NanoCarb

RCSI UNIVERSITY OF MEDICINE AND HEALTH SCIENCES

Probing the glycans accessibility of the nanoparticles biomolecular corona

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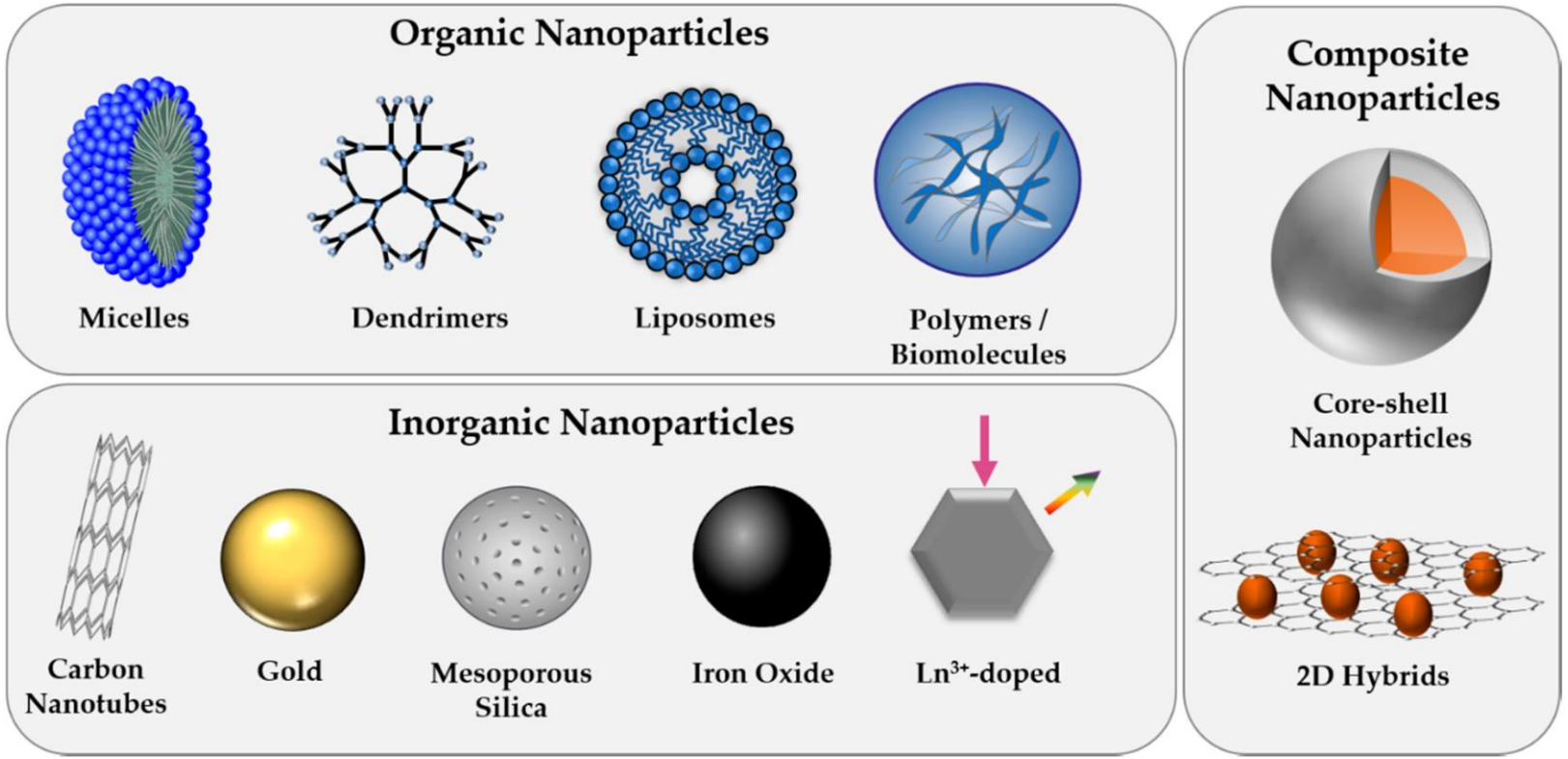
MARIE CURIE ACTIONS

TABLE OF CONTENT

NANOMATERIALS USED IN NANOMEDICINE

Nanoparticles (NPs) are materials with dimension in the range of 1-100 nm.



CLASSIFICATION

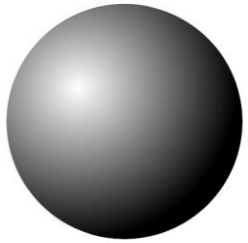
- Carbon-based
- Metal
- Polymeric
- Lipid-based
- Viral

PROPERTIES

- Nanoscale size
- High surface-to-volume ratio
- Surface modification
- Drug loading

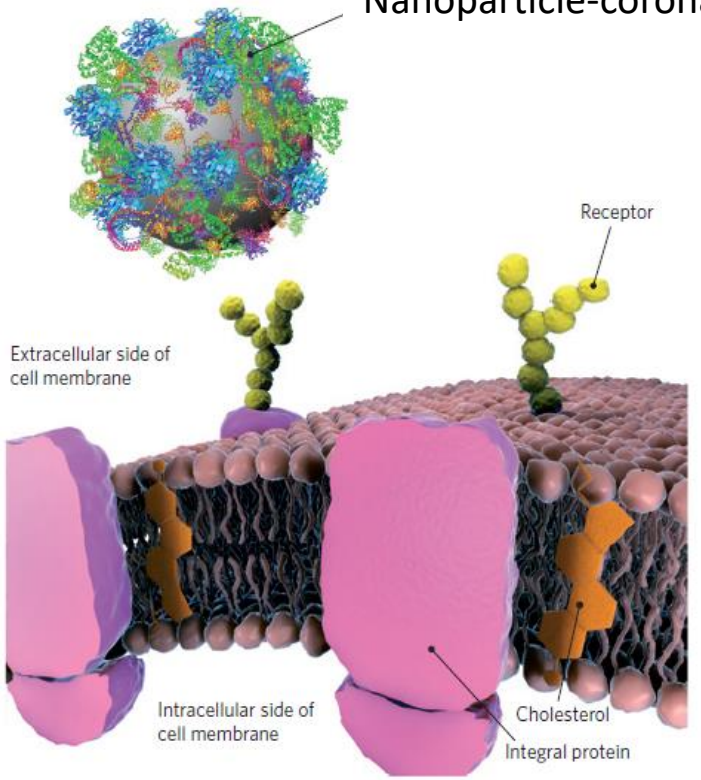
NANOPARTICLES IN BIOLOGICAL FLUIDS

The NPs surface properties and the colloidal stability are altered in biological mileu.



Human plasma

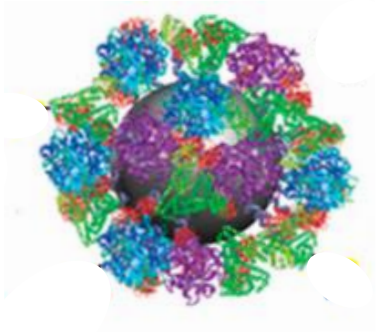
Nanoparticle-corona complex

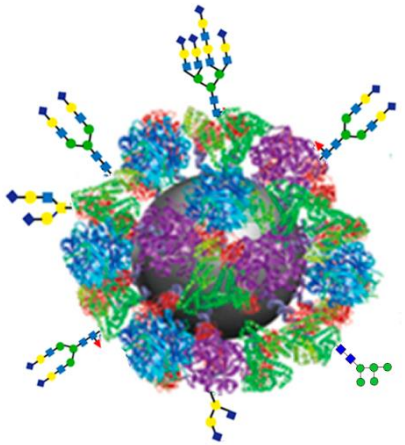


- NPs strongly associate with biomolecules with high affinity to the NPs surface.
- The biomolecular corona represents the new **BIOLOGICAL IDENTITY** of the system.

Monopoli, M. P.; Aberg, C.; Salvati, A.; Dawson, K. A., Biomolecular coronas provide the biological identity of nanosized materials. *Nat Nanotechnol* **2012,** *7* (12), 779-86.

