Raman spectroscopy characterization of multifunctional nanoliposomes for neurological disorders

Valentina Mangolini[1,2], Silvia Picciolini[1], Francesca Rodà[1,3], Alice Gualerzi[1], Luana Forleo[1], Aurora Mangolini[1], Pierfausto Seneci[4], Antonio Renda[5], Silvia Sesana[5], Francesca Re[5], Marzia Bedoni[1]

[1] IRCCS Fondazione Don Carlo Gnocchi ONLUS, 20148 Milan, Italy;
[2] Department of Molecular and Translational Medicine, University of Brescia, 25121 Brescia, Italy
[3] Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, 41121 Modena, Italy
[4] Chemistry Department, Università Degli Studi di Milano, 20133 Milan, Italy
[5] School of Medicine and Surgery, University of Milano-Bicocca, 20854 Vedano al Lambro, Italy

Drug delivery to the brain is still challenging because of the presence of the blood–brain barrier (BBB), which limits drugs' access to the brain.

Liposomes (LPs) have been proposed for their therapeutic potential that can improve the brain bioavailability of drugs, thanks to their versatile composition and structure.

How LPs can be characterized?

Raman Spectroscopy (RS) provides a specific spectrum with peaks related to the biochemical sample composition.

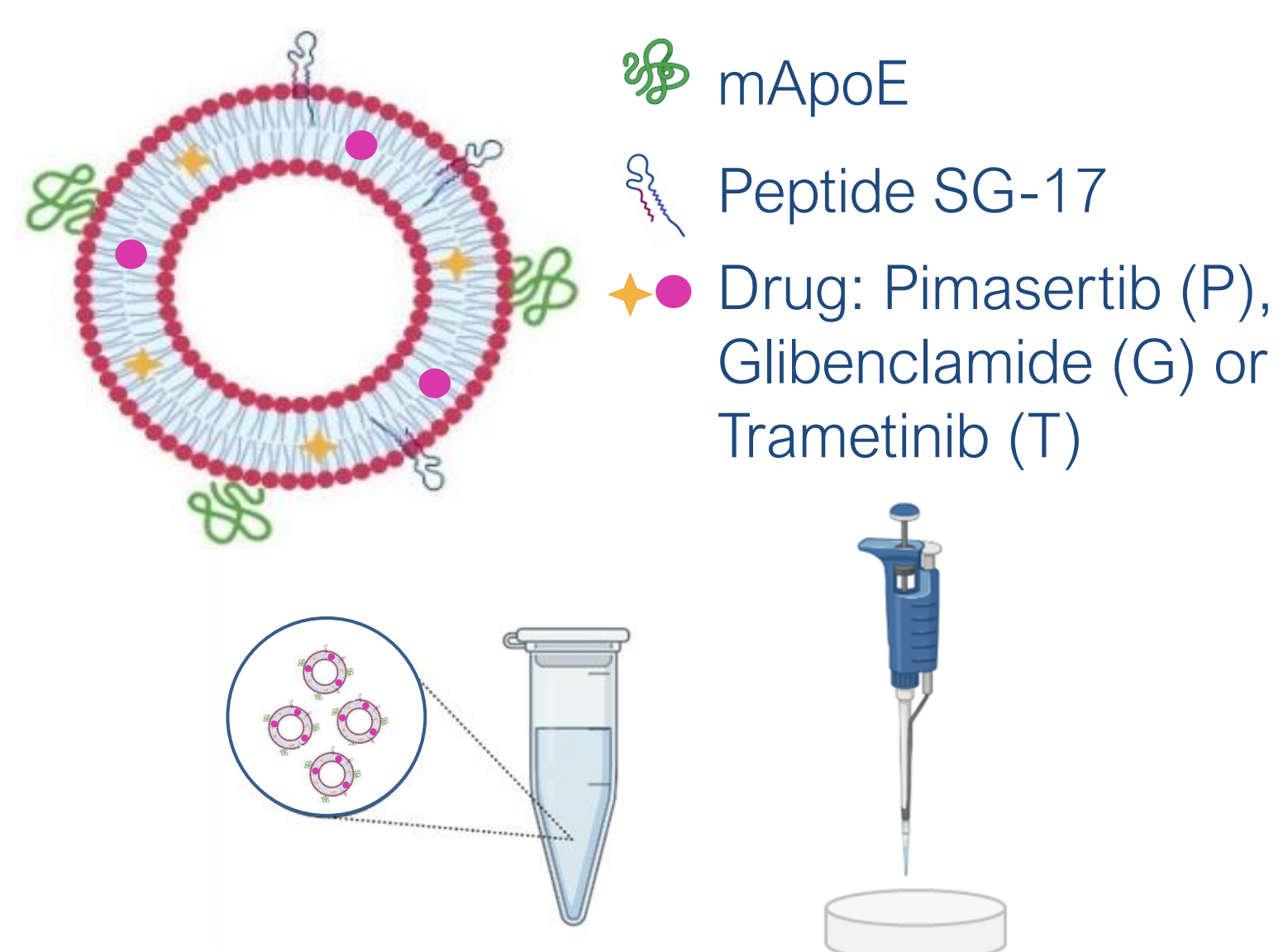RS advantages: small sample amount & fast analysis

