

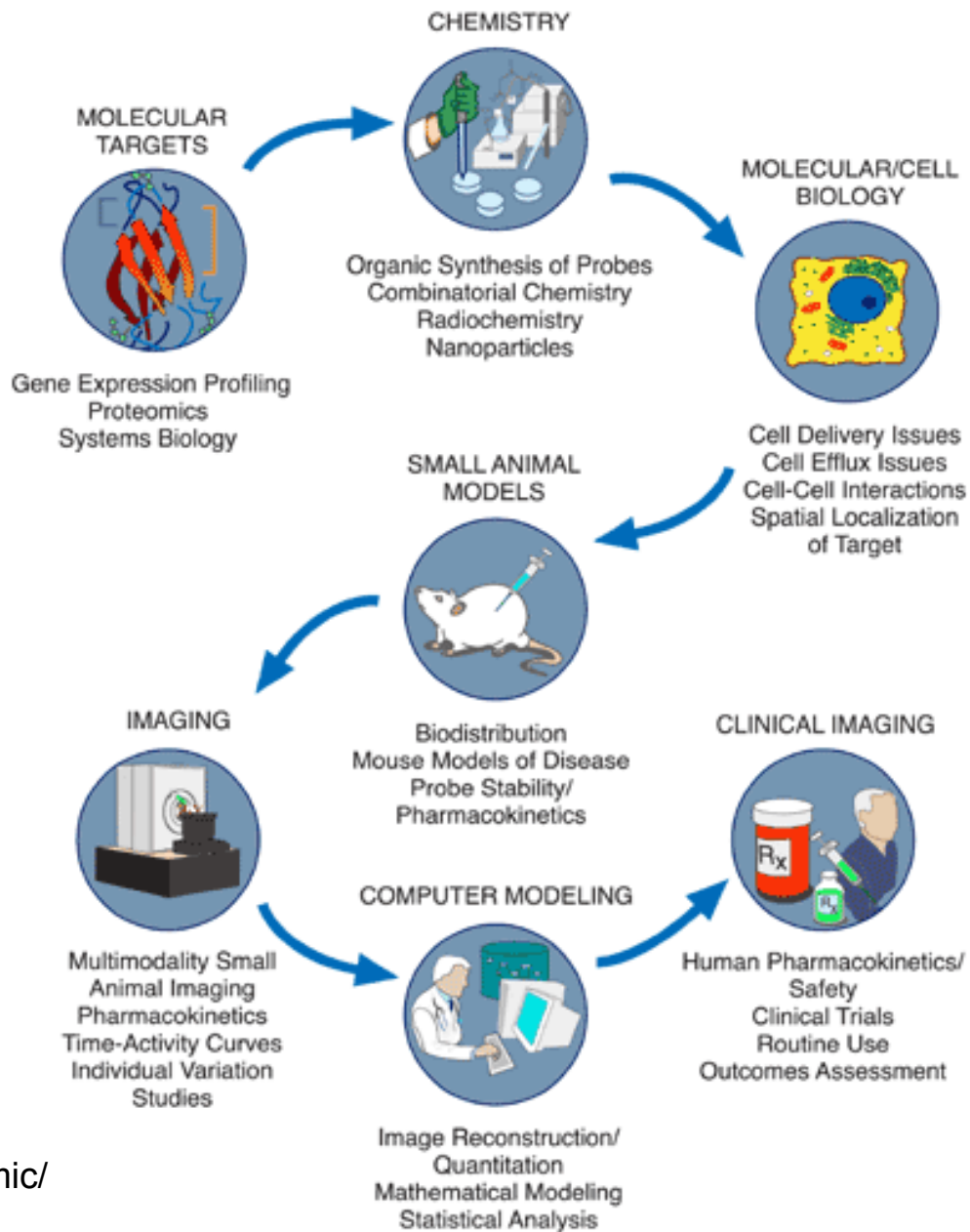
# Fluorescent Nanoparticles for In Vivo Molecular Imaging

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July 17, 2012  
Madrid, Spain

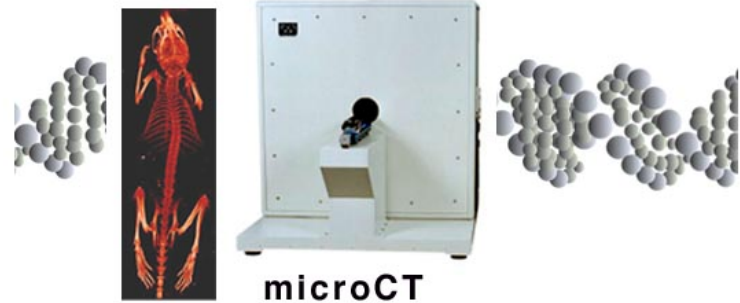
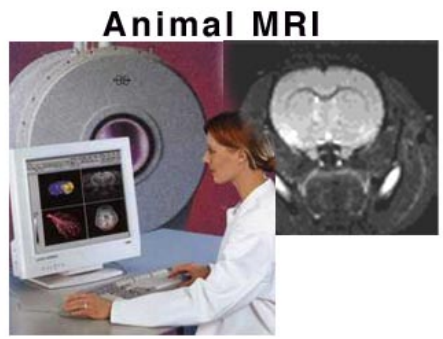
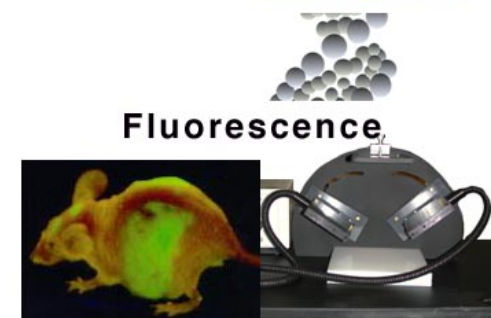
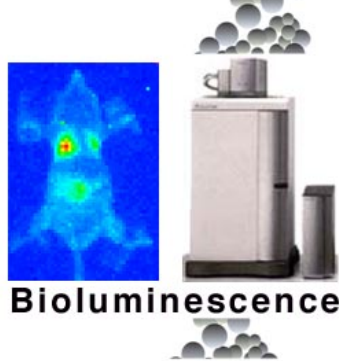
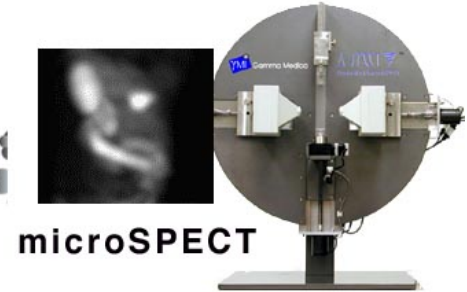
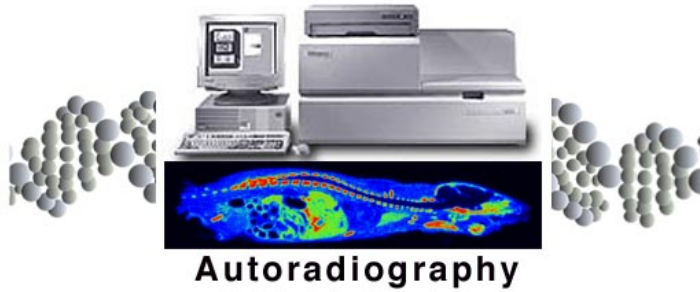
# Molecular Imaging in Living Subjects



# Why Molecular Imaging?

- Examining the molecular targets at their native environment
- Revealing spatiotemporal information
- Imaging gene expression, protein-protein interactions, metabolic pathway, cell tracking, differentiation, ...
- Facilitating drug development and target validation in pre-clinical animal model
- Disease detection and diagnostics: cancer, infectious diseases, cardiovascular diseases, neuro diseases, ...
- Treatment monitoring ...

# Molecular Imaging in Mice

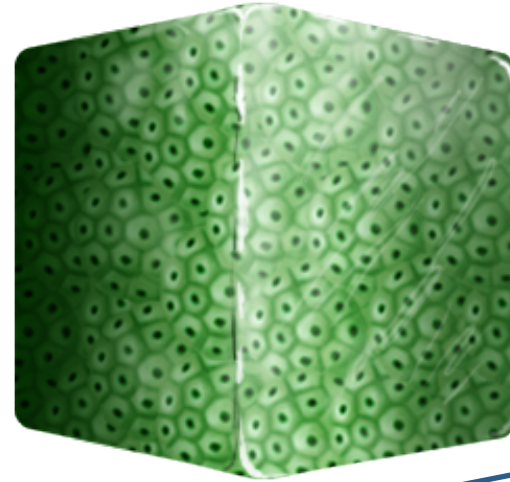
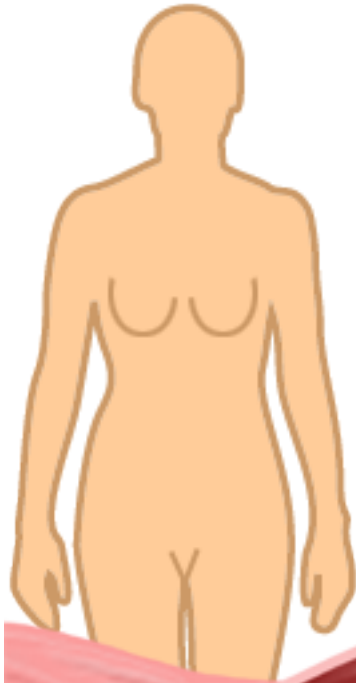




# Current Imaging Instrumentation

PROS AND CONS OF IMAGING MODALITIES								
Technique	Labels	Signal measured	Strengths	Weaknesses	Cost	Throughput	Sensitivity (moles of label detected)	Resolution
PET	Radiolabelled molecules	Positrons from radionuclides	Highly sensitive	Can detect only one radionuclide, requires radioactivity	High	Low	$10^{-15}$	1-2 mm
SPECT	Radiolabelled molecules	$\gamma$ -rays	Can distinguish between radionuclides, so more processes can be imaged at once	Requires radioactivity	High	Low	$10^{-14}$	1-2 mm
CT	None	X-rays	Fast, cross-sectional images	Poor resolution of soft tissues	High	Low	$10^{-6}$	50 $\mu$ m
MRI	Can use isotope-labelled molecular tracers	Alterations in magnetic fields	Harmless, high-resolution of soft tissues	Cannot follow many labels	High	Low	$10^{-9}$ - $10^{-6}$	50 $\mu$ m
Optical	Genetically engineered proteins and bioluminescent and fluorescently labelled probes	Light, particularly in the infrared	Easy, non-damaging technique readily adapted to study specific molecular events	Poor depth penetration	Low	High	$10^{-12}$	1-2 mm
Photoacoustic	Probes that absorb light and create sound signals	Sound	Better depth resolution than light	Information processing and machines still being optimized	Low	High	$10^{-12}$	50 $\mu$ m
Ultrasound	Microbubbles, which can be combined with targeted contrast agents	Sound	Quick, harmless	Poor image contrast, works poorly in air-containing organs	Low	High	$10^{-8}$	50 $\mu$ m

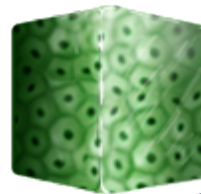
# Earlier Cancer Detection



10 mm

10 mm

$10^3 \text{ mm}^3$   
= 0.5-3 billion cells



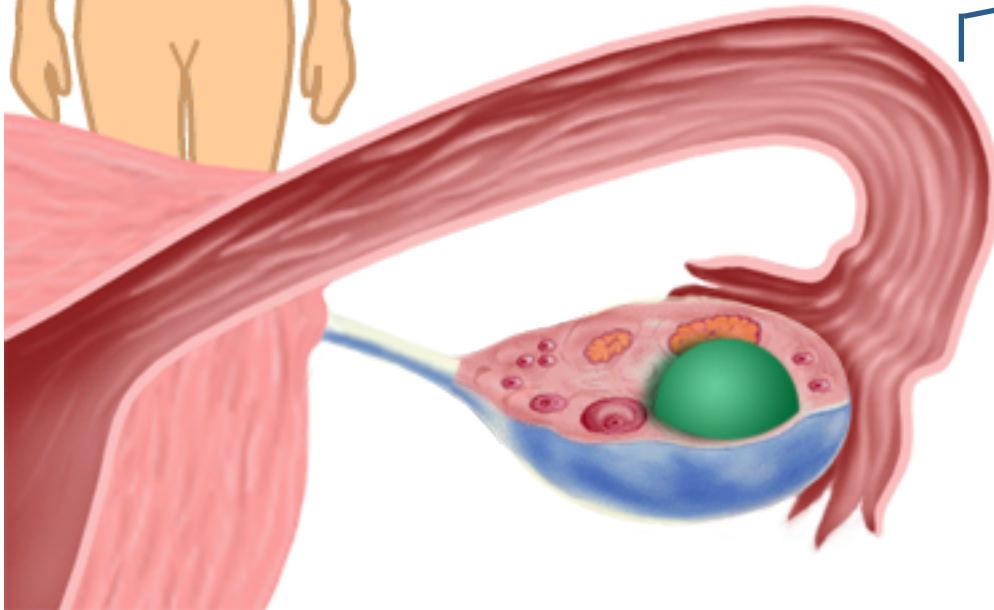
1 mm

1 mm

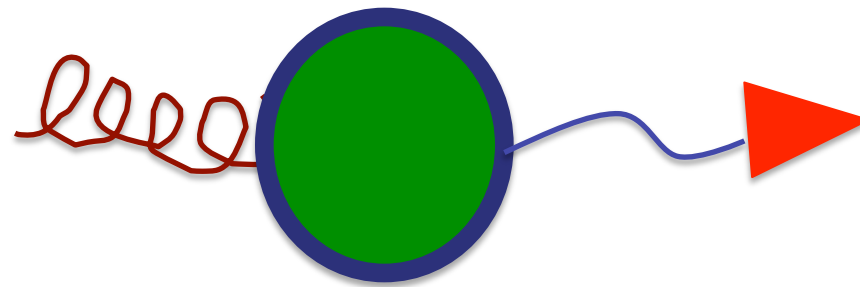
$1^3 \text{ mm}^3$   
= 0.5-3 million cells



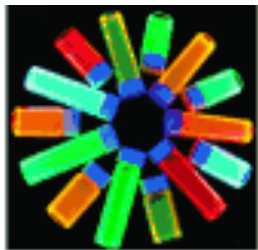
$0.1^3 \text{ mm}^3$   
= 500-3,000 cells



