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hic sunt futura



GIBB 2026

ANNUAL MEETING OF THE ITALIAN GROUP OF BIOMEMBRANES & BIOENERGETICS
UDINE, 11-13 JUNE 2026

Biotechnological production of extracellular vesicles from *Coffea arabica* L. cell suspension cultures: isolation and proteomic profiling

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COFFEE: HOW DO YOU SEE ME?

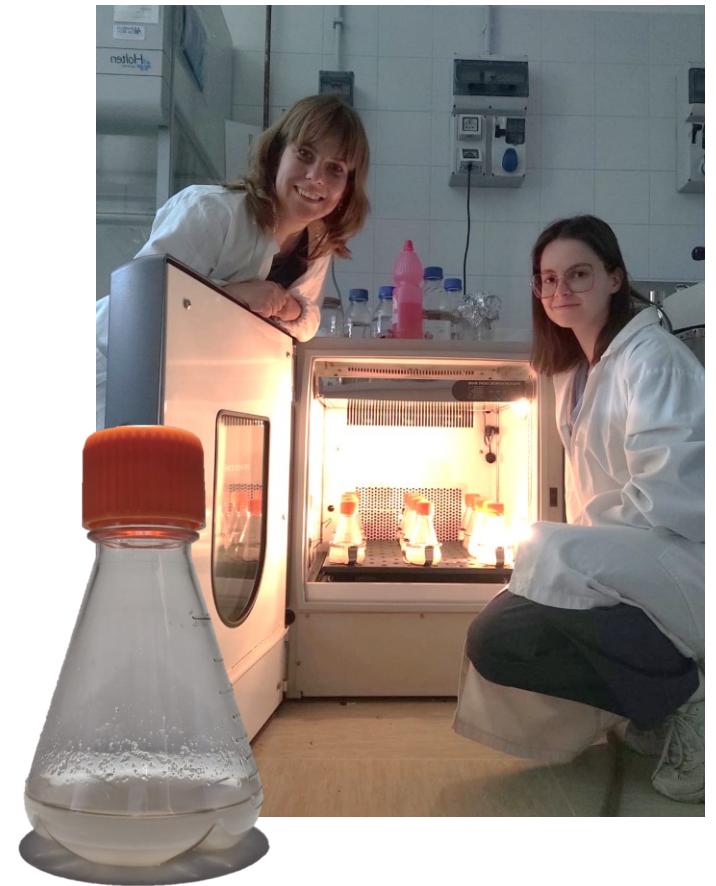
HOW EVERYONE SEES ME



HOW A PLANT PHYSIOLOGIST SEES ME

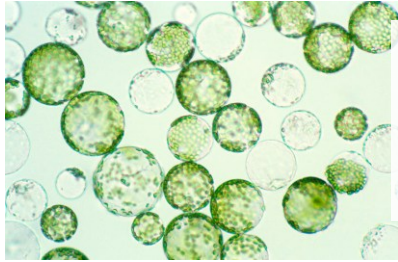


HOW DORA and AZZURRA SEE ME



PLANT TISSUE CULTURE: GREEN SOLUTION

PROTOPLAST
CULTURE

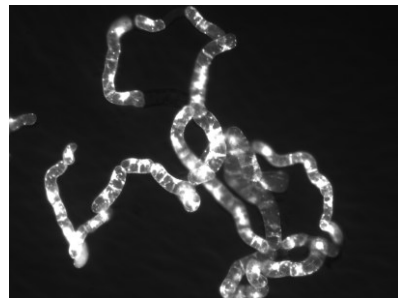


PLANT BREEDING/
CROP IMPROVEMENT

CALLUS CULTURE

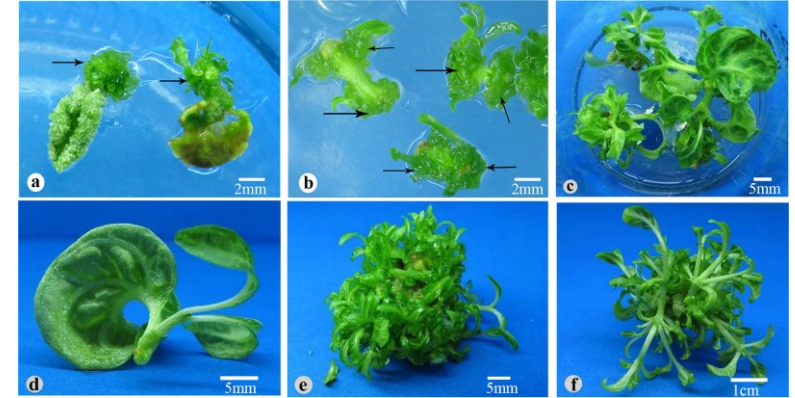


CELL SUSPENSION
CULTURE



BIOACTIVE
COMPOUNDS

TECHNIQUES &
PURPOSES



PLANT
REGENERATION

SOMATIC
EMBRYOGENESIS

ORGAN CULTURES

ORGANOGENESIS

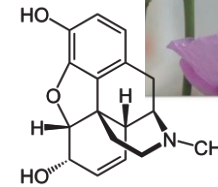
SECONDARY METABOLITES: HUMAN BENEFITS



- **80%** of the world population relies on herbal medicines
- **11% out of 250 drugs** essential for primary health care needs are of **plant-origin**

