Magnetic Nanoparticles & MR: From Imaging to Assays to Sensors

Lee Josephson

Center for Translational Nuclear Medicine
a division of Nuclear Medicine
& Molecular Imaging

Massachusetts General Hospital

Harvard Medical School

Disclosure for Lee Josephson

Consultant & Cofounder
T2 Biosystems, Inc.
(NMR Assays & Sensor)

Magnetic Nanoparticles & MR: An introduction

Nanoparticles As MR Contrast Agents

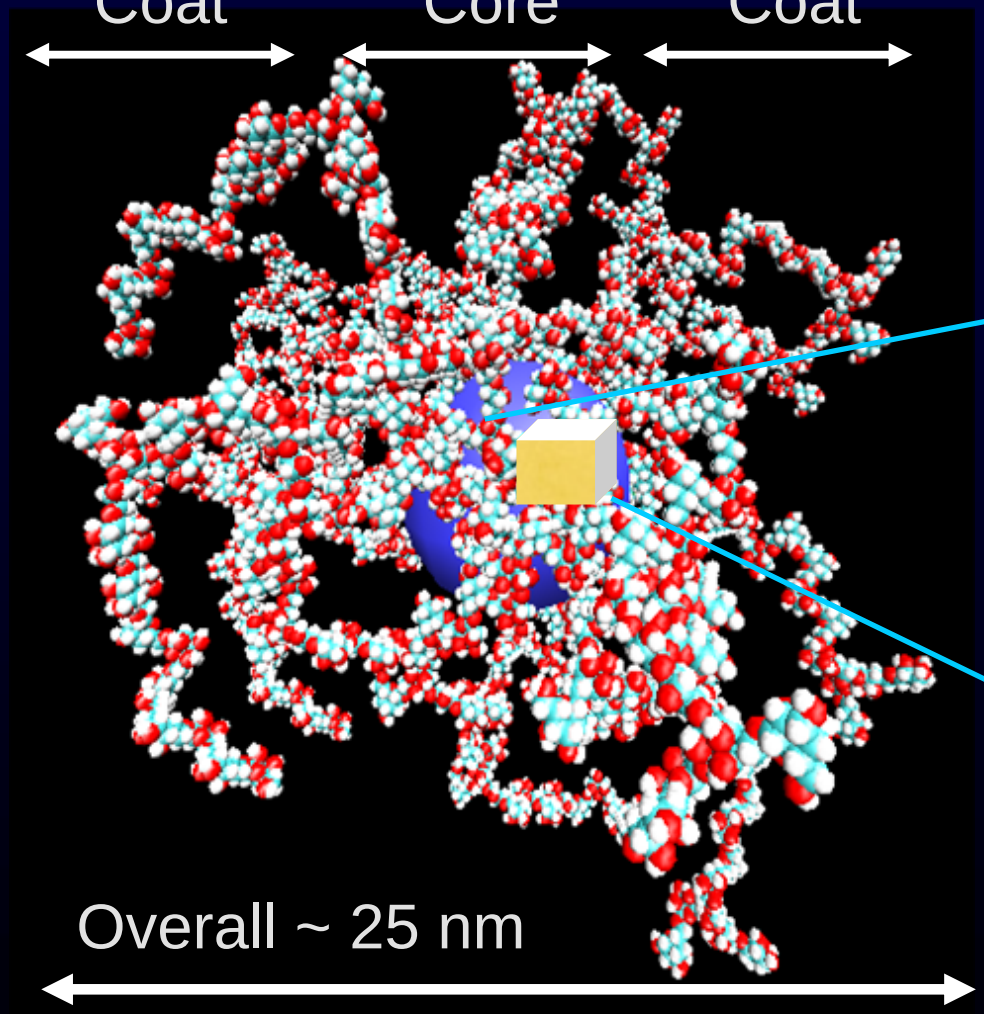
Nanoparticles for MR Based Assays

Nanoparticles & MR Based Implantable Biosensors

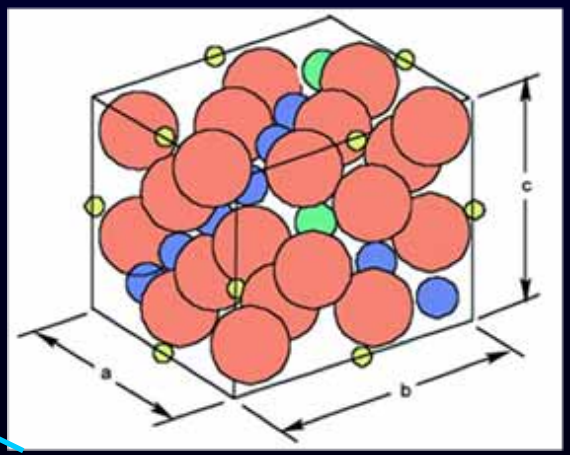
Polymer Coated Iron Oxides As Drugs

Drug	Type	Indication	Status
InFeD	Paramagnetic iron oxide/dextran	Fe anemia	Approved US
Dexferrum	Paramagnetic iron oxide/dextran	Fe Anemia	Approved US
Feridex IV	Superparamagnetic iron oxide/dextran	Short Circ/ Liver MRI	Approved US, Europe & Japan
Resovist	Superparamagnetic iron oxide/carboxy-dextran	Short Circ/ Liver MRI	Approved Europe & Japan
Combidex	Superparamagnetic iron oxide/dextran	Long Circ/ Lymph Node	Post phase III
Ferumoxytol	Superparamagnetic iron oxide/CM-dextran	Long Circ/ Fe anemia	Approved Us
Supravist	Superparamagnetic iron oxide/ Carboxydextran	Long Circ/ MR Angiograph.	?

Polymer Coated Iron Oxides: Crystal Based Superparamagnetism



Combidex/Ferumoxtran
Or
Feraheme/ferrumoxytol



Superparamagnetic Magnetite, Fe_3O_4 9000 Fe/crystal, 6000 Fe^{3+}(Ferric), 3000 Fe^{2+} (Ferrous)

- Fe^{3+} unpaired electron spins in two types of sites oppose each other

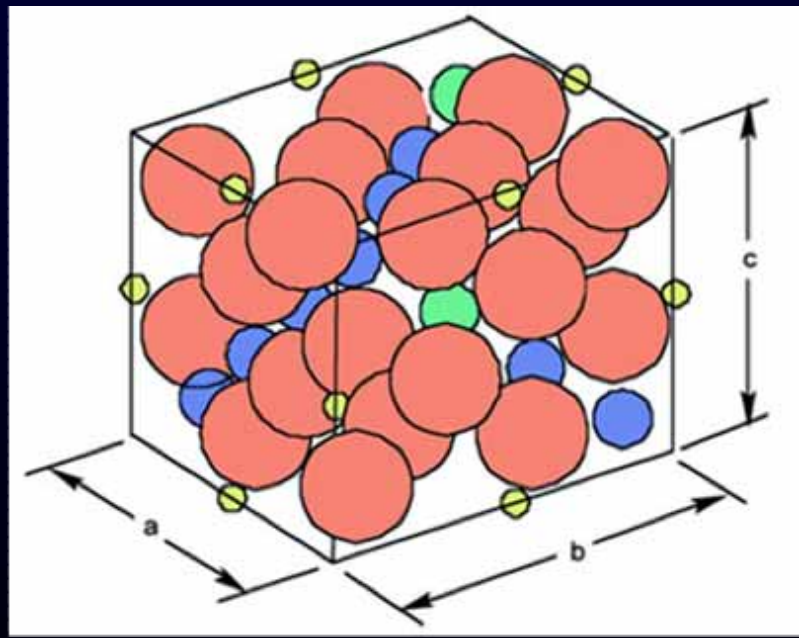
  ⇉ ⇇

  3000 Fe^{3+} 3000 Fe^{3+}

- 6000 Fe^{2+} spins are coupled

  ⇉

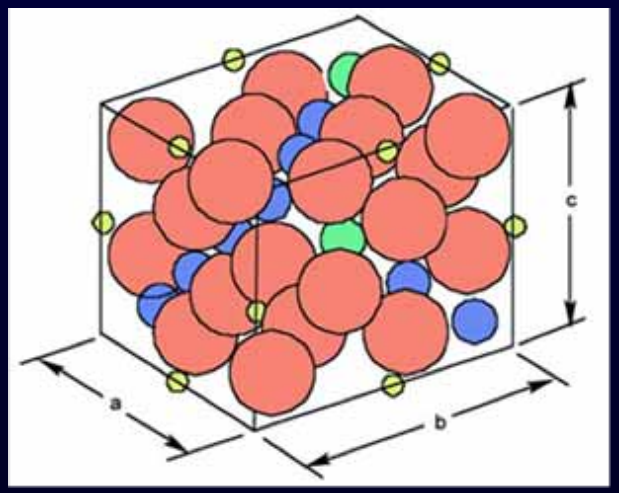
  3000 X 2 spins/Fe^{2+}



Unit cell of magnetite

Conversion of Superparamagnetic Iron Oxide Paramagnetic Ionic Iron

Crystalline
Superparamagnetic Iron



Dissolution
pH 4
Citric Acid

→

Ionic Iron

Fe^{2+}-Citrate
Fe^{3+} Citrate

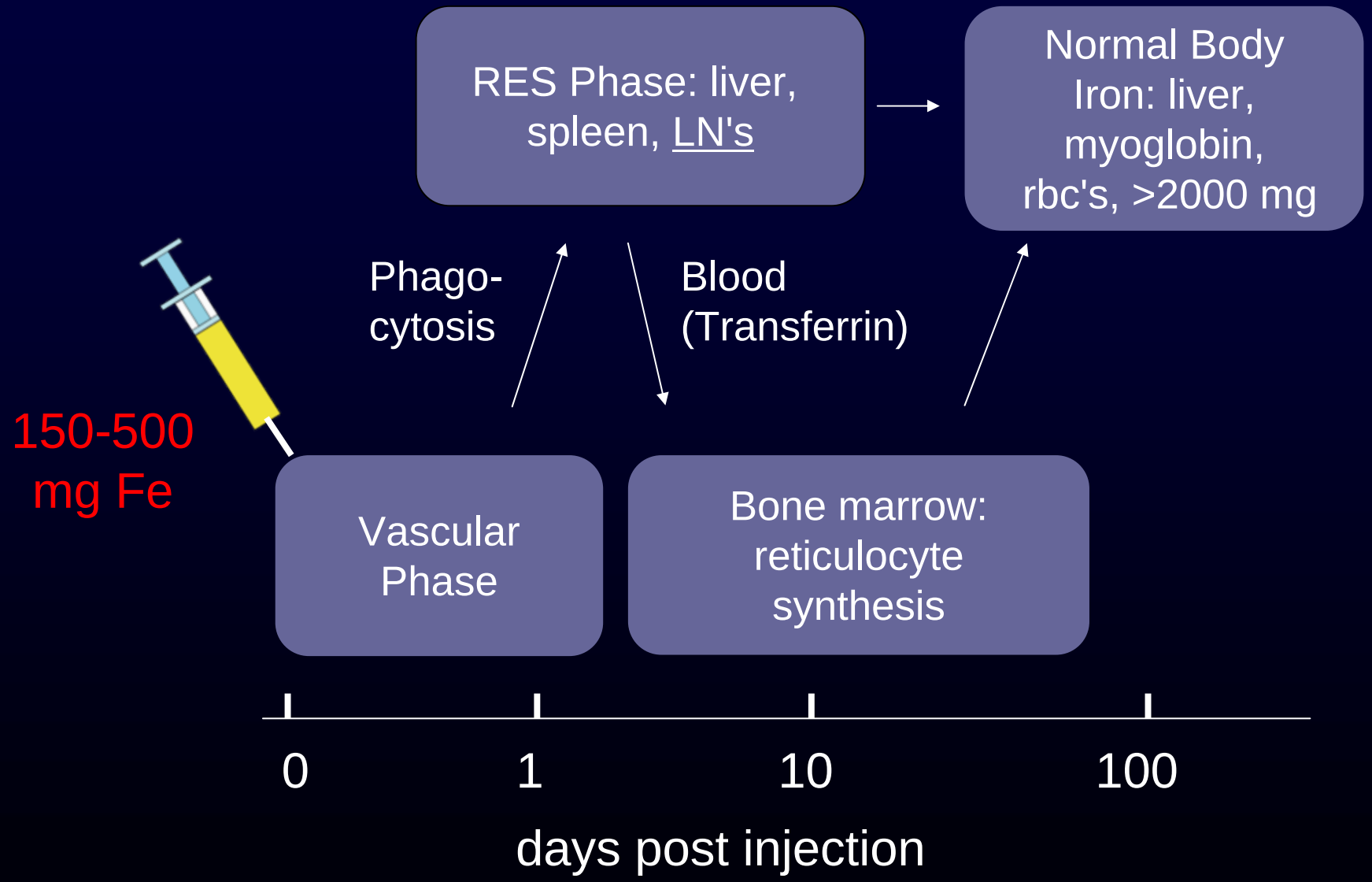
Unpaired ferrous electrons respond to a magnetic field in unison (coupled)

High Magnetism at 37 °C

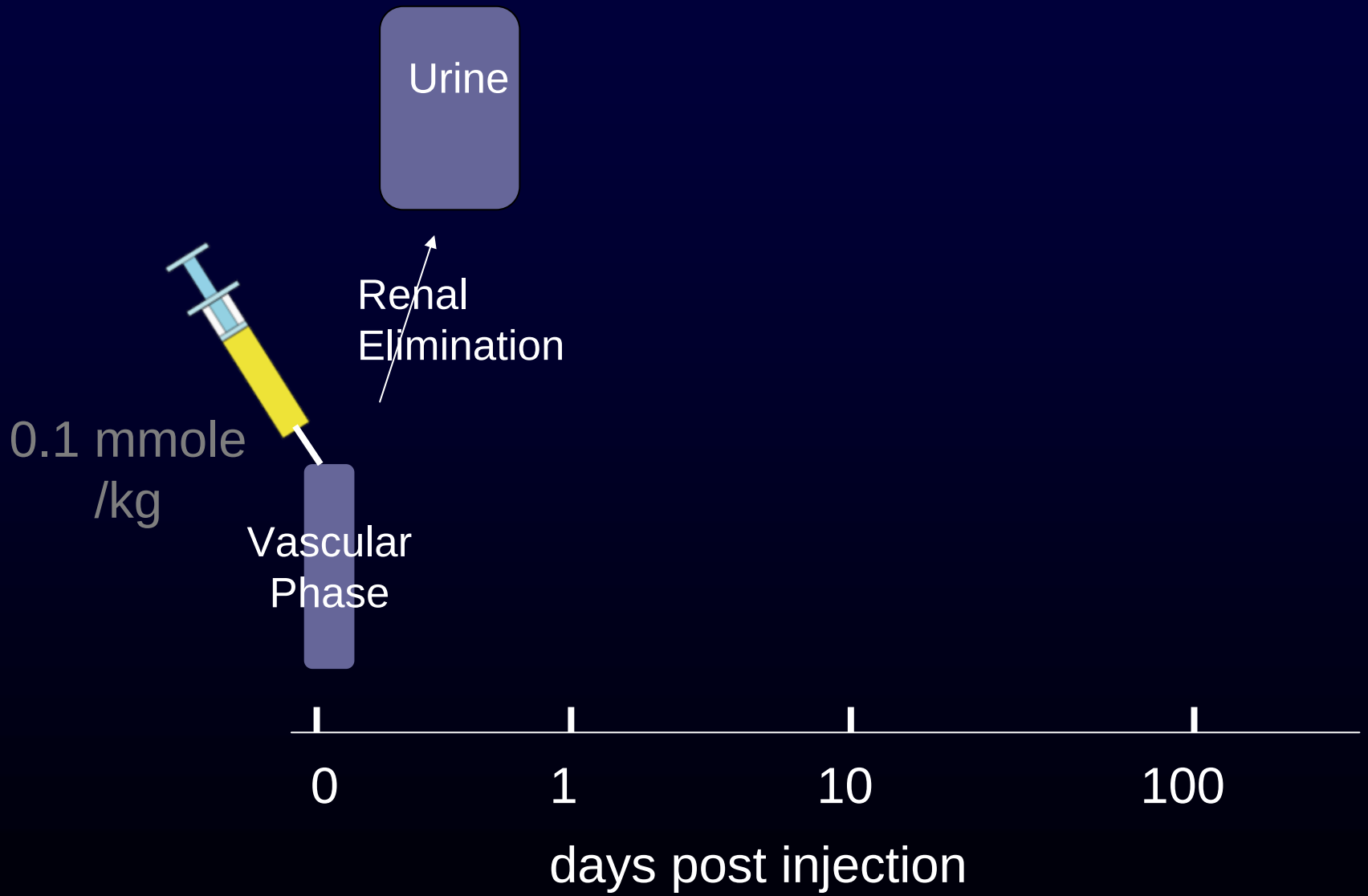
More unpaired electrons but uncoupled

Low Magnetism at 37 °C
V. High magnetism @ -77 °K

Combidex/MION & Ferumoxytol Nanoparticles: Prolonged Vascular Phase, LN Uptake

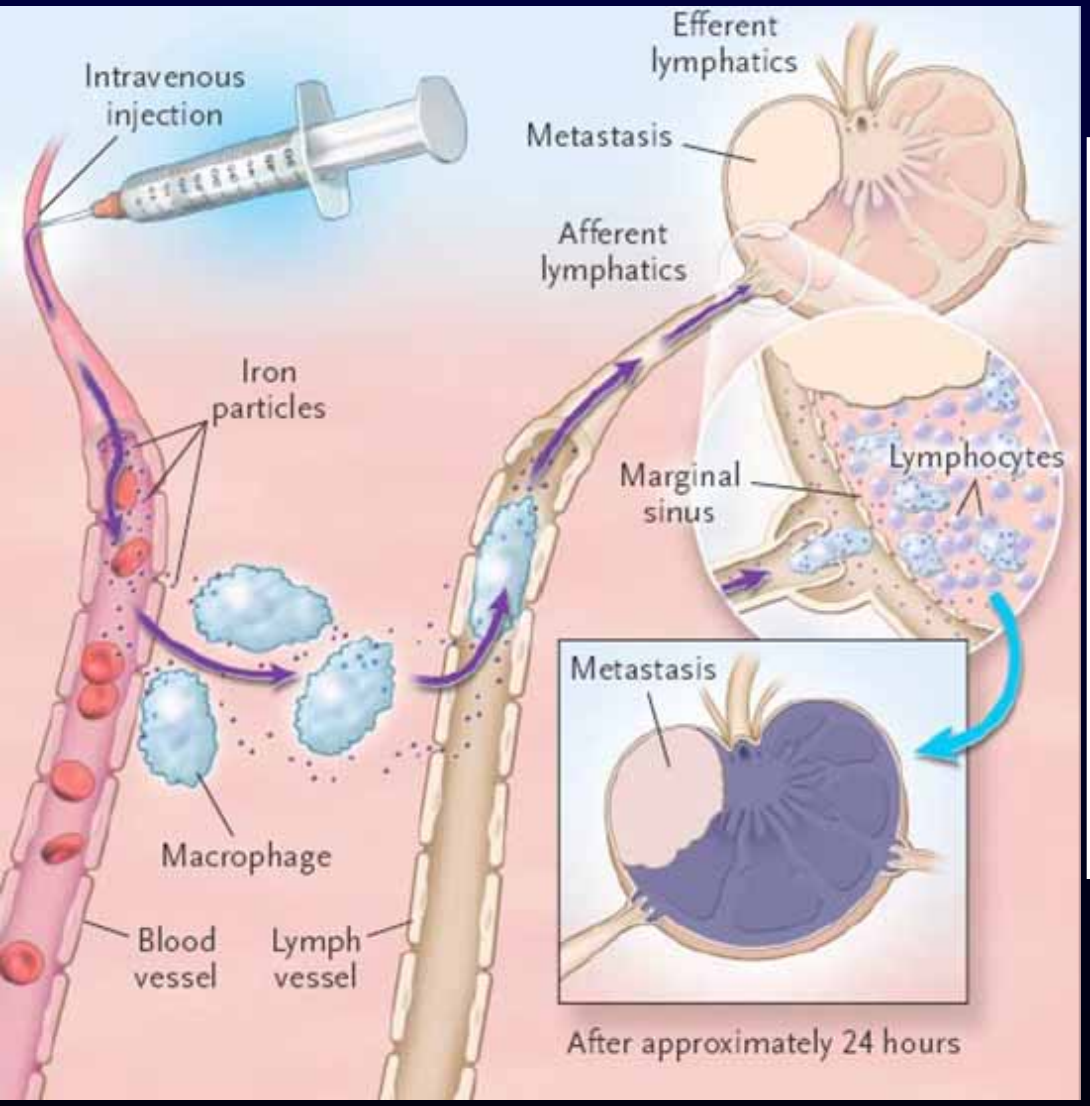


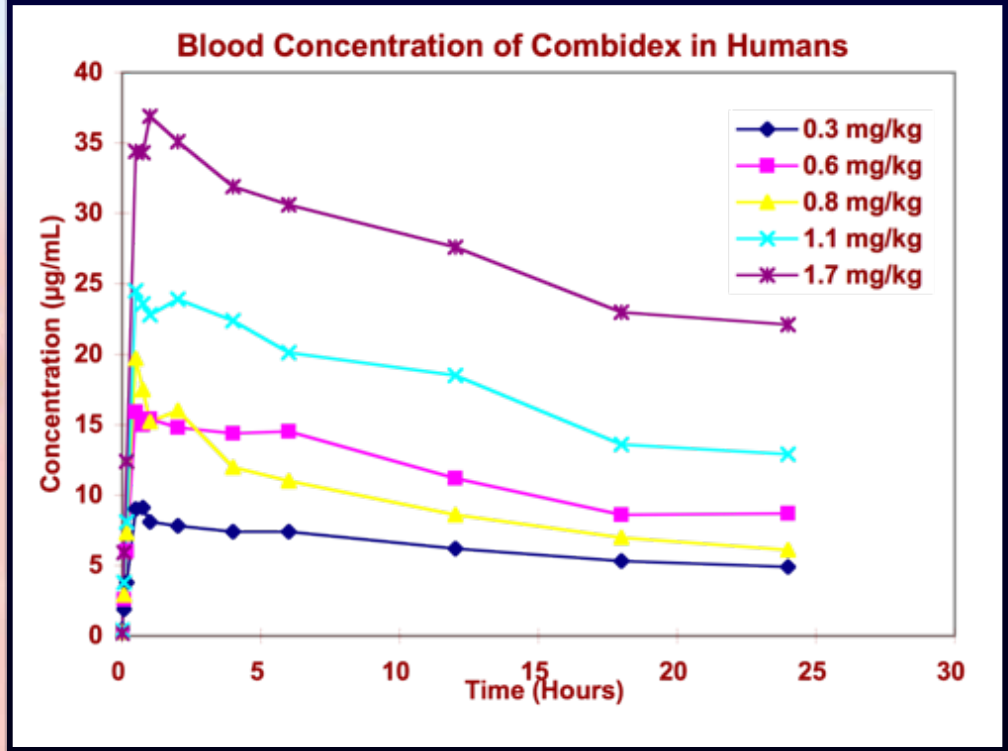
Gadolinium Chelates: Normal Renal Function



