Focused Ultrasound in Drug Delivery and Nanomedicine

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Thanks to:
Nathan MacDannold, BWH
Kathy Ferrara, UCD
Natalya Rapoport, U. Utah

HOUSTON Methodist RESEARCH INSTITUTE

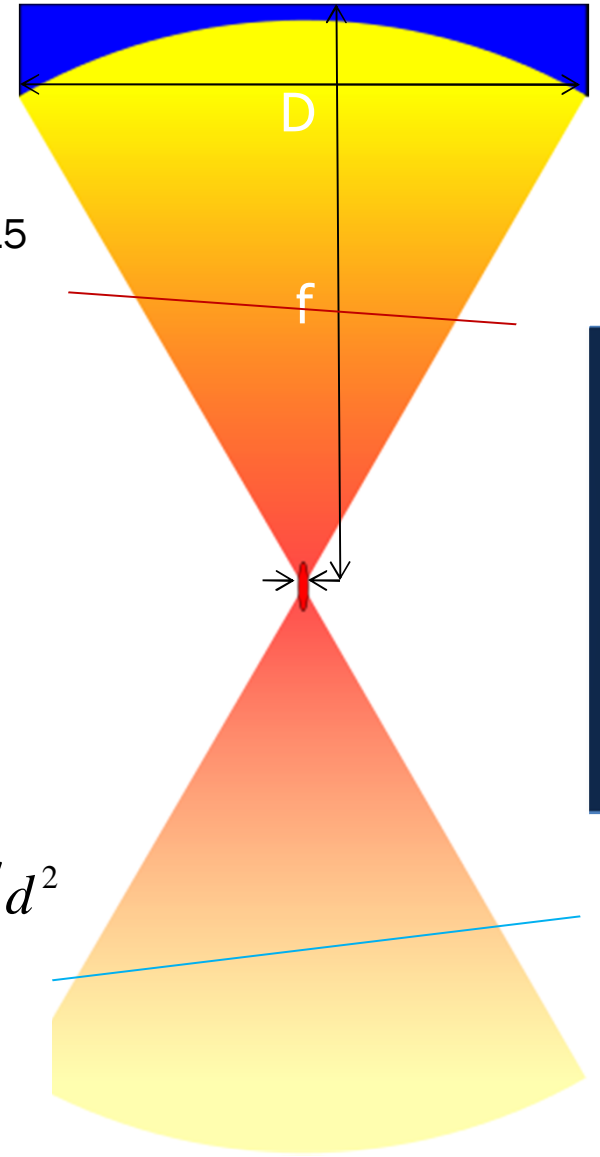
Motivation

- Delivering drugs exclusively to localized areas of disease should increase effectiveness and reduce side effects
- Nanoparticle drug carriers hold promise, but relying on natural targeting and drug release has failed to produce the expected results
- Focused ultrasound has advantages for “remote control” in tissue: deep penetration, accuracy on the mm scale, non-ionizing, feedback

Course Outline

- Mechanisms of Ultrasound-Material Interaction
- Ultrasound Alteration of Tissue Properties
  - Enhanced Delivery via Hyperthermia
  - BBB Disruption via Stable Cavitation
- Ultrasound Release from Drug Carriers
  - Induced Release from micelles and liposomes
  - Induced Release from microbubbles
  - Release from phase-change nanodroplets

Focused Ultrasound (HIFU or FUS)



- Diagnostic ultrasound: 1-2 cycle pulses (time resolution), 1-15 MHz (spatial resolution)
- Therapeutic ultrasound: 10^3-10^4 cycle pulses, 0.2-3 MHz
- Focused ultrasound: beam is directed to diffraction limited spot – ie. width ~ wavelength by geometry (single element), or electronic shift of phase (multiple element array)
- With sound speed ~ 1.5 mm/us, 1MHz ultrasound has wavelength 1.5 mm, so this is beam waist
- length depends on transducer diameter, for f=D, length~7 mm
- Intensity at focus is:

$$I_f = TAP / area = I_{xducer} \times D^2 / d^2$$

- For InSightec D = 15 cm, so focusing factor is 10^4
- I_{xducer} = 3 W/cm^2, so I_f < 3x10^4 W/cm^2

Effects of Focused Ultrasound

Thermal effects

- Hyperthermia (40-45 °C) -> altered blood flow, gene upregulation, inflammation, apoptosis
- Thermal Ablation (50+ °C) -> cell death through necrosis
- Thermal Dose:

$$TD_{43} = \int R^{T(t)-43}\, dt\,; R \approx 4\ (T < 43)|2(T > 43)$$

- Thermal Conduction:

$$\rho c\dot{T}(\boldsymbol{x}, t) = \dot{Q}(\boldsymbol{x}, t) + k\nabla^2 T - w_b c_b \Delta T_b$$

Effects of Focused Ultrasound

Mechanical effects

Cavitation (combination with bubbles)

$$MI \propto p_r / f^{0.5}$$

$$\omega_0 = \frac{1}{R_0}\left(\frac{3\gamma P_0}{\rho_0}(1 + \sigma \ldots)\right)^{0.5}$$

Sonoporation and sonolysis -> cell membrane damage

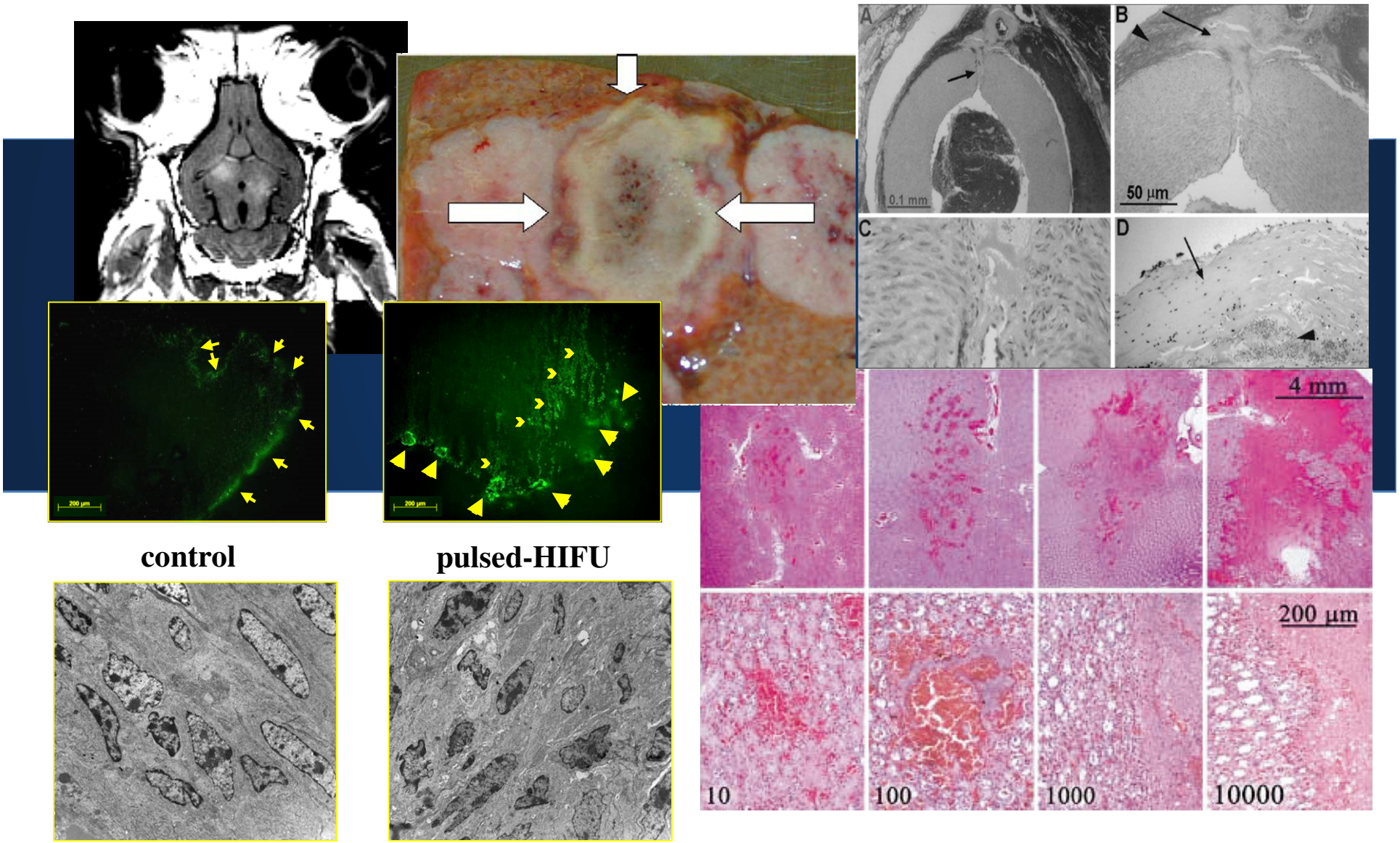
Sonochemistry -> ROS production

Radiation force/shear -> mechanotransduction, bioeffects

$$F = \alpha \frac{I(r)}{c_v}$$

Other??

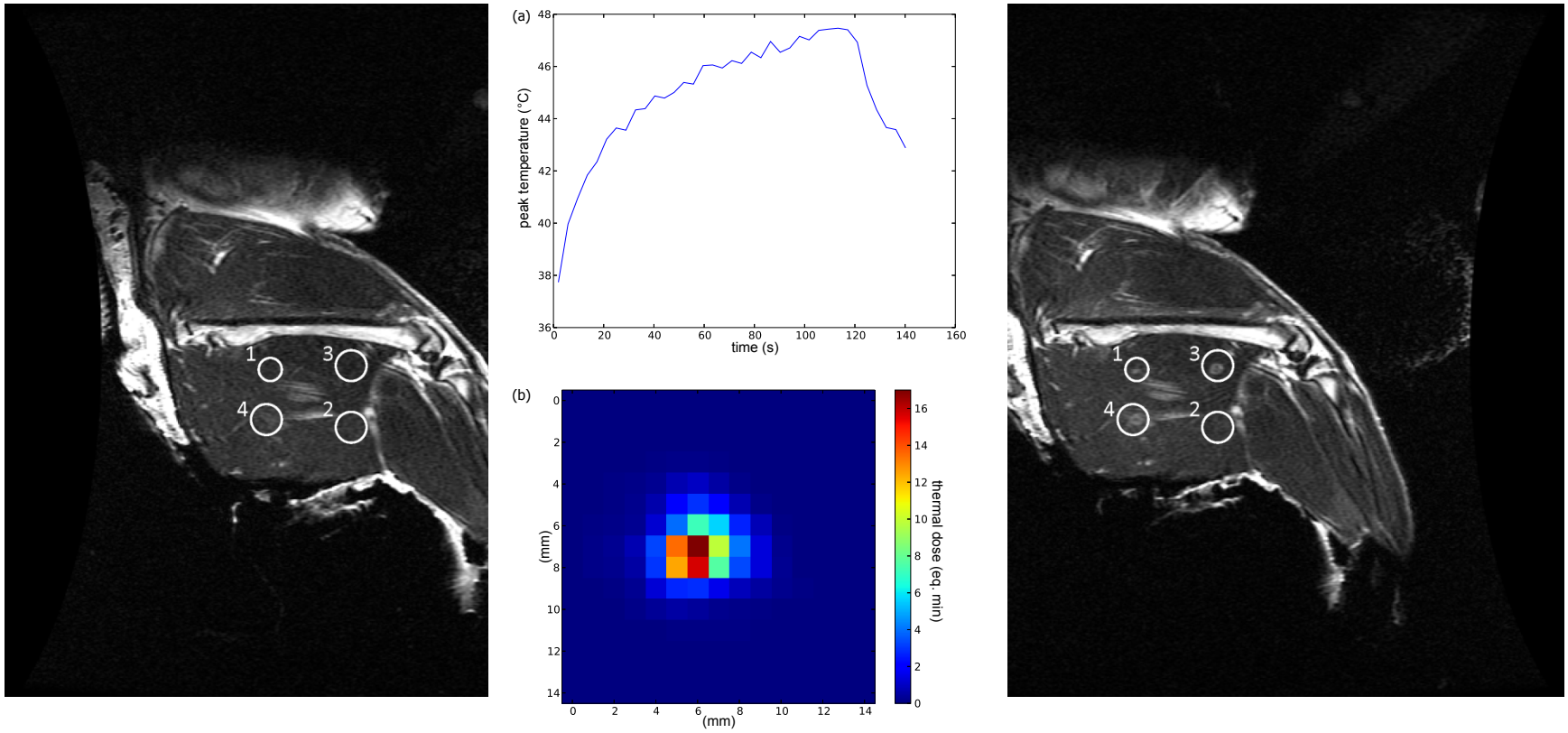
Therapeutic Effects



HIFU-Enhanced Transport

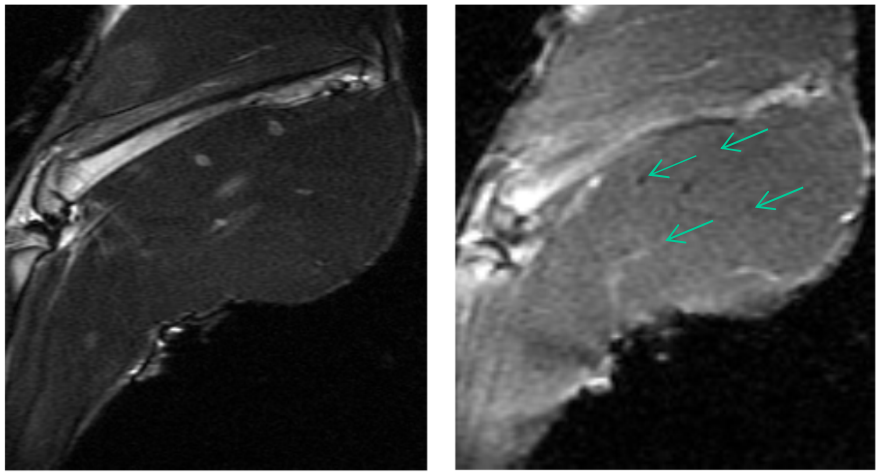
- Working with ultrasound only – very attractive because clinical translation of device is much easier
- Idea is that tissue transport properties (diffusion, permeability) are altered by HIFU
- 5+ years of work on mouse and rabbit models to understand mechanism

