Can Supersaturation Affect Protein Crystal Quality?

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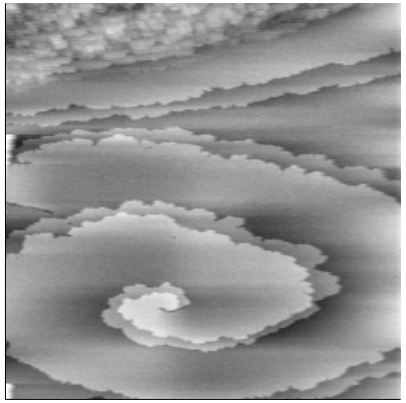
Overview

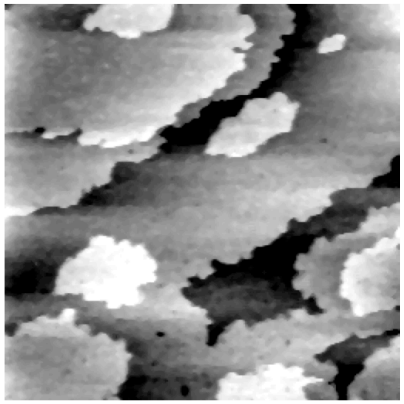
- Protein crystal quality infers X-ray diffraction quality (intensity or signal to noise ratio and resolution limit)
- There are a vast number (thousands) of articles published on the subject matter
- Protein crystal quality can be enhanced by any number of means (crystallization methodology is standardized):
    1) Various additives or precipitating agents
    2) Varying supersaturation (density, temperature, etc.)
    3) Minimizing physical "handling"
    4) Chemical/genetic modification of proteins
- Physical processes are suggested, but not readily verified

Scope

- Physical description for protein crystal growth
  1) Modes of crystal growth
  2) Kinetic roughening transition
  3) Growth rate data collection
  4) Growth rate beyond the roughening transition
  5) Critical roughening crossover supersaturation
  6) Implications of a critical supersaturation
- Speculations on microgravity effects
  1) Depletion zone formation
  2) Impurity partitioning

Modes of Crystal Growth





Growth by 2d nucleation (step generation) and addition to step edge

$$V_s(c,T) = Ac_{eq}^{1/3} \exp\left(\frac{2\Delta\mu}{3k_BT}\right)\left(\frac{\Delta\mu}{k_BT}\right)^{1/6}\left[\exp\left(\frac{\Delta\mu}{k_BT}\right)-1\right]^{2/3}\exp\left[-\frac{\pi\gamma^2}{3\Delta\mu k_BT}\right]$$

γ - effective step free energy (erg/molecule-cm)

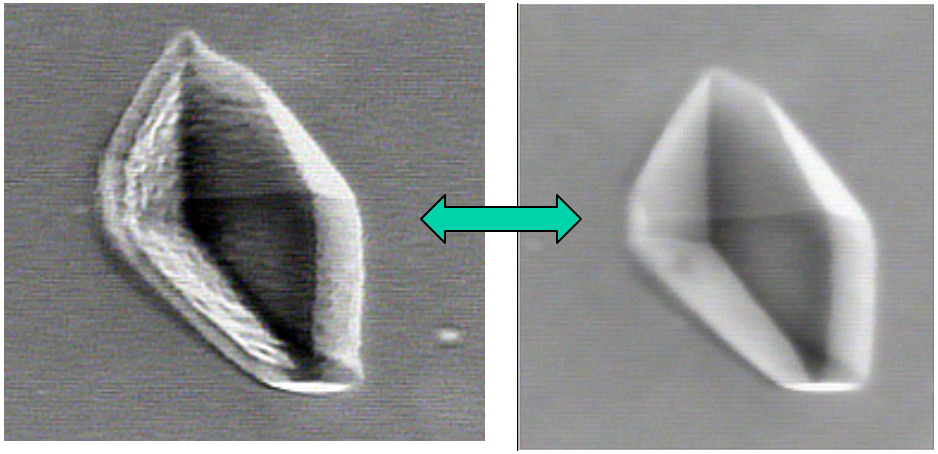
Growth by continuous addition anywhere on crystal surface

$$V_c(c,T) = B(c - c_{eq}(T))\exp\left[-E_c/k_BT\right]$$

E_c - energy barrier for continuous addition (erg/molecule)

