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NANO IN BIO 2022
Nanosciences for Life & Materials Sciences

Extracellular vesicles: grounds for hope in disease diagnostics and therapy

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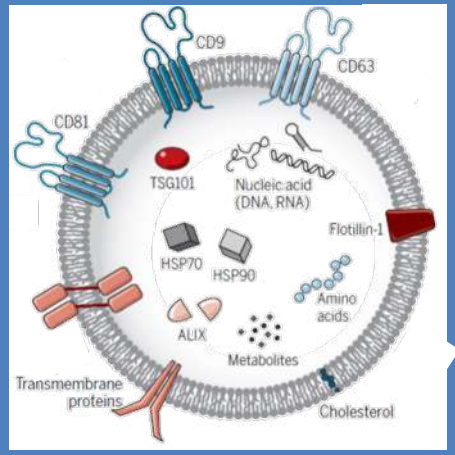
NanoInnovation Lab



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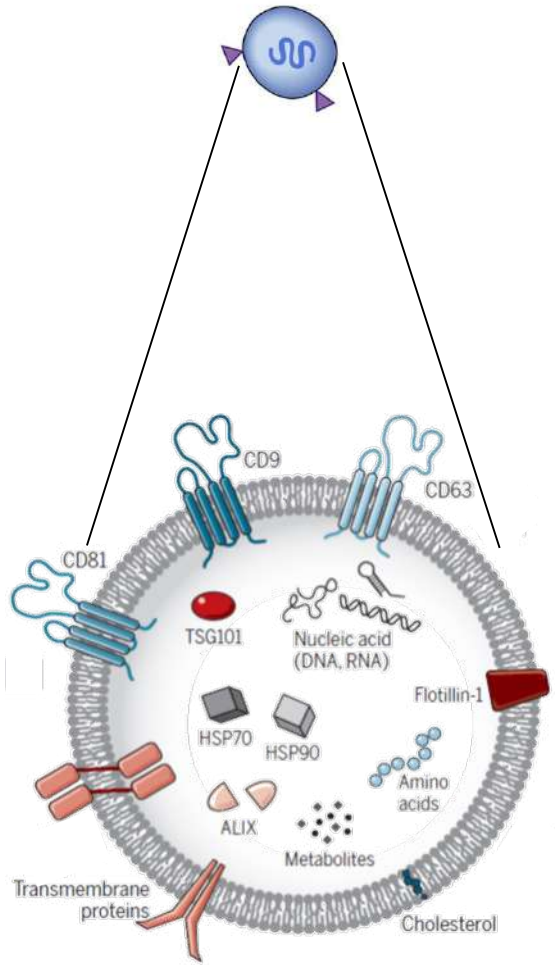
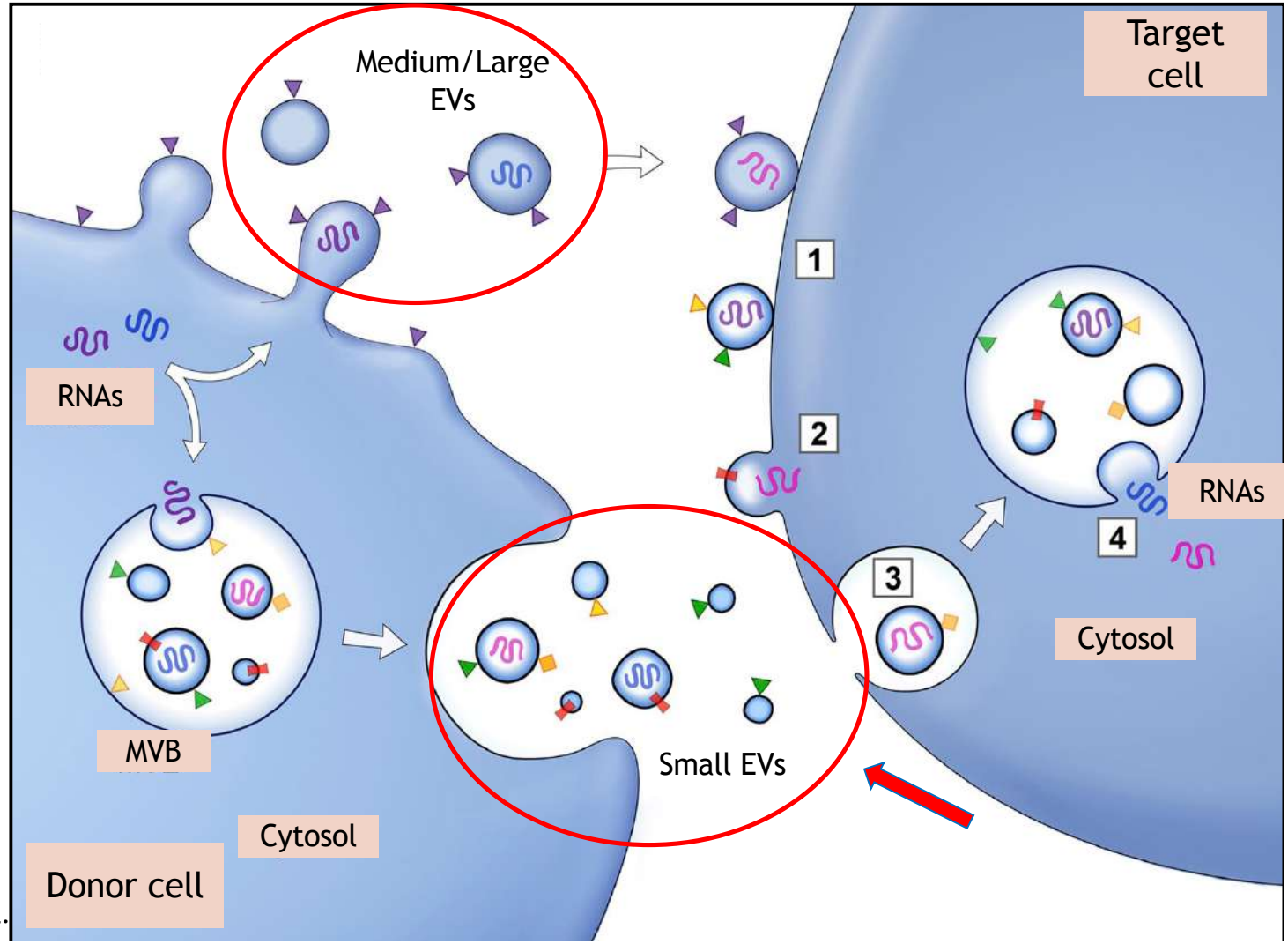
Extracellular Vesicles (EVs)

	m/l-EVs	s-EVs
Size (nm)	1000 ÷ 200	200 ÷ 30

ROLE
Transport of macromolecules throughout the body





Modified from Raposo et al. 2013, J Cell Biol.

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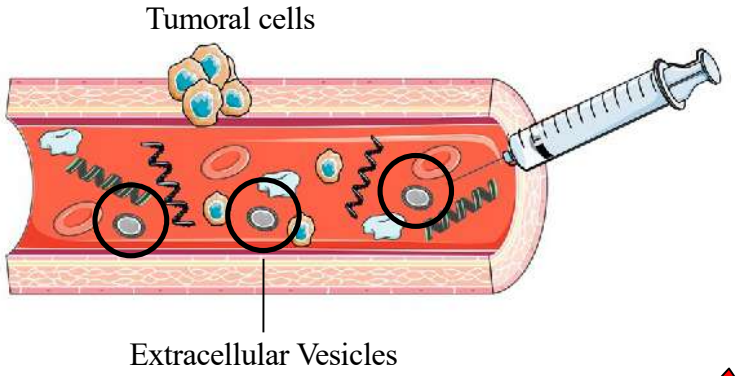
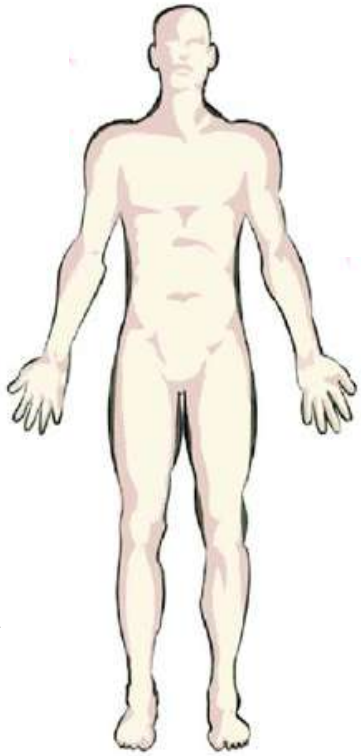
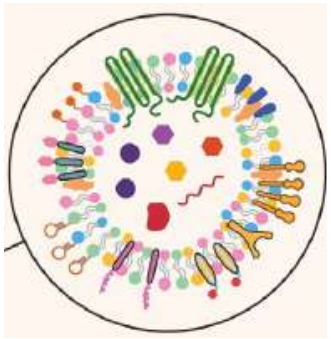
Small EVs in cancer

Diagnostics





Therapy



Specific signatures from originating cells

- Biomarkers

Influence on the fate of recipient cells

- Involved in disease spreading
- Therapeutic agents (regenerative medicine, drug delivery, ...)

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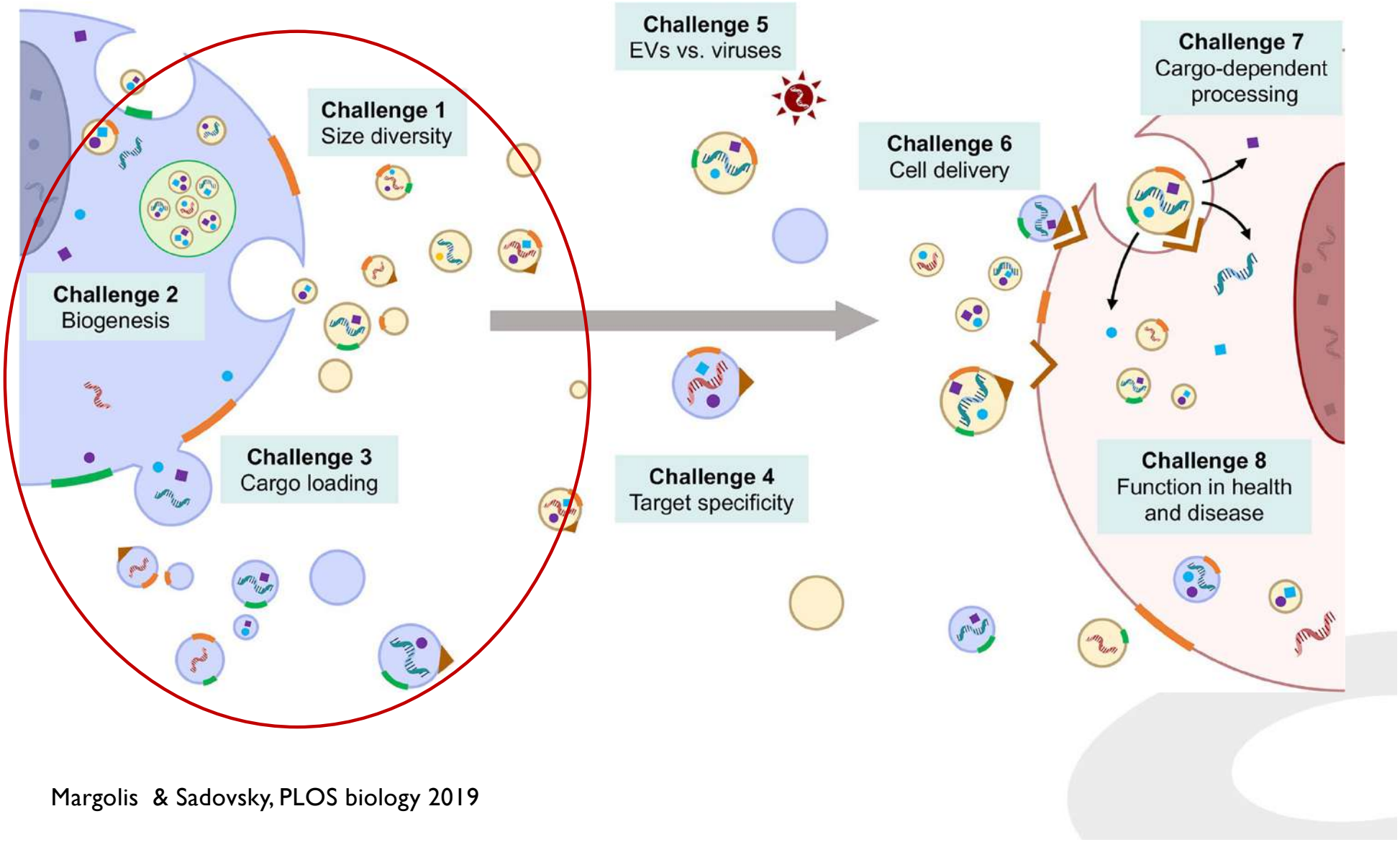
EVs: challenges



Margolis & Sadovsky, PLOS biology 2019

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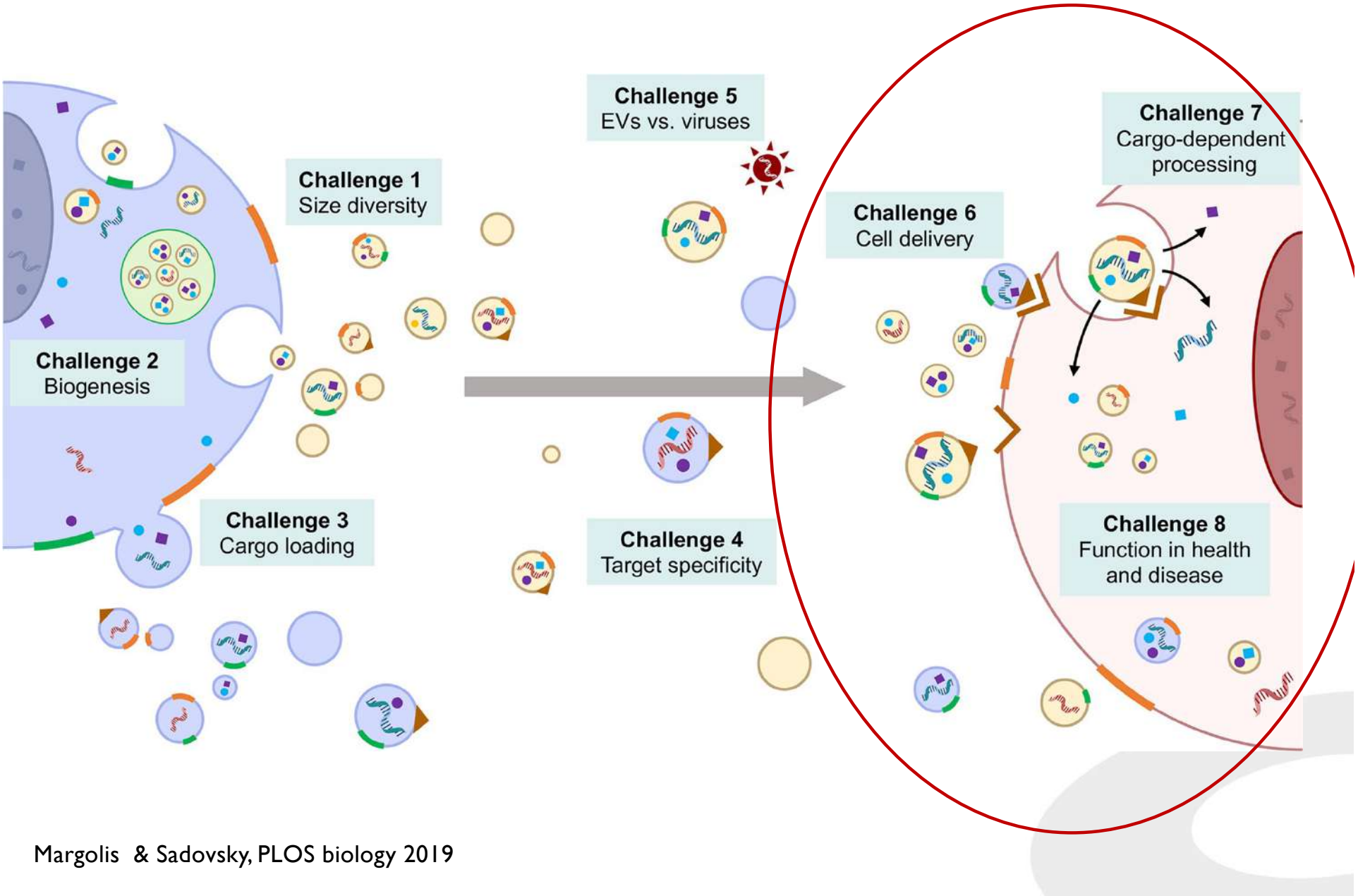
EVs: challenges



Margolis & Sadovsky, PLOS biology 2019

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AIMS

Exploring the role of small EVs in cancer spreading

- Role of pathological s-EVs in modulating physical properties of cells
- Uptake routes and dynamics: model membrane systems

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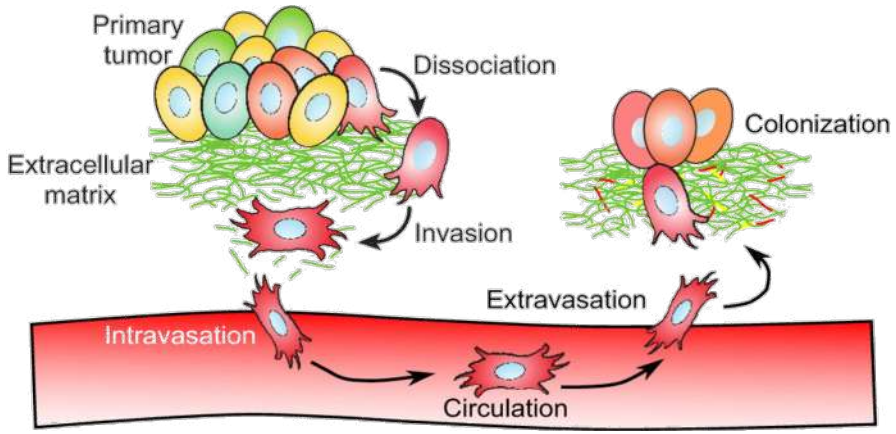
Part I: s-EVs in metastatic cancer



Albritton et al. 2018, Dis Model Mech.

