# From the user's perspective …


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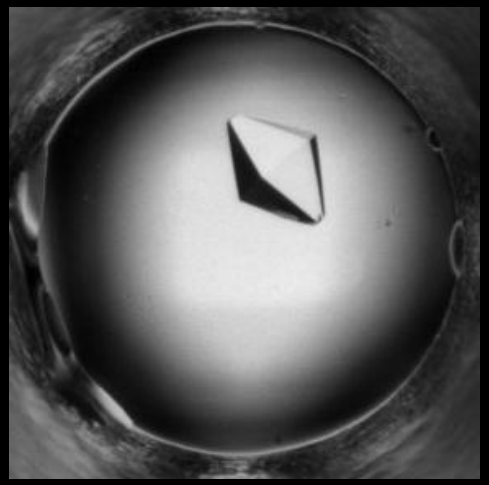
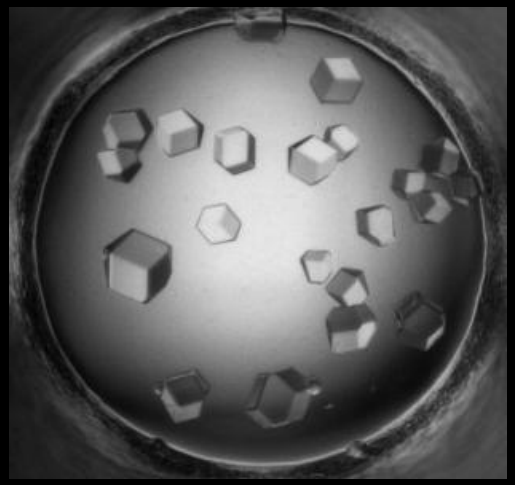
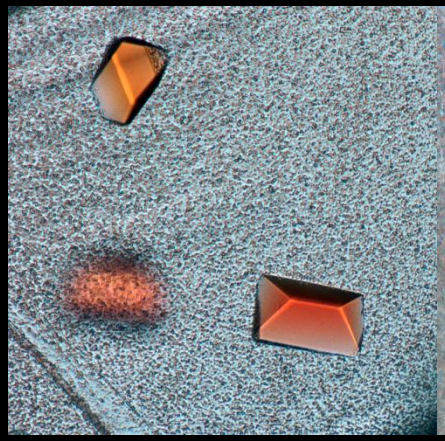
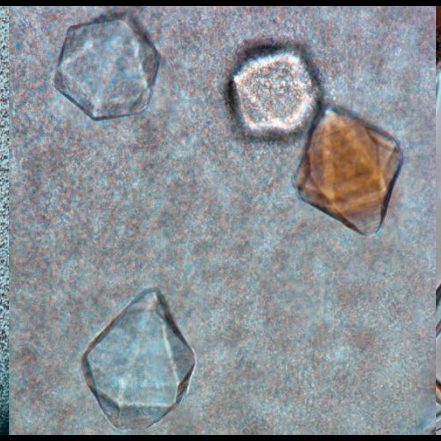
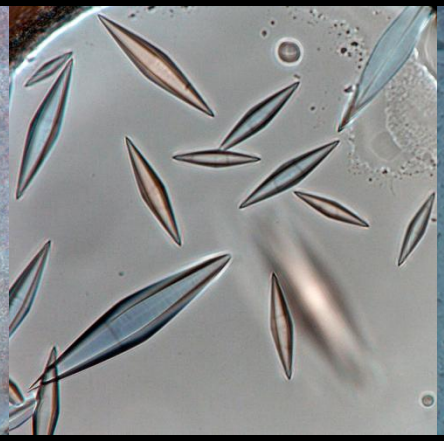
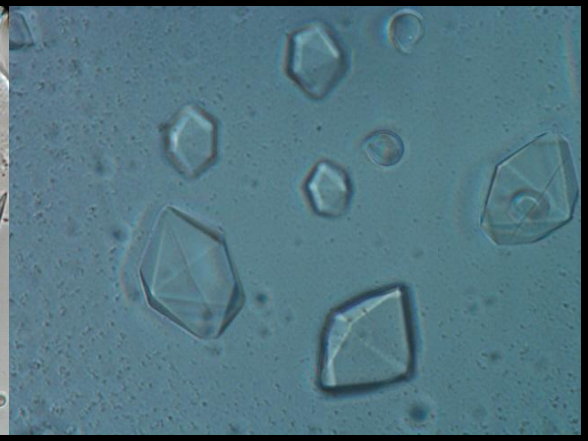
# Crystallization


# Crystallography Requires Crystals

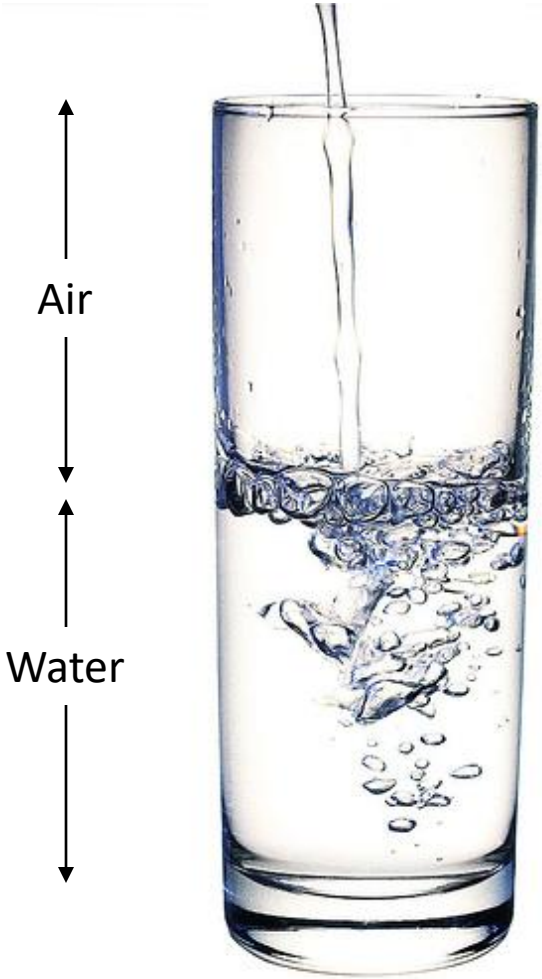





No crystal …

No crystallography ….

No crystallographer ….

# 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

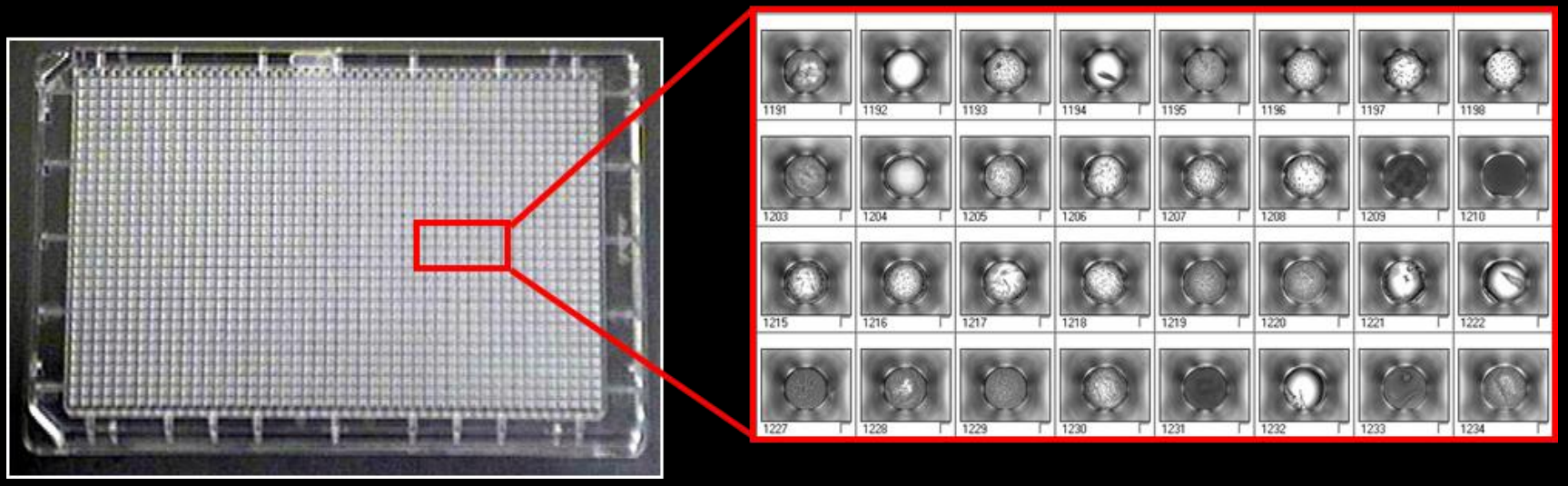
# The Crystallization Screening laboratory at the Hauptman-Woodward Medical Research Institute

Since February of 2000 the High Throughput Search (HTS) laboratory has been screening potential crystallization conditions as a high-throughput service

The HTS lab screens samples against three types of cocktails:

1. Buffered salt solutions varying pH, anion and cation and salt concentrations
2. Buffered PEG and salt, varying pH, PEG molecular weight and concentration and anion and cation type
3. Almost the entire Hampton Research Screening catalog.

The HTSlab has investigated the crystallization properties of over 15,000 individual proteins archiving approximately 140 million images of crystallization experiments.

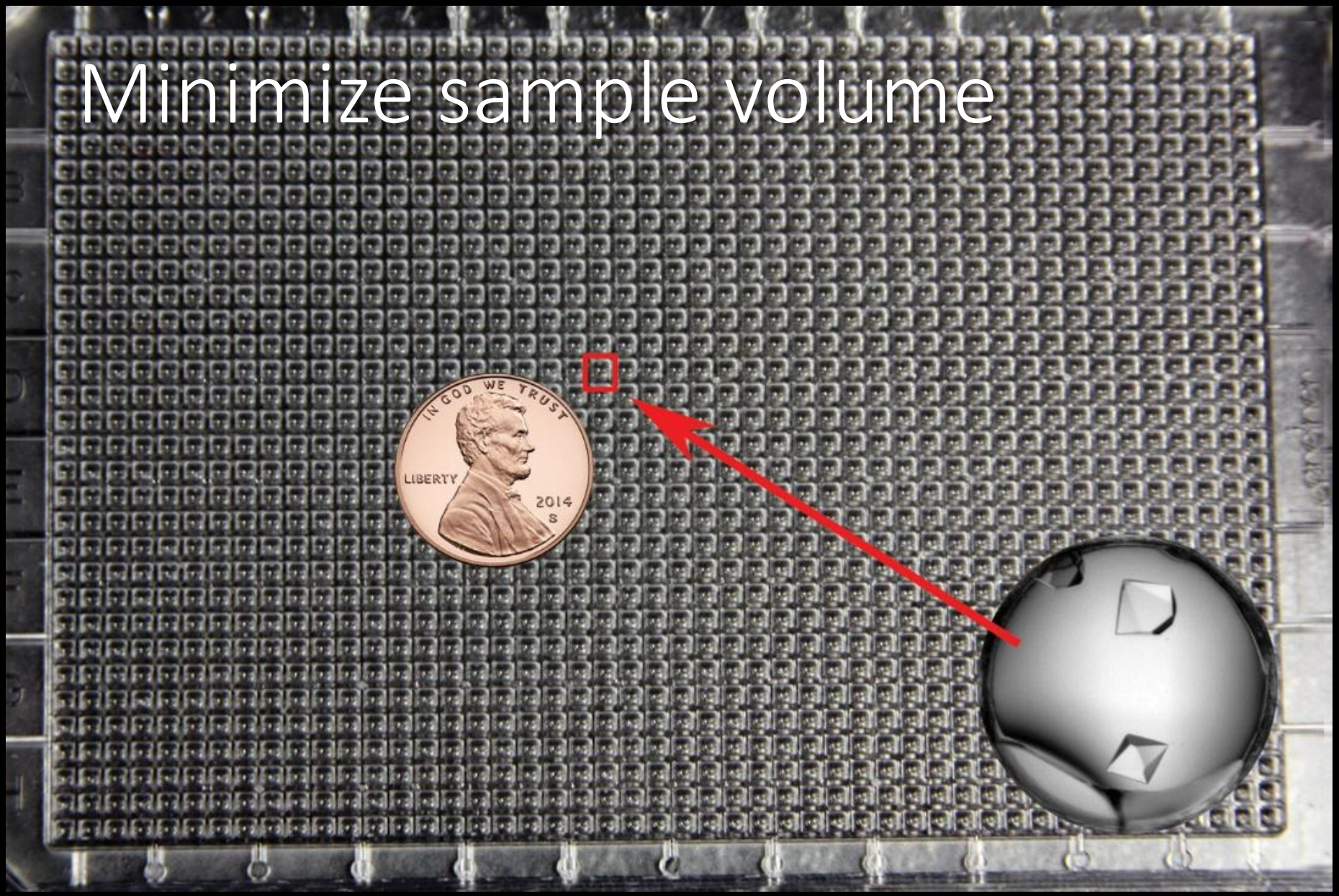



The crystallization method used is micro-batch under oil with 200 nl of protein solution being added to 200 nl of precipitant cocktail in each well of a 1536 well plate.

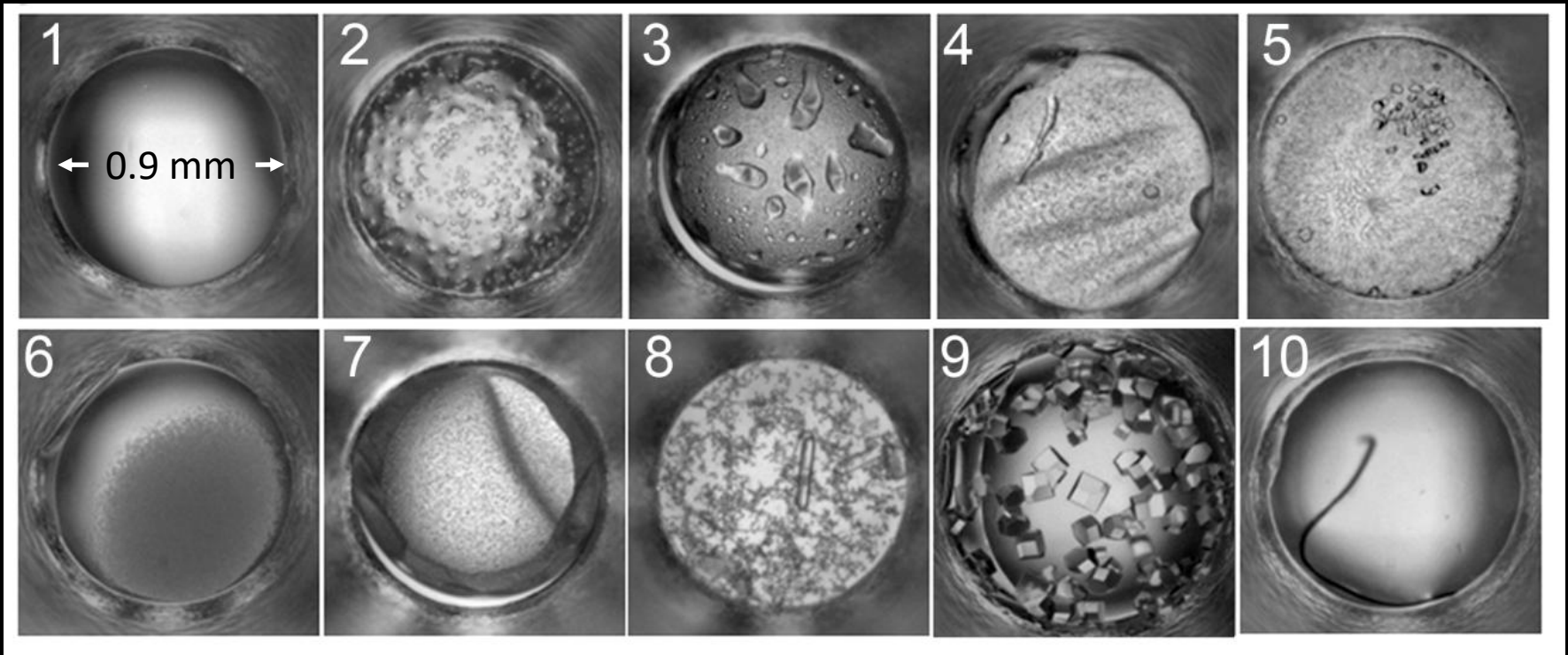
Wells are imaged before filling, immediately after filling then weekly for six weeks duration with images available immediately on a secure ftp server.

Several software utilities for viewing and analyzing data are available.

# Minimize sample volume



# Outcomes



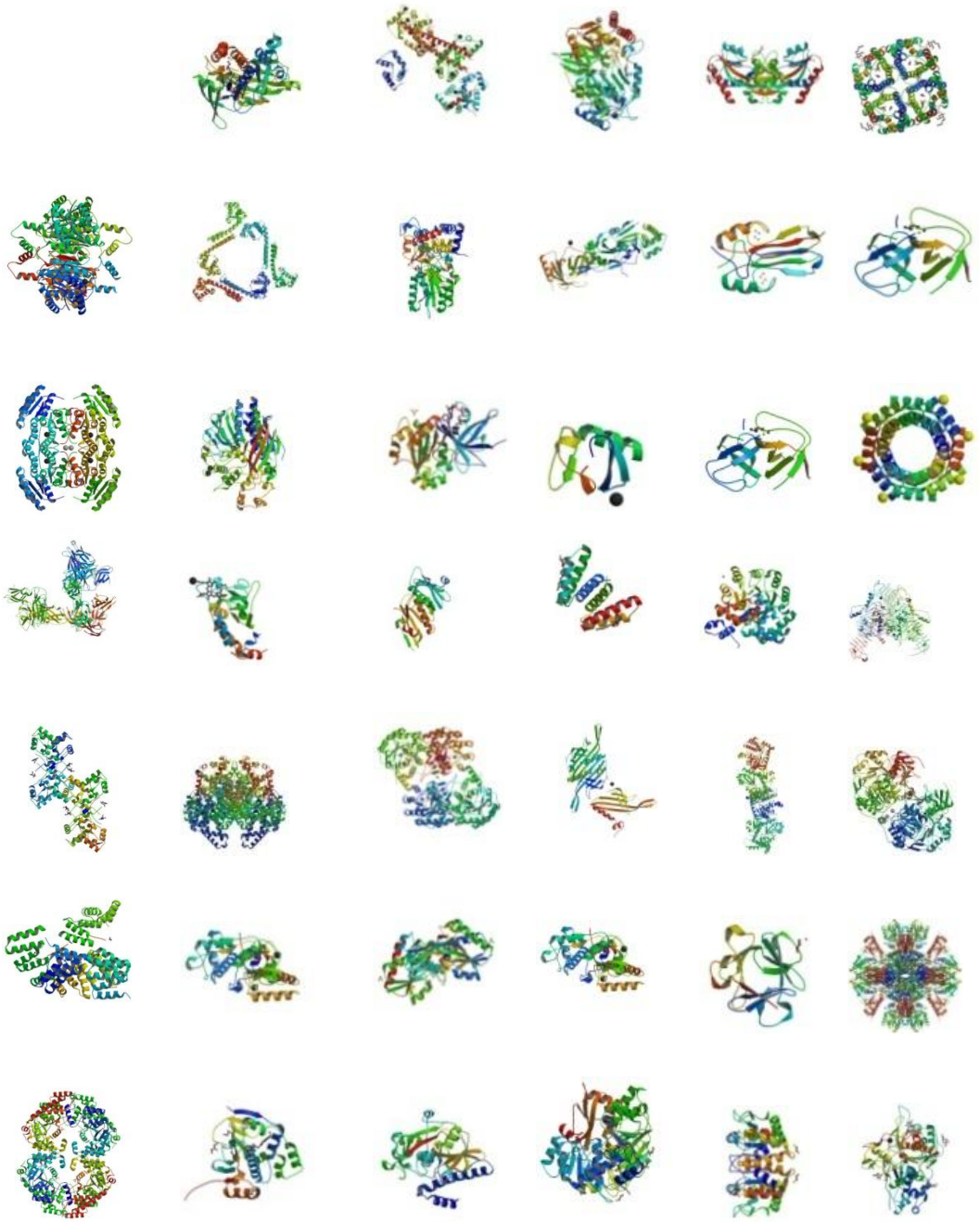
# Born in Buffalo

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.

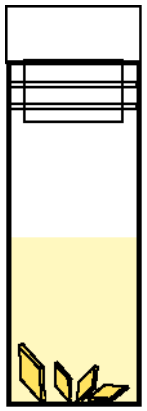
These images represent examples of structures from initial hits in the HTS laboratory.



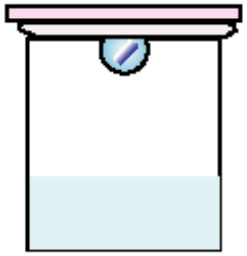
# Which method?

- Vapor diffusion (most common)
  - Dynamic – samples wide physical chemical space
  - Can use small volumes
  - Reproducible
  - Multiple experiments in one drop
- Microbatch under oil (used by our laboratory)
  - Static – initial conditions highly defined
  - Sealed in one setup
  - Transportable
- Dialysis (less common)
  - Larger volumes
  - Difficult automated setup

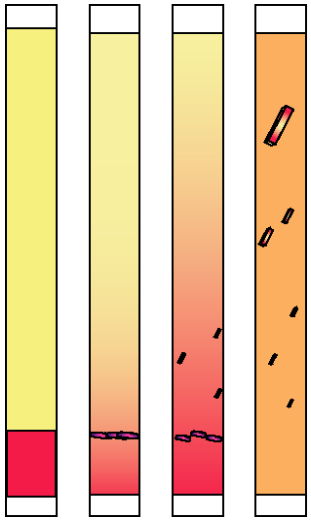
# Three basic methods: batch, vapor and liquid



Batch

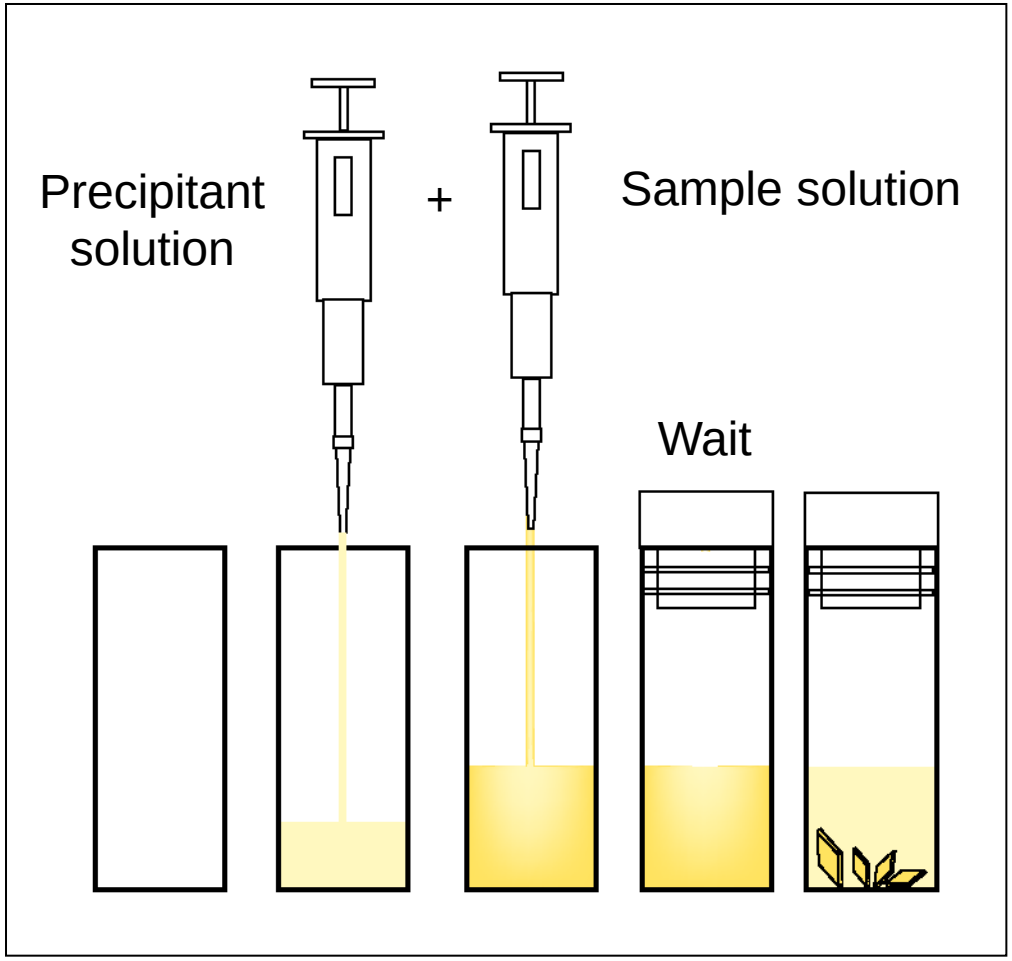


Vapor
(also known as vapour)

