Purdue University
Weldon School of Biomedical Engineering
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"The Importance of Zeta Potential for Drug/Gene Delivery in Nanomedicine"

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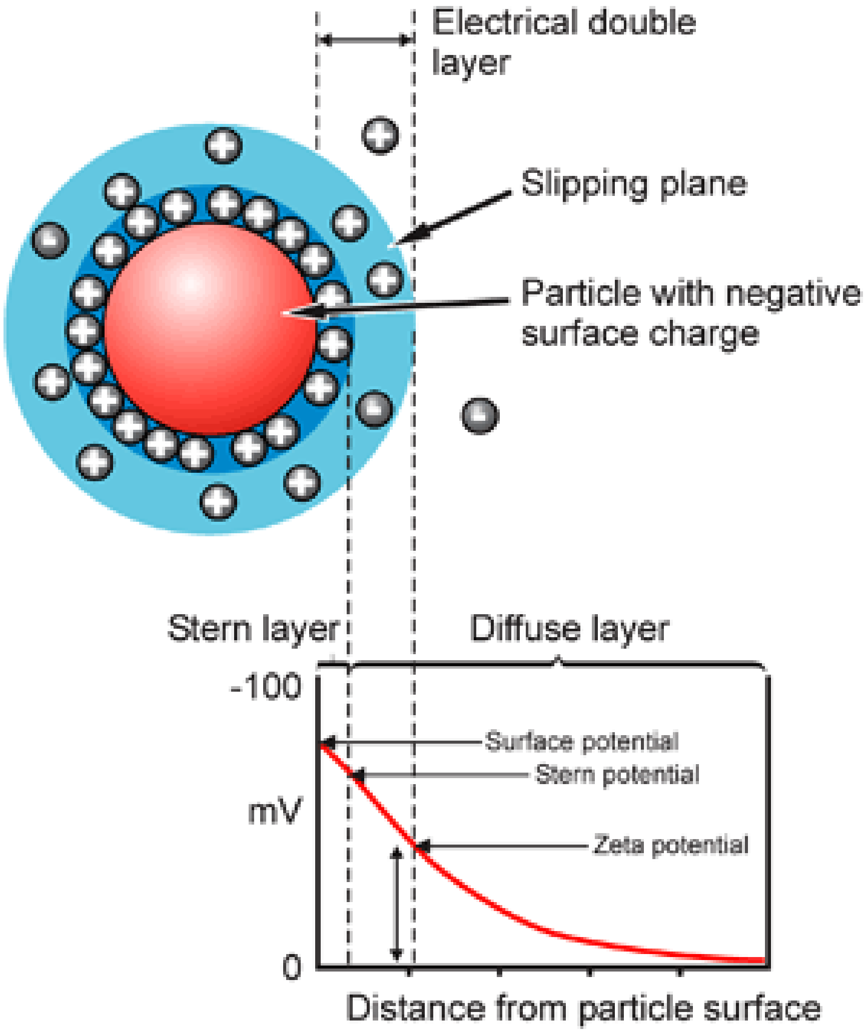
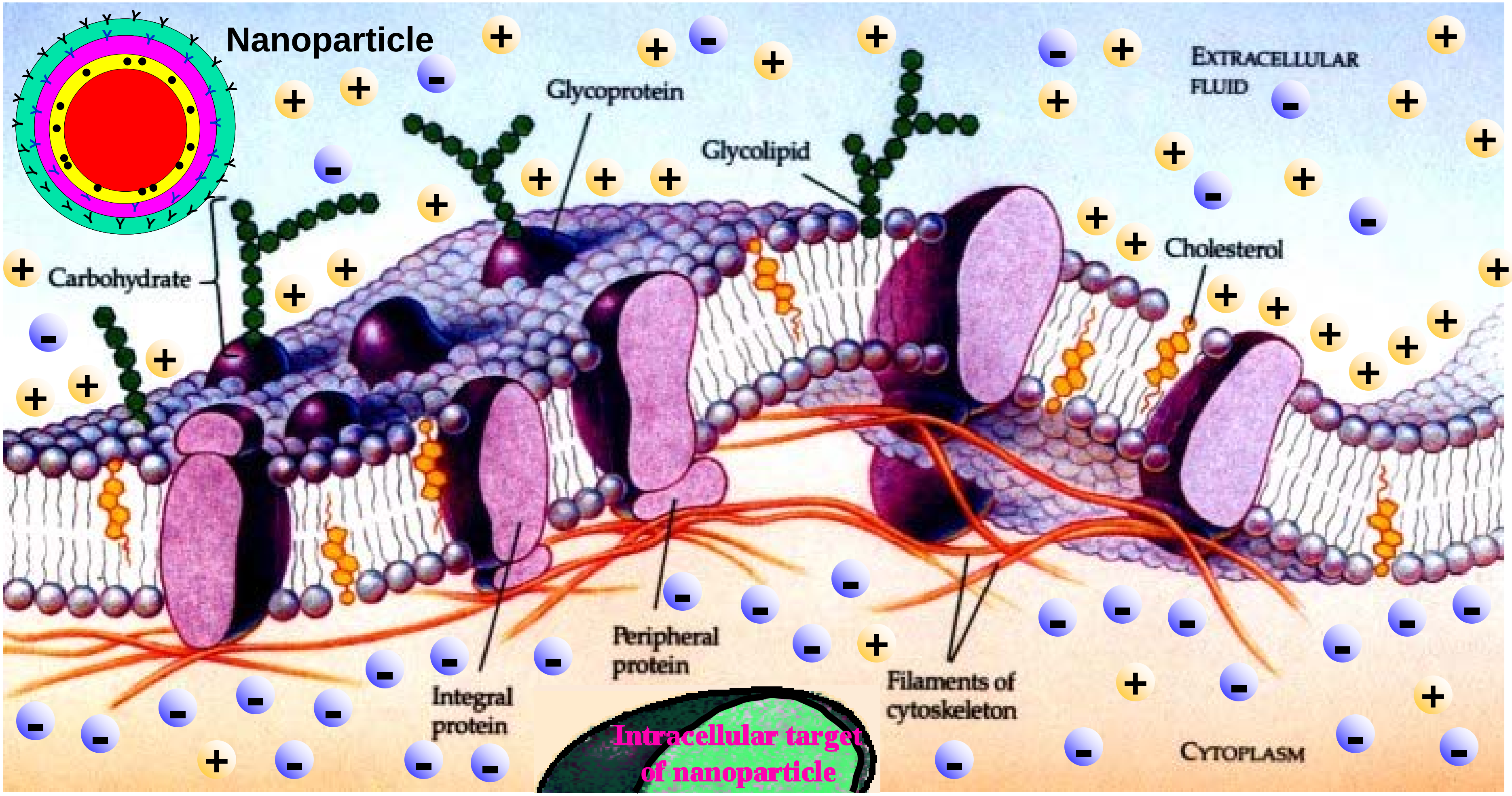
Zeta Potential – Electrostatics in Fluids



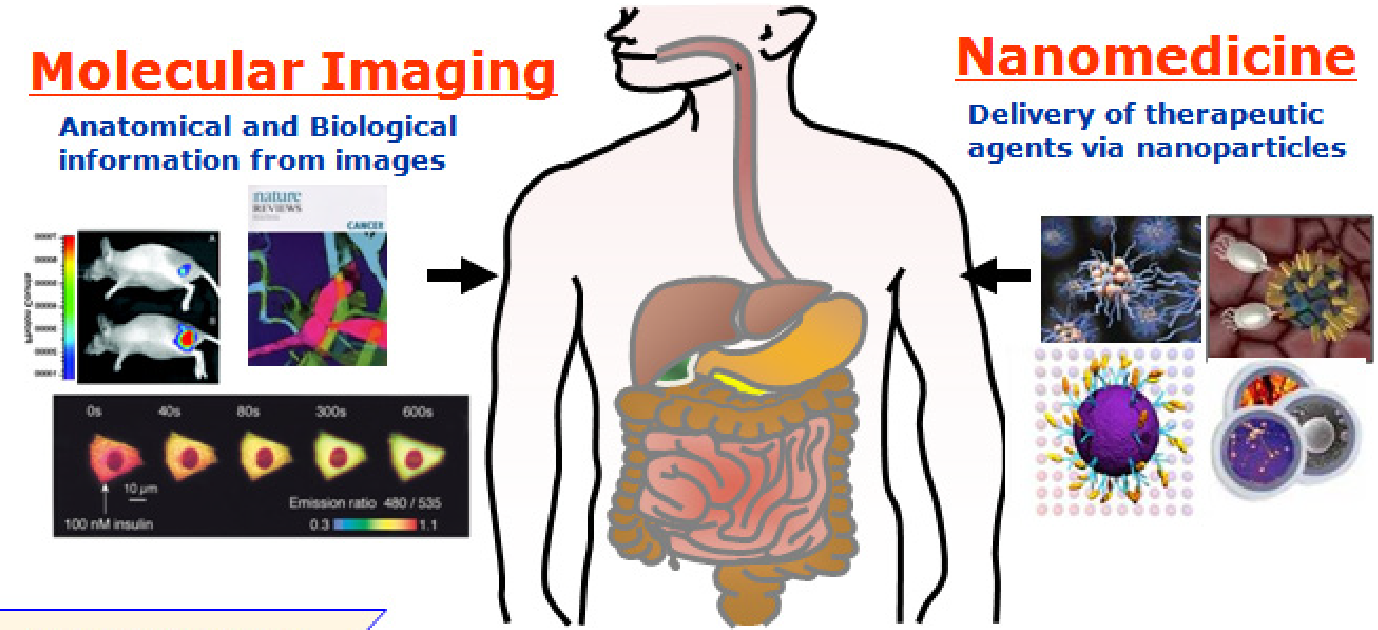
Zeta potential describes the electrostatic interactions of cells and particles in a fluid environment. The liquid layer surrounding the particle exists as two parts; an inner region (Stern layer) where the ions are strongly bound and an outer (diffuse) region where they are less firmly associated. Within the diffuse layer there is a notional boundary inside which the ions and particles form a stable entity. When a particle moves (e.g. due to gravity), ions within the boundary move it. Those ions beyond the boundary stay with the bulk dispersant. The potential at this boundary (surface of hydrodynamic shear) is the zeta potential.

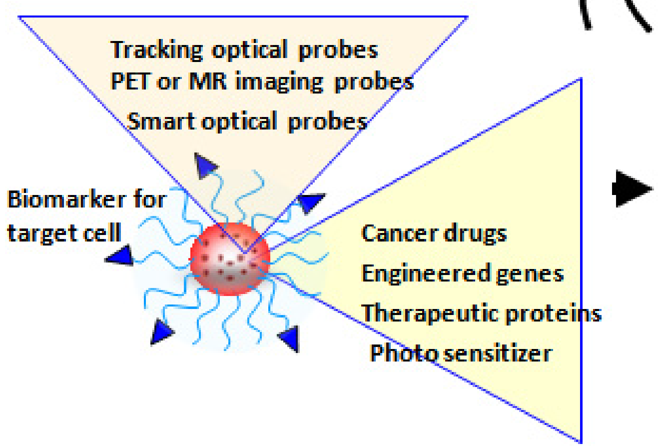
Interaction of Nanoparticles with the Cell Surface Based on Zeta Potential and Size



Adapted from Campbell, Neil A., and Jane B. Reece. Biology. 6th ed. San Francisco: Benjamin Cummings, 2002.

“Smart” Nanoparticles =drug + device*





- Molecular based diagnosis/therapy
- Early diagnosis
- Personalized therapy
- Real-time monitoring of therapeutic effects
- Predictive and preventive medicine

* FDA “combo device”

The importance of the zeta potential

A. nanoparticle-nanoparticle interactions
B. nanoparticle-cell interactions
C. part of the initial nanomedical system-cell targeting process
D. low zeta potential leads to low serum protein binding and potentially longer circulation

Characteristics of the zeta potential

- Zeta potential is the electrical potential at the hydrodynamic plane of shear.
- Zeta potential depends not only on the particle's surface properties but also the nature of the solution (e.g. Ionic strength, pH, etc.).
- Zeta potential may be quite different from the particle's surface potential.
- Small changes in ionic strength and pH can lead to large effects in zeta potential.
- Zeta potential can be used to predict the monodispersity (or agglomeration) of particles.
- High zeta potential (either positive or negative) (> 30 mV) lead to monodispersity. Low zeta potential (<5 mV) can lead to agglomeration.

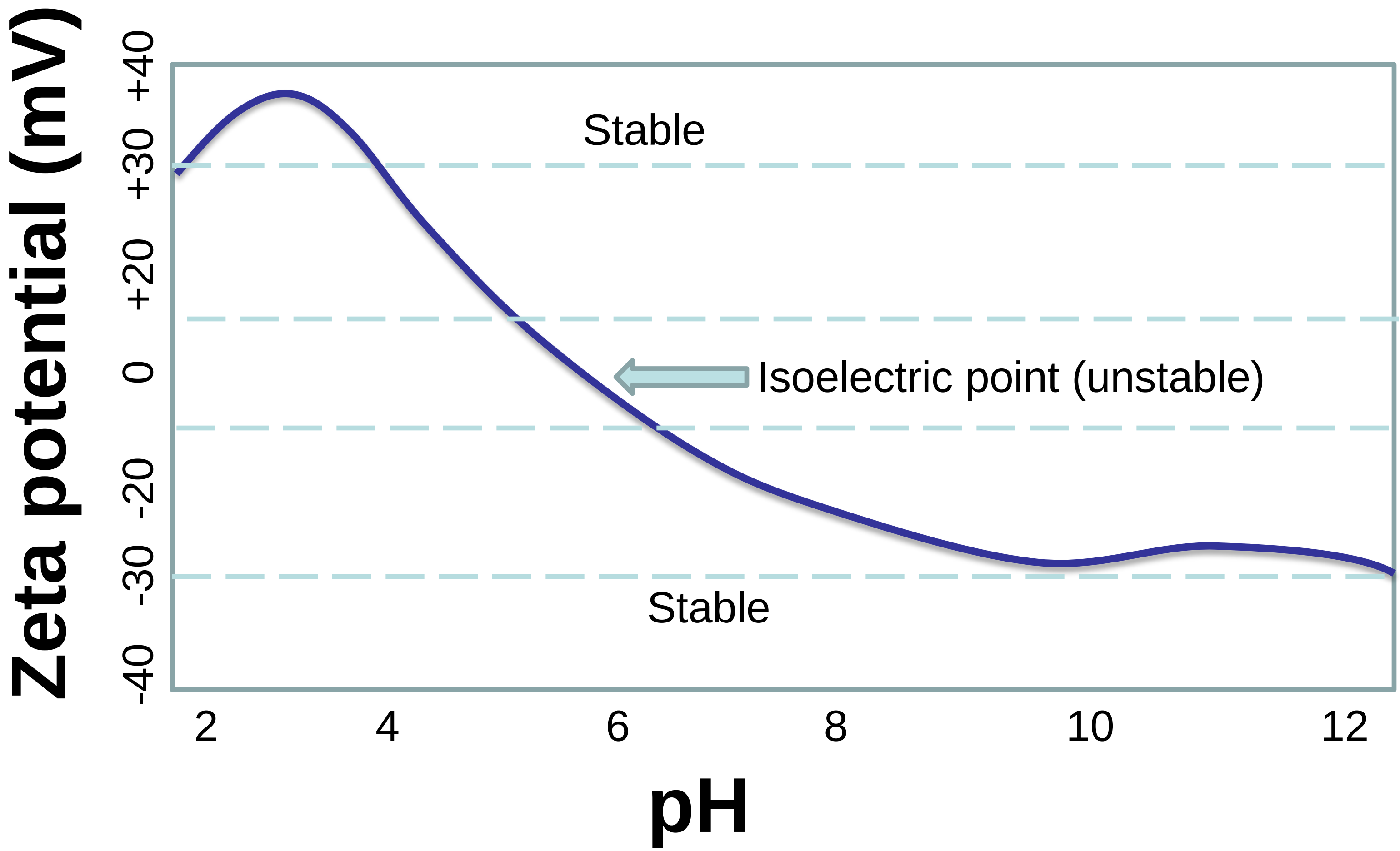
Most importantly, nanoparticles and cells interact according to the magnitude of each of their zeta potentials, not their surface charges!

