UNIVERSITÉ FRANÇOIS - RABELAIS TOURS

37th FACSS, Raleigh, 17-22 October 2010

Coupling Raman and fluorescence for confocal imaging of biological and pharmaceutical samples

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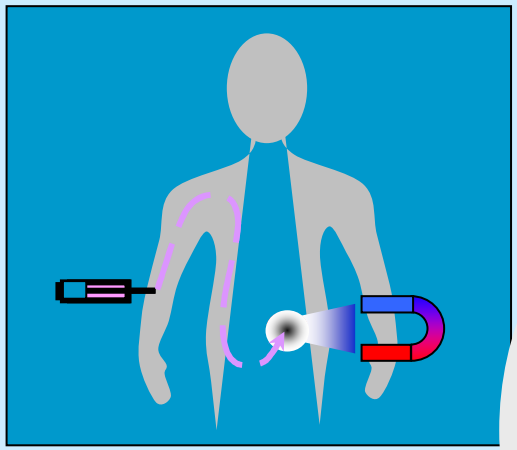
Faculty of Pharmacy,

Tours, France

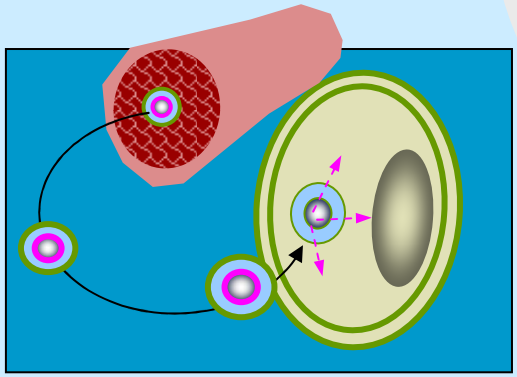
(http://www.pharma.univ-tours.fr/fmaa)

Nanoparticles for drug targeting and imaging

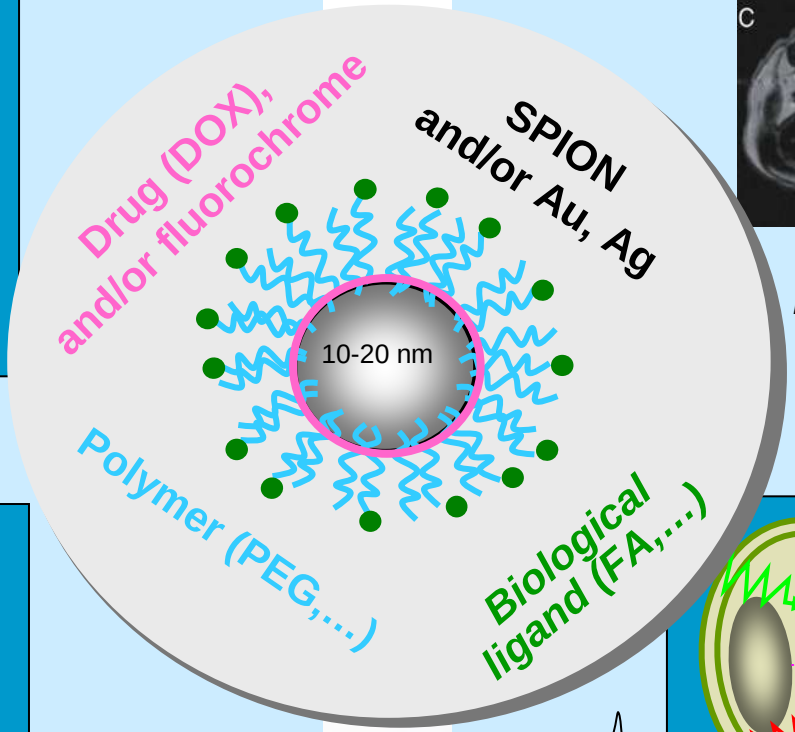
Anticancer drug targeting



external magnetic field

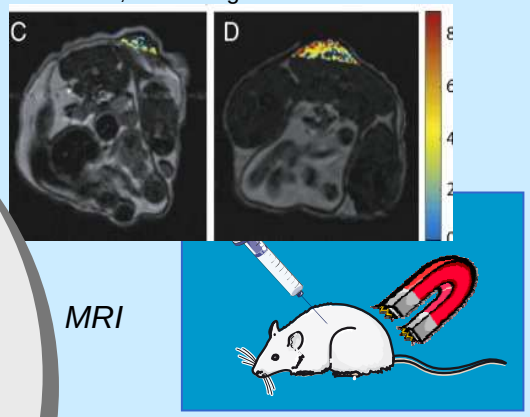


vascular permeability, biological recognition of cancer cells

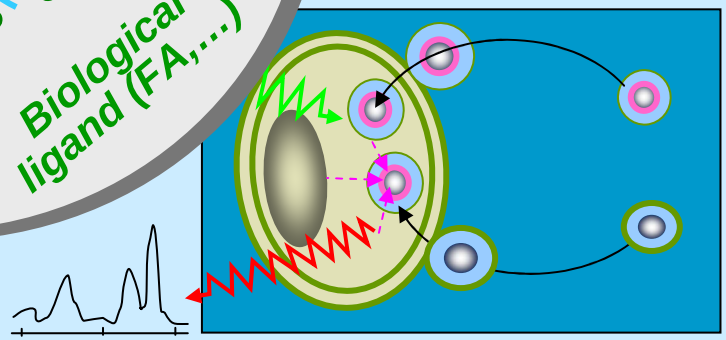


Imaging of anticancer drugs/cancer cells

Sun et al., Adv. Drug Deliv. Rev. 2008



MRI



SERS and/or fluorescence

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Nanoparticles for drug targeting and imaging