IMPORTANCE OF GLYCANS OF THE PROTEIN CORONA





PROTEIN CORONA – State of the art

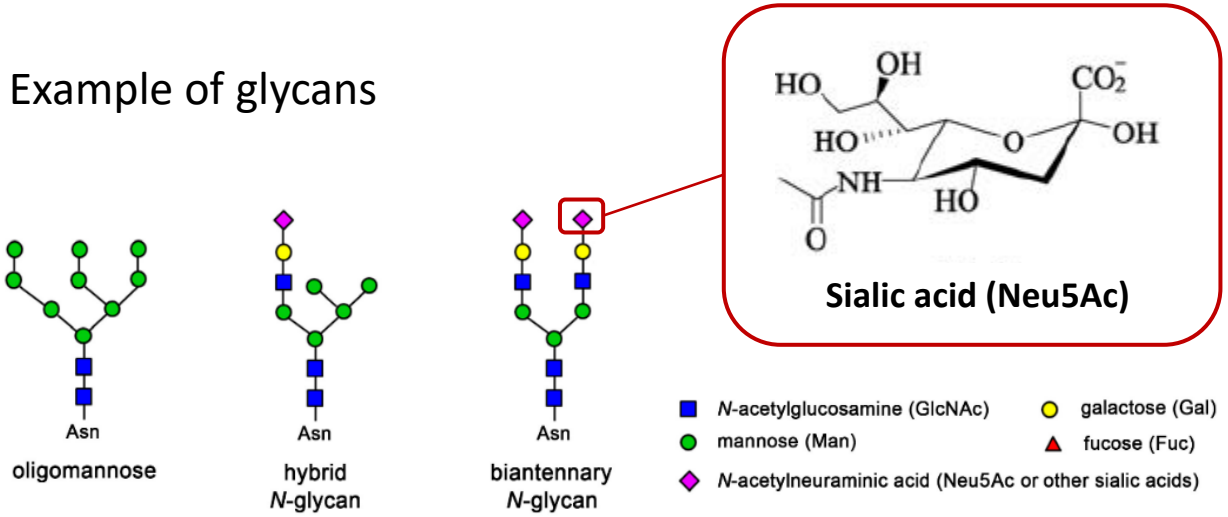
- Made of proteins with high affinity for the surface of NPs, but not necessary the most abundant in the biological milieu
- Proteins form strong/nearly irreversible bond
- Different proteins corona composition based on different NPs (Opsonins/diopsonins proteins)
- Exposure of new immunological epitopes

GLYCOSYLATED CORONA

Most proteins of human plasma are naturally glycosylated and the glycans are exposed at the bionanointerface.

IMPORTANCE OF GLYCANS OF THE PROTEIN CORONA

Example of glycans

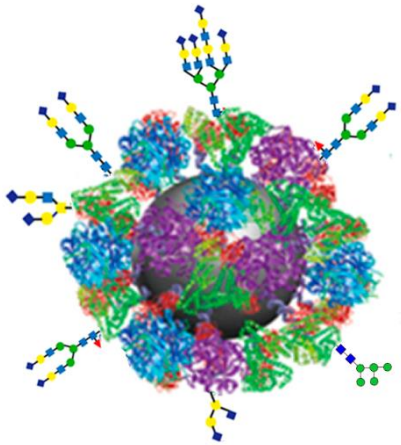


THEY AFFECT PROTEIN PROPERTIES

- Solubility
- Stability
- Folding

THEY ARE INVOLVED IN SEVERAL BIOLOGICAL PROCESSES

- Receptor interaction
- Immune response
- Protein secretion and transport



GLYCOSYLATED CORONA

Most proteins of human plasma are naturally glycosylated and the glycans are exposed at the bionanointerface.

PROPERTIES OF SIALIC ACID

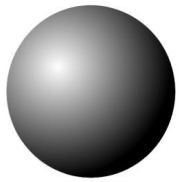
- Negative charge stabilize proteins
- Ligand for siglec
- Affect circulation half-life of proteins

AIM OF THE PROJECT

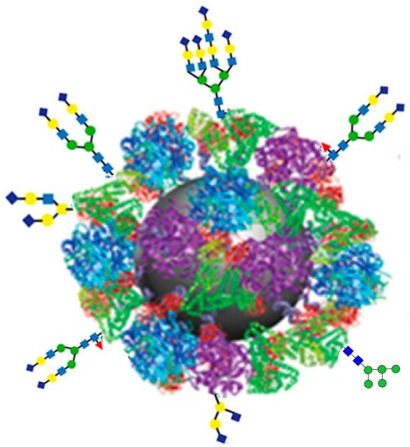
Characterize the biomolecular corona using gold nanospheres as model system, and study the role of the glycans of the corona in affecting the NP-corona stability and assess if they are biological accessible

- Detail characterization of the corona complex using simple analytical techniques (Physico-chemical characterization and proteomic/glycan analysis);
- Glyco-corona binding assay using glycan binding proteins (or lectins) that have specific affinity towards glycans.

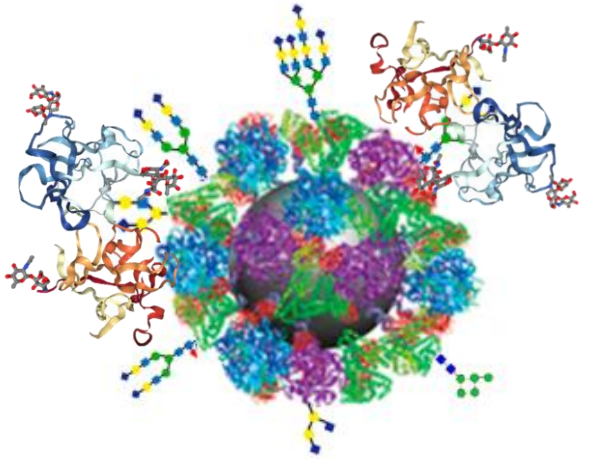
Bare Nanoparticle



Glyco-Corona (HC)



Glycan binding protein-corona complex



WORKFLOW

1. NANOPARTICLES PHYSICO-CHEMICAL CHARACTERISATION

Bare Nanoparticle

- Ultraviolet-visible spectroscopy (UV-vis);
- Dynamic light scattering (DLS);
- Differential centrifugal sedimentation (DCS).
- Fluorescence correlation spectroscopy (FCS)

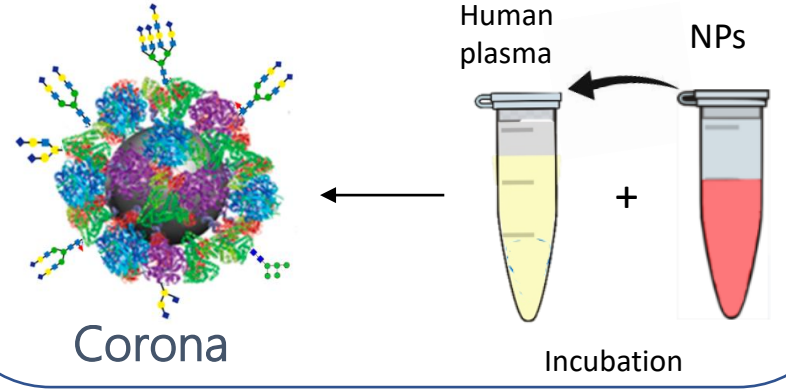
2. BIOMOLECULAR CORONA CHARACTERISATION

PROTEIN CHARACTERISATION
- Polyacrylamide gel electrophoresis (SDS PAGE);
- Mass spectrometry (MS);

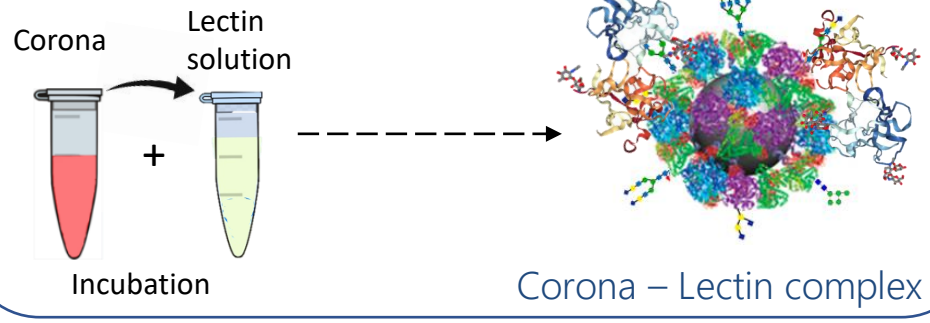
GLYCAN ANALYSIS
- Hydrophilic interaction liquid chromatography (HILIC)

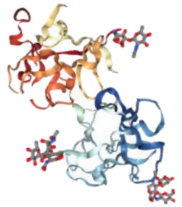
NP-HC FORMATION

Biological fluid: human plasma
10% - *in vitro* condition
80% - *in vivo* condition



