Corona Interactome

A key for deciphering protein adsorption kinetics on silica nanocarriers

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ICGM / MACS UMR 5253 – Université de Montpellier
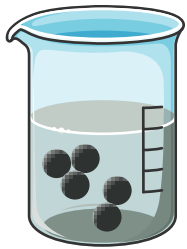CEA / DRF / BIAM – Site de Marcoule
France

Nanomedicine

Medical application of nanotechnologies

➢ Administrating therapeutic molecules with nanocarriers
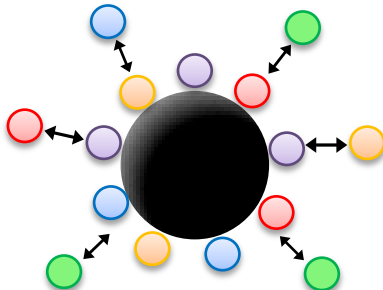
Advantages of nanocarriers:

- The delivery of low drug doses
- The limitation of drug side effects
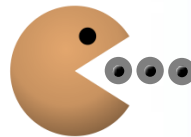- The use of low solubility molecules
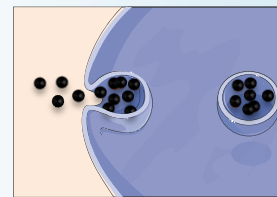
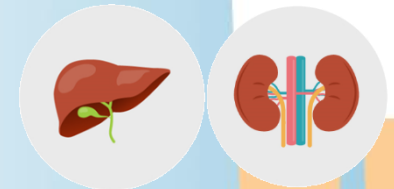## Issues for nanocarrier development:

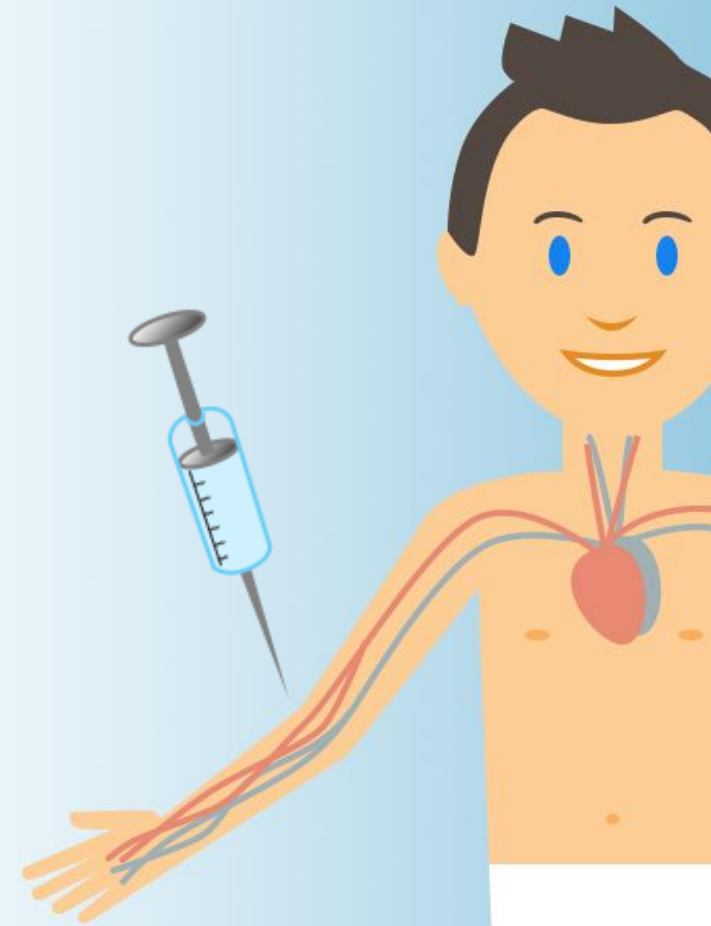| Aggregation | Protein coating « Corona » | Opsonization | Reaching the target site - cellular uptake | Biodegradation Elimination |

What is the Corona ?

➢ Protein layers formed around NPs in contact with biological fluids

➢ Some proteins within the corona could promote the opsonization process

>   *Opsonization is the biochemical process by which molecules cover an exogeneous body to promote its phagocytosis*

➢ Influences the biological fate and toxicity of NPs
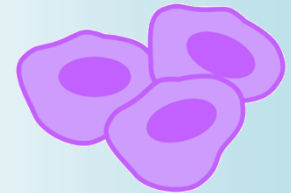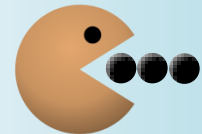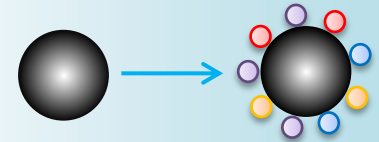
> Understanding the formation of the corona around nanocarriers is a major challenge in nanomedicine to be able to predict and control their fate.
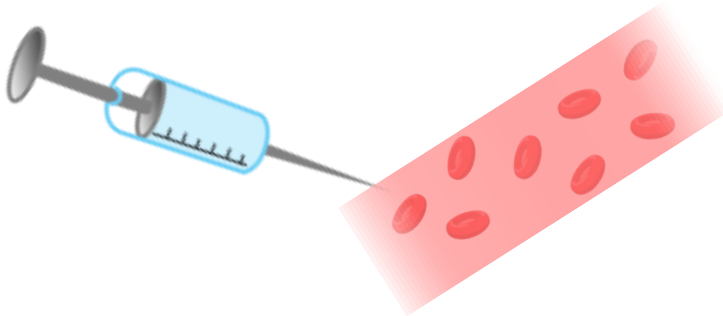
What was the aim?