Anticancer drug targeting

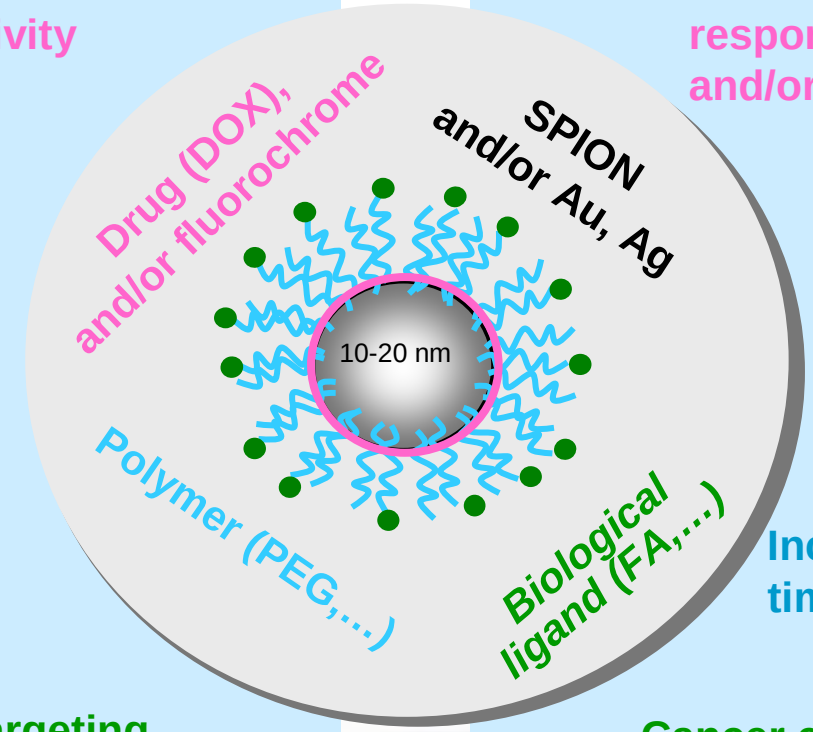
Anticancer activity

Magnetisation without remanence

Surface plasmons

Colloidal stability, biocompatibility, drug protection

Cancer cell targeting, intracellular action



Imaging of anticancer drugs/cancer cells

response in fluorescence and/or in Raman

MRI

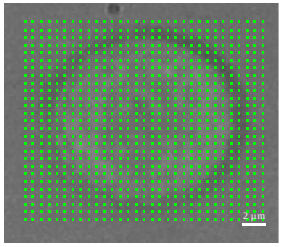
SERS

Increased circulation time in blood

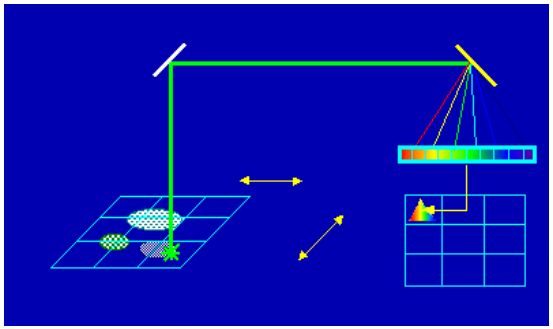
Cancer cell labelling

Analytical approach by confocal spectral imaging

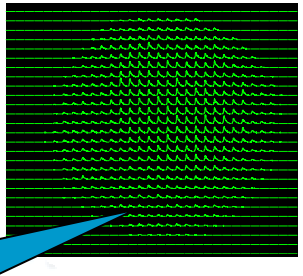
example: subcellular distribution of fluorescent drug MTX

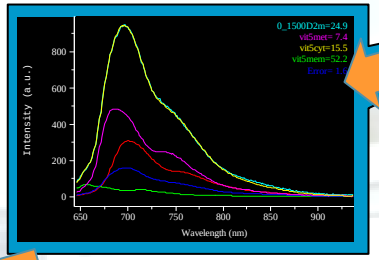


Selection of a treated cancer cell

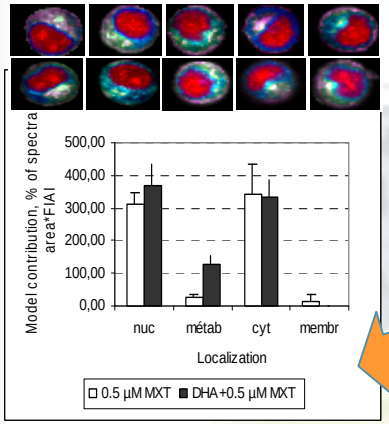


Spectral acquisition:
- 0.02 sec per spectrum

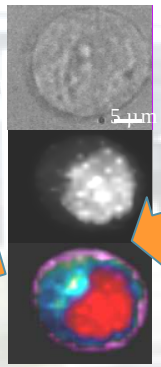




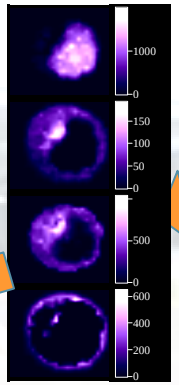
Multispectral map:
30x30=900 spectra



Statistics over >20 cells

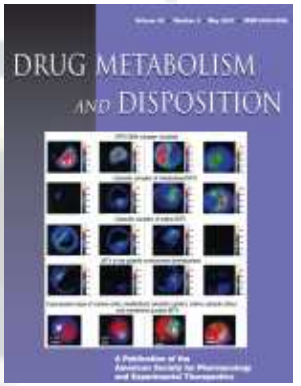


Co-localisation



drug-DNA
metabolite
drug-cytopl complex
drug-membrane

Treatment of the map :
1) Spectral : intensity / shape
2) Statistics: average, std deviation,...



Vibet et al., DMD, 2007

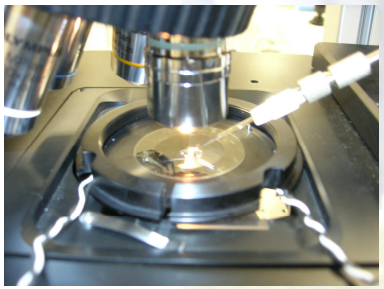
Instrumental (molecular optical spectroscopy)

Confocal microspectrometer LabRam (Horiba Jobin Yvon)

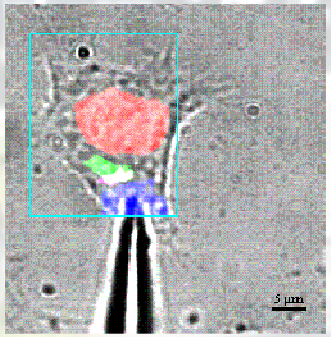
Olympus- BX40, obj. x100; x50, x50LWD, x10
Exc. 457, 488 et 514,5 nm (Ar+ laser)
Exc. 632,8 nm (He-Ne laser)
Exc. 785 nm (diode laser)

X-Y-Z motorised plate (0.1 µm / step, 0.5 µm in Z)
Gratings 300 ou 1800 ‘ / mm;
CCD 1024x256 pixels, air-cooled (–70 ℃)

Fast switch between excitation wavelength and between gratings



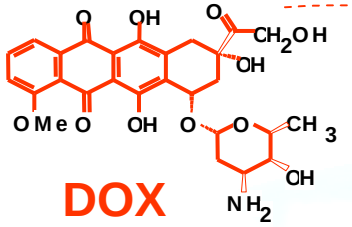
Accessories for live cell manipulation:



- Thermostated microscopy chamber
- Micro-manipulated femto-injector
  - (tip ext. diameter 0,9±0,3 µm)

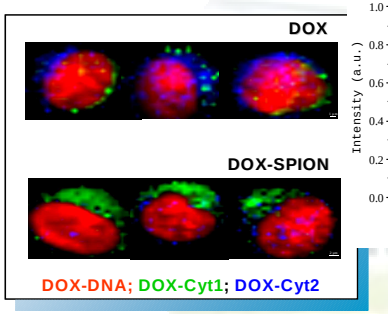
How do NP and drugs interact within a live cell?

