Nanomedicine and Nanotoxicology Group
Physics Institute of São Carlos - University of São Paulo

BIO-NANO INTERFACE MODELS APPLIED TO THE INVESTIGATION OF NANOPARTICLES CELL UPTAKE: PROOF OF CONCEPT USING REAL MEMBRANE MODELS

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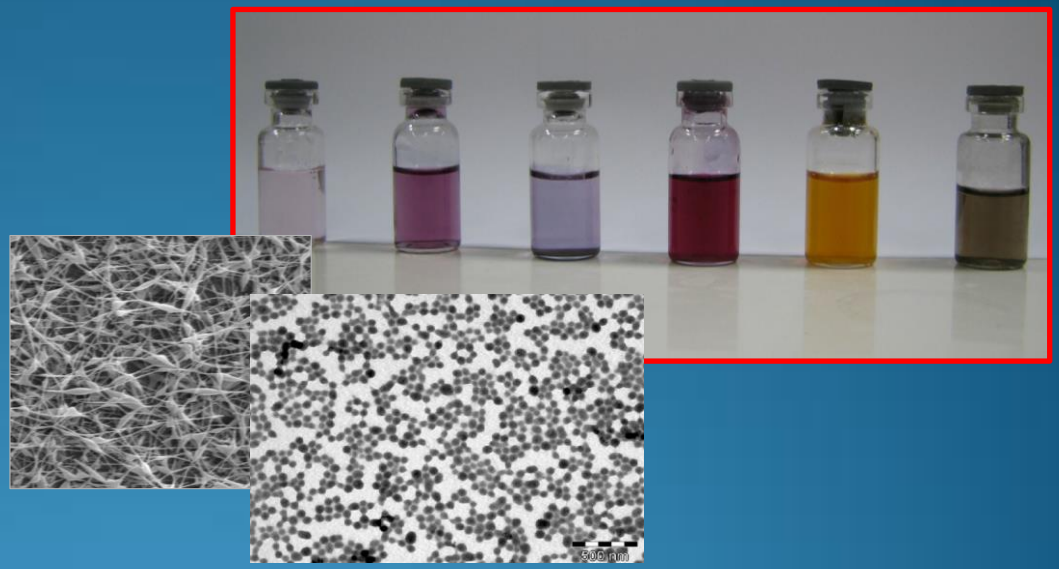
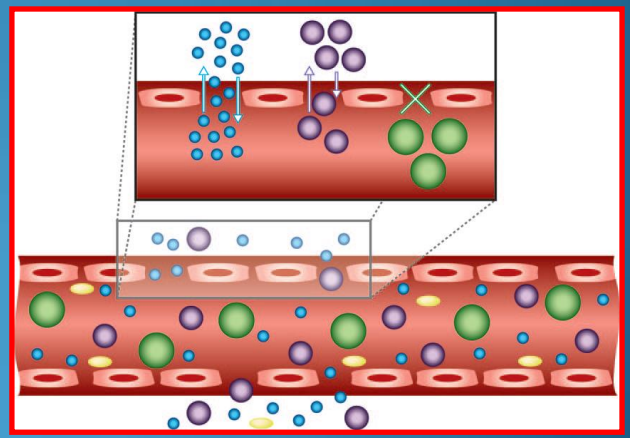
Potencial applications of nanomaterials in medicine





- Drug delivery
- Theranostics agents - detection and treatment
- Images
- Photothermal and photodynamic therapies

