

***In Vivo* Protein Corona on AuNPs** **of Various Size and Shape**

Rafaela García
PhD Student
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Presentation | Outline

1. Gold Nanoparticles
2. Protein Corona
3. Literature – AuNPs and Protein Corona
4. *In Vivo* Protein Corona Study
5. Conclusions



Gold Nanoparticles

Why Au?

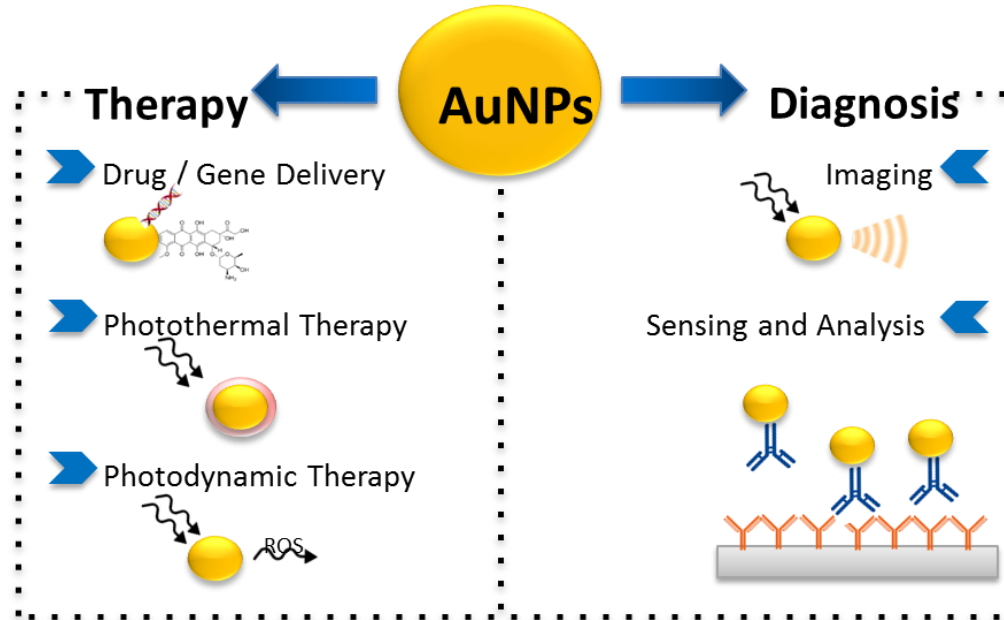
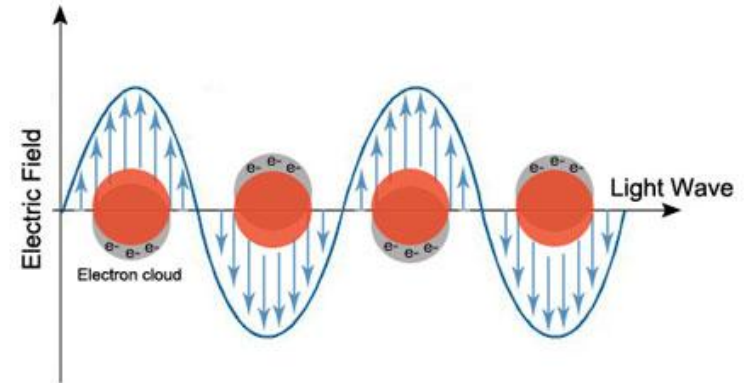
- Biocompatibility
- Biostability
- Low toxicity
- Functionalization
- Thermal properties
- Optical properties...

