Polyphenols Interaction with a biomimetic membranes system
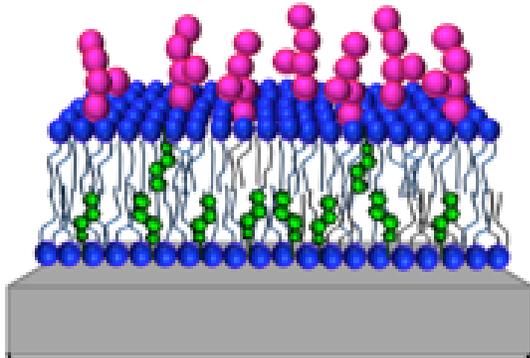
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# Objectives : polyphenol-biointerface interactions

Polyphenols can form hydrogen bonds with the lipids constituting the membranes, exhibiting an affinity for the lipid bilayer by binding to the lipid headgroups near the bilayer surface (adsorption) and/or penetrating into the bilayer interface (absorption).

Our primary goal is to deepen the understanding of

How variations in polyphenol *structures, characteristic and concentration* influence bio-interface interaction and the properties of the cell membranes

How composition properties of the polyphenolic mixtures from different sources impact these interactions and enhance their reactivity .

..with a specific focus on interactions with biomimetic lipid membrane assemblies containing cholesterol (DMPC/25% chol).
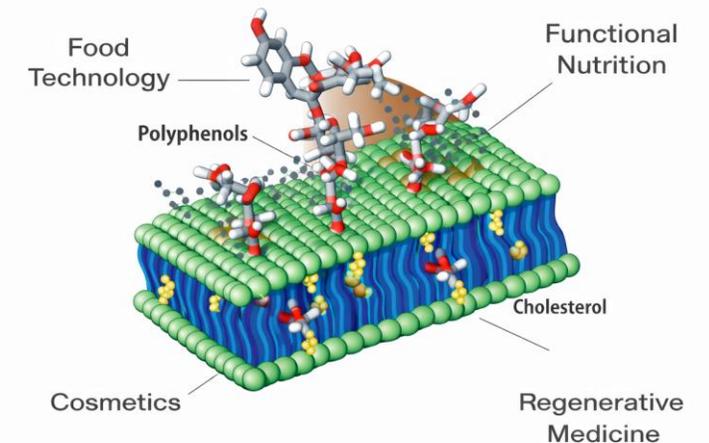
These effects can be accurately measured using *a non invasive techniques* such as **neutron reflectometry** and **small angle neutron scattering** providing characterization of the *structure* of solid-supported lipid bilayers in presence of polyphenols, and *of the structure in solution*.

Polyphenol extracts from agriculture waste

Vine canes of red (Pinot Noir, PN), and white (Fetească alba, FA) *Vitis* sp. varieties

& ## Mushroom : *Boletus edulis*

Main phenolic compounds based on HPLC analysis

PN Phenolic compounds	mg/L per 100g dw
Shikimic acid	572.81
Gallic acid	172.85
3,4-dihydroxybenzoic acid	106.92
Caffeic acid	184.32
Syringic acid	95.54
Ellagic acid	467.74
Catechin	502.37
Epicatechin	43.2
Isorhamnetin	502.37
Quercetin	467.74

FA Phenolic compounds	mg/L per 100g dw
Shikimic acid	567.36
Gallic acid	74.62
3,4-dihydroxybenzoic acid	70.71
Caffeic acid	232.74
Syringic acid	70.19
Ellagic acid	206.48
Catechin	388.86
Epicatechin	46.24
Isorhamnetin	99.43
Quercetin	206.48

Extract	TCP mg GAE/mL
FA raw	2.94
FA pur	**35.25**
PN raw	3.52
PN pur	**40.29**
Boletus pur	33.96
Boletus raw	**3.39**

Available in attractive amounts !!

