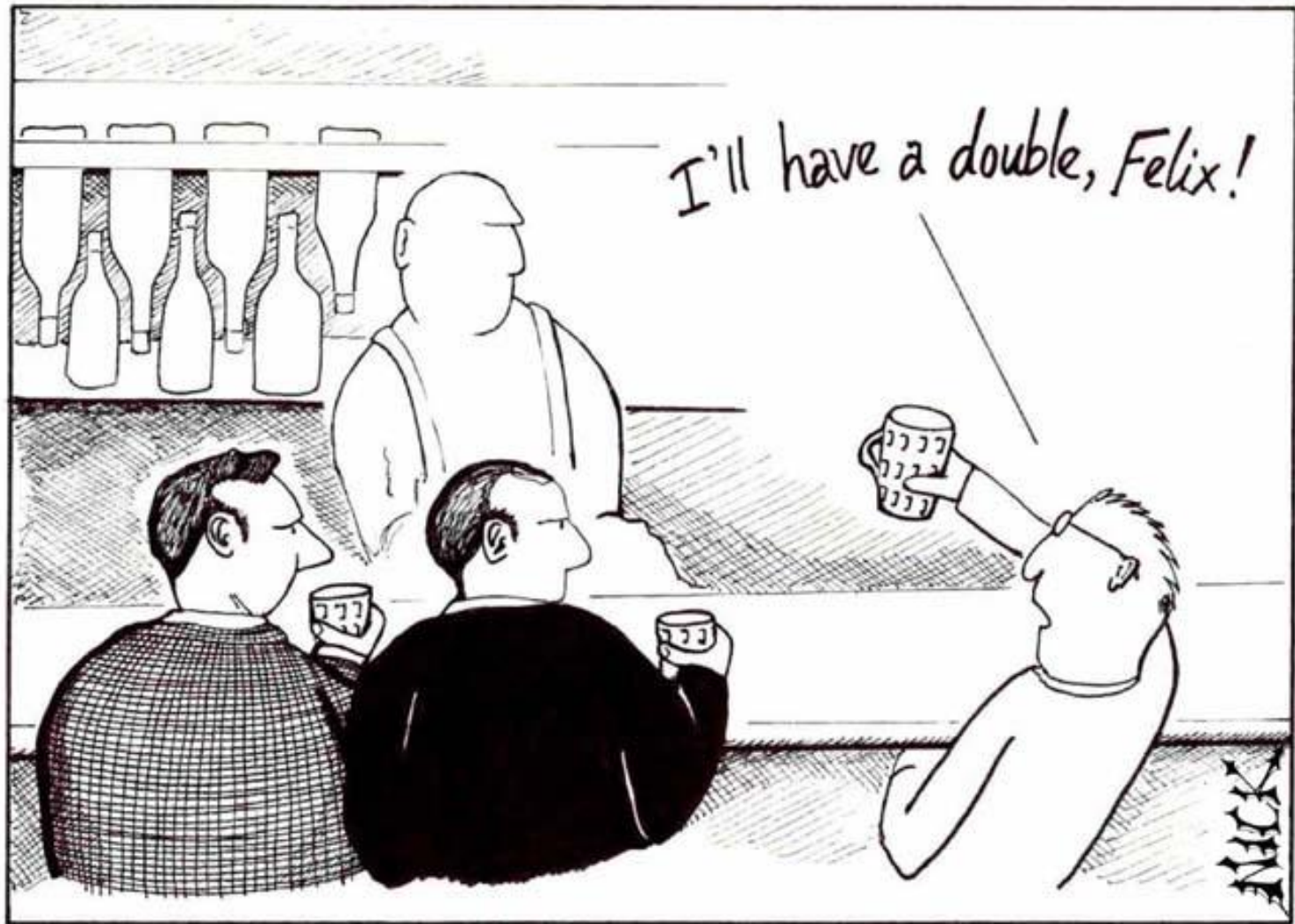


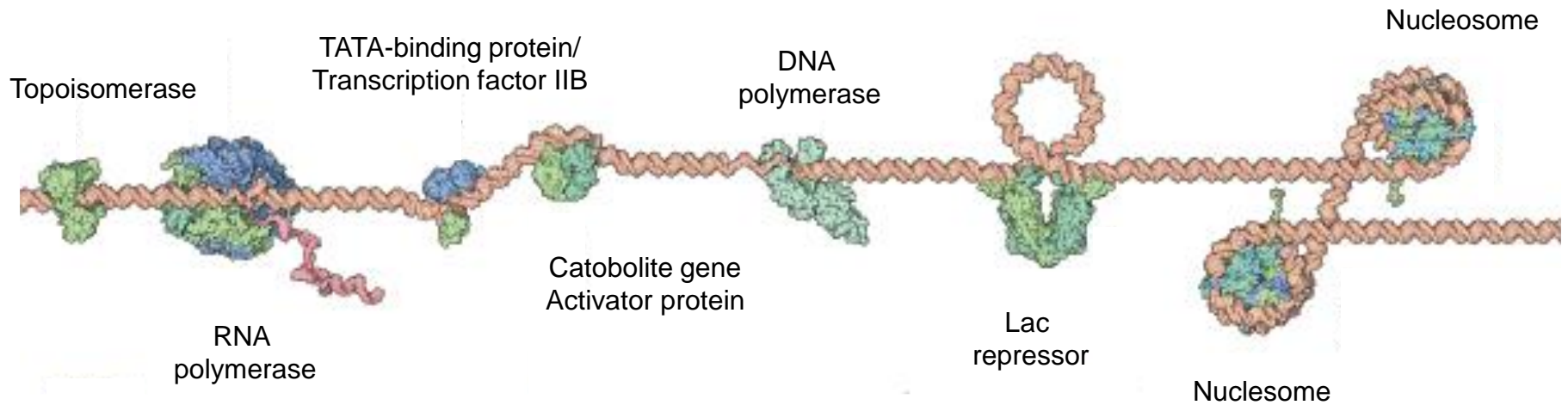
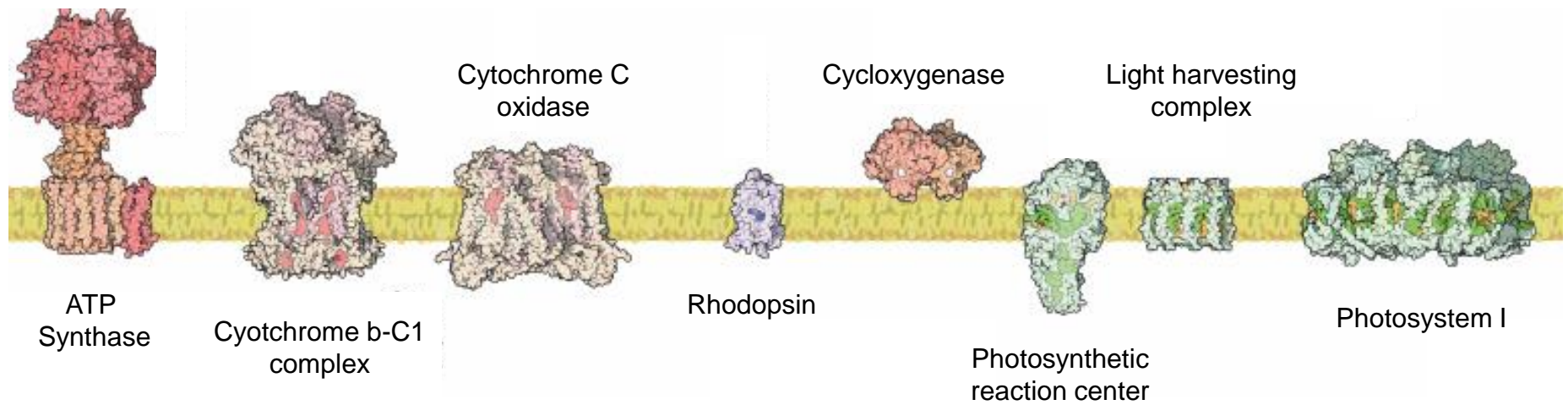
‘Where to go next and not loose ‘all’ your hair’



Edward Snell



Cambridge, 1953. Shortly before discovering the structure of DNA, Watson and Crick, depressed by their lack of progress, visit the local pub.



Form (or structure) gives a **clue** to the function.



SR-71



Space shuttle



B52



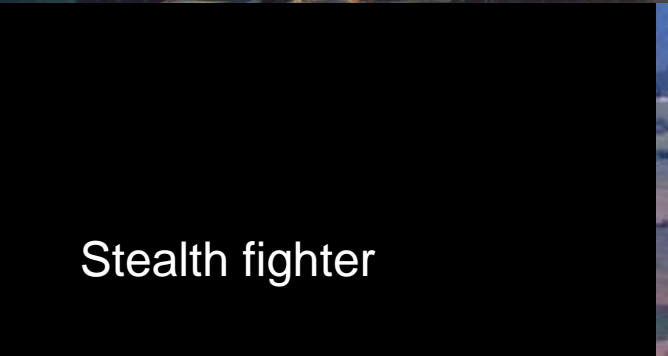
Biplane



Hot air balloon



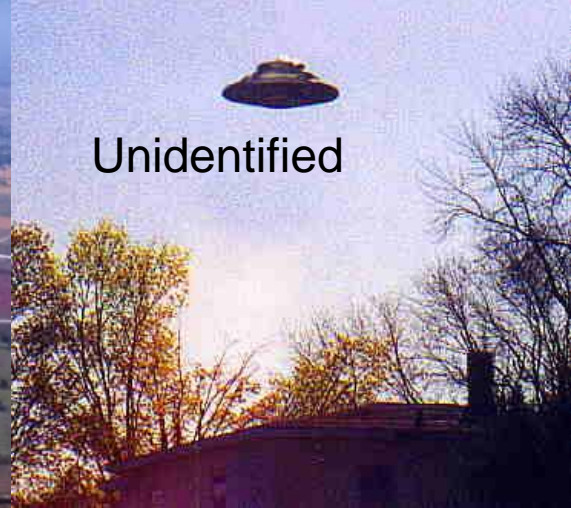
Hang-glider



Stealth fighter



Spitfire



Unidentified



Sleek,  
very mobile?



Only flies  
vertically?



Excretes numerous droppings



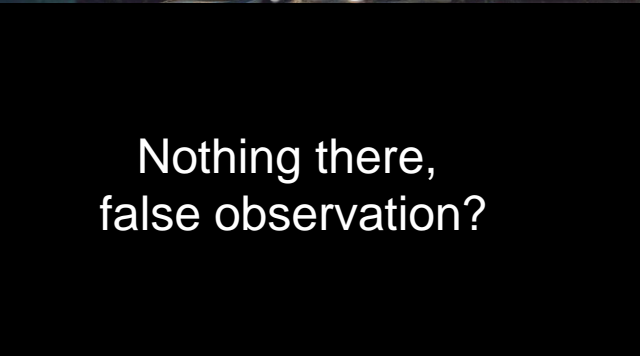
Two wings, must fly  
really high?



Needs others for  
reproduction



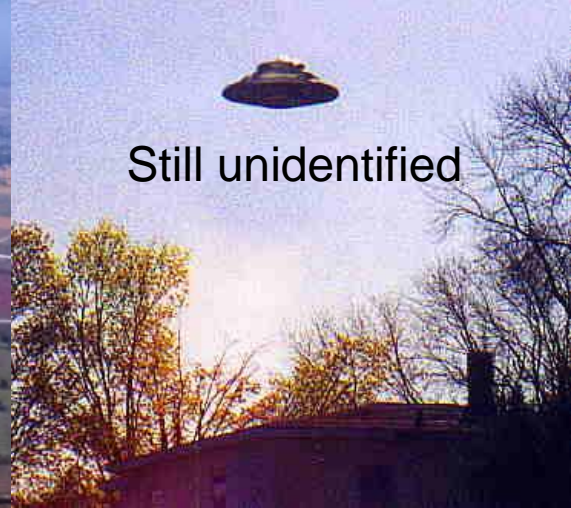
Symbiotic relationship?



Nothing there,  
false observation?



False eyes to scare predators



Still unidentified

Crystallographic information provides an average picture of the macromolecule

Biological information is also needed to explain that picture.

# The research proposal

Aim 1.

Aim 2.





**80% of crystallization  
is failure**



## Failure ...

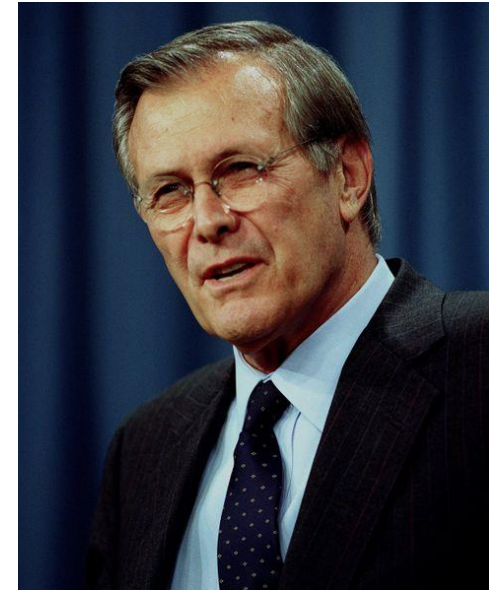
- I have not failed. I've just found 10,000 ways that won't work – Thomas Edison
- Failure is instructive. The person who really thinks learns quite as much from his failures as from his successes – John Dewey
- A life spent making mistakes is not only more honorable but more useful than a life spent in doing nothing – George Bernhard Shaw.
- Genius is one percent inspiration and ninety-nine percent perspiration – Thomas Edison.
- **Crystallization is one percent inspiration and ninety-nine percent optimization – Unknown crystal grower.**
- **Rule 2 – Be an optimist.**
- Garbage In, Garbage Out – From a syndicated article about the first stages of computerisation of the US Internal Revenue Service that appeared in several US newspapers on 1 April 1963.