3. LECTIN BINDING ASSAY





Lectin: *Sambucus nigra* (SNA)
Affinity: Sialic acid/GalNAc

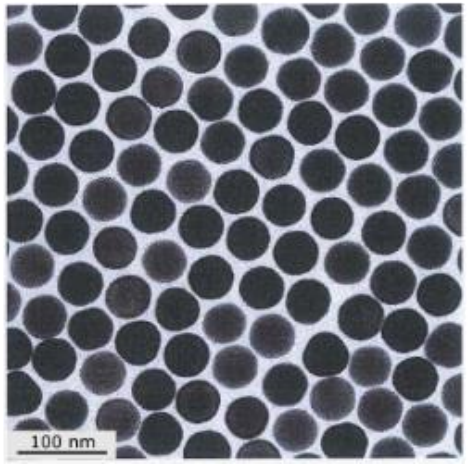
Lectin: *Wheat Germ Agglutinin* (WGA)
Affinity: GlcNAc/Sialic acid

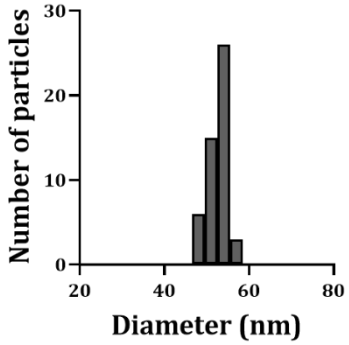
RESULTS

PYSICO-CHEMICAL CHARACTERISATION OF 50nm GOLD NANOSPHERES (synthesis in CICbioma GUNE, Spain)

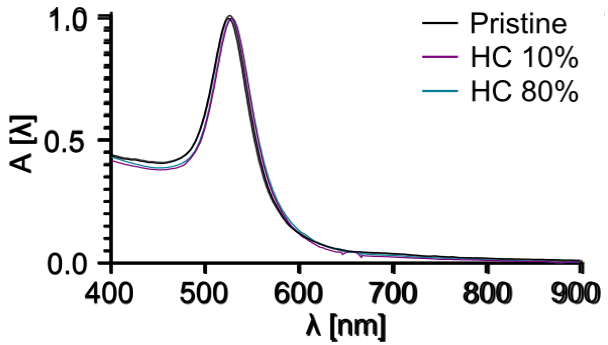
PYSICO-CHEMICAL CHARACTERISATION OF HC

TEM

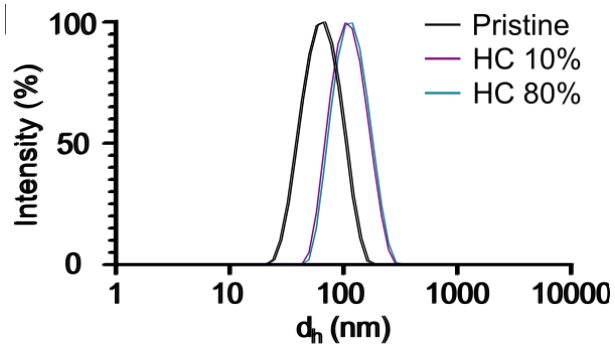




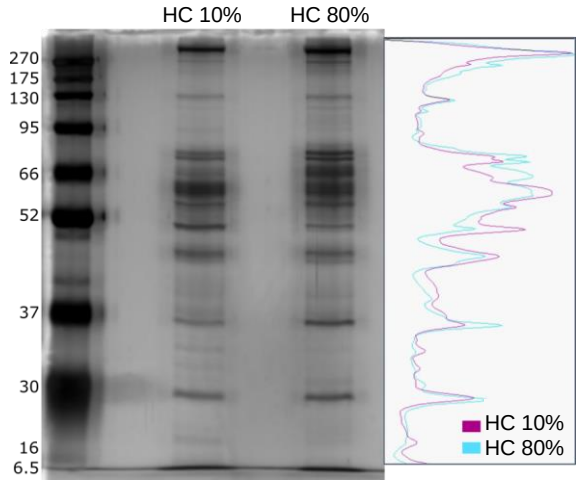
UV-vis



DLS



SDS-PAGE

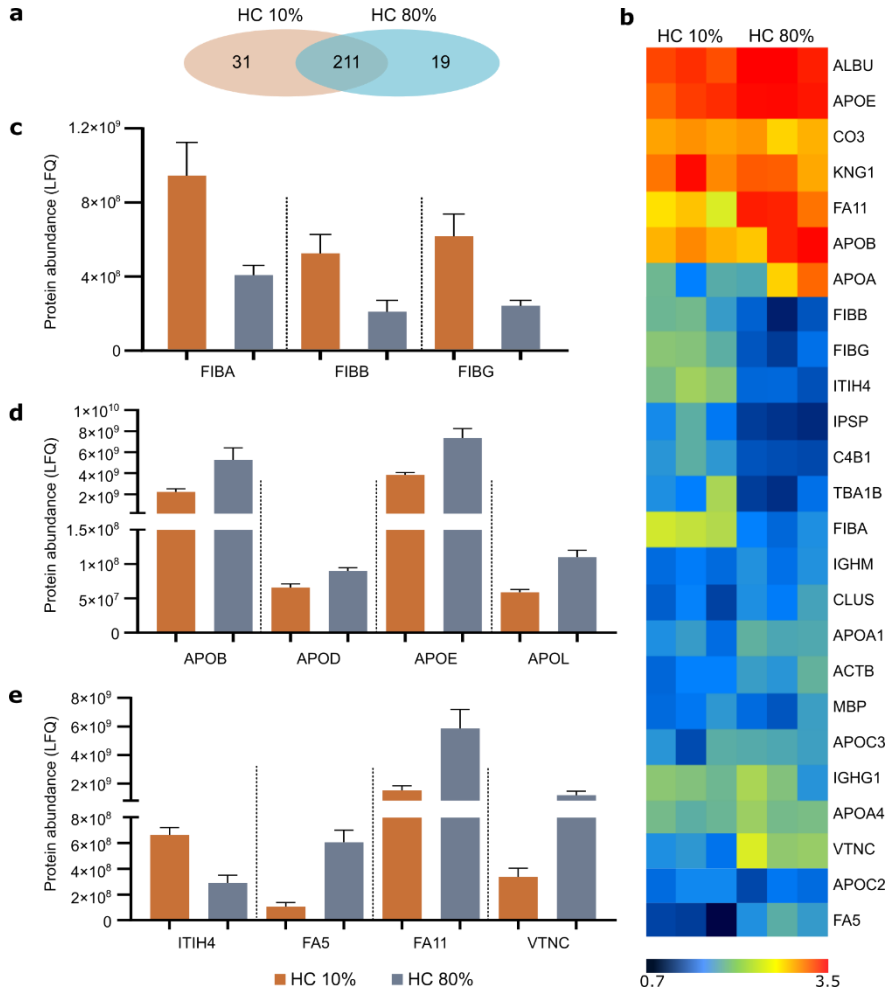


RESULTS – characterization of the proteins composition by Mass spectrometry

ANALYSIS OF PROTEIN CONTENT

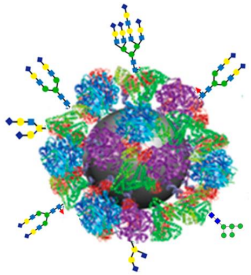
	HC 10 %			HC 80%	
	Protein name	**%**		**Protein name**	**%**
ALBU	Serum Albumin	12.81	ALBU	Serum Albumin	17.60
APOE	Apolipoprotein E	12.22	APOE	Apolipoprotein E	13.63
KGN1	Kininogen-1	11.54	FA11	Coagulation factor XI	10.87
CO3	Complement C3	7.41	APOB	Apolipoprotein B-100	9.76
APOB	Apolipoprotein B-100	7.12	KNG1	Kininogen-1	7.78
FA11	Coagulation factor XI	4.85	CO3	Complement C3	5.33
FIBA	Fibrinogen alpha chain	3.02	APOA	Apolipoprotein(a)	4.17
ITIH4	Inter-alpha-trypsin inhibitor	2.12	VTNC	Vitronectin	2.21
IGHG1	Ig gamma-1 chain C region	2.04	APOA4	Apolipoprotein A-IV	1.72
FIBG	Fibrinogen gamma chain	1.98	IGHG1	Ig gamma-1 chain C region	1.66
APOA4	Apolipoprotein A-IV	1.79	APOA1	Apolipoprotein A-I	1.30
FIBB	Fibrinogen beta chain	1.68	APOC3	Apolipoprotein C-III	1.24
APOA	Apolipoprotein(a)	1.50	ACTB	Actin, cytoplasmic 1	1.15
TBA1B	Tubulin alpha-1B chain	1.48	FA5	Coagulation factor V	1.12
C4B1	Complement C 4B1	1.35	CLUS	Clusterin	0.95
IPSP	Plasma serine protease inhibitor	1.18	IGHM	Ig mu chain C region	0.86
APOC3	Apolipoprotein C-III	1.11	FIBA	Fibrinogen alpha chain	0.76
VTNC	Vitronectin	1.08	MBP	Myelin basic protein	0.68
APOA1	Apolipoprotein A-I	1.08	TRFE	Serotransferrin	0.56
APOC2	Apolipoprotein C-II	0.91	APOC2	Apolipoprotein C-II	0.55
MBP	Myelin basic protein	0.90	ITIH4	Inter-alpha-trypsin inhibitor	0.54
ACTB	Actin, cytoplasmic 1	0.84	G3P	Glyceraldehyde-3-phosphate	0.52
IGHM	Ig mu chain C region	0.77	HBB	Hemoglobin subunit beta	0.51
G3P	Glyceraldehyde-3-phosphate	0.71	HRG	Histidine-rich glycoprotein	0.51
DESP	Desmoplakin	0.67	A1AT	Alpha-1-antitrypsin	0.48

