MATHEMATICAL MODELING AND OPTIMIZATION IN CRYOBIOLOGY

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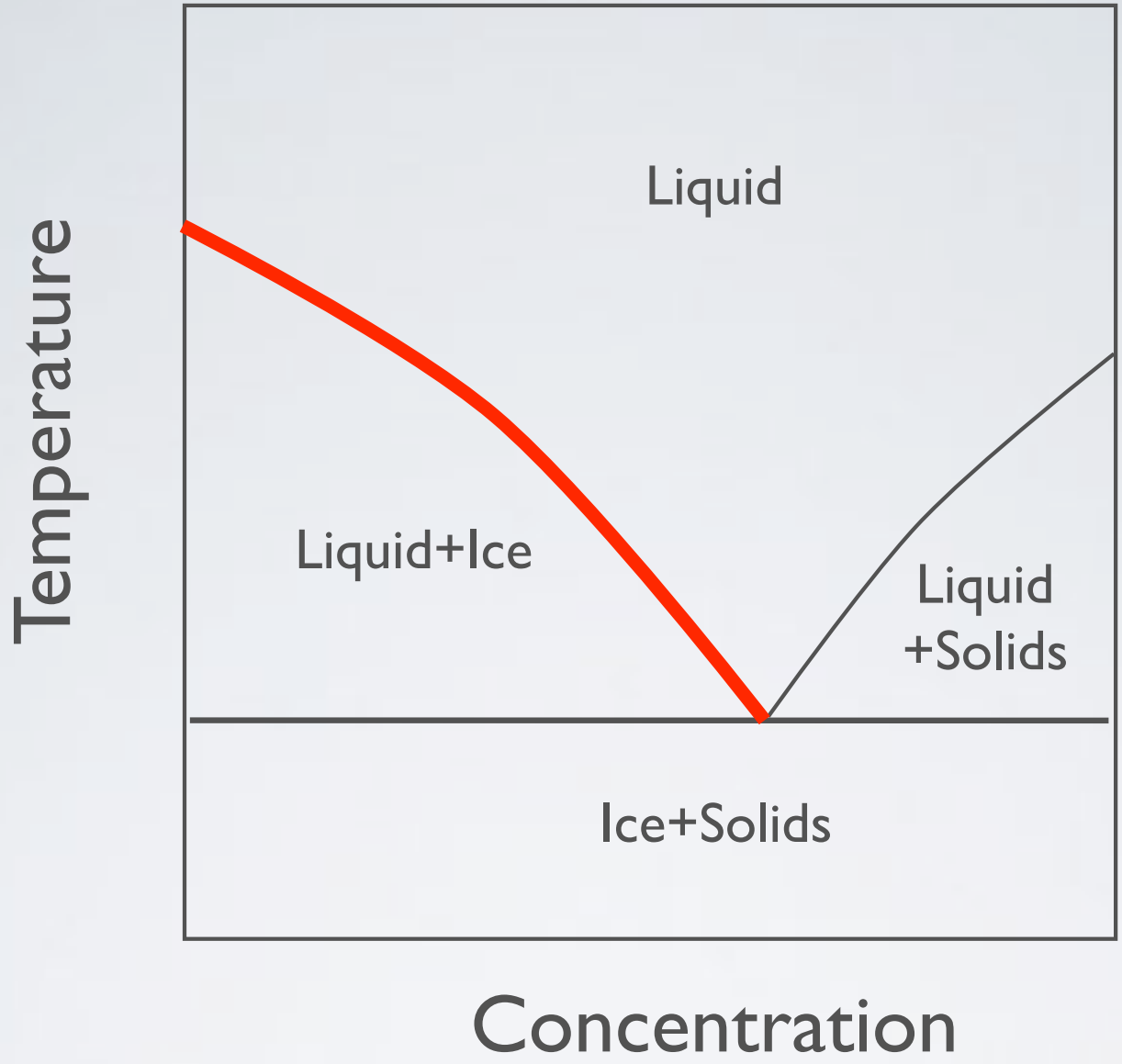
OUTLINE

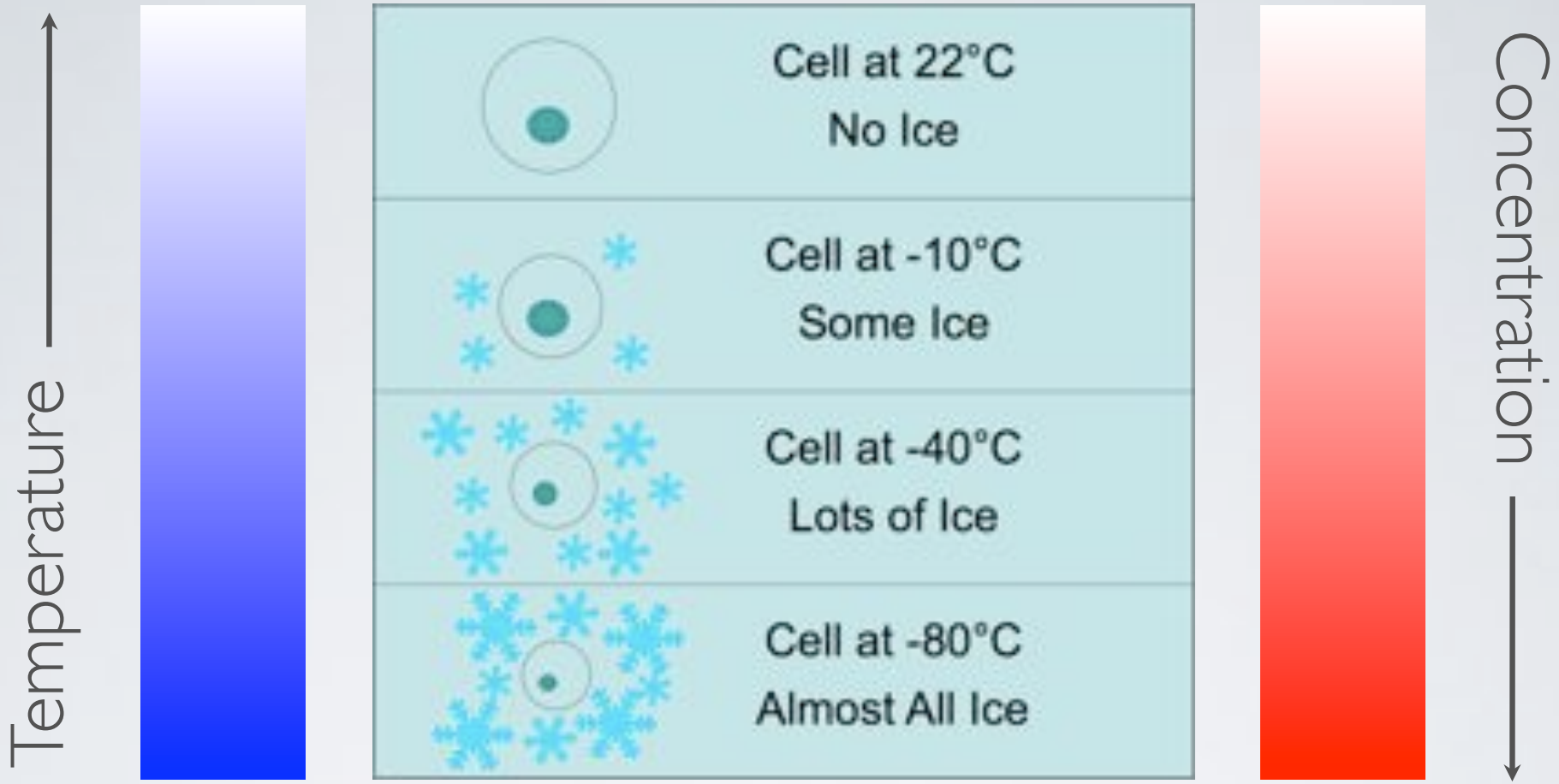
- Brief introduction to principles of Cryobiology
- Model development at three length scales
- Optimal control
- Current and future directions

WHY FREEZE BIOSPECIMENS?

- Colder temperatures mean longer storage: at least 100 years in LN
- Banking, distribution and testing of cells and tissues, maybe organs in the future
- Worldwide initiatives to preserve genetic samples
  - Millennium Seed Bank, Svalbard Seed Bank, UK Biobank (0.5M samples)
  - JAX Sperm bank (>10000 strains)
  - NCRR, MMRRC, MRRRC
  - NCI-Office of Biorepositories and Biospecimine Research
- Kill unwanted cells and tissues in living systems



HOW CELL FREEZING WORKS



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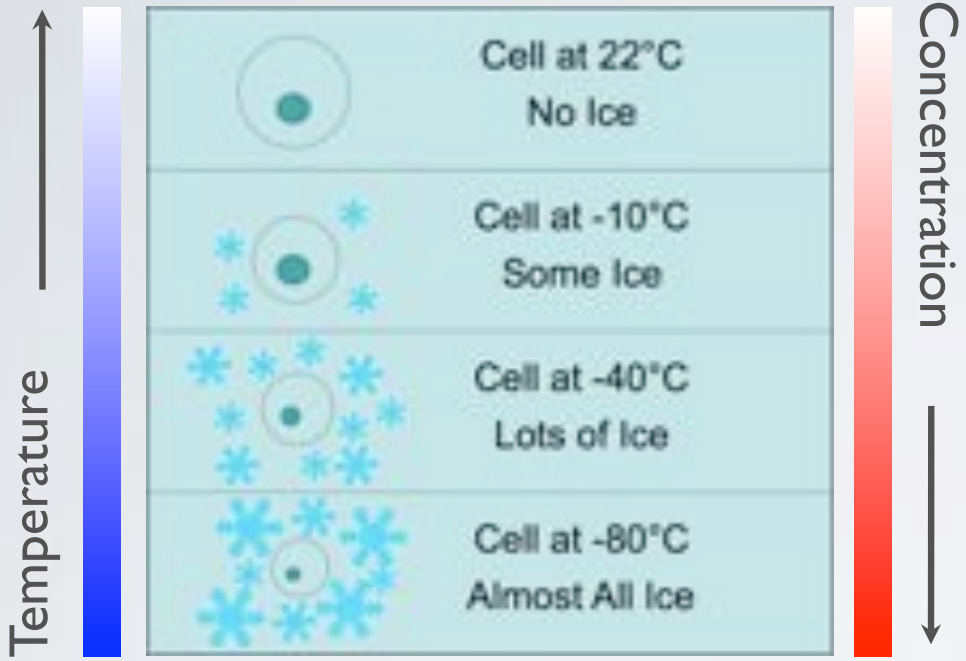
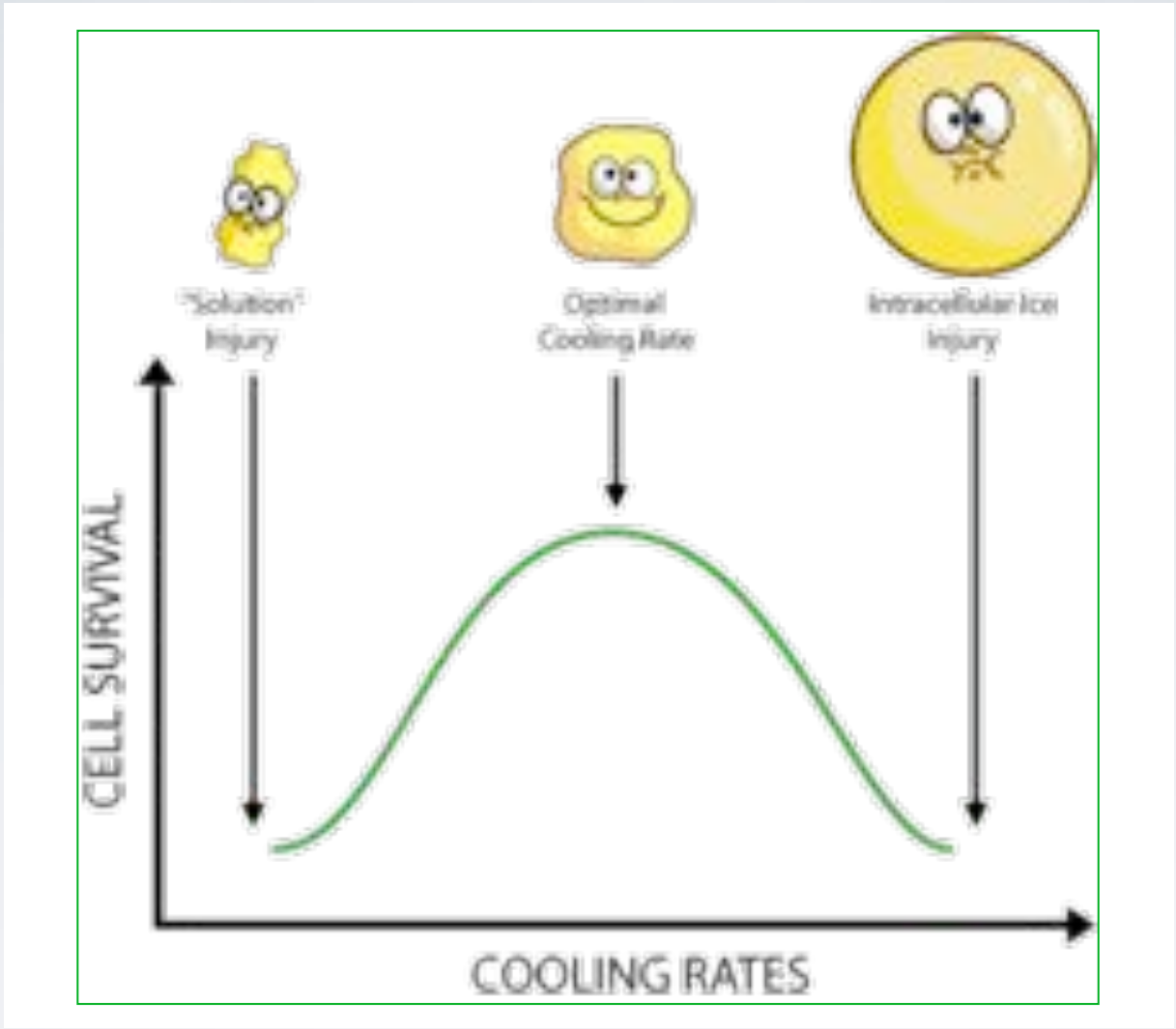
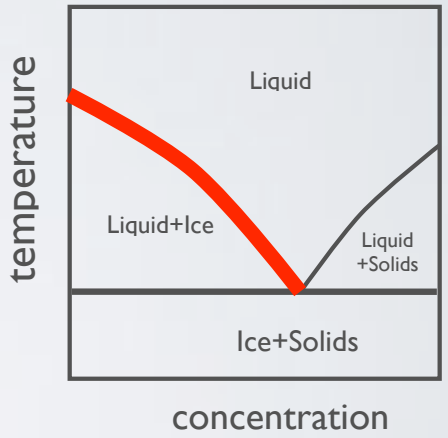


