



Institute of
Applied Sciences
and
Intelligent Systems



Hybrid inorganic nanoparticles for optical imaging and sensing

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Unit of Naples

National Research Council

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ISASI – Unit of Naples

29 staff members + 20 PhD students and postdocs

Units

ISASI- Headquarter (Pozzuoli-NA)

ISASI – Naples

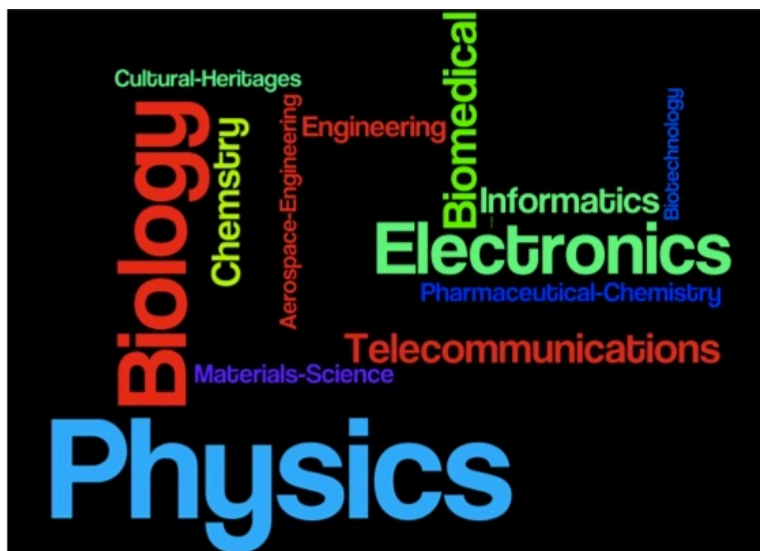
ISASI – Lecce

iMEG



Main Research Activities

- ☐ Photonics for Space, Avionics and Energy
- ☐ Optoelectronic Chemical and Biological Sensing
- ☐ Nanodevices for theranostics



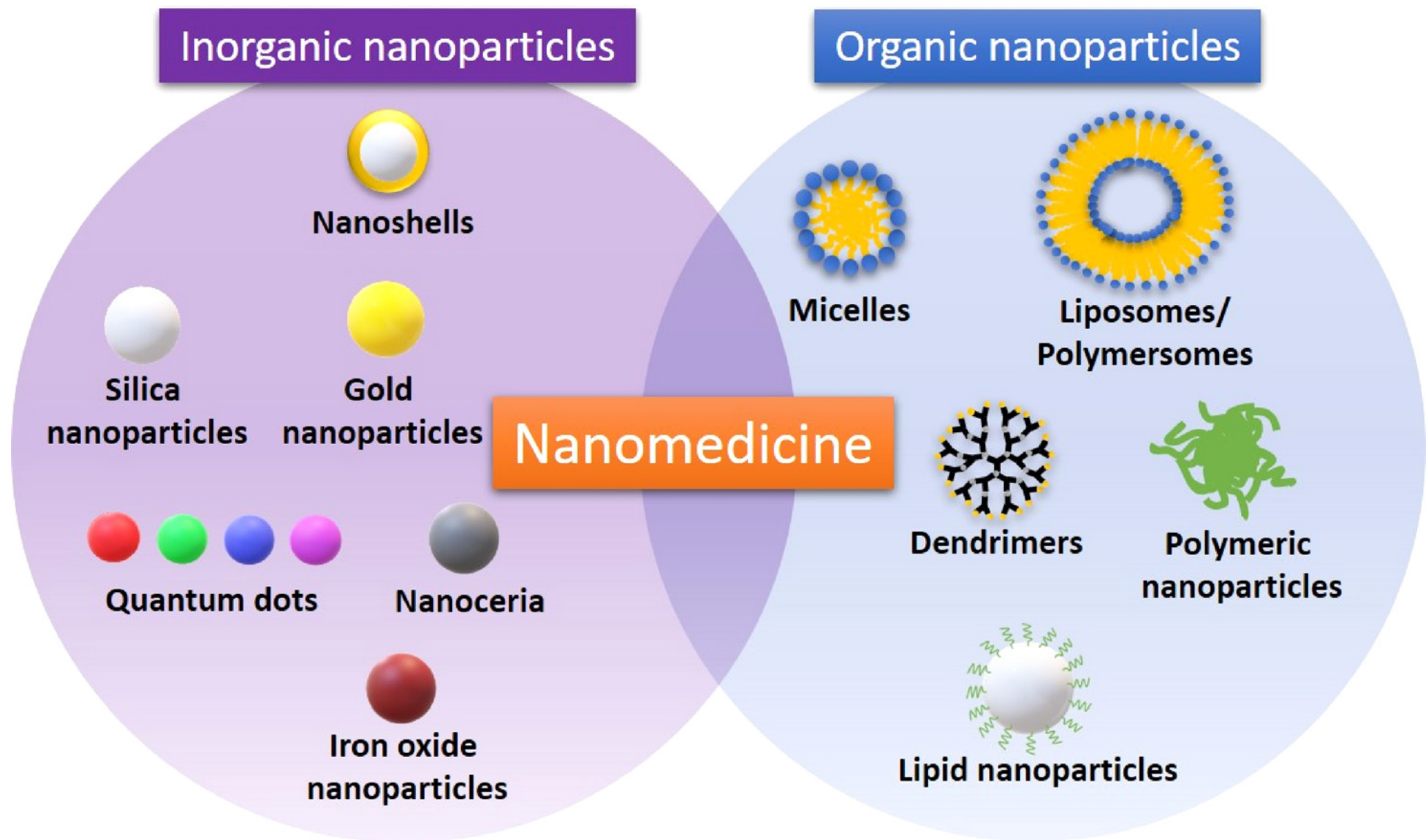


Collaborations



Luca De Stefano, PhD
Ilaria Rea, PhD
Principia Dardano, PhD
 R. Moretta, post Doc scientist
 C. Schiattarella, PhD
 C. Tramontano, PhD student
 B. Miranda, PhD student
 G. Chianese, fellowship
 C. Tammaro, PhD student
 M. Battisti, PhD (Materias)
 S. De Martino, PhD (Materias)

MATERIAS[®]
ideas come to life



Martinelli et al., APL Bioeng. **3**, 011502 (2019);

Hybrid Inorganic Nanomaterials

Fabrication
(top-down)

Synthesis
(bottom-up)

Metals

Polymers

New properties, features
and applications

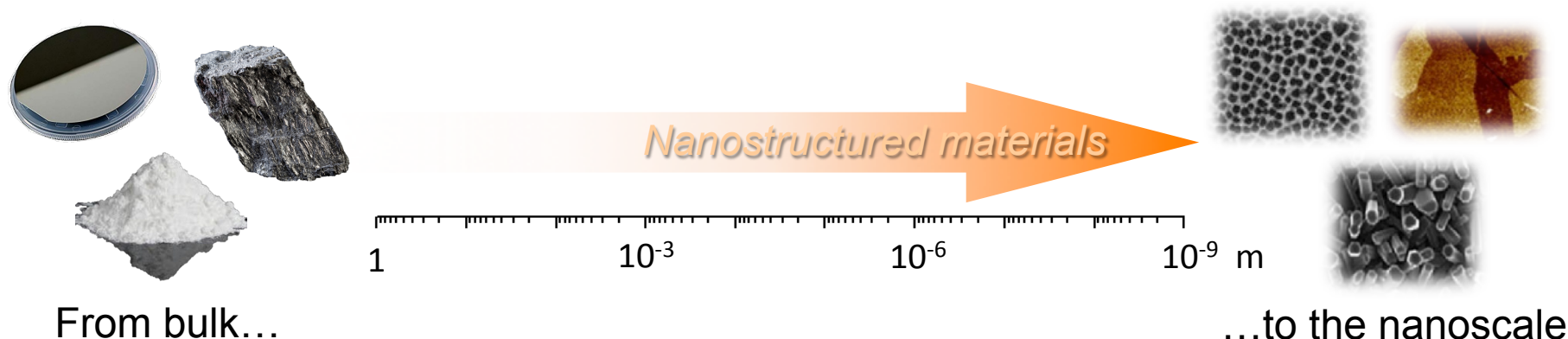
Semiconductors

Modification
(wet, dry)

Hydrogels

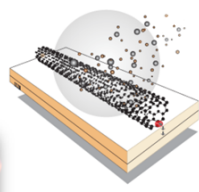
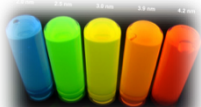
Characterization
(optical,
electrical)

Inorganic nanostructured materials



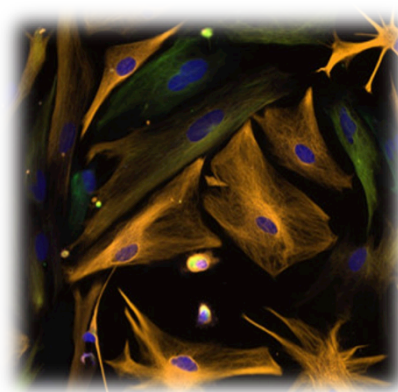
Appearance of quantum effects @macroscopic scale

- High surface area
- Efficient light emission
- Long photoluminescence lifetime
- Tunable optical response
- Biocompatibility



Biophotonics

(Molecular and medical diagnostics)

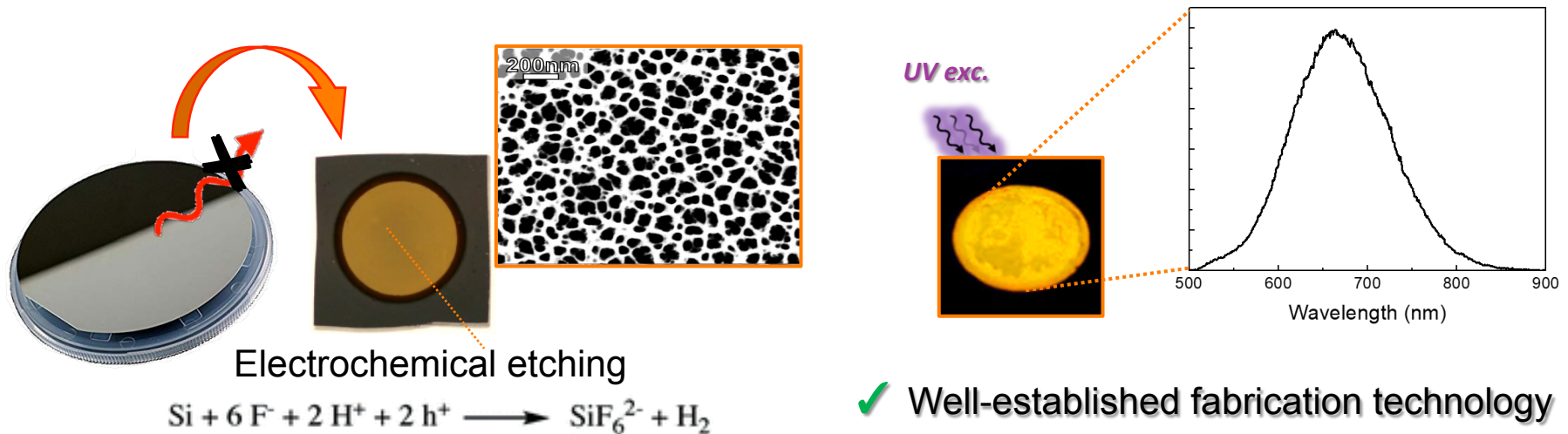


Bioimaging

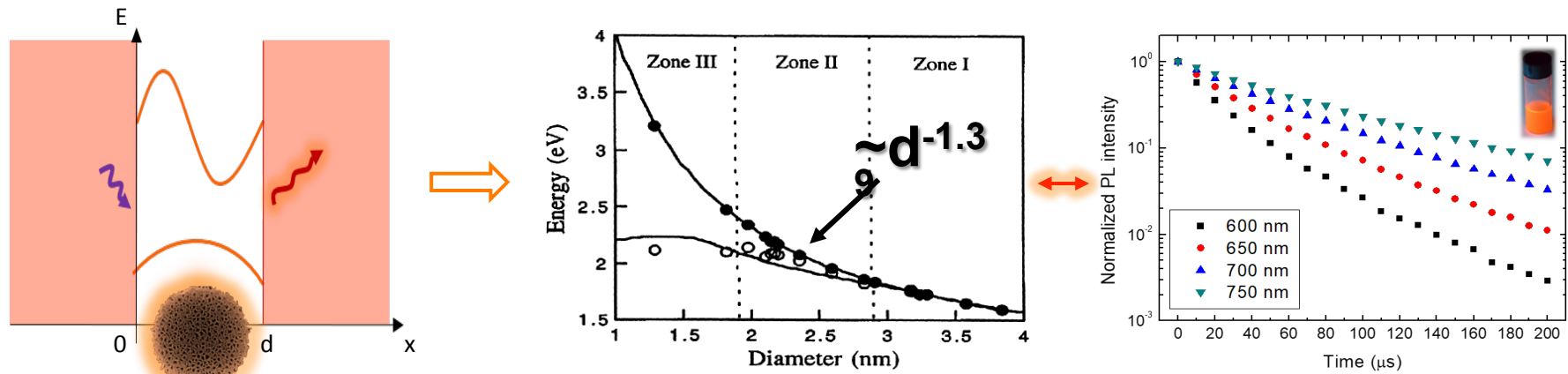


Biosensing

Porous silicon (PSi)



Quantum confinement model



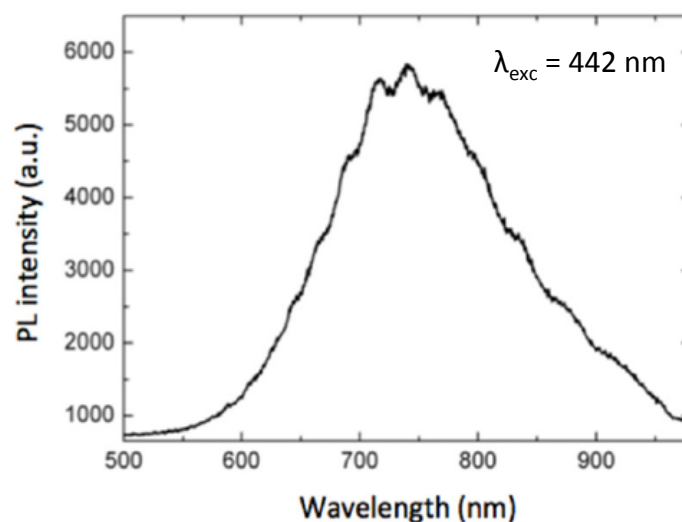
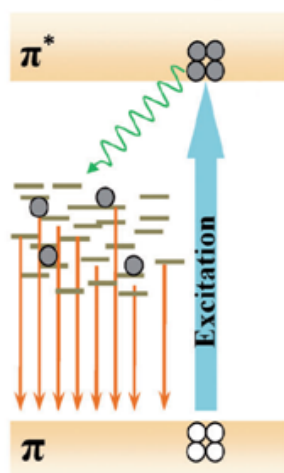
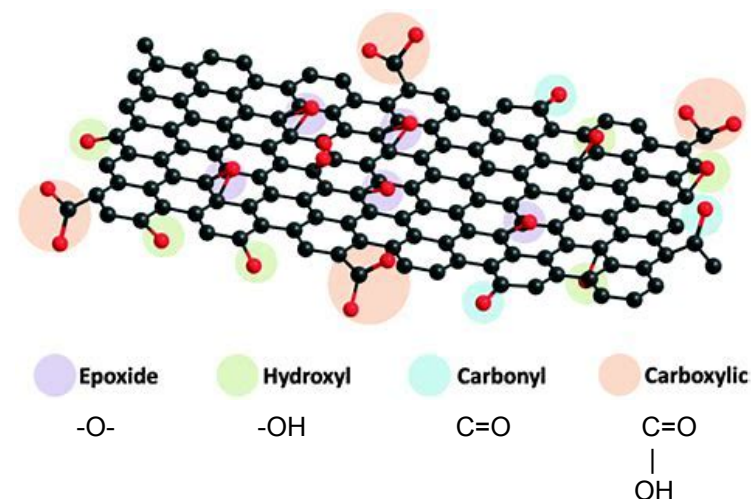
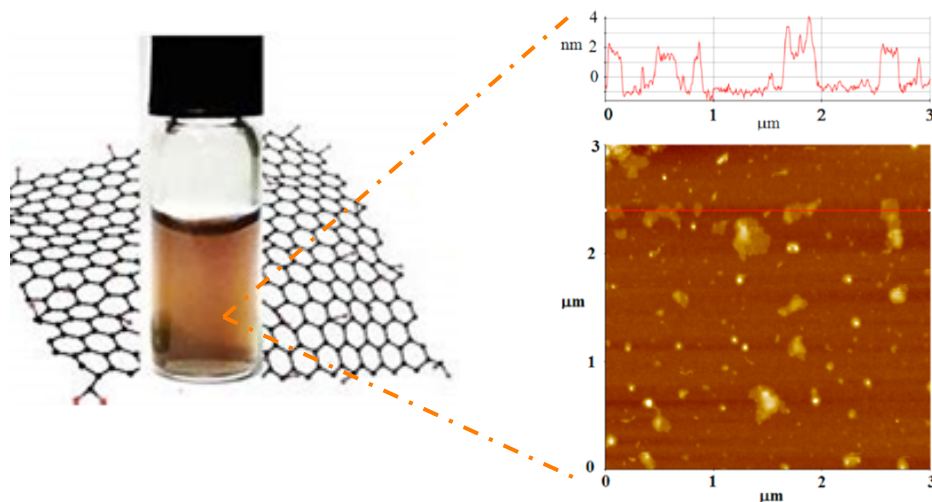
Size-dependent energy levels

Carrier relaxation dynamics

$$\frac{1}{\tau} = \frac{1}{\tau_r} + \frac{1}{\tau_{nr}} = \frac{16\pi^2}{3} n \frac{e^2}{h^2 m^2 c^3} E_0 |\langle i_{CB} | \hat{p} | f_{VB} \rangle|^2 \quad (1)$$

