Consiglio Nazionale delle Ricerche
Istituto per l'Energetica e le Interfasi

Conferenza di Istituto **Padova, 29 Febbraio – 1 Marzo 2016**

Interazione di nano-materiali con strati interfacciali modello: potenziali effetti su sistemi biologici e sviluppo di nuovi materiali per applicazioni biomediche

Francesca Ravera

U.O.S Genova

Sistemi molecolari e nanostrutture per la nanomedicina

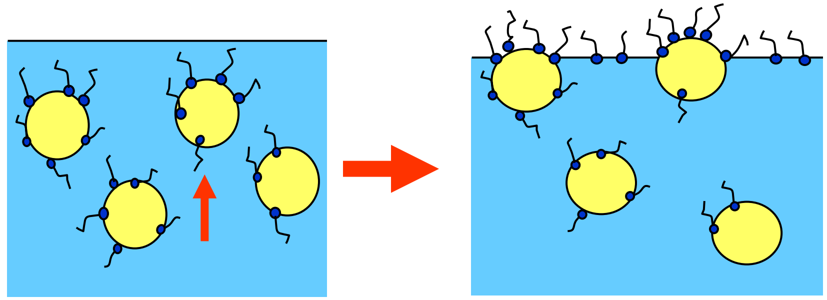
Tematiche di ricerca presso IENI-GE:

1. Interazioni di nanoparticelle solide con strati lipidici modello per sistemi biologici (surfattante polmonare, membrane cellulari).
2. ‘’Assembling’’ di micro-nanostrutture bidimensionali alle interfacce liquide
3. Proprietà interfacciali e citotossicità di derivati di antibiotici da irraggiamento laser in tessuti biomedicali
4. Sintesi di nanoparticelle con proprietà non lineari del secondo ordine per applicazioni nella diagnostica biomedicale.

1. Interazioni di nanoparticelle solide con strati lipidici modello per sistemi biologici (surfattante polmonare, membrane cellulari).

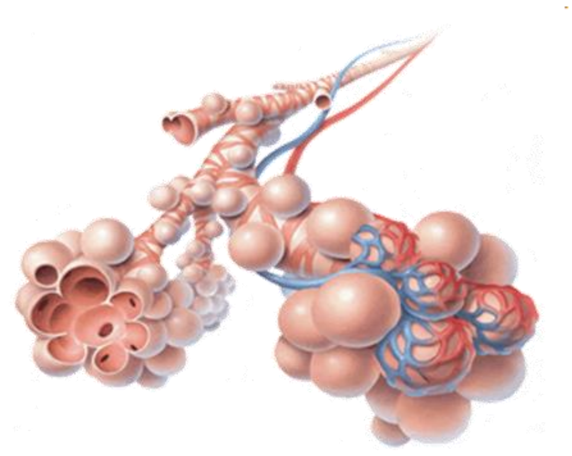
Background

- The transfer/incorporation of micro-nanosied solid particles into an adsorption interfacial layers may modify their mechanical, physico-chemical properties



- Lipid monolayers spread at water-air interfaces are good models for biologically relevant systems (lung surfactants, cell membranes

- Lung Surfactant: multi-component liquid film overlaying the alveola walls, responsible of the mechanical stability during the breathing cycles



- Surface Tension and dilational rheology are key properties for lung functionality

A **physical chemistry approach** to investigate the adverse effects of nanoparticles: the **NIPS** project

NIPS - Nanoparticle Impact on the Pulmonary Surfactant Interfacial Properties

Funded by IIT-Seed 2009 (2011-2014)
Participants: F. Ravera (resp.), L.Liggieri, M.Ferrari, E. Santini

Aim

Understanding of the NP effects on the PS interfacial properties relevant for lung functionality and identification of the key NP features responsible for them

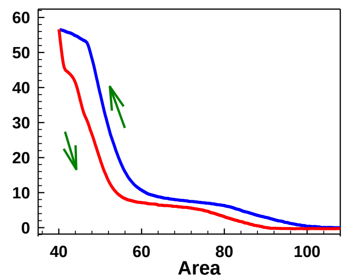
Motivations

- Increased presence of micro-nanoparticles in the environment
- Needs of evaluating the potential risks for health (ex. Respiratory physiology)
- Increasing need of protocols and in-vitro toxicological tests

Investigation Strategy

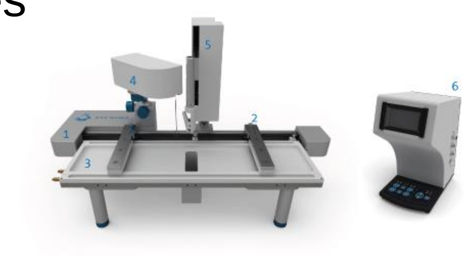
- **Selection of NP** relevant for the aim of the project
- Definition of **model lipid mixtures** relevant for mimicking PS functionality
- Study of the NP effects on the **mechanical and structural properties** of these layers
- Development of appropriate **experimental methodologies**

Techniques



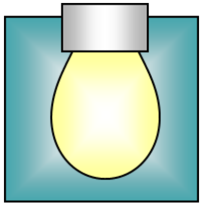
Langmuir trough and Drop/Bubble tensiometers:

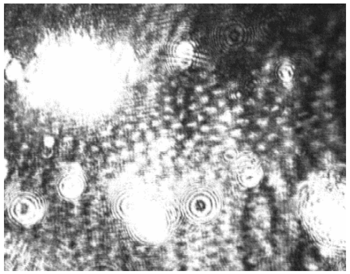
- 2D phase behaviour, Π-A isotherms
- low frequency dilational rheology
- Response to simulated respiratory cycles

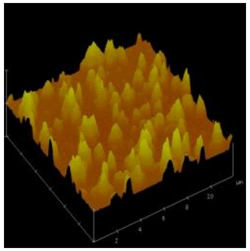


Brewster Angle Microscopy (BAM)

- 2D structure of the layer







Ellipsometry

- Layer thickness

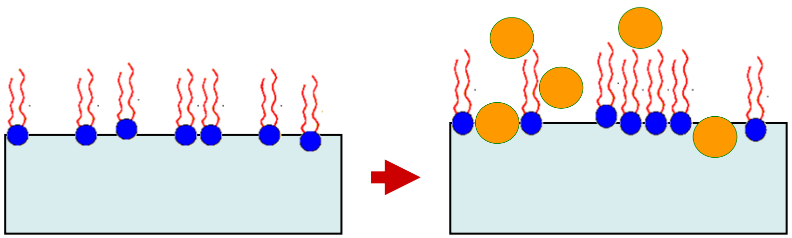
Langmuir-Blodgett Deposition + AFM

- Morphology of the aggregates

Nanoparticle incorporation into lipid monolayers

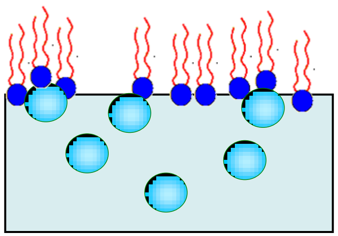
Lipid monolayers spread on aqueous sub-phases

Hydrophobic particles



- NP spread onto pre formed lipid monolayer

Hydrophilic particles



- Lipid spread on NP suspension
- NP transferred from aqueous sub-phase
- Process driven by NP - lipid molecule interaction

Methods of investigation

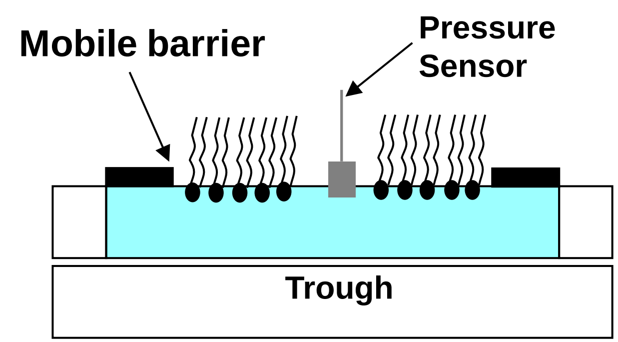
Langmuir trough:

2D phase behavior, Π-*A* isotherms

and low frequency dilational rheology

Brewster Angle Microscopy (BAM)