To kill cancer cells, doxorubicin (DOX) **needs to attain nucleus (DNA intercalation, action against the DNA topoisomerase)**



DOX-Fe^{2+} adsorbed on SPION

(pH-dependant release)

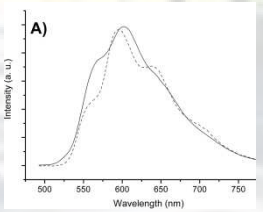


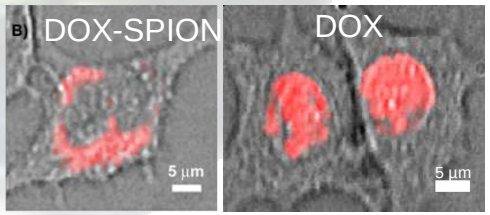
DOX covalently bound to SPION

(enzyme-dependent release)

Munnier et al.,
J. Nanopart. Res 2010
Int J Pharm 2008.

Shkilnyy et al.,
J. Phys Chem. C. 2010



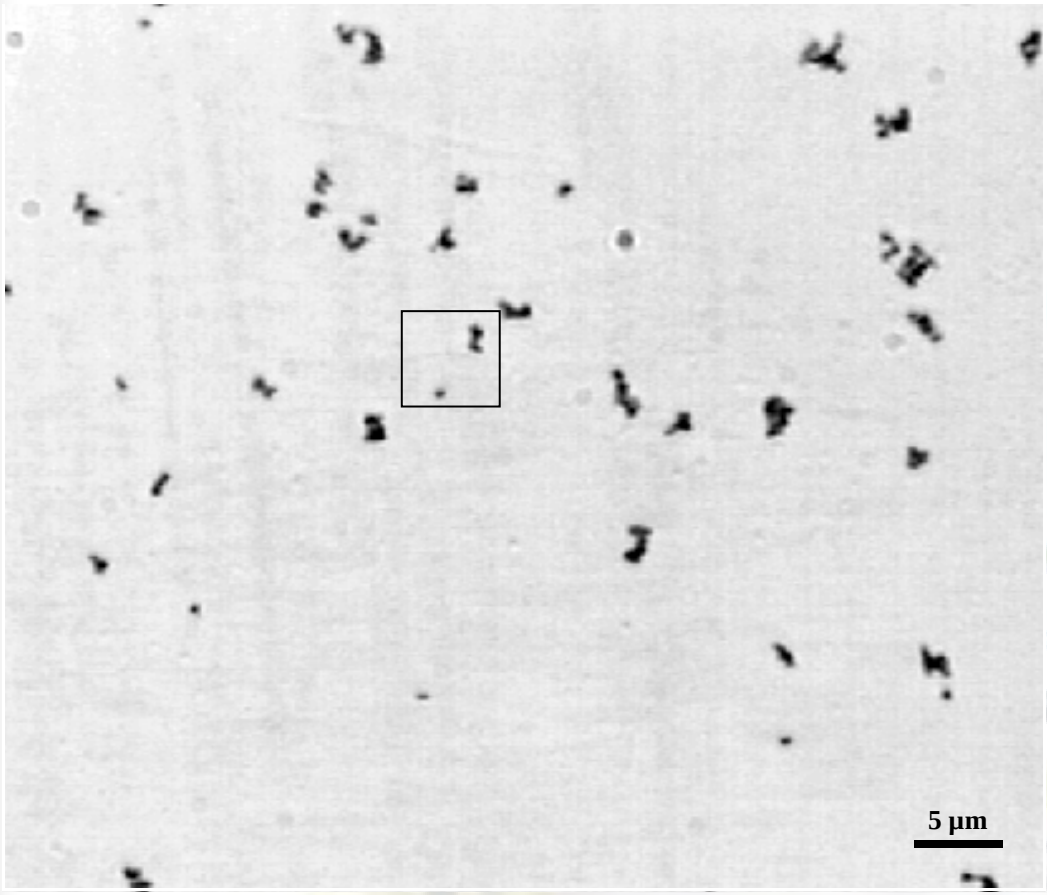


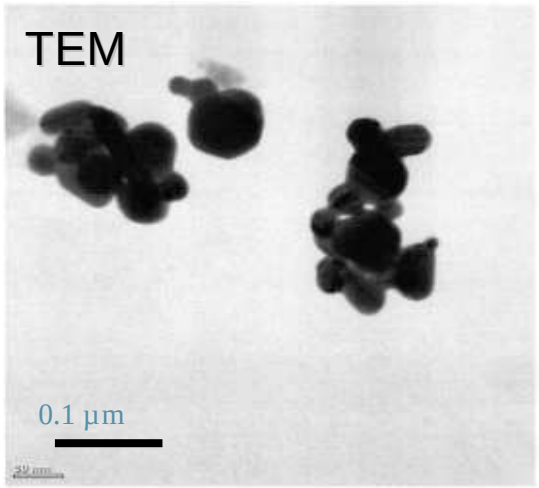
More specific molecular information from Raman spectra?

- Sensitivity of conventional Raman is low compared to anticancer drug concentration (<1µM)

- SERS (surface-enhanced Raman scattering) is sensitive enough, but needs a noble metal substrate

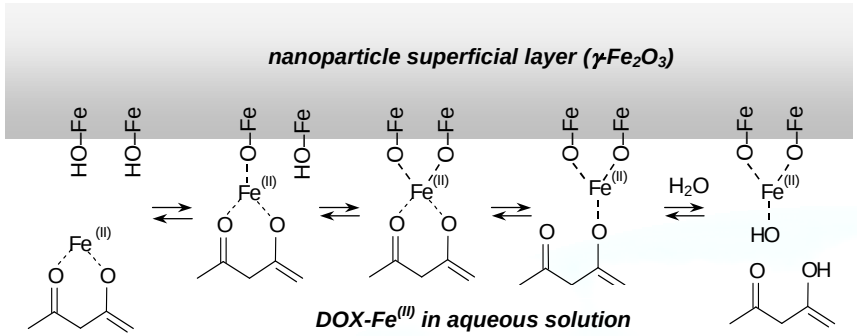
SERS substrates : plasmonic NP (Ag, Au)