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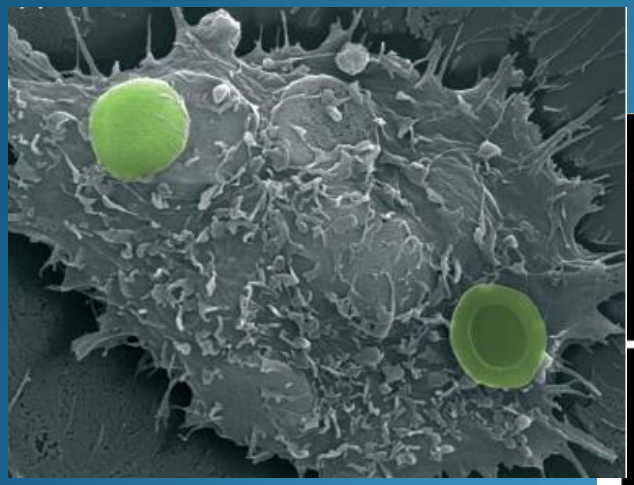
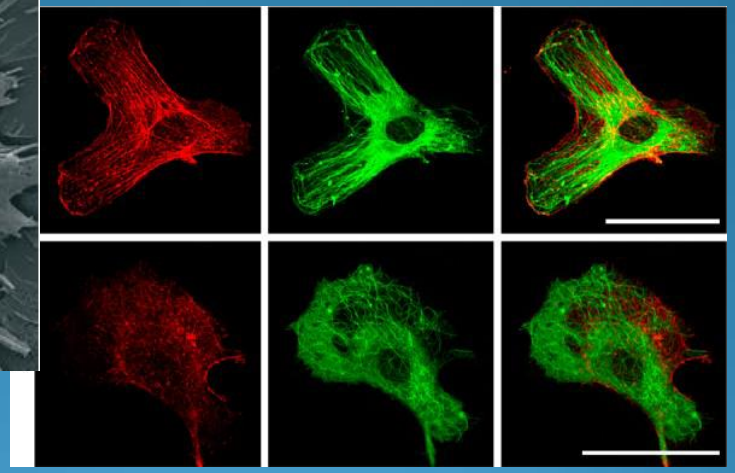
However there are potencial toxicology effects...

"nanotoxicology would make an important contribution to the development of a sustainable and safe nanotechnology".
Donaldson et al. (2004)



In vitro



In vivo

Combining Techniques!

Aiming to elucidate the mechanisms involved with nanotoxicology aspects!

Ferrari et al. *Cancer Science* v 102 2011 and Soenen et al. ACSNano v 6 2012

Motivation

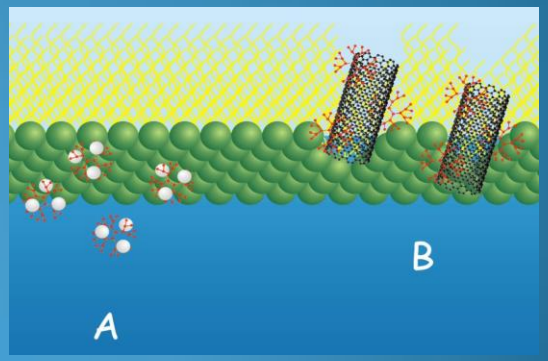
- Complexity of mechanisms involved in the bio-nano interface;
- Molecular level investigation.