2D structure of the layer



1. Role of the particle chemical nature

Carbon Black

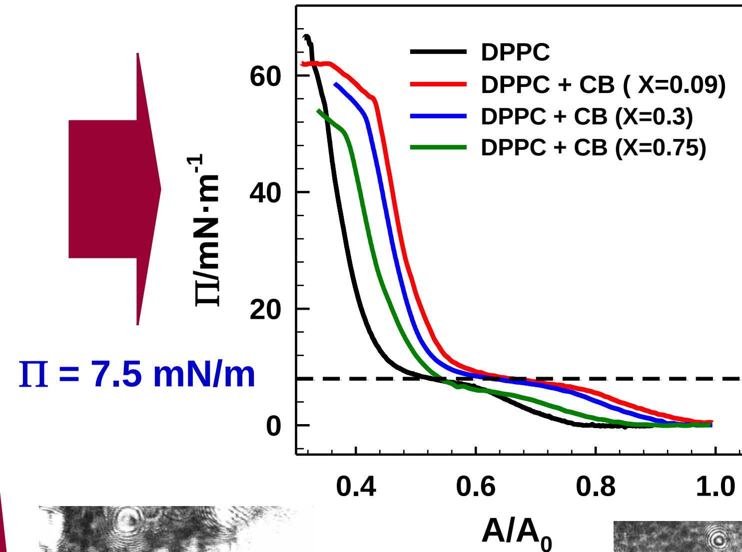
aggregates of spherical NP
Φ = 100-120 nm

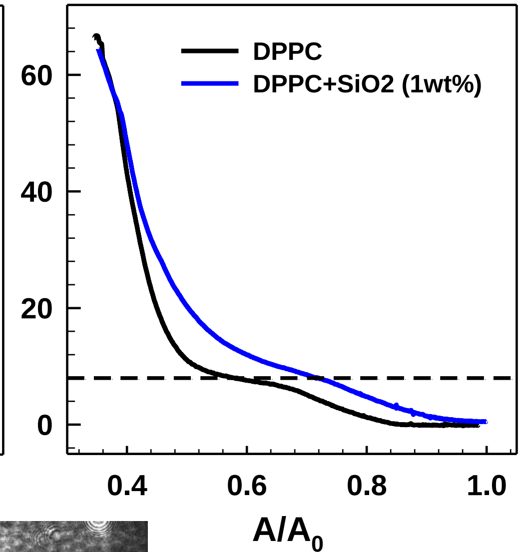
Silica NP

colloidal suspension
(**Levasil®** 200/30%)
Φ = 15 nm

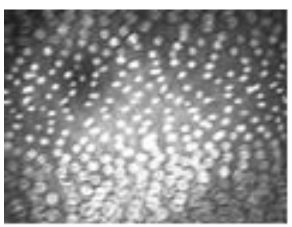
Effect on the DPPC phase behaviour

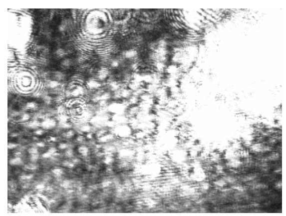
Π-A Compression Isotherm by the Langmuir trough

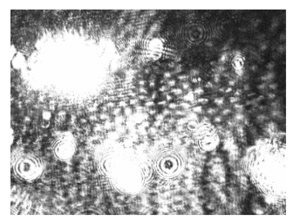


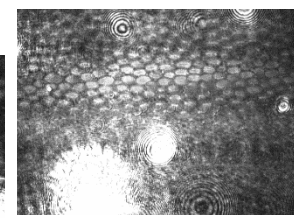


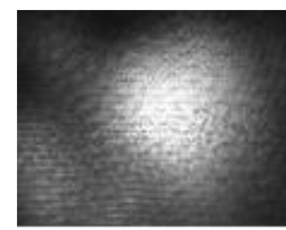
Effect on the LE-LC coexistence phase











Monitoring of the LC domain distribution by Brewster Angle Microscopy (BAM)

Carbon – DPPC hydrophobic interaction

- **Shift to higher areas (excluded area effect)**
- **Decrease of collapse pressure**

Silica NP– DPPC electrostatic interaction

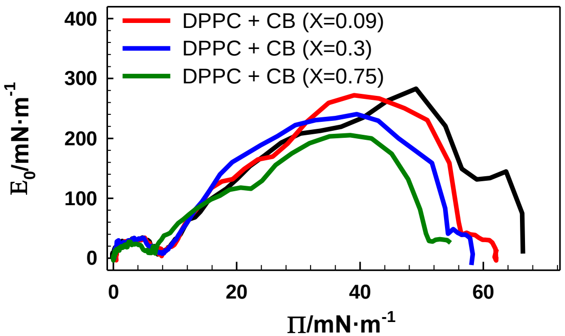
- **hundering of the LC domain growth**
- **Smaller and circular domains**

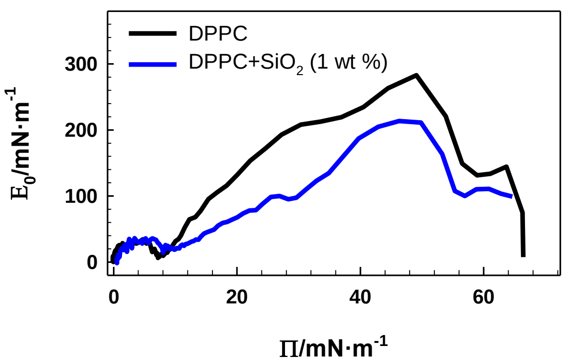
•*Guzmán et al., J. Phys. Chem. C, 115 (2011) 21715*
•*Guzmán et al., Colloids Surf. A 413 (2012) 280*

Dilational rheology

Quasi-static dilational elasticity from the compression isotherm

$$E_0 = -A\left(\frac{\partial \Pi}{\partial A}\right)_T$$



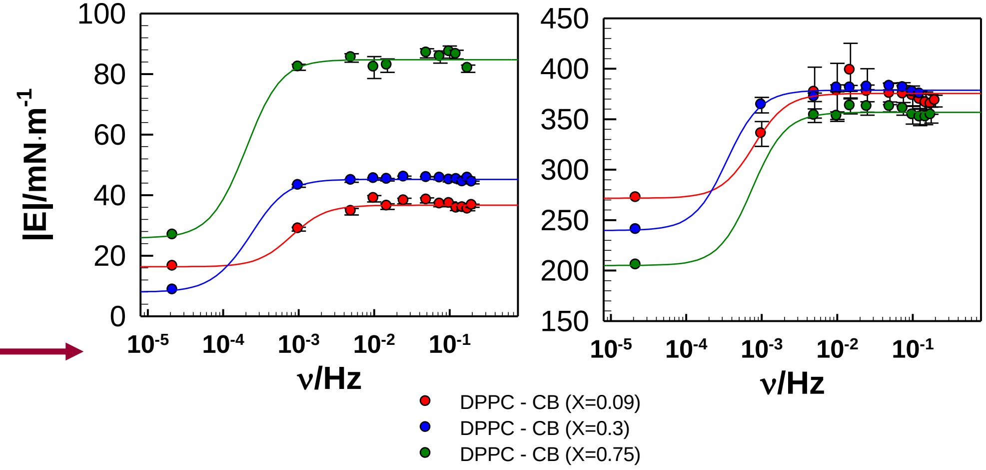


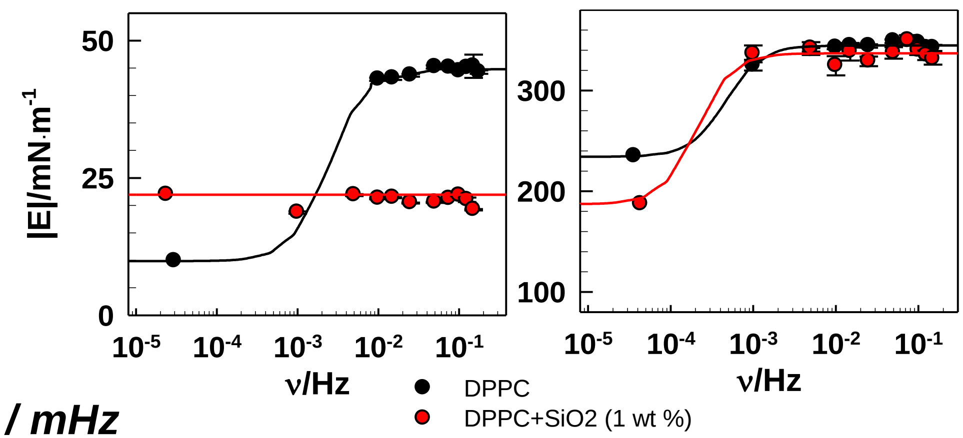
Carbon Black

Silica NP

Dilational viscoelasticity vs. frequency

Π=7.5 mN/m   Π=40 mN/m





ν_k / mHz

Π / mN/m	*DPPC*	*SiO2-DPPC*
7.5	*3.4*	*--*
40	*0.5*	*0.2*

Fitting equation

$$|E| = \sqrt{\frac{E_1^{\,2} + E_0^{\,2}\left(\nu/\nu_k\right)^2}{1+\left(\nu/\nu_k\right)^2}}$$

