Influence of protein corona on immune system activation and cellular uptake of nanoparticles by phagocytic cells

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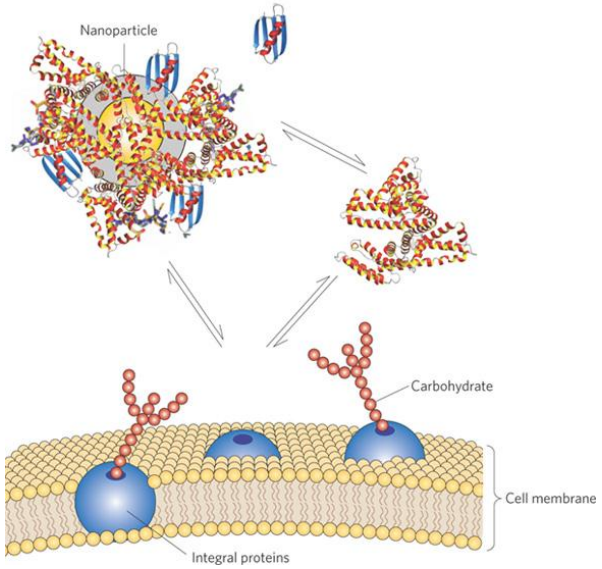
Introduction

Purpose of the visit

The aim of this project was to compare the efficiency of uptake and nanoparticle location for the different coronas in the 3 cell types (A549, Raw 264.7, THP 1) by flow cytometry, and confocal microscopy. In this way we tried to understand the different behaviour in specialised macrophages and connect it to the different properties of the nanoparticle-corona complexes. NPs were dispersed in serum free medium, medium supplemented with different content of Bovine serum, Human Serum and Human Plasma and NPs with a corona formed by albumin only, leading to different nanoparticle-protein complexes composition



Introduction

The "protein corona"

Once nanoparticles are dispersed in a biological fluid (plasma, or otherwise) they act as a scaffold for biomolecules such as proteins, lipids etc. which they rapidly adsorb to their "naked" surface, conferring a biological identity to the nanoparticle, leading to the so called protein "corona". During the nanoparticle internalization process into the cell, the adsorbed protein layer "corona" is what the cell will "see", therefore nanoparticle-protein complex composition and structure, etc. will influence not only cell uptake and trafficking, but also particle biodistribution within the cell.

Depending on the cellular morphology and its tissue origin, nanoparticles can be internalized by cells using different mechanisms, and this project aims to elucidate if typical cell surfaces will have great affinity for bare particles than to the protein "corona" layer surrounding the nanoparticles.

The systems investigated

Cell line	Type of cell line
A549	Human lung epithelial carcinoma
Raw 264.7	Mouse monocyte macrophage
THP 1	Human monocyte macrophage

Fluorescent size Kit, carboxylate-modified **polystyrene** microspheres

Size (nm)	DLS size(nm) in water	
40	68	72
	75	
	73	
200	275	270
	252	
	284	

Nanoparticles dispersed either in serum-free medium, or in cMEM (normal medium supplemented with different % of bovine serum (FCS), Human plasma (HP) and human serum (HS) see DLS data below)

5%, 10%, 50% and 100% of serum, plasma, albumin were used.

Different % of serum and plasma were calculated, according to their concentrations

(concentrations measured by BCA assay)

