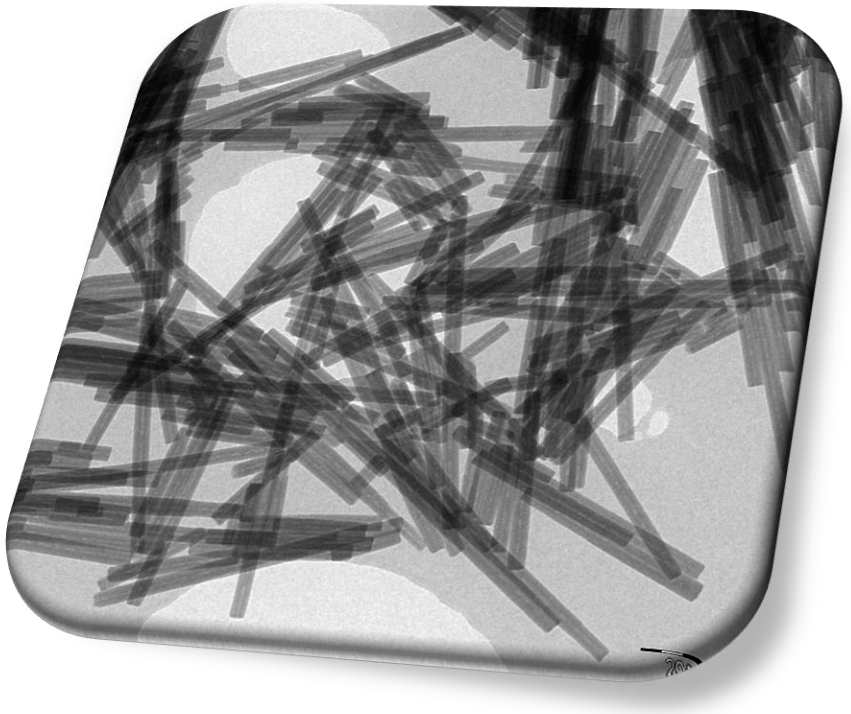
# Composite Functional Nanomaterials for Multimodal Imaging and Photo-Dynamic Therapy of Sick Tissues



Marcello **Campione**

**Università degli Studi di Milano Bicocca, Italy**

# The TEAM: Mineralogy + Materials Science + Medicine

## Fabricating a Composite Functional Nanomaterial

UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

***Department of Earth and Environmental Sciences***
Geomimetism: Chrysotile nanotubes

***Department of Materials Science***
Functionalization: Fluorescent and magnetic activity

Fondazione IRCCS Ca' Granda
Ospedale Maggiore Policlinico

***Department of Pathophisiology and Transplantation***
In-vitro and in-vivo tests

# **Geomimetism** – *Chrysotile Nanotubes*

## Transforming Asbestos Fibres in Medical Nanoparticles

**iron contamination** $[(Mg,Fe)_3(Si_2O_5)(OH)_4]$ imparts radical reactivity, hence toxicity, to any chrysotile outcrop

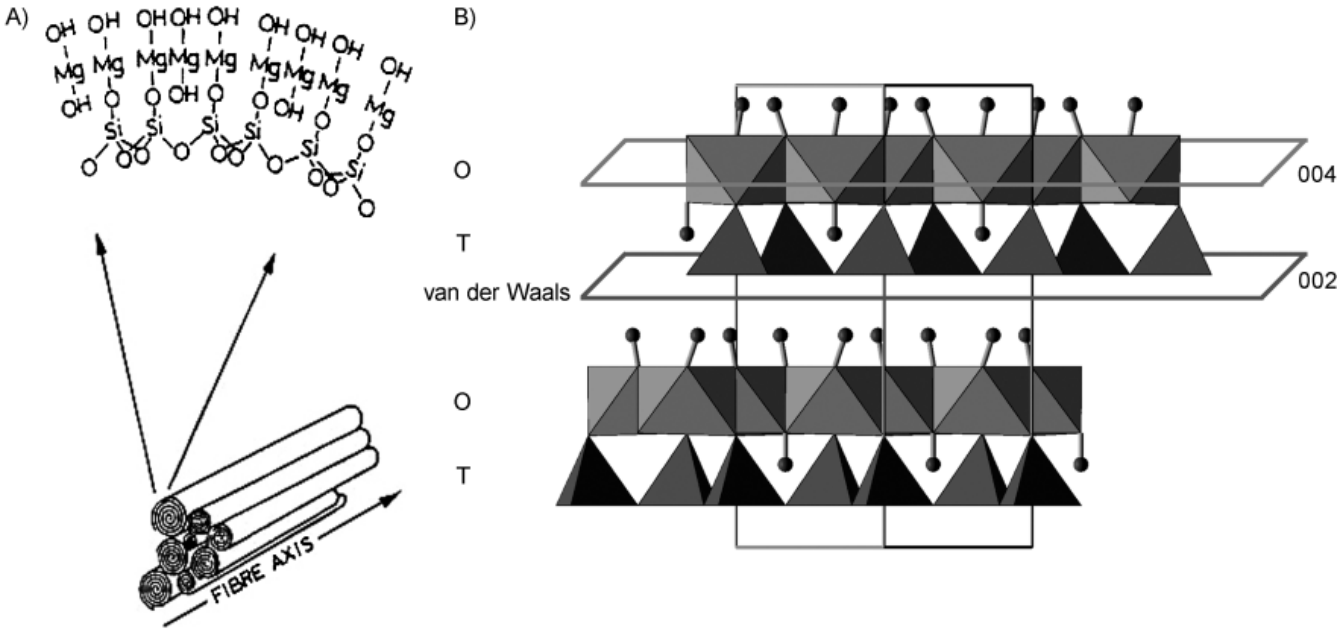
Synthetic **stoichiometric chrysotile** nanofibers $[Mg_3(Si_2O_5)(OH)_4]$ , devoid of iron or any other contaminant, did not exert genotoxic and cytotoxic effects

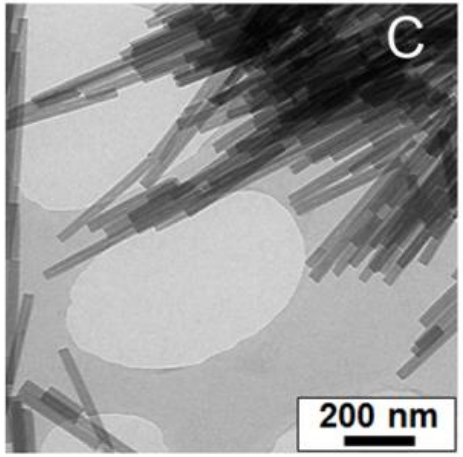
Gazzano *et al. Chem. Res. Toxicol*. **2007**, 20, 380-387

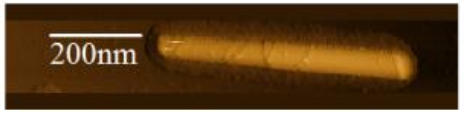
# **Geomimetism** – *Chrysotile Nanotubes*

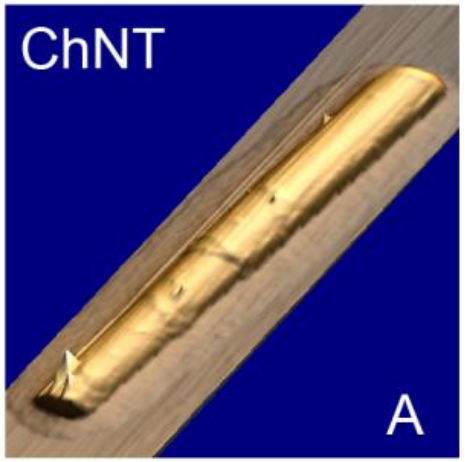
***Hydrothermal synthesis [T = 250-300 °C, P = 40-80 atm]***

$3MgCl_2 + 2SiO_2 + 6NaOH \rightarrow \mathbf{Mg_3Si_2O_5(OH)_4} + 6NaCl + H_2O$



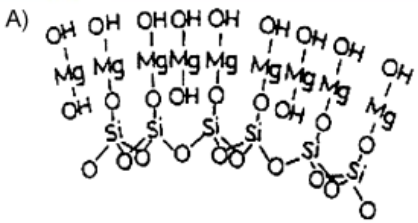



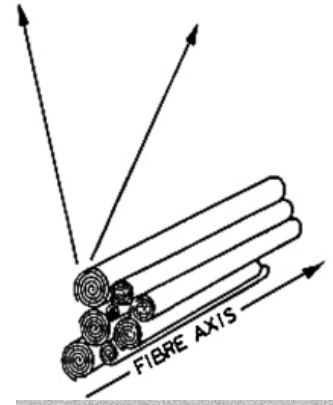



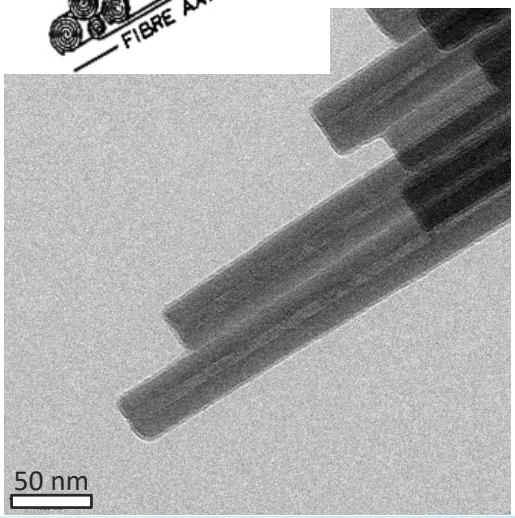



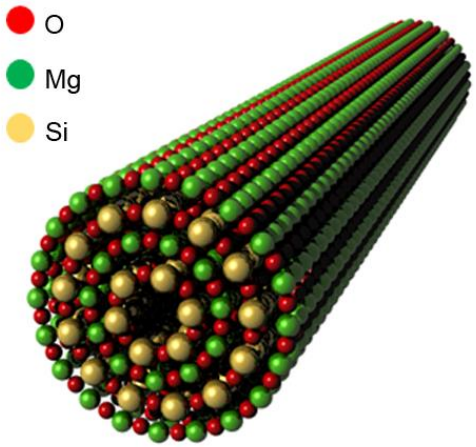

Turci *et al. Chem. Eur. J.* **2010**, 17, 350-358

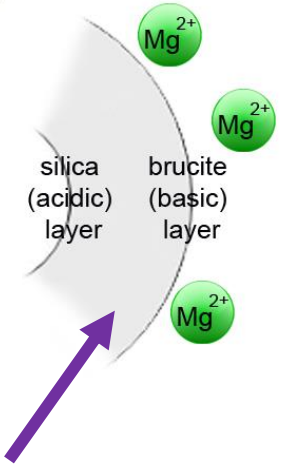
# **Geomimetism** – *Chrysotile Nanotubes*