Aim of the study

We propose RS for the characterization of LPs to be tested for the control of neuroinflammation and microglial dysfunctions in Glioblastoma multiforme and Alzheimer's disease, in order to test:
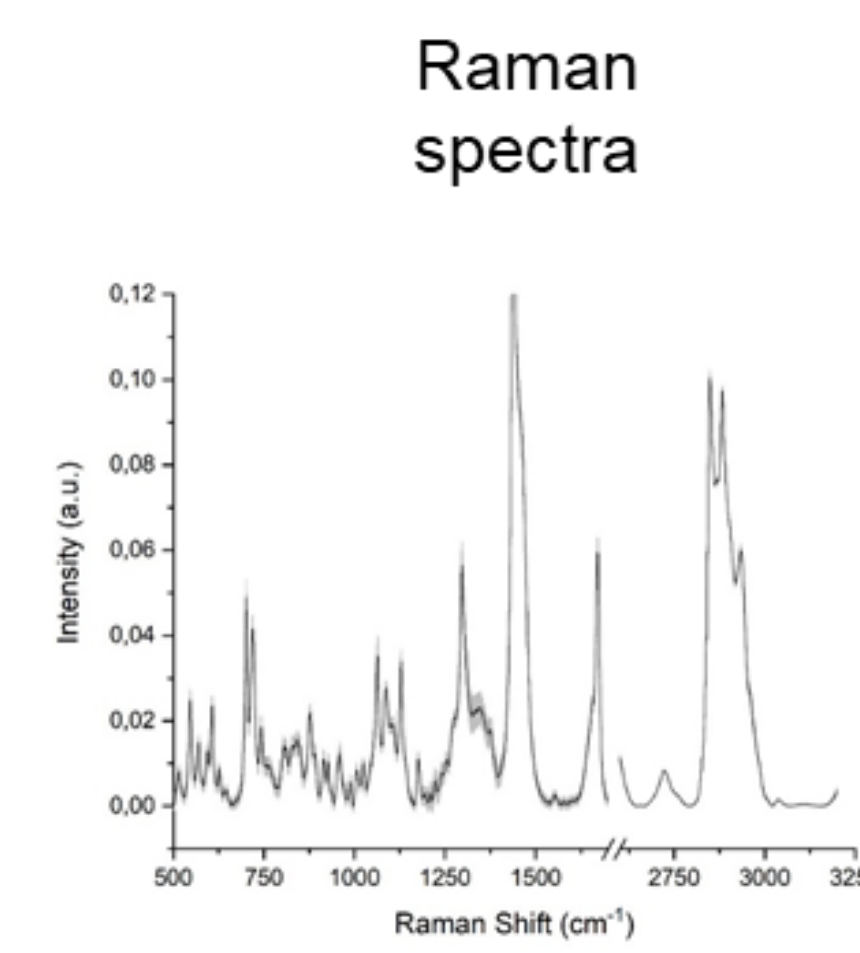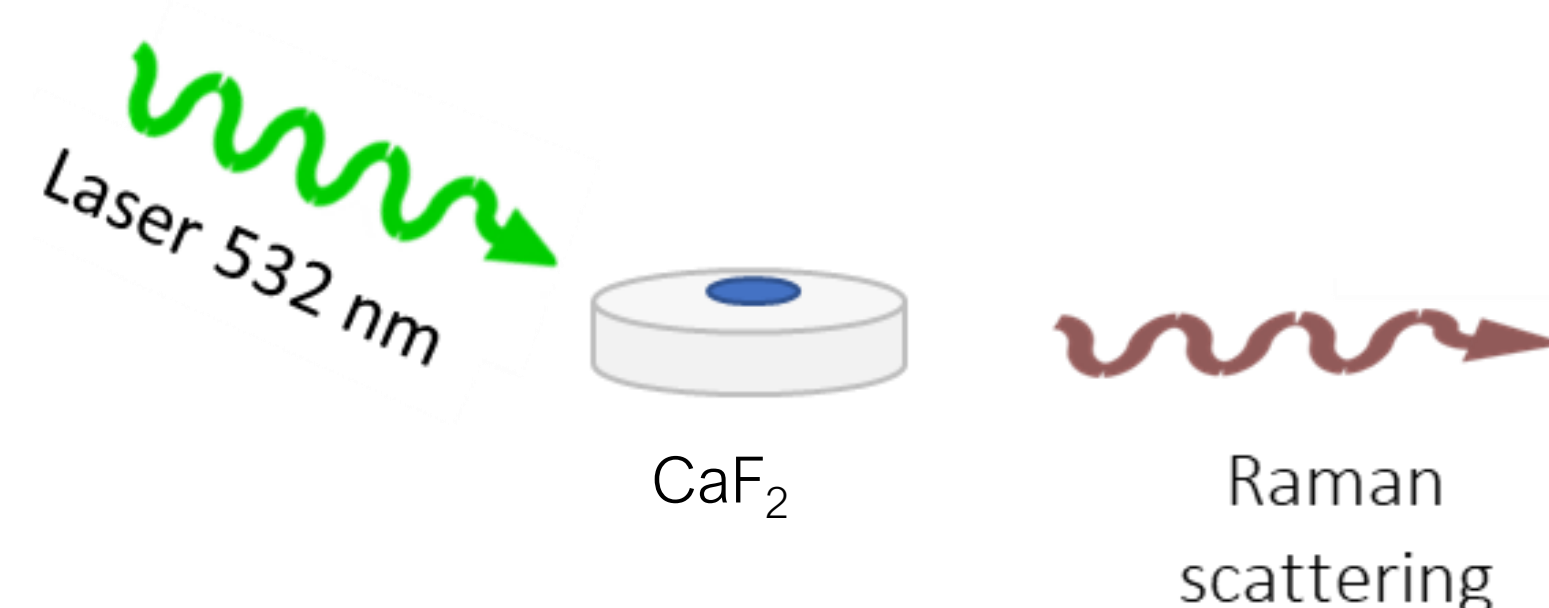
- their reproducibility
- the effective functionalization with selected ligands
- their stability