FetalBovineSerum (FCS) : 40mg/ml

Human Serum (HS): 85.2 mg/ml

Human Plasma (HP): 70 mg/ml

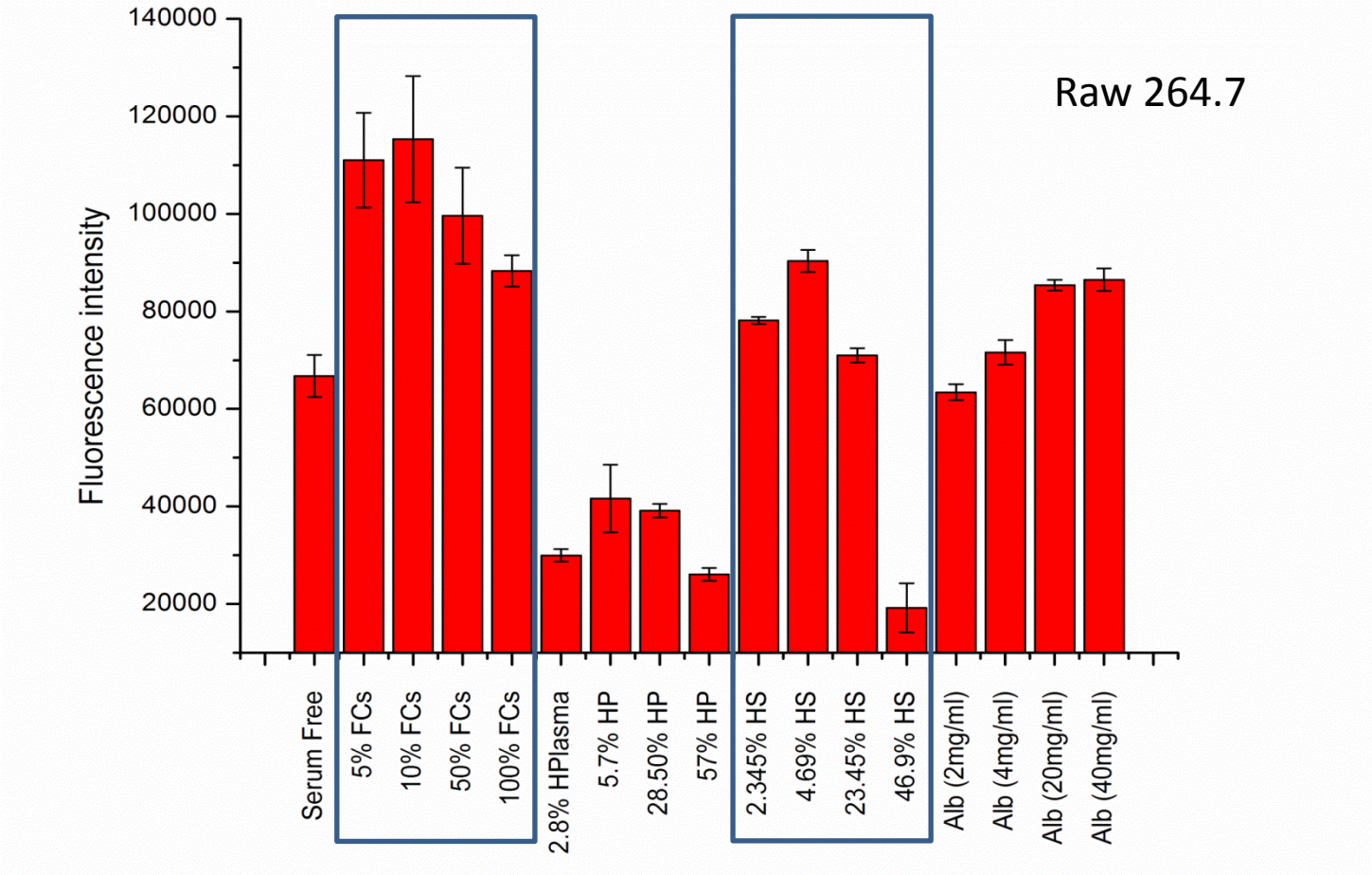
Raw 264.7

In Raw 264.7 results showed high reproducibility at early times (<8h), showing a strange behaviour once cell division starts to act (>12h) (see slide 12)

- Higher effiency on the Nps uptake when these are dispersed in Serum (either FCs or Human serum) when compared with plasma (human)
- Particles in serum free enter more then in plasma, which is not happening with when Nps are dispersed in serum
- Blood Serum is blood plasma without fibrinogen or the other clotting factors, so the differences on the uptake could be related to these factors
- 10% of FCS, HP, HS seems to be the “ideal %” for the uptake
- Strong cells dead when 100% of human serum is used

Serum/plasma dependence in RAW 264.7 (8h continuous uptake)

