Why do we need research in Preservation Science?

Allison Hubel, PhD

Director, Biopreservation Core Resource
(www.biocor.net)

Biospecimens

Biofluids
- Plasma/serum
- BALF
- Ascites
- Urine
- Etc.

Cells

Tissues

Biomarkers

DNA
Genome

RNA
Transcriptome

Proteins
Proteome

Sugars

Nucleotides

Amino Acids

Lipids

Metabolome

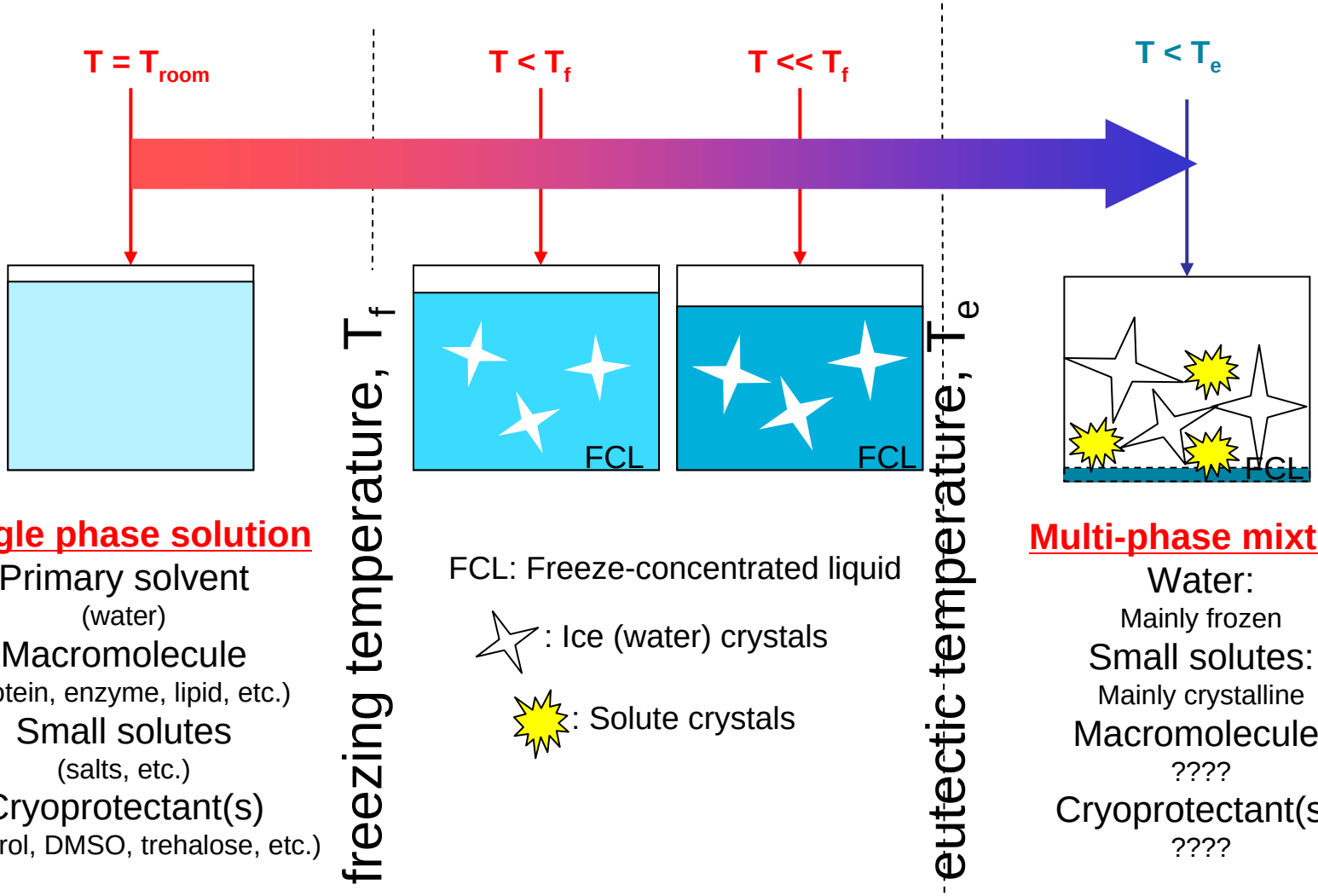
Candidates for stabilization

Isolated Biomarker Intact biospecimen

Myths about preservation

Myth #1: Preservation is a 'solved problem'. We have effective methods of preservation for all the biospecimens of interest

Stabilization of biomolecules



Single phase solution

Primary solvent
(water)
Macromolecule
(protein, enzyme, lipid, etc.)
Small solutes
(salts, etc.)
Cryoprotectant(s)
(glycerol, DMSO, trehalose, etc.)

