Biology Olympiad 2010 at Purdue University
On June 14, 2010

Nanomedicine – How Can Something so Small be so Huge for the Future of Healthcare?

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What is one of the biggest obstacles to progress in healthcare?

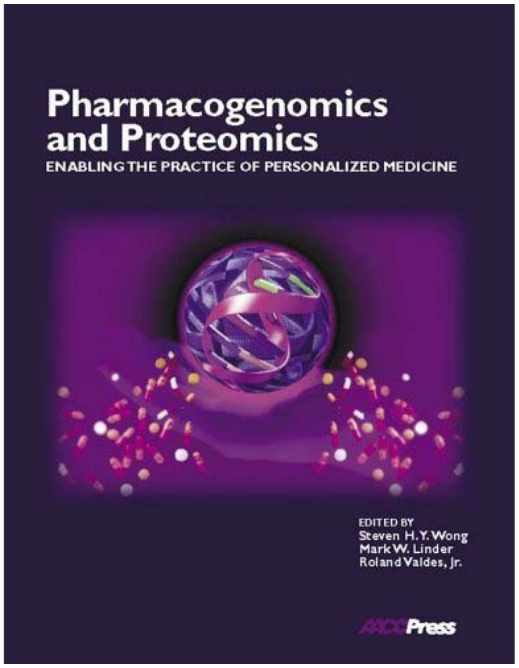
There is a need for targeted delivery of the right drugs to the right person to the right cells at the right dose.

We have great drugs that we cannot use because we do not know who should NOT get a particular drug and we cannot deliver that drug specifically to the diseased cells.

The Progression of Medicine

Conventional "Modern" Medicine

"Personalized" or "Molecular" Medicine



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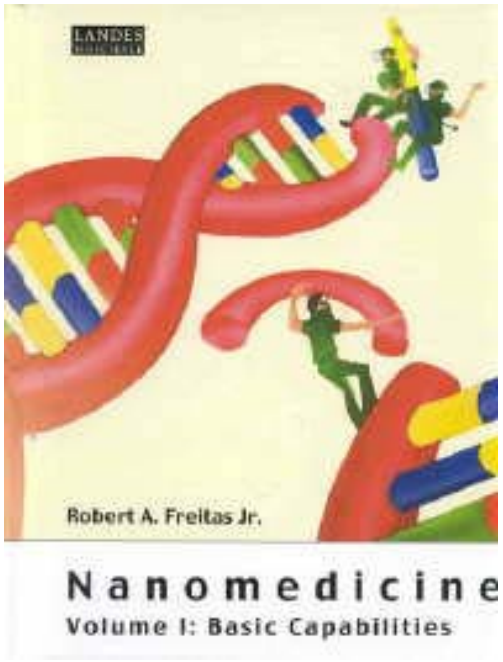
Nanomedicine Single-cell Medicine



“Personalized” Medicine

“Personalized Medicine” is based on the genetic characteristics of the patient, for example on:

- Specific gene rearrangements or mutations that have prognostic information that can affect choice of therapies

- Specific SNPs (“snips” - Single Nucleotide Polymorphisms) – mutation or deletion of a nucleotide at a specific region of a gene that can change that gene’s function (requires sequencing of specific genes of patient)

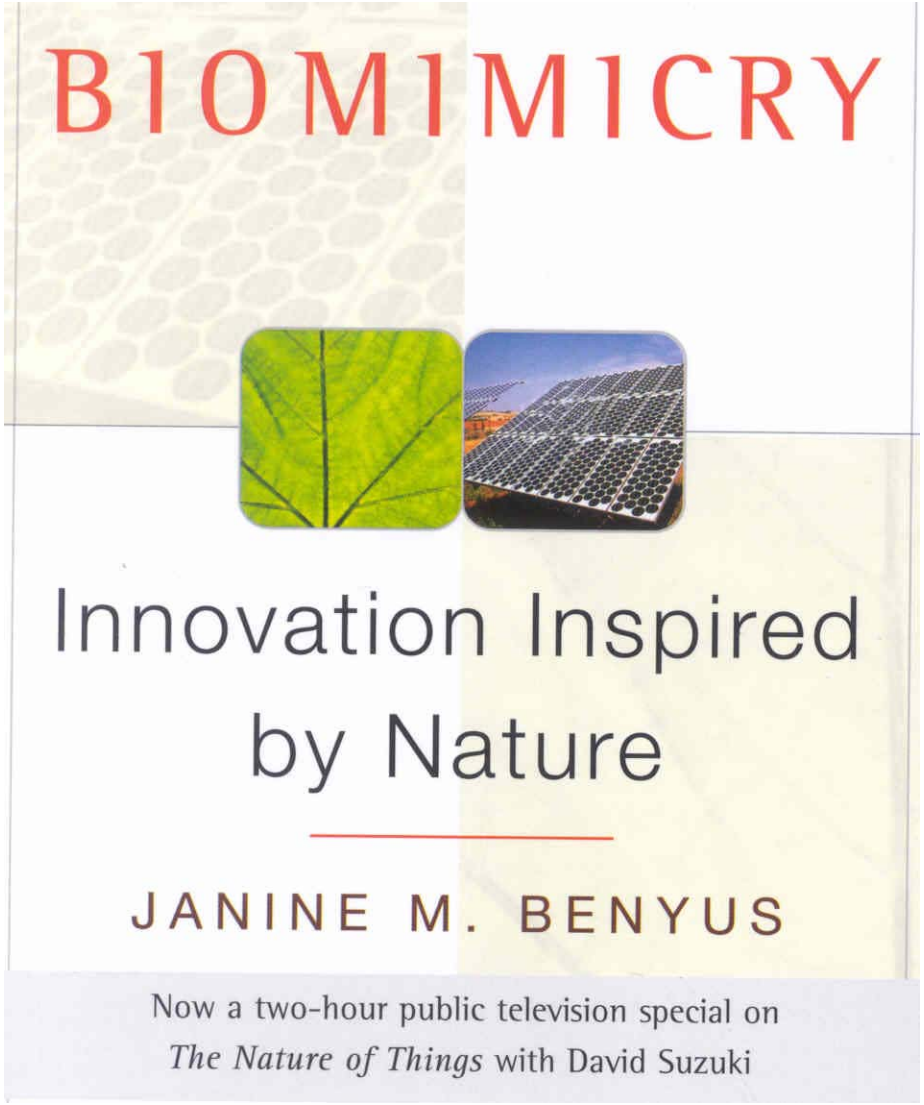
But personalized medicine only decides which patients get which therapies. In most cases it tells us nothing about personalized dose. Even different cells in the same patient need to get different doses.

Features of Nanomedicine

Beyond the obvious application of nanotechnology to medicine, the approach is fundamentally different:

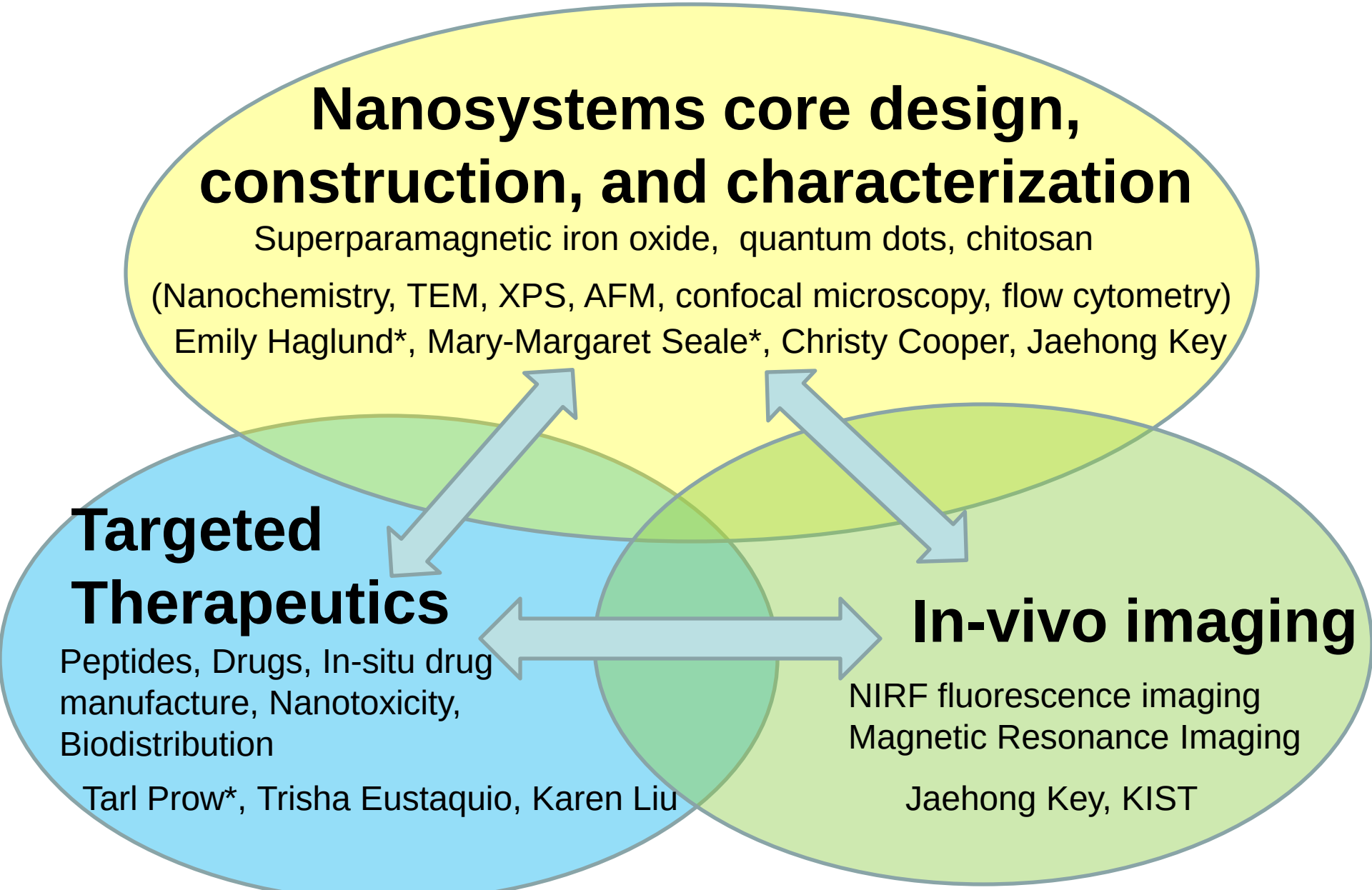
- Nanomedicine is a “nano-” approach NOT just due to the nano size. It is the nanotechnology “bottoms-up” rather than “tops-down” approach to medicine.
- Nanomedicine uses “nano-tools” (e.g. smart nanoparticles) that are roughly 1000 times smaller than a cell (knives to microsurgery to nanosurgery …)
- Nanomedicine is the treatment or repair (regenerative medicine, not just killing of diseased cells) of tissues and organs, WITHIN individually targeted cells, cell-by-cell.
- Nanomedicine typically combines use of molecular biosensors to provide for feedback control of treatment and repair. Drug use is targeted and adjusted appropriately for individual cell treatment at the proper dose for each cell (single cell medicine).

Biomimicry – Can Nature Provide Some of the Answers?



Viruses know how to perform a multi-step targeted process to infect cells, use the host cell machinery to produce gene products, and make copies of themselves. What if we could make a synthetic “good virus” that could deliver therapeutic gene templates to specific cells, and use the host cell machinery to produce therapeutic genes to perform regenerative medicine in a cell and cure disease at the single cell level (and NOT make copies of themselves!) ?

Design of Nanomedical Theragnostic Systems

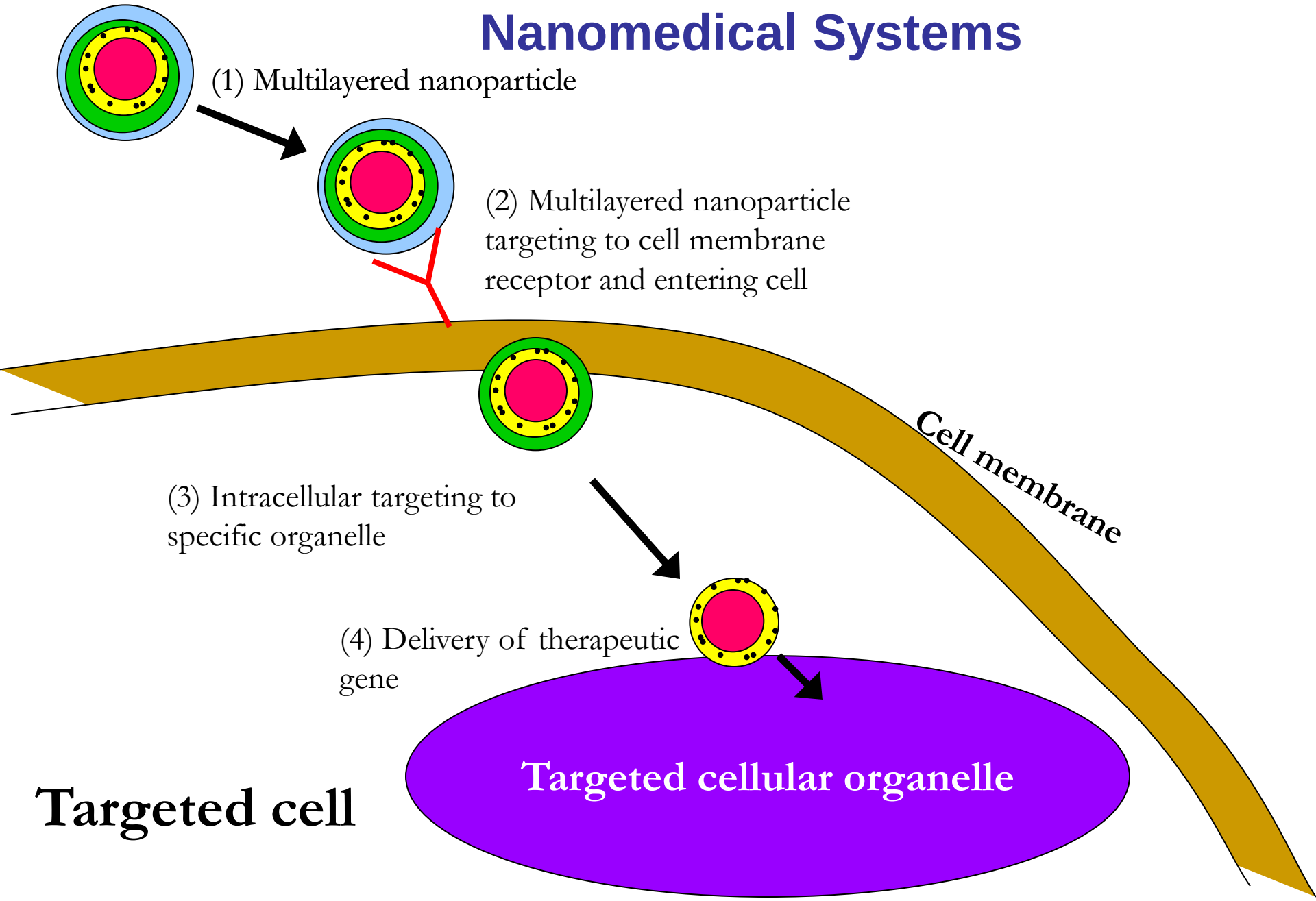


Types of Nanomedical Systems - Autonomous or Non-autonomous?