During metastasis cancer cells undergo different phenotypic changes

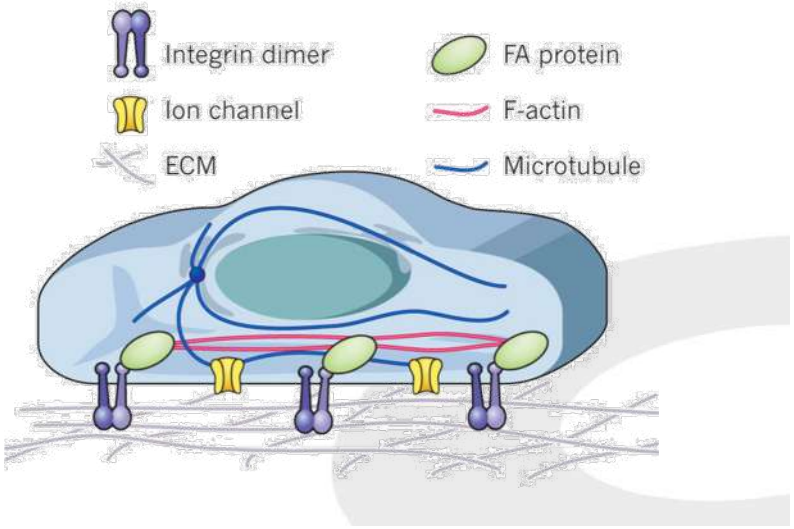
Cell rearrangements: cytoskeleton, nuclear morphology, focal adhesion

From literature:

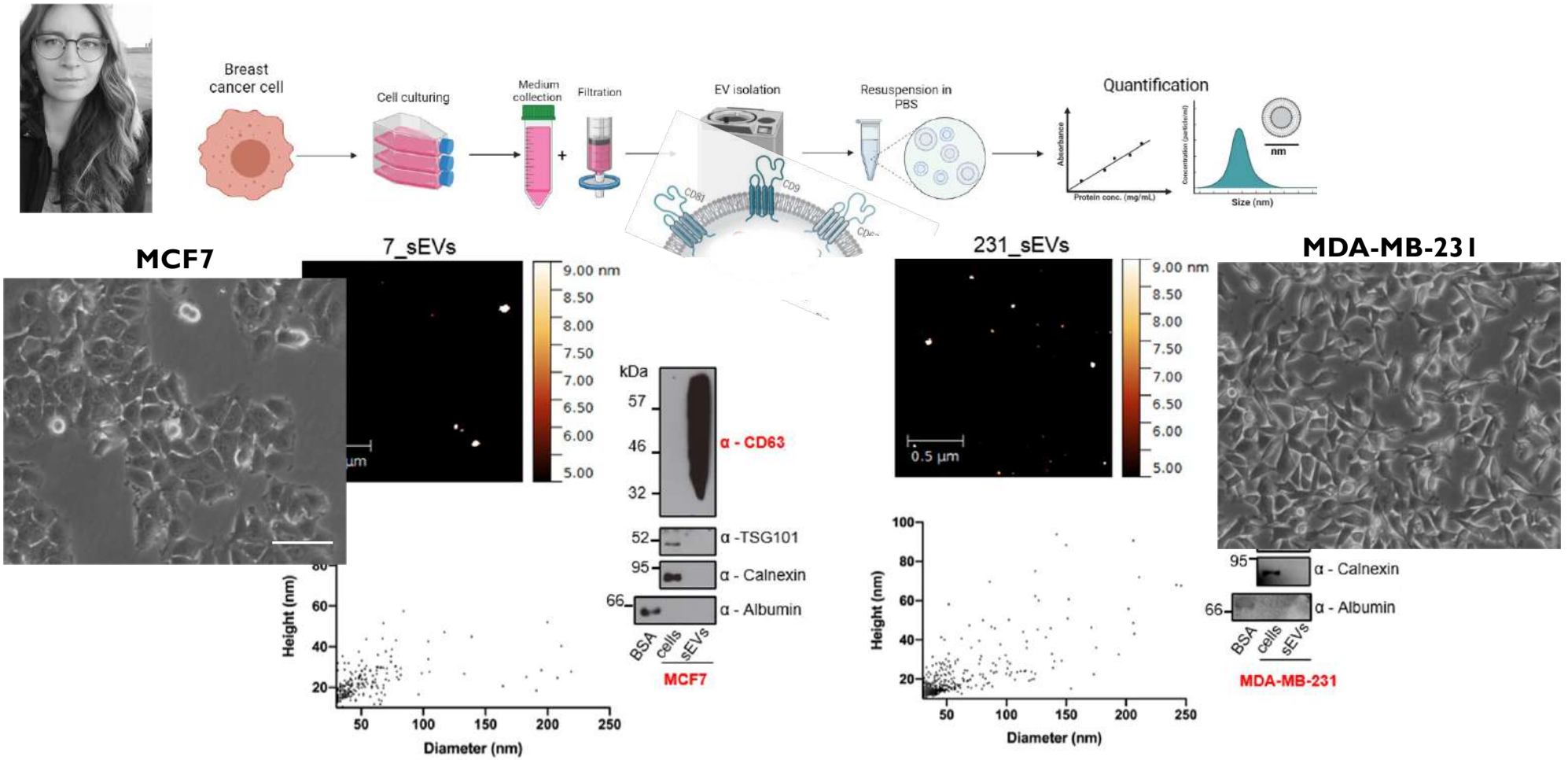
- metastatic potential of cancer cells requires specific biomechanical properties
- metastatic cancer cells transfer oncogenic proteins, mRNAs, and miRNAs to target cells through EVs

Questions:

- are s-EVs regulators of cell biomechanical changes?
- which type of phenotypic changes do they induce?



s-EVs from breast cancer cells



Triple negative breast cancer (TNBC): aggressive, fast spread, highly metastatic sEV extracted from TNBC cells (MB-MDA-231) with respect to the ones from non-metastatic breast cancer cells (MCF7)

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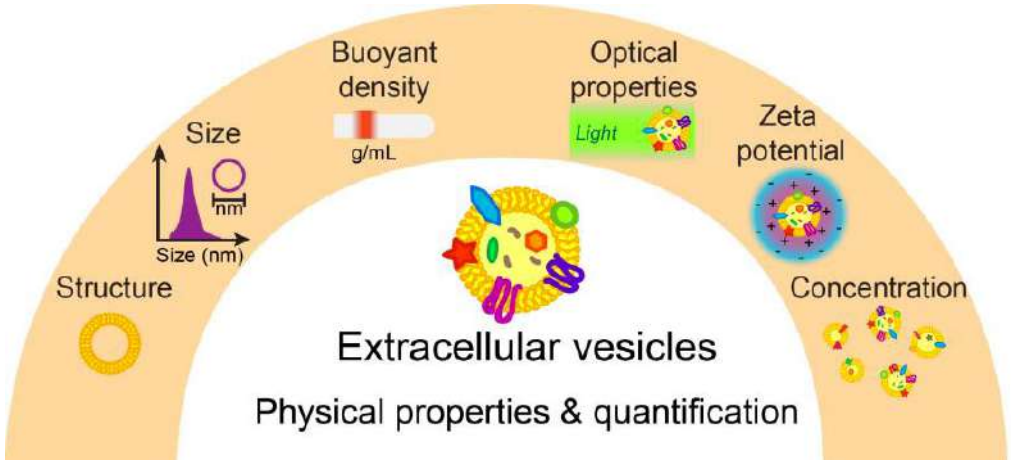
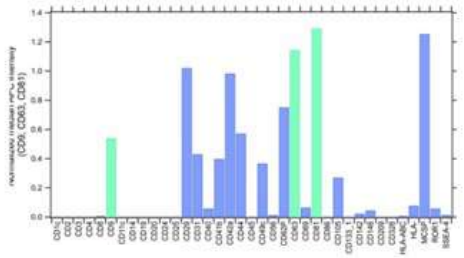
EVs characterization platform

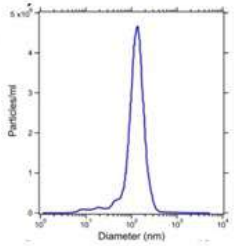


Rupert et al., BBA – Gen. Subjects (2016)

Surface markers

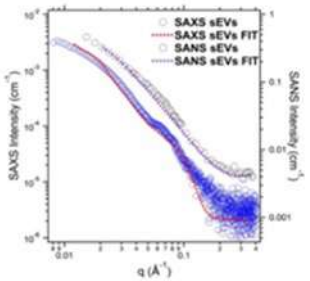
sEV/exosome markers (tetraspanins CD9, CD63 and CD81)

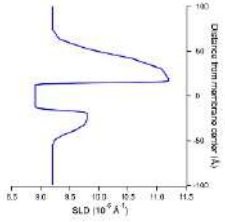




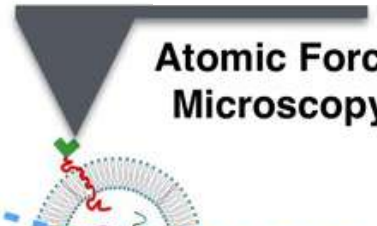
NTA

NanoparticleTracking Analysis

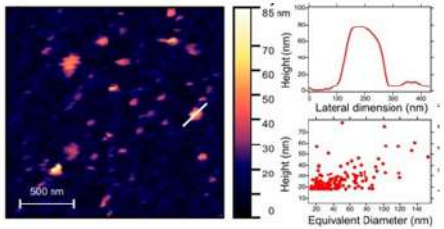


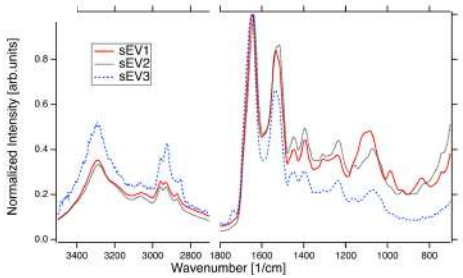


Small Angle X-Ray Scattering



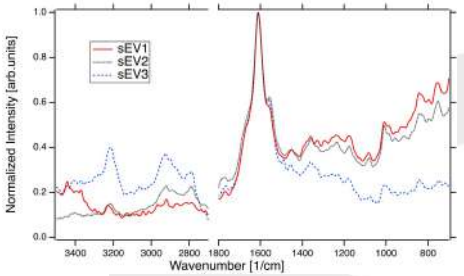
Atomic Force Microscopy





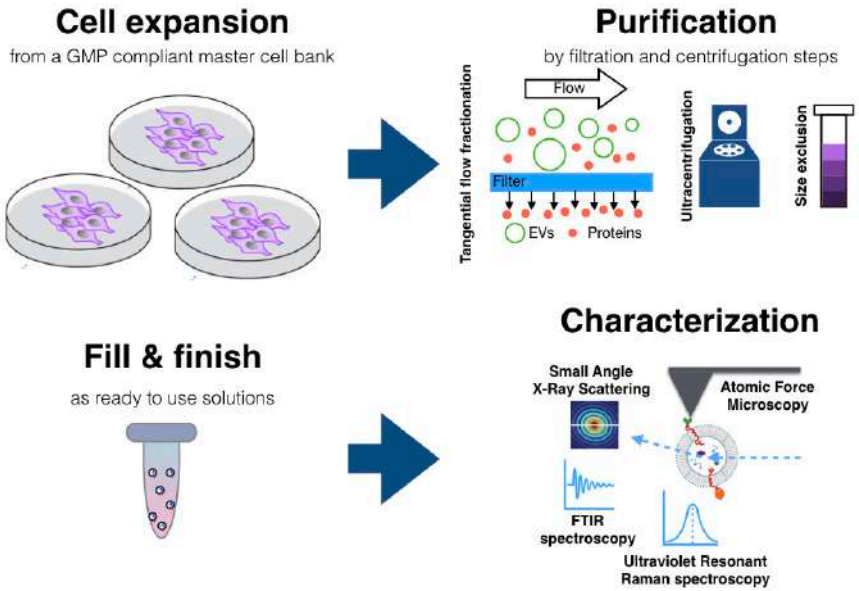
FTIR spectroscopy

Ultraviolet Resonant Raman spectroscopy

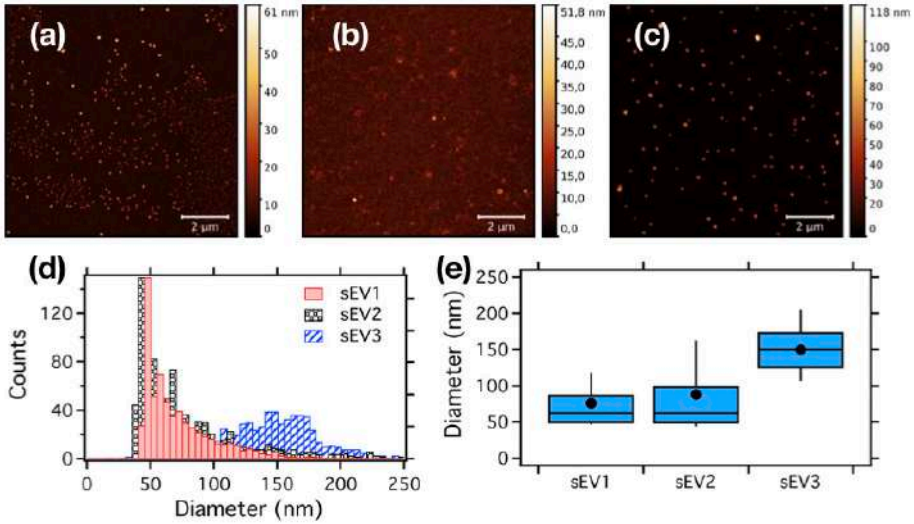


Perissinotto et al., Multi-technique analysis of EVs: not only size matters (2020)

IV. Extracellular Vesicles (EVs)



AFM

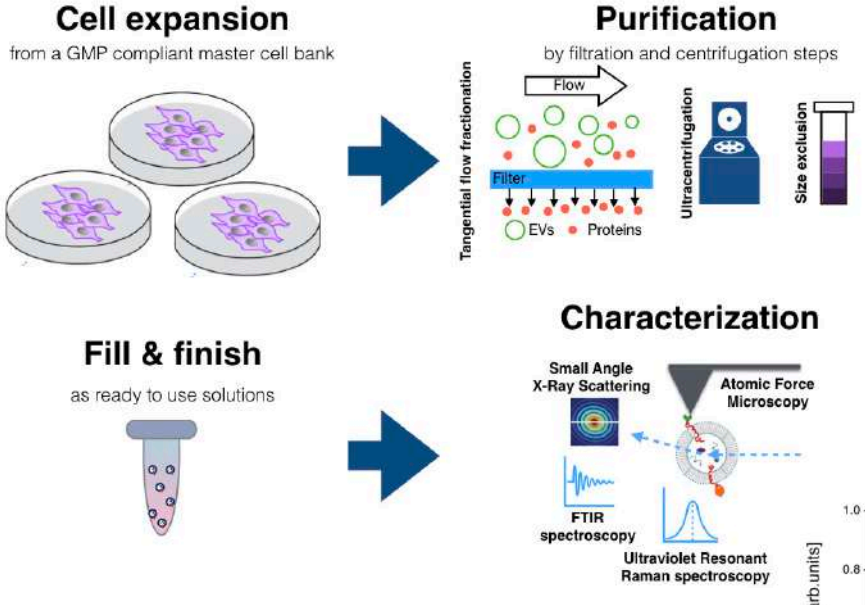


NTA

Name	EV isolation method	NTA Concentration (particles/ml)	NTA Average Size (nm)
sEV1	TFF 750 kD	1-4 x 10^{11}	100.8
sEV2	TFF 750 kD + UC	1-4 x 10^{11}	114
sEV3	TFF 750 kD +UC + SEC	0.8-2x10^{10}	116

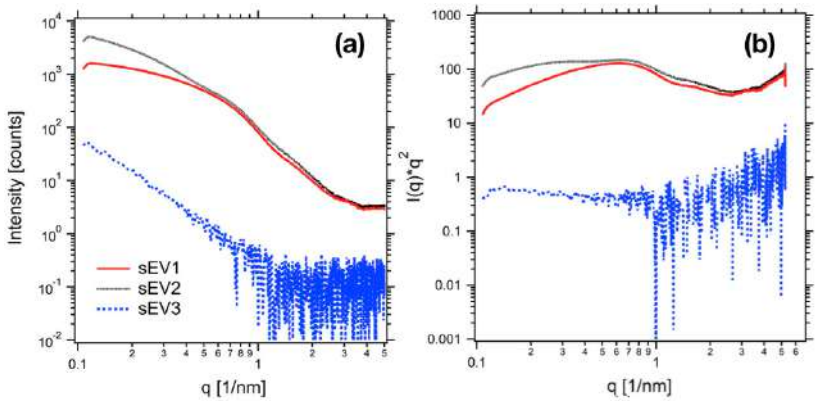
Table 1: Samples analysed with description of isolation method and concentration and average size measured my means of Nanoparticle Tracking Analysis.

IV. Extracellular Vesicles (EVs)

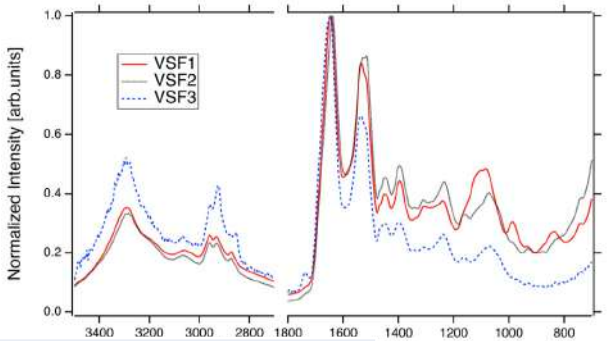


SAXS

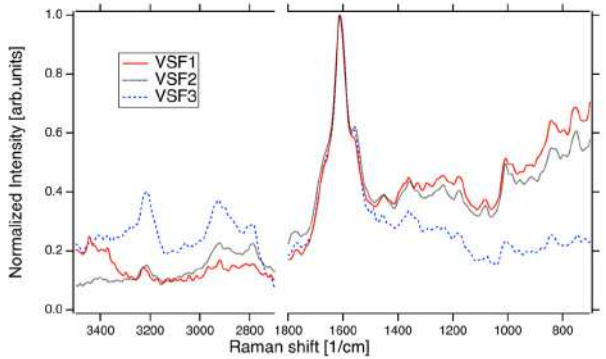
scattering intensity versus the momentum transfer q is function of the defined size d, as $q=2\pi/d$.



ATR-IR



UV RR



Samples	FTIR Protein/Lipid ratio	Raman Protein/Lipid ratio
sEV1	14+/-1	14+/-1
sEV2	13+/-1	4+/-1
sEV3	6+/-2	2.9+/-0.7

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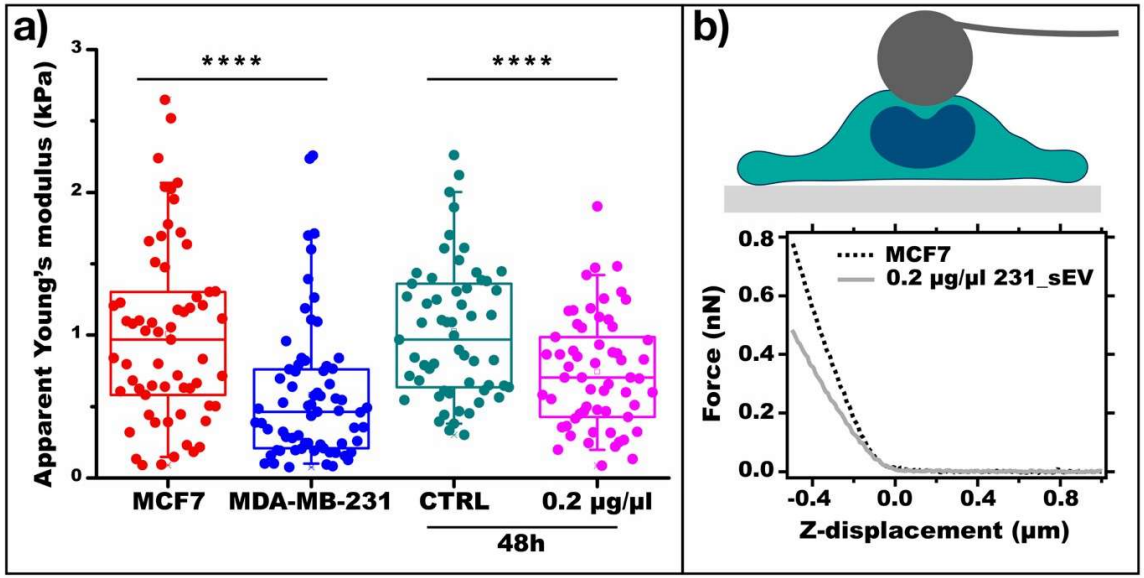
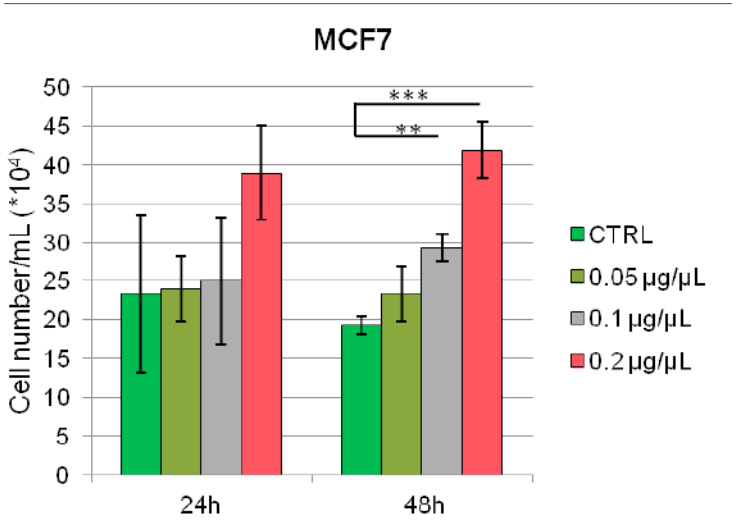
s-EVs uptake and cell biomechanics

Are sEVs modulators of biomechanics properties?