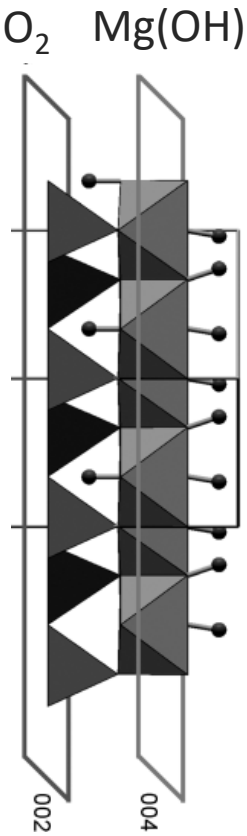



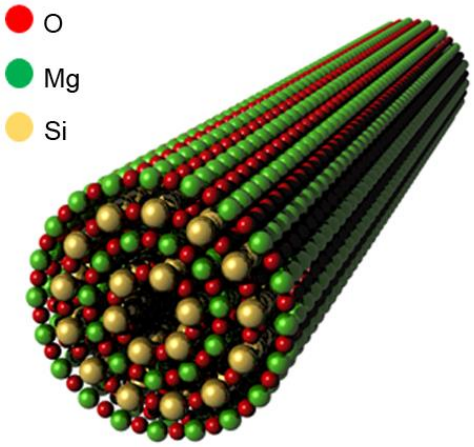



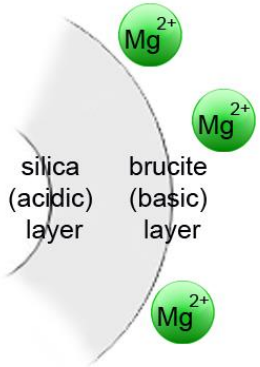




$\zeta$-potential: +30 mV @ pH = 7.0



# Functionalization – *Ionic Self Assembly*

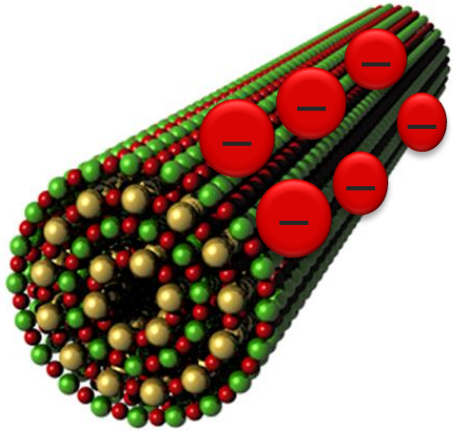


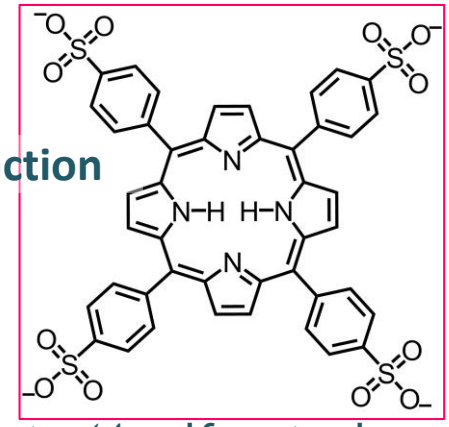




**ζ-potential: +30 mV @ pH = 7.0**

**anionic species**

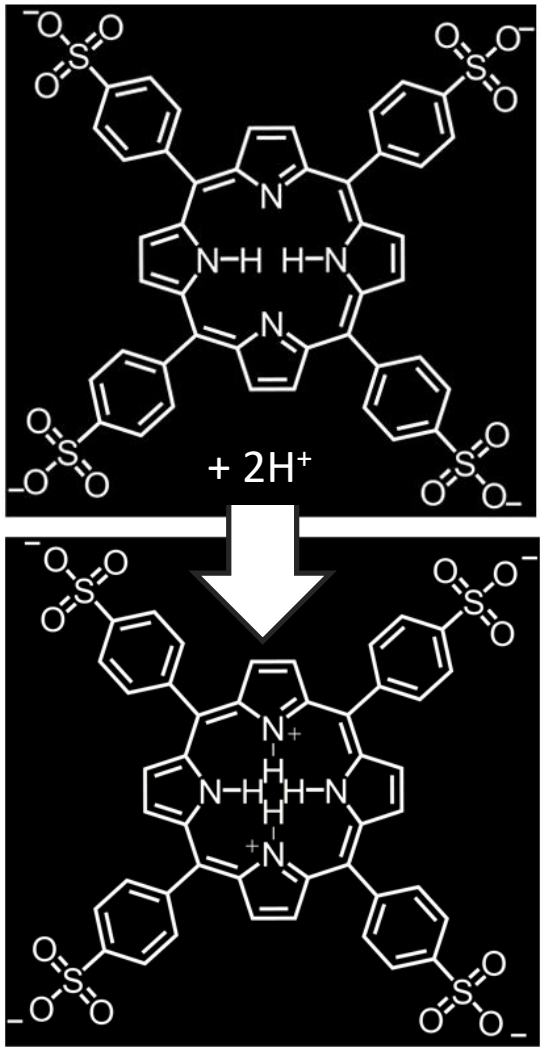
**Coulombic attraction**

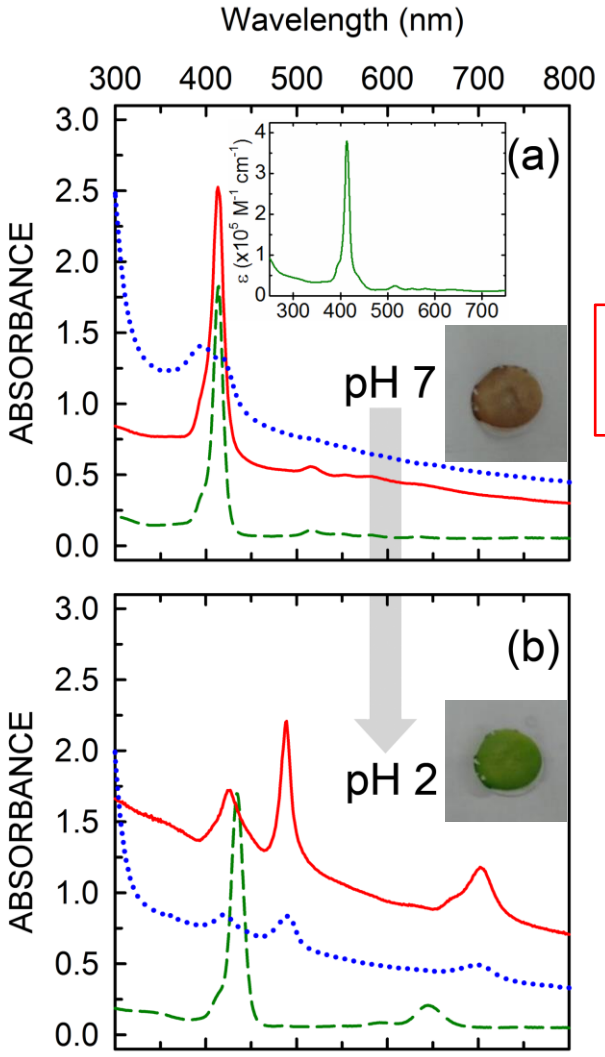




tetra(4-sulfonatophenyl) **porphyrin** ($H_2TPPS^{4-}$)

# Functionalization – *pH sensitive, fluorescent molecule*



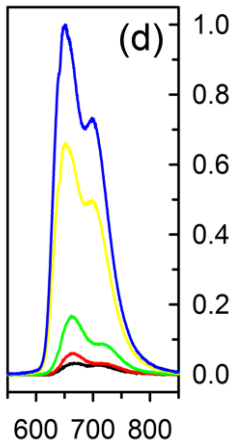

tetra(4-sulfonatophenyl) **porphyrin** ($H_2TPPS^{4-}$)

$NT:H_2TPPS^{4-}$ cast solid film

$NT:H_2TPPS^{4-}$ suspension

$H_2TPPS^{4-}$ solution

FLUORESCENCE ($\lambda_{ex}$ = 405 nm)

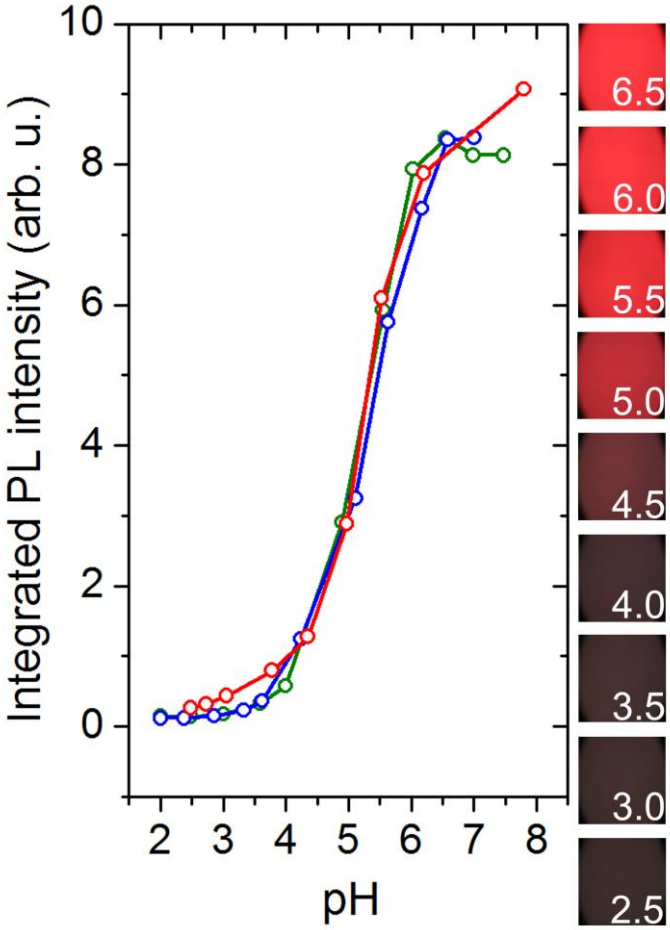



pH = 6.5

pH = 2.5

# Functionalization – *pH sensitive, fluorescent molecule*

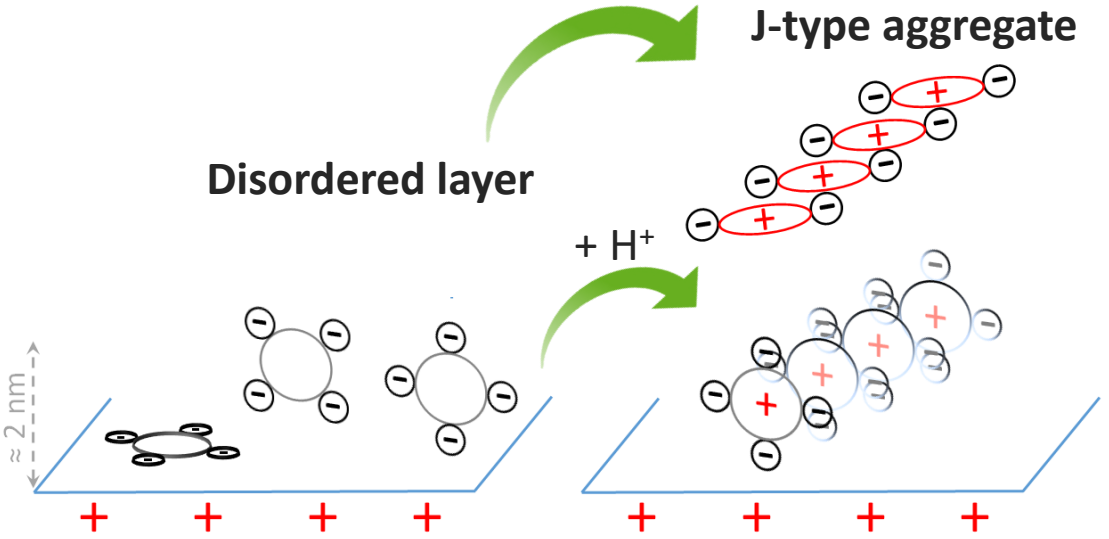
FLUORESCENCE ($\lambda_{ex}$ = 405 nm)  CH:$H_2TPPS^{4-}$ suspension