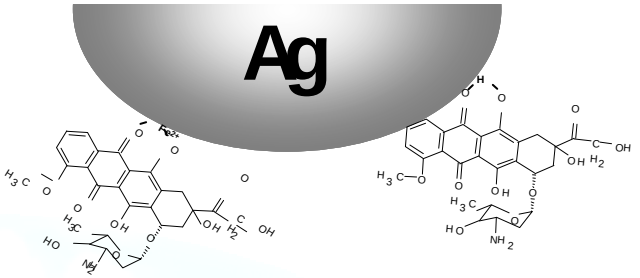


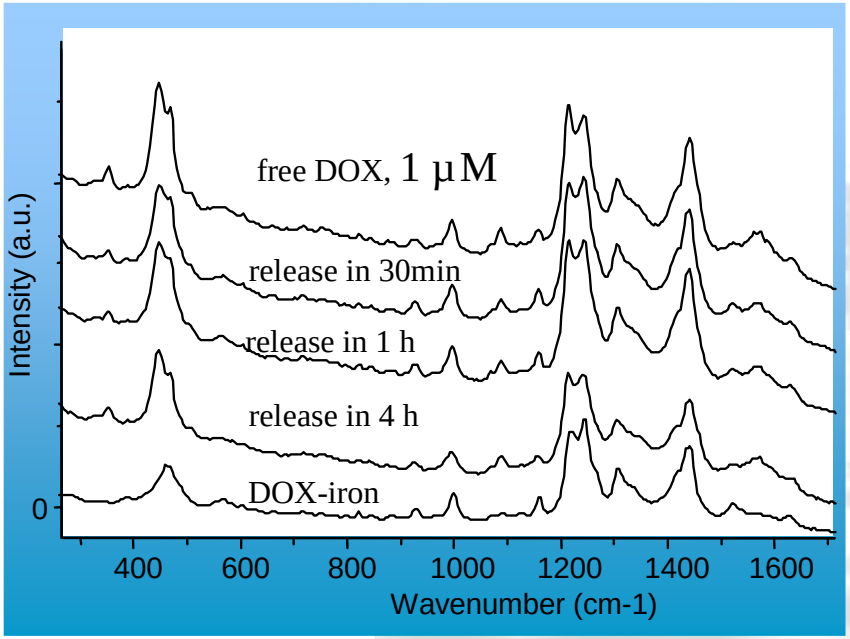


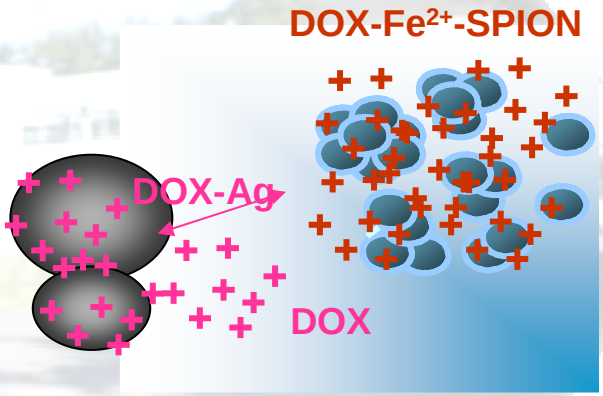
Ag-citrate NPs: aggregate in PBS and in cell

SERS spectroscopy on NP Ag-citrate :
analysis of anticancer drug doxorubicine (DOX) released from SPION



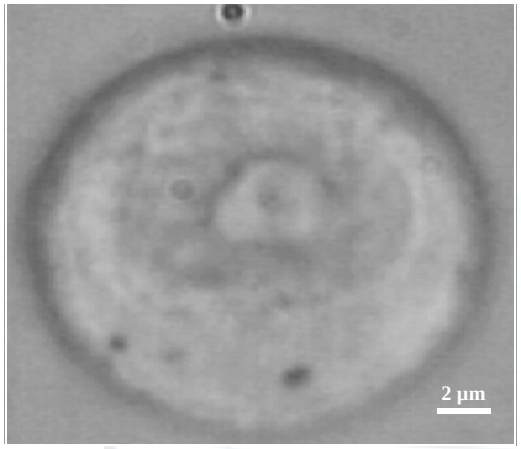




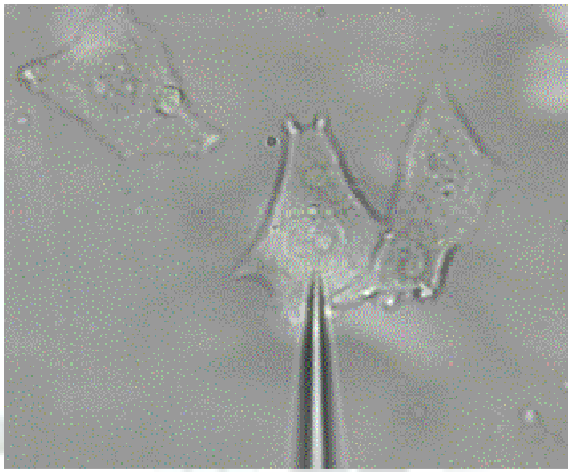


How to apply conventional NP-substrates for SERS spectroscopy and imaging of anticancer drug in live cells?

centrifugation



Micromanipulated femtoinjection



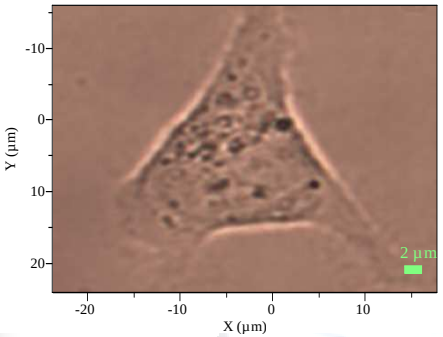
Problems with :

1 – Uncontrolled NP aggregation
2 – Uncontrolled NP distribution
3 – Perturbed cell environment (moderate if few small aggregates/cell)
4 – Limited mapping zones (spots around aggregates)
5 – Uncertain origin of the signal

What about coupling SERS with fluorescence ?

