

# SERS Intracellular Monitoring of Galunisertib Release from Porous Diatomite Nanoparticles in Colorectal Cancer Cells.

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Institute of  
Applied Sciences  
and  
Intelligent Systems



DIPARTIMENTO di FARMACIA



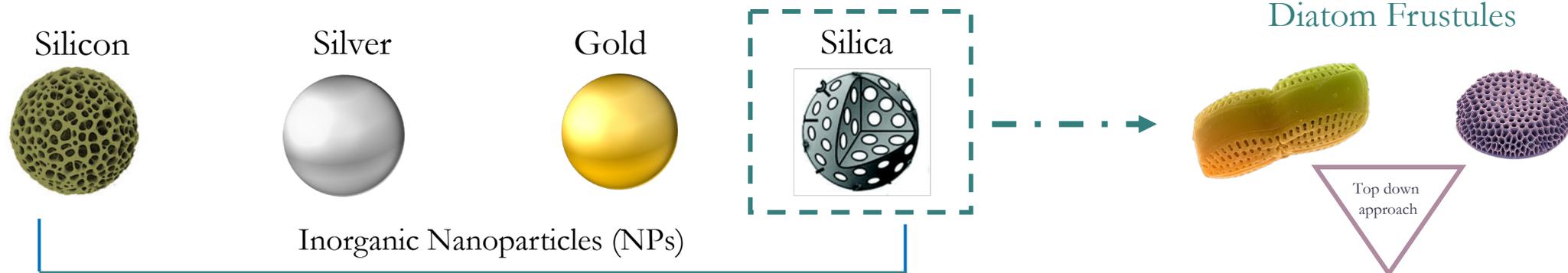
# Outline

- Introduction & Motivation : Hybrid Inorganic Nanoparticles for Colorectal Cancer
- Fabrication and Characterization of the Galunisertib Delivery System
- Monitoring of Galunisertib Release *via* SERS and HPLC Techniques
- Reversion of Metastatic Phenotype Induced by the Nanosystem in Colorectal Cancer Cell Line
- Conclusions and Future Perspectives

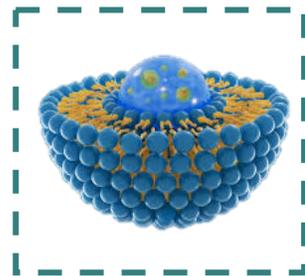
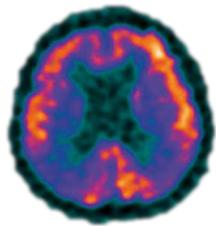
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# Inorganic Nanoparticles in Medicine



Diagnosis      Imaging      Drug delivery      Targeted Therapy



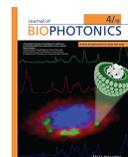
- ◇ Excellent biocompatibility of silica
- ◇ Non-toxicity
- ◇ High-Porous surface area
- ◇ Thermal stability
- ◇ Chemical inertness
- ◇ Tailorable surface chemistry
- ◇ Low-cost production of NPs