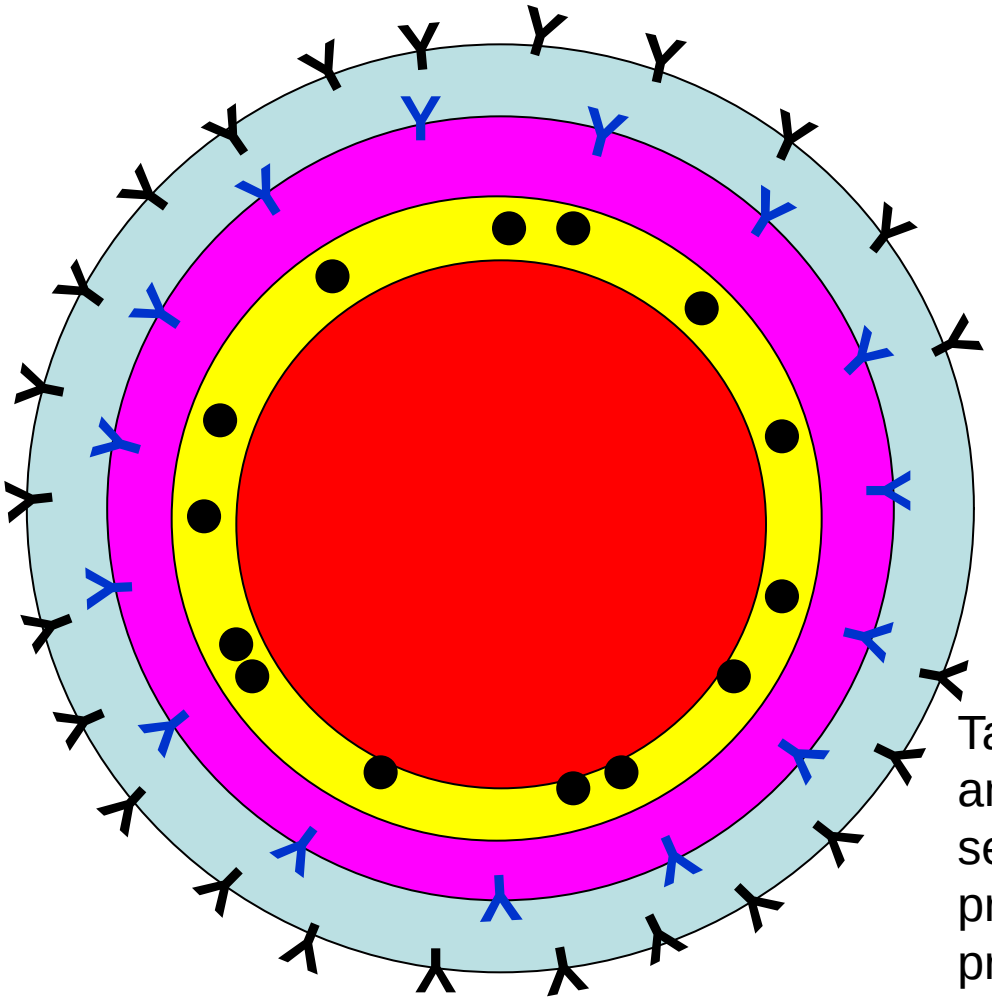
Both are self-guiding, adaptive, multi-component systems on the nanoscale for diagnostic and therapeutic prevention or treatment of disease. These “smart” nanomedical systems can deal with changing conditions, are error-correcting, and can provide proper dose of therapeutic response on a cell-by-cell basis.

Non-autonomous systems additionally require modulation from outside the body, e.g. heating from light or ultrasound; guiding or modulation by external electromagnetic fields; etc.

The Multi-Step Targeting Process in Nanomedical Systems



Designing “Programmable” Multifunctional Nanomedical Systems with Feedback Control of Gene/Drug Delivery within Single Cells



Cell targeting and entry

Intracellular targeting

Therapeutic genes

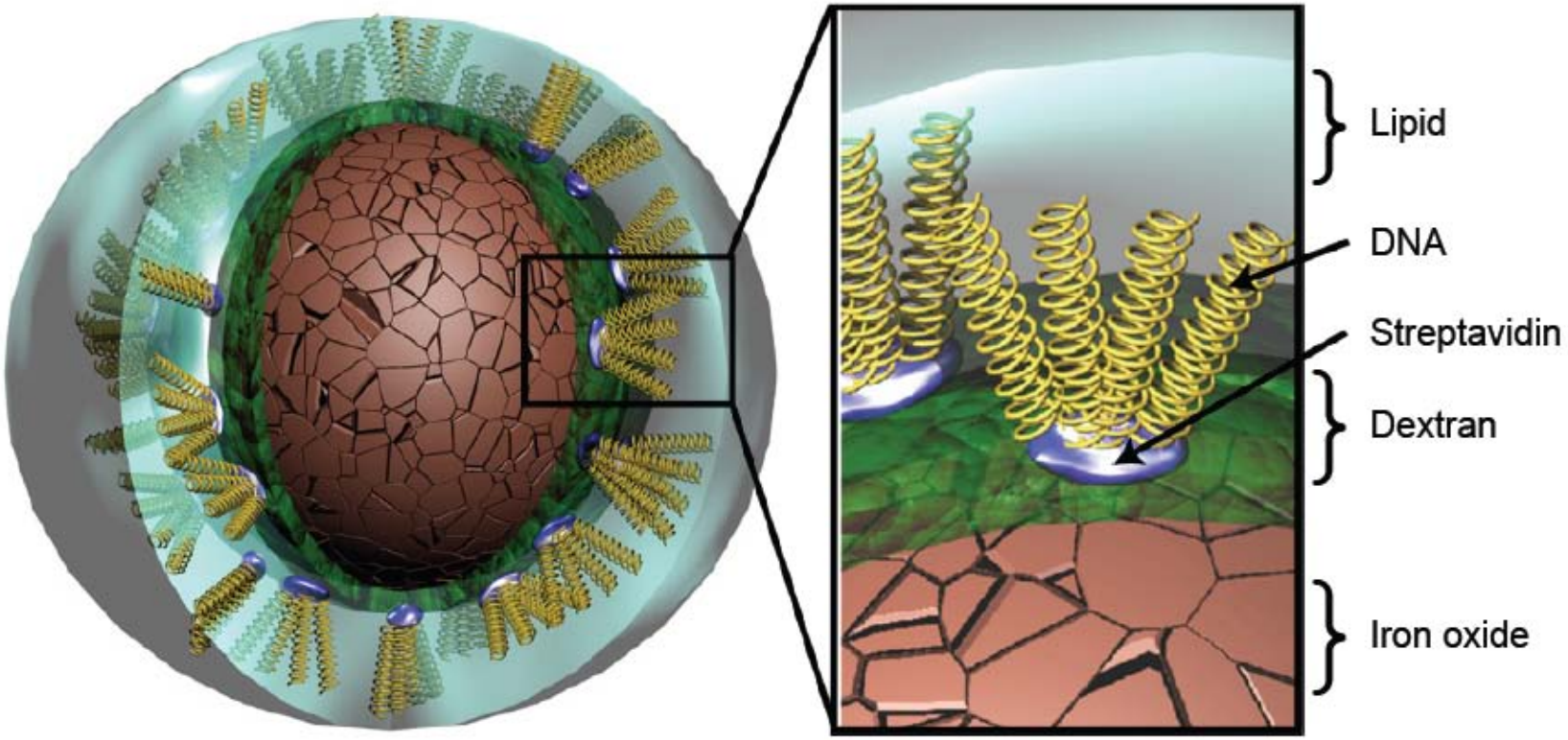
Magnetic or Qdot core (for MRI or optical imaging)

Biomolecular sensors (for error-checking and/or gene switch)

Targeting molecules (e.g. an antibody, an DNA, RNA or peptide sequence, a ligand, an aptamer) in proper combinations for more precise nanoparticle delivery

Leary and Prow, PCT (USA and Europe) Patent pending 2005

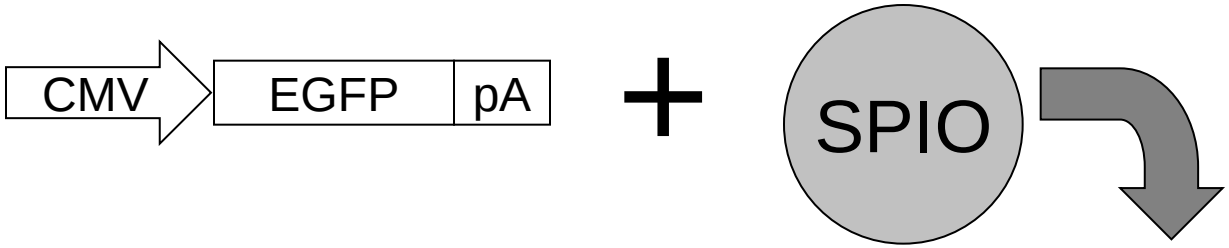
Example of multilayered magnetic nanoparticle for in-vivo use



Prow, T.W., Grebe, R., Merges, C., Smith, J.N., McLeod, D.S., Leary, J.F., Gerard A. Lutty, G.A. "Novel therapeutic gene regulation by genetic biosensor tethered to magnetic nanoparticles for the detection and treatment of retinopathy of prematurity" Molecular Vision 12: 616-625, 2006

Efficient Gene Transfer with DNA Tethered Magnetic Nanoparticles

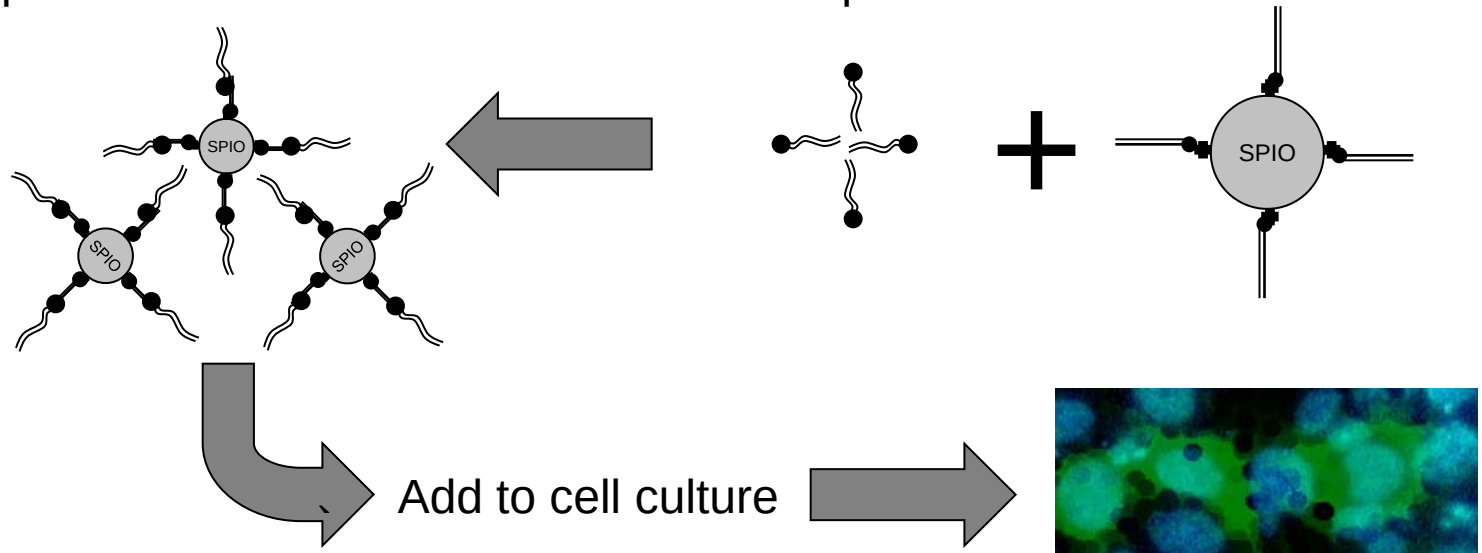
PCR product bioconjugated to magnetic nanoparticle



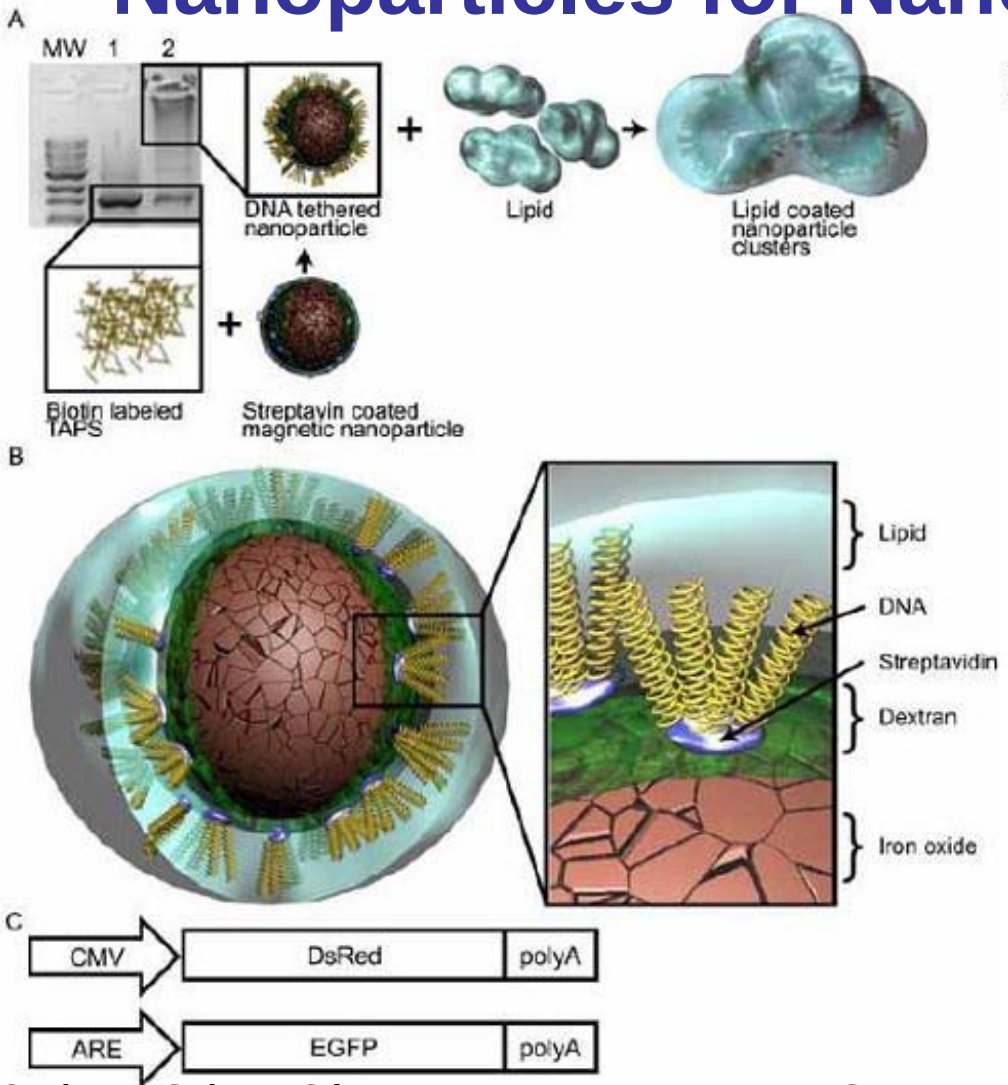
Lipid coated magnetic nanoparticles tethered with DNA

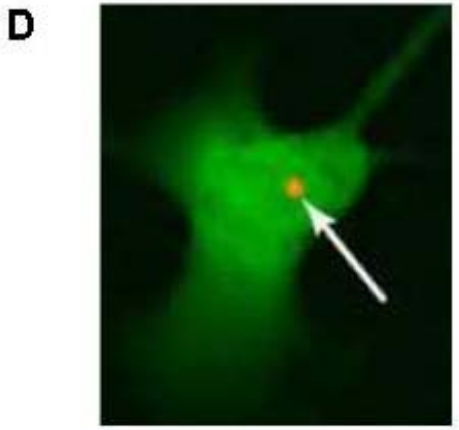
Lipid

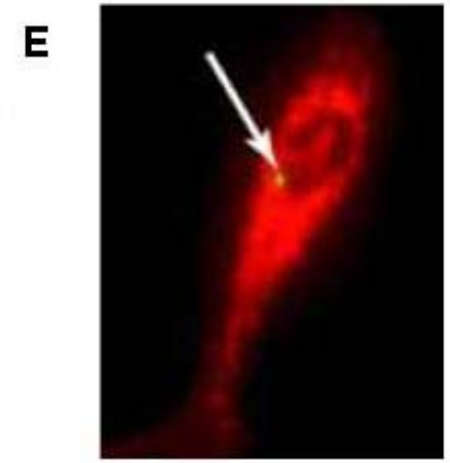
Magnetic nanoparticle tethered with DNA



Tethered Gene Expression on Magnetic Nanoparticles for Nanomedicine





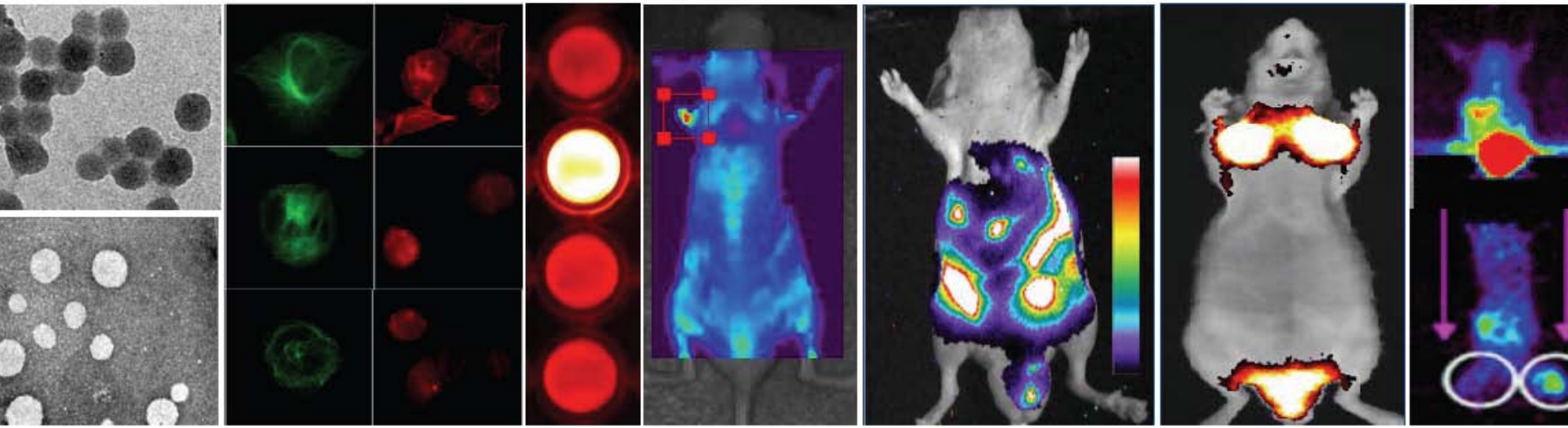


1. Prow, T.W., Smith, J.N., Grebe, R., Salazar, J.H., Wang, N., Kotov, N., Lutty, G., Leary, J.F. "Construction, Gene Delivery, and Expression of DNA Tethered Nanoparticles" Molecular Vision 12: 606-615, 2006a.