Raw extract contains proteins and polysaccharides

Purified extract will be a concentrated mix of polyphenols
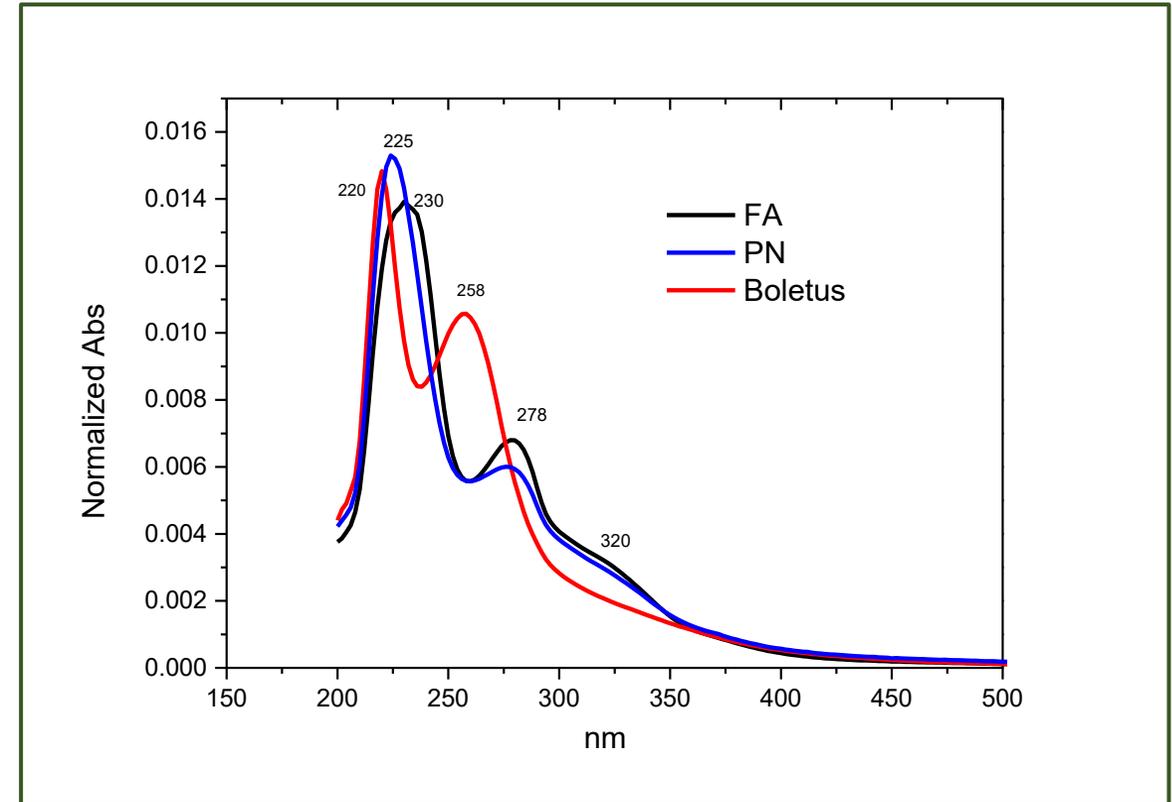
UV spectra

FA and PN extracts have a similar polyphenol content
Both differ from *Boletus* extracts.

The first peaks (220-230 nm) are typical
of $\pi \rightarrow \pi^*$ transitions found in **all polyphenols.**

258 nm cellular fingerprint (DNA/RNA…)

278 nm Indicative of **flavanols** (e.g. catechin, epicatechin)
or **condensed tannins** (proanthocyanidins)

320 nm **resveratrol** and **hydroxycinnamic acids**

General presence of phenolic compounds in all samples.

FA and PN seem **richer in phenolic acids and resveratrol**, while Boletus shows **simple phenolics and** flavonoids (shoulder >300 nm)

Dynamics light scattering

- Sharp peak**, around 100–150 nm** , suggesting a relatively homogeneous nanoparticle size distribution.

Purified extract will be a concentrated mix of polyphenols

- No significant signal above ~**300 nm**: no large aggregates or macromolecular complexes formation after filtering

- **Mushrooms** are rich in **β-glucans**, which can bind polyphenols non-covalently, it contains low molecular weight proteins or peptides that can bind phenolics.

- Such complexes may form **stable colloidal nanoparticles often <150 nm** (especially in aqueous extracts).

- The **FA** wine cane extract clearly forms **colloidal nanostructures** in aqueous solution.