Photostability

TUNABLE

Surface Plasmon Resonance

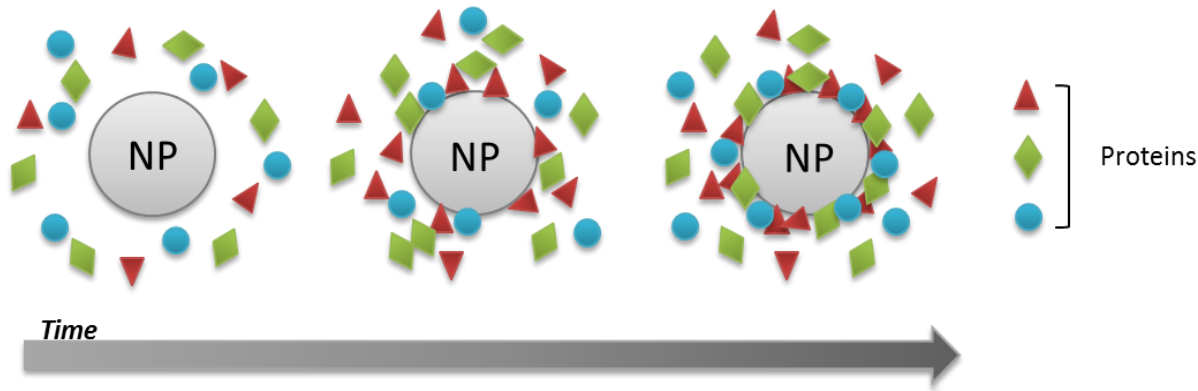
*Size, shape, morphology,
functionalization...*



Protein Corona

Why Protein Corona?

Protein corona term → 2007 – Dawson group (*Cedervall et al., PNAS, Vol. 104, no. 7, 2007*)



- Targeting → (*Salvati et al., Nature Nanotechnology, Vol. 8, February 2013*)
- Cell Uptake → (*Pozzi et al., J. Control Release, 148 2010*)
- Toxicity → (*Ge et al., PNAS, 108 2011*)
- Other...



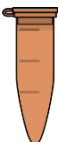
Literature | AuNPs and Protein Corona

Literature is focused on:

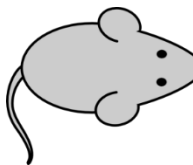
✓ 1. Spherical NPs 

✓ 2. Sizes 

✓ 3. Diff. media

✓ 4. *In Vitro* 

✓ 5. Separation → centrifugation

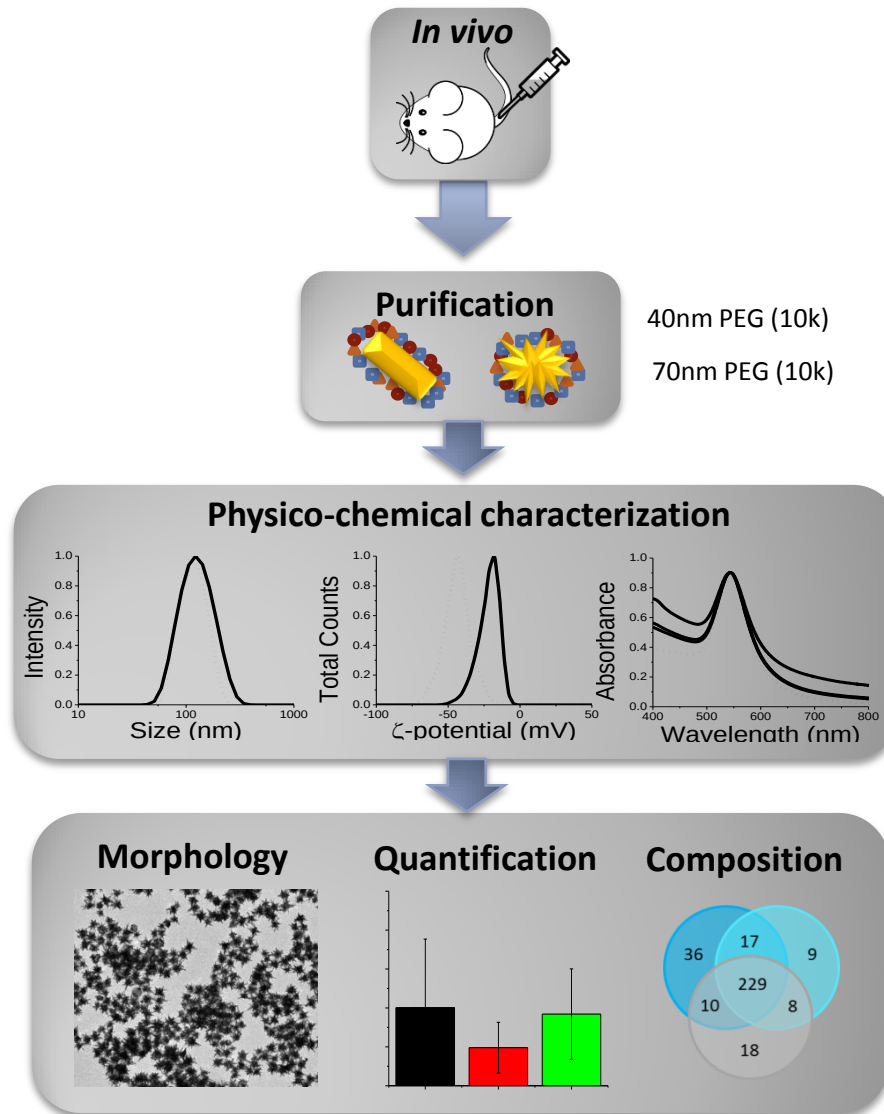
✗ 1. *In Vivo* 

✗ 2. Diff. shapes 

✗ 3. Diff. Separation method

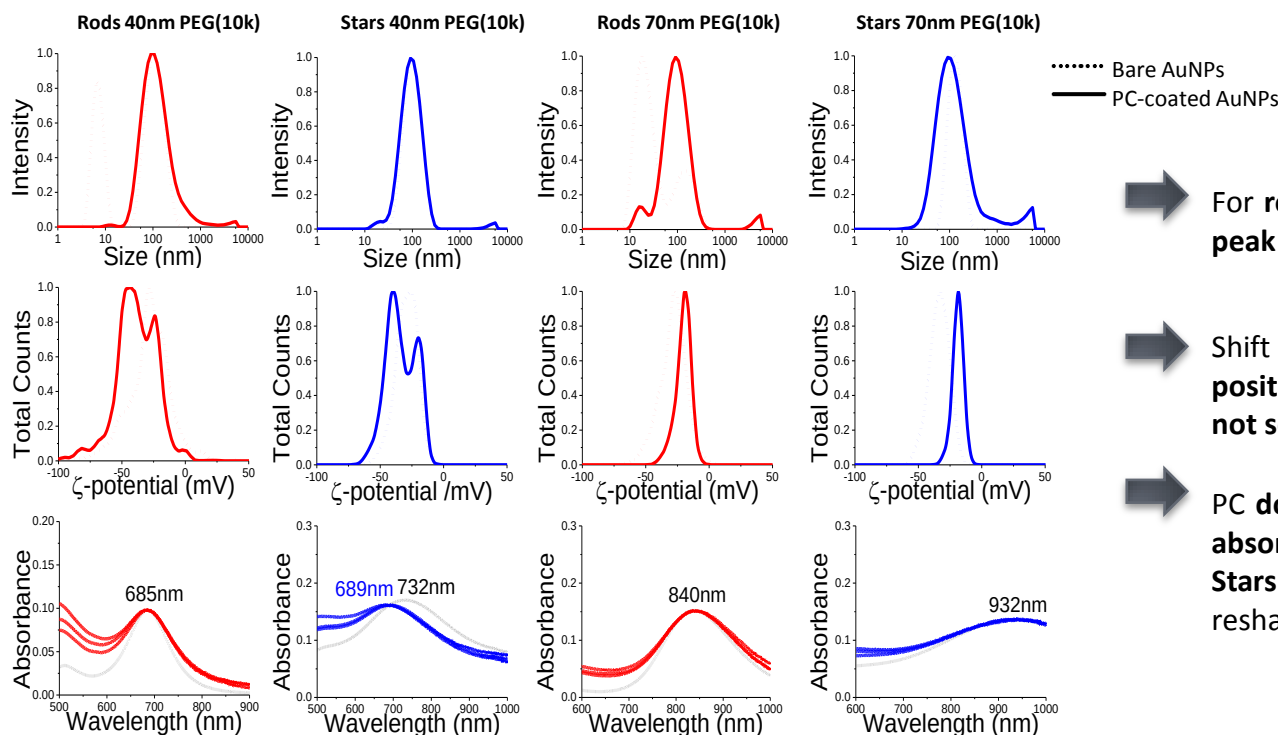


In Vivo Protein Corona Study on AuNPs

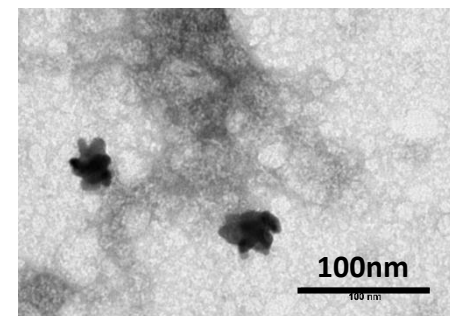


In vivo Protein Corona Study on AuNPs

Physicochemical characterization – DLS & UV-Vis