2. Prow, T.W., Grebe, R., Merges, C., Smith, J.N., McLeod, D.S., Leary, J.F., Gerard A. Lutty, G.A. "Novel therapeutic gene regulation by genetic biosensor tethered to magnetic nanoparticles for the detection and treatment of retinopathy of prematurity" Molecular Vision 12: 616-625, 2006b.

Global Research Lab Program

Molecular Imaging and Nanomedicine for Theragnosis using Nano-Biomaterials



http://www.nanohub.org/resource_files/2007/10/03388/2007.09.14-choi-kist.pdf

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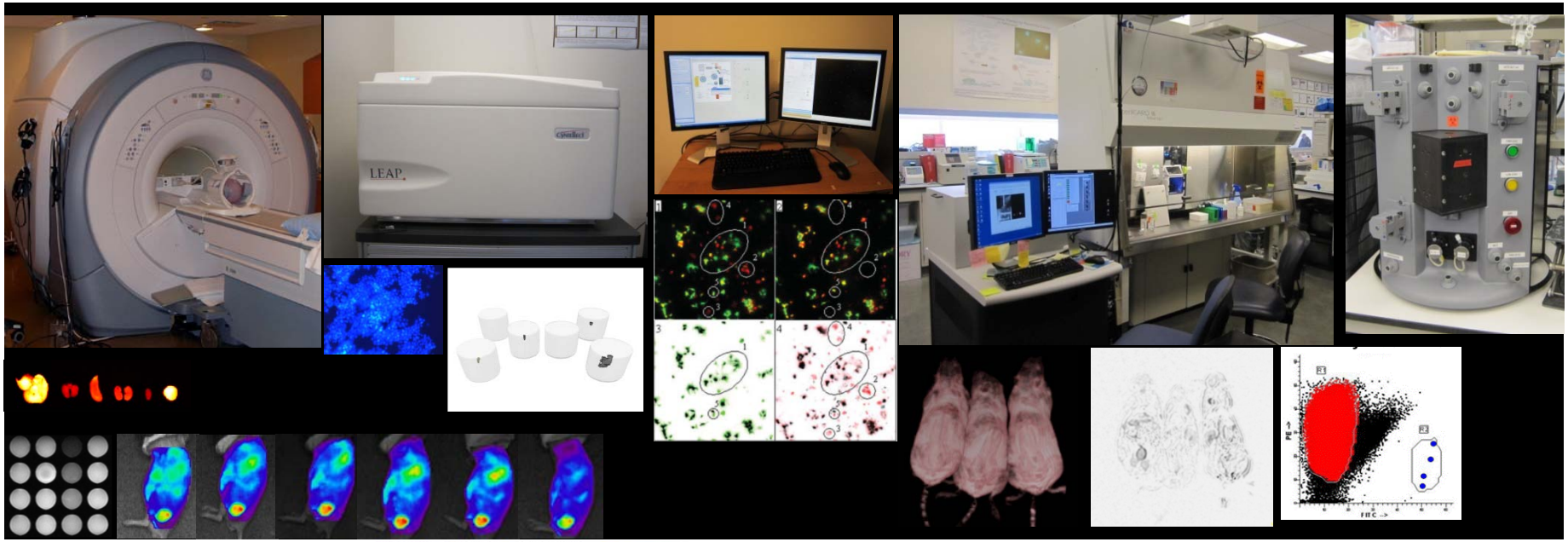
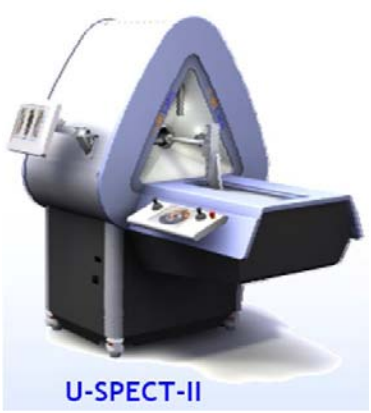
FPI: James F. Leary (Purdue Univ.)

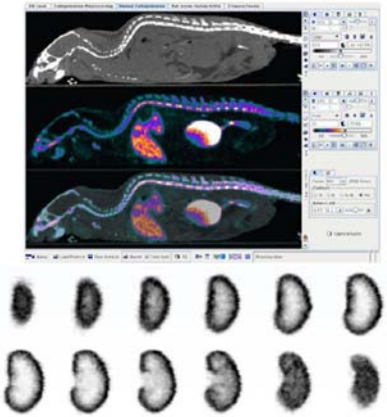
Korea Institute of Science and Technology

PURDUE

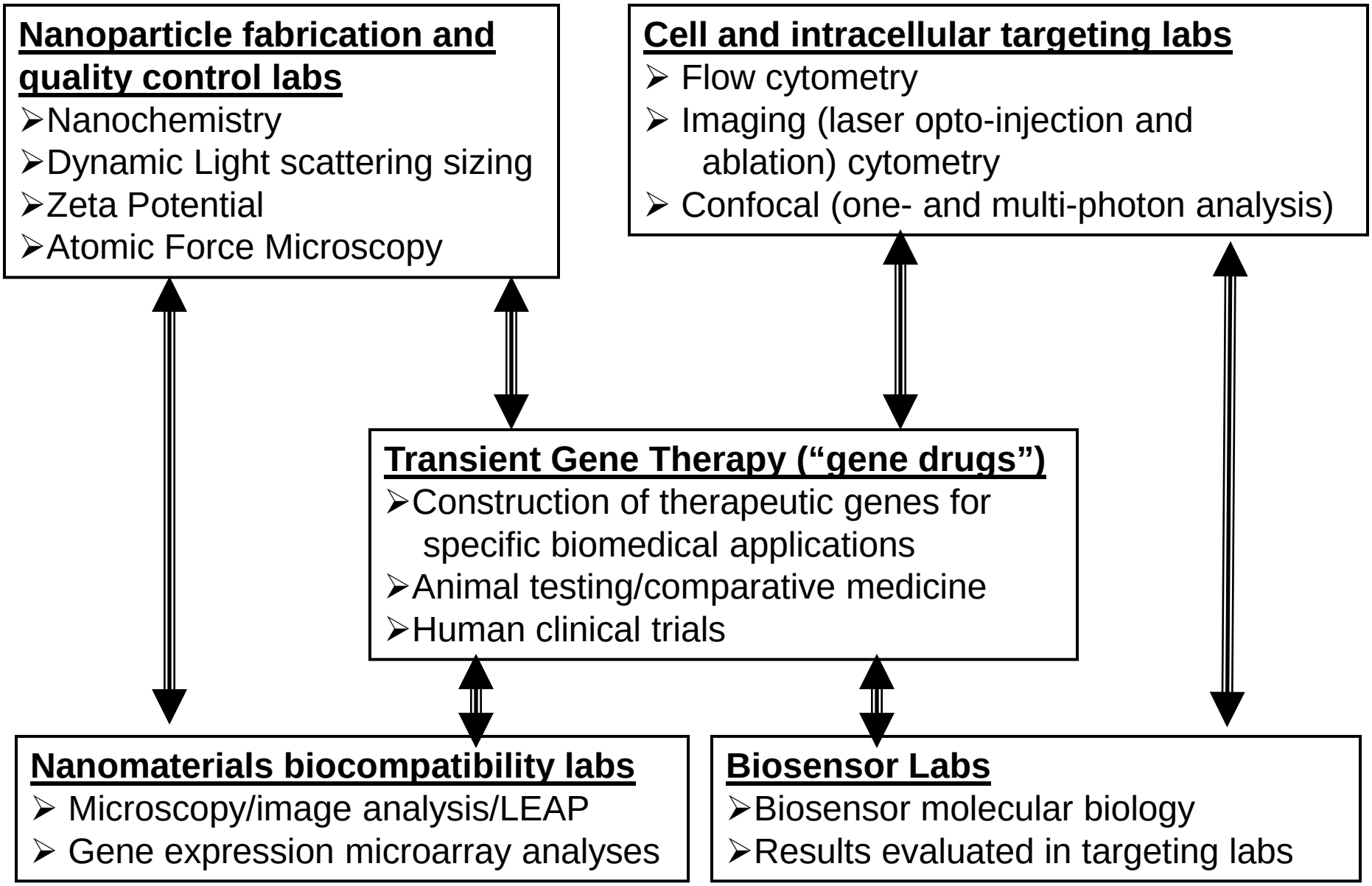
Purdue University's multidisciplinary core facilities being used by the Purdue Global Research Lab (GRL)







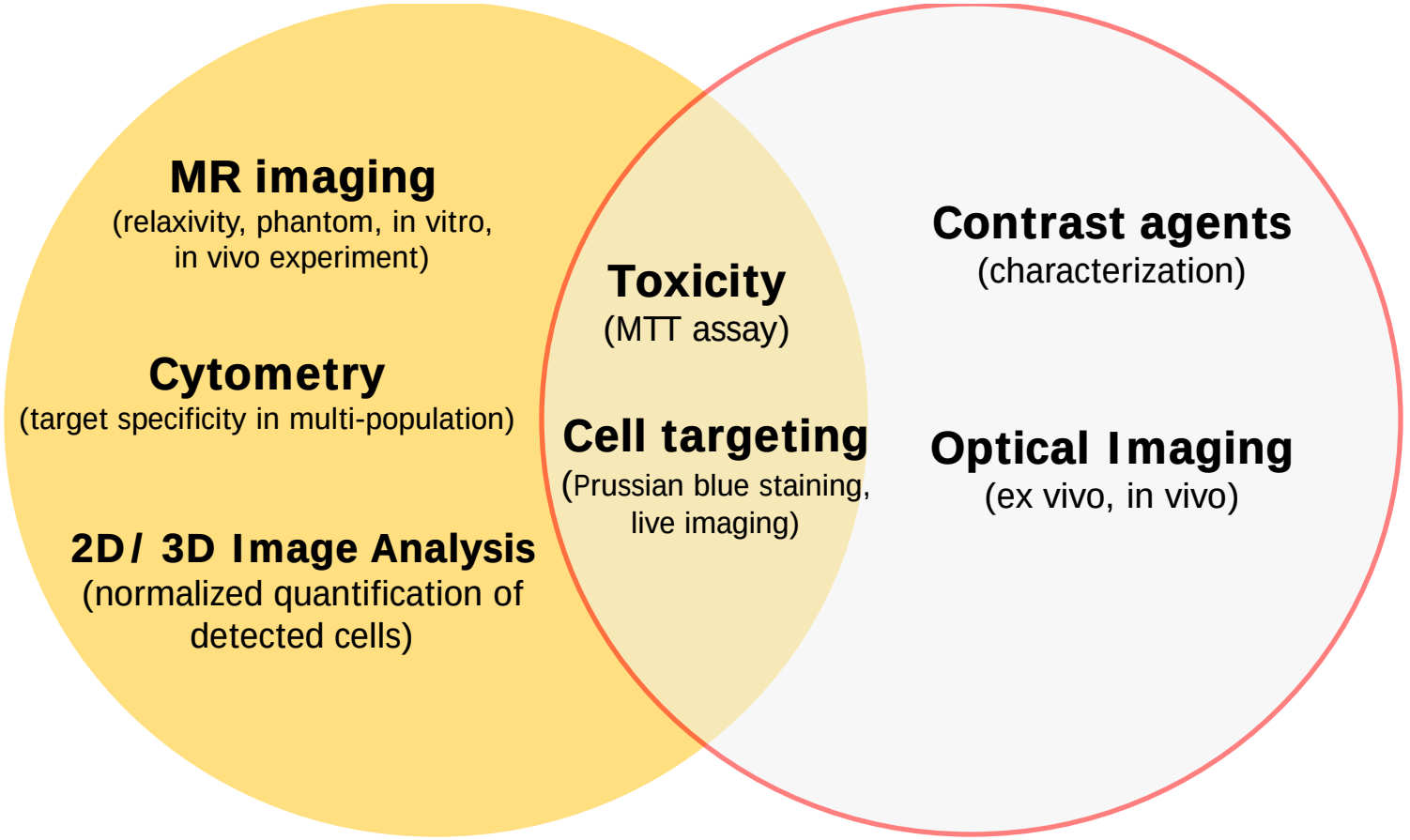
Interactions Between Technologies for Development of Nanomedical Systems



Evaluation of MR/NIRF imaging agents

PURDUE UNIVERSITY

KIST Korea Institute of Science and Technology 한국과학기술연구원



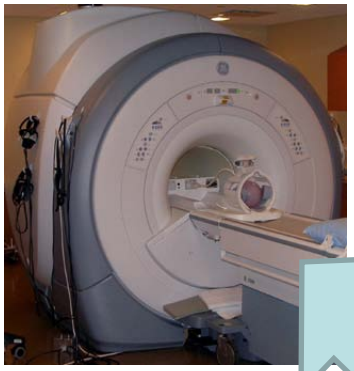
Imaging Systems

Imaging Technique	Spatial Resolution	Sensitivity[b]	Source of Imaging	Target	Tissue Penetrating Depth
MRI	> 7T, 25-300 µm Human 3T, 1mm	mM to µM (low)	Radiowave	Anatomical, physiological, molecular	No limit
CT	50-200 µm	not well characterized	X-ray	Anatomical, physiological	No limit
PET	1-2 mm	pM (high)	γ-ray	Physiological, molecular	No limit
Optical fluorescence Imaging	In vivo, 2-3 mm In vitro, sub-µm	nM to pM (medium)	Visible or near-infrared light	Physiological, molecular	< 1cm

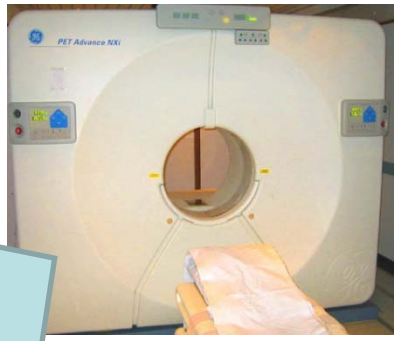
a. For high-resolution, small-animal imaging systems (Clinical imaging systems are different)
b. Sensitivity of detecting probe relative to background