Mass spectrometry

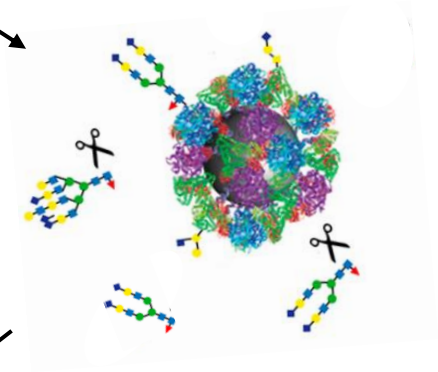


RESULTS – characterization of the glycans component by HILIC chromatography

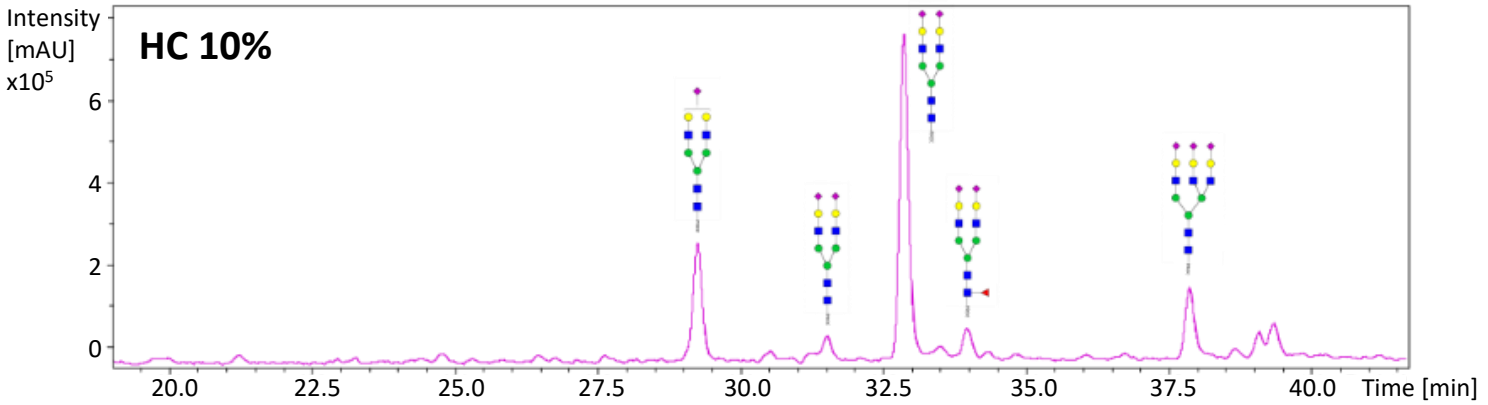
GLYCAN PROFILING

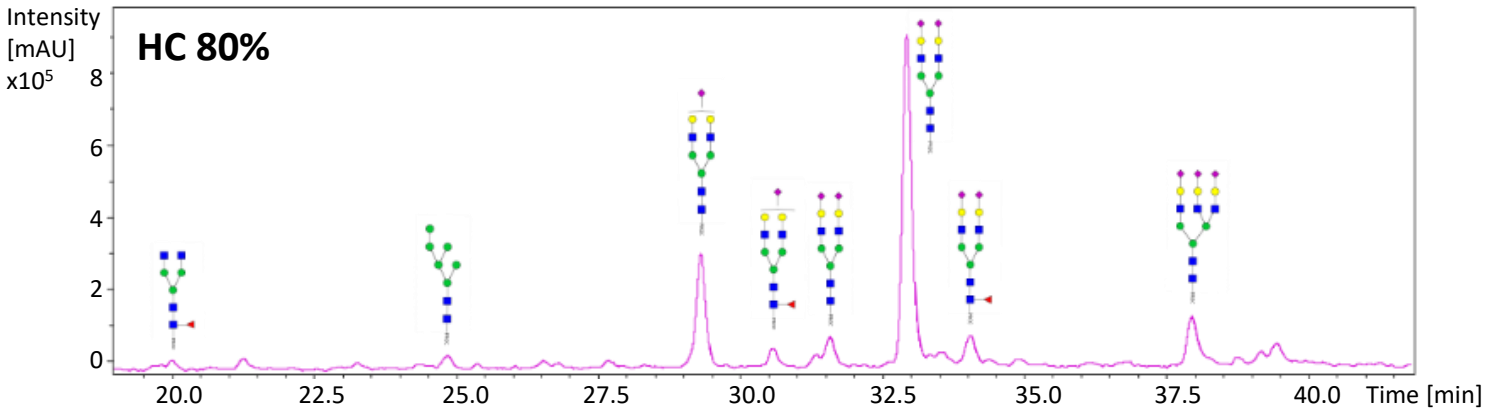


Deglycosylation



HILIC





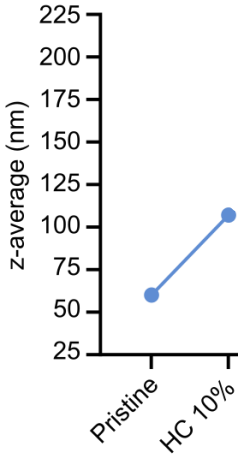
Secondment in Ludger Ltd, UK. Feb-Mar 2020

Ludger

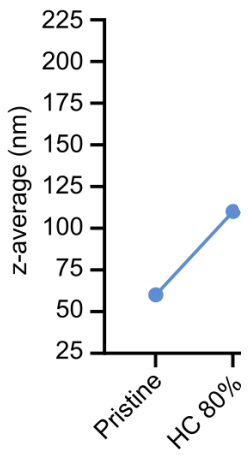
Protein corona retains colloidal stability