# Nanoparticles for Molecular Imaging



*As an imaging label, NPs possess high signal density*



Quantum Dot



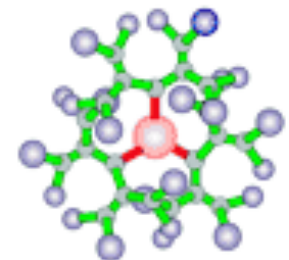
Nanoshell



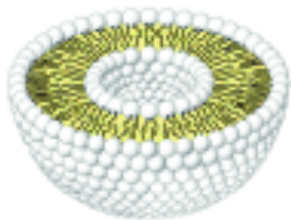
Gold



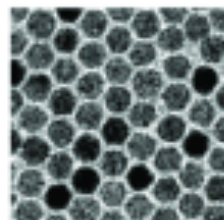
Nanotube



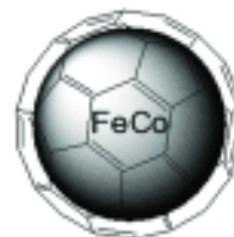
Dendrimer



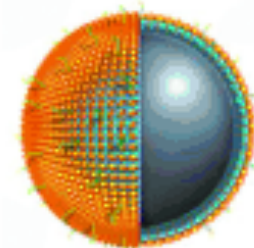
Liposome



Iron Oxide

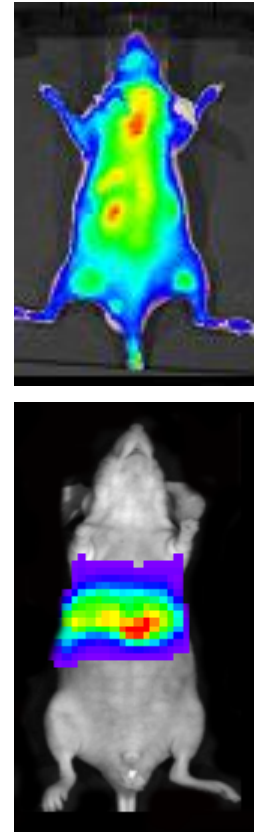


FeCo



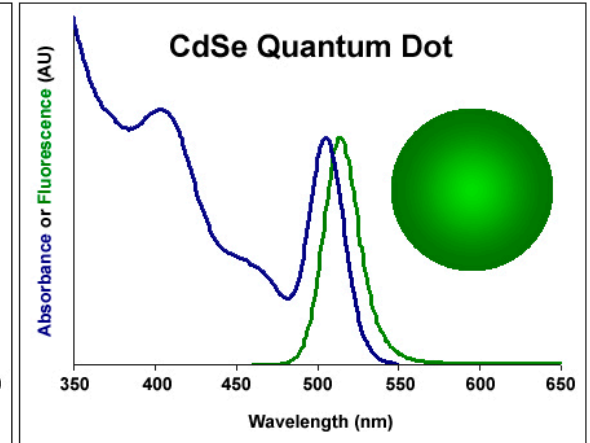
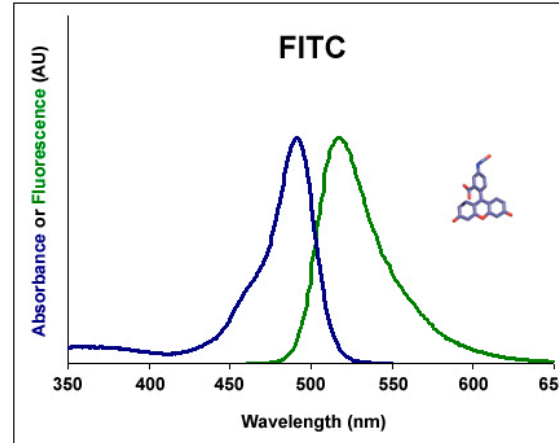
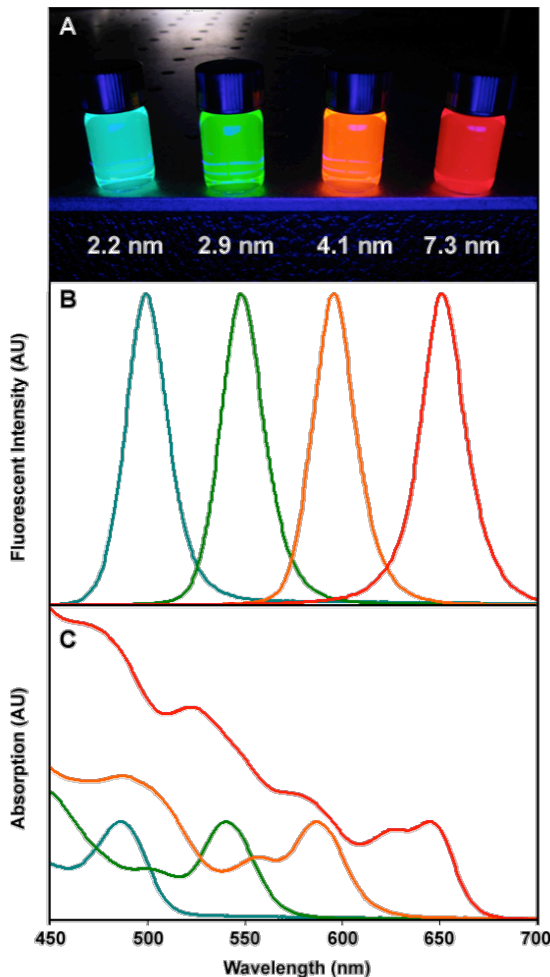
Perfluorocarbon

# Optical Imager for Small Animals



- Fluorescence: excitation light
- Bioluminescence: no excitation light, but requires the injection (i.v. or i.p.) of substrates (D-luciferin, coelenterazine) for each imaging

# Quantum Dots: Fluorescent Semiconductor Nanocrystals

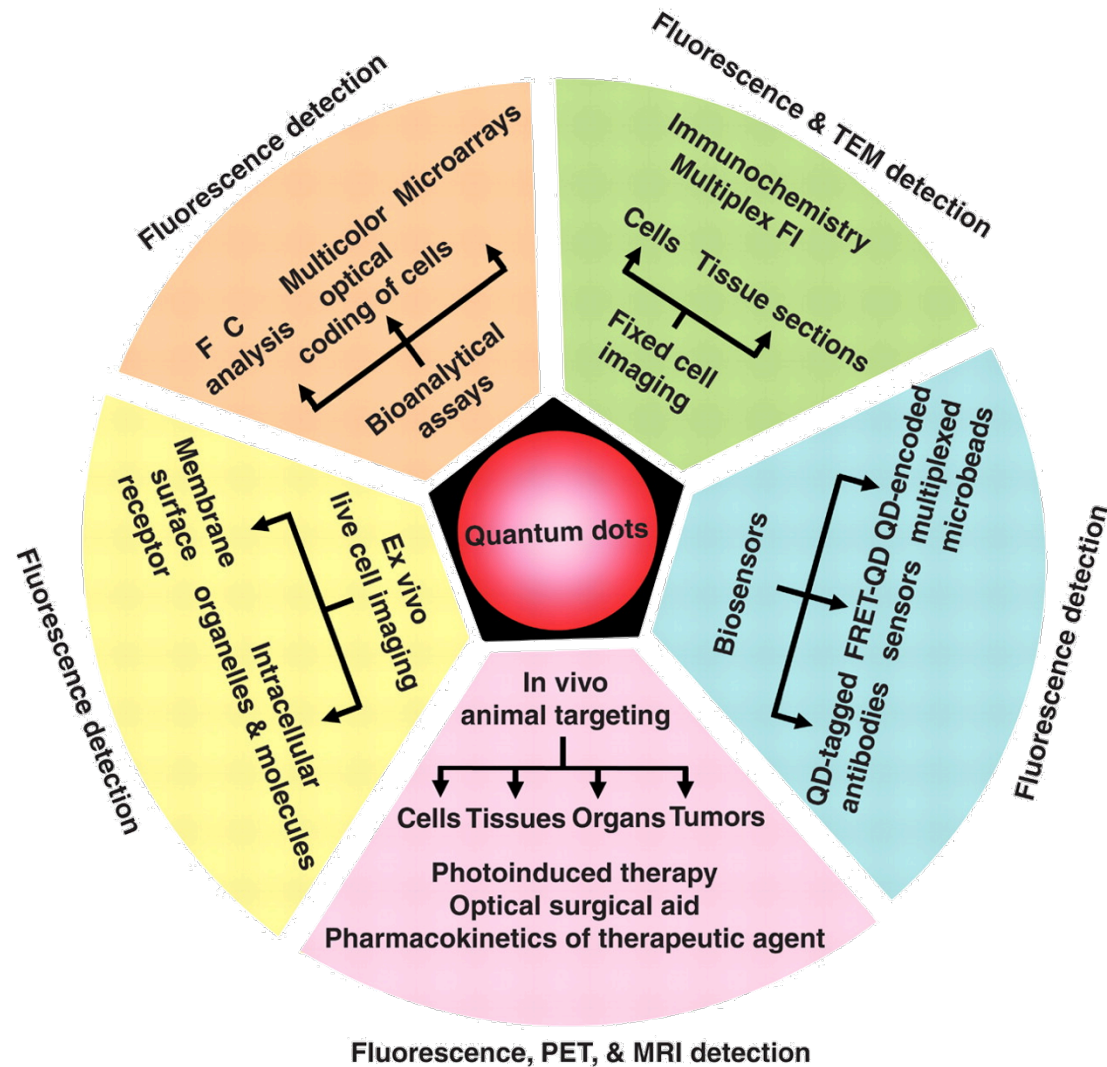


- ❑ Semiconductor nanocrystals (2-10 nm)
- ❑ Size/composition tunable emission spectra
- ❑ Narrow, symmetric emission spectra (FWHM: 20-30 nm), with continuous excitation spectra
- ❑ Highly resistant to photobleaching

➤ Superior probes for multiplex fluorescent labeling including in vivo imaging

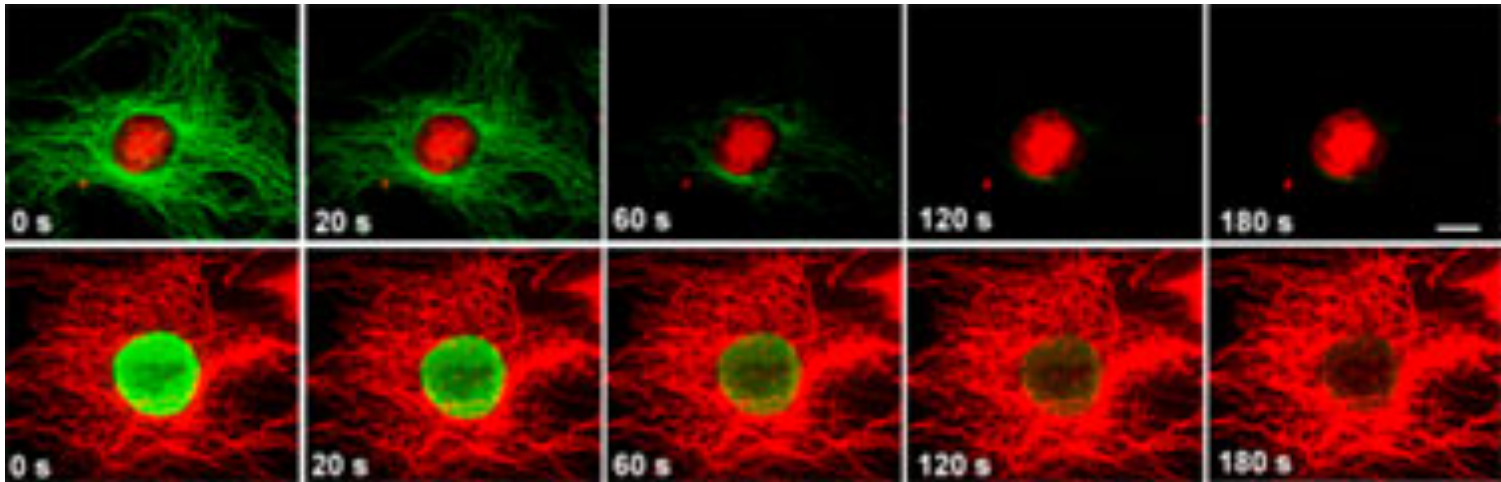
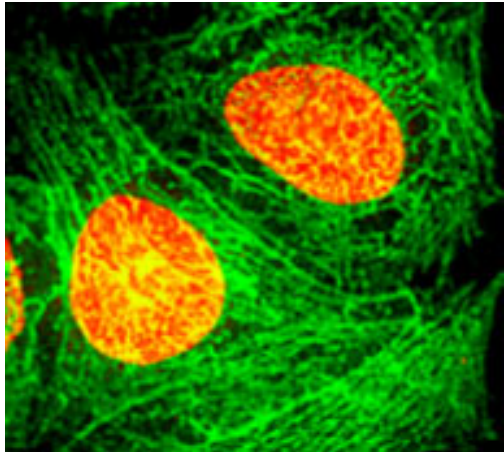
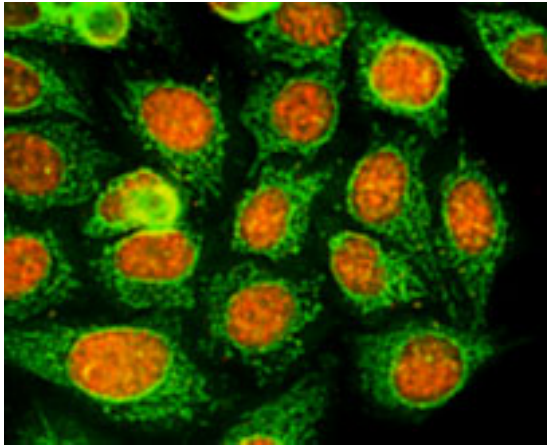