Some factors affecting the zeta potential that are important in nanomedicine

A. pH
B. ionic strength

The local pH and ionic strength can vary greatly in the different parts of the human body. These factors also change within different regions INSIDE human cells. So it is a challenge to design nanoparticles that have the optimal zeta potentials by the time they reach their final destinations.

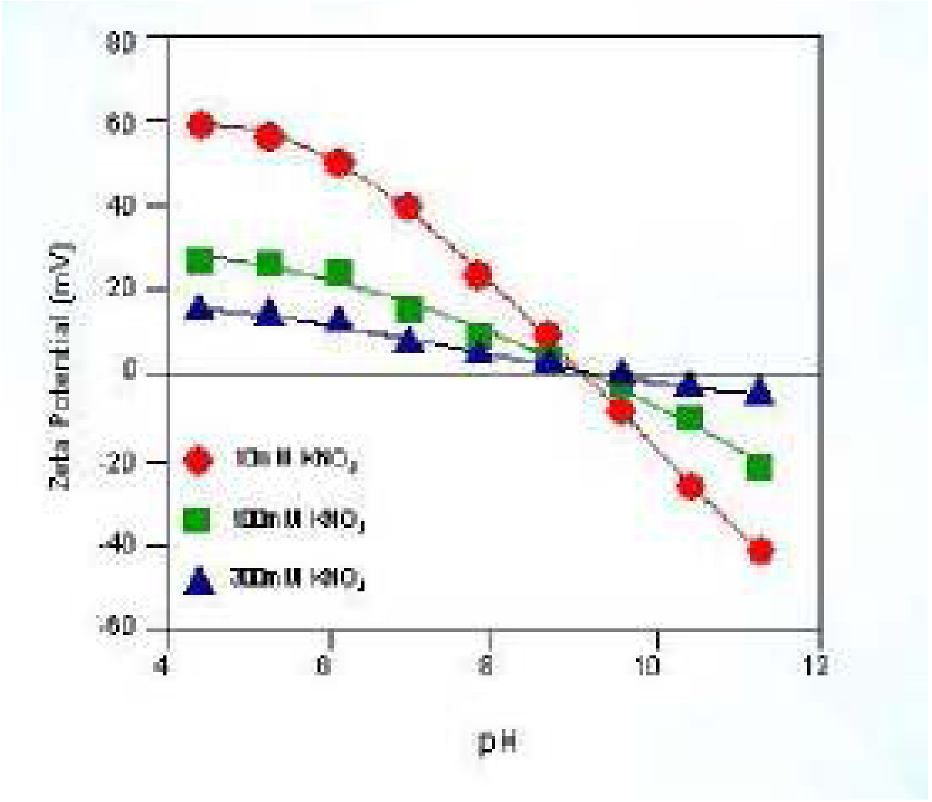
Zeta Potential and pH



Typical plot of zeta potential versus pH showing the position of the isoelectric point and the pH values where the dispersion would be expected to be stable

Effect of solution ionic strength or conductivity on zeta potential

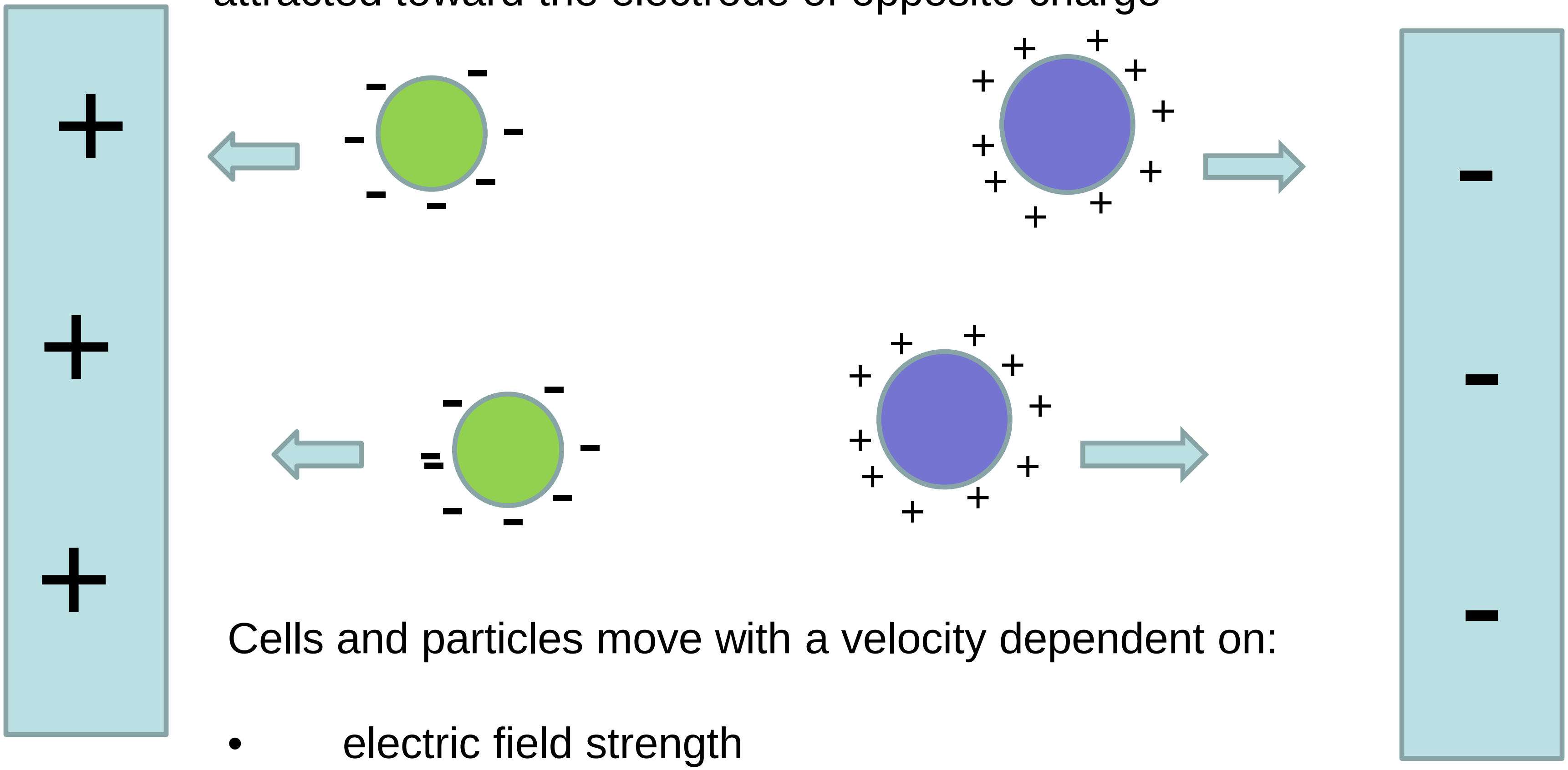
- Non-specific ion adsorption may, or may not, have an effect on the isoelectric point.
- Specific ion adsorption usually leads to a change in the isoelectric point



Source: http://www.malvern.com

Measuring zeta potential by electrophoresis

If an electric field is applied across a sample containing charged cells and/or particles, those cells and particles are attracted toward the electrode of opposite charge



Cells and particles move with a velocity dependent on:

- electric field strength
- dielectric constant of the medium
- viscosity of the medium
- zeta potential

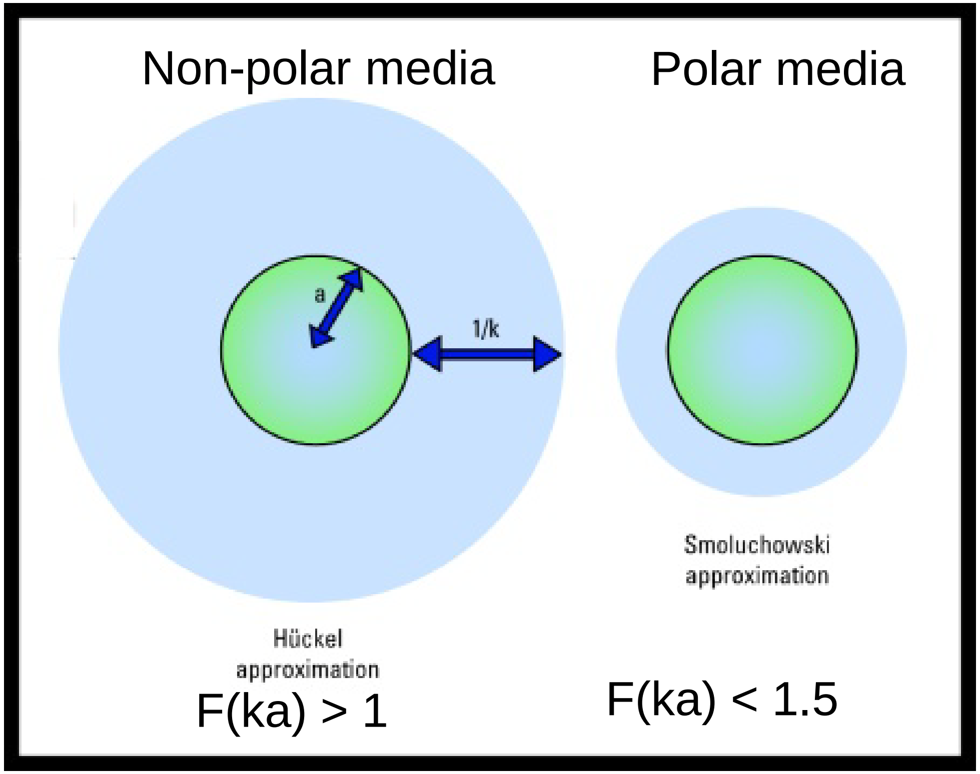
By measuring the velocity of a nanoparticle in an electric field its zeta potential can be calculated

The velocity of a particle in a unit electric field is referred to as its electrophoretic mobility. Zeta potential is related to the electrophoretic mobility by the Henry equation:

$$U_E = \frac{2\,\varepsilon\,z\,f(\kappa a)}{3\eta}$$

where UE = electrophoretic mobility, z= zeta potential, ε = dielectric constant, η = viscosity and f(κa) = Henry's function

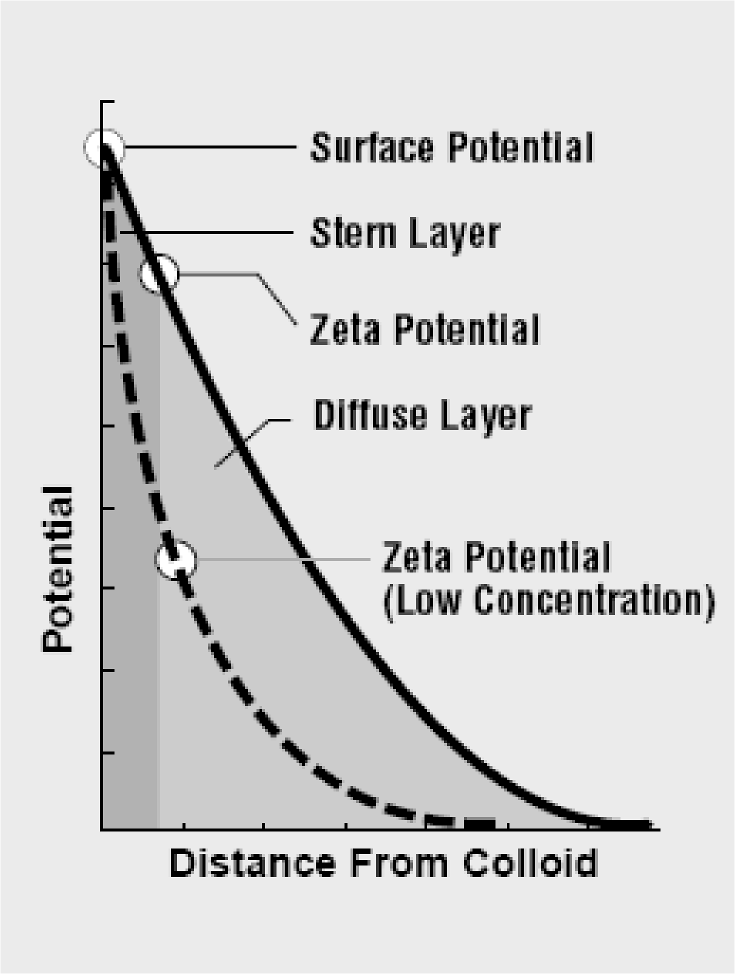
Assumptions about slip layer diameter when calculating Henry's function for the zeta potential



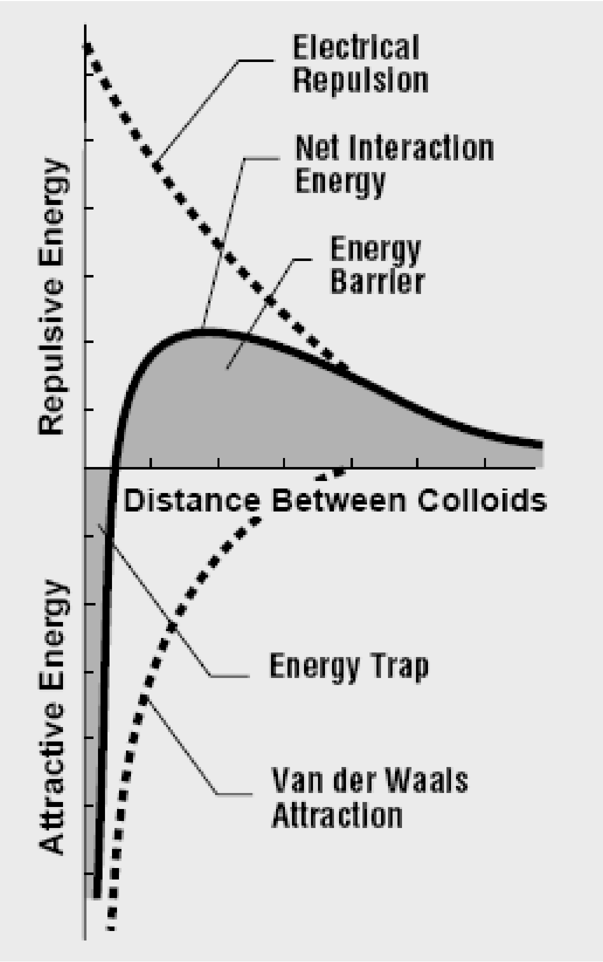
Schematic illustrating Huckel and Smoluchowski's approximations used for the conversion of electrophoretic mobility into zeta potential

Adapted from http://www.silver-colloids.com/Tutorials/Intro/pcs21.html

Zeta potential represents the potential barrier to cell-nanoparticle interactions



Zeta Potential vs. Surface Potential: The relationship between zeta potential and surface potential depends on the level of ions in the solution.



