


# Imaging Extracellular Vesicles: From Light Microscopy to Cryo-EM

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MUNI SCI

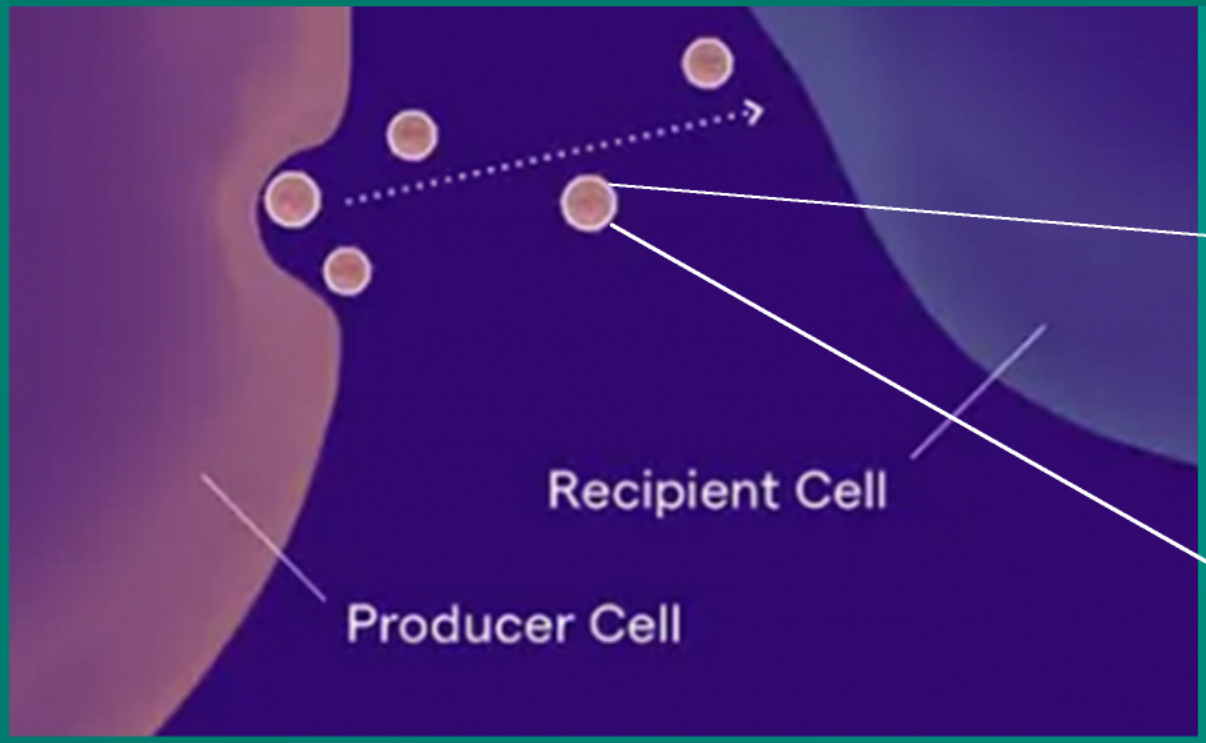
CzeSEV Czech Society for Extracellular Vesicles

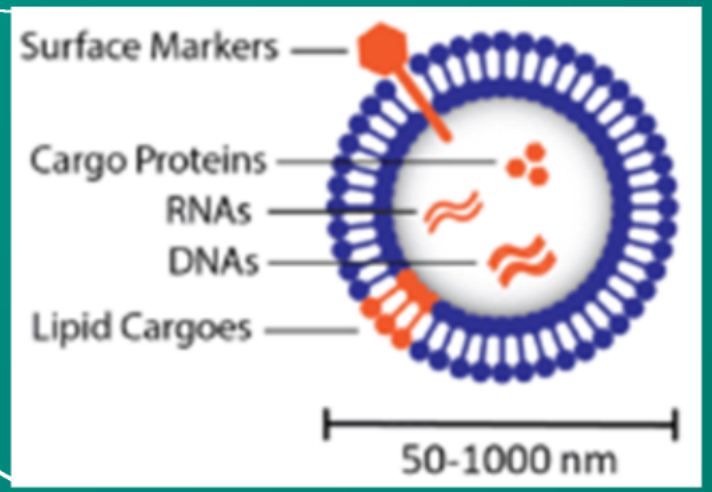
Multimodal Microscopy Workshop 2025-09-10

# Understanding Extracellular Vesicles (EVs)

EVs: nanoscale particles for intercellular communication

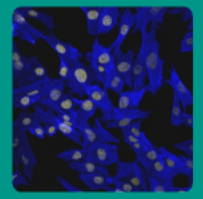
- Membrane-bound, secreted by all living cells
- Transfer bioactive cargo to recipient cells
- Influence the fate of recieving cells
- High potential in diagnostics & therapeutics



# Roles of EVs in Intercellular Communication and Theranostics

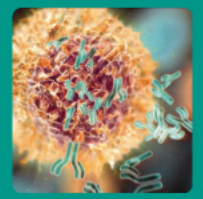
## Key Role in Cell Signaling



EVs are involved in every (patho)physiological process

- Influence signaling pathways, enhance cell interactions
- Facilitate long-distance communication
- Mediate communication between species
- Provide crucial insights for biomedical progress

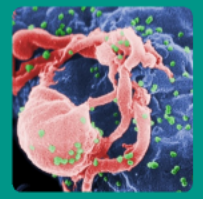
## Potential in Diagnostics



EVs carry molecular signatures of parent cells

- Indicate disease states - biomarkers
- Aid early detection, monitor disease progression
- Circulate in all body fluids - non-invasive biomarkers
- First FDA-approved EV-based urine test (ExoDx™ Prostate)

## Innovative Therapeutic Applications



Engineered EVs allow targeted drug/genetic delivery

- Enhance treatment efficacy and reduce side effects
- Significant breakthrough in personalized medicine

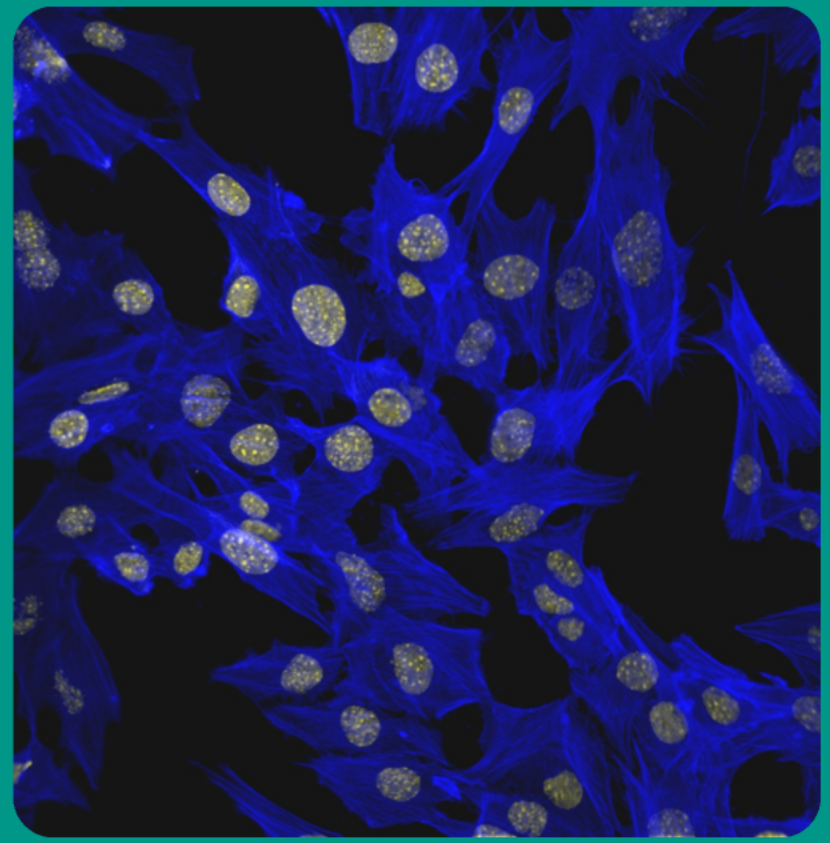
Intrinsic therapeutic activity - MSC-derived EVs