Rea, I. et al., *Adv. Healthcare Mater.*, 6



Terracciano et al., *Adv. Biosys.*, 3: 1970042

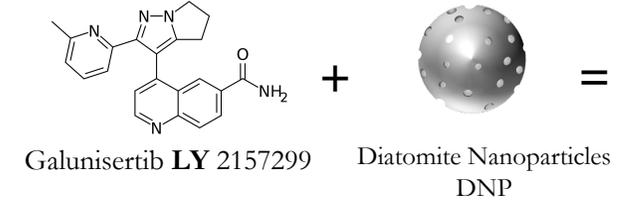
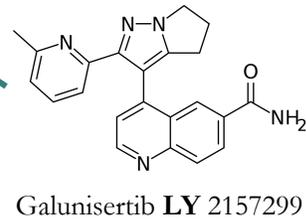
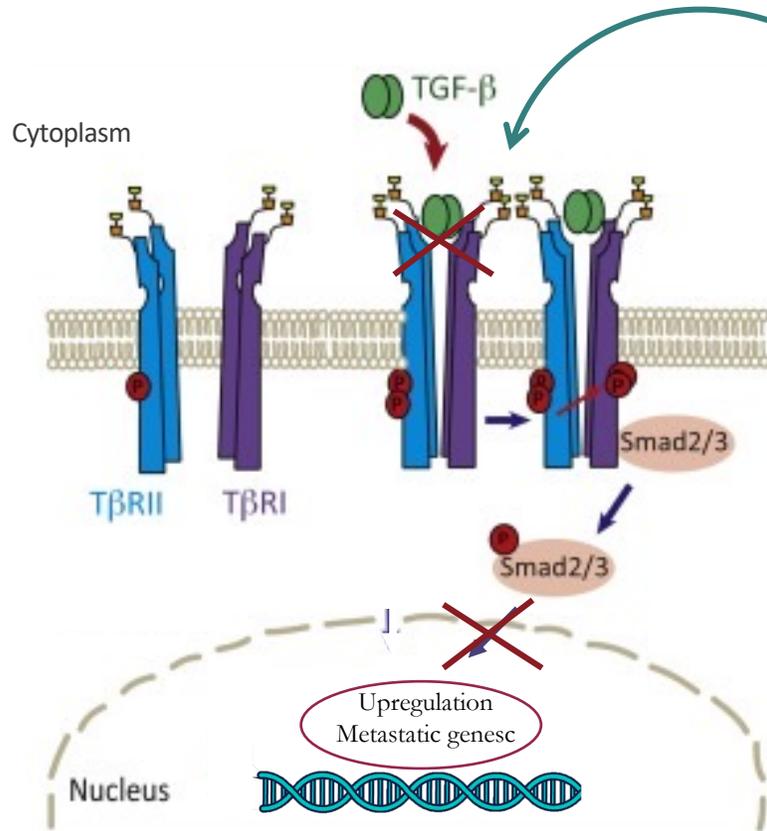