Acetate buffer

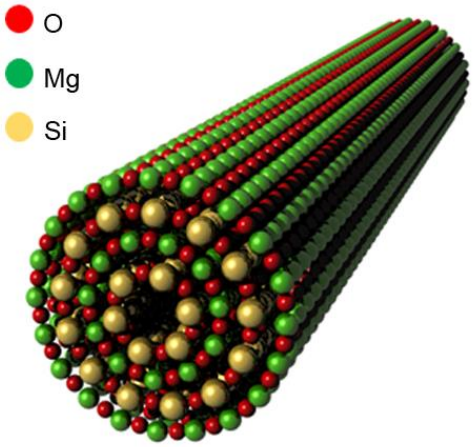
Phosphate buffer

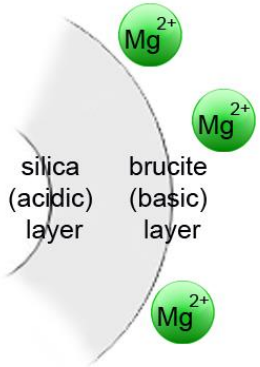
Phosphate buffer with enhanced ionic strength



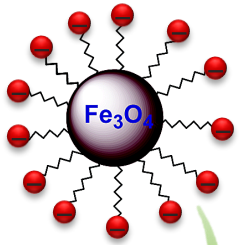

Monguzzi *et al., Phys.Chem.Chem.Phys.*, **2014**, 16, 2491

# **Functionalization** – *Magnetic Nanoparticles*

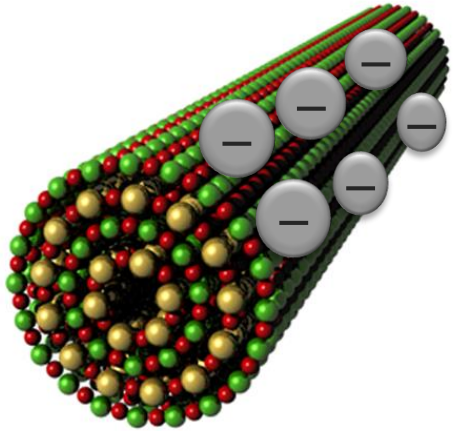



**ζ-potential: +30 mV @ pH = 7.0**



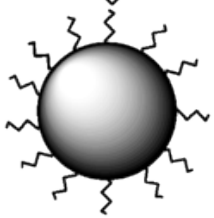

tetramethyl ammonium
**11-aminoundecanoate**
(TAU)-capped
**magnetite nanoparticles**



# **Functionalization** – *Magnetic Nanoparticles*

$Fe(acac)_3 + ROH + RCOOH + RNH_2 + Ph_2O$

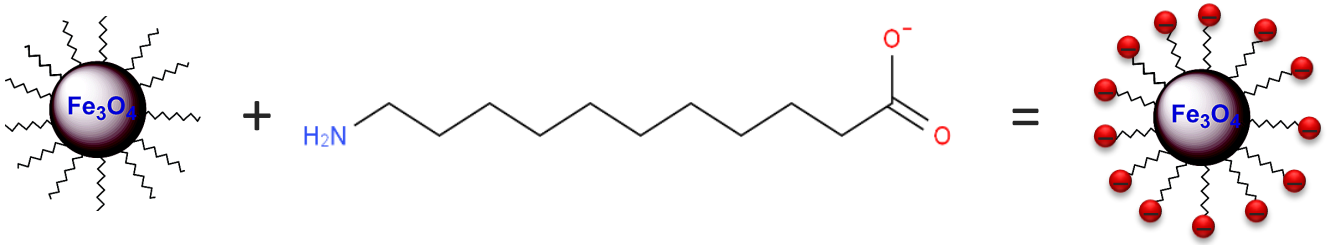
heating



Oleic acid-capped
**magnetite nanoparticles (MNP)**

Sun and Zeng, *J. Am. Chem. Soc.*, **2002**, *124*, 8204–8205

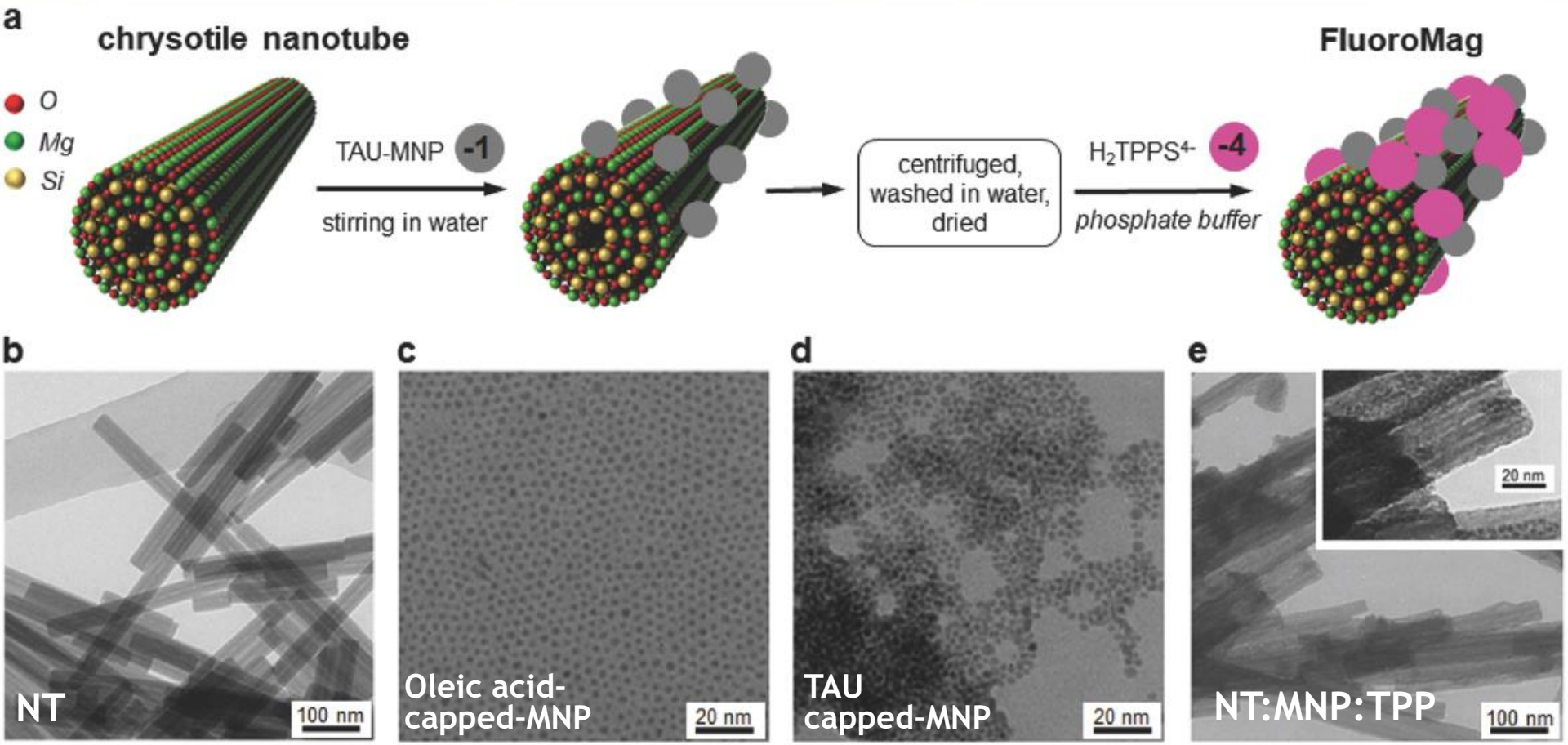
**ligand exchange** - A dispersion of MNPs in *n*-hexane was added to a suspension of tetramethyl ammonium 11-aminoundecanoate (TAU) in dichloromethane.




Water dispersible
(TAU)-capped
**MNP**

Dey *et al.*, *Langmuir*, **2010**, *26*, 9627–9631

# **Functionalization** – *Fluoro-Magnetic, pH sensitive NP*




Villa *et al., Adv. Funct. Mater.* **2018**, *28*, 1707582

# **Applications** – *Tracking & Diagnosis*

A pH decrease in tissue cells is indicative of a pathological state (exp. cancer)

