PERSONALIZED MEDICINE WITH LIGAND-TARGETED PERFLUOROCARBON NANOPARTICLES

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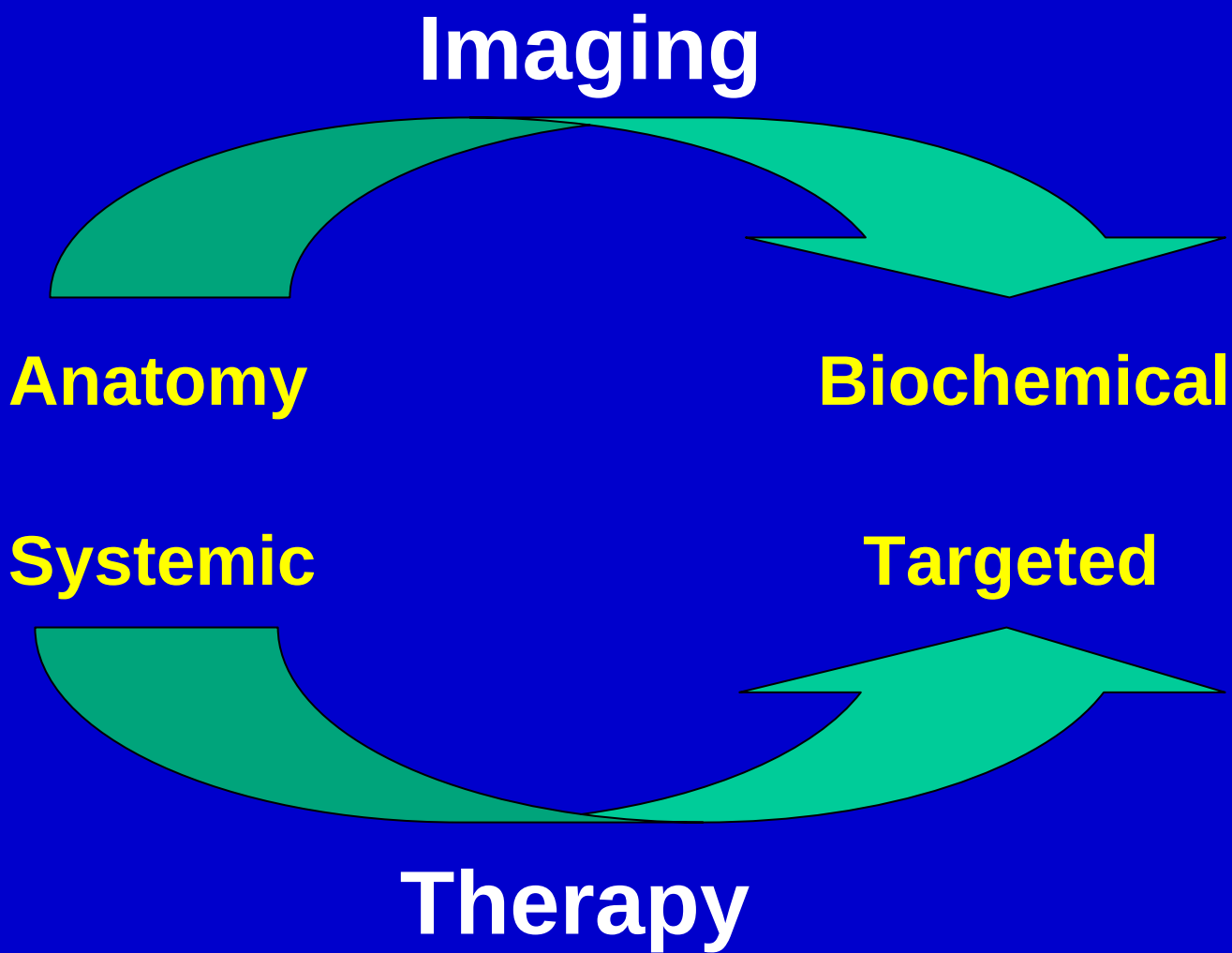
- **Financial Support: NHLBI, NCI**
- **Equipment Support: Philips Medical Systems**
- **Materials Support: Bristol-Myers Squibb Medical Imaging / Kereos, Inc**
- **Conflicts of Interest: Kereos Co-founder with < 5% common stock ownership**

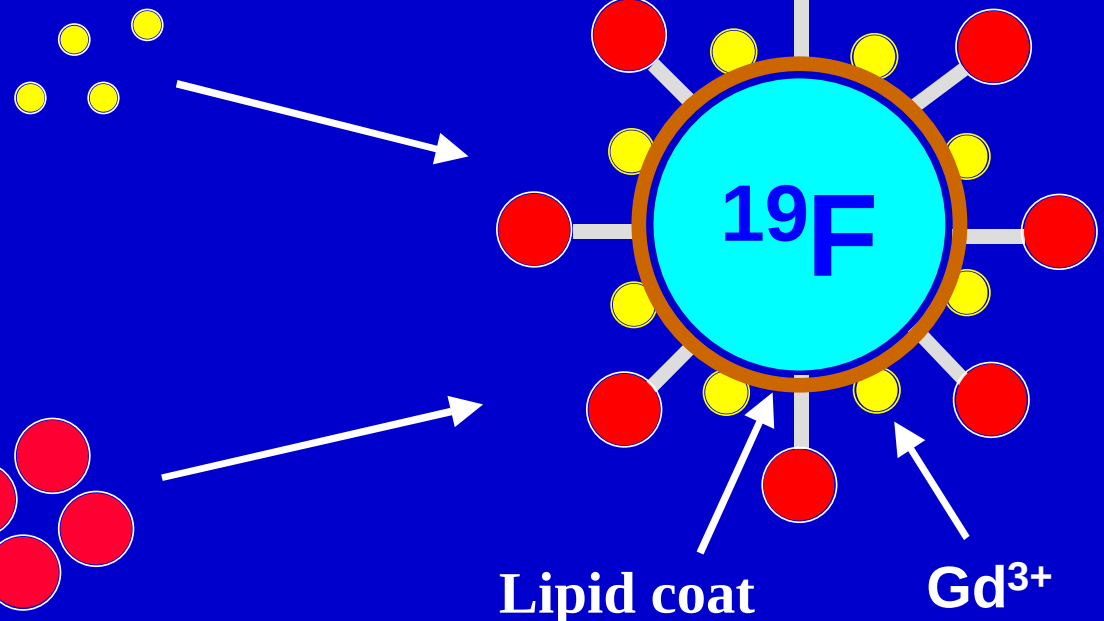
Evolving Paradigms in Medicine



GENERALIZED TARGETING PARADIGM

Payloads

Imaging agents (Gd^{3+})
Drugs
Radionuclides

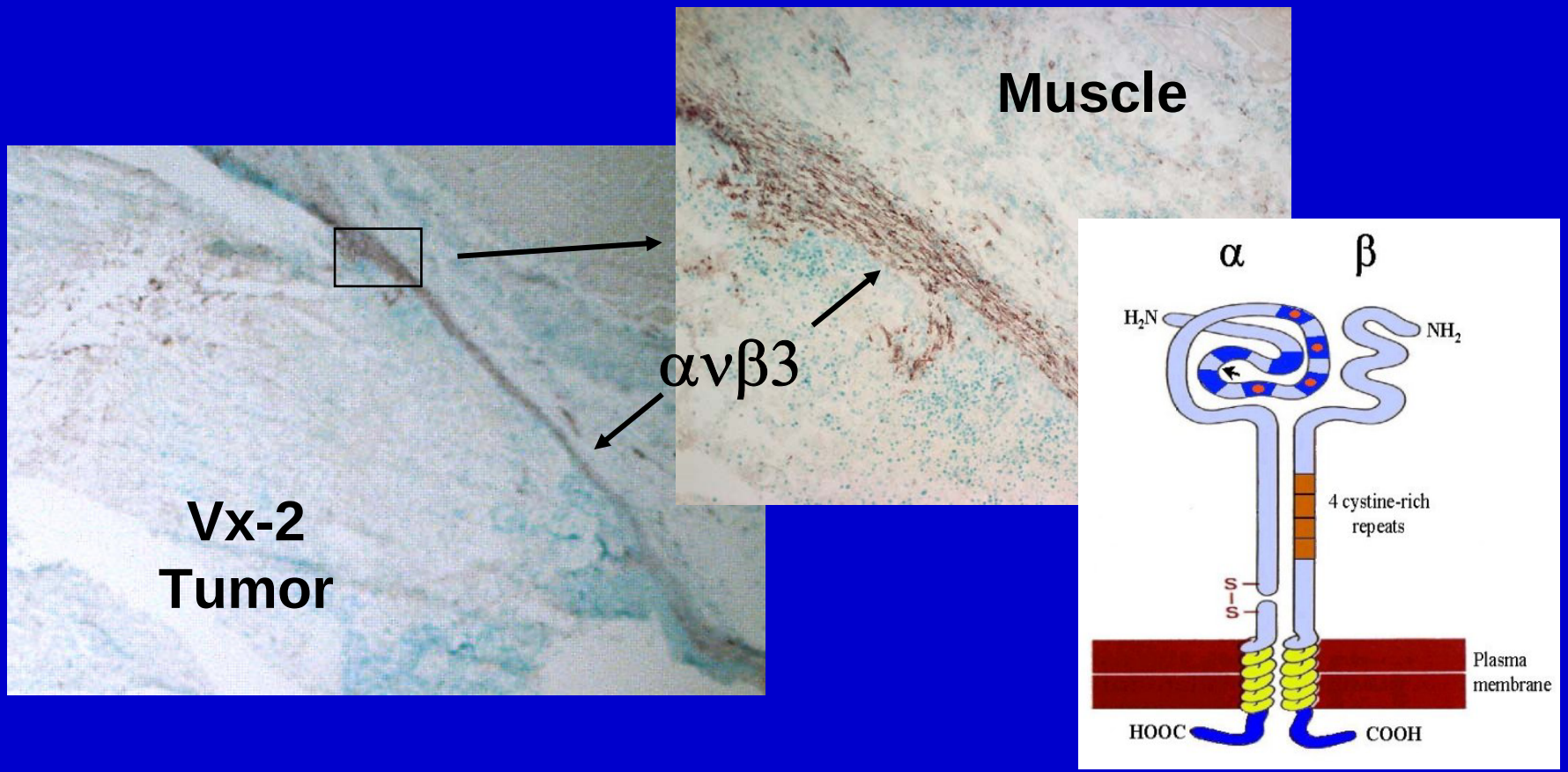


Anti-fibrin
Anti-tissue factor
Anti–$\alpha_v\beta_3$-integrin

Targeting system: "Molecular zip codes"

