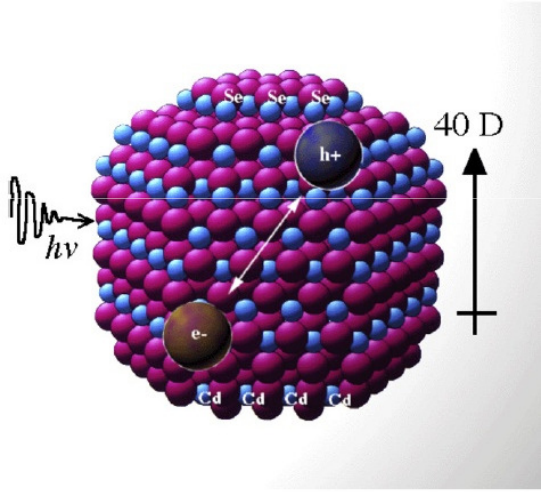
Rapid translocation of nanoparticles from the lung airspaces to the body

Nature Biotechnology **28**, 1300 (2010)



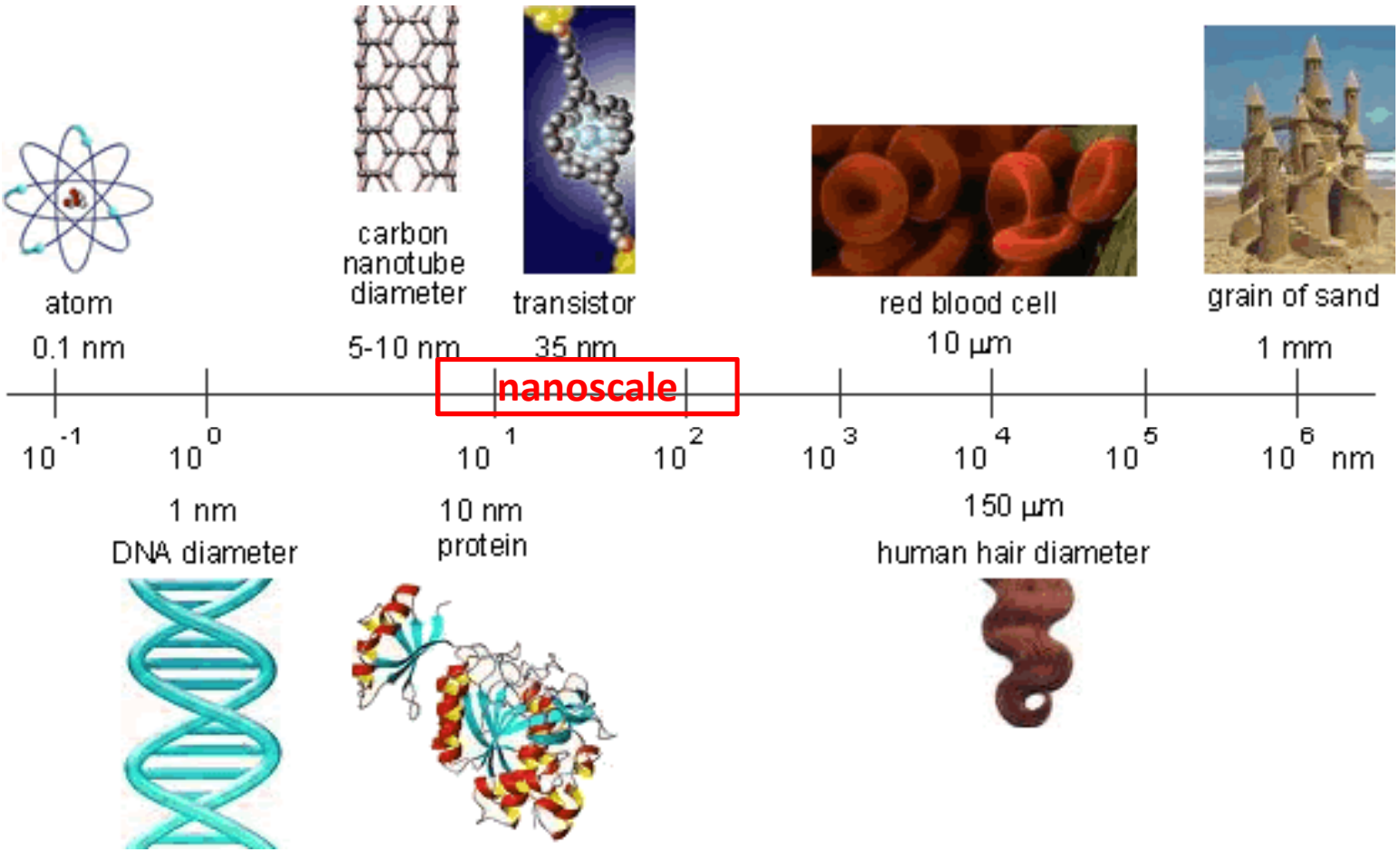
Speaker: Ching-Min Chang

Coach professor: Shih-Hsiung Wu

Date：31th, March, 2011

Introduction

What is nanotechnology??



A nanoparticle ≒ A football

Cell ≒ football field

Nanoparticles in the lung

large surface area
the air-blood barrier is rather thin

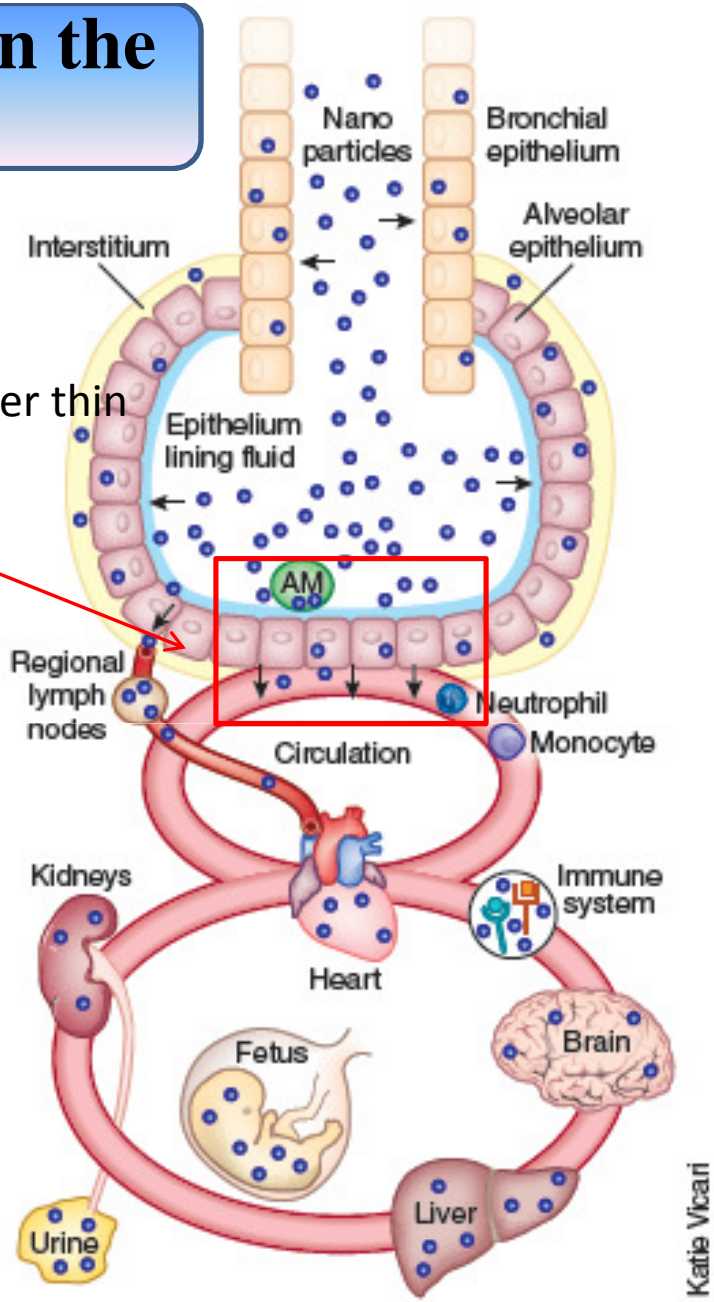
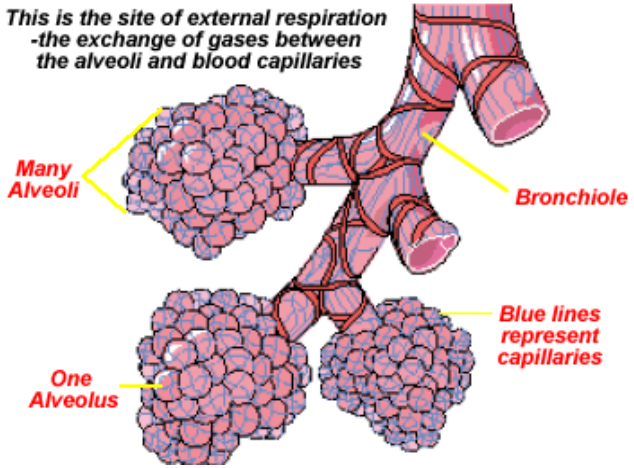
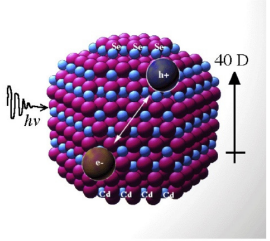


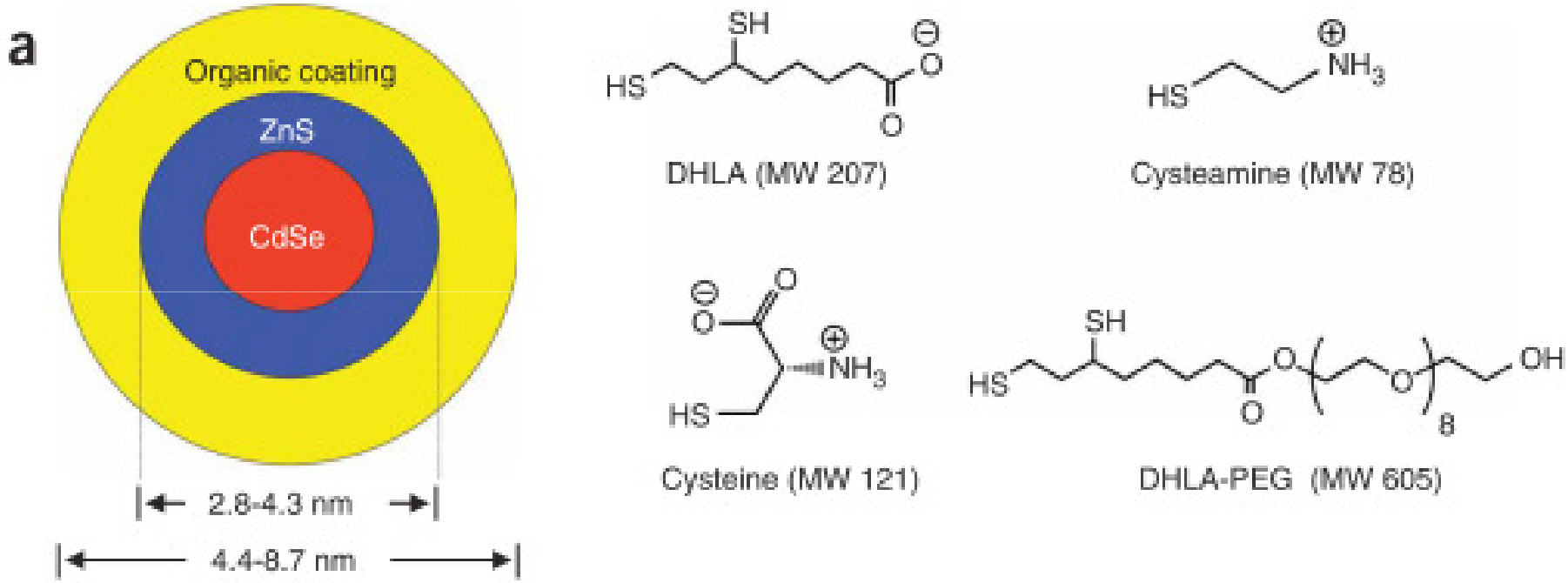
Figure 1 Schematic of nanoparticle translocation from the lung epithelium to regional lymph nodes and blood circulation. Once circulating