PYSICO-CHEMICAL CHARACTERISATION OF THE BIOMOLECULAR CORONA

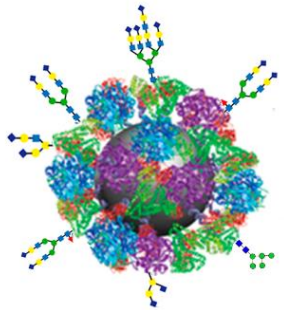
10%



80%

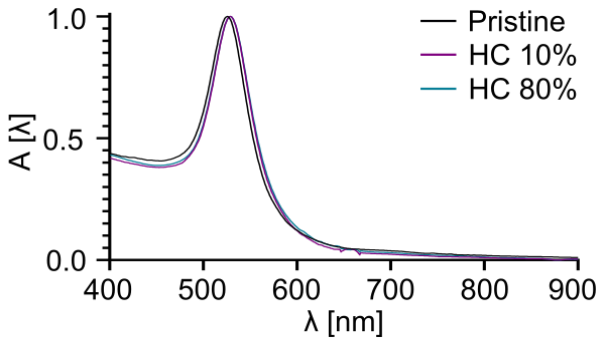


Glycosylated corona

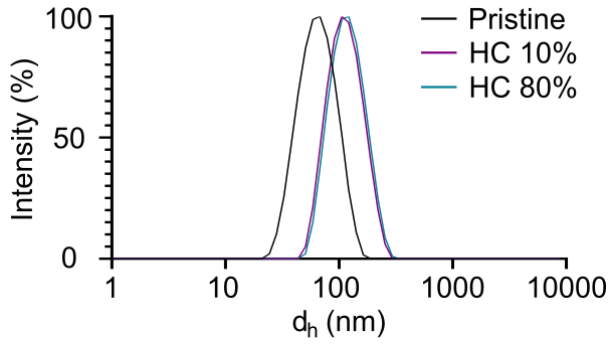


SAMPLE	Z-av (nm)	PDI
Pristine	60.0	0.12
HC 10%	107.0	0.13
HC 80%	110.1	0.14

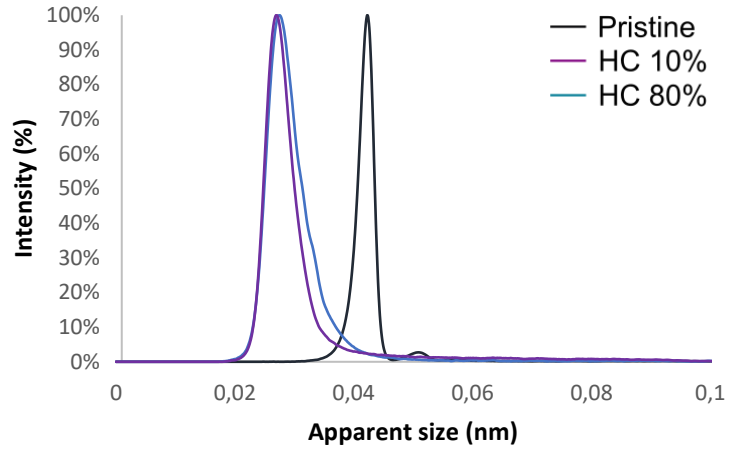
UV-vis



DLS

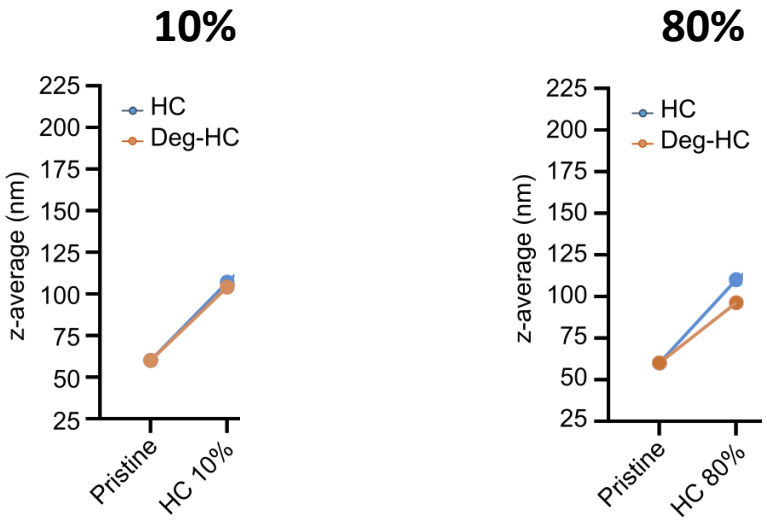


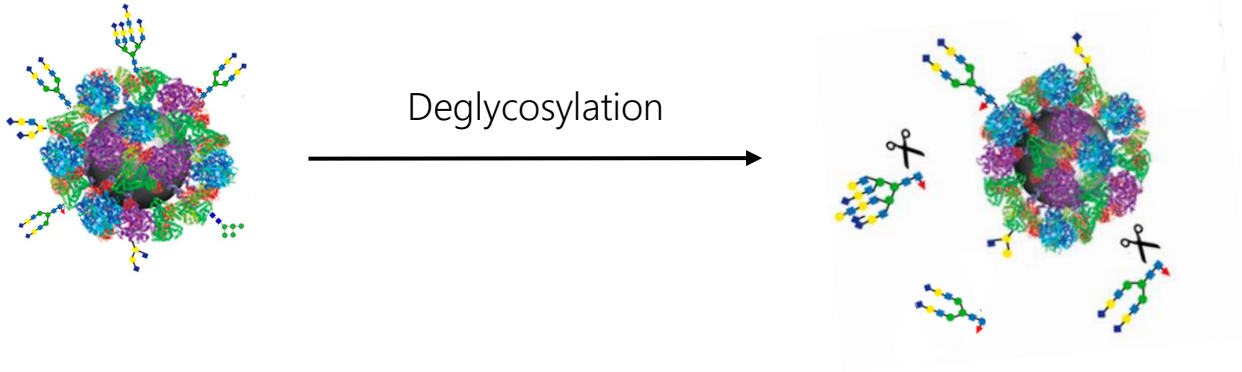
DCS



Deglycosylation of protein corona leads to an increase of polidispersity

PYSICO-CHEMICAL CHARACTERISATION OF DE-GLYCOSYLATED BIOMOLECULAR CORONA





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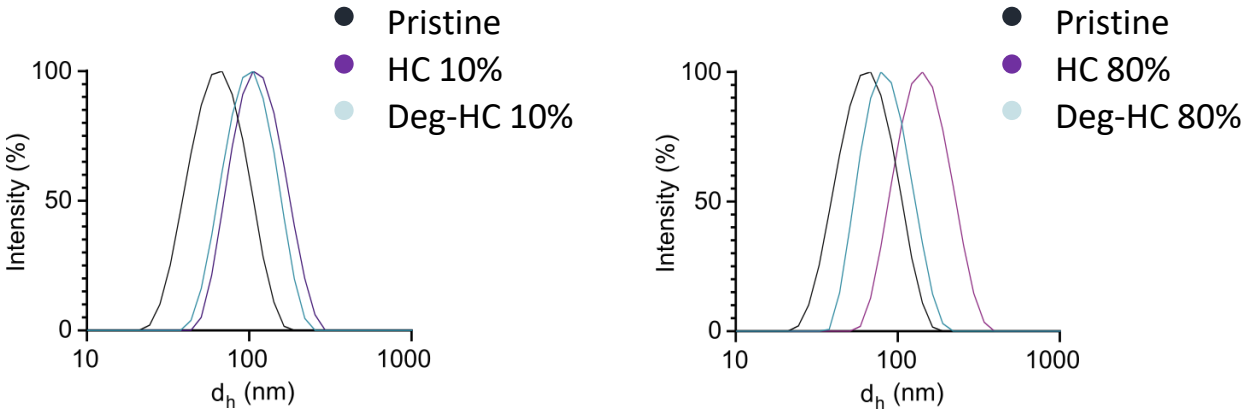
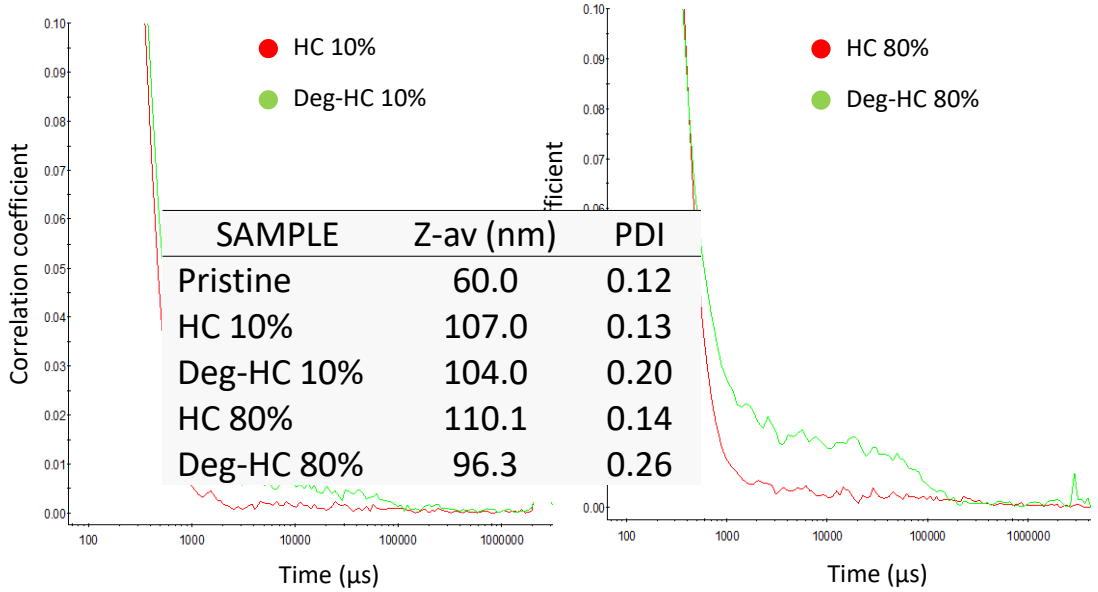