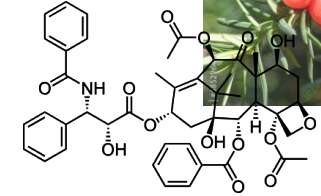
WHO, 2019

Papaver somniferum



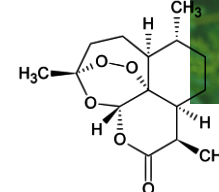
MORPHINE

Taxus brevifolia



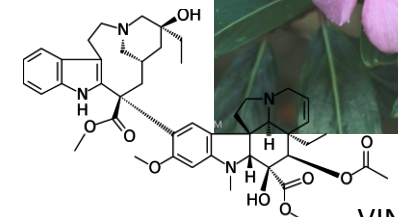
PACLITAXEL

Artemisia annua



ARTEMISIN

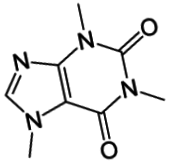
Catharanthus roseus



VINBLASTINE

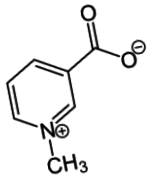
COFFEE: BEYOND THE BEVERAGE

ALKALOIDS



CAFFEINE

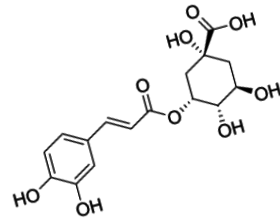
- Central nervous system stimulant
- Neurodegenerative disorders



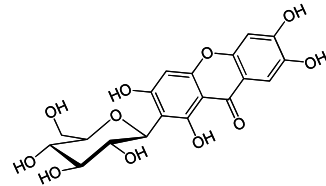
TRIGONELLINE

- Hypoglycemic and hypolipidemic

PHENOLIC COMPOUNDS



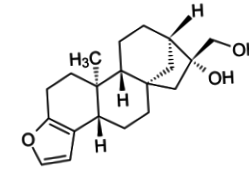
CHLOROGENIC ACIDS



MANGIFERIN

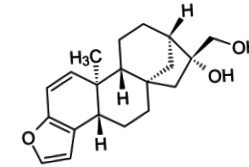
- Anti-inflammation
- Antioxidant

TERPENES



CAFESTOL

- Anticarcinogenic
- Antinociceptive
- Osteoclastogenesis



KAHWEOL



PLANT EXTRACELLULAR VESICLES

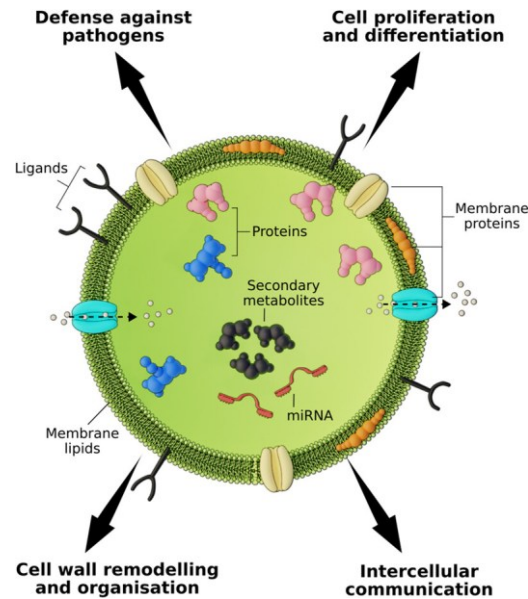
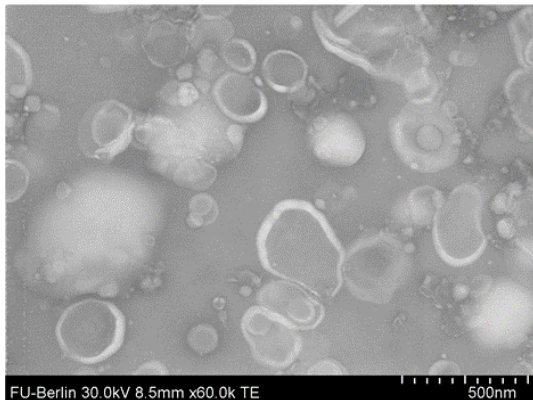


Image from Lian et al. (2022)



- Lipid bilayer with **HETEROGENEOUS CARGOES**: SMs, but also lipids, proteins and nucleic acids
- Several **FUNCTIONS**: intercellular communication, defense and toxic disposal.
- **POSSIBLE BIOTECH APPLICATIONS**:
 - Exploitation of inner **bioactive compounds**
 - **Drug delivery tools** (natural nano-cargoes)
- Plant-origin **ADVANTAGES** vs. animal-origin:
 - Easy extraction
 - High yield/low cost
 - Low immune and infection risk

AIMS & OBJECTIVES

More advantages associated to CSCs, so...



MIGHT COFFEE CSCs BE USED AS A SOURCE OF EXTRACELLULAR VESICLES (EVs) ?

