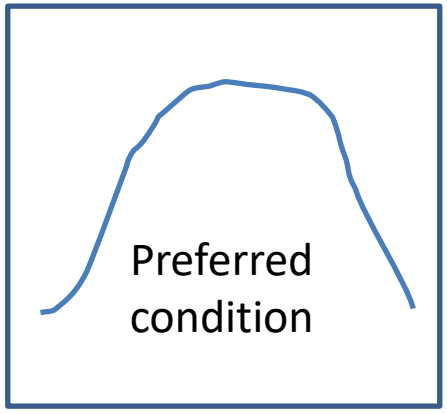
Structure	PO_4 buffer, pH 4.2 (%)	KAc buffer, pH 5.0 (%)	PO_4 buffer, pH 6.0 (%)	Cacod buffer, pH 7.0[a] (%)	PO_4 buffer, pH 9.0 (%)
α-Helix	32	38	37	38	30
β-Strand	21	20	22	20	25
Turns	15	14	11	18	15
Other	31	28	30	24	30

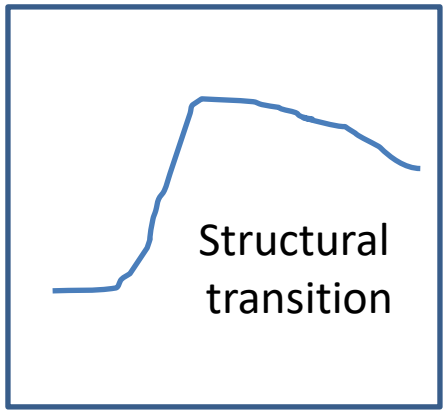
McCabe at al. Enzyme and Microbial Technology,36,70-74 (2005).

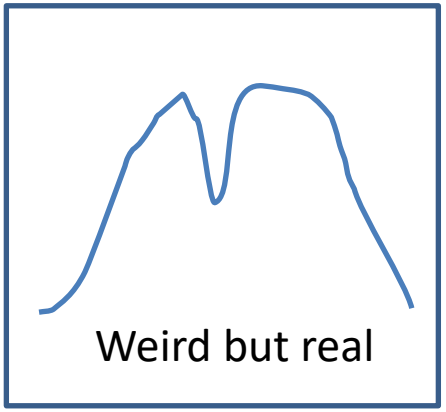
The pH screen has identified a structural transition. This is in agreement with CD data. Our structural knowledge is of the low pH form.

What signatures have been seen?









Samples to date.

On to crystals:

Introducing the cast

A typical crystallographer …

Wile E. Coyote (Genius)

Overconfidentii Vulgaris

(Cristali Coltivatore Optimista)

And the crystal of interest …

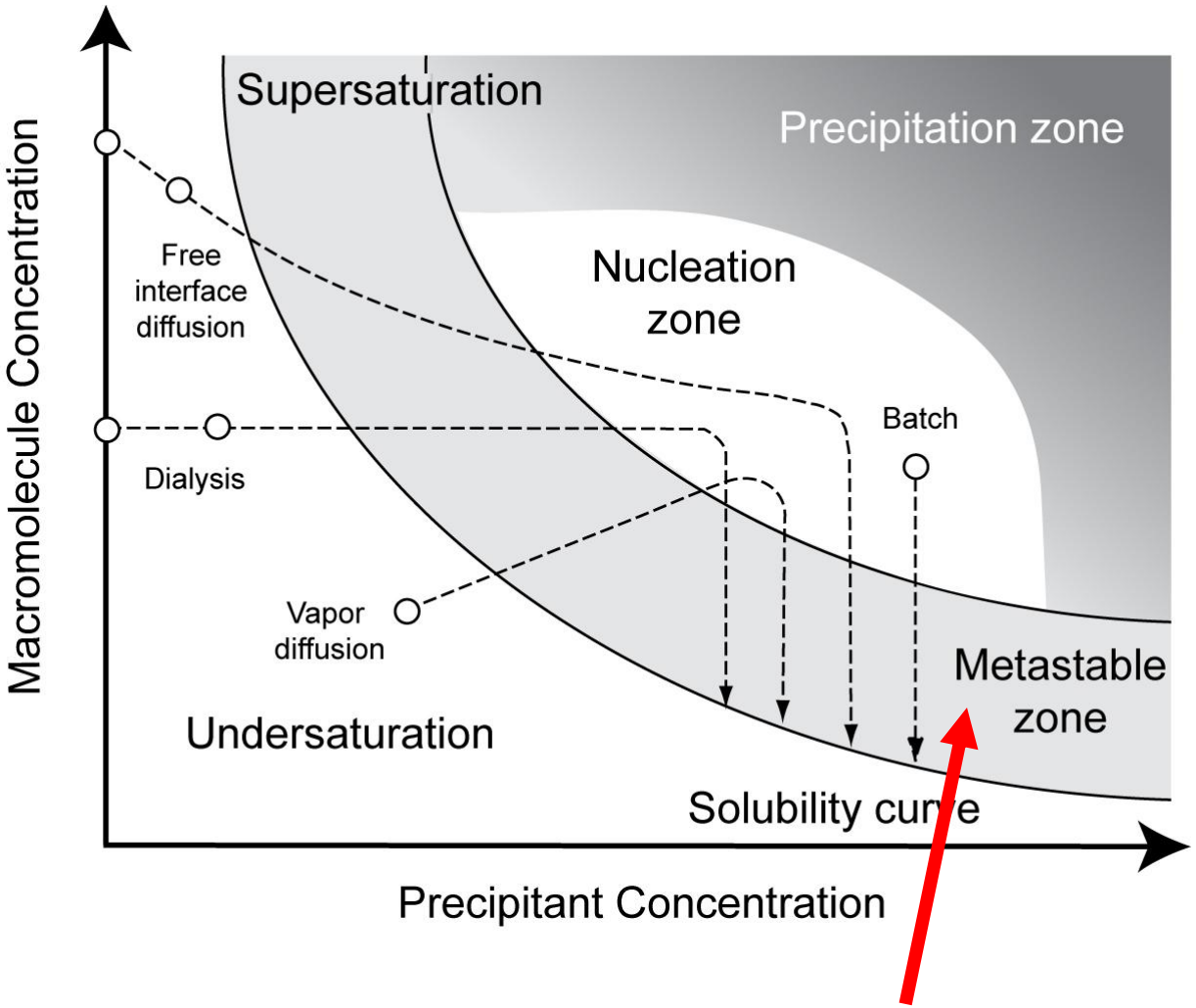
Road Runner
(Beep beep)

Disappearialis Quickius

(Cristallio Perfetto)

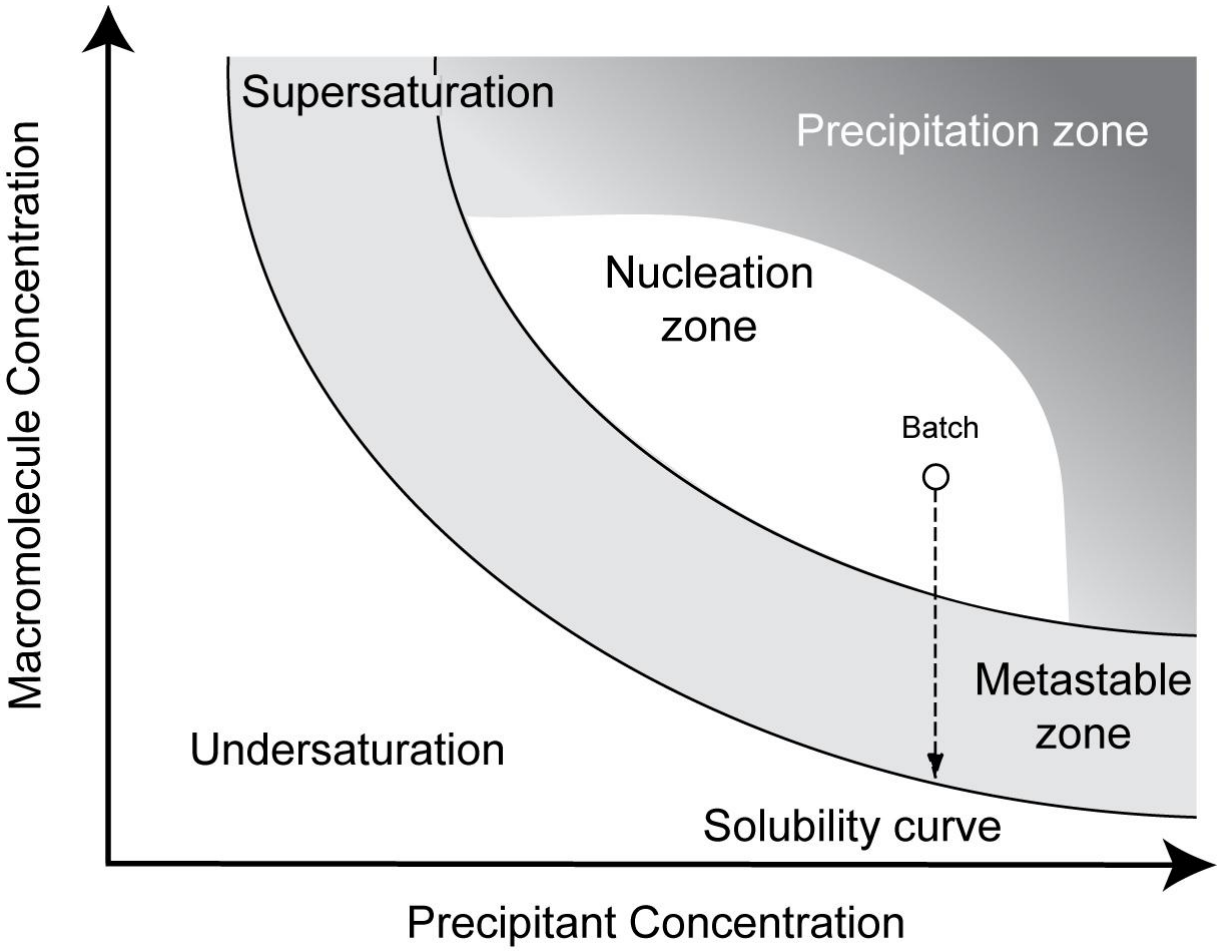
And the world they live in

Simplified phase diagram for crystallization

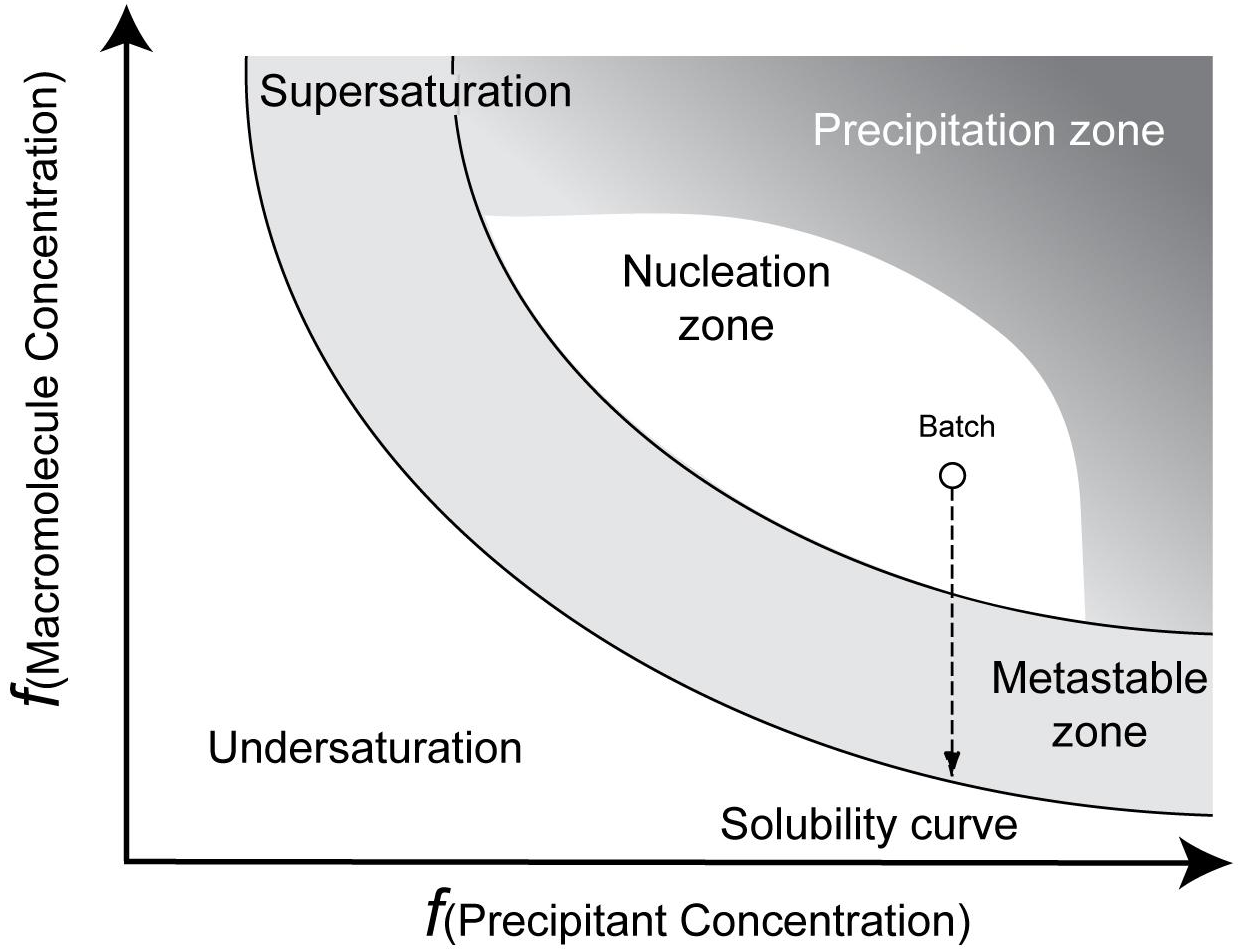


Note that the nice lines are actually a blurred probability gradient

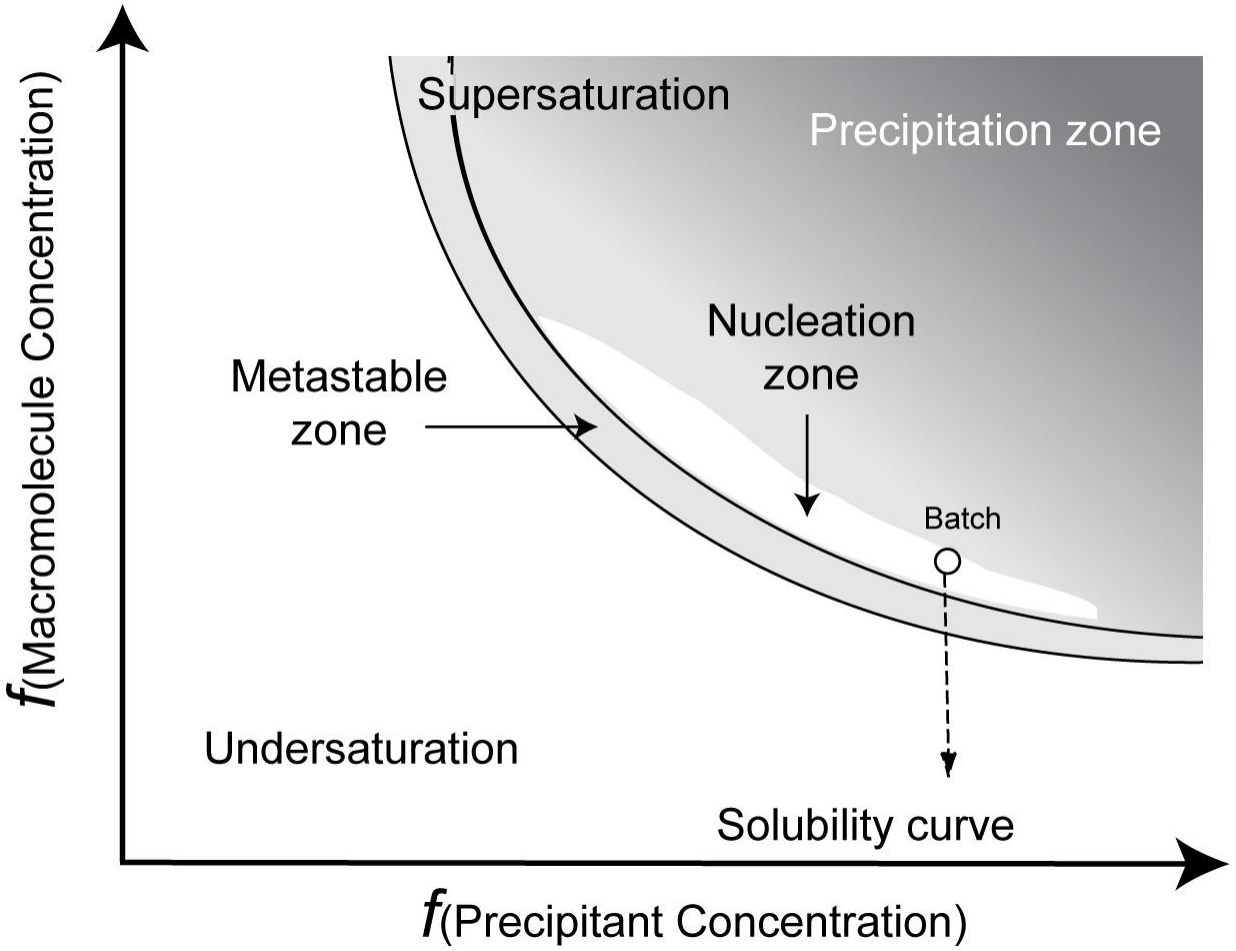
Even simpler phase diagram for crystallization



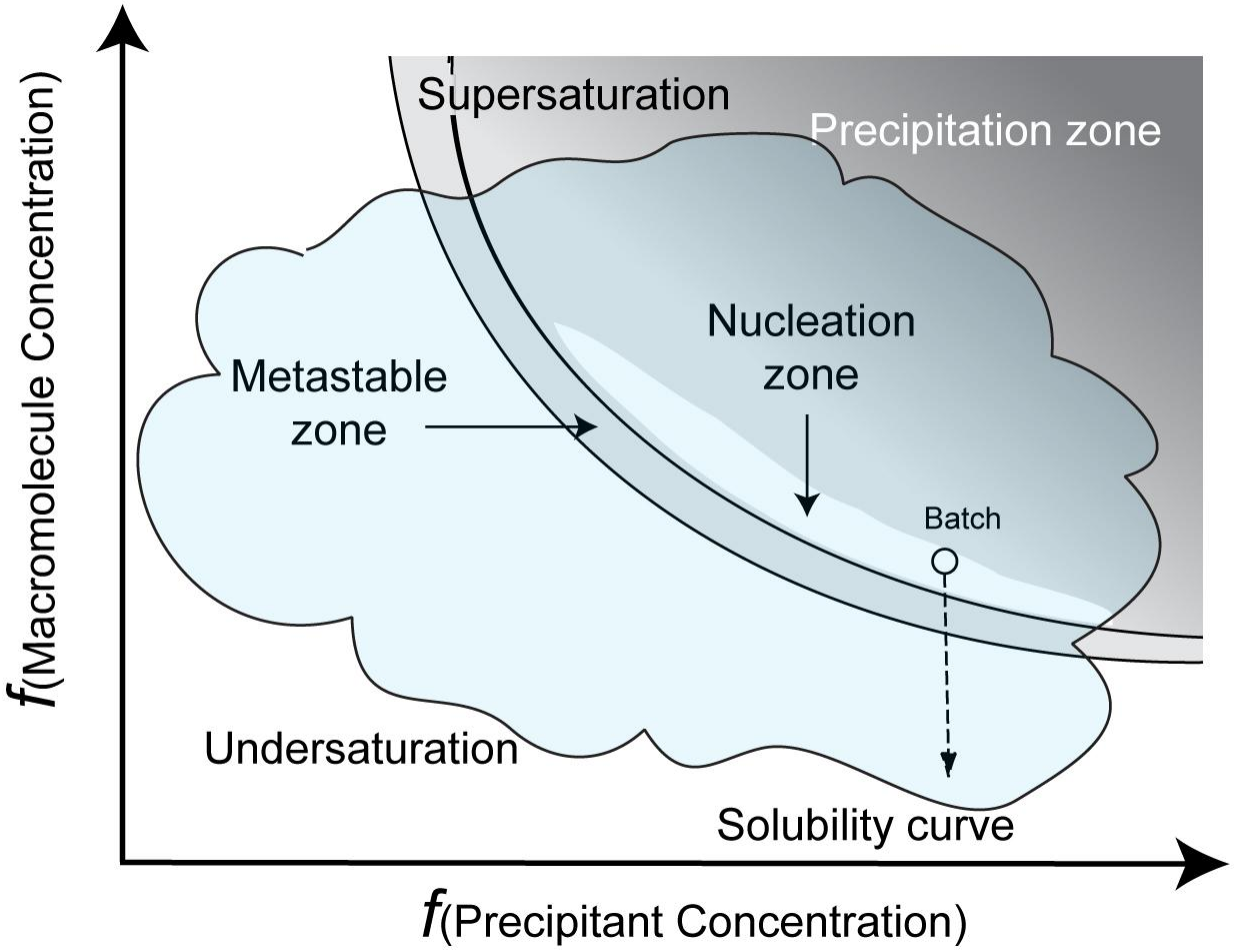
Start to throw some reality into the equation