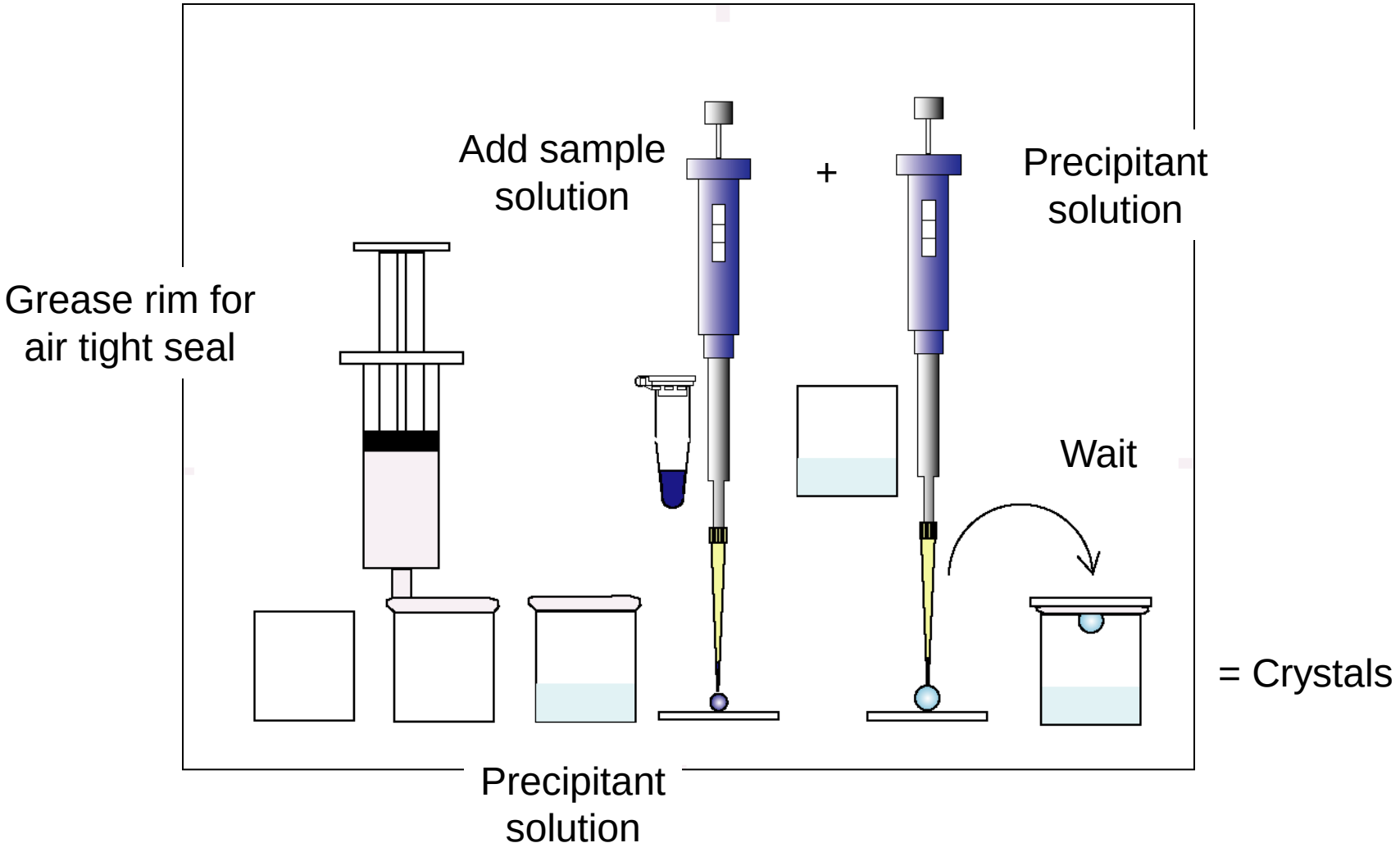


Liquid

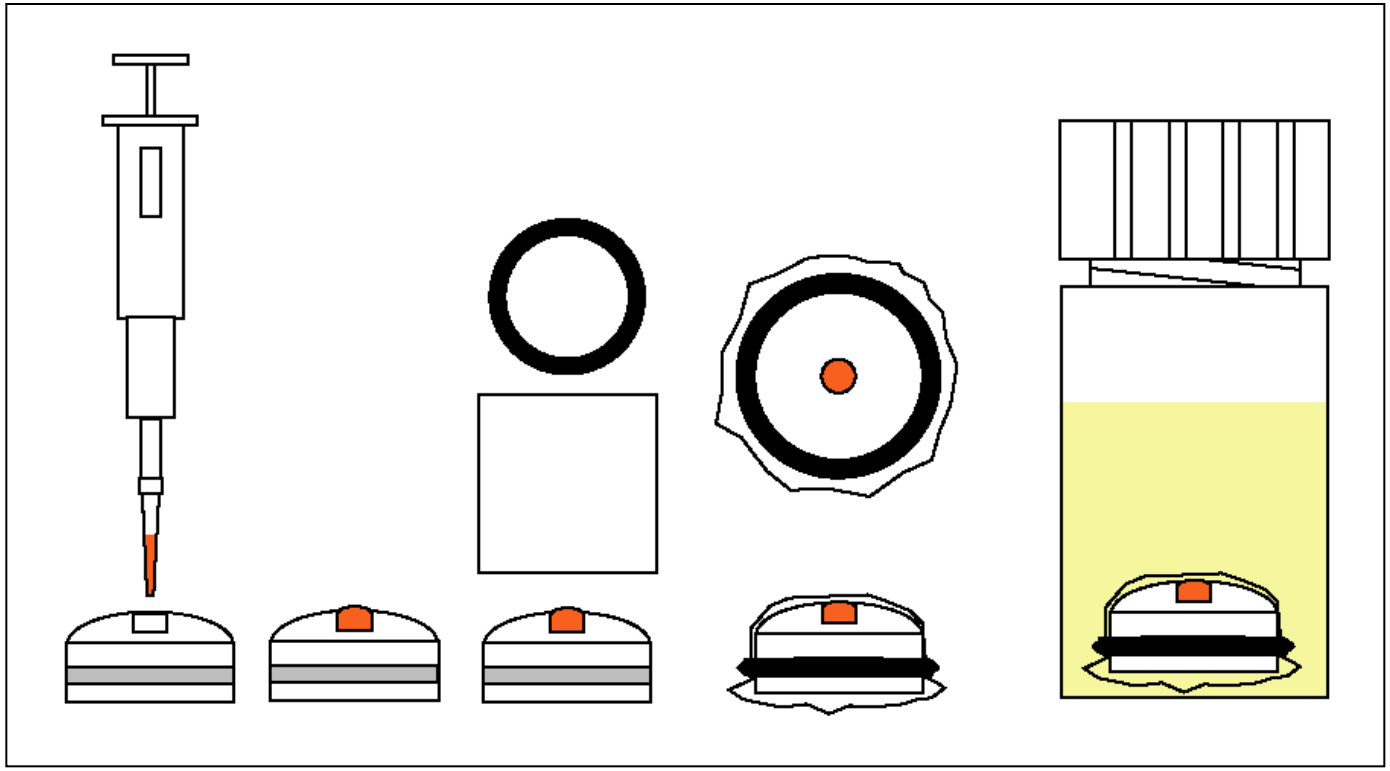
# Batch in a Vial: Set up



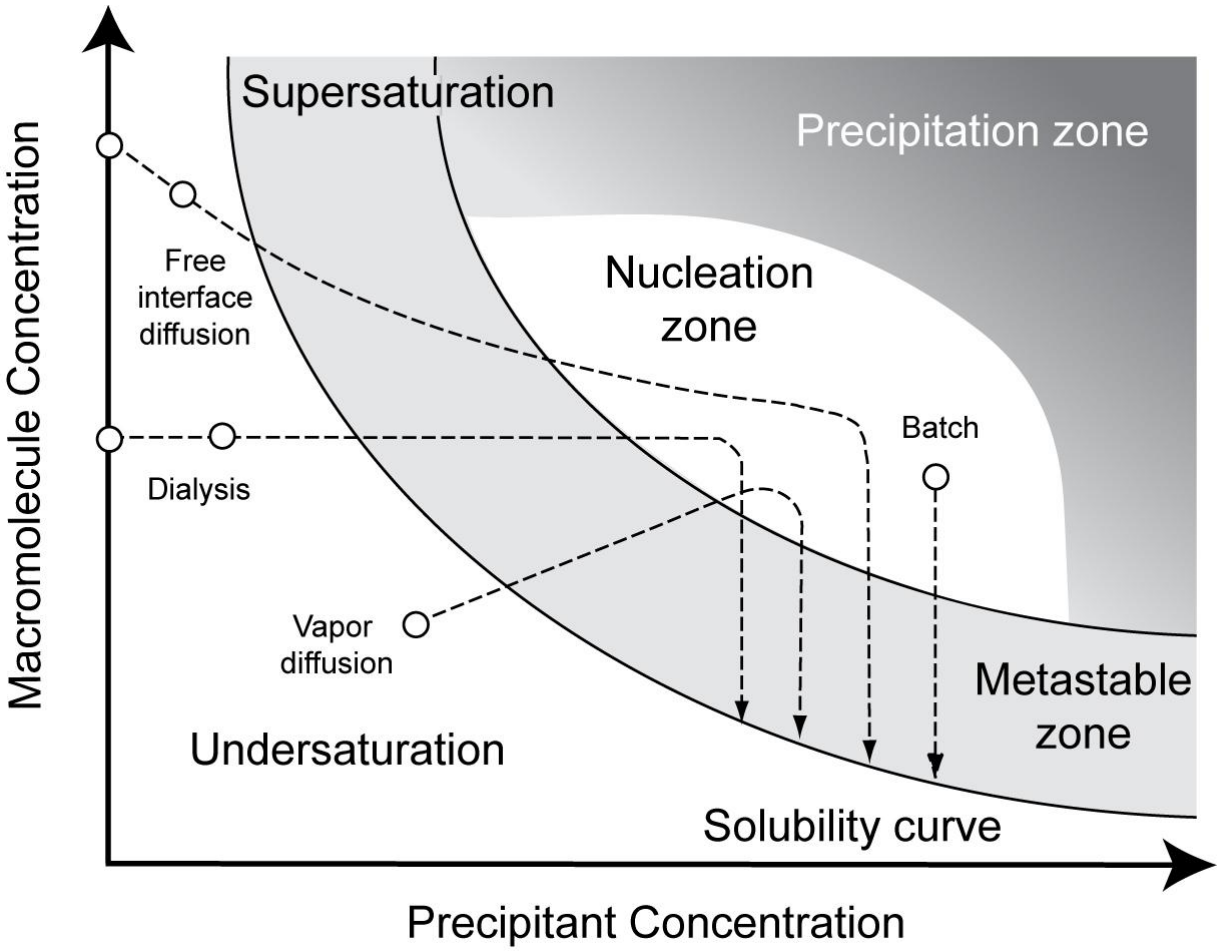
# Vapor Diffusion Setup



# Dialysis Experiments

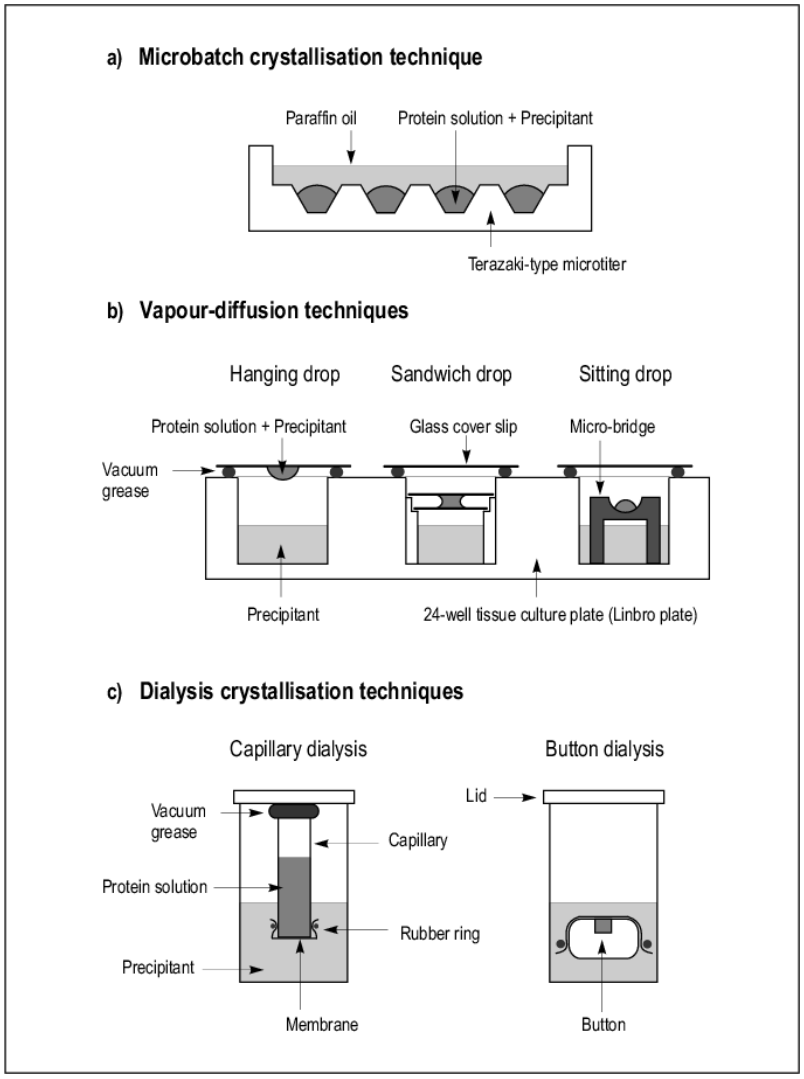


# Simplified phase diagram for crystallization




**Know this diagram by heart, but remember it's simplified.**

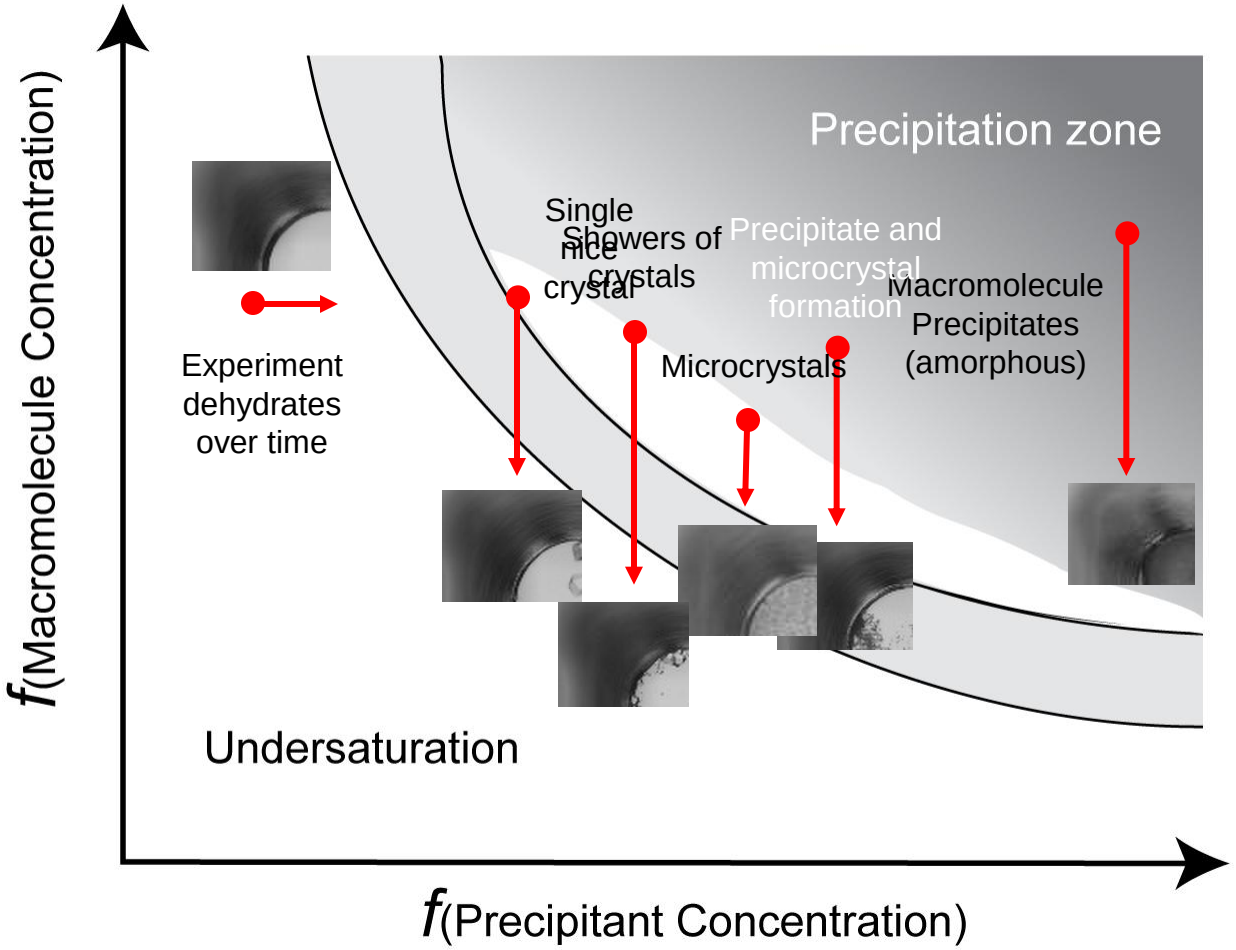
# Crystallizing Macromolecules



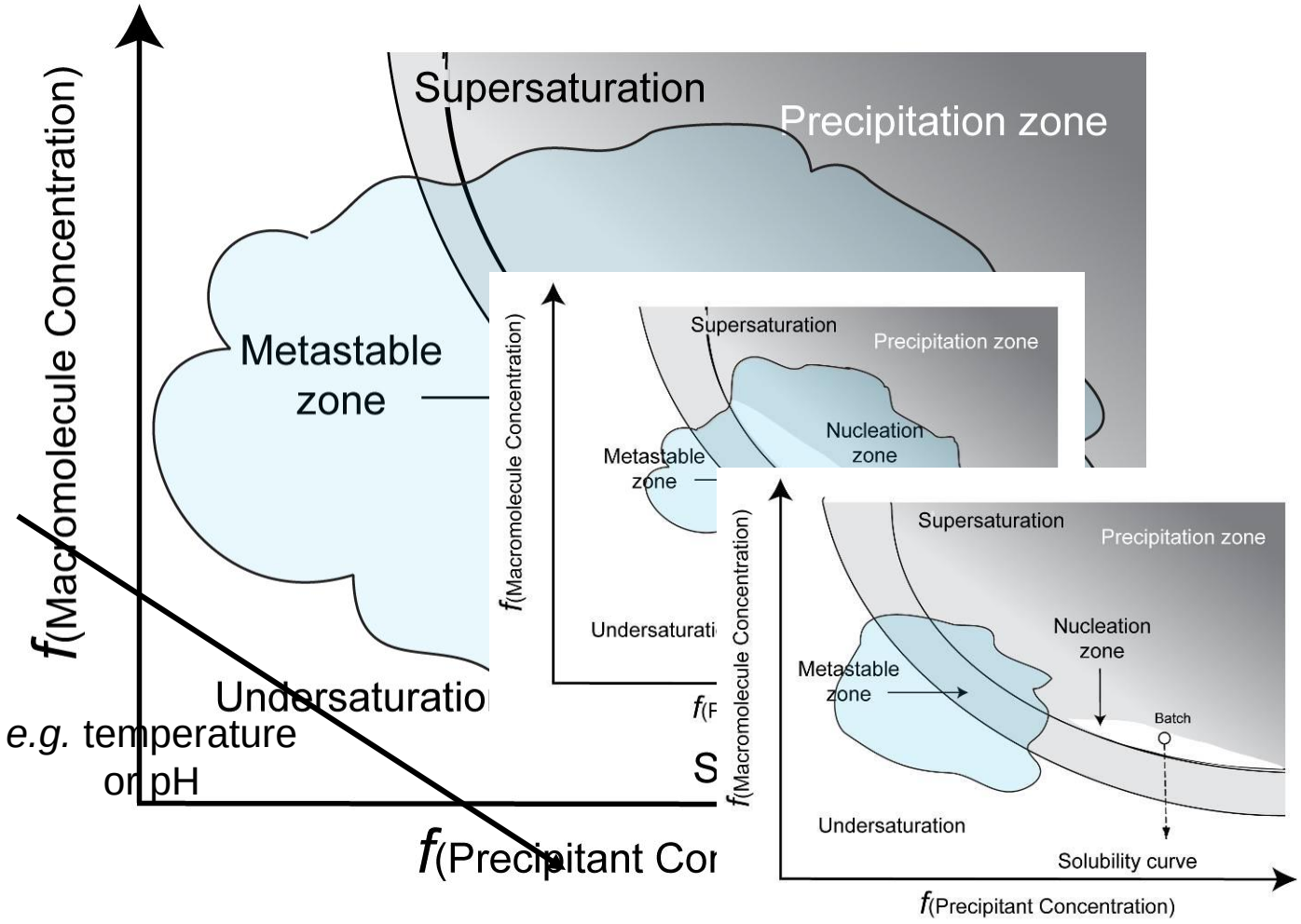

Many different methods but they all have things in common:

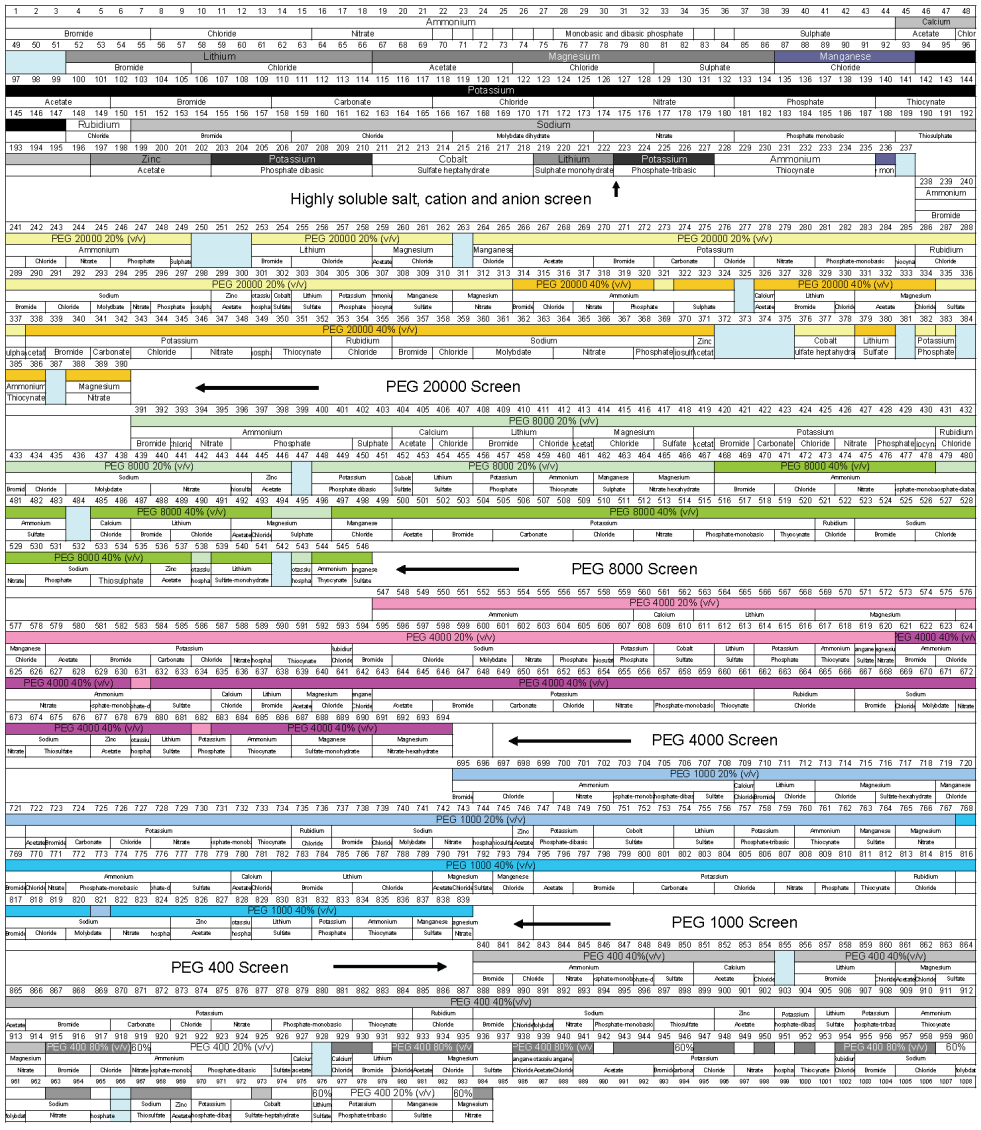
- They are designed to traverse the crystallization phase diagram.
- They use many different kinds of solutions to sample crystallization space at many points.

# What results can we expect to see?



# Typical situation, multidimensional area sampled



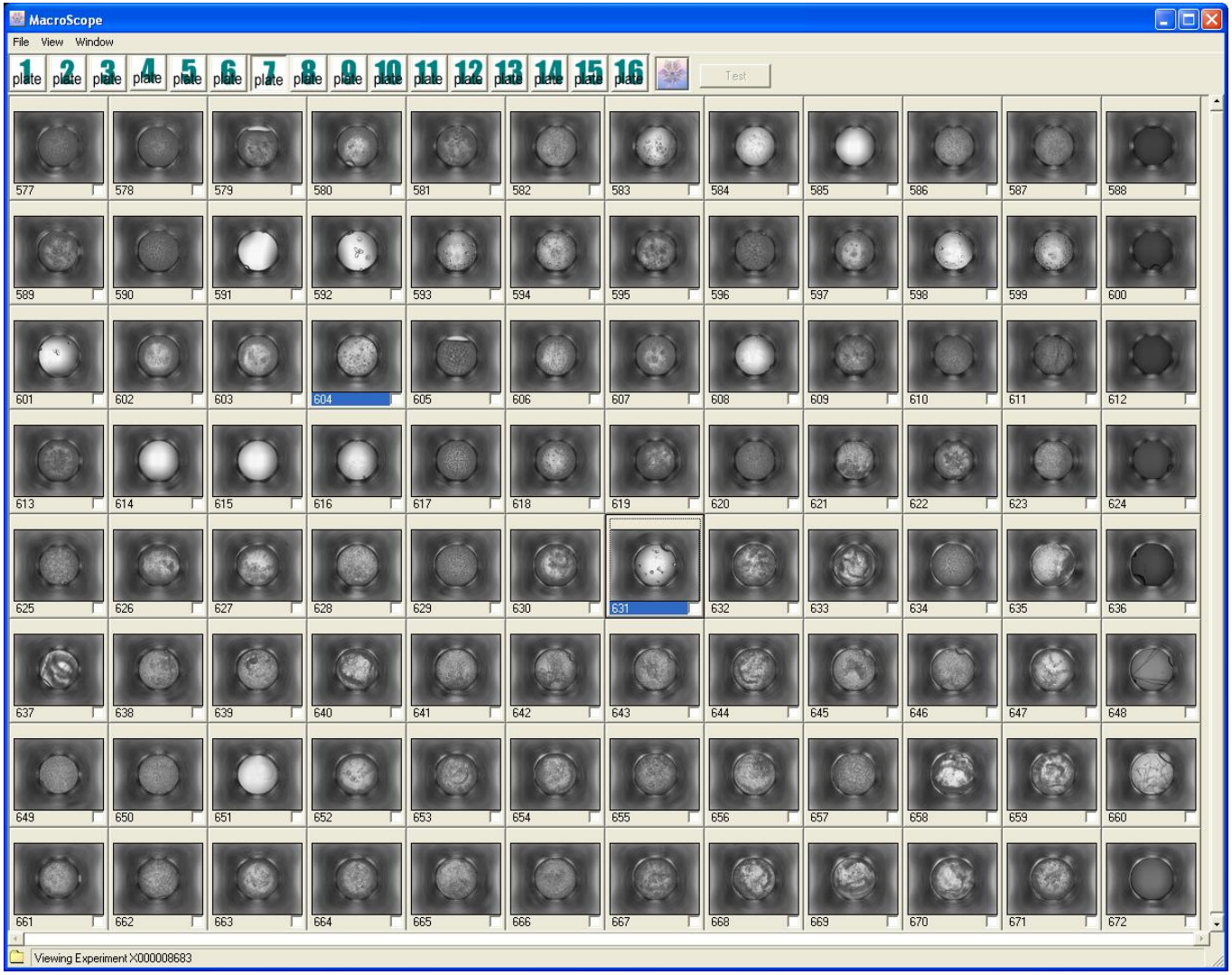



# The HWI crystallization cocktail screen.

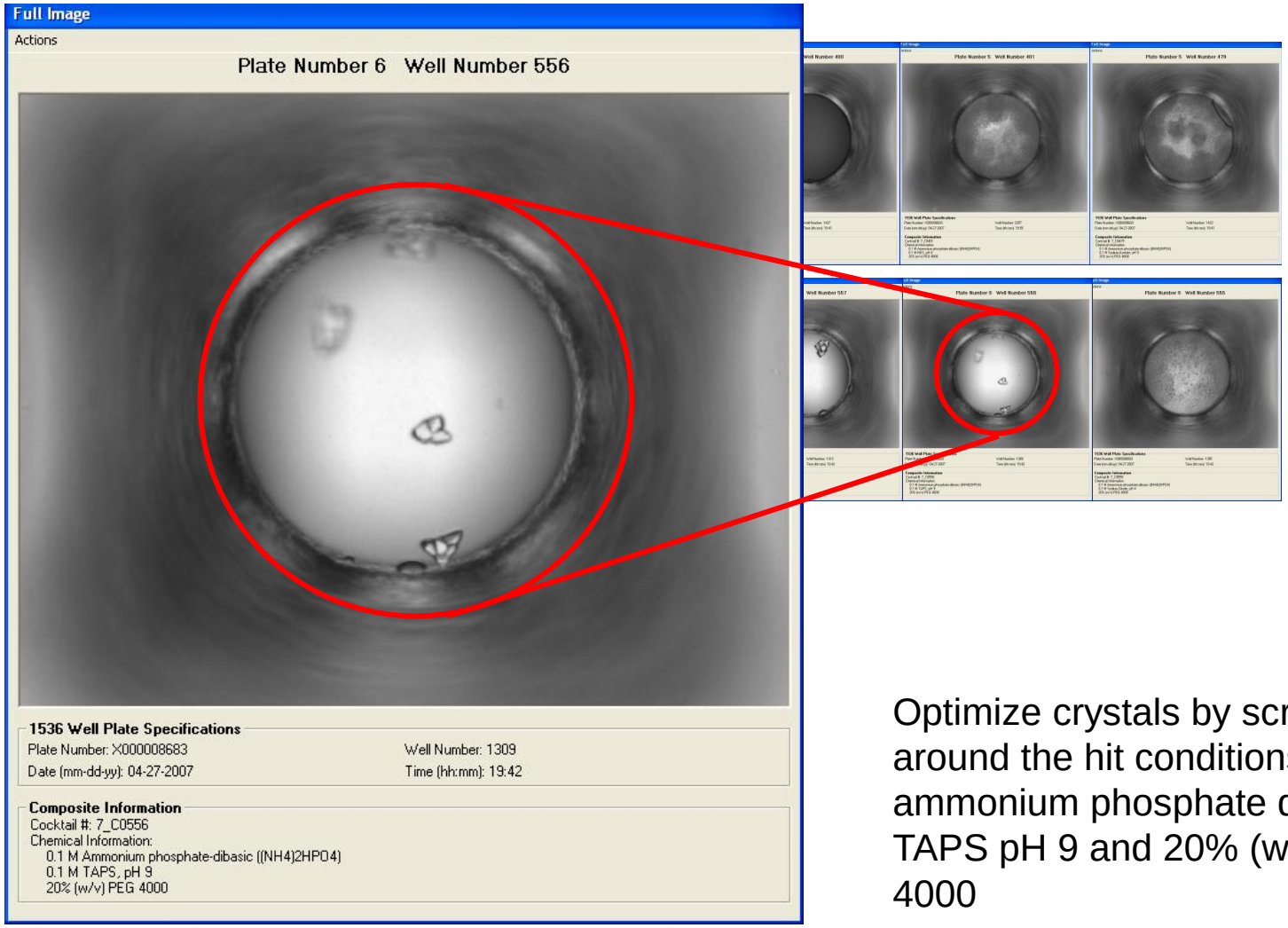
The 1536 diverse chemical cocktails (Luft et al., 2003). The 984 in-house conditions comprise an incomplete factorial sampling of 36 salts, eight buffers, and 5 different PEGs.

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.

