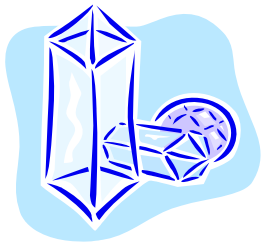
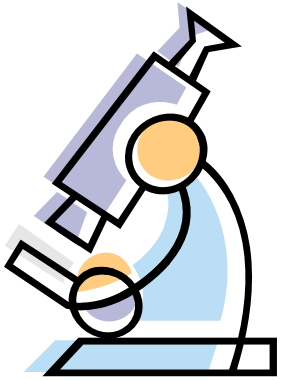
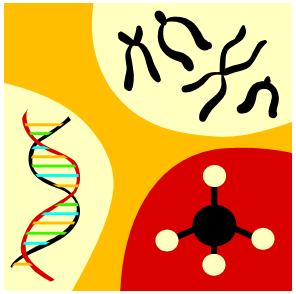
June 2008



Crystal Characterization

Enzyme Crystallization & Diffraction



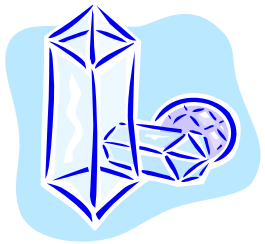


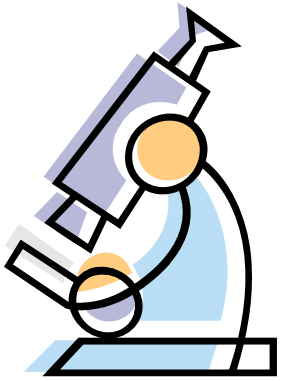
Dr. Thayumana Somasundaram

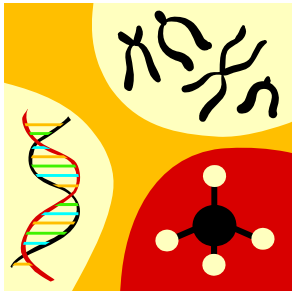
414 Kasha Lab, Florida State Univ

Overview

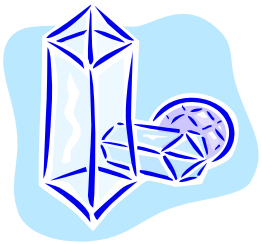
- Project Description
  - Objectives
- Project Methodology
  - Crystal Growth & Characterization
- Results
  - Findings
  - Results
- Conclusion





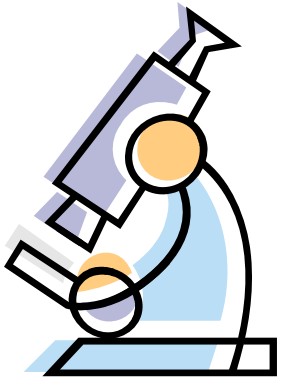


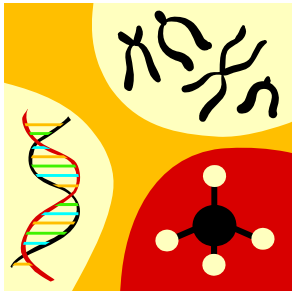
Project Description



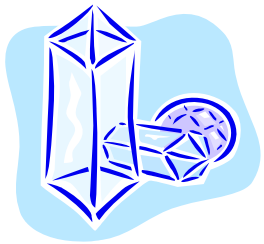
Objectives

- *Learn to handle biological samples*
- *Grow enzyme crystals*
- *Optically characterize crystals*
- *Vary crystal sizes*
- *Flash-cool crystals*
- *Diffract crystals using x-rays*
- *Record and analyze results*

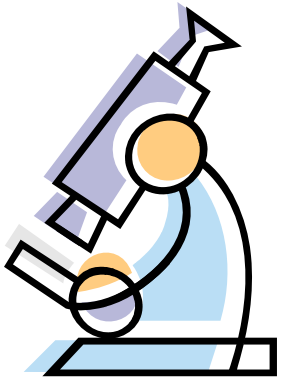


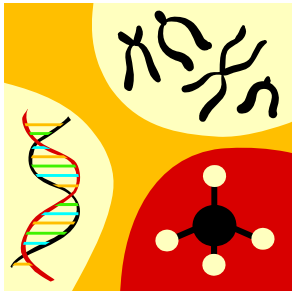


Project Methodology



- Prepare enzyme solutions in buffer
- Set-up crystal plates
- Observe crystals under microscope
- Vary conditions to get different size crystals
- Record results
- Flash-cool crystals in liquid nitrogen
- Diffract crystals with x-ray radiation
- Correlate crystal size to cooling rates and cell dimensions