✓ Biophysics interaction of nanomaterial in real cell membranes

✓ Using cellular membranes from cancer and health cells

✓ Langmuir and surface techniques

✓ Associate with *in vitro* studies



Methodology used

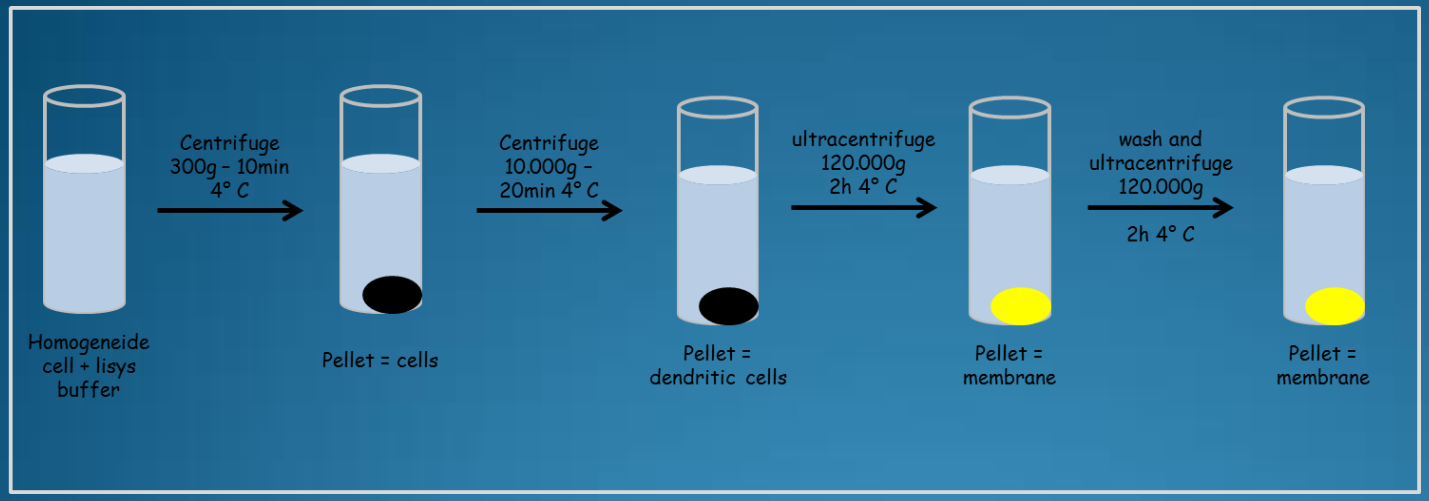
Nanoparticles characterization → Physical-chemical characterization of AuNP-PAH, AuNR-PAH and AuNR-PEG UV-Vis, DLS, zeta potential

Cell membrane extraction and characterization → Extraction of FC3-H and HTC cells membranes and characterization of the lipids and proteins extracted

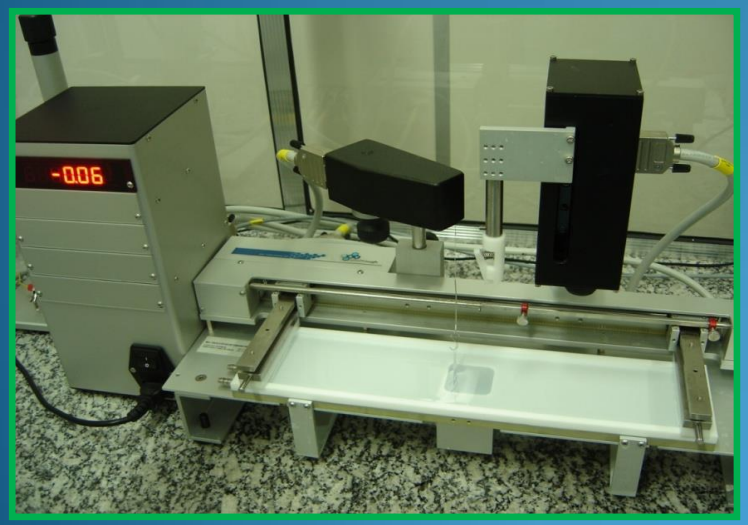
Membrane models → Reconstitution of extracted cell membranes on subphase with and without nanoparticles

Comparative studies → *In vitro* studies and ITC interactions to better understand

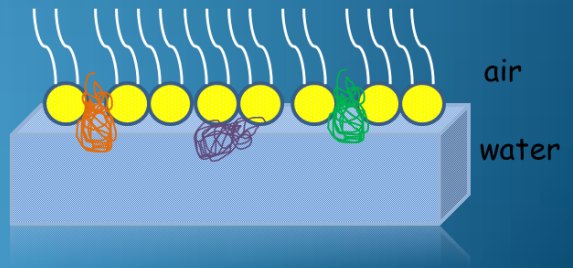
Membrane extraction and membrane model



membrane characterization



- ✓ simple model systems to investigate the physicochemical properties of biological membranes.
- ✓ molecules to come closer together to form a ordered monolayer.