Salt Alone	Salt	CPA
1%	1%	10%
10%	1.5%	15%
30%	3%	30%
-	4%	40%

- To reduce the effects of high salt concentrations and to aid in "glass formation" we add *cryoprotective agents* (CPAs)

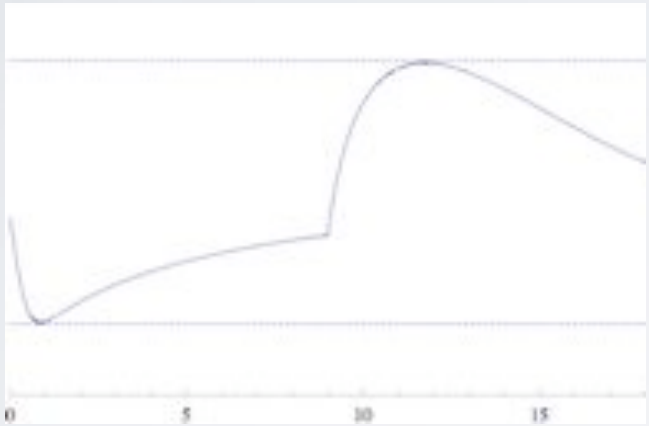




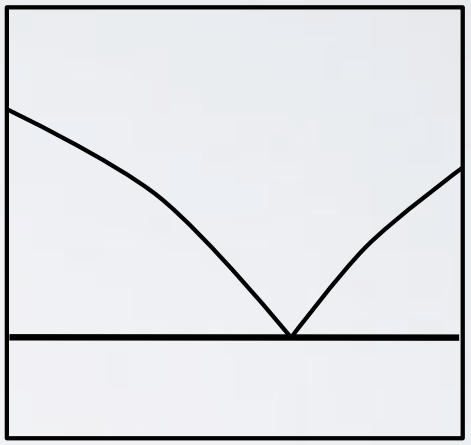
THE TWO FACTOR HYPOTHESIS

CRITICAL CRYOBIOLOGICAL QUANTITIES

Concentration



Heat

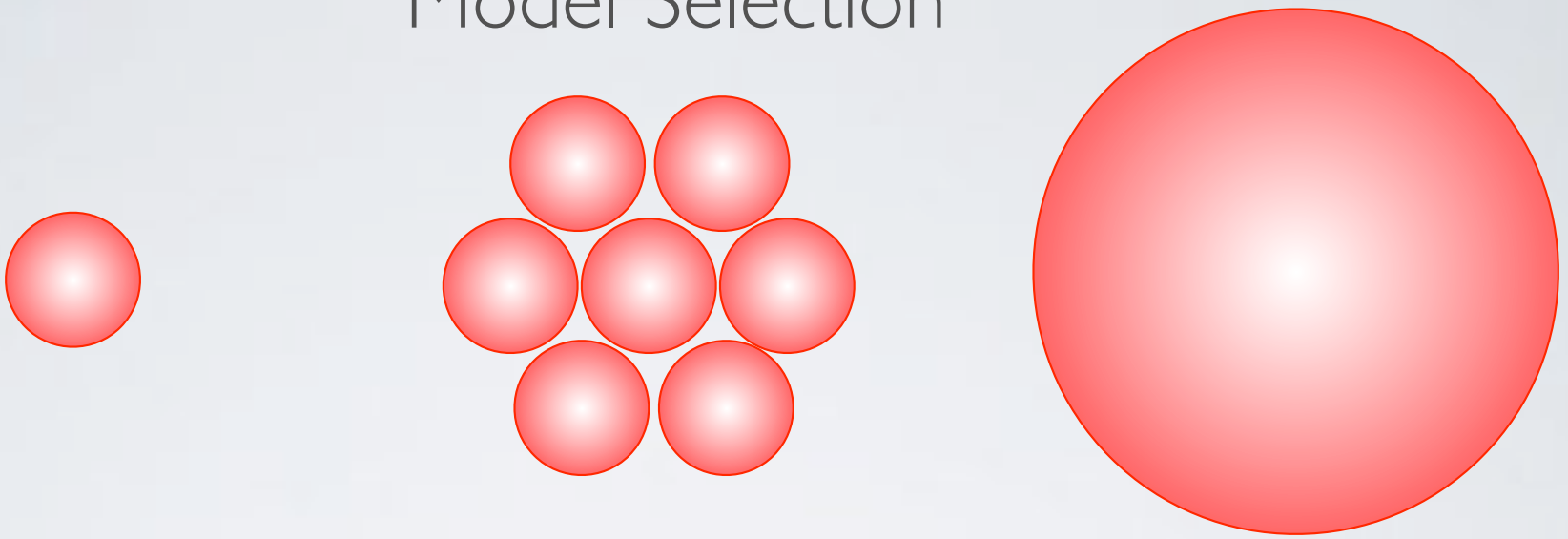


Above 0°C these quantities govern osmotically induced damage

Below 0°C these quantities govern the likelihood of intracellular ice

TRANSPORT PROBLEMS

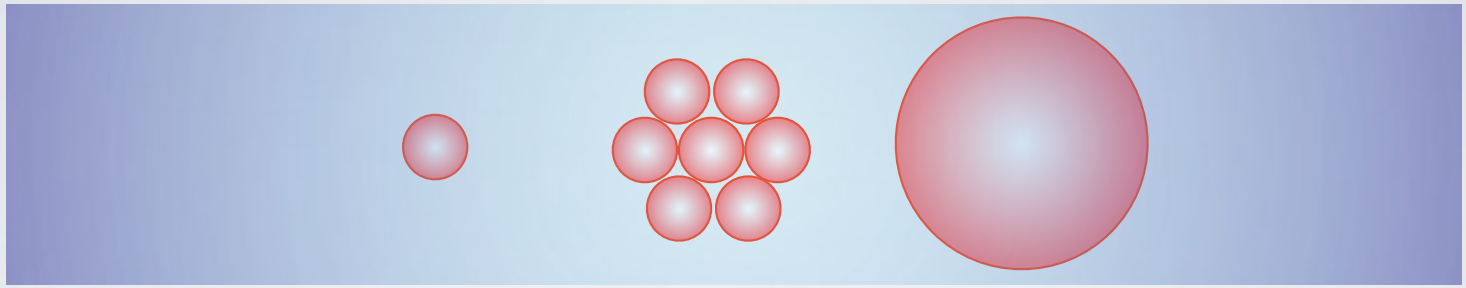
Model Selection



	Single Cell Suspensions	Multi Cell Tissues	Larger Tissues and Organs
Mass	ODE System	Hybrid ODE/PDE System	PDE System
Heat/ Solidi-fication	Stochastic ODE	Large Monte Carlo System	Nonlinear heat & stefan problem

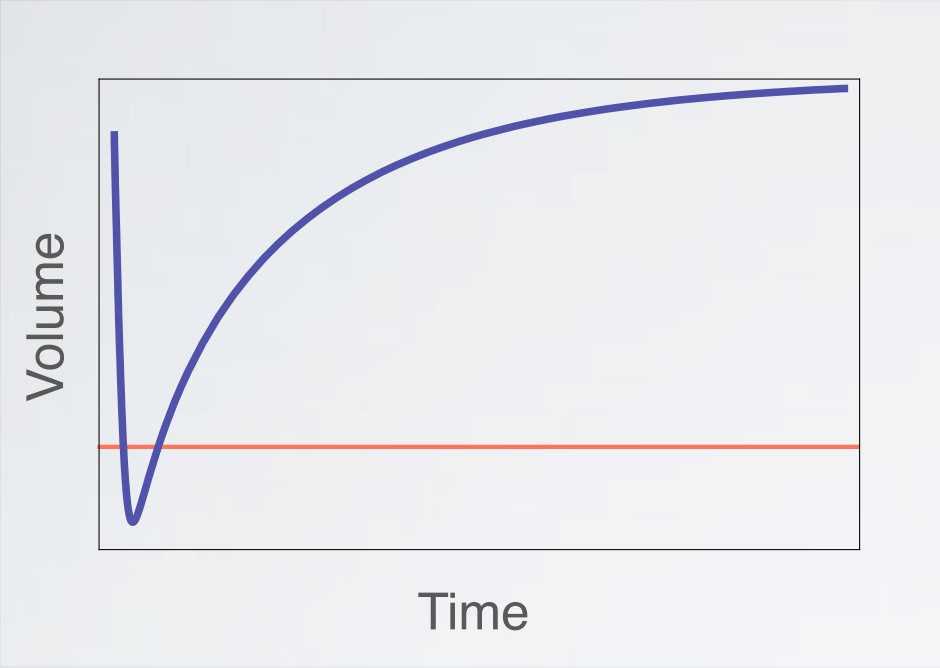
TRANSPORT PROBLEMS

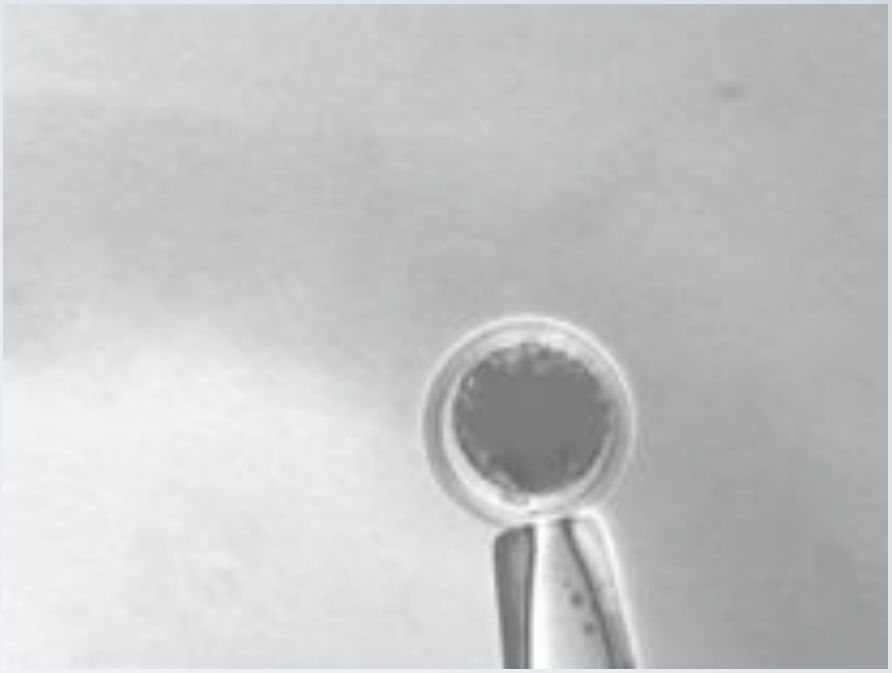
Model Selection



All models in cryobiology are coupled systems!