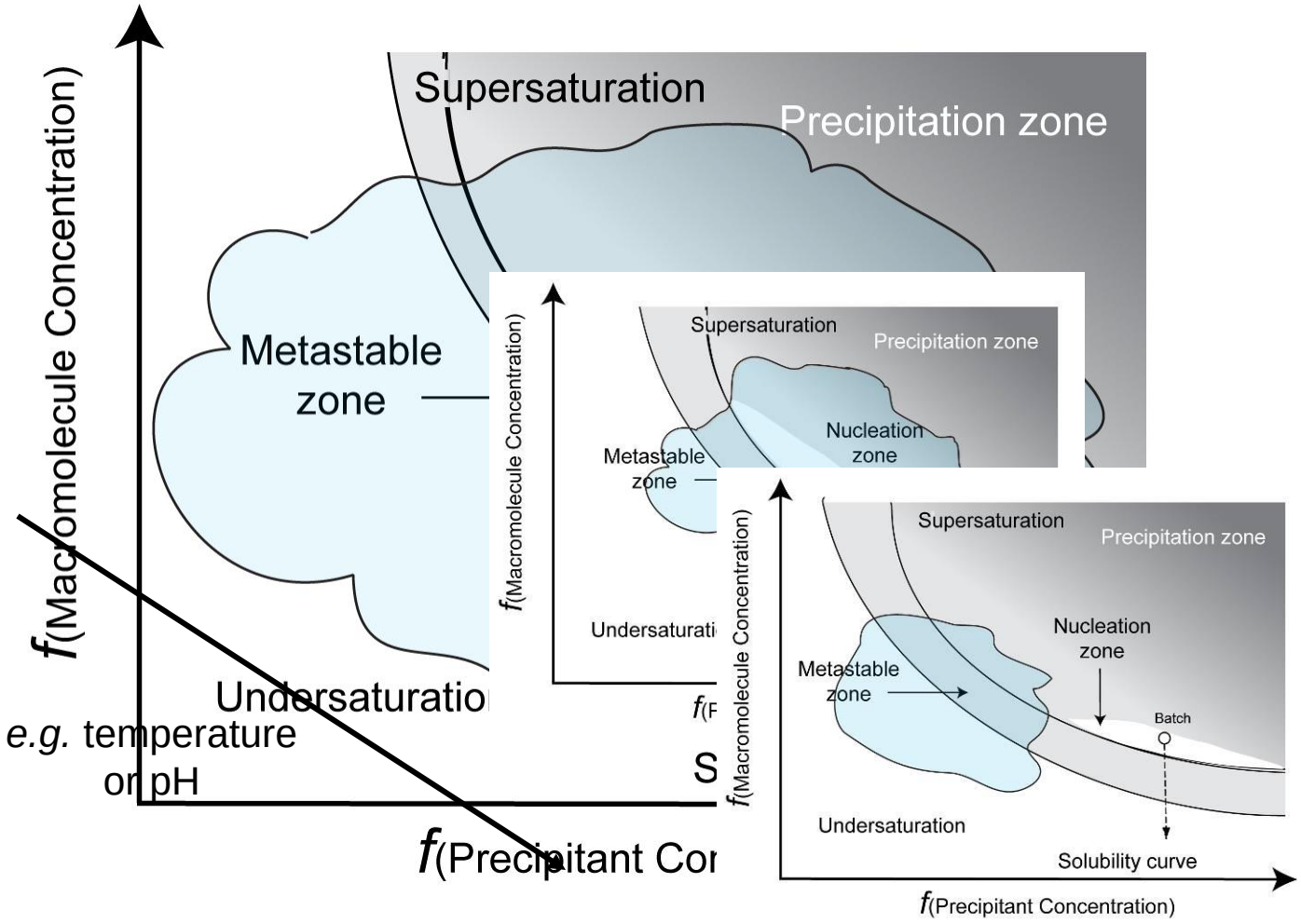
And reduce the chances of crystallization a little



Add the experimental space we sample

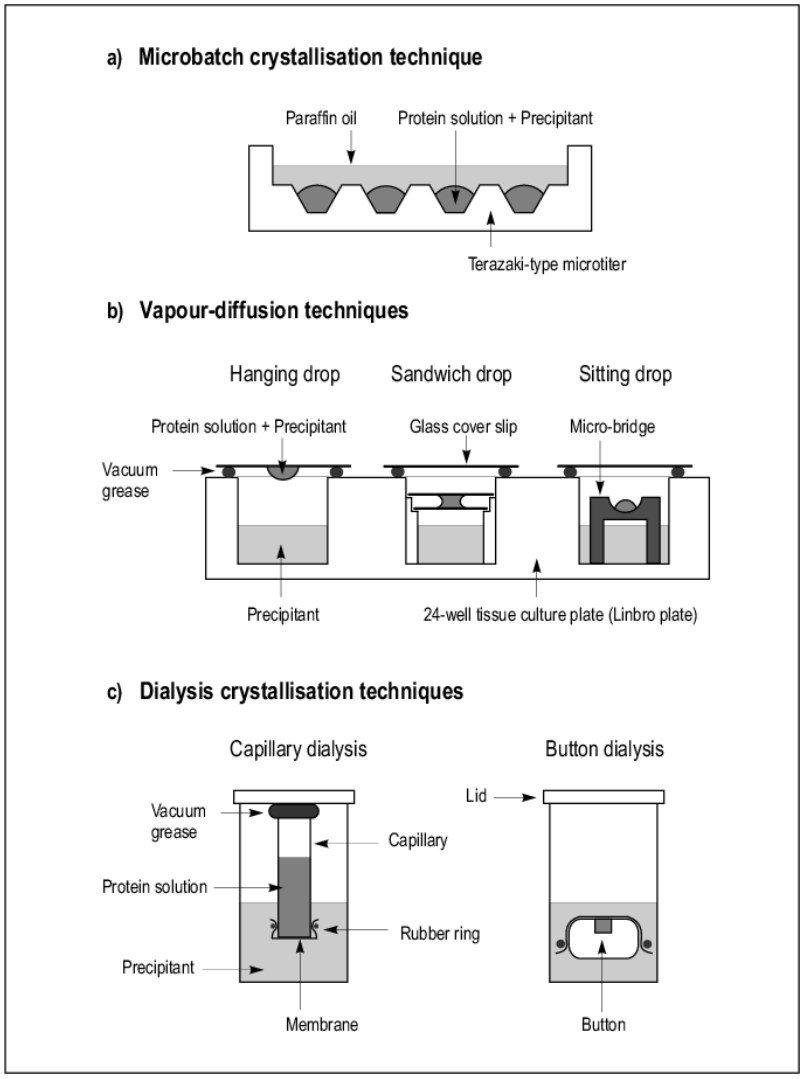


And the fact that it's not just two dimensions



The tools we have

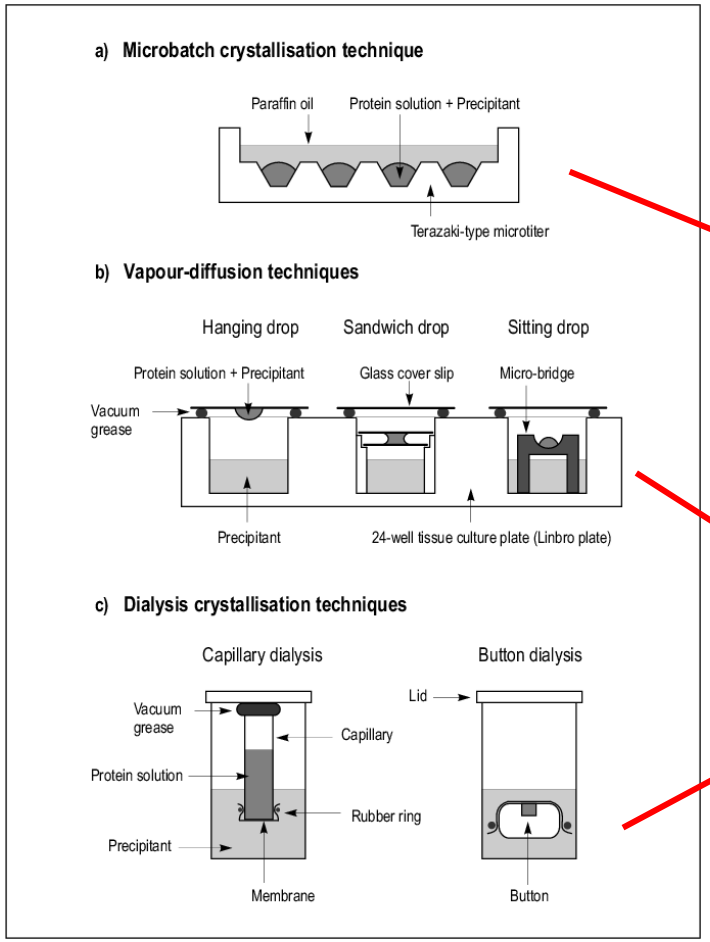
Crystallizing Macromolecules



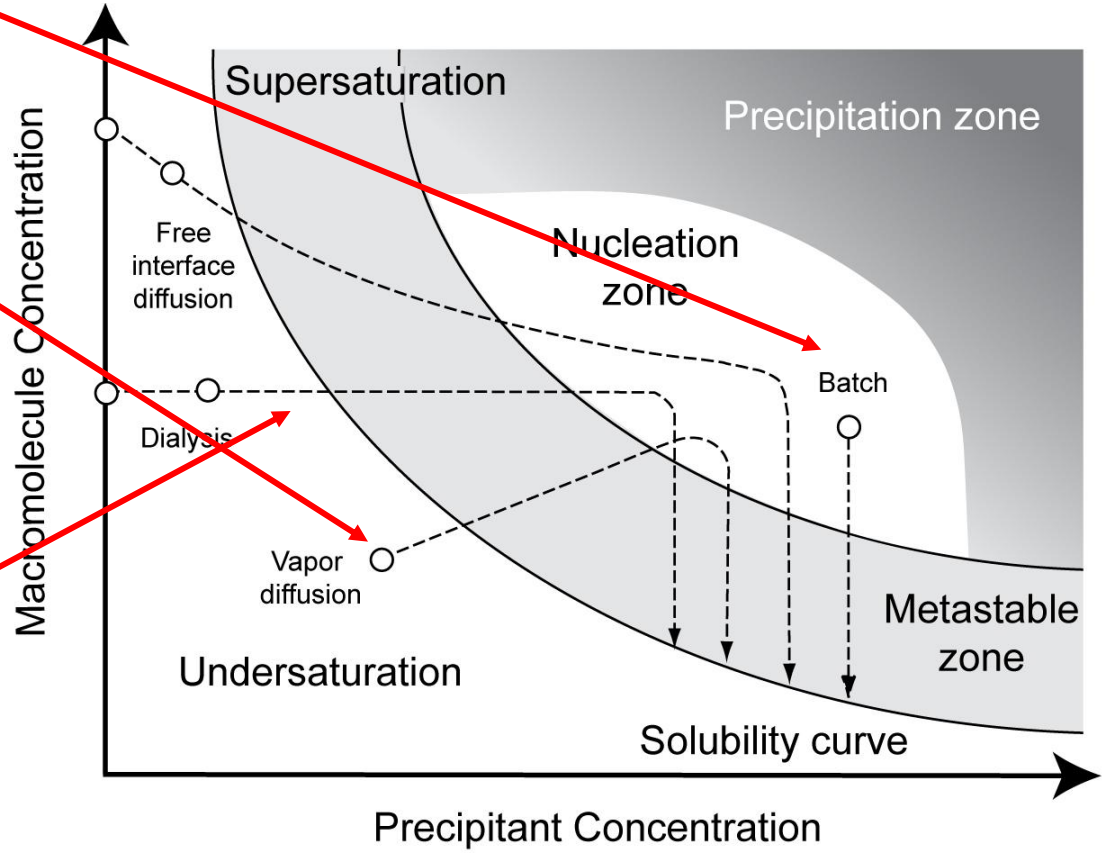
Many different methods but they all have things in common:

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

Simplified phase diagram for crystallization

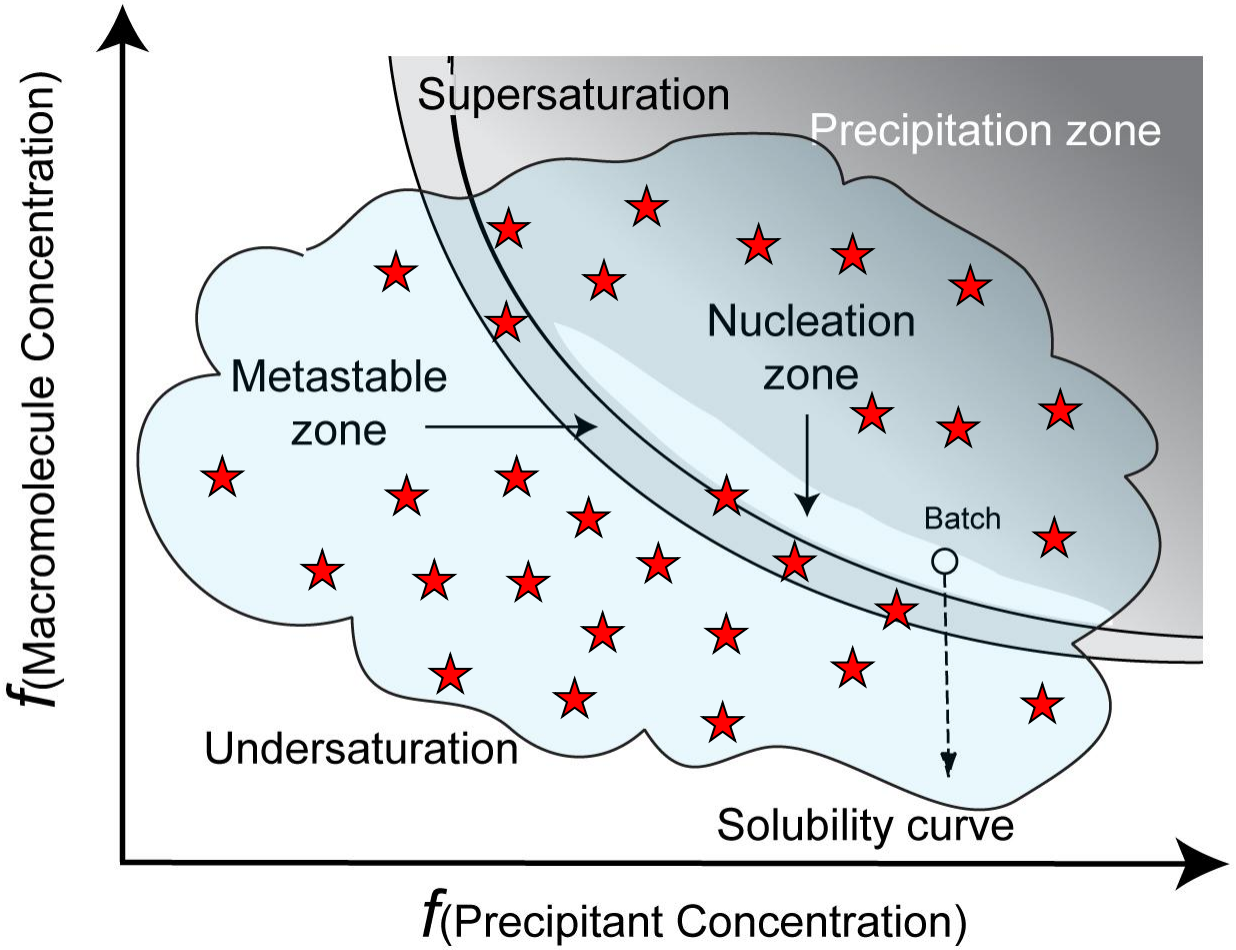


Different techniques traverse the crystallization space differently.



And finite resources

There is a lot of space we do not sample

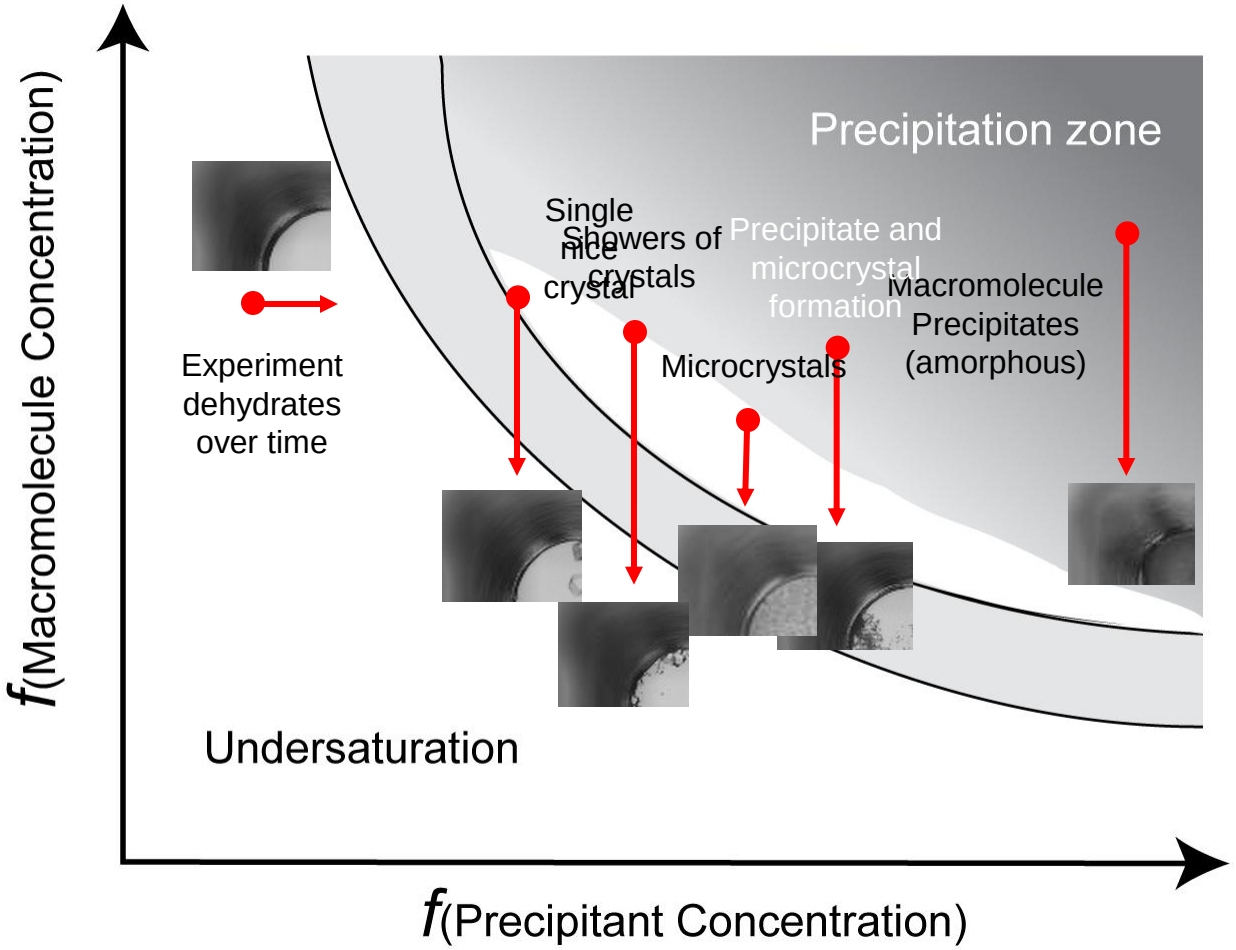


We only sample discrete points within the sampling space

With can't completely sample potential crystallization space

But we can get information from phase space

Phase space - What results can we expect to see?



A large area of space with finite sample

An introduction to the screening laboratory at the Hauptman-Woodward Medical Research Institute

Since February of 2000 the High Throughput Search (HTS) laboratory has been screening potential crystallization conditions for the general biomedical community and two Protein Structure Initiative large-scale structure production centers (NESG, Montelione, PI; SGPP/MSGPP, Hol, PI) and one PSI specialized PSI-2 center (CHTSB, DeTitta, PI).

The HTS lab screens samples against an incomplete factorial screen of two categories of crystallizing agents:

1. buffered (4<pH< 10), highly concentrated salts (35 salts total, sampling 18 different cations and 20 anions) – 229 conditions.
2. PEG/salt/buffer solutions (eight buffers (4<pH< 10), six molecular weight PEGs at three concentrations, and 35 salts at fixed 200 mM concentration) – 721 conditions.

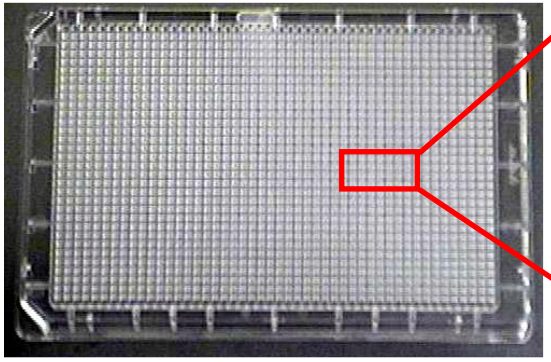
Added to this is a screen of some 586 conditions encompassing screens commercially available from Hampton Research.

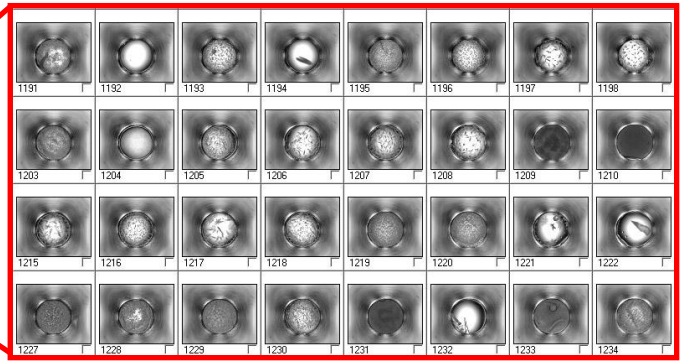
The crystallization method used is micro-batch under oil with 200 nl of protein solution being added to 200 nl of precipitant cocktail in each well of a 1536 well plate.