# The Essence of Crystallization

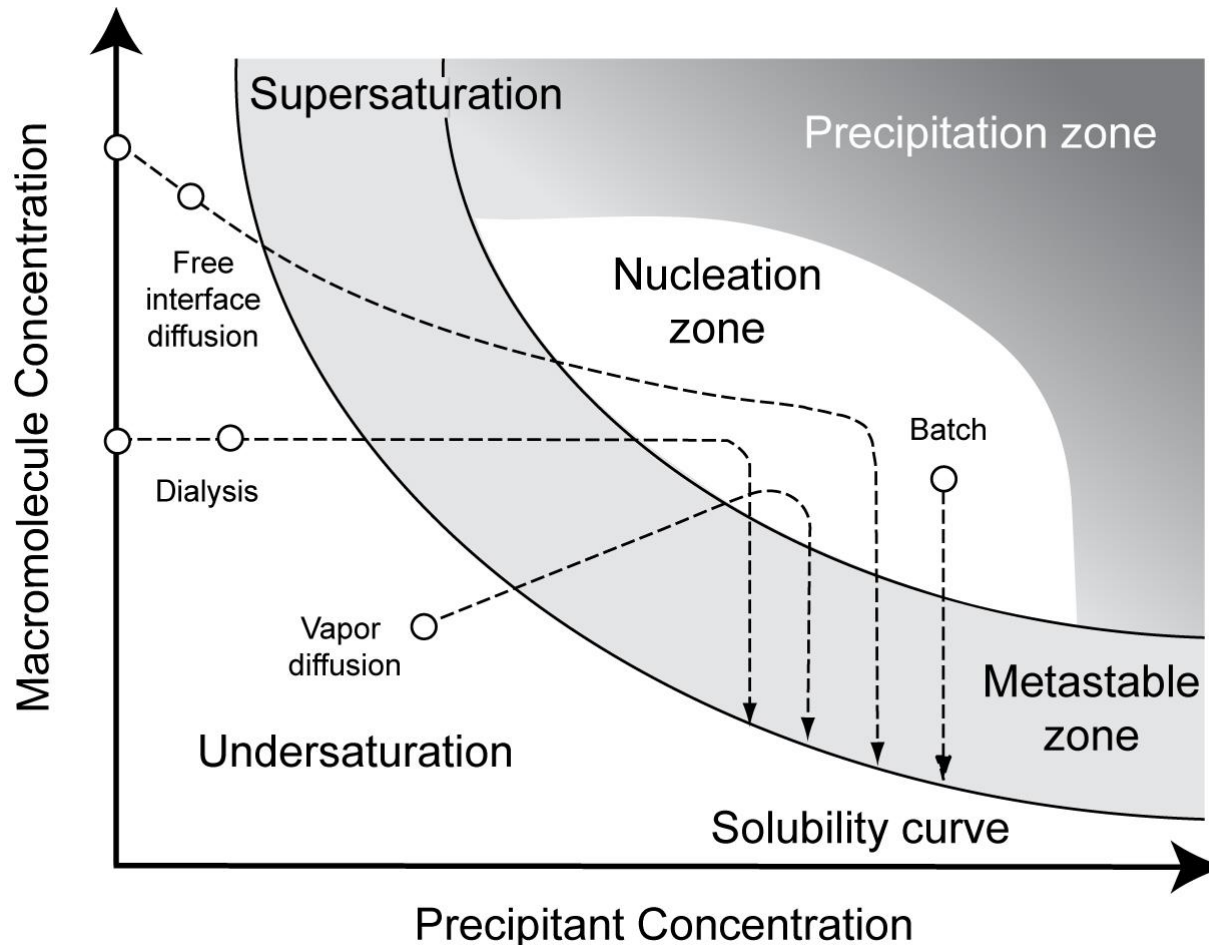
- There are known knowns. These are things we know that we know. There are known unknowns. That is to say, there are things that we know we don't know. But there are also unknown unknowns. There are things we don't know we don't know.

- Donald Rumsfeld, Feb 12<sup>th</sup> 2002.

- **Rule 3 – Write everything down.**



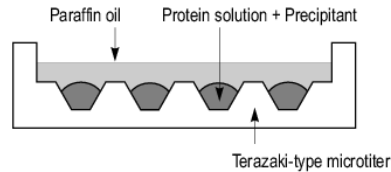
# Simplified phase diagram for crystallization



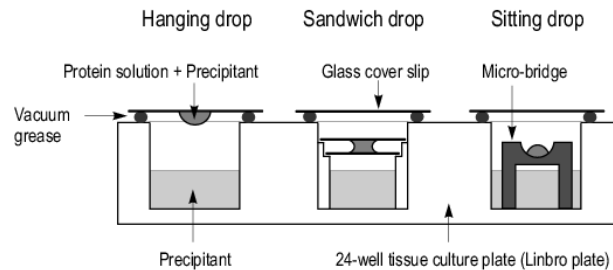
- **Rule 4 – Know this diagram by heart.**

# Crystallizing Macromolecules

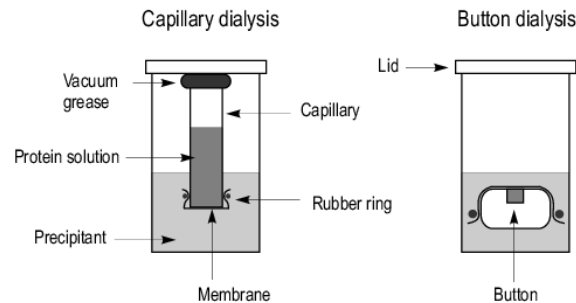
## a) Microbatch crystallisation technique



## b) Vapour-diffusion techniques



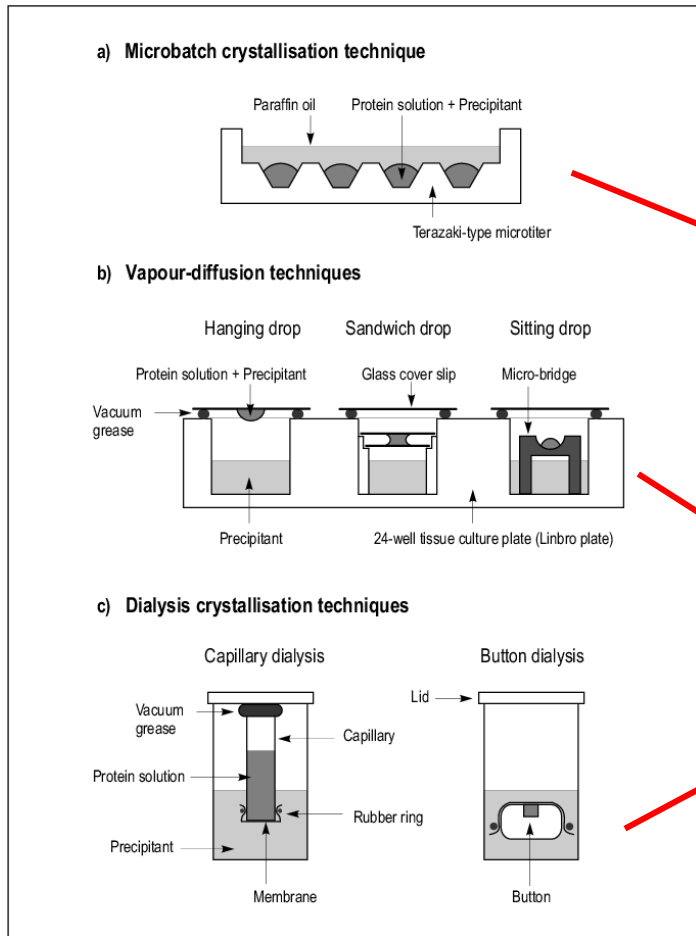
## c) Dialysis crystallisation techniques



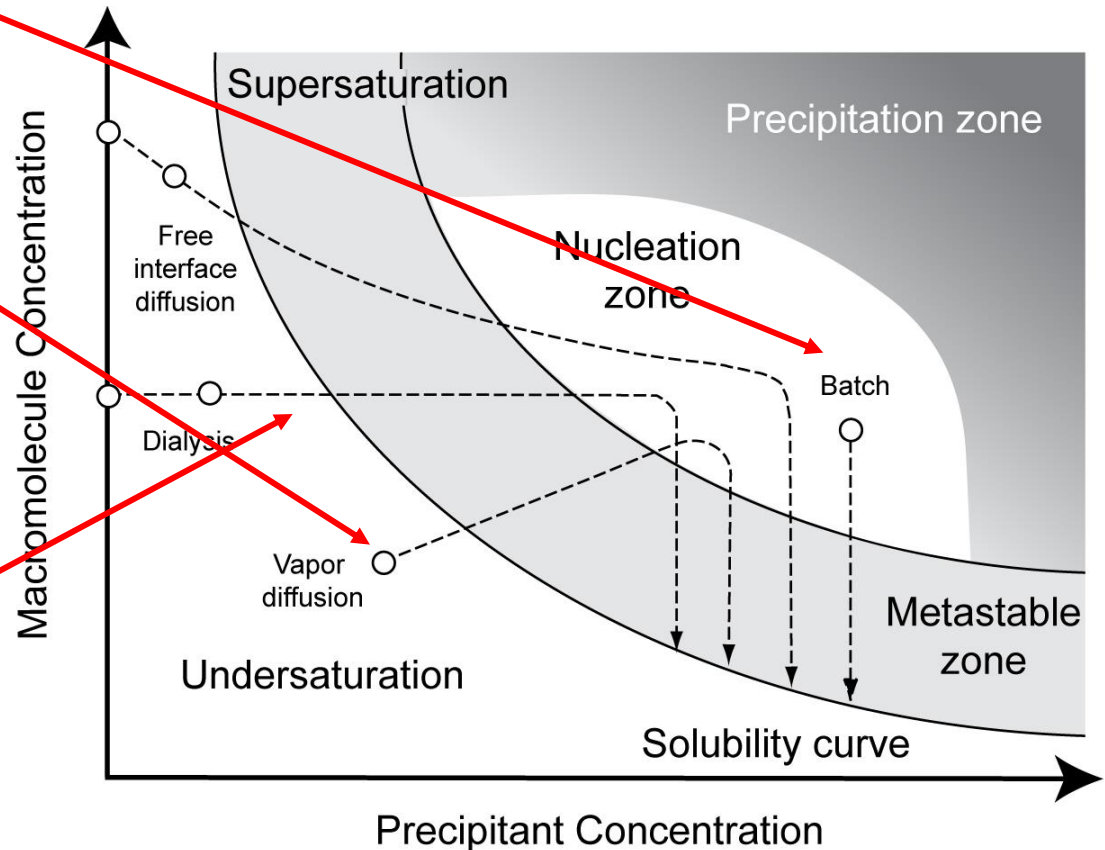
Many different methods but they all have things in common:

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

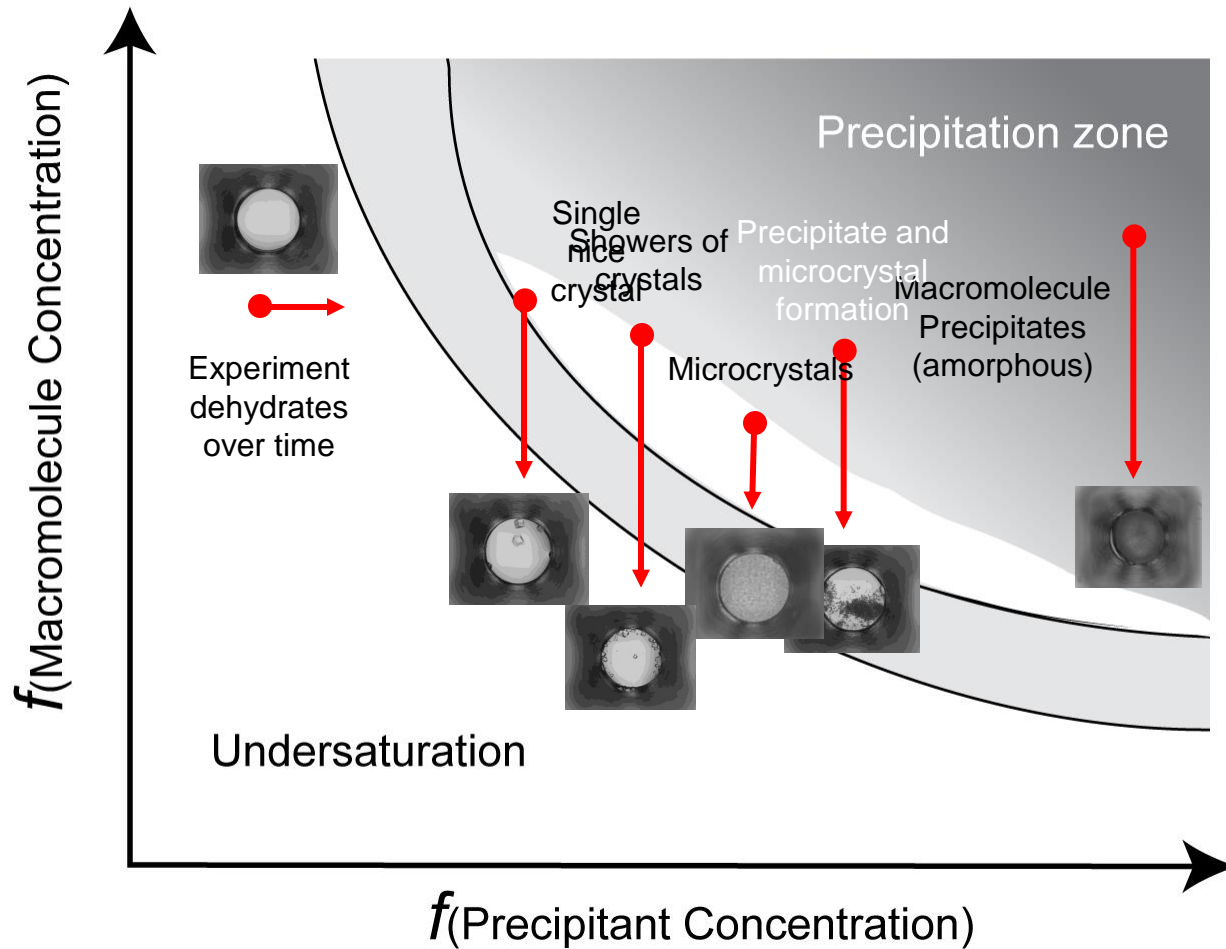
# Simplified phase diagram for crystallization