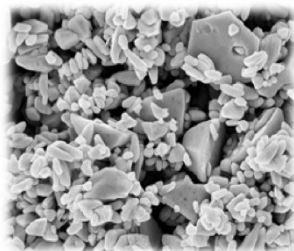
PSi is a disordered system: $I(t) = I_0 e^{-\left(\frac{t}{\tau}\right)^\beta} \quad (2)$

Graphene oxide (GO)

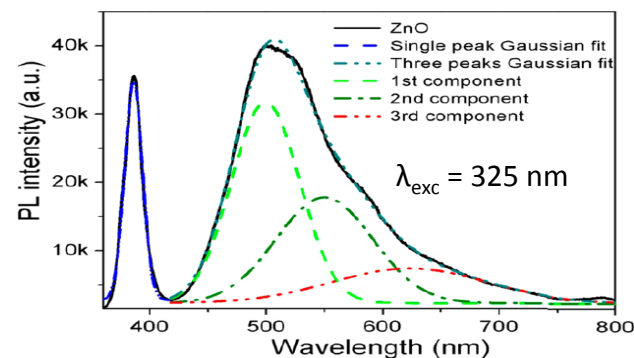


- ✓ Hydrophilic material
- ✓ Massive presence of functional groups
- ✓ Optical transitions from disorder-induced localized states lead to a broad excitation-dependent PL emission

Large bandgap hybrid semiconductors



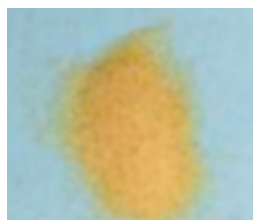
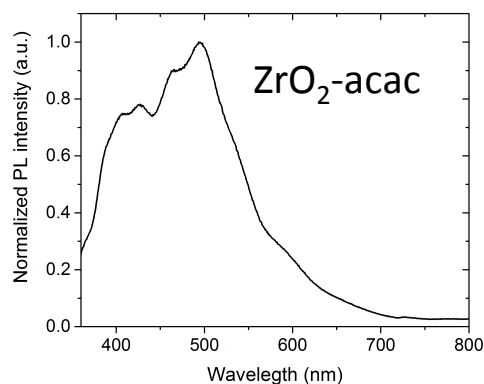
Fluorine doped-Zinc oxide (ZnO-F)



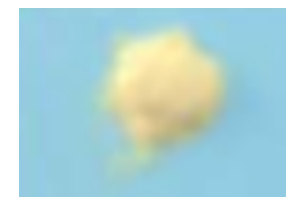
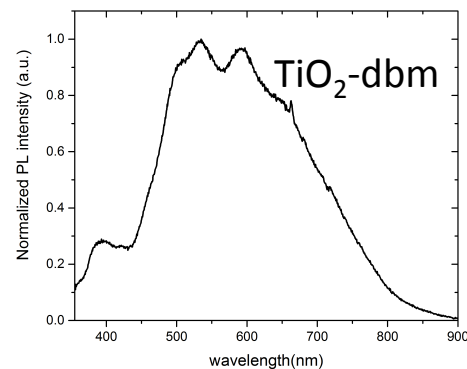
DI
C
Ma
PI

Prof. A. Aronne

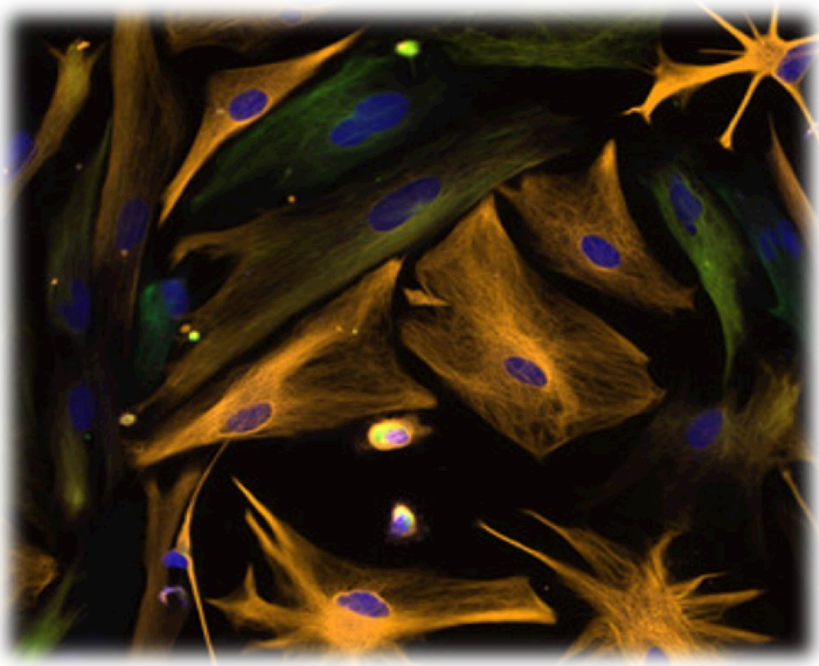
Zirconia-acetylacetonate (ZrO₂-acac)



Titania-dibenzoylmethane (TiO₂-dbm)



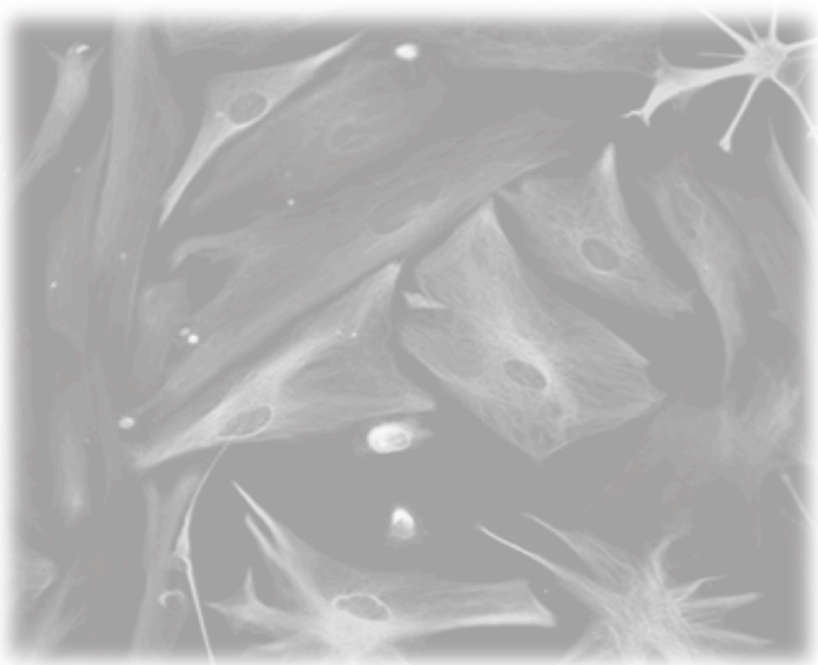
- ✓ Nanoparticles (average size=400 nm)
- ✓ EPR signal (ROS-generating)
- ✓ Characteristic efficient PL emission
- ✓ Colloidal stability in water-based solutions



Bioimaging



Biosensing



Bioimaging

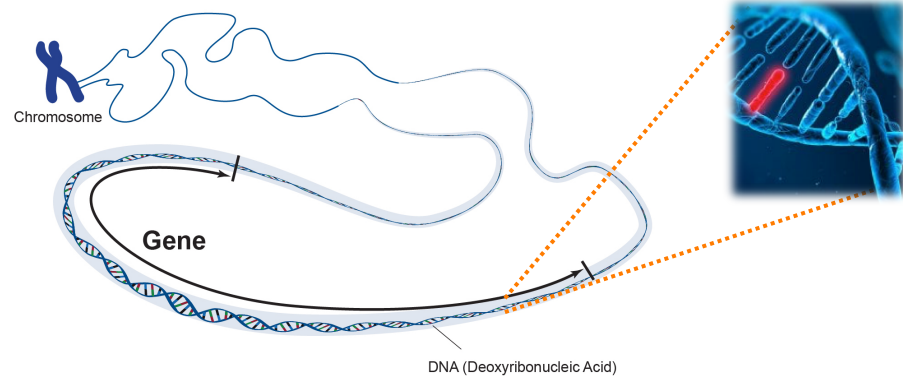
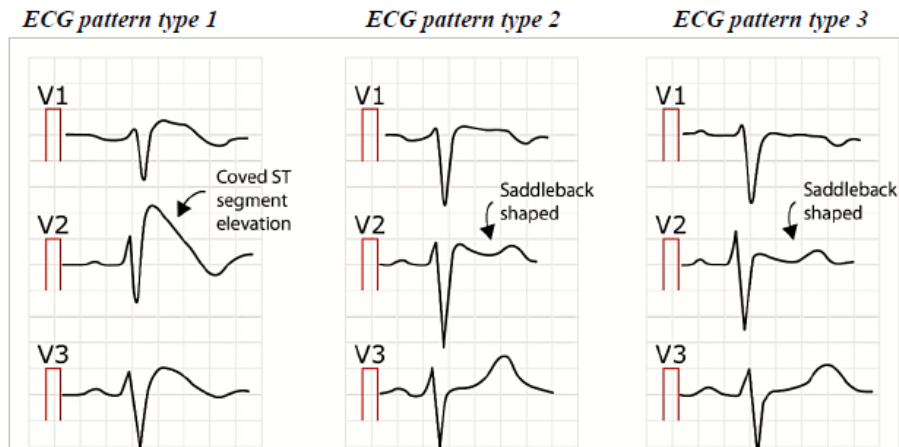


Biosensing

Graphene oxide/porous silicon device for early diagnosis of Brugada Syndrome

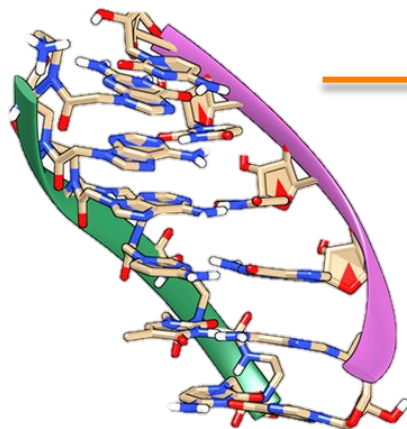
Nucleic acids-based technology for early diagnosis of genetic diseases

Brugada Syndrome (or Sudden Death Syndrome)

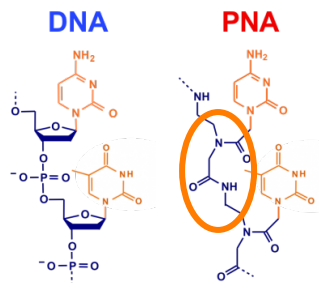


Point-mutations in the SCN5A gene

Nucleic acids (DNA, RNA, PNA) as biorecognition elements



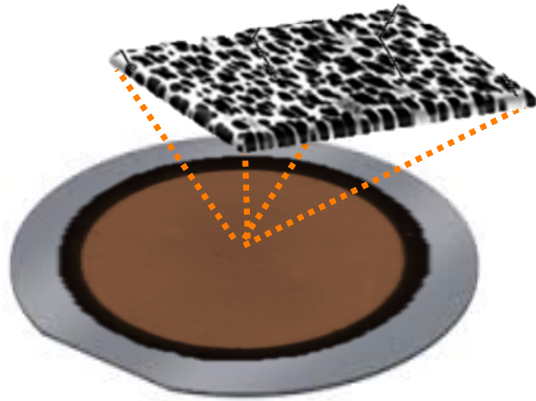
Detection specificity at the single-base level



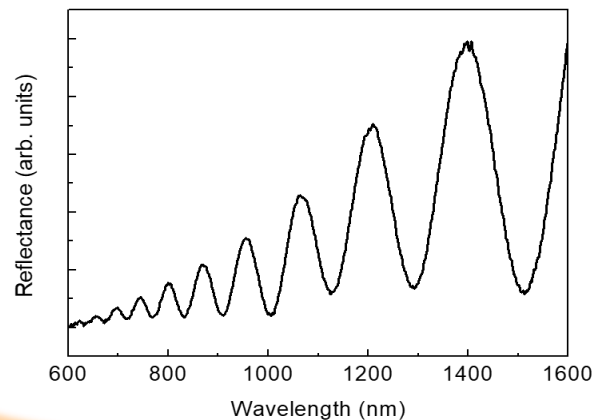
- PNAs are synthetic analogous of DNA
- The PNA/DNA hybridization is stronger than the one between DNA/DNA strands

Hybrid biosensing device based on porous silicon

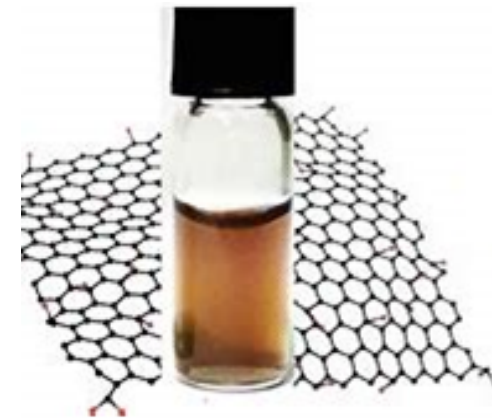
Non-luminescent PSi structure



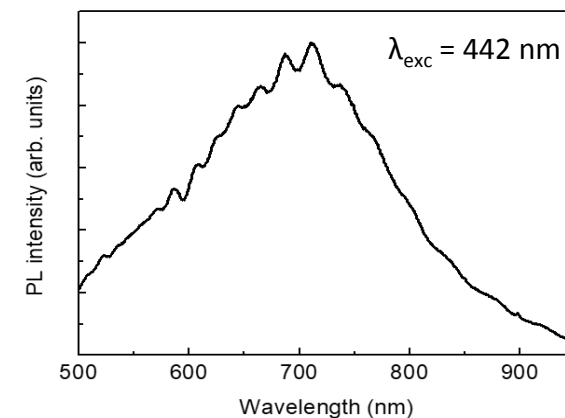
- ✓ Versatile surface chemistry
- ✓ Tunable photonic properties



Graphene oxide nanosheets



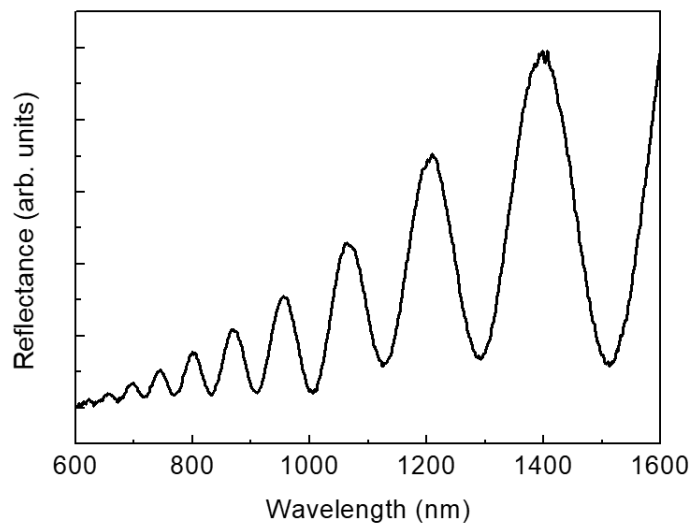
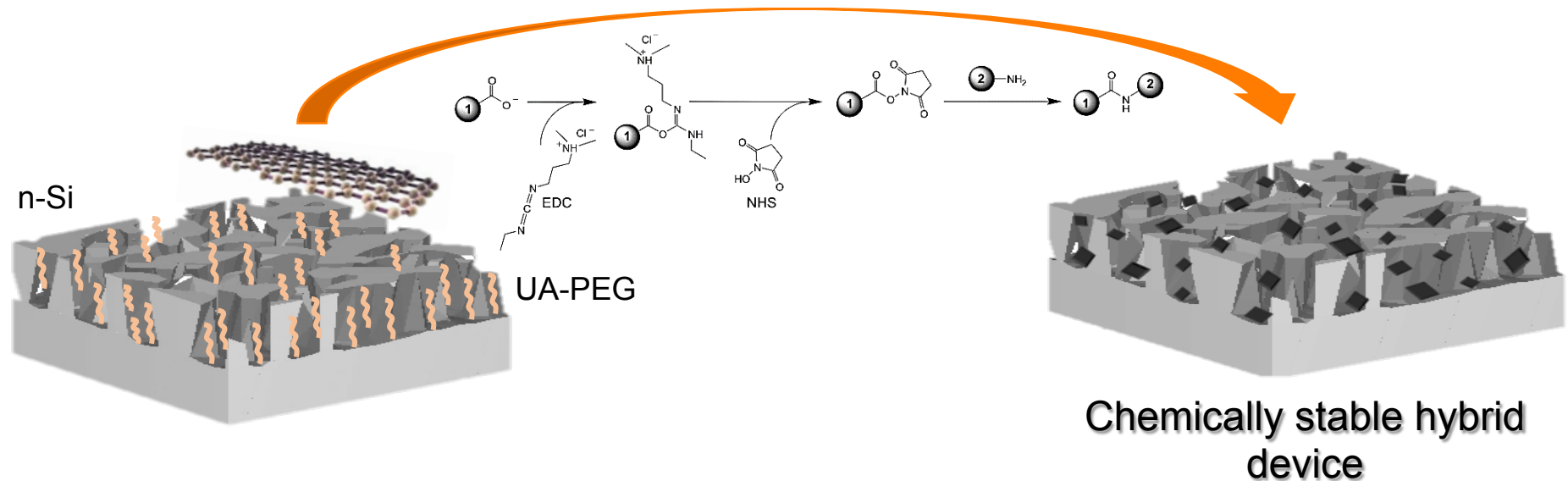
- ✓ High density of functional groups
- ✓ Characteristic PL emission