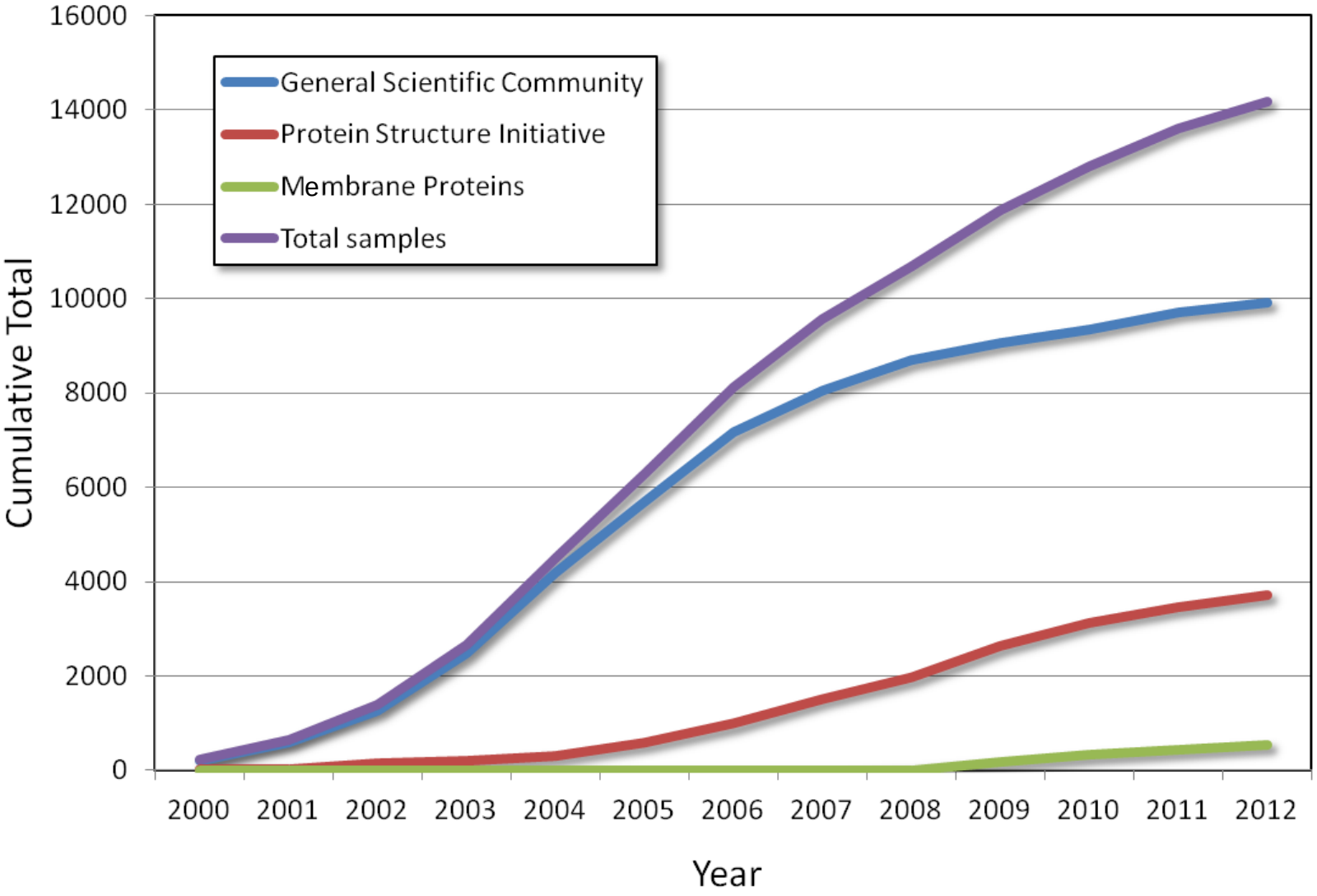
Wells are imaged before filling, immediately after filling then weekly for six weeks duration with images available immediately on a secure ftp server.

The HTSlab has investigated the crystallization properties of over 14,000 individual proteins archiving over 129,024,000 images of crystallization experiments.

The staff, instrumentation and crystallization plate used






16000
14000
12000
10000
8000
6000
4000
2000
0
Cumulative Total
General Scientific Community
Protein Structure Initiative
Membrane Proteins
Total samples
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
Year

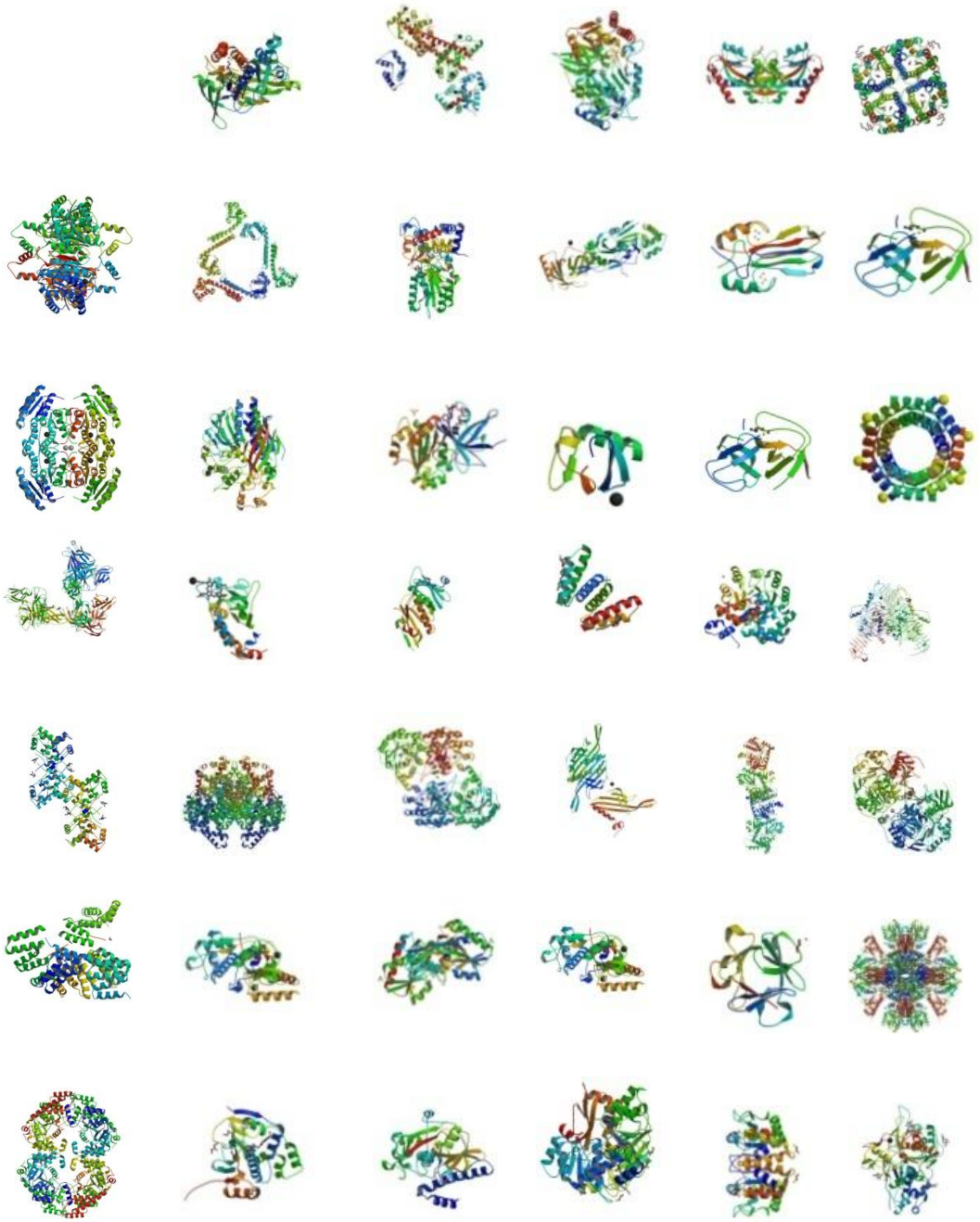
Born in Buffalo

Over 1,000 general biomedical laboratories world wide use the crystallization screening service with approximately 2,000 unique investigators.

Investigators are sent photographs of the results, analyze these images and perform their own optimization of any hits observed.

No information is released on targets. Progress is tracked by acknowledgements and citation searches. Currently no other metrics are used to measure success rates for the general biomedical community.

These images represent examples of structures from initial hits in the HTS laboratory.



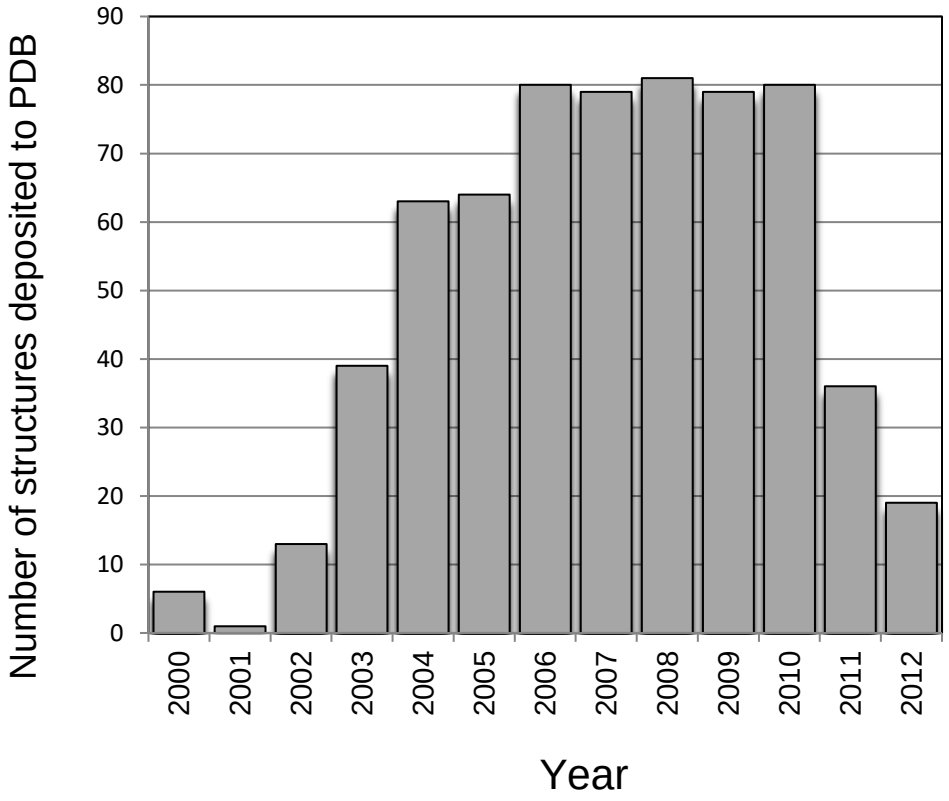
Where success is tracked

For our Protein Structure Initiative partners both success and failure is tracked. In the case of NESG our initial screening hits enable on average 80 structures per year to be deposited to the PDB.

The graph demonstrates the ramp up of operations with maximum success reached from 2006 onward.

Our success rate from protein in the door to a crystallization hit leading to a PDB deposition is **22%**.

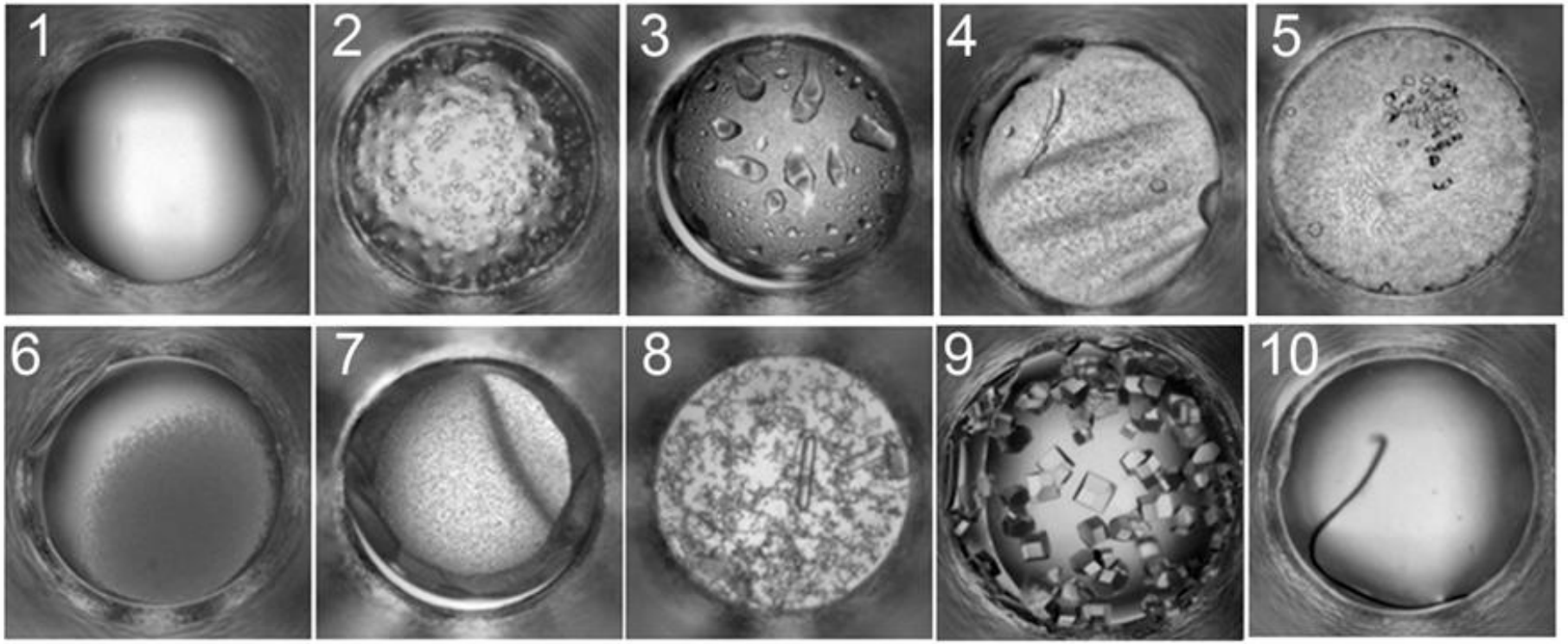
The NESG samples represent a special case in that they are well characterized beforehand – size exclusion chromatography, mass spec analysis and dynamic light scattering studies.



In 2011 we switched to PSI Biology – More difficult targets

Old graph!

Outcomes …



1 clear, 2 showing phase separation, 3 with phase separation and precipitate, 4 with phase separation and skin, 5 with phase separation and crystals, 6, with precipitate, 7 with precipitate and skin, 8 with precipitate and crystal, 9 with a crystal and 10, anything that is undefined or possible contamination.

What do outcomes tell us?

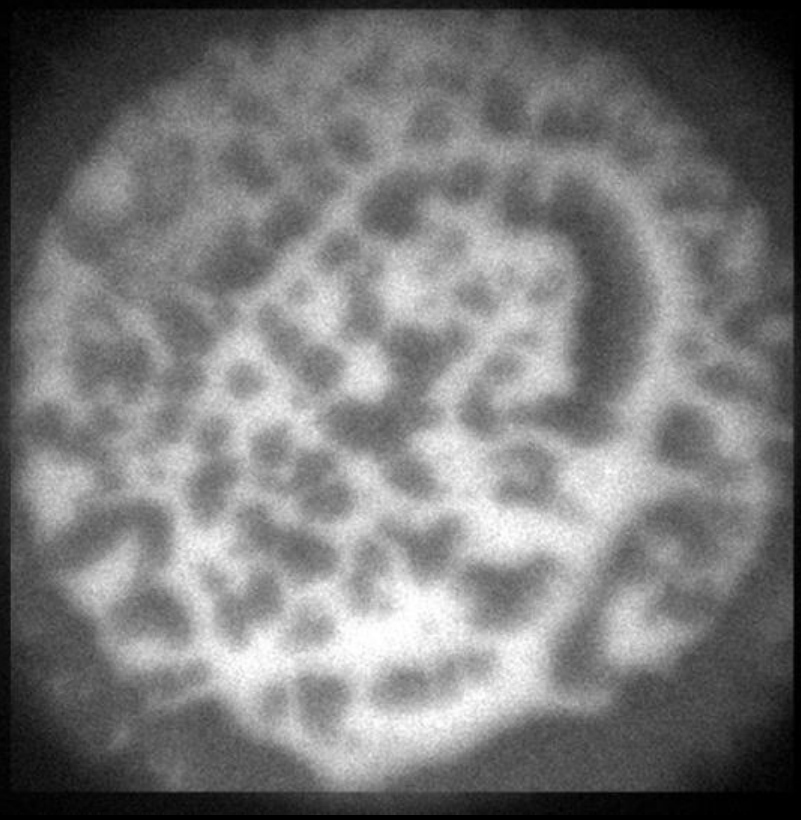
Clear Drops

Clear drops …

- The solution could be
  - (a) undersaturated (away from crystallization conditions)
  - (b) in a metastable supersaturated state (close to crystallization conditions)
- A clear drop in the undersaturated zone looks identical to a clear drop in the metastable zone.
- Clear drops in isolation provide limited information; undersaturated solutions have to be distinguished from metastable solutions.
- Those conditions that are undersaturated will largely slow clear drops in chemically related experiments
- Those conditions in metastable conditions, will show precipitate or even crystals in closely related chemical conditions.
- (It is important to define chemically related conditions)

Clear drops, in context, can be important

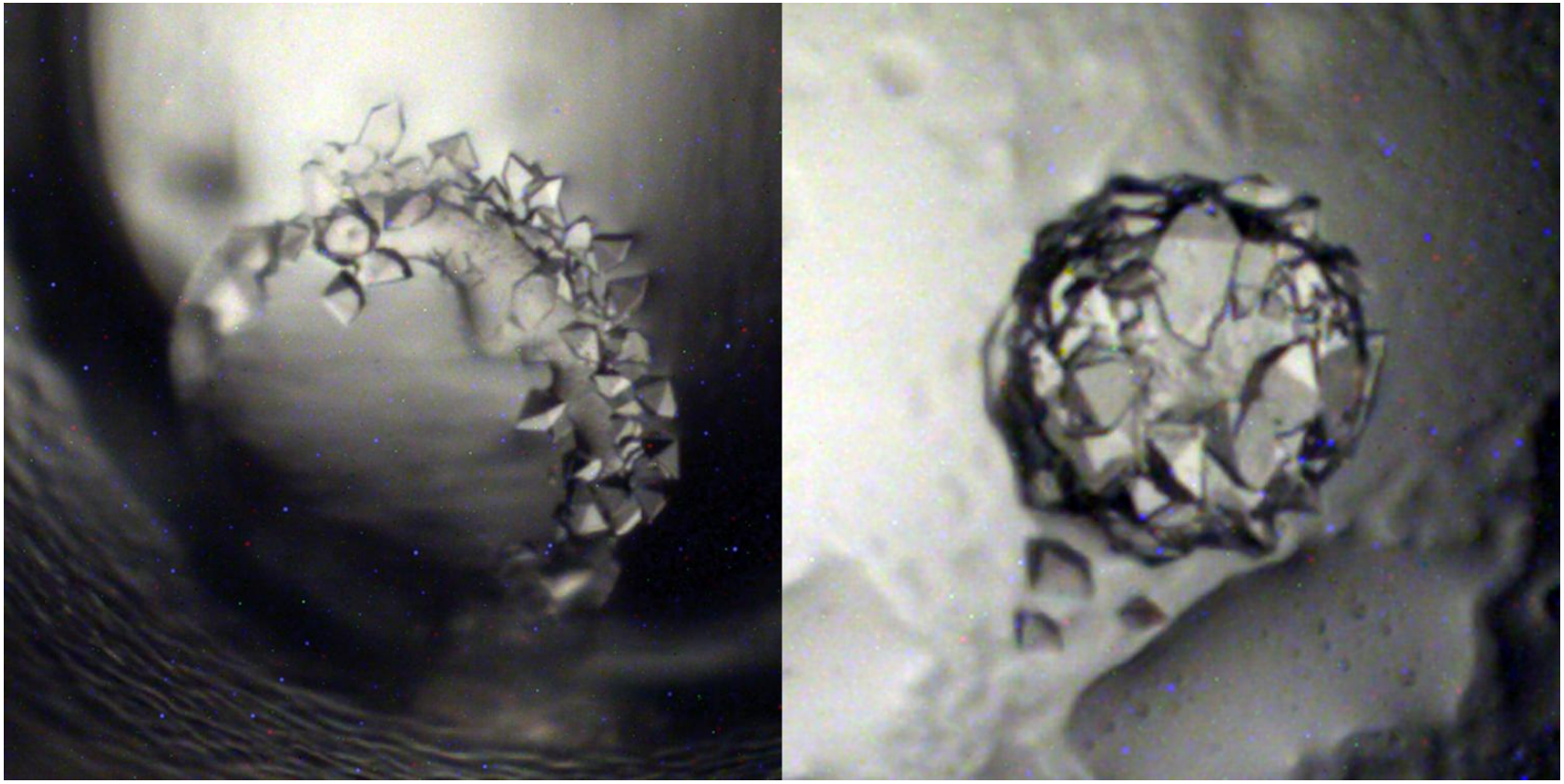
Phase Separation



UV imaging showing which component protein is present in

Phase separation

- Immiscible liquid-liquid phase separation forms only where there are short range, and/or highly anisotropic interactions between protein molecules.
- When a L-L phase separation is observed if one phase is protein-rich and the other protein-poor, then the system is very close to conditions that have the potential to produce crystals.
- An effective option to induce crystal formation is to drive the system towards a higher level of supersaturation, the labile state.
- Protein solubility is dictated by the combination of the protein and its chemical environment. The same protein can have increased solubility at higher temperatures in one chemical environment, and lower temperatures in a different chemical environment.
- Temperature can be used to drive phase separation into crystallization



Boundaries in phase separation can act as nucleation zones

Phase Separation is good, temperature can be used to drive it into crystallization

Precipitate

Good Precipitate

Good (microcrystalline).

Crystalline precipitation is protein aggregation where the chemical environment permits the native conformation to remain intact i.e. those likely to lead to a crystal with minimal optimization effort.

Crystalline precipitates are

- Patterned (can have a sandy appearance)
- typically but not always show bi-refringence (depending on the orientation and symmetry of the crystals)
- will re-dissolve
- will absorb dye
- can successfully act as seeds.