40nm COOH-Polystyrene



A549

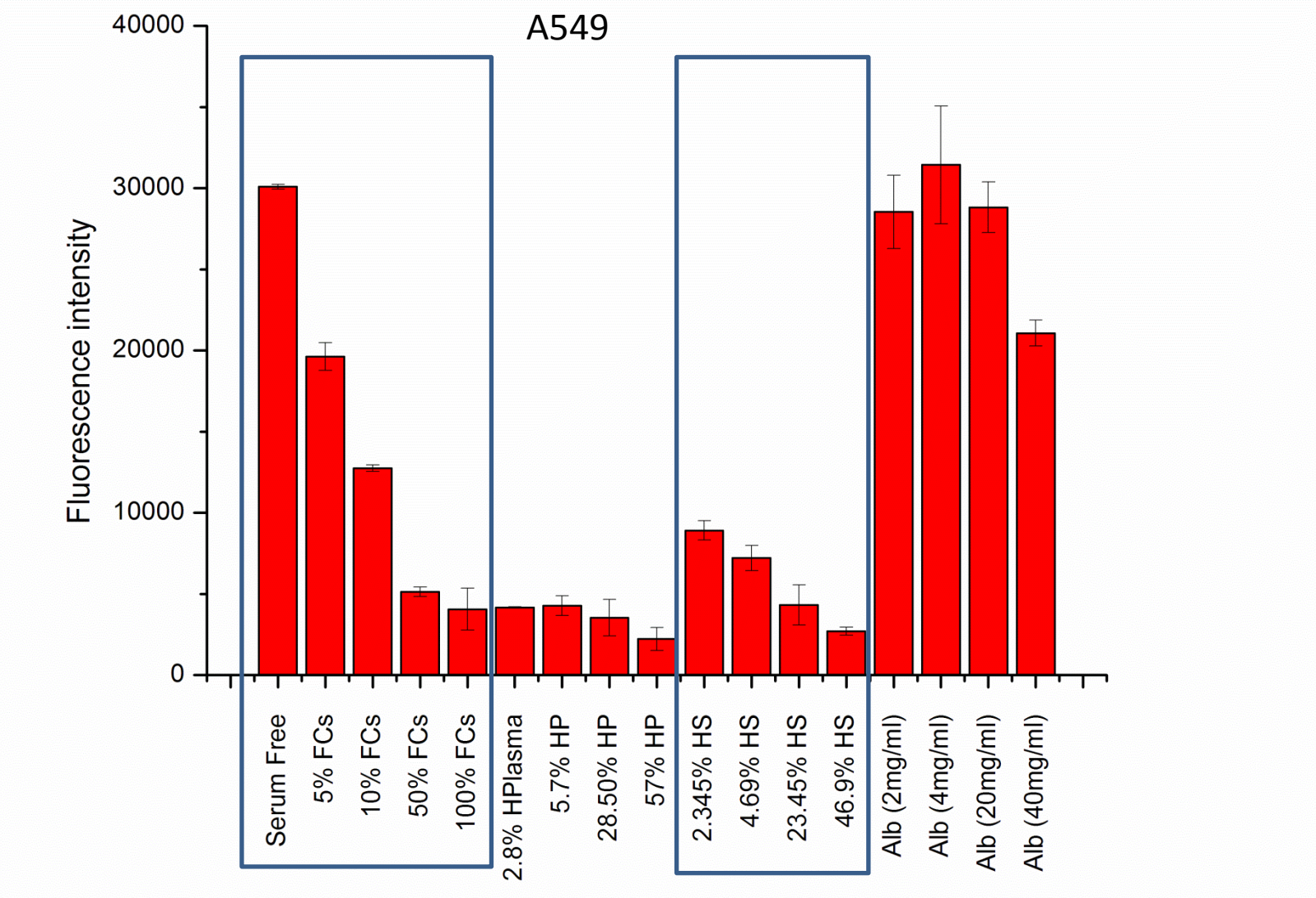
Uptake of serum free NPs showed to be always higher then in plasma or serum, when A549 cell line was used, showing an inverse behaviour of Raw 264.7