HR MAPPING OF pH IN TISSUES IS NECESSARY

MAGNETIC FUNCTIONALITY

FLUORESCENT FUNCTIONALITY

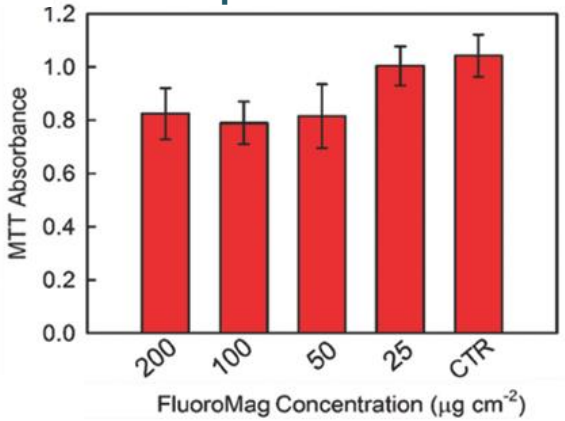
Tracking of tumor cell infiltration

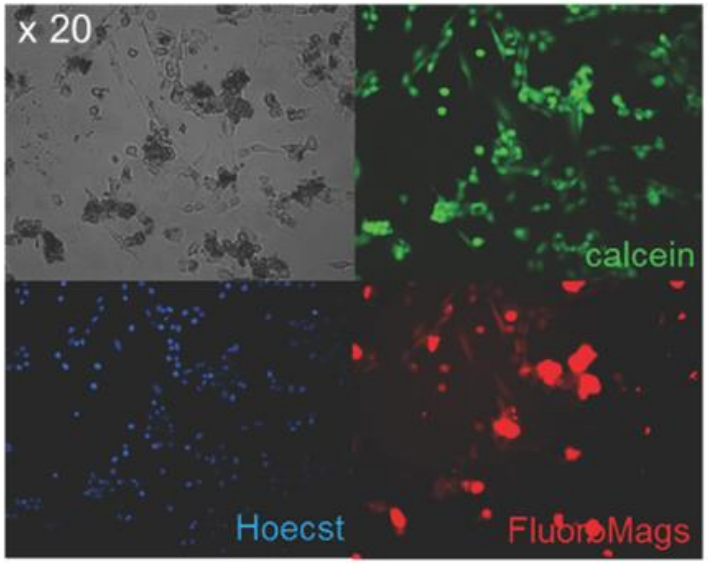
Biological environment dysregulation

**Cellular uptake of nanotubes by intravenous infusion must be verified**

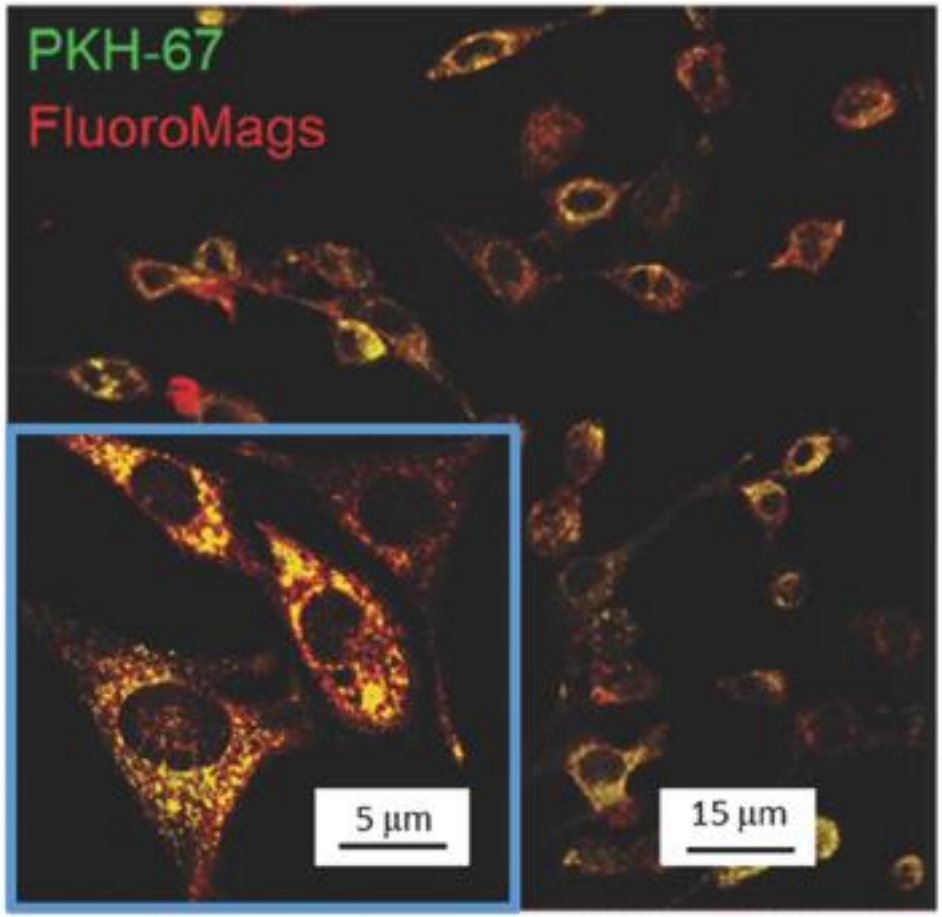
# In-Vitro Tests – *Cellular Uptake*

## NIH/3T3 fibroplast cell line after 24 h



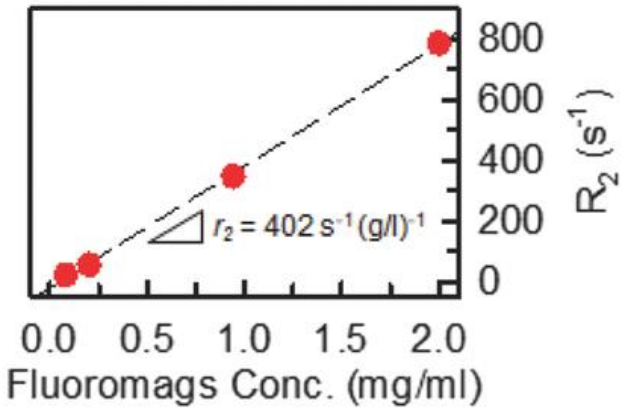




## Confocal fluorescence images of NIH/3T3 cells at pH = 7.4 under 410 nm laser excitation



# In-Vitro Tests – *Magnetic Resonance Imaging*

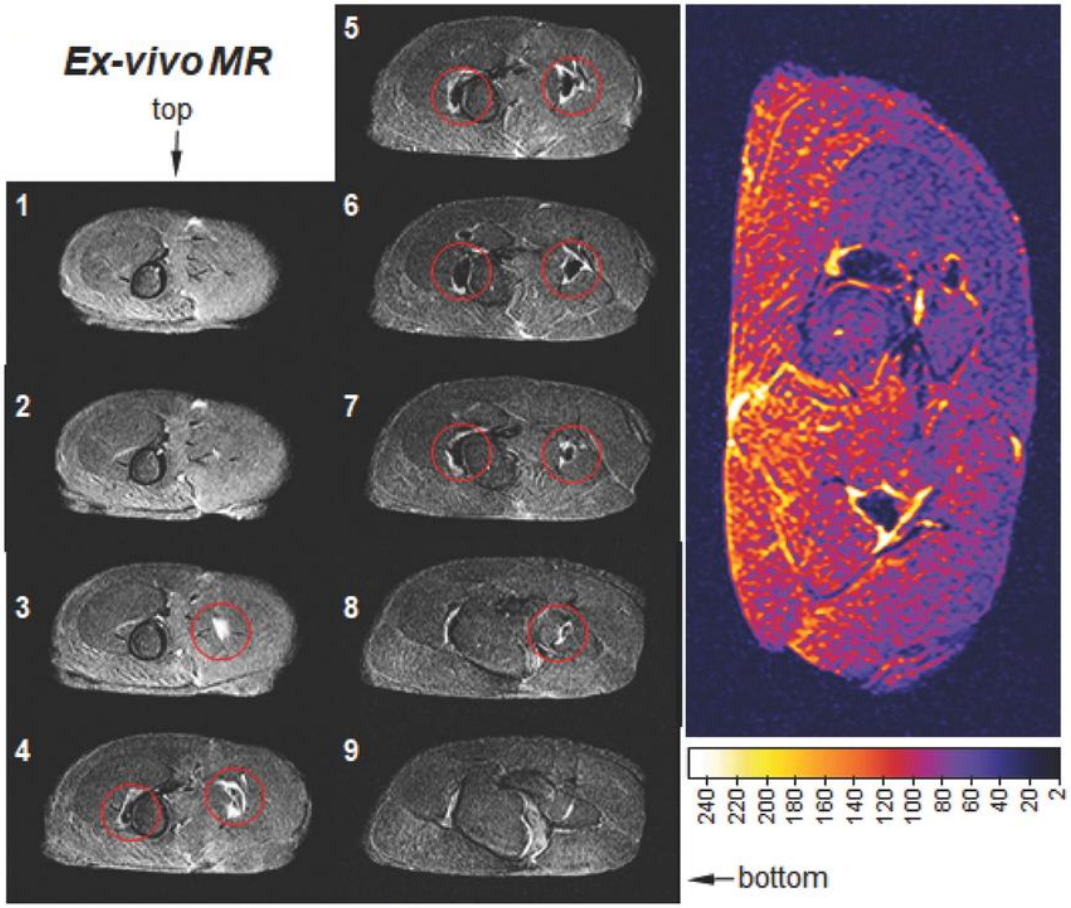
Transverse relaxation rate $R_2$




Transverse relaxivity $r_2$ = 402 $s^{-1}$ (g $l^{-1}$)$^{-1}$
Longitudinal relaxivity $r_1$ = 48 $s^{-1}$ (g $l^{-1}$)$^{-1}$
$r_2/r_1$ = 8.4 [**negative contrast agent**]

Villa *et al., Adv. Funct. Mater.* **2018**, *28*, 1707582

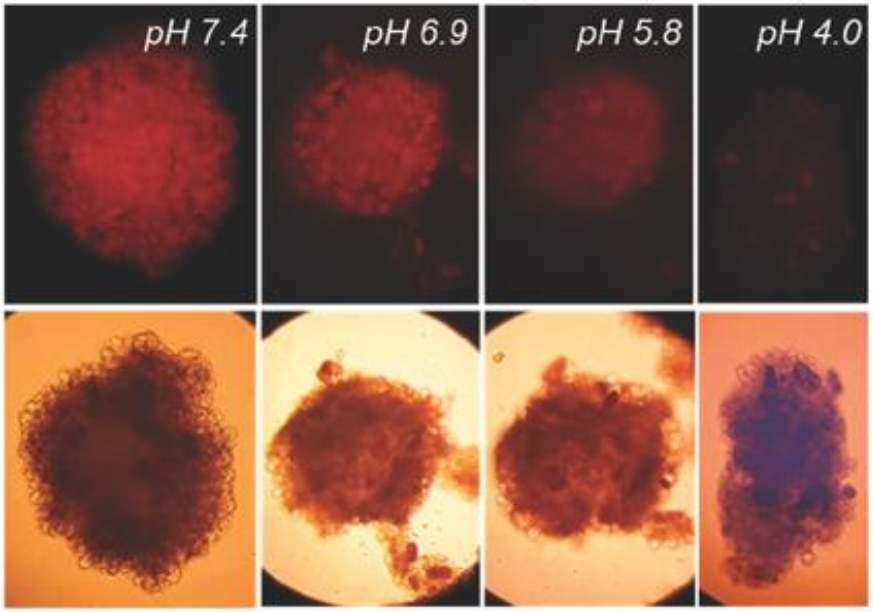
**$10^6$ FluoroMag-labeled NIH/3T3 fibroblast cells into a chicken breast (red circles)**

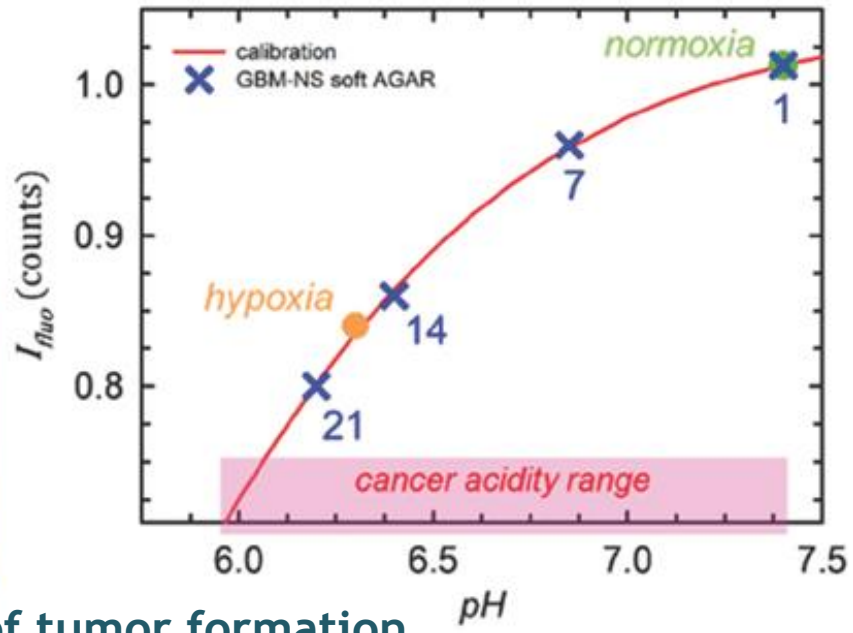


# In-Vitro Tests – *Glioblastoma Multiforme (GBM) Carcinogenic Model*

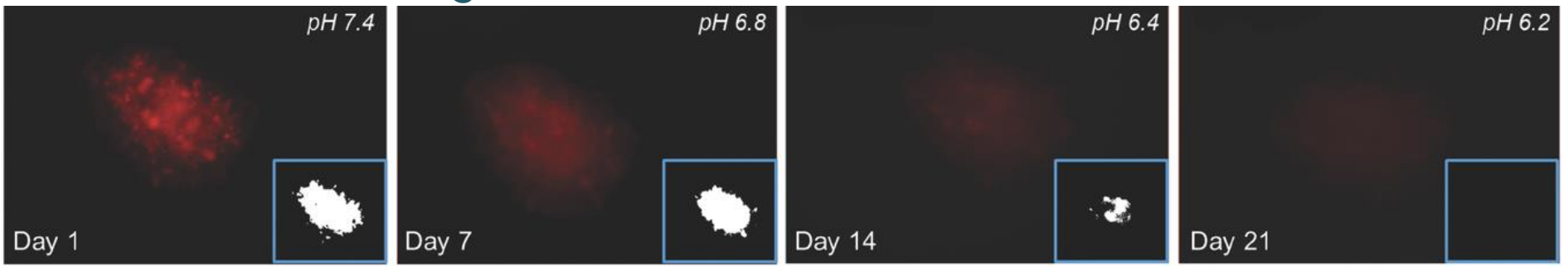
## Fluorescence monitoring as a function of pH