Multi-parametric PSi/GO nanocomposite

Fabrication of PSi/GO chip

Covalent grafting of GO on macroporous PSi chip via carbodiimide chemistry



Fabry-Pérot photonic structure

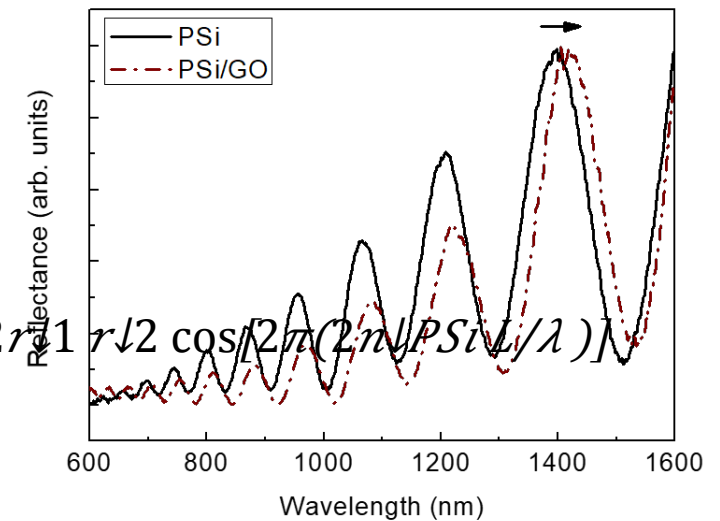
GO infiltration

$$R(\lambda) = r_1^2 + r_2^2 + 2r_1 r_2 \cos[2\pi(2n_{PSi}L/\lambda)]$$

$$r_1 = (n_{Air} - n_{PSi}) / (n_{Air} + n_{PSi})$$

$$r_2 = (n_{PSi} - n_{Si}) / (n_{PSi} + n_{Si})$$

$$m\lambda = 2n_{PSi}L$$

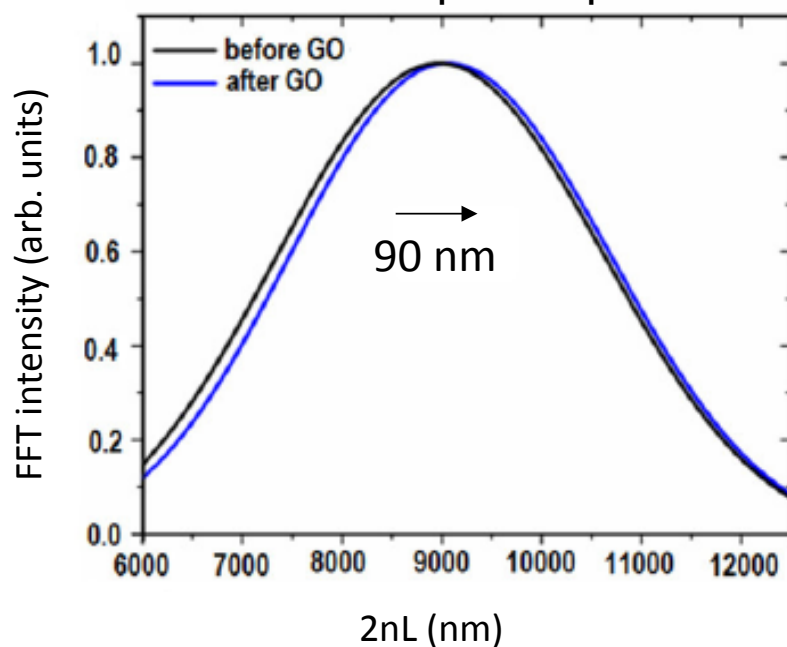


Optical characterization of PSi/GO chip

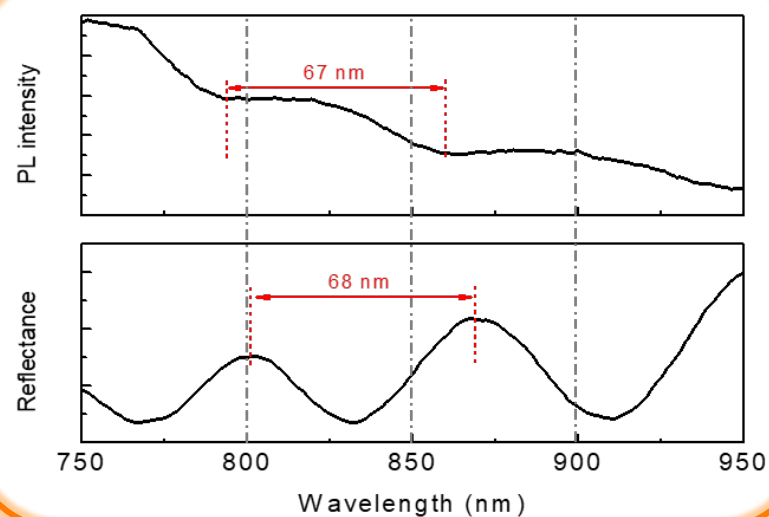
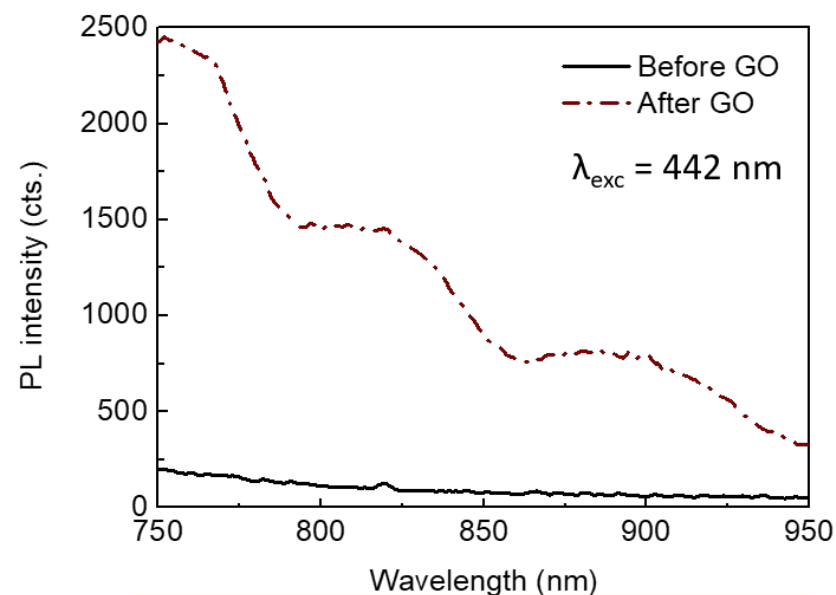
$$R(\lambda) = r_1^2 + r_2^2 + 2r_1r_2 \cos \left[2\pi \left(\frac{2n_{PSi}L}{\lambda} \right) \right]$$



Fast Fourier Transform (FFT)
of PSi/GO optical spectrum

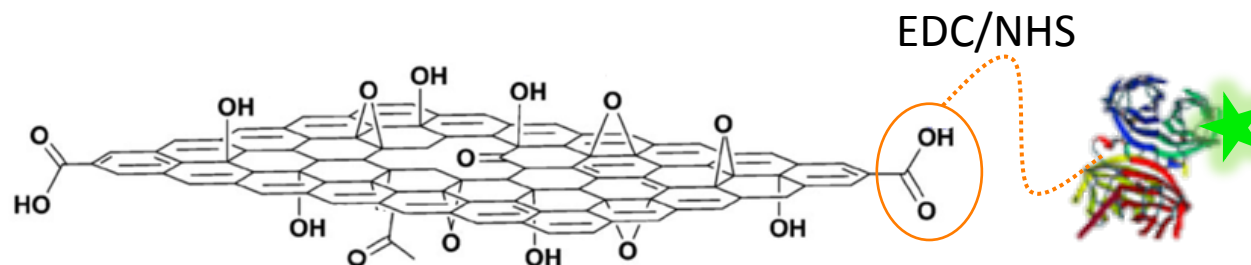


Steady-state PL analysis

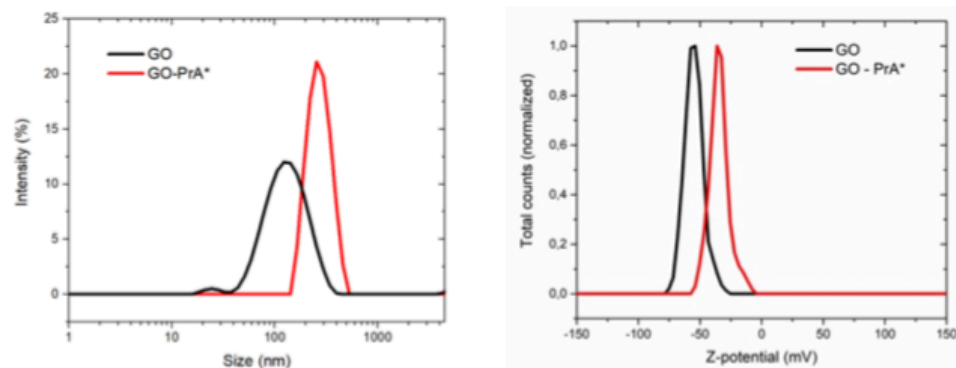


Preparatory study on a model bioprobe: protein A (1)

Protein A anchoring on GO nanosheets in aqueous suspension

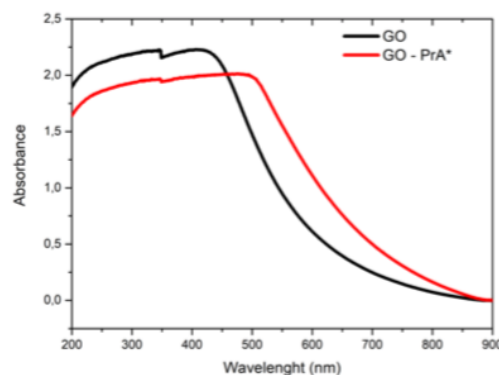


DLS analysis

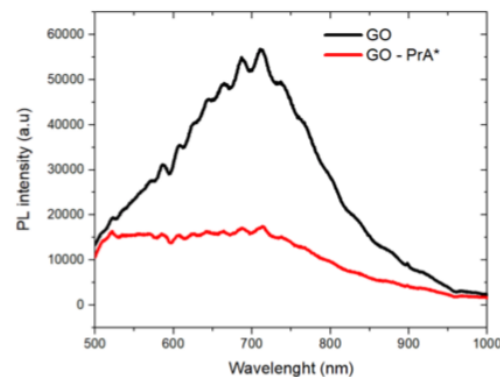


FITC-labelled
PrA
~ 0.3 mg/mL

UV-Vis

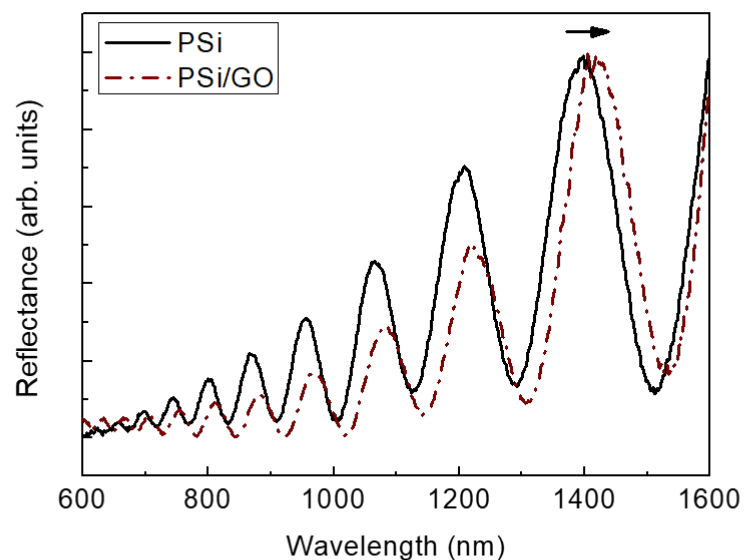
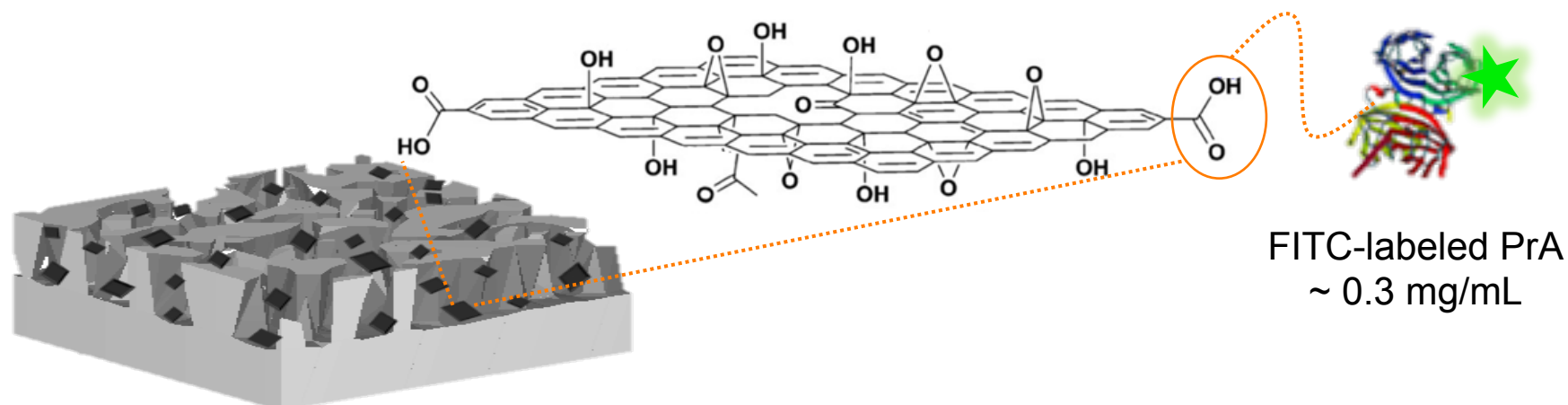


PL

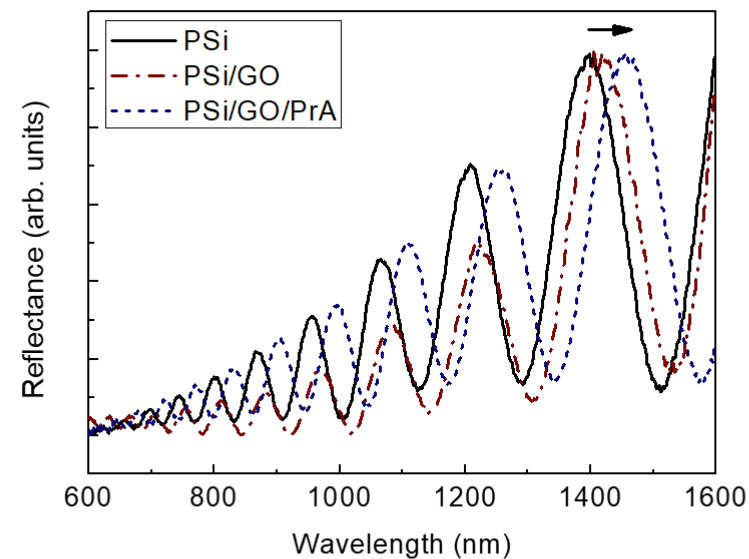


Preparatory study on a model bioprobe: protein A (2)

Protein A anchoring onto PSi/GO chip

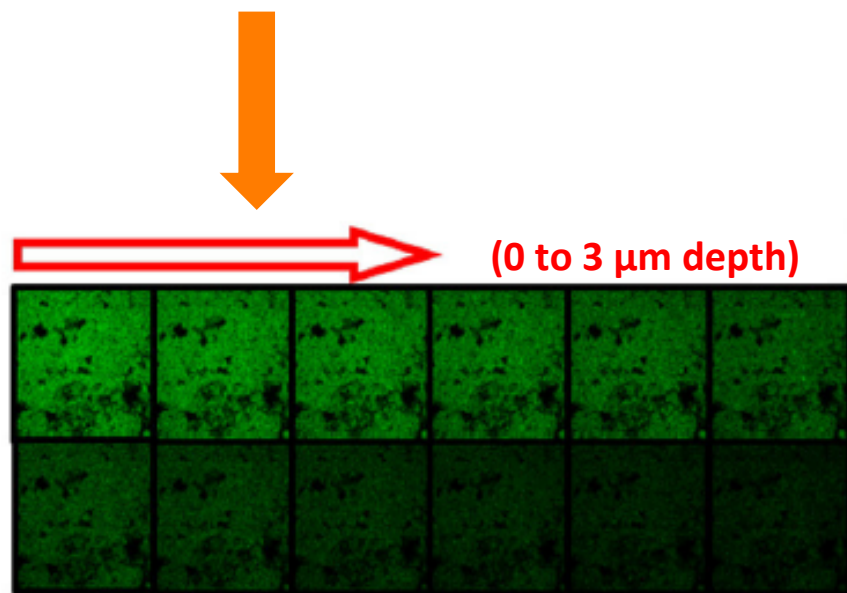
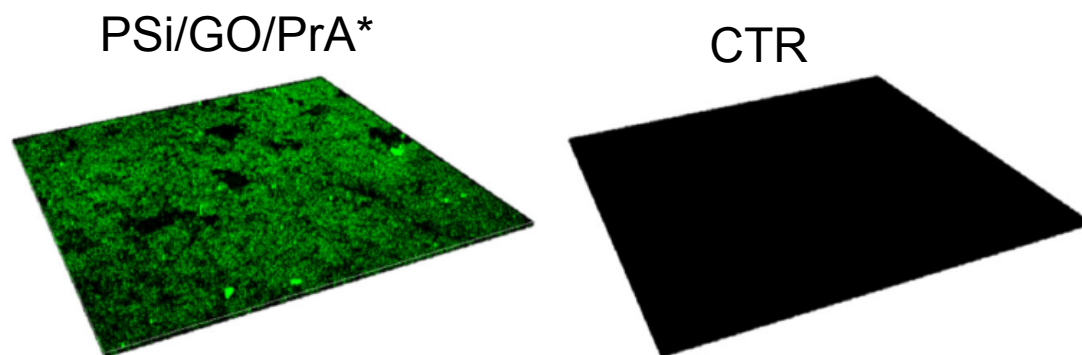