- ➢**Effect on the reorganization kinetics in the layer (ν_K and E_0,E_1)**
- ➢ **For Silica NP purely elastic layer (phase transition hindered)**

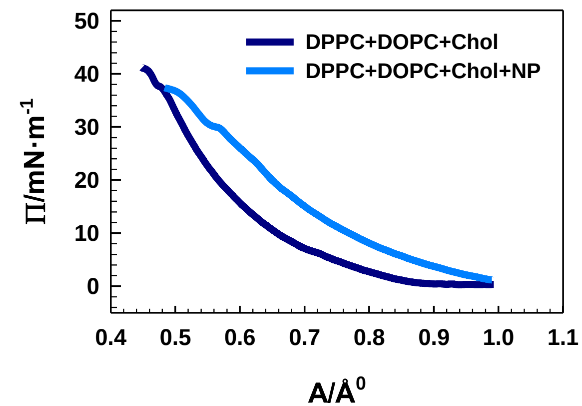
2. Silica NP into mixed DPPC-DOPC- Cholesterol layer

Relevant for interaction with biological systems

Effect on Phase Behavior

- ➢ **excluded area effect**
- ➢ **reduction of the collapse pressure and of quasi-equilibrium elasticity**

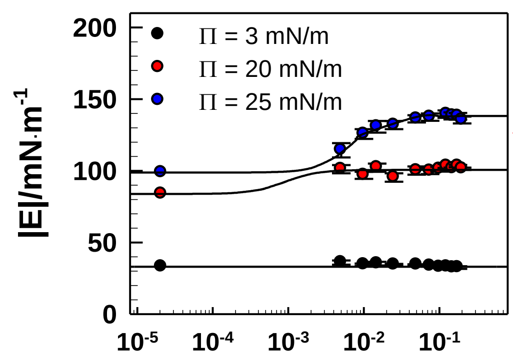
Π-A Compression Isotherm



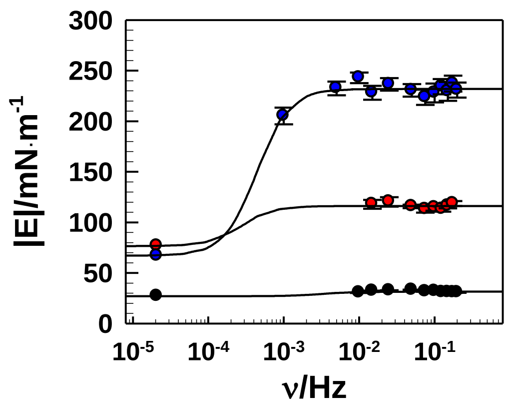
Effect on Dilational Rheology

Dilational viscoelasticity vs. frequency

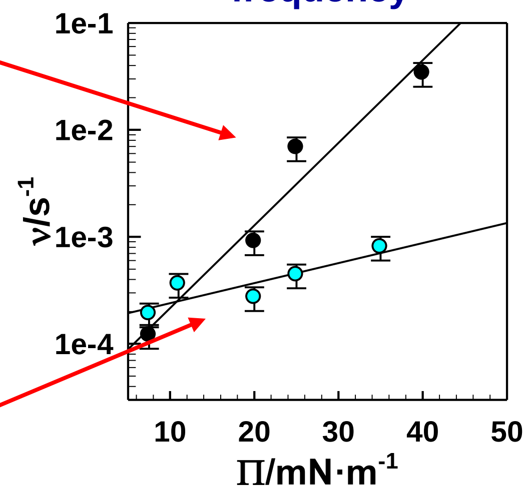
Without NPs



With NPs



characteristic frequency

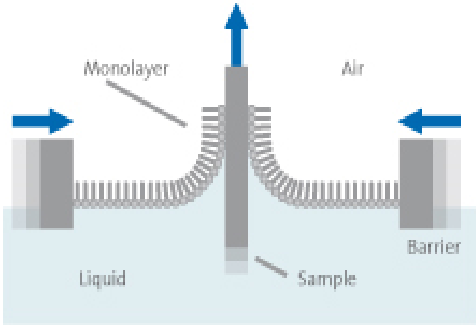


Fitting equation

$$|E| = \sqrt{\frac{E_1^{\,2} + E_0^{\,2}\left(\nu/\nu_k\right)^2}{1+\left(\nu/\nu_k\right)^2}}$$

➢ **Slowing down of the reorganization process in the layer**

Langmuir-Blodgett Deposition + AFM Analysis

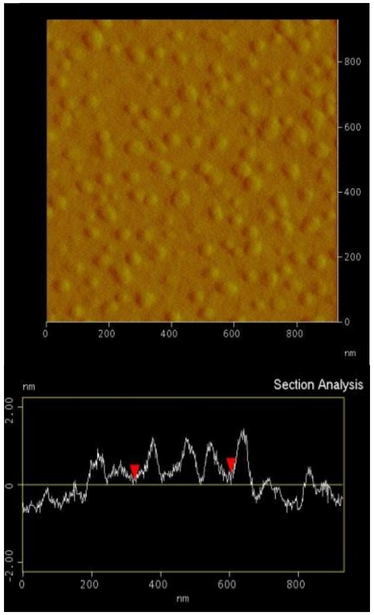


Layer Deposition on solid substrates

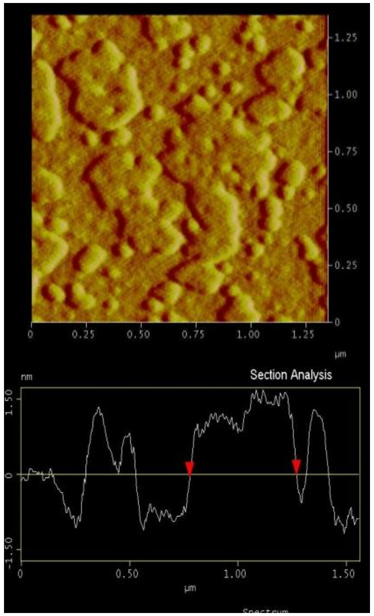
Layer transfer from fluid interface to solid substrates by vertical dipping at constant 1 mm/min speed.

Controlling of the surface pressure during dipping process.

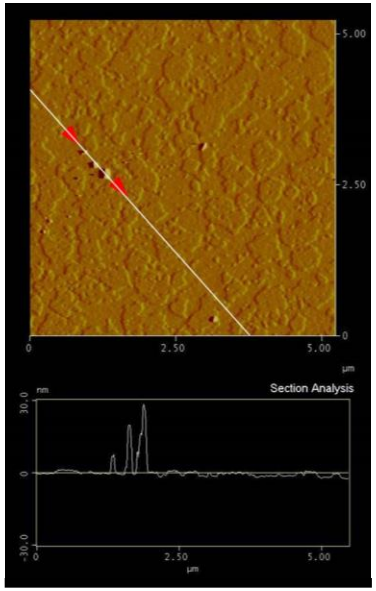
DPPC



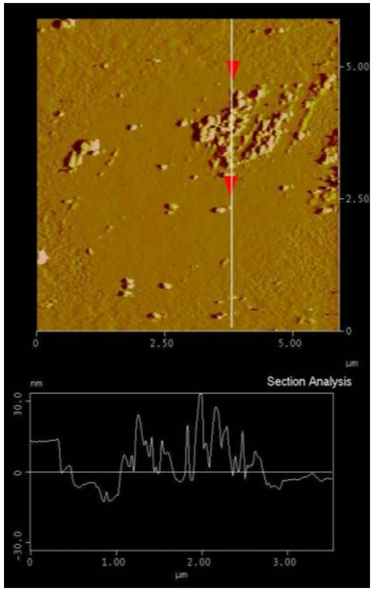
DPPC+DOPC



DPPC+NP



DPPC+DOPC+NP



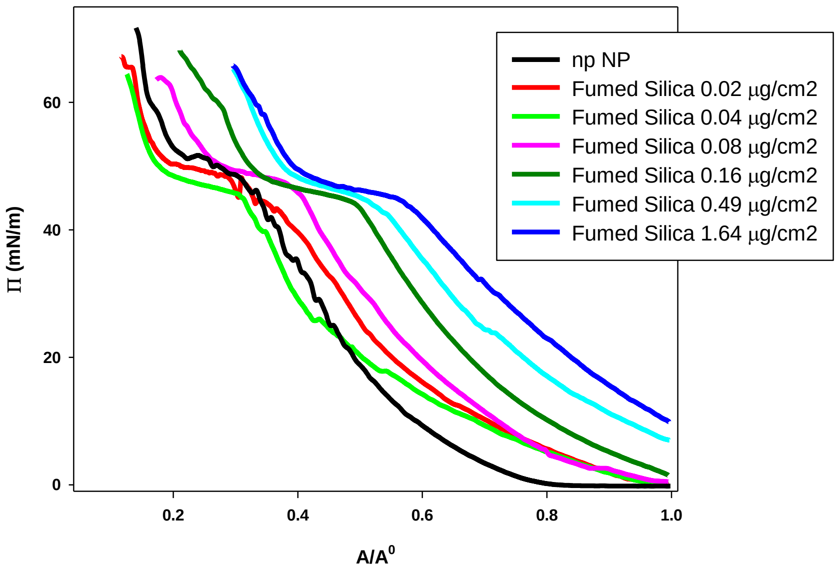
AFM images evidence the incorporation of NP on the lipid film

