Measurements of Protein Crystal Face Growth Rates

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Overview

• Slow growth improves protein crystal X-ray diffraction quality (signal to noise ratio and resolution limit)

• Protein crystal quality can be enhanced by any number of means:
  1) Various additives or precipitating agents
  2) Varying supersaturation (density, temperature, etc.)
  3) Minimizing physical “handling”
  4) Chemical/genetic modification of proteins
  5) Microgravity

• NASA/CASIS have several flight projects that address the quality of protein crystals grown in \( \mu \)-gravity environments.

• Ground based studies of physical processes that could determine crystal quality are also under investigation at MSFC.
Kinetic Roughening

Crossover Supersaturation

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

Kinetic Roughening of Lysozyme Crystals

Kinetic Roughening of Glucose Isomerase Crystals
M. Sleutel, D. Maes, L. Wyns, and R. Willaert

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


![Graph showing average resolution limit and supersaturation relationship](image)

**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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Face Growth Rate Apparatus

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Measurement of Growth Rates

Average growth rate $1.1 \pm 0.1 \times 10^{-6}$ cm/s
Crystal Growth Rates

Growth Rate (cm/s) vs. c/c_{eq}

- Log scale for both axes
- Data points and trend lines
- Units: cm/s
Protein Candidates

#1 – Inorganic PyroPhosphatase from *Thermococcus thioreducins*.

- Catalyzes cleavage of 1 pyrophosphate to 2x orthophosphate
  
  Found in all life
  
  Necessary for nucleic acid synthesis, any other reaction where a nucleotide triphosphate is converted to a nucleotide monophosphate + diphosphate.

- Hyperthermophile enzyme is active to 85°C

- Crystallizes very readily – current diffraction to ~1.1 Å

- Crystals currently grown on ISS for neutron diffraction studies.

- Very stable in X-ray beam at room temperature.

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#2 Proliferating Cell Nuclear Antigen – (PCNA)
• A processivity factor that is essential for nucleic acid replication.
• Both hyperthermophile and psychophile versions available.
• Both forms are facile crystallizers.

#3 Glyoxylate reductase
• Catalyzes reduction of glyoxyate to glycolate.
• Crystallizes readily, attractive for neutron diffraction studies.

#4 Haloacid Dehalogenase
• Catalyzes conversion of a 2-haloacid to 2-hydroxyacid + halide.
• May be useful in chemical waste treatment.
• Nascent collaborative effort between iXG and another laboratory.
Summary

• Protein crystal growth rates will be determined for several hyperthermophile proteins.

• The growth rates will be assessed using available theoretical models, including kinetic roughening.

• If/when kinetic roughening supersaturations are established, determinations of protein crystal quality over a range of supersaturations will also be assessed.

• The results of our ground based effort may well address the existence of a correlation between fundamental growth mechanisms and protein crystal quality.