Protein
infiltration

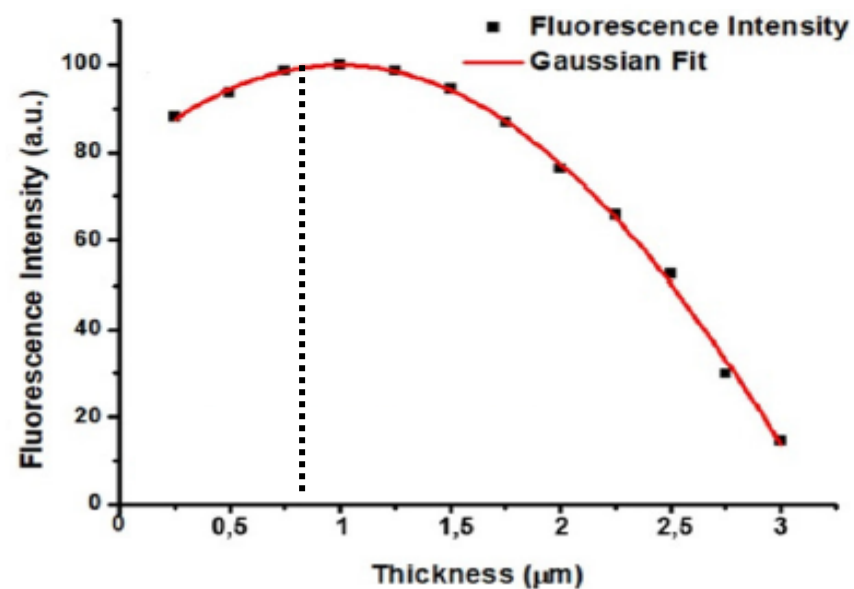
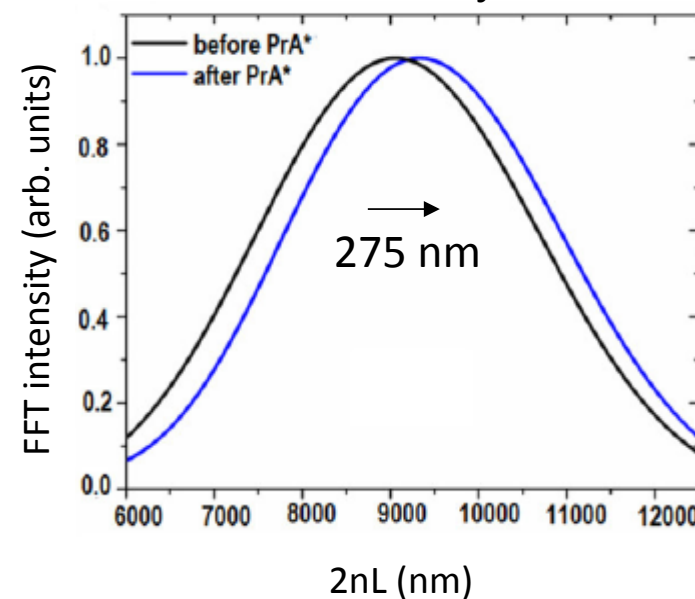


FITC-labeled PrA anchoring onto PSi/GO chip

Confocal fluorescence imaging



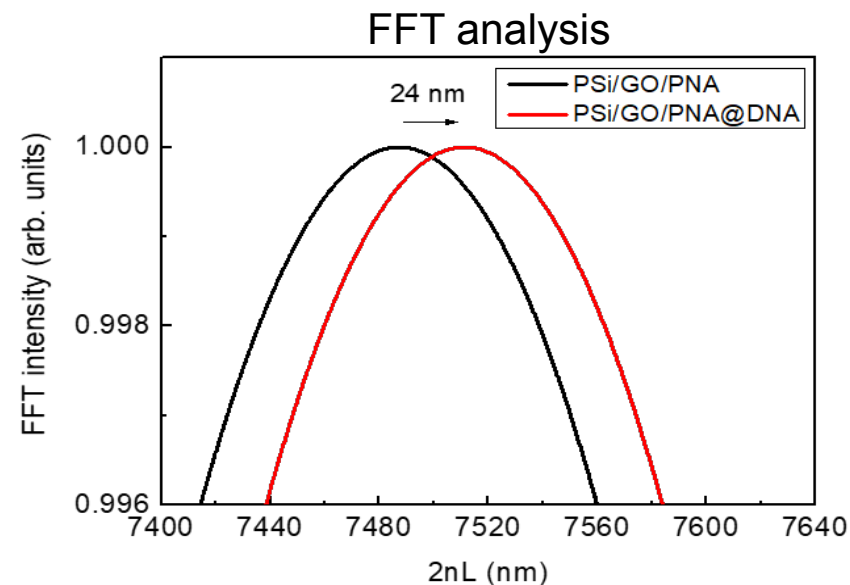
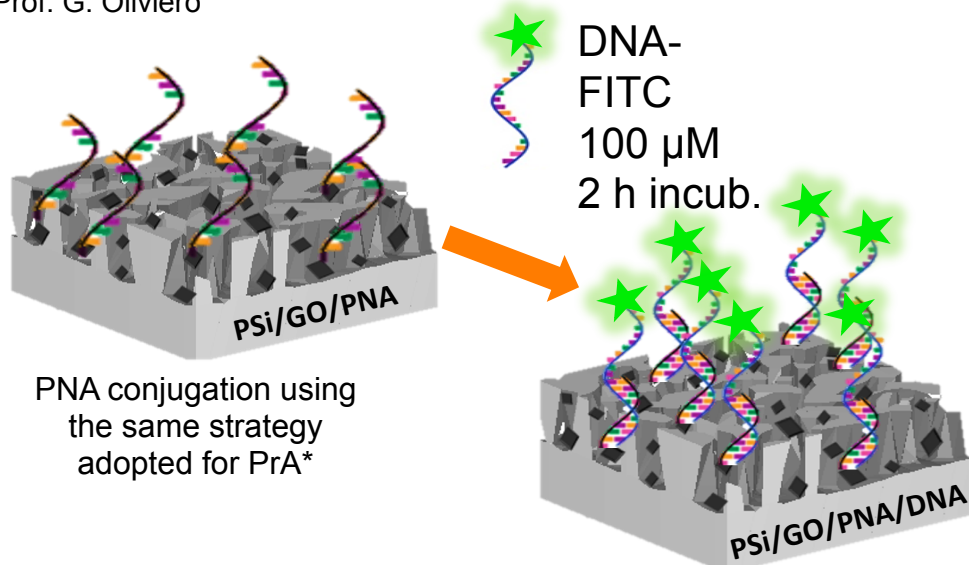
FFT analysis



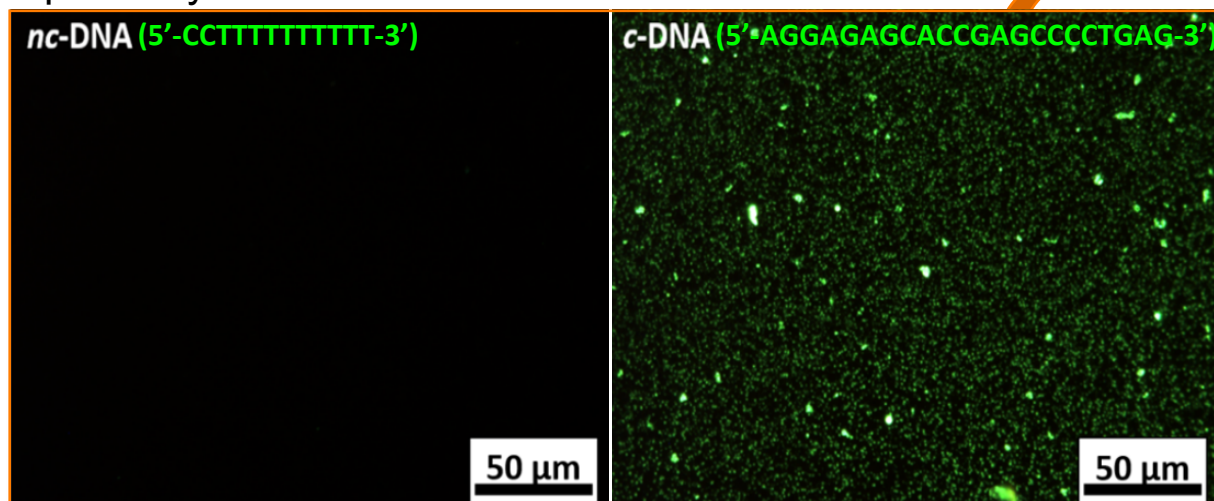
Preliminary PNA/DNA hybridization detection



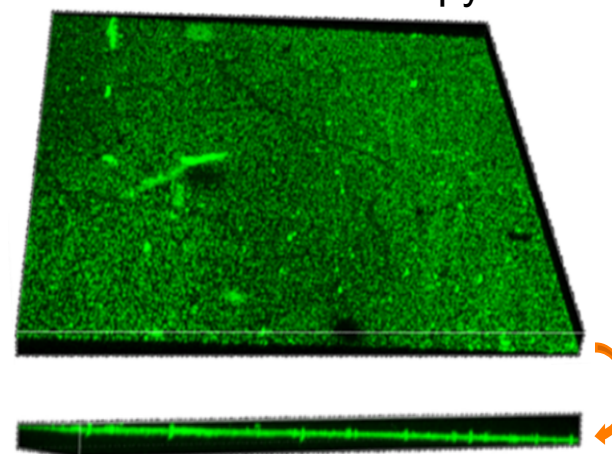
Dept. of Pharmacy
Prof. G. Oliviero



Specificity test

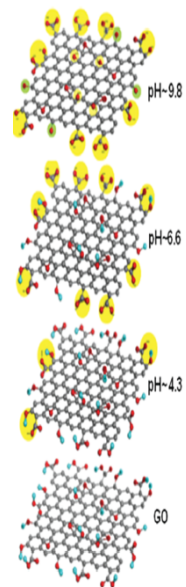
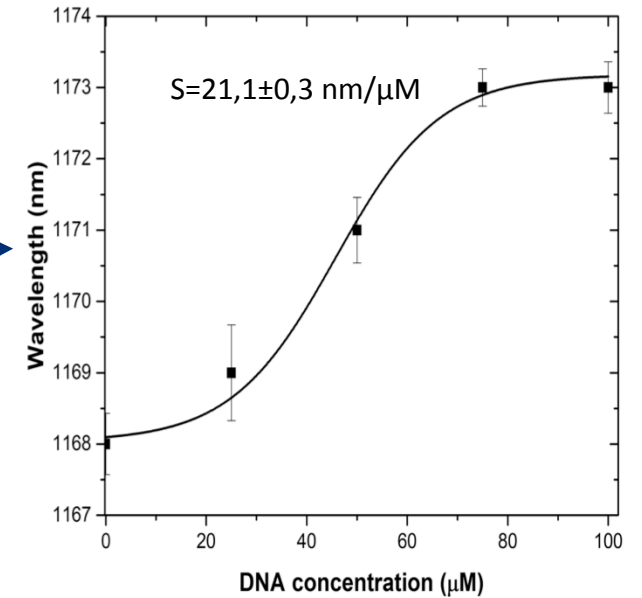
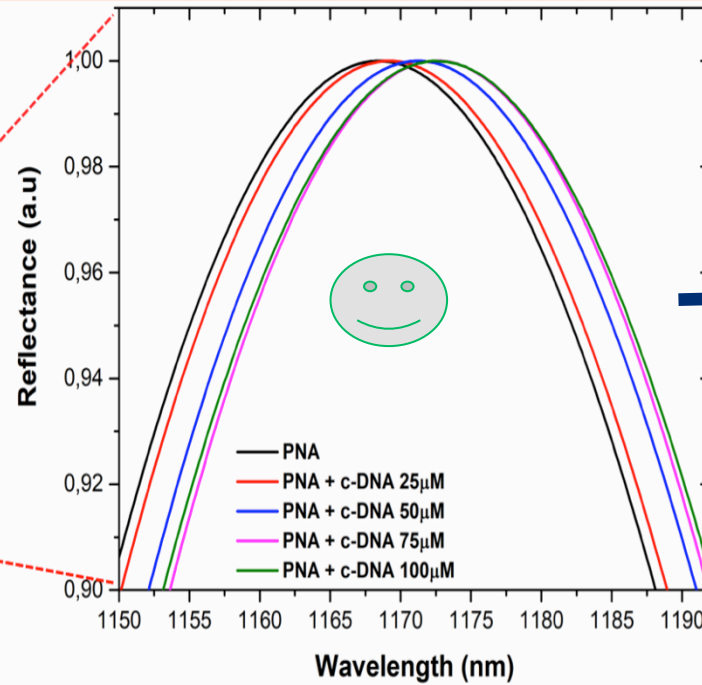
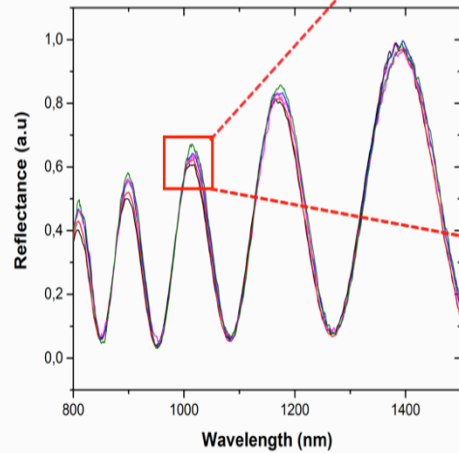


