

Subzero Organ Preservation: What We're Learning and What This Could Mean for Organ Utilization

TODAY'S PRESENTER



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Continuing Education Information

Evaluations & Certificates

Nursing

The Organ Donation and Transplantation Alliance is offering **1.0 hours of continuing education credit** for this offering, approved by The California Board of Registered Nursing, Provider Number CEP17117. No partial credits will be awarded. CE credit will be issued upon request within 30 days post-webinar.

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- Certificates should be claimed within 30 days of this webinar.
- We highly encourage you to provide us with your feedback through completion of the online evaluation tool.
- Detailed instructions will be emailed to you within the next 24 hours.
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- Group leaders, please share the follow-up email with all group participants who attended the webinar.



Deanna Fenton

Senior Manager, Program
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786-866-8730

Meet Our Moderator



Greg Veenedaal DNP, MS, RN, CCRN-K, NEA-BC

Director of Organ Clinical Services

LifeSource

ORGAN, EYE AND TISSUE DONATION



Meet Our Presenter



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Division of Transplantation



Cryobiology to Stabilize the Donor Organ

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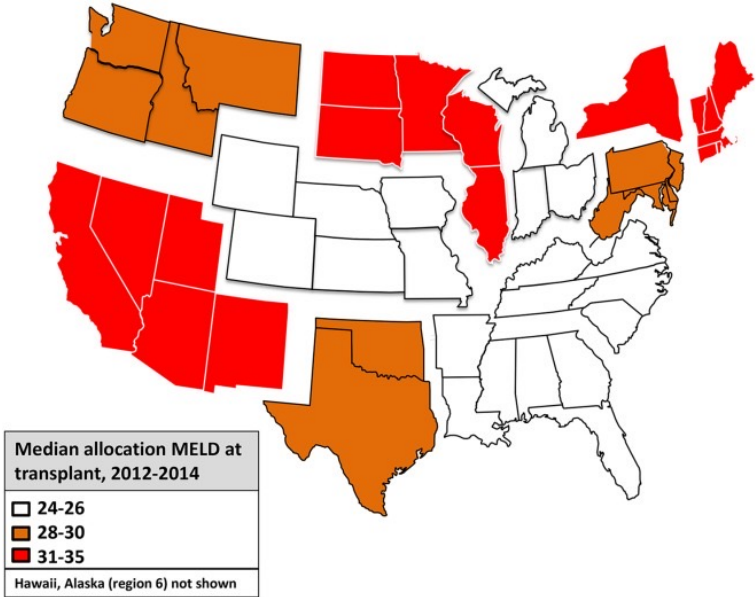
April 27, 2023

Disclosures: Existing patents on this technology

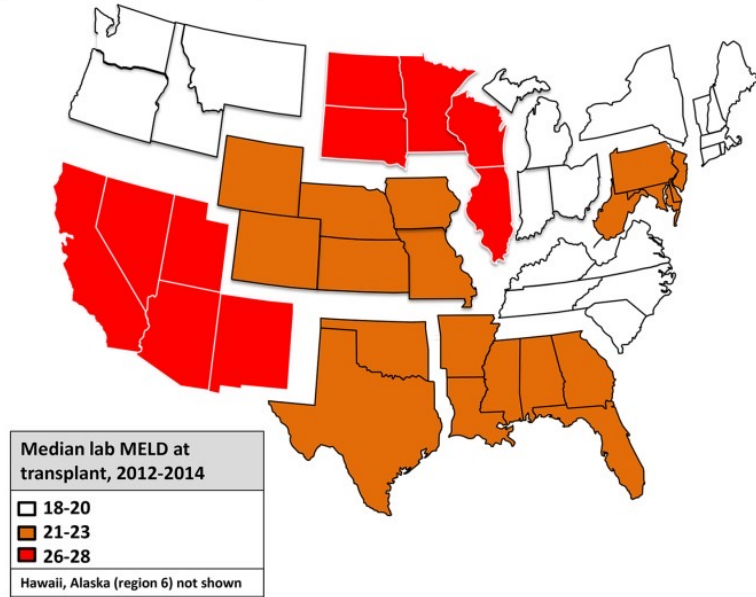


Geographic disparity in organ access reflects imbalance of supply and demand

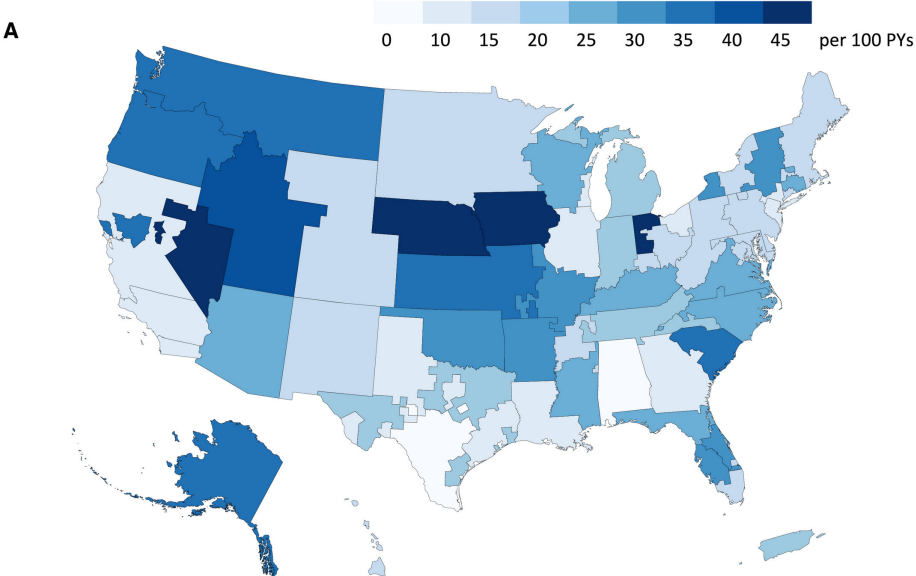
Median allocation MELD



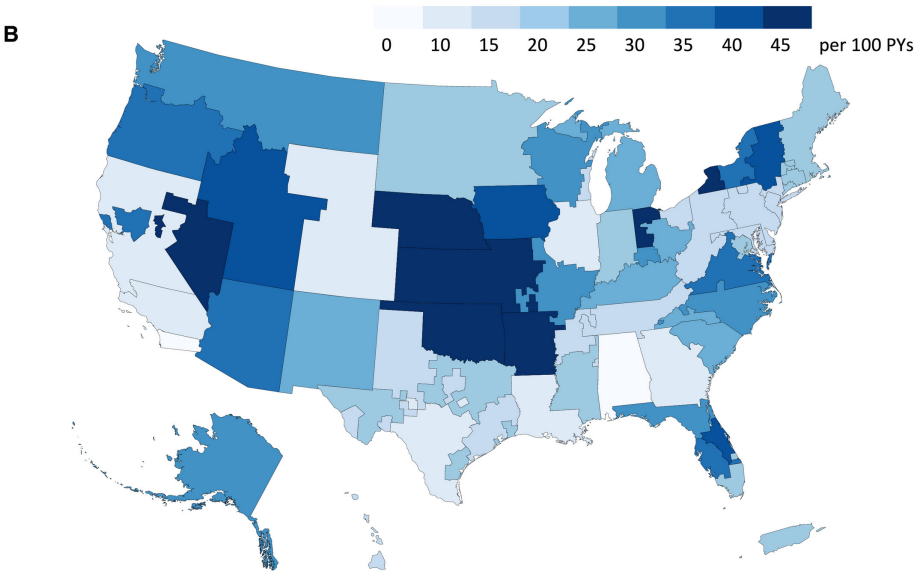
Median lab MELD



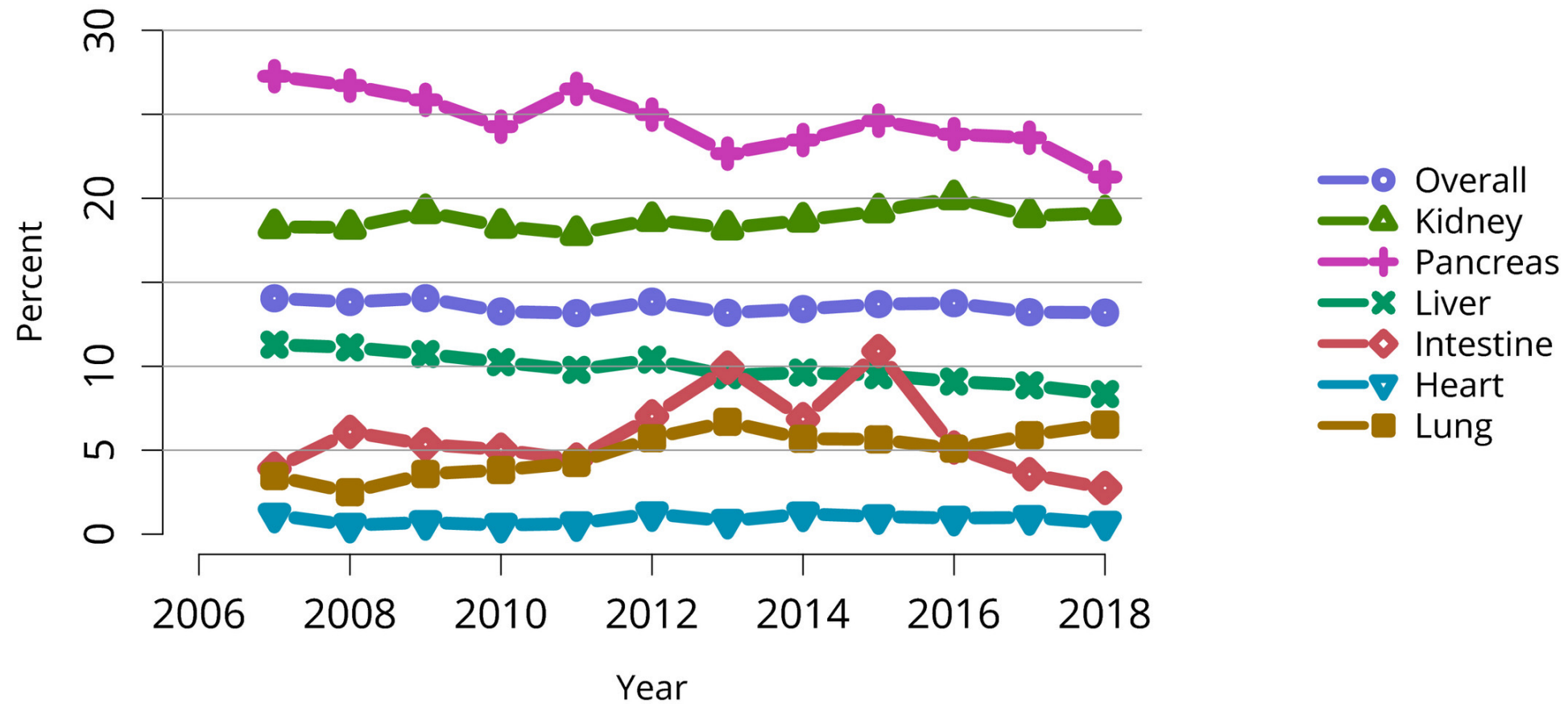
Pre-KAS transplant rate



Post-KAS transplant rate

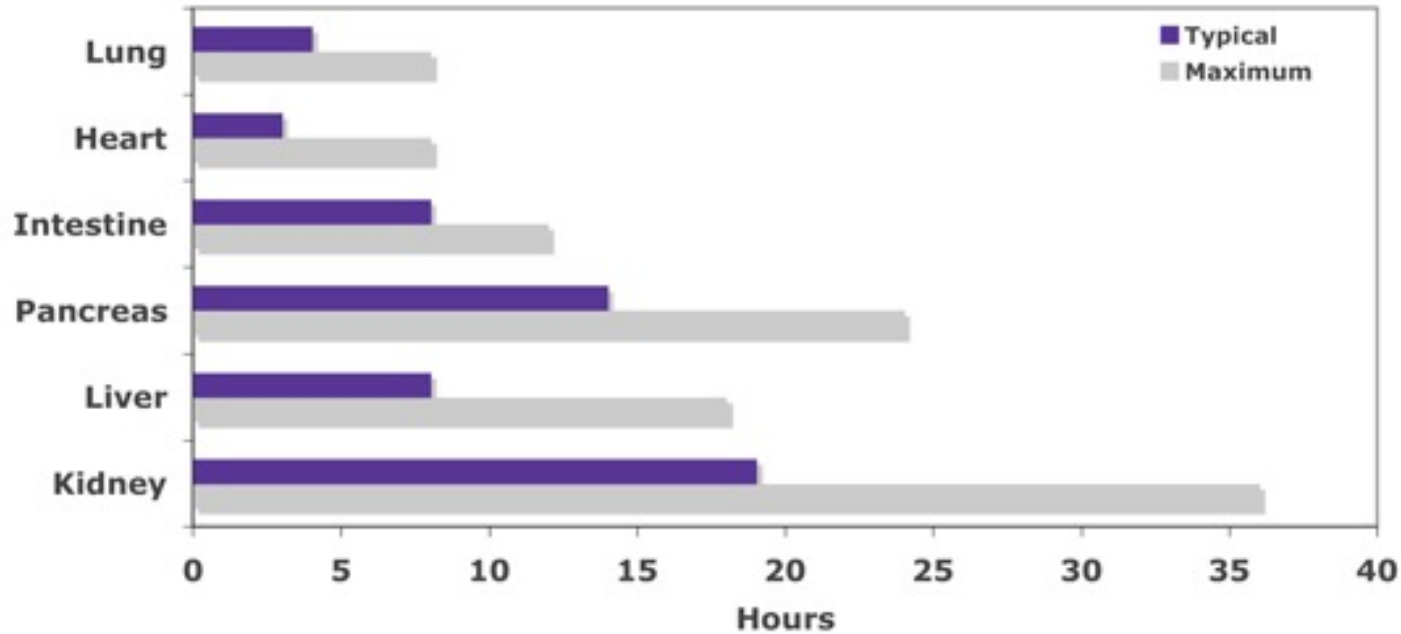


Organs recovered but not transplanted — a missed opportunity

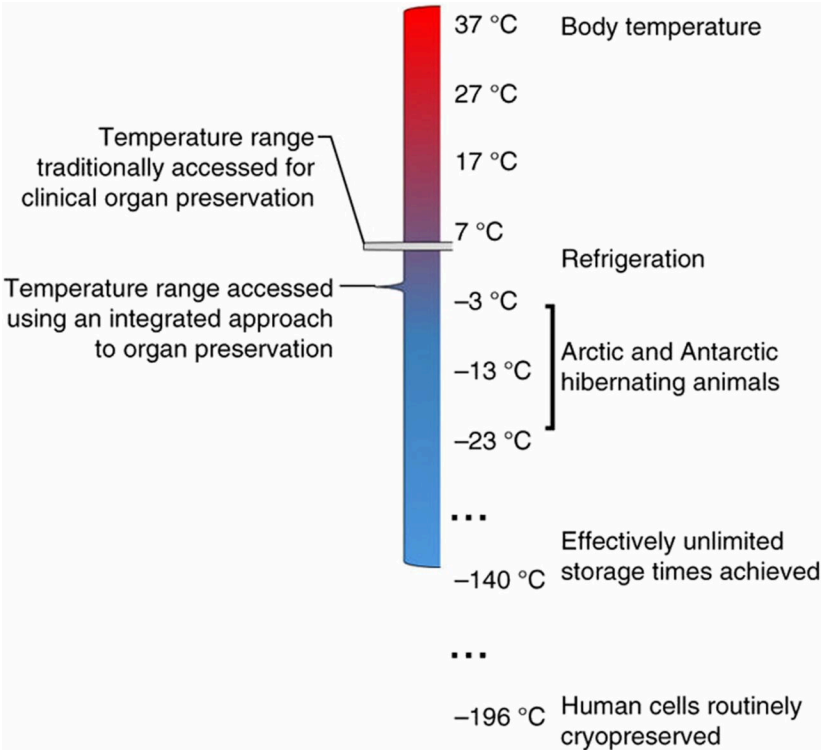


Standard static cold storage results in short acceptable preservation times

Typical and Maximum Preservation Times for Donated Organs



Tissue preservation: pick your temperature to change preservation limits



What would increasing preservation time mean?



Cold storage

- Time limited event
- Local allocation
- Regional sharing

Normothermic perfusion

- National sharing
- Daytime transplants

Partial freezing/ High subzero

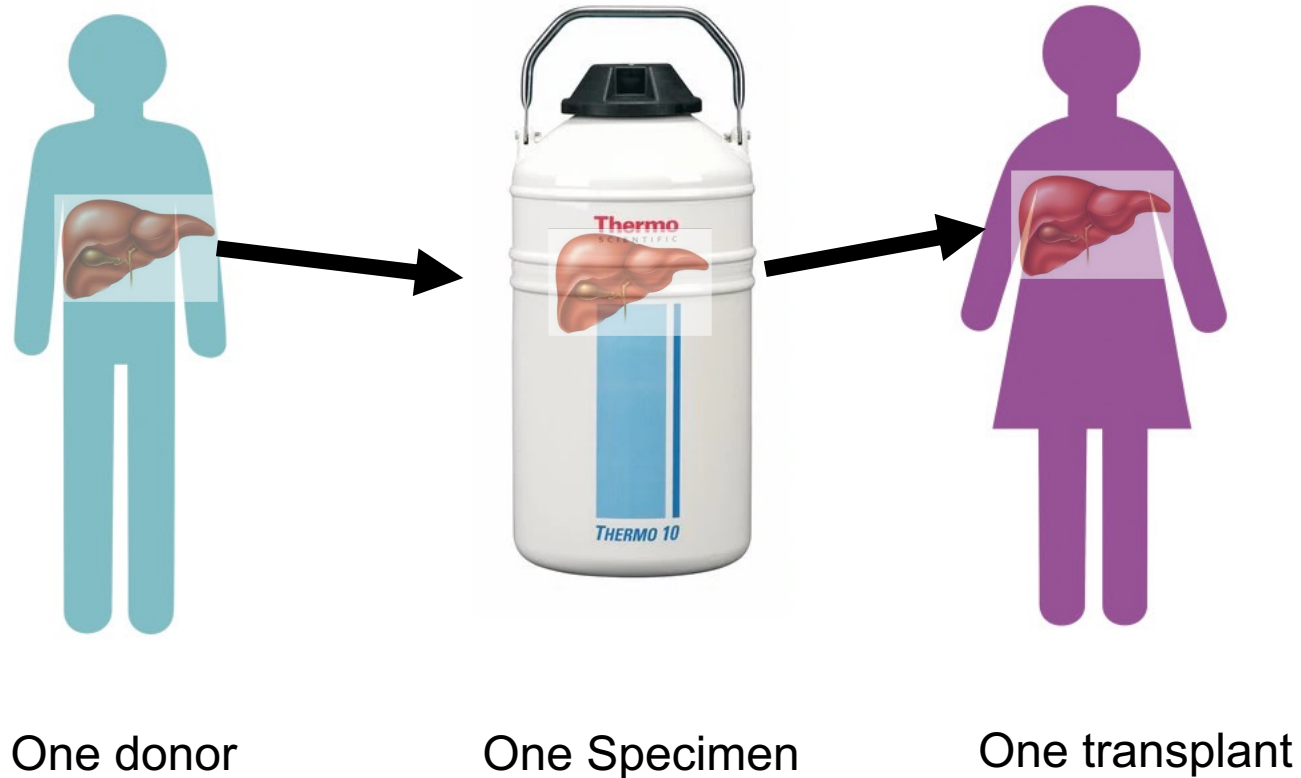
- International sharing
- Some tolerance protocols
- Improved patient preparation

