Models of cell membrane domains for surface interactions structural investigation

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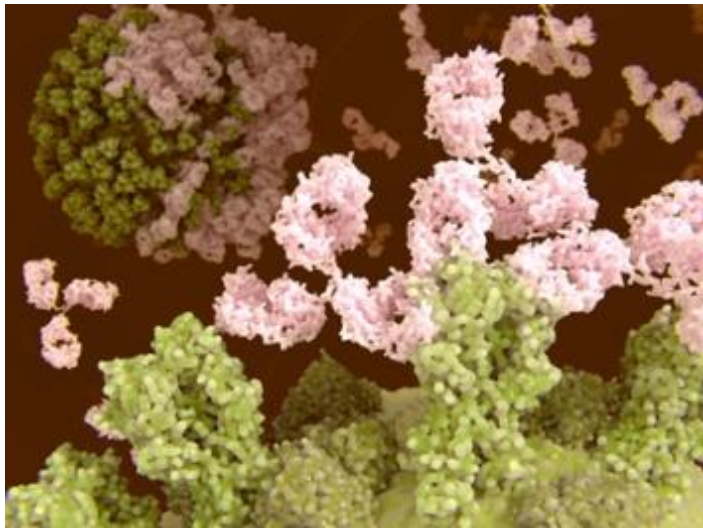
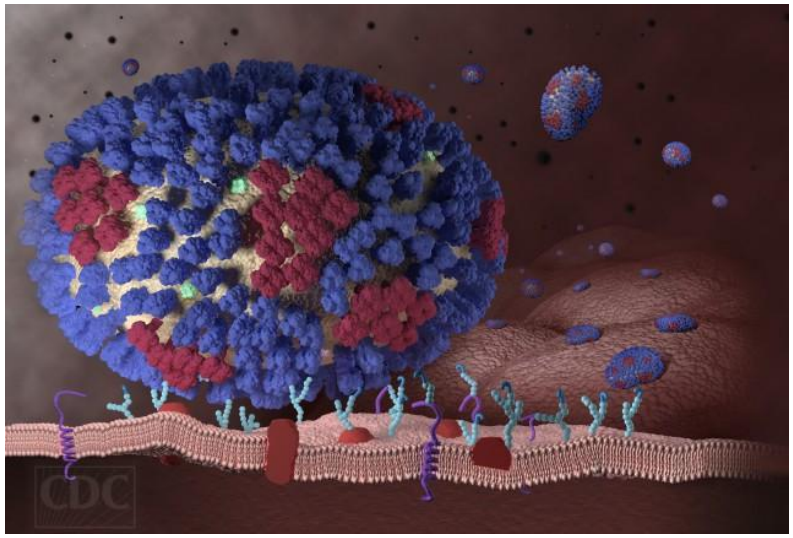


CELL MEMBRANE
INTERACTION WITH APPROACHING BODIES



Biology question is far...

what can we face?

→ ***simplified*** systems

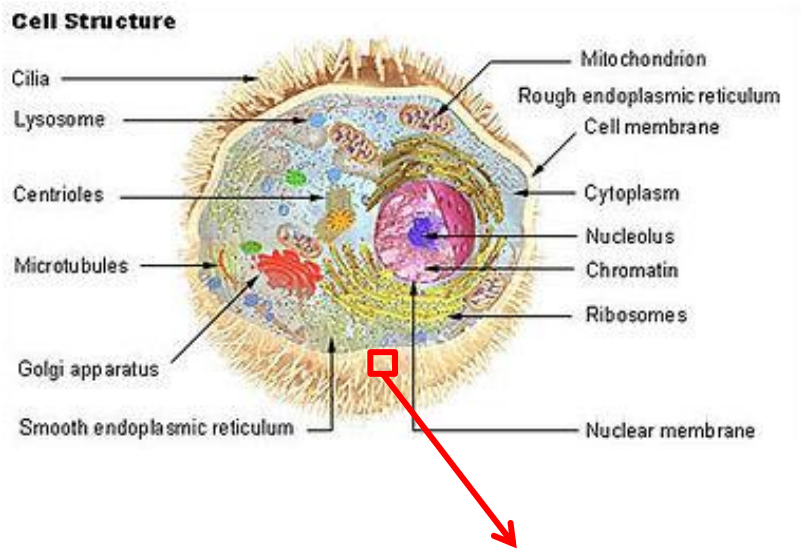
keeping the ***main 'bio'-features***

Challenging aspects *-> The model*

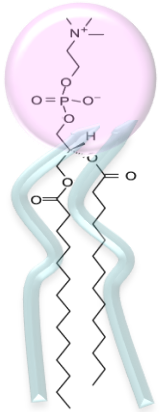
-> The possibility to investigate it (the technique)

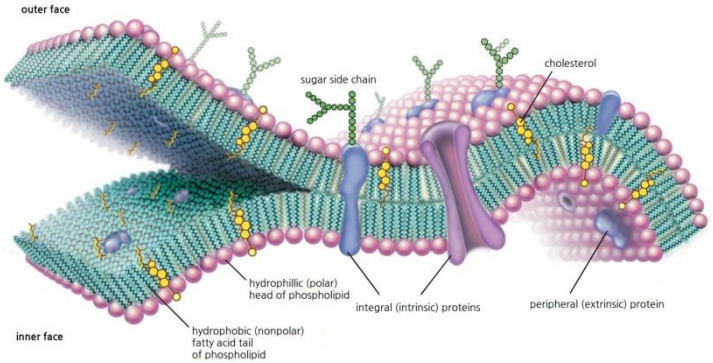
THE SYSTEM

Phospholipid membranes



Cell membrane





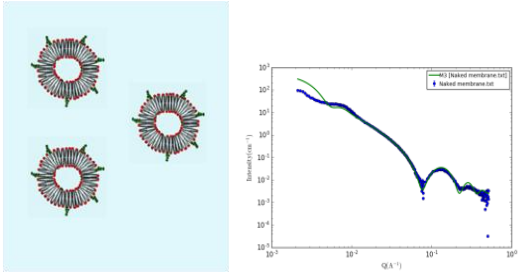
Structure

Dynamics

Activity

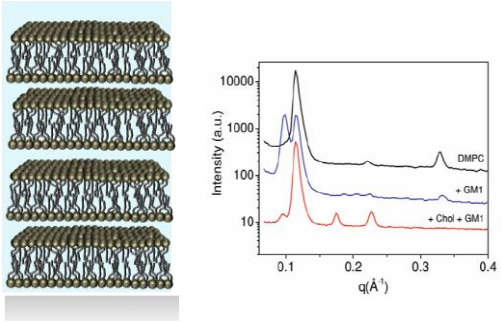
COMPLEMENTARY TECHNIQUES FOR MEMBRANE STRUCTURAL INVESTIGATION

SANS/SAXS from monolamellar vesicles



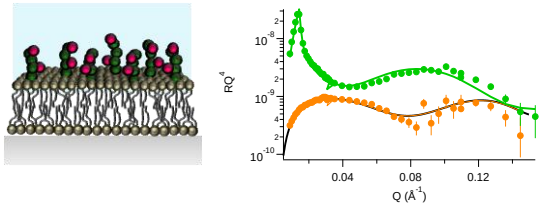
Membrane form factor
Aggregate shape
Structuring in solution

N/XD from membrane stacks



Membrane thickness/thicknesses (lateral domains)