Cheon J. et al., Acc Chem Res, 2008 Weissleder, Nature, 2008

Molecular Imaging Modalities



Magnetic Resonance Imaging



Positron Emission Tomography

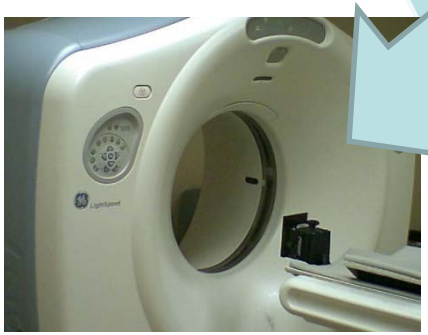
Resolution

Penetration Depth

Sensitivity

Information

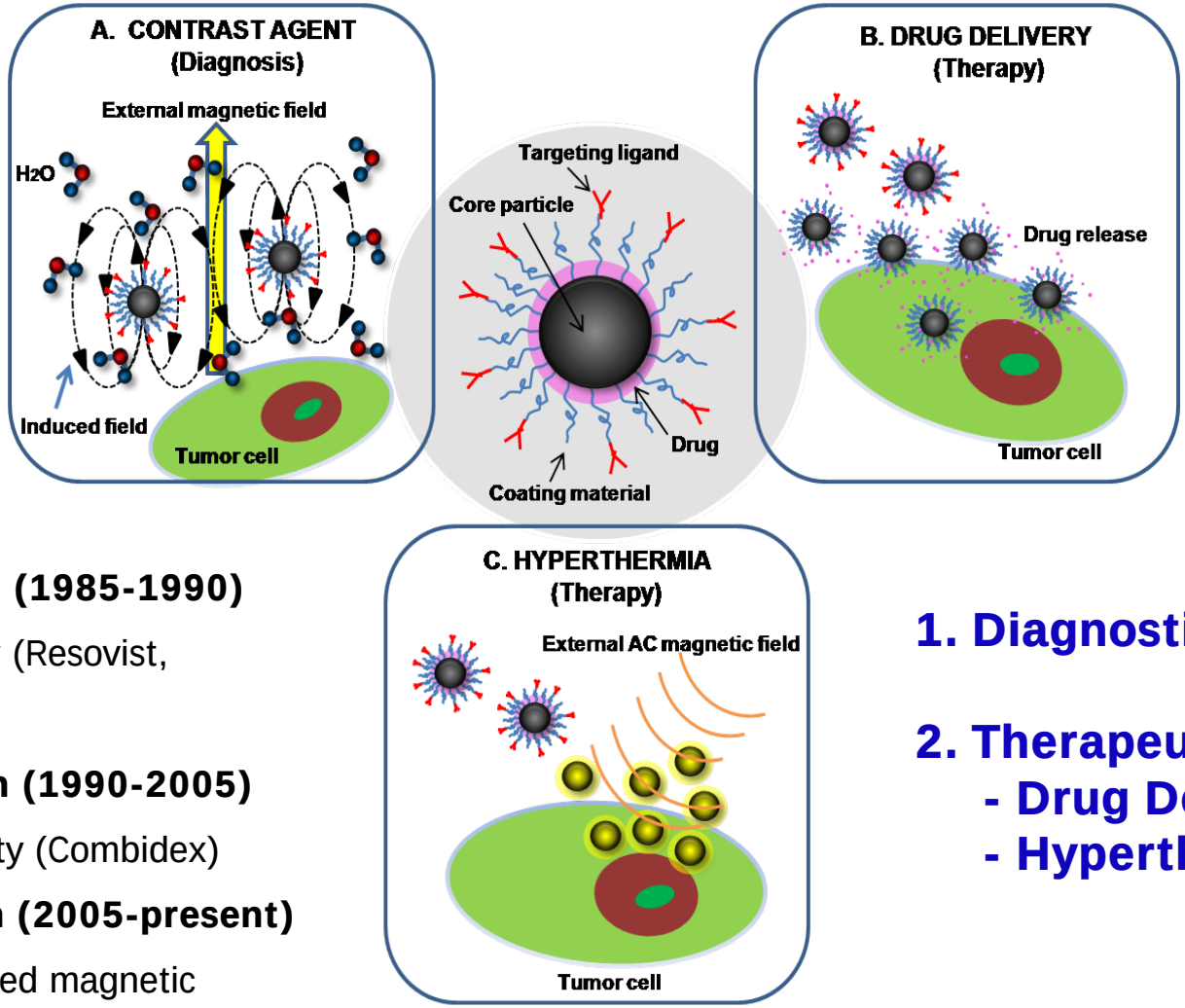
Clinical Use



Computed Tomography

Optical Imaging

Superparamagnetic Iron Oxide (T_2 agents)



1st generation (1985-1990)

: Poly-dispersity (Resovist, Ferridex)

2nd generation (1990-2005)

: Mono-dispersity (Combidex)

3rd generation (2005-present)

: Specific targeted magnetic nanoparticles, Sensors

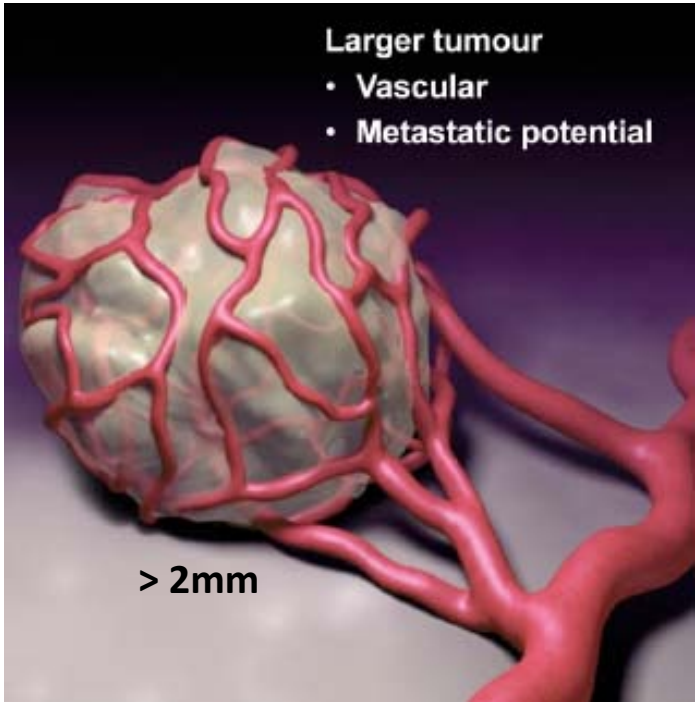
1. Diagnostic Agents

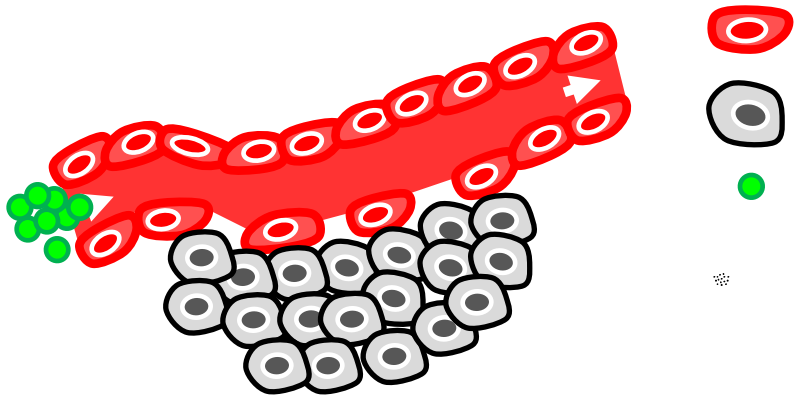
2. Therapeutic Agents
- **Drug Delivery**
- **Hyperthermia**

EPR Effect
(Enhanced Permeability And Retention)

- Most solid tumors possess unique characteristics

  : extensive angiogenesis, defective vascular architecture, impaired lymphatic drainage/ recovery system, increased permeability mediators

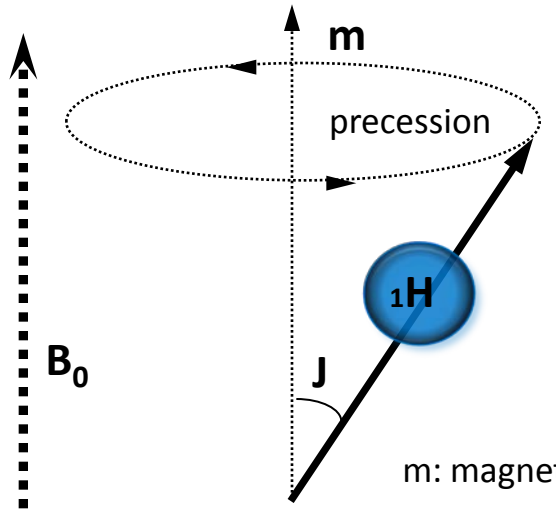




Tumor Targeting using EPR effect

World Cancer Report, 2008 Maeda et al., JCR, 2000

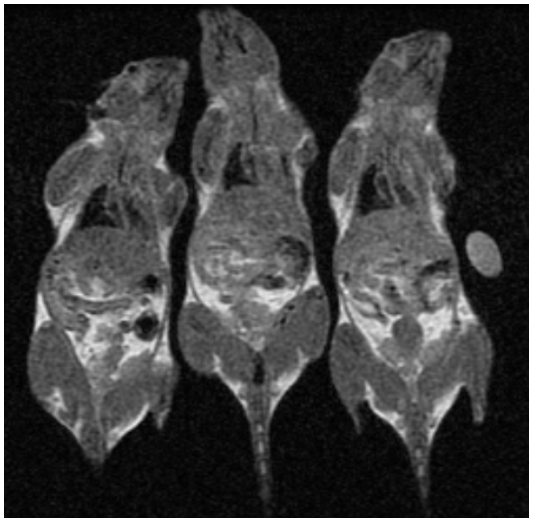
Magnetic Resonance Imaging

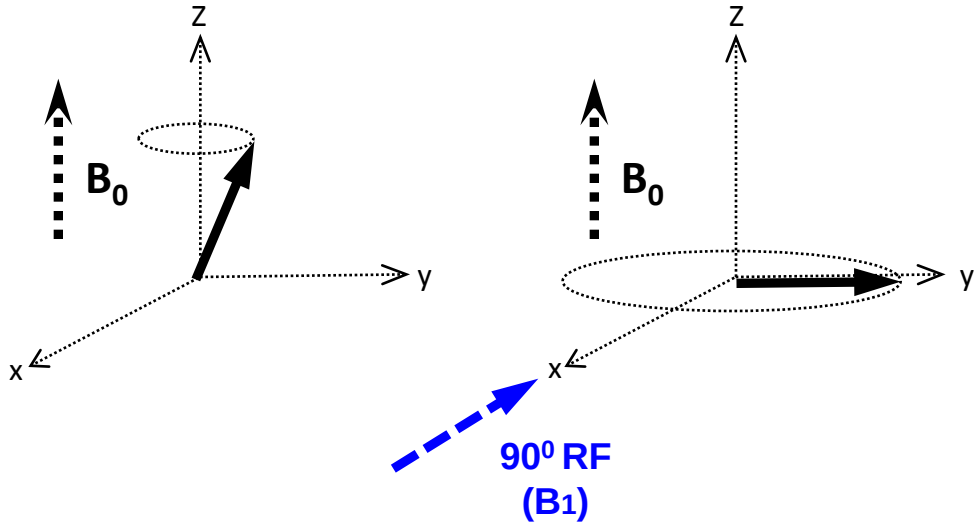


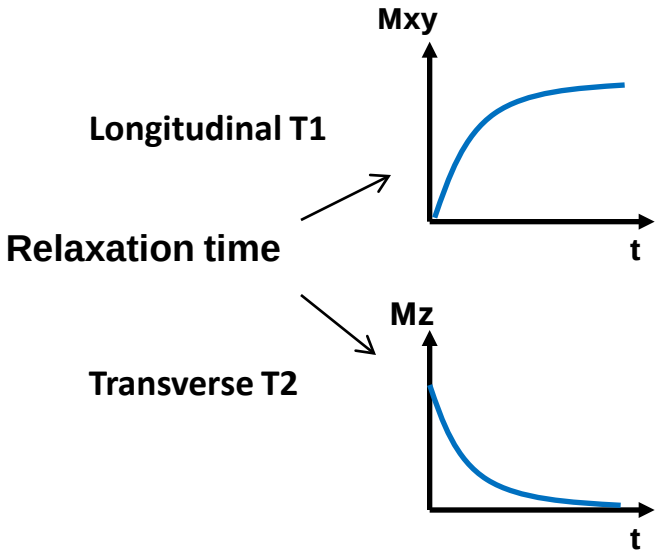
m: magnetic moment

J: spin angular momentum

MR image of mice

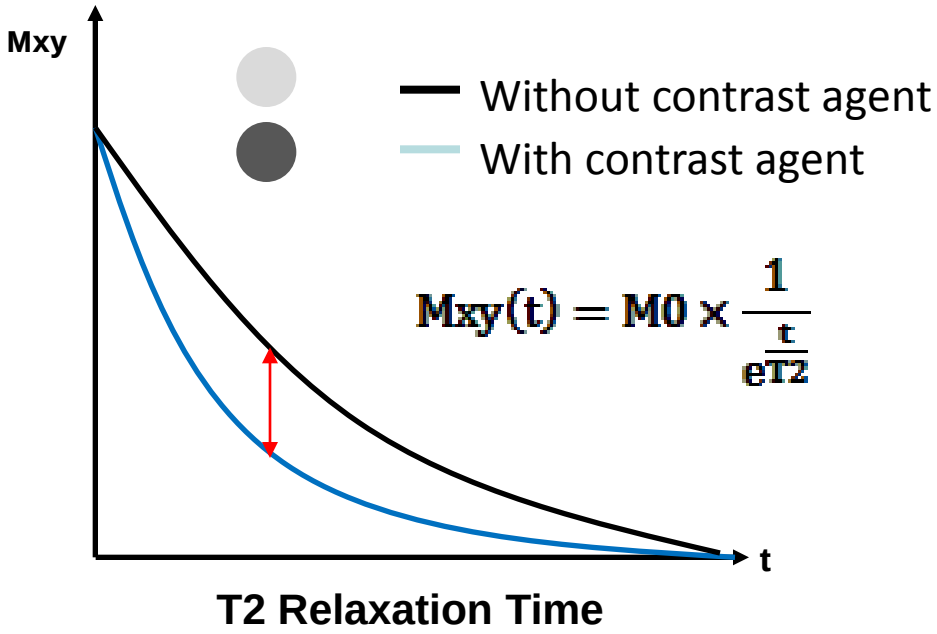






MR Contrast Agents

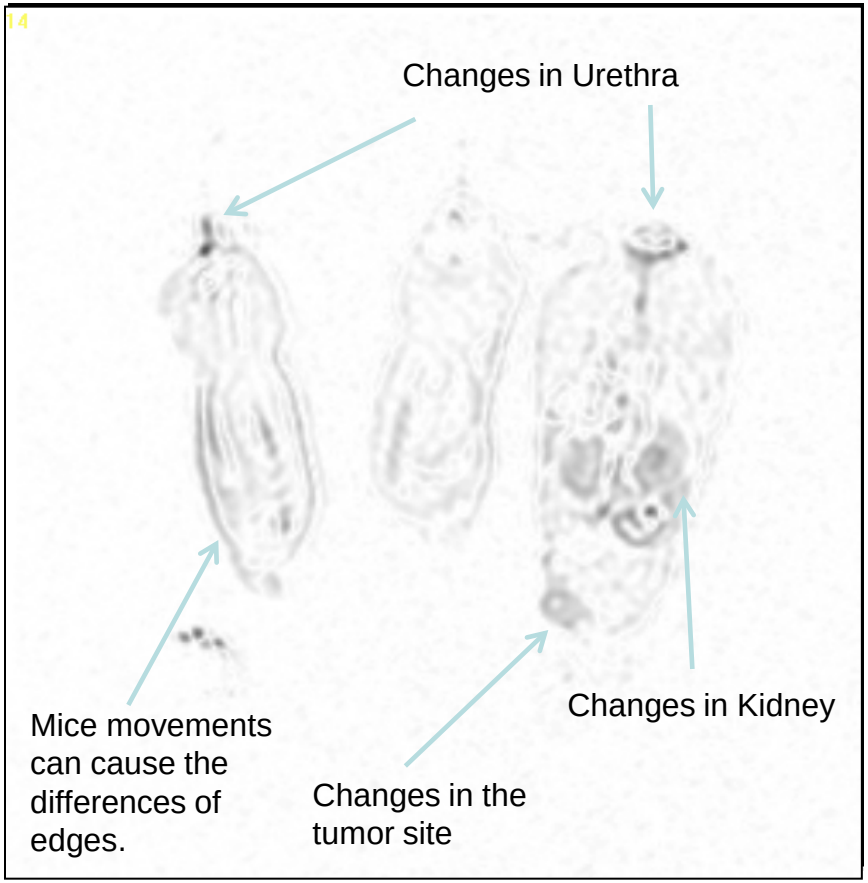
- MR contrast agents **shorten the relaxation times** of tissues and body cavities, which make a higher or lower signal.