- **Investigating the kinetics of corona formation**
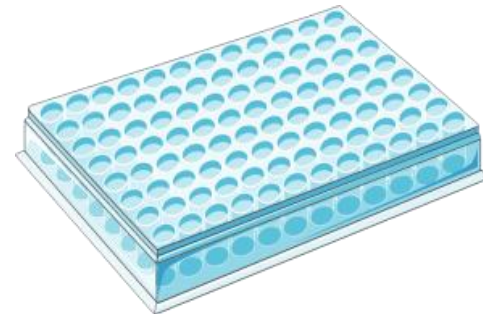
Strategy:

- Monitoring the adsorption of proteins in long term kinetics (from 30s to 7 days of contact)

- In two types of sera :

Human serum for Nanomedicine	Fetal bovine serum for Nanotoxicology

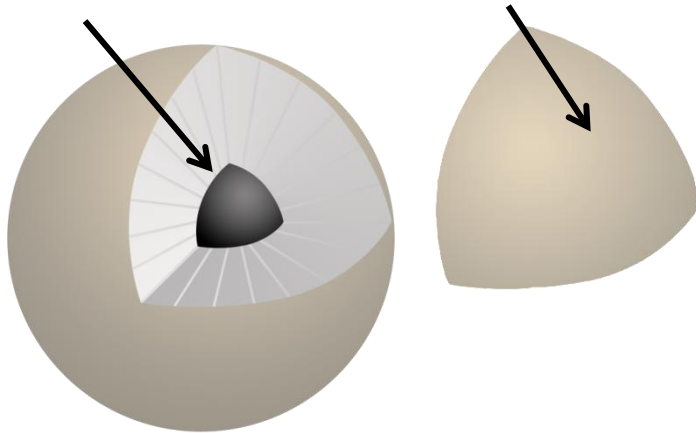Human serum because nanocarriers are intended to be injected in human blood

And in Fetal bovine serum because for *in vitro* nanotoxicology, is conventionally used in cell culture media
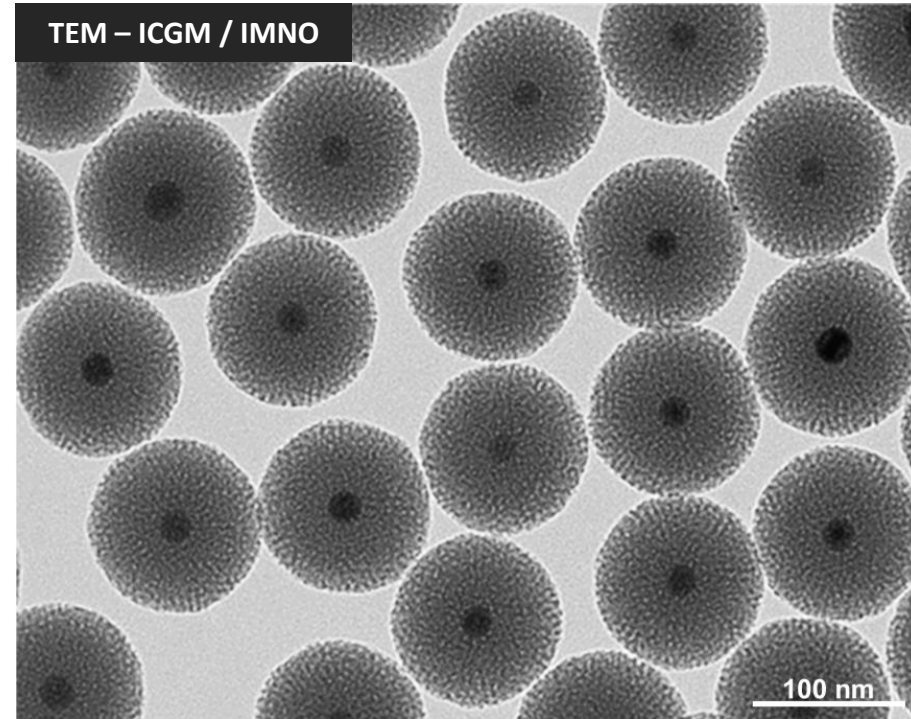
Nanoparticles