Ref: Y. Saito, *Statistical Physics of Crystal Growth* (World Scientific, Singapore, 1996)

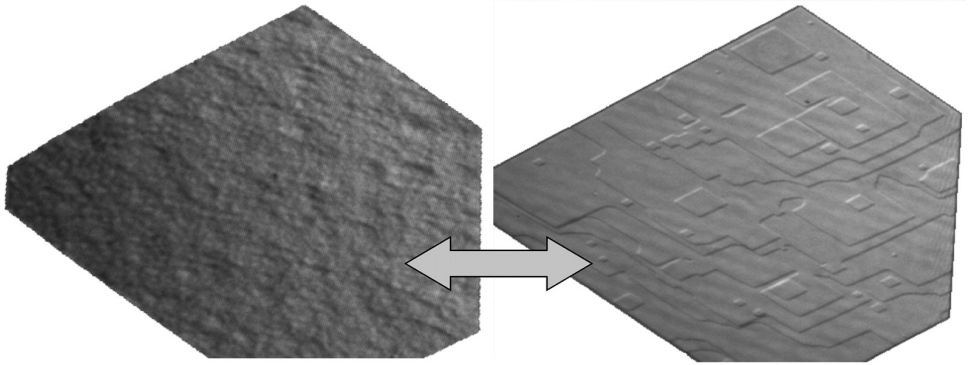
Kinetic Roughening



Kinetic Roughening of Lysozyme Crystals
S. Gorti, E.L. Forsythe & M.L. Pusey,
Cryst. Growth & Design (2004) 4:691-699
S. Gorti, E.L. Forsythe & M.L. Pusey,
Cryst. Growth & Design (2005) 5:473-482
S. Gorti, J. Konnert, E.L. Forsythe & M.L. Pusey,
Cryst. Growth & Design (2005) 5:535-545

<u>Crossover Supersaturation</u>

Lysozyme: $\sigma = 2.0 \pm 0.2$
Glucose Isomerase: $\sigma = 5.0 \pm 0.1$



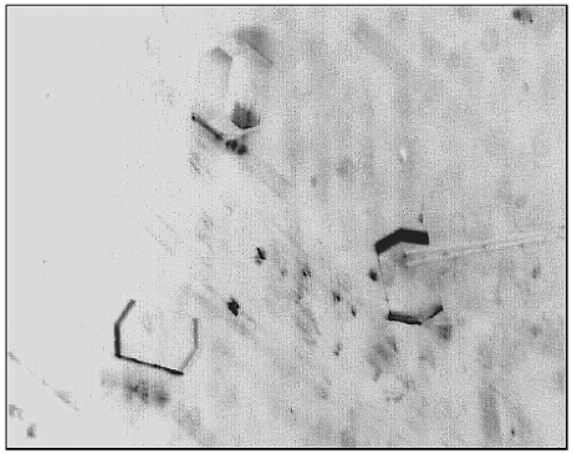
Kinetic Roughening of Glucose Isomerase Crystals
M. Sleutel, D. Maes, L. Wyns, and R. Willaert
Cryst. Growth Des., (2008) **8**:4409-4414

Kinetic Roughening Layer Growth

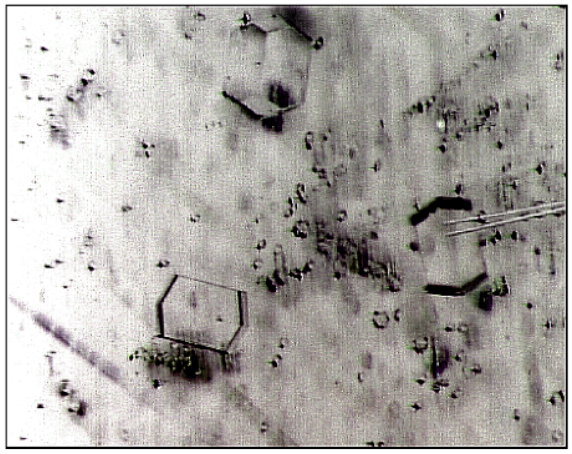
Note: A causal relationship between modes of crystal growth and X-ray diffraction "quality" has yet to be determined.