3. Effect of Silica NP on Natural PS

Natural lung extract (commercial sample) containing SP-B and SP-C

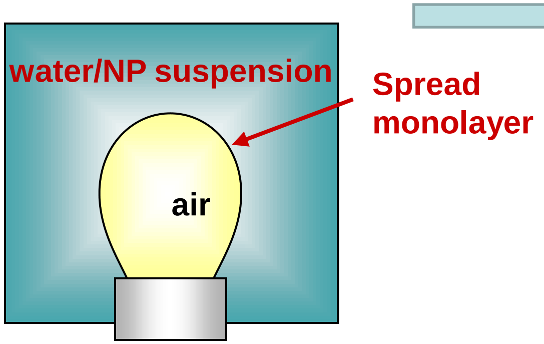
Dose-dependence of Fumed Silica NP (Hydrophobic)

Compression Π – A Isotherm

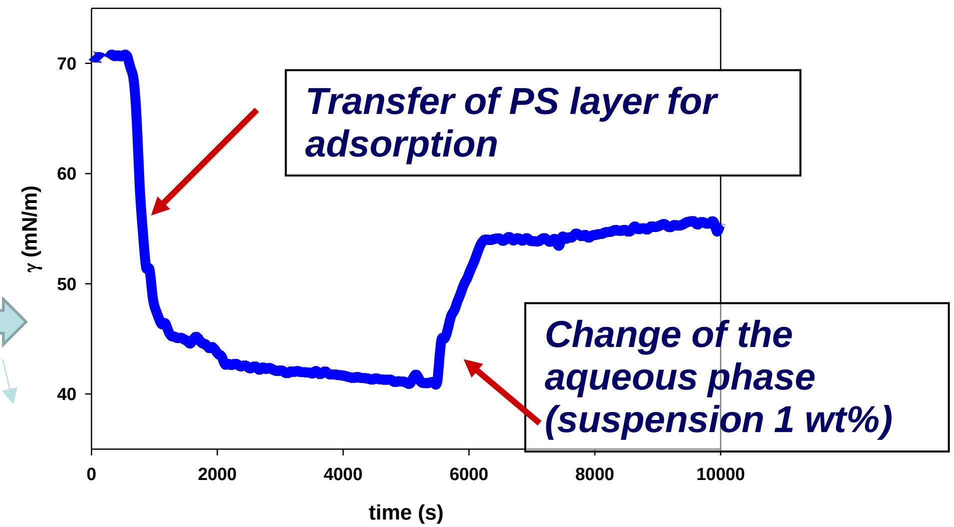


- Fumed Silica added onto the spread Lung Surfactant monolayer (Γ^0=0.16 mg/cm2)
- Appreciable effect for NP surface density larger than 0.02 μg/cm2

Bubble Shape Tensiometer



Colloidal Silica NP (Hydrophilic)



- Surface tension increase due to surfactant (proteins?) sequestration by SiO2 NP

➢ Key parameters relevant for PS functionality

1. **Collapse Pressure**
2. **Quasi-Equilibrium Dilational Rheology**

$$\varepsilon = -A\left(\frac{\partial \Pi}{\partial A}\right)_T$$

3. **Total Harmonic Distorsion (THD)**

$$THD = \frac{\sqrt{\sum_{k>1} \Delta\sigma_k^{\ 2}}}{\Delta\sigma_1}$$

4. **Normalized Hysteresis Area**

$$HA_n = \frac{\left[\int_{A_{min}}^{A_{max}} \Pi(A)dA\right]_C - \left[\int_{A_{min}}^{A_{max}} \Pi(A)dA\right]}{A_{max} - A_{min}}$$

5. **Stability Index**

$$SI = 2\frac{\gamma_{max} - \gamma_{min}}{\gamma_{max} + \gamma_{min}}$$

Summary of main Results and Conclusions

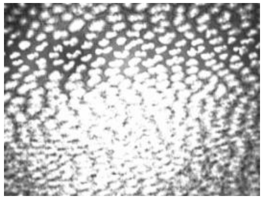
1. The interaction of NP with lipid layers may have different effects:
   - Modification of the surface phase behavior and structure of the layer
   - Change in the dynamic properties of the monolayer
   - Alteration of the composition (component sequestration)
2. Depending on the nature of NP, these effects can have different degree of importance
3. Definition of key physico-chemical parameters related to PS functionality
4. Evaluating the NP effects on these parameters provides criteria to quantify their potential adverse effect
5. These results obtained on model systems may be used to investigate the real systems

2. ‘’Assembling’’ di micro-nanostrutture bidimensionali alle interfacce liquide

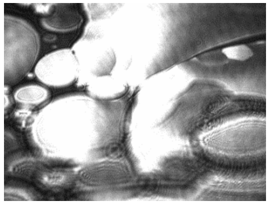
Background

- Lipid components (phospholipids, fatty acids) spread at liquid-air interfaces aggregate in 2D domain with specific 2D geometry

DPPC



Palmitic Acid

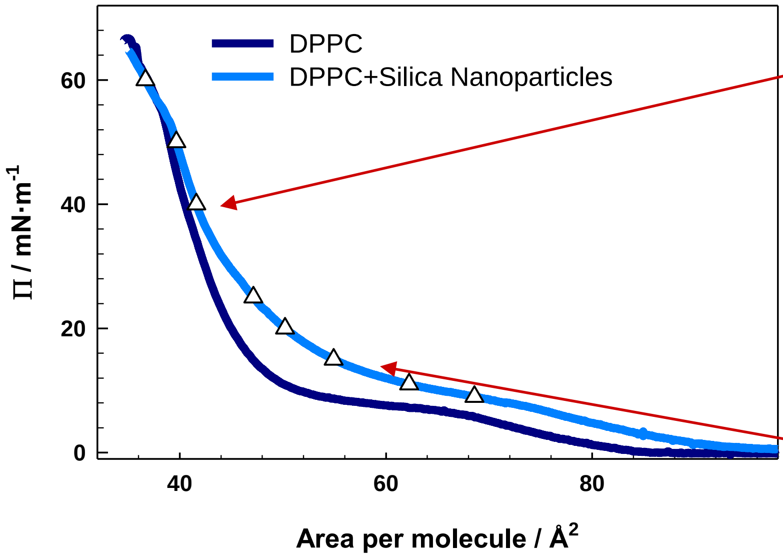


- It has been shown [1,2] that stable 2D structures with specific ordered geometrical characteristics are obtained driven by lipid molecules – nanoparticle interactions
- These structures are appealing for the development of new hybrid soft materials, membranes, capsules

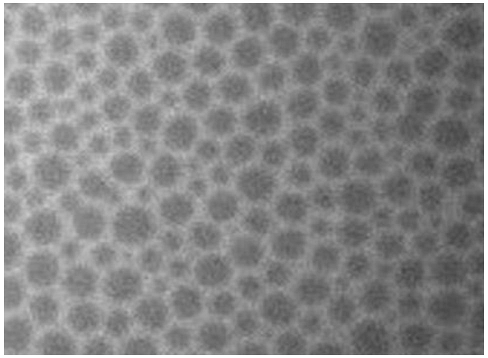
1. E. Guzmán, D. Orsi, L. Cristofolini, L. Liggieri, F. Ravera, "2D DPPC Based Emulsion–like Structures Stabilized by Silica Nanoparticles", Langmuir, 72 (2014), 127-138
2. D. Orsi, E. Guzmán, L. Liggieri, F. Ravera, B. Ruta, Y. Chushkin, T. Rimoldi, L. Cristofolini, "2D dynamical arrest transition in a mixed nanoparticle phospholipid layer studied in real and momentum spaces", Scientific Reports, 5(2015), Rticle 17930