Confocal
microscopy

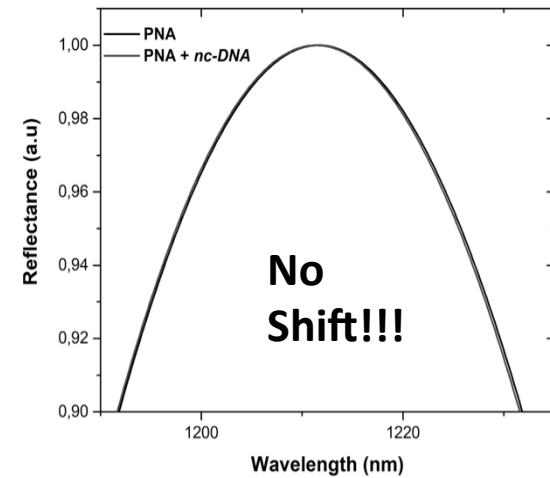


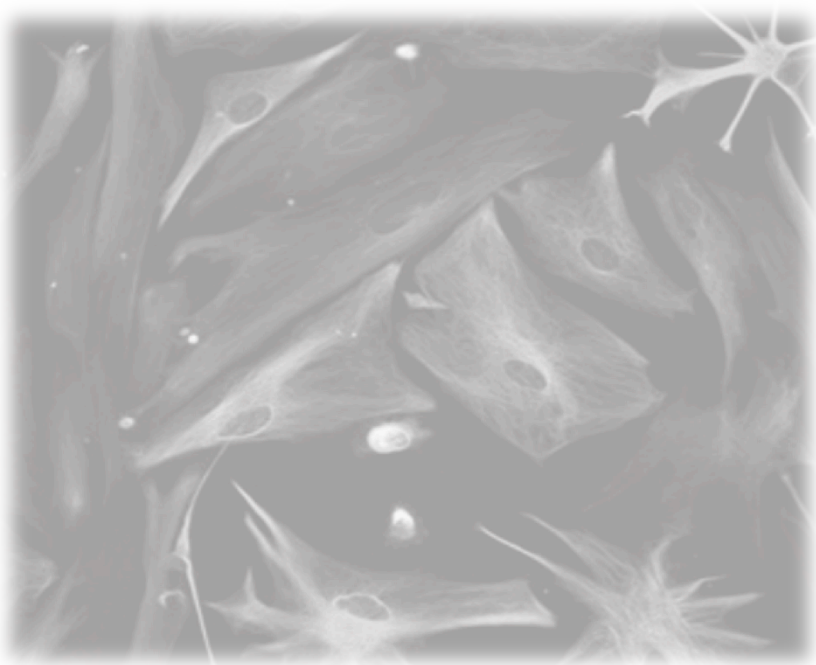
submitted to Nanomaterials

PNA/DNA hybridization detection at pH 5



Negative control





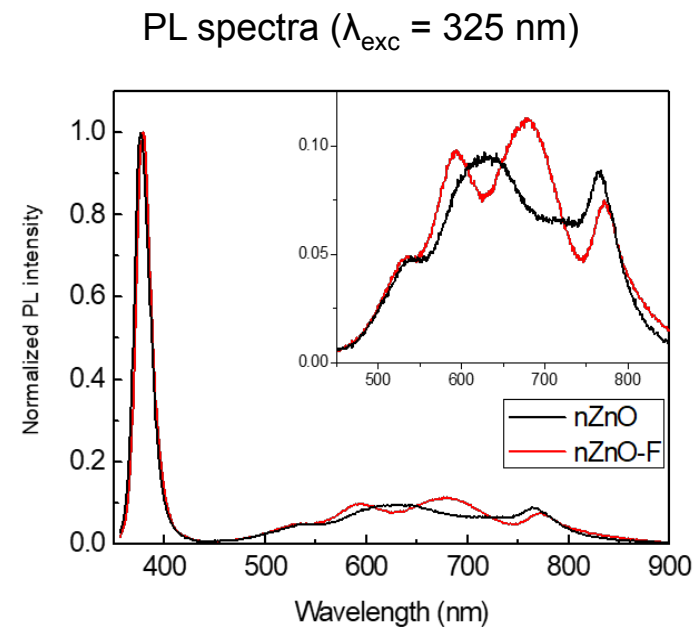
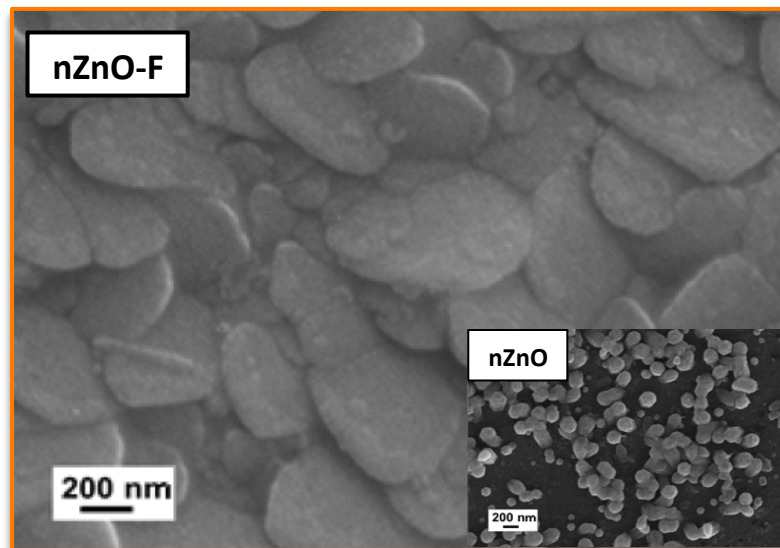
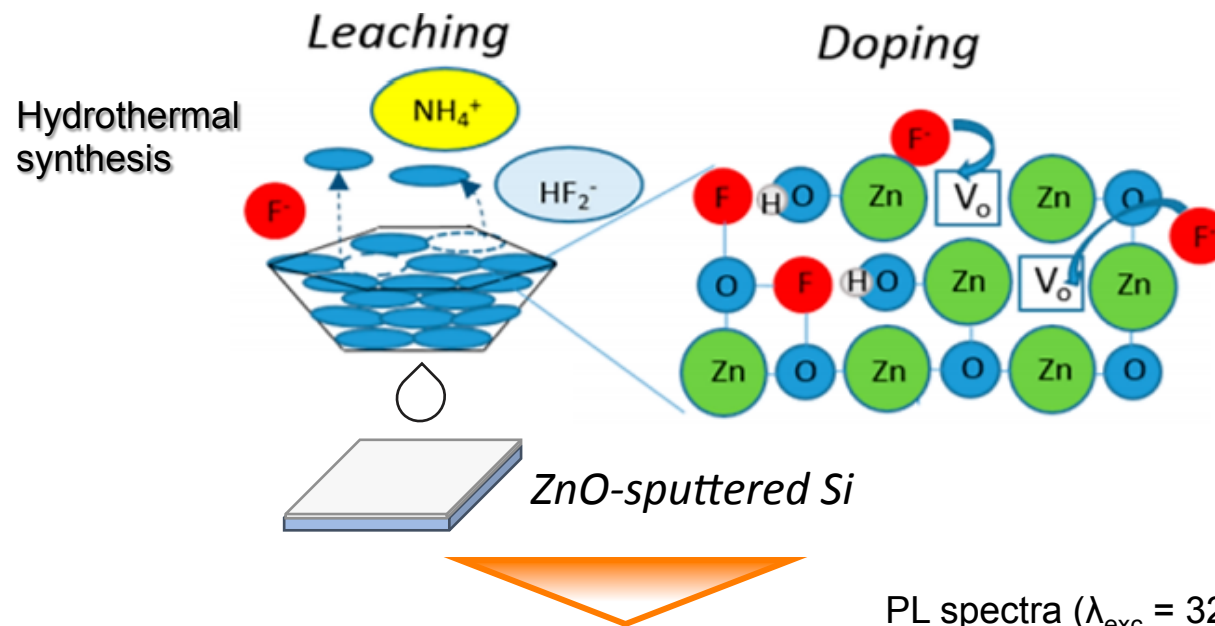
Bioimaging



Biosensing

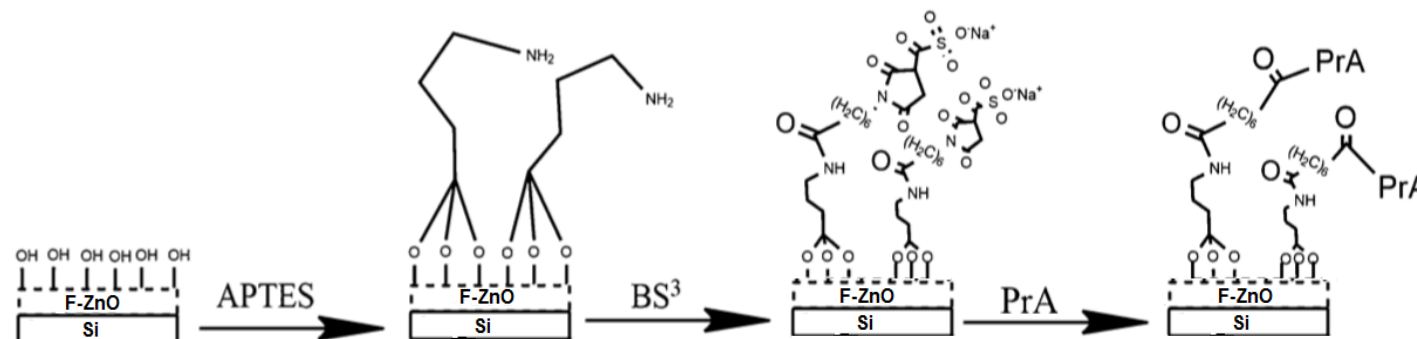
Towards label-free biosensing with fluorine-doped zinc oxide nanostructures

ZnO nanostructures synthesis and characterization

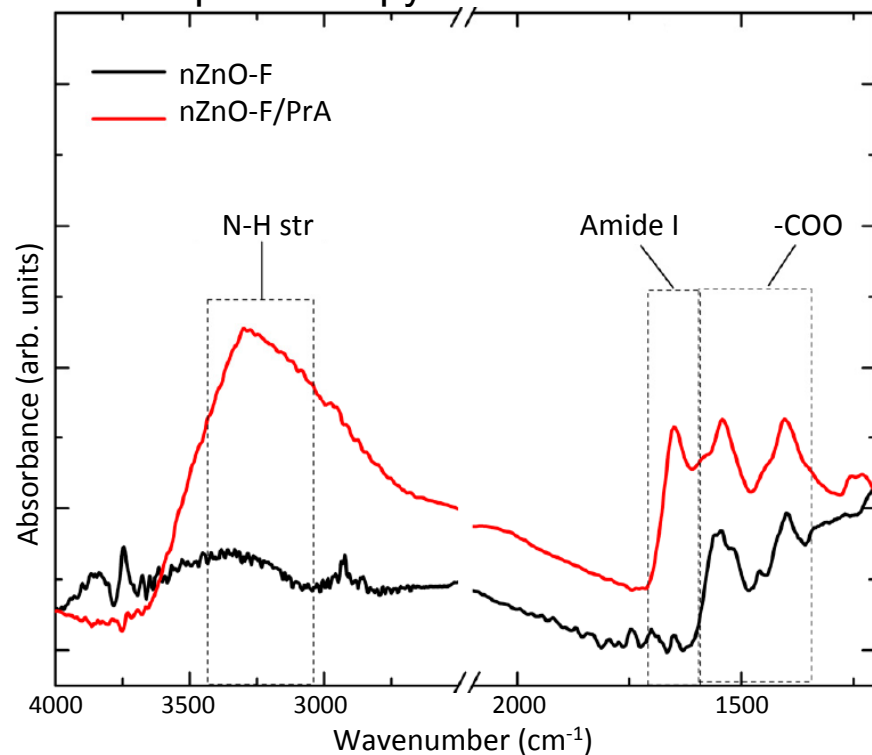


nZnO(-F) surface functionalization and bioprobe anchoring

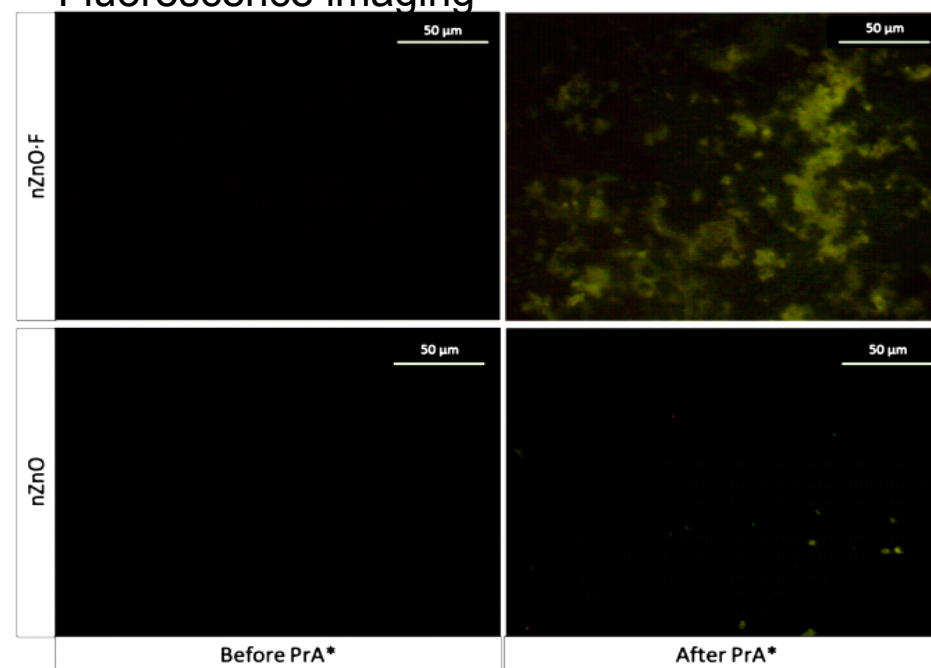
Covalent conjugation of PrA-FITC



FT-IR spectroscopy

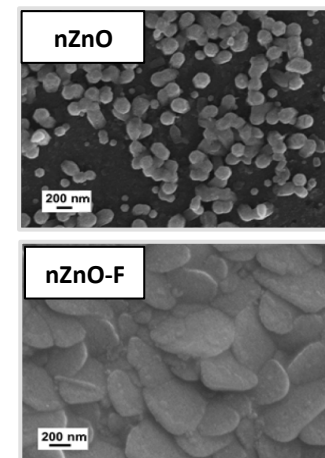
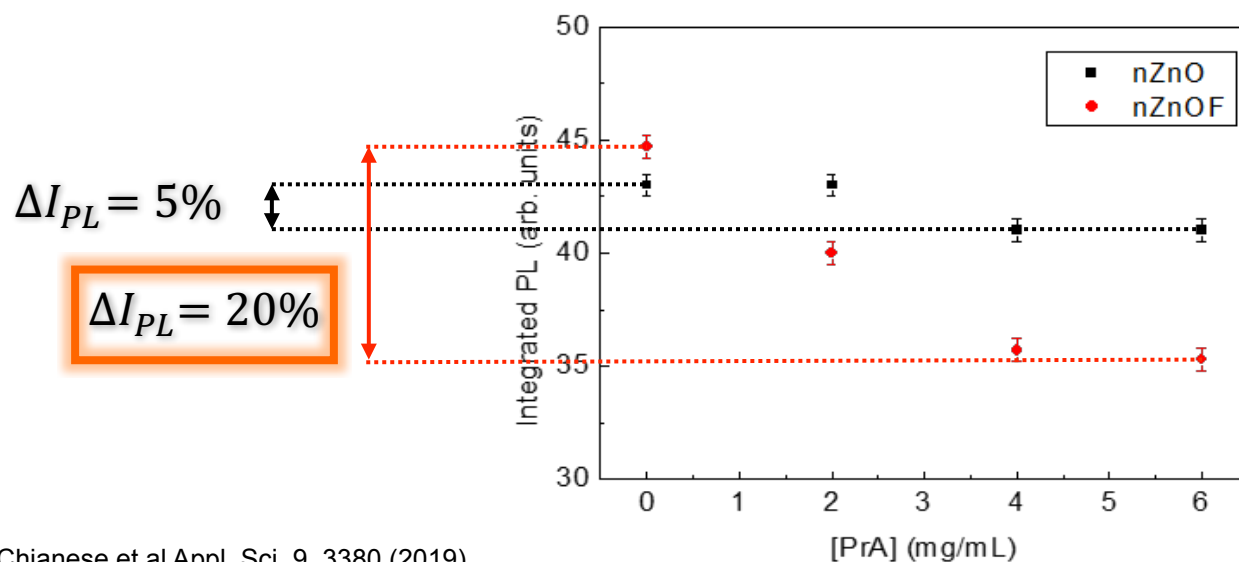
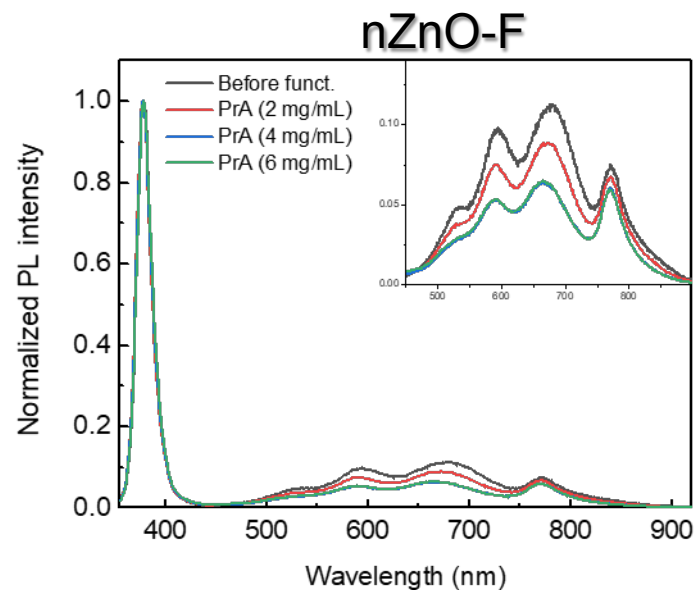
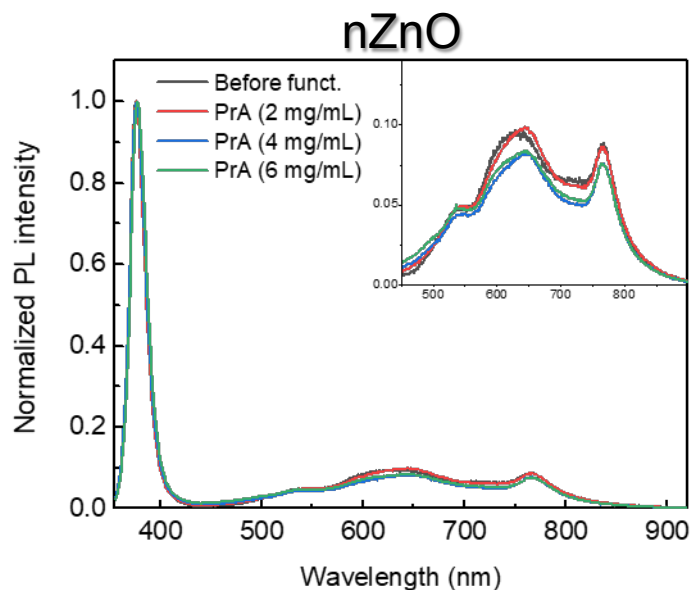


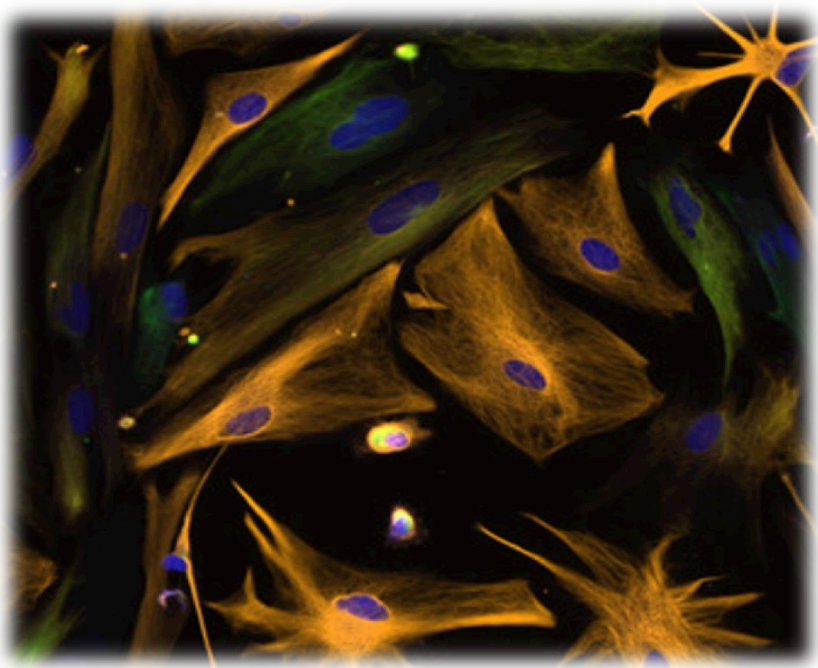
Fluorescence imaging



Towards label-free biosensing with ZnO-F nanostructures

Conjugation of PrA at different concentrations: $\Delta I_{PL}(\%) = \left| \frac{I_{PL}(6 \text{ mg/mL}) - I_{PL}(0)}{I_{PL}(0)} \right| \times 100$





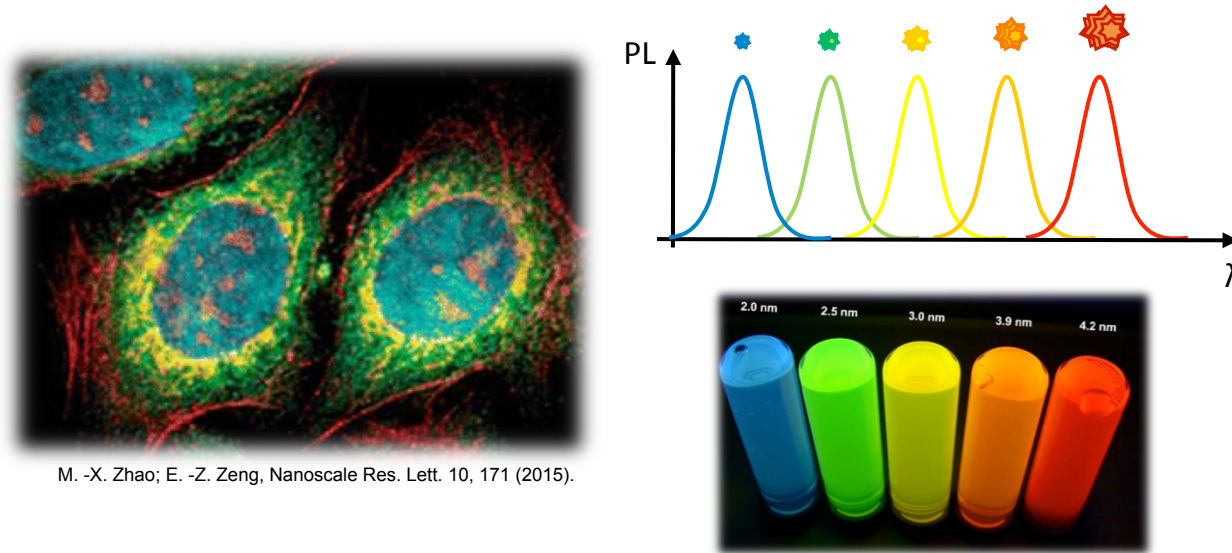
Bioimaging



Biosensing

Luminescent porous silicon nanoparticles for *in vivo* imaging

Luminescent probes for bioimaging based on semiconductor nanocrystals

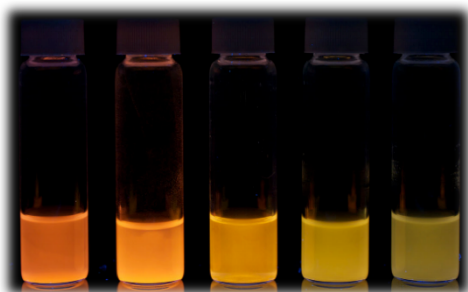
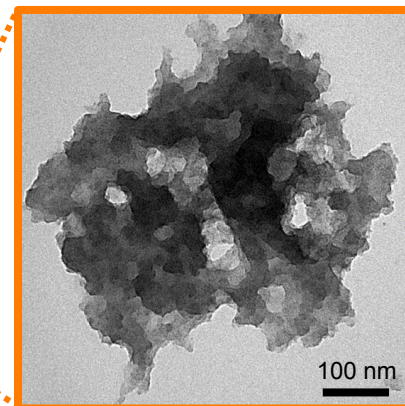