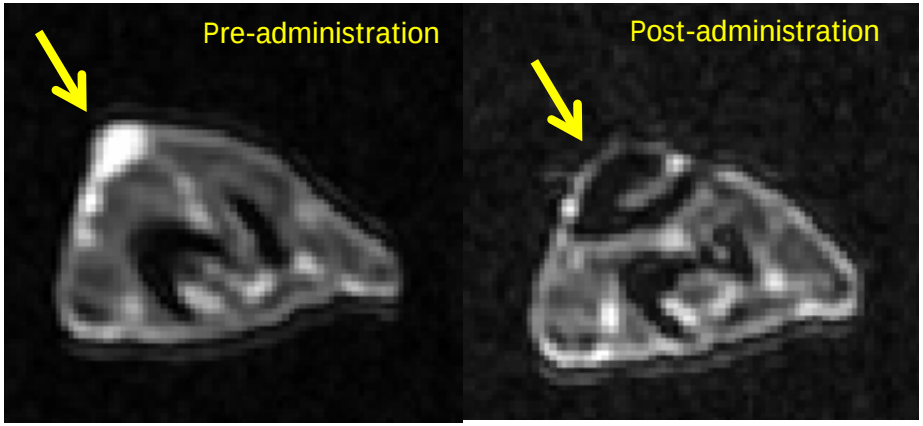
- Superparamagnetic iron oxide (SPIO) NPs as T2 contrast Agents

- A water insoluble iron oxide crystal containing thousands of paramag netic Fe ions - excessive T2 contrast effect

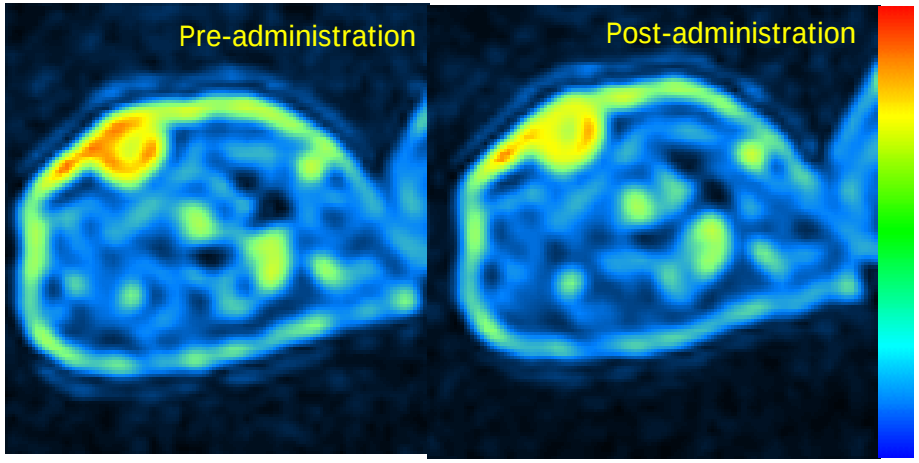
In vivo imaging of human tumors in nude mice



Positive Control

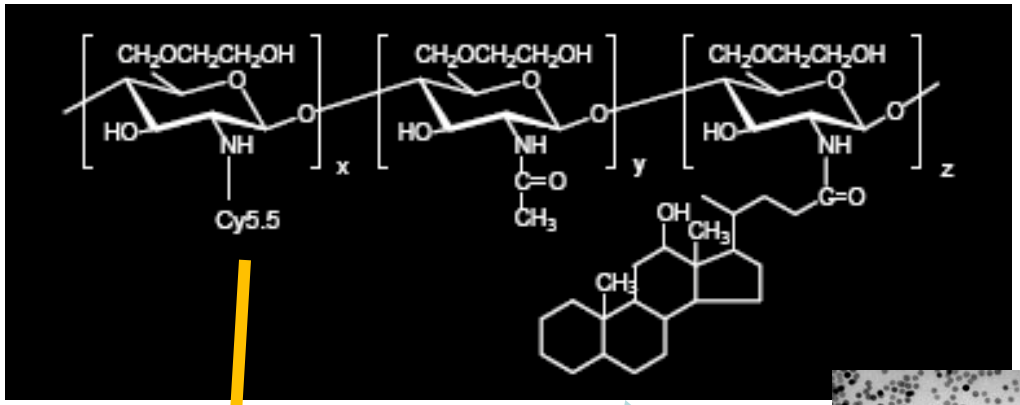


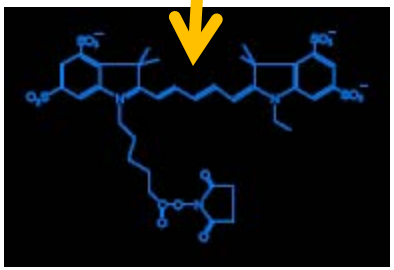
Tail Vein Injection



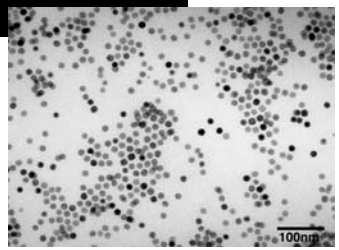
Hydrophobically Modified Glycol Chitosan

Glycol Chitosan



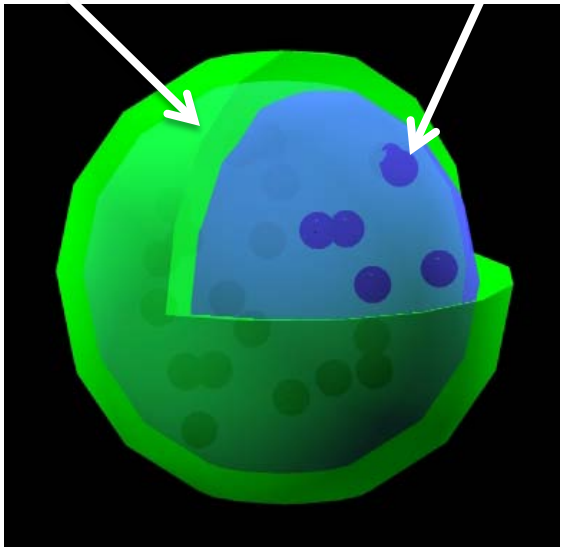


5β-Cholanic Acid
Hydrophobic Moiety



HGC-Cy5.5

SPIO NPs

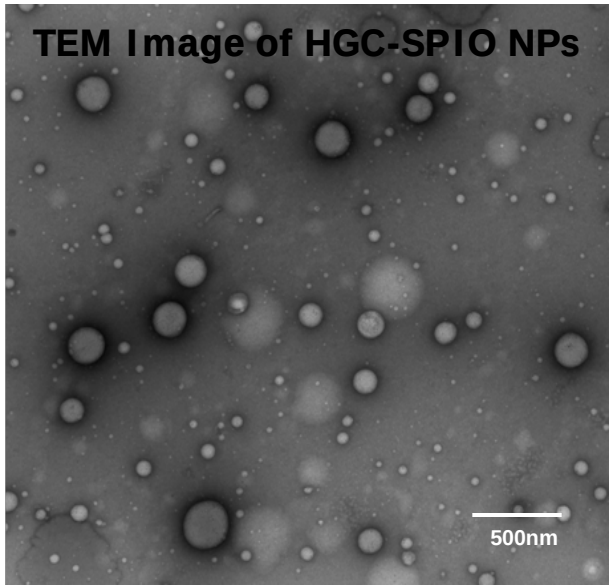


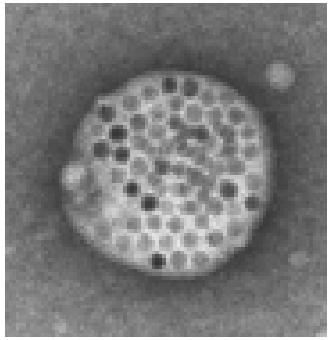
HGC-SPIO NPs

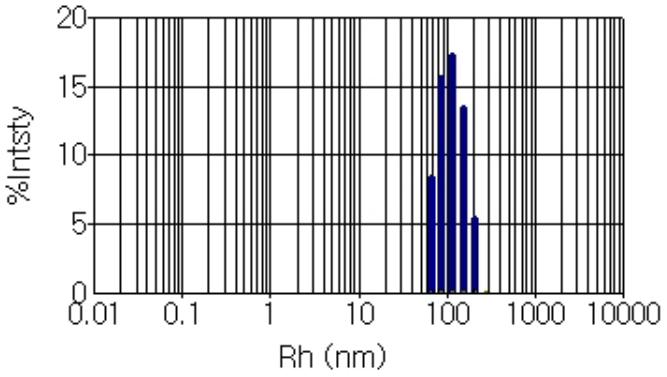
Near Infrared Fluorescence (NIRF) Dye
: visualization in deeper tissues (0.5mm-cm) with a low background signal

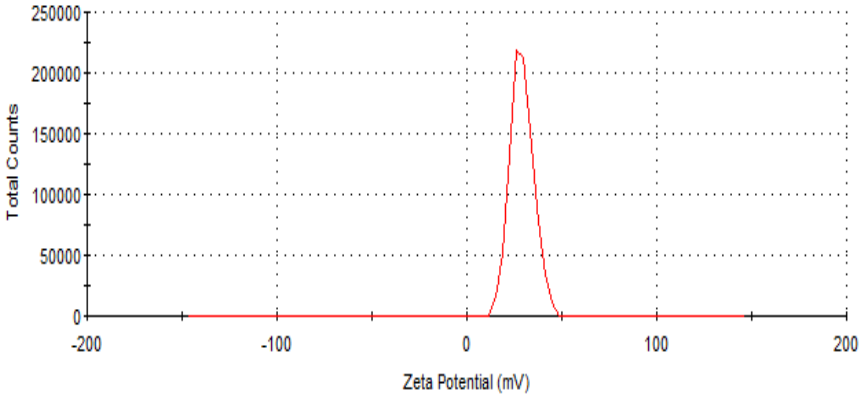
Nam, H.Y. et al., JCR, 2009

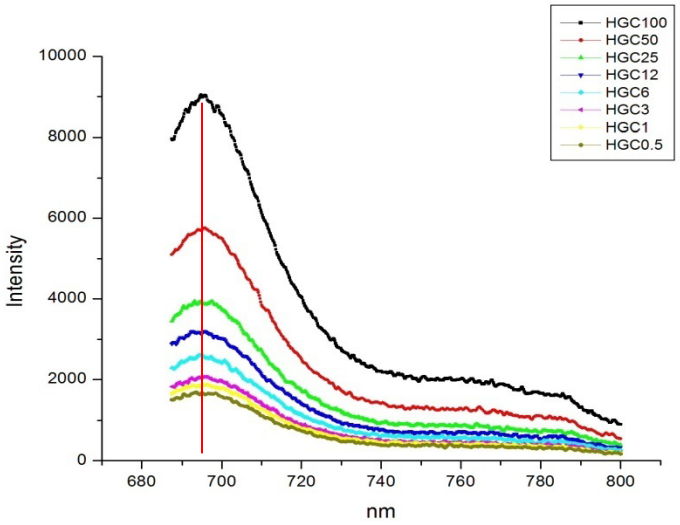
Characterization of HGC-SPIO NPs







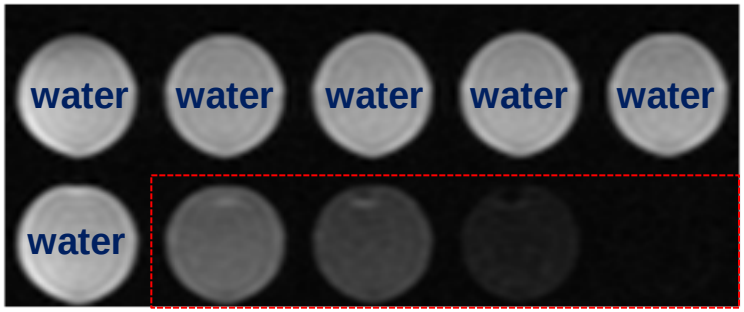




T_2 Contrast by HGC-SPIO NPs

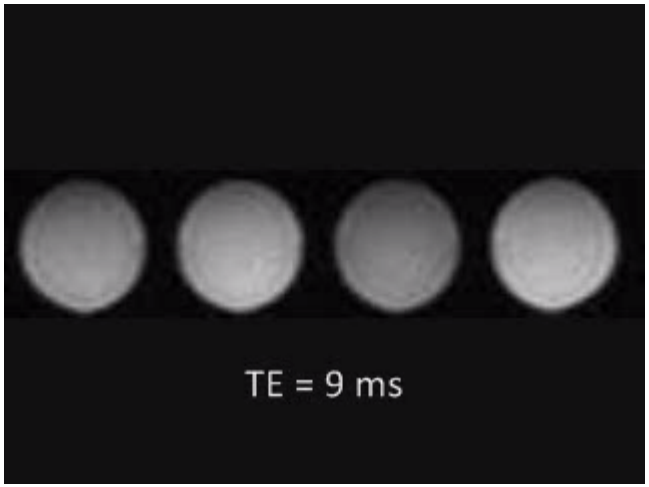
TR (repetition time): the time between successive RF pulses
TE (echo time): the time between the end of RF pulse and collection of MR signal