Journal of Proteome Research *2009, 8, 3078–3090*

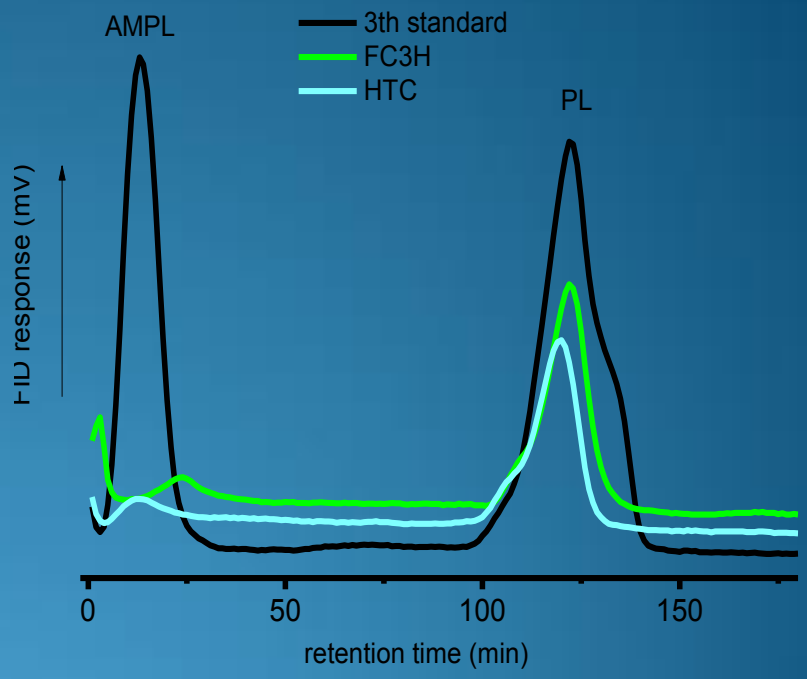
Membrane characterization

FC3-H

3% of triglycerides (TAG),
11% of free fatty acids (FFA),
15% of free aliphatic alcohol (ALC),
2% sterol (ST),
6% of mobile polar lipids in ketone (AMPL)
62% of phospholipids (PL)

HTC

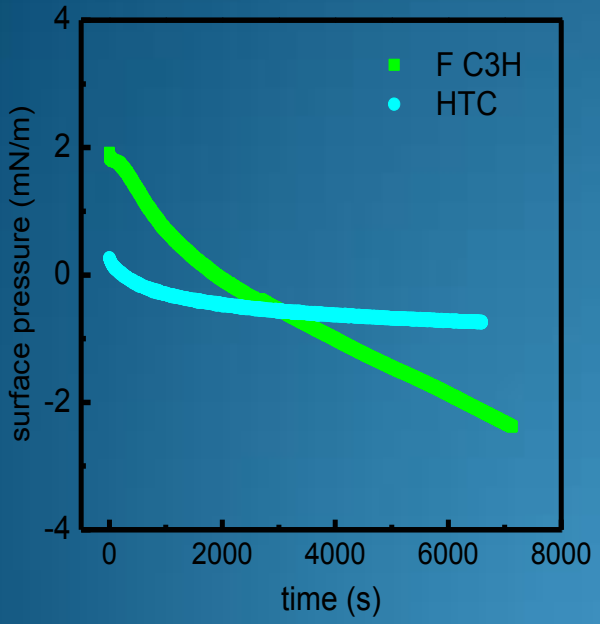
16% free aliphatic alcohol,
10% mobile polar lipids in ketone
74% of phospholipids.