THE SINGLE CELL PROBLEM





$$\dot{n}_1 = P_1(\mu_1^{ext} - \mu_1^{int})$$
$$\dot{n}_2 = P_2(\mu_2^{ext} - \mu_2^{int})$$

MASS TRANSFER

MASS TRANSFER
THE CHOICE OF μ

$$\Phi(T,P,N) = N_1\mu_0 + \sum_{i=2}^{n} N_i kT \ln\frac{N_i}{eN_1} + \sum_{i=2}^{n} N_i\psi_i + \frac{1}{2N_1}\sum_{i,j=2}^{n}\beta_{ij}N_iN_j$$

Differentiating with respect to N_1 or N_i and setting $\beta_{ij}/kT = (B_i + B_j)$

$$\mu_1 = \mu_0 - kT\left(\sum_{i=2} m_i + \frac{1}{2}\sum_{i,j=2}^{n}(B_i+B_j)m_im_j\right)$$

$$\mu_i = kT\left(\ln m_i + \psi_i^* + \sum_{j=1}(B_i+B_j)m_j\right).$$

JDB. Stability analysis of several non-dilute multiple solute transport equations. *J. Math. Chem.*, In press.

Specific Model: set $B_i = 0$ and $M_i \approx x_2/x_1$.

$$
\begin{aligned}
\dot{x}_1 &= \frac{x_{\mathrm{np}}}{x_1} + \sum_{j=2}^{k} \frac{x_j}{x_1} - \sum_{i=1}^{n} M_i, \\
\dot{x}_2 &= b_2 \left(M_2 - \frac{x_2}{x_1} \right), \\
&\vdots \\
\dot{x}_n &= b_n \left(M_n - \frac{x_n}{x_1} \right),
\end{aligned}
$$

Cellular Quantities

$x_1 =$ Water Volume

$x_{2,\ldots,n} =$ Moles of permeating solute

$x_{\mathrm{np}} =$ Moles of nonpermeating solute

$b_{2,\ldots,n} =$ Relative permeability

Extracellular Quantities

$M_1 =$ Nonpermeating solute molality

$M_{2,\ldots,n} =$ Permeating solute molality

$\bar{M}_i =$ Maximal ith solute molality

I. Katkov. A two-parameter model of cell membrane permeability for multisolute systems. *Cryobiology*, 40(1):64–83, Feb 2000.

SINGLE CELLS

Specific Model

$$
\begin{aligned}
\dot{x}_1 &= \frac{1}{x_1}\left(x_{\mathrm{np}} + \sum_{j=2}^{k} x_j - \sum_{i=1}^{n} M_i x_1\right), \\
\dot{x}_2 &= \frac{b_2}{x_1}\left(M_2 x_1 - x_2\right), \\
&\vdots \\
\dot{x}_n &= \frac{b_n}{x_1}\left(M_n x_1 - x_n\right),
\end{aligned}
$$

Cellular Quantities

$x_1 =$ Water Volume

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I. Katkov. A two-parameter model of cell membrane permeability for multisolute systems. *Cryobiology*, 40(1):64–83, Feb 2000.

We have a system of the form $\dot{x}(t) = \lambda(x(t))f(x(t))$, where $\lambda(x(t)) = 1/x_1(t)$ is a positive scalar function. In this case, we can define an invertible transformation

$$q(\tau) = \int_0^\tau \frac{1}{\lambda(x(s))} ds = \int_0^\tau x_1(s)\, ds$$

and a new system $w'(\tau) = f(w(\tau))$ such that

$$w(\tau) = x(q(\tau))$$

meaning that we may, without any penalty, linearize the system by removing the $1/x_1$ term.

JDB, C Chicone, J Critser. Exact solutions to a two parameter flux model and cryobiological implications. *Cryobiology,* **50**, 308-316, 2005

$$
\begin{aligned}
x_1'(\tau) &= x_{\mathrm{np}} + \sum_{j=2}^{n} x_j - \sum_{i=1}^{n} M_i(\tau) x_1, \\
x_2'(\tau) &= b_2 \left(M_2(\tau) x_1 - x_2 \right), \\
&\vdots \\
x_n'(\tau) &= b_n \left(M_n(\tau) x_1 - x_n \right).
\end{aligned}
$$

or

$$
x' = f(x, M) := A(M)x + x_{np} e_1,
$$

where

$$
A(M) = \begin{pmatrix}
-\sum_{i=1}^{n} M_i & 1 & 1 & \dots & 1 \\
b_2 M_2(t) & -b_2 & 0 & \dots & 0 \\
b_3 M_3(t) & 0 & -b_3 & \dots & 0 \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
b_n M_n(t) & 0 & 0 & \dots & -b_n
\end{pmatrix}.
$$

Define

$$D := \mathrm{diag}(1, (b_2 M_2)^{-1/2}, \ldots, (b_n M_n)^{-1/2})$$

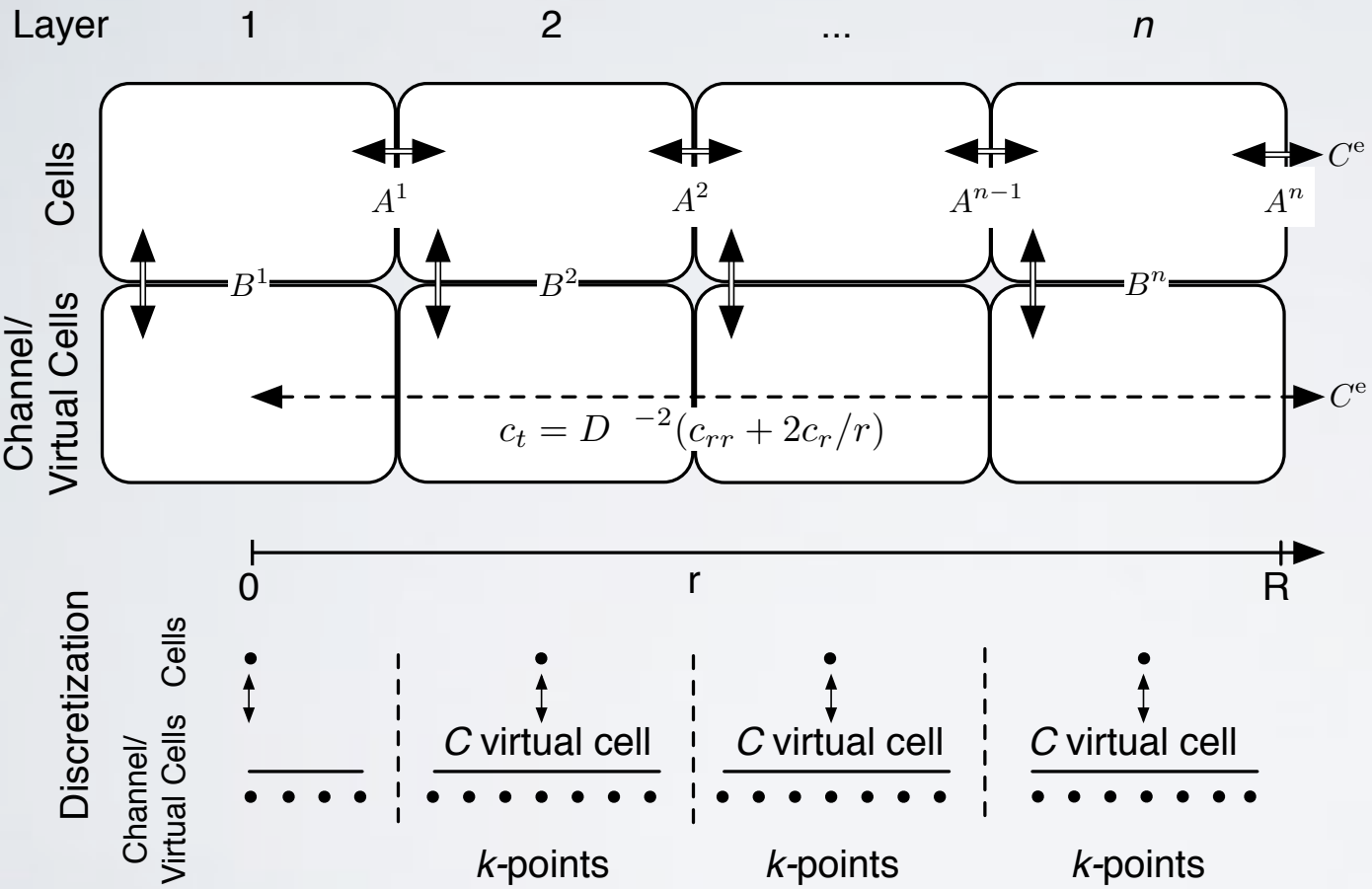
Then:

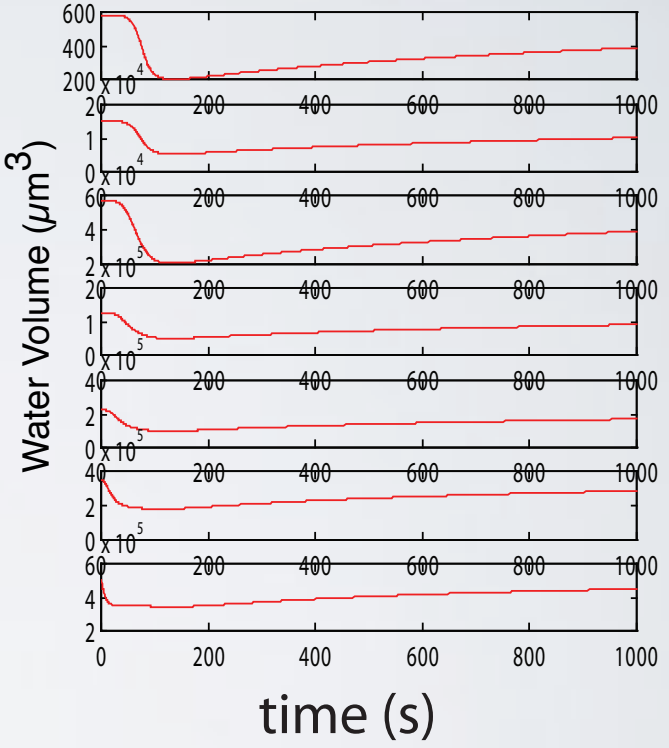
$$DA(M)D^{-1} = \begin{pmatrix} -\sum_i M_i & \sqrt{b_2 M_2} & \sqrt{b_3 M_3} & \ldots & \sqrt{b_n M_n} \\ \sqrt{b_2 M_2} & -b_2 & 0 & \ldots & 0 \\ \sqrt{b_3 M_3} & 0 & -b_3 & \ldots & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \sqrt{b_n M_n} & 0 & \ldots & 0 & -b_n \end{pmatrix}$$

is symmetric, negative definite, and our original n-dimensional nonlinear system is globally asymptotically stable.

JDB, C Chicone, J Critser. A general model for the dynamics of cell volume, global stability, and optimal control . *J. Math Bio.*, In press

MASS TRANSPORT IN SMALL TISSUES

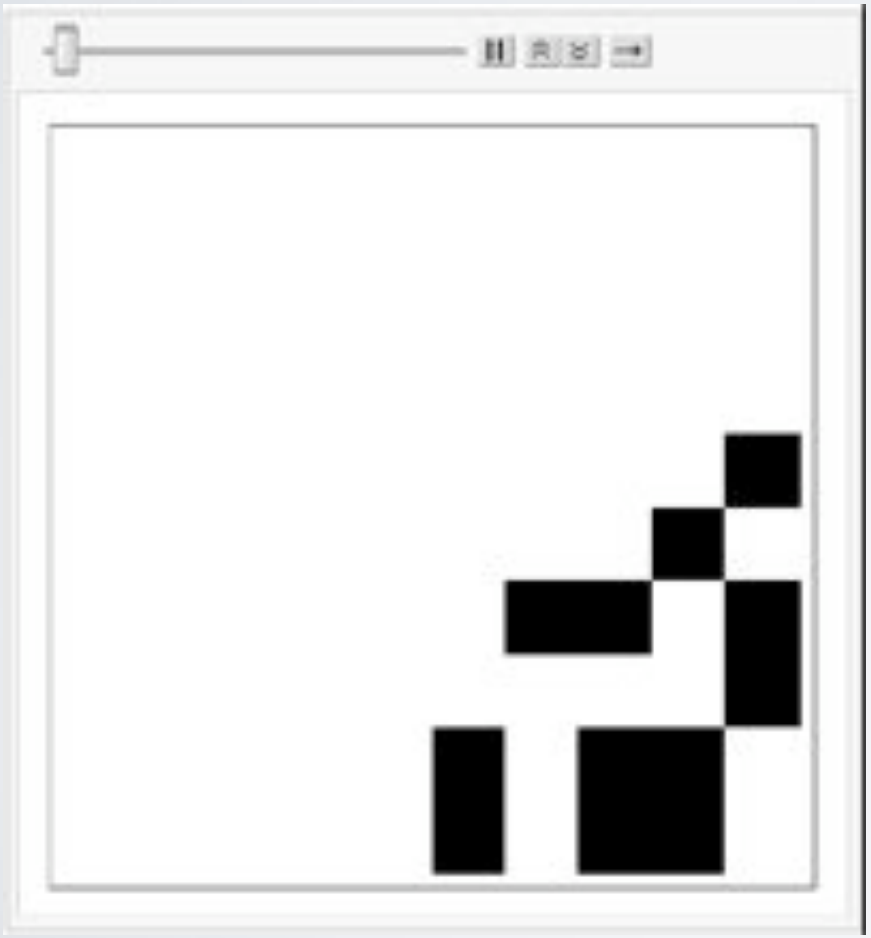




JDB, C Benson, J Critser. *Submitted to J. Biomech Eng.*

SOLIDIFICATION DURING COOLING, SMALL TISSUES:

Monte Carlo Simulation of IIF



$$\begin{aligned} p_j(\delta\tau) &\approx p_j^i + p_j^p \\ &\approx (1 + k_j\alpha)\delta t \end{aligned}$$

p_j is the probability of ice

p_j^i is the probability of ice forming spontaneously

p_j^p is the probability of ice propagating from neighbor

k_j is the number of icy neighbors

D Iremia, J Karlsson. *Biophys. J.* **88** 647-660, 2005.

