Lysozyme Crystallization

Presented by Maria Papamanousaki

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WHO AM I?

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📍 CURRENTLY A SUMMER STUDENT AT KEK

GR → ✈️ → JP

Some facts about me

- 🏝️ I'm from Crete, Greece's largest island
- 🌞 Grew up surrounded by sunshine
- 🧆 Spanakopita is one of my favorite Greek foods
- 🌊 This summer, I traded Greek sunshine for protein crystals
- 🛩️ Travelled 9,000 km to spend my summer in a laboratory
- 🙏 My patience has improved thanks to protein crystallization
- 🍣 Still learning how to use chopsticks properly

Greek Expectations vs Reality

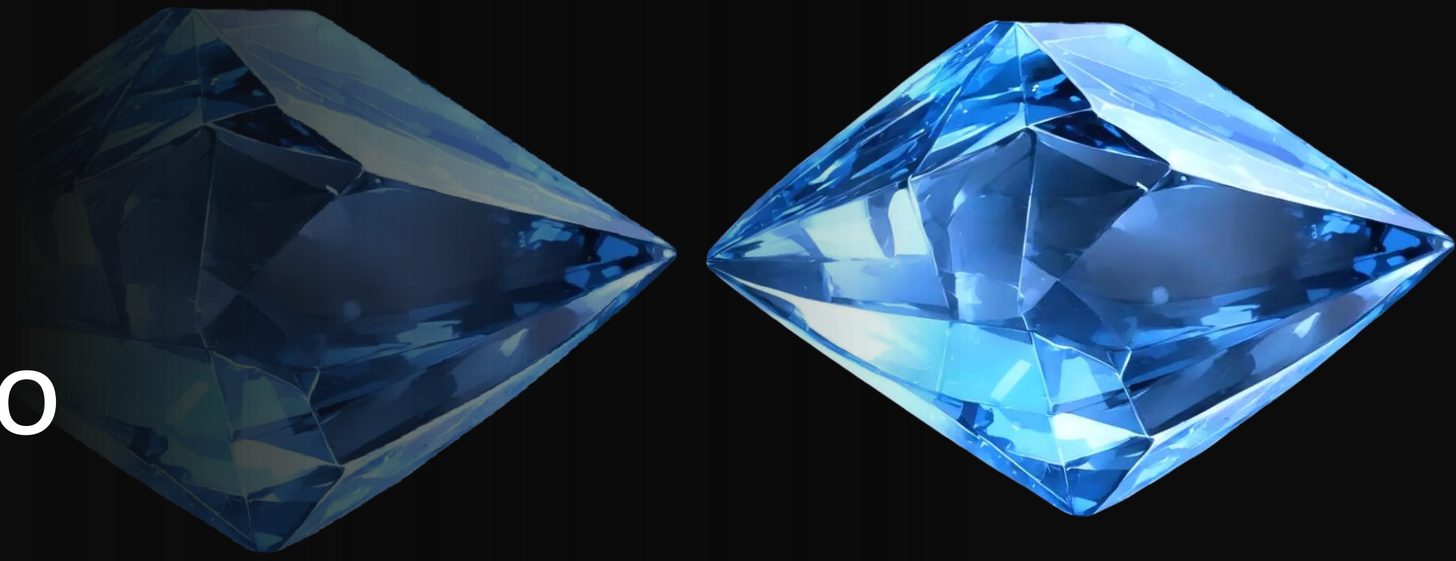
Things Greeks are known for

🏛️ Ancient history
🏖️ Beautiful beaches
🥙 Delicious summer food
☕ Spending hours over a single coffee

What I'm known for this summer

🔬 Pipetting 1 µL over and over again
💎 Looking for crystals
🫒 Missing olive oil as a desperate ex
☕ Drinking three coffees before lunch

Why is growing protein crystals so challenging?