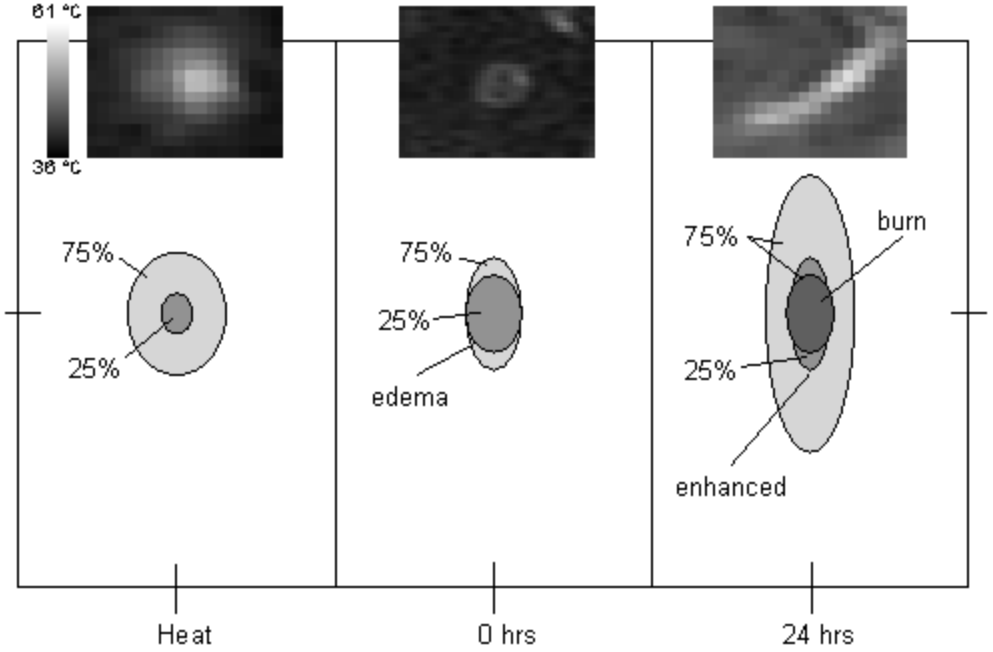
Pulsed-HIFU treatment



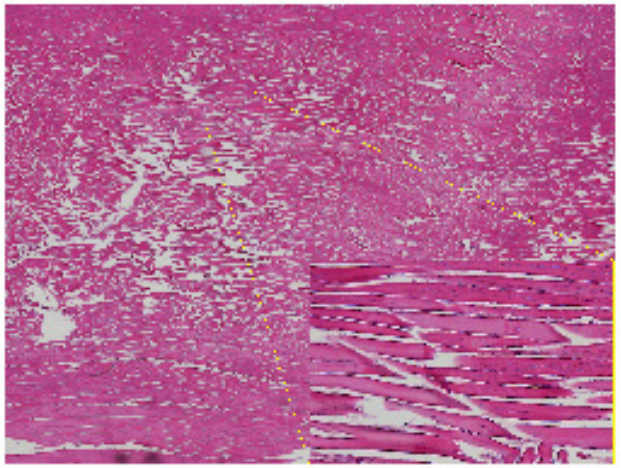
O'Neill, et al., JMRI 2013

Treatment Results

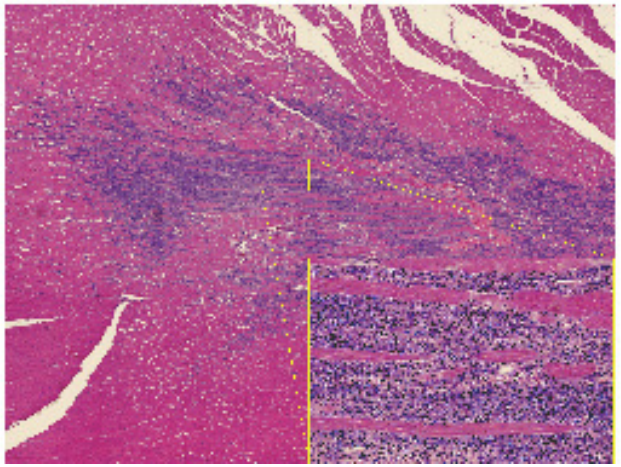




(a)

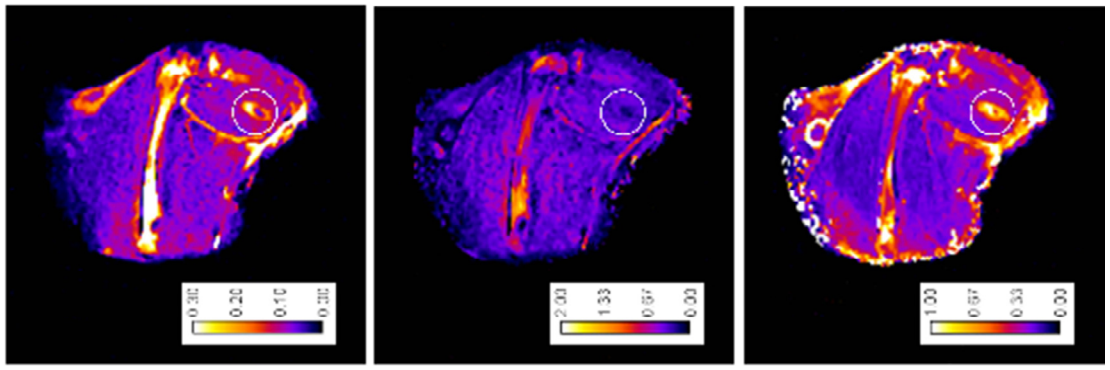


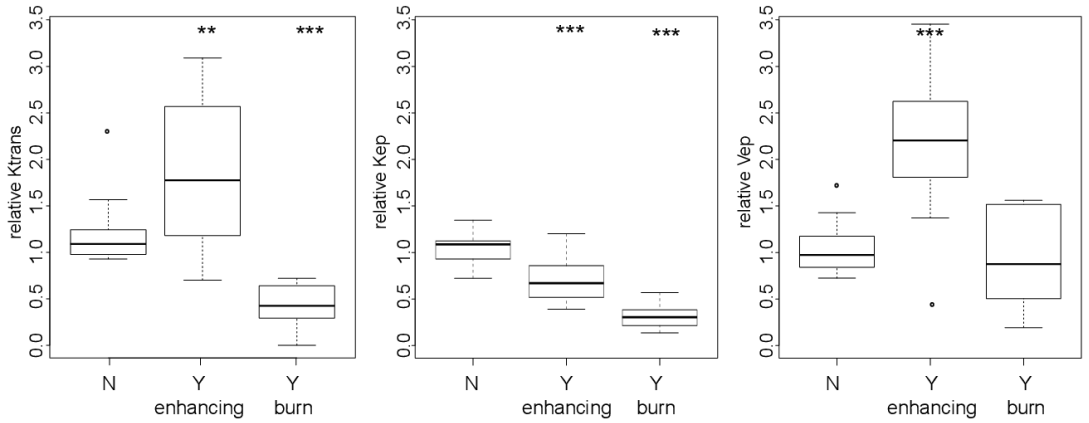
(b)

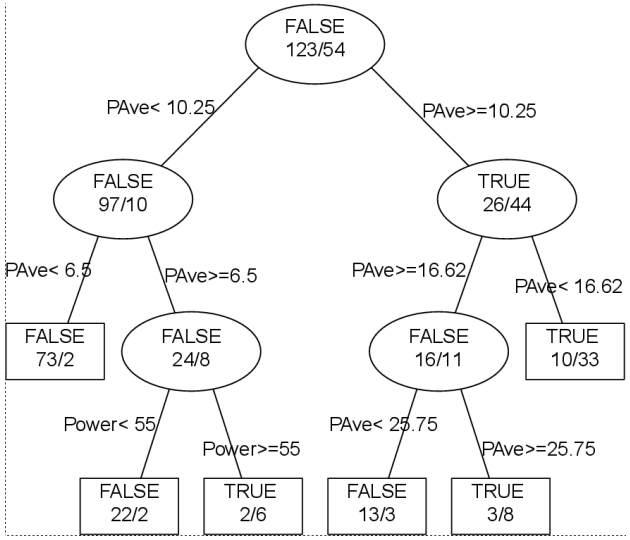


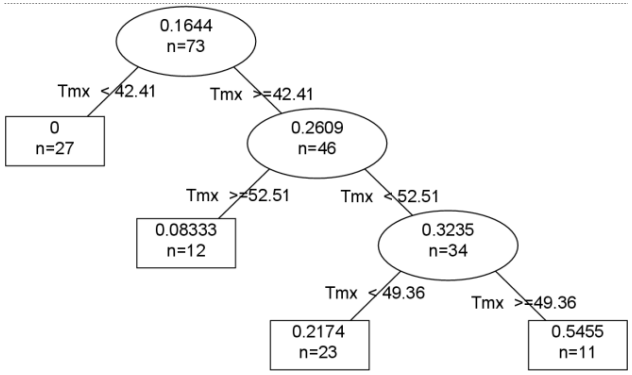
Transport at 24 hours

(conclusion: thermal effect)







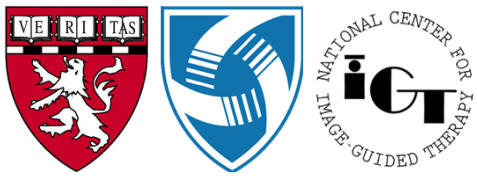


Ultrasound-mediated targeted drug delivery in the brain†

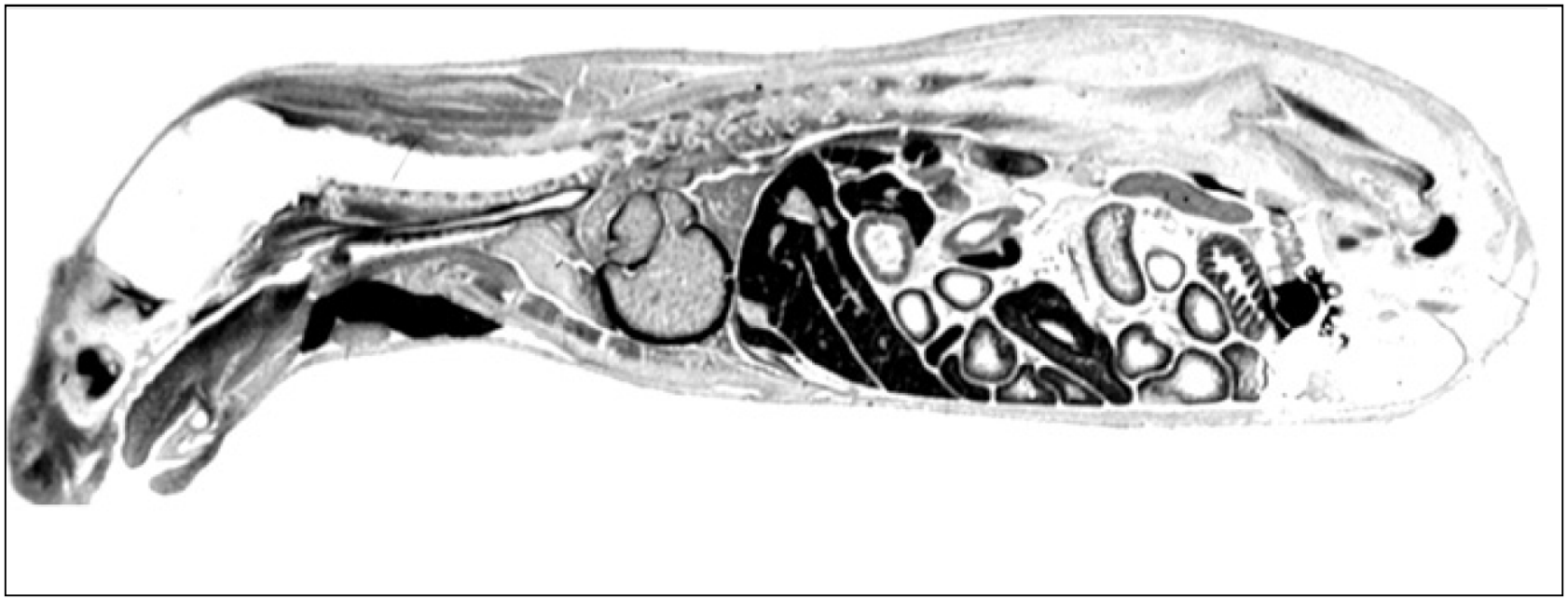
Nathan McDannold

Dept. Radiology, Brigham & Women's Hospital, Harvard Medical School, Boston, MA

†used with permission



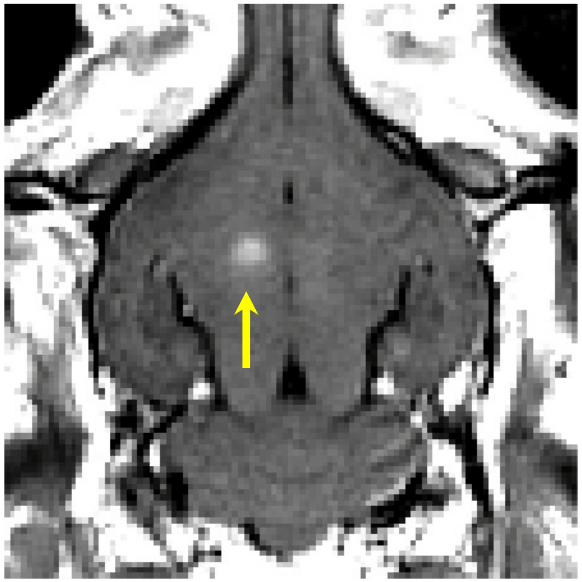
Blood-brain barrier (BBB)

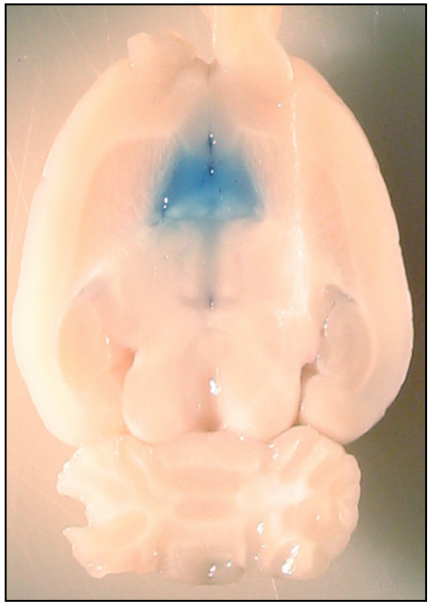


>98% of small molecule drugs do not cross the BBB	~100% of large molecule drugs do not cross the BBB	<1% of drug companies have a BBB drug targeting program	<1% of academic neuroscience programs emphasize BBB transport biology

BBB disruption with focused ultrasound

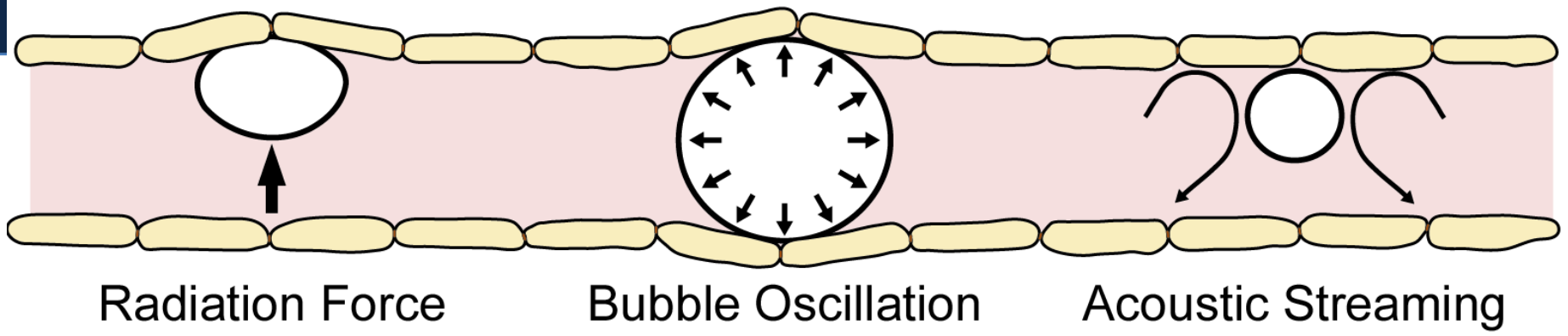
- Low-power, pulsed exposures
- Combined with ultrasound contrast agent (Optison, Definity)
- Temporary (~hours), localized, non-invasive





BBB disruption with Focused Ultrasound

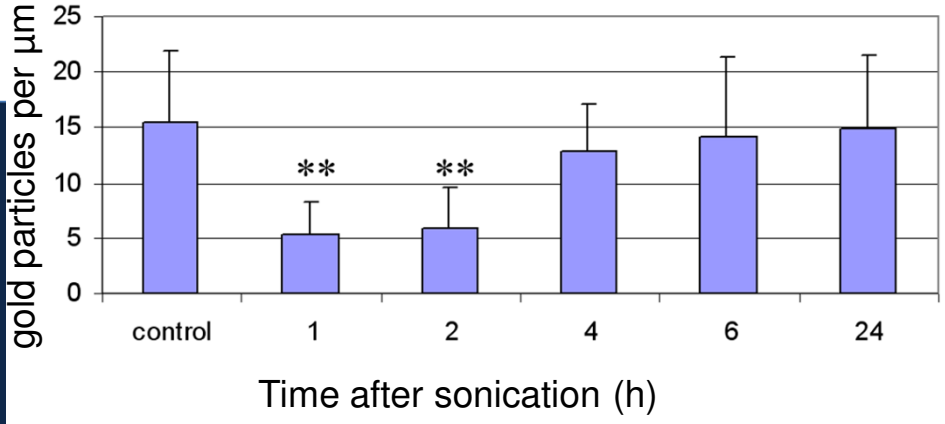
- Occurs due to mechanically-induced changes and/or stimulation to vasculature
- Caused by microbubble/US interaction
- Not due to heating
- Exact mechanism(s) not known



