LUND UNIVERSITY

The Fate of Nanoparticles in the GI Tract

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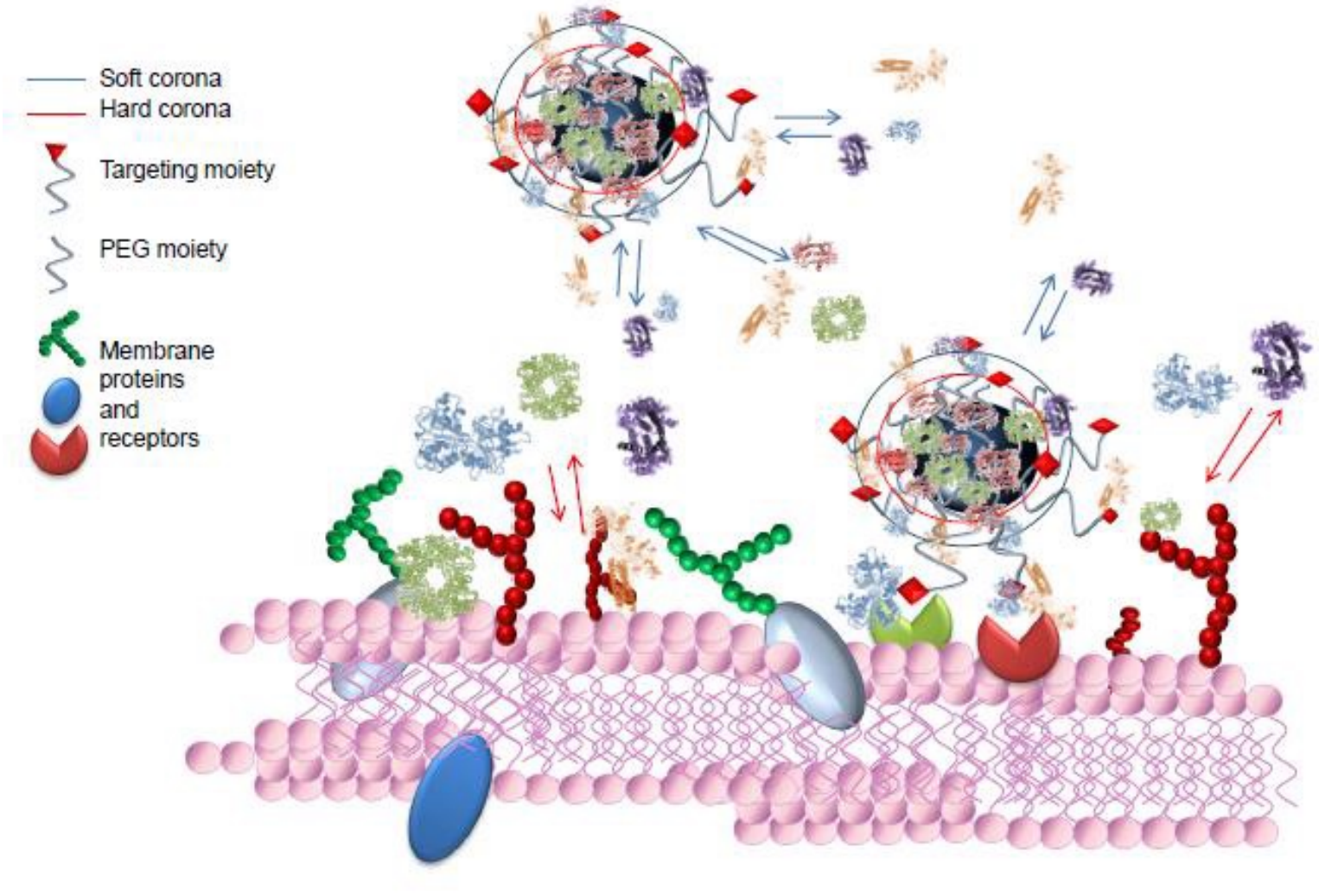
Introduction

- Nanoparticles (NPs) for medicine are an interesting tool able to carry out multiple tasks
- What happens to the NPs once they enter the body?
- What if they arrive as a result of contamination?



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UEA University of East Anglia

The nanoparticles (NPs) we used

NPs employed in QCM-D and Neutron Reflectance experiments described by size and Z-potential in PBS.

	d_H [nm]	PdI	Zp [mV]
PS-COOH20	32.3±0.4	0.10	-42.3±0.6
PS-COOH100	100.2±0.7	0.04	-33.7±1.4
PS-PEG	108.3±0.6	0.08	-2.1±1.0
Fe_3O_4	48.5±0.8	0.18	-22.3±1.4
Fe_3O_4-PEG1	34.1±1.4	0.15	-9.6±0.5
Fe_3O_4-PEG2	46.3±2.1	0.19	-1.2±2.3

NR used the D17 reflectometer in ILL in Grenoble with wavelengths in the range 2-20 Å. Two angles of incidence were used (θ = 0.8°-3°).

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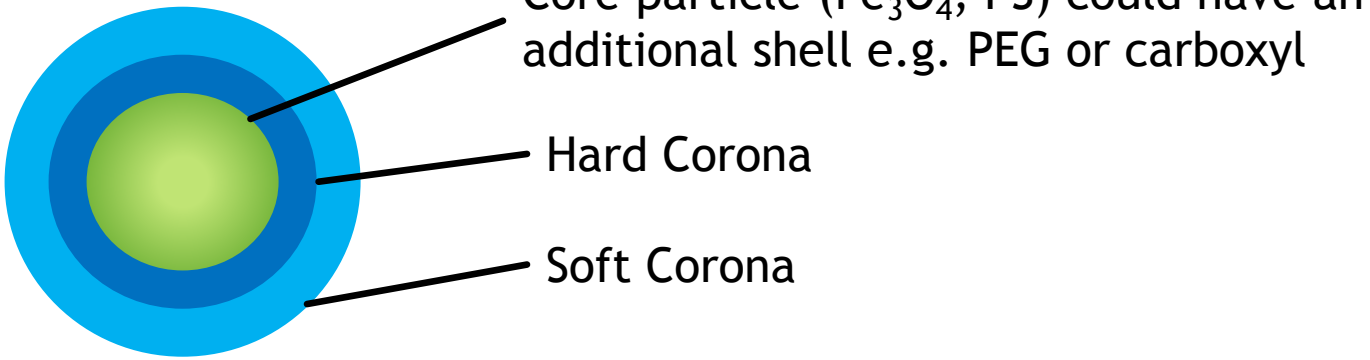
Core shell particles



The corona comprises adsorbed molecules e.g. proteins / peptides

In the next example the NPs were incubated in Skim Milk Powder

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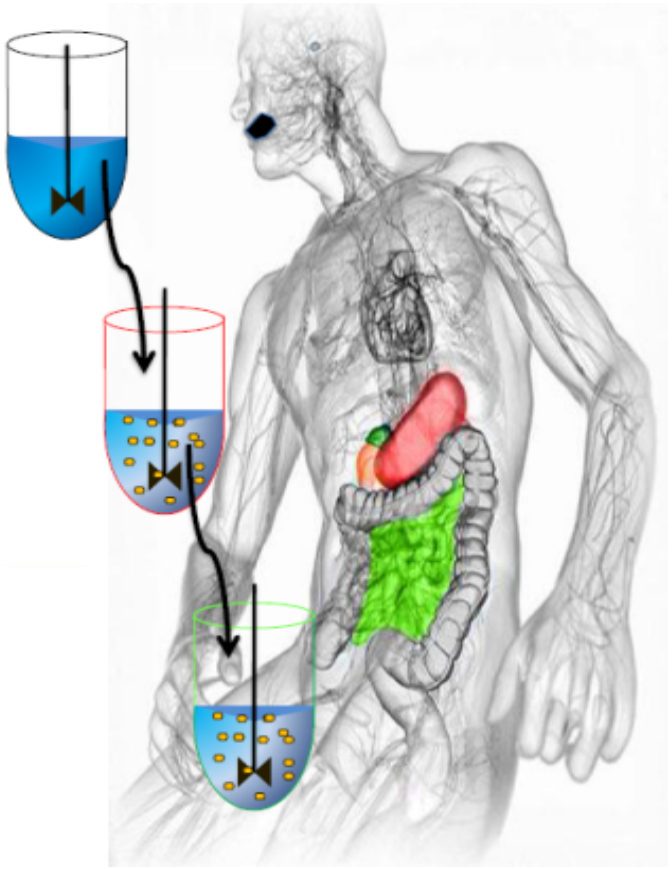
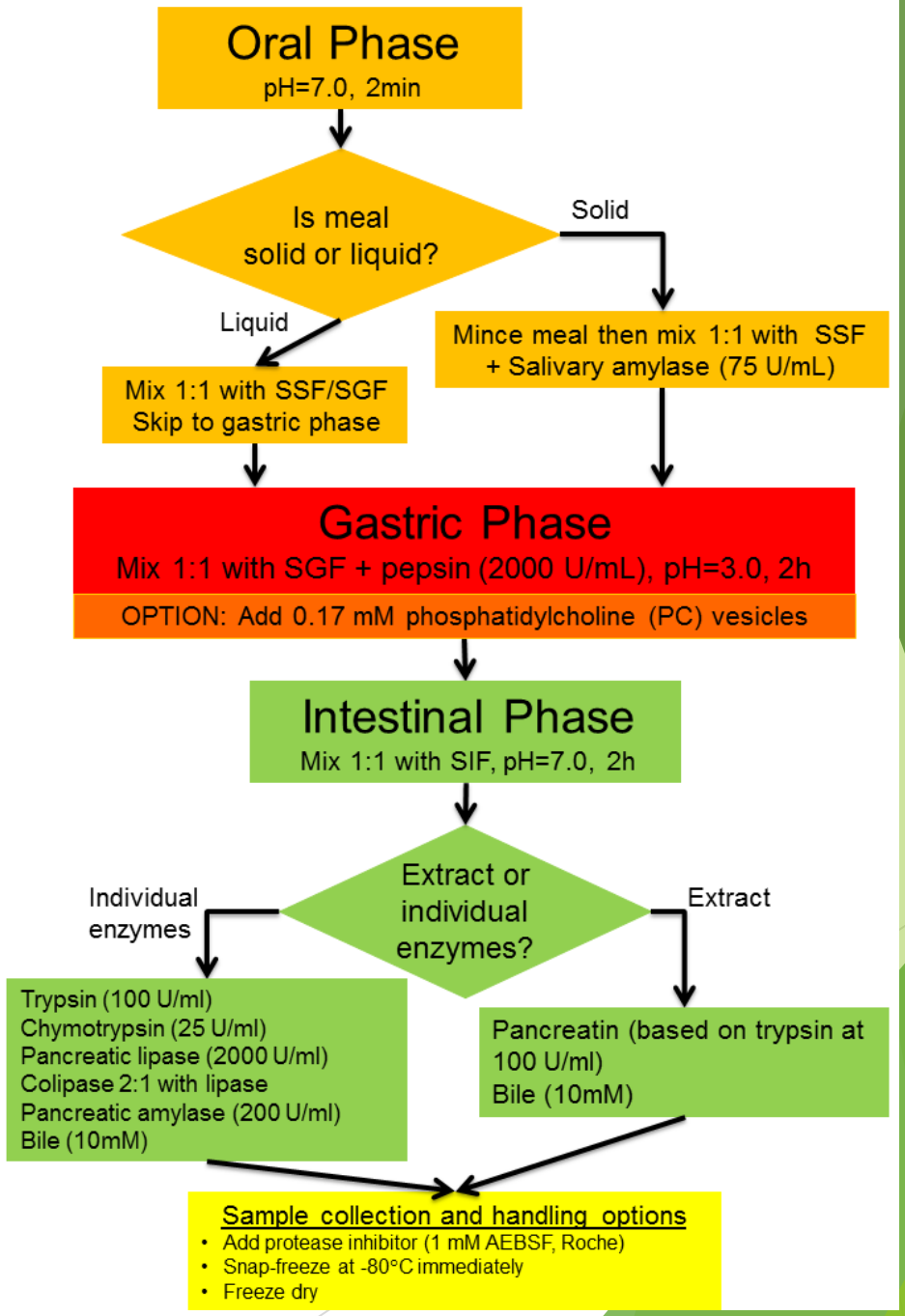
Digestion