Gadolinium Chelates: Poor Renal Function

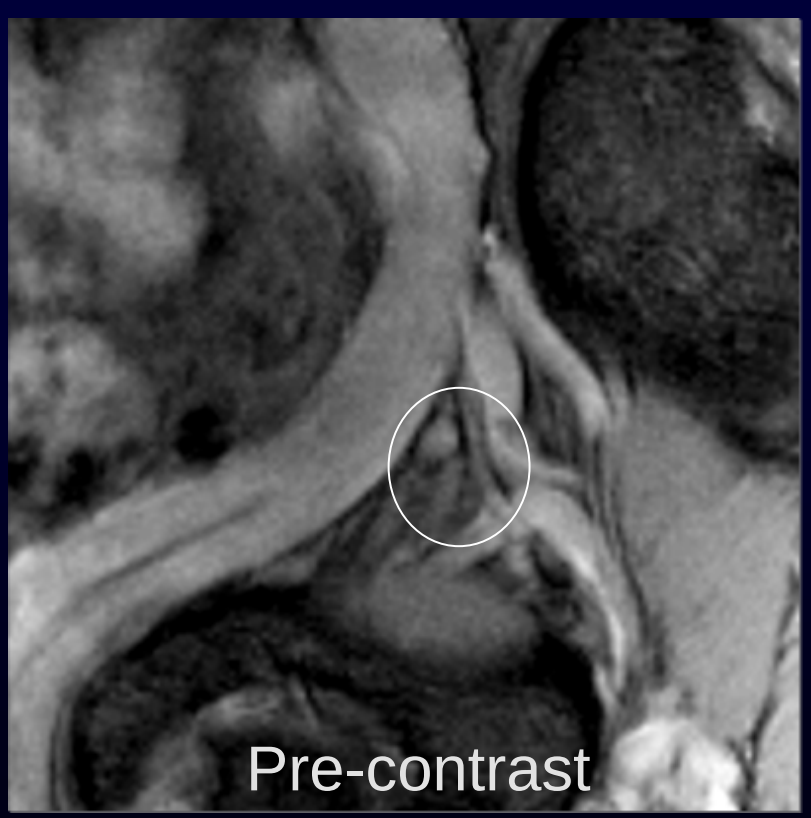
Long Circulating Magnetic Nanoparticles For Lymph Node Imaging

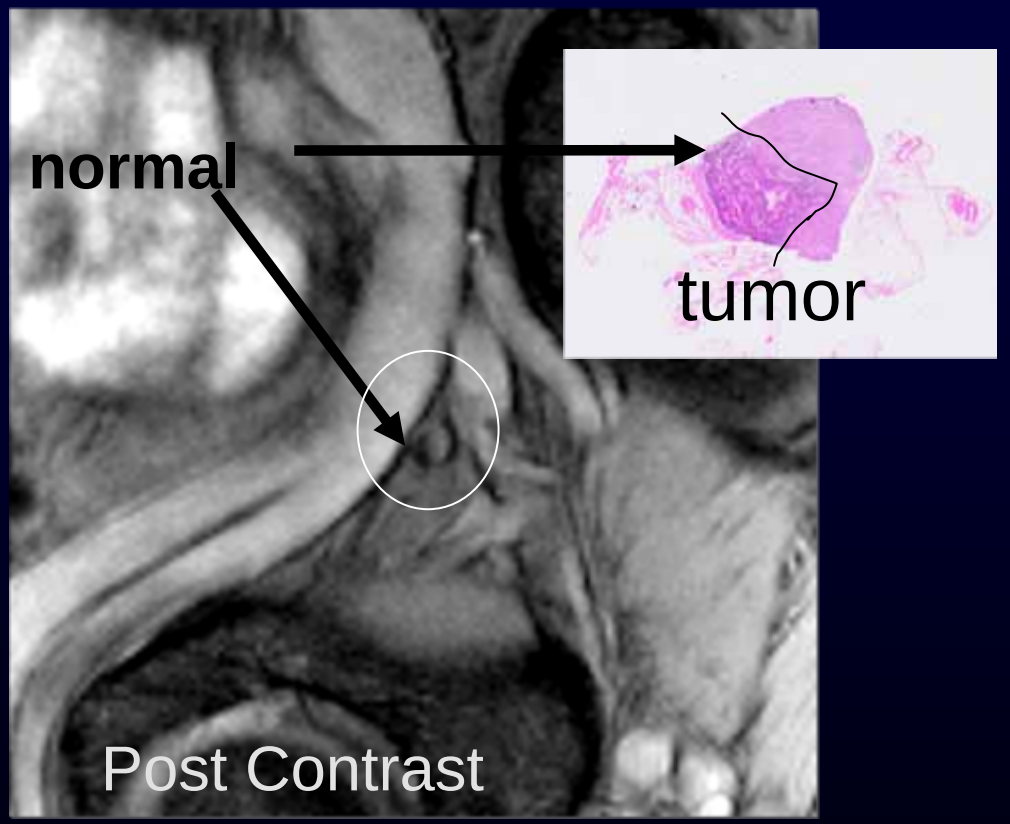




Harisinghani (2003) NEJM "Noninvasive detection of clinically occult lymph-node metastases in prostate cancer"

Combidex/MION Darkens Normal Lymph Nodes: T2 Weighted MRI





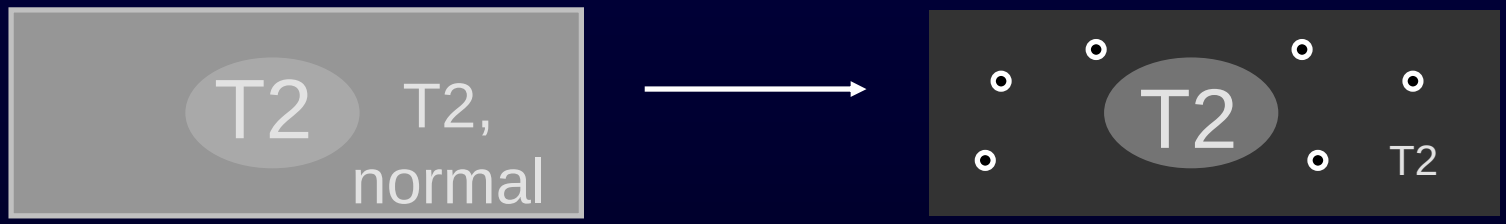
Magnetic NP's Can Be Targeted To Tumor or Normal Tissue

- Selective Normal Tissue T2 Shortening/Darkening

- Selective Tumor T2 Shortening Darkening

Magnetic NP's Can Be Targeted To Tumor Or Normal Tissue

- Selective Normal Tissue T2 Shortening/Darkening

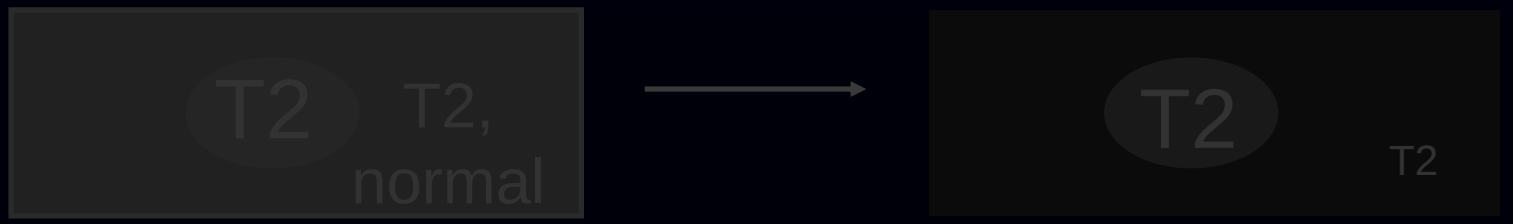


Pre-injection Contrast

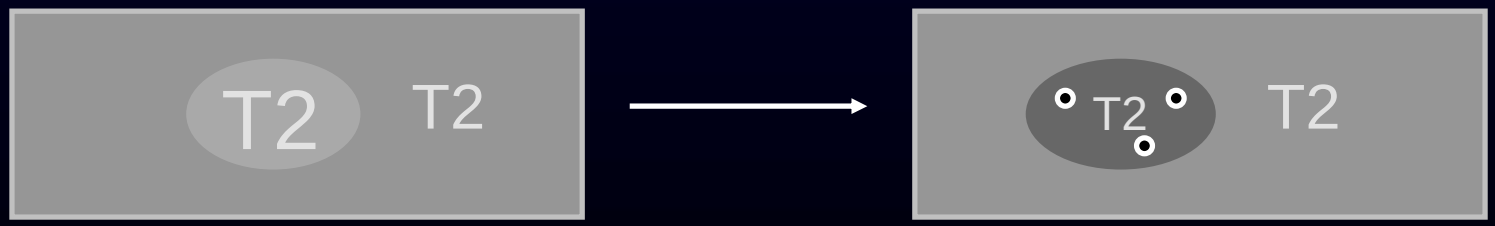
Post Injection Contrast

Magnetic Iron Oxide NP's Be Targeted To Tumor Or Normal Tissue

- Selective Normal Tissue T2 Shortening/Darkening



- Selective Tumor T2 Shortening/Darkening



Pre-injection contrast

Post Injection Contrast

MR Tumor Contrast Strategies With Magnetic Nanoparticles

Strategy	Molecular Target	Cellular Target	Contrast Strategy
Tumor Targeting	$a_V\beta_3$ integrin	Tumor Cell (BT-20)	Decr *Tumor* T2
Normal Tissue Targeting	Gastrin Releasing Peptide Receptor (GRP Receptor)	Normal Acinar cell of pancreas	Decrease *Normal* Pancreatic T2

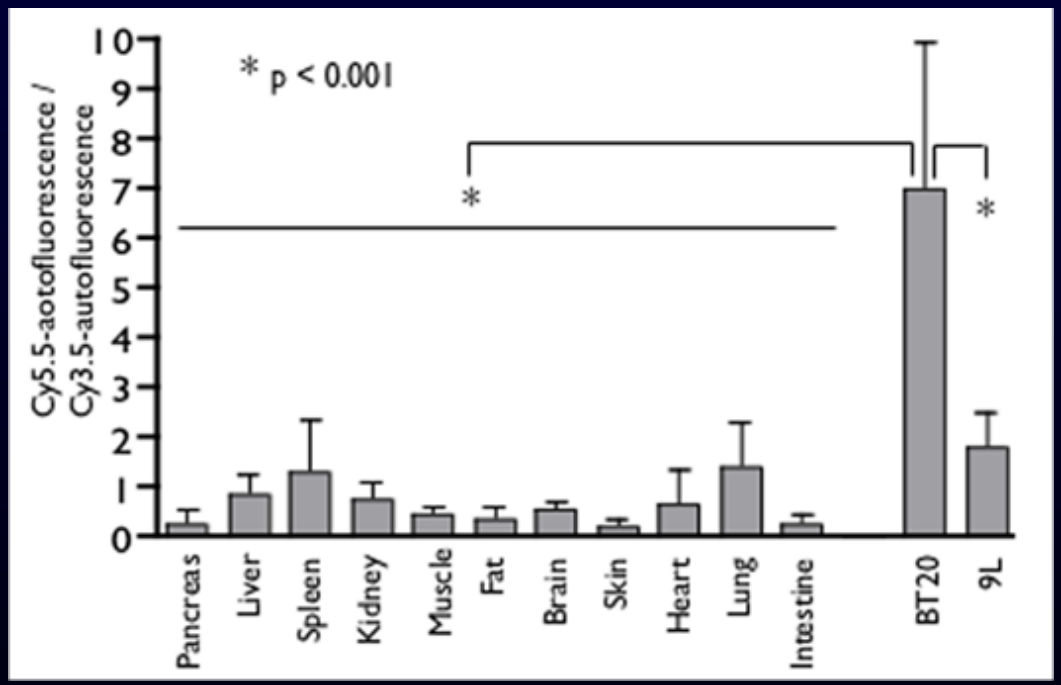
*BN= bombesin-like peptide binds GRP Receptor

Molecular Targeting vs. Tissue Macrophage Activity with Targeted NP's: scrambled peptide control NP

Target	Targeted NP	Control NP
Integrin BT-20 tumor	RGD-CLIO(Cy5.5)	DRG-CLIO(Cy3.5)
GRP Receptor Norm. Panc.	BN-CLIO(Cy5.5)	scrBN-CLIO(Cy5.5)

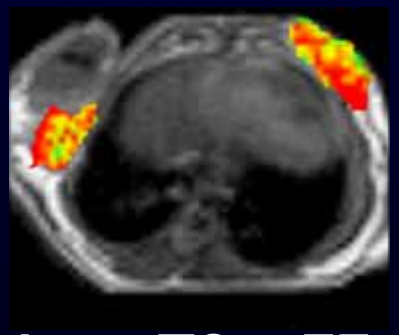
Imaging Tumor Integrin With RGD-CLIO(Cy5.5)

Targeting the BT20 tumor: Co-injection
Dual Fluorochrome Specificity Protocol

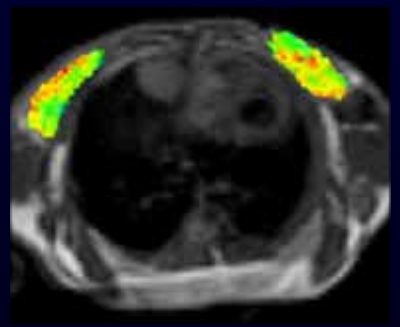


Fluorescence Molecular Tomography (FMT)

Pre



Post



Mean T2: 77 66 msec

T2 values, (msec)
(MRI)

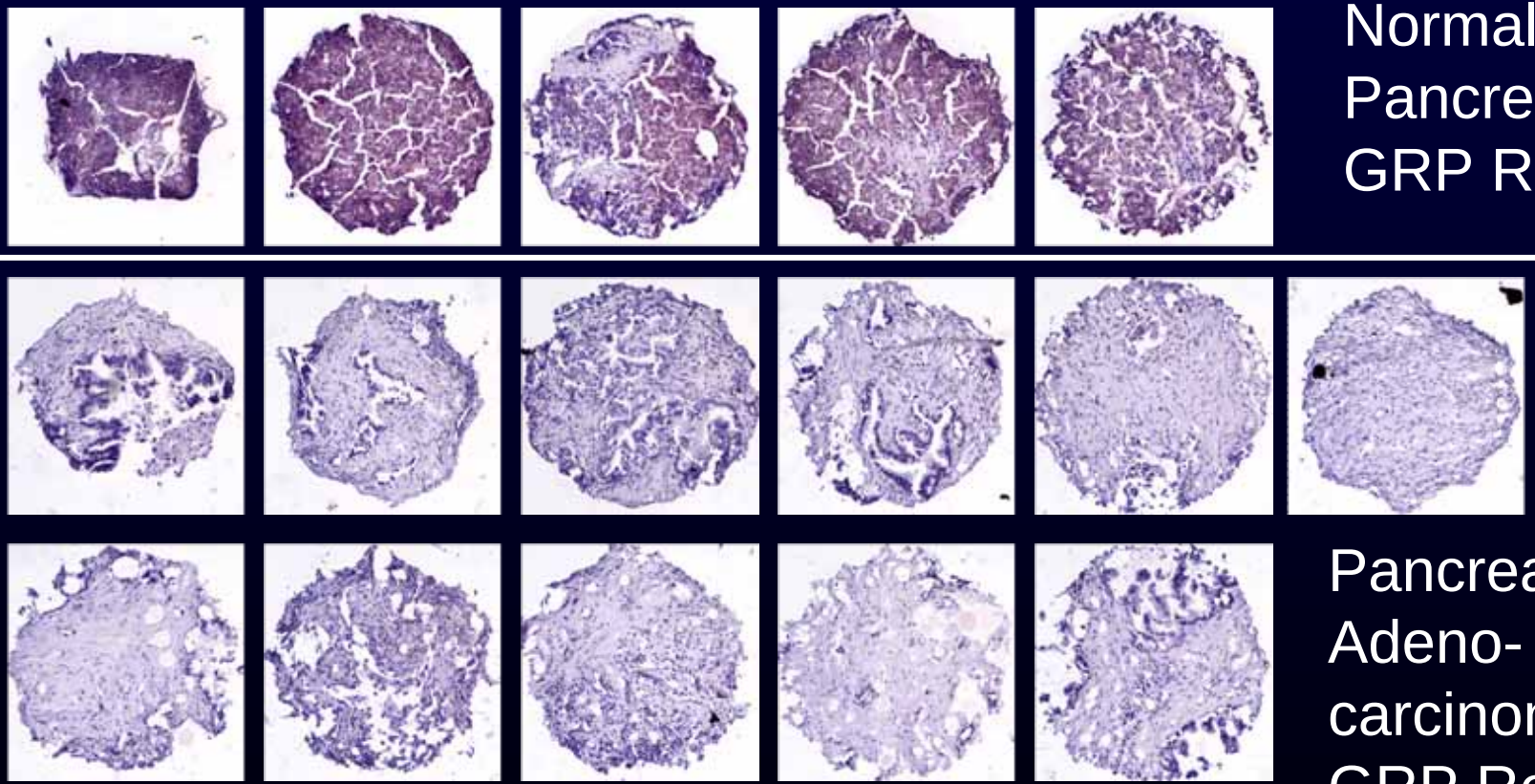
Montet (2006) Multivalent effects of RGD peptides obtained by nanoparticle display. J Med Chem 49, 6087.
Montet, (2006) Nanoparticle imaging of integrins on tumor cells. Neoplasia 8, 214.