# What do we see from the data?

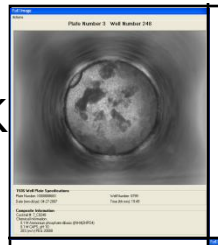
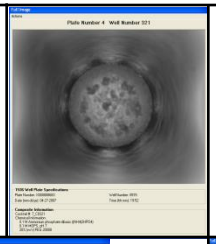
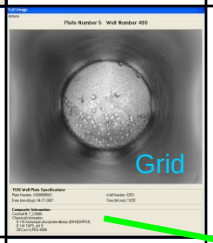
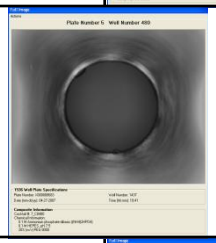
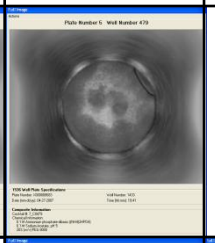
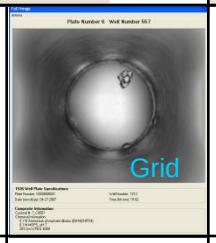
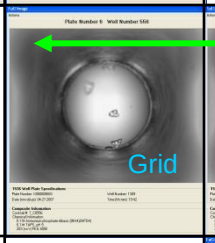
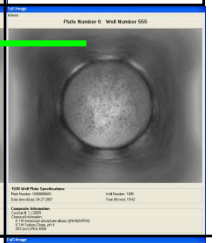
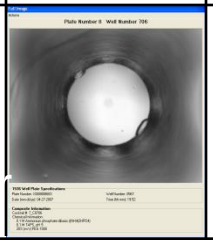
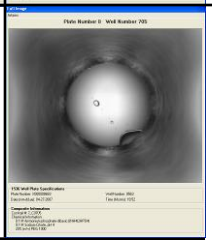


# What do we actually see?



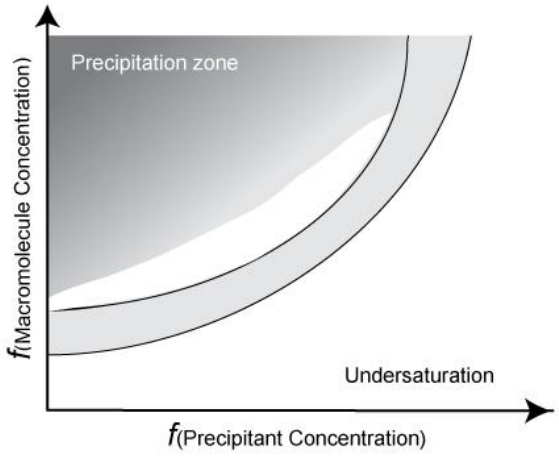

Optimize crystals by screening around the hit conditions, *i.e.* 0.1 M ammonium phosphate dibasic, 0.1 TAPS pH 9 and 20% (w/v) PEG 4000

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

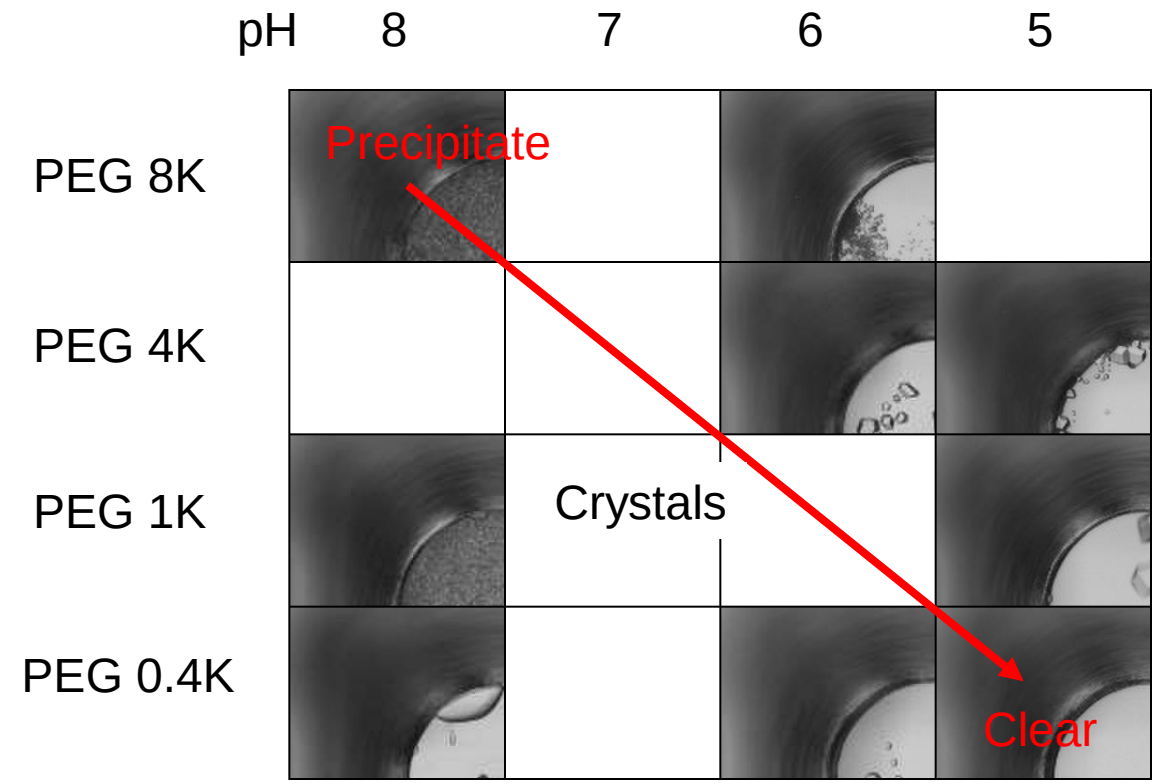
| pH | 10 | 9 | 8 | 7 | 6 | 5 | 4 |
|---|---|---|---|---|---|---|---|
| 20% PEG 20K |  | | |  | Bad | Bad | Bad |
| 20% PEG 8K | Small to large |  Grid |  | |  |  | Small to large |
| 20% PEG 4K | | Grid | Grid |  Grid | Grid |  Grid |  |
| 20% PEG 1K | |  | | Unknown | | |  |

# Chemical space provides a vector for optimization

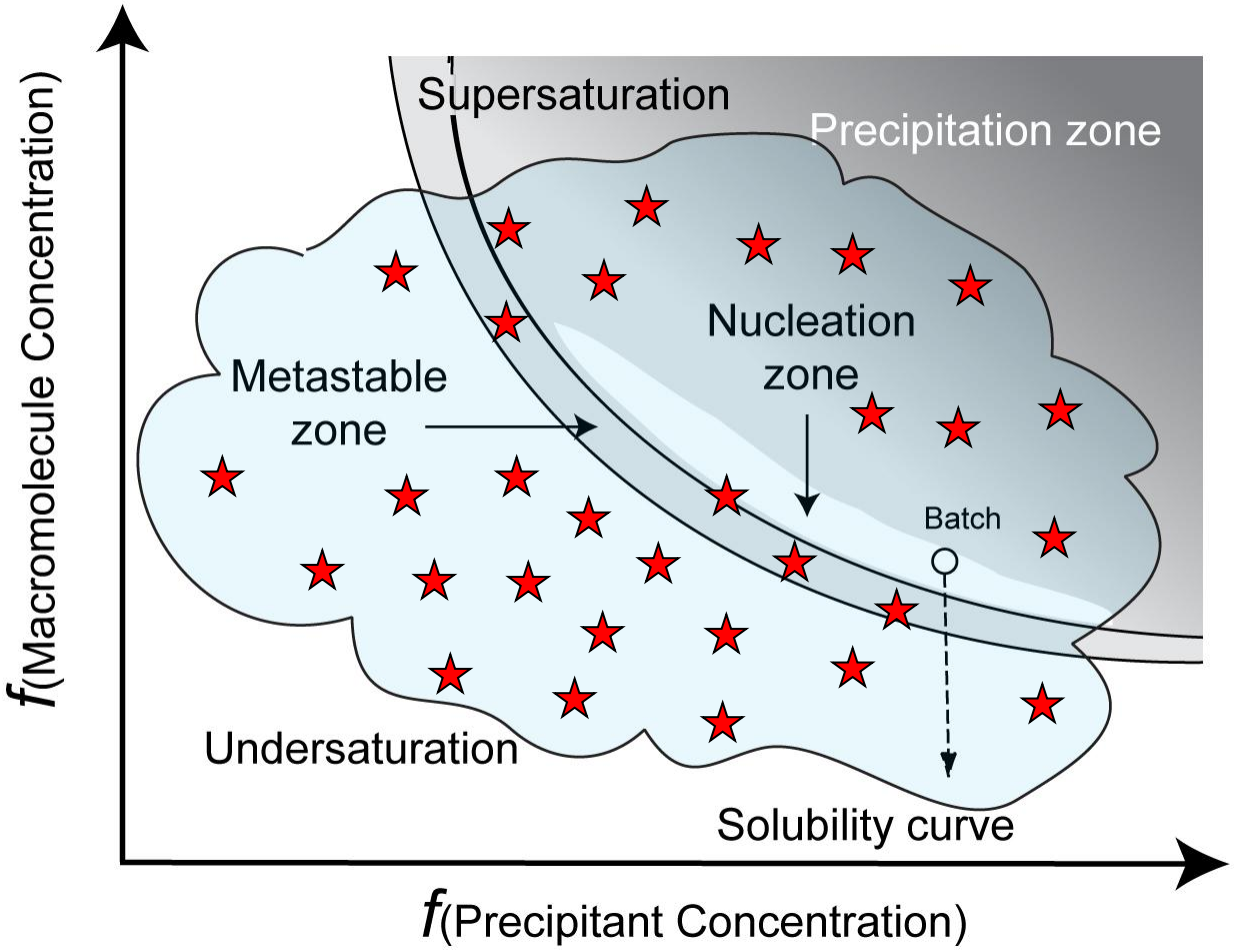
In this case the path from precipitate through crystals to clear is obvious. The phase diagram is reversed. Also clear are the number of chemical conditions that have not been sampled.



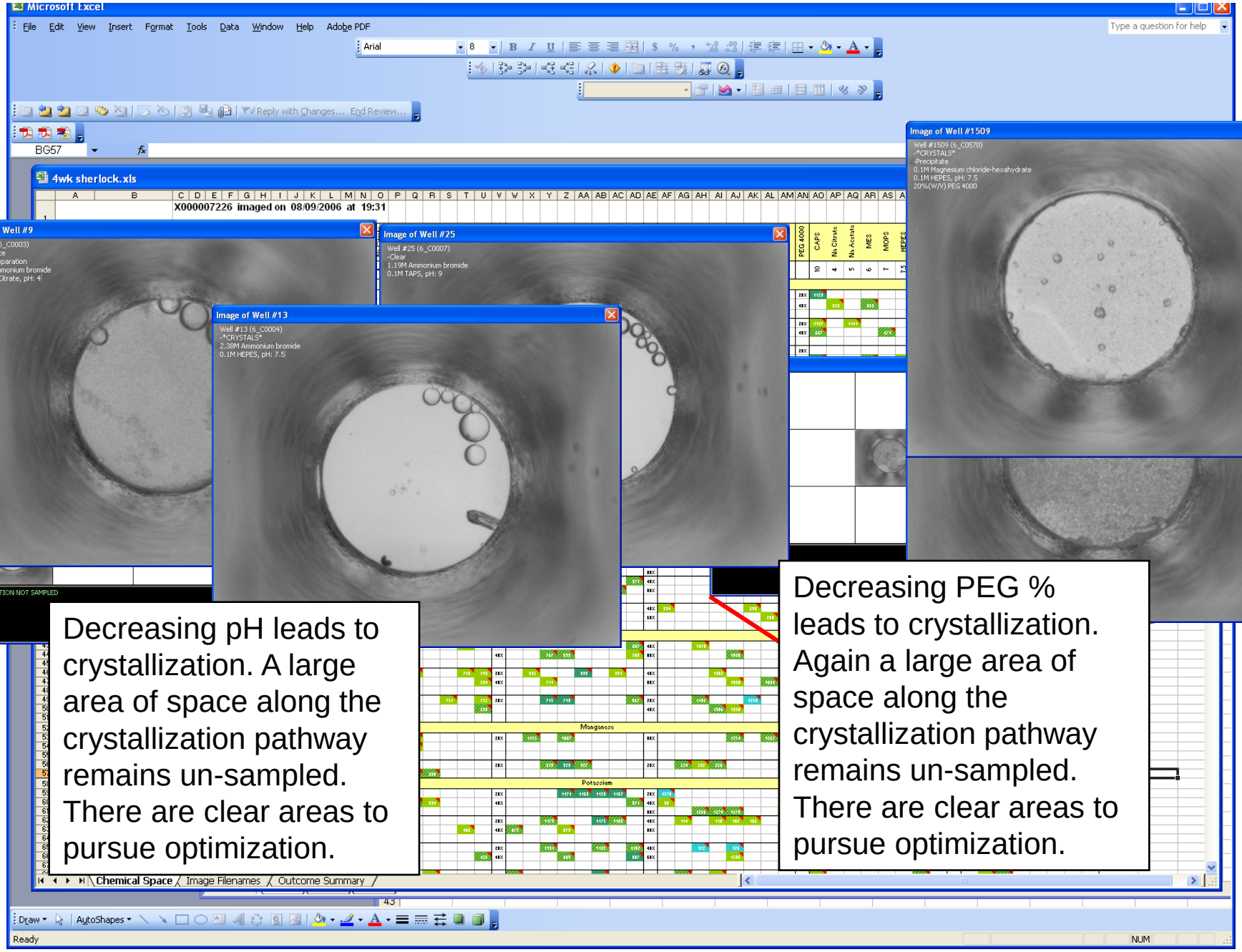

Ubiquitin, 40% PEG, 0.1M zinc acetate

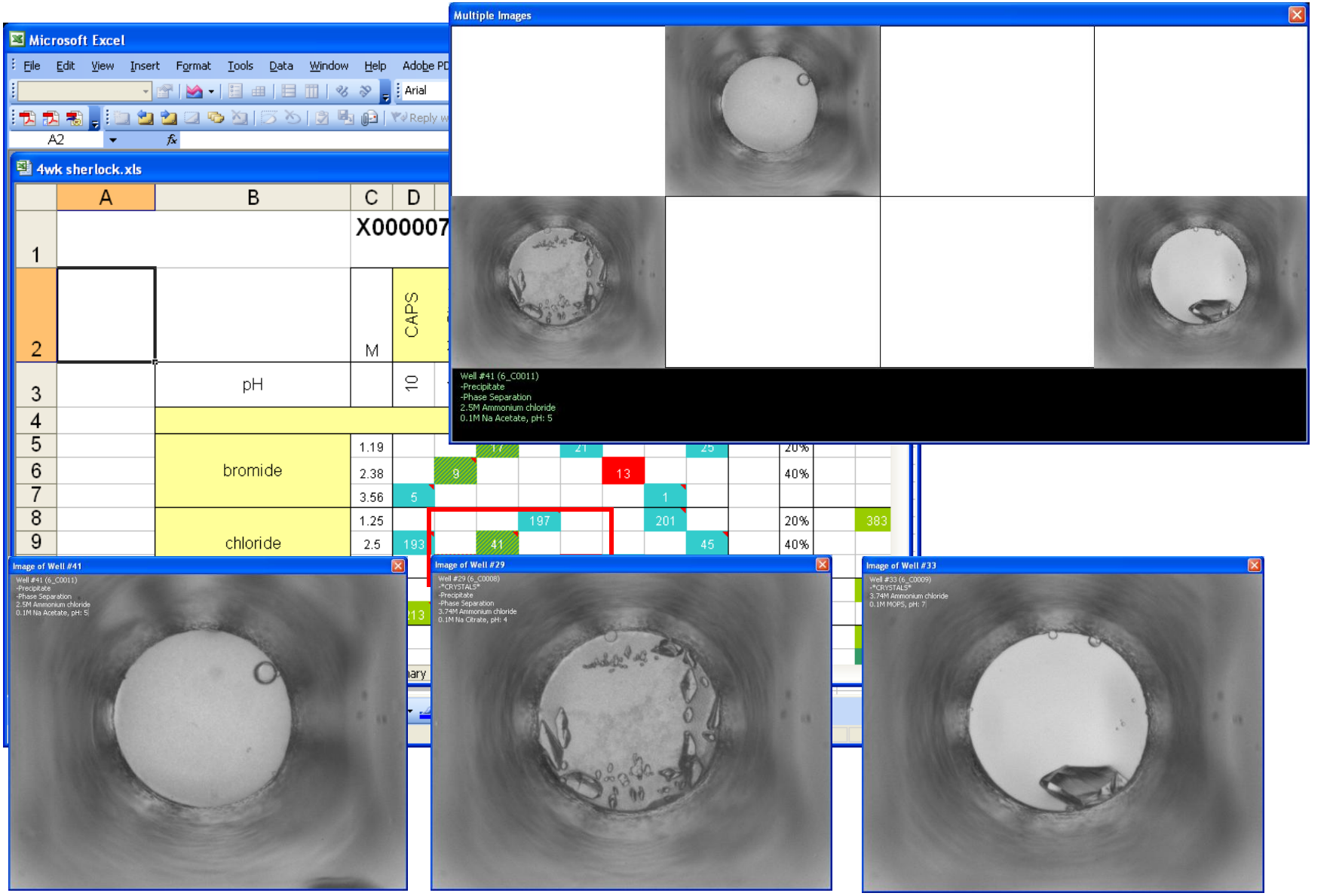


# It also illustrates the space we do not sample




We only sample discrete points within the sampling space


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.


Microsoft Excel
File Edit View Insert Format Tools Data Window Help Adobe PD
Arial
A2
4wk sherlock.xls
A B C D
X000007
M
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

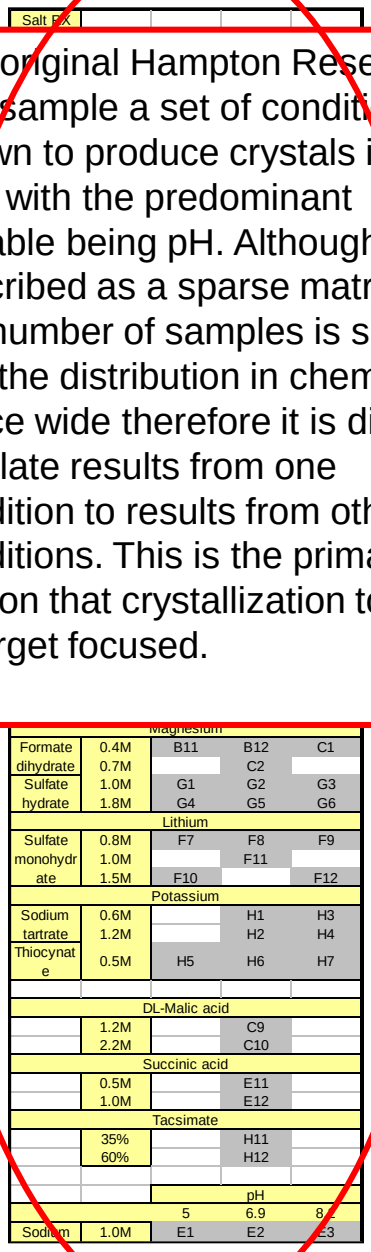
# The Commercial Screens in the HWI crystallization cocktails

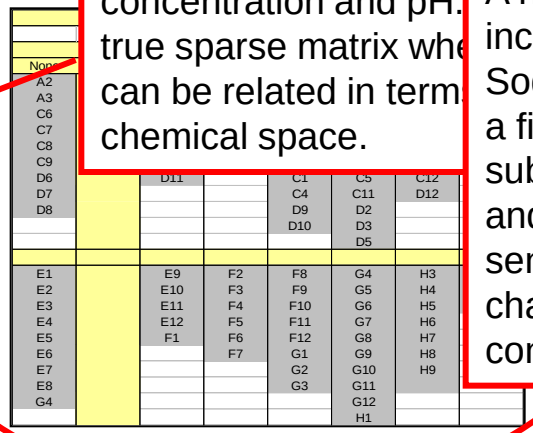
The original Hampton Research 1+2 sample a set of conditions known to produce crystals in the past with the predominant variable being pH. Although described as a sparse matrix the number of samples is small and the distribution in chemical space wide therefore it is difficult to relate results from 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**

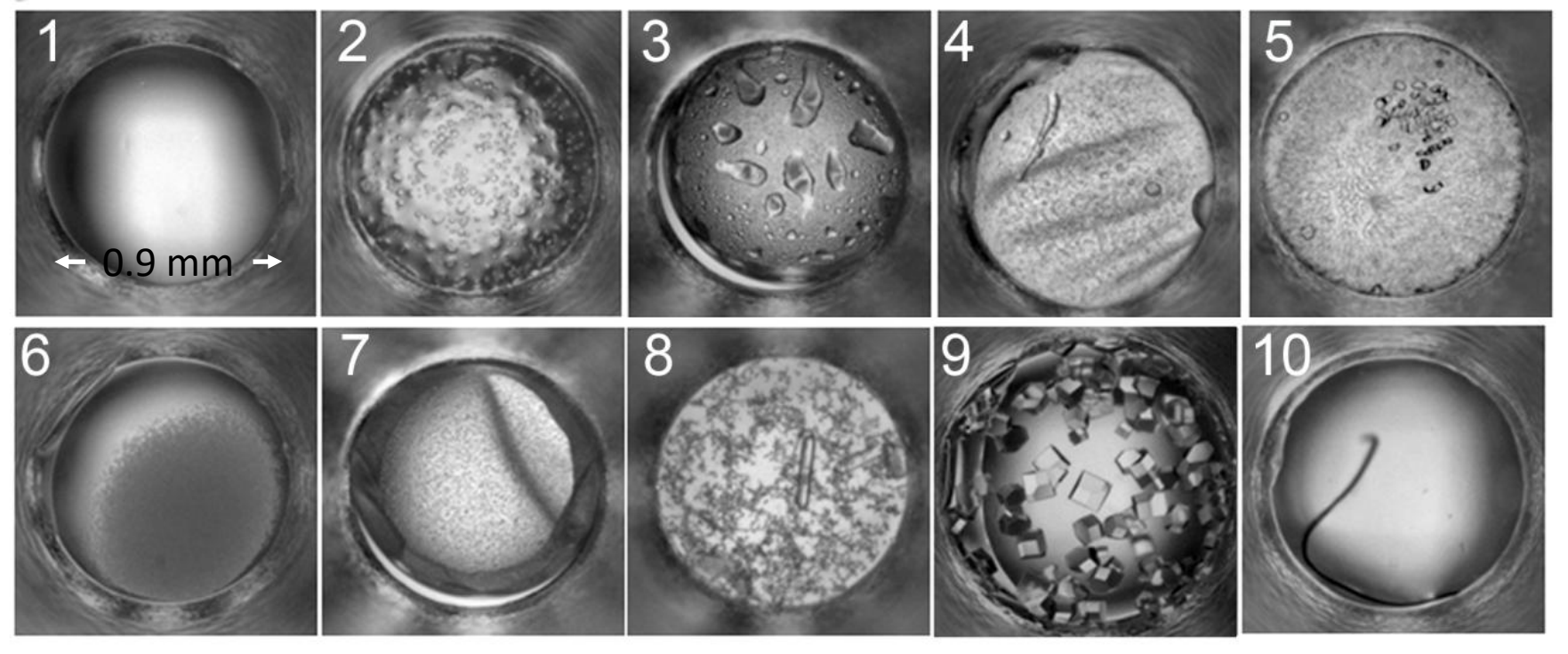
Note, the HT screen is not a convential screen as such. It is designed to sample a range of reagents and provide an indication of the appropiate chemical area and variables that would be appropiate for crystallization and should be used in this manner.

| pH | Ammonium Sulfate 2.0M | Sodium chloride 3.0M | Magnesium formate dihydrate 0.3M | Magnesium formate dihydrate 0.5M | Sodium phosphate pH | Neutralized organic acids (ph 7.0) pH | High supersaturation salt and low polymer pH | Low ionic strength systems pH | Non-volatile organics pH |
|---|---|---|---|---|---|---|---|---|---|
| 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

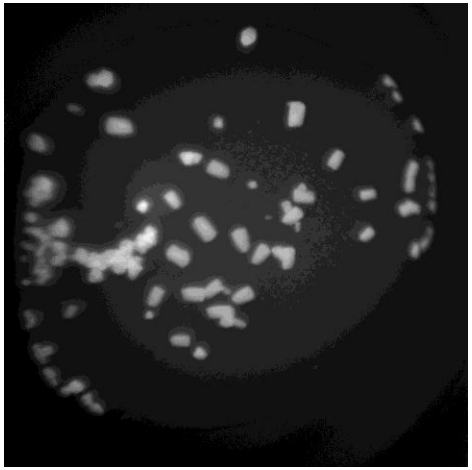
# Imaging

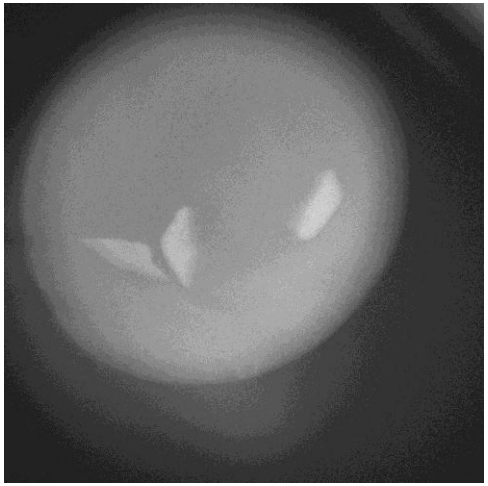


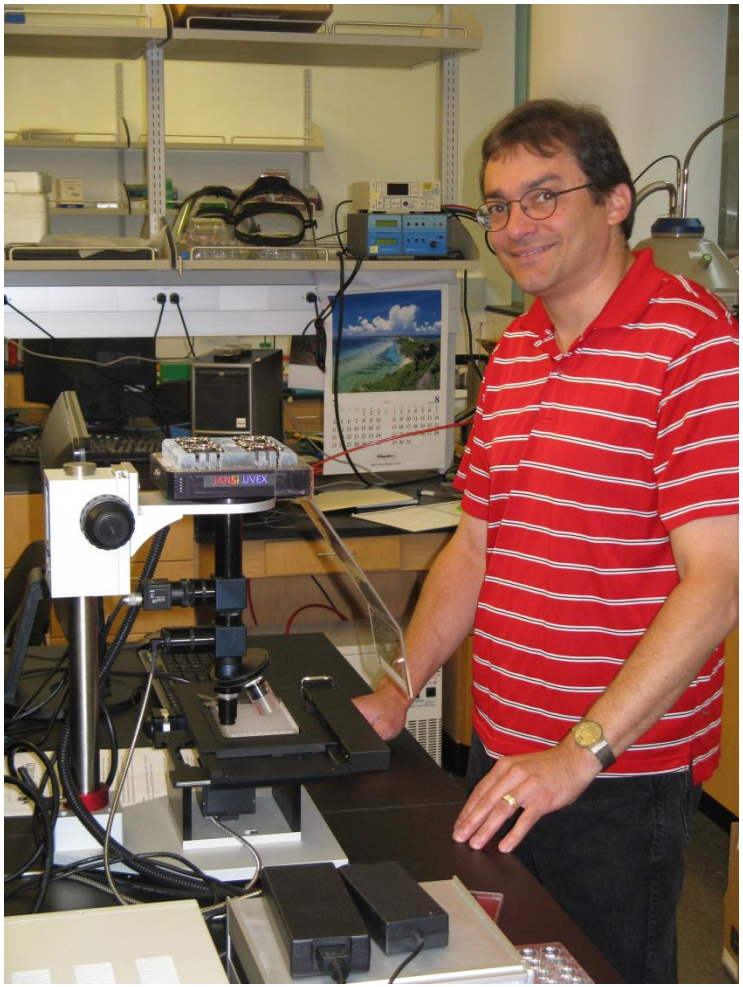

The volume is designed such that the complete drop is within the depth of focus.

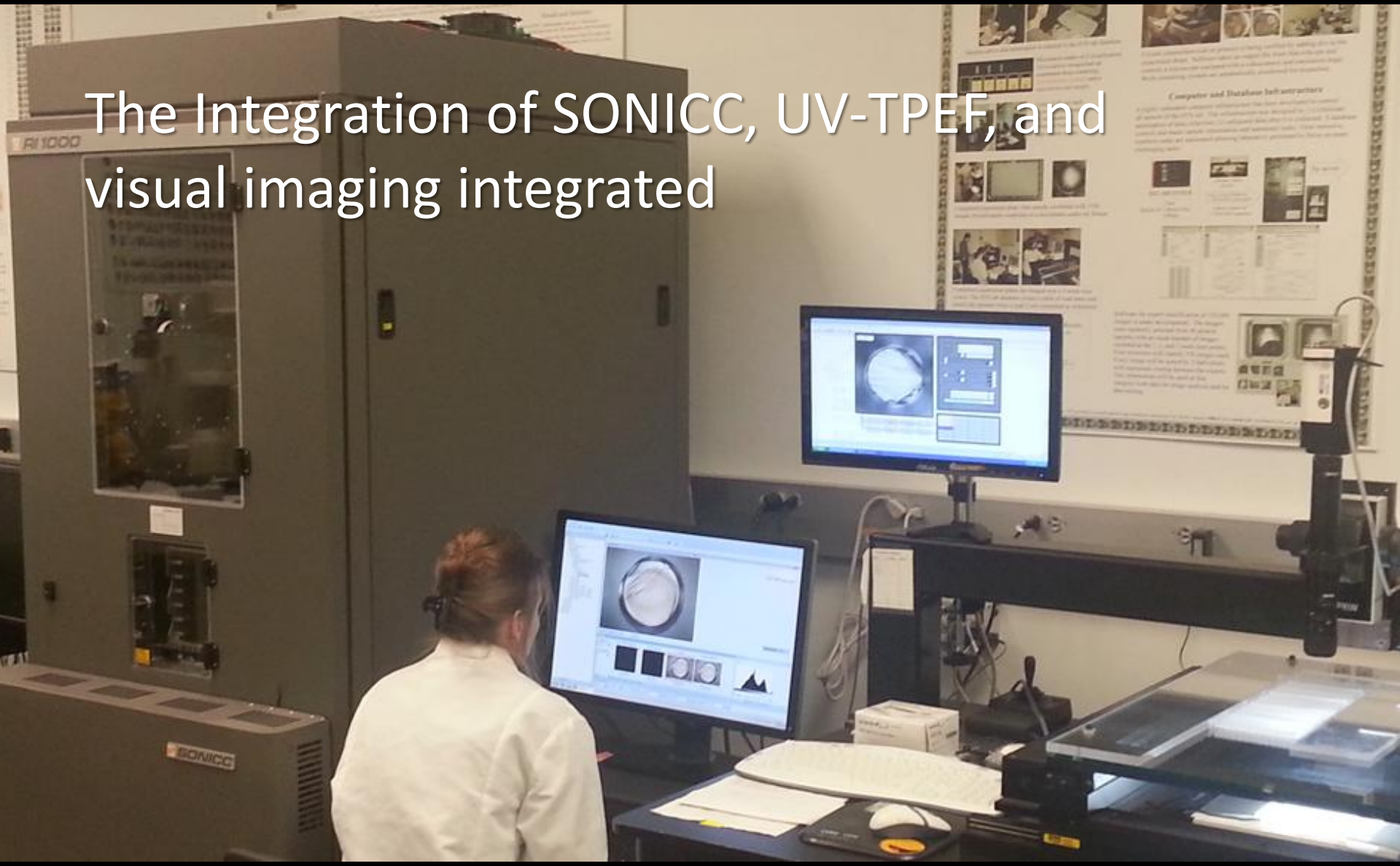
Imaging takes place before the protein is setup (a control), immediately after and then at one week intervals for 6 weeks.

