EPTRI

EUROPEAN PAEDIATRIC TRANSLATIONAL RESEARCH INFRASTRUCTURE

Nanoparticles for Target Therapy in Pediatric Brain Cancers

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CNR NANOTEC INSTITUTE OF NANOTECHNOLOGY

TecnoMedPuglia

Traditional medicine vs Precision medicine

Traditional treatments

SURGERY

RADIATION

CHEMOTHERAPY

- Neurotoxicity
- Disabilities (cognitive problems, neuroendocrine, and neurosensory deficits)

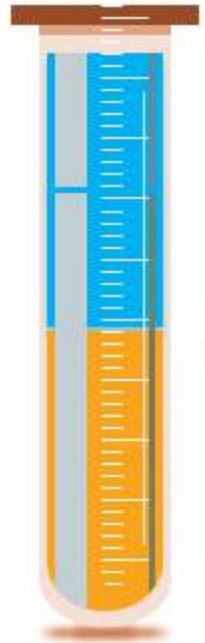
Advanced personalized treatments

- Promising as diagnostic tool
- Vectors for drug/gene delivery
- Abilities to cross BBB

POLYMERIC NANOPARTICLEs

Polycaprolactone (PCL) NPs

- Biocompatible polymer, approved by FDA
- Easy modulation of physical properties



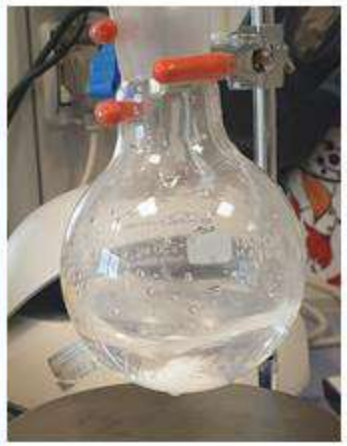
BIOMIMETIC COATING

Glioma cell membrane

- Overcoming BBB
- Targeting capabilities
- Camouflaging

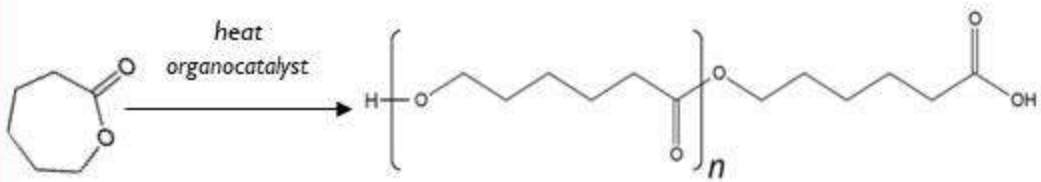
GLIOBLATOMA MEMBRANES COATED NPs

Carboxyl-terminated PCL



ROP: organo-mediated ring opening polymerization of ε-caprolactone

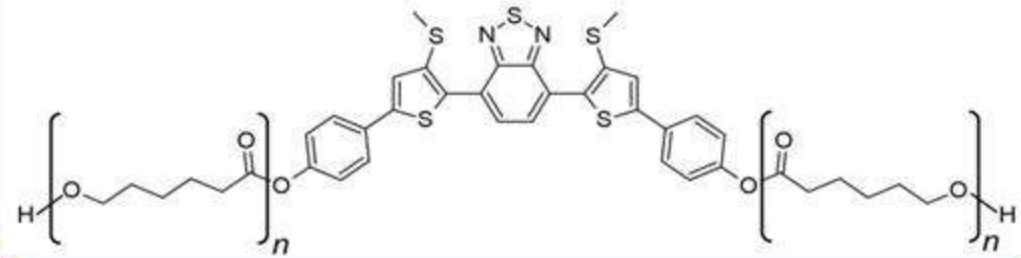
Green synthesis, no tin (VI)-based catalysts



Fluorescent PCL

PCL incorporating a fluorescent thiophene-based molecule

Avoiding aggregation-mediated fluorescence quenching



In collaboration with CNR ISOF, Bologna

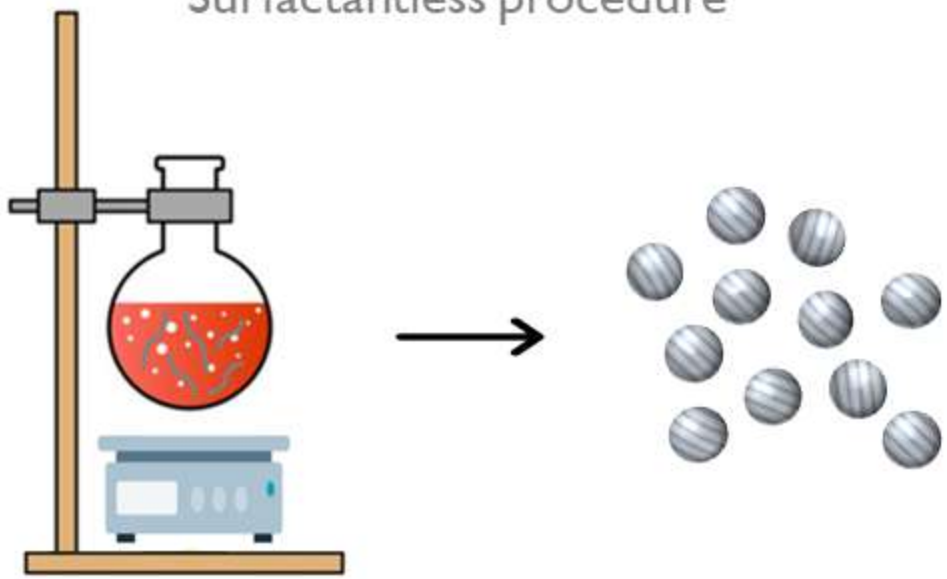
Unpublished results

Biomimetic Nanoparticles for Enhancing Homotypic Tumoral Targeting in Glioblastoma Therapy; Baldari et al., under submission

