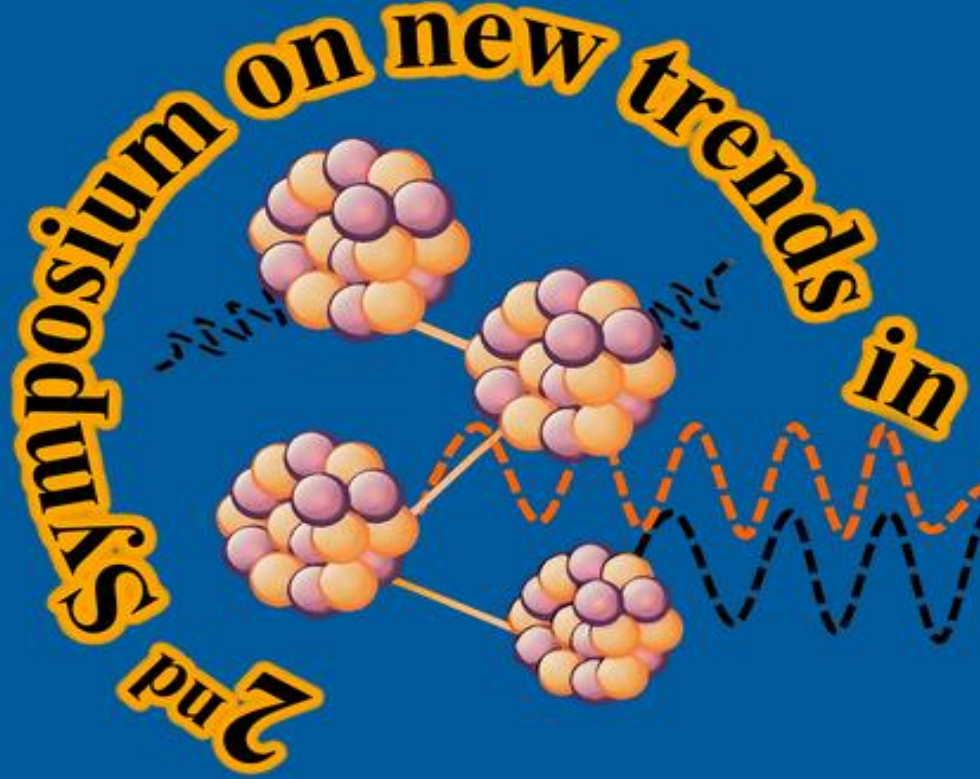

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RESEARCH UNIVERSITY

24-26 September 2025

Nuclear and Medical Physics

Faculty of Physics, Astronomy and Applied Computer Science,
Jagiellonian Universityin Krakow, Poland

Lipid Remodelling in Extracellular Vesicles from β-Cells under Hyperglycemic Stress - Multimodal Mass Spectrometry Approach

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Gdańsk University of Technology

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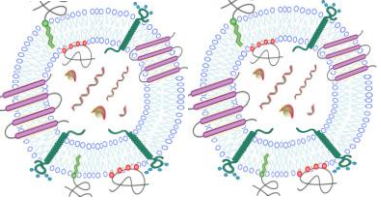
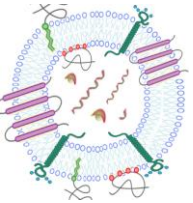
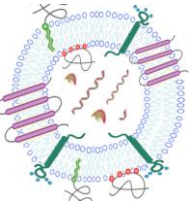
Background & Rationale