# UV imaging – is it protein?

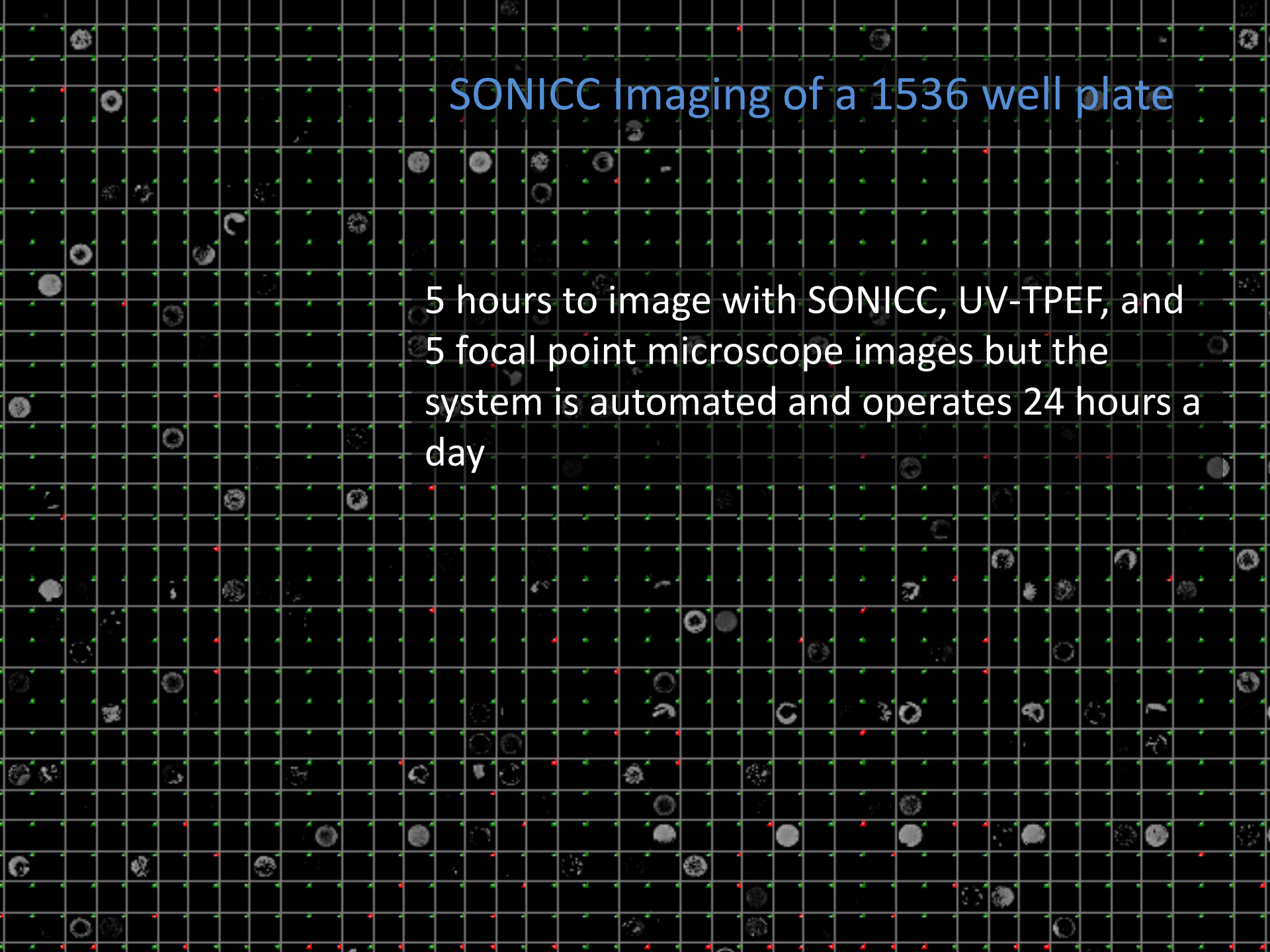







The Integration of SONICC, UV-TPEF, and visual imaging integrated

# SONICC Imaging of a 1536 well plate

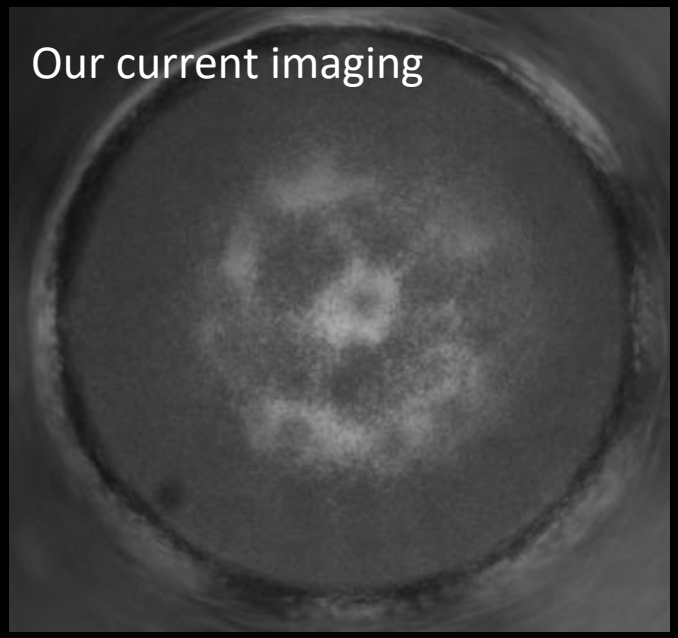


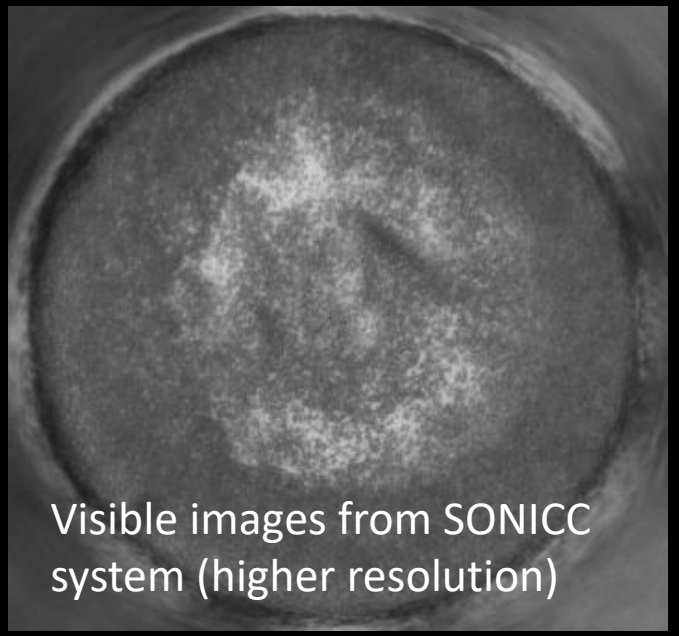
5 hours to image with SONICC, UV-TPEF, and 5 focal point microscope images but the system is automated and operates 24 hours a day

# One protein in detail to lay out the experiment

Protein 1, part of the pyruvate dehydrogenase protein complex

*Hampton Research PEGRx HT-F4,* 4% (v/v) 2-Methyl-2,4 pentanediol, 0.1 M Citric Acid pH=3.5 20% (w/v) PEG 1500 produced the following:



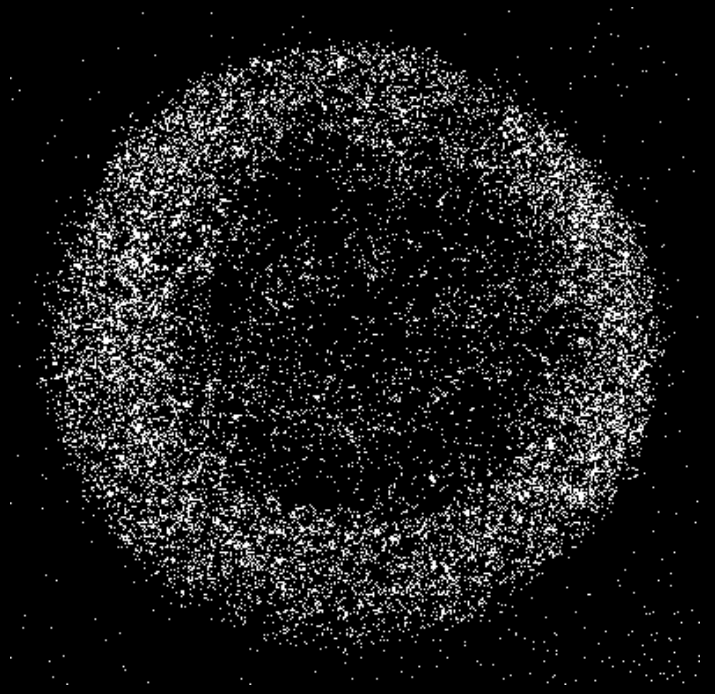




Immediately after the protein is added to the cocktail

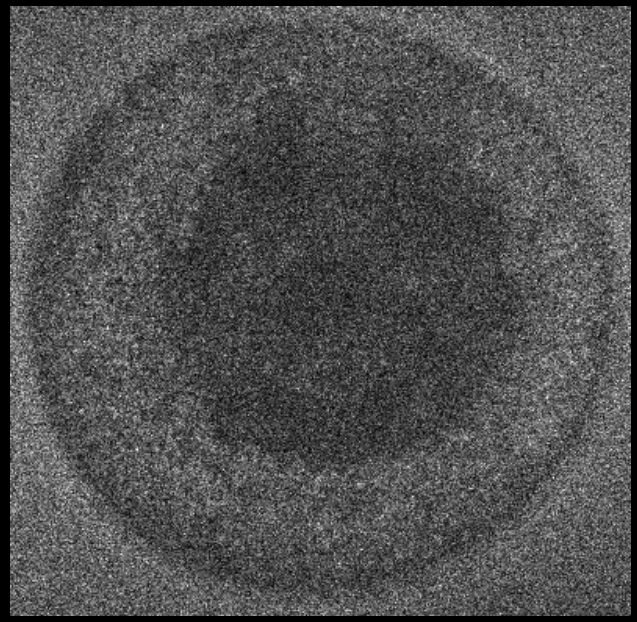
# Initial use of SONICC and UV imaging

Protein 1, part of the pyruvate dehydrogenase protein complex

*Hampton Research PEGRx HT-F4,* 4% (v/v) 2-Methyl-2,4 pentanediol, 0.1 M Citric Acid pH=3.5 20% (w/v) PEG 1500 produced the following:



SONICC SHG image



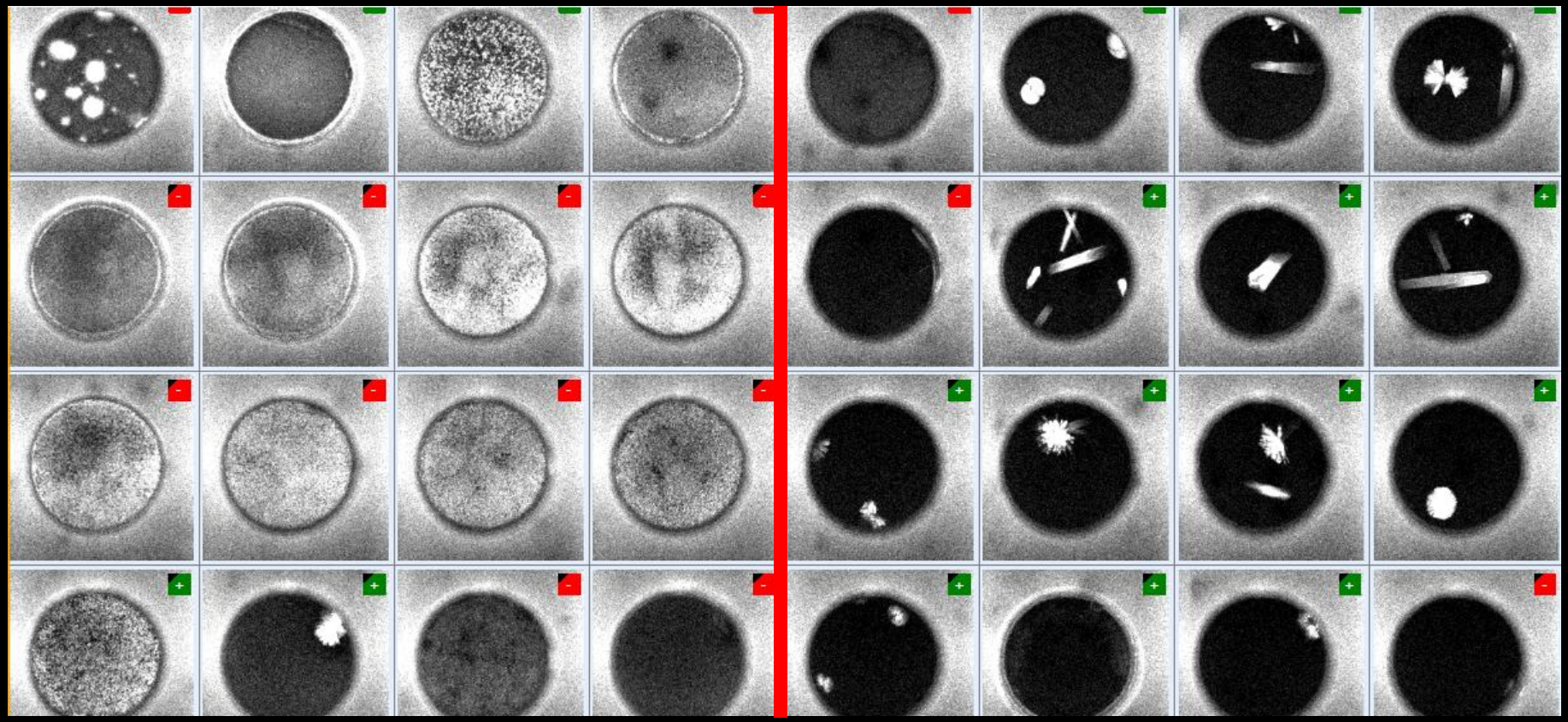
UV-TPEF image

# Protein 1, part of the pyruvate dehydrogenase protein complex

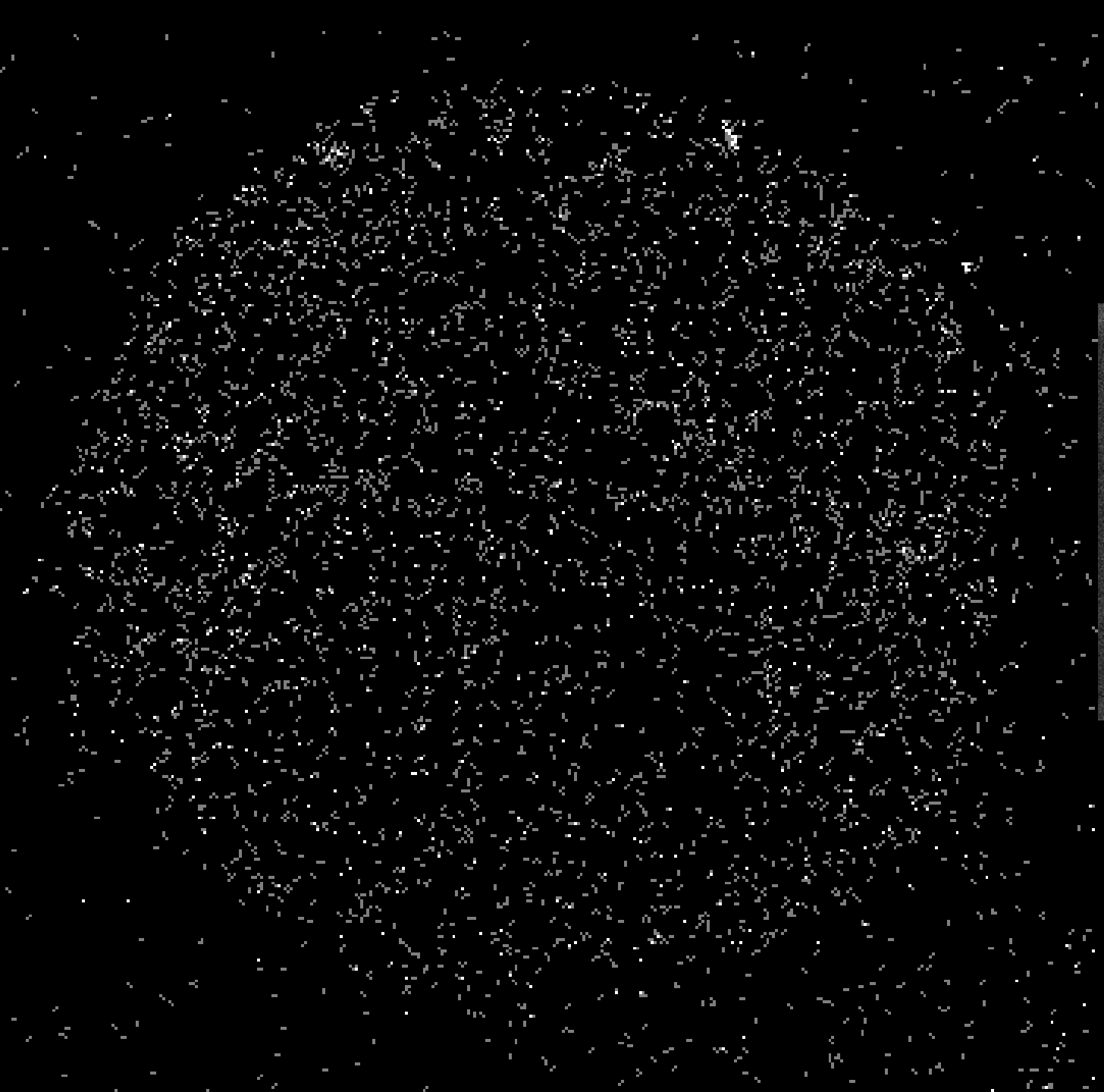
*Hampton Research PEGRx HT-F4,* 4% (v/v) 2-Methyl-2,4 pentanediol, 0.1 M Citric Acid pH=3.5 20% (w/v) PEG 1500

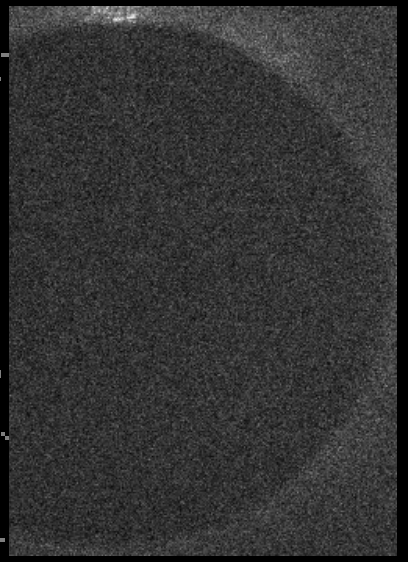
X14163- Full[P]- 10mg/ml

X14163- [P]/2- 5mg/ml



**UV-TPEF** at 4wk





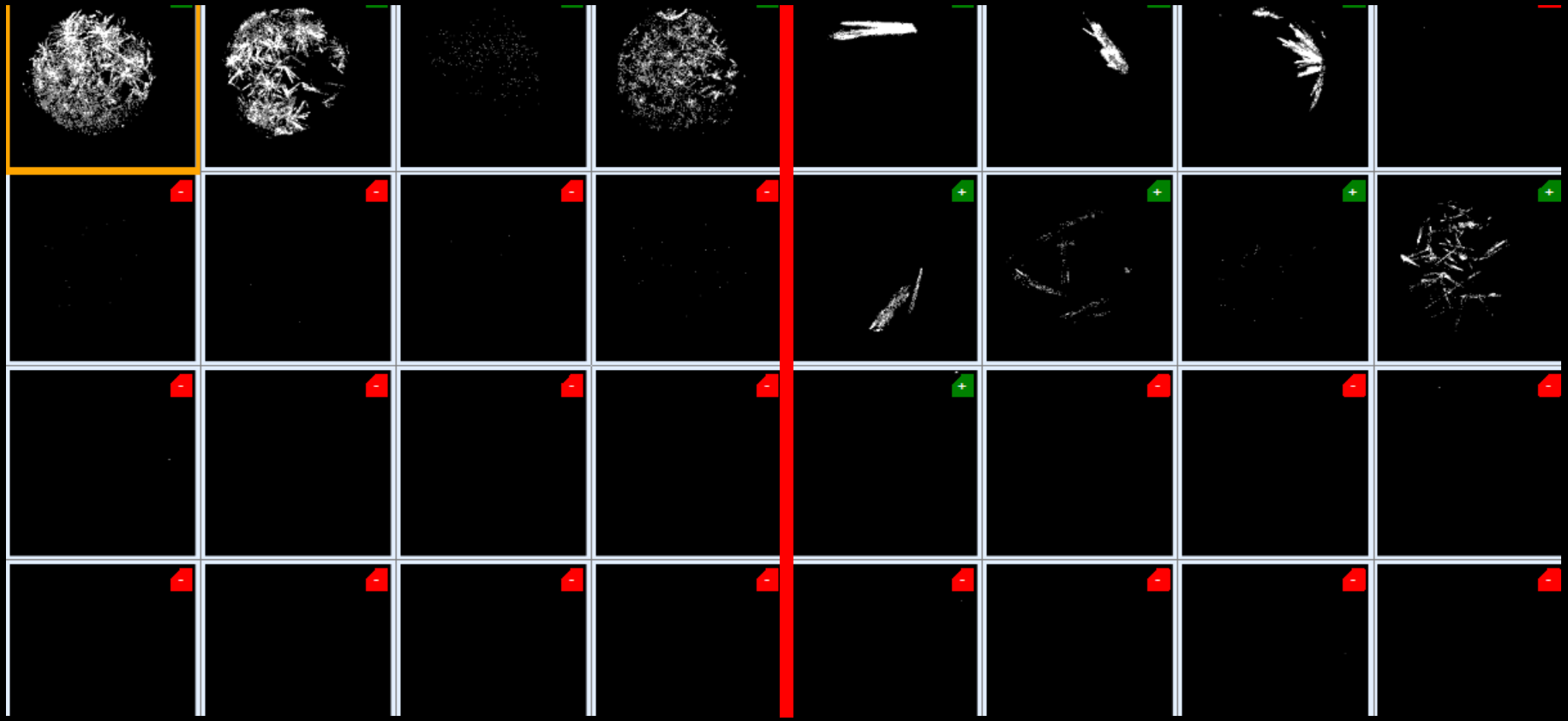
UV-TPEF

*Hampton Research Ionic Liquids*
5%(w/v) 1-Butyl-3-methyimidazolium dicyanamide

*Protein 2*

X14164- Full[P]-10 mg/ml

X14164- [P]/2- 5 mg/ml



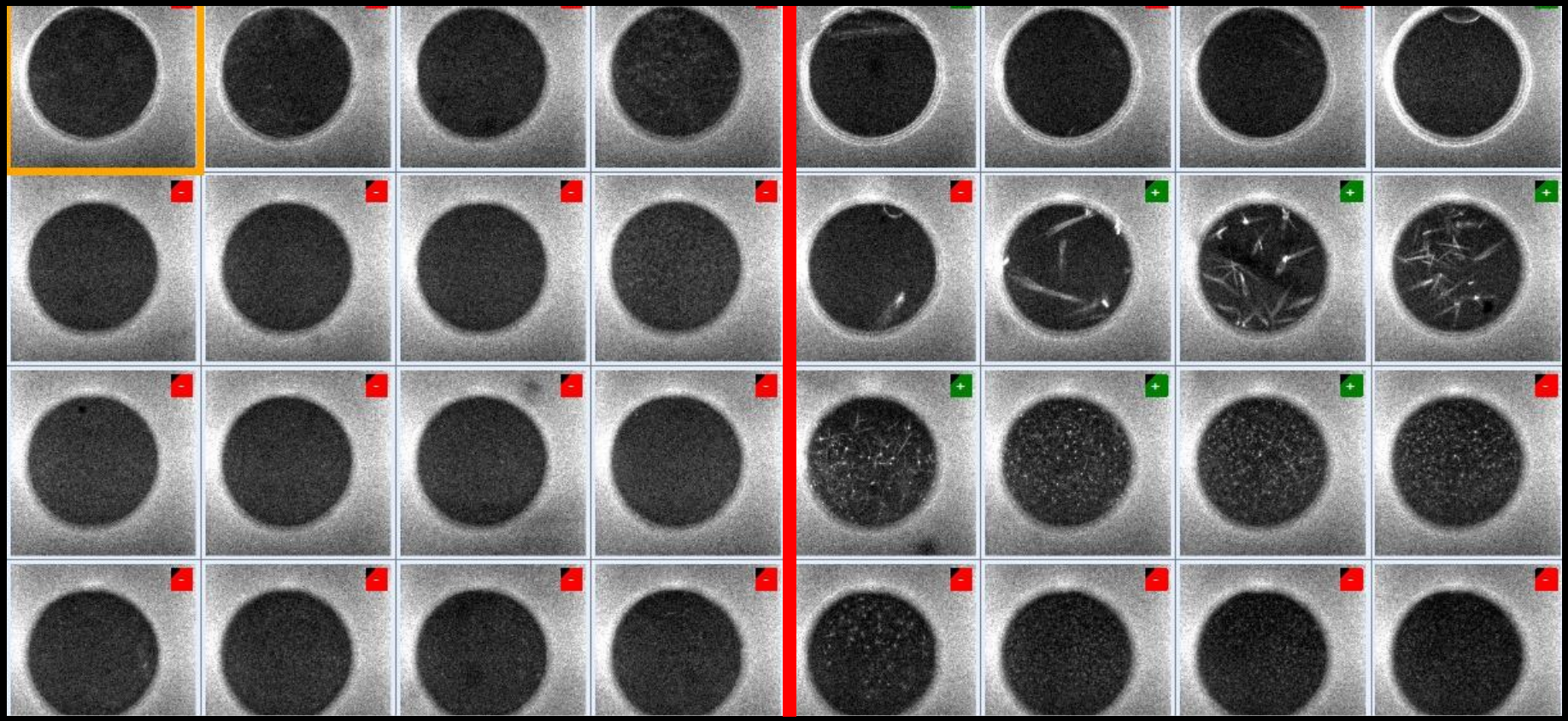
**SHG** at 4wk

*Hampton Research Ionic Liquids*
5%(w/v) 1-Butyl-3-methyimidazolium dicyanamide

*Protein 2*

X14164- Full[P]-10 mg/ml

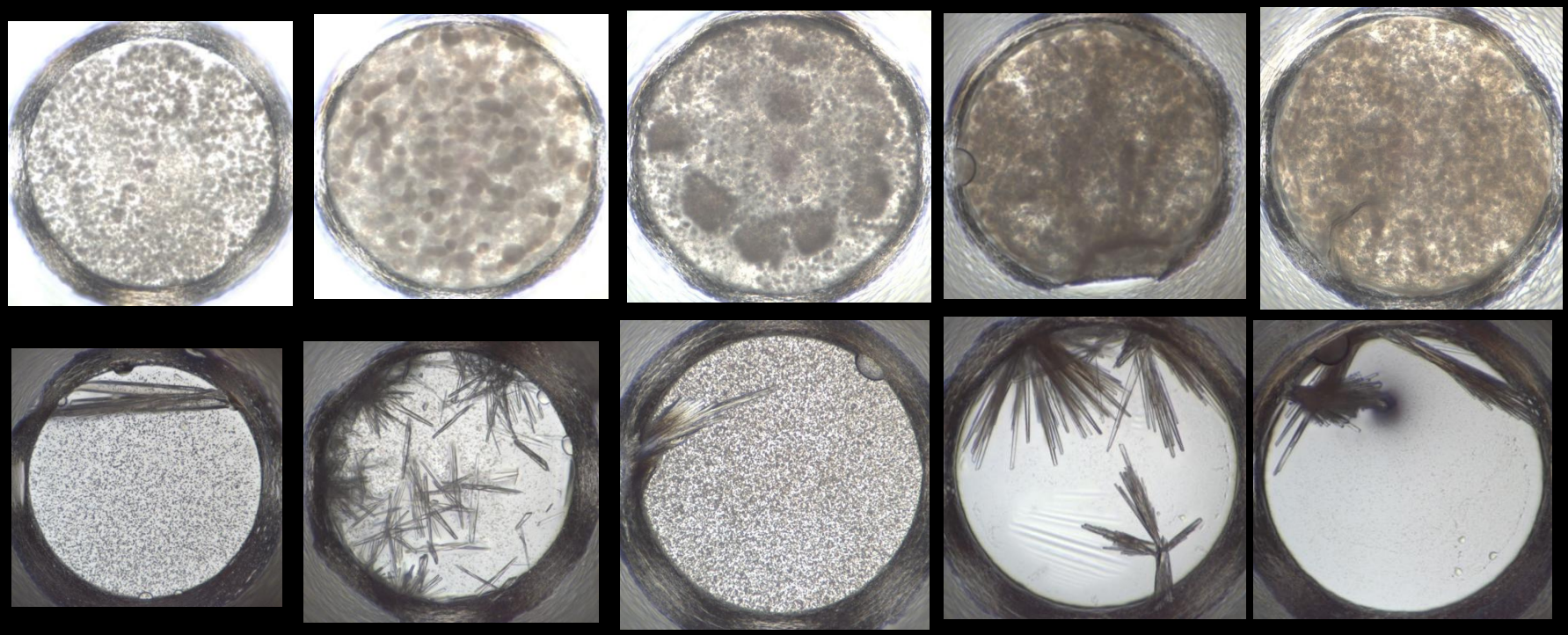
X14164- [P]/2- 5 mg/ml



**UV-TPEF** at 4wk

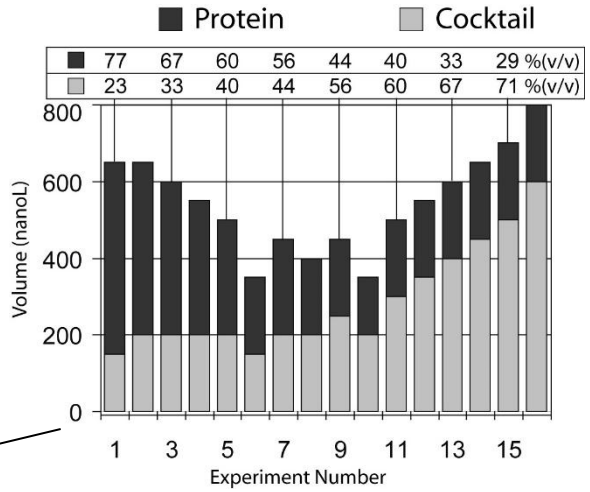
# Protein 2 (crystals identified visually in other conditions)

