AFM-IR enables single particle nanoscopic characterization and their label-free detection in cells

E. Pancani [1] , J. Mathurin[2], A. Deniset-Besseau[2], , S. Bilent[2], M-F. Bernet-Camard[3], K. Kjoller[4], C.B. Prater[4], A. Dazzi[2], R. Gref [1]

[1] Institut de Sciences Moléculaires d'Orsay (ISMO) CNRS UMR 8214, Univ. of Paris-Sud, Univ. Paris-Saclay, 91405 Orsay - France
[2] Laboratoire de chimie Physique , Univ. of Paris-Sud, Univ. Paris-Saclay, 91405 Orsay – France
[3] EA4043 "Unité Bactéries Pathogènes et Santé" (UBaPS), Univ. of Paris-Sud, Univ. Paris-Saclay, 92290 Châtenay-Malabry – France
[4] Anasys Instruments, Santa Barbara, CA - USA

Context

Aim:
Optimization of nanoparticles (NPs) for drug delivery necessitates a deep understanding of their morphology and structure → special emphasis on:

- **Drug location** (embedded in the core or adsorbed at the surface) → impact on the **release pattern: burst vs controlled**
- **Surface composition** → key role in the complex interactions with the living medium.

NPs characteristics:

Polymers used: **CORE→ polylactic acid (PLA) or polylactic-co-glycolic acid (PLGA)**
CORONA → polyvinyl alcohol (PVA)
Formulation technique: Nano-emulsion or nanoprecipitation
Characteristics: Hydrodynamic diameter ranging from 120 to 200nm, depending on formulation parameters

AFM-IR technique

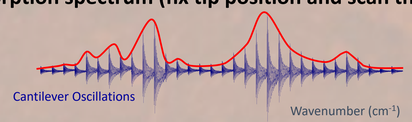
Atomic Force Microscopy (AFM) → high resolution but no chemical information
InfraRed (IR) spectroscopy → chemical signature of compounds but no resolution (bulk technique)

AFM-IR
combines the nanoscale spatial resolution of AFM and the chemical characterization offered by IR spectroscopy

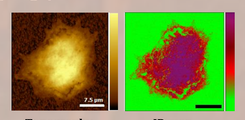
Cantilever tip acts as a sensor of the photothermal expansion induced in the sample when the excitation wavelenght = absorption wavelenght

Two acquisition modes:

→ Local absorption spectrum (fix tip position and scan the wavelength of the laser)



→ Chemical mapping (fix the laser wavelength and scan the surface with the tip)

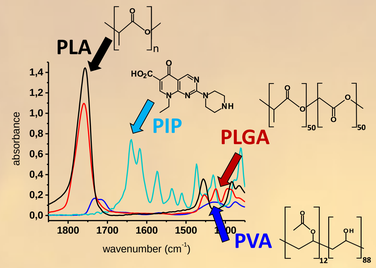


Example: amide I absorption in a cell

Employed laser: QCL → sensitivity ≤ 10 nm (depending on the selected setting: bottom-up/top-down, material of the AFM tip and of the sample support) .

A. Dazzi et al., ***Appl. Spectrosc.***, 66 , 1365 —1384 (2012).
Kurouski D., Dazzi A., Zenobi R., Centrone A., ***Chem. Soc. Rev.***,49, 3315-3347, *(2020)*.

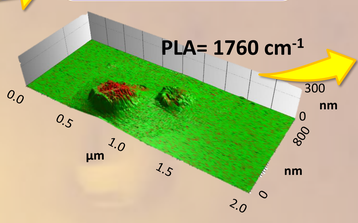
Particle-by-particle characterization



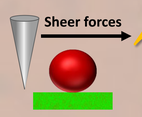
PLA PLGA + DRUG→PIP tried

AFM-IR in CONTACT MODE

BACKGROUND
In order to map the NPs component distribution, specific regions of the FTIR spectra of bulk materials were selected as fingerprints for PLA/PLGA, PVA and for the drug pipemidic acid (PIP) and were employed to specifically detect their distribution.