34 mg/ml SMP was used as the starting food





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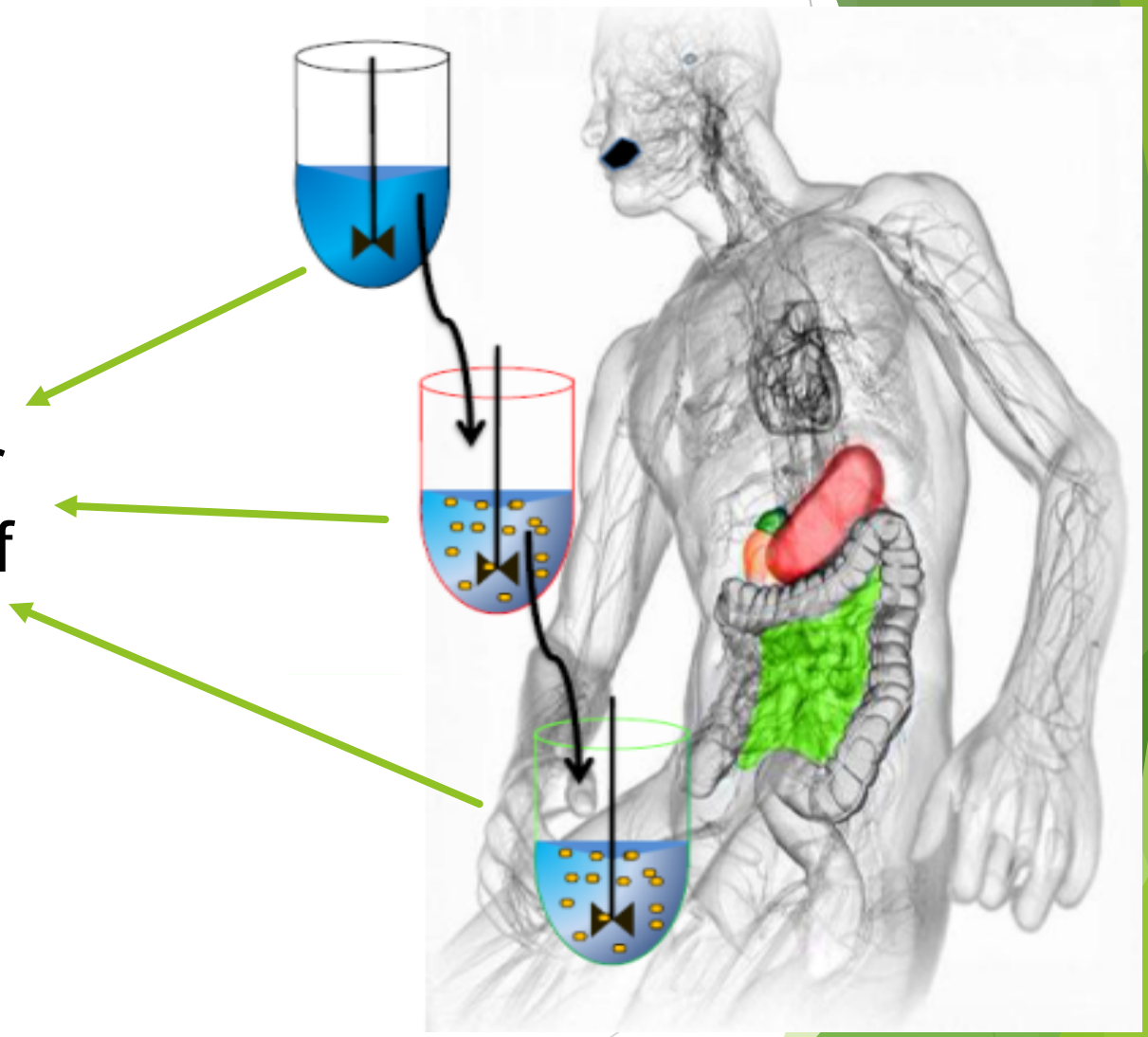
Digestion

Development of a protein corona

An aliquot of digesta was incubated with NPs for one hour at 37°C to get a concentration of $1.5x10^{13}$ NPs/ml



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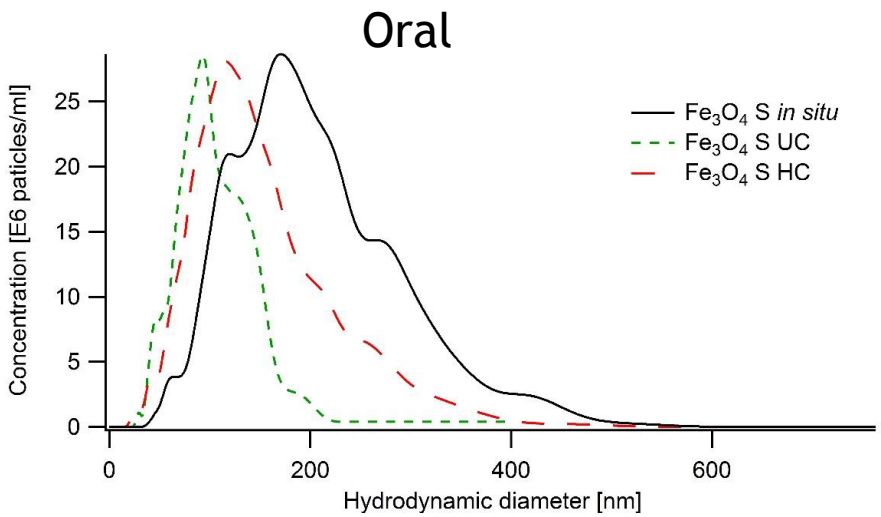
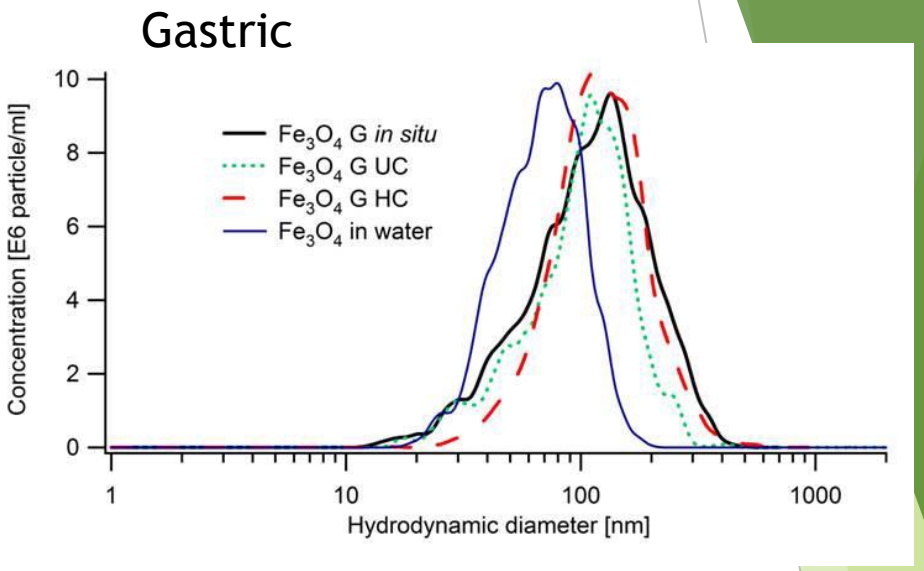
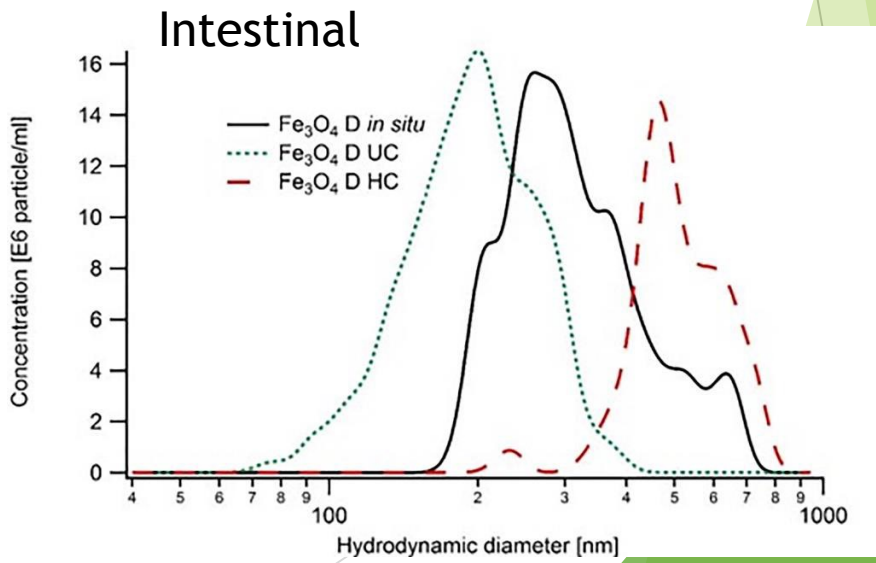
NPs in digesta





Size distributions obtained by NTA* for Fe_3O_4 NPs incubated with digestive fluids and protein corona complexes isolated by:

- Sucrose gradient ultracentrifugation (UC)
- Conventional centrifugation (HC).



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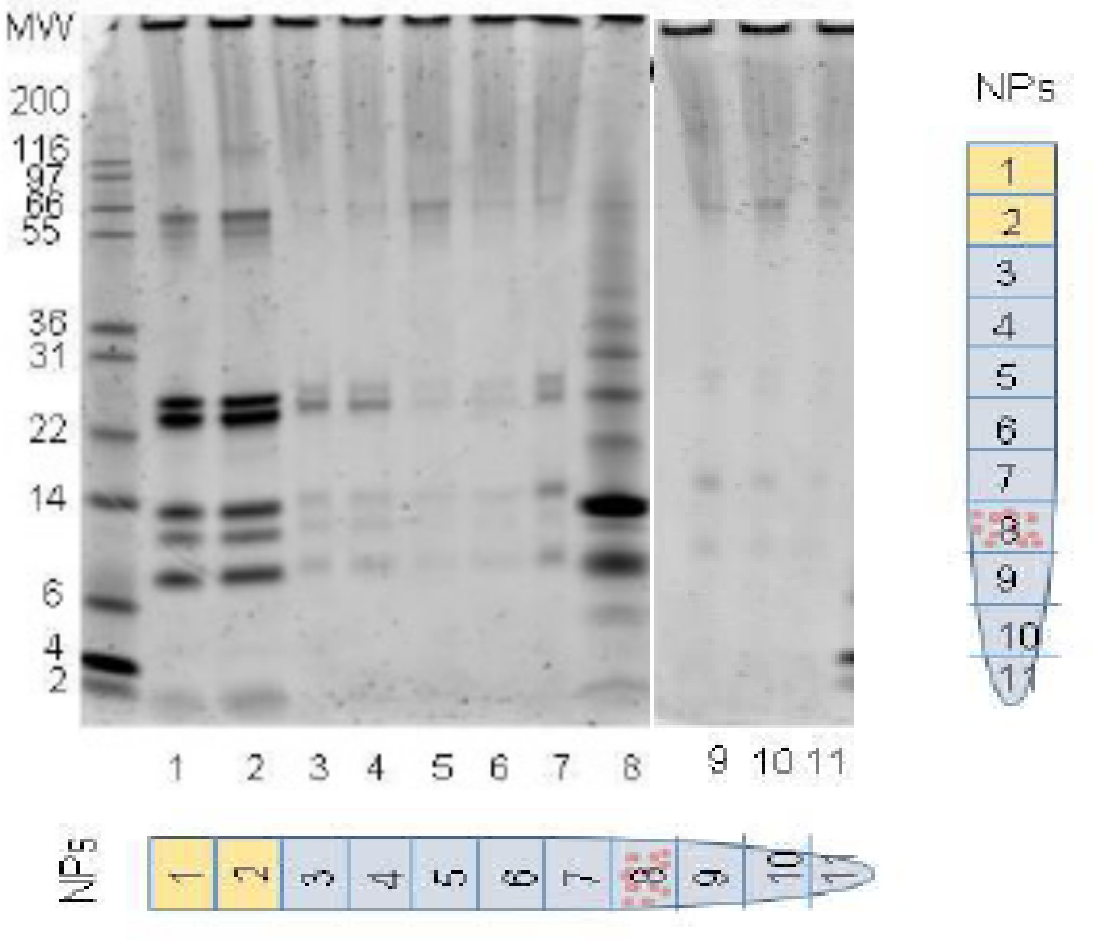
* Nanoparticle tracking analysis

NPs in digesta



SDS-PAGE of Fe_3O_4 NPs in SIF after separation by UC.

Lane 8 contains the NP complexes; lanes labelled HC and SC contained HC and SC complexes, respectively, isolated by conventional methods.