FCL: Freeze-concentrated liquid

: Ice (water) crystals

: Solute crystals

Multi-phase mixture

Water:
Mainly frozen
Small solutes:
Mainly crystalline
Macromolecule:
????
Cryoprotectant(s):
????

Stabilization of biomolecules

DNA, proteins

- Most highly studied
- Basic stability strategies have been developed

RNA

- Much less stable than DNA
- Commercial product: RNA later

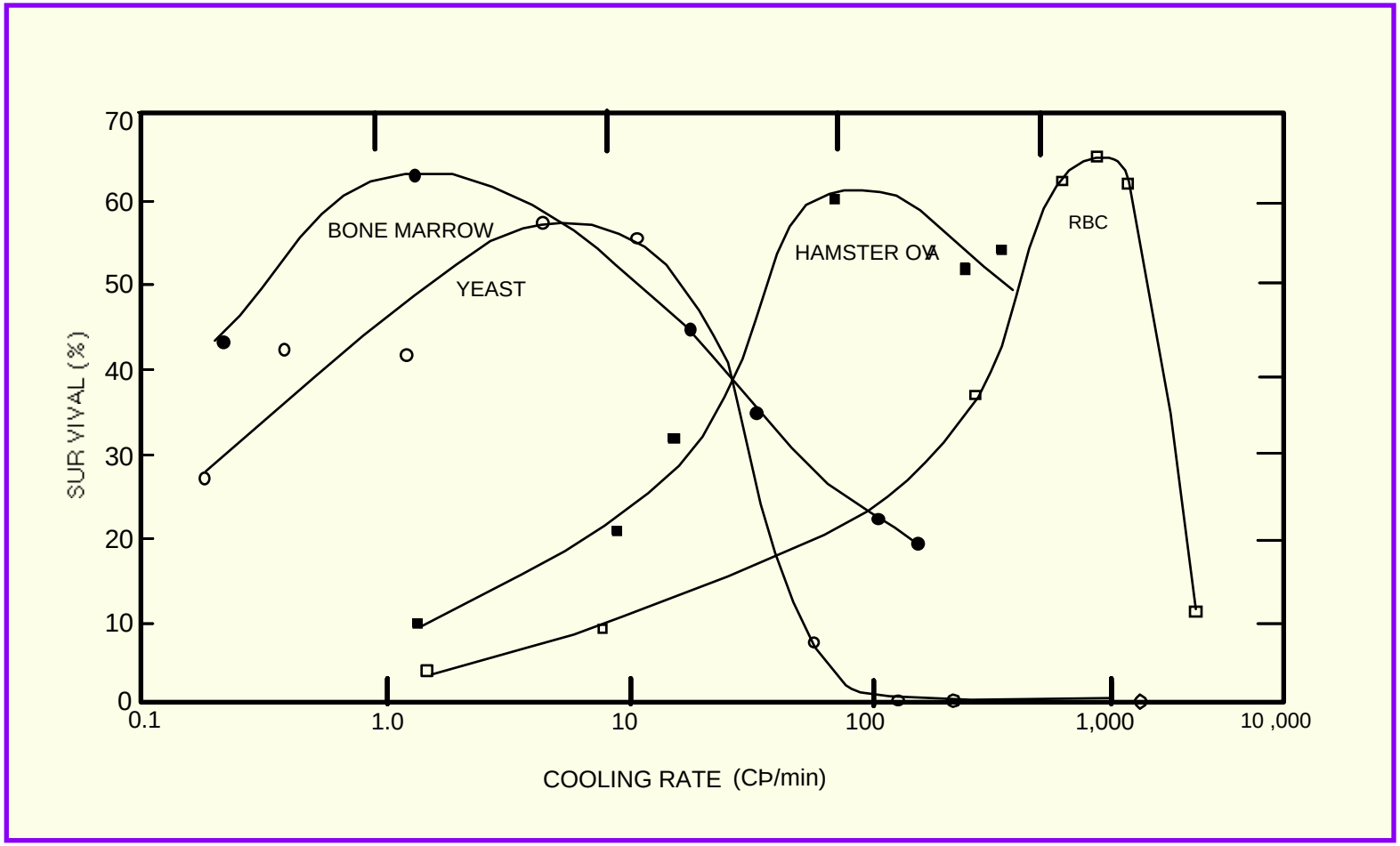
Lipids, amino acids, sugars, nucleotides

- Lipid stability studied only in association with cell membrane

Common issues

- Cold denaturation
- Molecule/ice interactions
- Molecule/molecule interactions
- Effects of co-solutes
- Effect of cooling rate
- Influence of pH shifts

Stabilization of cells



Stabilization of cells

Many cells cannot be preserved effectively: platelets, granulocytes, hESCs, iPS cells, gametes, etc.