Visual image where SHG/UV-TEV signal detected



Best optimized condition

# Optimization




$V_{sample} > V_{cocktail}$

$V_{sample} > V_{cocktail}$

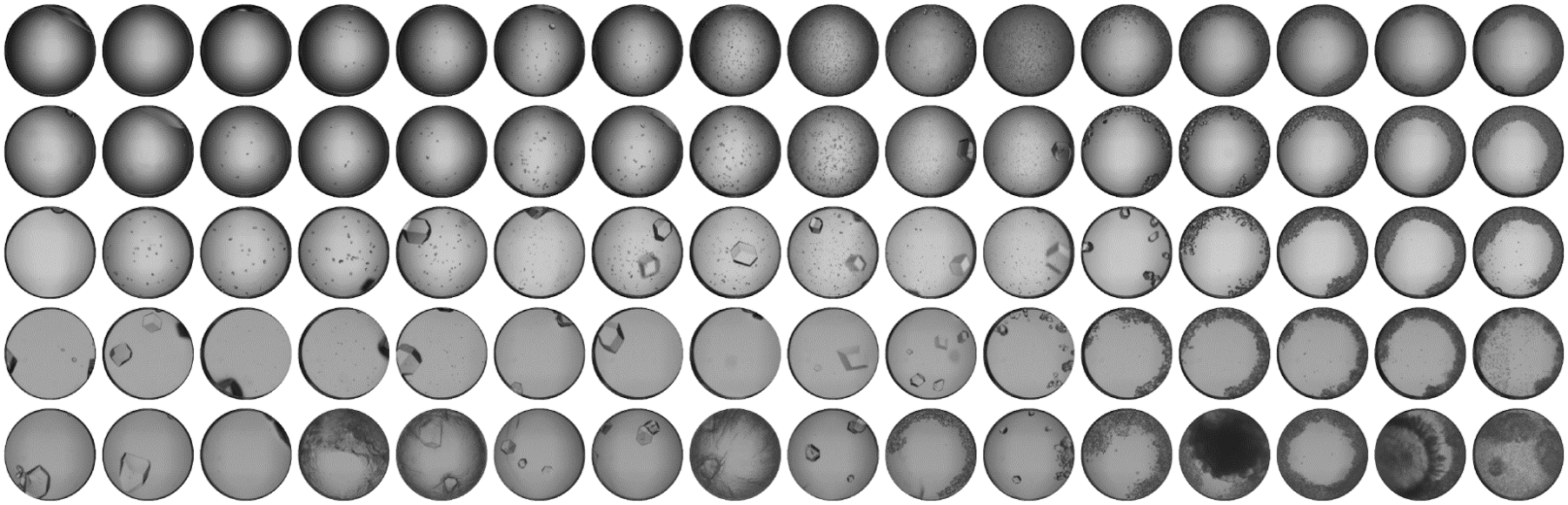
4 °C

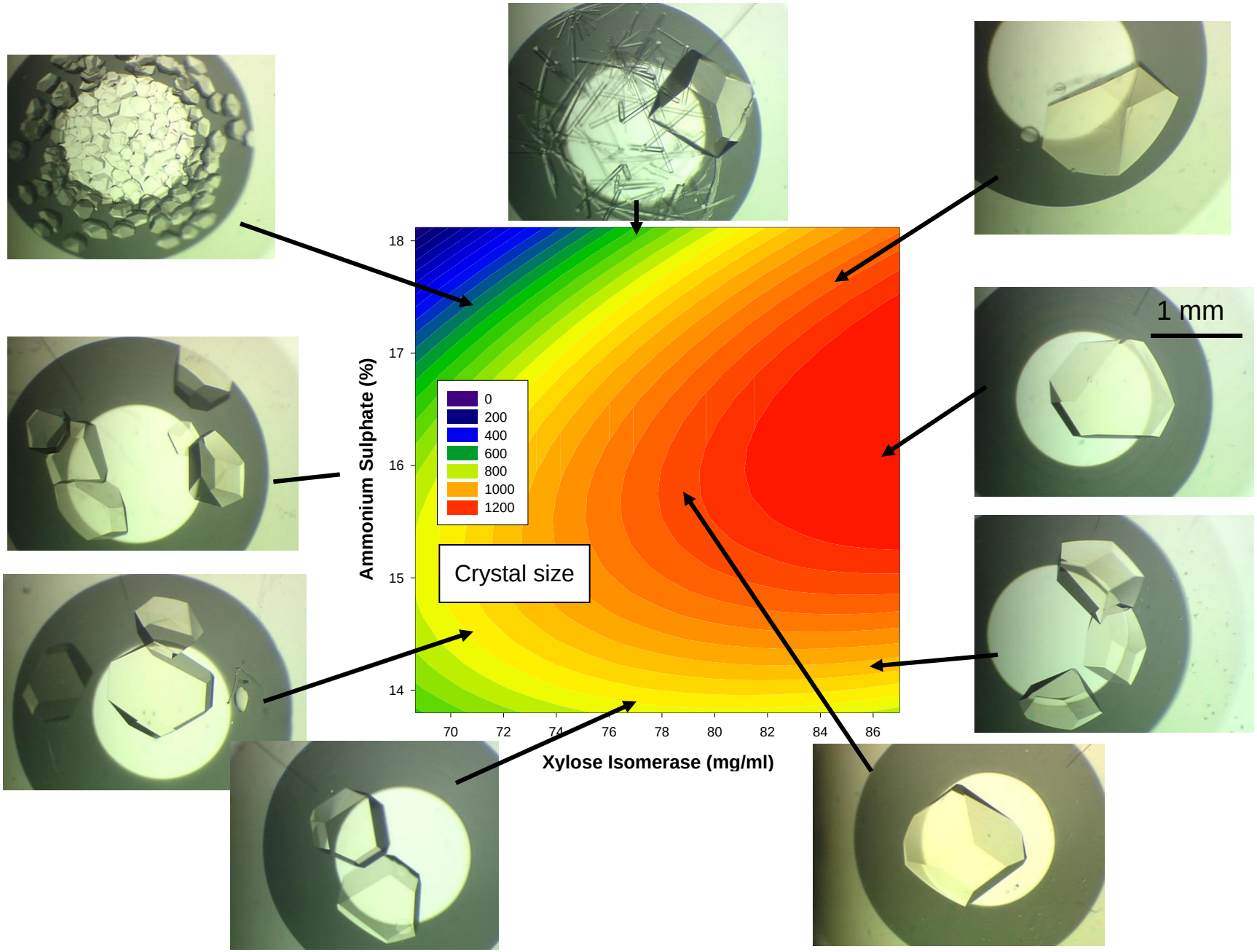
14 °C

23 °C

30 °C

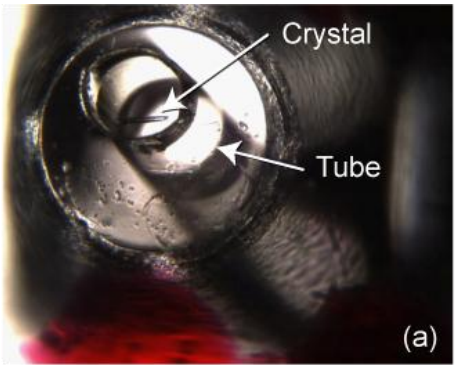
37 °C

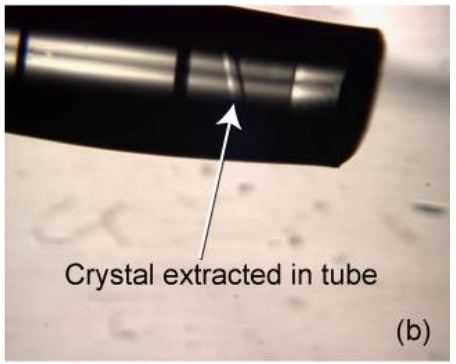


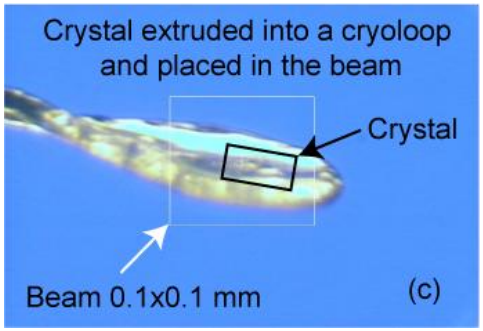

Ammonium Sulphate (%)
18
17
16
15
14
0
200
400
600
800
1000
1200
Crystal size
70
72
74
76
78
80
82
84
86
Xylose Isomerase (mg/ml)
1 mm

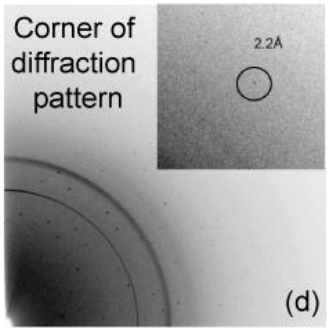
# Going from crystals to diffraction properties

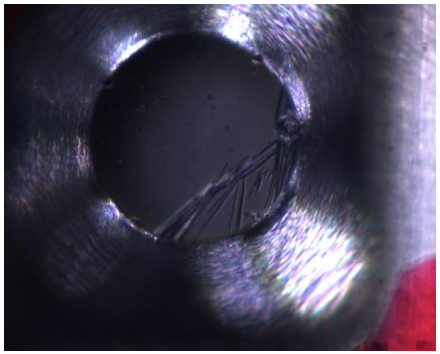
# Does it diffract? Screening before the synchrotron

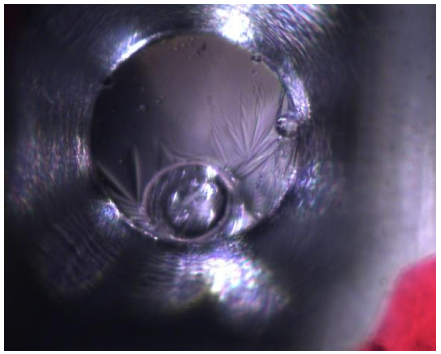


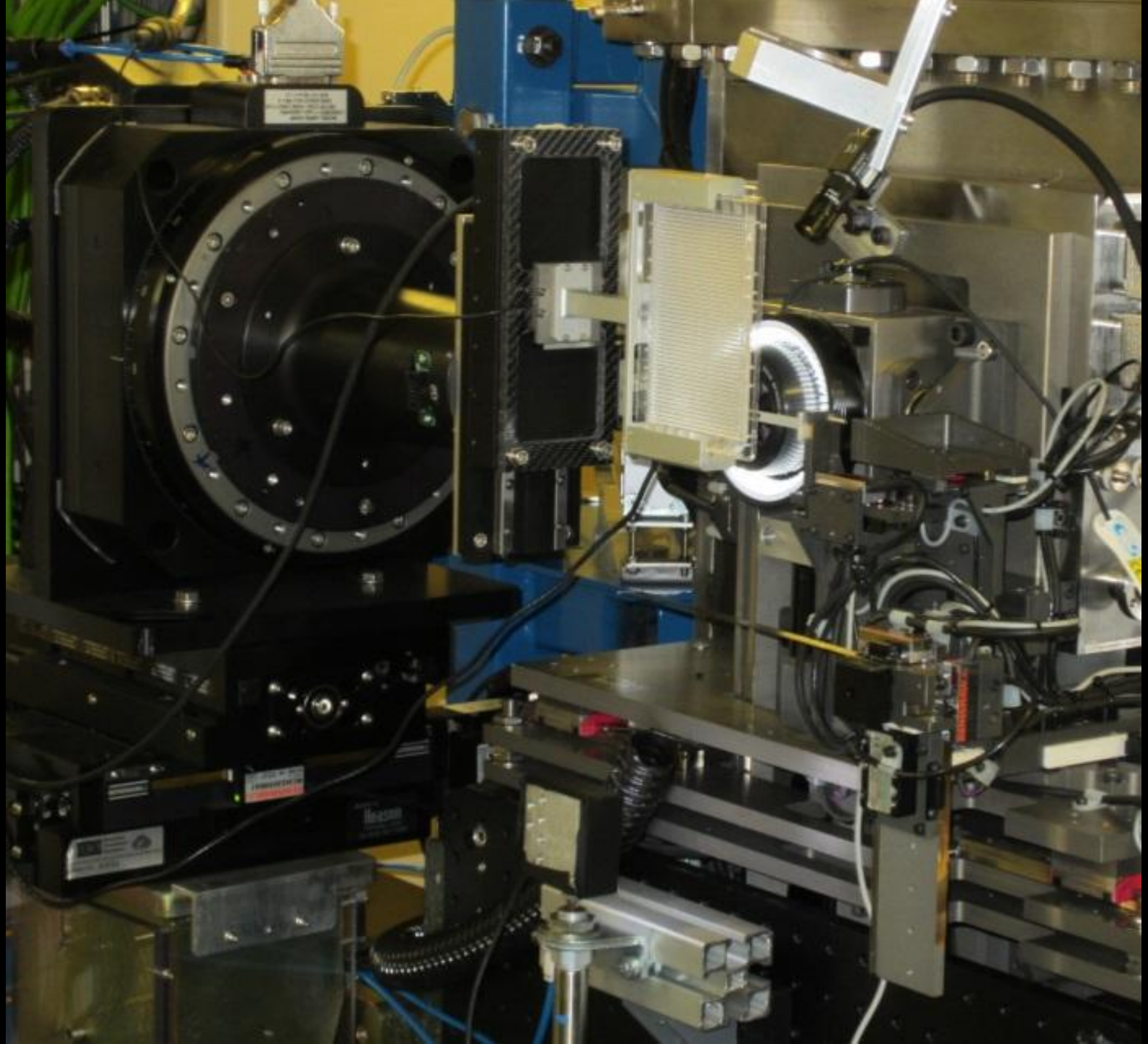



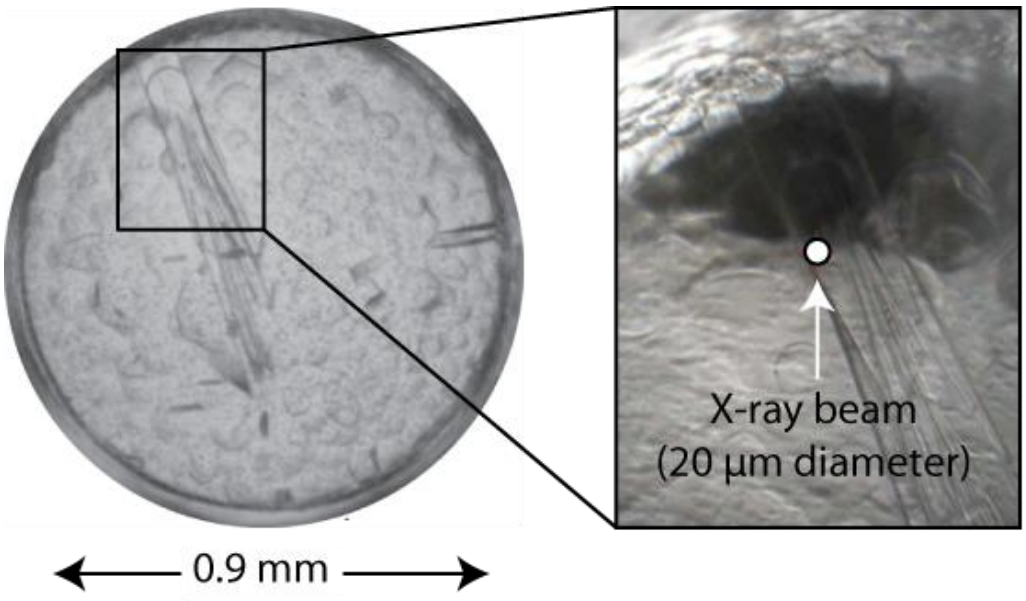



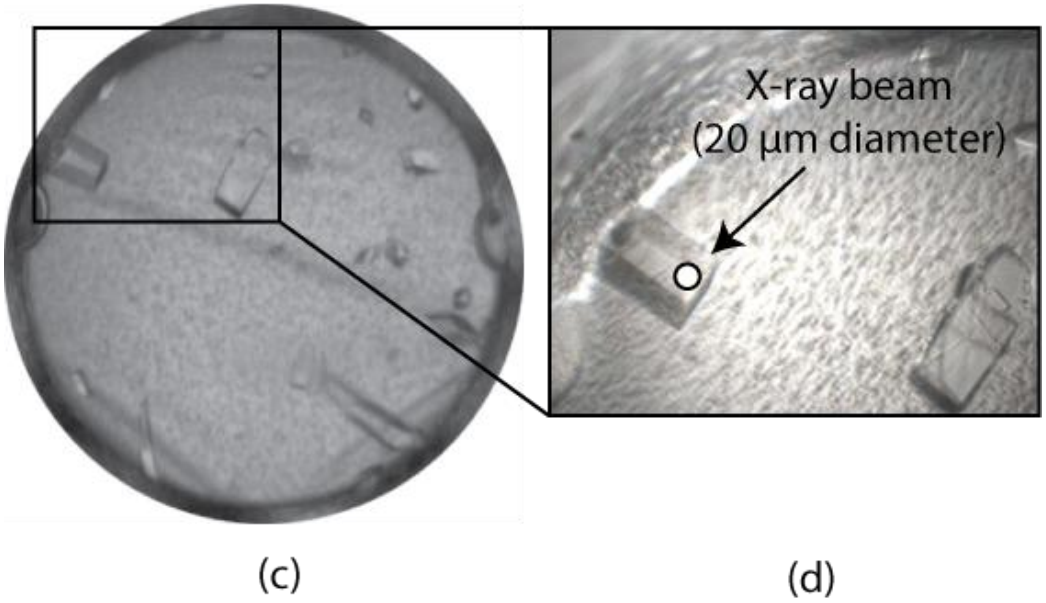







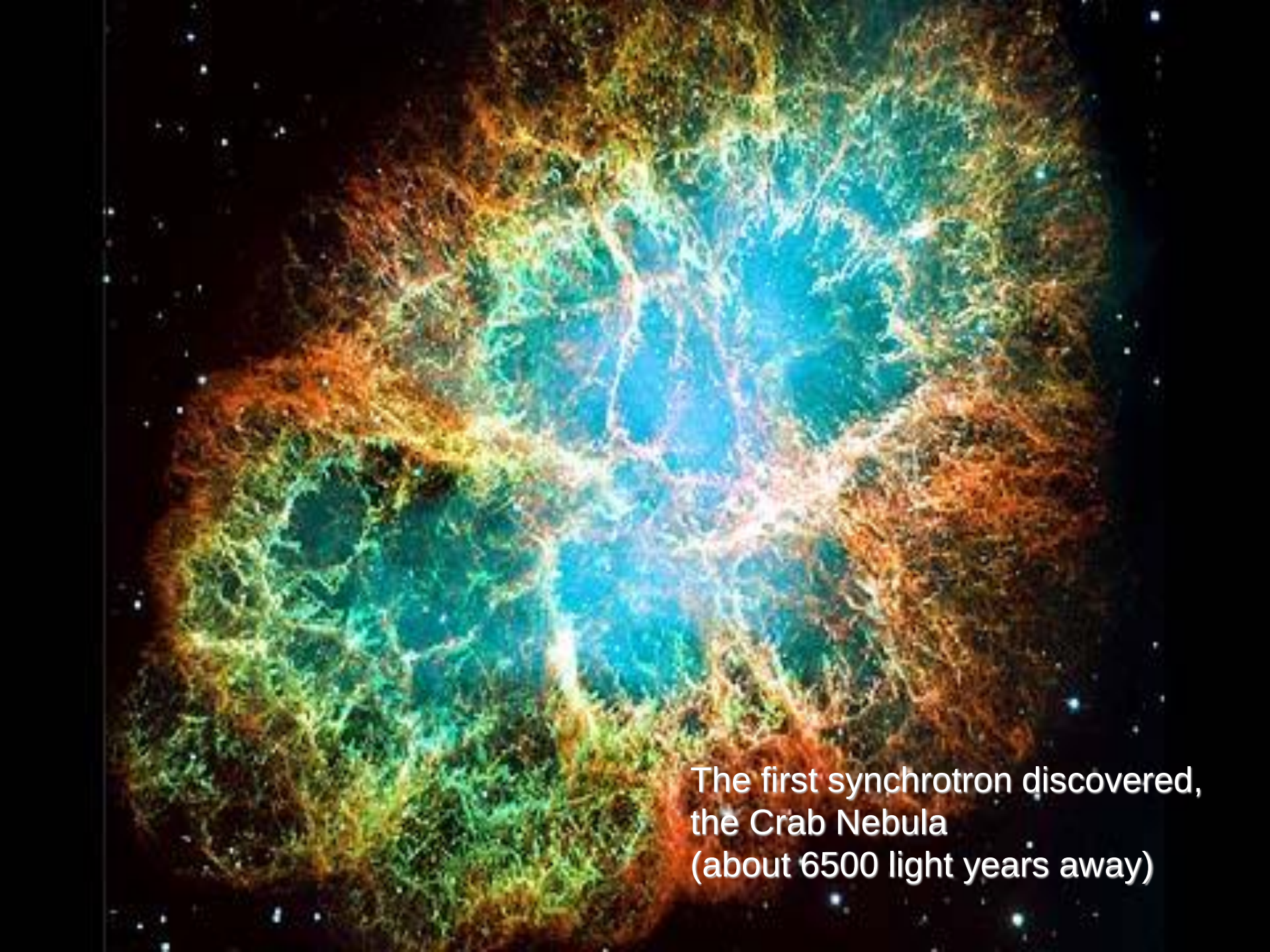


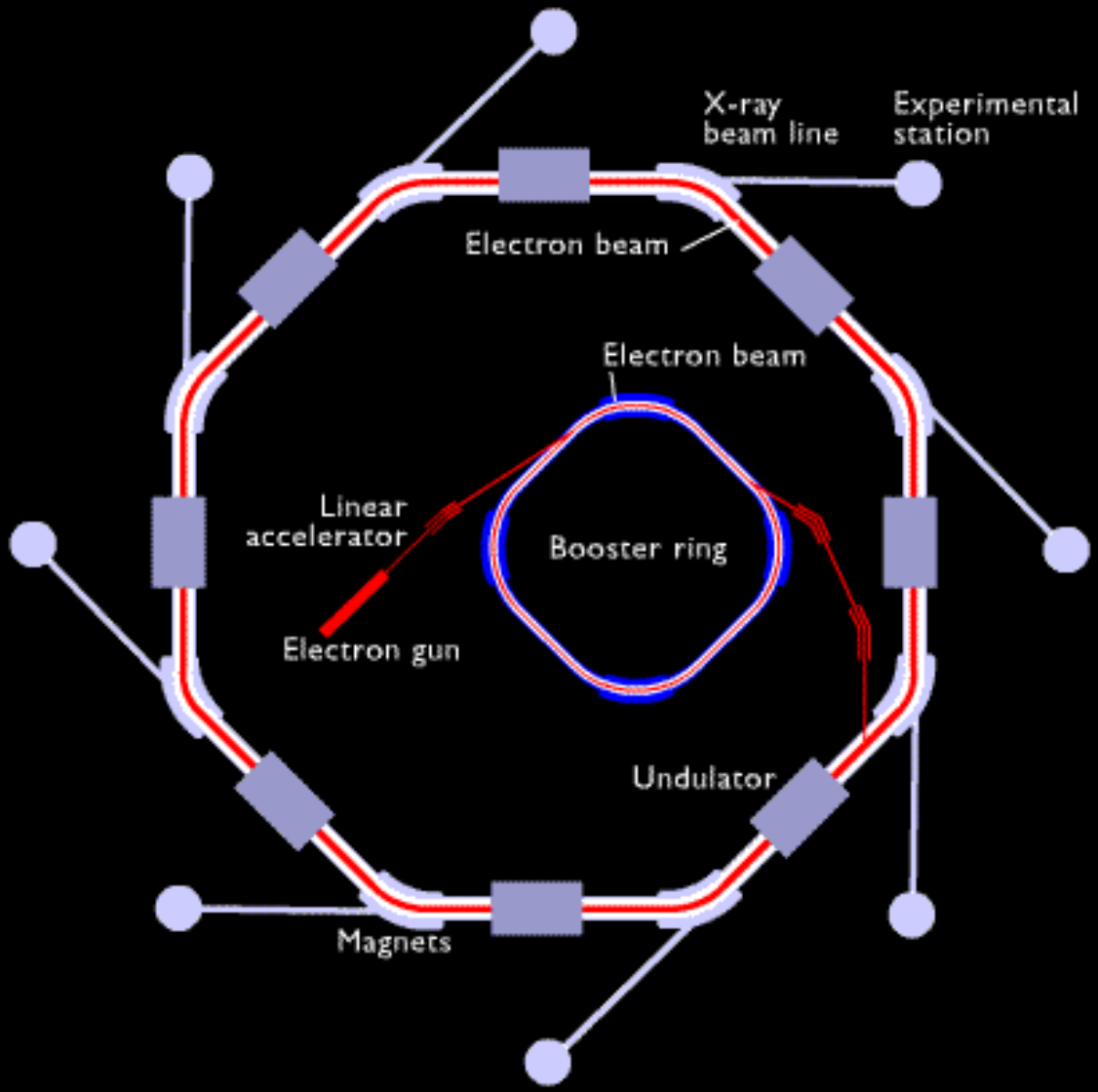



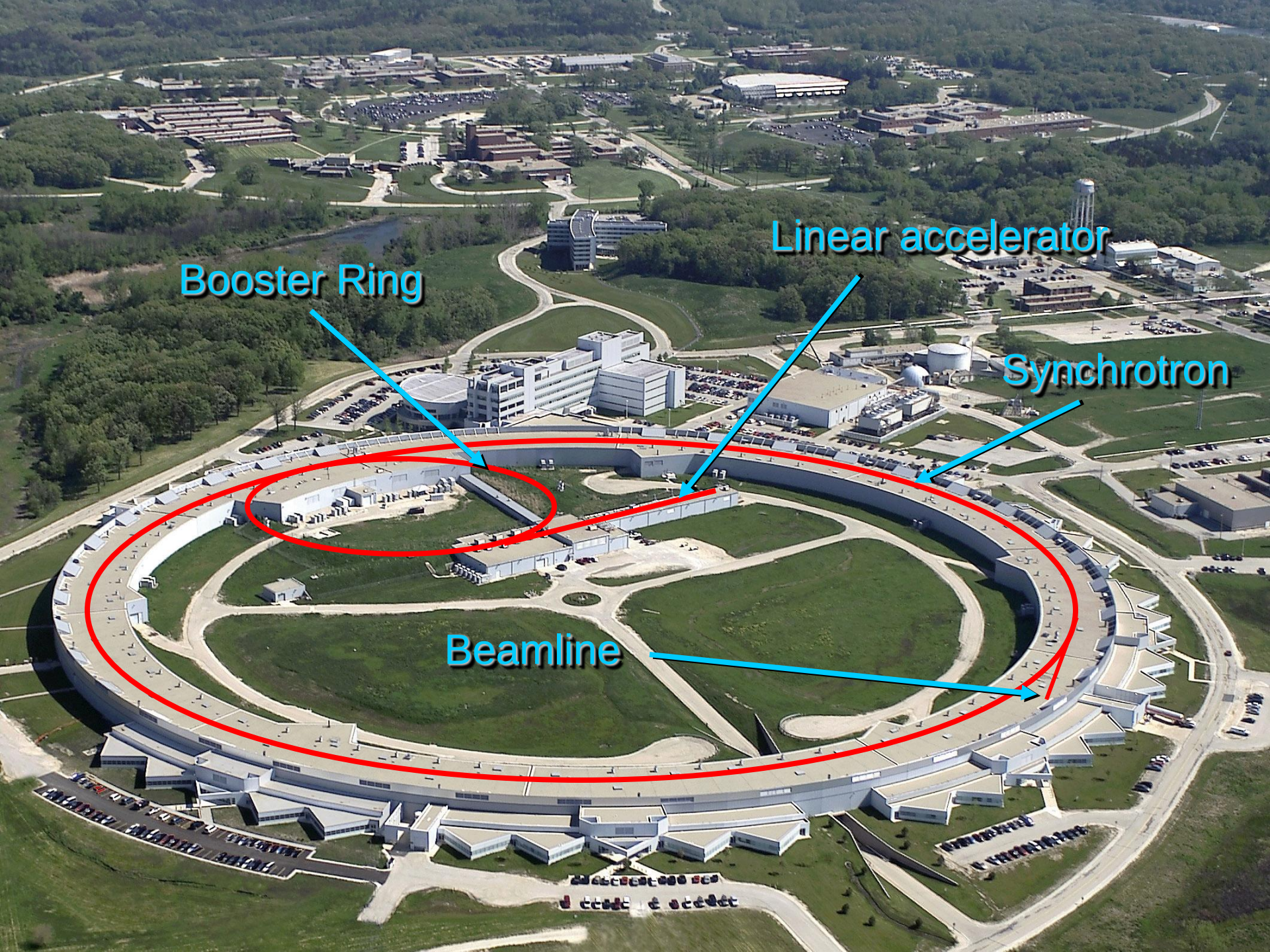

Crystal plates shipped by FedEx (Diamond and NSLS) and suitcase (Diamond)

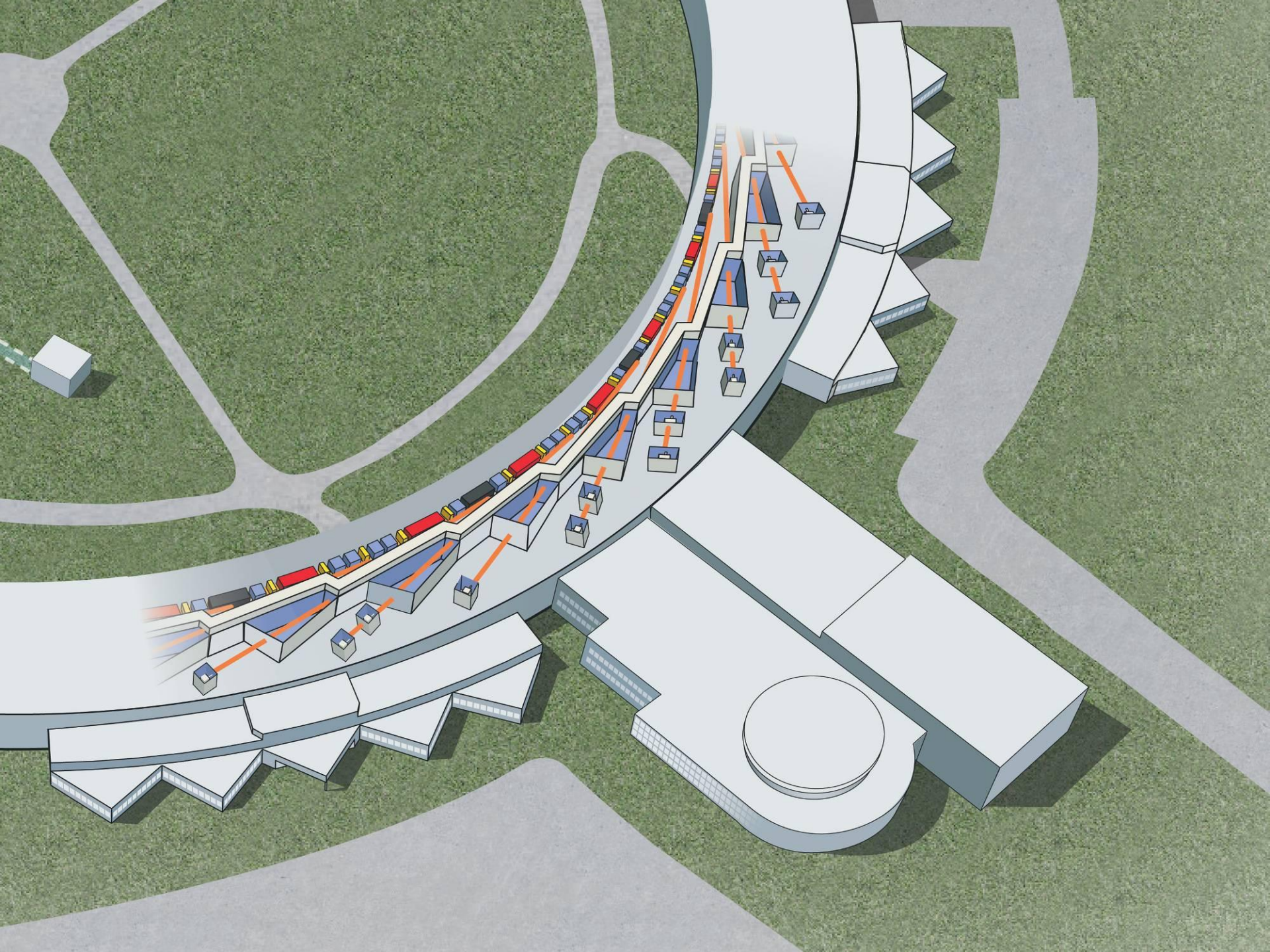
Crystals remained in place and diffracted.