Q1

ESTABLISHMENT OF ISOLATION PROTOCOL

Q2

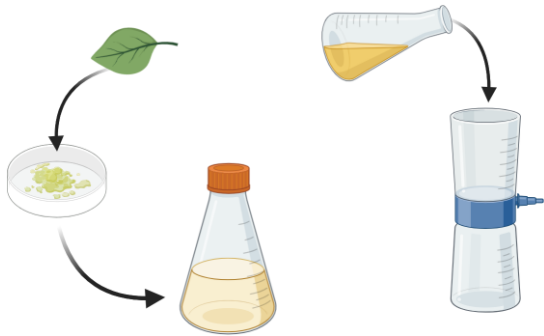
VESICLE CHARACTERIZATION

- Morphology
- Protein content

Q1: EVs ISOLATION

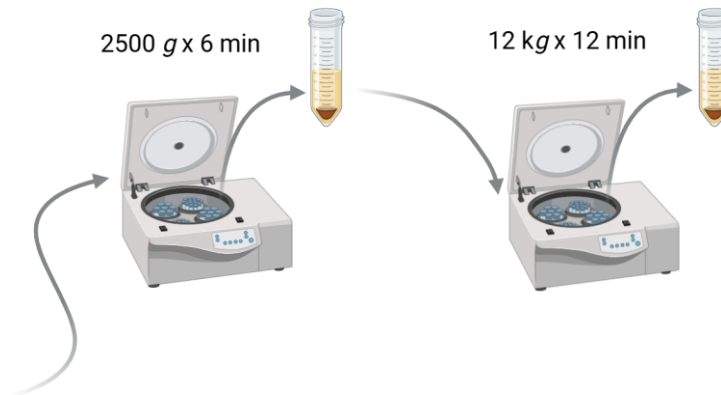
STEP 1

CULTURE MEDIUM FILTRATION



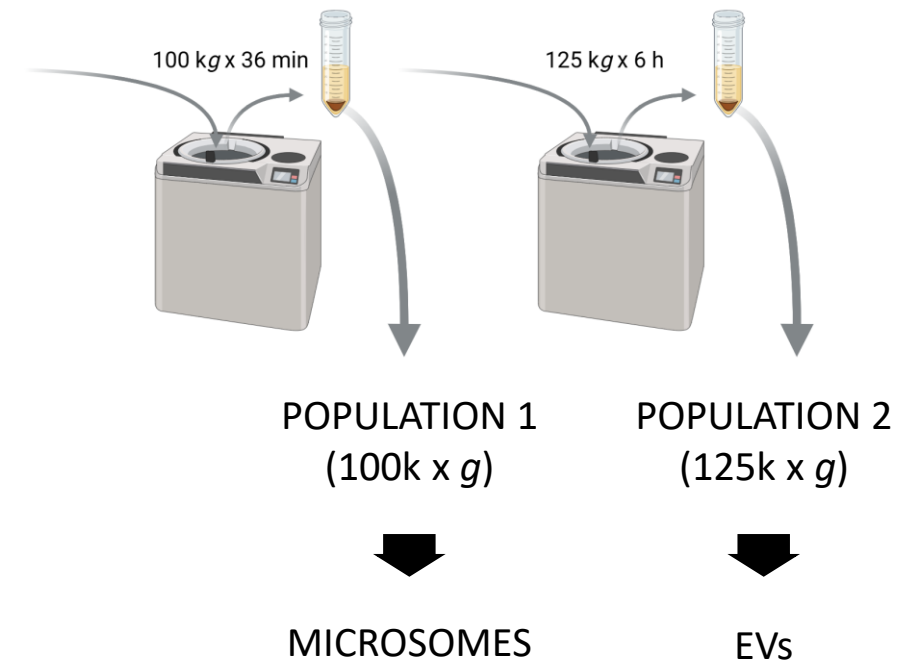
STEP 2

DEBRIS DISCARD

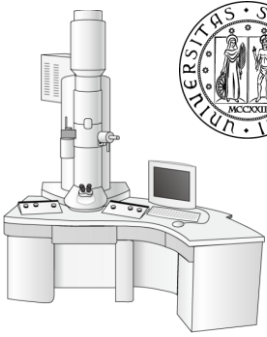


STEP 3

EVs ISOLATION by differential ultra-centrifugation

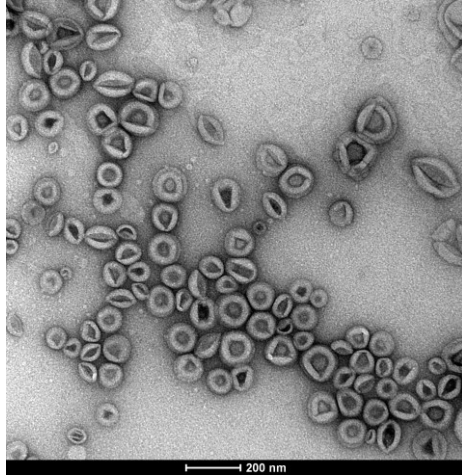


Q2: MORPHOMETRIC CHARACTERIZATION



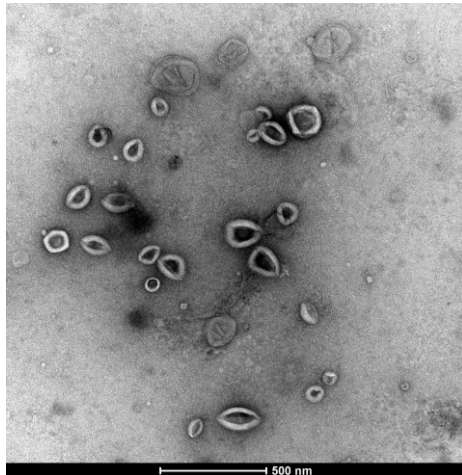
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POPULATION 1
(100k x g)



Avg Diameter $\pm$ SD
120.90 $\pm$ 37.84 nm

POPULATION 2
(125k x g)



Avg Diameter $\pm$ SD
117.81 $\pm$ 43.22 nm

- EVs are present in population 1



UNEXPECTED...