(**MAb**, aptamers, small peptides, peptidomimetics, polysaccharides, etc)

Integrins are Important Biosignatures of Neovascularity

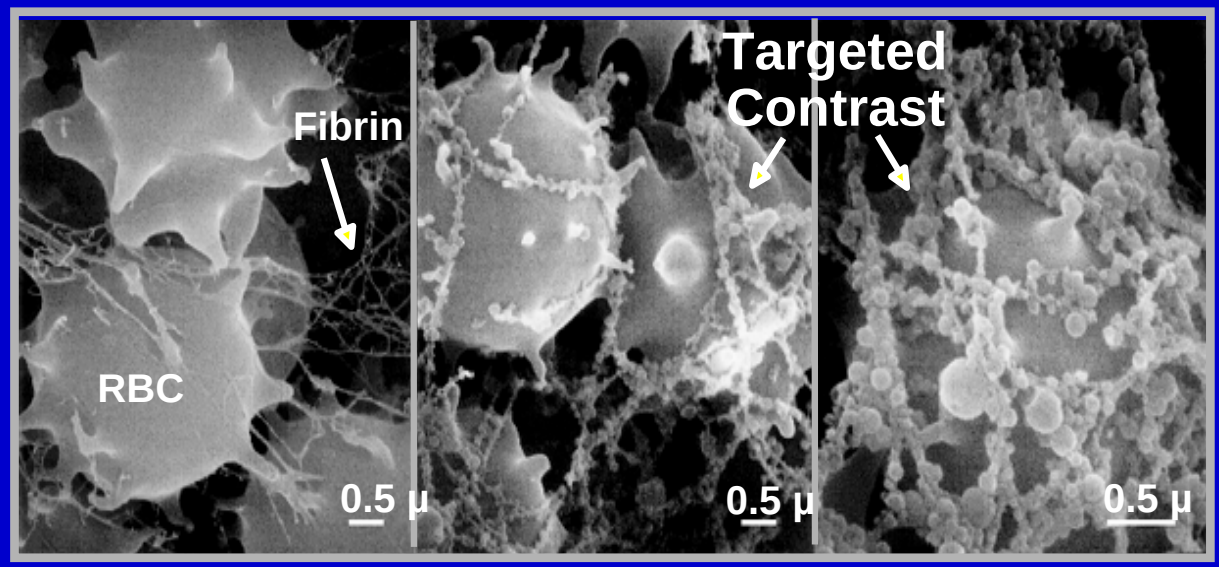


Helen J. Mardon, University of Oxford, http://www.medicine.ox.ac.uk/ndog/mardon

Fibrin Imaging

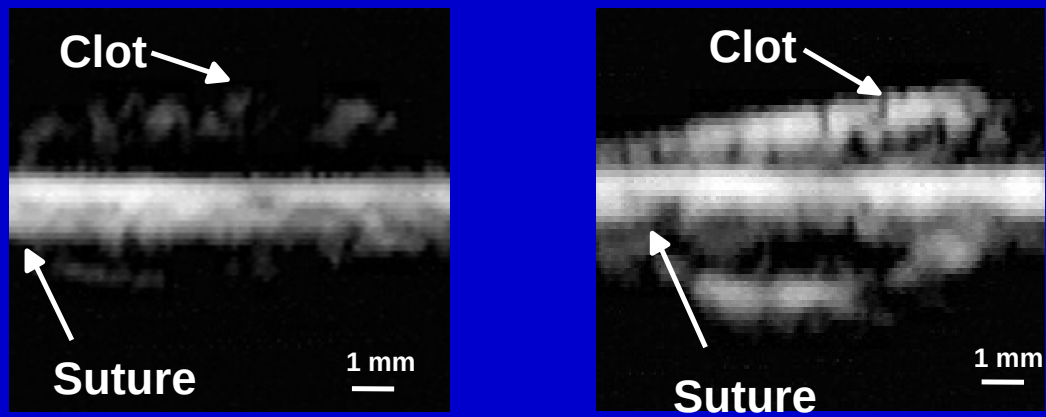
Scanning EM

clot labeled with fibrin targeted nanoparticles



Ultrasound contrast

enhancement of fibrin clot in vitro using conventional clinical scanner (7.5 MHz)