- Can overcome limitations of cell-based therapies

# Key Role in Cell Signaling

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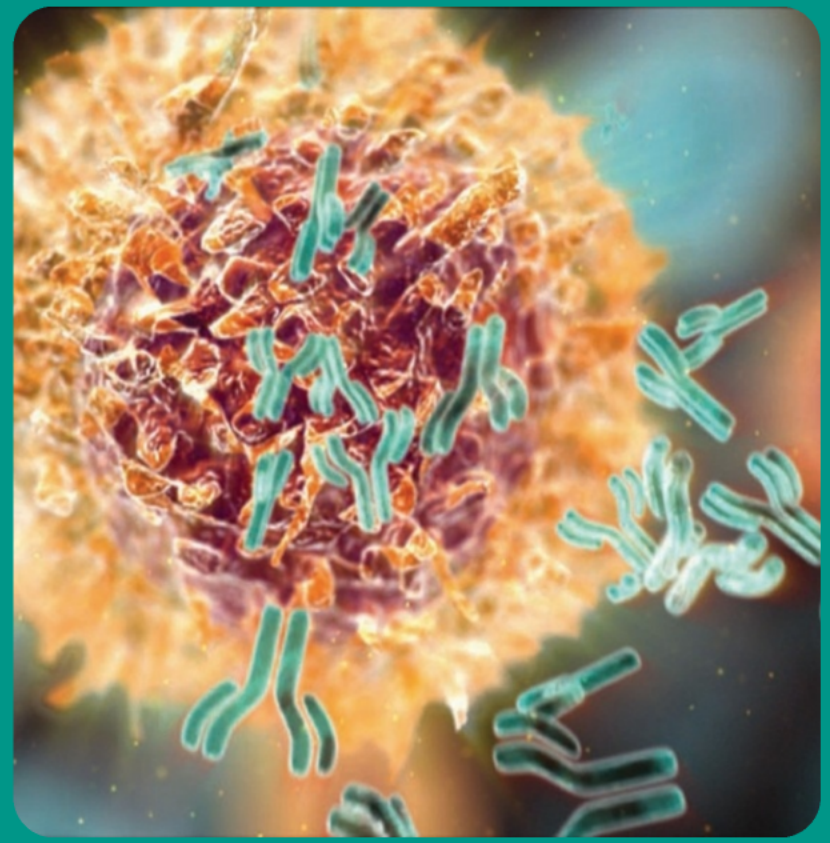
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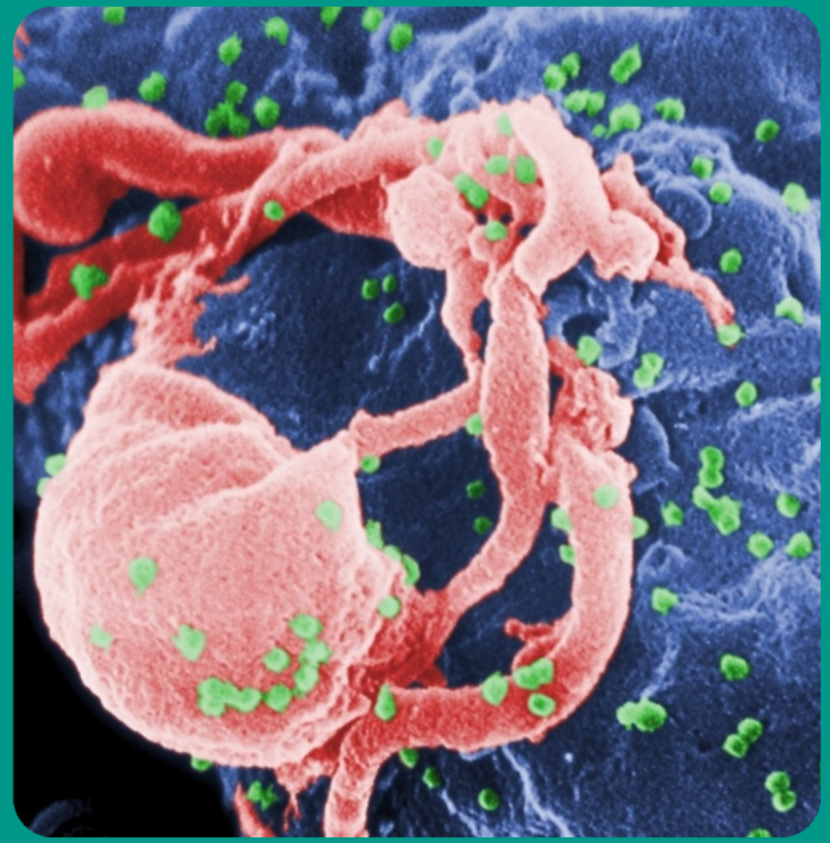
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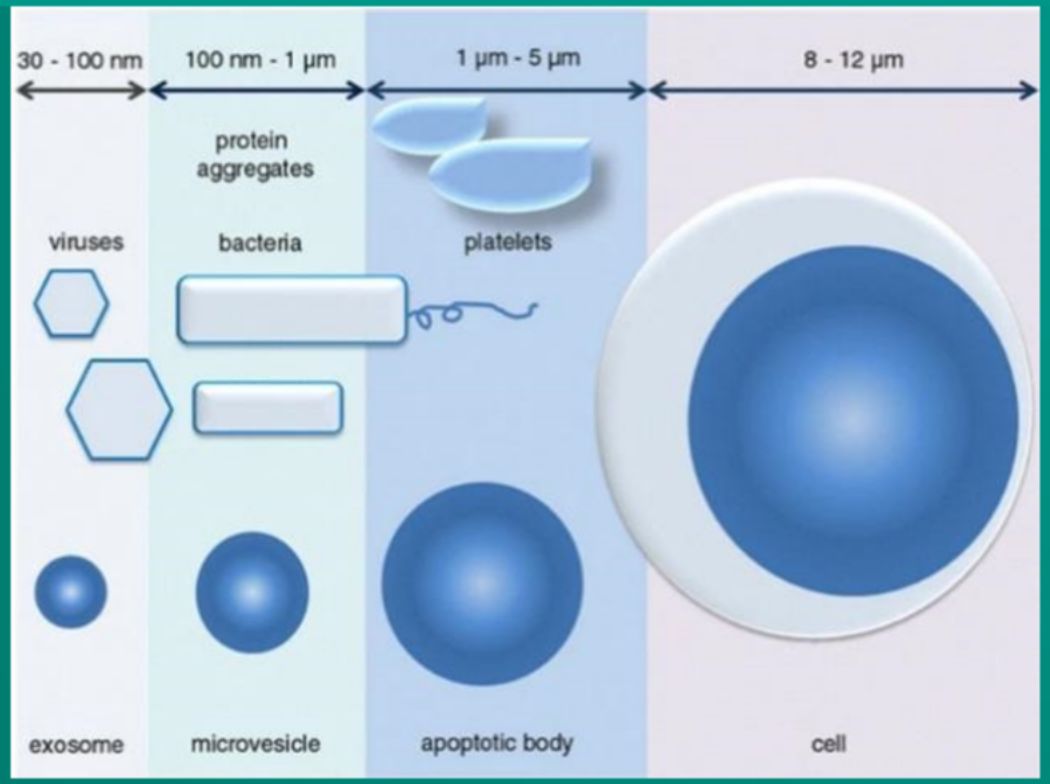
“