# Biological Applications of QDs



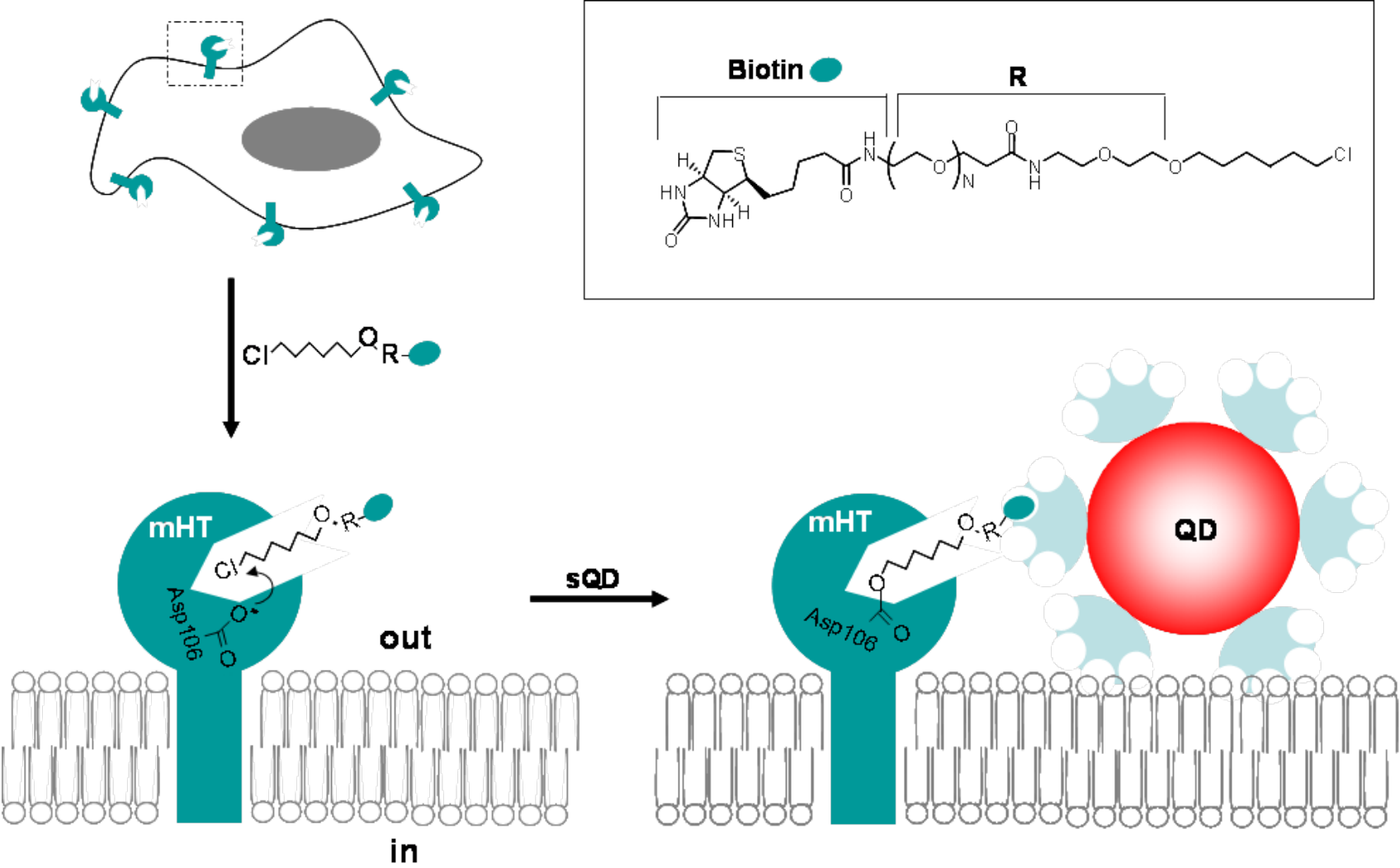
*(Michalet et al, Science 2005)*

# Fluorescent QDs for Fixed Cell Imaging

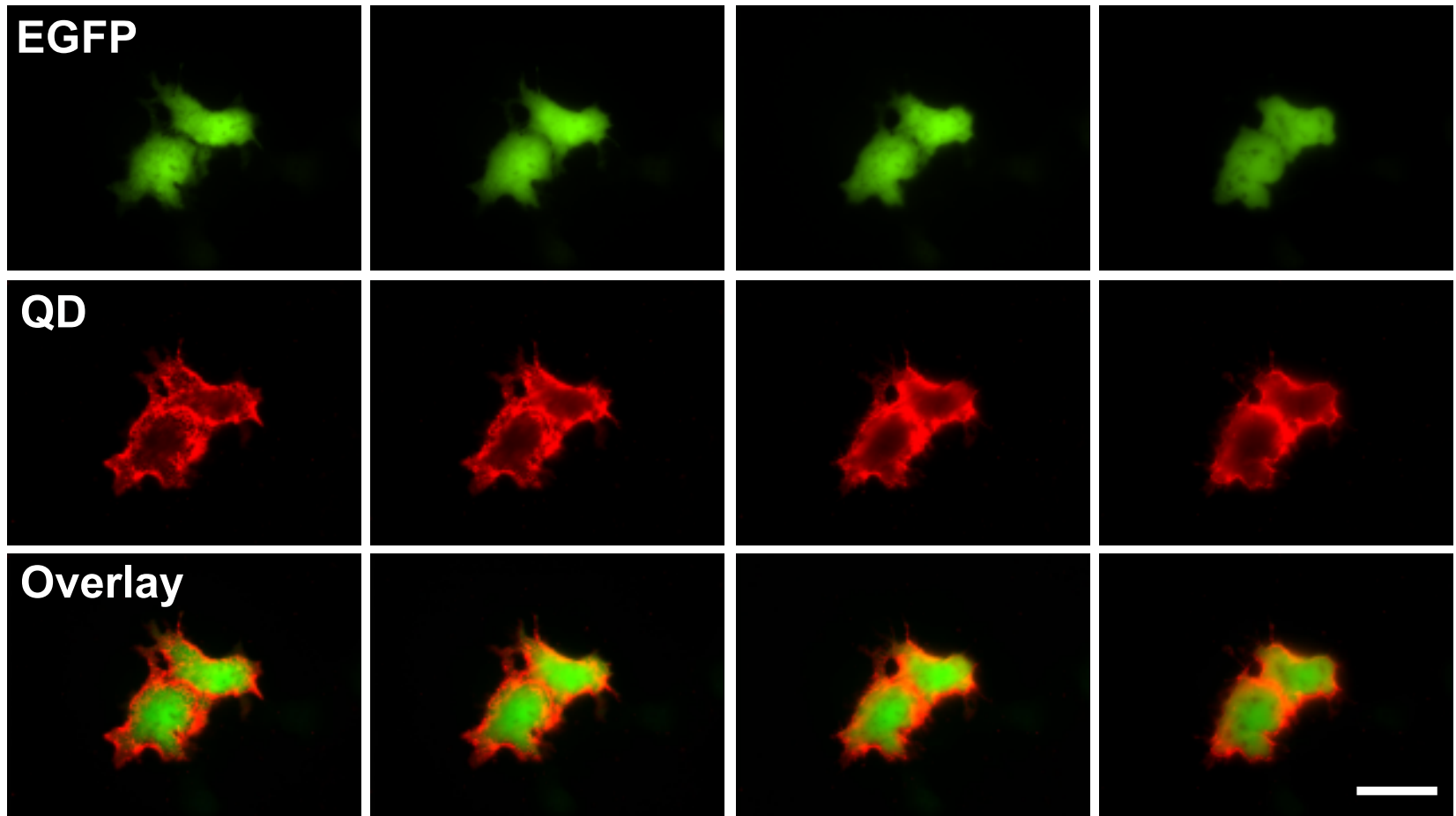




# Fluorescent QDs for Live Cell Labeling

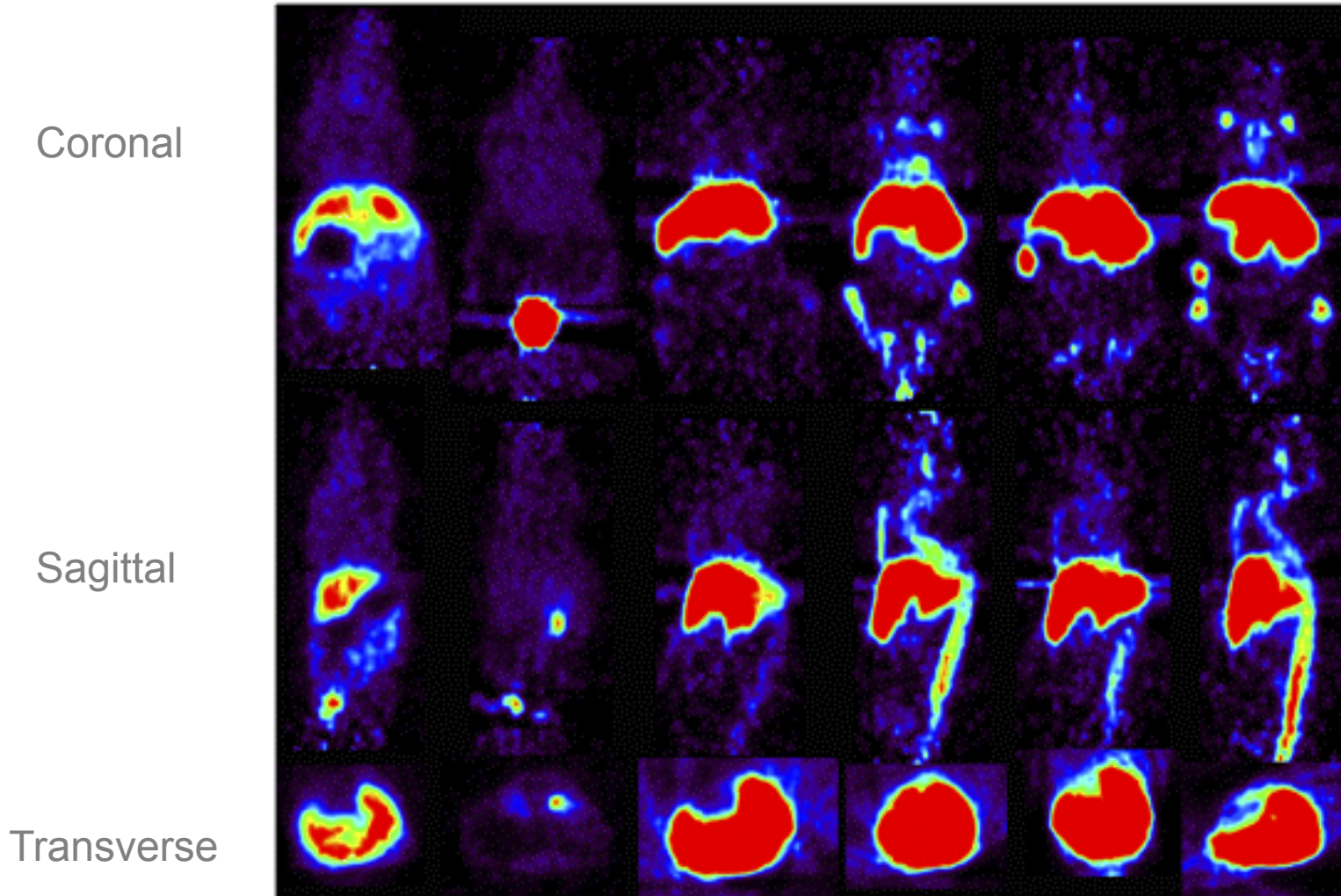


# Membrane Protein Labeling with QDs

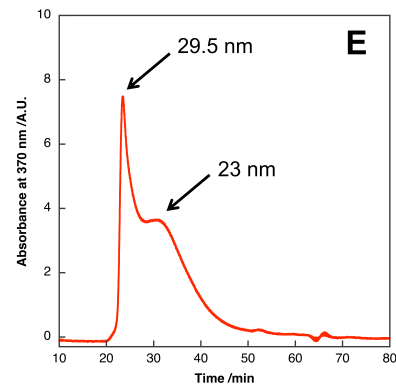
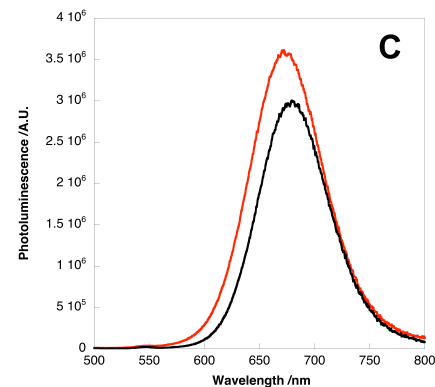
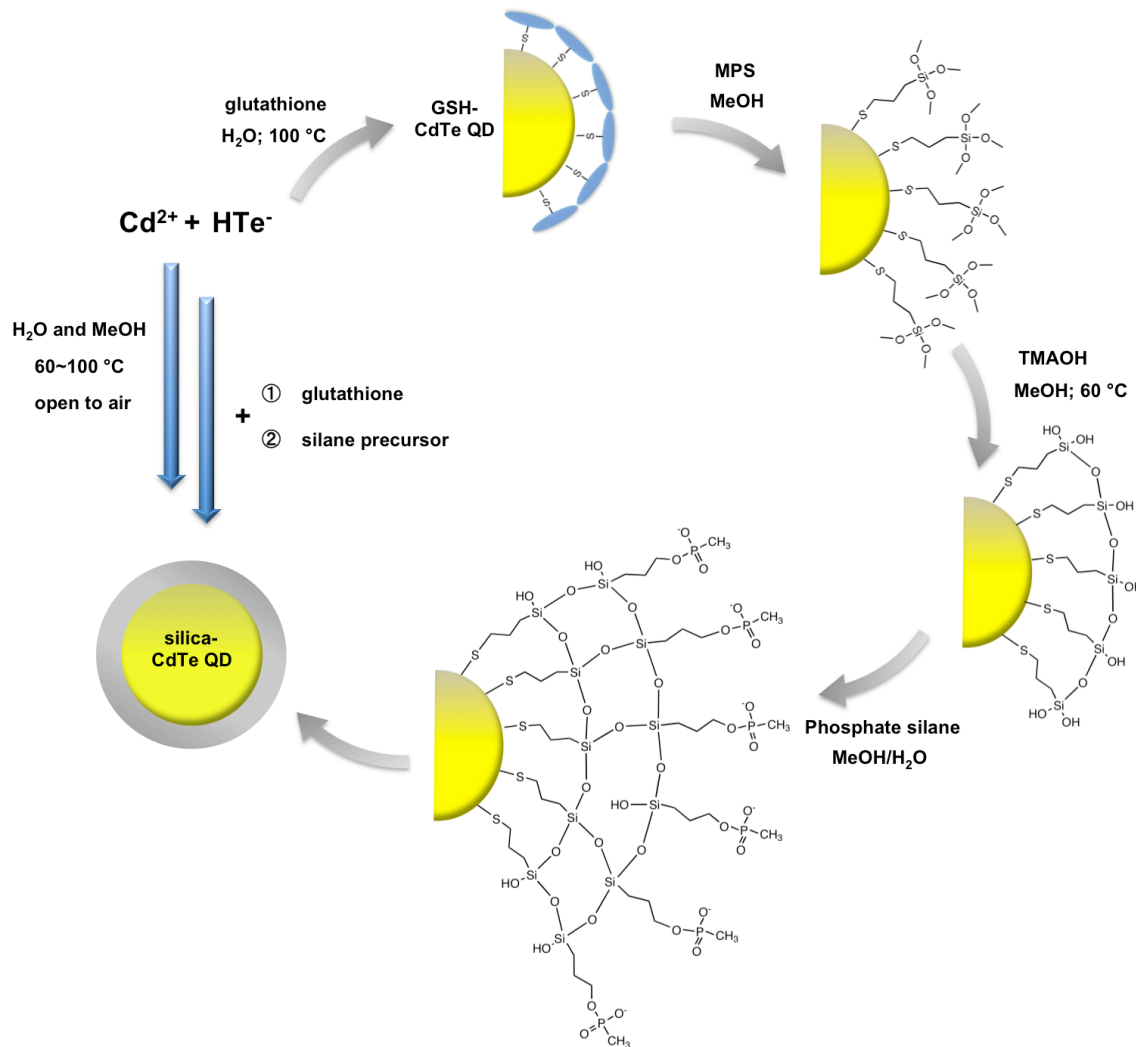


# In vivo Distribution of QDs by PET

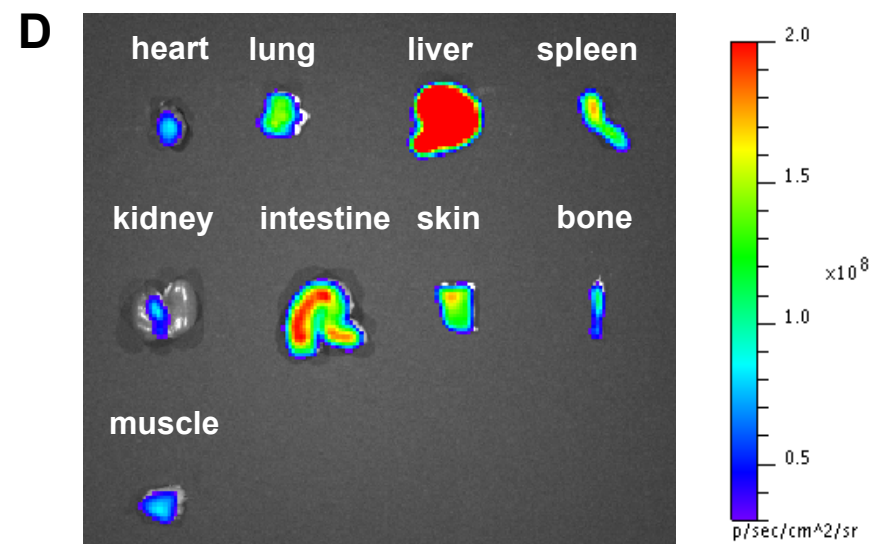
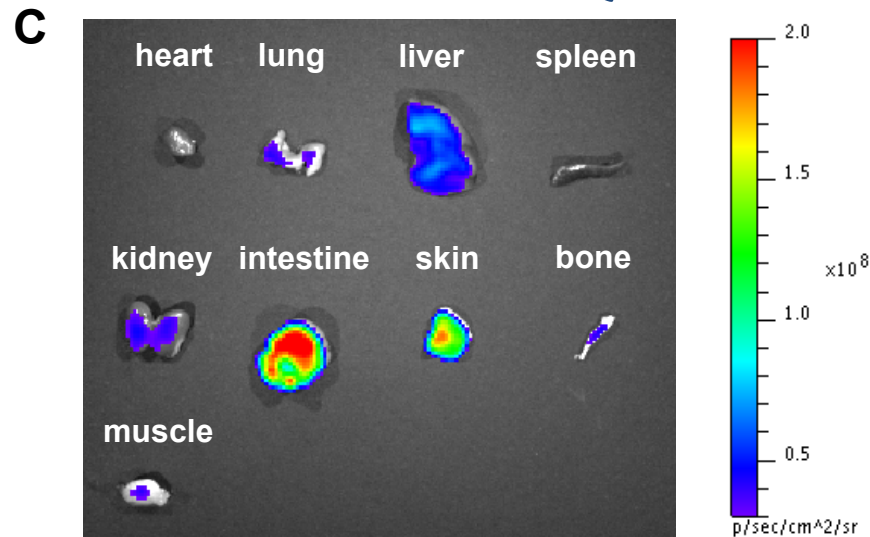
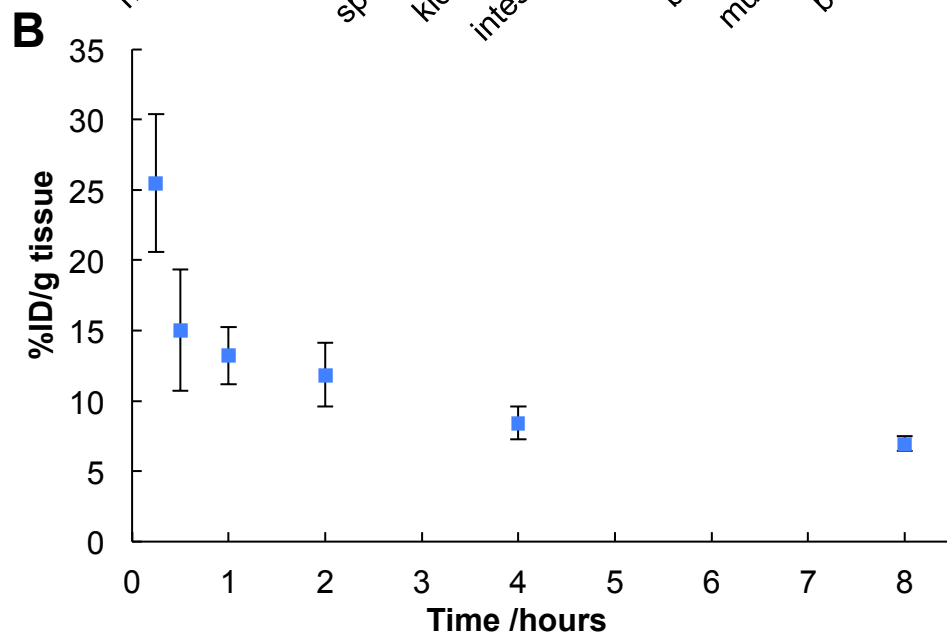
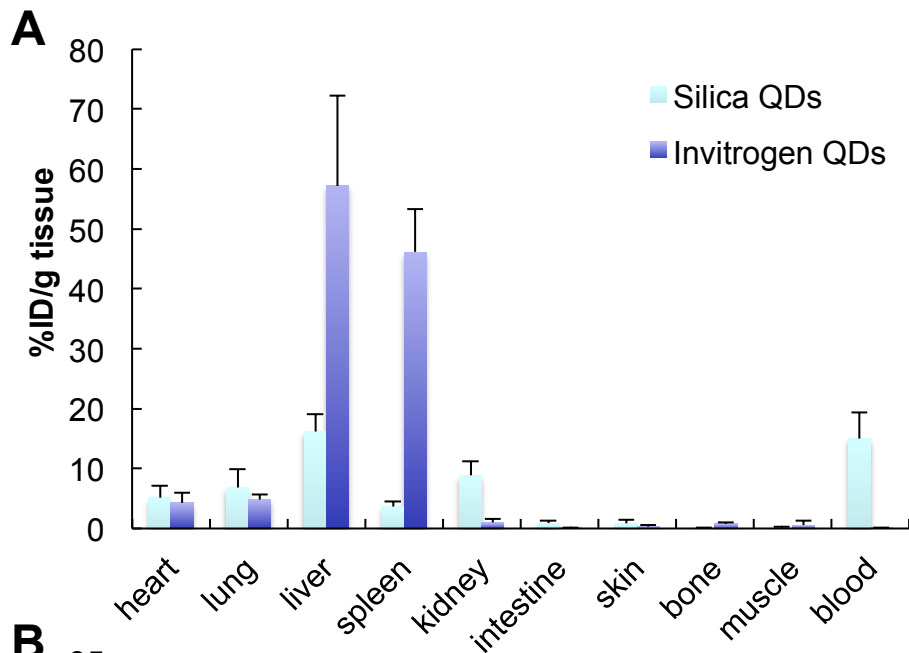
$^{64}\text{Cu}$   $\text{Cu}^{64}\text{-DOTA}$   $\text{QD}525$   $\text{PEG-QD}525$   $\text{QD}800$   $\text{PEG-QD}800$