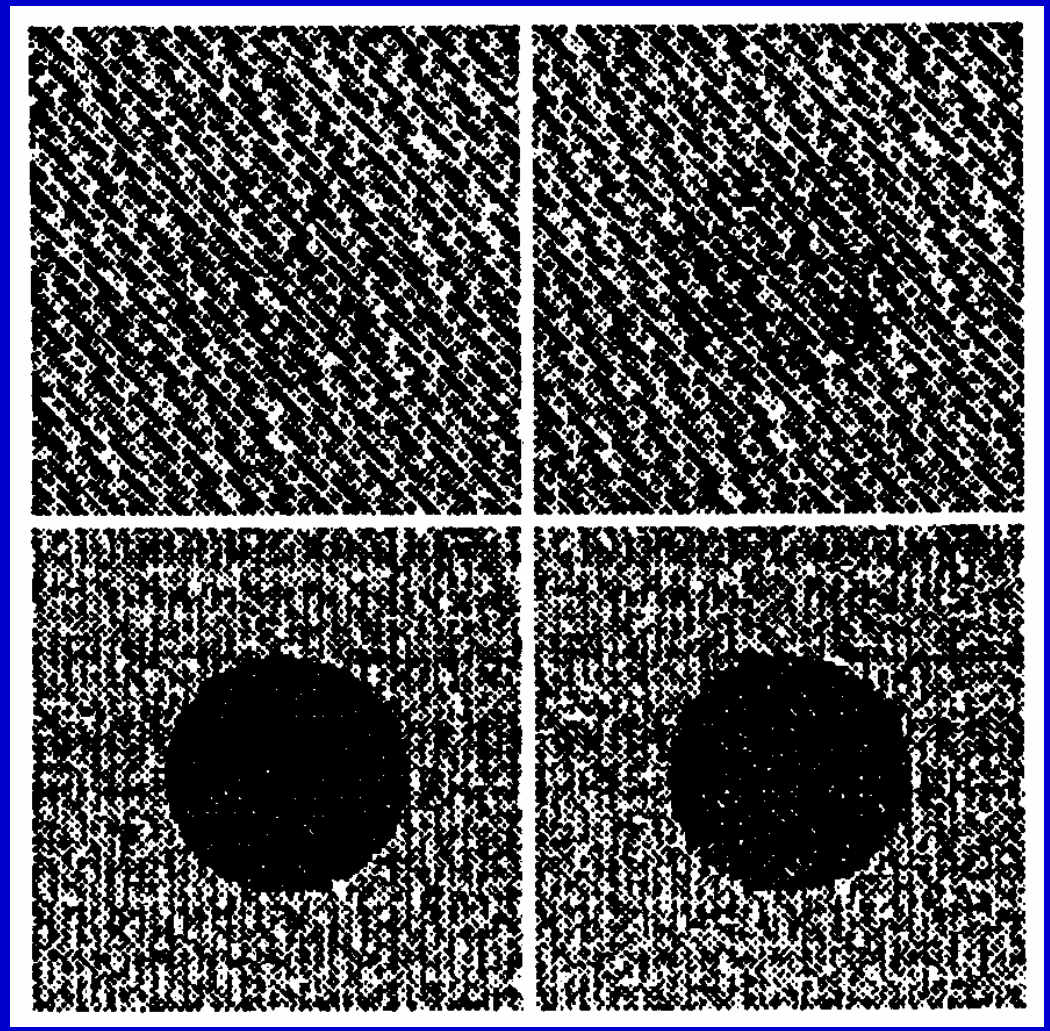


Control Thrombus Targeted Thrombus

Graphite Composite Images

P-P

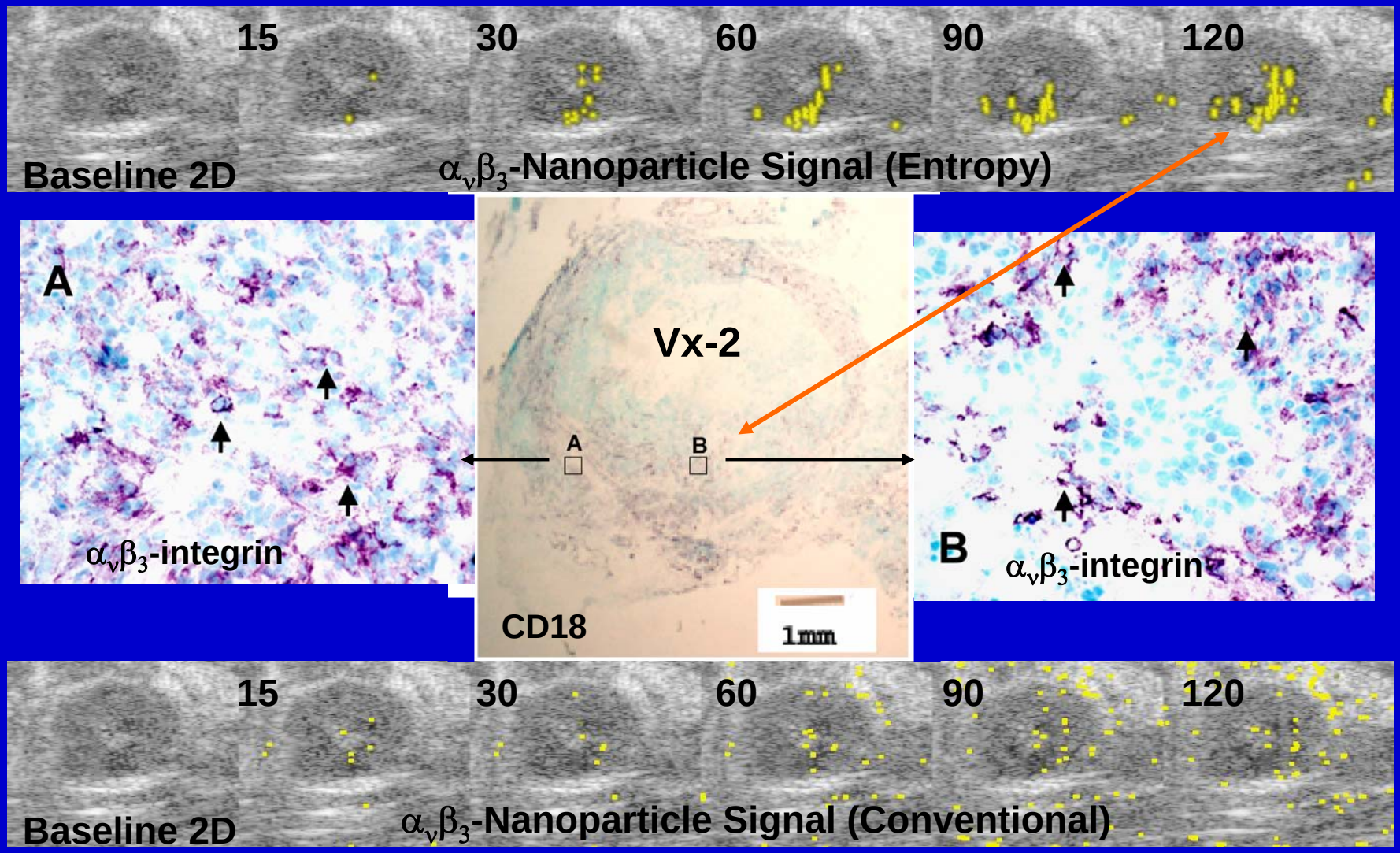
IBS



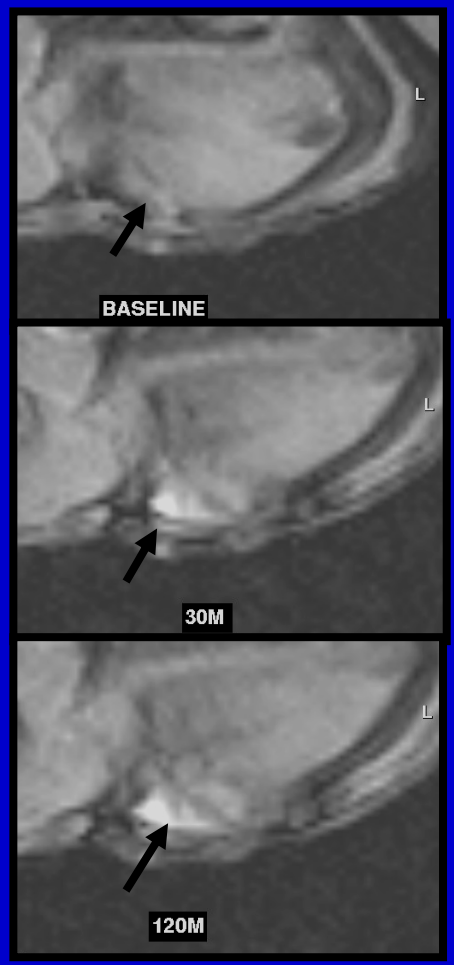
H

H_c

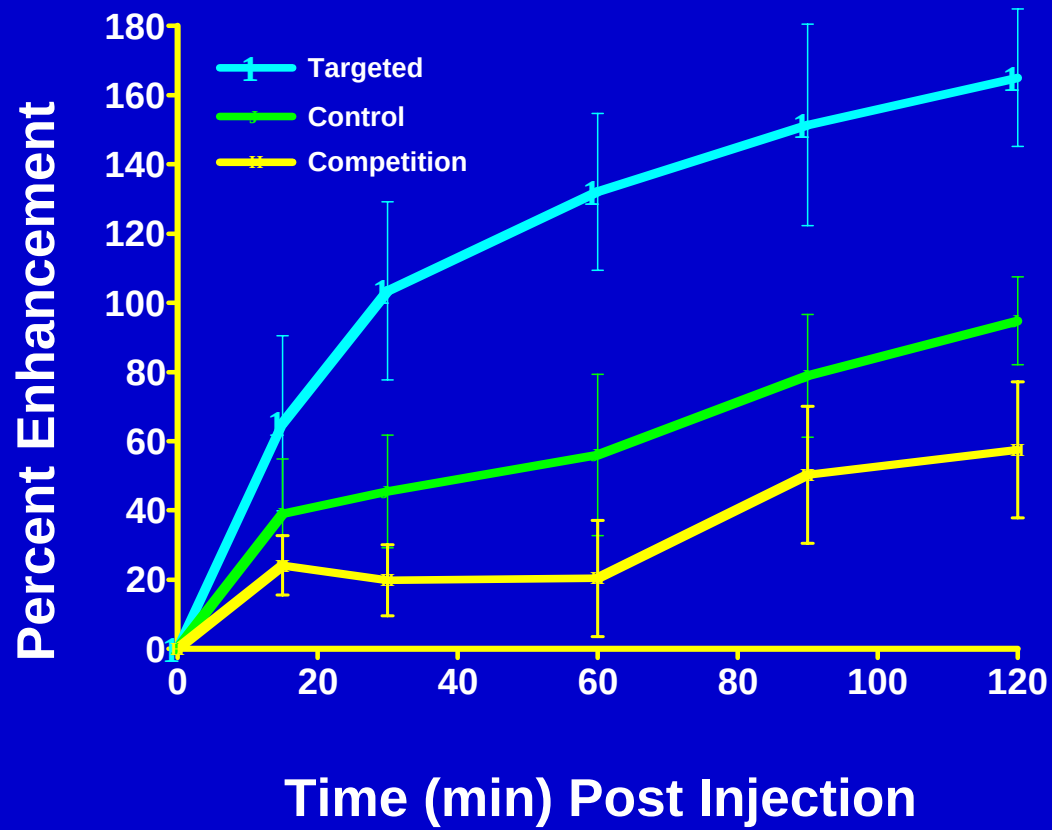
Improved Imaging of $\alpha_v\beta_3$-Nanoparticles Using Information Theoretic Detectors



Melanoma Angiogenesis: Detection With $\alpha_v\beta_3$ *Integrin-Targeted* Paramagnetic Nanoparticles *Mouse Imaging @ 1.5T*



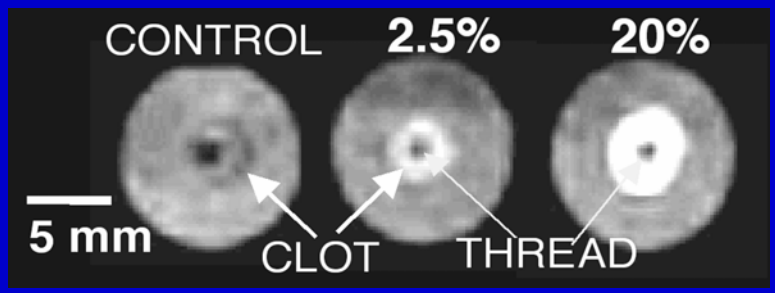
Time course

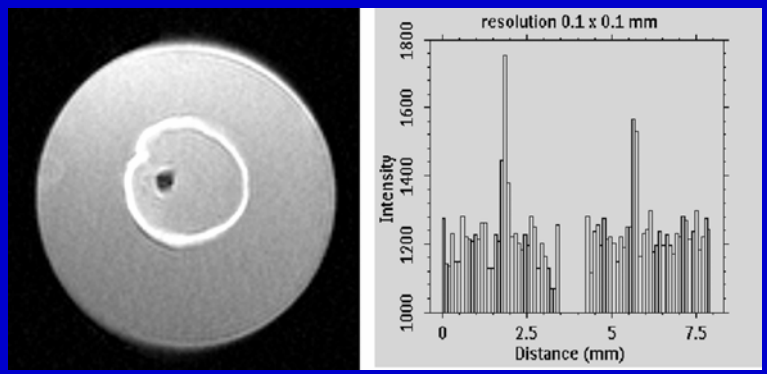


Schmeider, Wickline and Lanza et al MRM, 2005.

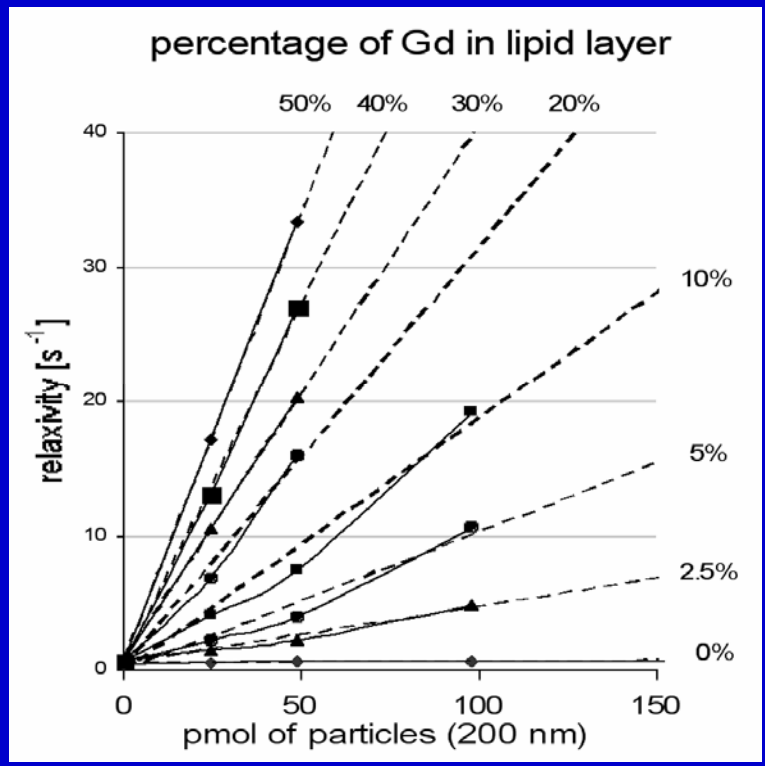
MRI Molecular Imaging (1.5T) of Thrombus

High Detection Sensitivity
0.7 x0.7mm



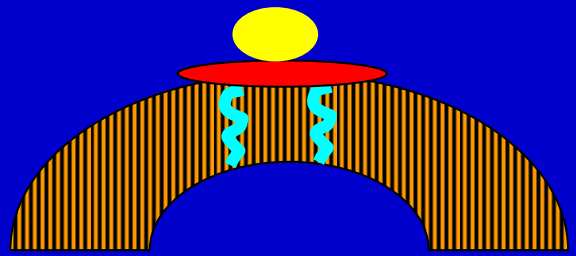


High Contrast and Resolution
0.1 x0.1mm

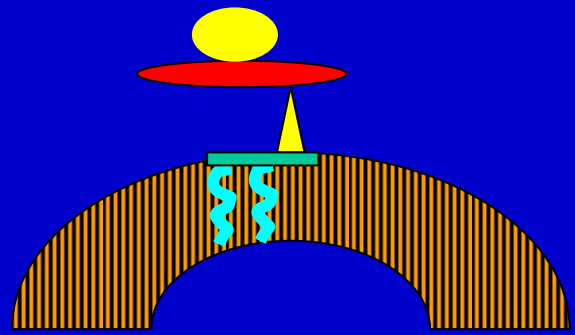


Increasing Payload
High Molecular Relaxivity

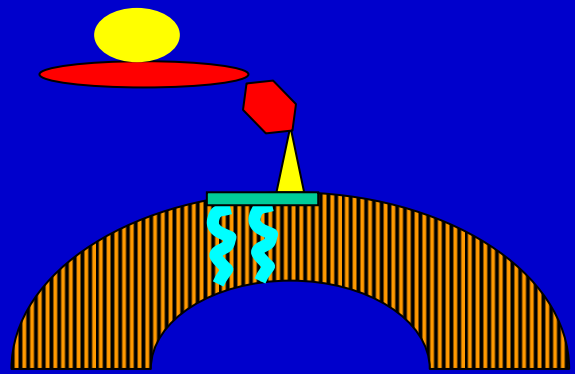
Optimize Paramagnetic Lipophilic Chelate for Nanoparticles