# Tiny, diverse, and elusive - EVs hide in plain sight.

## Small Size Complicates Detection

EVs: Nanoscale structures

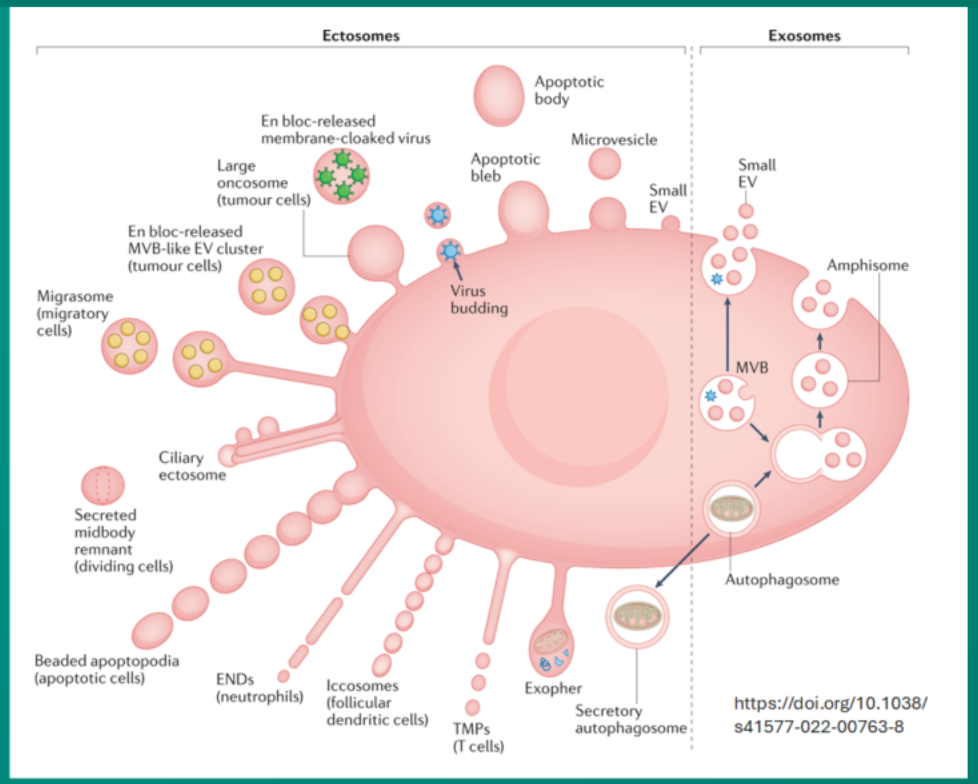
- Detection and characterization challenges
- Signal-to-noise issues
- Accurate characterization is crucial




## Heterogeneity Leads to Variability

EVs: Vary in size, content, and origin (Exosome ≠ EV)

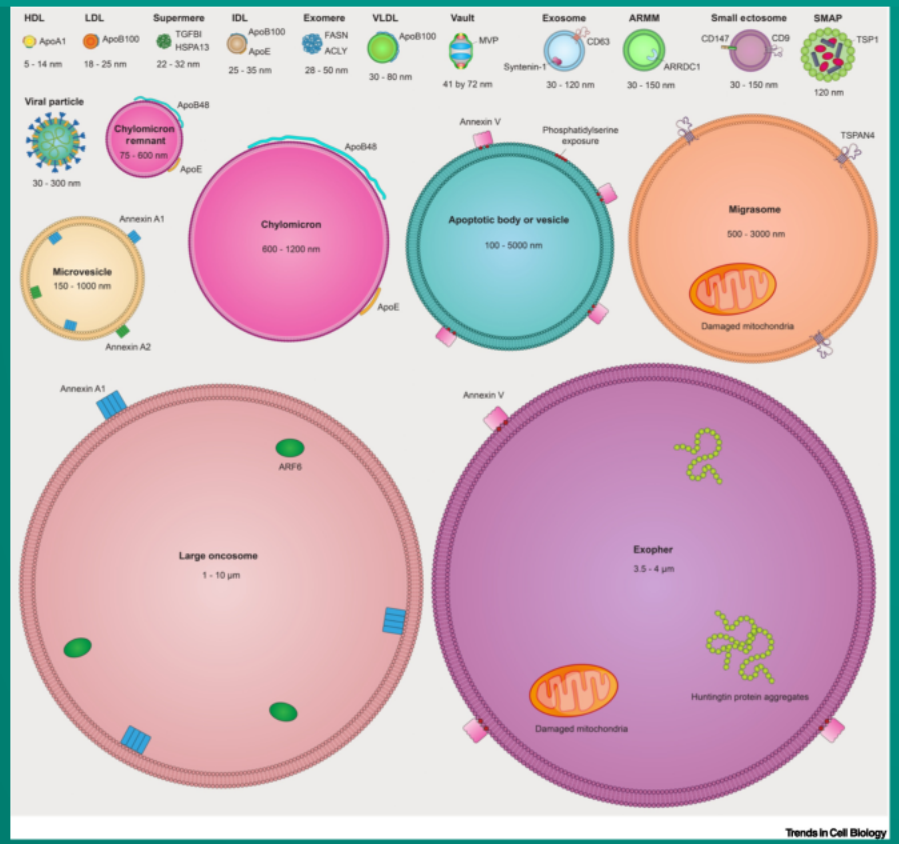
- This heterogeneity complicates research reproducibility
- Understanding EVs' biological roles and assessing their therapeutic potential is affected



# Low Abundance Requires Isolation

Non-vesicular extracellular particles (NVEPs):

- Much more abundant than EVs in body fluids
- EV enrichment needed in most cases
- Isolation issues - overlap of EV and NVEP properties

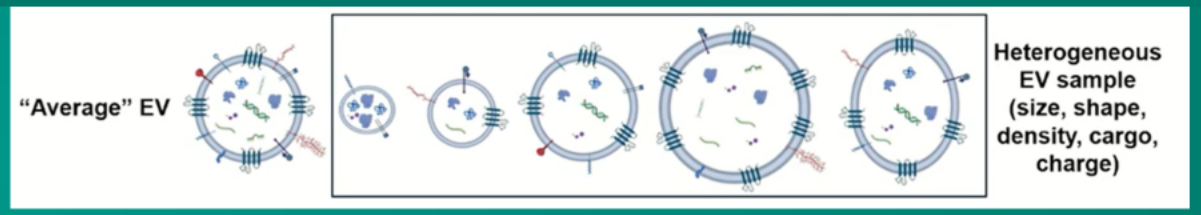


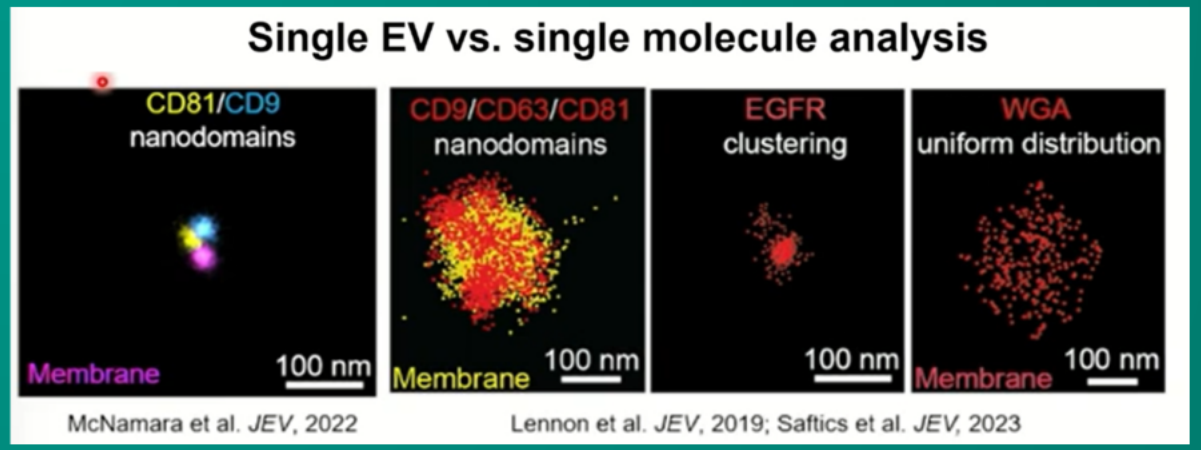

# Bulk vs. Single EV analysis

Bulk EV analysis: info about "average" state of sample

- Single-EV analysis: heterogeneity of individual EVs
- Single molecule analysis:

MISEV2023 guidelines for EV research and applications

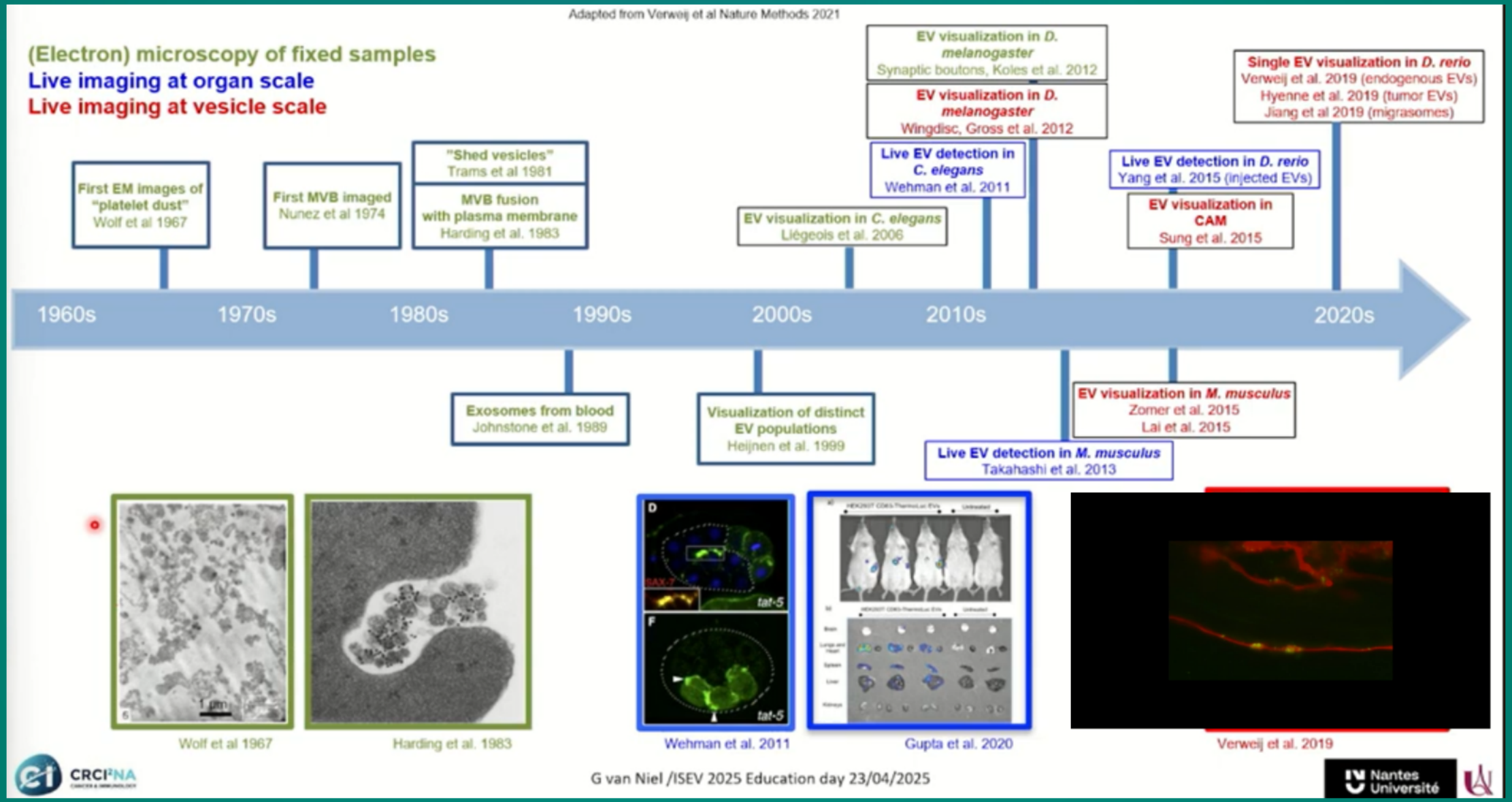


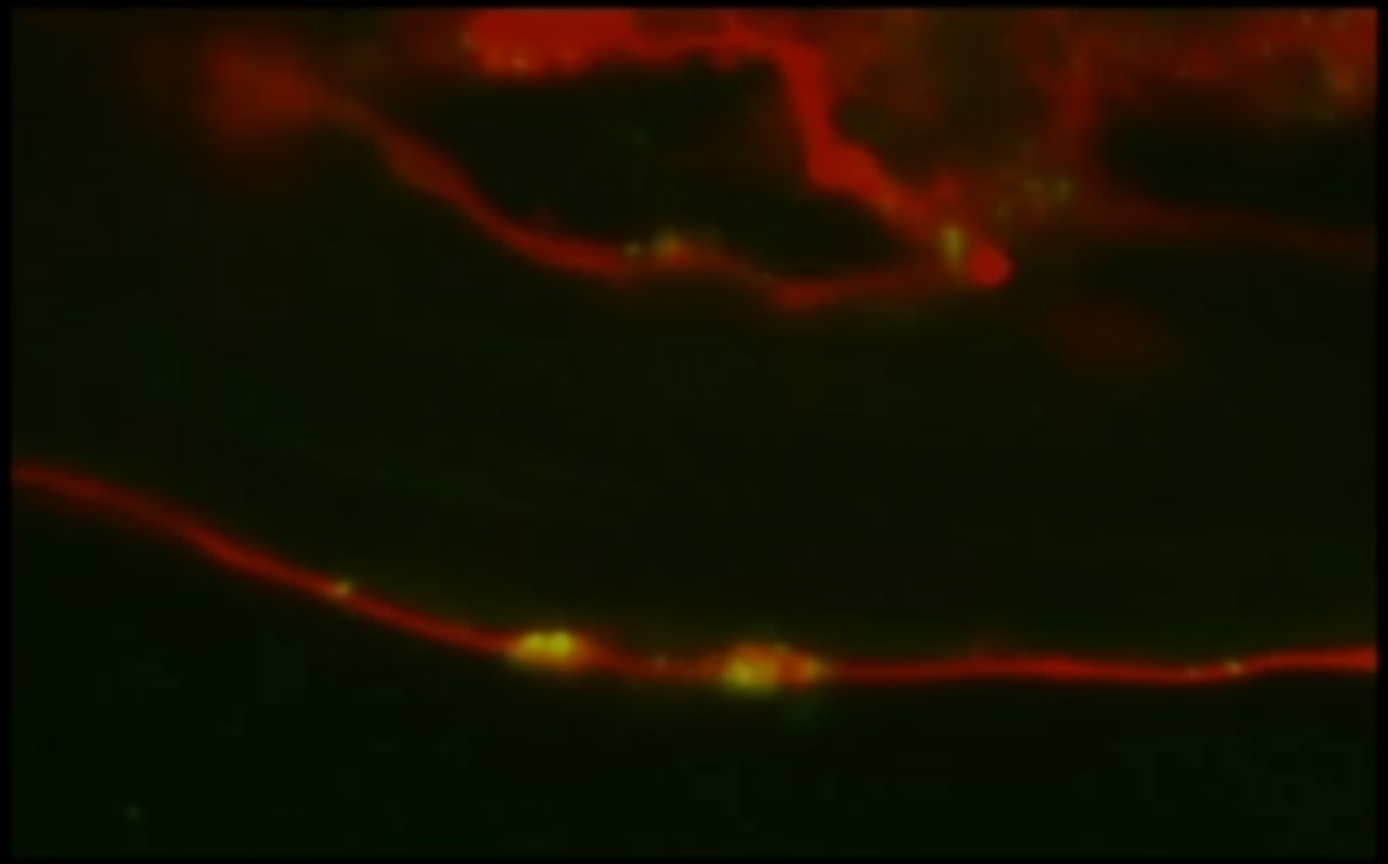




INTERNATIONAL SOCIETY for EXTRACELLULAR VESICLES
www.isev.org

MISEV2023
https://www.isev.org/misev2023

# Timeline of selected EV imaging milestones





# Overview of Imaging Techniques for EVs

Their unique contributions to understanding EV biology.