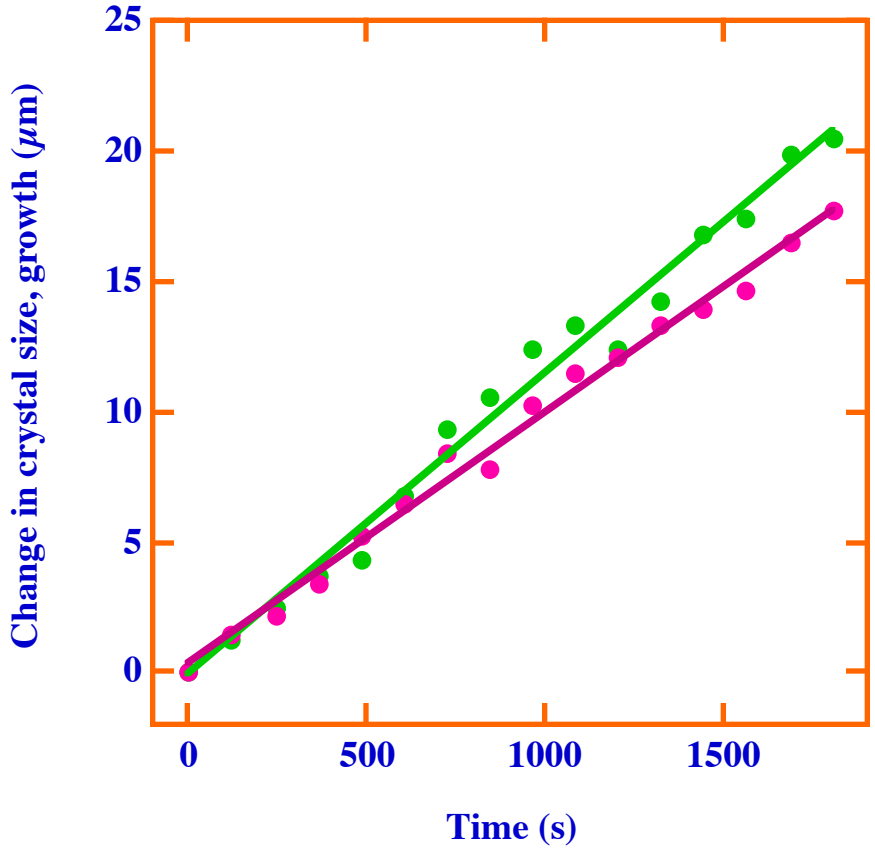
Measurement of Growth Rates

Start



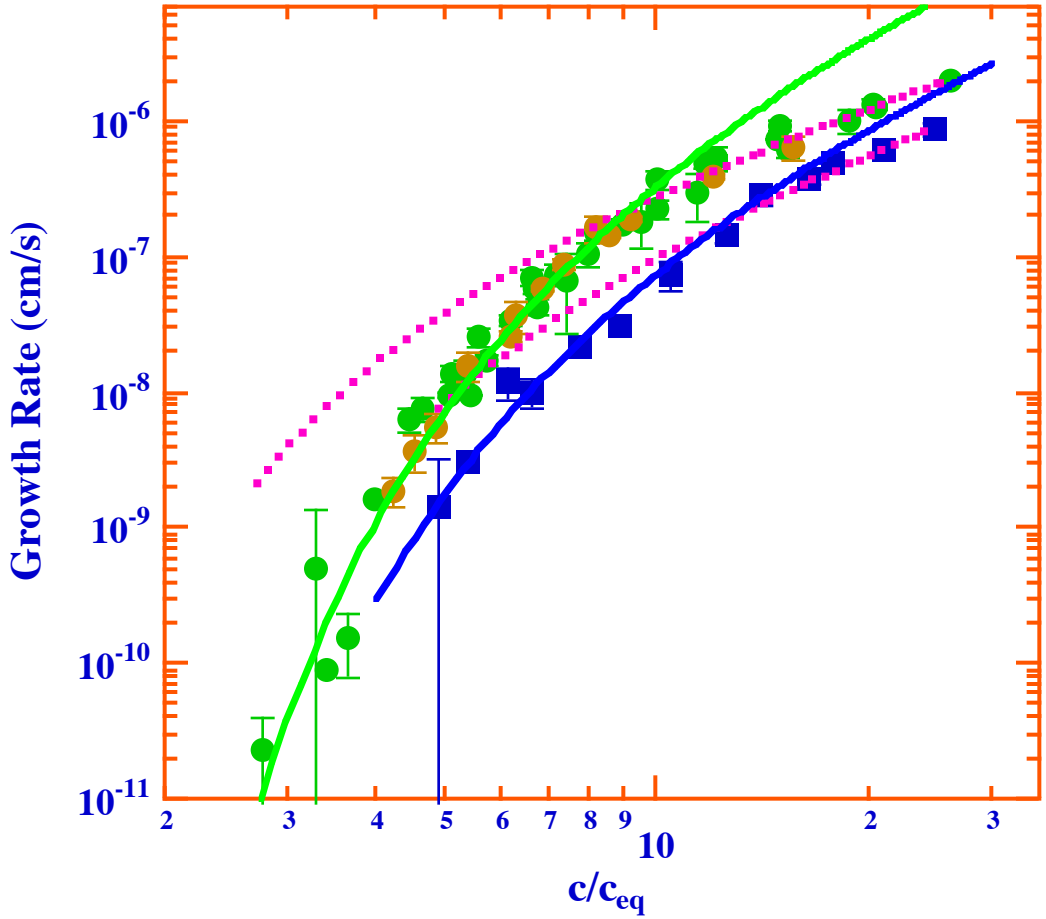
End



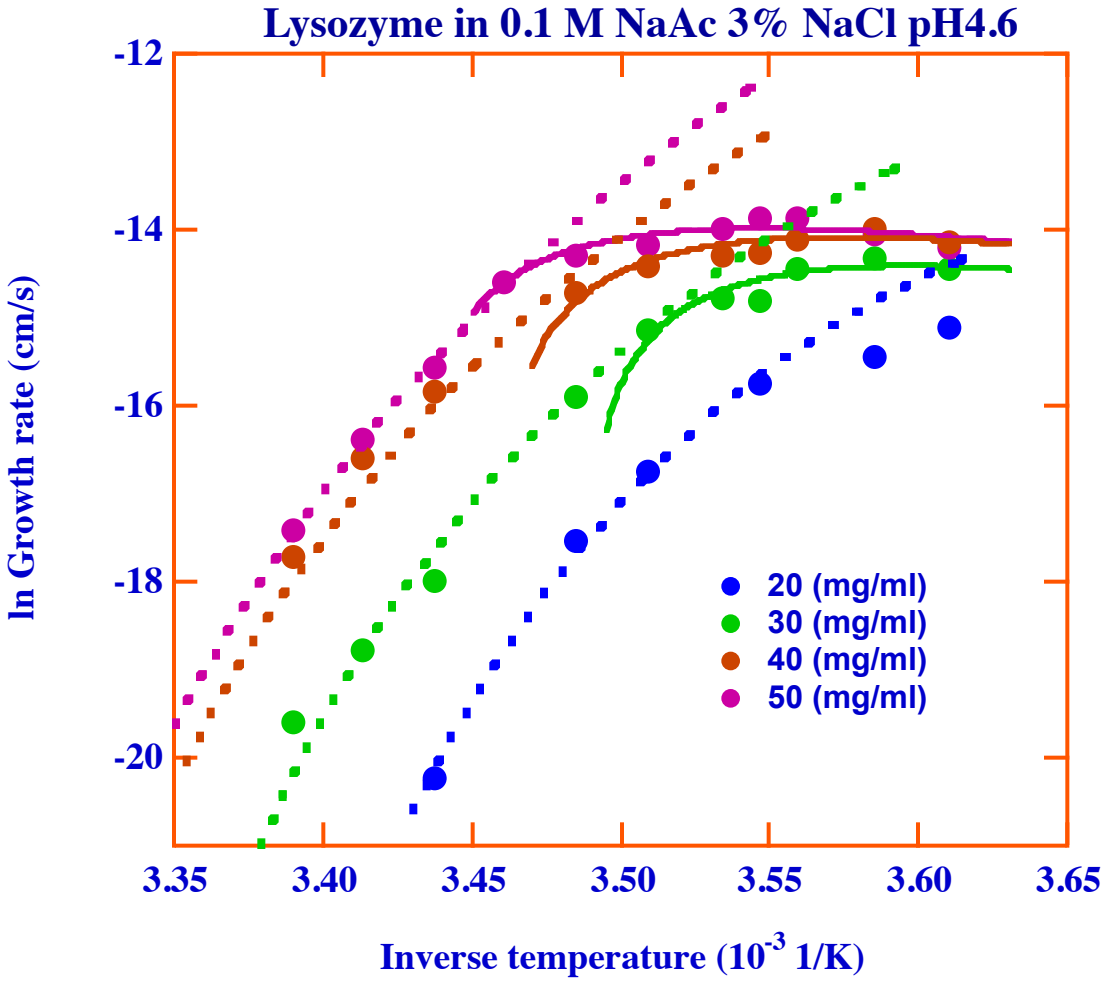


Average growth rate 1.1 ± 0.1 x 10^{-6} cm/s

Crystal Growth by Nucleation



Deviation: Kinetic Roughening

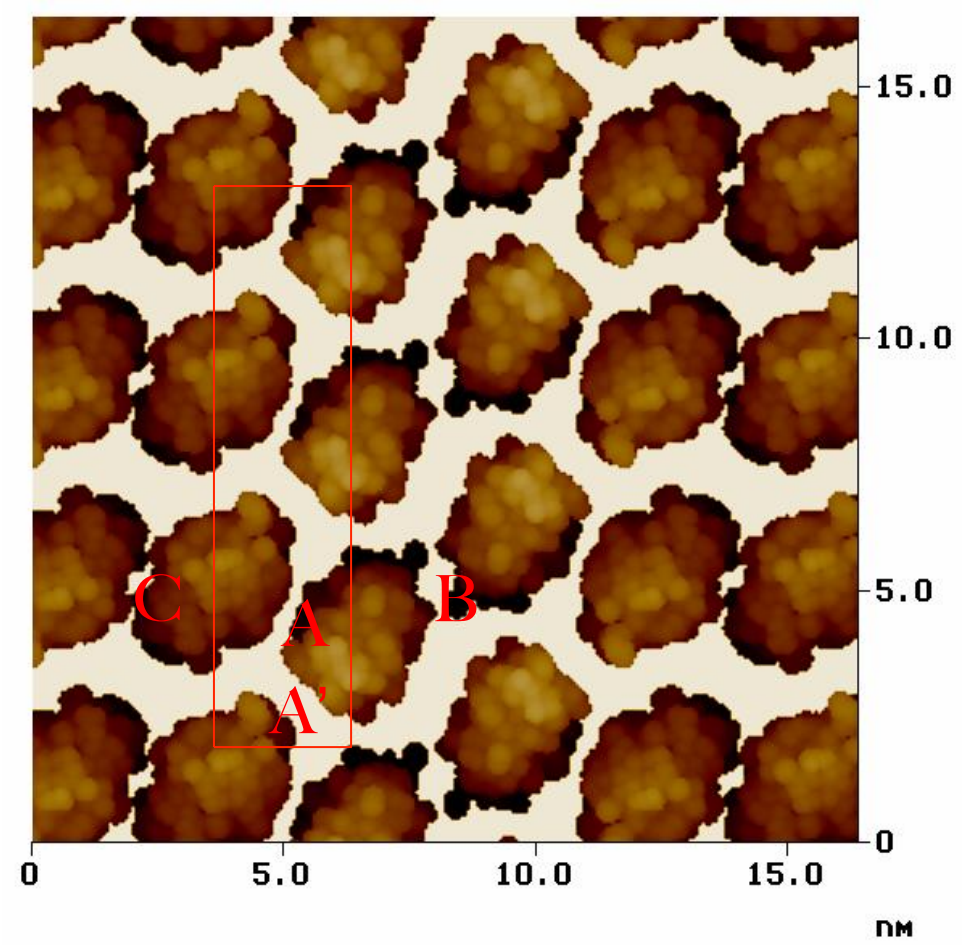


Estimates

Values of energy barriers for linear and 2d nucleation growth models