A single microscopic crystal can reveal the atomic structure of a protein

Abstract

Objective

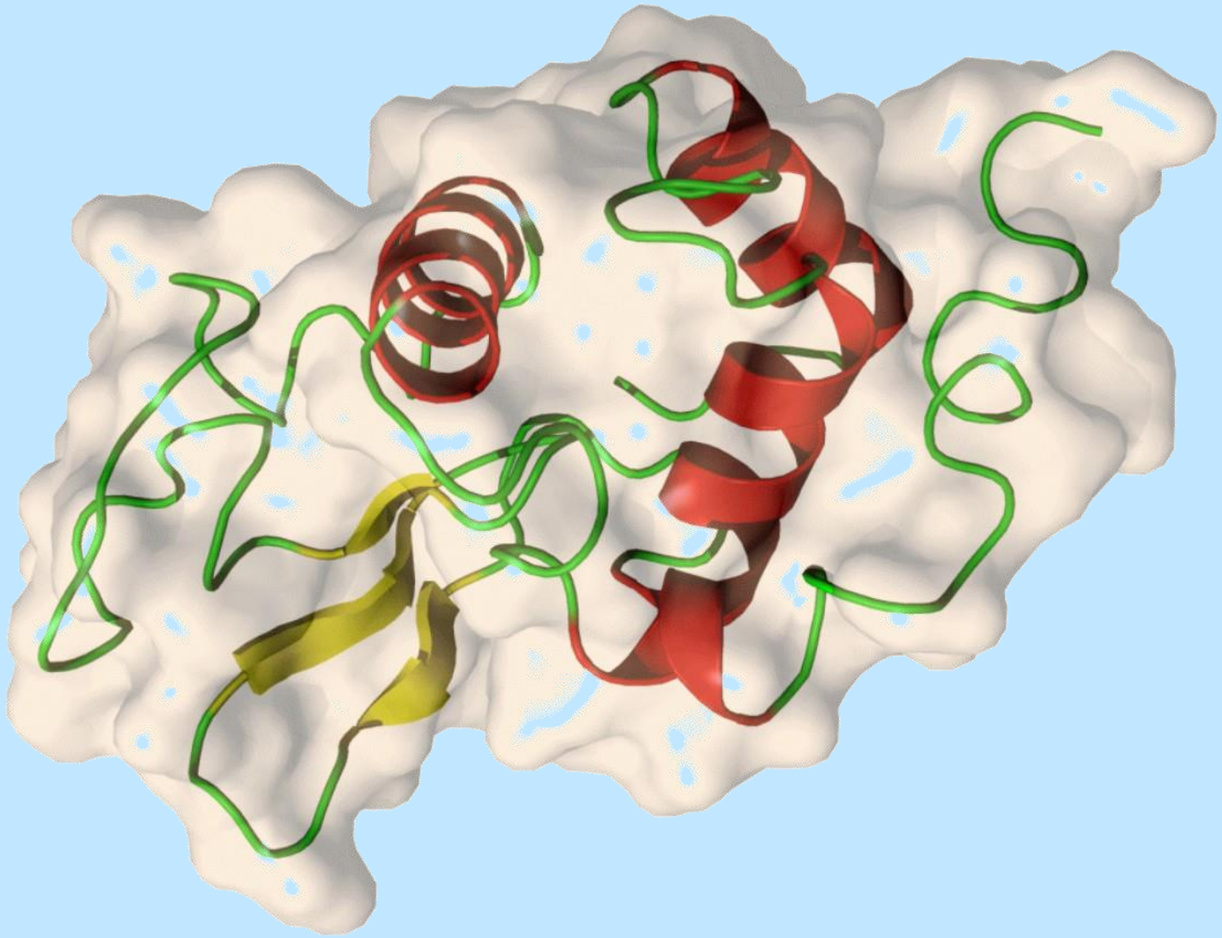
To grow high-quality lysozyme crystals suitable for structural studies.