Nature Biotechnology ***28***, 1275 (2010)

Nanoparticle



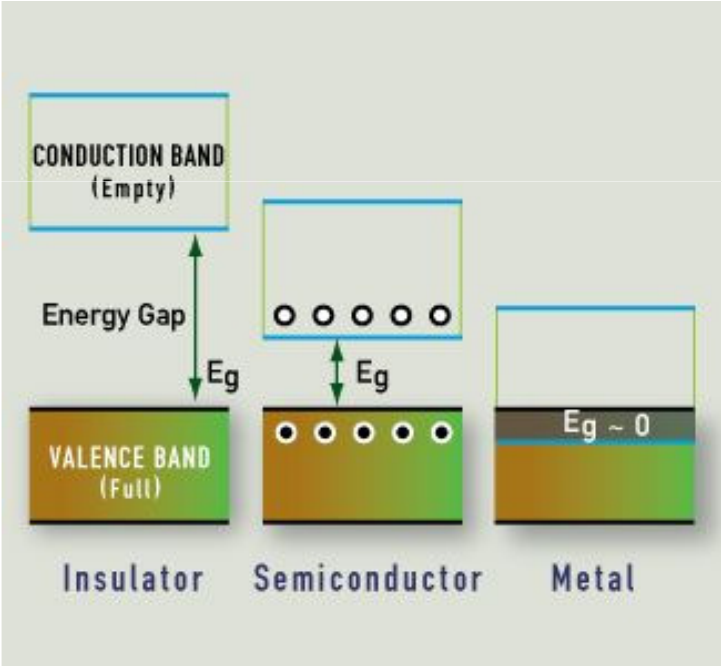


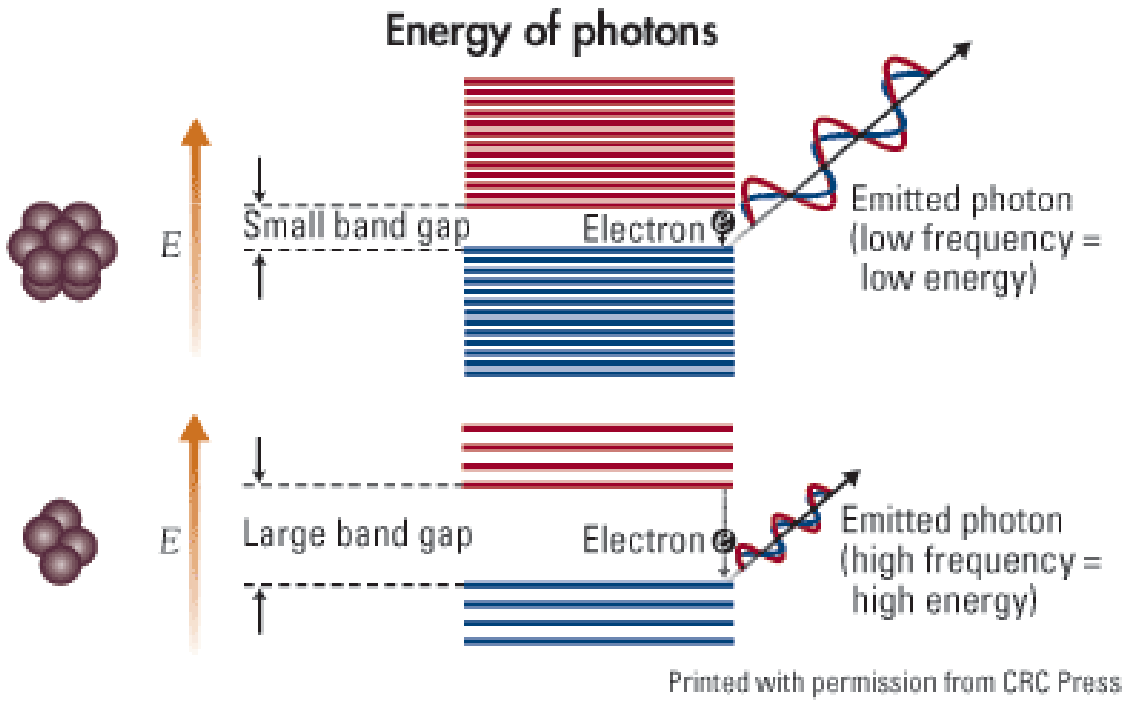
Nature Biotechnology **25** 1165 (2007)
Chemistry & Biology ***18***, 10 (2011)

Quantum dot

What are Quantum Dots?

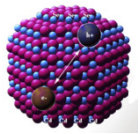
- Quantum dots are **semiconductors** that are on the nanometer scale.
- Obey quantum mechanical principle of quantum confinement.





What kind of nanoparticles can translocate from lung to the body?

What’s the pathways of nanoparticle biodistribution?



Size-dependent translocation of INPs from lungs to lymph nodes

↓

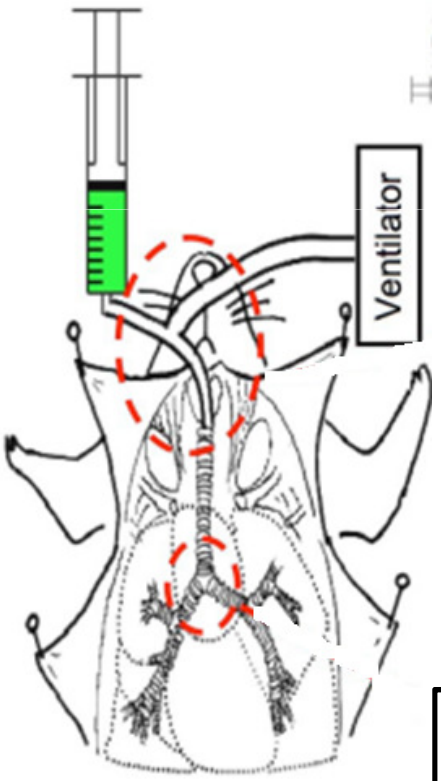
Charge-dependent translocation of nanoparticles from lungs to lymph nodes

↓

1.Real-time NIR fluorescence imaging (in vivo)
Biodistribution, clearance and histological analysis of INPs in **Sprague-Dawley rats**.
2. Histological analysis of tissues

To confirm the qualitative observations

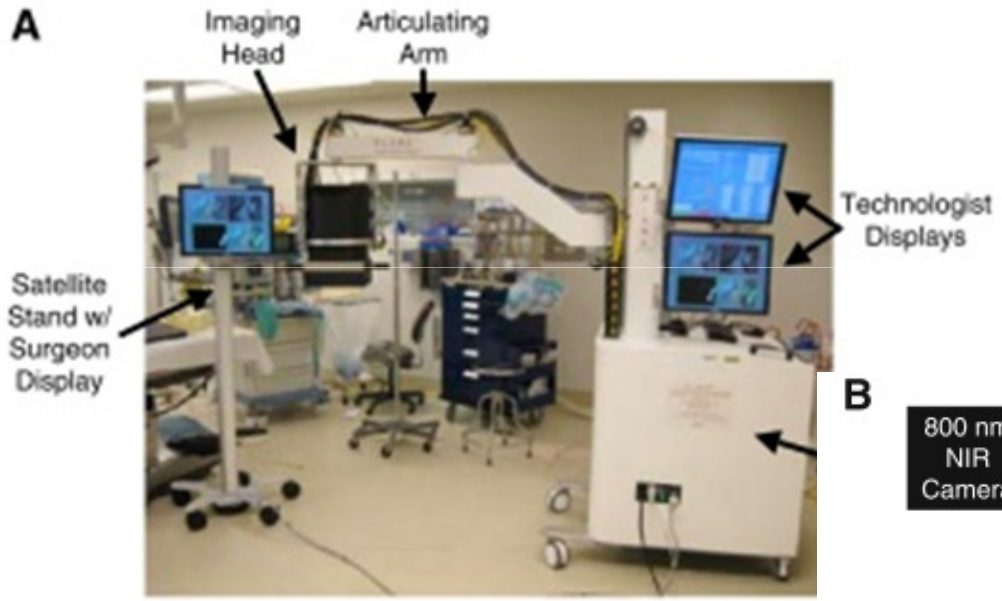
^{99m}Tc-labeling of nanoparticles and radioscintigraphic imaging

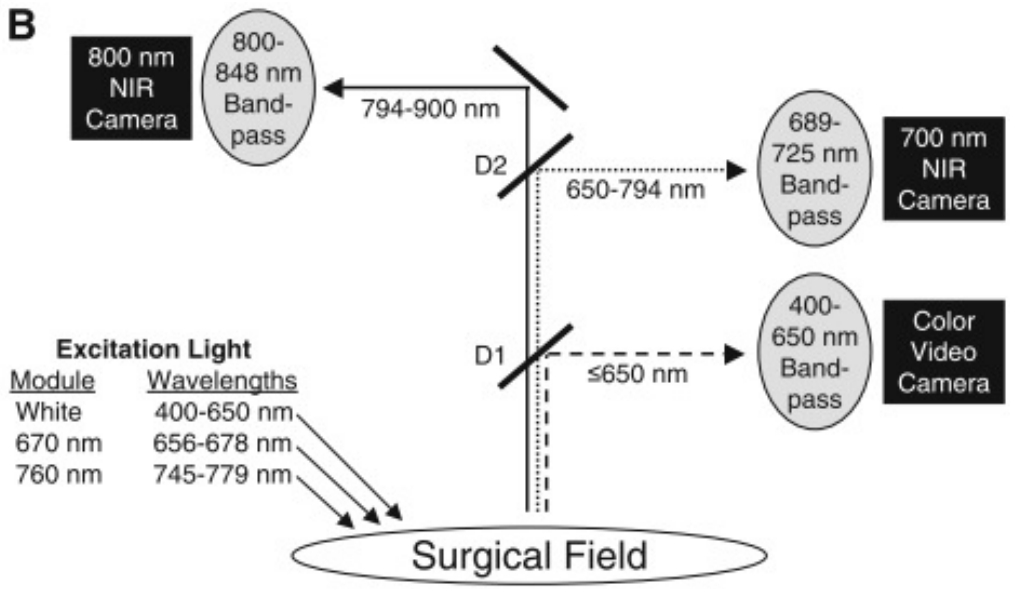


Double-lumen balloon catheter

NIR & FLARE

intraoperative fluorescence-assisted resection and exploration (FLARE) imaging system, which permits two independent channels (700 nm and 800 nm) of NIR fluorescence images to be acquired simultaneously with color video images in real time.