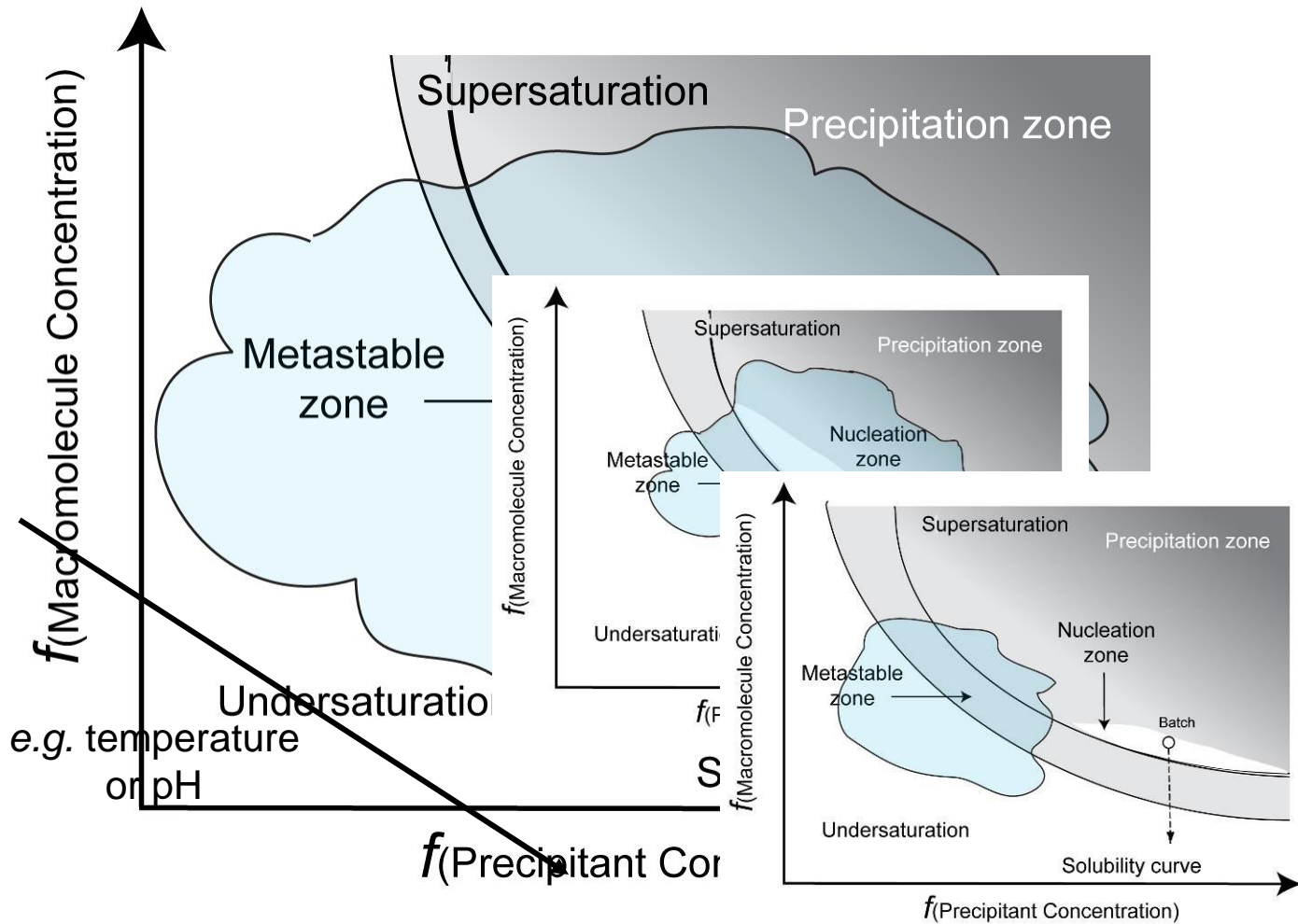
Different techniques traverse the crystallization space differently.



# What results can we expect to see?



# Typical situation, multidimensional area sampled



# The HWI crystallization cocktail screen.

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Highly soluble salt, cation and anion screen																																																																					
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Sodium Chloride										Sulfate										Carbonate										Chloride										Nitrate										Phosphate										Zinc									

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 Matrix Screen, Quick Screen, Nucleic Acid Screen, Sodium Malonate Grid, Ammonium Sulfate Grid, Sodium Chloride Grid, HT Screen, Index and the SaltRx screen.



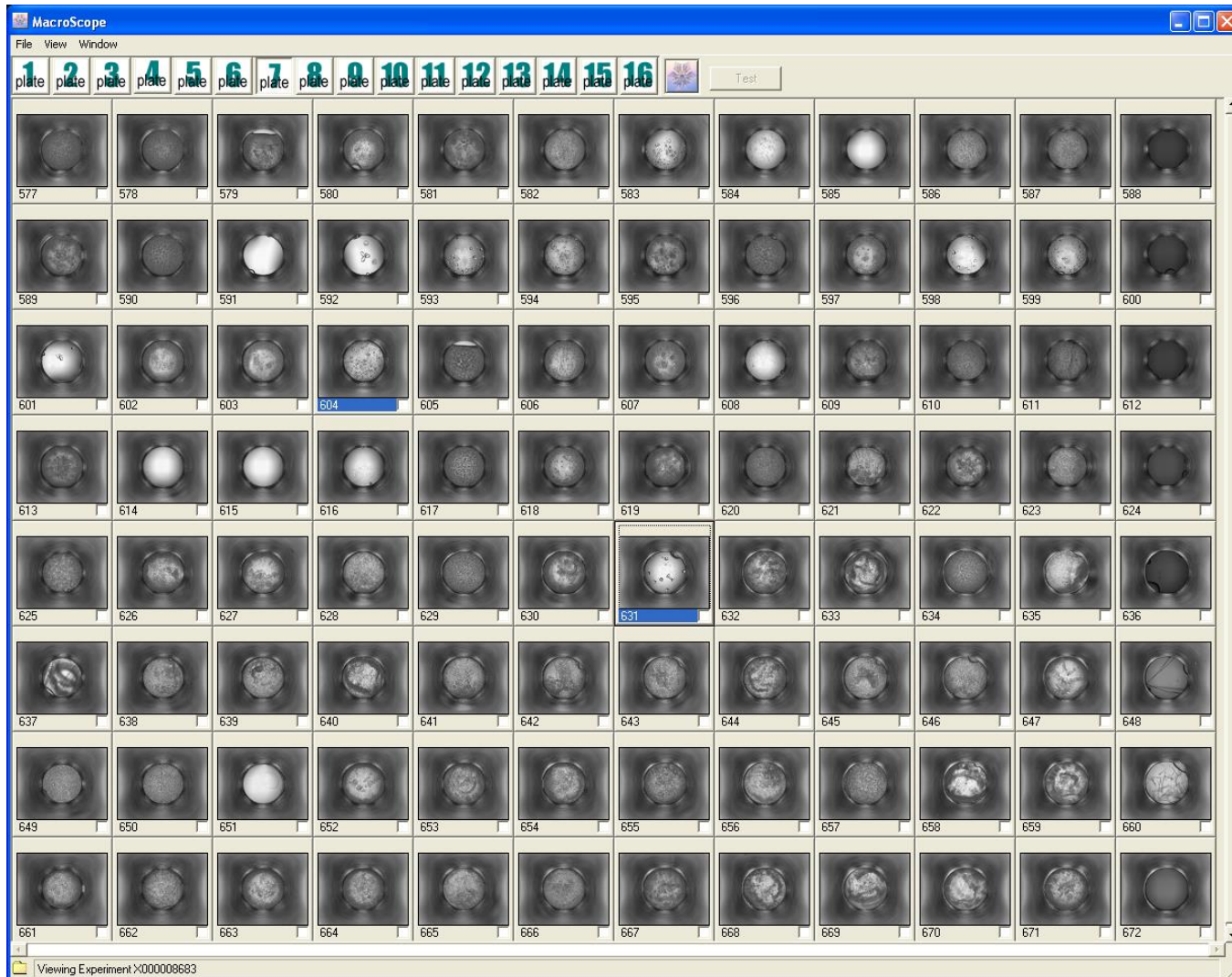
# The HWI crystallization cocktail screen.

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Sodium										Zinc										Potassium										Cobalt										Lithium										Potassium										Magnesium										Sulfate									

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 Matrix Screen, Quick Screen, Nucleic Acid Screen, Sodium Malonate Grid, Ammonium Sulfate Grid, Sodium Chloride Grid, HT Screen, Index and the SaltRx screen.

# What do we see from the data?

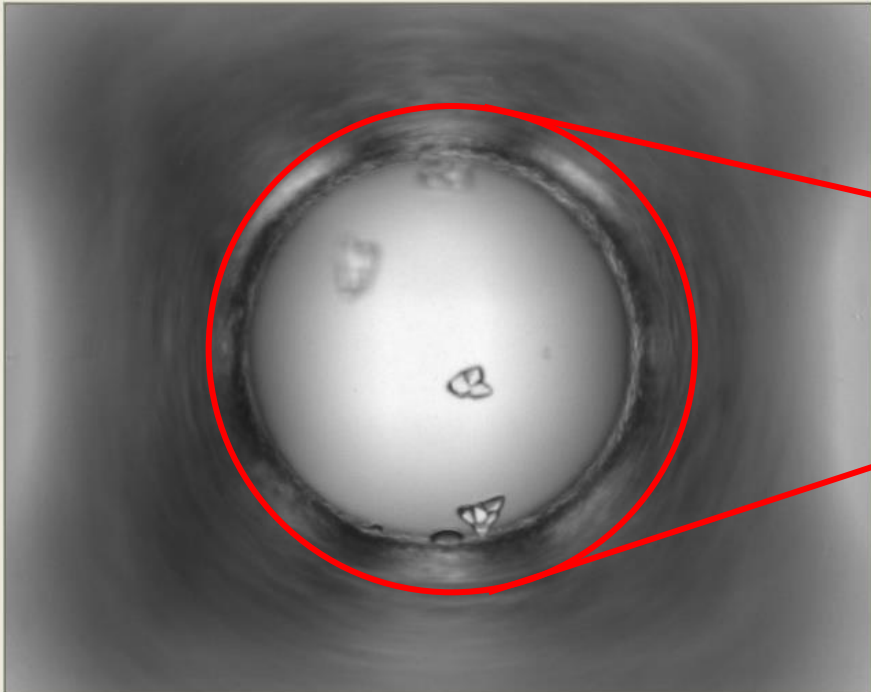


# What do we actually see?

Full Image

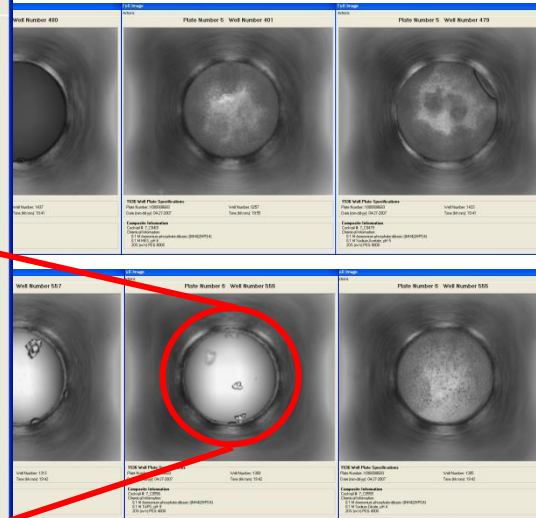
Actions

Plate Number 6 Well Number 556



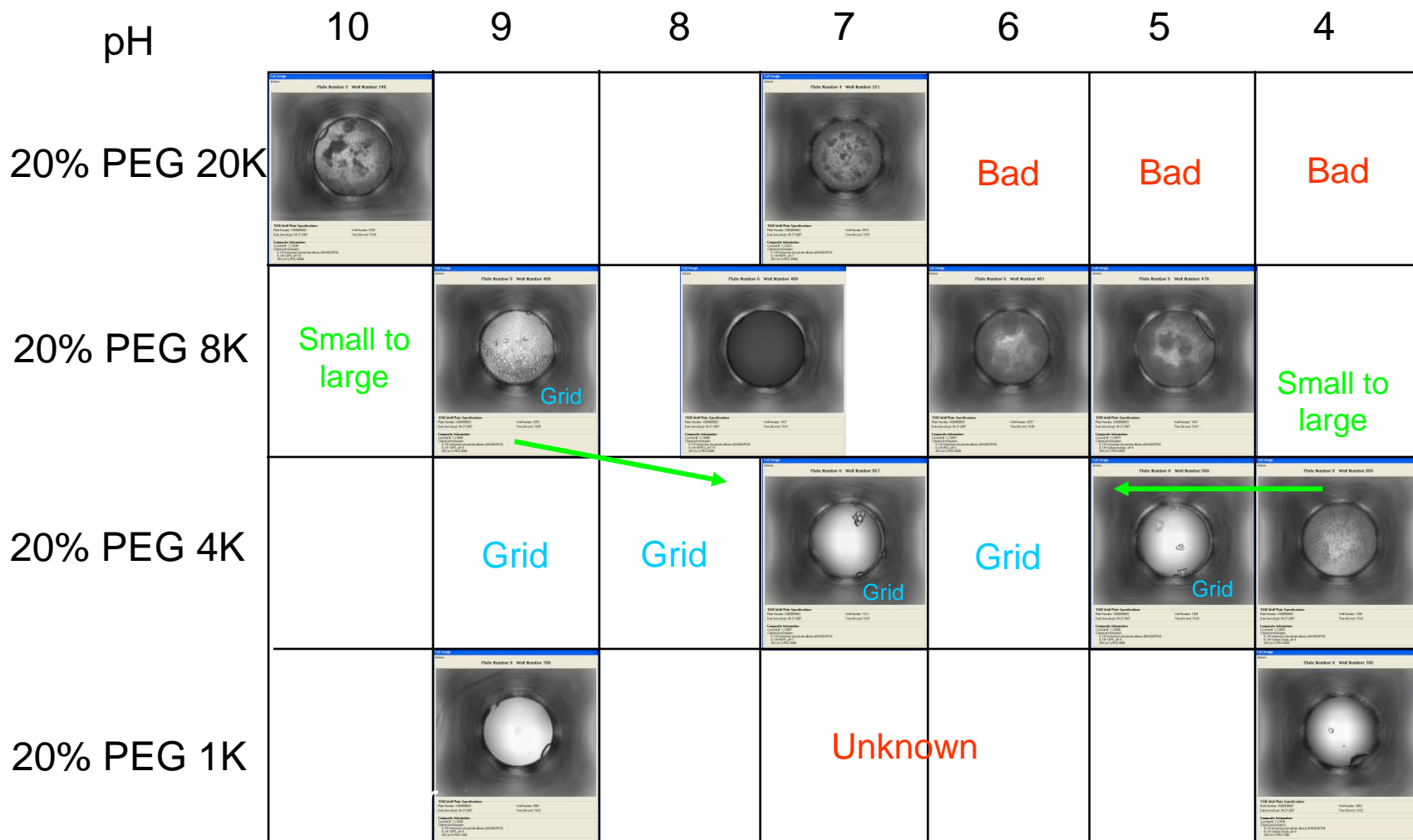
**1536 Well Plate Specifications**  
Plate Number: X000008683 Well Number: 1309  
Date (mm-dd-yy): 04-27-2007 Time (hh:mm): 19:42