Solution Condition	E_c (erg/molecule)	γ (erg/molecule)	σ_c
3% NaCl, pH 4.6	$5.8 \pm 0.2 \times 10^{-13}$	$1.2 \pm 0.1 \times 10^{-13}$	2.0 ± 0.2
4% NaCl, pH 5.0	$7.4 \pm 0.2 \times 10^{-13}$	***	2.0 ± 0.1
5% NaCl, pH 5.0	$7 \pm 1 \times 10^{-13}$	$1.3 \pm 0.1 \times 10^{-13}$	1.9 ± 0.2
3% NaCl, 6 °C pH 4.0 - 5.4	$6.0 \pm 0.1 \times 10^{-13}$	$1.0 \pm 0.2 \times 10^{-13}$	1.9 ± 0.1
5% NaCl, 14 °C pH 4.0 - 5.4	$6.0 \pm 0.2 \times 10^{-13}$	$1.3 \pm 0.1 \times 10^{-13}$	2.1 ± 0.2
5%, 22 °C pH 4.0 - 5.4	$5.9 \pm 0.2 \times 10^{-13}$	$1.4 \pm 0.3 \times 10^{-13}$	1.9 ± 0.2
8 °C pH 4.4 2% - 7% NaCl	$5.8 \pm 0.4 \times 10^{-13}$	$1.0 \pm 0.1 \times 10^{-13}$	2.0 ± 0.2
14 °C pH 4.8 2% - 7% NaCl	$6.2 \pm 0.4 \times 10^{-13}$	$1.3 \pm 0.2 \times 10^{-13}$	2.2 ± 0.1
18 °C, pH 4.0 2% - 5% NaCl	$6.1 \pm 0.5 \times 10^{-13}$	$1.5 \pm 0.5 \times 10^{-13}$	1.9 ± 0.2

Crystallographic Bond Energies



Approximate
Macro Bond
Strengths

A = 4.3×10^{-12} erg
A' = 9.7×10^{-12} erg
B = 4.9×10^{-12} erg
C = 7.2×10^{-12} erg

Numerical Estimates

Free Energy for Crystal Growth:

Varies with ΔG^* for $c > c_{eq}$:

$$\Delta G_{nuc} = -\Delta\mu N + \gamma\sqrt{4\pi N}$$

@ Crossover supersaturation σ_c (T, pH, NaCl):

