NANOTECHNOLOGY IN BIOMEDICINE

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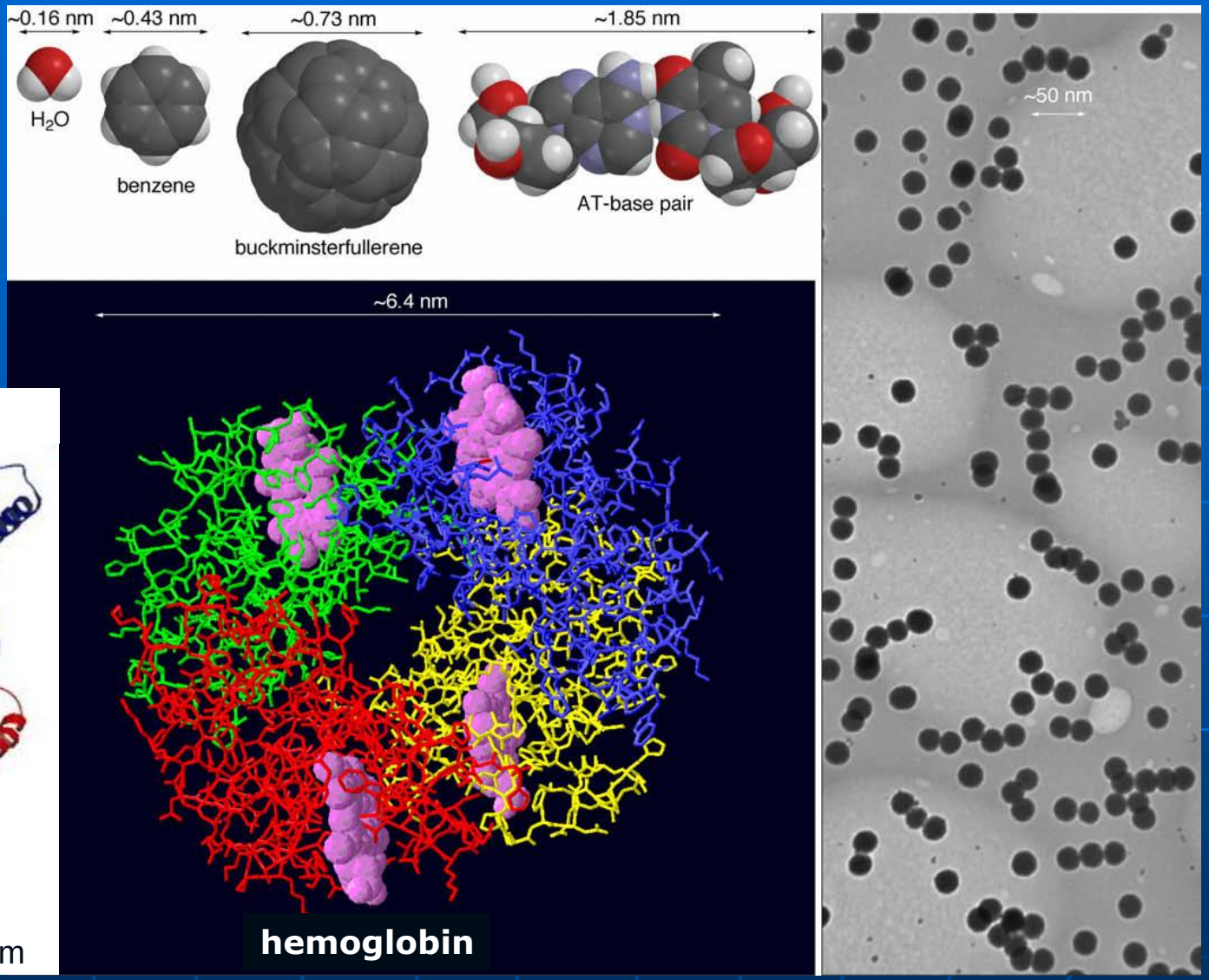
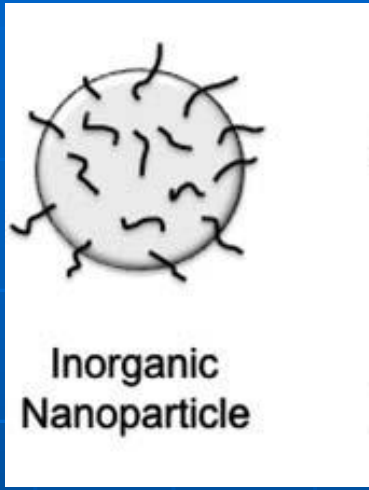


Sizes of organic molecules and biological macromolecules (left) in relation to silica nanoparticles (right).

nanoparticles (NPs) used in biomedical research



Gold NPs → bio-sensing and bio-imaging

Boisselier E, Astruc D. Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. Chem Soc Rev. 2009 Jun;38(6):1759-82. Epub 2009 Apr 21.

Hainfeld JF, Dilmanian FA, Slatkin DN, Smilowitz HM. Radiotherapy enhancement with gold nanoparticles. J Pharm Pharmacol. 2008 Aug;60(8):977-85. Links

→Used for Tumor directed drug delivery

Paciotti GF, Myer L, Weinreich D, Goia D, Pavel N, McLaughlin RE, et al. Colloidal Gold: A Novel Nanoparticle Vector for Tumor Directed Drug Delivery 2004; 11:169

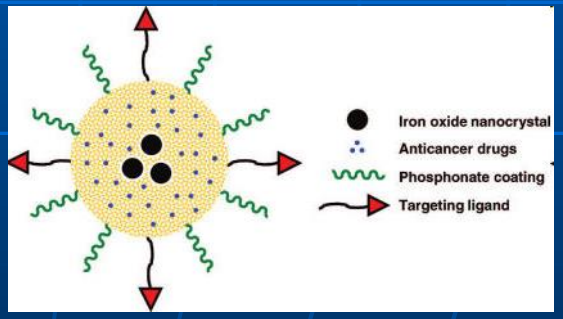
O'Neal DP, Hirsch LR, Halas NJ, Payne JD, West JL, Photo-thermal tumor ablation in mice using near infrared-absorbing nanoparticles Cancer Lett 2004; 209:171

Iron oxide NPs → bio-imaging---- MRI

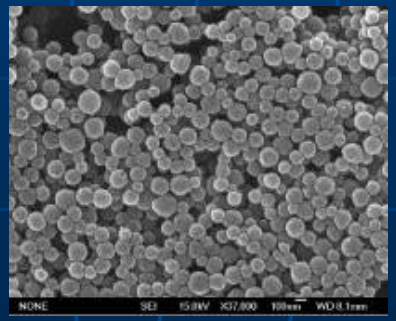
Kharisov BI, Kharissova OV, Yacamán MJ, Ortiz M U. State of the art of the bi- and trimetallic nanoparticles on the basis of gold and iron. Recent Pat Nanotechnol. 2009;3(2):81-98.

Xie J, Huang J, Li X, Sun S, Chen X. Iron oxide nanoparticle platform for biomedical applications. Curr Med Chem. 2009;16(10):1278-94.

Liong M, Lu J, Kovochich M, Xia T, Ruehm SG, Nel AE, Tamanoi F, Zink JI. Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. ACS Nano. 2008 May;2(5):889-96.



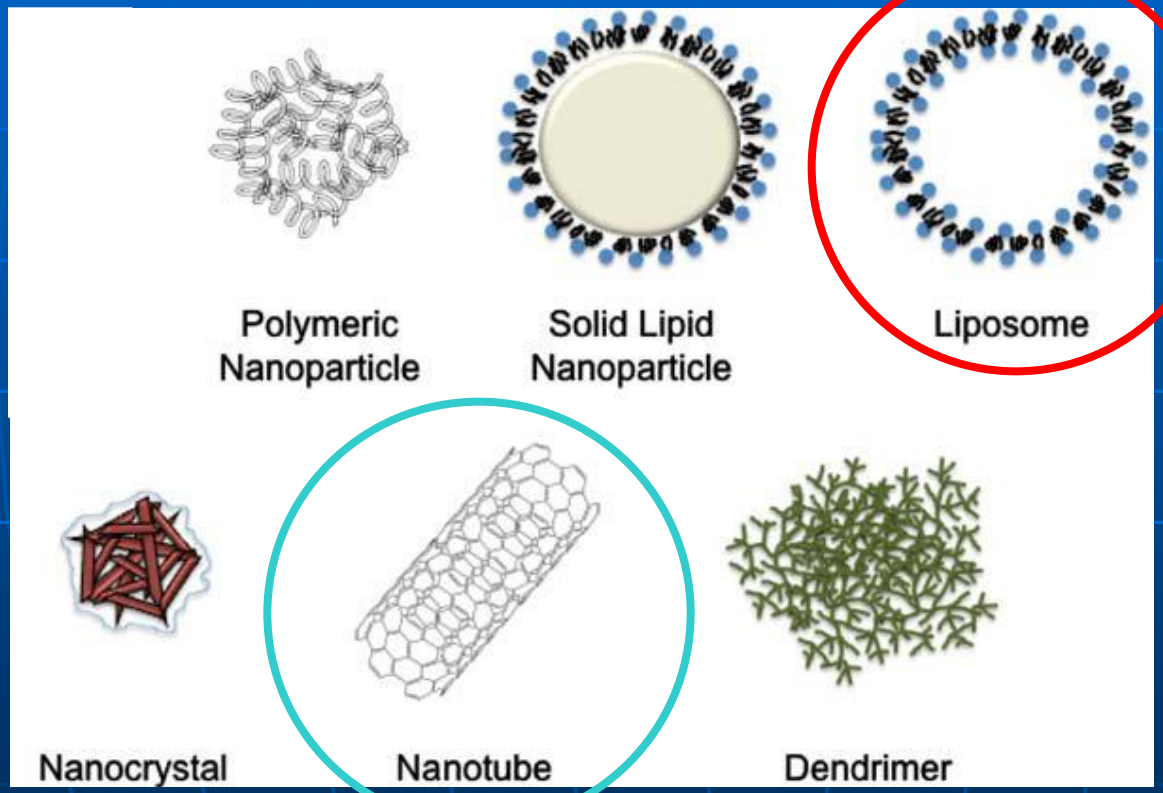
Silica NPs → surface pores



nanoparticles used in biomedical research

Ease of surface modification

Good biocompatible profile



Low biological stability

↓

Have not a high medical impact

Large internal volumes –drug delivery-

External surface easly functionalized –targeting-

Biocompatibility and toxicity reports are not clear

characteristics to define BIO-nanoparticles:

Distinctive features of BNPs, in comparison to synthetic nanoparticles

- Monodispertion and uniform size distributions
- controlled composition and surface properties in a genetic meaning
- High stability
- Economic large-scale production in grams and kilograms quantities

Bio-Nanoparticles pharmacokinetics

it is very important to monitor the pharmacokinetics (PK)
of NP
to understand and predict

their **efficacy** and
side effects

Bio-Nanoparticle pharmacokinetics (1)

Biodistribution

When a pharmaceutical agent is introduced into the circulatory system it is distributed systemically from the vascular and lymphatic systems in a tissue

A passive targeting mechanism involves in the biological distribution profile is the size of the nanoparticle

- Very small nanomaterials, **1–20 nm**, not have long circulatory residence time but they extravase and accumulate into interstitial spaces

- larger sizes, **20–100 nm**, avoid leakage into capillaries, but they are small enough to avoid reticuloendothelial or mononuclear phagocyte clearance

- over **100 nm** have a long permanence in circulating blood and tend to deliver in anatomical districts with leaky vascular beds or tumors or inflamed sites

Bio-Nanoparticle pharmacokinetics (2)

PK profile is determined by their chemical and physical properties:
size, solubility, surface charge and surface functionality

1 an hydrophilic drug → renal filtration into the urine

cutoff size for renal excretion is around **5.5 nm**
size > 5.5 nm (neutral charge) long permanence in circulating blood

2 an hydrophobic drug → serum protein binding

The hydrophobic drugs are often transformed into hydrophilic metabolites in the liver and excreted into the bile or eliminated into the urine

Bio-Nanoparticle pharmacokinetics (3)

1. Opsonins = The serum proteins that bind the nanoparticles

2. The Opsonins-bionanoparticles complex is recognized by receptors on the surface of the macrophages (reticuloendothelial system -RES- or mononuclear phagocyte system -MPS-)

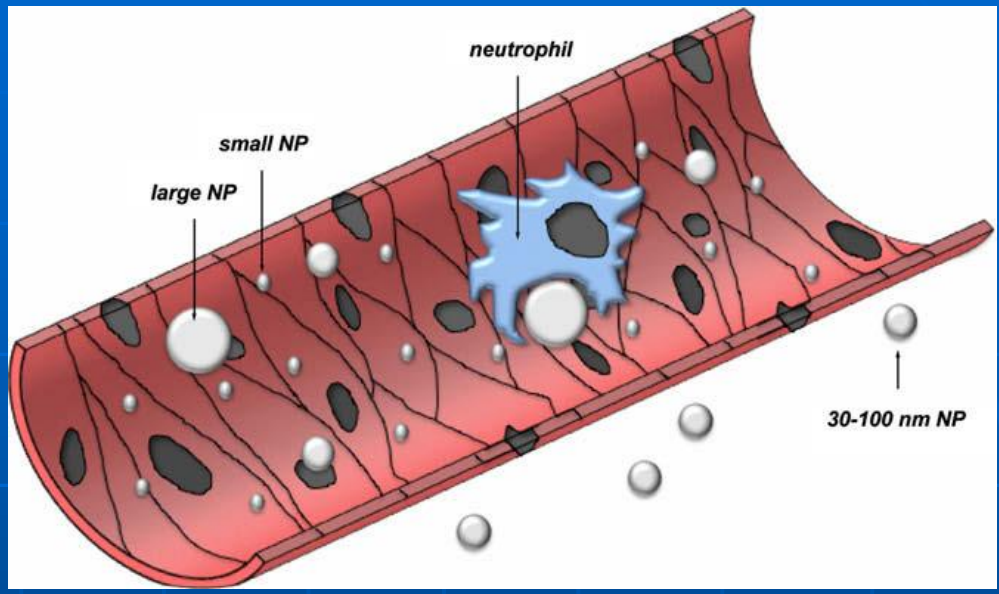
Opsonization is the major factor that induces RES uptake of nanoparticles

↓

the most commonly used strategy to minimize the degree of opsonization is to conjugate the polyethylene glycol (PEG) polymer onto the surface of the nanoparticles

Bio-Nanoparticle pharmacokinetics (4)

Tissue Selectivity of Nanoparticles



endothelial wall in the tissue
=
primary delivery barrier

leaky endothelial wall
=
liver (100nm), spleen, bone marrow

and pathological situation:
tumor and inflamed sites

NB The increased rate of tumoral uptake of nanoparticles is based on a phenomenon termed the "enhanced permeability and retention" (EPR) effect due to the increased capillary permeability in the tumor tissue.

enhanced uptake in the liver, spleen, and bone marrow is attributed to the macrophages residing in the tissues, responsible for clearing circulating nanoparticles

