Cryopreservation of woody plants

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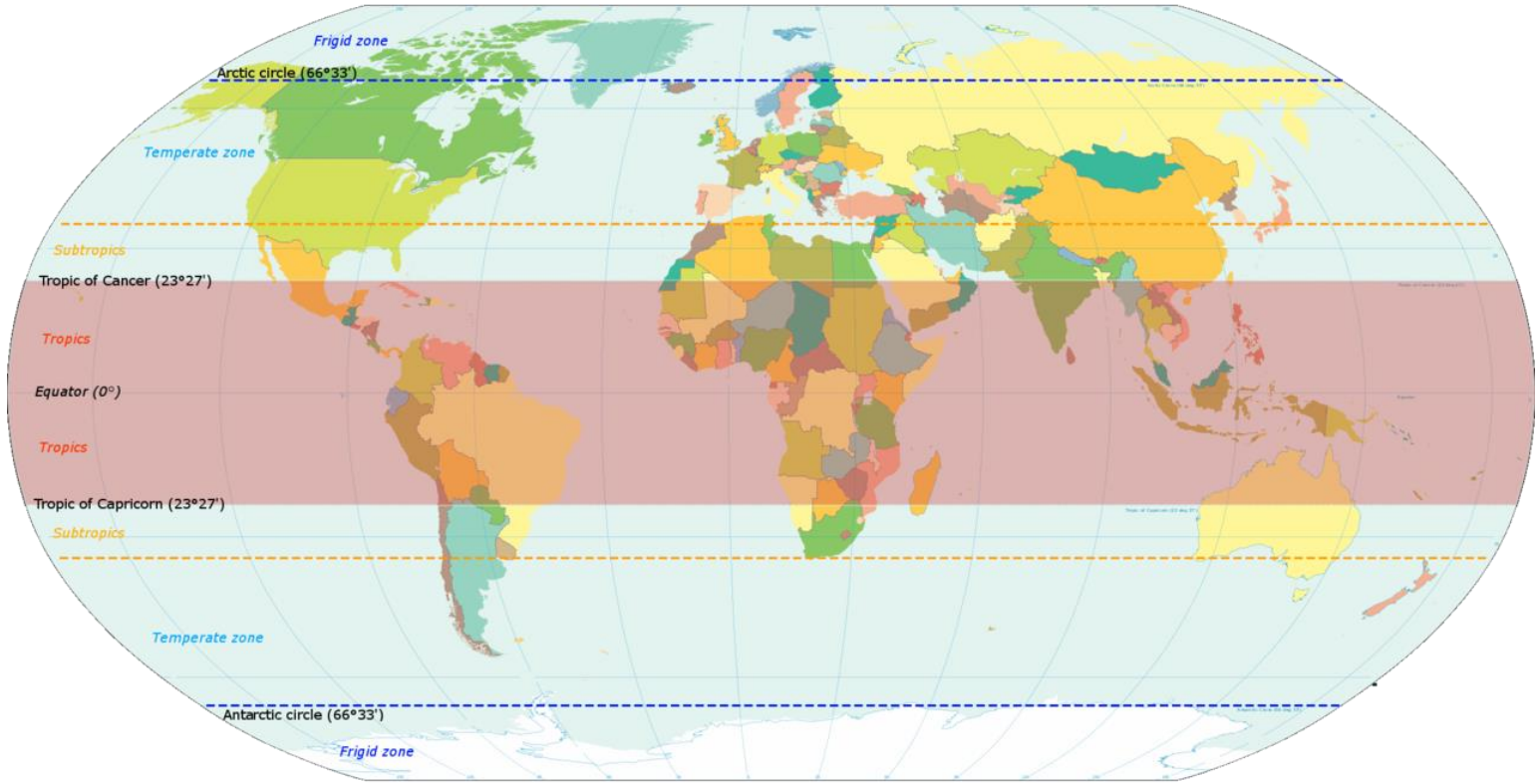
Cryopreservation of woody plants

Distribution on Earth

- Tropical
- Subtropical
- Temperate

Usage

- Forest
- Ornamental
- Medicinal
- Fruit



Cryopreservation of woody plants

In vitro

- meristems
- somatic embryos
- zygotic embryos
- clumps, stem disc-bearing adventitious buds

Ex vitro

- pollen
- orthodox seeds
- dormant buds

Cryopreservation of woody plants

CRYOPRESERVATION METHODS – MAIN STEPS:

I. Acclimation – can be skipped in some methods

II. Dehydration - obligatory

III. Cooling – specific ways

IV. Warming – specific ways - usually fast

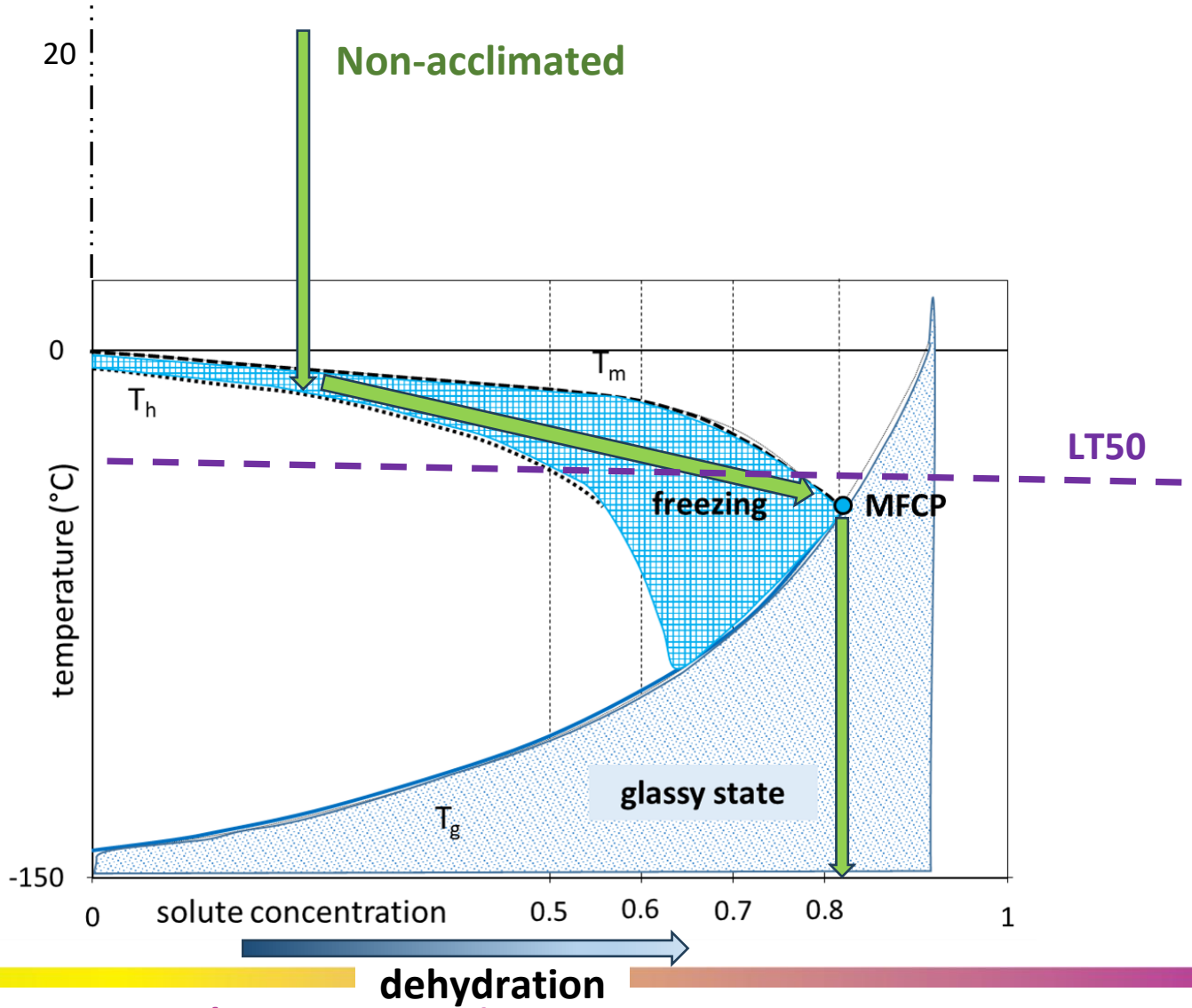
CRYOPRESERVATION METHODS:

Risk of low temperature damage:

- **Non-acclimated plants**

>>> damage by ice crystals

Acclimation **improves tolerance to low temperatures.**



Cryopreservation of woody plants

Cryopreservation methods – main steps:

- ***Acclimation – can skiped in some methods***

- ***Dehydration - obligatory***

- ***Cooling – specific ways***

- ***Warming – specific ways - usually fast***

Cryopreservation of woody plants

CRYOPRESERVATION METHODS :

- **Dehydration before cooling**
  - encapsulation-dehydration (encapsulation-vitrification, D cryo plate)
  - vitrification (droplet vitrification, V cryo plate, cryo-mesh)

air-dehydration

osmotic-dehydration

- **Dehydration during cooling**
  - two-step (controlled) freezing method
  - droplet freezing method (DMSO – improves/modifies the freezing-dehydration)

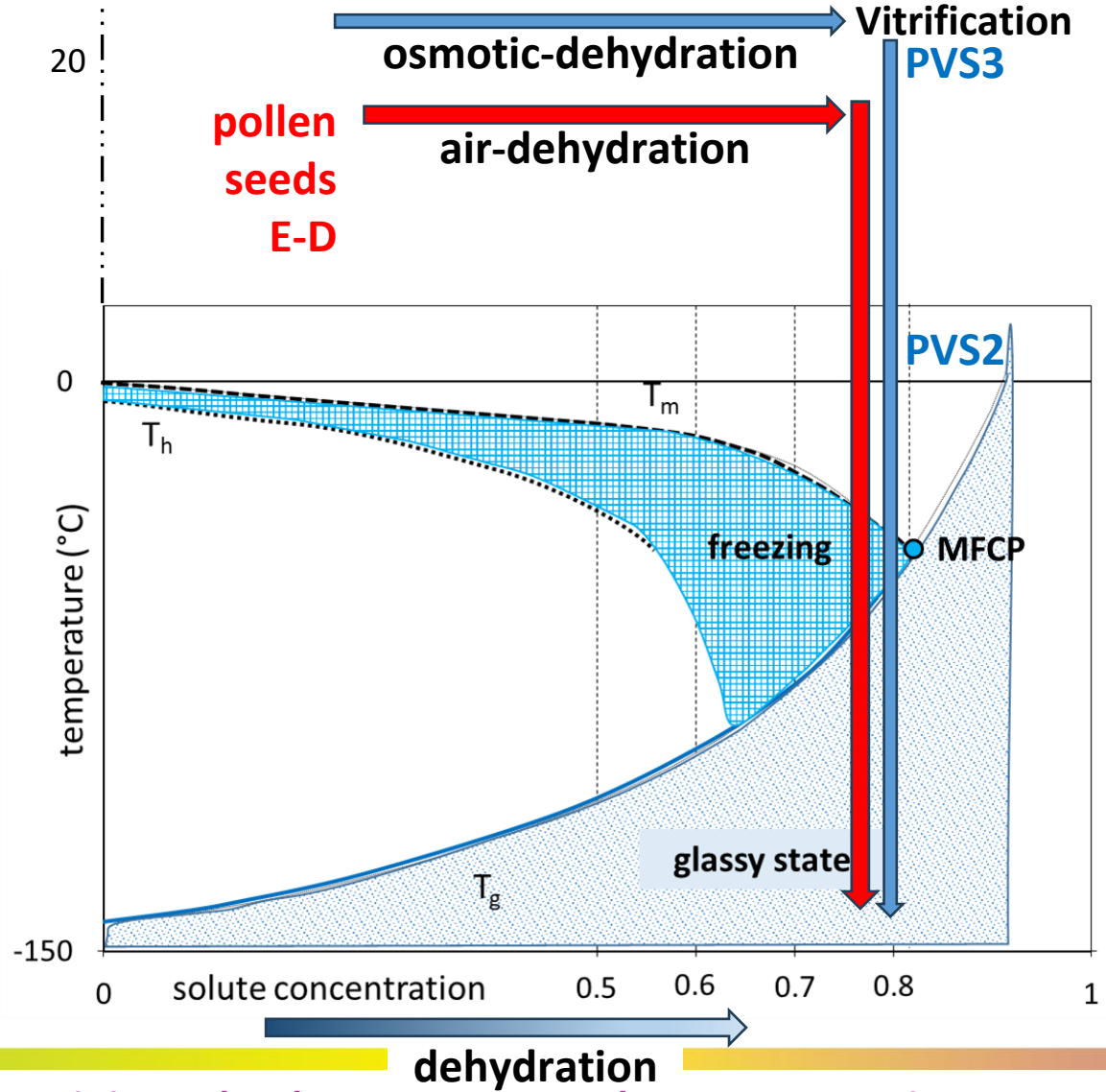
freezing-dehydration

DEHYDRATION:

- before cooling

air-dehydration

osmotic-dehydration



Cryopreservation of woody plants

DORMANT BUD CRYOPRESERVATION

- temperate deciduous trees

Prerequisites:

DORMANCY of the leaf buds during winter time

- development of embryonic shoot is stopped by phytohormons and / or enviromental conditions
- increases the frost tolerance of buds during winter time

Cryopreservation of woody plants

DORMANT BUD CRYOPRESERVATION

- temperate deciduous trees

Frost tolerance:

- induced by low temperatures
- genetically determined
- developmentally dependent

Cryopreservation of woody plants

DORMANT BUD CRYOPRESERVATION

- temperate deciduous trees

Cryotolerance:

- benefits from the natural ability of temperate deciduous trees to overcome adverse winter conditions
- cryopreservation method overcomes natural limits of frost tolerance

DORMANCY >>>> FROST TOLERANCE >>>> CRYOTOLERANCE

natural ability + cryoprotocol

Cryopreservation of woody plants

DORMANT BUD CRYOPRESERVATION

- temperate deciduous trees

Advantage:

- simple and fast
- avoiding explant cultures (TC lab and staff)

Disadvantage:

- seasonality – limited period during a year
- health status unreliable
- depends on environmental conditions
- programable freezer required

Cryopreservation of woody plants

DORMANT BUD CRYOPRESERVATION

- temperate deciduous trees

MAIN STEPS:

- material collection in mid-winter from orchard (acclimation)
- twigs dehydration in freezer at -4 – -5°C to 25 – 40 % WC in FW
- controlled cooling (1°C/hour, 0.25°C to 0.5°C/min., 5°C/day) to desired temperature from -25 to -40 °C (alternatively vitrification)
- rapid cooling to the final temperature (glass transition~ vitrification)
- storage in liquid or vapor phase of nitrogen
- warming to temperature above zero (slow vs. fast)
- rehydration at 2 – 4 °C
- grafting/*in vitro* recovery