Gd-DTPA-BOA



Gd-DTPA-PE

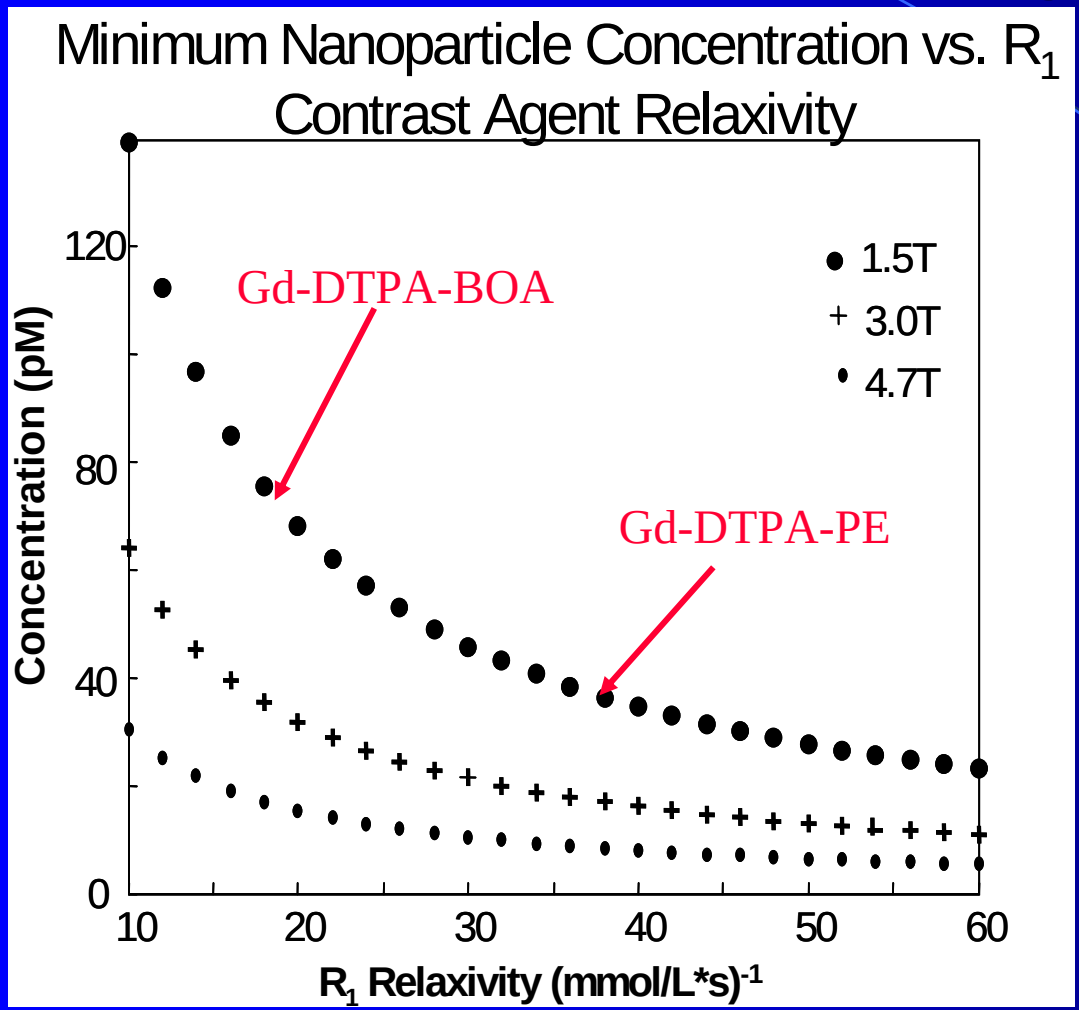


Gd-DOTA-MeO-PE

Impact of Lipophilic Chelate on Relaxivity

Magnetic Field	Paramagnetic Chelate	Ion-Based Relaxivity $(s*mM)^{-1}$		Particle-Based Relaxivity $(s*mM)^{-1}$	
		r_1	r_2	r_1	r_2
0.47 T	Gd-DTPA-BOA	21.3 ± 0.2	23.8 ± 0.3	1,210,000 ± 10,000	1,350,000 ± 20,000
	Gd-DTPA-PE	36.9 ± 0.5*	42.3 ± 0.6*	2,710,000 ± 40,000*	3,110,000 ± 50,000*
1.5 T	Gd-DTPA-BOA	17.7 ± 0..2	25.3 ± 0.6	1,010,000 ± 10,000	1,440,000 ± 30,000
	Gd-DTPA-PE	33.7 ± 0..7*	50 ± 2*	2,480,000 ± 50,000*	3,700,000 ± 100,000*
4.7 T	Gd-DTPA-BOA	9.7 ± 0.2	29.4 ± 0.3	549,000 ± 9,000	1,670,000 ± 20,000
	Gd-DTPA-PE	15.9 ± 0.1*	80.0 ± 0.7*	1,170,000 ± 6,000*	5,880,000 ± 50,000*

Particle Concentration Requirements *for CNR=5*: Dependence on Relaxivities and Field Strengths

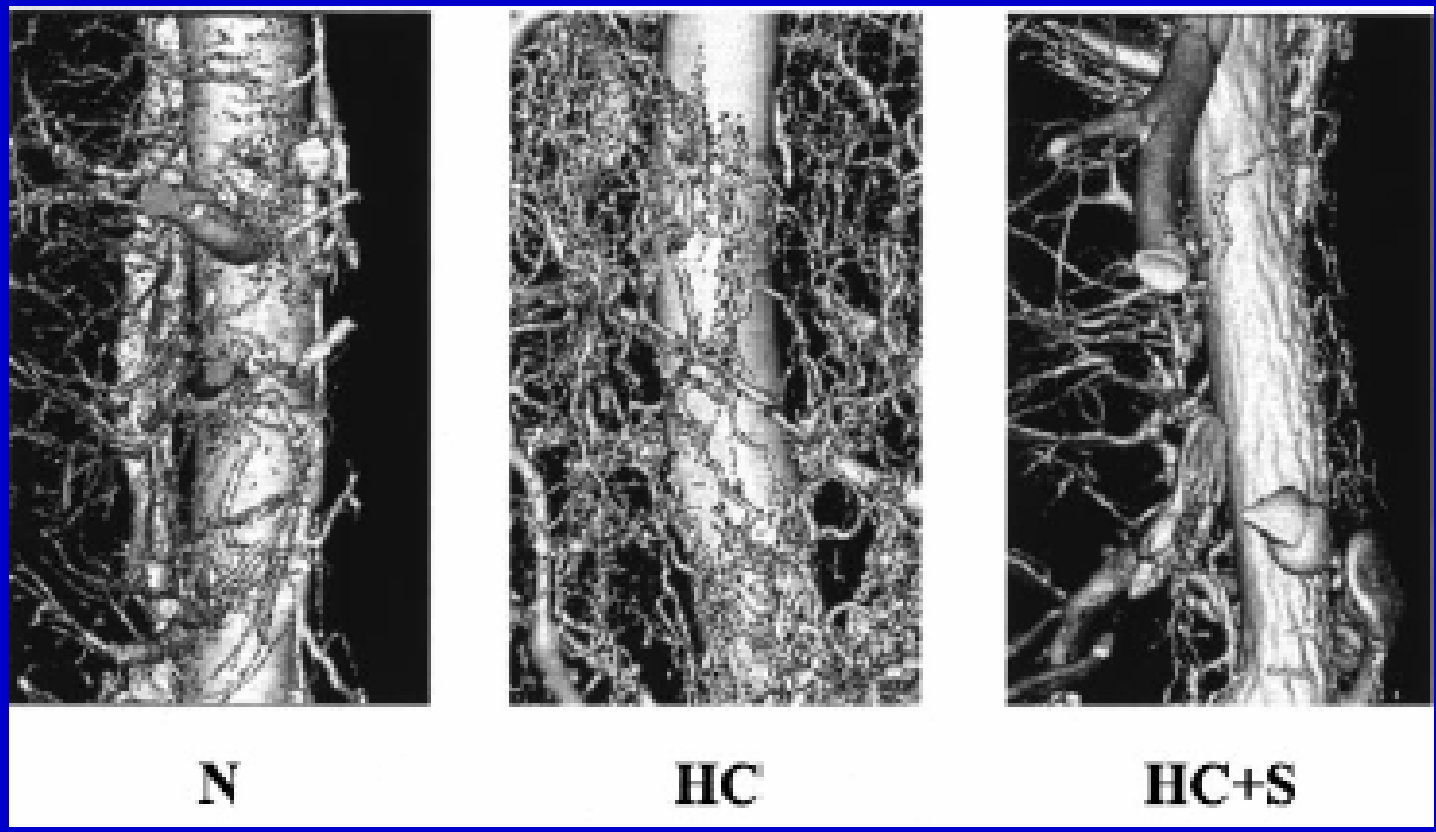


- Model simulations: blood vs targeted tissue
- Gd^{3+}/particle=85,350
- Spin echo sequence (T1w)
  - TE:10/TR: 1100-1600
- SA=8
- Voxel volume=0.3125 µl
- R2 adjusted for field

$$CNR = \left| \frac{I_b - I_a}{N} \right|$$

Atherosclerotic Expansion of the Vasa Vasorum

Wilson, et al. Circulation 105:415, 2002.