Bio-nanoparticles in biomedical imaging

Ferromagnetic Bio-nanoparticles

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Magnetic Resonance Imaging

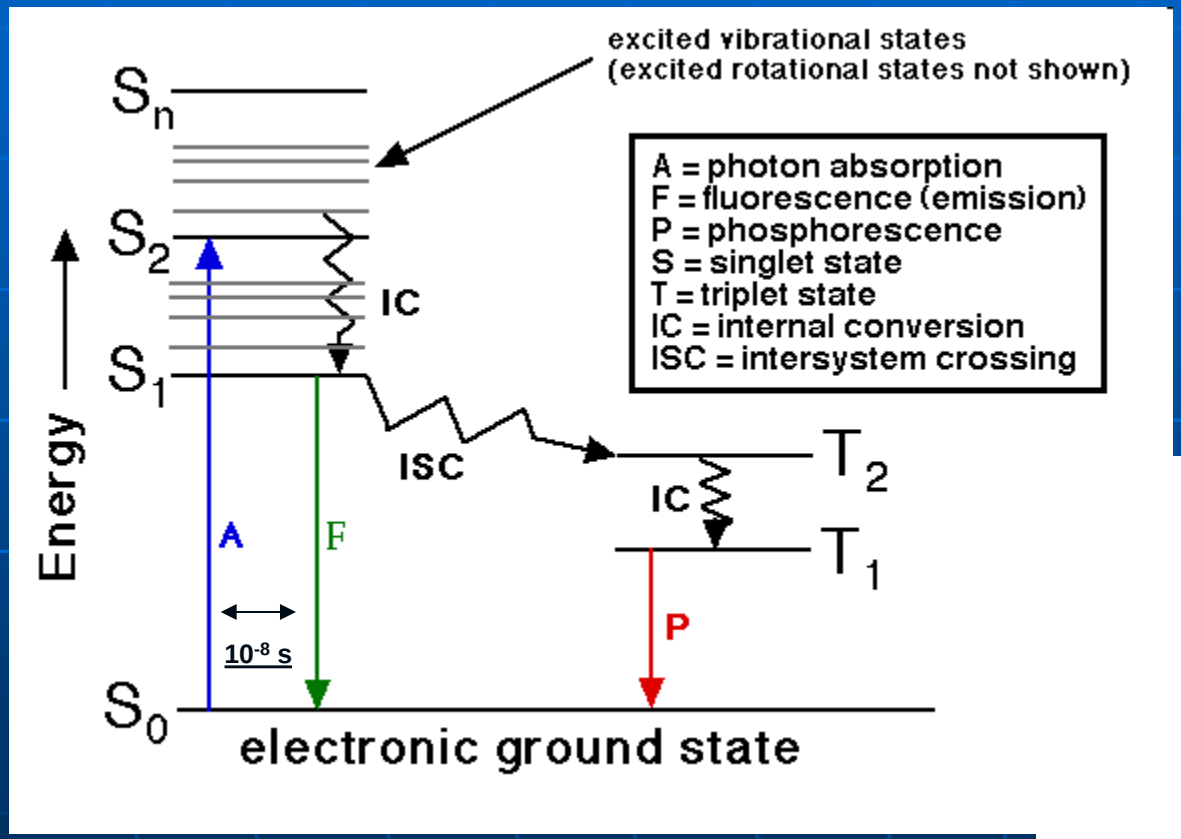
Fluorescent Bio-nanoparticles

↓

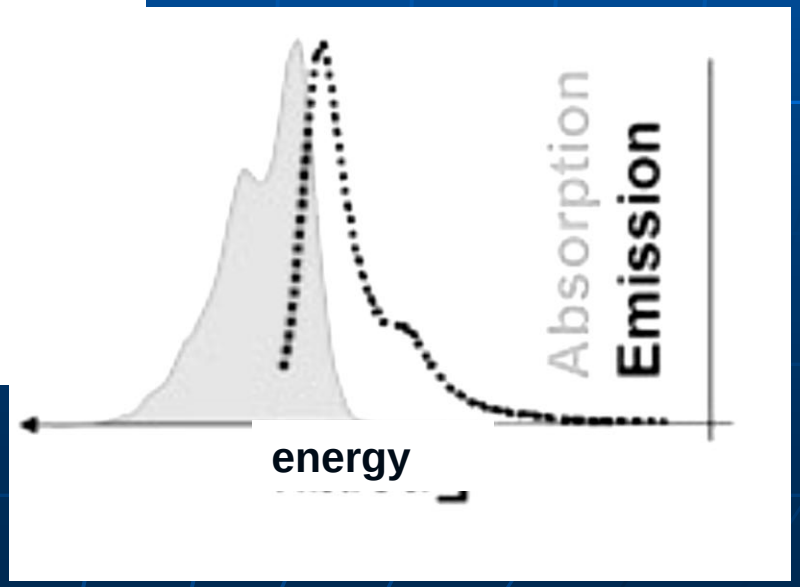
Optical Imaging

Fluorescent nanoparticles

Classical Fluorophores



electronic structure composed of **discrete electronic states**

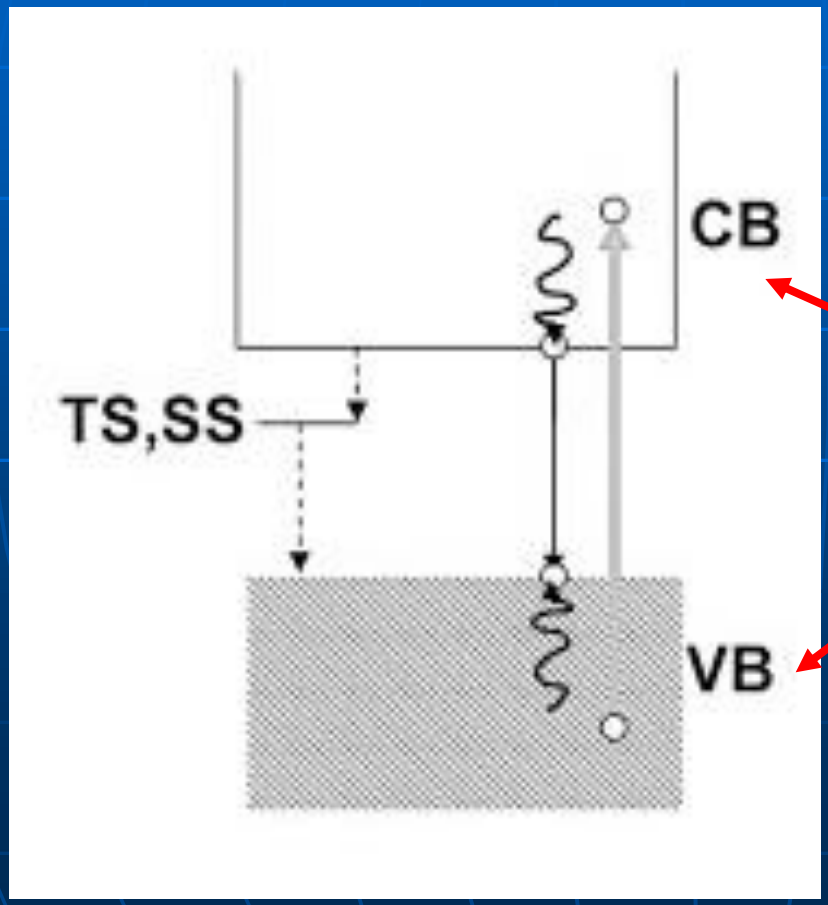


Jablonski diagram for organic dye

Fluorescent nanoparticles: Quantum dots

Fluorescent systems that differ markedly from organic dyes

quantum dots are nanocrystals made of semiconductor materials



Electronic states merge

into bands:

conduction band

valence band

separated by a **bandgap**

Fluorescent nanoparticles: Quantum dots

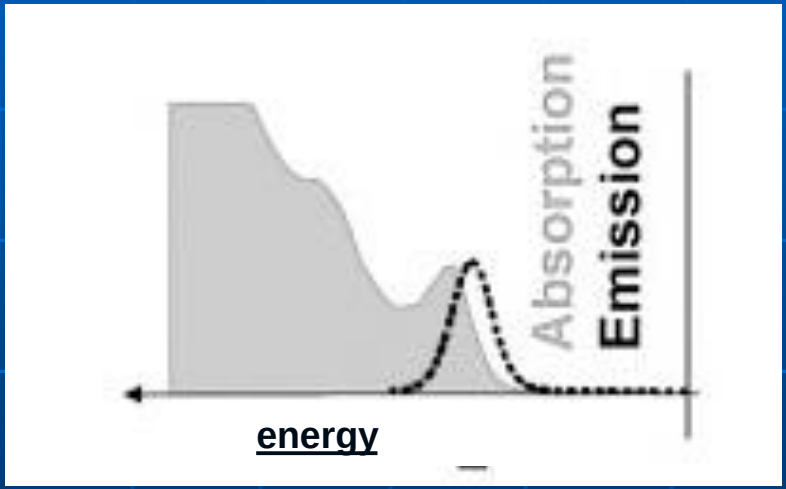
1. The absorption spectra is a continuum like the continuous nature of the conduction band

Advantages for biomedical imaging,

The excitation wavelength could be far from

the emission wavelength

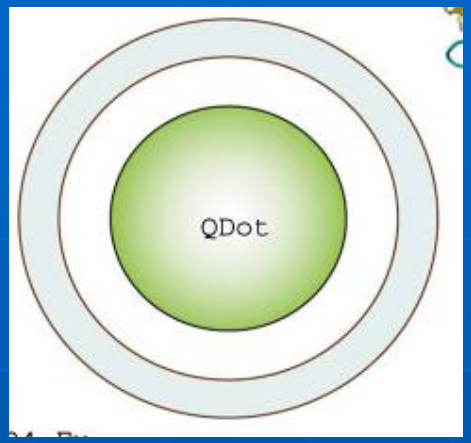
=> Great improve to fluorescent detection



2. Bangap is correlated with diameter of nanoparticles

quantum dots fluorescence is tunable with the size

Fluorescent nanoparticles: Quantum dots



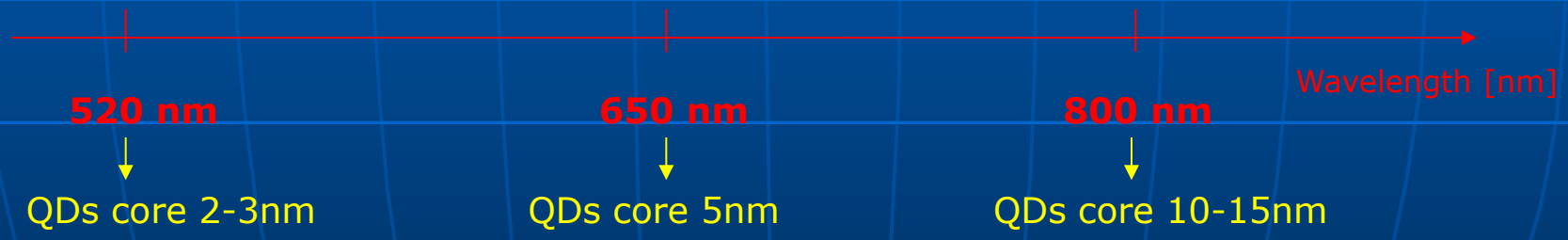
Quantum dots is costituted by

core (CdSe-CdTe) -→ core size determines bandgapa

shell (ZnS) that protects the core and enhances the optical properties

PEG coating → solubility and biocompatibility

→ Emission of quantum dots cover the visible spectrum from green and the nearIR spectrum



→ Quantum dots have a high extinction coefficient
→ a lower amount of light is needed to excite them

→ Quantum dots bioconjugation and targeting is quite easy
→ surface attach of biomolecules (antibody, peptides, enzyme)

Applications of quantum dots in biomedicine:

Quantum dots as bio-probe -> monitor cellular function

1. **Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, Weiss S. Quantum dots for live cells, in vivo imaging, and diagnostics.S**cience. 2005 Jan 28;307(5709):538-44.

2. **Pinaud F, Michalet X, Bentolila LA, Tsay JM, Doose S, Li JJ, Iyer G, Weiss S. Advances in fluorescence imaging with quantum dot bio-probes.** Biomaterials. 2006 Mar;27(9):1679-87. Epub 2005 Nov 28.

3. **Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. Quantum dot bioconjugates for imaging, labelling and sensing. Nat Mater. 2005 Jun;4(6):435-46.**

Quantum dots to target tissue specific vascular markers

Akerman ME, Pilch J, Peters D, Ruoslahti E. Angiostatic peptides use plasma fibronectin to home to angiogenic vasculature. Proc Natl Acad Sci U S A. 2005 Feb 8;102(6):2040-5.