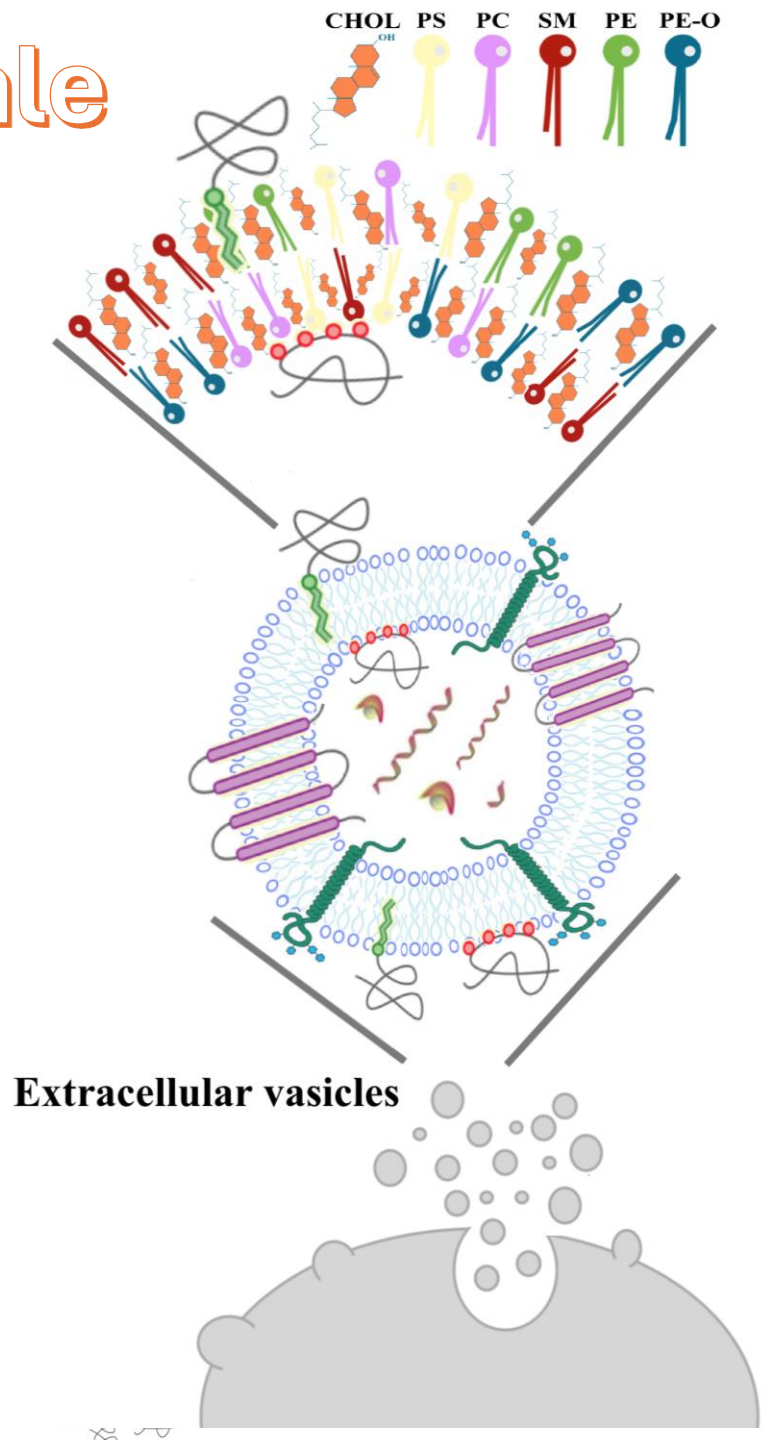


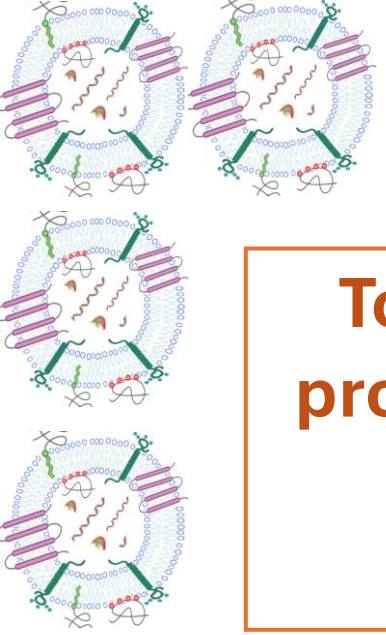
EXTRACELLULAR VESICLES (EVS) AS BIOMARKERS

EVs are spherical structures surrounded by a lipid bilayer and contribute to a variety of functions in biological systems.
Their basic classification distinguishes three subpopulations based on their size and biogenesis:

- exosomes (50–150 nm) = SMALL EVs,
- ectosomes (100–1000 nm) = LARGE EVs
- apoptotic bodies.

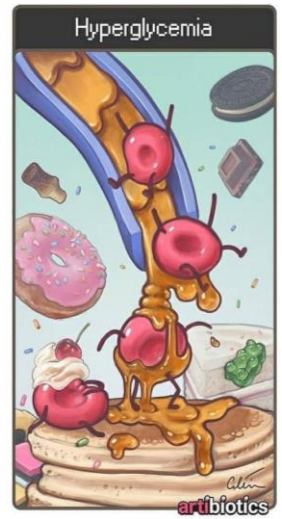
EV content varies according to the cell or organ of origin and the microenvironment at the time of their generation, determining in this manner their fate and biological activity.