MR Tumor Contrast Strategies With Magnetic Nanoparticles

Strategy	Molecular Target	Cellular Target	Contrast Strategy
Tumor Targeting	$a_V\beta_3$ integrin	Tumor Cell	Decr *Tumor* T2
Normal Tissue Targeting	Gastrin Releasing Peptide Receptor (GRP Receptor)	Normal Acinar cell of pancreas	Decrease *Normal* Pancreatic T2

*BN= bombesin-like peptide binds GRP Receptor

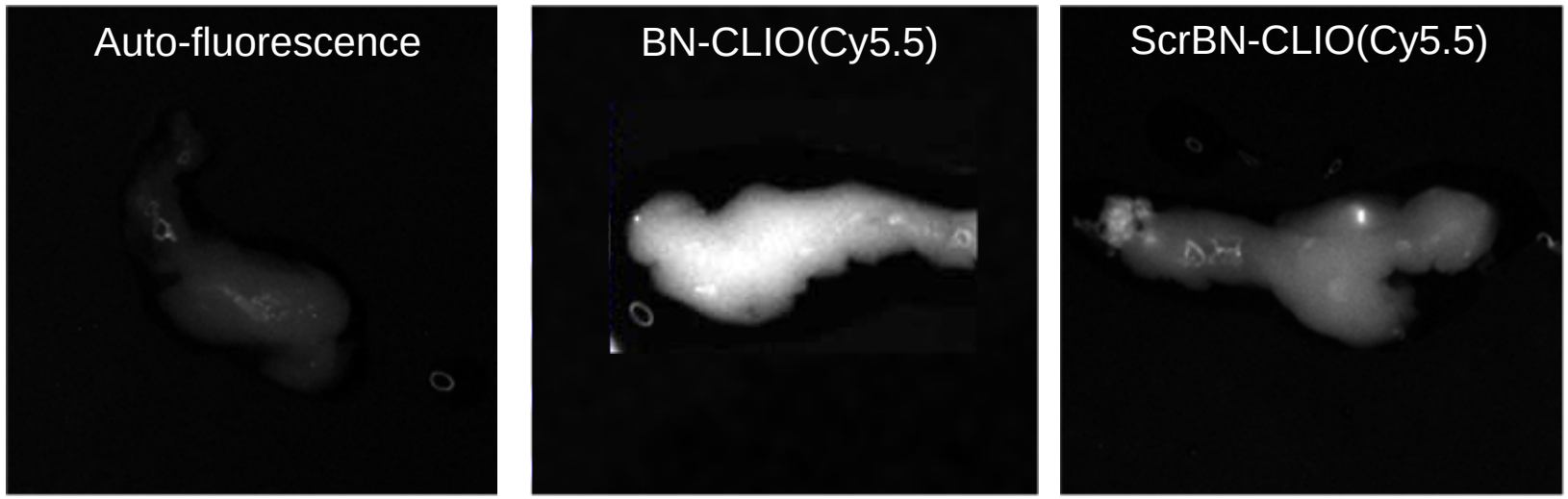
Human Tissue Microarray: BN Peptide Binds GRP Receptor on Normal Pancreas (not tumor)

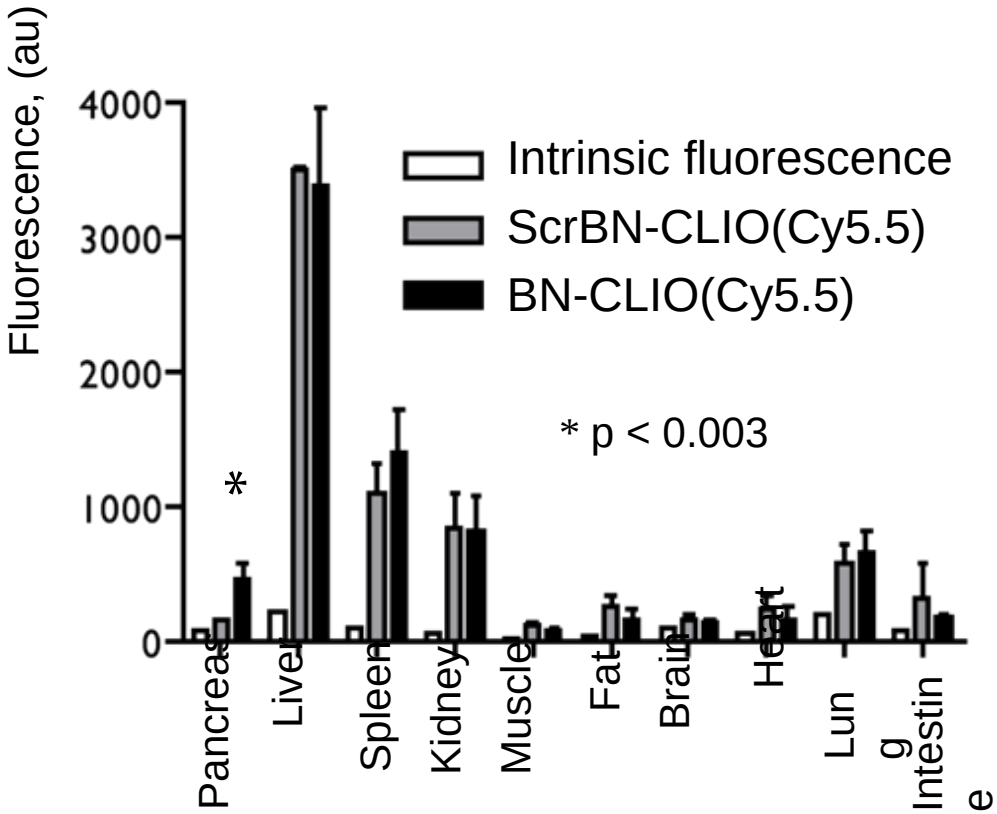


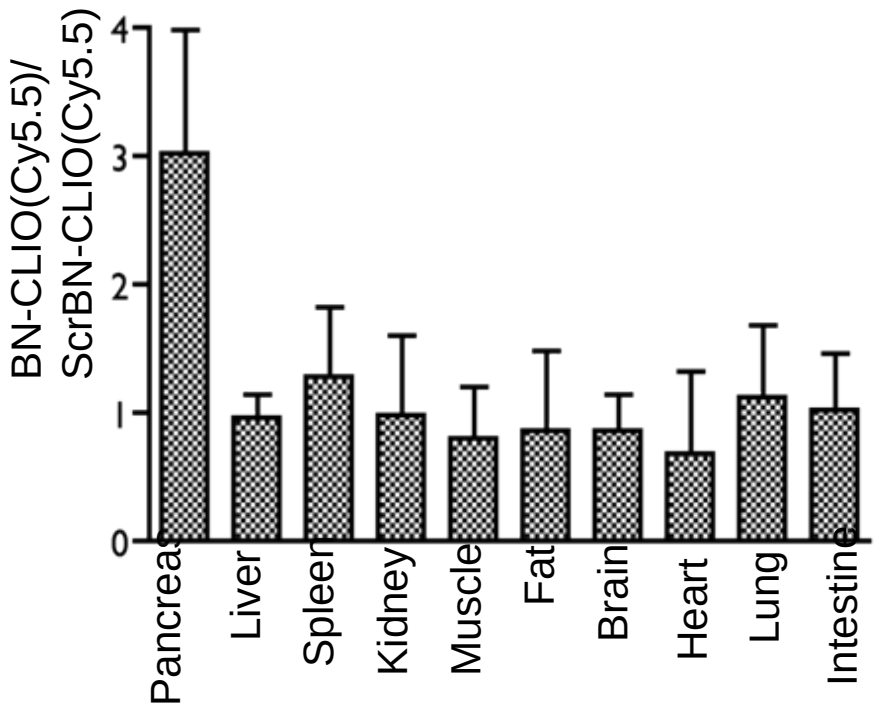
Normal Pancreas GRP Receptor (+)

Pancreatic Ductal Adeno-carcinomas, GRP Receptor (-)

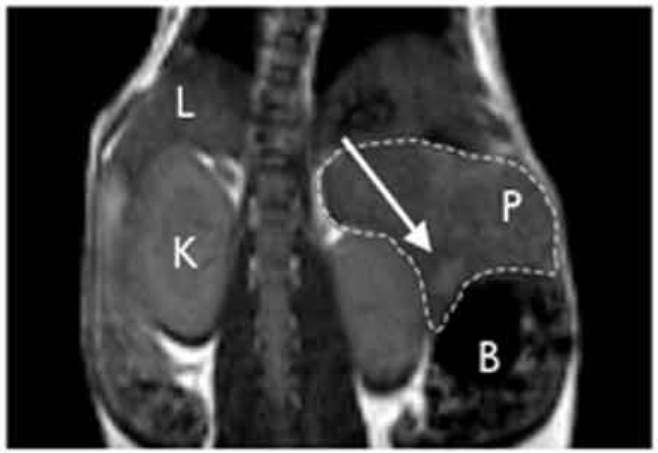
Montet, (2006) Imaging pancreatic cancer with a peptide-nanoparticle conjugate targeted to normal pancreas. Bioconjug Chem 17, 905.


Auto-fluorescence
BN-CLIO(Cy5.5)
ScrBN-CLIO(Cy5.5)

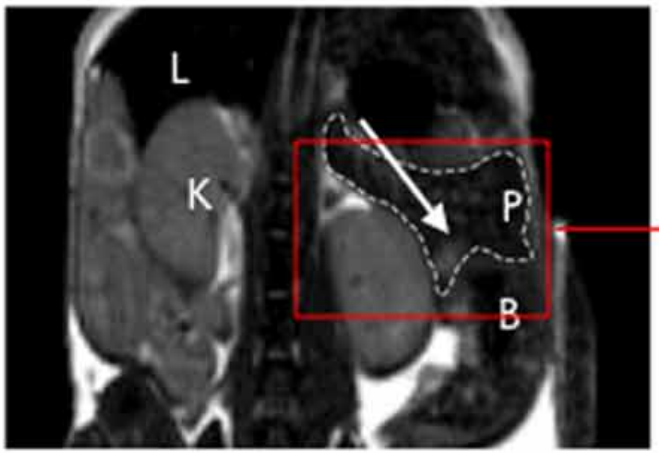

Fluorescence, (au)
4000
3000
2000
1000
0
Intrinsic fluorescence
ScrBN-CLIO(Cy5.5)
BN-CLIO(Cy5.5)
* p < 0.003
*
Pancreas
Liver
Spleen
Kidney
Muscle
Fat
Brain
Heart
Lung
Intestine


BN-CLIO(Cy5.5)/
ScrBN-CLIO(Cy5.5)
4
3
2
1
0
Pancreas
Liver
Spleen
Kidney
Muscle
Fat
Brain
Heart
Lung
Intestine

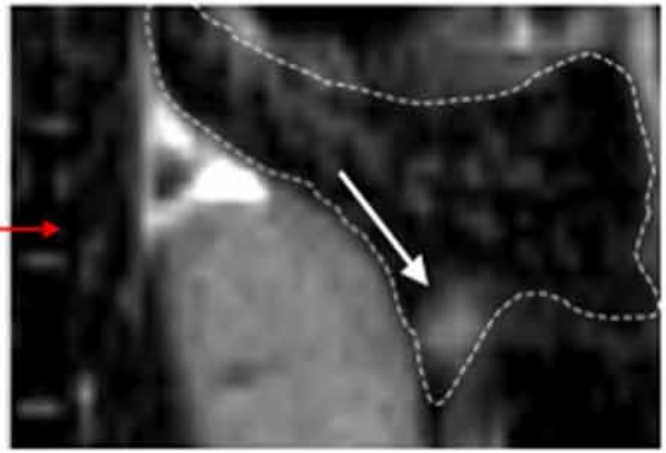
Imaging Pancreatic Tumor With BN-CLIO(Cy5.5): GRP Receptor on Normal Acinar Cells



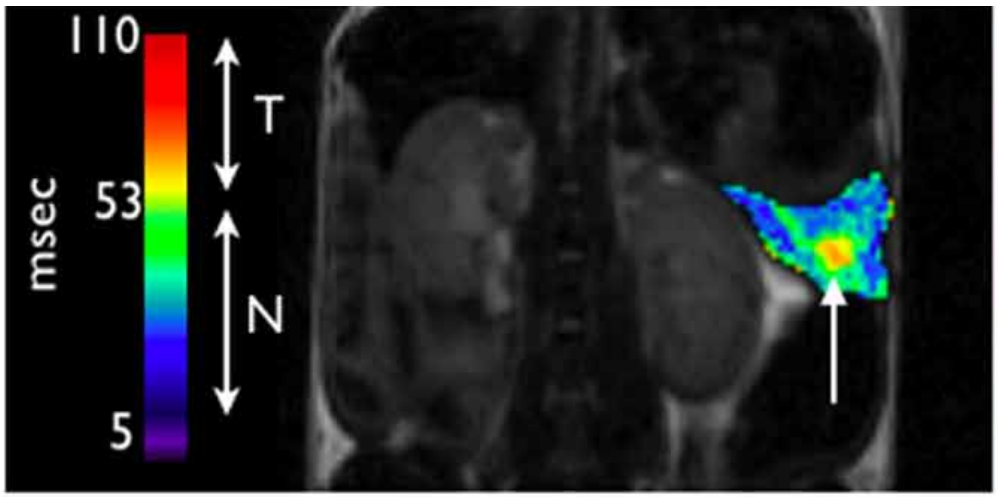
Pre-contrast

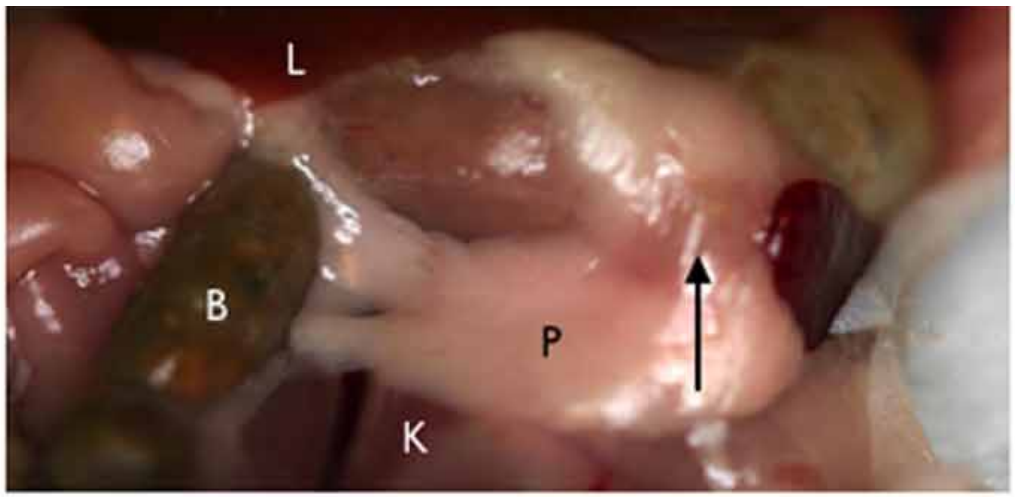


Post-contrast



Post Contrast





Why Magneto/fluorescent Nanoparticles (MFNP's) For Tumor Margin Delineation?

- Enhance Tumor/Normal Contrast In Two Modalities With The Same Nanoparticle
  - MRI: pre-operative
  - Fluorescence: intra-operative

- MFNP's Are Internalized by Cells & Feature Slowly Metabolized

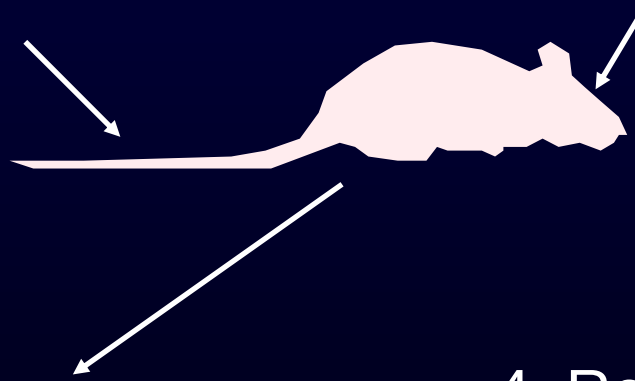
Classes Of Margin Delineating Agents

Gd Chelates & Fluorescent Dyes	Magneto/fluorescent Nanoparticle
MW < 0.8kDa	MW ~ 1000 kDa
Rapid wash in, wash out where BBB is disrupted.	Internalization and slow metabolism by cells (if BBB is disrupted)
Blood half-life: 20 min (human)	Blood half-life: 24 hr
Contrast Half-life: Variable: 10 -200 min	Contrast half-life: 3-4 d

Visualization of brain tumor with magneto/fluorescent nanoparticle

2. Inject MFNP

1. Implant Tumor, Grow 5-10 d

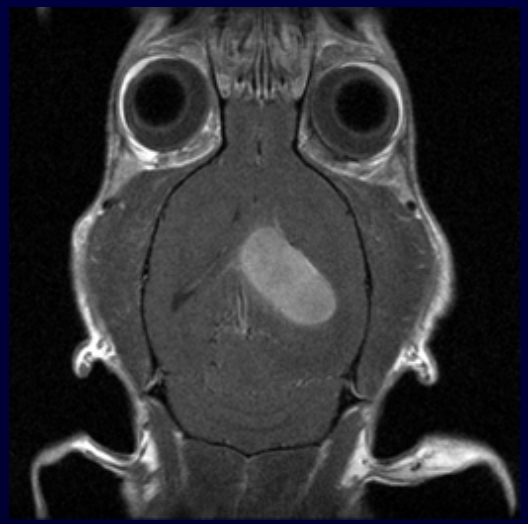


3. MRI @ 24 h Post Inj. "Pre-operative"

4. Remove cranium, Fluorescence reflectance image @ ~ 26 h Post Inj. "Intra-operative"

Pre-operative MR, Intra-operative Fluorescence Imaging

T1w & Gd

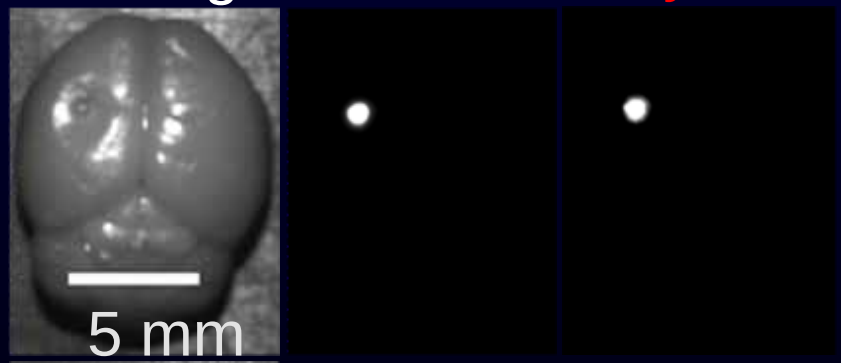


T2w & MFNP

"CLIO(Cy5.5)"

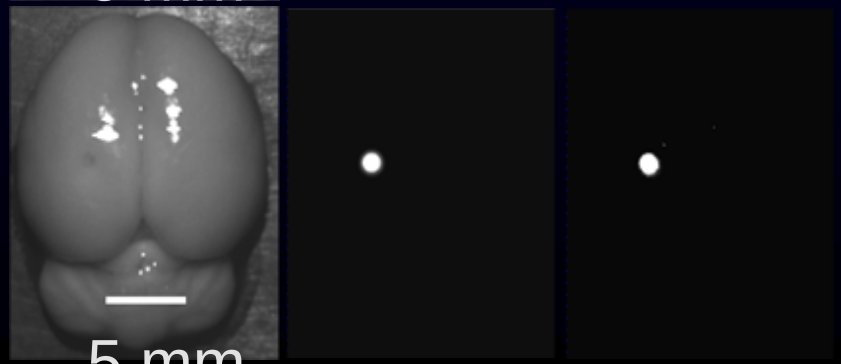
While Light GFP Cy5.5

Nude Mouse



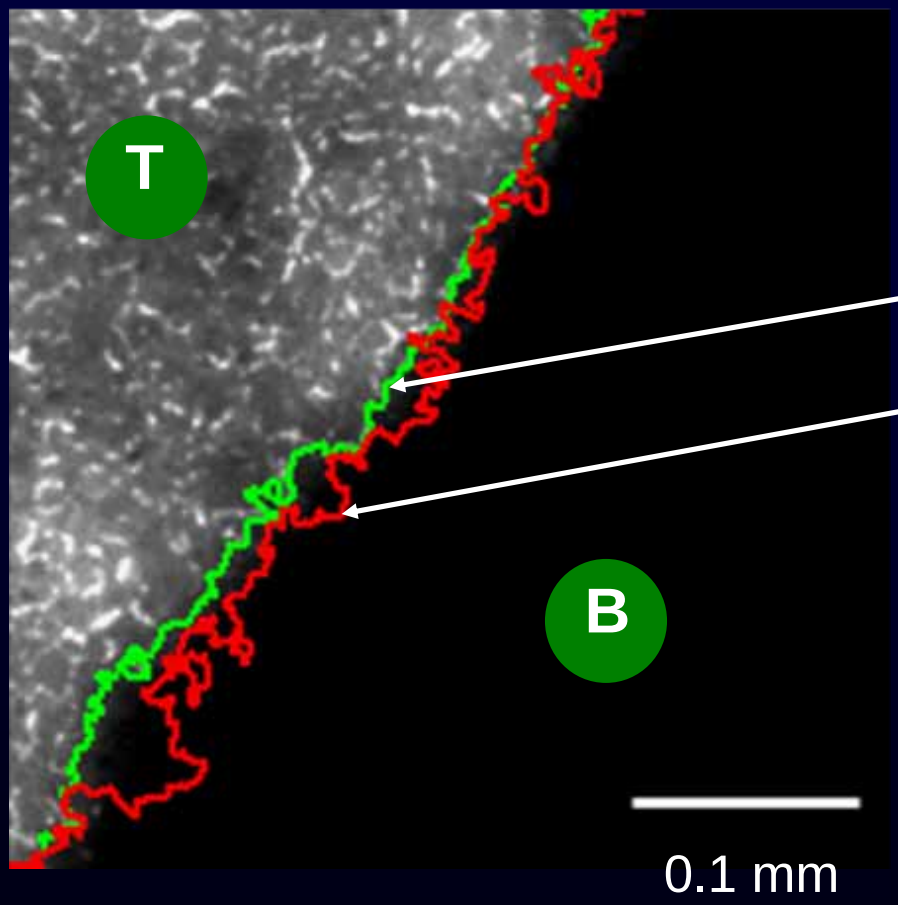
Fluorescence Reflectance Image

Rat



Objective Measurement Of Nanoparticle Fluorescence To Determine Tumor Margin

GFP Fluorescence



- Obtain tumor margin for each fluorochrome:

M = B+0.5(T)

M = B+0.5(T)

Kircher (2003) A multimodal nanoparticle for preoperative magnetic resonance imaging and intraoperative optical brain tumor delineation. *Cancer Res 63*, 8122.