Quantum dots to image lynph nodes

Kim S, Lim YT, Soltesz EG, De Grand AM, Lee J, Nakayama A, Parker JA, Mihaljevic T, Laurence RG, Dor DM, Cohn LH, Bawendi MG, Frangioni JV. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nat Biotechnol. 2004 Jan;22(1):93-7. Epub 2003 Dec

Optical Imaging

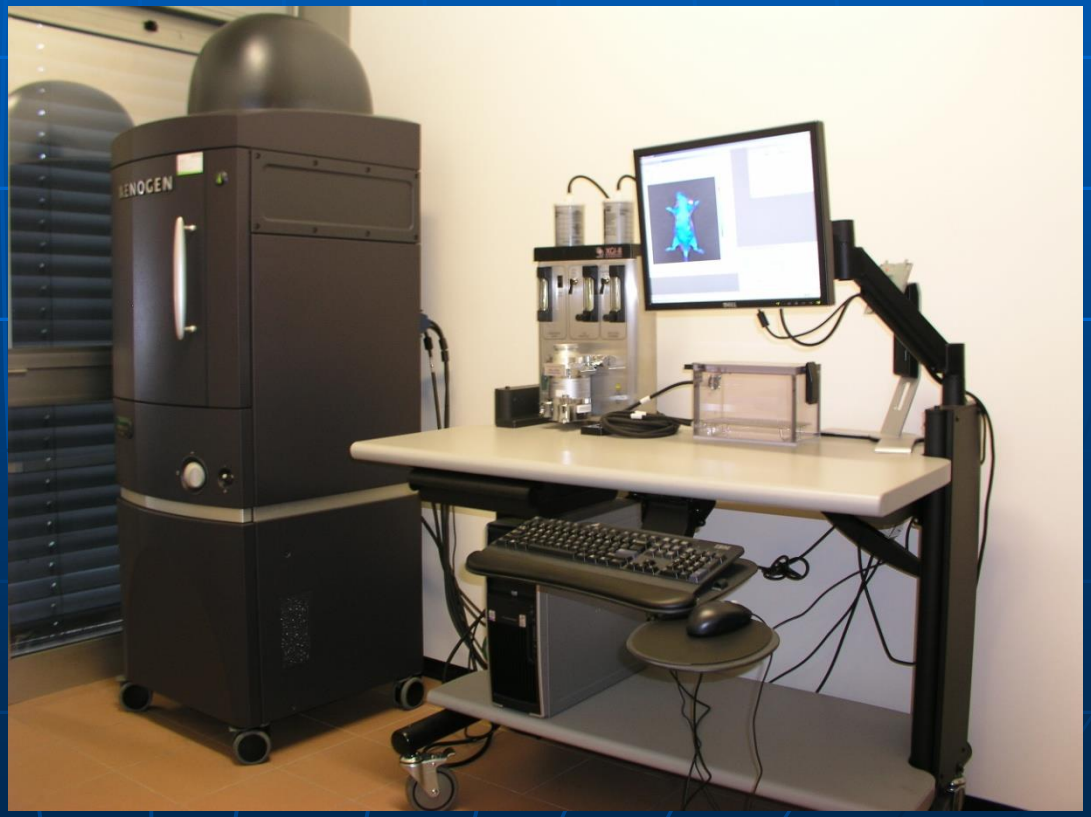
OPTICAL IMAGER

VivoVision Systems, IVIS® 200 Series, Xenogen (Xenogen Corporation, Alameda USA) Imaging system for laboratory animals

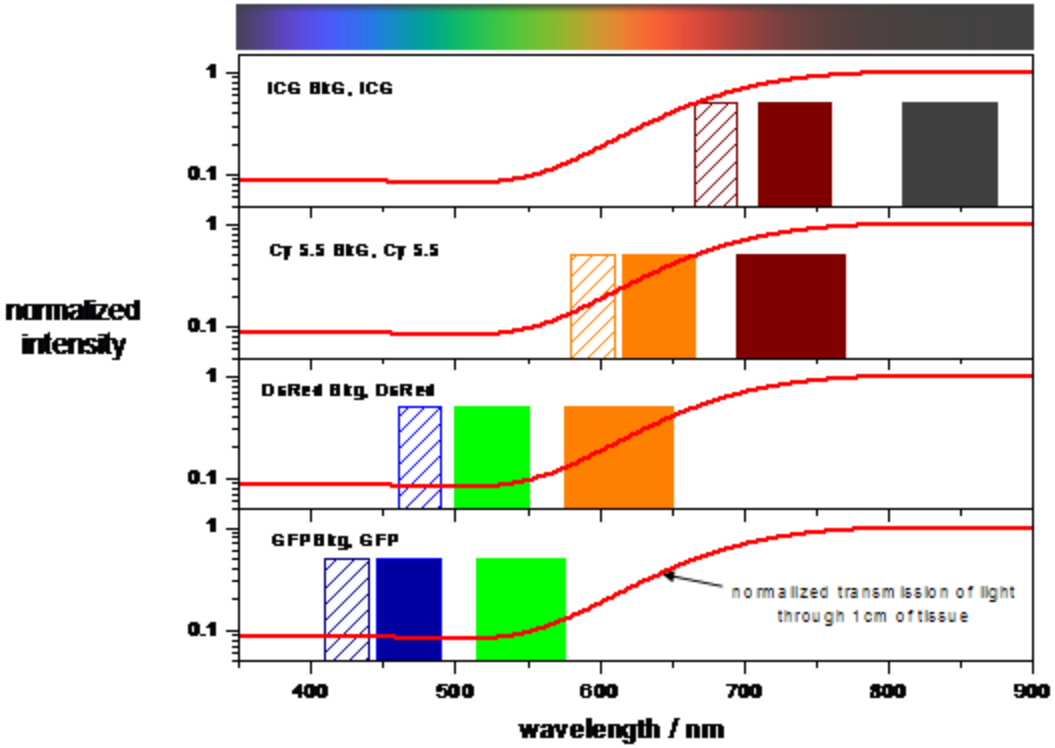
constituted by:

- a camera sensor CCD 1 (2.7 x 2.7cm, cooled at -90°C),
- a 150W Quartz halogen, 3250° Kelvin lamp
- minimal image pixel resolution: 20µm,
- pixel dimension 13.5µm, (imaging pixels 2048 x 2048)
- 8 excitation filters, 11 emission filters
- quantum efficiency > 85% between 500 and 700nm, > 30% between 400 and 900nm.

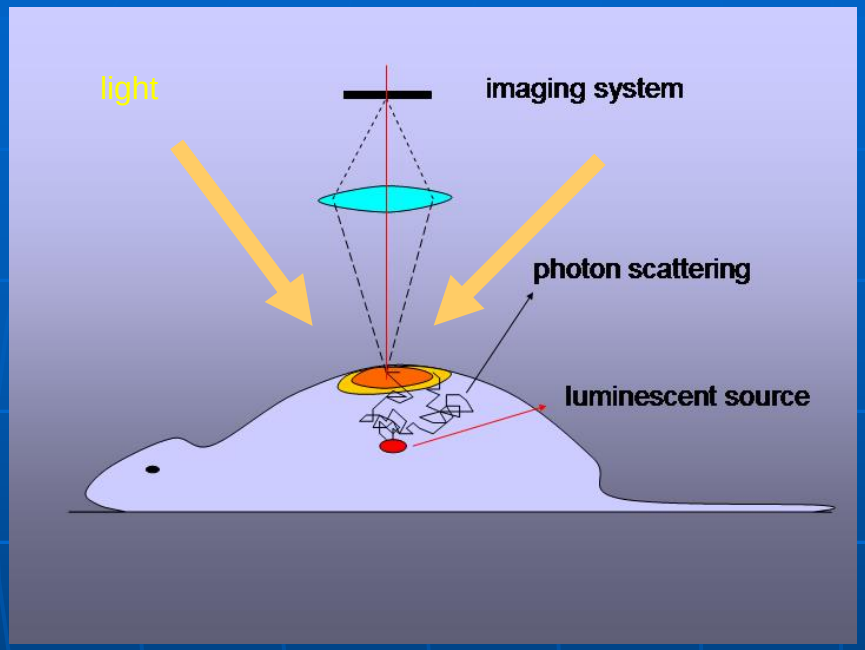
Two different acquisition modality: fluorescence and bioluminescence



Excitation and Emission filters set



Optical Imaging technique: Fluorescence

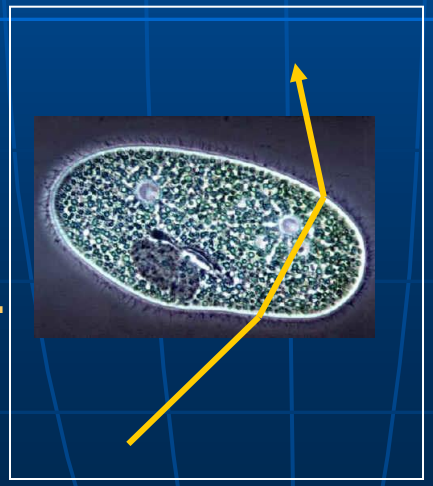


- an *in vivo* imaging tecnique,
- not invasive
- no ionizating radiation,
- high sensitivity,