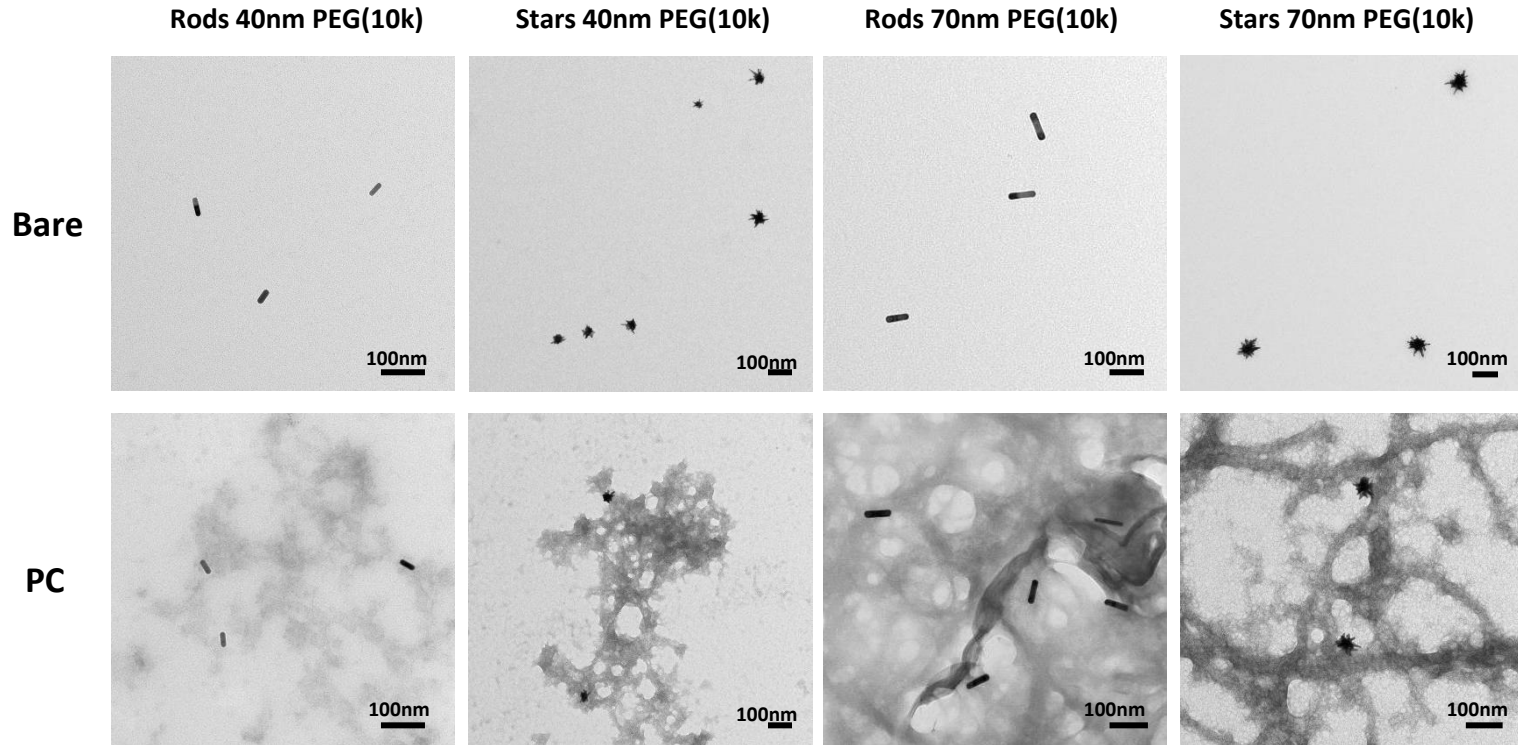


	Rods 40		Rods 70		Stars 40		Stars 70	
	No PC	PC	No PC	PC	No PC	PC	No PC	PC
Size (nm)	21.25±0.03	83.28±7.50	24.91±0.34	75.67±6.24	79.98±0.70	82.09±2.70	122.00±1.31	93.36±4.84
ZP (mV)	-28.57±0.55	-36.76±7.05	-28.77±0.38	-20.32±0.95	-29.33±0.23	-31.78±8.89	-33.63±0.21	-18.88±0.44
PDI	0.786±0.002	0.315±0.088	0.378±0.003	0.388±0.110	0.245±0.008	0.232±0.080	0.101±0.007	0.388±0.017



In vivo Protein Corona Study on AuNPs

Morphology - TEM