Managò, S. et al., *Biophotonics*, 11

# Diatomite Nanoparticles For Colorectal Cancer

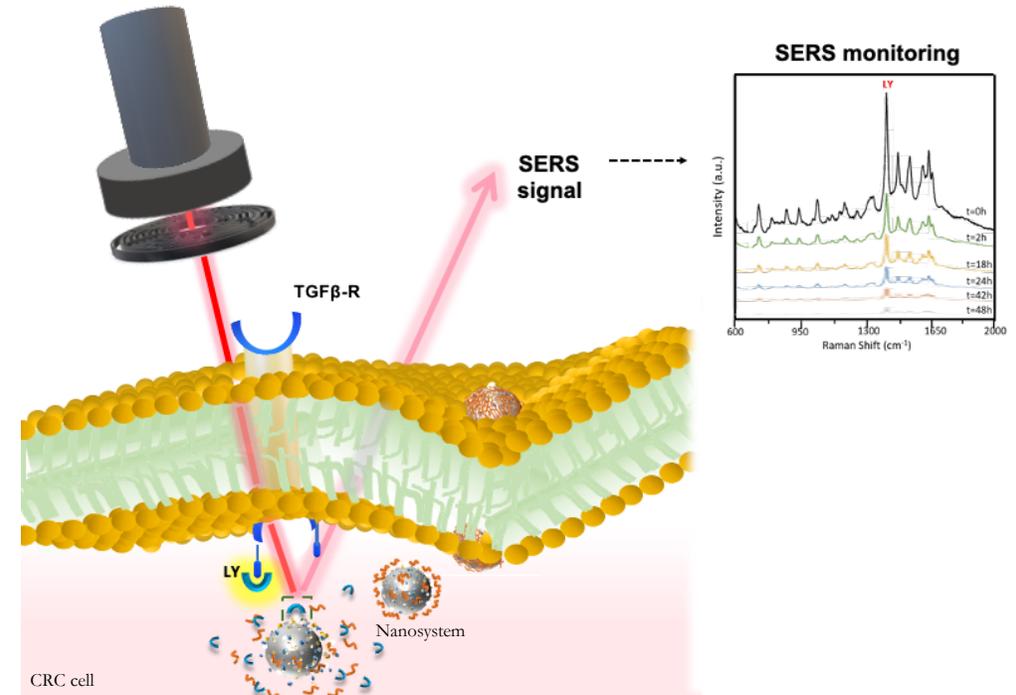
**CANCER TODAY**

Colorectal Cancer Incidence  
**10.6%**



Lowering Toxicity  
Improving Efficacy  
Targeted Therapy

Motivation



GIOBOCAN 2020 <https://gco.iarc.fr>

**CSAC 2021**

NBSY

3

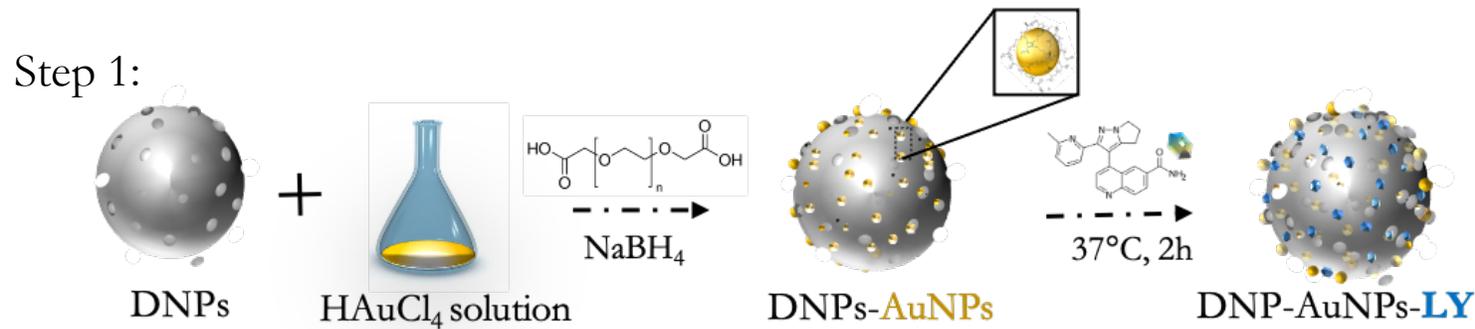
S. Managò et al. Manuscript Peer Reviewed by Small, 2021

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- ☞ Conclusions and Future Perspectives

# Nanosystem Fabrication and Characterization

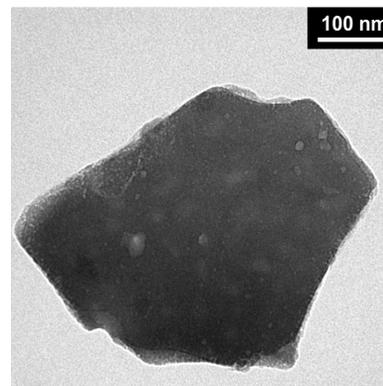
First step of functionalization



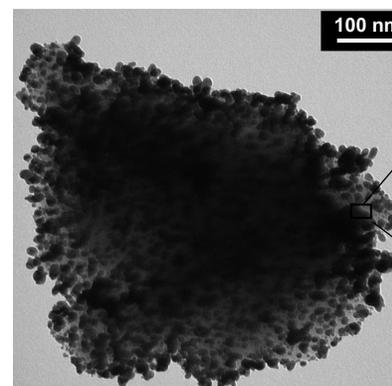
## Nanosystem Characterization

### TEM Characterization

	Size (nm) (DS)	Z-Potential (mV) (DS)
DNPs 	400 (50)	+20 (5)
DNP-AuNPs 	400 (50)	-15 (10)