Uptake of Nps in serum (either bovine and human) is higher then plasma (cells are really stressed when especially 100% plasma and serum are used)

High uptake of NPs dispersed in albumin

Serum/plasma dependence in A549 (8h continuous uptake)

40nm COOH-Polystyrene



THP 1 (differentiated)

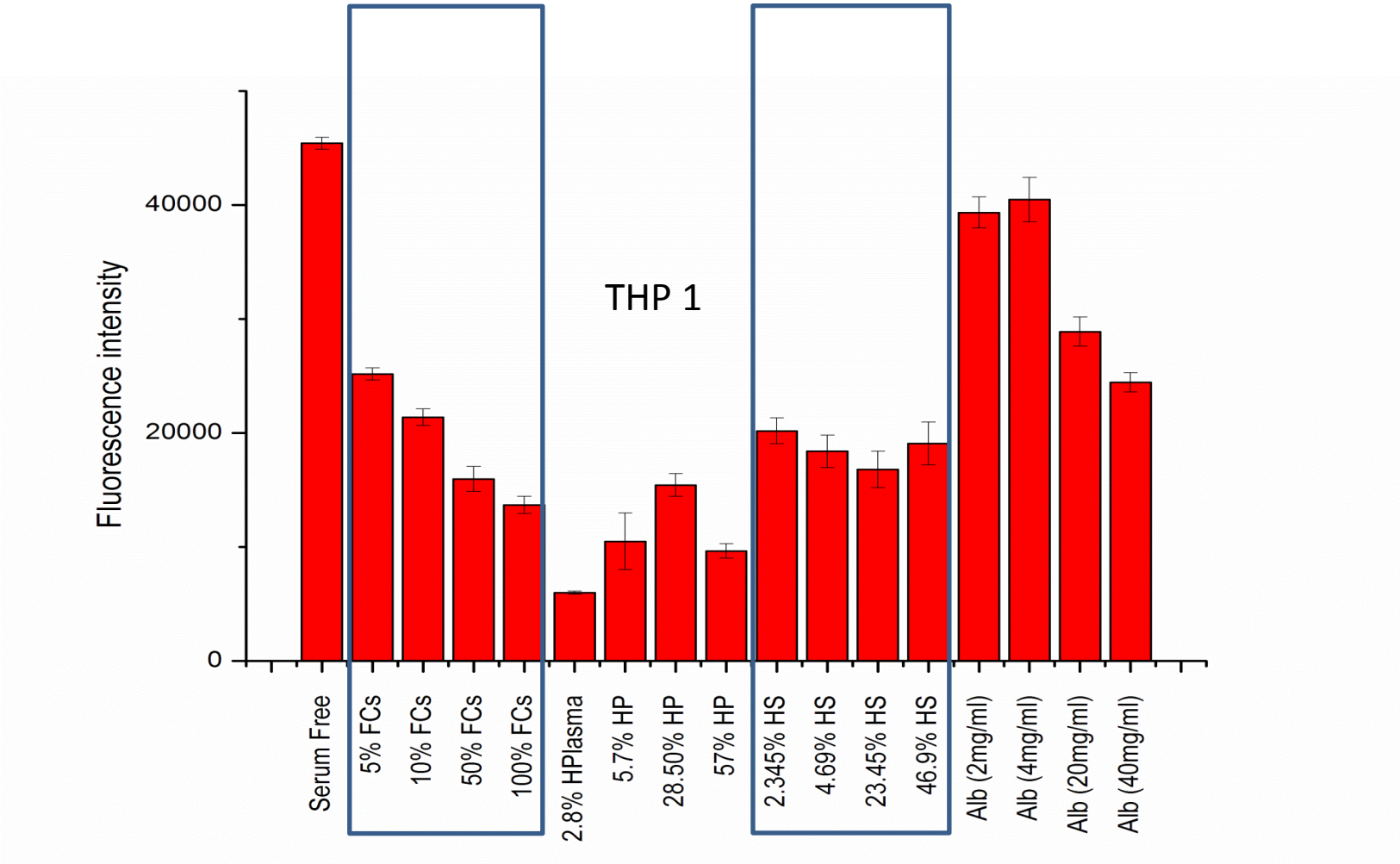
These cells grow in suspension, so phorbol-12-myristate-13-acetate (PMA) needs to be added in order to get them attached to the plate. It also allows differentiation of these cells into macrophages

20nM of PMA during 24h, was used in order to guarantee good diferentiation

Cells behave similarly to A549, with serum free NPs having higher uptake then in plasma/serum...