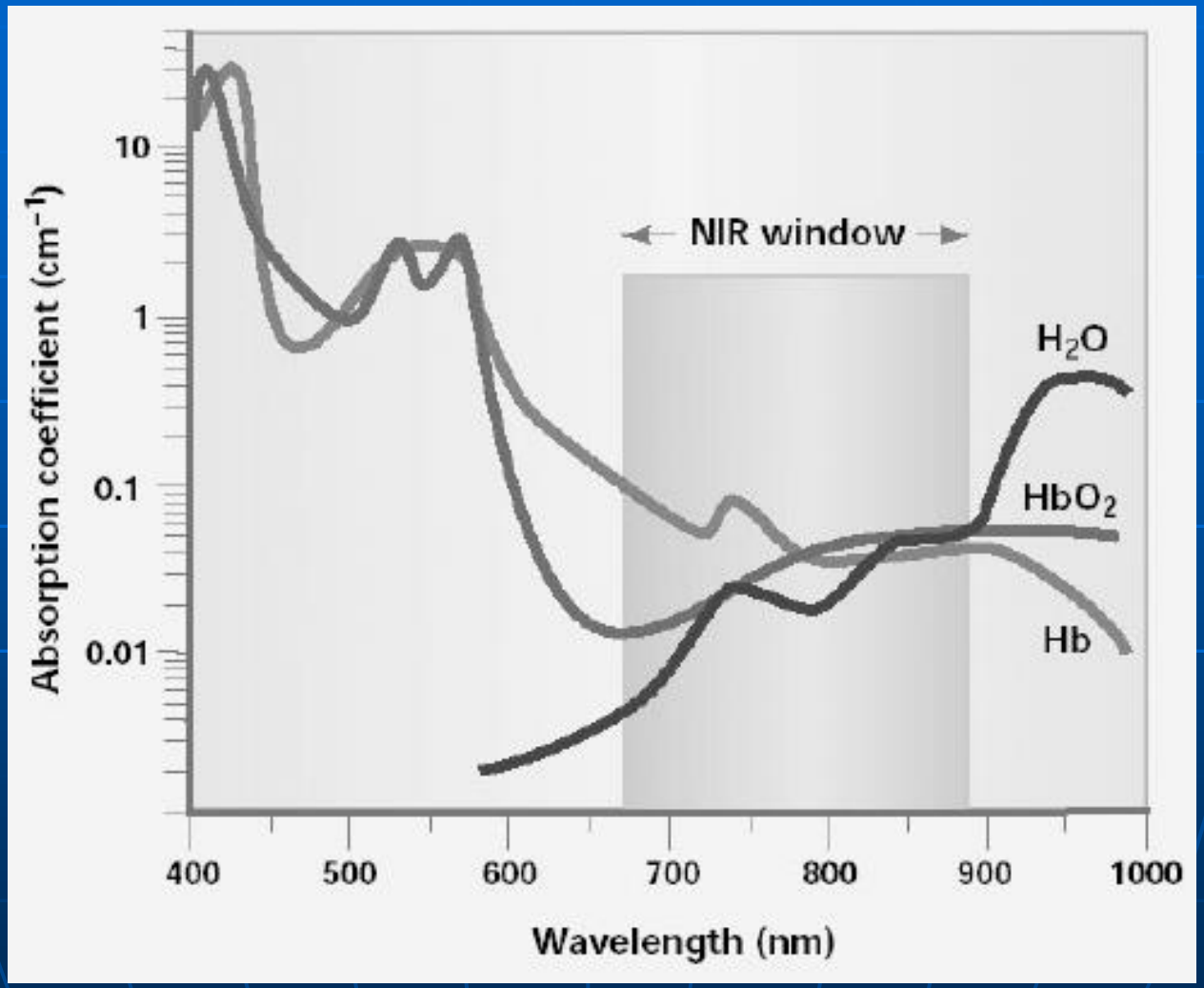
- low resolution (scattering),
- 2D images

- low penetration depth,

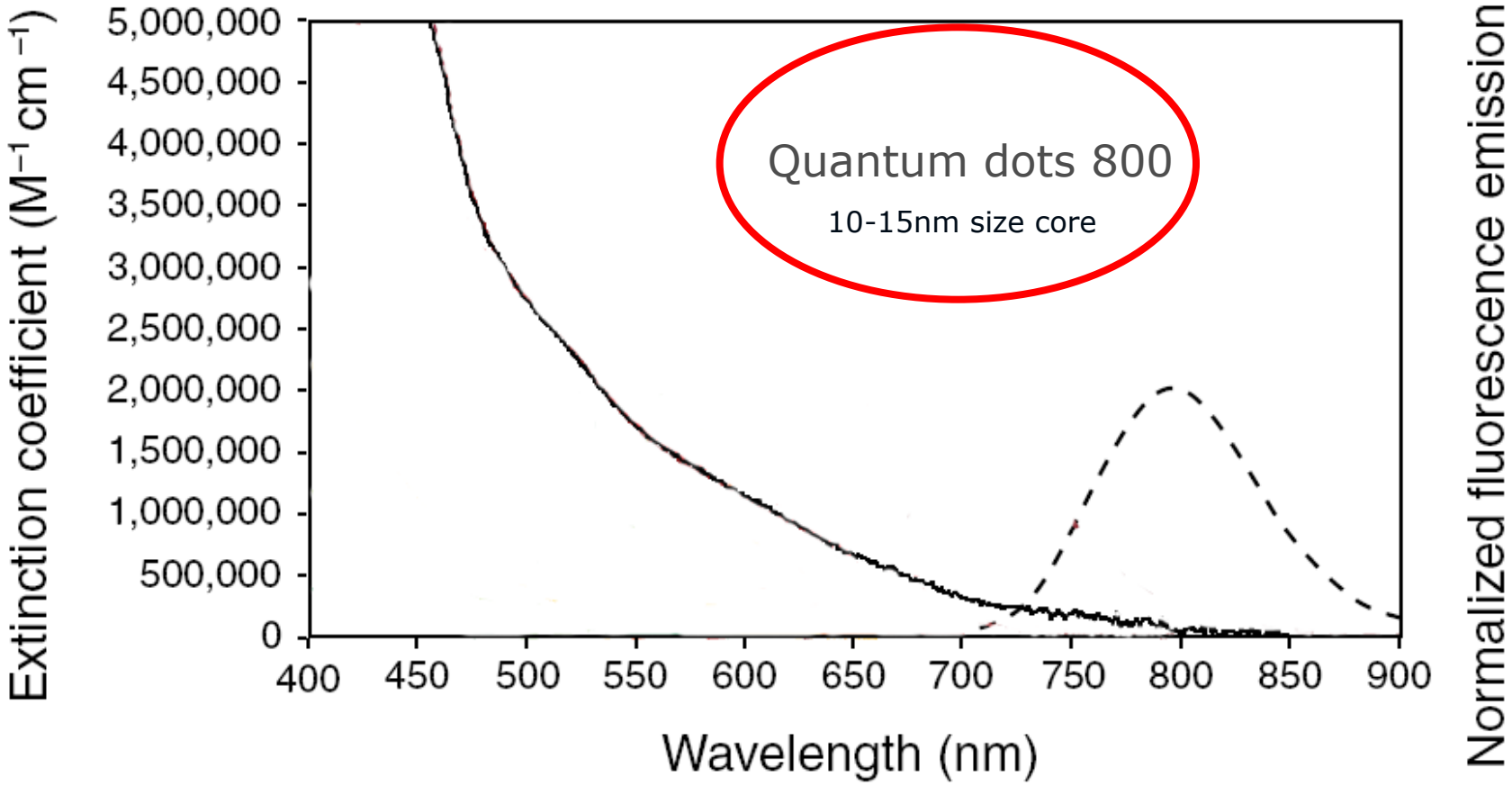
Scattering:
deviation of the light
from a straight trajectory.



Wavelengths window of tissue transparence



Commercial Quantum dots by Invitrogen



Quantum dots biodistribution in vivo

Aim :

to study with an in vivo imaging modality the biodistribution kinetics after the i.v. administration of quantum dots, Qtracker® 800 non-targeted QDs800, InvitrogenTM Milan, Italy.

Animal model:

- we have studied

  two different stock of laboratory animals
  - Athymic nude mice, female 4-5 weeks old
  - Balb C mice, female 4-5- weeks old

- two different dosages of quantum dots 800 solution: 1:5 and 1:10 dilution of commercial solution, 0.01ml/1g body weight, i.v. administration

- three different biodistribution kinetics time points:

3 hours immediatey after i.v. administration, 24 hours and 1 week after i.v. administration.

Acquisition protocol:

For all experiments we have used fluorescent modality, with the acquisition set of excitation filters:

GFP (445-490nm),

DsRed (500-550nm),

Cy5.5 (615-665nm),

ICG background (665-695nm),

ICG (710-760nm);

and the emission filter ICG (810-875nm).

A 150W Quartz halogen 3250° Kelvin lamp at high intensity; binning factor = 8; pixel resolution = 0.125x0.125; field of view = 12.8cm; exposition time = 1sec; opening of diaphragm (f/stop) = 2.

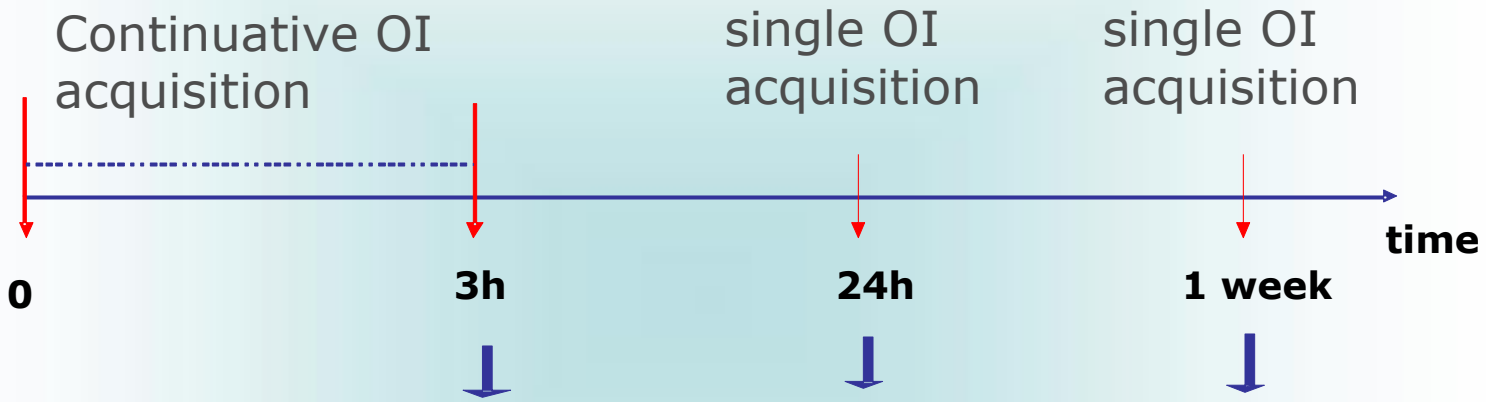
Images were acquired and analyzed with Living Image 2.6 software, Living Image 3D software (Xenogen Corporation, Alameda USA) and Matlab 7.1. Data was analyzed with ROI (region of interest) method.

Acquisition protocol was about a first pre acquisition and 30 post tracers i.v. administration acquisitions.