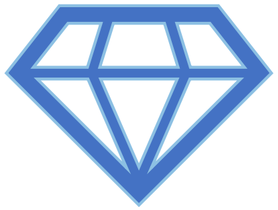
Method

Lysozyme crystals were grown using the hanging-drop vapor diffusion method under two sodium chloride concentrations (1.0 M and 2.0 M). For each crystallization condition, three micro-seeding strategies (original seed, 1:100 diluted seed, and no seed) were evaluated.

Outcome

Crystal formation was assessed by light microscopy, and the combined effects of sodium chloride concentration and micro-seeding on crystal nucleation and growth were compared.





Protein crystallization

is a delicate balance

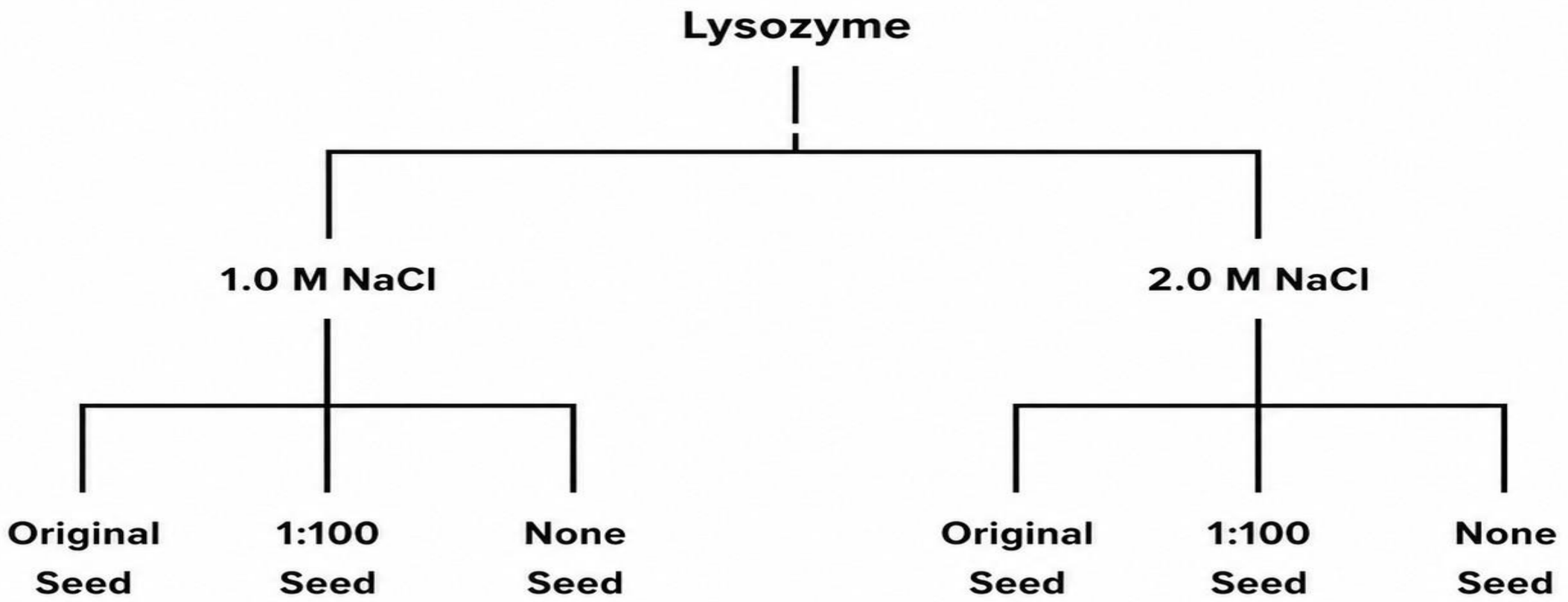
Too soluble ⟶ **No crystals**