Serum/plasma dependence in THP-1 (8h continuous uptake)

40nm COOH-Polystyrene

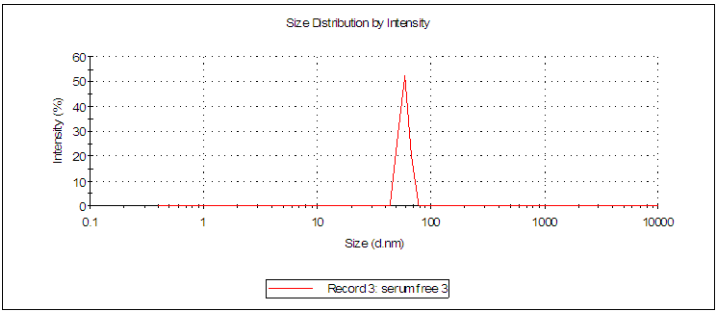


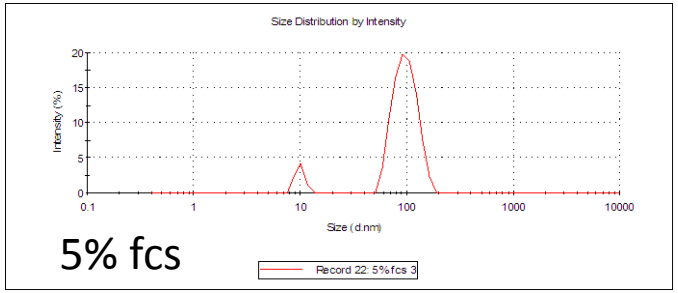
DLS data (40nm)

Huge protein aggregation, for higher % (100, 50) of Fserum, Hserum, Hplasma...

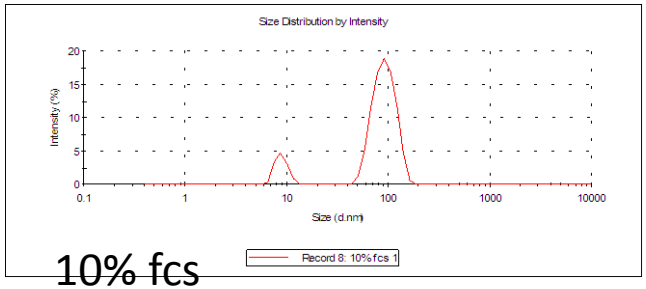
FCS	Pk1 (nm)	Pk2 (nm)	Pk3 (nm)
Serum free	59		
5% Fcs	10	95	
10% Fcs	11	96	
50% Fcs	7	30	108
100% Fcs	8	30	102