➡ **PC is formed** onto the surface of all the AuNPs .

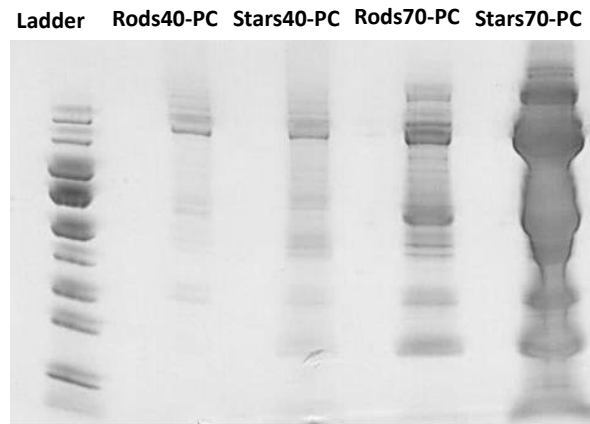
➡ **PC does not affect** the morphology of **Rods and 70nm Stars**.



In vivo Protein Corona Study on AuNPs

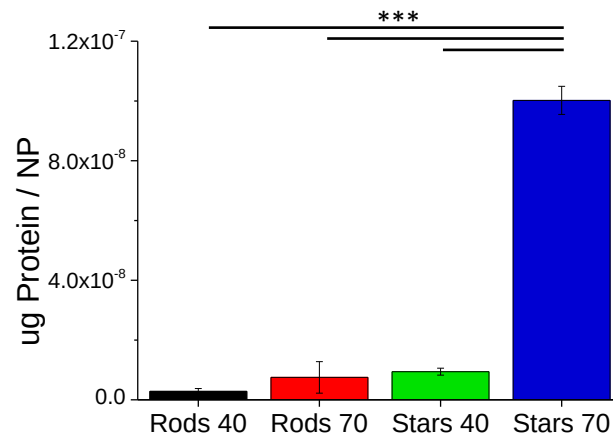
Quantification

SDS-PAGE Gel



- ➔ Amount of proteins adsorbed onto the AuNPs surface is affected by the shape and size of the NP.
- ➔ Bigger NPs absorb higher amount of proteins – opposite to *In vitro* studies.
- ➔ Stars absorb more proteins than Rods .