Cell proliferation assay

Effect of sEVs from Triple Negative Breast Cancer cell lines (MDA-MB-231) on the biomechanics of non metastatic breast cancer cells (MCF7)

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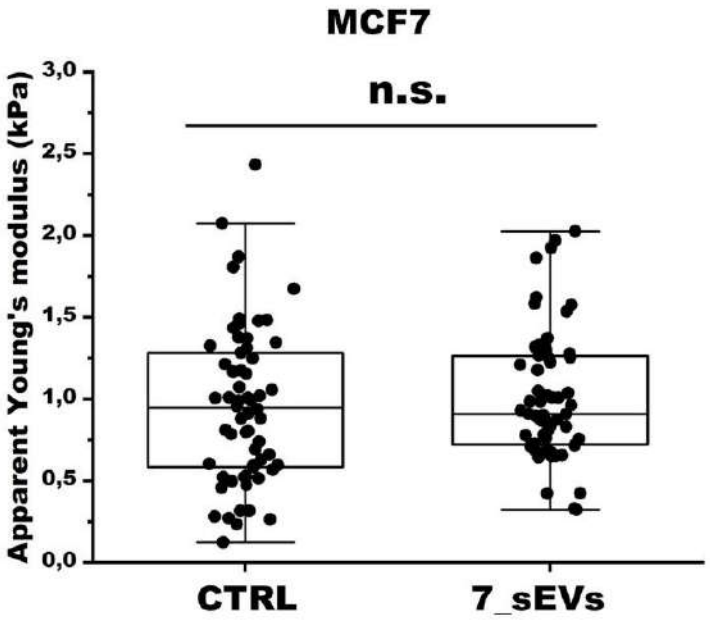
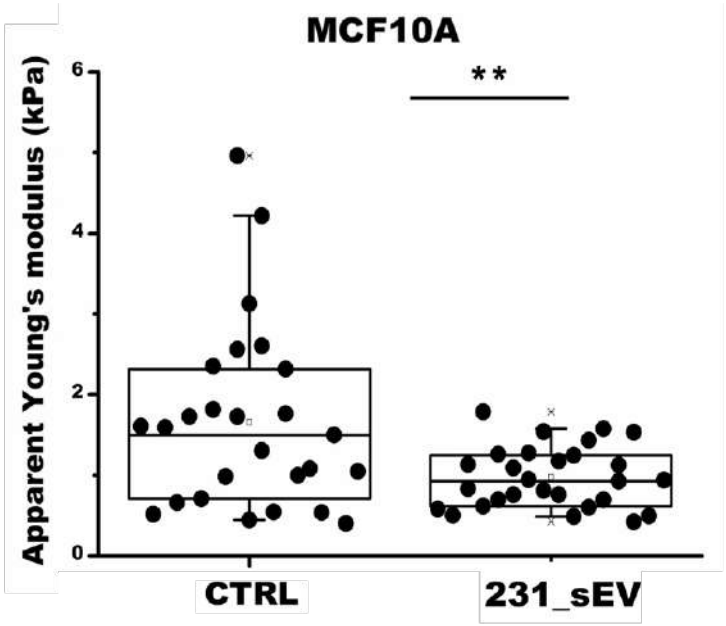
s-EVs uptake and cell biomechanics

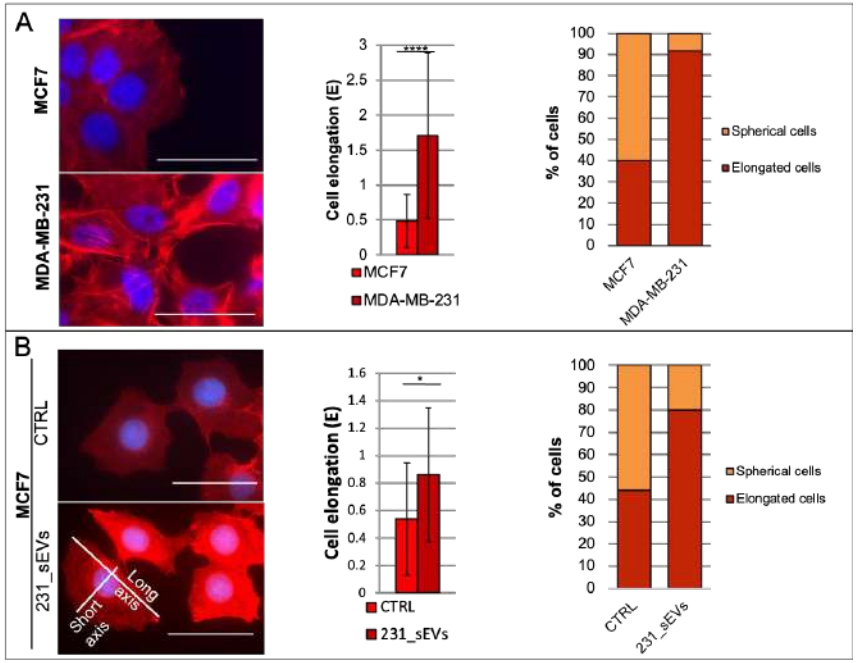
Are sEVs modulators of biomechanics properties?





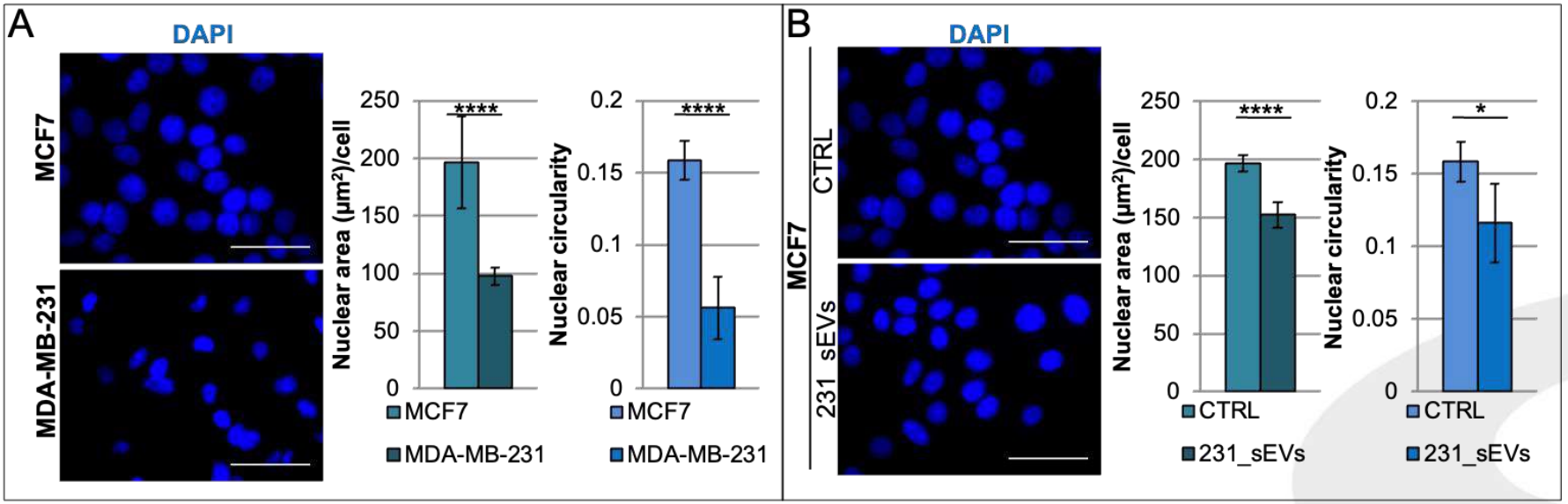
breast cell line (MCF10A)

Stiffness modulation: where from?



$$E = A_L/A_S - 1$$

E<0.5 spherical cells
E>0.5 elliptical cells



* = p < 0.05; ** = p < 0.01; *** = p < 0.001; **** = p < 0.0001

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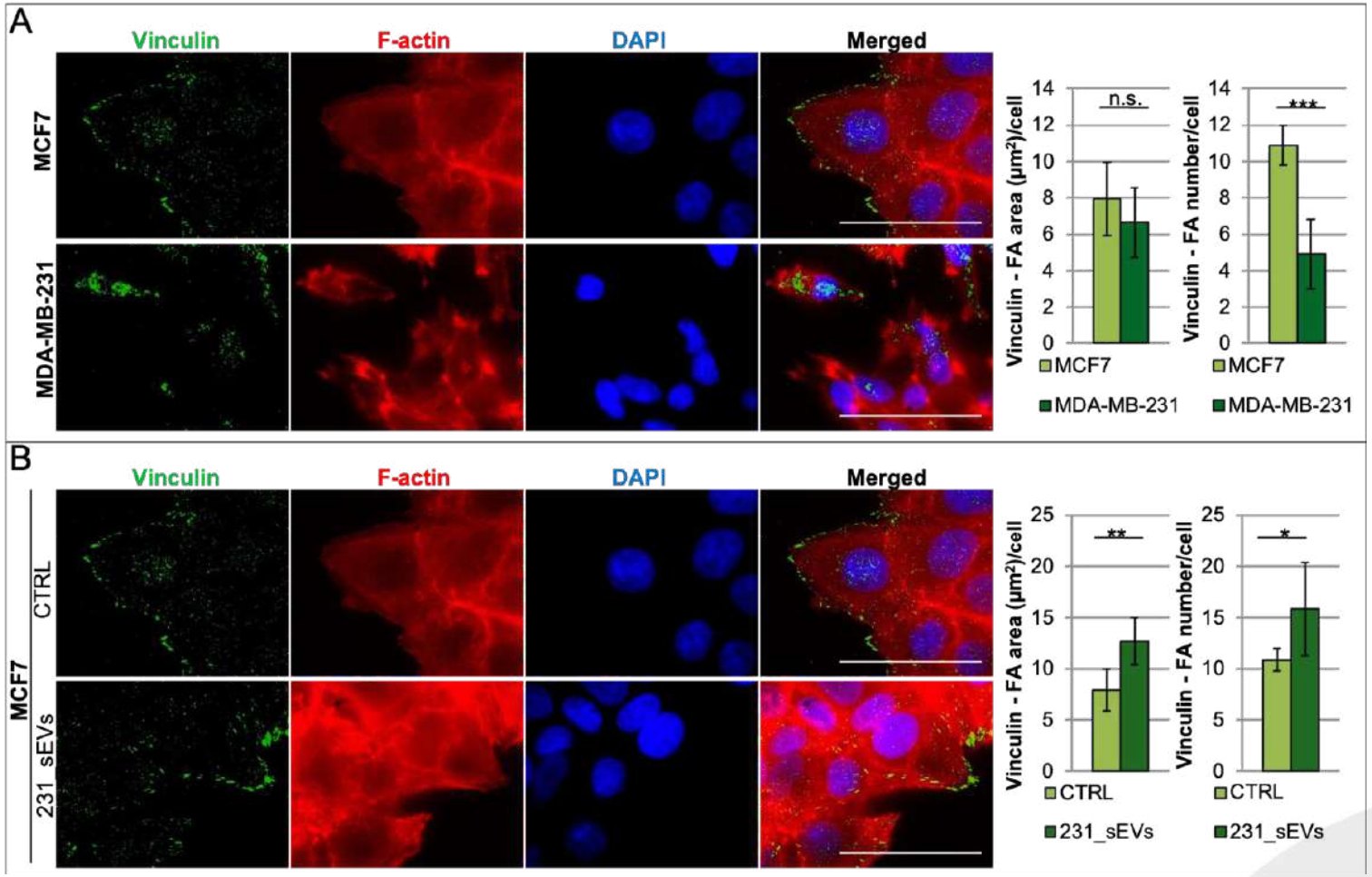
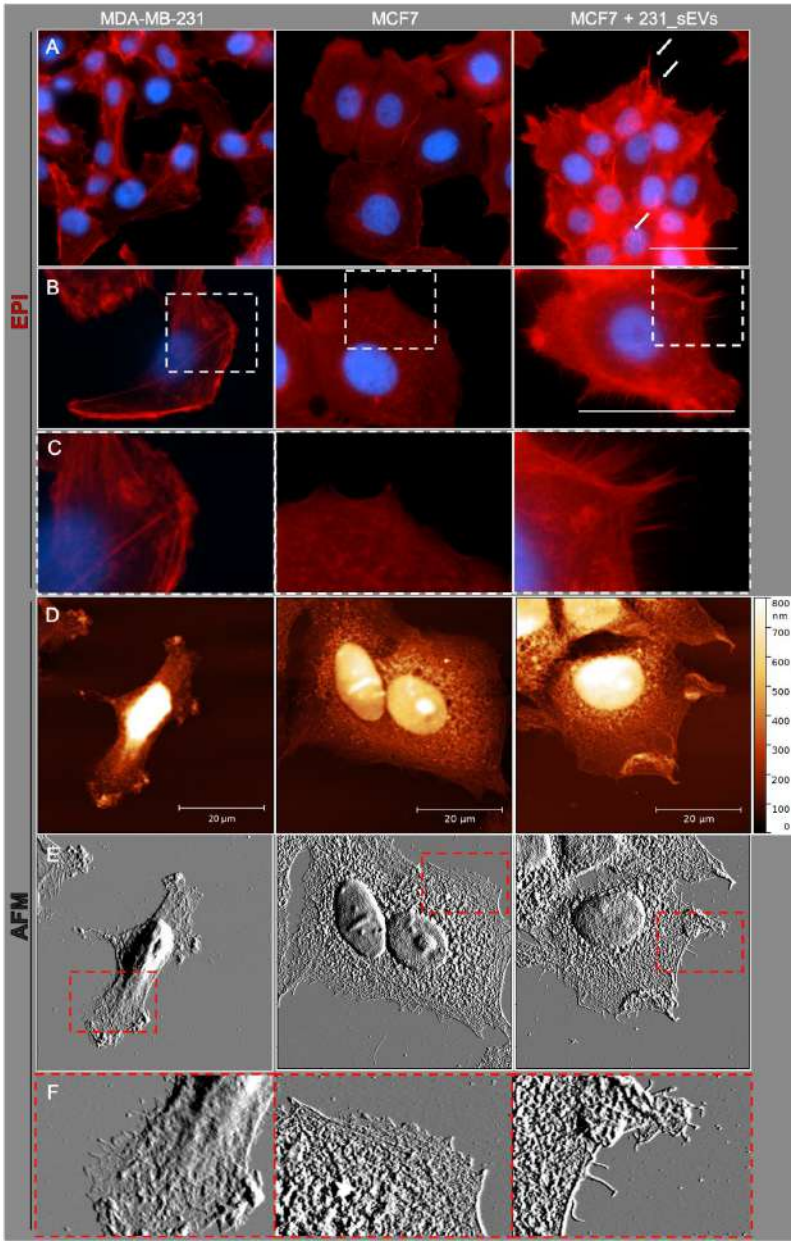
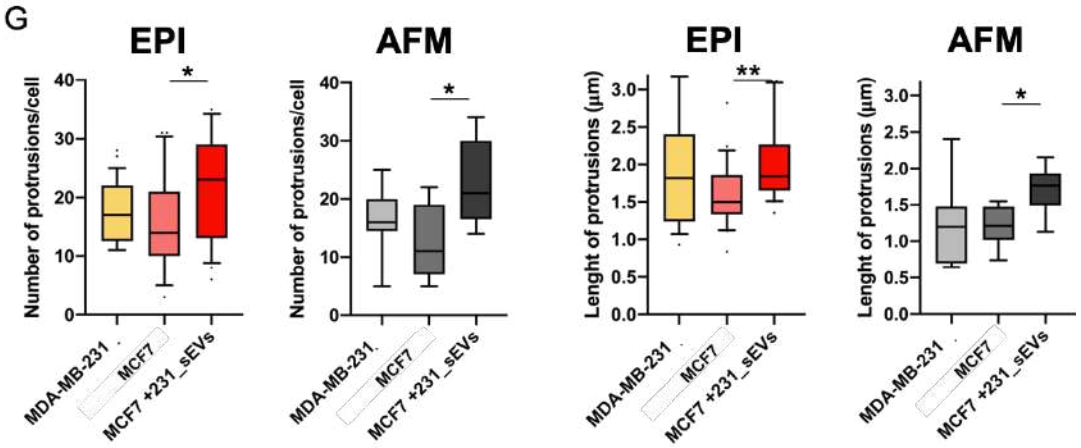
Stiffness modulation: where from?



Stiffness modulation: where from?

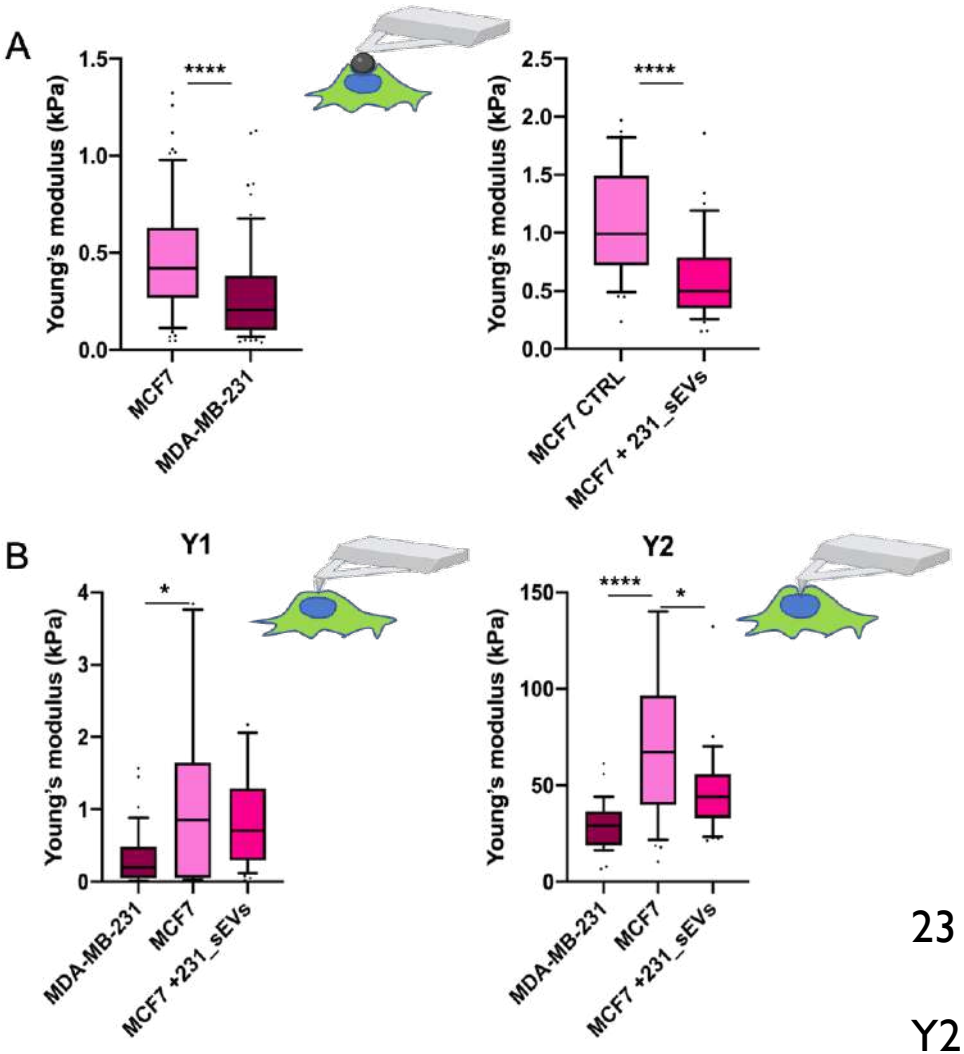


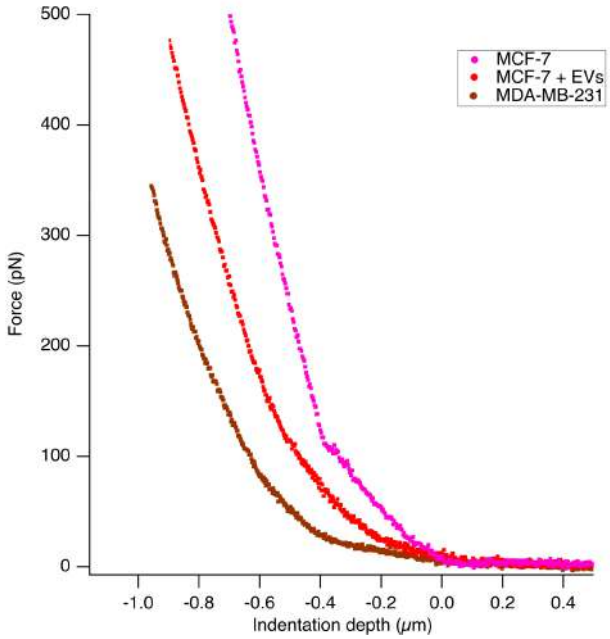


* = p < 0.05; ** = p < 0.01; *** = p < 0.001; **** = p < 0.0001

Stiffness modulation: where from?

