RAMC – Bichenberg 2013

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Free Energy $\delta F$ For Formation of the Crystalline Aggregate

Size of the Aggregates, $R$

$\delta F_c$

$\mu_1 > \mu_2$

$R_c$
Tangential and Normal Growth

Creation of new steps - Normal Growth

Requires 2D nucleation
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
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)
H₂O

\[ [\text{ppt}]_{\text{drop}} = \frac{[\text{ppt}]_{\text{reservoir}}}{2} \]

Reservoir Solution
pH range over which crystals grow

Beginning of range over which crystals may or may not grow

pH below which crystals never observed

pH above which crystals never observed
<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>3.5-4.4</td>
</tr>
<tr>
<td>Sodium citrate tribasic dihydrate</td>
<td>3.6-4.5</td>
</tr>
<tr>
<td>Sodium acetate trihydrate</td>
<td>3.7-4.9</td>
</tr>
<tr>
<td>DL-Malic</td>
<td>4.7-5.9</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>4.8-6.0</td>
</tr>
<tr>
<td>Sodium cacodylate trihydrate</td>
<td>5.2-6.4</td>
</tr>
<tr>
<td>MES monohydrate</td>
<td>5.3-6.5</td>
</tr>
<tr>
<td>BIS-TRIS</td>
<td>5.7-6.9</td>
</tr>
<tr>
<td>ADA</td>
<td>5.8-7</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6.2-7.4</td>
</tr>
<tr>
<td>BIS-TRIS propane</td>
<td>6.4-7.3</td>
</tr>
<tr>
<td>MOPS</td>
<td>6.5-7.7</td>
</tr>
<tr>
<td>HEPES sodium</td>
<td>6.6-7.5</td>
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<tr>
<td>HEPES</td>
<td>6.8-7.7</td>
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<tr>
<td>TRIS hydrochloride</td>
<td>7.2-8.1</td>
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<tr>
<td>Tris</td>
<td>7.3-8.5</td>
</tr>
<tr>
<td>Tricine</td>
<td>7.4-8.6</td>
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<tr>
<td>BICINE</td>
<td>7.5-8.7</td>
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<tr>
<td>BIS-TRIS propane</td>
<td>8.5-9.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>8.6-9.5</td>
</tr>
<tr>
<td>AMPD</td>
<td>8.7-9.6</td>
</tr>
</tbody>
</table>
• Glucose isomerase
• Lysozyme
• Elastase
• Concanavalin A
• Insulin
• Subtilisin
• Papain
• Thermolysin
• Ubiquitin
• Trypsin

Crystallized using only pH & buffer type
pH Ranges of Crystallization of Proteins Using Slice

- Citric Acid
- Papain
- Succinic Acid
- GI
- Ubiquitin Cacodylate
- HEPES
- Bis-Tris
- Imidazole
- Peroxidase
- Papain
- Con A Glycine
- Elastase Glycine
- Trypsin AMPD
- Thermolysin Bis-Tris
- Papain
- Cacodylate
- HEPES
- GI
- Succinic Acid
- GI
- Ubiquitin Cacodylate
- HEPES
- Bis-Tris
- Imidazole
- Peroxidase
- Papain
- Con A Glycine
- Elastase Glycine
- Trypsin AMPD
- Thermolysin Bis-Tris
pH 8.6  pH 8.9  pH 9.2  pH 9.5

1.0 M Glycine
Con A
<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIS-TRIS propane</td>
<td>6.4</td>
<td>Clear</td>
</tr>
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<td></td>
<td></td>
<td>Ubiquitin</td>
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<tr>
<td>ADA</td>
<td>6.4</td>
<td>Precipitate</td>
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<td>Ubiquitin</td>
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<tr>
<td>Sodium cacodylate</td>
<td>6.4</td>
<td>Crystal</td>
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<tr>
<td></td>
<td></td>
<td>Ubiquitin</td>
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</table>
Glycine pH 8.6
AMPD pH 8.7
BIS-TRIS propane pH 8.8
Glycine pH 8.9
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
Introducing **Protein Crystals**

"The taste is amazing... I can't stop eating this stuff!"

Benny Carmino, Jersey City, NJ

**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, Crystallized 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 Crystallized 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

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

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

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