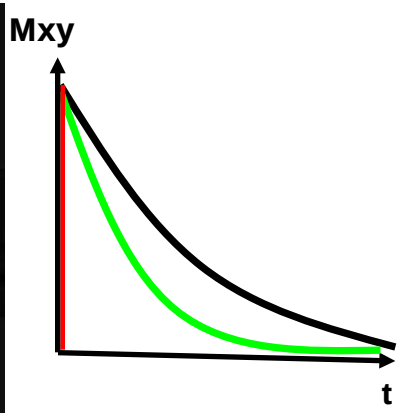
TR = 1500 ms TE = 80 ms

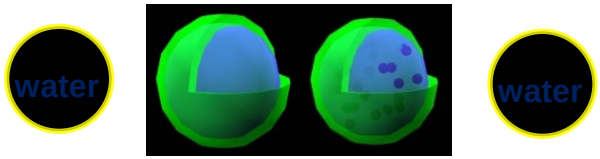


HGC-SPIO
½ dilution

TR = 1500 ms TE = 9~72 ms



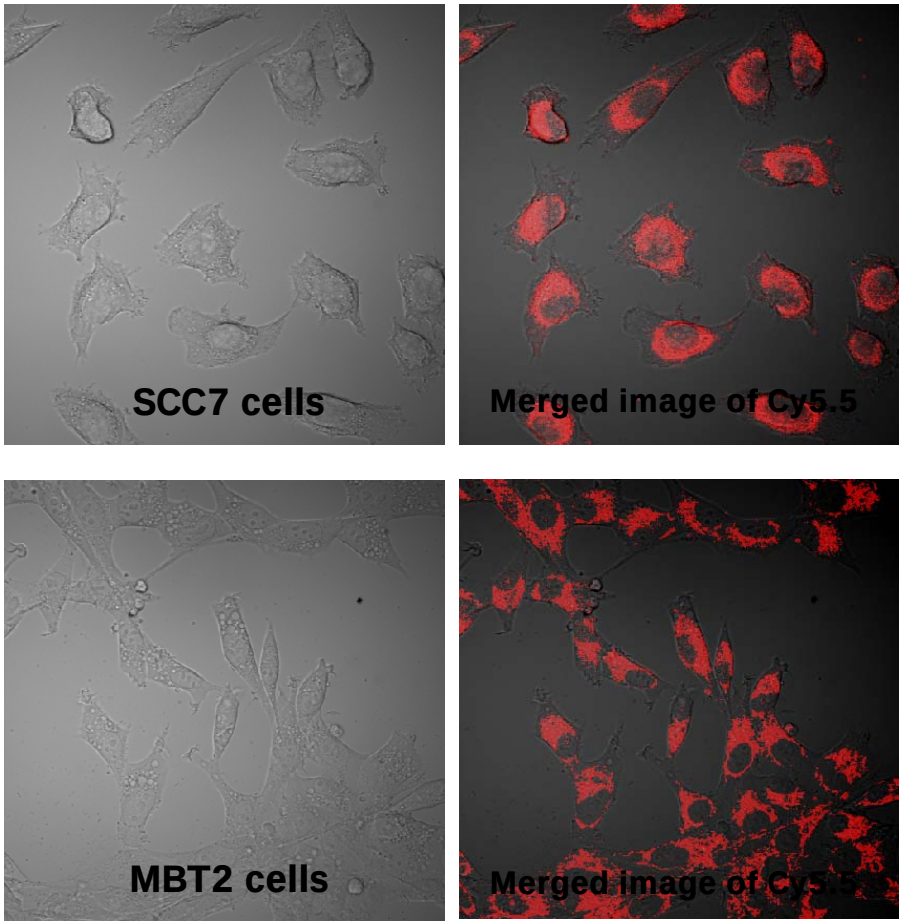




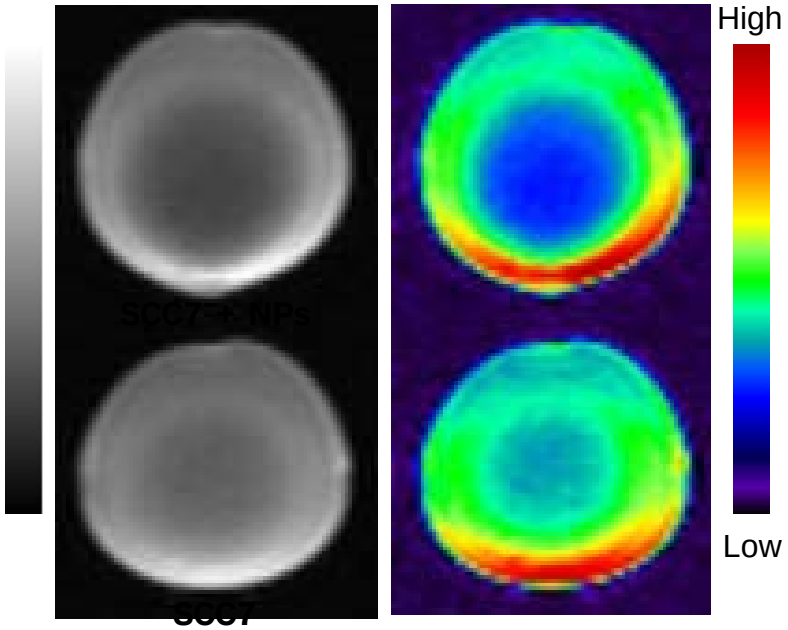
HGC
HGC
SPIO

Intracellular Targeting of SCC7/MBT2 Cells by HGC-SPIO NPs

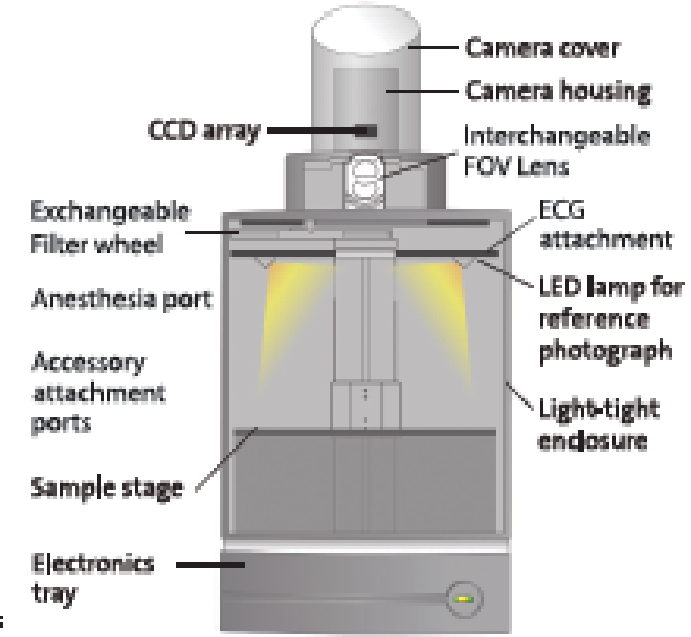
Confocal Images of HGC-SPIO NPs

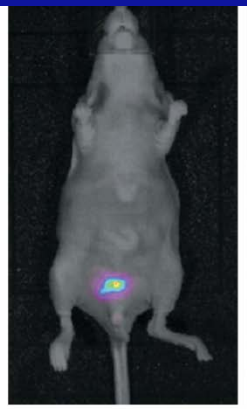


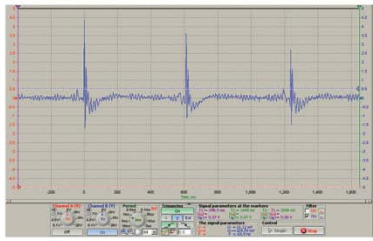
In vitro MR Images of HGC-SPIO NPs

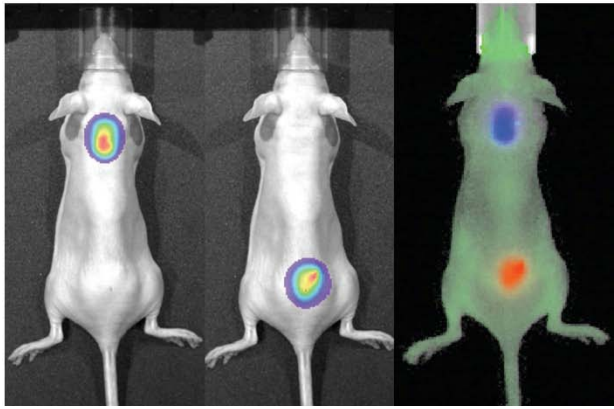


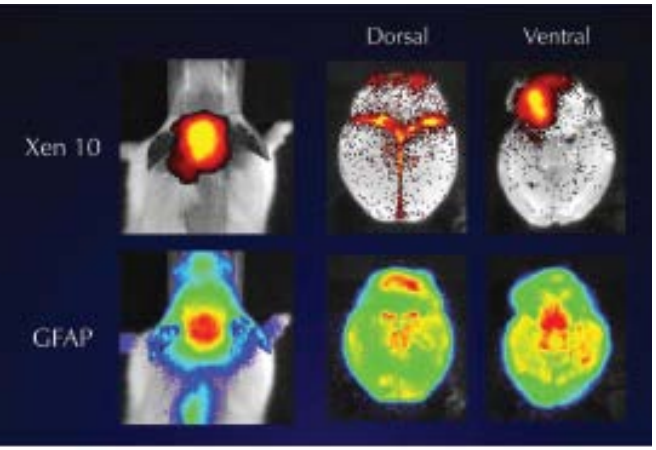
Near Infrared Fluorescence Imaging



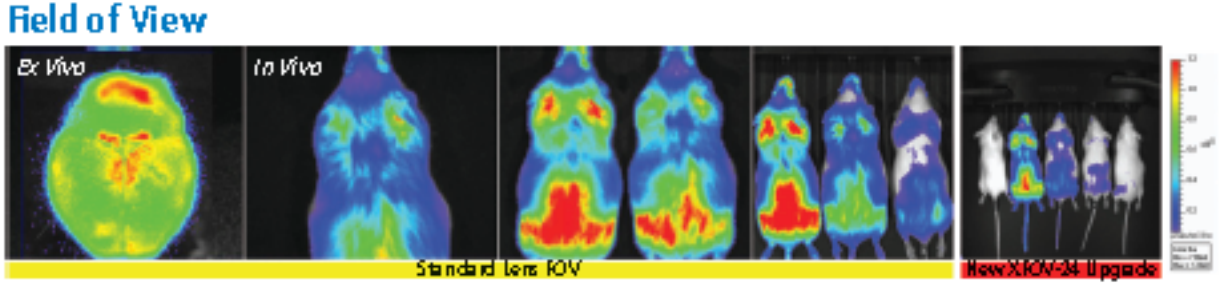








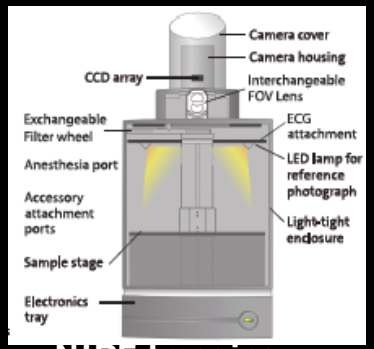
Dual Reporter Imaging - High Resolution Ex Vivo Applications



The IVIS Lumina II Imaging System provides 5 fields of view.

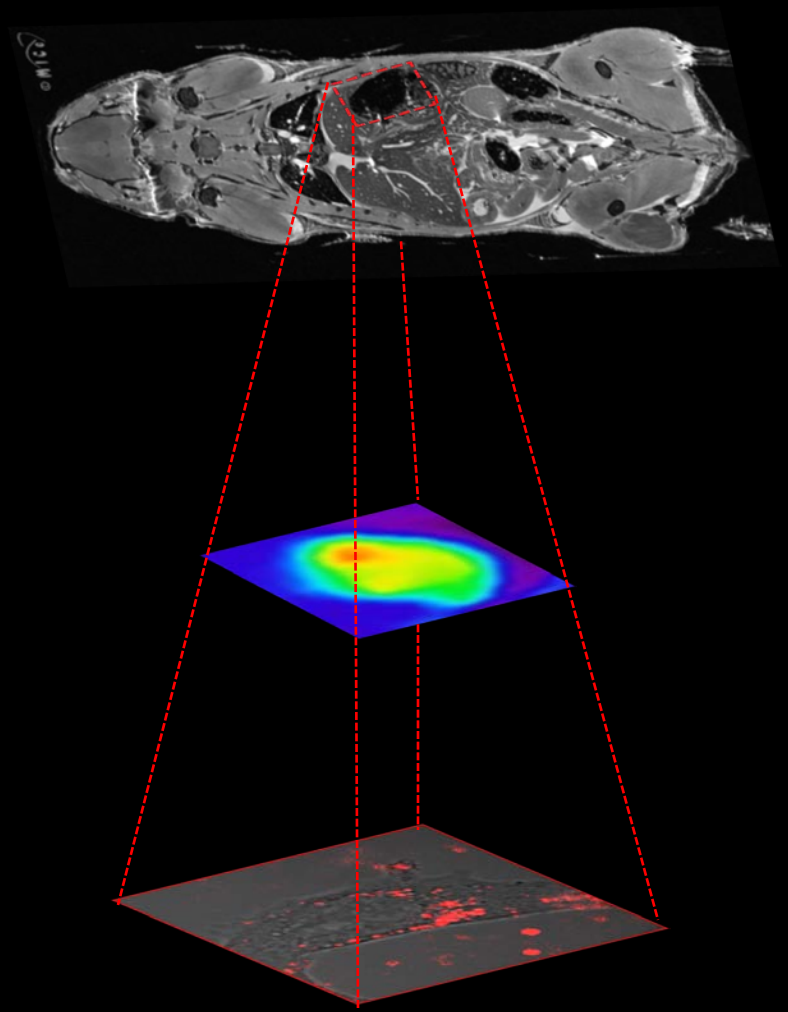
Combination Technology with MR, NIRF, and Confocal Images