# Synchrotrons


The first synchrotron discovered,
the Crab Nebula
(about 6500 light years away)

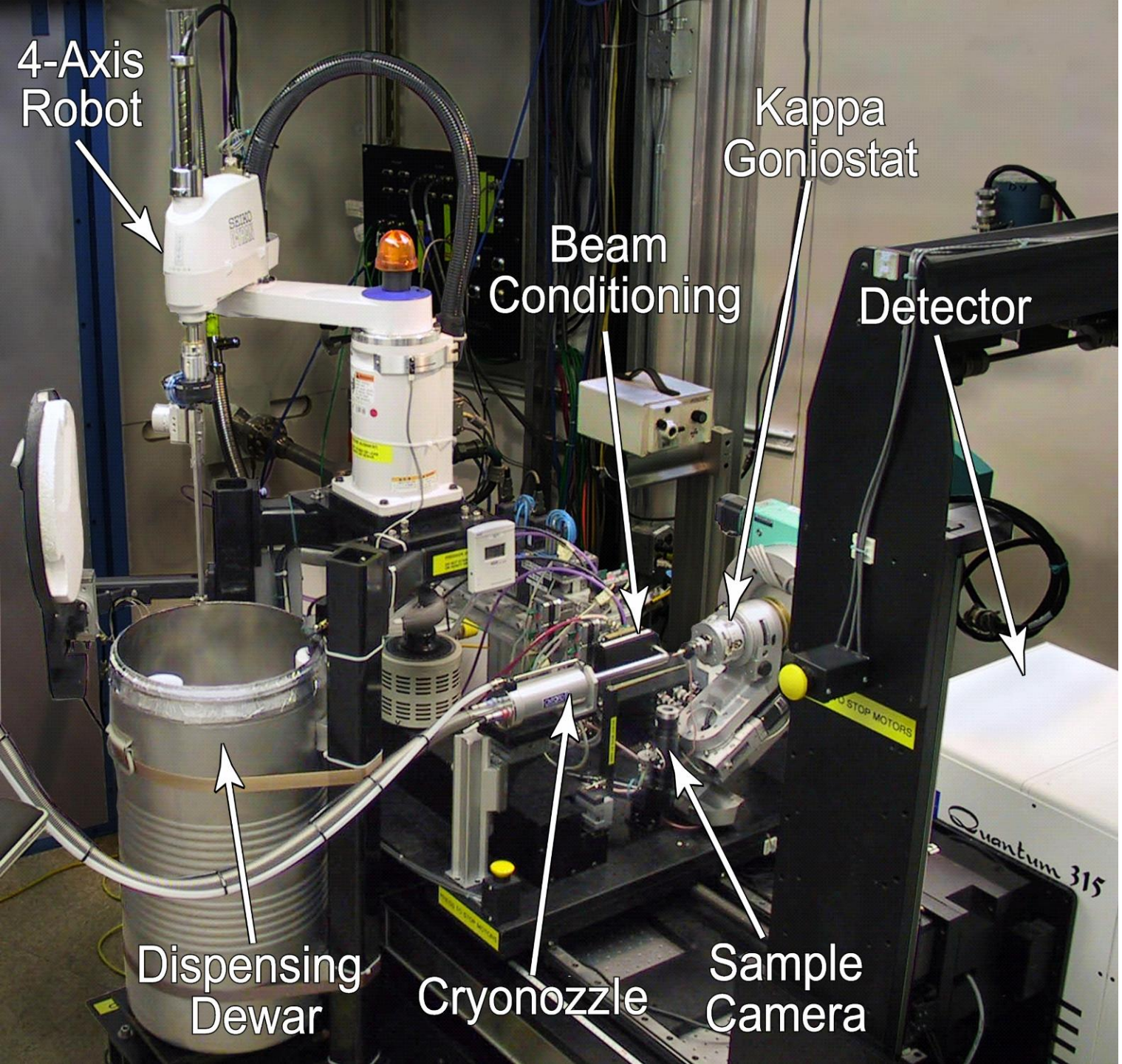

X-ray
beam line
Experimental
station
Electron beam
Electron beam
Linear
accelerator
Booster ring
Electron gun
Undulator
Magnets

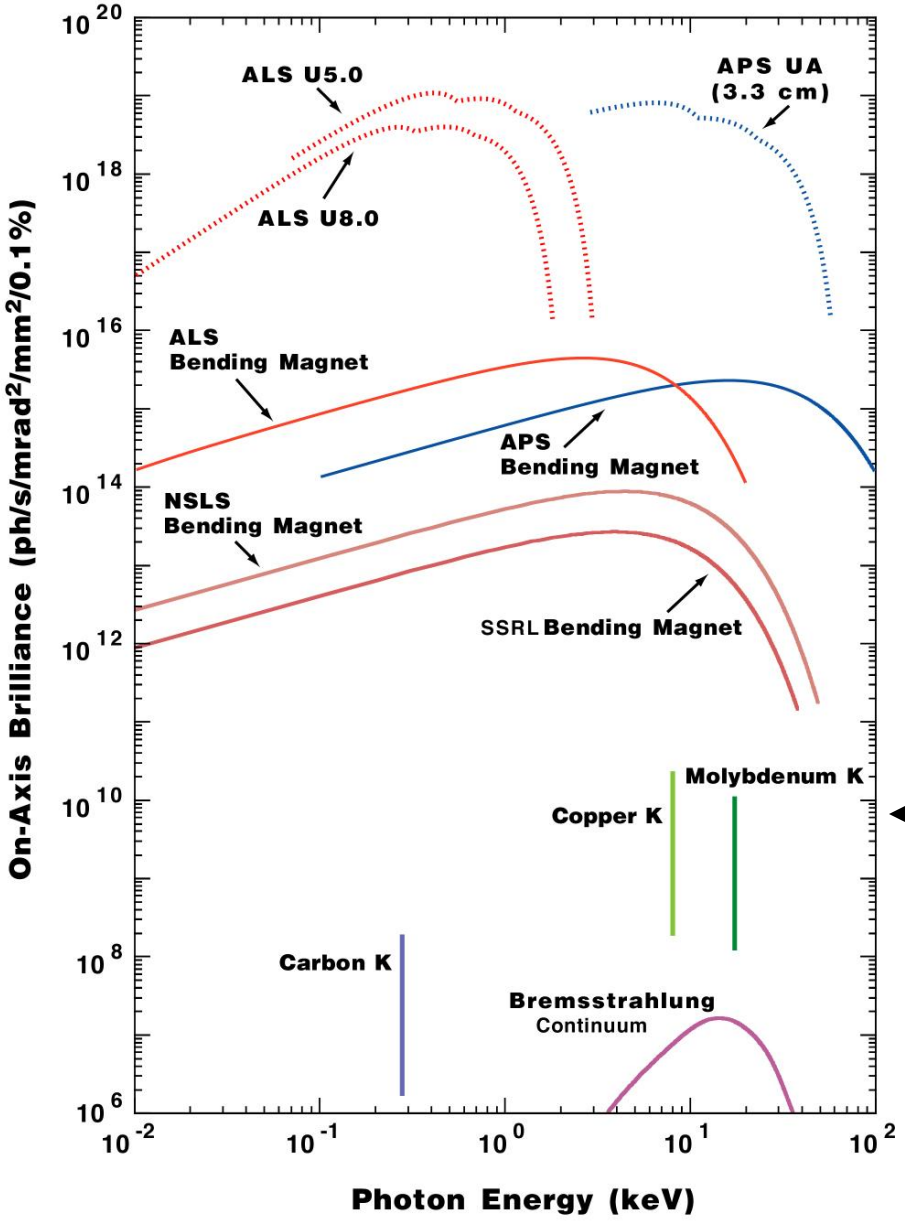

Booster Ring
Linear accelerator
Synchrotron
Beamline



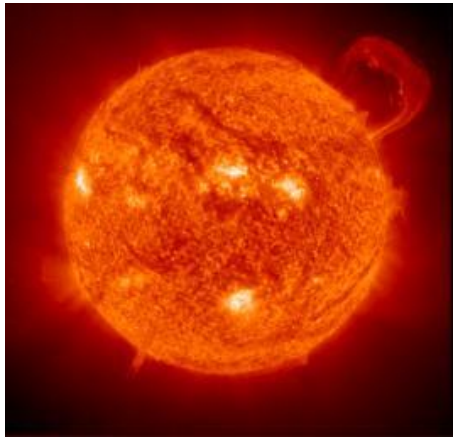

B200

# Inside the Hutch






Synchrotron radiation is $10^9$ times
More brilliant than the sun
and about 100 million miles closer



# Cryocooling

# Processes of radiation damage

Primary, secondary, direct and indirect radiation-damage events in a protein crystal.

The incoming X-ray photons cause primary damage events, represented by darker stars. The paths of secondary radicals are shown by dotted arrows, and the damage events they induce are represented by lighter stars. Direct events occur on the protein molecules, and indirect events occur in the solvent region.

Primary effects are a fact of life, we cannot prevent them. Secondary effects can be reduced by cryocooling.

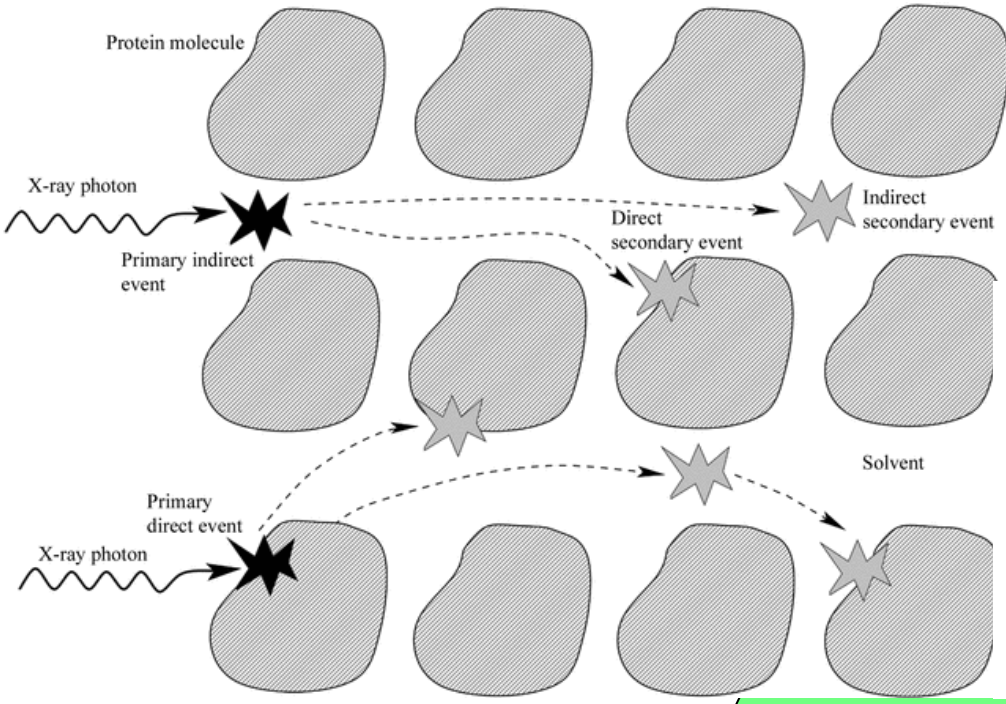
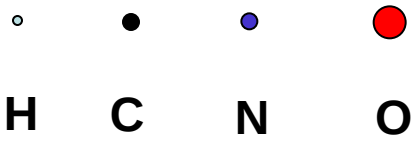
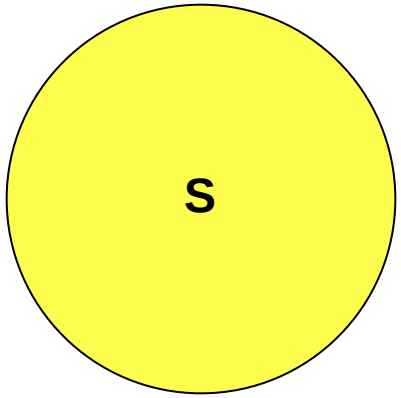
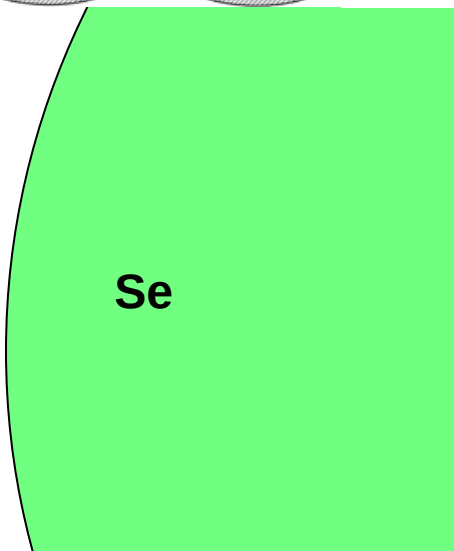



Image from Elspeth Garman

Effect on Other atoms



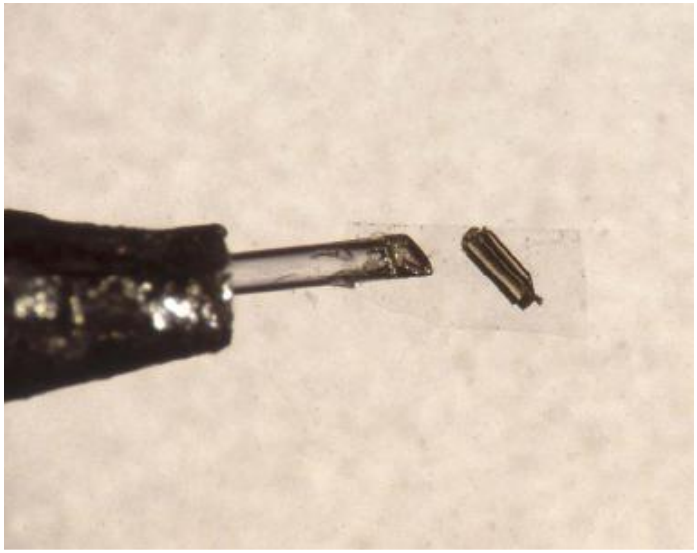



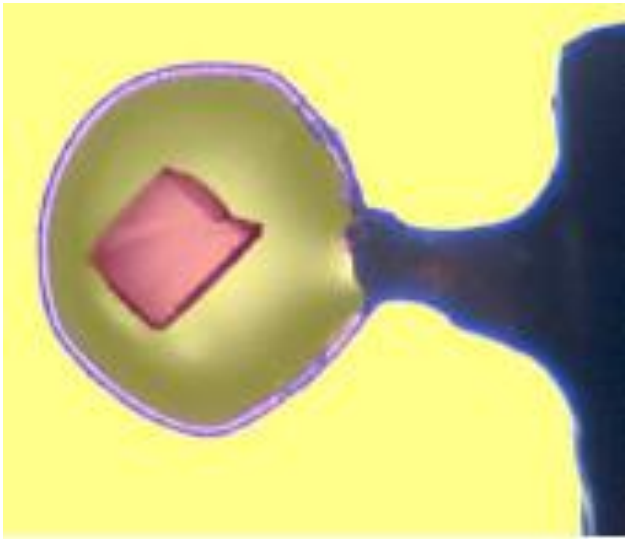
# Development of cryocooling

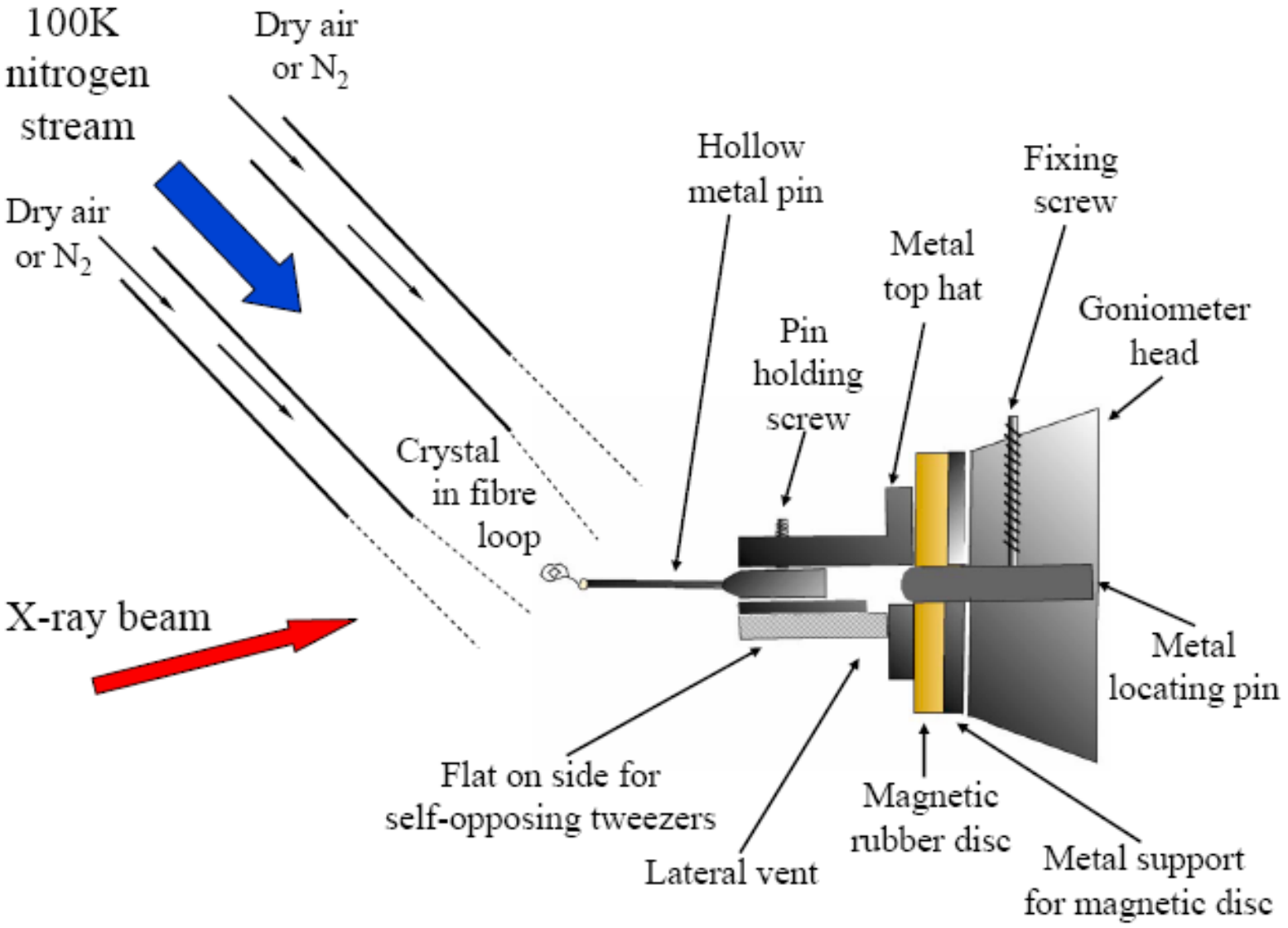
Hope Acta Cryst. B44, 22-26 (1988) at 130K with oil and spatulas.

Loop mounting, Teng J. Appl. Cryst. 23, 387-391 (1990) first introduced a metal loop which is now the nylon loop we know today.

Cryocooling with loops is younger than everyone that uses it!



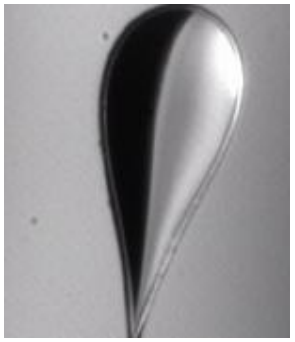





Courtesy of Elspeth Garman

# Cryocooling – How?

- Cool the crystal fast enough so that amorphous ice rather than crystalline ice is formed (vitrification).
- To vitrify water cooling has to occur in $10^{-8}$ s.
- Cryoprotectants extend this time to 1-2 s.
- A cryobuffer is the buffer the crystal is grown in with the cryoprotectant added.
- The cryoprotectant replaces water, it does not dilute the solutions.
- Visually clear is usually a good indication of a good cryoprotectant condition.
- Collect data below 130 K, preferably as low as possible but never above 140K.



Good cryobuffer

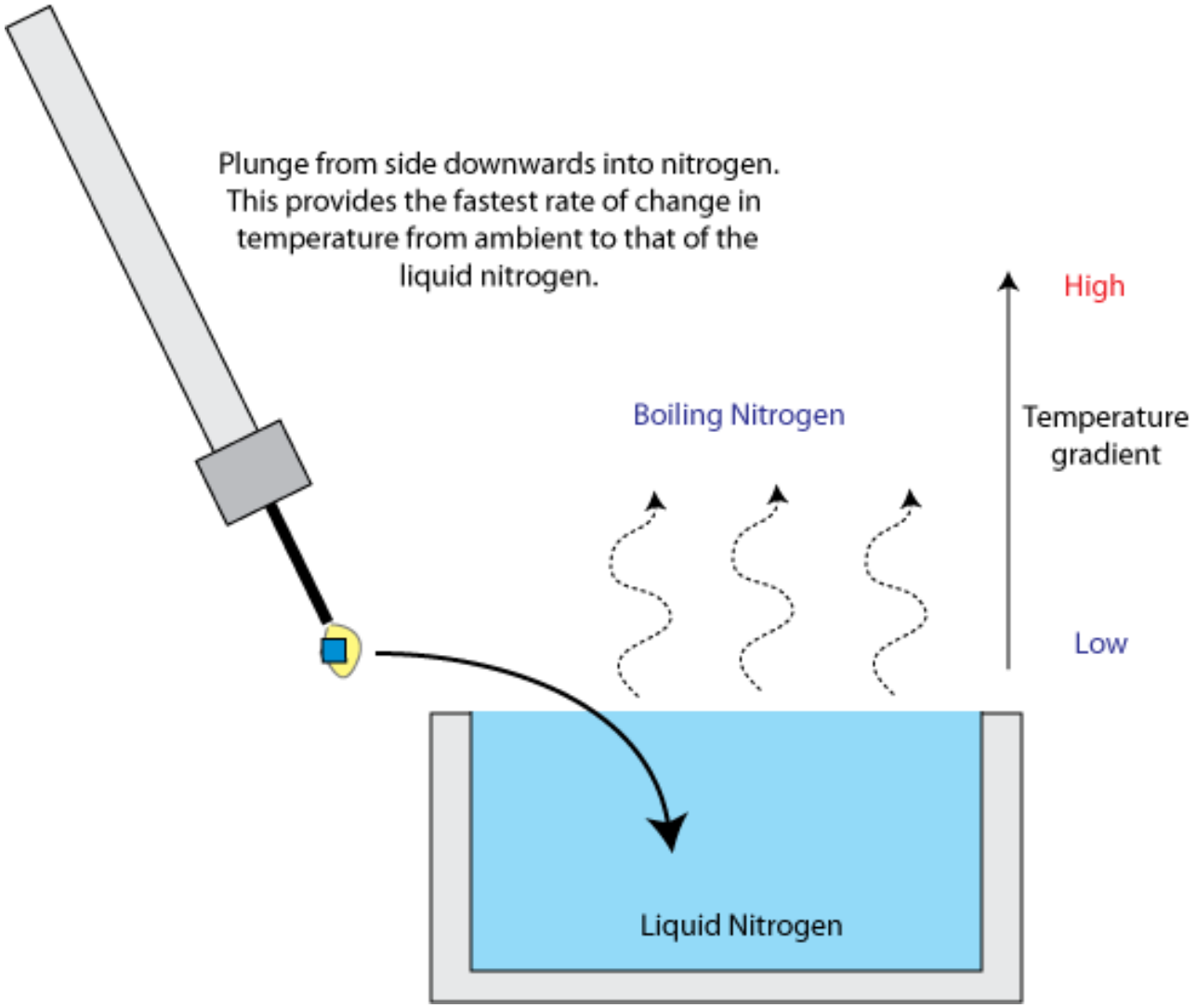
Bad cryobuffer

Read any of the papers by Elspeth Garman listed at http://biop.ox.ac.uk/www/garman/publications.html

# Cryo-buffers (make your own)

- Look for similar crystallization conditions in other publications.
- PEG < 4K – increase PEG, add small PEGs
- PEG > 4K – add small PEGs
- 30% of cases – add 15-25% glycerol
- MPD – increase MPD concentration
- Salt – add MPD and/or ethylene glycol or glycerol
- Salt – increase concentration/add salt
- Salt – exchange salts
- Note low salt concentrations need greater concentration of cryoprotectant than higher salt concentrations.
- Other cryoprotectants, DMSO, propanediol etc.
- Butanediol is very effective but expensive.
- (with thanks to Elspeth Garman for many tips).

# Flash cooling by plunging



# Remote data collection – practical tips

# Caveat Emptor

The tips, experiences and opinions presented are those from the user side after many remote data sessions involving many investigators. They do not necessarily represent the views of the user support at the synchrotron, are frequently being revised, and may change with time, more practice and better sleep. They are in addition to and not replacing those tips available on the user guides at SSRL. The tips and experience may be obvious or new, hopefully they will be useful!

If you have any tips to add they would be most welcome.

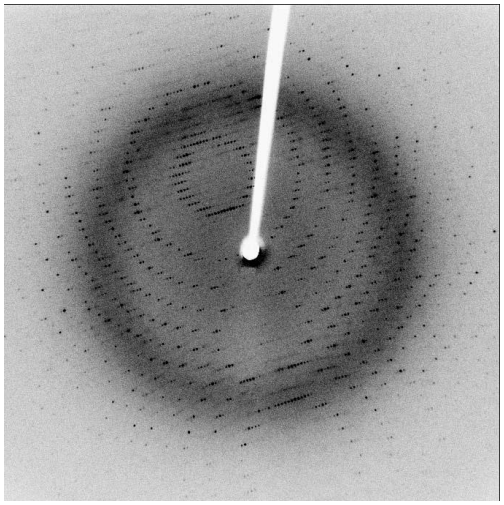
# Does it diffract?

You cannot tell how well a crystal will diffract X-rays by looking at it.

There are many ugly ducklings.

The only way to tell is to put your crystal in front of an X-ray beam … find the friendly crystallographer.

Or, screen crystals on the SSRL robot – not the ideal use of beamtime but very rapid, ~3-4 mins per sample.



# Getting your beamtime

**Note:** First time users are currently requested to collect data at the beamline once or to attend a training workshop at the SSRL before they perform the experiment remotely.

# Ways to get your beamtime

1. **Submitting a proposal**. Proposals can be submitted for single experiments, for a program (i.e. different research problems or projects scientifically linked or related to each other) or for rapid access ("hot" new projects).
2. Setting up **Participating Research Team** (PRT access)
3. **Scientific collaborations with SSRL staff**

# A successful proposal

- A successful proposal has three components:
    1. Important structural target/s, and/or a novel and useful experiment
    2. Sound justification of the need for synchrotron time
    3. Experience and previous results (can you carry out the experiment)
- Make your proposal strong by balancing all three components.
- The strongest are careful to address the ‘why a synchrotron’.
- Contact the support staff beforehand for advice if you have any questions to ask. Most, with rare exceptions, do not bite.
- Make a reasonable estimate of time and a determination the appropriate beamlines that could be used. How much time does it take to screen, how much time does it take to collect data, how many samples do you have then ask for one shift extra?
- Look at other successful proposals if you can.

# Justifications for Synchrotron Time

Examples include:

- A spectrum of energy (wavelength) unavailable in the laboratory. Required for anomalous diffraction experiments.
- An increased intensity to push the resolution limit further. What resolution do you have, why do you need to extend it further? What question cannot be answered at the current resolution?
- The ability to resolve longer unit cells. What can the system at home resolve, what can you expect to resolve at the synchrotron? Look into the beamline instrumentation, do a quick calculation.
- In the robotic case, study many samples much faster than at home. What percentage of crystals provide good diffraction, how many do you need to screen efficiently? What time would that take at home. Do you have facilities at home?
- Does the experiment require many measurements in a rapid as possible time?
- Why remote time? Standard experiments, limited travel budget, save the funding agency money, enable you to do more?

Once you have your beamtime …

# Planning:

- Preparation – Things to do beforehand
- Filling – Putting samples in the cassette
- Shipping – Options and tips
- Screening – Quick look data collection
- Planning – Going from screening results to data collection
- Data collection – Tips and tricks
- Data backup – Getting it home
- Finishing – Acknowledgements, lessons and feedback

- Proper Preparation and Planning Prevents Poor Performance (PPPPPP)
- Ice is the enemy
- Liquid nitrogen boils off
- Keep it simple stupid (KISS)

# Preparation

Robotic beamtime is no substitute for the real thing. To use robotic beamtime to its fullest potential you should try and ensure that at least one member of the team is competent in data collection at the synchrotron and understands the differences from home source collection.

Personal contact with the beamline staff is also very useful for transferring the latest ideas, advice etc. In the case of Stanford this has the added benefit of having very nice restaurants….

# Know you timeline

- Know which samples you plan to send (possibly pre-cool and store in cryovials to mount later). Crystal positioning make a device for longterm storage of pucks in lab Dewars.
- Inventory pins, loops, mounting equipment and do a dry run sufficiently ahead of the beamtime so that you can procure any missing items if necessary (two weeks).
- Check that you have liquid nitrogen available, a shipping dewar and a means to ship it (one week).
- Make up a log of samples as they are loaded, add this to the spreadsheet (several days).
- Keep the spreadsheet handy to upload immediately before data collection.