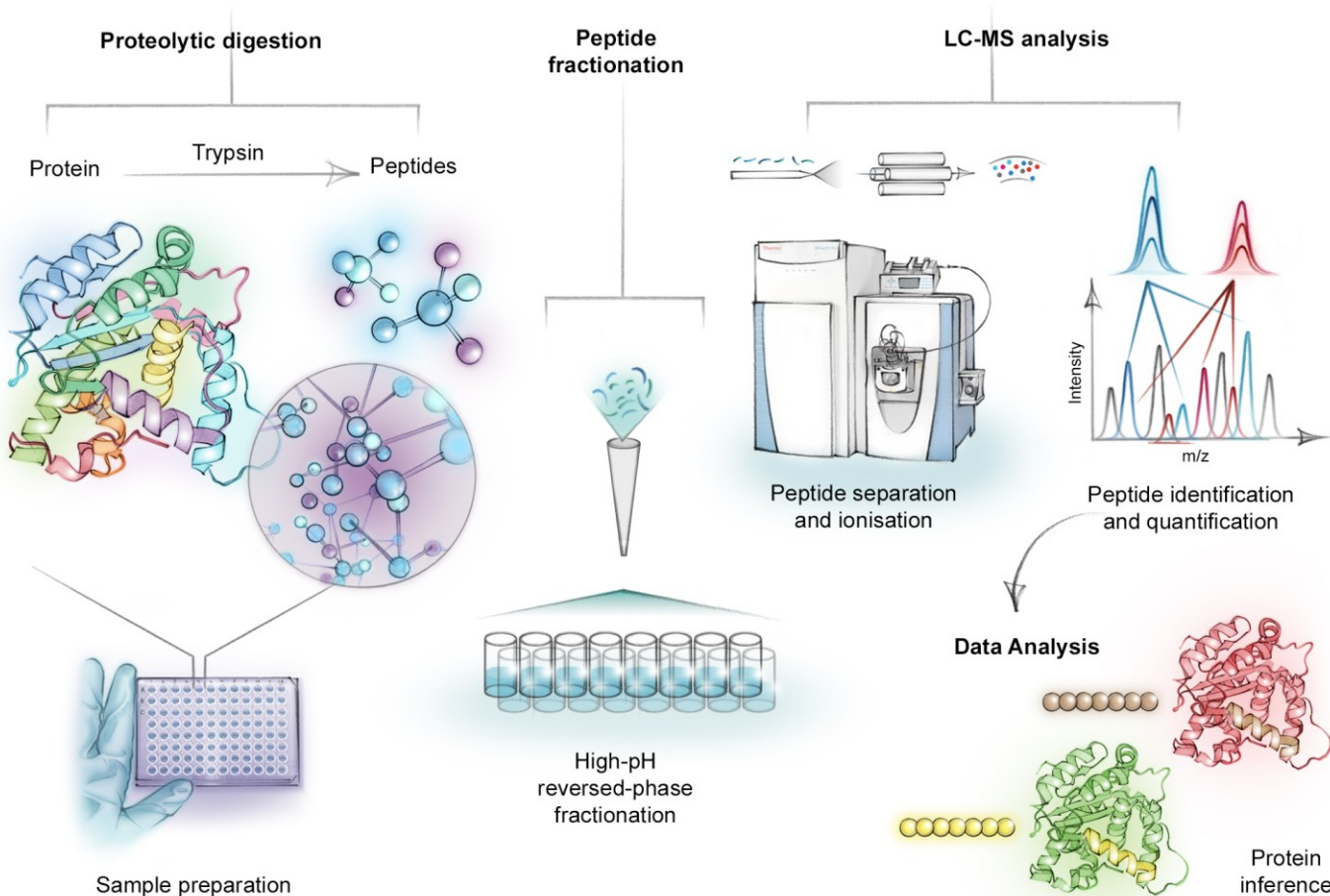
Hypothesis? Uncomplete
pelleting

- Average diameter within the size range typically associated with EVs (30 – 150 nm)
- Round or elliptical shape
- Occasionally surrounded by a double-lipid membrane