**Composite Information**  
Cocktail #: 7\_C0556  
Chemical Information:  
0.1 M Ammonium phosphate-dibasic ((NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>)  
0.1 M TAPS, pH 9  
20% (w/v) PEG 4000



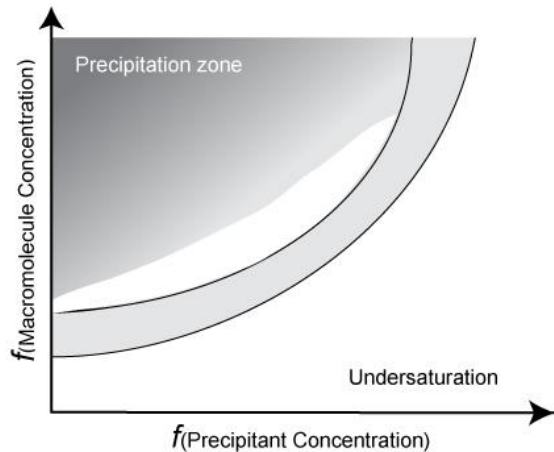
Optimize crystals by screening around the hit conditions, *i.e.* 0.1 M ammonium phosphate dibasic, 0.1 TAPS pH 9 and 20% (w/v) PEG 4000

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

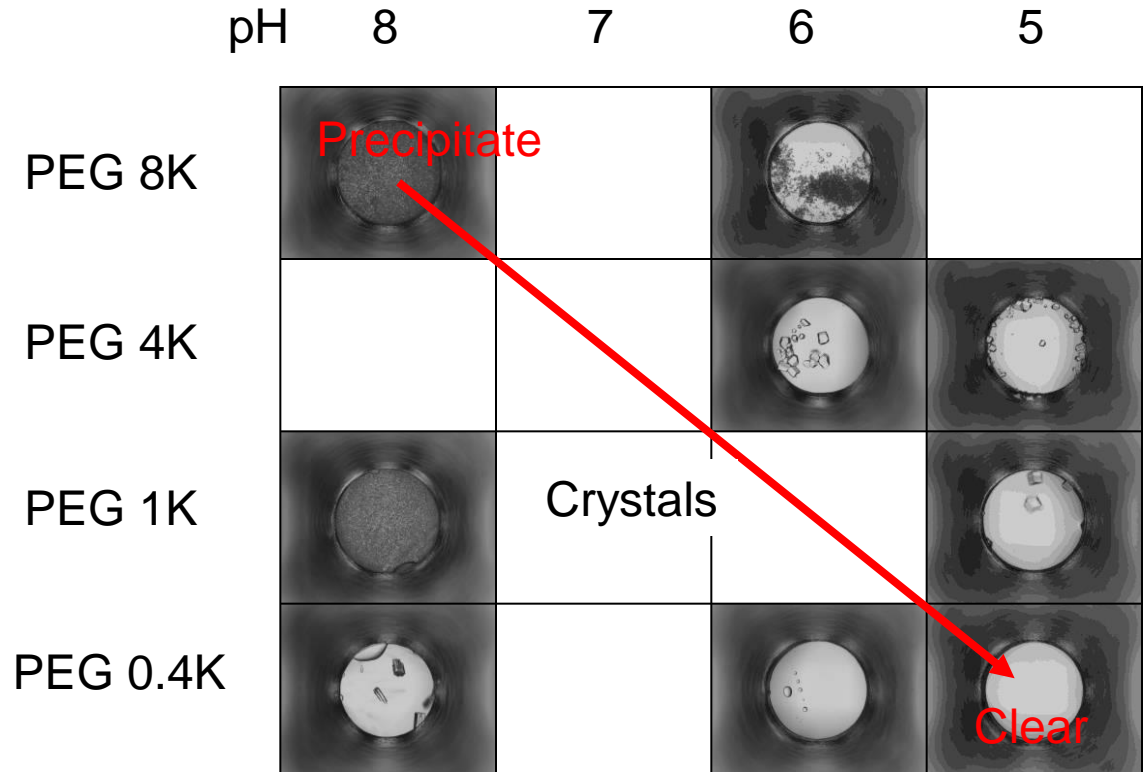


# Chemical space provides a vector for optimization

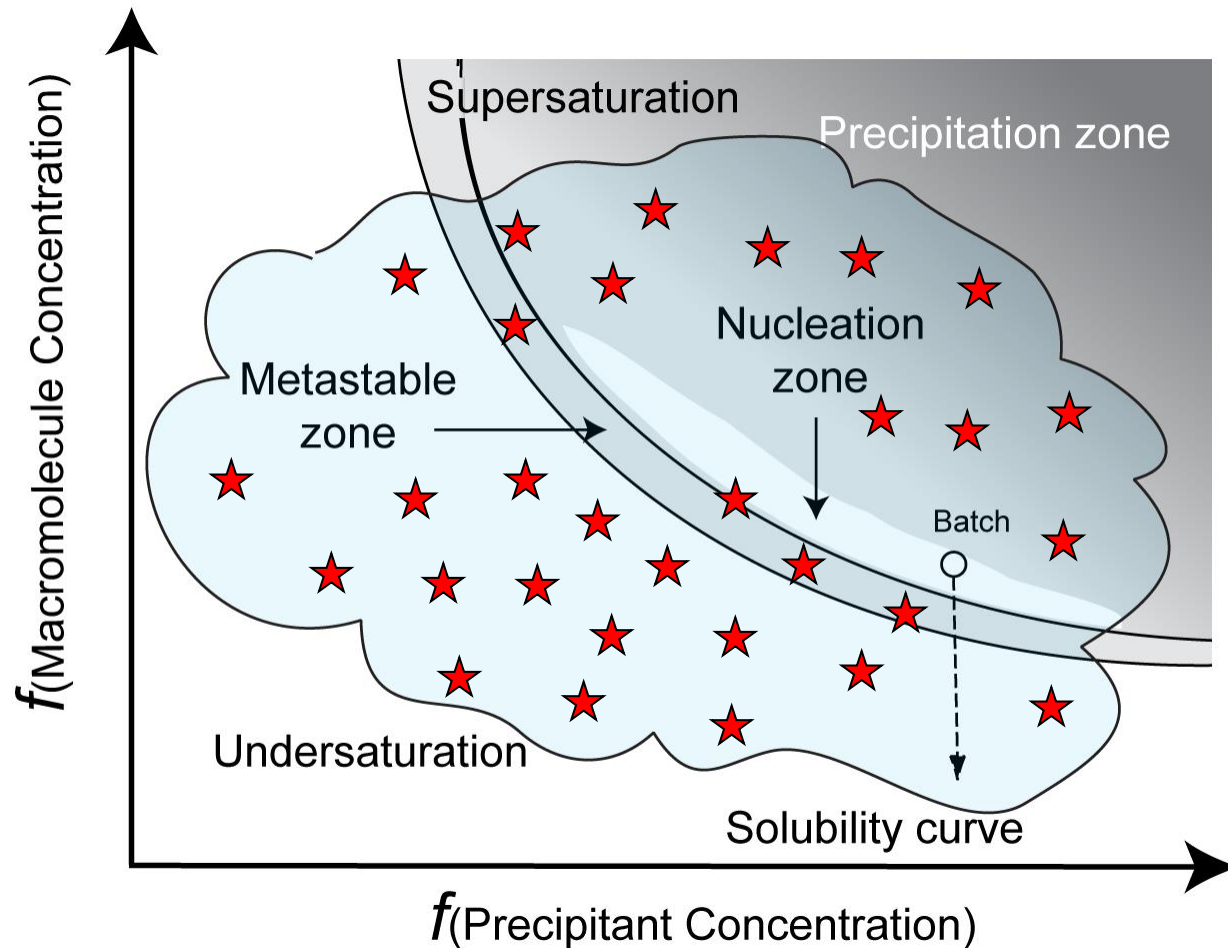
In this case the path from precipitate through crystals to clear is obvious. The phase diagram is reversed. Also clear are the number of chemical conditions that have not been sampled.



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

Numbers – the quantity of data

# AutoSherlock

TM



`"Because scientists would rather spend less time organizing their data, and more time learning from it."`

As of the New Year:

Over 850 laboratories sending samples (multiple samples from PSI centers)

Over 9,500 different macromolecules to date

Over 14.5 million images

Over 3000 years of computing time spent analyzing 1% of those images

A difficult task to easily visualize results.

Develop automated procedures.

Microsoft Excel

File Edit View Insert Format Tools Data Window Help Adobe PDF

Arial 8

BG57

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

PEG 4000	CaPS	Na Citrate	Na Acetate	MES	MOPS	HEPES
10	4	5	6	7	15	
28X	1113					
48X	515	515				
48X	107	1141				
48X	417				274	
28X						

CONDITION NOT SAMPLED

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.

Chemical Space / Image Filenames / Outcome Summary

Ready



Microsoft Excel

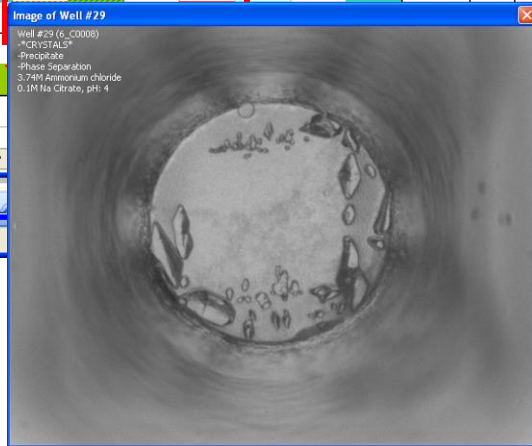
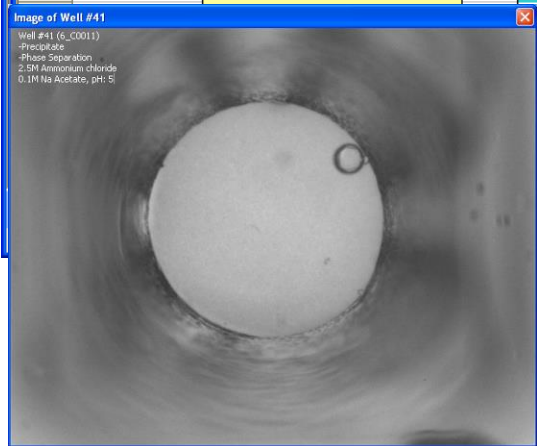
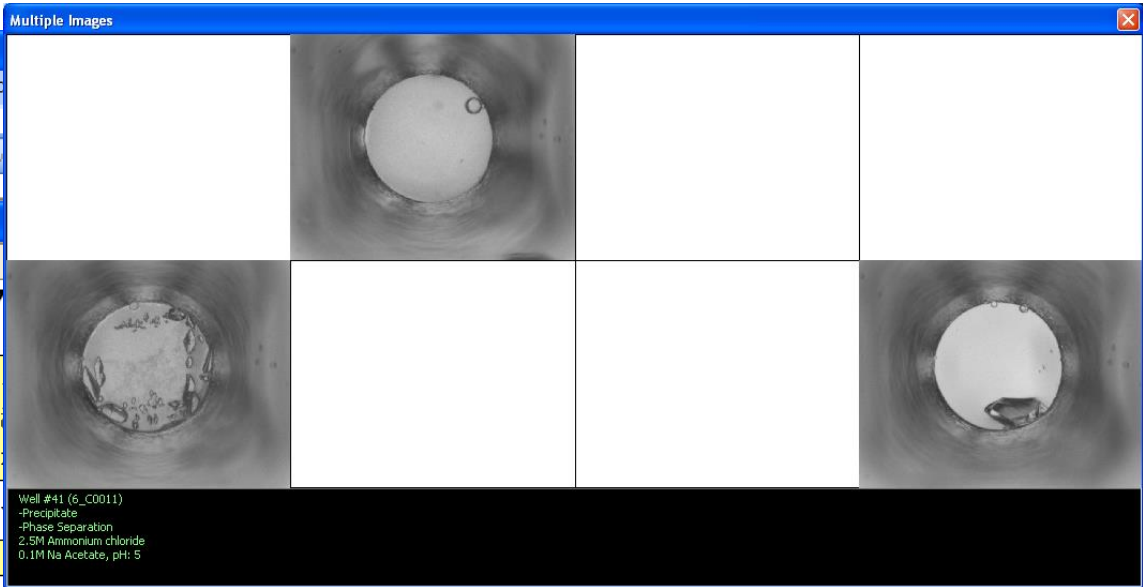
File Edit View Insert Format Tools Data Window Help Adobe PD

Arial

A2

4wk sherlock.xls

	A	B	C	D
1			X000007	
2			M	CAPS
3		pH		10
4				
5			1.19	
6		bromide	2.38	9
7			3.56	5
8			1.25	
9		chloride	2.5	193





# A special case – The Hampton Research Index Screen

Hampton Research Index Screen																				
Note, the HT screen is not a conventional screen as such. It is designed to sample a range of reagents and provide an indication of the appropriate chemical area and variables that would be appropriate for crystallization and should be used in this manner.																				
pH	Ammonium Sulfate 2.0M		Sodium chloride 3.0M		Magnesium formate dihydrate		Sodium phosphate		Neutralized organic acids (pH 7.0)		High supersaturation salt and low polymer		Low ionic strength systems		Non-volatile organics					
	pH				0.3M	0.5M	pH				pH		pH		pH					
3.5	A1	A7					5.6	B5		B9		5.5	C8	3.5	D4		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		E1			
6.5	A3	A10				B2				B12			C9		D7		E3			
7.5	A5	A11			B3					C1		7	C10	6.5	D10	6.5	E6			
8.5	A6	A12				B4				C2			C11		D11		E9			
										C3					D2		E10			
	Classic salt versus pH										C4			7	D3		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			
																7.5	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 chlorides	%	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	Magnesium 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

Automagic?

