RAMC – Bichenberg 2013

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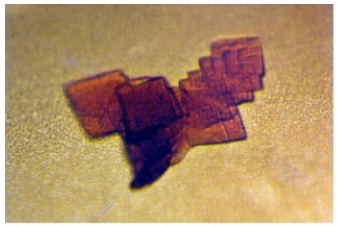
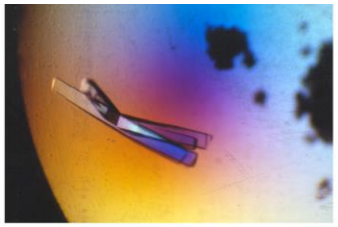
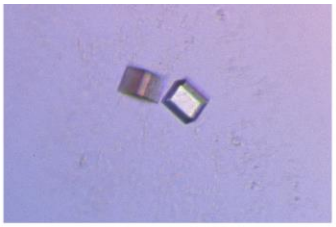
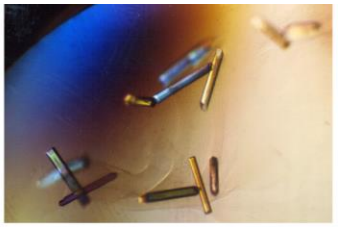
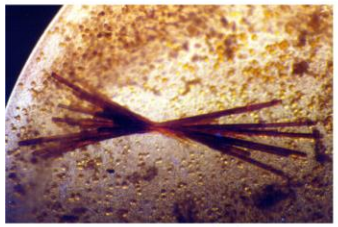
University of California, Irvine