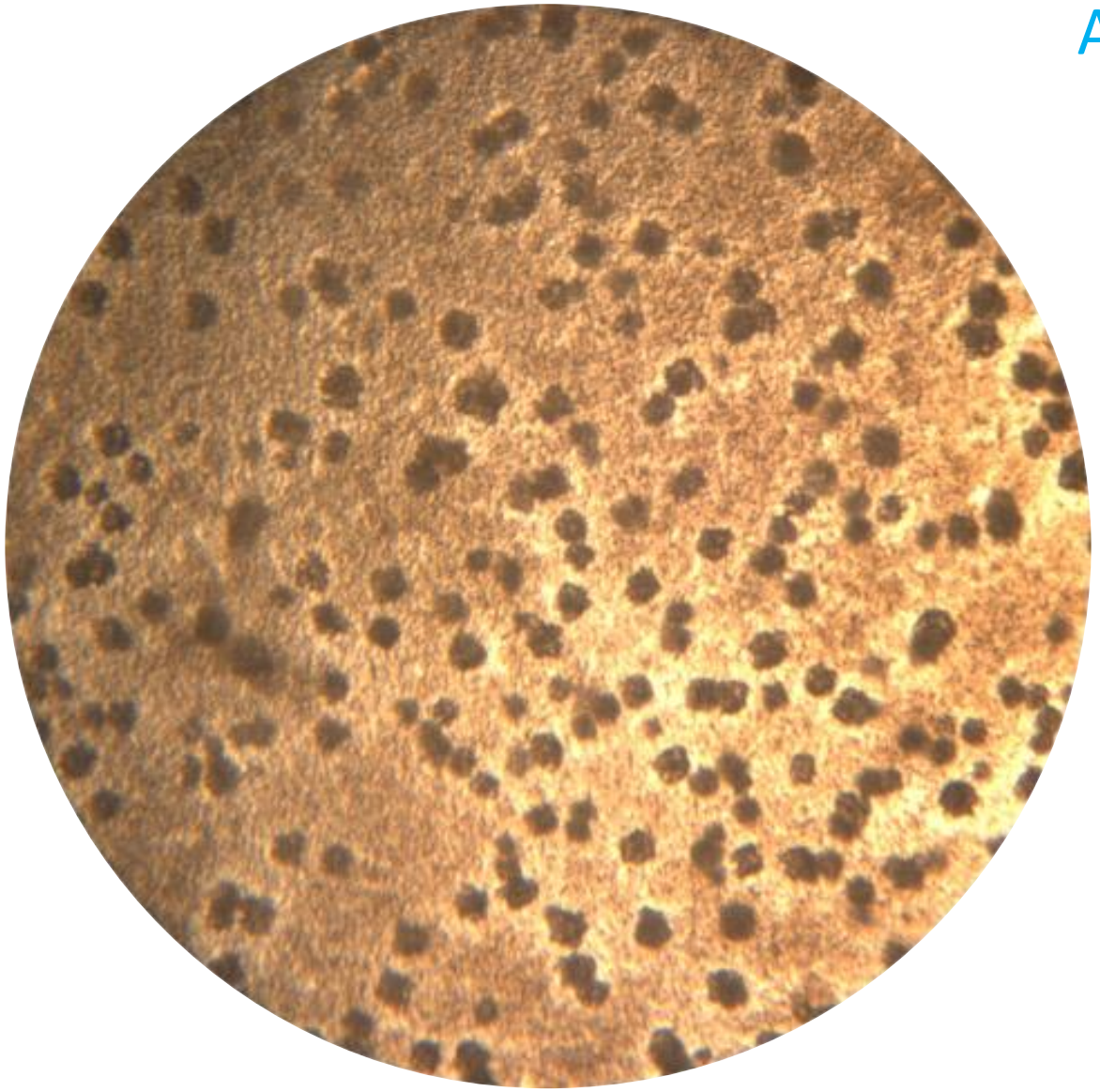
Bad Precipitate

Bad (Amorphous) precipitate occurs due to non-native protein aggregation.

Signatures are:

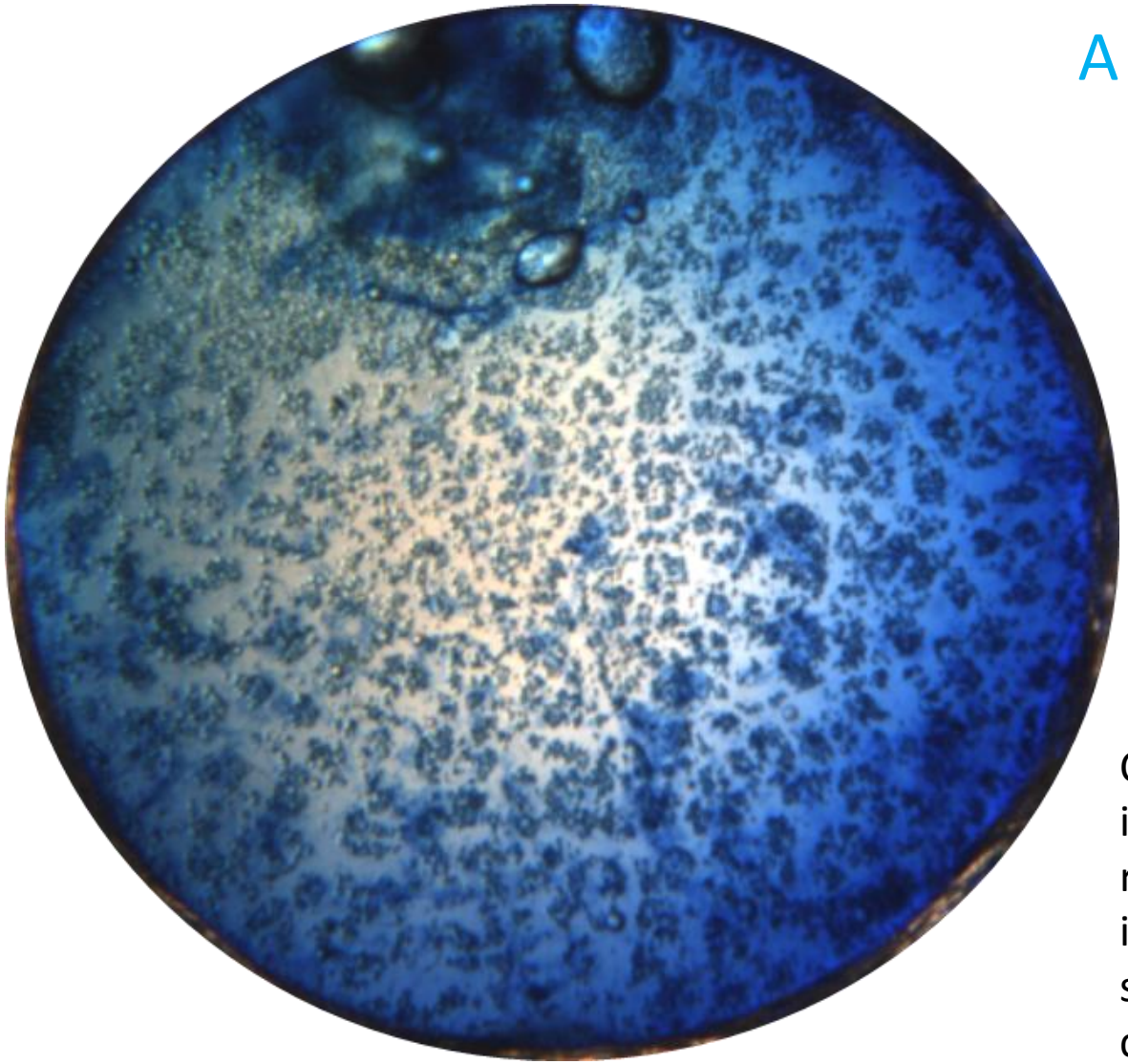
- Frequently brownish in color
- Often associated with a skin
- It will not redissolve
- It will not absorb dye
- It will not act as a successful seed

Good Precipitates



A Good Precipitate

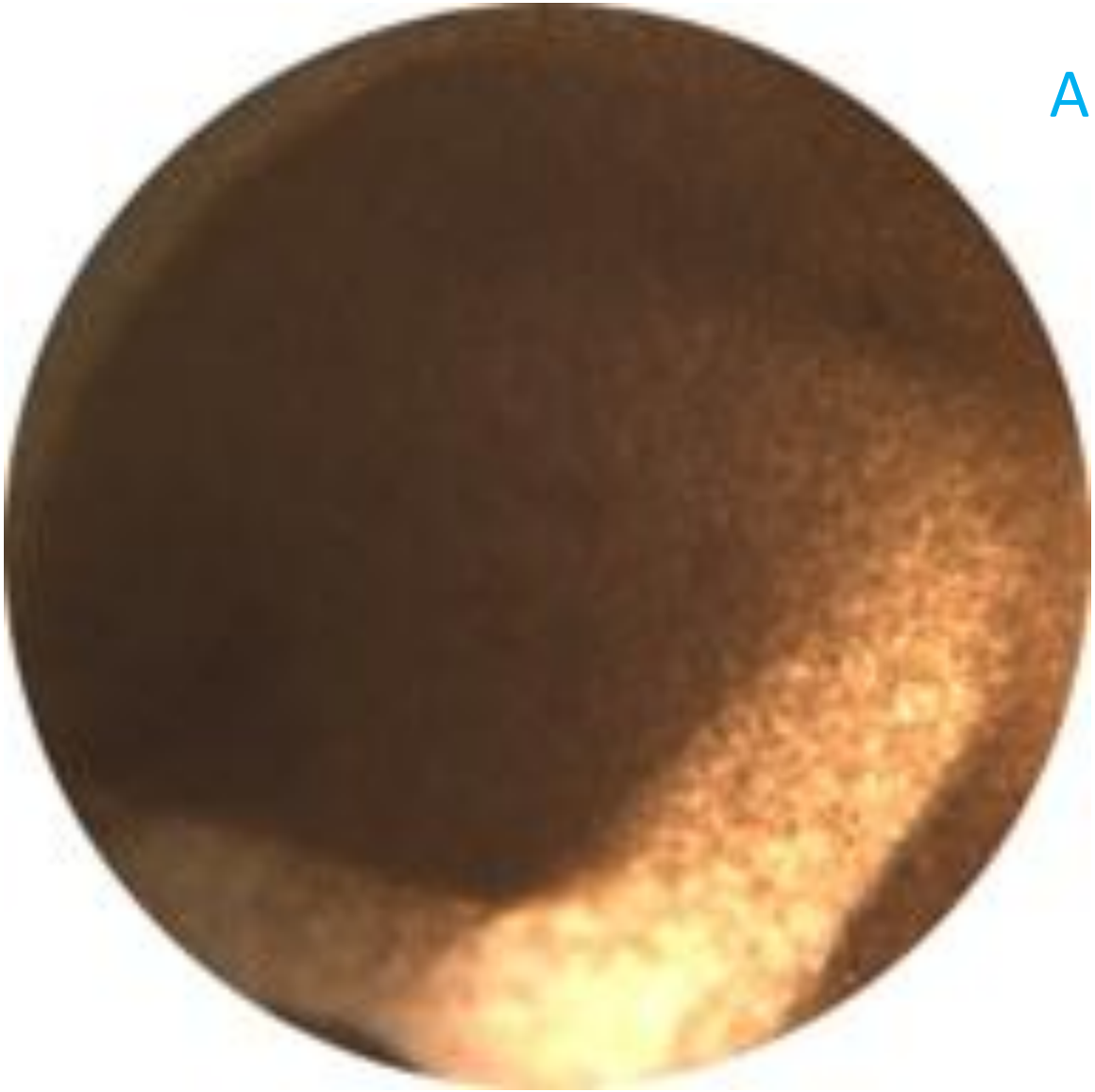
This is patterned (and has a sandy appearance). There are features present and on enlarging these features resolve themselves into crystals.



A Good Precipitate

Initial examination classified this as a precipitate. It was patterned and took up dye. When enlarged using a better microscope evidence of crystal formation was seen.

Crystalline precipitate can be identified by a sufficiently high resolution microscope. This is an investment each laboratory should make especially considering that X-ray data can now be collected from crystals as small as 5 micron routinely.



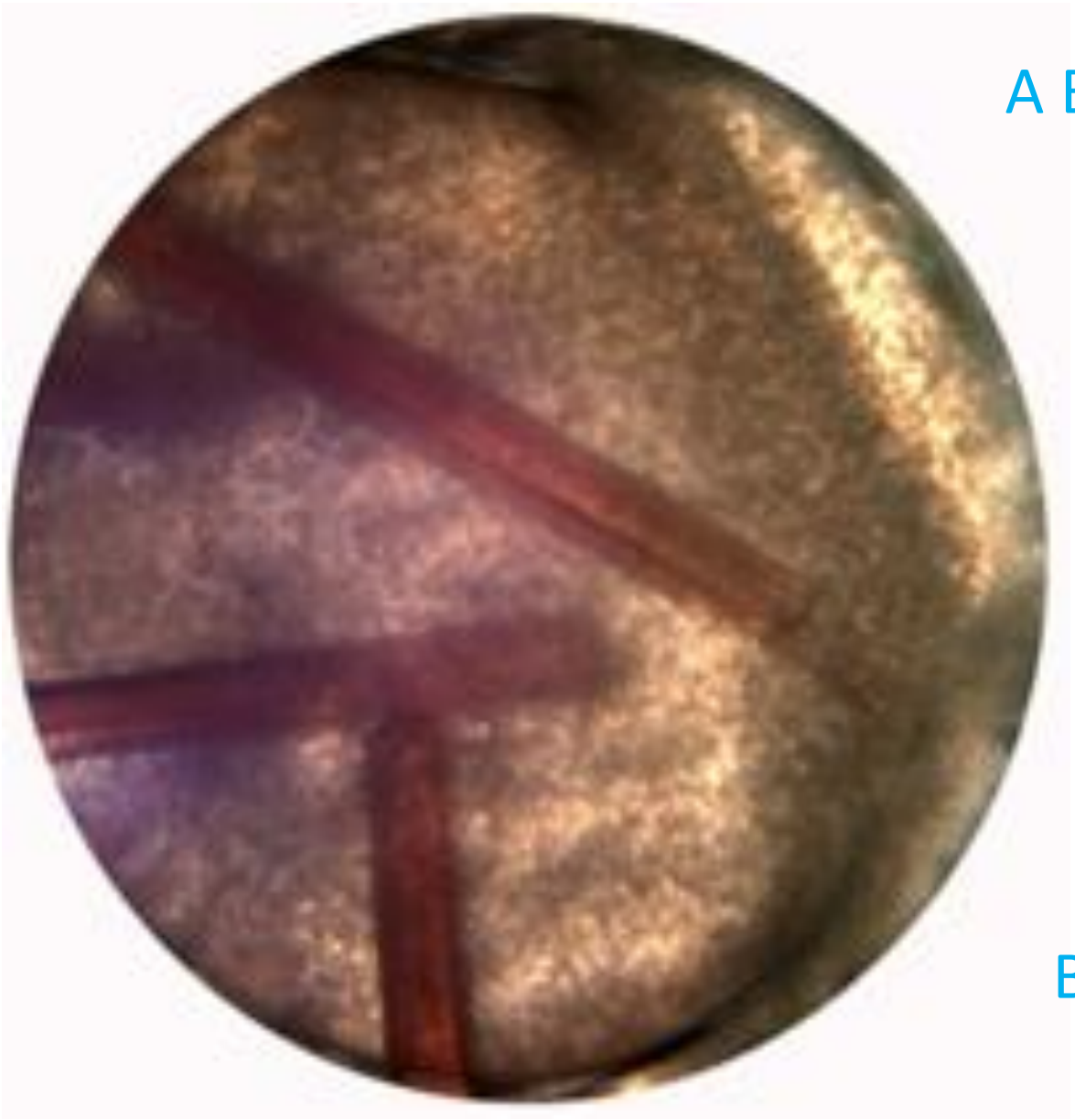
A Good Precipitate

Initial examination classified this as a precipitate. It was patterned. When enlarged using a better microscope and **focused correctly** evidence of crystal formation was seen.

A Good Precipitate

Initial examination classified this as a precipitate. It was patterned and took up dye. When enlarged using a better microscope evidence of crystal formation was seen.

Bad Precipitate



A Bad Precipitate gone good

Bad can be good

A Bad Precipitate trying to look good

Amorphous, ‘bad’ precipitate

Generally bad

Precipitate can be either good or bad.

Further characterization is needed to determine which.

However, with a good microscope there is a little good in a lot of bad.

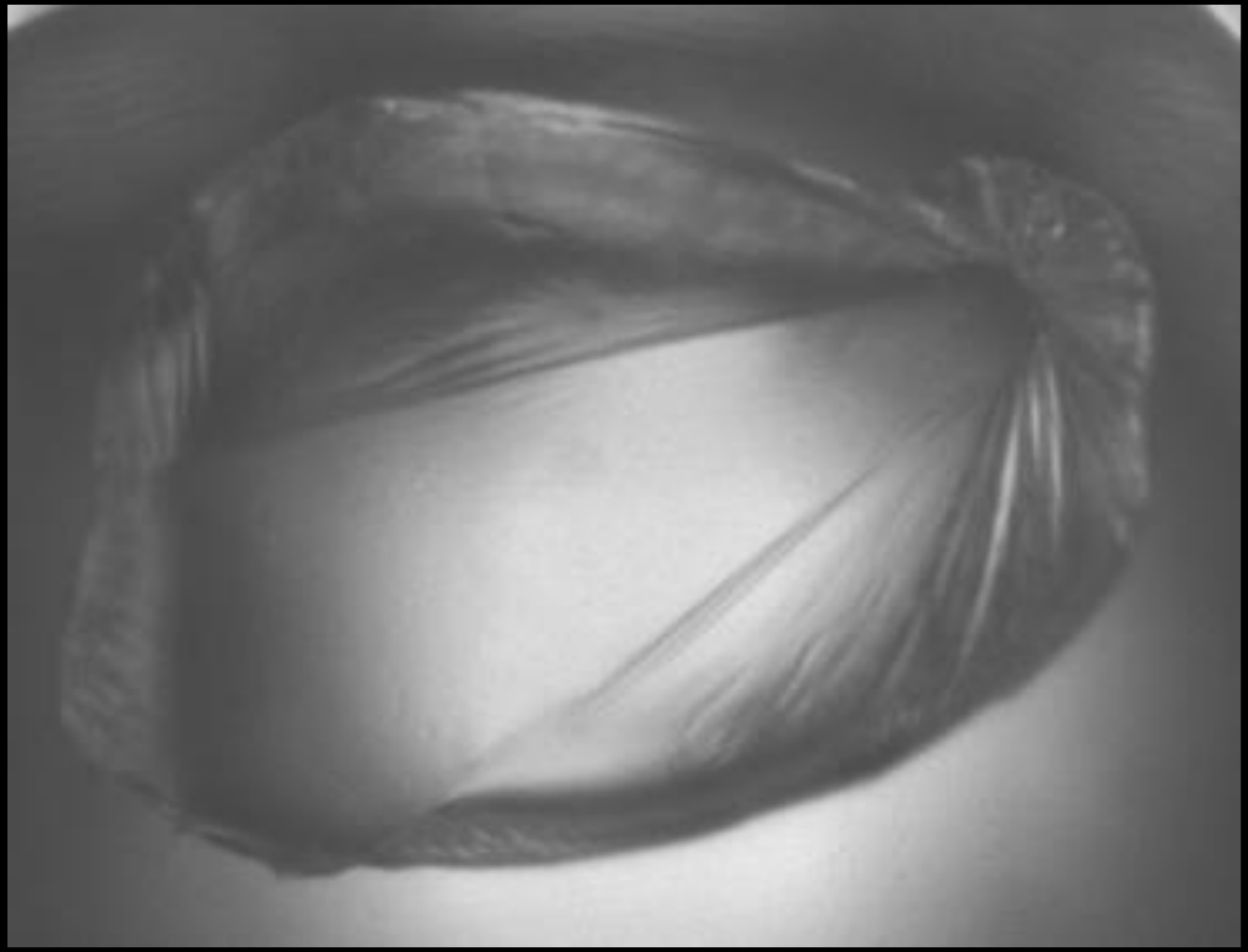
Skin Formation

Skin Formation

- Skin is a form of interfacial adsorption of the protein onto the interface whether it is solution/oil or solution/air, or solution/surface such as the plastic and glass materials that typically support the protein drop
- There is an induction period or lag-time involved in this process due to diffusive and convective transport as the protein begins to concentrate at the interface
- For protein solutions with concentrations greater than 50 to 100 µg/mL, two orders of magnitude less than typical crystallization experiments, the lag-time is not resolved, having a time-scale faster than the experimental technique used for observation

Skin Formation

- Layers of protein molecules undergo conformational changes, proteins aggregate, form branches, and can produce a viscoelastic, gel-like network structure (skin) that is often an irreversible process permanently denaturing the protein.
- Contemporary anecdotal reports that suggest higher concentrations of reducing agents, such as 25mM dithiothreitol, can sometimes alleviate skin formation on crystallization drops.
- Silicon grease35 and Fluorinert36 have been deposited onto a surface to float the crystallization drop to prevent crystals from adhering. While intended to ease removal of the fragile crystals that form on the substrate's surface, these protocols also change critical interfacial properties which can affect crystallization.



Skin is bad ….

