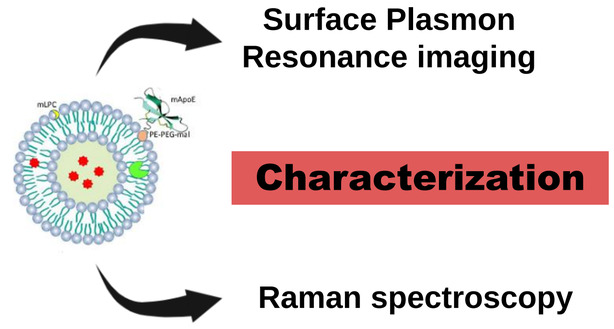
Characterization of dual functionalized - drug loaded liposomes by biophotonics techniques

F. Rodà [1], S. Picciolini [1], A. Gualerzi [1], A. Antoniou [2], S. Giofré [2], P. Seneci [2], F. Re [3], M. Bedoni [1]

[1] IRCCS Fondazione Don Carlo Gnocchi, Laboratory of Nanomedicine and Clinical Biophotonics (LABION), Milan, Italy
[2] Department of Chemistry, University of Milan, Milano, Italy
[3] School of Medicine and Surgery, University of Milano Bicocca, Monza, Italy

Neurological diseases are poorly treated diseases with a considerable social and economic impact. Drug delivery to the brain is still challenging because of the presence of the blood-brain barrier (BBB) that limits the access of drugs. **Liposomes** (LPs) have been used to improve the brain bioavailability of several molecules, demonstrating their therapeutic potential as drug delivery systems.

We propose the **biophotonic techniques**, ***Surface Plasmon Resonance Imaging*** (SPRi) and ***Raman Spectroscopy*** (RS) as innovative tools for the characterization and validation of multi-functionalized LPs for the control of neuroinflammation and associated microglial dysfunctions in Glioblastoma and Alzheimer's Disease.
Drug-loaded LPs have been functionalized with mApoE to cross the BBB, and with a protease sensitive peptide to guarantee the effective and localized release of the candidate drugs (Pimasertib/Trametinib/Glibenclamide) in diseased areas where some specific proteases are overexpressed.

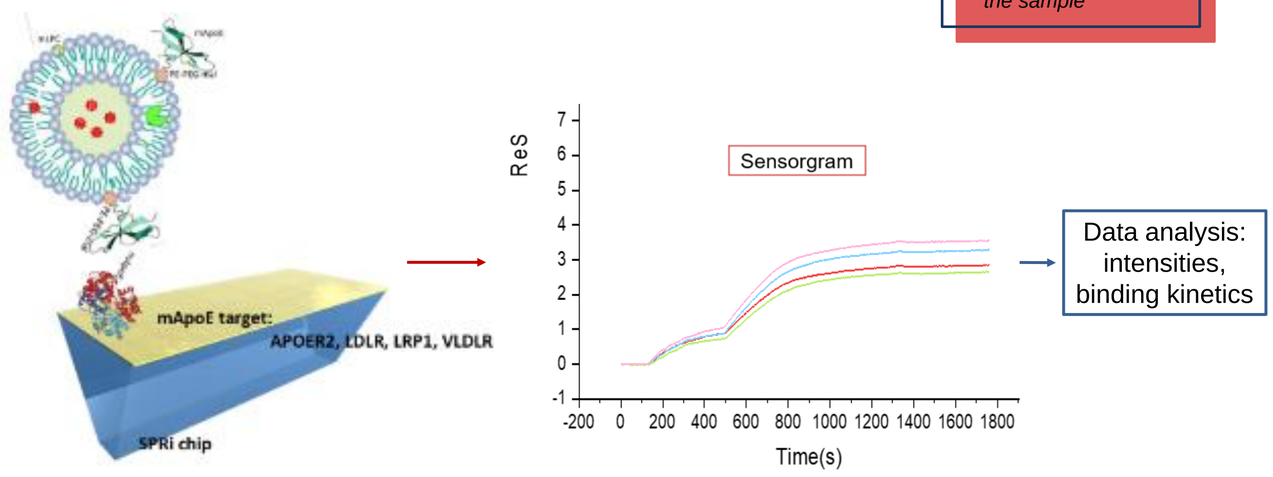


Surface Plasmon Resonance imaging

SPRi is an optical detection technique used to monitor and analyzed biomolecular interactions between an analyte in solution and ligands immobilized on a gold chip.