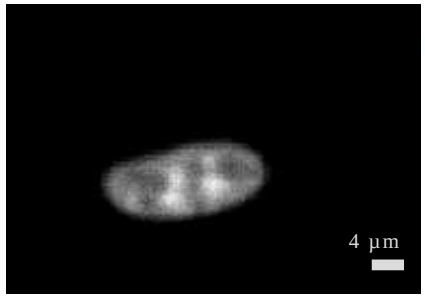
Instrumental coupling : Combined band-pass and spectral imaging modes on the same microscope

Video capture



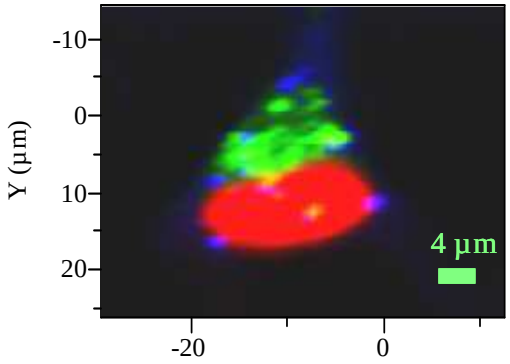
MCF-7 cancer cell
Treated with DOX

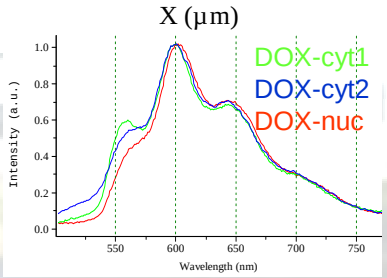
Band-pass confocal microscopy mode



Most intense DOX fluorescence is in the nucleus

Spectral imaging mode : fluorescence, Raman





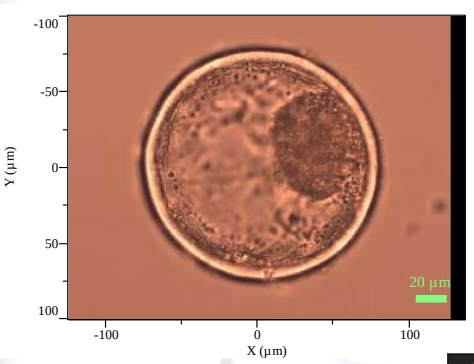
Spectral analysis reveals drug molecular interactions

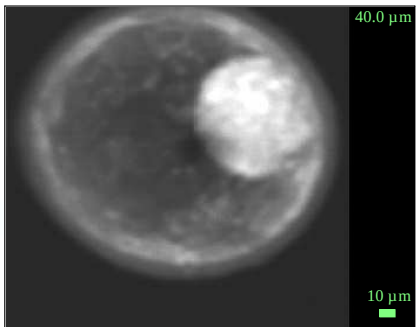
Instrumental coupling : Combined band-pass and spectral imaging modes on the same microscope

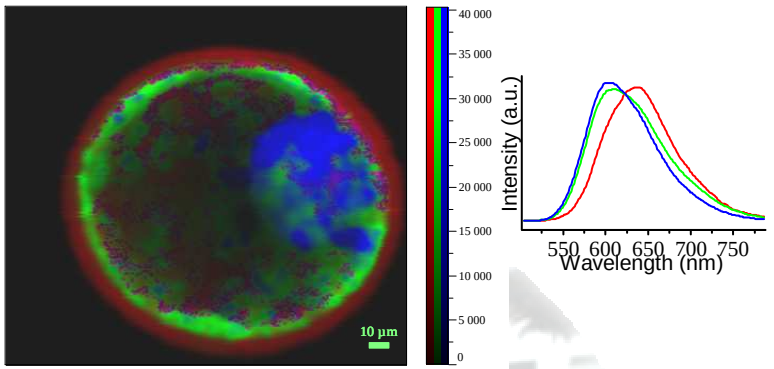
Video capture

Band-pass confocal microscopy mode

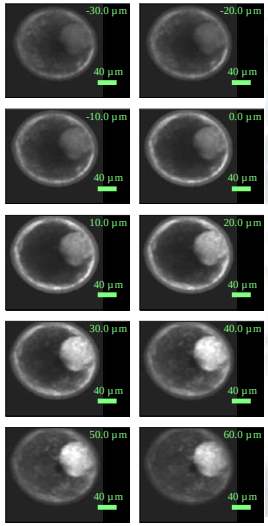
Spectral imaging mode : fluorescence, Raman







Bovin embryo incubated with Nile Red fluorochrome



Fast selection of slice of interest

400x400 points
4 sec per slice

Spectral analysis reveals lipidic content of the sample

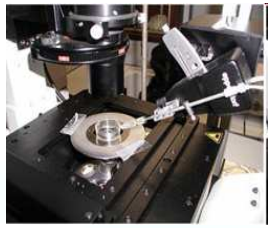
~35000 spectra recorded in 3 min, (SWIFT™ acquisition mode, Horiba Jobin Yvon)

Details of the instrumental innovation

- **Combined band-pass and spectral modes for fast confocal 3D imaging**

XploRA™ *INV*

HORIBA Scientific



Open inverted microscope

- dedicated for LifeScience and biomedical applications (cells, tissues…)

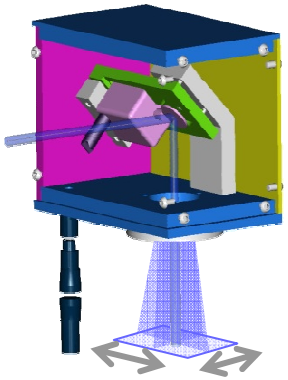
- easy integration of AFM module or injection devices

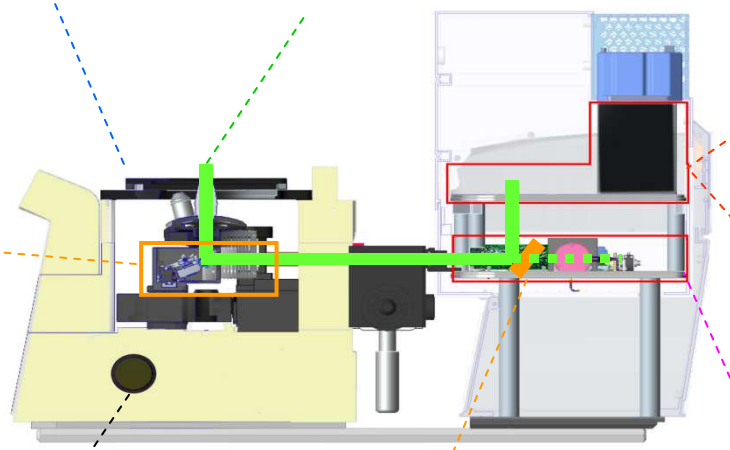
3 integrated laser sources

large samples variety

DuoScan™ option

laser scanning device





spectral confocal mapping module

compact and rugged

SWIFT™ acquisition mode

rapid spectra acquisition

band-pass confocal microscopy module

fast 3D imaging option

Z drive

(3D scan)