Schematic drawing of the sucrose layers in the UC tube showing where NPs and proteins were located

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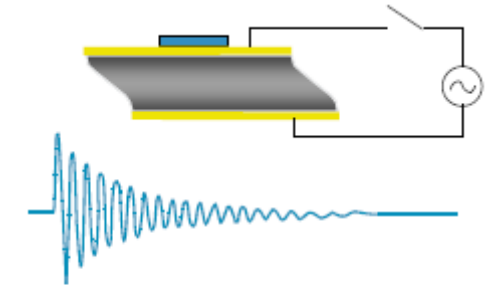
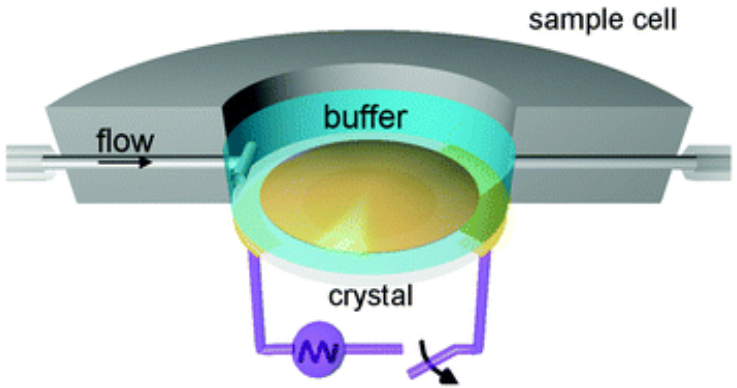
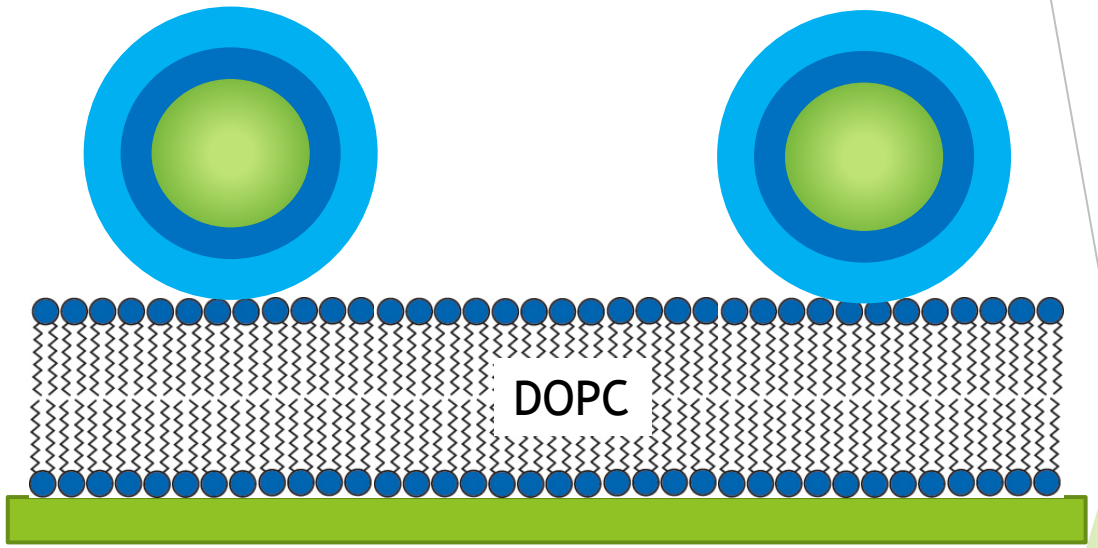
Quartz crystal microbalance (QCM-D)



Δf is related to the mass of the attached film (Sauerbrey relation)





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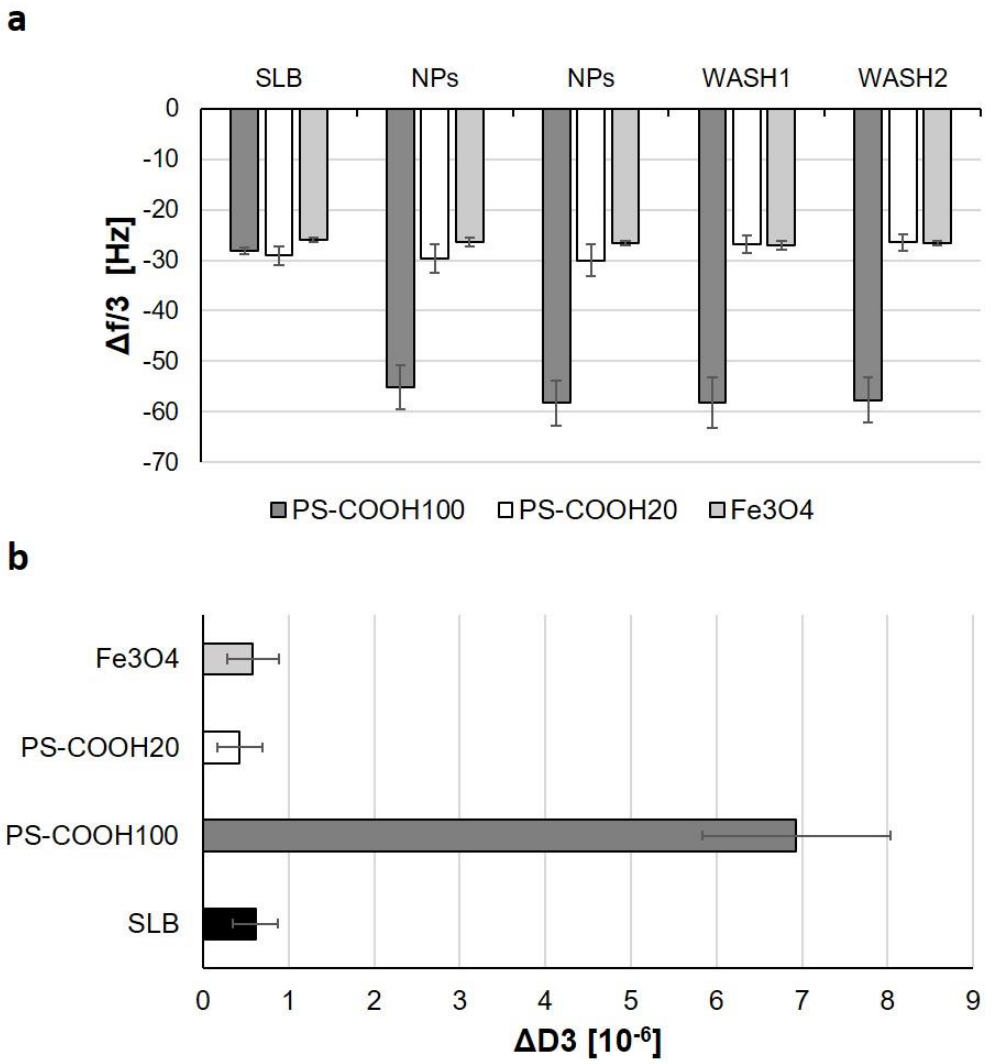
QCMD: Interaction with a lipid bilayer (SLB)

Frequency shift (a) after SLB formation, nanoparticle injection and washing

Dissipation (b) after SLB formation or at the end of the experiment



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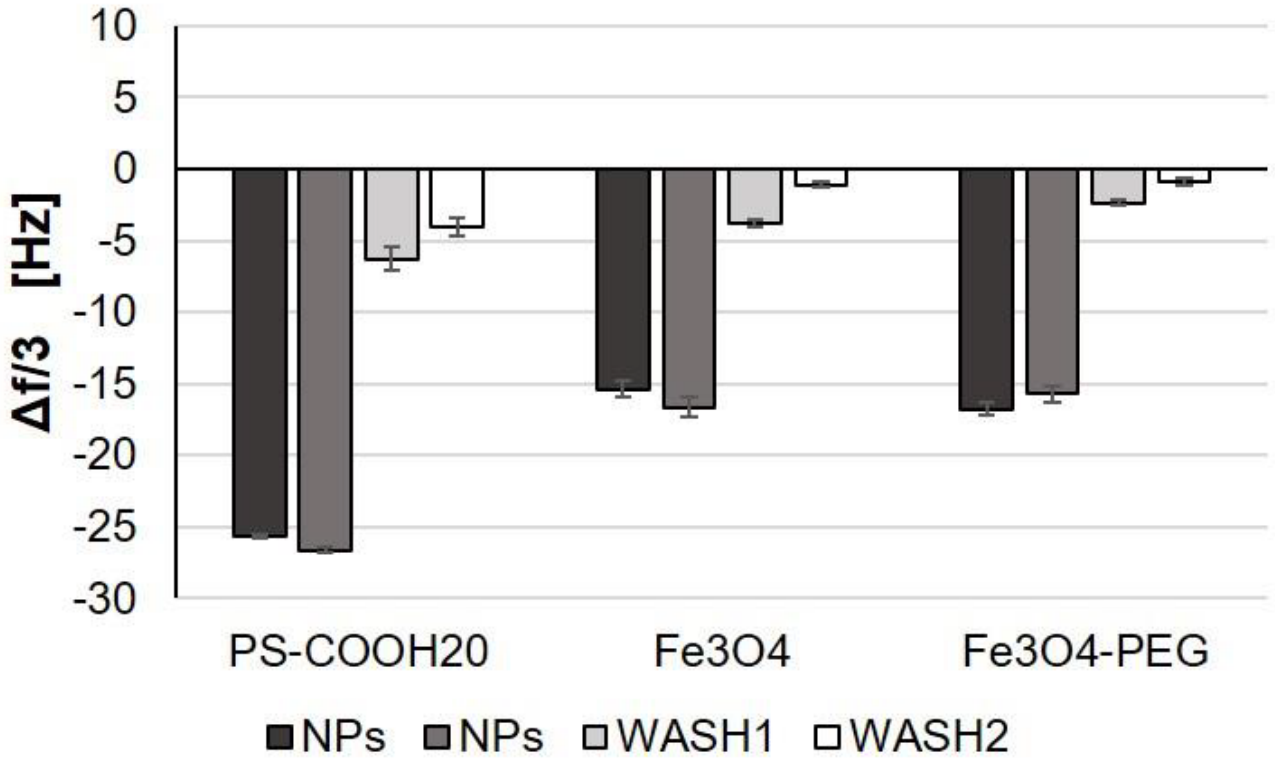
QCMD: Interaction with SLB + corona



NPs were added in excess of FBS proteins after one hour of incubation at 37°C, left to equilibrate on the SLB

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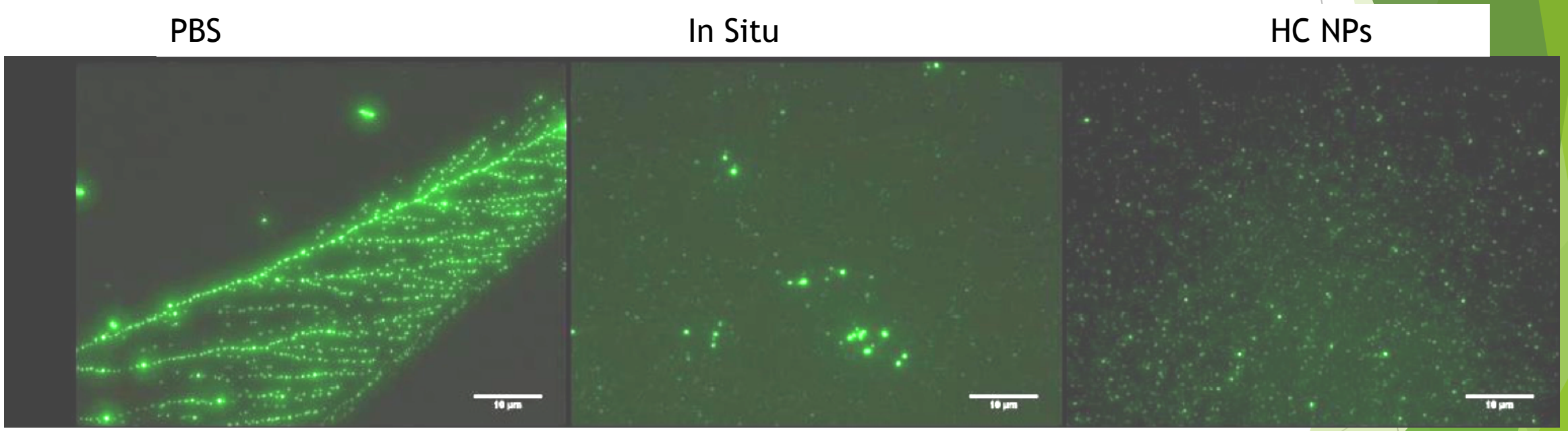
Do the NPs adsorb to the bilayer?