MR Imaging



NIRF Imaging

Confocal Imaging

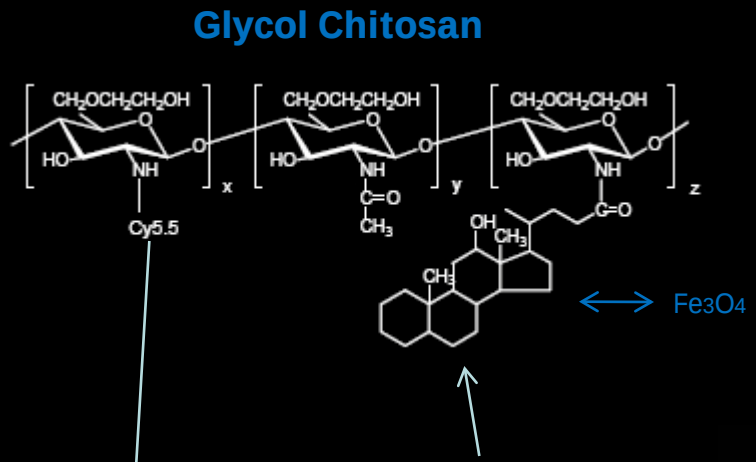


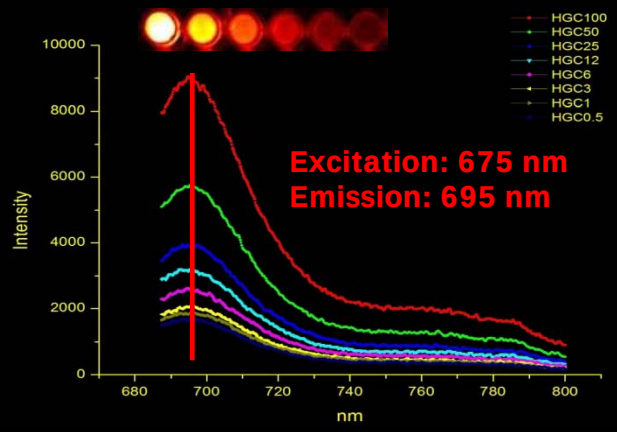
A Whole Body Imaging

Specific tumors

Nanoparticles in each tumor cell

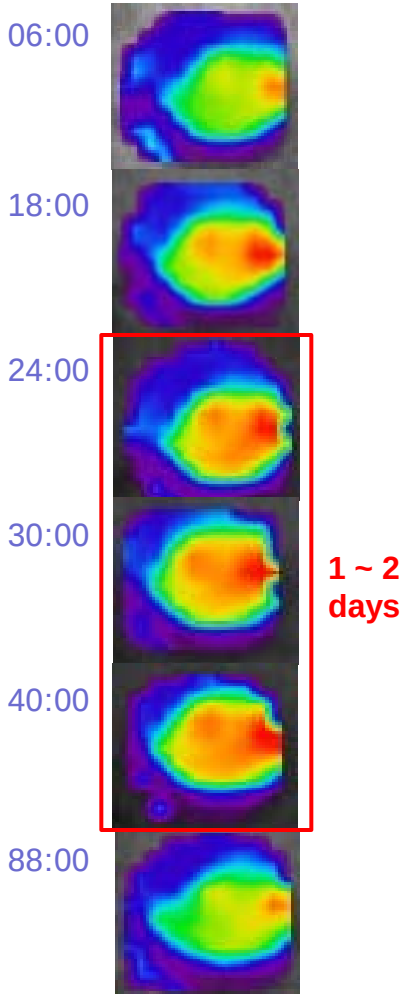
HGC - Cy5.5 - SPIO Nanoparticles

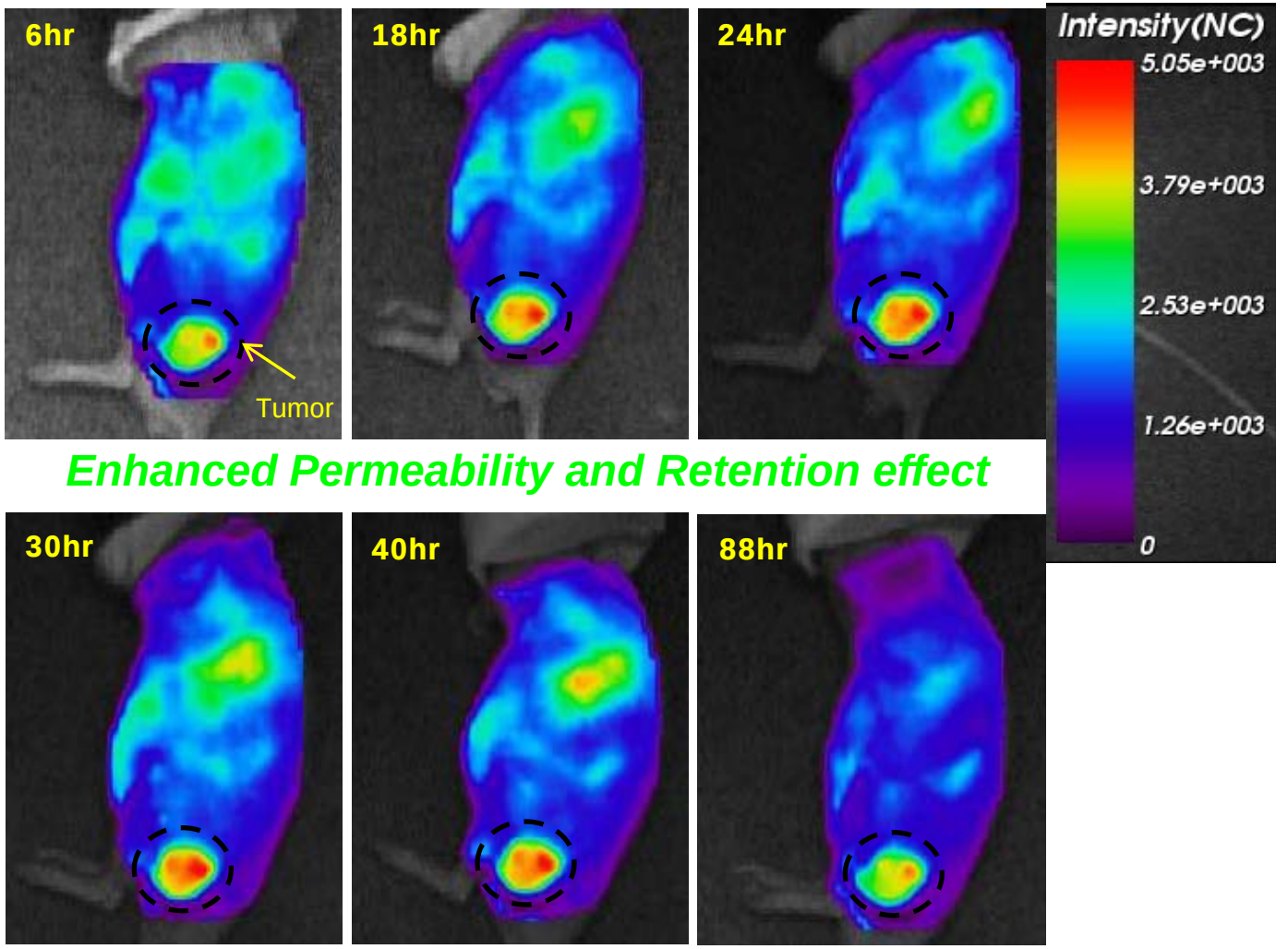




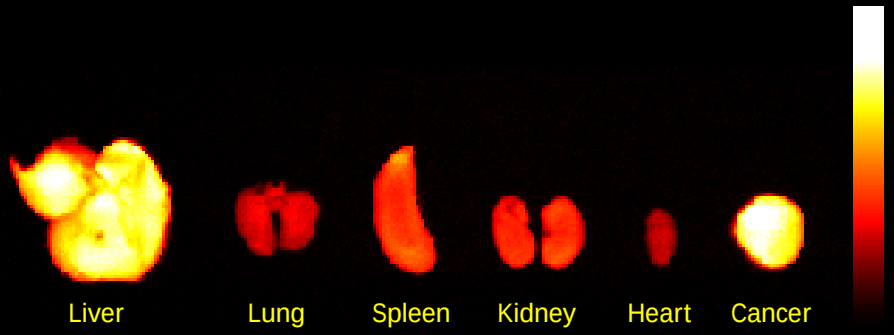
Amphiphilic glycol chitosan-cholanic acid conjugates self-assembled to form glycol chitosan nanoparticles (HGC NPs) in aqueous solution. SPIOs were loaded into HGC NPs by hydrophobic interactions.

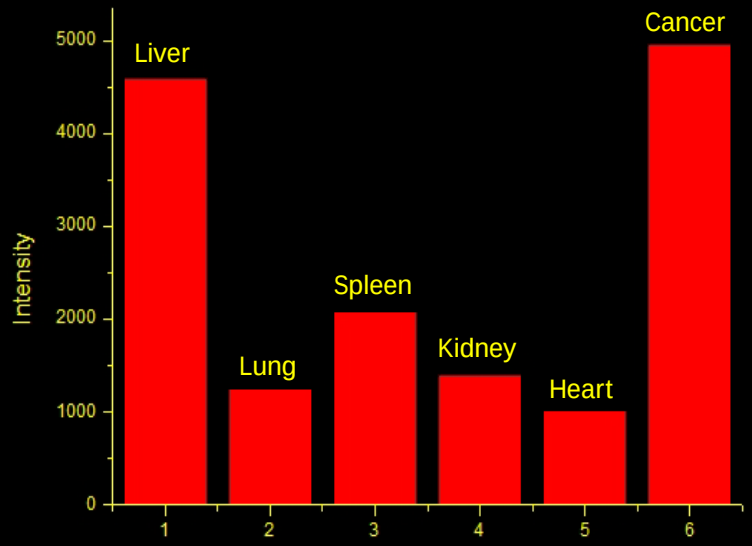
In Vivo NIRF Imaging of Skin Cancer





Ex vivo results of HGC-Cy5.5-SPIOs

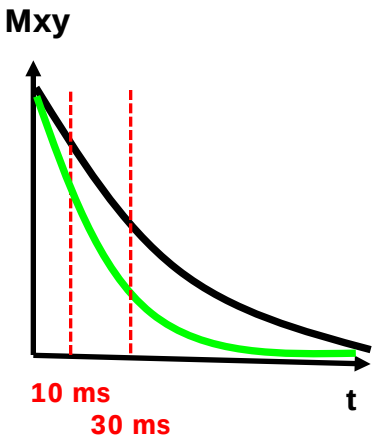




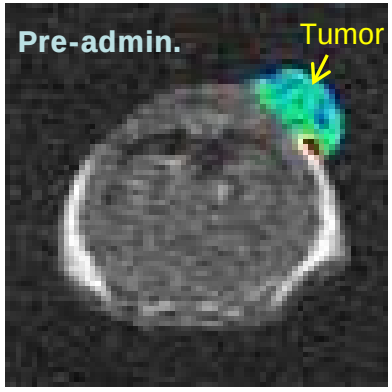
Ex vivo results means that most NPs were accumulated in cancer and liver. The accumulation in liver is a problem still remained. It might cause by large size or less flexibility of the NPs. However, when comparing current drugs available, it is still meaningful in terms of that the NPs were mostly accumulated in cancer.

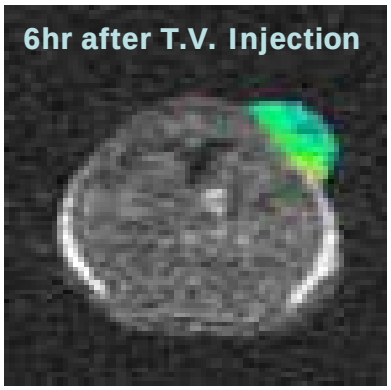
In Vivo MRI about HGC-SPIO NPs

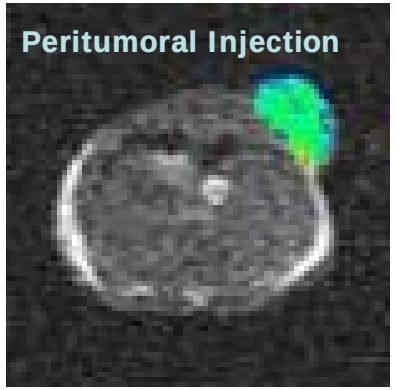
In Vivo MR Merging Images of SCC7 Targeting by HGC-SPIO NPs



TR/TE = 1500 ms / 10 ms







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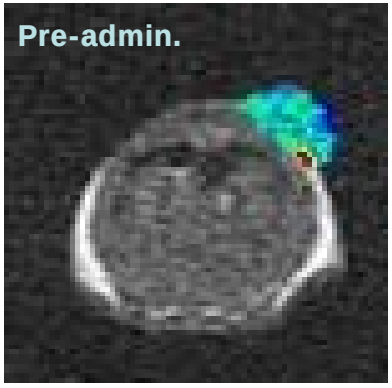
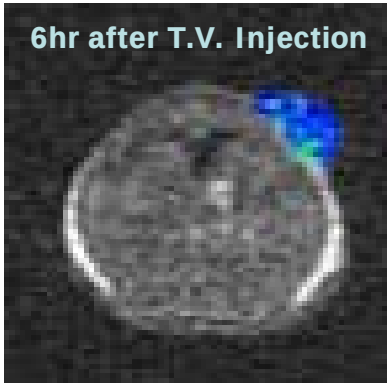
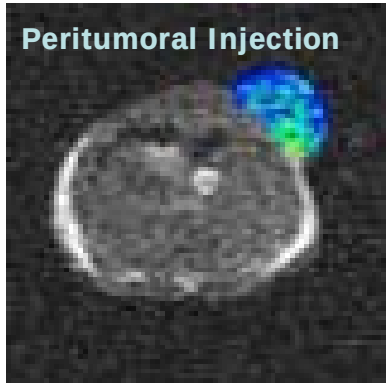
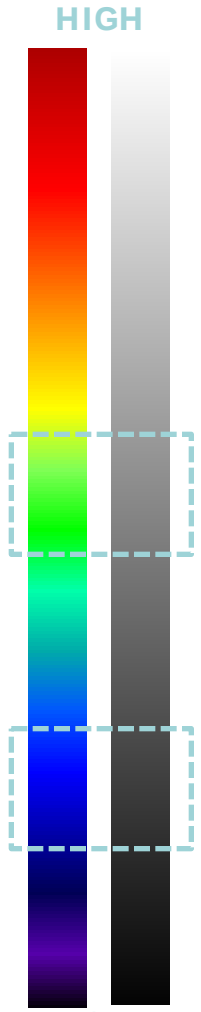