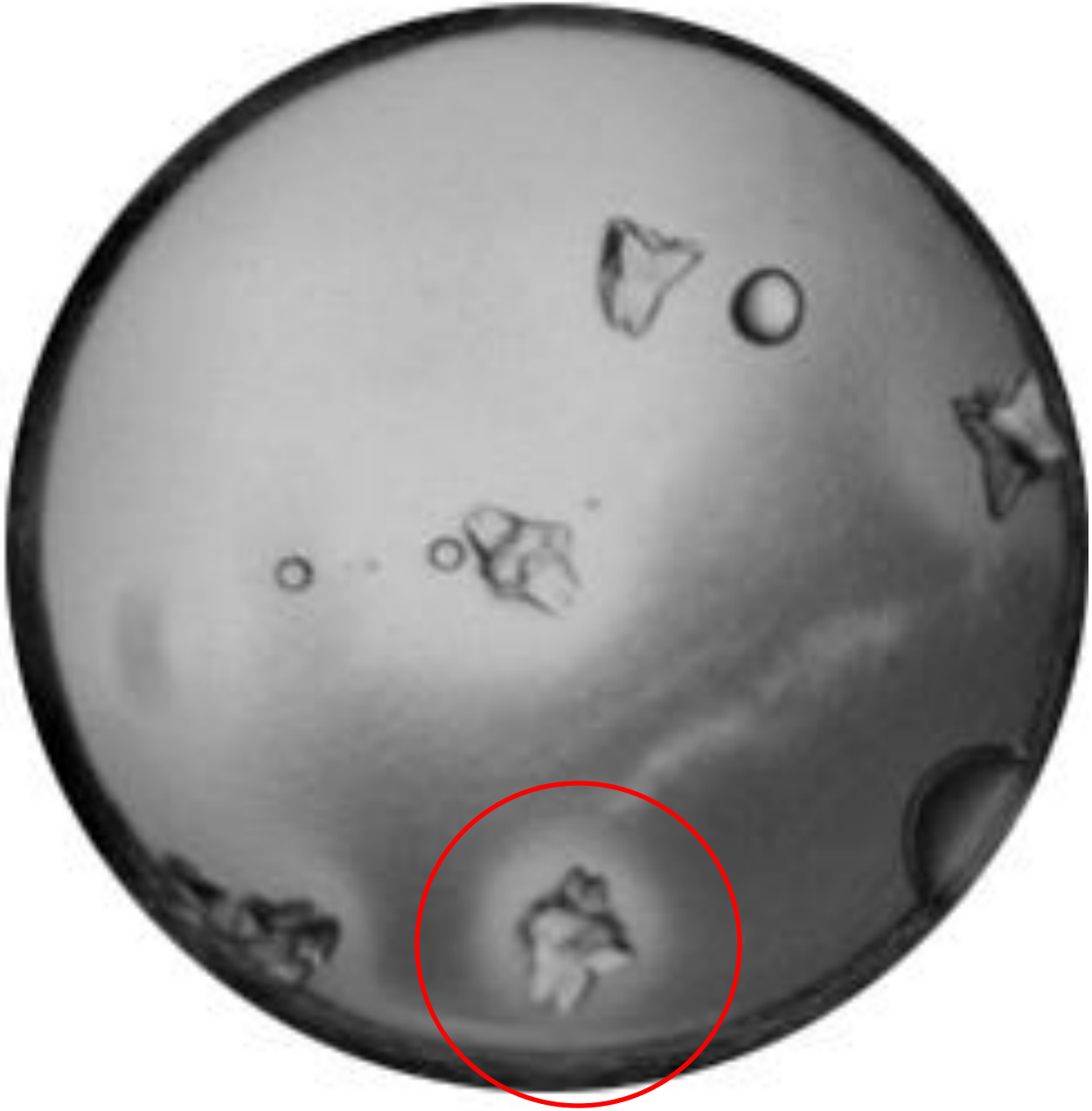
Time

Time is an important factor

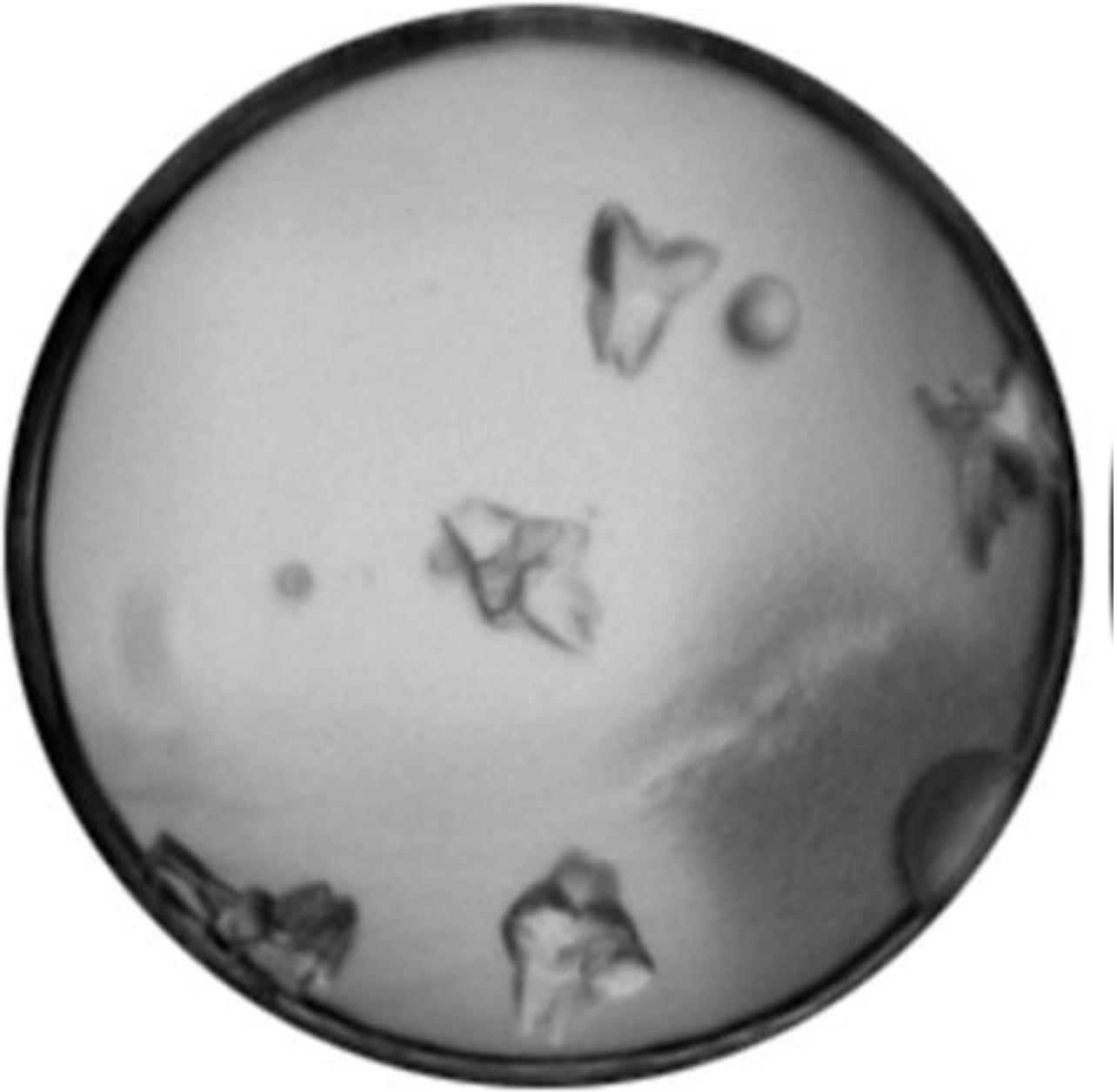
- In some cases amorphous precipitate may be observed, followed over time by a small crystal which slowly grows while the precipitate recedes.
- This is described by Ostwald's rule of stages which simply states, "When leaving a given state and in transforming to another state, the state which is sought out is not the thermodynamically stable one, but the state nearest in stability to the original state."
- In terms of crystallization, this means that the least soluble solid state will be the first to come out of solution.
- The next form to appear is not necessarily the most thermodynamically stable, but rather the form that is closest in energy to the first material to phase separate from the solution.
- This process continues, with a series of intermediate metastable forms, whose appearance is dependent on kinetics and not solely thermodynamics, until the formation of the most thermodynamically stable state, the form with the lowest Gibb's free energy.

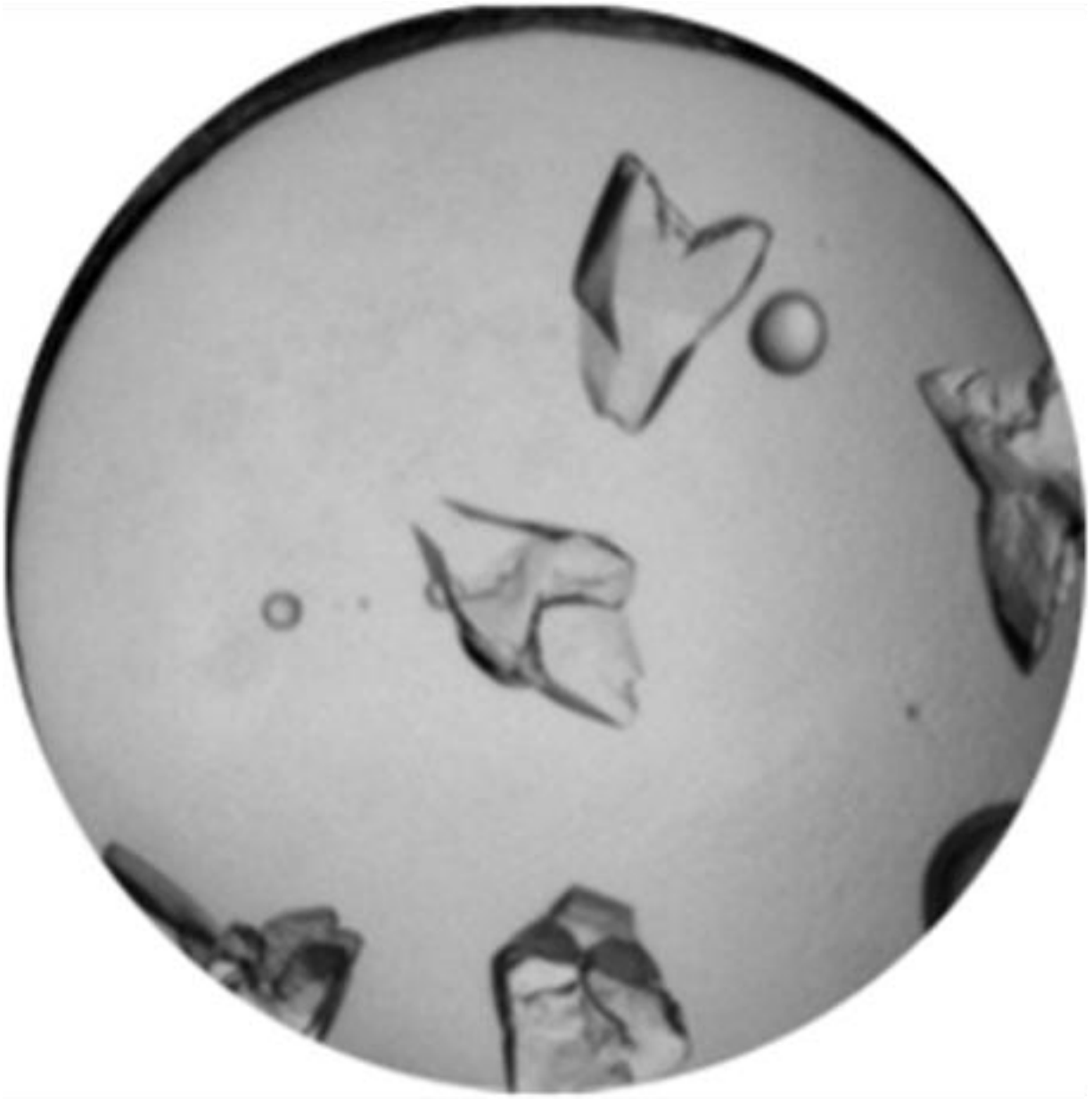
Time is an important factor

- Eloquently stated by Threfall, "The very existence of different forms at a given temperature is proof of the triumph of kinetics over thermodynamics".
- Another common example is when different morphologies of a crystalline protein are observed in a single drop where they can co-exist for some time.
- However over time, one crystal form, the most thermodynamically stable form, will increase in size at the expense of the other.
- Ostwald's rule of stages is not the same as Ostwald ripening. Ostwald's rule of stages transitions between different states to decrease the free energy of the system, while Ostwald ripening will decrease the surface free energy of a system of single small crystals through mass-controlled transport to larger crystals of the same form.



Crystal growing from precipitate



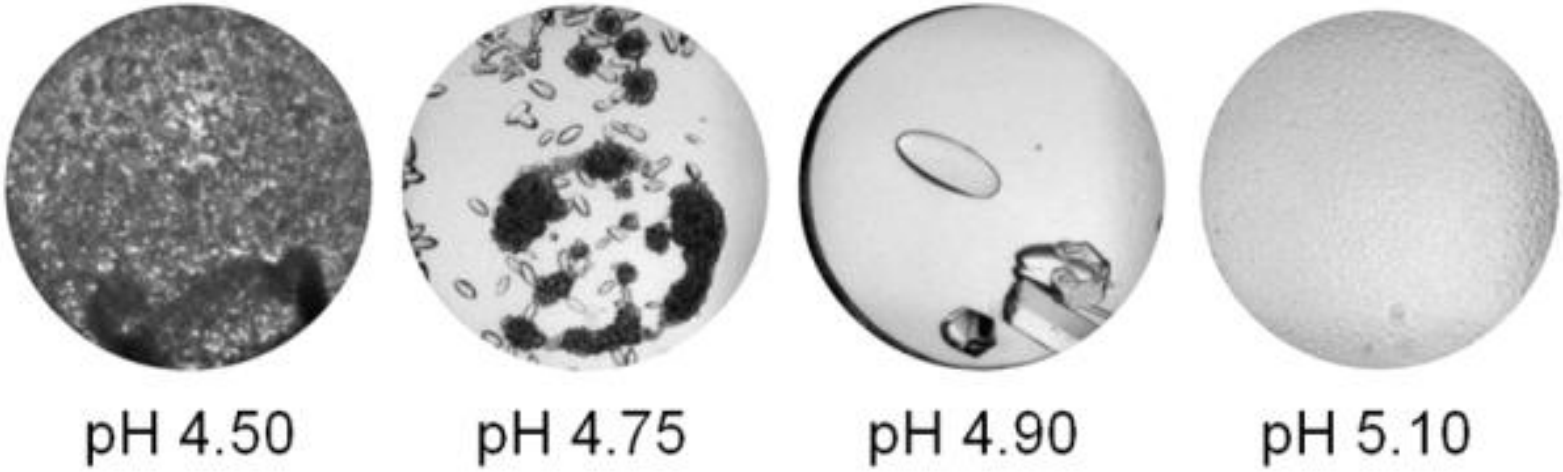


Observation over time is critical to understanding the process
(but not getting the structure)

Optimization

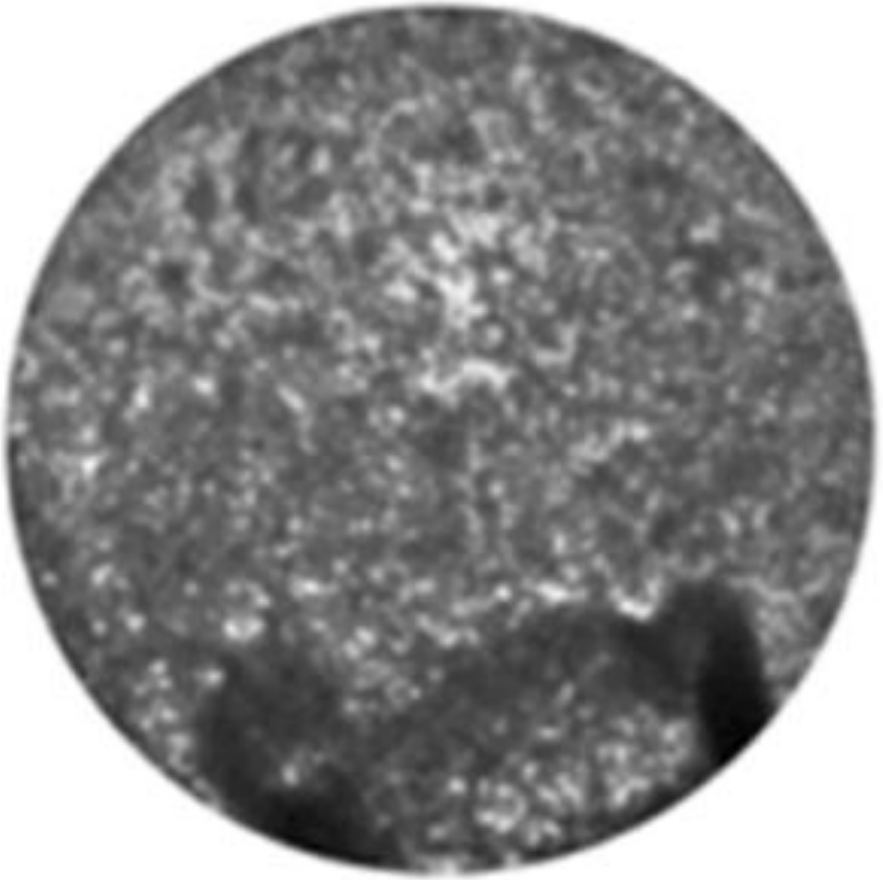
Small changes can have big effects

pH can drive crystallization



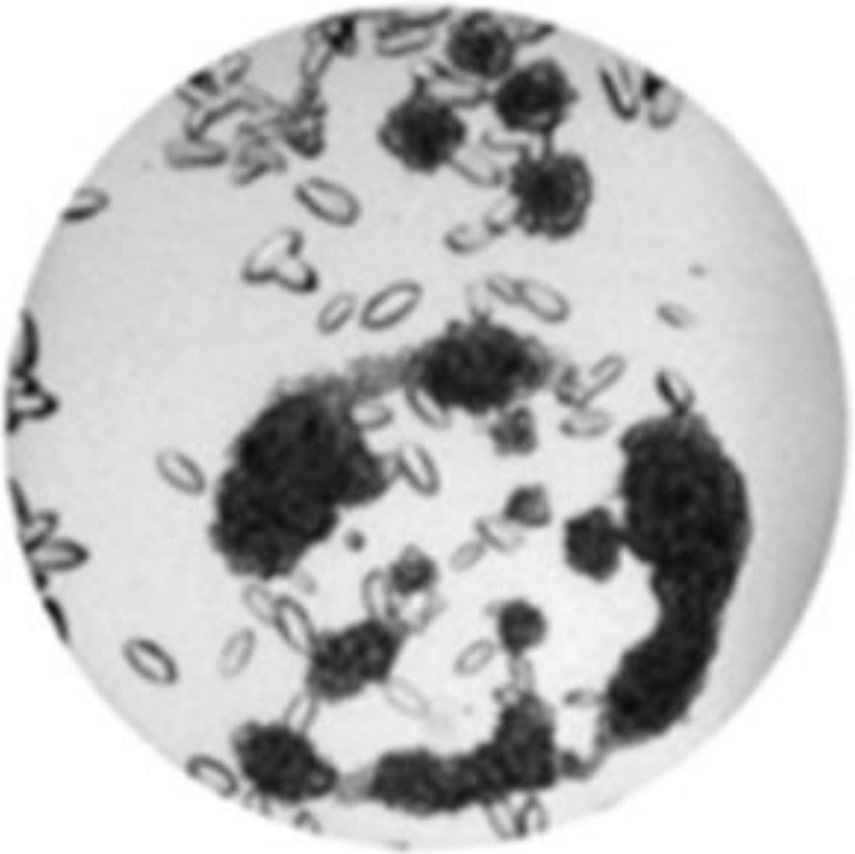
Note the good precipitate, crystal, phase separation progression

