



Soluble Proteins: Phase Diagrams & Crystal Size

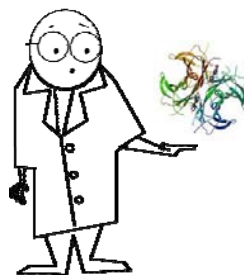


Joseph R. Luft
Hauptman-Woodward Institute

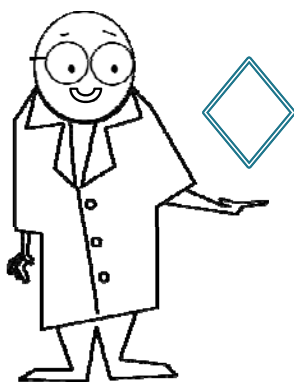


A crystallographer uses crystals to visualize structures

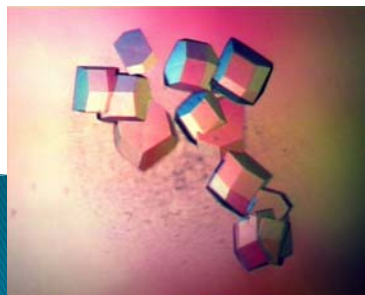
- ▶ The structure helps us to understand how the machinery functions



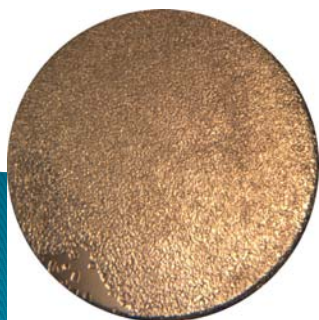
Crystallization = Sample Preparation



Typically, we try to identify initial crystallization conditions, then optimize to produce a small number of large crystals for diffraction studies



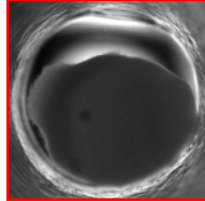
For XFEL, we try to identify initial
crystallization conditions, then
optimize to produce a
large number of small crystals for
diffraction studies



Methods to distinguish
microcrystals from
amorphous precipitate



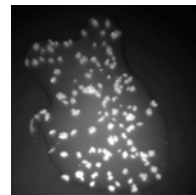
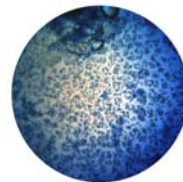
Characteristics of amorphous precipitate




- ▶ Will not re-dissolve with addition of water
- ▶ Will not absorb dye
- ▶ Will not show up with UV-fluorescence

These are characteristics of a microcrystalline 'precipitate'

- ▶ Patterned
- ▶ Sand-like appearance
- ▶ Most show birefringence
- ▶ Will re-dissolve with addition of water
- ▶ Will absorb dye
- ▶ UV-fluorescence
- ▶ SONICC signal

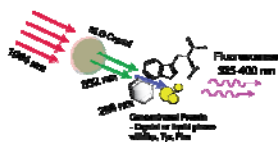


An ultraviolet fluorescence-based method for identifying and distinguishing protein crystals. R. A. Judge, K. Swift and C. González, *Acta Cryst.* (2005). D61, 60-66.



SHG

Detection and verification of submicron crystals with SONICC



UV-TPEF


▶ The combination of Second Harmonic Generation (SHG) and UV–Two Photon Excited Fluorescence (UV–TPEF) imaging can detect and verify submicron protein crystals

Madden JT, DeWalt EL, Simpson GJ. Two-photon excited UV fluorescence for protein crystal detection. *Acta Cryst* (2011). **D67**, 839–846.

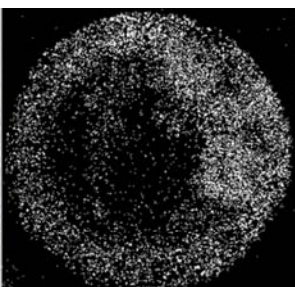
Hapert LM, Simpson, GJ. Screening of protein crystallization trials by second order nonlinear optical imaging of chiral crystals (SONICC) (2011), *Methods*, **55**(4)379–386

<http://www.formulatrix.com/products/protein-crystallography-tools/sonicc/how.html>

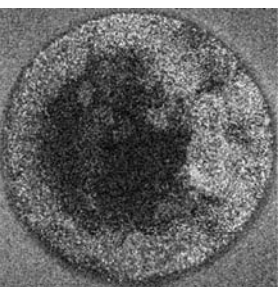
Vis



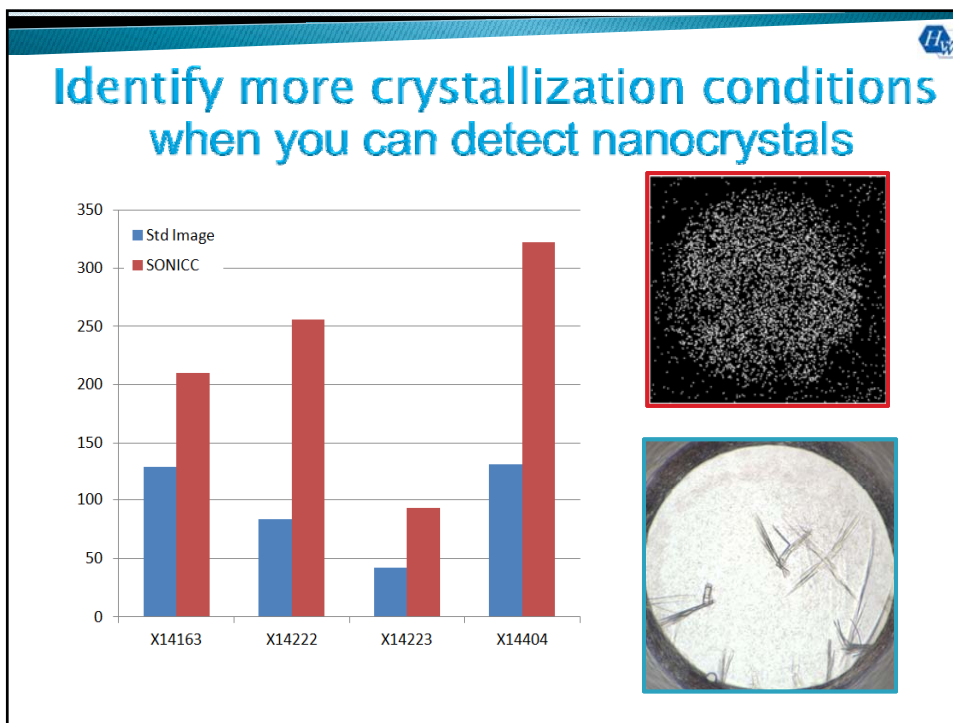
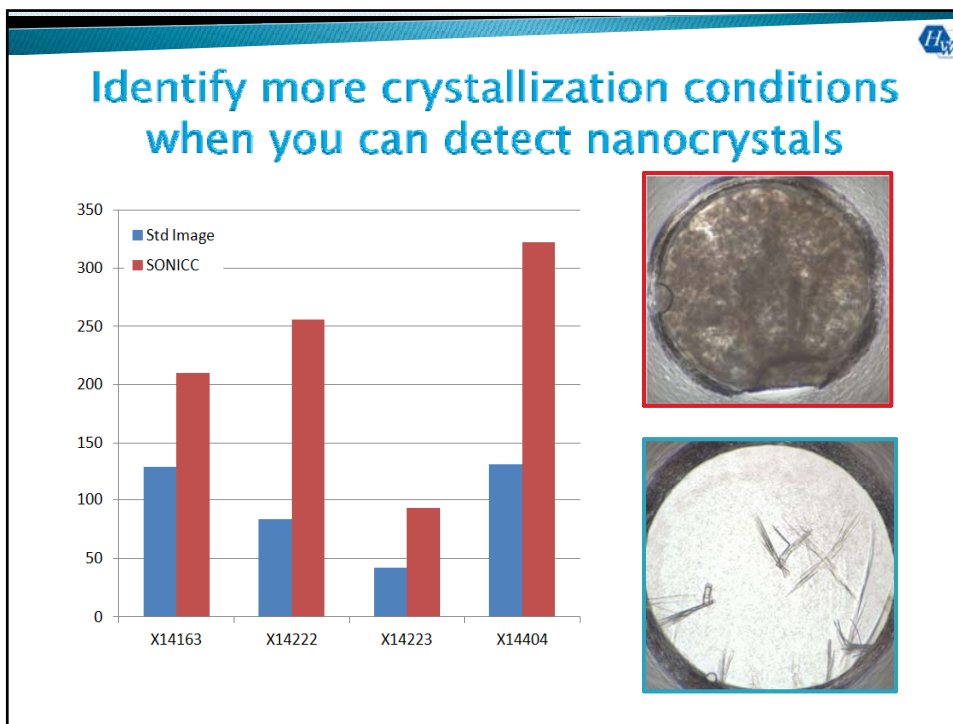
SHG



UV-TPEF



Identifying, studying and making good use of macromolecular crystals. Calero G, Cohen AE, Luft JR, Newman J, Snell EH. *Acta Crystallogr F Struct Biol Commun*. 2014 Aug 1;70(Pt 8):993-1008. Epub 2014 Jul 25. PMID: 2508437



Preparing biological macromolecules for crystallization

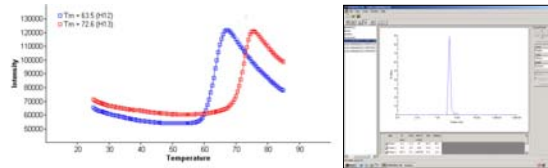
The protein is the most important variable!

The same rules apply for the optimization of large or nanocrystals

- ▶ Pure protein
 - ▶ Knowledge of crystallization
 - ▶ Rational approaches
 - ▶ Attention to details
- ▶ These tools will reduce frustration, increase efficiency and lead to reproducible crystals

A protein that is pure and stable will crystallize more reproducibly

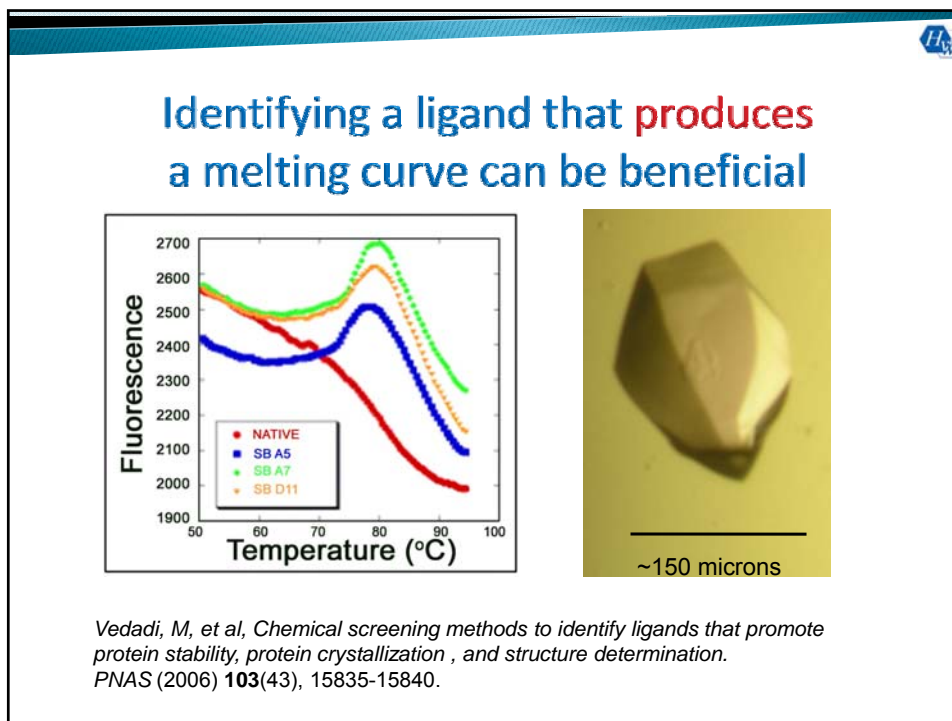
- ▶ Soluble
- ▶ Stable
- ▶ Pure/monodisperse
- ▶ Active (if activity can be measured)
- ▶ Prepared in a “minimal buffer”




Chemicals that have been used to stabilize a protein for storage or crystallization

Protein Methods, D.M. Bollag, M. D. Rozycki, and S. J. Edelstein, Wiley-Liss Publishing (1996)

- ▶ Ligands
 - ▶ Co-factors
 - ▶ Salts
 - ▶ Reducing agents (TCEP is the longest lasting)
 - ▶ Protease Inhibitors
 - ▶ Chelating agents (EDTA)
 - ▶ Buffers (control surface charge distribution)
 - ▶ Detergents (attach to hydrophobic patches)
 - *Acta Cryst.* (1994). D50, 414–423
 - ▶ Preservatives (stop microbial growth)
 - ▶ Glycerol (protects solution and crystal cryoprotectant)
 - *Methods* 32 (2004) 415–423
- Can reduce conformational flexibility –test with DSF





Maximizing protein solubility with buffers and salts can improve crystallization success

- ▶ Increasing the protein solubility can increase the range of chemical space suitable for crystallization
- ▶ You can gain insight from screening outcomes or ancillary experiments
- ▶ *further reading:*
Acta Cryst. (2006) D62, 833–842.
Acta Cryst. (2005) F61, 1035–1038.
Acta Cryst. (2004) D60, 1674–1678.
Acta Cryst. (2004) D60, 1670–1673.



General recommendations: protein storage for crystallization

- ▶ Do not lyophilize your sample
- ▶ If your protein is stable at room temperature, or refrigerated don't freeze the sample
- ▶ If you must freeze your sample freeze and thaw quickly
- ▶ Aliquot your sample to minimize freeze thaw cycles
- ▶ Optimize storage of your protein
 - ▶ Determine best storage formulation and temperature
 - ▶ DLS, SDS-PAGE, SEC, Activity

Deng, J.P., et al. An improved protocol for rapid freezing of protein samples for long-term storage. (2004) *Acta Cryst.* **D60**, 203-204

*Screening:
Identifying initial
crystallization conditions*

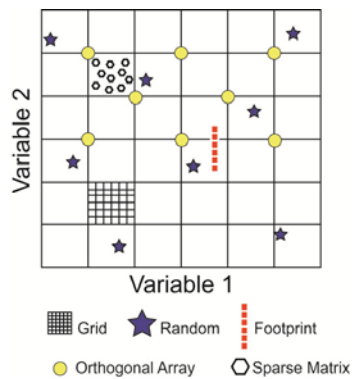
The most common method to supersaturate a protein is to use chemical cocktails to initiate crystallization

- ▶ A cocktail is made up of chemical components:
 - 1 - Buffer
(to control pH)
 - 2 - Precipitating agent
(to supersaturate the protein)
 - 3 - Chemical additive
(to promote favorable intermolecular interactions)

Note: You can have more than one of any of these components

The identification of initial crystallization conditions is a chemical sampling problem

- ▶ Challenge the protein with a diverse set of chemical reagents to identify the finite set that produce crystals

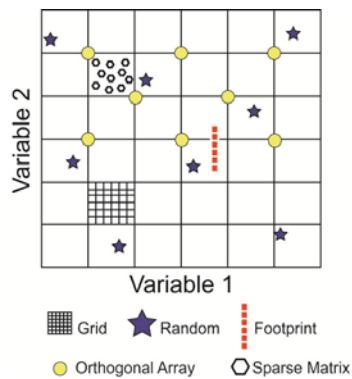


Crystallization screening: the influence of history on current practice. Luft JR, Newman J, Snell EH. (2014) *Acta Cryst. F* **70**(Pt 7):835-53.

If crystallization fails, alter the protein

- ▶ Challenge the protein with a diverse set of chemical reagents to identify the finite set that produce crystals

If crystallization conditions are not identified, then investigate protein formulation and constructs



Crystallization screening: the influence of history on current practice. Luft JR, Newman J, Snell EH. (2014) *Acta Cryst. F* **70**(Pt 7):835-53.

Optimization of initial crystals



The same variables determine optimum conditions to produce large or nanocrystals

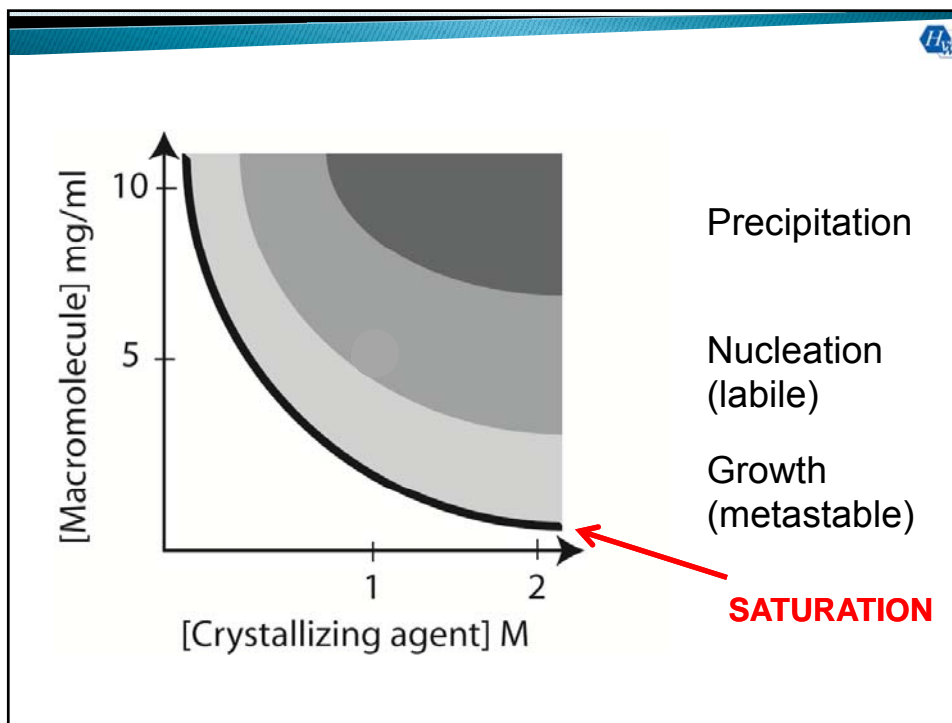
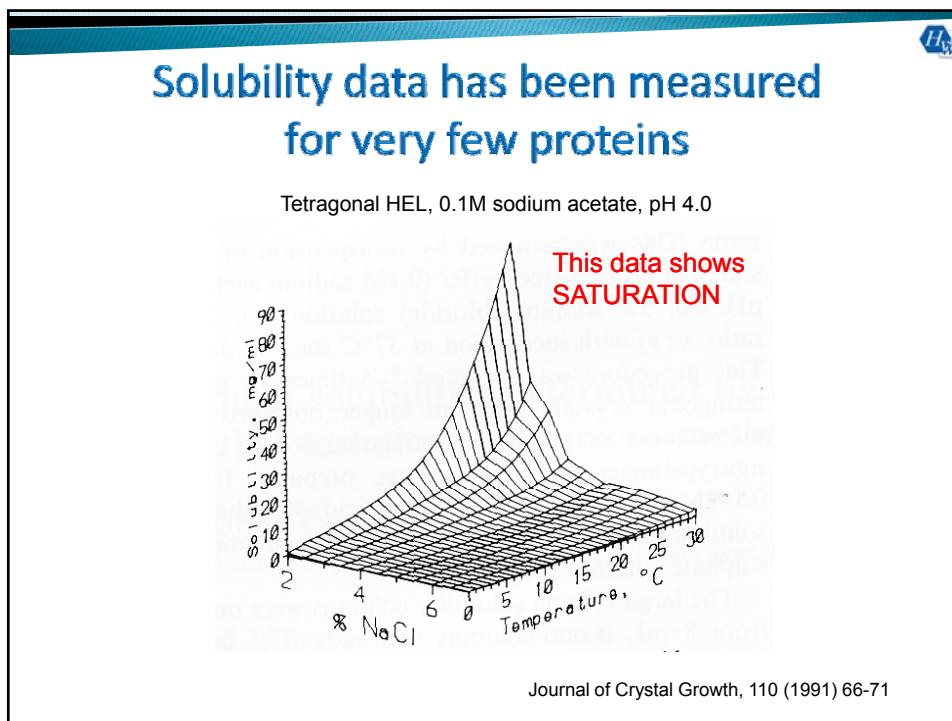
“In preparing large crystals, many details of technique were important. Preliminary exploration of the optimum conditions for maximum yield of small crystals as a function of *pH, salt concentration, protein concentration and temperature* was always necessary”

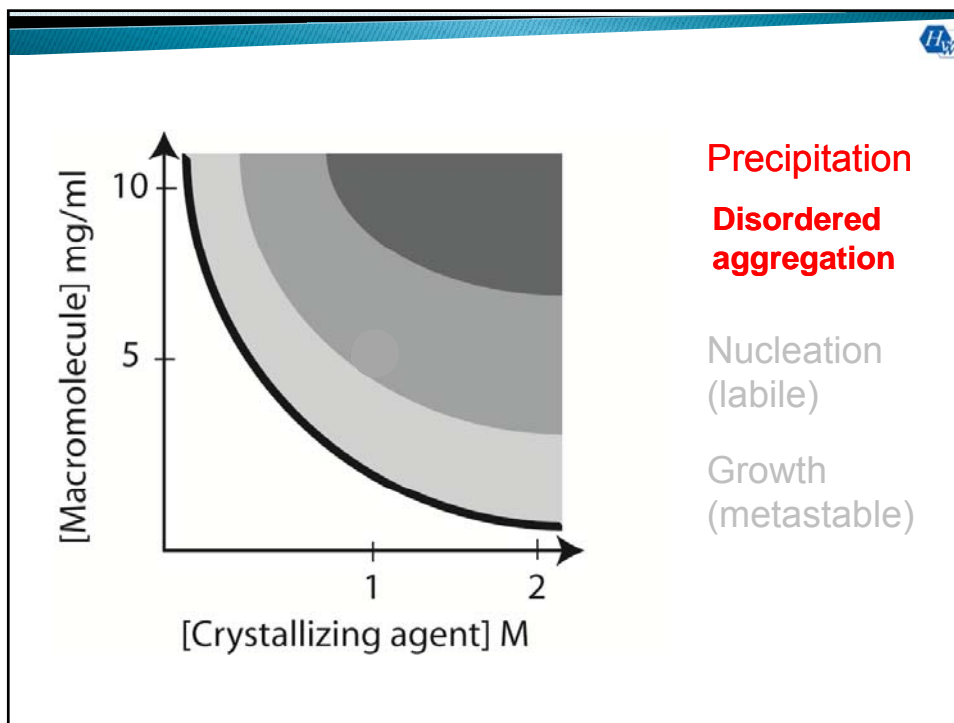
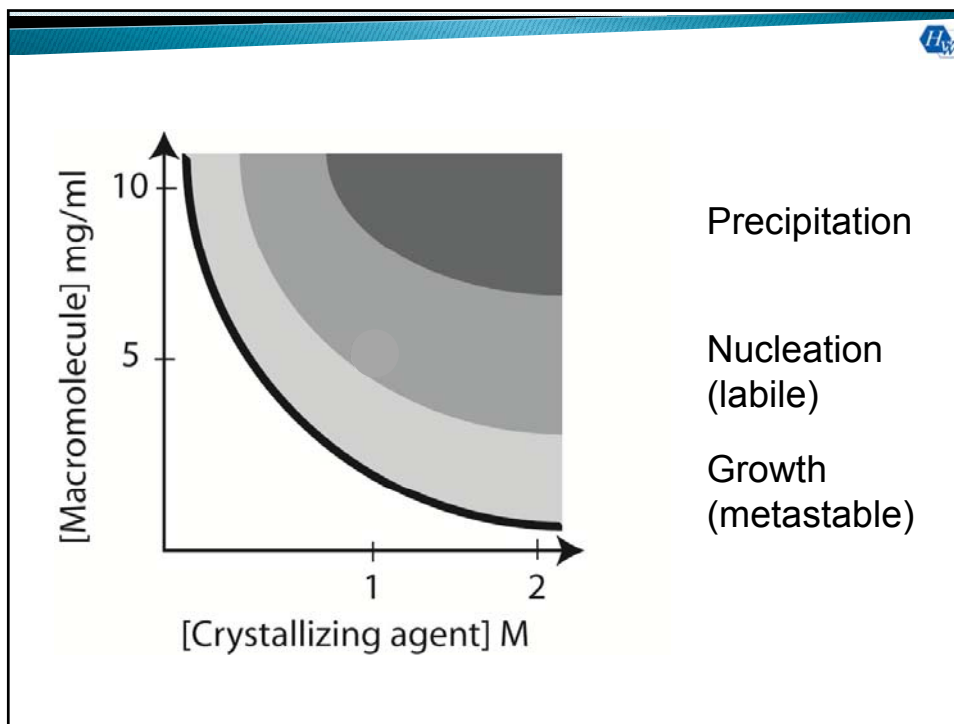
Lewin J: Preparation and Properties of Serum and Plasma Proteins. XXX. Crystalline Derivatives of Human Serum Albumin and of Certain Other Proteins. *Journal of the American Chemical Society* 1951, 73:3906–3911.

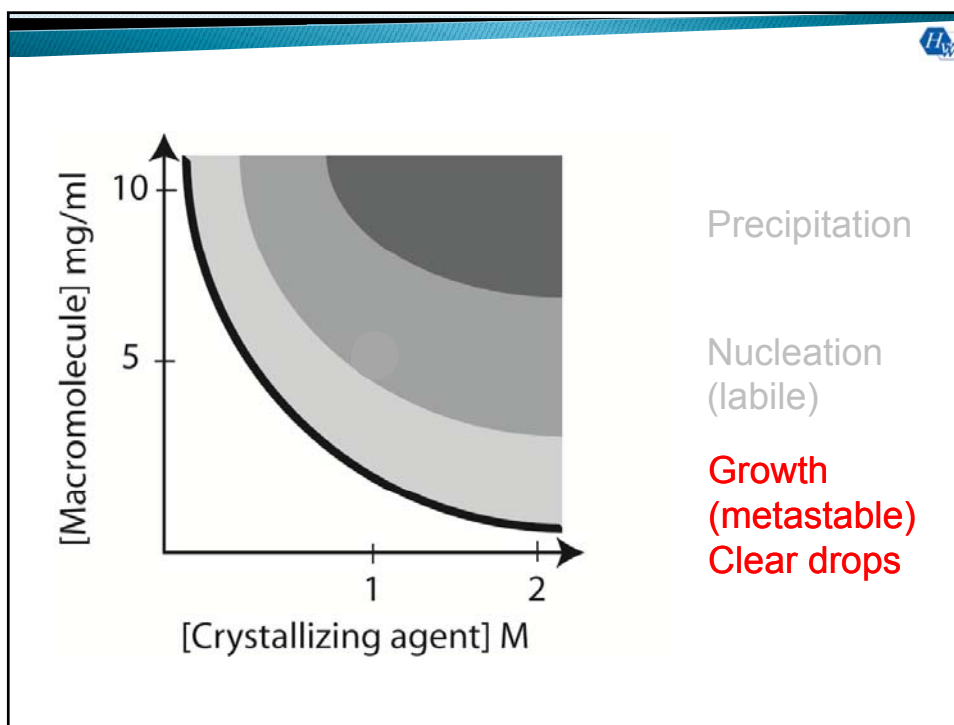
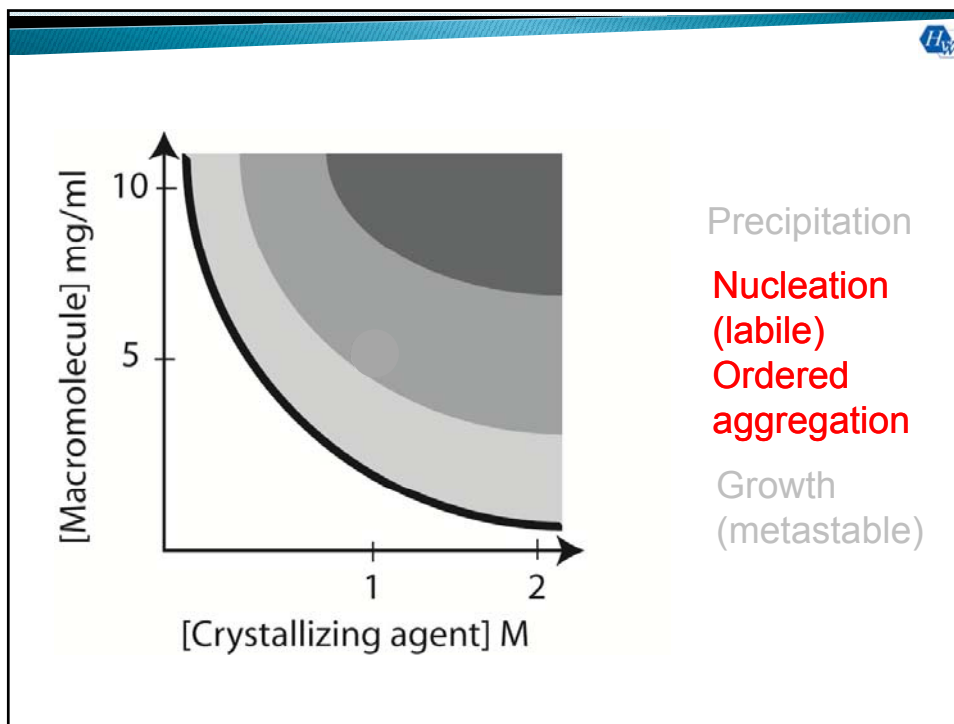
Phase diagrams

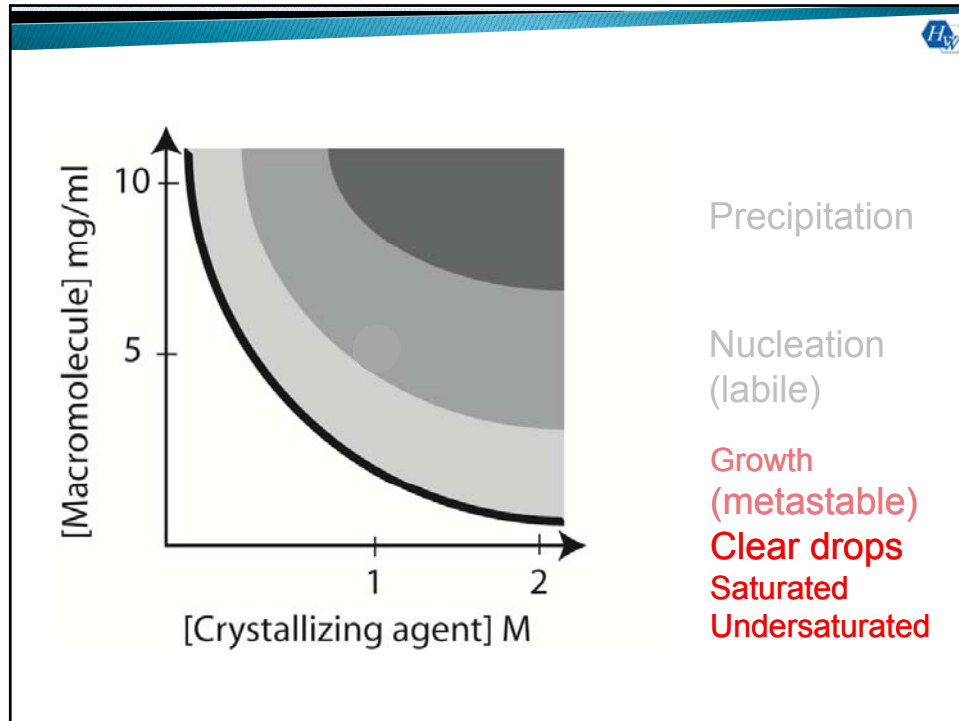
A guide to optimization

Seeing the forest, not just the trees









In simplest terms,
the phase diagram is a collective view of
experiments' outcomes
as they relate to solubility



Consider the **chemical design** of the crystallization experiments and their respective **outcomes** as two related, but distinct sets of data.