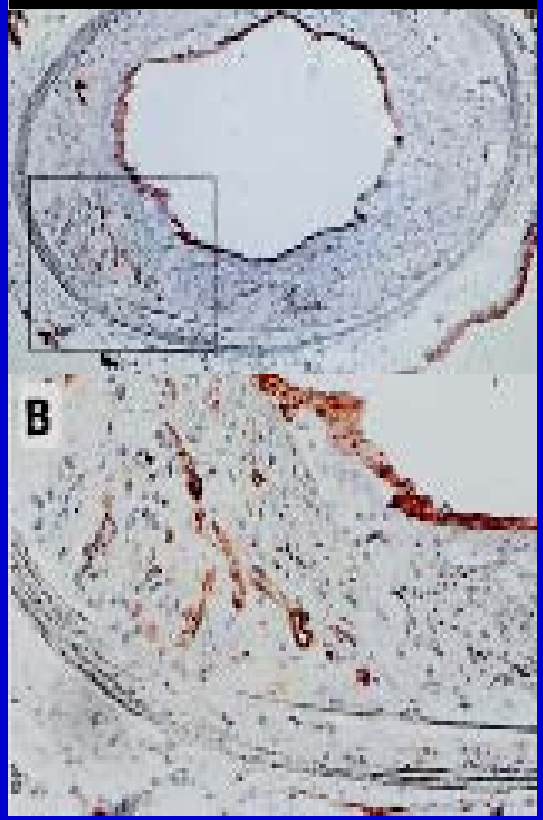


Micro CT of Isolated Pig Coronary Artery

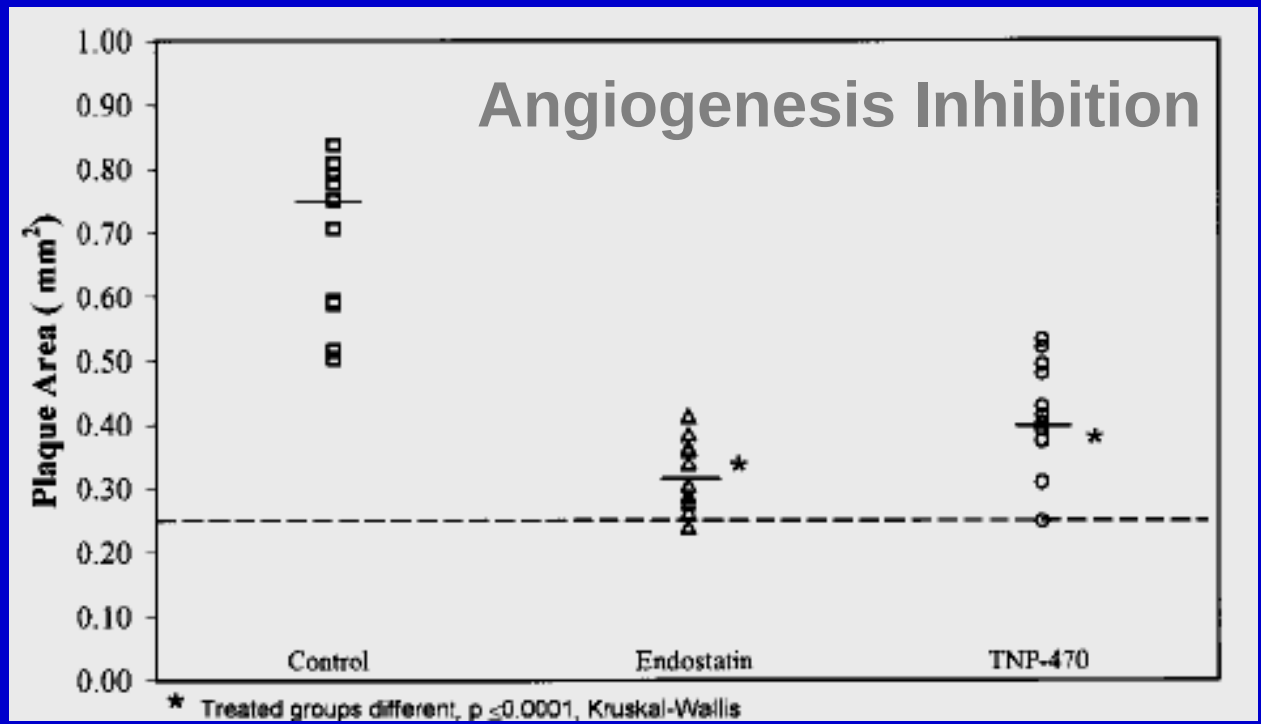
Anti-Angiogenesis in the ApoE Mice

Moulton, et al. Circulation 99: 1726, 1999.

Anti-CD31 staining



Cholesterol-Fed ApoE-Deficient Mice

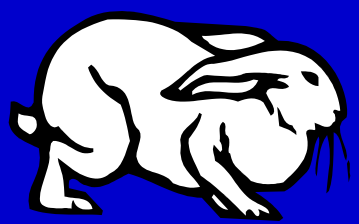


13 weeks @ 30 mg/kg/QOD (45 doses) ~ 1.6 g/kg

Animal Model

~40 Trillion
Nanoparticles
In 1 ml I.V.

New Zealand
White Rabbit



Cholesterol-Fed
1 % for ~ 80 days

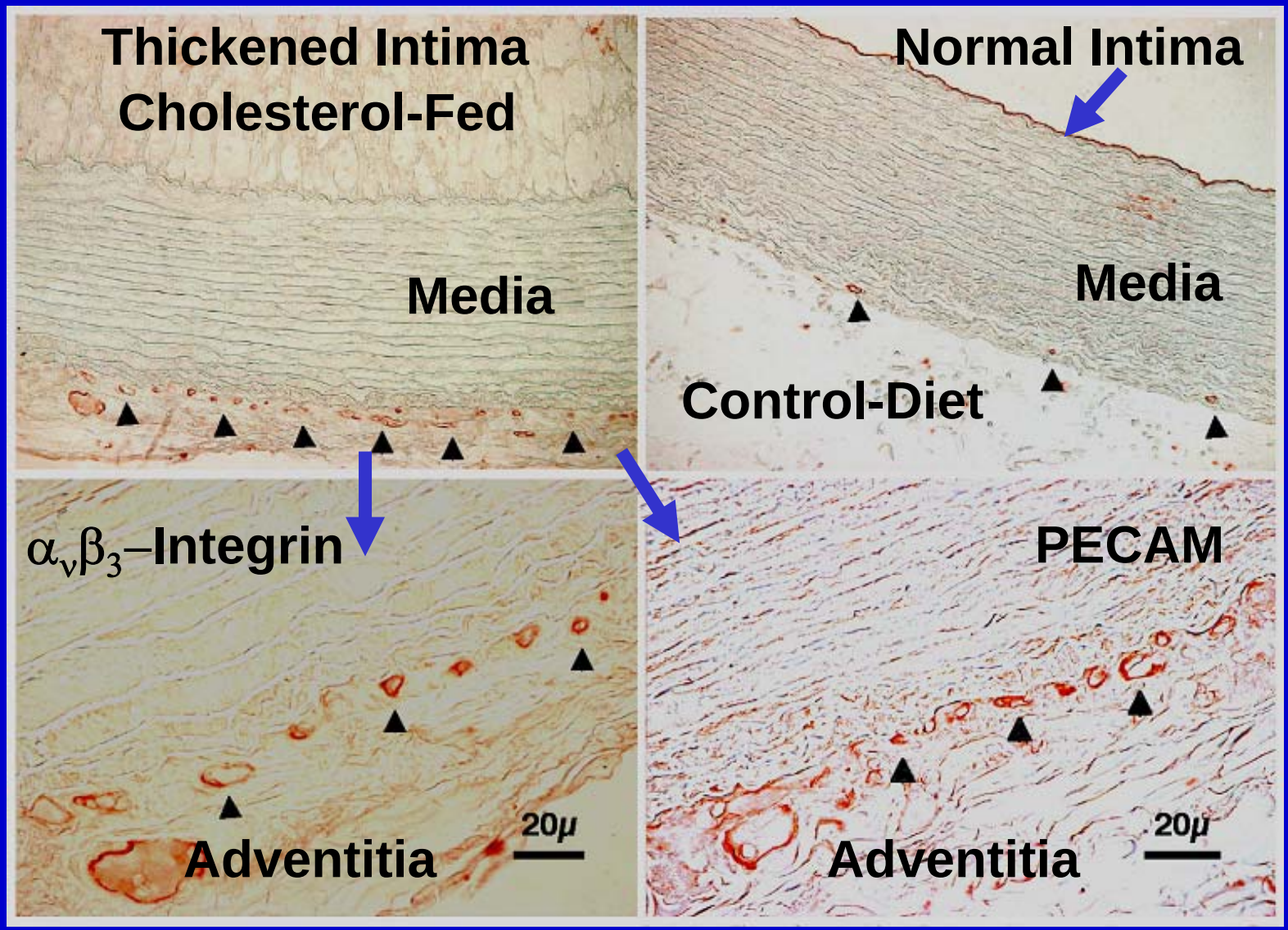
$\alpha_v\beta_3$-Targeted
Nanoparticles
n = 5

