Cryobiology

ONPRC Saturday Academy
Part 1

OREGON NATIONAL PRIMATE Research Center
OHSU

http://www.ars.usda.gov/News/docs.htm?docid=15744&page=2

OREGON HEALTH & SCIENCE UNIVERSITY
OHSU

What is Cryopreservation?

- **Cryopreservation** is a process where cells or tissues are preserved by **cooling** to very low temperatures (usually the temperature of liquid nitrogen, -196°C).

- At this temperature, almost all biological activities, including the ones that would lead to cell death, are stopped. Sometimes biological activity can be restored upon warming.

http://www.biopoliticaltimes.org/img/original/egg%20freezing.jpg

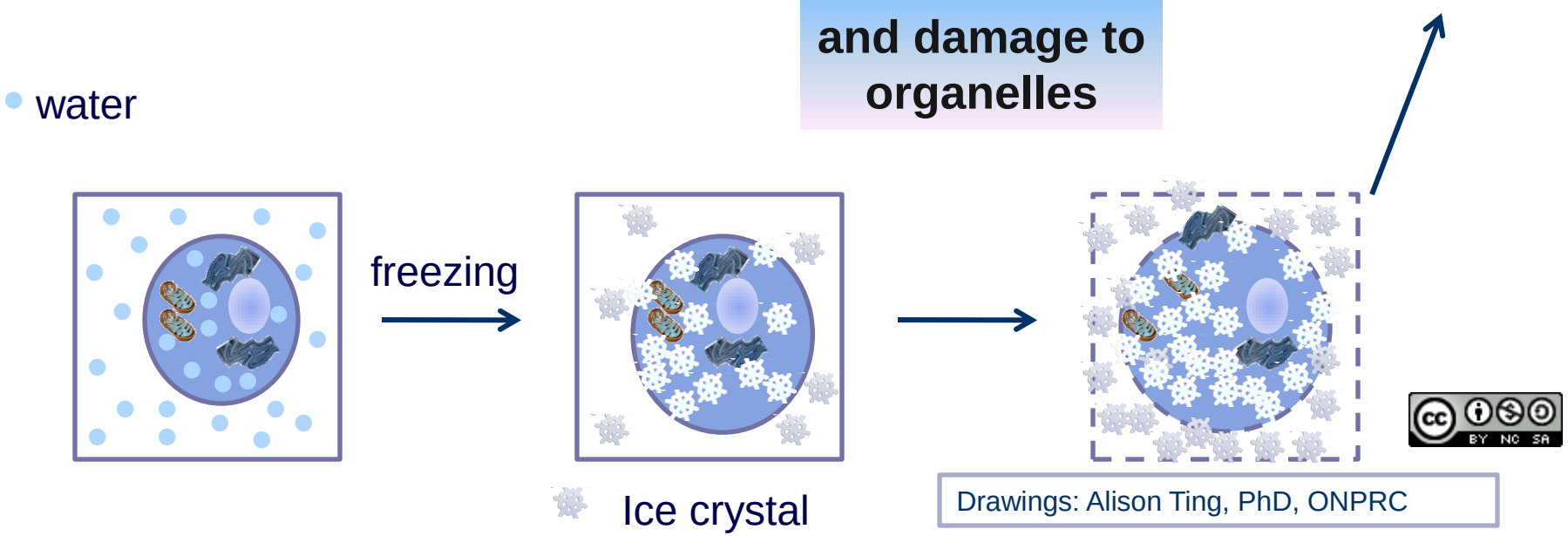
The goal of cryopreservation

To preserve the integrity of the living cell by avoiding ice formation inside the cell

Most of a cell is water

Freezing will lead to the formation of ice crystals in the cell which expand and can burst the cell.

Freezing temp. → **Ice crystal** → **Rupture of cell membranes and damage to organelles** → **Cell death**



Two Theories About the Cause of Damage to Cells As They Freeze

1. Freezing living tissue cause ice to form inside of cells which damages fragile membranes of organelles in the cell and can potentially rupture cell membranes causing cell death. Dehydration of tissues could address this issue.

2. Damage comes from the secondary effect caused by the concentrating of solutes left in solution as the water freezes. Cryoprotectants added to the solution could help solve this problem.

Cryoprotectants

- Cryoprotectant agents (CPAs) are compounds that are added to cells to increase the total concentration of all solutes in the system and thus reduce the amount of ice that could from at any temperature.
- Cryoprotectants should be able to diffuse or osmose into and out of the cell and have low toxicity.
- Cryoprotectants include sugar, glycerol, and other sugar alcohols.

Long before the scientists figured out how to achieve cryopreservation…

Cryopreservation in Nature

Survival strategies exist in bacteria, fungi, plants, insects and other animals during harsh winters.