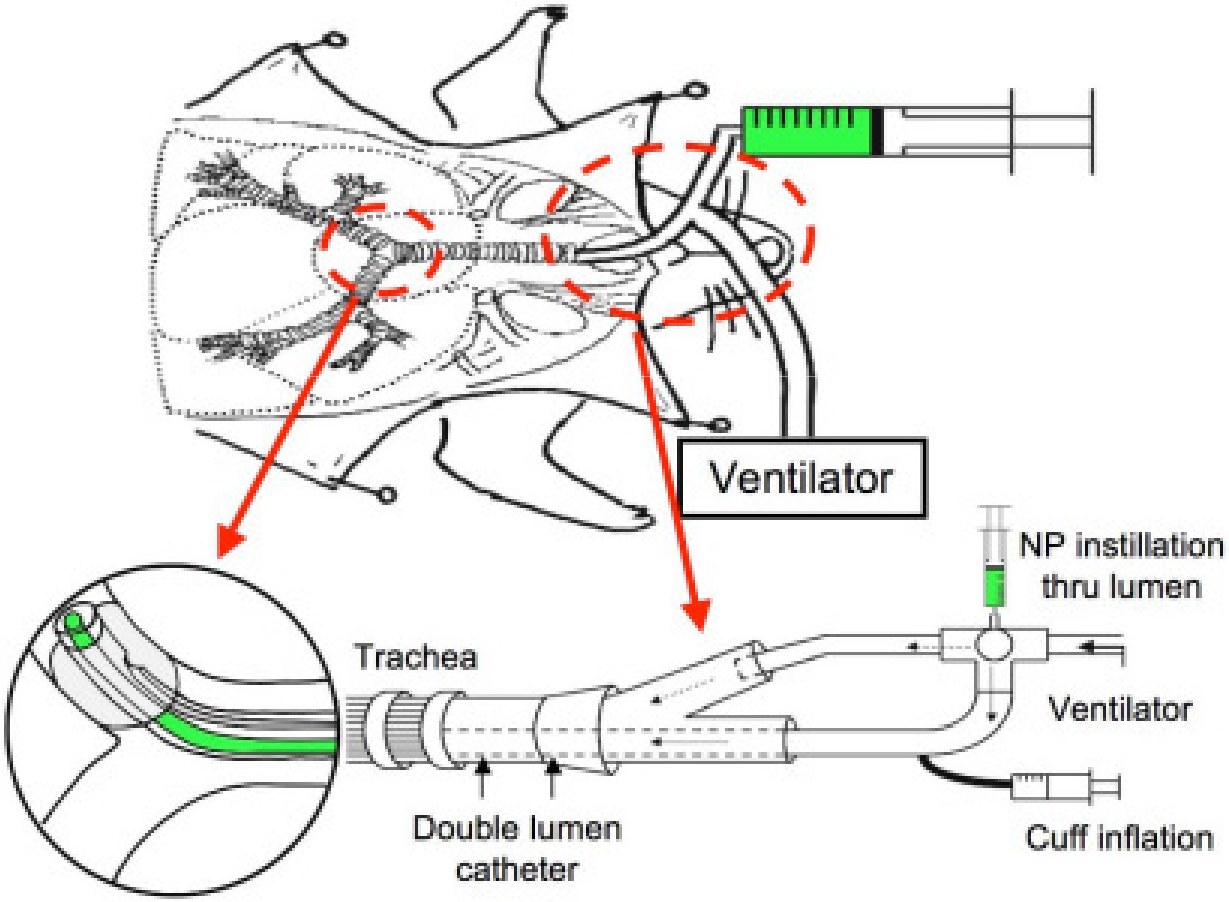




Annals of Surgical Oncology **16,** 2943(2009)

Materials & Methods

(Supplementary Fig. 3).



Supplementary Figure 3 – Experimental Setup and Operation of the Double-lumen Balloon Catheter: Gray indicates the balloon used to block mucociliary flow back into the trachea while the animal is being ventilated through the thru lumen. Green indicates the NP solution.

Results

Table 1 Chemical and physical properties of inorganic/organic hybrid nanoparticles (INPs) and organic nanoparticles (ONPs)

	Nano-particle	Core(shell)	Organic coating	HD[a] (nm) In PBS	HD[a] (nm) In serum
Inorganic/organic hybrid nanoparticles (INPs)	INP1	CdSe(ZnCdS)	Cys-CW800	5	5
	INP2	CdSe(ZnCdS)	PEG-CW800	9	9
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	INP4	CdTe(ZnS)	PEG-NH2	16	29
	INP5	CdTe(ZnS)	PEG-COOH	23	38
	INP6	Silica/CdSe(ZnS)	CW800	52	56
	INP7	Silica/CdSe(ZnS)	CW800	110	110
	INP8	Silica/CdSe(ZnS)	CW800	130	130
	INP9	Silica/CdSe(ZnS)	CW800	320	320
Organic nanoparticles (ONPs)	ONP1	HSA	Cy5.5	7	7
	ONP2	mPEG20k	Cy5.5	9	9
	ONP3	PS-PAA	Cy5.5	21	34
	ONP4	PS-PAA	Cy5.5	35	48
	ONP5	PS-PAA	Cy5.5	51	68
	ONP6	PS-PAA	Cy5.5	97	120
	ONP7	PS-PAA	Cy5.5	220	270

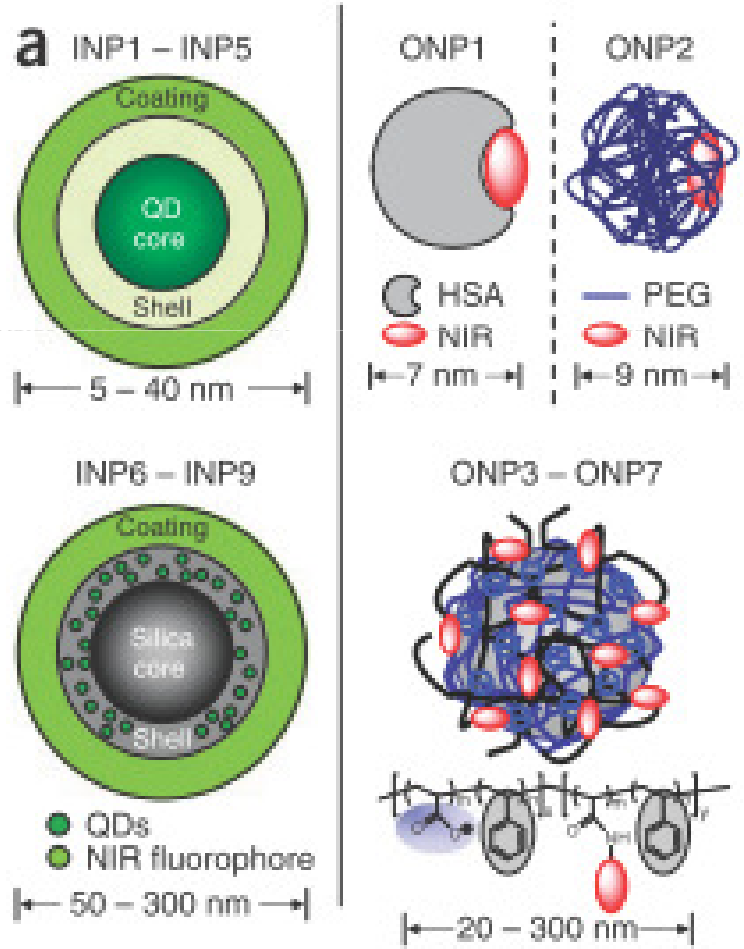
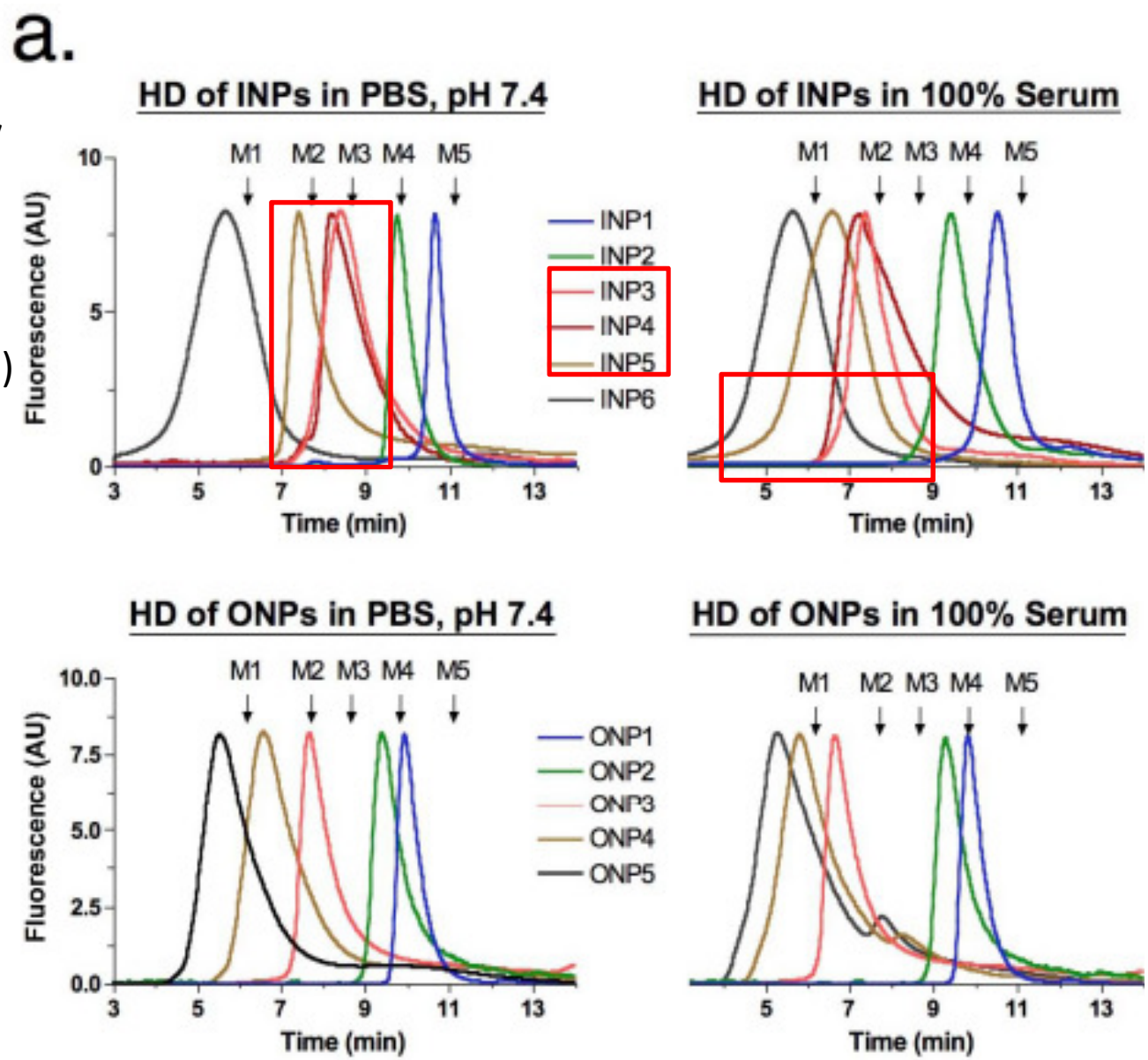