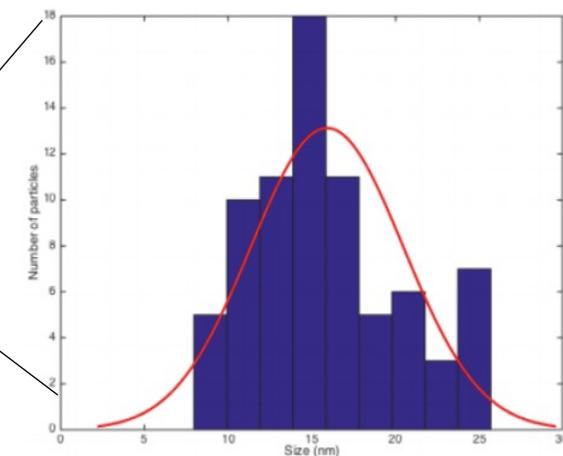


DNP



DNP-AuNPs

### Size Distribution Analysis



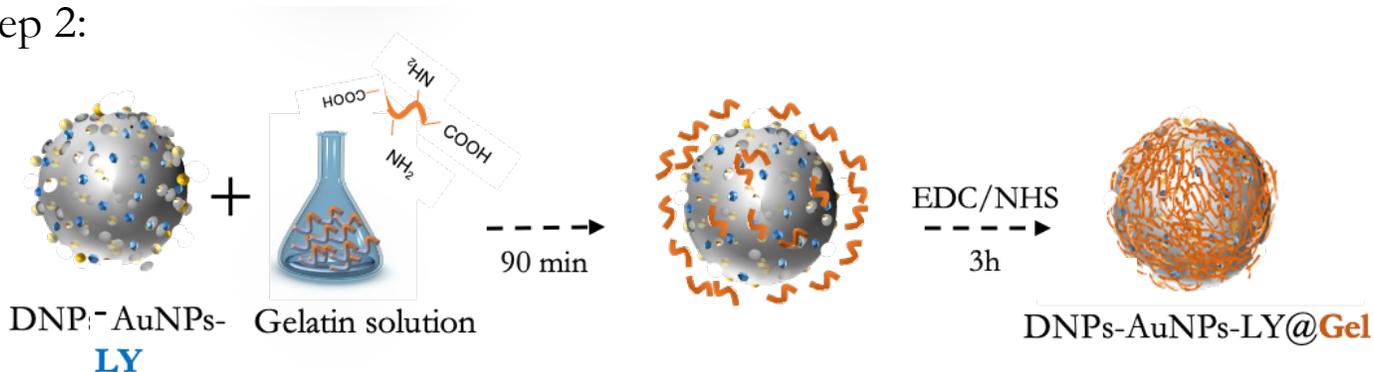
AuNPs Size 16 (5) nm

The mean radius of the AuNPs grown on the DNP was 16(5) nm. The overall complex had a size of 400 (50) nm and a negative surface charge, due to the pegylated AuNPs

# Fabrication and Characterization of the Nanosystem

Second step of functionalization

Step 2:

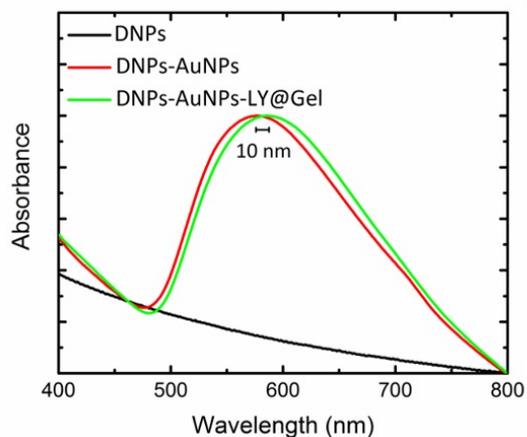


Nanosystem Characterization

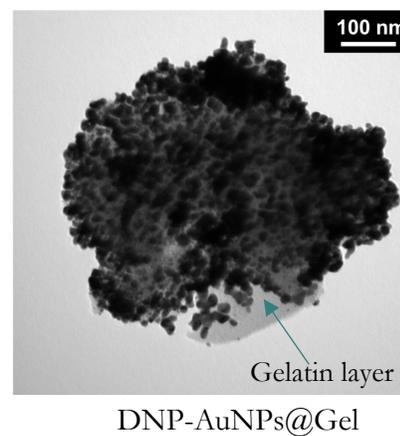
Dynamic Light Scattering (DLS)  
Characterization

	Size (nm) (DS)	Z-Potential (mV) (DS)
DNP-AuNPs	400 (50)	-15 (10)
DNP-AuNPs-LY@Gel	450 (50)	-7 (8)