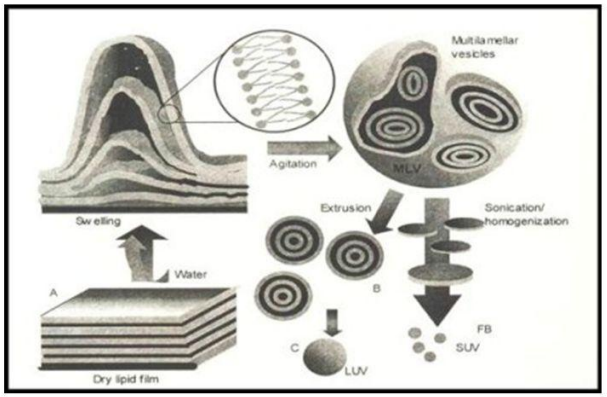
N/XR from single supported membranes

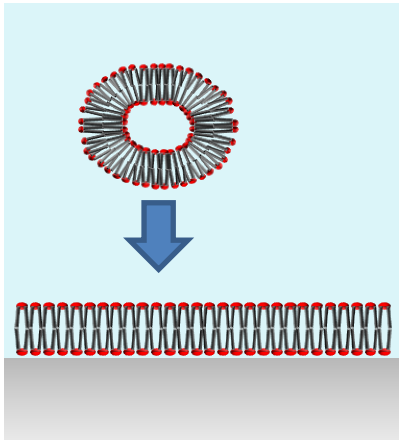


Membrane transverse structure

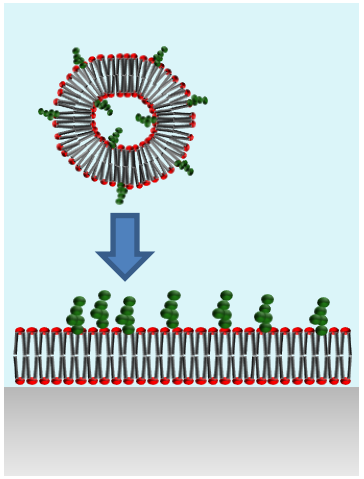
DIFFERENT METHODS FOR MEMBRANE DEPOSITION
Vesicle Fusion

Liposomes Preparation



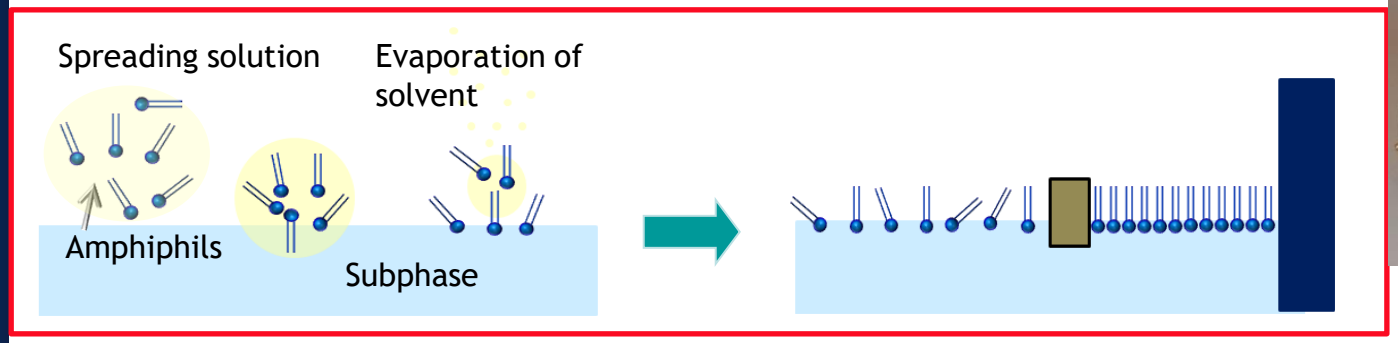


Fusion on solid supports

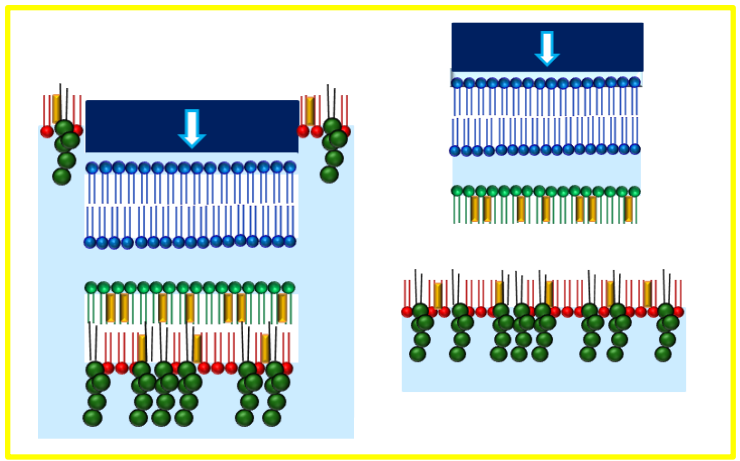


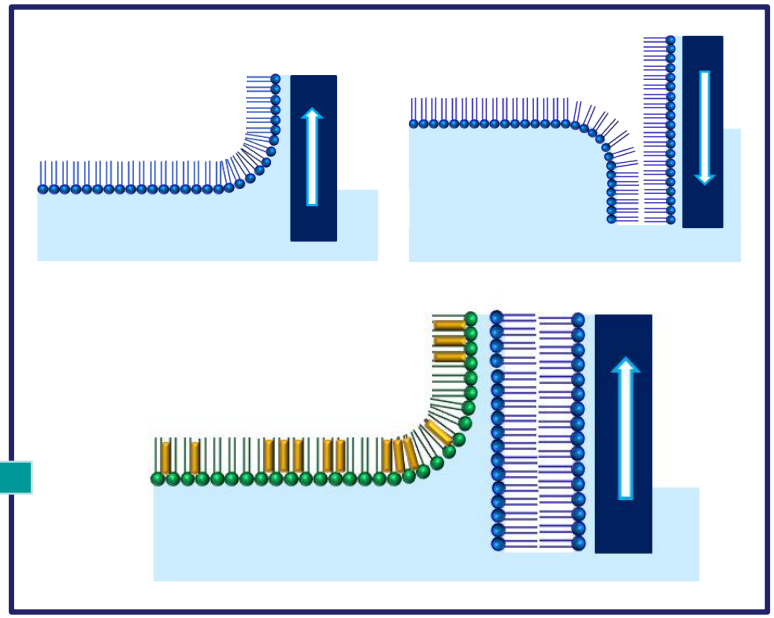
Multicomponent vesicle fusion

V. Rondelli et al., Biophys Chem (2017)



Langmuir Blodgett – Langmuir Schaefer technique





HOMOGENEOUS DEPOSITION (***5 nm thick***) OVER **LARGE AREAS (*5x8 cm²*)** OF MONOLAYERS CONTROLLED IN **NUMBER** AND **COMPOSITION**

BIOMETRA

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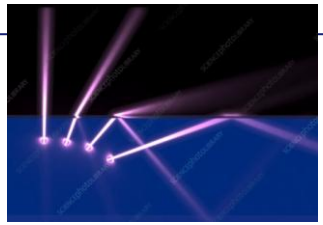
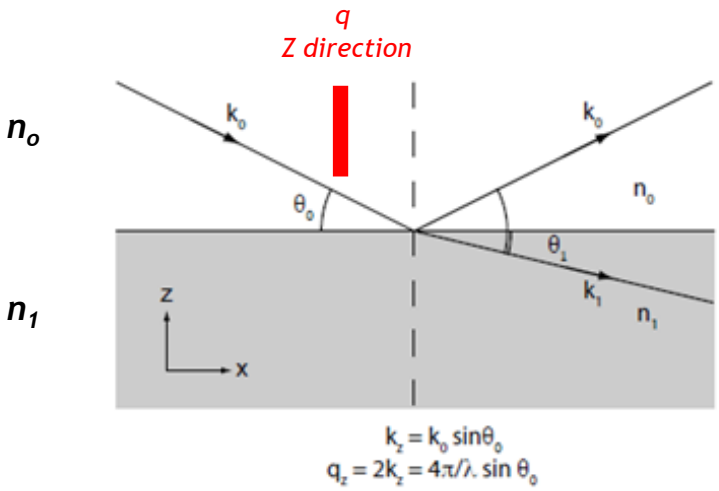
SPECULAR REFLECTION FROM FLAT INTERFACES