Interaction: The net interaction curve is formed by subtracting the attraction curve from the repulsion curve.

http://www.malvern.com

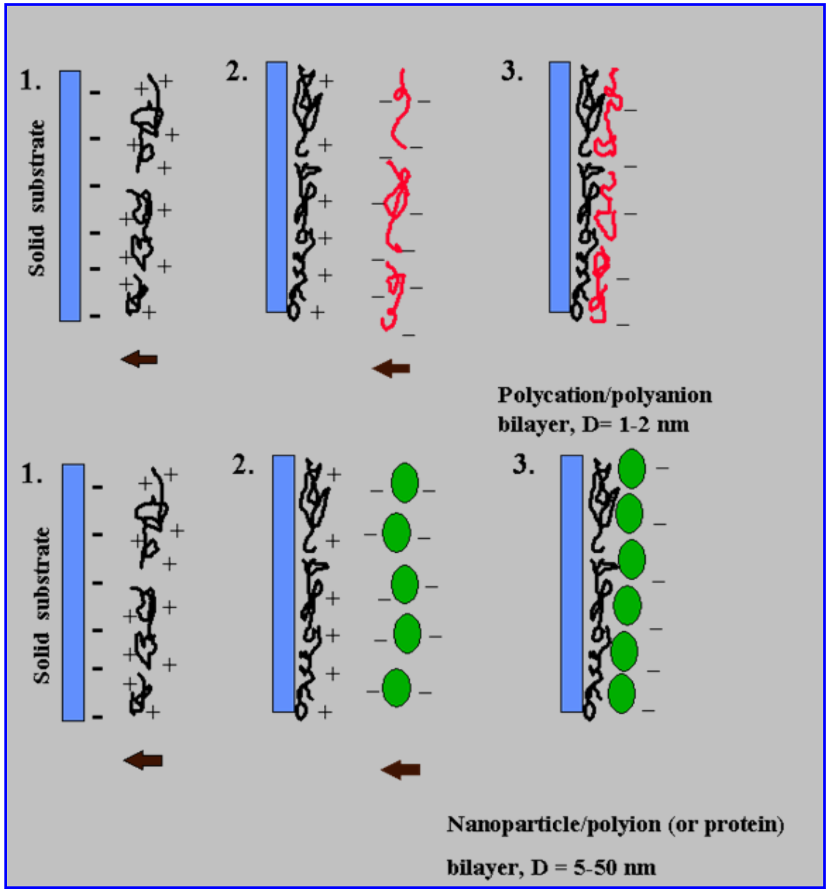
What is the best zeta potential to have for nanomedical systems?

That is not a simple question, but in general it is good to have a zeta potential of approximately -5 to -15 mV. Since most biological cells have zeta potentials in this range you want your nanomedical systems to also be slightly negative zeta potentials so that they do not stick non-specifically to cells but interact through a receptor-mediated interaction that allows binding of nanoparticles only when there is a receptor-ligand bond strong enough to overcome a modest electrical repulsion.*

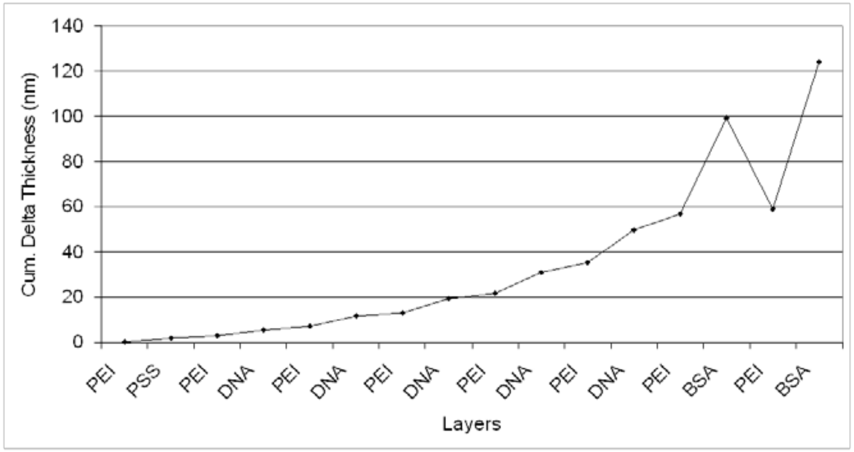
* If all you want is to have nanoparticles stick to cells in tissue culture for transfection, the zeta potential can be positive. Just pay attention to the zeta potential of the tissue culture plate surfaces!

Size and Zeta Potential Changes During LBL Construction of Nanoparticles

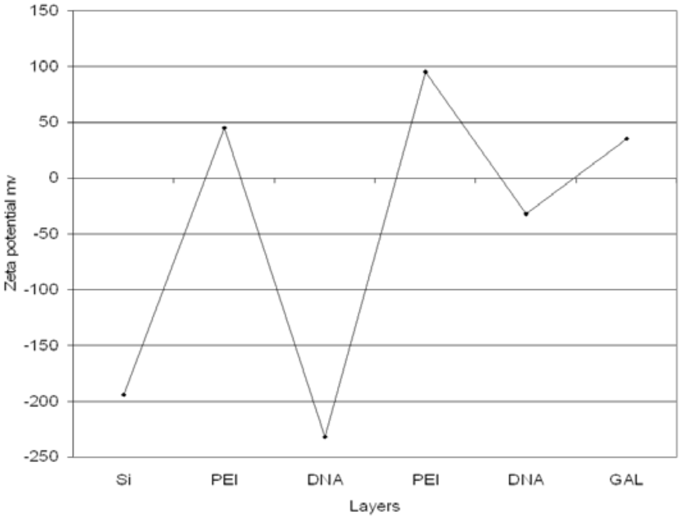
Layer-by-layer (LBL) assembly of NP with charged polymers



Increase in NP size with layers

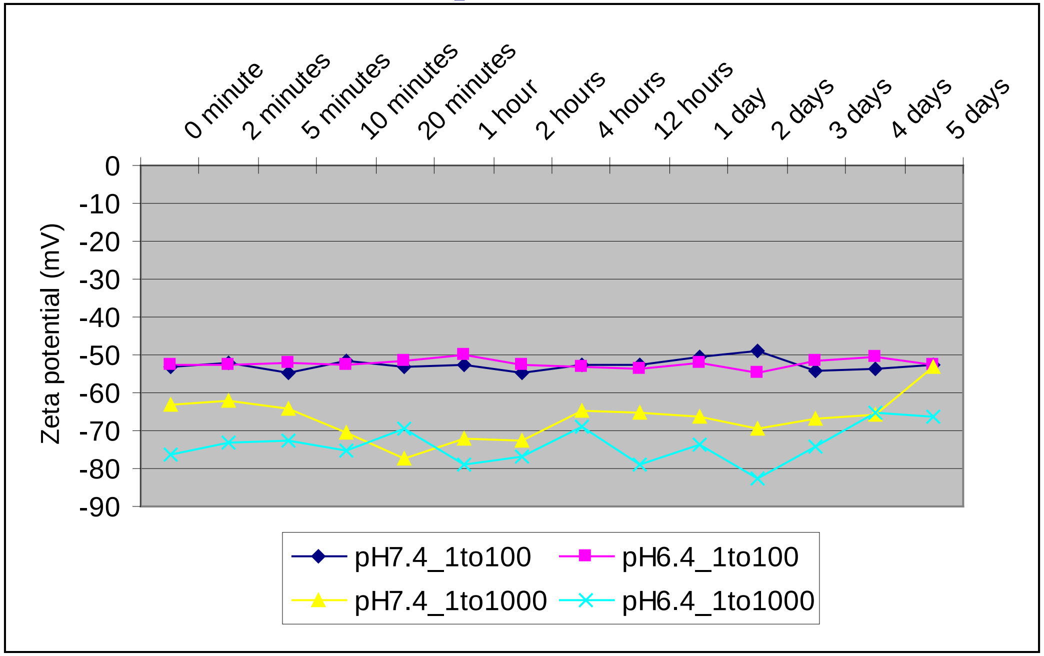


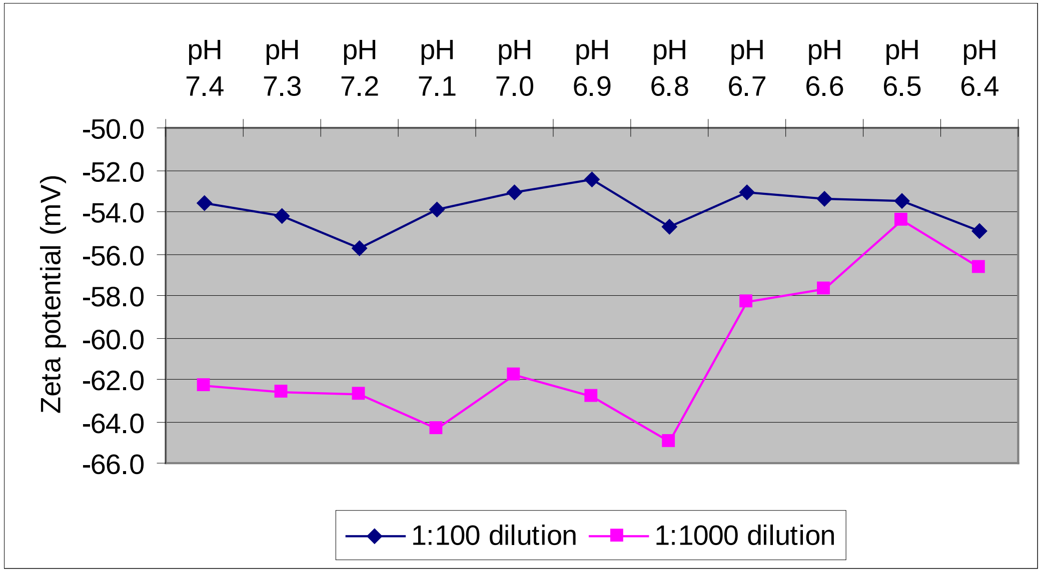
Change in NP zeta potential with additions of layers



Source: Prow et al. 2005.

Effects of pH and dilution on NP zeta potential





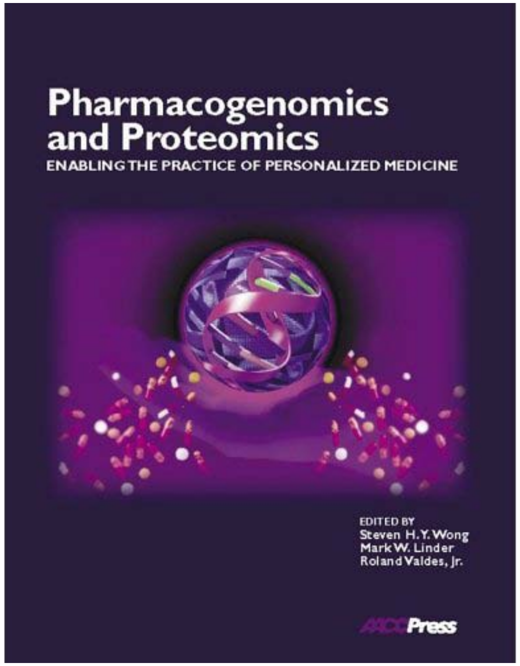
Zeta potential measurements of 40-50 nm silica particles tested over a 5 day time period at two different pH values and two different dilutions with distilled/ deionized water.

Source: Prow et al. 2005.

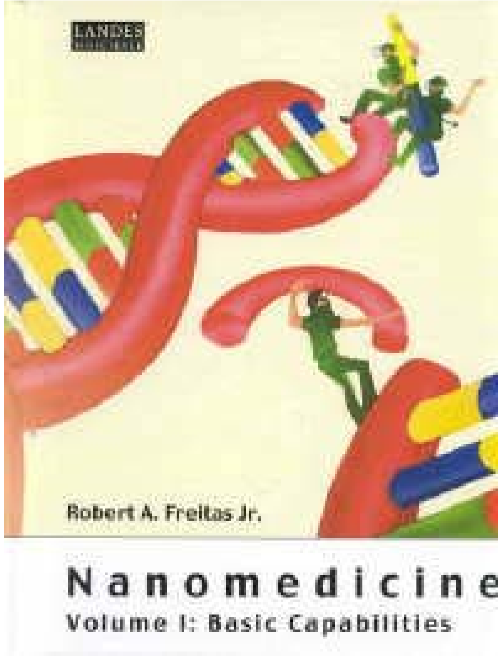
The progression of medicine and the evolution of nanomedicine

Conventional "Modern" Medicine → "Personalized" or "Molecular" Medicine + Nanomedicine Single-cell Medicine

Best guess on how to treat this particular patient…



Should this patient receive this drug? Predictive medicine based on genomic info.



How can we target that drug to single cells to reduce side effects?

Features of Nanomedicine

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

- Nanomedicine uses "nano-tools" (e.g. smart nanoparticles) that are roughly 1000 times smaller than a cell (knives to microsurgery to nanosurgery …) to treat single cells

- 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).