A process carried out automatically in such a clever way that the result appears to be magic

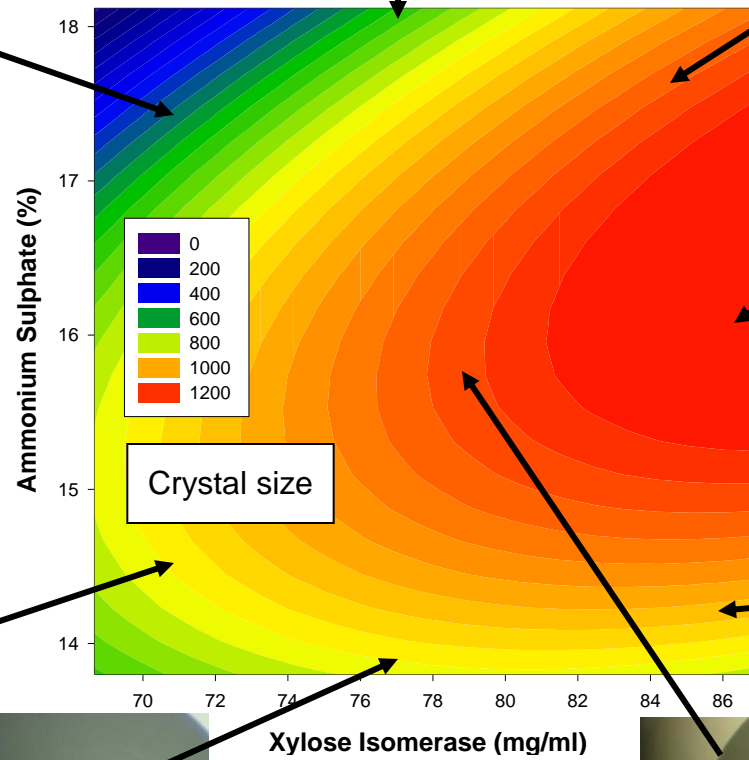
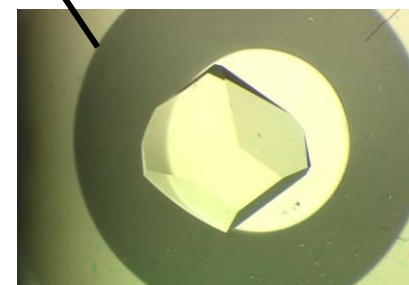
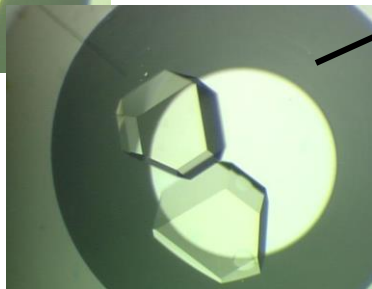
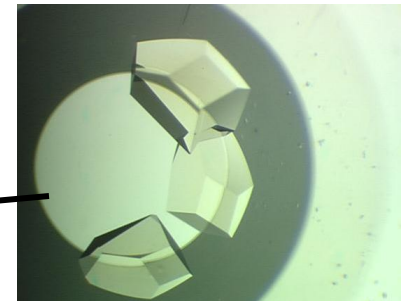
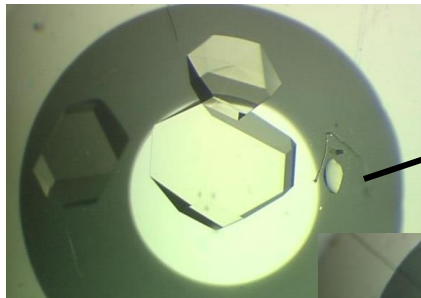
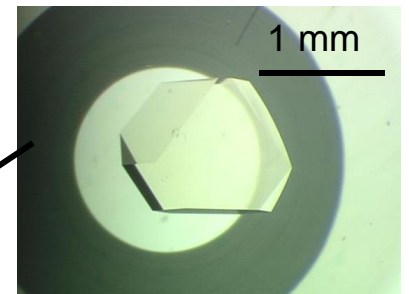
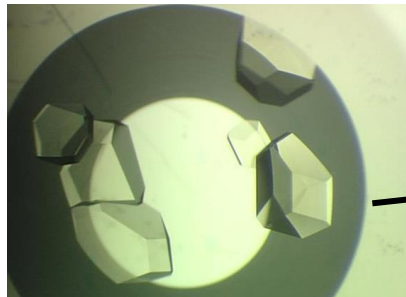
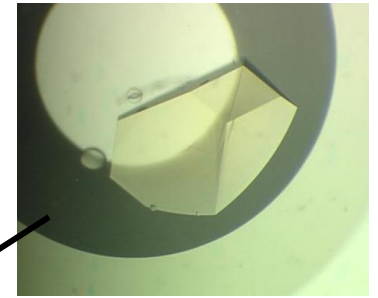
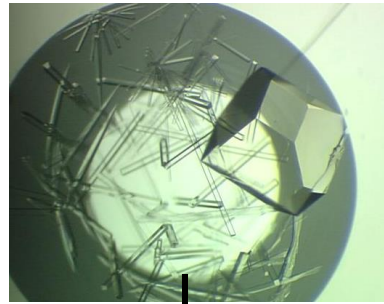
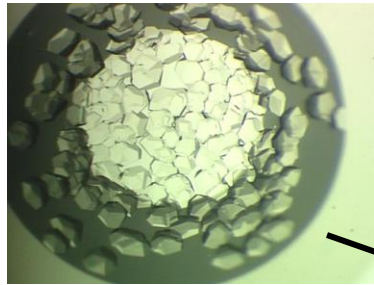
Not yet....

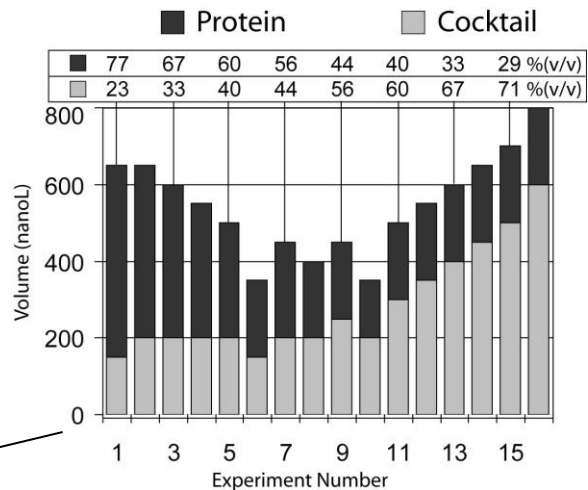
Crystallize  
Now

Rule 1: Think

- Remember, Garbage In, Garbage Out

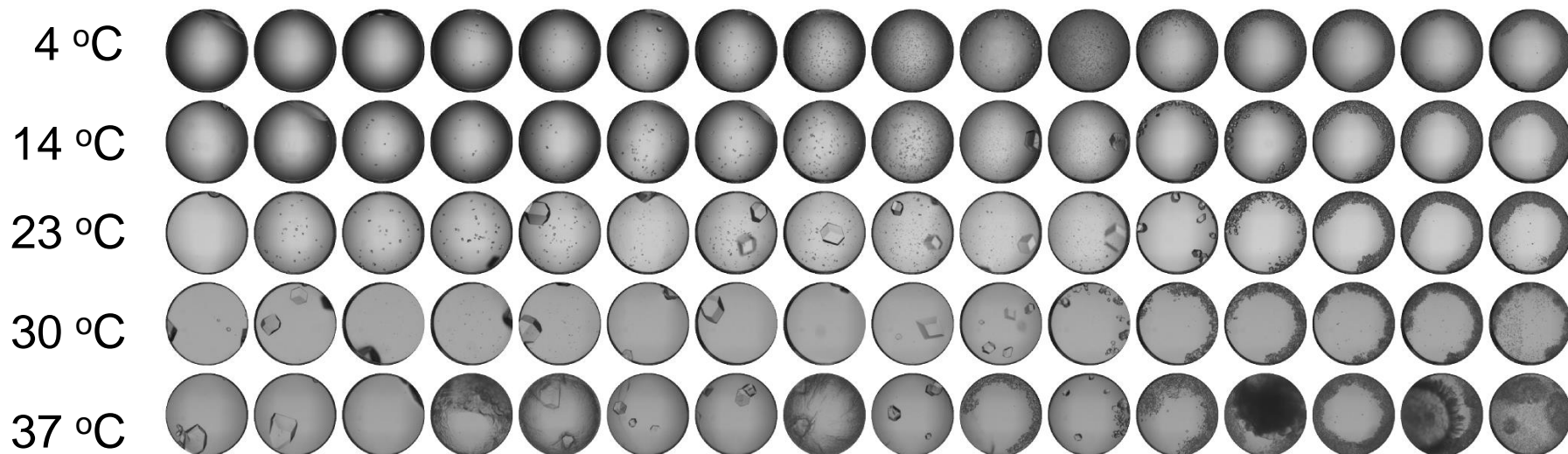
- Crystallization is one percent inspiration and ninety-nine percent optimization – Unknown crystal grower.
- **Rule 2 – Be an optimizer**



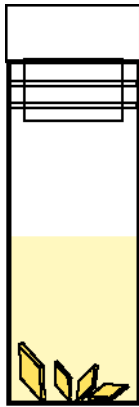


$V_{\text{sample}} > V_{\text{cocktail}}$

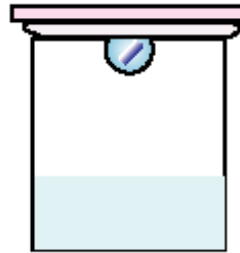
$V_{\text{sample}} > V_{\text{cocktail}}$



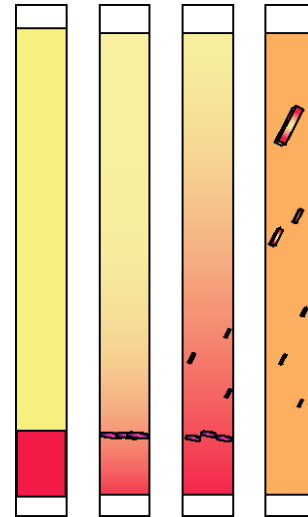
# Three basic methods: batch, vapor and liquid



Batch



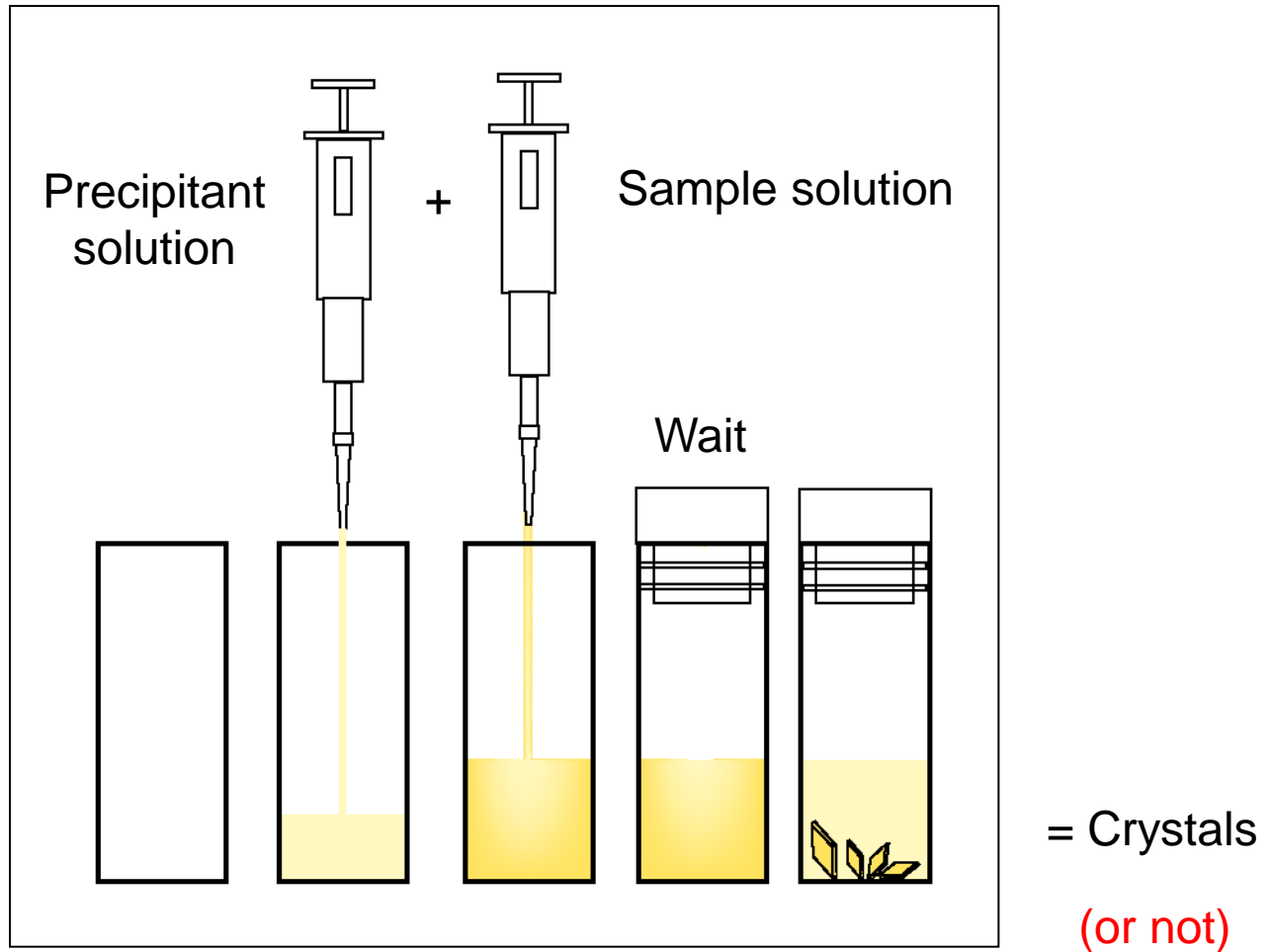
Vapor  
(also known as vapour)