Q2: PROTEOMIC ANALYSIS

Samples analyzed by LC-MS/MS

= Liquid Chromatography Tandem Mass Spectrometry



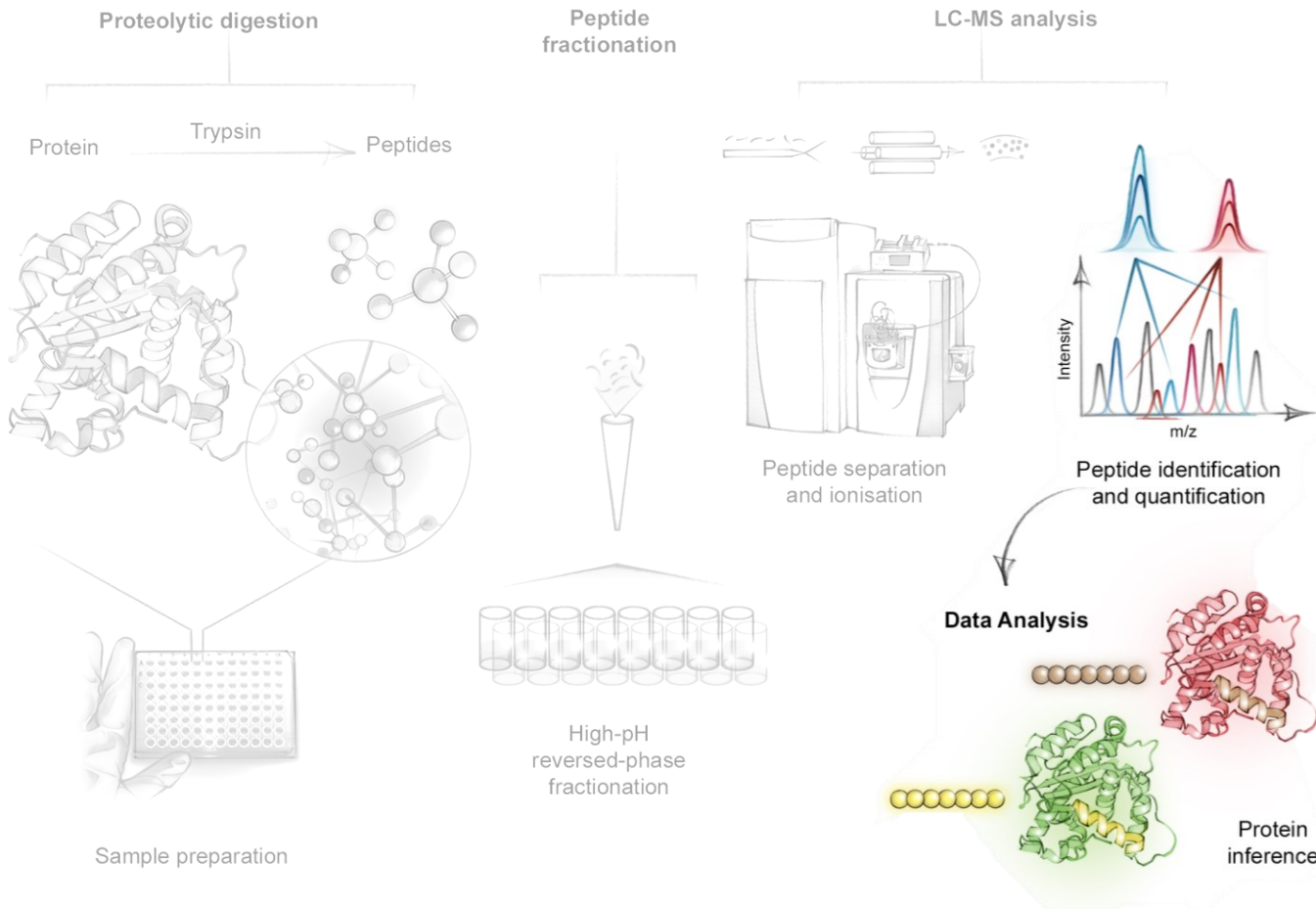
OUTPUT: PEPTIDE

- Comparison against the *C. arabica* proteome (Proteome Discoverer 2.2 + MASCOT engine)
→ WHICH PROTEINS ARE PRESENT?
- Gene ontology (GO) enrichment and prediction of protein localization (STRING + DeepLoc-2.1)
→ WHICH LOCALIZATION IS ASSOCIATED TO A SPECIFIC PROTEIN?

Q2: PROTEOMIC ANALYSIS

Samples analyzed by LC-MS/MS

= Liquid Chromatography Tandem Mass Spectrometry



From Fredman et al. (2021)



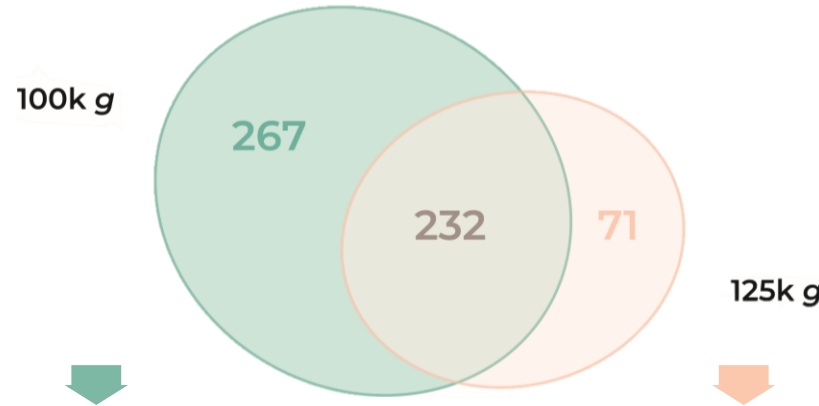
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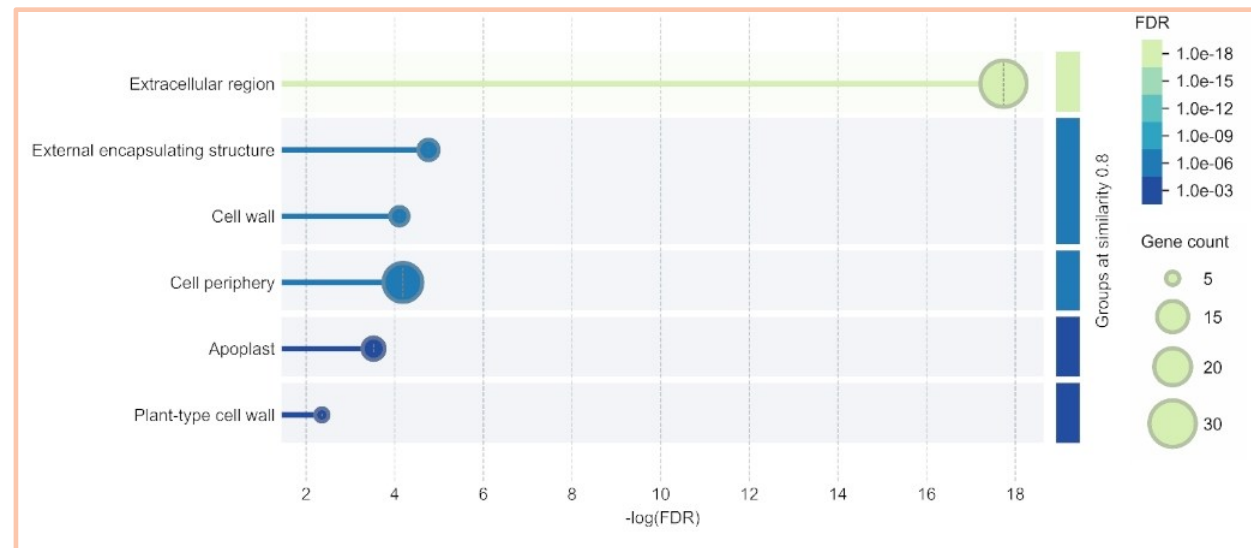
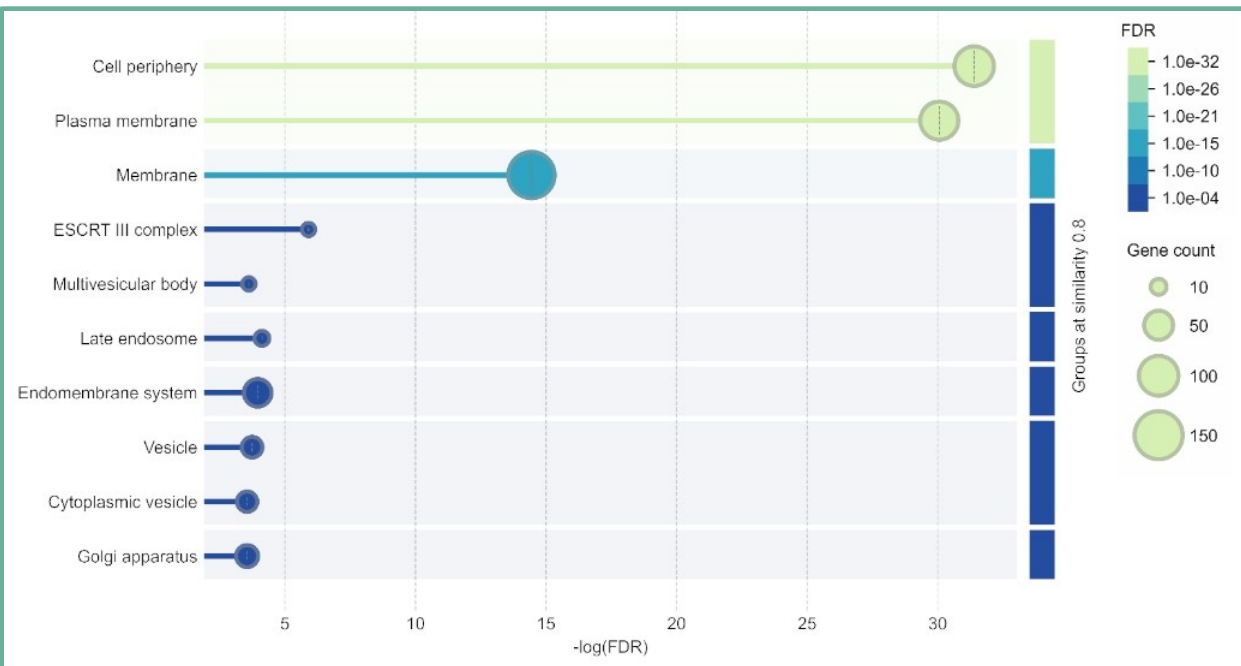
Q2: PROTEOMIC ANALYSIS