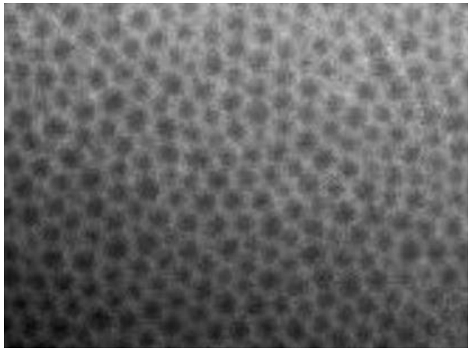
2D foam–like structures at water-air Interface stabilized by Silica NP

Compression Isotherm of DPPC monolayer by Langmuir trough

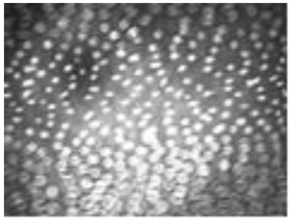


Fluorescence Microscopy





BAM images in the coexistence phase



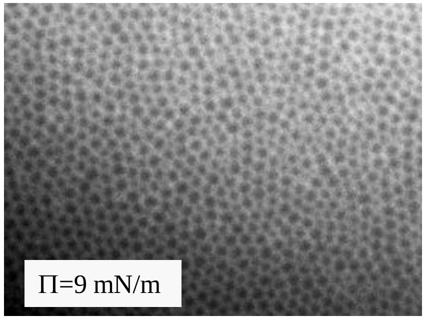
DPPC

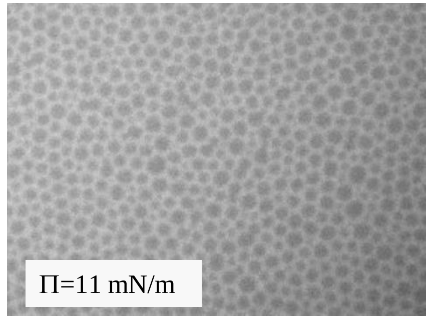
DPPC + NP

- In presence of NP, LC domains less elongated and of reduced size
- the transition to a total condensed phase not observed

- *E. Guzmán et al, Langmuir, 30 (2014), 1150*

Fluorescence microscopy at different compression degree





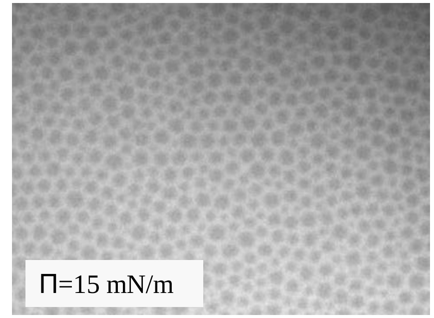
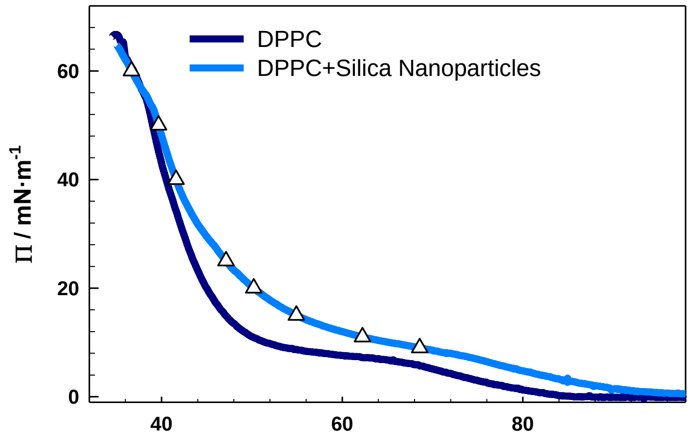
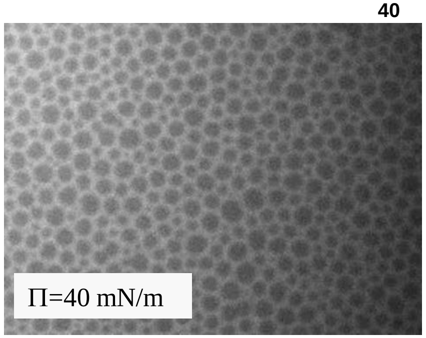
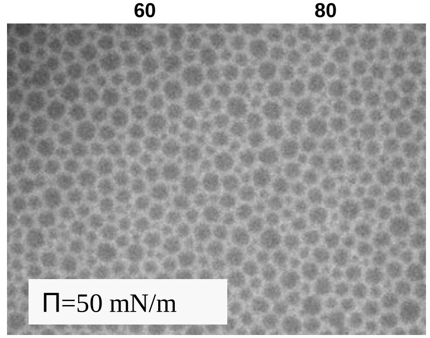


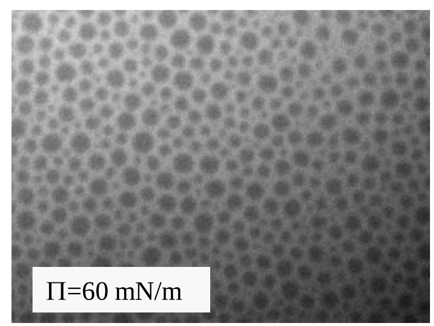
Image size:
280 x 200 μm²



➢ **Highly improved visualization of the domains**



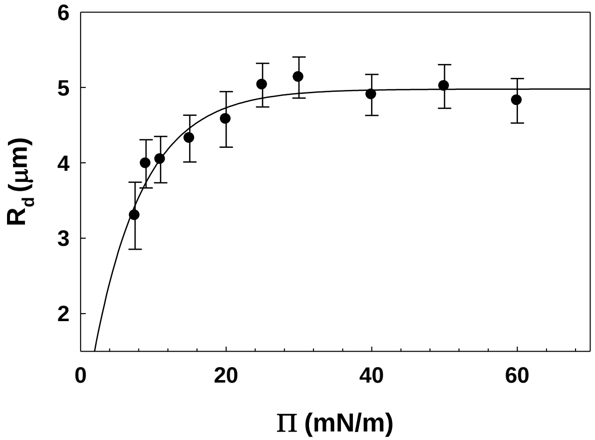




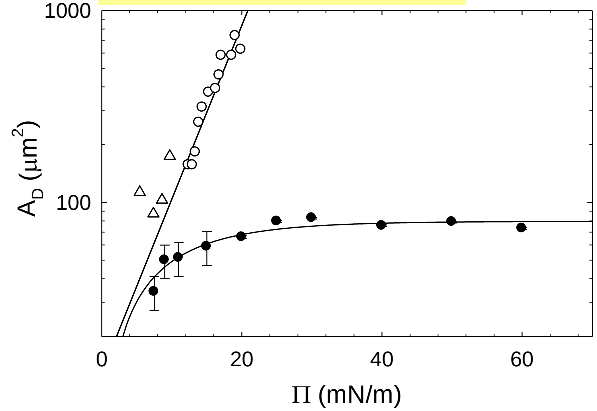
- **Study performed at Univ. of Parma (L. Cristofolini)**