Optimal supersaturation ⟶ **Crystal growth**

Too much supersaturation ⟶ **Precipitation**

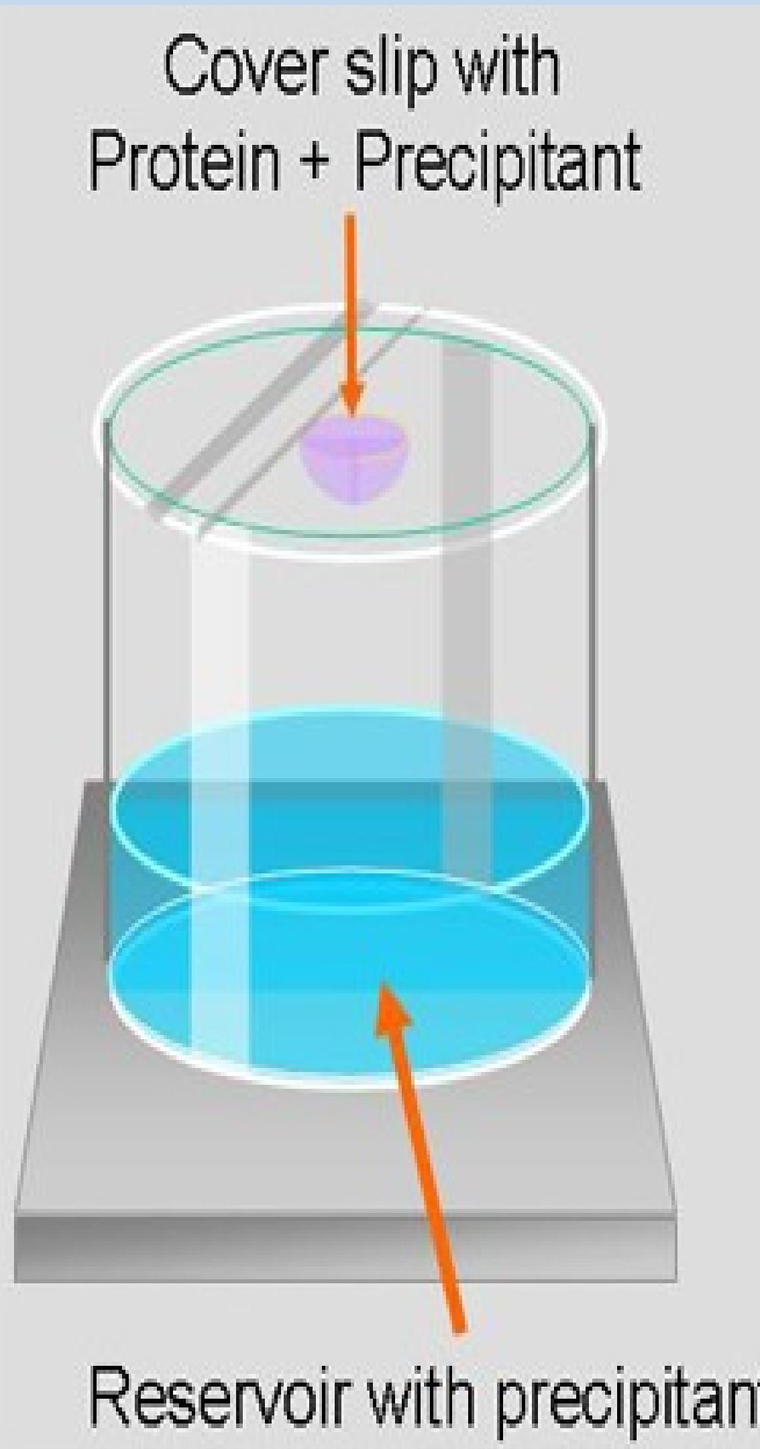
!!! Small changes in crystallization conditions can determine whether crystals form or not

Experimental Design



Materials

- ❑ Lysozyme 125mg/mL (dissolved with Milli-Q)
- ❑ micro-seed solution stocked into freezer
- ❑ Crystallization solution
- ❑ Crystallization plate
- ❑ Pipet (P1000, P2)
- ❑ Siliconized Cover Slides



Experimental Procedure

- **Step 1 – Prepare the hanging drop**

Mix **1 µL lysozyme solution** with **1 µL crystallization solution** on a siliconized coverslip.