Trehin, (2006) Fluorescent nanoparticle uptake for brain tumor visualization. *Neoplasia 8*, 302.

Magnetic Nanoparticles As MR Contrast Agents

Established safety and metabolism

Enable two targeting and tumor contrast enhancing strategies

Magnetofluorescent NP’s can be pre-operative MR and intraoperative fluorescent imaging agents

Magnetic Nanoparticles & MR: An introduction

Nanoparticles As MR Contrast Agents

Nanoparticles for MR Based Assays

Nanoparticles & MR Based Implantable Biosensors

MR Proton Relaxometry: Pulse & Listen

Pulse

- Sample in homogeneous magnetic field
- Radiofrequency pulse excites protons ($\boldsymbol{H}_2O$) to higher energy levels and synchronizes them

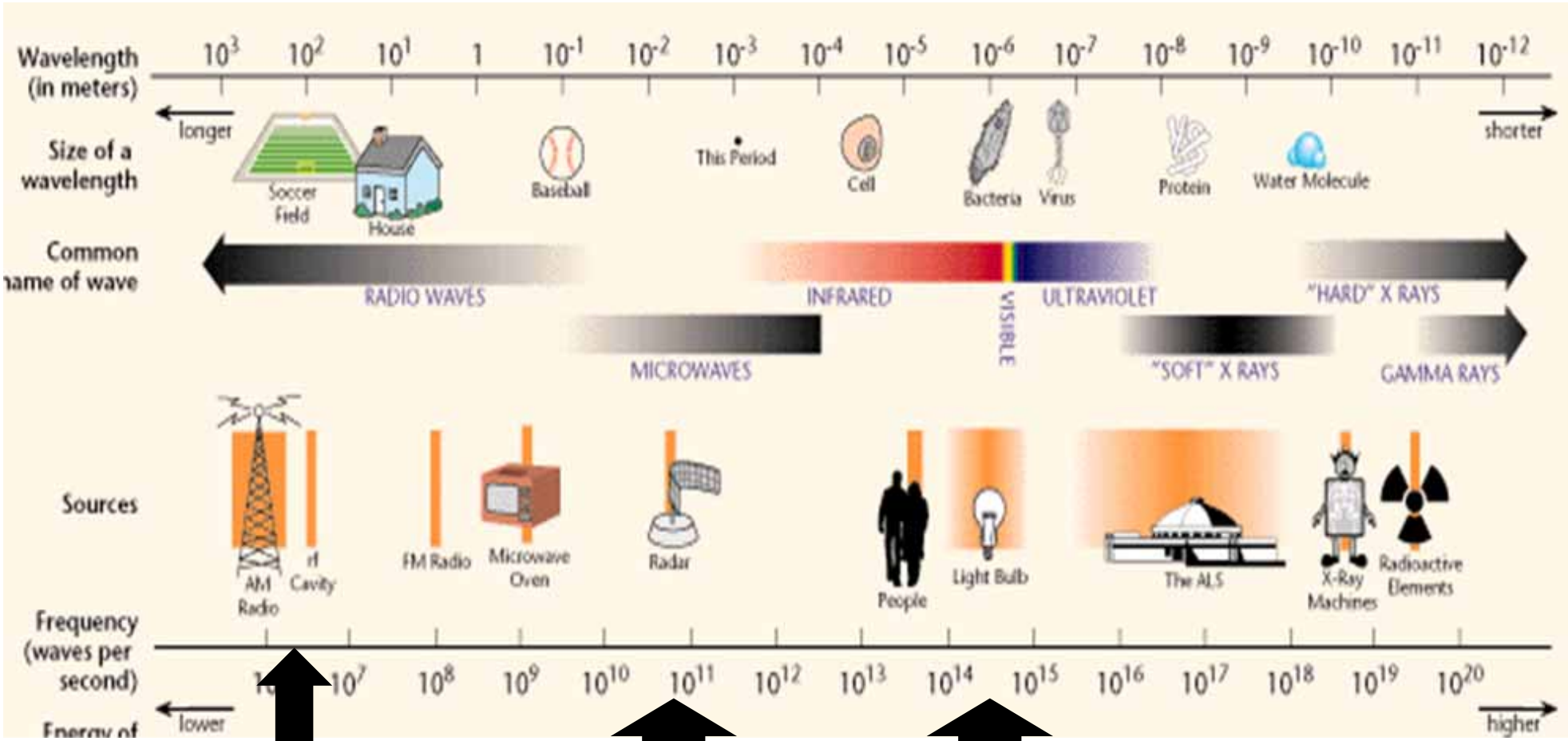
Listen

- Emitted radiofrequency signal from protons

Larmour relationship: frequency of absorbed radiation proportional to magnetic field:

e.g. Bo= 0.47 Tesla, 20 MHz

MR Relaxometry: Radiofrequency Interrogation of Water Protons



NMR
20 MHz

ESR

Visible

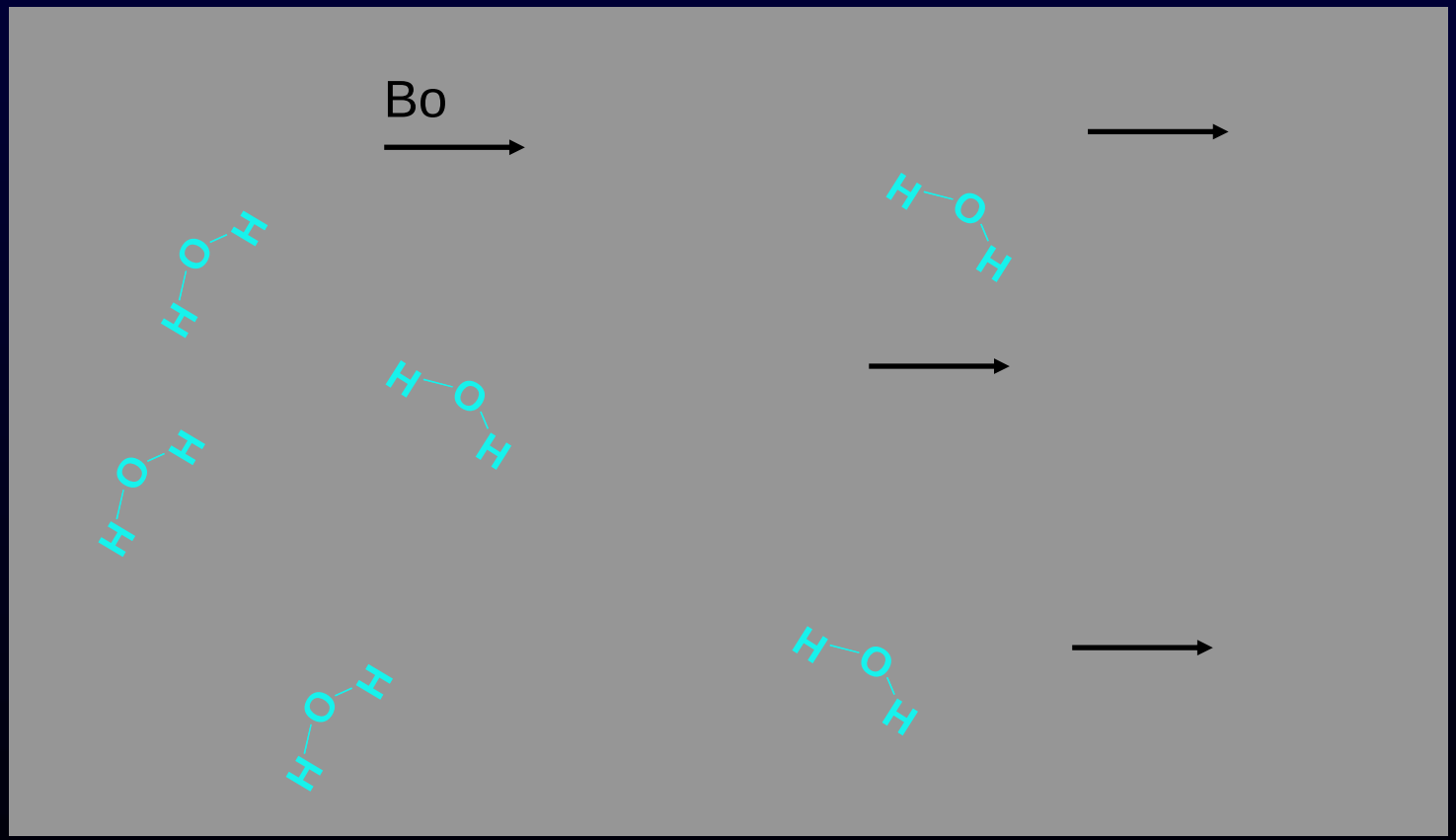
Magnetic Particles Enhance (speed up) Proton Relaxation

Mechanisms Of Proton Relaxation

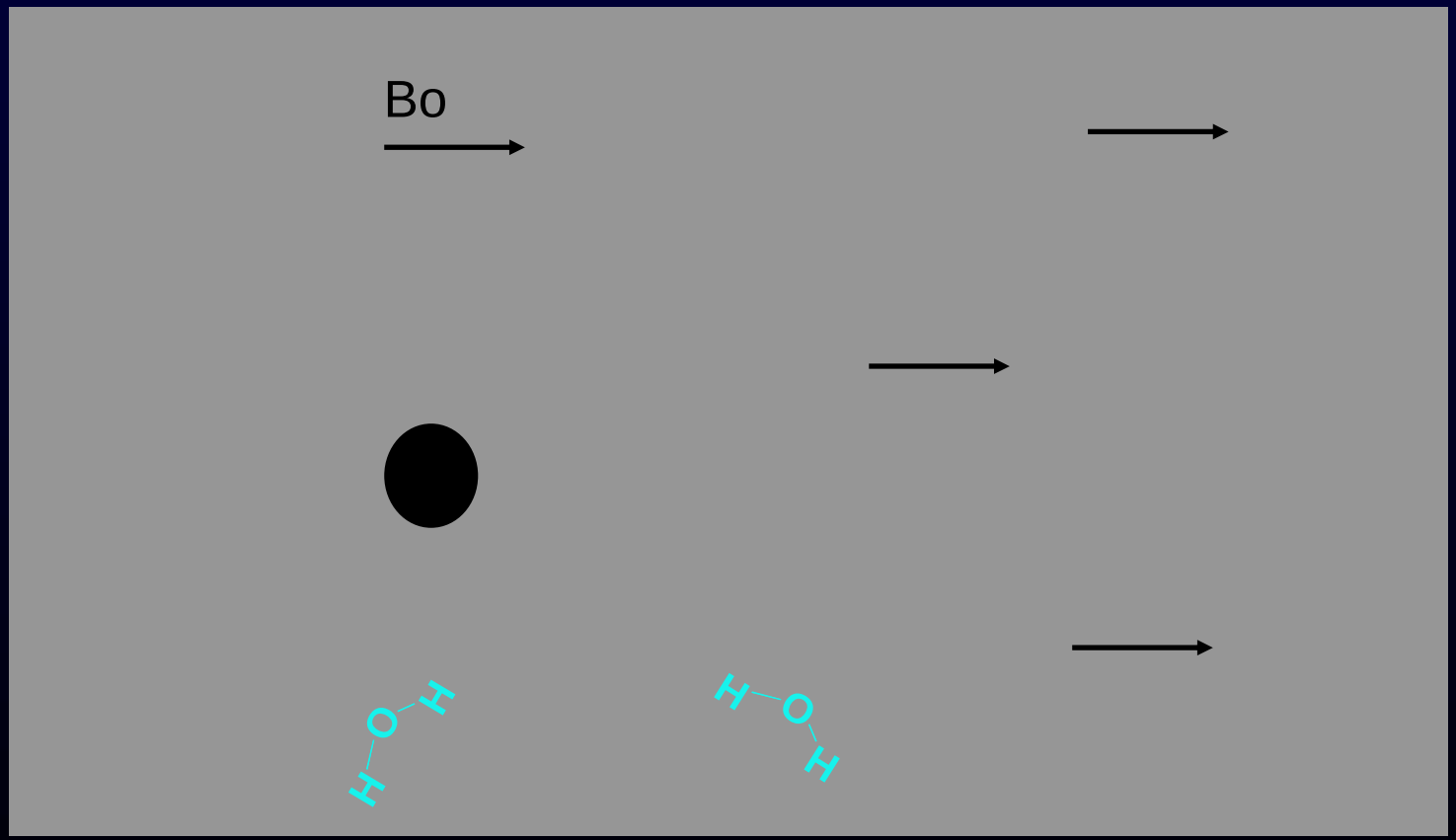
- T1 or spin-lattice relaxation: energy transfer from an excited proton to other materials
- T2 or spin-spin relaxation: spin dephasing due to magnetic field inhomogeneity

T1 Spin Lattice Relaxation

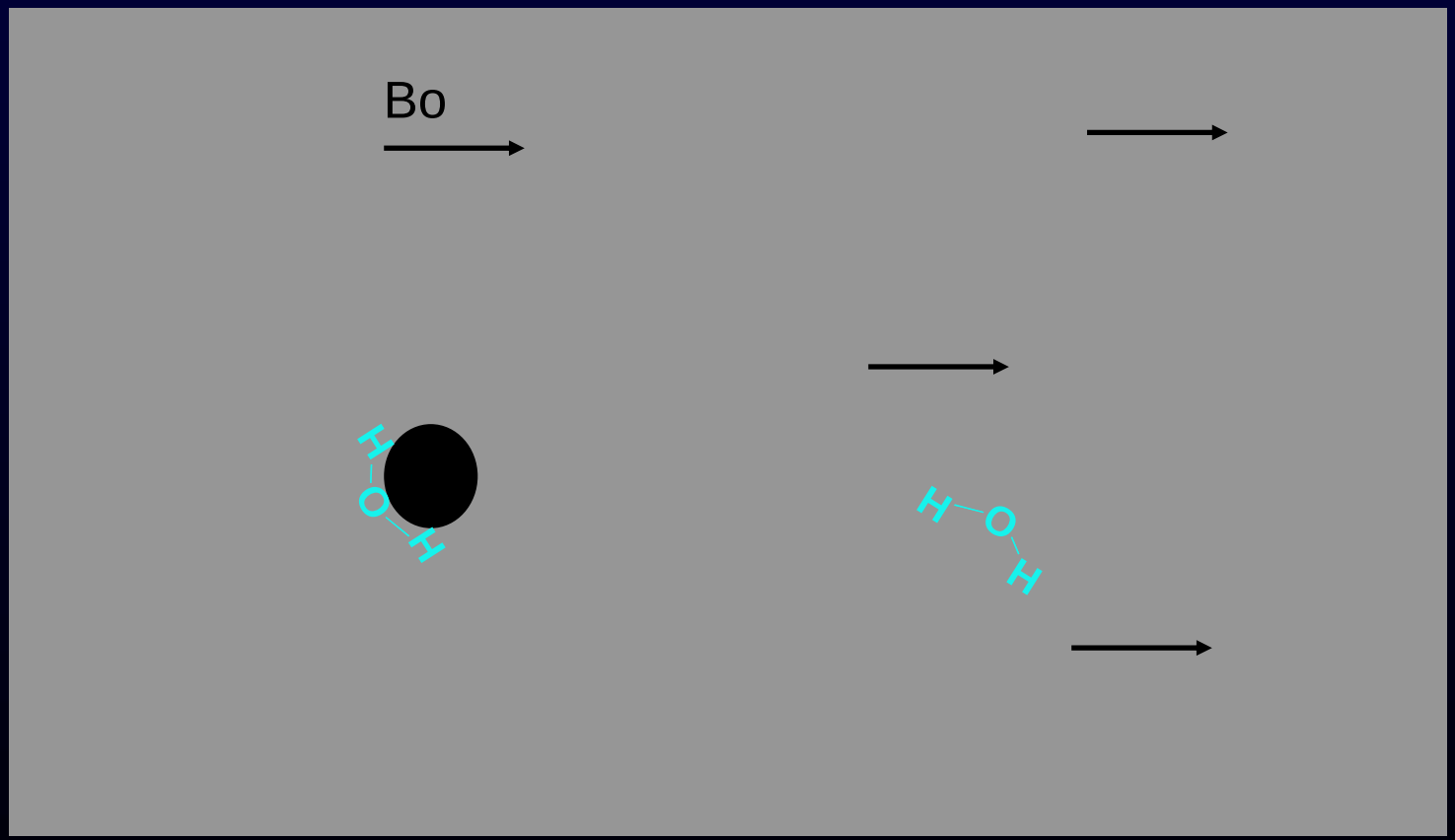
Water in a homogeneous magnetic field,
Induced Bo Is Constant In Sample



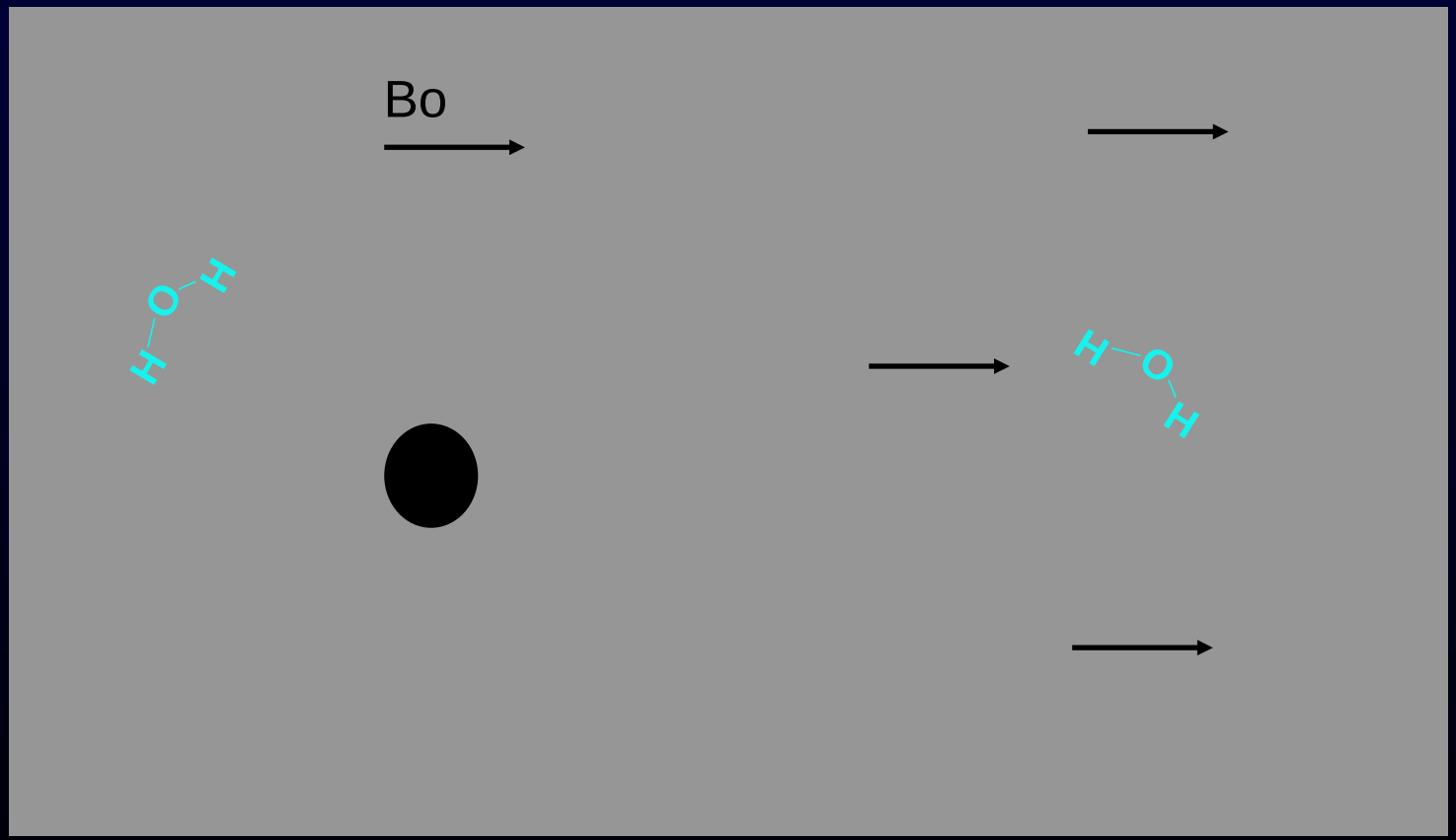
T1: Water diffuses & makes contact with a magnetic surface