Magnetic (Fe_3O_4) mesoporous silica nanocarriers

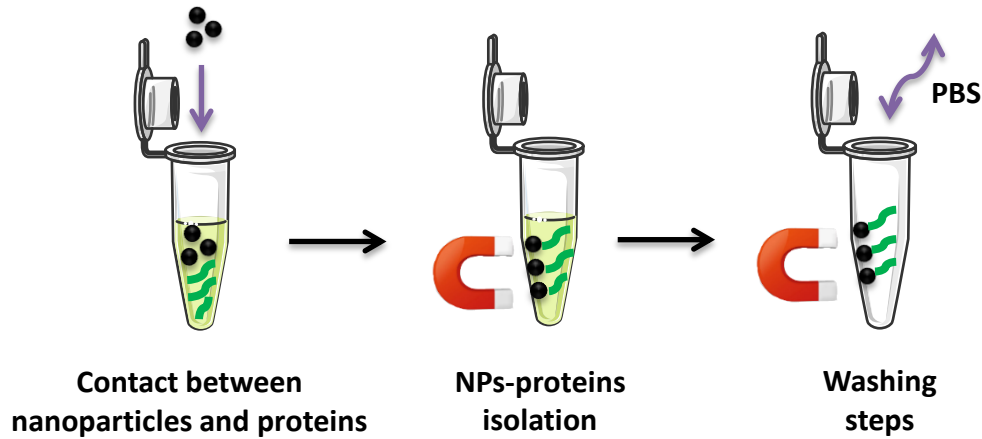Fe$_3$O$_4$ Nanocrystal core

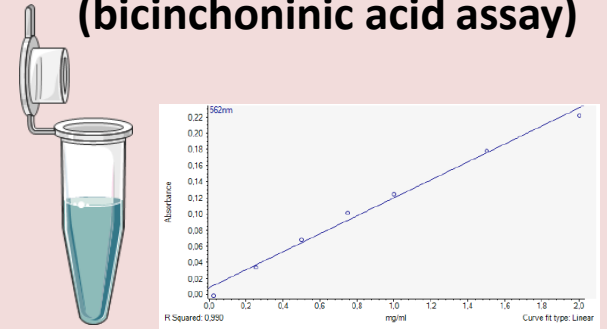Mesoporous silica shell

TEM – ICGM / IMNO

100 nm

- Diameter: **100 nm** (TEM)

- Hydrodynamic diameter: **160 nm** (DLS)

- Zeta potential pH 7.4: **-39 mV** (DLS)

- Specific surface area: **640 m²/g** (BET)
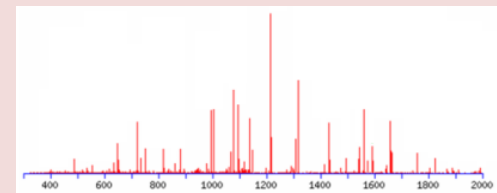
- Pore diameter: **3 nm** (BET)

Contact between
nanoparticles and proteins

NPs-proteins
isolation

Washing
steps

PBS

**Quantification by BCA
(bicinchoninic acid assay)**

Identification by nano-LC MS/MS

Quantification

BCA Assay (Nanodrop)

Chart: Protein concentration (mg/ml) vs Incubation time (min) in LOG10 scale.
Legend: M-MSN - HS corona; M-MSN - FBS corona

AFM – CEA / ICSM

AFM – CEA / ICSM
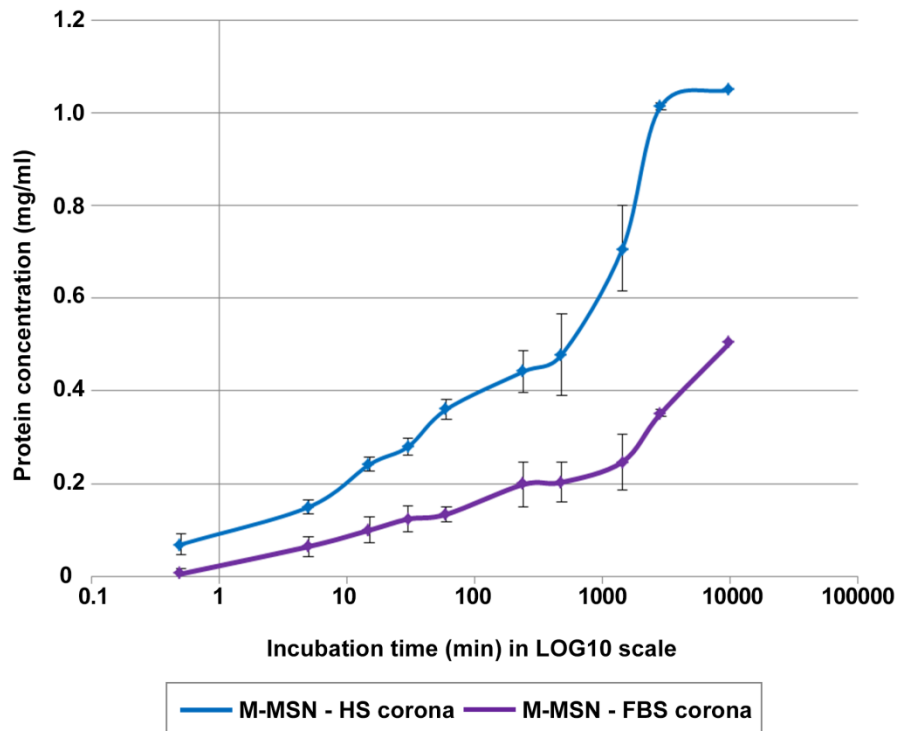
- ➤ Time range: from 30 s to 7 days