Figure 1

Measure the size of nanoparticles

Gel-filtration chromatography

M1 (dextran, 43.4 nm HD)
M2 (thyroglobulin, 18.8 nm HD)
M3 (γ-globulin, 11.1 nm HD)
M4 (ovalbumin, 6.1 nm HD)
M5 (myoglobin, 3.8 nm HD



Supplementary Fig. 2

Supplementary Figure 2a – Physical and Optical Properties of NPs in PBS and 100% Serum:

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	ONP4	PS-PAA	Cy5.5	35	48	Anionic	700	<0.02	<5.0
	ONP5	PS-PAA	Cy5.5	51	68	Anionic	700	<0.02	<5.0
	ONP6	PS-PAA	Cy5.5	97	120	Anionic	700	<0.02	<5.0
	ONP7	PS-PAA	Cy5.5	220	270	Anionic	700	<0.02	<5.0

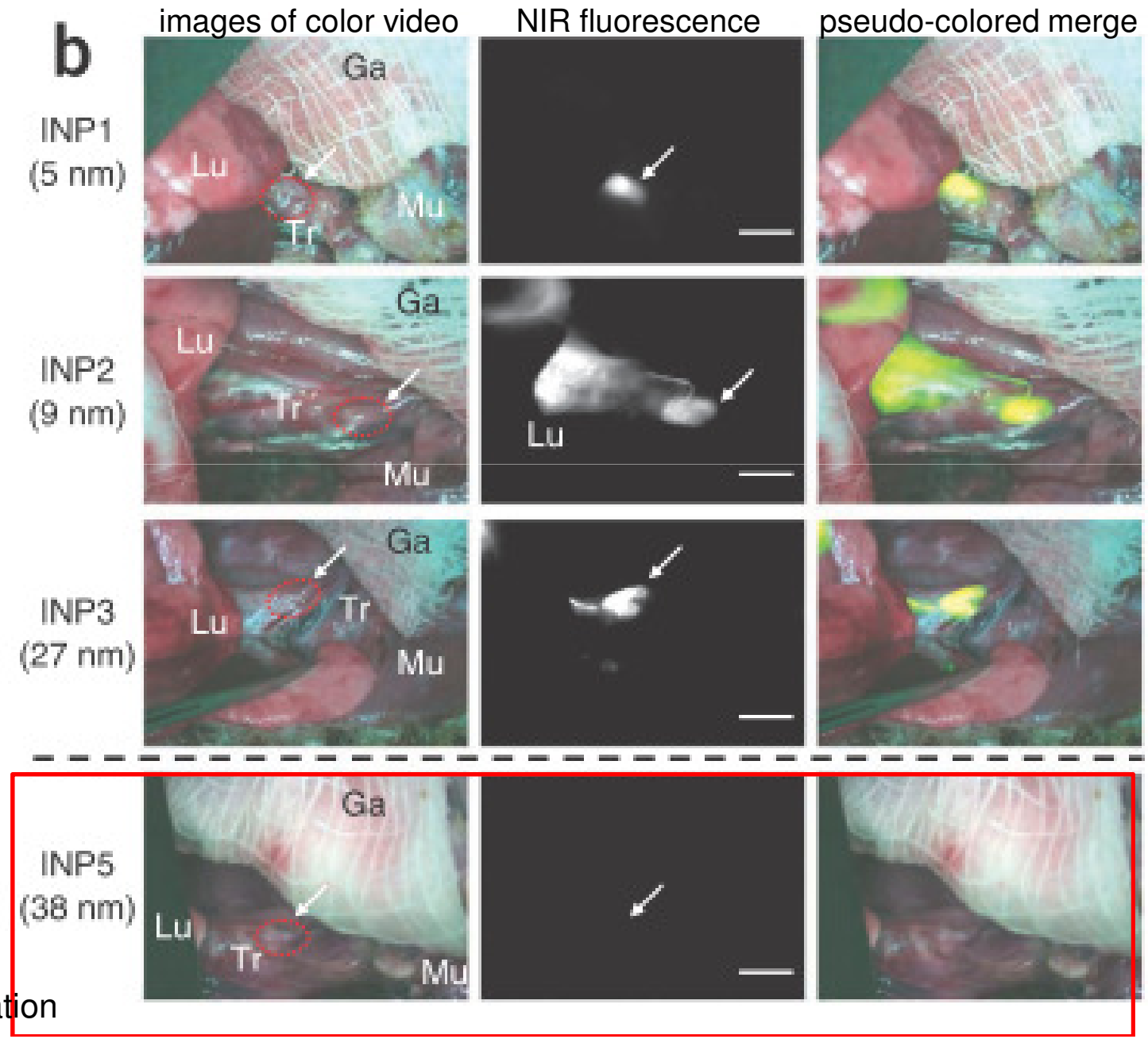
1. Charge particles will absorb some protein within serum
2. HD in serum can represent the real size in animals.

To investigate the role of the “”sizes”” in the translocation of nanoparticles

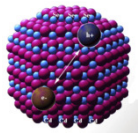
FIGURE 1

Ga:gauze
Lu:lung
Mu:muscle
Tr:trachea

Arrows and red dotted circles indicate lymph node



30 min after administration



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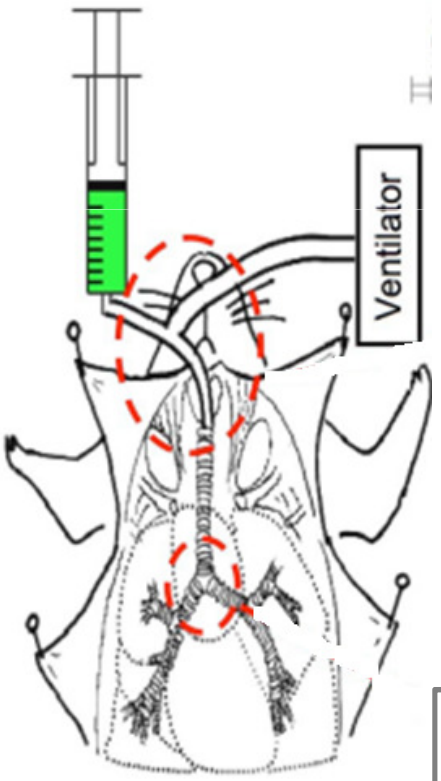


Double-lumen balloon catheter

The surface charge of nanoparticles is also critical for rapid translocation

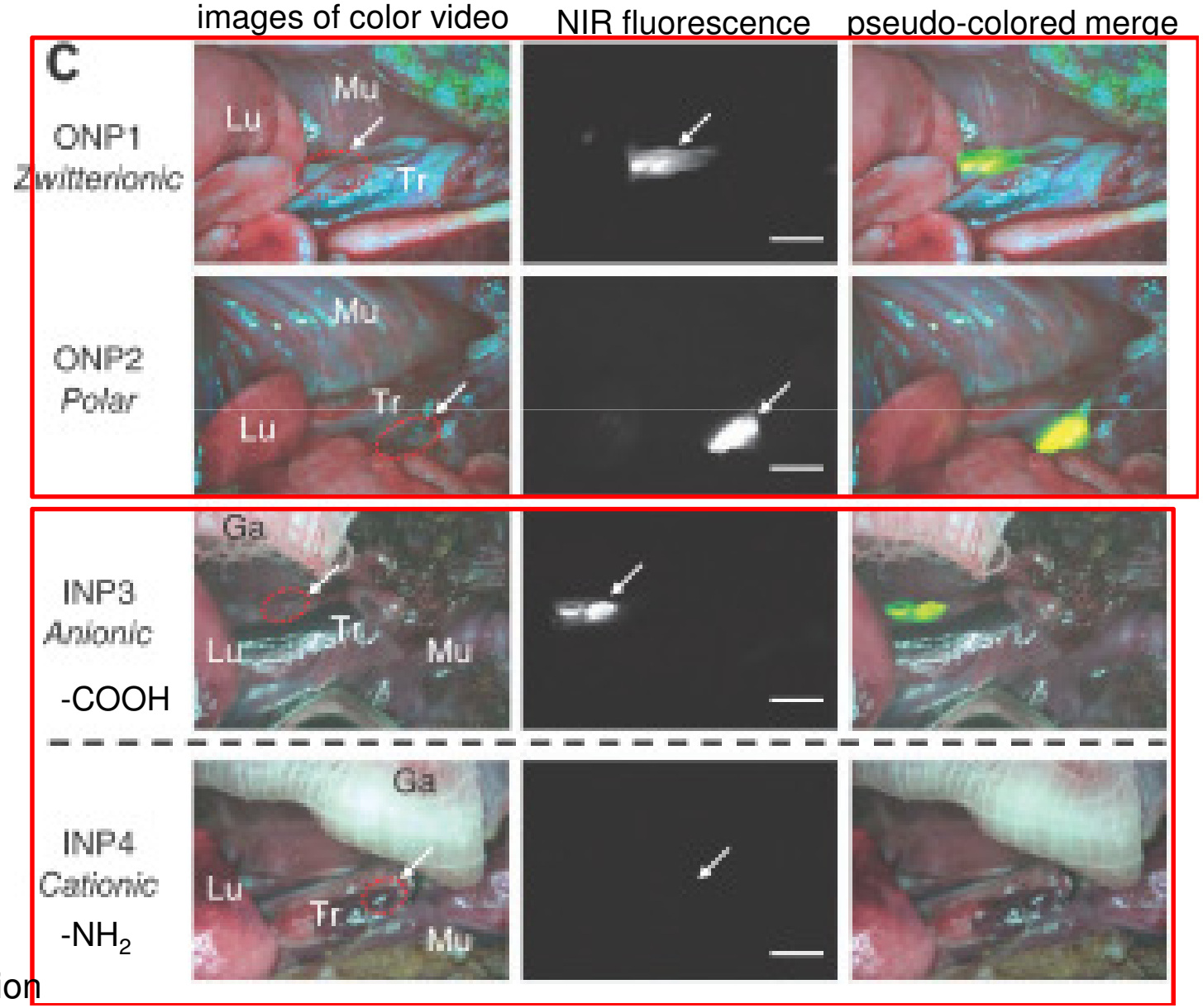
Neutral and polar ONP1,2 didn't absorb serum proteins.