Methods

SAMPLE PREPARATION

- mApoE
- Peptide SG-17
- Drug: Pimasertib (P), Glibenclamide (G) or Trametinib (T)

RAMAN ANALYSIS

Laser 532 nm → CaF$_2$ → Raman scattering → Raman spectra

Raman acquisition/data analysis parameters

| Parameter	Value
Drop volume	3 µl
Laser source	532 nm
Objective	100x
Spectral range	500–1800 cm^{-1} 2700–3200 cm^{-1}
Acquisition time	25s x 2 accumulations
Diffraction grating	1800 grooves/mm
Entrance slit	200 µm
Hole	400 µm
Number of spectra	15-20 spectra per drop
Post-processing	Polynomial baseline subtraction and unit vector normalization

Results

Ref: Rodà F et al. 2023 Nanomaterials.

LPs RAMAN SPECTRA

SG-17 mApoE LPs + G / SG-17 mApoE LPs + P / SG-17 mApoE LPs + T
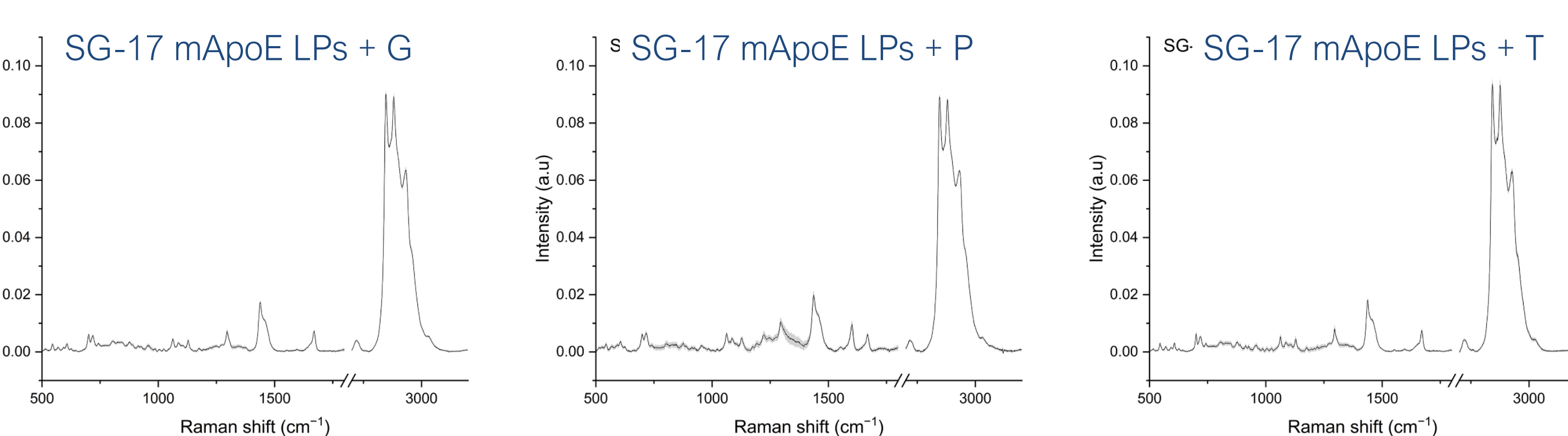
LP Raman spectra are a **combination of the signals of single components**, with prominent peaks confirming their composition