# Silica-Coated QDots

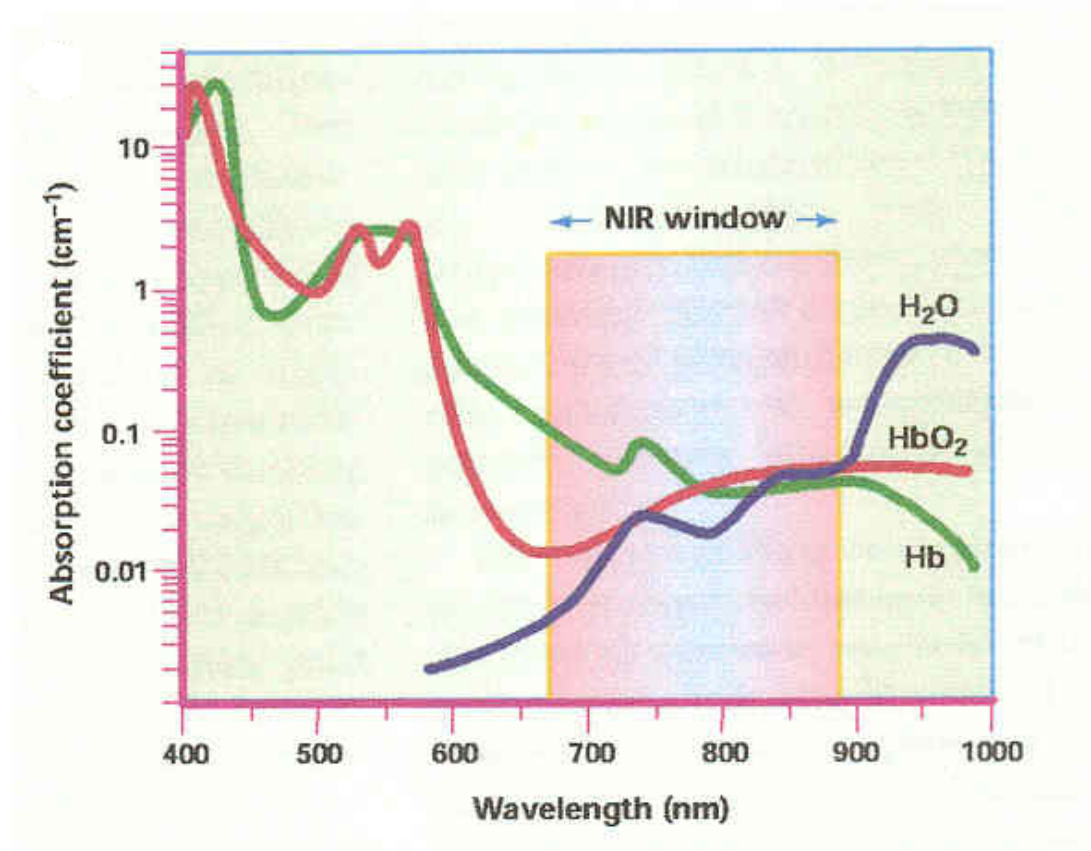


# Biodistribution of Silica-Coated QDs



# NIR Fluorescence *in vivo* Imaging

- The NIR window is ideally suited for *in vivo* imaging b/o minimal light absorption by hemoglobin (<650 nm) and water (>900 nm)
- NIR light travels 10 cm through breast tissue, and 4 cm of skull/brain tissue or deep muscle

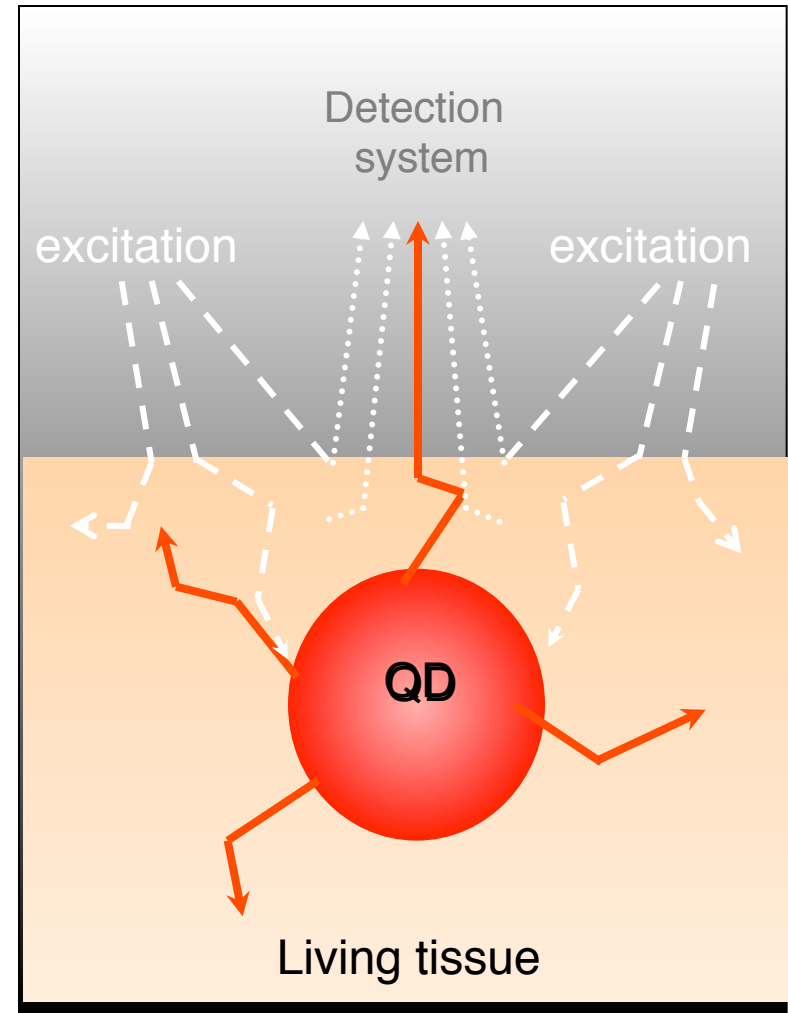


Weissleder, R. *Nat. Biotech.* 2001, 19, 316.



# Challenges of Fluorescence *in vivo* Imaging

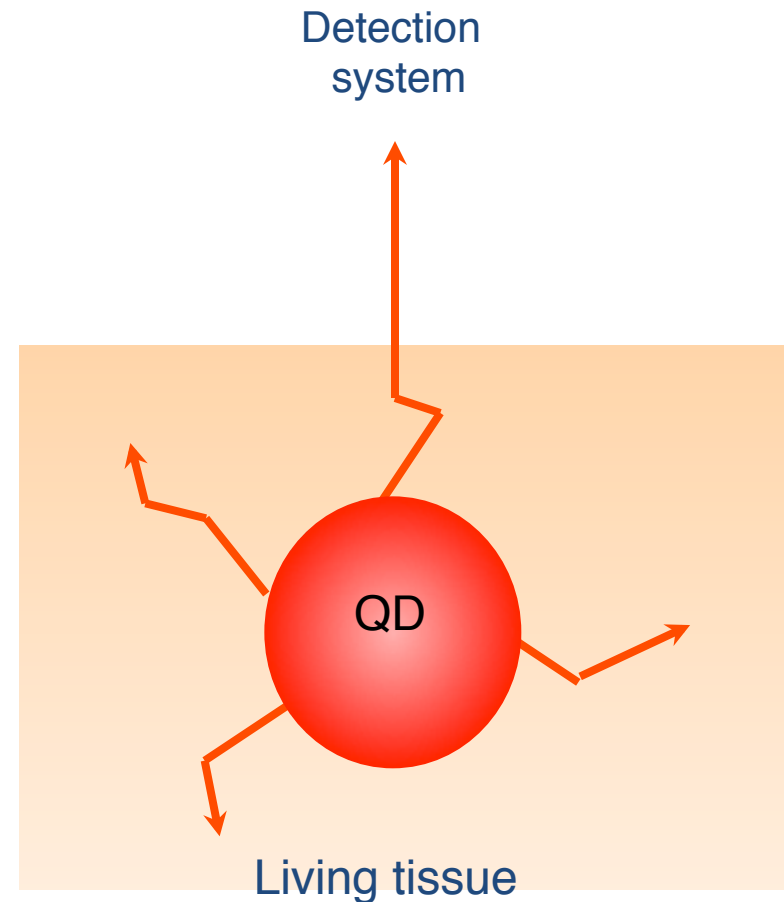
- **Require external illumination**  
→ **Produces strong background autofluorescence**
- **Absorption and scattering of optical photons in tissues**  
→ **Little light is available for QD excitation at non-superficial locations**



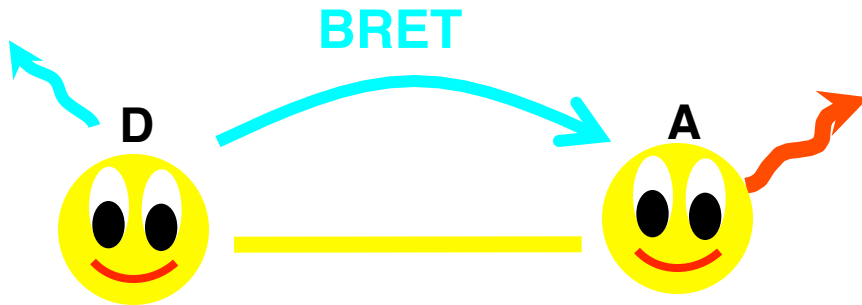


# Can QDs Emit Without External Light Excitation?

- **No external illumination;  
No strong background autofluorescence**
- **More light is available for QD excitation at non-superficial locations**



# Bioluminescence Resonance Energy Transfer (BRET)

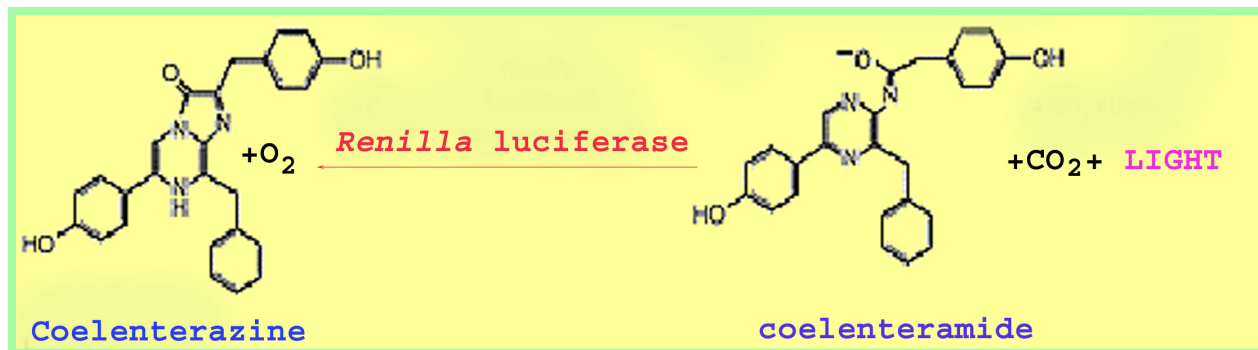


**Donor: luminescent protein (D),  
e.g. *Renilla luciferase***

**Acceptor: fluorophores (A),  
e.g. green fluorescent protein**

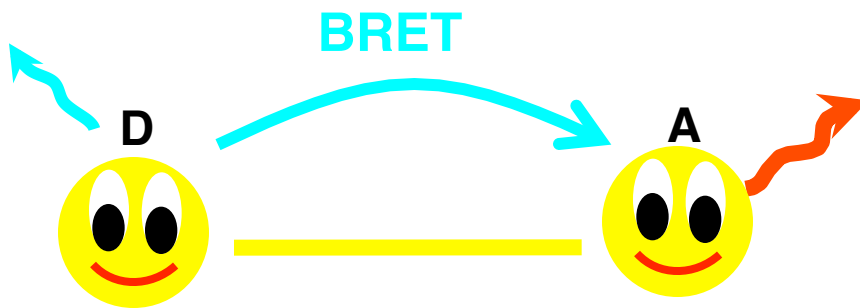


Sea  
Pansy



480 nm

# Bioluminescence Resonance Energy Transfer (BRET)

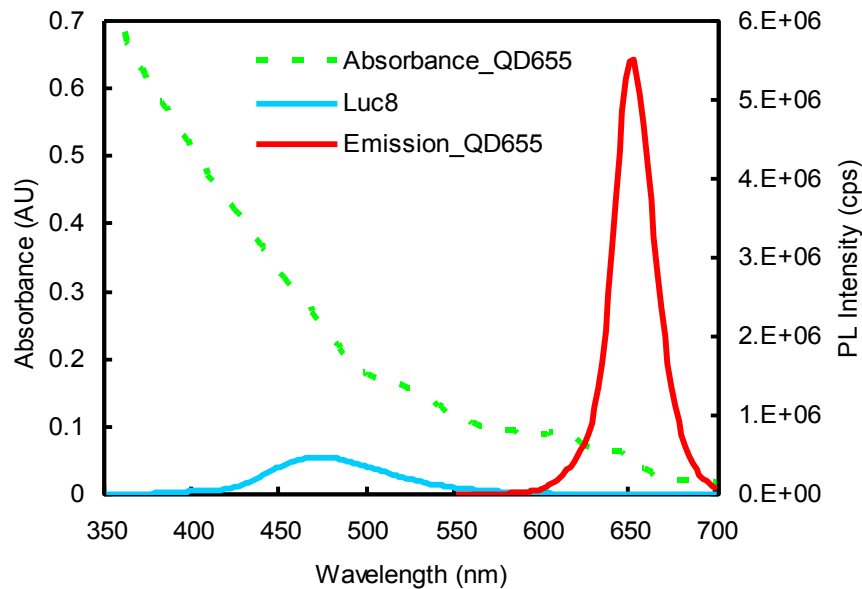
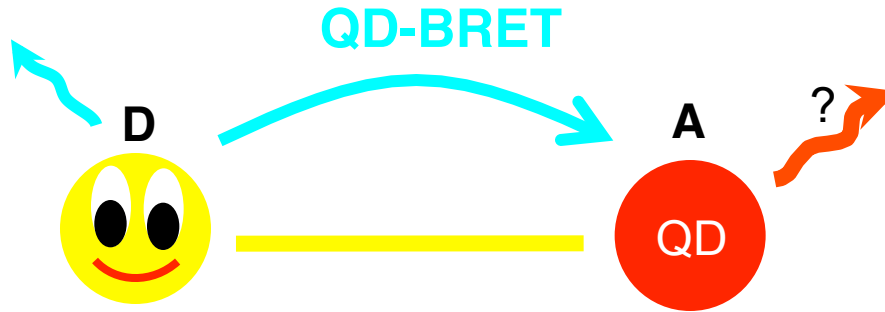