Glioblastoma derived neurospheres (GBM-NS)



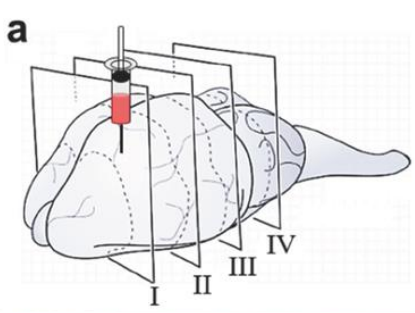



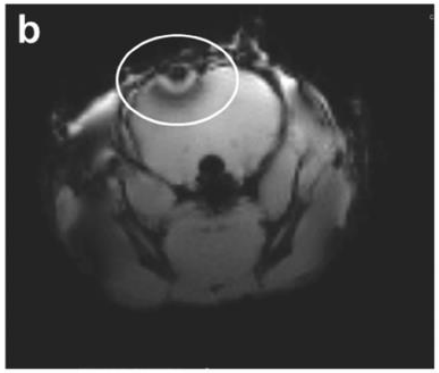

## Fluorescence monitoring as a function of tumor formation

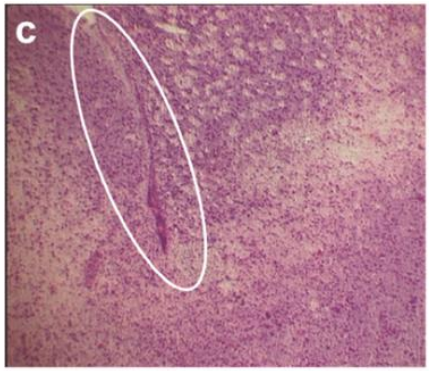


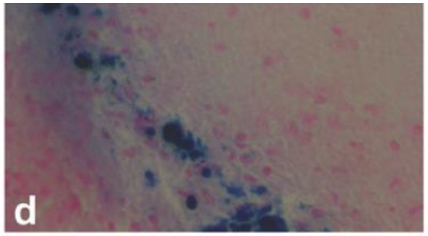
# In-Vivo Tests – *Glioblastoma Multiforme (GBM) Carcinogenic Model*

## In-vivo injection of FluoroMag-stained GMB-NSs in mouse brain

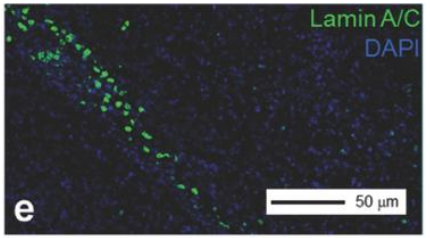


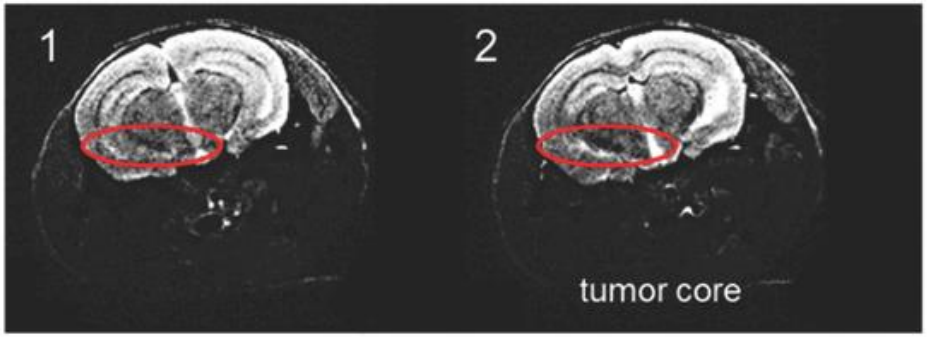

Prussian Blue MNP staining (optical microscope)



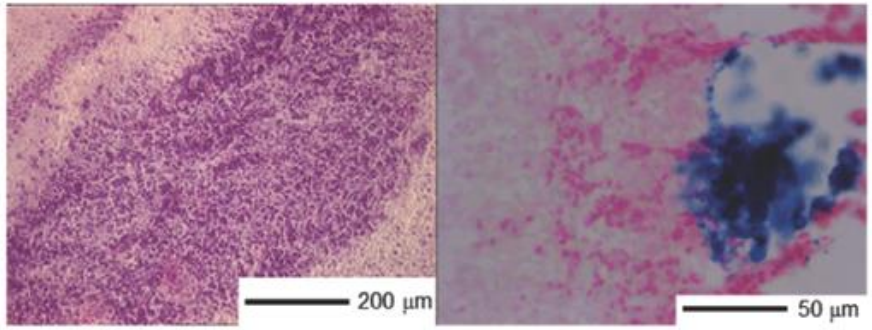

Fuorescence

## … After 90 days

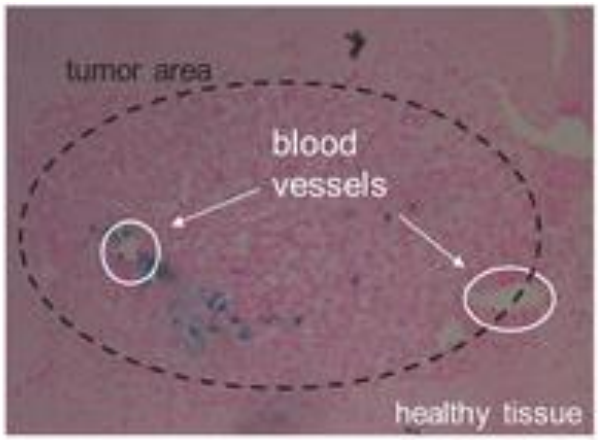
a




b

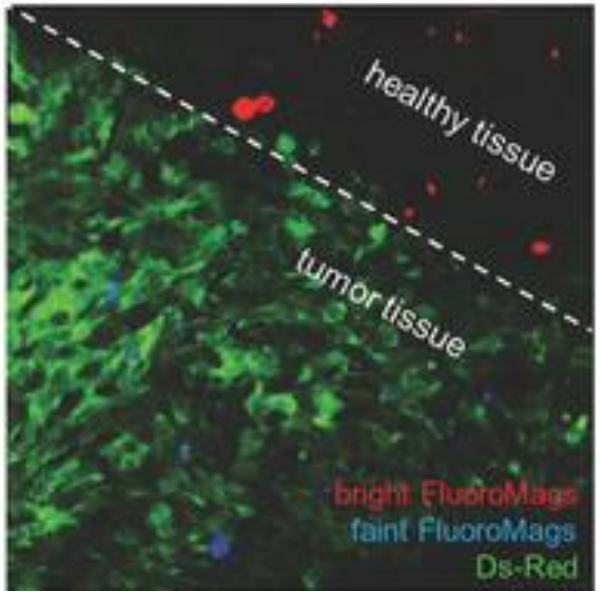


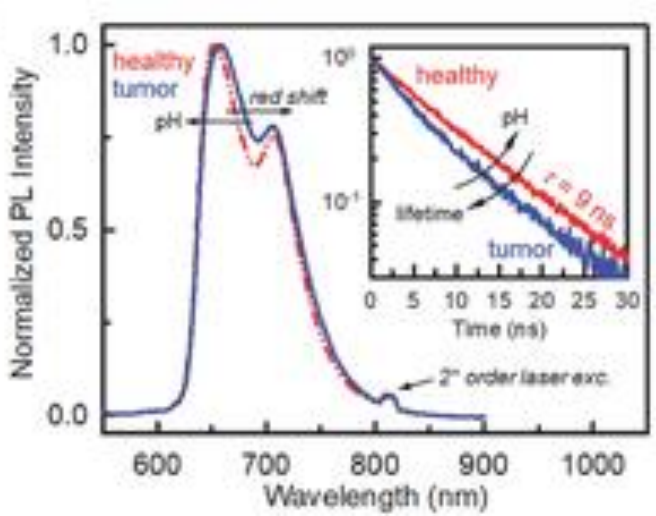
# In-Vivo Tests – *Crossing of the Brain Blood Barrier*




nanotubes can penetrate the BBB and reach the brain

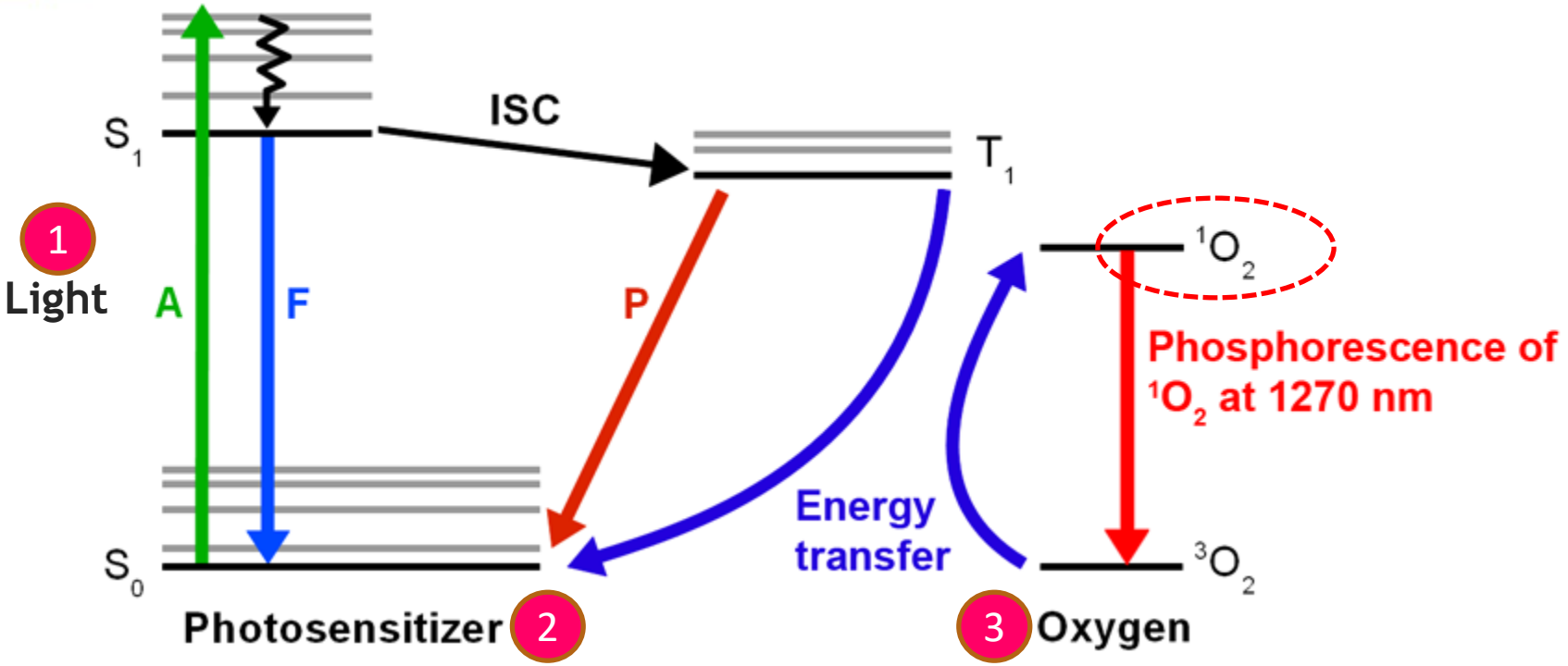
The peculiar tumor vessel fenestrated structures may exert dimensional tumor selectivity toward our nanoprobes