Cryopreservation

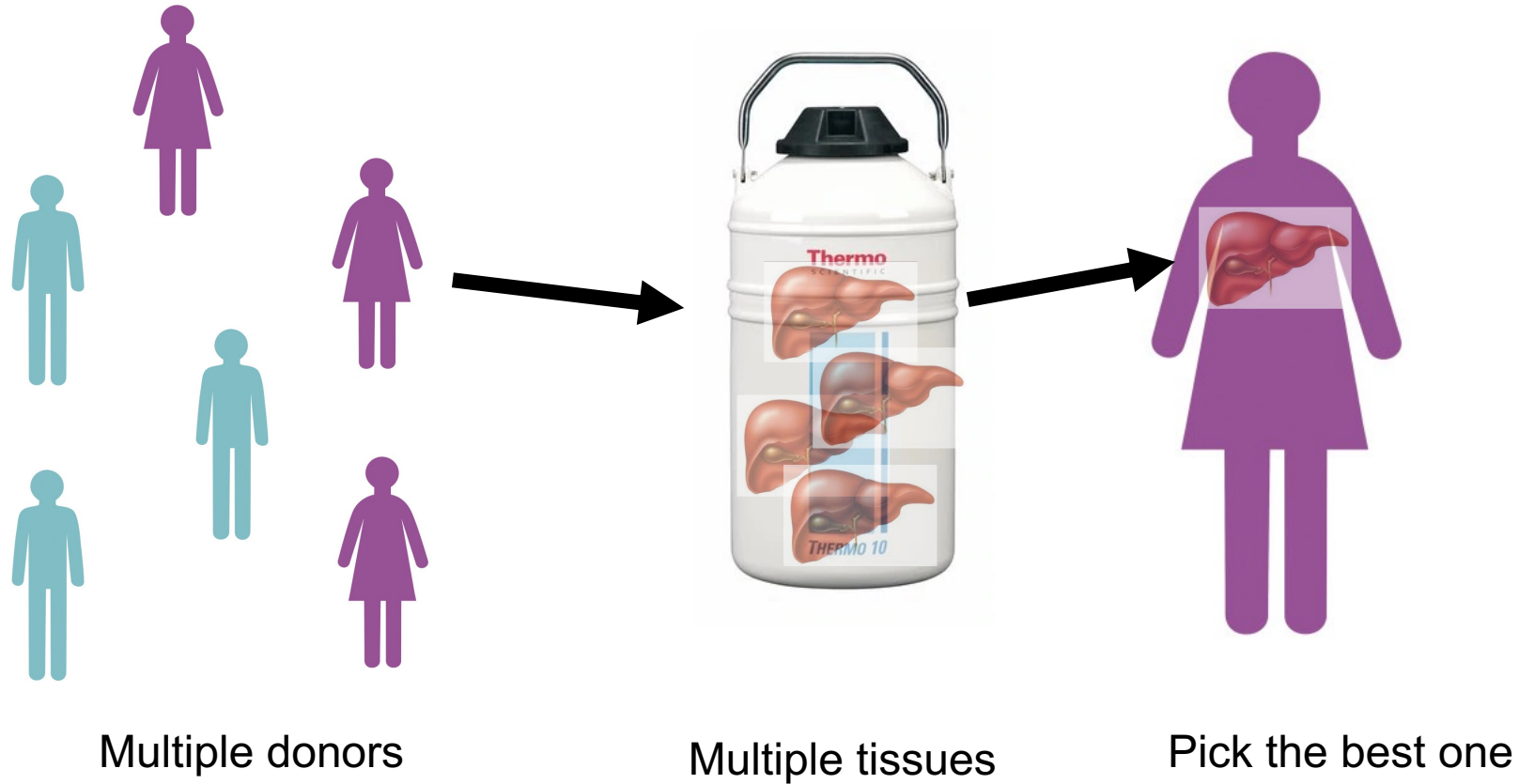
- Donor/recipient matching
- Tolerance protocols
- Elective procedures
- On demand organ supply (true organ bank)



Cryopreservation of organs for transplant



Organ banking:



Ice is the enemy -- how to avoid it, or, how to manage it



Rana sylvatica – wood frog

They can stay for weeks in a dormant state until spring when they breathe and resume



Freeze tolerance in nature – strategies for ice avoidance

Portal fish – antifreeze proteins



Wood frog – accumulate urea



Tardigrade (water bear) – dehydration



Molecular Adaptations Supporting Freeze Tolerance

- ### Hypometabolism & stress response
- Prepare by acquiring sufficient fuel reserves for long term survival without feeding
 - Lower metabolic rate to 1-30% of normal resting rate
 - Coordinate suppression of ATP-expensive cell functions: e.g. transcription, translation, cell cycle, active transport
 - Suppress enzyme function by post-translational modifications
 - Epigenetic controls: e.g. DNA methylation, histone modifications, microRNA inhibition of mRNA transcripts
 - Up-regulate cytoprotective mechanisms: e.g. chaperone proteins, antioxidant defense, anti-apoptosis, innate immunity defenses
 - Stress-specific gene/protein expression & protein/enzyme regulatory modifications

- ### Anoxia Tolerance
- Optimize anaerobic ATP production
 - Minimize development of acidosis
 - Anoxia-specific gene/protein/enzyme controls

- ### Dehydration Tolerance
- Endure wide changes in cell & body water content
 - Manage aquaporins & other solute carriers
 - Accumulation of urea or other osmolytes for volume regulation
 - Dehydration-specific gene/protein/enzyme controls

- ### Adaptations for Cold Hardiness
- Carbohydrate cryoprotectants
 - Antifreeze proteins & glycolipids
 - Membrane phospholipid changes
 - Cold-specific gene/protein/enzyme controls

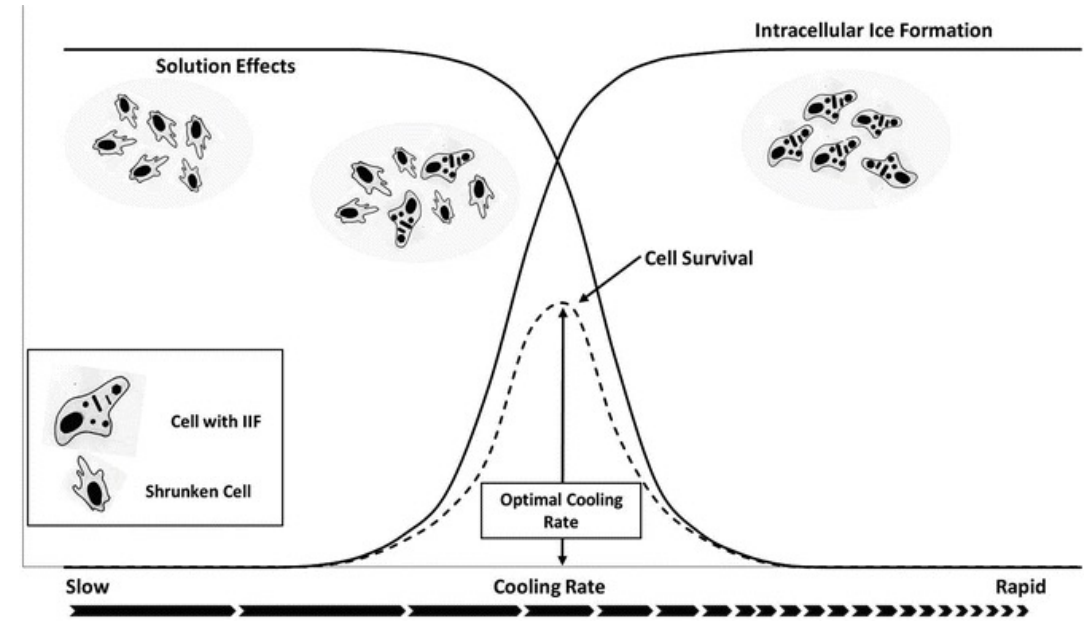
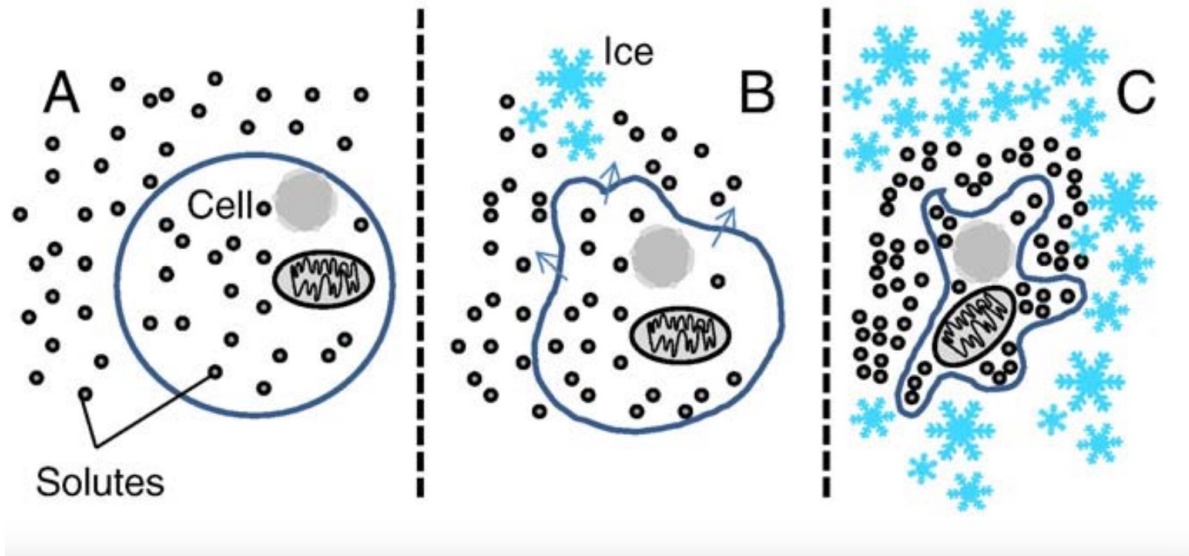
- ### Adaptations Unique to Freeze Tolerance
- Restrict ice to extracellular spaces & inhibit recrystallization
 - Initiate ice nucleation at high subzero temperatures
 - Ice binding proteins & novel proteins
 - Ischemia-tolerance & controls to halt/restart vital functions over freeze/thaw
 - Freeze-specific gene/protein/enzyme controls



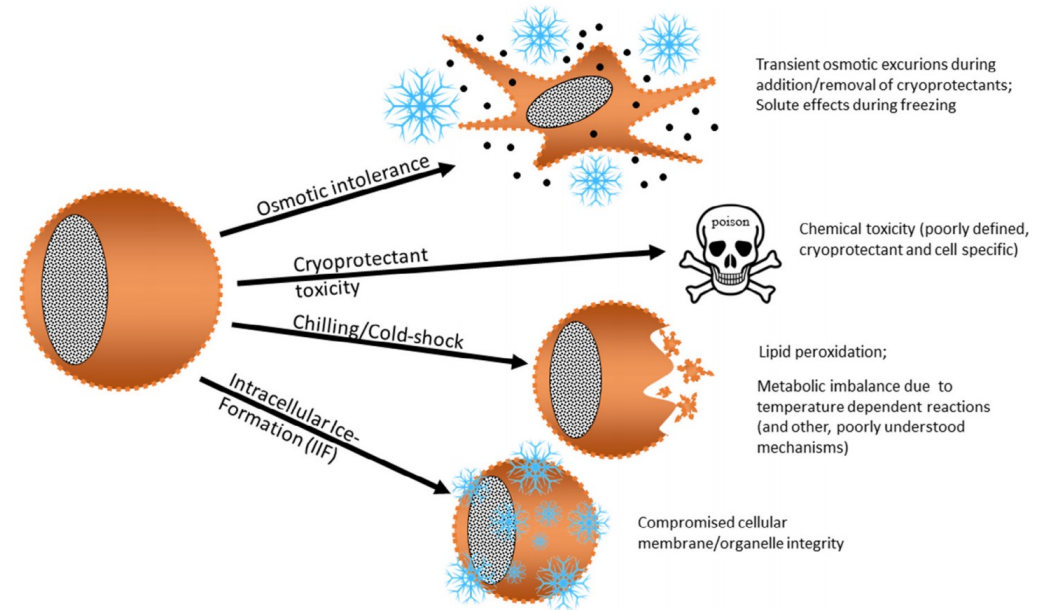
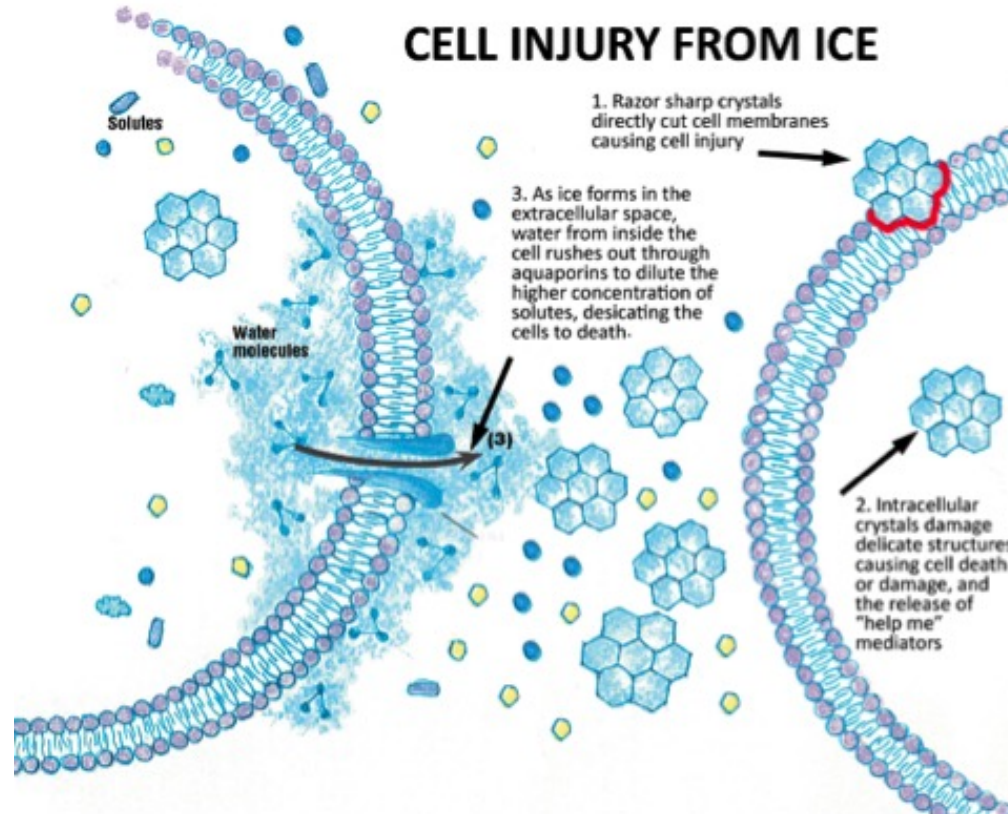
Freeze Tolerance



Conventional cryopreservation by slow cooling results in extracellular ice



Conventional cryopreservation leads to cell injury



Limitations of conventional cryopreservation



Works for cells in suspension and small aggregates (embryos)



Ice still forms – cell injury occurs



Macroscopic destruction of tissue architecture

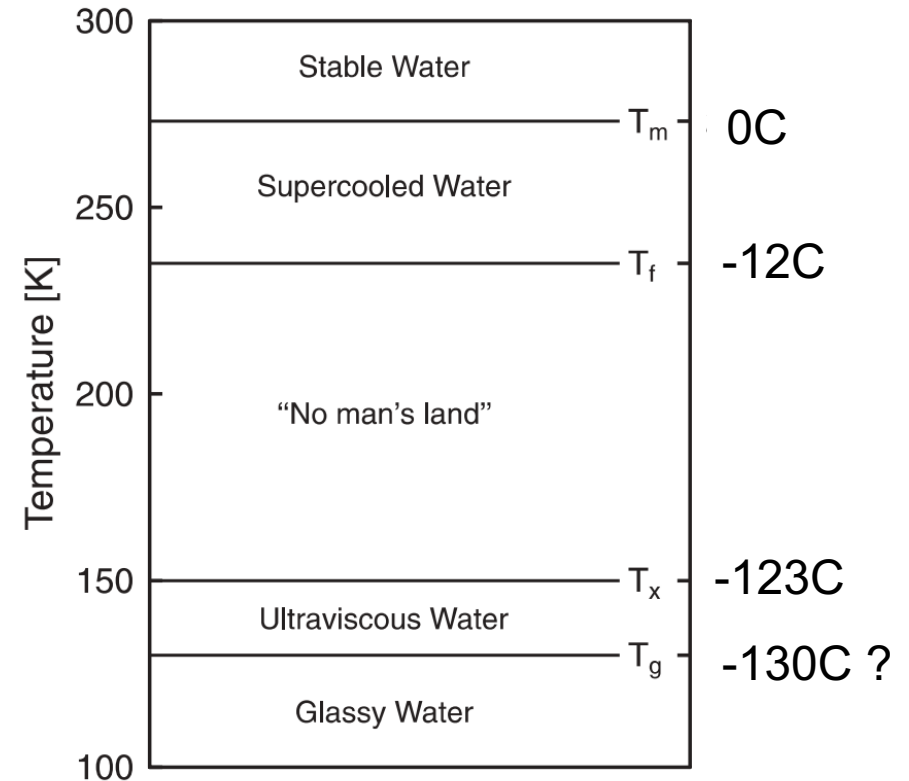
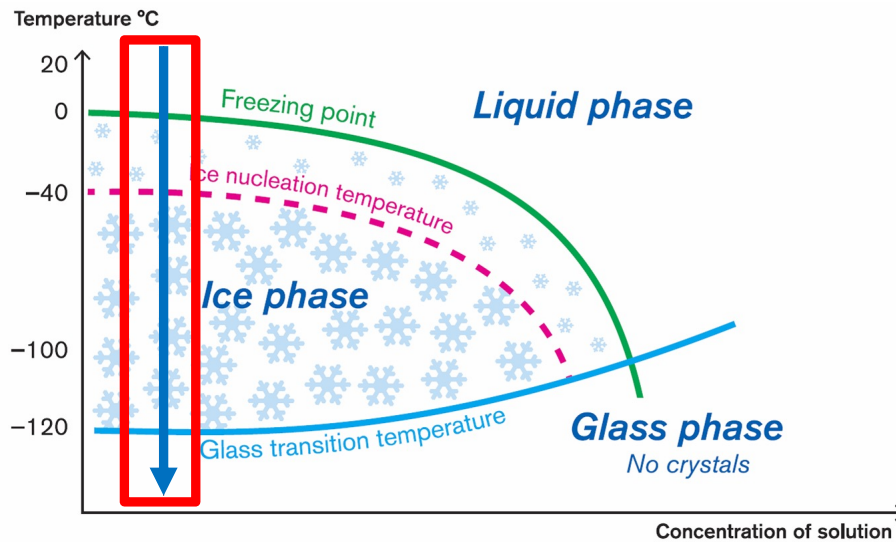


Some cells and tissues don't tolerate cryoprotective agents (CPA, ie, DMSO)

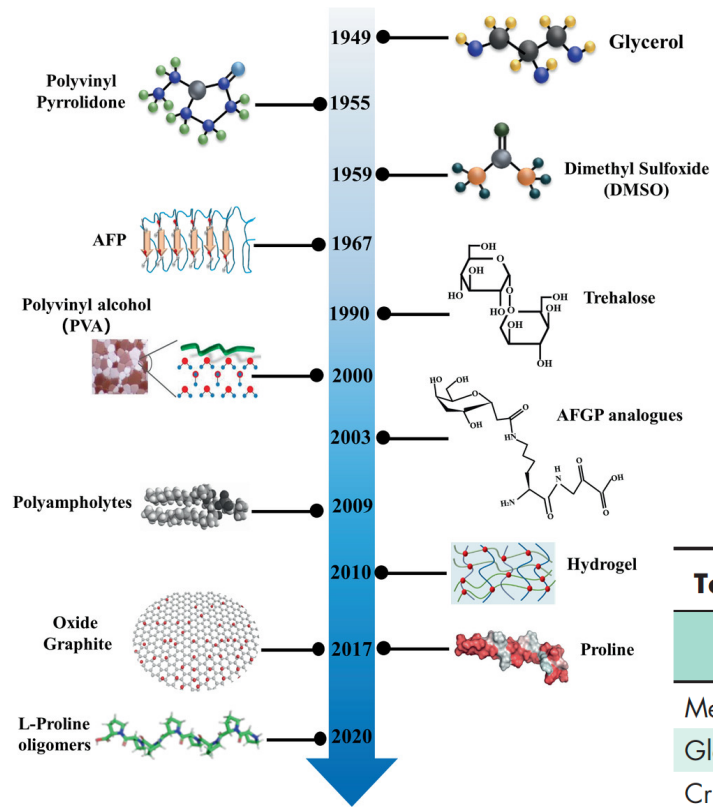


Fails in larger tissues and organs

Vitrification (from Latin vitreum, "glass" via French vitrifier) is the transformation of a substance into a glass, that is to say a non-crystalline amorphous solid.