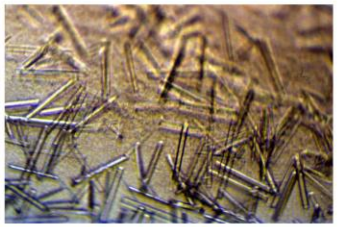

Sheep Hemoglobin


Carbonic Anhydrase

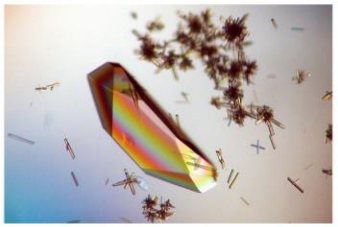

Concanavalin B


Concanavalin A

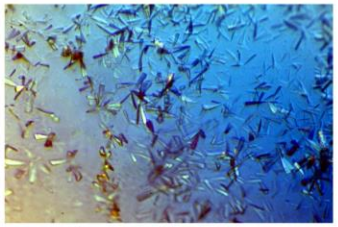

Goat Hemoglobin


Bence Jones Protein KWR

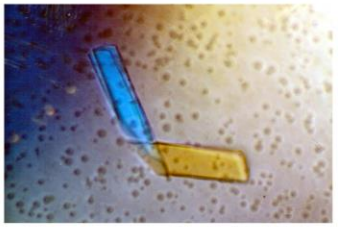

Fungal Lipase

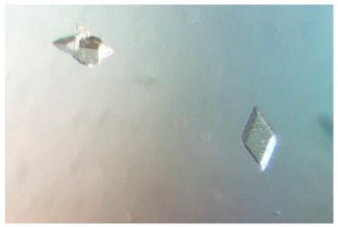

Lysozyme

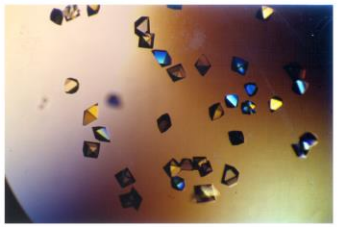

α Lactalbumin

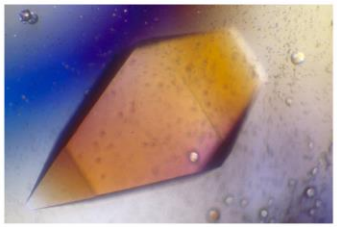

Bacterial Xylanase

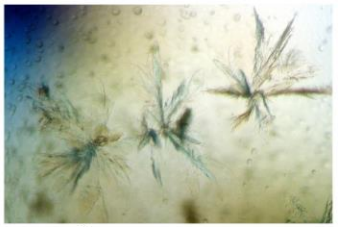

RNase A

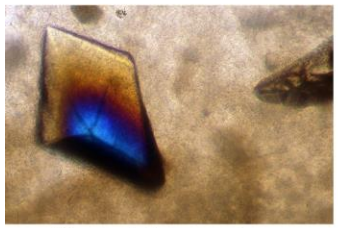

Bence Jones Protein MLE

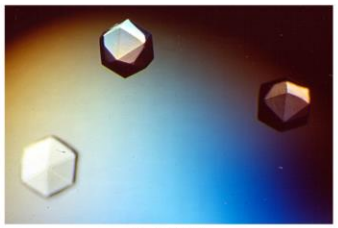

SPMV

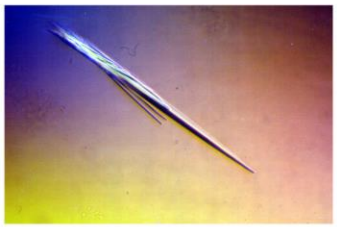

Proteinase K


Porcine Trypsin


β Lactoglobulin

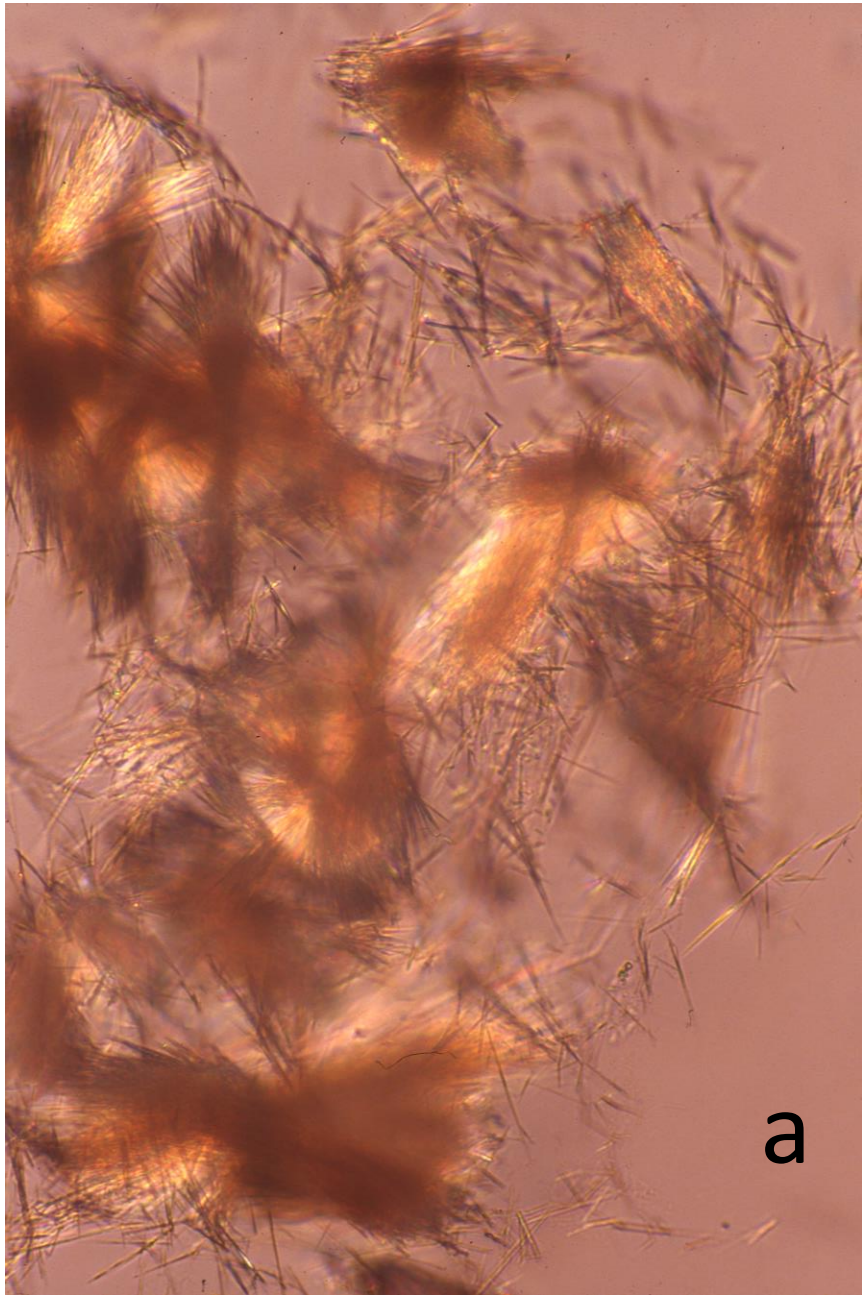

Bacterial α-amylase


TYMV


Elastase


Alcohol Dehydrogenase

experiment I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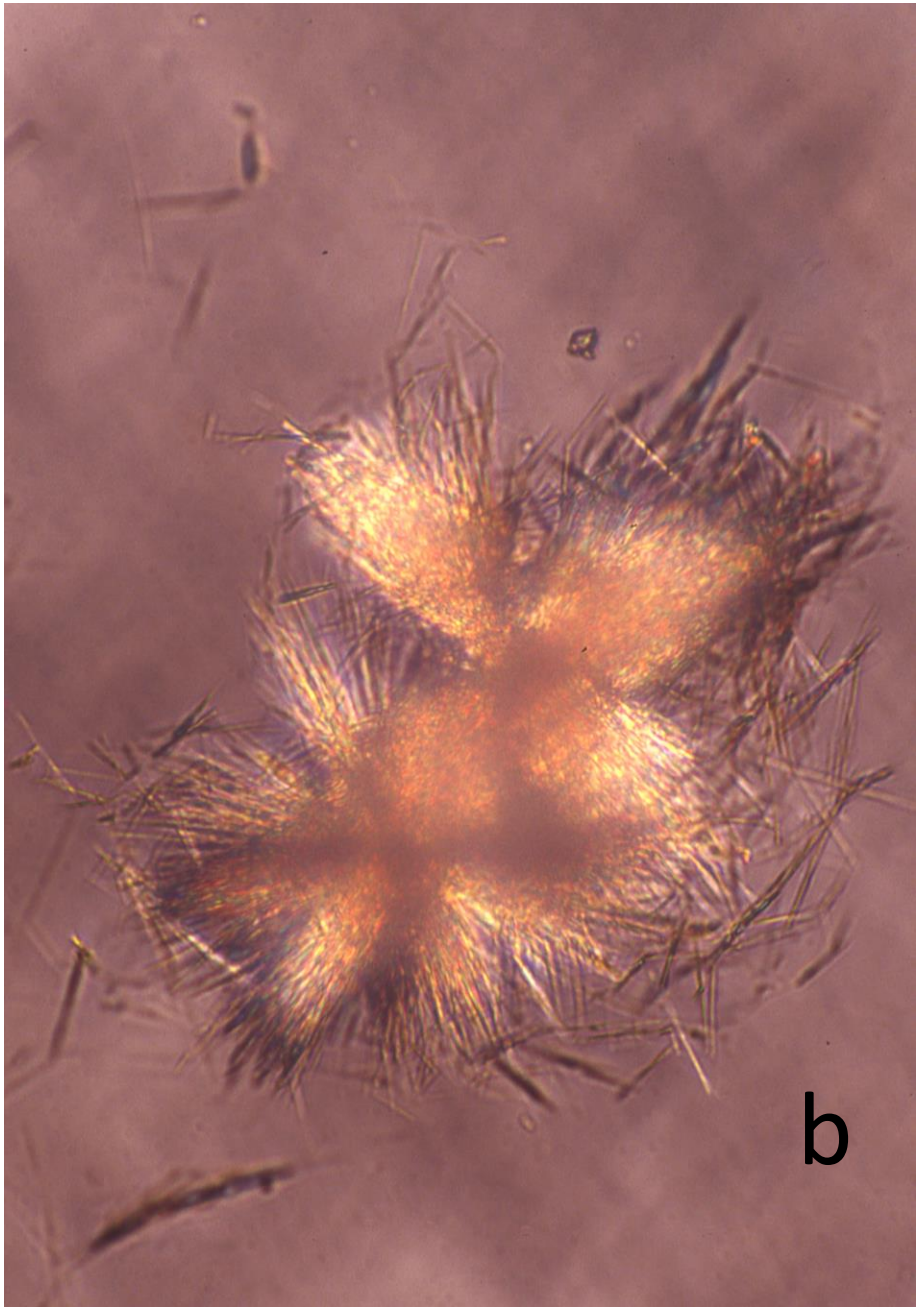
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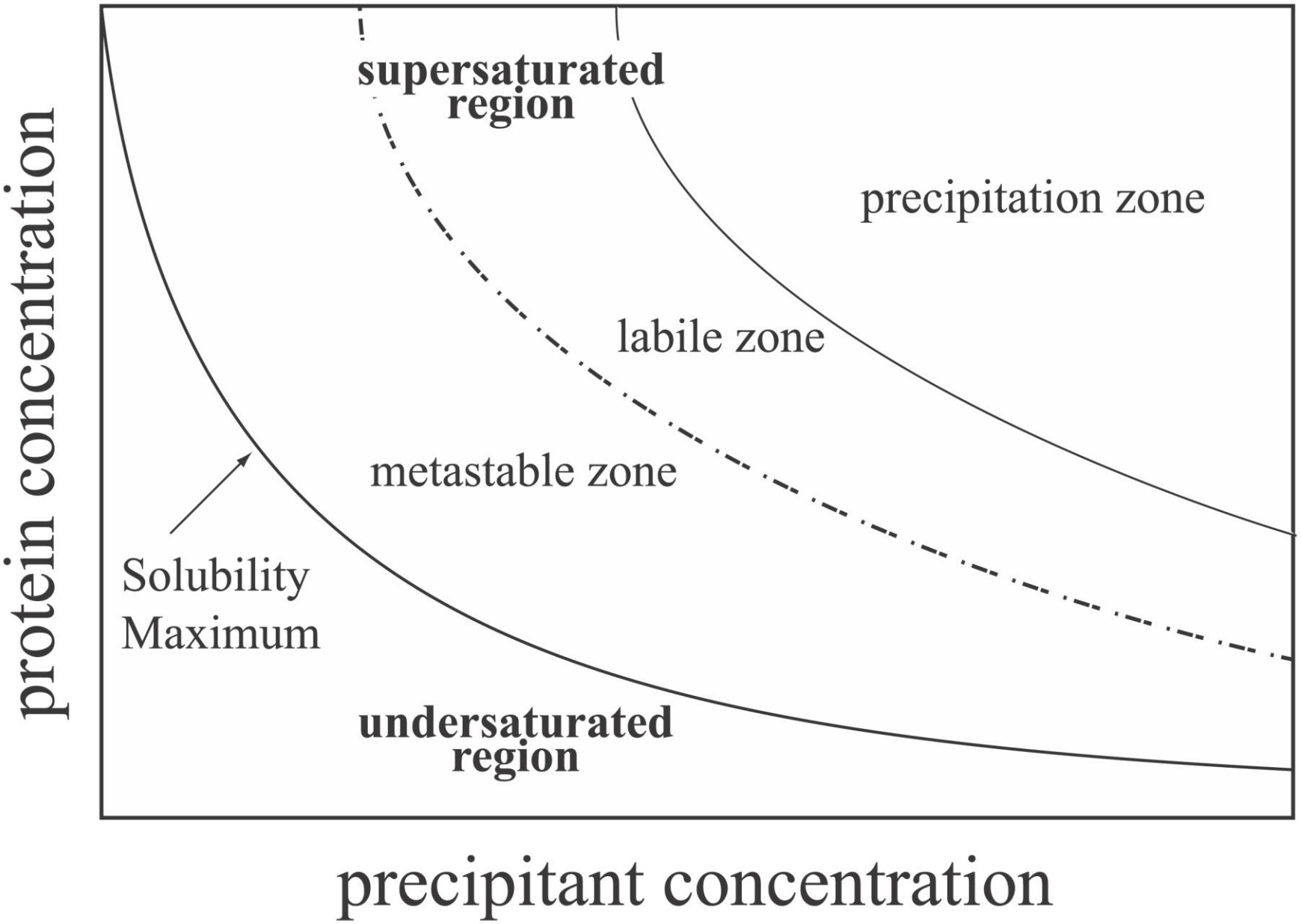
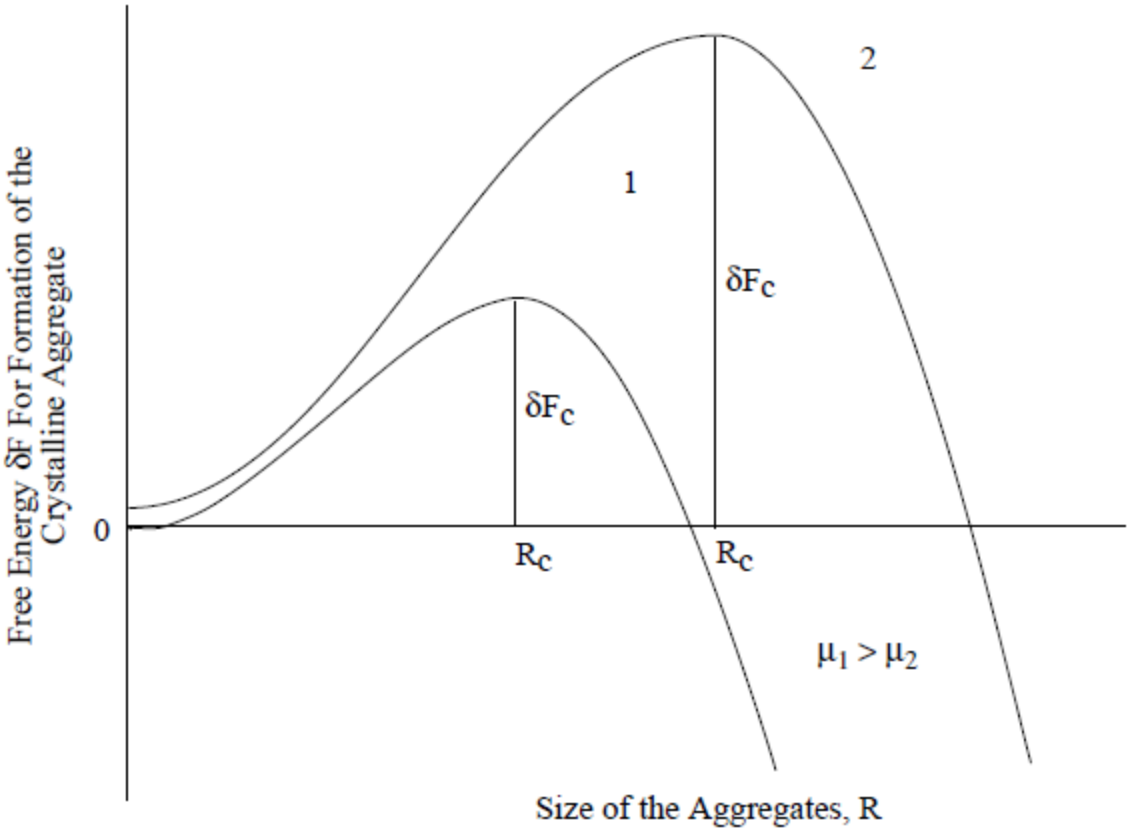
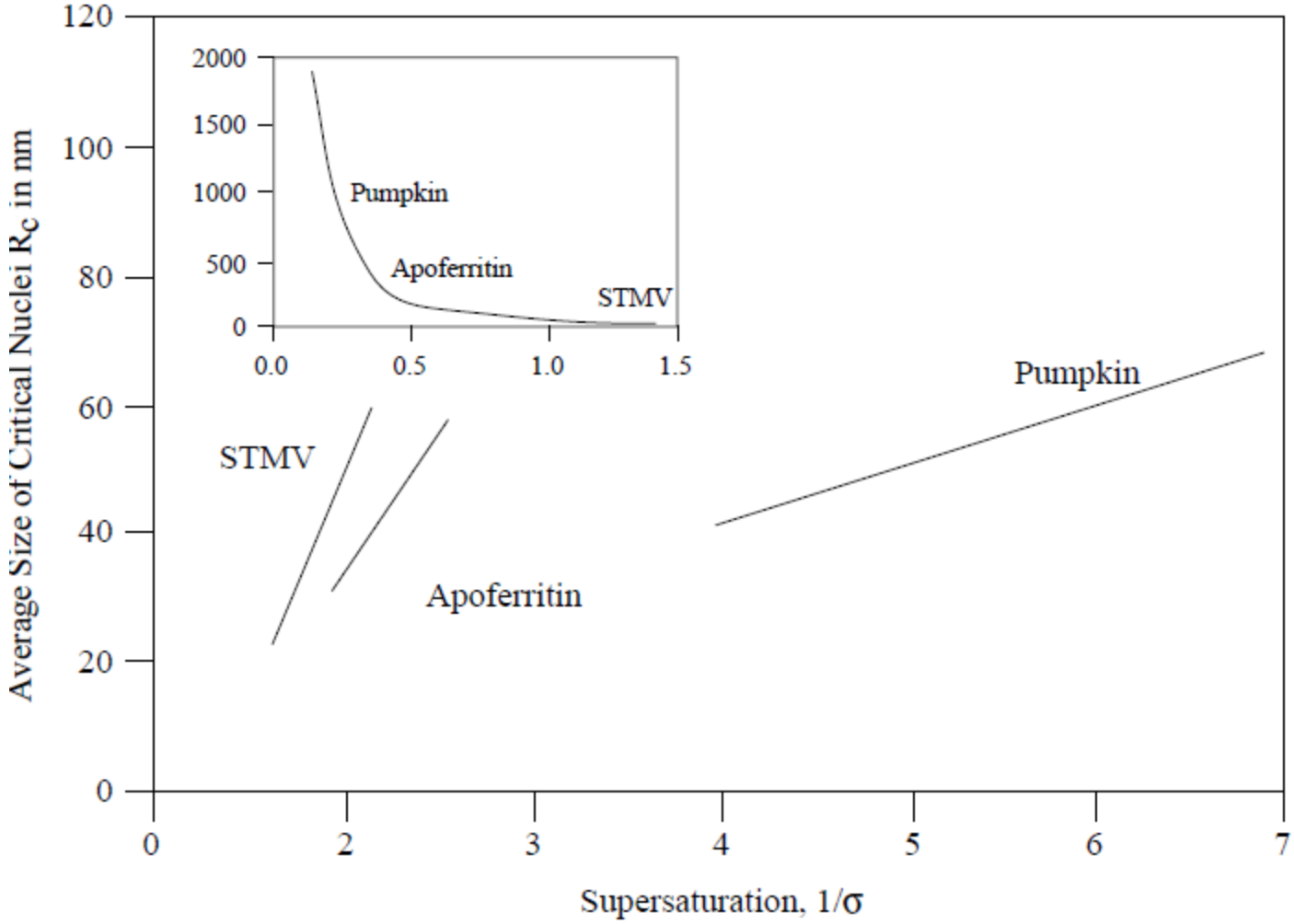
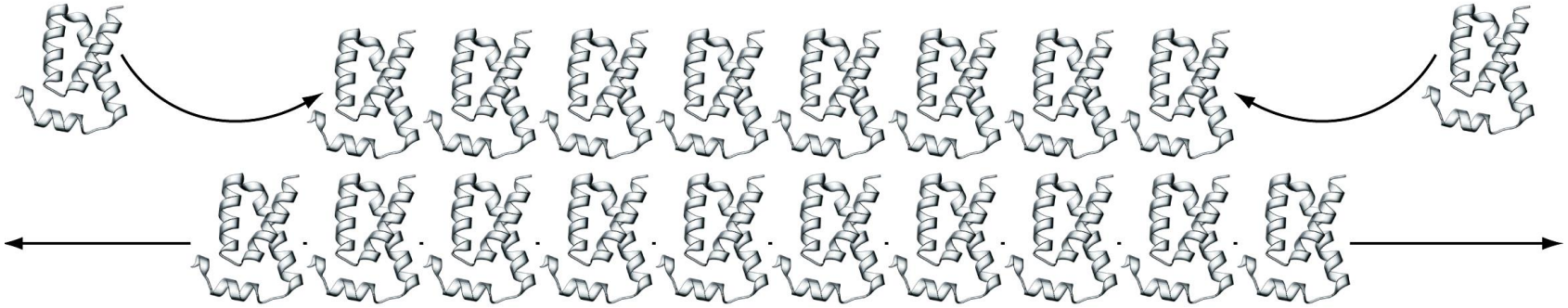
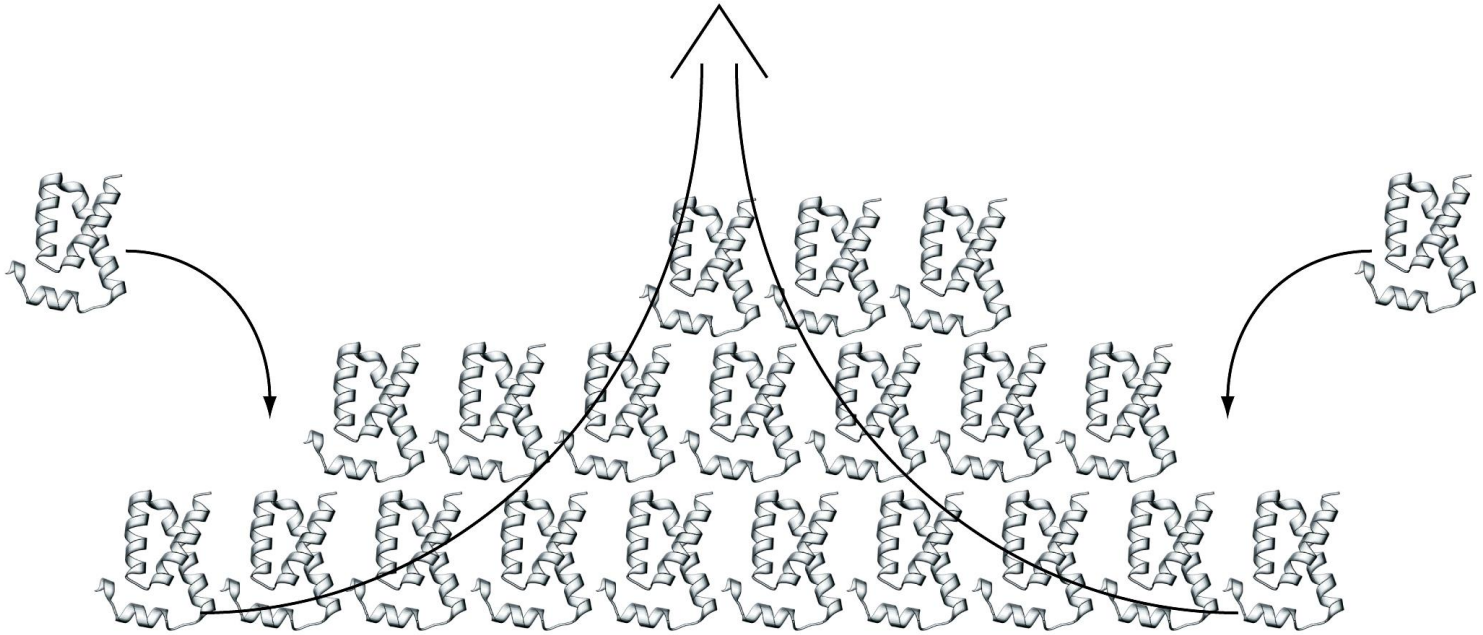

supersaturated
region
precipitation zone
labile zone
metastable zone
Solubility
Maximum
undersaturated
region
protein concentration
precipitant concentration


Free Energy δF For Formation of the Crystalline Aggregate
0
1
2
δFc
δFc
Rc
Rc
μ1 > μ2
Size of the Aggregates, R


Average Size of Critical Nuclei R_c in nm
120
100
80
60
40
20
0
0
2
3
4
5
6
7
Supersaturation, 1/σ
2000
1500
1000
500
0
0.0
0.5
1.0
1.5
Pumpkin
Apoferritin
STMV
STMV
Apoferritin
Pumpkin