The chemical design of a crystallization experiment is like a deck of cards

- ▶ You can look at cards separately, one at a time...

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The chemical design of a crystallization experiment is like a deck of cards

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The chemical design of a crystallization experiment is like a deck of cards

- ▶ You can look at cards separately, one at a time...

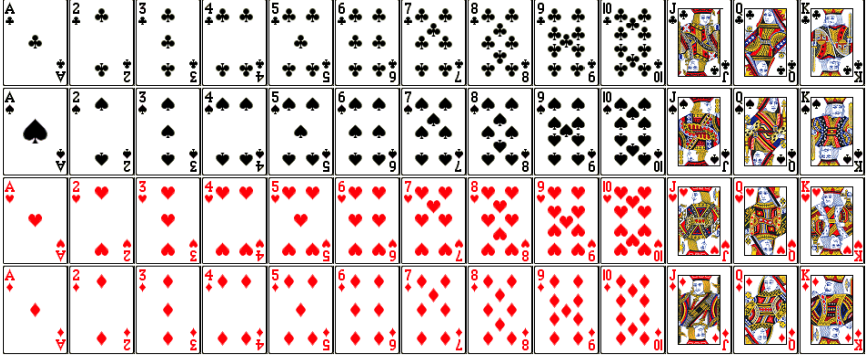



You can randomly arrange cards with no regard for how they relate





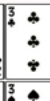























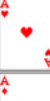










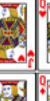

















Or, you can organize the cards according to how they relate










Let's represent the cards using a simple coordinate system

	A	2	3	4	5	6	7	8	9	10	J	Q	K
♣													
♠													
♥													
♦													








Let's represent the cards using a simple coordinate system


	A	2	3	4	5	6	7	8	9	10	J	Q	K
													
													
													
													




Let's represent the cards using a simple coordinate system

	A	2	3	4	5					10	J	Q	K
													
													
													
													







Let's represent the cards using a simple coordinate system

	A	2	3	4	5				
♣									
♠									
♥									
♦									

Let's represent the cards using a simple coordinate system

	A	2	3	4	5				
♣									
♠									
♥									
♦									

Let's represent the cards using a simple coordinate system

	A	2	3	4	5		10	J	Q	K
♣										
♠										
♥		●								
♦										

Let's represent the cards using a simple coordinate system

	A	2	3	4	5		10	J	Q	K
♣										
♠										
♥		●								
♦										

Let's represent the cards using a simple coordinate system

	A	2	3	4	5	6	7	8	9	10	J	Q	K
♣													
♠													
♥		●											
♦													

We can represent the entire deck of cards

	A	2	3	4	5	6	7	8	9	10	J	Q	K
♣	●	●	●	●	●	●	●	●	●	●	●	●	●
♠	●	●	●	●	●	●	●	●	●	●	●	●	●
♥	●	●	●	●	●	●	●	●	●	●	●	●	●
♦	●	●	●	●	●	●	●	●	●	●	●	●	●

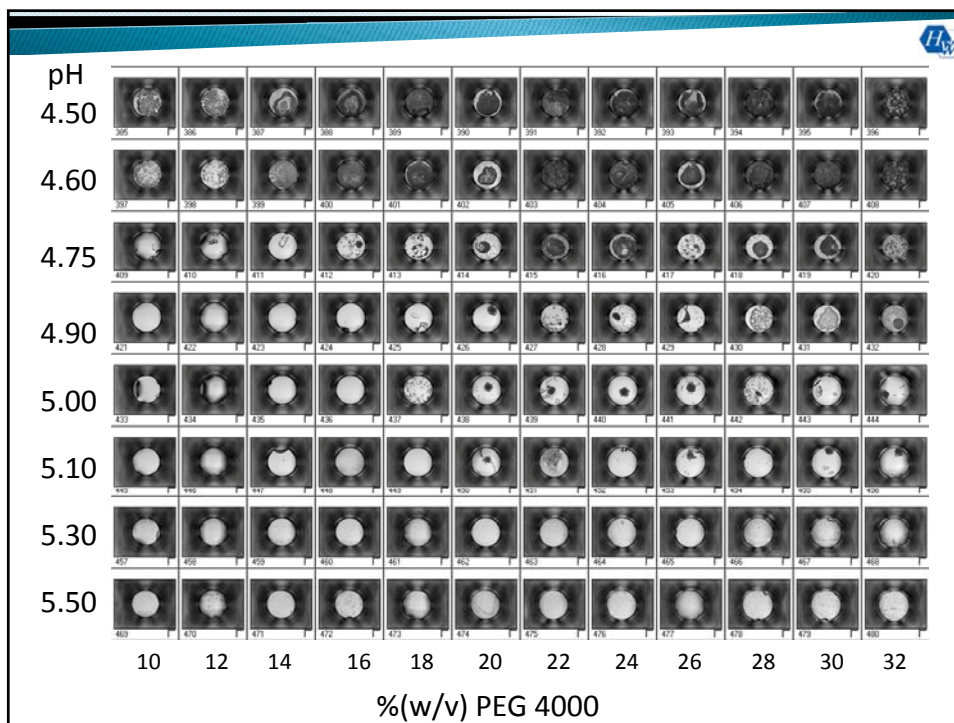
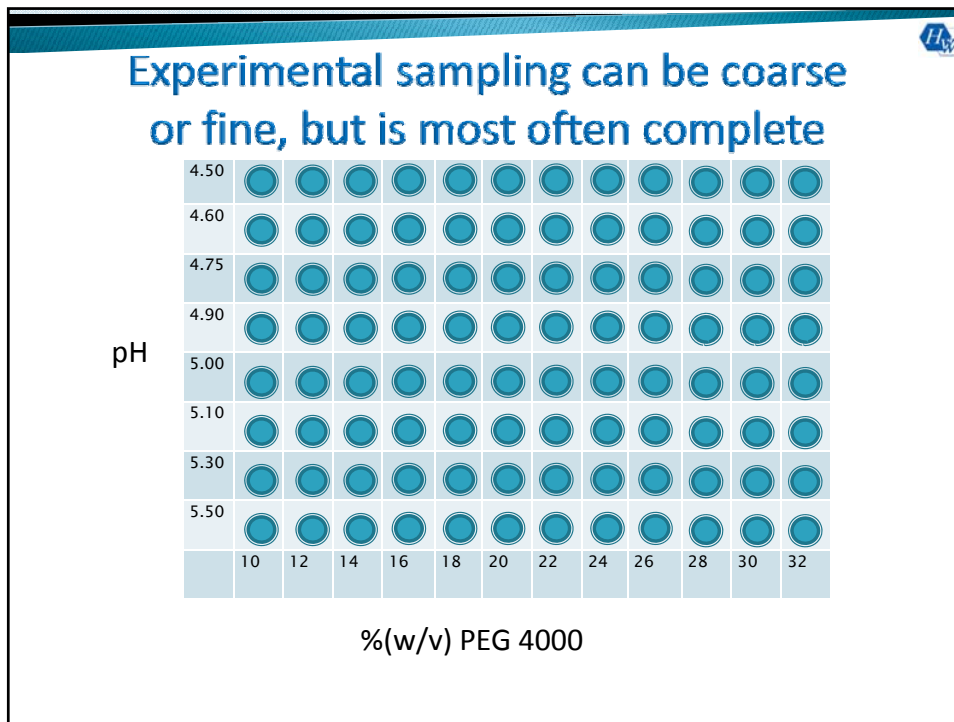
We can represent the entire deck of cards
This is a 'grid screen'

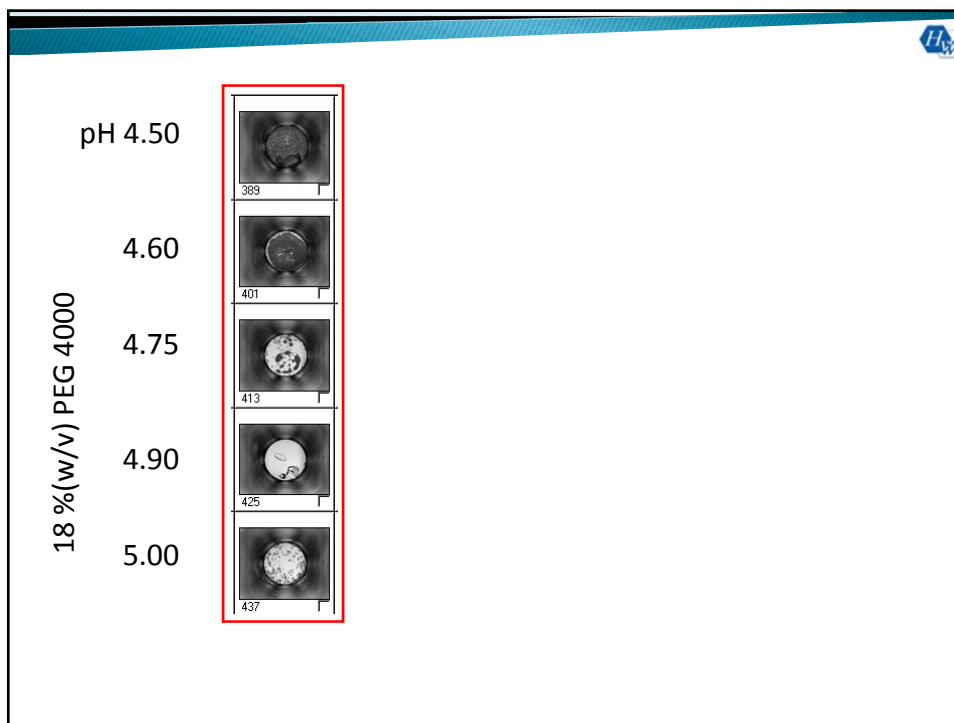
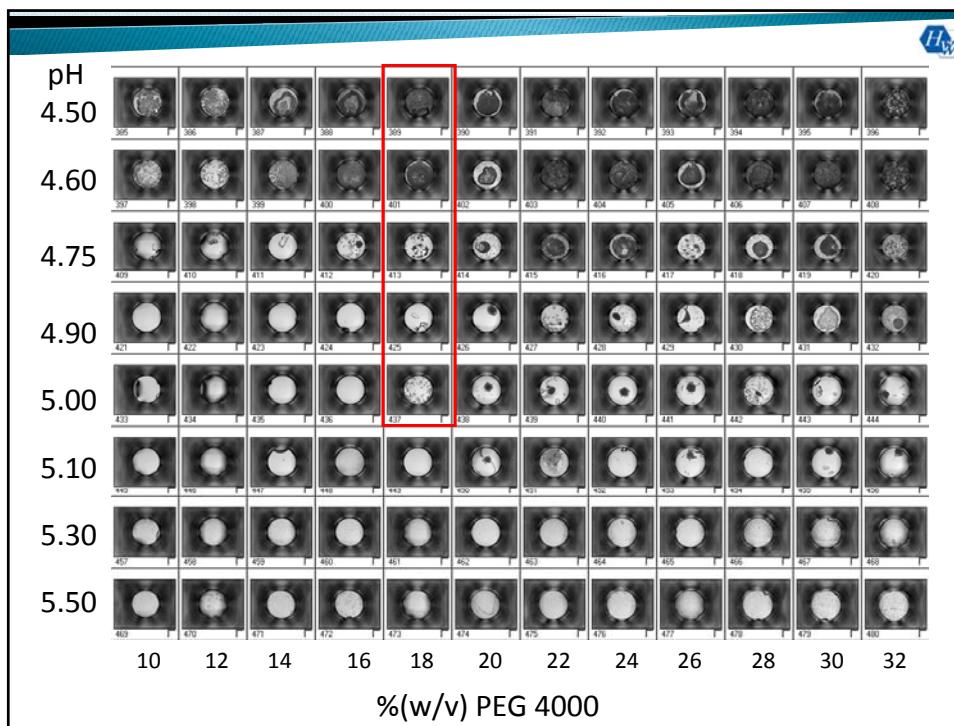
	A	2	3	4	5	6	7	8	9	10	J	Q	K
♣	●	●	●	●	●	●	●	●	●	●	●	●	●
♠	●	●	●	●	●	●	●	●	●	●	●	●	●
♥	●	●	●	●	●	●	●	●	●	●	●	●	●
♦	●	●	●	●	●	●	●	●	●	●	●	●	●

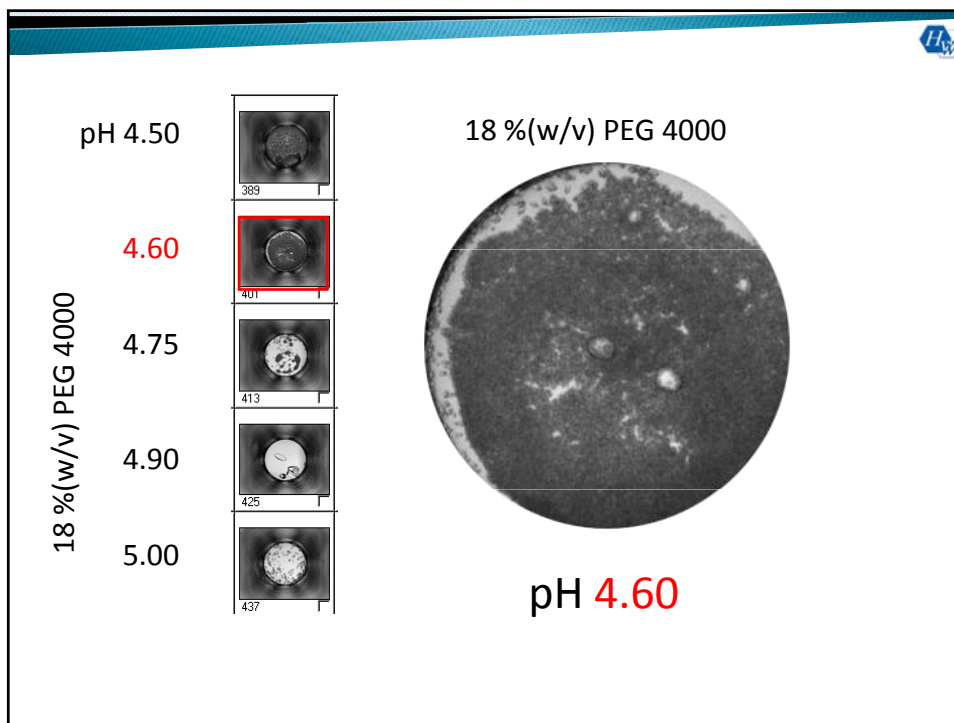
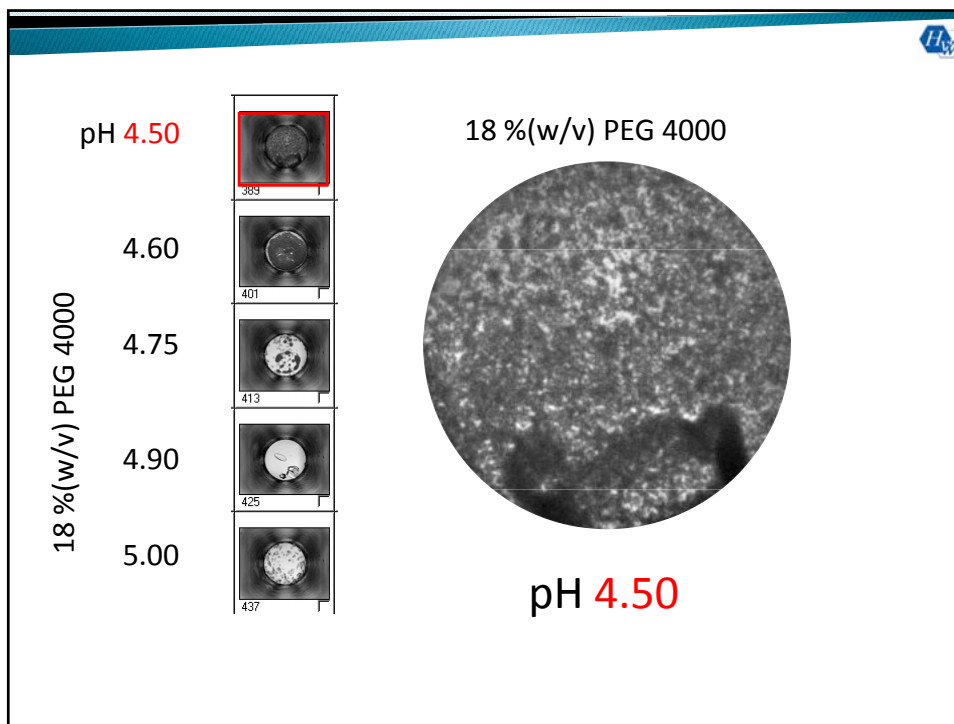
A grid screen incrementally samples a range of pH values and [precipitant]

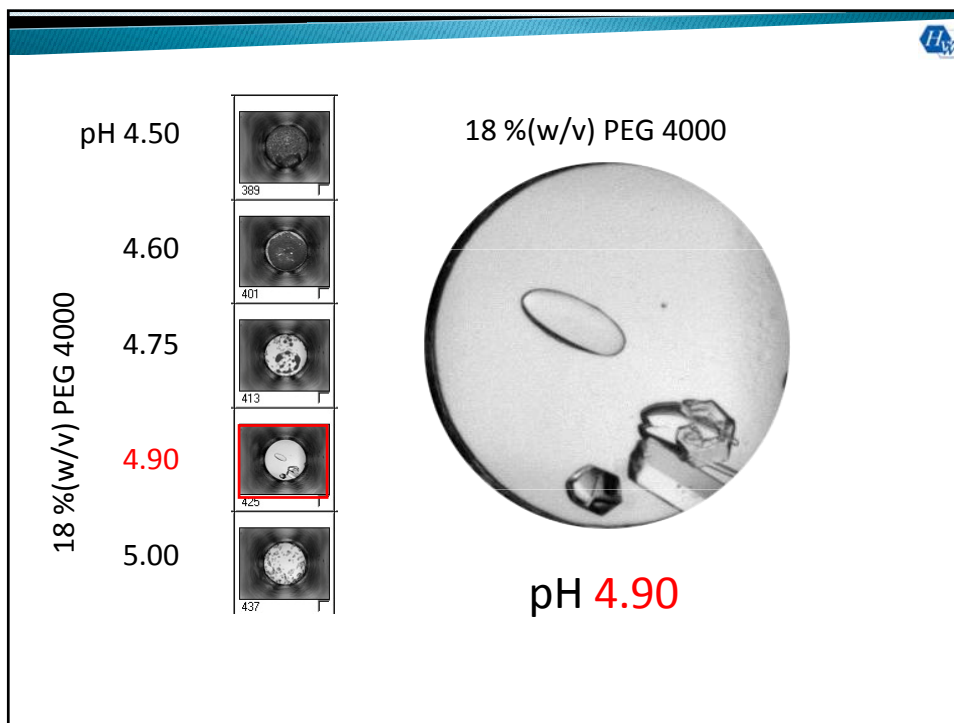
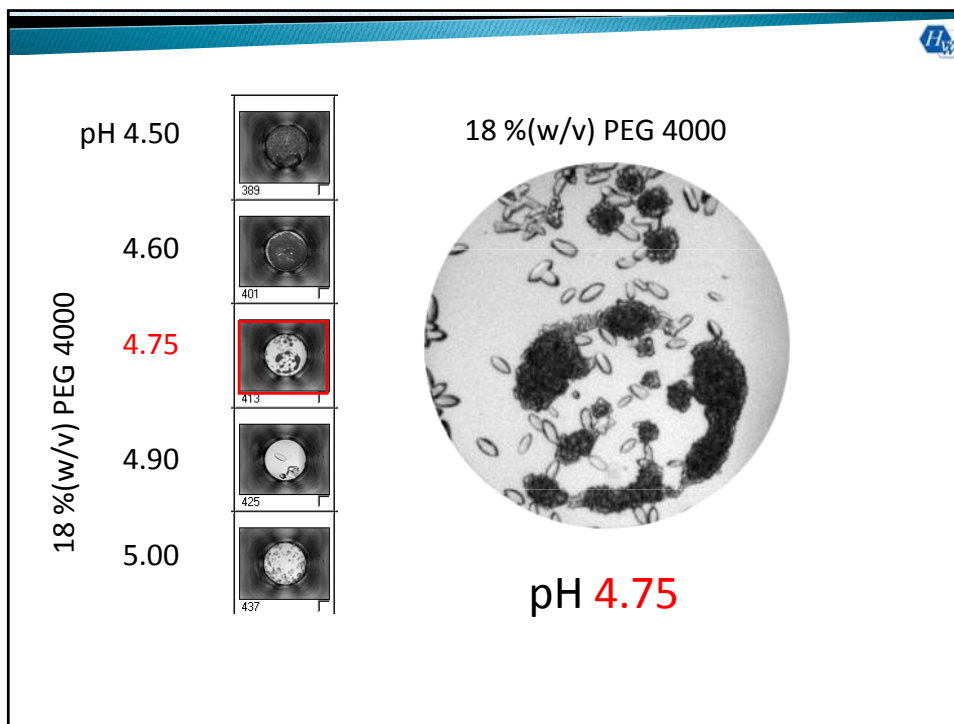
pH	10	12	14	16	18	20	22	24	26	28	30	32
4.50												
4.60												
4.75												
4.90												
5.00												
5.10												
5.30												
5.50												

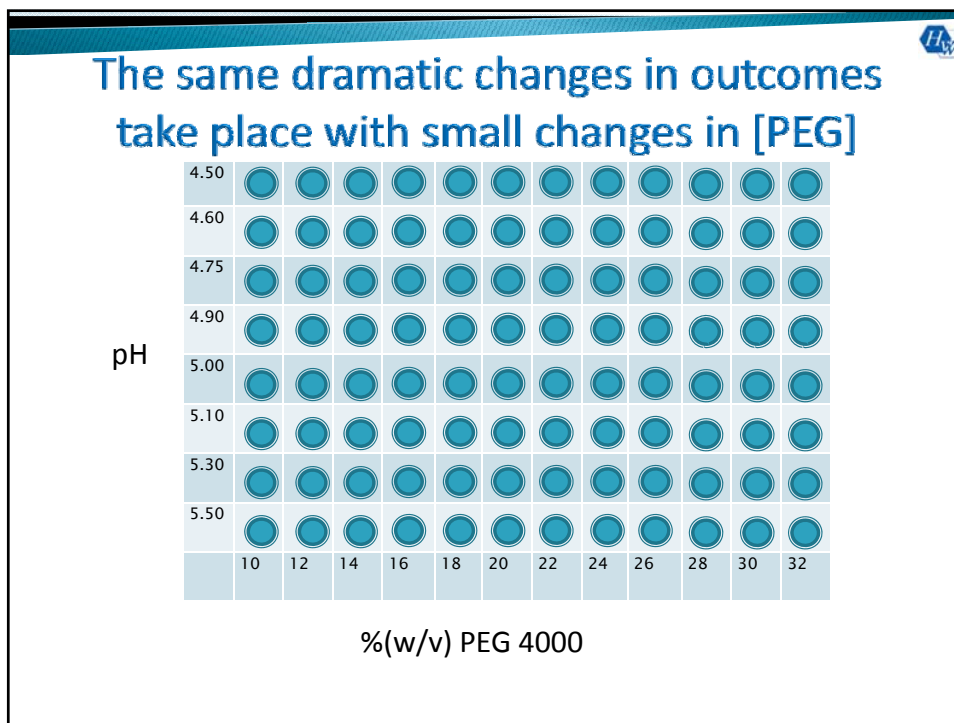
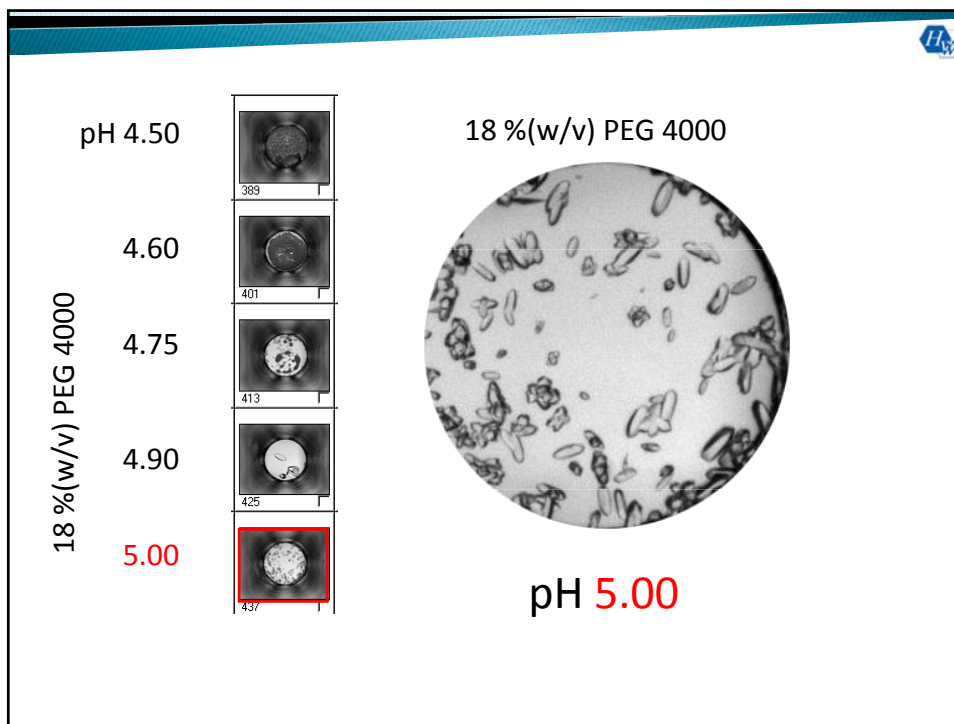
Methods: A Companion to Methods in Enzymology 1 (1), (1990) pp. 31-37.