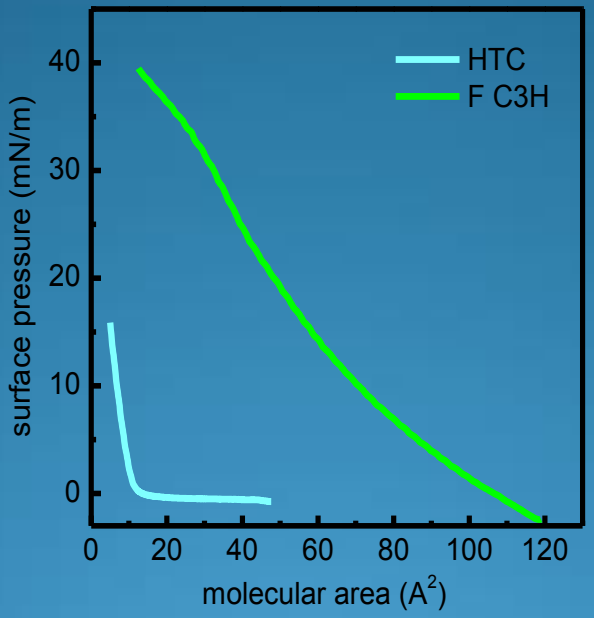


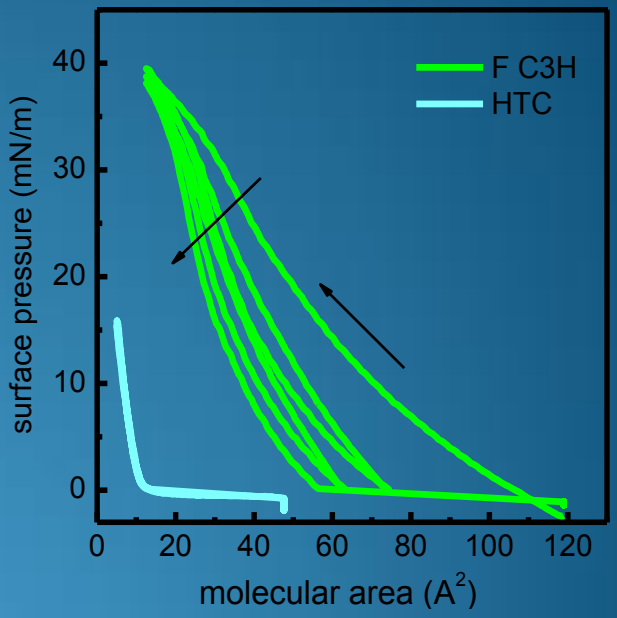
	Zeta potential	[membrane protein]
FC3-H	-10.9 mV	428 µg/mL
HTC	-12.9 mV	590 µg/mL

Membrane Models

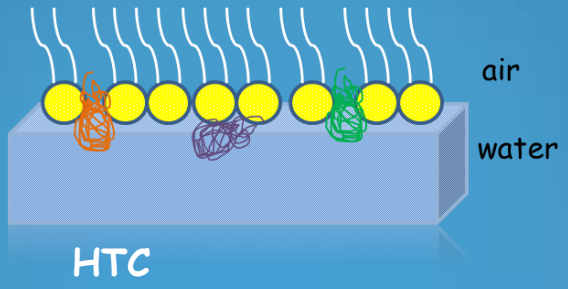
How looks FC3-H and HTC membrane cells at the subphase...

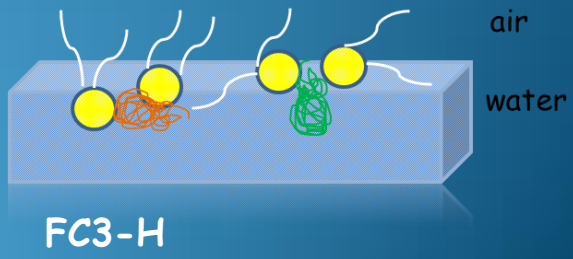






What is important to know?