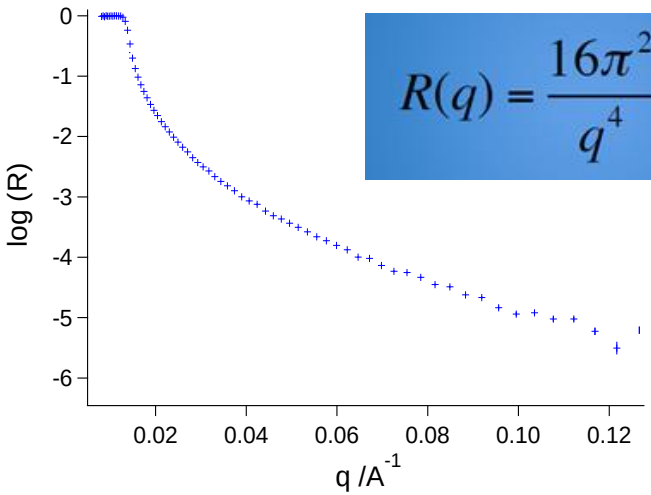




If $\theta_i = \theta_r$

Specular reflection

The intensity of the reflected radiation depends on n_o and n_1



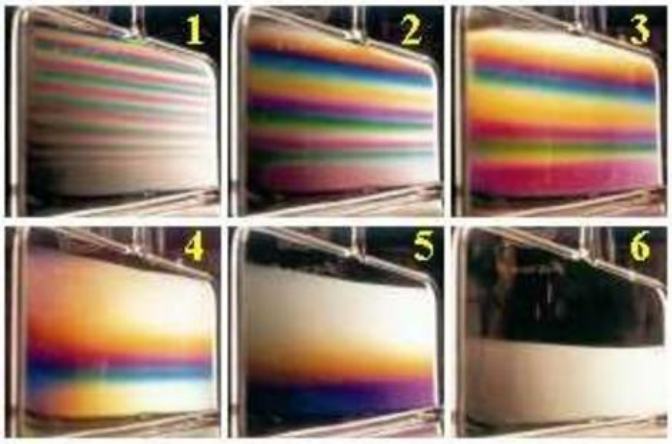
$$R(q) = \frac{16\pi^2}{q^4}\left|N_b'(q)\right|^2$$

$$N_b = \frac{\sum_i n_i b_i}{V}$$

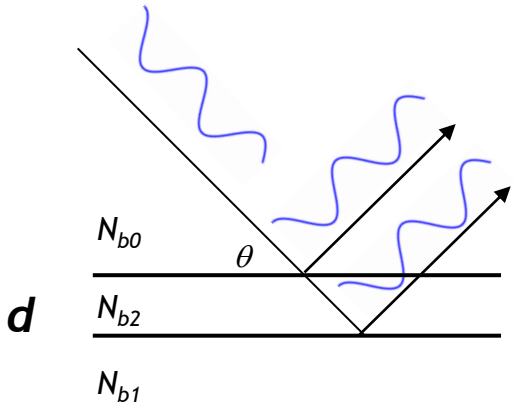
Scattering Length Density (SLD)

SPECULAR REFLECTION FROM MULTILAYERS

More than 1 interface:

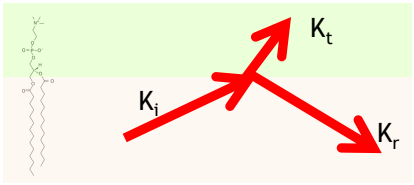


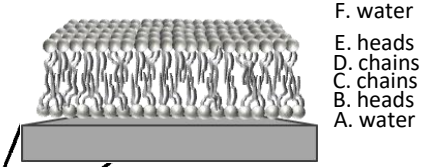
Newton, 1675

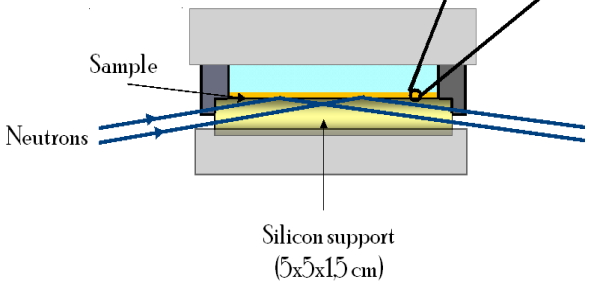


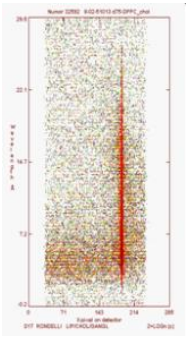
Interference depends on **thickness** ***d*** *as well as on the* **refractive indexes** N_{bi}

REFLECTOMETRY ON LIPID MEMBRANES



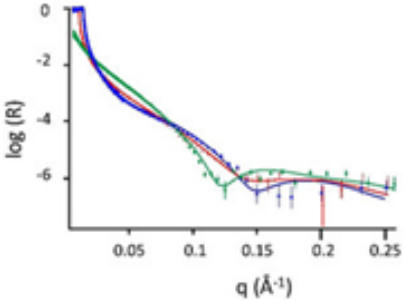


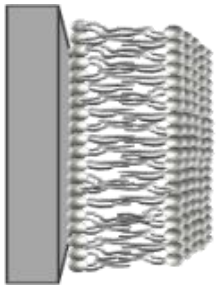


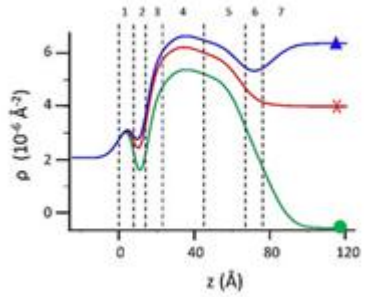


$$R \approx \left(\frac{16\pi^2}{q^4} N_b^2 \right) e^{-q_z^2 \sigma^2}$$

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







SAMPLE CROSS PROFILE

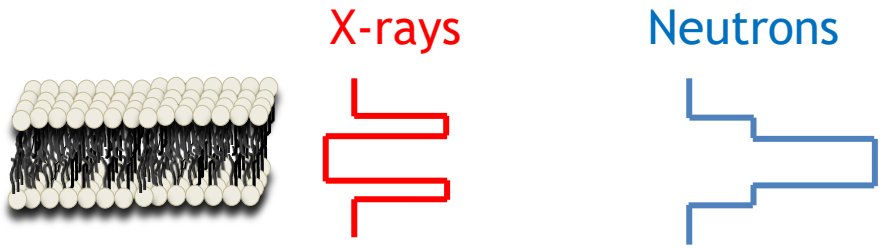
Information about the transverse structure of the sample, layer by layer:

thickness, composition, compactness, roughness

WHY DIFFERENT RADIATIONS?

$$I(q) \div c\ M\ P(q)\ S(q)\ \text{contrast}^2$$

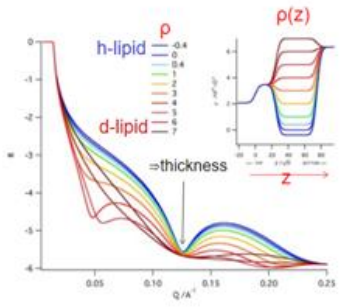
X-ray — **Good contrast for SUGARS (high electron density)**



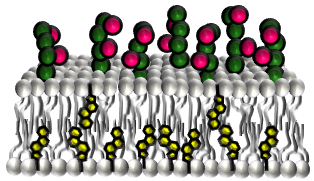
Neutron

Hydrogen coherent scattering length : -3.74×10^{-5} Å
Deuterium coherent scattering length : $+6.67 \times 10^{-5}$ Å

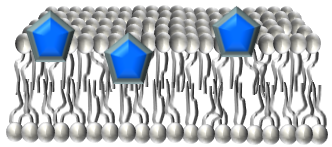
Playing with selective deuteration **<u>protiated molecules can be evidenced in the deuterated phospholipid matrix</u>**



H. Wacklin, ESS



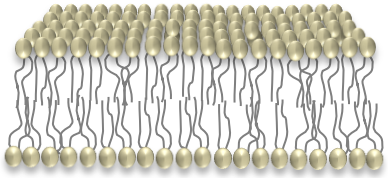
Membrane components distribution



External interacting molecules distribution

MEMBRANE MODELS

phospholipids



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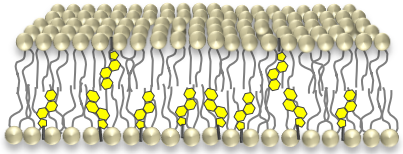
cholesterol