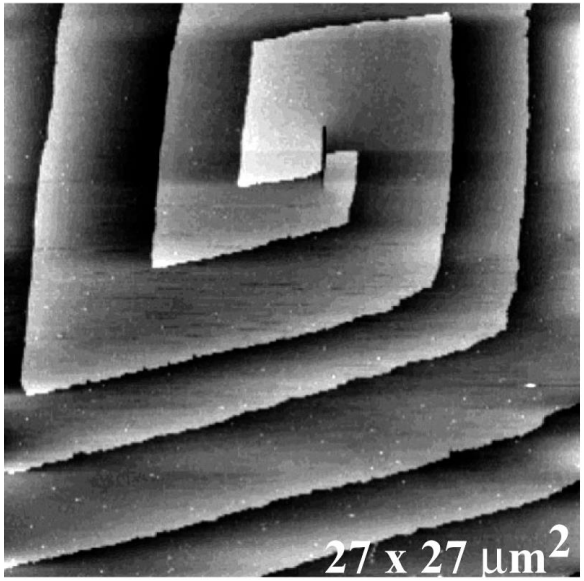
Tangential and Normal Growth





Creation of new steps - Normal Growth

Requires 2D nucleation



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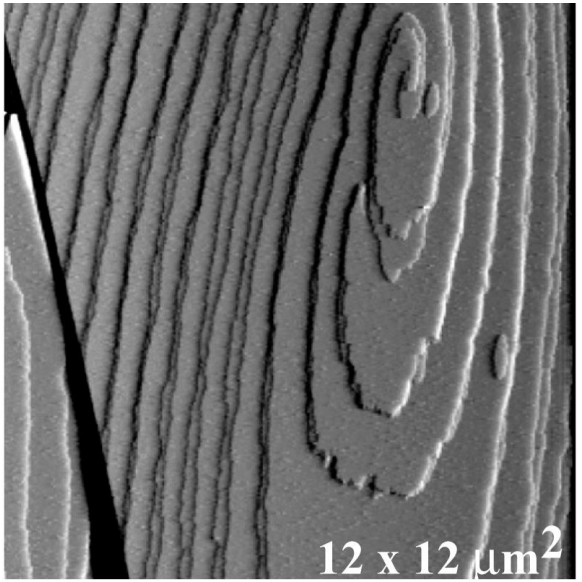

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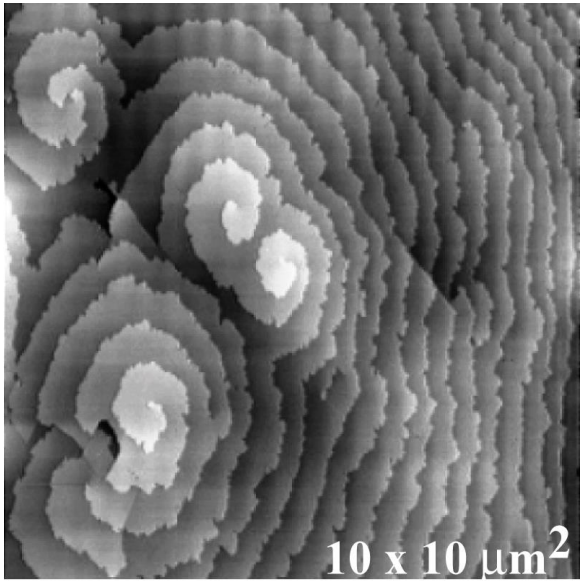
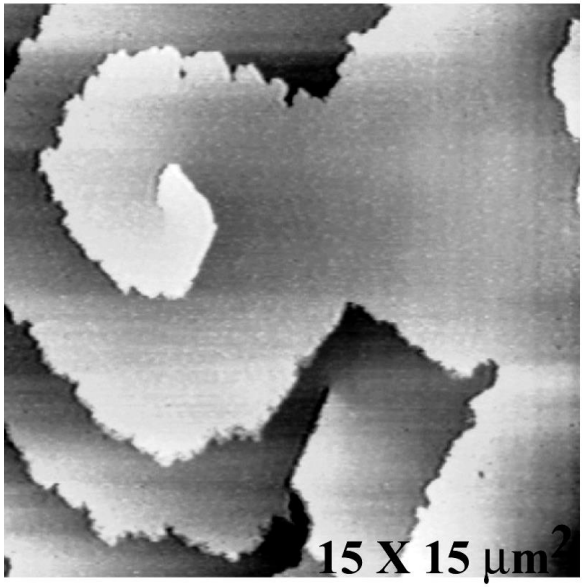
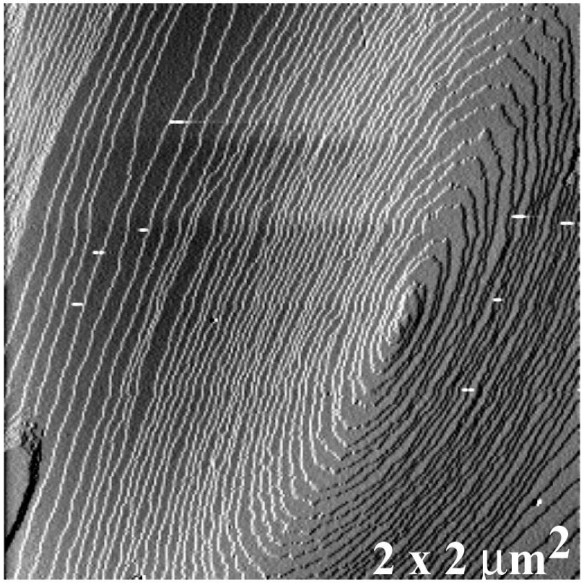


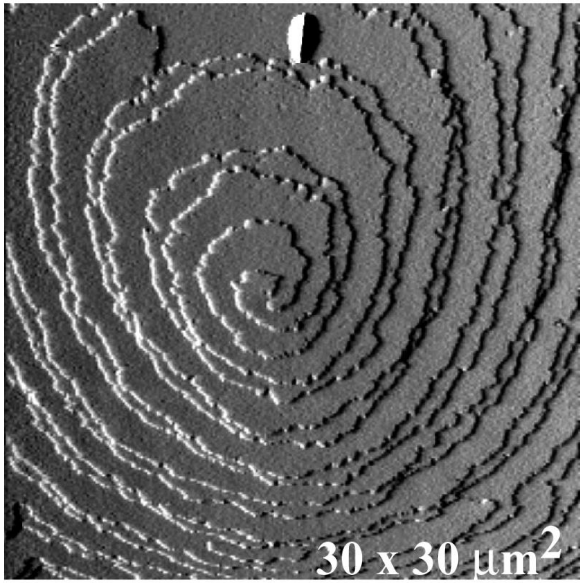
c



d



e



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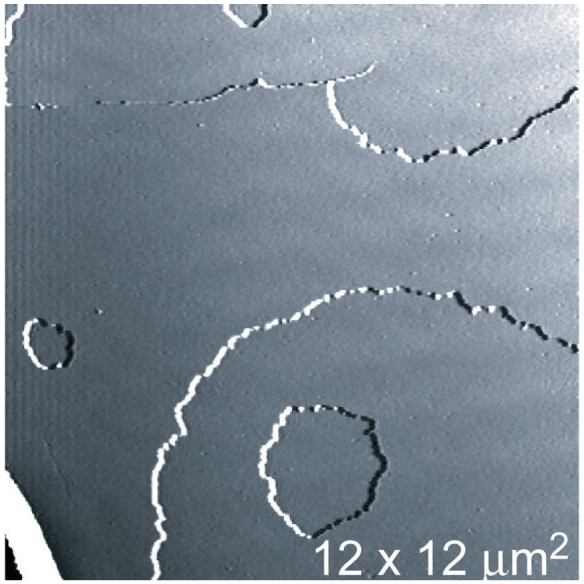

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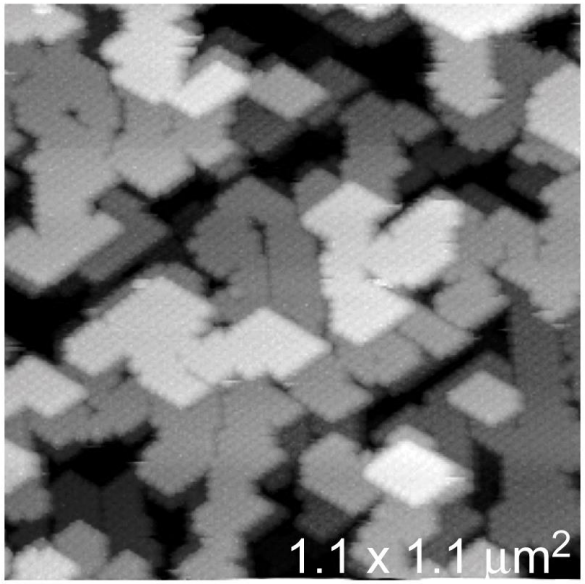

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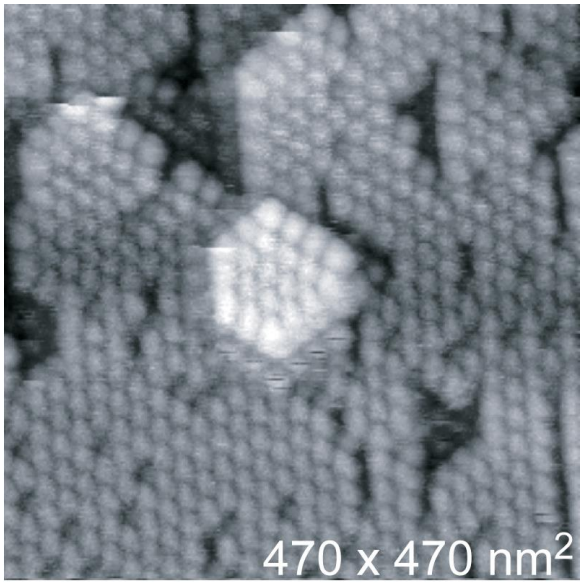

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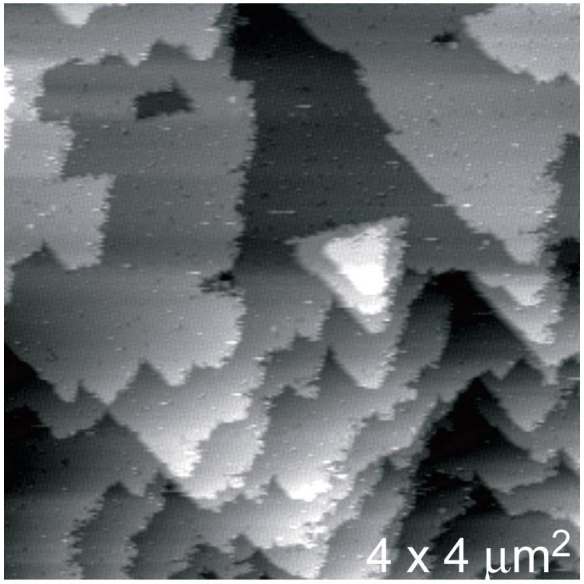

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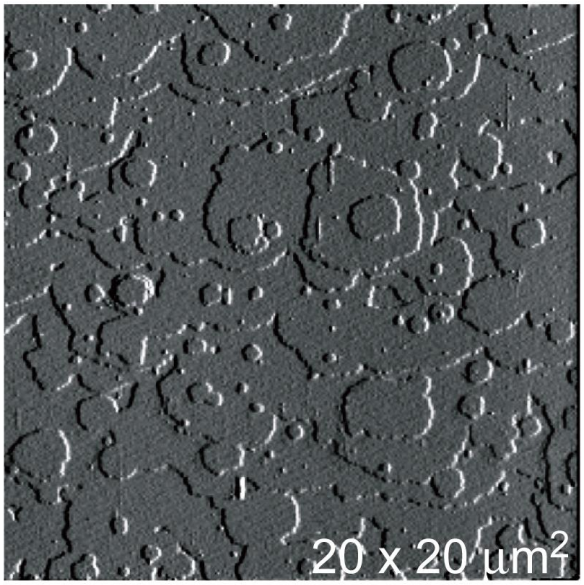

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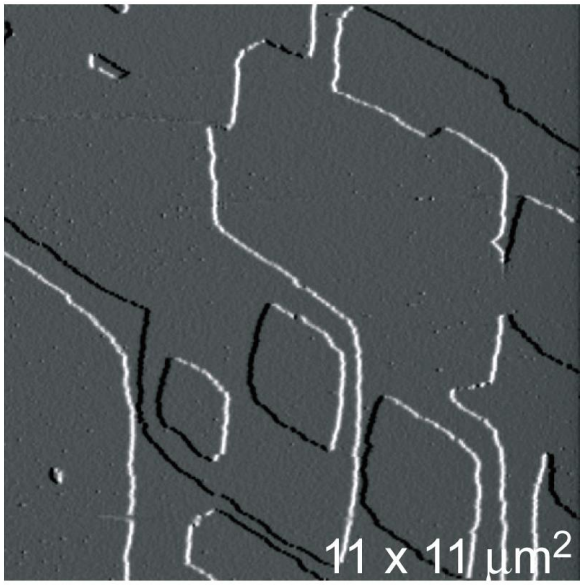