REPRODUCIBILITY OF LP SYNTHESIS

SG-17 mApoE LPs + G / SG-17 mApoE LPs + P / SG-17 mApoE LPs + T
(Batch 1, Batch 2)
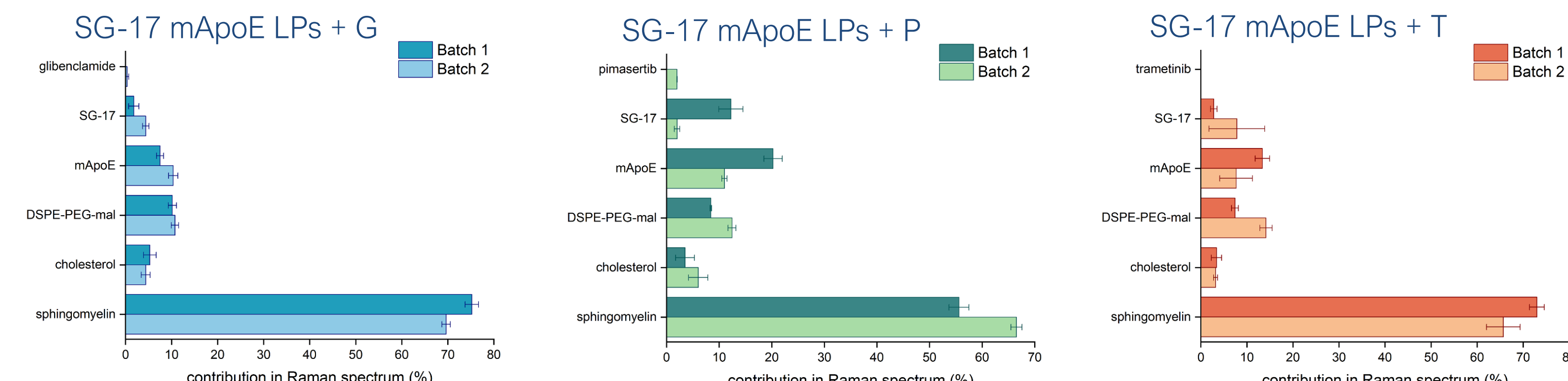
CLS analysis revealed **a good level of synthetic