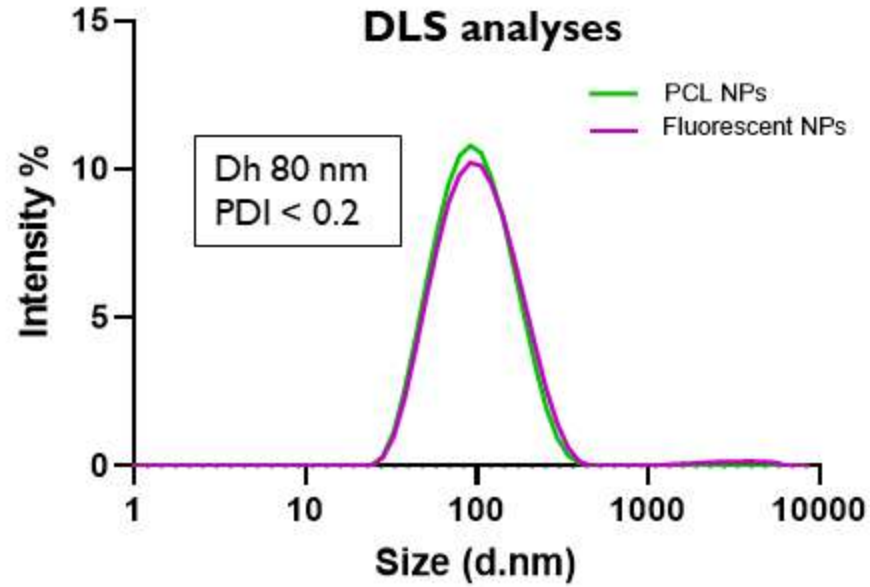
Polymeric nanoparticles production and characterization

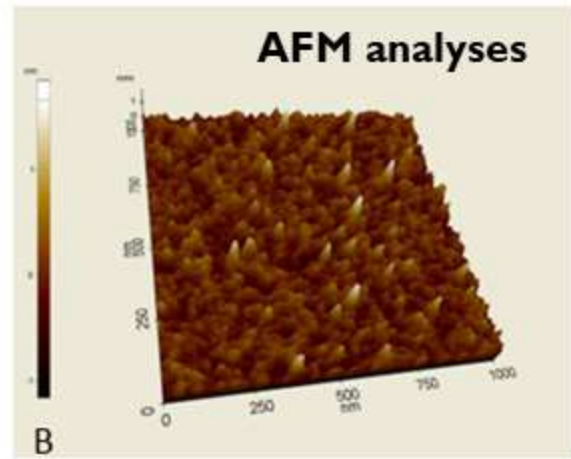
Fabrication of PCL NPs

Solvent evaporation method
Surfactantless procedure



- ✓ Monodisperse NPs
- ✓ NP size less than 100 nm





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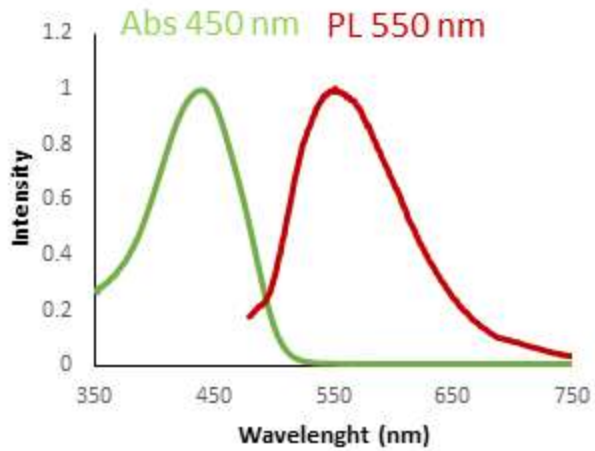
Drug encapsulation in polymeric nanoparticles