- ➤ Growth of the corona for both types of sera

- ➤ Quantity of human proteins > bovine proteins
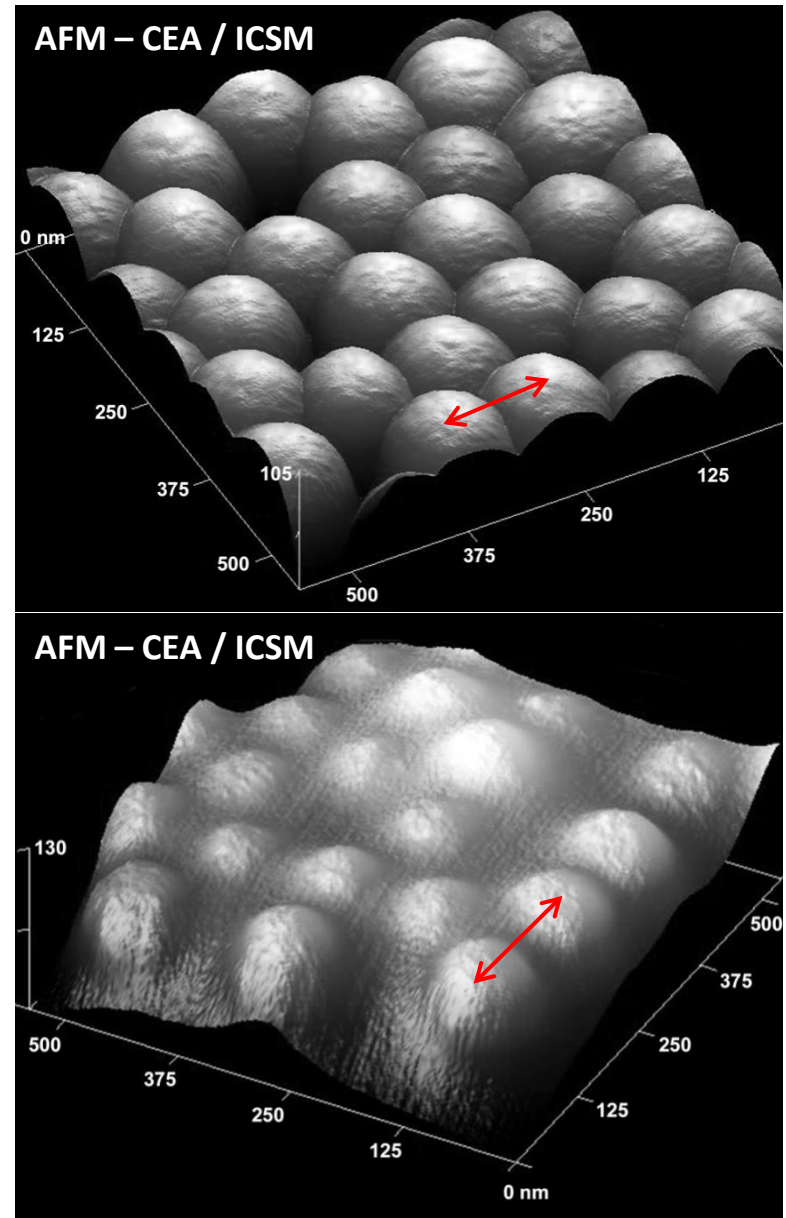
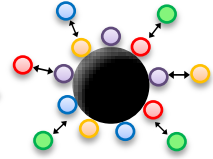## Identification by Mass Spectrometry

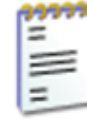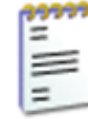Kinetics of the corona formation