* = p < 0.05; ** = p < 0.01; *** = p < 0.001; **** = p < 0.0001





Pyramidal tips k = 0.200 N/m, tip curvature < 20 nm;
two-slope modified Hertz-Sneddon model to fit the force curves

231_sEVs-treated MCF7 cells:

Y2 significantly lower than MCF7 cells
Y1 comparable to MCF7 cells.

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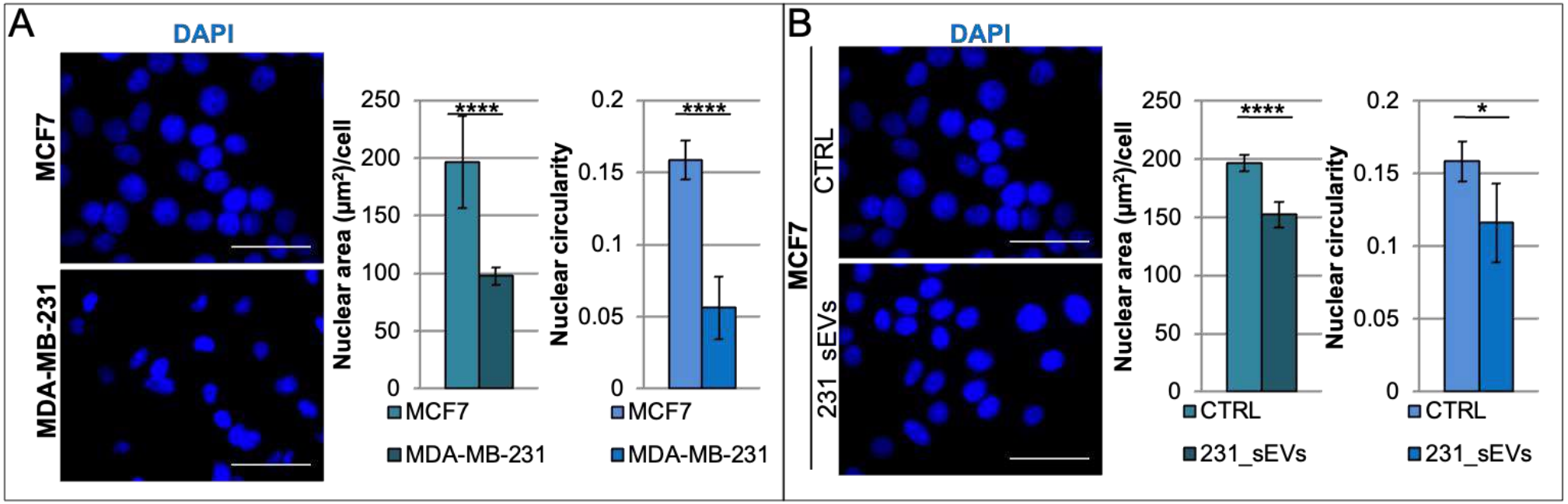
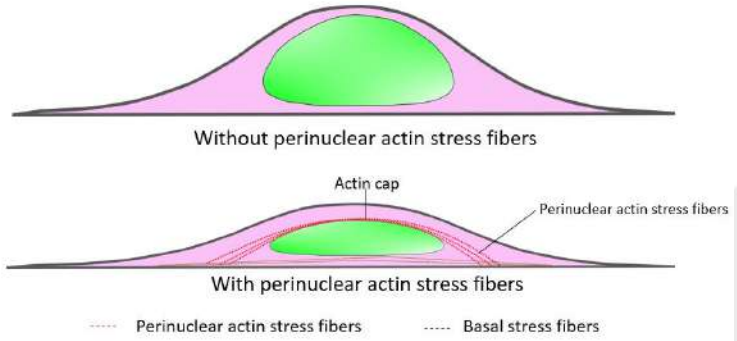
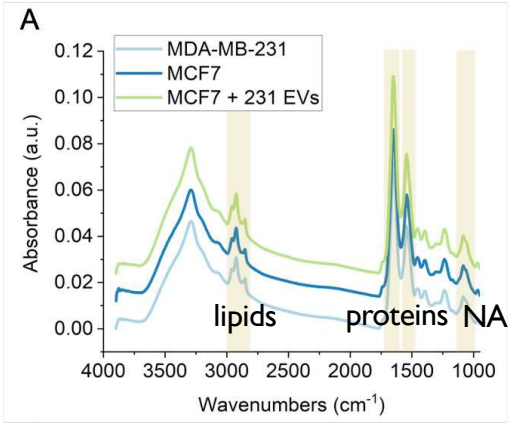
Stiffness modulation: where from?



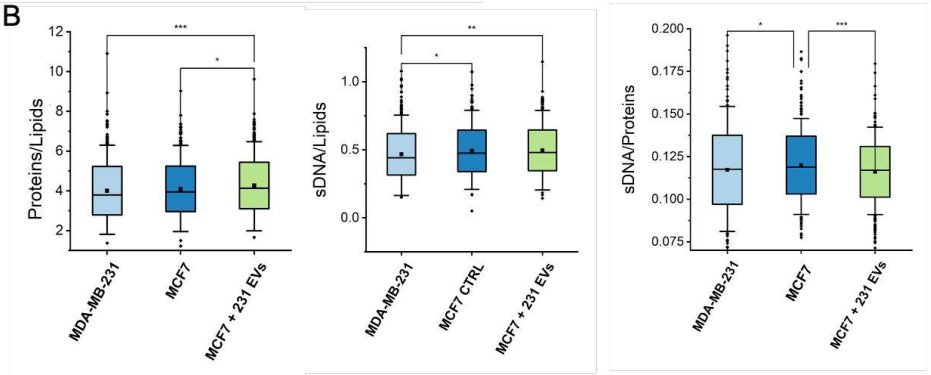
* = p < 0.05; ** = p < 0.01; *** = p < 0.001; **** = p < 0.0001

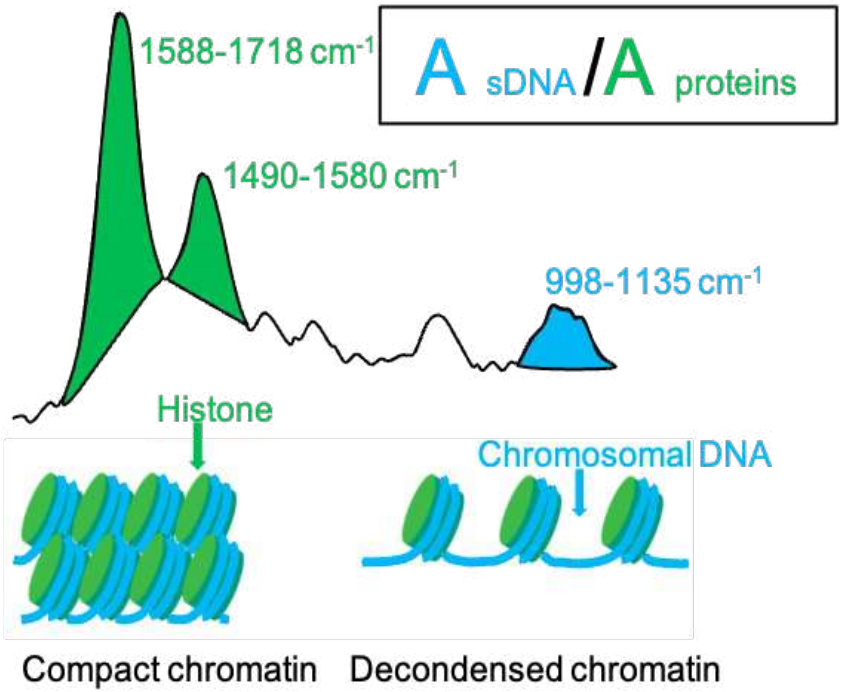


Stiffness modulation: where from?



FTIR





	DNA/Lipid	DNA/Protein	Protein/Lipid
231_sEVs+MCF7/MCF7	=	-	+

PROTEIN CONTENT INCREASES IN TREATED CELLS
MDA-MB-231 have more lipids than both MCF7 cells

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Part 1: conclusions

- s-EVs soften non-metastatic breast cells
- s-EVs induce modification of nuclear shape and size, actin fiber formation
- The Young modulus changes at the level of nucleous, not membrane-associated cortical fibers
- Chromatin decondensation might play a relevant role in biomechanical changes

Inducede biomechanical changes on model cell cultures by patient-derived s-EVs as a new functional assay in metastatic cancer diagnosis and therapy.

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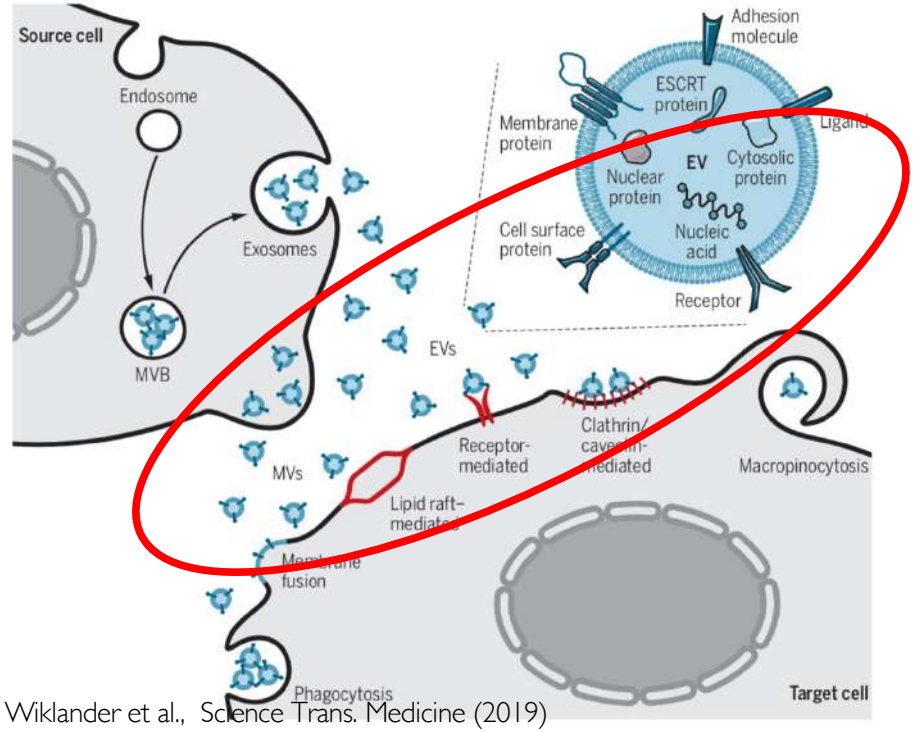
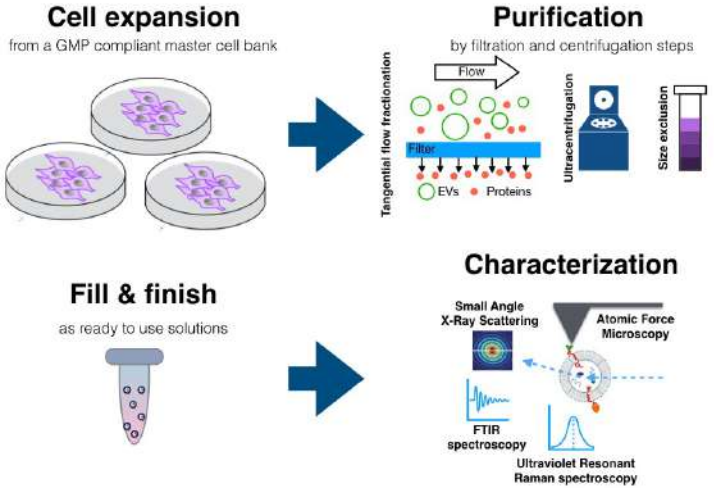
Part II: s-EVs uptake mechanism



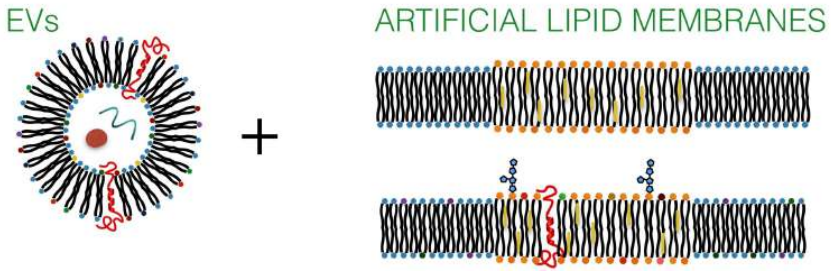
Wiklander et al., Science Trans. Medicine (2019)

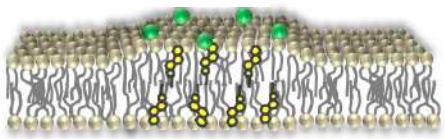
Model system:

✓ EVs from Umbilical Cord multipotent Mesenchymal StemCells



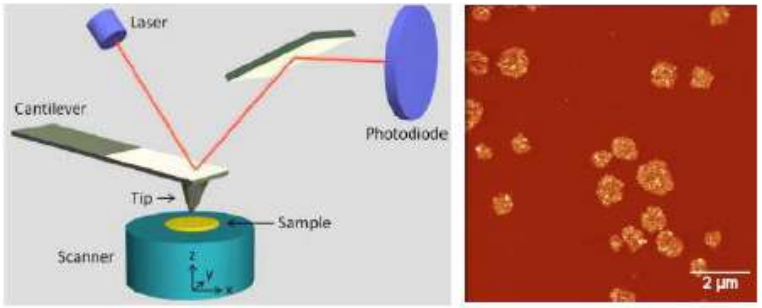
✓ Model Plasma Cell Membrane with Lipid Rafts





s-EVs uptake mechanism

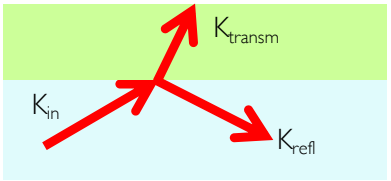
AFM



Atomic Force Microscopy

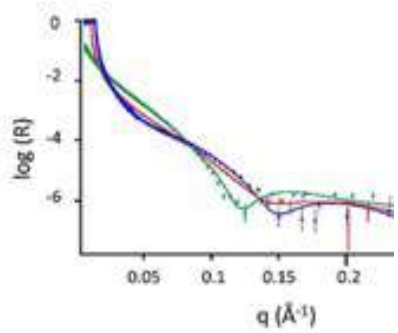
- ✓ **Morphological/mechanical characterization**
- ✓ **(Sub)Nanometer resolution**
- ✓ **Liquid environment**

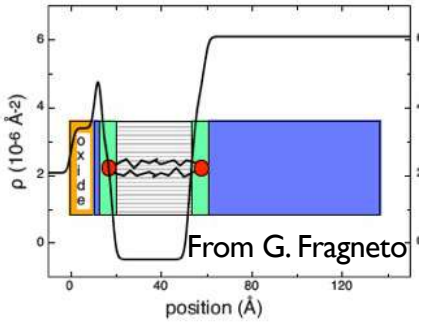
Neutron Reflectometry



$$R \approx \left(\frac{16\pi^2}{q^4} N_b^2 \right) e^{-q_z^2 \sigma^2}$$

The Reflectivity spectrum obtained is given by the interference of the waves reflected from the top and bottom of each layer



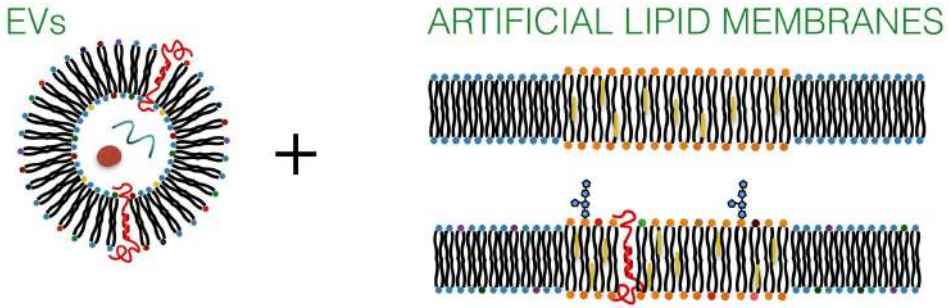


- ✓ Sample cross sectional profile extracted from data analysis
- ✓ Sensitive to light elements (H,O,N,C..)
- ✓ Sample cross-sectional profile: thickness, composition, compactness, roughness

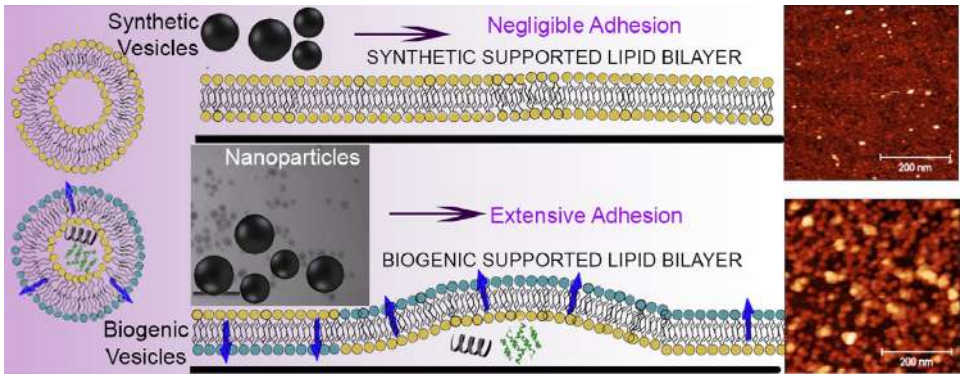
Playing with **selective deuteration**, protiated molecules can be evidenced in a ghost phospholipid matrix