We do not understand why certain cells can be effectively preserved and others not

We do not understand why survival of the same cell type varies from species to species

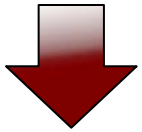
➔ We cannot develop scientific protocols to preserve cells refractive to current approaches

Stabilization of tissues

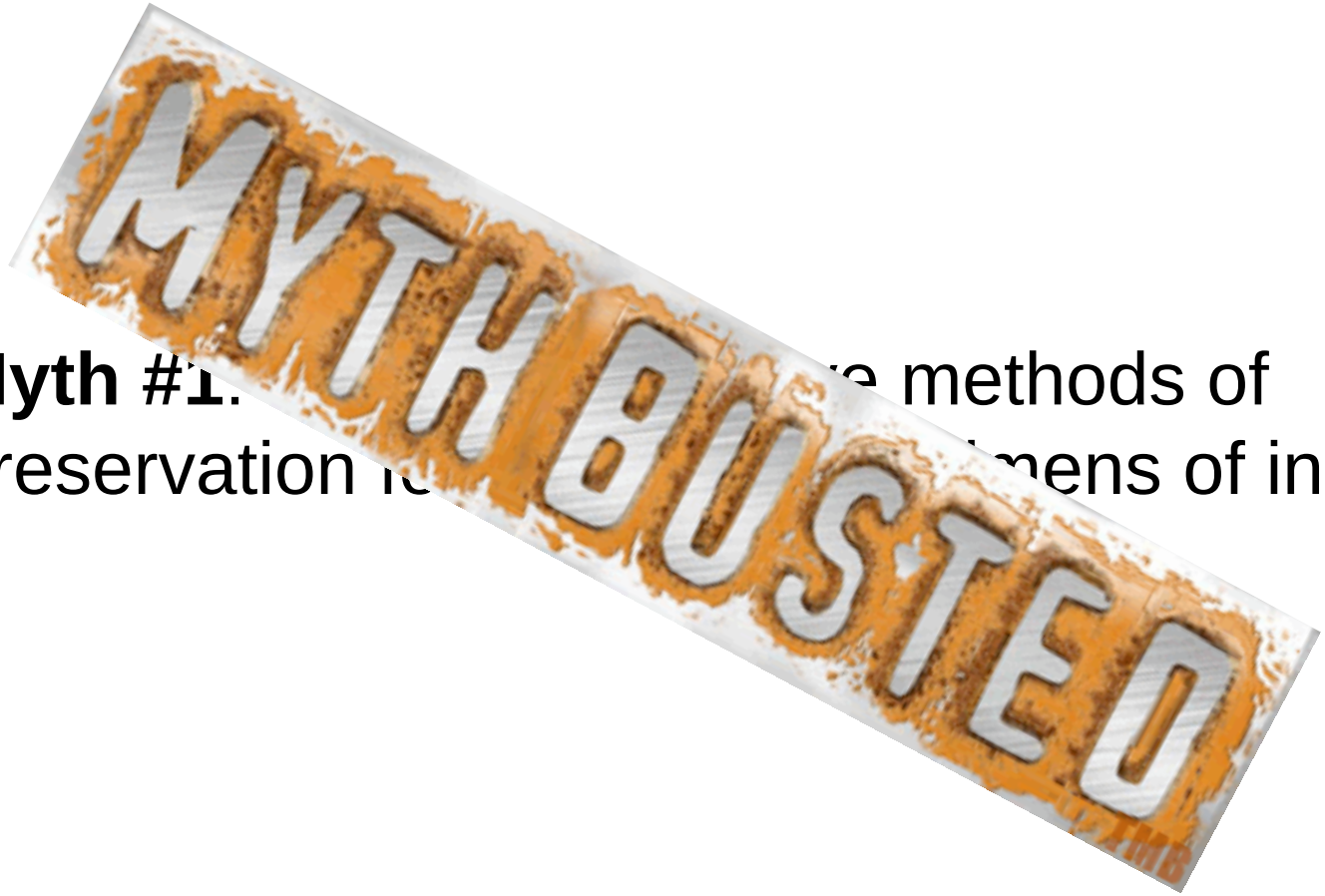
Viability:

- We can preserve islets of langerhans and achieve post thaw function
- Preserving other tissue involves preservation of scaffold with little post thaw cellular function
- Cells isolated from a tissue survive much more readily than cells embedded in a tissue

Biomarkers:
Genes and proteins can change significantly within 30 mins of ischemia