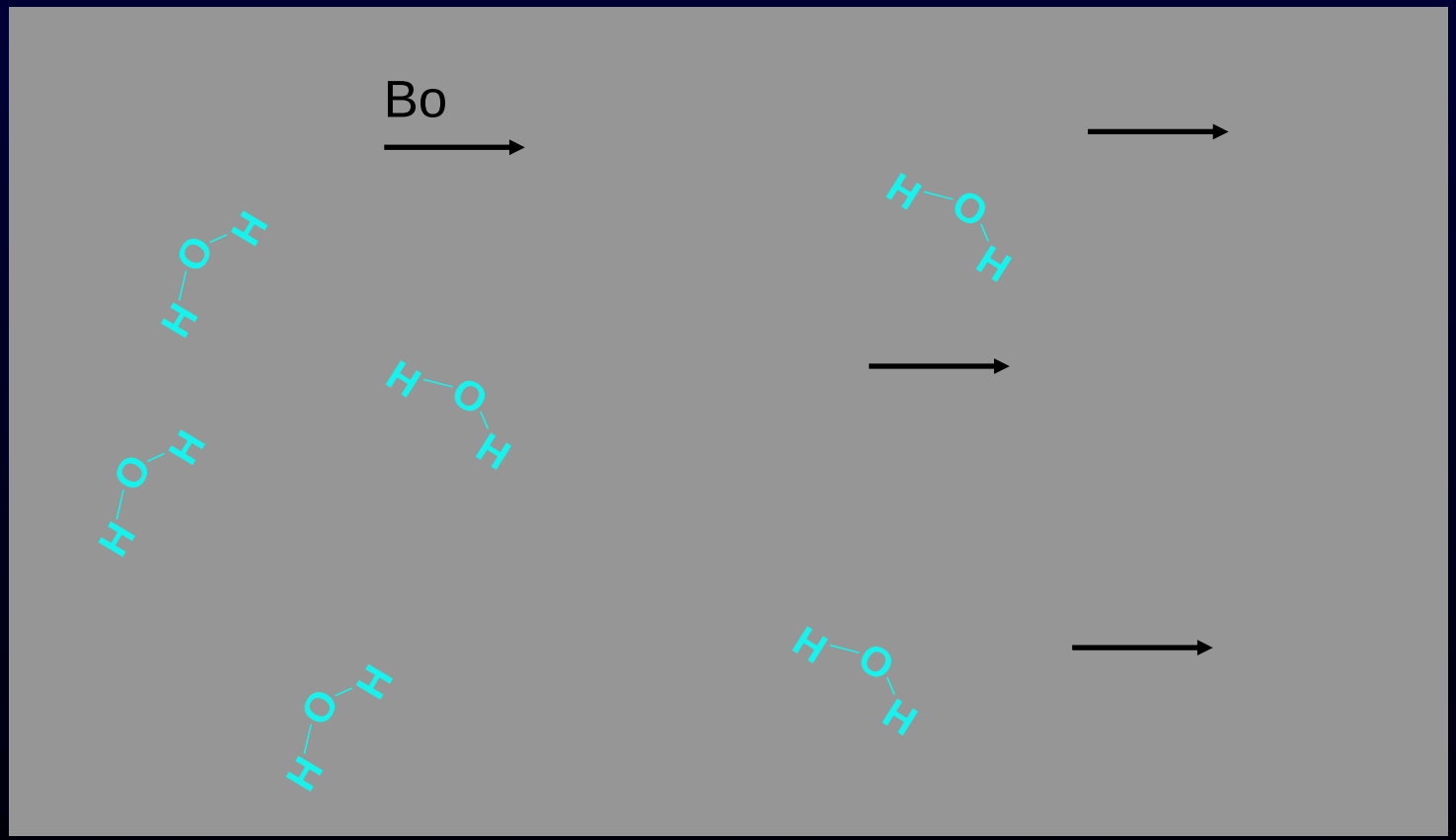
T1: Water diffuses & makes contact with a magnetic surface



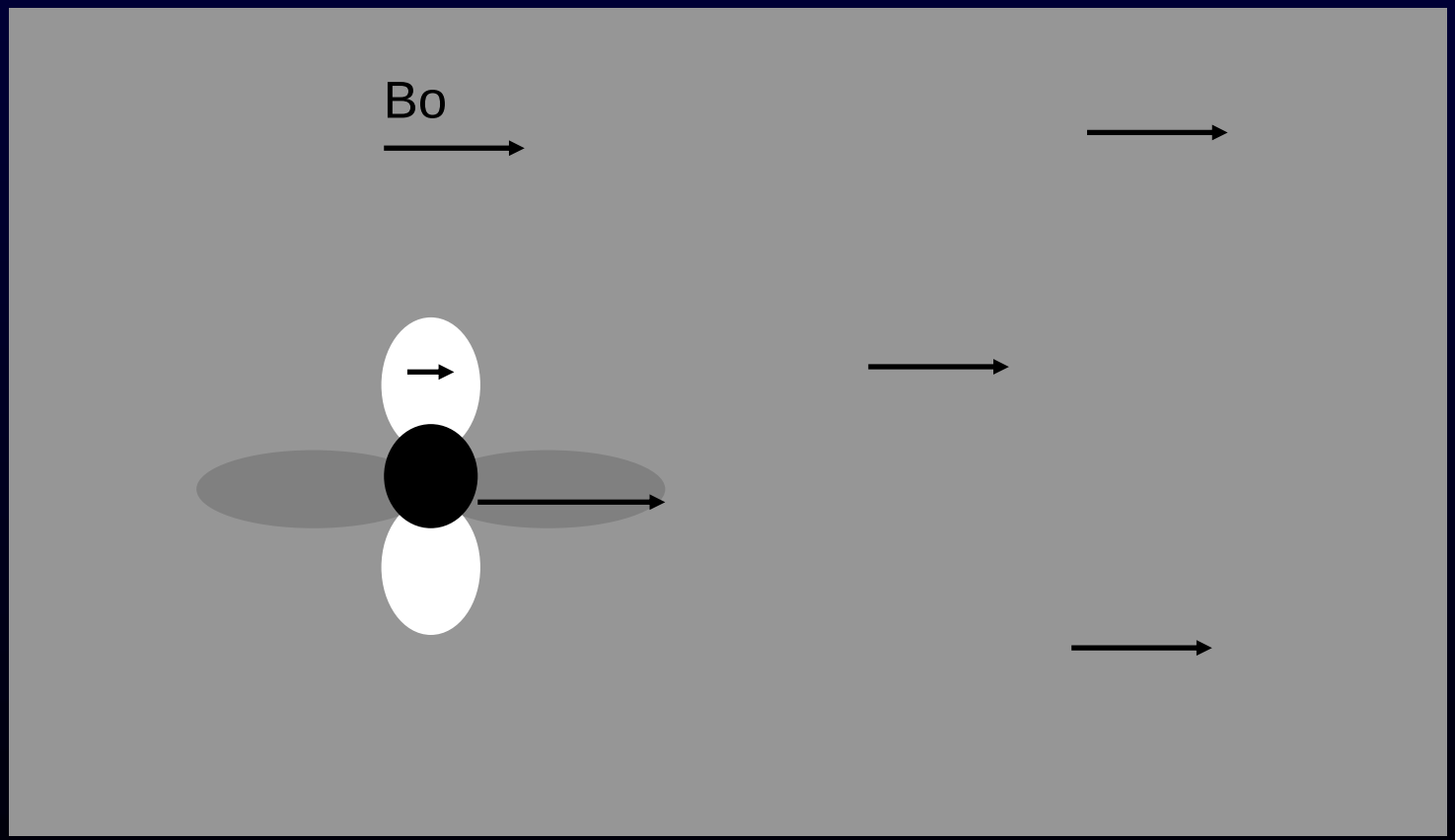
T1: Water diffuses & makes contact with magnetic surface



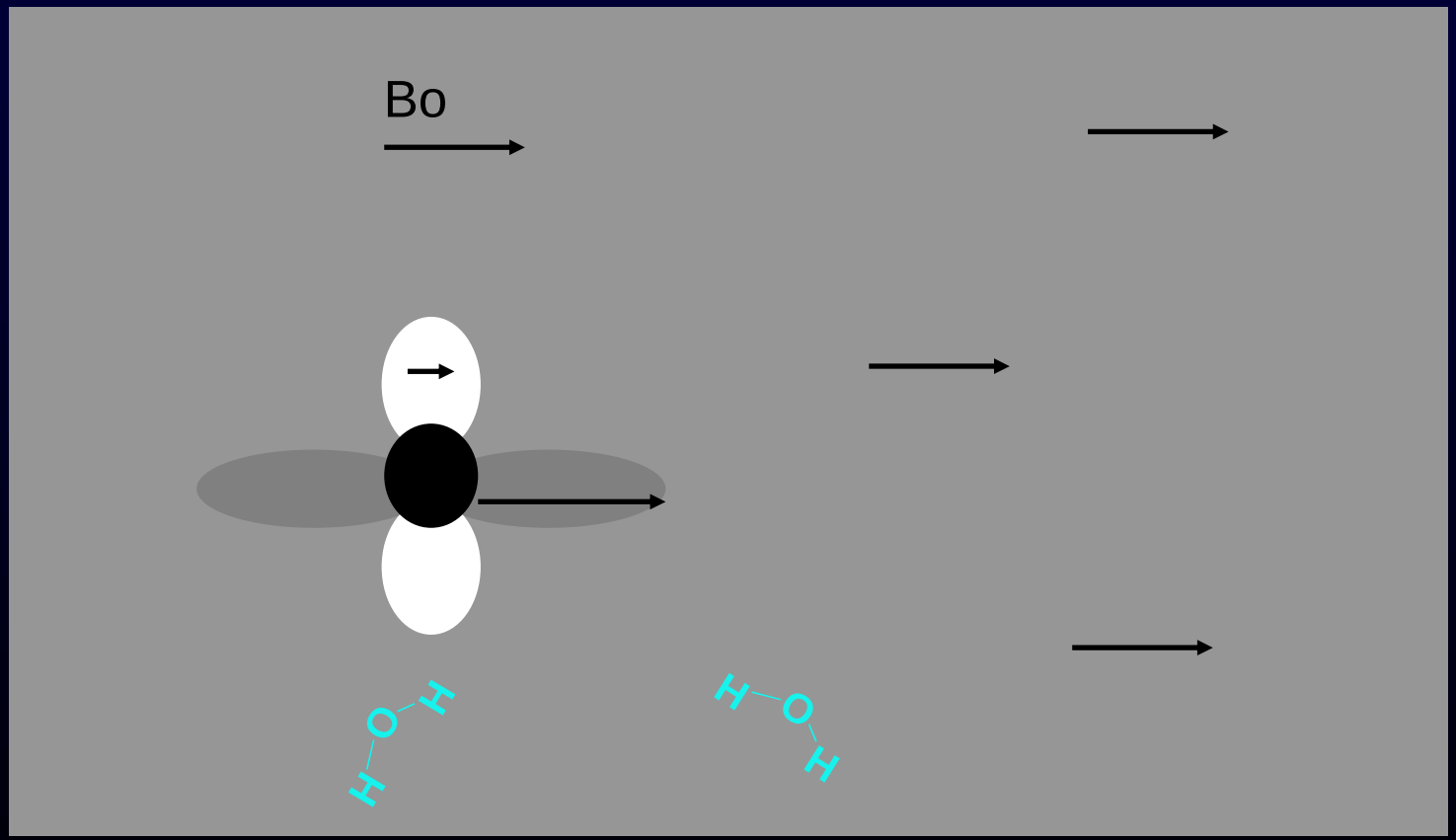
T2 Spin-spin Relaxation



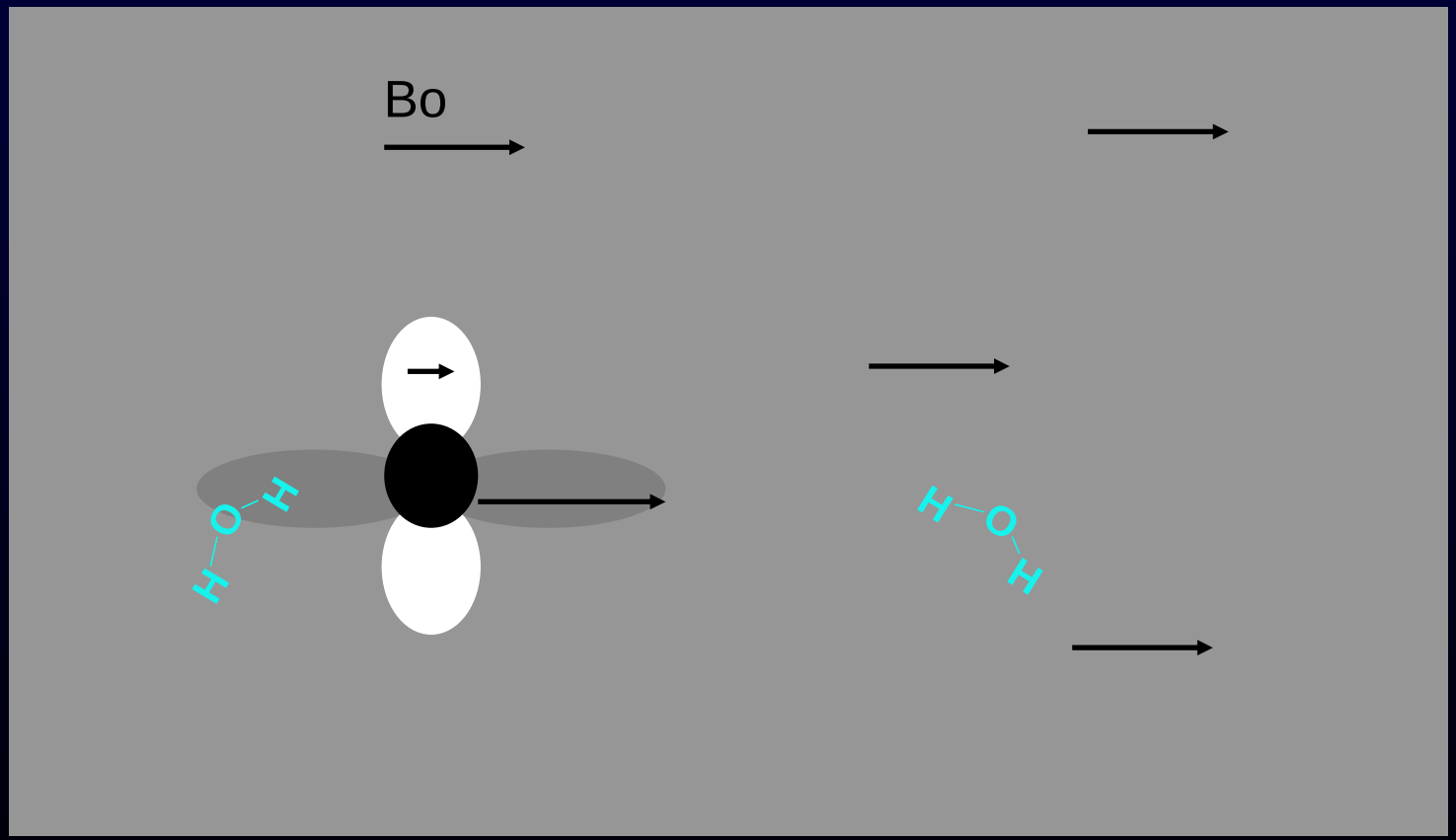
T2 Spin-spin Relaxation: A magnetic particle creates regions of local magnetic field inhomogeneity



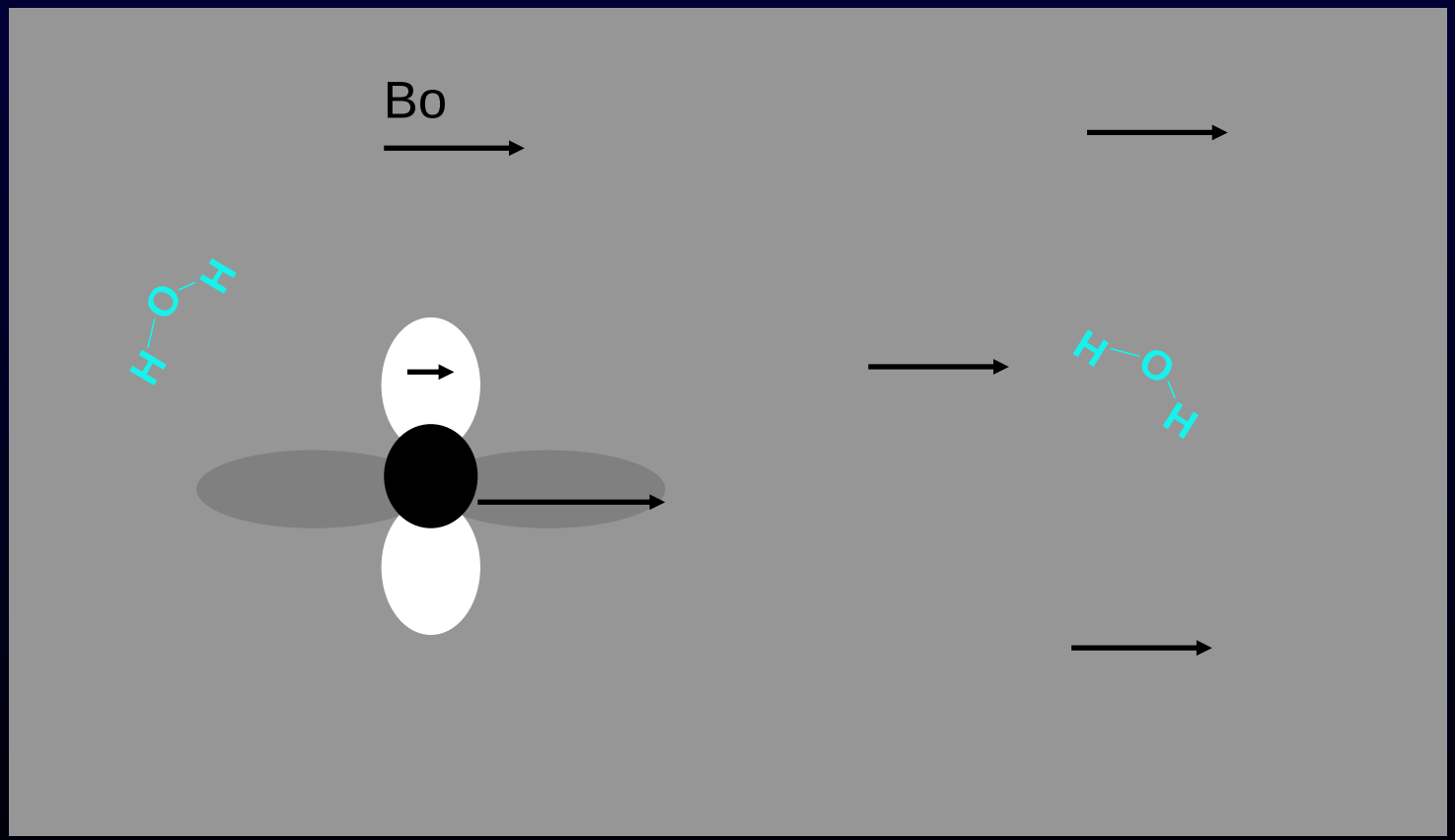
T2 Spin-spin Relaxation: Water diffuses through volumes where magnetic field is not Bo