Nanomedicine Concept of Regenerative Medicine "Fixing cells one cell at-a-time"

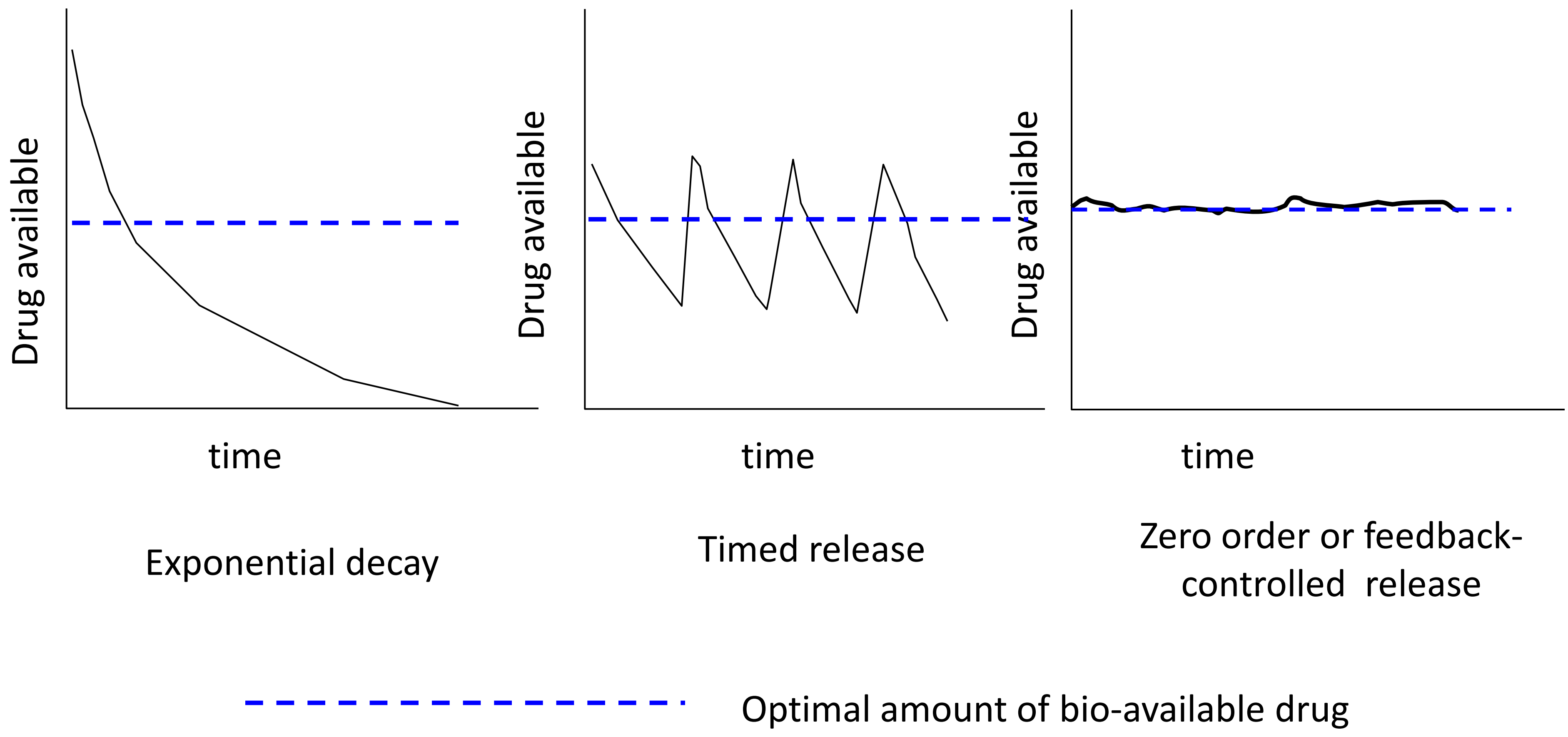
- Nanomedicine attempts to make smart decisions, pre-symptomatically, to either remove specific cells by induced apoptosis or repair them one cell-at-a-time.
- Single cell treatments will be based on molecular biosensor information that controls subsequent drug delivery at the appropriate level for that single cell.

Why does Nanomedicine Represent a Huge Promise for Health Care?

Earlier diagnosis increases chances of survival. By the time some symptoms are evident to either the doctor or the patient, it may be already too late, in terms of irreversible damage to tissues or organs.

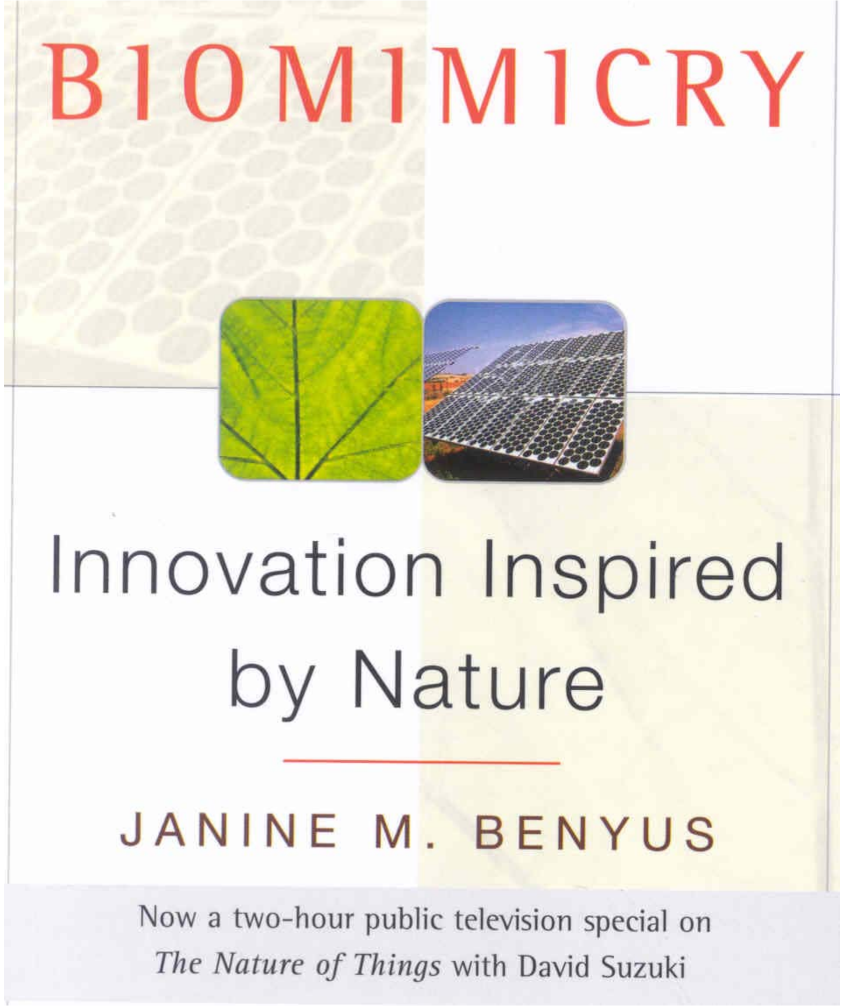
Nanomedicine will diagnose and treat problems at the molecular level inside single-cells, prior to traditional symptoms and, more importantly, prior to irreversible tissue or organ damage.

Drug Delivery Systems with and without Zeroth Order Nanopores or Feedback Control Biosensors



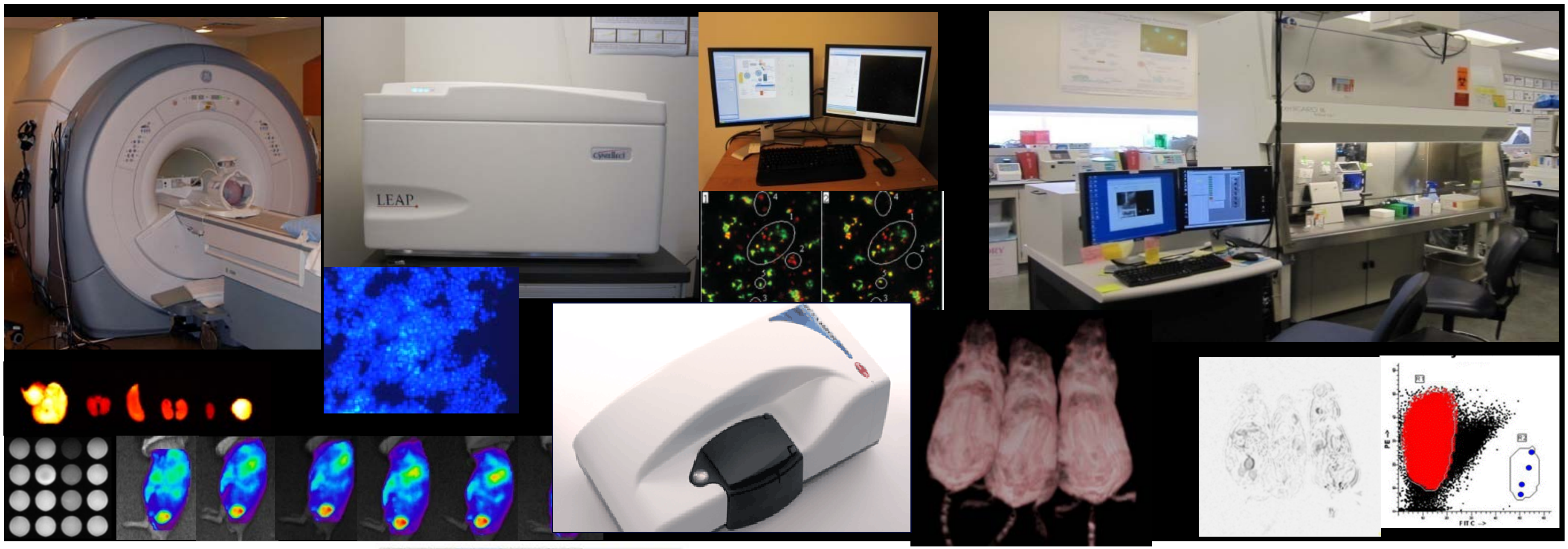
How can we build and evaluate these nanomedical devices ?

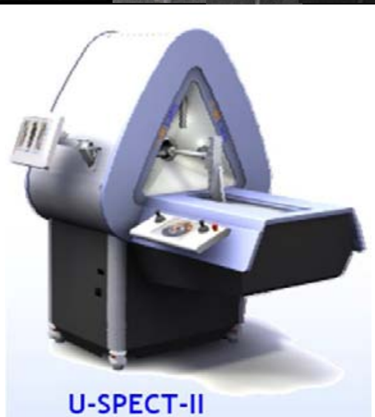
Biomimicry – Let nature provide Some of the Answers!

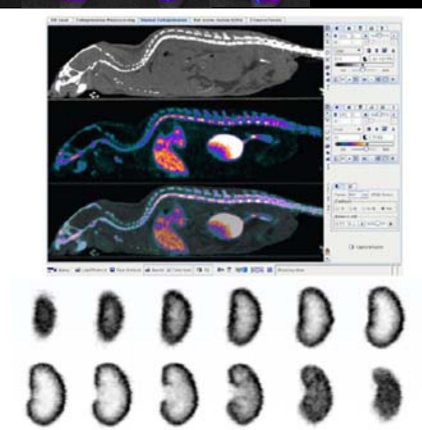


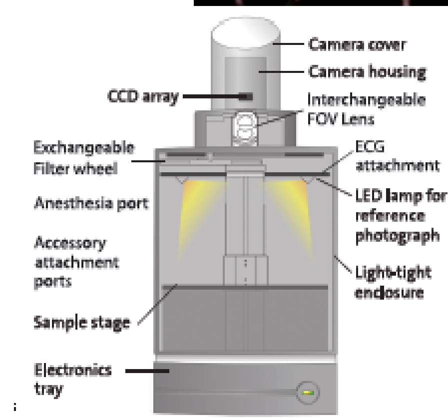
Viruses know how to perform a multi-step targeted process to infect cells, use the host cell machinery to produce gene products, and make copies of themselves. What if we could make a synthetic, self-assembling, “good virus” that could deliver therapeutic gene templates to specific cells, and use the 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!) ?

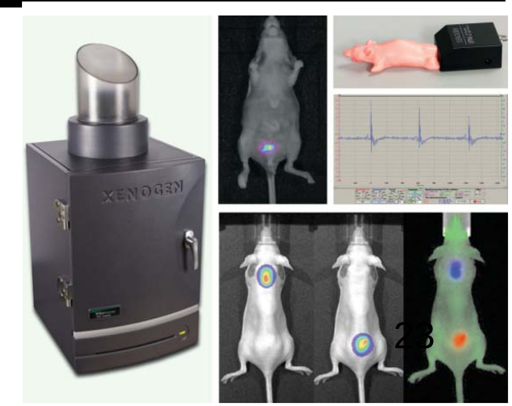
"Nanotools" for Development and Evaluation of Nanomedical Devices











Interactions Between Technologies for Development of Nanomedical Systems

Nanoparticle fabrication and quality control labs

- Nanochemistry
- Dynamic Light scattering sizing
- Zeta Potential
- Atomic Force Microscopy

Cell and intracellular targeting labs

- Flow cytometry
- Imaging (laser opto-injection and ablation) cytometry
- Confocal (one- and multi-photon analysis)

Transient Gene Therapy ("gene drugs")

- Construction of therapeutic genes for specific biomedical applications
- Animal testing/comparative medicine
- Human clinical trials

Nanomaterials biocompatibility labs

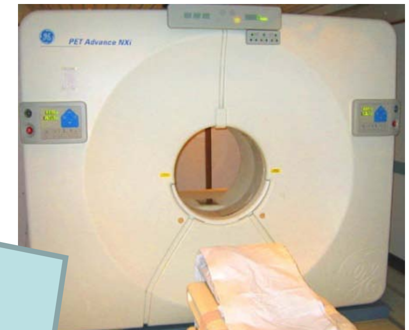
- Microscopy/image analysis/LEAP
- Gene expression microarray analyses