Development of CPAs



Permeating agents	Nonpermeating agents	
	Small molecules	Sugars
<ul style="list-style-type: none"> • Dimethyl sulphoxide • Ethylene glycol • Propylene glycol • Glycerol • Methanol • Ethanol • Glycine betaine 	<ul style="list-style-type: none"> • Sucrose • Trehalose • Raffinose • Mannitol • Glucose • Galactose 	<ul style="list-style-type: none"> • Polyethylene glycol • Polyvinyl pyrrolidone • Hydroxy ethyl starch • Ficoll • Serum proteins (mixture) • Milk proteins (mixture)

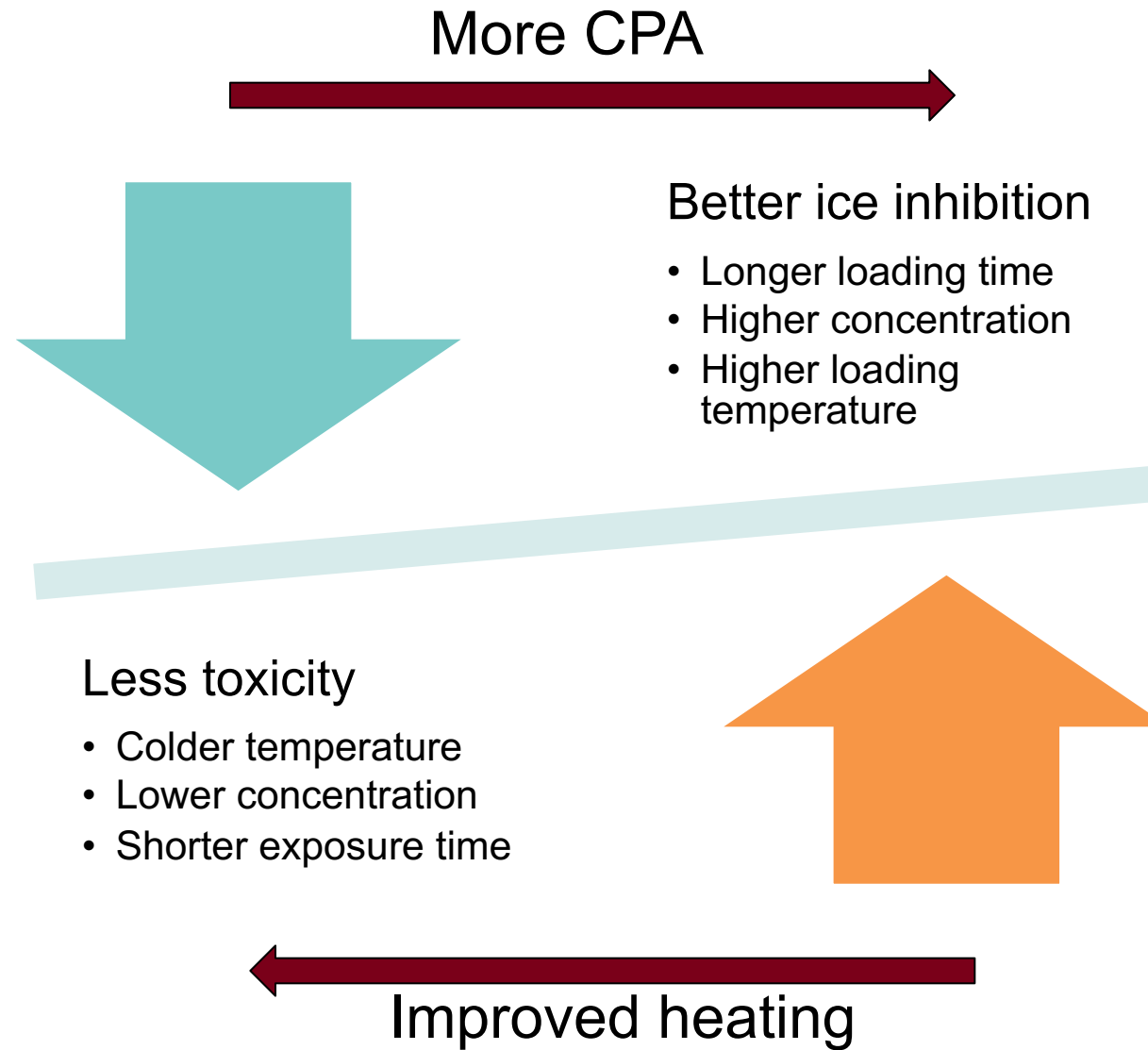
Table 1. Physical properties of cryoprotective agent cocktails^a

	6M Glycerol	DP6	VS55	M22 (VS 22)
Melt temp (T_m)	-26°C	-29.8°C	-38°C	~ -59°C
Glass transition (T_g)	~ -100°C	-119°C	-123°C	~ -122°C
Critical cooling rate	85°C/min	~40°C/min	2.5°C/min	0.1°C/min
Critical warming rate	3.2 x 10 ⁴ °C/m	~200°C/min	50°C/min	0.4°C/min
Concentration (mol/l)	6	6	8.4	9.3

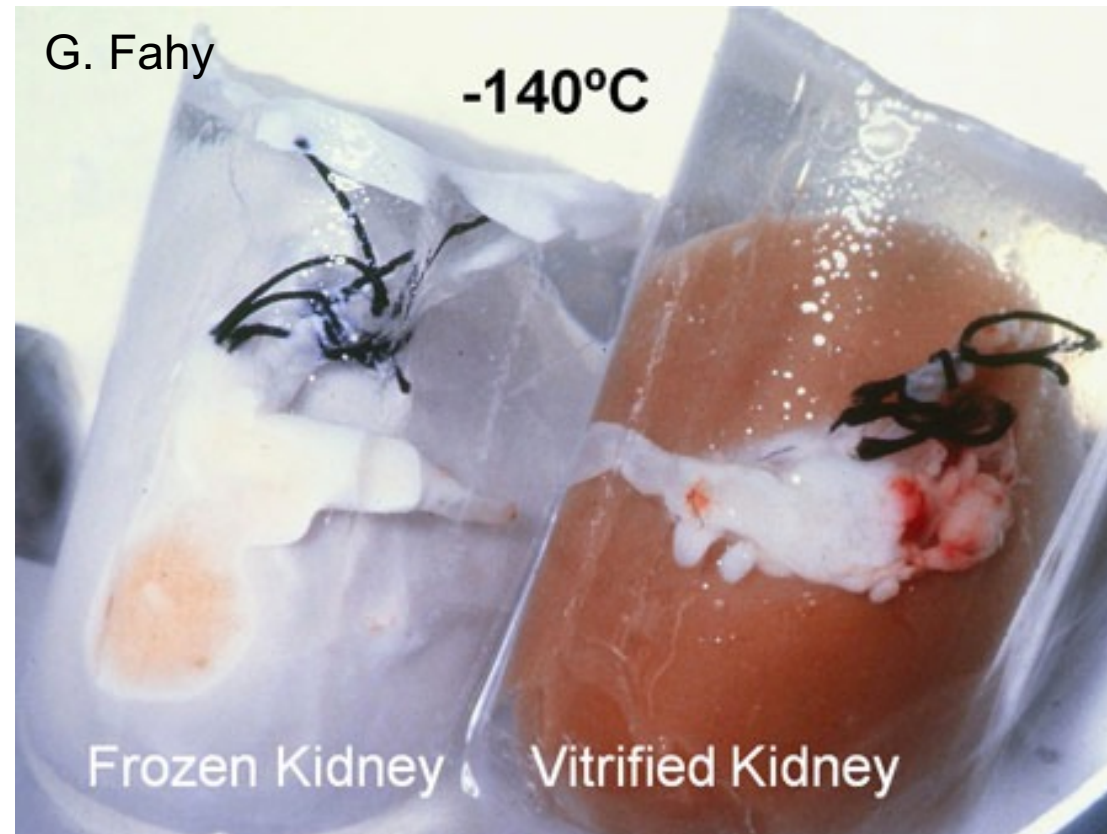
^aReferences: [17,18,58].



Balancing CPA toxicity v. efficacy



Vitrification (from Latin vitreum, "glass" via French vitrifier) is the transformation of a substance into a glass, that is to say a non-crystalline amorphous solid.



Rewarming Large Systems is Still a Problem



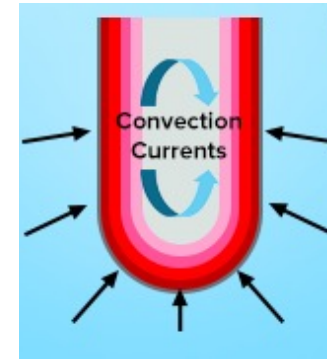
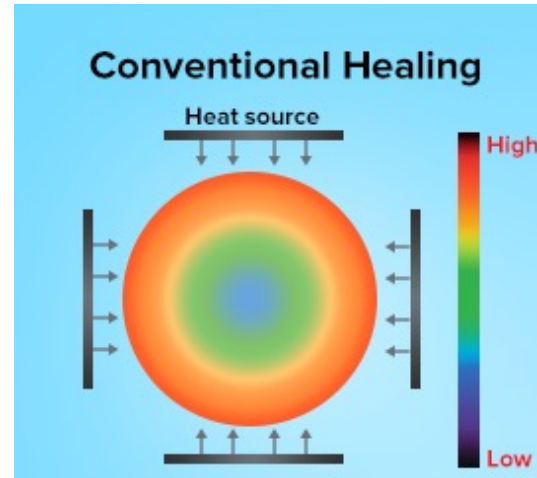
But to successfully warm we need...

Speed up warming

Uniformly warm to avoid cracks

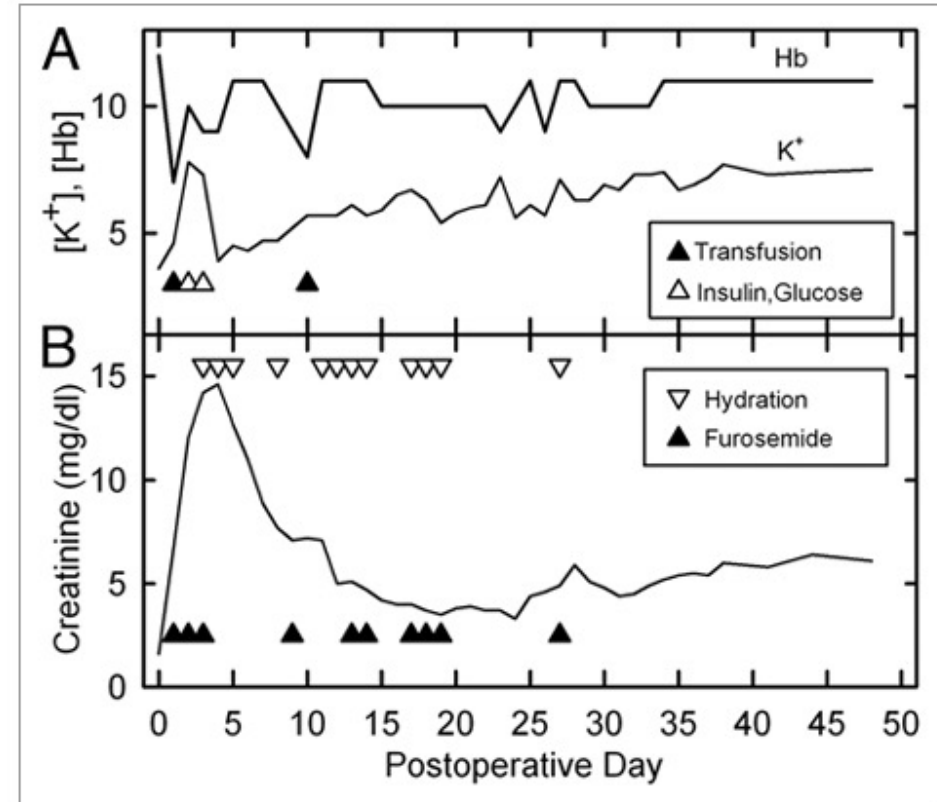


Convective rewarming

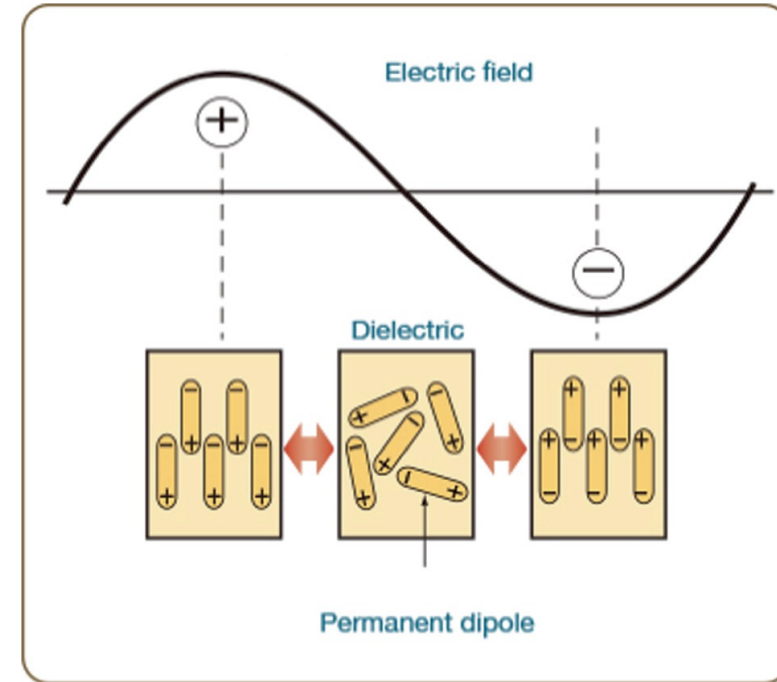
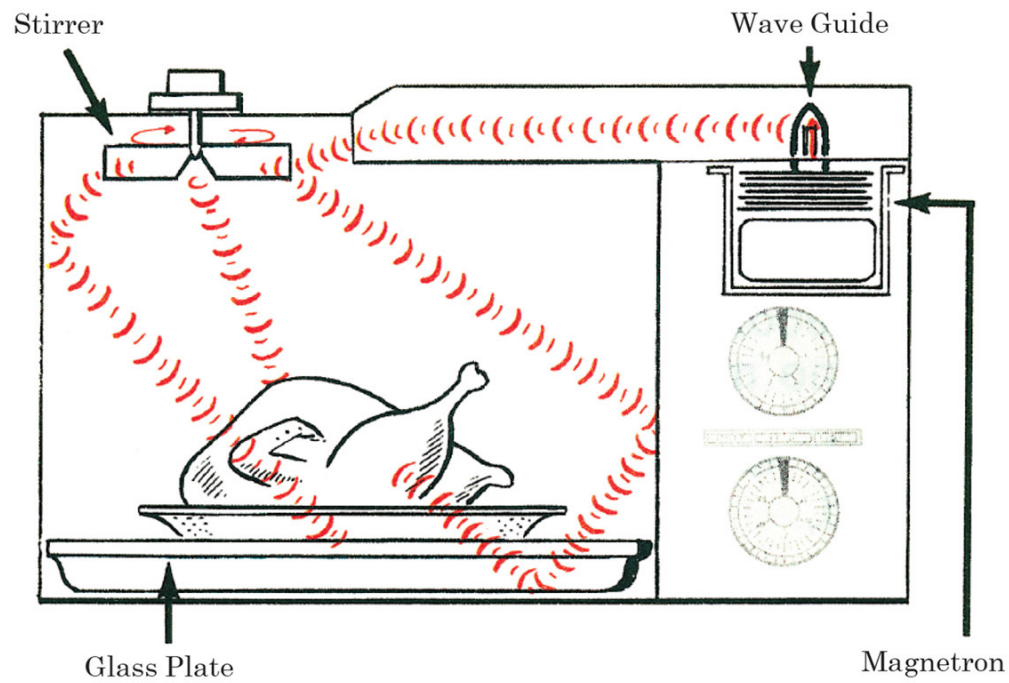


State of the art vitrification and rewarming 1984 to 2021

- Only one rabbit kidney has ever been vitrified and rewarmed with function in vivo (survival for 48 days).
- The kidney reperfused immediately and made urine
- Creatinine rose to ~14 mg/dL, but then improved.
- Creatinine never fell below 3.3
- Anemia and hyperkalemia
- Never been repeated

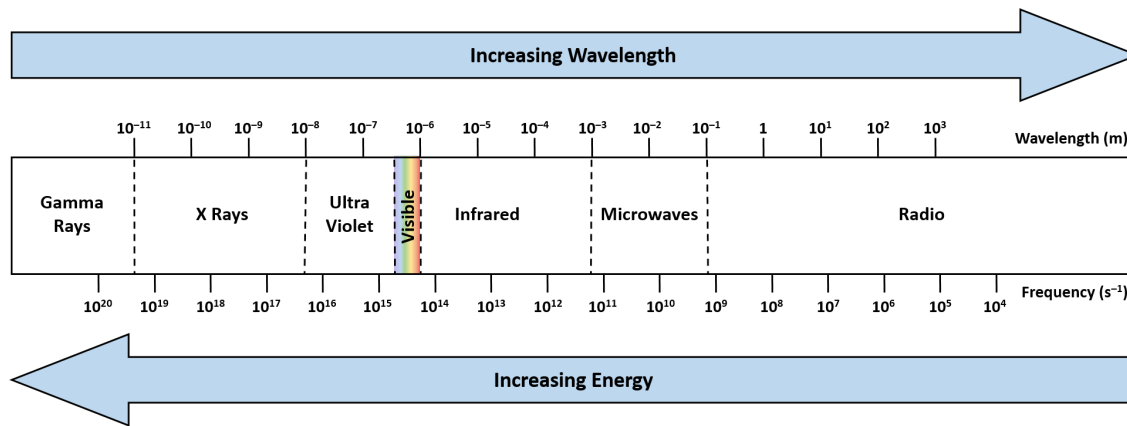


Microwave oven

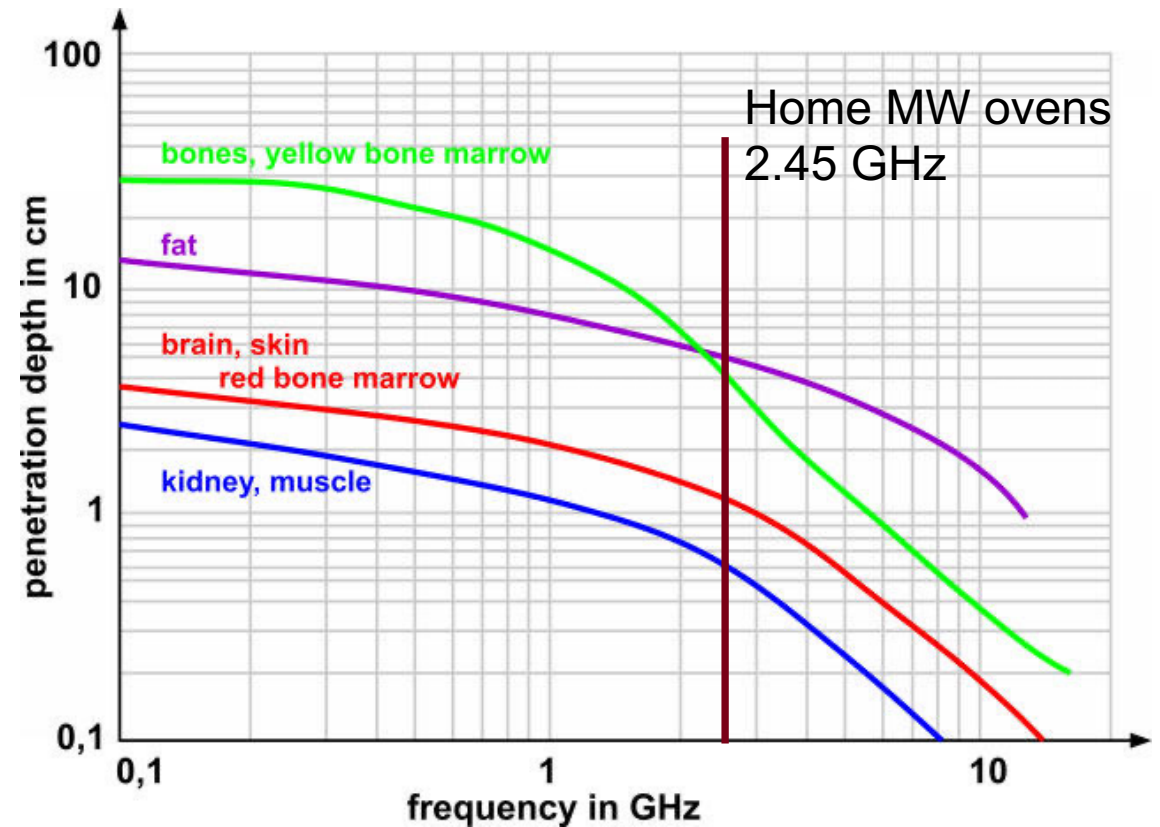


EM wavelength, energy, and penetration

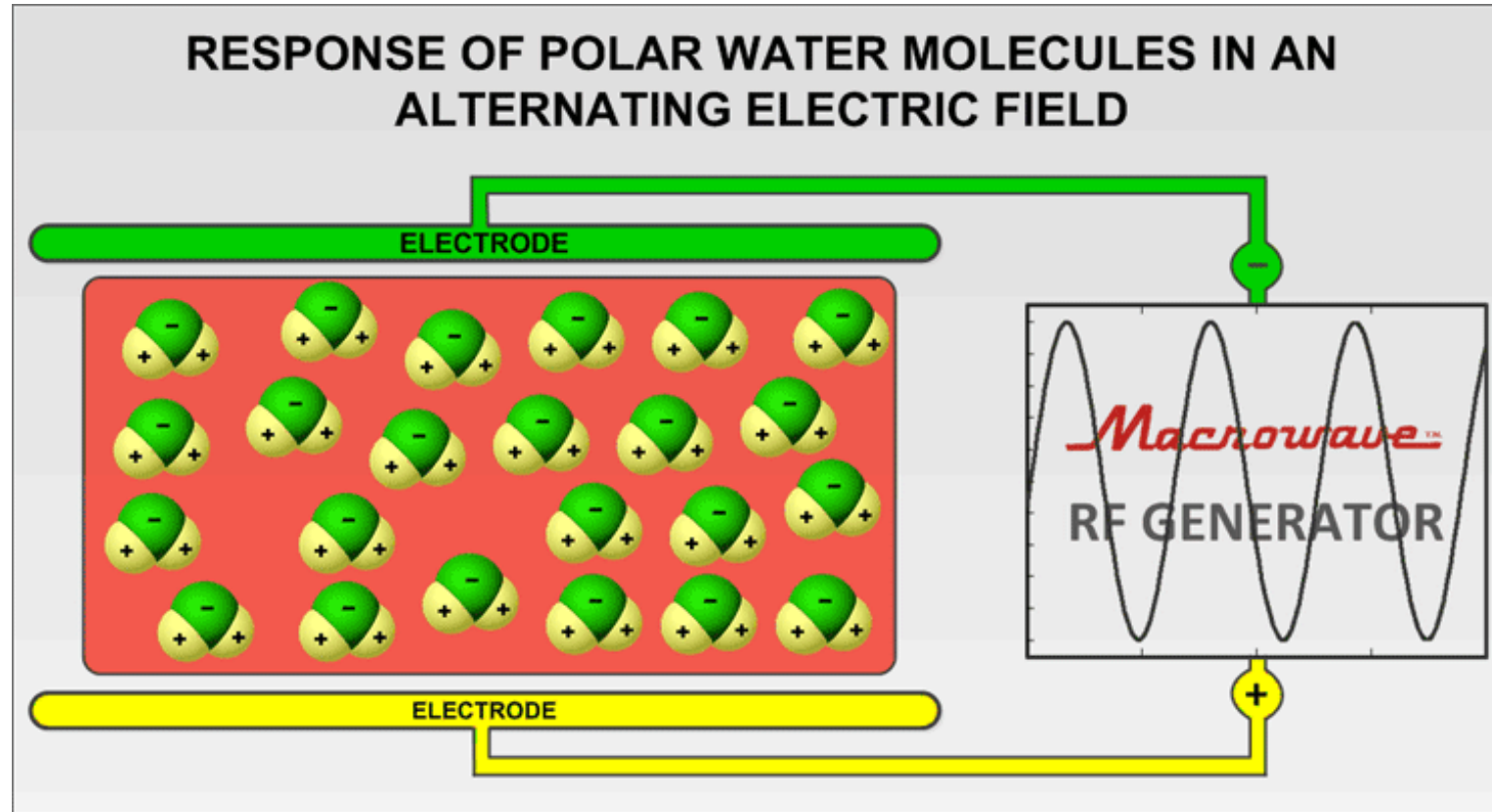
EM spectrum:



Penetration in biologic tissue:

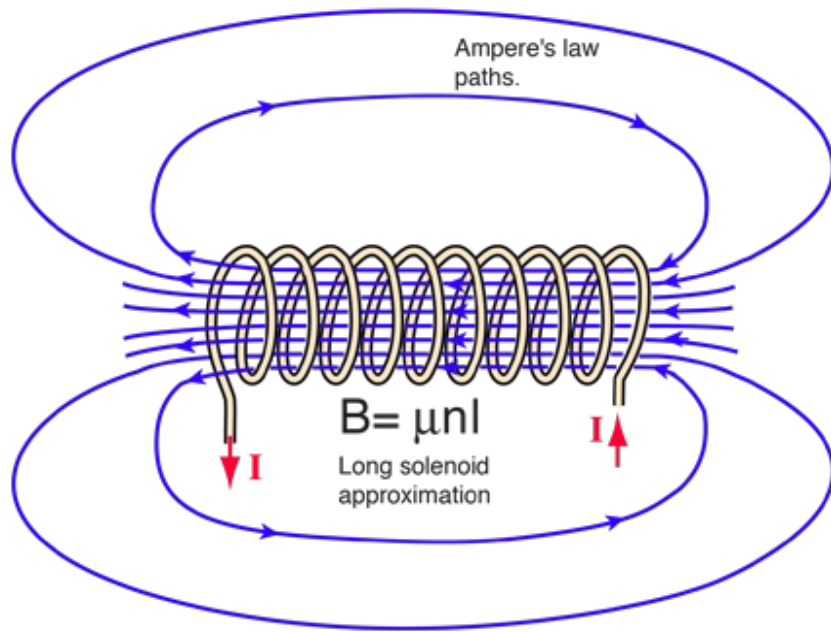


Dielectric heating



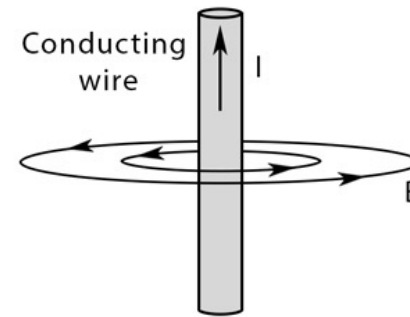
Material does not
contact electrodes
(does not conduct)

Solenoids and Ampere's Law

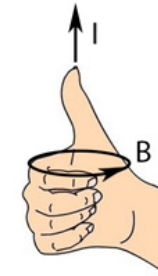


The magnetic field is concentrated into a nearly uniform field in the center of a long solenoid. The field outside is weaker and the lines representing the magnetic field are further apart.

Ampere's Law



Right hand thumb rule



Integral form: $\oint \vec{B} \cdot d\vec{l} = \mu_0 I$

Differential form: $\nabla \times \vec{B} = \mu_0 \vec{J}$

I : Electric current

B : Magnetic field

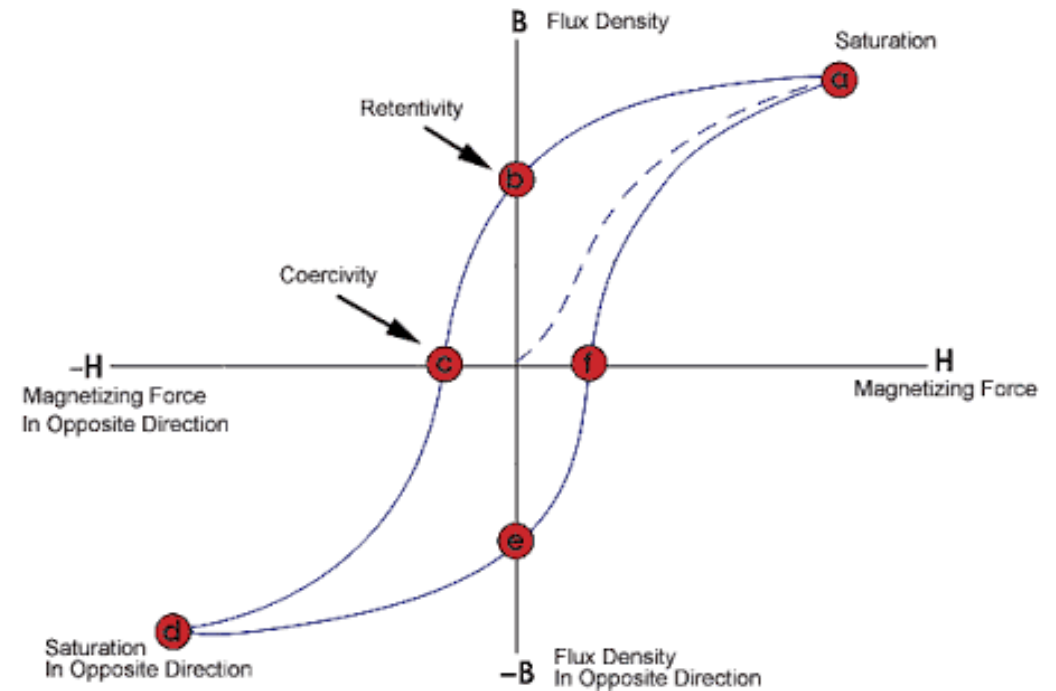
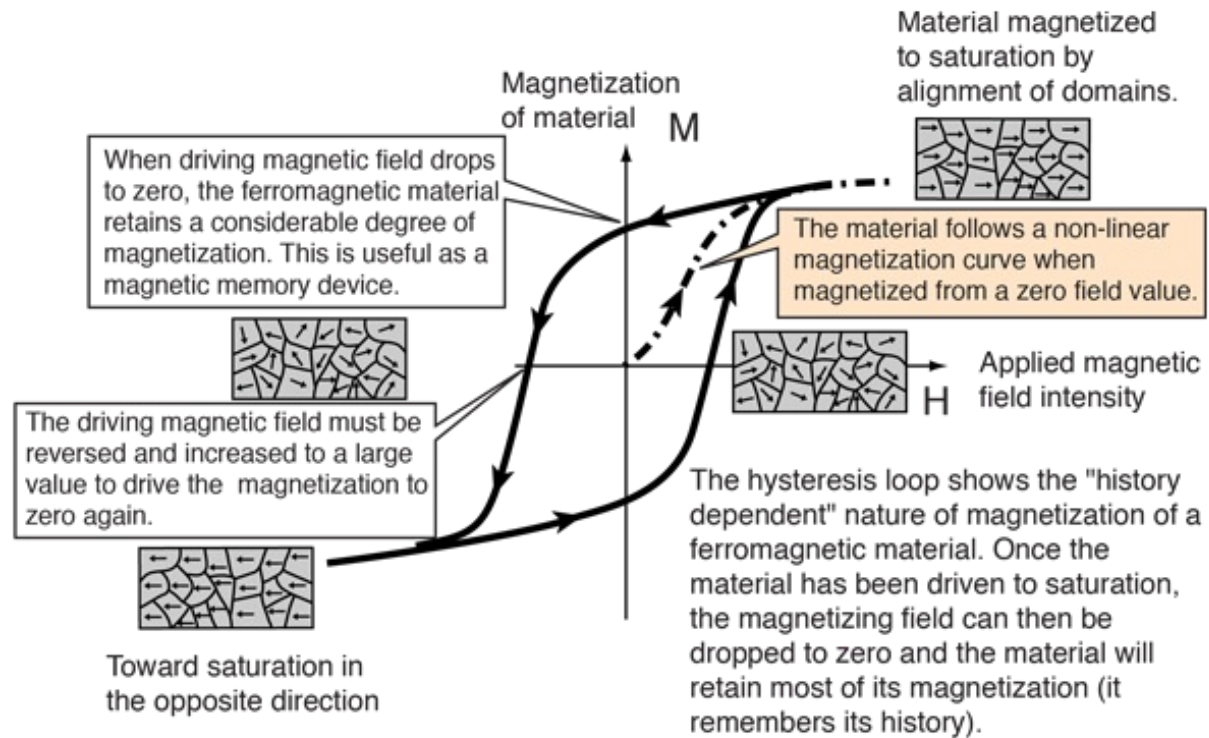
μ_0 : Permeability of free space

J : Current density

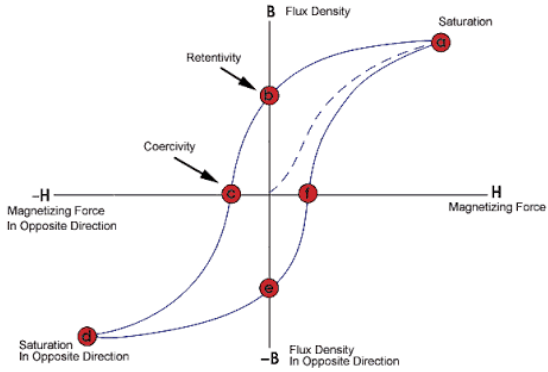
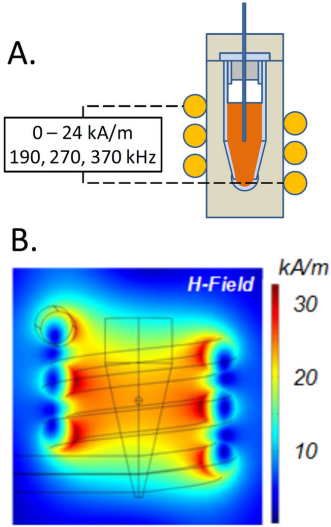
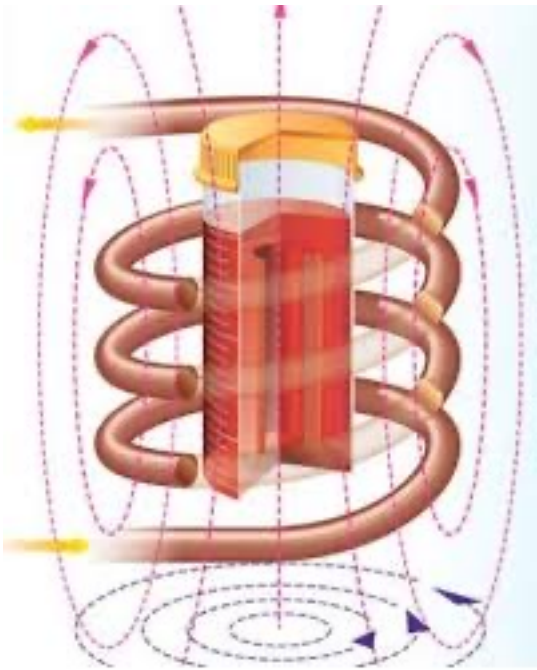
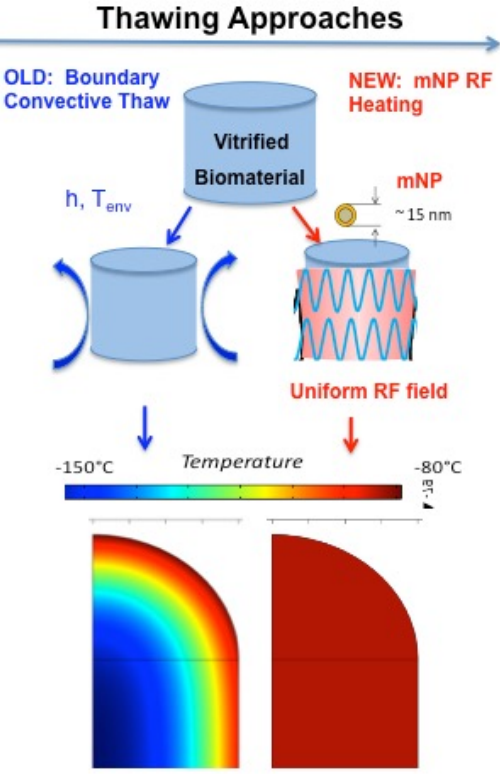
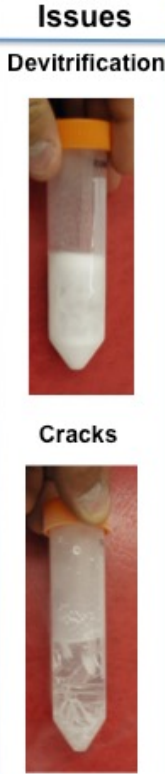
Thumb points in the direction of the electric current and fingers curl around the current indicating the direction of the magnetic field

Science Facts

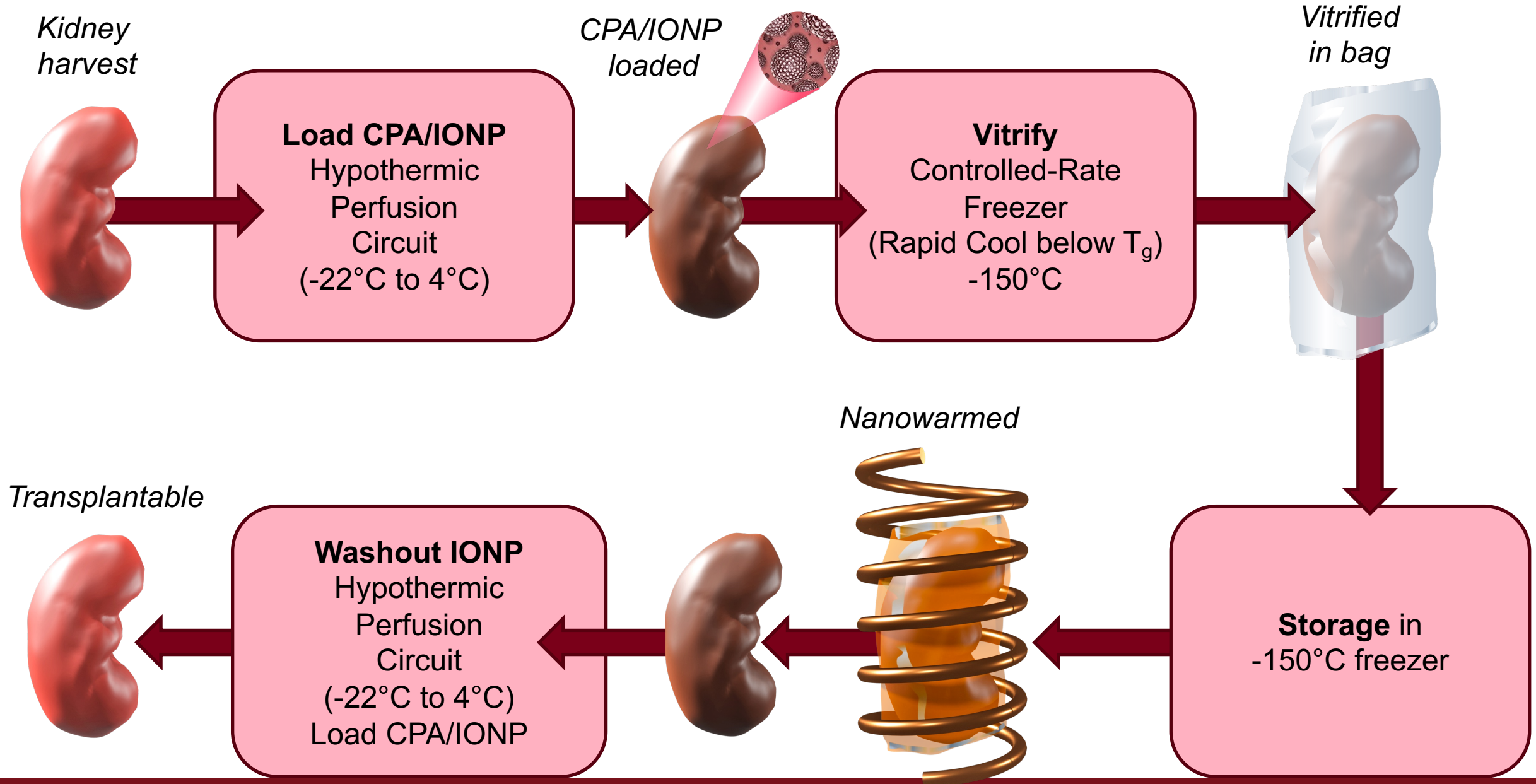
Radio frequency inductive heating through hysteresis losses



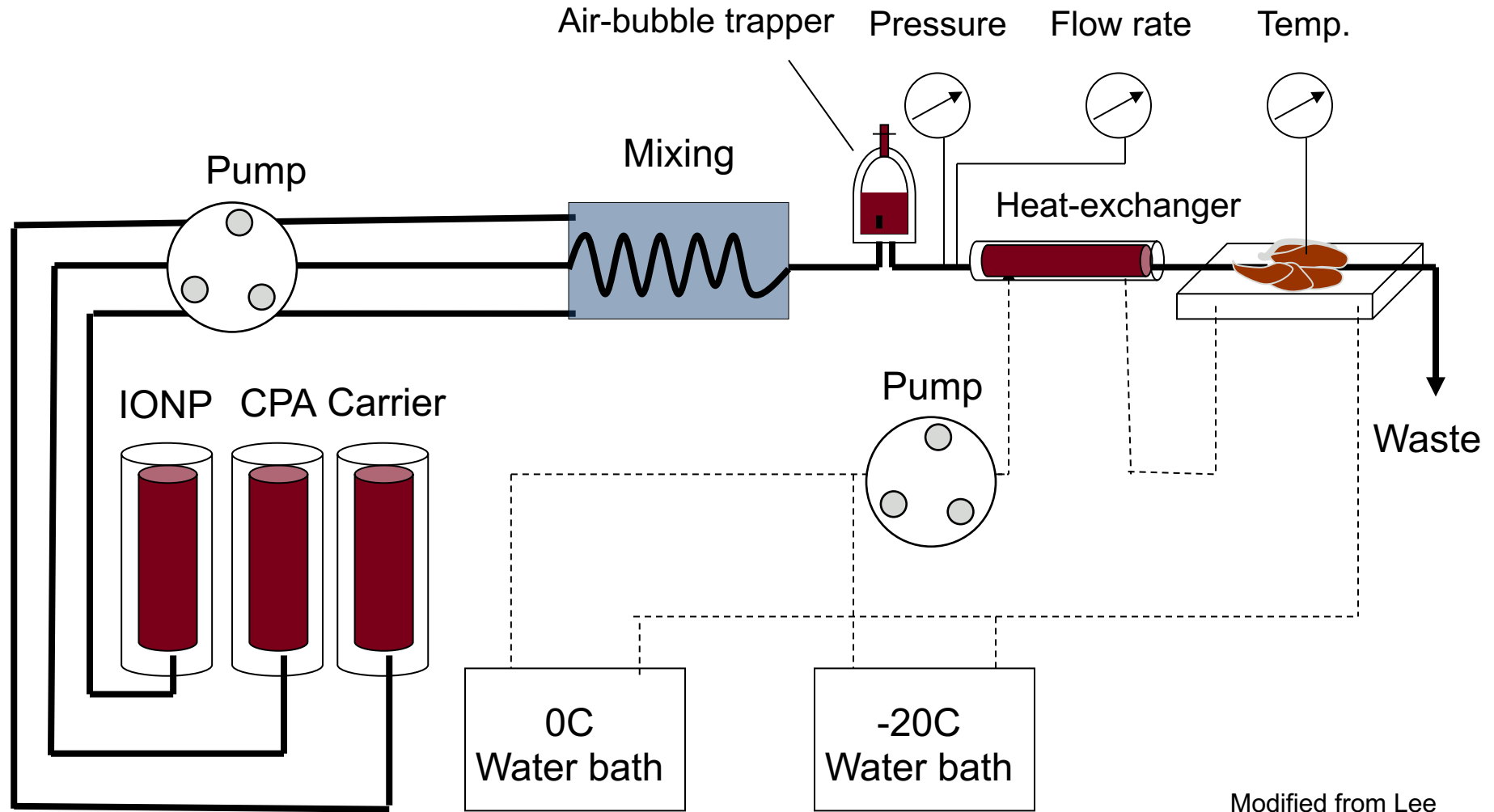
Nanowarming: rapid and uniform rewarming of vitrified material