Serum free

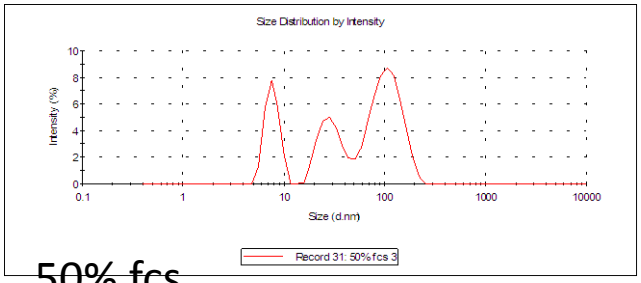




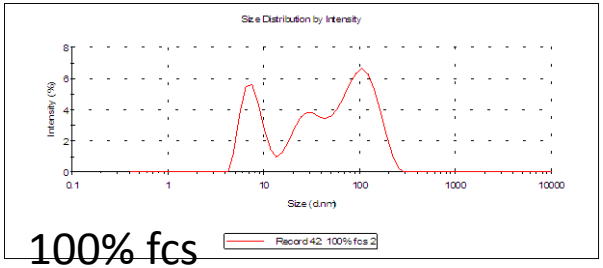
5% fcs



10% fcs



50% fcs

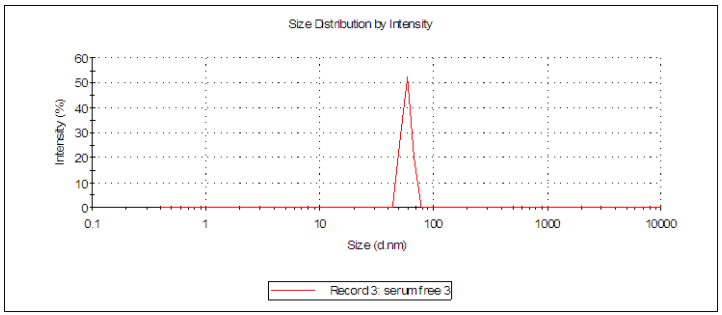


100% fcs

DLS data
40nm

Human plasma	Pk1 (nm)	Pk2 (nm)	Pk3 (nm)	Pk4 (nm)
Serum free	59			
5% Fcs	8	25	105	5300
10% Fcs	10	32	141	
50% Fcs (high coagulation)				
100% Fcs	10	52	267	3200

Serum free



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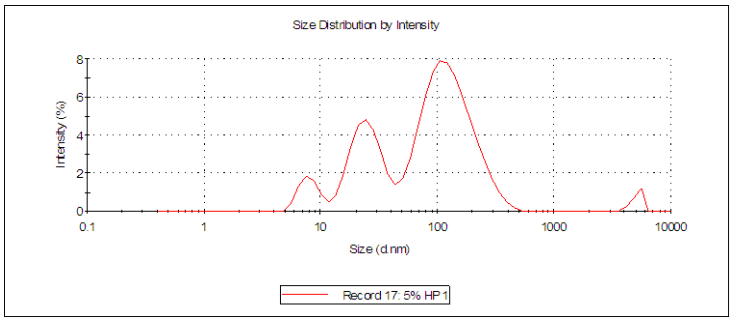

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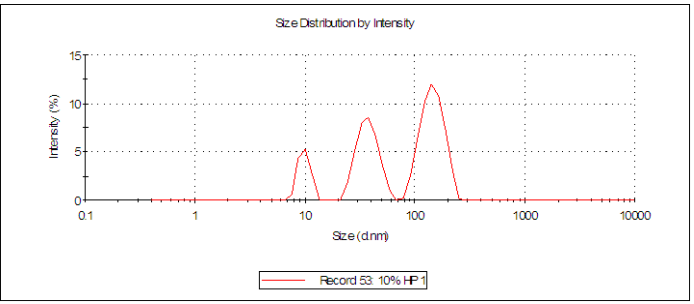
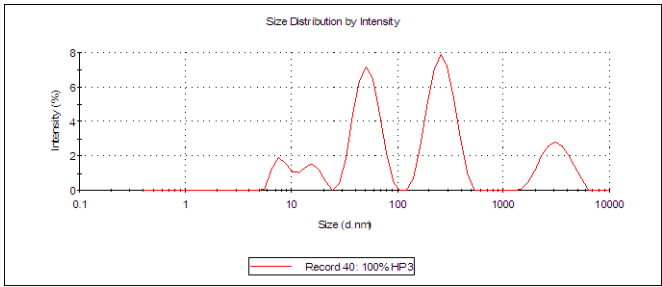