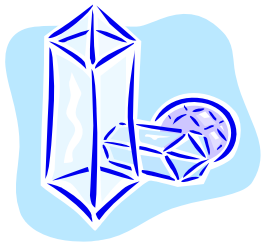


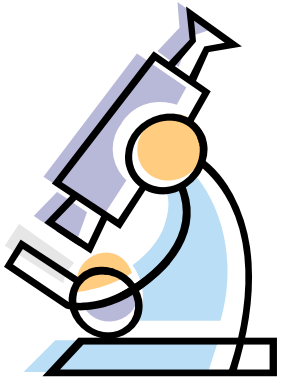
Key Assumption

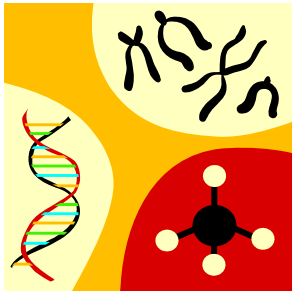
- *Crystal size should play a role in cooling rates.*

Procedure: Enzyme Preparation

- Learn to handle biological samples
- Prepare buffer solutions
- Adjust the pH
- Weigh correct amount of enzyme
- Dissolve enzyme in buffer solution
- Dilute solutions as needed

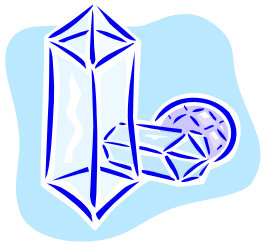


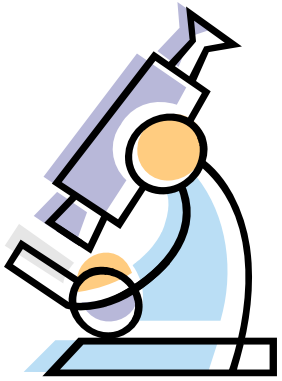


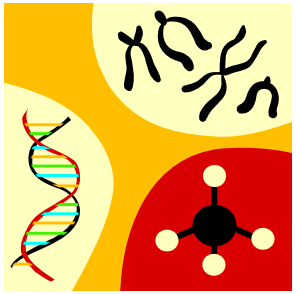


Procedure: Crystal Set-up

- Label and ready crystal plates
- Clean the cover slips
- Dispense well solutions
- Dispense enzyme on cover slips
- Set-up the cover slips
- Store at appropriate temperature

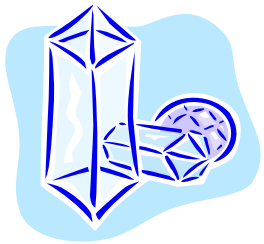


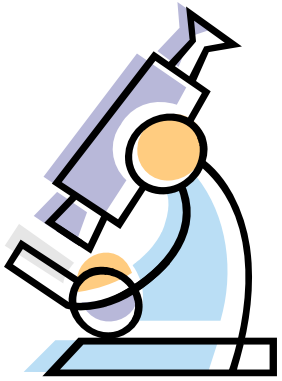


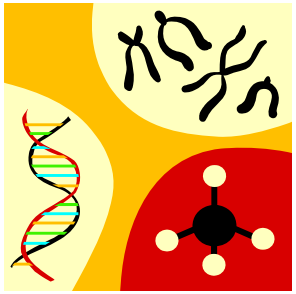


Procedure: Observation

- Examine wells under microscope
- Record “clear” and “crystal” conditions
- Capture images of crystals
- Modify conditions
- Set-up next batch of plates
- Store at appropriate temperature