Non-Targeted
Nanoparticles
n = 4

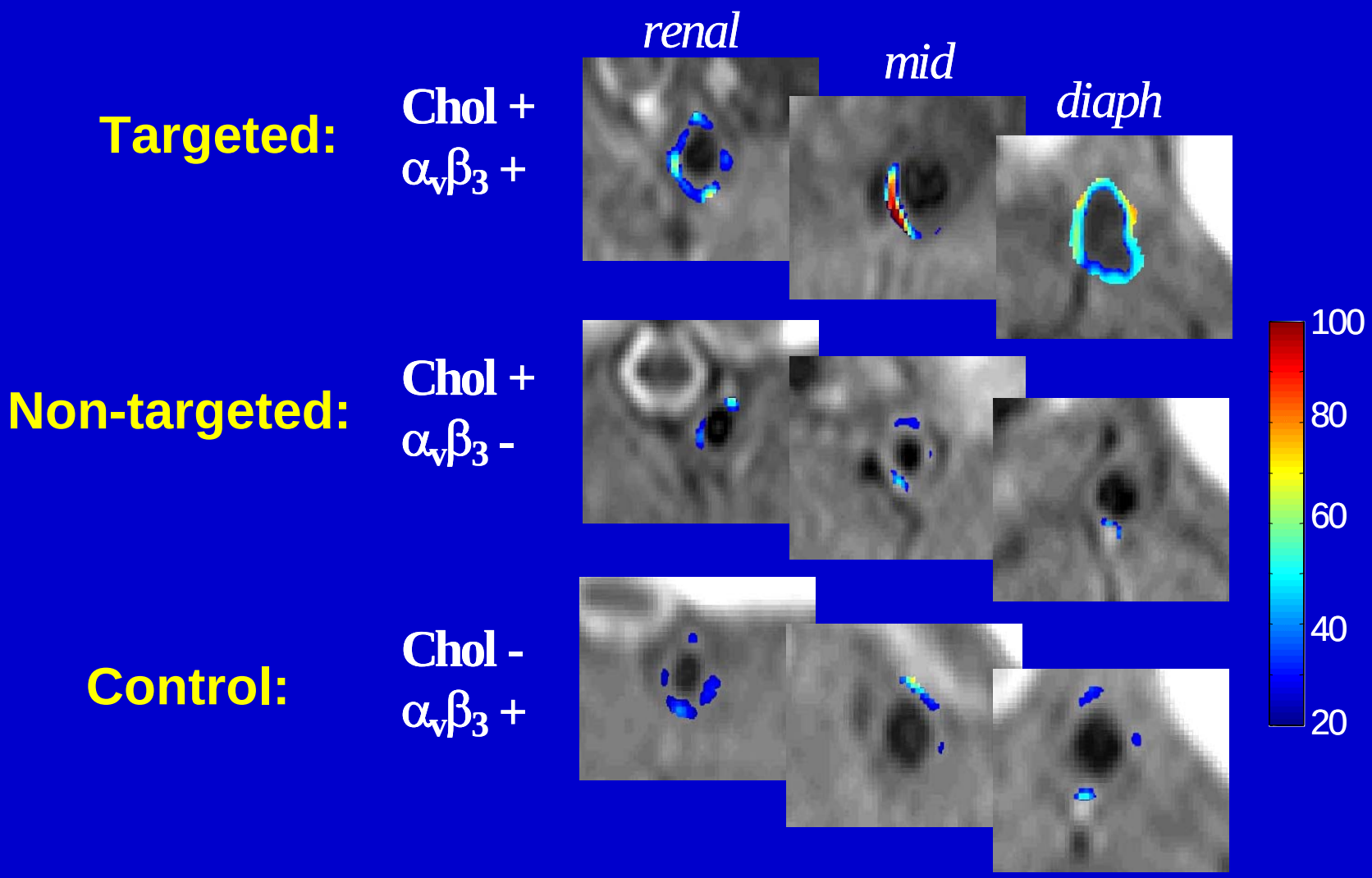
Control Diet
~ 80 days

$\alpha_v\beta_3$-Targeted
Nanoparticles
n = 5

Histological Corroboration of Angiogenesis

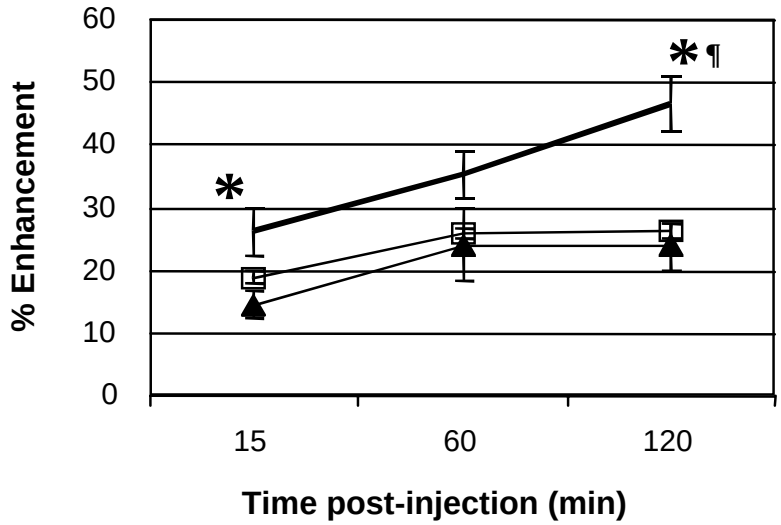


Signal Enhancement in Selected Transverse Slices

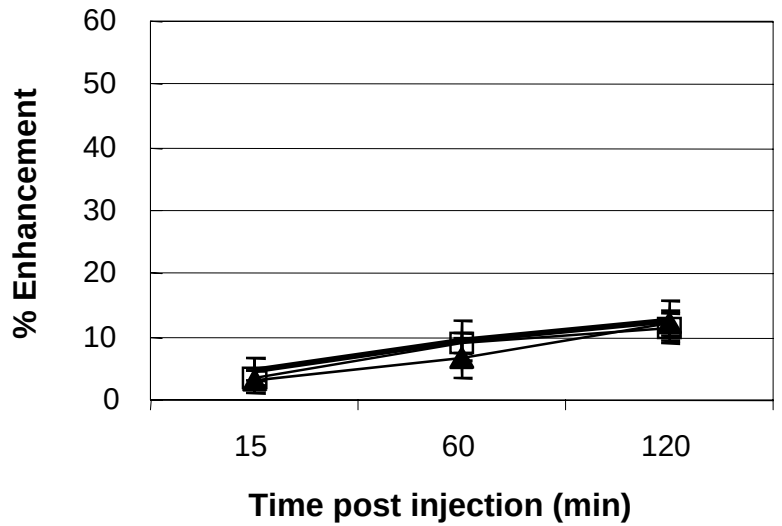


Relative contrast enhancement for aorta vs skeletal muscle

AORTA *all levels, all aortas averaged*



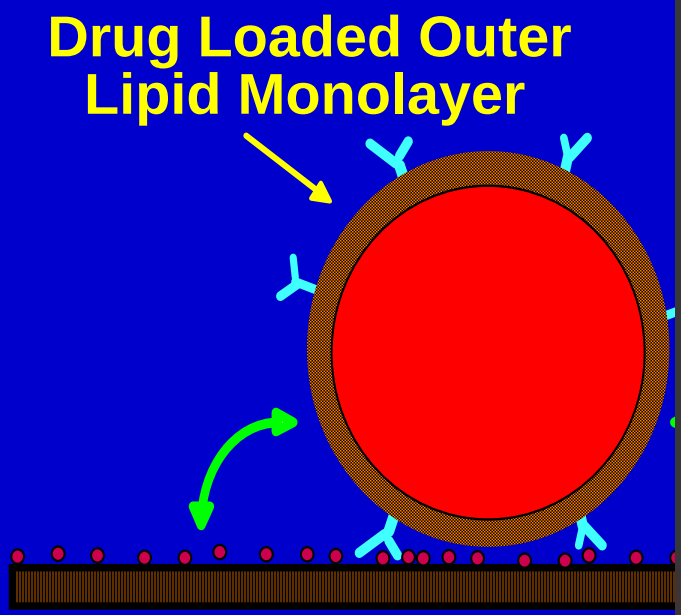
MUSCLE *single ROI's*



—: **Chol targeted** ▲ **: Control targeted** □ **: Chol nontargeted**