Examples:

“The Living Dead”: Wood frogs make increased levels of glucose in cells of vital organs as temperatures approach freezing. A partially frozen frog will stop breathing, and its heart will stop beating. It will appear quite dead. But when the weather warms up, the frog's frozen portions will thaw, and its heart and lungs resume normal activity.

“Beetle-juice antifreeze”: Arctic beetles achieve their protection due to the glycerol and other sugar alcohols produced by their livers. Glycerol is an "antifreeze“ and reduces ice formation and lowers freezing point.

Clip art: Microsoft

http://www.pbs.org/wgbh/nova/nature/costanzo-cryobiology.html **(video)**

How is cryopreservation achieved in a laboratory?

- **Cryoprotective agents (CPAs) or anti-freeze agents**

  Common CPAs: glycerol, ethylene glycol, propylene glycol, dimethyl sulfoxide, sugars

- **Dehydration**

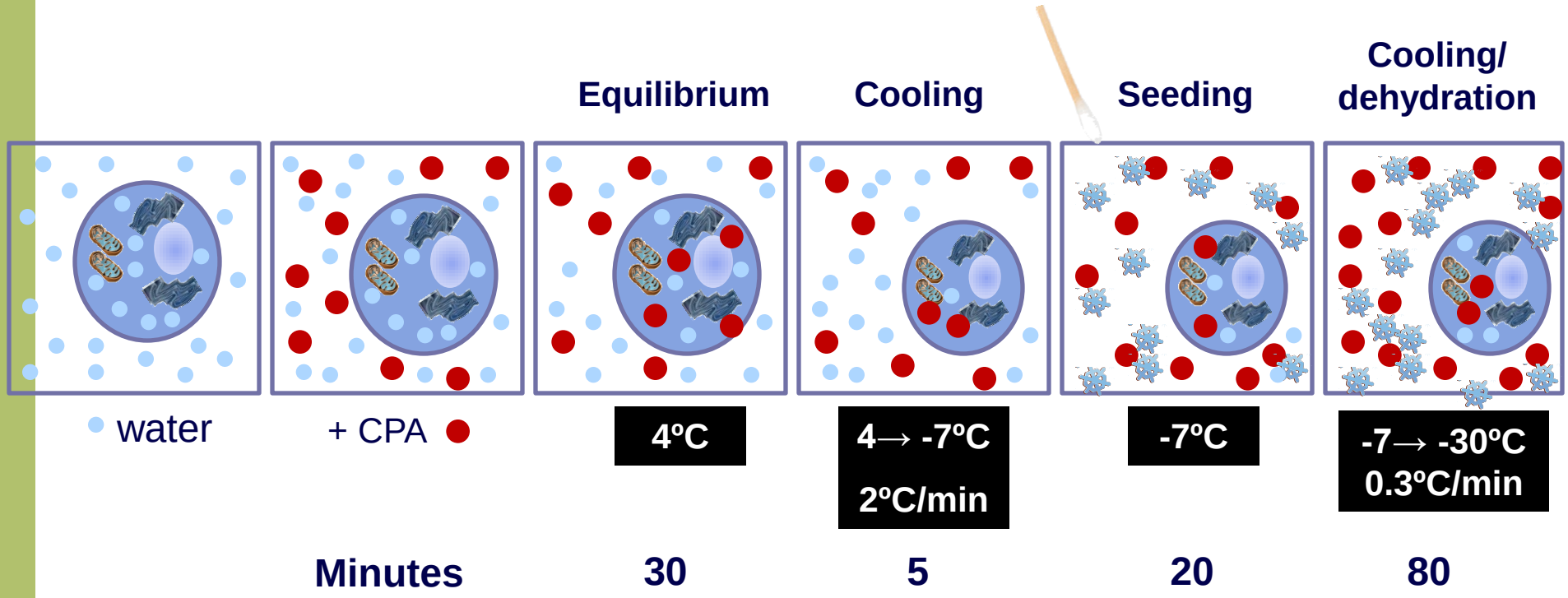
  Get rid of the water in the cell → no ice formation

Two Methods of Cryopreservation

- Slow rate freeze
- Vitrification (super rapid freezing)

Slow Rate Freezing

Avoids ice formation inside the cell by making water leave the cell. Ice still forms outside the cell.