**Donor: luminescent protein (D),  
e.g. *Renilla* luciferase**

**Acceptor: fluorophores (A),  
e.g. green fluorescent protein**

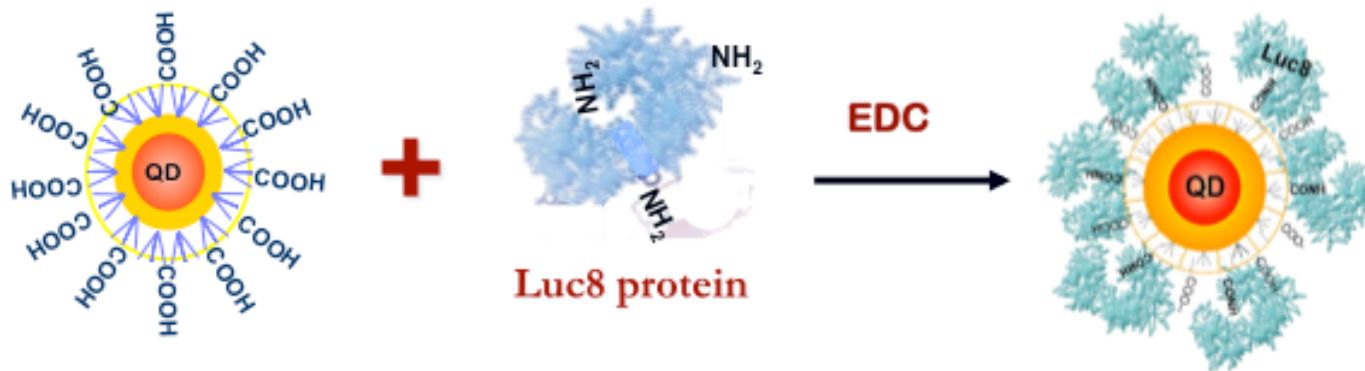
- Substantial overlap between emission spectrum of the donor and the absorbance spectrum of the acceptor
- Close distance between donor and acceptor (within 10 nm)
- Excited state dipole-dipole interaction

# QD-BRET: *Renilla* Luciferase as the Donor, QD as the Acceptor

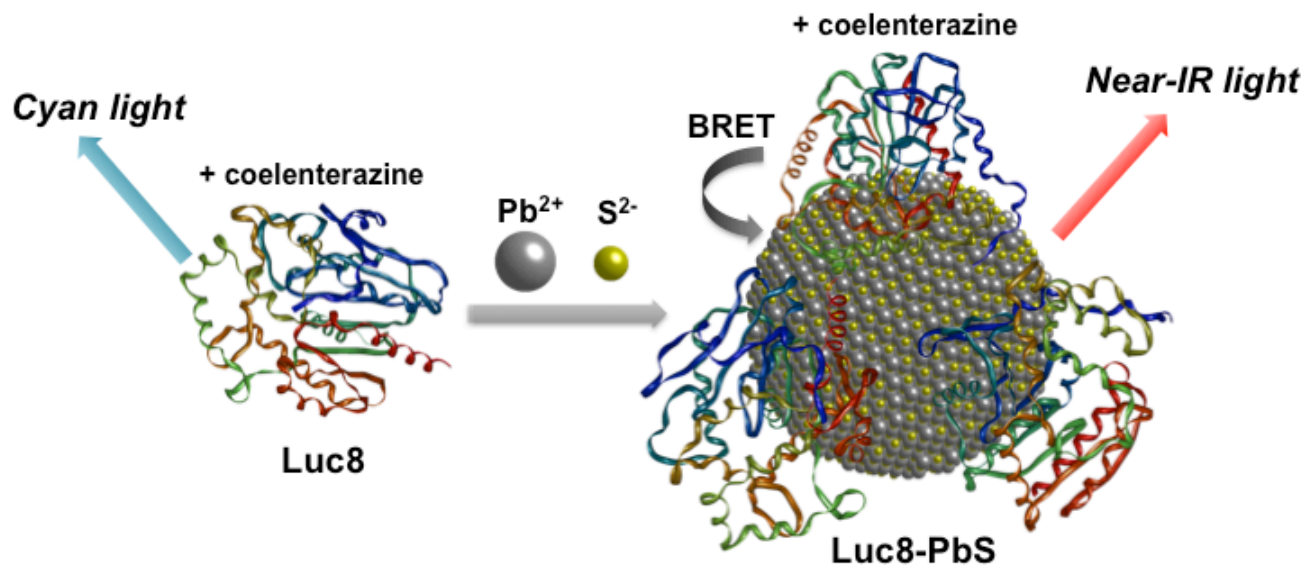


# Strategies for Making QD BRET NPs

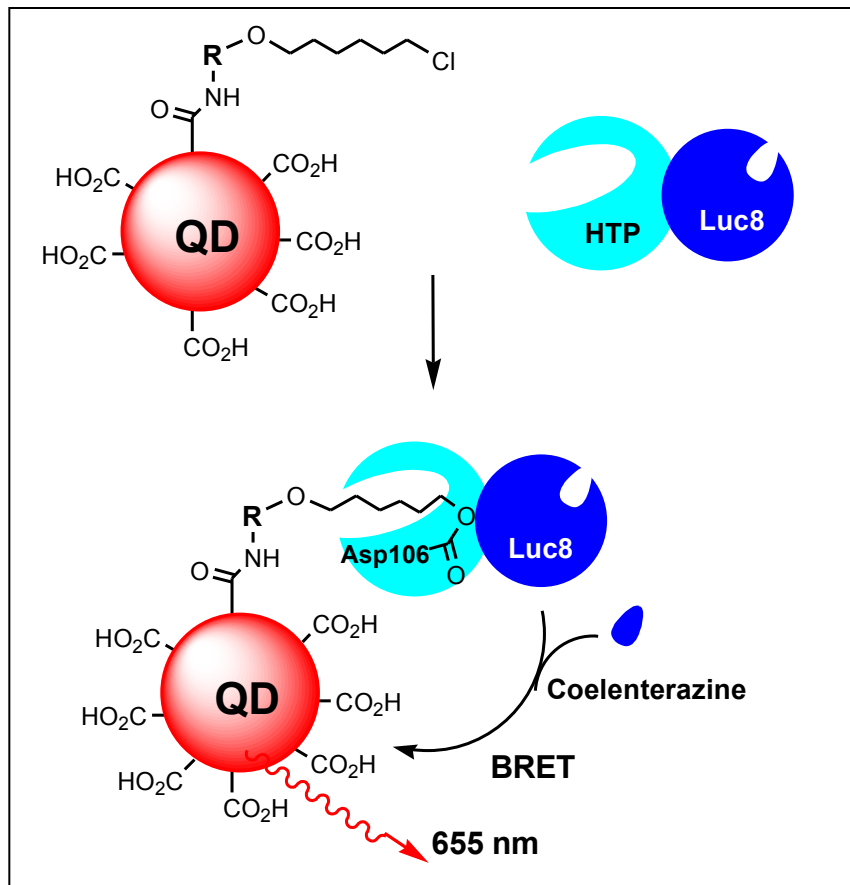
## Bioconjugation



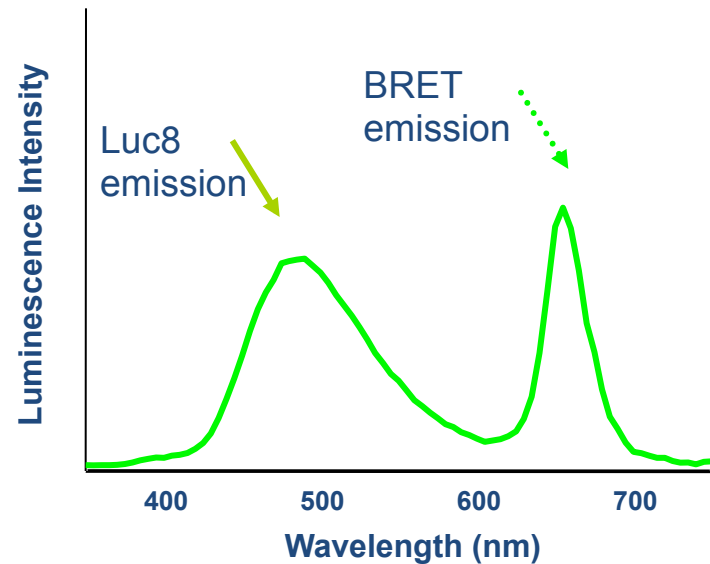
## Biomineralization



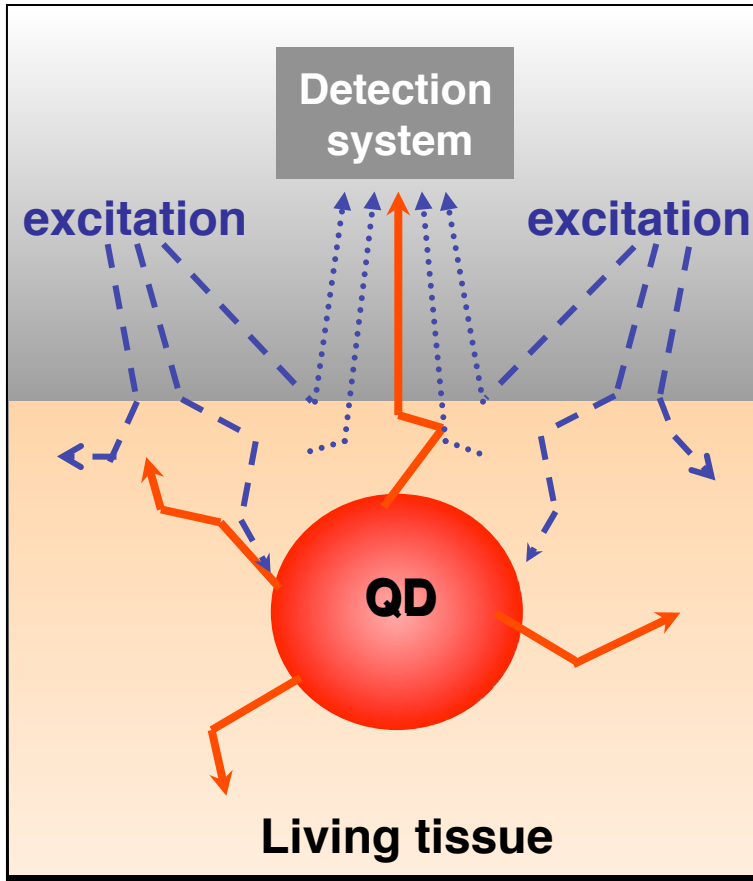
# Strategies for Making QD BRET NPs



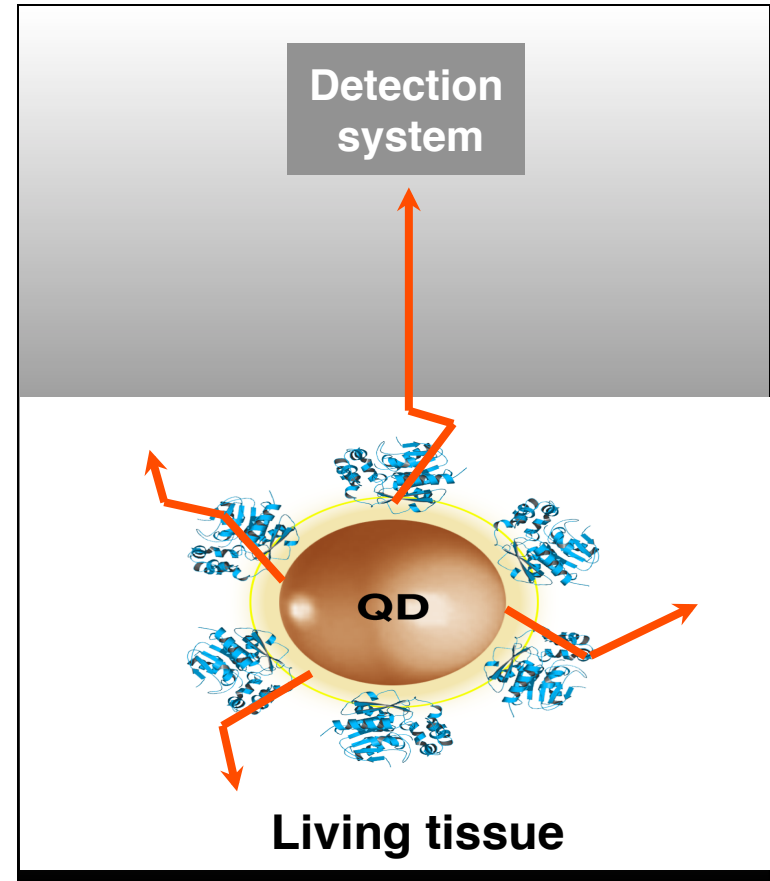
## Protein-Ligand Binding



# Advantages of QD-BRET for In Vivo Imaging



vs.

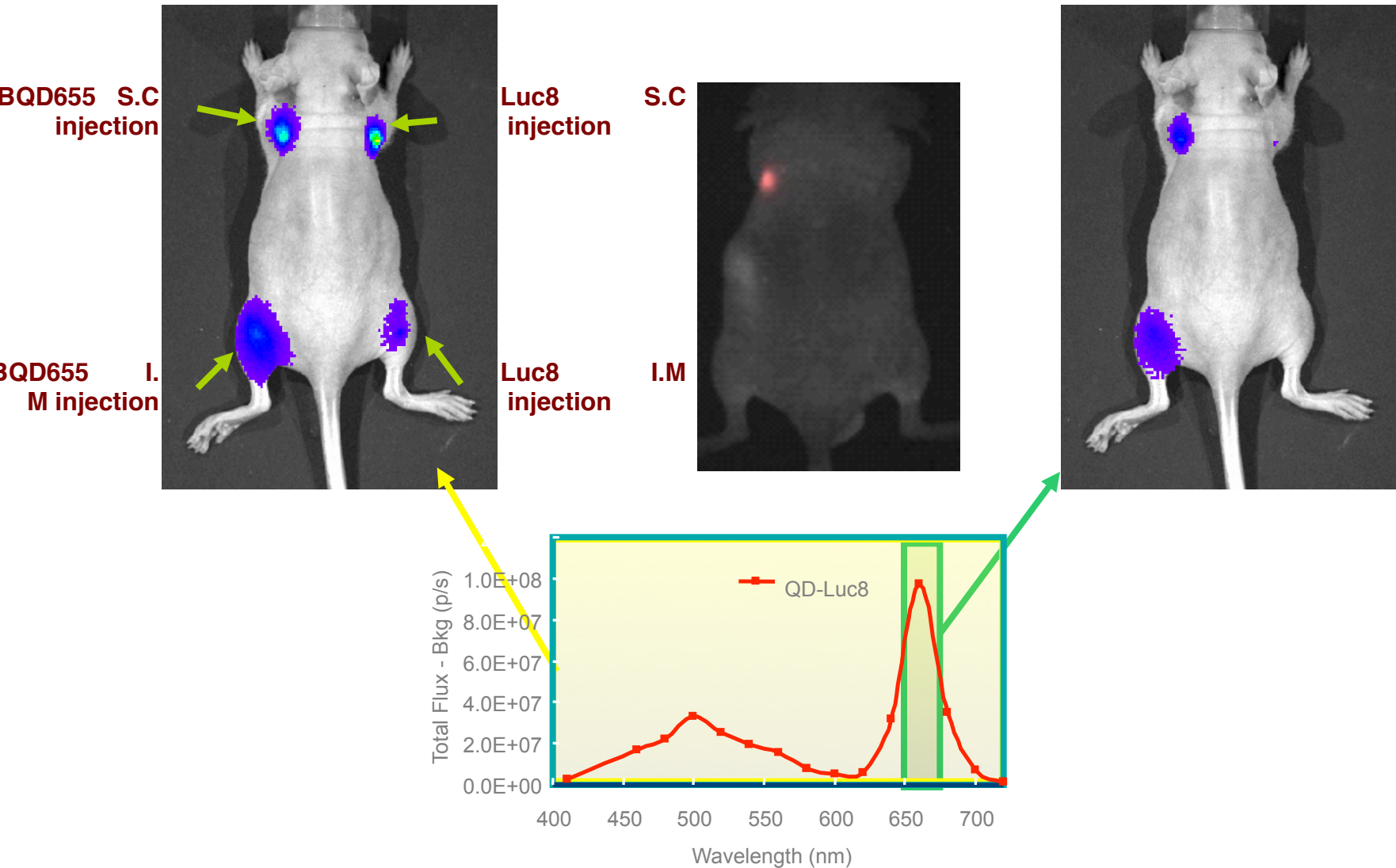


- Strong background autofluorescence
- Absorption and scattering of optical photons in tissues