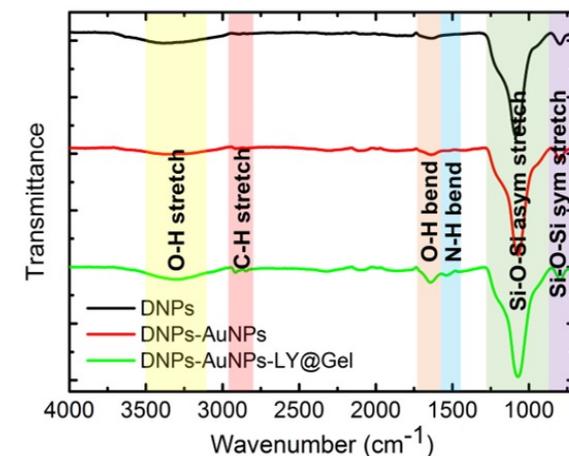
Optical Characterization



TEM Image



FTIR analysis

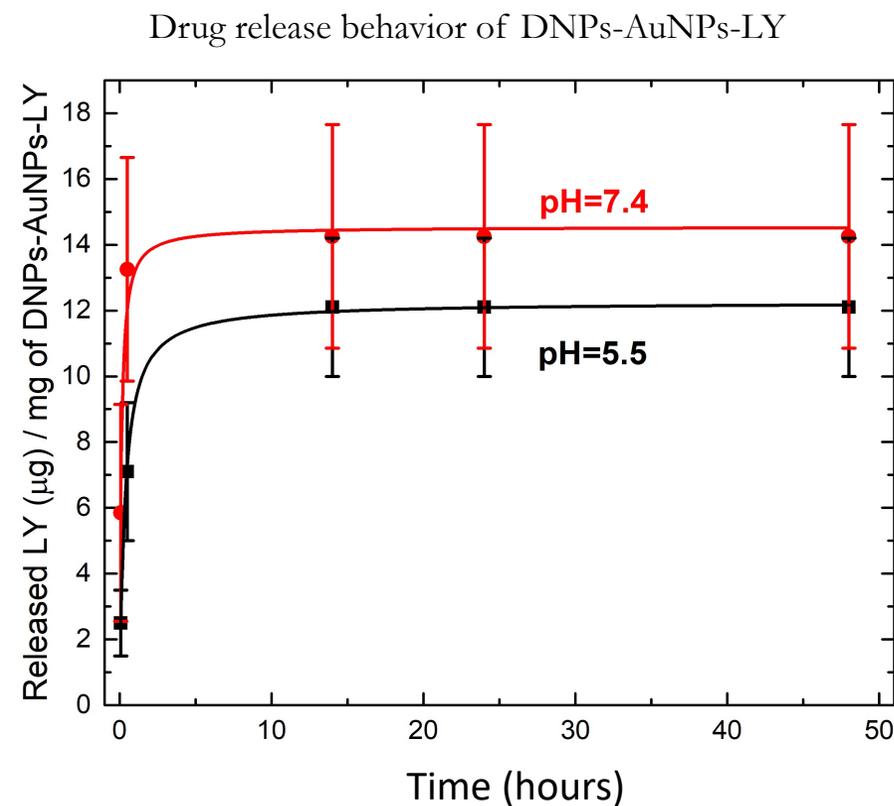
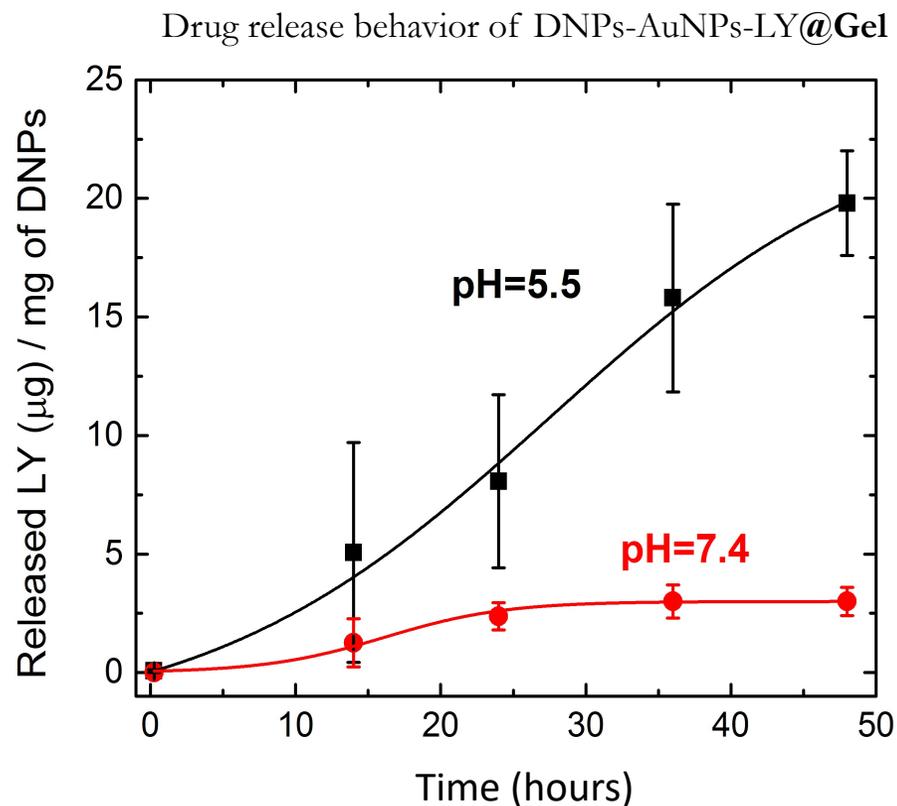


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# *In vitro* Drug Release Studies by HPLC Technique