Experimental plan

Note: OI= optical imaging

Group A 1:5 diluition Qds800 dosage
Group B 1:10 diluition Qds800 dosage
Group C control group



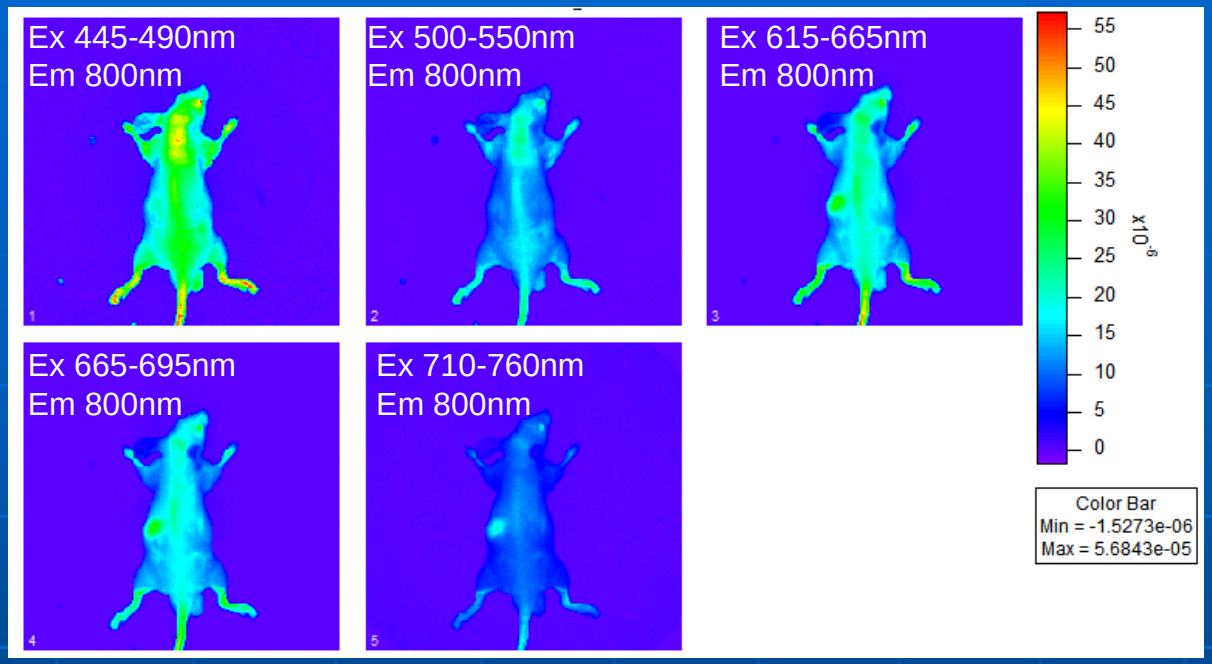
After 3h OI acquisition
- N=2 group A sacrified and surgically extracted organs
- N=2 group B sacrified and surgically extracted organs
- N=2 group C sacrified and surgically extracted organs
- N=1 group B sacrified, perfused with PBS, surgicaly extracted organs and collected blood

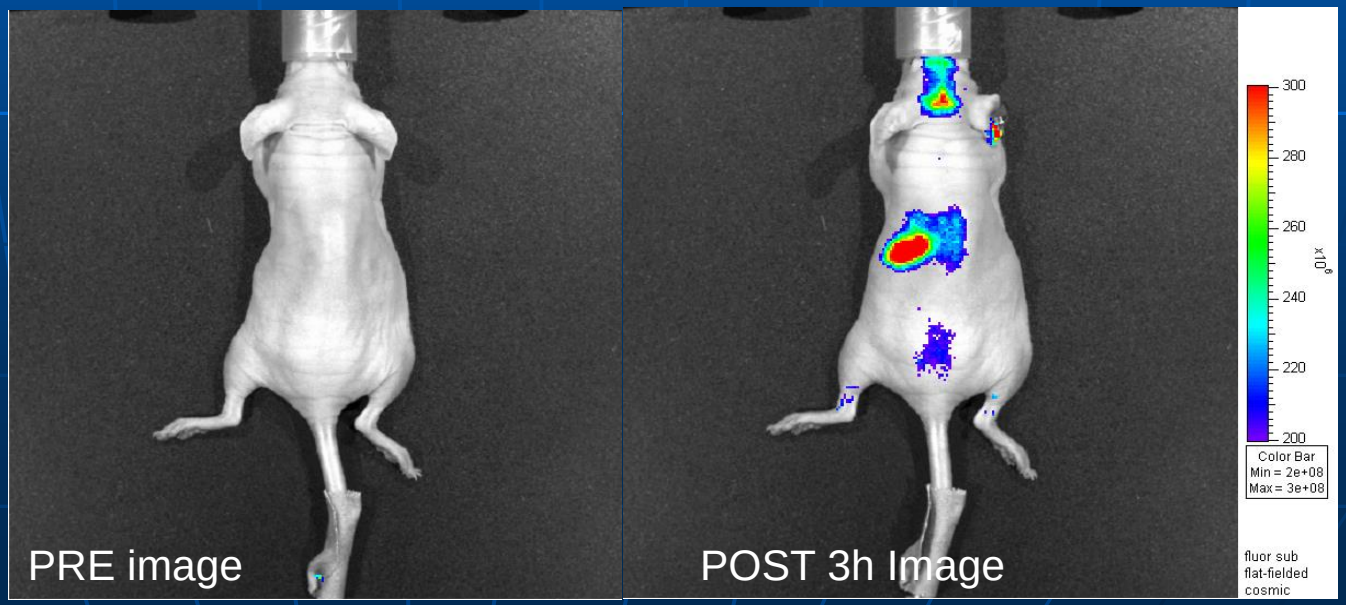
After 24h OI acquisition
- N=2 group A sacrified and surgically extracted organs

N=2 group B sacrified and surgically extracted organs
- N=2 group C sacrified and surgically extracted organs

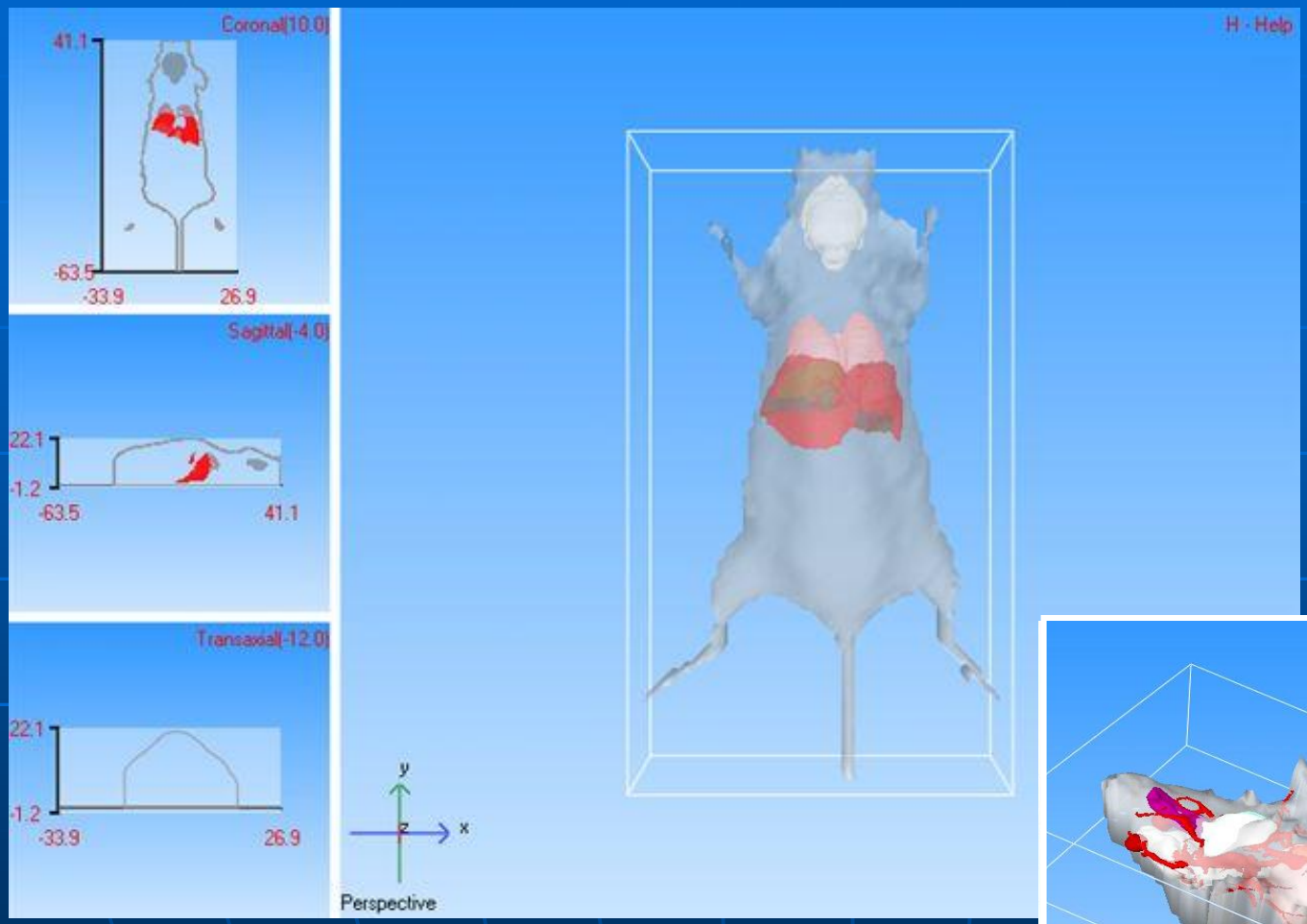
After 1 week OI acquisition
- N=2 group A sacrified and surgically extracted organs
- N=2 group B sacrified and surgically extracted organs
- N=2 group C sacrified and surgically extracted organs