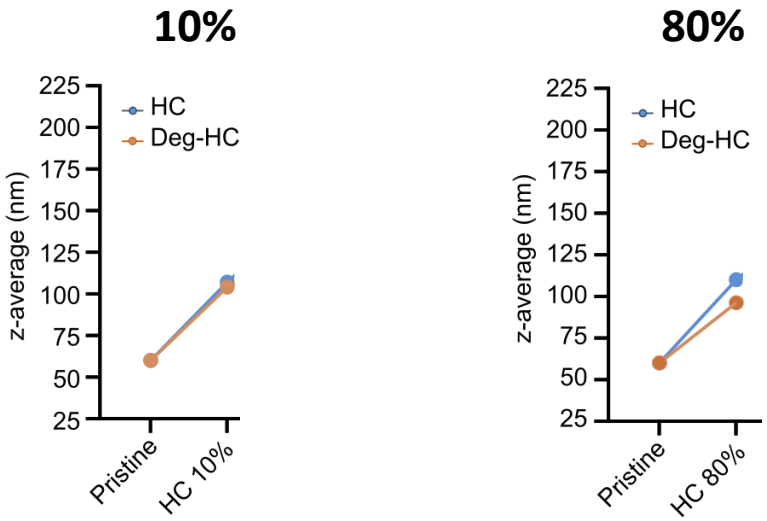
DLS – Correlogram

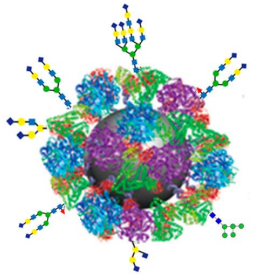


SAMPLE	Z-av (nm)	PDI
Pristine	60.0	0.12
HC 10%	107.0	0.13
Deg-HC 10%	104.0	0.20
HC 80%	110.1	0.14
Deg-HC 80%	96.3	0.26

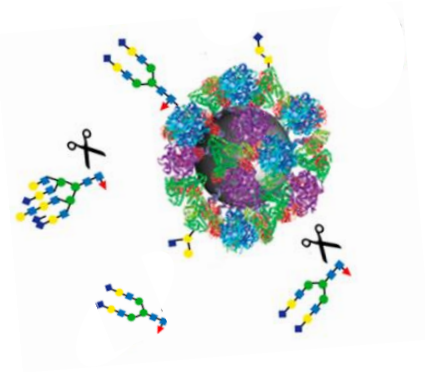
Deglycosylation of protein corona leads to an increase of polidispersity

PYSICO-CHEMICAL CHARACTERISATION OF DE-GLYCOSYLATED BIOMOLECULAR CORONA





Deglycosylation



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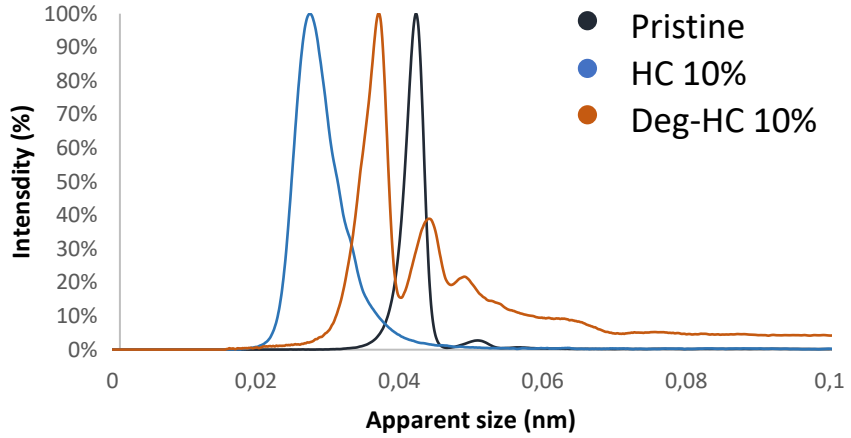
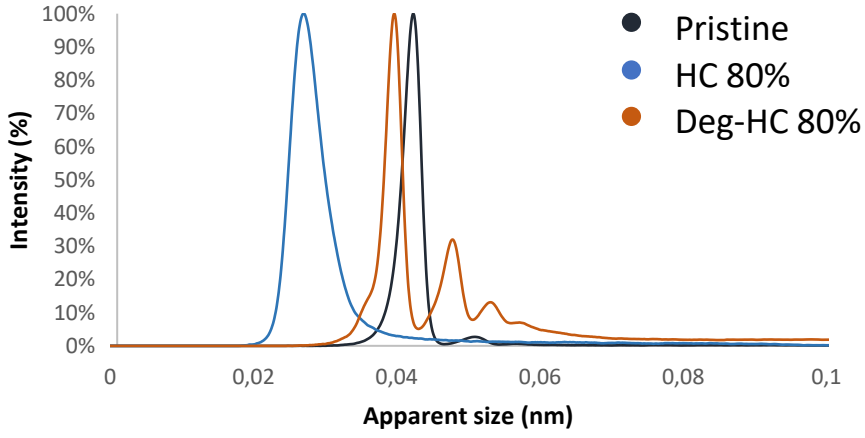
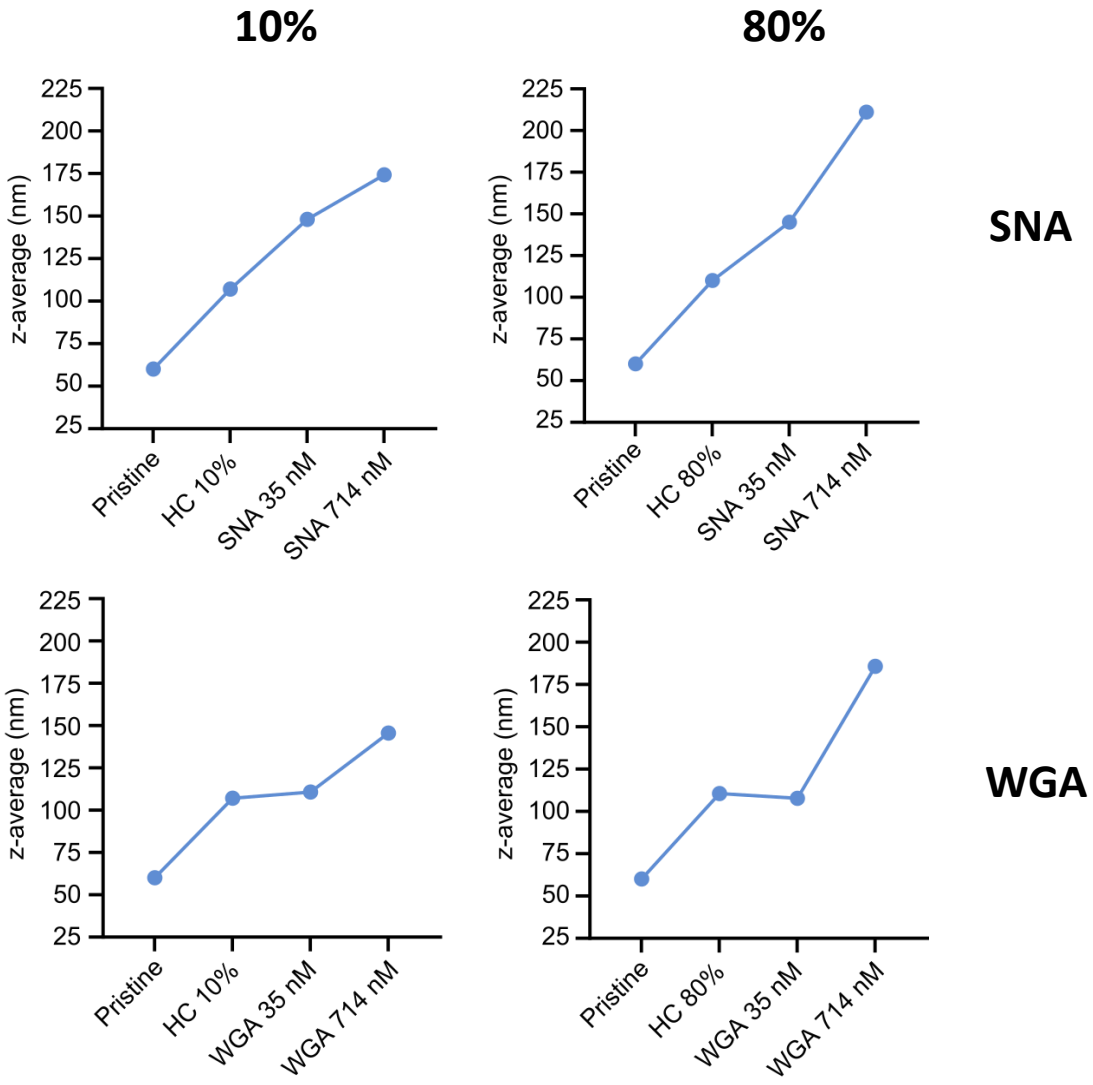