Biosensor Labs

- Biosensor molecular biology
- Results evaluated in targeting labs

Molecular Imaging Modalities

Magnetic Resonance Imaging



Positron Emission Tomography

Resolution

Penetration Depth

Sensitivity

Information

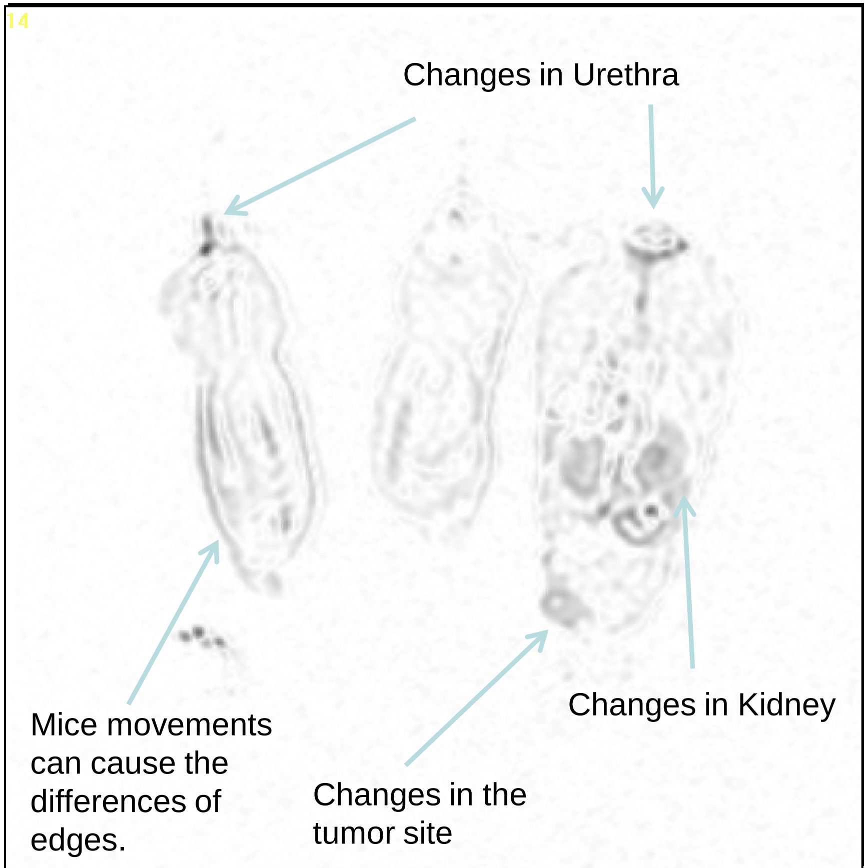
Clinical Use

Computed Tomography

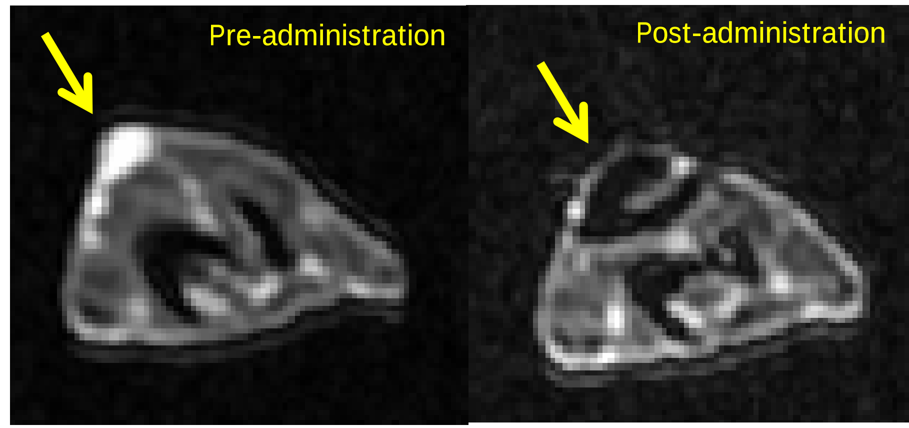
Optical Imaging

Work of Jaehong Key

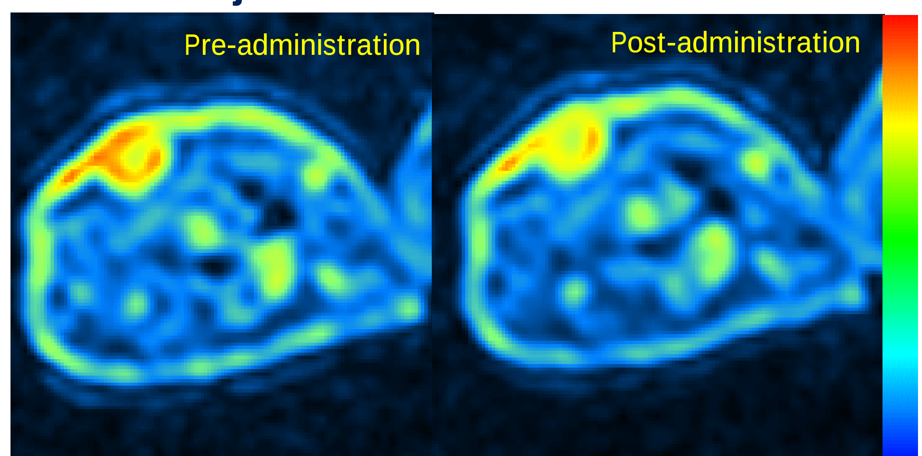
In vivo imaging of human tumors in nude mice



Positive Control



Tail Vein Injection



But remember, “real animals (and most humans) have active immune systems!

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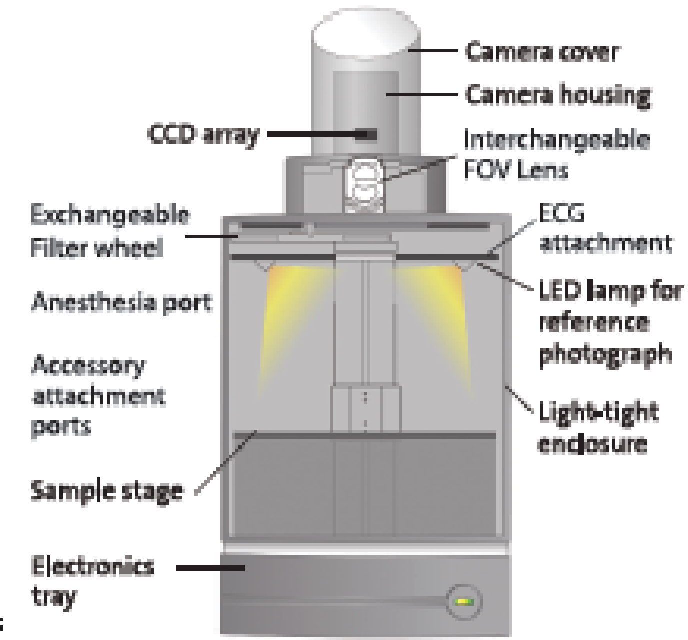
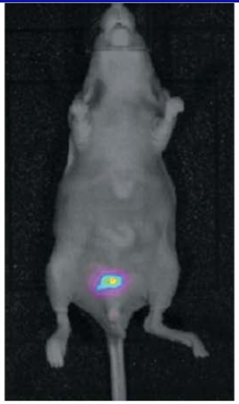
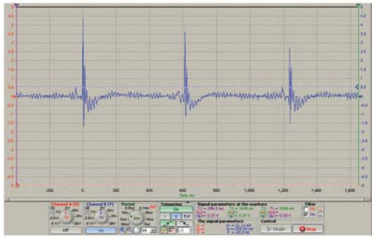
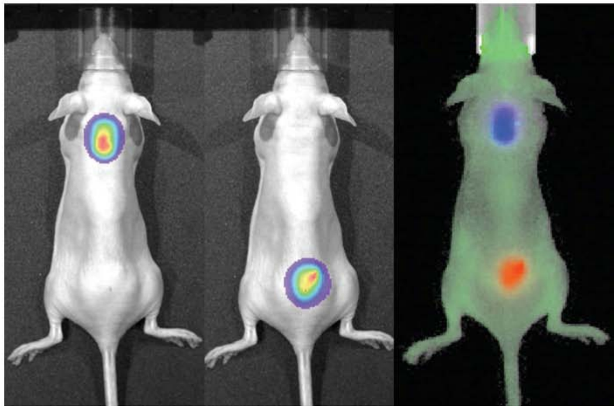
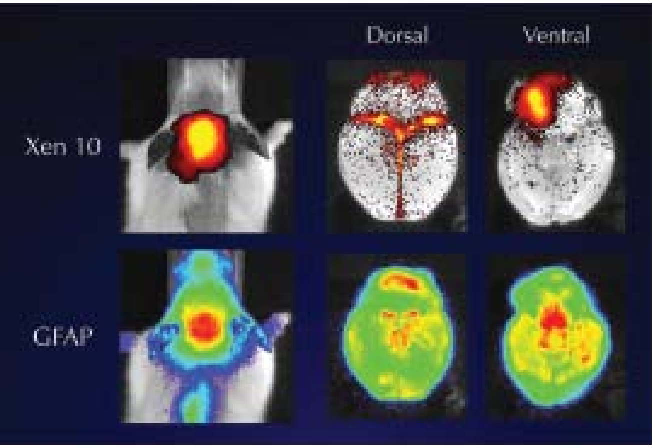
Near Infrared Fluorescence (NIRF) Imaging





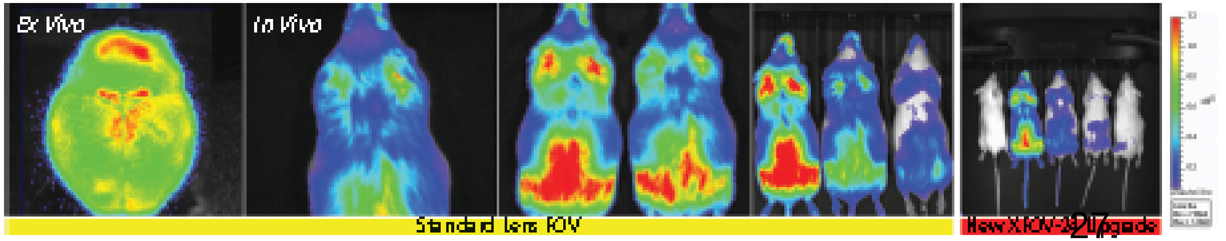






Dual Reporter Imaging - High Resolution
Ex Vivo Applications

Field of View



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

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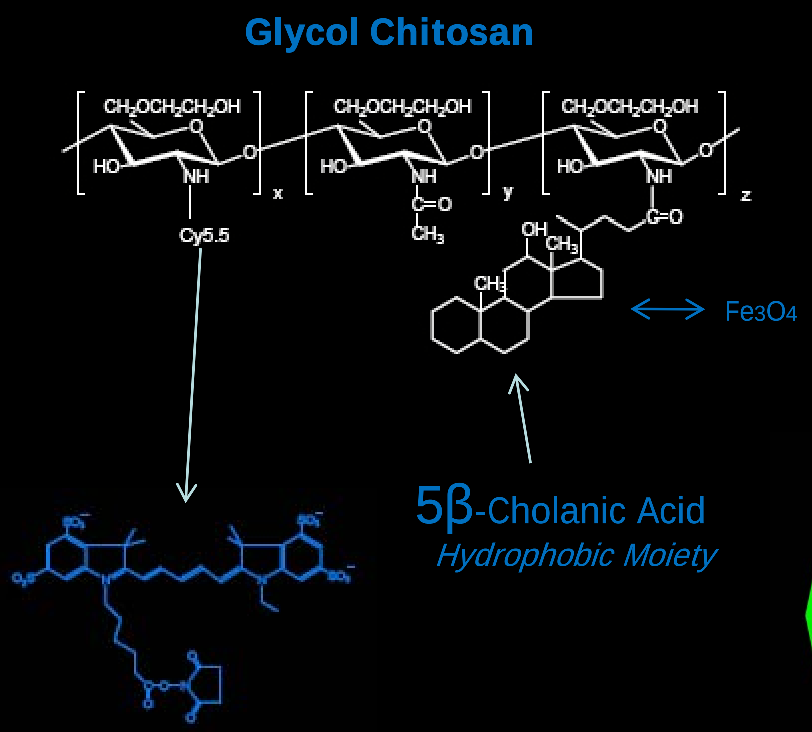
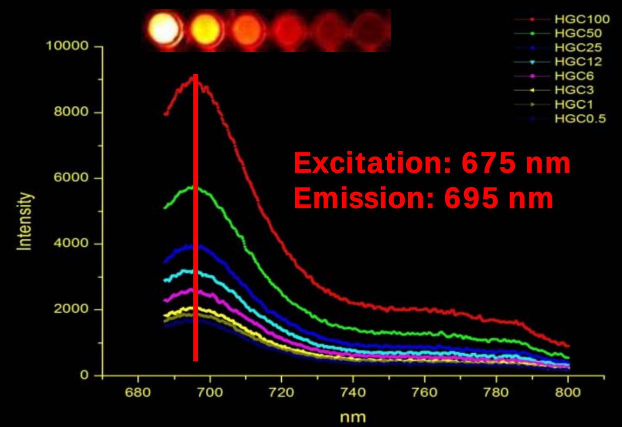
HGC - Cy5.5 - SPIO Nanoparticles



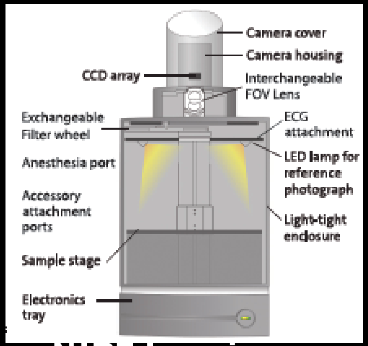


Amphiphilic glycol chitosan-cholanic acid conjugates self-assembled to form glycol chitosan nanoparticles (HGC NPs) in aqueous solution. SPIOs were loaded into HGC NPs by hydrophobic interactions.

Work of Jaehong Key

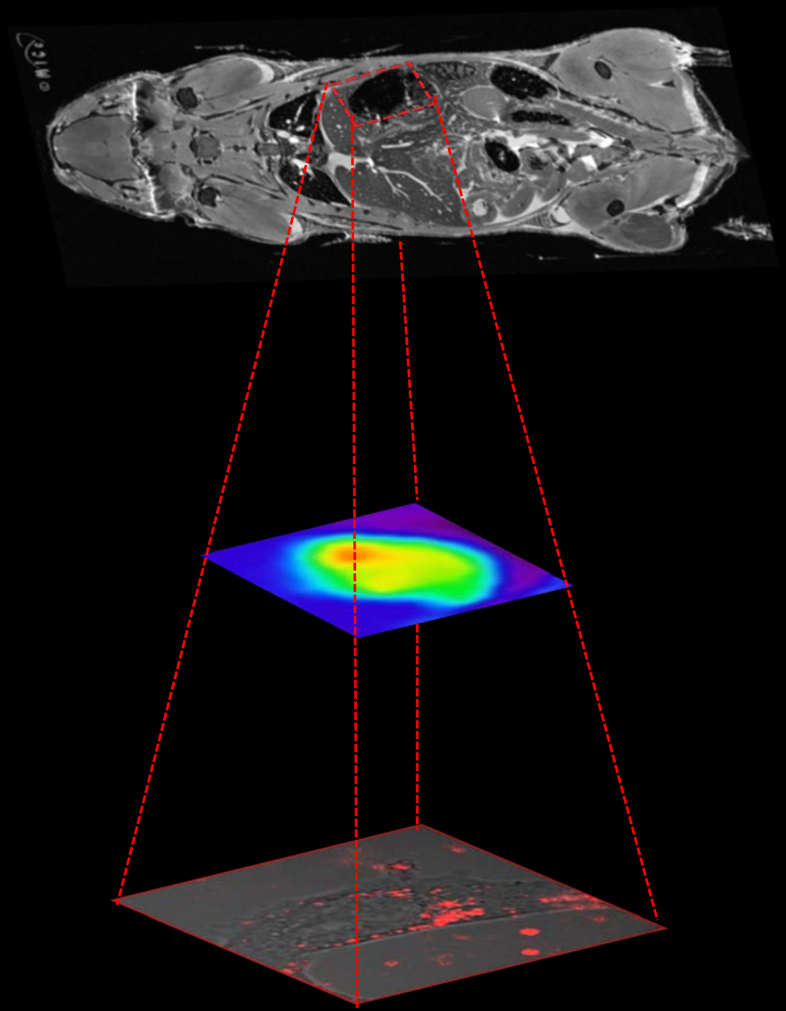
Combination Technology with MR, NIRF, and Confocal Images