| 0.5min | 5min | 15min | 30min | 1h | 2h | 8h | 1d | 2d | 7d |

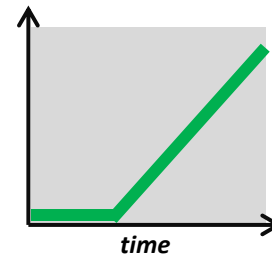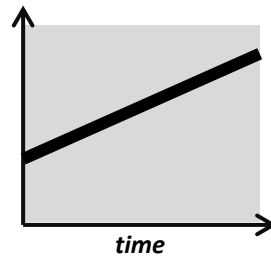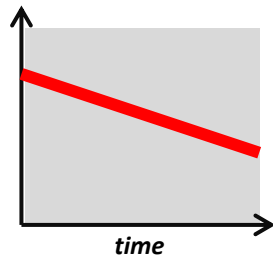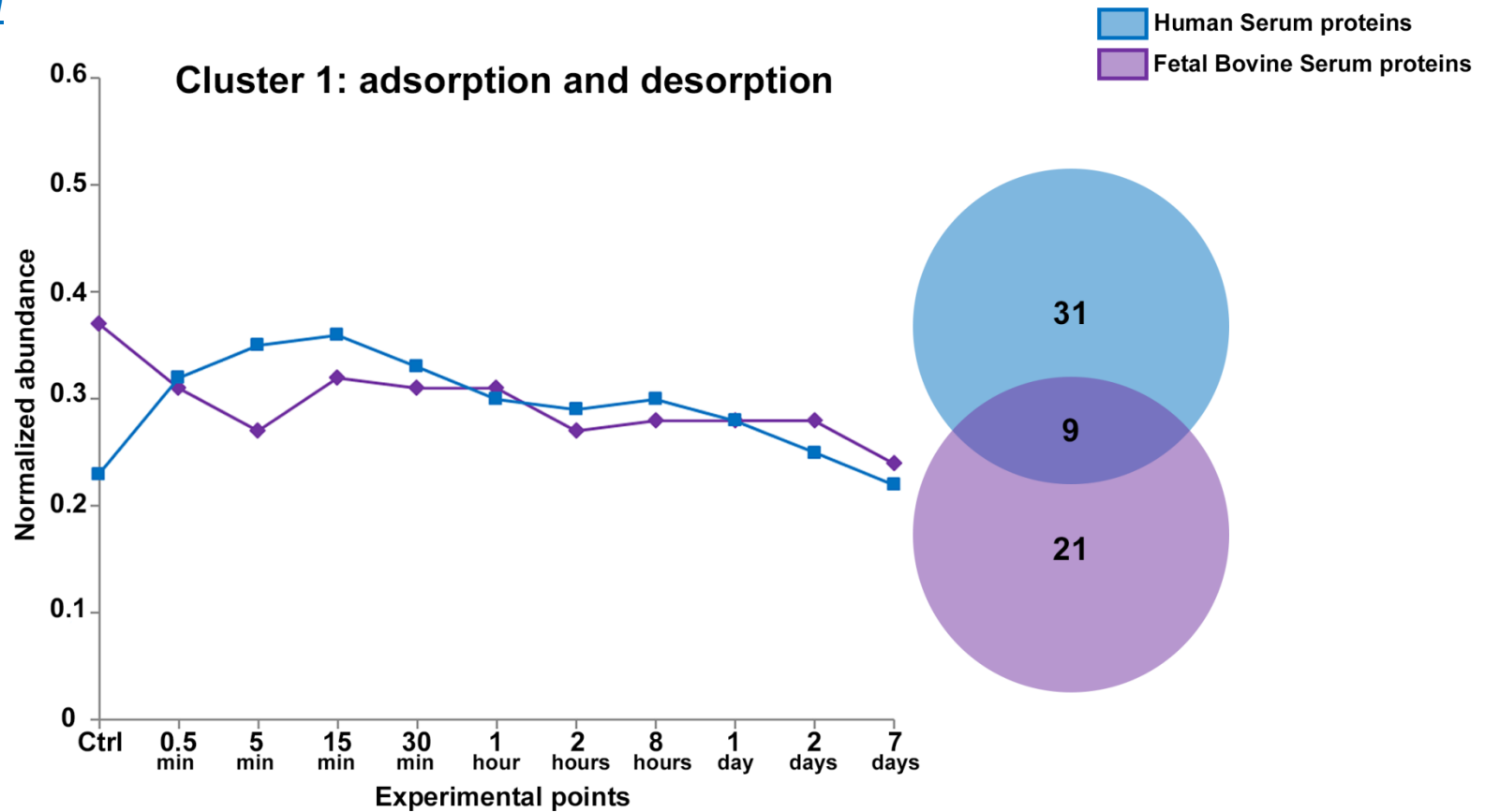**Bioinformatics tools**

Determination of 3 main trends of behavior for protein adsorbed around nanoparticles

Same clusters for human and fetal bovine coronas
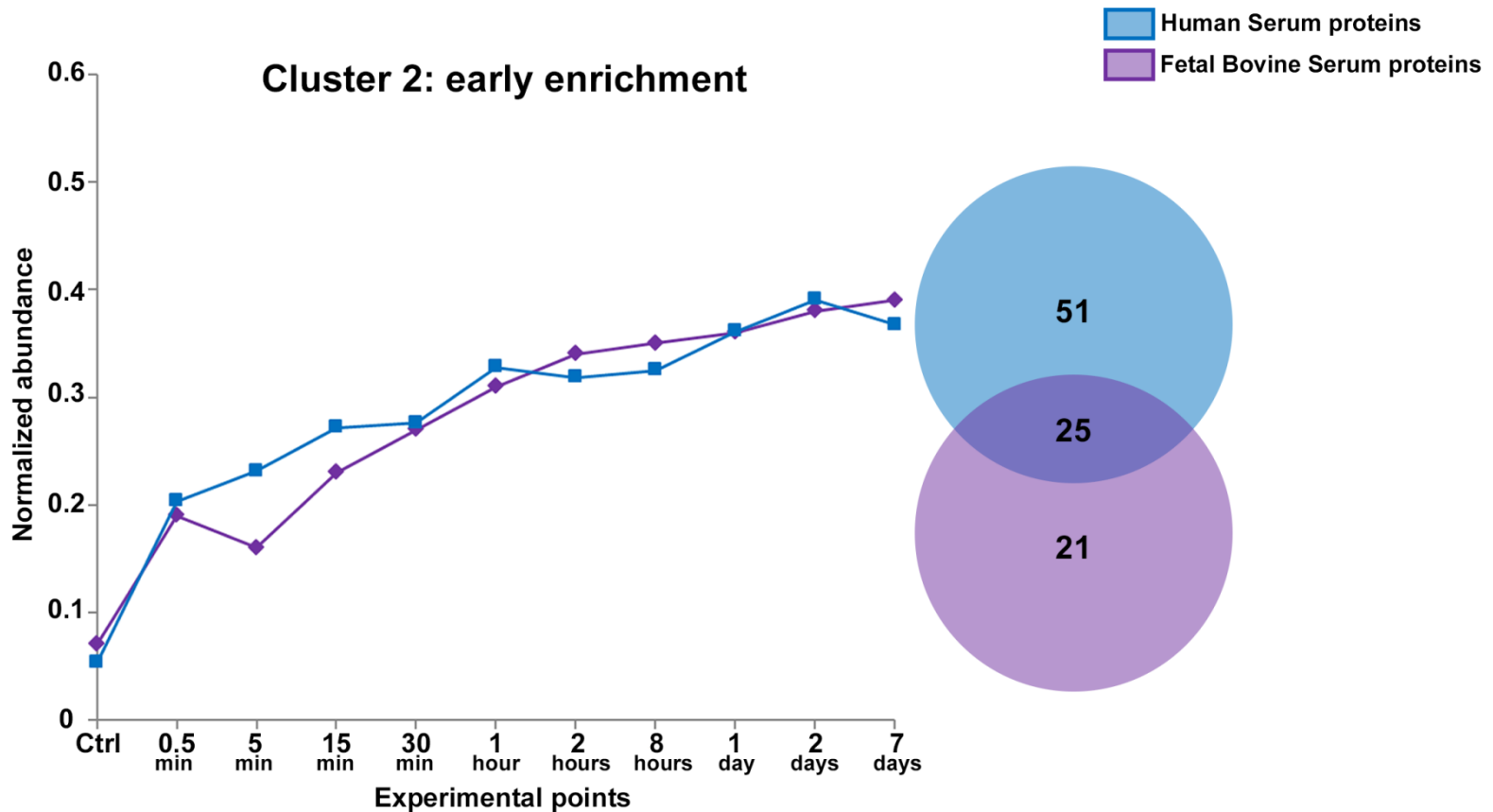
Clusterisation