Liquid

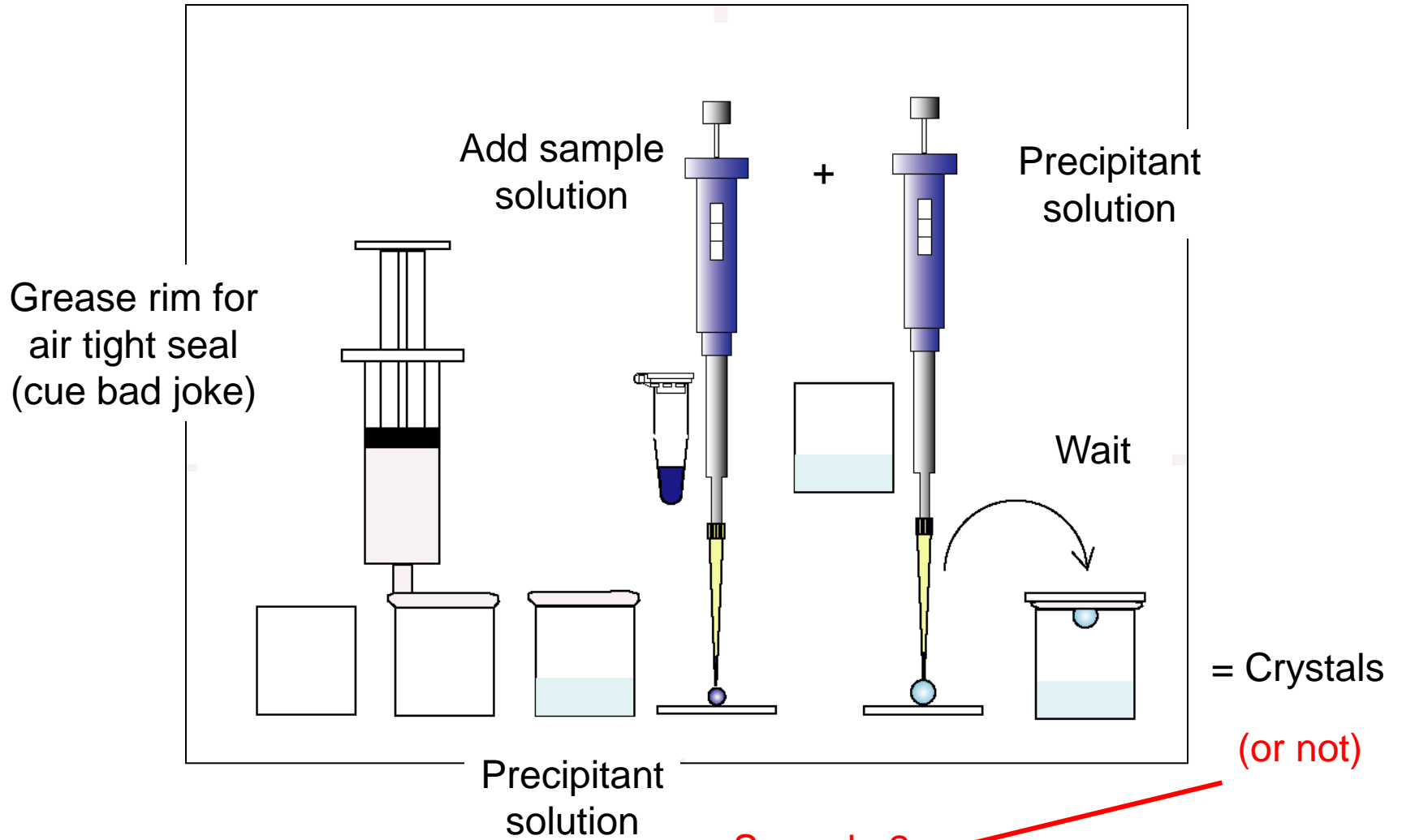


# Batch in a Vial: Set up



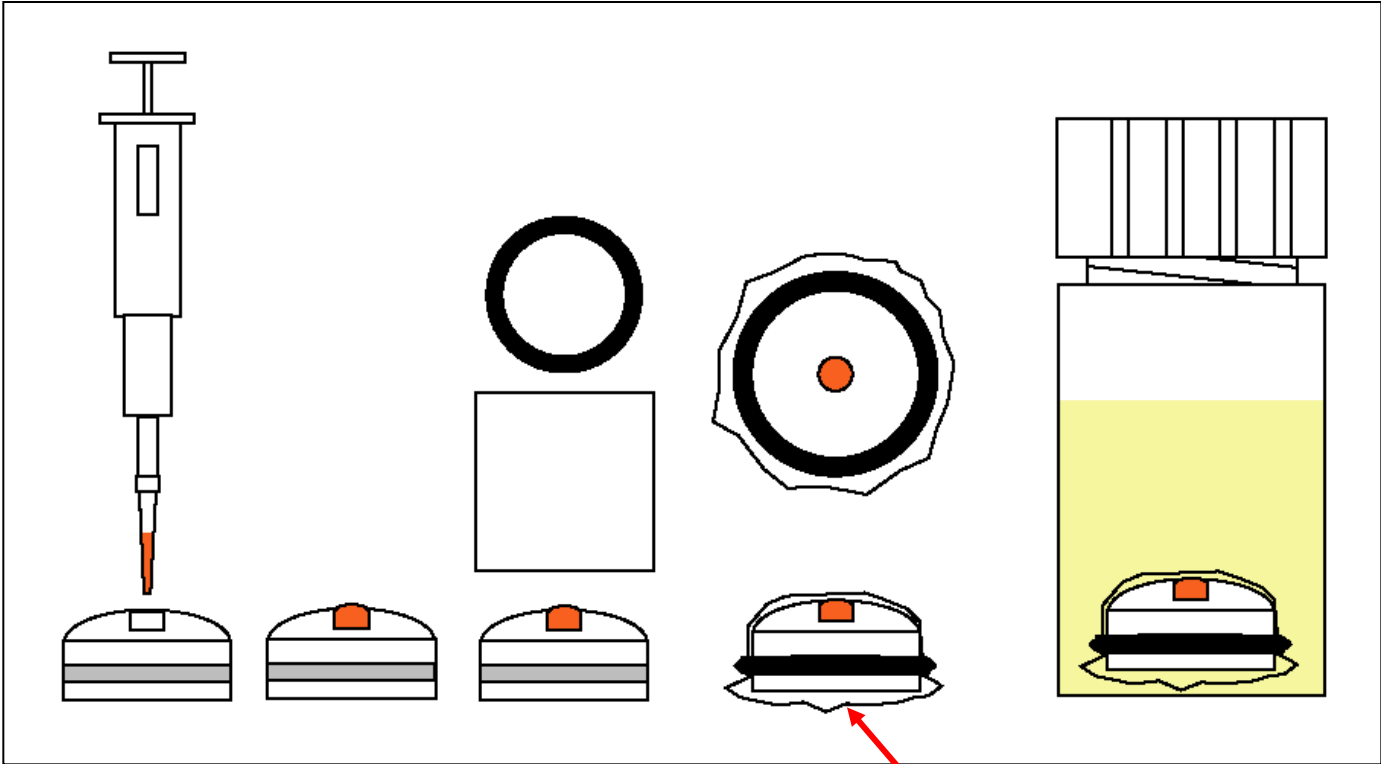
See rule 2

# Vapor Diffusion Setup



See rule 2

# Dialysis Experiments



See rule 1

Not a hamburger

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Table of frequently used crystallization buffers and their useful pH range.
- Crystallization Scoring Sheet**  
24 well plate format scoring sheet for screening and optimization.
- Preliminary Sample Preparation**  
What to do and what not to do while preparing your sample for crystallization.
- Crystal Growth Techniques**  
There are several techniques for setting up crystallization experiments (often termed "trials") including sitting drop vapor diffusion, hanging drop vapor diffusion, sandwich drop, batch, microbatch, under oil, microdialysis, and free interface diffusion. Here we offer an overview of these crystallization techniques.
- Hanging Drop Vapor Diffusion Crystallization**  
The hanging drop vapor diffusion technique is the most popular method for the crystallization of macromolecules. The principle of vapor

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7:00 am to 5:00pm  
Monday - Friday  
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Internet 100%

Useful websites:

[www.hamptonresearch.com](http://www.hamptonresearch.com)

http://hamptonresearch.com/documents/growth\_101/3.pdf - Windows Internet Explorer

http://hamptonresearch.com/documents/growth\_101/3.pdf

# Hanging Drop Vapor Diffusion Crystallization

HAMPTON RESEARCH  
Solutions for Crystal Growth

## Crystal Growth 101

The hanging drop vapor diffusion technique is the most popular method for the crystallization of macromolecules. The principle of vapor diffusion is straightforward. A drop composed of a mixture of sample and reagent is placed in vapor equilibration with a liquid reservoir of reagent. Typically the drop contains a lower reagent concentration than the reservoir. To achieve equilibrium, water vapor leaves the drop and eventually ends up in the reservoir. As water leaves the drop, the sample undergoes an increase in relative supersaturation. Both the sample and reagent increase in concentration as water leaves the drop for the reservoir. Equilibrium is reached when the reagent concentration in the drop is approximately the same as that in the reservoir.

Figure 1  
Process of vapor diffusion.

**Benefits of Hanging Drop Crystallization**

- Can be cost effective.
- Sample and reagents in contact with a silicized glass surface.
- Relatively easy access to crystals.
- Can perform multiple drops (experiments) with a single reservoir.

**Using the VDX Plate**

The VDX Plate is a 24 well plate manufactured from clear polystyrene. The VDX Plate is typically sealed with High Vacuum Grease (HR3-510) and Silicized 22 mm Circle or Square Glass Cover Slides. The VDX Plate is also available pregreased. Rows of the plate are labeled A-D and columns are labeled 1-6 on the VDX Plate.

**VDX Plate Tips**

- Note the VDX Plate has a raised cover to protect the cover slides during transport and storage.
- To access a drop and/or reservoir simply grasp the edge of the cover slide with forceps or fingertips, twist and pull gently.
- VDX Plates can be stacked for convenient storage.
- One can pipet multiple drops onto the cover slide. This technique is often useful when screening additives since one can use the same reservoir with multiple drops with each drop containing a different additive. This technique can also be used to screen different drop sizes and ratios versus the same reservoir. Use care not to avoid mixing the drops during pipetting, plate transport, and plate viewing.

**Using the Q Plate**

1. Pipet 1.0 milliliter of crystallization reagent into reservoir A1 of the Q Plate. (*Note: Recommended reservoir volume is 0.5 to 1.0 milliliters*)

2. Clean a Silicized 18 or 22 mm Circle Cover Slide by wiping the cover slide with lens paper and blowing the cover slide with clean, dry compressed air. Pipet 2 microliters of sample into the center of a Silicized 18 or 22 mm Circle Cover Slide. (*Note:*

5. Holding the cover slide with forceps, the Pen Vac, or on the edge between your thumb and forefinger, carefully yet with out delay invert the cover slide so the drop is hanging from the cover slide.

6. Position the cover slide onto the bead of grease on reservoir A1. Gently press the slide down onto the grease and twist the slide 45° to ensure a complete seal.

7. Repeat for reservoir 2 through 24.

1 of 2

Nice 101 technical notes on many basic techniques.