MASS TRANSFER IN LARGE TISSUES

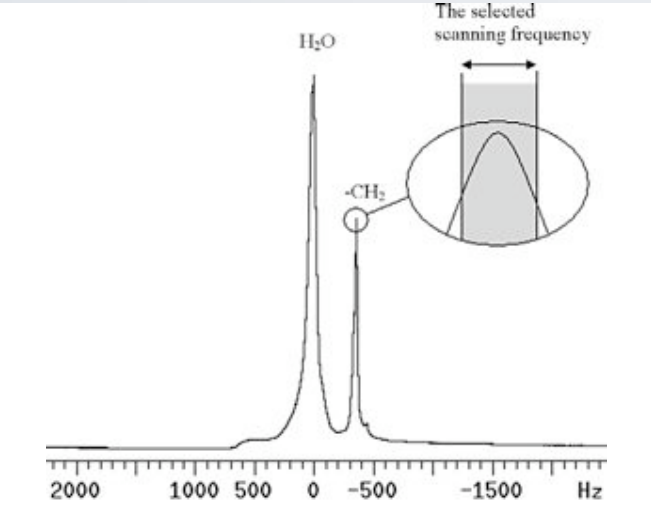


Fig. 2. The proton MR spectrum at 7 T from the sample holder loaded with two ovaries and 40% (w/w) EG solution. The excitation frequency was centered at the resonance frequency for the $-CH_2$ group in EG molecules.

$$\frac{\partial c}{\partial t} = \nabla \cdot (D \nabla c)$$

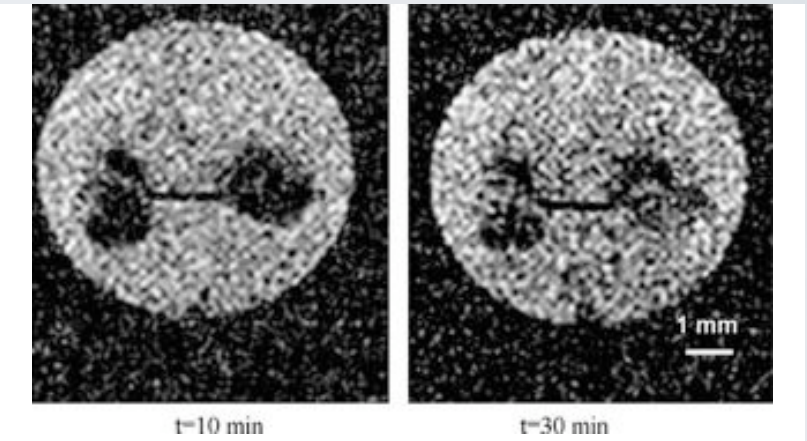


Fig. 5. Two sample MR images, with water signal saturated, showing the increasing EG concentration in ovaries during perfusion.

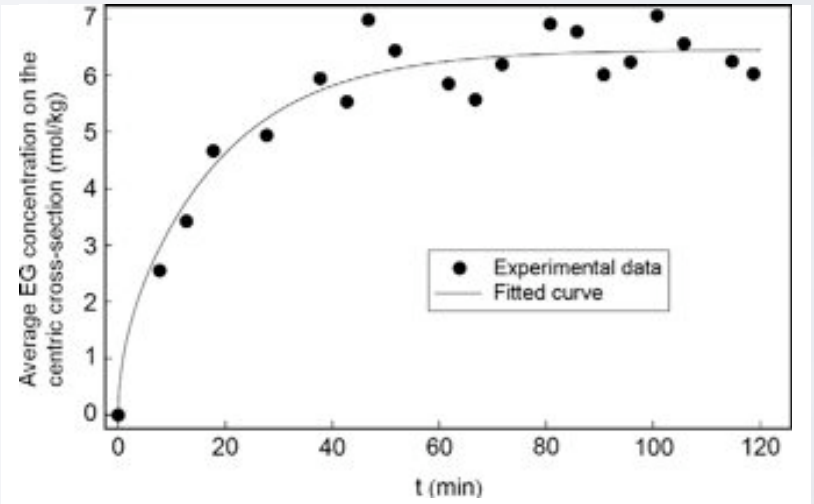
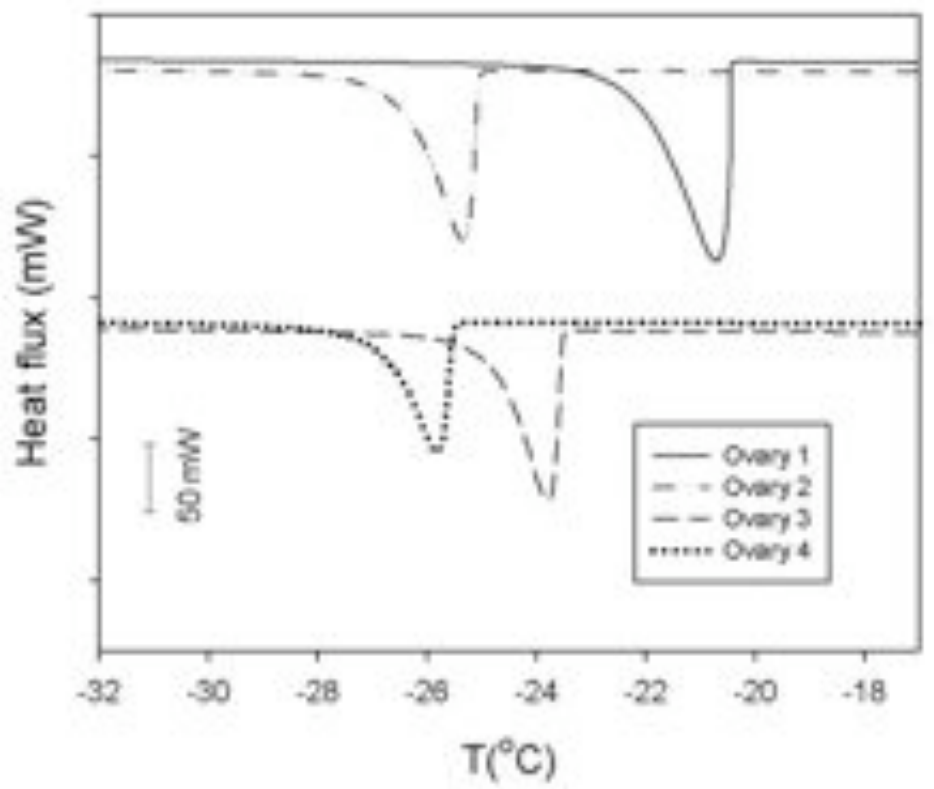
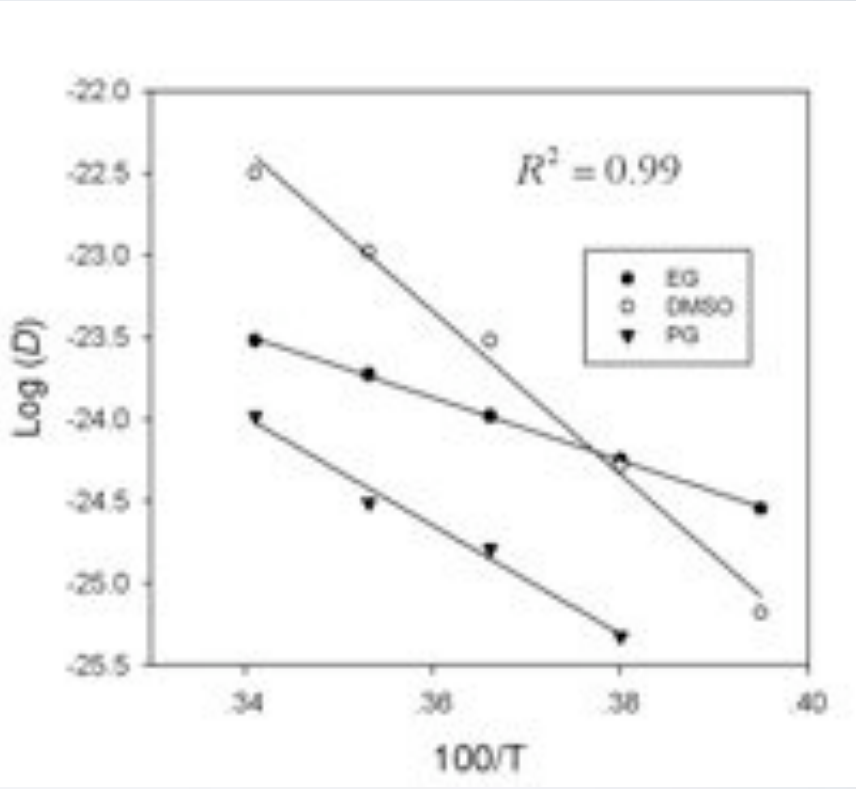


Fig. 6. The experimental data with their fitted curve for the average EG concentration change on the centric cross-section of an ovary with 1.1 mm as its identical radius.