No significant translocation was observed for the cationic INP4

FIGURE 1

Ga:gauze
Lu:lung
Mu:muscle
Tr:trachea

Arrows and red dotted circles indicate lymph node



30 min after administration

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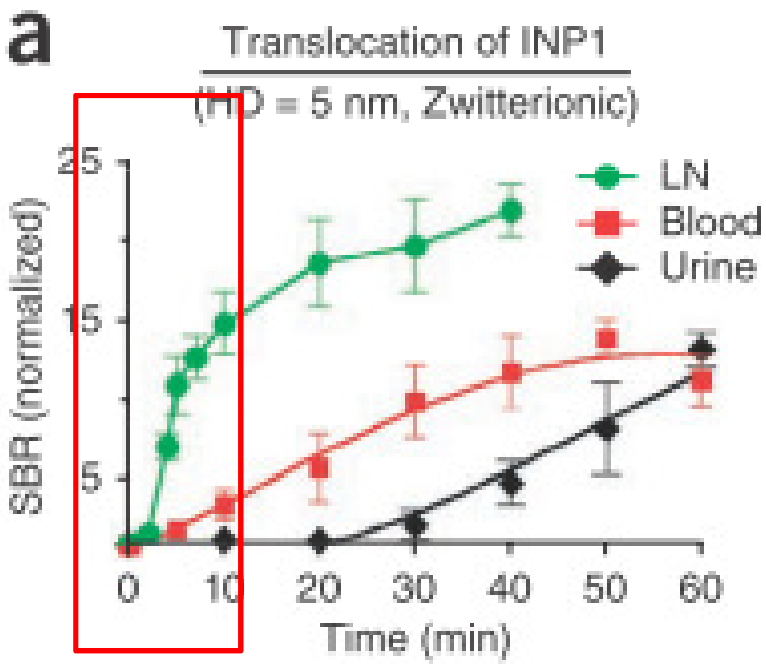
	ONP7	PS-PAA	Cy5.5	220	270	Anionic	700	<0.02	<5.0

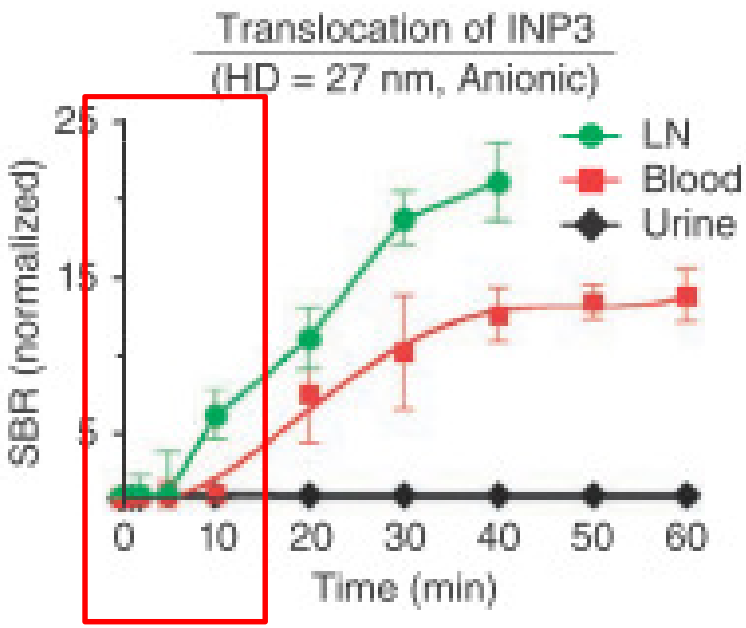
Below the size threshold of 34 nm, the surface charge of nanoparticles is also critical for rapid translocation from lungs to regional lymph nodes.

Biodistribution, clearance of INPs in Sprague-Dawley rats (quantitative result from NIR-FLARE imaging system)

FIGURE 2

slower accumulation into lymph nodes (≈10 min) and blood (≈20 min)





migration to the lymph nodes was very fast

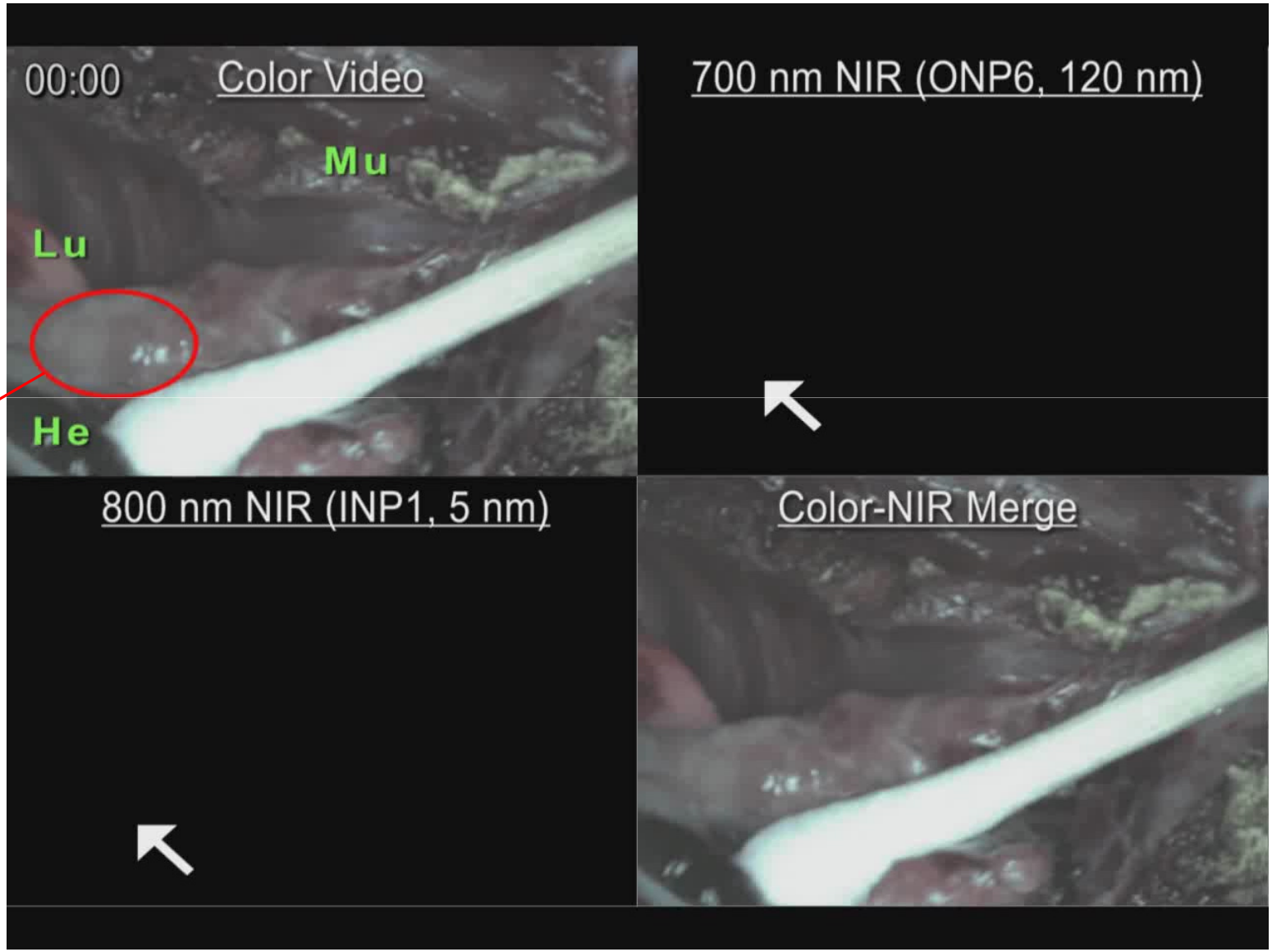
Real-time translocation of NPs from the lung to a mediastinal lymph node.

1 h post-administration

He,:heart;
Lu:lung;
Mu: muscle

Lymph node

INP1: green
ONP6: red



histological analysis of INPs in Sprague-Dawley rats

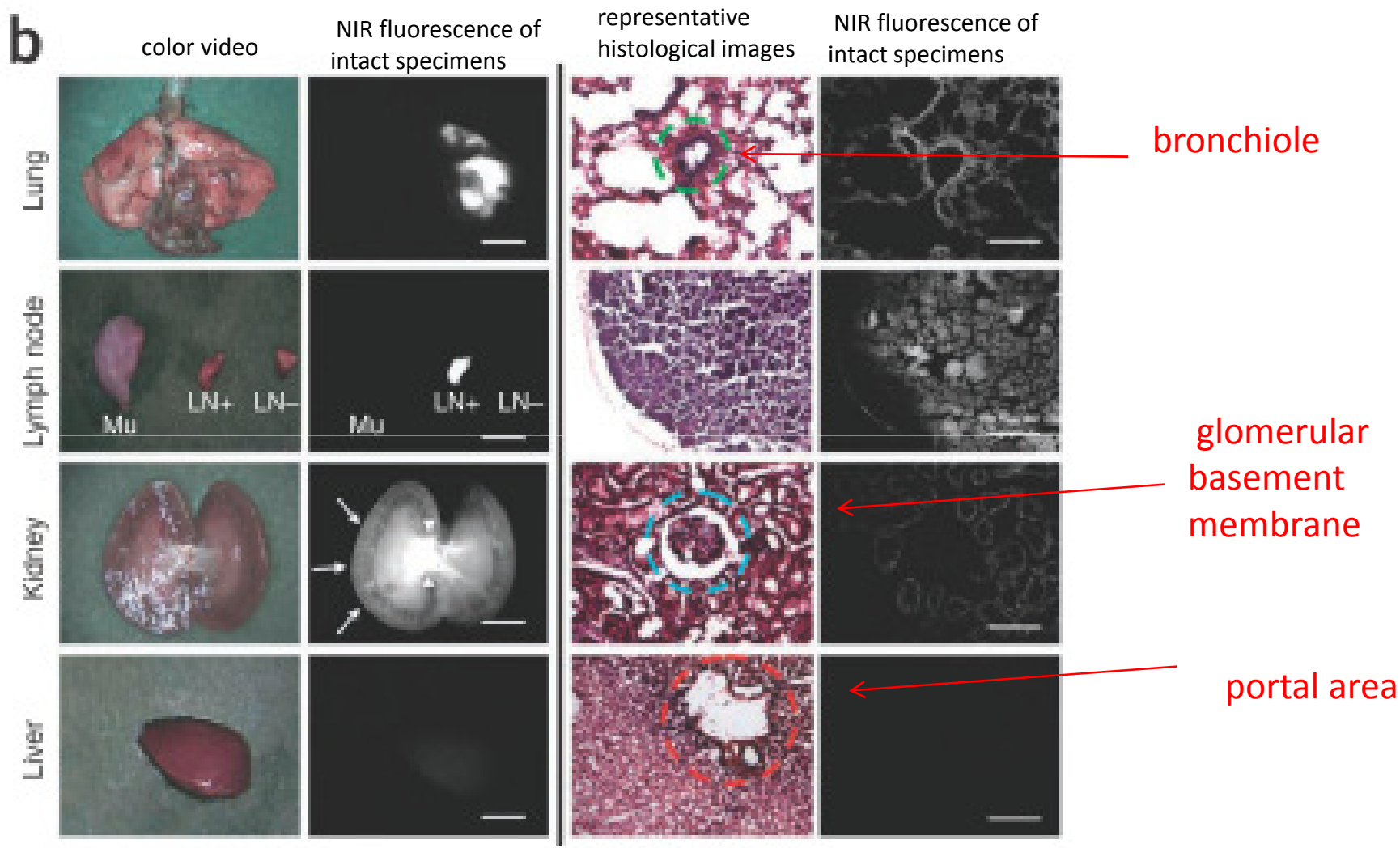


FIGURE 2

Frozen sections at 1 h after instillation (INP1)

Mu, muscle
LN+, posterior mediastinal lymph node
LN−, negative para-aortic lymph node