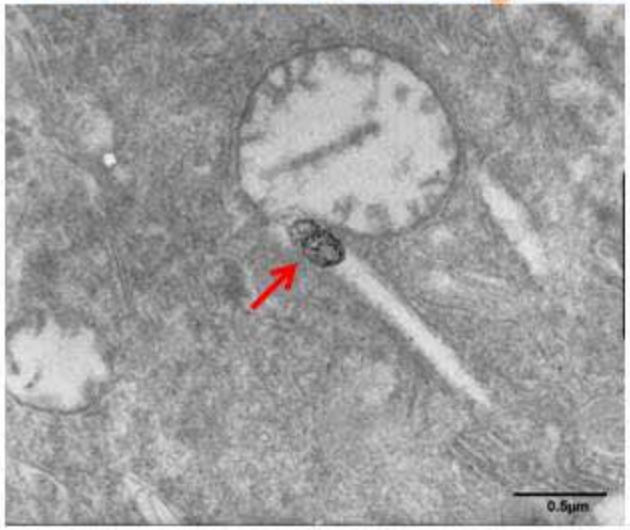


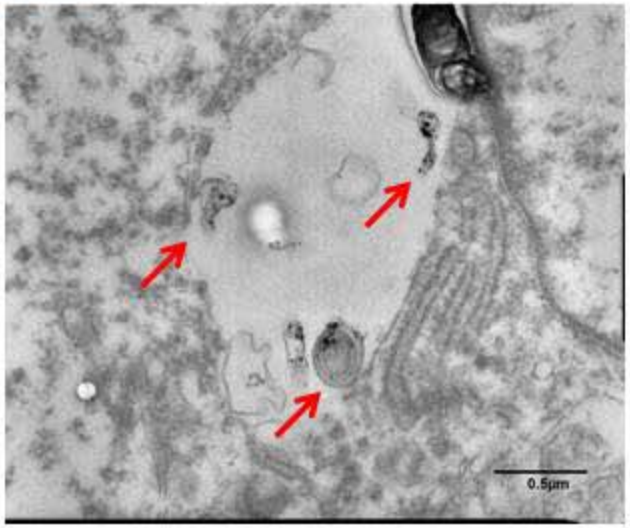


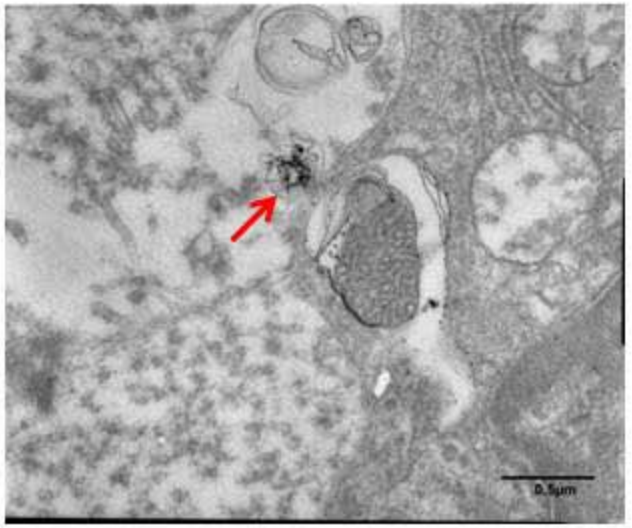


Ex Vivo Results Validated by TEM

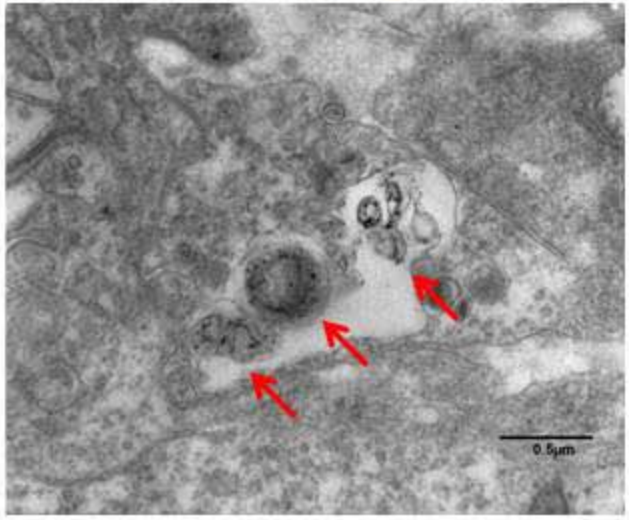
Peritumoral Injection

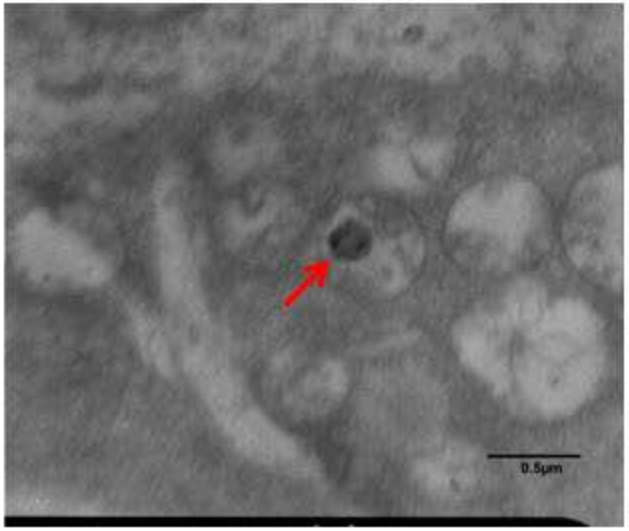


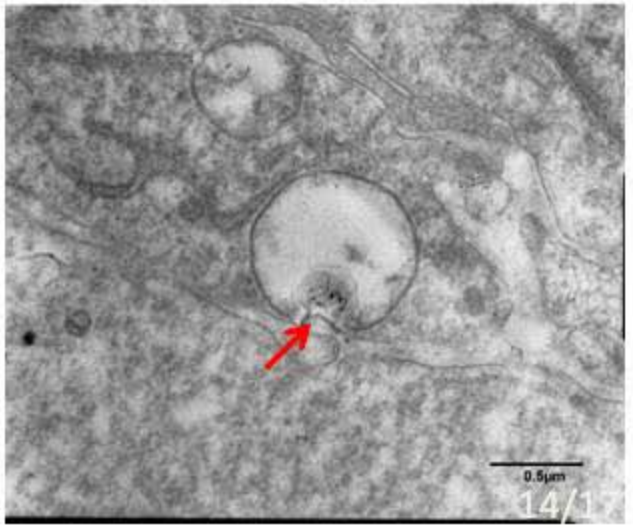




Tail Vein Injection







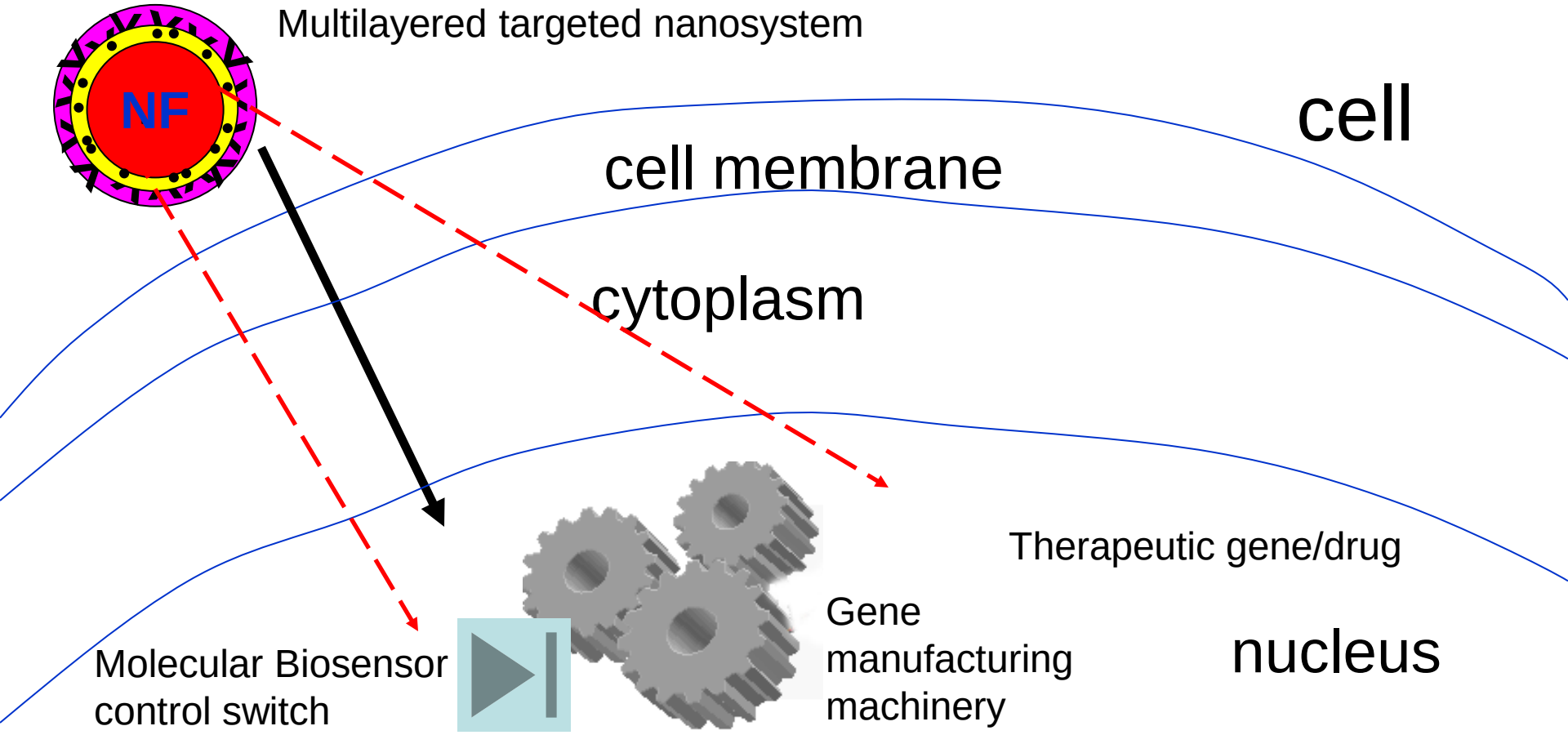
Future Medicine: Nanomedicine

- Medicine performed at the single cell level – advanced targeted drug therapy
- Possible repair, rather than just elimination, of diseased cells at the single cell level (“regenerative medicine”)
- Sufficiently early diagnosis and treatment of disease that the distinction between prevention and treatment is blurred

The challenge of precise drug delivery and dosage per cell

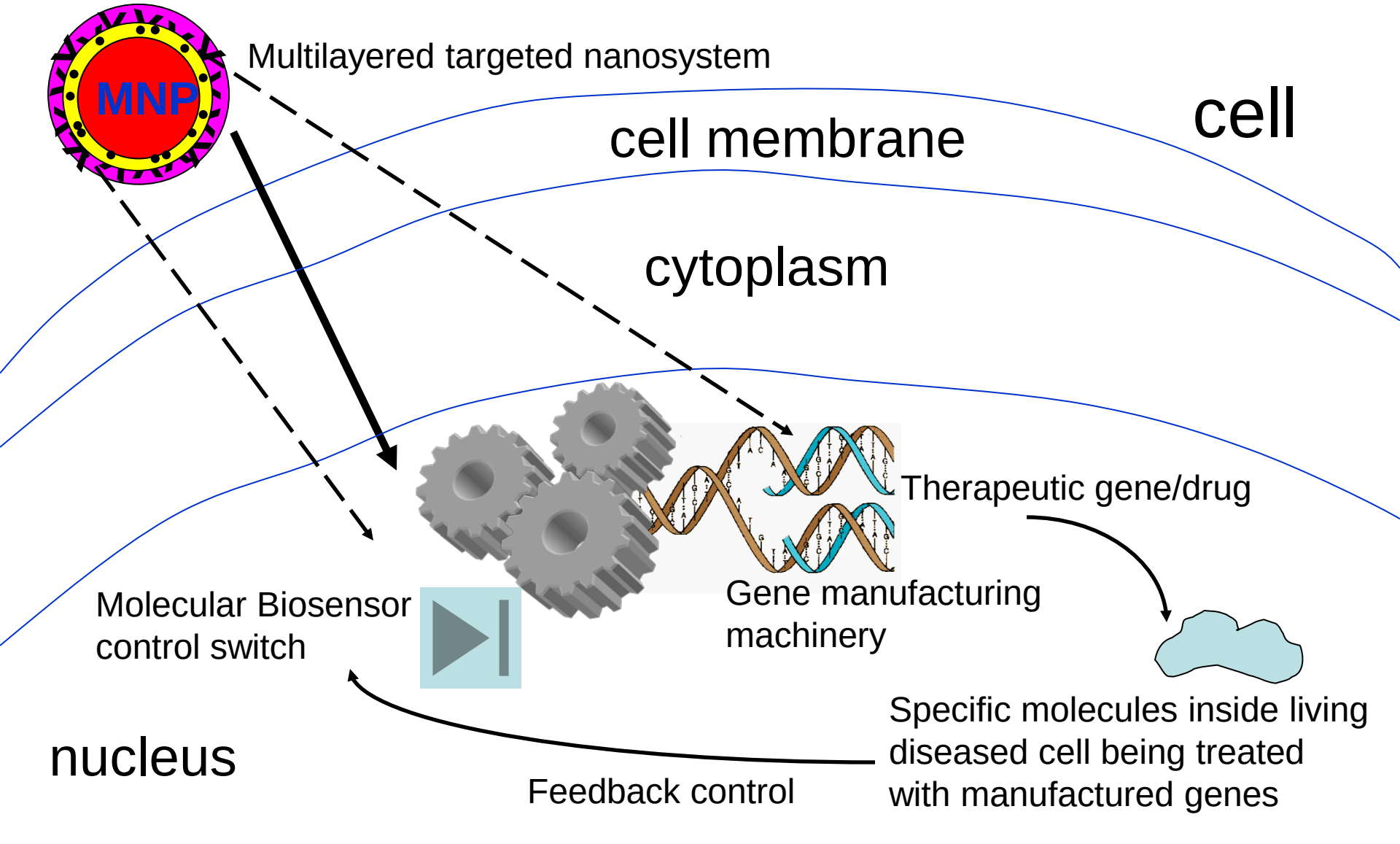
It is impossible to control the number of nanoparticles that will bind and be active in a given cell. For regenerative nanomedicine the drug/gene needs to be created in-situ and controlled in feedback loops. This is possible to do with biomolecular sensors controlling down-stream transient gene therapy inside living cells.

Dealing with the dosing problem: Concept of nanoparticle-based "nanofactories" –feedback-controlled manufacturing of therapeutic genes inside living cells for single cell treatments using engineered nanosystems



The nanoparticle delivery system delivers the therapeutic gene template which uses the host cell machinery and local materials to manufacture therapeutic gene sequences that are expressed under biosensor-controlled delivery.

Dealing with the dosing problem: Concept of nanoparticle-based "nanofactories" –feedback-controlled manufacturing of therapeutic genes inside living cells for single cell treatments using engineered nanosystems



Regenerative Nanomedicine

Nanomedical devices will provide individual cell dosing with a specific patient. The control of dosage of therapy at the single cell will be a fundamental requirement for “regenerative nanomedicine”. Diseased cells will not necessarily be killed but rather re-programmed to less dangerous, if not completely normal, cell types.

Important question: Can nanomedical systems be "bionanomanufactured" under cGMP principles?

Our MCF Team and Current Collaborators

Our MCF Team and Current Collaborators

Nanochemistry
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Combinatorial chemistry/ Drug Discovery
David Gorenstein (UTMB)
Xianbin Yang (UTMB)
Andy Ellington (UT-Austin)

Nanoparticle technology
Nick Kotov (Univ. Michigan)
Kinam Park (Purdue)
Alex Wei (Purdue)

Nanotoxicity studies
Debbie Knapp (Purdue)
James Klaunig (IU-SOM)

MRI Imaging
Tom Talavage (Purdue)
Charles Bouman (Purdue)

Image/confocal/SPR
Paul Robinson (Purdue)
Joseph Irudayaraj (Purdue)

Funding from NIH, NASA, and Army Breast Cancer Program

Molecular Cytometry Facility
Director: James Leary

Lisa Reece (SVM) – flow cytometry/ BioMEMS; tissue culture

Christy Cooper (SVM) - bioanalytical chemistry, nanochemistry, XPS, AFM

Meggie Grafton (BME) - BioMEMS

Emily Haglund *(BME) – multilayered Qdots for ex-vivo nanomedicine

Mary-Margaret Seale-Goldsmith* (BME) – multi-layered magnetic nanomedical systems

Michael Zordan (BME) – prostate cancer, rare cell flow/image cytometry

Trisha Eustaquio (BME) – gene silencing/therapy; interactive imaging

Jaehong Key (BME)- 3D/MRI imaging

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Deepika Dhawan (Purdue)
Sophie Lelievre (Purdue)
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High-Energy TEM
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Robert Hannemann (Purdue)

Magnetic Cell Sorting
Paul Todd (Techshot, Inc)

LEAP Interactive Imaging
Fred Koller (Cyntellect, Inc.)

BioMEMS/Microfluidics
Kinam Park (Purdue)
Pedro Irazoqui (Purdue)
Huw Summers (Cardiff Univ, UK)

A Few Relevant Recent References

1. Prow, T.W., Smith, J.N., Grebe, R., Salazar, J.H., Wang, N., Kotov, N., Lutty, G., Leary, J.F. "Construction, Gene Delivery, and Expression of DNA Tethered Nanoparticles" Molecular Vision 12: 606-615, 2006
2. Prow, T.W., Grebe, R., Merges, C., Smith, J.N., McLeod, D.S., Leary, J.F., Gerard A. Lutty, G.A. "Novel therapeutic gene regulation by genetic biosensor tethered to magnetic nanoparticles for the detection and treatment of retinopathy of prematurity" Molecular Vision 12: 616-625, 2006
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6. Kim S, Lim CK, Na J, Lee YD, Kim K, Choi K, Leary JF, Kwon IC. "Conjugated polymer nanoparticles for biomedical in vivo imaging" Chem Commun (Camb).46(10):1617-1619 (2010).
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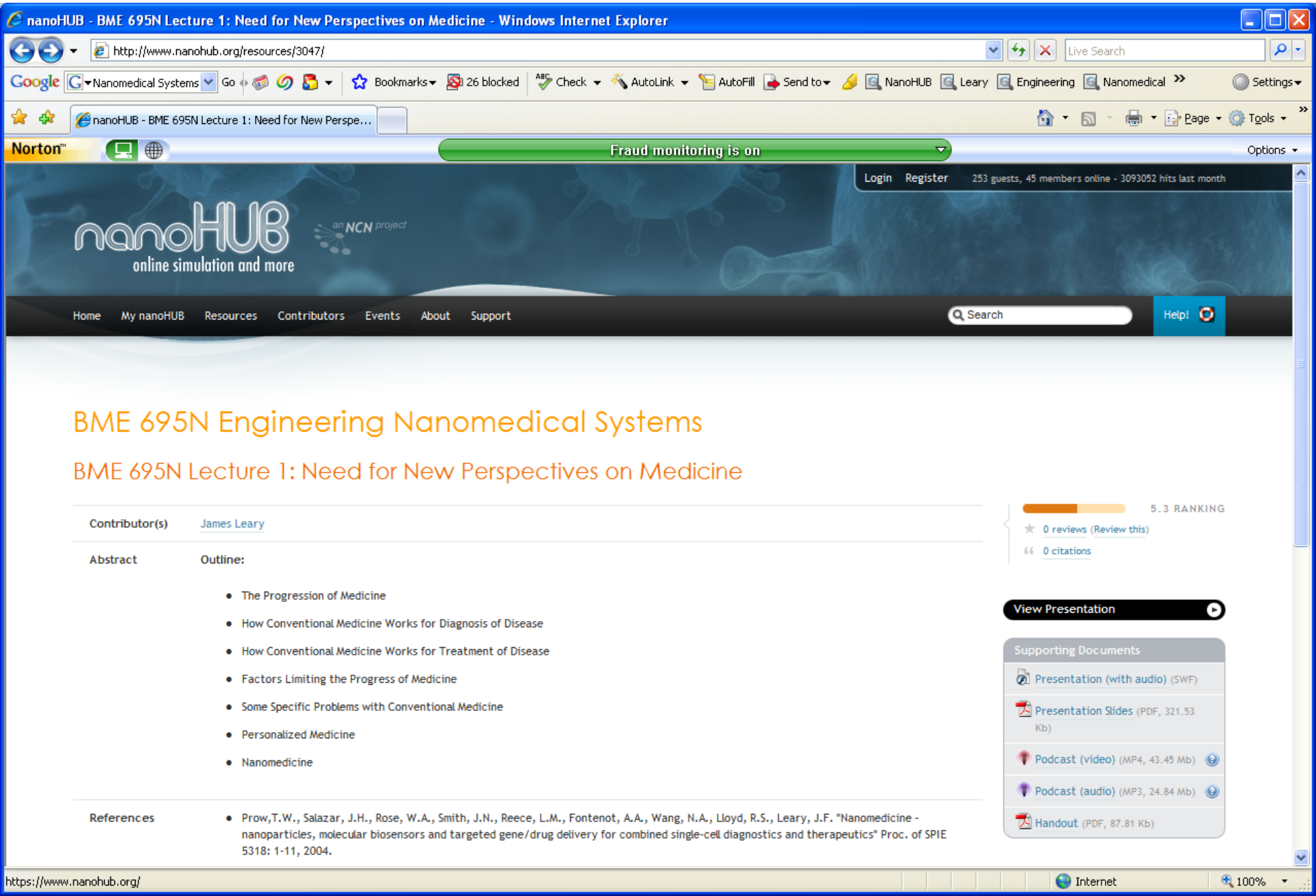
9. Zarbin, M.A., Montemagno, C., Leary, J.F., Ritch, R. "Nanomedicine in Ophthalmology: The New Frontier" American Journal of Ophthalmology 2010 (In Press).

http://www.nanohub.org/courses/nanomedicine



Q & A