The QCM-D suggests little absorption



Fluorescence images of the QCM-D chips after experiments using PS-COOH20

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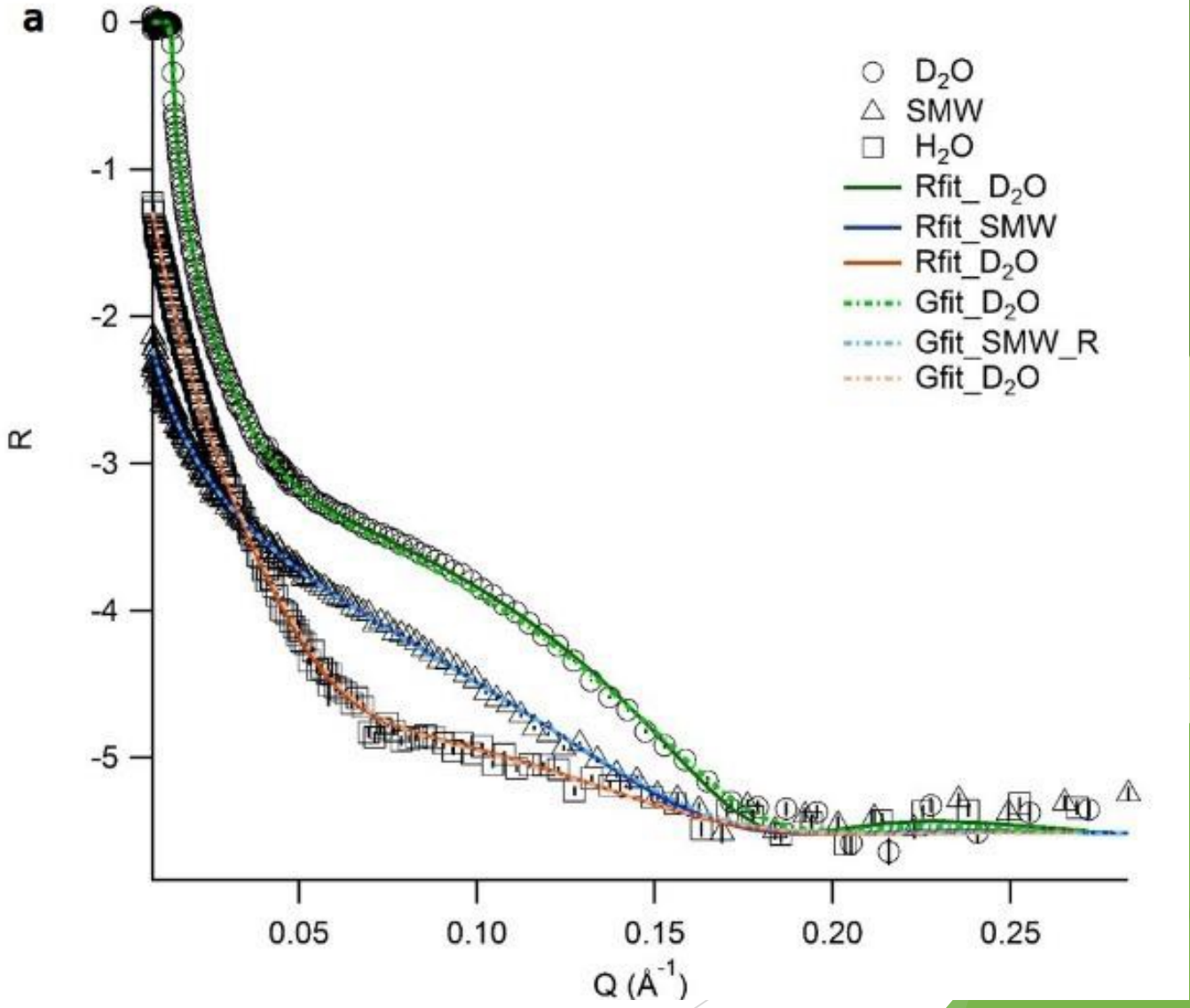
Analysis of absorption by Neutron Reflectometry

Three spectra from the SLB based on:

- D_2O
- H_2O
- Silica Matched Water



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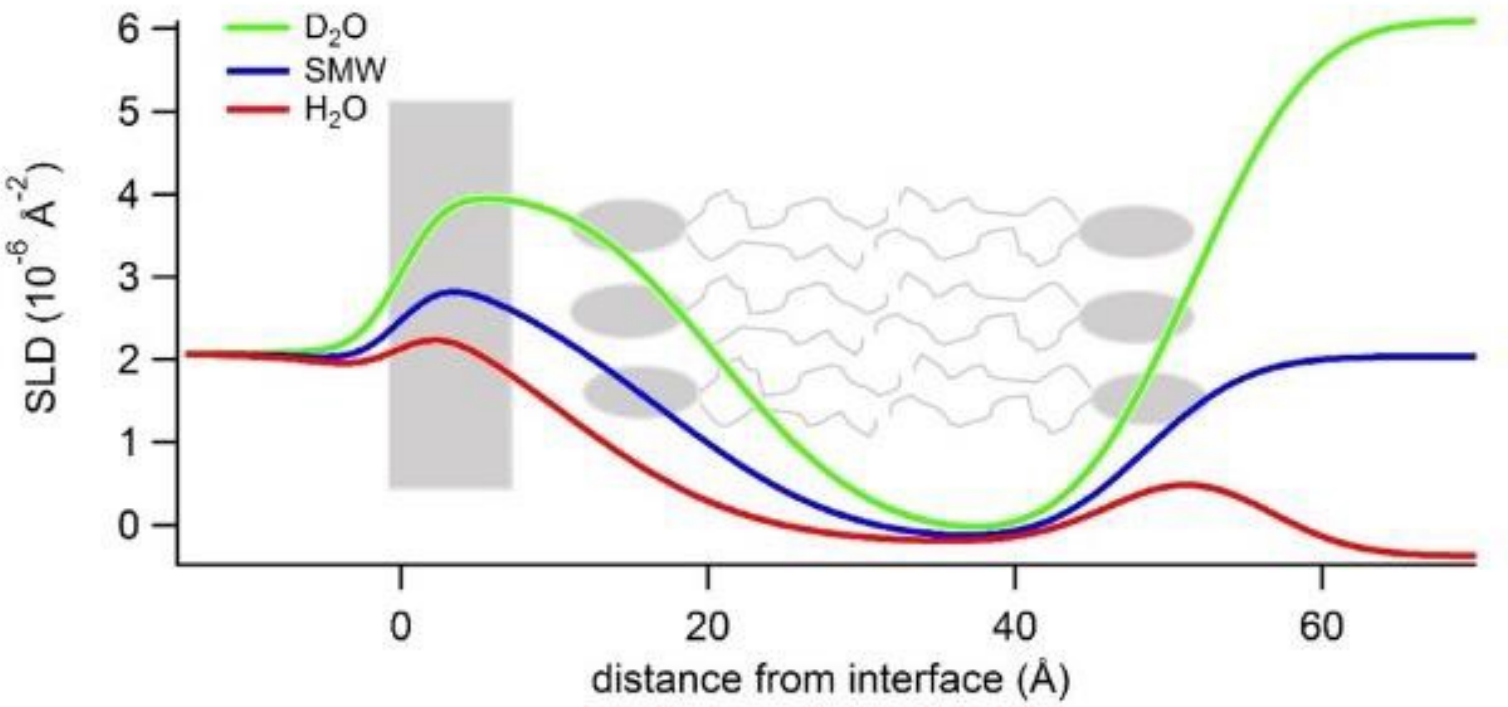
Scattering length density for the SLB



The head groups and acyl regions are clearly shown

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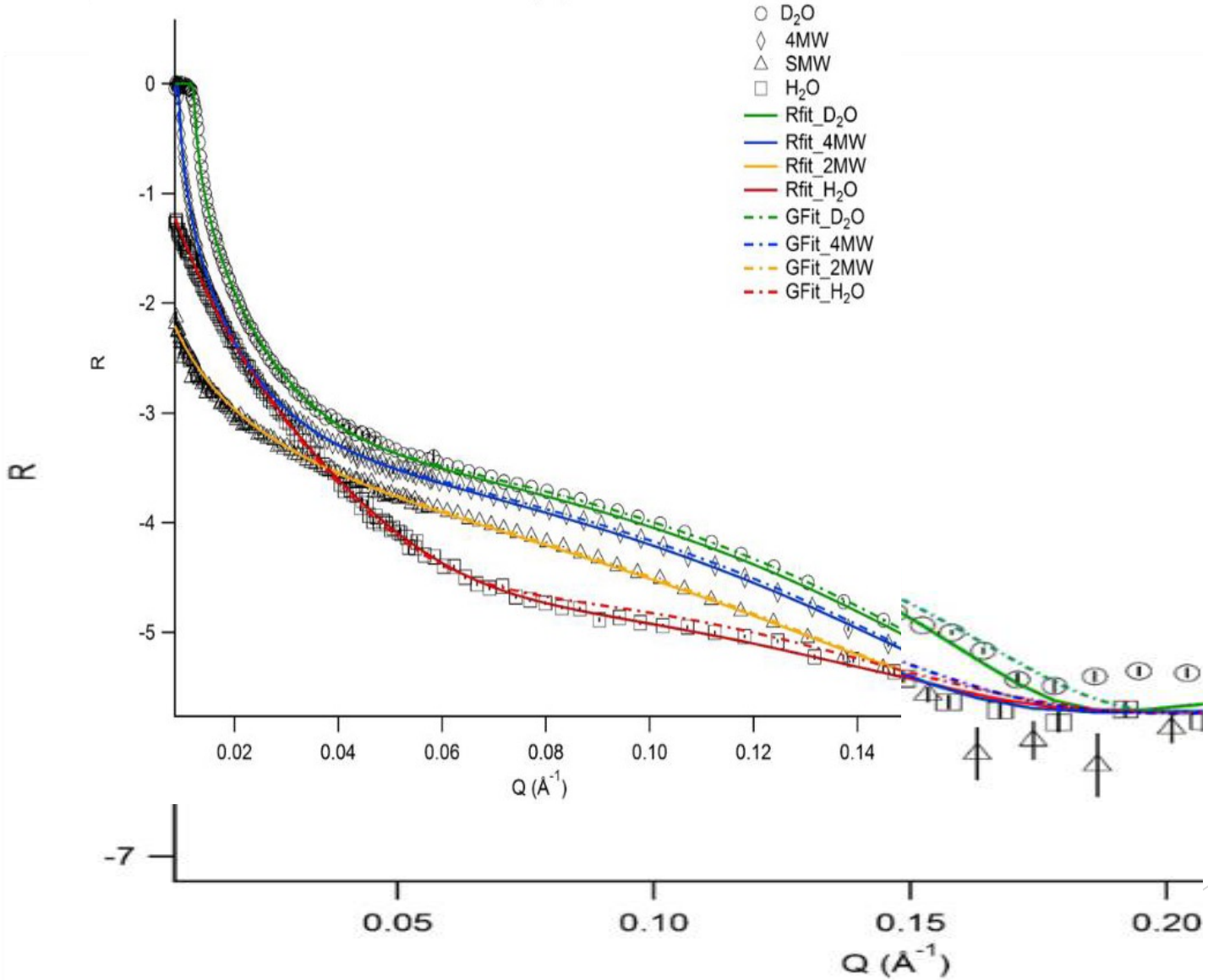
Addition of pristine NPs to the SLB



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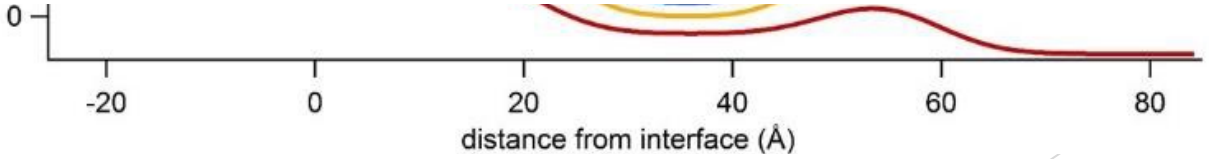
The Numbers

PS-COOH20 induced structural changes on SLB. The parameters describing the SiO_2 layer and the roughness of the layers were kept constant to evaluate the effects on thicknesses (t) and solvent penetration degrees (φ).

Layer	t(Å)		φ	
	SLB	NPs	SLB	NPs
2	11.6	12.4	61.7	64.4
3	13.7	14.8	0.3	7.6
4	13.7	14.8	0.3	10.7
5	6.4	6.6	30.4	34.9

Layer 2 is the inner head group, layer 3 is the inner tail group, layer 4 is the outer tail group and layer 5 is the outer head group.