We simply cannot preserve tissues and organs and we do not understand why



Myth #1: e methods of preservation re mens of interest

<u>Myth #2</u>*:* *Conventional methods of preserving biospecimens are suitable for new and emerging applications*

DMSO as a stabilization agent

Most cellular biospecimens are preserved with DMSO

DMSO has well documented toxicity (cellular and infusion)

DMSO is also associated with epigenetic events and may irreversibly denature macromolecules

➔ Alternative stabilizing agents are needed

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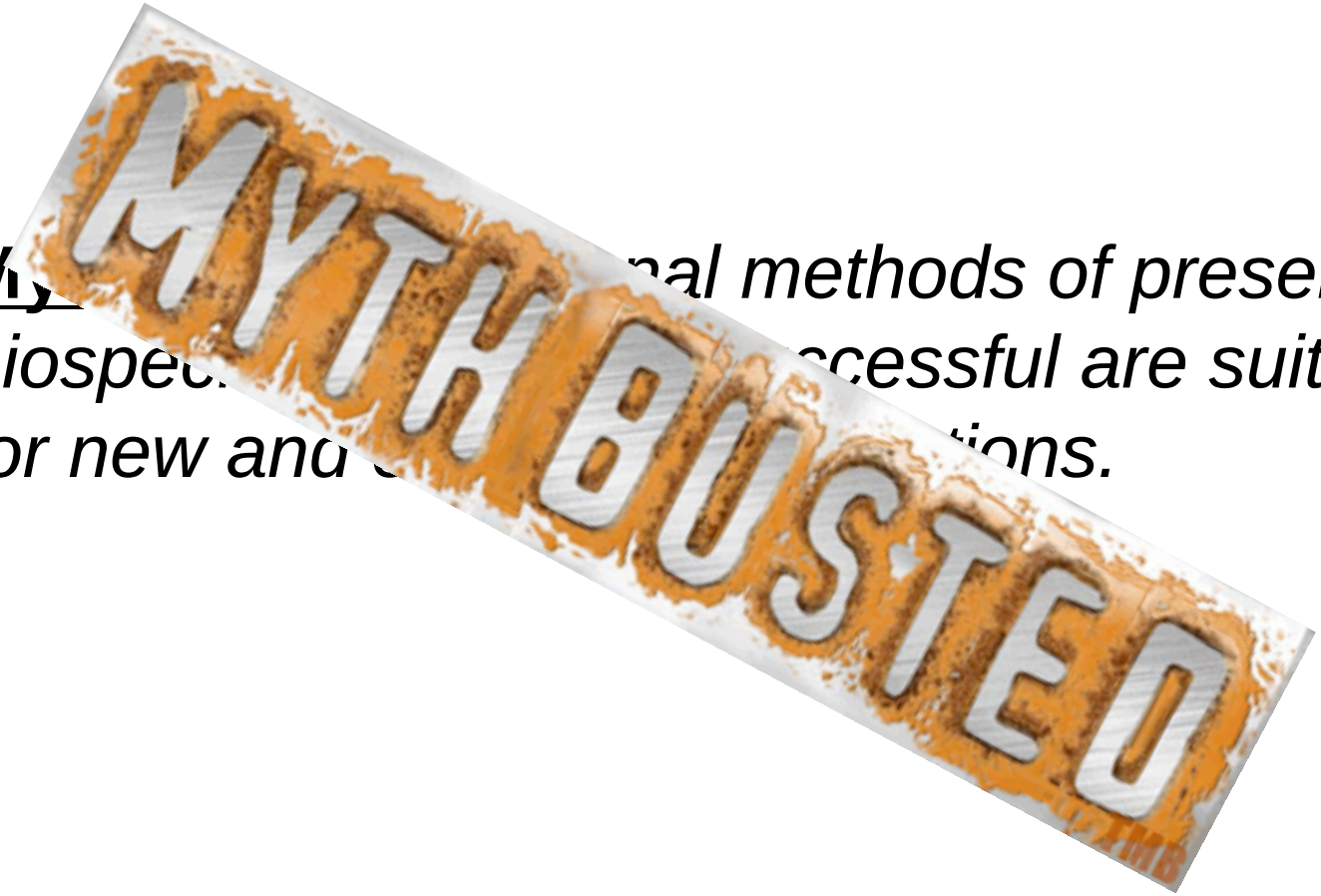
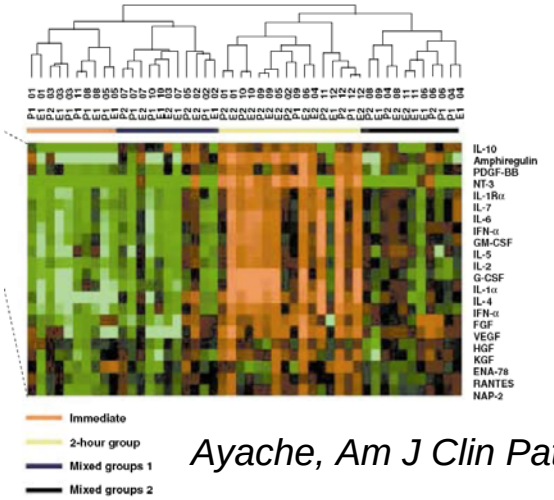
nal methods of preserving biospec[illegible]ccessful are suitable for new and [illegible]ions.