- ✓ Tunability of absorption and emission wavelengths
- ✓ Excellent resistance to photobleaching
- ✗ High toxicity of commonly employed semiconductors

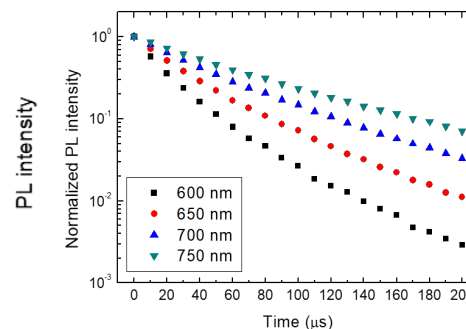
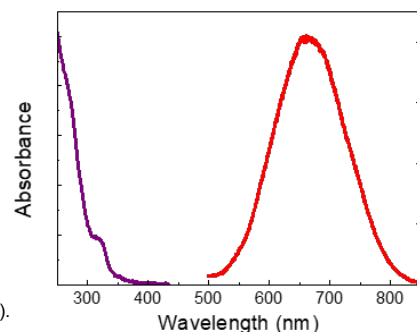
Luminescent porous silicon nanoparticles for bioimaging

Electrochemical etching of p-type Si:

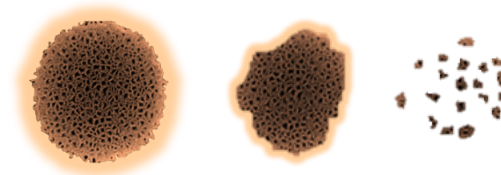
- Mechanical size reduction and sonication in isopropanol
- Storage in isopropanol



A. Mughal et al., Phys. Chem. Chem. Phys. 16, 25273 (2014).

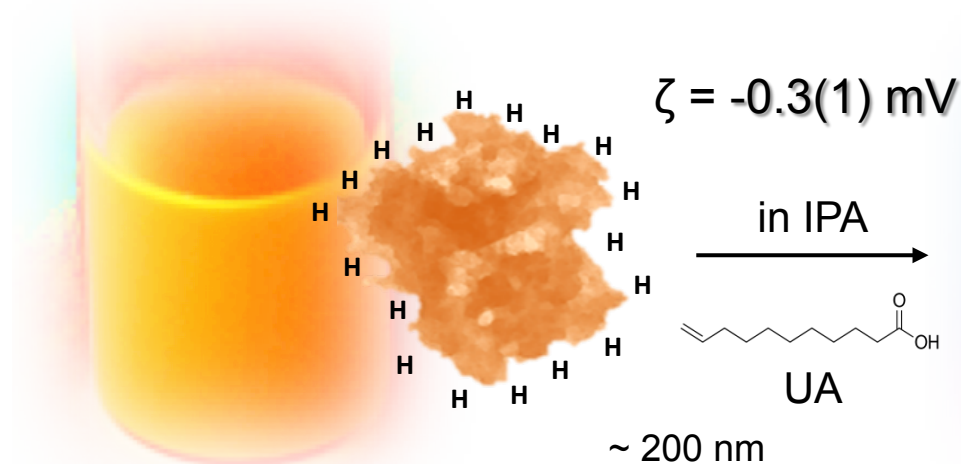
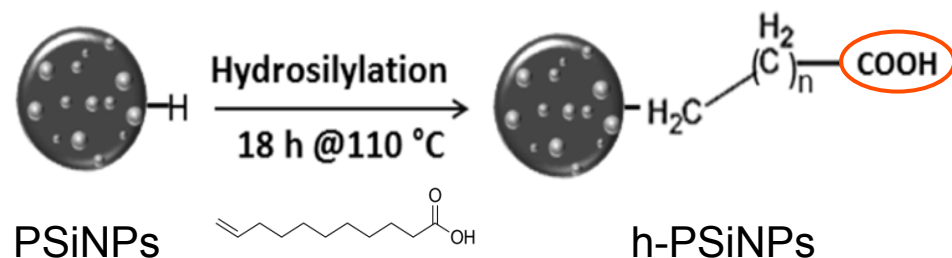


PSi degrades into harmless $\text{Si}(\text{OH})_4$ in physiological environment!!!



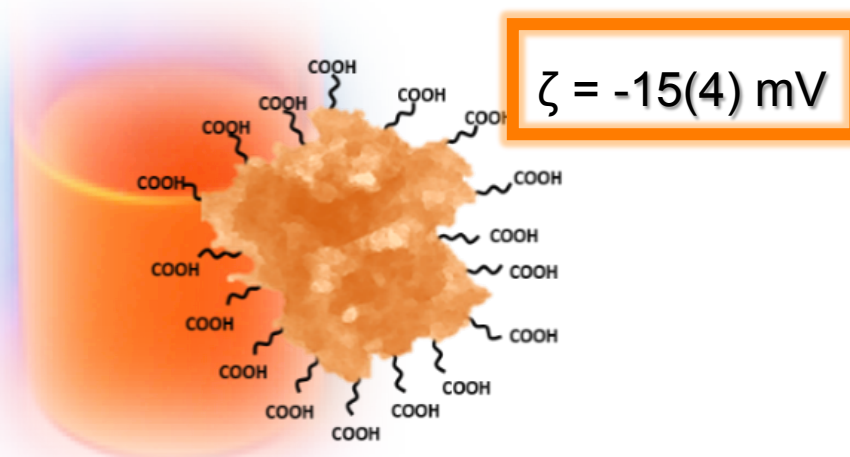
Modification of PSi nanoparticles for bioimaging

Thermal hydrosilylation with undecylenic acid (UA):



Electrochemical etching of p-type Si (H-terminal NPs)

PSiNPs

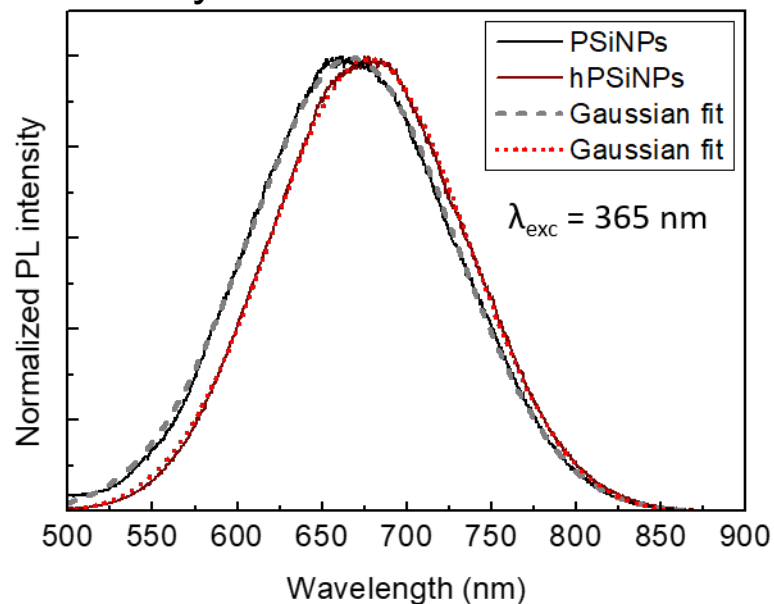


Thermal hydrosilylation with undecylenic acid (COOH-terminal NPs)

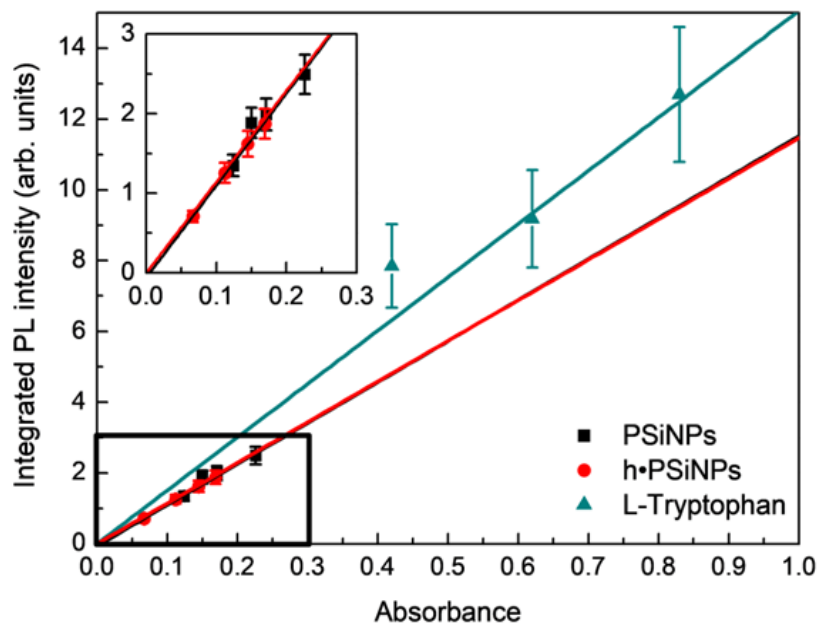
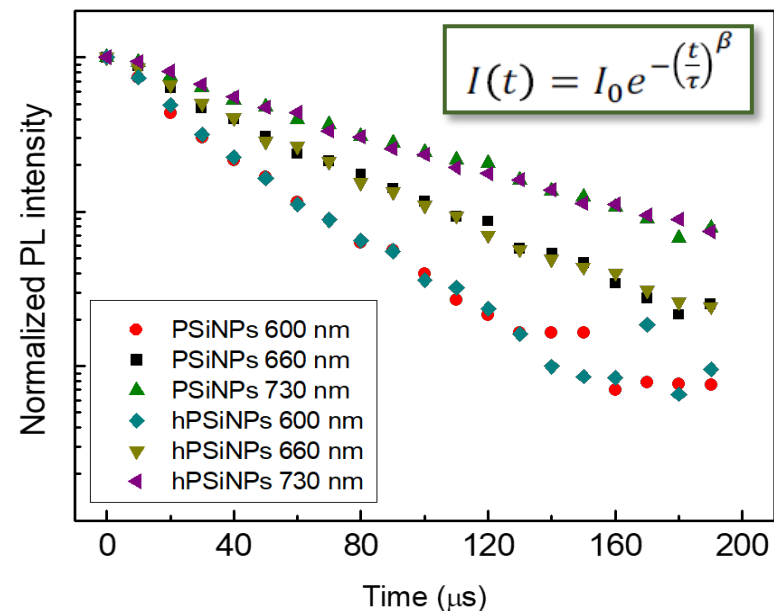
h-PSiNPs

(h-)PSiNPs optical characterizations in IPA medium

Steady-state PL



Time-resolved PL



Material	PL lifetime @ 650 nm	Stretching factor β
PSiNPs	$31.8 \pm 0.6 \mu\text{s}$	0.86 ± 0.01
h-PSiNPs	$31.5 \pm 0.3 \mu\text{s}$	0.90 ± 0.02

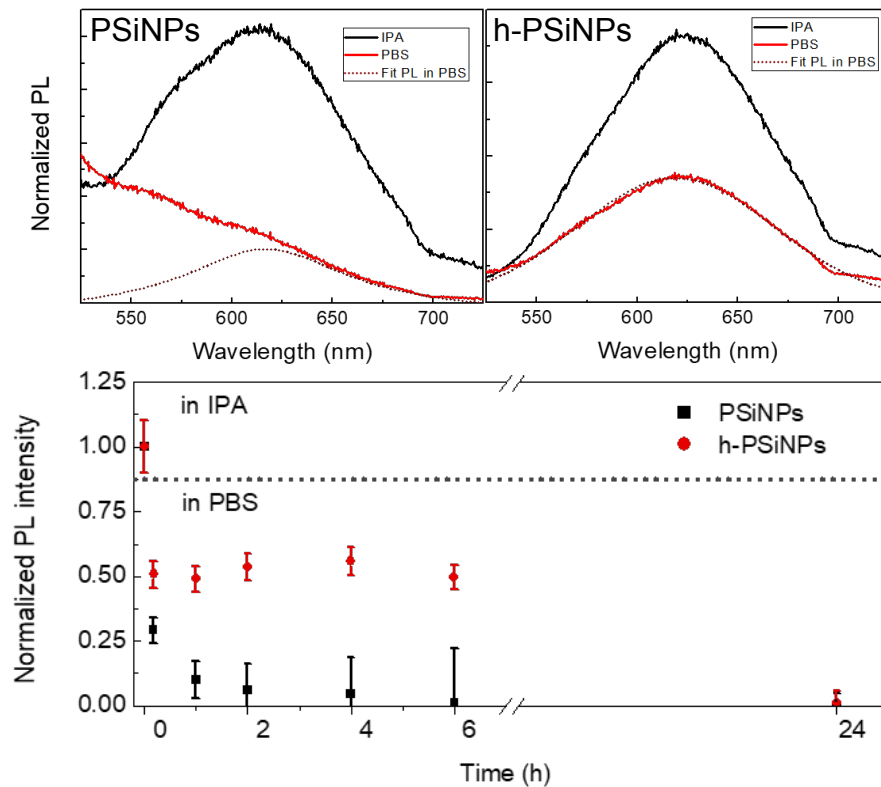
PL quantum yield

$$\left. \frac{QY_{NPs}}{n_{IPA}^2 \alpha_{NPs}} \right|_{\lambda_{ex}} = \left. \frac{QY_{L-T}}{n_{H_2O}^2 \alpha_{L-T}} \right|_{\lambda_{ex}} \left\{ \begin{array}{l} QY_{PSiNPs} = 12(5)\% \\ QY_{h-PSiNPs} = 13(5)\% \end{array} \right.$$

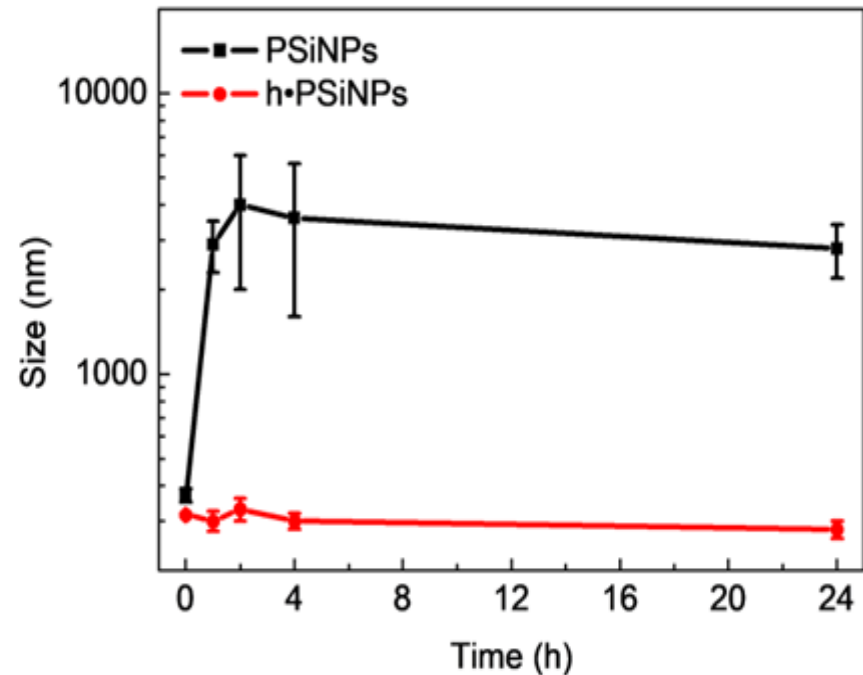
Optical and morphological stability in physiological medium