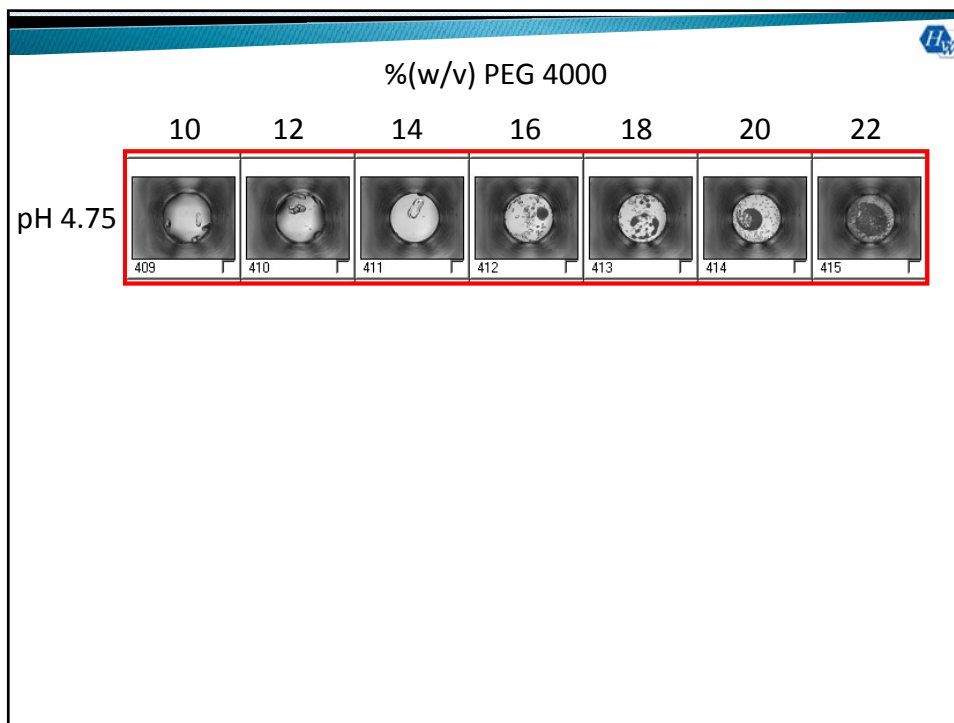
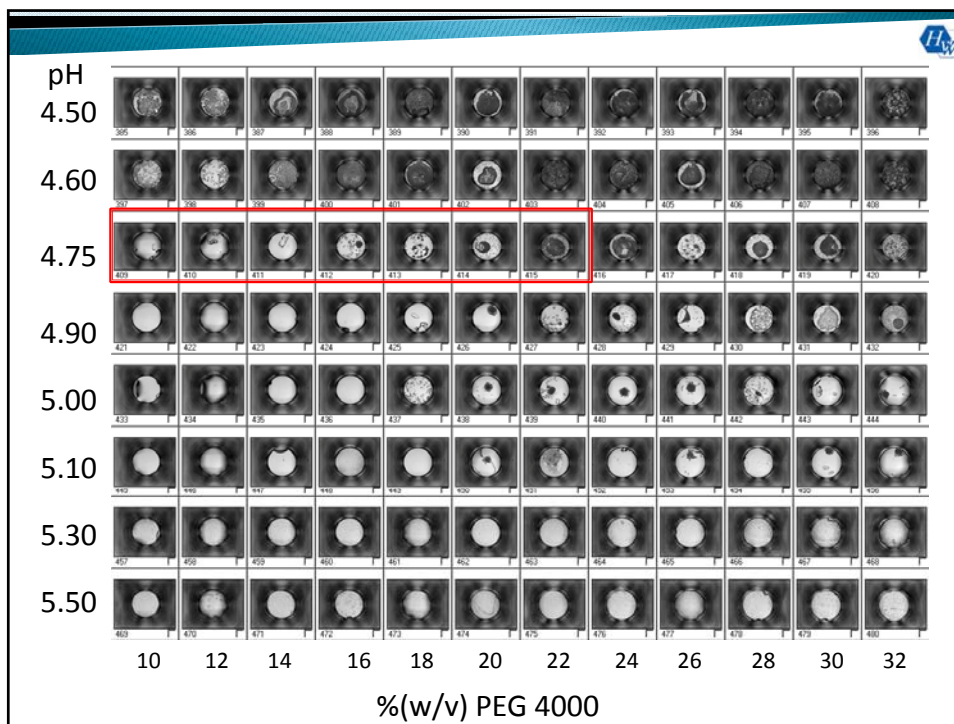


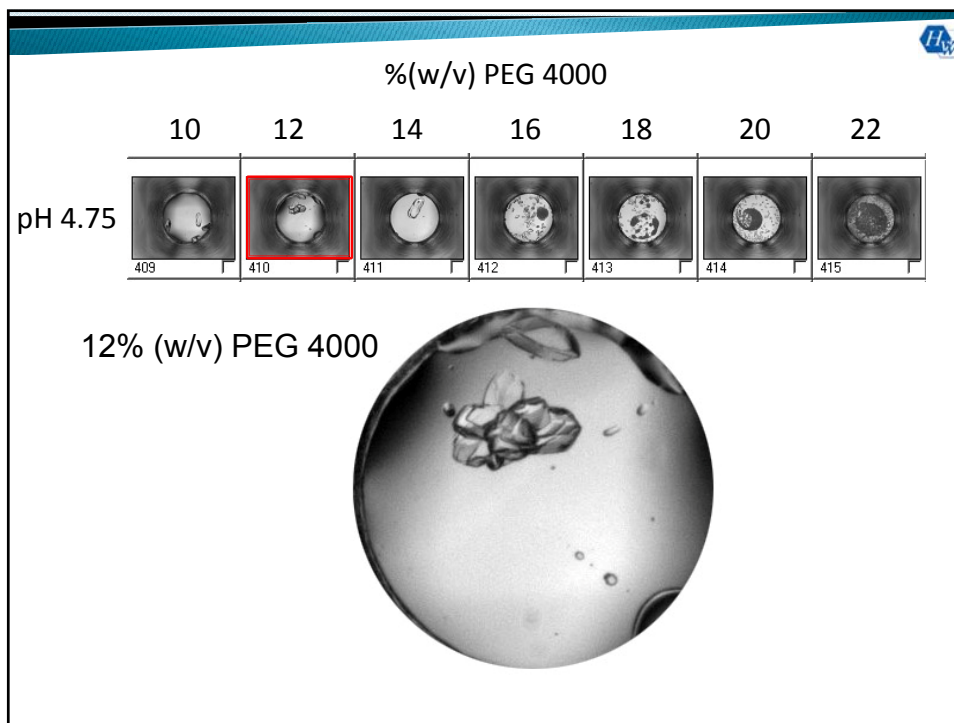
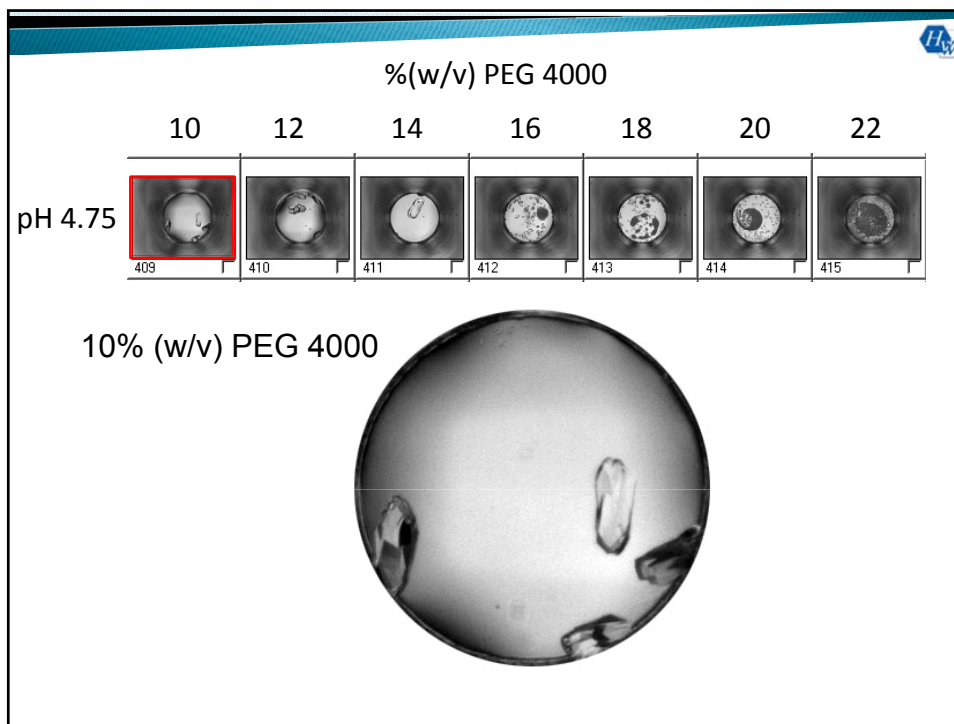


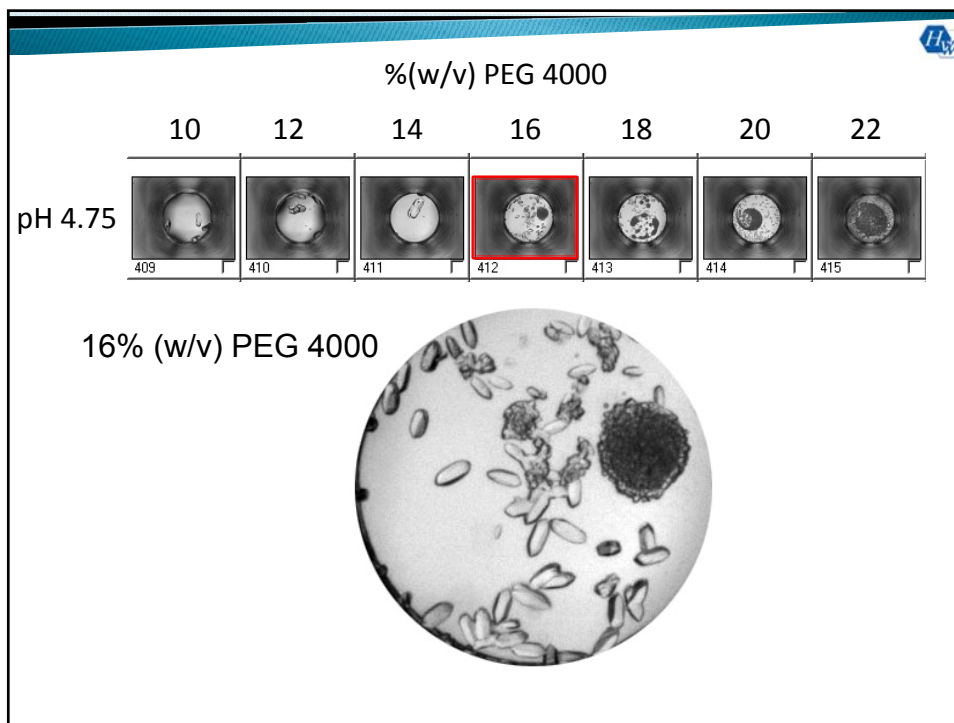
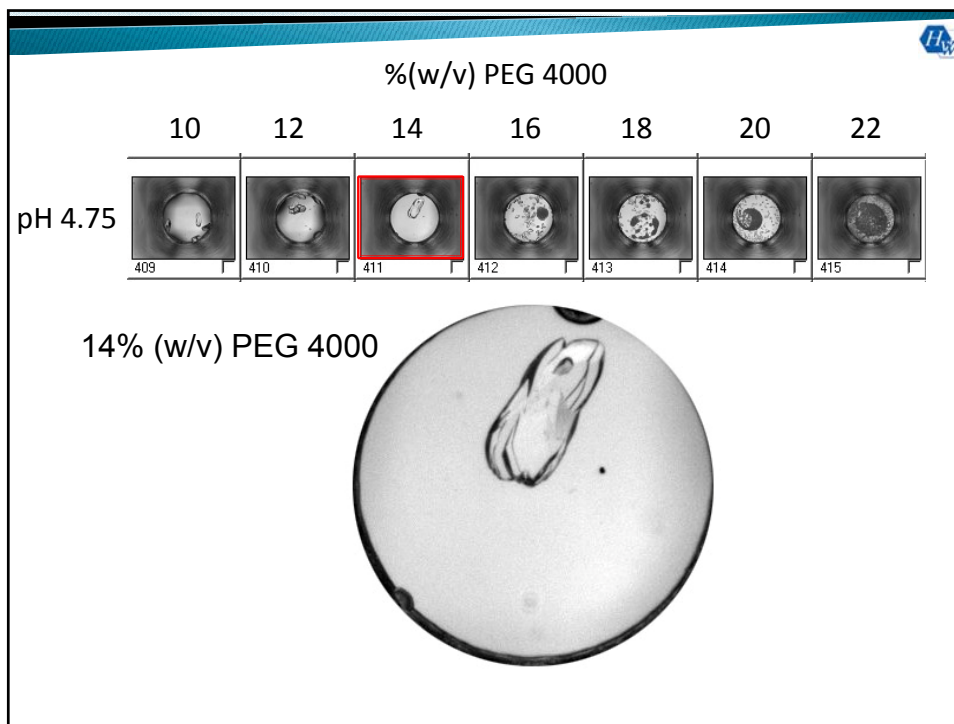


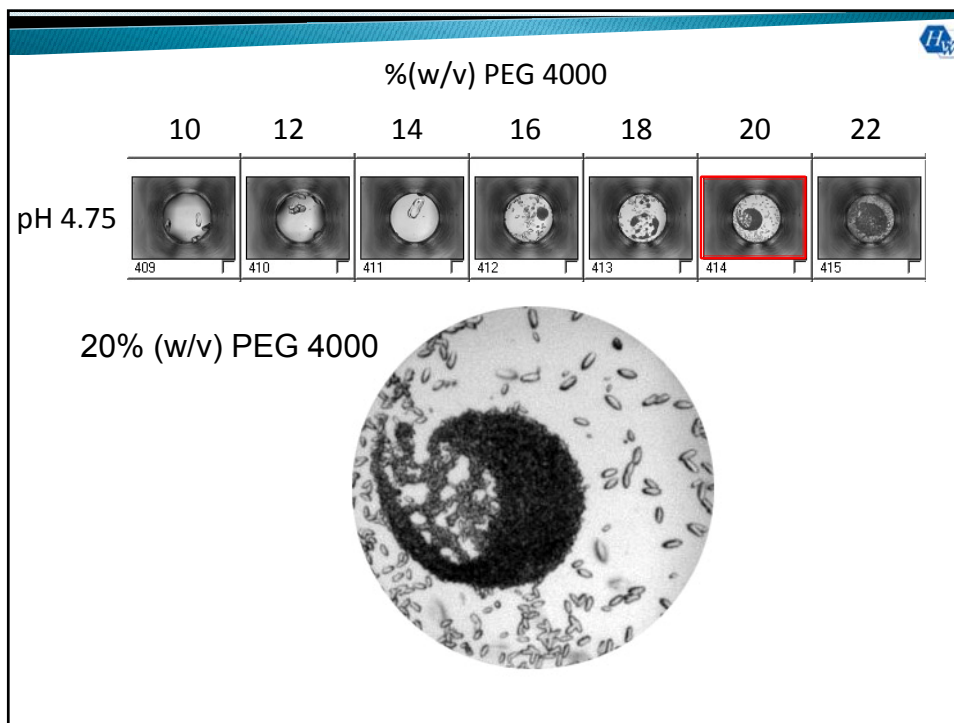
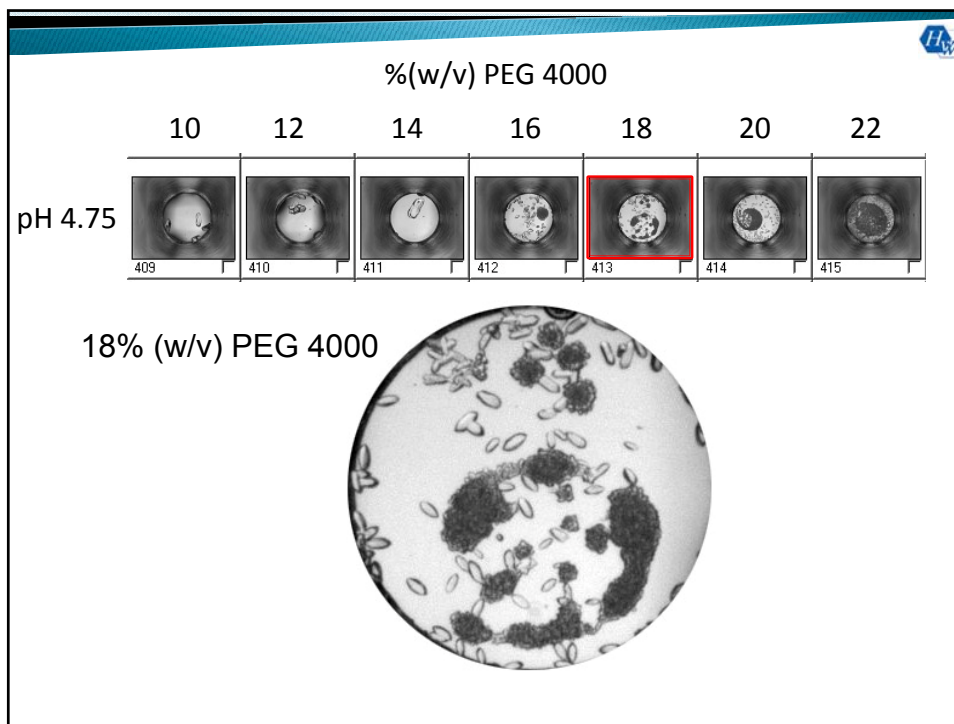


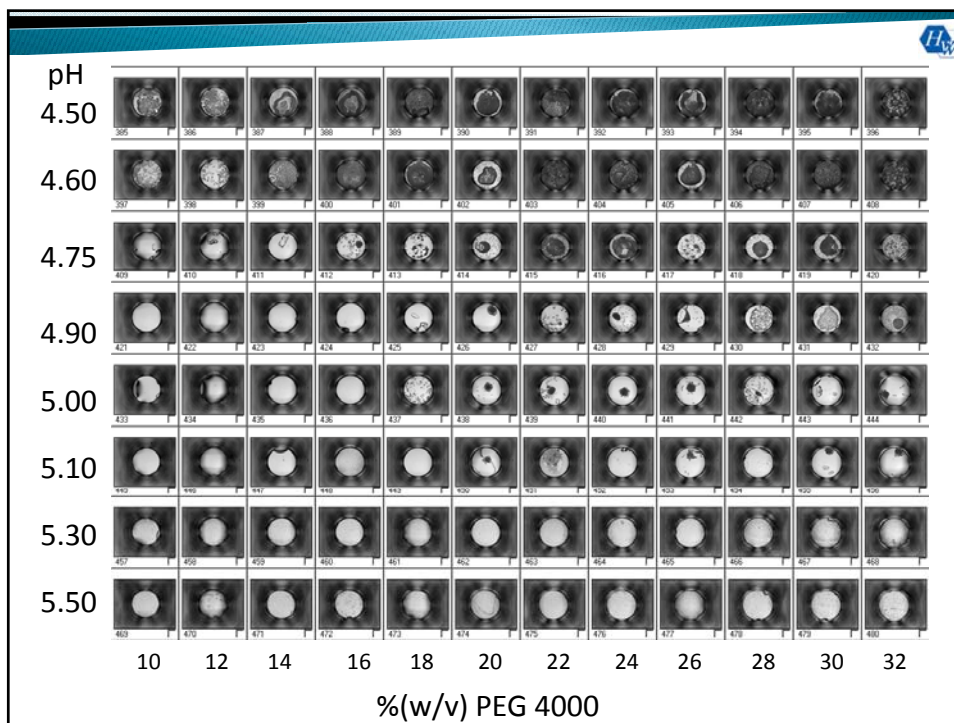
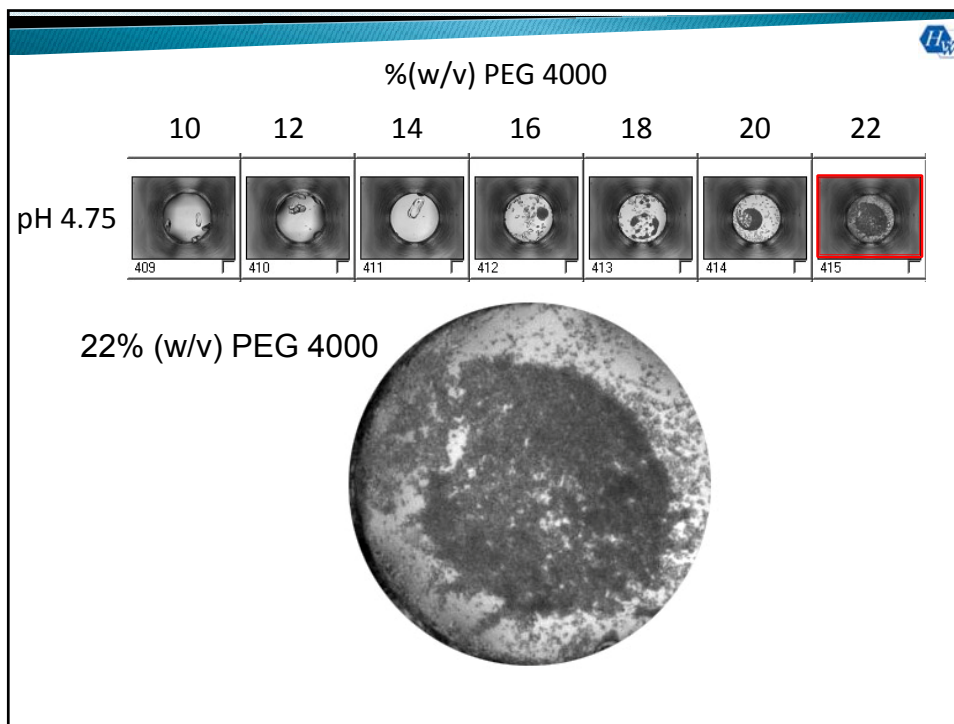


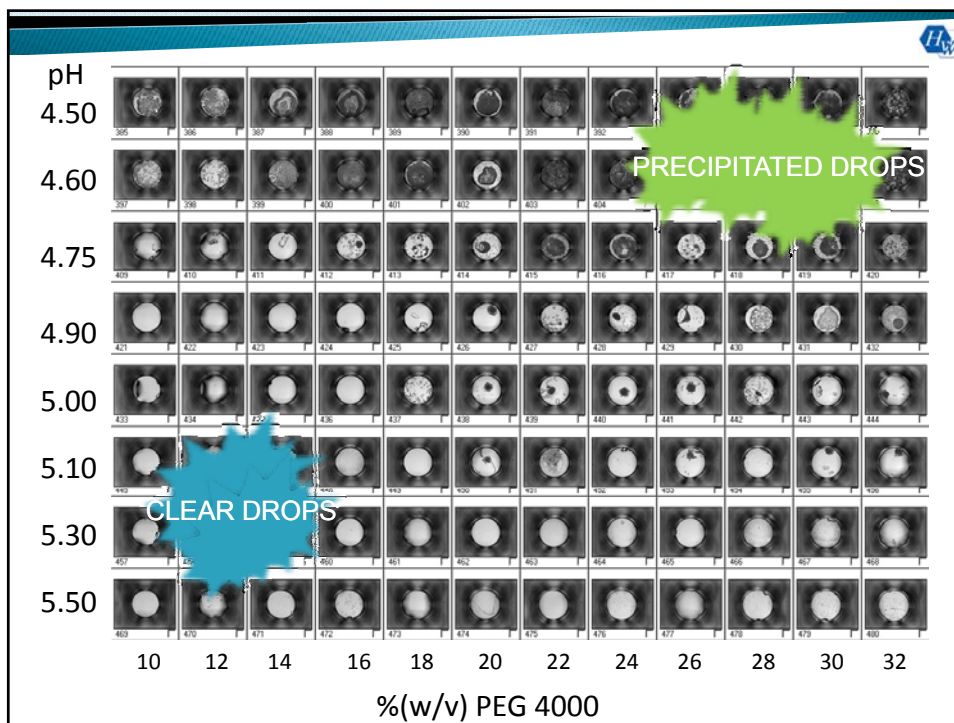
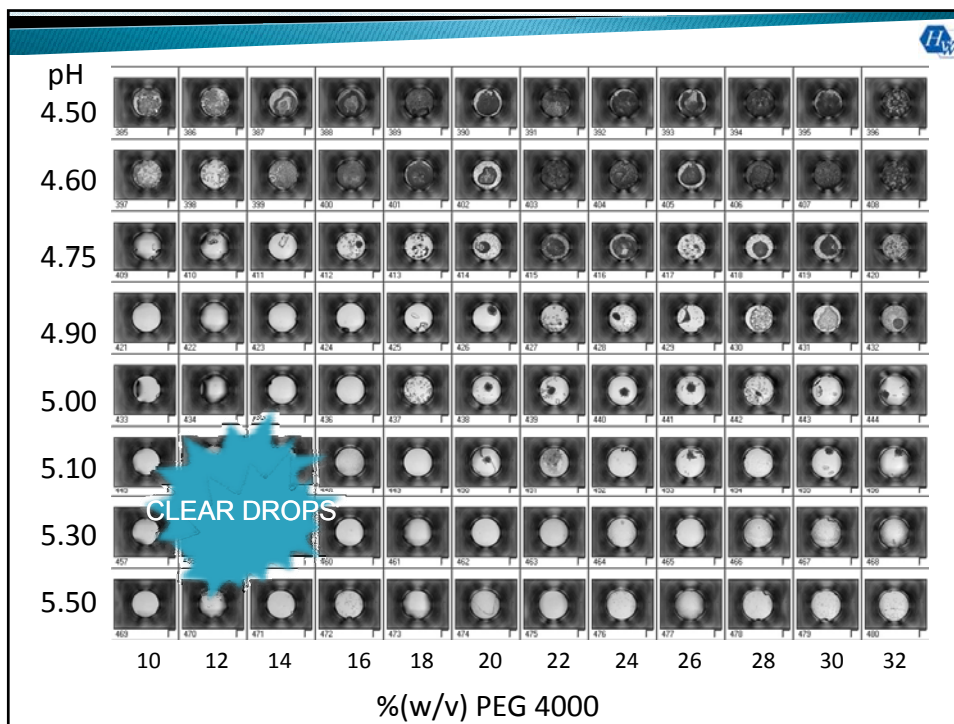


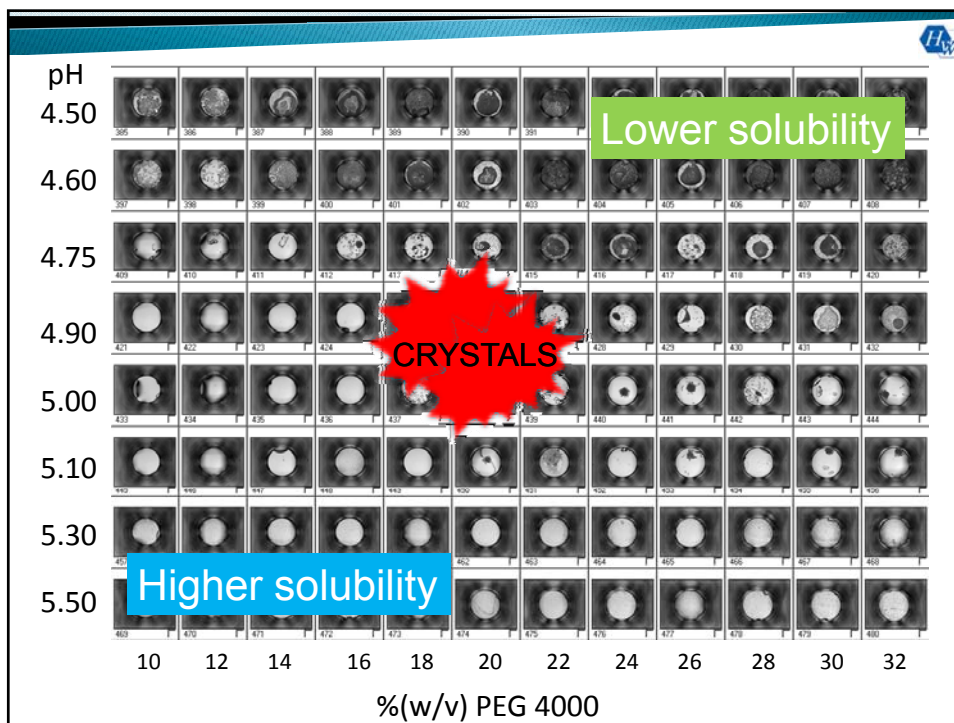
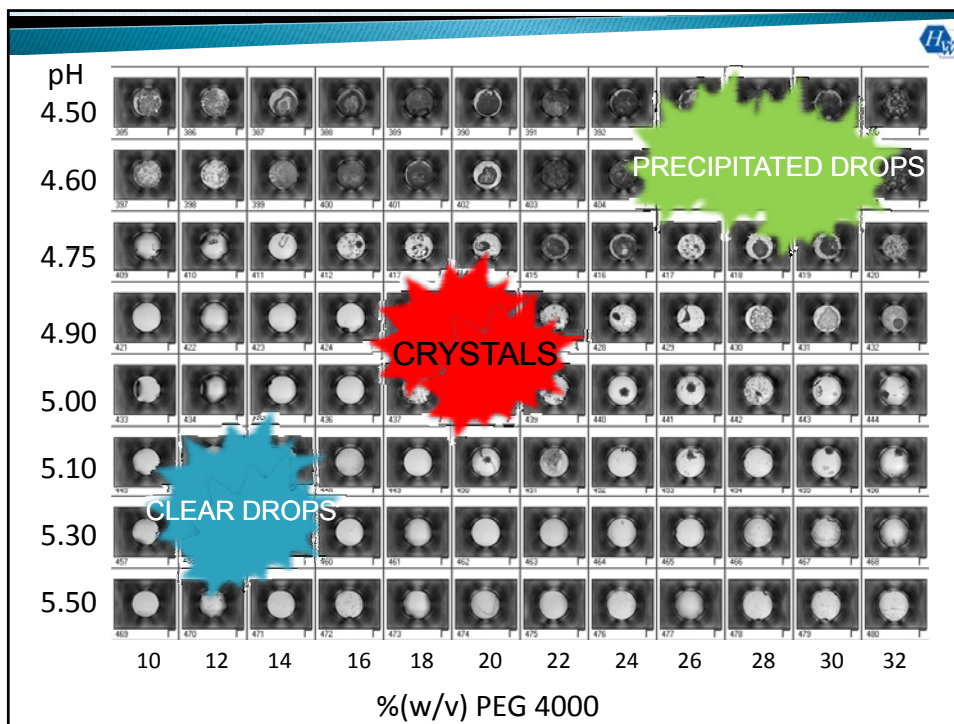


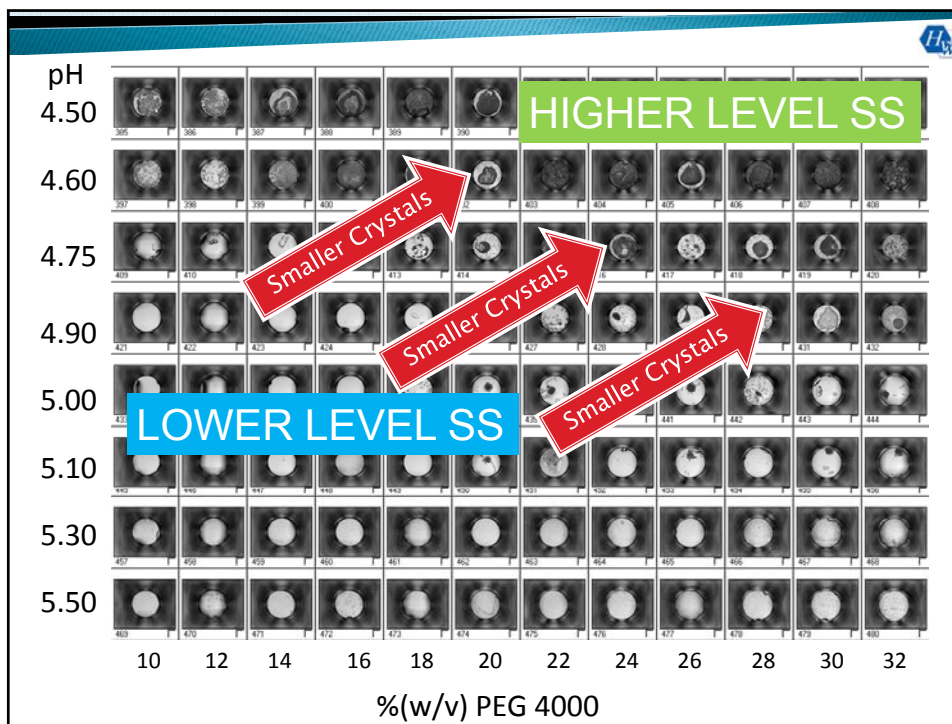












In most instances, there is an incomplete grid sampling of chemical space

We can incompletely sample the deck of cards and still understand the layout

	A	2	3	4	5	6	7	8	9	10	J	Q	K
♣	●		●				●		●		●		
♠	●			●				●	●			●	●
♥		●				●		●		●	●	●	
♦		●	●		●		●		●	●			●

Let's use the card analogy to represent the chemical components in the cocktails

	PEG 400	Na Citrate	Na Acetate	MES	Bis-Tris Propane	HEPES	Tris	TAPS	CAPS
	Lithium								
pH		4.2	5	6	7	7.5	8	9	10
bromide	20%								
	40%								
	80%								

Nagel, R. M., Luft, J. R., and Snell, E. H. (2008). AutoSherlock: a program for effective crystallization data analysis. *J. Appl. Cryst.* **41**: 1173-1176.