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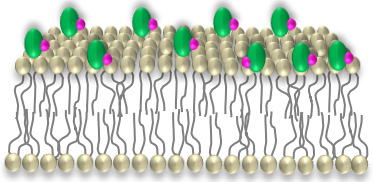
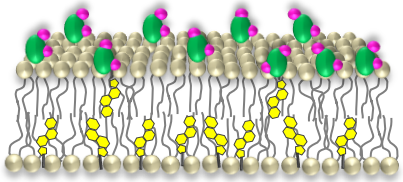
glycolipids



raft models



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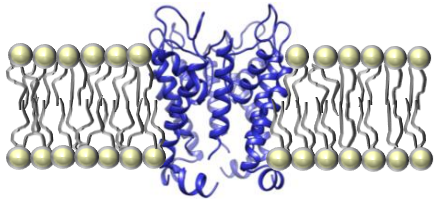
transmembrane protein (K^+ channel)



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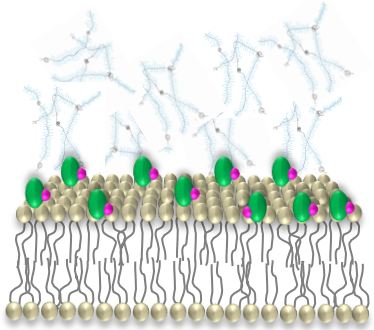
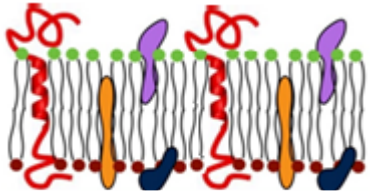
gel forming proteins (mucin)