Carboxyl-terminated PCL

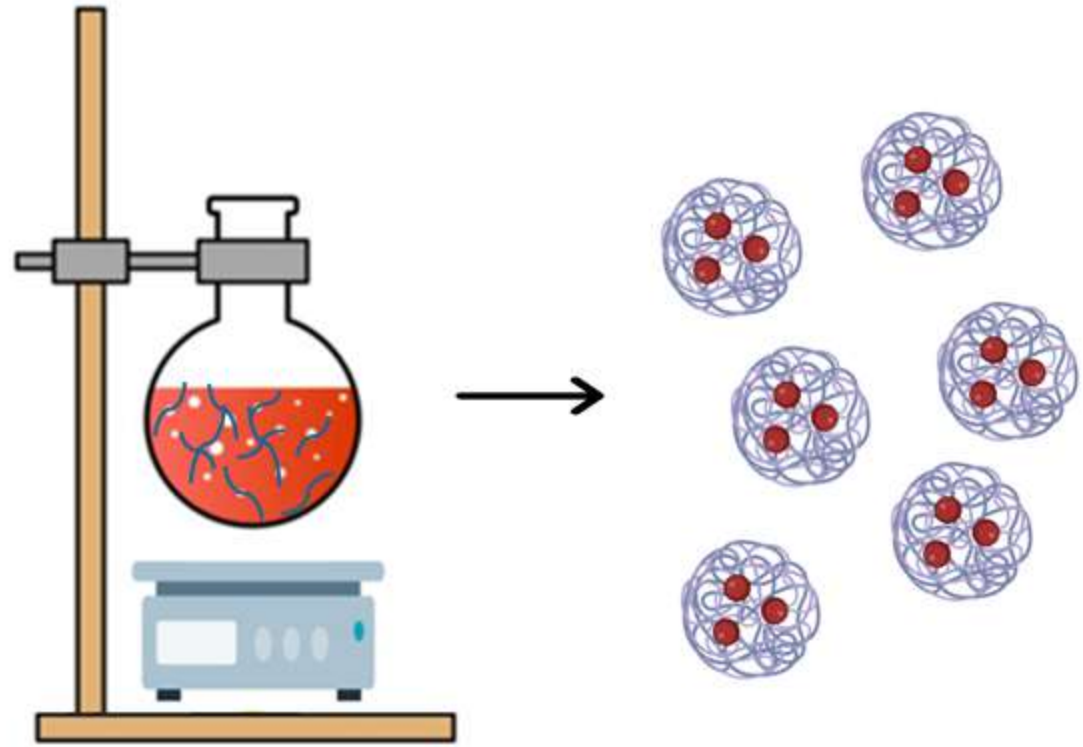
Palamà et al, 2022

T3PhSO

High cytotoxic fluorescent molecule



Fabrication of T3PhSO loaded PCL NPs



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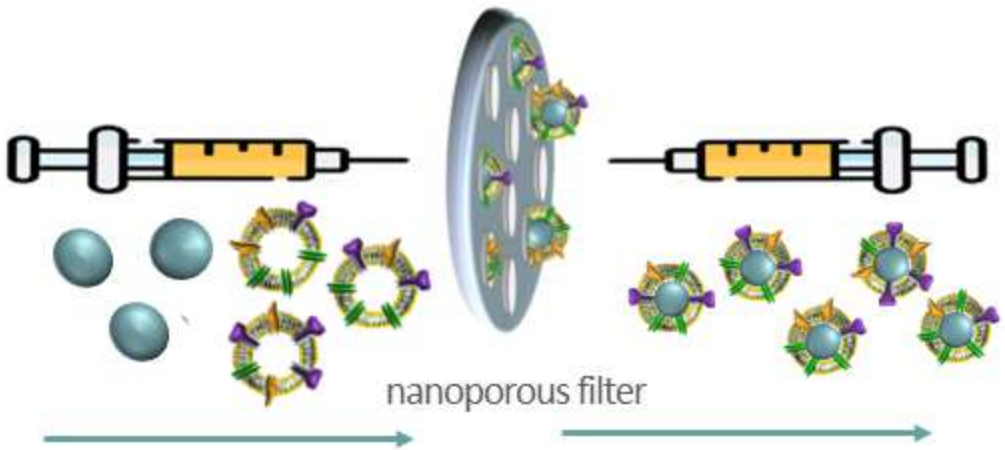
Preparation of cell membranes coated biomimetic nanoparticles

1. Cell lysis

2. Differential centrifugation

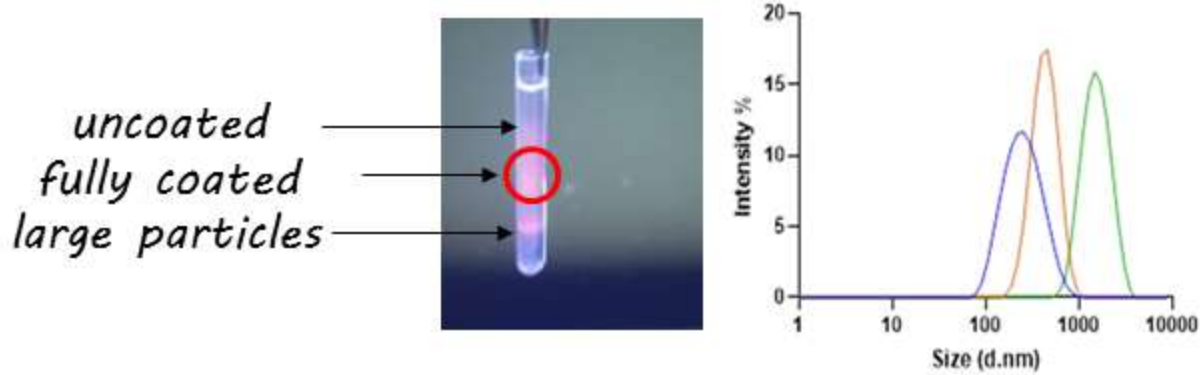
3. Membrane vesicle formation

4. Co-extrusion procedure



5. Purification of cell membrane coated NPs

density gradient ultracentrifugation



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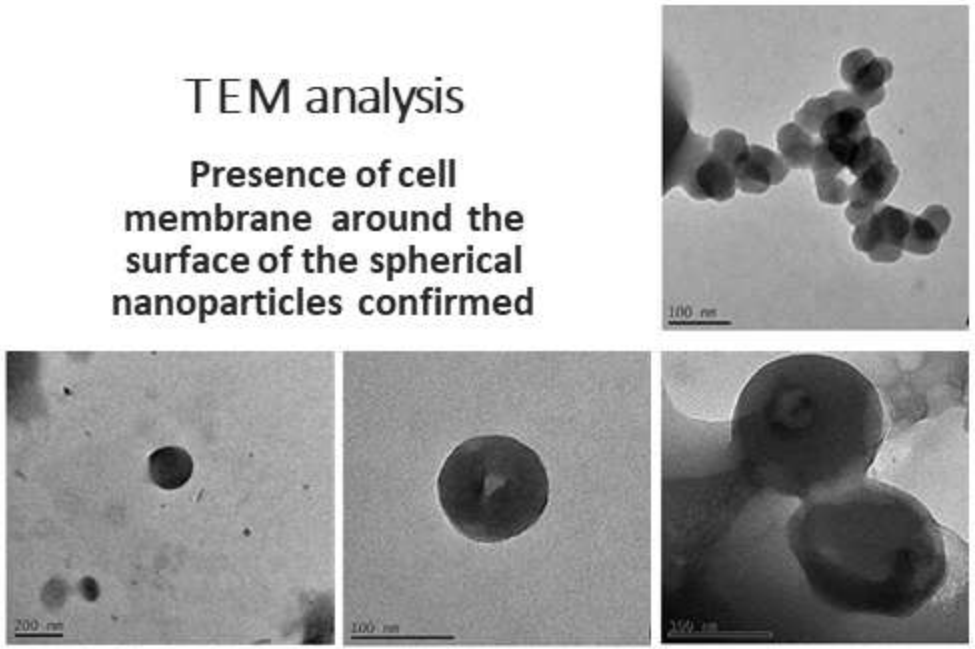
Structural characterization of biomimetic nanoparticles

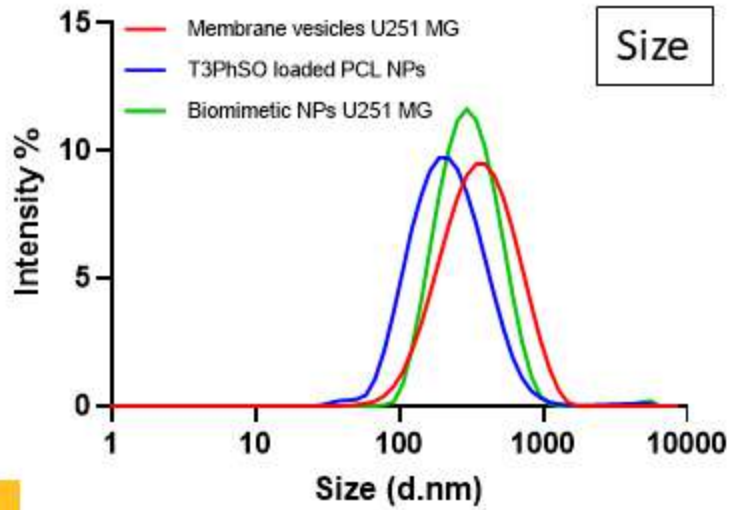
TEM analysis

Presence of cell membrane around the surface of the spherical nanoparticles confirmed