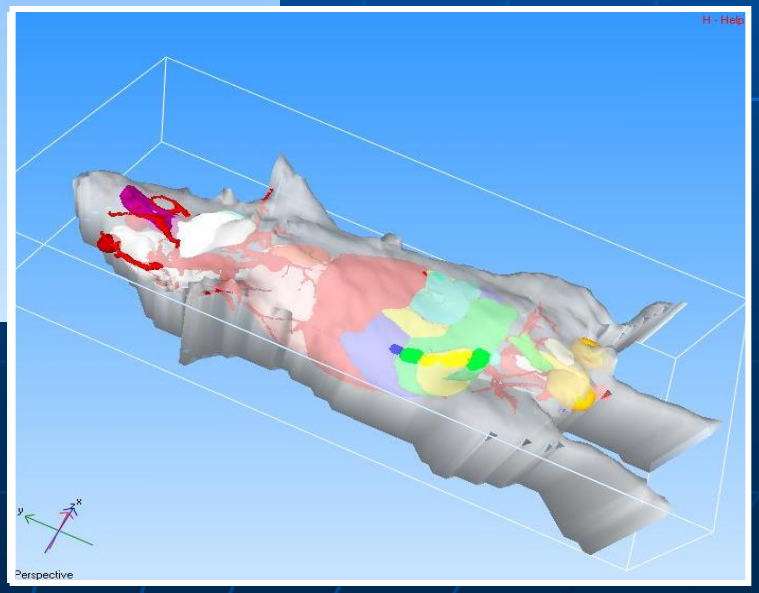
In vivo biodistribution kinetic in athymic nude mice

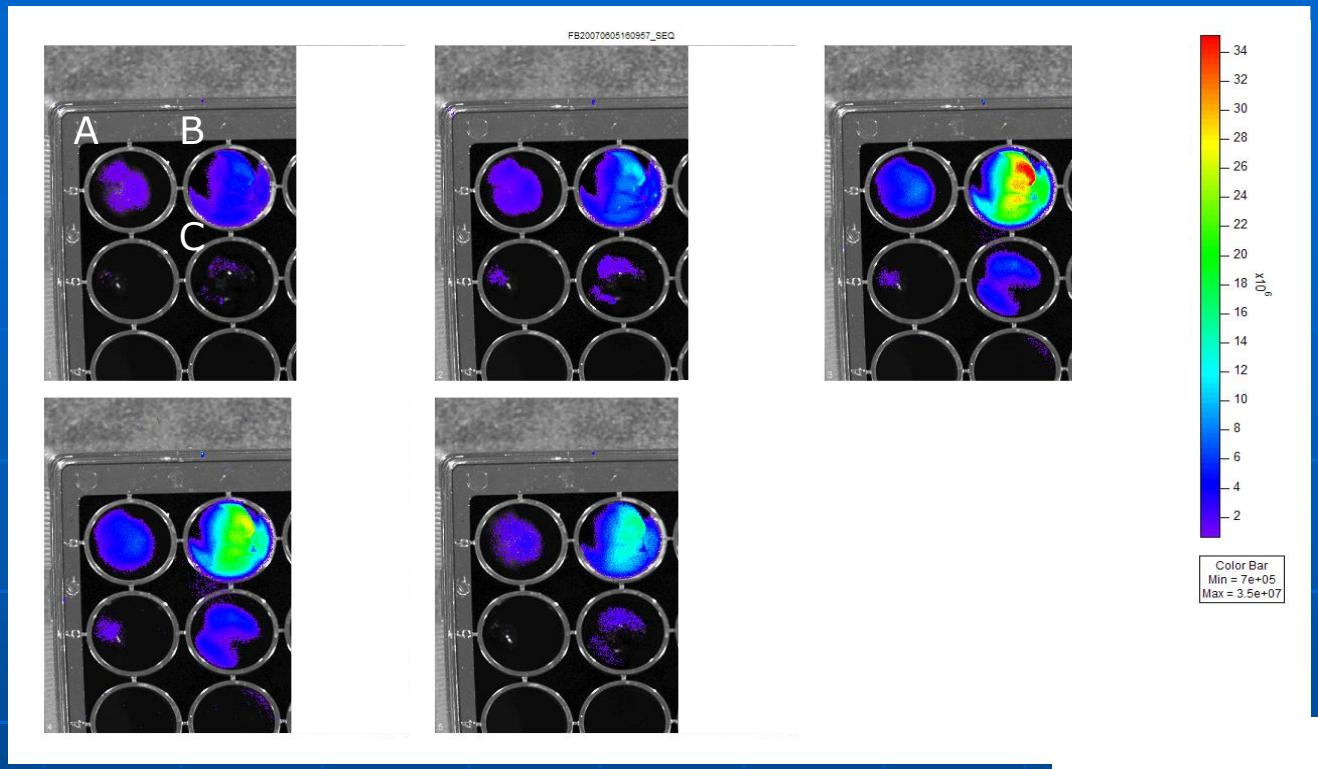




3D anatomical atlas

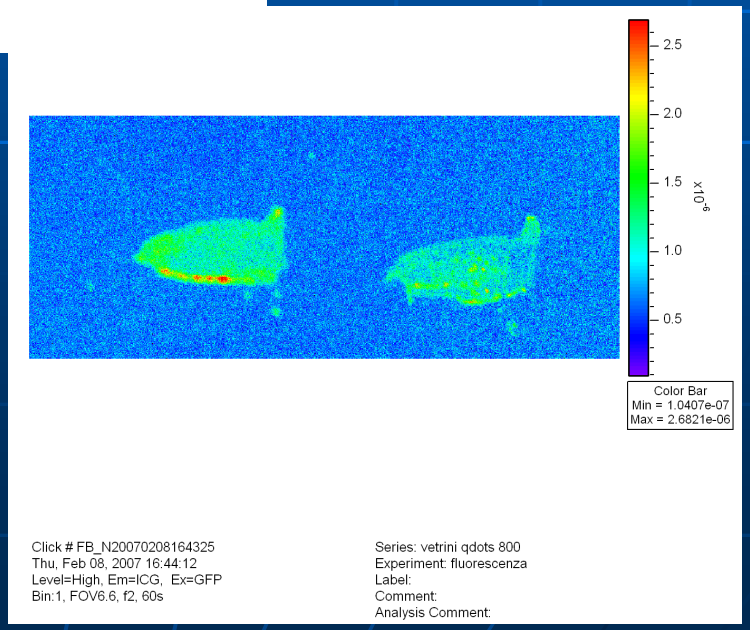






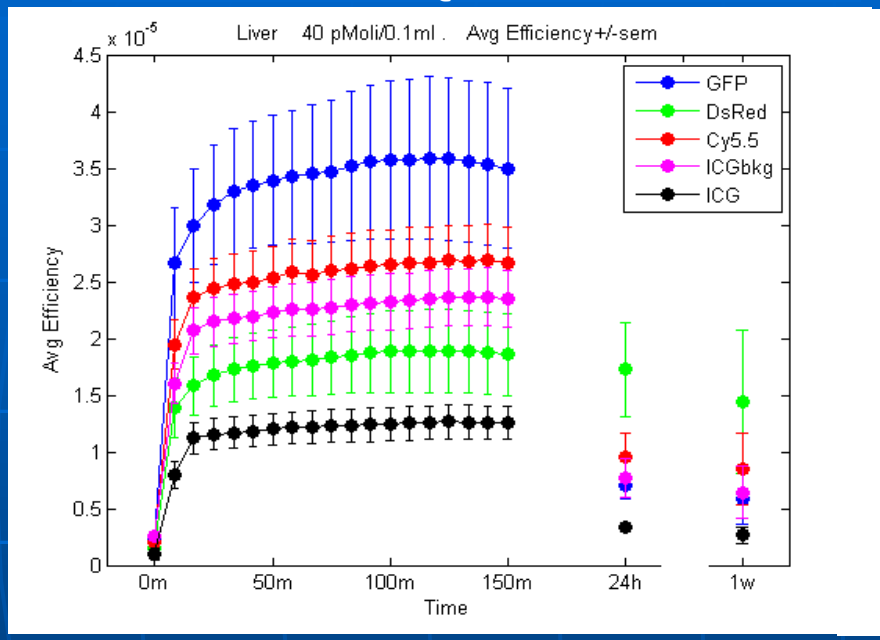
OI acquisition of organs after surgical extraction
→ 3h after an i.v. administration of Quantum Dots 800 solution
(1:10 dilution of commercial solution).
A brain, B liver, C lungs

OI acquisition about animal liver slices (10 µm of thickness)
Organ was exstracted after 3h from i.v. administration of Quantum Dots 800 solution
(commercial solution dilution 1:10) and -80°C frozen and cut with a cryostat