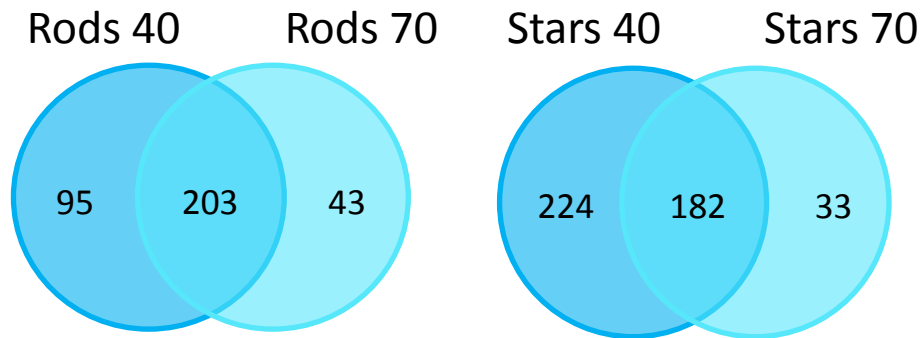
*BCA Assay
ICP-MS*



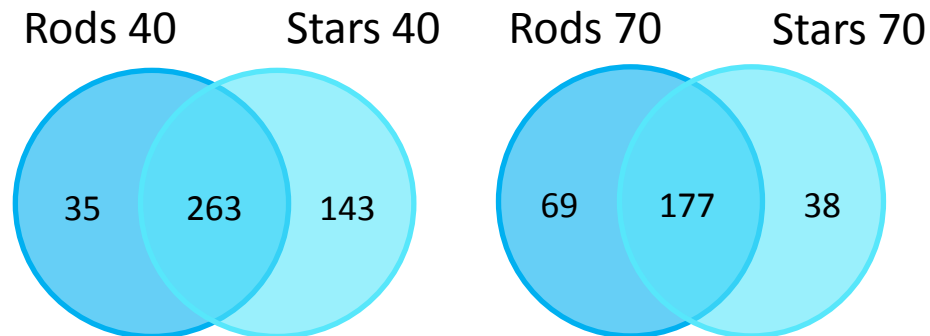
In vivo Protein Corona Study on AuNPs

Composition

- Size comparison



- Shape comparison



- ➡ Smaller NPs exhibit **more unique proteins**.
- ➡ **Stars 40nm** present **more unique proteins** than **Rods 40nm**.
- ➡ **Rods 70nm** present **less unique proteins** than **Stars 70nm**.
- ➡ **PC composition of AuNPs is affected by the shape and size of the NP.**



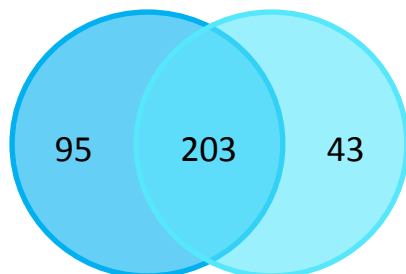
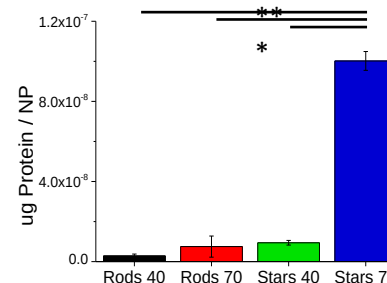
In vivo Protein Corona Study on AuNPs

List of Top 20 identified proteins (Most-abundant)