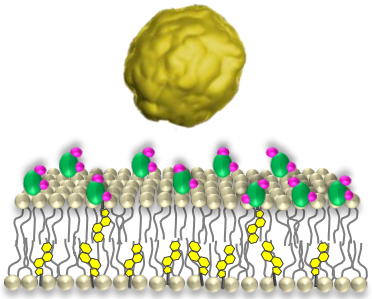


from natural vesicles/membrane extracts

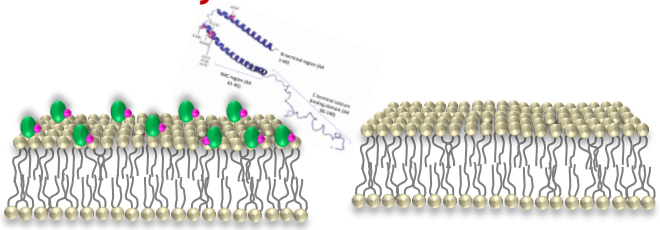


WE CAN MODEL INTERACTIONS AT MEMBRANE SURFACE

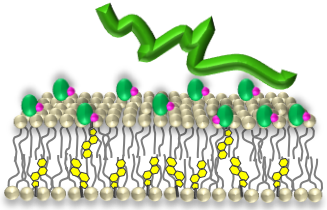
Enzymatic digestion on membrane surface



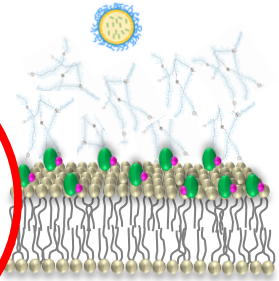
α-synuclein interaction



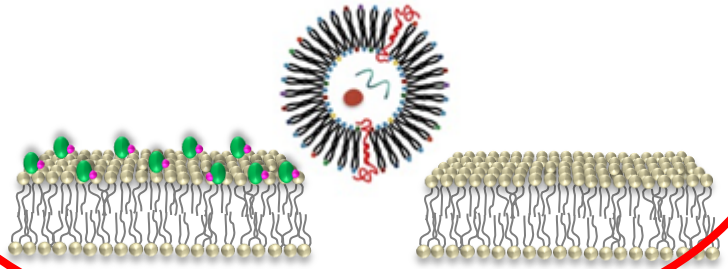
Aß Peptide interaction



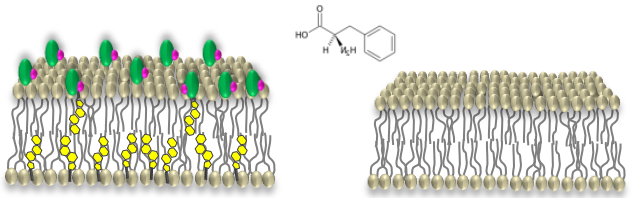
Transmucosal delivery



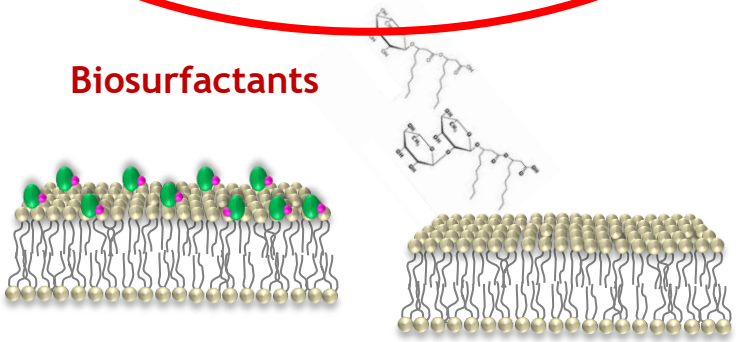
Extracellular vesicles interaction



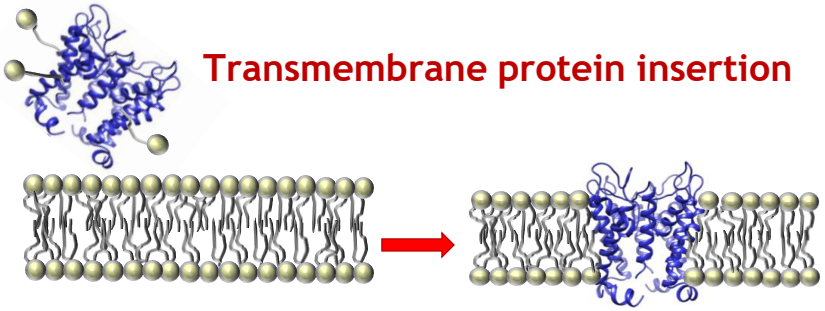
Amino acid interaction



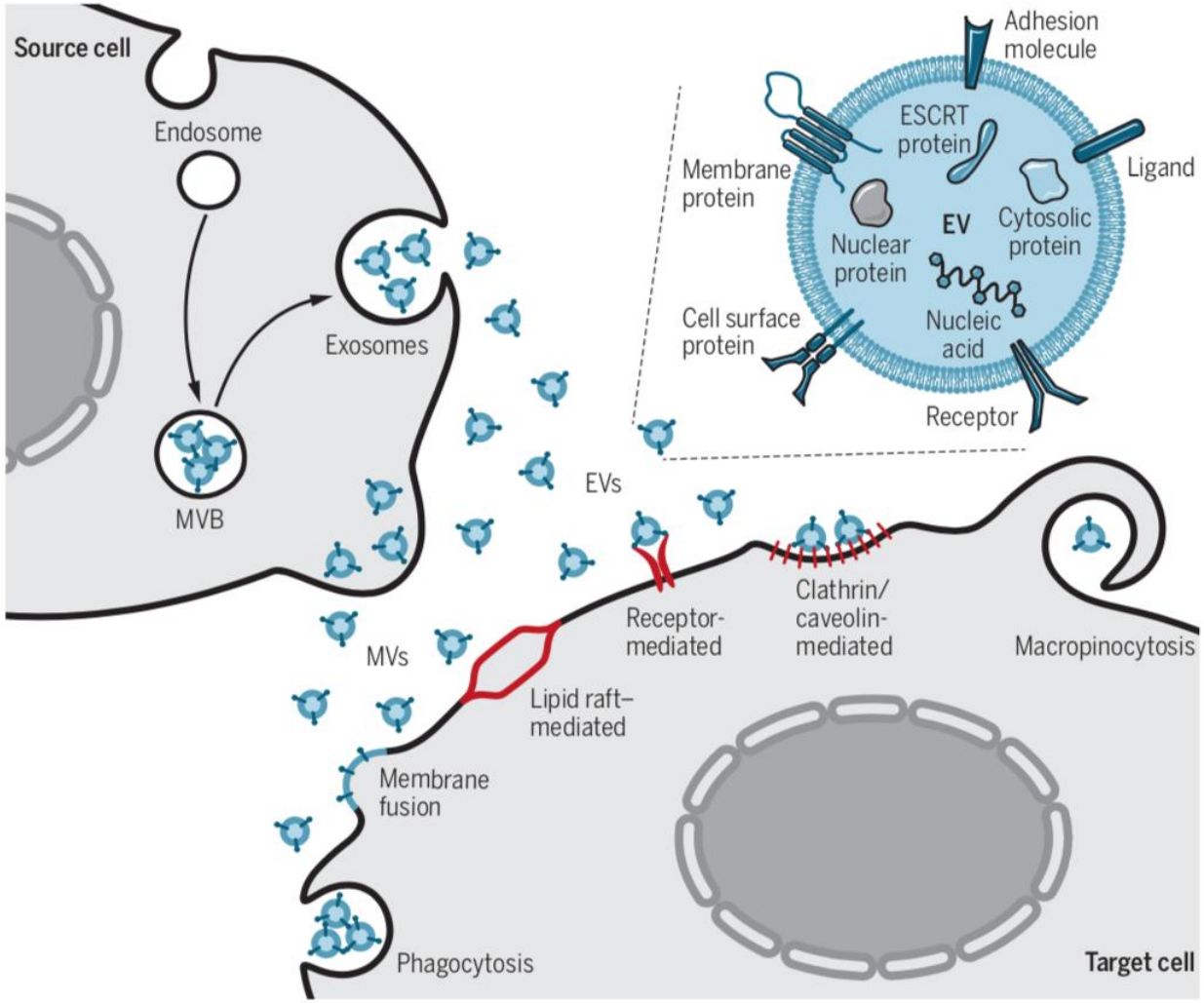
Biosurfactants



Transmembrane protein insertion



EXTRACELLULAR VESICLES



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

GAPS

- ✓ Nanoscale spatio-temporal details on how different EVs interact with target cells
- ✓ Factors influencing the biogenesis and release of the molecular cargo

KEY CONCEPTS

→**Uptake** dynamics and mechanisms are tightly related to the **potency and function of EVs**

→**Evs** play a key-role in **diseases spreading**

? **Understanding the molecular basis of diseases (eg. cancer, neuro-degenerative)**

→ **devise of EVs-based therapies**

BIOMETRA

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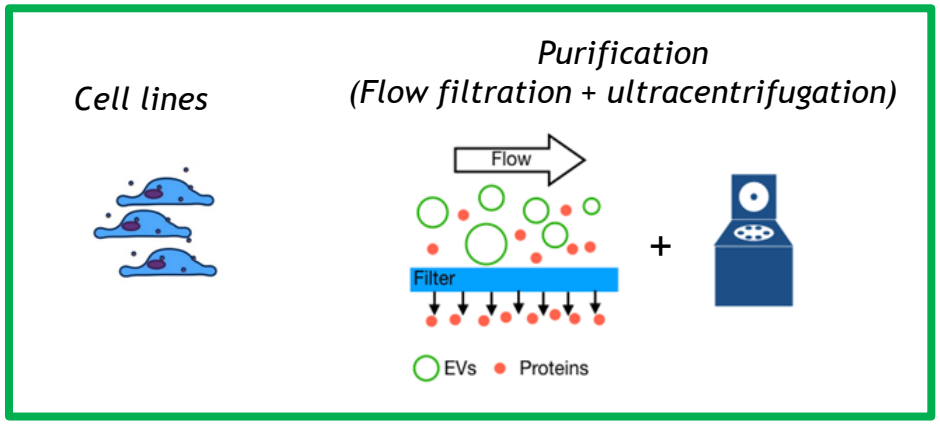
OUR STRATEGY

sEVs

Standardized protocols and Good Manufacturing Practice conditions to derive **highly stable vesicles** of **defined size** and **reproducible molecular profiles** from Umbilical Cord multipotent Mesenchymal Stem (Stromal) Cells (MSCs)

1. **Isolation** and **Characterization** of sEVs



- ✓*Nanoparticle Tracking Analysis*
- ✓*Light Scattering*
- ✓*Surface marker analysis*
- ✓*Cryo-Electron Microscopy*
- ✓*Small Angle X-Ray Scattering*
- ✓*Small Angle Neutron Scattering*
- ✓*Atomic Force Microscopy*
- ✓*Neutron Reflectometry*

2. **Investigation** of sEVs uptake mechanisms

- ✓*Atomic Force Microscopy*
- ✓*Small Angle Neutron Scattering*
- ✓*Neutron Reflectometry*

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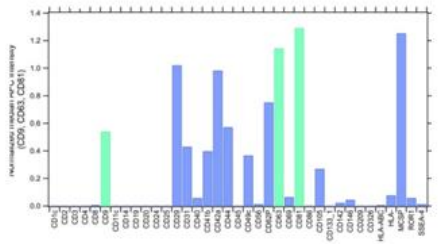
sEVS CHARACTERIZATION

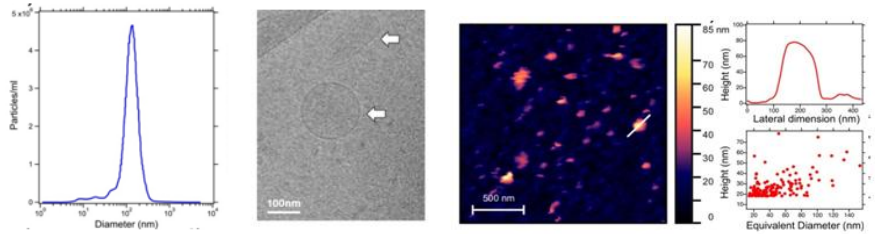
Surface marker analysis

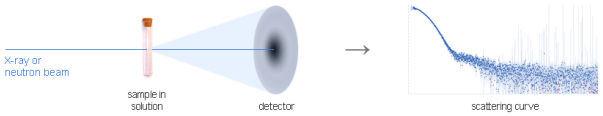
typical sEV/exosome markers (tetraspanins CD9, CD63 and CD81)



DLS
NTA
Cryo-EM
AFM

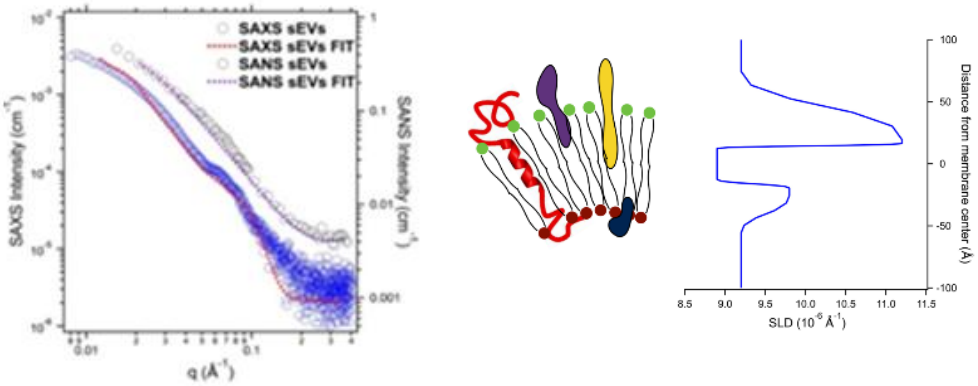
particles sized less than 50 nm + sEVs sized ~ 100 nm

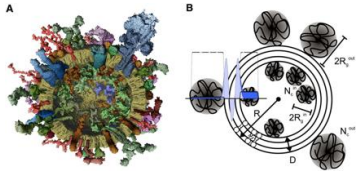




SAXS
SANS

3-layered membrane model, accounting for the proteomic component extending in the extra-vesicular solution.