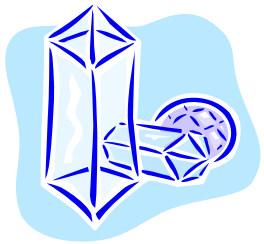


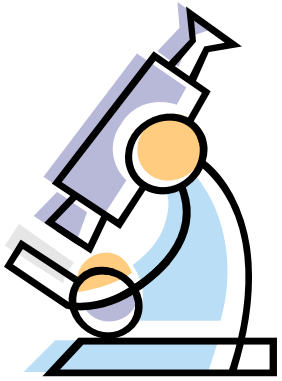


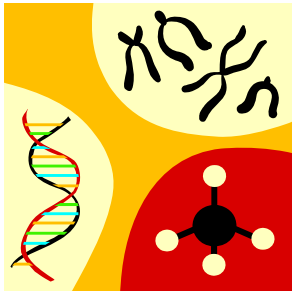


Procedure: Flash-Cool

- Learn about cryo conditions
- Safe handling of liquid nitrogen
- Retrieve a good crystal
- Flash-cool with liquid/gas nitrogen
- Store at cryo temperature

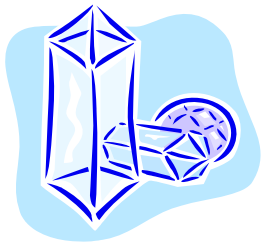


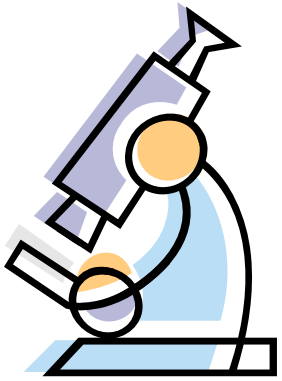


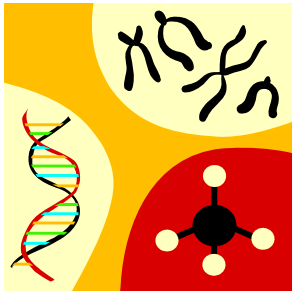


Procedure: Diffraction

- Learn about diffraction
- Safety of x-ray radiation
- Mount a good crystal
- Record a diffraction pattern
- Analyze and note the results

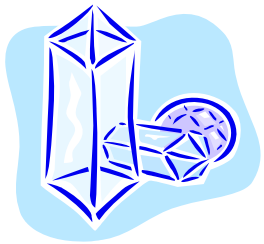


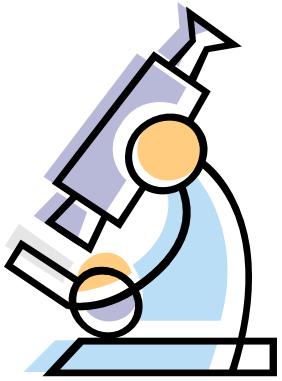


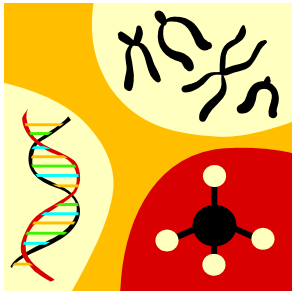


Results

- What is the role of crystal size on
  - Cooling rates
  - Cell dimensions
- Can we make a statement?
- How will it affect future work?

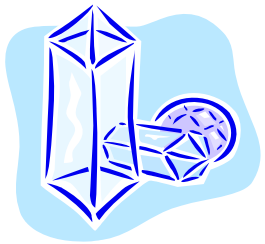


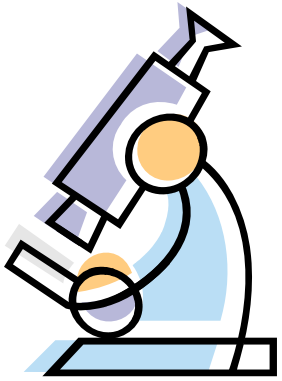


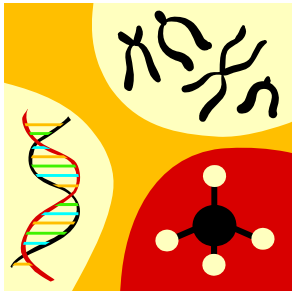


Conclusion

- What conclusions can we draw?
- What future experiments can be plan?







Questions
&
Discussion