reproducibility**, showing <14% variations in the contribution of each component in the Raman spectrum of the same LP
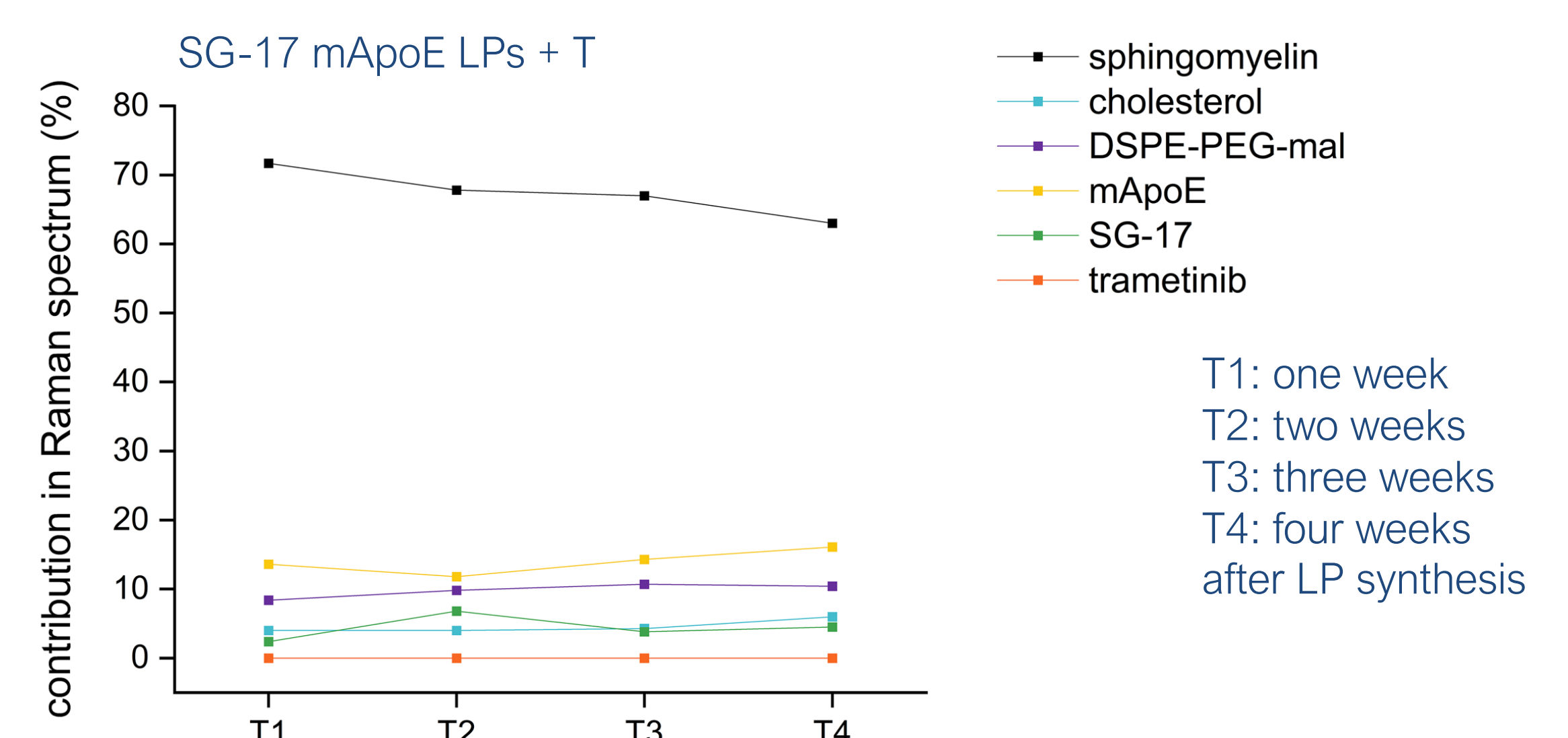
DISCRIMINATION OF LPs WITH DIFFERENT FUNCTIONALIZATION PATTERNS