Changes in both size and surface charge confirm the correct coating with cell membranes

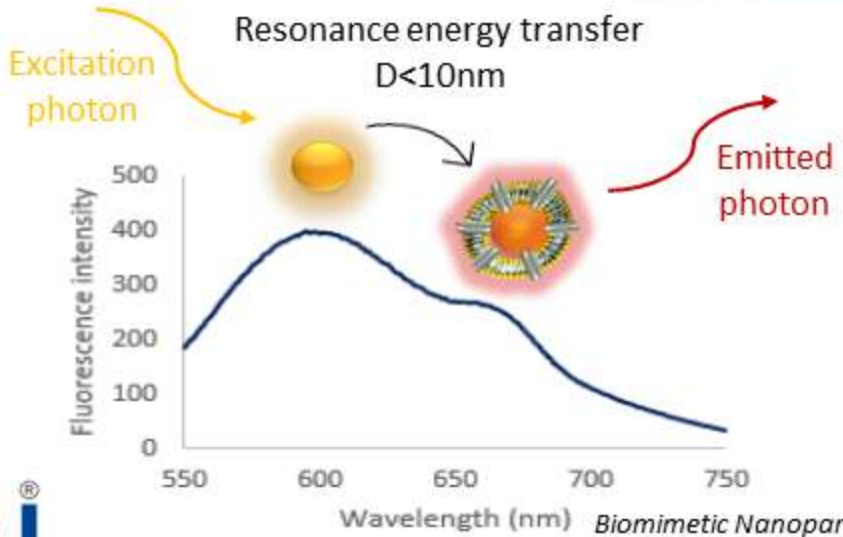
DLS analysis

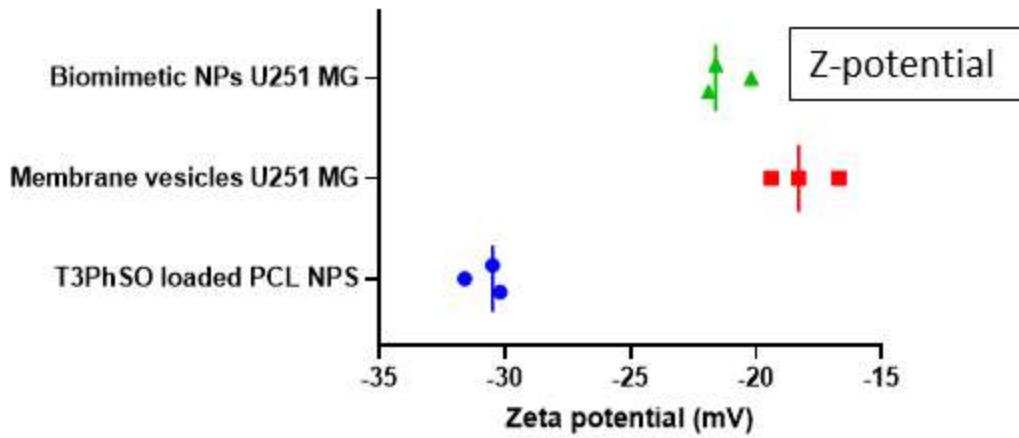


Right assembly of biomimetic NPs

FRET studies

DiD doped membranes and fluorescent PCL are FRET pairs





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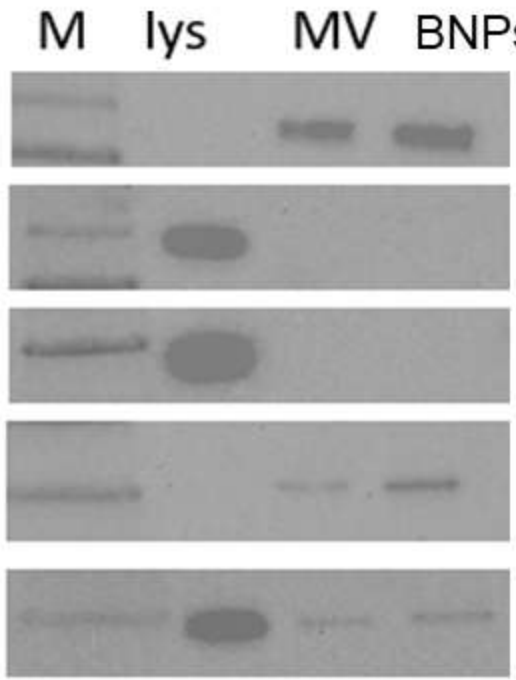
Biological characterization of biomimetic nanoparticles

Western Blotting



M marker
Lys intracellular lysate
MV membrane vesicles
BNPs Biomimetic NPs

- Na/K ATPase in membrane vesicles and biomimetic NPs
- Golgin 97 (Golgi), GRP78 (ER), ATP5A (mitochondria) present only in lysate
- GAPDH in intracellular lysate

Correct and pure cell membrane isolation and proteins retention in biomimetic NPs