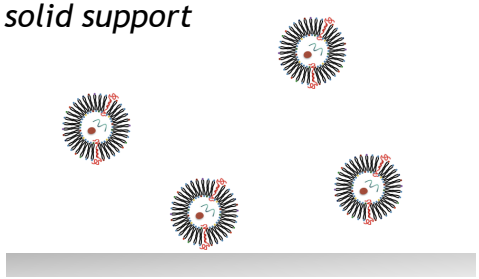


Castorph et al. Biophysical Journal (2010)

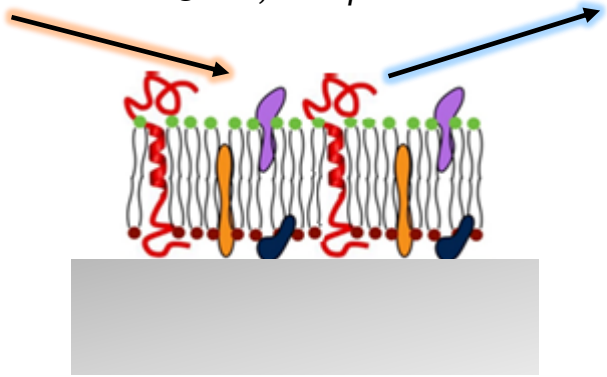
(Synaptic vesicles)

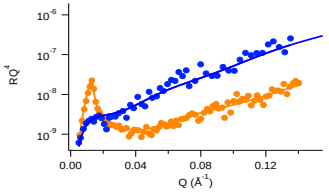
NEUTRON REFLECTOMETRY ON sEVS-DERIVED SUPPORTED BILAYERS

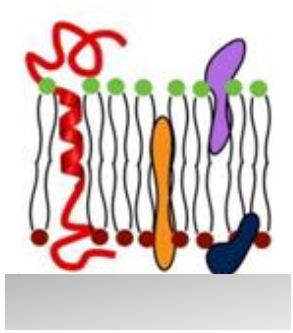
Extracellular Vesicles fusion on solid support

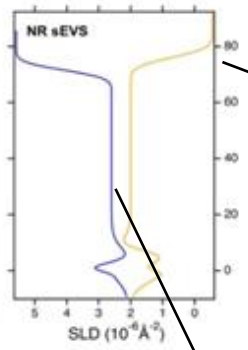


Neutron reflectometry @BNC, Budapest









Thickness:

6.9 ± 0.2 nm

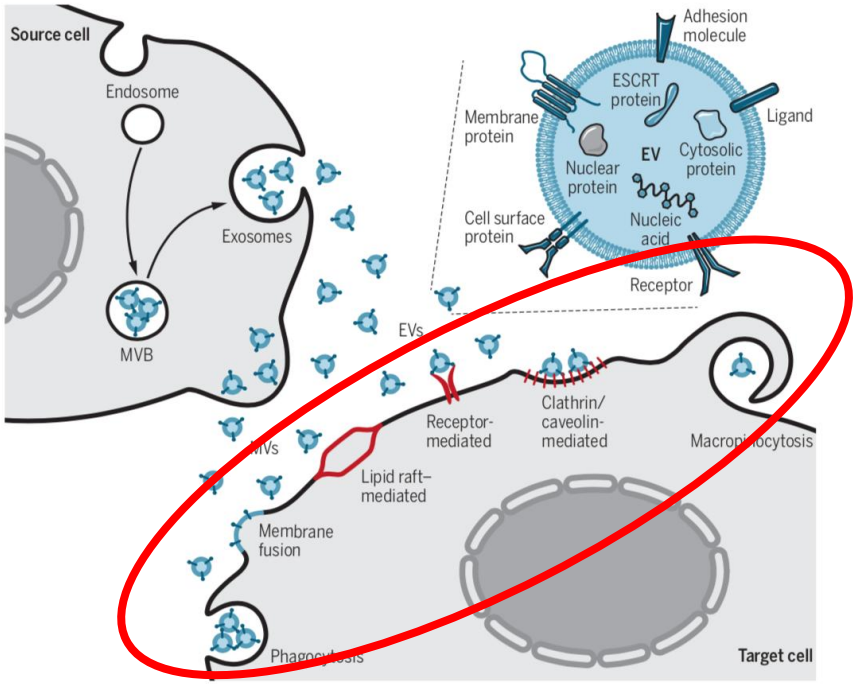
single bilayer containing molecules other than lipids, as **large proteins**

SLD 2 ± 0.2 x10^{-6} Å^{-2}

lipid : protein
22 : 78
(by volume)

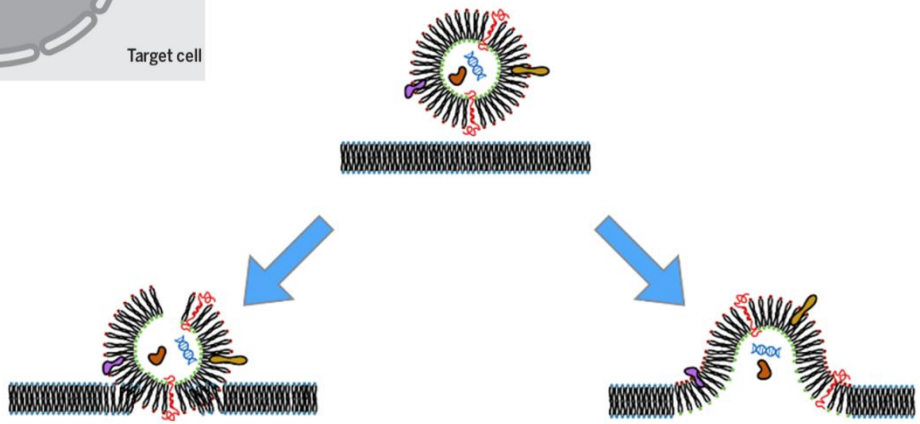
$$R \approx \left(\frac{16\pi^2}{q^4} N_b^2 \right) e^{-q_z^2 \sigma^2}$$

sEVS UPTAKE MECHANISMS



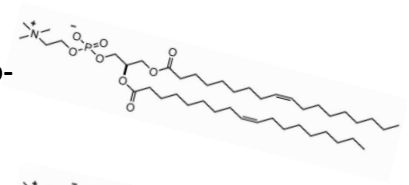
Wiklander et al., Science Trans. Medicine (2019)

Cargo release may be favoured or prevented

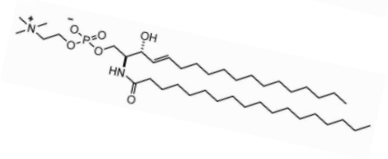


PREFERENTIAL SITE OF INTERACTION

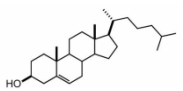
1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC)



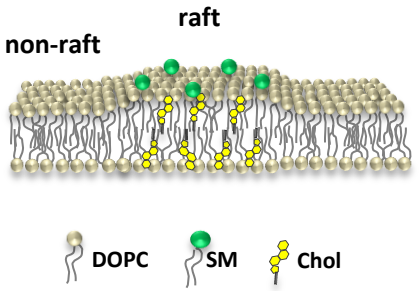
Sphingomyelin (SM)



Cholesterol (Chol)