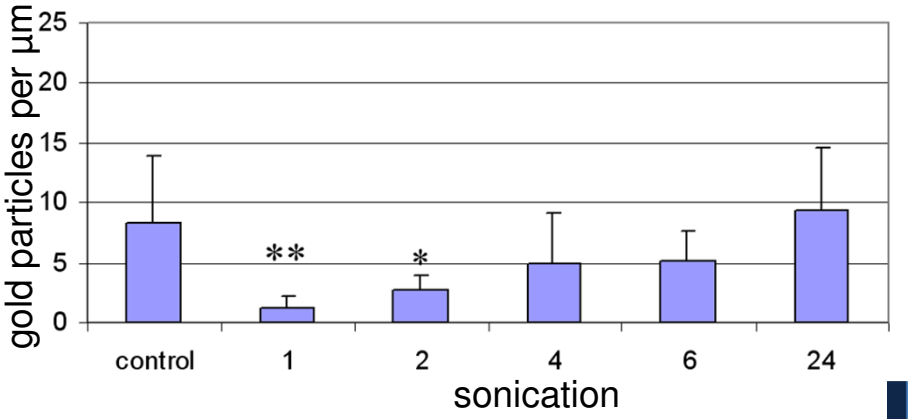
Electron microscopy study: tight junctions

Results: # TJ proteins reduced after BBBD; restored at 4h

ZO-1



Claudin 5



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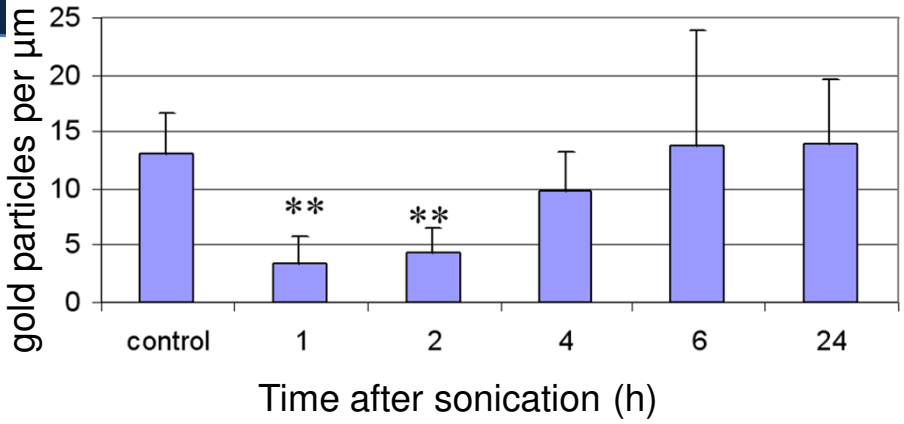
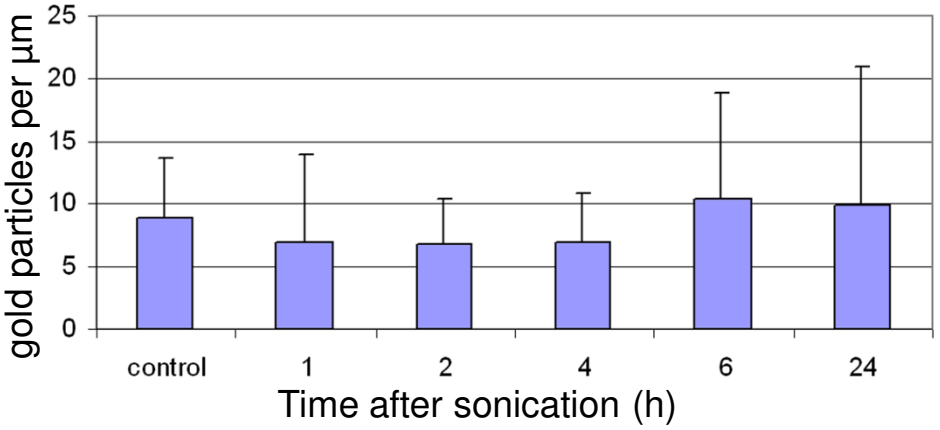


Claudin-1



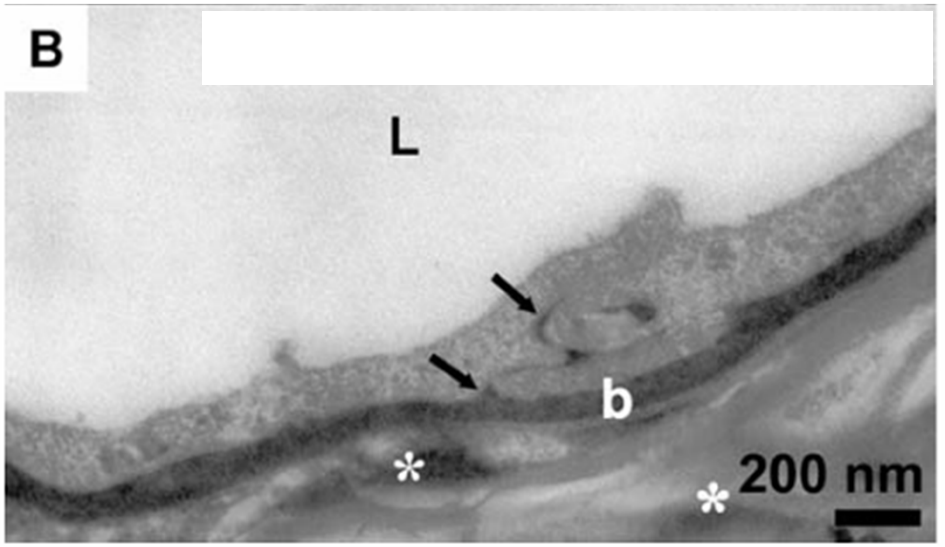
Sheikov et al. Ultras. Med Biol (2008)

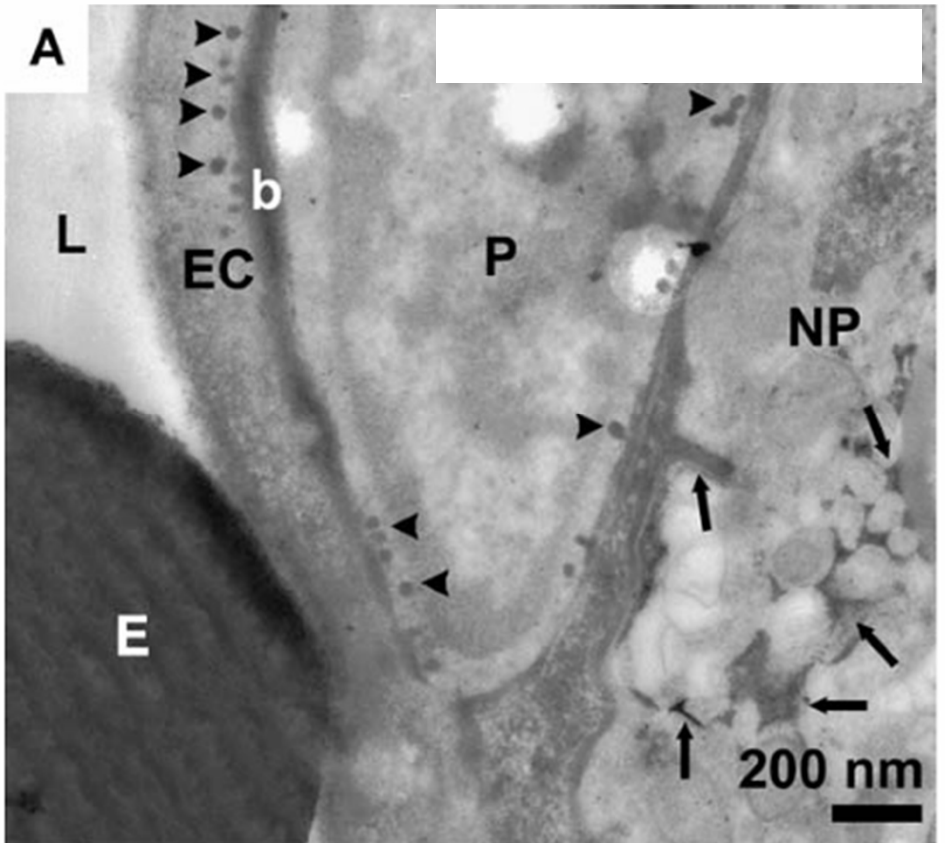
BBB disruption with focused ultrasound

Mechanical interaction between US, microbubbles, and vessel walls results in:

- Transient disassembly of tight junction proteins
- Stimulation of active transport

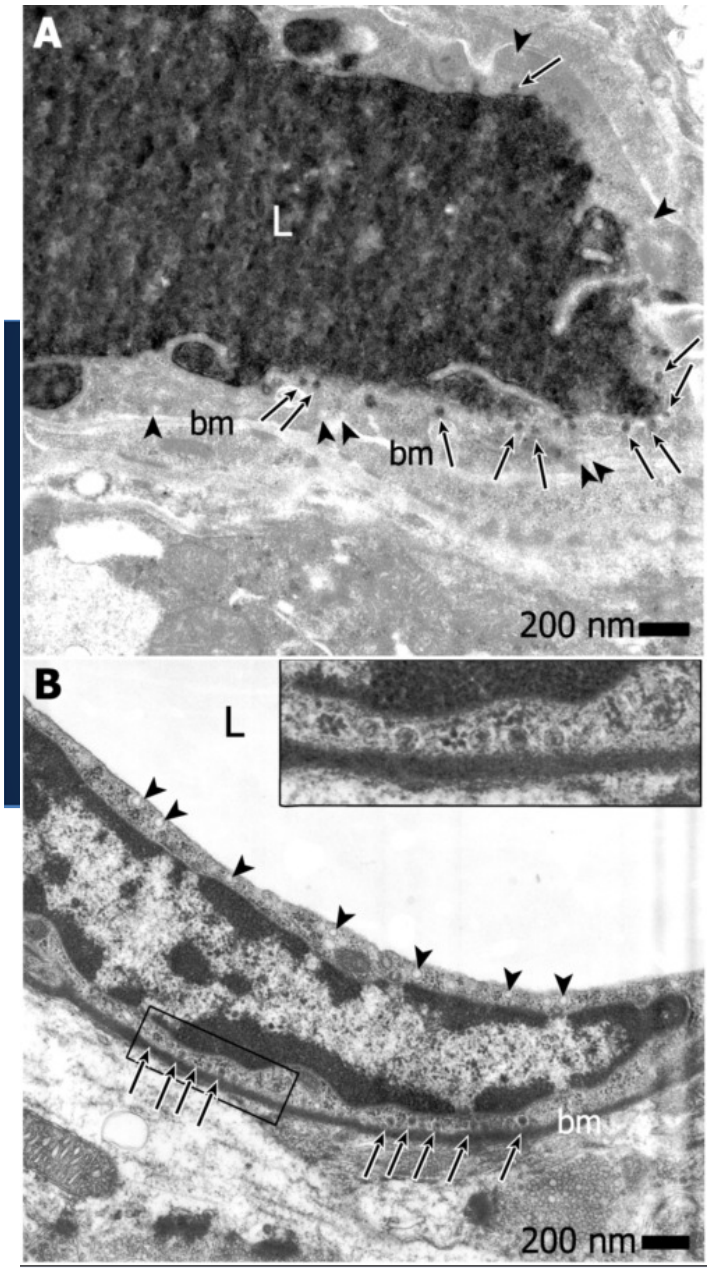
At higher exposure levels, inertial cavitation occurs, leading to vessel damage





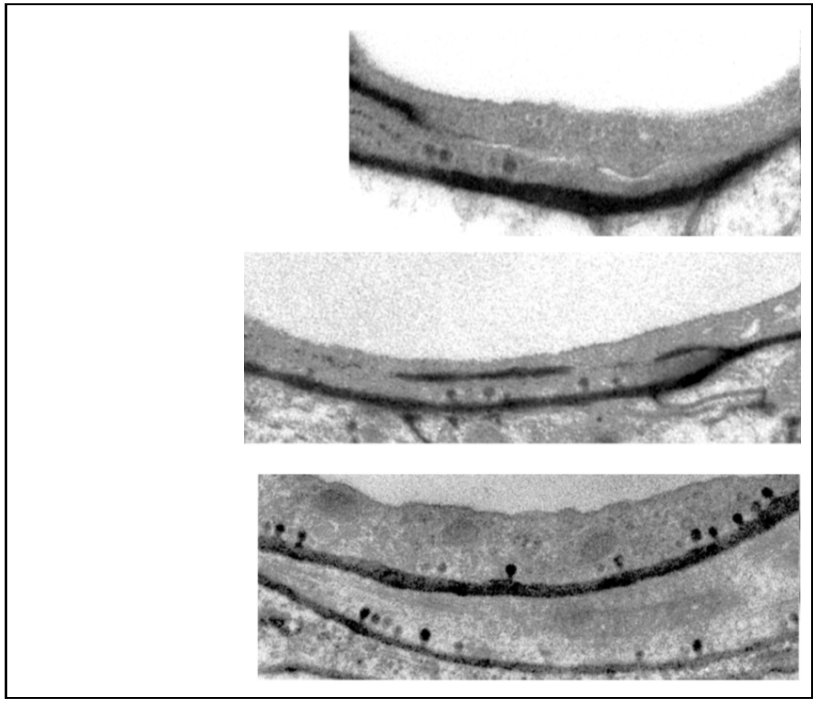
Hynynen et al., Neuroimage 2004

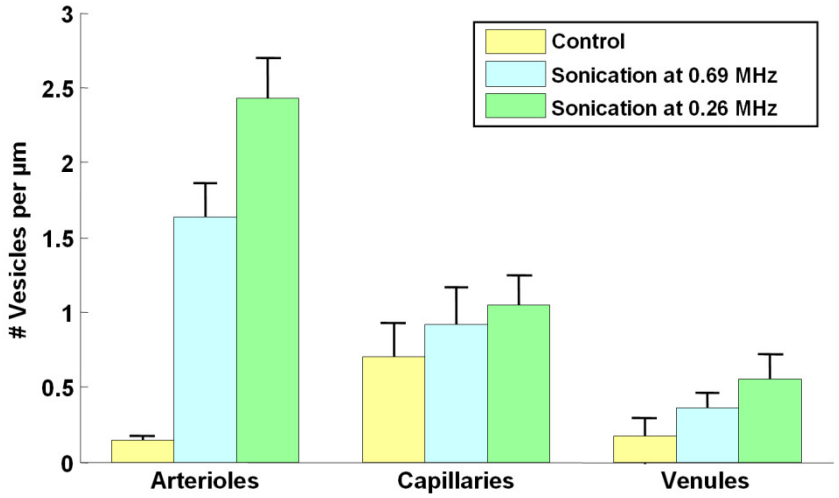
Electron microscopy study: active transport



5 min after FUS

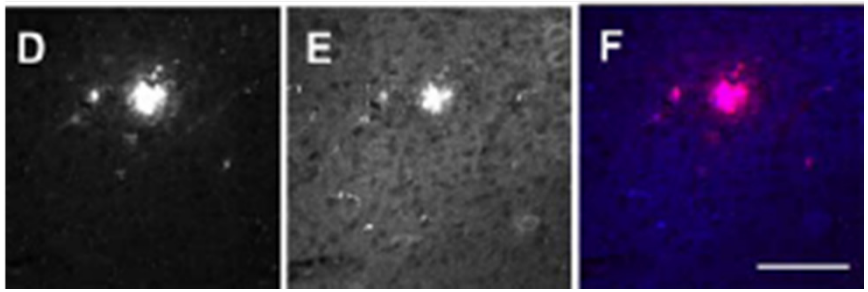
1h after FUS





N. Sheikov et al. Ultrasound Med. Biol. 2008

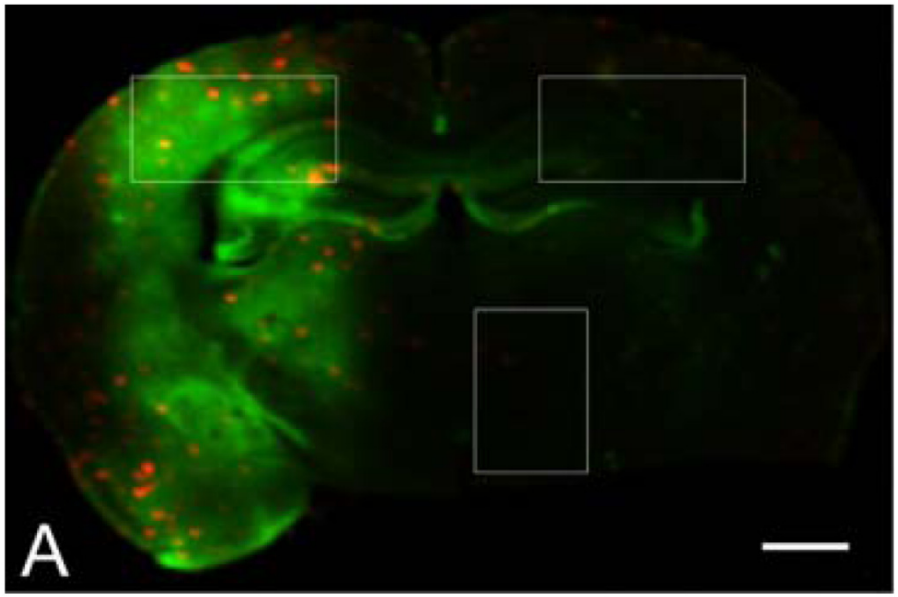
BBB disruption with focused ultrasound



Small animal studies:

- Reliably induce BBB disruption without tissue damage
- Deliver a range of molecules to the brain, including therapeutics
- Improve outcomes in animal disease models

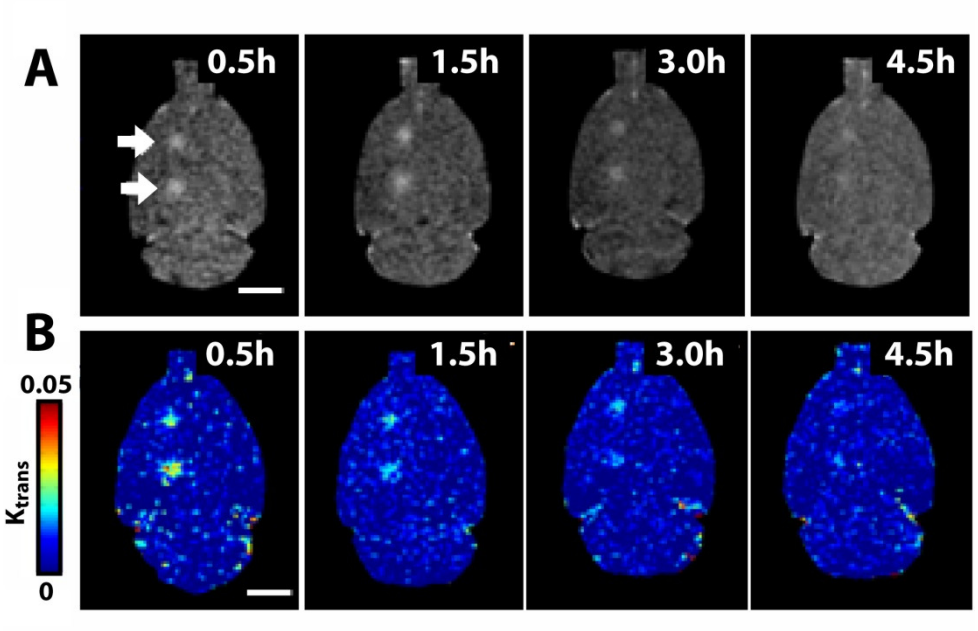
  Glioma, Alzheimer's



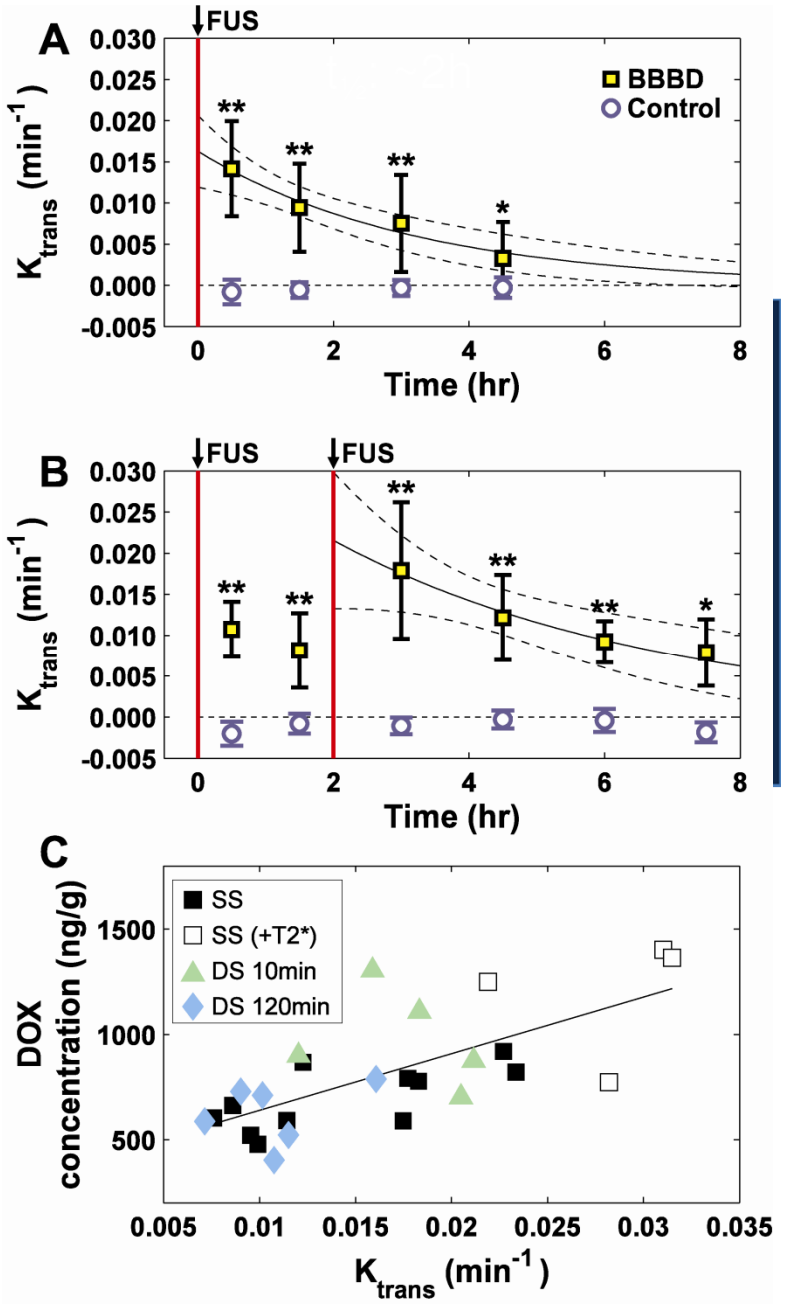
Alzheimer's model mouse
Endogenous IgG (green)
+Trypan blue bound to Amyloid plaque (red)

Raymond et al., PLoS One 2008

Characterizing BBBD with dynamic contrast enhanced MRI



J. Park et al. J Control Release. 2012



Summary of therapeutic agents delivered via FUS-BBBD

- **Chemotherapy**
  - BCNU, methotrexate, doxorubicin, liposomal doxorubicin
- **Antibodies**
  - Herceptin, BAM10 (Alzheimer's)
- **Nanoparticles**
  - Magnetic nanoparticles
  - Gold nanoparticles
- **Neuroprotective agent**
  - BDNF, GDNF (Parkinson's, stroke, traumatic brain injury)
- **Viruses**
  - siRNA for Htt (Huntington's disease)
- **Cells**
  - Neural precursor cells (stem cells)
  - Natural killer cells
- **Nothing!**
  - BBBD *alone* might help Alzheimer's disease, induce neurogenesis

FUS Induced Release from Nanoparticles

Two general approaches: Thermal and Mechanical

Thermal: Competes with many other modalities: RF, laser, AMF

Relies of heat sensitive liposomes, heat sensitive polymers – maybe reversible

Mechanical: based on cavitation

Microbubbles, nanodroplets – not reversible

Thermally sensitive liposomes

Liposomes are spherical lipid bilayers that can be used for carrying hydrophilic drugs

Problem: either too leaky or too stable

Sol'n: Lipid bilayers undergo gel to liquid phase transition with temperature dependent on composition. Leaky during transition due to phase mismatch

LTSL: developed at Duke, now used many places

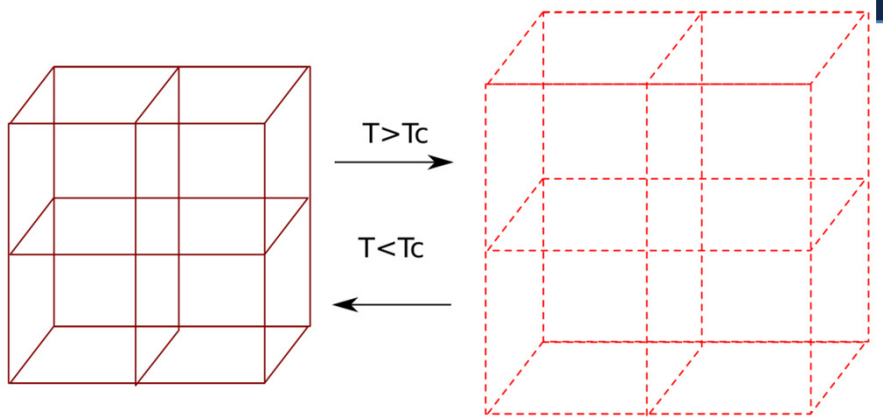
Mills & Needham, BBA Biomembranes, 2005; 1716(2):77–96

Thermal sensitive polymeric NPs

Many kinds of nanoparticles built of polymers as drug carriers – generally slow diffusion

Some polymers undergo conformational phase change that alters solubility in water (expansion, collapse, micelle formation, disassociation…)

Huge potential, barely scratching surface



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Ablation + long circulating LTSL†

16 element annular array (IMASONIC)