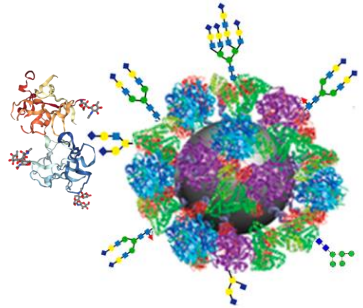


RESULTS – exposure to lectin leads to an increase of size

LECTIN BINDING ASSAY - DLS



Glycosylated corona

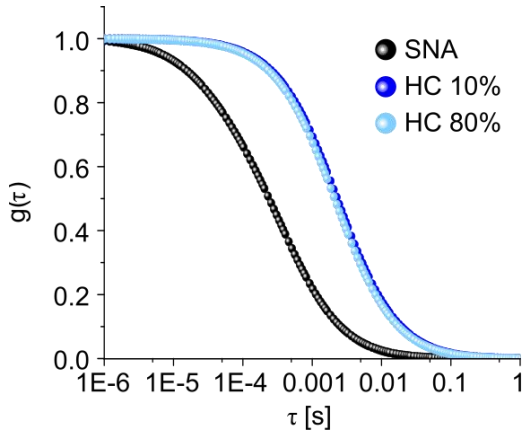


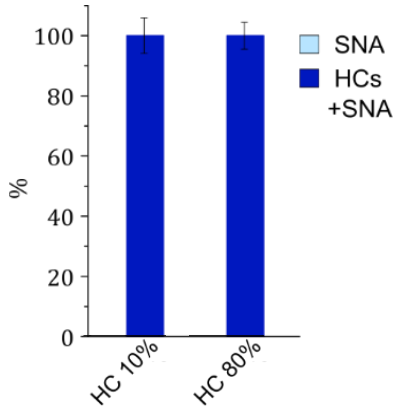
Lectin concentration
- 35 nM
- 714 nM

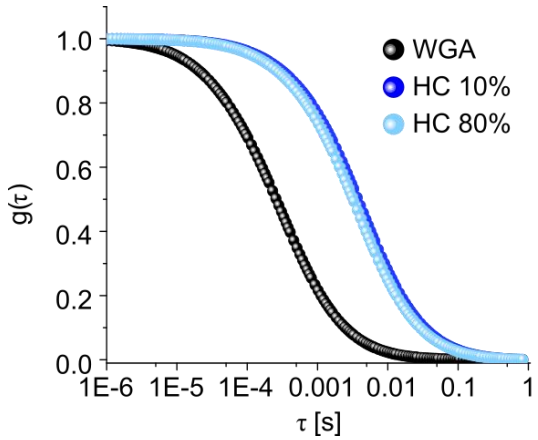
SAMPLE	Z-av (nm)	SD	PDI
Pristine	60.0	0.07	0.12
HC 10%	107.0	0.86	0.13
HC 10% + SNA 35 nM	148.0	1.20	0.21
HC 10% + SNA 714 nM	174.2	2.25	0.18
HC 10% + WGA 35 nM	110.8	3.65	0.15
HC 10% + WGA 714 nM	145.5	9.78	0.19
HC 80%	110.1	1.36	0.14
HC 80% + SNA 35 nM	145.0	2.60	0.19
HC 80% + SNA 714 nM	211.0	3.69	0.21
HC 80% + WGA 35 nM	107.8	4.05	0.14
HC 80% + WGA 714 nM	185.7	10.00	0.25

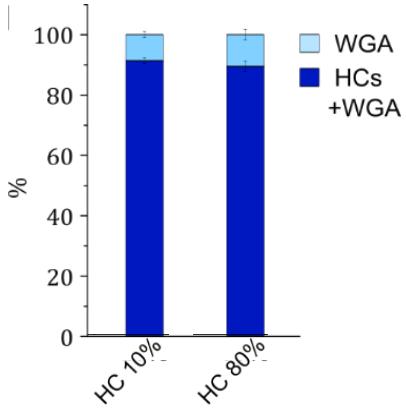
RESULTS - exposure to lectin leads to an interaction

FCS – CICbioma GUNE, Spain

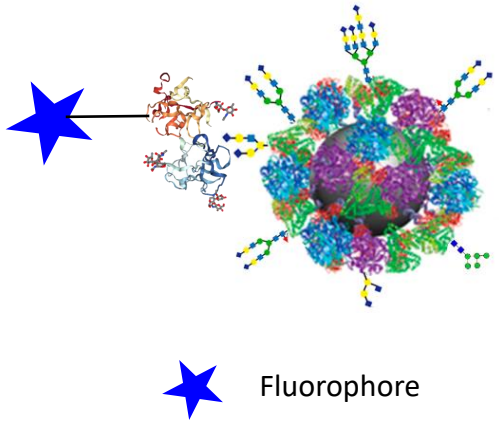








Glycosylated corona

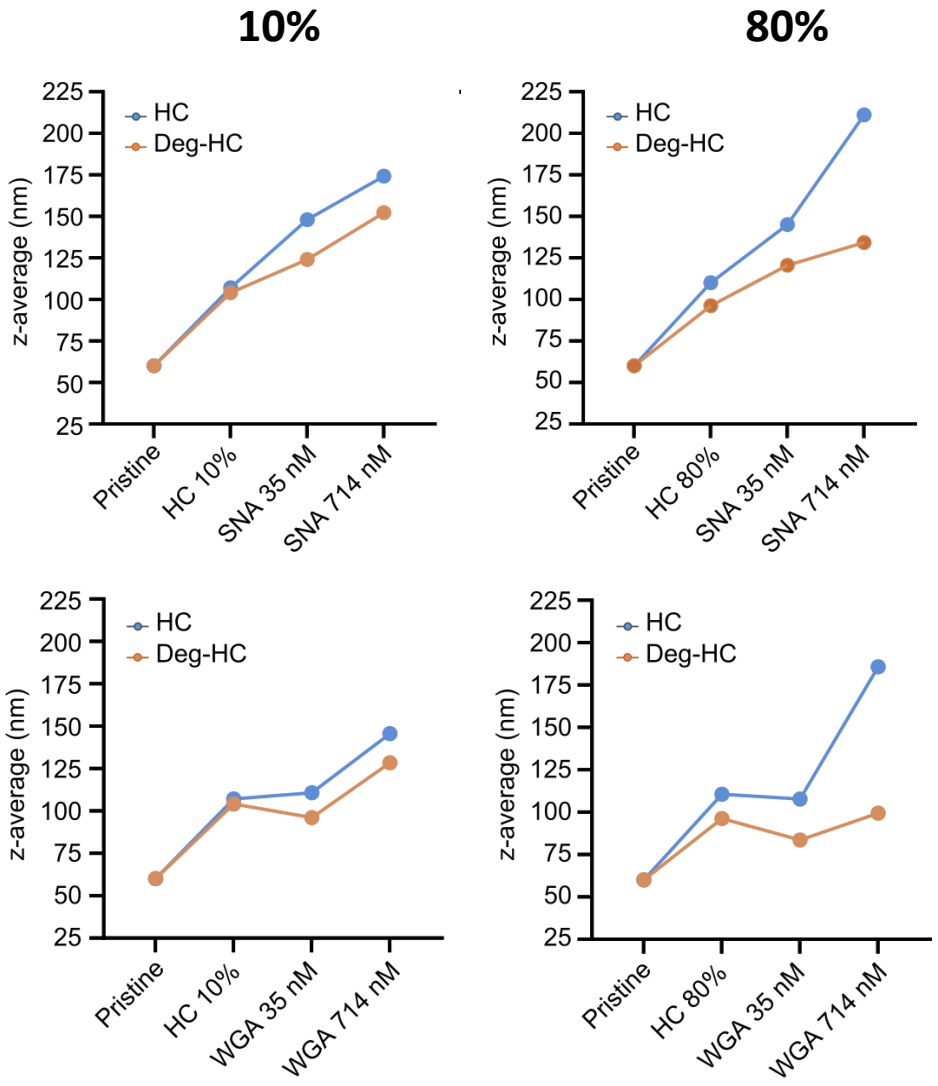


Lectin concentration
- 35 nM

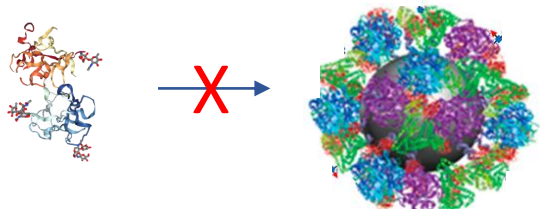
	τ [µs]	D_c [µm²·s⁻¹]
Free SNA	428 ± 8	33.5 ± 0.6
GNP-HC 10%-SNA	2365 ±132	6.07 ± 0.37
GNP-HC 80%-SNA	2139 ±143	6.72 ± 0.45
Free WGA	206.6 ± 1.5	75.6 ± 0.5
GNP-HC 10%-WGA	2397 ± 76	6.52 ± 0.21
GNP-HC 80%-WGA	2469 ± 85	6.32 ± 0.17

RESULTS – exposure to lectin after glycans removal leads to a loss of lectin interaction