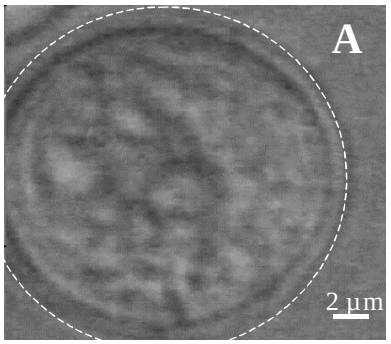
Splitting optics

(switch between modes)

Methodological coupling: Simultaneous co-detection SERRS-fluorescence on the same spectral image

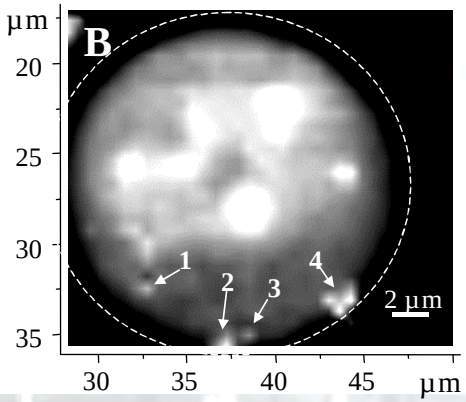
Chourpa et al., Chem. Soc. Rev. 2008

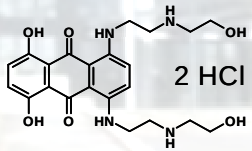
Video capture



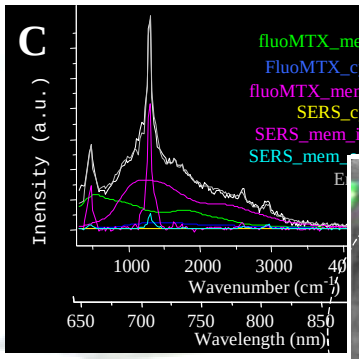
MCF-7 cancer cell treated with 1 µM MTX and incubated with NP Ag-citrate

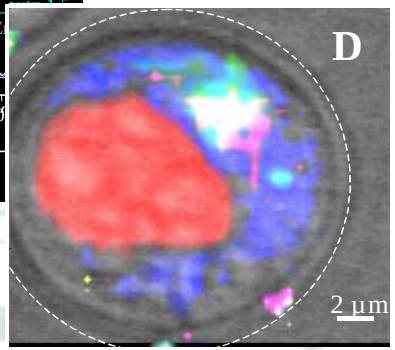
Spectral intensity



MTX :  2 HCl

Spectral analysis : SERRS and fluorescence





Rem 1:

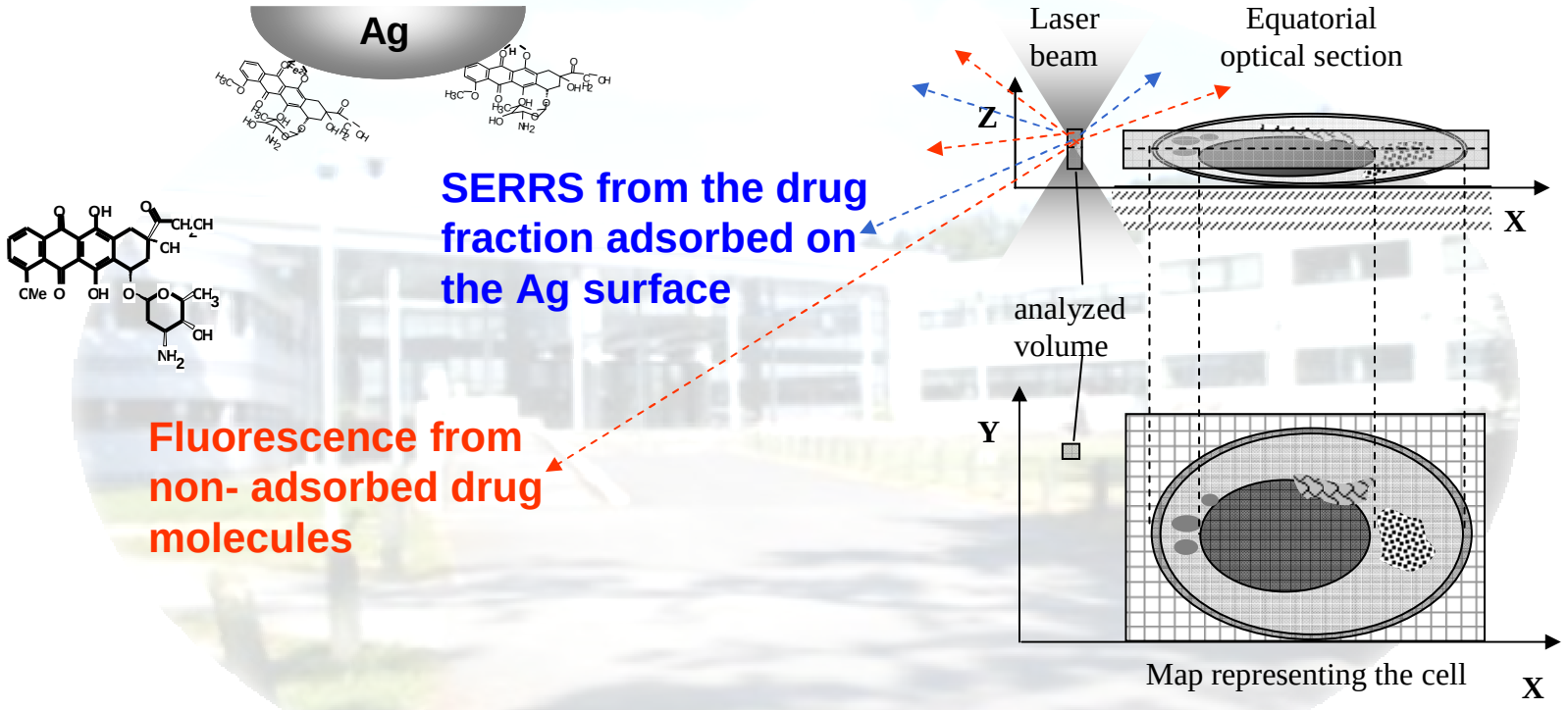
Aggregates ≤ 1 µm are detectable

Rem 2 :

SERRS and fluorescence spectral informations are complementary

Details of the methodology

- Few intracellular Ag NPs
- Resonance excitation wavelength to co-detect SERRS (resonance SERS) and fluorescence of the drug