🧪 + 💧 → ⚗️

- **Step 2 – Set up the hanging drop**

Invert the coverslip and seal it over the reservoir well.

💧 ⬇️ 🔵

- **Step 3 – Apply micro-seeding**

Add **0.2 µL seed solution** to the selected crystallization drops.

🌱 ➜ 💧

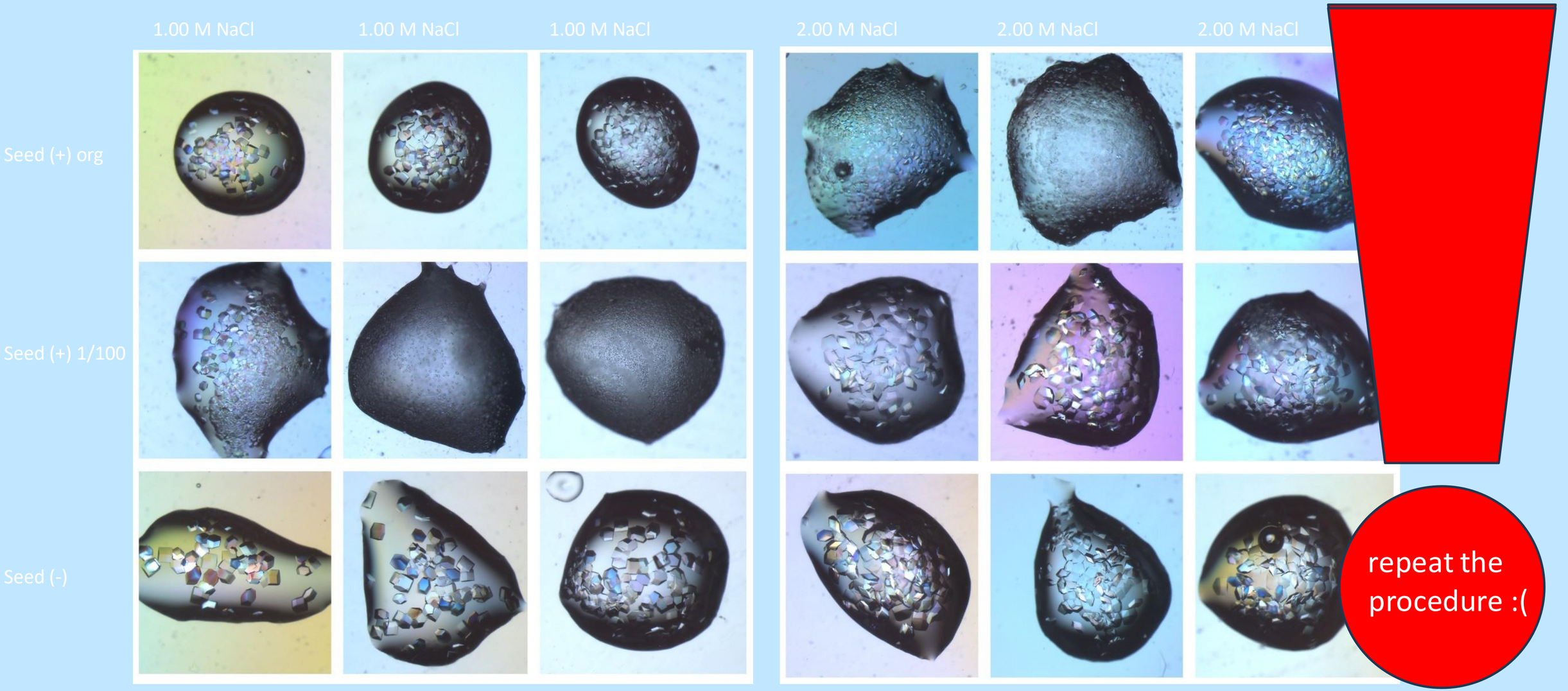
- **Step 4 – Incubation and observation**

Incubate the plates and monitor crystal growth under a light microscope.