## Atomic Force Microscopy (AFM)

AFM enables label-free morphometry and nanomechanical profiling of EVs, offering insights into their structural properties.

## Fluorescence Microscopy

A range of fluorescence methods, from diffraction-limited to super-resolution, allow visualization of EV release, uptake, and molecular composition.

## Cryo-Electron Microscopy (Cryo-EM)

Cryo-EM uniquely preserves the native morphology of EVs, providing clear visualization of their double lipid bilayer, distinguishing them from lipoproteins.

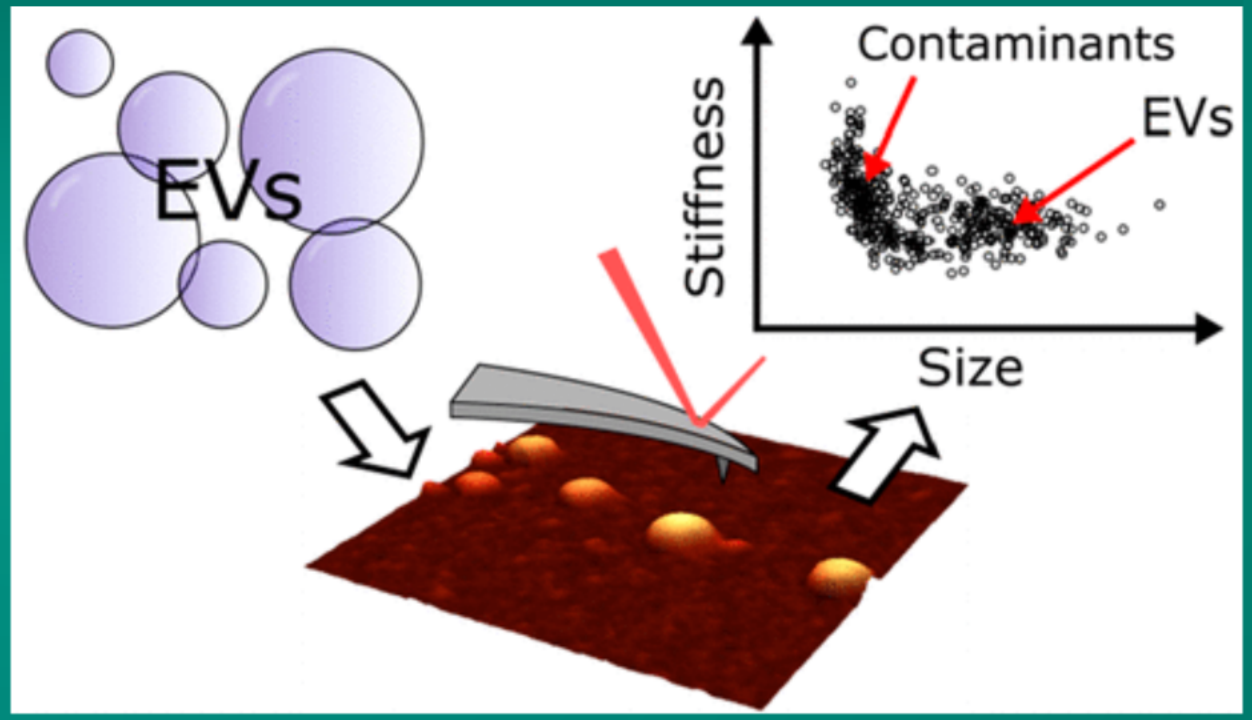
# Atomic Force Microscopy (AFM): Key Insights into EV Characteristics

AFM: Label-free EV analysis

- Detailed morphometry & nanomechanical profiling
- Liquid AFM allows native condition analysis
- Key properties: size & shape
- Insights into EV biological functions

Limitations:

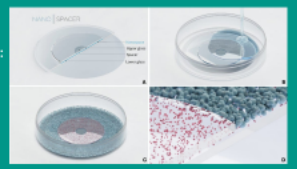
- Samples need to be immobilized
- Remains low-throughput (time-consuming); excels in detailed nanoscale characterization
- Best for specific, intricate studies, not broad investigations and in combination with other approaches



## Diffraction-Limited Imaging

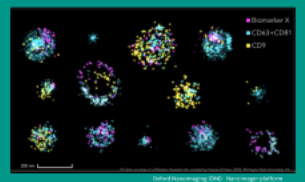
Diffraction-limited fluorescence microscopy:

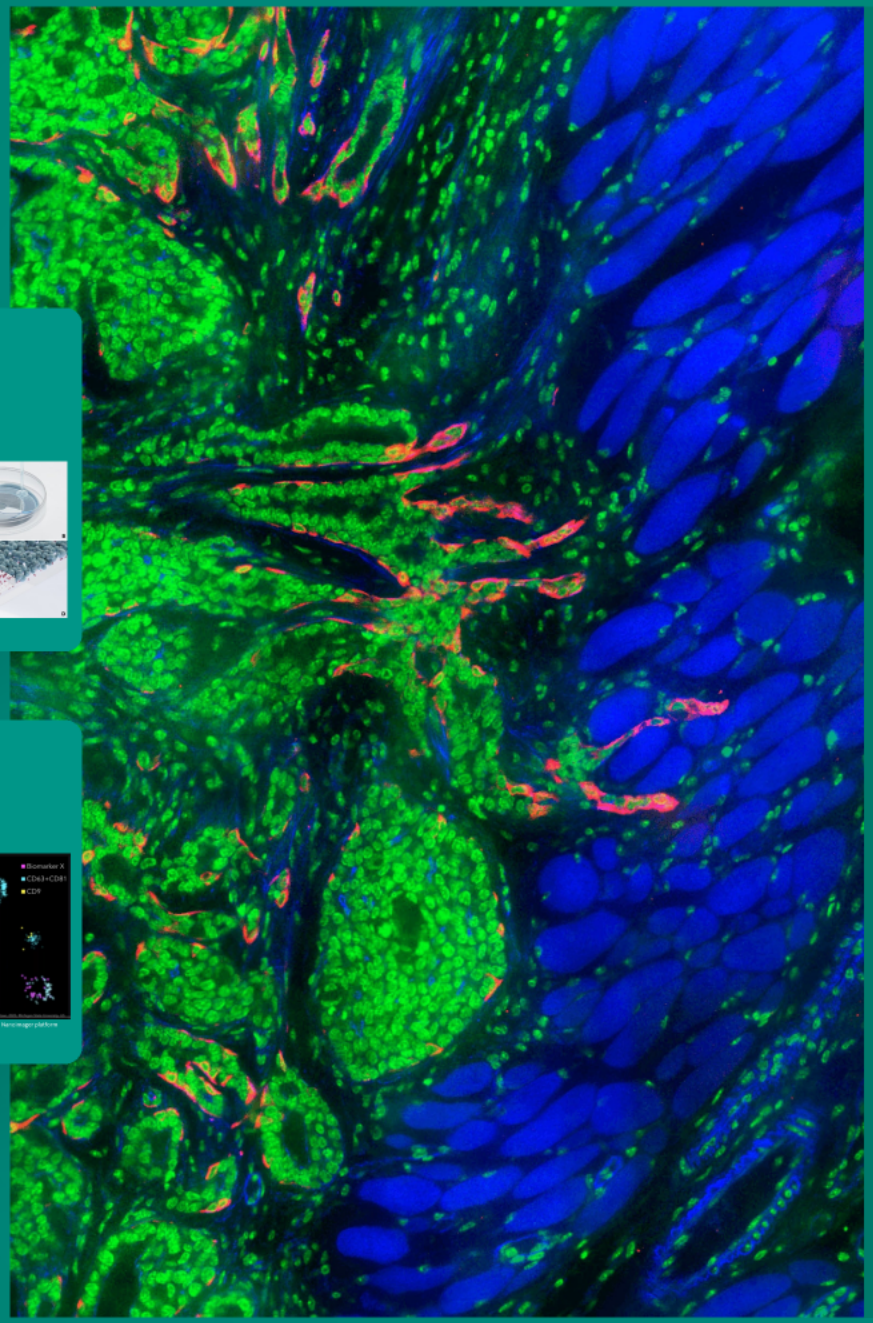
- Visualizes EVs in vivo
- Monitors release & uptake in live cells
- Uncovers spatial distribution during interactions
- Enhances understanding of EV dynamics