Model lipid bilayers

Multicomponent artificial lipid bilayers for mimicking cellular membranes



Nanoparticles + artificial membranes/EVs



Montis et al., **Journal of Colloid and Interface Science (2020)**

Model lipid bilayers

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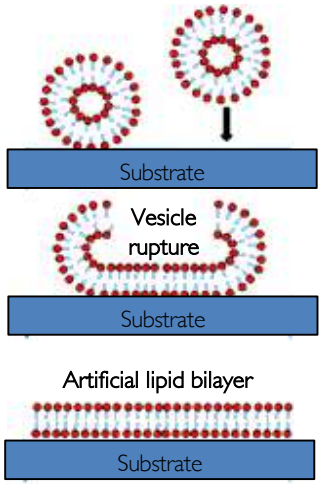
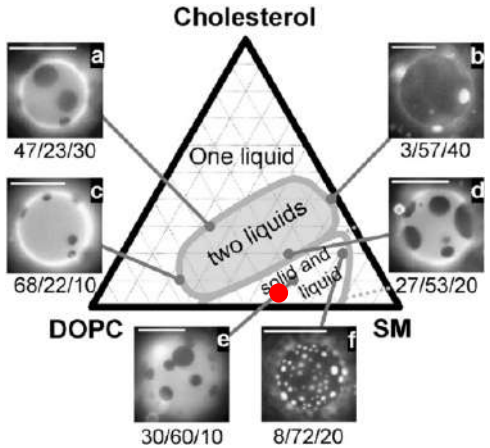
Vesicle fusion method



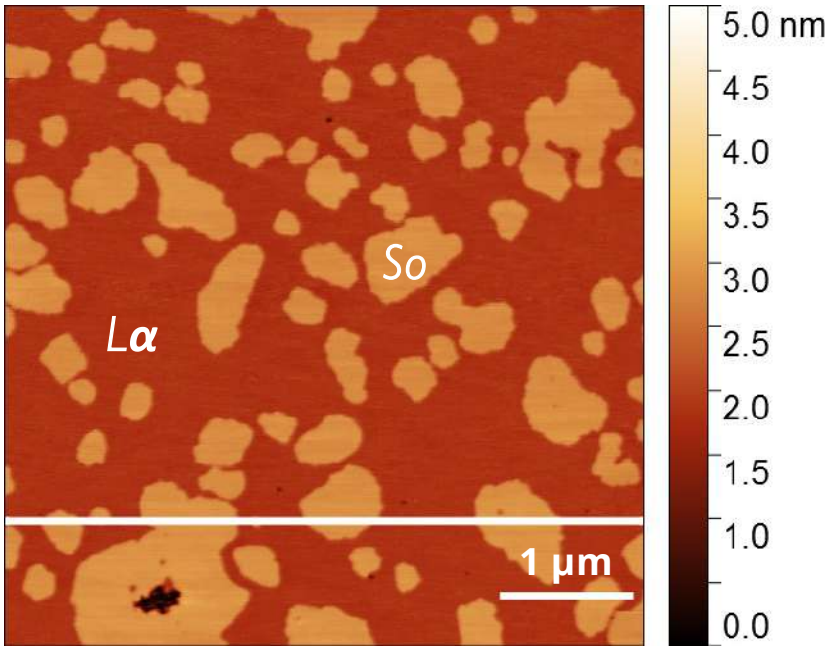


Adapted from Veatch and Keller. *Phys. Rev. Lett. (2005). 94, 148101*

➢DOPC + SM (66:33) + 5% Chol

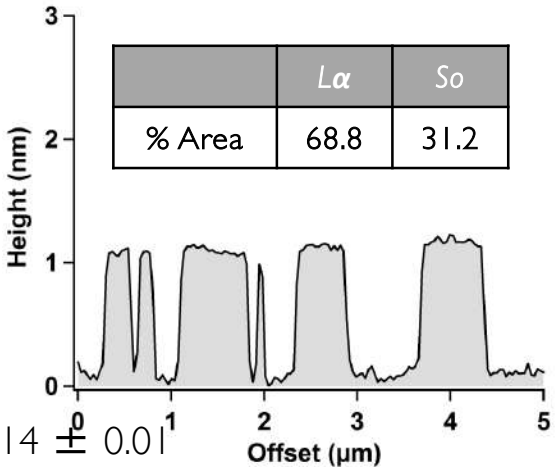
DOPC
1,2-dioleoyl-sn-glycero-3-phosphocholine
SM
Sphingomyelin
Chol
Cholesterol

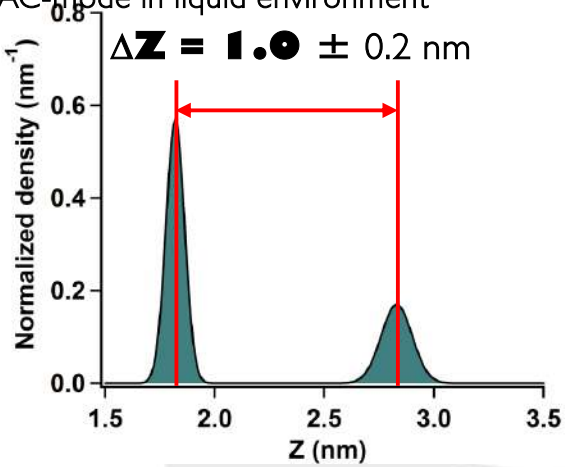
Lα=Liquid-disordered /fluid
So=Solid-ordered/gel



AFM imaging in dynamic AC-mode in liquid environment

	Lα	So
% Area	68.8	31.2





surface roughness: Lα=0.16 ± 0.01 nm, So=0.14 ± 0.01

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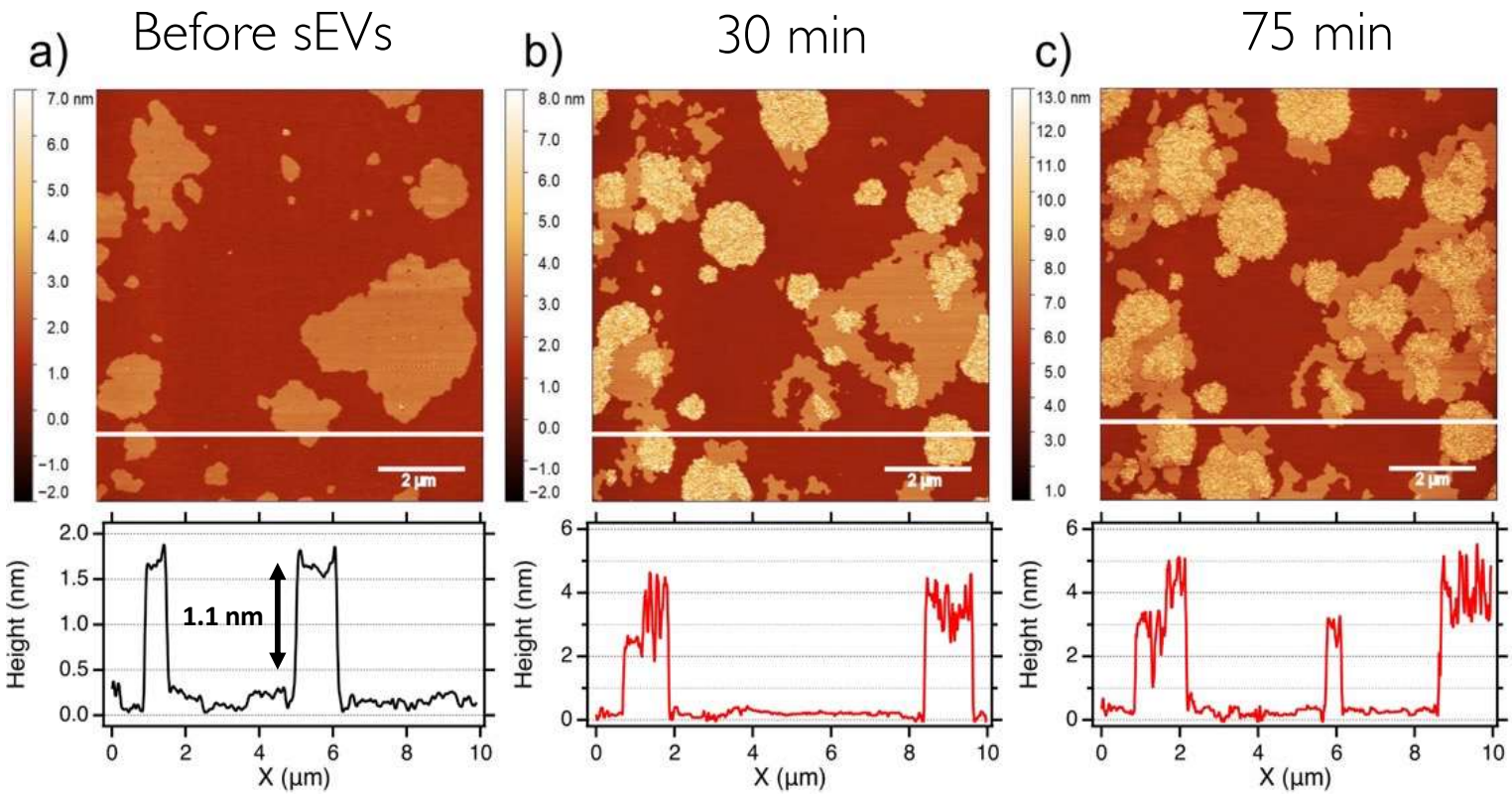
s-EVs interaction with membrane



Patches protruding 3-4 nm above SLB tend to expand in a more favourable fashion in L_d phase

sEVs preferentially dock and break at phases borders!

F. Perissinotto et al., Nanoscale, 2021, 13, 5224

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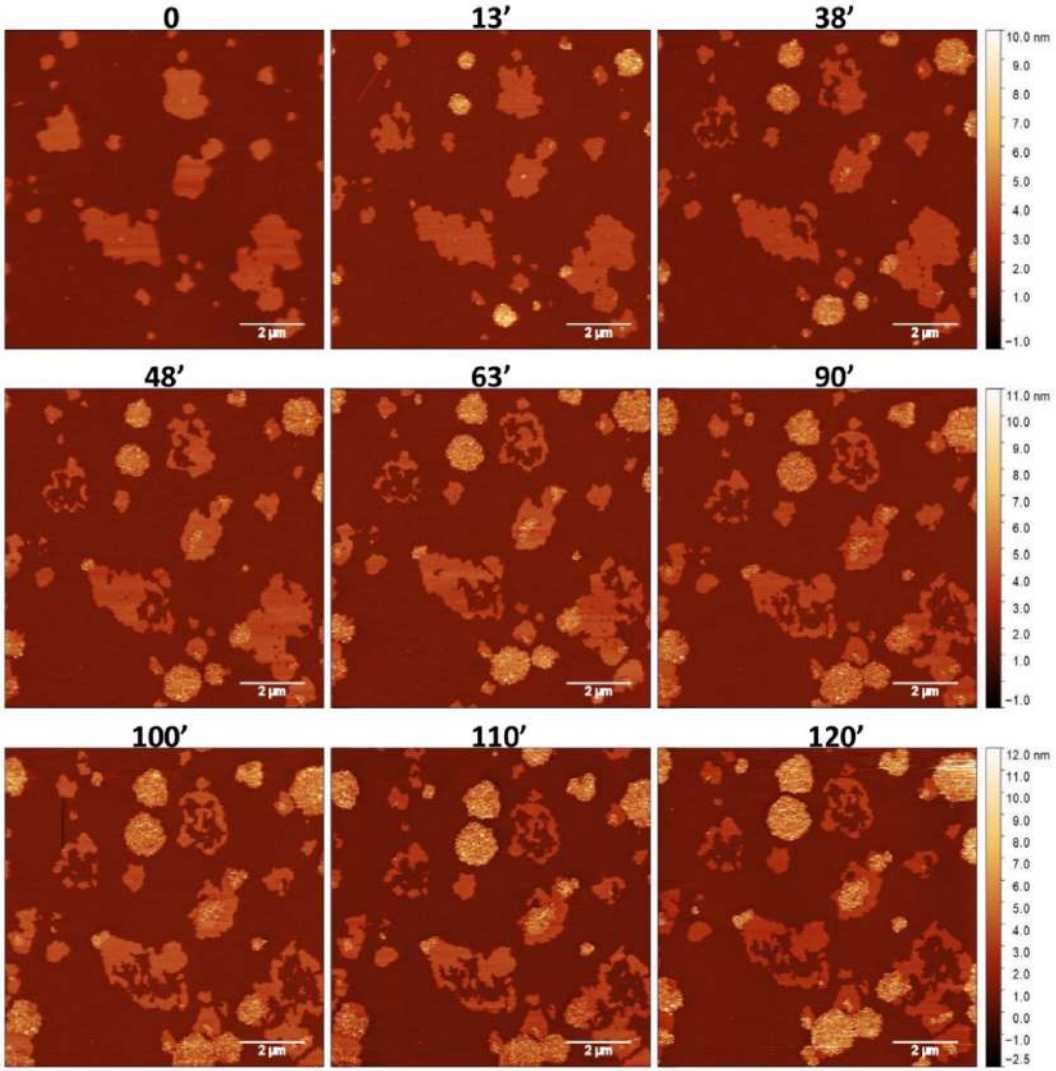
s-EVs interaction with membrane

L_o phase re-shaping, borders granularity



From literature:
-> cholesterol depletion
-> components redistribution

Distinct processes occur:

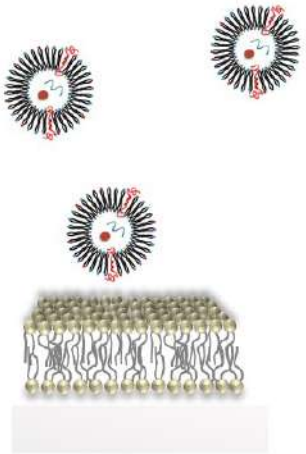
1. Fast diffusion of lighter elements laterally migrating along phase boundaries

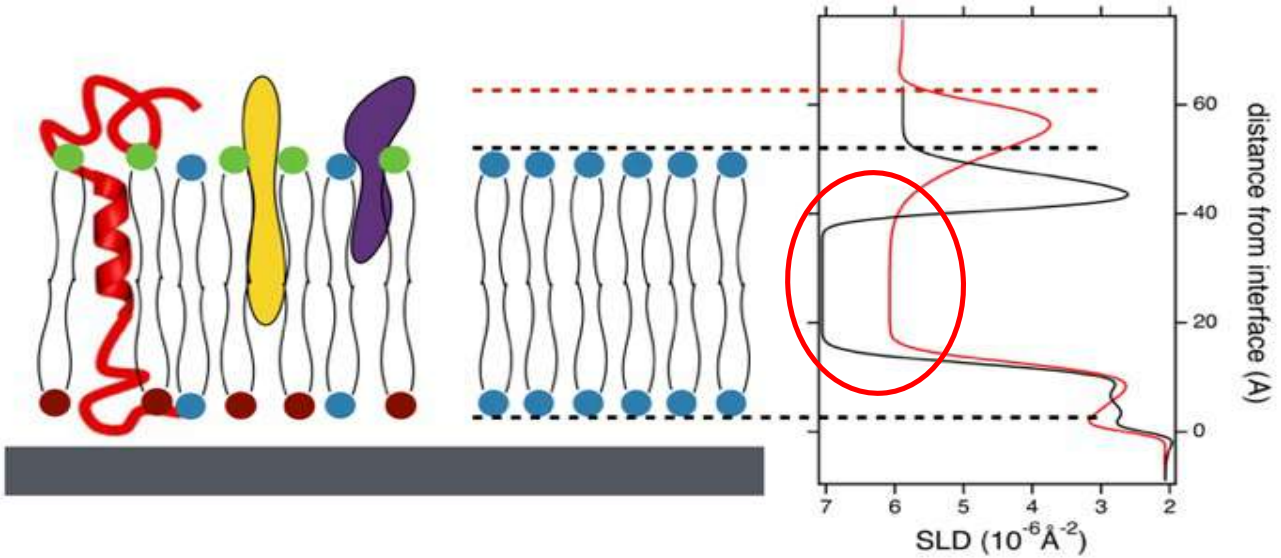
2. Diffusion of bulkier sEVs components mixing with target membrane

The uptake process is different from 'standard' membrane fusion

s-EVs interaction with membrane

$$R \approx \left(\frac{16\pi^2}{q^4} N_b^2 \right) e^{-q_z^2 \sigma^2}$$





Playing with selective deuteration: protiated molecules in a ghost phospholipid matrix

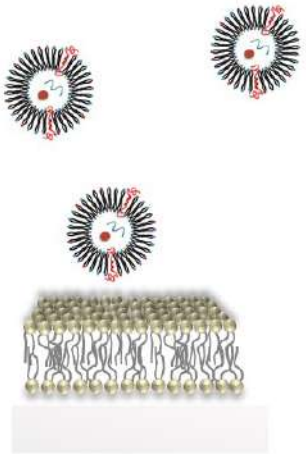
- 20% volume penetration
- Change in contrast spans whole membrane thickness
- Asymmetry

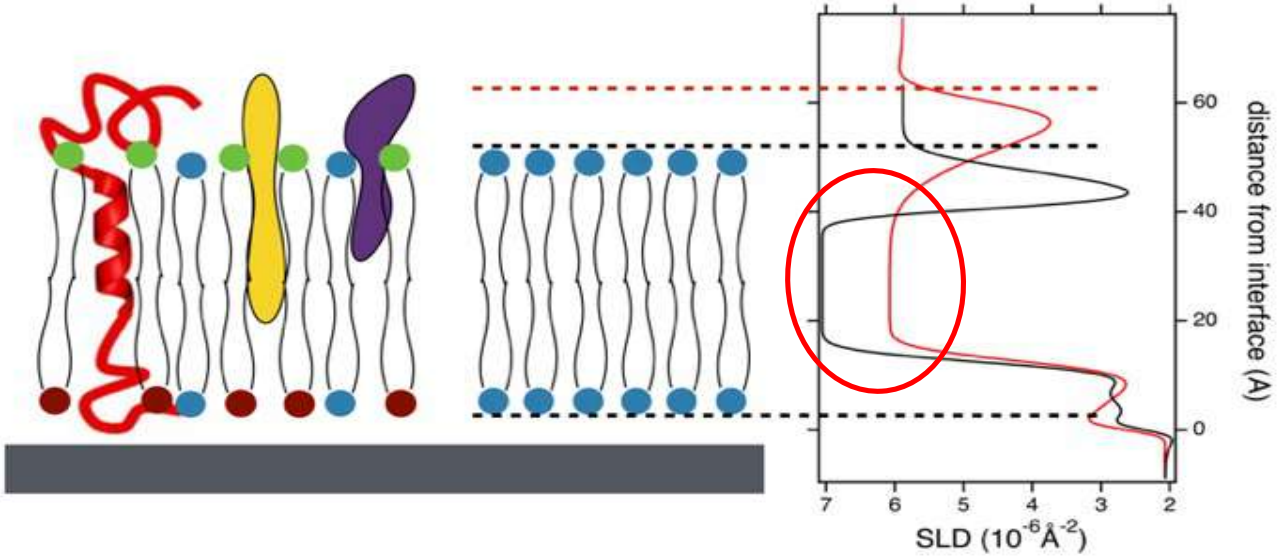
	AFM ΔZ (nm)	NR h (nm)
PC	5.1 ± 0.6	4.2 ± 0.3
PC+EVs	6 ± 2	5.4 ± 0.3

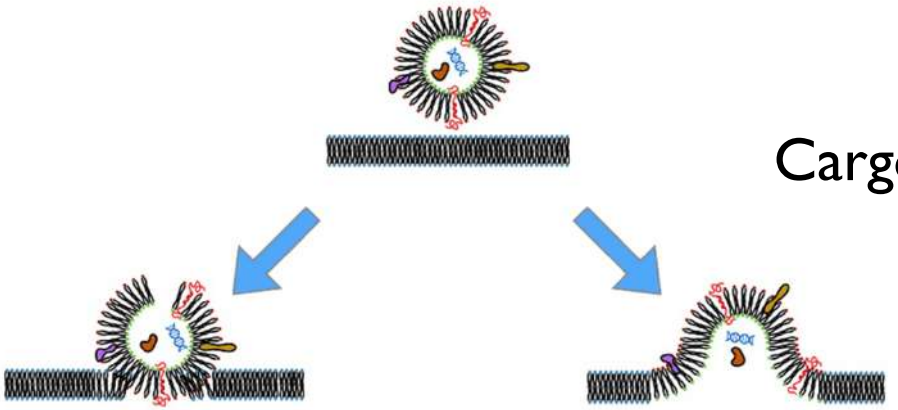
F. Perissinotto et al., Nanoscale, 2021, 13, 5224

s-EVs interaction with membrane

$$R \approx \left(\frac{16\pi^2}{q^4} N_b^2 \right) e^{-q_z^2 \sigma^2}$$







Cargo release seems favoured!