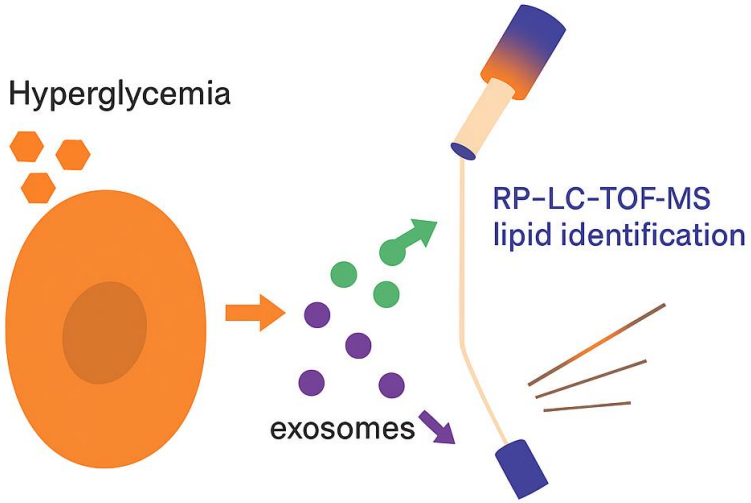


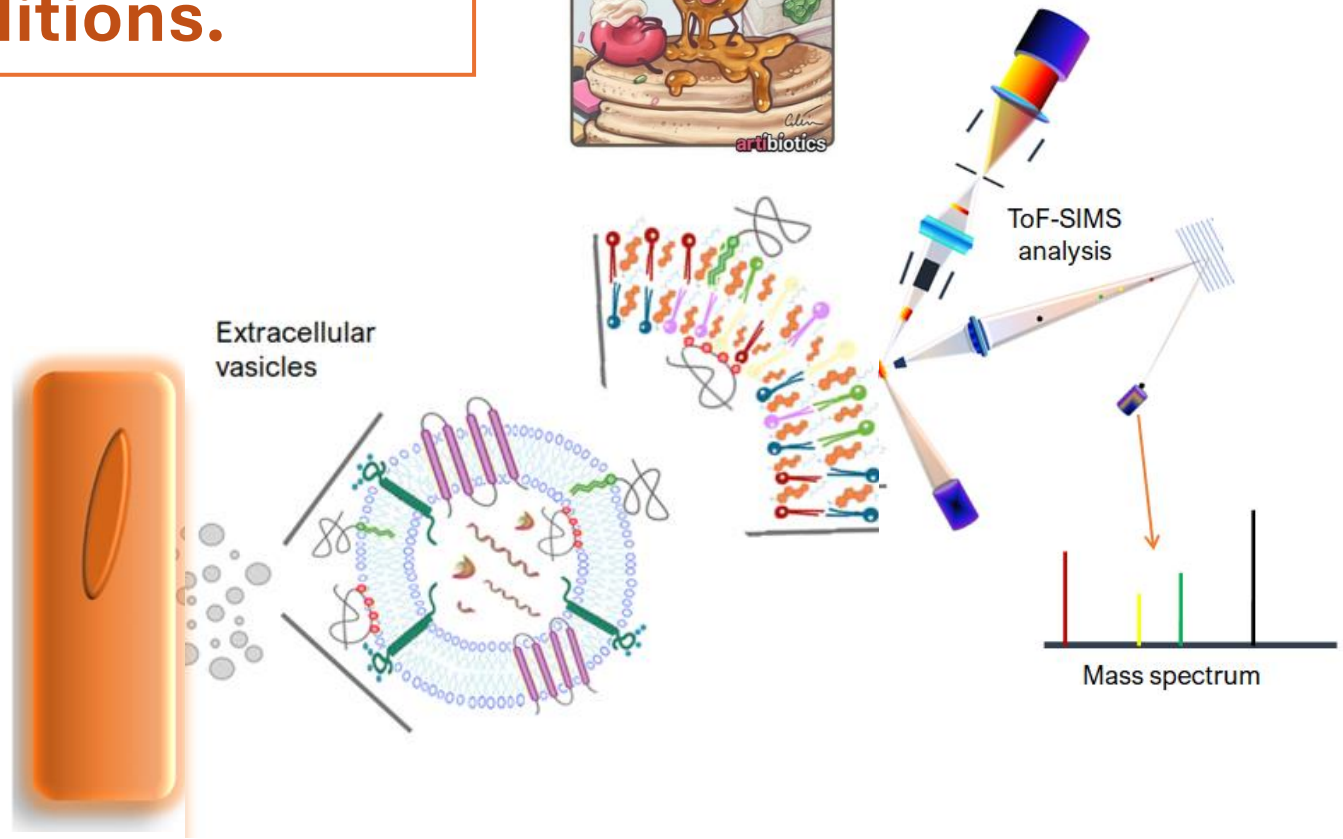


Impact of hyperglycemia on β-cell EV lipid composition

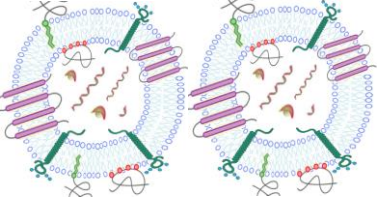
To compare and discover molecular profile changes of EVs subpopulations derived from cells culltured in hyperglycemic conditions.

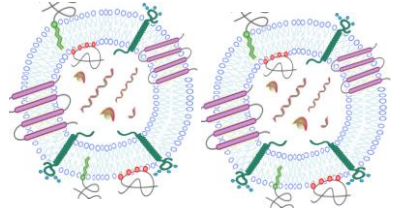


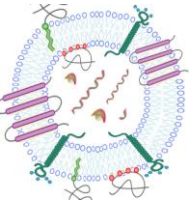




Analytical Techniques

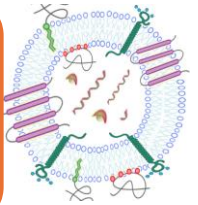






Reversed-Phase Liquid Chromatography – Quadrupole Time-of-Flight Mass Spectrometry
(RP-LC-Q-TOF-MS)
high-resolution lipid identification

Time of Flight – Secondary Ion Mass Spectrometry
direct surface mapping of lipids



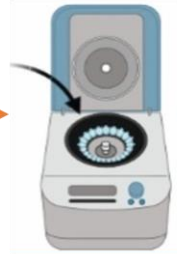
	RP-LC-Q-TOF-MS	ToF-SIMS
Purpose	High-resolution lipid ID	Surface mapping of lipids
Advantages	Accurate mass, broad lipid coverage, quantitative	Sub-micron imaging, minimal prep, label-free
Limitations	Requires extraction, no spatial info	Semi-quantitative, fewer intact lipids

Experimental Design

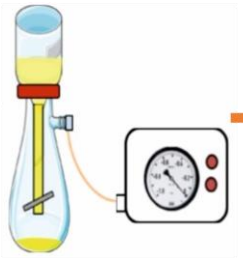
I STEP

Extracellular Vesicle Isolation

MEDIUM SAMPLE



SPINNING
3350 x *g*
30 MIN, 21 °C



LOW-PRESSURE FILTRATION

ULTRAFILTRATION
150 000 *x g*, 1.5H, 4 °C

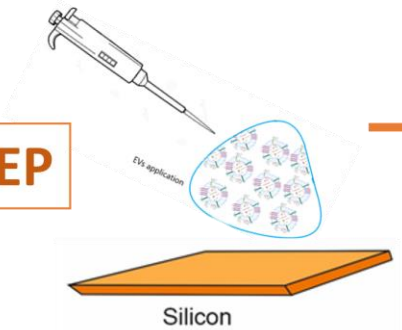
EXTRACELLULAR VESICLES (EV) SAMPLE

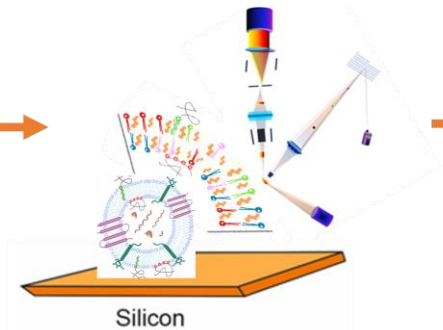
II STEP

EXTRACELLULAR VESICLES (EV)

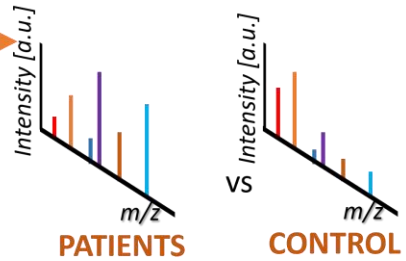
EVs characterization by microBCA colorimetric test and qNano apparatus