- ➤ Most abundant serum proteins are in this cluster.
  Ex: Albumin (HS&FBS); Serotransferrin (HS&FBS)
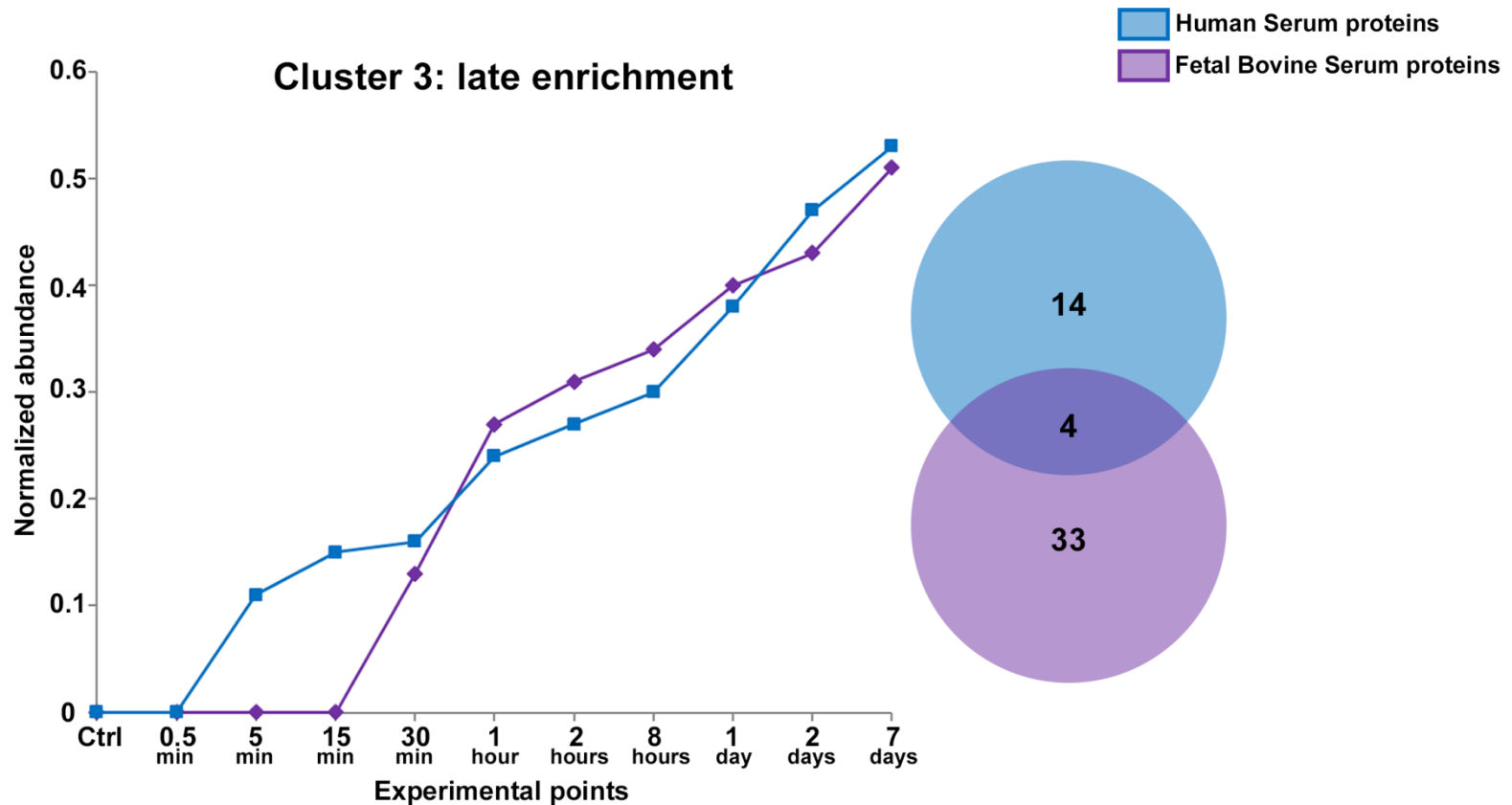
- ➤ Human cluster contains also all immunoglobulins