DORMANT BUD CRYOPRESERVATION

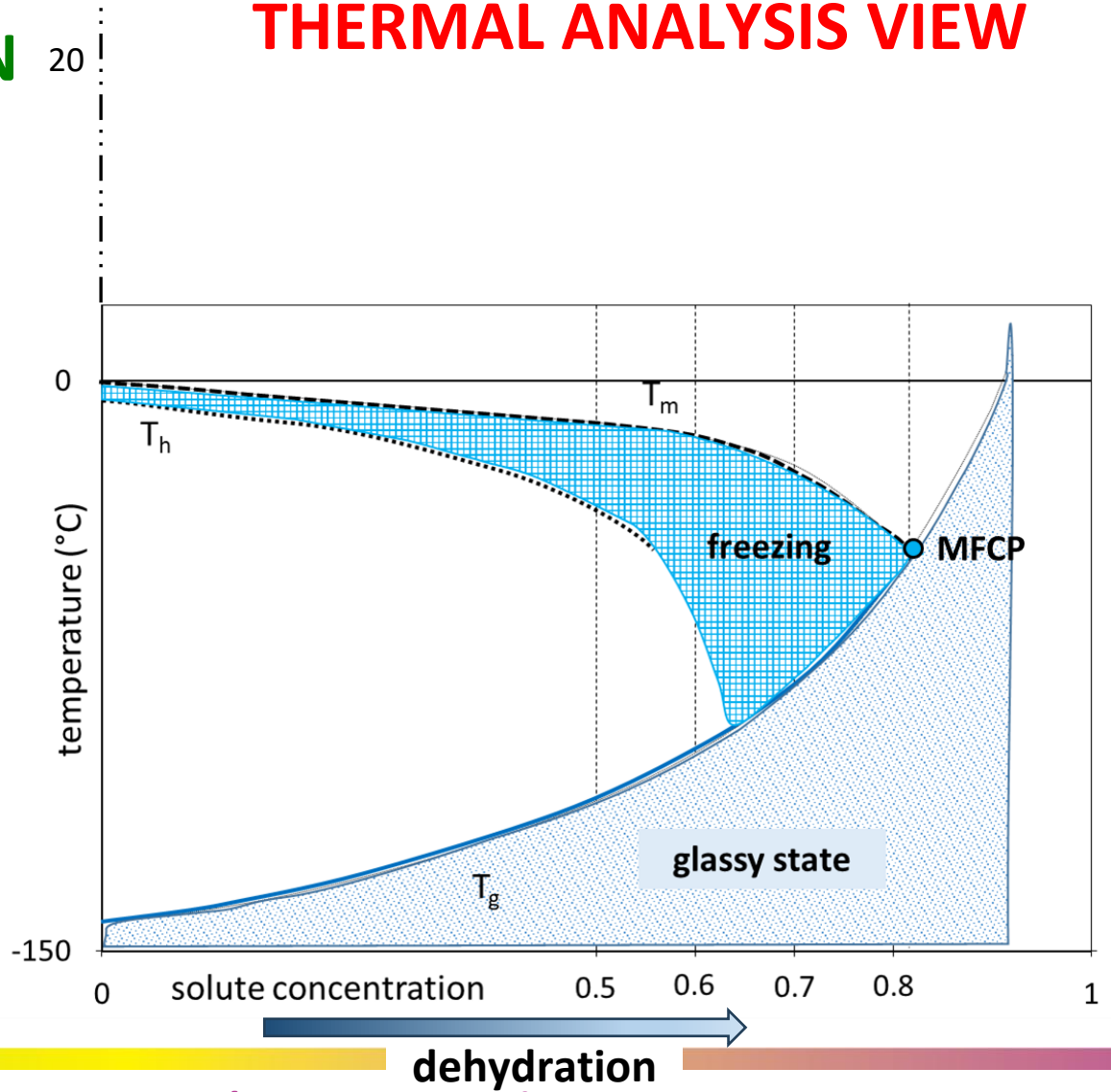
by two-step freezing method:

MAIN STEPS:

- **ACCLIMATION**
- **DEHYDRATION**
  (during cooling)
- **CONTROLLED COOLING**
  (freezing)
- **RAPID COOLING**
  (glass transition~ vitrification)

RAPID WARMING
(freezing avoidance)

THERMAL ANALYSIS VIEW



DORMANT BUD CRYOPRESERVATION

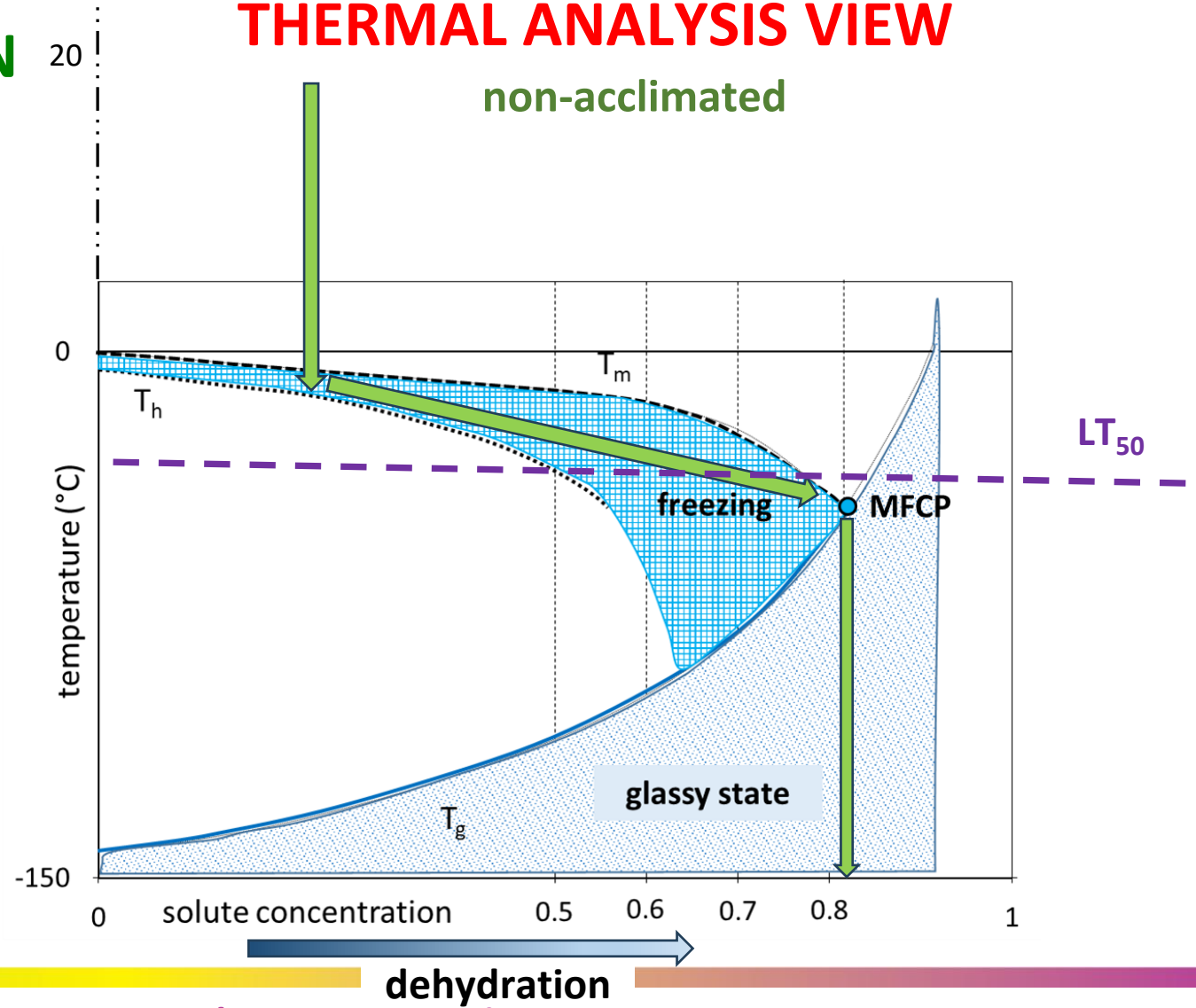
by two-step freezing method:

ACCLIMATION

- **Non-acclimated plants**
  >>> damage by ice crystals

THERMAL ANALYSIS VIEW

non-acclimated



DORMANT BUD CRYOPRESERVATION

by two-step freezing method:

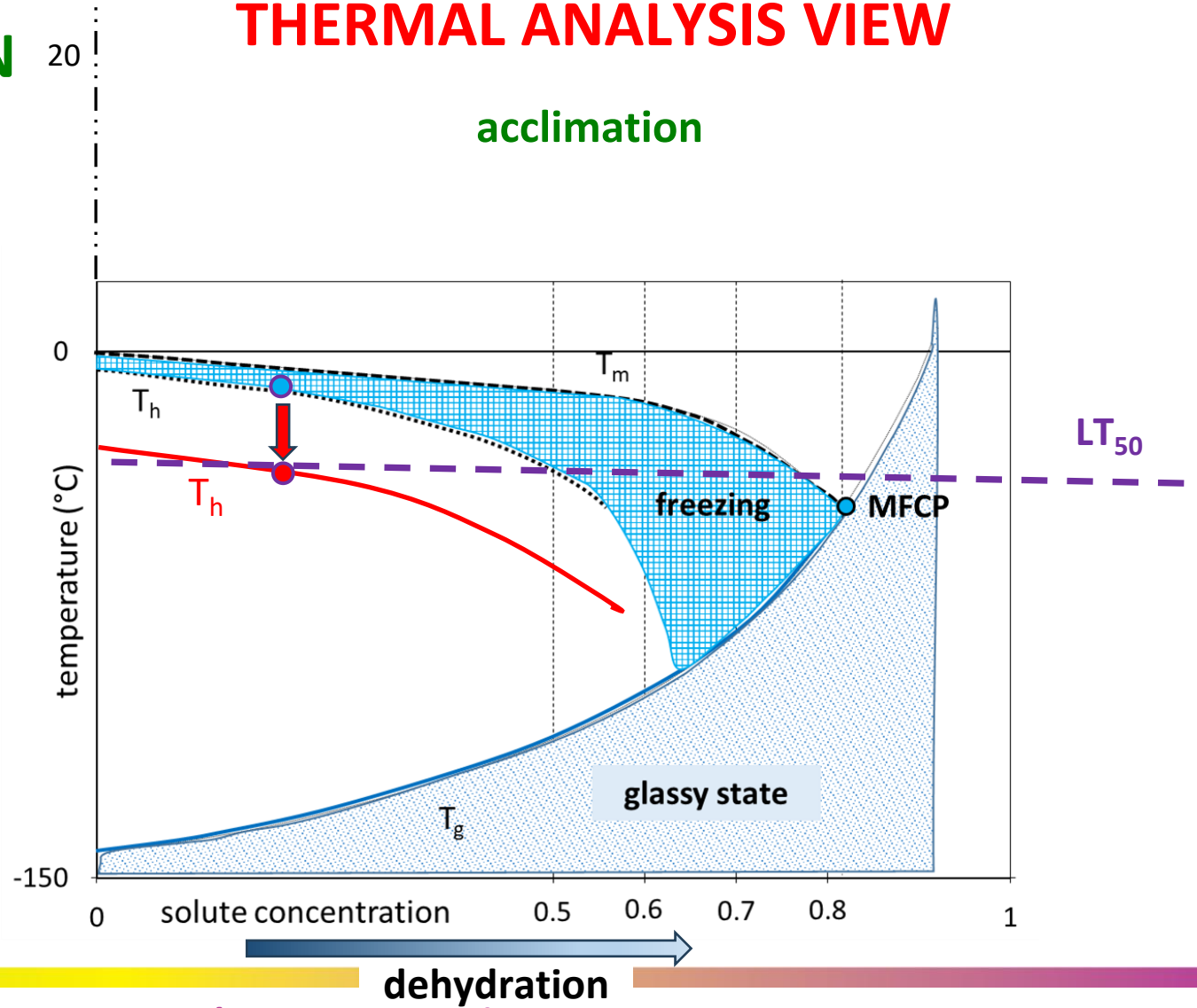
ACCLIMATION

>>> Decrease in Tm and Th

~ water activity (AW) increase

THERMAL ANALYSIS VIEW

acclimation



DORMANT BUD CRYOPRESERVATION

by two-step freezing method:

ACCLIMATION

>>> Decrease in Tm and Th

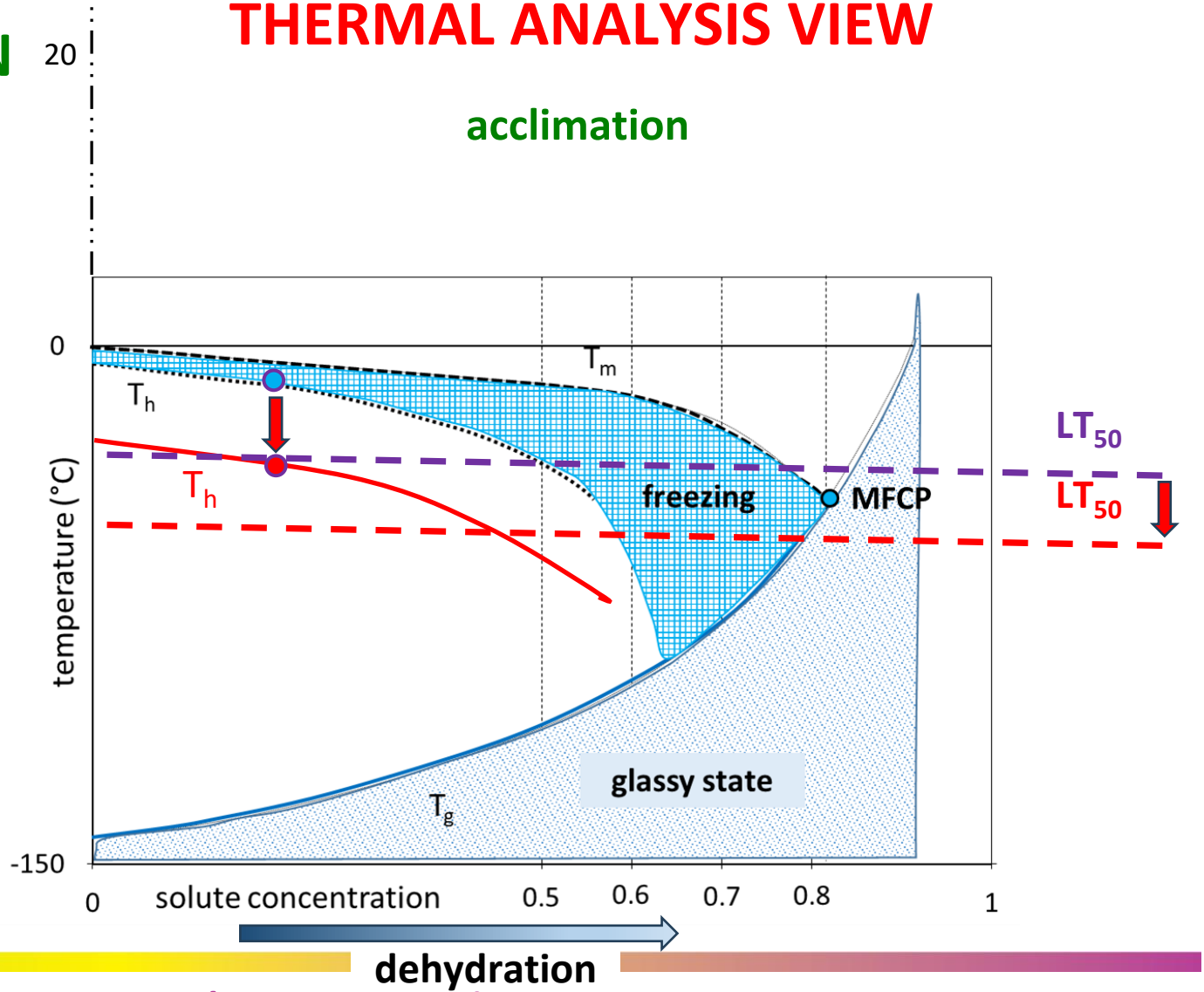
~ water activity (AW) increase

>>> Decrease LT_{50}

(freezing tolerance increase)

THERMAL ANALYSIS VIEW

acclimation



DORMANT BUD CRYOPRESERVATION

by two-step freezing method:

ACCLIMATION

>>> Decrease in Tm and Th

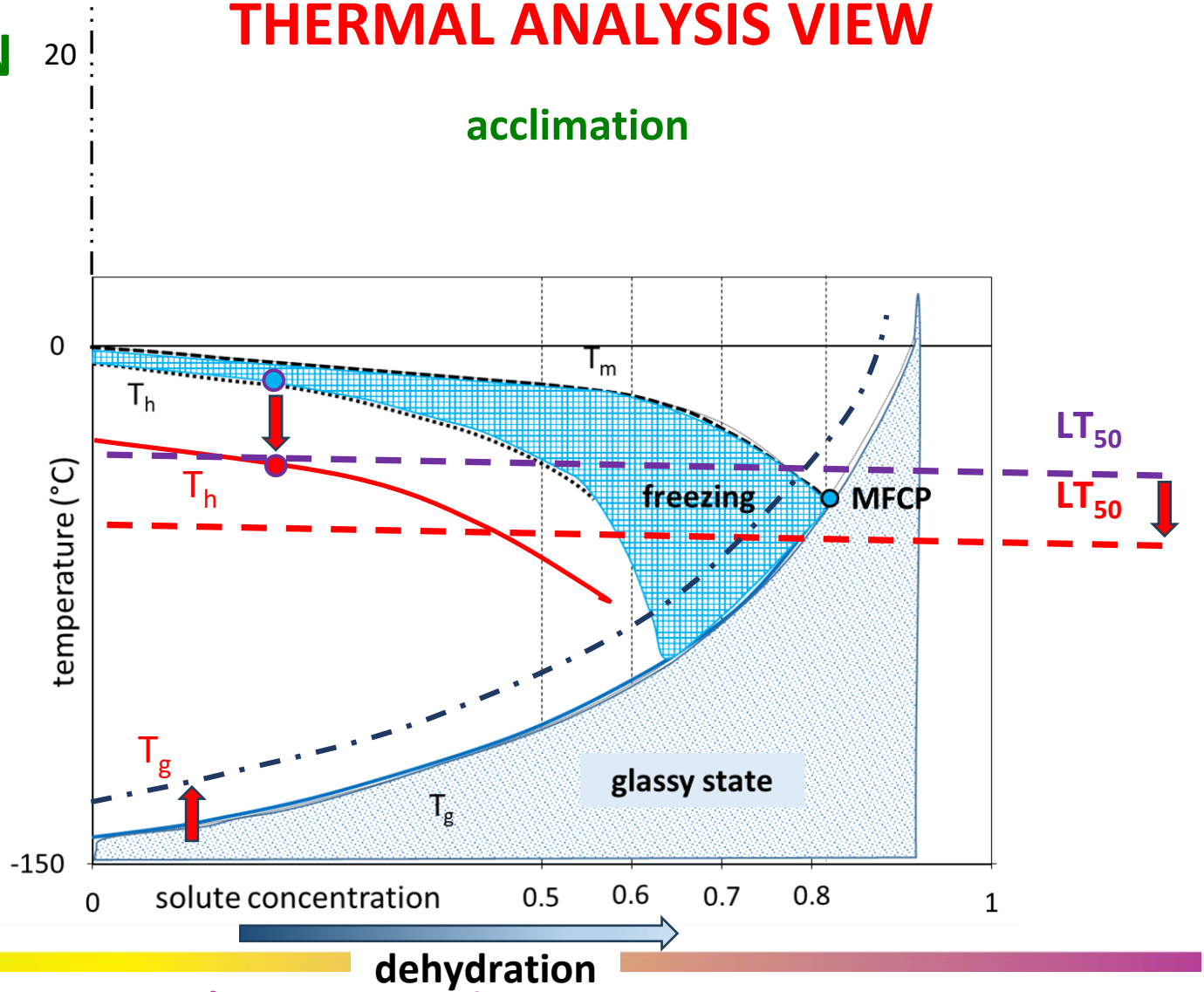
~ water activity (AW) increase

>>> Decrease LT_{50}
(freezing tolerance increase)

>>> Tg increase

THERMAL ANALYSIS VIEW

acclimation



DORMANT BUD CRYOPRESERVATION

by two-step freezing method:

DEHYDRATION

- **by freezing -4 – -5°C**
  (alternatively by air dehydration)

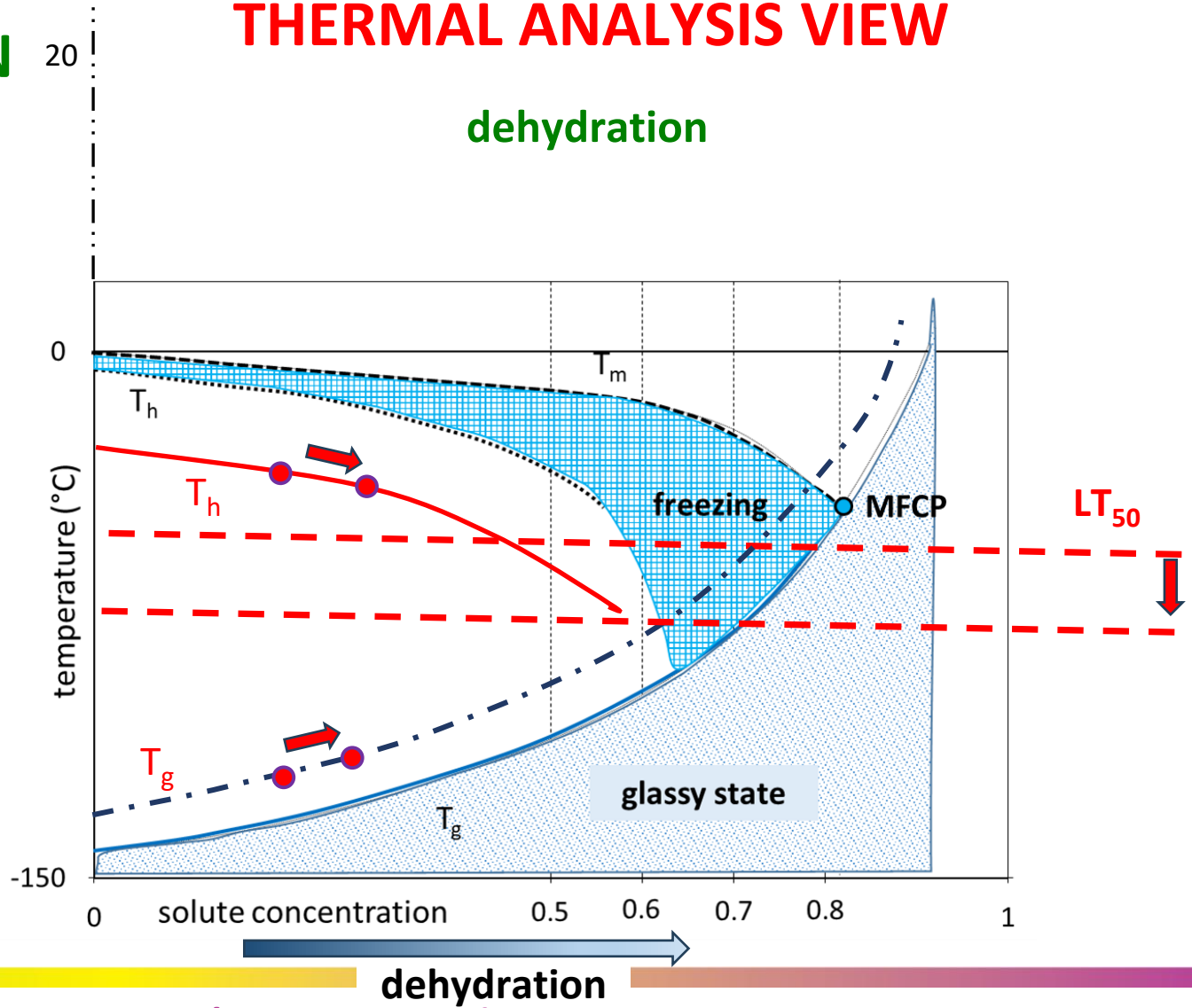
>>> Decrease in Tm and Th
(result of dehydration)

>>> Tg increase

>>> LT_{50} decrease
(freezing tolerance increase)

THERMAL ANALYSIS VIEW

dehydration



DORMANT BUD CRYOPRESERVATION

by two-step freezing method:

FREEZING

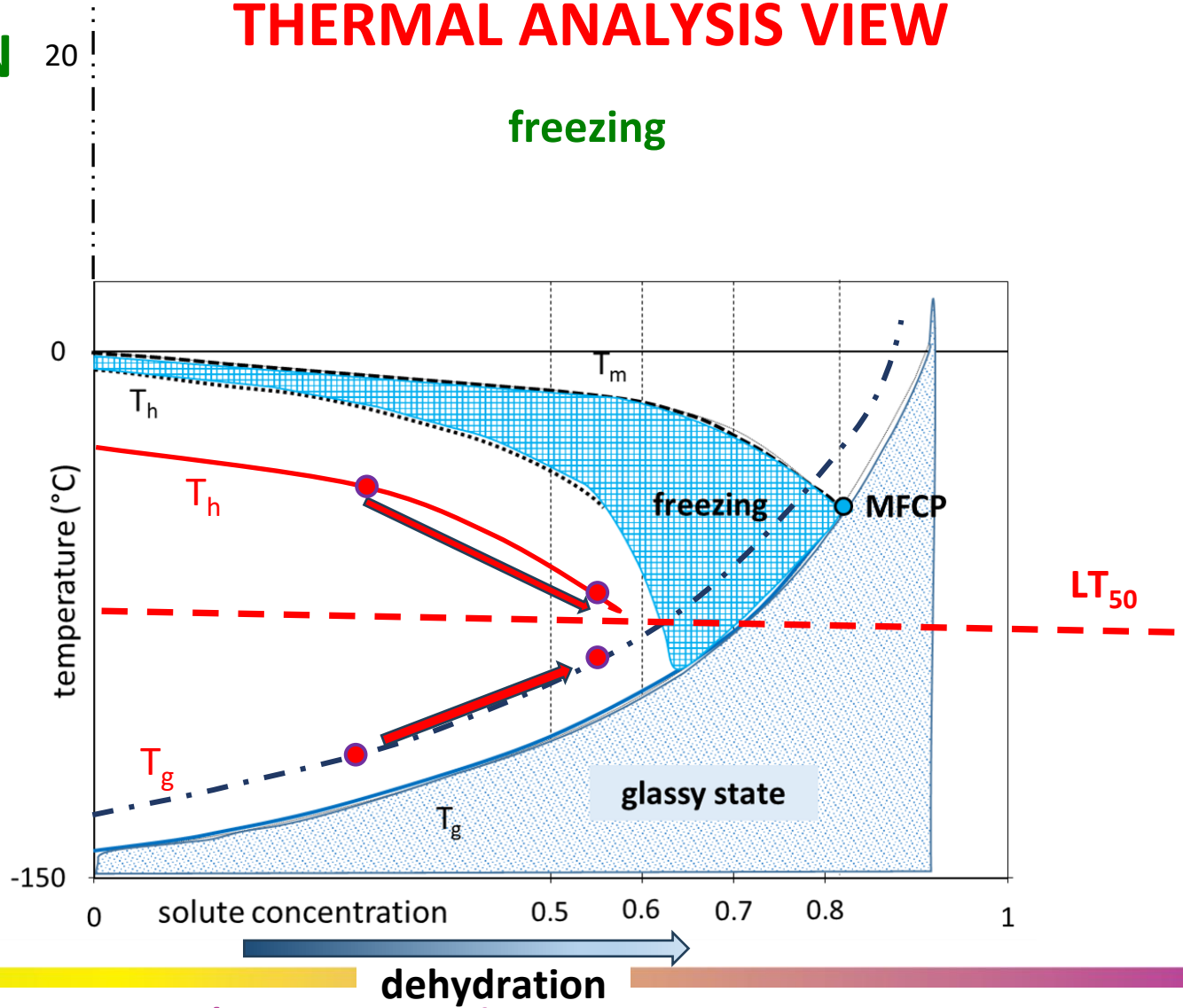
- controlled cooling rate

>>> Decrease in Tm and Th
(result of dehydration)

>>> Tg increase

>>> LT_{50} decrease
(cryotolerance increase)

THERMAL ANALYSIS VIEW



DORMANT BUD CRYOPRESERVATION

by two-step freezing method:

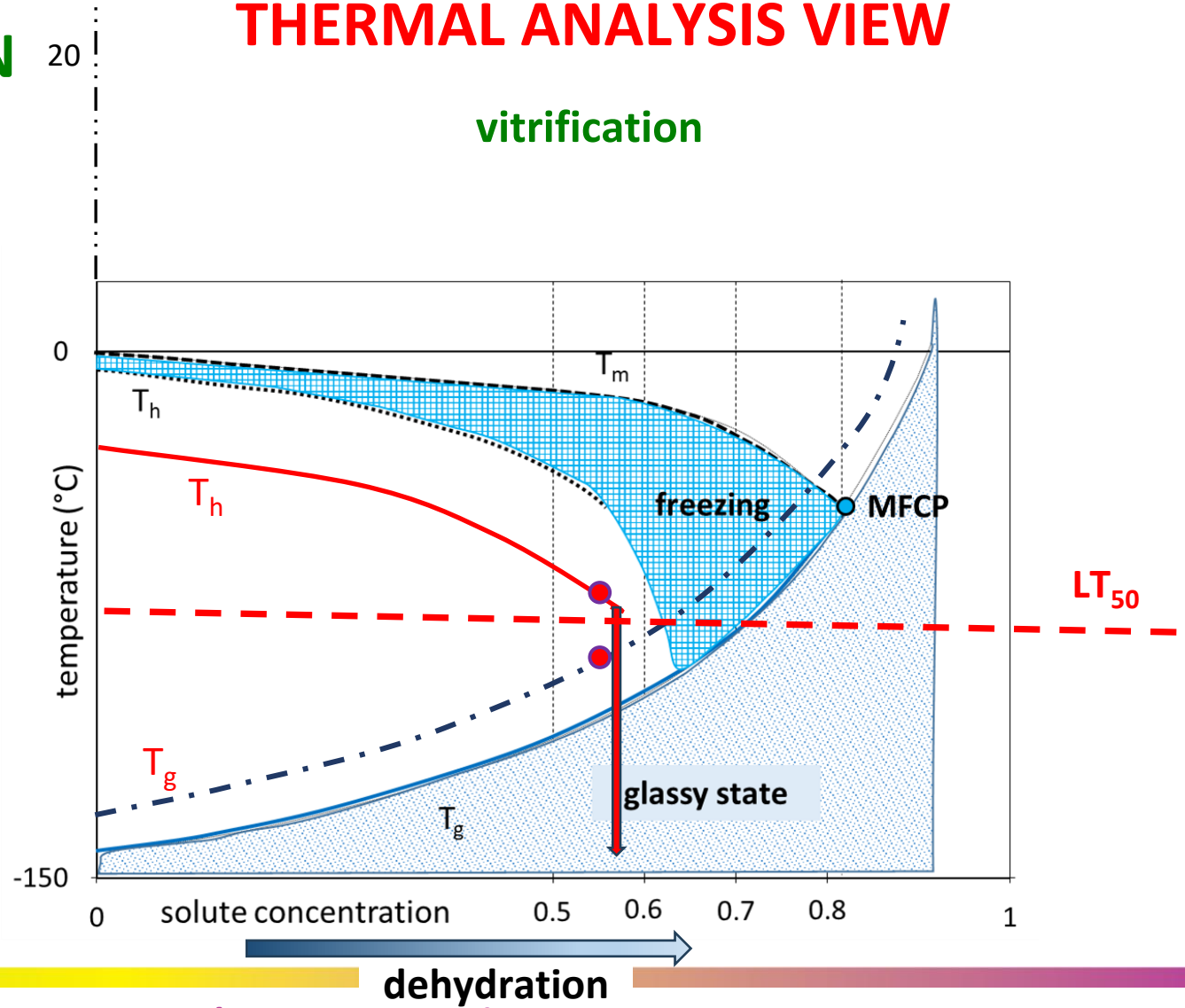
VITRIFICATION

- **rapid cooling**

>>> glassy state development

THERMAL ANALYSIS VIEW

vitrification



DORMANT BUD CRYOPRESERVATION

by two-step freezing method:

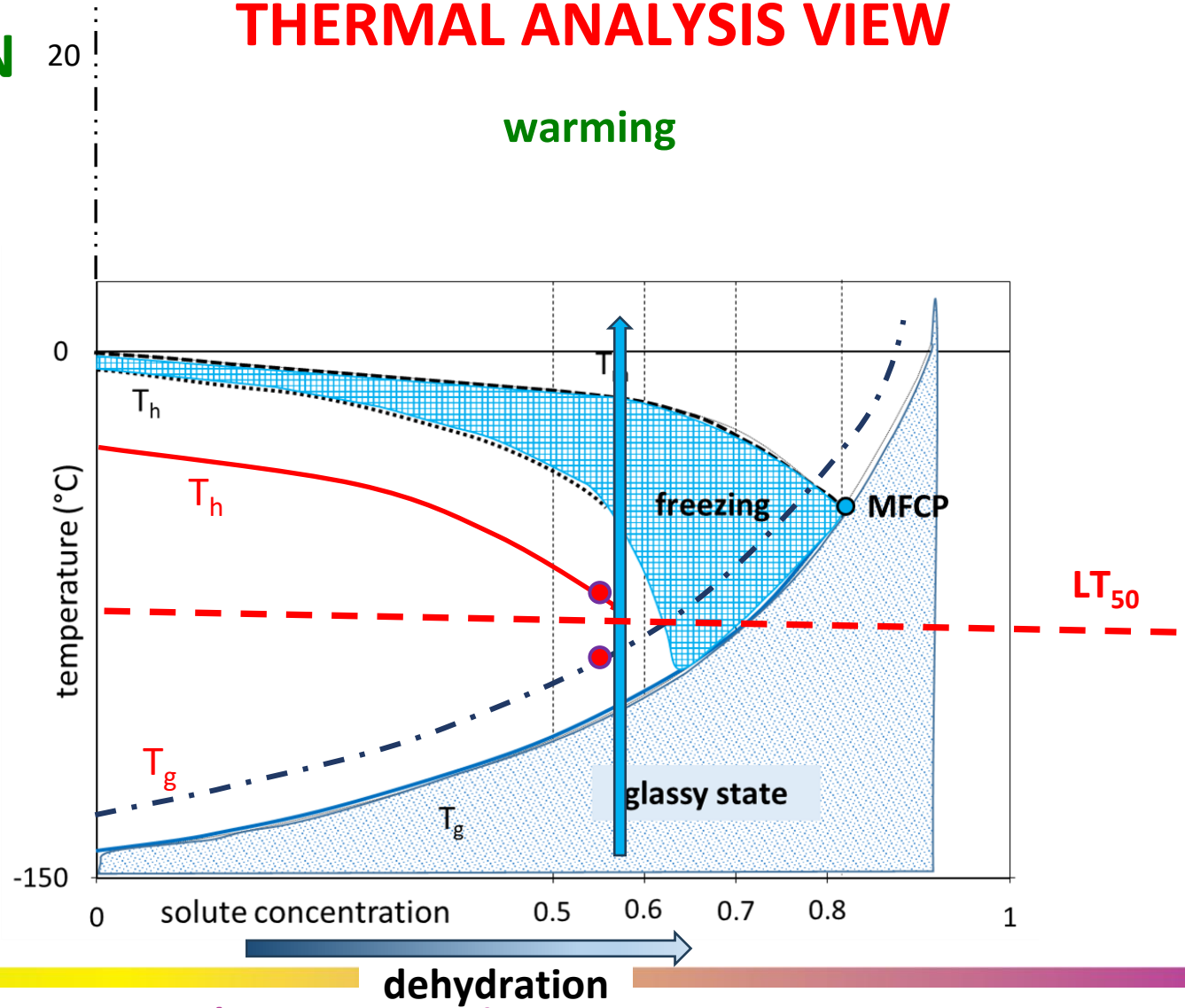
WARMING

- **small amount of freezable water >> risk of damage during warming!**

>>> rapid warming rate
(freezing avoidance)

THERMAL ANALYSIS VIEW

warming



DORMANT BUD CRYOPRESERVATION

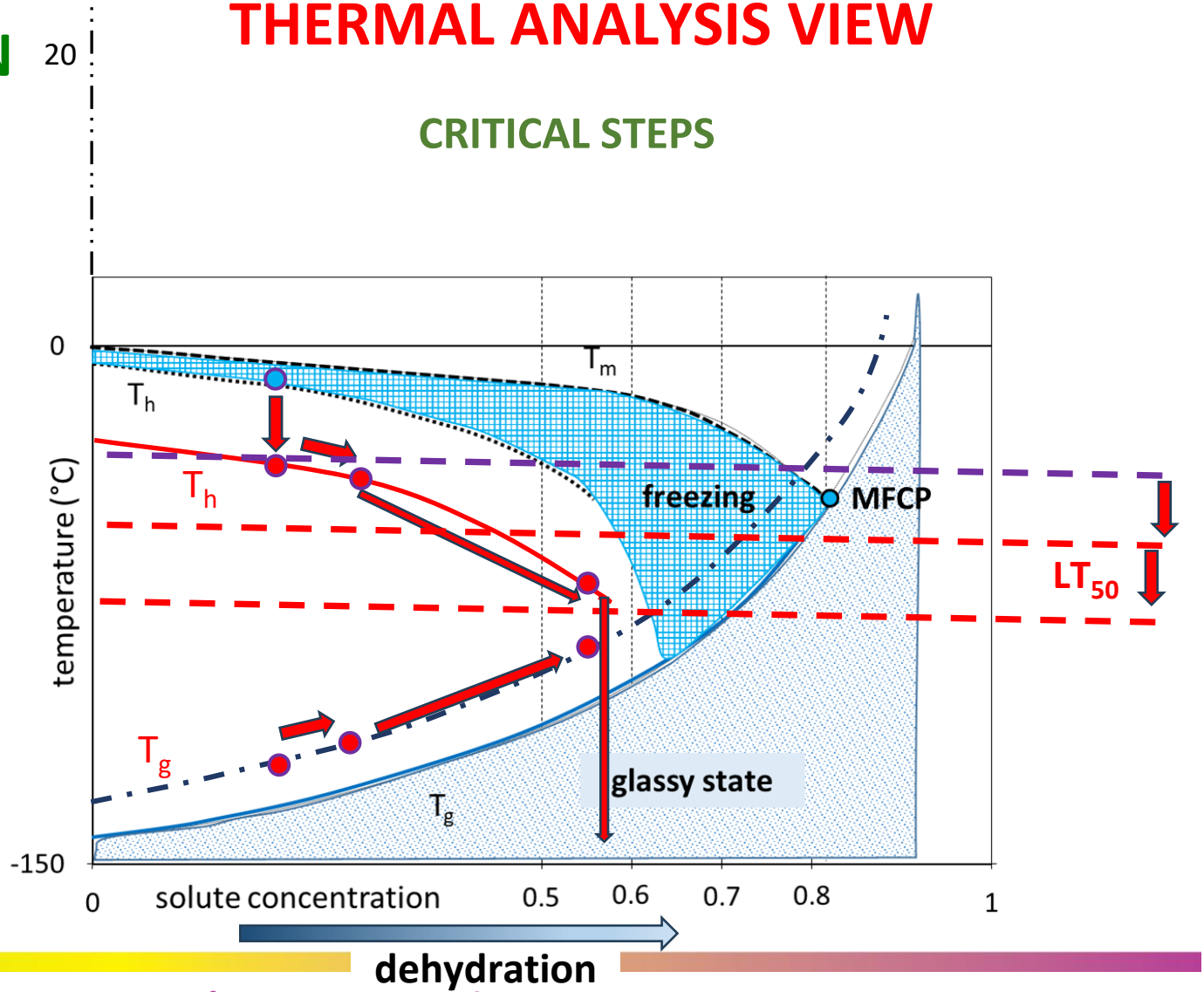
by two-step freezing method:

CRITICAL STEPS:

- **Acclimation** – temperature, collection period, dormancy, LT_{50}
- **Dehydration** – freezing temperature, period, final water content, LT50
- **Freezing** – controlled rate, final temperature, equilibrium
- **Glass transition** – fast cooling

THERMAL ANALYSIS VIEW

CRITICAL STEPS



DORMANT BUD CRYOPRESERVATION

by two-step freezing method:

CRITICAL STEPS:

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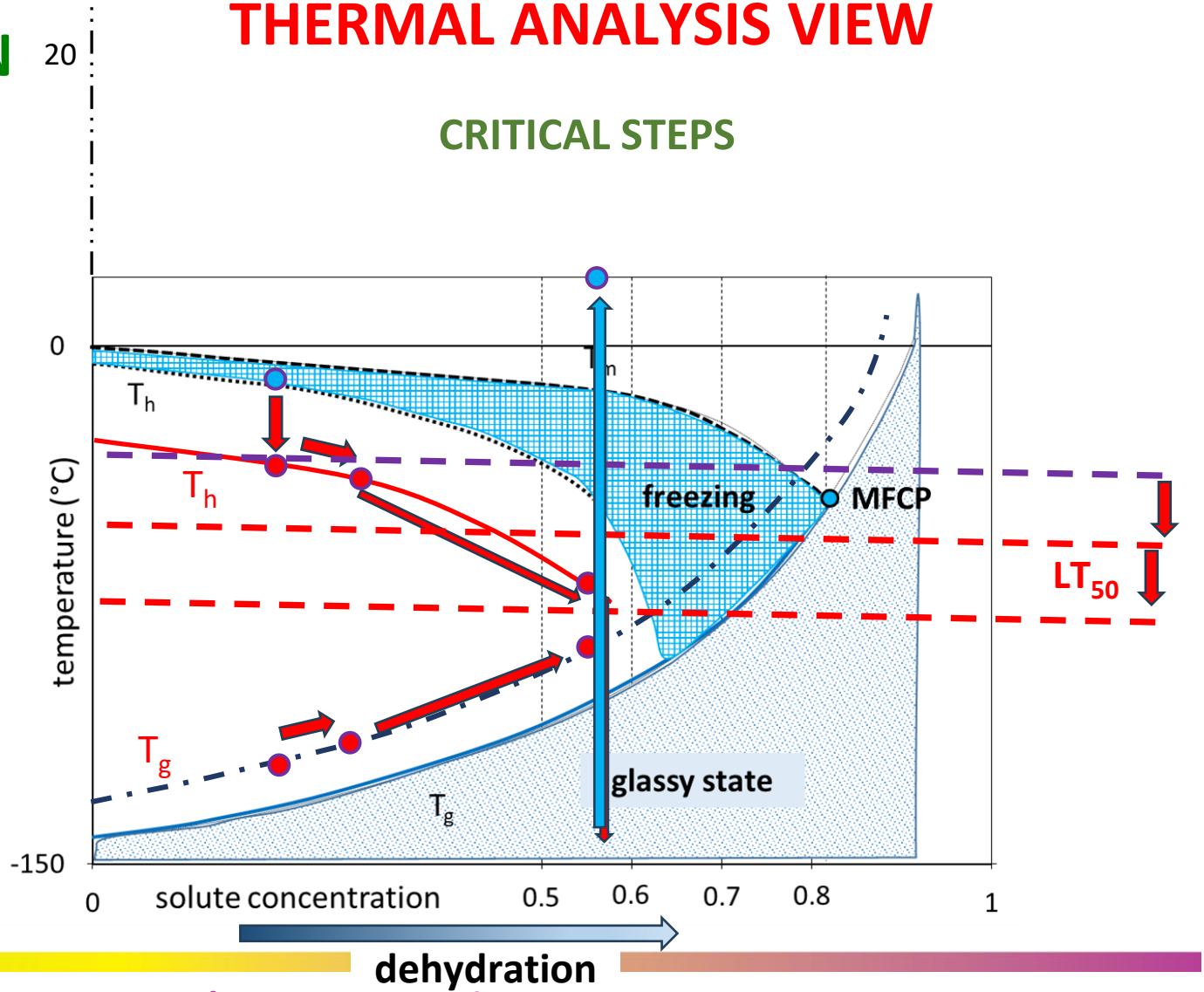
- **Warming** – rate, conditions

THERMAL ANALYSIS VIEW

CRITICAL STEPS



DORMANT BUD CRYOPRESERVATION

by two-step freezing method:

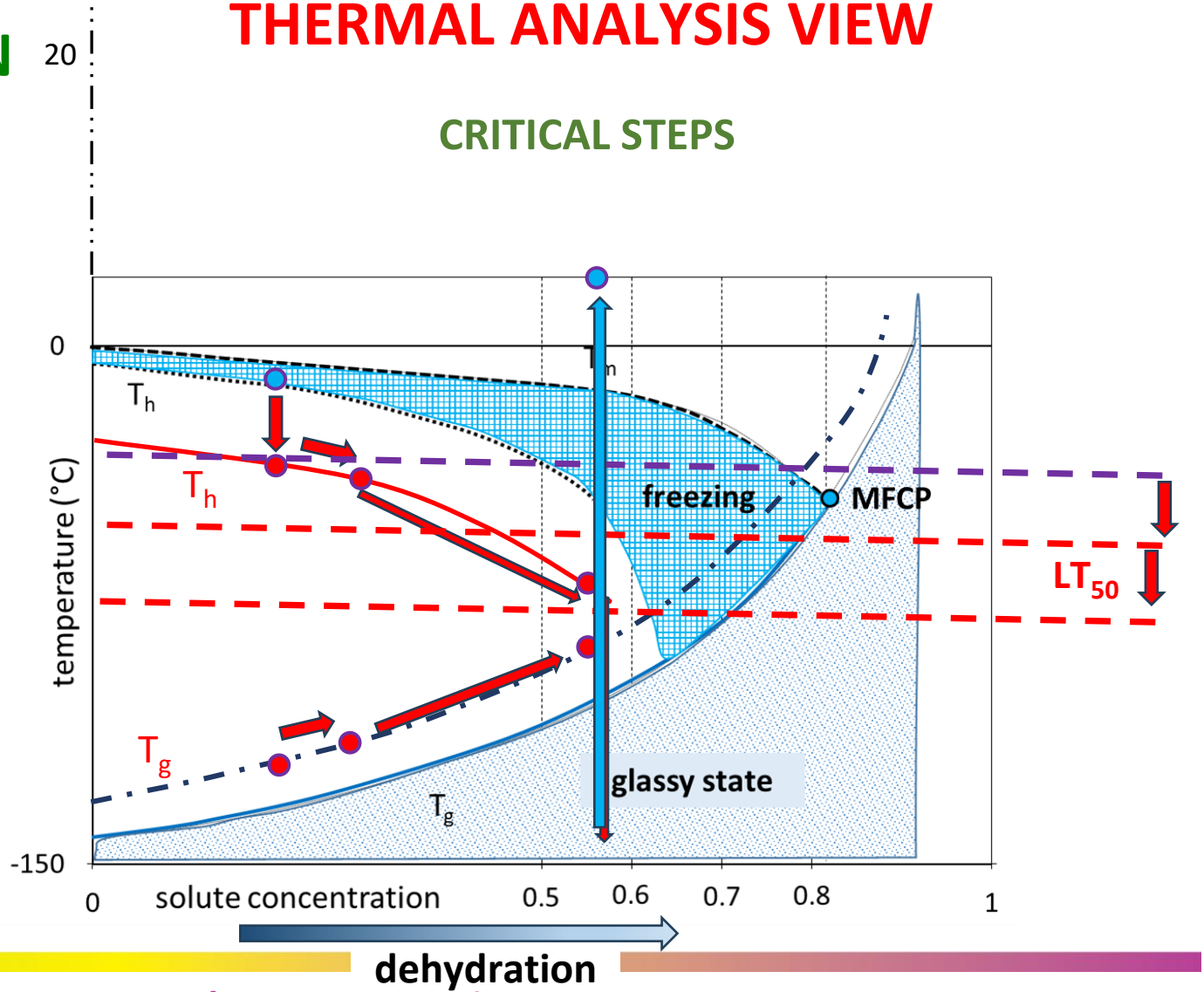
CRITICAL STEPS:

- **Acclimation** – temperature, collection period, dormancy, LT_{50}
- **Dehydration** – freezing temperature, period, final water content, LT50
- **Freezing** – controlled rate, final temperature, equilibrium
- **Glass transition** – fast cooling
- **Warming** – rate, conditions

- **Rehydration, grafting, recovery evaluation**

THERMAL ANALYSIS VIEW

CRITICAL STEPS



Thank you for your attention!