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The effect of different NPs

PS-COOH20 NPs

Layer (L)	t [Å]		Φ	
	SLB	NPs	SLB	NPs
2	11.6	12.4	61.7	64.4
3	13.7	14.8	0.3	7.6
4	13.7	14.8	0.3	10.7
5	6.4	6.6	30.4	34.9

Fe_3O_4 NPs

Layer (L)	t [Å]		φ	
	SLB	NPs	SLB	NPs
2	10.3	10.7	57.1	58.5
3	14.1	15.1	3.1	9.8
4	14.1	15.1	3.0	9.7
5	6.4	11.0	31.3	59.7

Fe_3O_4-PEG NPs

Layer (L)	t [Å]		Φ	
	SLB	NPs	SLB	NPs
2	12.0	9.6	63.0	54.2
3	14.3	14.3	4.7	4.4
4	14.3	14.3	4.6	4.3
5	6.3	6.1	30.2	27.6

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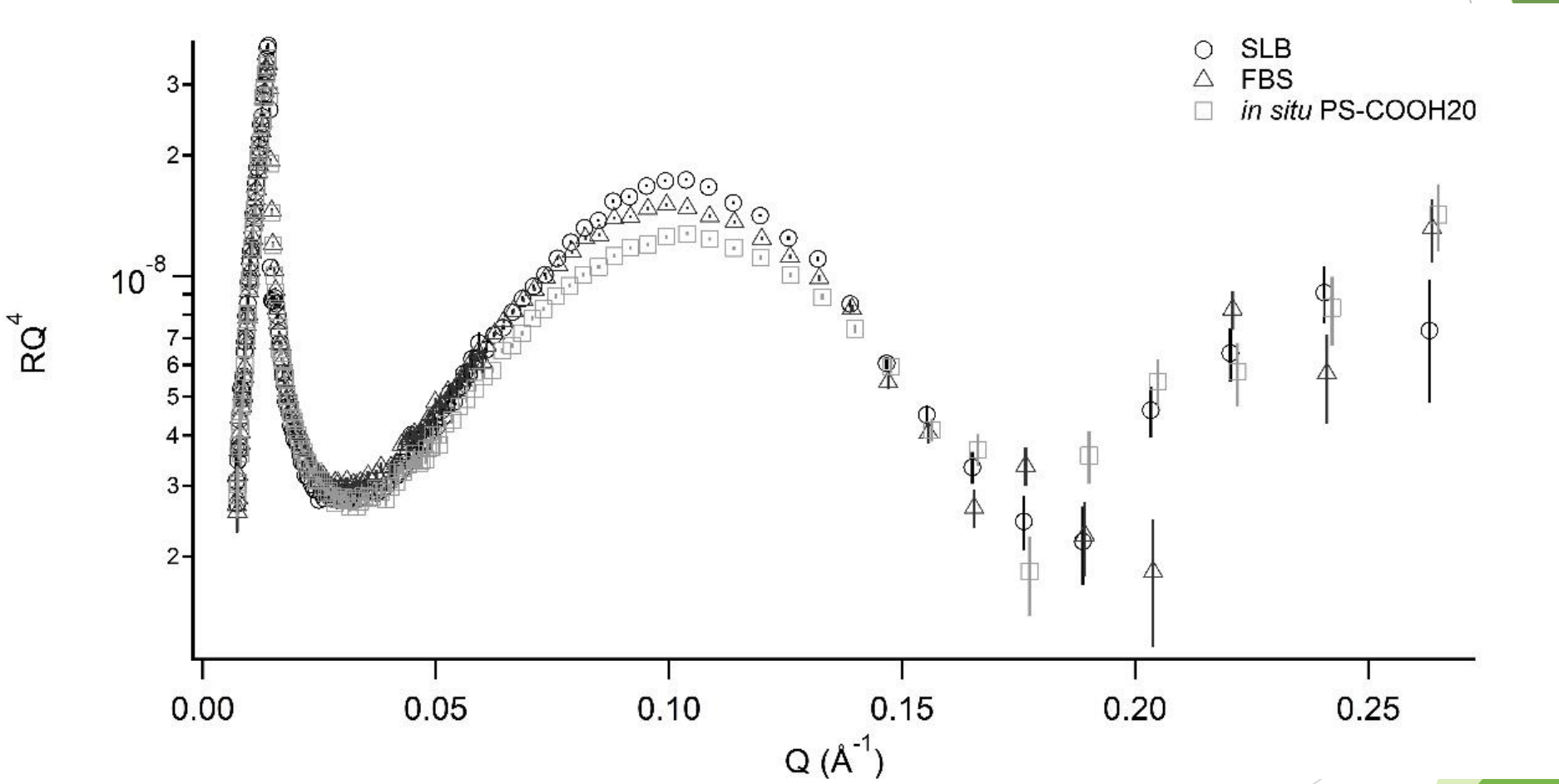
Addition of NPs with a corona to the SLB



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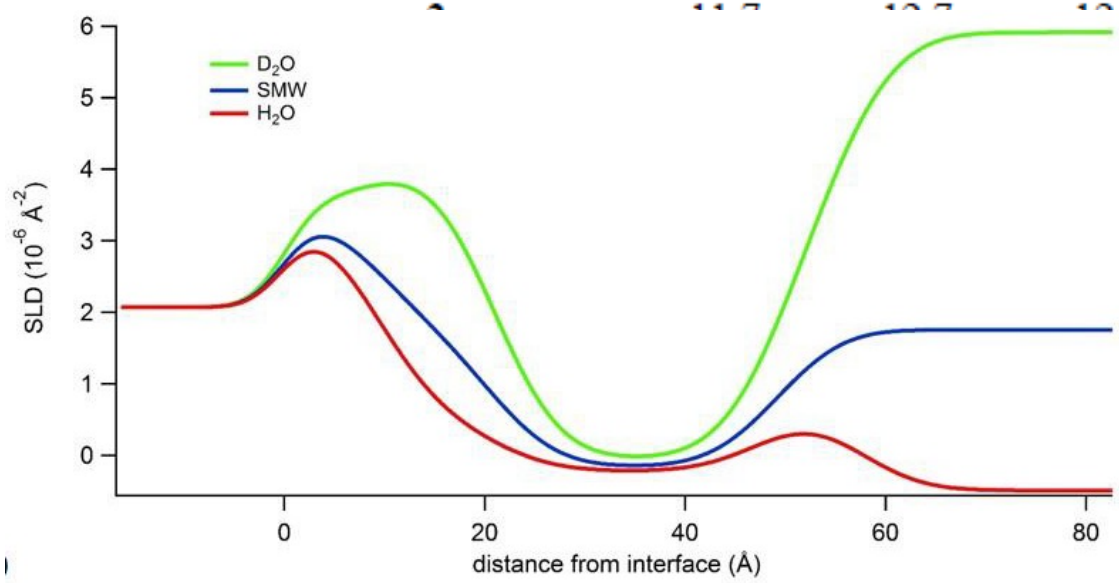
Addition of NPs and protein to the SLB

Layer (L)	t [Å]			Φ		
	SLB	FBS	IS NPs	SLB	FBS	IS NPs



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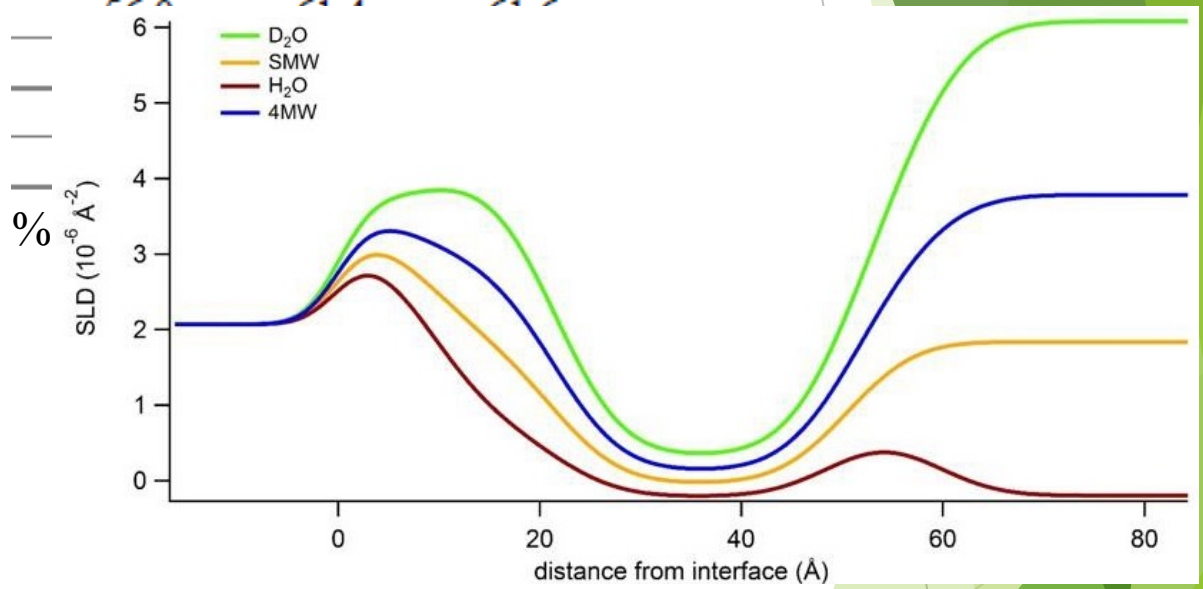

The lipid bilayer was characterized in D_2O, 4MW (H_2O:D_2O 34:66), SMW (H_2O:D_2O 62:38), H_2O.

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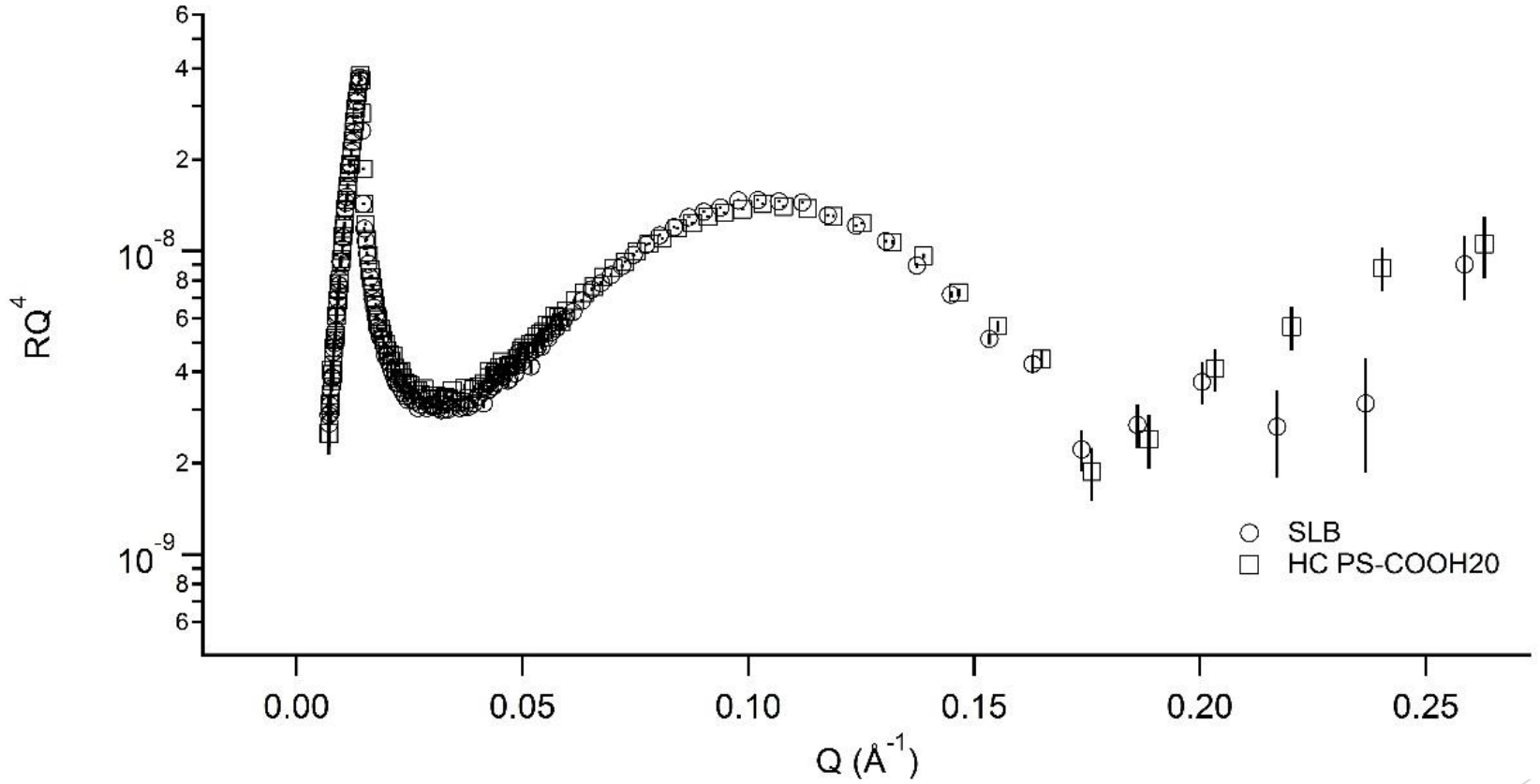
Addition of NPs with a corona to the SLB