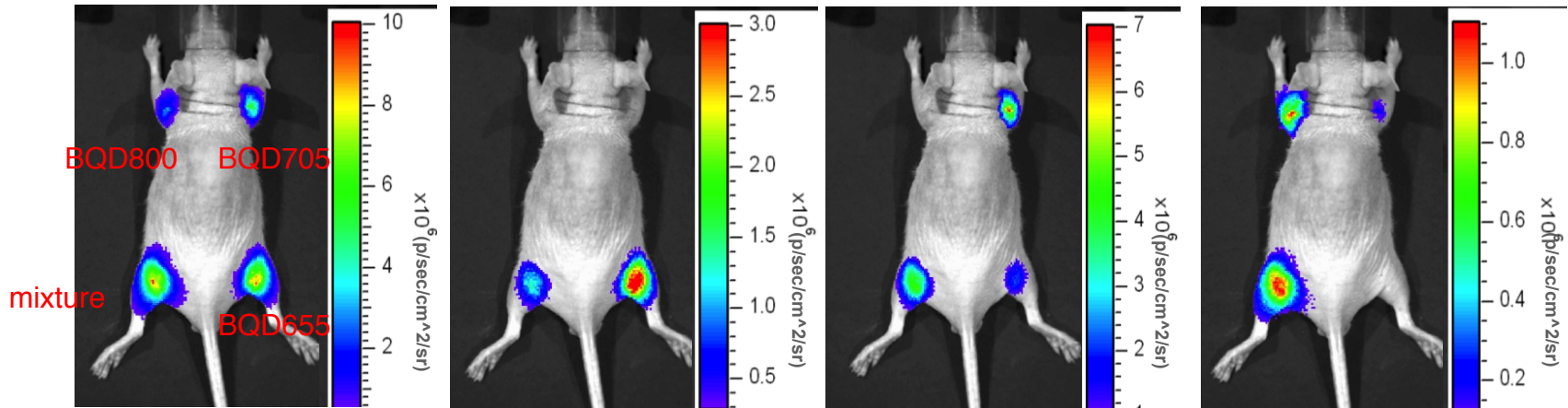
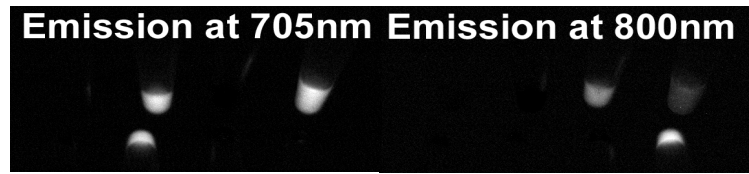
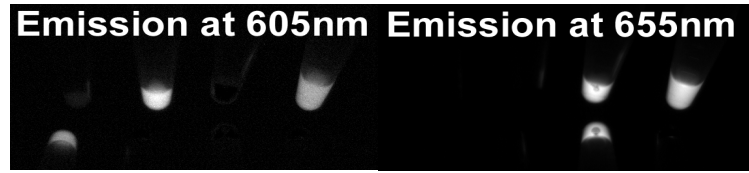
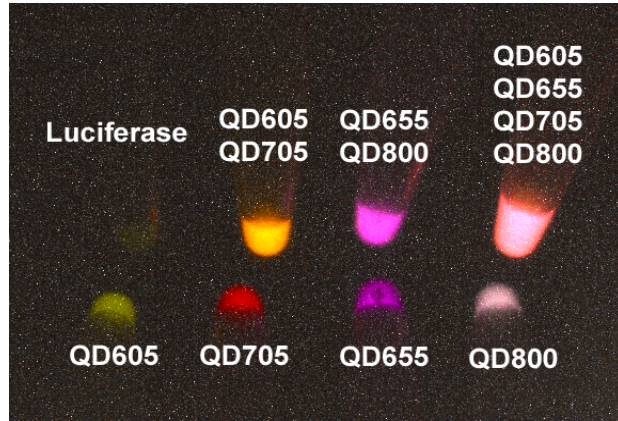
- No external illumination; No strong background autofluorescence
- More light is available for QD excitation at non-superficial locations



# QD BRET: *In vivo* Imaging in Mice

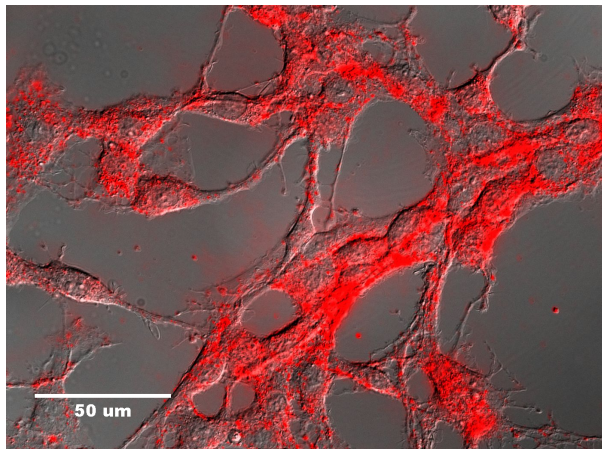


# QD BRET: Multiplexed imaging

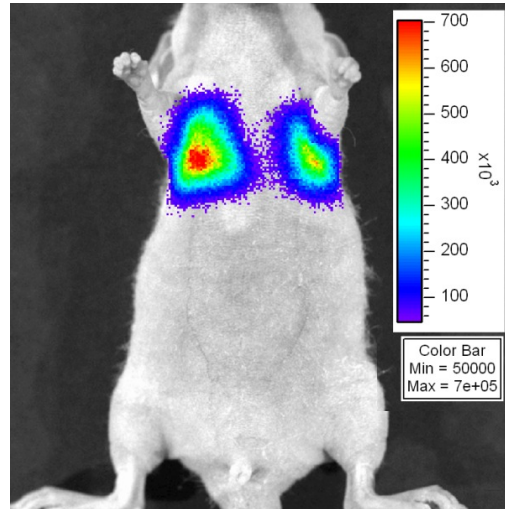


(So et al, Nat Biotech 2006)

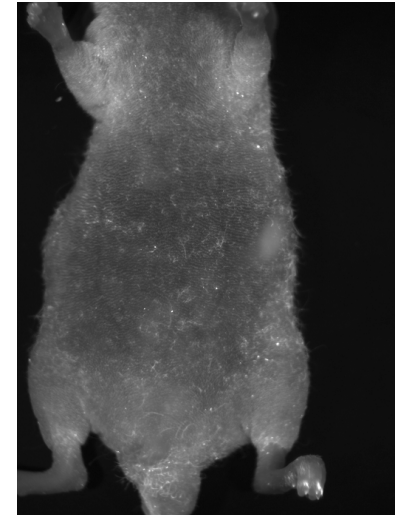
# In vivo Imaging QD-Labeled Cells



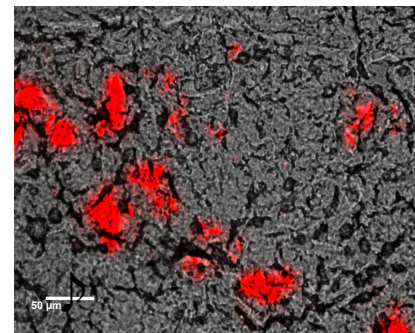
**Fluorescence/DIC C6 cell images**



**No excitation light**



**Fluorescence:  
Excitation 503-555nm**



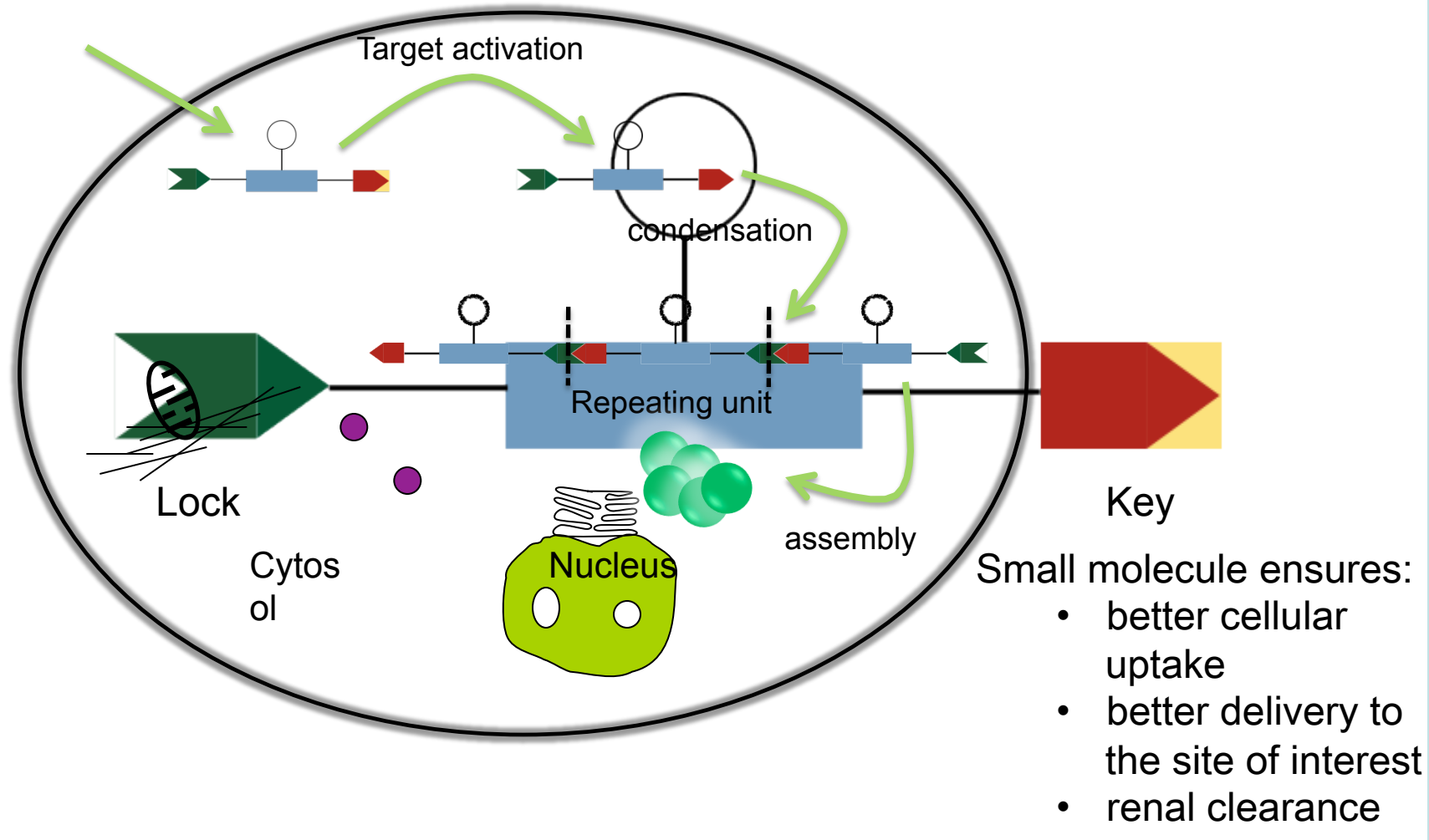
**Histology:  
Lung slice**

# A New Approach to imaging intracellular targets ...

instead of using the *pre-synthesized nanoparticles*, deliver **small building blocks** and synthesize NPs in target cells

# In Cellulo Synthesis of Nanoparticles

In cellulo synthesis of the nanoparticles using small molecules as the building blocks

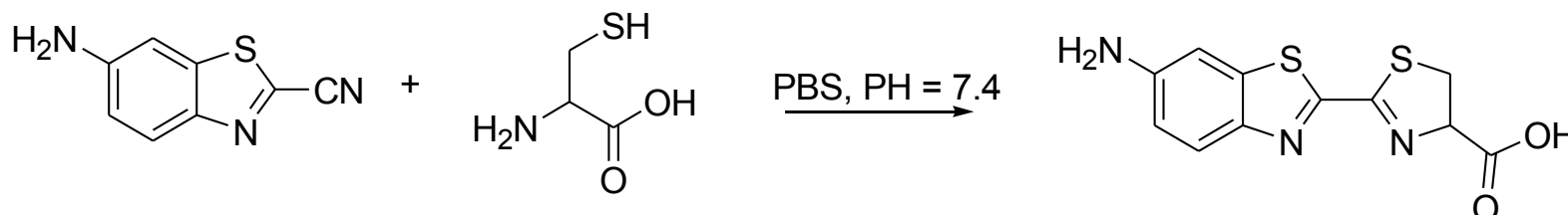
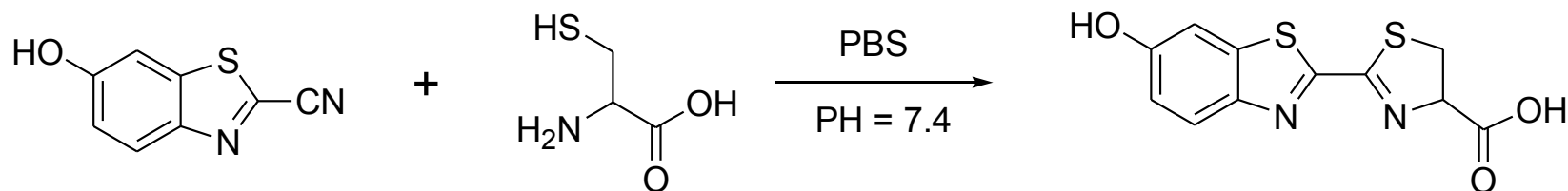
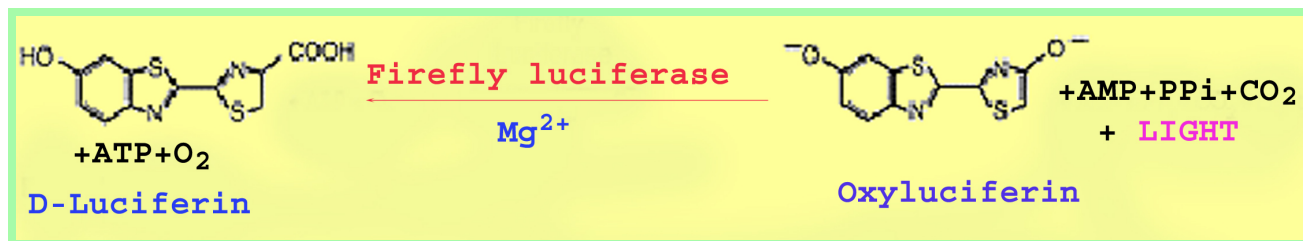




# Biocompatible Condensation Reaction

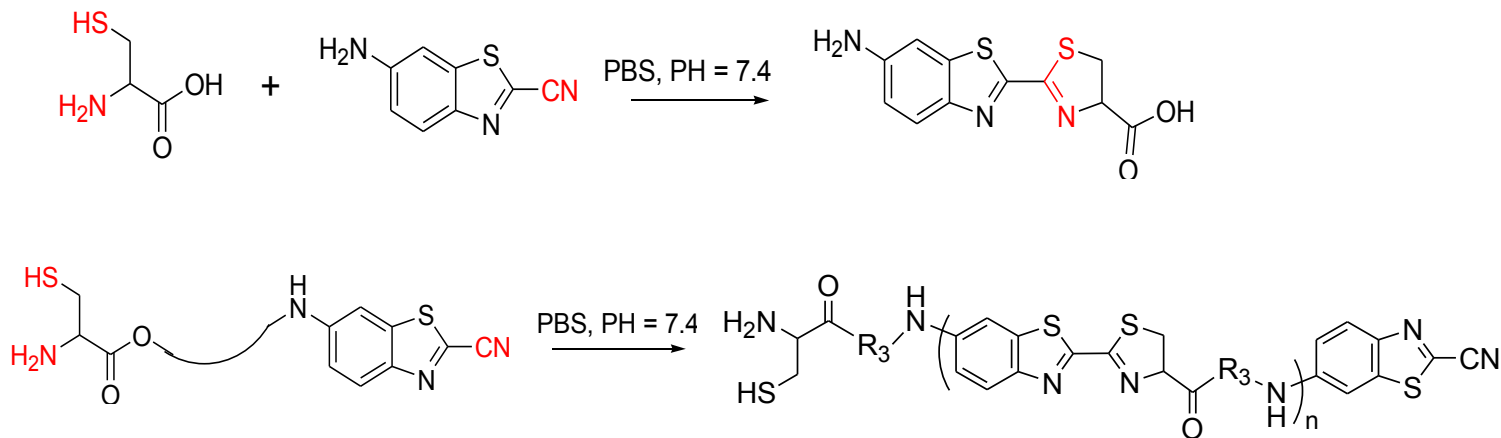


Firefly luciferase

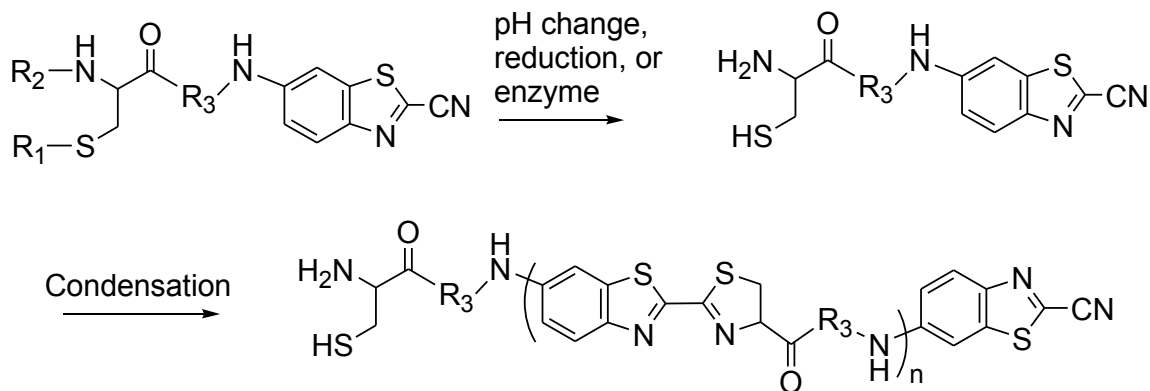


Specific reactivity!