- *Multiplexing*
- *Label free technology*
- *High specificity*
- *Real time imaging*
- *Low amount of sample required*
- *Minimal preparation of the sample*

- Preparation of SPRi array and injection of liposomes

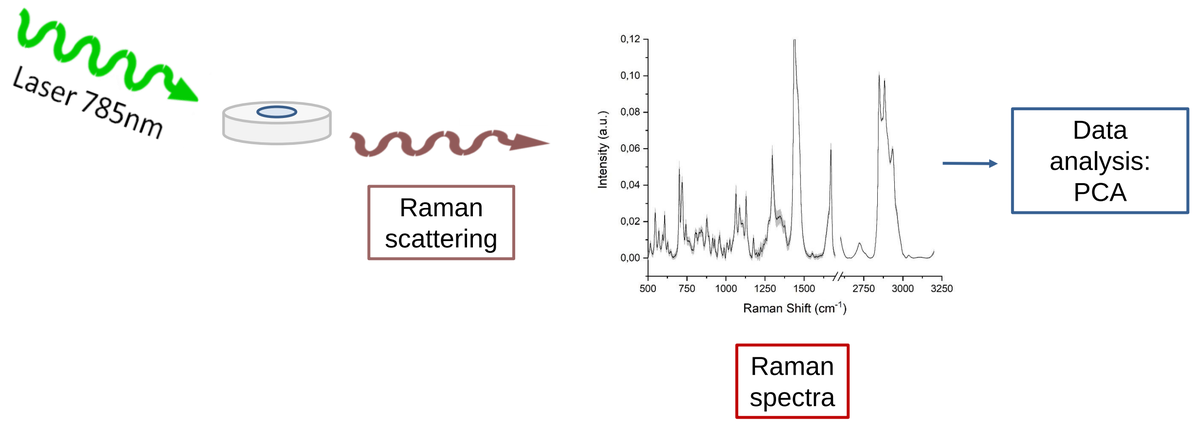


Raman Spectroscopy

RS is a vibrational investigative methodology, able to provide qualitative and quantitative information regarding the chemical components of the analyzed target.

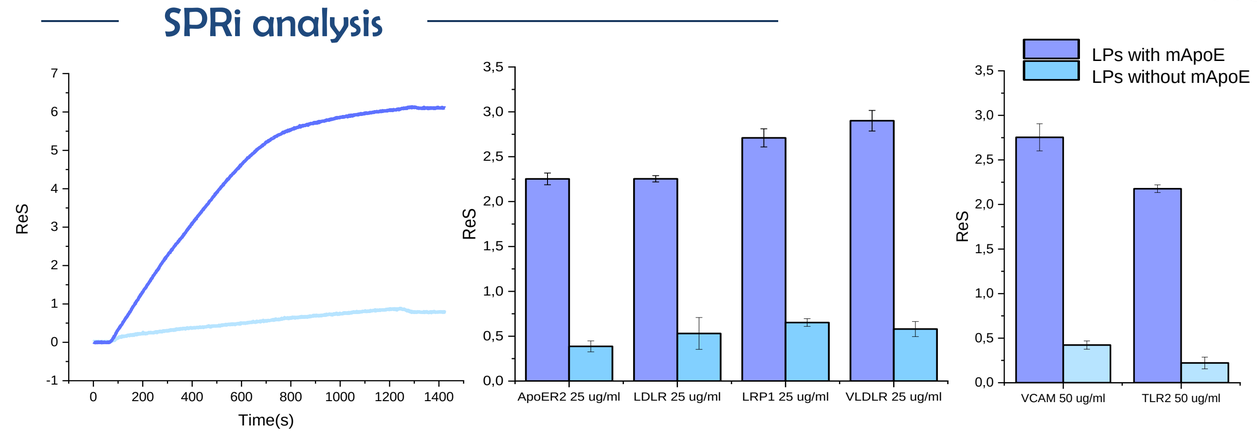
- *Label free technology*
- *Low amont of sample required (3 ul)*
- *No preparation of the sample*
- *Fast analysis*

- Deposition of a drop of sample on a CaF_2 disk



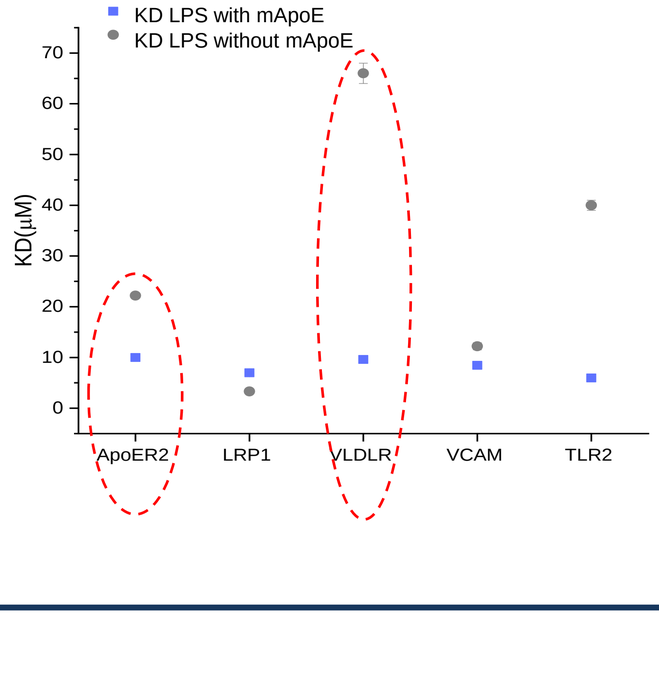
Results

SPRi analysis



LPs functionalized with mApoE generate higher SPR signals referred to the interactions between mApoE and different receptors compared to non functionalized LPs.