F. Perissinotto et al., Nanoscale, 2021, 13, 5224

s-EVs interaction with membrane

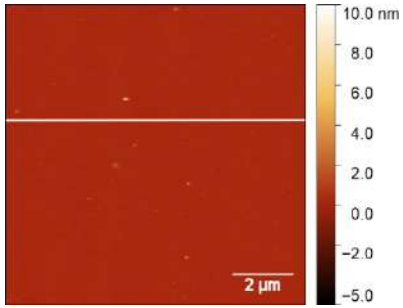
DOPC

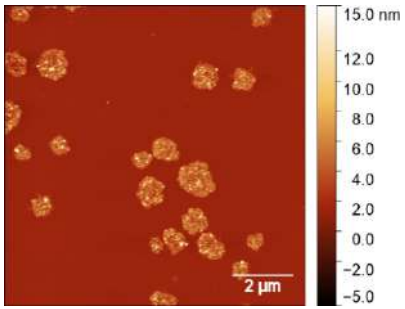
+ sEVs

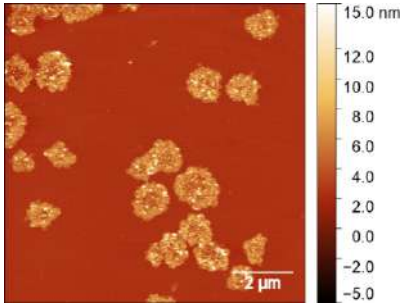
t = 30 min

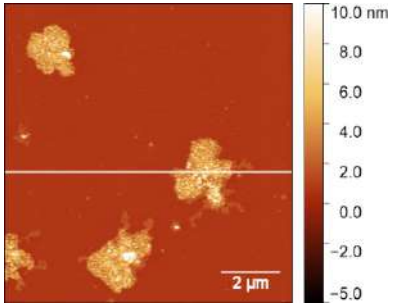
t = 90 min

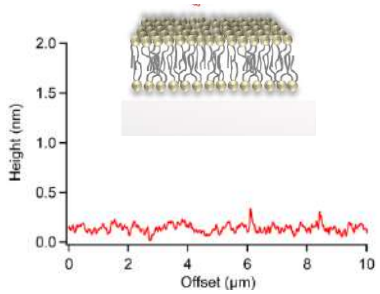
t = 180 min

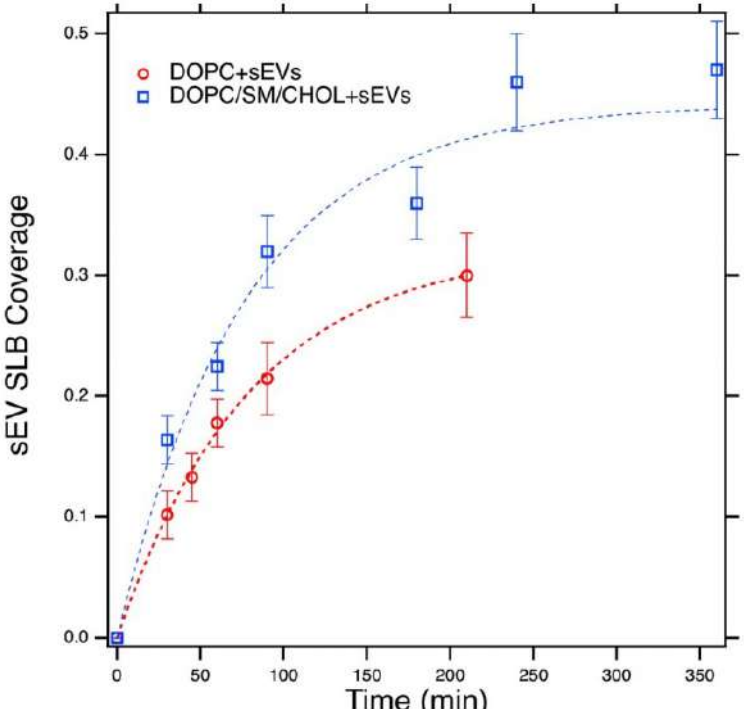


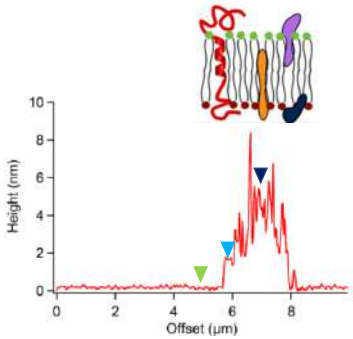












Protrusion of patches 3.4+- 0.7 nm

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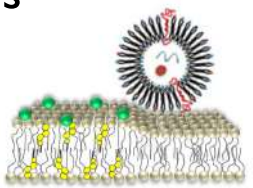
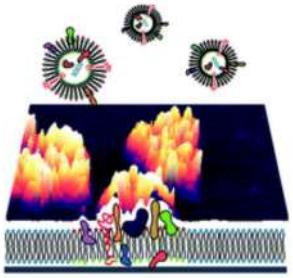
Part II: conclusions

- s-EVs on artificial lipid bilayers break and form EVs-membrane domains

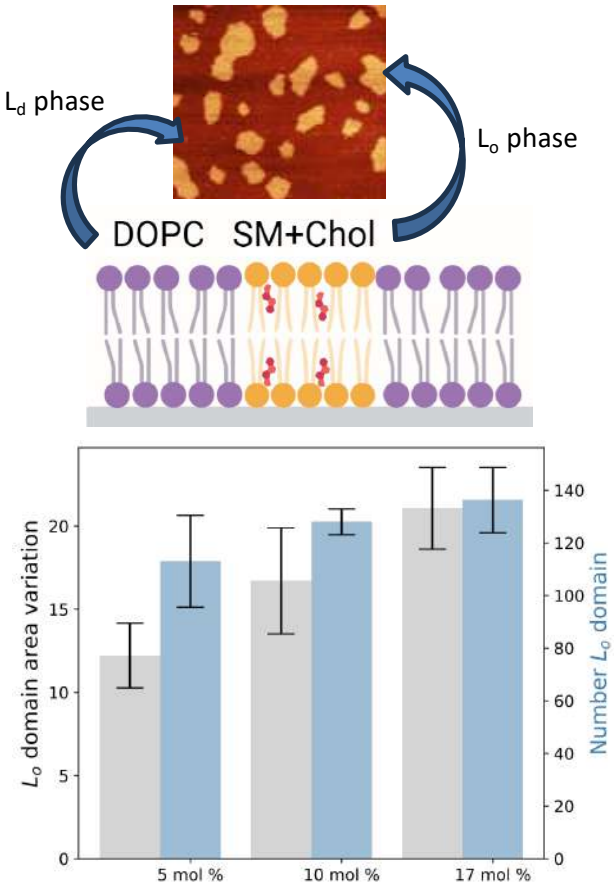


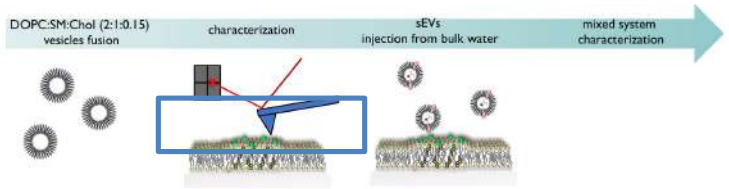
- Phase borders are docking sites
- Different s-EVs components spread with different kinetics
- The area of s-EVs-membrane domains increases over time: initial nucleation seeds act as docking sites for other s-EVs from solution

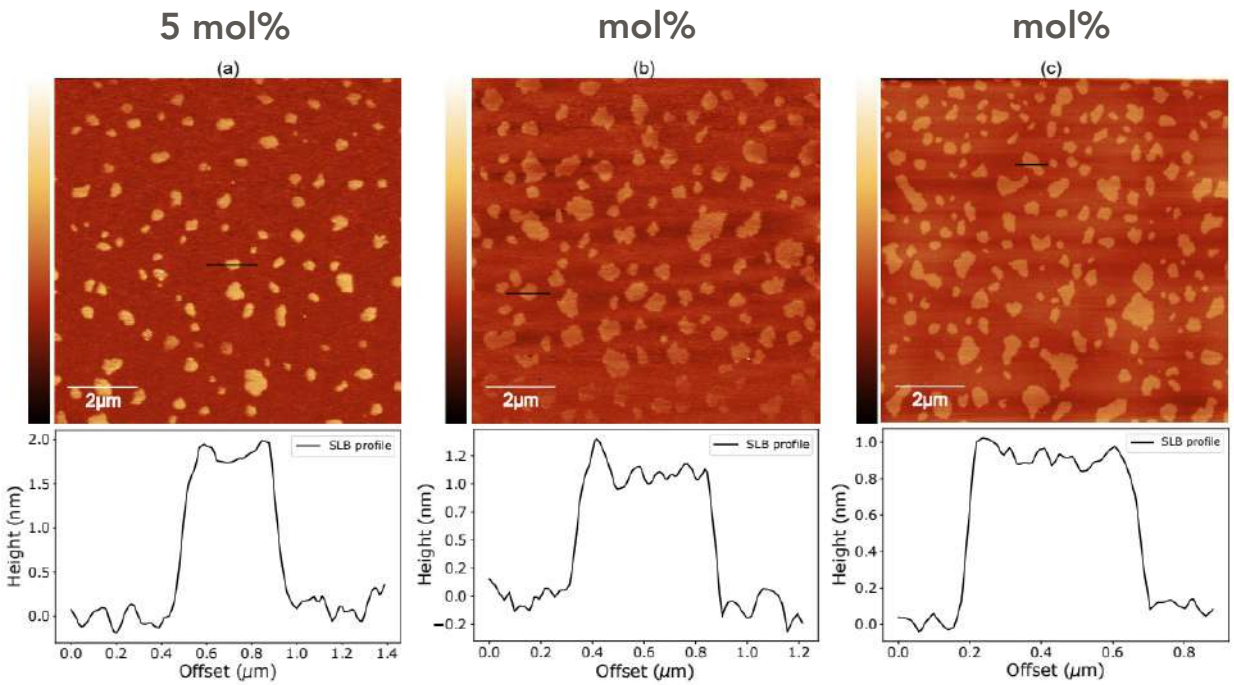


- The final membrane is asymmetric
- Cargo release in this case seems favoured

WHAT about cholesterol?







Paba, et al. Accepted at JCIS – 19.08-23

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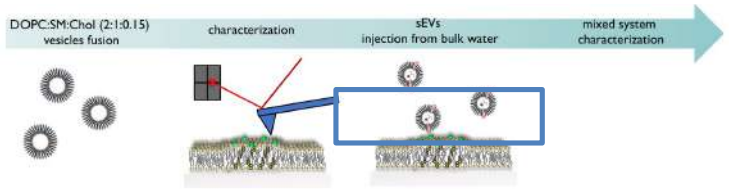
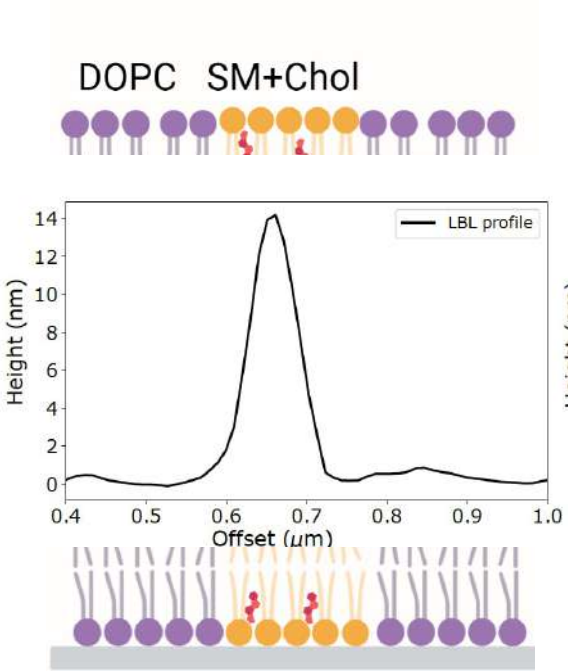
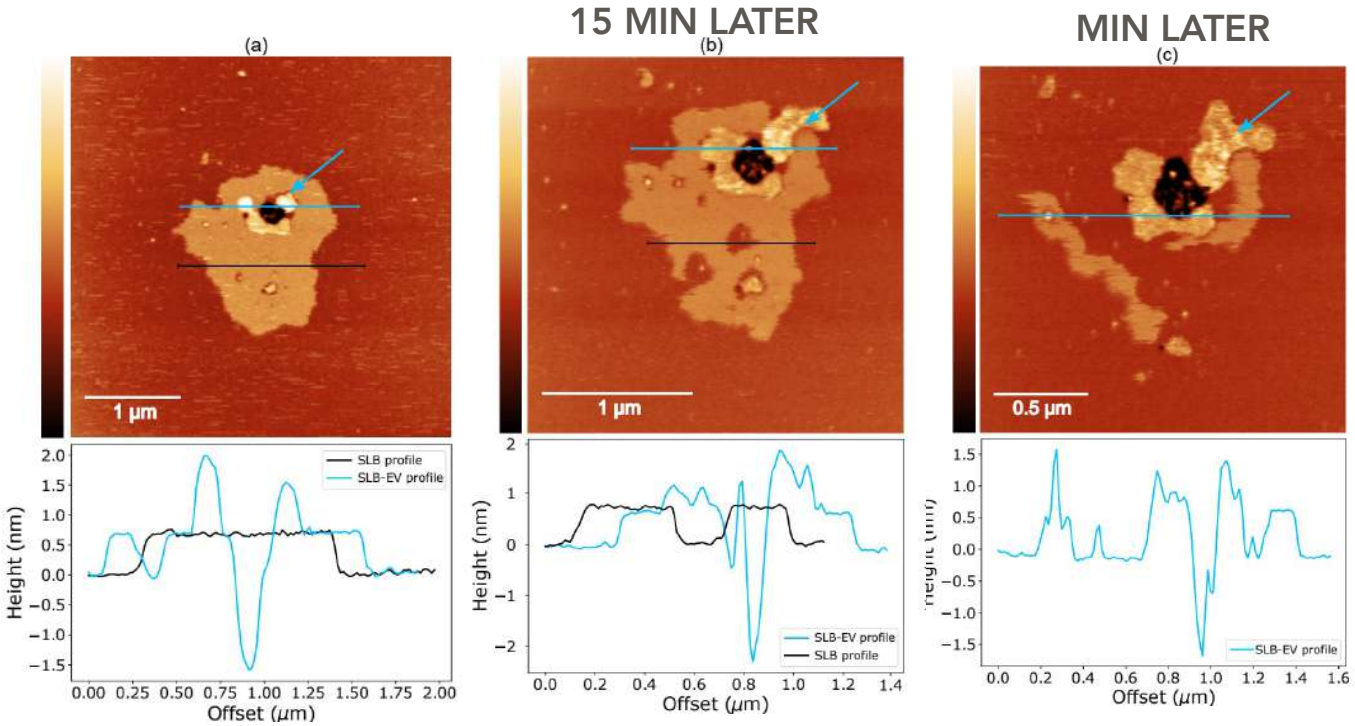
EV FROM TRBC*: ADSORPTION

*







LbL composition: DOPC, SM (2:1) with 17 mol% CHOL

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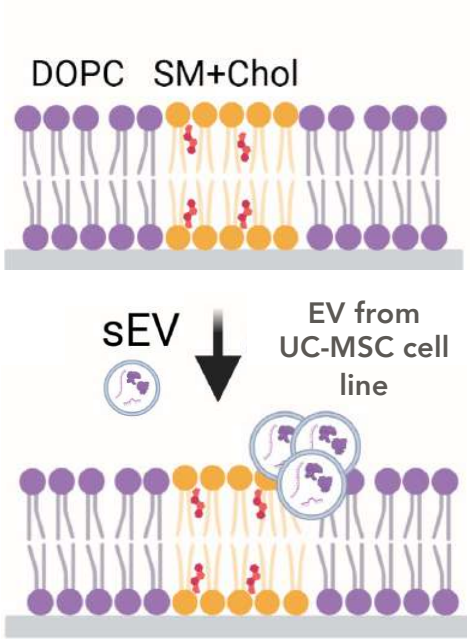
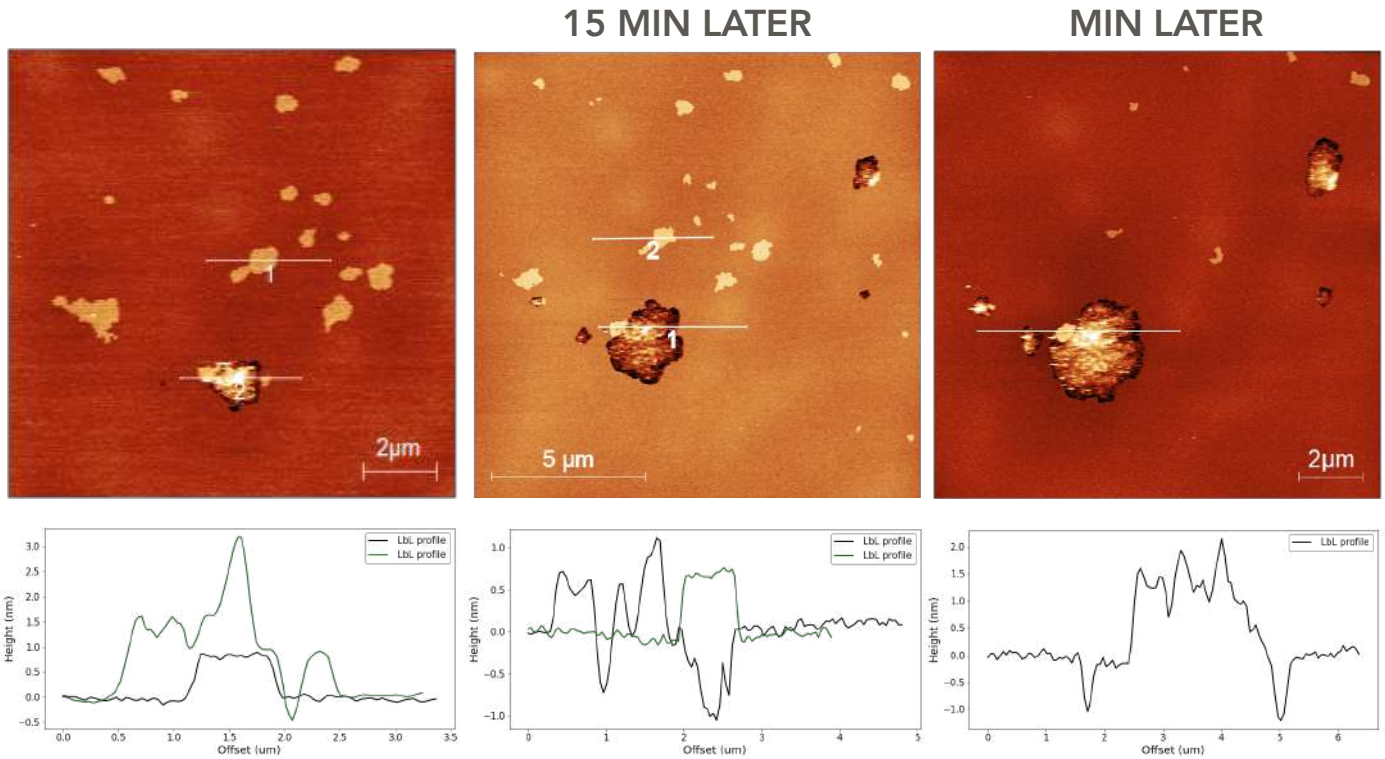
. . . AND IF WE CHANGE ev CELL ORIGIN*?