<u>Myth #3</u>*: All biospecimens are equal**

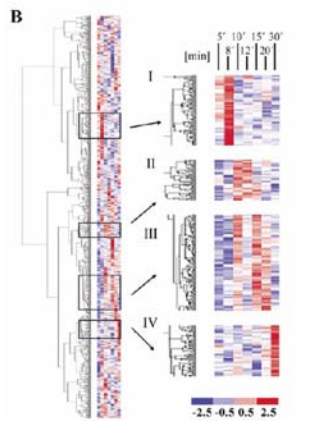
**well some are more equal than others…..*

Rapid changes in biomarkers



Ayache, Am J Clin Path, 174, 2006.

- Whole blood stored at RT for 2 h
- 35 different factors differed between the two samples
- Protease inhibitors had little effect
- Effects were cell mediated



By 15 min: 10%–15%
By 30 min: 20%
Detectable genes and proteins differed significantly from the baseline values.

Spruessel, BioTechniques, 36:1030, 2004

Biospecimen procurement
(e.g. pre-analytical variables)

Controlling and specifying pre-analytical variables is important.

But is it sufficient??

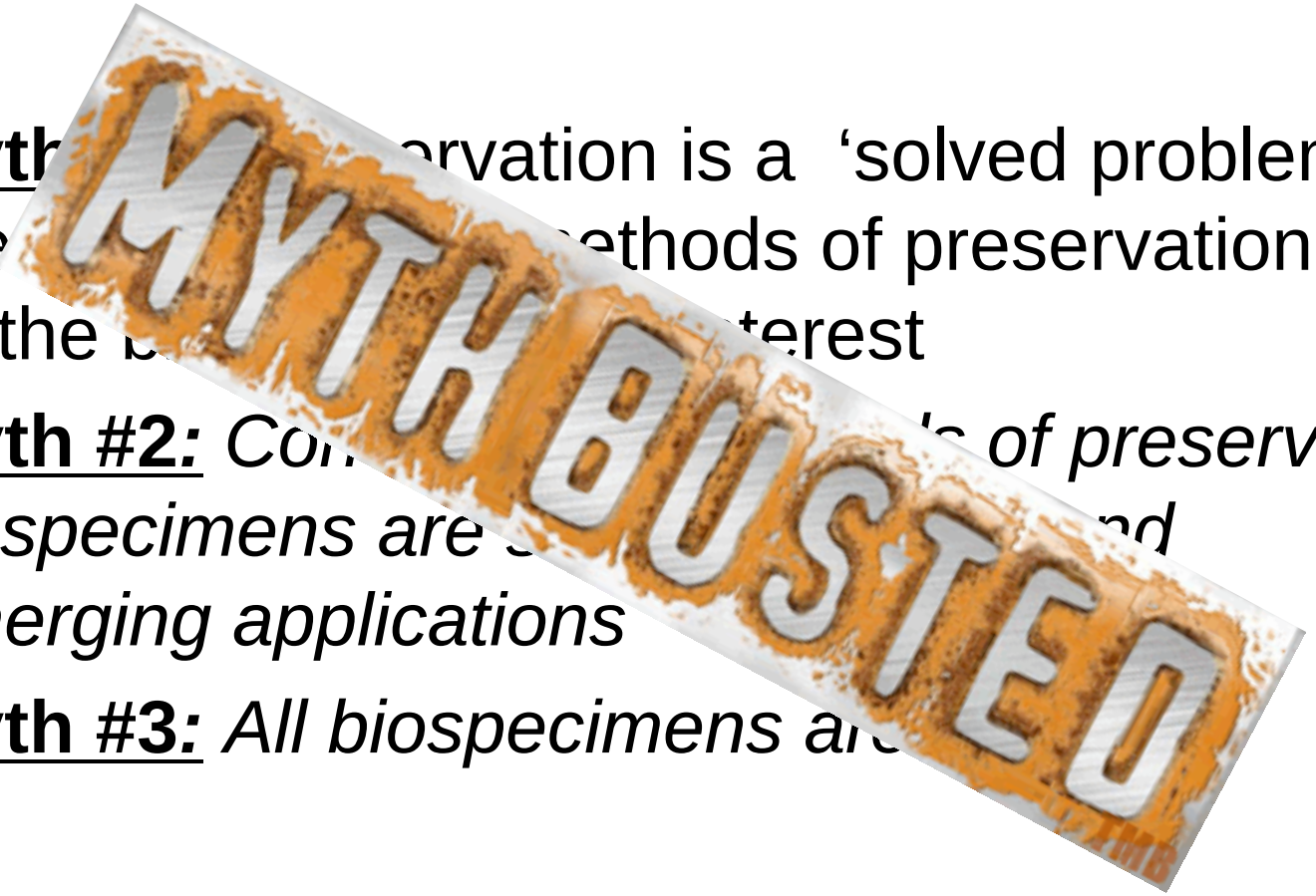
Other elements of the protocol are also important (cooling rate, storage conditions, etc).