Overall strategy for cryopreservation and Nanowarming of kidneys



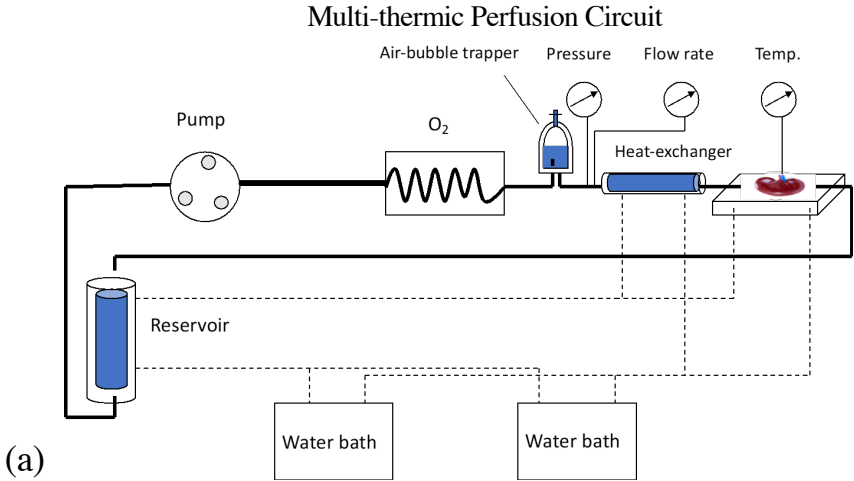
Hypothermic Machine Perfusion Set-up



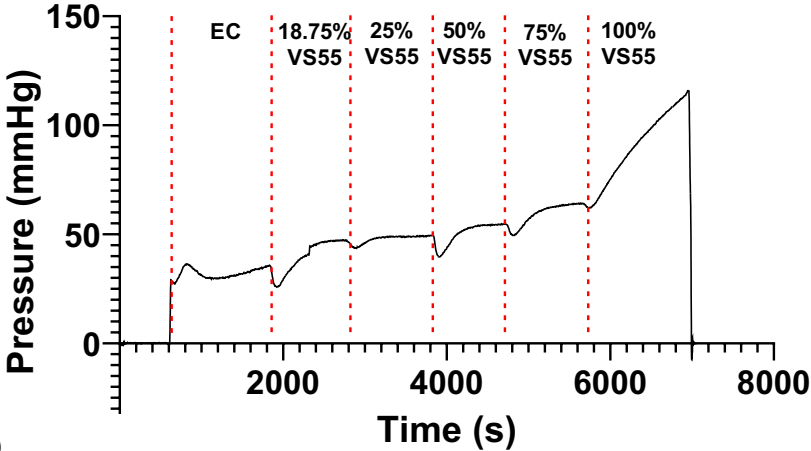
Modified from Lee



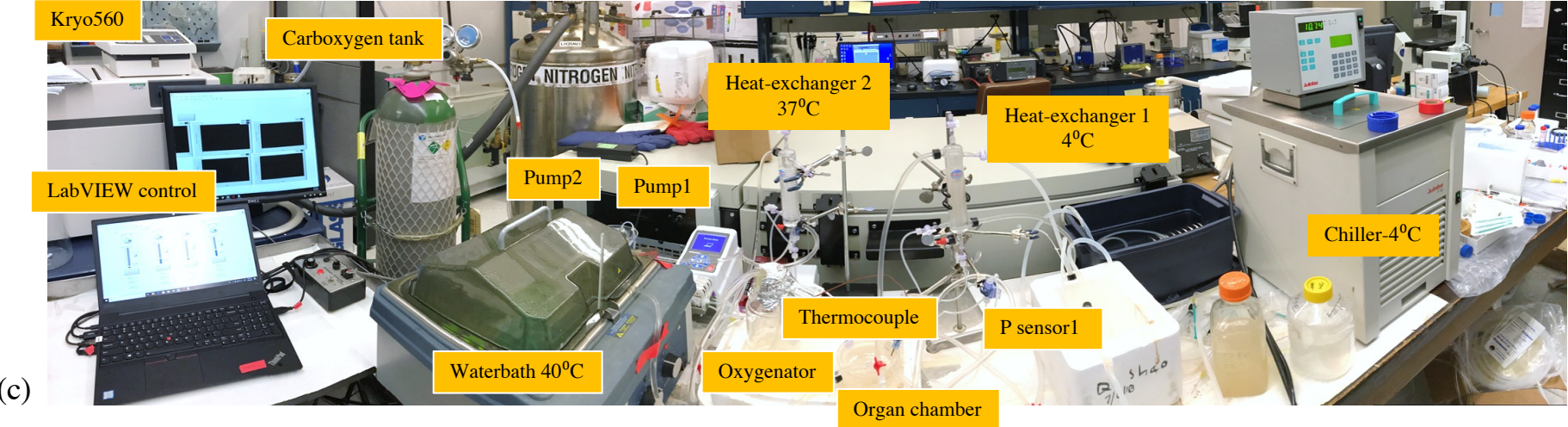
Rat kidney perfusion



(a)

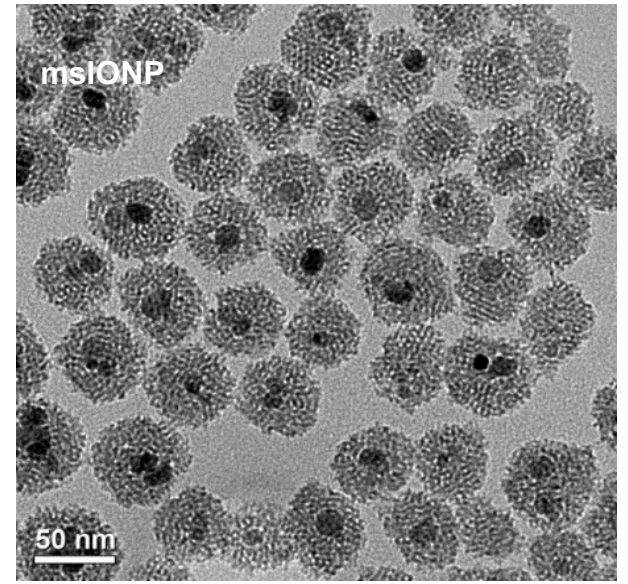
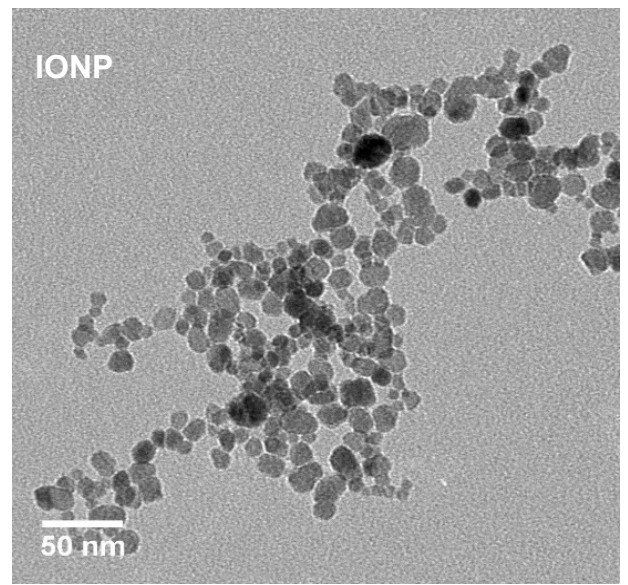
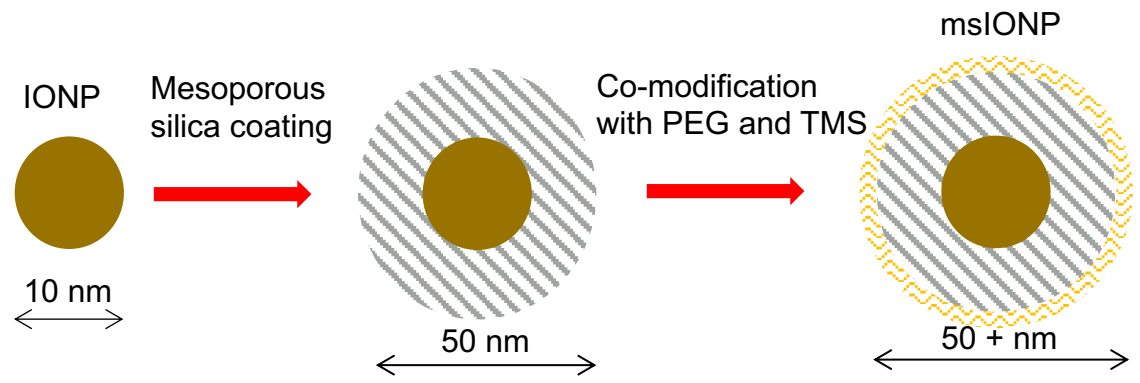


(b)

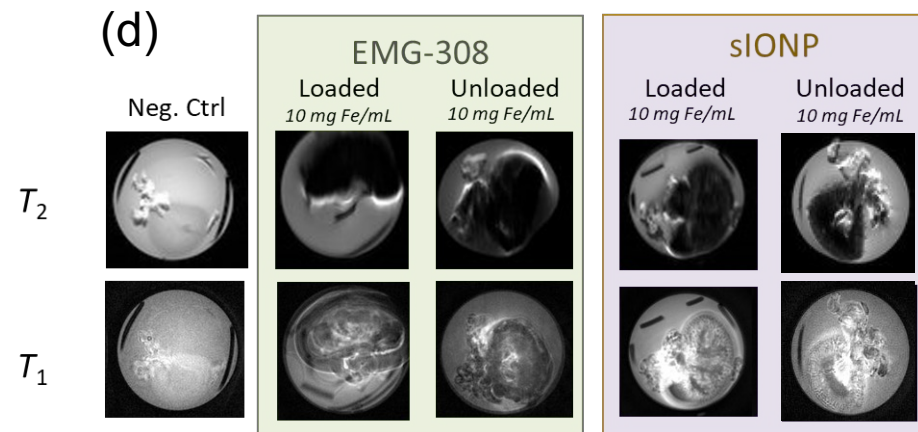
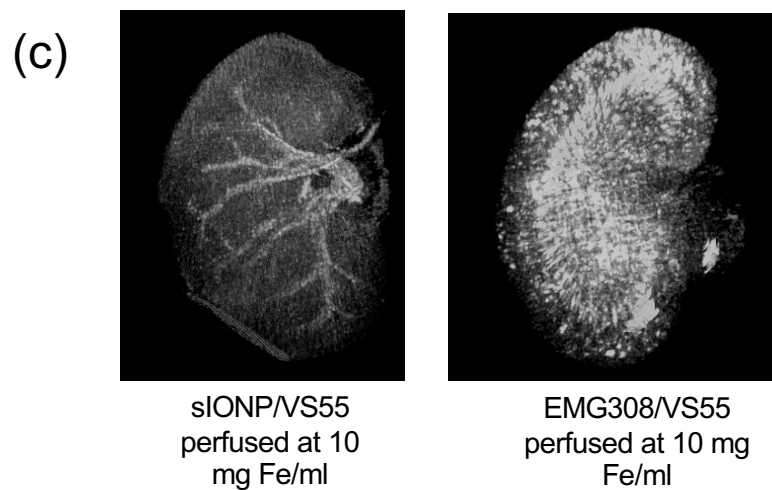
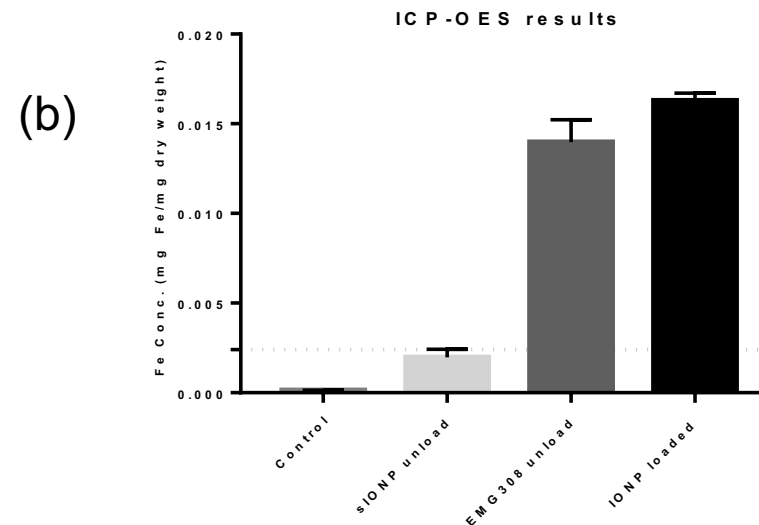
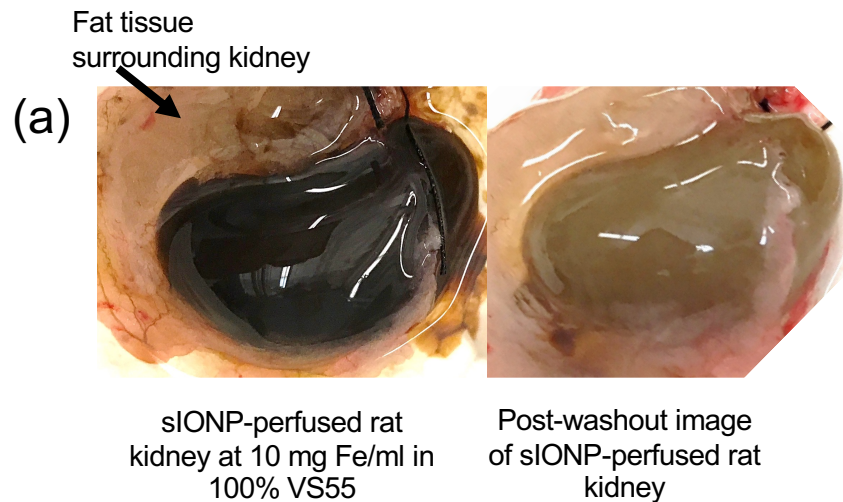


(c)

Nanoparticles: Colloidally stable, high heating, and biocompatible

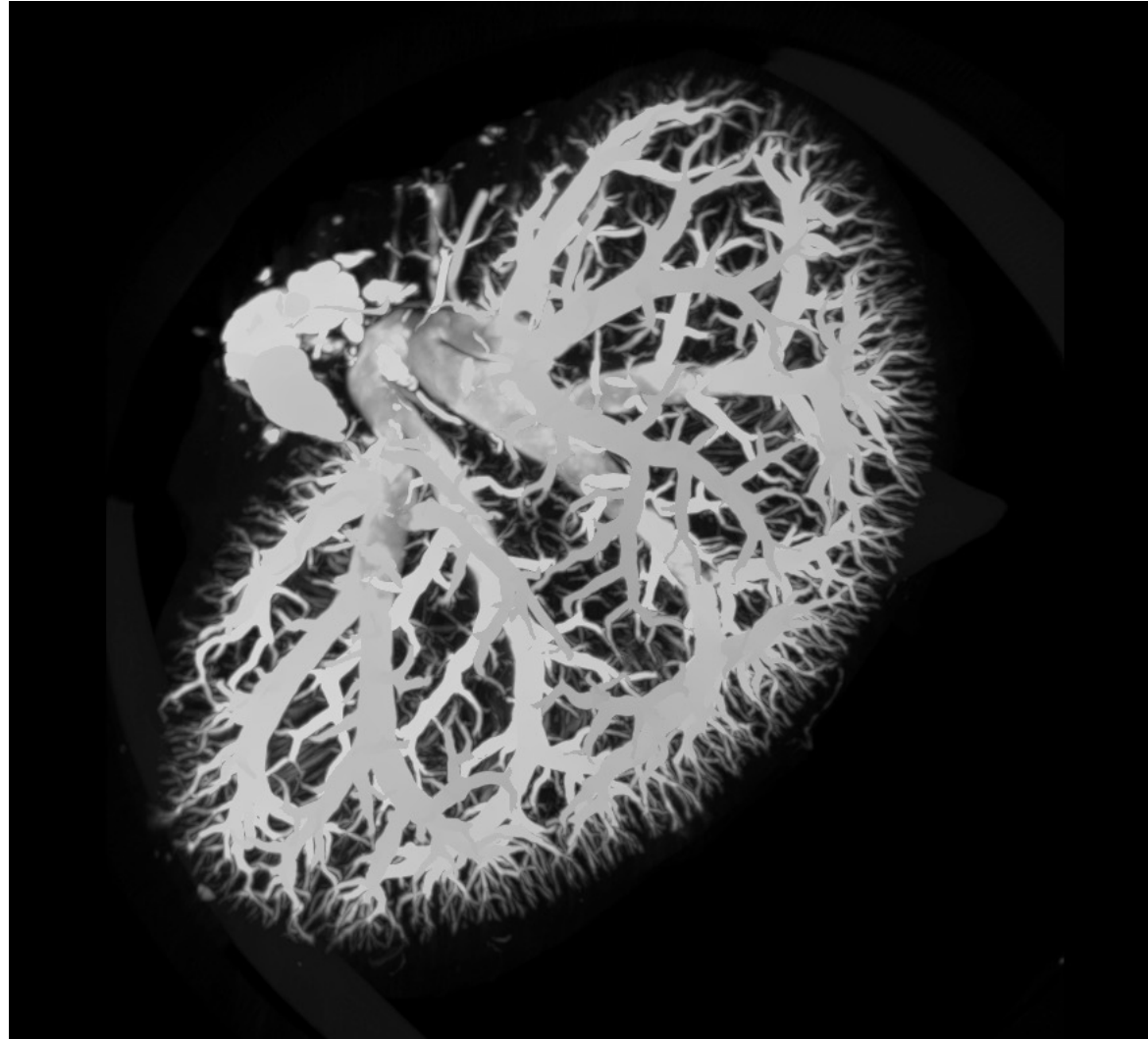


New sIONP Perfuses in and Washes Out

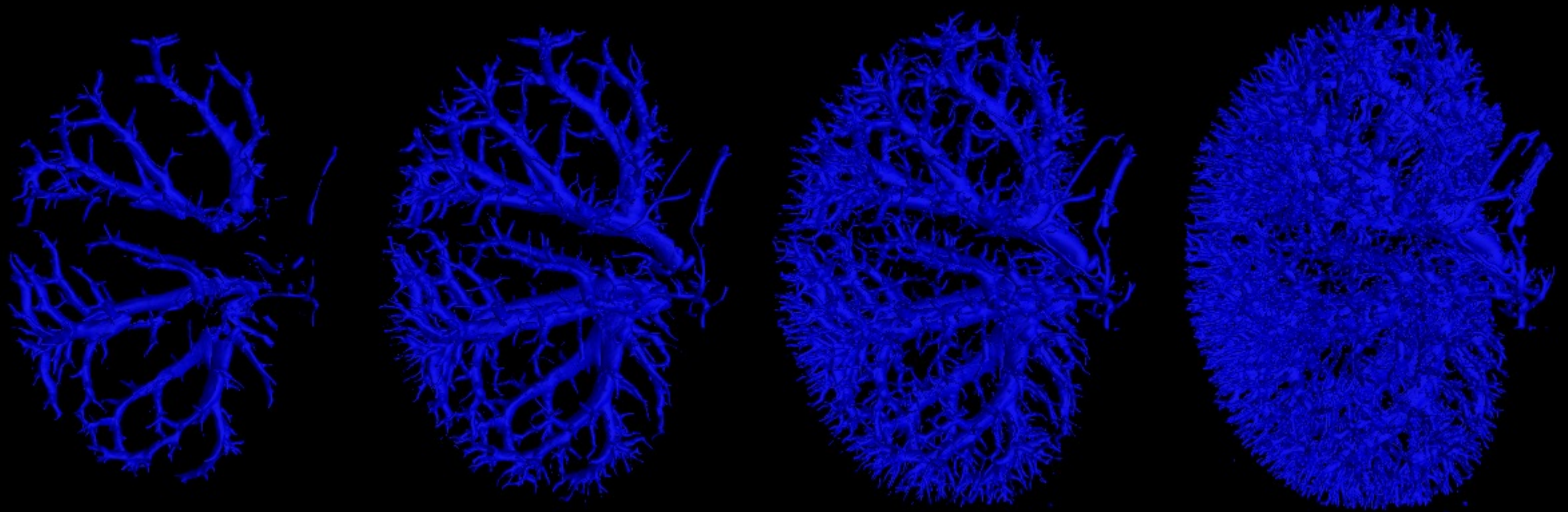


Rat kidney

31 x 31 x 31 μm^3

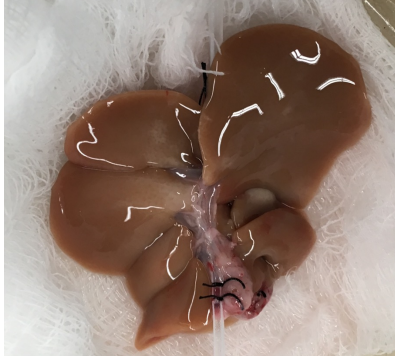


Rat kidney ($31 \times 31 \times 31 \mu\text{m}^3$)