⏳ → 🔬 → 💎

Results

Lysozyme plate No.001 260623 crystallized 1day/Lot.125mg/ml seed(+) (-) /20°C incubator/Reservoir: 1.0-2.0 M sodium chloride, 50 mM sodium acetate pH 4.5, 25%(v/v) glycerol



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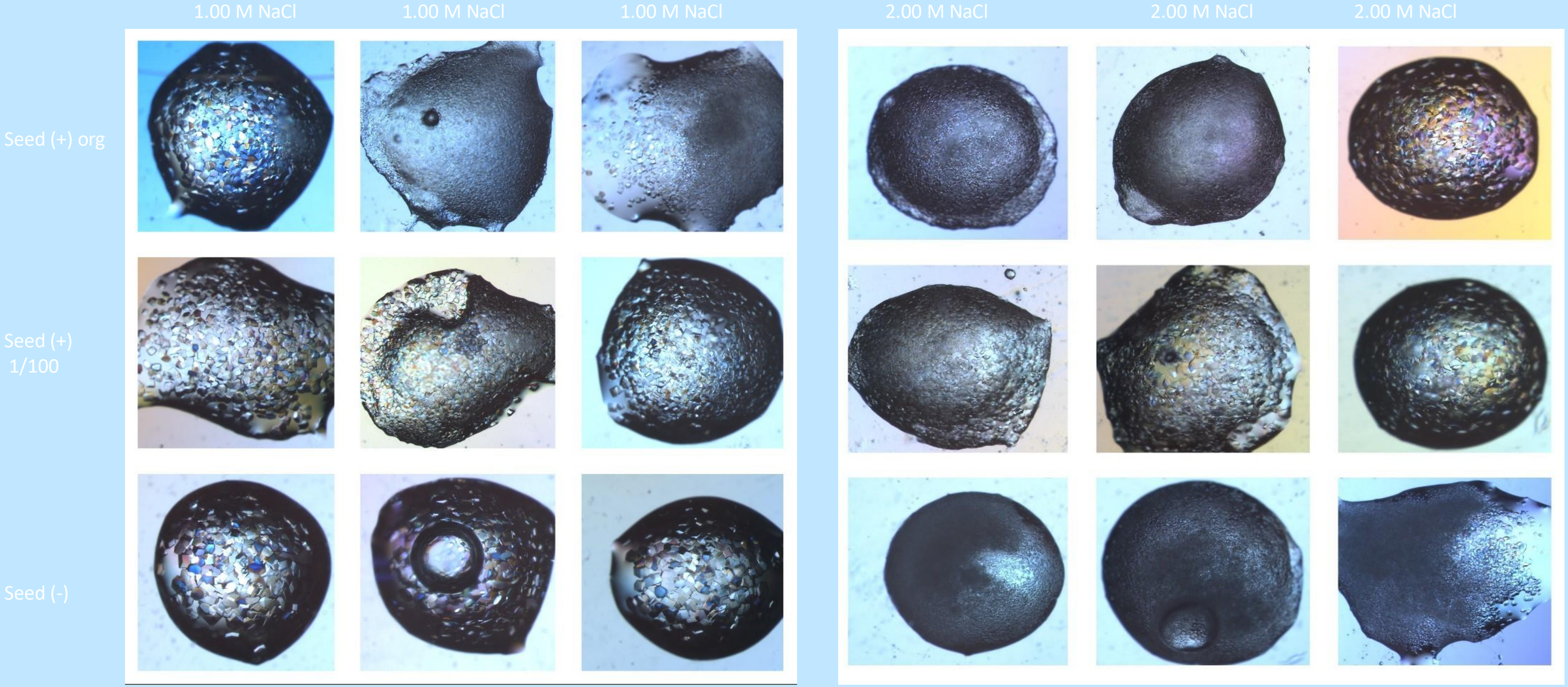


Discussion

- ✓ Lysozyme crystallization was successfully achieved under all experimental conditions.
- ✓ Original seeding resulted in extensive nucleation, producing a high density of microcrystals.
- ✓ Dilution of the seed suspension (1:100) appeared to reduce nucleation density and promoted more controlled crystal growth.
- ✓ Drops without seed generally showed reduced crystal formation, highlighting the role of micro-seeding in initiating nucleation.
- ✓ Differences between the two NaCl concentrations suggest that salt concentration also influenced crystallization behaviour, although its effect was less pronounced than that of the seeding strategy.