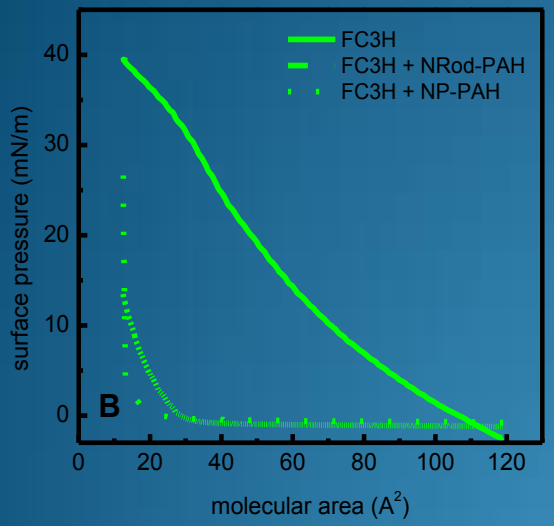


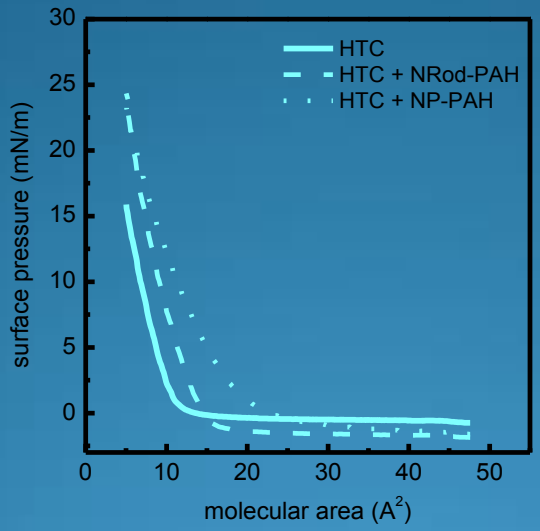


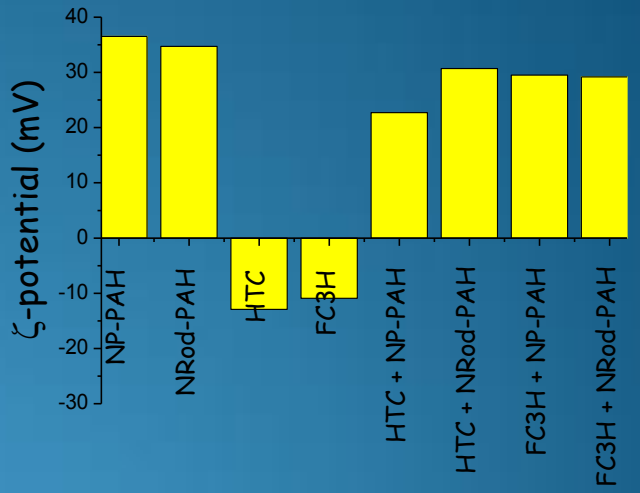
Membrane Models

Has morphology influence the uptake process in FC3-H and HTC membrane cells at the subphase?