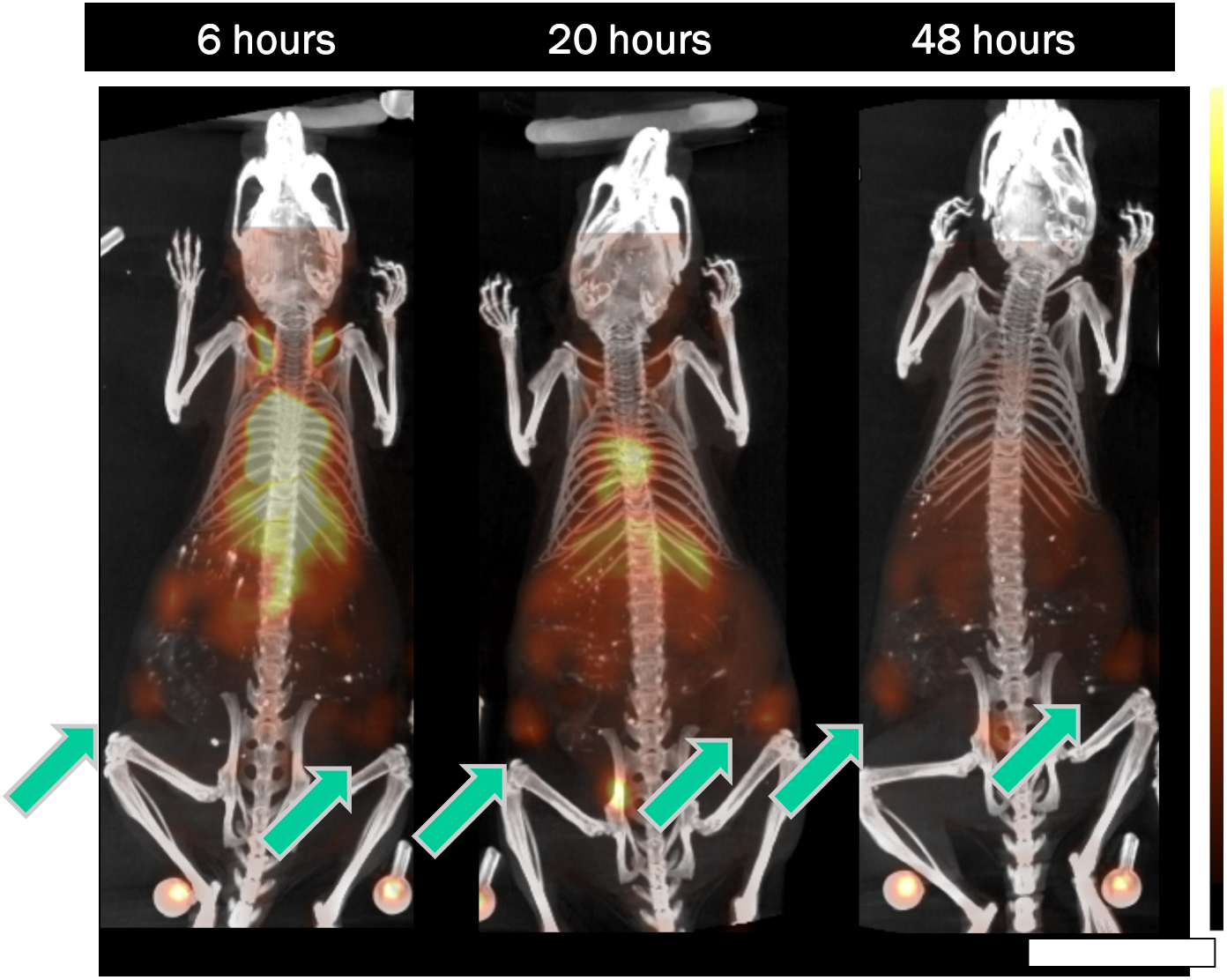
3 MHz center frequency

14 MPa PPP, -7.7 MPa PNP

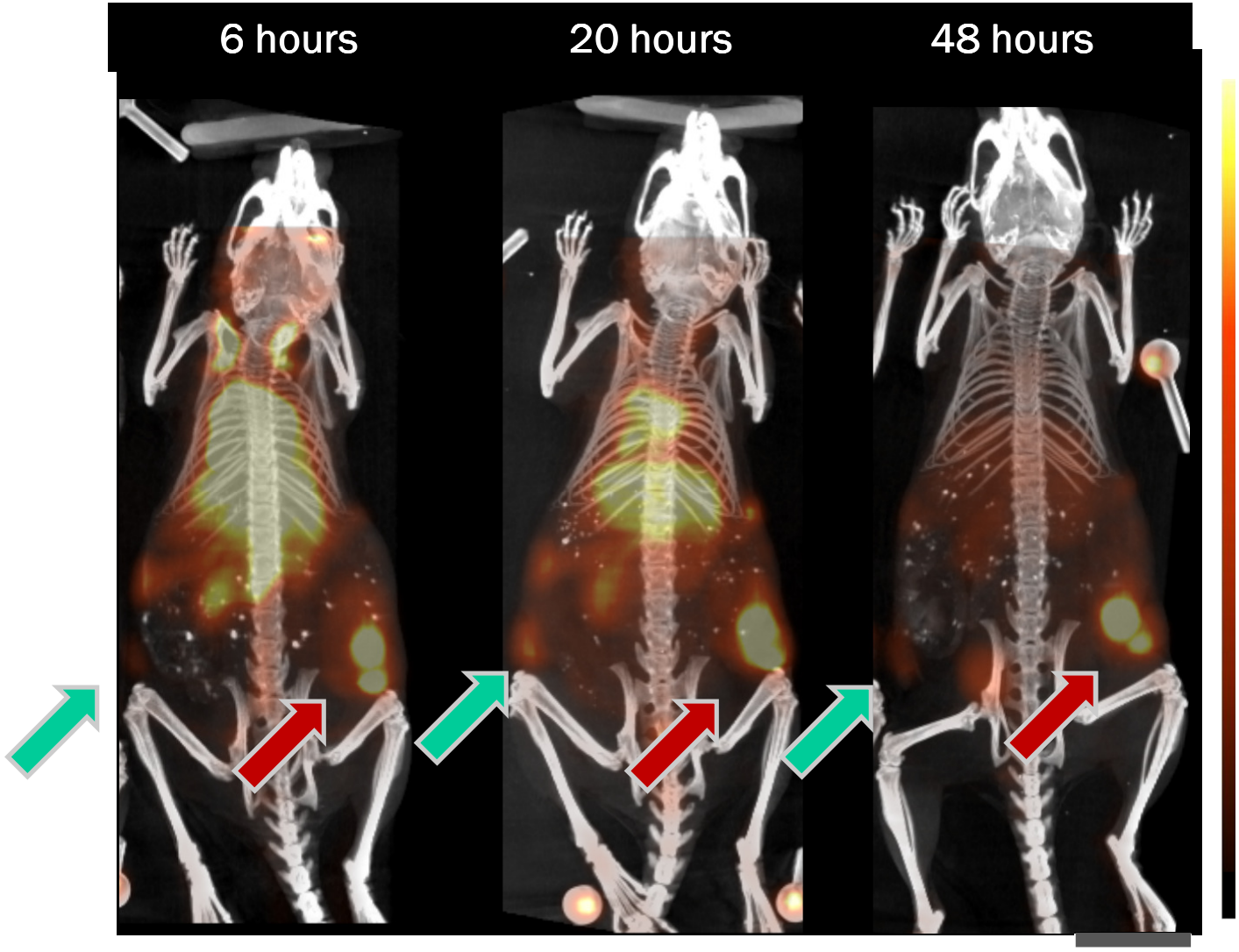
7 s CW, single spot >65 °C

†used with permission

^{64}Cu-LCL – no US



MRgFUS + ^{64}Cu-LCL



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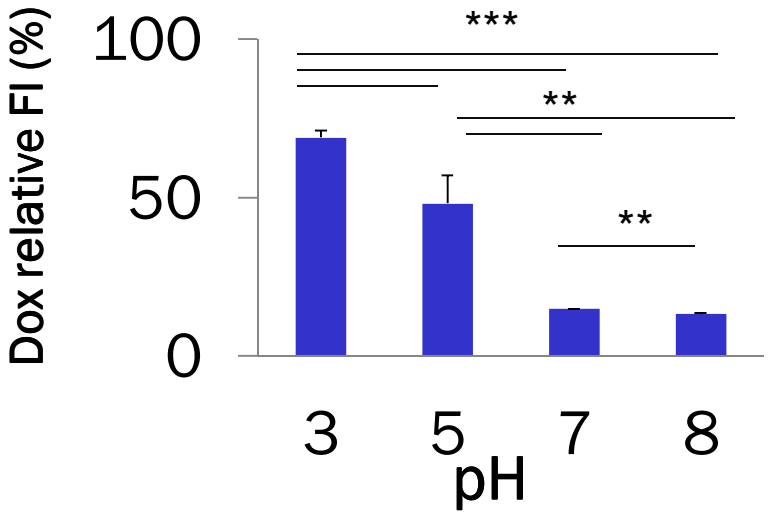
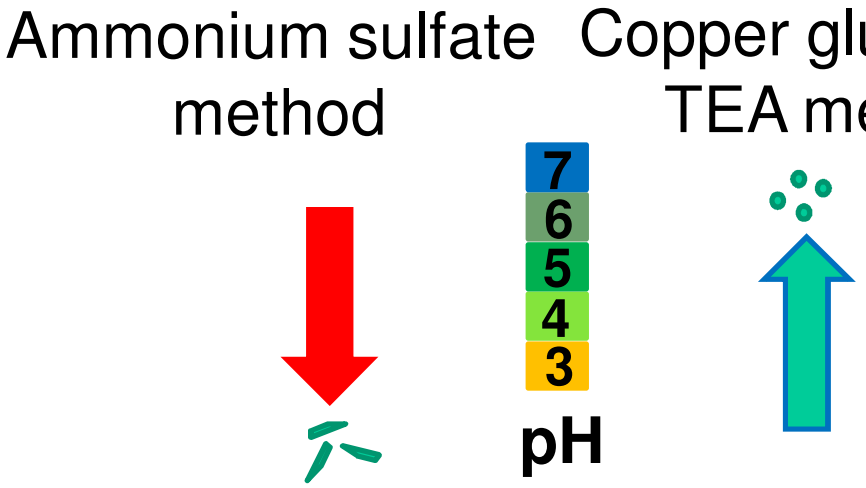
Complexation of Cu(II) and Dox within liposomes

Problem: Even liposomal doxorubicin has substantial cardiac toxicity and dose cannot exceed 500 mg/m^2 in lifetime.

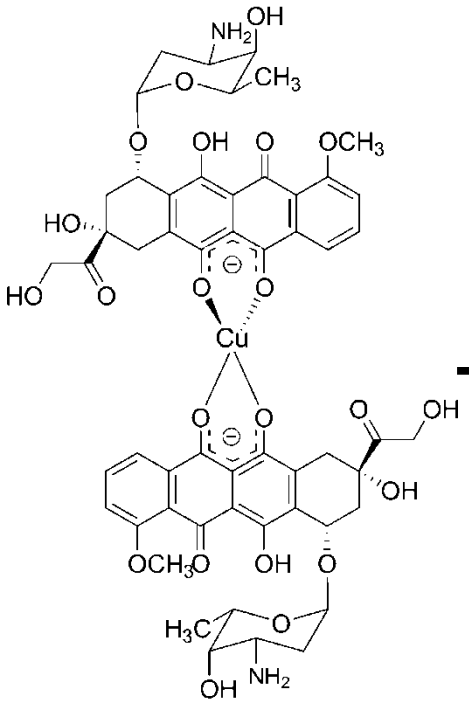
Solution: Create a doxorubicin salt that is very stable in circulation



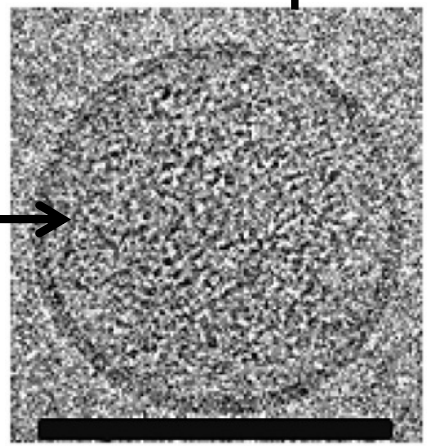


Kheirolomoom et al Molecular Pharmaceutics

Complex of Cu(II) & Dox with liposomes

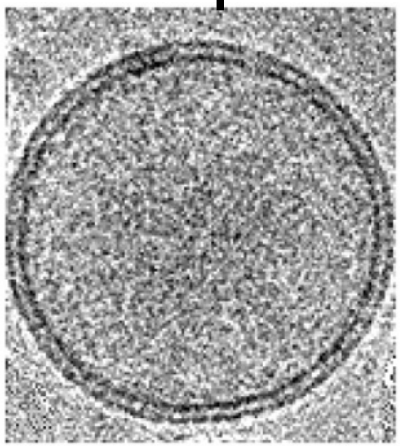


CuDox-lipo



100 nm

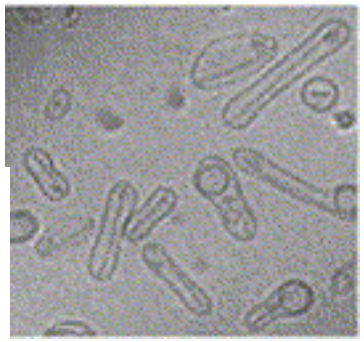
Cu-lipo



Kheirolomoom et al
Molecular Pharmaceutics
2010

100 nm

Doxil



Lasic, D.D. et al., Biochimica Biophysica Acta (1995) vol. 1239, 145-156

Tumor growth

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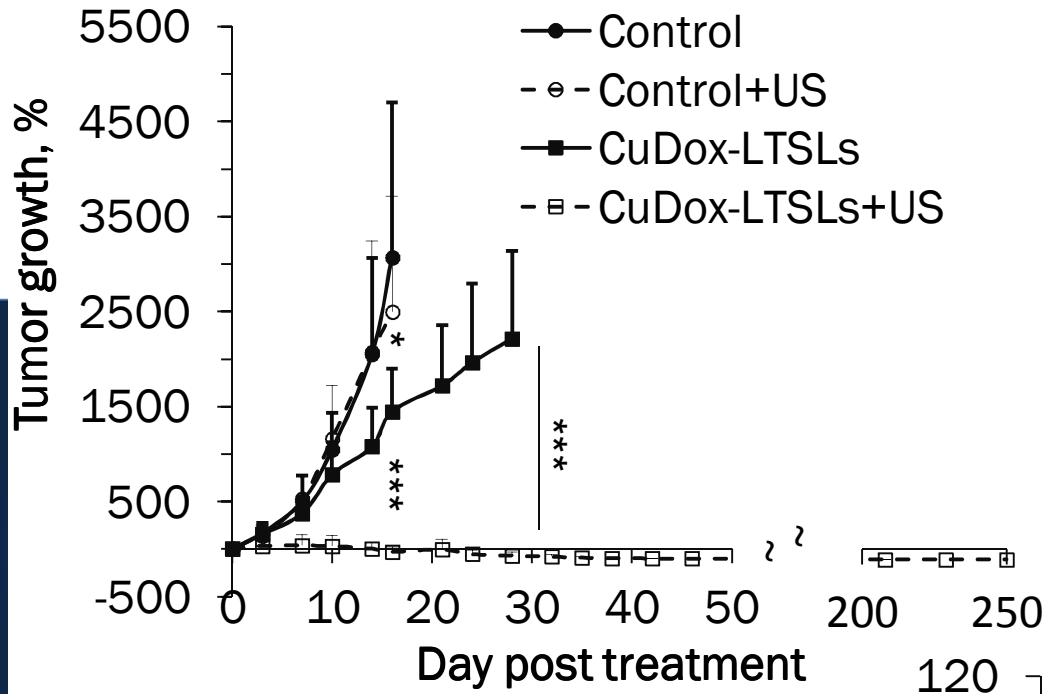
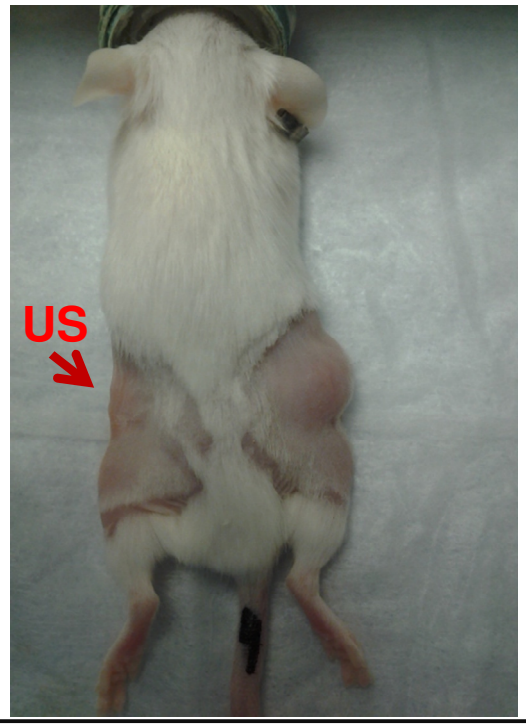
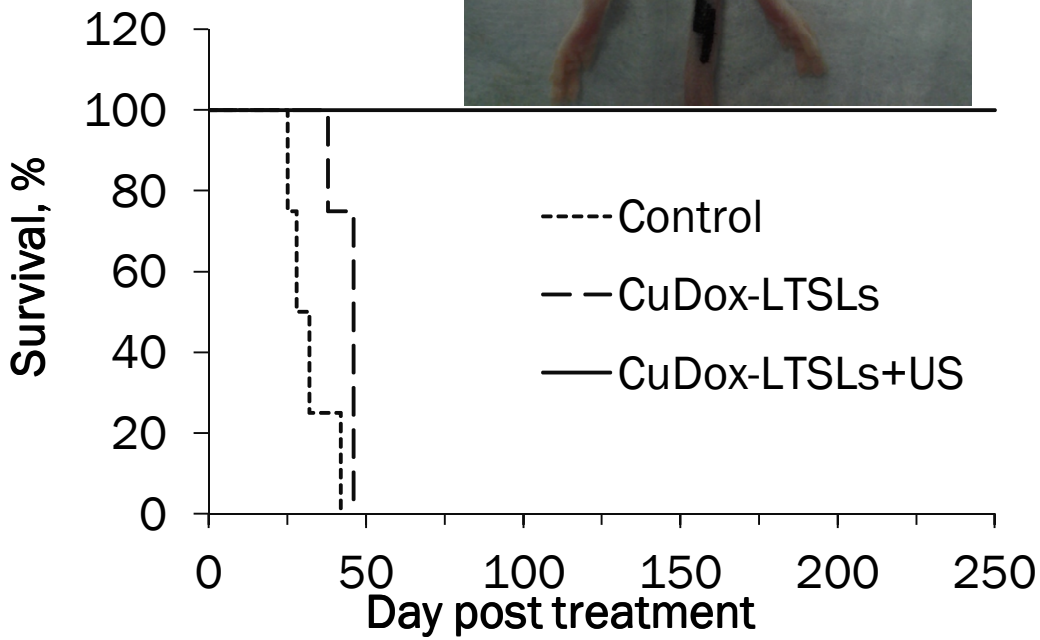


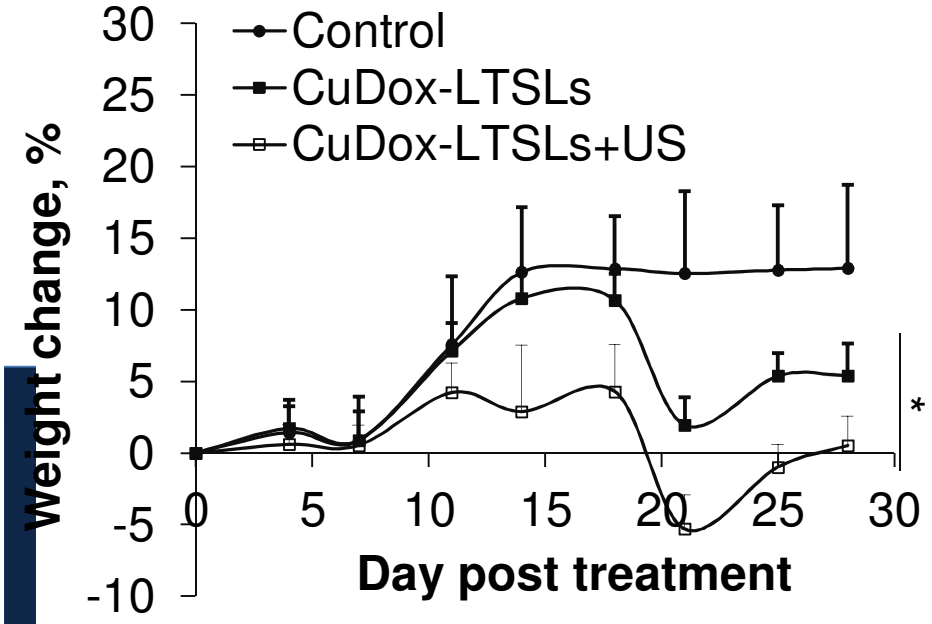


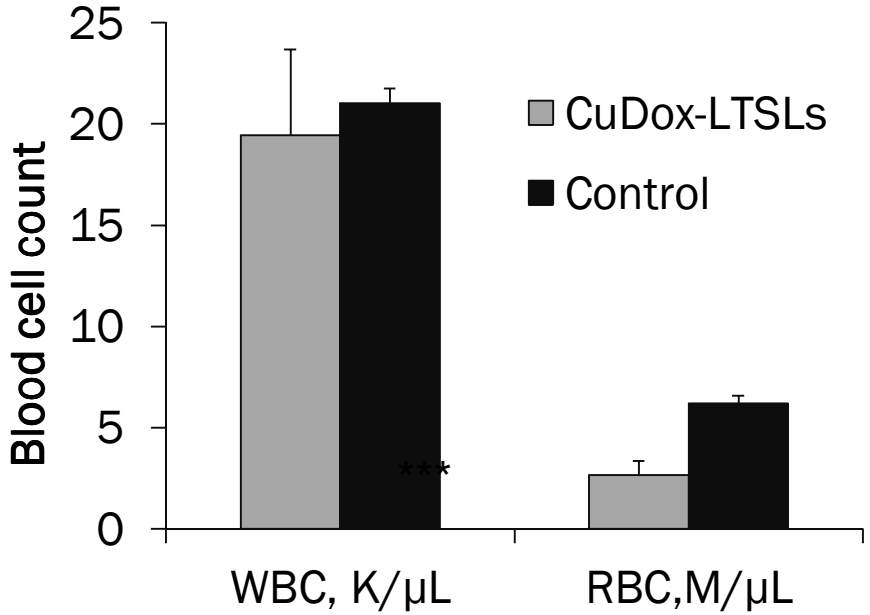
Treat 2x/week, 4 weeks,
6 mg/kg



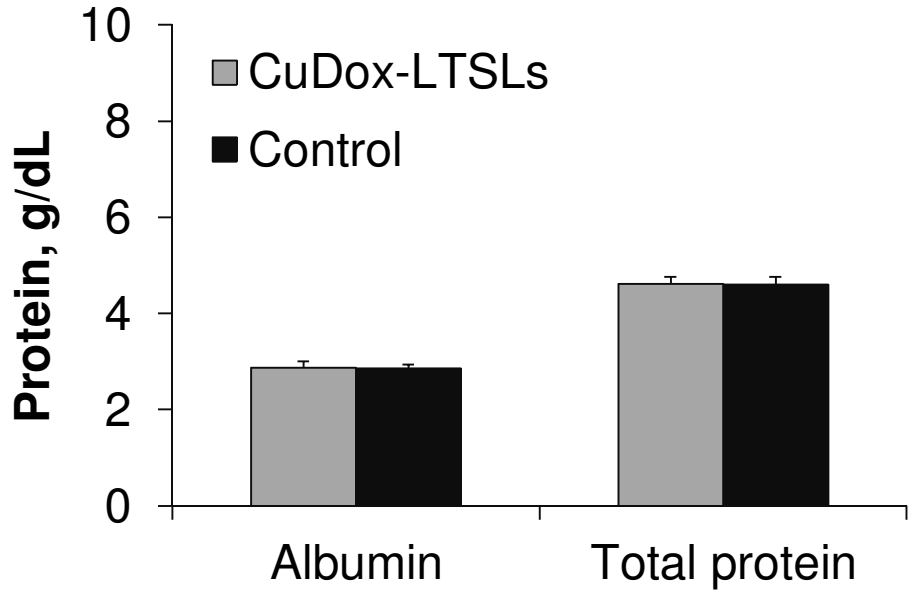
* p<0.05 compared to control
*** P<0.001 compared to control