X Han, L Ma, A Brown, JDB, J Critser. *Cryobiology*, **58** (3), 2009

MASS TRANSFER IN LARGE TISSUES

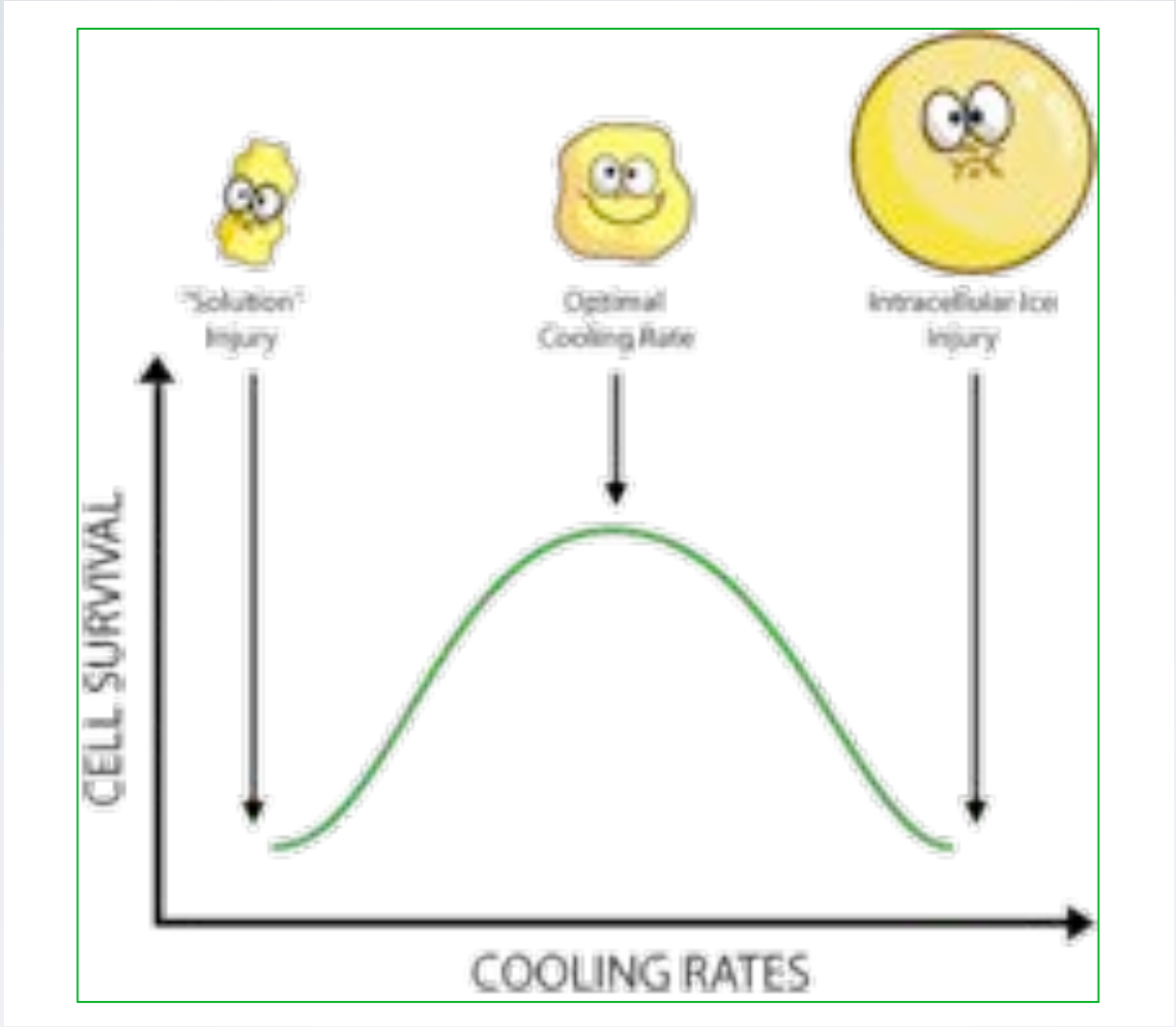


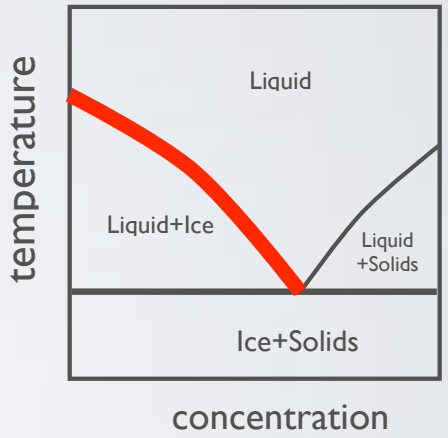


$$\frac{\partial c}{\partial t} = \nabla \cdot (D(T)\nabla c)$$

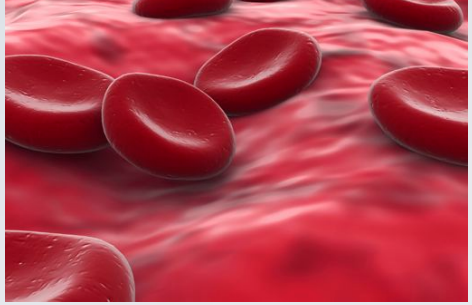
$$D(T) = \exp(-E_a/RT)$$

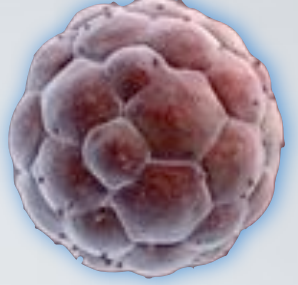
X Han, L Ma, A Brown, JDB, J Critser. In review: *IJHMT*

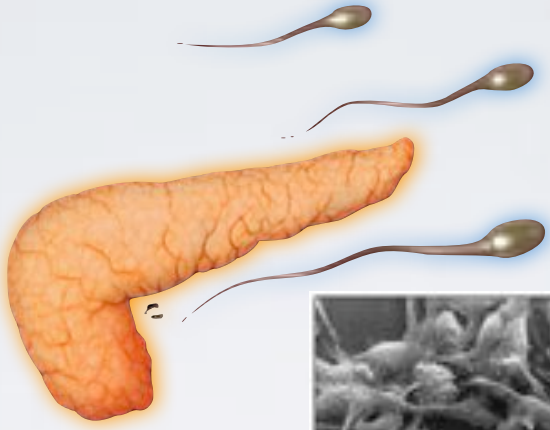


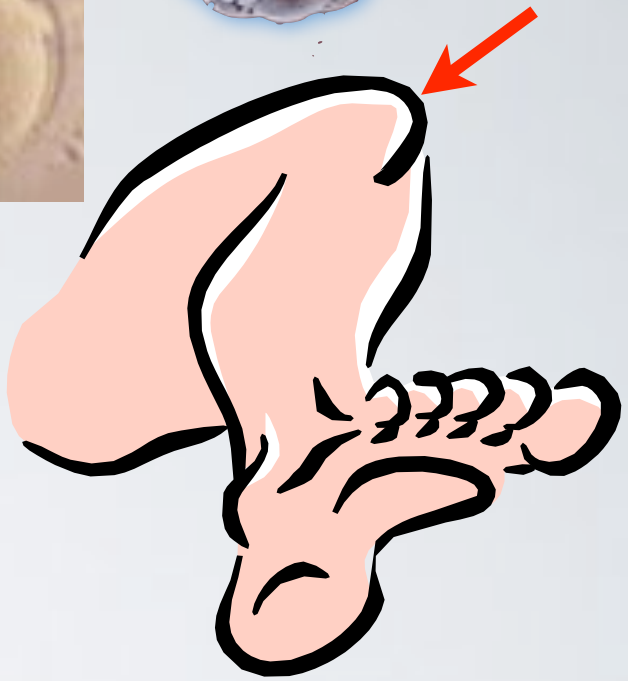


WHAT CAN WE CONCLUDE FROM THE ABOVE MODELS?









HEAT AND MASS TRANSFER LIMIT THE SIZE OF FREEZABLE TISSUE!

DOES MODELING IN CRYOBIOLOGY WORK?

Available online at www.sciencedirect.com

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Cryobiology 56 (2008) 120–130

CRYOBIOLOGY

www.elsevier.com/locate/ycryo

An improved cryopreservation method for a mouse embryonic stem cell line ☆

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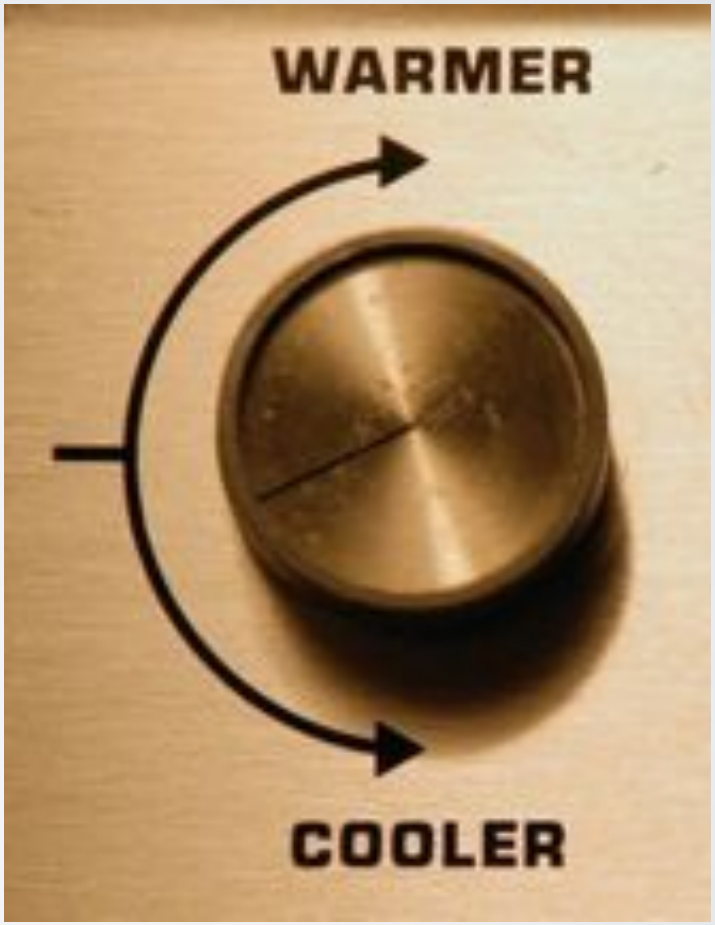
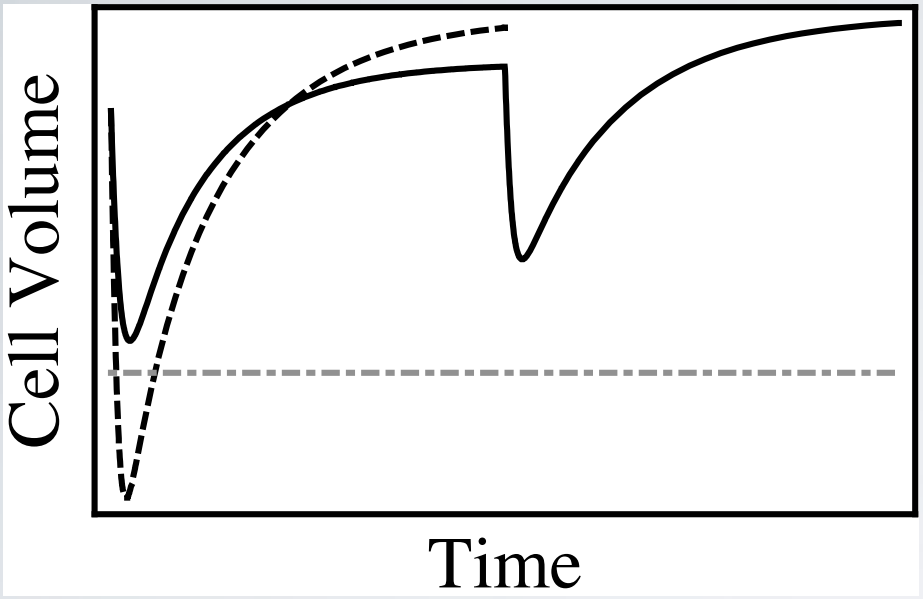
Received 15 May 2007; accepted 3 December 2007
Available online 14 January 2008

- Previous best protocol: 31% recovery
- "Optimally" defined new best protocol: 64% recovery

OPTIMAL CONTROL IN CRYOBIOLOGY:

- control quantity to minimize cost J (e.g. time, energy, stress, P_{IIF} or combinations.)
- subject to exact and inequality constraints:
  - exact constraints: governing physical system, (e.g. 2P model, heat equation, diffusion, etc).
  - inequality constraints: state or control constraints, (e.g. cell volume > 0).





$$\min_{M \in \mathcal{A}} s_f \quad \text{subject to}$$

$$\dot{w}_1 = \frac{x_{\text{np}}}{w_1} + \frac{w_2}{w_1} - M_1 - M_2,$$

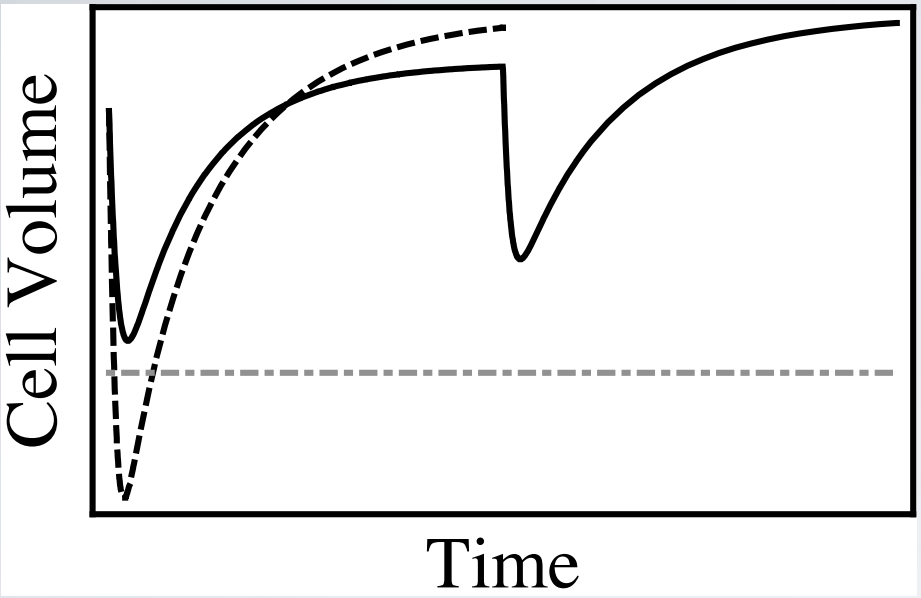
$$\dot{w}_2 = b_2 \left(M_2 - \frac{w_2}{w_1} \right),$$

and

$$w_1 + \gamma w_2 - k^* \leq 0,$$

$$k_* - w_1 - \gamma w_2 \leq 0.$$

FIRST CONTROL PROBLEM



$$\min_{M \in \mathcal{A}} s_f = q(t_f) = \int_0^{t_f} x_1(\tau)\, d\tau$$

subject to

$$\begin{aligned} \dot{x}_1 &= -(M_1 + M_2)x_1 + x_2 + x_{\mathrm{np}} \\ \dot{x}_2 &= bM_2 x_1 - bx_2 \end{aligned}$$

and

$$x_1 + \gamma x_2 - k^* \leq 0,$$
$$k_* - x_1 - \gamma x_2 \leq 0.$$

Bilinear state equation (in controls and state) give:

Existence ✓ Controllability ✓

OPTIMAL CONTROL

$$H(x^*, p^*, M^*) = \max_{M \in CP} (A(M)x + x_1 e_1) \cdot p - x_1$$

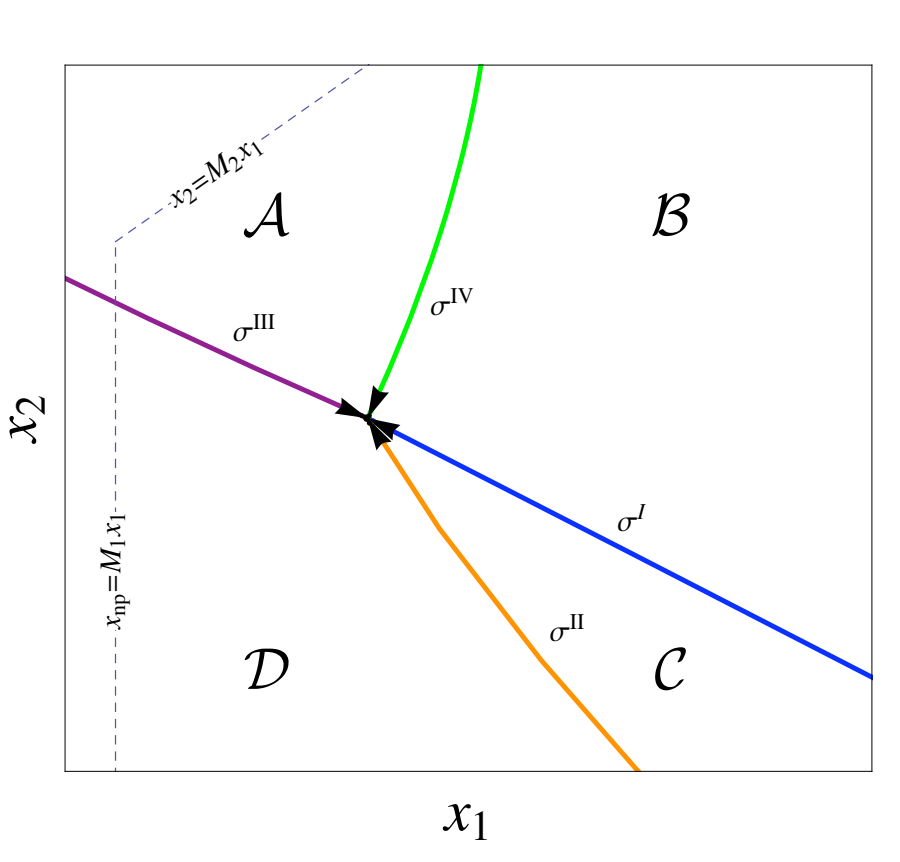
$$= \max_{M \in CP} \Big(- M_1 x_1 p_1 + x_1 \sum_{i=2}^{n} M_i (b_i p_i - p_1)$$

$$+ \text{terms with no } M \Big)$$

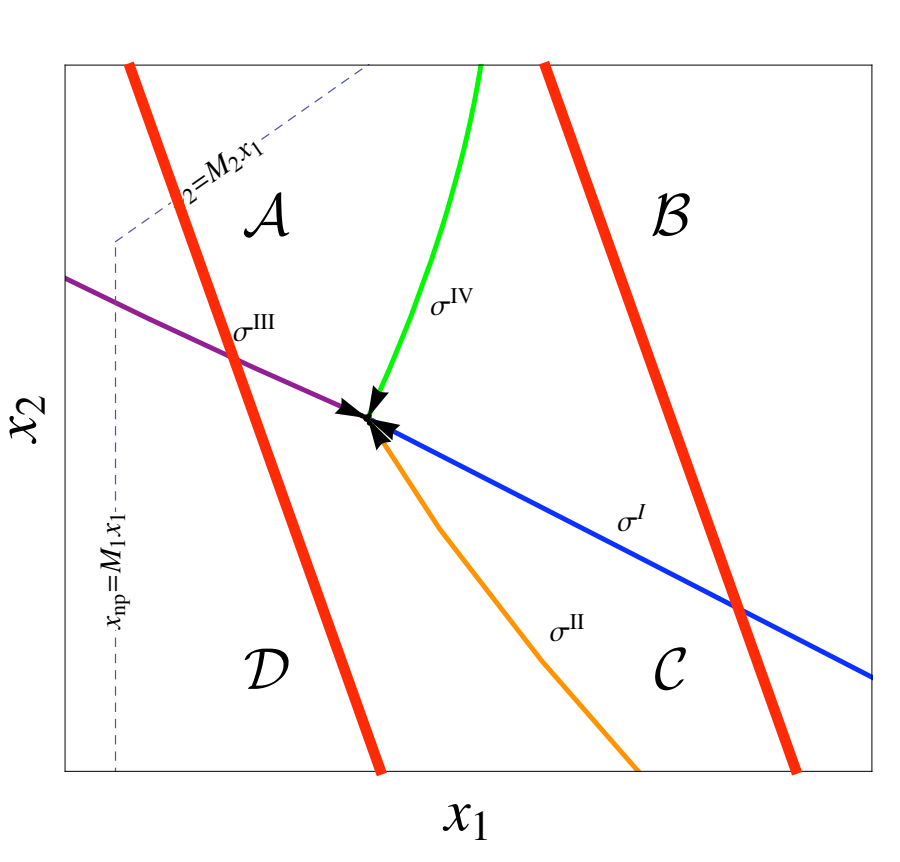
$$M_1(t) = \begin{cases} 0, & p_1 > 0 \\ \bar{M}_1, & p_1 \leq 0 \end{cases}$$

$$M_i(t) = \begin{cases} 0, & b_i p_i - p_1 < 0 \\ \bar{M}_i, & b_i p_i - p_1 \geq 0 \end{cases} .$$

Optimal controls maximize the Hamiltonian



WHY GEOMETRIC OPTIMAL CONTROL?



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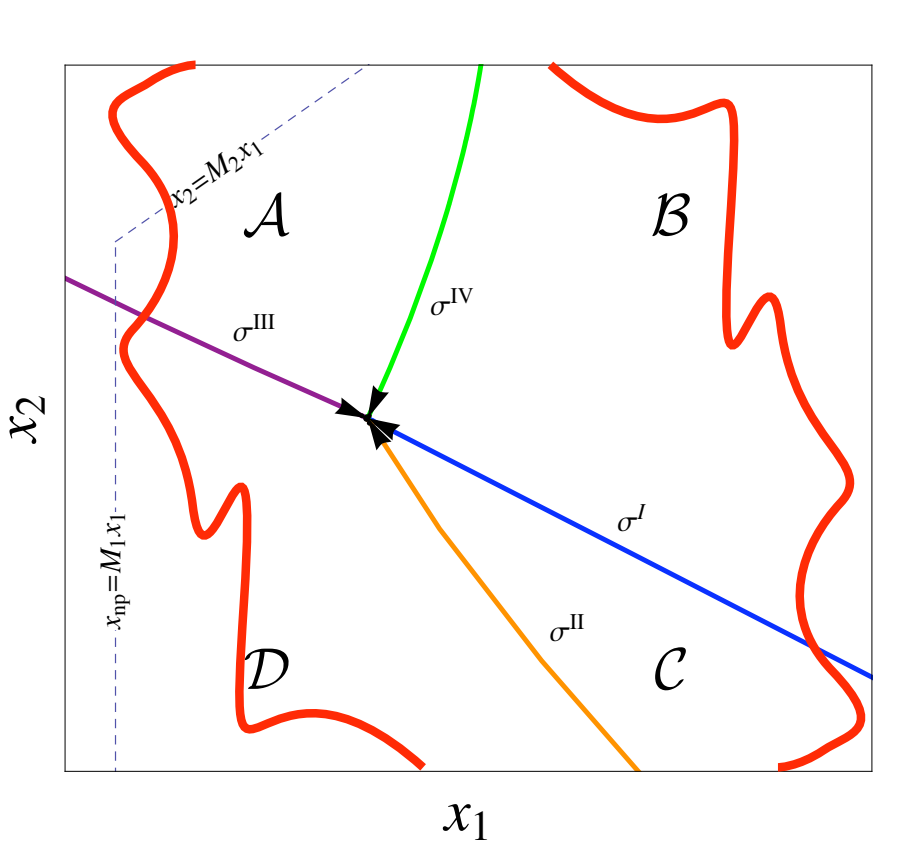
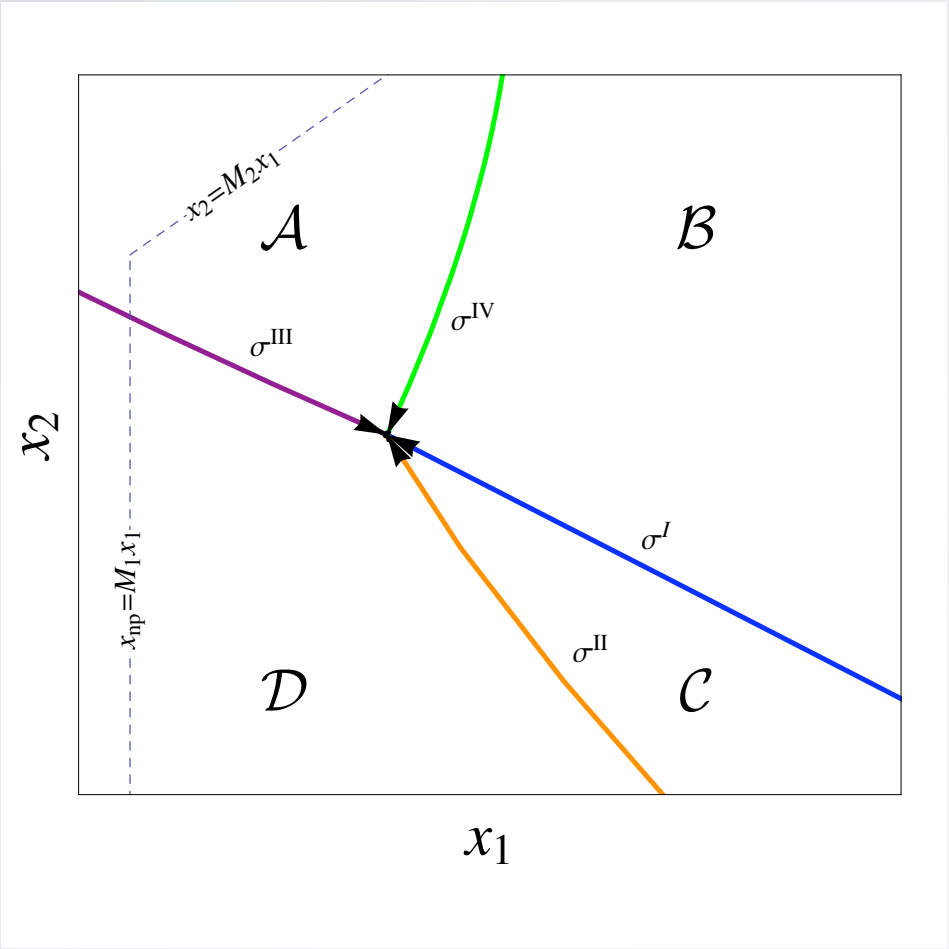


WHY GEOMETRIC OPTIMAL CONTROL?

Boltayanskii sufficiency theorem: a "regular, distinguished" trajectory defined by a state dependent control function $v(x)$ is optimal.