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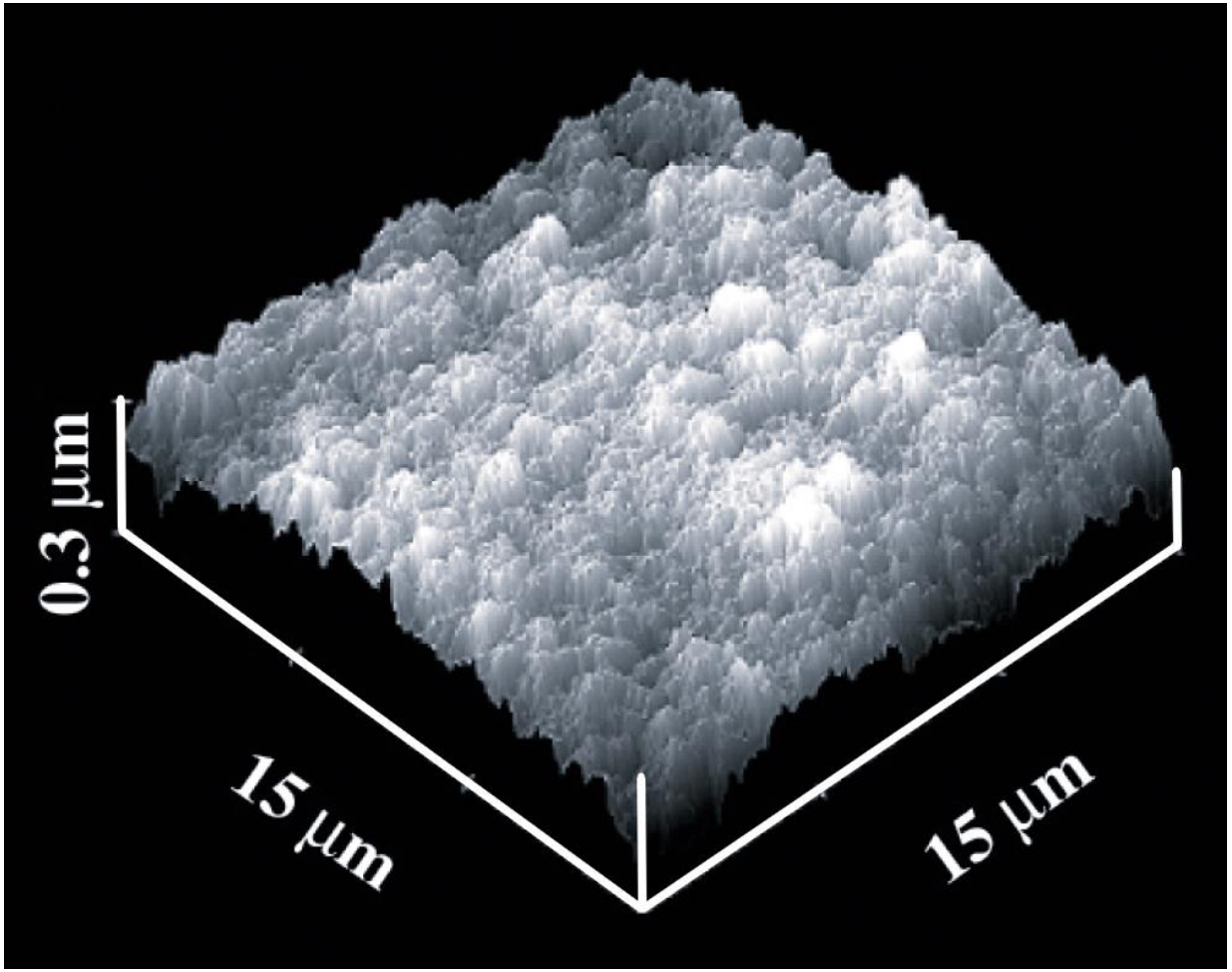
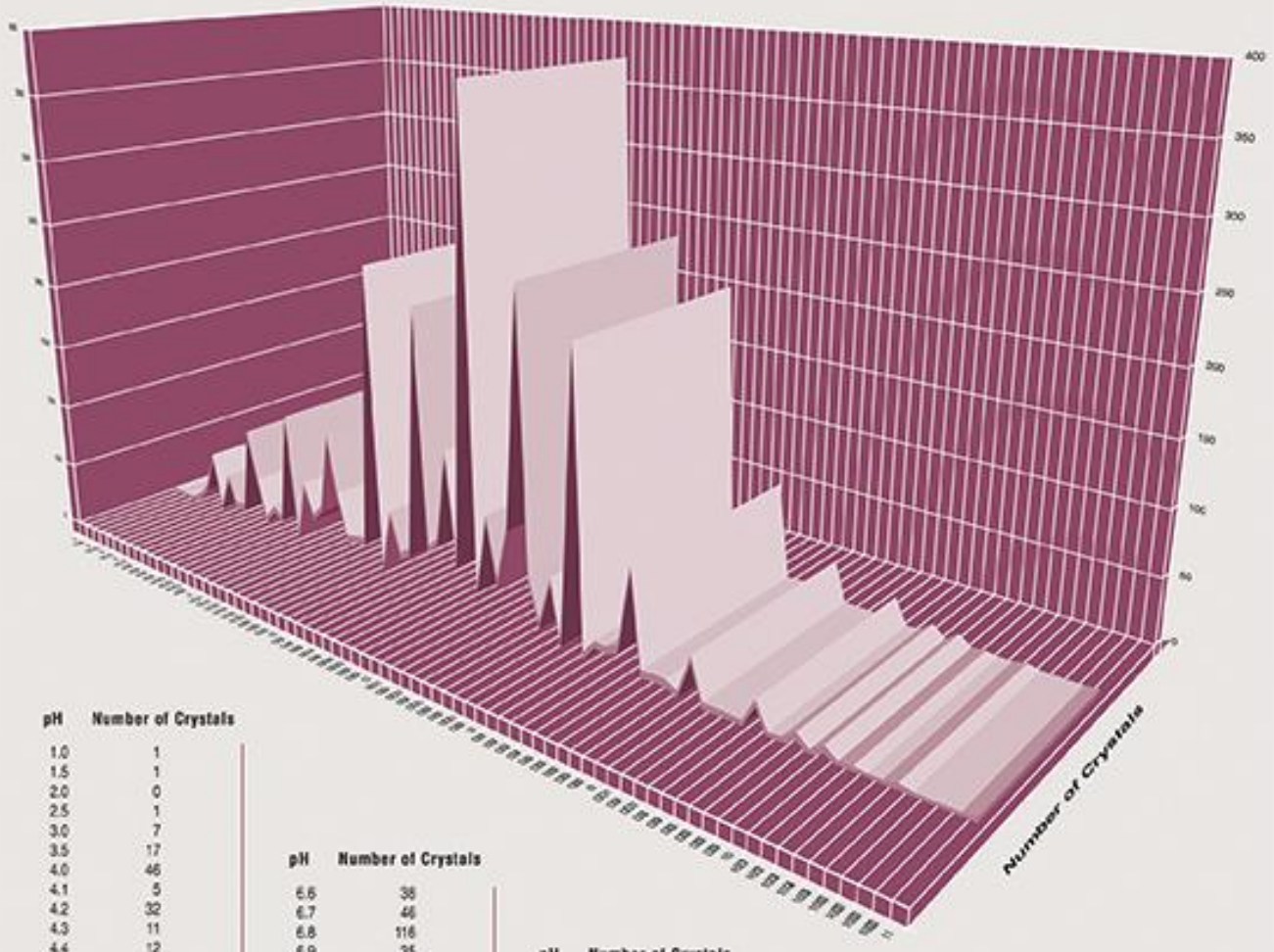


The surface of a ferritin crystal growing at very high supersaturation

Strategies for Creating Supersaturation

- Direct mixing to immediately create a supersaturated condition (Batch Method)
- Alter temperature
- Alter salt concentration (salting in or out)
- Alter pH
- Add a ligand that changes the solubility of the macromolecule
- Alteration of the dielectric of the medium
- Direct removal of water (evaporation)
- Addition of a polymer that produces volume exclusion
- Addition of a cross bridging agent
- Concentration of the macromolecule
- Removal of a solubilizing agent

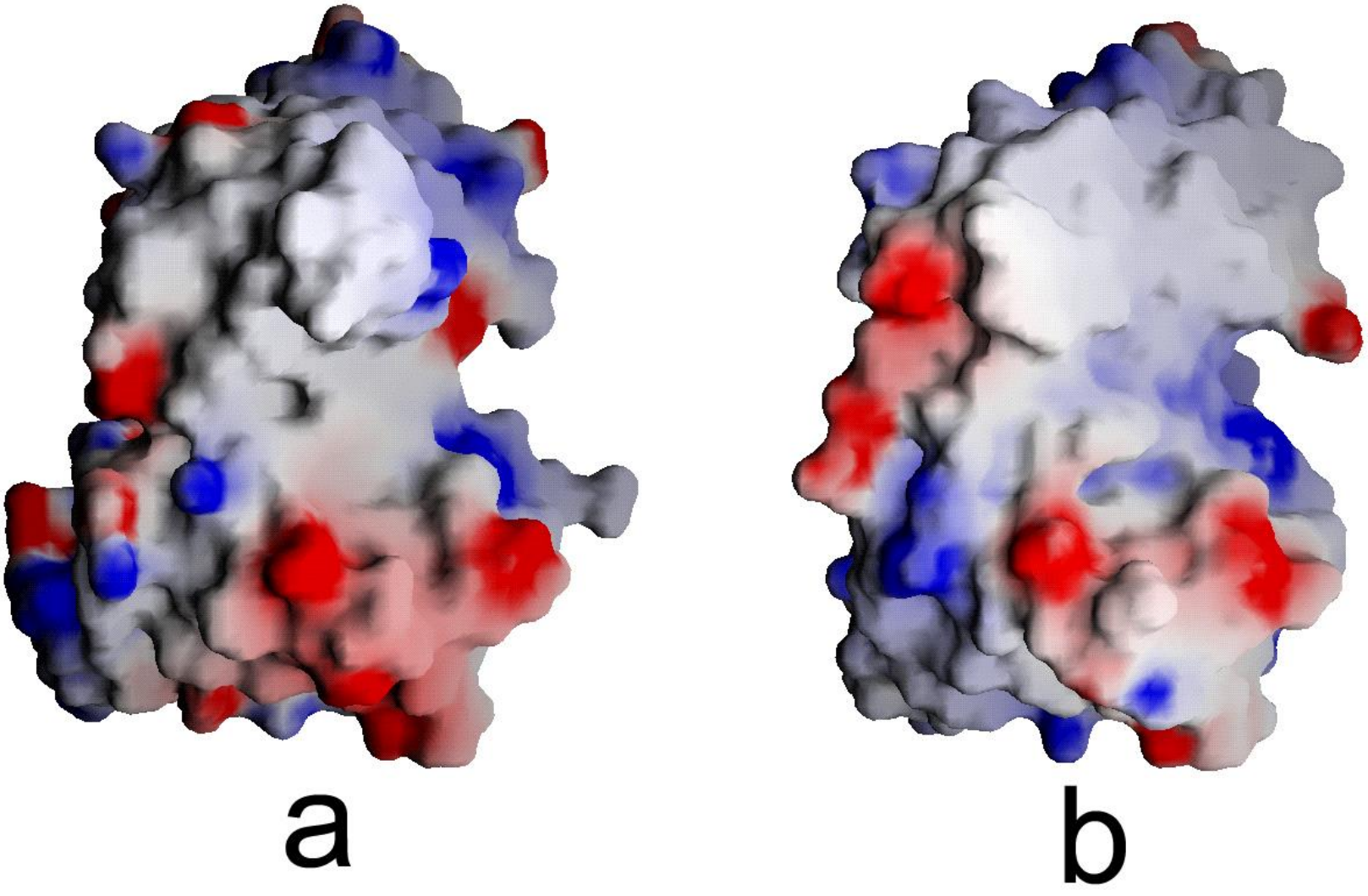


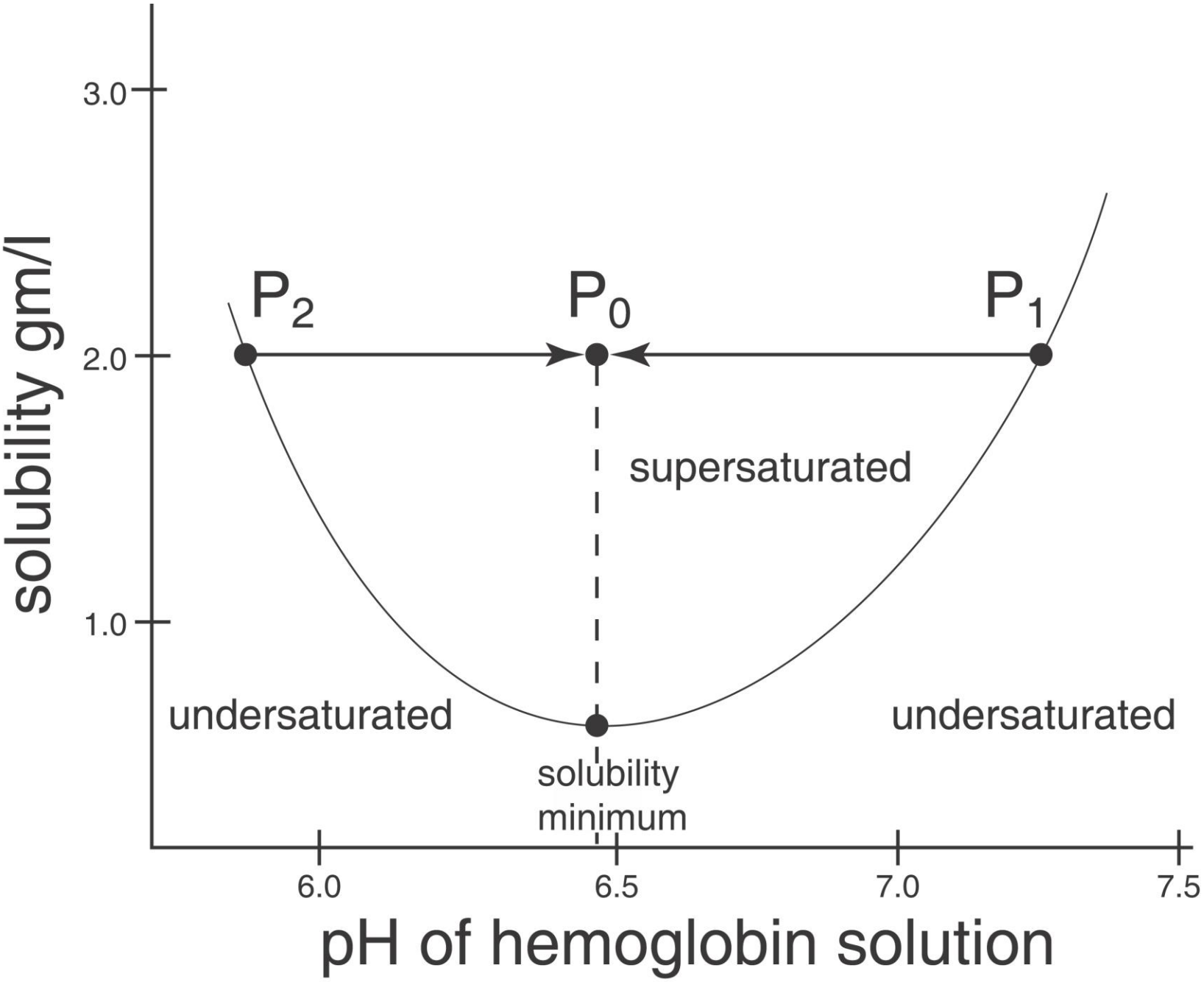
pH	Number of Crystals
1.0	1
1.5	1
2.0	0
2.5	1
3.0	7
3.5	17
4.0	46
4.1	5
4.2	32
4.3	11
4.4	12
4.5	78
4.6	39
4.7	10
4.8	21
4.9	16
5.0	102
5.1	8
5.2	51
5.3	28
5.4	37
5.5	98
5.6	53
5.7	21
5.8	23
5.9	24
6.0	239
6.1	9
6.2	54
6.3	24
6.4	29
6.5	217

pH	Number of Crystals
6.6	38
6.7	46
6.8	116
6.9	35
7.0	379
7.1	21
7.2	78
7.3	32
7.4	77
7.5	251
7.6	45
7.7	13
7.8	49
7.9	6
8.0	220
8.1	3
8.2	15
8.3	14
8.4	18
8.5	79
8.6	7
8.7	8
8.8	8
8.9	3
9.0	32

pH	Number of Crystals
9.1	2
9.2	2
9.3	2
9.4	1
9.5	19
9.6	0
9.7	1
9.8	8
9.9	0
10.0	7
10.1	0
10.2	1
10.3	0
10.4	0
10.5	2
10.6	0
10.7	0
10.8	0
10.9	0
11.0	1

pH versus number of crystals grown for 2,953 biological macromolecules reported from the BMCD.