Loading and unloading of nanoparticles in rat livers

Control



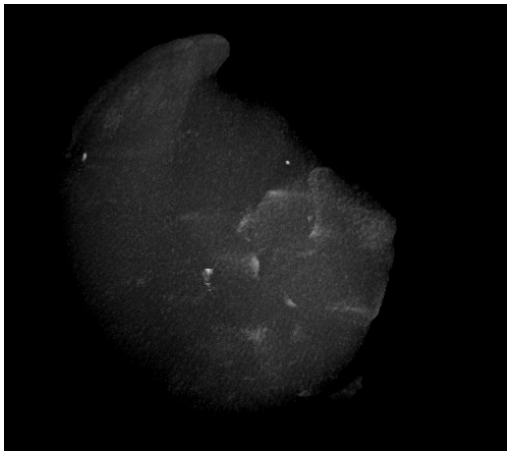
CPA+IONP loaded



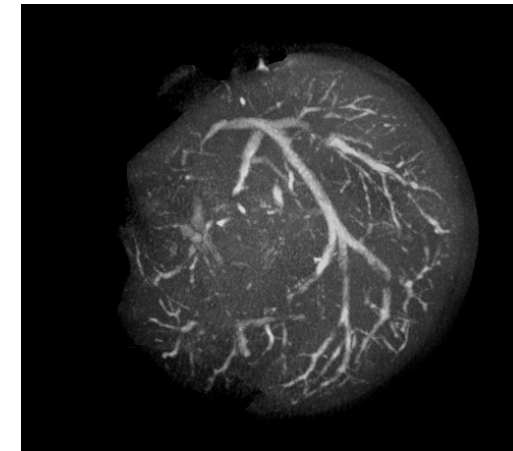
CPA+IONP washout



Neg. Ctrl (CPA loaded)

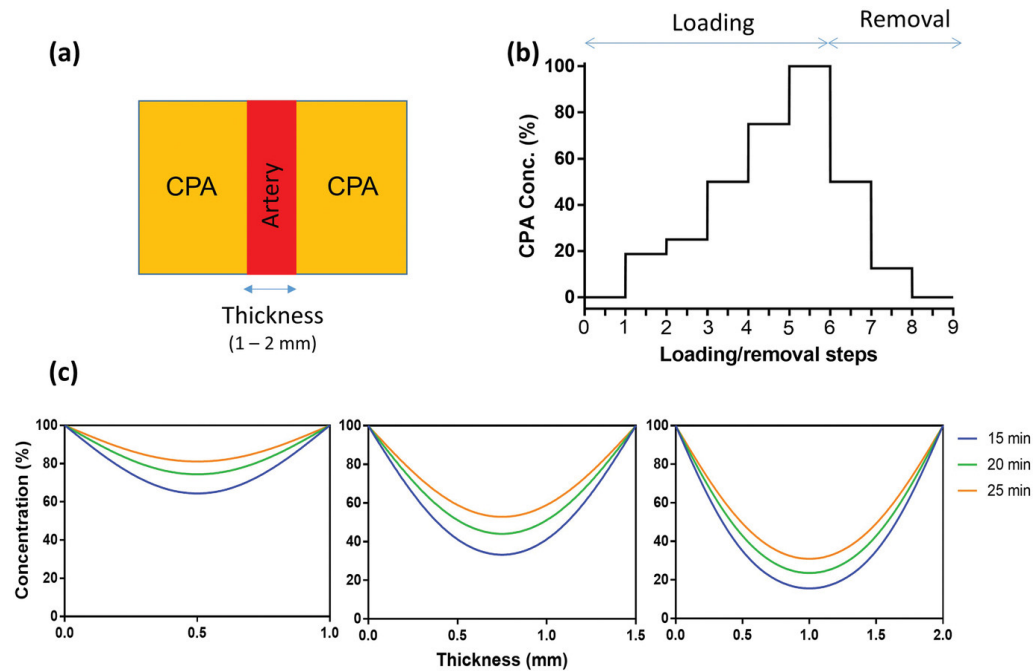


10mg Fe/mL sIONP Loaded



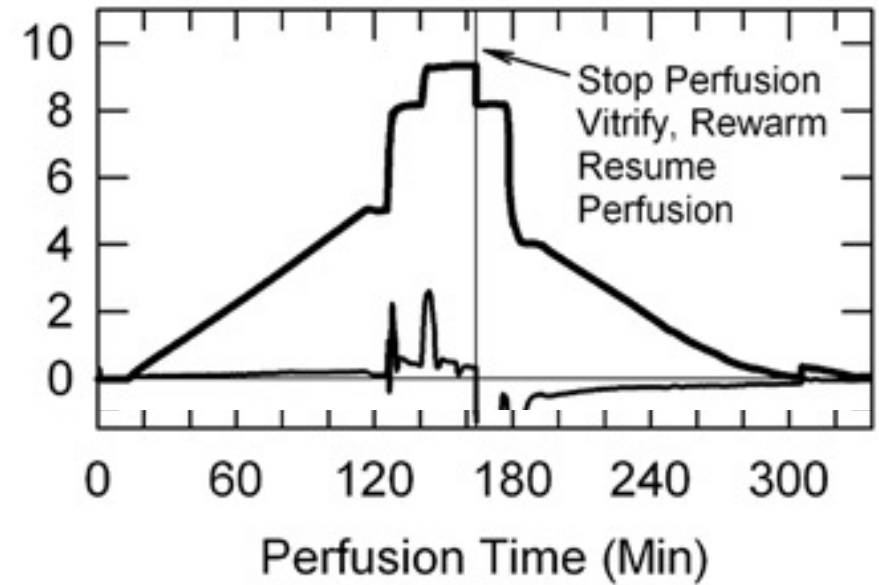
CPA loading and unloading must be gradual to avoid osmotic injury

Step loading

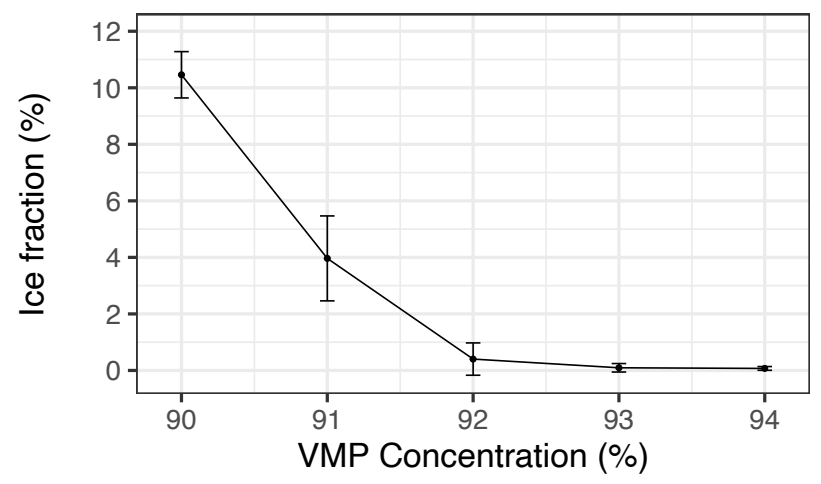
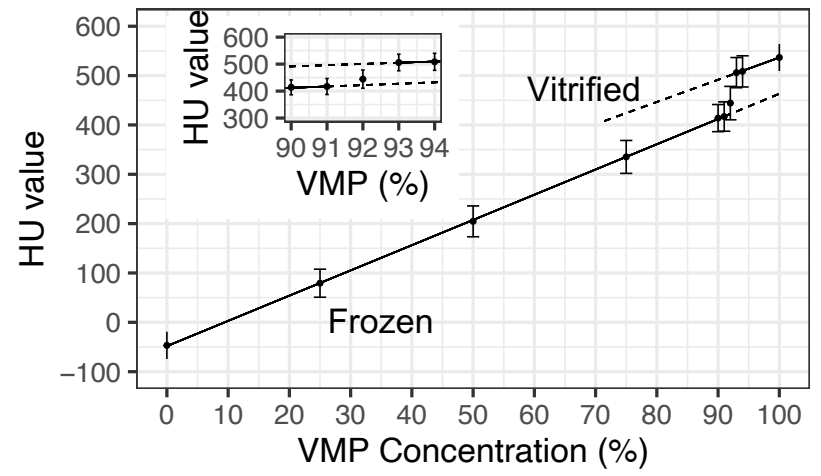
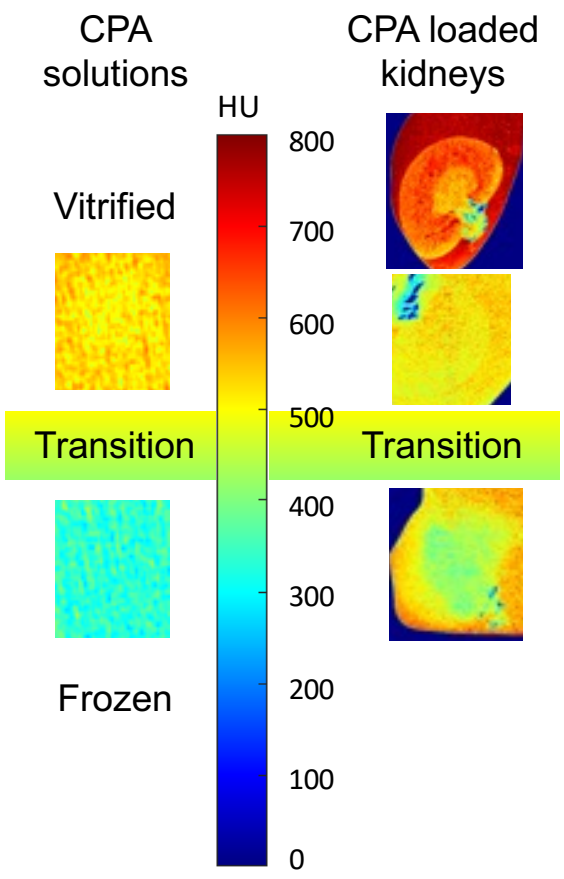
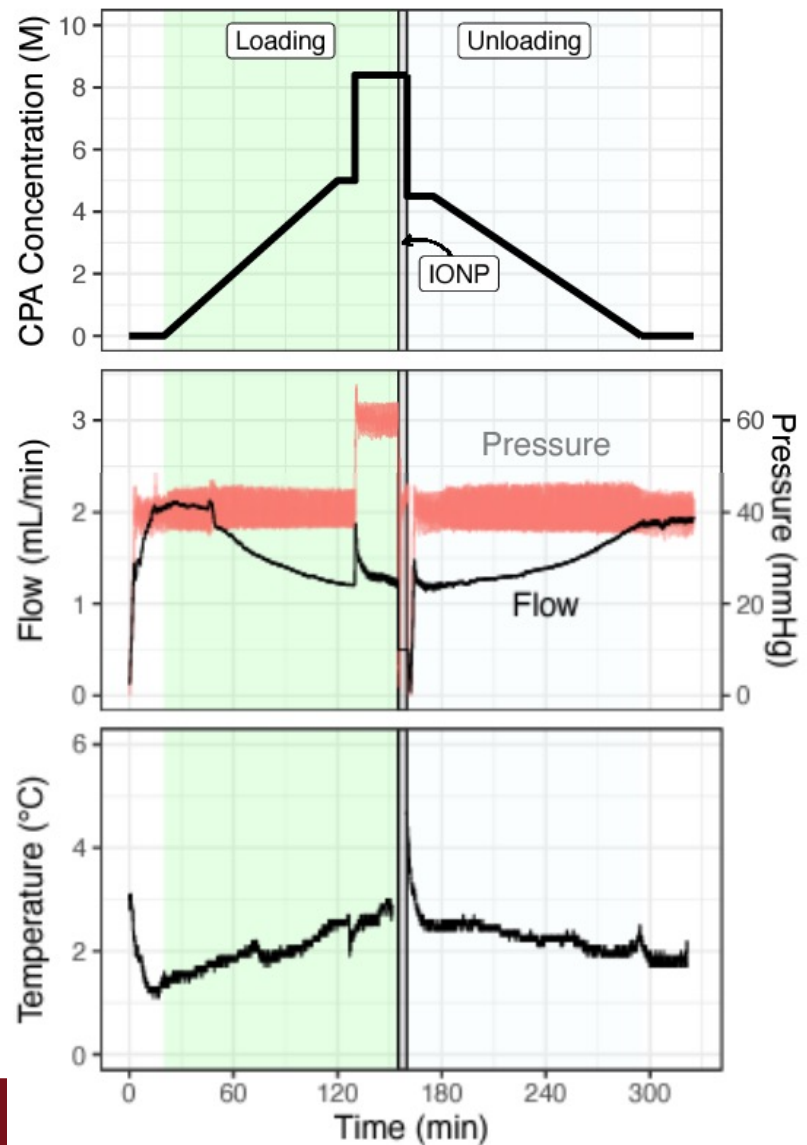


Ramp/step hybrid loading

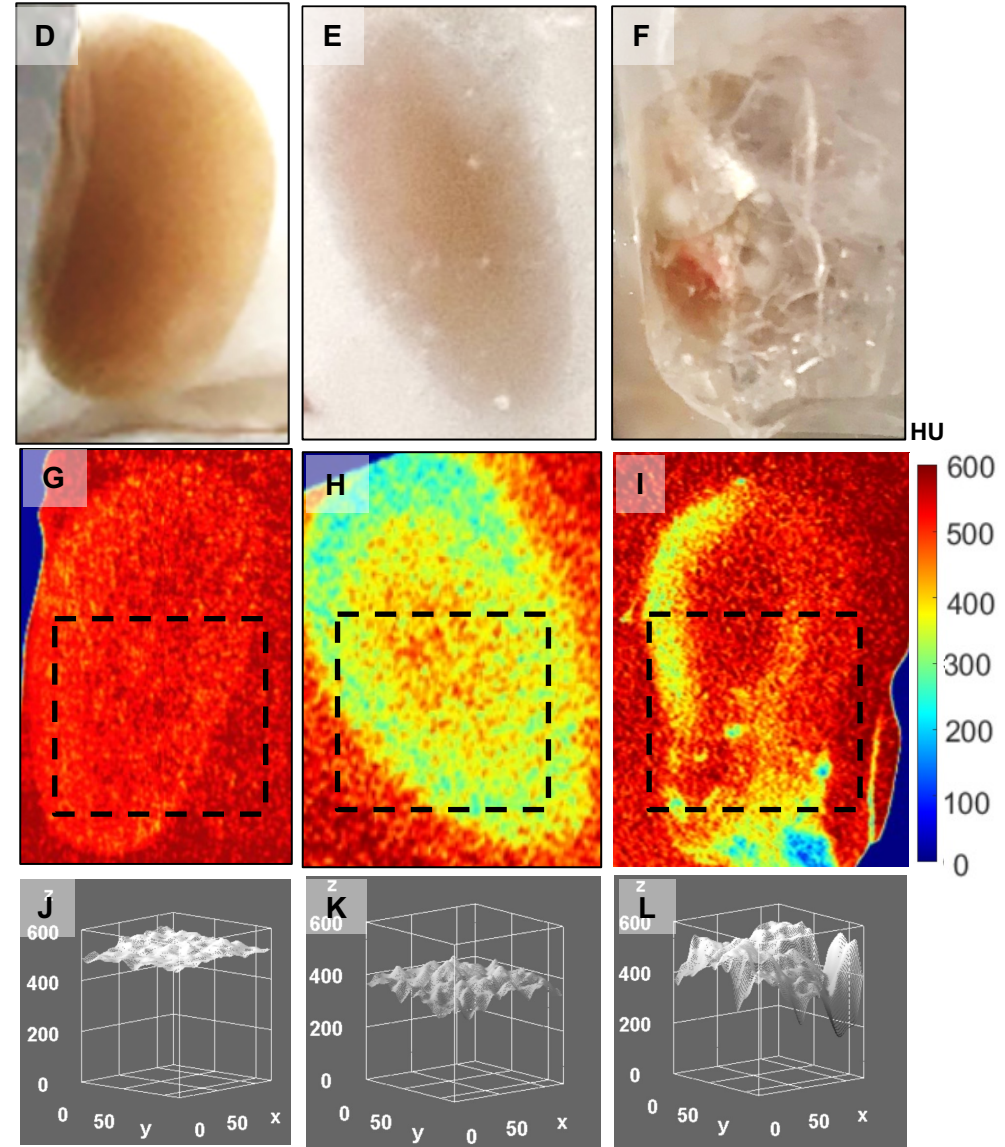
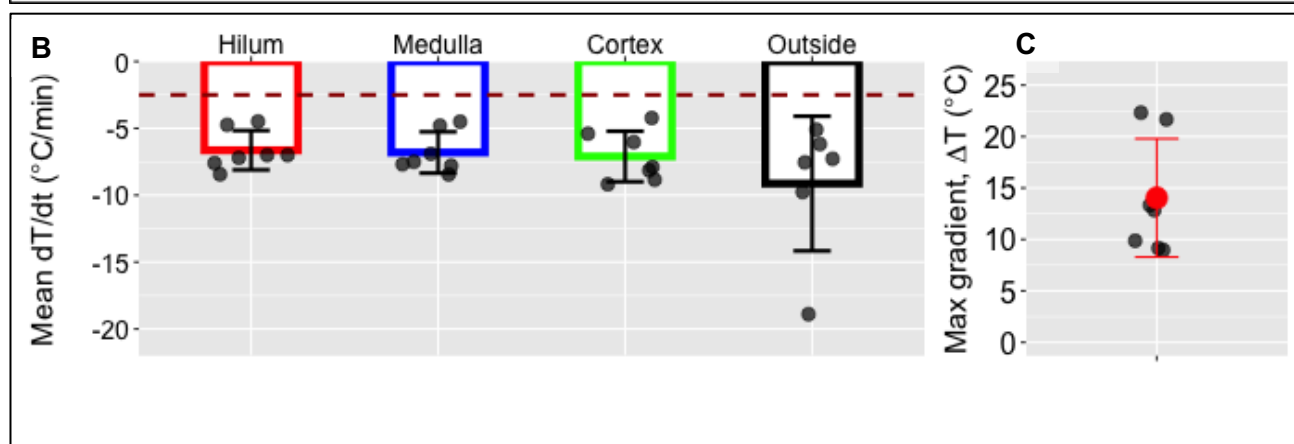
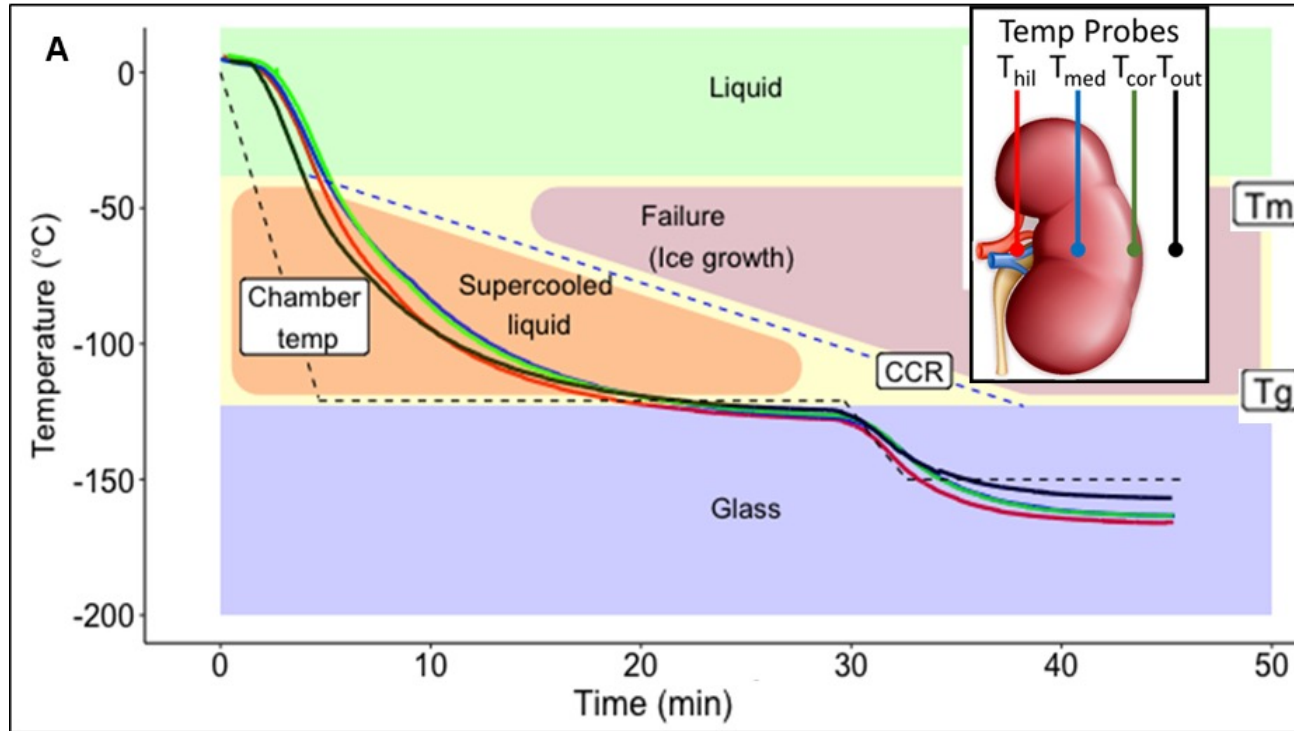
A
M (arterial)
A-V (M)