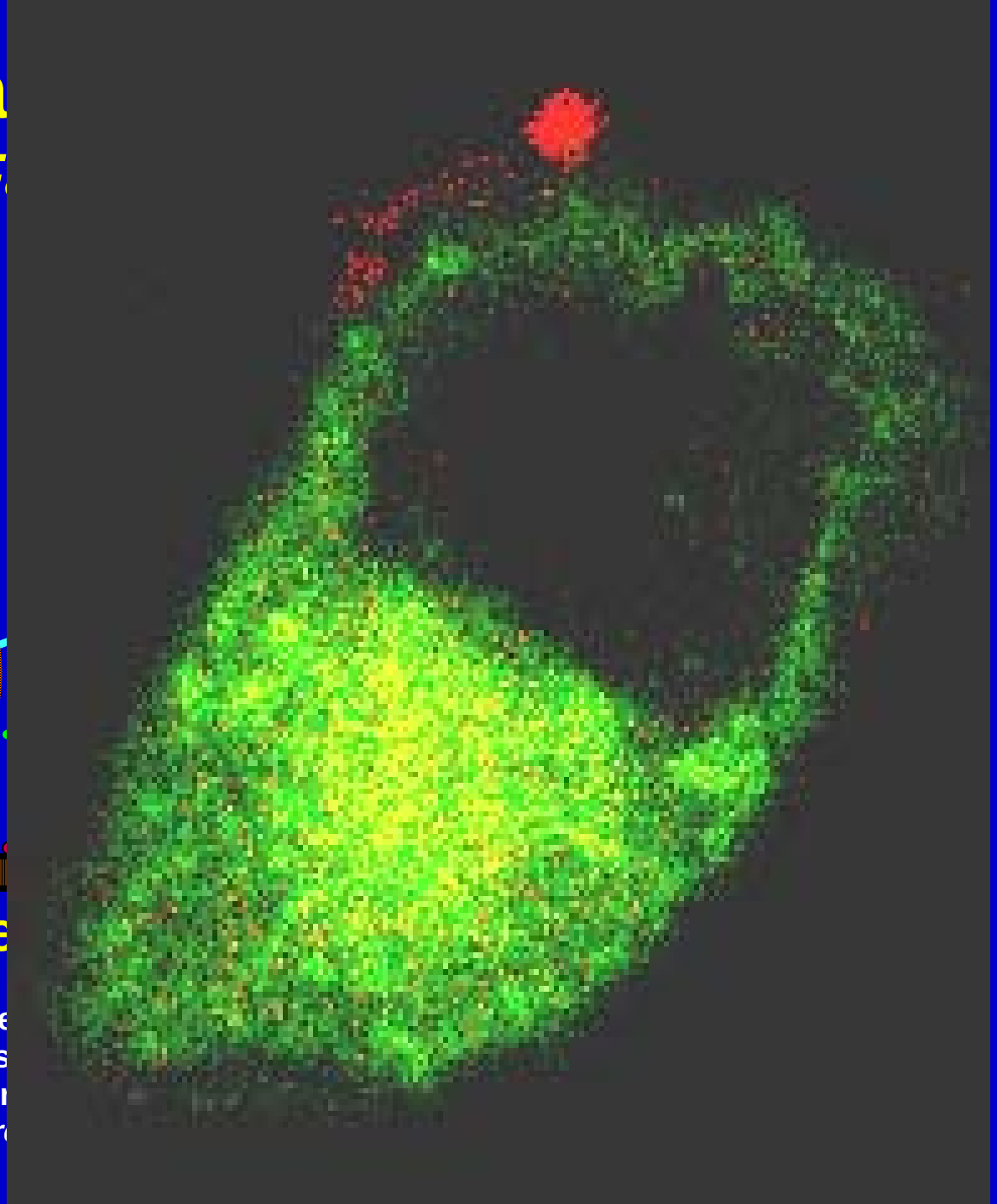
Novel Mecha

"Contact F

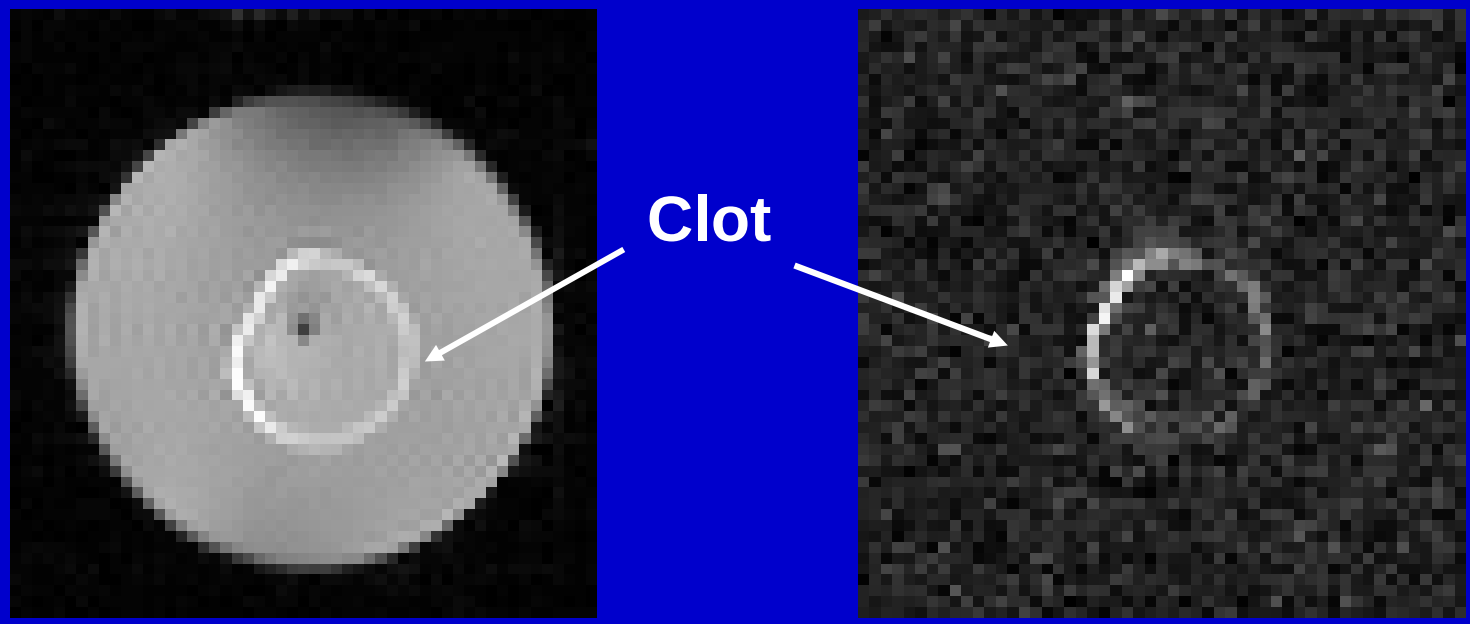


Target Cell Me

*Lanza GM, Wickline SA et al: Targete
drug delivery to vascular smooth mus
resonance imaging nanoparticle contr
for rational therapy of restenosis. *Cir*
2847



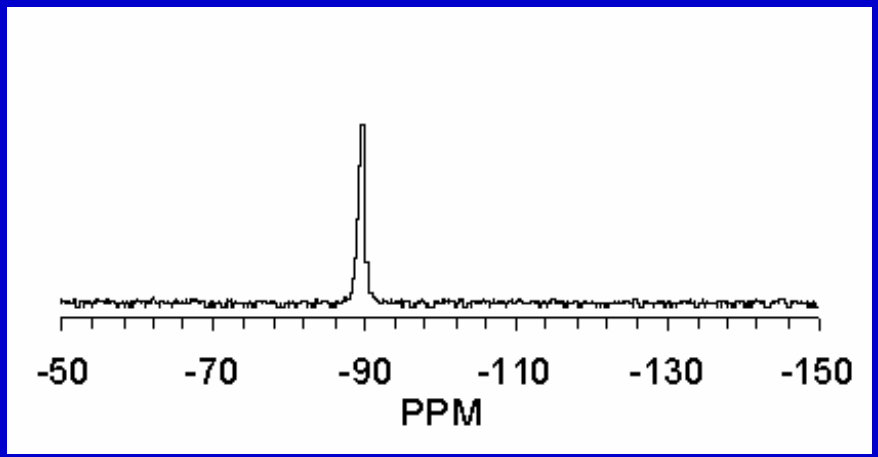
Proton and Fluorine Imaging at 4.7T

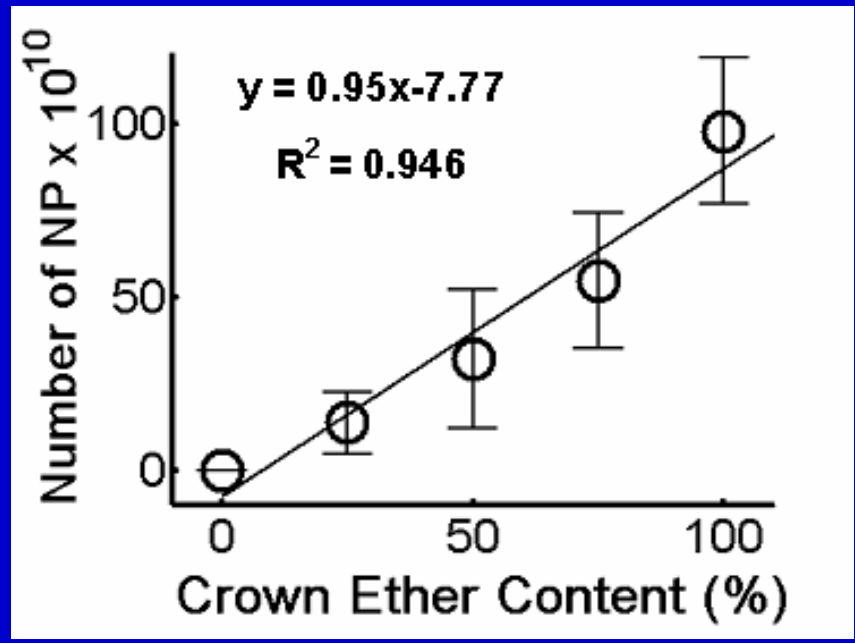


Proton Imaging

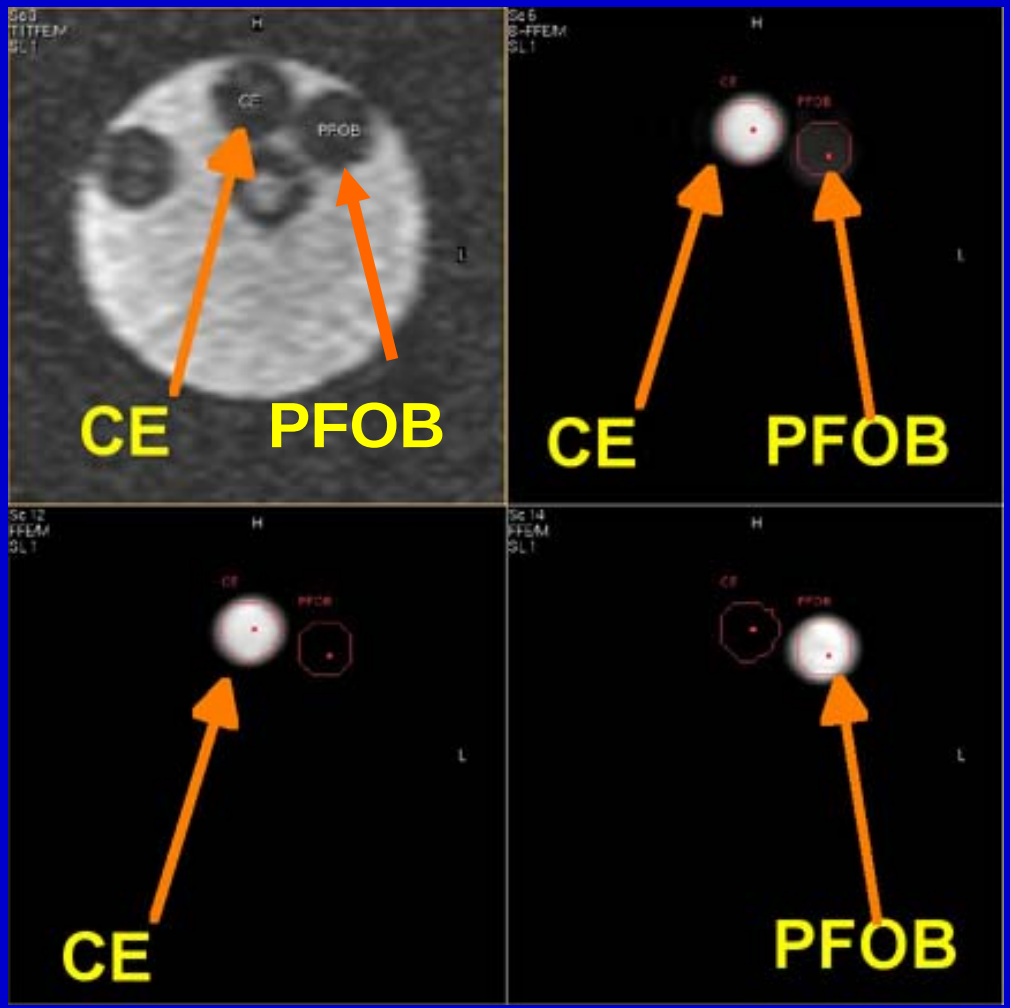
Fluorine Imaging

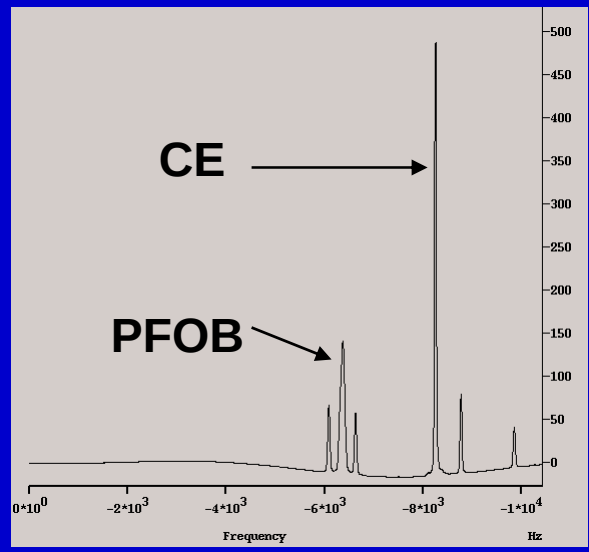
Measured ^{19}F Signal is Linear with Crown-Ether (CE) Content