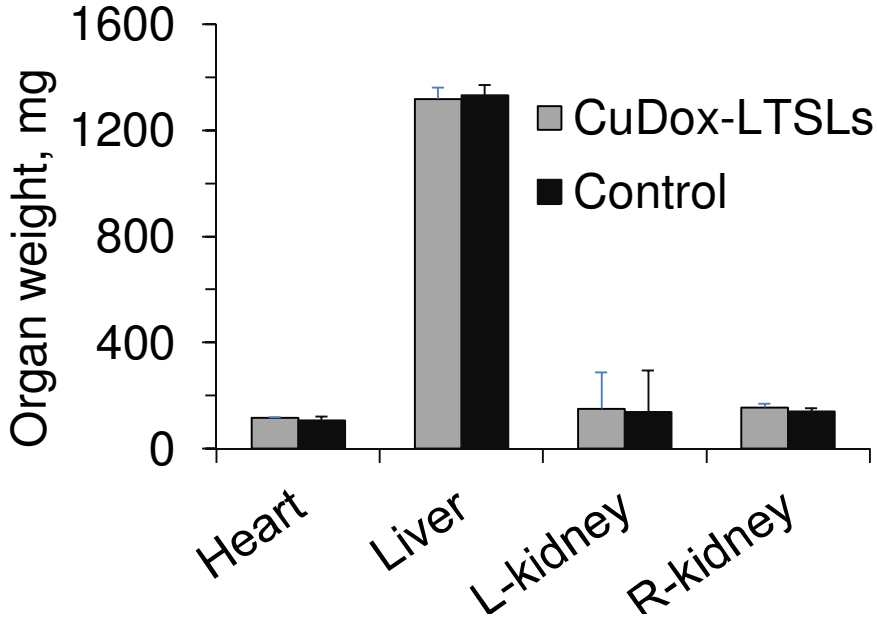
Kheirolomoom et al, JCR 2013





Toxicity is low





Why do we favor thermally-sensitive nanoparticles?

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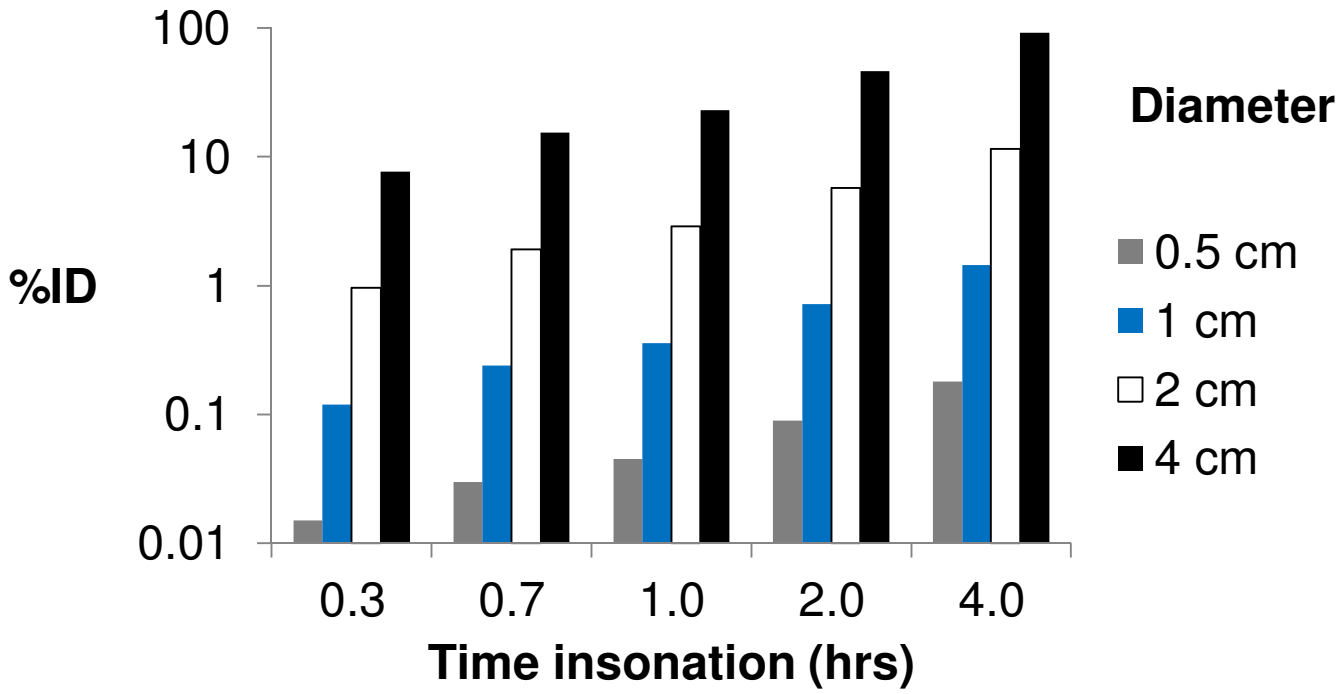
%ID delivered
depends on volume insonified,
time of insonation



Assumes 5% blood volume in tumor
10 sec tumor blood refresh
5 L blood volume

Non-Thermal Release

- Release driven via pressure changes, cavitation – rapid release, no change in T
- Types include drug loaded microbubbles, gas containing liposomes, liposomes attached to microbubbles, phase shifting ‘nanodroplets’
- The latter are PFC with bulk liquid-gas transitions around body temperature that are held together by Laplace pressure: $\Delta P = \frac{2\sigma}{R}$

Liposomes or oil carriers on bubbles

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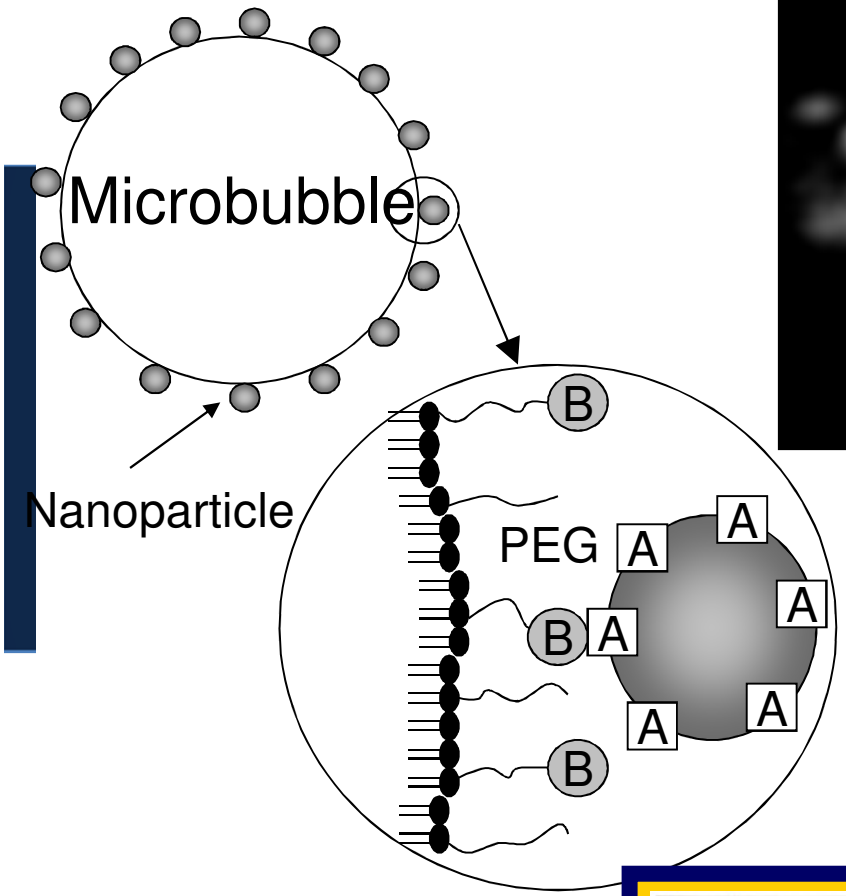
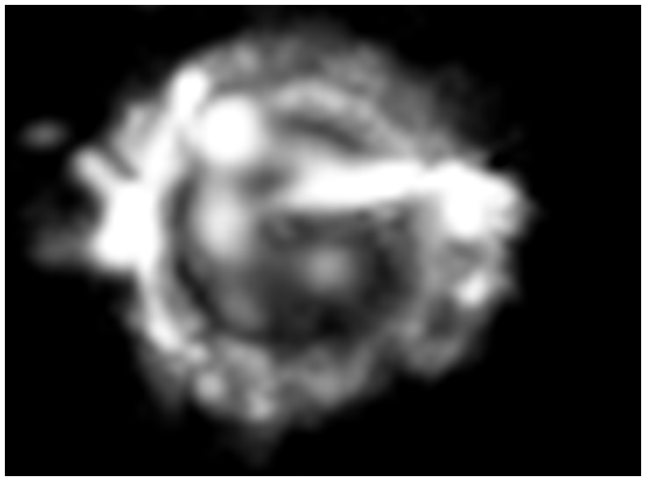
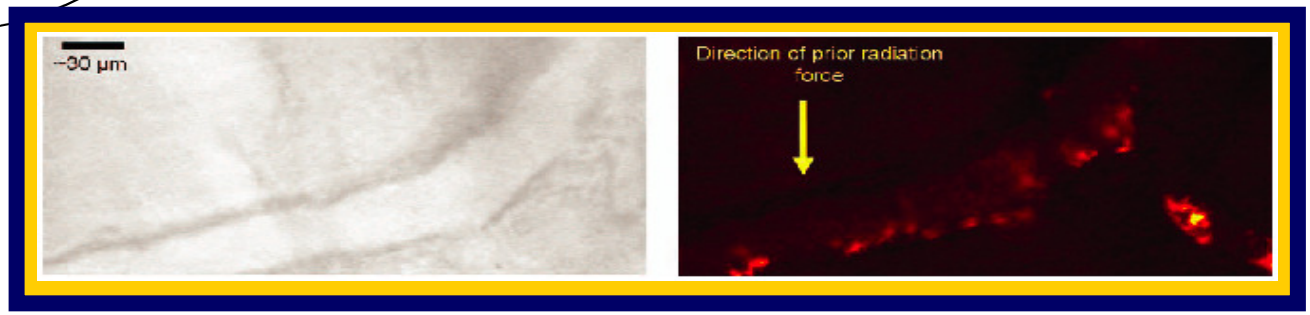




10^5 10^6 10^7

Fluid Concentration (Bubble-bead aggregates/mL)



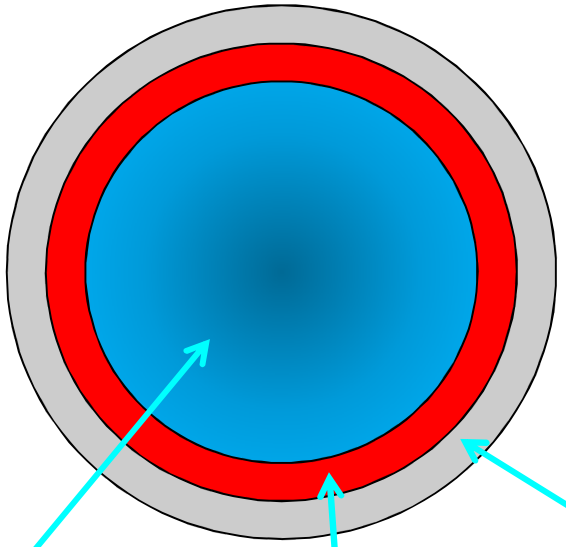
UMB 2006,
JCR, 2006 and 2007

Nanodroplets† (Courtesy of N. Rapoport, U. of Utah)

Versatile structures with properties that depend on the core and shell compositions