T2 Spin-spin Relaxation: Water diffuses through volumes where magnetic field is not Bo

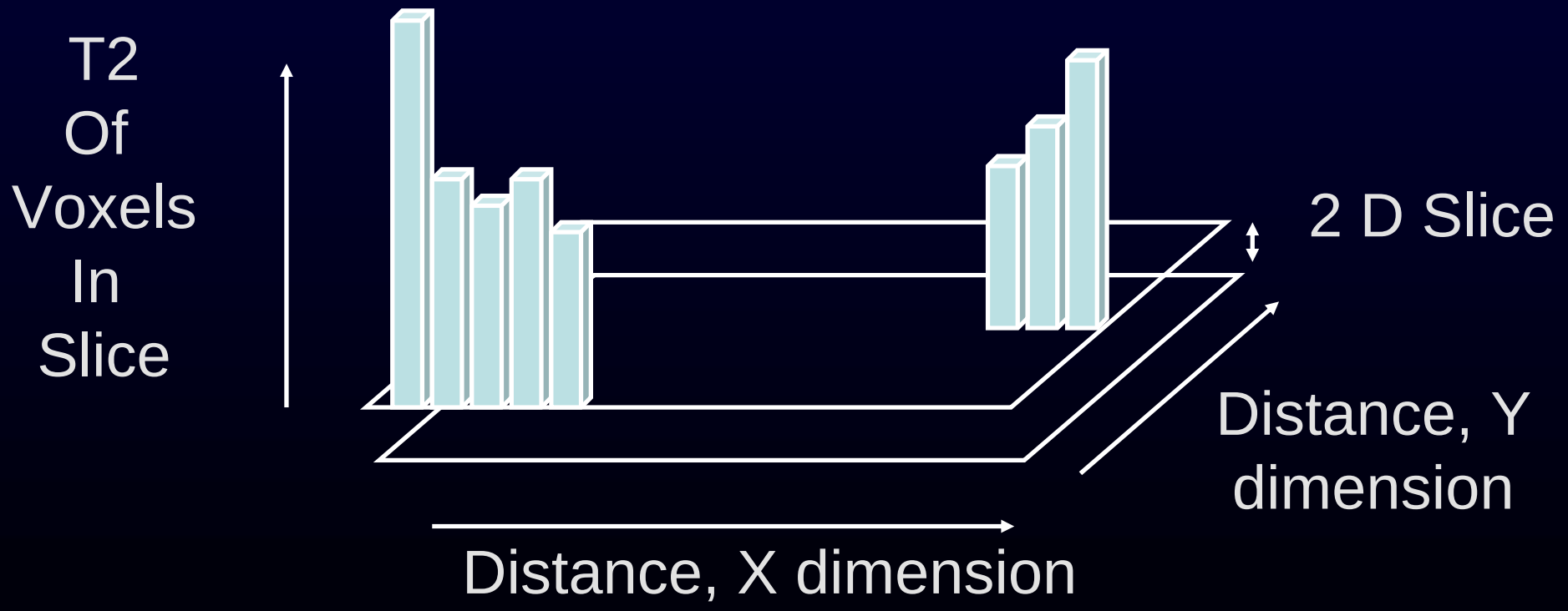


T2 Spin-spin Relaxation: Water diffuses through volumes where magnetic field is not Bo



MRI: T2 From Different Points In Matrix

Sagital T2w Image, Lumbar Spin
Single slice, 2D image

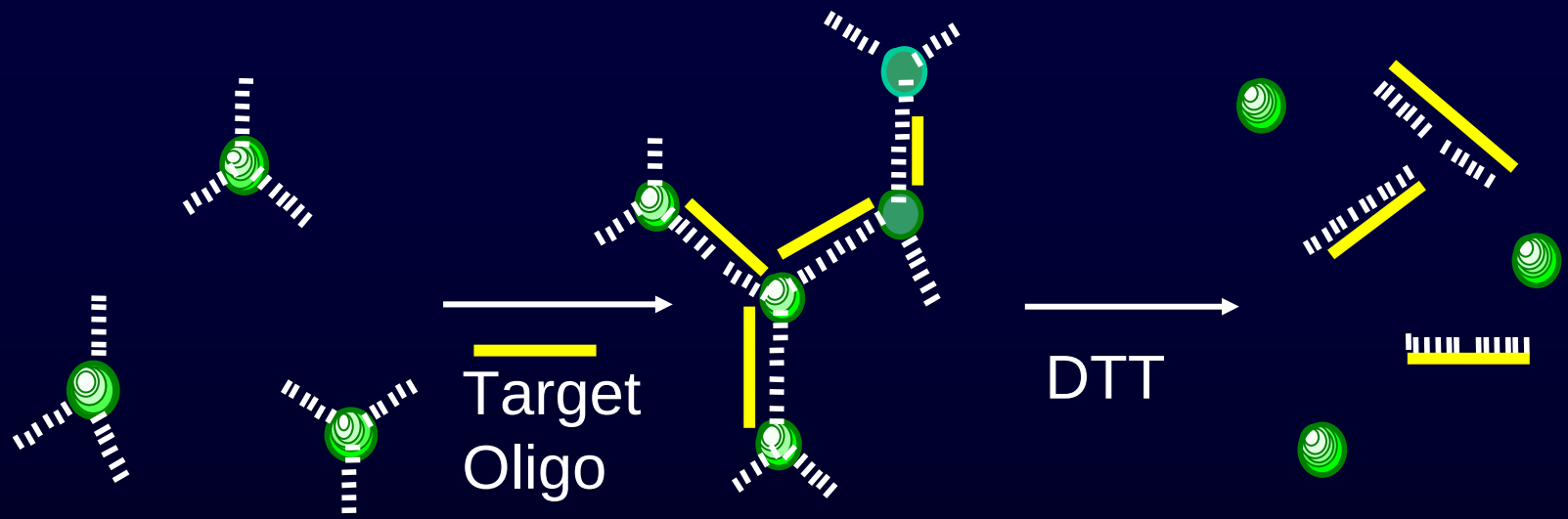


Magnetic Relaxation Switch (MRSw's) Assays

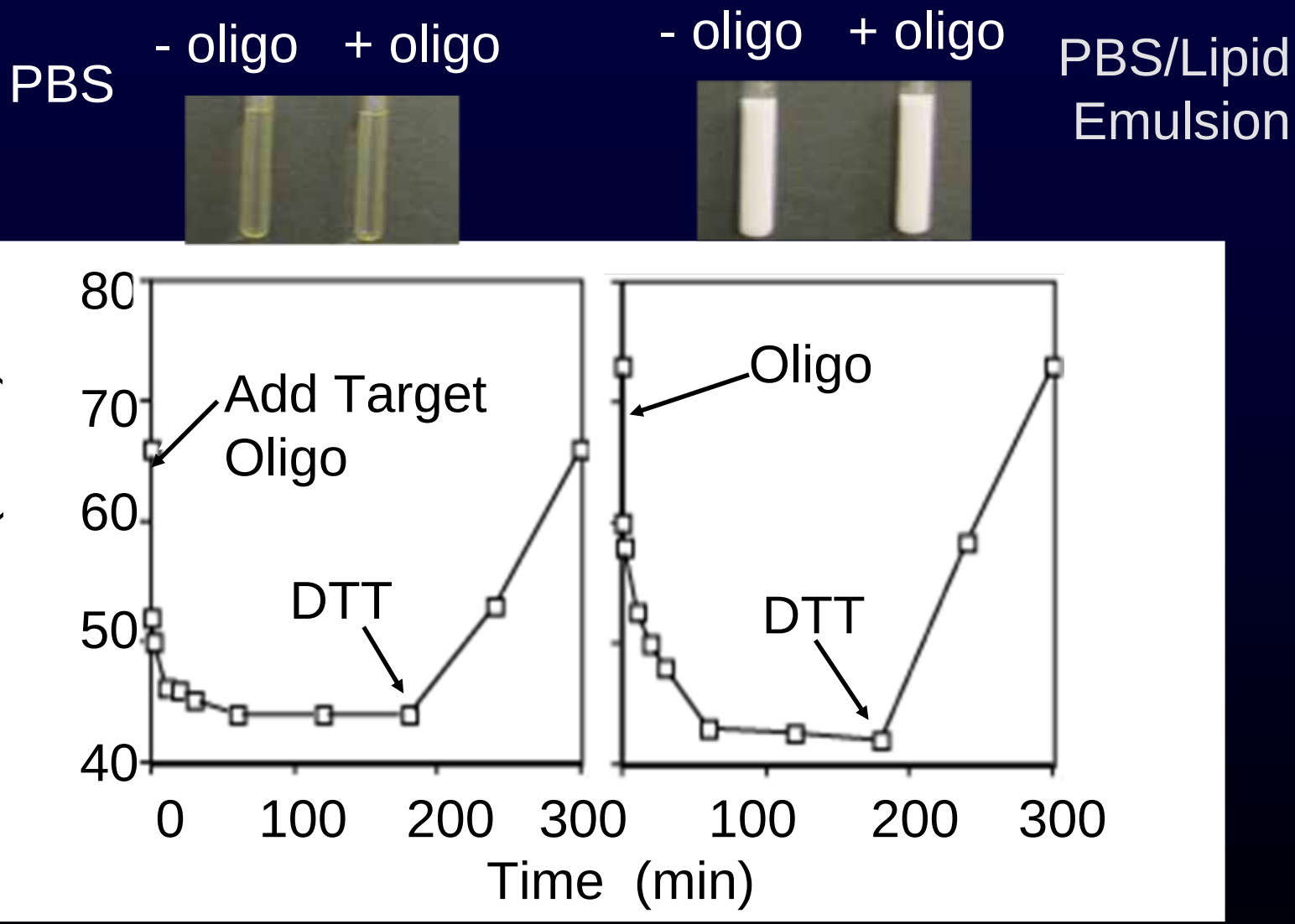
- At a given concentration of magnetic particles, T2 depends on the "microdistribution" of magnetic field inhomogeneities, the "uneveness" of magnetic moments

- T2 depends on whether particles are aggregated or dispersed.

Magnetic Nanoparticles As Magnetic Relaxation Switches (MRSw's)



MRSw Assay Of Target Oligonucleotide: Indifference To Light

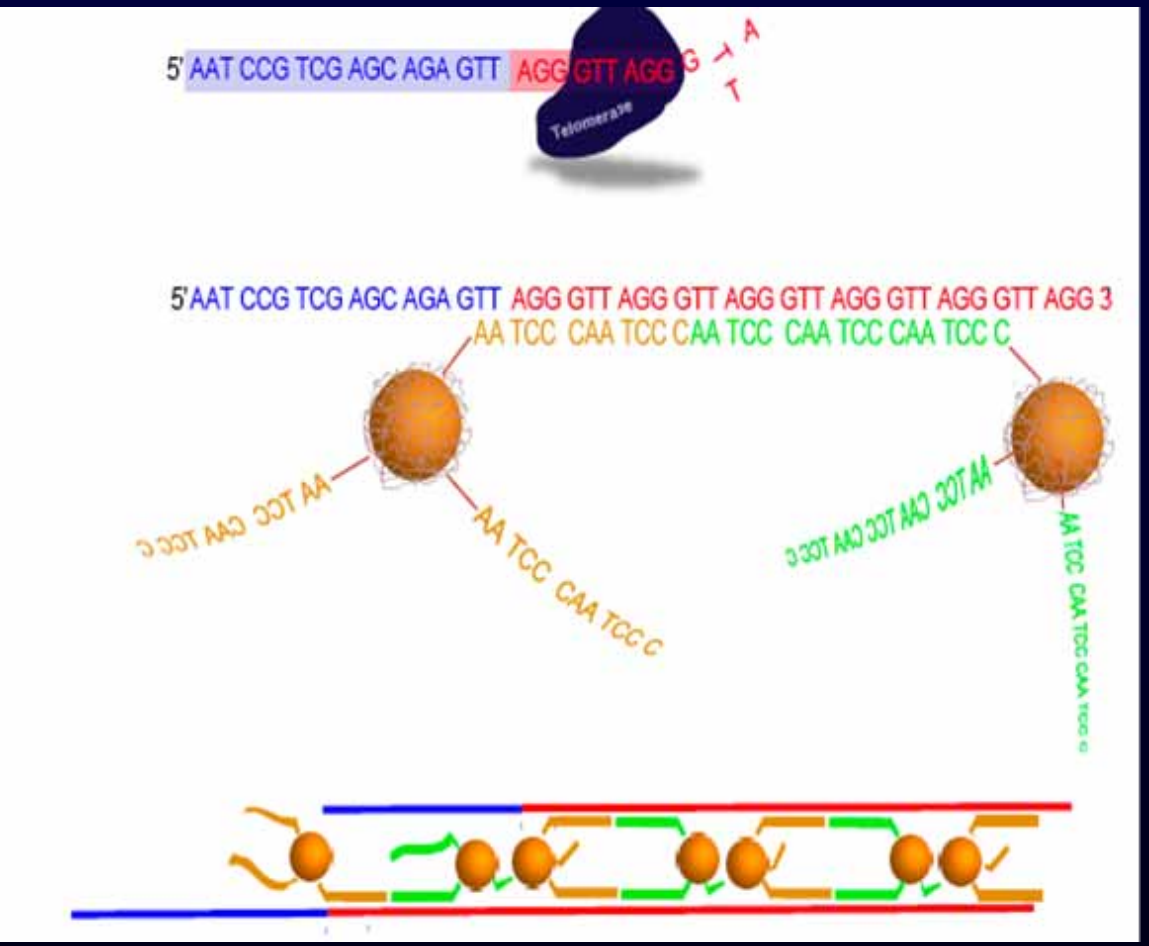


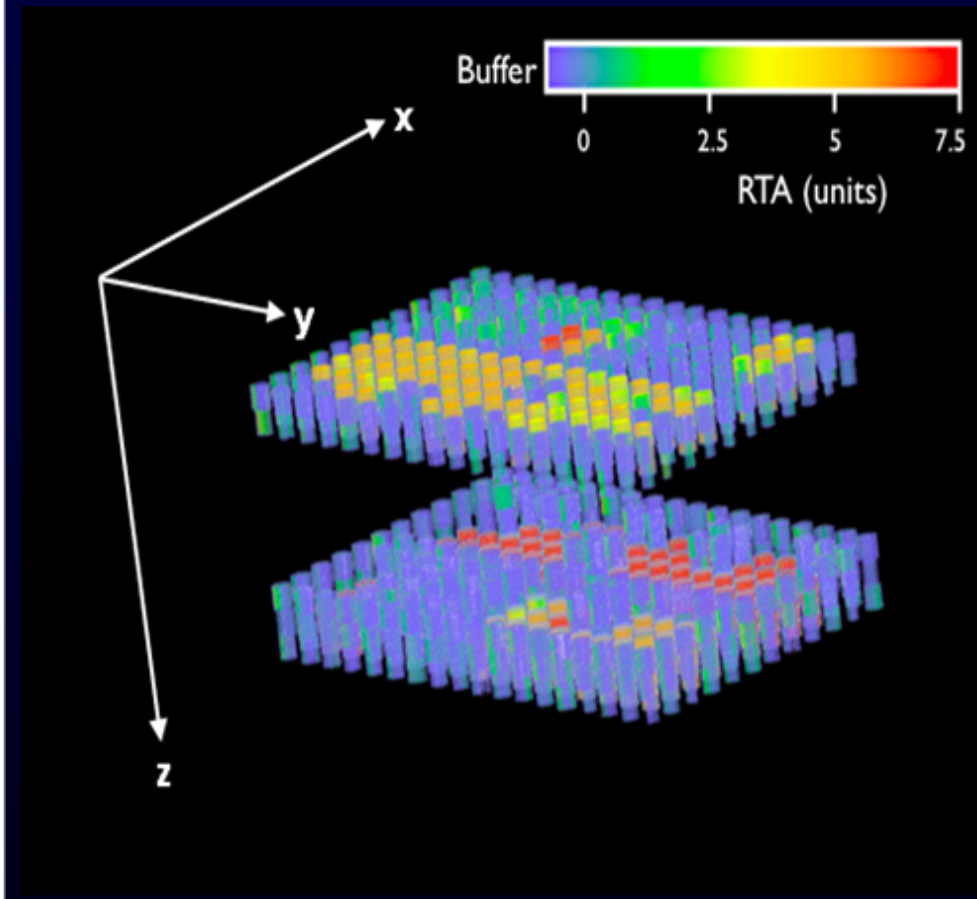
Perez, (2002) Magnetic relaxation switches capable of sensing molecular interactions. *Nat Biotechnol 20*, 816.

MRSw Assays Measure Diverse Analytes (MGH Group Assays Only)

Target/Analyte	Reference
DNA, proteins	(2002) Nature Biotech 20:816
DNA Methylases	(2002) JACS 124:2856
Proteases	(2003) Angew Chem. 42:1375
Viruses	(2003) JACS 125:10192
Polymerases	(2004) Cancer Res. 64:639
Glucose, Folate	(2006) Small 2:1144
Bacteria, cells	(2007) Bioconj Chem.18:2028
Anti viral antibody	(2008) Angw Chem 47:4119
Bacteria, cells	(2008) Nat. Med. 14:869.

MRSw Assay For Telomerase Activity





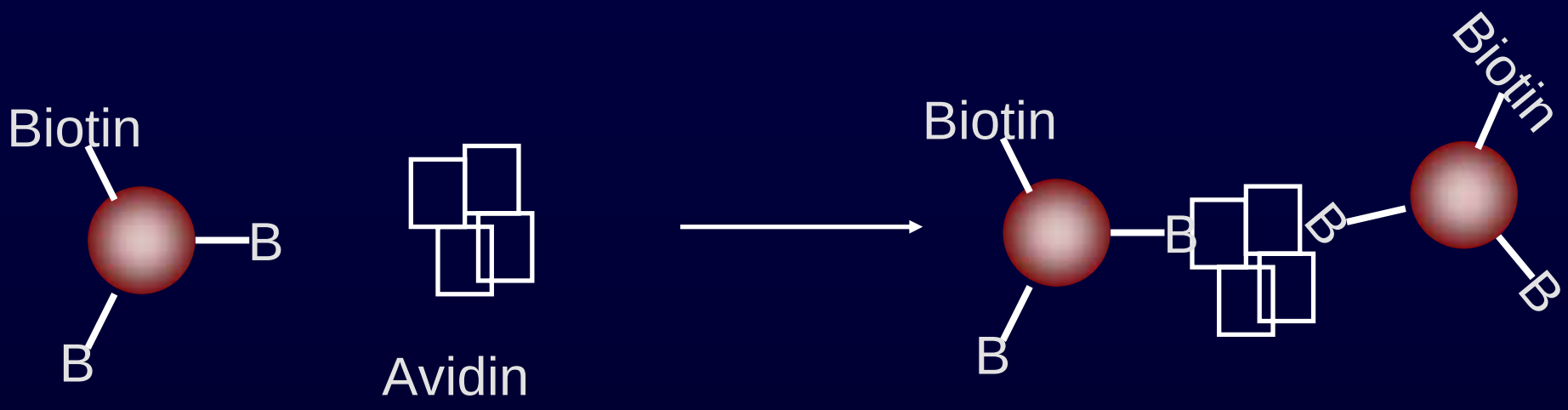
Grimm, (2004) Novel nanosensors for rapid analysis of telomerase activity. *Cancer Res 64*, 639.