NPs loosely adhere to the support



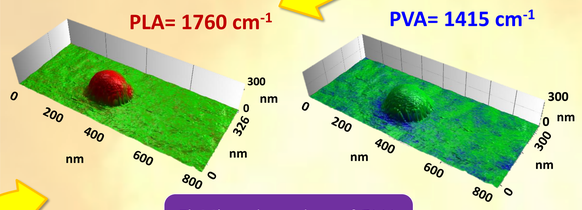
Displaced and/or crushed by AFM tip

NEW DEVELOPED AFM-IR TAPPING MODE

Despite non-linear interaction between the tip and the sample

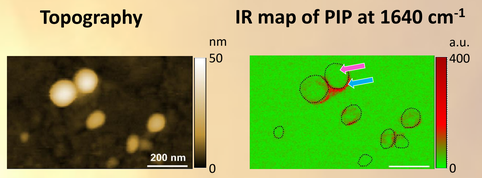
Thermal expansion ∝ Absorbance

Tapping AFM-IR allowed imaging the NPs without displacing or crushing them



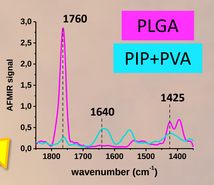
Fist time imaging of PVA corona around NPs

The IR absorption of PLA and PVA is represented through a color code in the 3D topographies, green corresponds to no IR absorption, whereas red and blue signals are attributed to PLA and PVA IR absorption, respectively.

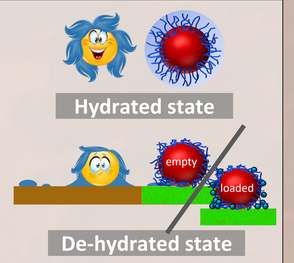


Fist time imaging of drug location:
PIP is found to be located on NPs surface
Colocalization of PVA and PIP in NPs corona

IR spectra recorded on PVA-PIP corona of 15 nm thickness



Note: drying effect on NPs



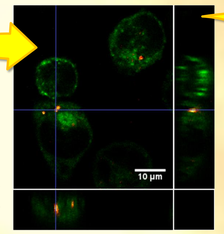
PVA/PIP layer on top of NPs is too thin to be detected and PLA/PLGA signal prevail.

Mathurin J.*, **Pancani E.*** *et al.* , ***Analyst*** *143, 5940-5949 (2018).* doi:10.1039/C8AN01239C.

Label free detection in cells

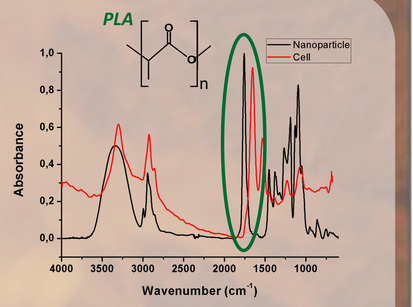
BACKGROUND
The **visualization** of organic polymeric NPs inside cells normally **requires grafting either fluorescent or electron dense probes** to perform fluorescence or transmission electron microscopy experiments enabling to follow NPs intracellular fate.

- Rhodamine(Rho)-labelled NPs
- Lipid rafts of THP-1 macrophage membrane tagged with Cholera-ToxinB-AlexaFluor488

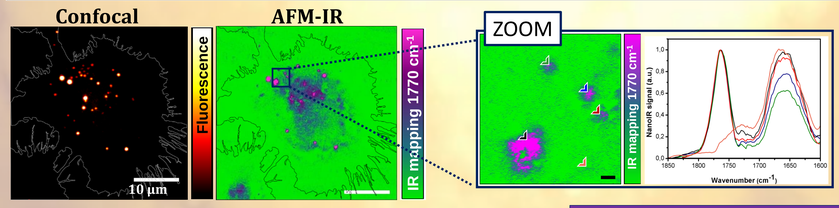


but

FTIR studies on bulk NPs and cells
Exploit
PLA → characteristic IR signature at 1760 cm-1
different from cellular components
TO PERFORM AFM-IR



Correlative microscopy → distribution of nanoparticles within macrophages



Correspondence between fluorescence and IR signal

Possibility to acquire spectra on a single nanoparticle → **allow to**:
- **Evaluate the state of the phagocytosis**
- **Evaluate the influence of the local environment on NPs**
- **Clearly discriminate the NPs from the other cellular compounds (spectrum in orange in zoom section)**

Detection of PLA NPs using the **intrinsic** signal of the polymer →
DIRECT OBSERVATION OF NPs → No need of labelling to obtain AFM-IR imaging

Pancani E. *et al..* ***Part. Part. Syst. Charact.*** *1700457 (2018).* doi:10.1002/ppsc.201700457.

Acknowledgements

Financial support for carrying out this work was provided by the European Community through the Marie Curie ITN "CycloN Hit"
Current funding → H2020-MSCA-IF-2018-843014 "POLAR STAR" / Current affiliation →CNR-ISOF, Via Piero Gobetti 101, 40129, Bologna, Italy