$$N^* = \pi\gamma^2 / \Delta\mu^2$$ ~ 8 ±5 molecules in adatom

$$\Delta G^*_{nuc} = \pi\gamma^2 / \Delta\mu$$ ~ 7 x 10^{-13} erg/molecule

$$\sigma_c = \pi\gamma^2 / k_B^2 T^2 \approx 32$$ For single molecule addition

Crystal Quality

Journal of Crystal Growth 237–239 (2002) 295–299

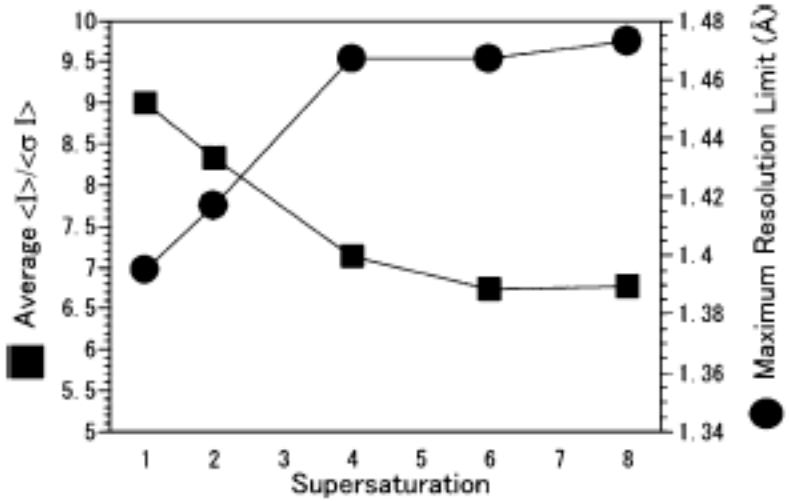


Fig. 1. Average maximum resolution limit (the line with circles) and average $\langle I \rangle / \langle \sigma I \rangle$ (the line with squares) of crystals from each supersaturation condition. Note that both the resolution limit and the average $\langle I \rangle / \langle \sigma I \rangle$ value are higher in lower supersaturated solution.

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Crystal Growth Summary

- A kinetic roughening transition occurs.
- Step-Energy barrier γ for 2d growth ~$3.2 \pm 0.4\ k_B T$
- Energy barrier E_c for continuous growth ~$15 \pm 2\ k_B T$
- Analyses assuming a critical crossover concentration
  - Energy barrier E_c is invariant with temperature up to 12 °C
  - -~1 $k_B T$ increase in pH range 4.0 - 5.4
  - -~2 $k_B T$ decrease between 2.0-7.0 %NaCl
- Measured critical crossover supersaturation $\sigma_c \sim 2.0 \pm 0.2$
- At crossover, crystallizing nuclei contain ~ 3-13 molecules
- Kinetic roughening transition has been shown to exists in two protein systems (lysozyme and glucose isomerase)

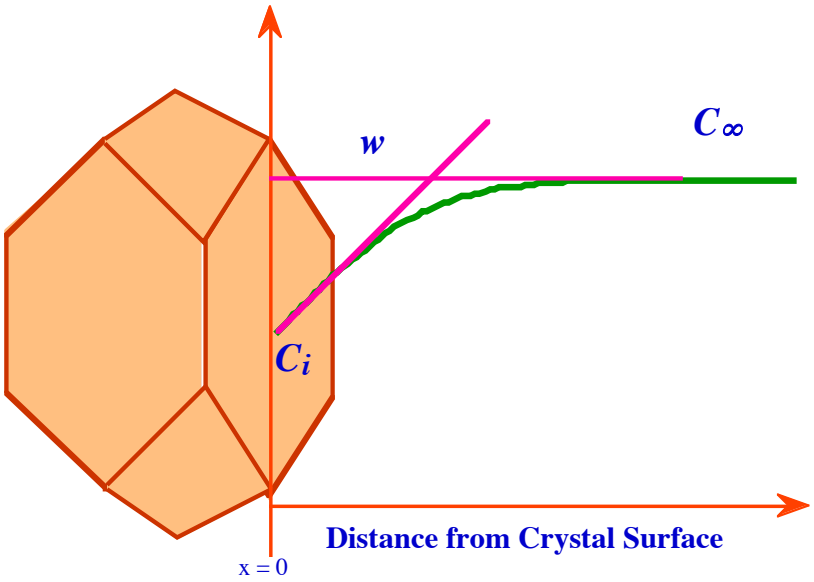
μ-gravity Environment

- The only known physical process affecting protein crystal growth is the formation of a depletion zone due to limited sedimentation and density driven convection.

- It is speculated that impurity partitioning can occur also due to the formation of a depletion zone, provided that the "size" of impurities are much larger than crystallizing molecules.

Depletion Zone Formation

Expected Concentration Profile



Solutal flux in x-direction:

$$j_{in} = D\text{—}c = D\Delta c/w \ ,$$

where $w \sim (Dt)^{1/2}$ mean diffusion width.