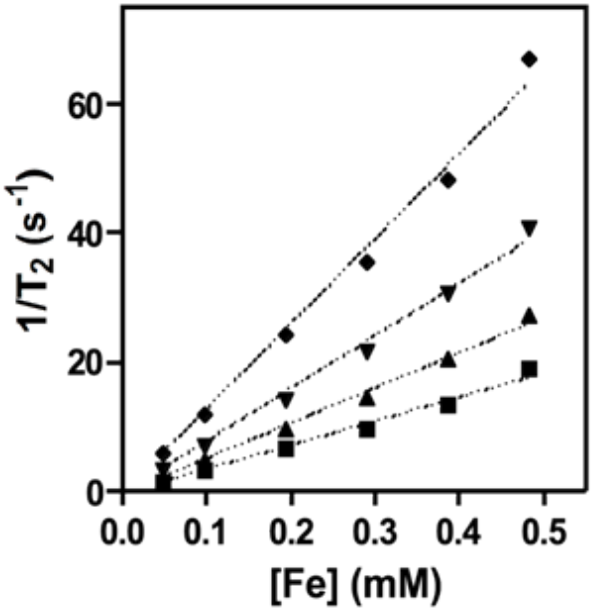
Magnetic Nanoparticles & Proton Relaxation

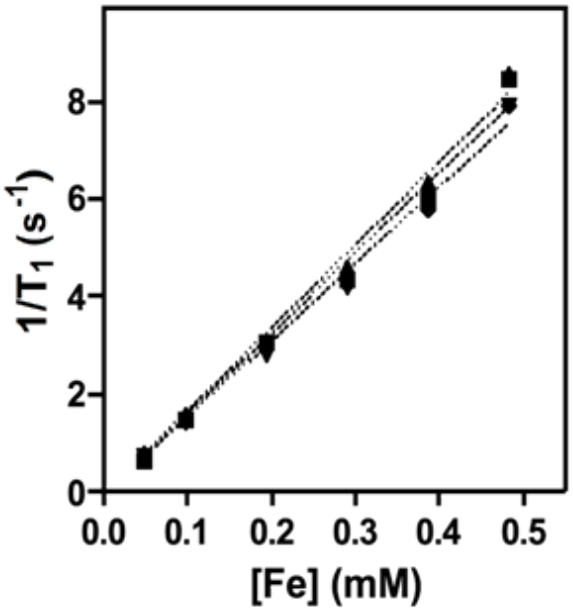
Spin-lattice, T1 relaxation: Water contacts surface of NP's and looses energy to its surroundings

Spin-spin, T2 relaxation: Water diffuses through the magnetic field of the NP, and those spins are knocked out of phase with spins that have never "experienced" the magnetic field

Multi-pulse Sequence MRSw Interrogation



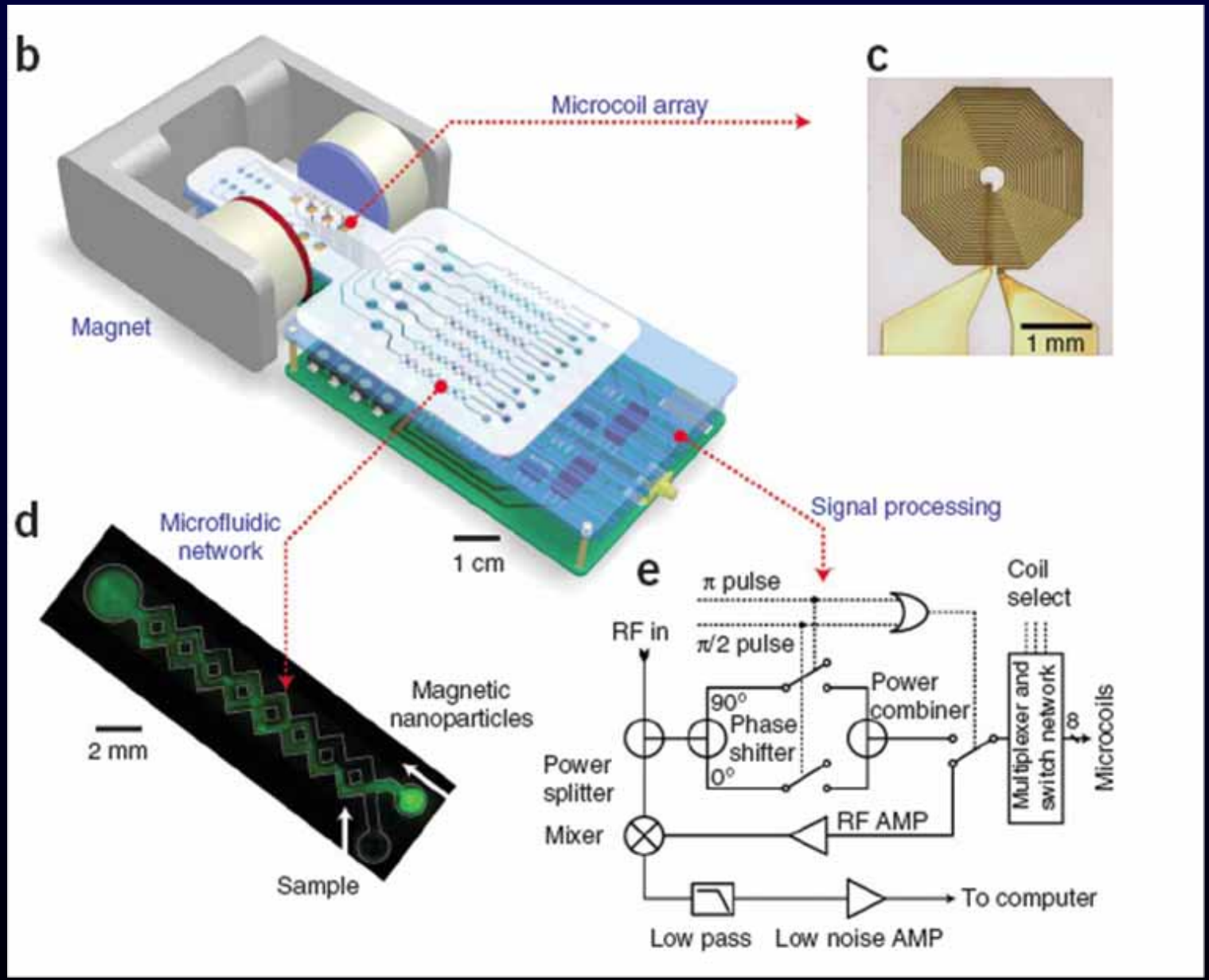




Adding Avidin, Increases R2 But Not R1

Taktak (2007) Multiparameter magnetic relaxation switch assays. *Anal Chem 79*, 8863.

Miniaturized, Multiwell Relaxometer, 8 wells, 10 uL/well



Lee (2008) Nat Med "Chip NMR biosensor for detection and molecular analysis of cells"

Magnetic Nanoparticles As MRSw Assays

Homogeneous mix & read but Indifferent to light

Targets: viruses, cells, proteins, nucleic acids, etc.

High sensitivity: microspheres & magnetic field assisted chemical reactions

Multipulse sequence interrogation permits corrections for unknown reagent (NP) concentration

Miniaturized relaxometer designs

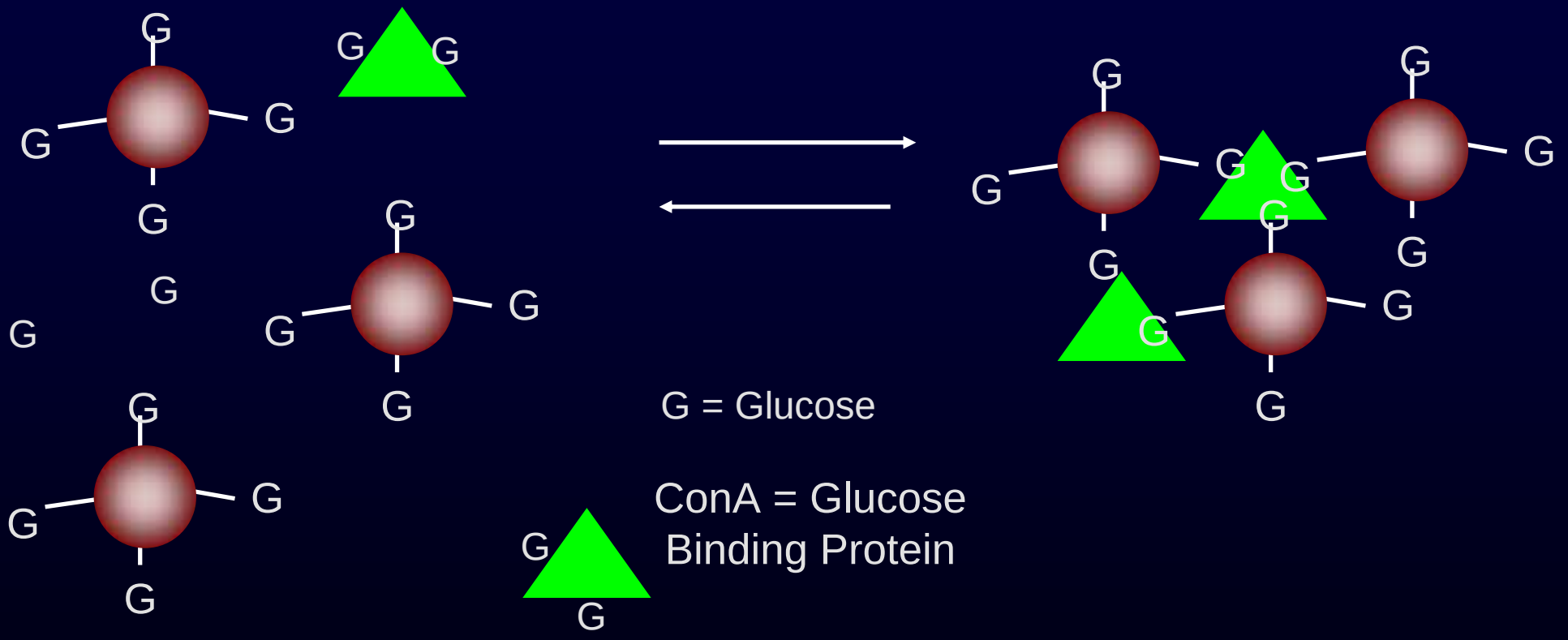
Magnetic Nanoparticles & MR: An introduction

Nanoparticles As MR Contrast Agents

Nanoparticles for MR Based Assays

Nanoparticles & MR Based Implantable Biosensors

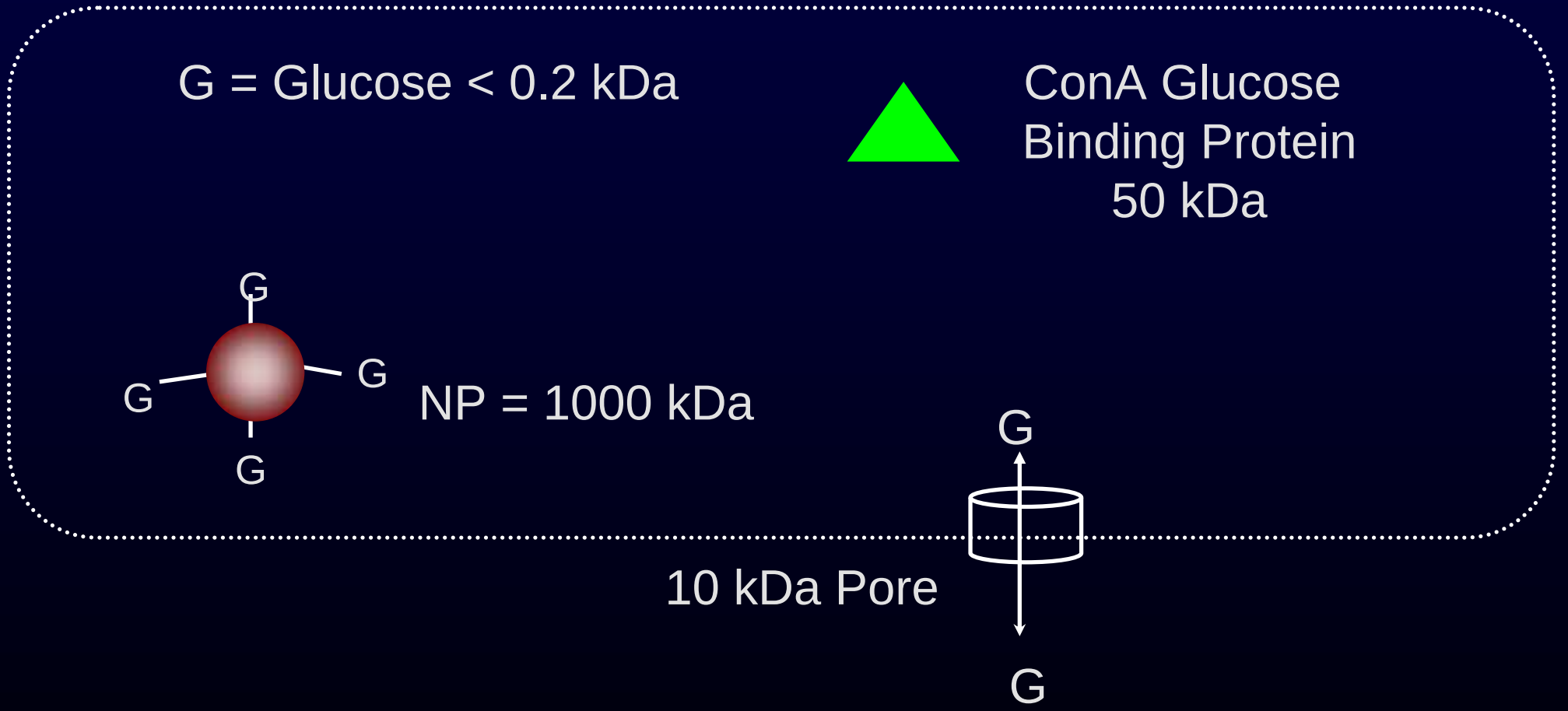
Competitive, *Reversible* MRSw Assay For Glucose



High Glucose
High T2

Low Glucose
Low T2

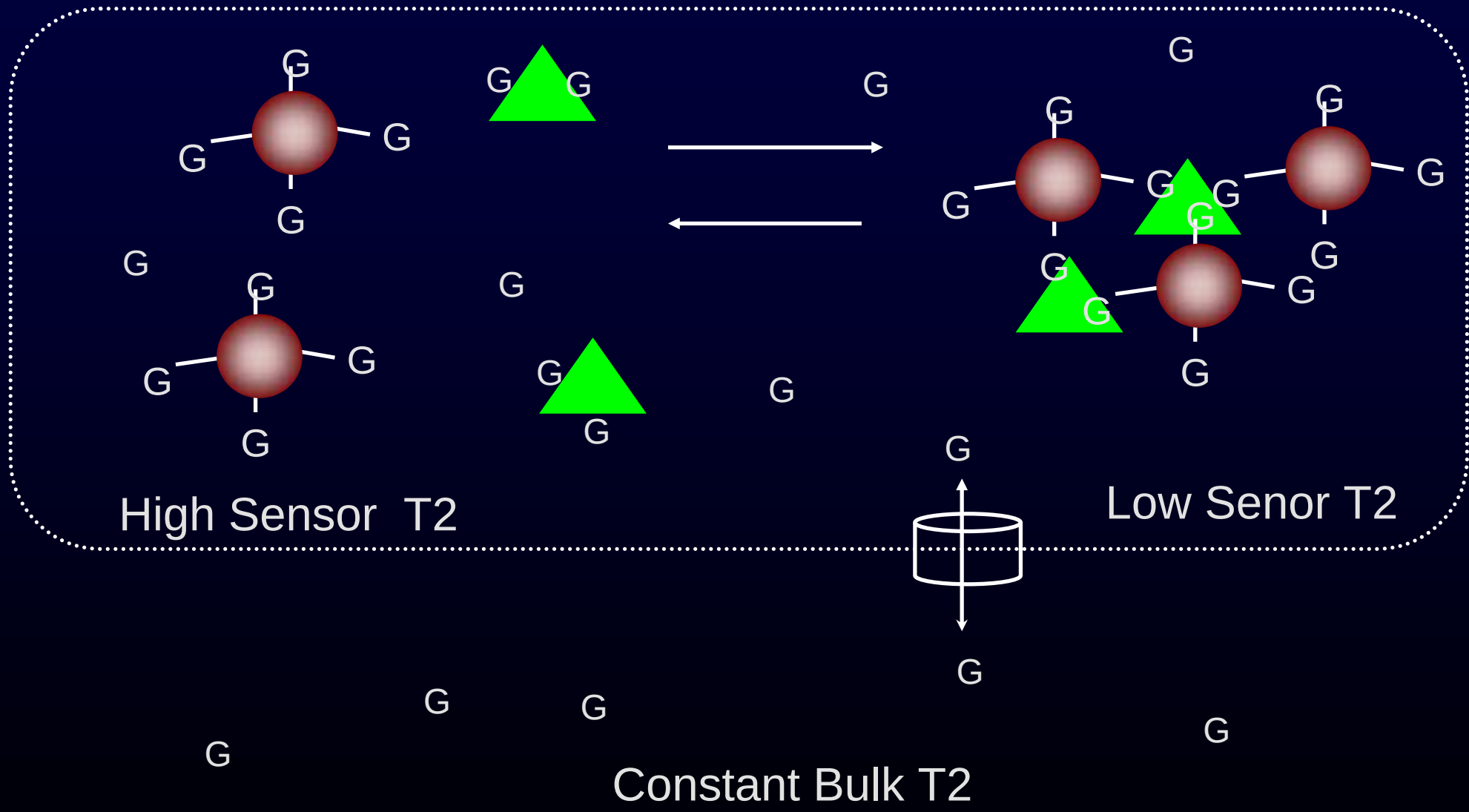
MRSw Glucose Sensor Format: Glucose Enters & Leaves While Reagents Are Confined



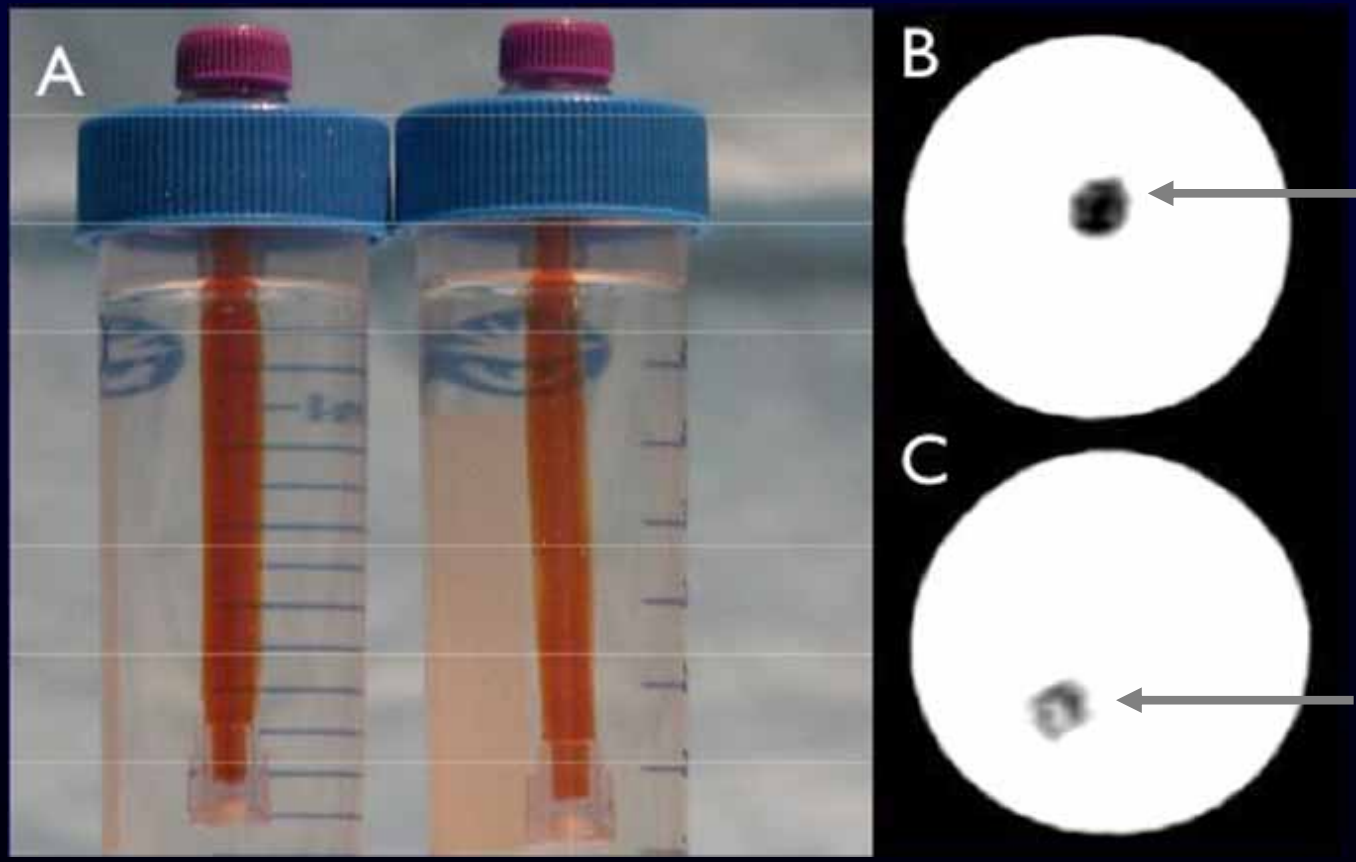
Sun. (2006) Continuous analyte sensing with magnetic nanoswitches.