Rods 40nm PEG (10k)		Stars 40nm PEG (10k)		Rods 70nm PEG (10k)		Stars 70nm PEG (10k)	
Identified Protein	RPA	Identified Protein	RPA	Identified Protein	RPA	Identified Protein	RPA
Serum albumin	5.16±2.39	Serum albumin	3.71±2.85	Serum albumin	7.52±0.87	Serum albumin	8.19±0.84
Alpha-2-macroglobulin	1.30±0.64	Alpha-2-macroglobulin	3.70±1.24	Alpha-2-macroglobulin	6.13±0.37	Alpha-2-macroglobulin	7.80±0.32
Fibrinogen beta chain	2.29±0.90	Serine protease inhibitor		Serine protease inhibitor		Serine protease inhibitor	
Apolipoprotein A-I	2.27±0.22	A3K	2.44±1.81	A3K	4.83±0.86	A3K	6.01±1.01
Complement factor H	2.12±0.26	Fibrinogen beta chain	2.37±0.53	Apolipoprotein A-I	3.24±0.47	Fibrinogen beta chain	3.99±1.71
Serine protease inhibitor		Apolipoprotein E	2.31±0.37	Fibrinogen beta chain	2.51±0.92	Alpha-1B-glycoprotein	3.11±0.36
A3K	2.07±1.06	Fibrinogen gamma chain	2.07±0.25	Fibrinogen gamma chain	2.28±0.92	Fibrinogen gamma chain	2.99±1.48
Ig mu chain C region (Fragment)	1.71±0.29	Apolipoprotein A-I	1.98±1.21	Complement C3	2.10±0.34	Apolipoprotein A-I	2.57±0.80
Fibrinogen gamma chain	1.64±0.56	Ig mu chain C region (Fragment)	1.87±0.69	Apolipoprotein E	1.71±0.23	Apolipoprotein E	1.98±0.67
Argininosuccinate synthase	1.62±0.54	Complement factor H	1.73±0.17	Murinoglobulin-1	1.71±0.10	Fibrinogen alpha chain	1.94±0.74
Plasminogen	1.61±0.20	If kappa light chain (Fragment)	1.61±0.05	Complement factor H	1.68±0.38	Murinoglobulin-1	1.93±0.17
Protein Ighv7-1 (Fragment)	1.55±0.28	Complement C3	1.33±0.84	Ig mu chain C region (Fragment)	1.67±0.28	Complement factor H	1.63±0.34
Apolipoprotein E	1.50±0.45	IgM heavy chain VDJ region (Fragment)	1.29±0.54	Alpha-1B-glycoprotein	1.35±0.31	Apoa1 protein	1.55±2.69
Complement C3	1.49±0.93	Beta-globin	1.13±0.25	Fibronectin	1.28±0.31	Complement C3	1.31±0.18
Beta-globin	1.25±0.08	Fibrinogen alpha chain	1.08±0.22	Plasminogen	1.15±0.10	Ig mu chain C region (Fragment)	1.31±0.58
Betaine--homocysteine S-methyltransferase 1	1.18±0.45	Protein Ighv7-1 (Fragment)	1.04±0.52	Alpha-1-antitrypsin 1-3	1.13±0.18	Ceruloplasmin	1.25±0.21
Apolipoprotein C-III	1.18±0.32	Argininosuccinate synthase	1.01±0.45	Serpina1a protein	1.12±0.07	Fibronectin	1.25±0.35
Plasma kallikrein	1.17±0.35	Complement factor properdin	0.94±0.54	Fibrinogen alpha chain	1.09±0.38	Serine protease inhibitor	
Murinoglobulin-1	1.08±0.12	Plasminogen	0.92±0.26	Beta-globin	1.09±0.27	A3N	1.20±0.24
Apolipoprotein C-I	1.04±0.45	Plasma kallikrein	0.86±0.24	Protein Ighv7-1 (Fragment)	1.01±0.22	Murinoglobulin-2	1.18±0.28
Fructose-bisphosphate aldolase B	1.04±0.47	Fibronectin	0.85±0.20	Ceruloplasmin	0.97±0.19	Alpha-1-antitrypsin 1-3	1.16±0.04
						Serpina1a protein	1.12±0.03

Conclusions

- ❖ Amount of protein adsorbed is affected by the size and shape of the NP.



- ❖ Protein corona composition is affected by the size and shape of the NP.

- ❖ *In vivo* Protein Corona composition of AuNPs presents in high abundance a protein not detected among the top 20 list under *In vitro* conditions.

Rods 40nm PEG (10k)		Stars 40nm PEG (10k)		Rods 70nm PEG (10k)		Stars 70nm PEG (10k)	
Identified Protein	RPA	Identified Protein	RPA	Identified Protein	RPA	Identified Protein	RPA
Serum albumin	5.16±2.39	Serum albumin	3.71±2.85	Serum albumin	7.52±0.87	Serum albumin	8.19±0.84
Alpha-2-macroglobulin	1.30±0.64	Alpha-2-macroglobulin	3.70±1.24	Alpha-2-macroglobulin	6.13±0.37	Alpha-2-macroglobulin	7.80±0.32
		Serine protease inhibitor		Serine protease inhibitor		Serine protease inhibitor	





Thanks for your attention

Questions?