Kaput

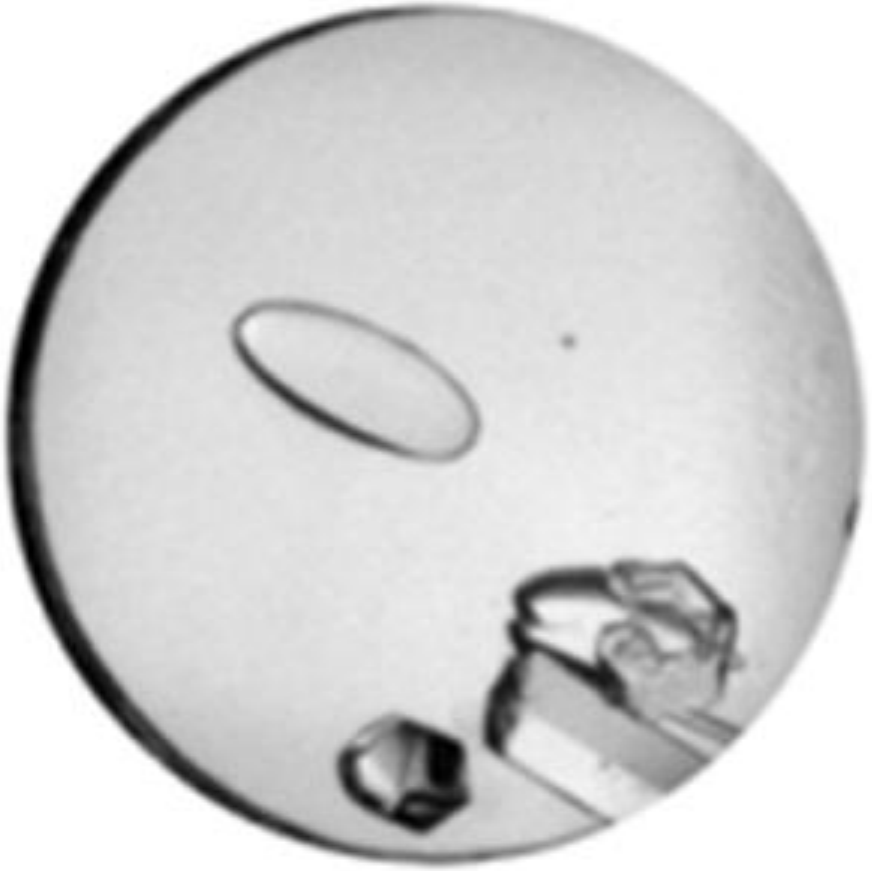


pH 4.50

Crystals



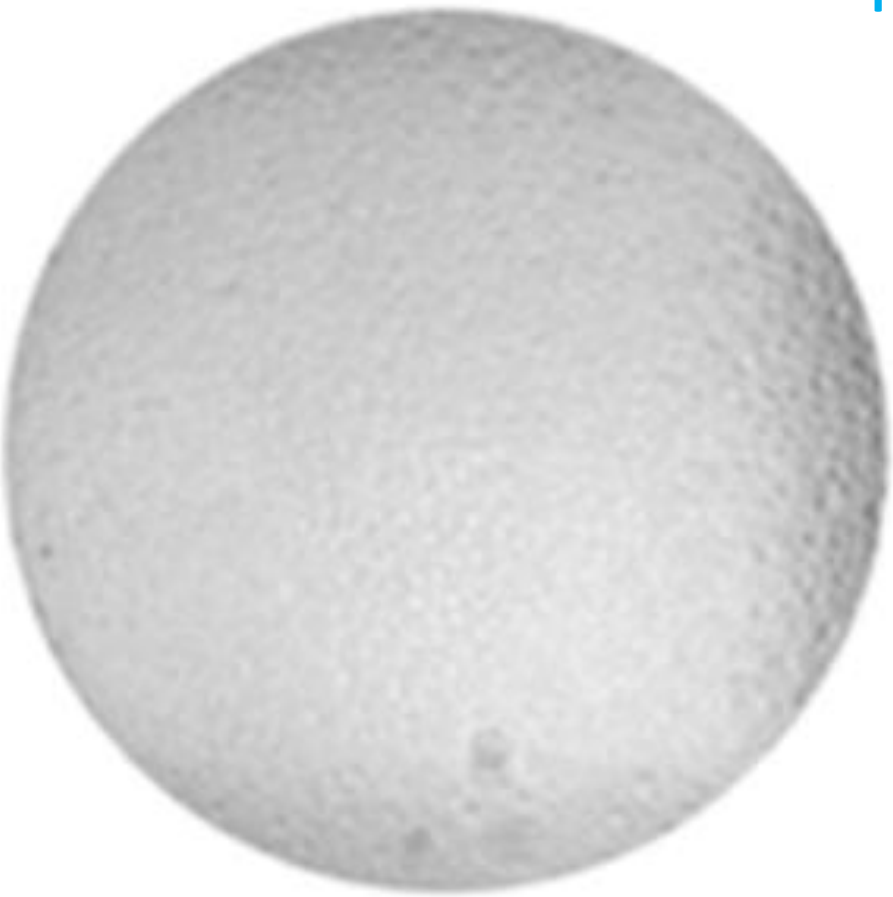
pH 4.75



pH 4.90

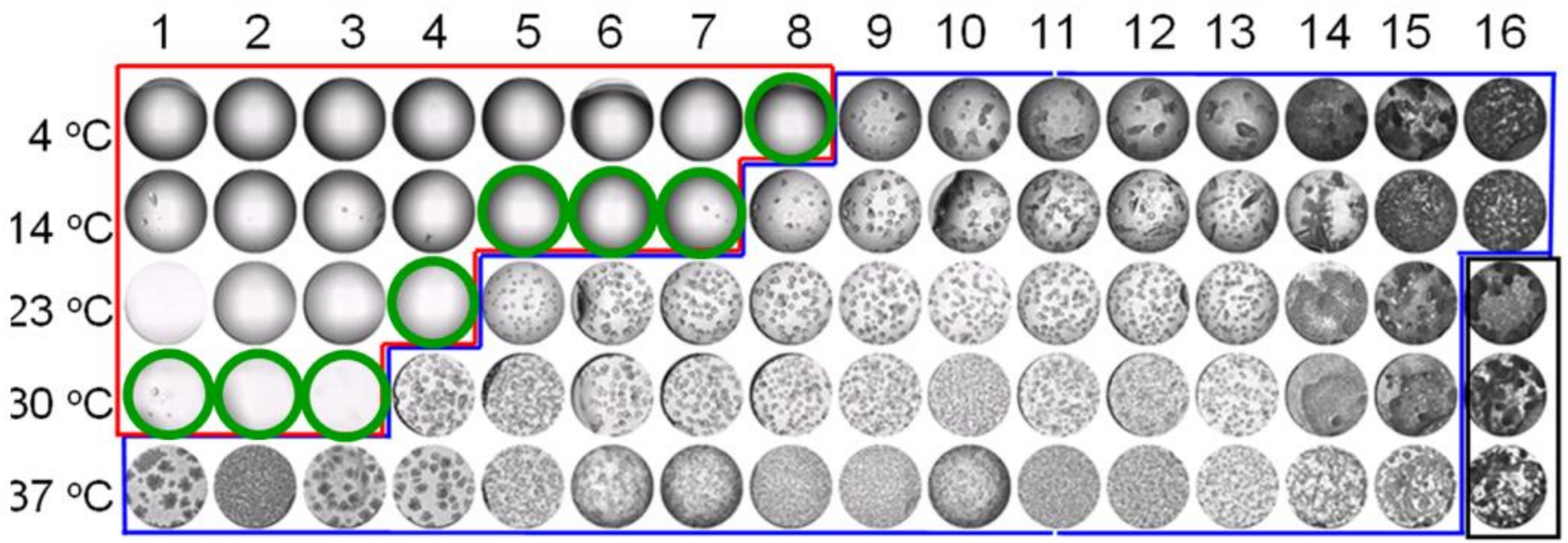
Crystals (note the curved edge on the top)

Phase separation



pH 5.10

Careful optimization design
(with simple parameters)
can yield the phase diagram

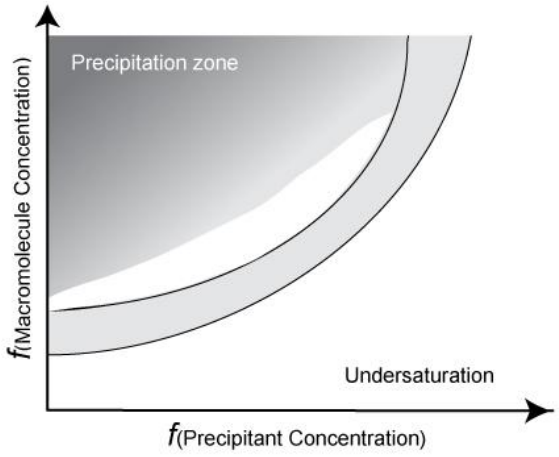


- Efficient optimization of crystallization conditions by manipulation of drop volume ratio and temperature Joseph R. Luft, Jennifer R. Wolfley, Meriem I. Said, Raymond M.
- Nagel, Angela M. Lauricella, Jennifer L. Smith, Max H. Thayer, Christina K. Veatch, Edward H. Snell, Michael G. Malkowski, and George T. DeTitta. Protein Sci. 2007 April;
- 16(4): 715–722.

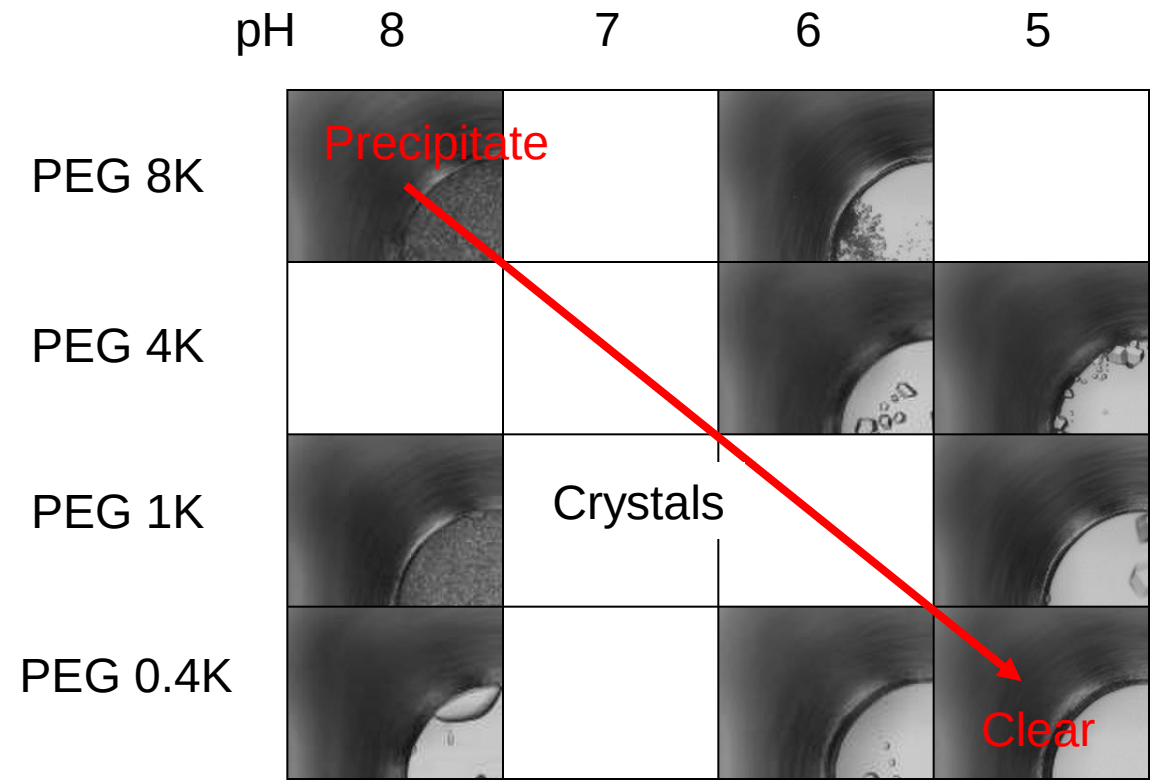
How do we use this information?

Chemical space provides a vector for optimization

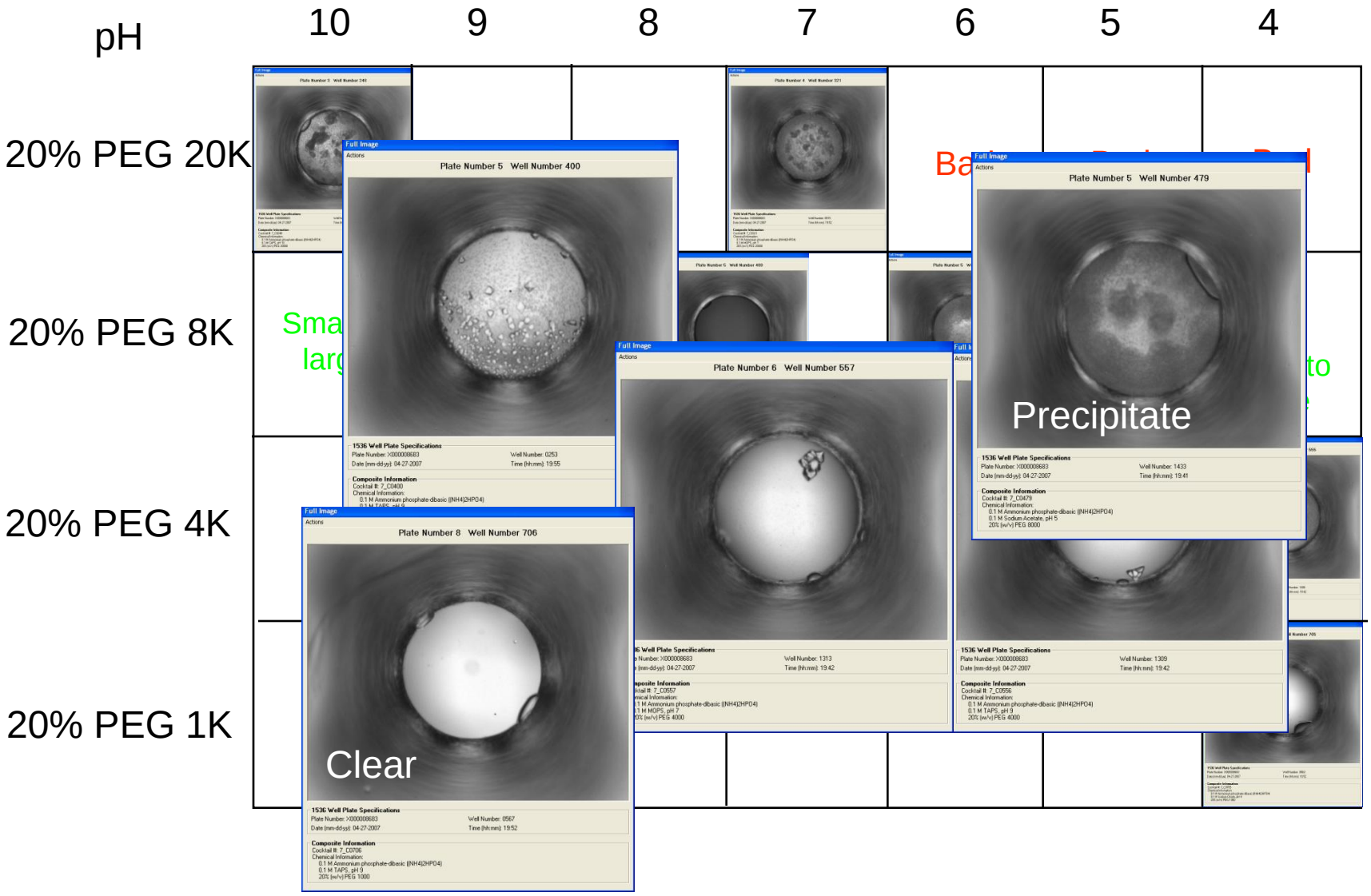
In this case the path from precipitate through crystals to clear is obvious. The phase diagram is reversed. Also clear are the number of chemical conditions that have not been sampled.

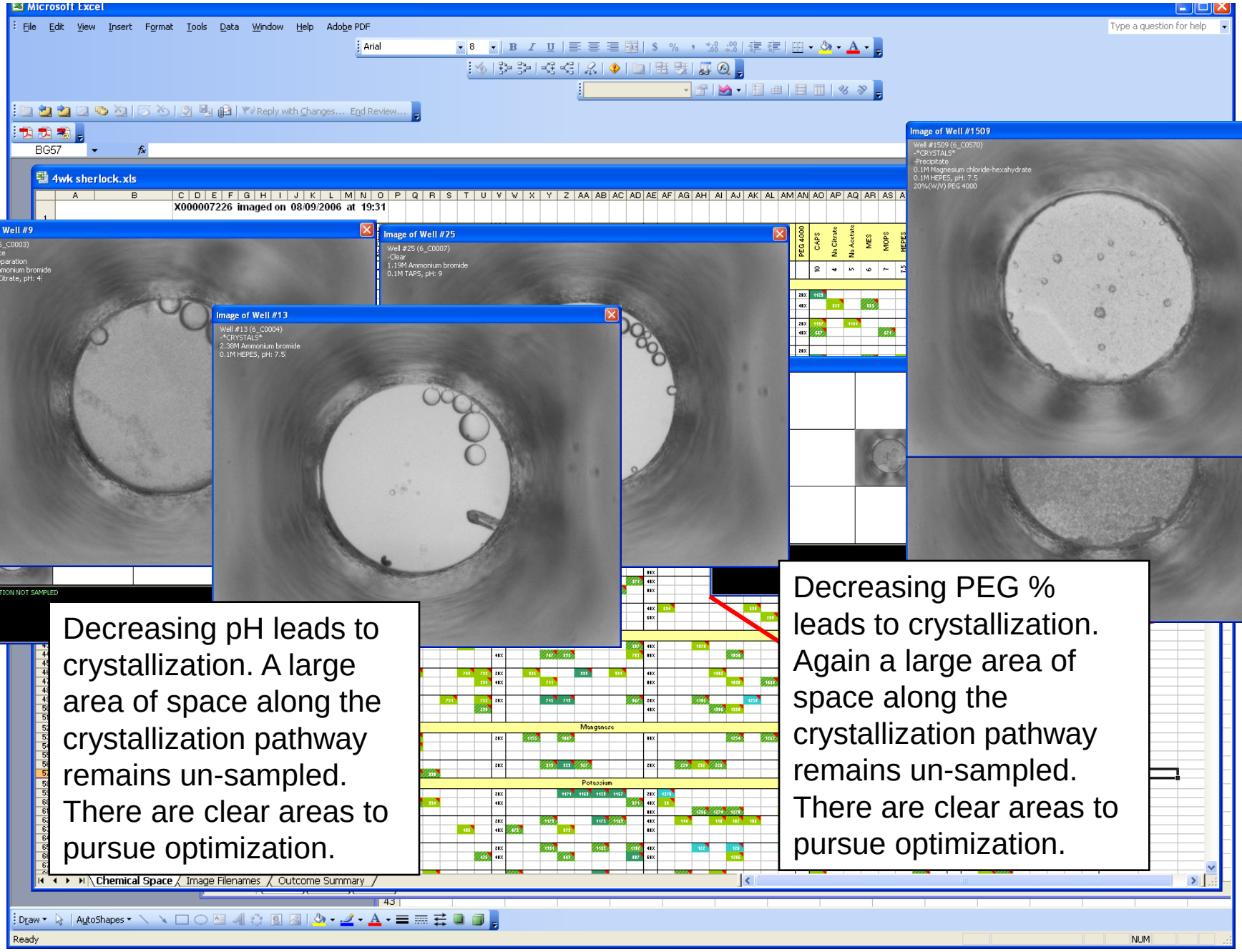


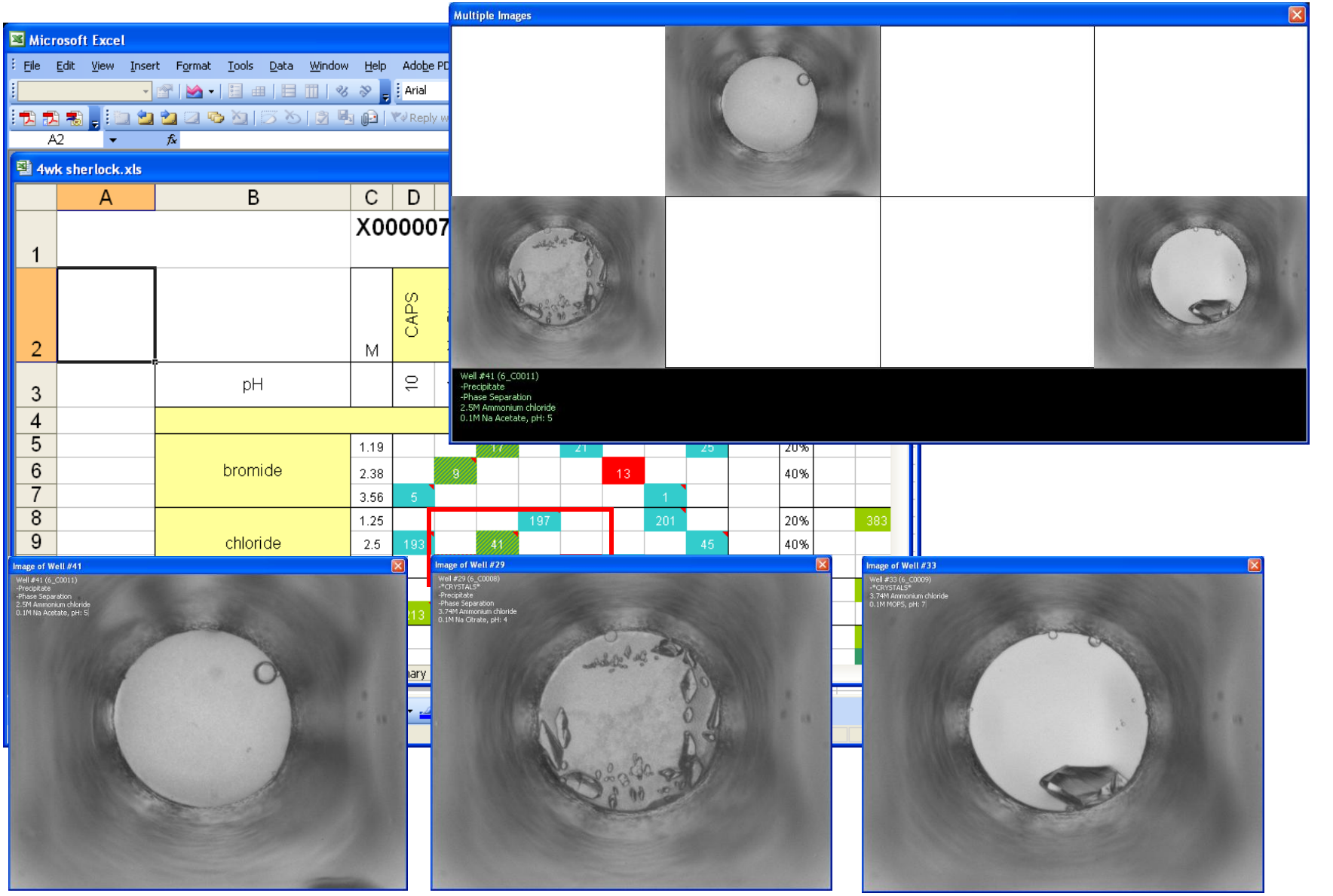
Ubiquitin, 40% PEG, 0.1M zinc acetate



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




Microsoft Excel
4wk sherlock.xls
X000007226 imaged on 08/09/2006 at 19:31
Image of Well #9
Well #9 (6_C0003)
-Precipitate
-Phase Separation
2.38M Ammonium bromide
0.1M Na Citrate, pH: 4
Image of Well #25
Well #25 (6_C0007)
-Clear
1.19M Ammonium bromide
0.1M TAPS, pH: 9
Image of Well #13
Well #13 (6_C0004)
-*CRYSTALS*
2.38M Ammonium bromide
0.1M HEPES, pH: 7.5
Image of Well #1509
Well #1509 (6_C0570)
-*CRYSTALS*
-Precipitate
0.1M Magnesium chloride-hexahydrate
0.1M HEPES, pH: 7.5
20%(W/V) PEG 4000
CONDITION NOT SAMPLED
Manganese
Potassium
Chemical Space
Image Filenames
Outcome Summary
Ready
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.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File Edit View Insert Format Tools Data Window Help Adobe PD
Arial
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CAPS
pH
10
bromide
chloride
1.19
2.38
3.56
1.25
2.5
Multiple Images
Well #41 (6_C0011)
-Precipitate
-Phase Separation
2.5M Ammonium chloride
0.1M Na Acetate, pH: 5
Image of Well #41
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

What information is available in typical screens?