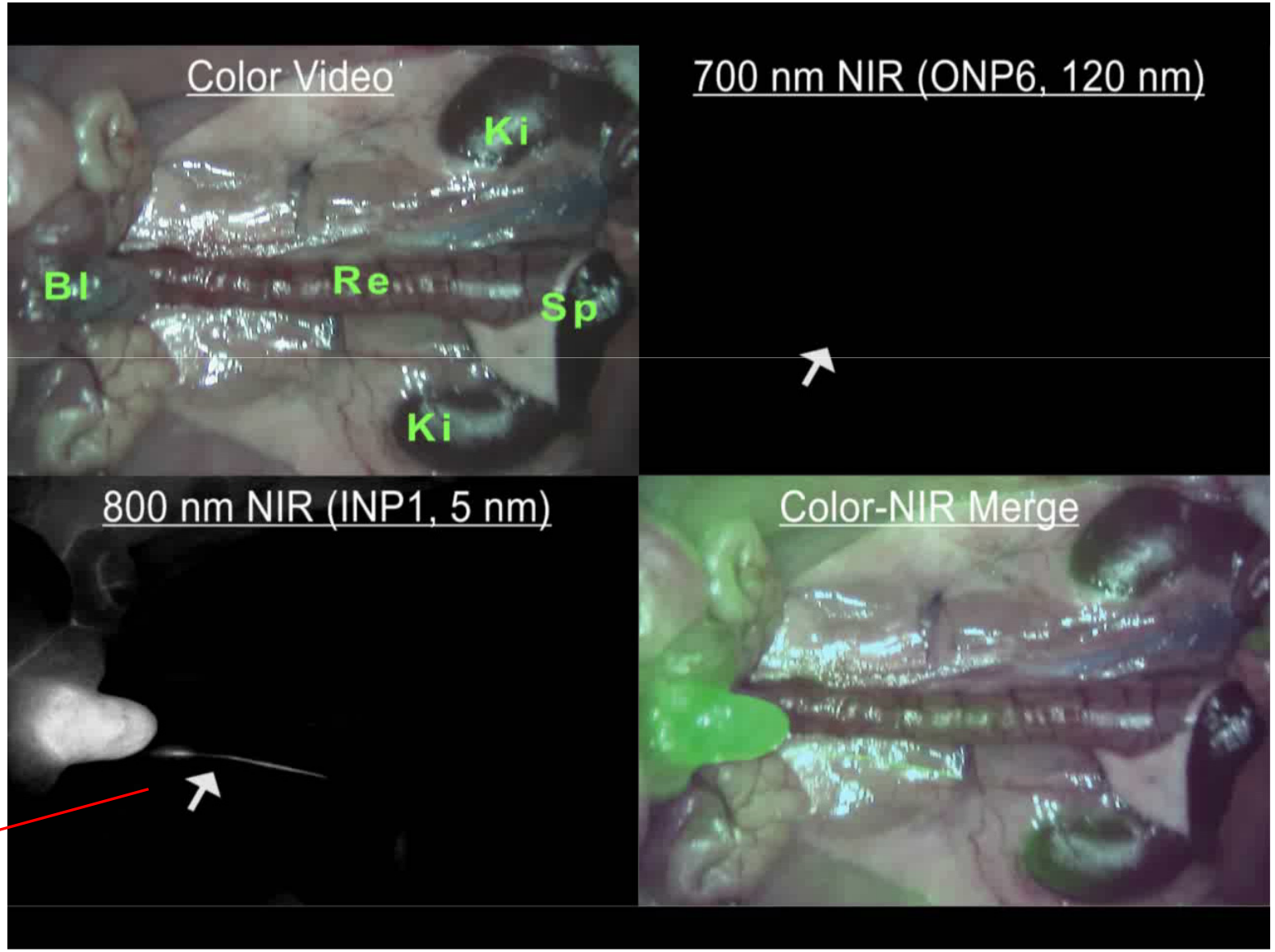
Real-time clearance of NPs from kidneys to bladder

1 h post-administration.

Bl:bladder;
Ki,:kidney
Re:rectum
Sp:spleen.

INP1: green
ONP6: red

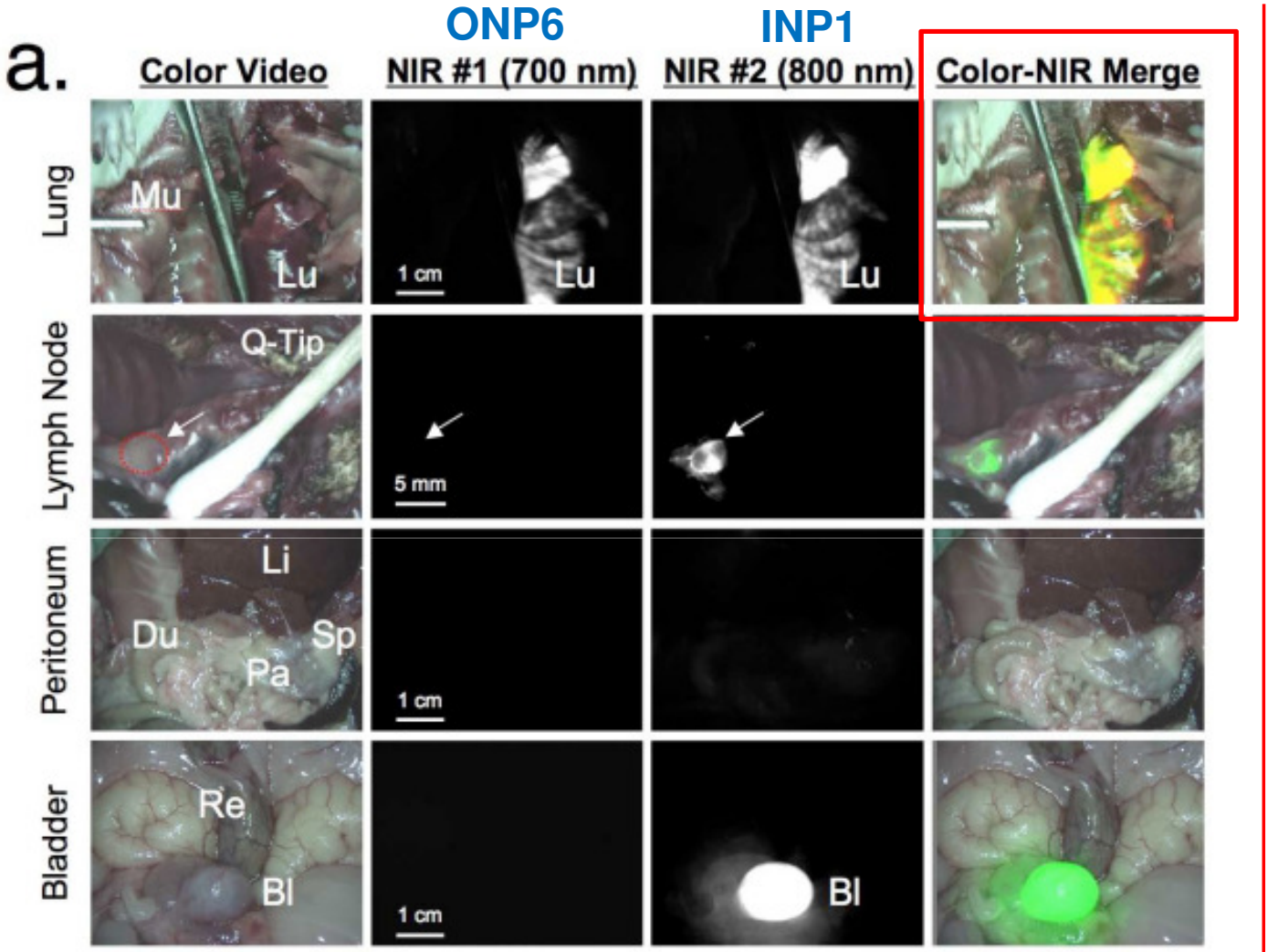
ureter



Biodistribution of INP1 (5 nm HD) and ONP6 (120 nm HD)

Bl: bladder
Du: duodenum
Li: liver
Lu: lung
LN: lymph node
Mu: muscle
Pa: pancreas
Sp: spleen
Re: rectum

INP1: green
ONP6: red



Supplementary Figure 4

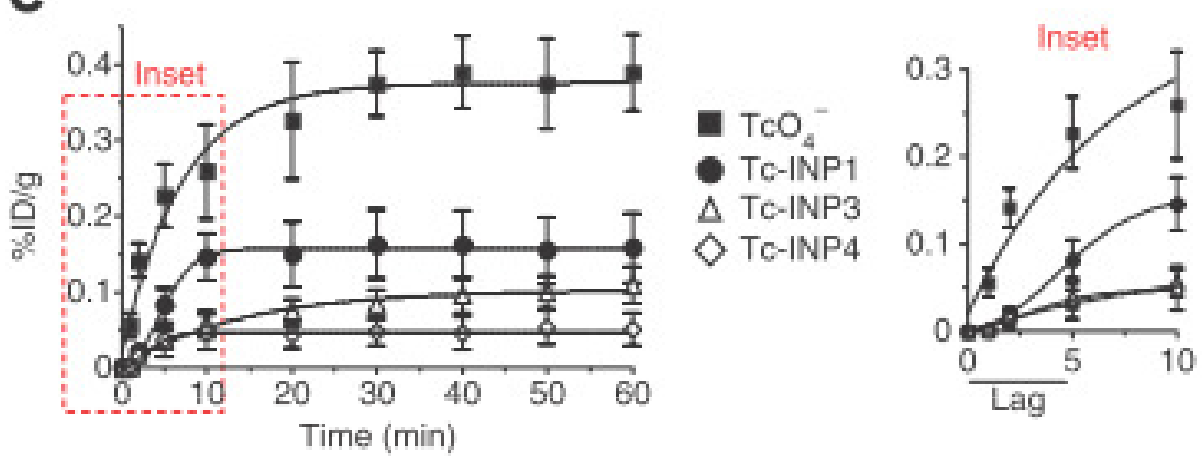
pseudo-colored merge of the three taken 1 h post-instillation.