DOPC:SM:Chol 2:1:0.15 mol

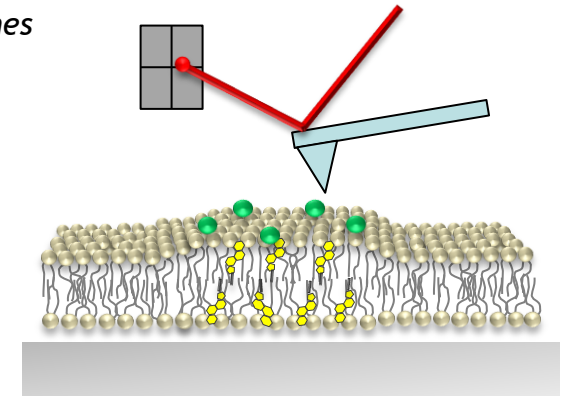


AFM

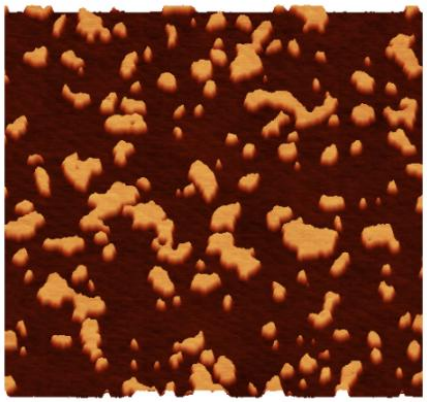
@ ELETTRA, Trieste

Fabio Perissinotto
Pietro Parisse
Loredana Casalis

Supported membranes by vesicle fusion

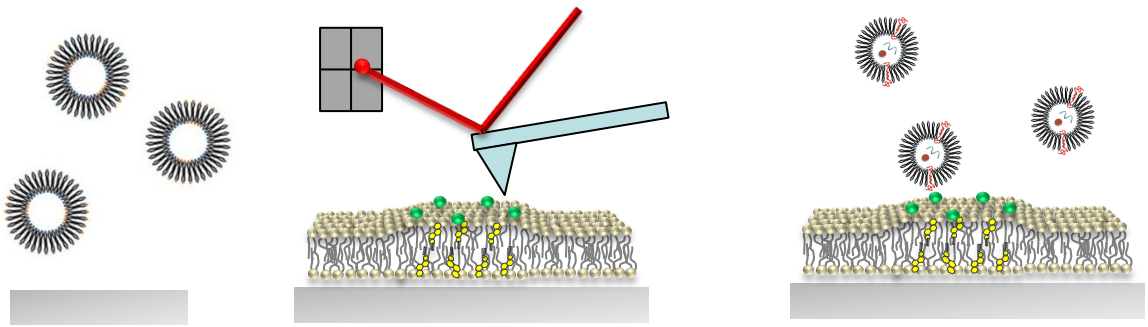


Lipid phase separation

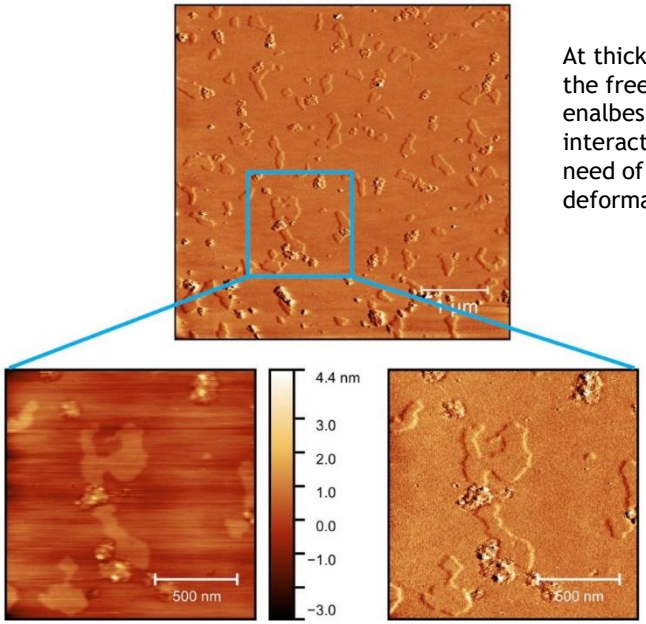


PHASE BORDERS ARE DOCKING SITES

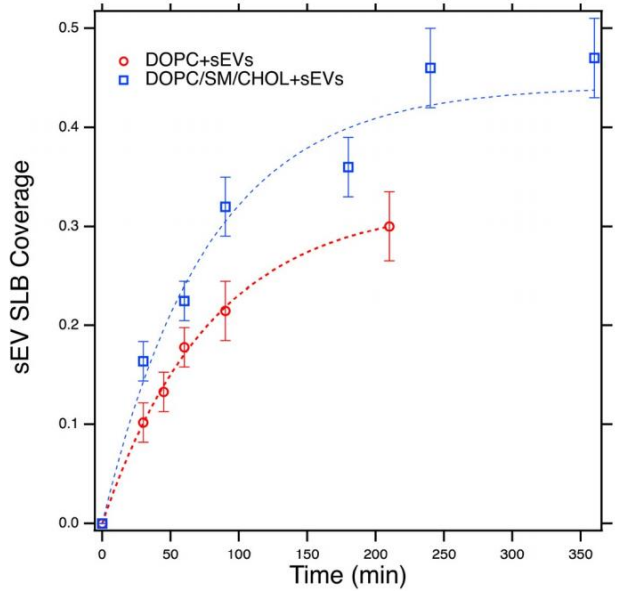
DOPC:SM:Chol (2:1:0.15) vesicles fusion → *characterization* → *sEVs injection from bulk water* → *mixed system characterization*



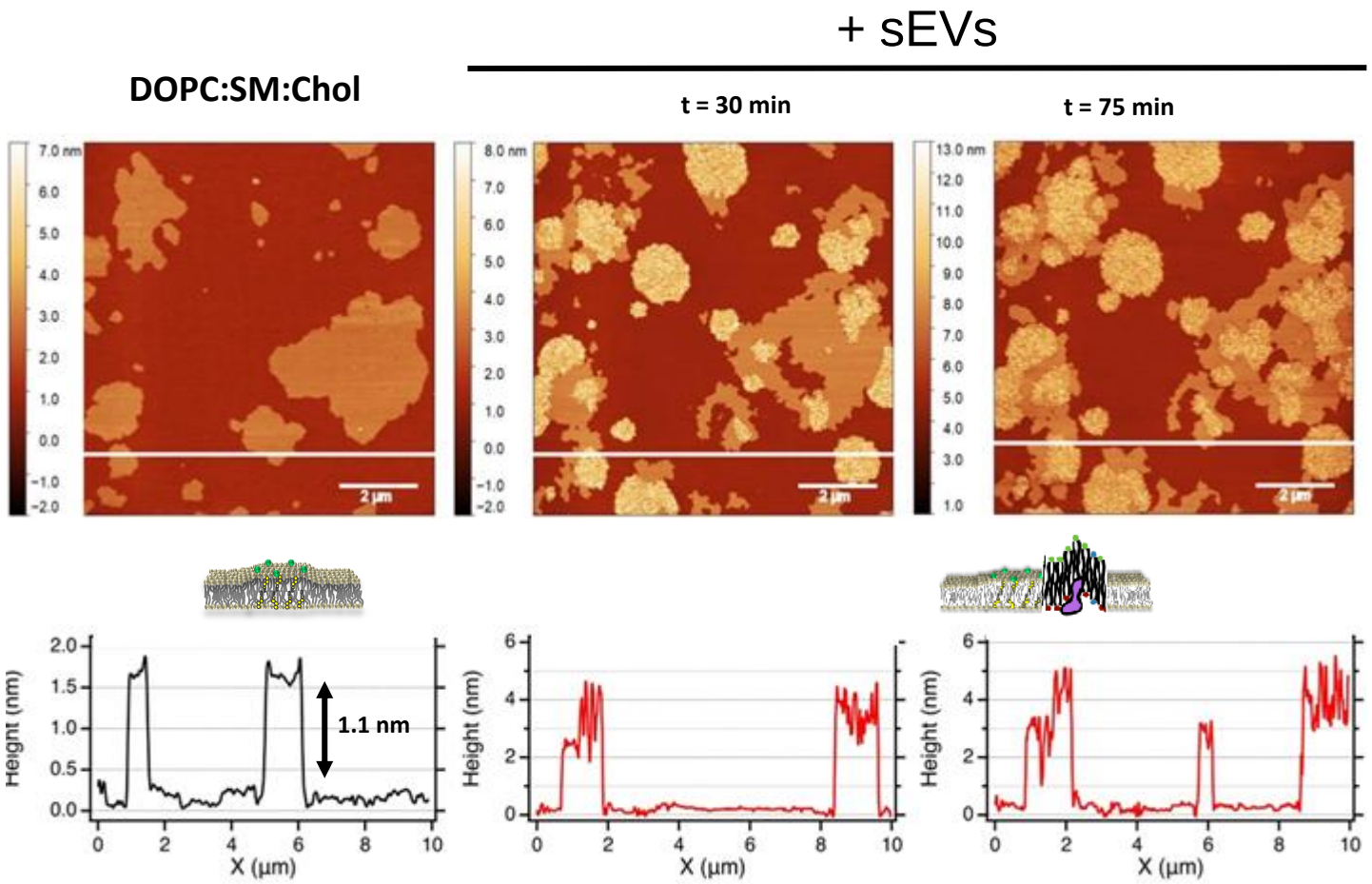
SLB 5 minutes after addition of sEVs



At thickness mismatches the free energy minimum enalbes favourable interactions without the need of large curvature deformations.