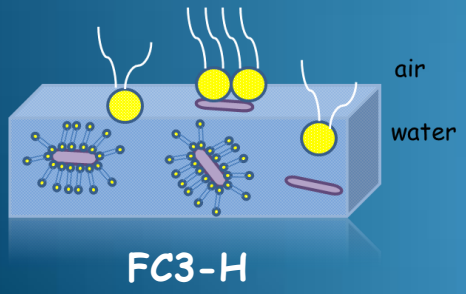
+ AuNR-PAH and AuNP-PAH

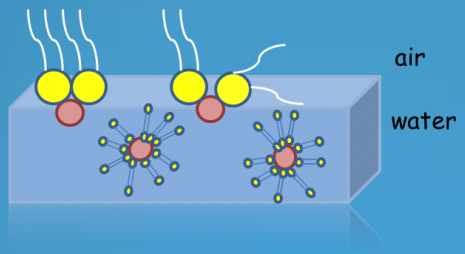


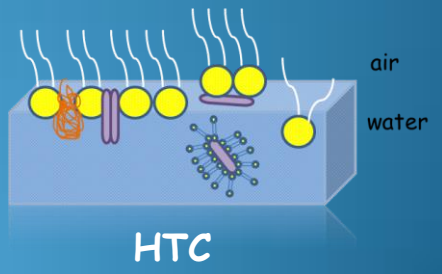




Types of interaction



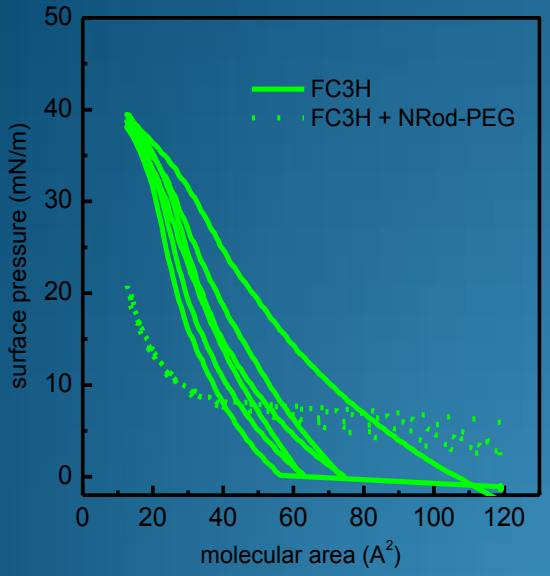


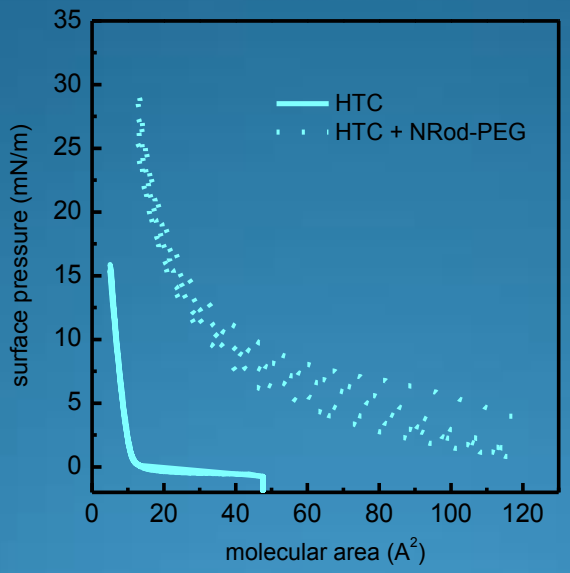


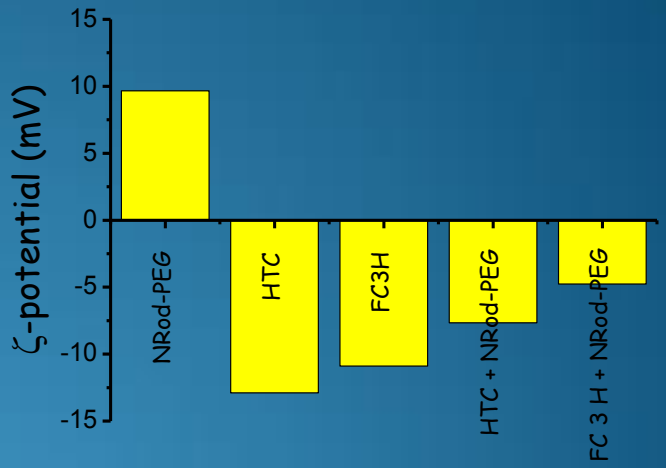
Membrane Models

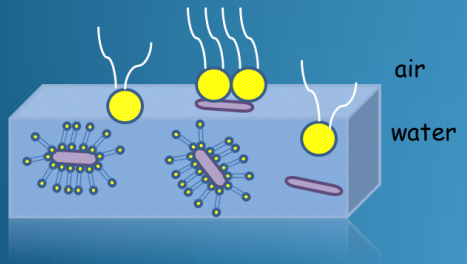
+ AuNR-PEG

... and charge influences?