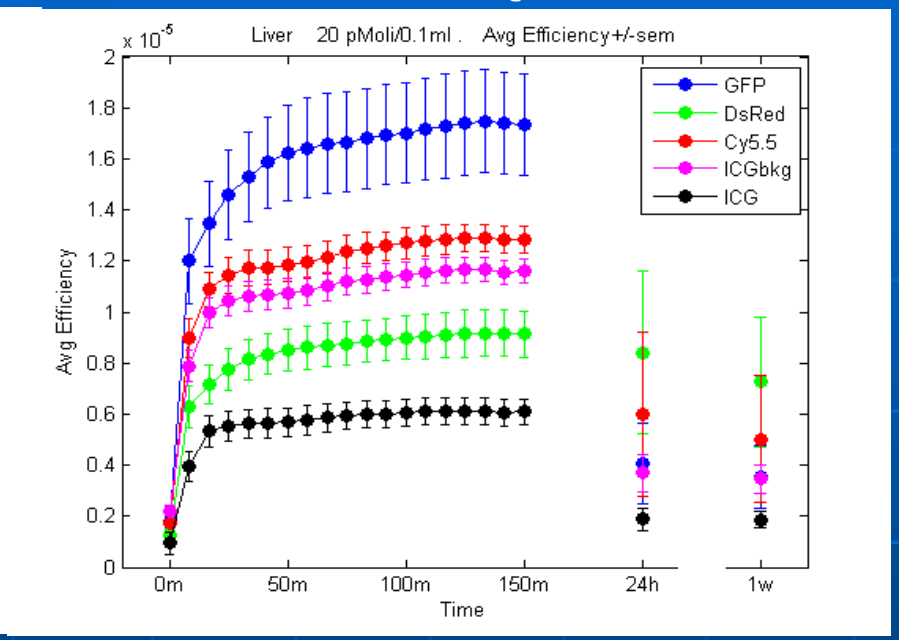


Hepatic region

1:5 dilution of QDs800 solution



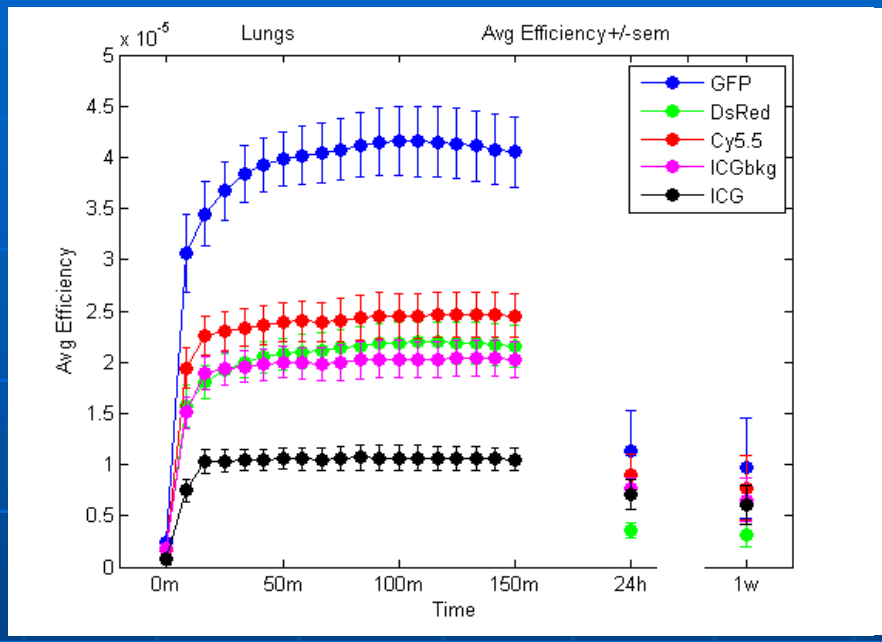
1:10 dilution of QDs800 solution



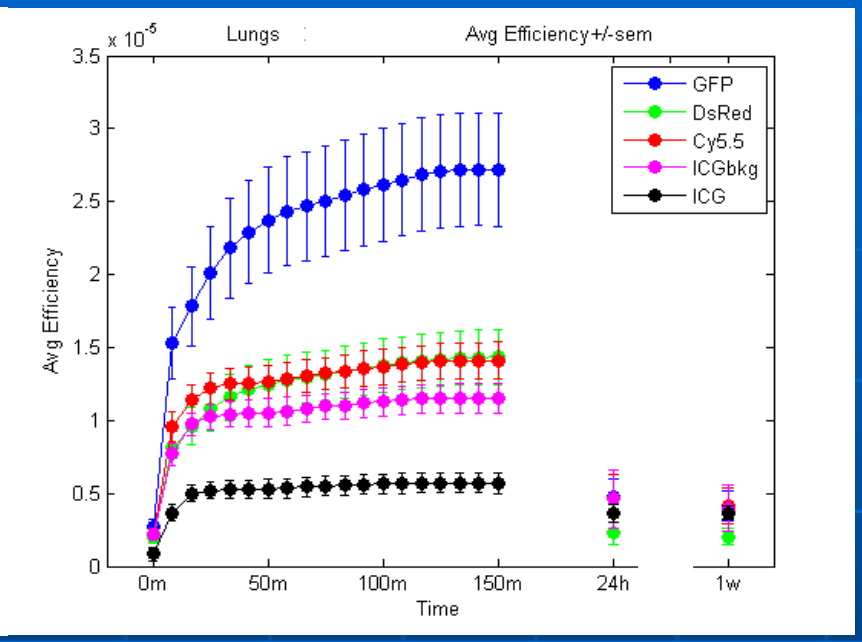
Average efficiency of fluorescence emission about accumulation kinetic of QDs800 on hepatic region: a 3h continuous acquisition immediately after i.v. administration; 24 hours and 1 weeks after i.v. administration. Excitation filters used: GFP, DsRed, Cy5.5, ICGbkg e ICG. Emission filter used: ICG

Pulmonary region

1:5 dilution of QDs800 solution



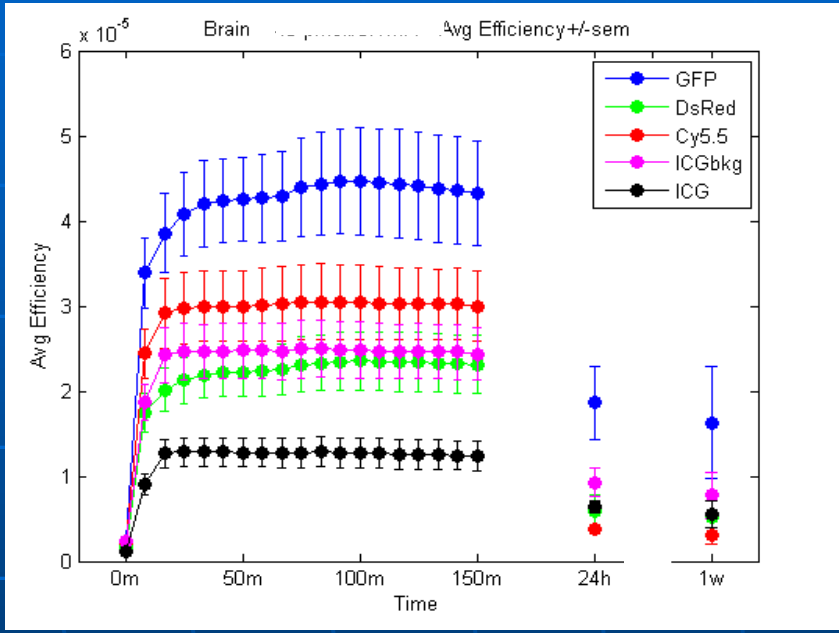
1:10 dilution of QDs800 solution



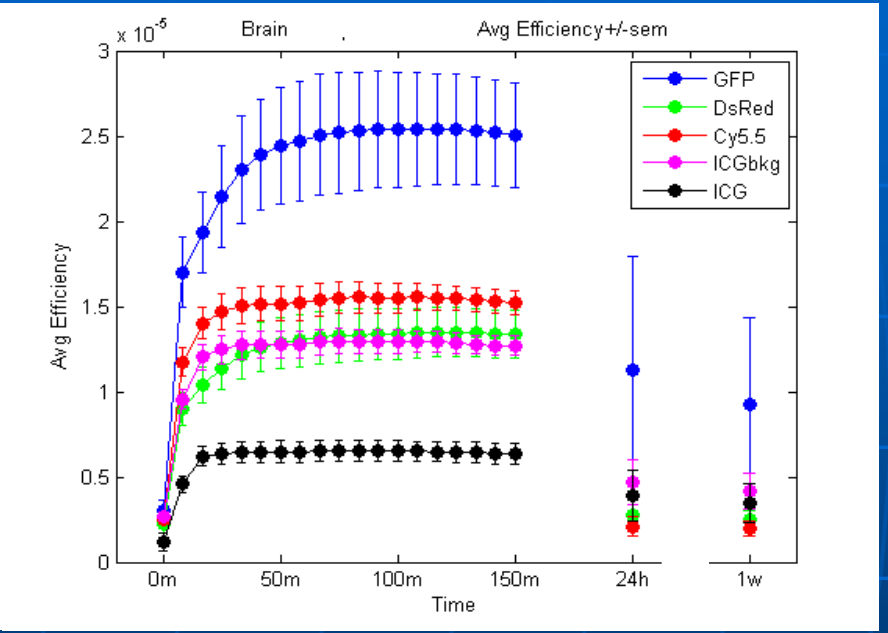
Average efficiency about kinetic of QDs800 accumulation on pulmonary region: a 3h continuous acquisition immediately after i.v. administration; 24 hours and 1 weeks after i.v. administration. Excitation filters used: GFP, DsRed, Cy5.5, ICGbkg e ICG. Emission filter used: ICG

Cerebral region

1:5 dilution of QDs800 solution



1:10 dilution of QDs800 solution



Average efficiency about kinetic of QDs800 accumulation on cerebral region: a 3h continuous acquisition immediately after i.v. administration; 24 hours and 1 weeks after i.v. administration. Excitation filters used: GFP, DsRed, Cy5.5, ICGbkg e ICG. Emission filter used: ICG

BEHAVIOUR

Spontaneous locomotor activity

Proximity to food/water dispenser

ELECTROENCEPHALOGRAM (EEG)
signal telemetry

Wake/sleep cycle

EEG features

<u>Thank you to</u>:

Optical imaging group:

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Behavioural group

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Veneto Nanotech group

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