- Associated to CELLULAR BOUNDARY, PLASMA MEMBRANE and intracellular compartments
- FUNCTION: cellular signaling and intracellular transport

NUMBER OF PROTEINS

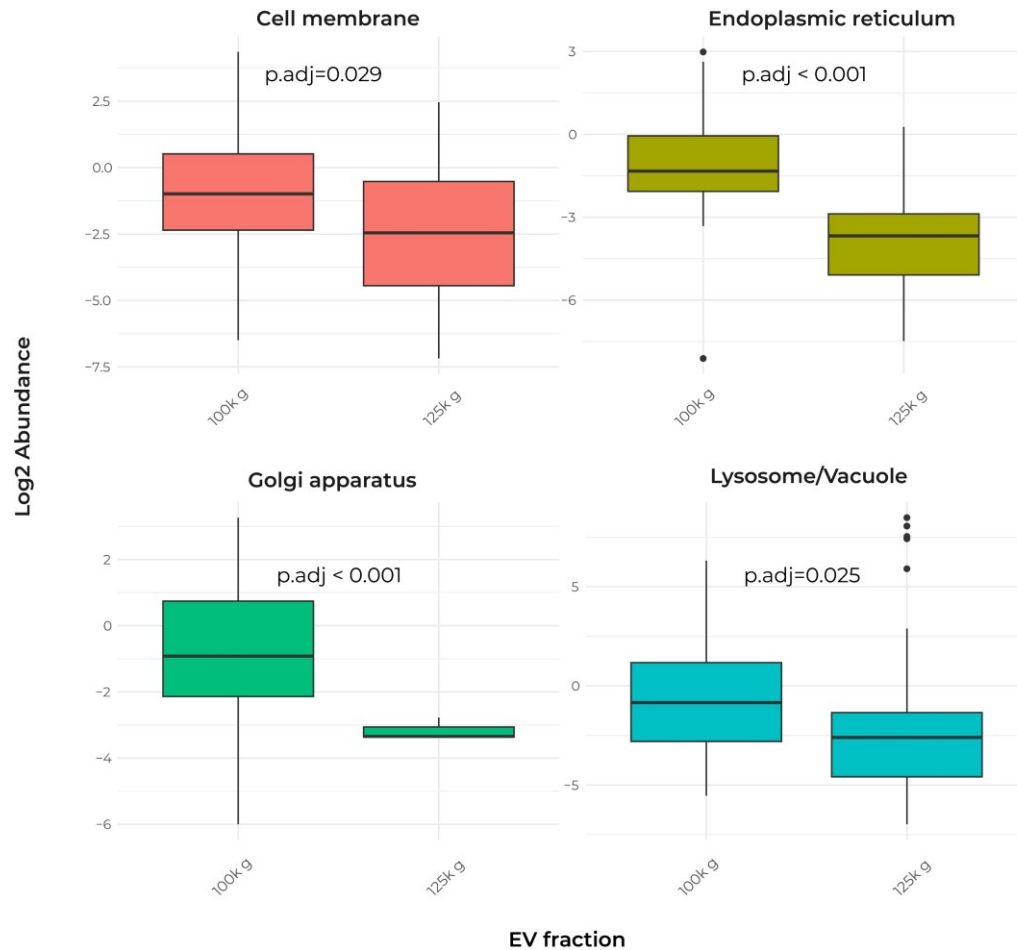


- Associated to EXTRACELLULAR REGION
- FUNCTION: cell wall remodelling and response to abiotic/biotic stress