III STEP

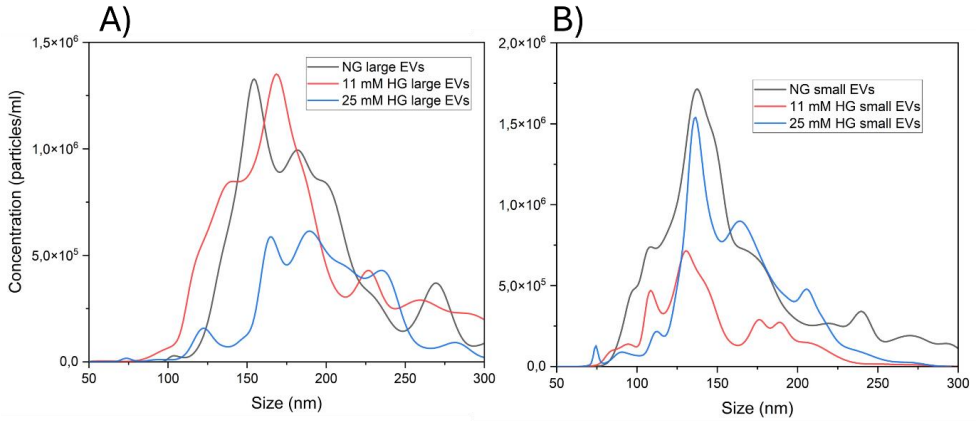


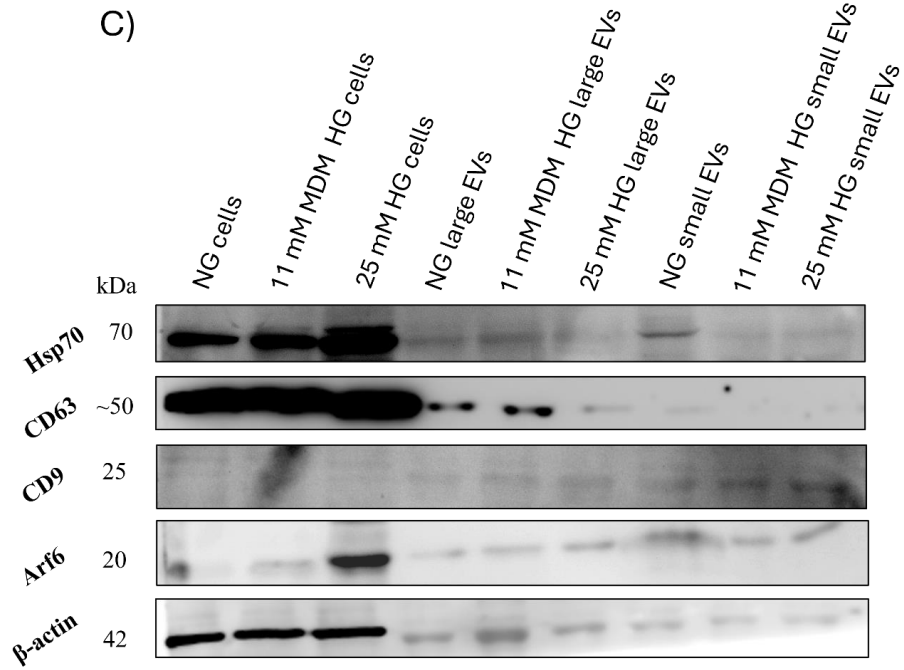


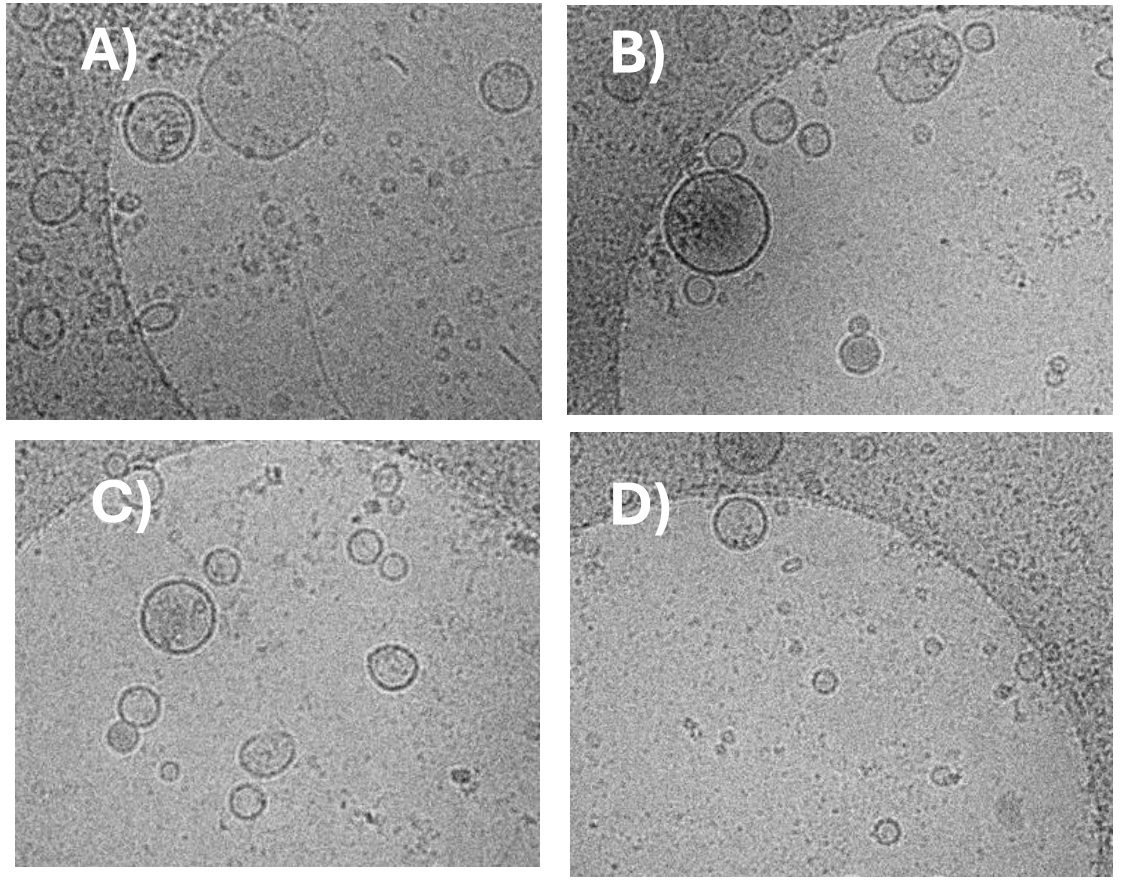
ToF-SIMS comparative analysis