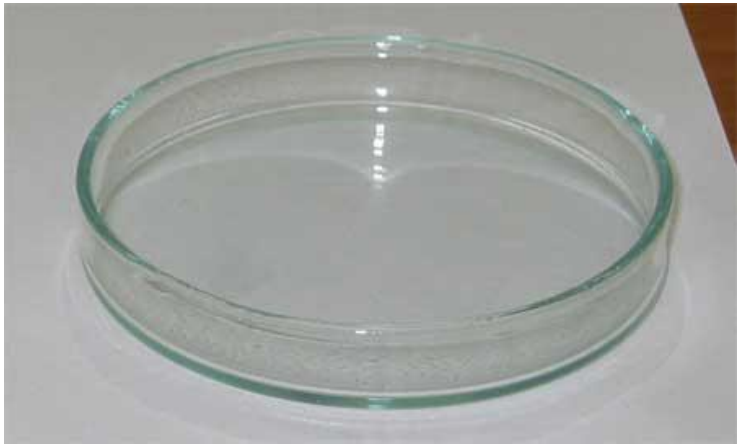
Drawings: Alison Ting, PhD, ONPRC

Vitrification (Super Rapid Freezing)

Avoids overall ice formation inside and outside the cell.

- Pure water can be vitrified (no ice formation) if cooled at a rate of millions of degrees Celsius per second.

Not Vitrified (Ice crystal)



Vitrified (glass-like)

http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm197586.htm

http://en.wikipedia.org/wiki/Glass#mediaviewer/File:Szalka_petriego.jpg
http://creativecommons.org/licenses/by-sa/3.0/ No changes were made.

Vitrification (Super Rapid Freezing)

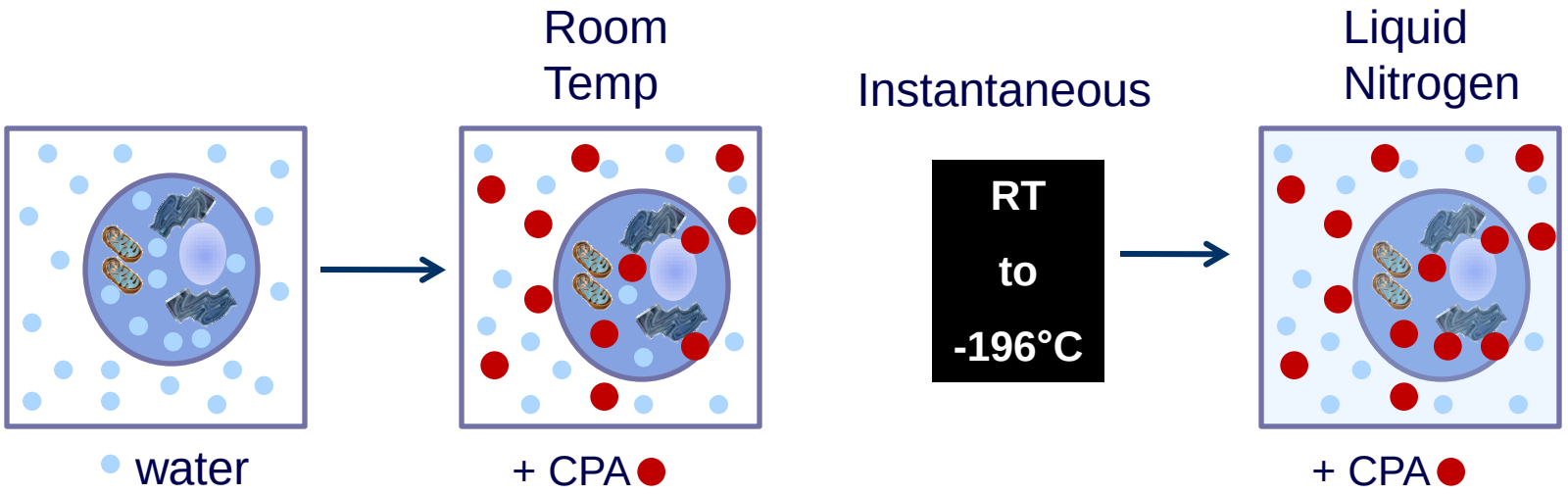
Avoids overall ice formation inside and outside the cell.

Requires

- **High cryoprotective agent (CPA)**
- **Super rapid cooling in liquid nitrogen**

Vitrification (Super Rapid Freezing)

Avoids overall ice formation inside and outside the cell.



Drawings: Alison Ting, PhD, ONPRC

How can cryopreservation be used for fertility preservation in male cancer patients?

- **Sperm Freezing**
- **Embryo Freezing**
- **Testicular Tissue Freezing**
- **Whole Testicle Freezing**

Shiver

First rhesus monkey in the world born from *in vitro* fertilization, frozen-thawed embryo

ONPRC
October 31, 1989

Photo: ONPRC

Arnold and Danny

First rhesus monkey twins in the world born from *in vitro* fertilization, frozen-thawed embryos

ONPRC
1991

Photo: ONPRC