Geometrical characteristics of the domain

average radius



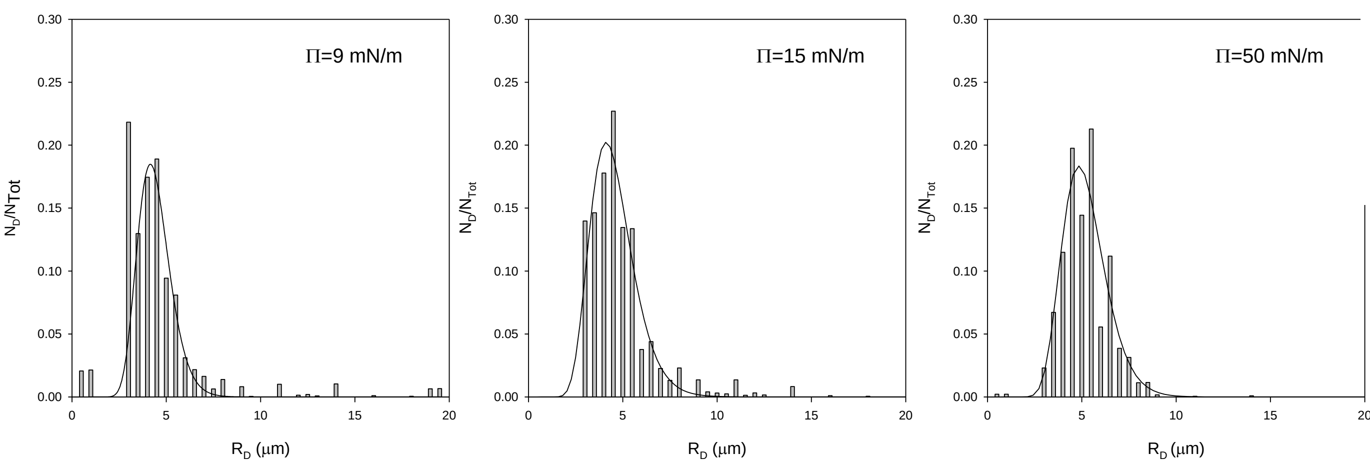
average area



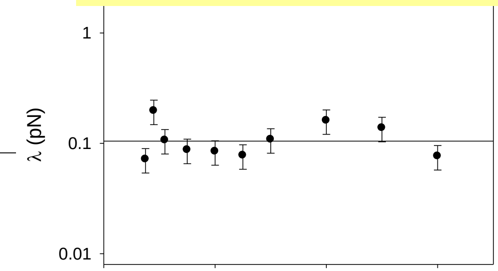
- The domain size ncreases for low surface pressure till reaching a plateau value for Π > 30 mN/m
- the presence of NPs hinder the domains to overcome the size of the initial LC nucleus

** compared with those of pure DPPC by Arriaga et al, J. Phys. Chem , 2010*

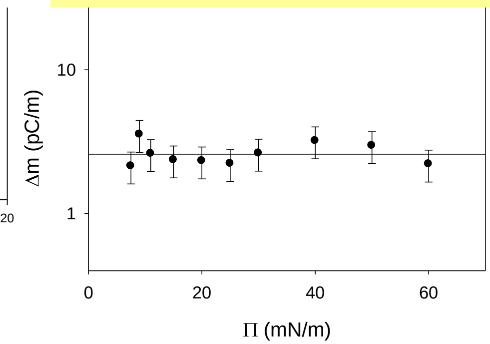
- Normalized domain size distribution



Line tension

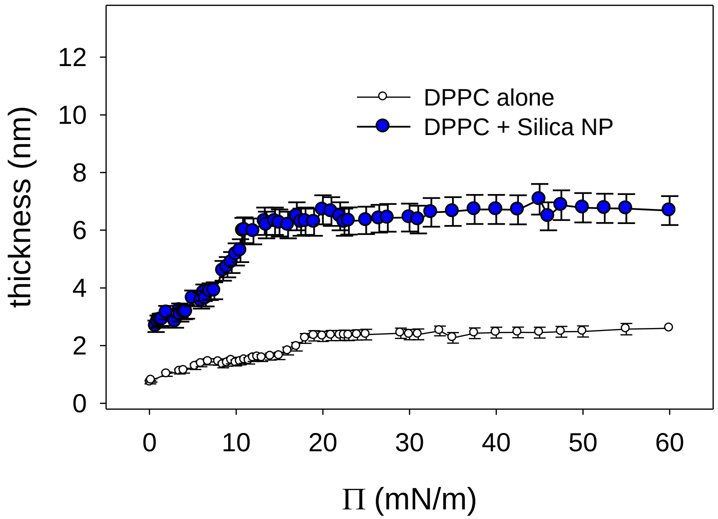


Dipole density difference

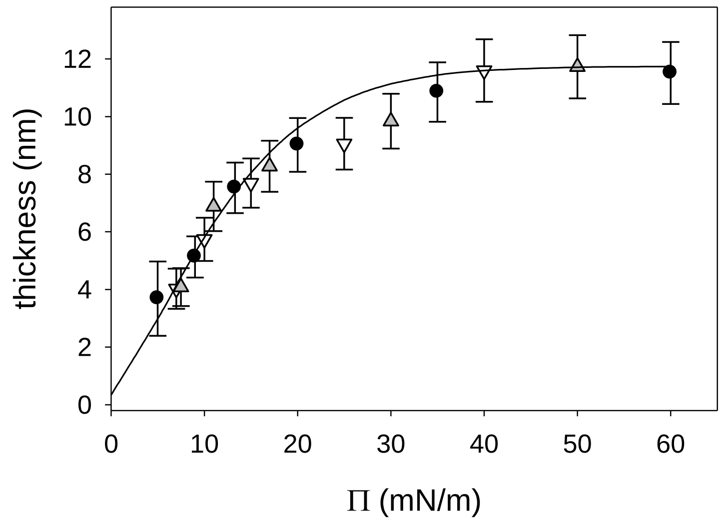


Elipsometry Analysis

Thickness of the mixed DPPC Silica NP layer



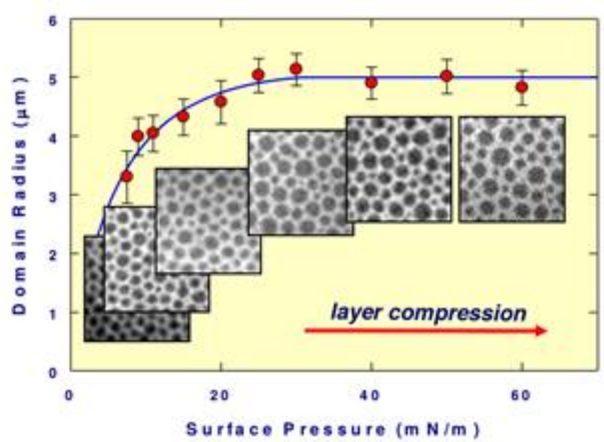
Thickness of the matrix layer surrounding the domains



On the bases of this multi technique analysis:

- Silica NP are mainly distributed in the LE phase
- LC domains grow with compression before being surrounded by DPPC decorated NP
- DPPC decorated NP prevent domains coalescence

- After the incorporation of Silica NP, the DPPC monolayer assumes the characteristics of a 2D NP stabilized Foam

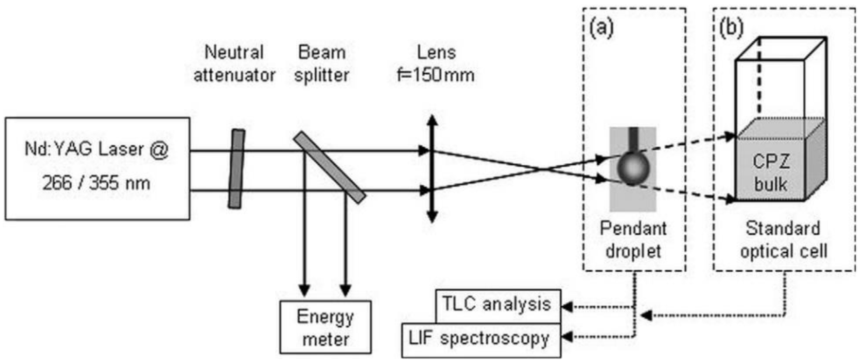


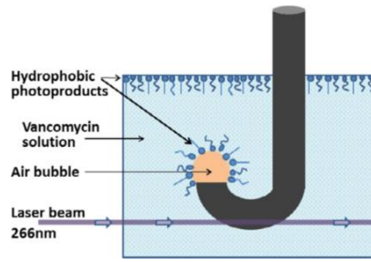
3. Proprietà interfacciali e citotossicità di derivati di antibiotici da irraggiamento laser in tessuti biomedicali

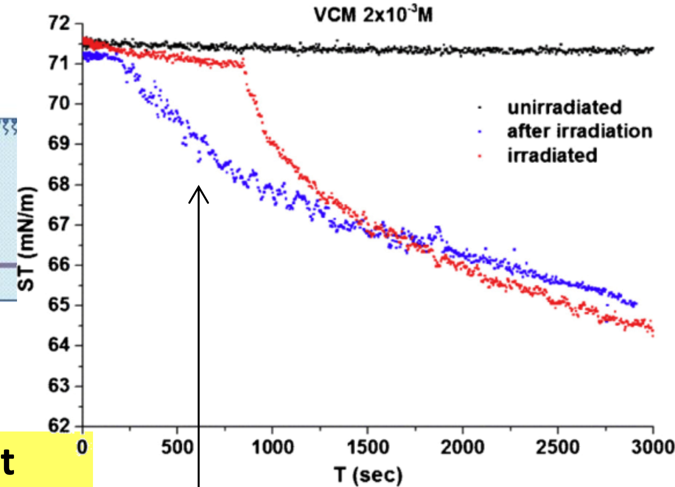
INTERFACIAL PROPERTIES AND CITOTOXICITY OF LASER IRRADIATED ANTIBIOTIC DERIVATIVES IN BIOMEDICAL FABRICS

Bilateral Project: Italia (M.Ferrari)- Romania National Institute for Laser, Plasma and Radiation Physics (M.L. Pascu)

- Multiple drug resistance requires a flexible approach to find medicines able to overcome it.
- The exposure of existing medicines to ultraviolet laser beams generates photoproducts efficient against bacteria and/or malignant tumors.