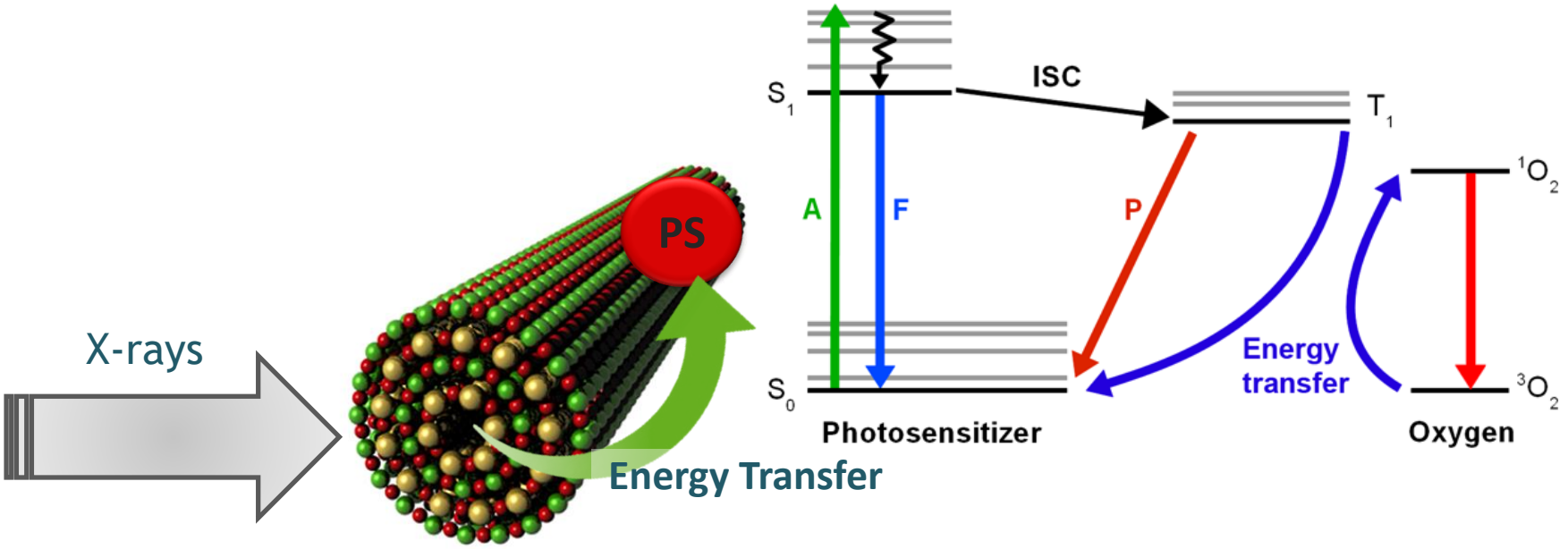

fluorescent nanotubes internalized in the tumor have been visualized with a blue pseudocolor

# Photodynamic Therapy (PDT) – *Generation of Cytotoxic Species*



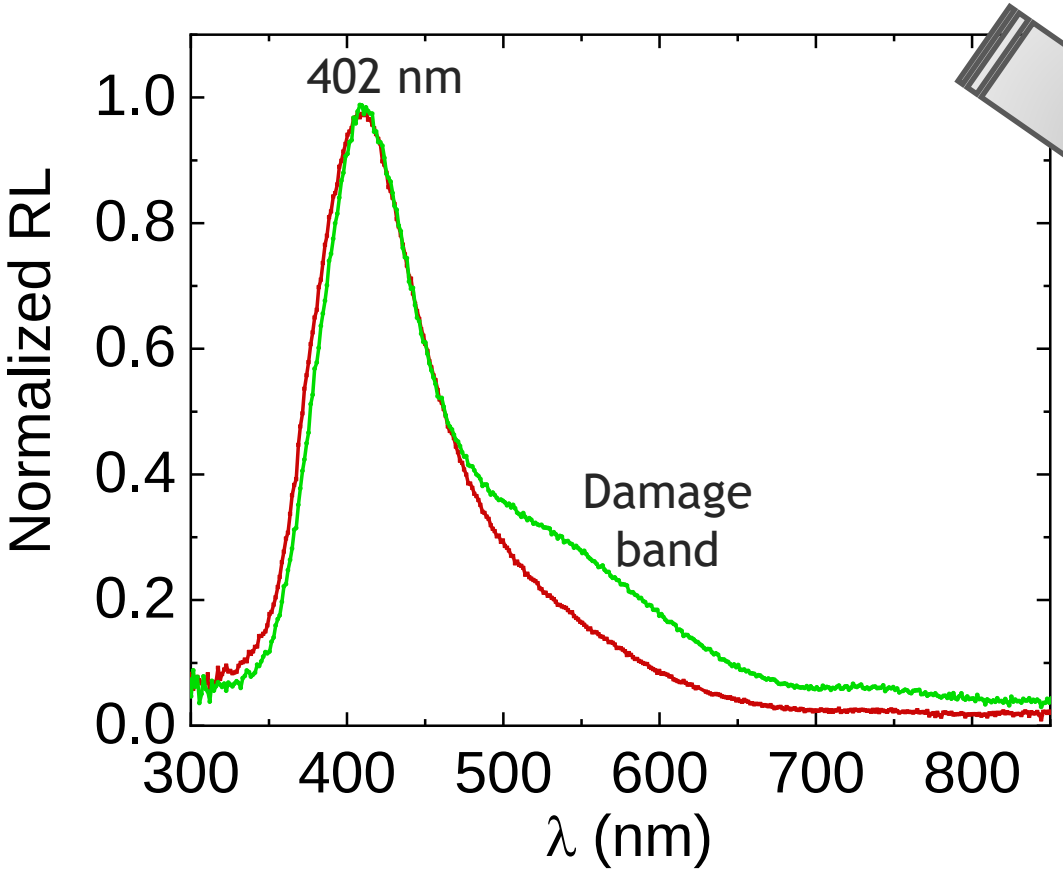

! Visible light is absorbed and scattered by skin and biological liquids
! Band of transparency of tissues (700-1300 nm)
! Even with NIR radiation, penetration depths are few millimeters
! Established treatment only for **shallow tumors**

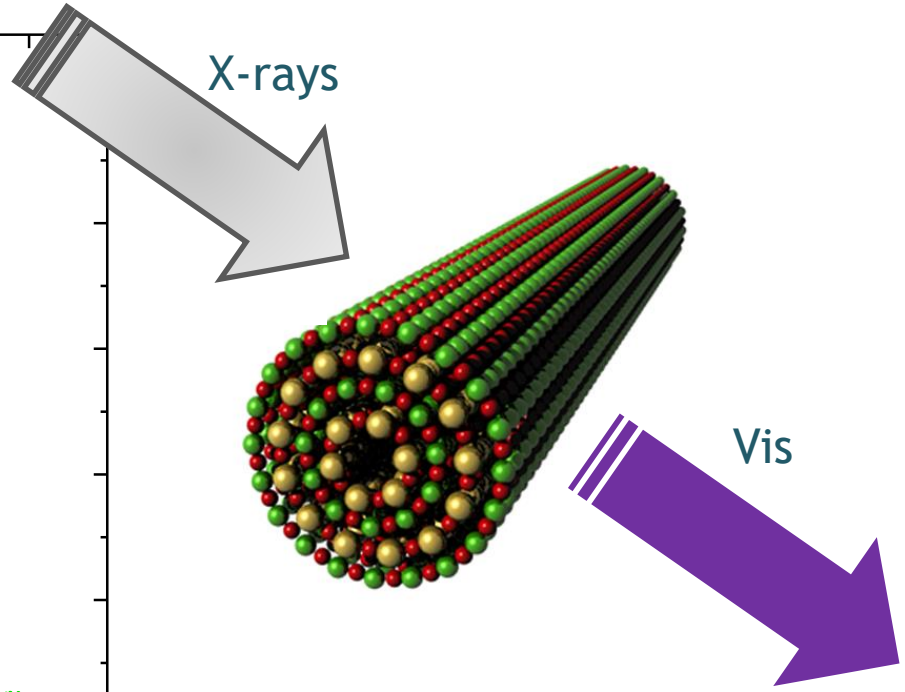
# X-Ray Activated Photodynamic Therapy (X-PDT)




1. DEEP PENETRATION INTO TISSUES
2. CELL APOPTOSIS CAUSED BY CYTOTOXIC SPECIES
3. DNA DAMAGE CAUSED BY X-RAYS

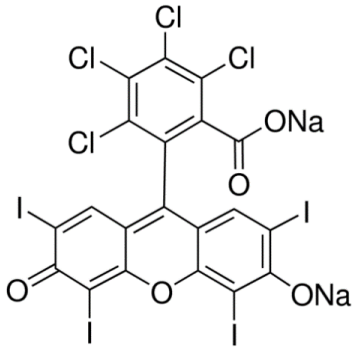
# **Scintillation** – *Radioluminescence of Chrysotile Nanotubes*