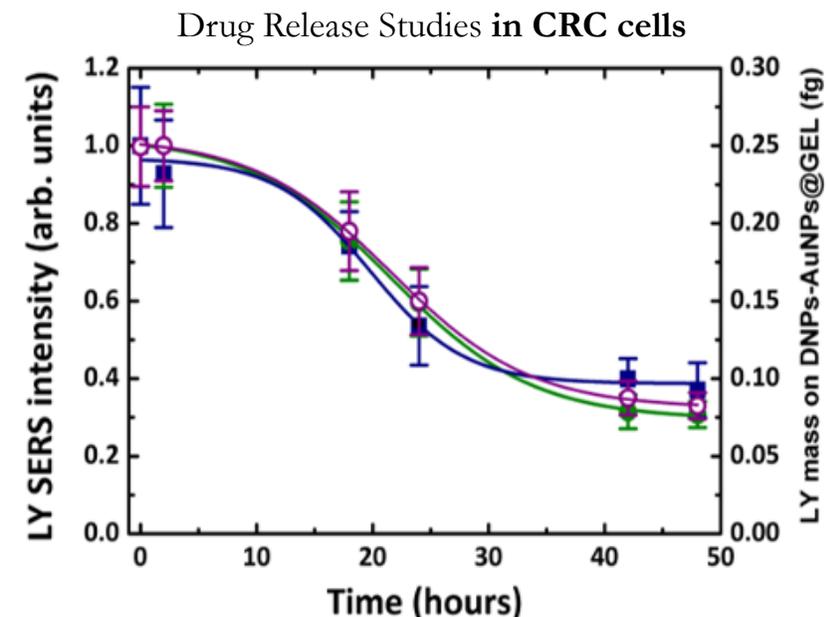
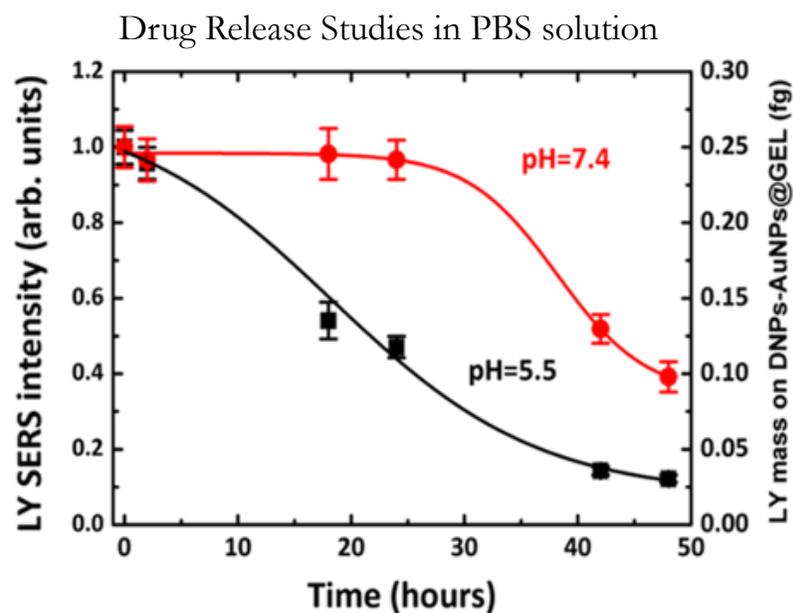
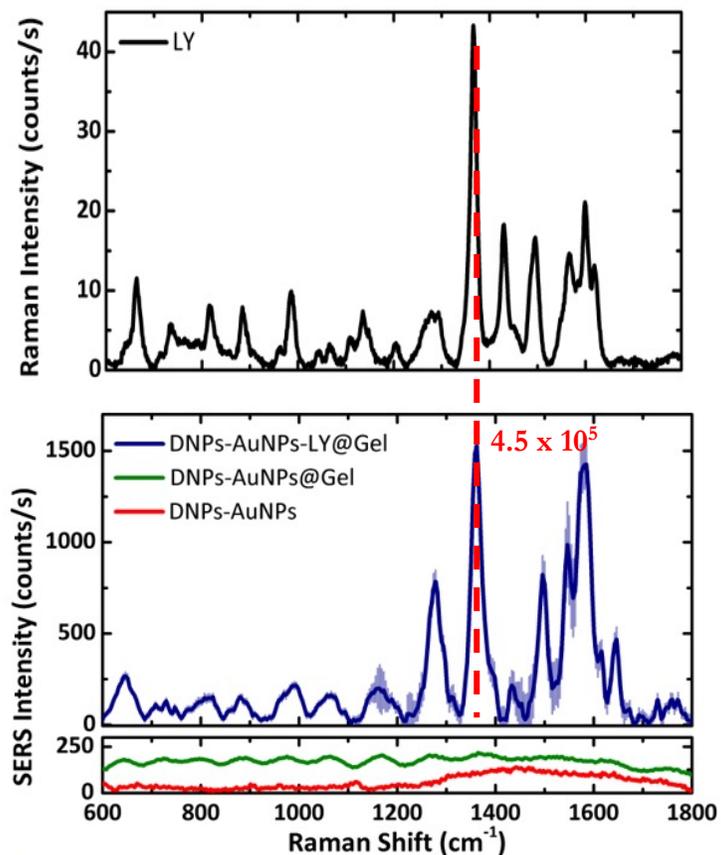
To highlight the advantages of the gelatin capping, the release behavior of both the DNP-AuNPs-LY@Gel complex and DNP-AuNPs-LY (without the gelatin layer) was studied by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC).