PATCHES EXPANSION FAVOURED IN L_d PHASE



Patches protruding 3-4 nm above SLB

STRUCTURAL DETAILS OF MIXING: NEUTRONS

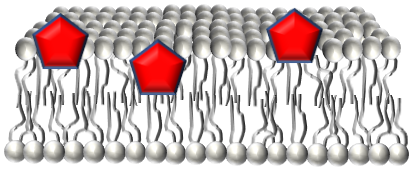
Reflectometry

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

Small Angle Scattering

$$I(q) \div c \; M \; P(q) \; S(q) \; (\Delta\rho)^2$$

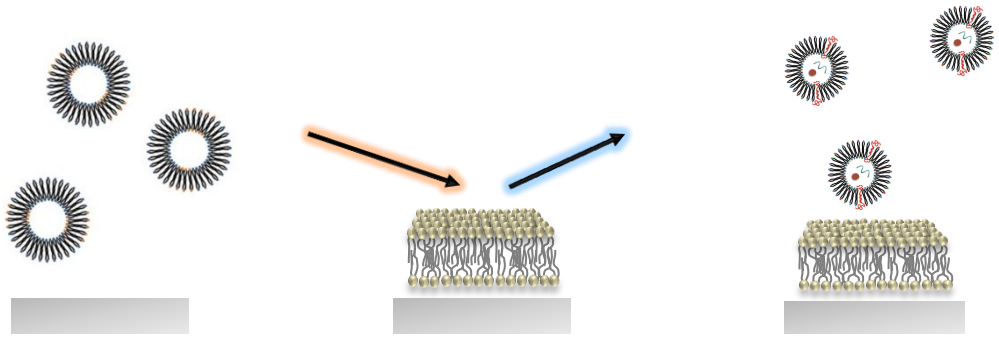
Selective deuteration → **H-bringing molecules can be evidenced in a deuterated phospholipid matrix**



External molecules interaction and distribution within membrane leaflets

NEUTRON REFLECTOMETRY

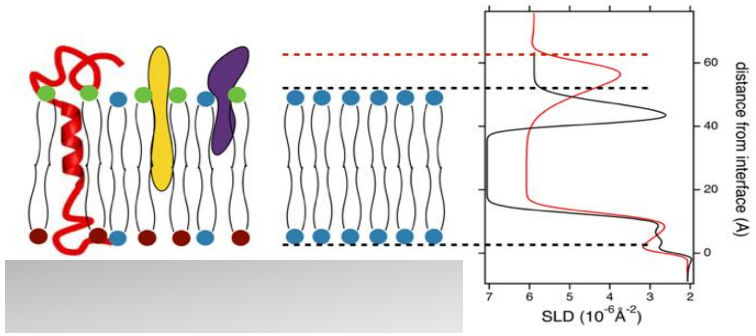
dDMPC vesicles fusion → *dDMPC characterization* → *sEVs injection from bulk water* → *mixed system characterization*



	AFM ΔZ (nm)	NR h (nm)
PC	5.1 ± 0.6	4.2 ± 0.3
PC+EVs	6 ± 2	5.4 ± 0.3
EVs	9 ± 3	6.9 ± 0.3

0.5nm water

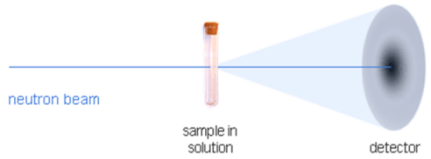
?

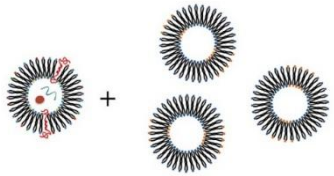


- 20% volume penetration
- Change in contrast spans whole membrane thickness
- Asymmetric

SANS

sEVs : dDMPC Vs
1: 15000
1:3000
1:2700



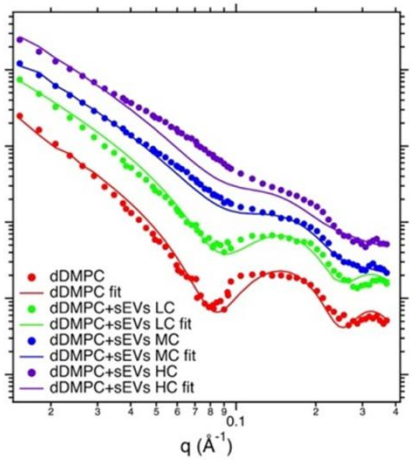


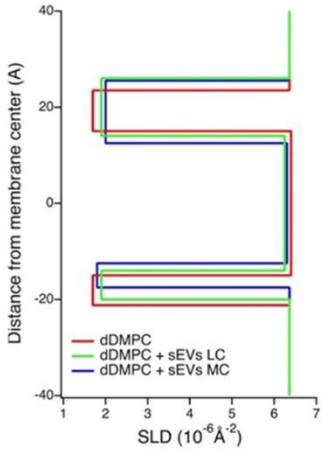
FITTING MODEL
Multilayered spherical form factor

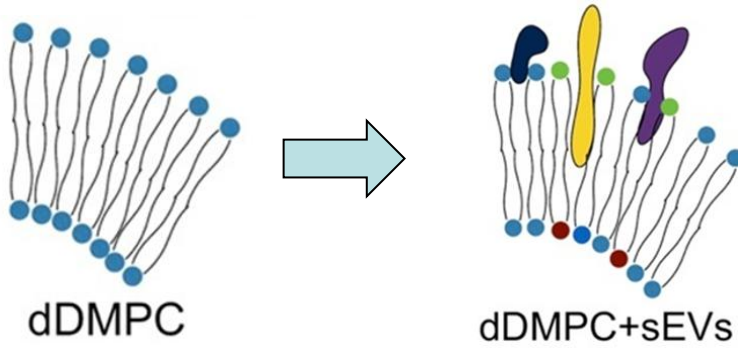
$$P(q) = \frac{\text{scale}}{V} F^2(q) + \text{background}$$

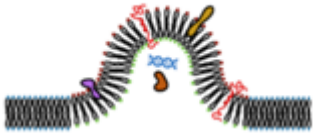
$$F(q) = \frac{3}{V_s}\left[V_c(\rho_c - \rho_s)\frac{\sin(qr_c) - qr_c\cos(qr_c)}{(qr_c)^3} + V_s(\rho_s - \rho_{\text{solv}})\frac{\sin(qr_s) - qr_s\cos(qr_s)}{(qr_s)^3}\right]$$

(Guinier, 1955)



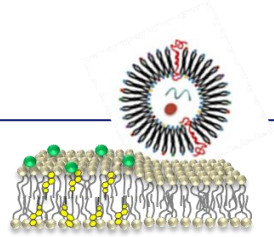




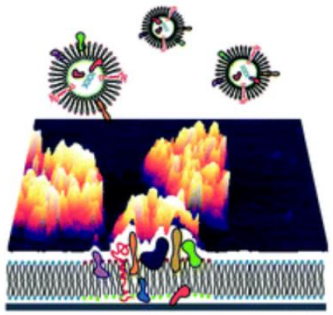


- Change in contrast spans whole membrane thickness
- Asymmetric

- ✓ Phase borders as docking sites, favouring sEVs uptake
- ✓ Separate processes can be distinguished
- ✓ l_o borders granularity increases after fusion
- ✓ Patches expansion is favoured in l_d phase
- ✓ Final membrane is asymmetric



F. Perissinotto & V. Rondelli et al., Nanoscale (2021)



The uptake process departs from the espected ‘simple’ fusion

We can identify specific vesicle-cell uptake routes

…eventually tunable for therapeutic needs

Miriam Grava - Sezione V - Comunicazioni
DSC unveils new aspects of extracellular vesicles mixing with model membranes

Conclusion

Complex systems can be built up and characterized

X-ray and neutron small angle scattering and reflectometry are essential complementary techniques for cross-structural investigations down to the nanoscale of self assembled systems and of thick interfaces

Possibility to work in physiological conditions

Possibility for in-situ interaction studies

Perspectives in biology (and not only….) are numerous

Acknowledgements

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