*



LbL composition: DOPC, SM (2:1) with 17 mol% CHOL



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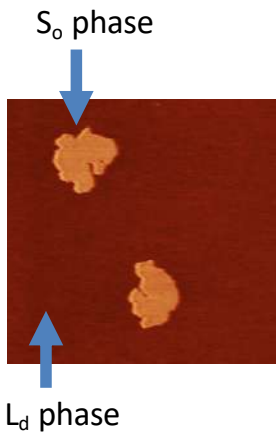
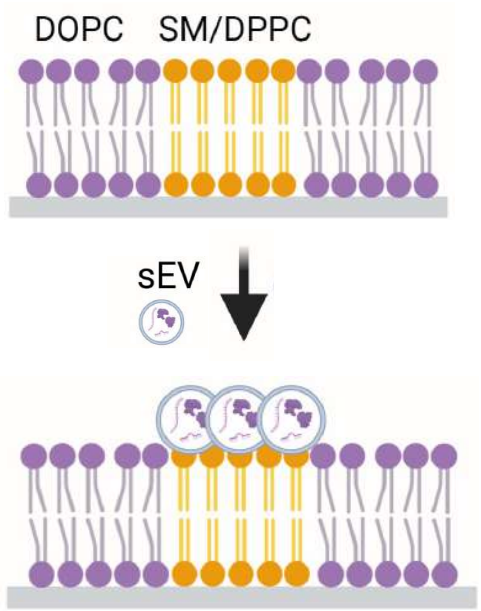
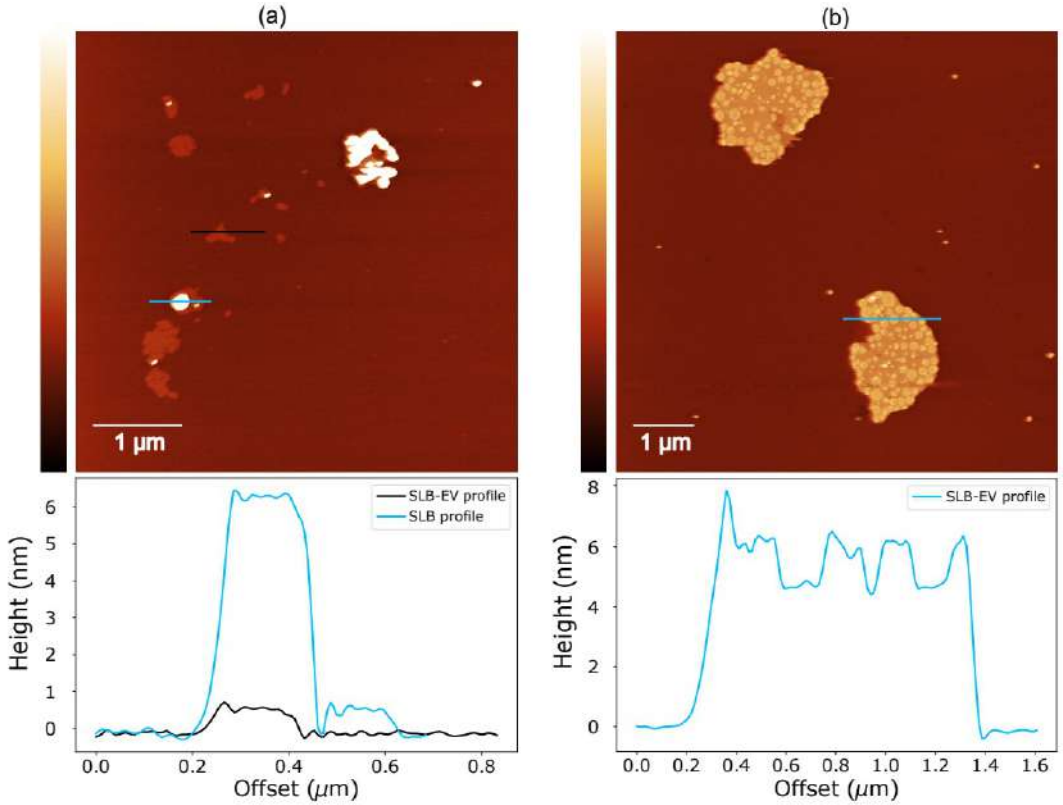
. . . WHAT if we remove chol?

*



EV from MDA-MB-231 cell line





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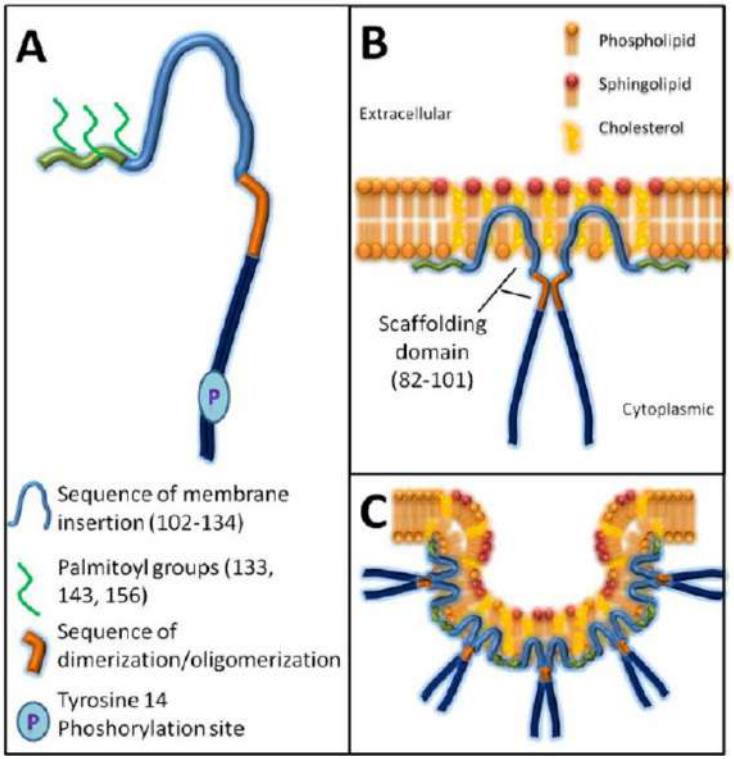
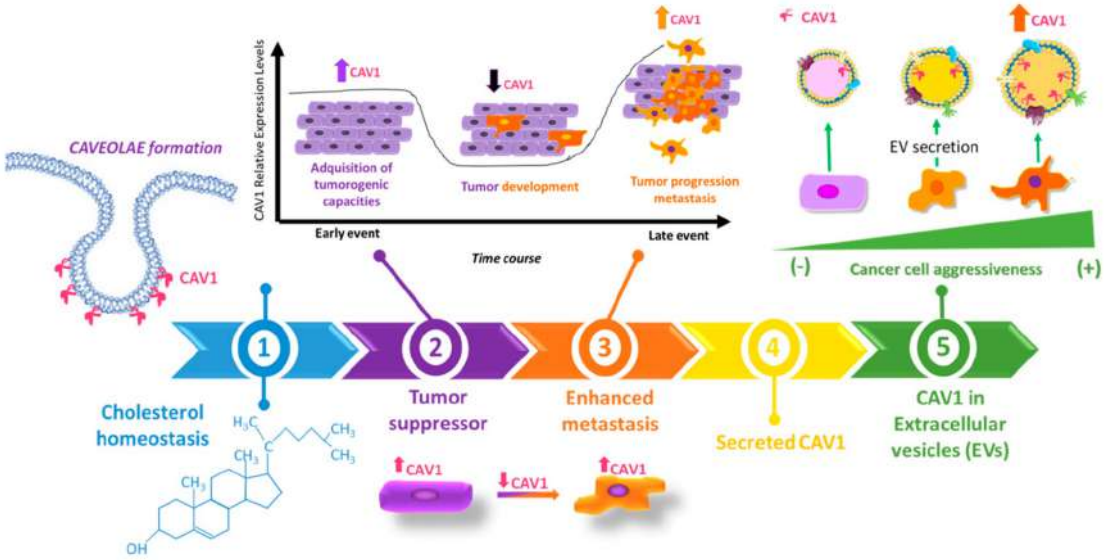
FUTURE PLANS: CAVEOLIN-1





Campos, A., et al., Biomolecules, 2019.

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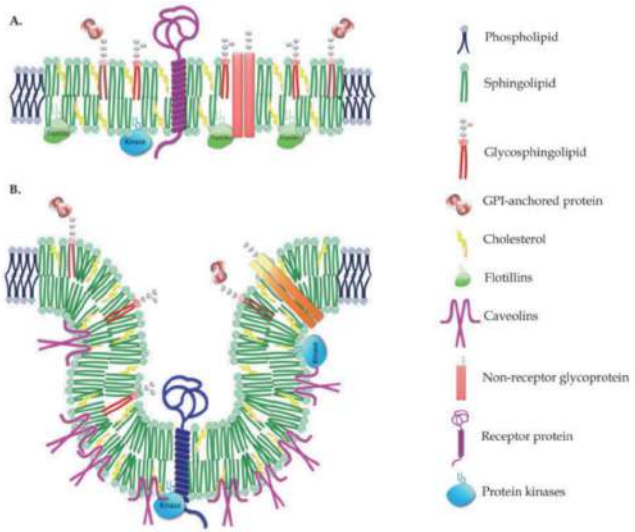
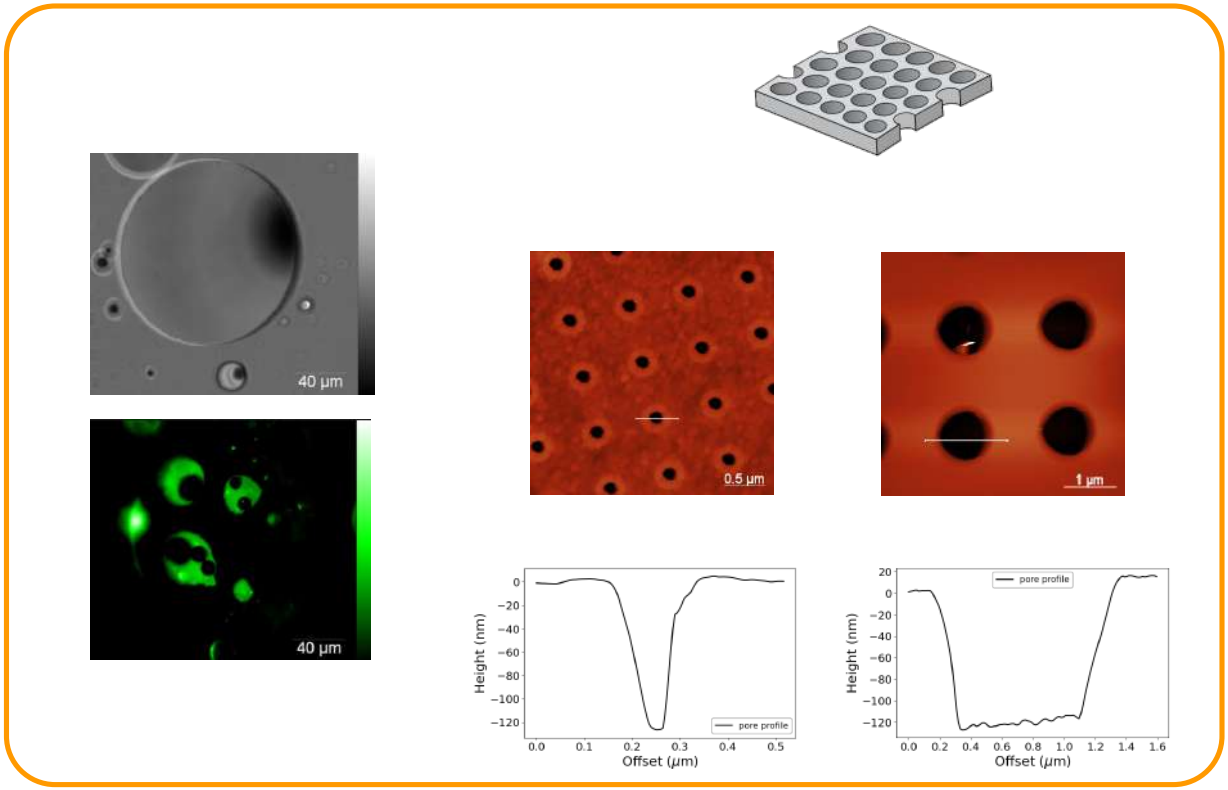
PORE SPANNING MEMBRANE





LUO, Yitian, et al., Structure, 2021.
Zhang, Y., et al., Molecules, 2021.
Gunduz, M., et al., 2011.

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Elettra Sincrotrone Trieste

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Interreg Italia-Österreich
European Regional Development Fund
EUROPEAN UNION

EXOTHERA

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Consortium

Thank you for your attention!

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Intl Institutes homed in Trieste

Users coming for beamtime

All researchers coming to Elettra for a beamtime proposal that has been approved by the Proposal Review Panel.
Access request.

https://www.elettra.trieste.it/userarea/access-request.html BEAMTIME expenses covered for EU scientists

OWSD PhD Fellowship offered to women scientists from science- and technology-lagging countries (STLCs) to undertake PhD research in the natural, engineering and information technology sciences

U. Of the French West Indies might be interested to participate to the programme.

IUPAP Centenary YEARS 1922 - 2022

UNESCO

twas THE WORLD ACADEMY OF SCIENCES

ORGANIZATION FOR WOMEN IN SCIENCE FOR THE DEVELOPING WORLD

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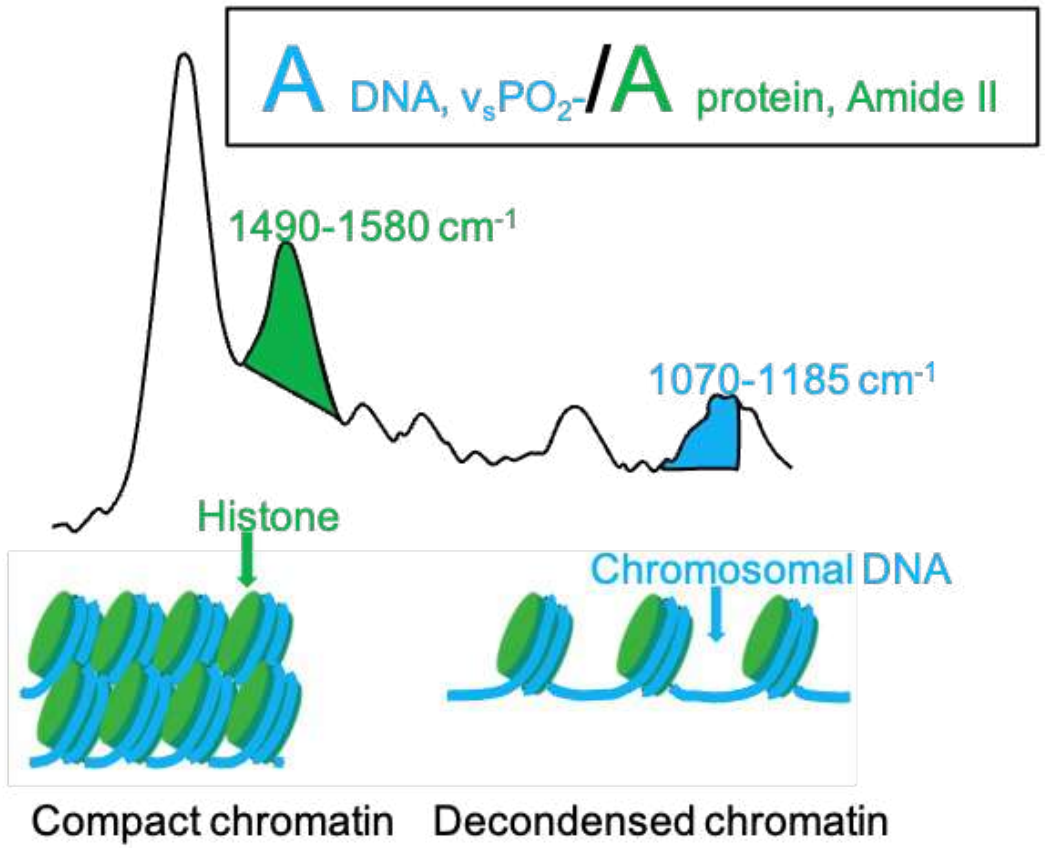
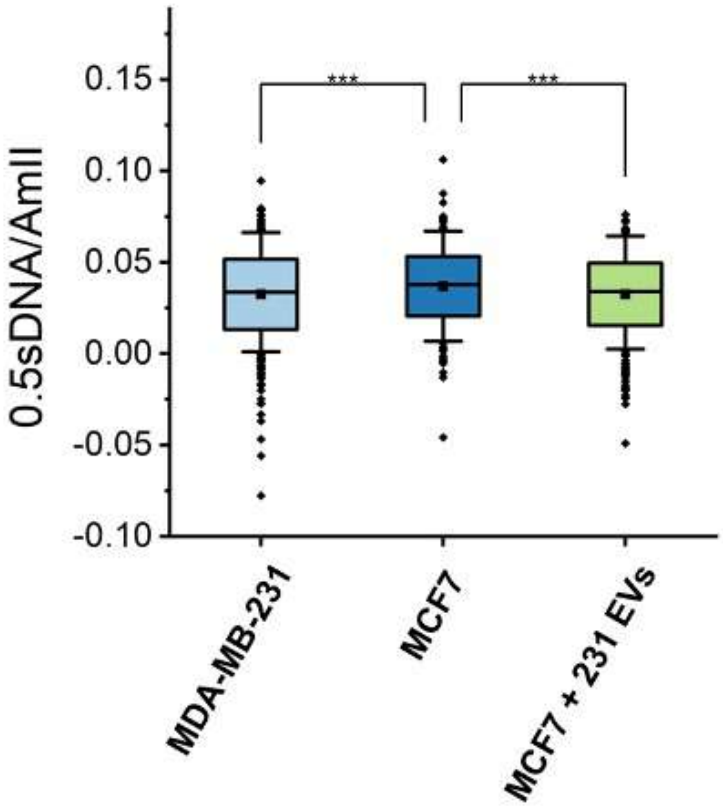
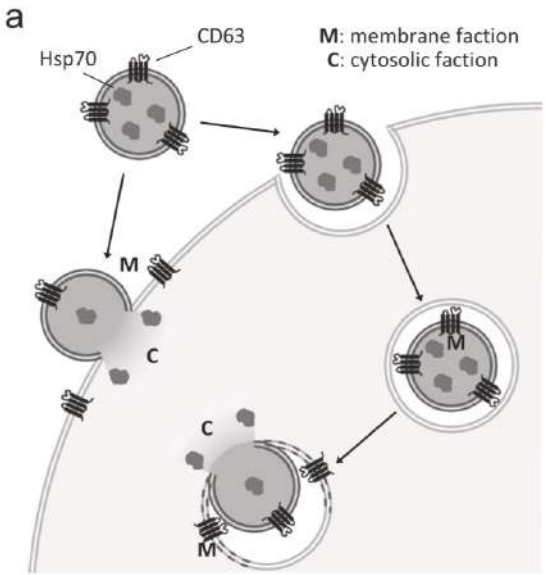
A