# Controlled Polycondensation



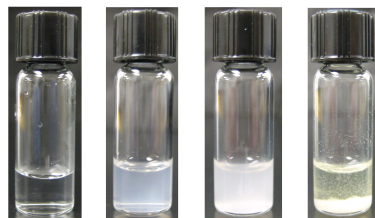
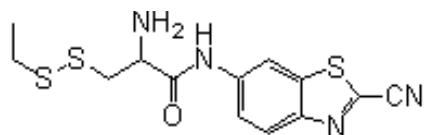
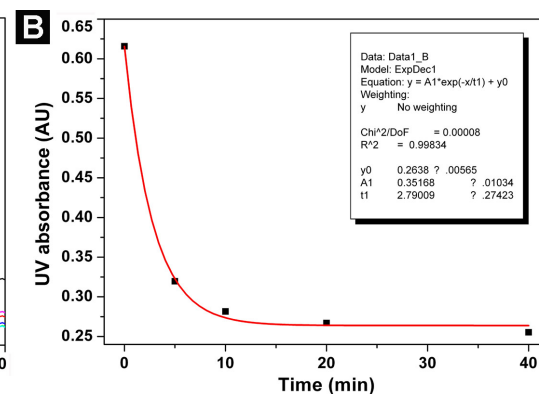
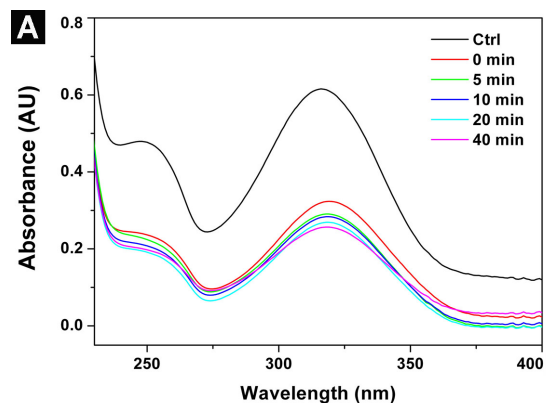
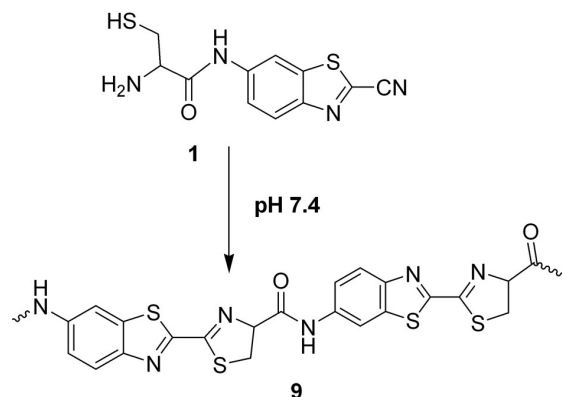
Spontaneous, uncontrolled!



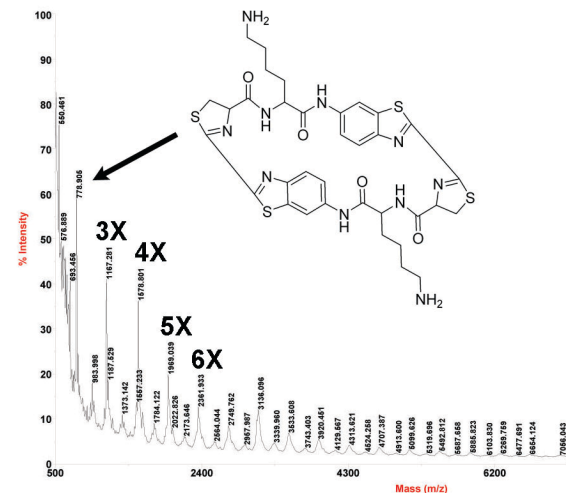
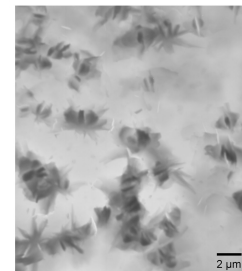
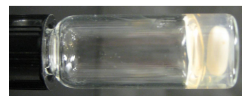
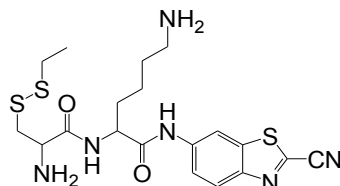
Control the activity of thiol and/or amino groups!



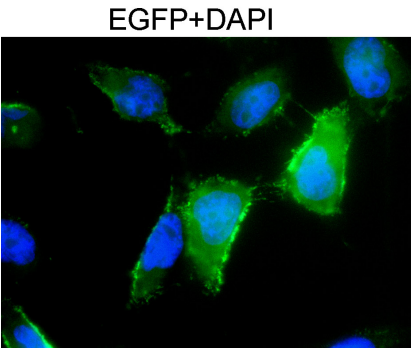
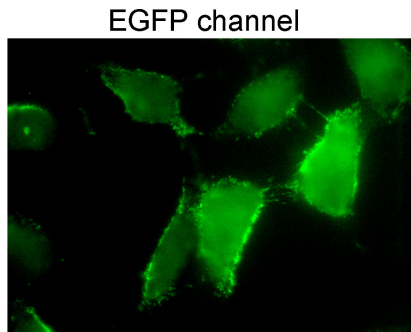
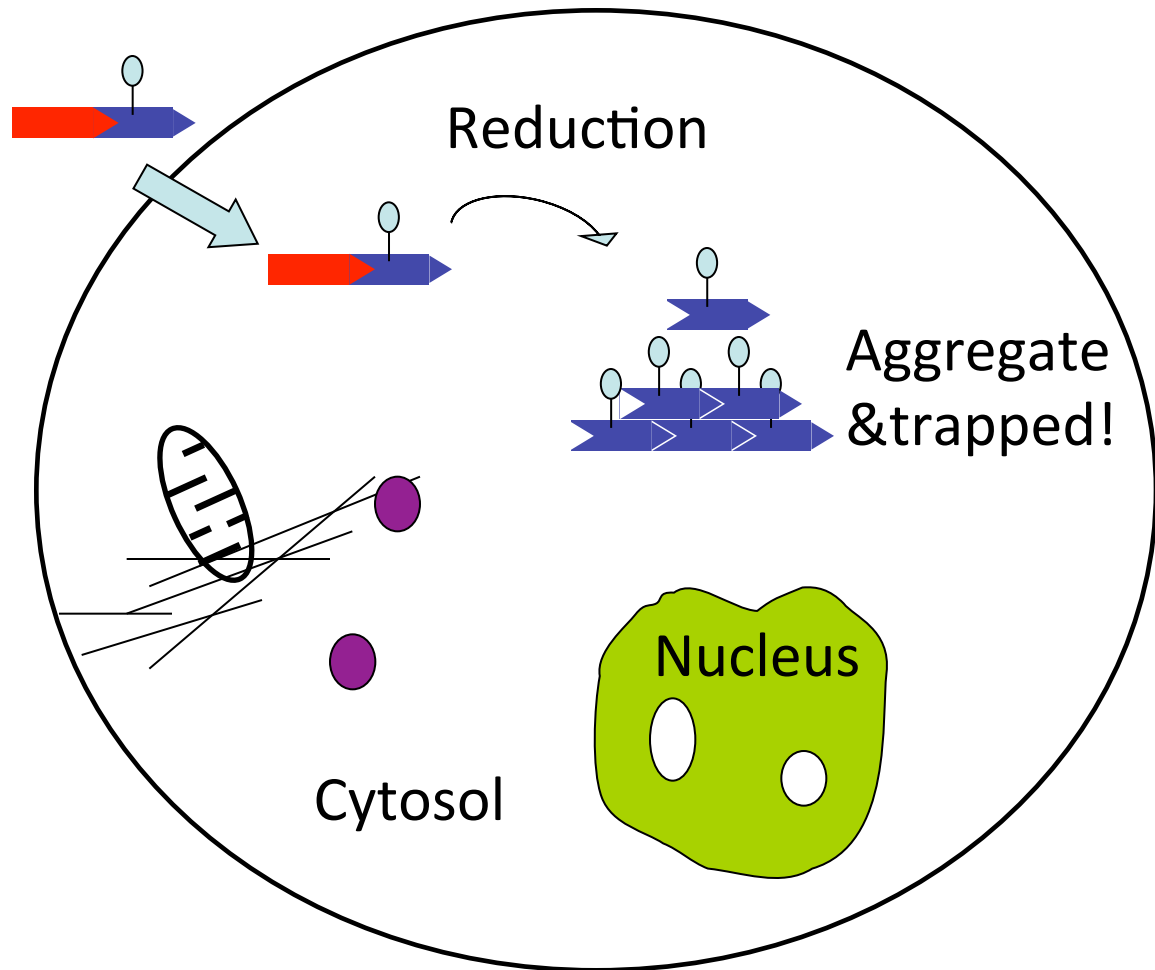
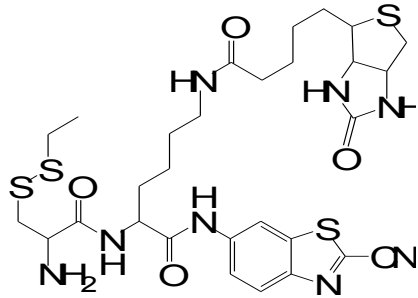
# pH or Reduction Triggered Polycondensation



0 min    5 min    10 min    24 h

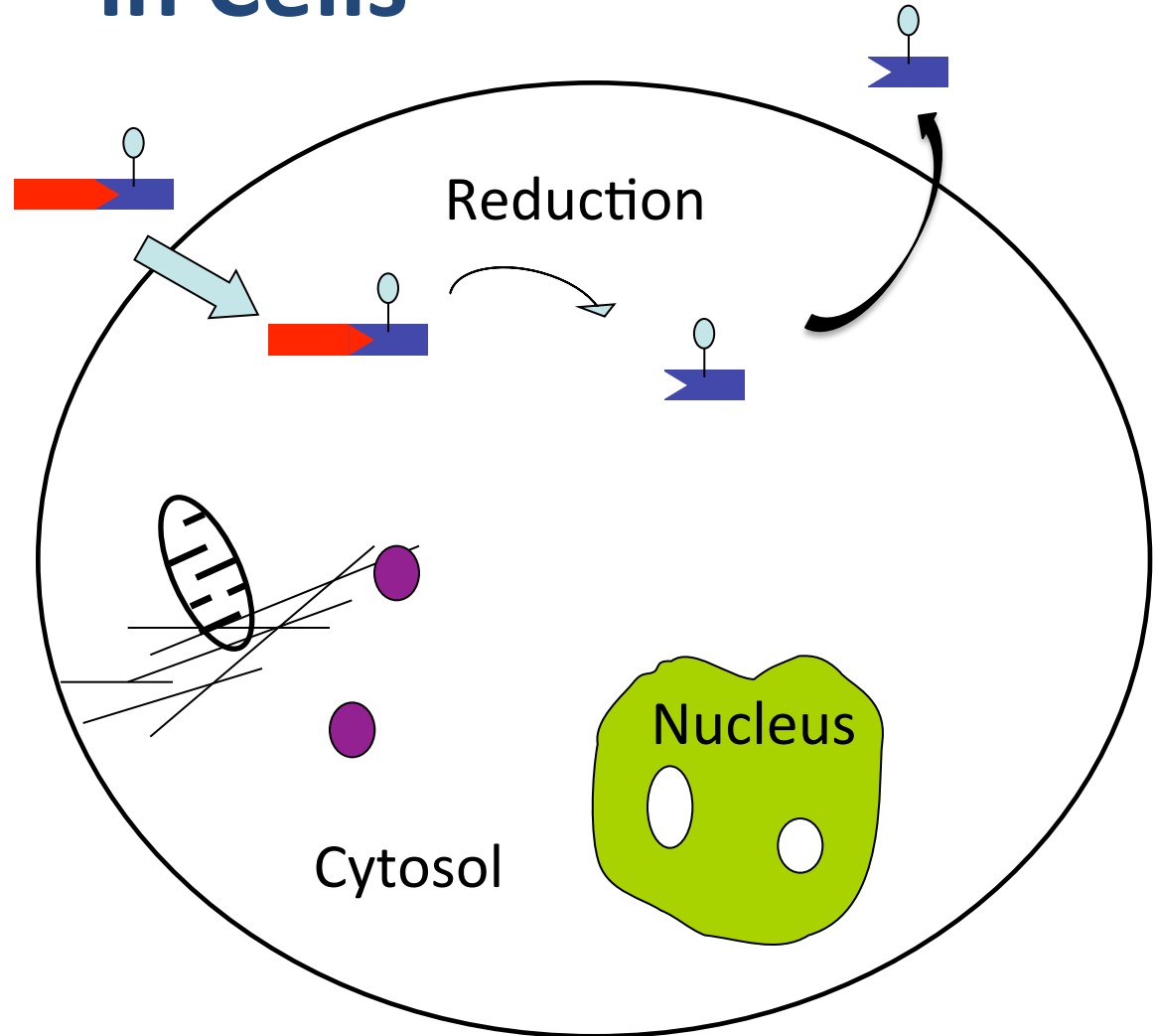
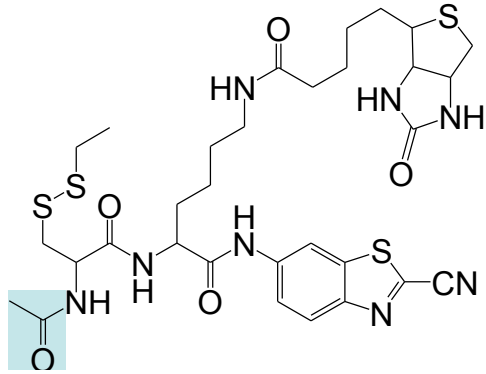


# Reduction-Triggered Polymerization in Cells

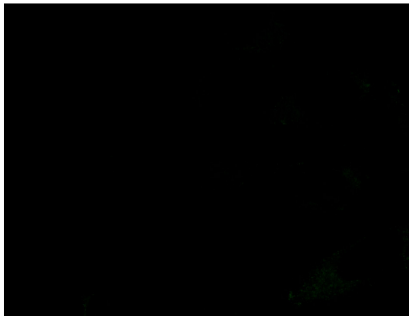


Wash, permeabilize and stain with FITC-Streptavidin

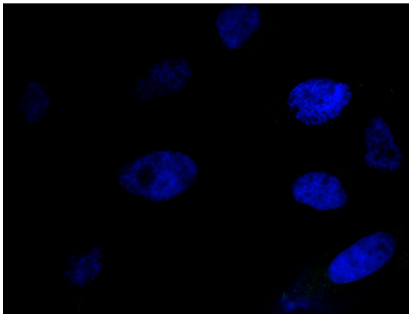
# Reduction-Triggered Polymerization in Cells