## Super-Resolution Techniques

- Enable the study of molecular composition of EVs - mostly STORM, STED, or PALM
- Single-particle tracking and live-cell imaging
- Limitations: need fo labelling (specificity of probes and the efficiency of labeling), which may alter EV behavior
  - the potential for photobleaching



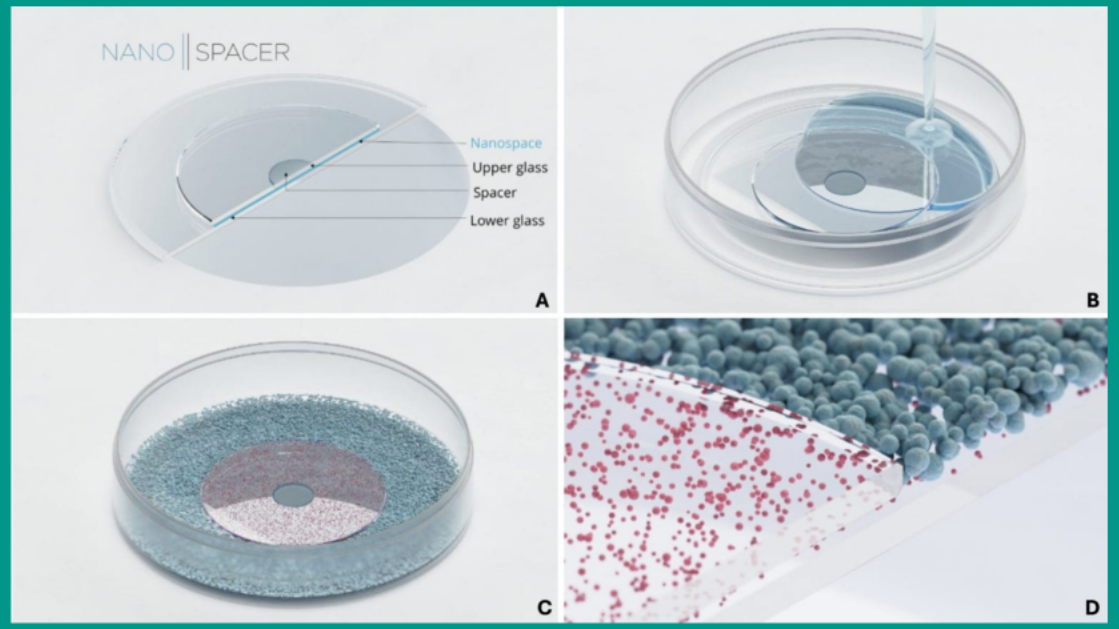


# Fluorescence Microscopy: Techniques and Applications

# Diffraction-Limited Imaging

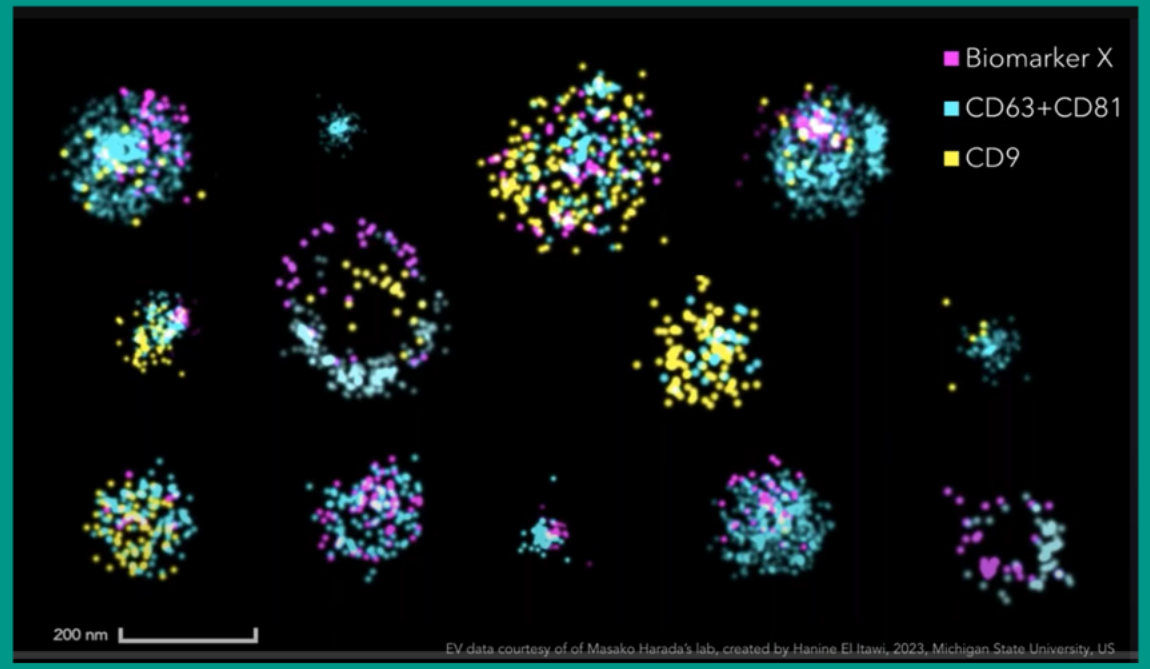
Diffraction-limited fluorescence microscopy:

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Oxford Nanoimaging (ONI) - Nanoimager platform

# Electron Microscopy: The Power of Cryo-EM



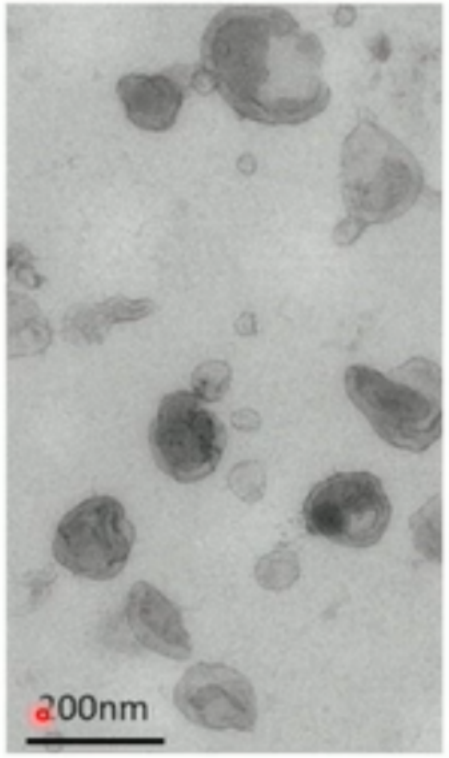
Cryo-Electron Microscopy

COre Facility CEITEC MU

- Preserves native morphology
- Visualizes double lipid bilayer
- Distinguishes from lipoproteins
- Essential for EV characterization
- Enhances understanding of EV roles