Nanosystem Loading Capacity: 20 µg of LY/mg of DNPs

# Intracellular Galunisertib Release Monitoring *via* SERS analysis

The enhancement factor of the LY Raman signal provided by the DNP-AuNPs-LY@Gel complex was studied by SERS before investigating the release profile of the developed hybrid nanosystem in colorectal cancer (CRC) cells.



An efficient SERS intracellular tracing of LY was performed up to 48 hours in living CRC and quantified to provide a LY sensing resolution down to  $7.5 \times 10^{-18}$  g.

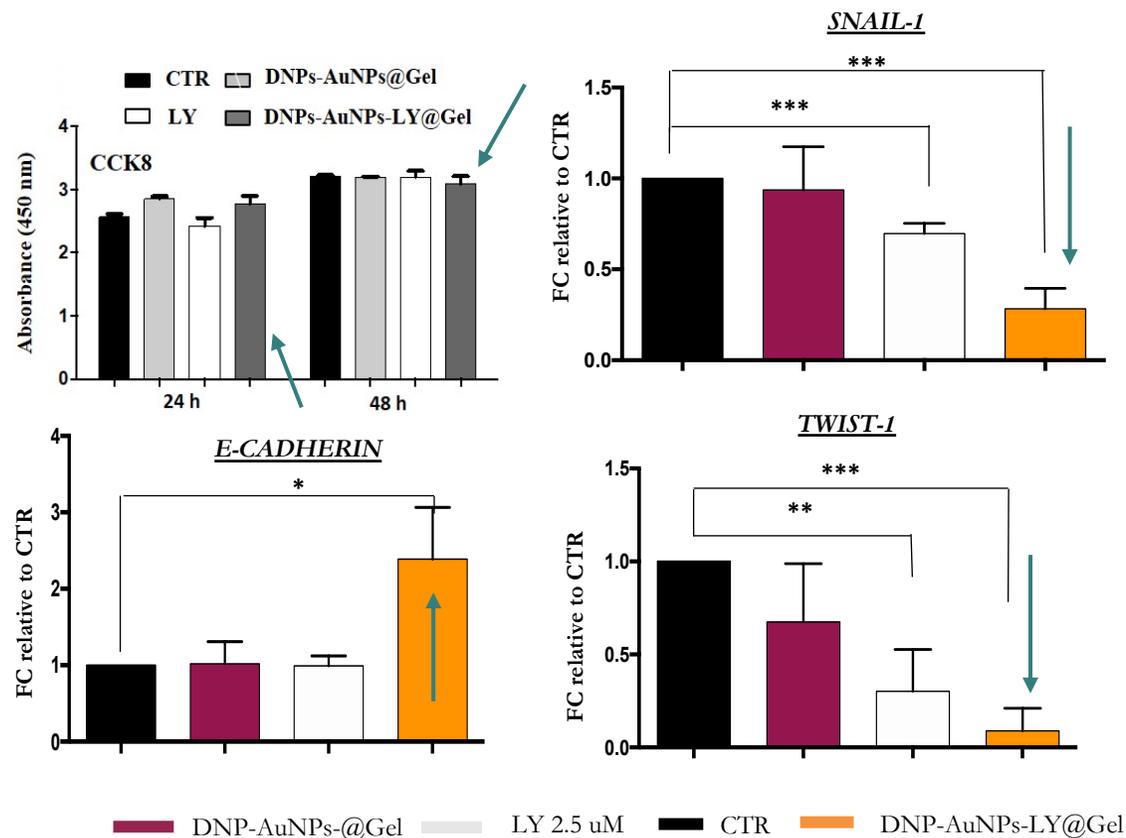
The most intense SERS vibration was found at  $1360 \text{ cm}^{-1}$  and was used for monitoring the LY intracellular release from the developed platform.