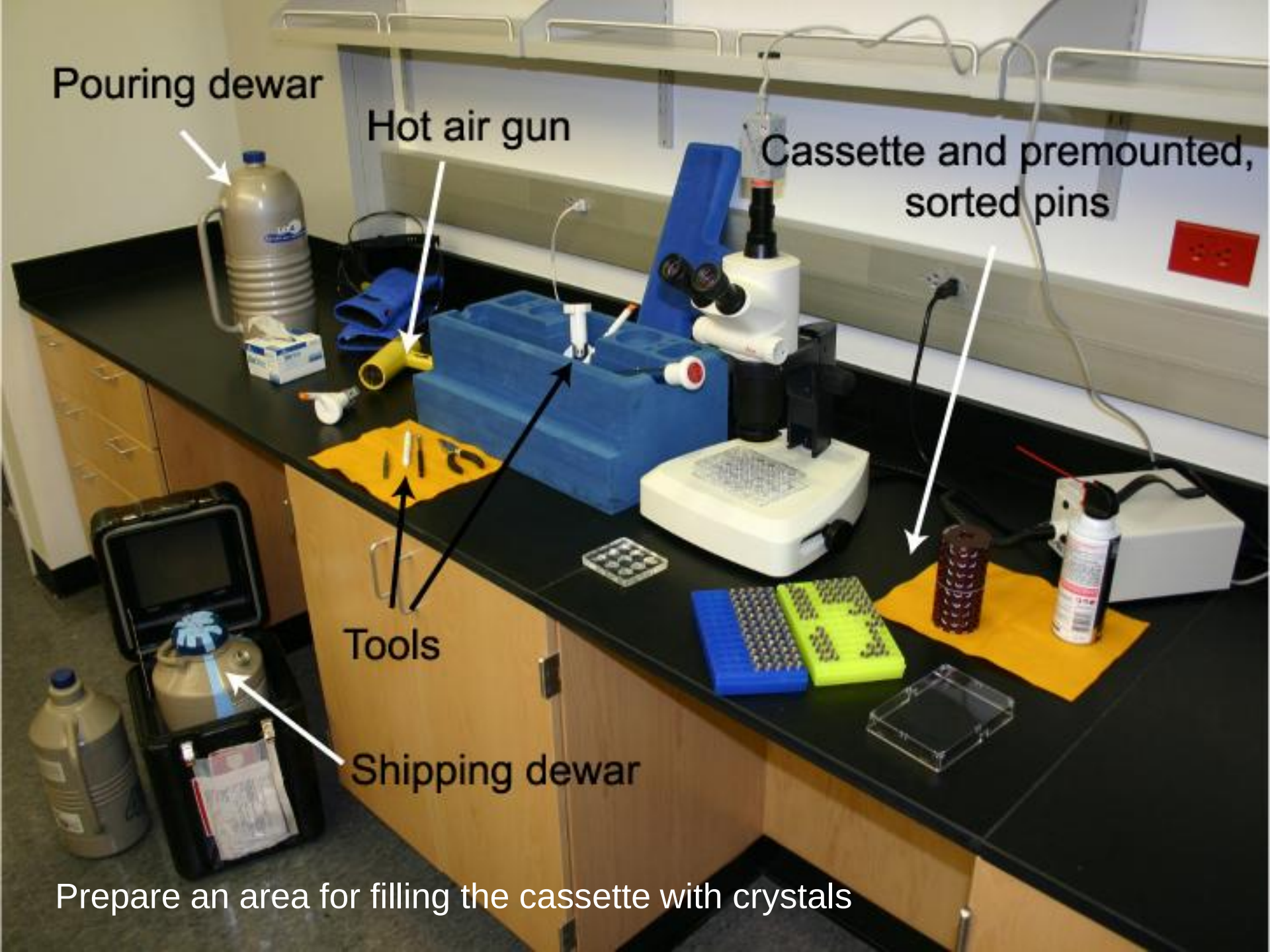


# Preparing pins

- Prepare a selection of pins and loops to suit crystal size.
- Prepare several at once.
- Select an appropriate loop to put in the pin.
- Glue the loop in the pin, 5 minute epoxy on the bottom of the loop holder to be inserted into the pin works well. Avoid getting epoxy on the outside of the copper mount.
- Check the loop under a calibrated microscope, make sure it is intact then write the size on the magnetic base.

# Preparing for filling

- Make sure you have an excess of different size loops in standard height  pins. Loops and pins can be cleaned and reused.
- Have loops and pins organized by size ready for mounting (see the 96 well organizers).
- Make sure the loop size can be read.
- If feasible, switch your entire laboratory over to SSRL compatible height pins. This will save problems later. Loops that are too short confuse the automatic centering and stop the process.
- Make sure the loops and pins are clean and the loop is solidly held in the pin (test and verify). A pair of pliers is a useful tool to have around.



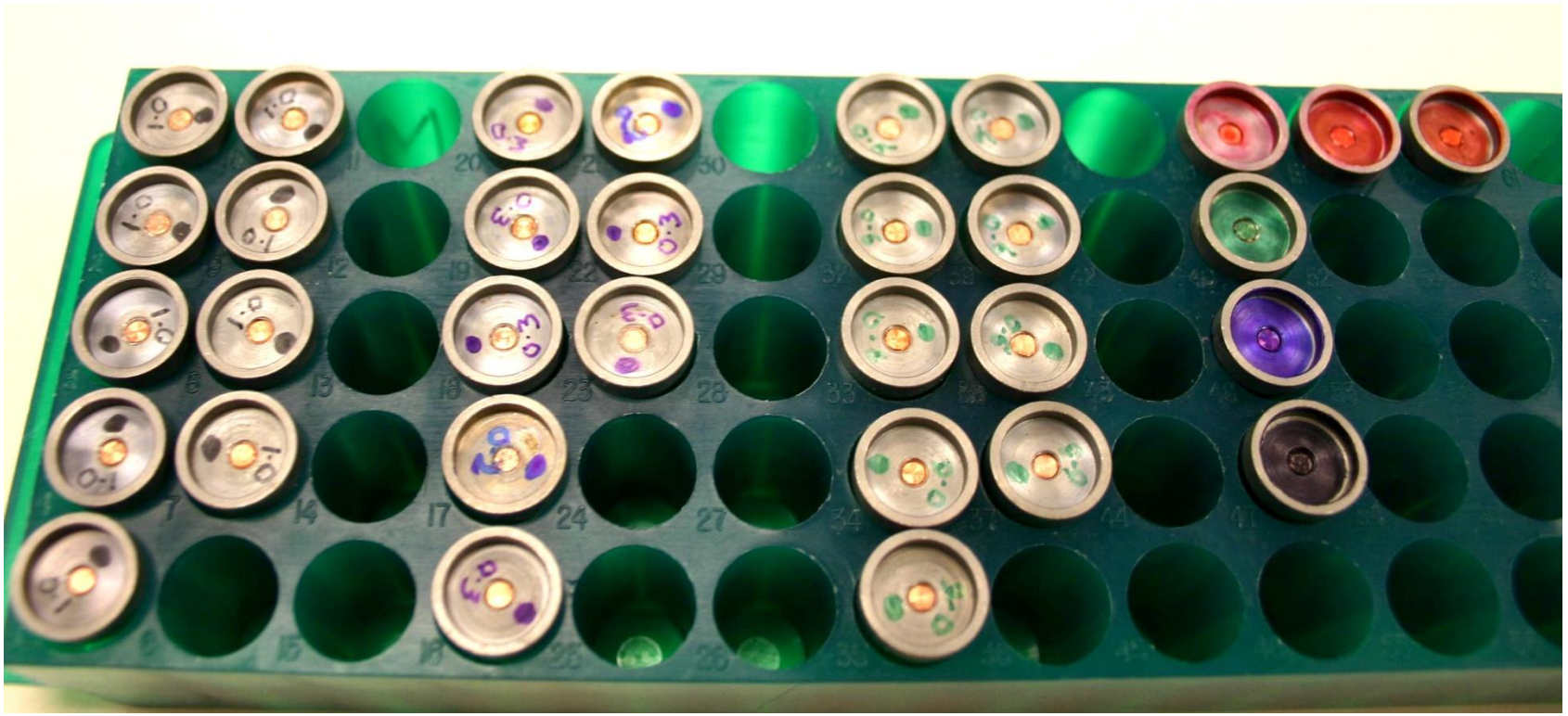

Prepare an area for filling the cassette with crystals

# Testing Loops

- Use forceps to test that the loop posts are firmly seated in the magnetic base
- If not, extract the loop with a pair of pliers and if the post is clean either re-glue the original loop or put a new one in its place.
- Test all loops before each data collection if reusing them (see last section).

# Color code or identify loop sizes

- Better to use size indicator with color spot – less to remember
- Keep the loops organized for easy selection.



# Preparing for filling

- Make sure liquid nitrogen is available for the filling.
- Use a phase separator on the liquid nitrogen delivery tube. These are available for approximately US $50 and reduce the amount of gas released when transferring liquid nitrogen. They are good for both safety and economy reasons as the amount of nitrogen used seems lower with these.
- With practice and the use of a phase separator, a single 100 liter liquid nitrogen Dewar is sufficient for the filling of two shipping Dewars and four cassettes with nitrogen left over (commercially the mainland cost of nitrogen for this is about US $100).
- Have safe handling equipment available; gloves, face shield and safety glasses as appropriate to your institutions liquid nitrogen handling policy. .
- Have two or more pouring Dewars ready and a suitable funnel to help fill them.
- Have a cassette (cryocane) Dewar ready. This is useful for washing the cassettes and brief storage when emptying the shipping Dewar of liquid nitrogen.

# Preparing for filling (continued)

- If the shipping Dewars show evidence of ice allow them to warm and dry before use. Sometimes ice can build up if the Dewars are left dry but cold for a prolonged period.
- Before filling make sure the shipping Dewars have been fully charged and have not lost vacuum. Follow the manufacturers instructions for filling.
- Keep them filled with liquid until ready for shipping.
- Make sure the handle on the Dewar insert is firmly attached and the Dewar insert can be easily removed and replaced in the Dewar. The handle can come loose, this is not a disaster but it is useful to know before ‘discovering it’ during filling.
- Keep a spare microscope bulb and know where to find it.
- Keep a check list and follow it and amend it as necessary.
- Have a small sieve for removing ice from the liquid nitrogen in the blue reservoir.

A phase separator is a useful piece of equipment to fit on the end of a transfer hose. It cuts down significantly on the gas rather than liquid that is transferred, only costs about $50 and seems to make our large Dewar last longer between refills.

Liquid should flow fairly rapidly. If not ice has formed inside the separator and it needs to be warmed and dried.

# General Preparation

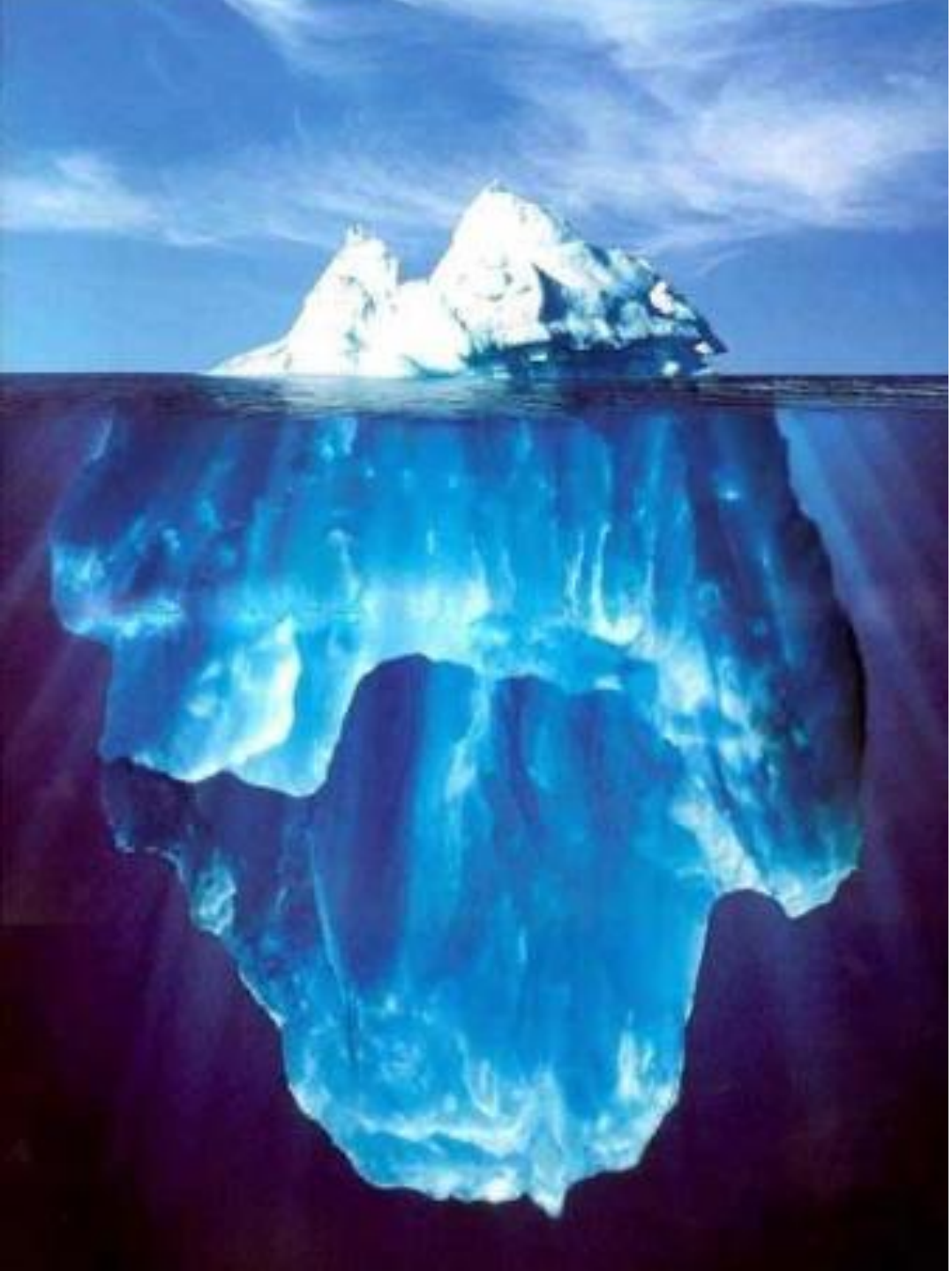
- Know where to find, read and understand the user guides.
  - These contain very useful practical tips and are updated regularly from comments received by users.
- Get details on the software available on the beamline and make sure you know how to use it. Print out the manuals and have them to hand.
- Make sue your remote access software works in advance and that you have an alternate plan if you should have network problems
- Know how to develop a good data collection strategy, how to integrate and scale the data. Be prepared to do this rapidly during screening and data collection.
- Know your crystal, screen it or others in the laboratory beforehand. If possible know the space groups and expected unit cells.
- Think about the sequence of experiments assuming that the experiment could end unexpectedly at any time (one advantage of robotic beamtime is that your sample can usually be saved for later if necessary).

# Preparing for filling

- Have control crystals available, *e.g.* cryoprotected lysozyme, for each cassette. These can be used to tell if something untoward happened to the Dewar during shipping, e.g. if it were opened or tipped and warmed a little.
- A dry Dewar will keep below 100K for over two weeks if unopened (but don't let it).
- If available fill in a fume hood to create an updraft of boiling nitrogen and reduce ice formation.
- Have a log book ready to note down crystal details and position.
- Decide on the order of filling and cassettes to use.
- If shipping an odd number of cassettes remember that the styrofoam insert is not trash! If you loose this ship an empty cassette on top of the full one.
- Practice dry and if possible under liquid nitrogen.
- Have the microscope next to the filling reservoir.
- Make sure the cassette or cassettes are empty and clean. Sometimes the loop holder can come out of the pin and is still lodged in the cassette. Filling this position will cause severe problems.

# Filling the cassette

(The first point where things can go wrong)



The number one practical tip:

Ice is the Enemy

**Filling should be done as rapidly as possible.**

# Filling – Ice is the Enemy, Liquid Nitrogen Boils off

- Note the puck or cassette number and order to be used.
- Make sure the shipping Dewar is still full of liquid nitrogen, top it off, keep it closed as much as possible.
- At all times assume the cassette could fall during transfer. Use cryogloves beneath it, not to hold it, but to catch it just in case.
- Each cassette contains 96 experiments. If several people are filling the result could only be as good as the worst skilled
- Make sure the nitrogen level is maintained.
- The blue lid covers the filling reservoir, it does not prevent boil off of the liquid nitrogen. If loading in a fume hood it is better not to use it.
- If floating ice appears put the cassette in the full shipping Dewar, empty and dry the reservoir and then replace the nitrogen.
- If in doubt, use fresh nitrogen.
- If frost appears on the cassette wash it by pouring liquid nitrogen over it.
- Do not leave for a prolonged period, nitrogen boils off fast.

# Filling (continued)

- Keep the pouring Dewar filled.
- Use good illumination.
- Make notes during the filling and transfer them to the spreadsheet as soon as possible. Even better, enter them as filling.
- Cover the strong magnet on the wand with tape to remind you not to use it.
- Use two tool sets if you have them. Dry one set as you use the other set. Swap over every 4 crystals or sooner if needed.
  - Ice on the end of the tool can cause the pin to stick to the tool.
  - Ice in the guide tool can cause the crystal to hit the cassette edge.
- If the pin will not dismount push it back in then angle both the wand and guide tool. The pin should remain in the cassette (see next slide).




The magnet head can sticks to the wand due to ice between the two. To overcome this push the magnet in with the wand following the guide tool, then tip both the wand and guide tool together. The magnet will remain in the cassette. New tools with a push release can also develop the same problem (with a similar solution).

# Filling (continued)

- Mount several samples of each crystal.
- If possible use a little bit more cryoprotectant than needed.
- Empty the shipping Dewar at the last moment before you ship.
- Make sure all the liquid is emptied (follow the manufacturers instructions, e.g. invert, stand upright for some time, invert again etc.) A little liquid left can cause an amazing paperwork mountain if discovered. Future shipments would be jeopardized not to mention possible civil and criminal penalties – in the US they'll send you to Cuba.
- Wash the cassettes in liquid nitrogen if necessary to remove ice and then put then in the shipping Dewar.
- Tape the Dewar lid and write "Contains sample" on the tape (advice from the FedEx guy). Anyone who opens the lid will immediately assume you are shipping prize racehorse or bull products and leave it alone.

# Shipping the cassette

(Another major point where things can go wrong)

# Shipping – Contact the shipping company first!

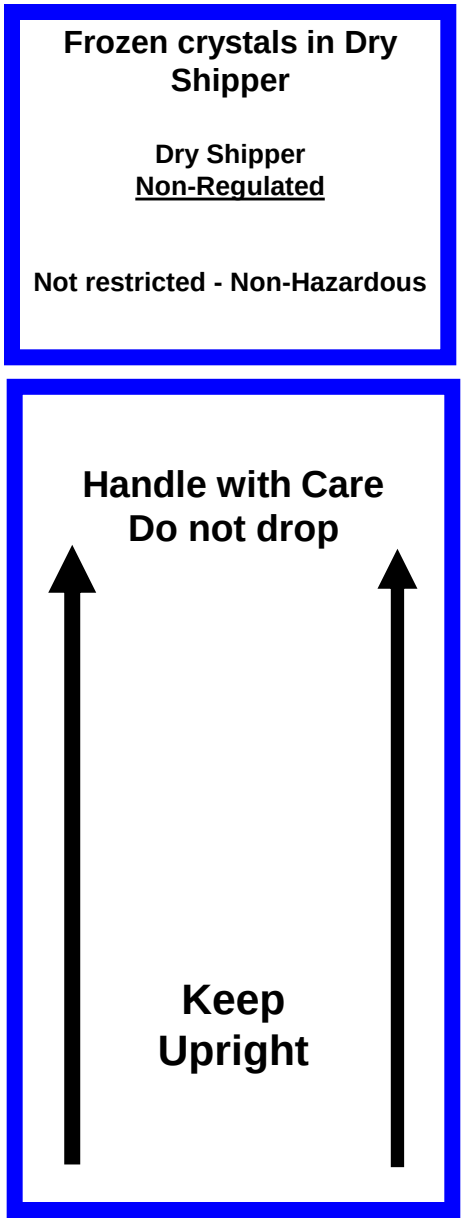
There are several ways to ship e.g.

- Overnight in a dry shipper (the usual method)
- Overnight in a Dewar or dry shipper with liquid nitrogen.

- Shipping liquid nitrogen, *i.e.* in a Dewar or with the dry shipper filled with liquid requires lots of paperwork and a training course in the US. Check with your shipper.


# Shipping Dry (magic words)

- To avoid delays in shipping your Dewar (shipping dry) should have a label with the magic words “Non-regulated”, “Not-restricted” and “Non-Hazardous”.
- Any label signifying liquid nitrogen should be removed if shipping dry (your Dewar will be returned to you if this is not removed).
- For shipping in liquid nitrogen the rules are very different and beyond the scope of this workshop – please ask me later if you are interested in these details.
- Ship to arrive at least a day in advance of the beamtime and arrange to have the Dewar filled on arrival. At SSRL note that shipments are not accepted over the weekend so if your beamtime starts Monday aim to have the Dewar arrive on the Friday.



# Shipping (continued)

- Track the shipment to ensure no surprises. First time Dewar shipments can be like a boomerang until local shipping office becomes used to the sight of those strange boxes.
- Label the Dewar with your name, similarly label the shipping box on the outside.
- Put the return shipping label inside the shipping box.
- Note: Use a control crystal or several for the first few times. This will validate your cryocooling and Dewar preparation (and is also nice when you have a series of poor diffracting samples and your lysozyme, xylose isomerase or ribonulcease sample comes up and diffracts beyond 1A).

# Avoiding Icing During Mounting

- One method that we use is to fill in a fume hood, fill rapidly and before inserting the cassette into the shipping Dewar wash the cassette with liquid nitrogen.
- Another method is to keep the cassette ports filled with dummy pins and only extract those pins before filling that port with a sample.
- A more complex but more useful technique is to pre-cool samples and store them under nitrogen using a cryo vial. These can then be transferred to the cassette rapidly on the day of filling. This is my preferred method of mounting as I can record a picture of the crystal cooled and check to see if ice buildup has happened as a result of shipping.

# Planning

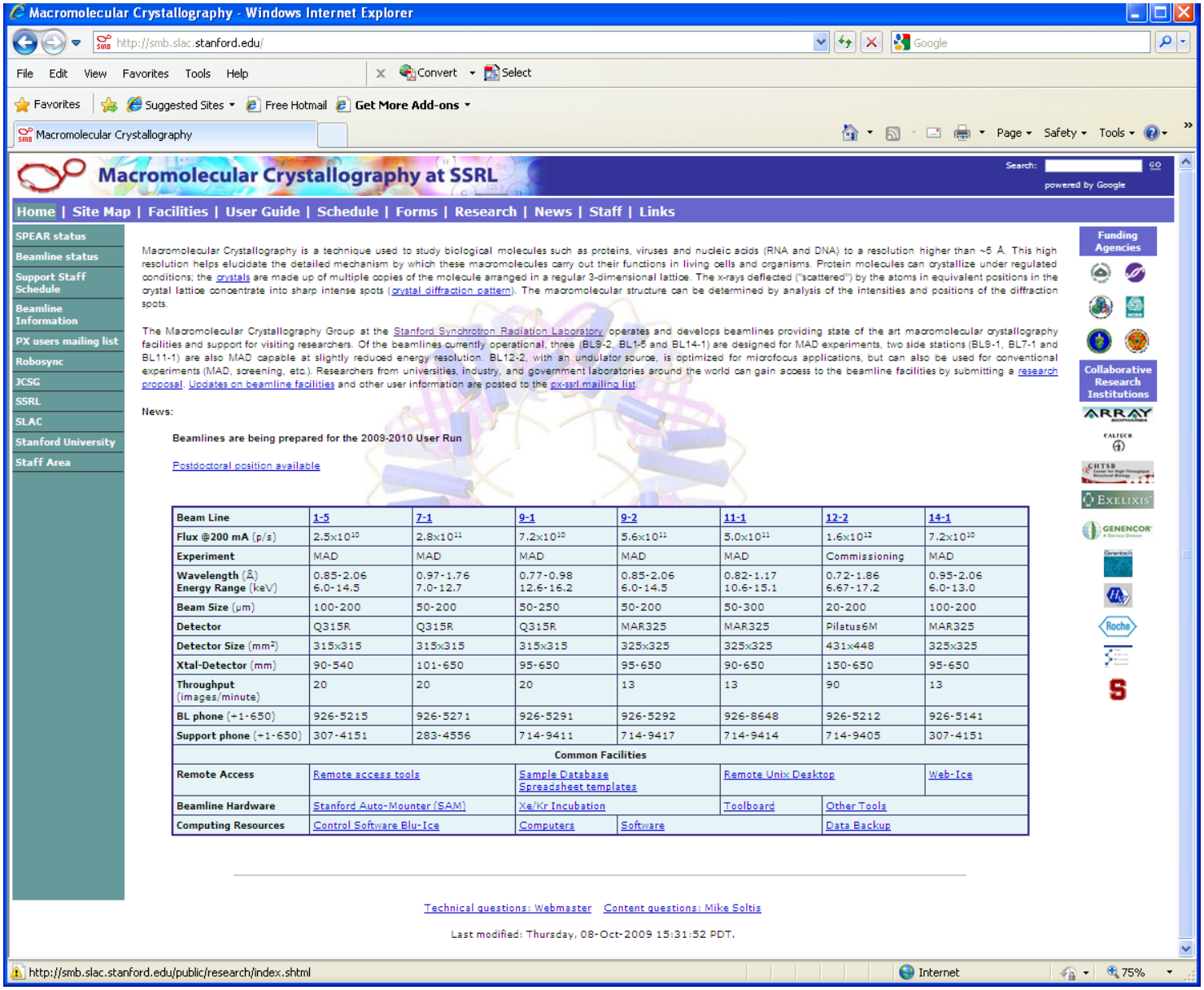
(This will help make your experiment efficient)

# Planning – Communication

- Contact the support staff before the run, let them know how many cassettes are planned.
- Share all the contact details of the team with each other and the support staff. Try and have one or at most two numbers where the person collecting data can be contacted at all times.
- Get the cell phone number for the support staff (tip, look on the website at SSRL using the browser available in the remote software).
- During available hours, 9:00 am – 9:00 pm California, don't be afraid to contact the support staff. Check if they will be taking calls outside these hours and if not, do not call.
- If you fail to contact your staff support look on the schedule to see who else is on support and try them next … if it is urgent.
- Have a good communication plan within the team collecting data, especially if many different locations are being used. Instant messenger has worked quite well but a chat window available in Blu-Ice would be lovely ☺

# At the synchrotron

(Setting up at home, screening and data collection)




| Beam Line | 1-5 | 7-1 | 9-1 | 9-2 | 11-1 | 12-2 | 14-1 |
|---|---|---|---|---|---|---|---|
| Flux @200 mA (p/s) | $2.5x10^{10}$ | $2.8x10^{11}$ | $7.2x10^{10}$ | $5.6x10^{11}$ | $5.0x10^{11}$ | $1.6x10^{12}$ | $7.2x10^{10}$ |
| Experiment | MAD | MAD | MAD | MAD | MAD | Commissioning | MAD |
| Wavelength (Å)<br>Energy Range (keV) | 0.85-2.06<br>6.0-14.5 | 0.97-1.76<br>7.0-12.7 | 0.77-0.98<br>12.6-16.2 | 0.85-2.06<br>6.0-14.5 | 0.82-1.17<br>10.6-15.1 | 0.72-1.86<br>6.67-17.2 | 0.95-2.06<br>6.0-13.0 |
| Beam Size (µm) | 100-200 | 50-200 | 50-250 | 50-200 | 50-300 | 20-200 | 100-200 |
| Detector | Q315R | Q315R | Q315R | MAR325 | MAR325 | Pilatus6M | MAR325 |
| Detector Size ($mm^2$) | 315x315 | 315x315 | 315x315 | 325x325 | 325x325 | 431x448 | 325x325 |
| Xtal-Detector (mm) | 90-540 | 101-650 | 95-650 | 95-650 | 90-650 | 150-650 | 95-650 |
| Throughput (images/minute) | 20 | 20 | 20 | 13 | 13 | 90 | 13 |
| BL phone (+1-650) | 926-5215 | 926-5271 | 926-5291 | 926-5292 | 926-8648 | 926-5212 | 926-5141 |
| Support phone (+1-650) | 307-4151 | 283-4556 | 714-9411 | 714-9417 | 714-9414 | 714-9405 | 307-4151 |
| Common Facilities | | | | | | | |
| Remote Access | Remote access tools | | Sample Database<br>Spreadsheet templates | | Remote Unix Desktop | | Web-Ice |
| Beamline Hardware | Stanford Auto-Mounter (SAM) | | Xe/Kr Incubation | | Toolboard | Other Tools | |
| Computing Resources | Control Software Blu-Ice | | Computers | Software | | Data Backup | |

Know your beamline

http:\\smb.slac.stanford.edu

# Setting up at home

- It is possible to efficiently run the screening and data collection from a single computer but it is much easier to use two or three.
- One computer should be dedicated to the Blu-Ice control software. The other can be used for data processing, structural solution, refinement, web surfing etc.
- If using a computer with a small screen or low resolution the effective screen size needs to be increased so that software such as HKL2000 will run.
- Different session names should be used. If you have multiple users it is good to incorporate their name into the session name. That way you do not accidentally terminate their session instead of your own.
- If you loose connectivity the current process will not stop. Log in again and hit passive to gain control of the software.
- You cannot log into Blu-Ice until the support staff enable you and your beamtime.
- However, you can log into the “SSRL” computers and create a directory for the data collection.


Blu-Ice for data collection with HKL2000 processing
Web-Ice and beamline video system
Structural data processing (CCP4)
Strong Coffee
Phone to call staff

# Screening

- Make sure your spreadsheet is uploaded and the appropriate directory is set for screening.
- One person needs to monitor screening throughout – failure of auto centering can stop the screening, crystals may be shot out of the beam etc.
- Scoring is fairly accurate however it should be checked for choosing the top samples to study:
  - Bad crystals may not be too bad. Icing can produce bad statistics but washing or even returning the crystal and remounting it effectively removes small amounts of surface icing.
  - Good crystals can be bad. Ice crystals can mislead scoring.
- The screening images can be integrated and a strategy determined however if the crystal is returned to the Dewar and remounted the crystal position often changes. Strategy will have to be run again.
- If it is a critical experiment collect data as soon as the screening shows a good crystal (see tips in data collection).