RESULTS – Evs characterisation





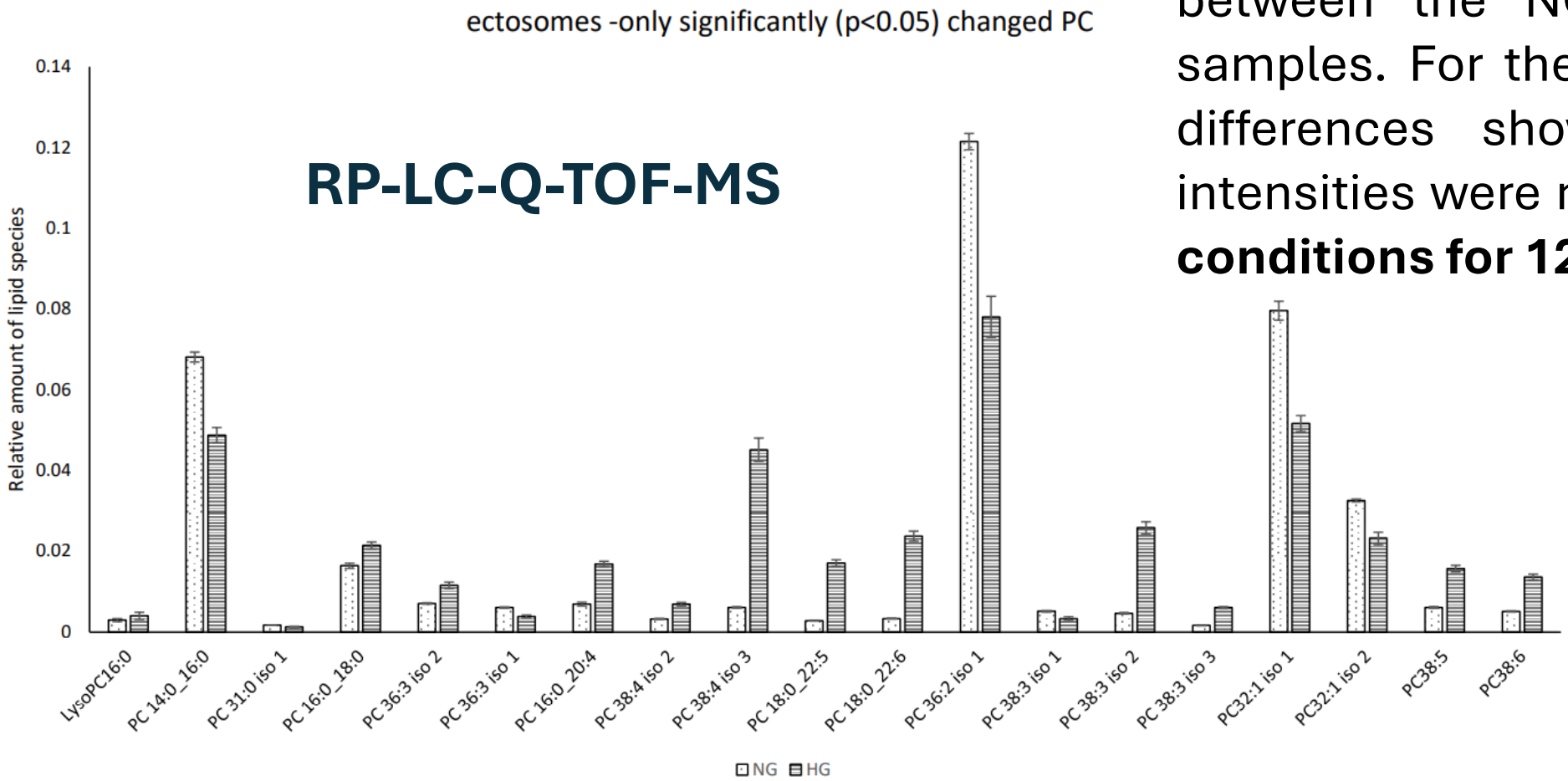


The cryo-TEM image of A) large HG-EVs, B) large NG-EVs, C) small HG-EVs, D) small NG-EVs.