Conundrum: setting the bar too high has its downsides.

Myth rvation is a ‘solved problem”. We ethods of preservation for all the b terest

Myth #2: *Co of preserving biospecimens are nd emerging applications*

Myth #3: *All biospecimens ar*



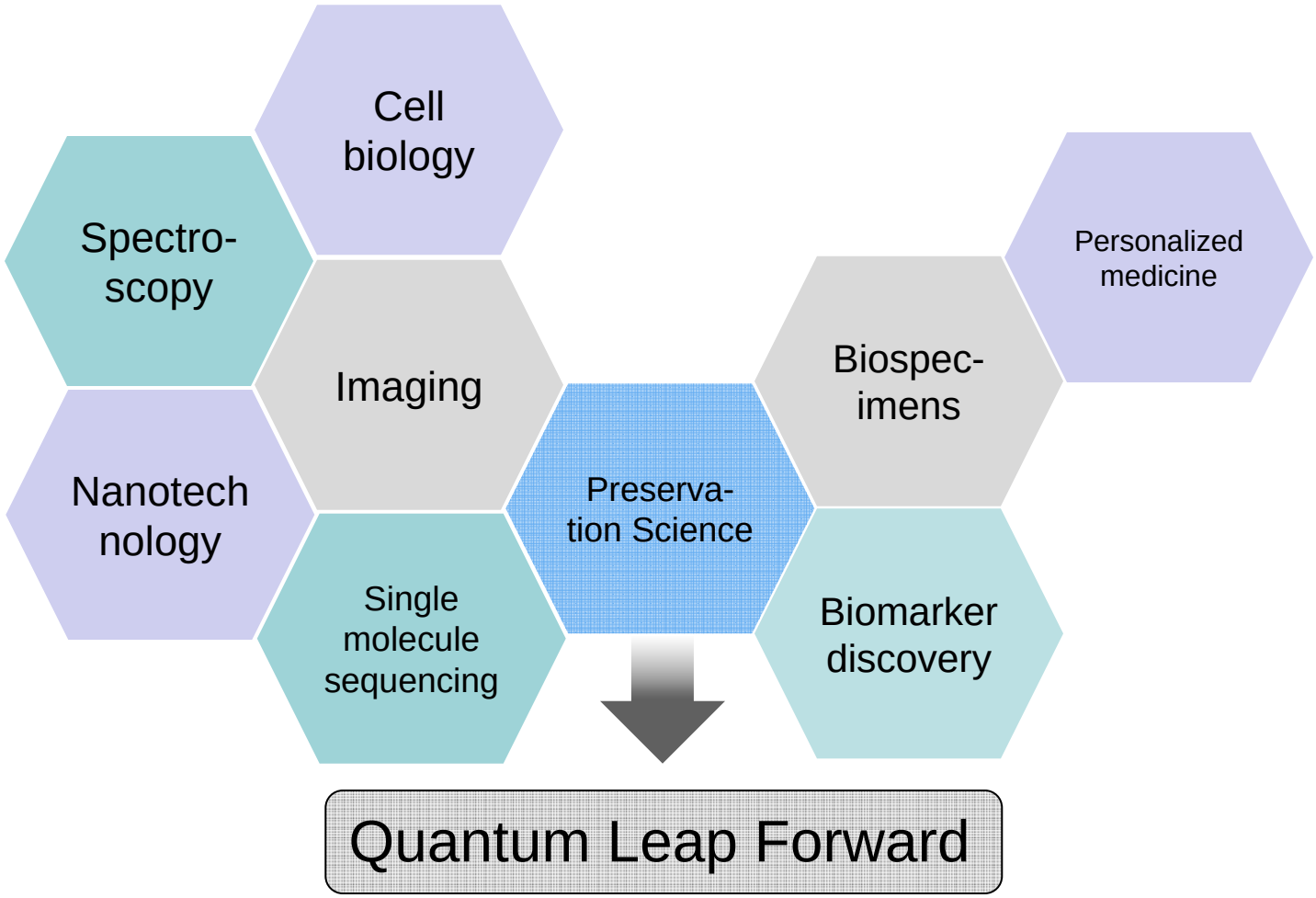
Freezer Farms

Millions of samples are in storage (with more added every day)

What (if any) usefulness do these specimens have?