Hypothermic Perfusion of CPA and sIONP – Vitrification – Nanowarming – sIONP and CPA washout

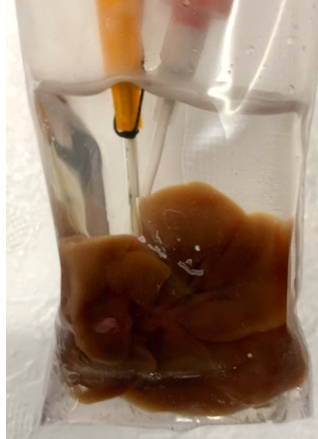


Cooling to a vitrified state



Vitrified organs and failure states

A. Vitrified



B. Vitrified



C. Vitrified with sIONP



D. Liver is frozen, solution is vitrified



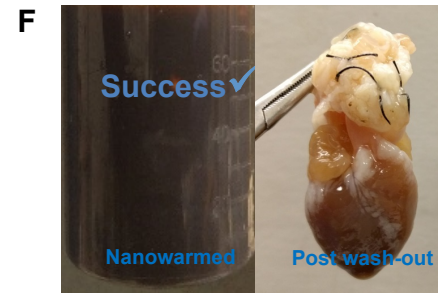
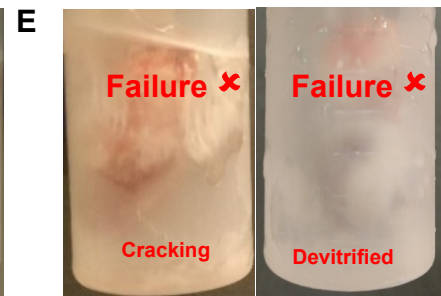
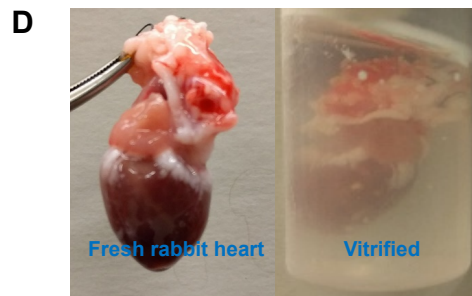
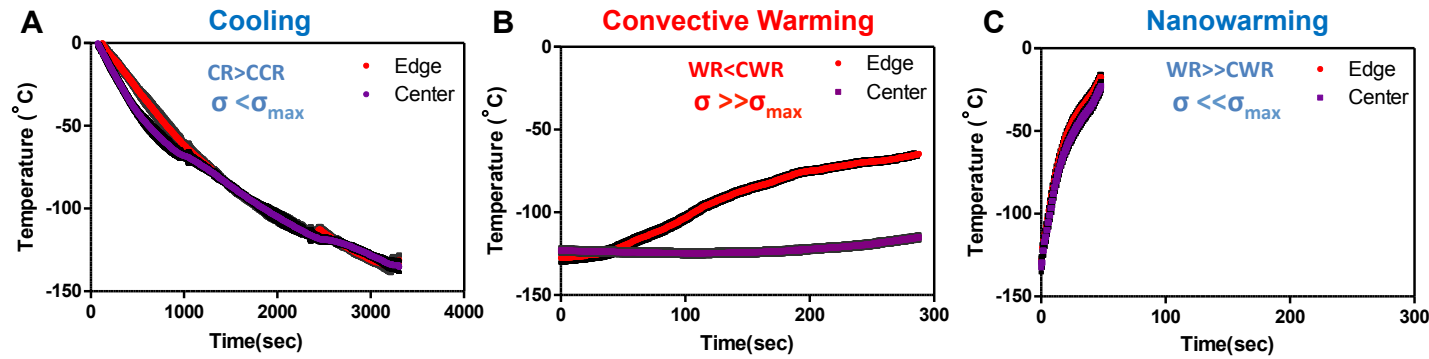
E. Liver and solution frozen



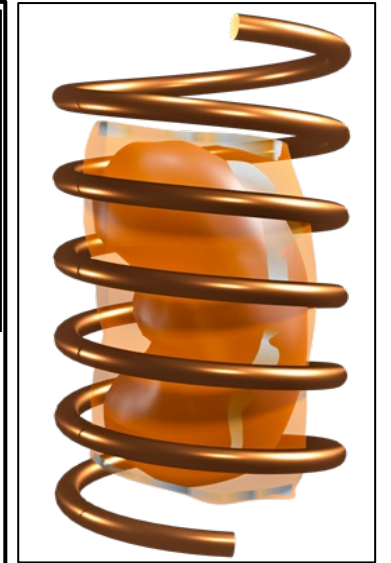
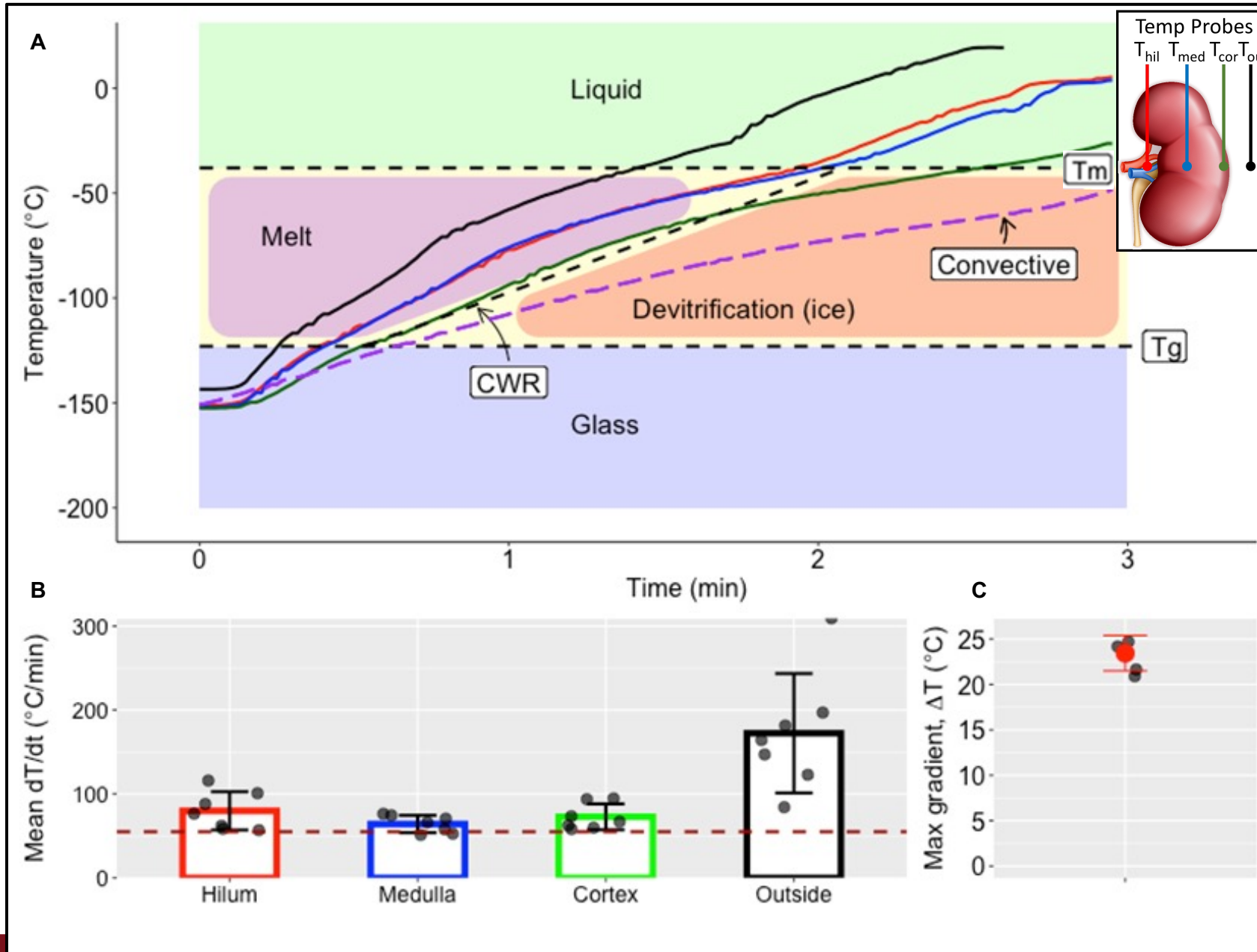
F. Liver and solution frozen with cracks



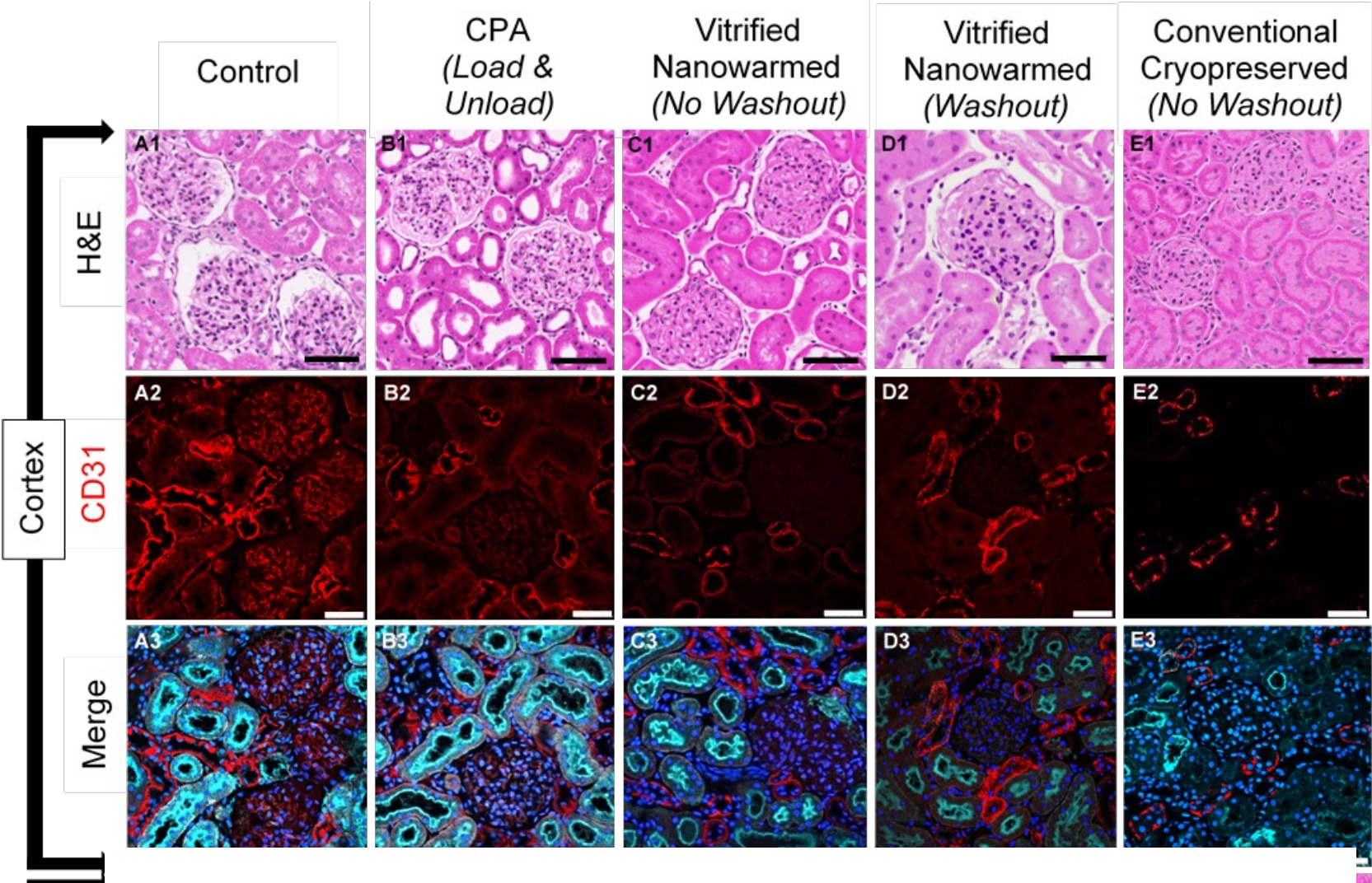
Cooling and heating performance in rabbit hearts



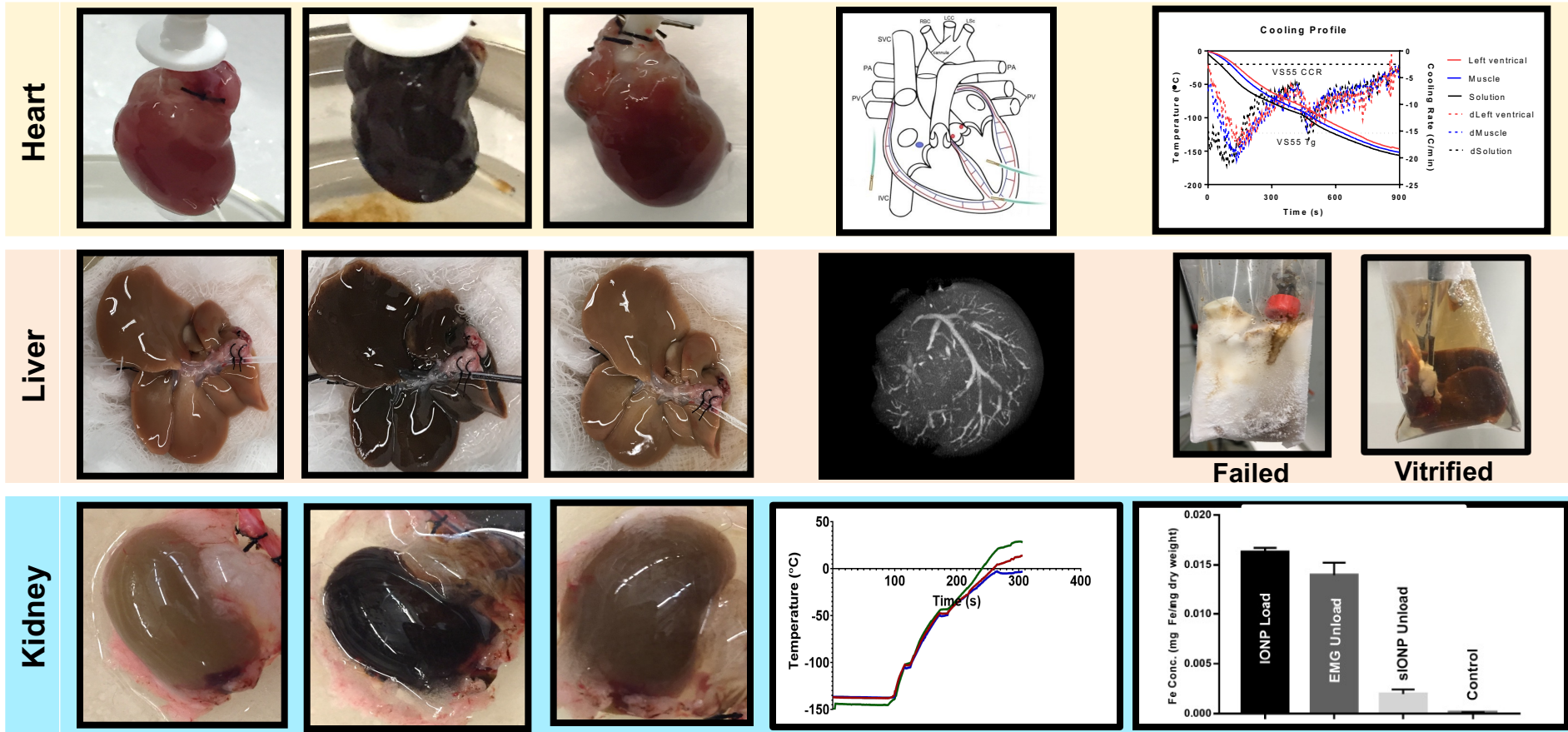
Nanowarming



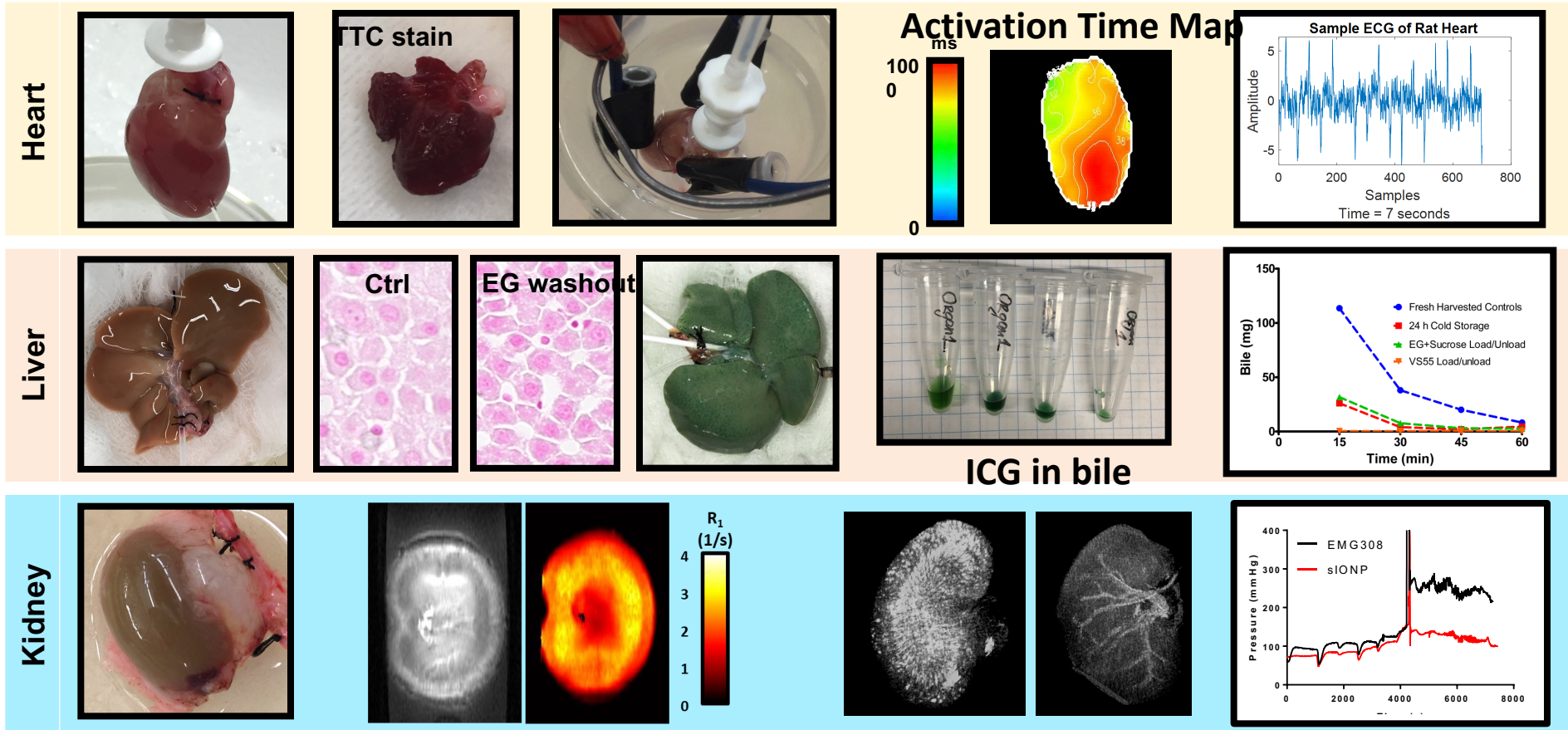
Nanowarming is superior to conventional cryopreservation but suffers from CPA-induced injury



Nanowarming achieves physical success in multiple organ systems in rat and rabbit