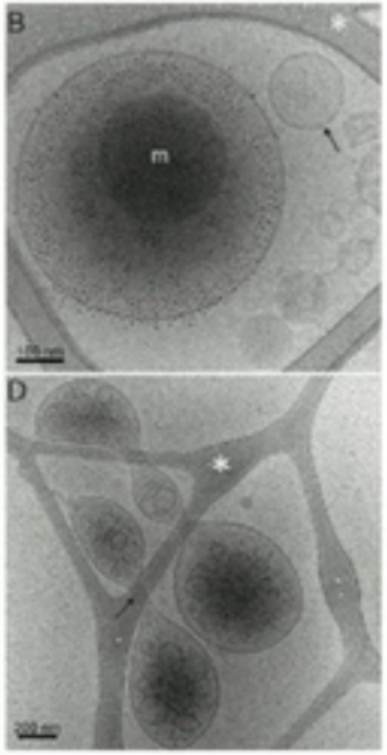
# SINGLE EV ELECTRON MICROSCOPY

## Transmission EM (TEM)



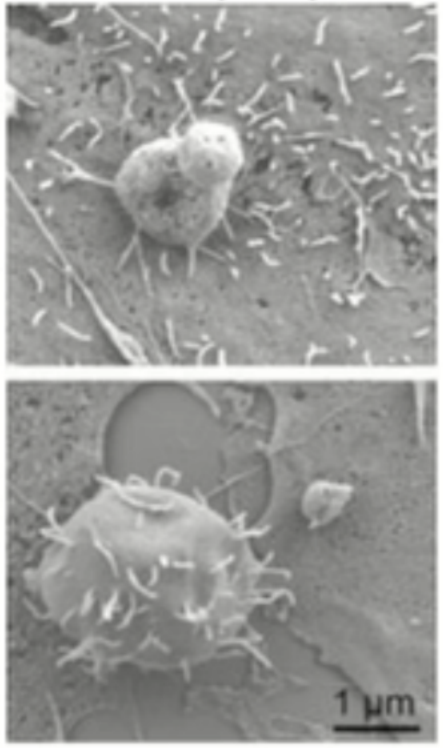

G. Raposo/G van Niel

## Cryo-EM



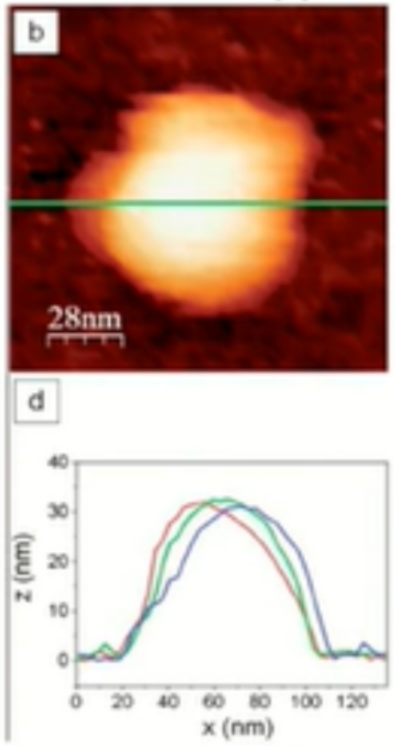

A. Brisson

## Scanning EM (SEM)




K. Rilla

## Atomic Force Microscopy

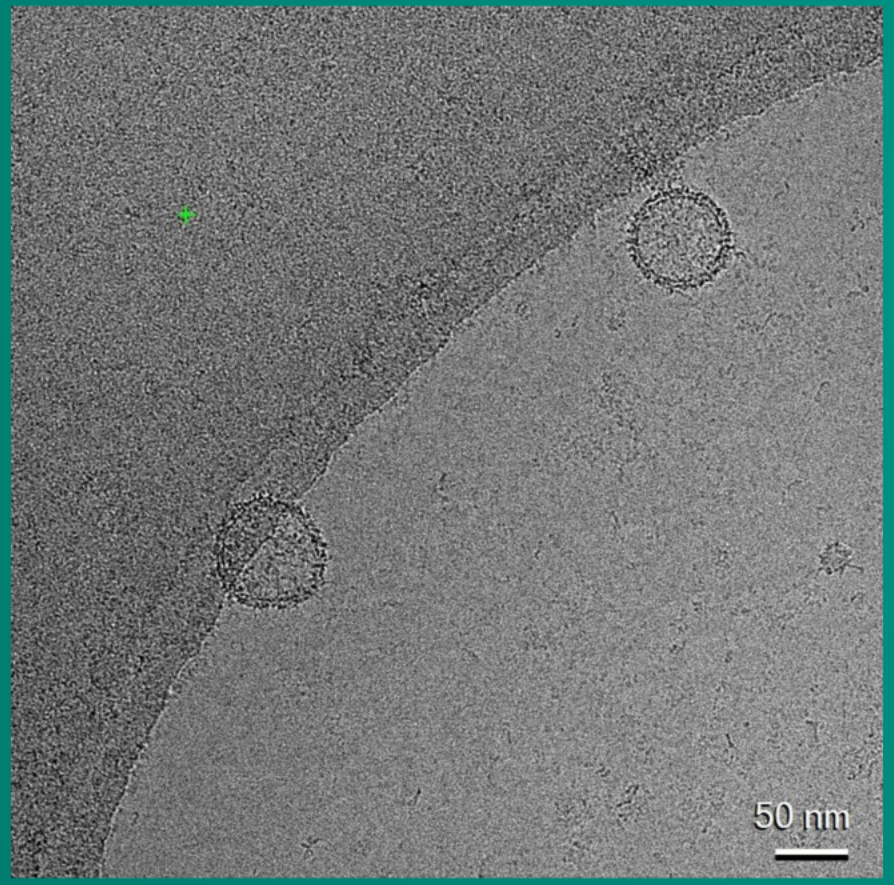


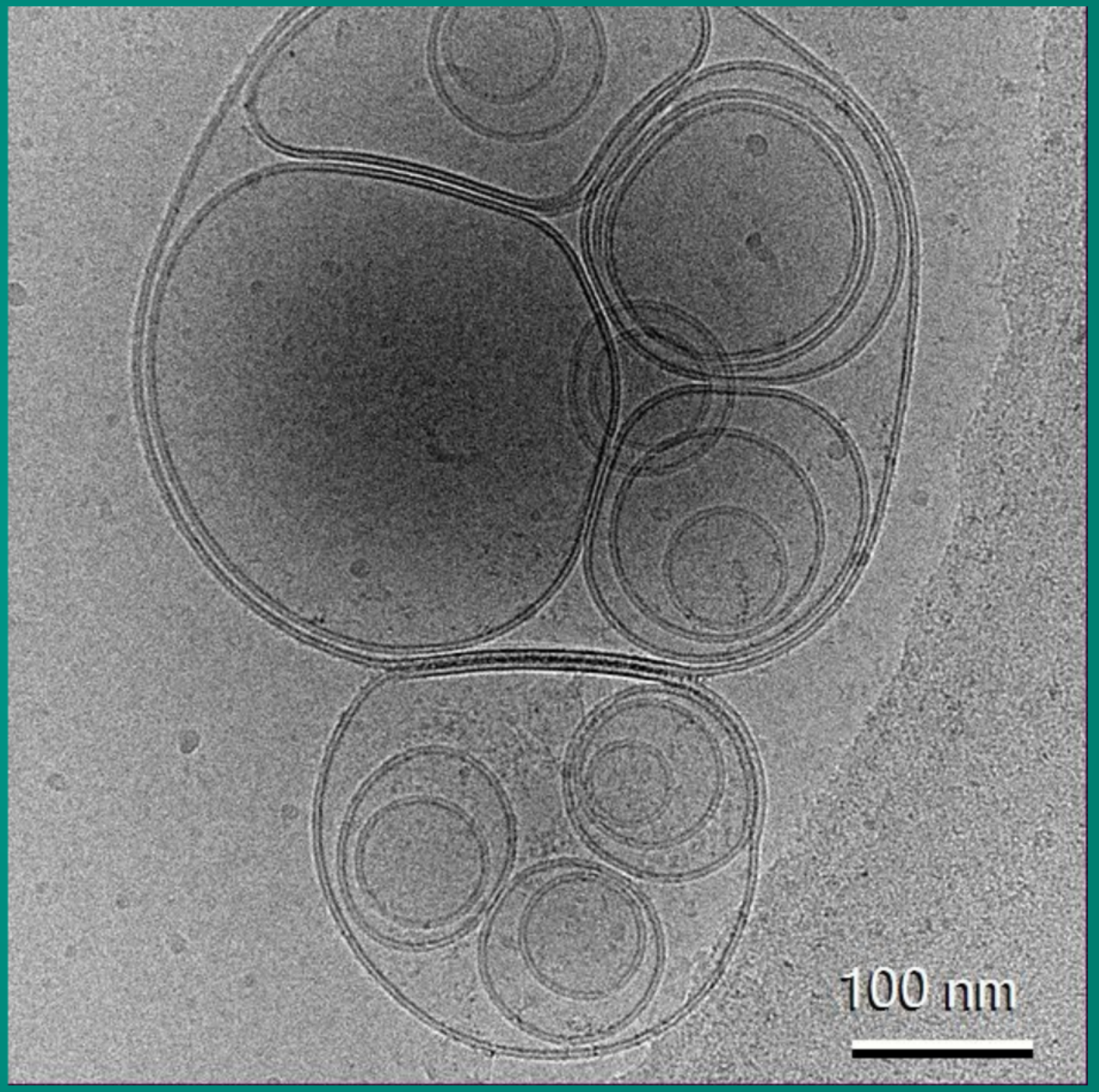

W. Roos

| | | | |
|---|---|---|---|
| Chemical Fixation | Snap Freezing | Chemical Fixation | No Fixation |
| Few nm | Less than 1nm | 5-10 nm | Few nm |
| Labeling +++ | Labeling + | Labeling + | Labeling ? |
| Isolated and in situ EV | Isolated EV | Isolated and in situ EV | Isolated EV |

**EV Definition:**

- particles released from cells
- delimited by a lipid bilayer
- cannot replicate on their own



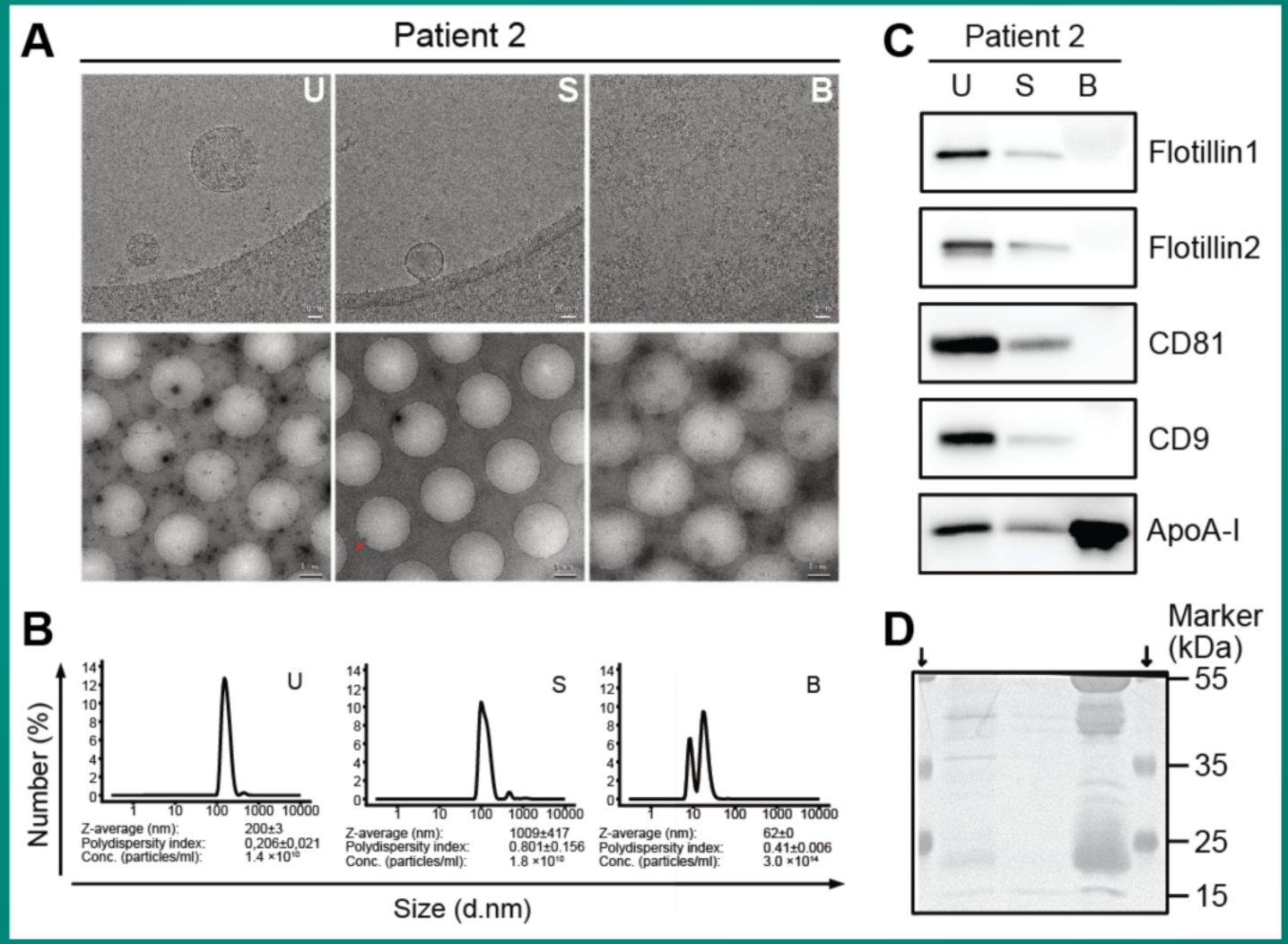
# Cryo-EM is complementary to other EV characterisation methods

A) Cryo-Electron Microscopy - shape and contaminants

B) Dynamic Light Scattering (DLS) - size and concentration

C) Western Blotting (WB) - EV markers and "negative" markers

D) Total protein staining - highly abundant serum proteins and lipoproteins in B



# Take home messages

- EVs = active messengers, not debris, lipid bilayer membrane
- Diagnostics: non-invasive biomarkers of disease
- Therapeutics: engineered delivery or intrinsic healing (e.g., MSC-EVs)
- Challenge: nanoscale size & heterogeneity
- Solution: multimodal imaging — with cryo-EM as structural gold standard

> **Seeing is believing**

# Thank you!

Laboratory for
Ovarian Cancer &
Extracellular Vesicles

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MUNI MED | InGA Interní Grantová Agentura

NATIONAL INSTITUTE FOR CANCER RESEARCH

- To all collaborators and patients

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