Small 2, 1144. "

Glucose Sensor: Glucose Enters & Leaves While Reagents Are Confined



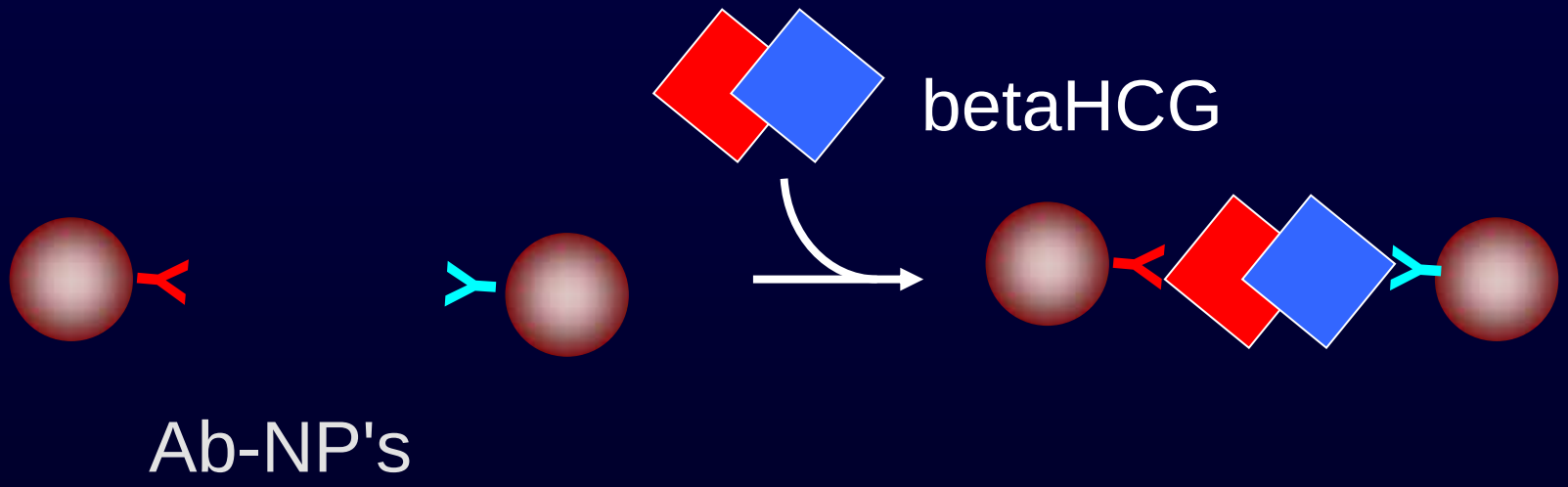
MRSw Sensor: Nanoparticle Sensor Responds to External Glucose With T2 Change (MRI)



0 mg/mL Glucose
NP Clustered
Low T2
Dark Sensor

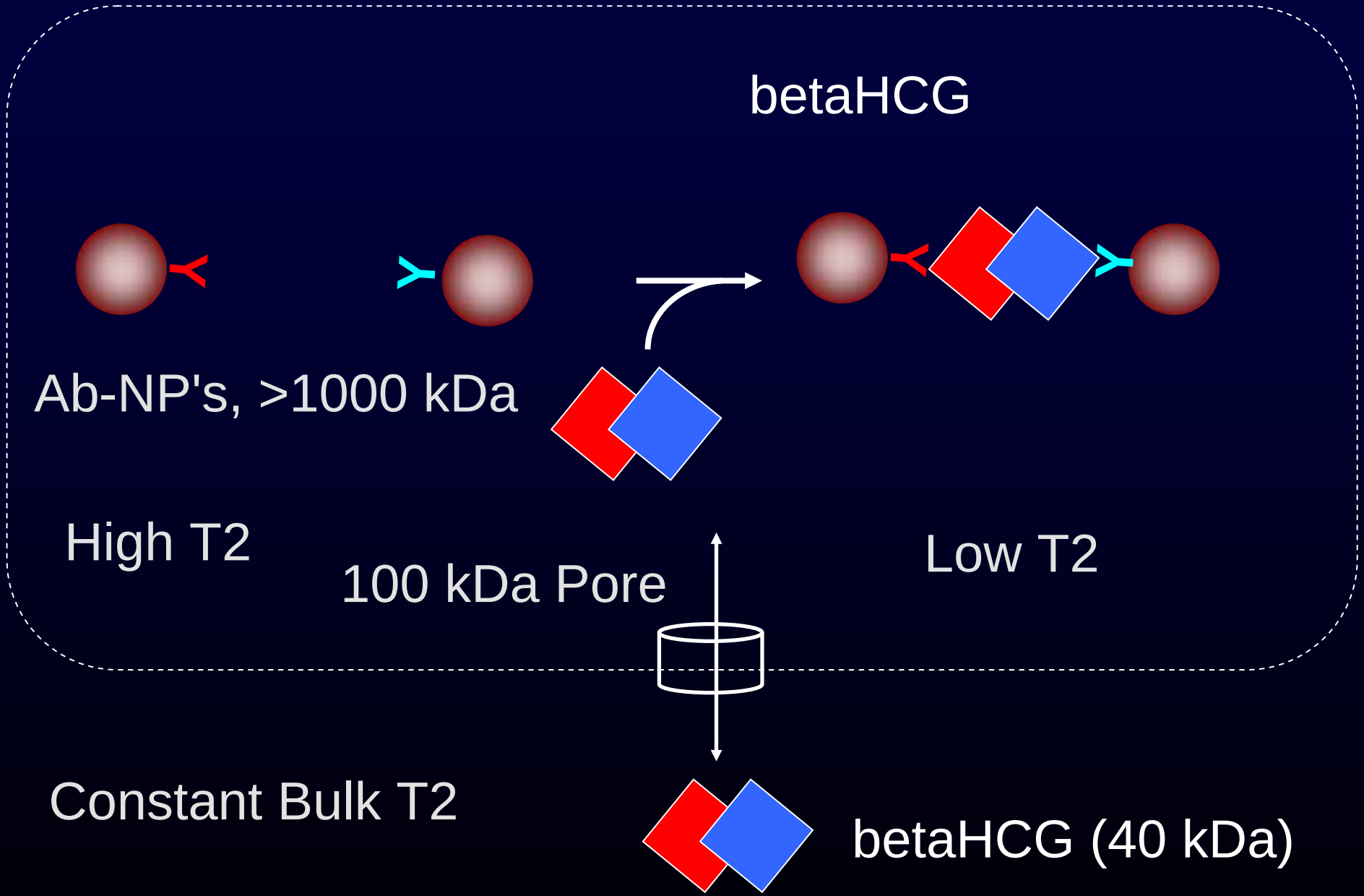
2 mg/mL Glu
NP Dispersed
Hi T2
Bright Sensor

Irreversible MRSw Assay For hCG

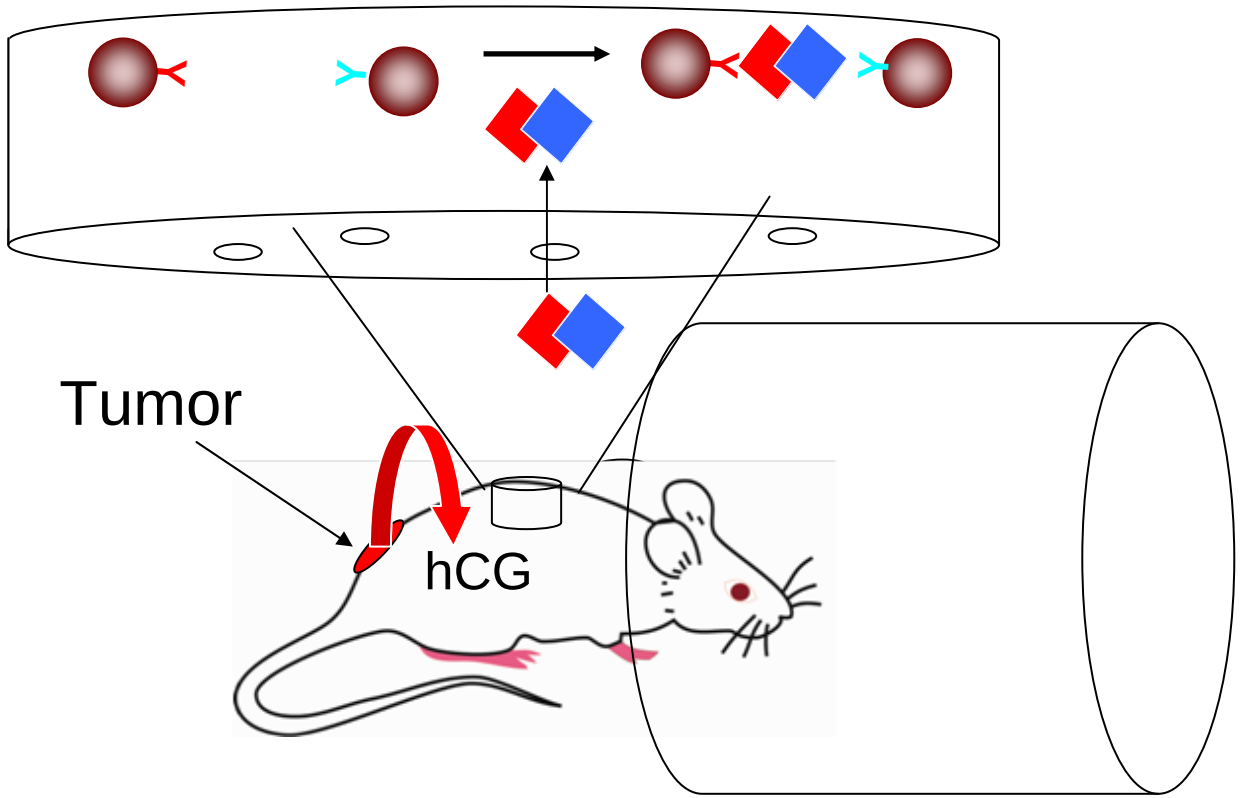


Kim (2007) Magnetic relaxation switch detection of human chorionic gonadotrophin. *Bioconjug Chem 18*, 2024.
"

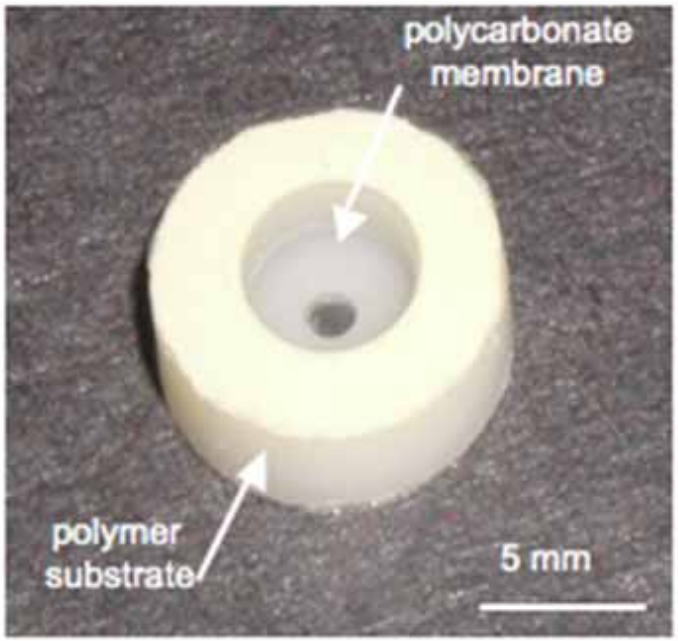
MRSw Sensor Chemistry For hCG

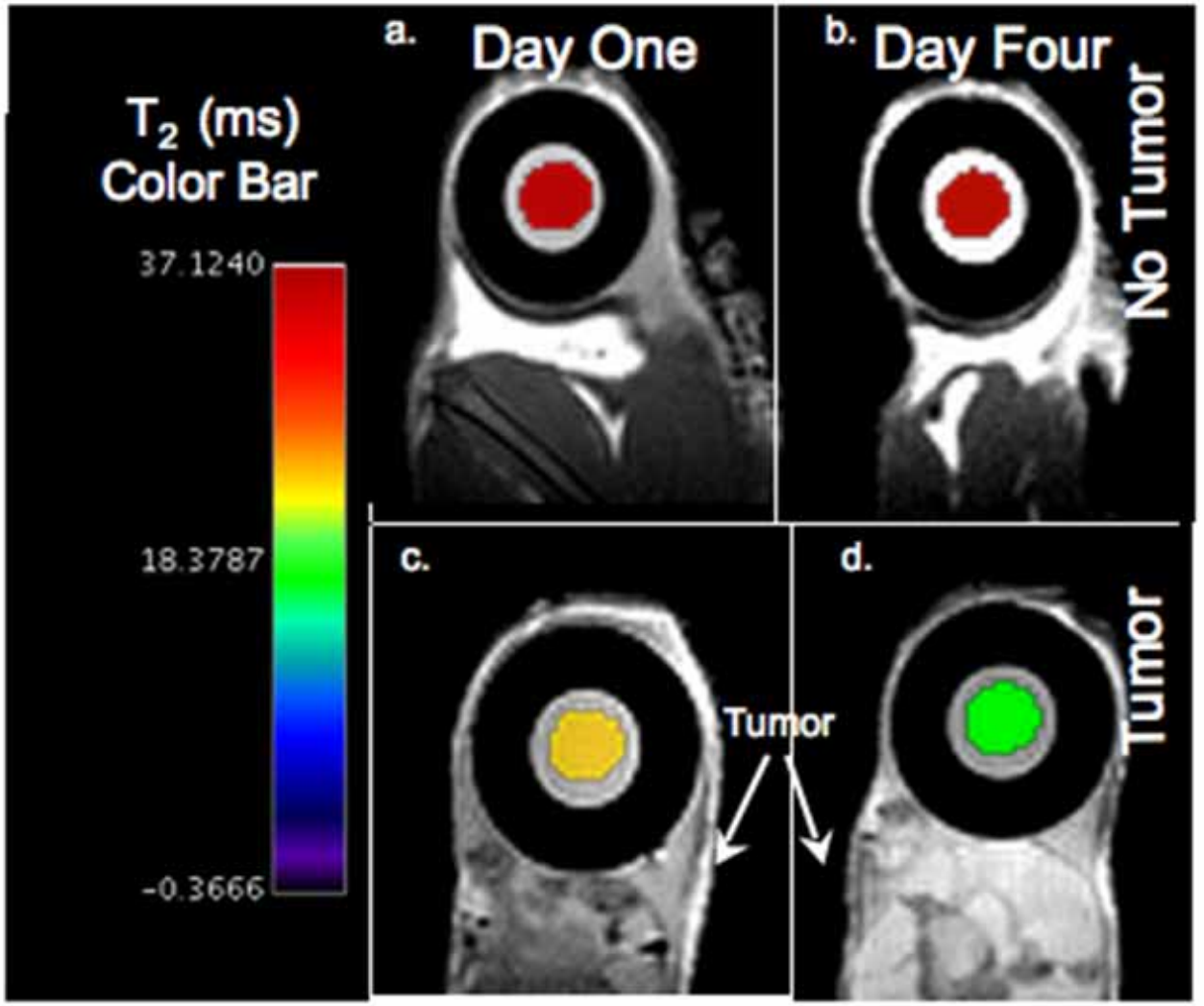


Implantable MRSw Sensor For Tumor Excreted hCG



Implantable MRSw Sensor for Tumor Excreted hCG, MRI @ 4.7T





Daniel (2009) "Implantable diagnostic for cancer monitoring…" Biosens. Bioelect.

Summary MRSw Sensors

- Indifferent to light
- Emit Rf radiation but no power supply
- Simple MR Instrumentation: Discriminate Sensor T2 From Bulk T2, No MR image Needed
- Multianalyte MRSw Sensor Capability Based on MRSw Multianalyte assays
- Use Multiparameter Sample Interrogation

Future Magnetic NP / MR Technology

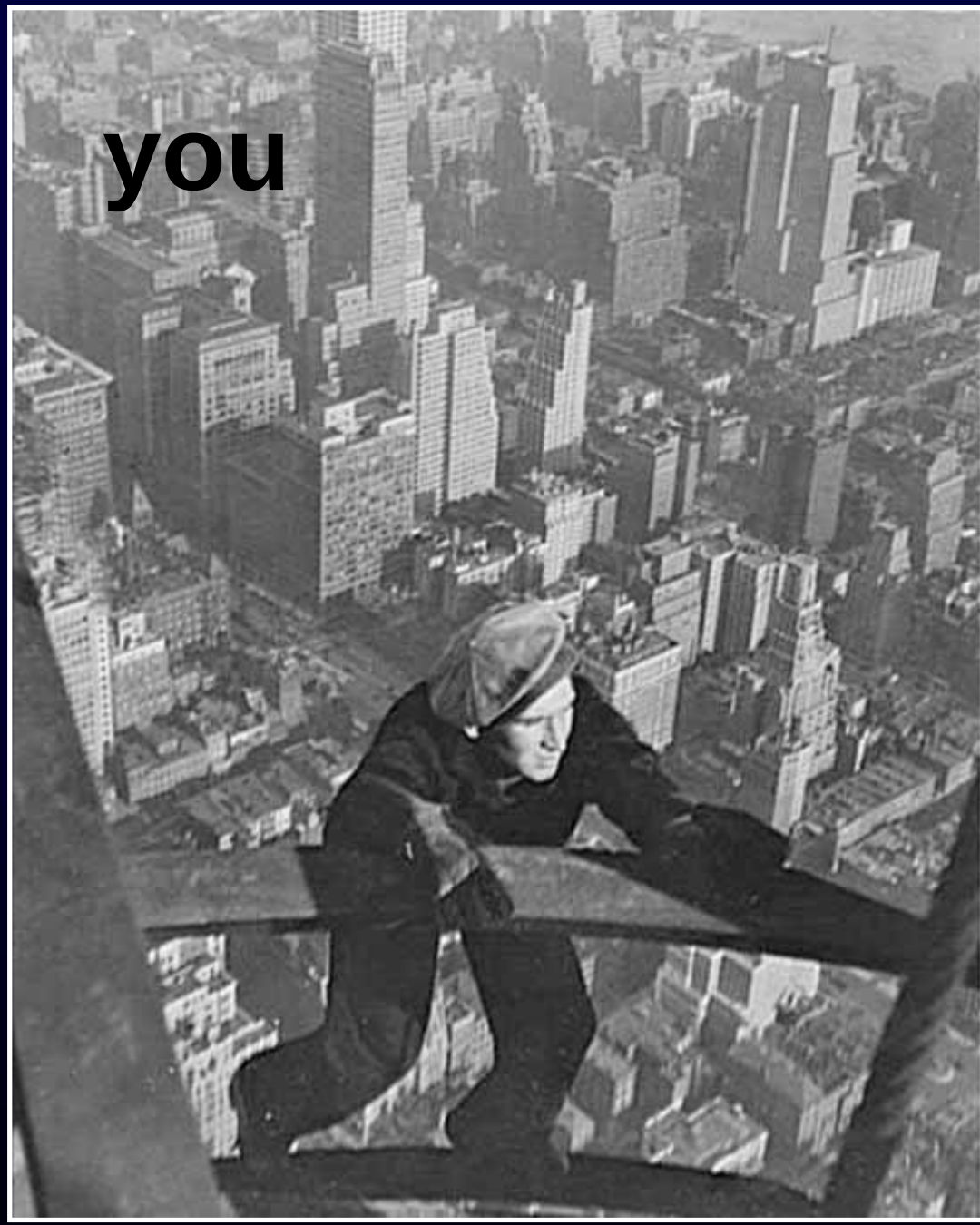
- Magnetofluoroescent NP's for Precontrast MR and Intraoperative Fluorescent Images

- MRSw Assays
  – Intraoperative measurement of biomarkers (cells or proteins), e.g. tissue aspirates
  – Biomarkers in stool homogenates

- Implantable MRSw Sensors
  - Irreversible NP aggregation: Cumulative biomarker measurement over time…
  - In situ measurements of intratumoral drug, pH, biomarker measurements (clinical research)

Acknowledgements

- Manny Perez: MRSw assays
- Xavier Montet: Targeted NP's as MR Contrast Agents
- Eric Sun: Continuous Glucose Sensor
- Jan Grimm: MRSw Telomerase Activity
- Sonia Taktak: Multiparameter MRSw's
- Grace Kim, Karen Daniels, Michael Cima: hCG Sensor
- Hakko Lee: Miniature Relaxometer
- Dr. Ralph Weissleder (CMIR Director)



Lewis Hine (1931) Construction of the Empire State Building, Manhattan, NYC