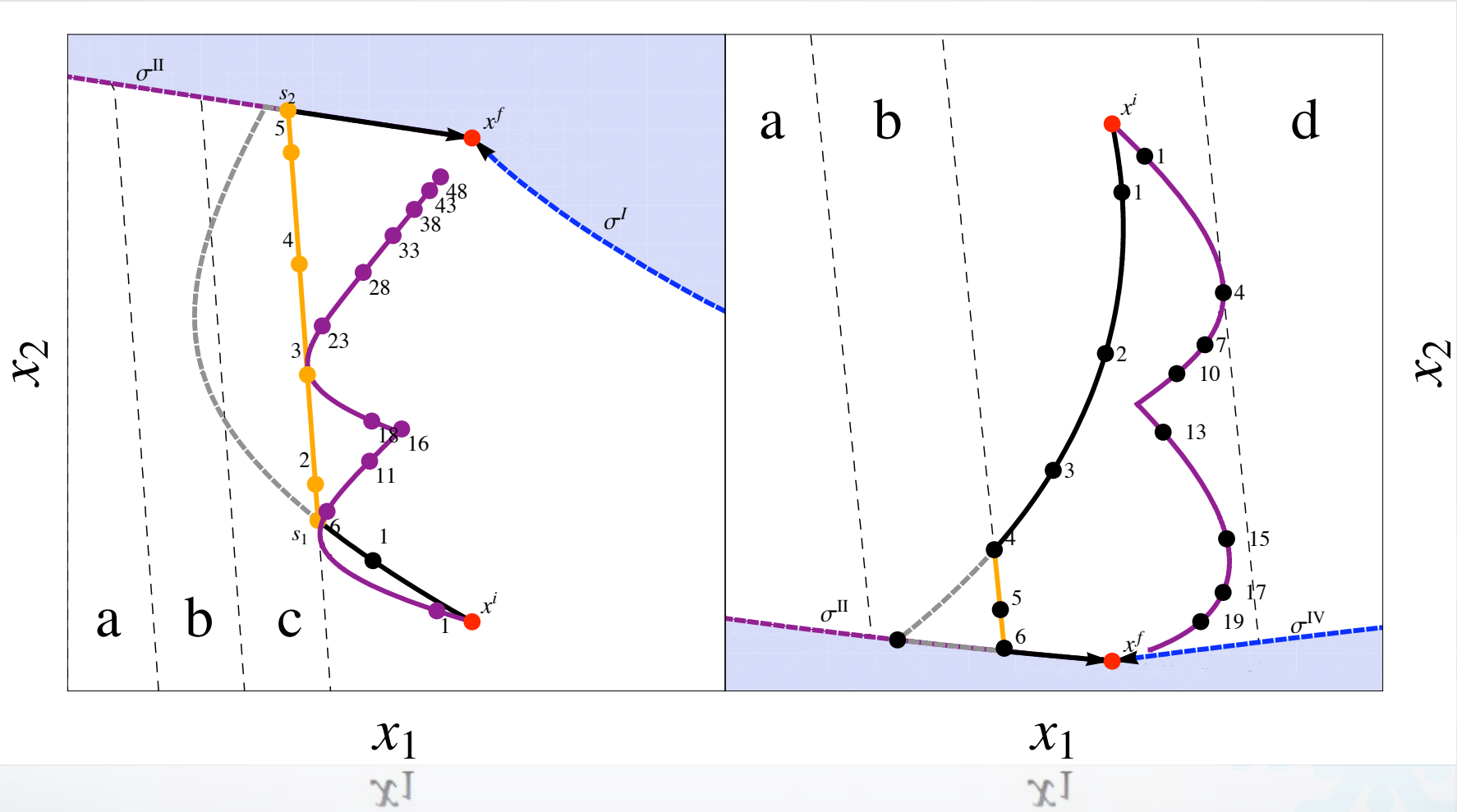
Region	Control Scheme	M_1	M_2
σ^I	M^I	$\bar{M}_1$	$\bar{M}_2$
C, D, σ^{II}	M^{II}	0	$\bar{M}_2$
σ^{III}	M^{III}	0	0
A, B, σ^{IV}	M^{IV}	$\bar{M}_1$	0

SUFFICIENCY

RESULTS

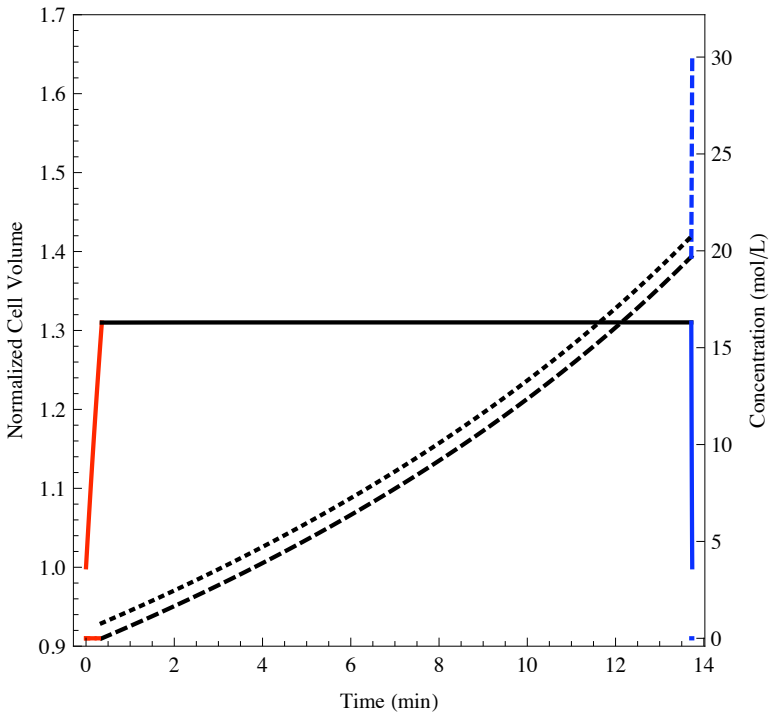
CPA Addition

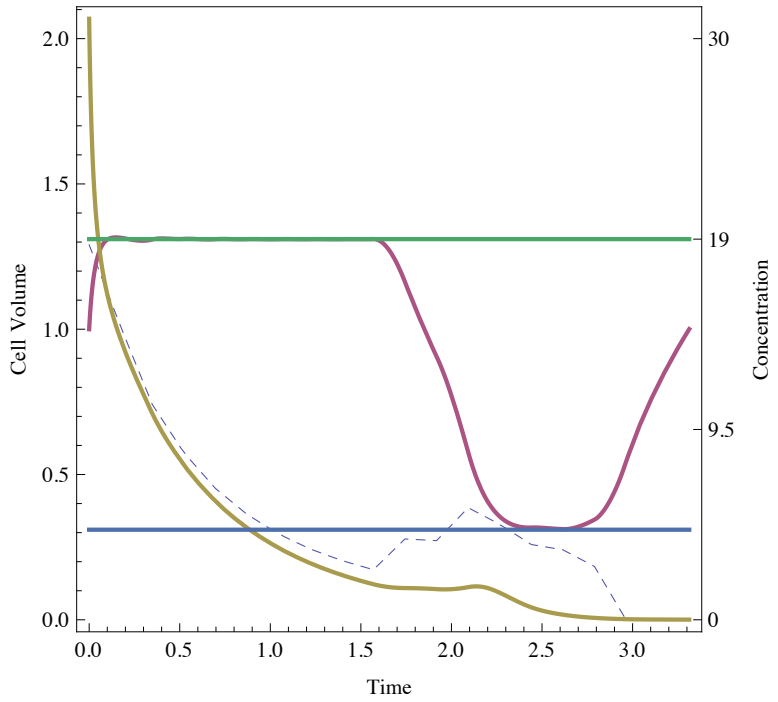
CPA Removal



Addition

Removal





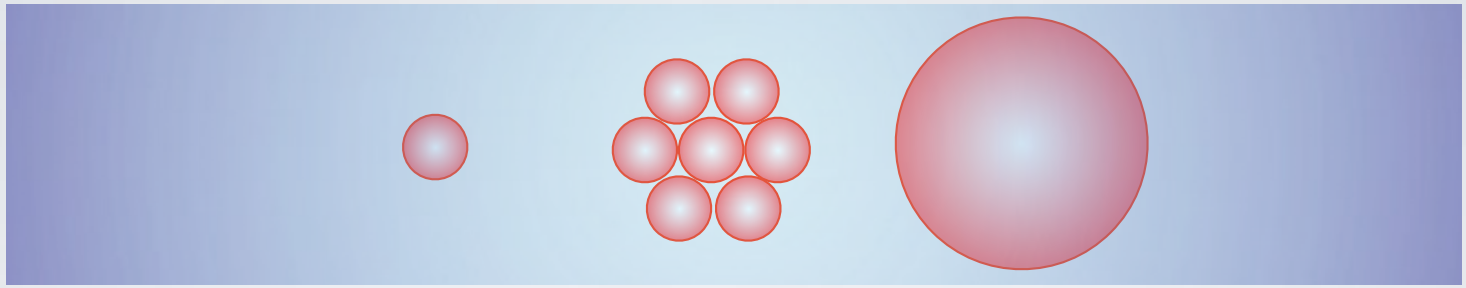
Cost function:

$$J = \int_0^T C_{\text{cell}}(t)^2 \, dt$$

Solved with a direct method: parametrize system with piecewise linear controls, minimize constrained system with a truncated-Newton approach to the augmented Lagrangian

OPTIMAL CONTROL

System Coupling



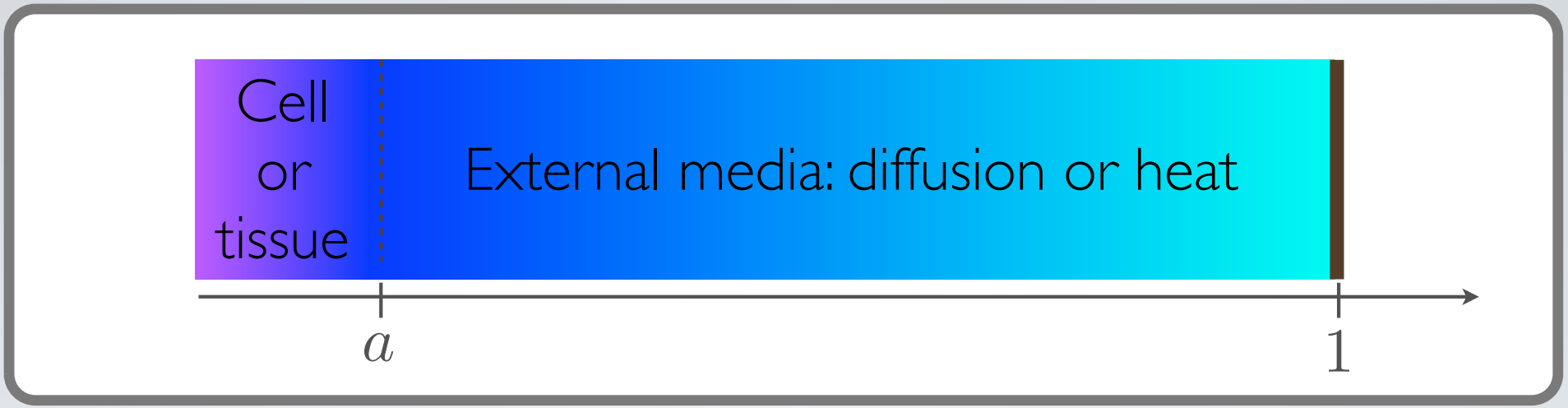
All models in cryobiology are coupled systems!

	Before cooling	During cooling
Mass & Heat	Parabolic System	Thermal convection/mushy layers/etc...