Snell, E. H., Nagel, R. M., Wojtaszyk, A., O'Neill, H., Wolfley, J., and Luft, J. R. (2008). The application and use of Chemical Space Mapping to interpret crystallization screening results. *Acta Cryst.* **D64**: 1240-1249.

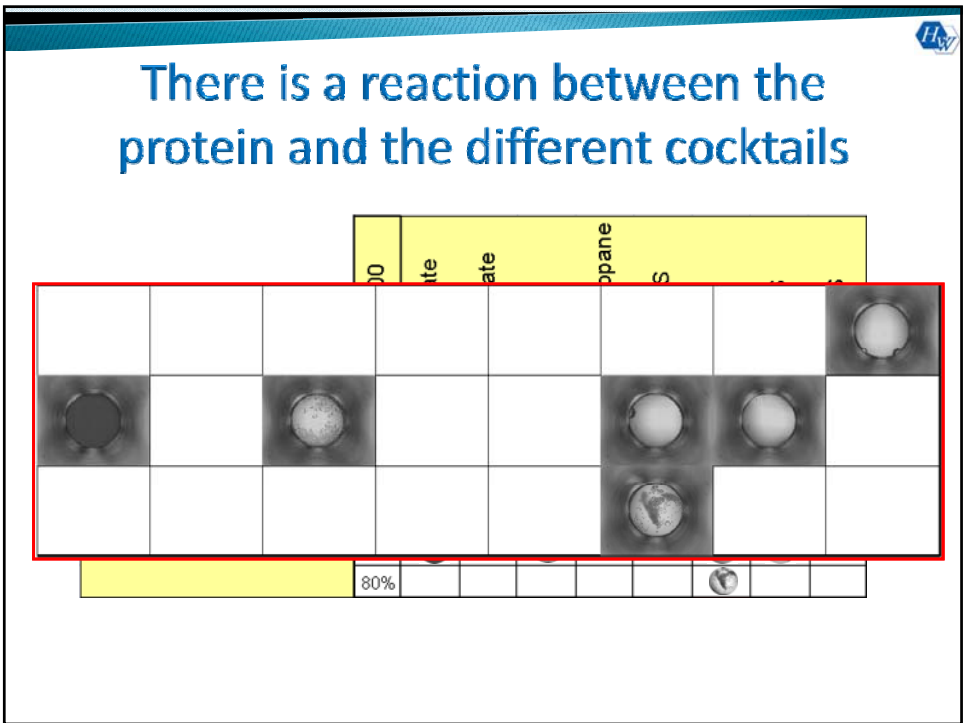
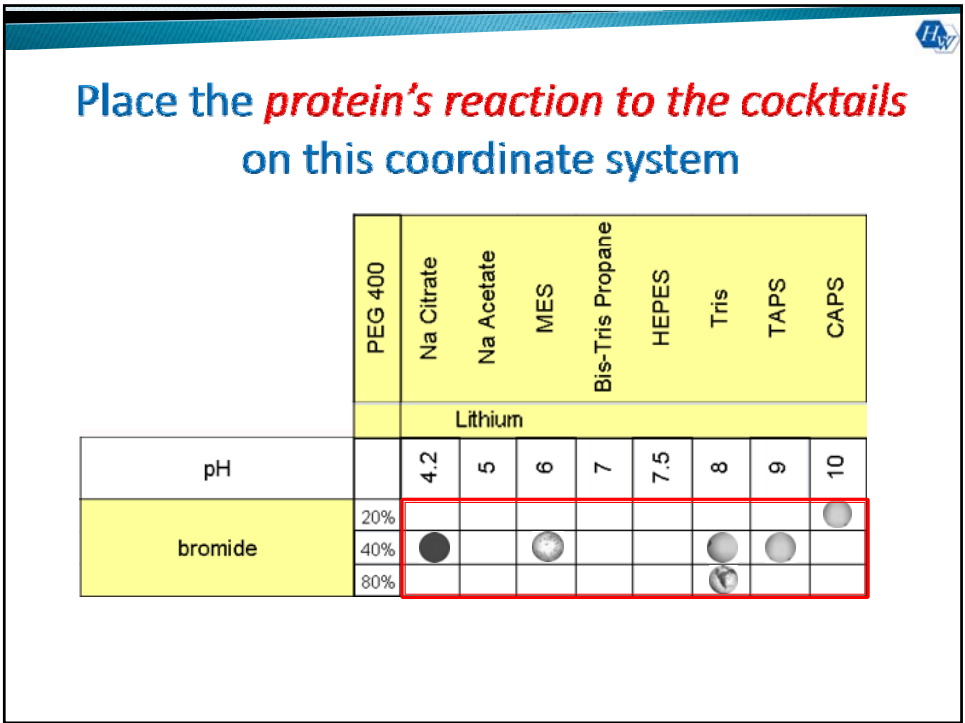
We can represent the cocktails on a coordinate system

	PEG 400	Na Citrate	Na Acetate	MES	Bis-Tris Propane	HEPES	Tris	TAPS	CAPS
		Lithium							
pH		4.2	5	6	7	7.5	8	9	10
bromide	20%								●
	40%	●		●			●	●	
	80%						●		


Note there is a lot of white space (chemistries we did not sample).

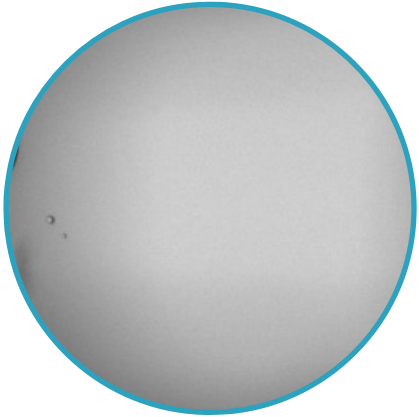
Place the *protein's reaction to the cocktails* on this coordinate system.

	PEG 400	Na Citrate	Na Acetate	MES	Bis-Tris Propane	HEPES	Tris	TAPS	CAPS
		Lithium							
pH		4.2	5	6	7	7.5	8	9	10
bromide	20%								●
	40%	●		●			●	●	
	80%						●		




Color-coding these reactions
simplifies interpretation

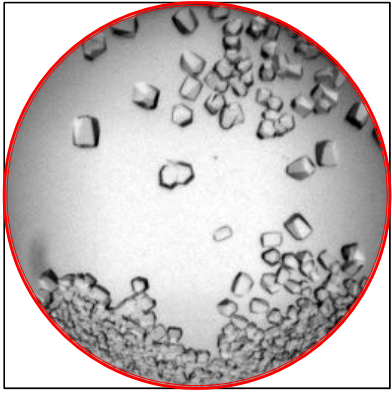
 Clear



A circular inset showing a clear, colorless liquid in a test tube, representing a 'Clear' reaction result.


Color-coding these reactions
simplifies interpretation

 Crystals

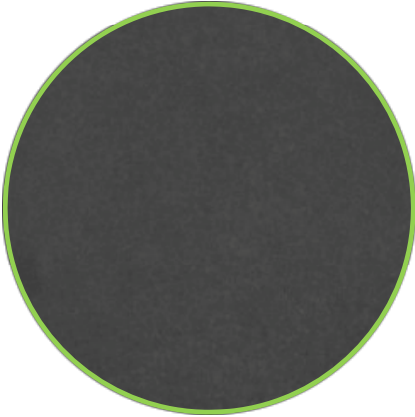


A circular inset showing a test tube containing a white precipitate of crystals, representing a 'Crystals' reaction result.







Color-coding these reactions simplifies interpretation

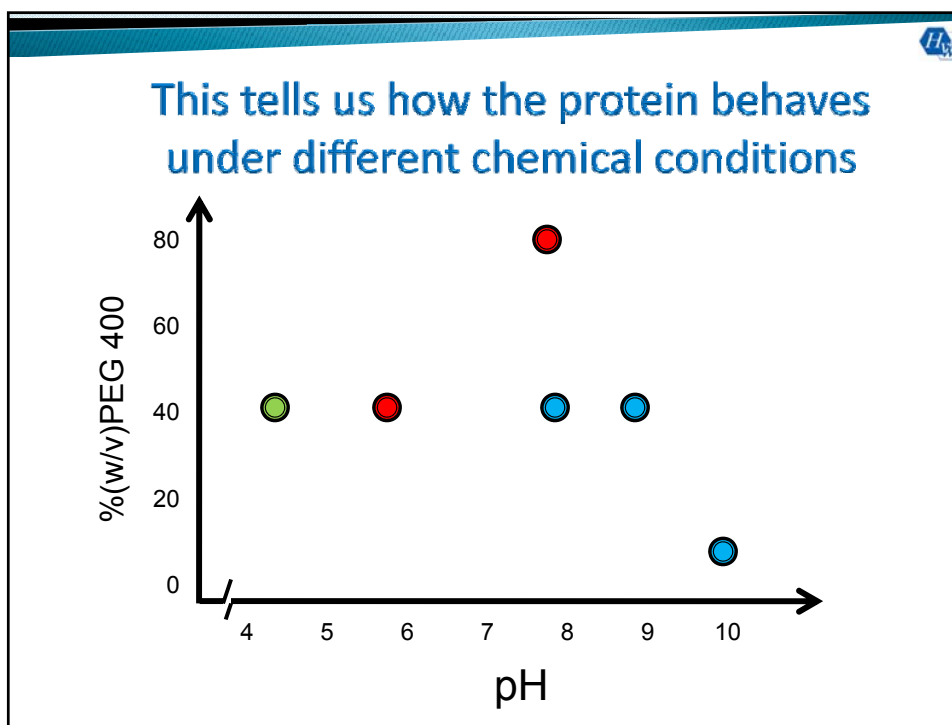
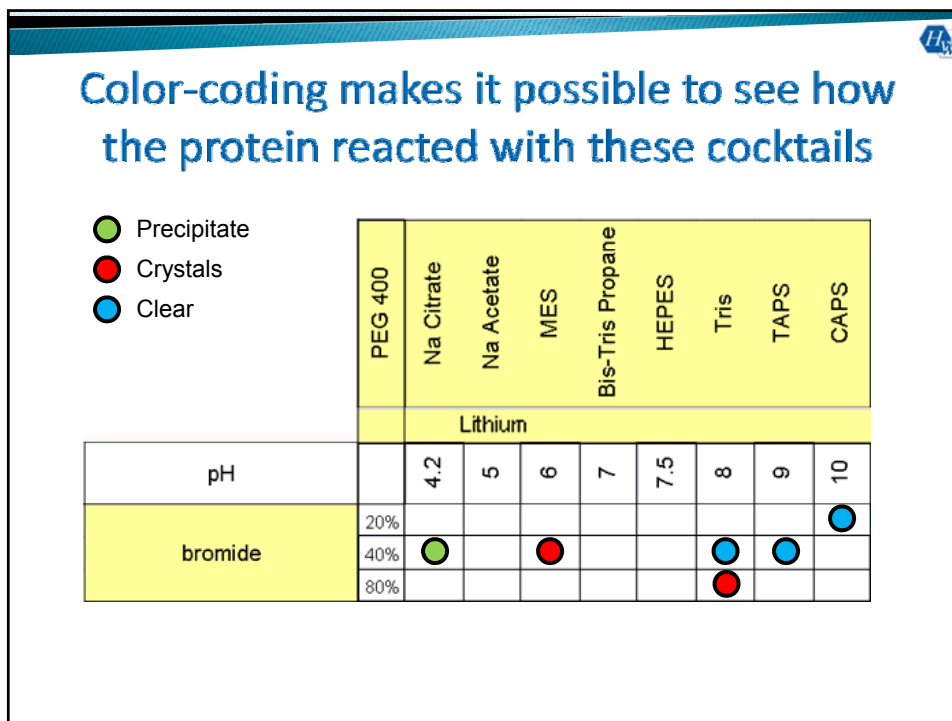


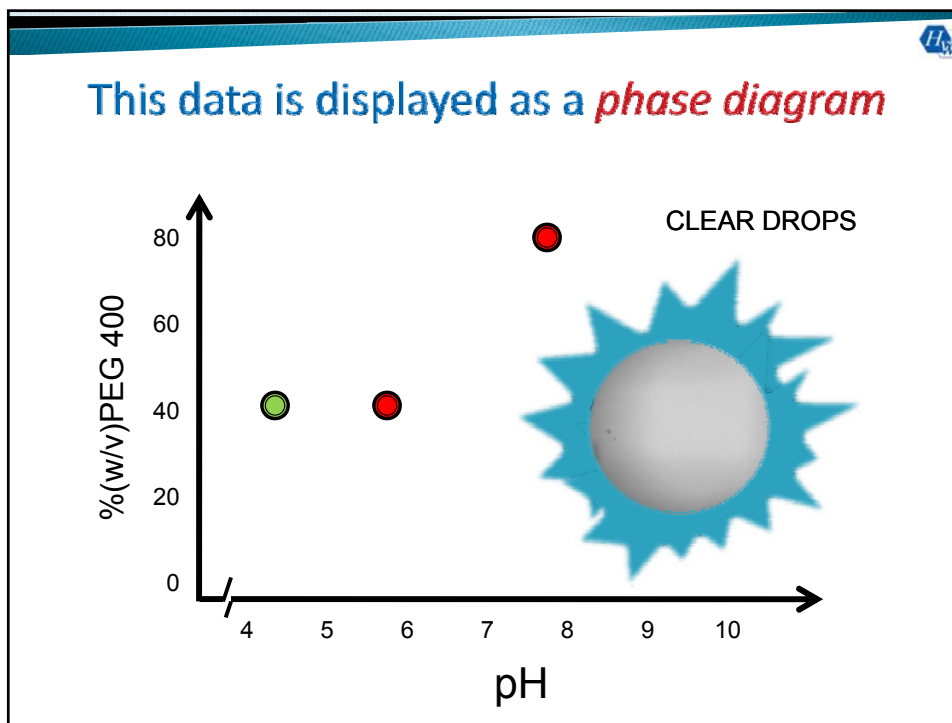
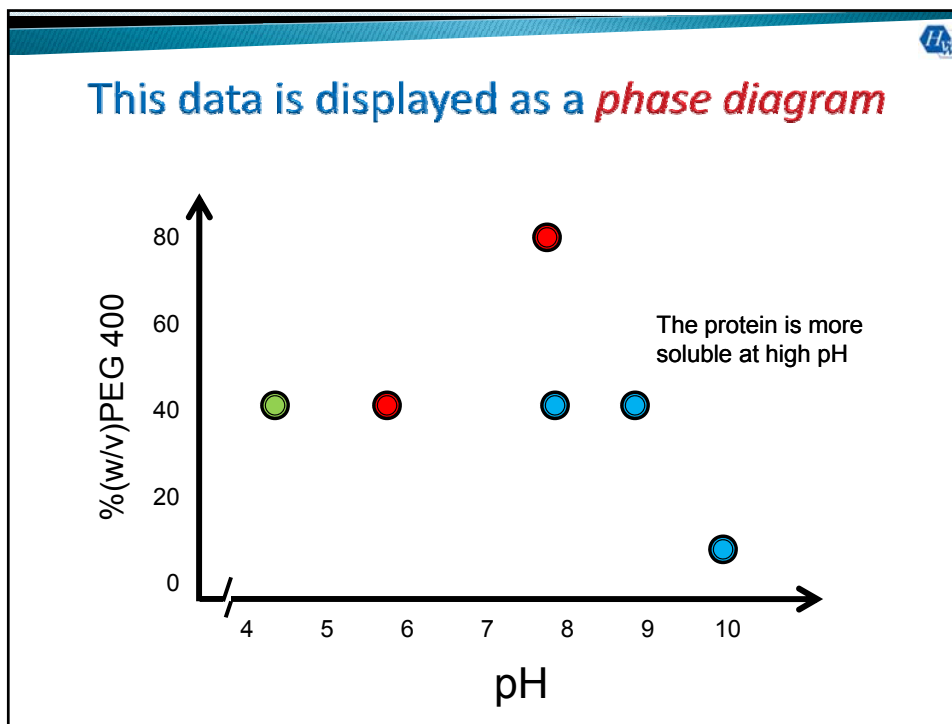
Precipitate

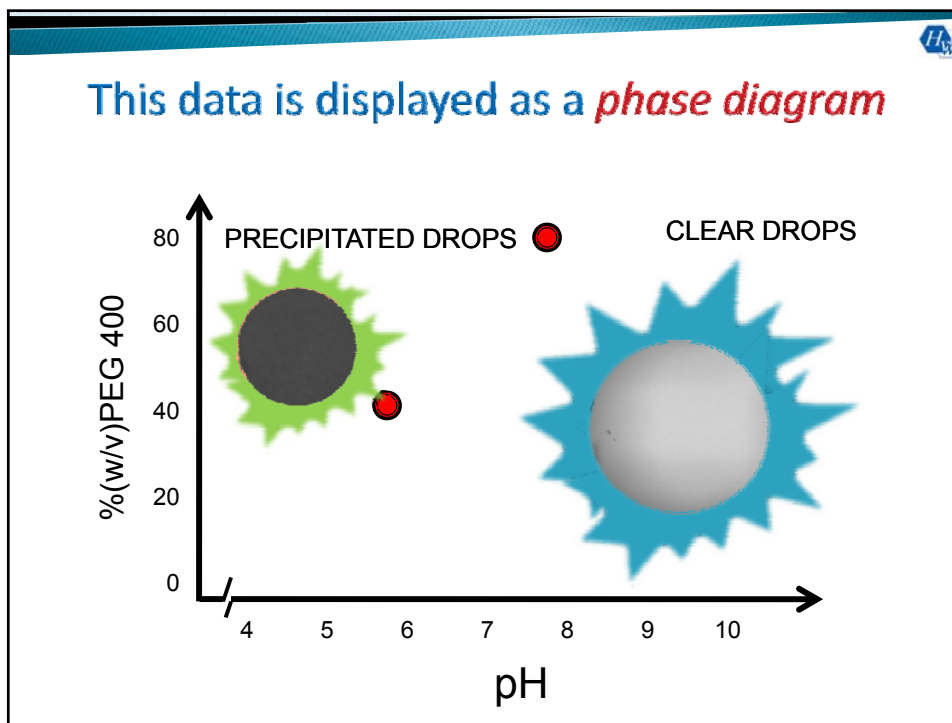
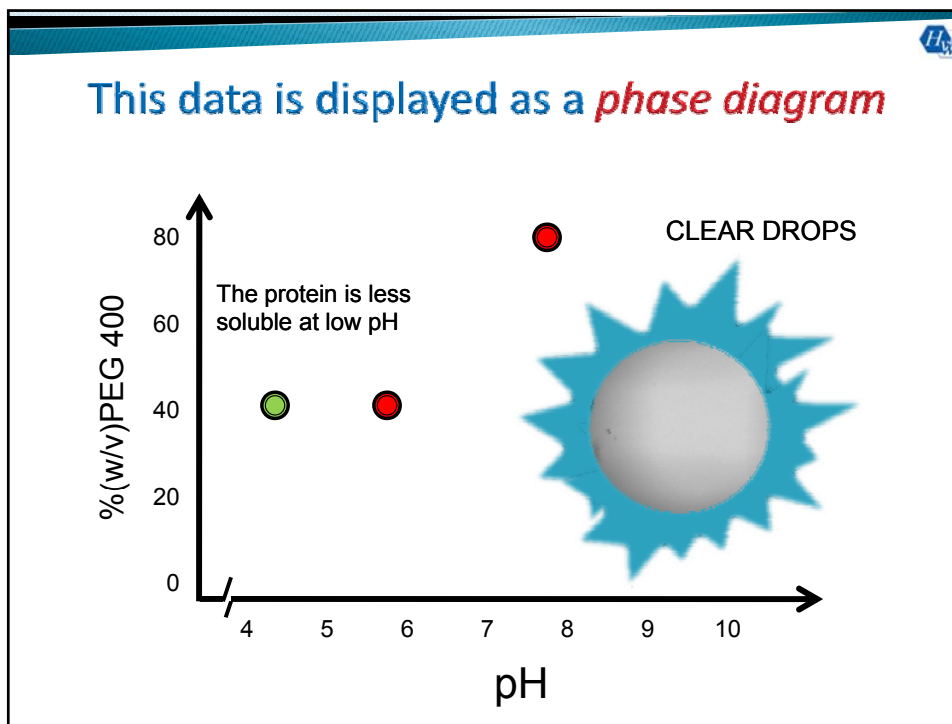


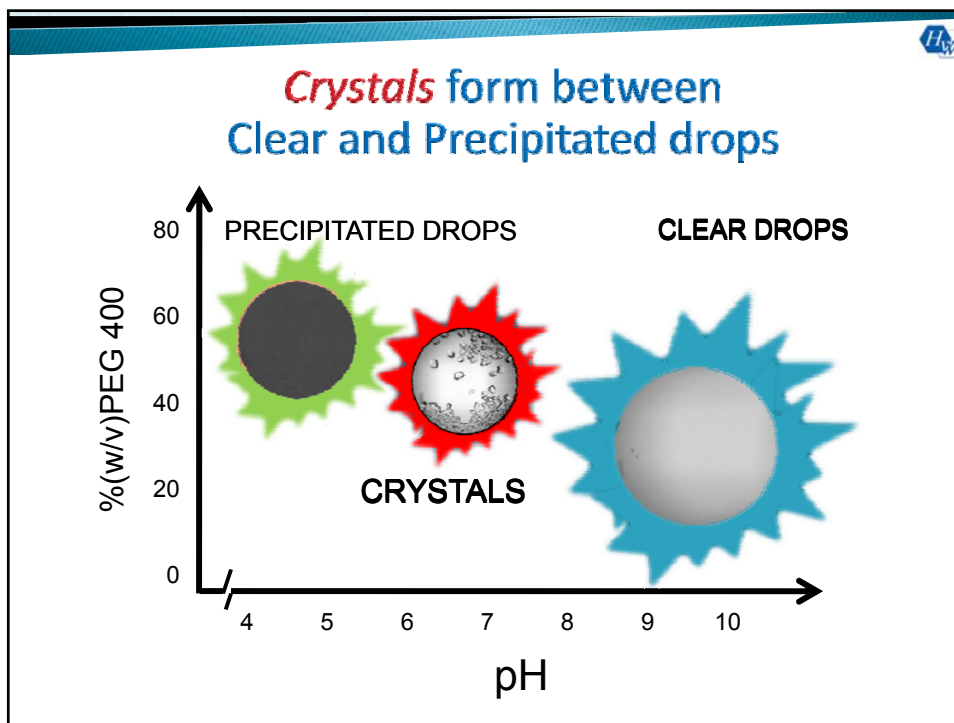
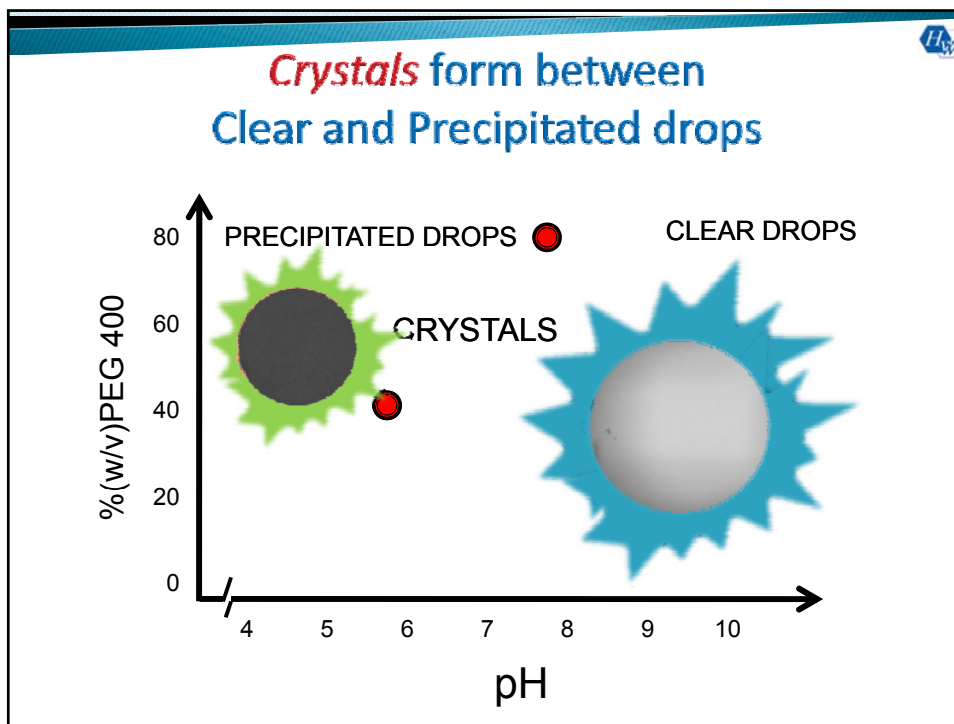
The original map only provided chemical information about the cocktails

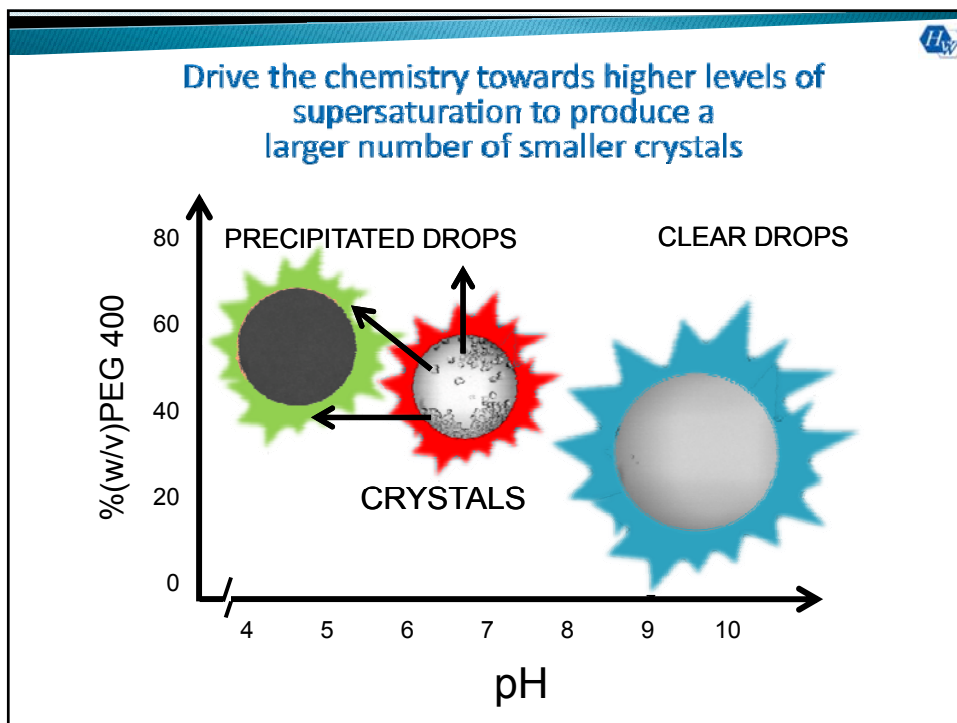
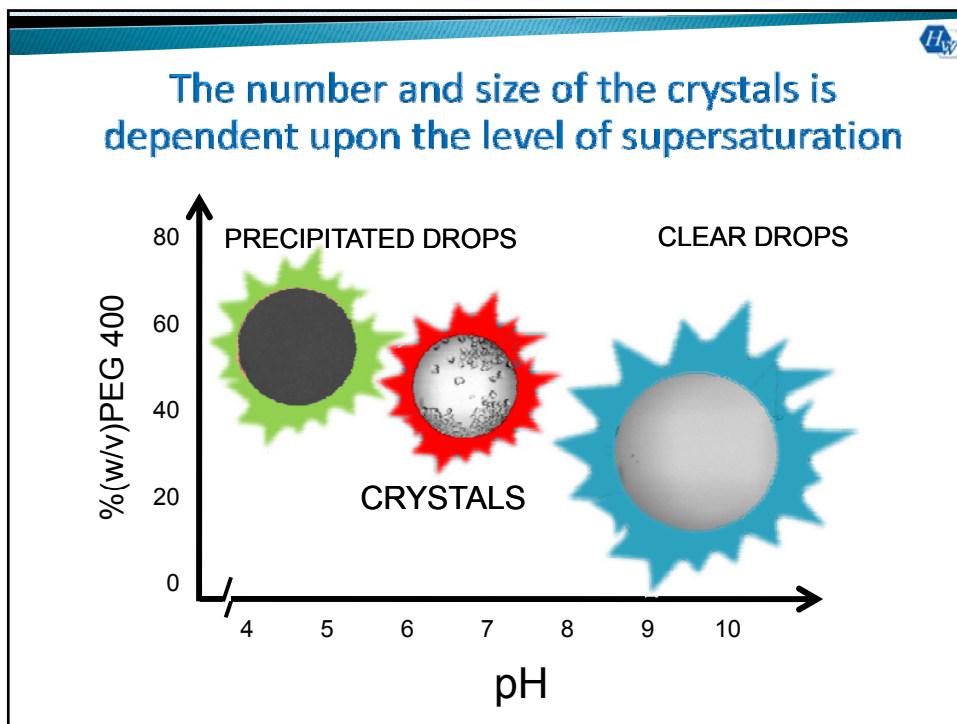
	PEG 400	Na Citrate	Na Acetate	MES	Bis-Tris Propane	HEPES	Tris	TAPS	CAPS
		Lithium							
pH		4.2	5	6	7	7.5	8	9	10
bromide	20%								
	40%								
	80%								