Advancing preservation science

Advancing preservation science: driving forces



How do we advance preservation science?

Education

Preservation is not a 'cold black box'

<u>Understanding current scientific principals is critical to:</u>

- Improving/harmonizing biospecimen quality and best practices
- Filling in the gaps in our knowledge

Education

Preservation of molecular, cellular and tissue biospecimens

May 18-20, 2010 Minneapolis, MN

Topics covered:

Liquid storage of biospecimens.
Fundamentals of cryopreservation
Protocol development
Quality systems
Clinical cell cryopreservation
Repository design
Tissue preservation
Preservation of biomarkers
Regulatory issues for cell/tissues

Lecturer

Allison Hubel, University of Minnesota
Charles Lee, U of North Carolina, Charlotte
Ian Pope, CoreCryolab, Toronto
Amy Skubitz, University of Minnesota
Fran Rabe, University of Minnesota
Alptekin Aksan, University of Minnesota
David McKenna, University of Minnesota
Diane Kadidlo, University of Minnesota

This course has been endorsed by ISBER

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Service

A series of ‘little things’ may make the difference

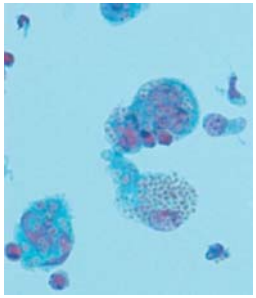
➔ Ask the BioCoR expert

➔ Have BioCoR develop a protocol for your biospecimen

Research

Bronchoalveolar Lavage Fluid

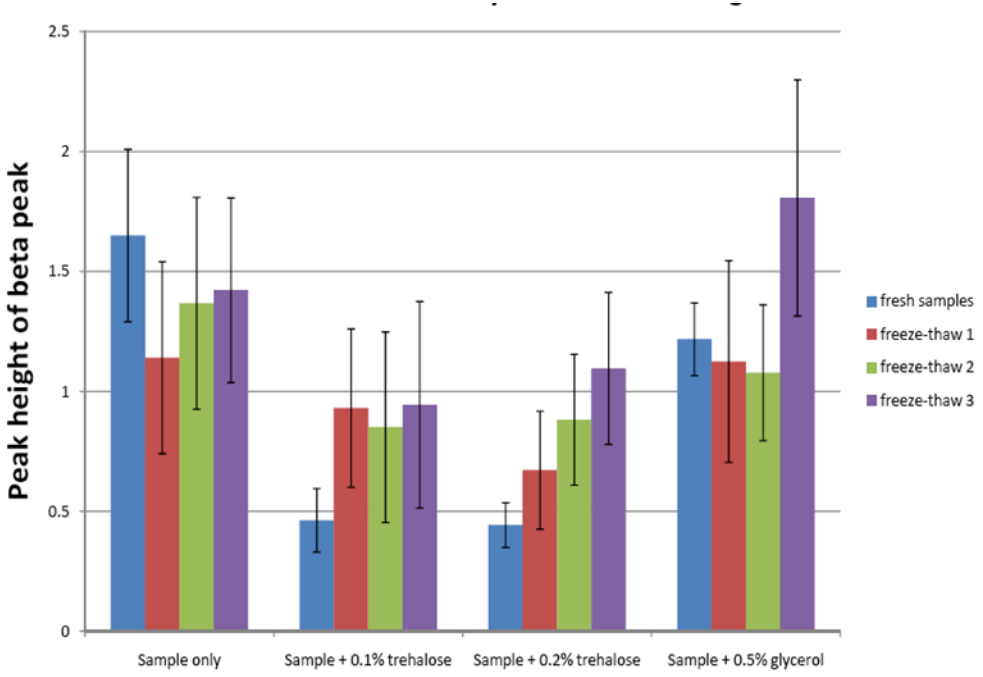
- In contact with affected organ
- Potential biomarkers identified: proteins, cells, lipids
- Banked but not used for diagnosis monitoring treatment



Alveolar macrophages
J. Resp. Dis, 29:1, 2008.