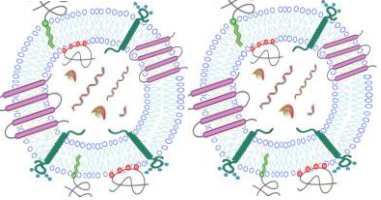
ECTOSOMES

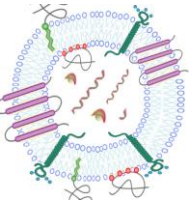
Of the 28 analysed PCs, 19 were statistically different between the NG and HG samples. For the significant differences shown, higher intensities were noted in **HG conditions for 12 lipids.**

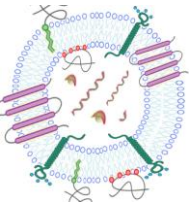
RP-LC-Q-TOF-MS

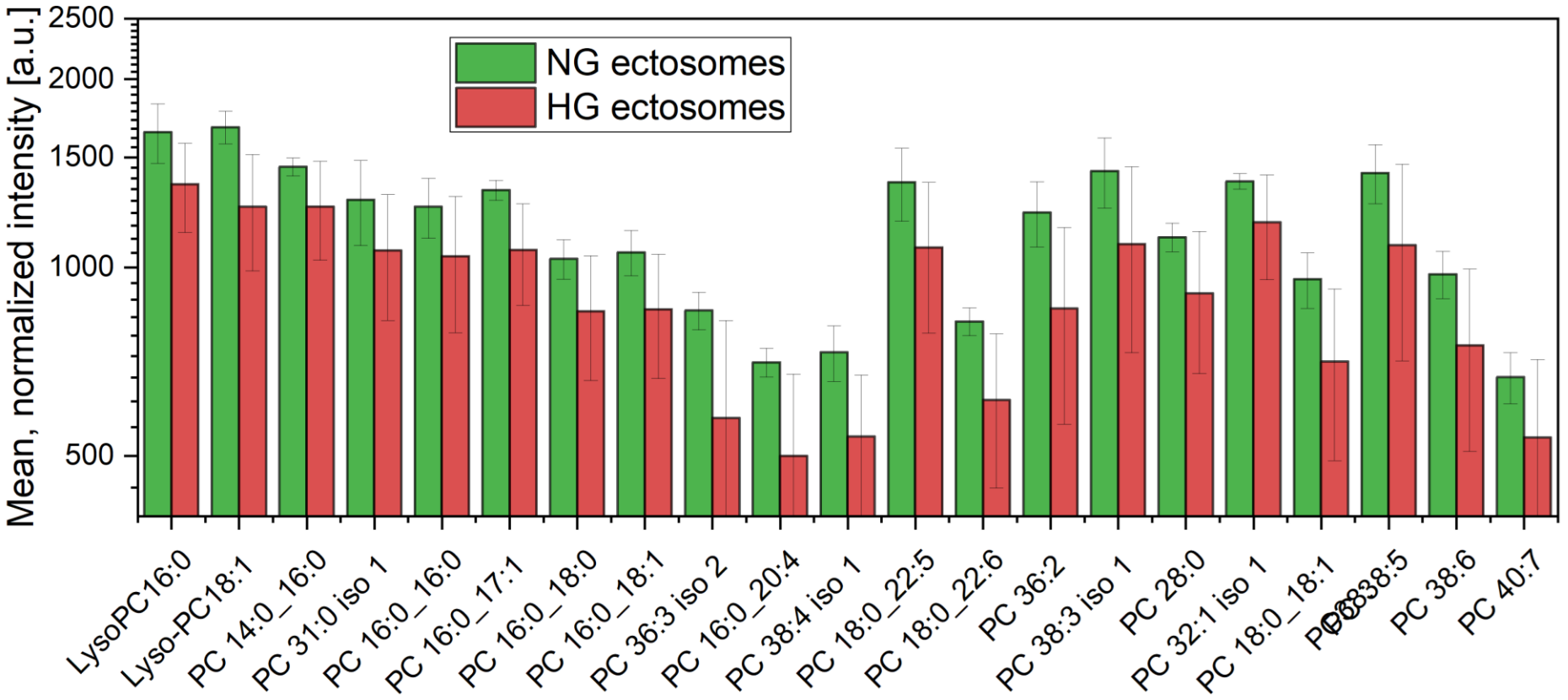


Comparison of PCs in NG and HG conditions. The error bars indicate SD corresponding to 3 independent biological replicates.