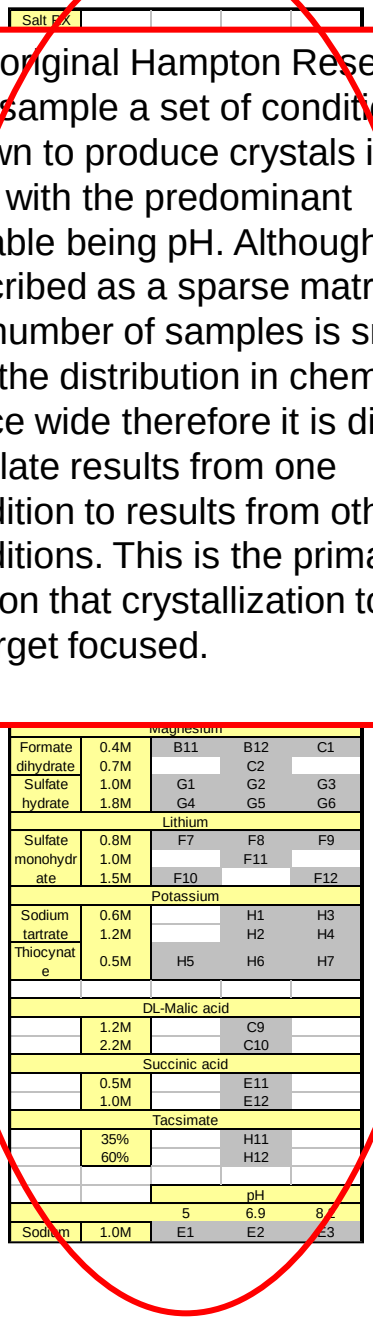
The Commercial Screens in the HWI crystallization cocktails

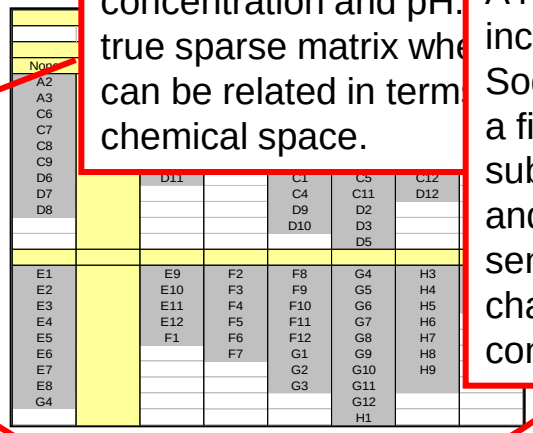
The original Hampton Research 1+2 sample a set of conditions known to produce crystals in the past with the predominant variable being pH. Although described as a sparse matrix the number of samples is small and the distribution in chemical space wide therefore it is difficult to relate results from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

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

The SaltRx screen samples 22 crystallization salts with varying concentration and pH. true sparse matrix whe can be related in term chemical space.

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 would 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	pH	Sodium phosphate	Neutralized organic acids (ph 7.0)	pH	High supersaturation salt and low polymer	pH	Low ionic strength systems	pH	Non-volatile organics
3.5	A1	A7			5.6	B5	B9	5.5	C8	3.5	D4	5.5	D12
4.5	A2	A8			6.9	B6	B10	6.5	C6	4.5	D5		E2
5.5	A3	A9	B1		8.2	B7	B11	8.5	C7	5.5	D6	6.5	E1
6.5	A3	A10		B2			B12	7	C9	6.5	D7		E3
7.5	A5	A11	B3				C1		C10		D10		E6
8.5	A6	A12		B4			C2		C11		D11		E9
							C3		C12	7	D2		E10
	Classic salt versus pH						C4				D3	7.5	E4
							C5			7.5	D8		E7
	Hits here indicate that a variation of salt concentration and pH in a grid screen has a strong potential for crystallization									8.5	D9		E8
													E11
												8.5	E5
													E12

PEGs and Salts as a function of pH								PEG 3350 and salts										
	3.35K					10K	3.35K											
pH	Ammonium sulfate	Sodium chloride	Lithium sulfate monohydrate	Ammonium acetate	Magnesium Chloride hexahydrate	Ammonium acetate	Mixed chloridehydrates	%	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
5.5	F6	F10	G2	G6	G10	F5		15				H5			H8				
6.5	F7	F11	G3	G7	G11			20	H2	H3	H4		H6	H7		H9	H10		
7.5	F8	F12	G4	G8	G12		F4	25											
8.5	F9	G1	G5	G9	H1			30										H11	H12

Coarse test for chemical conditions likely to produce crystallization

D'Arcy and Cudney

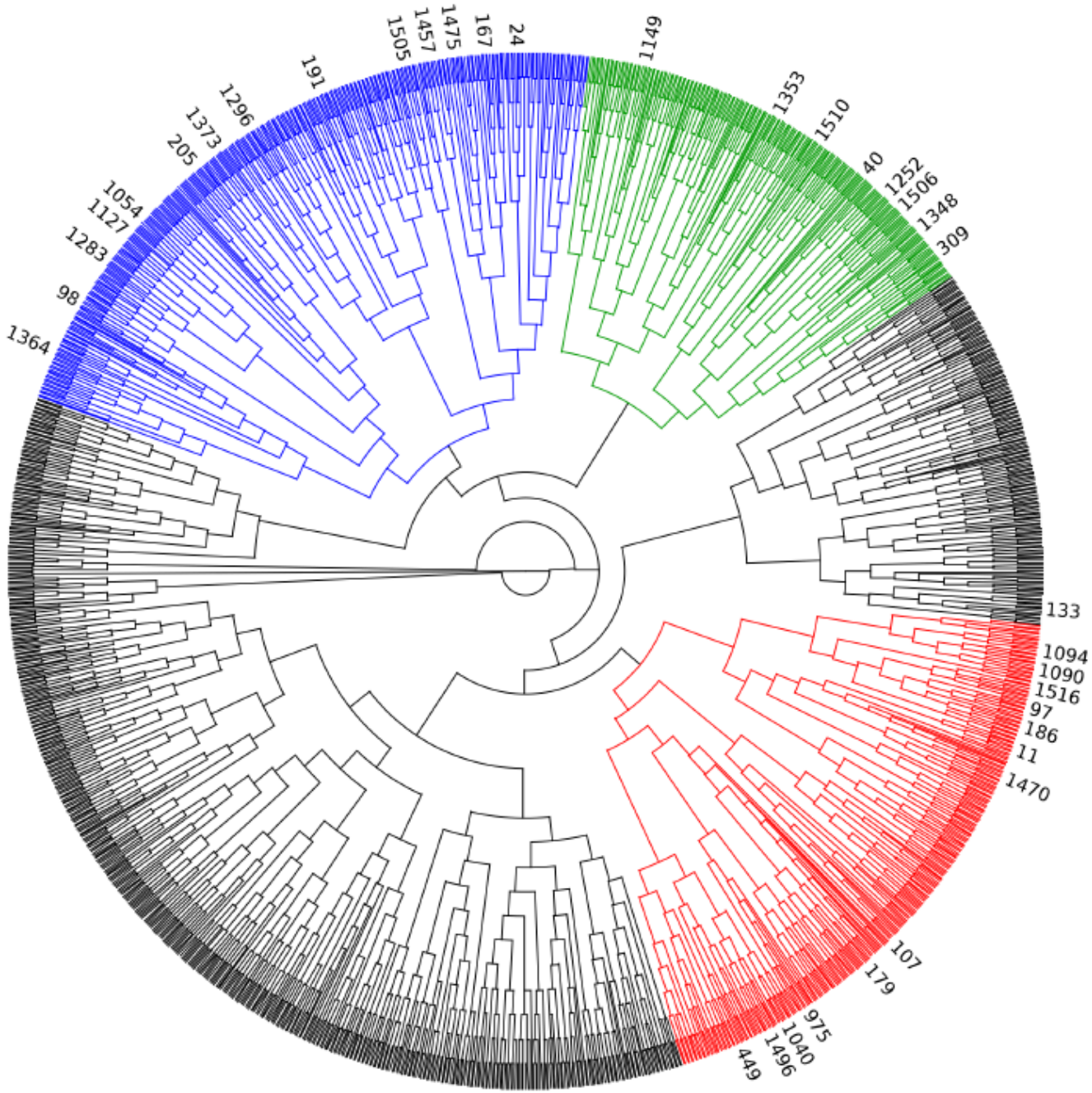
Know “why” your screen is designed.

Can we use screening to describe biology?

Fundamentally important work

C6 by the Newman Lab

The C6 Web Tool: A resource for the rational selection of crystallization conditions. J. Newman, V.J. Fazio, B. Lawson, T.S. Peat Crystal Growth & Design 01/2010; 10:2785-2792. pp.2785-2792


24
167
1475
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1296
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1516
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186
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1470
107
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975
1040
1496
449

Group 3							
1094	1	Sodium malonate	1.5			6.0	
1095	2		1.9			6.0	
1090	3		2.4			5.0	
1255	4	Sodium acetate	0.2	Sodium cacodylate	0.1	6.5	
1516	5	Magnesium sulfate	1.8	Sodium acetate		4.6	
176	6	Sodium nitrate	2.6			5.0	
1483	7		1.50			4.6	
97	8	Potassium nitrate	5.76			5.0	
26	9	Ammonium phosphate-monobasic	0.96			5.0	
1490	10	Ammonium dihydrogen phosphate	1.8			4.6	
186	11	Sodium phosphate-monobasic	1.1			5.0	
1456	12	di-Ammonium hydrogen citrate	1.8			4.6	
1449	13	Sodium Chloride	2.2			4.6	
11	14	Ammonium chloride	2.5			5	
1470	15	Sodium Formate	3.5			4.6	
1282	16		2.0	Sodium Acetate trihydrate		4.6	
1467	17		2.0	Sodium Acetate		4.6	
1100	18	Sodium malonate	1.5			7.0	
1101	19		1.9			7.0	
107	20	Potassium bromide	1.33	Sodium Citrate	0.1	4.2	
179	21	Sodium nitrate	1.3			4.2	
130	22	Potassium nitrate	0.88	Tris		8	
975	23	Cobalt sulfate heptahydrate	0.1	Sodium Citrate		4.2	
1124	24	Sodium dihydrogen phosphate monohydrate	0.27	Potassium phosphate dibasic	0.27	7.5	
1040	25		0.9		0.1	5.6	
1052	26		1.62		0.18	5.6	
1046	27		1.26		0.14	5.6	
1045	28		1.372		0.028	5.0	
1051	29		1.746		0.036	5.0	
1496	30	Sodium/Potassium Phosphate	1.8			5.0	
1317	31	Sodium phosphate monobasic monohydrate	0.1	MES monohydrate	0.1	6.5	2M Sodium chloride and 0.1M Potassium phosphate
449	32	Potassium phosphate dibasic	0.1	Sodium Citrate	0.1	4.2	20% PEG 8000
24	18	Ammonium phosphate-monobasic	1.92	MES	0.1	7	
						6.0	

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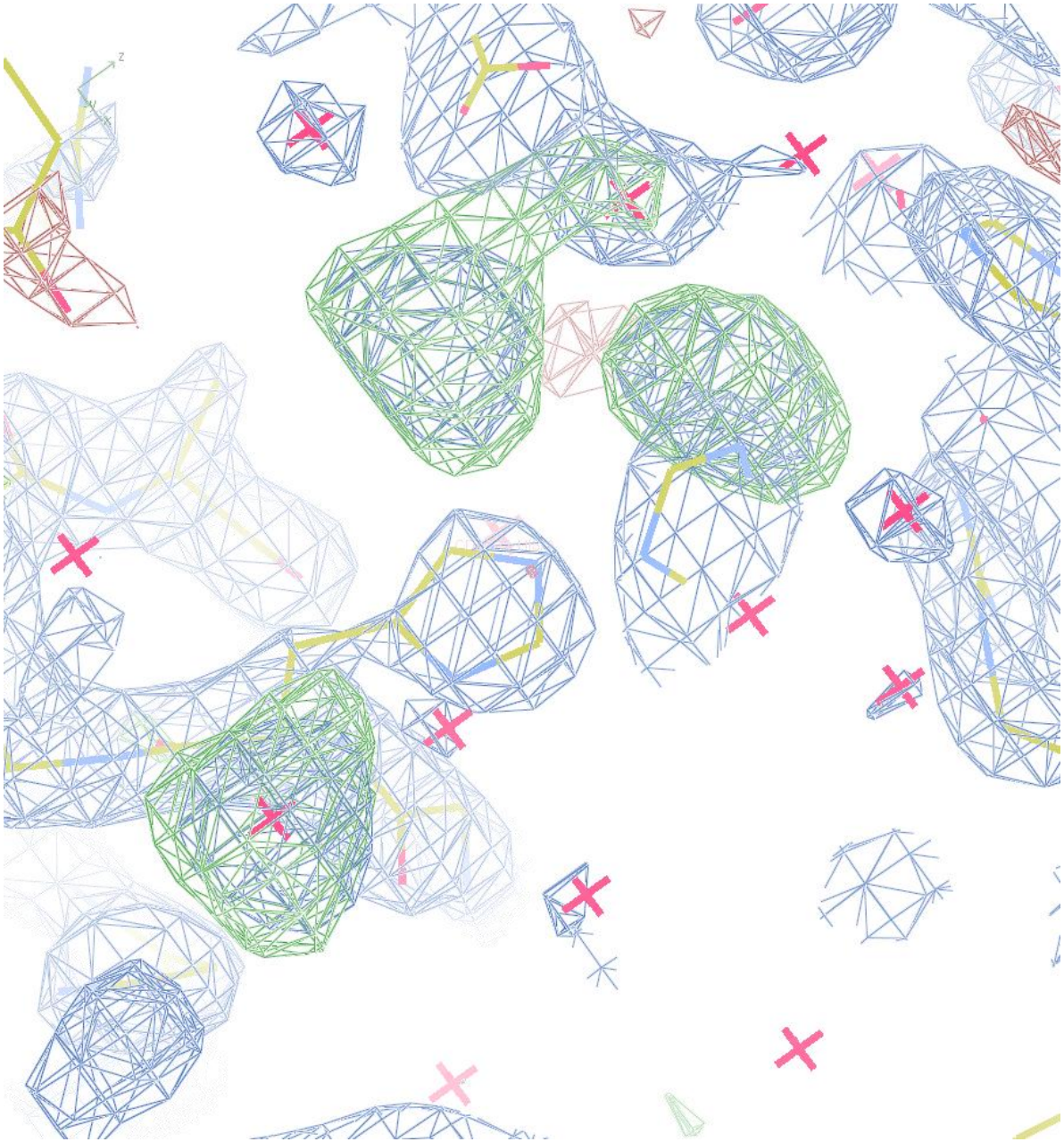
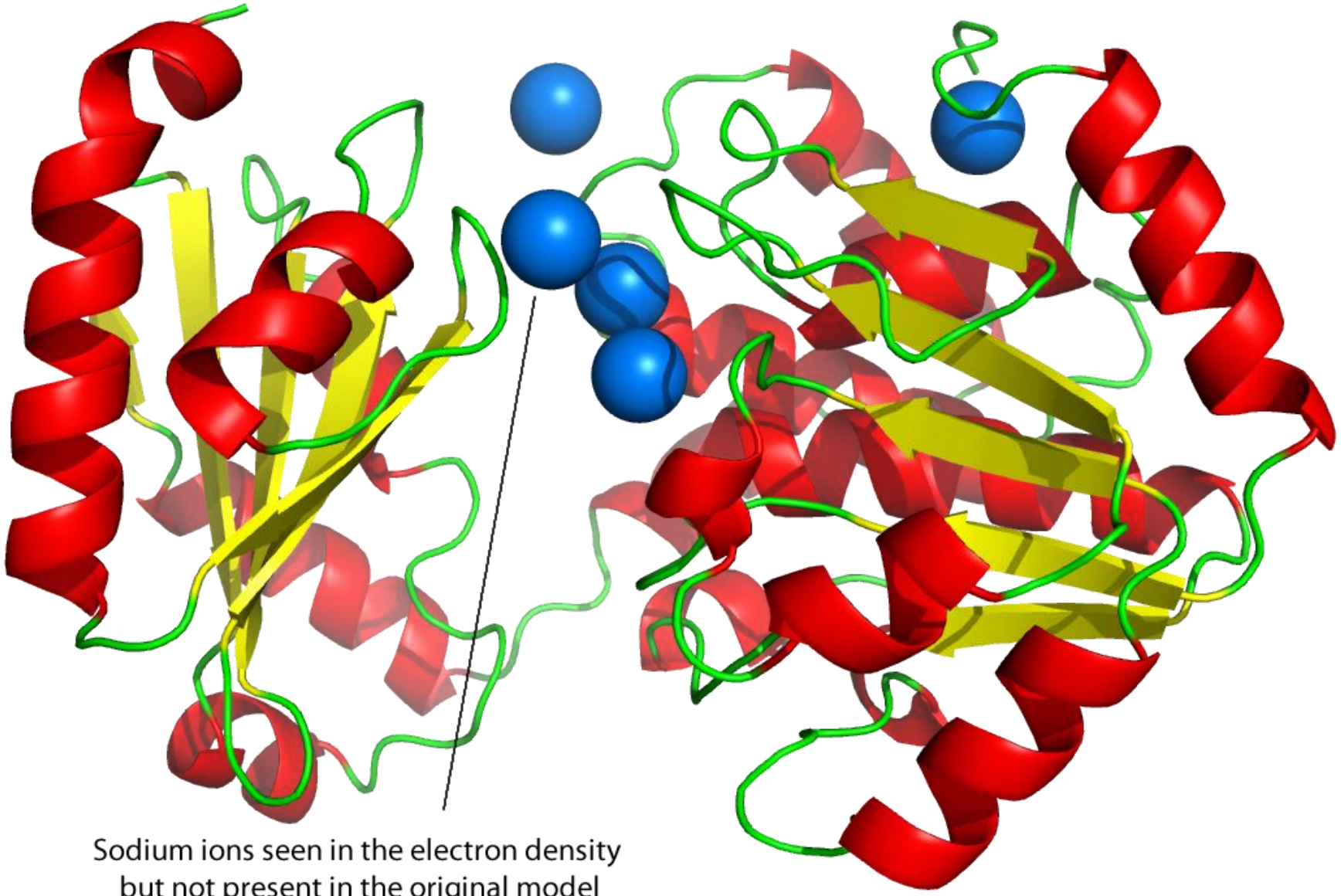
Cocktail #	Salt		Buffer		pH	Classification
1480	Ammonium Nitrate	6.0 M	Sodium acetate	0.10 M	4.6	Clear
176	Sodium nitrate	2.60 M			5.0	Clear
1483		1.50 M			4.6	Crystals
1486		4.60 M			4.6	Precipitate
97	Potassium nitrate	5.76 M			5.0	Crystal and precipitate
126		0.88 M			5.0	Crystal
129		1.77 M			5.0	Crystalline precipitate

Cocktail #	Salt	conc	Buffer	conc	pH	Classification
1522	di-ammonium tartrate	1.2 M	Sodium acetate	0.1 M	4.6	Clear
1099	Sodium malonate	1.0 M			7.0	Clear
1100		1.5 M				Crystals
1101		1.9 M				Small crystals
1104		3.4 M				Precipitate
1103		2.9 M				Precipitate

Cocktail #	Salt		Buffer		pH	Classification
1480	Ammonium Nitrate	6.0 M			4.6	Clear
176	Sodium nitrate	2.60 M			5.0	Clear
1483		1.50 M			4.6	Crystals
1486		4.60 M	Sodium acetate	0.10 M	4.6	Precipitate
97	Potassium nitrate	5.76 M			5.0	Crystal and precipitate
126		0.88 M			5.0	Crystal
129		1.77 M			5.0	Crystalline precipitate

Cocktail #	Salt	conc	Buffer	conc	pH	Classification
1522	di-ammonium tartrate	1.2 M	Sodium acetate	0.1 M	4.6	Clear
1099	Sodium malonate	1.0 M			7.0	Clear
1100		1.5 M				Crystals
1101		1.9 M				Small crystals
1104		3.4 M				Precipitate
1103		2.9 M				Precipitate





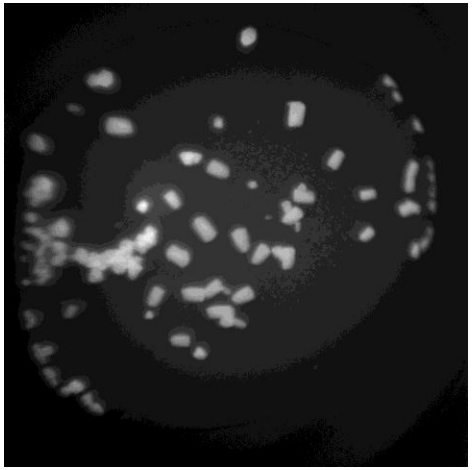
Sodium ions seen in the electron density
but not present in the original model

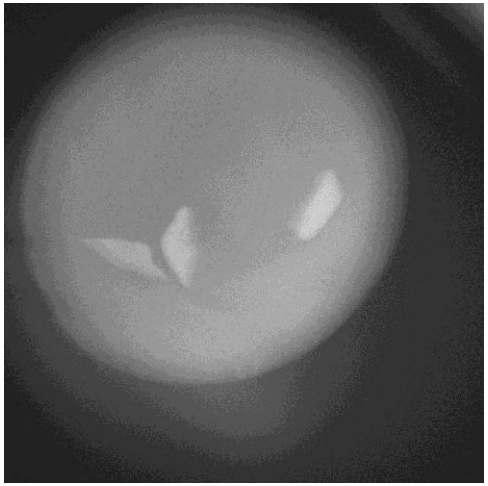
Crystallization screening is informative about biology

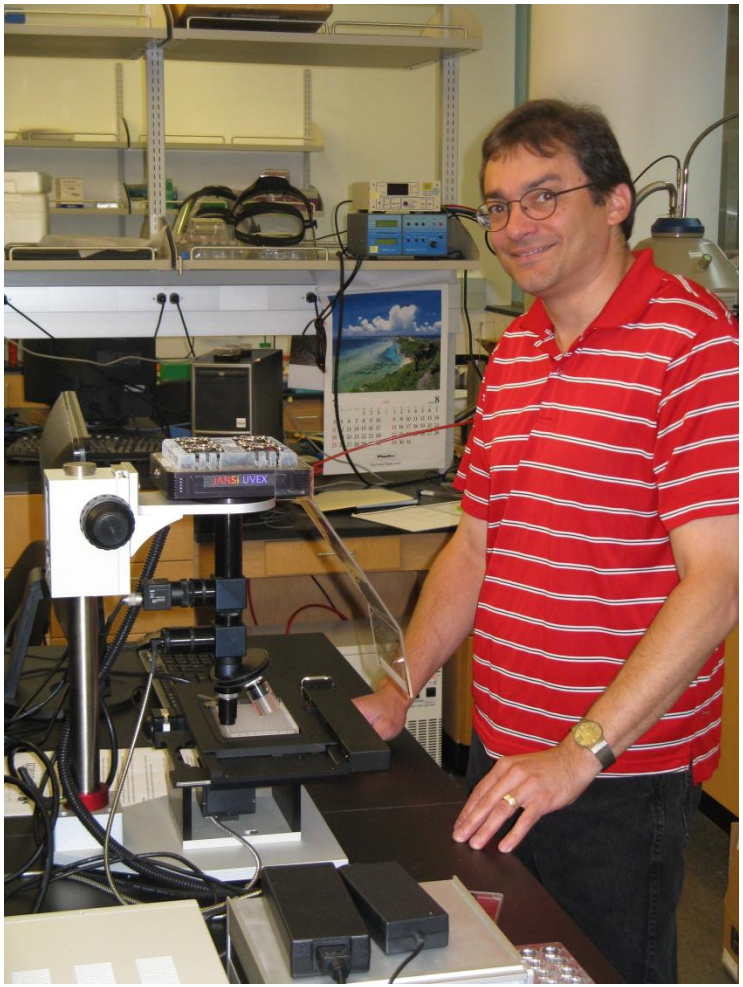
Plug for UV imaging …

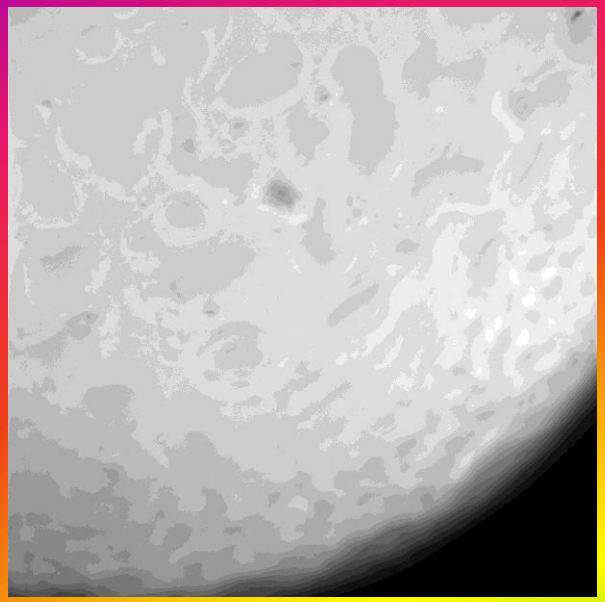
Or “the Good,
the Bad and the Ugly”

UV imaging – is it protein?