Clusterisation

➢ This cluster represents a stable layer called ''hard corona''

➢ Contains well-known protein families
   Apolipoproteins, Complement components, Coagulation factors

Clusterisation

Cluster 3: late enrichment

- Human Serum proteins
- Fetal Bovine Serum proteins

➤ Some proteins are not detected in CTRLs but are detected within the corona

➤ Similarities with cluster 2: *some apolipoproteins and complement factors*

Investigate the involvement of protein-protein interactions in the formation of the corona

We implemented System Biology tools to construct a protein-protein network
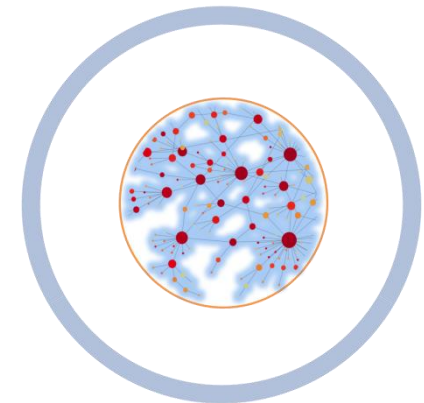
- **NetworkAnalyst software**

NetworkAnalyst is an online tool which enables the construction and visualization of networks from gene or protein lists.

This tool uses the high quality protein-protein interaction database from InnateDB

NetworkAnalyst.ca

- **Database : InnateDB**

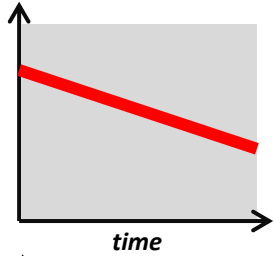InnateDB is a publicly available database of experimentally-verified interactions. It is a manually-curated database.
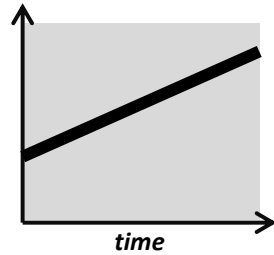
Results