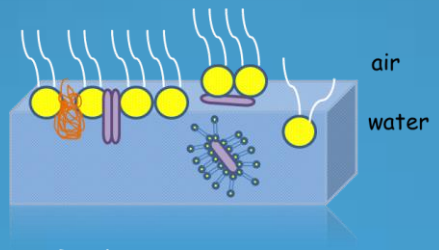








FC3-H



HTC

Types of interaction

Membrane model vs *in vitro* results

FC3-H

uptake of nanoparticles

no inhibition to adhesion action

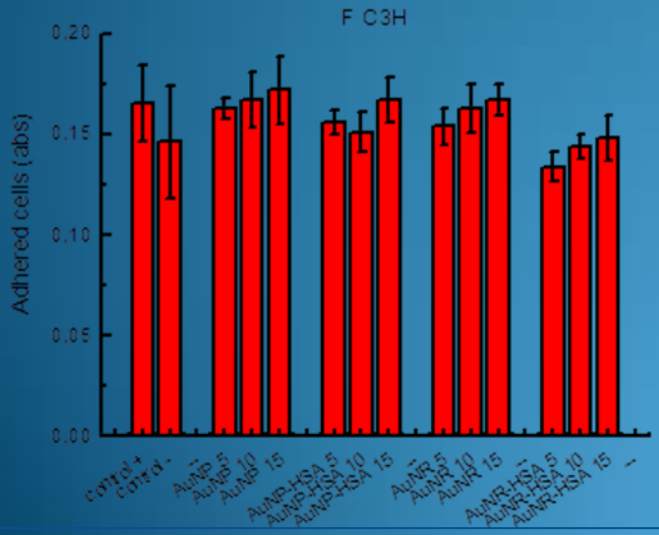
vesicles formation = uptake

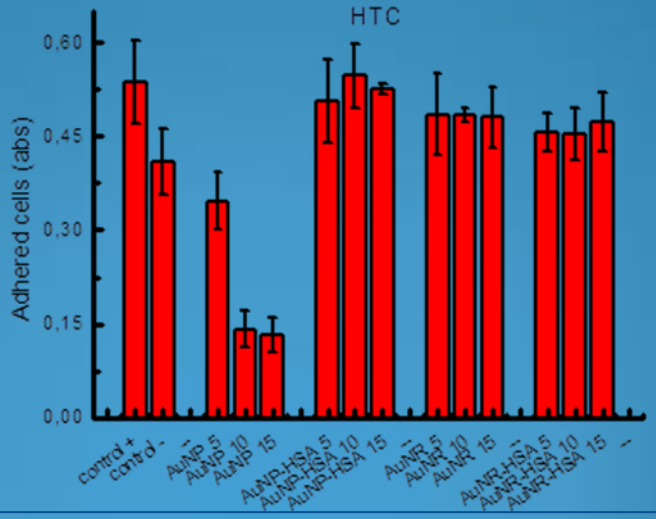
HTC

uptake of nanoparticles

high inhibition of adhesion

incorporation through the monolayer





Composition of the cell membrane had the major influence

Cell adhesion results

Summary

✓ The extracted membranes from FC3-H and HTC cells were characterized and they revealed high differences in their composition.

✓ The reconstitution of the membranes on the subphase showed that HTC formed more stable monolayers compared to FC3-H .

✓An expansion or decrease of the molecular area were indicatives that NP or NR affect packing of the lipids.

✓ The models revealed that not morphology but charge is mandatory in uptake

✓ FC3-H cell allows NPs uptake through the cell more easily, while HTC probably adsorbs NPs on the cell surface before uptake.

✓ Such investigation may be of great importance to understanding toxicity of nanomaterials at molecular level.

Acknowledgements

Thank you for your attention!

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