# Screening – Keep it Simple

- Use good names for data directories. Process in a separate directory with a related name. Write these down in a logbook.
- Use the video cameras to troubleshoot – check the ion chambers, that the phi axis is correct and matches the software setting. Make sure the beam is coming through, *i.e.* no attenuation etc. then call the support staff. If out of hours call a more experienced person in the lab to have a look. If this does not do the trick go home and get some sleep and come back when the support staff are on call again.
- Generate a plan for data collection priority as screening progresses
- Each sample takes about ~40 seconds (for a few seconds exposure time and two images). The largest part of this is mounting and dismounting.
- *It is quicker to center manually if you can keep up the pace and are ready to do this immediately after mounting.*
- Make sure you know what is happening and keep stuff happening, It is easy to be whiling away the time while screening has stopped for any number of reasons. Make notes on diffraction that looks good, compare with the automatic scoring routine.

# Data Collection

- Follow a plan and know how to process the data before you collect it – practice with other data sets.
- Check the directory before collecting into it. Check the directory again, check you have the correct crystal, check the crystal and the directory!
- Plan on having rested before data collection. On a long run the person screening should get some sleep before starting data collection.
- Don’t be greedy with resolution if collecting a MAD data set.
- Collect in dose mode but if you change beam size, dose needs renormalizing.
- Use a low (possibly medium) and high-resolution pass in that order if the data needs it. Low resolution can use a much shorter exposure time than high and can also use a wider oscillation range. Use about 20% resolution overlap depending on the number of reflections (a sufficient number must be common to scale the sets).
- Understand how to use distance, wavelength, oscillation and offset and the consequences in terms of data completeness where appropriate. Never think of the detector as square but use it as a round one (with no offset). Know the spectrum of the beamline and how to use it most effectively.

# Data Collection (continued)

- For MAD data save the MAD scan data. Note down the wavelengths, and f’ and f’’. Know the sequence, molecular weight and number of sites expected. Keep careful notes – this will make structural solution much easier!
- Excitiation scans are always useful for identifying metals.
- Know how to make a good data strategy and the differences between an anomalous and a completeness strategy.
- Use a strategy program, e.g. Best from Webice. Choose the appropriate starting point but collect 180 degrees of data if possible.
- Collect complete data sets, if more time is available collect even more data.
- Process your data as soon as it is collected even if the processing is a quick and dirty job. Make full use of the scripts available (have 2 people working during data collection). If cannot be immediately processed resolve the problem with the help of the support staff.
- There is no advantage to having the detector any closer than the edge of the diffraction. However the edge is difficult to determine by eye.
- If several people are collecting have a good communication plan.
- Let the support staff know if you are going to finish early.

# Being polite
# (several projects during the same beamtime)

- If there are several projects collecting data remember that any waste of time by you eats into time that could be used productively by someone else.
- Don’t waste time and have a plan beforehand on the priority of samples.
- Let others know the approximate time you will finish.
- Keep an eye on the data collection and process continuously so that any problems can be identified early on.
- If this is your critical experiment, only when you are happy should you remove that crystal (and take a lower priority next time).
- Those who send salt crystals shall be shot ☺

# Finishing up

(Data transfer, sample return, pin cleaning etc.)

# Transferring data

- It is best to process on the SSRL computers and ship the integrated intensities and log files back. The data will follow assuming you have requested it.
- It will take a few weeks for CD/DVD's to be received from the experimental run.
- Use descriptive names for the CD/DVD's. You, hopefully, will end up with a lot of them.

# Choices to be made – sample return

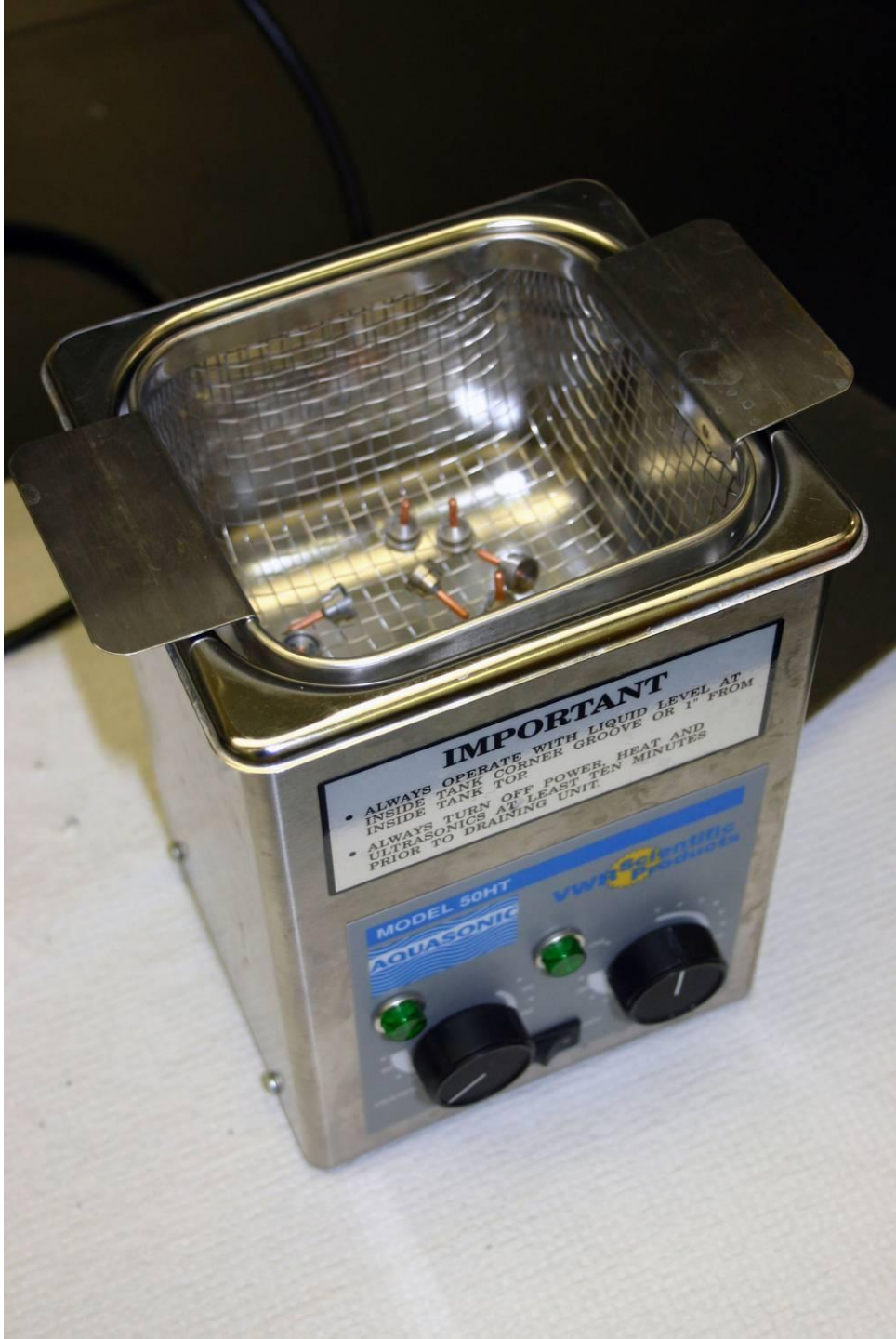
- Cassettes or pucks can be left at SSRL. Remember this when collecting data. It may be better to spend more time collecting the best data set from one sample than several marginal datasets from another ….
- But, the more manipulation of the crystal that takes place the more chances to lose it!

# After the run

- Make notes of any problems and suggestions. Send them in the end of run report.
- Contact the support staff directly if you wish (email is best).
- A lot of effort goes into making the robotic system work well and ensuring the resulting data is the best possible -
  - acknowledge the support staff who helped out
  - reference appropriate publications about the robotic system and Blu-Ice
  - acknowledge SSRL or any synchrotron you use.
  - Get to know the habits of your support staff. When you see them encourage those habits, e.g. buy a beamline scientist a beer (or anything chocolate for particular individuals), say hello at meetings etc.
- Publish the paper, acknowledge the developments at SSRL that made it possible.
- Look forward to new developments.
- Tell others!

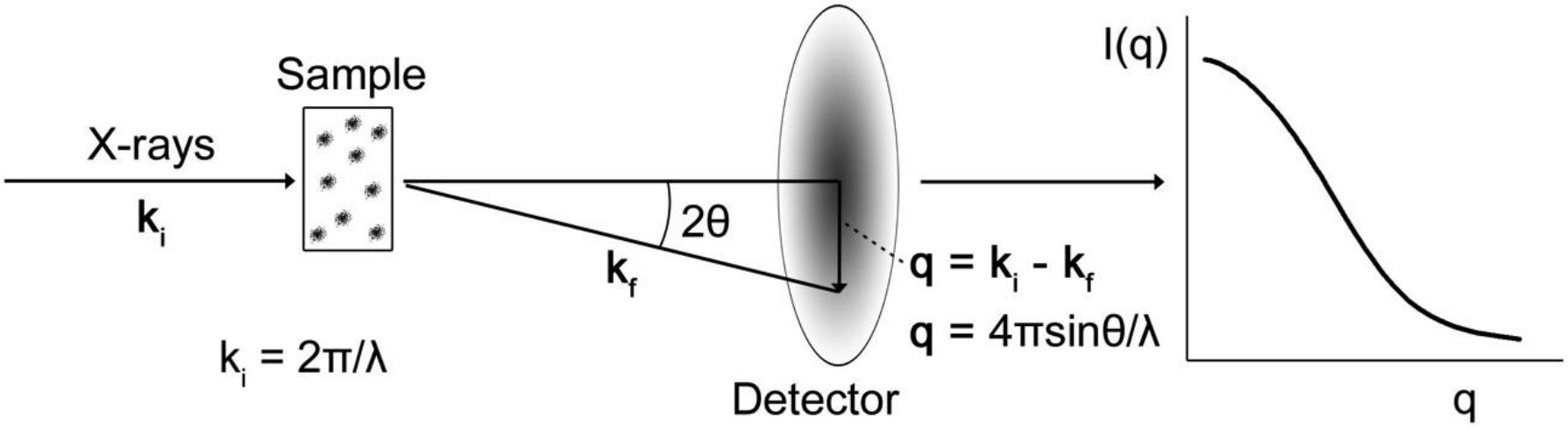
# Cleaning pins

- Pins and loops can be easily cleaned using a sonicator bath.
- We use a water wash followed by a 30% ethanol wash.
- The pins and loops are then dried and examined.
- Broken loops are pulled out of the pin and replaced with new loops.
- Loops still dirty are washed again.
- Best results are achieved using only a single layer of pins.
- About 80% of loops are reusable.



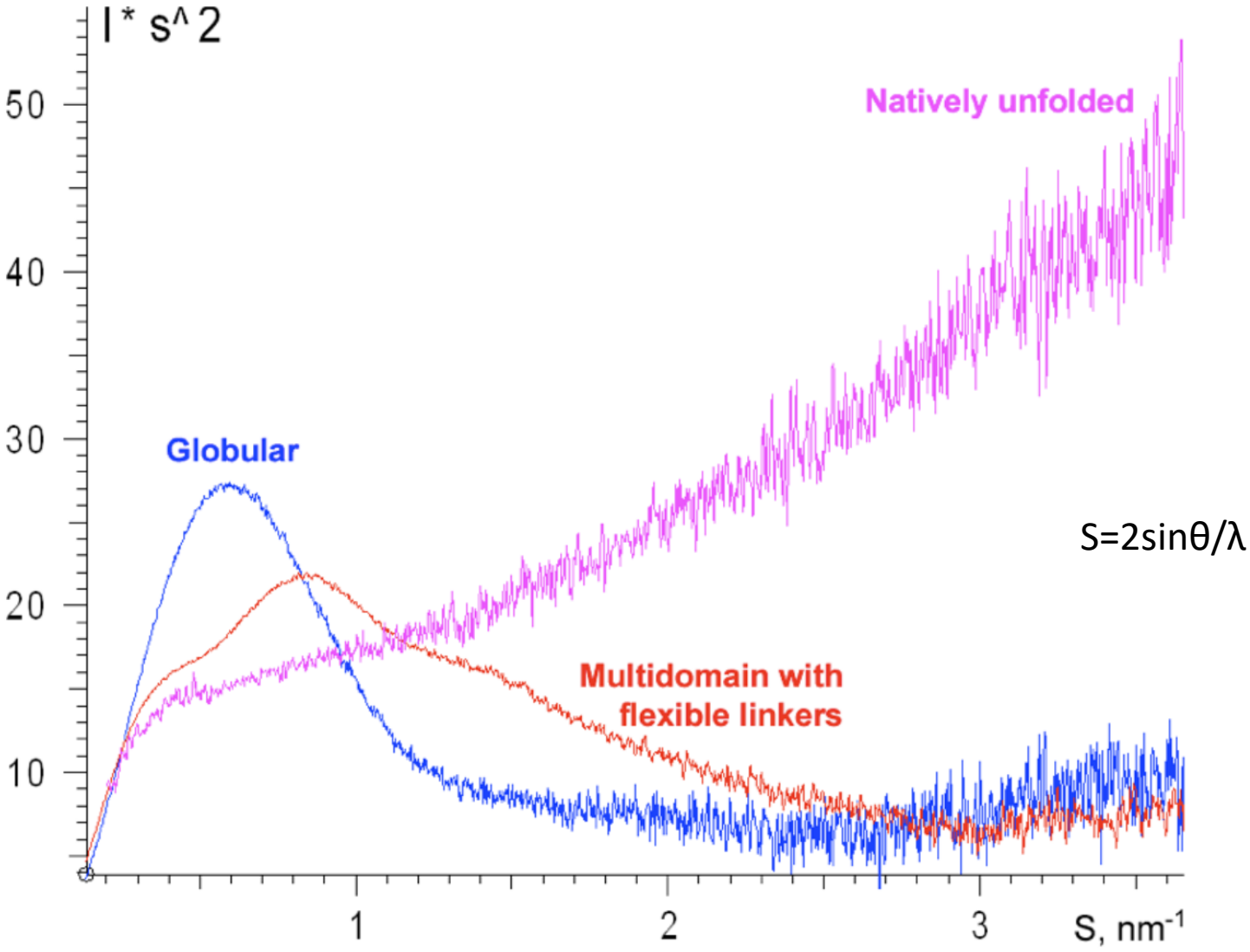
No crystal, no diffraction …

# SAXS images everything behind the beamstop




- Particles in solution tumble – spherically averaged intensity is recorded.
- Radial integration results in one dimensional SAXS profile.
- Larger particles scatter at smaller angles.
- Analysis of the 1D profile  yields information about size and shape.

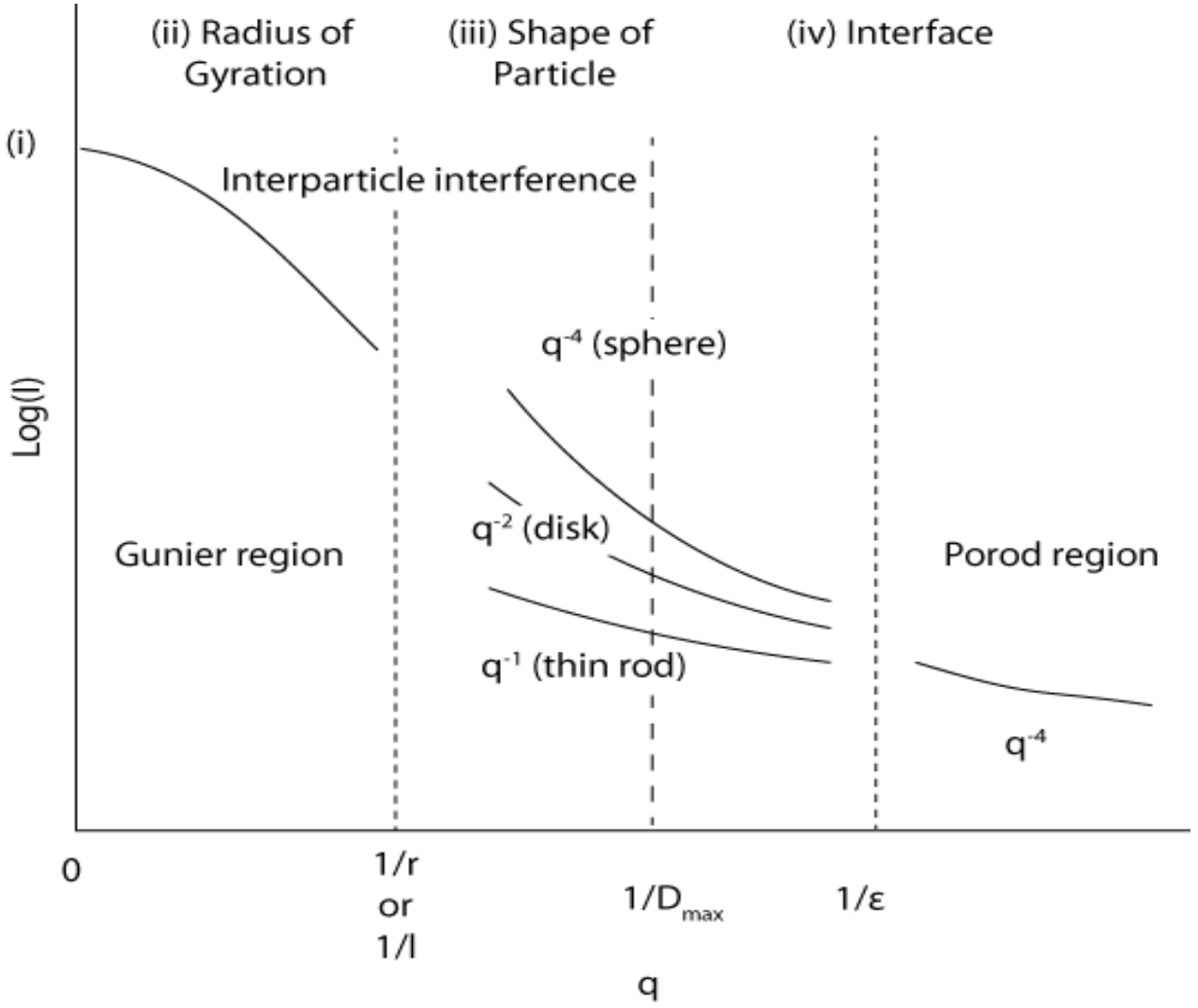
# Kratky analysis reveals dynamics



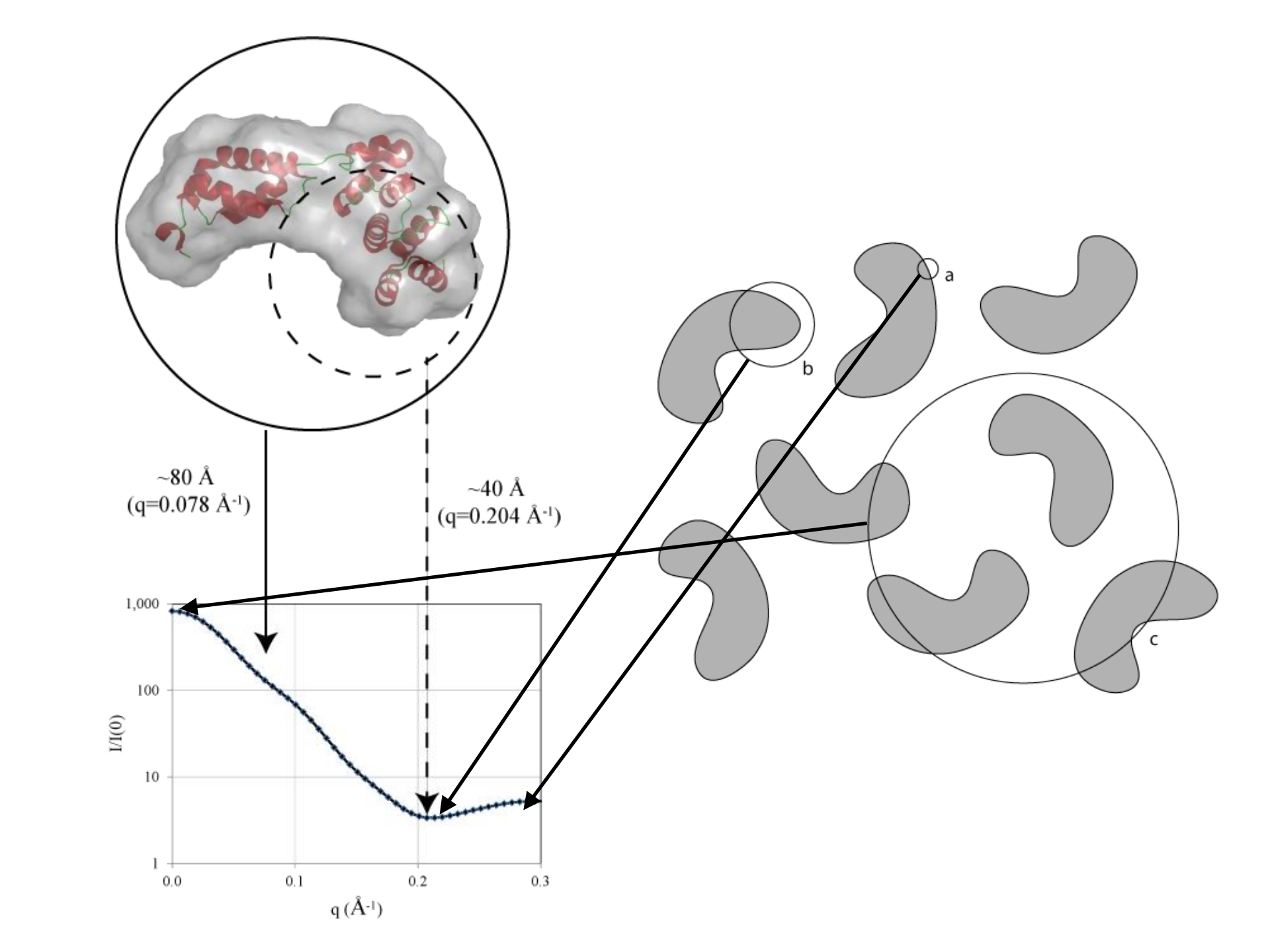

S=2sinθ/λ

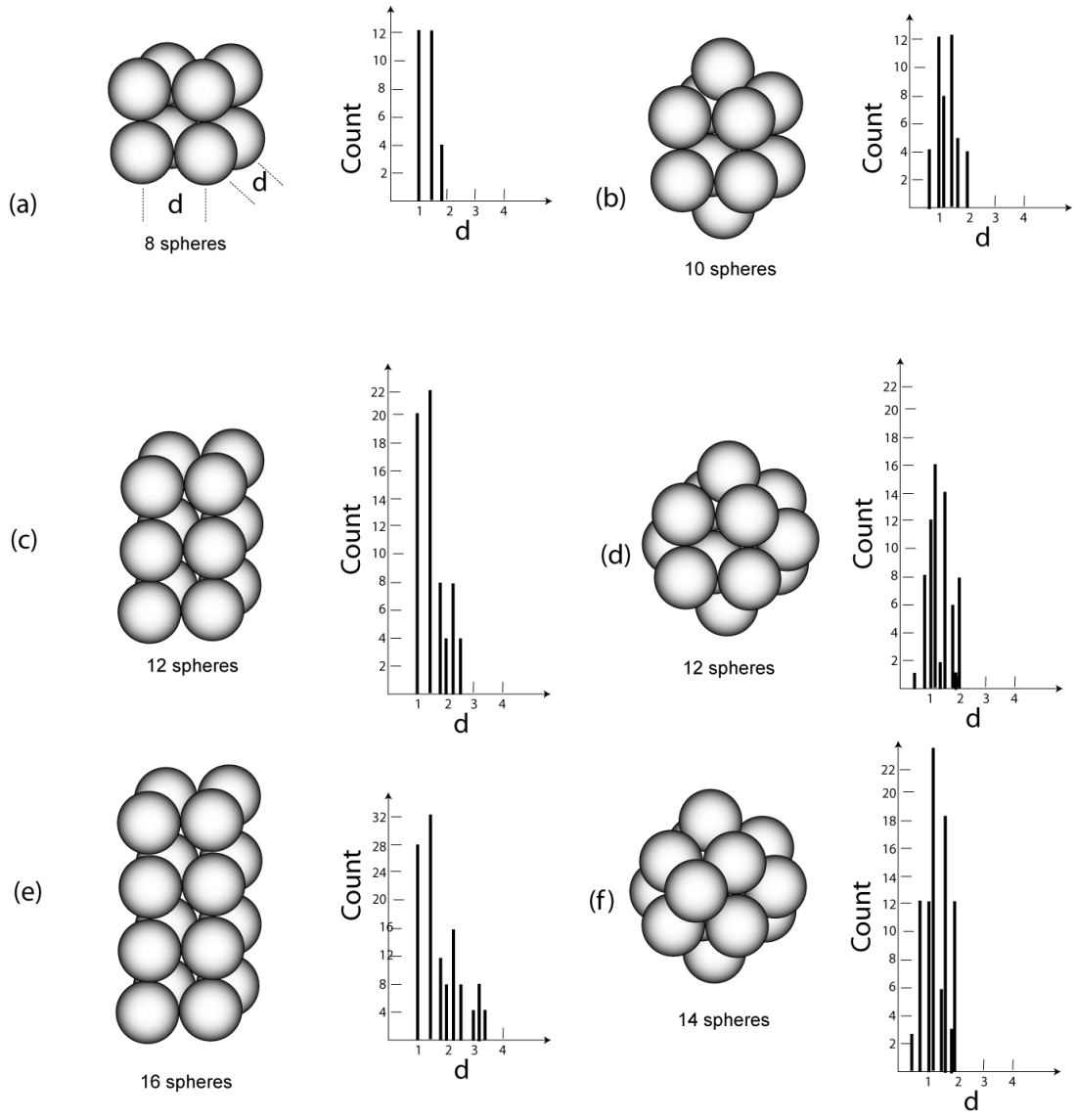
http://www.saxier.org/forum/viewtopic.php?t=337

The Shape of the Scattering Curve is important but not the absolute intensity




Only concentration information is contained in the intensity values


~80 Å
(q=0.078 Å-1)
~40 Å
(q=0.204 Å-1)
a
b
c
1,000
100
10
1
I/I(0)
0.0
0.1
0.2
0.3
q (Å-1)

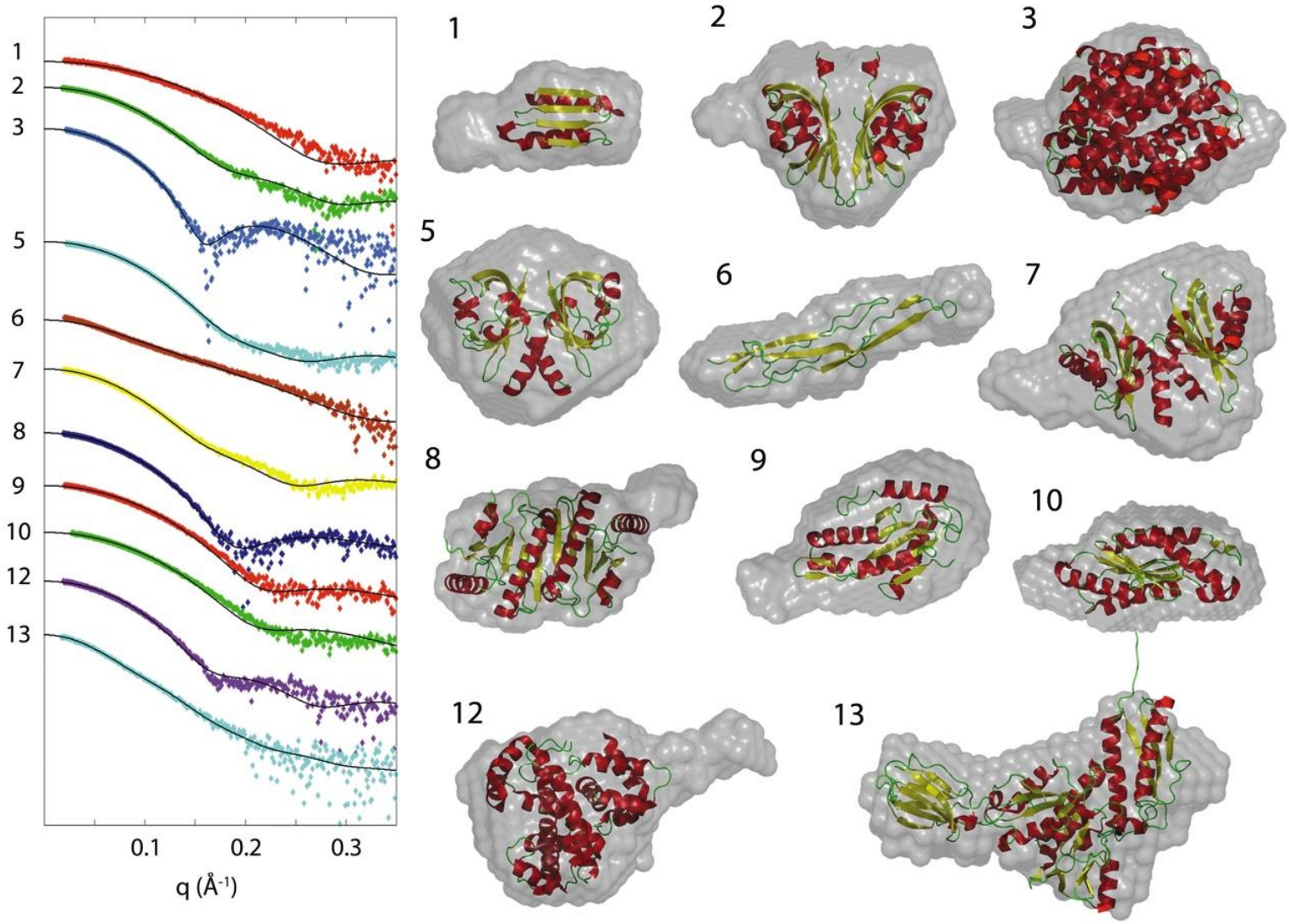



Fourier space yields frequency of interatomic scattering vectors as a function of the length of the vector

P(r) {**Pair distribution function**) plot is simply the histogram of interatomic scattering

Larger compact molecules have a high distribution at lower angle (consider detector distance etc.)

# Comparing X-ray structures



# Thank you and questions?

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