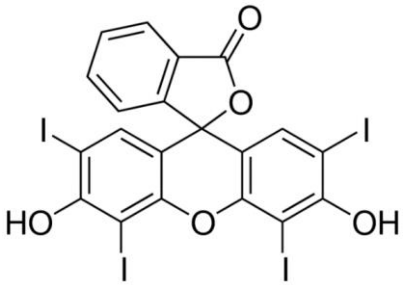



# **Functionalization** – *Photo-Sensitizer*

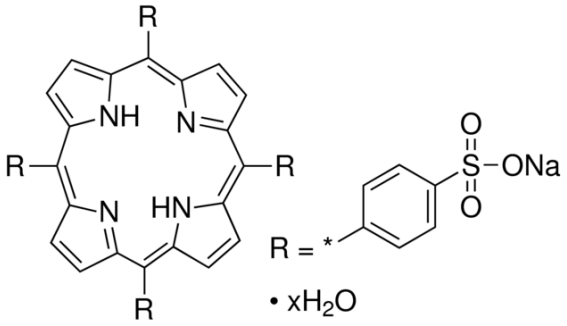
Bengal Rose

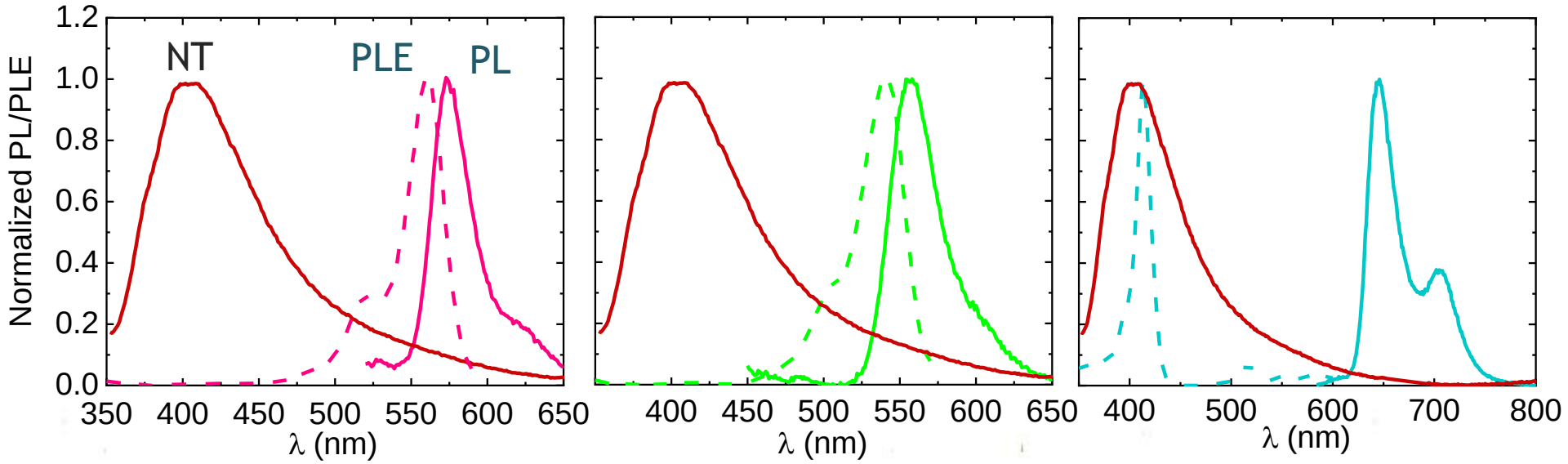



Erythrosine B



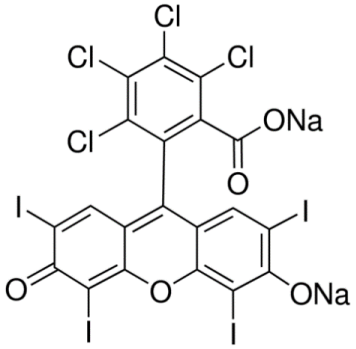

$TPPS_4Na_4$



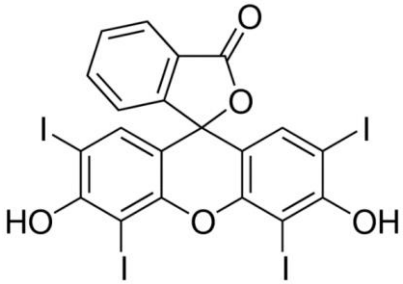



# **Energy Transfer** – *Functionalized NT for X-PDT*

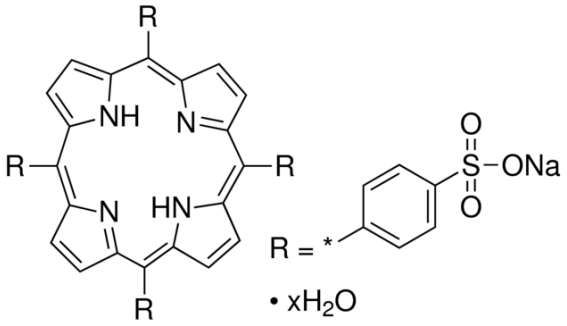
Bengal Rose

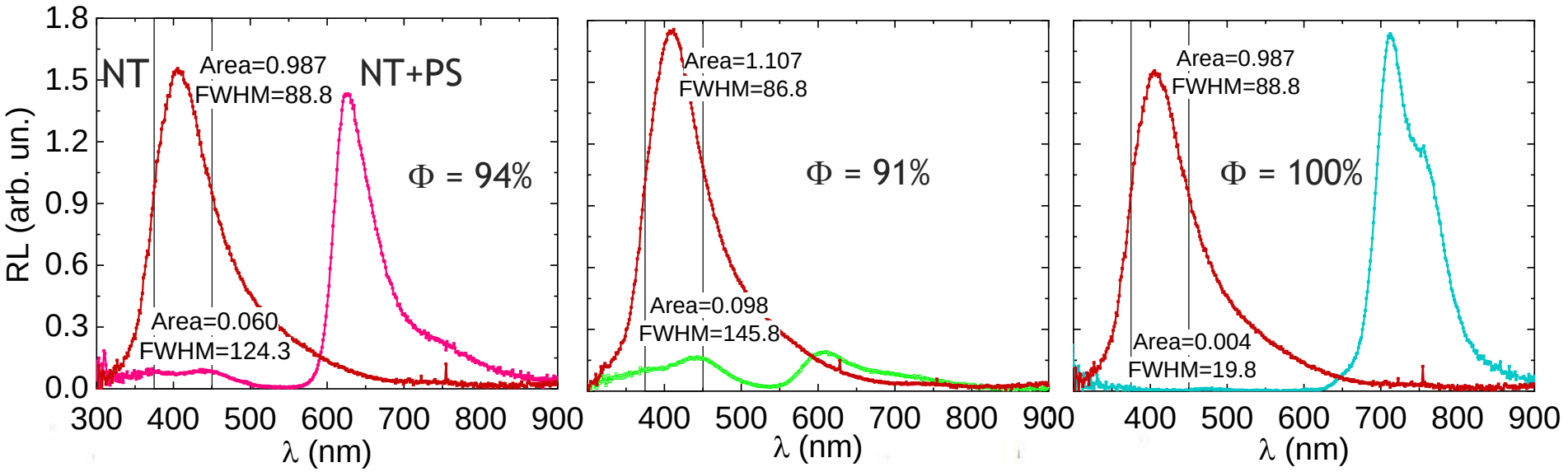



Erythrosine B

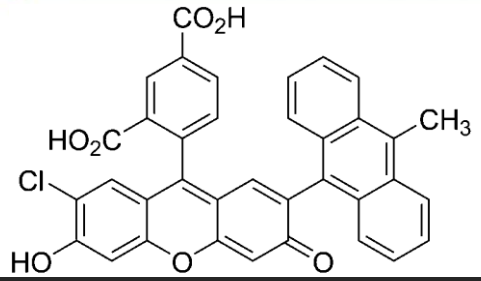



$TPPS_4Na_4$



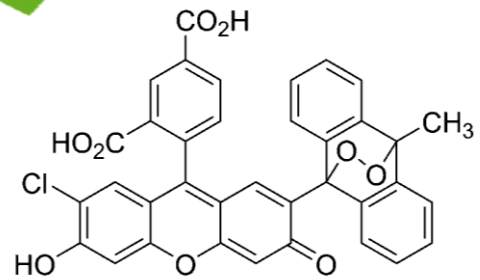



# Singlet Oxygen Generation – *Functionalized NT for X-PDT*

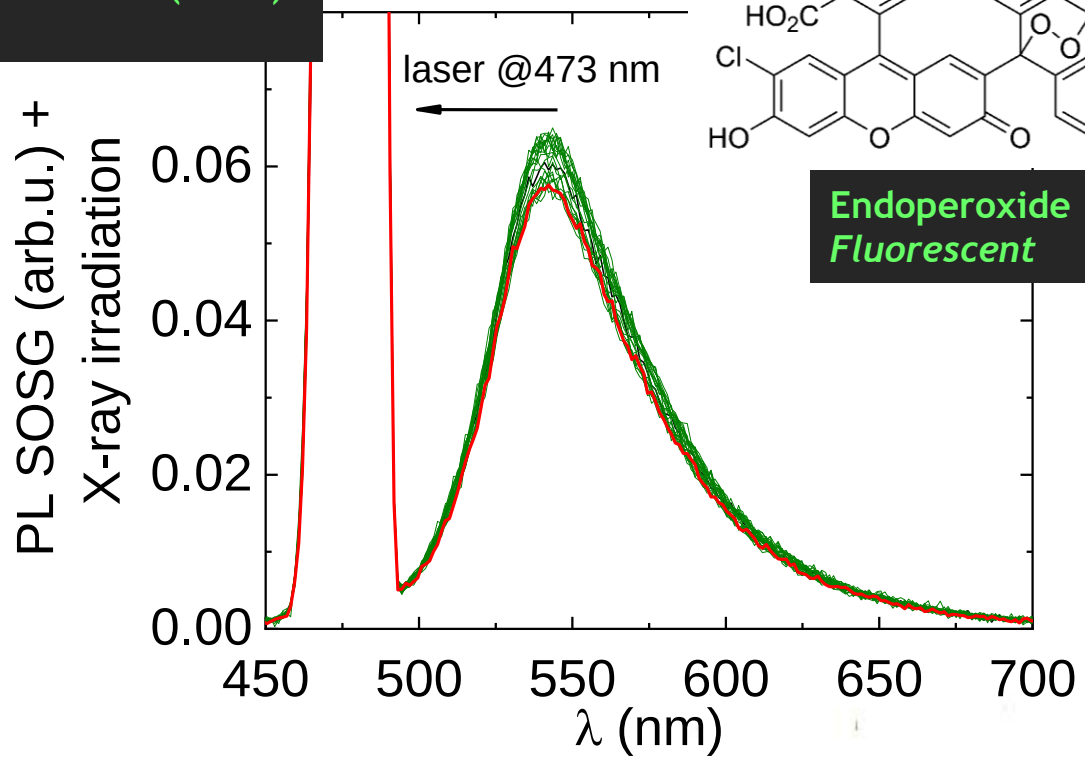



**Singlet Oxygen Sensor Green (SOSG)**
***Non-fluorescent***

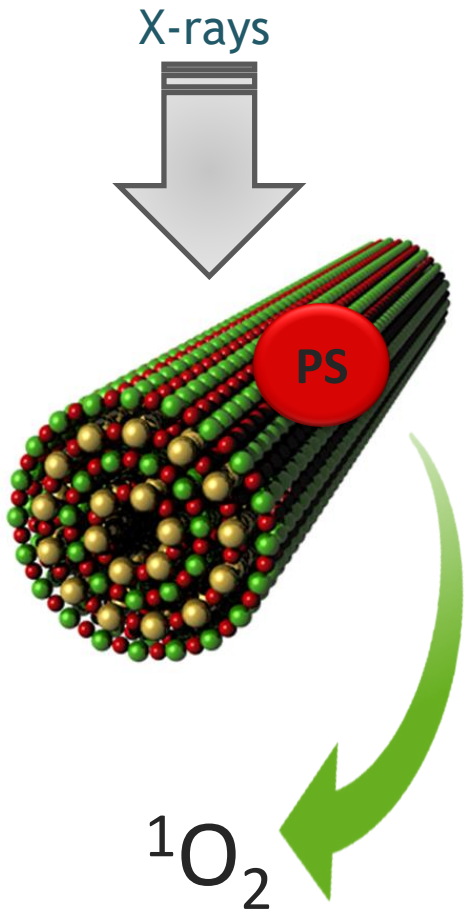
$^1O_2$

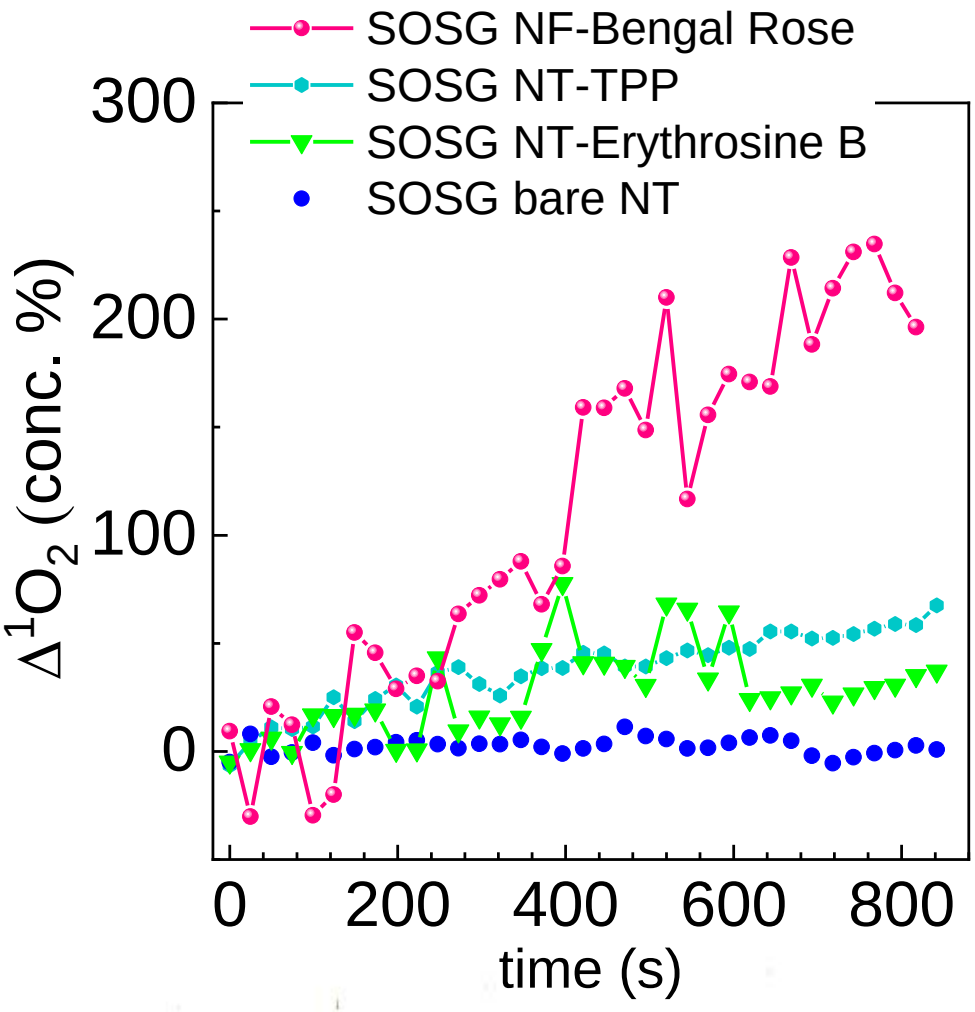



**Endoperoxide**
***Fluorescent***



# Singlet Oxygen Generation – *Functionalized NT for X-PDT*



# CONCLUSIONS

- FluoroMags can be effectively included in GBM-NS