Integrated team: preservation scientists, biomedical researchers searching for biomarkers and clinicians collecting biospecimens

Influence of freezing on protein structure



BALF samples were analyzed using FTIR

Condition: fresh, frozen, repeated FT cycles

Data: height of β-peak

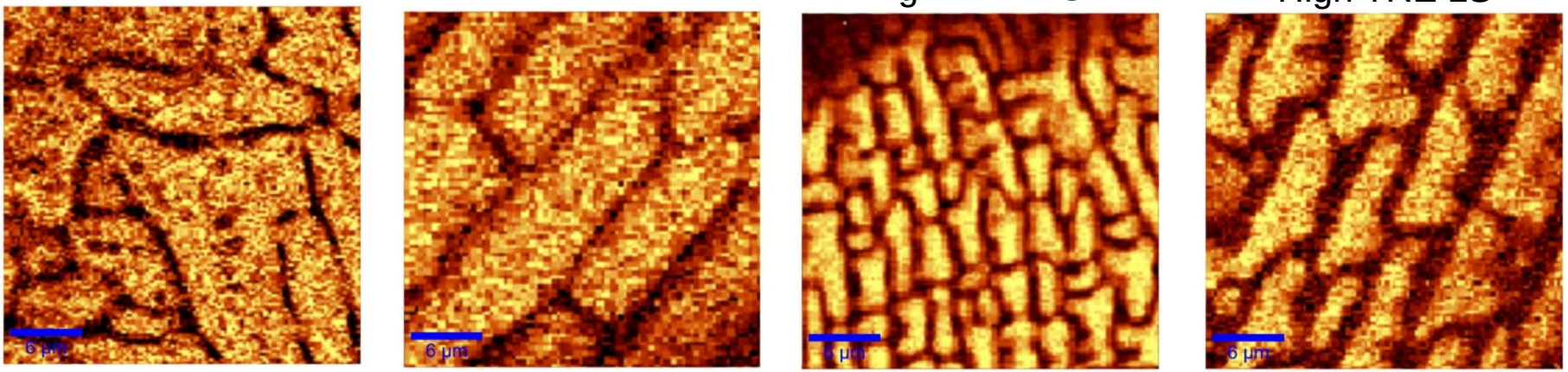
Stabilizers: trehalose and glycerol

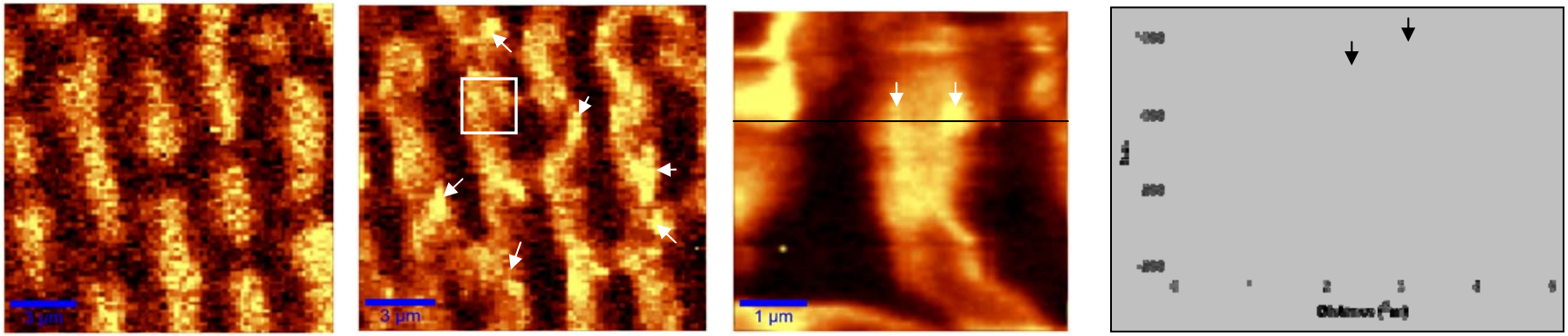
- Proteins in frozen samples exhibit higher levels of β-sheet
- Trehalose/glycerol reduced changes in proteins
- Repeated freeze thaw cycles influenced protein in sample

Molecular Mechanisms of Protein Damage

Microcompartmentalization in Frozen Protein Solutions

Low TRE HS | Low TRE LS | High TRE HS | High TRE LS





Ice
FCL

Ice
LYS+TRE

Ice
LYS+TRE

Dong, Hubel, Bischof, Aksan
"Freezing-Induced Phase Separation and Spatial Microheterogeneity in Protein Solutions"
J. Phys. Chem. B *published online on 07/02/09* DOI: 10.1021/jp809710d

Acknowledgments

Funding:
Academic Health Center
Medical School
Institute of Technology
Medical Device Center
Institute for Engineering
in Medicine

Collaborators:
Alptekin Aksan
Chris Wendt

Jinping Dong
Xiao Zhong