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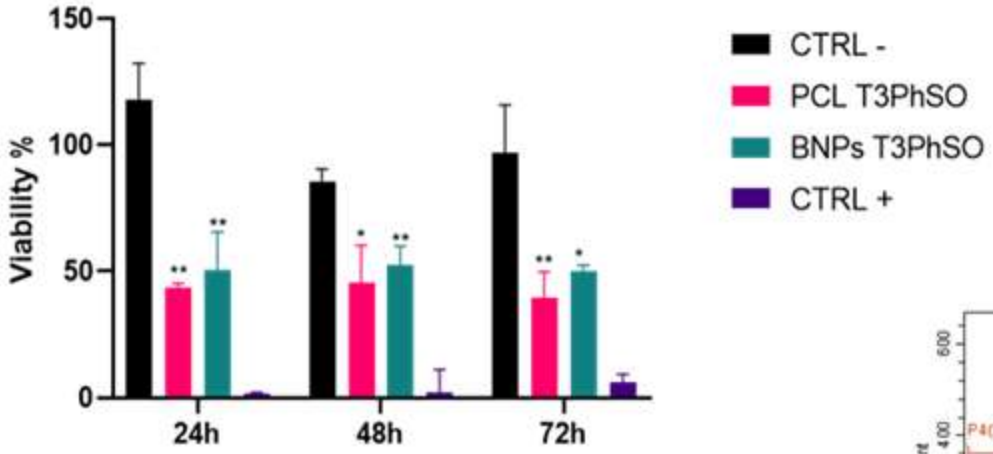
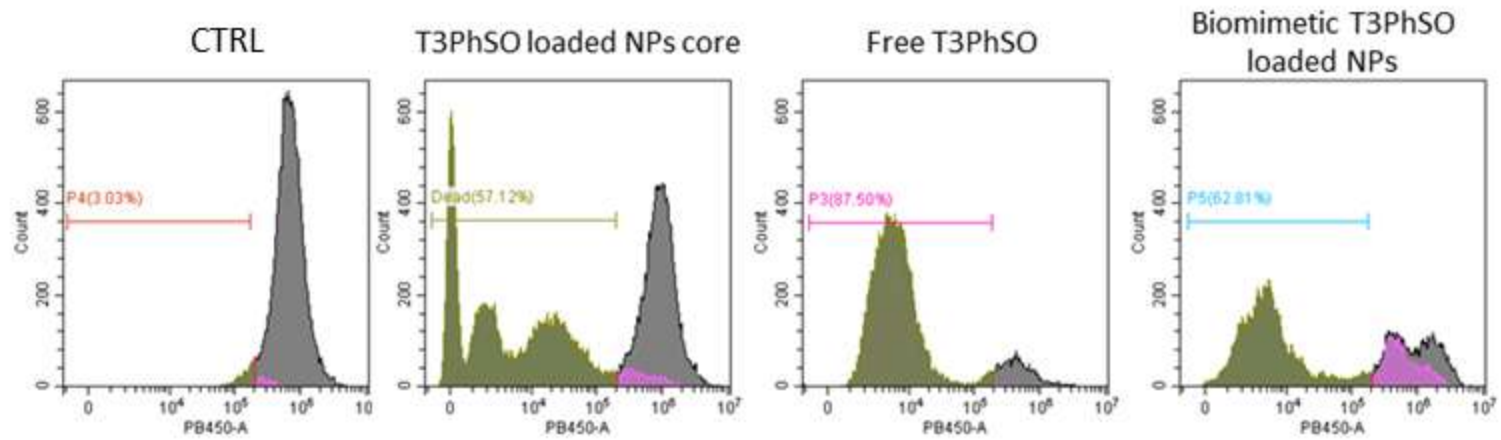
Cell viability 2D by MTT assay



Viablility 2D by flow cytometry
Calcein blue assay 72h



T3PhSO, free or incapsulated reduce cell viability of U251 MG cells more than 50%

Unpublished results

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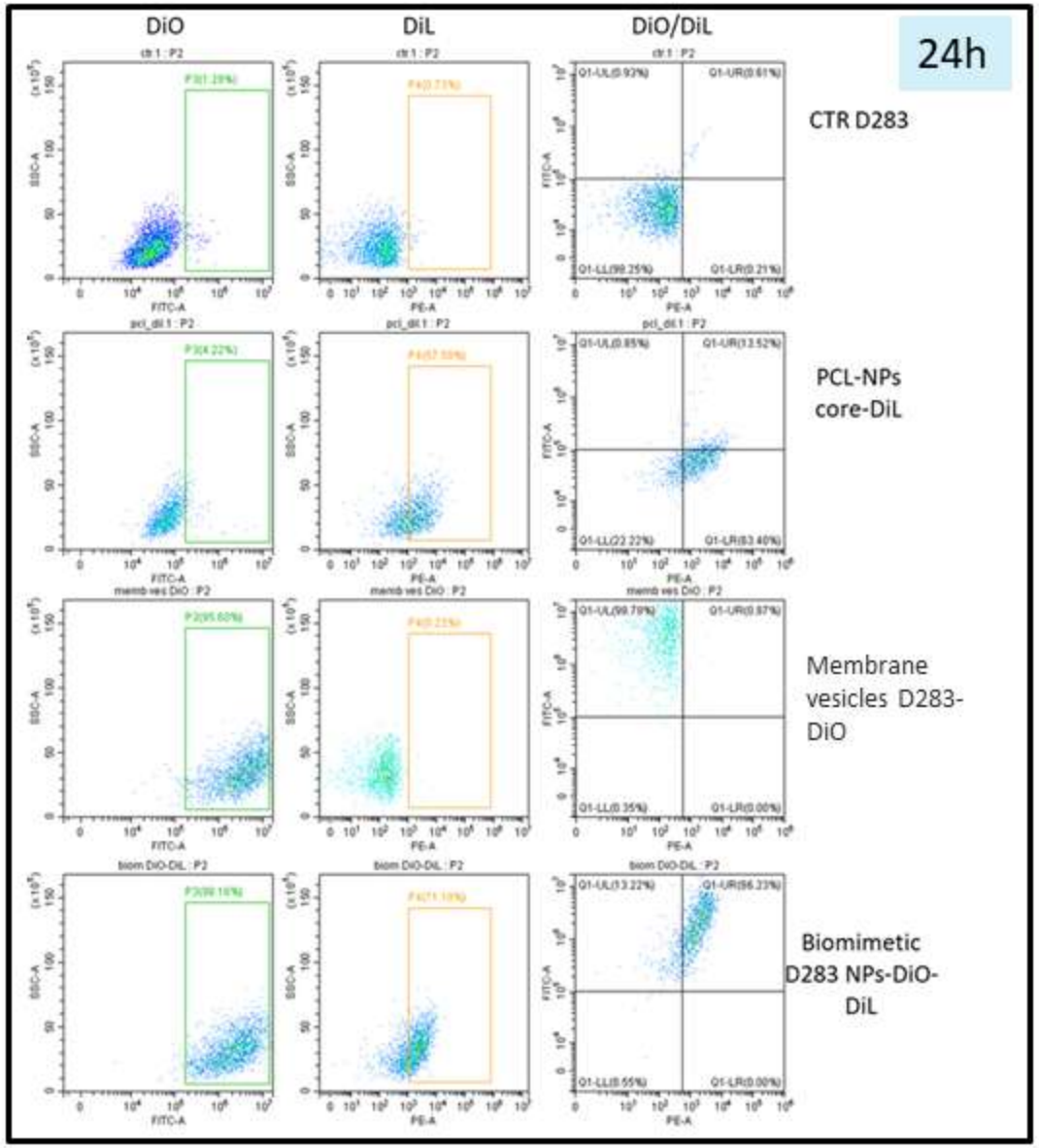
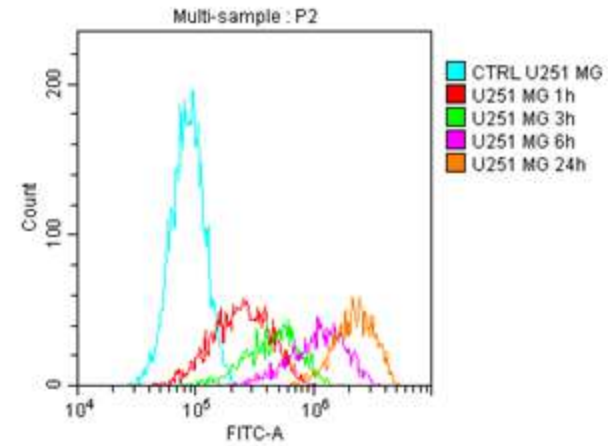
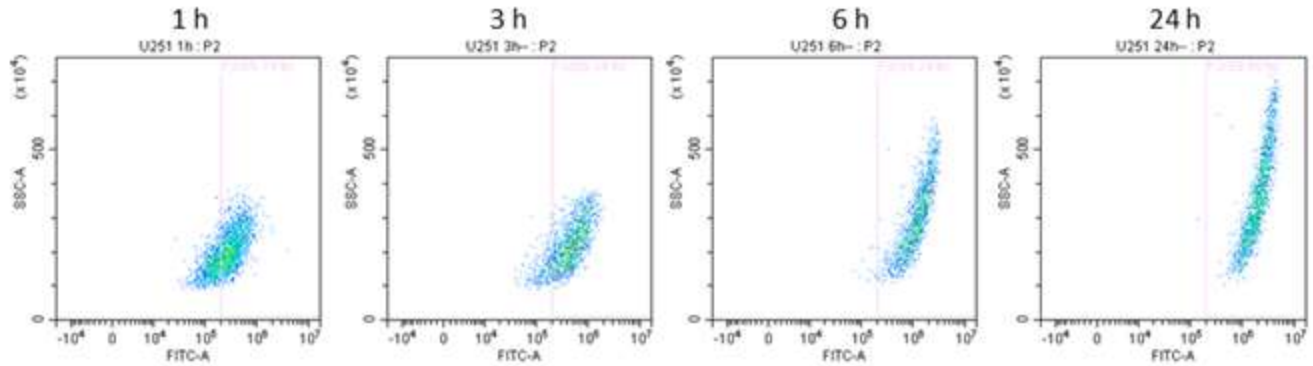
Cellular uptake in 2D cell culture by flow cytometry