- The size range is **consistent with protein–polyphenol or tannin aggregates**, known to self-assemble into particles between **80–150 nm**.
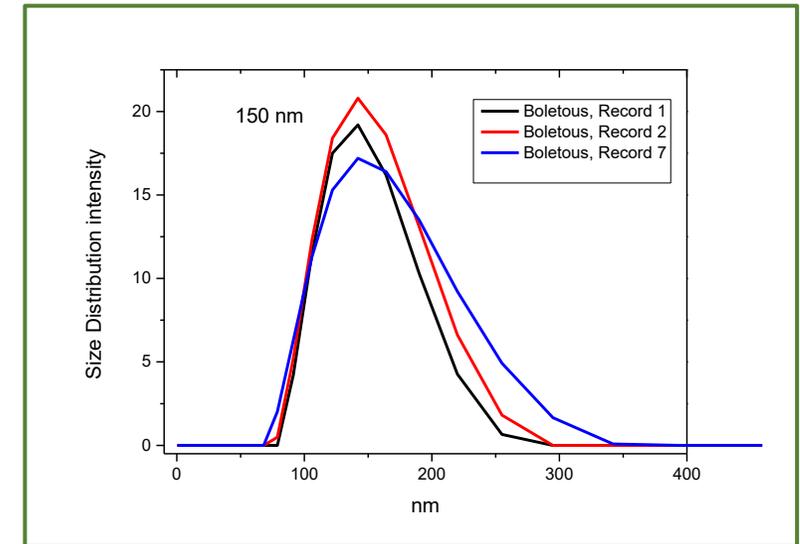
Raw extracts FA wine cane
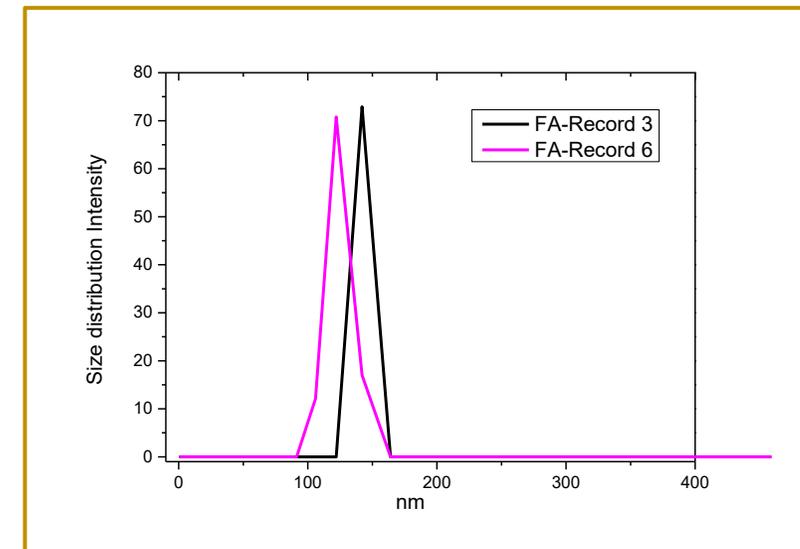
DMPC-20% cholesterol vesicles : bilayer on one silicon block