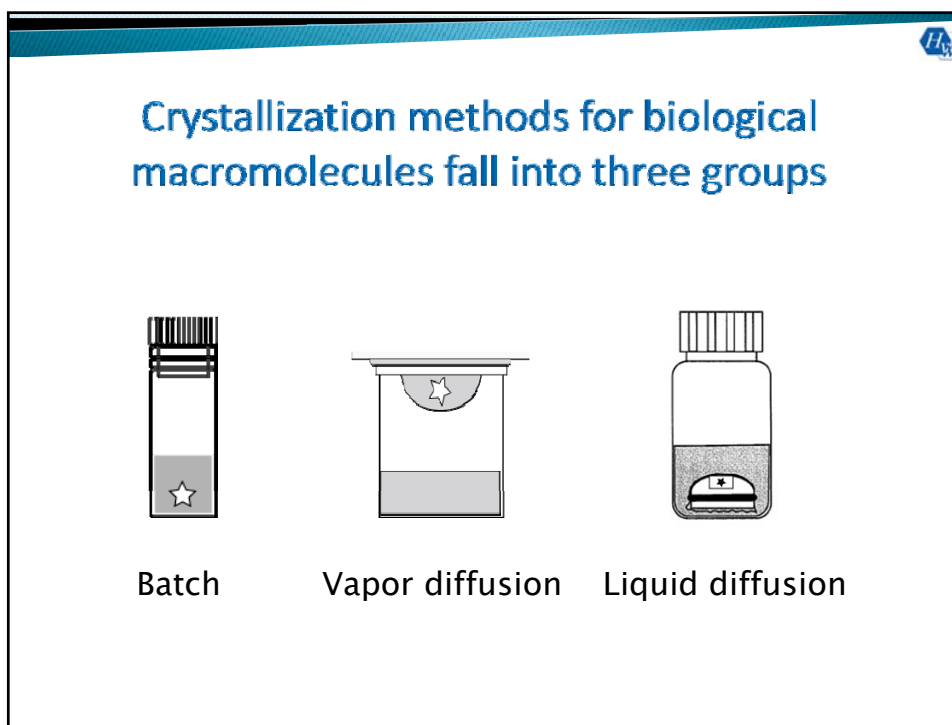
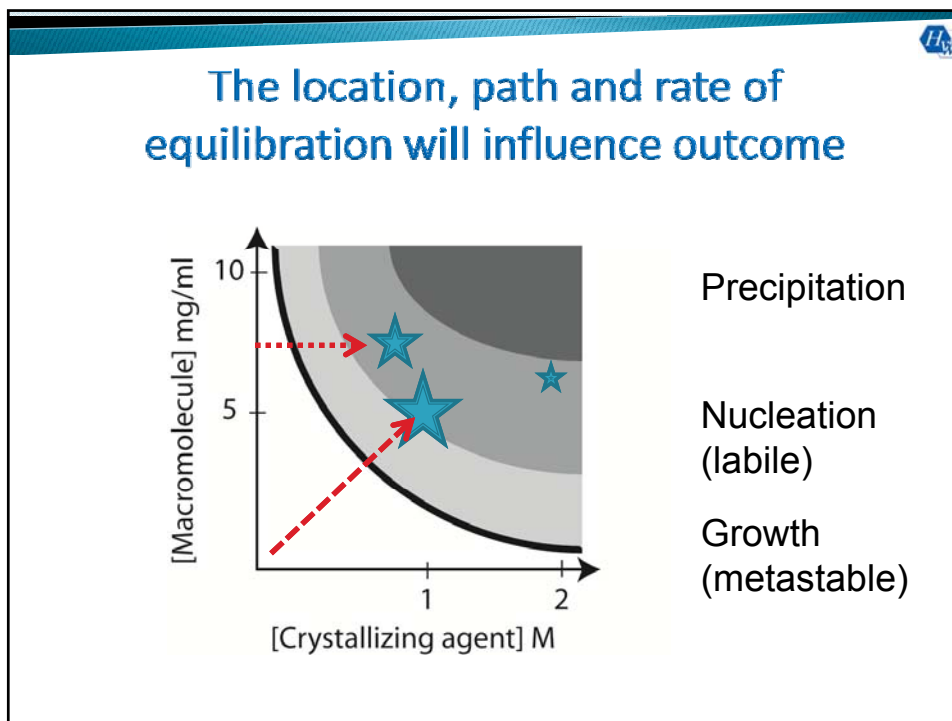
Crystallization methods


The path and the rate to supersaturation

Crystallization methods physically and chemically supersaturate a protein solution

- ▶ Supersaturation is achieved through chemical and/or physical manipulation of the solution environment
- ▶ Methods target specific variables for optimization
- ▶ Methods have unique trajectories through the phase diagram
- ▶ Most methods have a set endpoint
- ▶ Kinetics of equilibration can often be controlled


$$f(\text{Crystallization Method}) = (\text{Supersaturation}_{\text{kinetics}}) \cdot (\text{Supersaturation}_{\text{path}})$$






Batch crystallization

The experiment has to be supersaturated at the onset

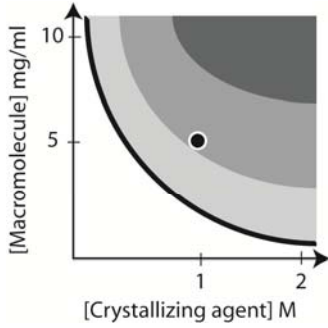


Classical batch experiments are initiated at labile supersaturation

pre-nucleation



Batch



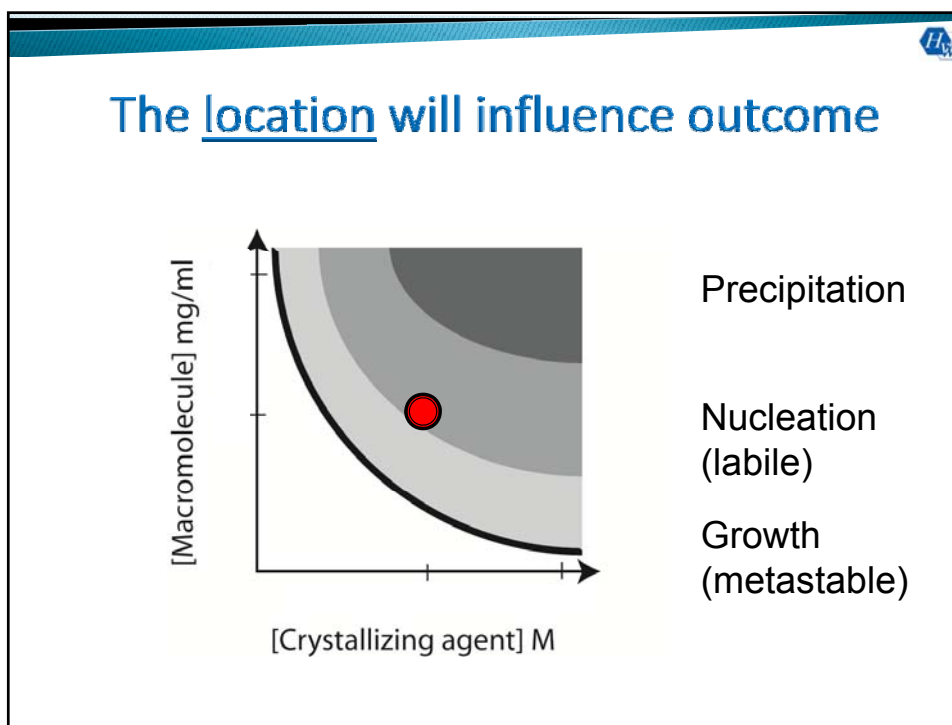
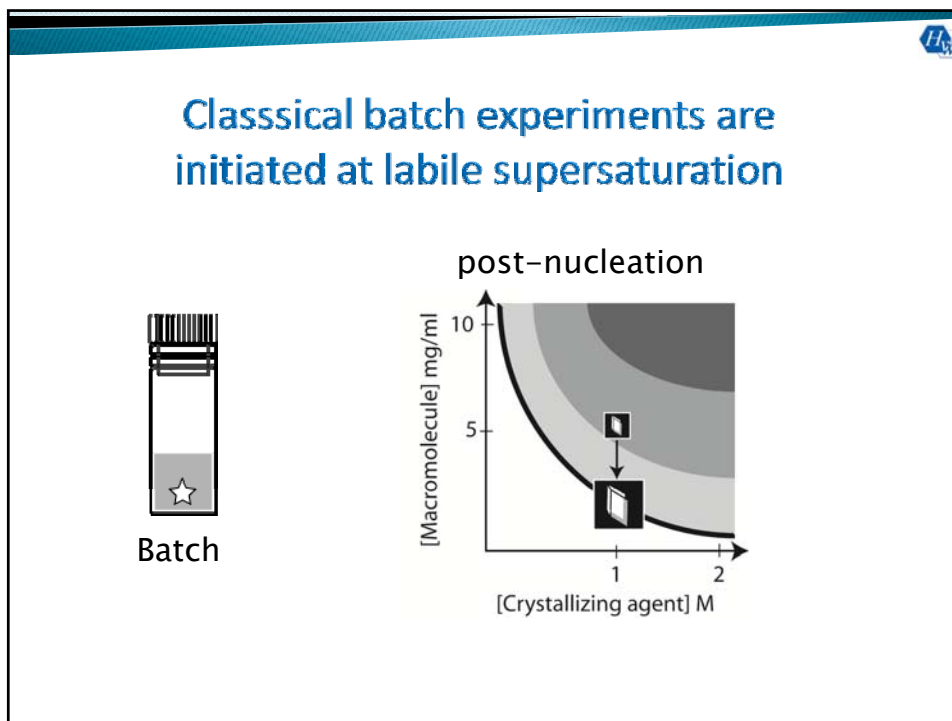
[Macromolecule] mg/ml

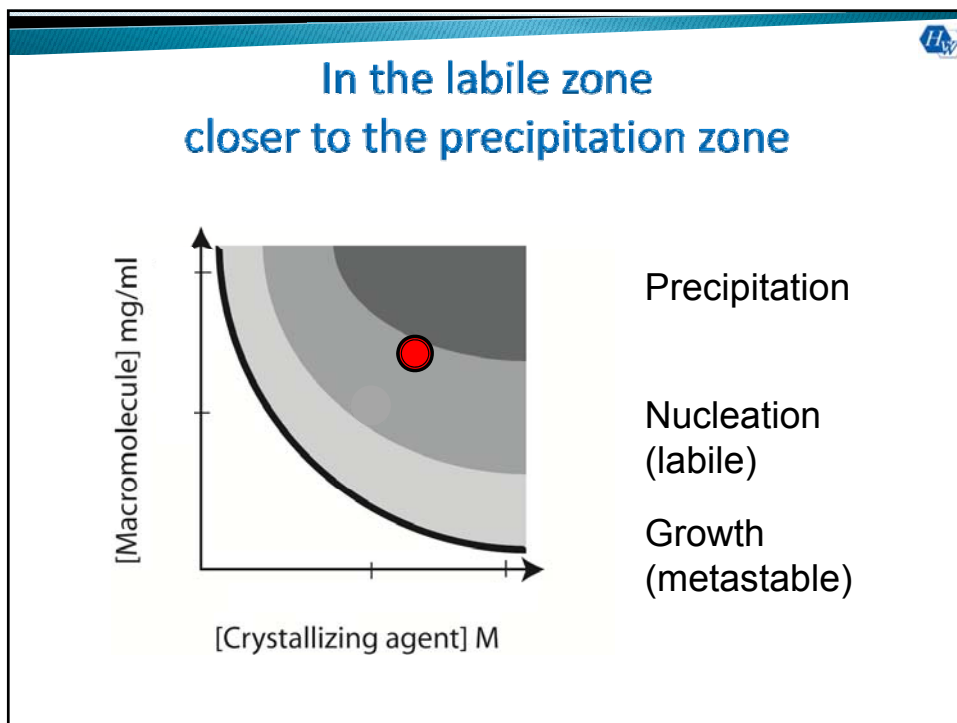
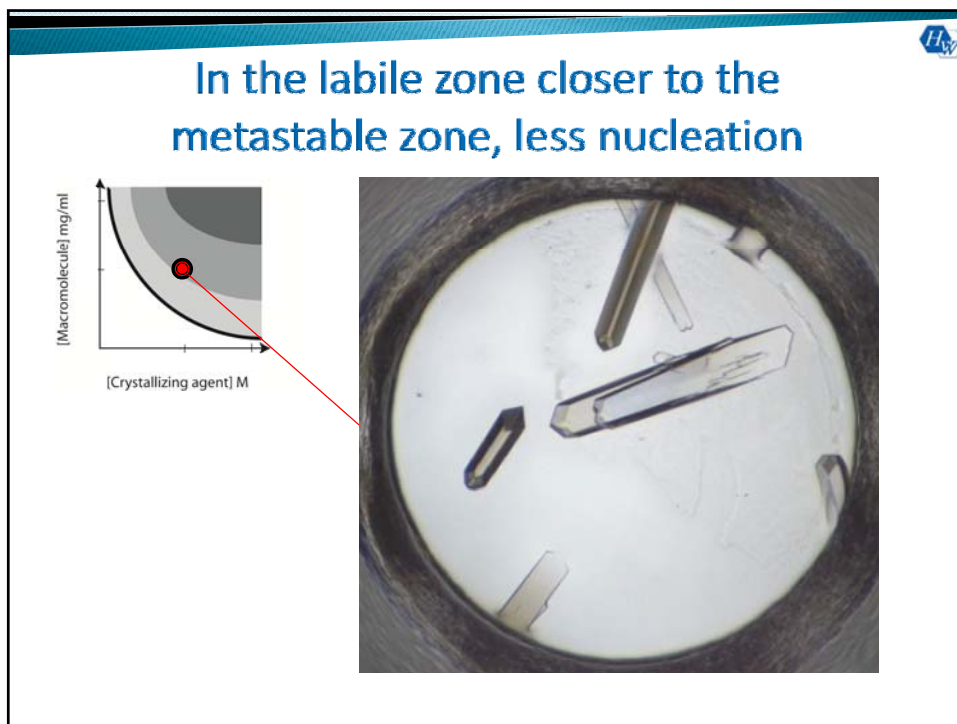
[Crystallizing agent] M

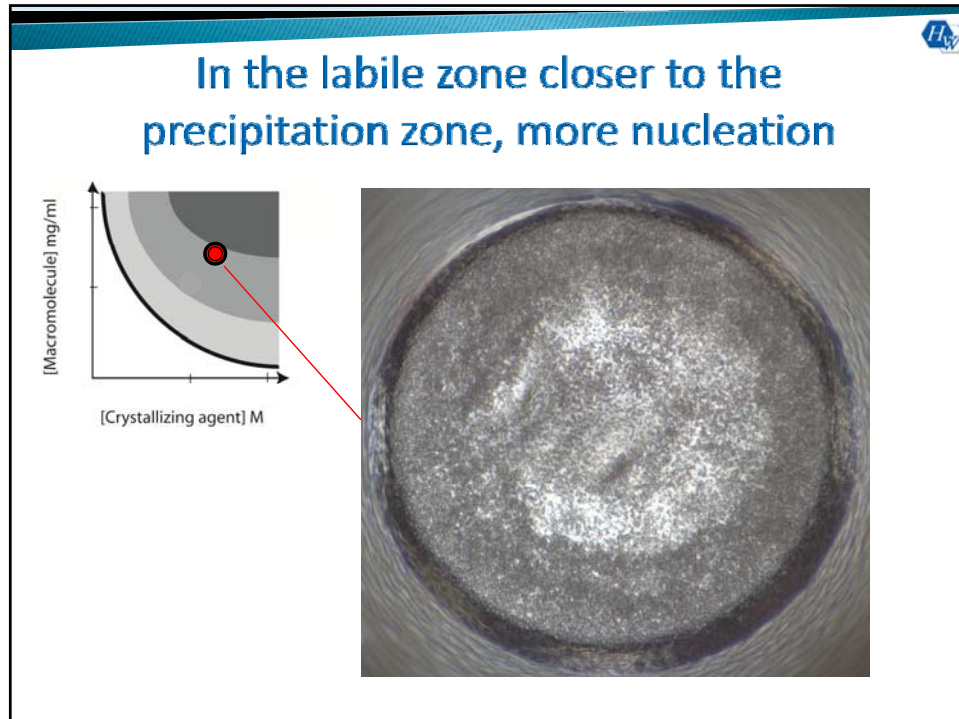
Precipitation

Nucleation (labile)

Growth (metastable)

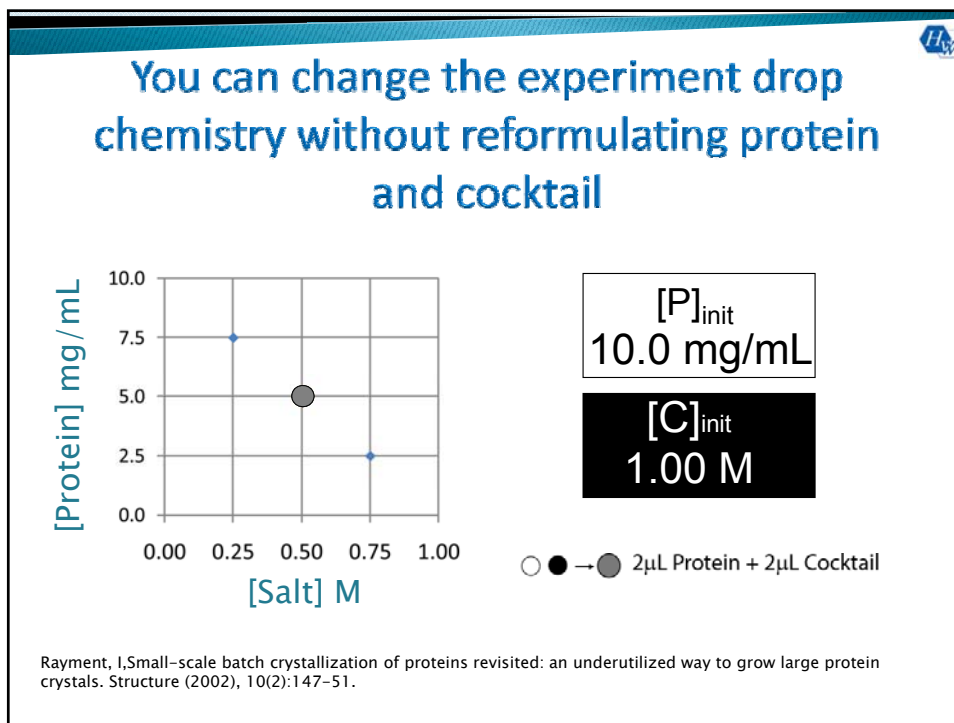
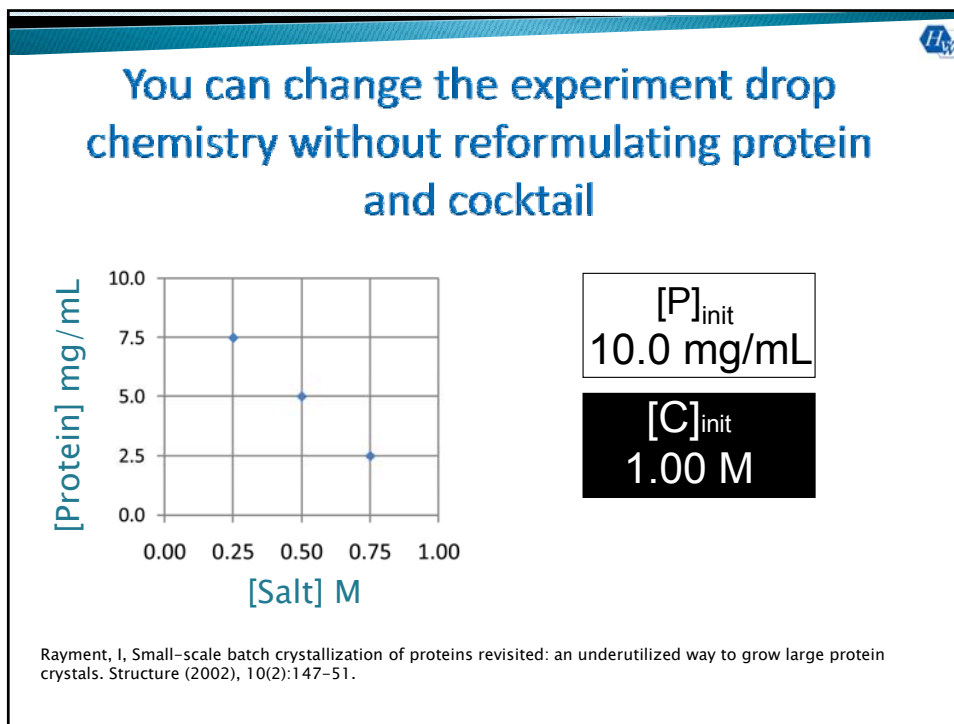


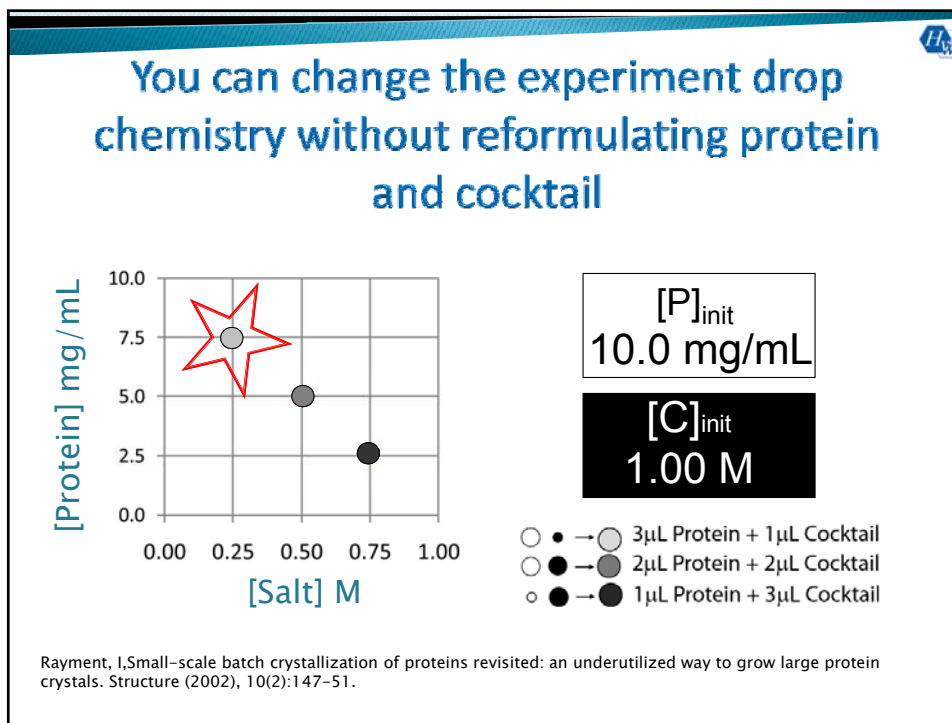
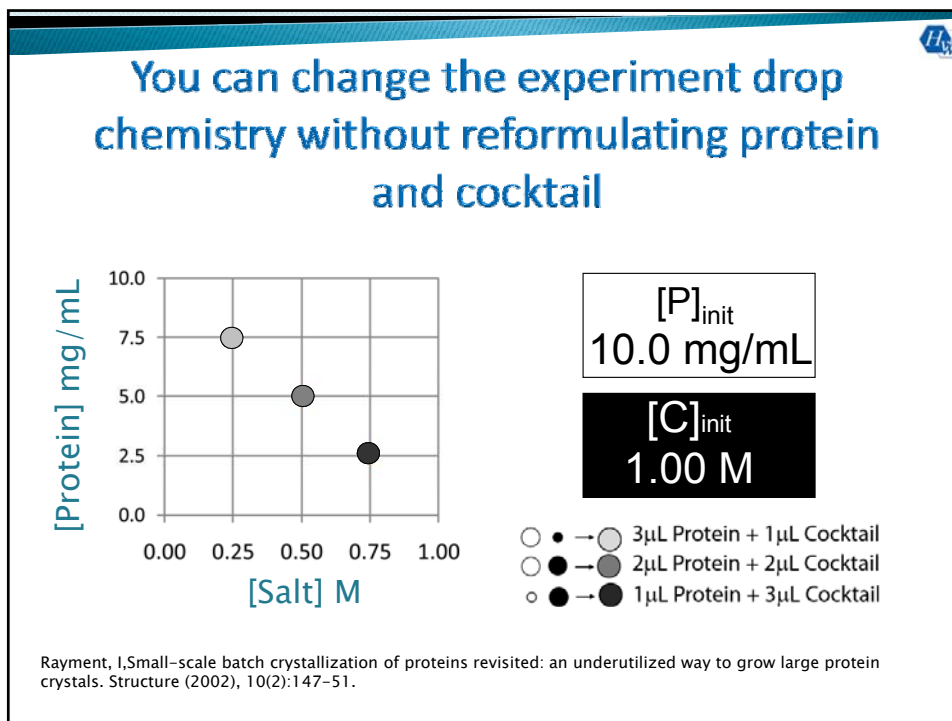


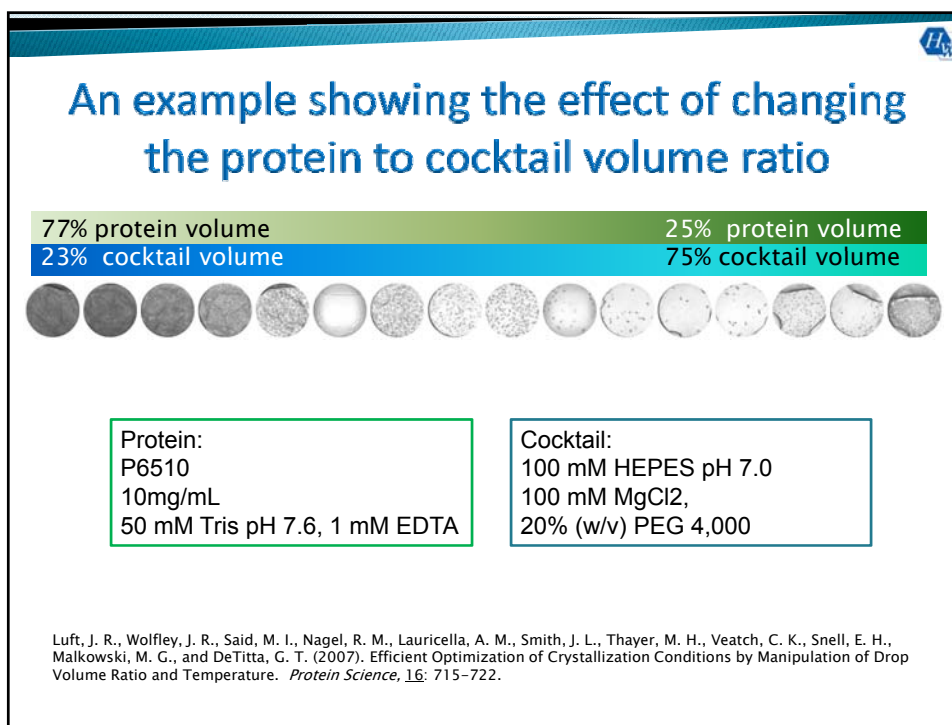
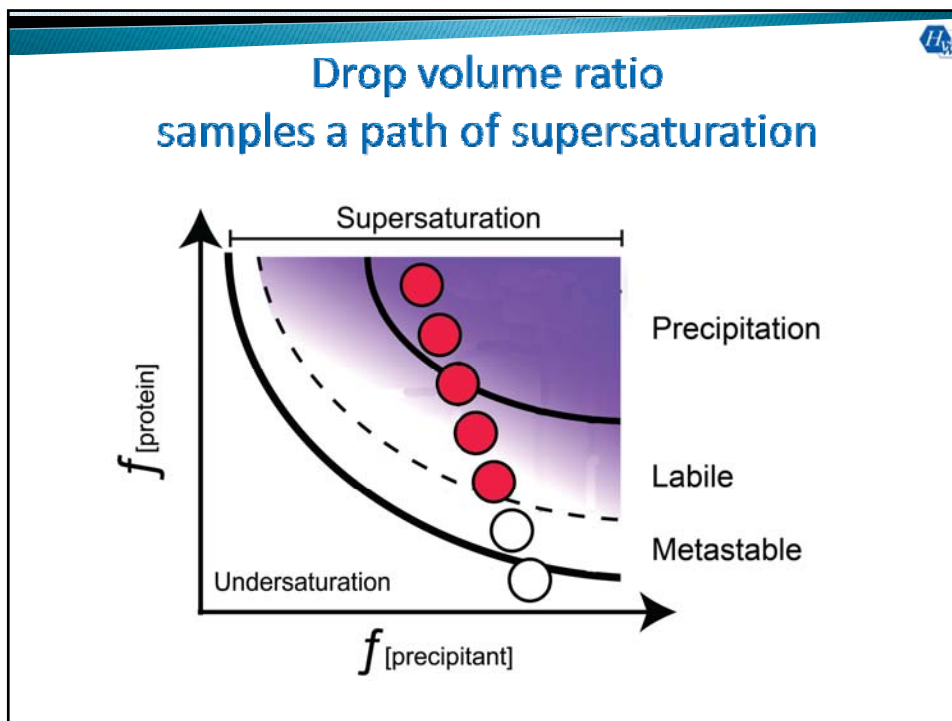