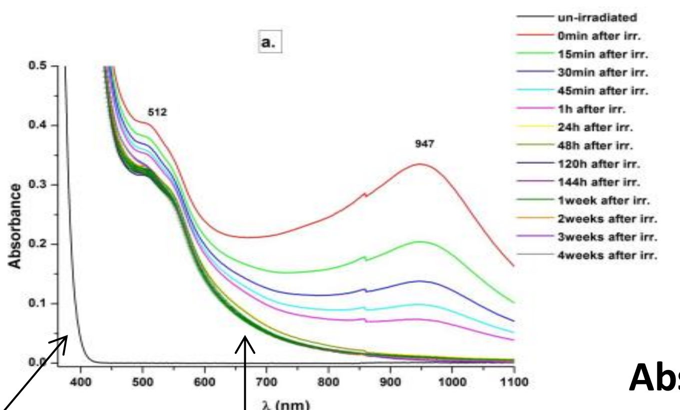






Set-up for exposure **to UV laser light and surface tension measurement**

The formation of <u>surface active compounds</u> improves the <u>wetting</u> action of the solution for application on <u>surfaces or fibers</u>



Before irradiation

After irradiation

Absorption spectra of CPZ measured immediately after exposure to UV laser radiation (irradiated 4h; laser beam energy 6,5mJ; laser beam wavelength 266nm)

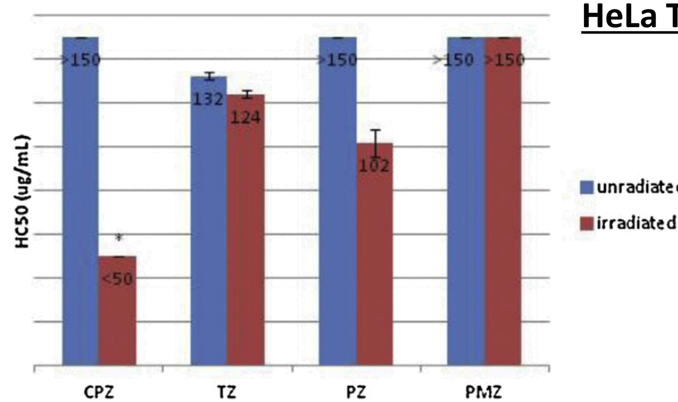
In collaboration with UB Farmacy Faculty Fisiology Dept. (C.Moran)

CYTOTOXICITY

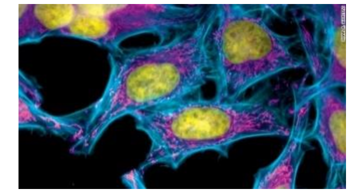
- Animal testing in toxicology and pressure from both the general public and government accounts for developing alternatives to *in vivo* testing

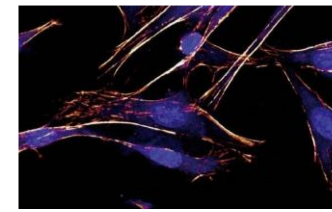
- **HEMOLYTIC ASSESSMENTS Red Blood cells**

- **CYTOTOXIC ASSESSMENTS on <u>3T3 Healthy</u>**



<u>HeLa Tumoral cell lines</u>

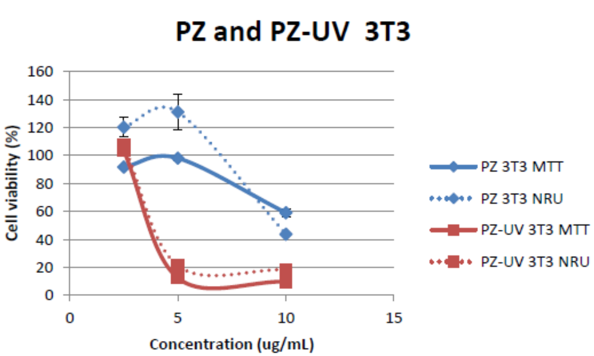


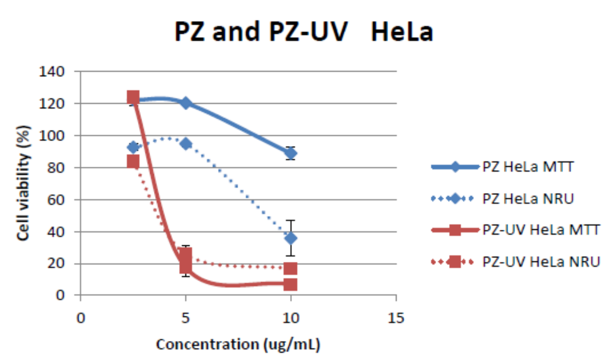


- ***In vitro* cell-based models** may be more attractive for preliminary testing of new materials.

- Their direct use in solutions or on tissues or impregnation with them of **materials applied in treatments of biological surfaces** is of great interest

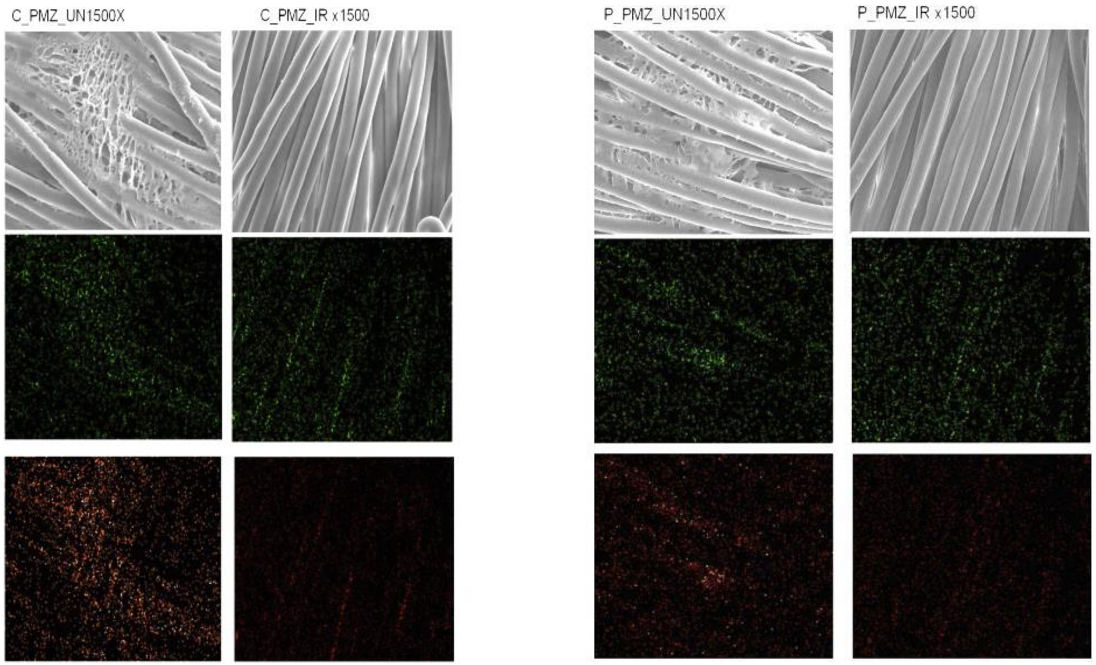
- Laser irradiated CPZ and PZ are more efficient against some cell cultures





INTERACTION WITH FABRICS

SEM morphology (1500X) and **EDS maps** for S (green) and Cl (red) for **irradiated and unirradiated PMZ** (20mg/mL) on **different fabrics** (C for Cotton, P for Polyester)



EDS maps distribution of the characteristic element present in the medicines acting as tracing agents coupled with morphology observations. In this case S and Cl maps have been extracted from the whole spectra.