Crystal flux:

$j_{crys} = V_x c_x$, where $V_x = ß(c_0 - c_{eq})$

Assume c_∞, $c_0 > c_{eq}$:

$$c_i = \frac{c_\infty}{1 + \beta c_x \sqrt{t/D}}$$

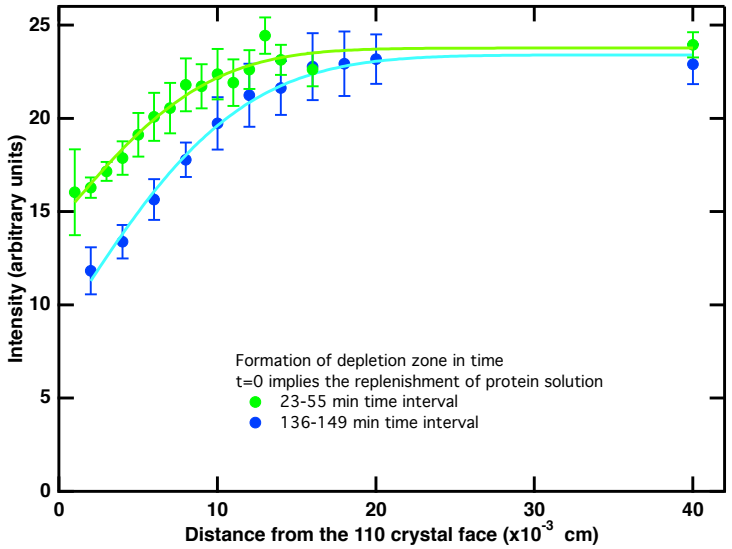
Observations times for non-linear growth measurement:

$$t = \frac{D}{V_x^2}\left(\frac{c_\infty}{c_x}\right)^2 \sim 6000s$$

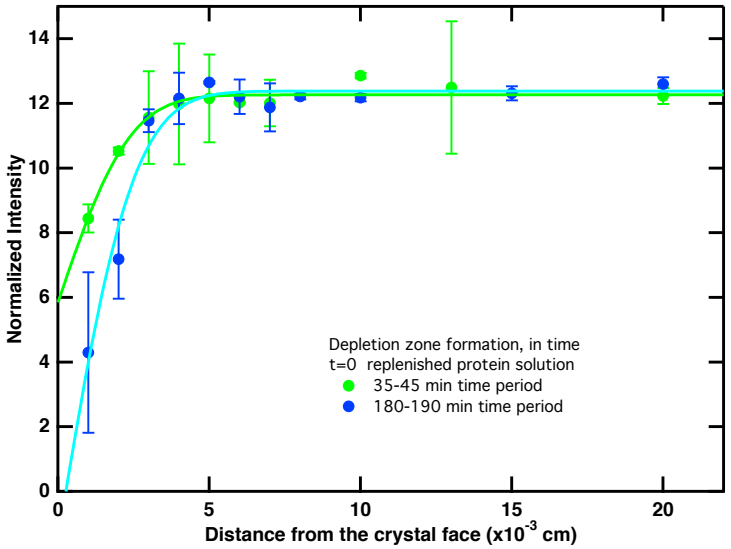
For $D \sim 1 \times 10^{-6}$ cm^2/s, $V_x \sim 1 \times 10^{-6}$ cm/s, $c_x \sim 800$ mg/ml and $c_\infty \sim 60$ mg/ml

Measurement of Depletion Zone Formation

Lysozyme 110 Crystal Face



Glucose Isomerase Crystal



Measurements were made using microscope laser light scattering spectroscopy

Conclusion

- In quiescent environments (microgravity, capillary tubes, gels) formation of a depletion zone is to be expected, due either to limited sedimentation, density driven convection or a combination of both.

- The formation of a depletion zone can:
  - ❖ Modify solution supersaturation near crystal
  - ❖ Give rise to impurity partitioning

- It is conjectured that both supersaturation and impurity partitioning affect protein crystal quality and size.

- Further detailed investigations on various proteins are needed to assess above hypothesis.