Volume ratio of protein to cocktail

A powerful and efficient optimization tool






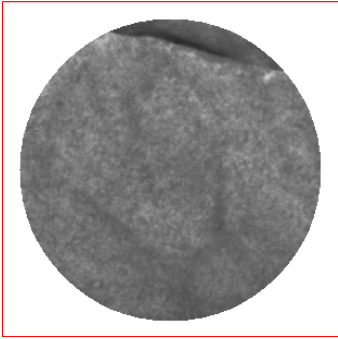


An example showing the effect of changing the protein to cocktail volume ratio

77% protein volume 25% protein volume
23% cocktail volume 75% cocktail volume

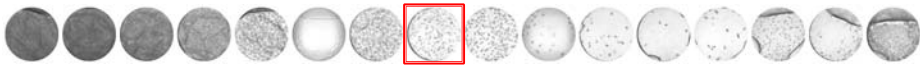


77% Protein 23% Cocktail

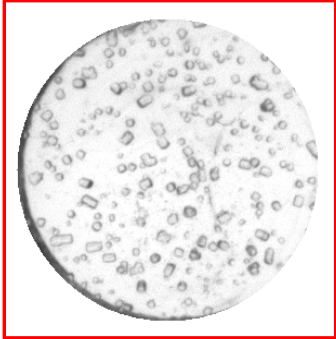


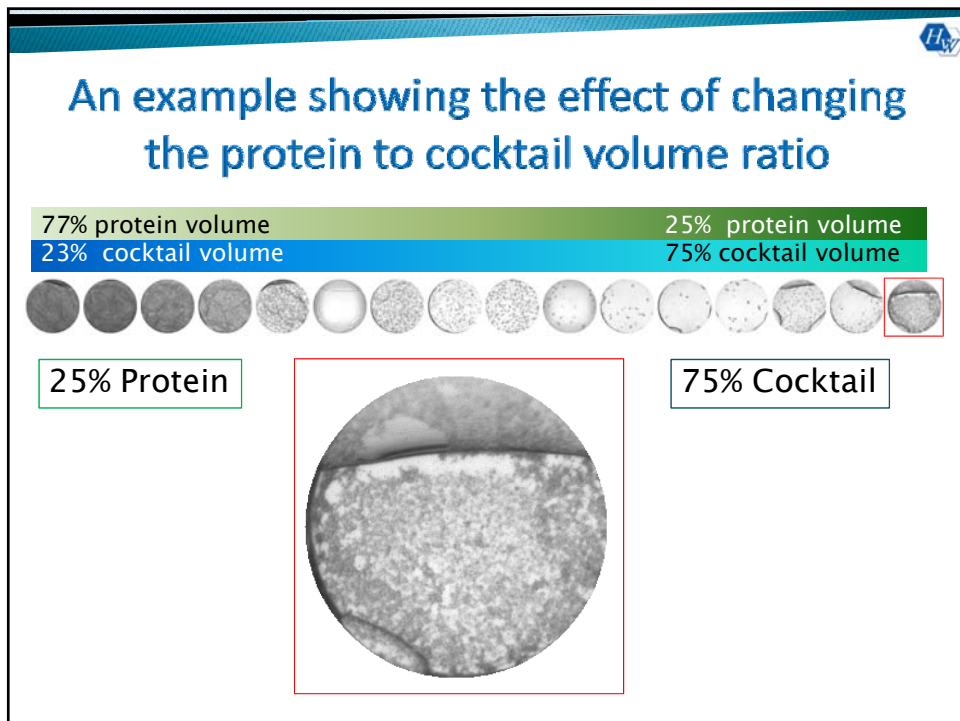
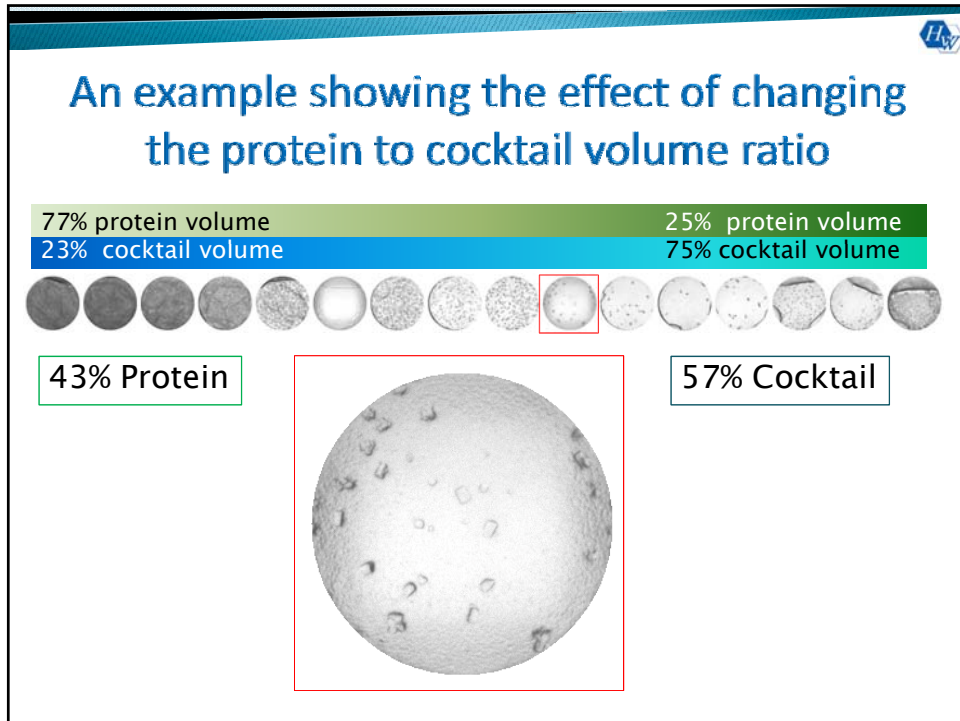
An example showing the effect of changing the protein to cocktail volume ratio

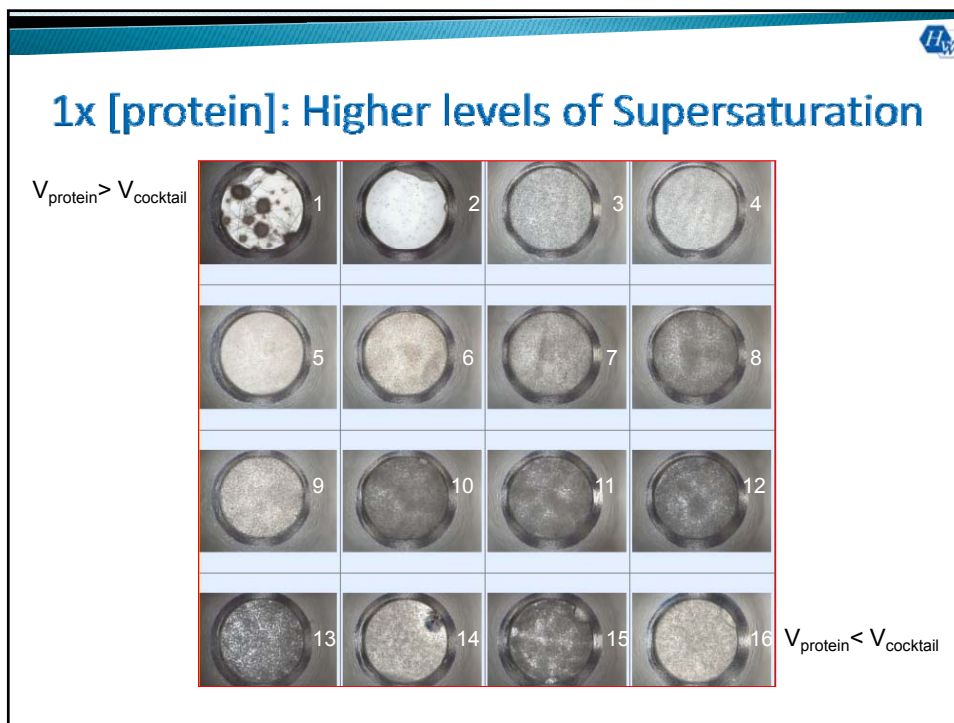
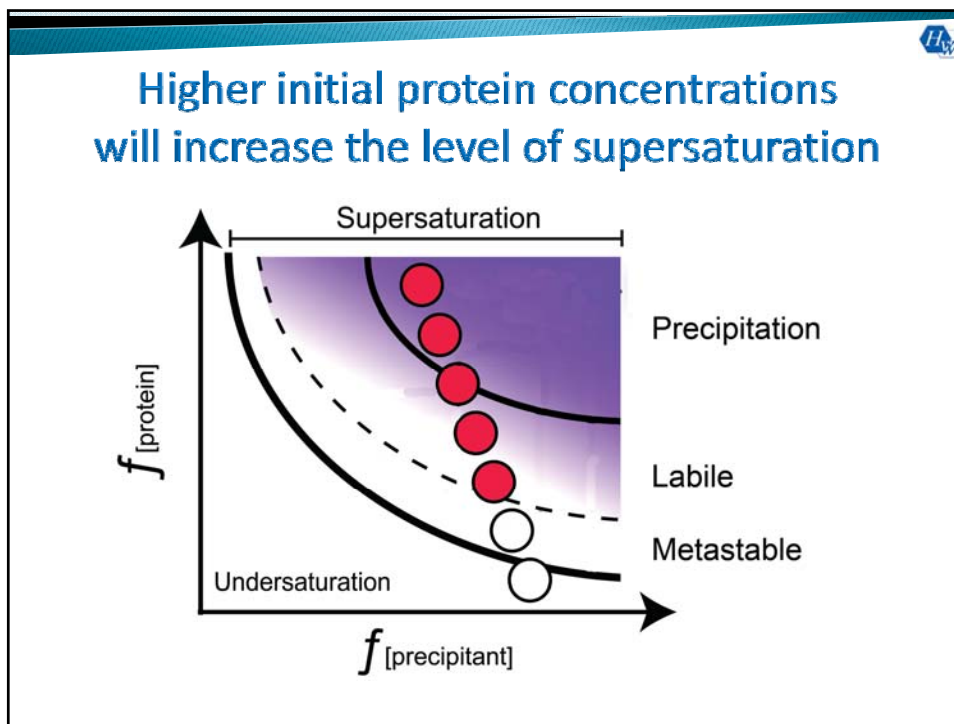
77% protein volume 25% protein volume
23% cocktail volume 75% cocktail volume

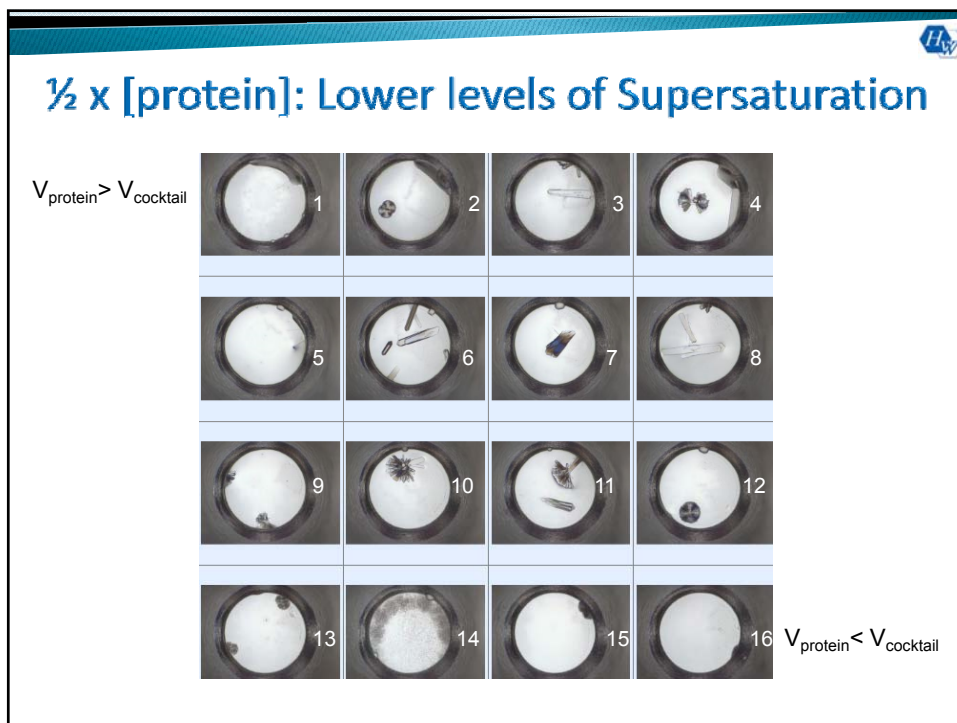
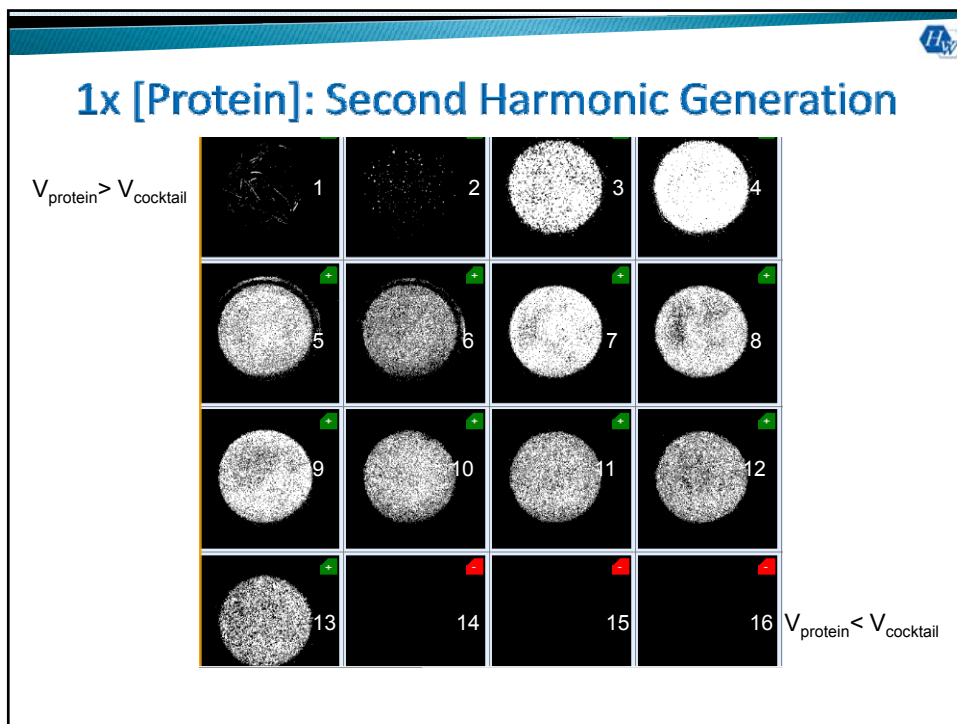


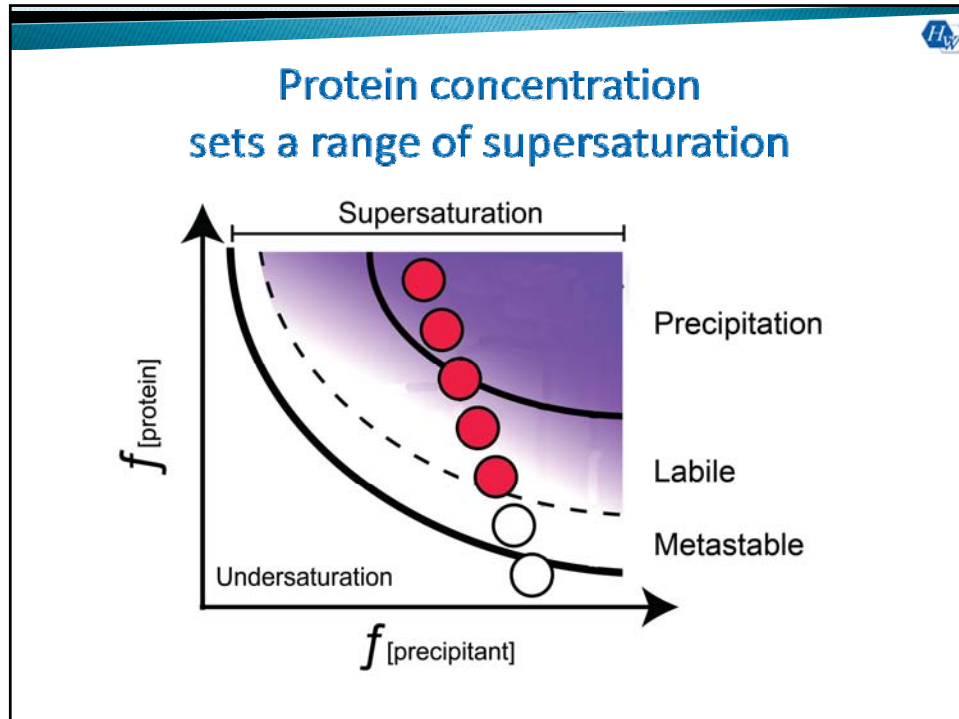
50% Protein 50% Cocktail









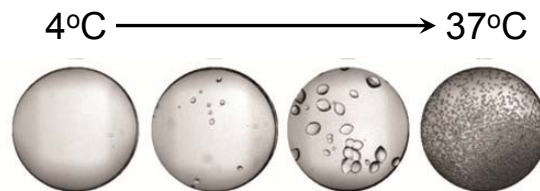


Temperature effects

Effective, but technically challenging

Temperature often alters solubility

- ▶ Temperature changes can alter the solubility of a protein (solvent-dependent)
- ▶ Protein solubility can be directly (normal) or inversely (retro) related to temperature
- ▶ The impact of temperature on solubility is most significant at low ionic strength



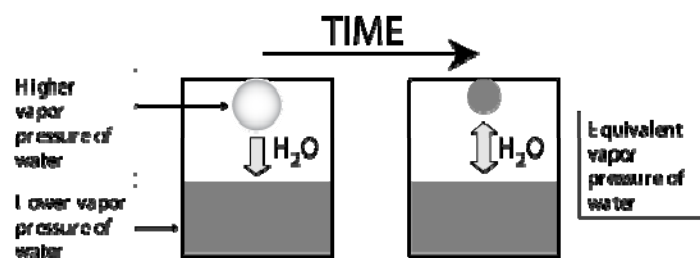
Christopher GK, Phipps AG, Gray RJ, Temperature-dependent solubility of selected proteins
Journal of Crystal Growth 1998, **191**:820-826.

Protein Science (2007) 16(4): 715-722.

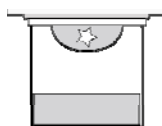
Vapor diffusion crystallization

The experiment dehydrates and drives the protein to a supersaturated state

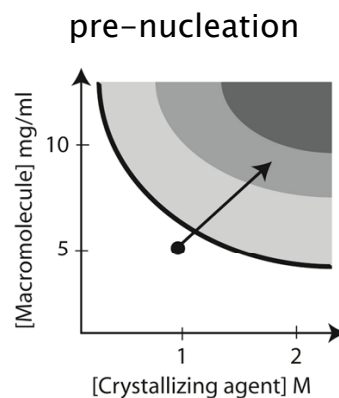
Vapor diffusion crystallization experiments use a reservoir solution to dehydrate the experiment drop and set the endpoint of dehydration

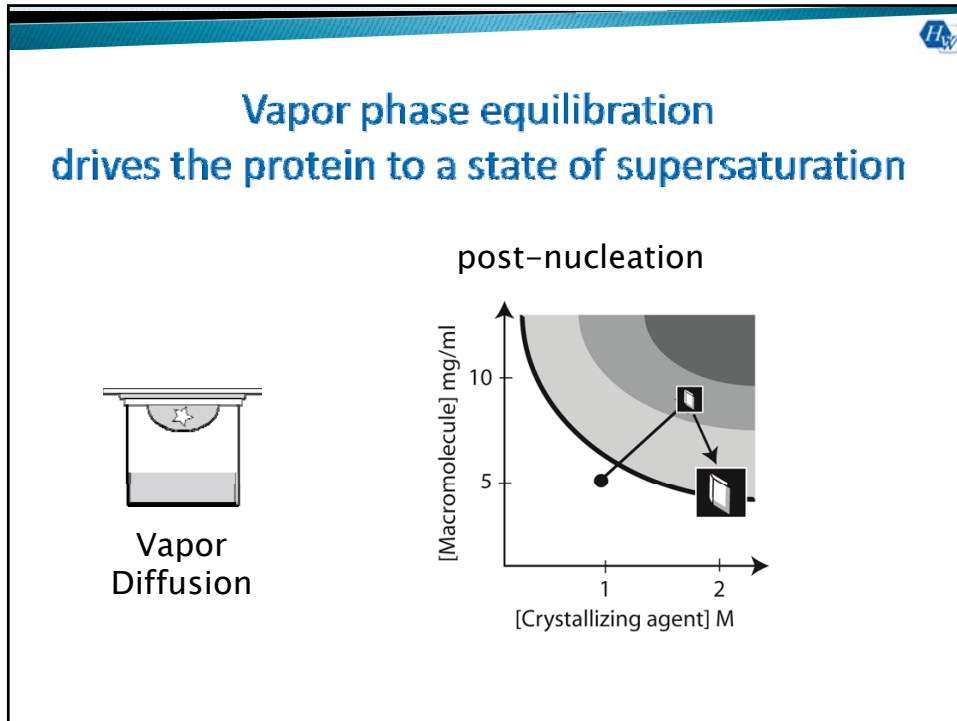


Vapor diffusion experiments are undersaturated when initiated



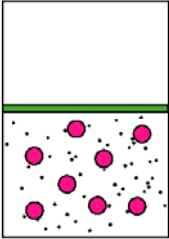
Vapor Diffusion





Dialysis membranes are available with different pore sizes

- ▶ Molecules smaller than the MWCO pass
- ▶ Molecules larger than the MWCO do not pass

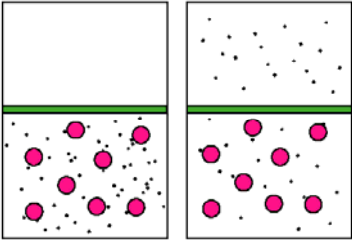


Dialysis can be used to **remove** solutes from your protein

The diagram shows a rectangular dialysis bag with a green horizontal line representing the membrane. Below the membrane, the bag contains several large pink circles representing protein molecules and many small black dots representing smaller solutes. The top half of the bag is empty, representing the dialysate solution.

Dialysis membranes are available with different pore sizes

- ▶ Molecules smaller than the MWCO pass
- ▶ Molecules larger than the MWCO do not pass

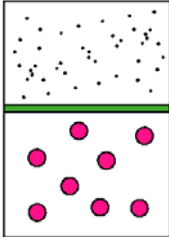


Dialysis can be used to **remove** solutes from your protein

The diagram shows two side-by-side rectangular dialysis bags, each with a green horizontal line representing the membrane. The left bag contains several large pink circles (protein) and many small black dots (solutes). The right bag shows the same large pink circles, but the small black dots have moved from the bottom half to the top half, above the membrane, indicating they have been removed from the protein solution.

Dialysis membranes are available with different pore sizes

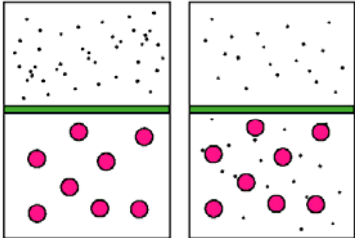
- ▶ Molecules smaller than the MWCO pass
- ▶ Molecules larger than the MWCO do not pass



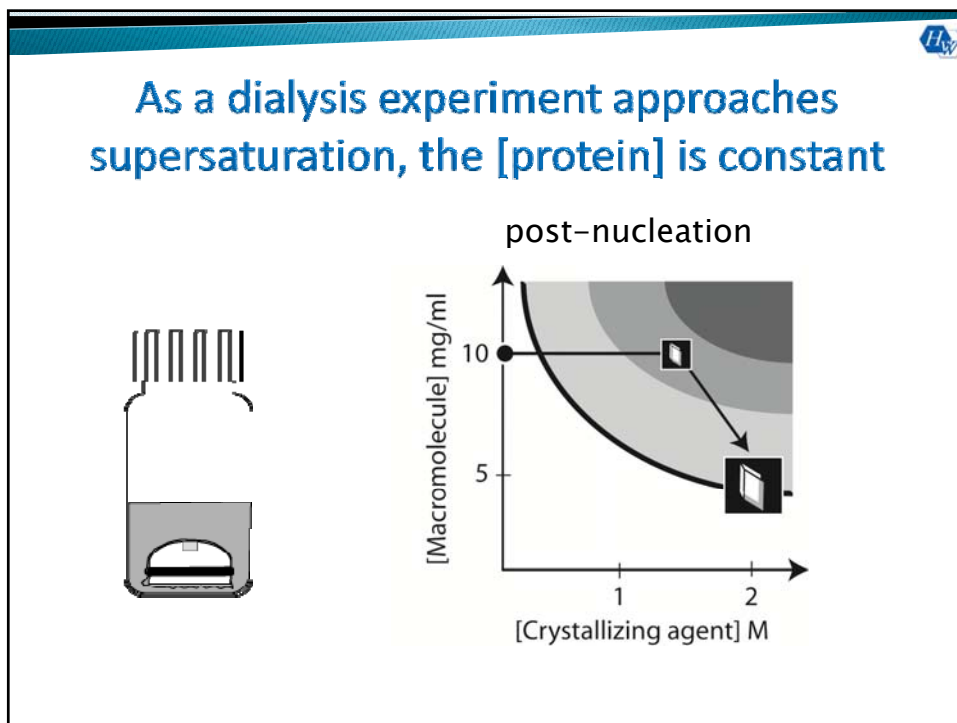
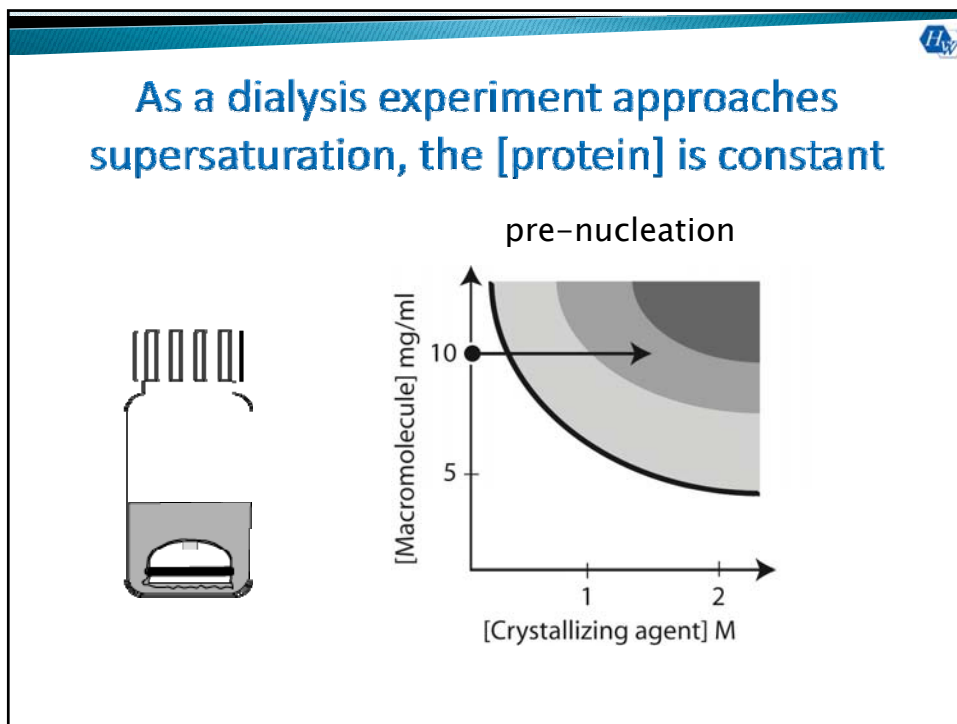
Dialysis can be used to **add** solutes to your protein


Dialysis membranes are available with different pore sizes

- ▶ Molecules smaller than the MWCO pass
- ▶ Molecules larger than the MWCO do not pass




Dialysis can be used to **add** solutes to your protein



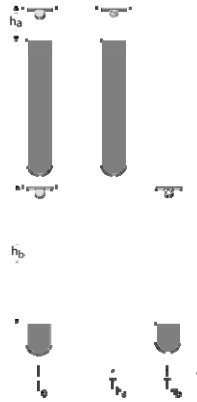


Rate of equilibration

Faster equilibration, smaller crystals



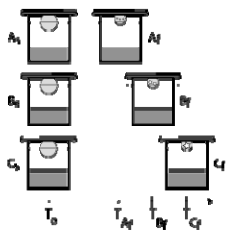
Rapid equilibration rates typically produce smaller crystals



Time to Equilibration

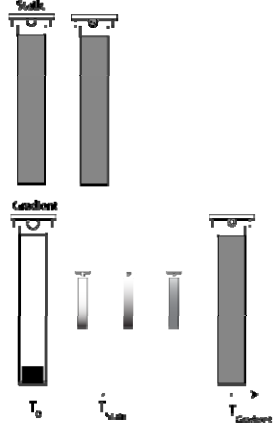
Distance

N.E. Chayen. A novel technique to control the rate of vapour diffusion, giving larger protein crystals
J. Appl. Cryst. (1997). 30, 198-202.



Time to Equilibration

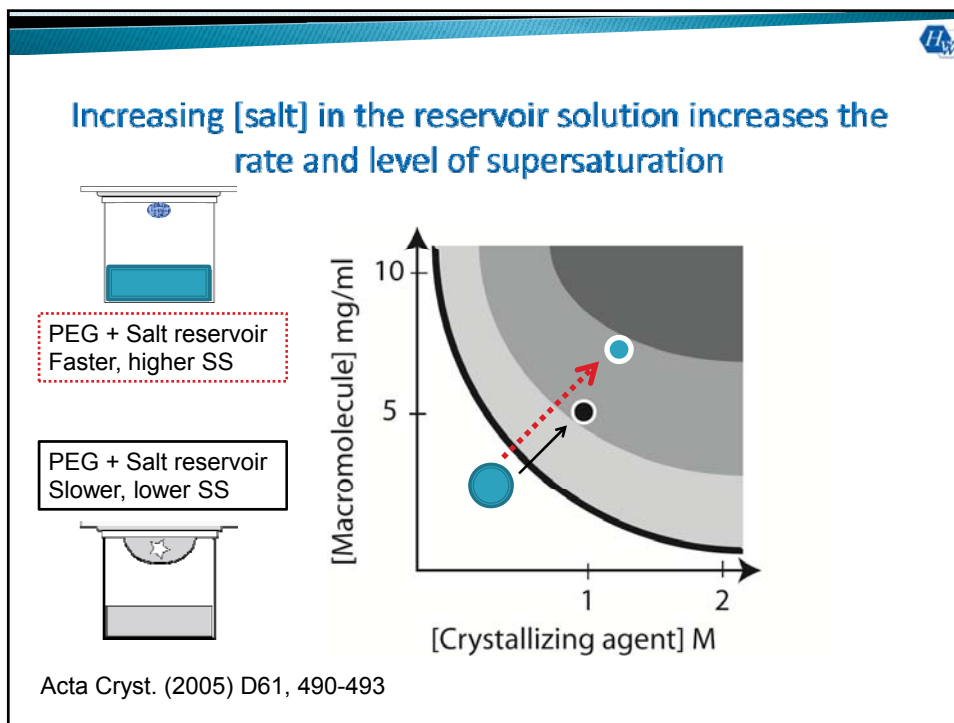
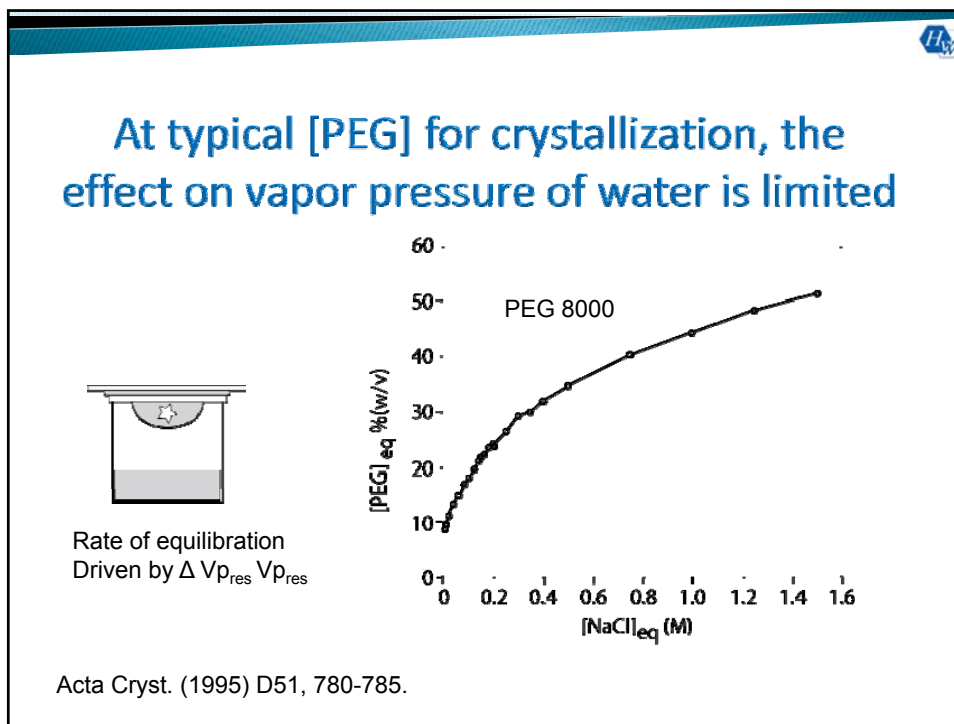
Oil layer



Time to Equilibration

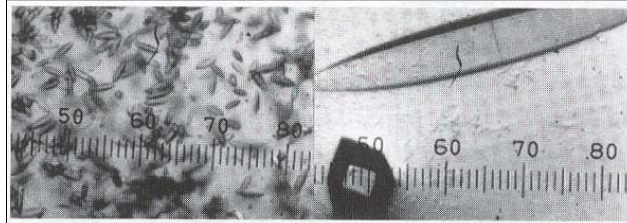
Diffusion gradient

Luft, J.R. and DeTitta, G.T., Kinetic Aspects of Macromolecular Crystallization, *Methods of Enzymology*, 276, 110-131 (1997).