homogeneous DMPC bilayers can be obtained in well-known and reproducible ways

polar head

DMPC

tails

Tm = 23 C

160 nm

size distribution Intensity

nm
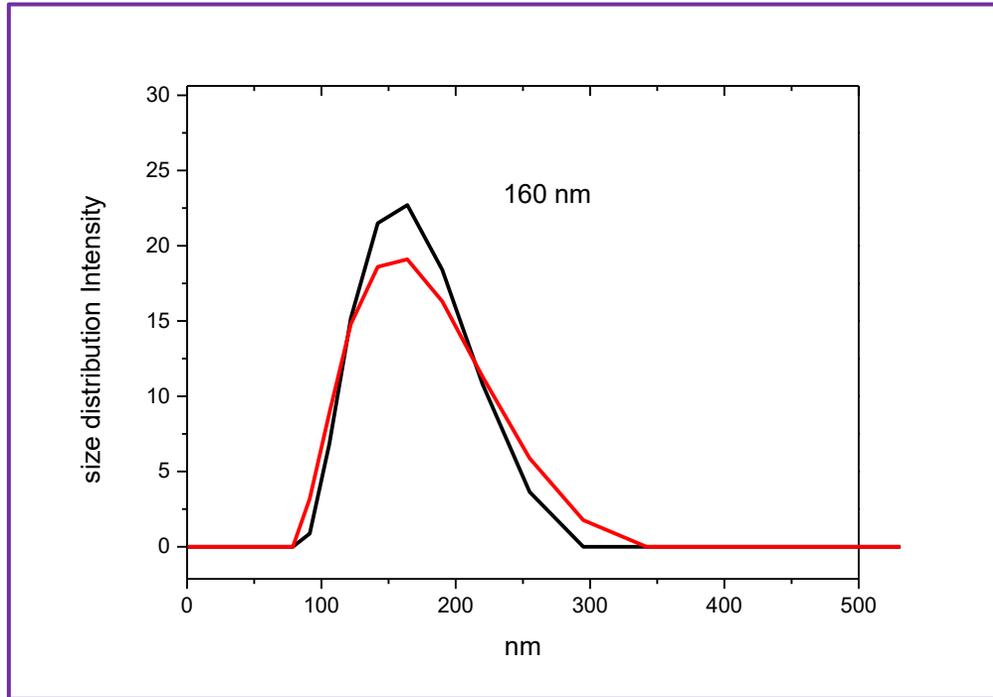
For vesicle rupture we prepared 0.2 mg ml-1 solution of vesicles in D2O. Bath sonicating for 30 minutes (above 30 degrees) and tip sonicating for 10 minutes (2s on 1s off) to produce small unstable vescicles.
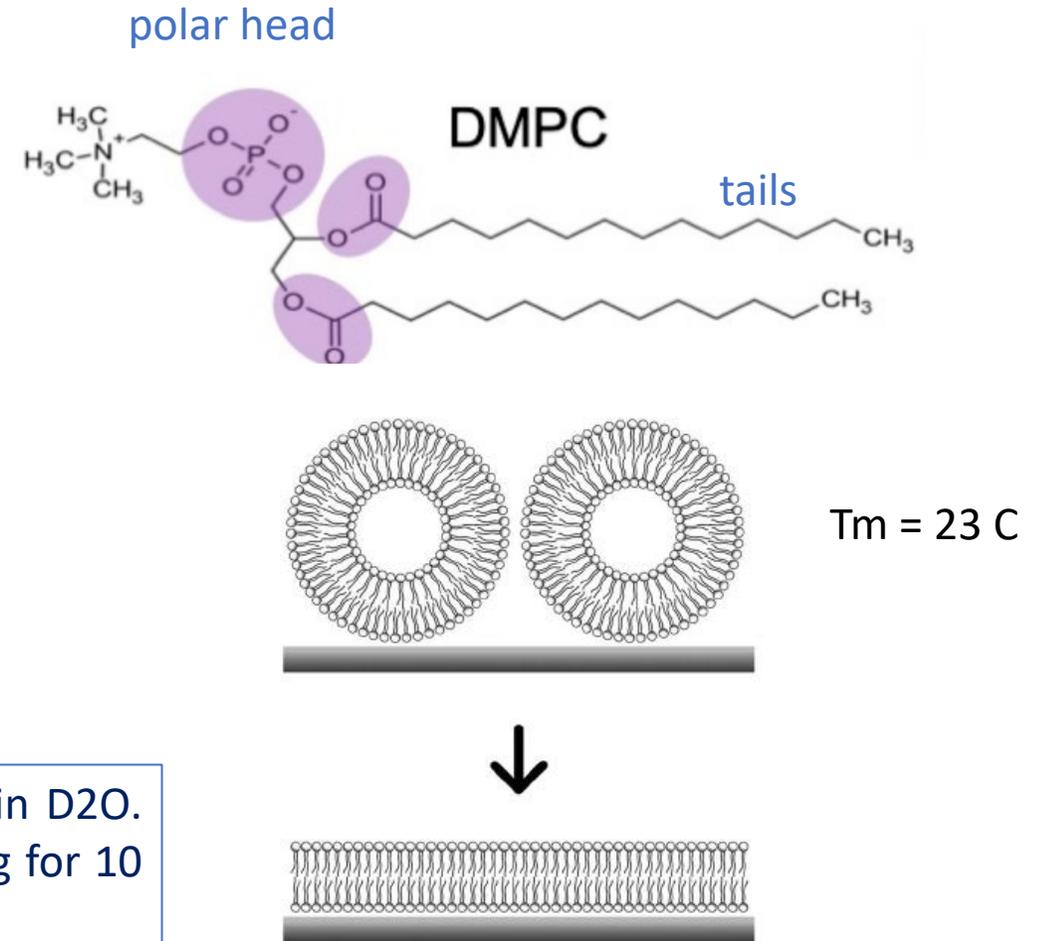
Tip sonication is done right before addition to the solid liquid flow cells for reflectometry experiment

Why Neutron Reflectometry ?