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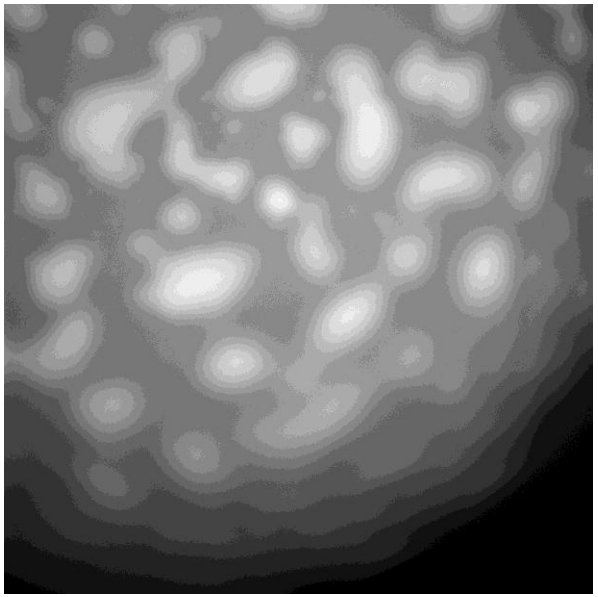
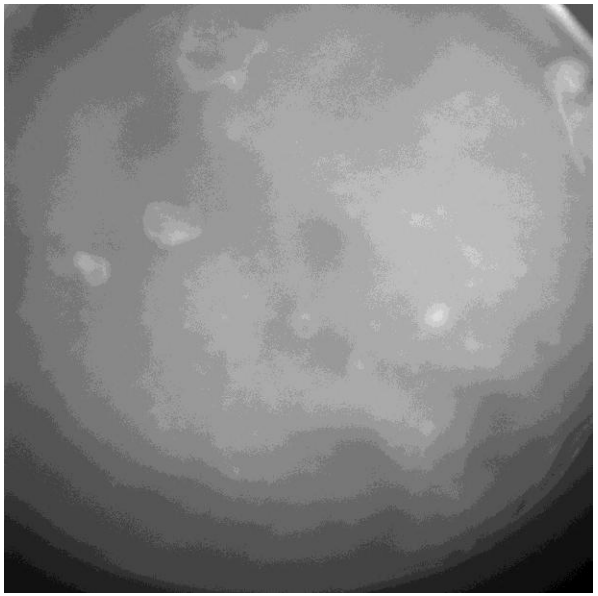
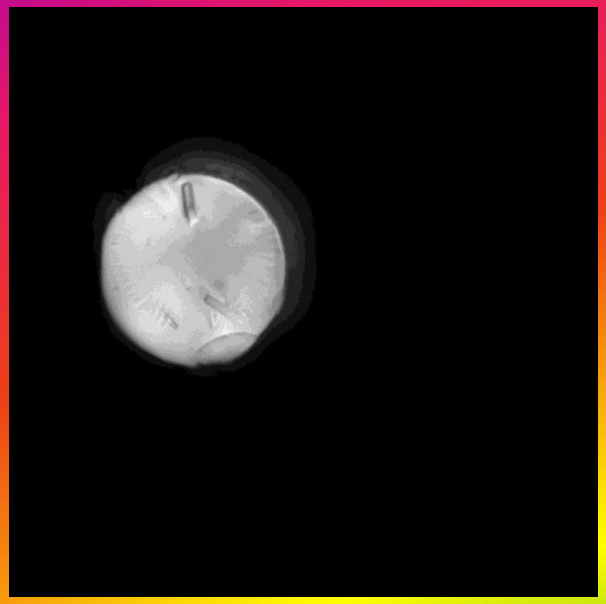


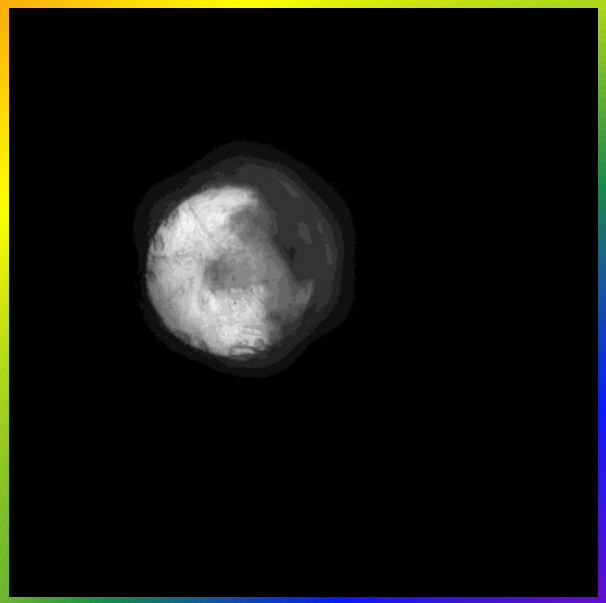


UV

Protein phase

Protein crystal





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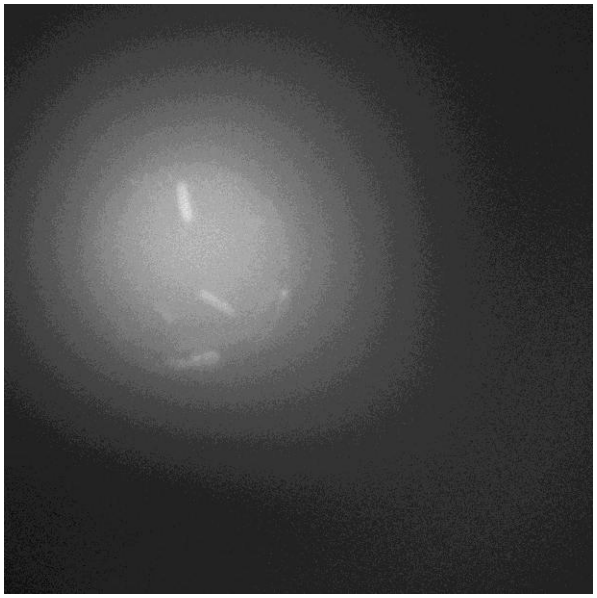
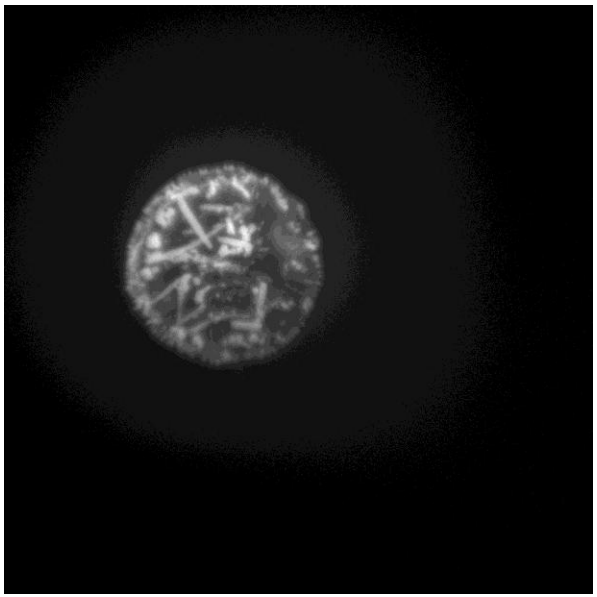
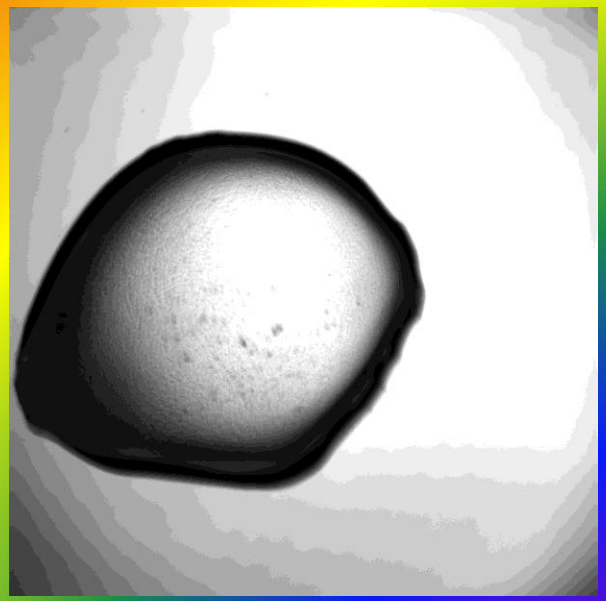


Protein crystal



Protein crystal

UV



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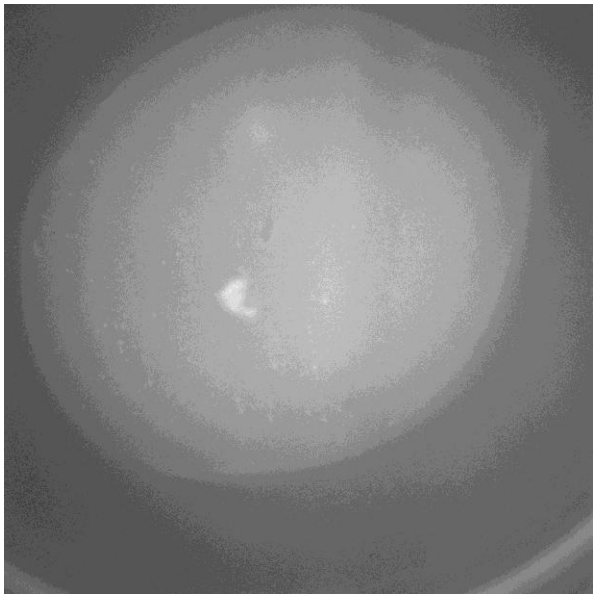
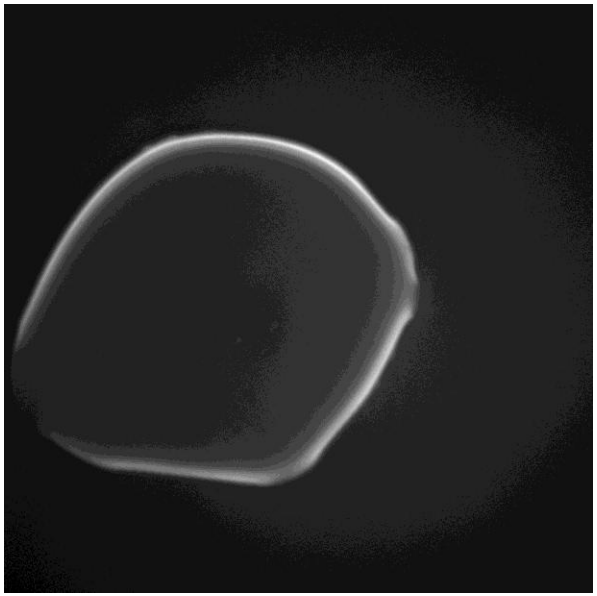
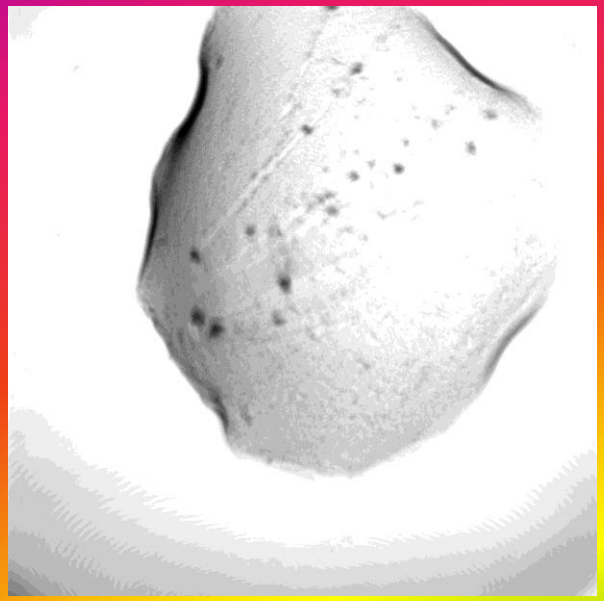


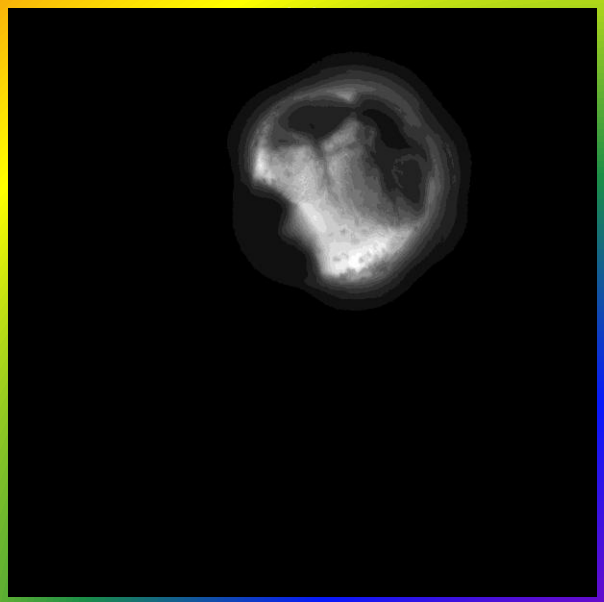


UV

Protein crystal

Salt crystals





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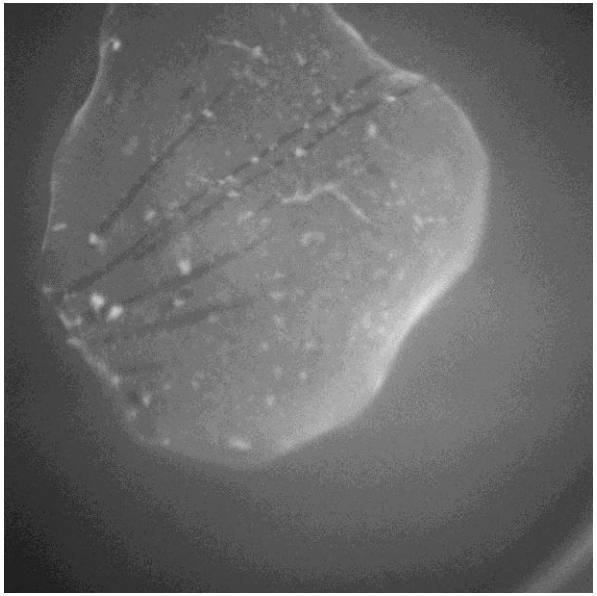
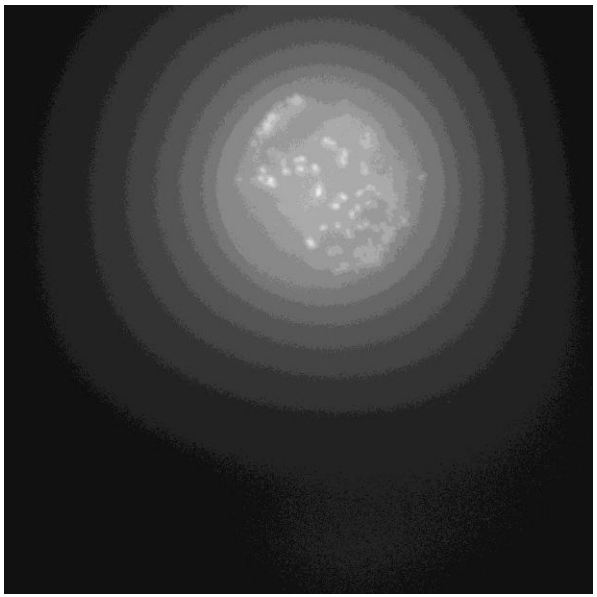


Protein crystals



Protein crystals

UV

Summary

- Crystallization should not be thought of as a binary process (crystal or not).
- Every result tells you something.
- Analyzing the results over time tells you something.
- Try and think about why you got each result with a crystallization phase diagram in mind

Importance of Summary

- No crystal
- No crystallography
- No crystallographer

- While the outcome may be bad, all screening results are good – use them all to get a complete picture

Reference to the old stuff

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What's in a Drop? Correlating Observations and Outcomes to Guide Macromolecular Crystallization Experiments

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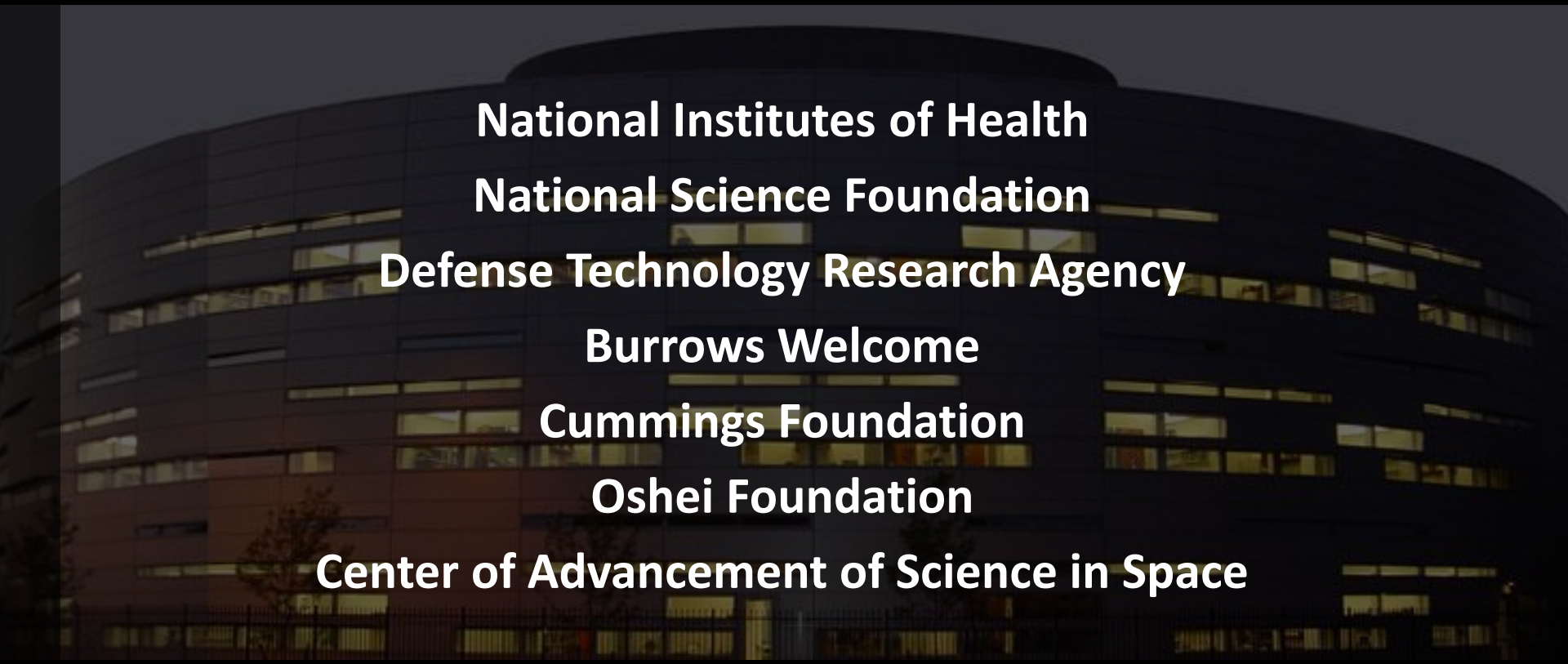

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Thank you and questions?

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