(a) LPs / mApoE LPs / SG-17 LPs / SG-17 mApoE LPs
(b) PCA plot
(c) Mann Whitney test, *p>0.05
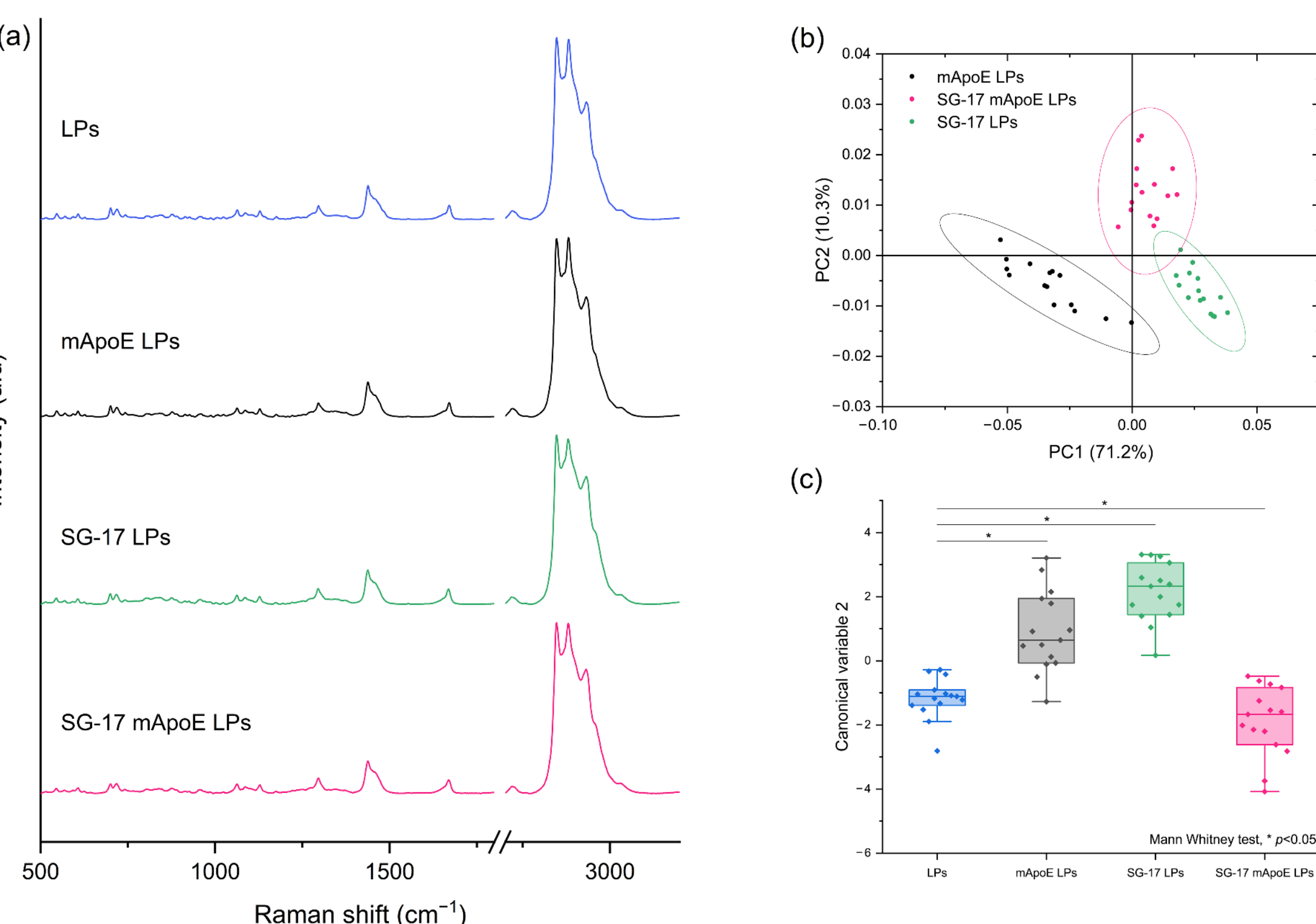
Each molecular component has an influence in the Raman spectrum of the final LP formulation

RS is able to **differentiate the differently functionalized LP formulations**

STABILITY OF LPs WITH TRAMETINIB

SG-17 mApoE LPs + T

Legend: sphingomyelin, cholesterol, DSPE-PEG-mal, mApoE, SG-17, trametinib

T1: one week
T2: two weeks
T3: three weeks
T4: four weeks
after LP synthesis

The variability is < 5% between subsequent weeks and < 9% within 1 month from the synthesis, **confirming the stability of these formulations for at least 1 month**

Conclusions

RS represents a valuable tool for **a fast, sensitive and label free biochemical characterization of LPs** that could be used for **quality control** of nanoparticle-based therapeutics
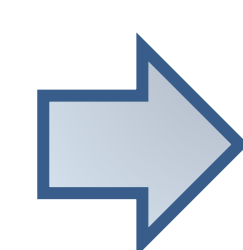
This innovation in the characterization of LP-based drug-delivery systems can be extended to different types of nanoparticles for the treatment of many diseases

Valentina Mangolini
IRCCS Fondazione Don Carlo Gnocchi
Laboratory of Nanomedicine and Clinical Biophotonics (LABION)

vmangolini@dongnocchi.it
labion@dongnocchi.it
@labion_
www.labion.eu
@labion.eu