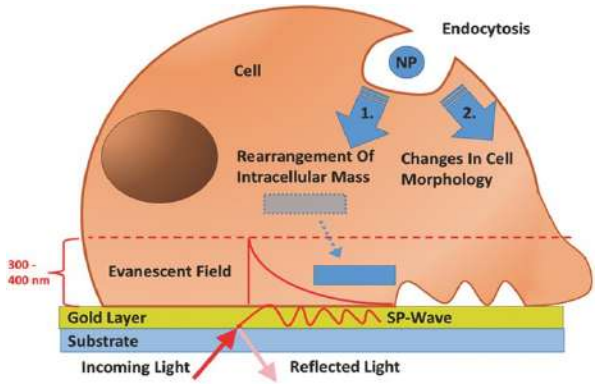
B



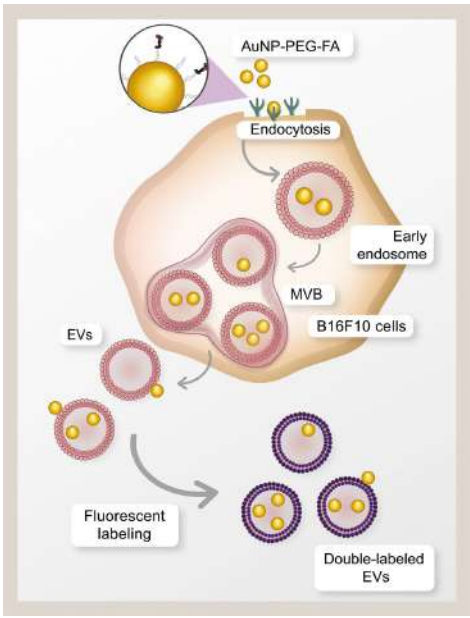
Mechanisms of EVs uptake



Bonsergent et al., Nat Commun 2021, 12, 1864



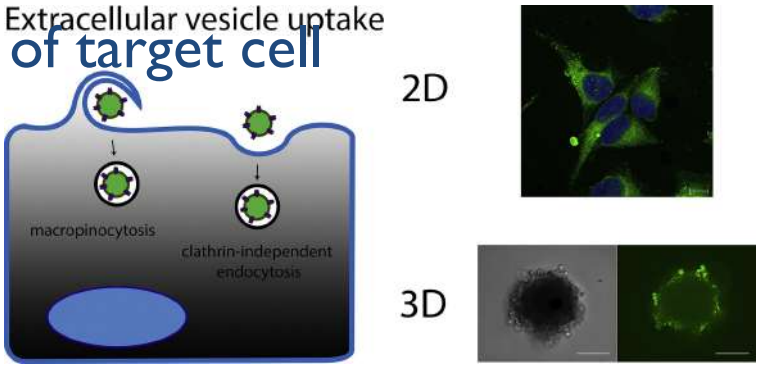
Koponen et al., Biosens. Bioelectronics, 2020, 168, 112510
Suutari et al., Small, 2016, 12, 6289-6300



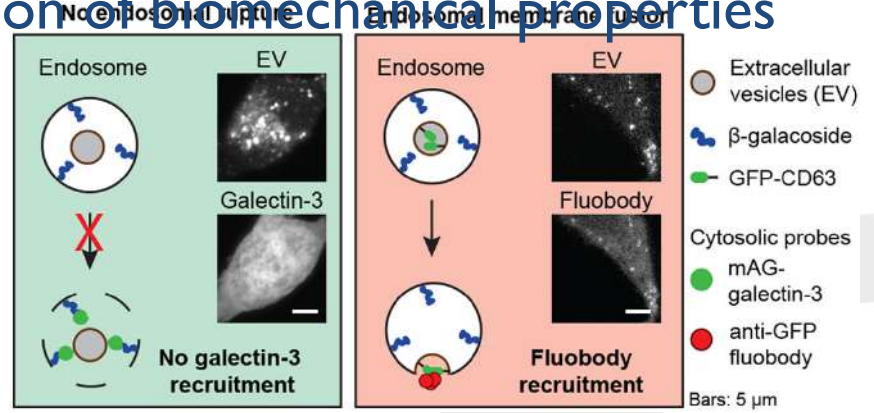
Lara et al.., J Nanobiotechnol 18, 2020, 20

P69 Senigagliesi Beatrice (Wednesday)

Role of Extracellular Vesicles in modulation of biomechanical properties



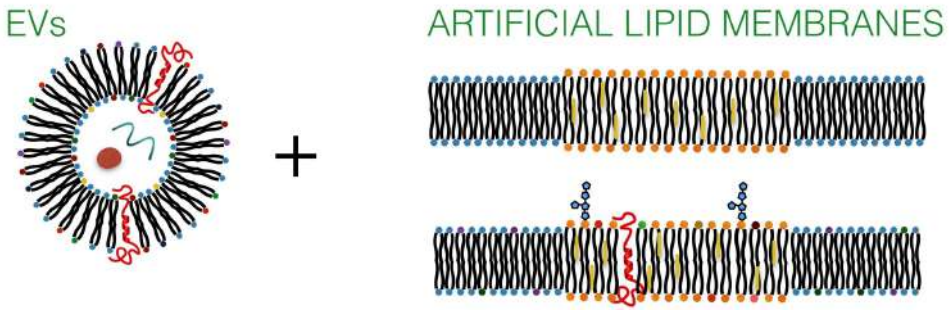
Verdera et al., Journal of Controlled Release, 2017, 266,100-108



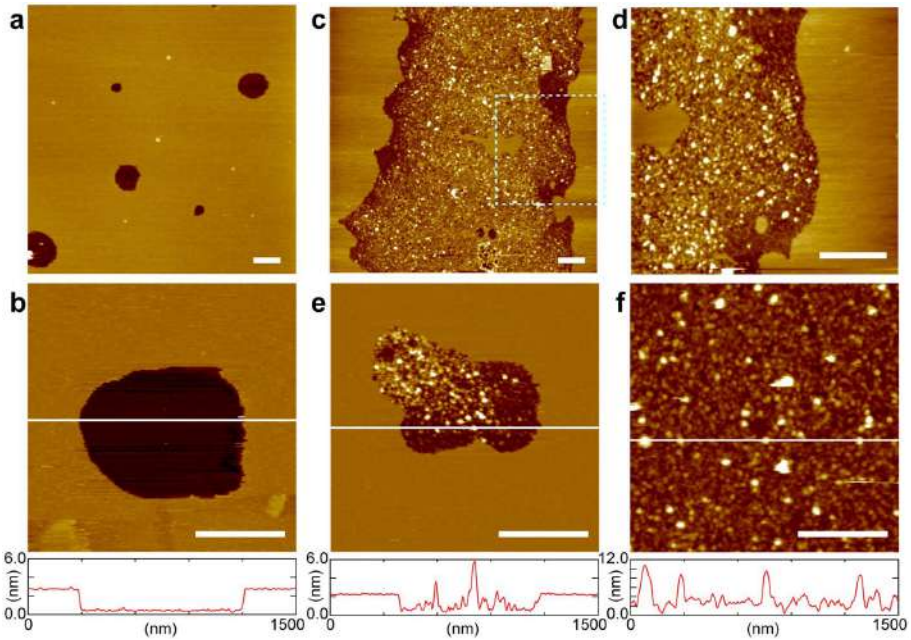
Joshi et al., ACS Nano 2020, 14, 4444–4455

EVs + Model membranes

Multicomponent artificial lipid bilayers for mimicking cellular membranes

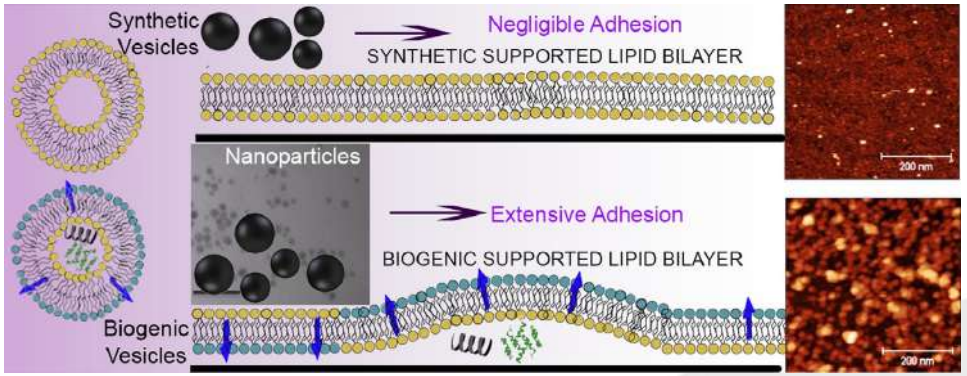


Proteoliposomes + artificial membranes



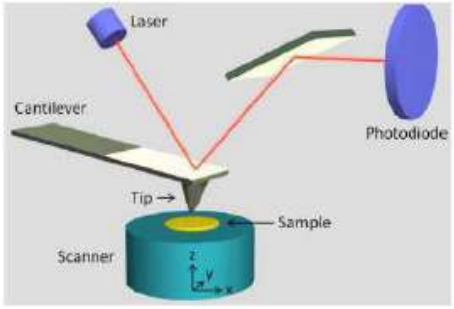
Tero et al., Scientific Reports (2017)

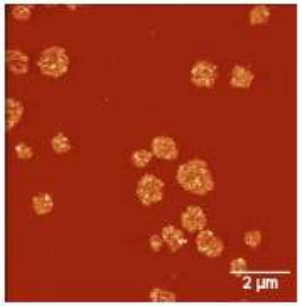
Nanoparticles + artificial membranes/EVs



Montis et al., **Journal of Colloid and Interface Science (2020)**

How do EVs interact with cell membranes? An integrated biophysical approach

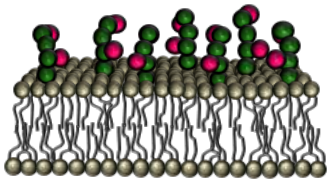




Atomic Force Microscopy

- ✓ **Morphological/mechanical characterization**
- ✓ **(Sub)Nanometer resolution**
- ✓ **Liquid environment**

X-ray



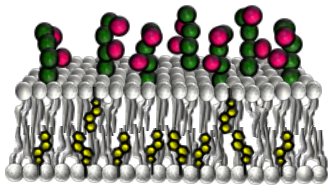
Good contrast for molecules with high electron densities (eg. SUGARS)

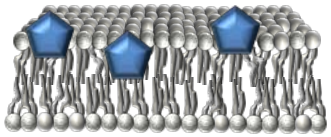
Neutron

Playing with selective deuteration, protiated molecules can be evidenced in a ghost phospholipid matrix

- ✓ Probe relevant length (Å to µm) and time (*ps* to *hr*) scales
- ✓ Non-destructive
- ✓ Possibility of selective deuteration to play with contrast
- ✓ Deep material penetration (buried systems)

Membrane components distribution

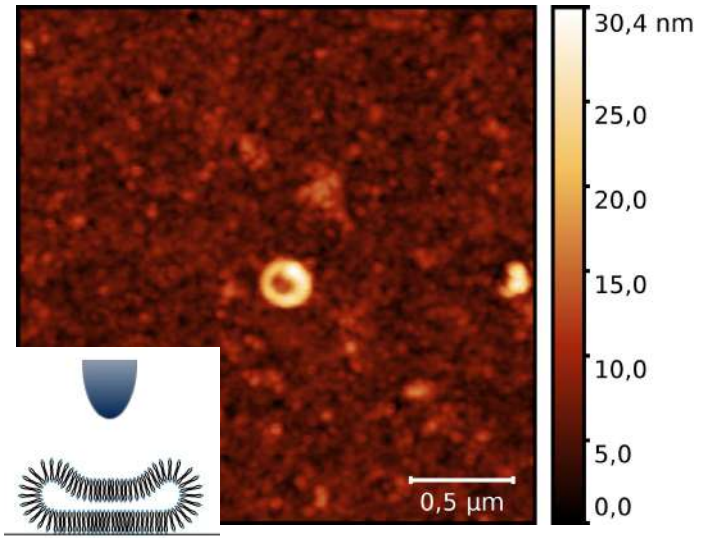




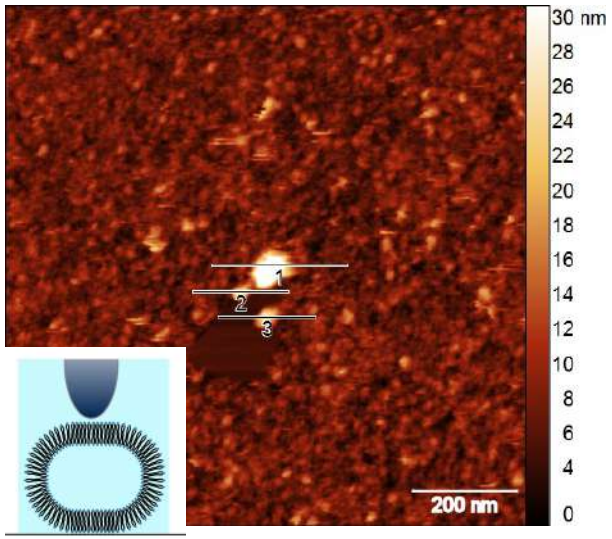
External interacting molecules distribution

AFM imaging of single EVs

Air

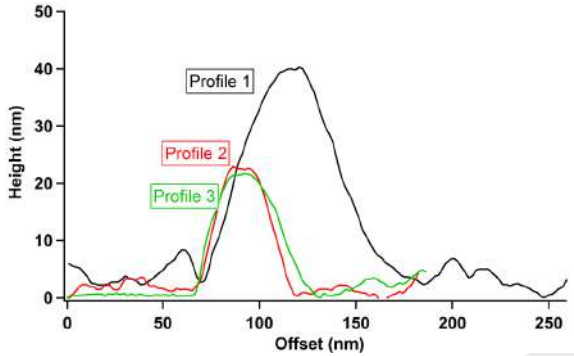


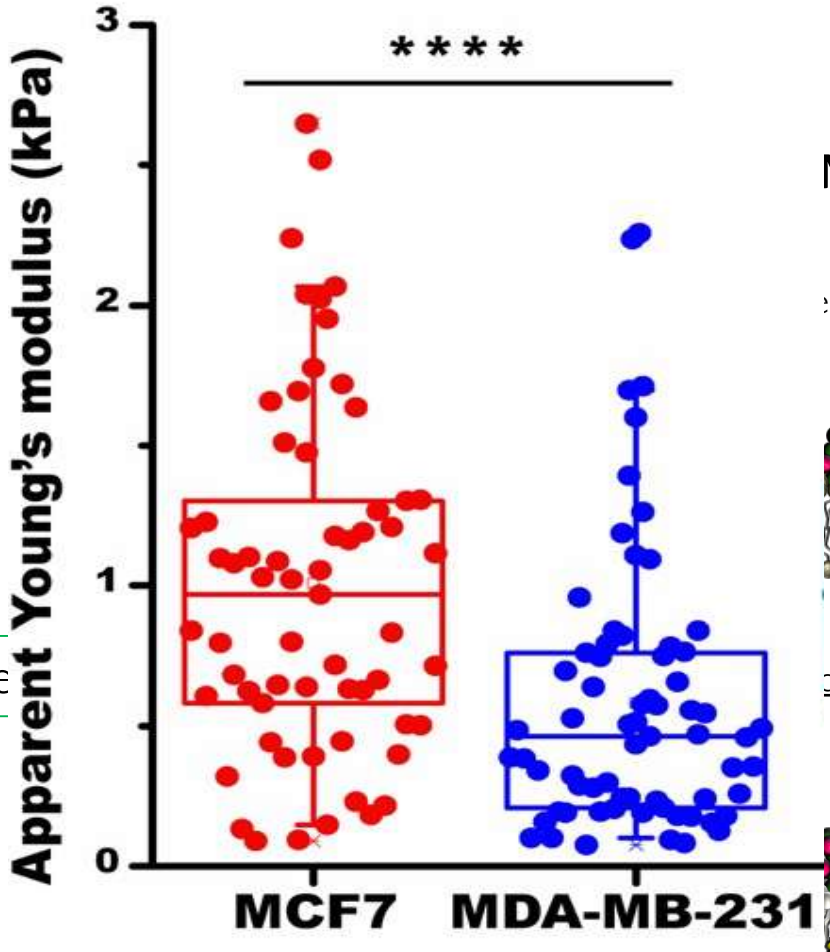
Liquid



EVs derived from Umbilical cord Stem Cells

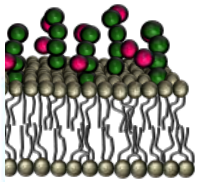
- Tangential Flow Fractionation
  +
  Ultracentrifuge





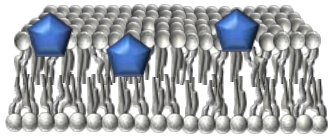
NS WITH LIPIDS?

ecules with high electron densities (eg. SUGARS)

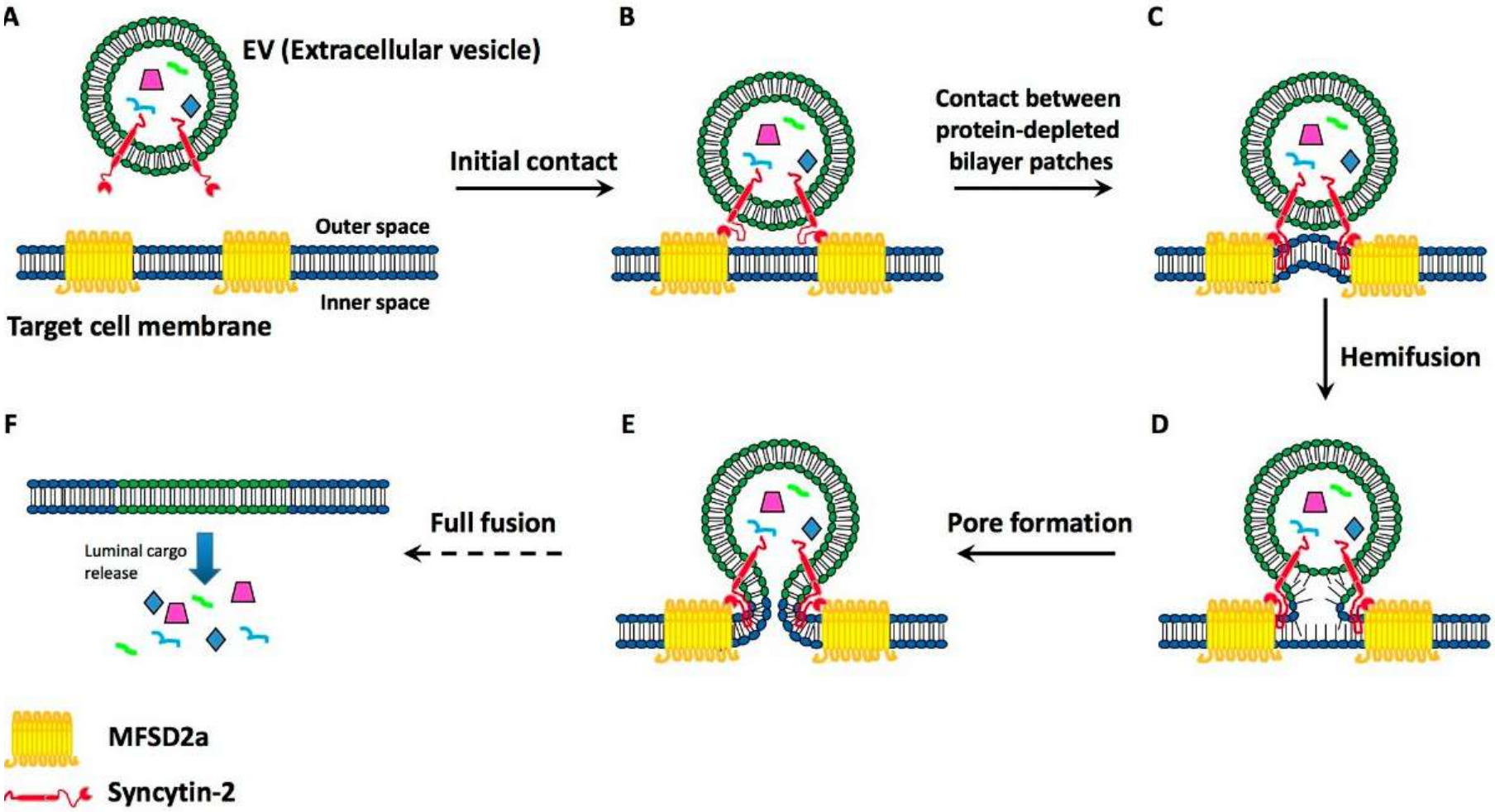


Ne d molecules can be evidenced in a ghost phospholipid matrix and vice versa

Membrane components distribution



External interacting molecules distribution



I.Prada, J. Meldolesi, Int. J. Mol. Sci. 2016