Uptake evaluation in medulloblastoma (in collaboration with Bambino Gesù children hospital in Rome) and glioblastoma cell lines





Increase of internalization rate over time (1h, 3h, 6h, 24h)

Unpublished results

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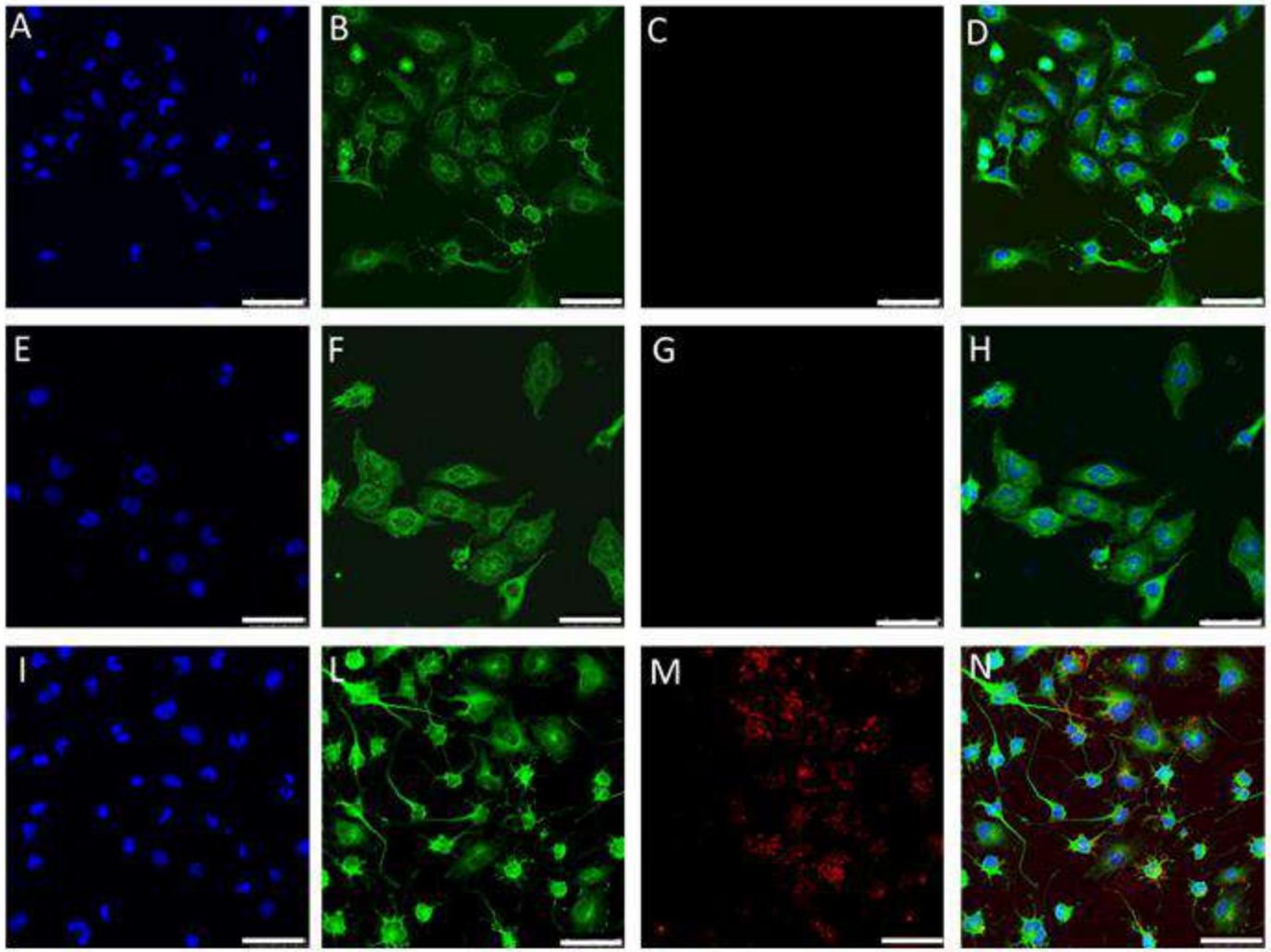
Cellular uptake in 2D cell culture by CLSM