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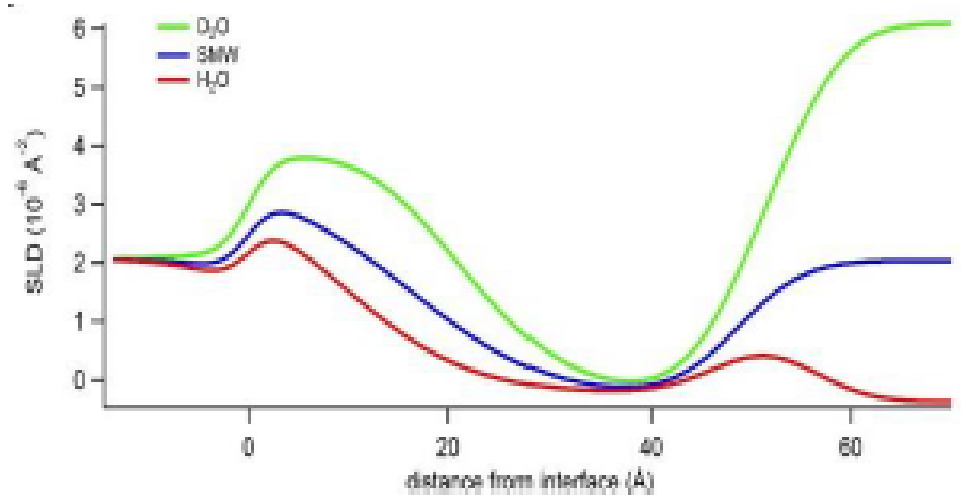
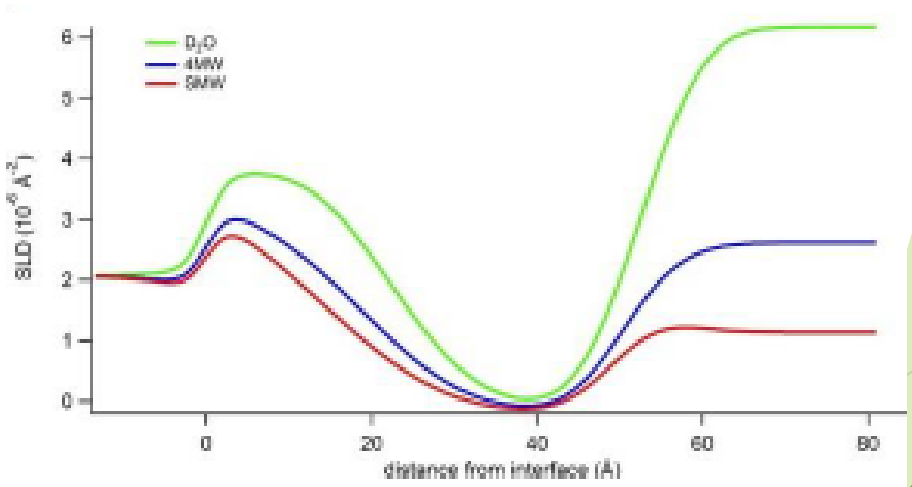
Addition of NPs with a corona to the SLB

Layer (L)	t [Å]		φ	
	SLB	NPs	SLB	NPs
2	11.7	11.9	62.0	62.7
3	13.7	13.7	0.6	0.4
4	13.7	13.7	0.5	0.3
5	6.0	6.4	26.3	30.5





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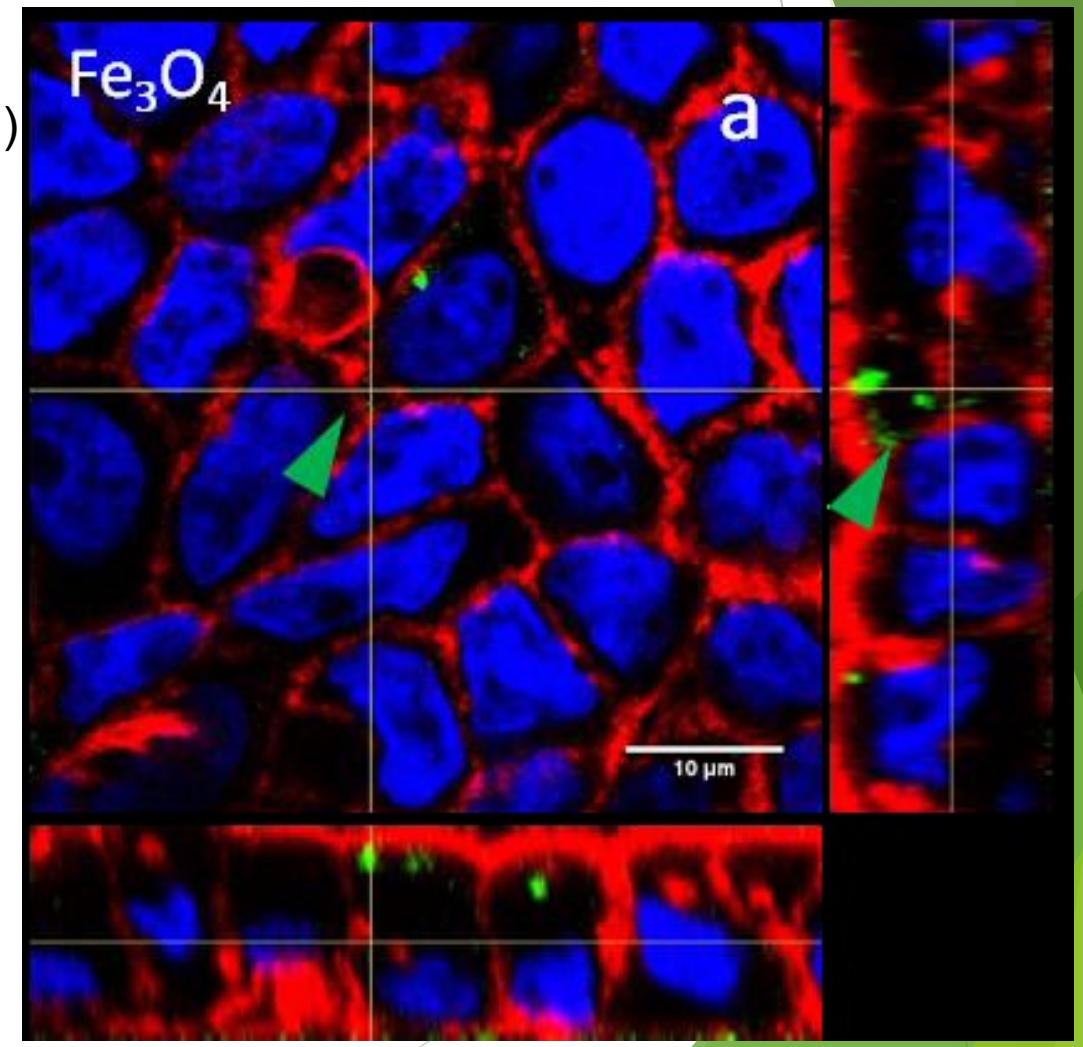
Addition of NPs to intestinal cells

Pristine NPs incubated monolayer.

- Actin filaments -> Phalloidin- Texas Red (591/608 nm)
- Nuclei -> Hoechst 33342 (350/461 nm)
- Fe_3O_4 NPs -> BODIPY FL-EDA (500/510 nm)



NPs seemed to accumulate at the intersections among adjacent cells and induce alteration in the tight junctions.

The amount of NPs taken up was quite low and some clusters are visible in the upper part of the cytoplasm.

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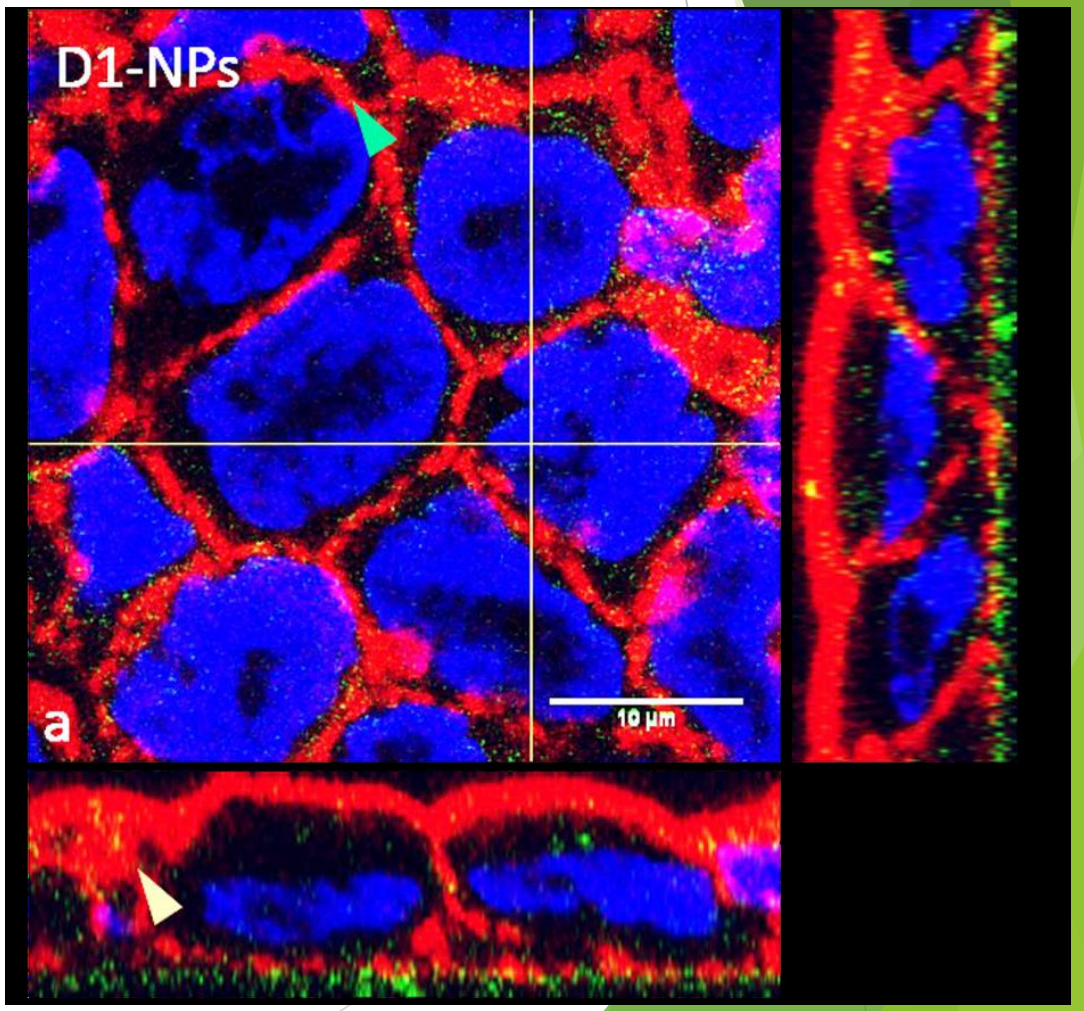
Addition of NPs to intestinal cells

D1-NPs were isolated from a duodenal environment

D1-NPs were present in the cells mainly as clusters enclosed in vesicles formed from the apical membrane as shown with an orthogonal stack and a top view of the monolayer.

However, most of the NPs are located between the basal and the support membranes and at the top of the porous channels



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Conclusions

- The QCM-D results were not conclusive and coupling with Neutron Reflectance was necessary. Although agreement between QCM-D and NR results was found, NP adsorption was not confirmed.
- The effects of NP corona on uptake and translocation across a Caco-2 cell monolayer were evaluated. It was confirmed that the corona enhanced the uptake of magnetite NPs in agreement with previous results in the literature for other NPs bearing plasma or serum derived coronas.

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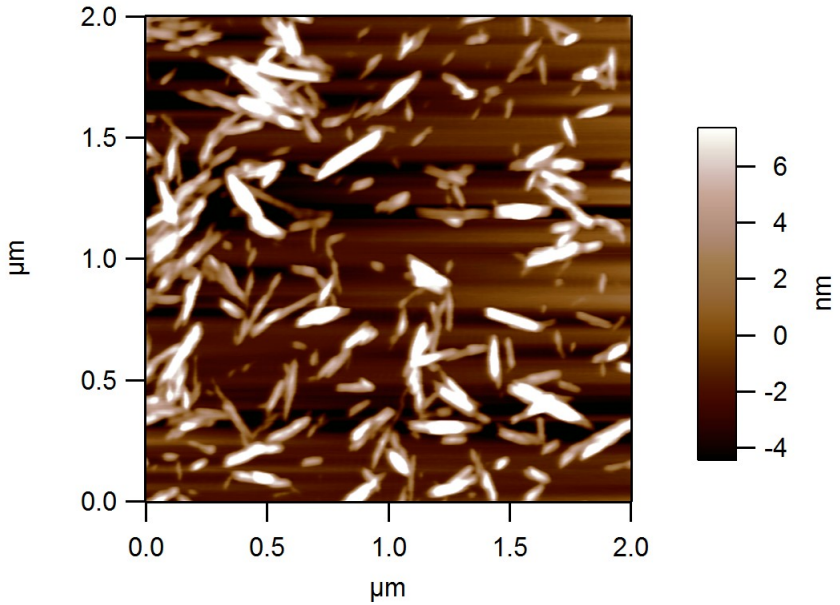
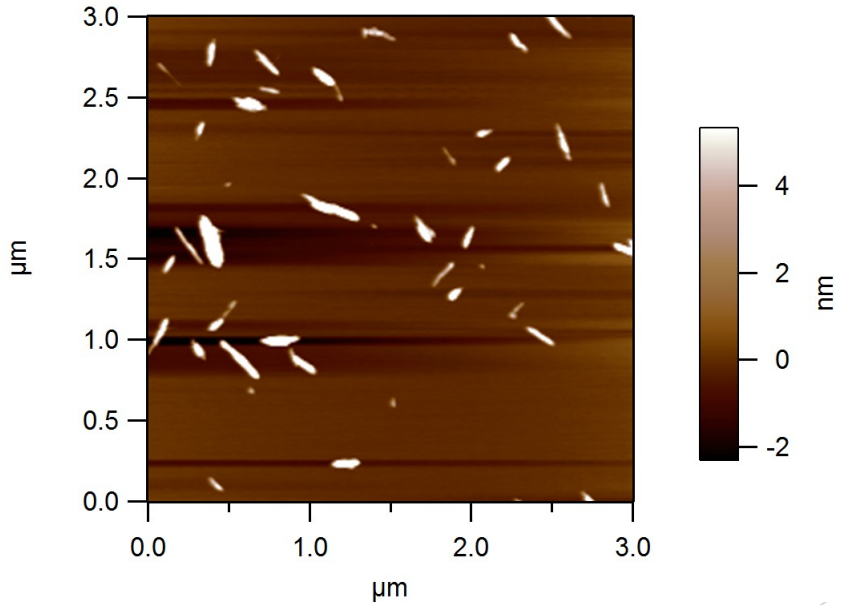
Cellulose nanocrystals (CNCs)