Corona interactome

Cluster 1

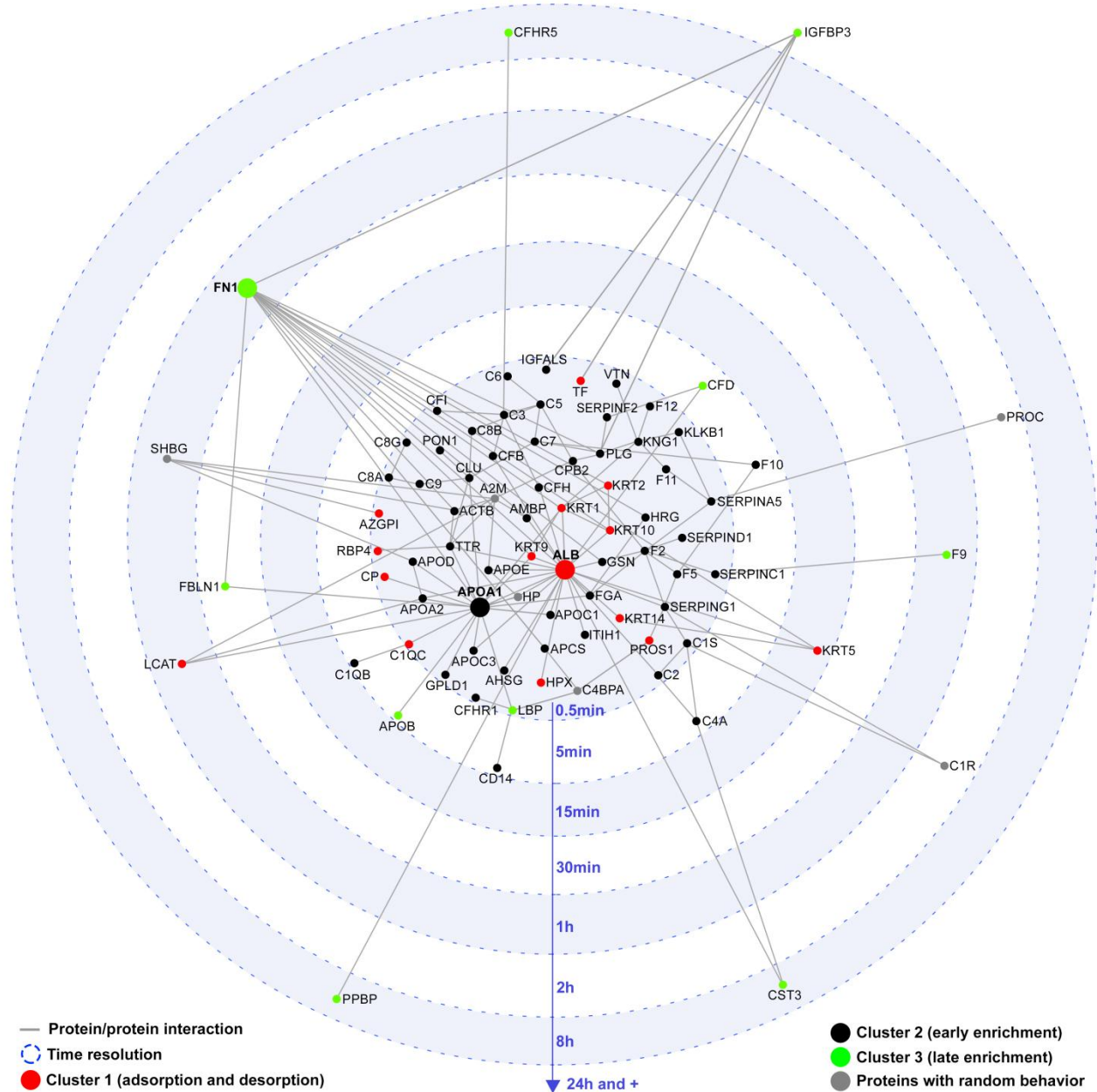*time*

Cluster 2

time

Cluster 3

time

— Protein/protein interaction
○ Time resolution
● Cluster 1 (adsorption and desorption)

● Cluster 2 (early enrichment)
● Cluster 3 (late enrichment)
● Proteins with random behavior

0.5min
5min
15min
30min
1h
2h
8h
24h and +

Corona interactome

English translated - Le génie des alpages. © Dargaud, by F'Murr.
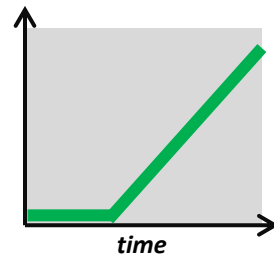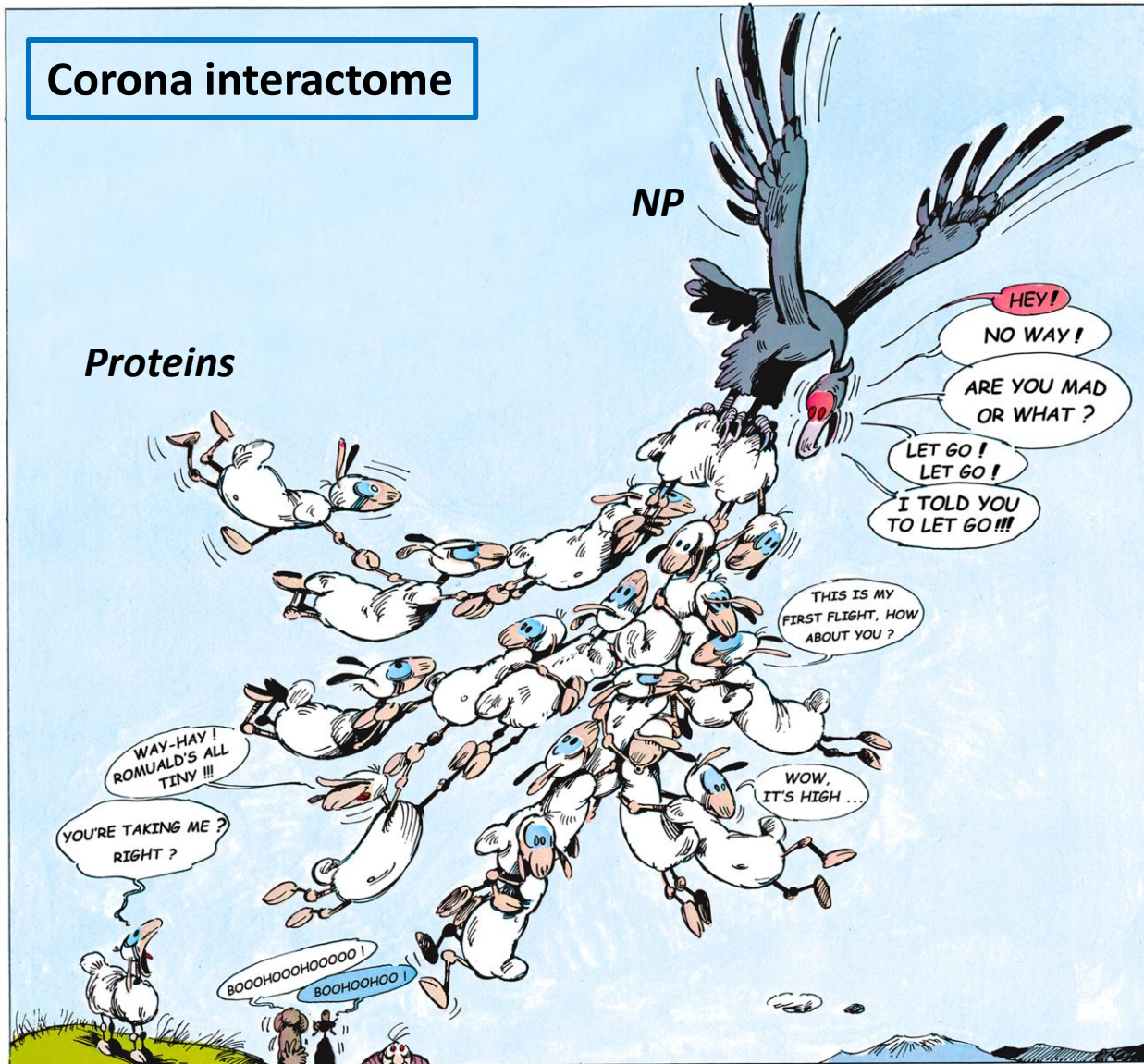
Thank you for your attention

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