a
b

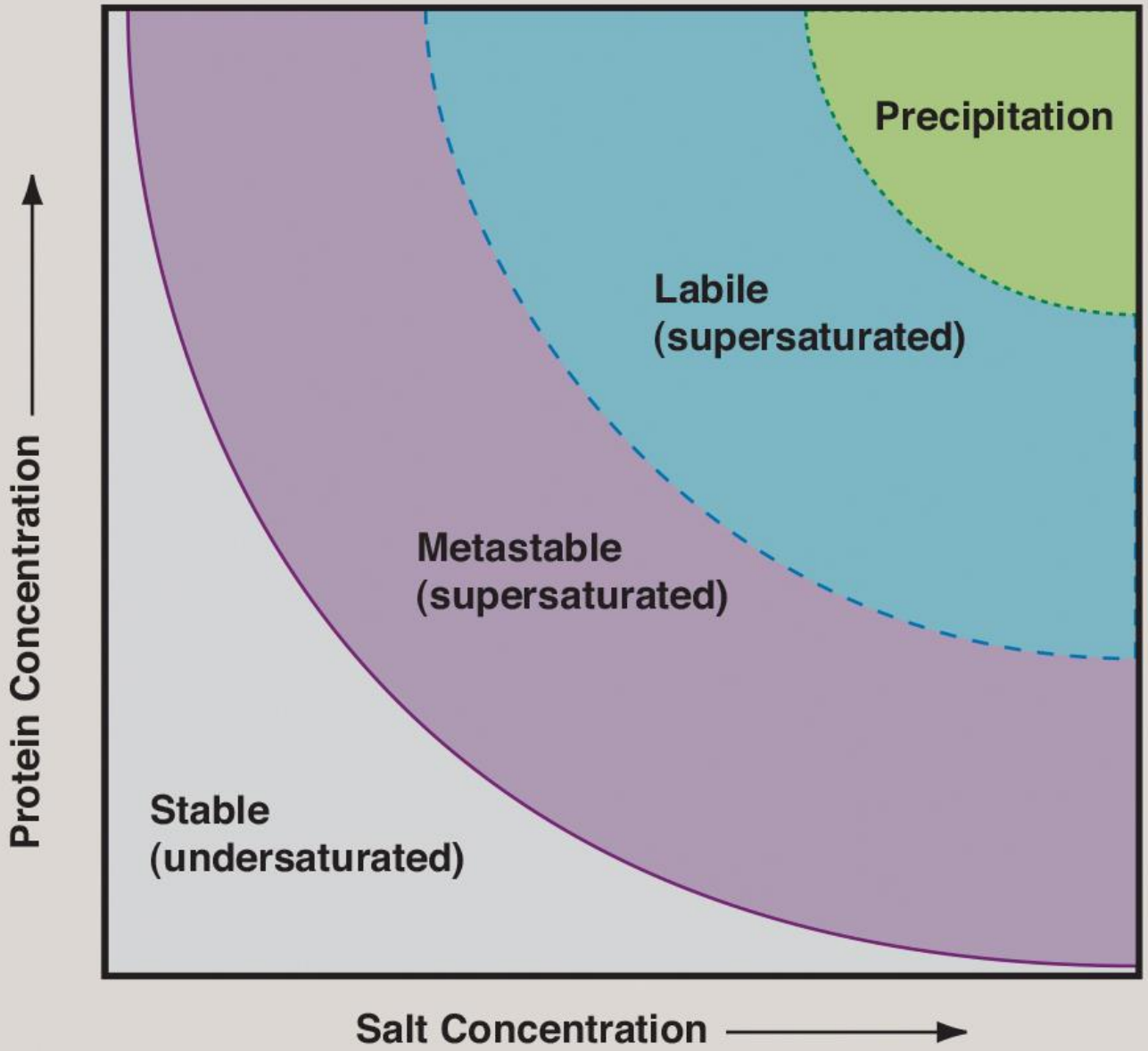

3.0
2.0
1.0
solubility gm/l
P2
P0
P1
supersaturated
undersaturated
undersaturated
solubility
minimum
6.0
6.5
7.0
7.5
pH of hemoglobin solution

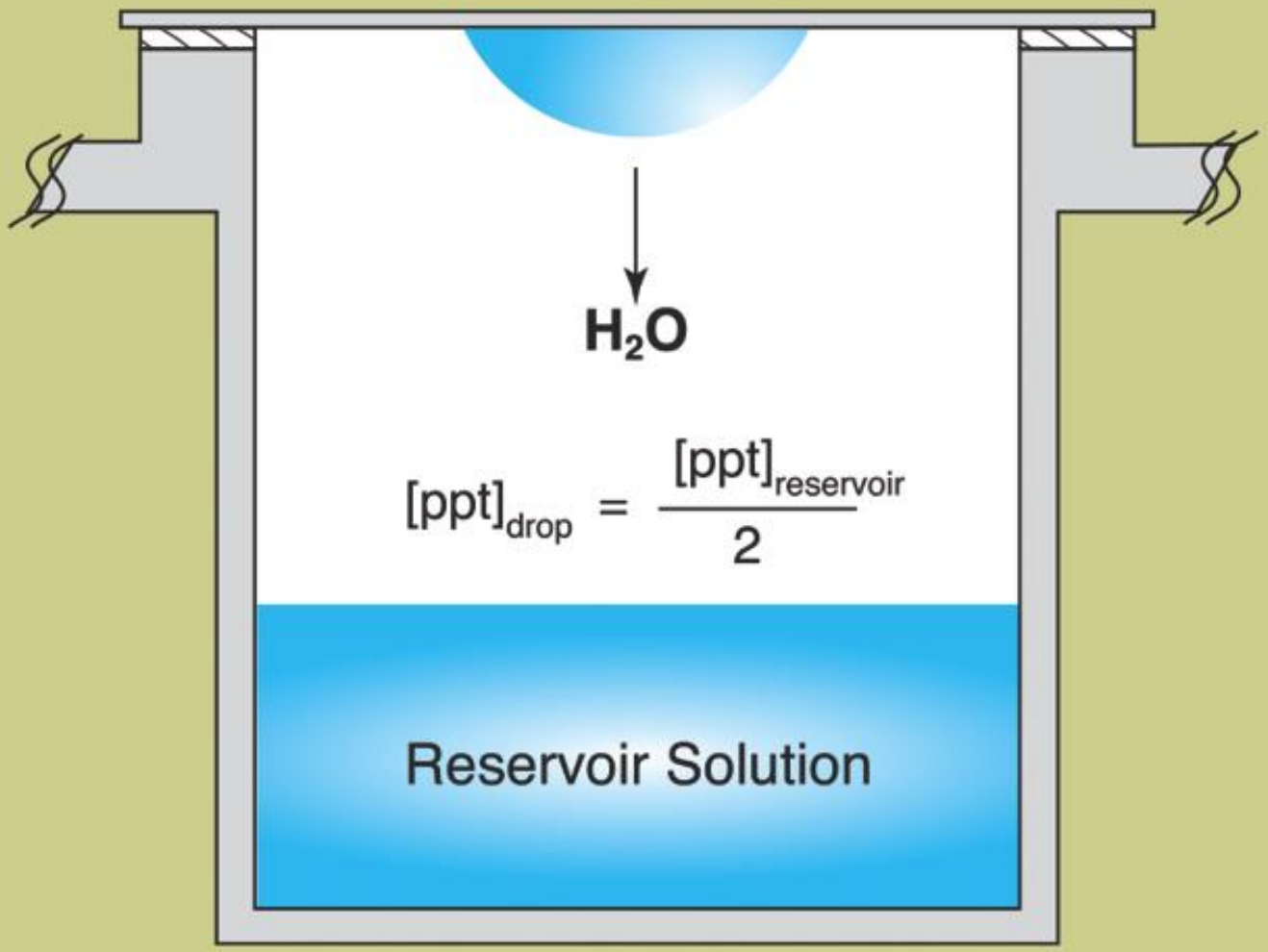
pKa's of ionizable side chains

- Aspartic and Glutamic Acid 3.5 – 4.5
- Histidine 5.5 – 6.5
- Cysteine 7.5 – 8.5
- Lysine and Arginine 8.5 – 10.0

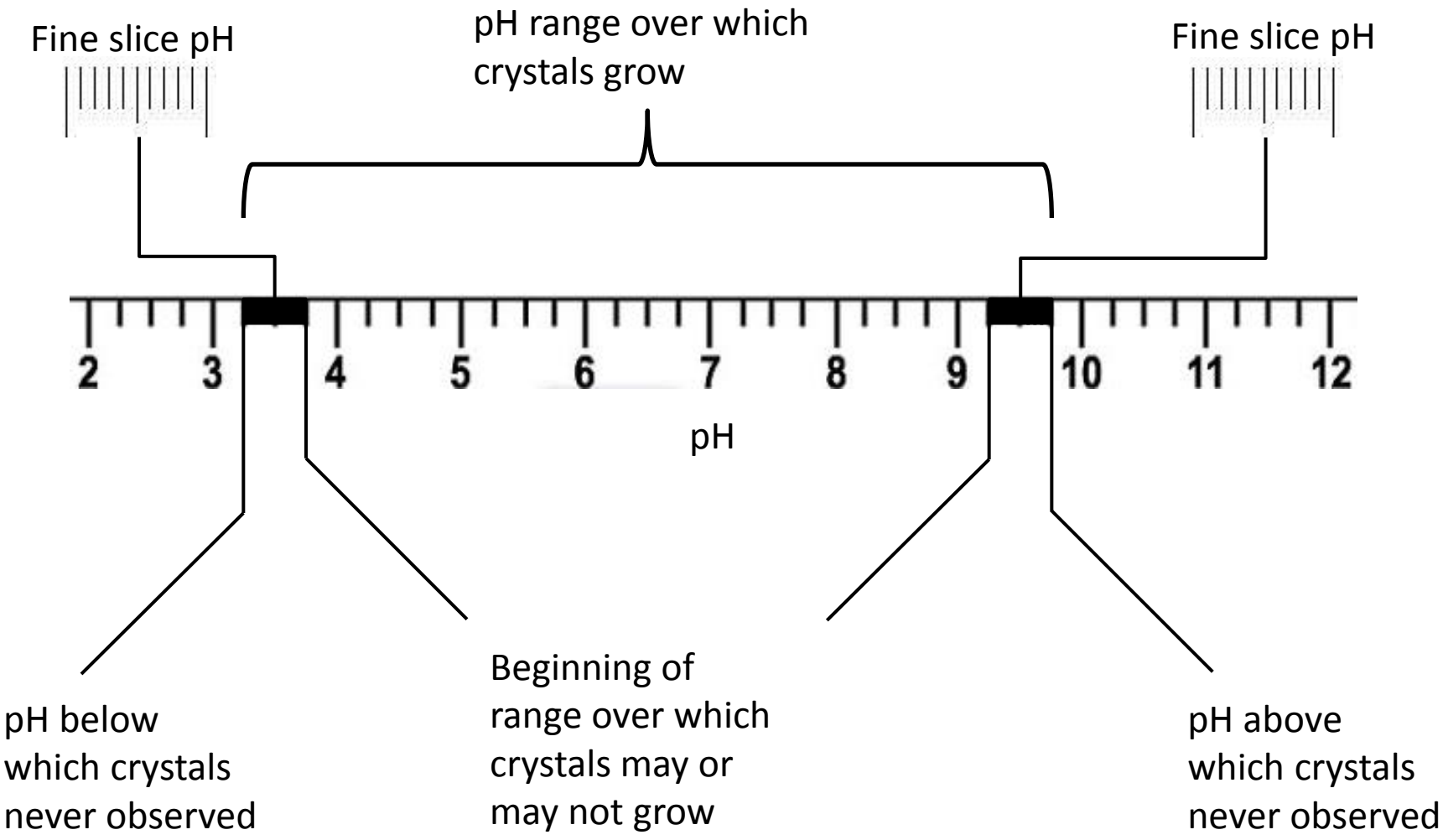
Shifting pH using volatile acids and bases

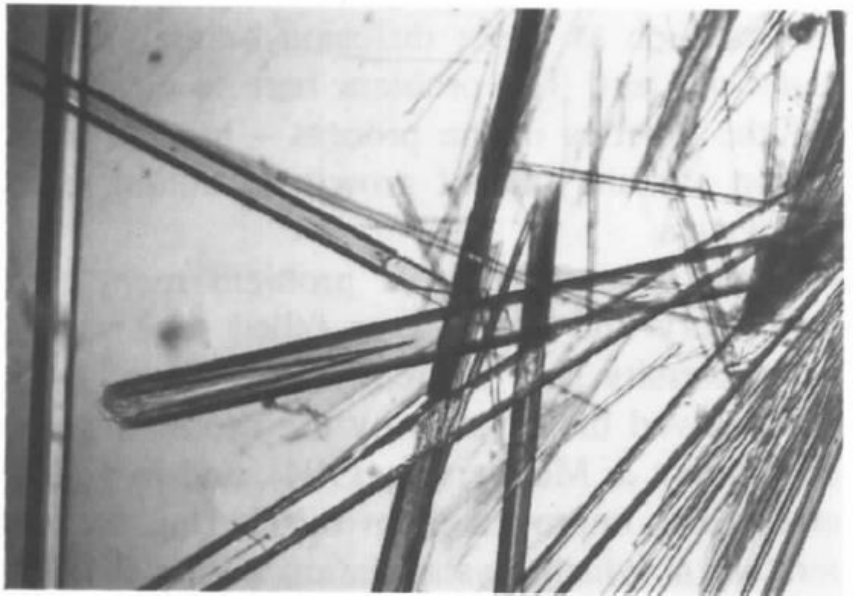
- Acetic acid
- Ammonium hydroxide
- Bicarbonate
- Dry ice (carbon dioxide)

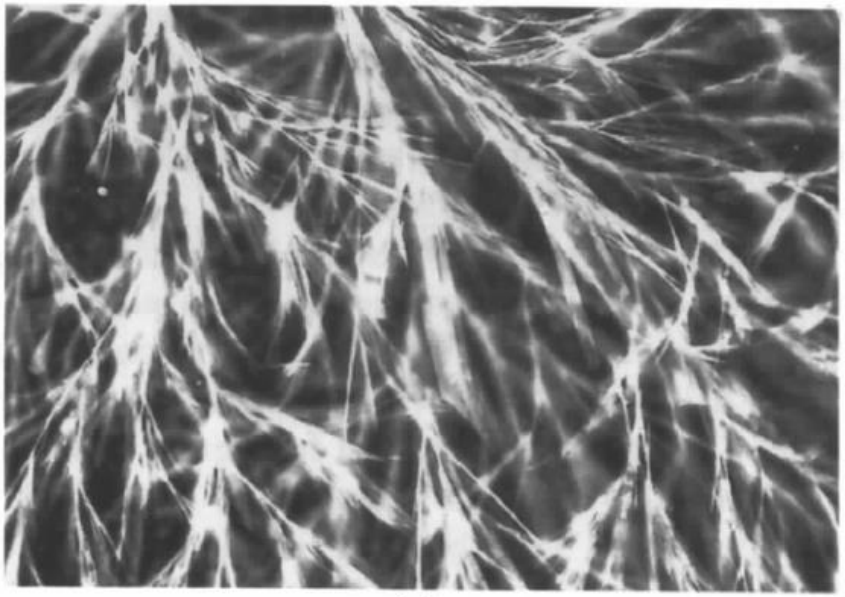

Precipitation
Labile
(supersaturated)
Metastable
(supersaturated)
Stable
(undersaturated)
Protein Concentration
Salt Concentration