50% fcs

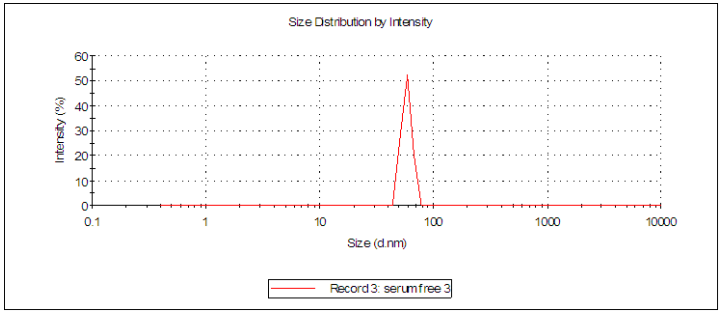
100% fcs



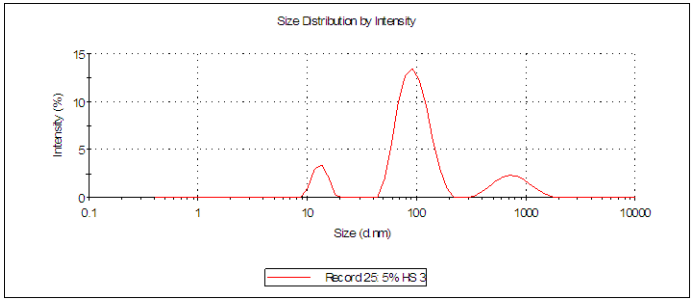
DLS data
40nm

Human serum	Pk1 (nm)	Pk2 (nm)	Pk3 (nm)	Pk4 (nm)
Serum free	59			
5% Fcs	13	91	712	
10% Fcs	8	15	79	220
50% Fcs	11	43	220	
100% Fcs	28	164	2305	

Serum free



5% fcs



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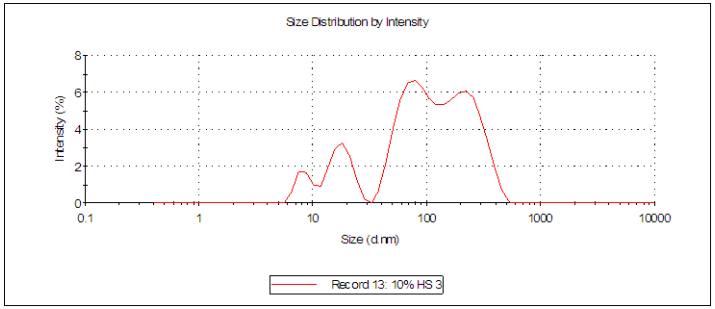

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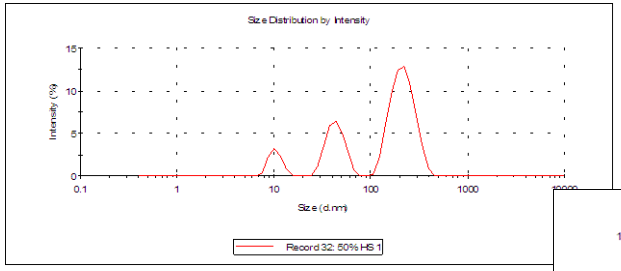
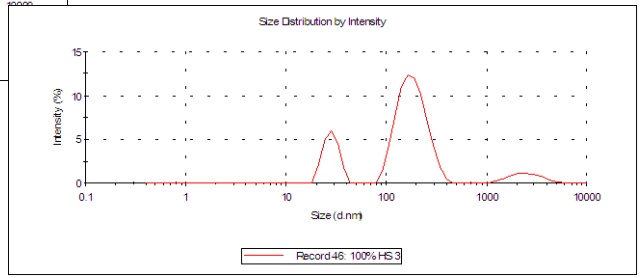