Core:
PFP, $T_b = 29\ ^\circ C$
PFCE, $T_b = 140\ ^\circ C$

Shell:
PEG-PDLA
PEG-PLLA
PEG-PCL
Pluronic



PFC droplet

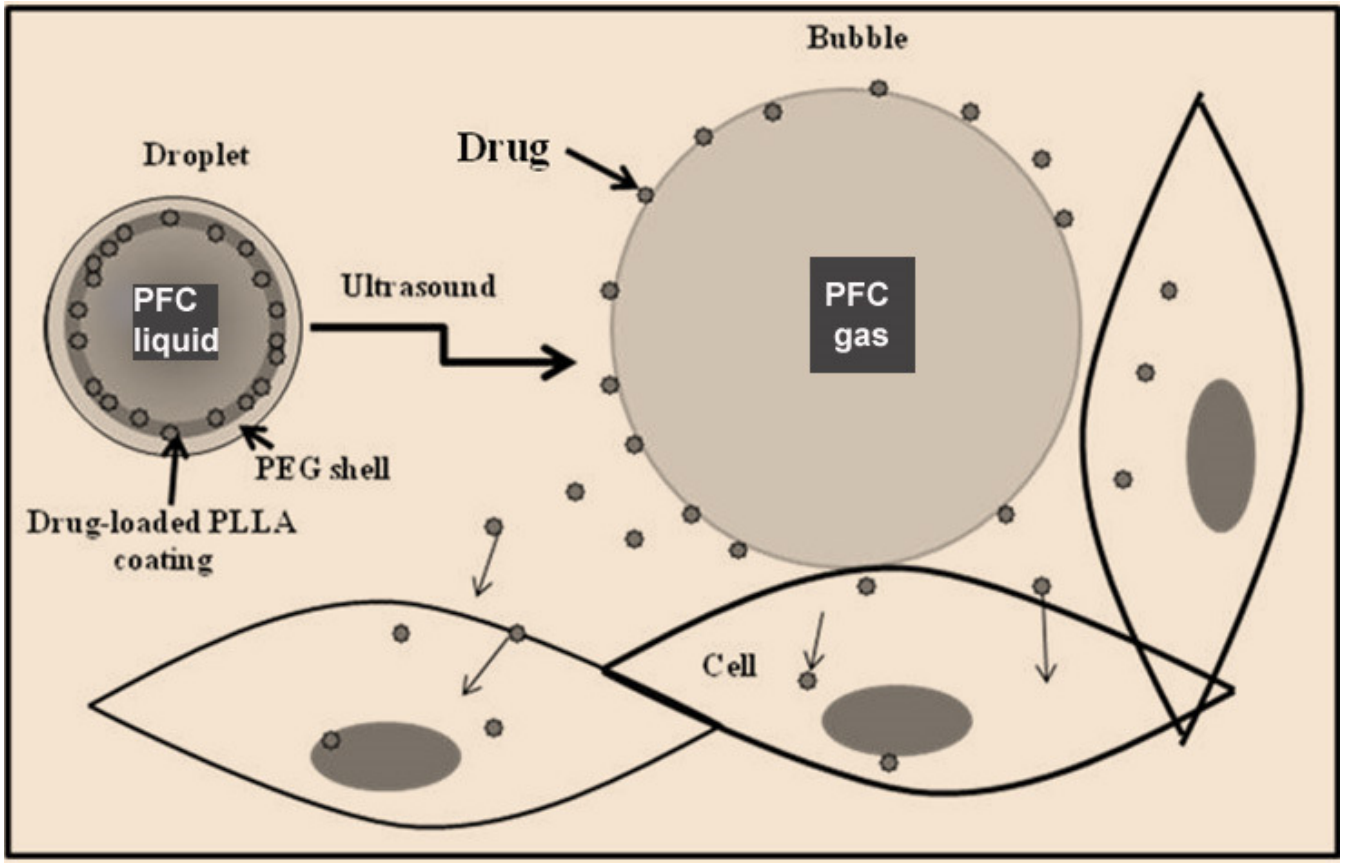
Hydrophobic part of the shell with drug

Hydrophilic corona

†used with permission

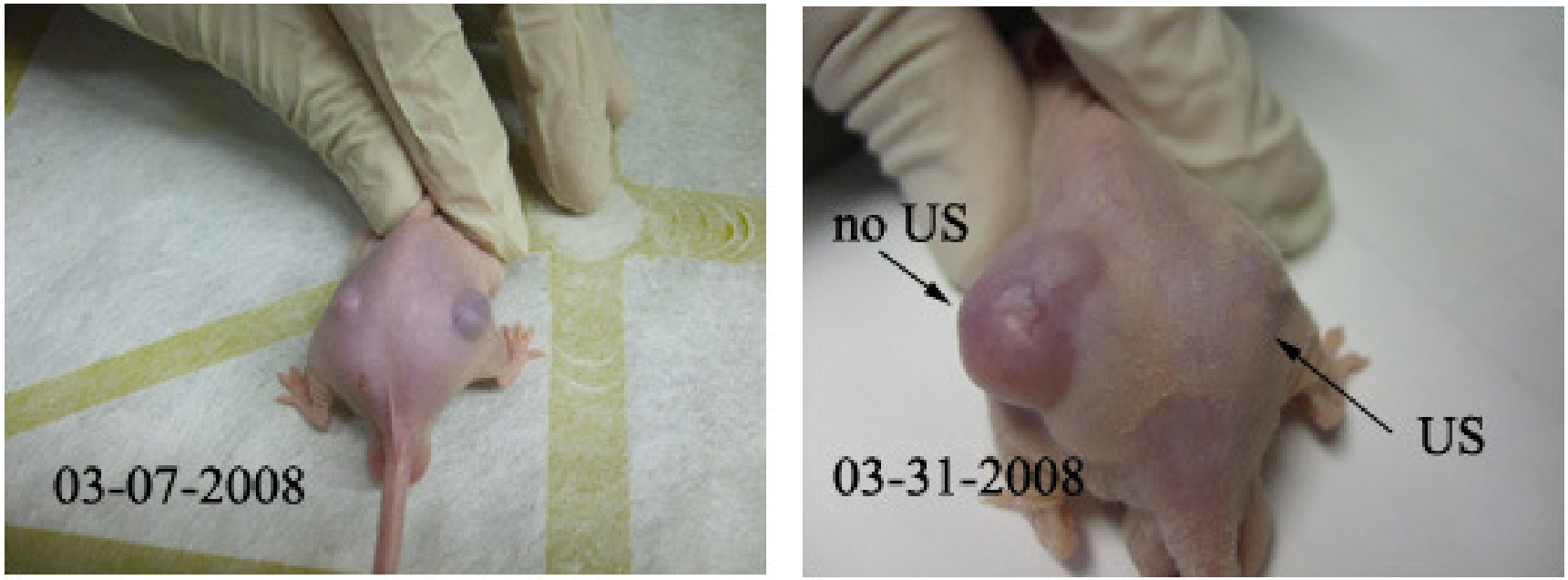
Ultrasound effect on the nanodroplet

Scheme of the ultrasound-induced drug release



Ovarian Carcinoma Model

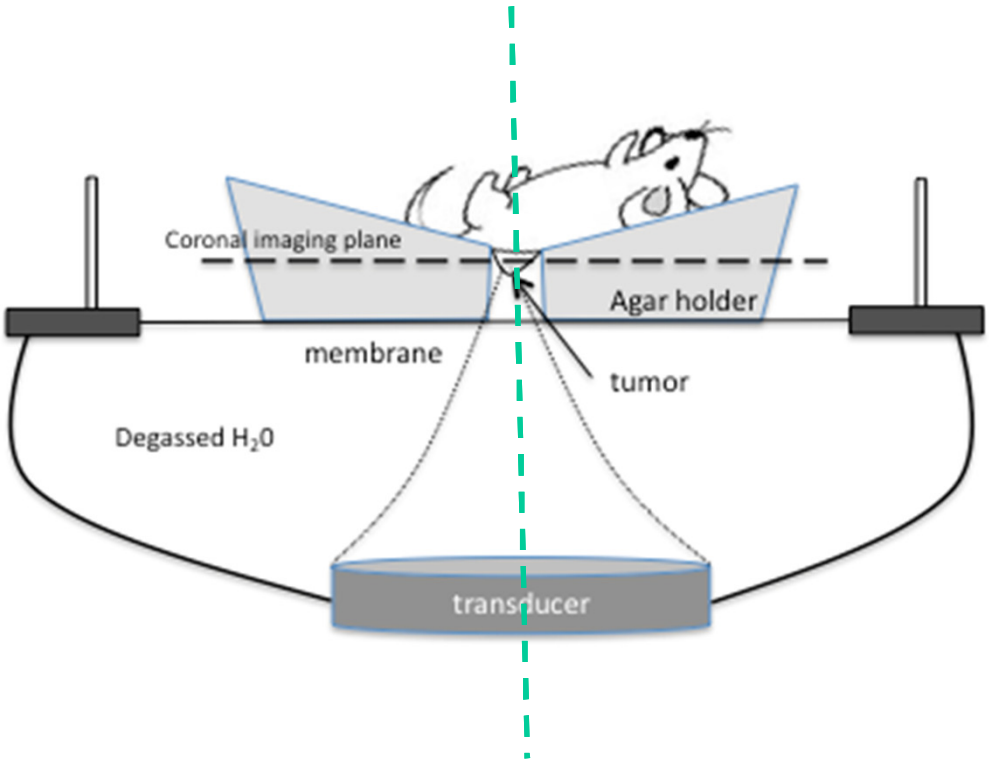
- Chemotherapy by PTX/PFP/PEG-PLLA nanodroplets and ultrasound

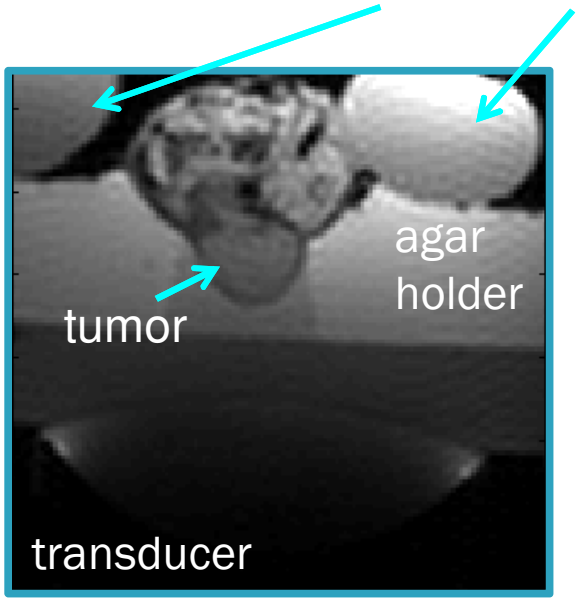


Rapoport, N. et al., J Control Release 2009; 138(3): 268-276

MRgFUS

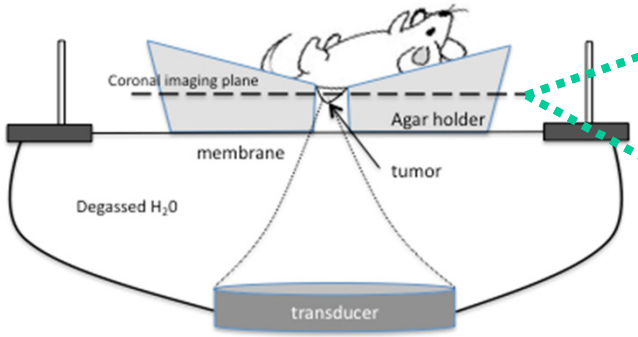
- Small Animal LabFUS System (Image Guided Therapy, Inc.)
  - 16-element annular transducer, f = 3 MHz, r_c = 3.5 cm

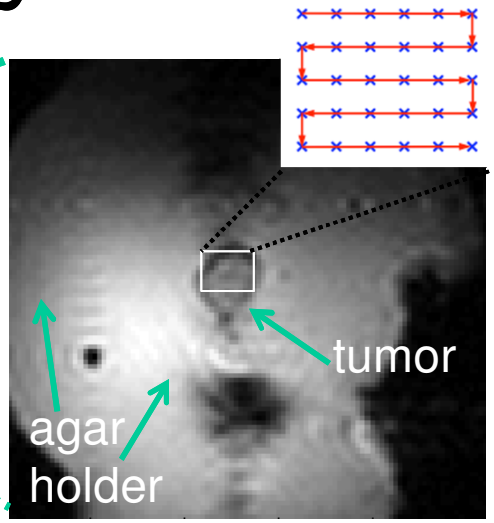




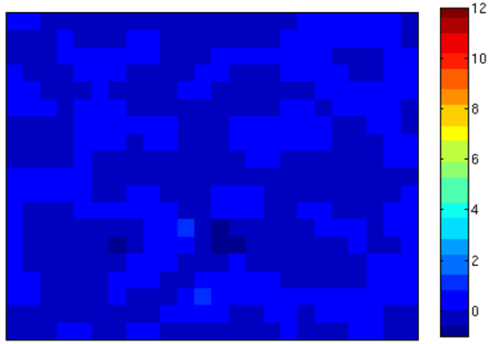
Rapoport, N. et al., J Control Release 2011; 153(1): 4-15

Treatment monitoring: MR Thermometry





Coronal slice orientation

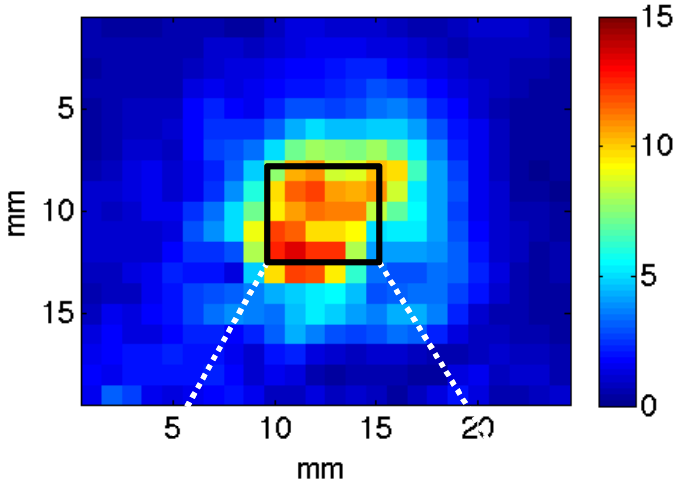


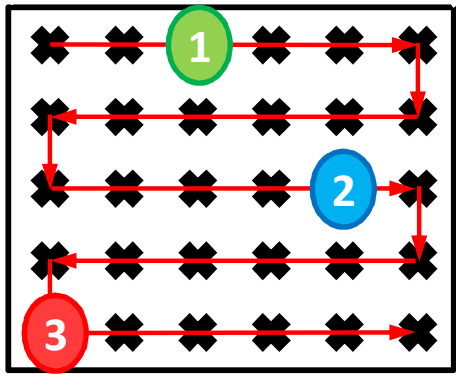
Ultrasound Parameters

- 3-MHz
- P = 3.4 MPa
- 1 x 3 mm focal spot
- Grid trajectory, 4 x 5 mm
- 5 minute sonication time

MR thermometry response

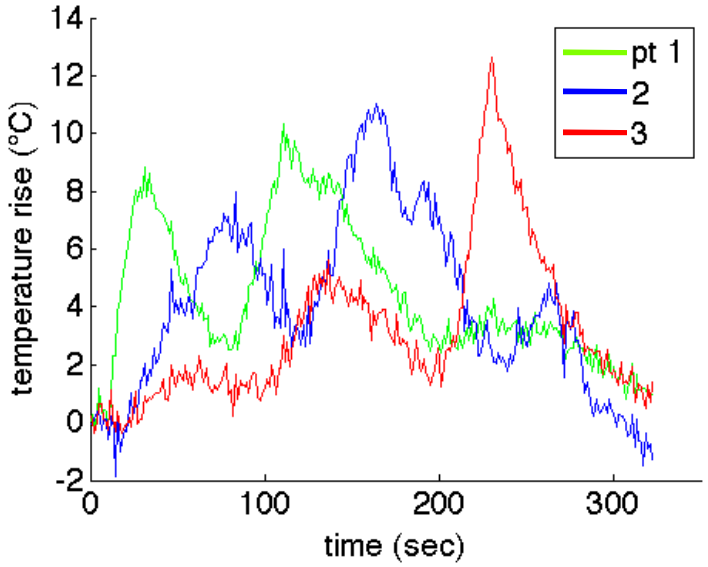
Maximum temperature projection in time



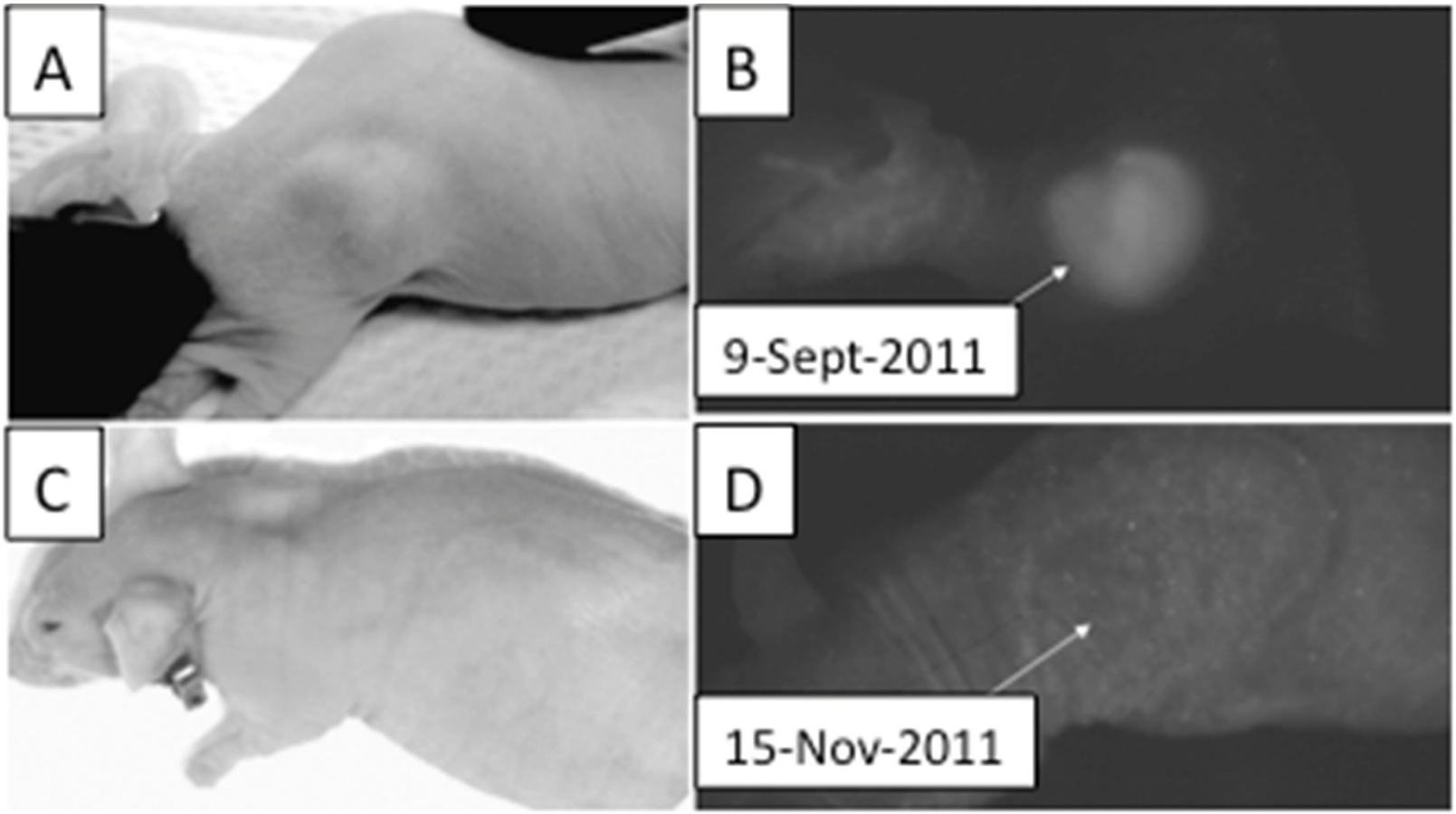


MR Parameters

- SegEPI sequence, EPI=3
- 2x2x3 mm (ZFI to 1x1x3mm)
- 1.3 seconds
- TR/TE = 60/10 ms
- Flip angle = 15°
- 752 Hz/pixel
- Referenceless reconstruction