Further confirmation of previous data

- ^{99m}Tc-conjugated INPs (intratracheally)
- TcO_4^- was used as a control

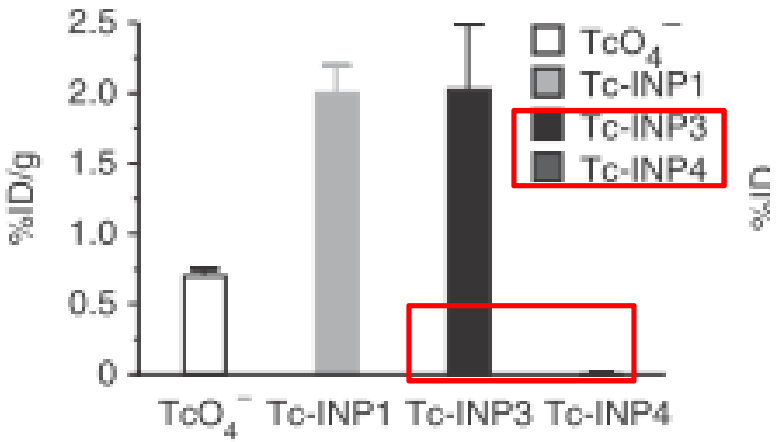
FIGURE 2

Translocation from lung to blood

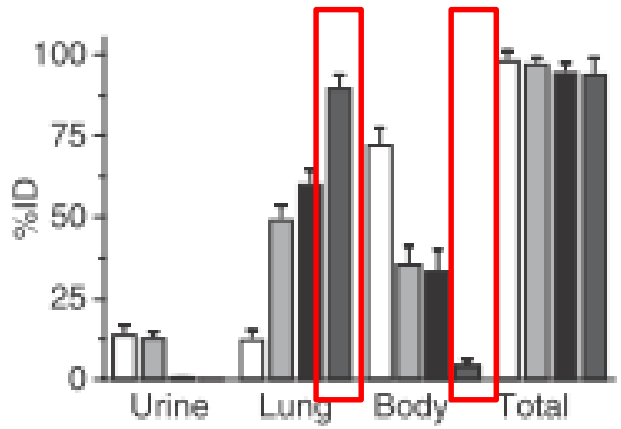


INP3: -COOH
INP4: -NH_2

Translocation from lung to regional lymph nodes



Recovery of injected dose in urine, lung, body



CONCLUSION

(i) A size threshold of ≈34 nm

a. rapid transepithelial translocation of nanoparticles from the alveolar luminal surface into the septal interstitium

b. quick translocation to the regional draining lymph nodes (bloodstream could occur)

(ii) HD <34 nm, **surface charge** is a major factor

a. zwitterionic, anionic and polar surfaces being permissive

b. cationic surfaces being restrictive in the lung

(iii) HD is <6 nm and surface charge is zwitterionic, nanoparticles can enter the bloodstream quickly from the alveolar airspaces and can ultimately be cleared from the body by means of renal filtration.

Summary

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Application

Drug delivery:

The results might prove useful to investigators trying to engineer inhaled nanoparticle-based drugs.

a. The sizes of cationic liposomes for drug dilvery might belown the threshold size of NP.
(increase the absorption of drug into blood circulation)

b. Engineering nanoparticle-based drugs to be zwitterionic and <6 nm in HD should result in rapid and high levels of drug in the bloodstream.

c. Noncationic nanoparticles ≤34 nm and ≥6 nm
1.high levels of drug to pulmonary lymph nodes

Limitation of nanotechnology

Many challenges still remain to be resolved prior to widespread use of nanobiotechnology in clinical field.

1. The risk of nanoparticles depends on their type;some are toxic, whereas others have negligible toxicity and some even have a tissue-protective effect.

2. There is some concern about the toxicity of nanoparticles, and extensive investigations are in progress to resolve this issue.

3. Risk evaluation presents challenges because of a lack of data, the complexity of nanomaterials, measurement difficulties and undeveloped hazard assessment frameworks.

A major difference favoring QDs over organic fluorophores

1. QD (Quantum dot) is resistant to to photobleaching.

2. the fluorescence is not easily quenched or dampened by the surrounding medium

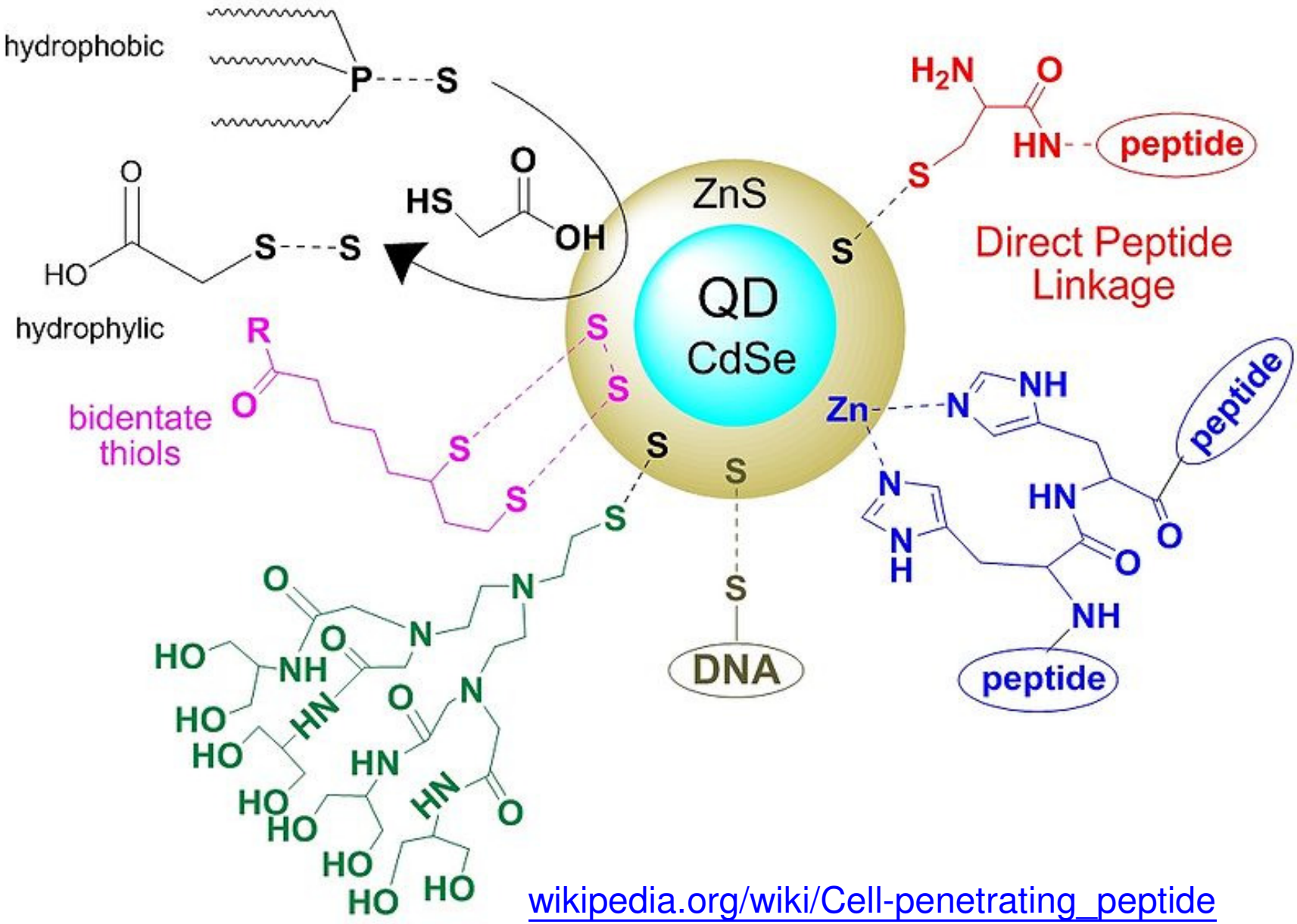
3. the color emitted by the quantum dot depends on its size

4. the QD do not degrade easily

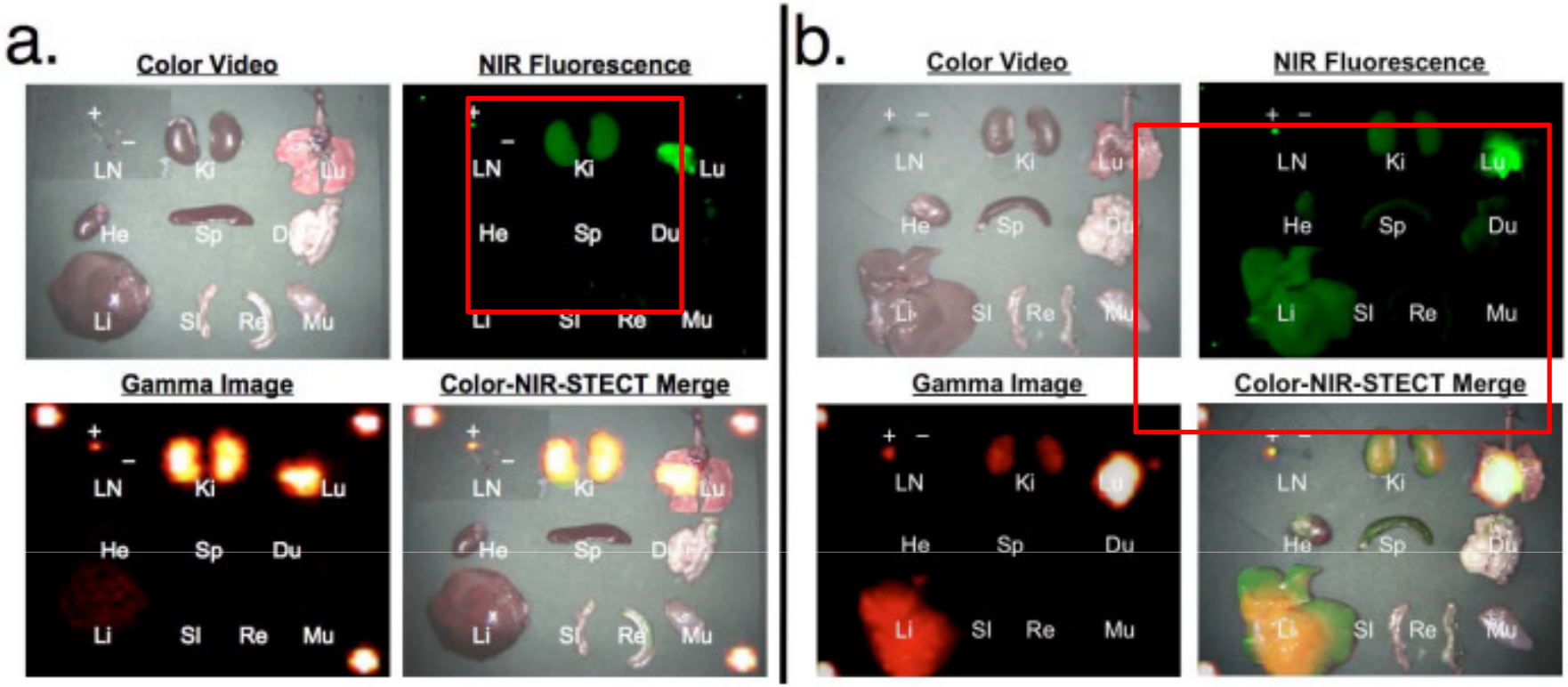
Current Opinion in Chemical Biology **7,** 626 (2003)