MR Imaging



NIRF Imaging

Confocal Imaging



A Whole Body Imaging

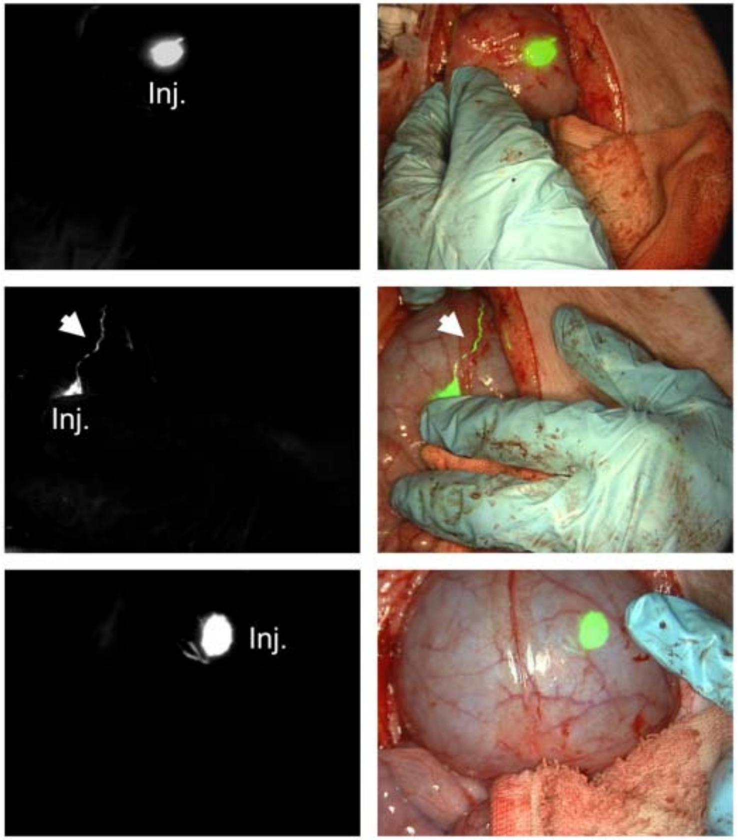
↓

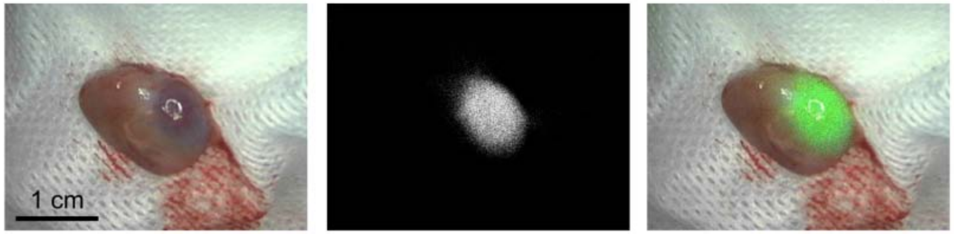
Specific tumors

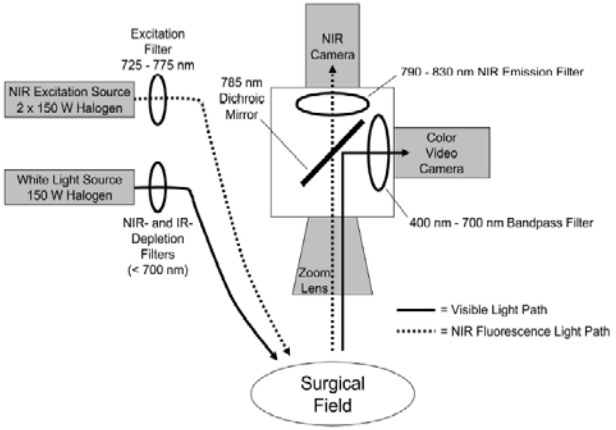
↓

Nanoparticles in each tumor cell

Near Infrared Fluorescent Imaging for fluorescence-guided surgery

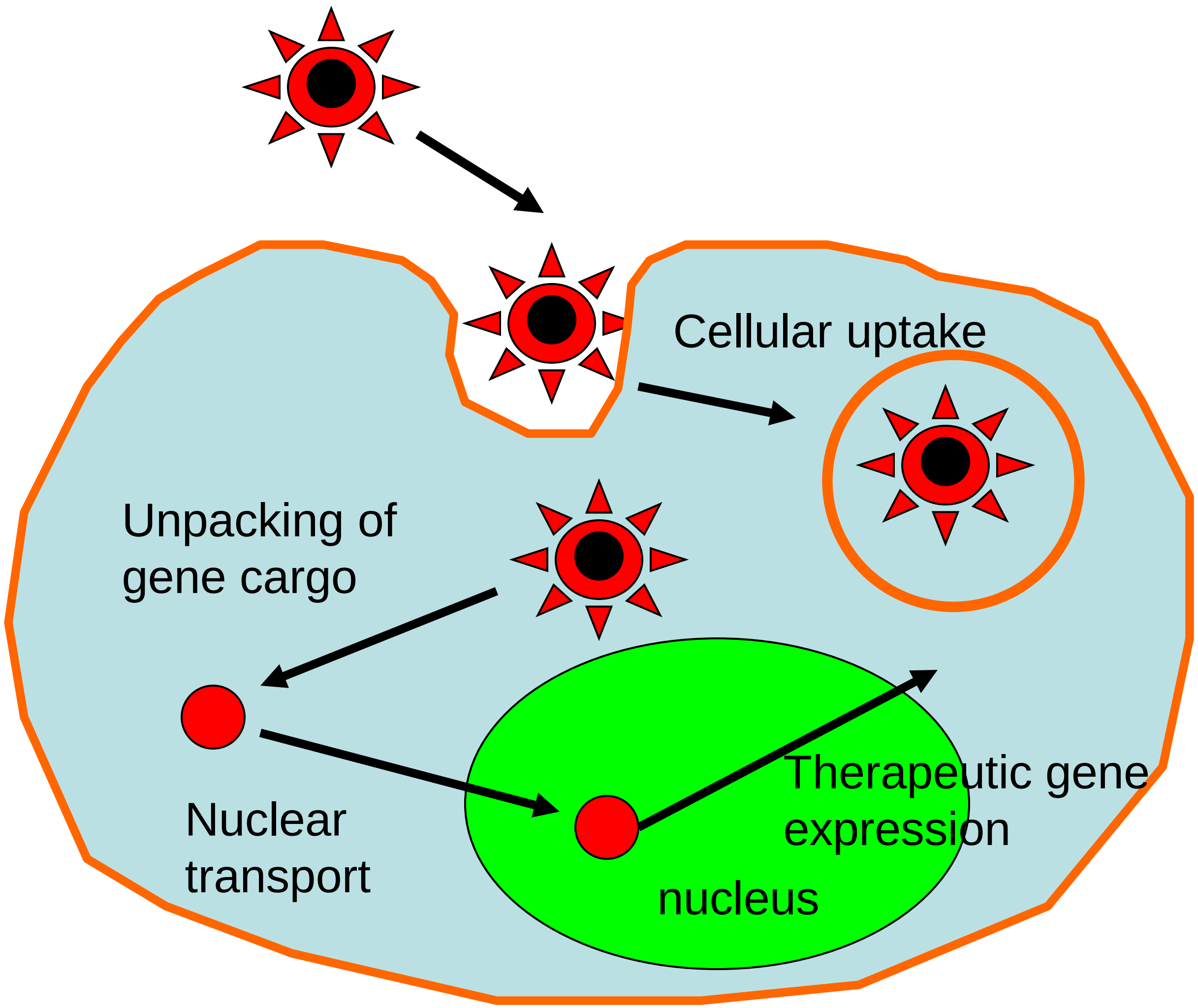




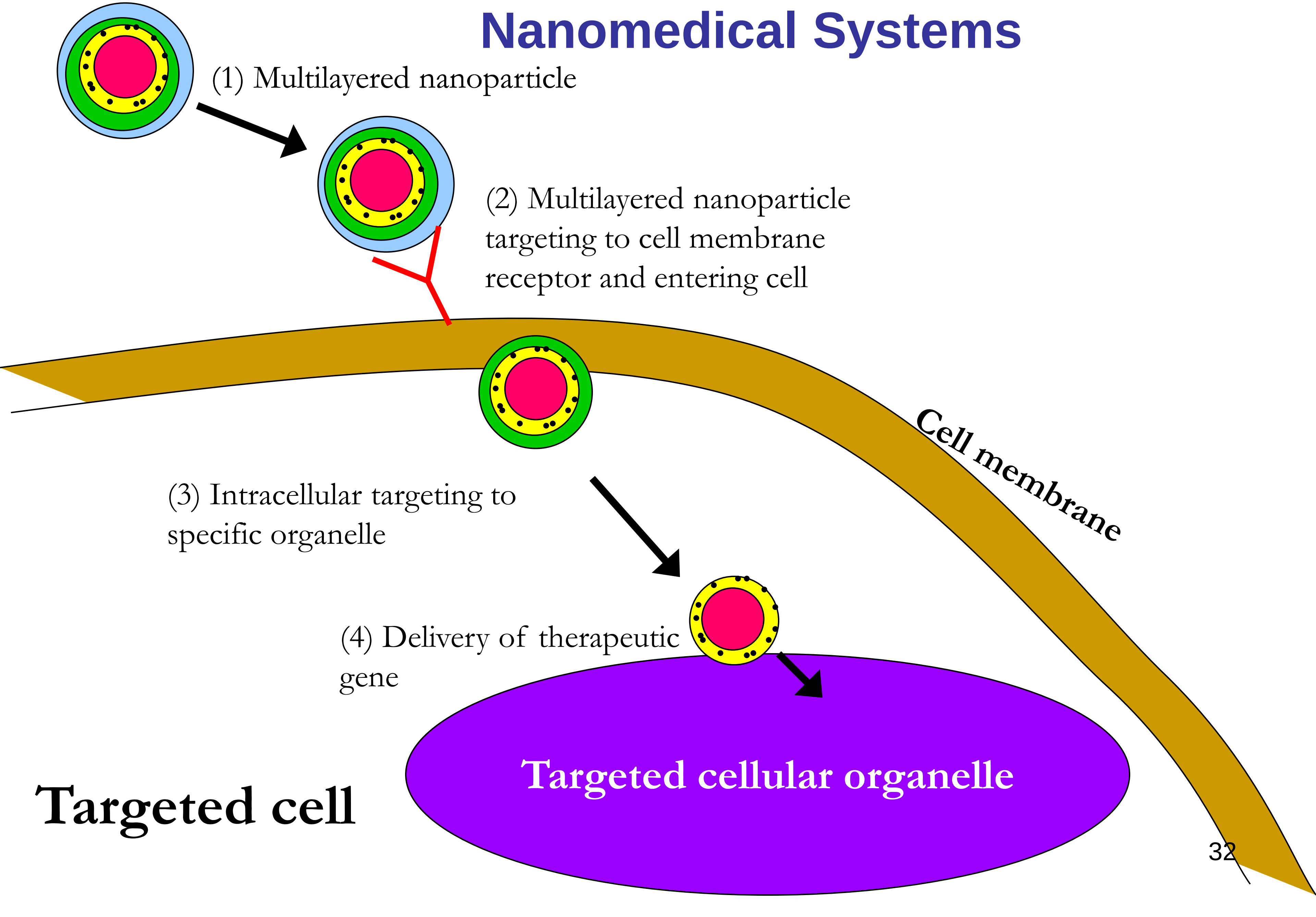


Deborah W. Knapp, European Urology, 2007

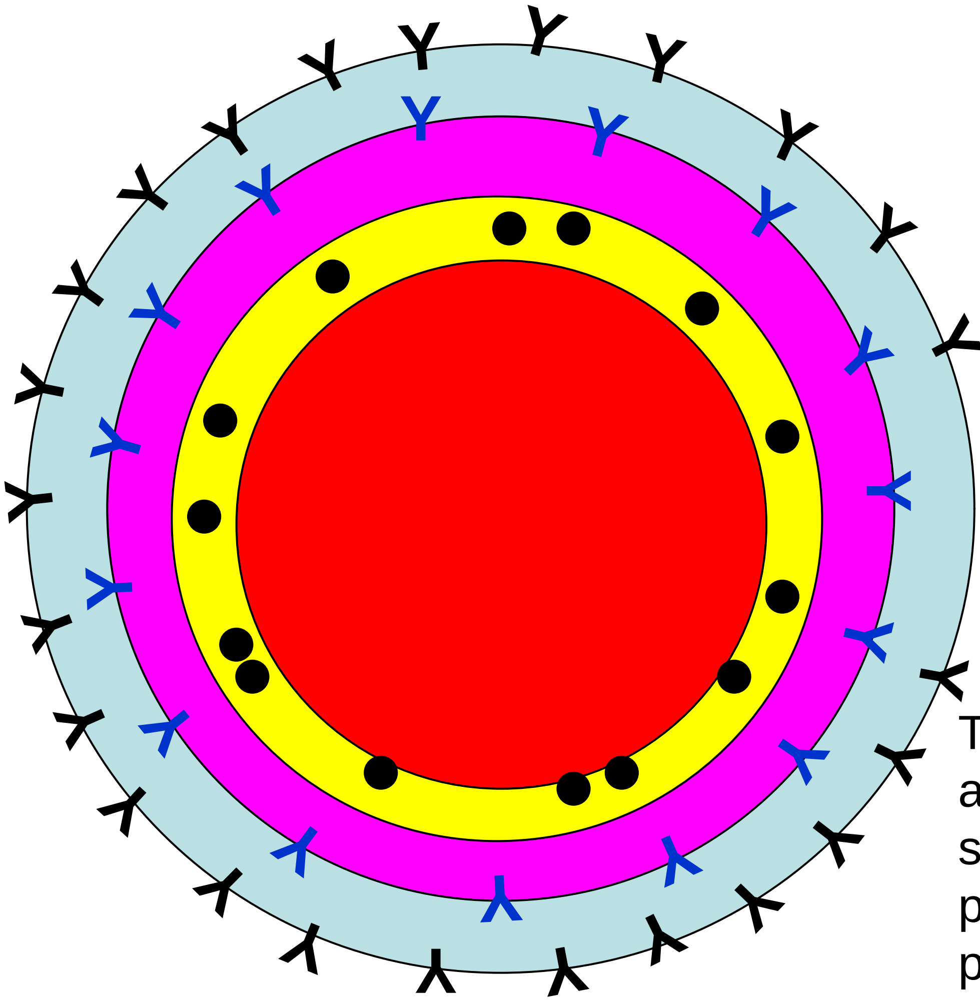
Multi-step Gene Delivery Process in Cells



The Multi-Step Targeting Process in Nanomedical Systems



Building Smart Nanomedicine Systems for Multi-step Control of Gene/Drug Delivery within Single Cells



Cell targeting and entry

Intracellular targeting

Therapeutic genes

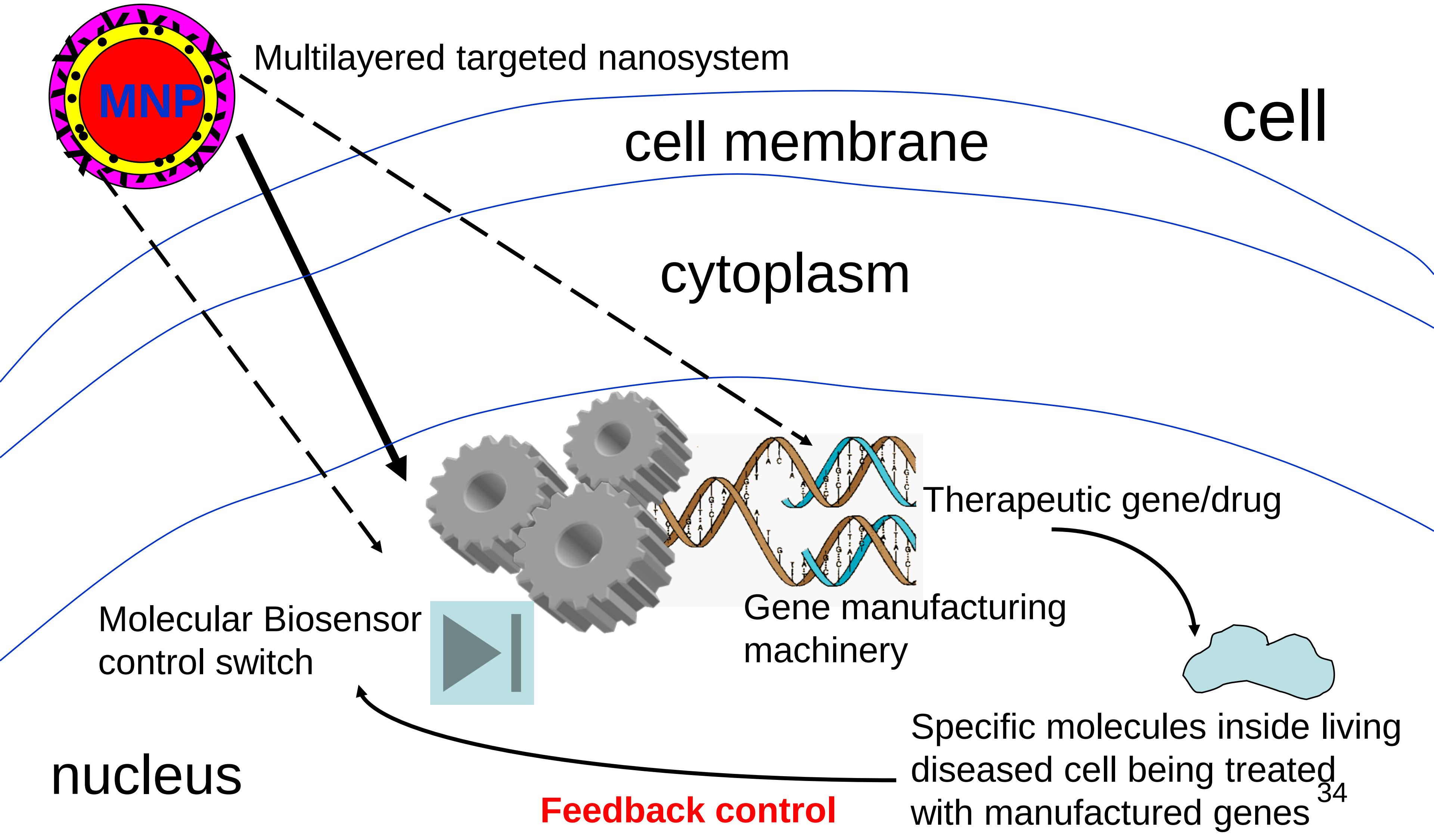
Magnetic or Qdot core (for MRI or optical imaging)