(h-)PSiNPs behavior in PBS (pH = 7.4) over time

Integrated PL

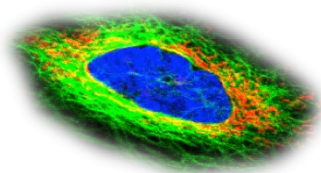


Hydrodynamic diameter

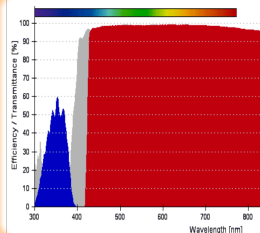
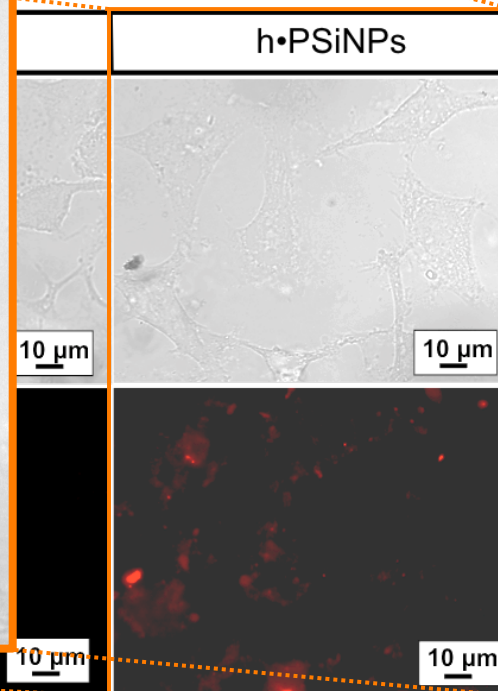
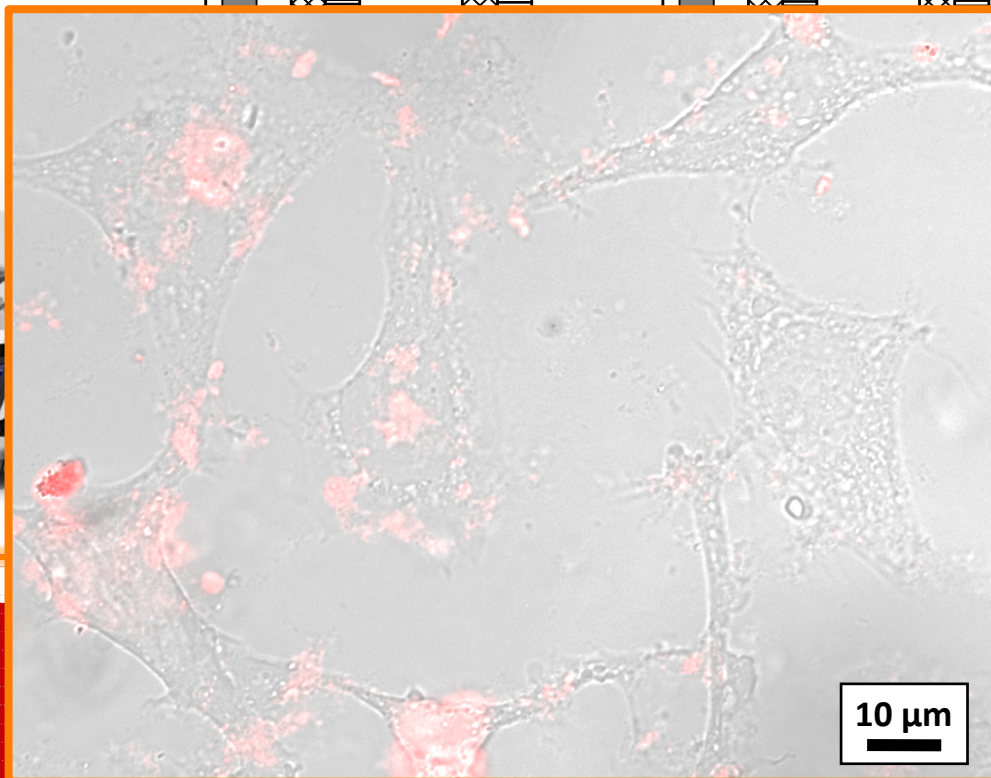
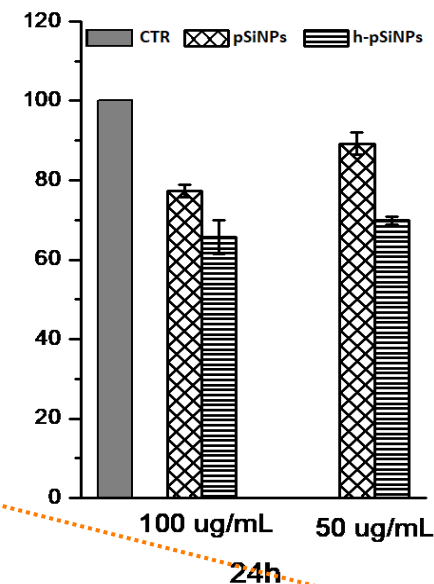
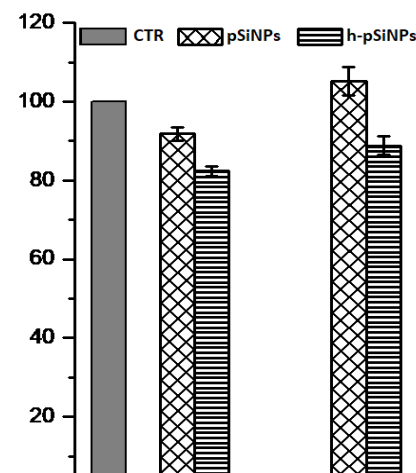
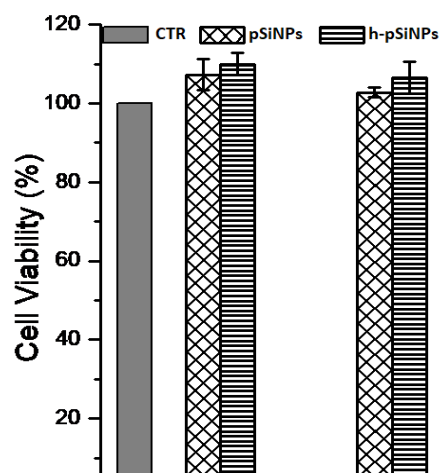


Next step: *in vitro* study

In vitro study: viability and fluorescence imaging of HeLa cells

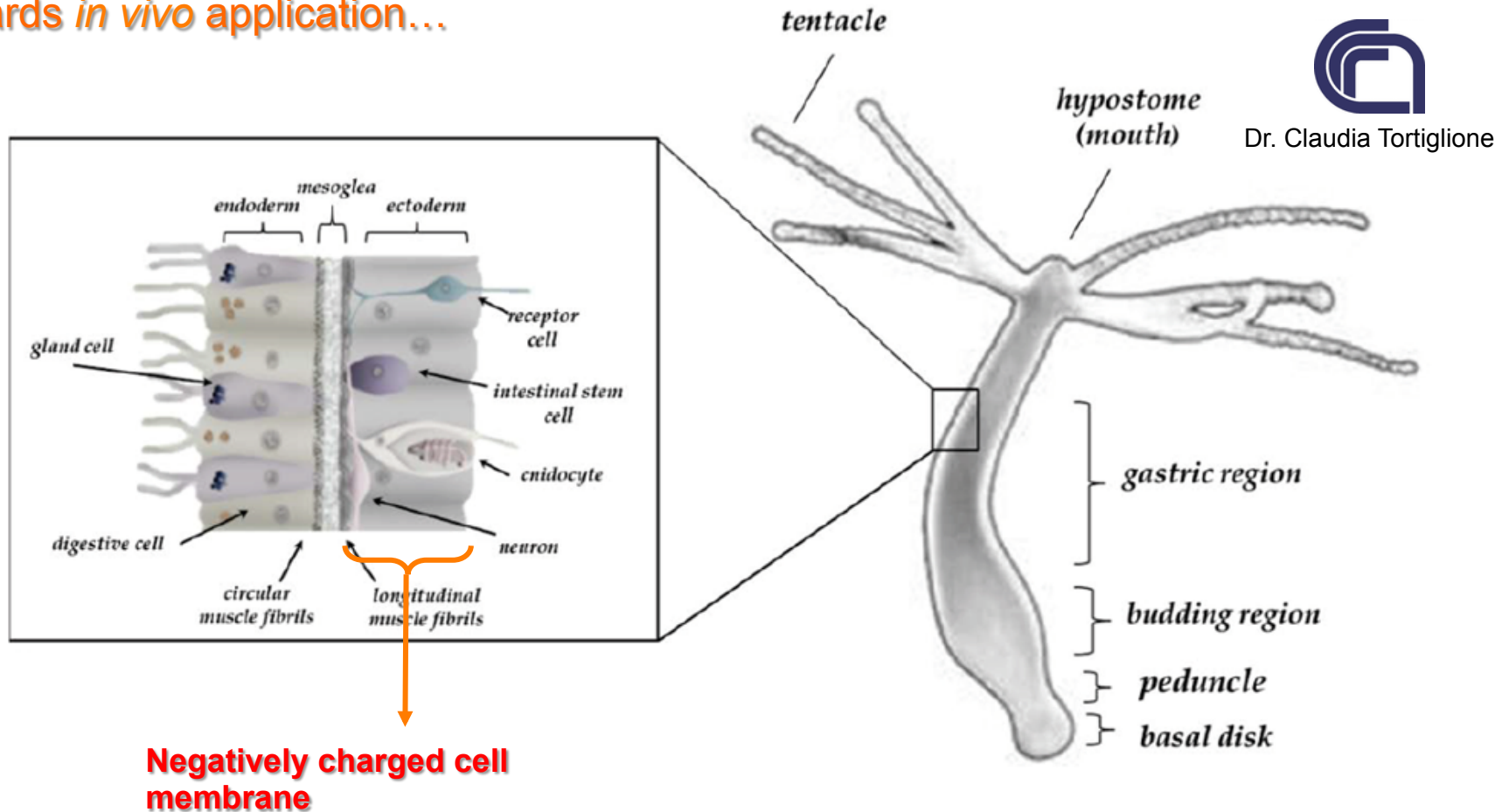


MTT assay
(insufficient)



Hydra vulgaris as *in vivo* model

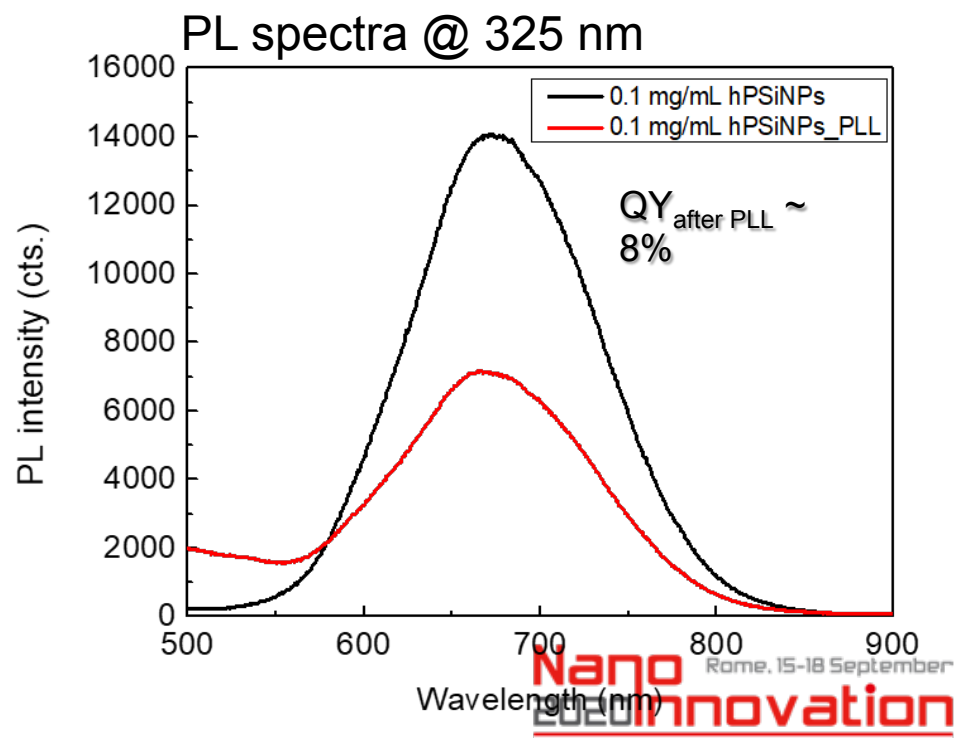
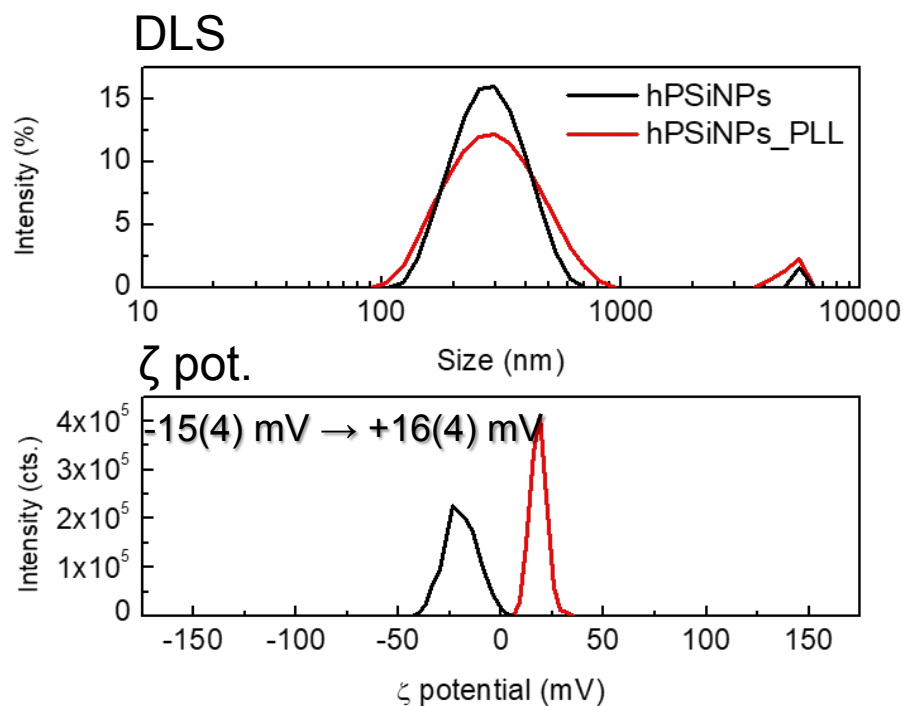
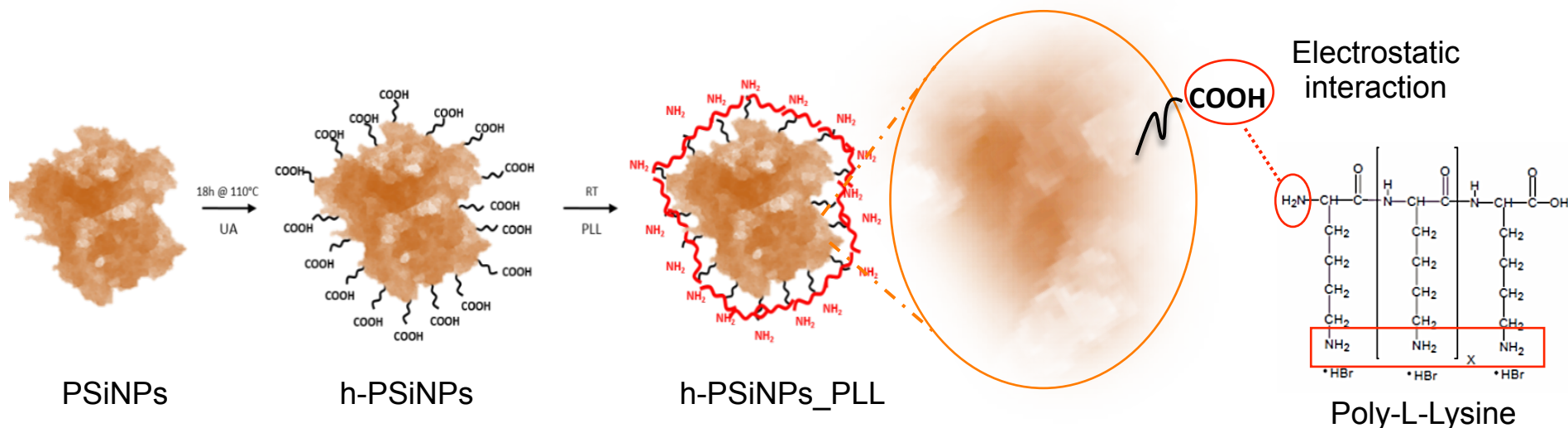
Towards *in vivo* application...