CTRL U251 MG cells

Free T3PhSO U251 MG cells

T3PhSO loaded PCL-NPs U251 MG cells

DAPI-nuclei
Red-T3PhSO
FITC-β-tubulin

- U251 MG glioblastoma cell line
- T3PhSO 30 ug/mL loaded NPs (red)

NPs maximize the concentration of the drug at the intended

Unpublished results

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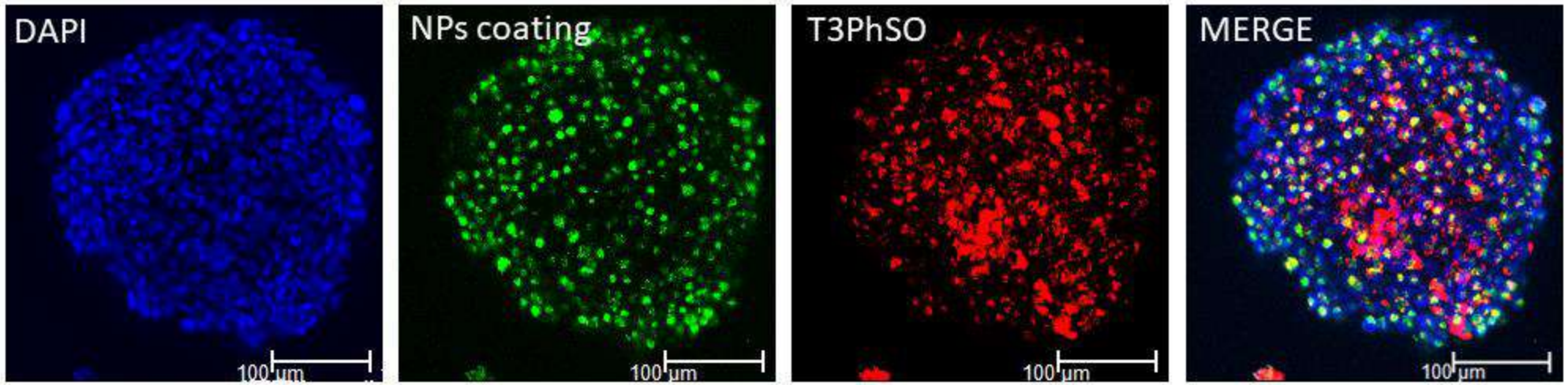
Cellular uptake in 3D cell culture by CLSM

- Spheroids of U251 MG
- T3PhSO loaded biomimetic NPs (red)



Scale bar 100 µm

DAPI-nuclei
Red-T3PhSO
DiO-Biomimetic NPs coating

NPs uptake is verified also in 3D cell culture

Unpublished results

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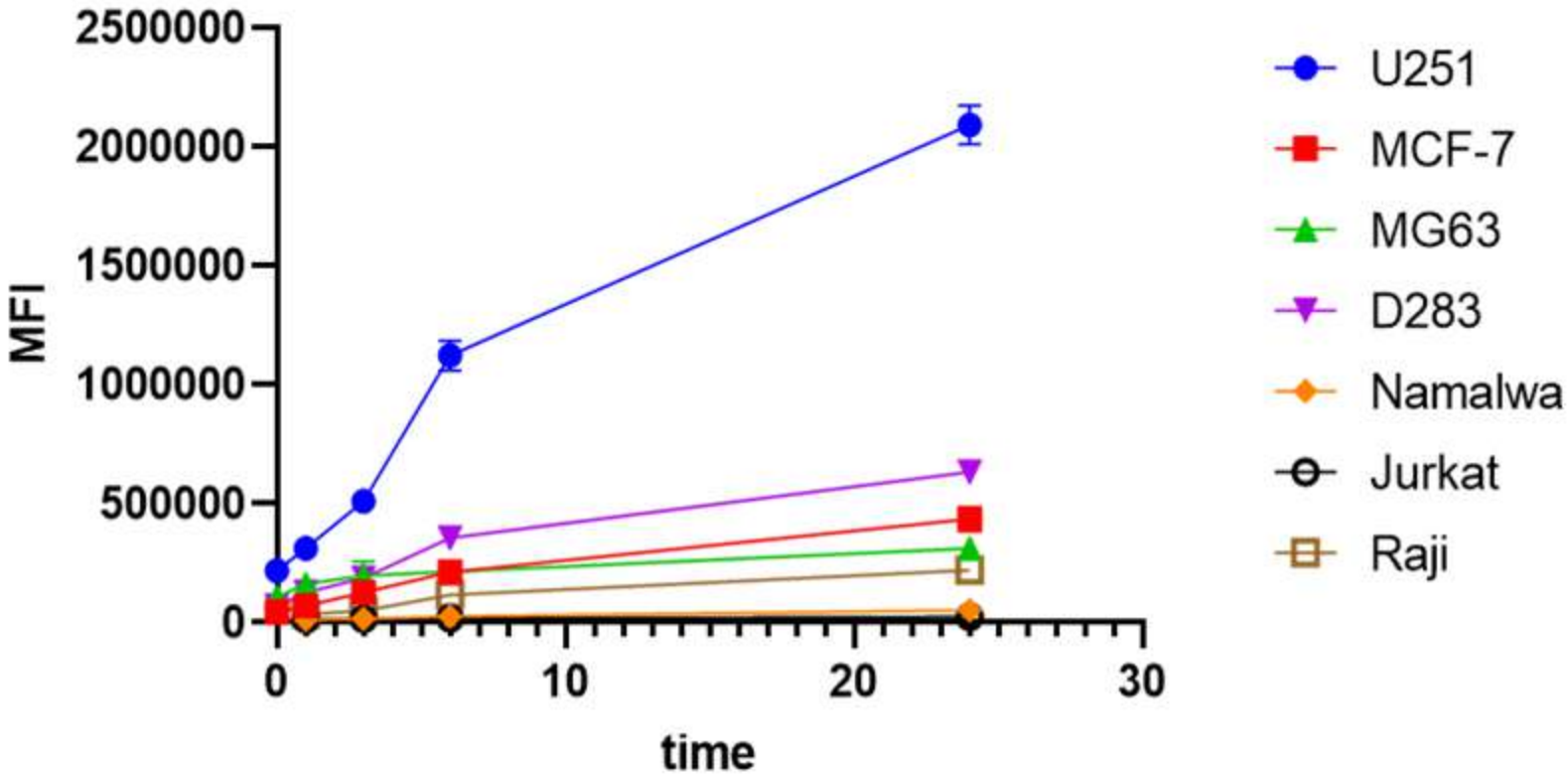
Flow cytometry evaluation of targeting properties of glioma BNPs