- They keep their ferromagnetic properties upon in-vivo transplantation
- Their pH-sensitivity can be used to map disease evolution

- Bengal Rose photosensitizer is able to produce $^1O_2$ with an increment of 200% after 15 minutes. An optimal system for X-PDT.
  → in vivo tests
- Causes that make Erythrosine B and TPP ineffective producers of $^1O_2$ must be investigated

→ Doping of NT with luminescent ions acting as color centers

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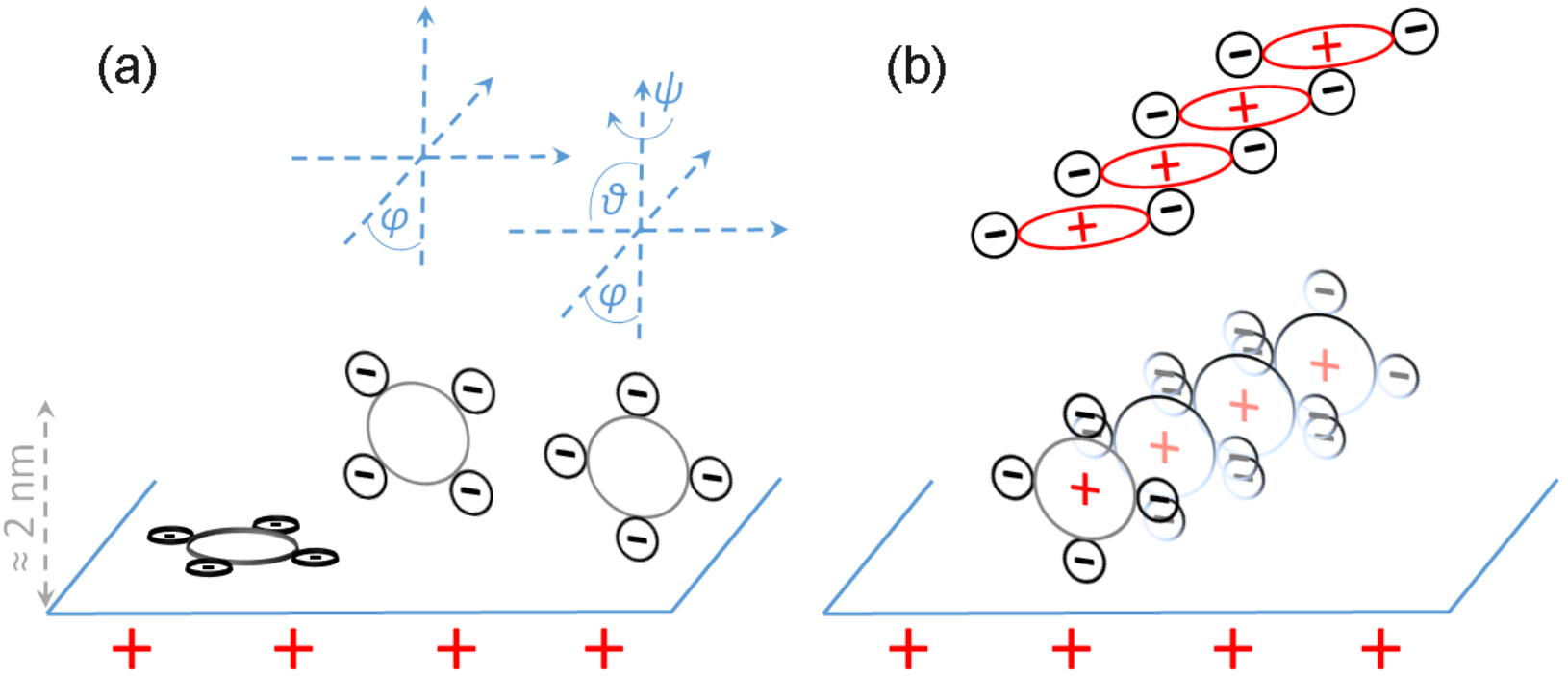
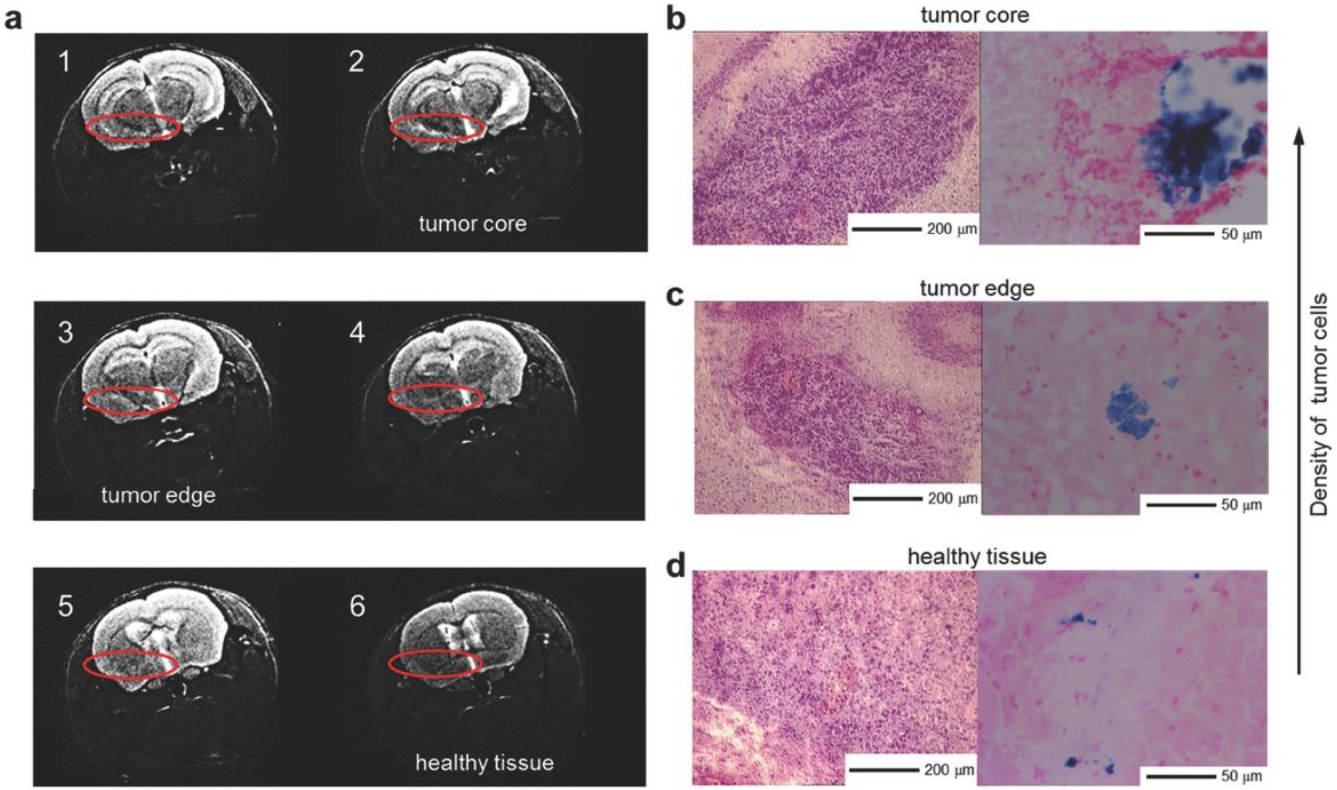
Fondazione I.R.C.C.S. Istituto Neurologico Carlo Besta, Milano, Italy

# **Functionalization** – *pH sensitive, fluorescent molecule*




a
1
2
tumor core
3
4
tumor edge
5
6
healthy tissue
b
tumor core
200 µm
50 µm
c
tumor edge
200 µm
50 µm
d
healthy tissue
200 µm
50 µm
Density of tumor cells