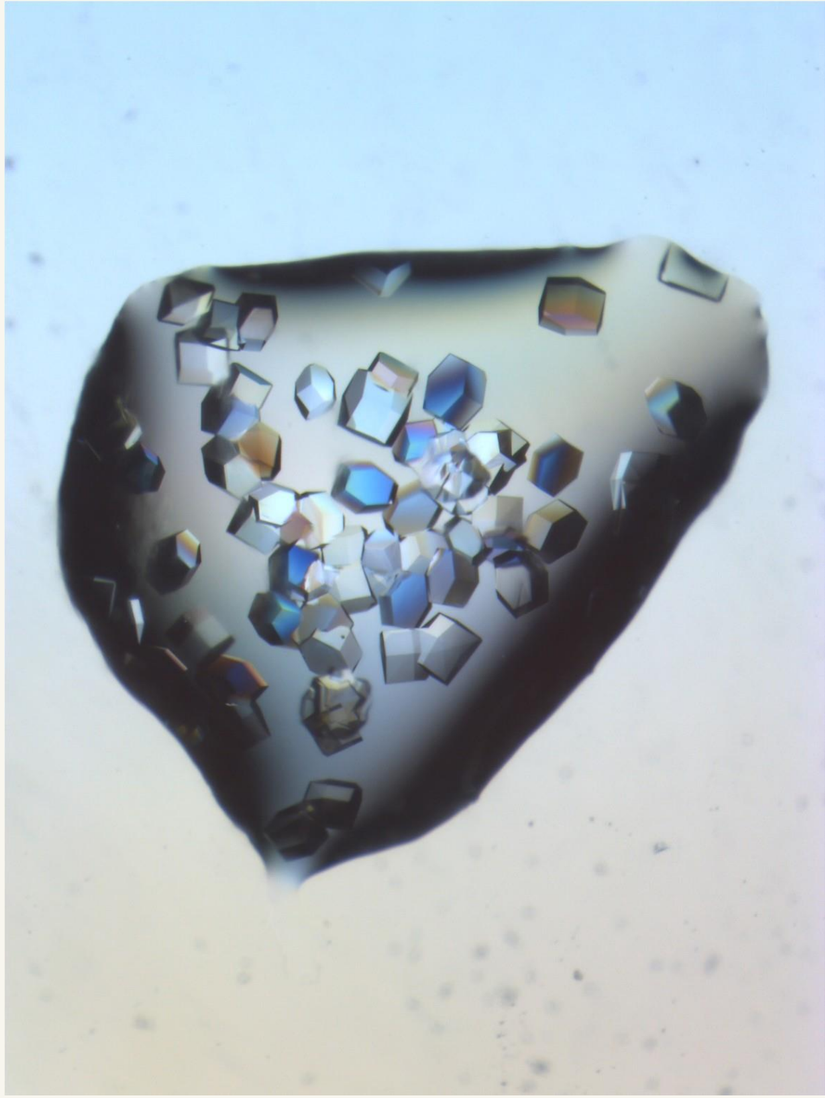
Conclusions

✓ Successful crystallization of lysozyme was achieved

✓ Crystal morphology depended on both **NaCl concentration** and **micro-seeding**

✓ Controlled nucleation through **micro-seeding optimization** is crucial for improving crystal size

✓ Protein crystallization requires careful optimization to obtain crystals suitable for structural analysis




Thank you for your attention
The end of the presentation