# Outline

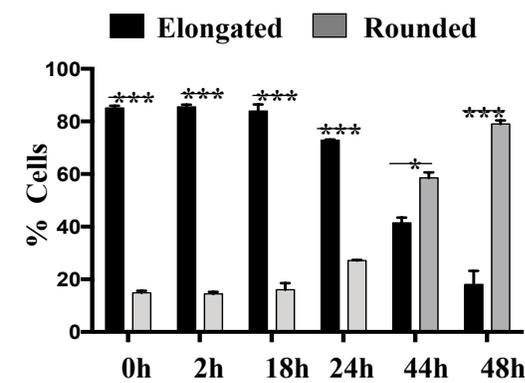
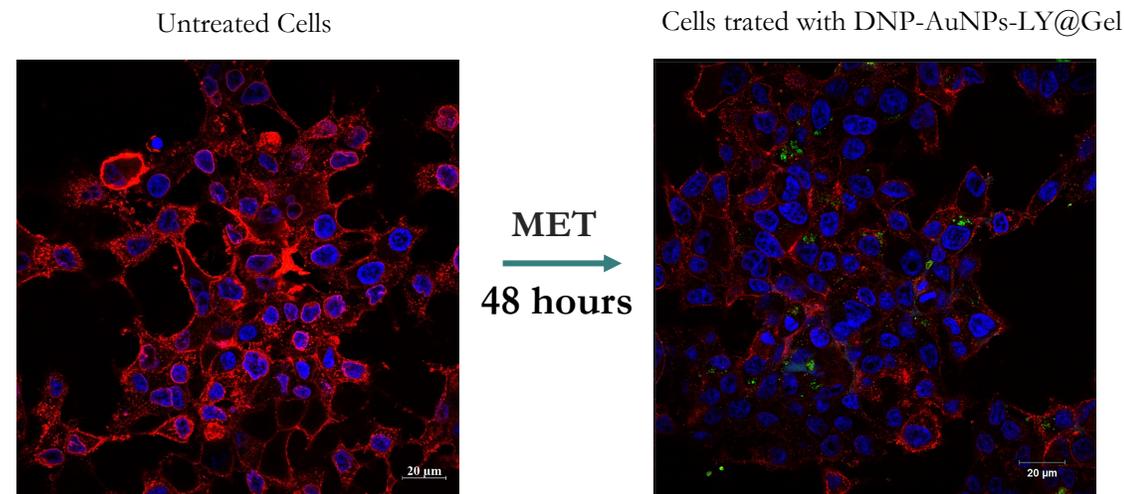
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# Nanosystem-Induced Reversion of CRC Metastatic Phenotype

## Modulation of Metastatic Genes in the LS-174T Cell line



## Internalization of Alexa-488-labeled nanosystem and Epithelial Transformation of CRC cells



\*Nanosystem concentration was 50  $\mu\text{g}/\text{mL}$  in all the studies. According to the HPLC analysis, 50  $\mu\text{gM}/\text{mL}$  of DNP-AuNPs-LY@Gel contains 2.5  $\mu\text{M}$  of LY

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# Conclusions

## Achievements



- ✓ Development of a pH-responsive hybrid Galunisertib delivery nanosystem with a size of 450 (50) nm and a drug loading capacity of  $20 \text{ ug}\cdot\text{mg}^{-1}$
- ✓ Real-time monitoring of Galunisertib release in living cells thanks to the high-sensitivity of the hybrid DNP-AuNPs-LY@Gel complex.
- ✓ Enhancement of the therapeutic effect of and reversion from metastatic to epithelial phenotype in CRC cells after 48 hours of treatment with DNP-AuNPs-LY@Gel.



## Work in Progress

- Functionalization of the complex DNP-AuNPs-LY@Gel with Anti-L1CAM antibodies to address Galunisertib release in malignant cells overexpressing L1-CAM.
- Assessing the therapeutic effects of the newly developed system on different cell lines (pancreatic and CRC cell lines)



*Thank you for your  
kind attention*



## Acknowledgments

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**Dr. Giovanna Chianese, ISASI-CNR**  
**Dr. Enza Lonardo, IGB-CNR**  
**Dr. Donatella Delle Cave, IGB-CNR**  
**Dr. Gianluigi Zito, ISASI-CNR**  
**Dr. Monica Terracciano, UNINA**