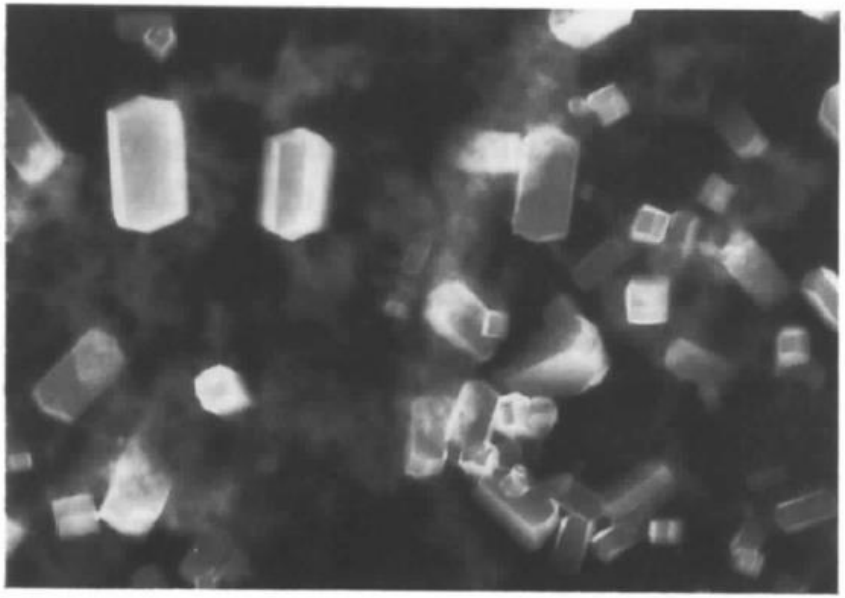

H_2O
$[ppt]_{drop} = \frac{[ppt]_{reservoir}}{2}$
Reservoir Solution

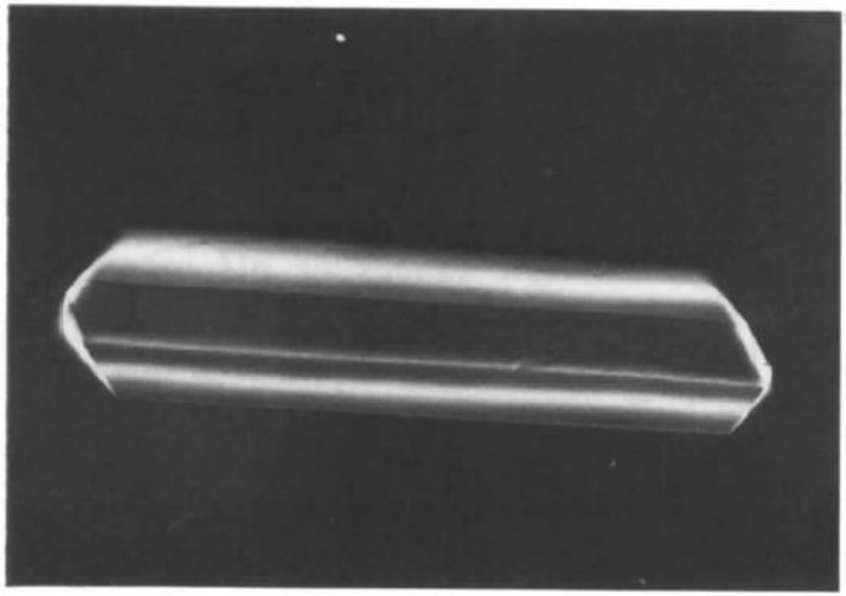
- McPherson A. (1995) Increasing the size of microcrystals by fine sampling of pH limits, Journal of Applied Crystallography 28, 362-365


Fine slice pH
pH range over which crystals grow
Fine slice pH
2
3
4
5
6
7
8
9
10
11
12
pH
pH below which crystals never observed
Beginning of range over which crystals may or may not grow
pH above which crystals never observed





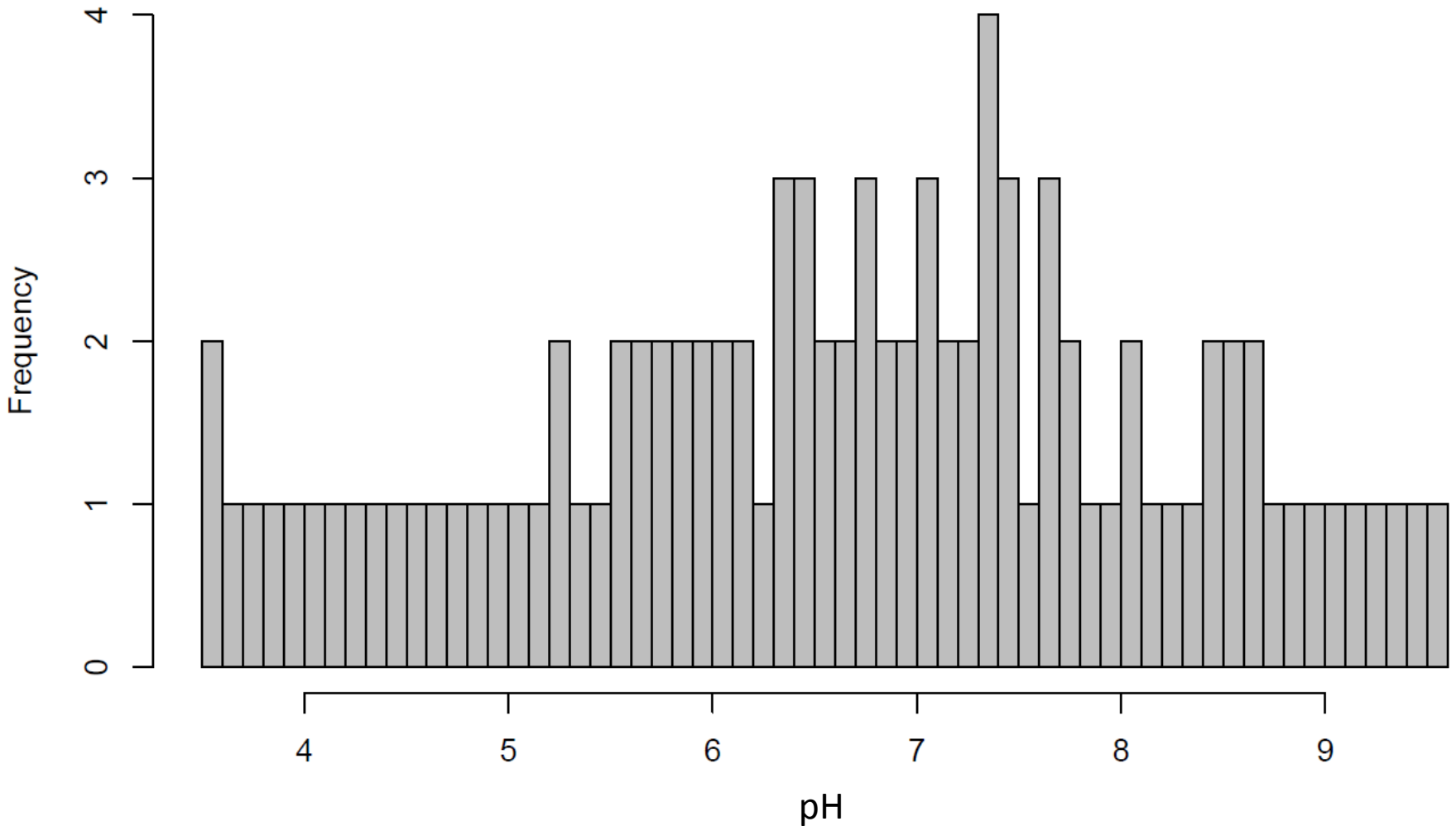




SLICE Buffers

Citric acid pH 3.5-4.4
Sodium citrate tribasic dihydrate pH 3.6-4.5
Sodium acetate trihydrate pH 3.7-4.9
DL-Malic pH 4.7-5.9
Succinic acid pH 4.8-6.0
Sodium cacodylate trihydrate pH 5.2-6.4
MES monohydrate pH 5.3-6.5
BIS-TRIS pH 5.7-6.9
ADA pH 5.8-7
Imidazole pH 6.2-7.4

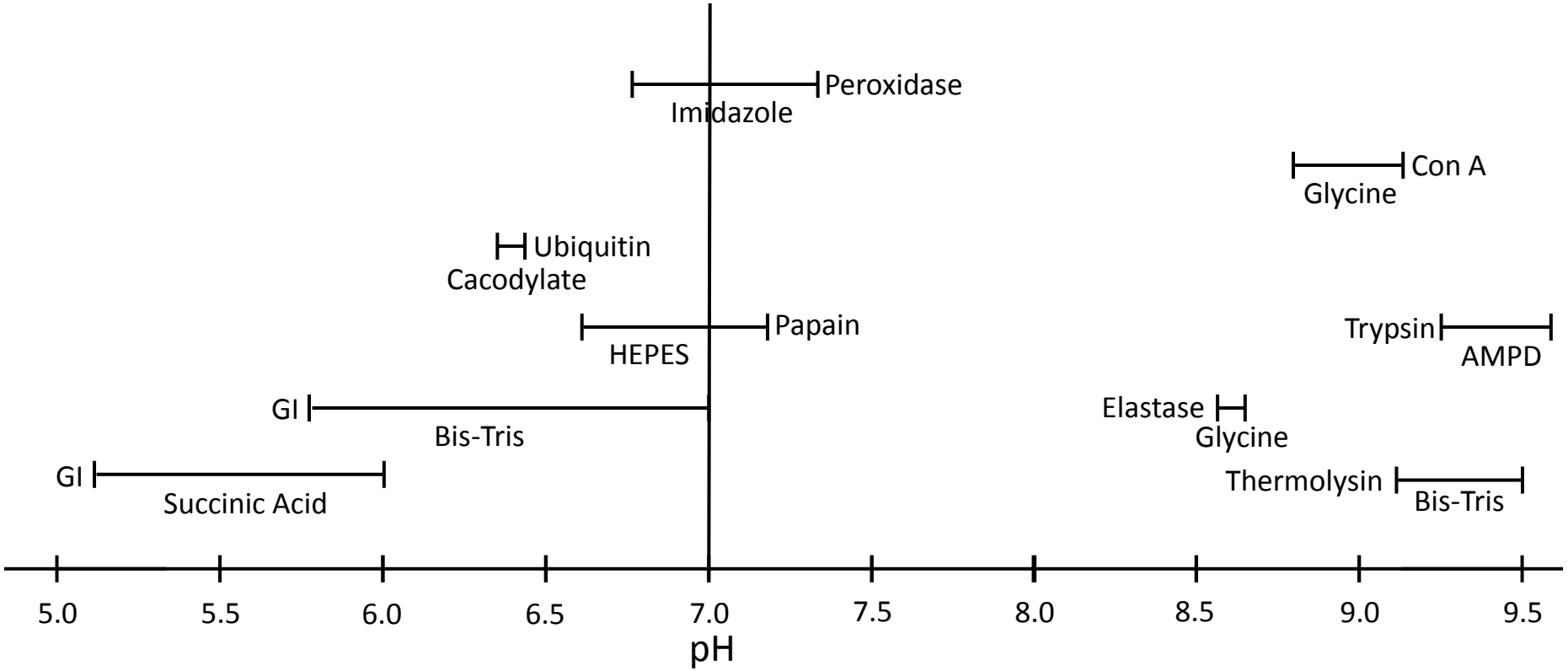
BIS-TRIS propane pH 6.4-7.3
MOPS pH 6.5-7.7
HEPES sodium pH 6.6-7.5
HEPES pH 6.8-7.7
TRIS hydrochloride pH 7.2-8.1
Tris pH 7.3-8.5
Tricine pH 7.4-8.6
BICINE pH 7.5-8.7
BIS-TRIS propane pH 8.5-9.4
Glycine pH 8.6-9.5
AMPD pH 8.7-9.6

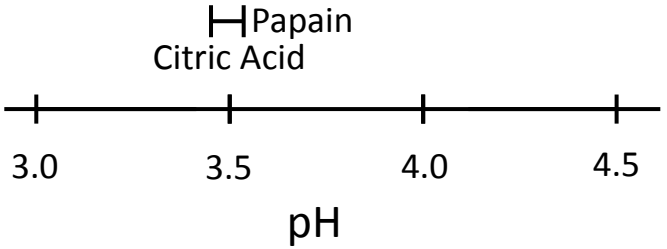

pH Sampling Range For Slice
Frequency
0
1
2
3
4
4
5
6
7
8
9
pH

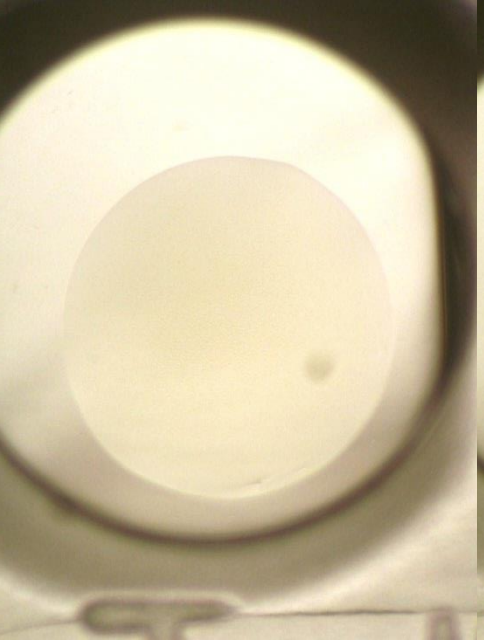
Crystallized using only pH & buffer type

- Glucose isomerase
- Lysozyme
- Elastase
- Concanavalin A
- Insulin
- Subtilisin
- Papain
- Thermolysin
- Ubiquitin
- Trypsin