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

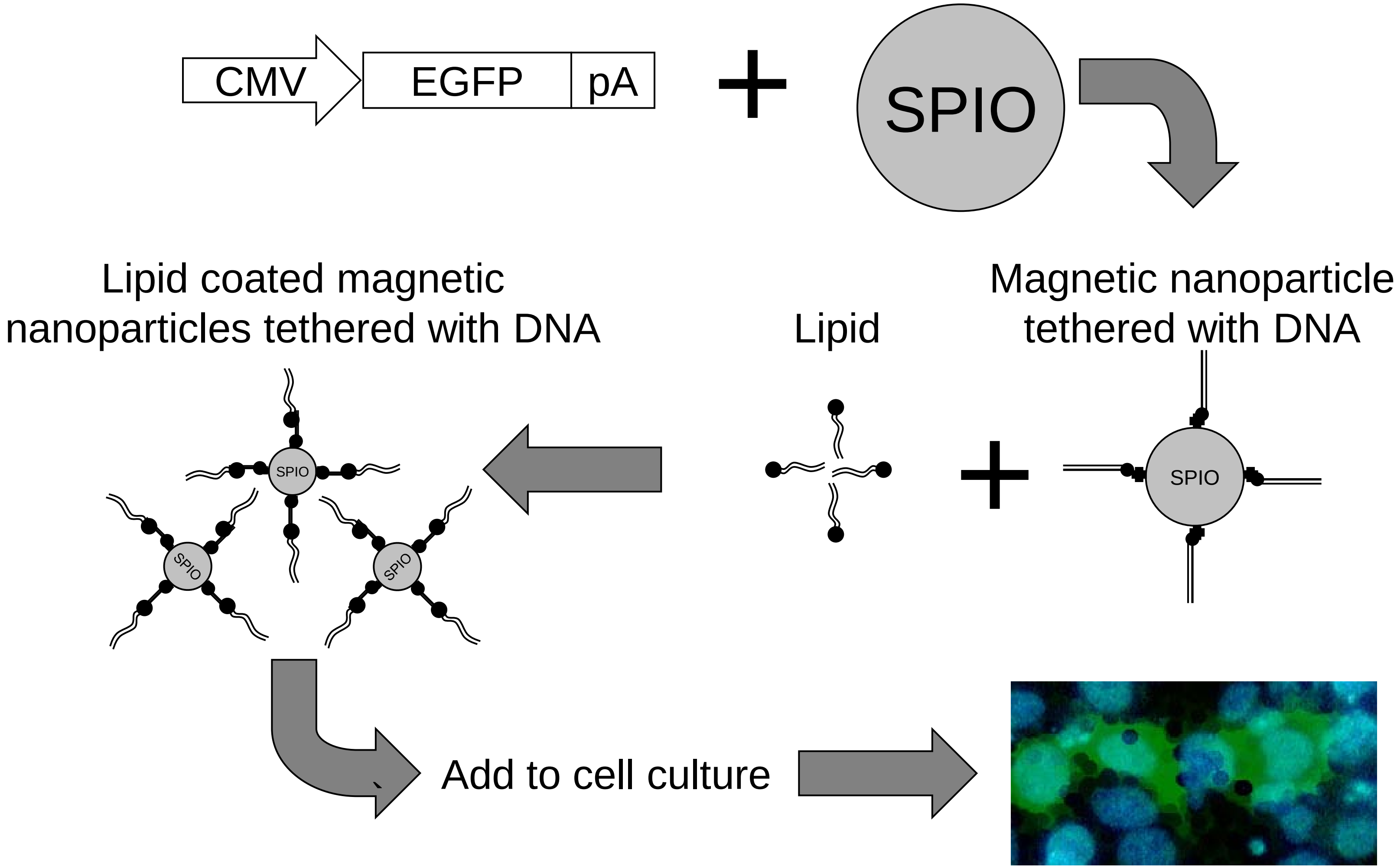
N.B. These nanodevices thermodynamically self-assemble under the proper experimental conditions and disassemble in-vivo in a predictable pattern.

Manufacturing Therapeutic Agents Inside Living Cells

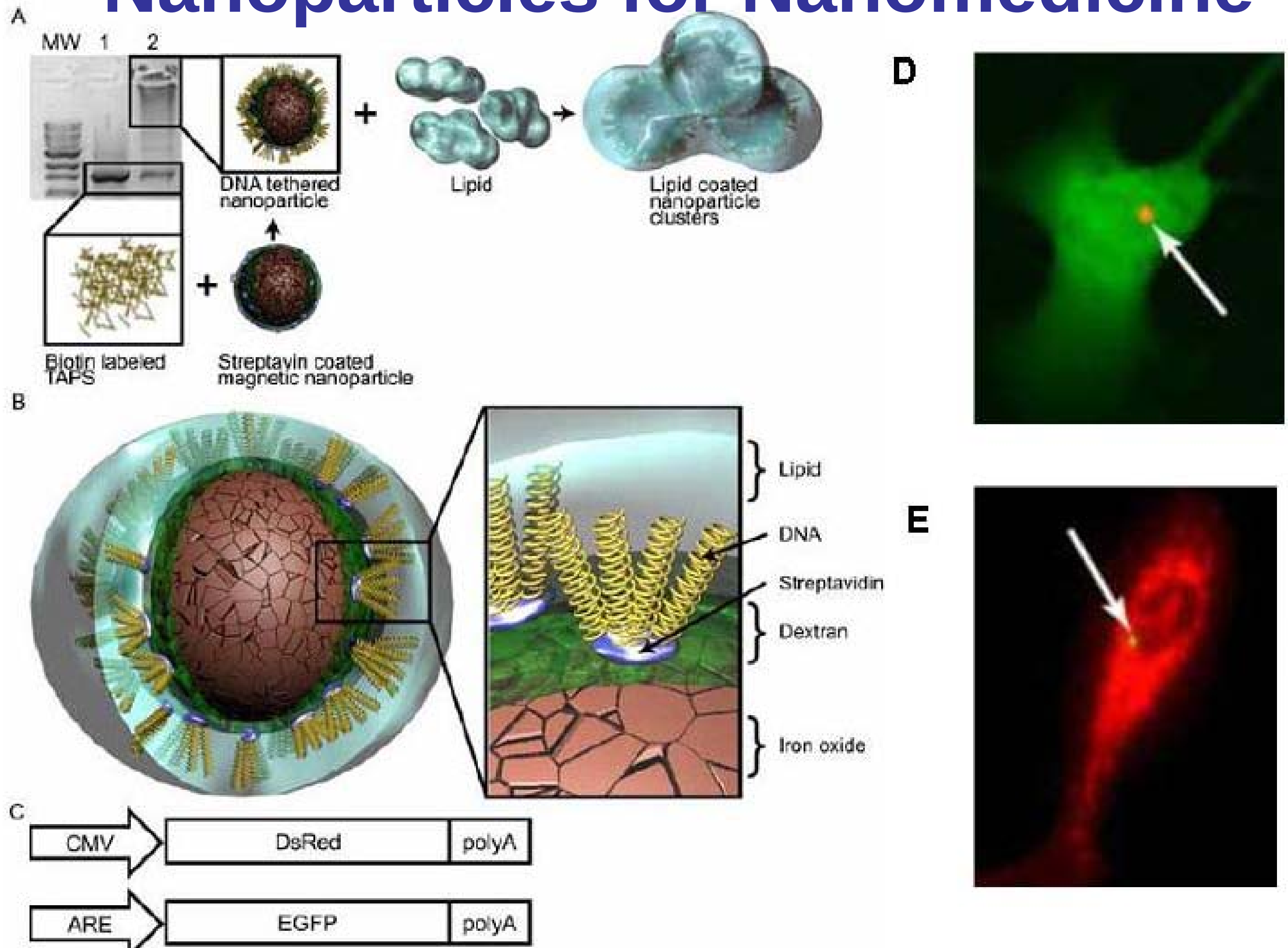


Efficient Gene Transfer with DNA Tethered Magnetic Nanoparticles

PCR product bioconjugated to magnetic nanoparticle



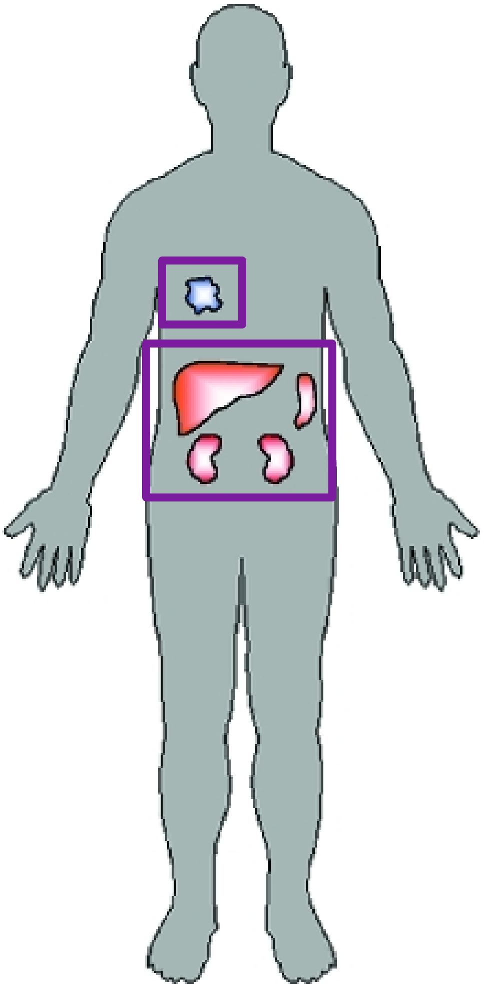
Tethered Gene Expression on Magnetic Nanoparticles for Nanomedicine



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.

Where do the nanoparticles (NPs) go in the body?



<u>Diseased Organ (Tumor):</u>

-Improve biodistribution in tissue through passive (EPR) and active (ligand functionalization) targeting

- Smaller particle size increases accumulation and enhances diffusion within tissue

<u>Liver and Spleen</u>

- Clearance by phagocytic uptake and hepatic filtration

- Improve circulation half-life through particle sizes $\leq$ 100 nm and negative or neutral surface charge

<u>Kidneys</u>

- Clearance through excretion

- Improve circulation half-life through particle sizes $\geq$ 10 nm

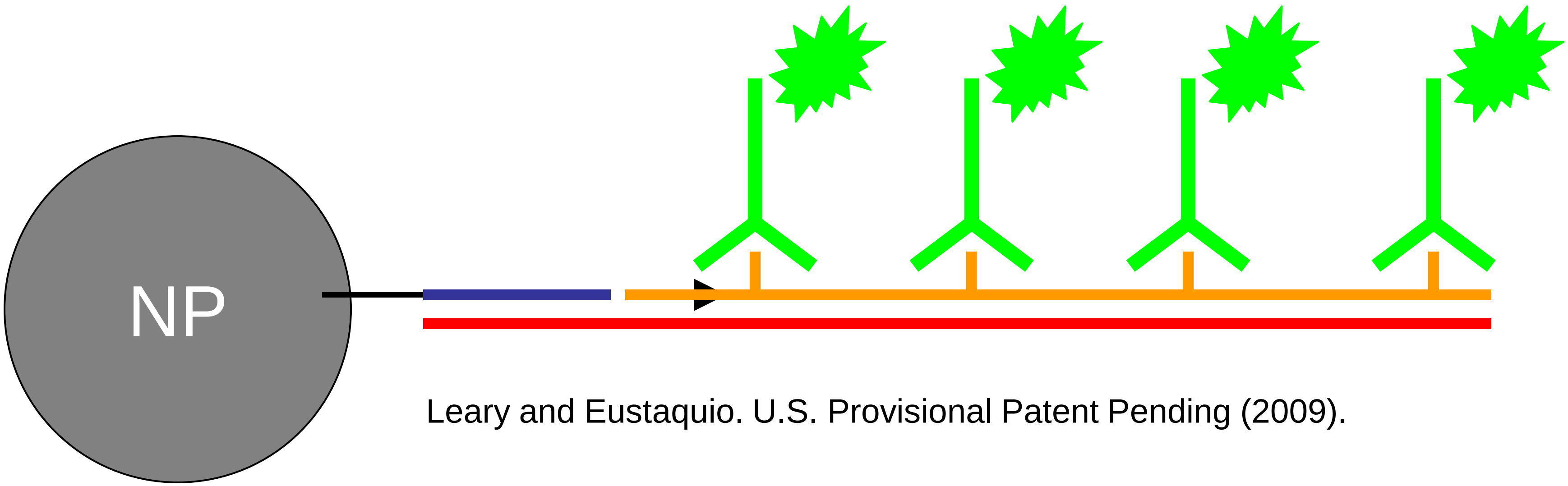
Alexis *et al.* (2008)

NP biodistribution =

Method of tracking where NPs travel in an experimental animal or human subject

The *in situ* PCR technique can be adapted to the detection of single NPs inside single cells