Organ Nanowarming: Physical and Functional Assessment



44

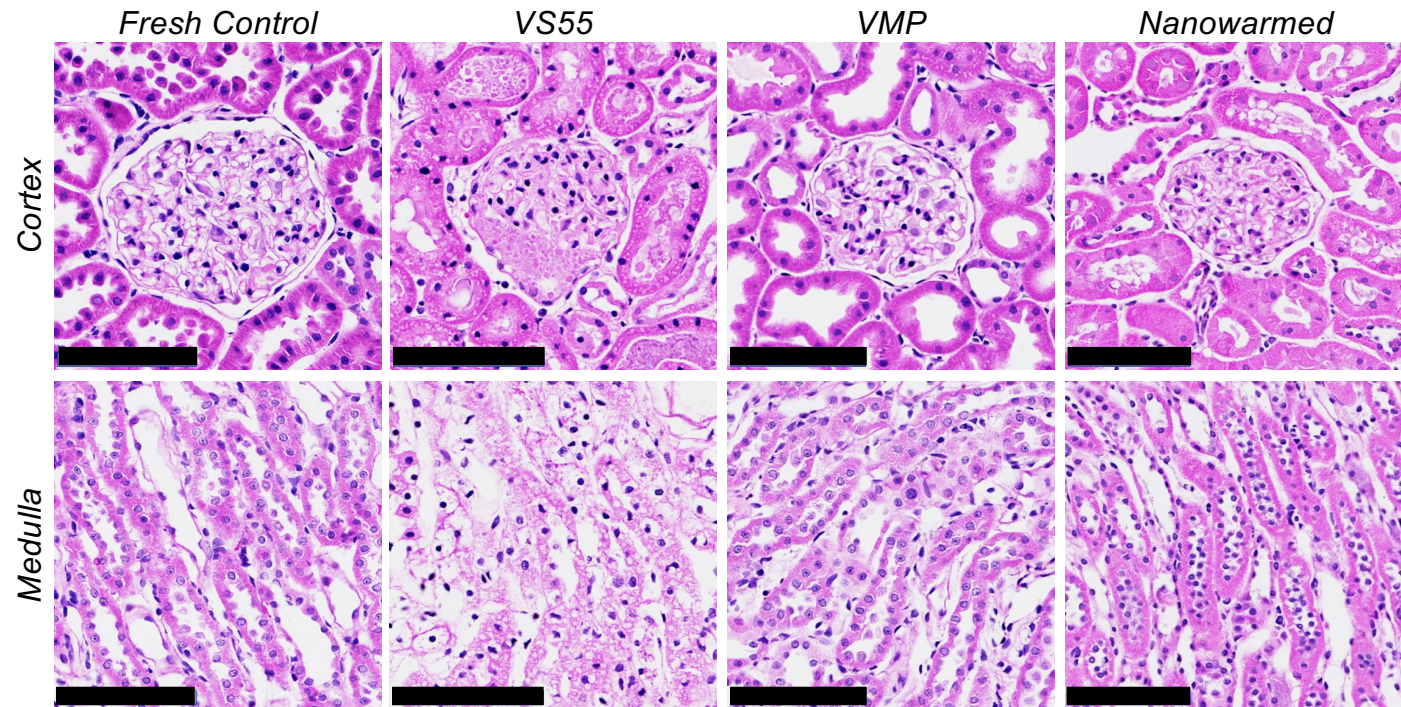


Alternative CPA with less toxicity

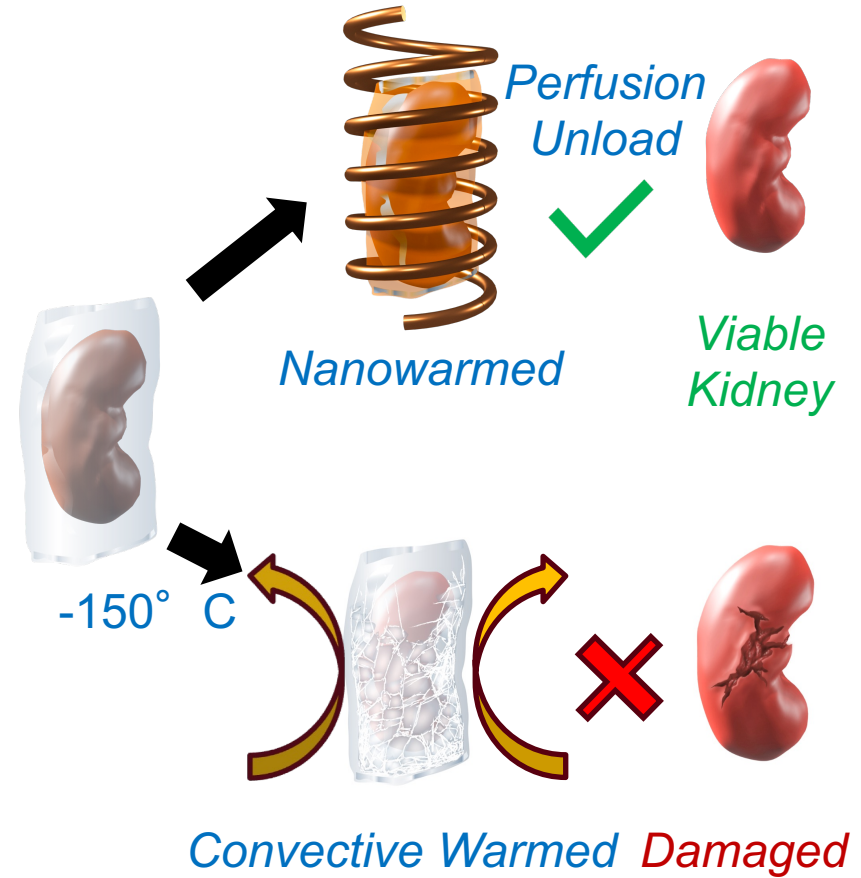
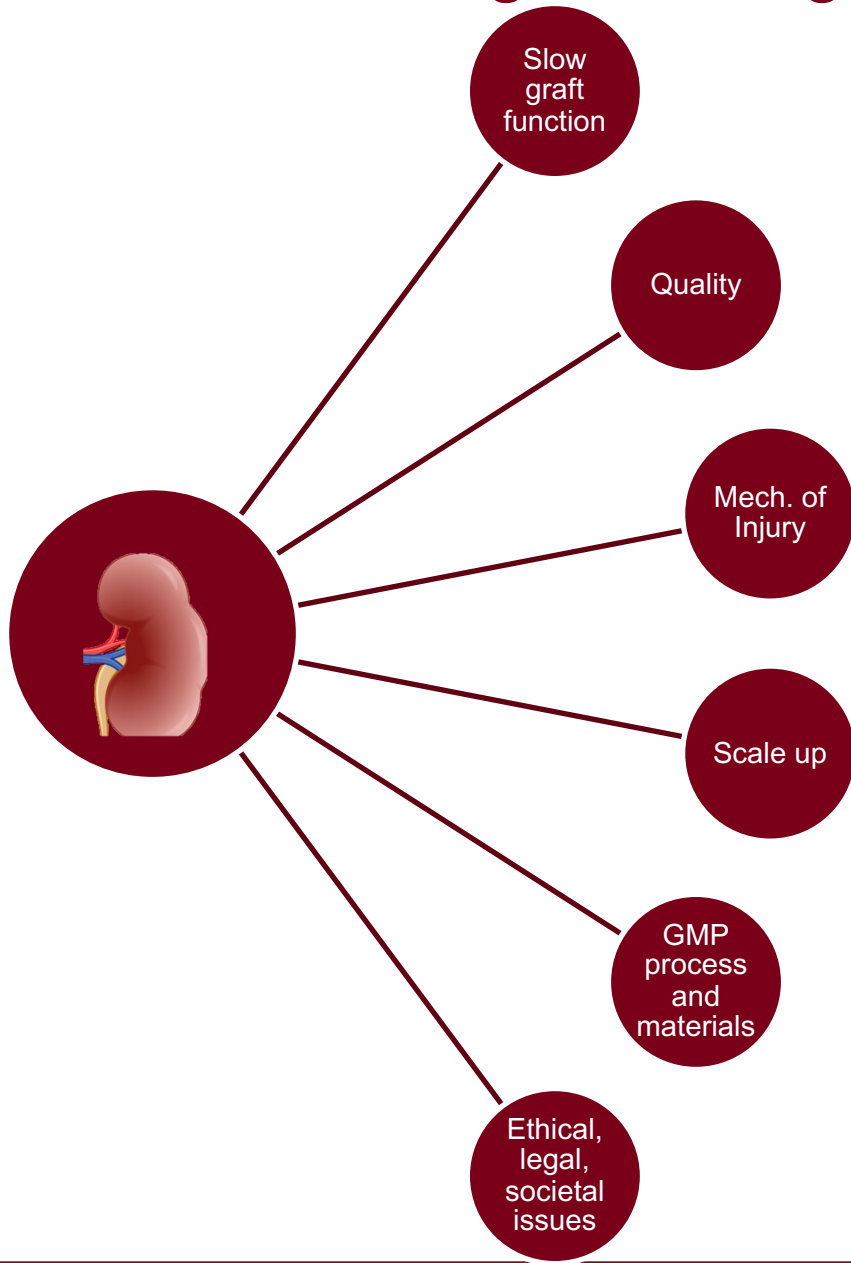
CPA candidates:

Components (gm/L)	VS55	VMP	VM3	CPR-S	M22
Ethylene Glycol		168.4	168.40	168.4	168.4
Formamide	139.6	128.6	128.60	128.6	128.6
DMSO	242.1	223.00	223.00	223.0	223.0
PVP (5000 kDa)			70		28
1,2-Propanediol (PG)	168.4				
X-1000 (polyvinyl alcohol)		10	10	10	10
Z-1000 (polyglycerol)		10	10	20	20
N-Methylformamide (g/L)				30	30
3-Methoxy, 1,2-propanediol				40	40

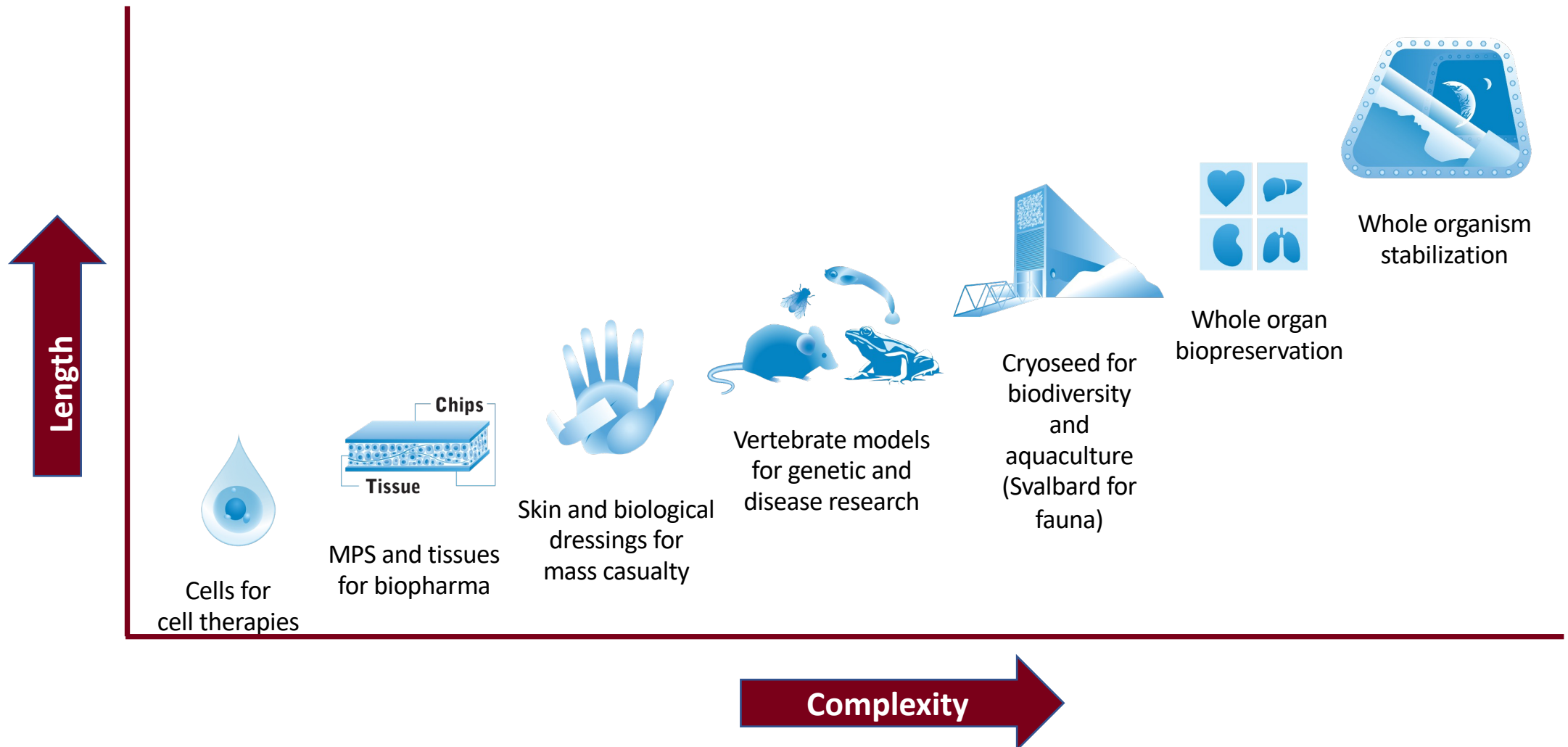
Compare VMP to VS55:



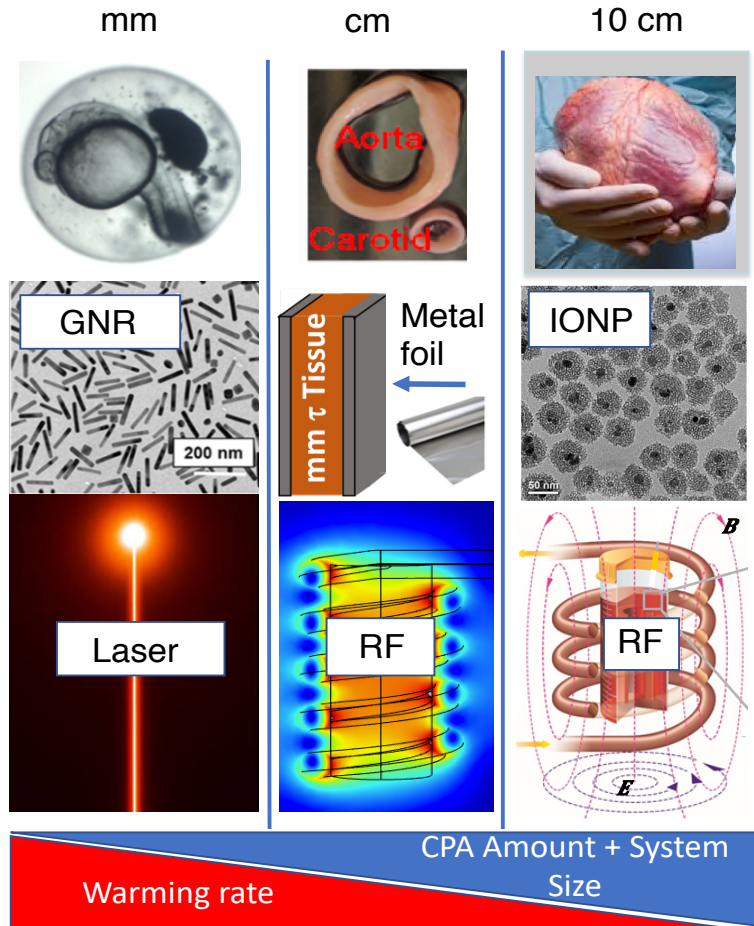
Challenges for organ vitrification and nanowarming



Increasing system size leads to increased complexity in cryopreservation



Applications



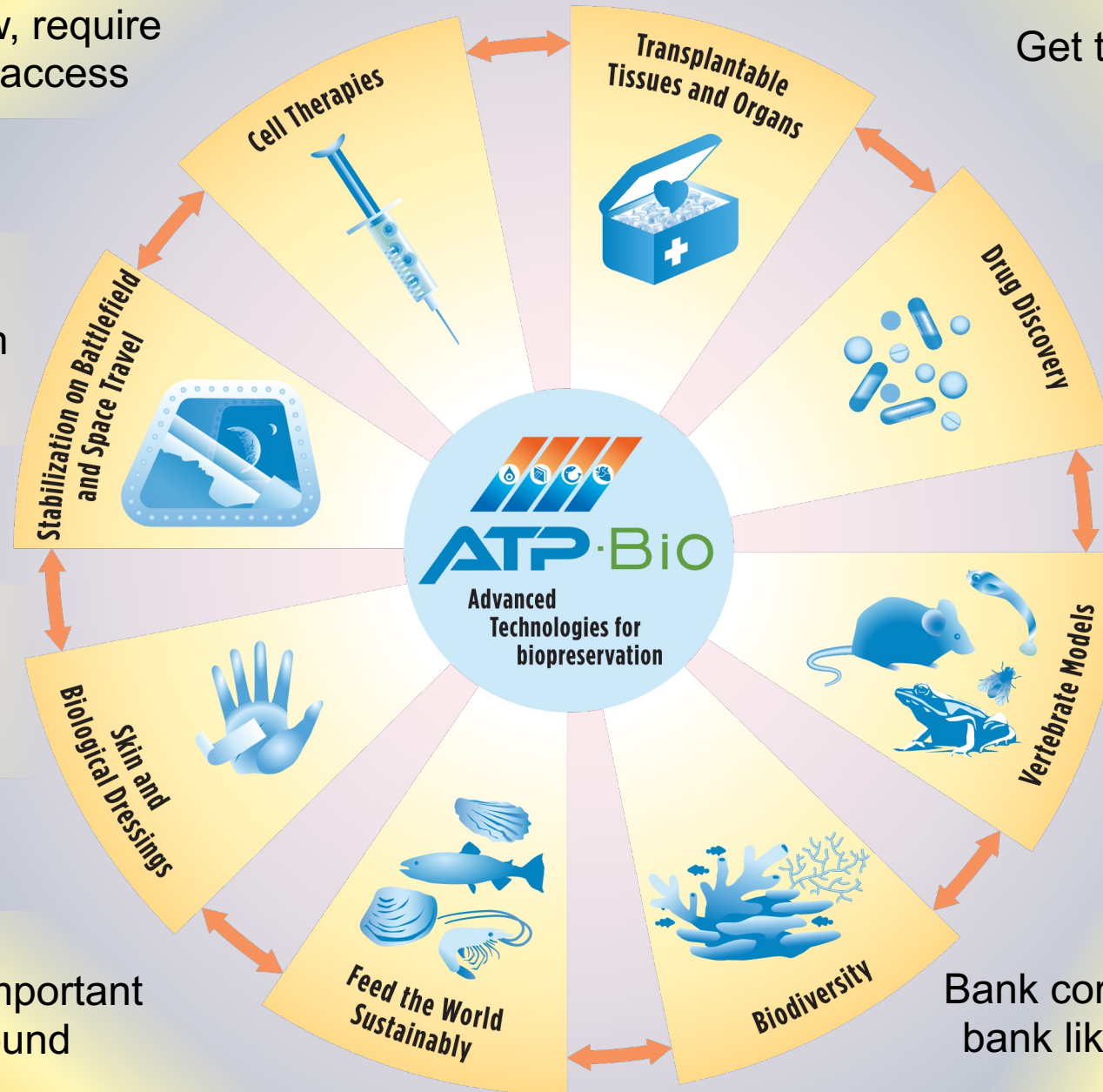
- Organs (Heart, kidney, ovary, liver)
- Flat or annular substances (Blood vessels, skin, cartilage, tissue slices)
- Cell clusters (Islets Shrimp, Zebrafish embryos, coral) embryos

Cells, the drugs of tomorrow, require banking to increase patient access

Improve trauma and battlefield injury stabilization and space travel

Bank tissue and bio-dressings for use in mass casualty events

Create cryo-seed to grow important aquaculture species year round



Get the right organ or tissue to the right person at the right time

Shrink the cost of > \$2 B / drug with greater cell and tissue availability and testing

Bank transgenic lines, thereby stabilizing important disease models

Bank coral reefs and aspire to a fauna bank like Svalbard is for seeds (flora)

UMN Center for Organ Preservation/BRI/ATP-bio

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Funding:

- UMN Faculty Research and Development
- NIH NHLBI
- NIH NIDDK
- Regenerative Medicine Minnesota
- National Science Foundation
- Organ Preservation Alliance



Convergent team science

Cell Biology
Molecular Biology
Genetics
(Bio)Chemistry
Metabolism
Regenerative Medicine
Surgery
Immunology
Ecology

Thermodynamics
Heat Transfer
Mass Transfer
Nucleation Physics
Chemical Kinetics
Metabolic Eng
Microfluidics
MEMS
Nanotechnology
Tissue Engineering



Overarching challenges in stopping biological clock

A Special Thanks to Our Presenter



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Q & A

QUESTIONS & ANSWERS