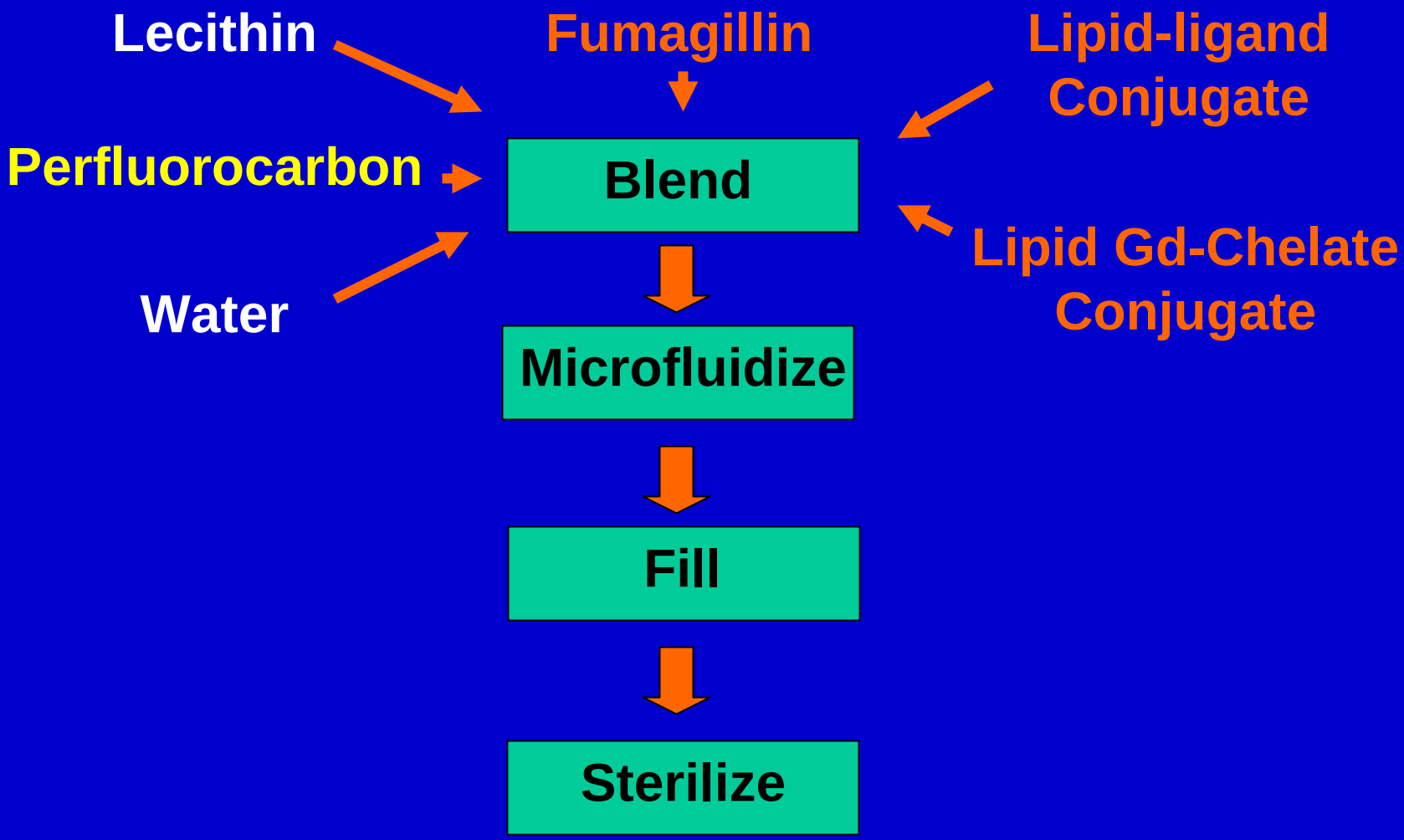




Selective Imaging of PFCs

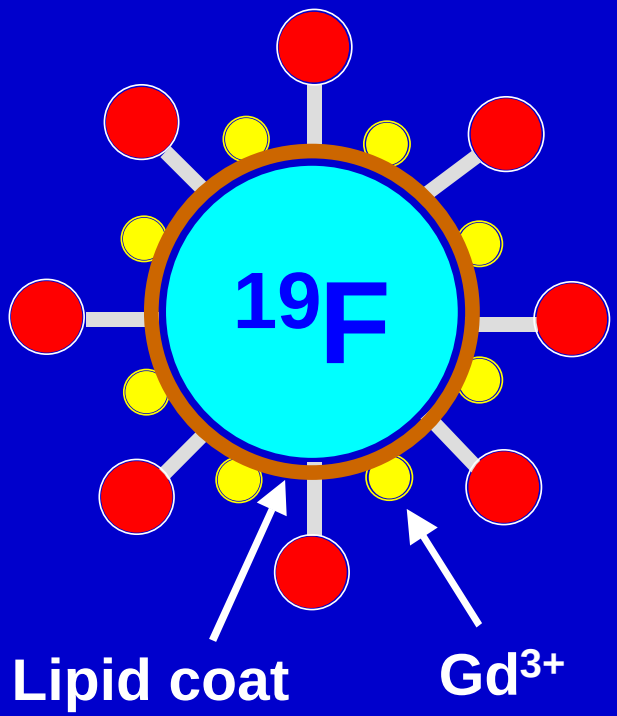






Salad Dressing

Homing Ligands

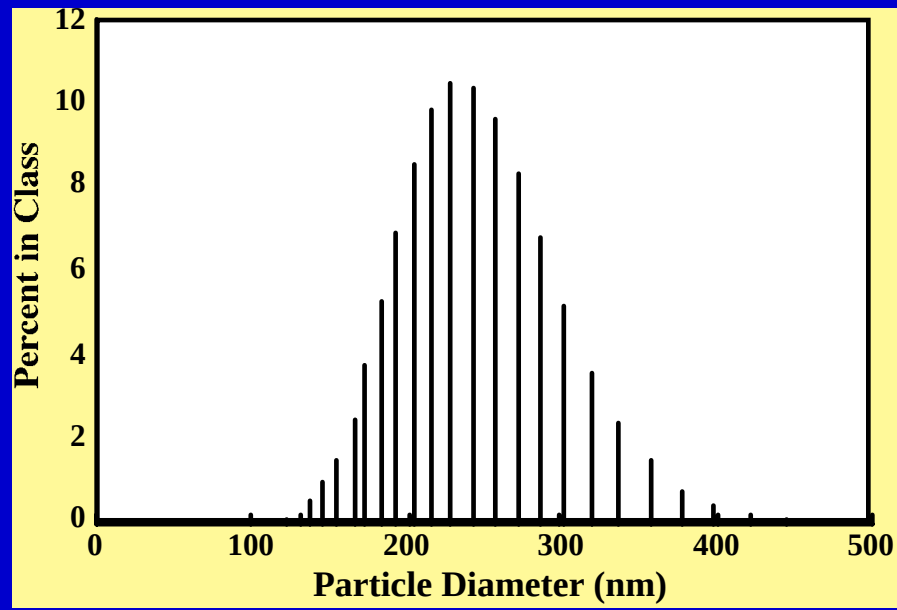


Three-Dimensional

Multiple API
(Active Pharm. Ingredients)

Complicated Syntheses
(32+ Steps)

Polydispersed Colloid

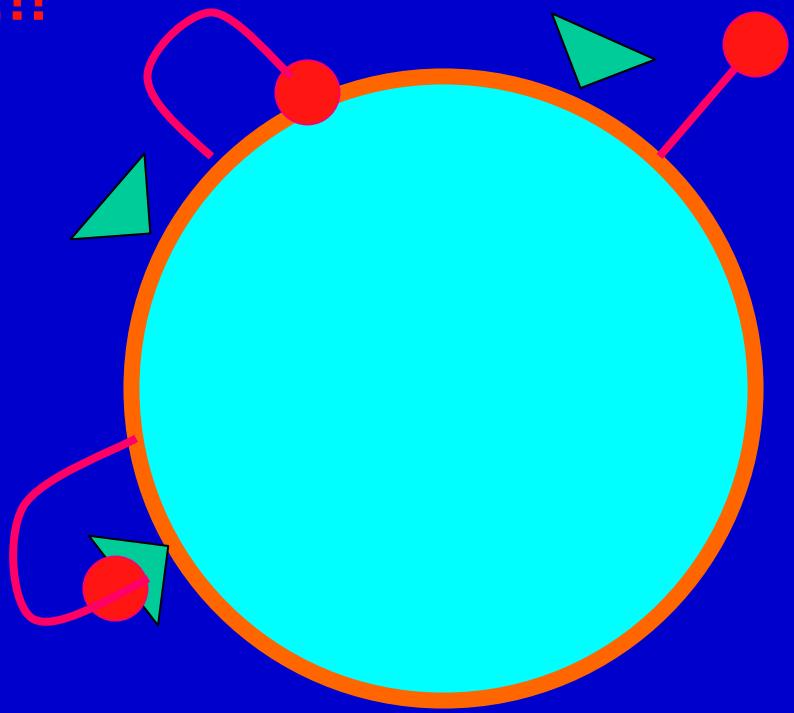


Homing Ligand Interacts with Surface

No Targeting!!!

Properly Oriented

Function Independently



Homing Ligand Interacts with Chelate

No Targeting!!!

Gd Chelate

Homing Ligand

Ratio of Components Must be Controlled

The wrong ratio of Surfactants to Oil Phase

Too Much:

- Liposome formation
- Steals surfactant ingredients
- Increases emulsion particle size
- Increases polydispersity
- Decreases stability
- Inhomogeneity of lipid distribution

Too Little:

- Increases emulsion particle size
- Increases polydispersity
- Decreases stability

Source of Components Must be Determined

Natural or synthetic lipids:

- Packing of lipids effects particle size and temperatures (I.e., fluidity)
- Lysolecithin contaminants
- Unwanted proteins
- Phosphatidylserine content (apoptotic marker)
- Cholesterols (Sheep derived Prions?)
- Solvent impact e.g., Chloroform Residues
- Cost to purchase
- Analytical Cost and Specifications
- Shrinkage

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Multiple API = Complicated Process

- Complex GMP decisions/strategy in the synthesis relating to when raw materials become active ingredients
- Chemical and functional specifications
- Chirality
- Ratio of active ingredients
    - Minimum per particle vs Nominal
    - Multi-level titrations
    - Impacts on safety
    - Independence
    - Homogeneity
- Analytical Standards
- Blood/Tissue analytical methods
  Sensitivity of detection
- Pre-post formulation analyses

Analytical Tools Are Pivotal for the Pharmaceutical Development of Nanoparticle Technology

- Essential in throughout R&D to provide insight into reality versus expectation.
- Often analytical insight challenges fundamental assumptions
- Critical for process development, stability, metabolism, residue, safety, …..
- Critical for bioequivalence as processes evolve

And For Biomedical Nanotechnology

Harder than ever because: Complex, Parenteral, 3D

And, that is just the tip of the Iceberg

Complimentarity of the imaging system - optimized for detection and presentation of tiny targets in high volume clinical situations

Pharmacology and pharmacokinetics - right dose, right patient population, right drug, …

Safety

Drug interferences

Administration dynamics

Economics

Clinical use, packaging and marketing

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