Vapor diffusion: Faster equilibration smaller crystals, slower equilibration larger crystals

Rat transthyretin co-crystallized with a plant flavonoid



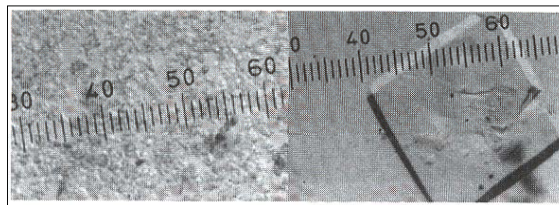
Faster

Slower

Luft, J.R. and DeTitta, G.T., Kinetic Aspects of Macromolecular Crystallization, *Methods of Enzymology*, **276**, 110-131 (1997).

Dialysis: Faster equilibration smaller crystals, slower equilibration larger crystals

- Crystals of the insecticidal bacterial δ -endotoxin Cry3Bb1 from *Bacillus thuringiensis* grown using gradient microdialysis. Larger crystals were grown by slowly reducing the concentration of NaBr.



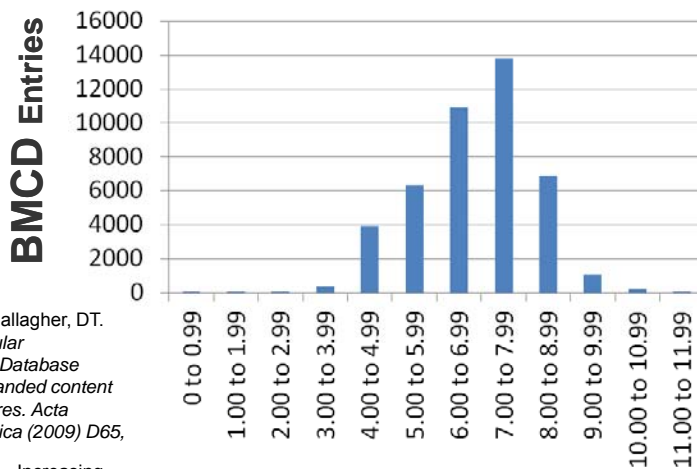
Faster

Slower

N. Galitsky, V. Cody, A. Wojtczak, D. Ghosh, J. R. Luft, W. Pangborn and L. English, Structure of the insecticidal bacterial delta-endotoxin Cry3Bb1 of *Bacillus thuringiensis* *Acta Cryst.* (2001). D57, 1101-1109


pH as a crystallization variable

Crystallization screens use pH as a variable: Controls the protein surface charge distribution



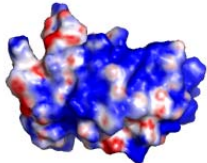
Tung, M and Gallagher, DT.
The Biomolecular Crystallization Database version 4: expanded content and new features. Acta Crystallographica (2009) D65, 18-23
McPherson, A. Increasing the size of microcrystals by fine sampling of pH limits *J. Appl. Cryst.* (1995). **28**, 362-365.

pH range




Crystallization Cocktails: pH, a critical variable for crystallization, influences the surface charge distribution of the protein

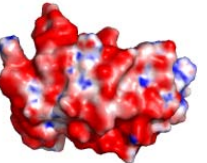
1Z66 domain III of the E protein of tick-borne Langat flavivirus, pI 7.01



pH 4




pH 7



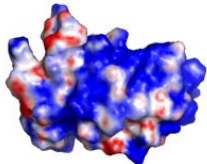
pH 11

<http://kpwu.wordpress.com/tag/pymol/>

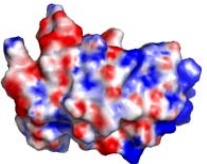


If the pH is less than the pI, then the protein will have a net positive surface charge

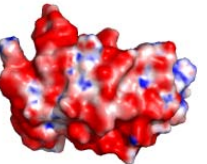
1Z66 domain III of the E protein of tick-borne Langat flavivirus, pI 7.01



pH 4




pH 7



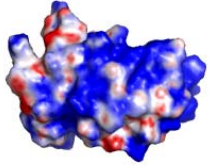
pH 11

pH < pI
Positive
net charge

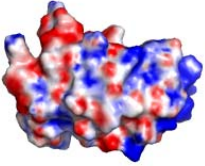


If the pH is greater than the pI, then
the protein will have a net negative surface charge

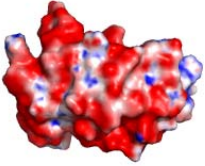
1Z66 domain III of the E protein of tick-borne Langat flavivirus, pI 7.01



pH 4




pH 7



pH 11


pH > pI
Negative
net charge

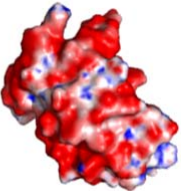


At a pH greater than the pI the protein
will have a net negative surface charge

1Z66 domain III of the E protein of tick-borne Langat flavivirus, pI 7.01

pH 11 > pI 7
Negative
net charge



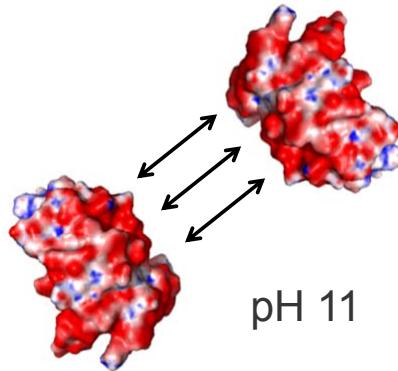


pH 11

Like charges repel

1Z66 domain III of the E protein of tick-borne Langat flavivirus, pI 7.01

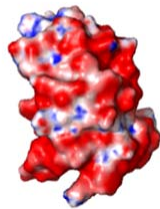
pH 11 > pI 7
Negative
net charge



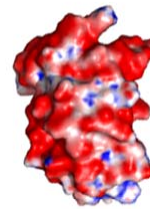
pH 11

Proteins tend to be more soluble when they have a highly negative or positive net charge

1Z66 domain III of the E protein of tick-borne Langat flavivirus, pI 7.01



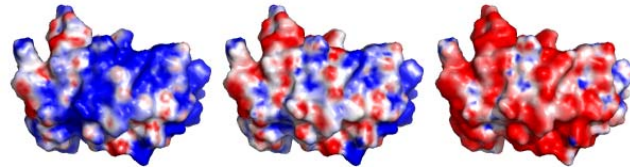
Like charges repel
Protein solubility high
(assumes no denaturation,
or other chemical effects)



pH 11

If the pH is equal to the pI, then
the protein will have a net zero surface charge

1Z66 domain III of the E protein of tick-borne Langat flavivirus, pI 7.01



pH 4

pH 7

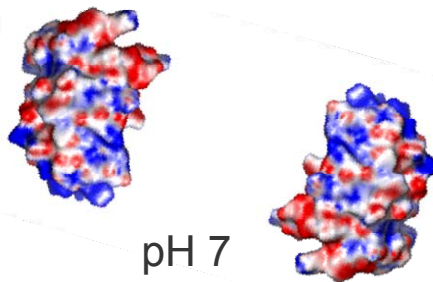
pH 11

pH = pI
Zero
net charge

At a pH equal to the pI the protein
will have a net zero surface charge

1Z66 domain III of the E protein of tick-borne Langat flavivirus, pI 7.01

pH 7 = pI 7
Zero
net charge

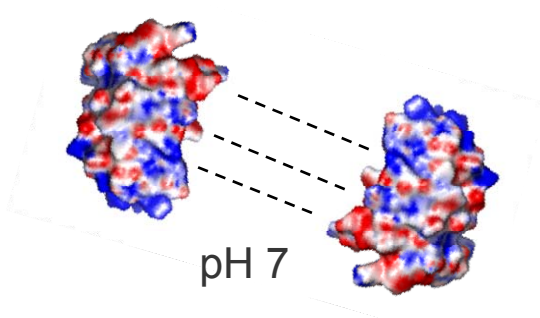


pH 7

Flv

Opposite charges attract

1Z66 domain III of the E protein of tick-borne Langat flavivirus, pI 7.01



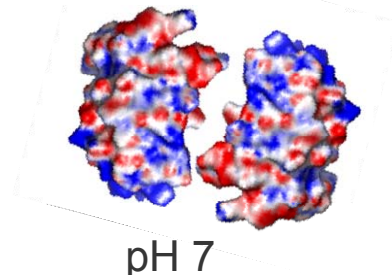
pH 7

Flv

Proteins tend to be less soluble near their pI

1Z66 domain III of the E protein of tick-borne Langat flavivirus, pI 7.01

Opposite charges attract
Protein solubility low



pH 7



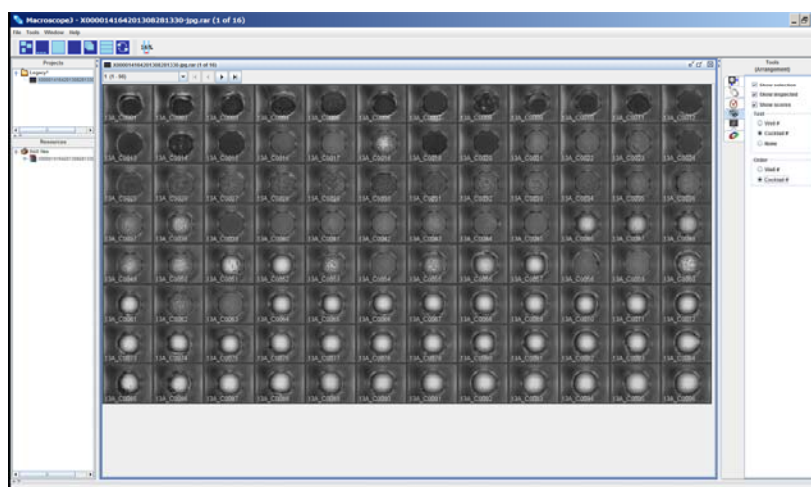
The Hampton Research Slice pH screen samples very fine pH increments

- 96 pH-titrated 1.0 M buffer cocktails
- $3.5 \leq \text{pH} \leq 9.6$
- Increments of 0.1 pH unit
- 20 chemically distinct buffers
- Overlaps between pH and buffer types

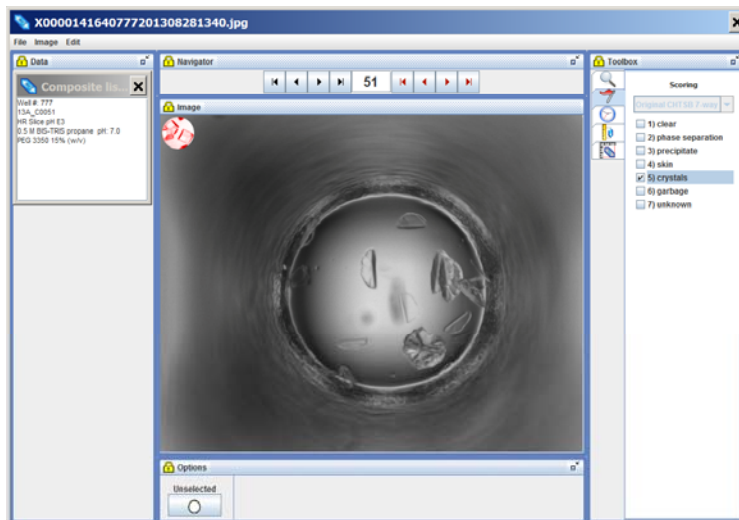
http://hamptonresearch.com/product_detail.aspx?cid=1&sid=200&pid=616



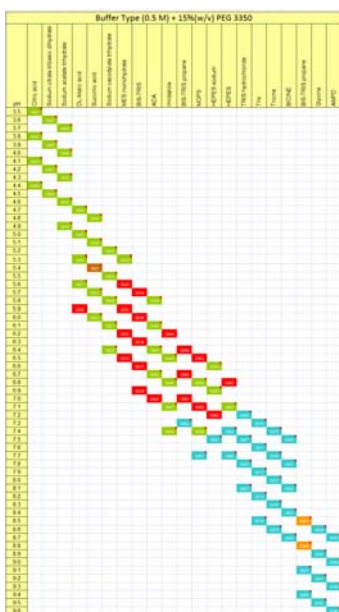
A standard way to view outcomes for Hampton Research Slice pH

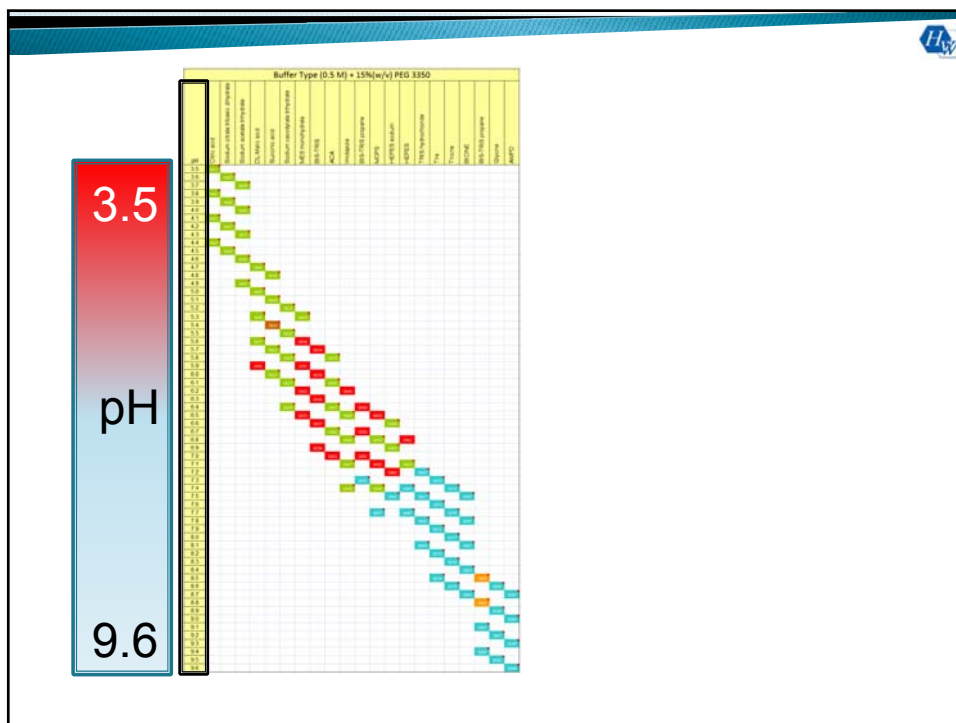
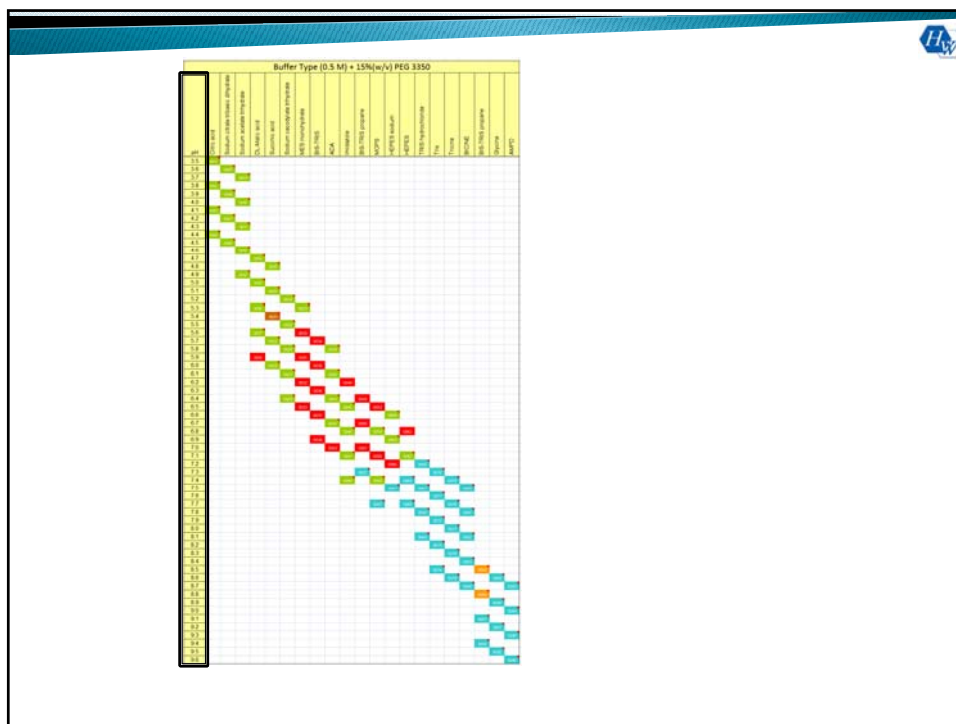


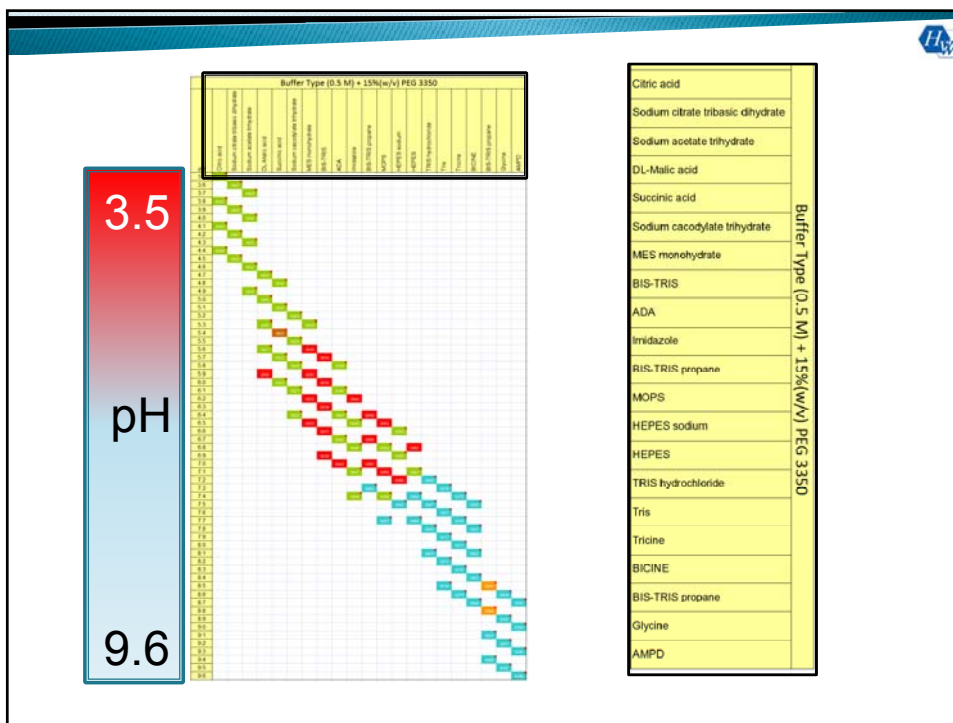
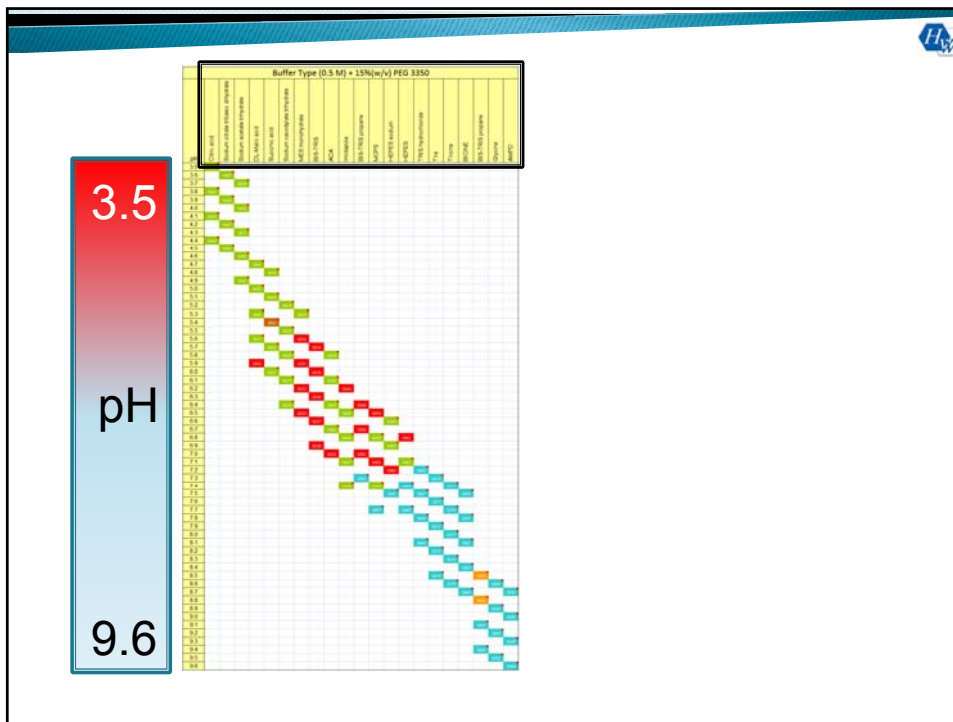
Single outcomes are consecutively reviewed and outcomes classified

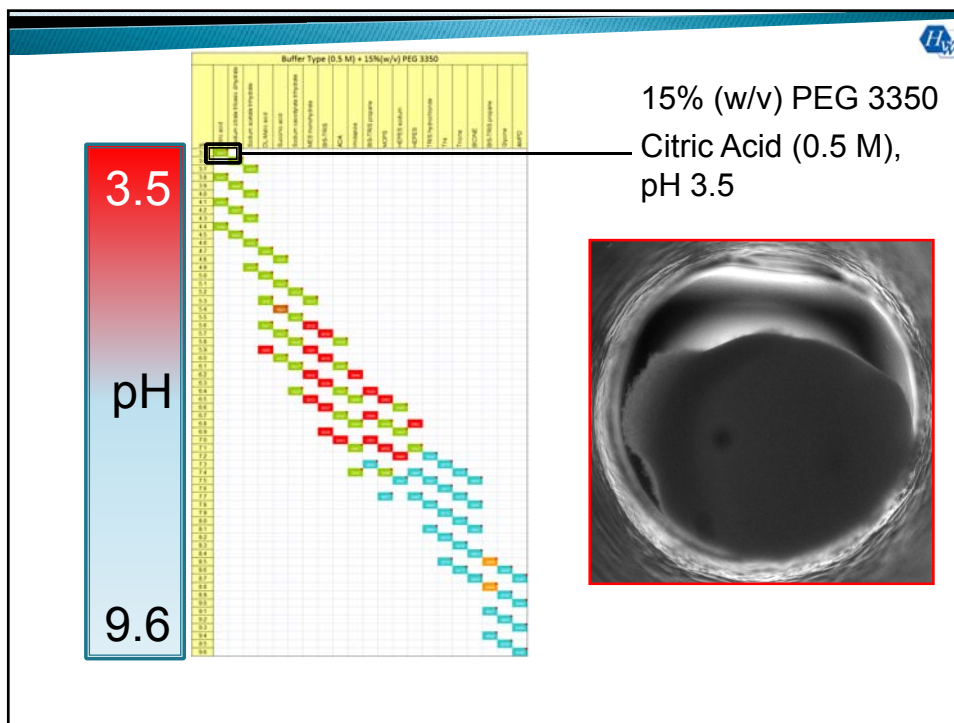
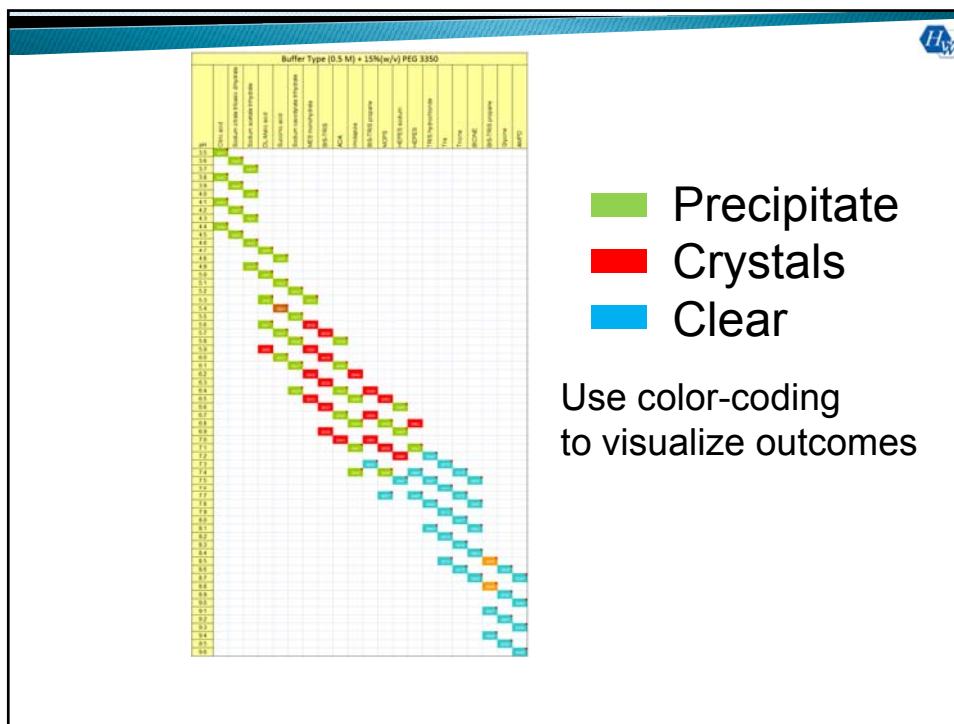


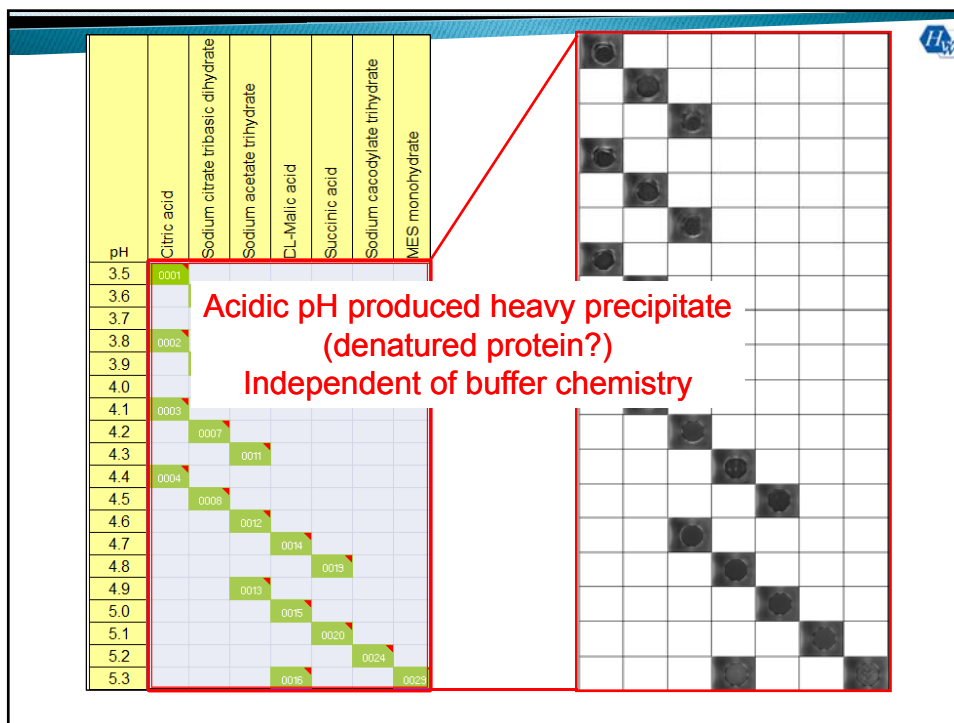
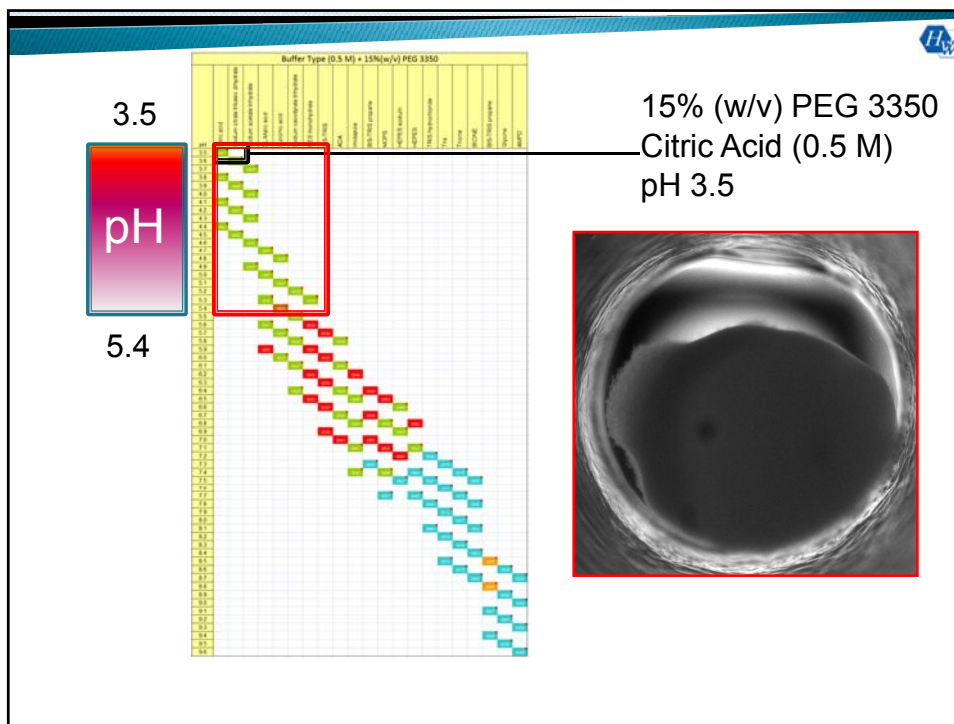
Use a grid to review outcomes as they relate chemically