Dr. Claudia Tortiglione

Simpler than vertebrates with central nervous system and specialized organs but more complex than cultured cells

Poly-L-lysine conjugation to surface-modified PSiNPs



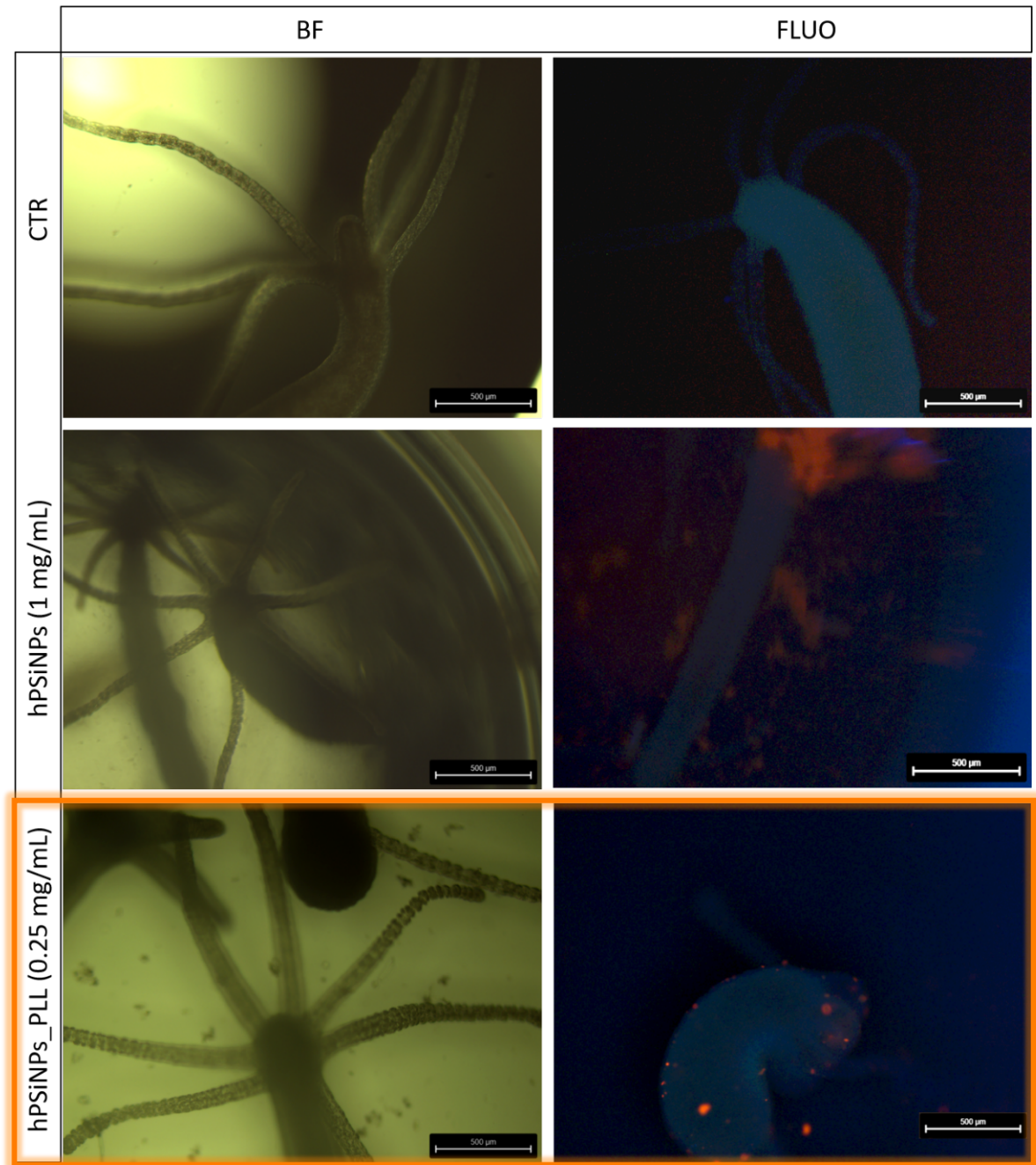
Fluorescence imaging of luminescent PSiNPs in *Hydra*



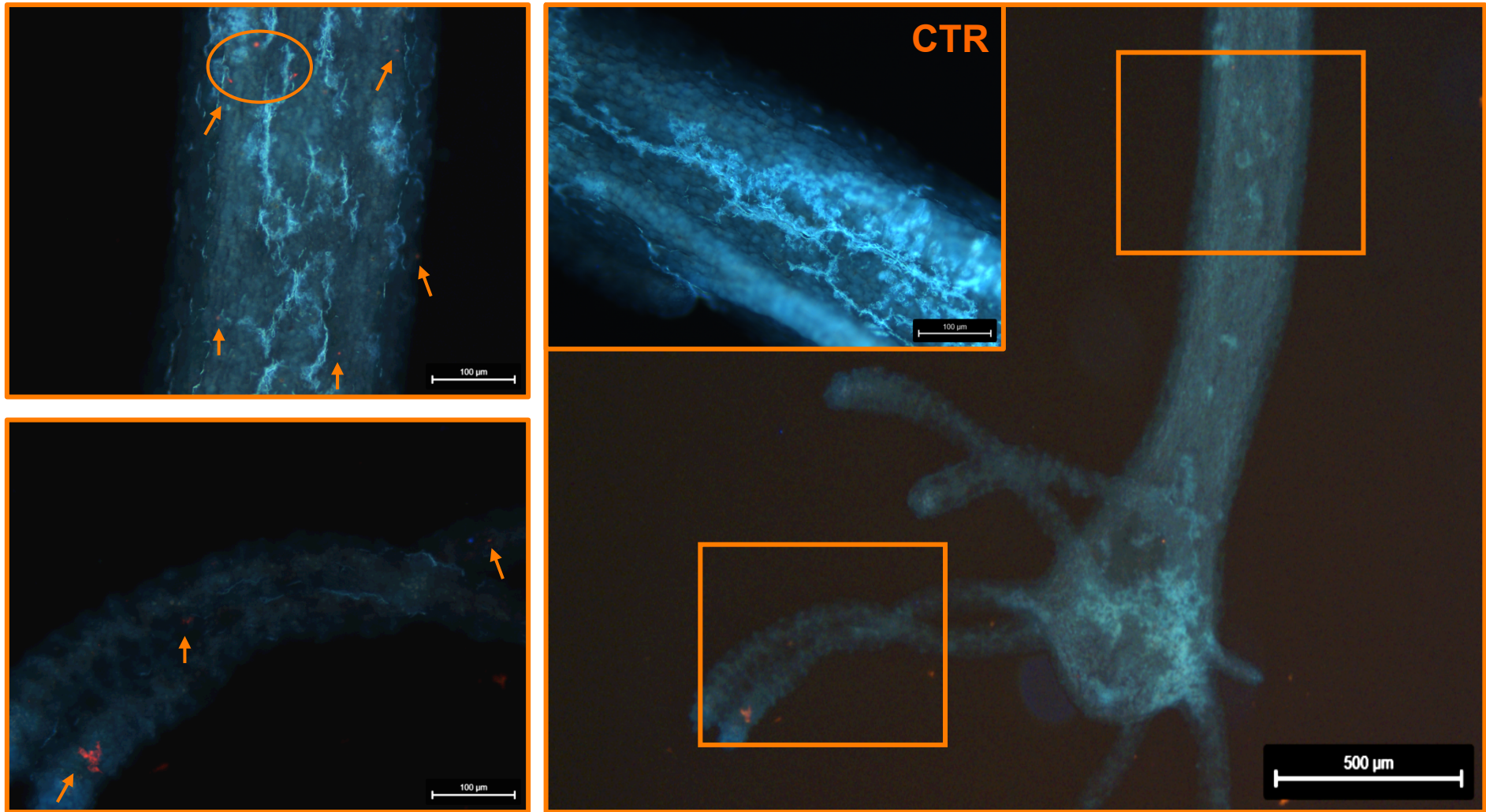
(365 nm excitation)

✗ ζ potential < 0

✓ ζ potential > 0



Fluorescence imaging of luminescent PSiNPs in fixed *Hydra*



Endogenous emission represents a **crucial obstacle** when carrying out fluorescence imaging studies on more complex tissues/organisms



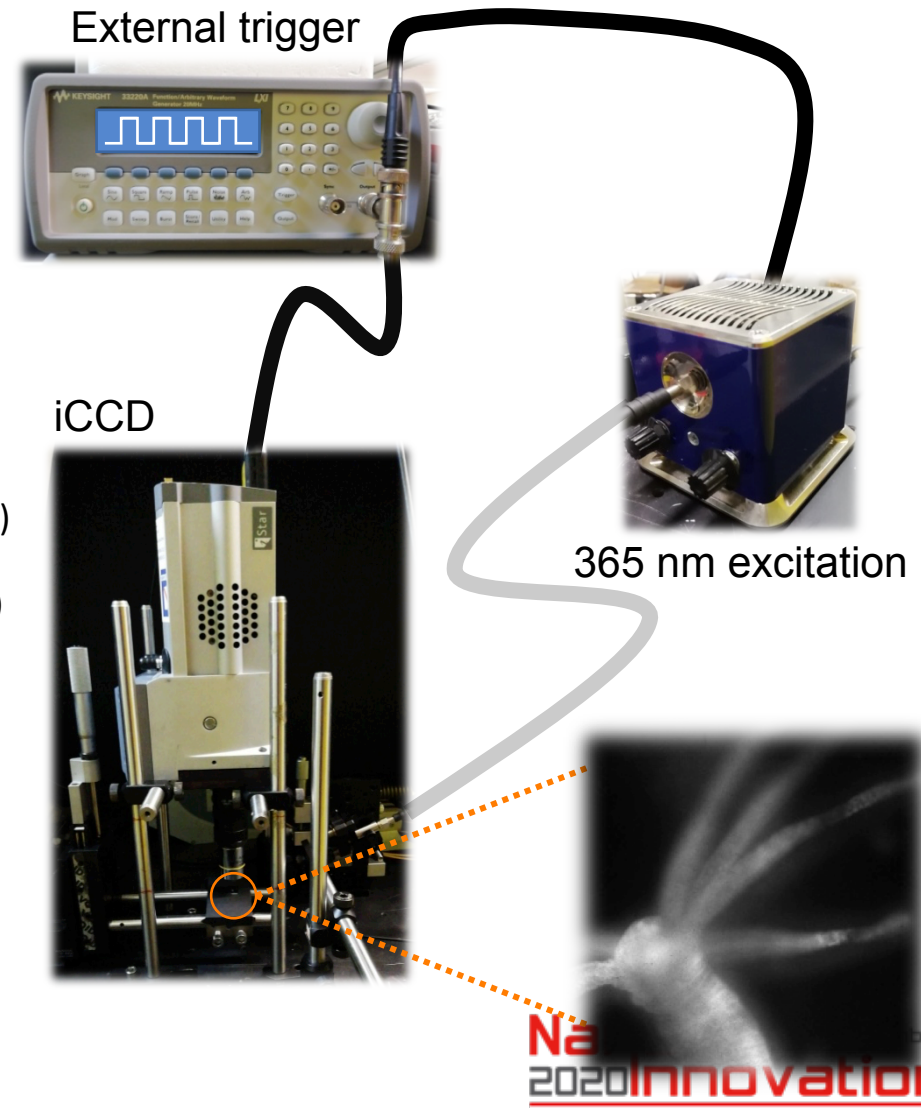
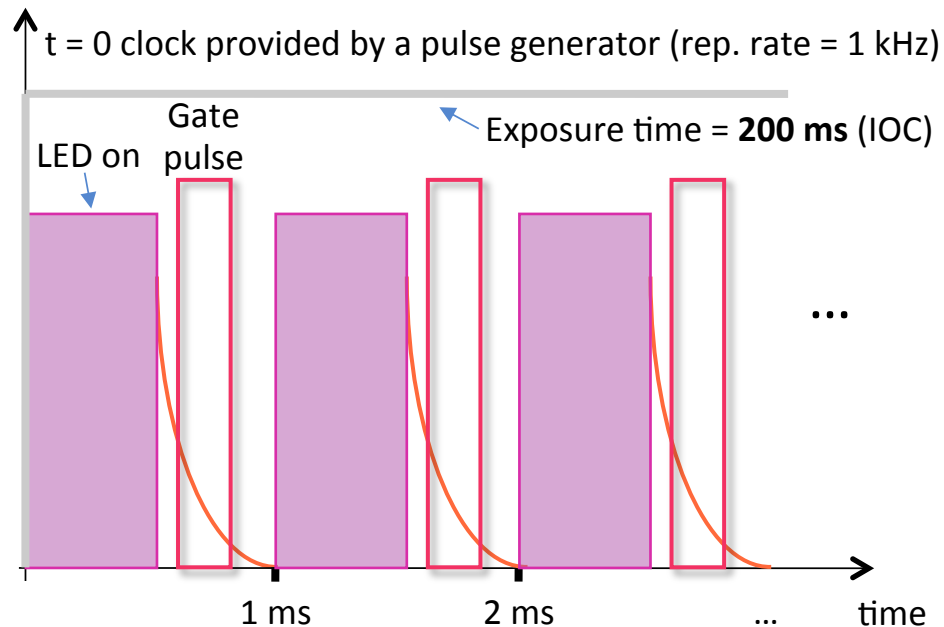
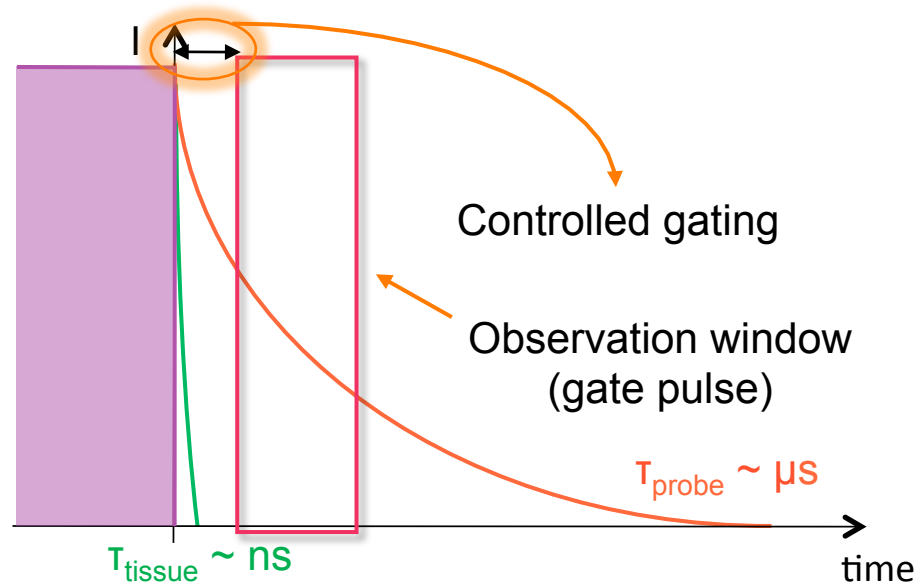
Time-gated luminescence imaging

Time-gated fluorescence imaging technique

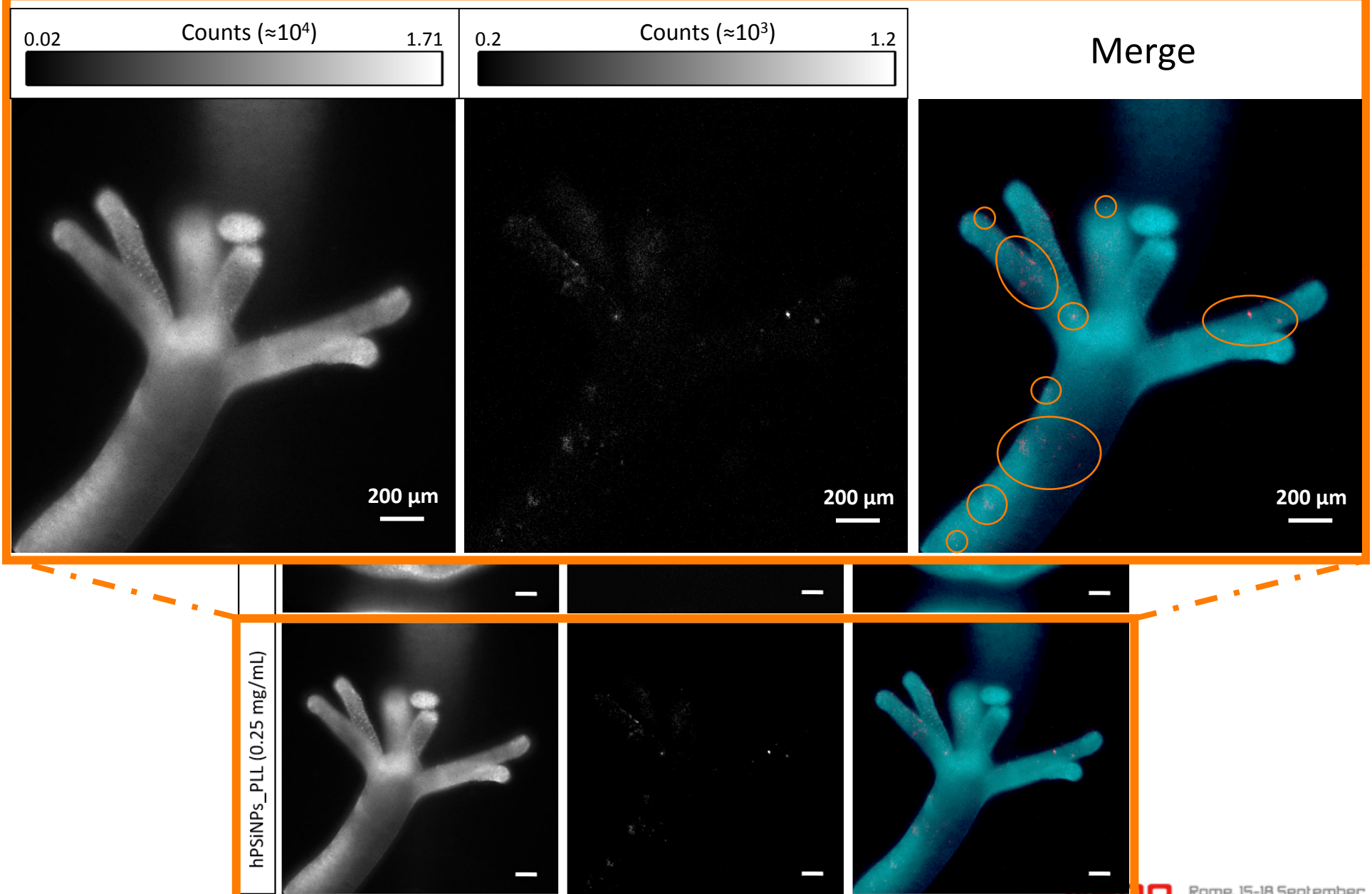


Prof. M. J. Sailor

Design and setting up



In vivo time-gated fluorescence imaging of *Hydra vulgaris*



Conclusions

Luminescent porous silicon nanoparticles as label-free probes for bioimaging

- ✓ The optical and stability properties of highly luminescent PSiNPs have been investigated and characterized before and after surface functionalization
- ✓ Time-gated PL imaging ***in vivo*** has proved PSiNPs uptake in *Hydra vulgaris* organism, overcoming the issue of endogenous emission

Graphene-oxide/porous silicon device for Brugada Syndrome diagnosis

- ✓ A robust hybrid chip that combines the photonic properties of a macroporous PSi monolayer and the characteristic PL emission from GO has been developed
- ✓ The PSi/GO nanocomposite has been tested for the detection of the SCN5A gene point-mutation associated to Brugada Syndrome

Towards label-free biosensing with fluorine-doped ZnO nanostructures

- ✓ An analysis of the optical behavior of ZnO and fluorine-doped ZnO nanostructures has been carried out
- ✓ The higher specific surface area of F-doped ZnO resulted in an enhancement of the response of the device with respect to the undoped case after bioprobe conjugation

Conclusions

Luminescent porous silicon nanoparticles as label-free probes for bioimaging

- ✓ The optical and stability properties of highly luminescent PSiNPs have been investigated and characterized before and after surface functionalization
- ✓ Time-gated PL imaging *in vivo* has proved PSiNPs uptake in *Escherichia coli* organism, overcoming the issue of endogenous emission

Graphene oxide/porous silicon device for Brugada Syndrome diagnosis

- ✓ A robust hybrid chip that combines the photonic properties of a macroporous PSi monolayer and the characteristic PL emission from GO has been developed
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MAY 9, 2019
VOLUME 123
NUMBER 10
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