LECTIN BINDING ASSAY - DLS



De-glycosylated corona



Lectin concentration
- 35 nM
- 714 nM

SAMPLE	Z-av (nm)	SD	PDI
Pristine	59.98	0.07	0.12
Deg-C 10%	104.0	3.79	0.20
Deg-C 10% + SNA 35 nM	124.8	1.42	0.16
Deg-C 10% + SNA 714 nM	152.2	4.15	0.28
Deg-C 10% + WGA 35 nM	96.0	1.67	0.20
Deg-C 10% + WGA 714 nM	128.4	1.85	0.27
Deg-C 80%	96.3	3.97	0.26
Deg-C 80% + SNA 35 nM	120.5	3.03	0.23
Deg-C 80% + SNA 714 nM	134.3	0.42	0.25
Deg-C 80% + WGA 35 nM	83.6	1.96	0.23
Deg-C 80% + WGA 714 nM	99.4	3.61	0.27

RESULTS – exposure to lectin after glycans removal leads to a loss of lectin interaction

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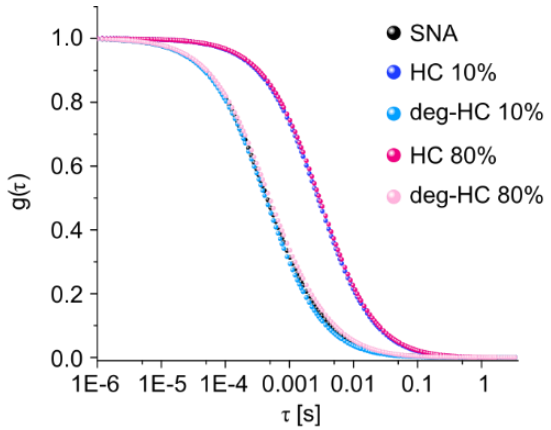
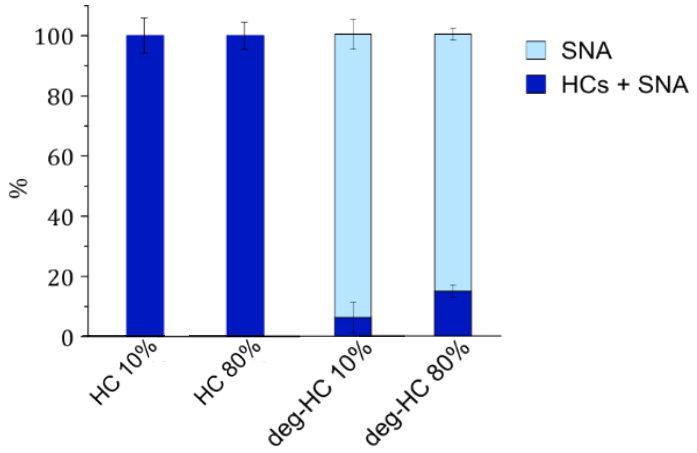
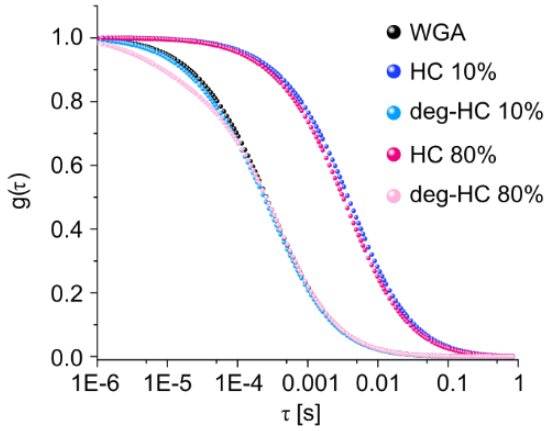
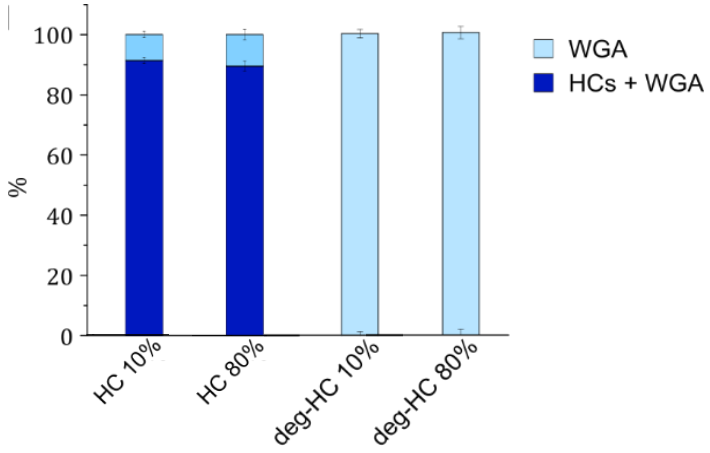
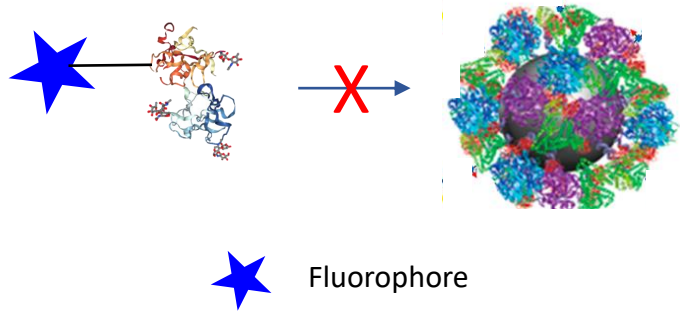








De-glycosylated corona



Fluorophore

Lectin concentration
- 35 nM

CONCLUSIONS

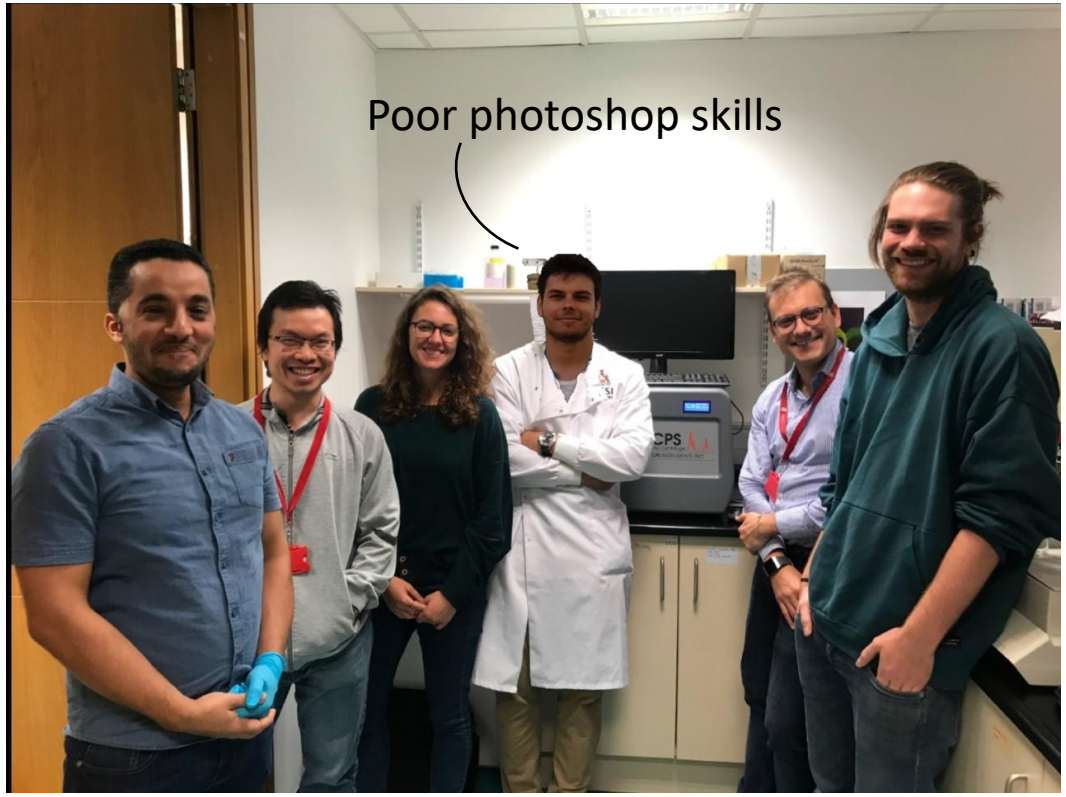
- The NPs protein corona composition changes depending on the concentration of the biological fluid during the incubation time, but the glycan composition is the same.

- The corona is abundant in bisected sialilated N-glycans.

- The glycans are biological accessible to lectins and are likely to have an effect when interacting with the immunological system.

- Loss of glycans results in decrease of colloidal stability and loss of lectins interaction.

ACKNOWLEDGMENTS



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Thank you

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