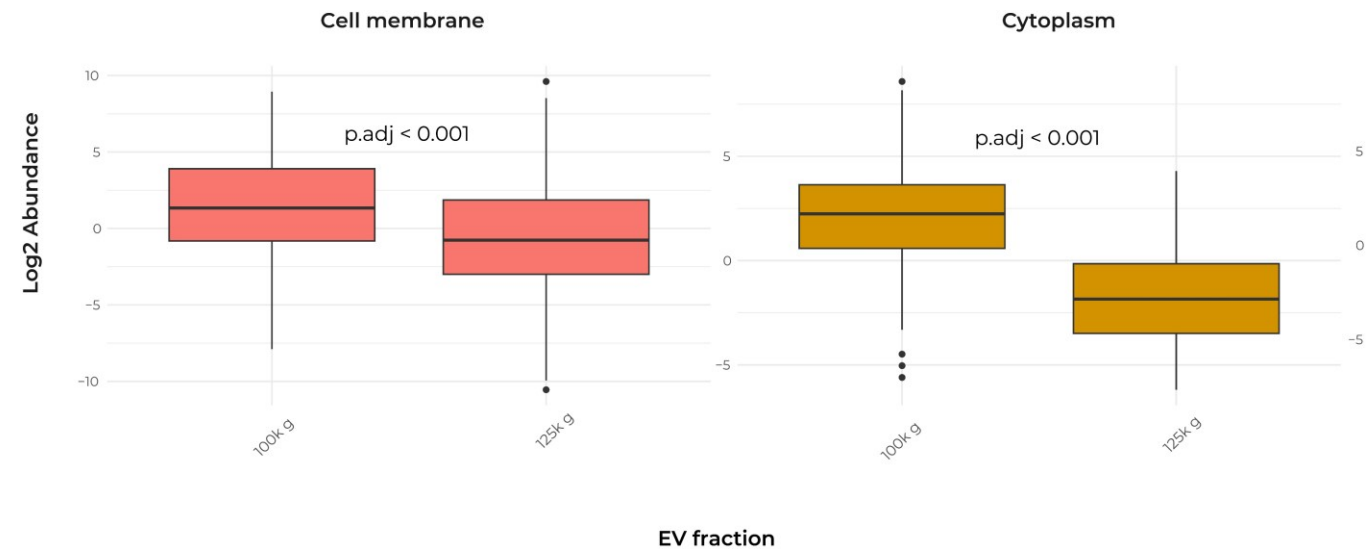
Q2: PROTEOMIC ANALYSIS

PROTEINS **EXCLUSIVE** TO THE 100k *g* OR 125k *g* FRACTION



Higher abundance of proteins associated to cell membrane and intracellular compartments in the 100k *g* fraction

PROTEINS **SHARED** BY THE 100k *g* AND 125k *g* FRACTIONS



Hypotheses on **multiple pathways** of origin → See more here: **Di Bonaventura *et al.*, 2026**

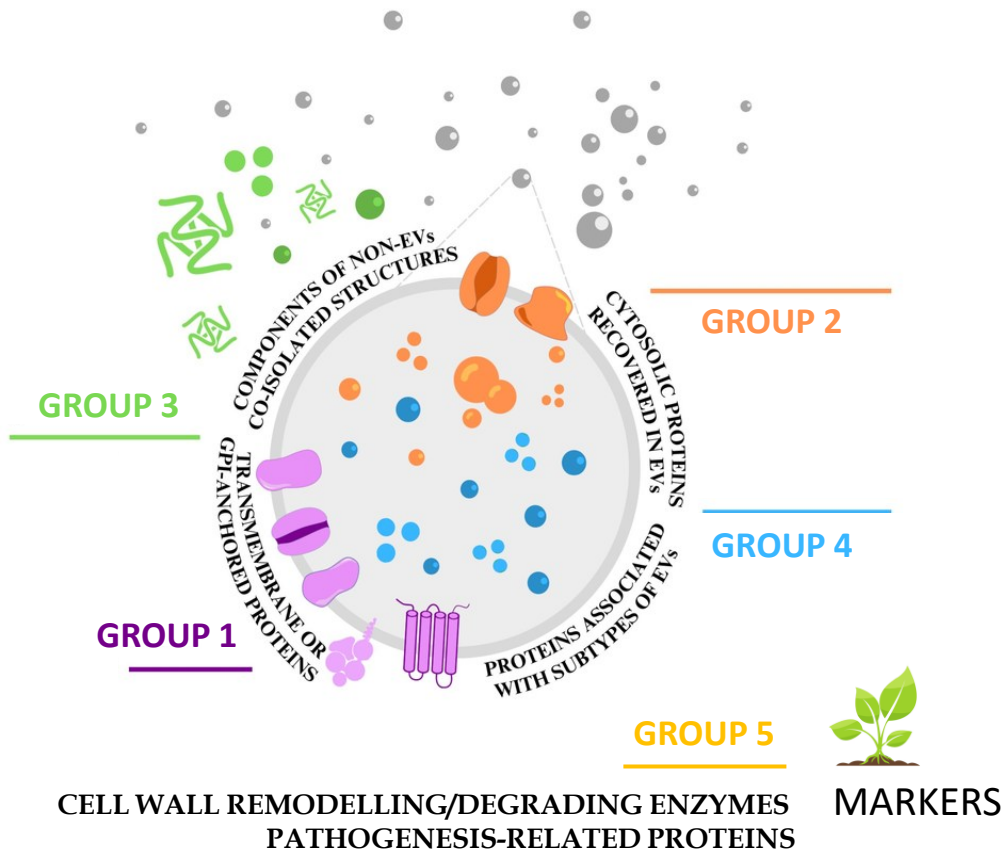


Q2: PROTEOMIC ANALYSIS

How to validate EVs characterization?

PRESENCE OF SPECIFIC MARKERS

see Welsh et al. (2024)