- Interaction of laser irradiated phenothiazines with fabrics show CPZ and PMZ **<u>improved wetting properties.</u>**

- CONCLUSIONS

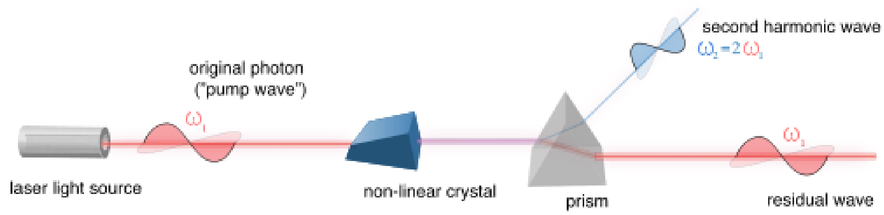
  - Correlation of these two groups of properties shows that CPZ appears to be a more recommended compound for applications on tissue using fabrics as medicine transport vectors.
  - The reported results concern stability study of phenothiazines solutions to know the time limits within which they are stable and may be used.

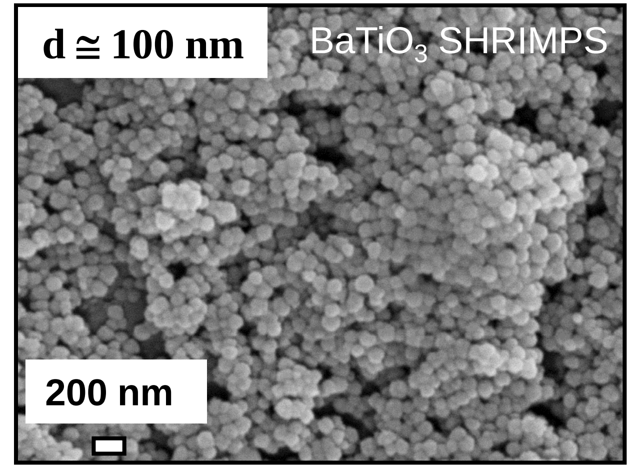
4. Sintesi di nanoparticelle con proprietà non lineari del secondo ordine per applicazioni nella diagnostica biomedicale.

$BaTiO_3$ nanoparticles as Second Harmonic Radiation IMaging Probes (SHRIMPs)

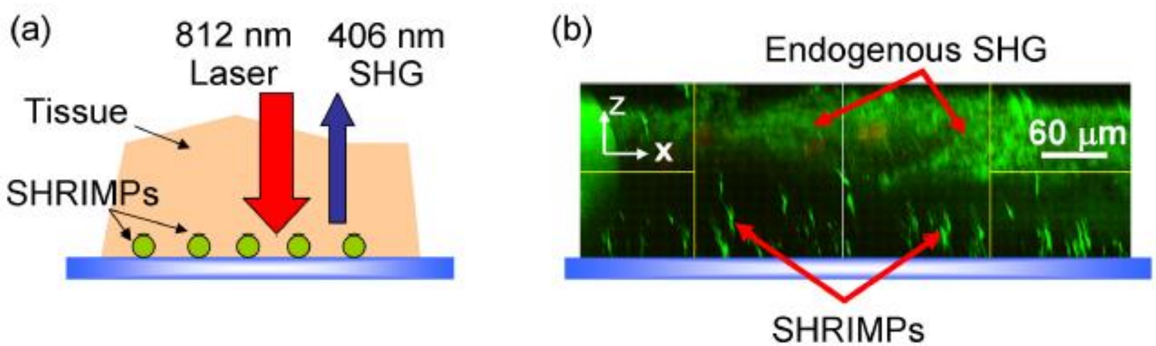
Collaboration IENI-GE (V. Buscaglia) and Univ. Of Uena, Germany

Second harmonic generation (SHG) concept





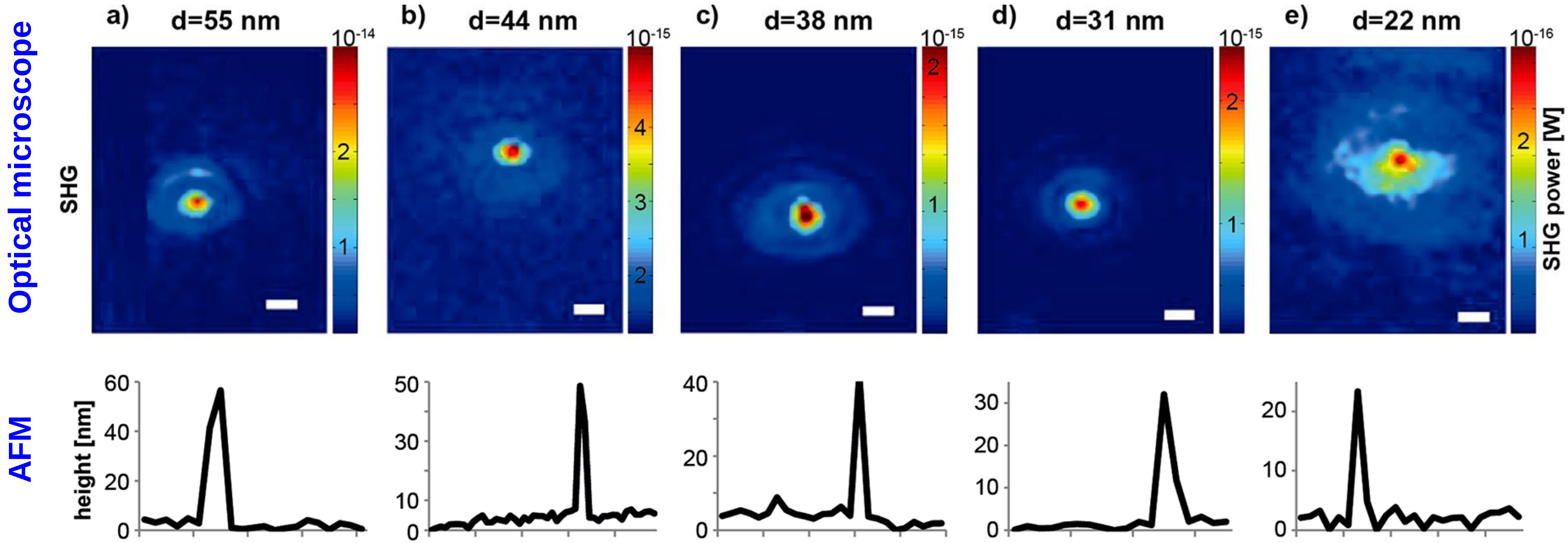
An example of $BaTiO_3$ SHRIMPS prepared by an hydrothermal-like process used as contrast markers in in-vitro and in-vivo experiments



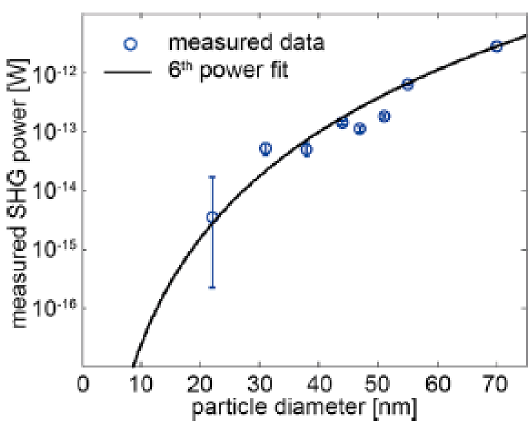
Scanning confocal imaging of 300 nm $BaTiO_3$ SHRIMPs embedded 120 µm below an in vitro mouse tail tissue

R. Grange et al., *Biomedical Optic Express* **2**, 2532 (2011)

Influence of particle size on the SHG of $BaTiO_3$



Total SHG power $\approx V^2$ ($\approx d^6$)



E. Kim et al., *ACS Nano* **7**, 5343 (2013)

Conclusioni & prospettive

Competenze acquisite e risultati ottenuti:

1. Sviluppo di metodi sperimentali per la valutazione quantitativa dell'impatto di NP su interfacce modello.
2. Studio di strutture ordinate 2D di composizione ibrida per self-assemblng alle superfici liquide
3. Applicazione delle competenze sulla chimica fisica dei tensioattivi per l'ottimizzazione di farmaci e medicamenti
4. Sintesi di nanoparticelle per il bio-imaging
5. Applicazioni e potenziali sviluppi:
   - Sviluppo materiali per il drug delivery, micro-nanocapsule e membrane
   - nano-tossicologia e/o effetti avversi di NP su sistemi bio
   - Markers per la diagnostica medica