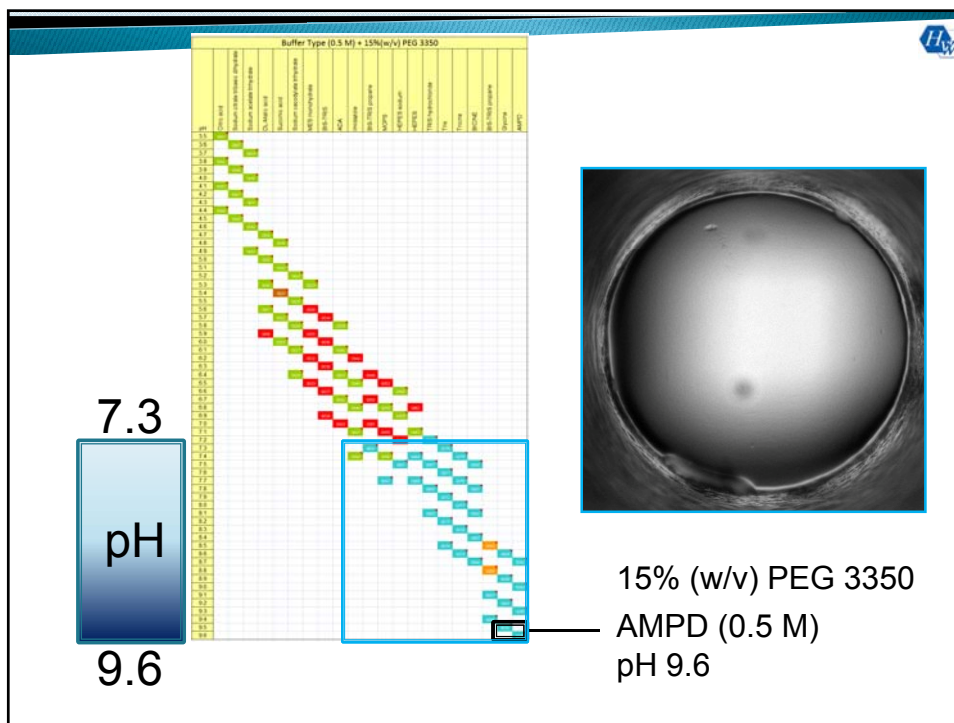
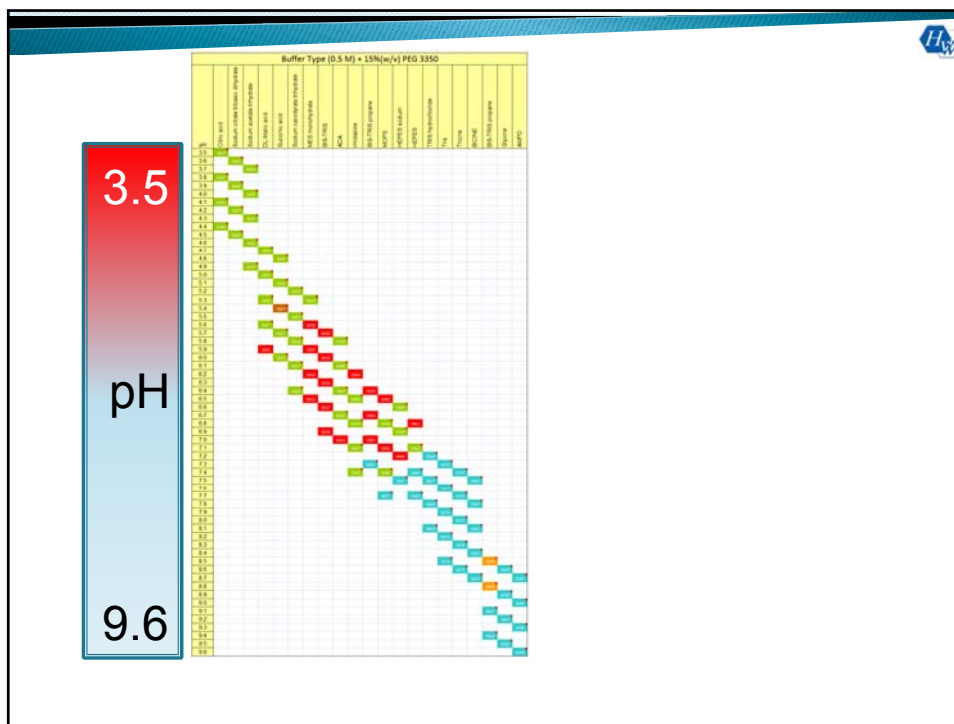


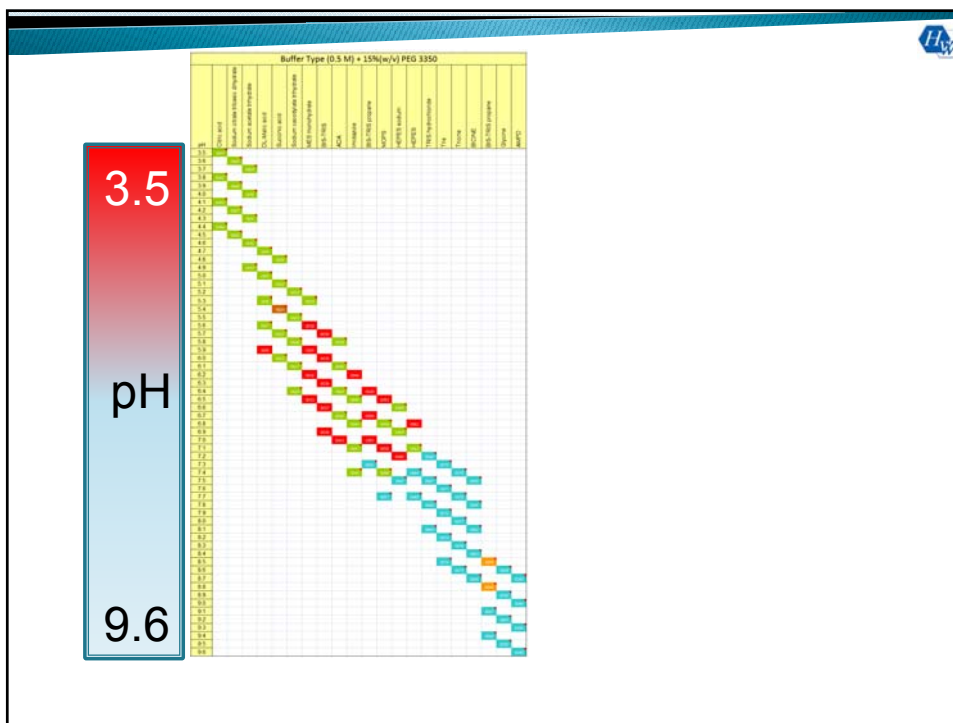
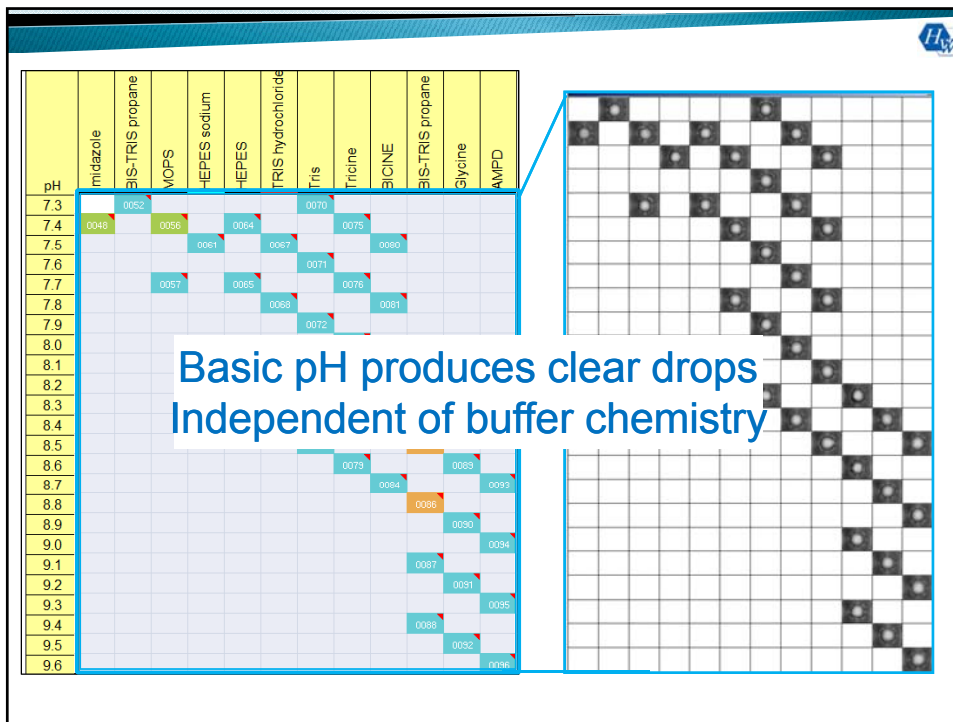


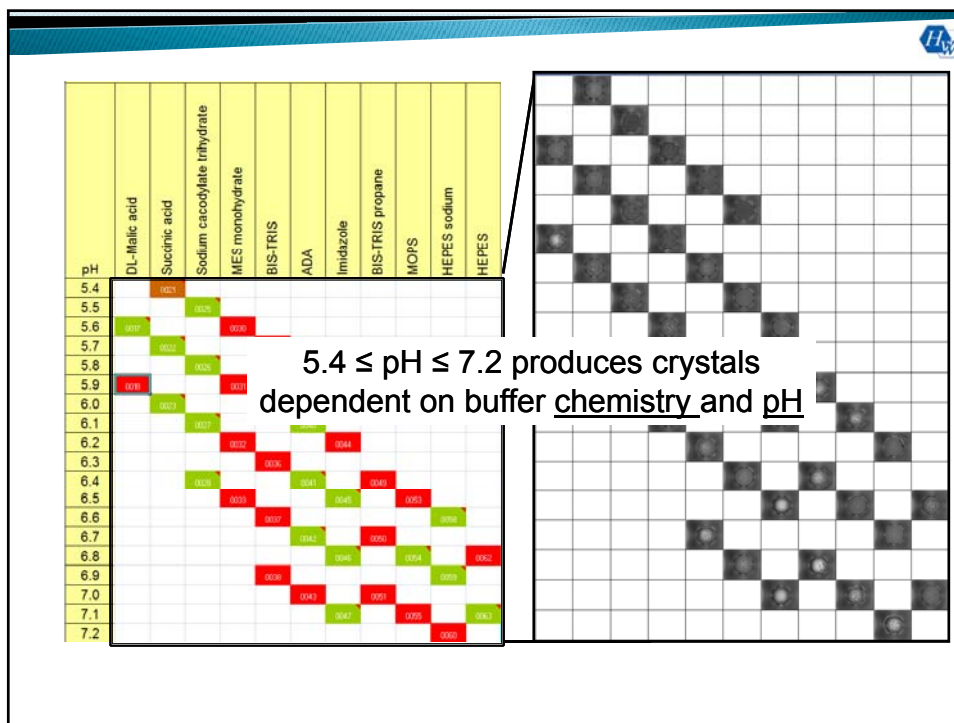
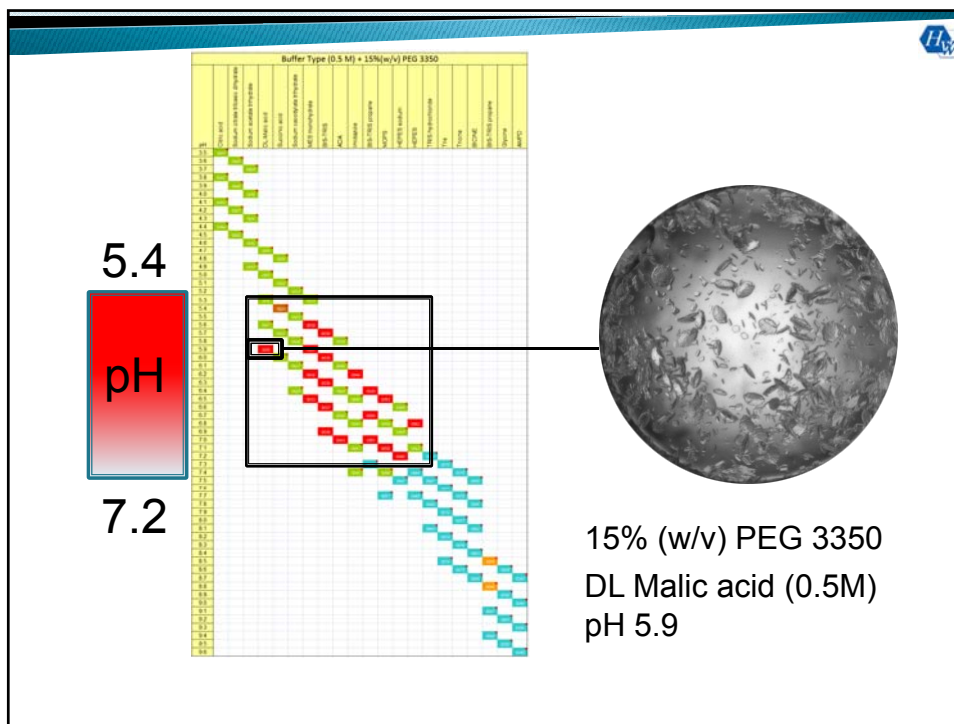


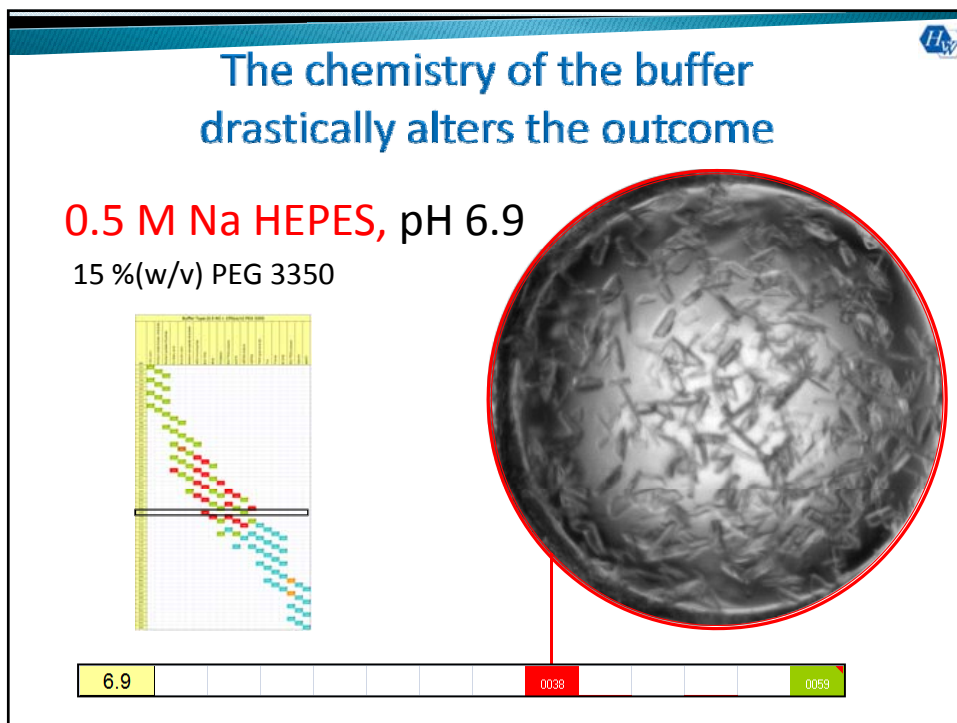
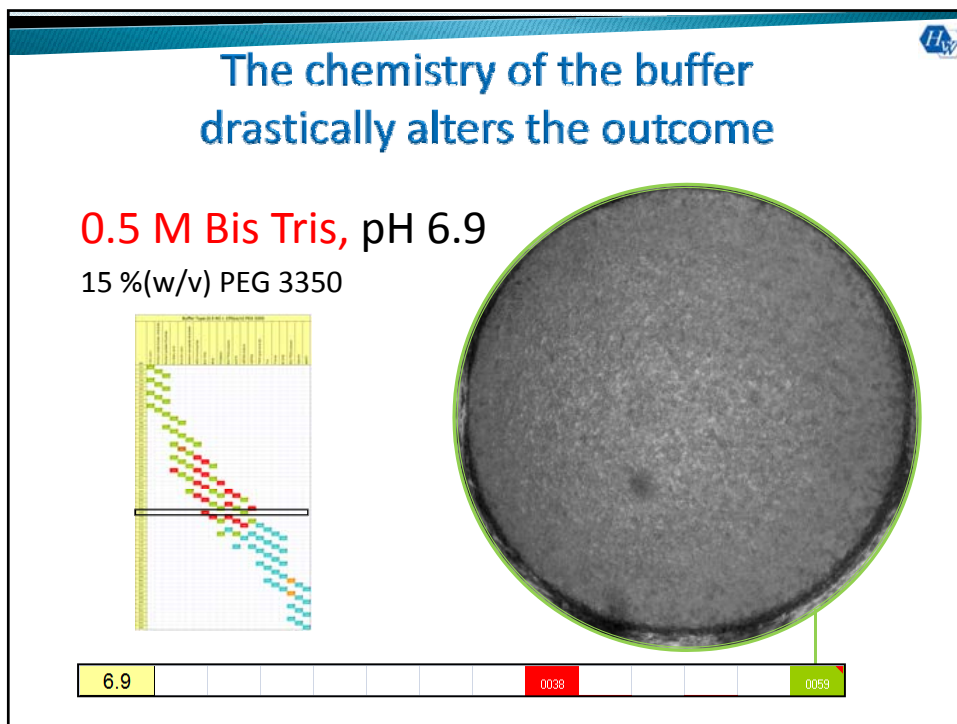







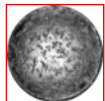








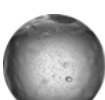



Buffer type held constant with variable pH

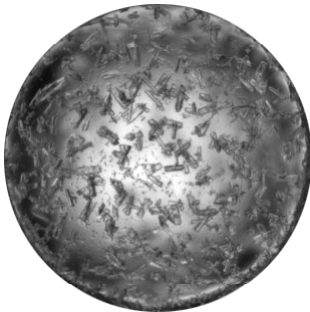
pH 6.5 

pH 6.8 

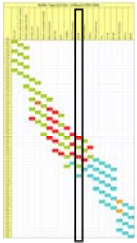
pH 7.1 


pH 7.4 

pH 7.7 

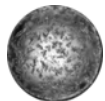


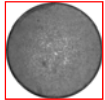
0.5 M MOPS, pH 6.5
15 %(w/v) PEG 3350




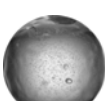



Buffer type held constant with variable pH

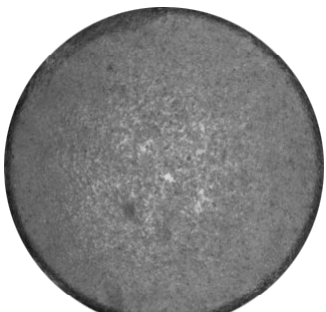
pH 6.5 

pH 6.8 

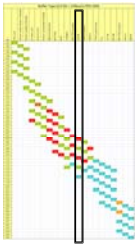
pH 7.1 

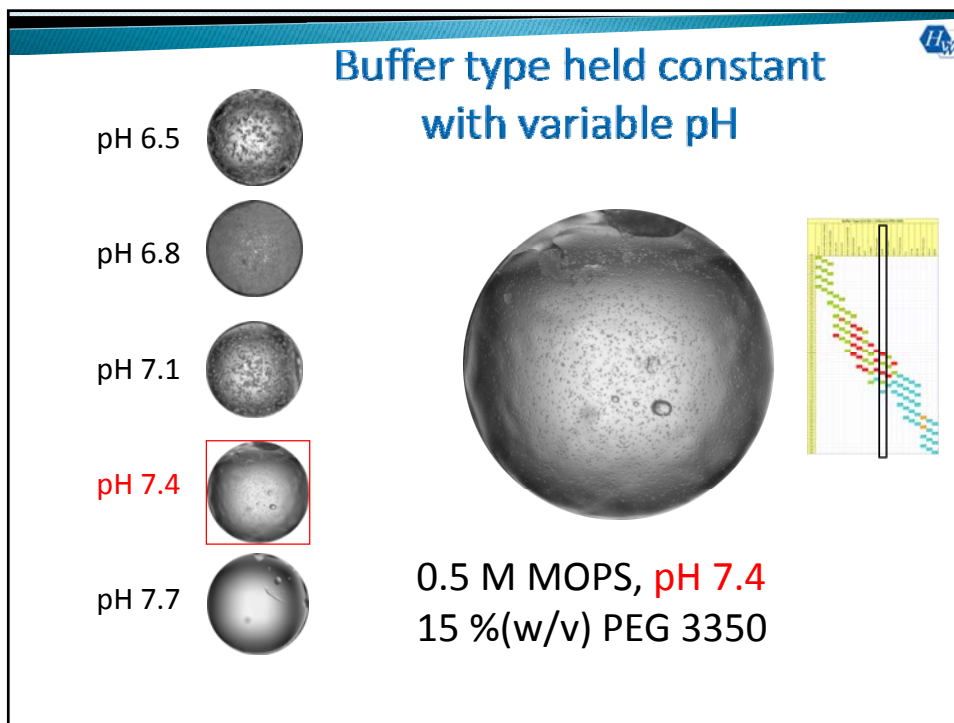
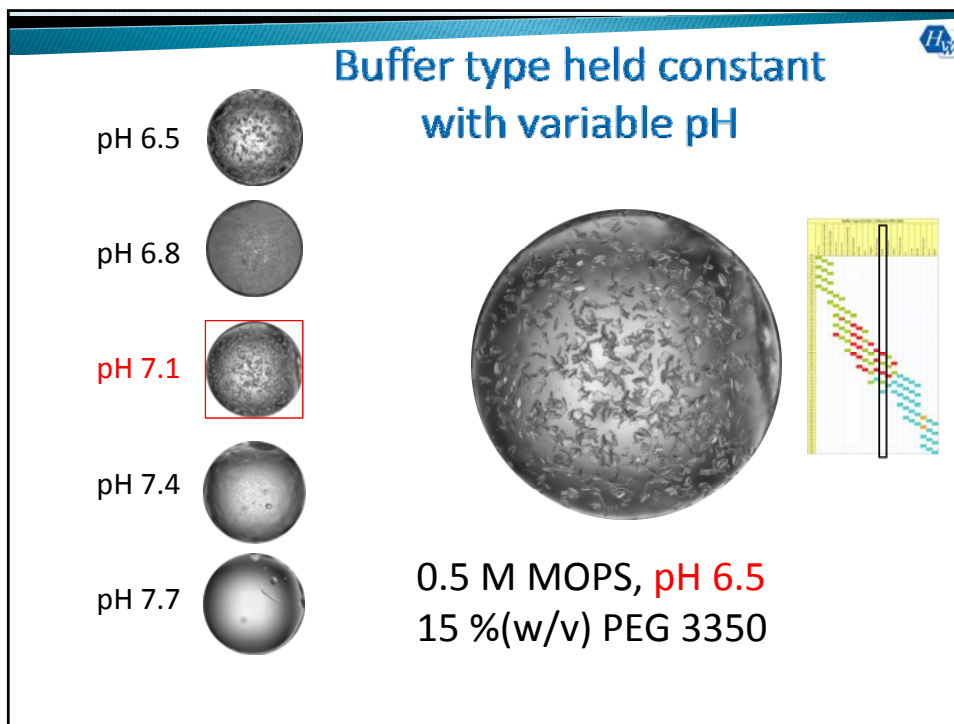
pH 7.4 

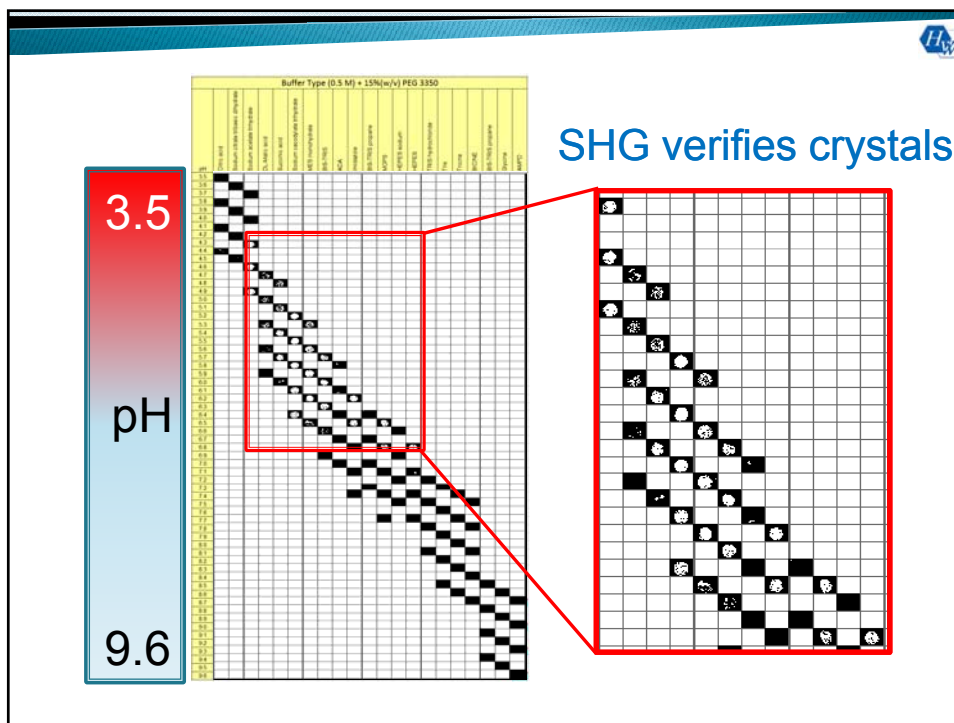
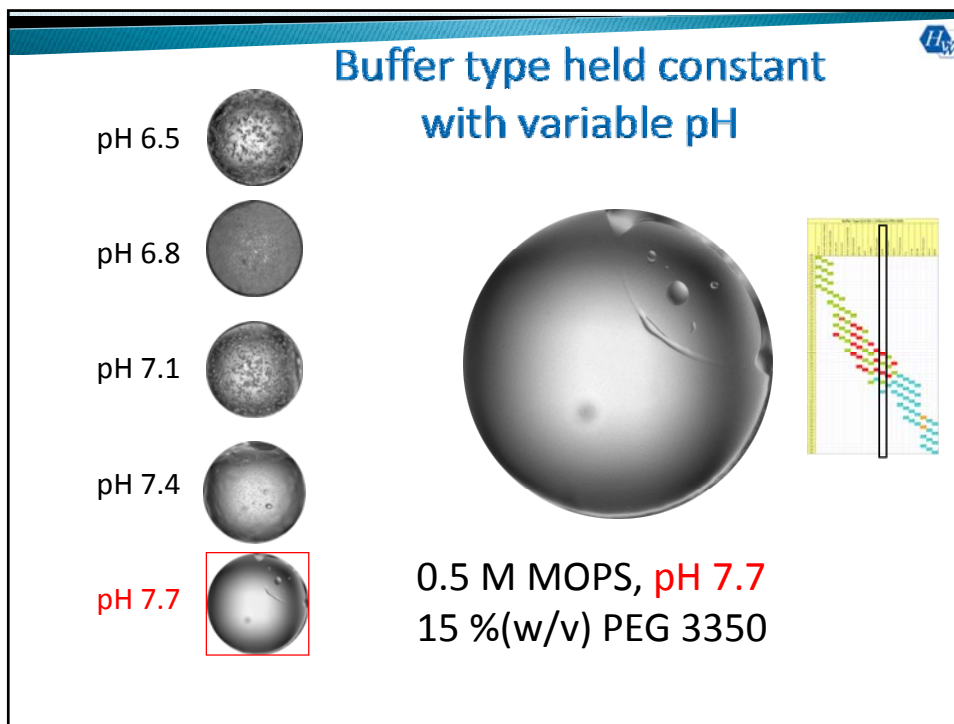
pH 7.7 



0.5 M MOPS, pH 6.8
15 %(w/v) PEG 3350



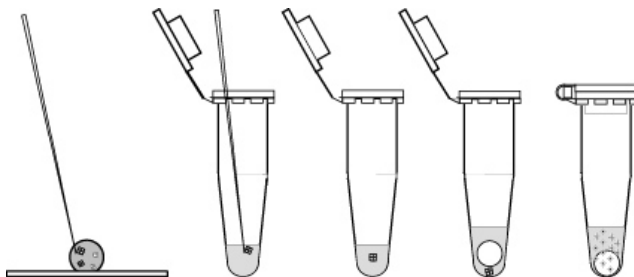




The sledgehammer

Crushing crystals using a seed bead

- ▶ Take crystal(s) and add to a stabilizing solution in a 1.5 ml microcentrifuge tube
- ▶ Add Teflon bead
- ▶ Vortex mix to produce micro-crystals



Acta Cryst. (1999). **D55**, 988-993

Final thoughts

The same variables will determine
optimum conditions to produce
large or nanocrystals

“...*pH, salt concentration, protein
concentration and temperature* ...”

Lewin J: Preparation and Properties of Serum and Plasma Proteins. XXX.
Crystalline Derivatives of Human Serum Albumin and of Certain Other
Proteins. *Journal of the American Chemical Society* 1951, 73:3906-3911.