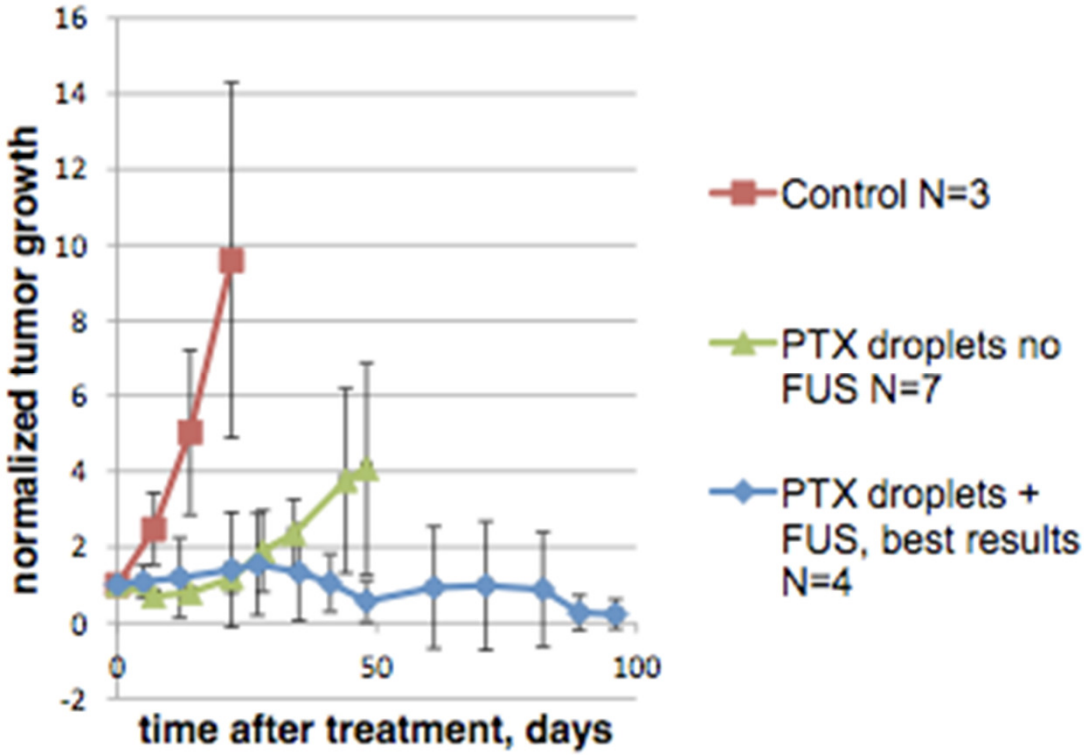


Tumor Resolution



Tumor cells were transfected with RFP; only viable cells generated fluorescence

Growth Curves
Pancreatic Cancer



Lifespan results

Treatment Group	Average Life Span, weeks (mean ± std)
Control (N=7)	3.5 ± 0.5
No injection, MRgFUS (N=6)**	4.8 ± 2.3
Empty droplets, MRgFUS (N=6)**	3.5 ± 2.1
PTX droplets, no MRgFUS (N=7)	7.0 ± 0.8
PTX droplets, MRgFUS (CW, injection-MRgFUS time=8 hrs, N=8)***	10.3 ± 1.6

**Mice that died within several days after treatment (P>4.2 MPa) were excluded

***Survivors (N=2 for the grid trajectory) were excluded

Results **Courtesy of N. Rapoport, U. of Utah**

- PTX-loaded nanodroplets + MRgFUS dramatically decrease pancreatic tumor growth
- MR guidance improves treatment outcome
  - Detailed anatomic visualization
  - Tumor targeting and treatment planning
  - Real-time MR temperature imaging
- Treatment success is a function of ultrasound parameters
- In the absence of drug, hyperthermic conditions could increase perfusion and inflammation thus accelerating tumor growth.

Study Participants

Natalya Rapoport

Allison Payne

Christopher Dillon

Jill Shea

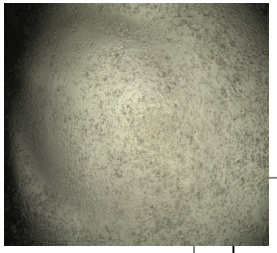
Roohi Gupta

University of Utah, Salt Lake City, Utah, USA

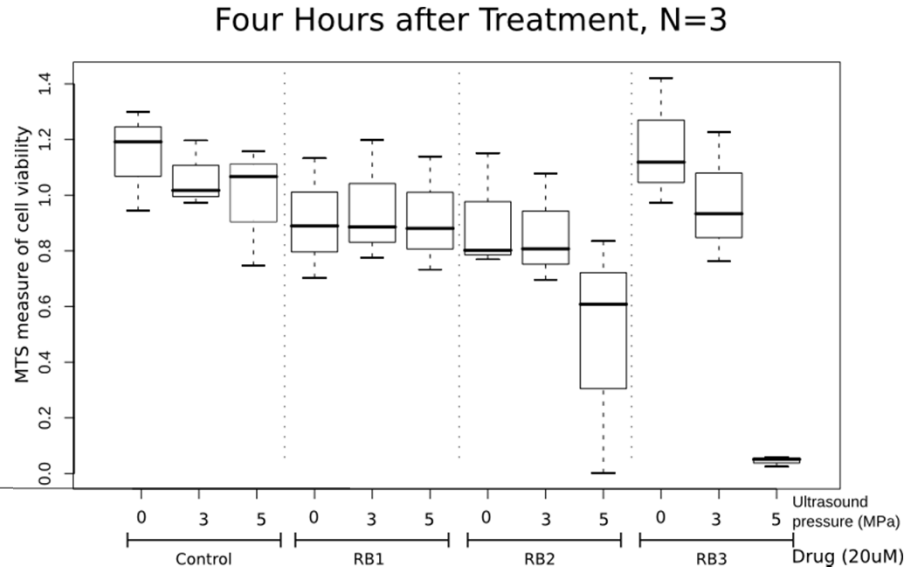
Understanding Ultrasound/Drug Synergy

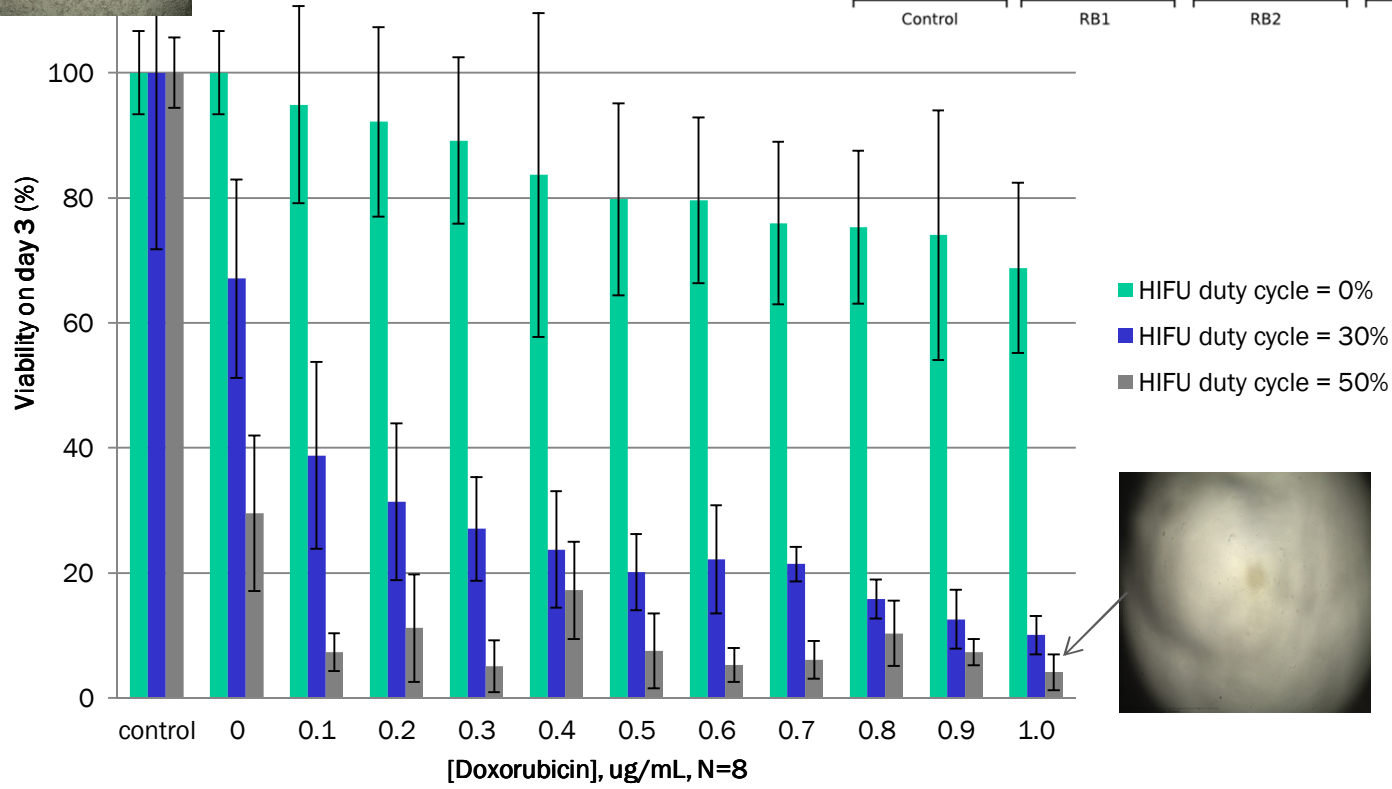
- Going beyond the anecdotal evidence
- Look at thermal, mechanical interactions independently
- Understand biological mechanism
- Clues to what drugs might work best

HIFU Treatment with Drugs (Sonodynamic Therapy?)

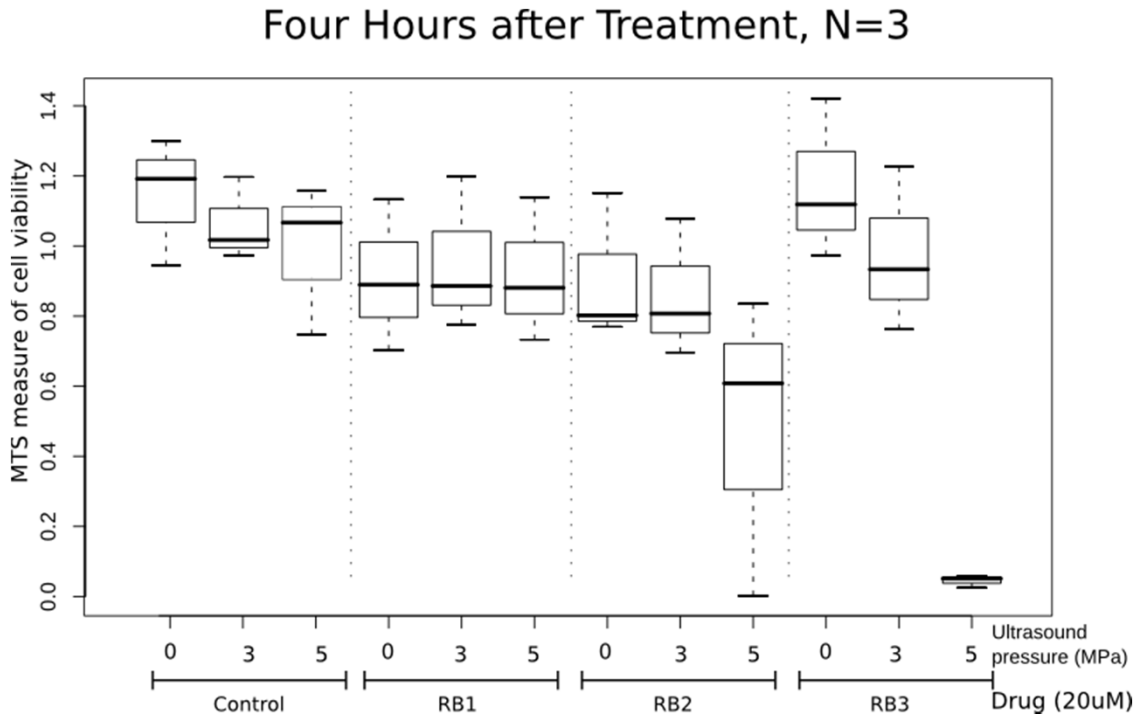


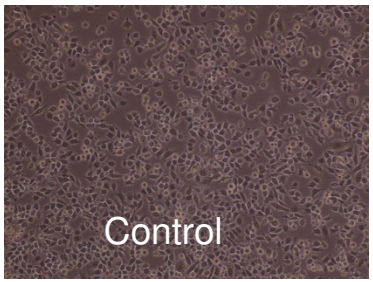
control

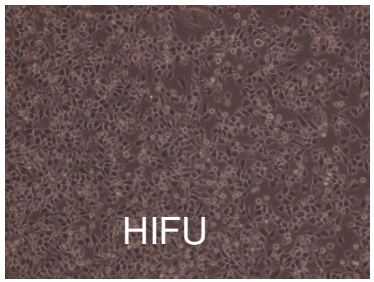


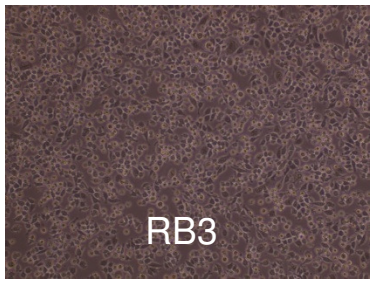


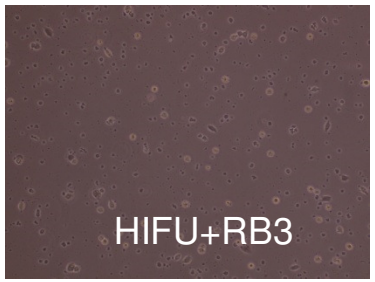
‘Sonodynamic Therapy’











Conclusions

Use of ultrasound to alter tissue properties or drive release from nanocarriers is a very promising approach to targeted drug delivery

Challenges: need to visualize the target before you can hit it (metastatic disease problem)

- regular ultrasound limitations: no penetration in air, little through bone

Potential Areas for Application

Cancer – large or infiltrative tumor

Cardiac – plaques or thrombii

Neuro – target drugs to specific sites of the brain, spine

Orthopedic – joints, near surface bone lesions

Ophthalmology – drugs to the retina, through cornea

Others?