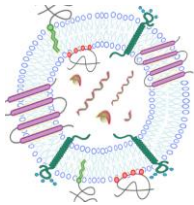


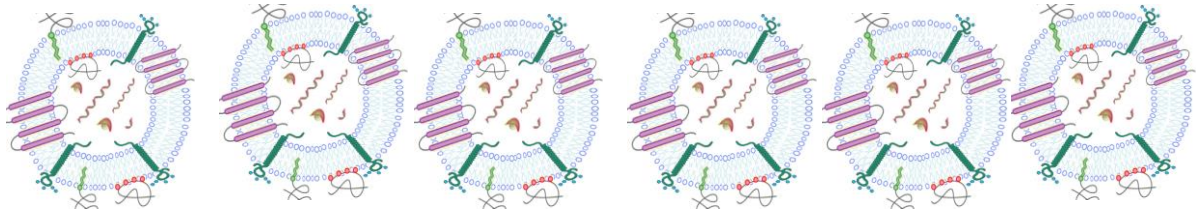






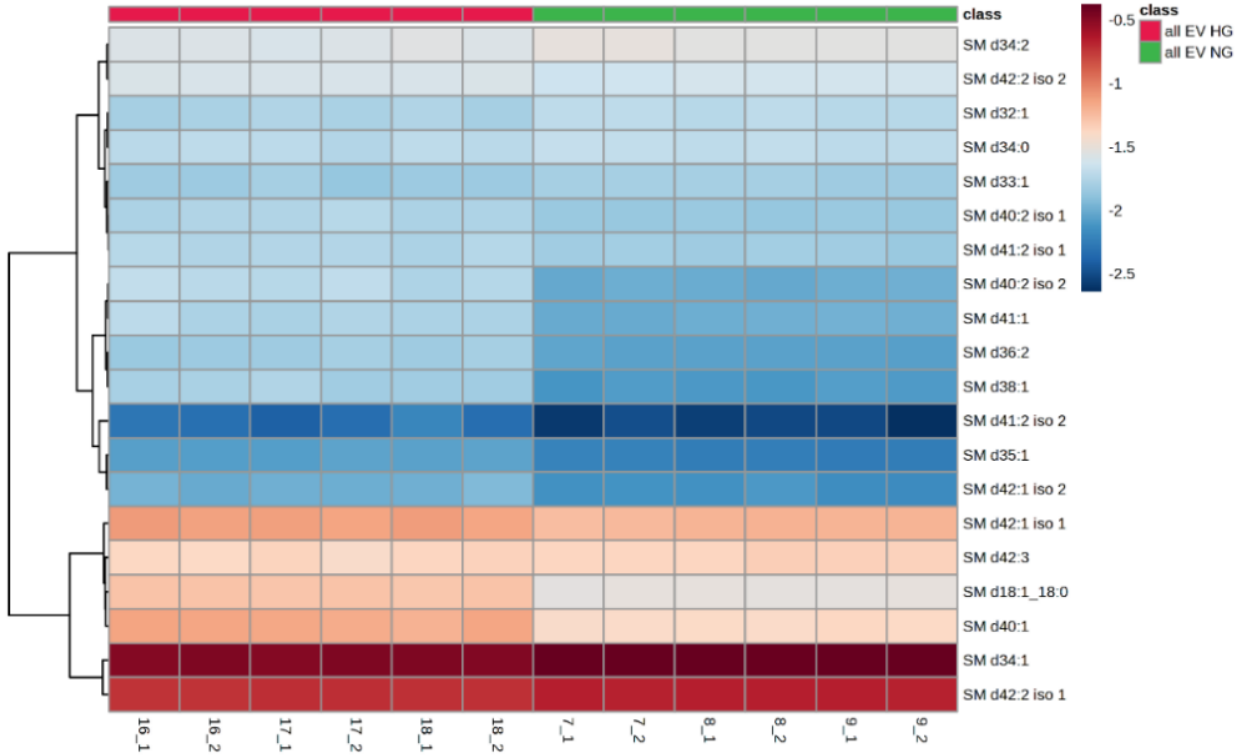
There are no statistical changes in the ectosome population.



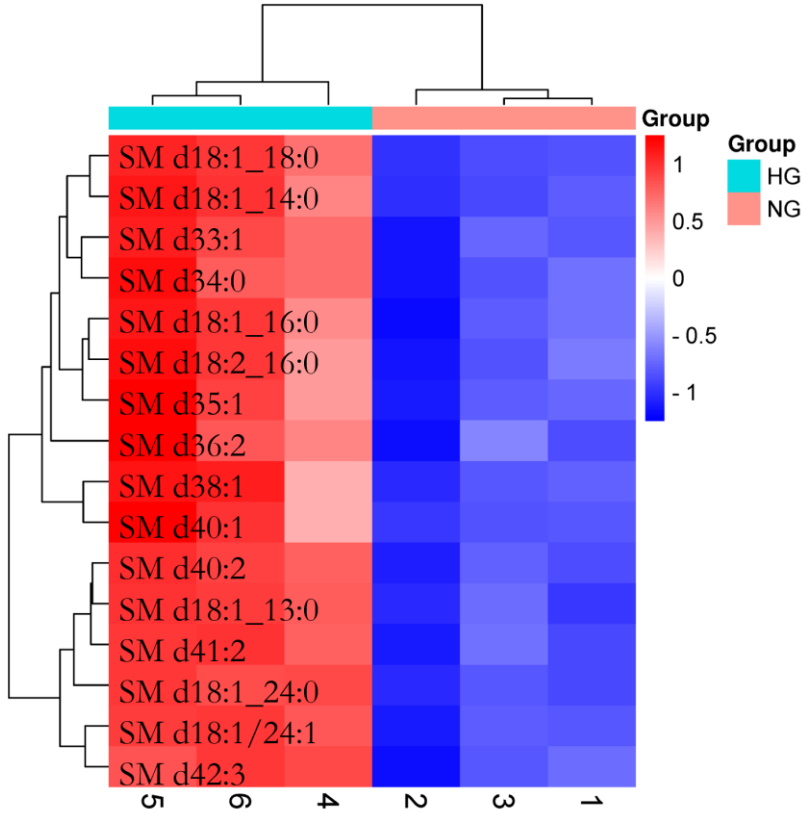


Heatmaps presenting the sphingomyelins (SM) lipid profile in NG and HG conditions