Other sites include:

<http://www.moleculardimensions.com/>

<http://www.emeraldbiosystems.com/>

Rules of thumb to convert vapor diffusion to batch:

<http://www.douglas.co.uk/convert.htm>

Choosing a starting point for pH

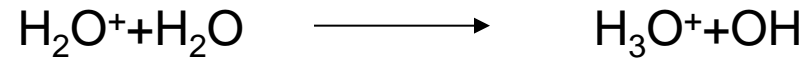
<http://www.ruppweb.org/cryspred/default.html>

General crystallization phenomena

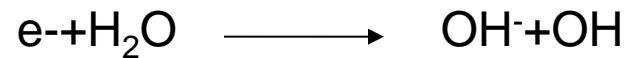
<http://xray.bmc.uu.se/~terese/crystallization/library.html>

## Bad news on X-rays

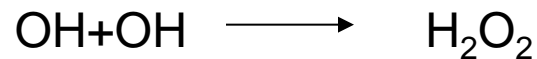
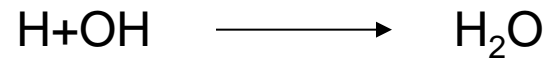
Ionizing radiation can remove an electron from water:



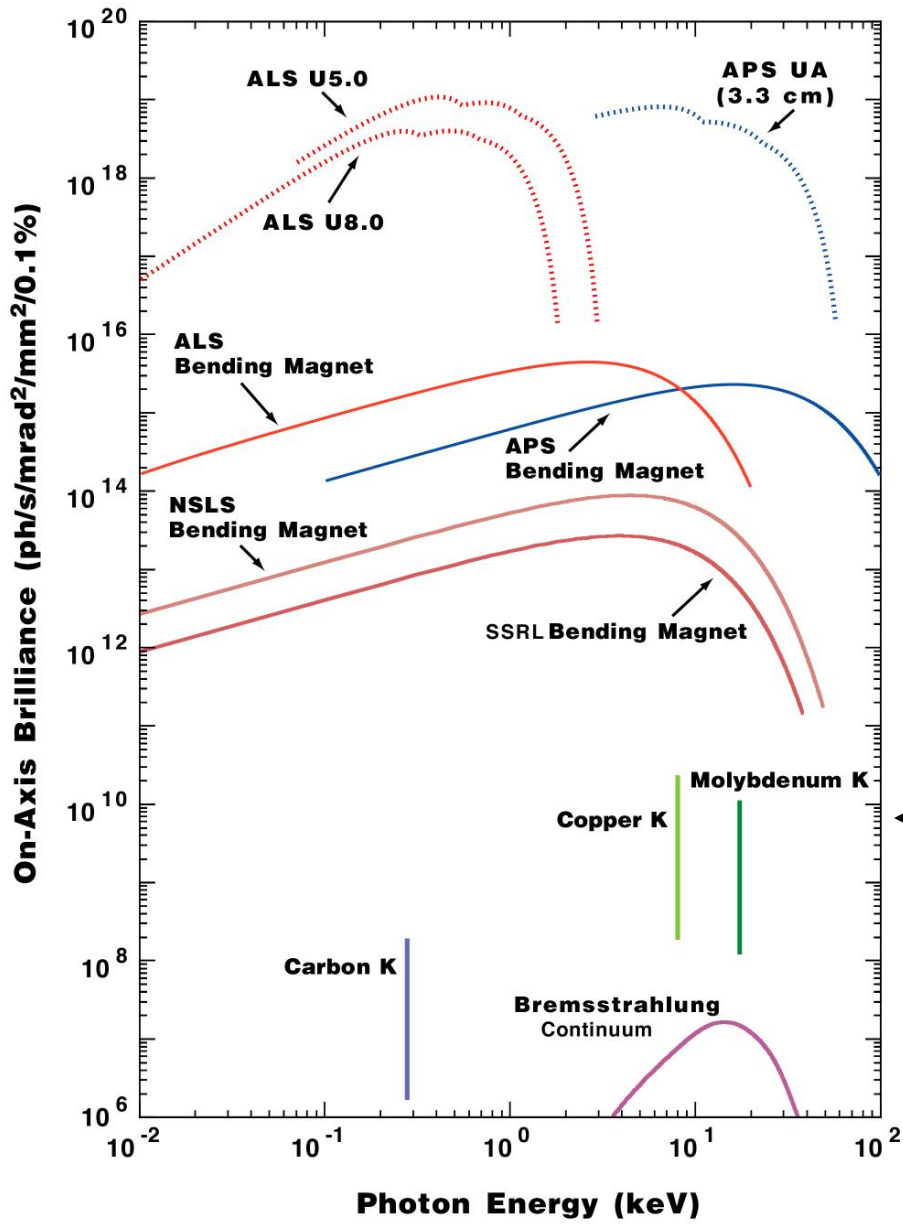
And the ejected electron



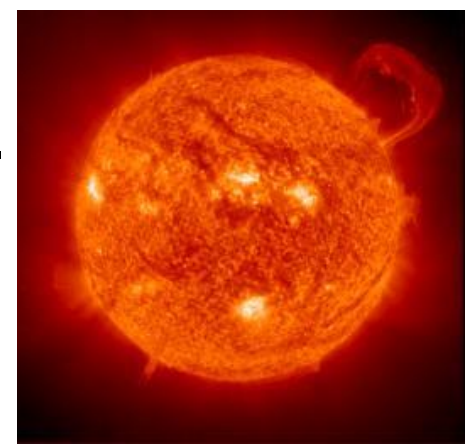
The simultaneous formation of H and OH free radicals gives further reactions



Your body, cells, protein crystals contain water, the body ~66%, crystals 30-70%.



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



# 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.

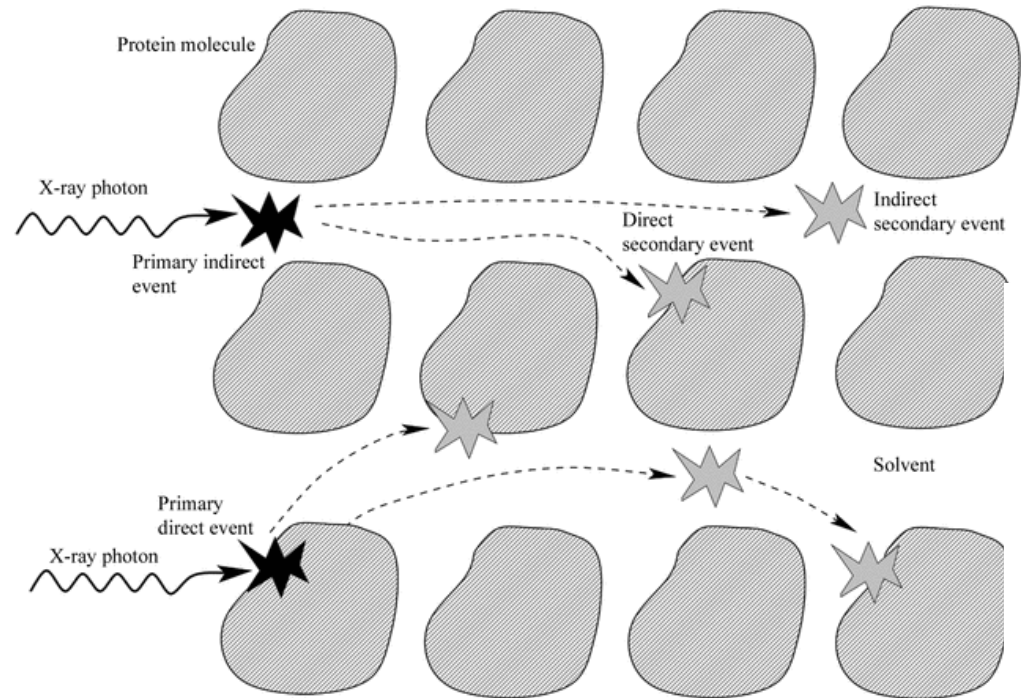
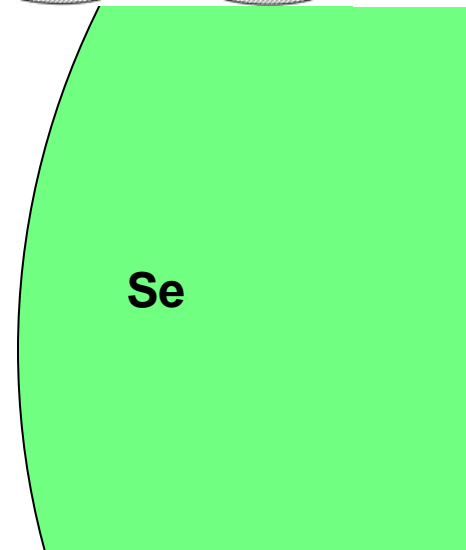
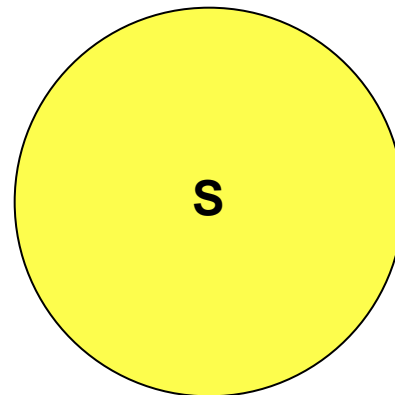


Image from Elspeth Garman

Effect on  
Other  
atoms

◦	●	●	●
H	C	N	O



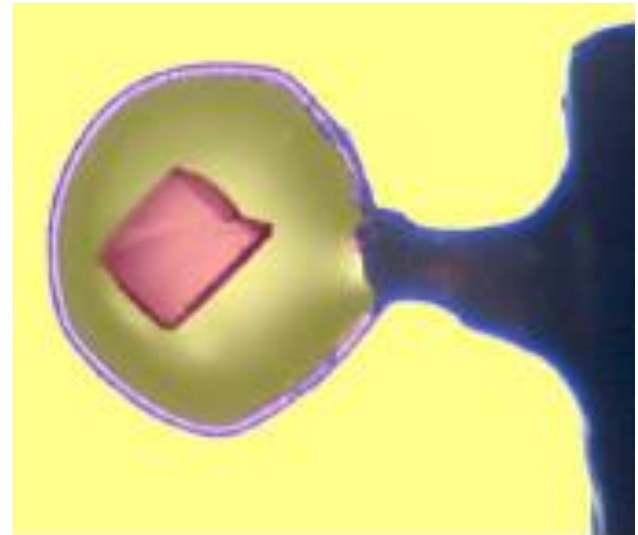
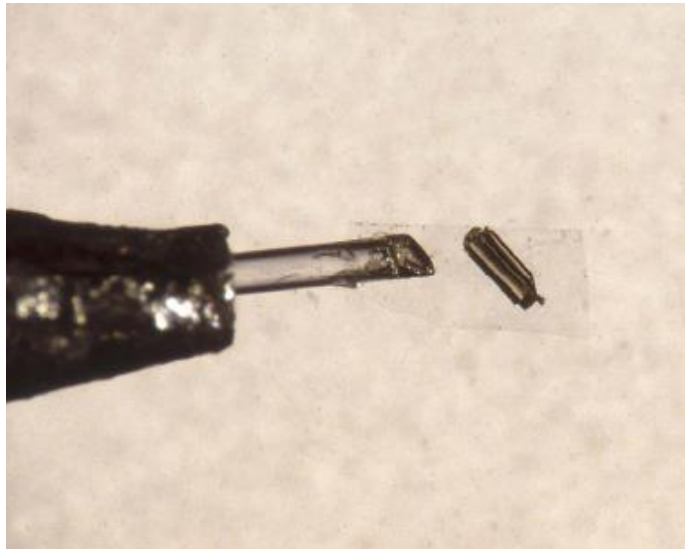


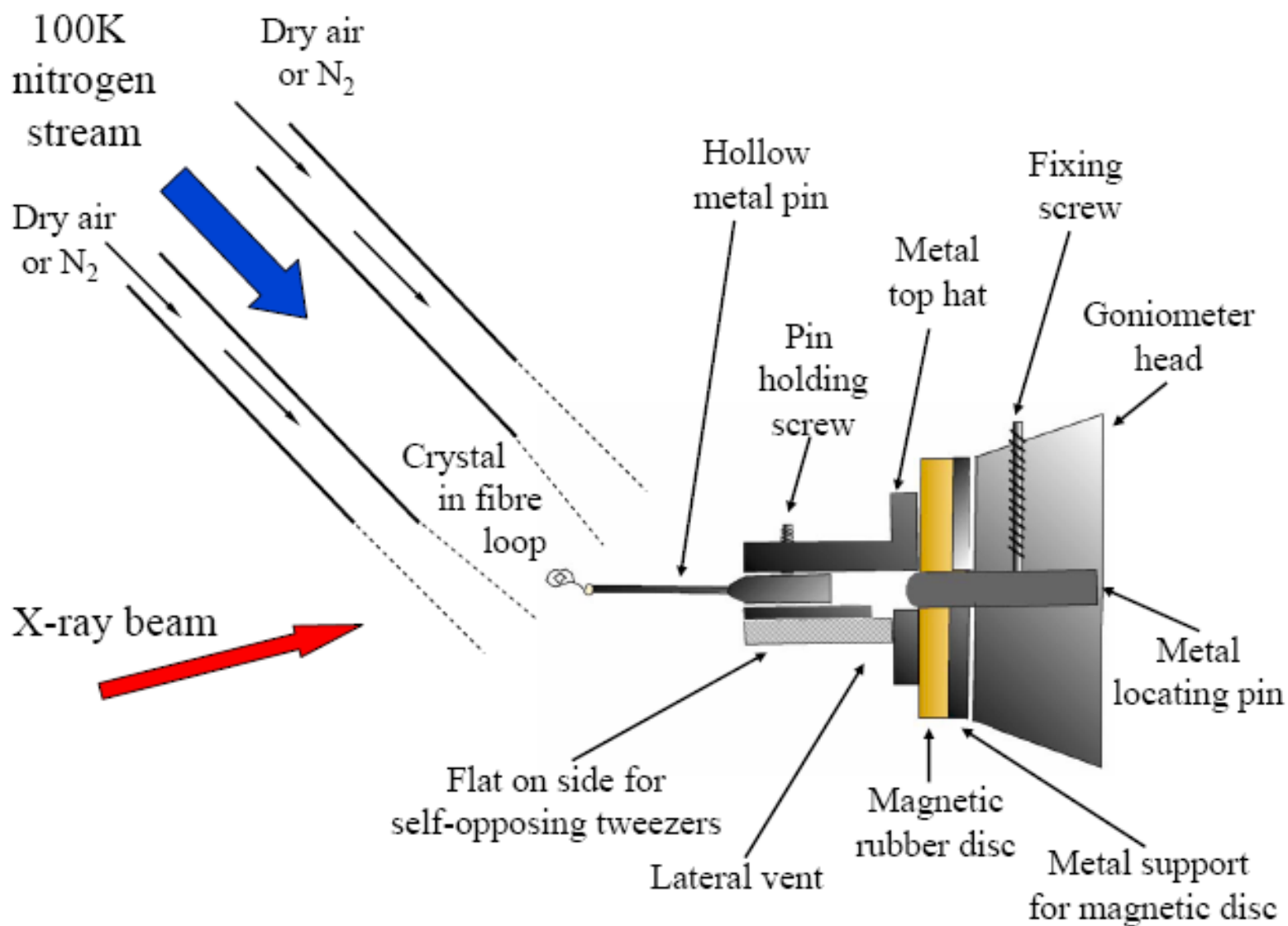
## Development of cryocooling

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.