EGFP channel

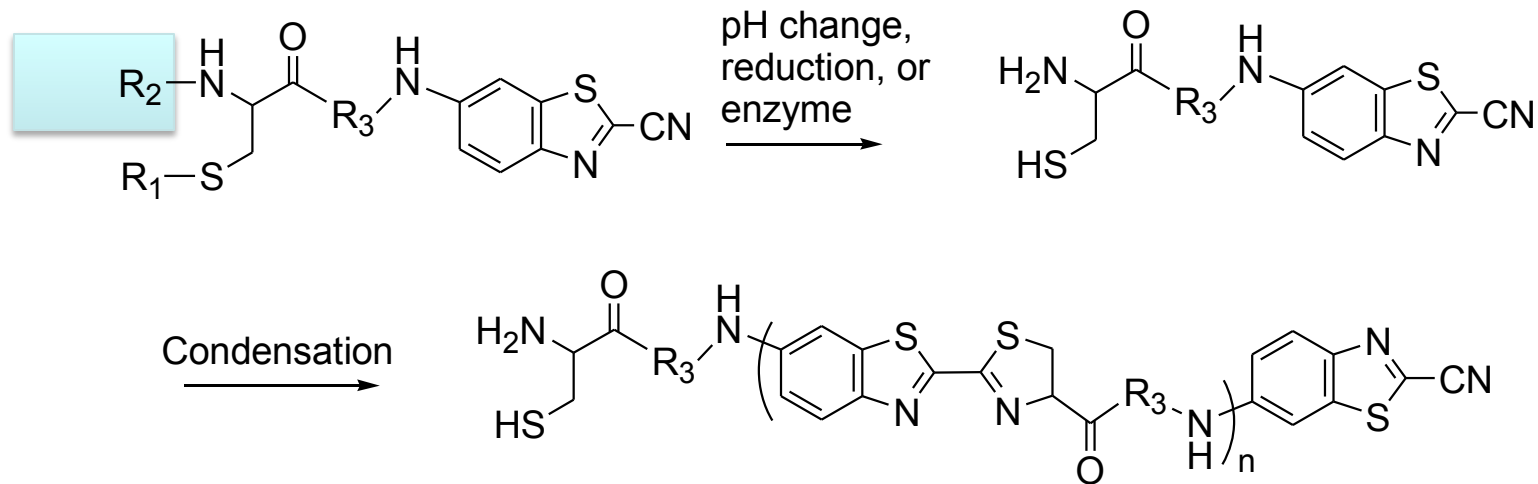


EGFP+DAPI



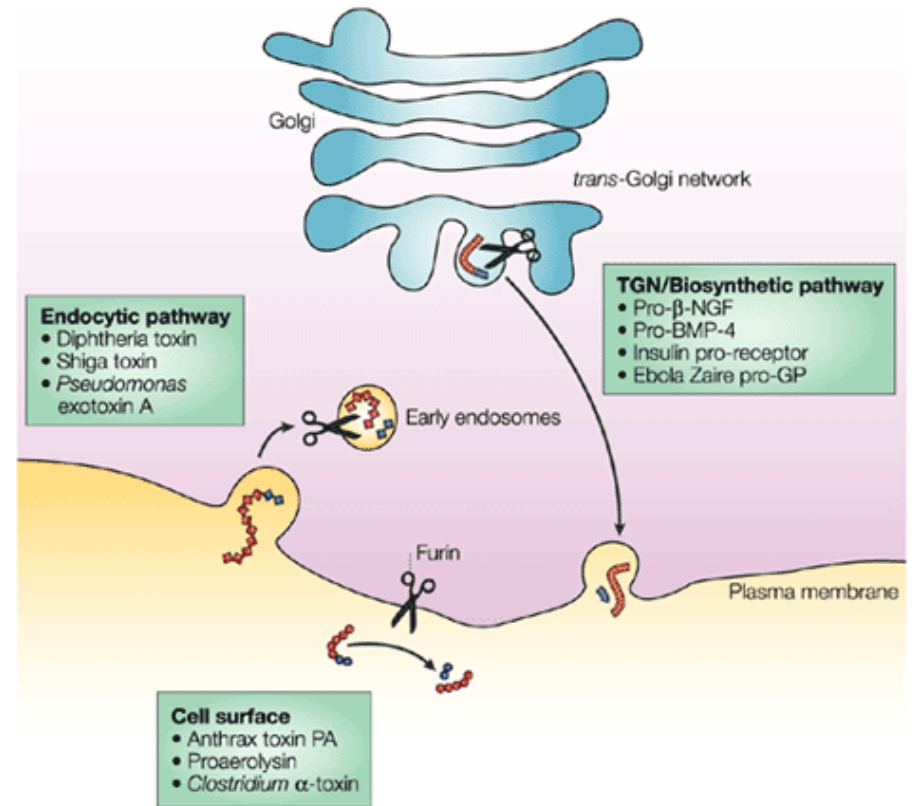
Wash, permeabilize and stain with FITC-Streptavidin

# How to control the NP formation in tumor cells not in normal cells?

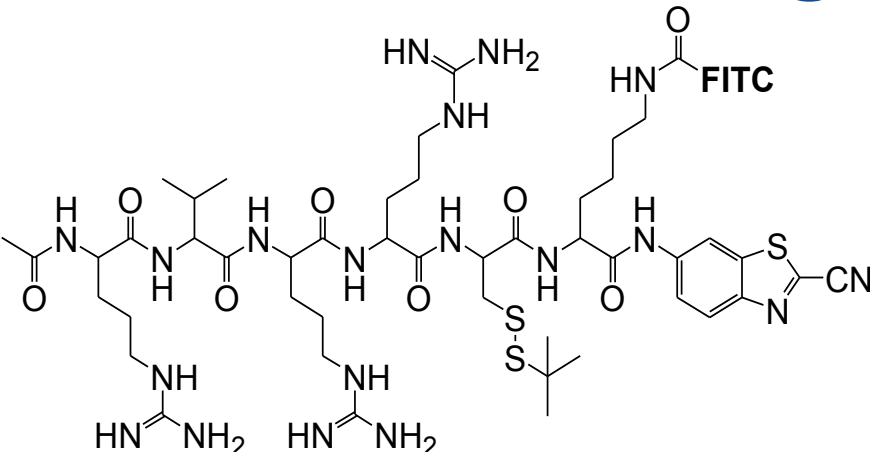


# Furin Activity

- Ubiquitous subtilisin-like proprotein convertase
- 96 kDa transmembrane glycoprotein
- Human furin - cleaves growth factors and hormones, cell surface receptors, matrix metalloproteinases, bacterial toxins, and viral glycopeptides.
- Over-expressed in cancer and various inflammatory conditions

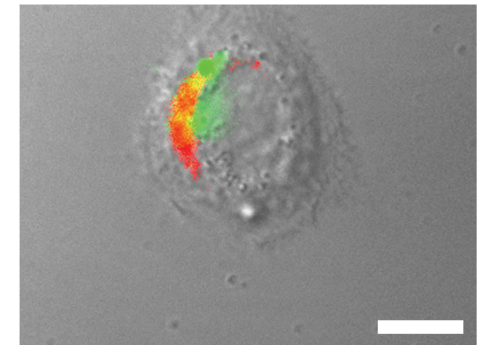
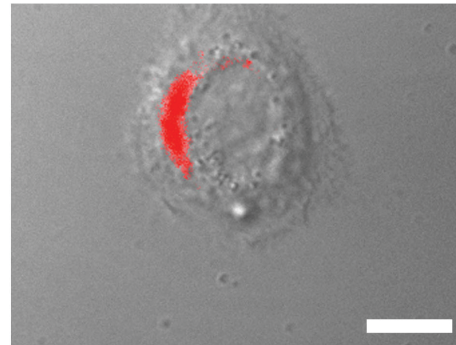
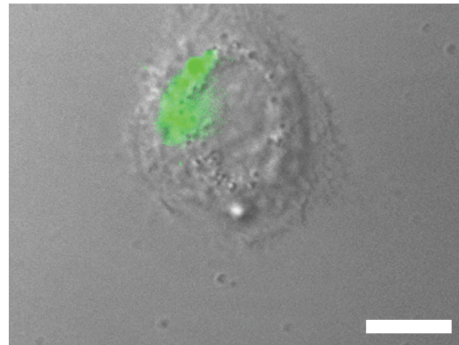
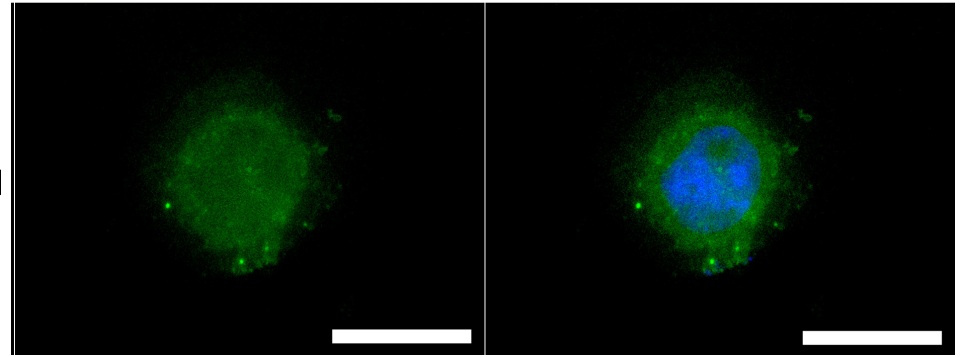


# Live-Cell Imaging of Furin Activity

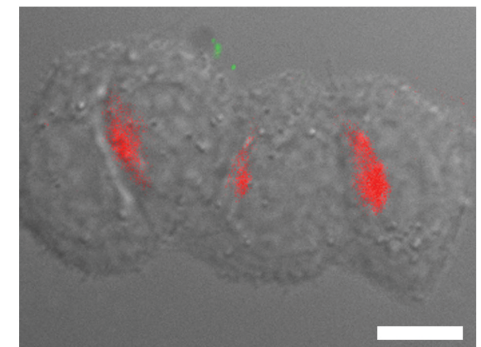
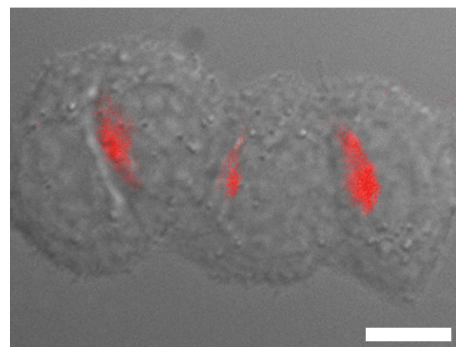
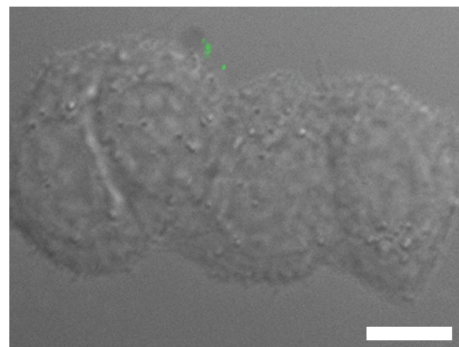


EGFP channel

DAPI + EGFP



Scrambled  
Control  
Probe



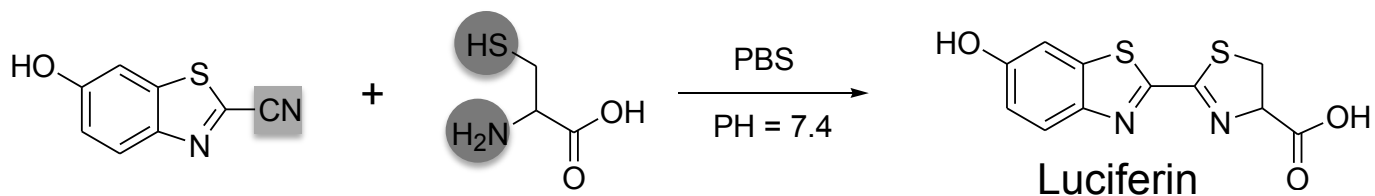
FITC

Golgi Staining

Overlay

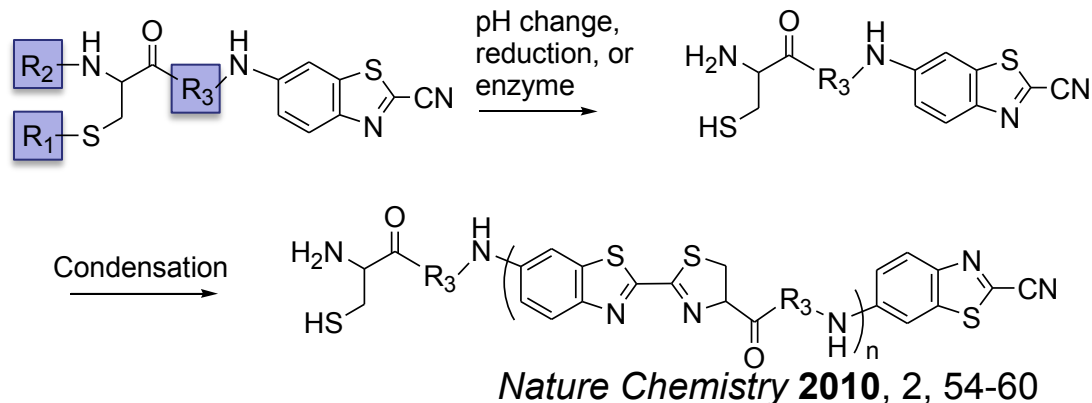


# Biocompatible Condensation Reaction



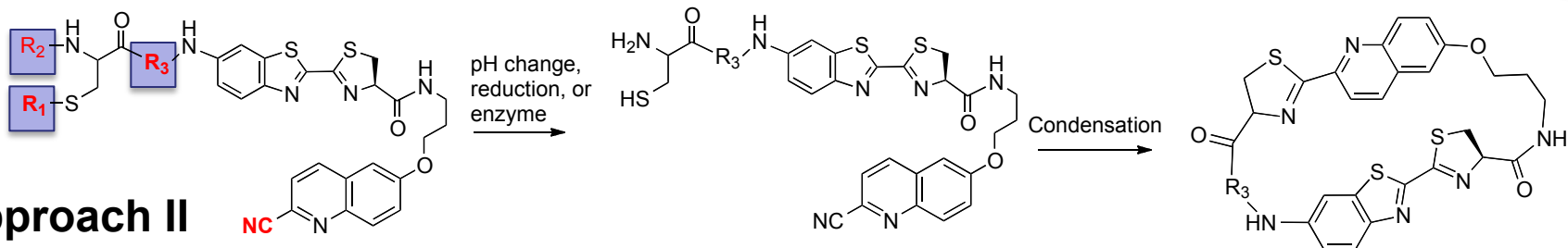
## Approach I

- Potential competition from intracellular free cysteine
- Condensation products are a mixture of oligomers



## Approach II

- Not subject to free intracellular cysteine
- Simple cyclization products
- larger molecule, harder to make



# What May It Be Used for

- Imaging

- Potential targets in many cellular processes

redox potential in cells

deacetylase

hydrolytic enzymes: proteases

- Modalities

Magnetic Resonance Imaging

PET/SPECT

Optical: Fluorescence; Photoacoustics

... ..

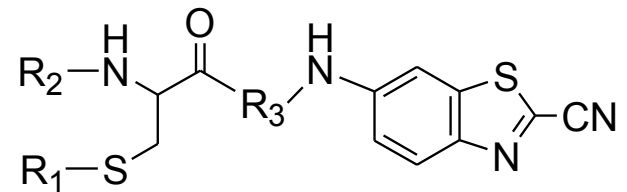
- Beyond imaging

- Drug delivery for cancer molecular therapeutics: an anticancer drug as the payload

- Radio-therapeutics

- Hyperthermal ablation for cancer treatment: Au NPs as the payload

... ..



# Acknowledgements

## ***Current lab members***

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Jongho Jeon, Ph.D.

Kyung Hyun Lee, Ph.D.

**Kanyi Pu, Ph.D.**

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Mikael Palner, Ph.D.

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**Adam Shuhendler, Ph.D.**

Lina Cui, Ph.D.

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## ***Former lab members***

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Heike Daldrup-Link (Stanford)

Jeffrey Cirillo (Texas A&M)

**Brian Rutt (Radiology/Stanford)**

W. E. Moerner (Stanford)

**Fred Chin (Radiology/Stanford)**

## **Financial Support**

Burroughs Wellcome Fund

NCI Center for Cancer Nanotechnology Excellence (CCNE)

NCI ICMIC@Stanford

DoD BRCP

NIBIB

NCI

NIGMS

Bill & Melinda Gates Foundation

Human Science Frontier Program