EXTENSION TO TISSUES, SYSTEMS

- Find $c^e(t)$ such that $f(t)$ approximates the desired independent control $u_D(t)$, known a priori
- Can use "inverse problem" techniques to solve analytically
- This gives a tool to develop numerical schemes for completely novel optimal control problems

*A Carasso, *SIAM J App. Anal.* 198



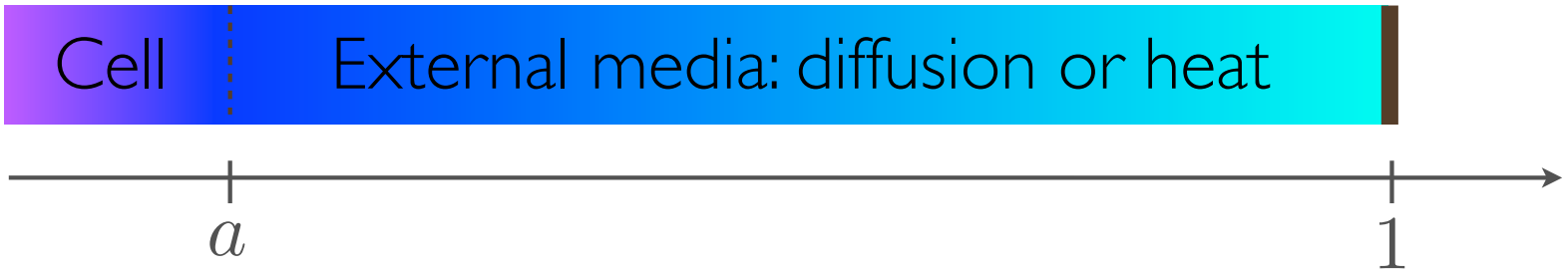
In Media

$$
\left|
\begin{aligned}
c_t &= D\left(c_{rr} + \frac{2}{r}c_r\right) \quad (r,t) \in (a,1) \times [0,\\
\frac{\partial c}{\partial r} &= k(c^c - c), \quad (r,t) \in \{a\} \times [0,\infty),\\
c &= c^e, \quad (r,t) \in \{1\} \times [0,\infty),\\
c &= c_0, \quad (r,t) \in [0,1] \times \{a\},
\end{aligned}
\right.
$$

In Cell

$$
\left|
\begin{aligned}
\dot{x} &= h(x, c(a,t))\\
c^c &= \mathcal{M}c(a,t)
\end{aligned}
\right.
$$

$$
\begin{aligned}
\mathcal{M} &= M_1 M_2,\ M_1 : (x,y) \to x/y,\\
d(M_2 c(a,t))/dt &= h(M_2 c(a,t), c(a,t))
\end{aligned}
$$



After Laplace transform, we may solve for

$$\bar{f}(s) = \bar{h}_1(s)\bar{c}^e(s) + \bar{h}_2(s)\bar{c}^c(s)$$

where $\bar{h}_1(s),\ \bar{h}_2(s)$ are modified spherical Bessel functions*. Thus,

$$\begin{aligned} f(t) &= \int_0^t c^e(\tau)h_1(t-\tau) + c^c(\tau)h_2(t-\tau)\,d\tau, \\ &:= K_1 c^e + K_2 c^c. \end{aligned}$$

Define $K = K_1[I - K_2 M_2]^{-1}$.

*DLMF: http://dlmf.nist.gov/10.47.E2

Lemma: $\mathcal{M}$, M_2 exist and are bounded.

Lemma*: K_1 and K_2 are compact linear operators with zero spectral radius and unbounded inverse.

Formally:

$$\begin{aligned} c^e &= K_1^{-1} K_2 c^c + K_1^{-1} f \\ &= K_1^{-1}(K_2 \mathcal{M} + I) f \end{aligned}$$

which exists for f with sufficient decay.

*A Carasso, *SIAM J App. Anal.* 1982

Problem

Define $J_1(v) = \{T : |(M_2 v)(T) - x^d| = 0\}$.

Define the cost

$$J(c^e) := T + \epsilon_1 |(M_2 K c^e)(T) - x^d|^2 + \epsilon_2 \|c^e\|^2.$$

Find $\min_{c^e \in \mathcal{A}} J(c^e)$ subject to above PDE-ODE system and with state constraints $\Gamma \cdot x \leq 0$, $\Gamma \in \mathbb{R}^2$.

Proposition: Let $\varepsilon_2 = 0$. Then there exists an ε_1 such that

$$\begin{aligned} c_j^e(t) &= K_1^{-1}(K_2 M + I) f \\ &:= K^{-1} f \\ &= \operatorname{argmin} J(c^e) \end{aligned}$$

From above, we recall that f has step changes, and thus the frequency spectrum will not exponentially decay.

Theorem: The PDE-ODE system has no exact optimal controls.

We must use approximate controls.

Define

$$J_2(v) := \|c(a,t) - Kv\|^2 + \left(\frac{\epsilon}{M}\right)^2 \|v\|^2.$$

Theorem: The unique minimizer of J_2 is

$$v(t) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} e^{i\xi t} \frac{\hat{h}_1^{-1}(\xi)\left(\hat{h}_2(\xi)\widehat{M_2 v(\xi)} + \hat{v}(\xi)\right)}{1 + \omega^2 \hat{h}_1^{-1}(\xi)\left(\hat{h}_2(\xi)\hat{M_2 v}(\xi)\right)} d\xi$$

Pf: Solve the overdetermined system

$$Kv = c^\rho, \quad \omega v := \frac{\epsilon}{M} v = 0,$$

in the frequency domain and take inverse FT.

Define

$$J(c^e, t) := t + \epsilon_1 |(M_2 K c^e)(t) - x^d|^2 + \epsilon_2 \|c^e\|^2.$$

Theorem: Fix $t^f = J_1(f)$. Then there exist ε_1 and ε_2 and $\omega(\epsilon_1, \epsilon_2, M_2, t^f)$

$$K_\omega^{-1} f = \operatorname{argmin} J(c^e, t^f).$$

Pf:

$$\begin{aligned} |(M_2 K c^e)(t) - x^d|^2 &= |(M_2 K c^e)(t) - (M_2 K K^{-1} f)(t)|^2, \\ &= |(M_2 K c^e)(t) - (M_2 f)(t)|^2, \end{aligned}$$

Since T is fixed, and M_2 and K are bounded, there exists $\varepsilon_3 > 0$ (depending on T, M_2), such that

$$| \; M_2 K c^e \;\; t \;\; - \;\; M_2 f \;\; t \; |^2 \geq \epsilon_3 \| K c^e - f \|_{L^2}^2,$$

and

$$J(c^e, t^f) - t^f \geq \epsilon_1 \epsilon_3 \left(\| K c^e - f \|^2 + \omega_2 \| c^e \|^2 \right) > 0.$$

Thus, $\begin{aligned} \operatorname{argmin} J(c^e, t^f) &= \operatorname{argmin} \| K c^e - f \|^2 + \omega_2 \| c^e \|^2 \\ &= K_{\omega_2}^{-1} f. \end{aligned}$

Now note that

$$\|M_2\|^2\|Kc^e - f\|_{L^2}^2 \geq |(M_2Kc^e)(t) - (M_2f)(t)|^2,$$

and thus

$$\begin{aligned}\|M_2\|^2\|(KK_\omega^{-1} - I)\|^2\|f\|_{L^2}^2 &\geq \|M_2\|^2\|(KK_\omega^{-1} - I)f\|_{L^2}^2 \\ &\geq \|M_2\|^2\|KK_\omega^{-1}f - f\|_{L^2}^2 \\ &\geq |(M_2KK_\omega^{-1})(t) - (M_2f)(t)|^2,\end{aligned}$$

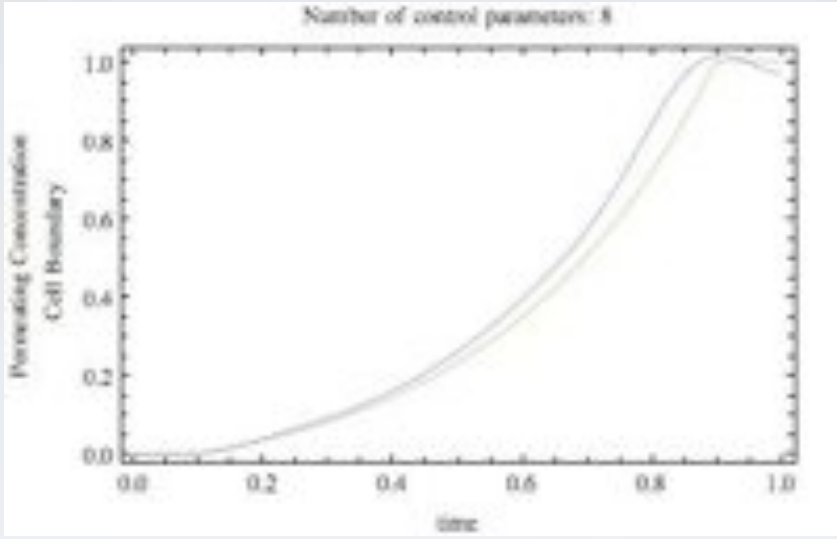
and with $\|M_2\|\|f\| < k < \infty$, given $\delta(\omega) > 0$ there exists an $\omega > 0$ such that

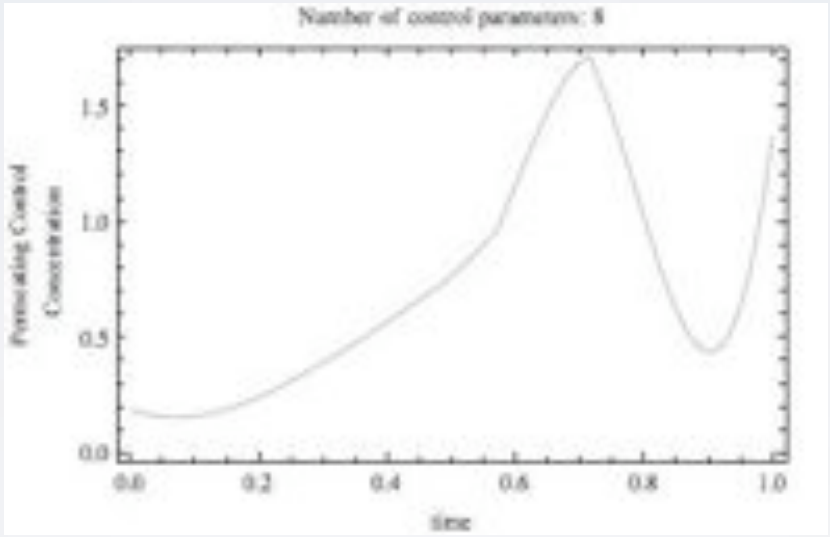
$$\begin{aligned}\delta &\geq \|KK_\omega^{-1} - I\| \\ &\geq k^{-2}|(M_2KK_\omega^{-1})(t) - (M_2f)(t)|^2.\end{aligned}$$

NUMERICS

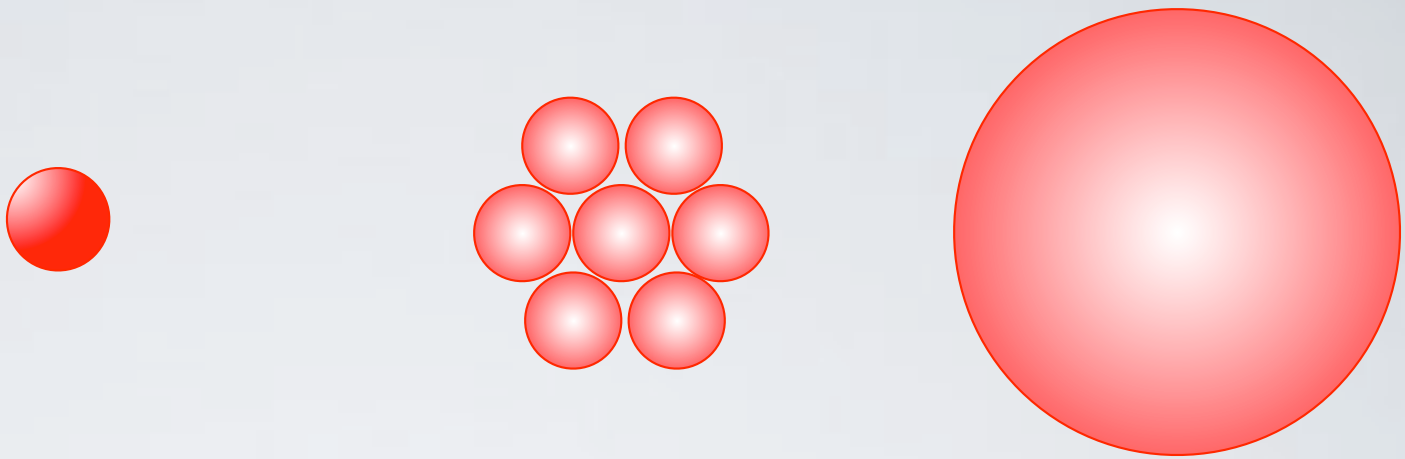
- PHAML: hp-adaptive multilevel elliptic solver
- Implicit-Filtering minimization algorithm: adaptive secant approximation to gradient

W Mitchell, *J. Par. Dist. Comp., 2007*: P Gilmore, T Kelley, *SIAM J Opt.* 1995


Number of control parameters: 8
Forwarding Concentration
Cell Boundary
0.0
0.2
0.4
0.6
0.8
1.0
time


Number of control parameters: 8
0.0
0.5
1.0
1.5
0.2
0.4
0.6
0.8
1.0
time

Model scaling shows where future work lies:



MODELS	Mass	ODE System ✓	Hybrid ODE/PDE System ✓	PDE System ✓
	Heat	Stochastic ODE ✓	Large Monte Carlo System ✓	nonlinear heat equation
CONTROLS	Mass	ODE System ✓	Hybrid ODE/PDE System	PDE System
	Heat	Stochastic ODE	Large Monte Carlo System	nonlinear heat equation

CURRENT AND FUTURE PROBLEMS

- Develop cost functions for entire cryo-protocol
- Extend 'inverse' approach to 2D and 3D systems
- Model multiphase ternary solidification and interaction with biomaterials
- Iterative optimization of freezing protocols
- Optimal design of counter-current dialysis devices

QUESTIONS?