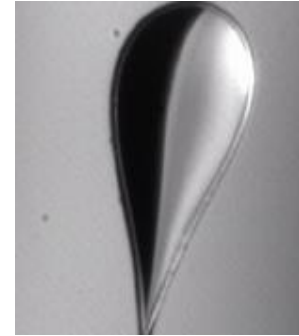
Cryocooling with loops is younger than everyone that uses it!





## 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



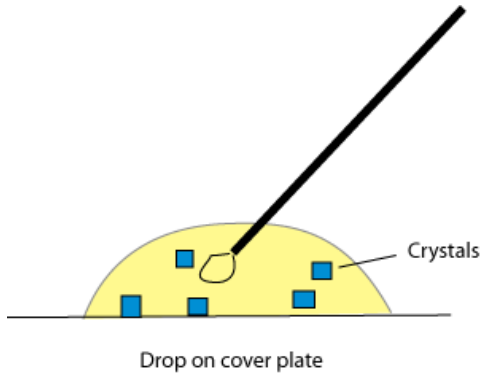
Bad cryobuffer

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

## Cryo-buffers (make your own)

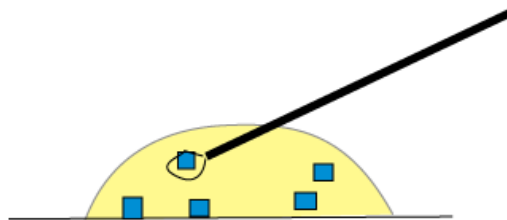
- Look for similar crystallization conditions in other publications.
- PEG < 4K – increase PEG, add small PEGs
- PEG > 4K – add small PEGs
- 30% of cases – add 15-25% glycerol
- MPD – increase MPD concentration
- Salt – add MPD and/or ethylene glycol or glycerol
- Salt – increase concentration/add salt
- 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).

# Getting the crystal

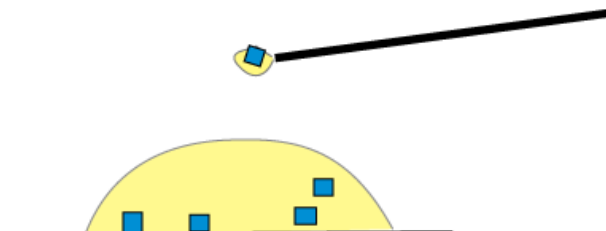


Ideally minimize the amount of liquid around the crystal while maintaining the crystal covered by the liquid

The loop size should be slightly larger than the crystal and used perpendicular to the liquid.



The loop should be brought up from below the crystal to capture the crystal.

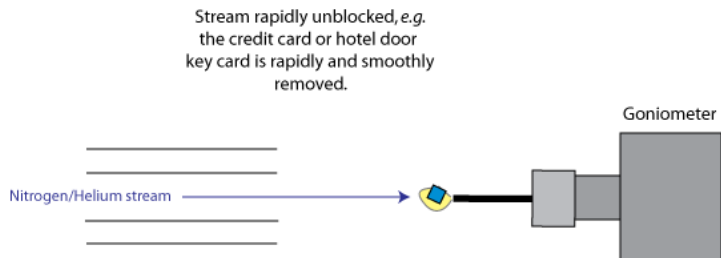
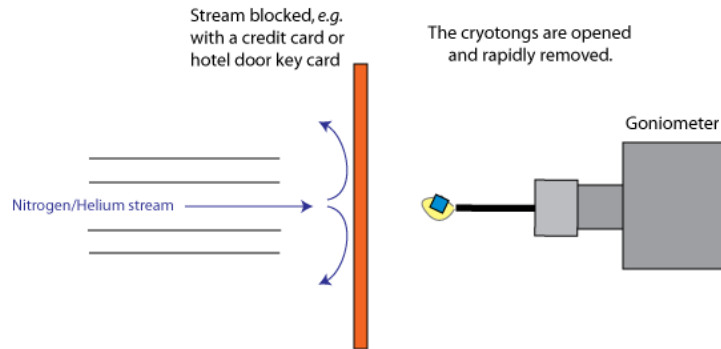
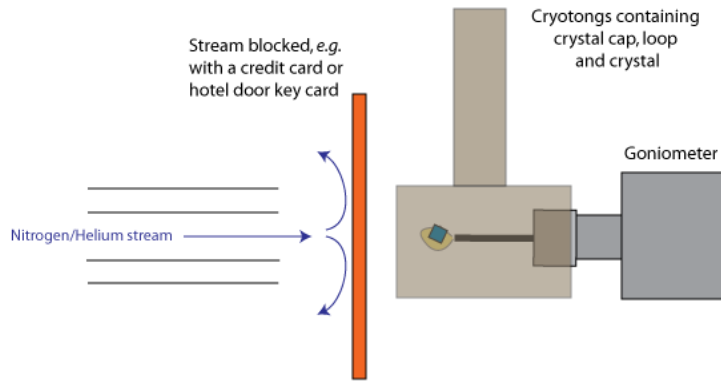


The loop containing the crystal is rapidly raised and as quickly as possible transferred to the cold stream or plunged into liquid nitrogen/propane.

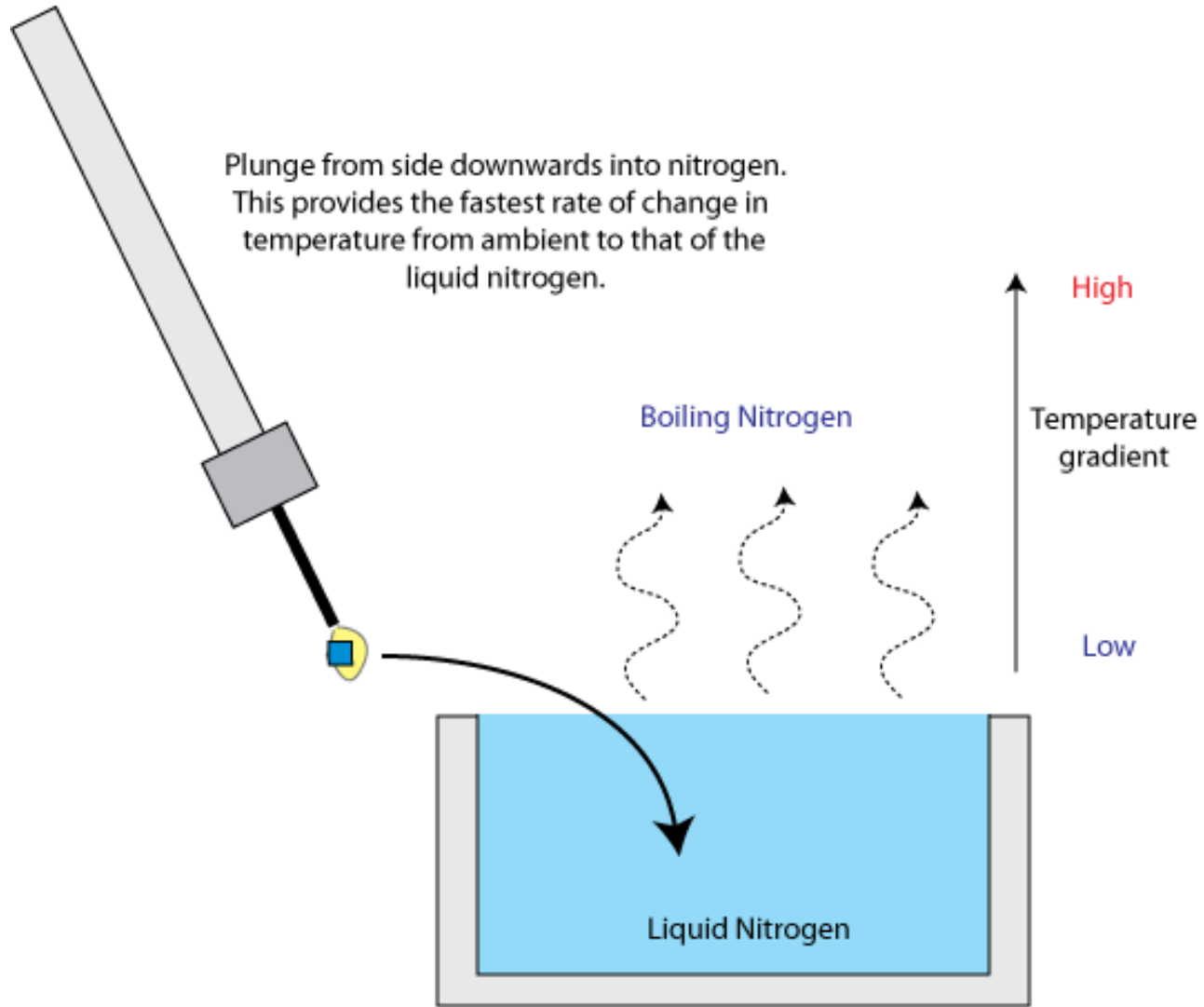
If more than one crystal is available more than one should be mounted and saved.

Note: Every step must be as rapid and smooth as possible to prevent turbulence

# Flash cooling in the gas stream



# Flash cooling by plunging

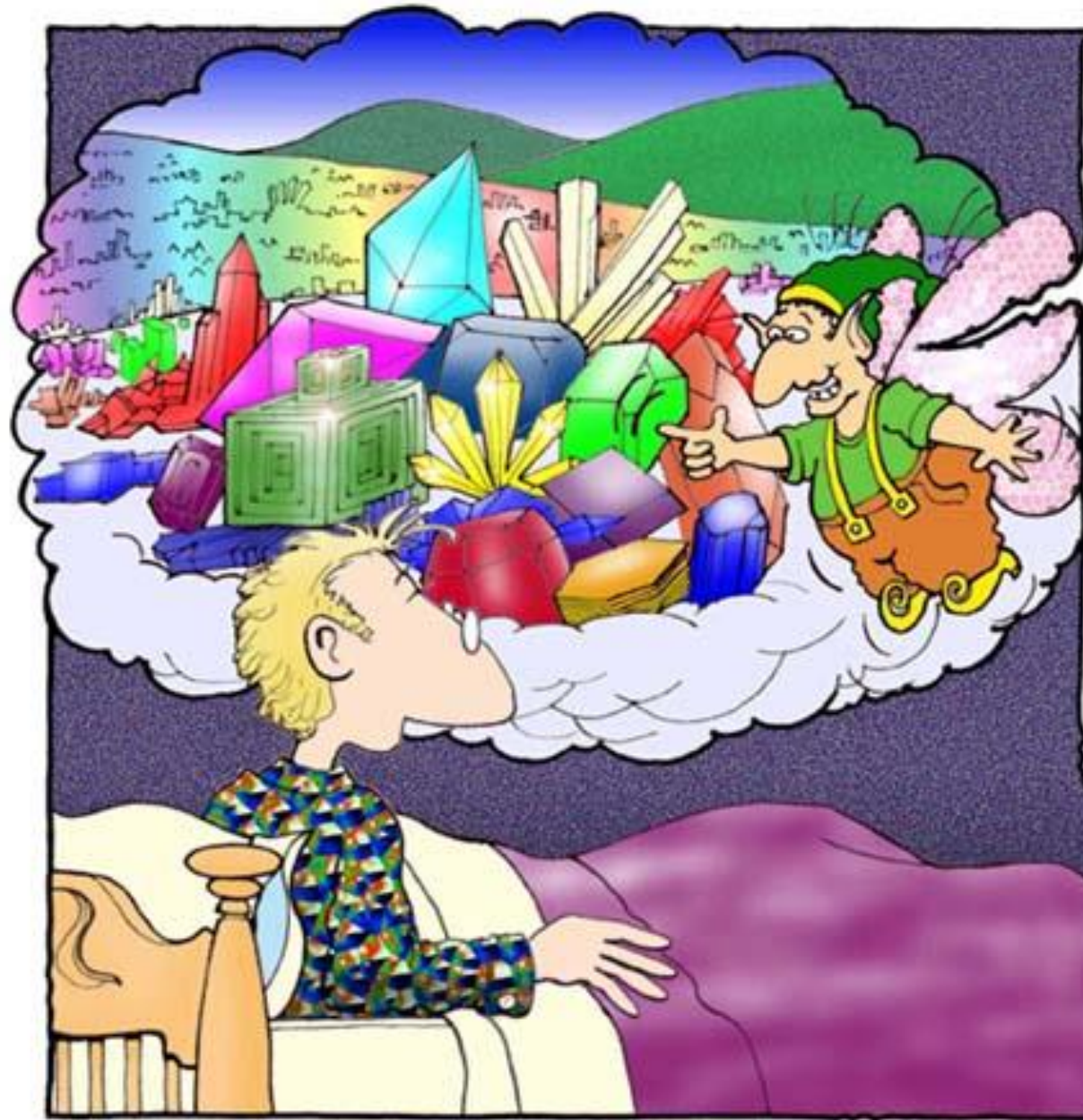


# Summary

- **Rule 1: Think**
- **Rule 2: Be an optimist (or an optimizer)**
- **Rule 3: Write everything down**
- **Rule 4: Know the phase diagram by heart.**

**Most of all, try. If you get a crystal and get stuck at that point there are plenty of willing hands to help out and many other research opportunities open up.**





Have you ever wondered why, no matter what you do, you can never get some of your reaction products to crystallise? Well, I've got news for you, Eric....They DO crystallise!! But just as soon as the crystals form, we SPIRIT them away! We put them HERE, Eric..! They're all HERE...!! HA HA HA HAAA...!!!

THE EVIL PIXIE OF REACTION MIXTURES TAUNTS ANOTHER CHEMIST

NICK