Selected ligands
Specific for mApoE: ApoER2, LDLR, LRP1, VLDLR
Other receptors: VCAM1, TLR2

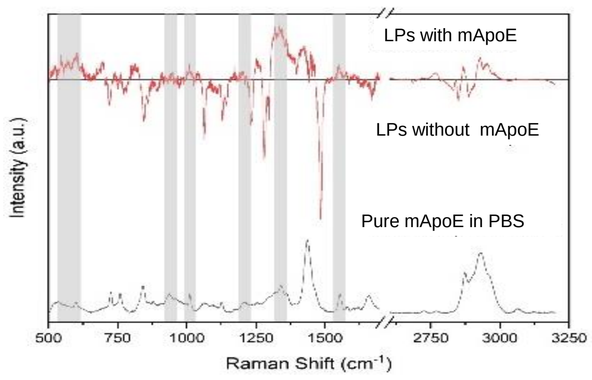


The kinetics analysis of LPs showed that LPs mApoE have a higher affinity (low KD) for APOER2 and VLDLR compare to LPs non functionalized. For the other receptors the differences are less significant.

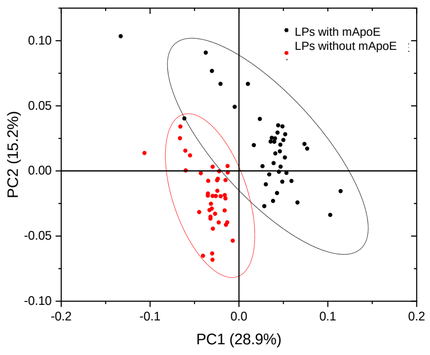
Preliminary data ⚠

Raman Analysis

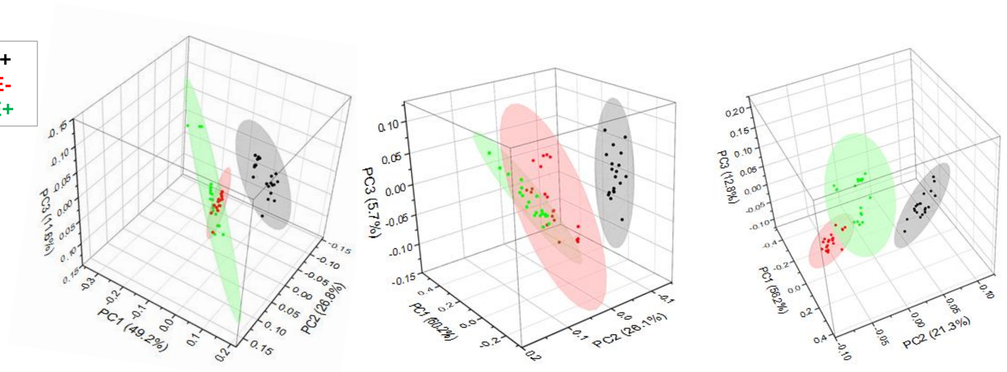
The direct spectral comparison of mApoE with LPs functionalized and not functionalized with mApoE, highlighted slight modifications in the signal of the LPs conjugated with the protein (grey bands).



The Principal Component Analysis (PCA), on the Raman dataset, is able to statistically discriminate the spectra collected from the LPs functionalized and not functionalized with mApoE .



LPs drug-, mApoE+
LPs drug+, mApoE-
LPs drug+, mApoE+



To verify the encapsulation of the drugs, PCA has been performed.
The 3-D distribution of PC showed that drugs have a strong influence on Raman spectra.

Conclusions

- **Surface Plasmon Resonance imaging** allows to verify the preservation of the binding affinity of mApoE.
- The obtained Raman data can identify **statistically significant differences** among the different liposomal formulations**.**

In conclusion SPRi and RS could become excellent techniques for the validation and characterization of liposomes.

Acknowledgments

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Francesca Rodà
Laboratory of Nanomedicine and Clinical Biophotonics
froda@dongnocchi.it
labion@dongnocchi.it
www.labion.eu
@labion_
@labion.eu