- U251 MG coated biomimetic NPs-DiO stained
- Time points: 1h, 3h, 6h, 24h

Selectivity of biomimetic NPs for the source cells (U251 MG): homotypic properties

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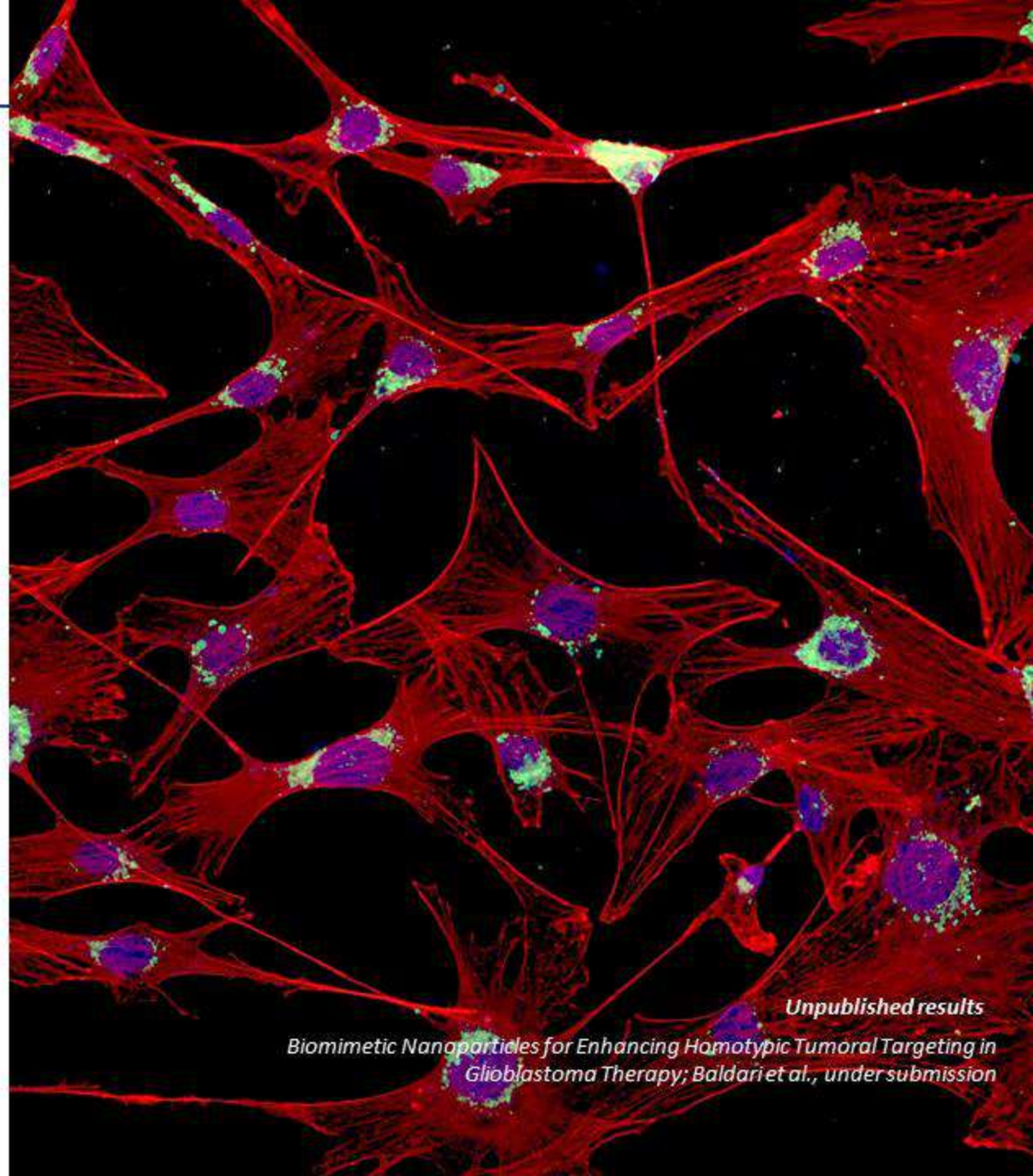
Conclusions and future perspectives

- PCL-NPs core perform better compared to NPs made employing commercial PCL
- PCL-NPs can encapsulate and deliver an active fluorescent compound inside cells
- Extracted cell-membranes are pure and retain surface biomarkers
- Self recognition and the preferentially interaction with cancer source cells

<u>Efficient tool in precision medicine for the possibility to employ patient-derived cell membranes</u>



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Acknowledgements

Prof. Giuseppe Gigli, Dr. Ilaria E. Palamà and Dr. Gabriele Maiorano research group

FUNDING:

- "Tecnopolo per la medicina di precisione" (TecnoMed Puglia) - Regione Puglia
- PON ARS01_00906 "TITAN - Nanotecnologie per l'immunoterapia dei tumori"
- EU funding within the MUR PNRR "National Center for Gene Therapy and Drugs based on RNA Technology"
- Life-Science Hub, PNRR–Terapie Avanzate