AFM images of the CNCs on mica





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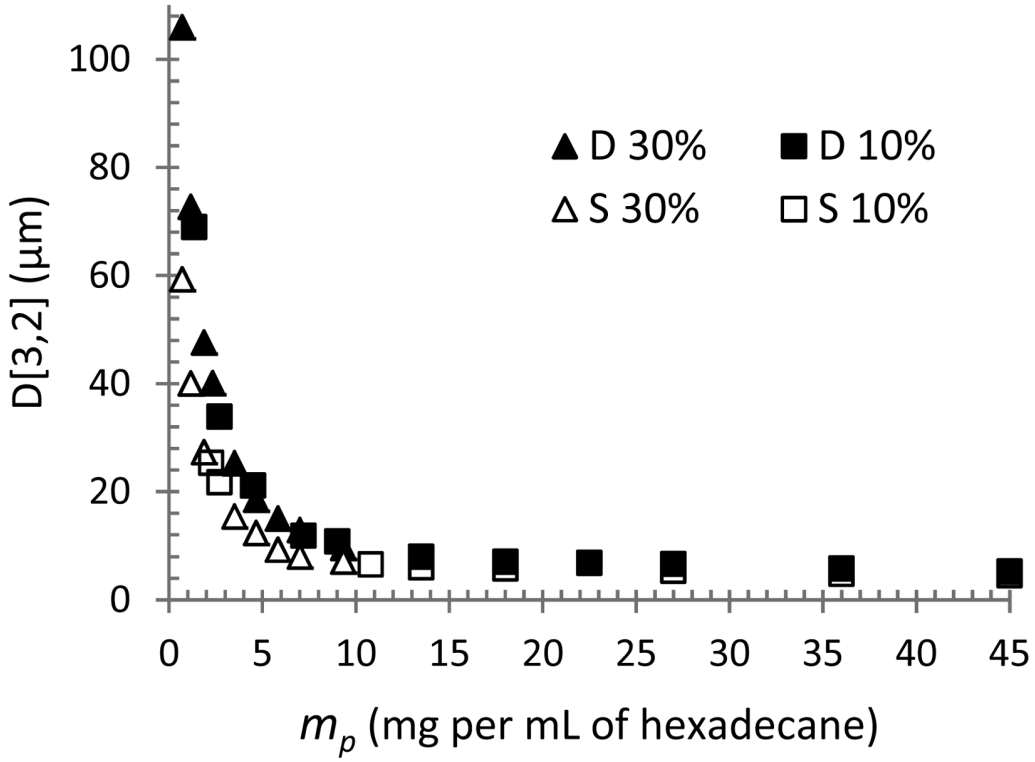
Nanoparticles for Pickering stability

Thanks to Isabelle Capron

UR1268 Biopolymères Interactions Assemblages, INRA, F-44316 Nantes, France



- 10% oil in water
- 10 mg CNCs /mL of oil
- Emulsified by sonication

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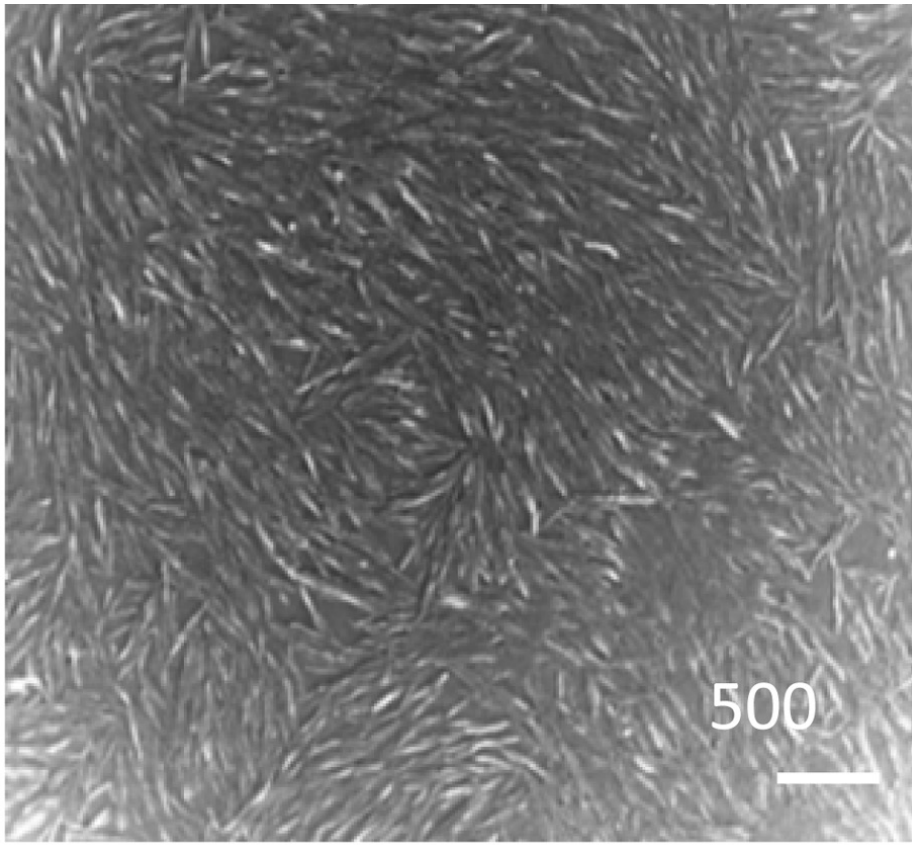
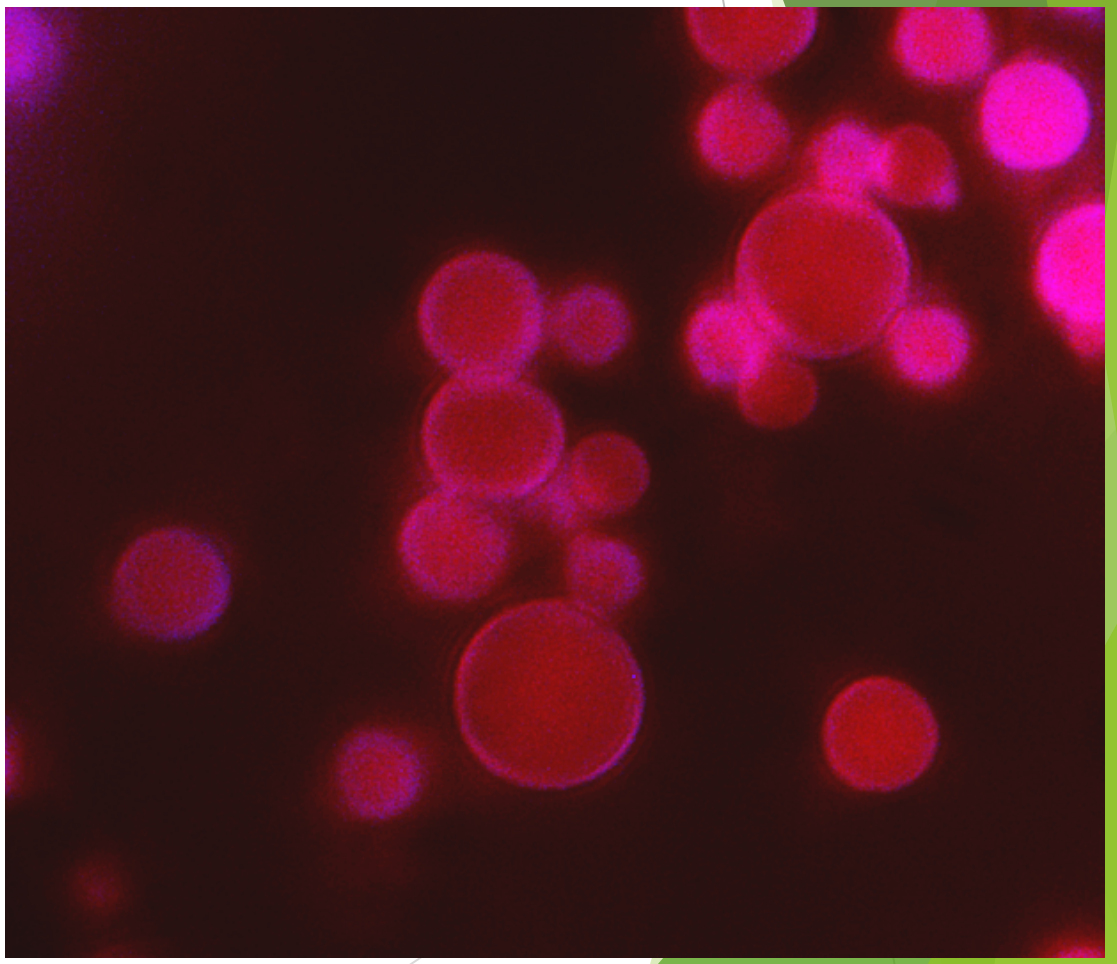
Cherhal et al. Biomacromolecules 2016, 17, 496−502