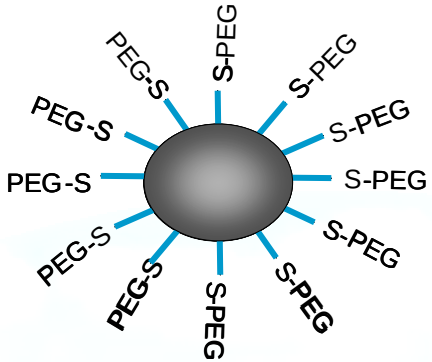


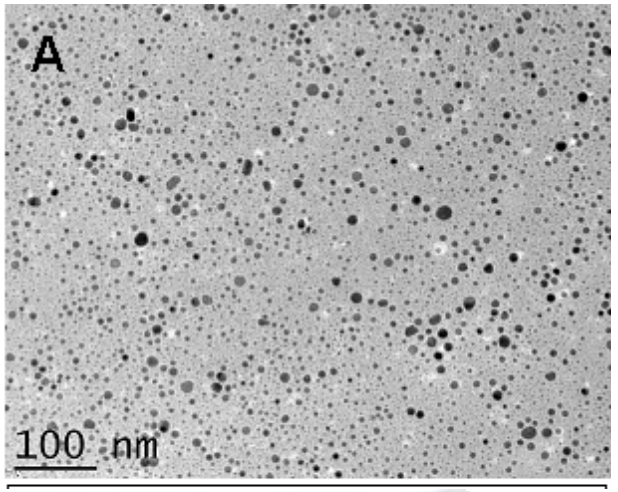
Nanotechnology benefits: use polymer-coated SERS substrates

Ex: $AgPEG_{5000}$, Shkilnyy et al., Analyst 2009

One-pot synthesis

$$AgNO_3 + NaBH_4 + PEG\text{-}SH \xrightarrow[N_2]{\text{ethanol}}$$





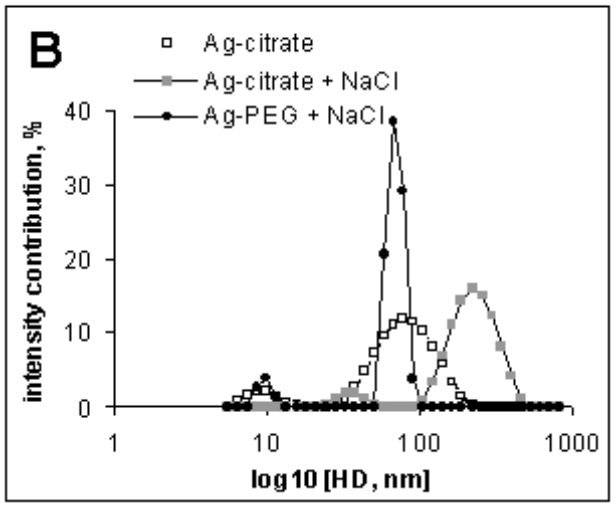
Homogeneous and small size : 10-20 nm

Aggregation-free system

Biocompatible, stealthy coating (PEG_{5000})

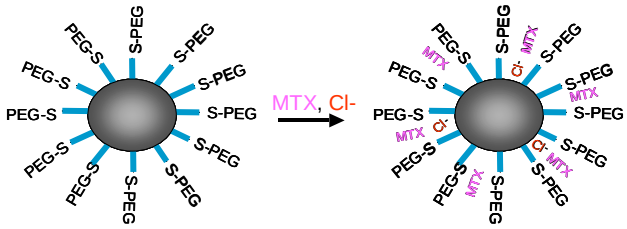
Polymer can carry biological ligands

enhancement?



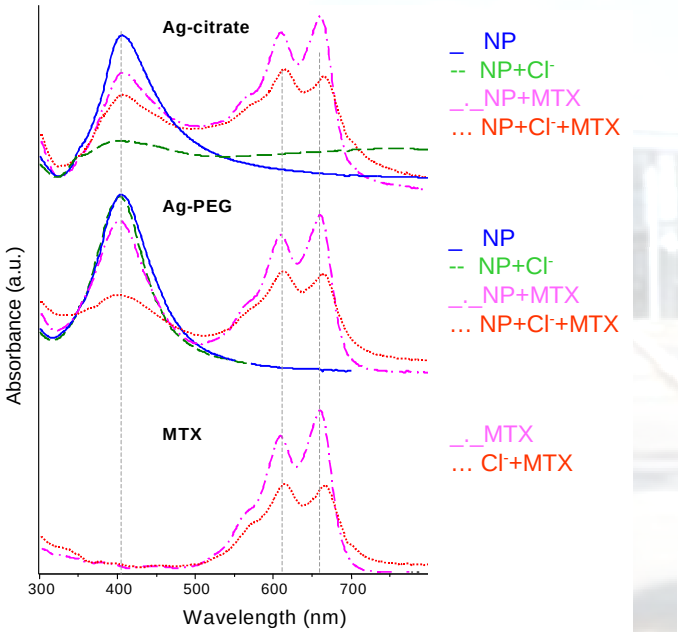
Detail of the methodological approach:

SERS of anticancer drug MTX on NP AgPEG$_{5000}$

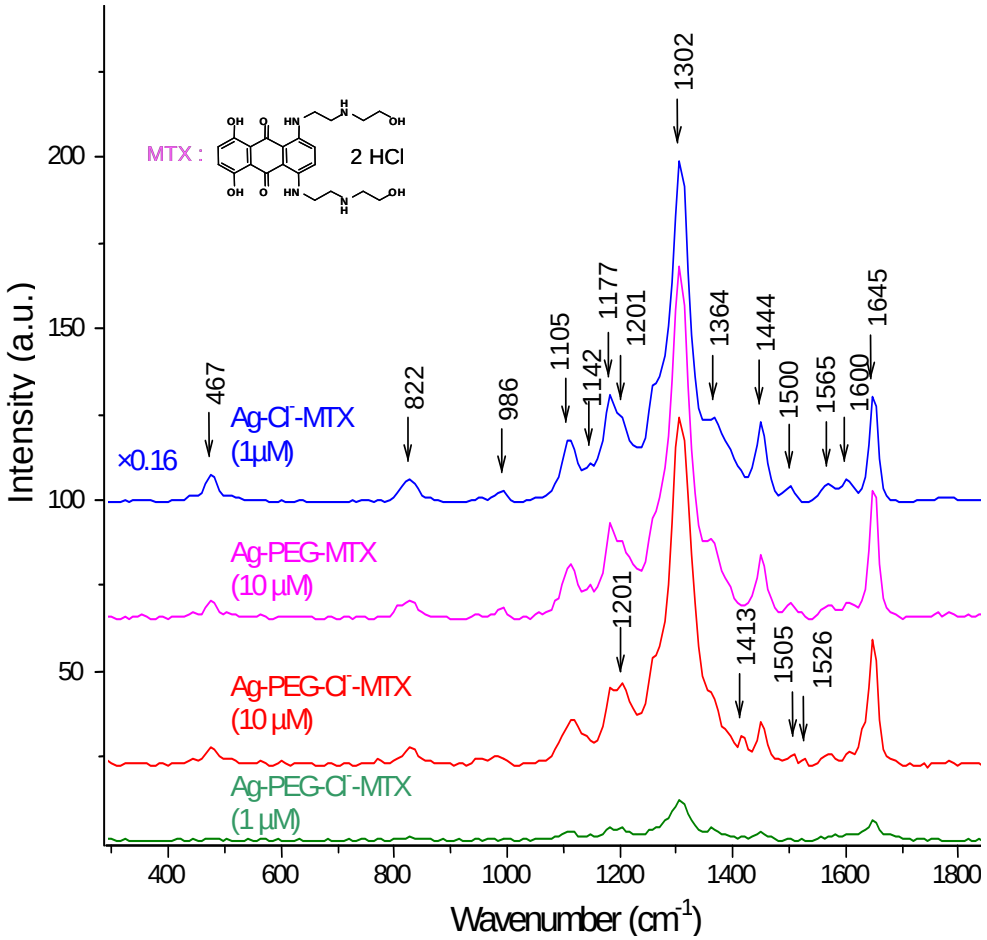


Shkilnyy et al. Analyst 2009

Drugs and ions diffuse through the PEG layer to the Ag surface

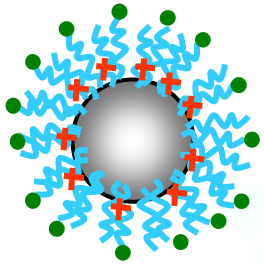


« hot spot-free » SERS is detected, enhancement is ~10^2 fold lower

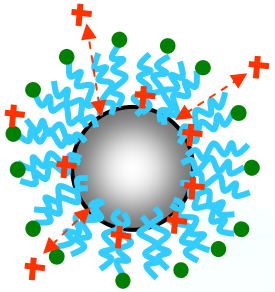


Development of novel, polymer-coated NP-substrates for intracellular SERS spectroscopy and spectral imaging

labelling



analytical spectroscopy



drugs, Ions, ..

Challenges:

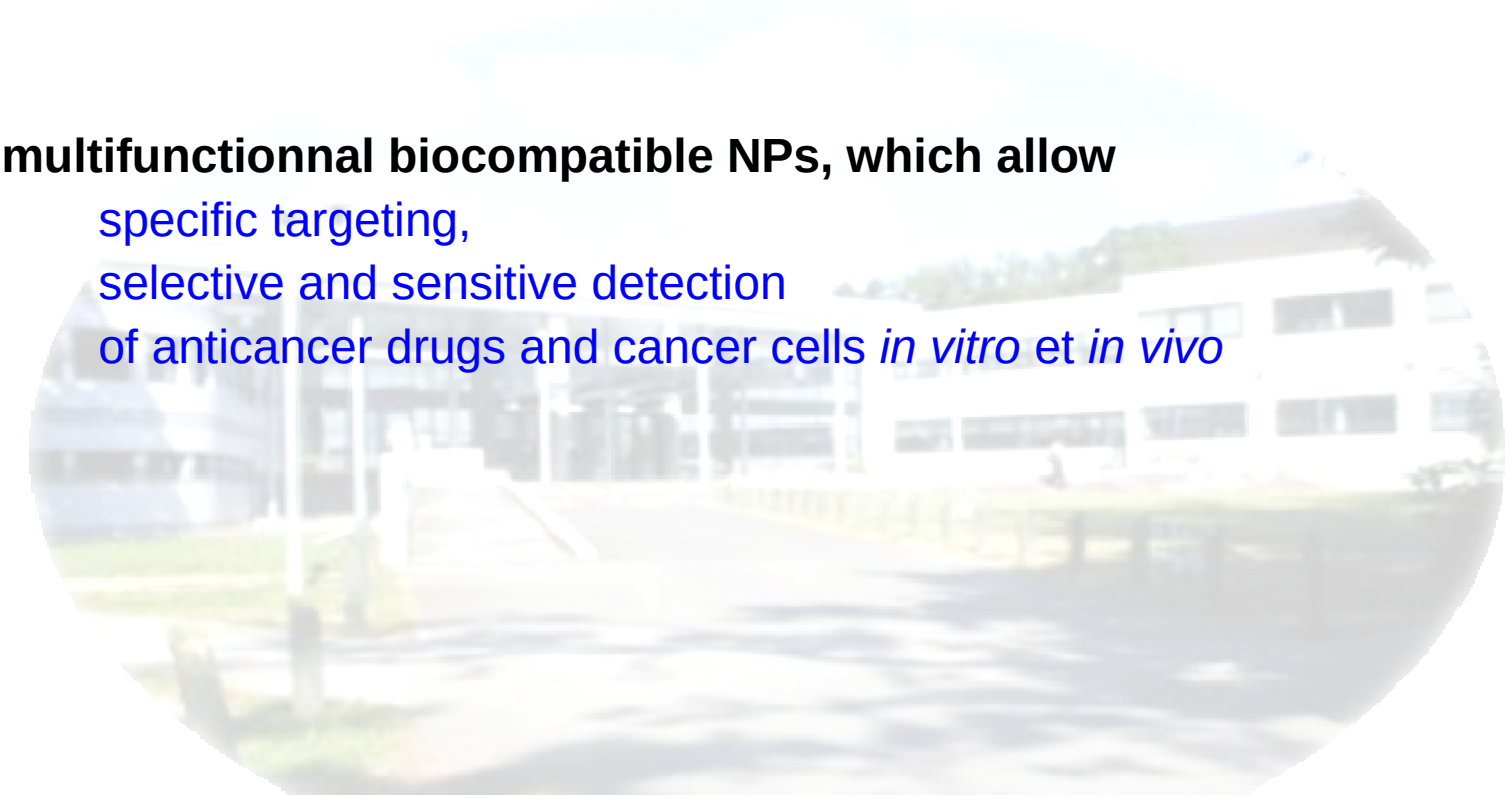
- **Selective adsorption/enhancement of chromophores**
- **Aggregation-free enhancement**
- **Biological and/or magnetic targeting of NP to cancer cells and selective binding to intracellular compartments**
- **=> Converging to nanocarrier technology**

=> composite multifunctional NP

Conclusion and perspectives

- **Performant bio-analytical approaches can be developed**
  by means of confocal imaging, spectral and conventional,
  coupling of several techniques (SERS, fluorescence, MRI)
  and based on…

- **... multifunctionnal biocompatible NPs, which allow**
  specific targeting,
  selective and sensitive detection
  of anticancer drugs and cancer cells *in vitro* et *in vivo*



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