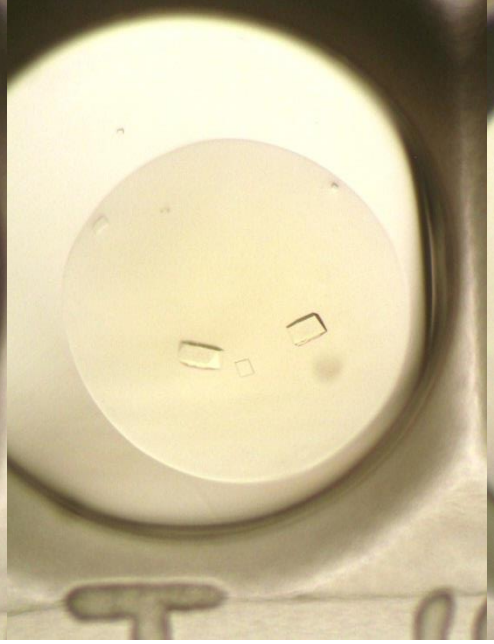
pH Ranges of Crystallization of Proteins Using Slice



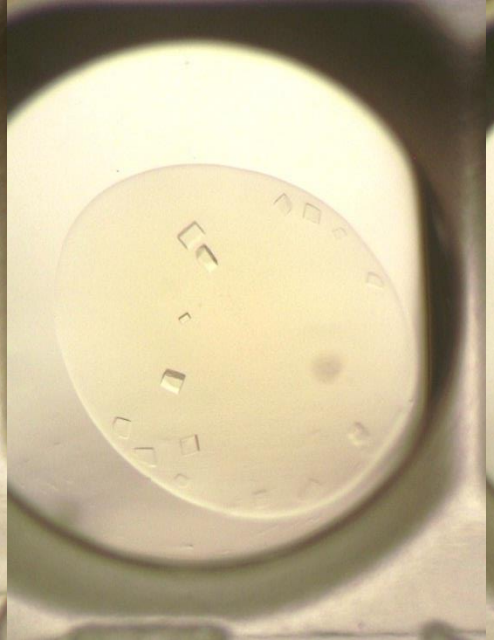




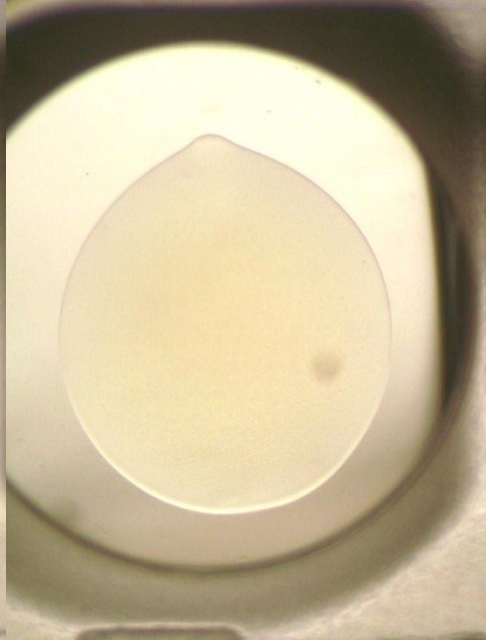
pH 8.6



pH 8.9

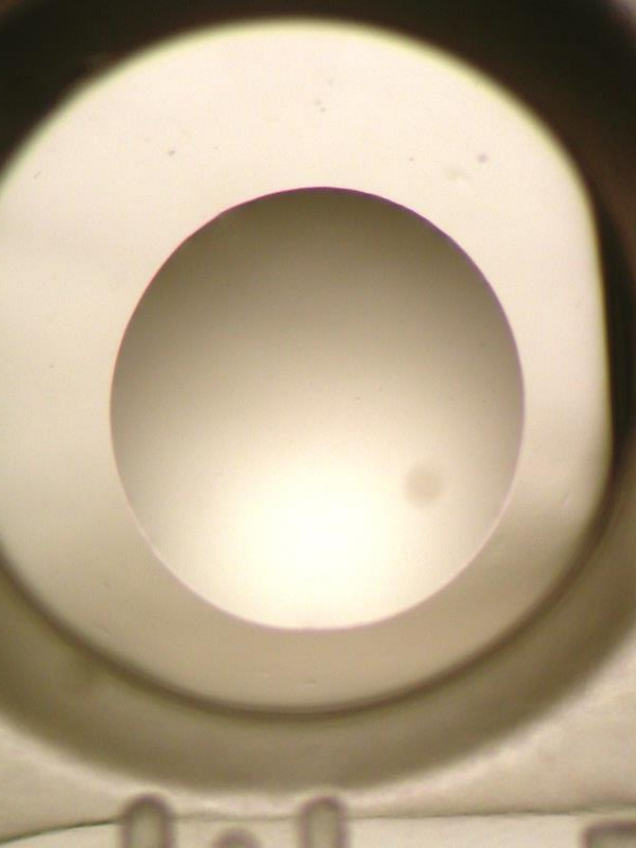


pH 9.2

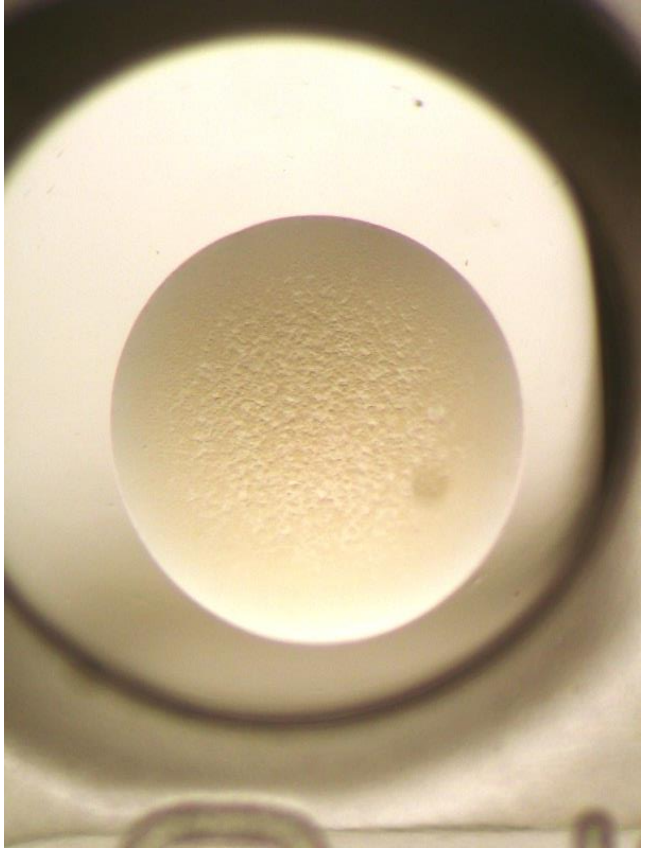


pH 9.5

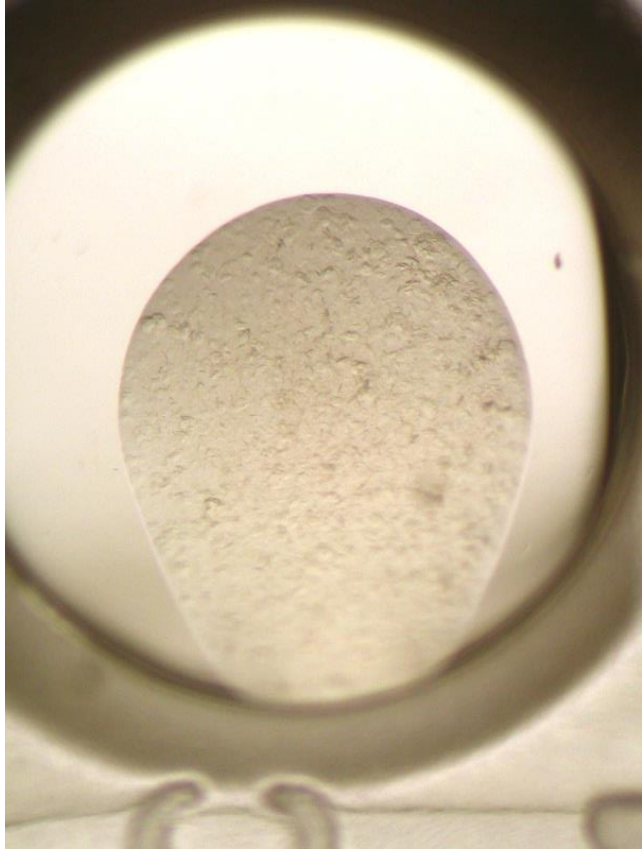
1.0 M Glycine
Con A



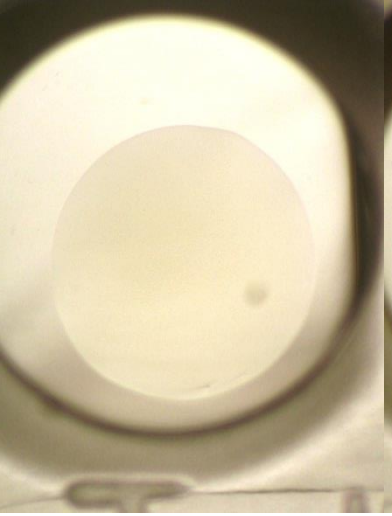
BIS-TRIS propane pH 6.4
Clear
Ubiquitin



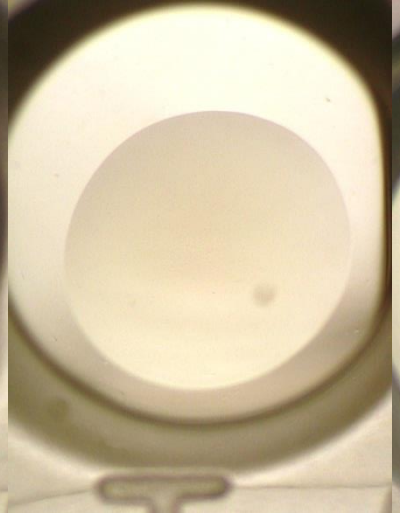
ADA pH 6.4
Precipitate
Ubiquitin



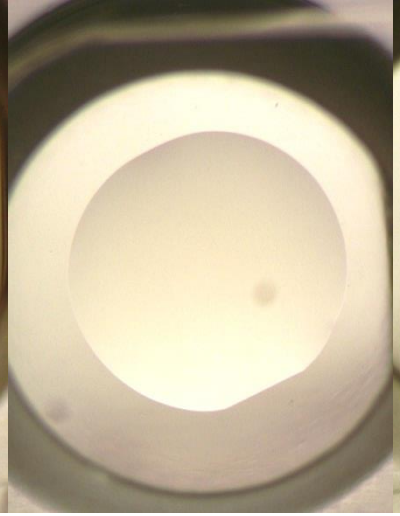
Sodium cacodylate pH 6.4
Crystal
Ubiquitin