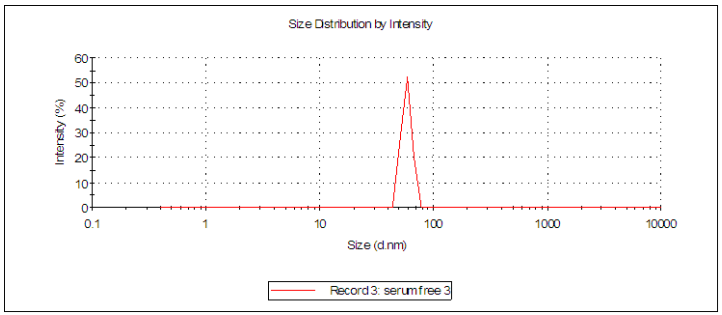
100% fcs



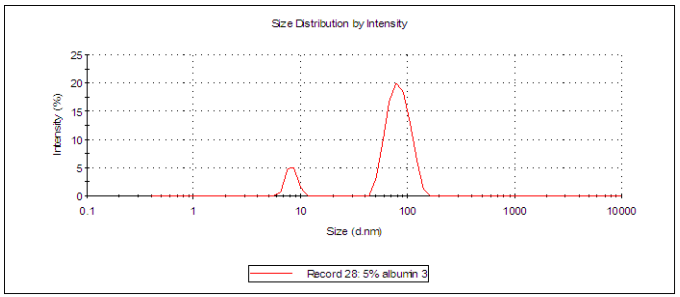
DLS data
40nm

Albumin	Pk1 (nm)	Pk2 (nm)	Pk3 (nm)	Pk4 (nm)
Serum free	59			
5% Fcs	9	78		
10% Fcs	8	79		
50% Fcs	7	79		
100% Fcs	7	50	220	

Serum free



5% fcs



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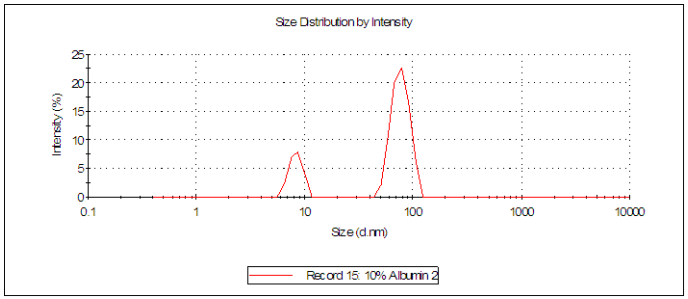

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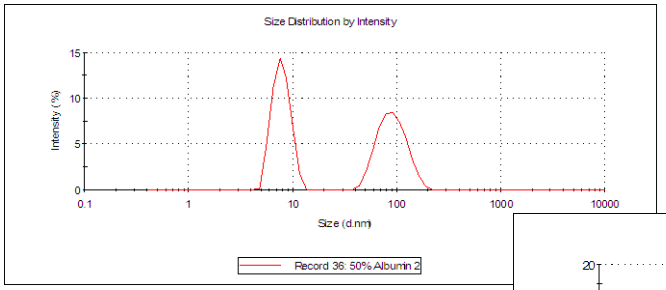

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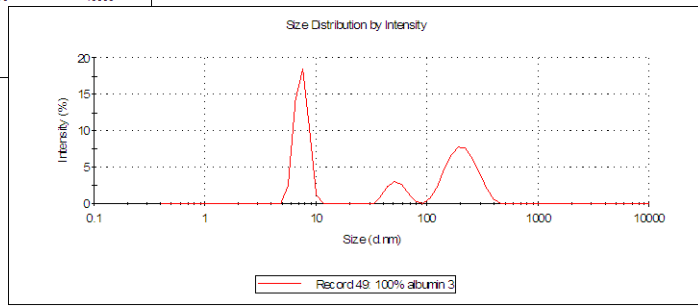

Conclusions

A549 (non-phagocytic cell type), showed that nanoparticle uptake is higher in serum free conditions, for all sizes studied.

High surface hydrophobicity of bare nanoparticles will increase the adherence to the cell membrane, increasing the uptake

In the case of the macrophages (Raw 264.7) in serum free conditions a higher uptake efficiency is observed for nanoparticles covered with proteins on their surface, as opposed to the same material in serum free conditions.

The specificity of some protein on the nanoparticle surface, can able to
activate the cellular machinery in these phagocytic cells, when nanoparticles are dispersed in the biological medium.

Future work

- Optimization of THP 1 differentiation protocol
- Protein corona composition analysed by 1D- SDS page gels in order to identify the presence of protein capable to activate phagocytic cells (such as complement proteins)
- CPS analyzis