RP-LC-Q-TOF-MS

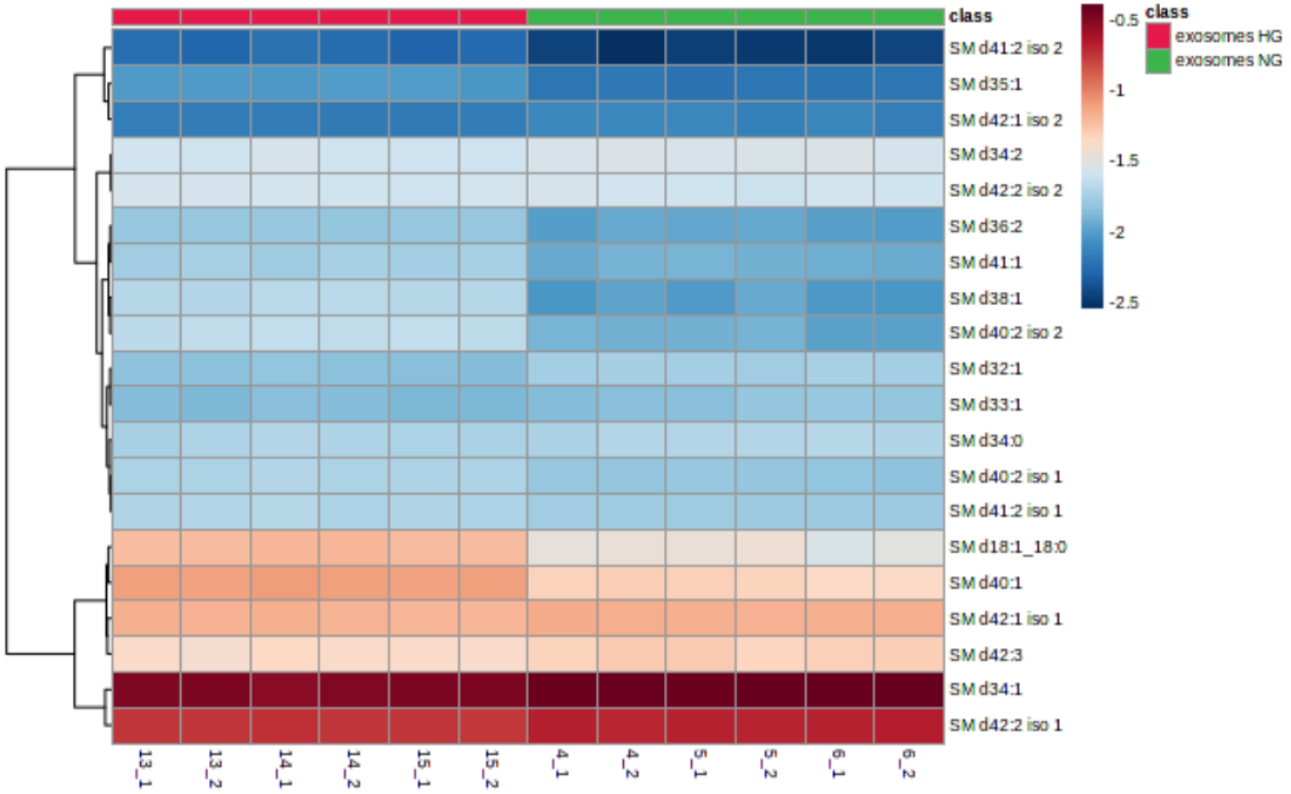


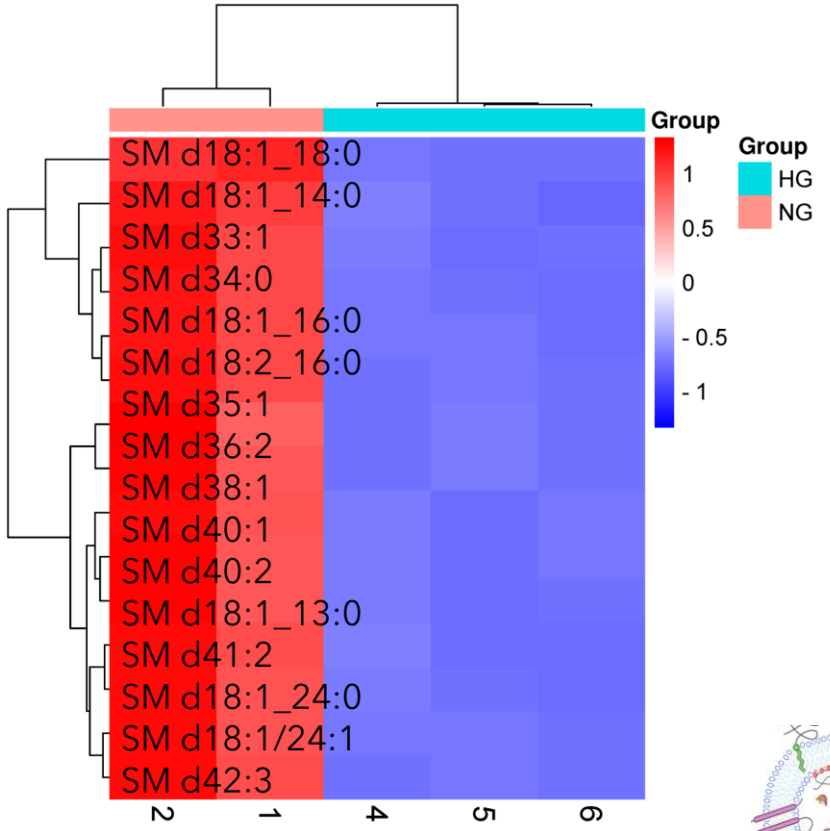
ToF-SIMS



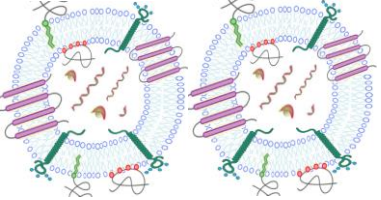
ectosomes

Heatmaps presenting the sphingomyelins (SM) lipid profile in NG and HG conditions

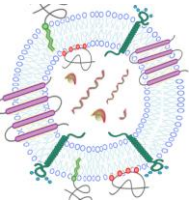




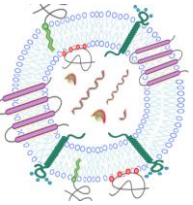
exosomes



CONCLUSION



Exosomes – RP-LC-Q-TOF-MS revealed significant increases in **8/20 sphingomyelins** and **19/28 glycerophospholipids** under hyperglycemia (HG). ToF-SIMS showed the same trend for surface SM but no major structural changes.

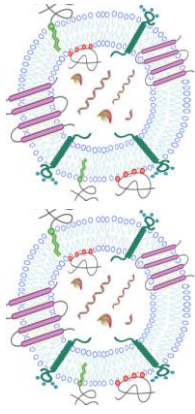


Ectosomes – LC-MS detected changes in **10/20 sphingomyelins** and **19/28 phosphatidylcholines** (12 higher in HG). ToF-SIMS again showed **no significant surface differences**.

Overall Insight

Hyperglycemia enriches the internal lipid pool of EVs (especially SM and PC) **while leaving the outer membrane largely unchanged**.

Take Home Message **Multimodal analysis** is essential:
– **LC-MS/MS** provides detailed molecular profiles.
– **ToF-SIMS** maps surface composition.
Together they reveal **bulk lipid remodelling without surface disruption**, highlighting EV lipids as potential **biomarkers of metabolic stress**.



THANK YOU FOR YOUR KIND ATTENTION

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