~Thanks For Your

Attention~



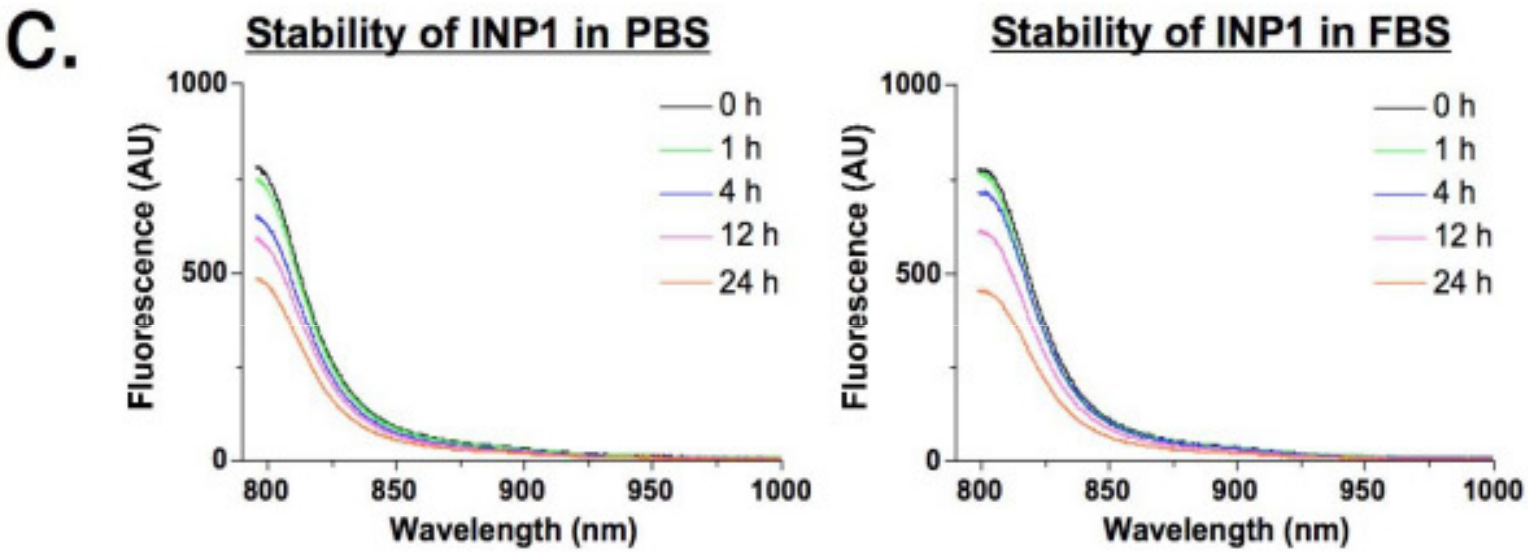
wikipedia.org/wiki/Cell-penetrating_peptide



Supplementary Figure 5 – Biodistribution of ^{99m}Tc-Conjugated INP1 (a) and ONP1 (b) into SD Rats: Shown are the color video (top left), NIR fluorescence (top right), gamma camera images (bottom left), and merged image of the three (bottom right) of resected organs at 1 h post-instillation. Abbreviations used are: LN+, posterior mediastinal LN; LN-, negative para-aortic LN, Ki, kidneys; Lu, lungs; He, heart; Sp, spleen; Du, duodenum; Li, liver; SI, small intestine; Re, rectum; and Mu, muscle.

This suggests that ONP1 was being excreted through the hepatobiliary clearance route at 1 h post-administration.

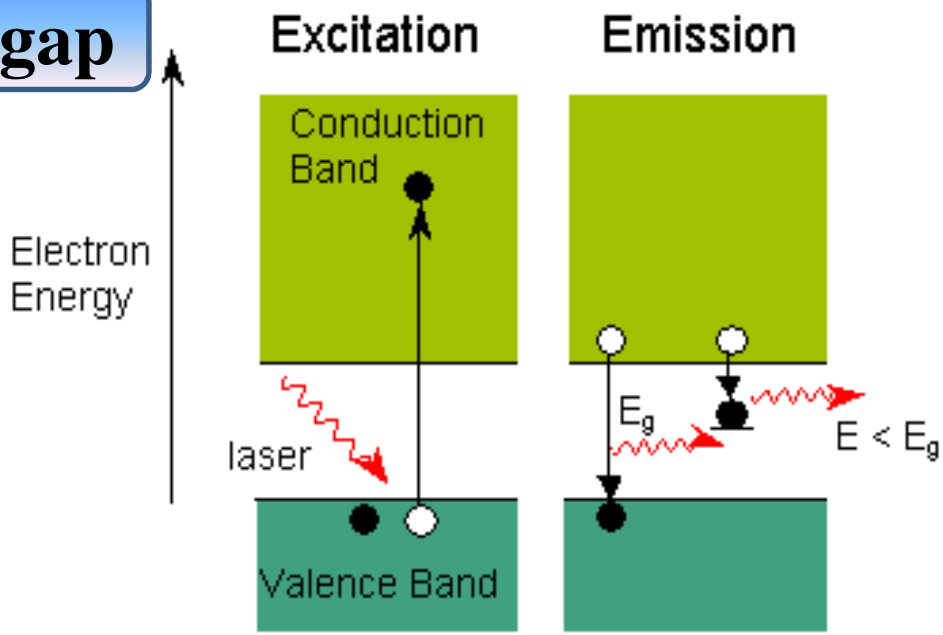
Stability of INP1 in PBS and in FBS

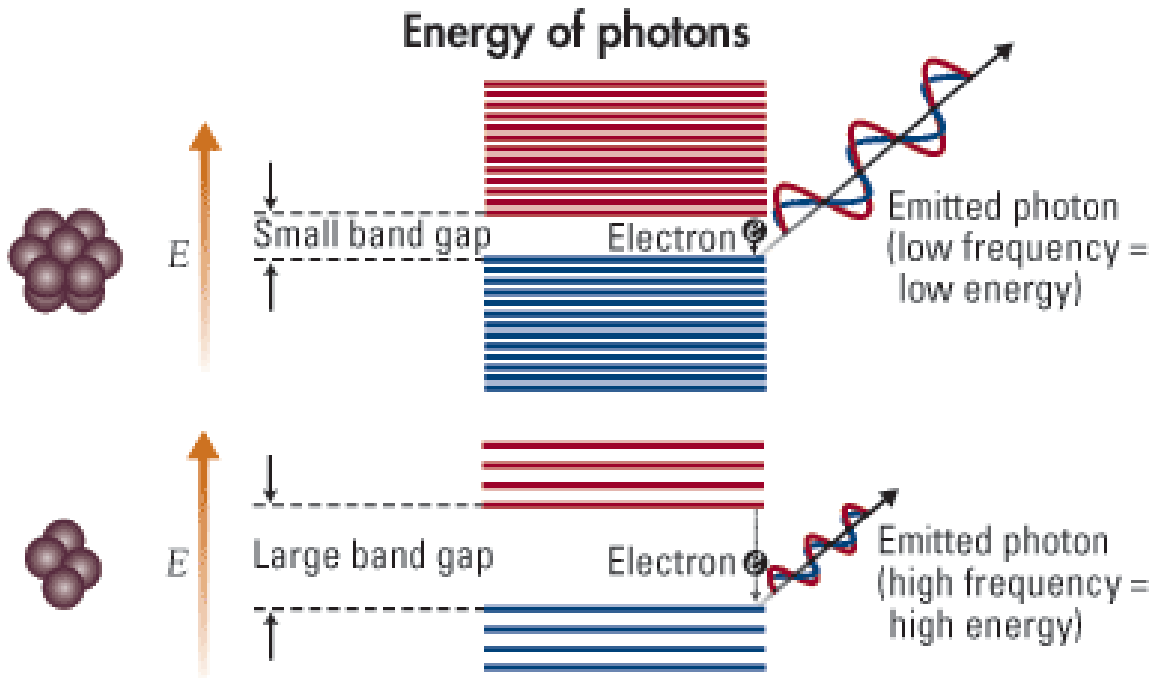


Stability	0 h	1 h	4 h	12 h	24 h
INP1 in PBS (%)	100.00	95.18	82.86	75.40	62.59
INP1 in FBS (%)	100.00	95.22	91.68	77.18	58.82

Supplementary Figure 2c – Physical and Optical Properties of NPs in PBS and 100% Serum: Optical stability of INP1 in PBS and 100% fetal bovine serum (FBS) over 24 hr at 37 °C.

Energy band gap





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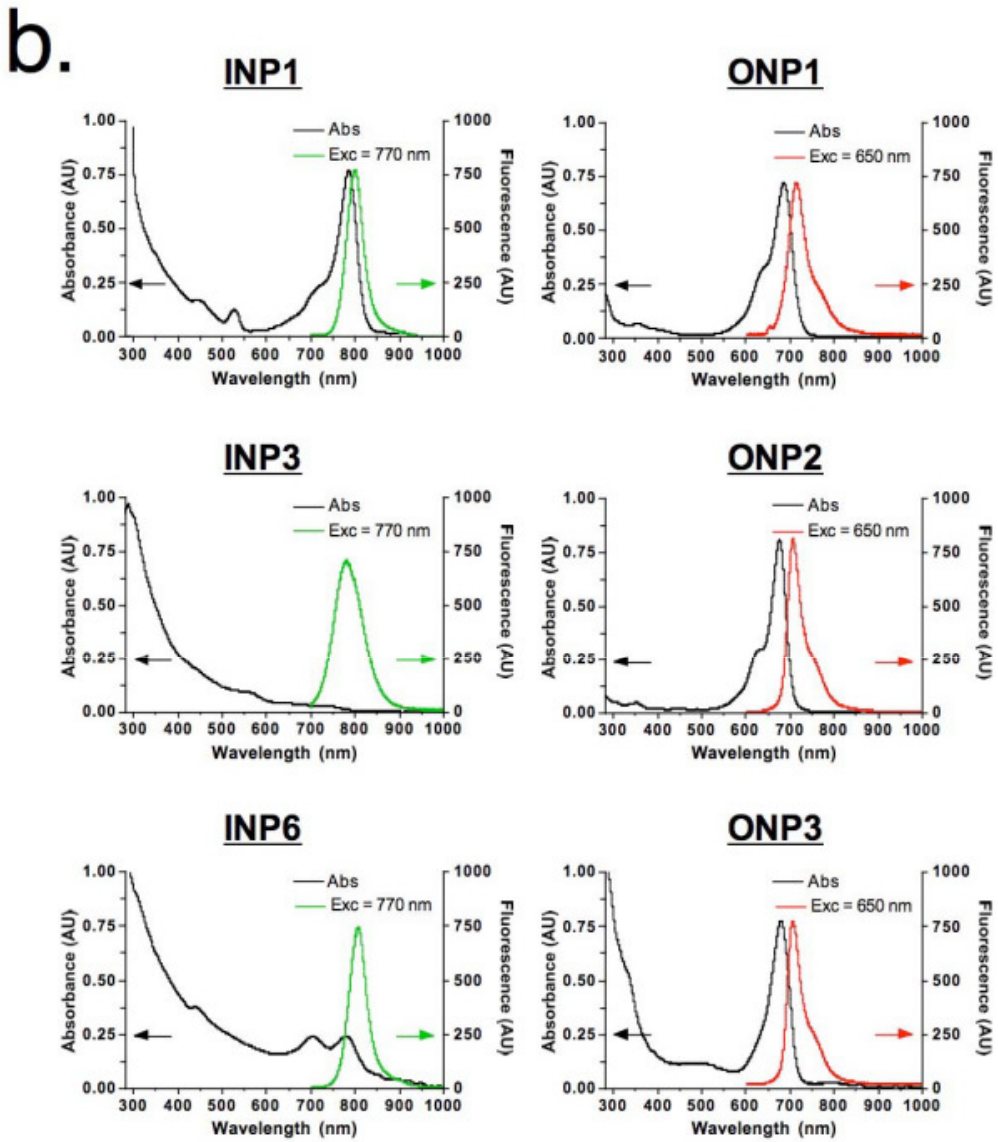

Supplementary Figure 2b – Physical and Optical Properties of NPs in PBS and 100% Serum: Absorption and emission fluorescence spectra of INPs (excitation = 770 nm) and ONPs (excitation = 650 nm) in 100% serum.