Characterising the surface layer





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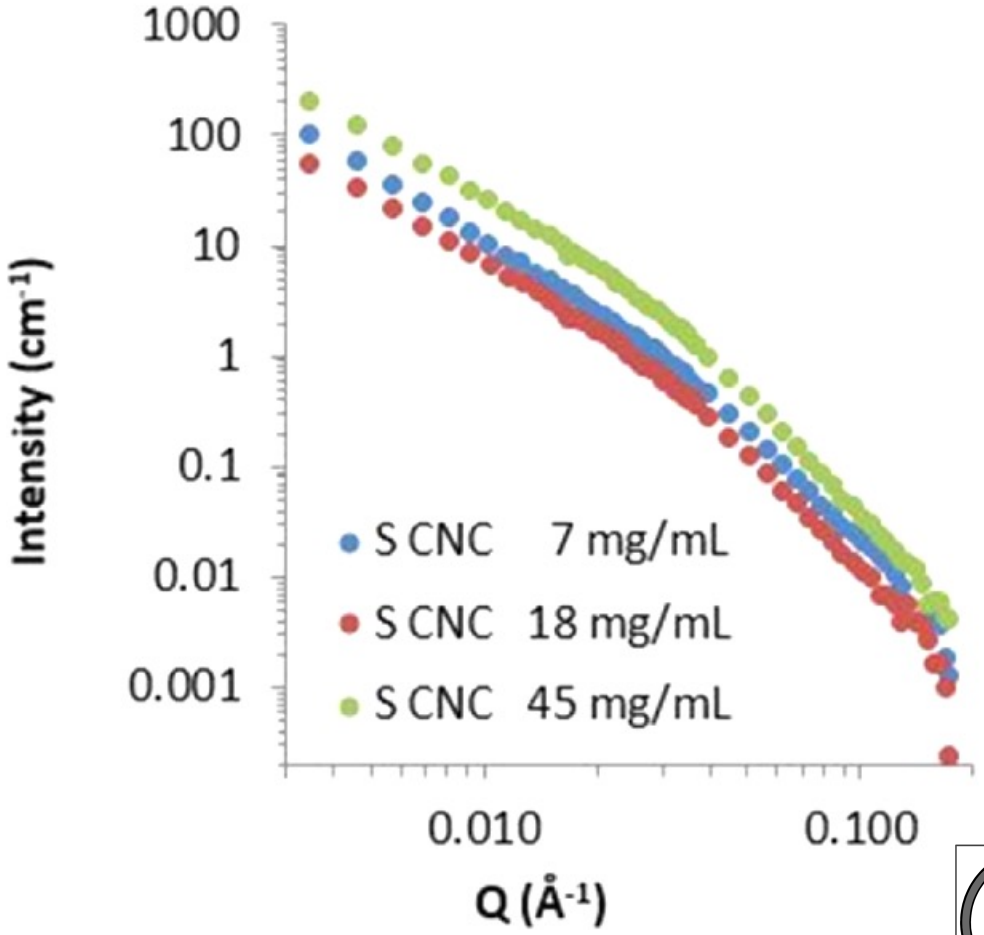
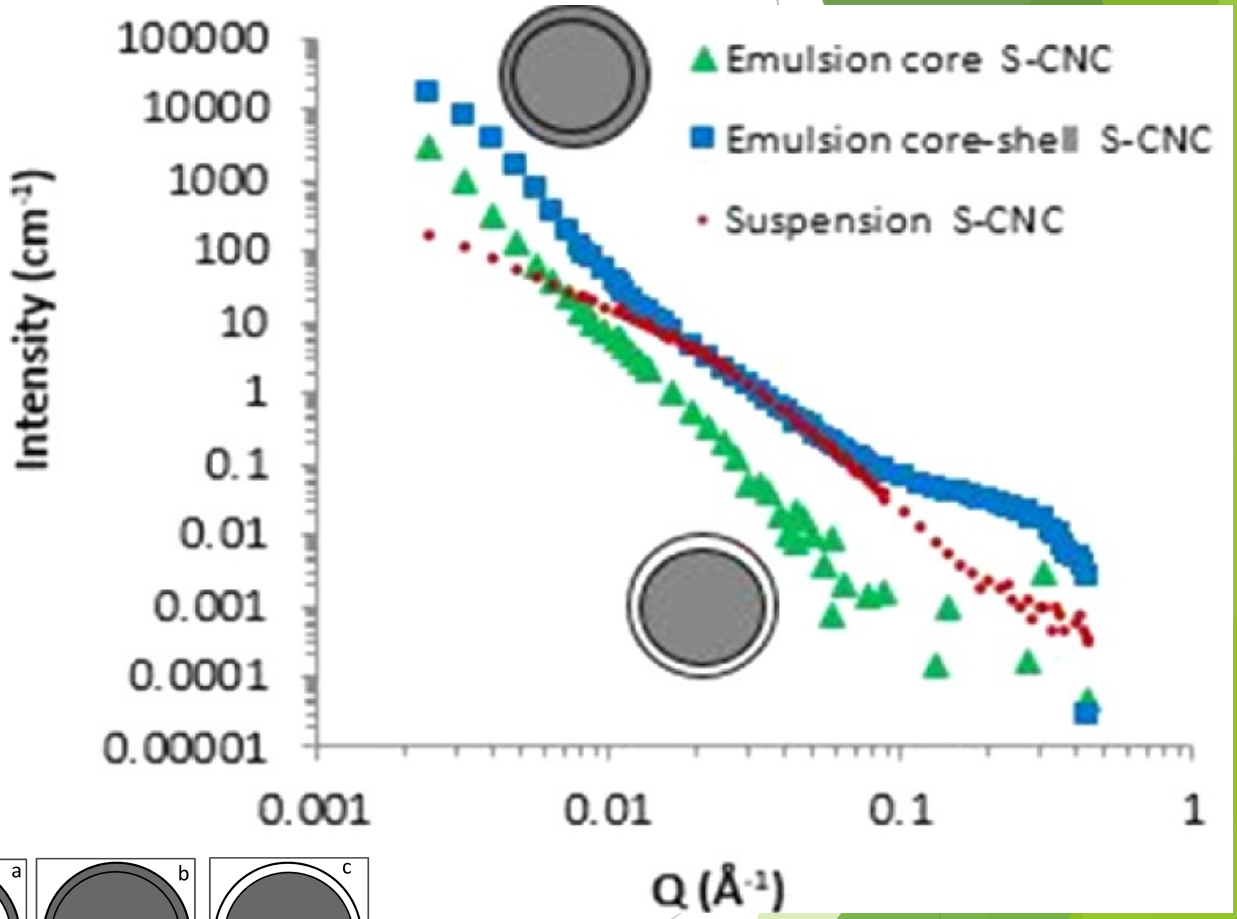
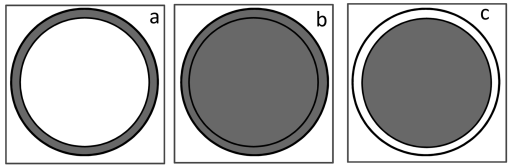
SANS data from hexadecane in water emulsions







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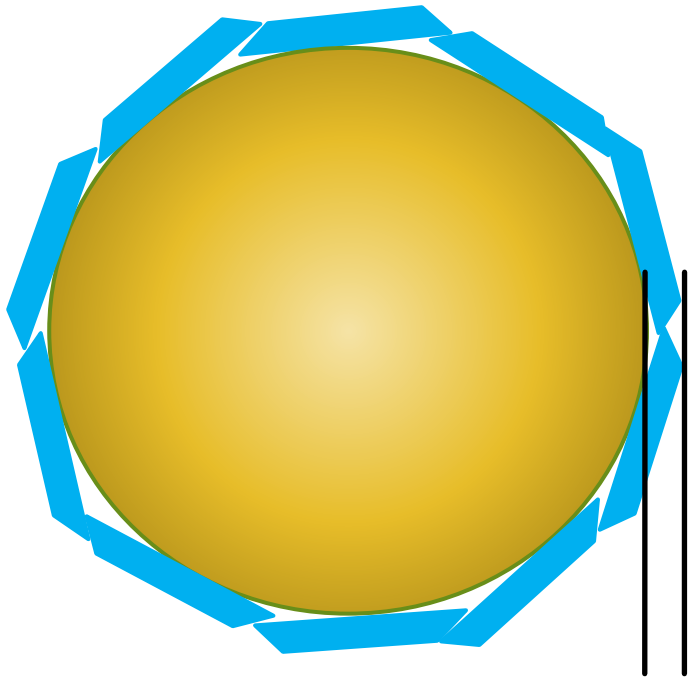
Results of the analysis



Layer thickness = 7 nm

The results strongly suggests that the (2 0 0) crystalline plane of the CNC directly interacts with the interface without deforming it. As a result, only surface interactions occur between the CH of the CNC and the alkyl chain of hexadecane.

This experiment shows clearly that rigid nanoparticles can be densely adsorbed at the oil/water interface without deforming it at the nanoscale.

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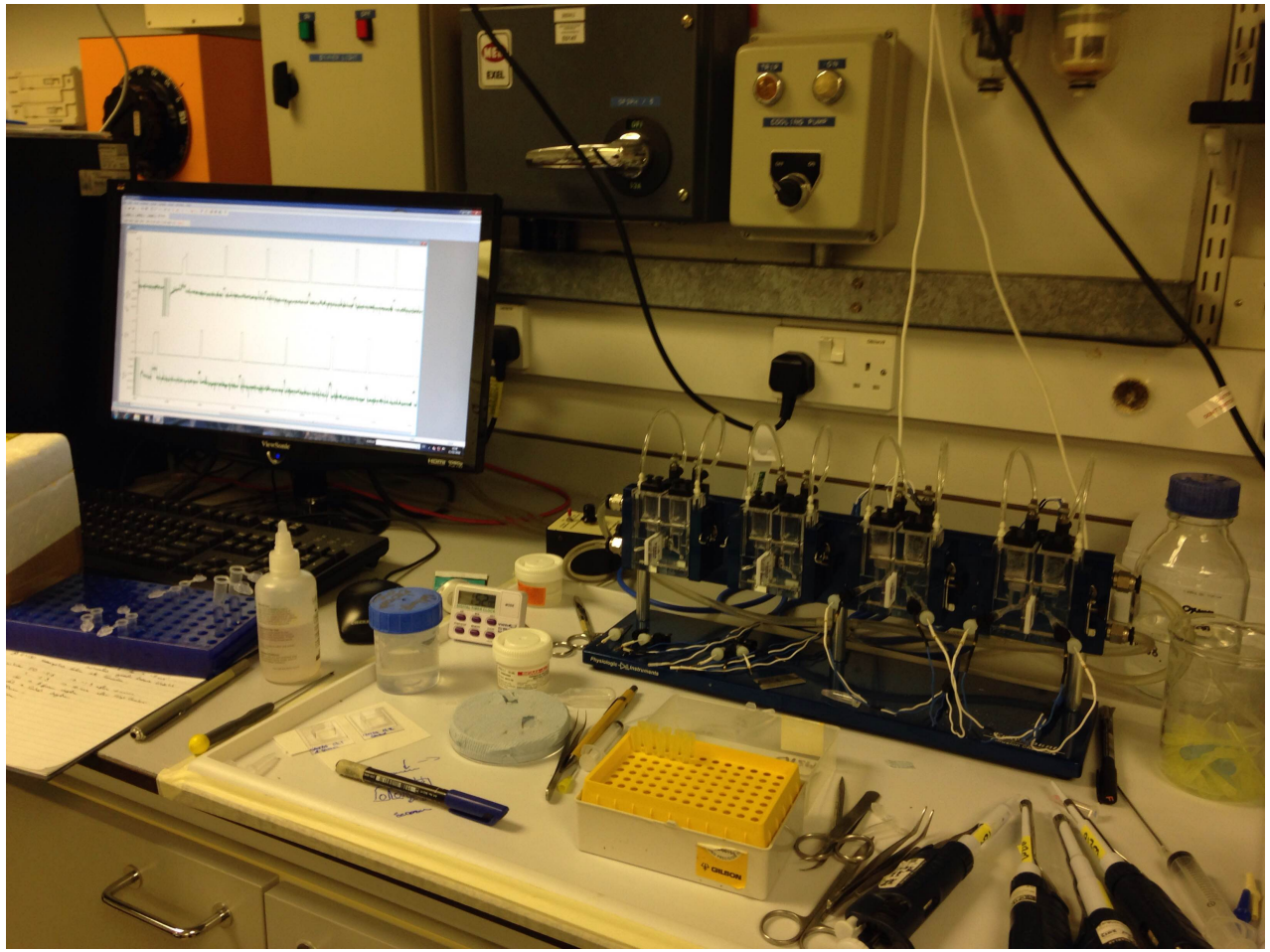
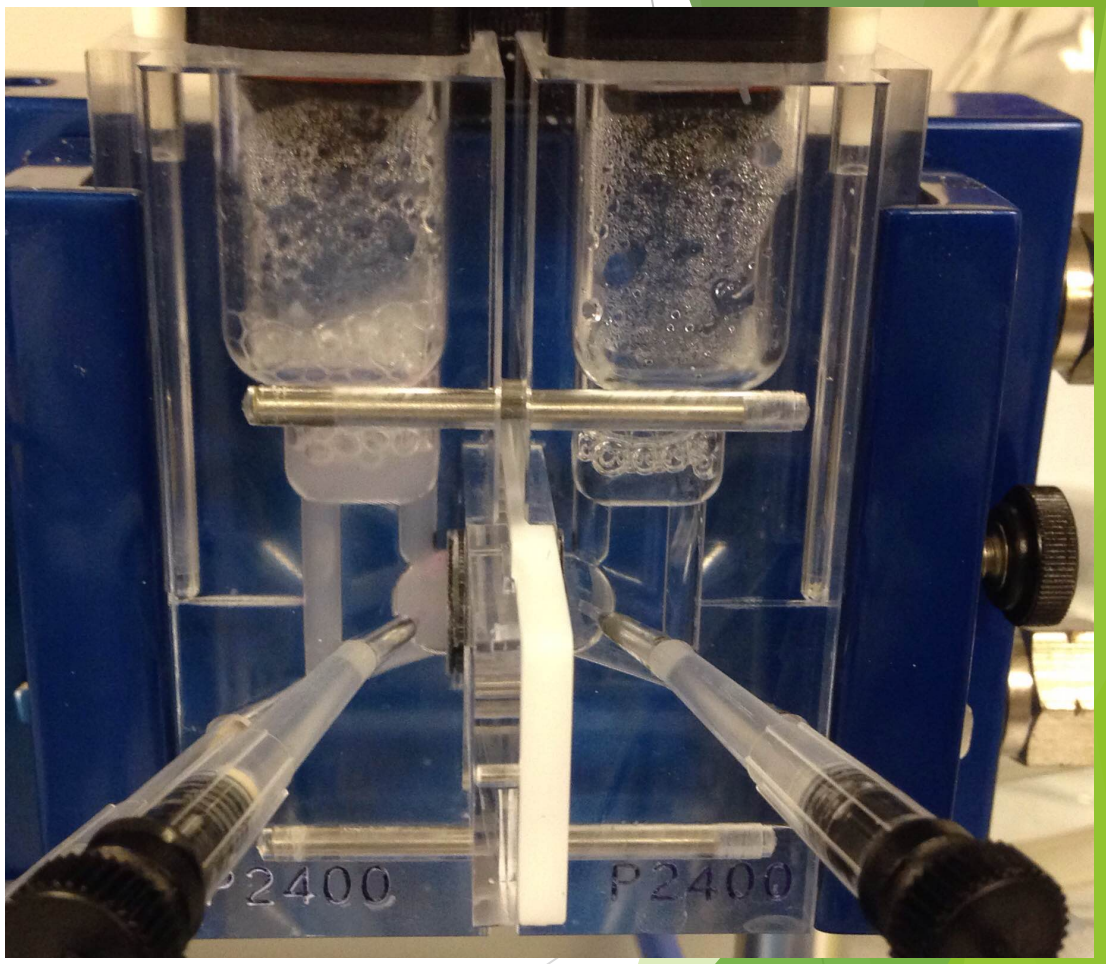
Future plans





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The future

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