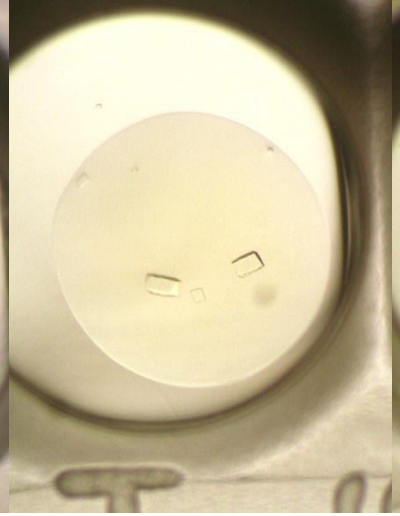
Glycine pH 8.6



AMPD pH 8.7

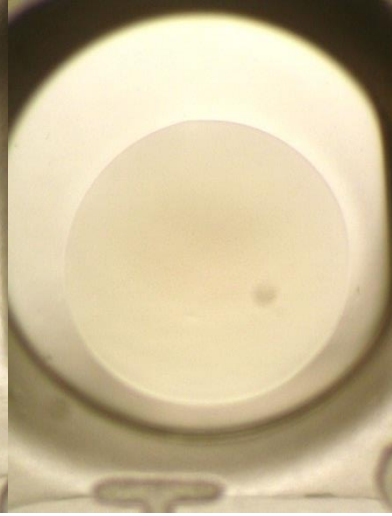


BIS-TRIS propane pH 8.8

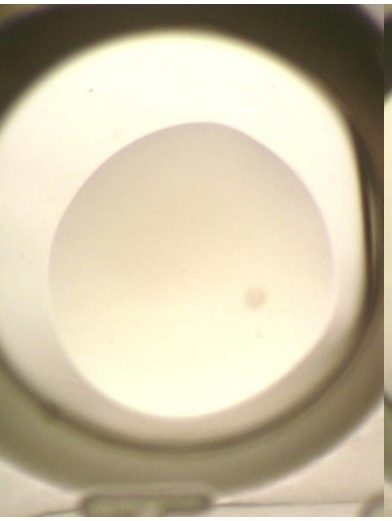


Glycine pH 8.9

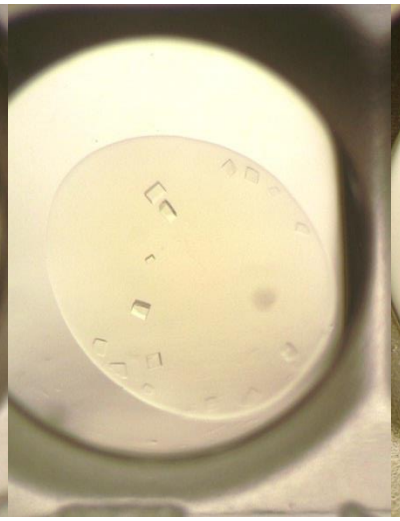
Crystals



AMPD pH 9.0

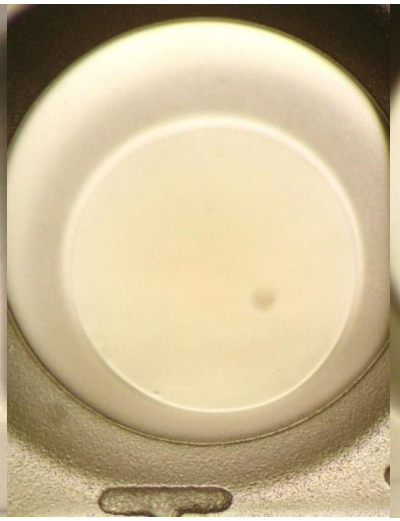


BIS-TRIS propane pH 9.1

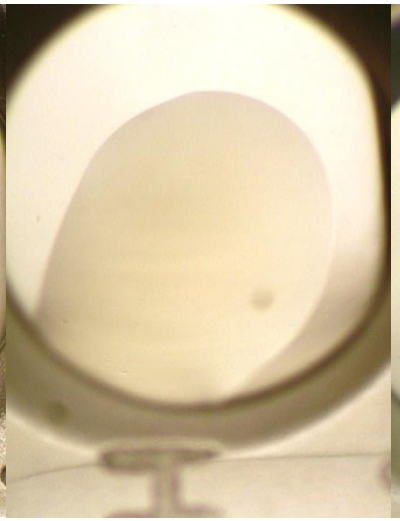


Glycine pH 9.2

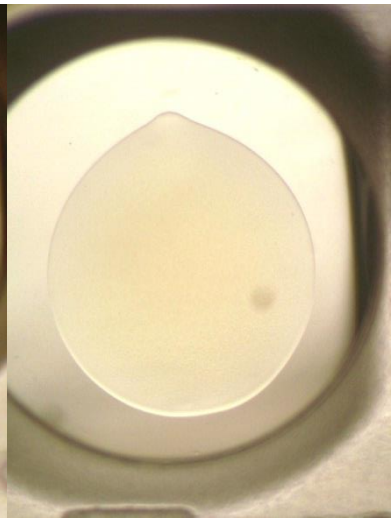
Crystals



AMPD pH 9.3

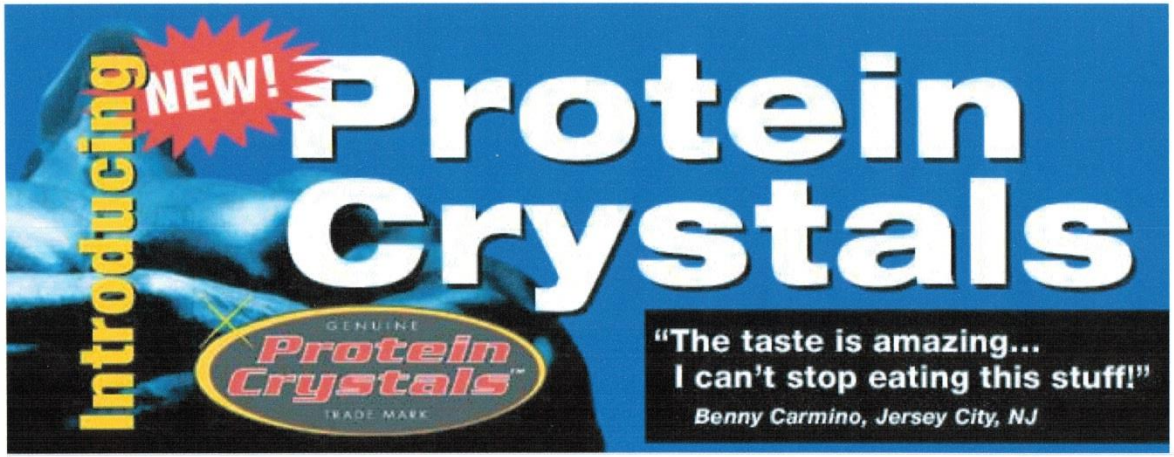


BIS-TRIS propane pH 9.4



Glycine pH 9.5

Con A



Pop it in your mouth... it tastes better than Pixy-Stix.®** Drop it in water... it tastes better than Kool-Aid.®‡ Either way, Protein Crystals™ is the best-tasting, most convenient, fastest-assimilating, 100% whey isolate protein you've ever had. You don't need water. You don't need a shaker. You don't need a blender. You definitely don't need a refrigerator. Heck, you don't even need to chew. It's the absolute, most incredible breakthrough in protein supplementation since the invention of... well... protein supplements.

Remember Pop Rocks®* or Pixy Stix®?** Those sugar-filled candies you used to get at the candy store? The patent-pending technology that produces, what we call, Crystalized Protein™† makes it possible for protein to taste just as sugary-sweet and flavorful as that incredibly delicious candy you used to enjoy as a kid... but without all that sugar (Protein Crystals™ is sweetened with calorie free sucralose). One taste of Protein Crystals™ and you'll never have to force yourself to eat your necessary daily supply of protein again.

Don't Let the Great Taste Fool You... Powerful Protein Supplementation

Protein Crystals™ is the first product ever to contain the patent-pending Crystalized Protein™ technology... delivering an incredible 20 grams of 100% Whey Protein Isolate per serving, with just 6 grams of total carbohydrates. That's right. Simply tear open a QuikPak™ of Protein Crystals™ and pop the sweet-tasting, textured granules directly into your mouth. You'll immediately feel a slight effervescence as your salivary glands begin to dissolve Protein Crystals™, immediately dissipating Whey Protein Isolates directly across your buccal membranes

and into your bloodstream.

Instant Oral Absorption

Oh sure, you could mix Protein Crystals™ with water for a cool, refreshing Black Cherry drink that tastes better than Kool-Aid®‡. But for ultra-quick absorption, just hold Protein Crystals™ in your mouth for a few short seconds. That way, the 100% whey isolate protein (loaded with branched chain amino acids [BCAAs] like valine, leucine and isoleucine, as well as a full and complete spectrum of Essential, Nonessential and Conditionally Essential Amino Acids) can immediately begin going to work to help rebuild and grow lean muscle tissue, and up-regulate your metabolism. Instant oral absorption means less amino acid catabolism (waste) because less protein passes through your gut and digestive tract. You get immediate amino acid availability and the best-tasting protein product ever developed.

The Ultimate in Great Taste and Convenience

Protein Crystals™ is more than just a great-tasting, rapidly assimilated, totally convenient protein supplement. Protein Crystals™ contains not only 100% whey isolate protein, the purest form of protein you can buy, but a 100% cross-flow, micro-filtrated protein extraction (ion-exchange), so the protein in Protein Crystals™ is undenatured (which means that all of the growth factors and… powerful immune-supporting protein fractions found in pure whey… such as immunoglobulins and lactoferrin… are intact and in high supply). No more worrying about whether you have hot water, cold water, a blender, or a cup. All you need is a resealable Protein Crystals™ QuikPak™ and you're locked and loaded with the one supplement that keeps you anabolic and metabolic all day long… no matter where you go, no matter how busy you get.

"Muscle Candy™"

Revolutionary Protein Crystals™ is scientifically engineered to increase amino acid absorption, cell volumization (larger, fuller muscles), strength, muscle recovery and dramatically decrease muscle catabolic stress and oxidation.

Most importantly, Protein Crystals™ tastes great right out of the bag or instantly mixed with water. Protein Crystals™ is an incredible innovation in taste, assimilation and convenience… high quality protein in a revolutionary formula that just happens to taste amazingly better than any other protein supplement ever! Pop some Protein Crystals™ in your mouth and you're good to go. Protein Crystals™ is so great tasting, one company is even calling it "Muscle Candy™."

No powders to mix, no gritty, watered-down sludge… no gas, no bloating, no embarrassment. Just total convenience and ultimate taste. Although Protein Crystals™ tastes like candy in your mouth, this powerful ion exchange formula is serious supplementation — delivering "instant" protein for

Finally there's a powerful protein supplement with taste that's off the charts... "Muscle Candy™" that delivers high quality, body-building protein in a revolutionary, great-tasting formula. Just pop Protein Crystals™ into your mouth and you're good to go... anytime, any place!

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**Pixy Stix® is a registered trademark of Societe Des Produits Nestle S.A.
‡Kool-Aid® is a registered trademark of Kraft Foods Holdings, Inc.
†Crystalized Protein™ is a trademark of Basic Research®, LLC.

Crystal Pro

10 - 36 gm packets
Out of Stock

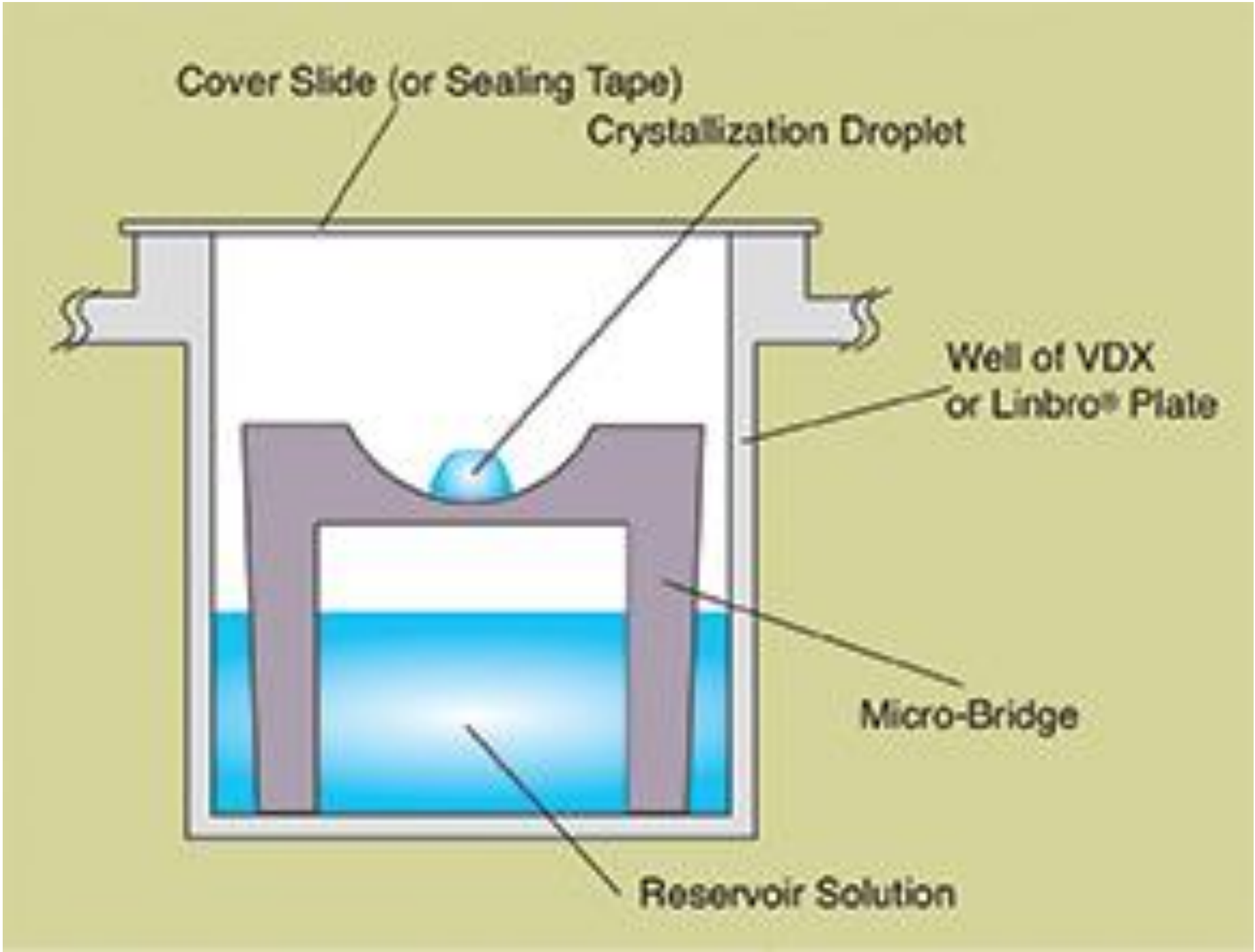
20 - 36 gm packets
Out of Stock

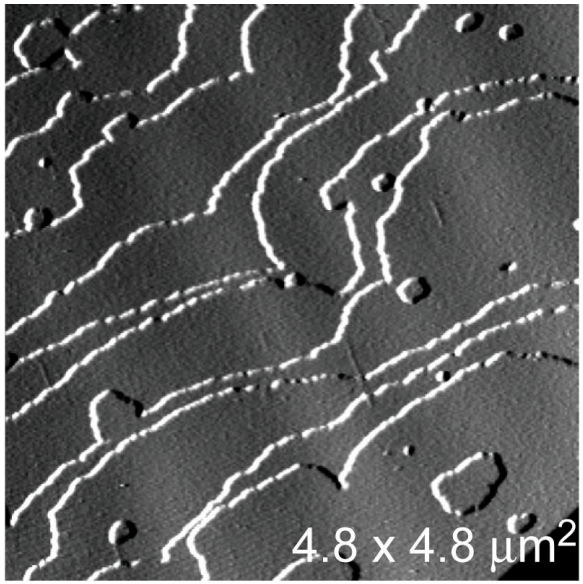
URBAN BIOLOGICS

Related Products:
Nirto ATP | Methoxybolic | MegaTropin | Isolate-X | Kynovar | Protein Crystals | TestroGel | Testosterole

Drastically improve performance, new break through potent formulation, 100% Corpustestrolic Rigimax

Home


Cover Slide (or Sealing Tape)
Crystallization Droplet
Well of VDX
or Linbro® Plate
Micro-Bridge
Reservoir Solution



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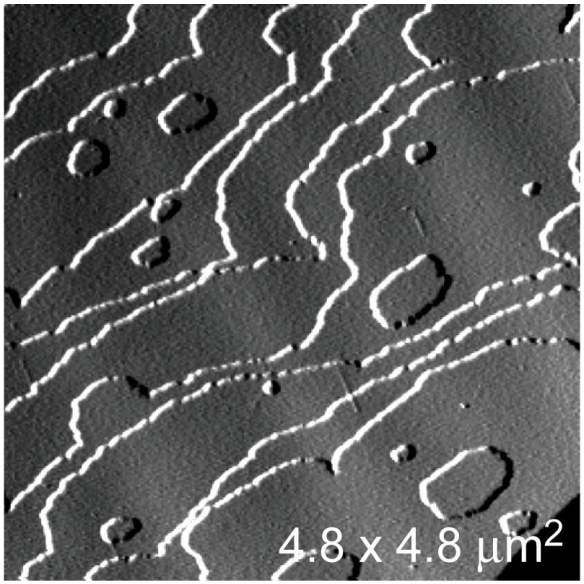

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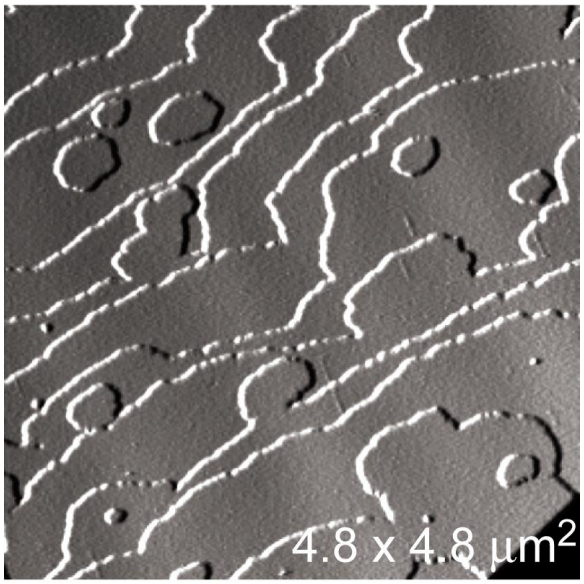

c

Images at two-minute intervals showing the development of the surface of a growing thaumatin crystal