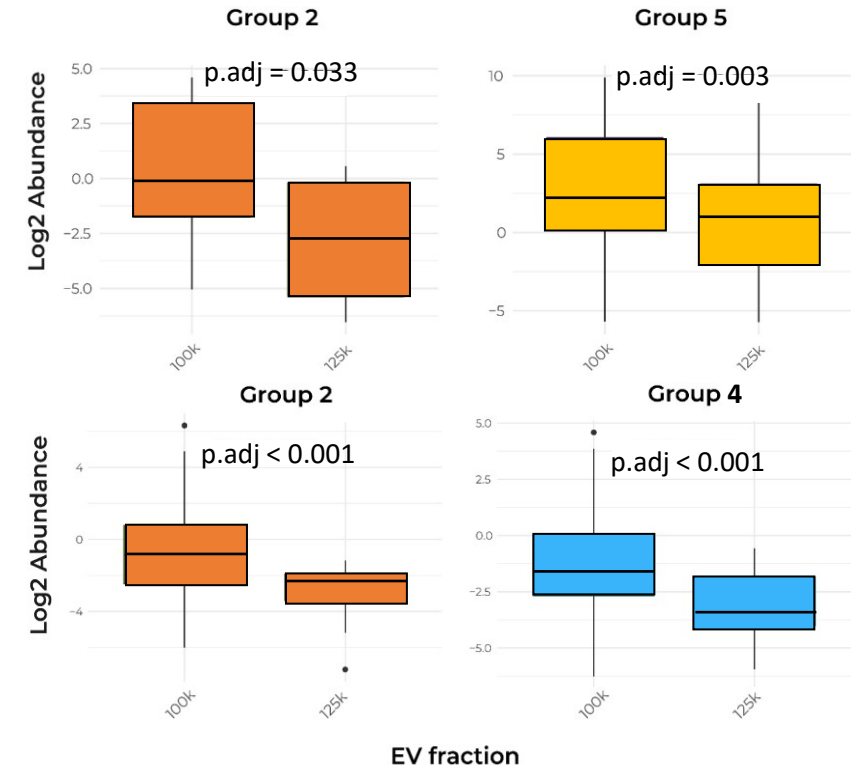


modified from Kolenc and Maličev (2024)

- Overall, 25% ca. of the proteins are EVs markers, up to 50% in the 125k g fraction
- No protein from Group 3 found

PROTEINS
SHARED

PROTEINS
EXCLUSIVE

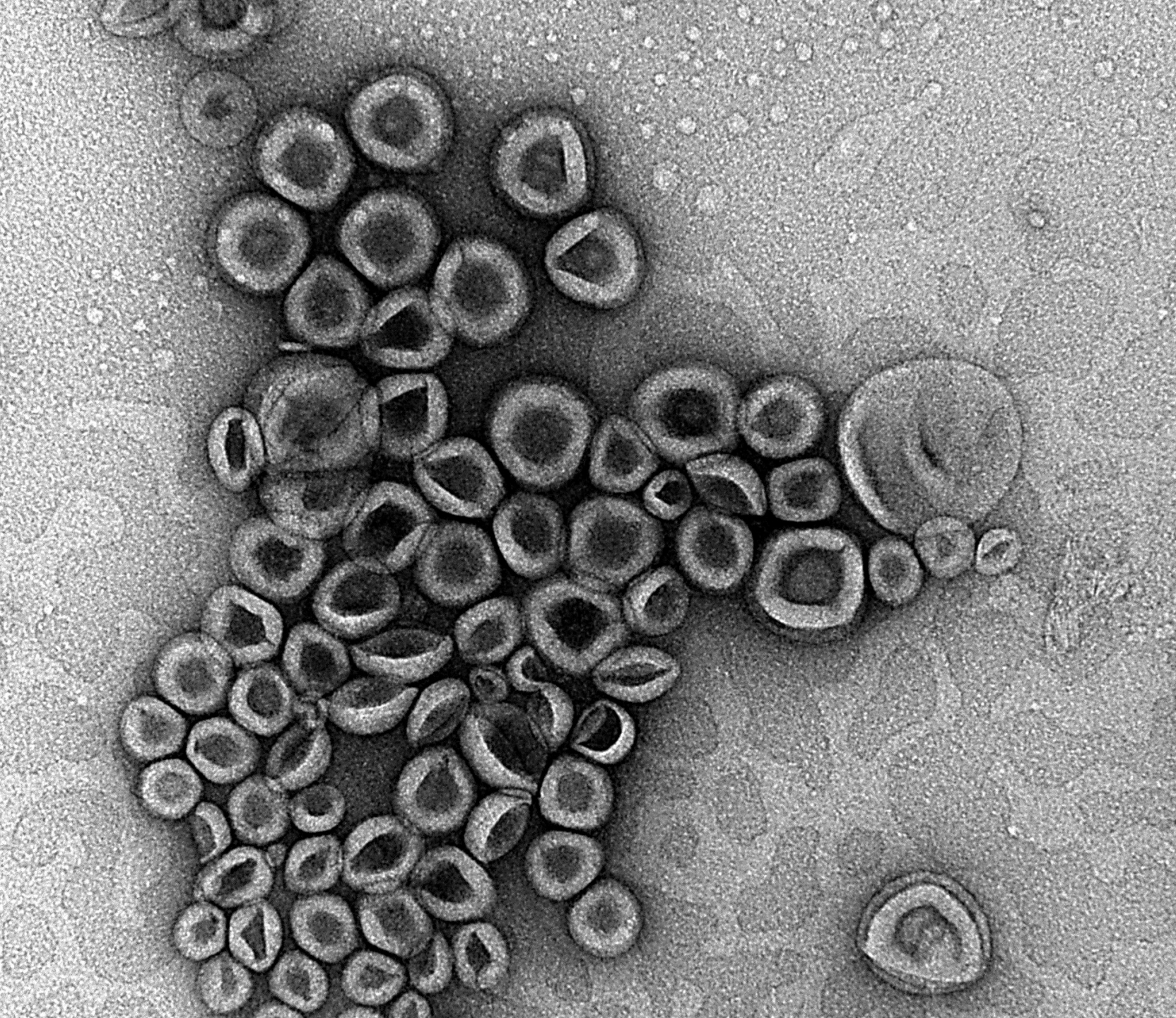


CONCLUSIONS

...YES, COFFEE CSCs REPRESENT A VALUABLE SOURCE OF EXTRACELLULAR VESICLES



- WHAT HAS BEEN DONE
 - Isolation protocol for **"true" Evs** from "waste"
 - Discoveries about biogenesis and potential role in cells
- WHAT'S NEXT
 - Further *omics* characterization and more...
 - Potential applications: **disease treatment?**
 - **Exploitation of the natural bioactive molecules already present**
 - **Biotech manipulation for drug transport**
 - **Is it safe and effective to scale up?**



THANK YOU FOR YOUR ATTENTION!

Special thanks to:

Azzurra Di Bonaventura, Giacomo Trotta,
Stefano Marchetti, Elisa Petrusa, Enrico
Braidot, Luciano Navarini, Marco Zancani



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