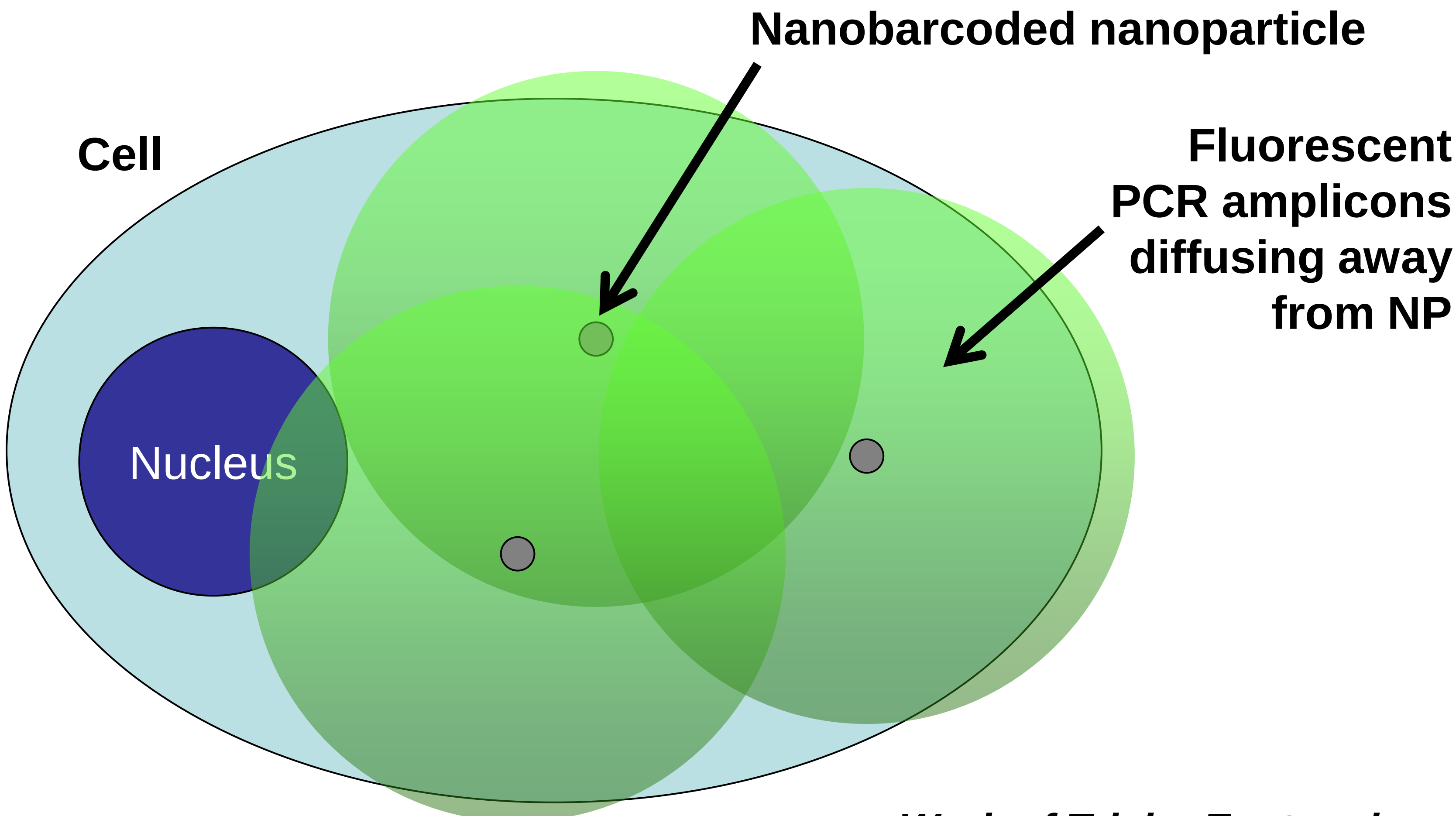
We have invented a novel method for single NP detection called "nanobarcoding" that incorporates an oligo on the NP surface for use as a unique "nanobarcode" (NB).



Leary and Eustaquio. U.S. Provisional Patent Pending (2009).

Work of Trisha Eustaquio

Labeled amplicons drift and form diameter of detectable signal around each NP



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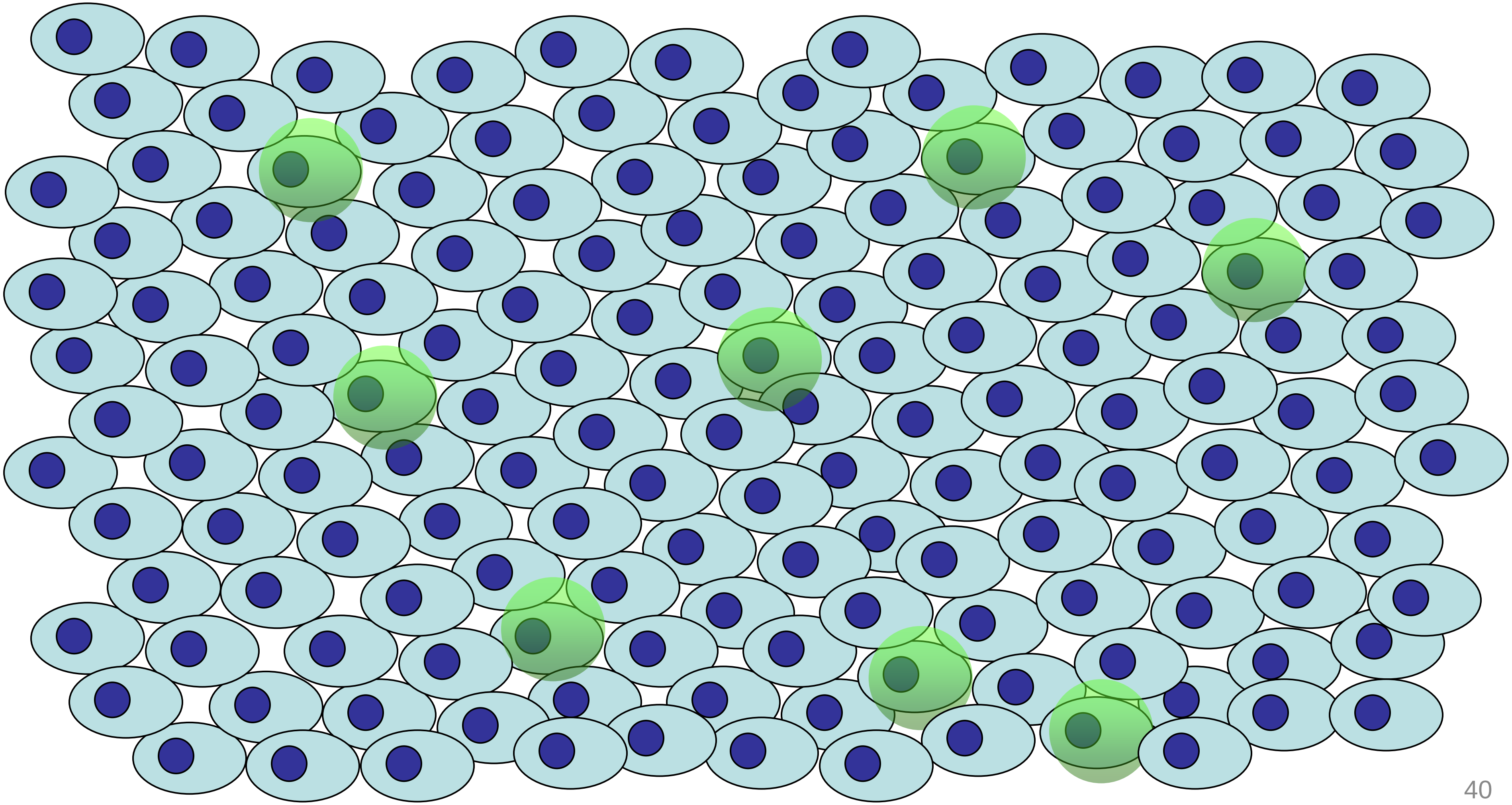
A researcher can quickly determine which cells in a tissue section contain internalized NPs and can analyze by high-throughput imaging



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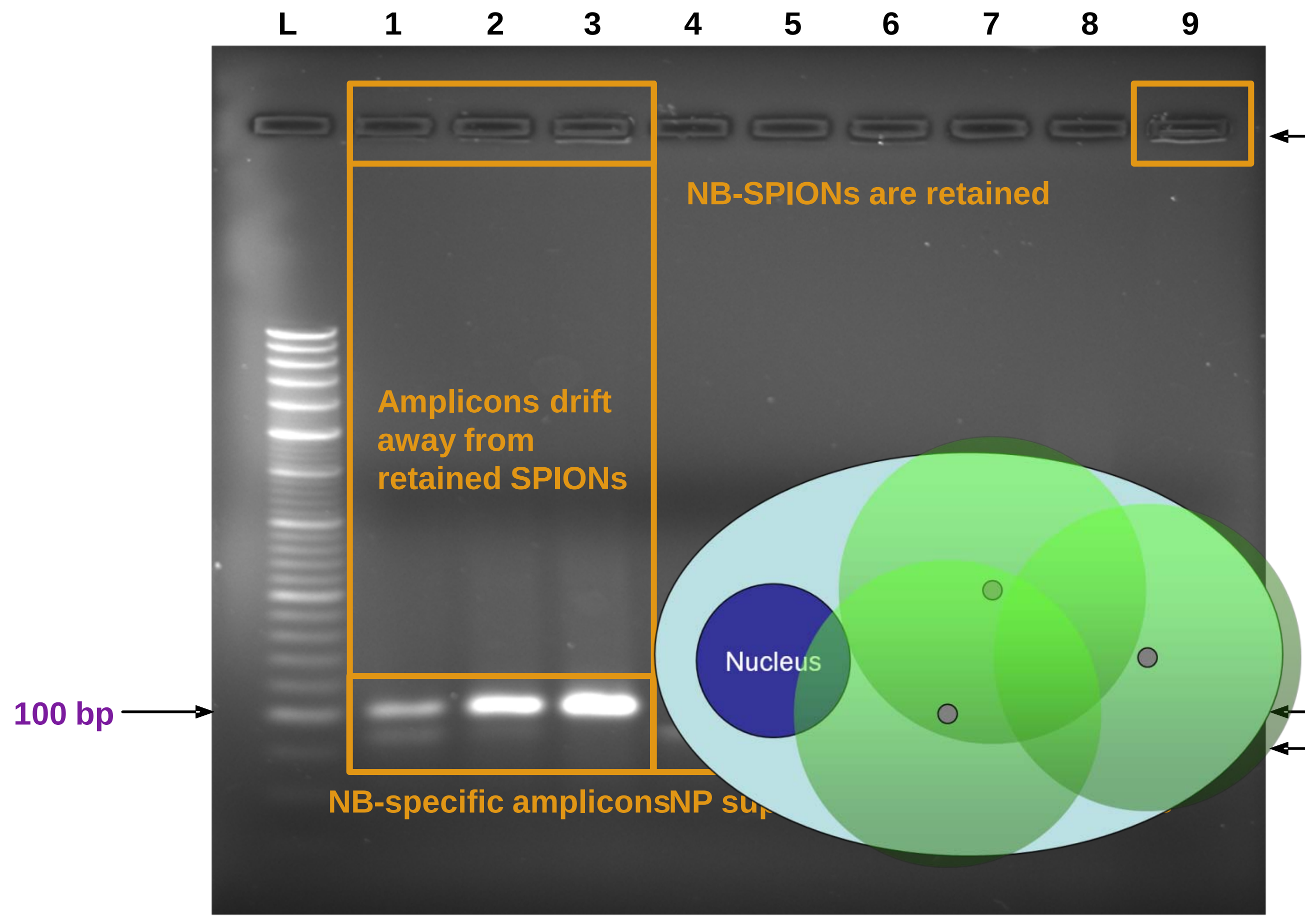
Specification: Amplified signal is specific to NP
No non-specific amplification from NB-SPION supernatant



L	100-bp marker
1-3	NB-SPIONs
4-6	NB-SPION supernatant
7	H_2O (no NB control)
8	Free NB (positive control)
9	NB-SPIONs (no PCR control)

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Summary: Importance of Nanobarcoding

- Ability to rapidly locate sub-optical nanoparticles over large areas in tissues and organs

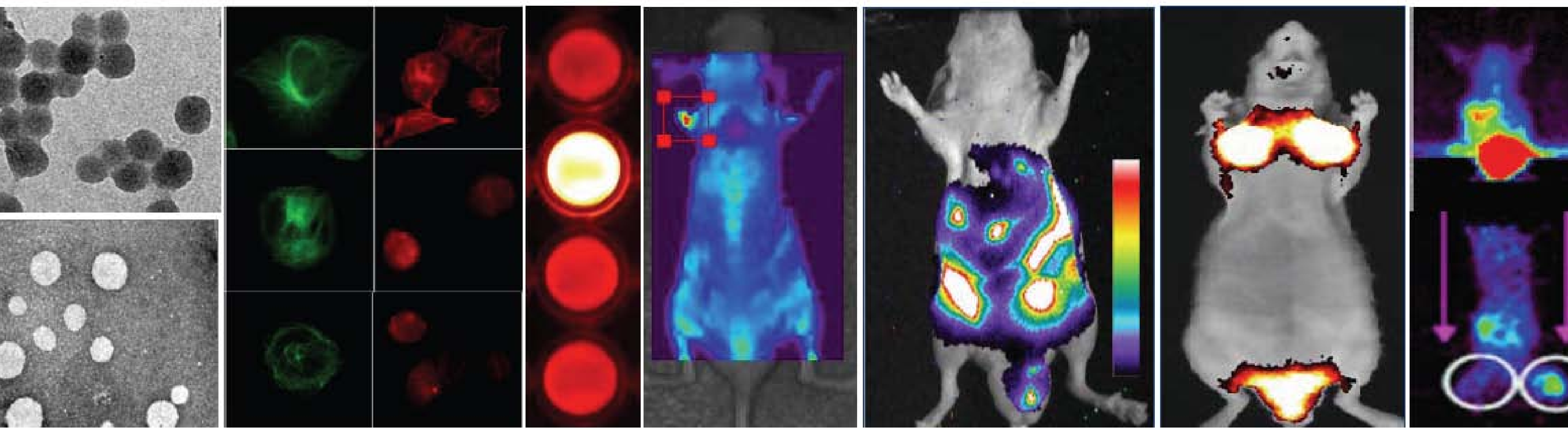
- A method to allow for high-throughput imaging assays for semi-quantitative biodistribution studies

- Can use different nanobarcodes encoding different experimental information (e.g. different targeting, different drugs, different times of administration, …) to perform multiple experiments in a single animal to reduce animal-to-animal variations.

- Allows more information to be obtained from each animal thereby minimizing the number of animals used for experiments

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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PURDUE

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Kinam Park (Purdue)
Alex Wei (Purdue)

Nanotoxicity studies
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James Klaunig (IU-SOM)

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

Mol. Imaging/Theranostics
Kuiwon Choi (KIST)
Ich Chan Kwon (KIST)
Kwangmeyung Kim (KIST)

KIST=Korean Institute of Science and Technology (many others):
*Recently graduated ** Former student

Director: James Leary

Lisa Reece (SVM) – nanomedicine repair of traumatic brain injury

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)-MRI imaging
Teimour Maleki, PhD – micro- and nanofabrication; BioMEMS
Desiree White (BSDT): nanomedical systems for treating spinal cord injury
Michael Walls (BMS): nanomedical systems for treating spinal cord injury
Abigail Durkes (SVM/CPB) tissue pathology for nanomedicine

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Atomic Force Microscopy
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Steve Wereley (Purdue)
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