Neutron reflectometry examines the way in which **surfaces** or interfaces reflect neutrons in *order to provide information on their organization, thickness, composition and roughness (irregular surface)*
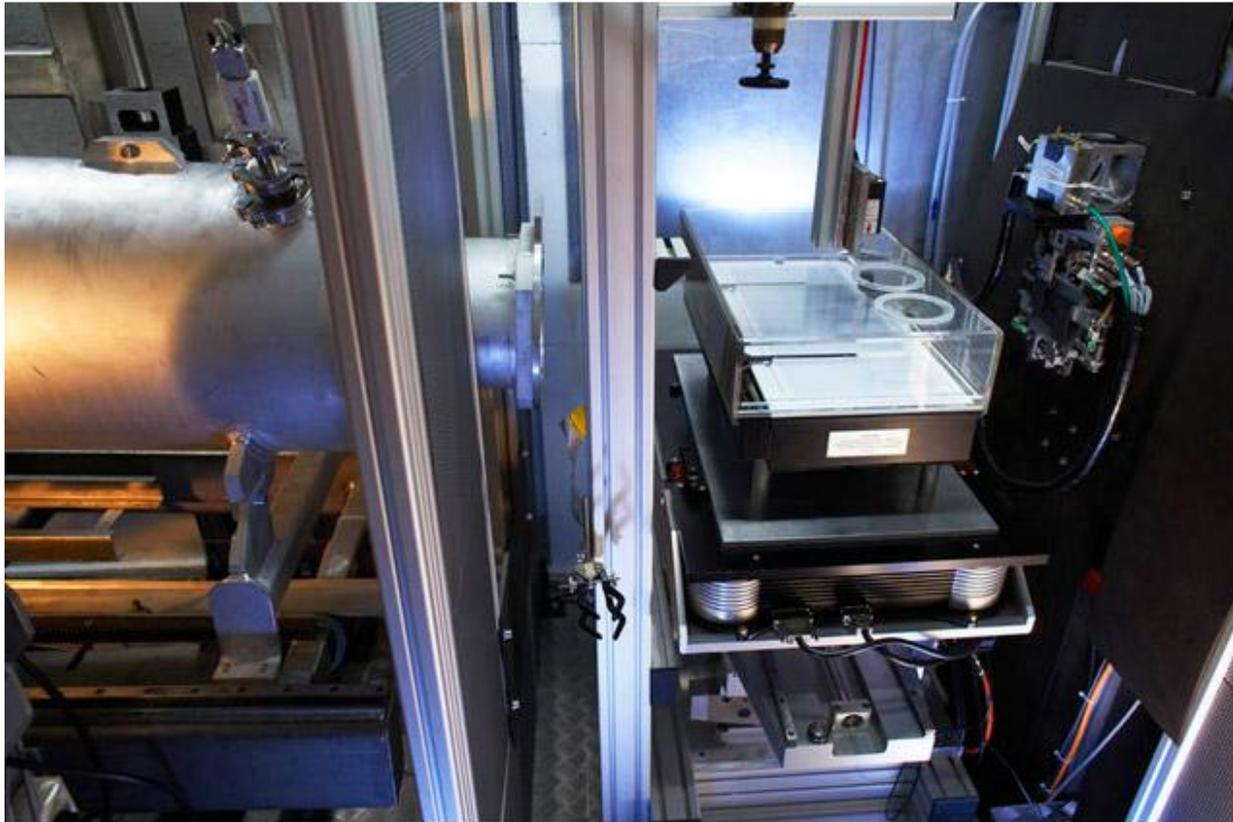
Neutron reflectometry bases on the reflection of a neutron beam at interfaces.
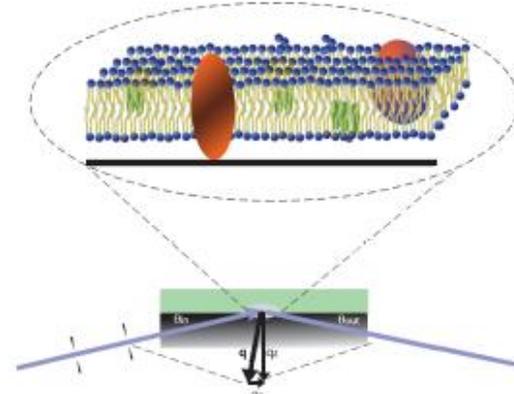
Reflectivity (R) is defined as the ratio between the measured intensities of the reflected and incident neutron beams at a given scattering vector,
$q_z = (4/\lambda) \sin\theta$

λ = wavelength, of the neutron and the θ= grazing angle of incidence,
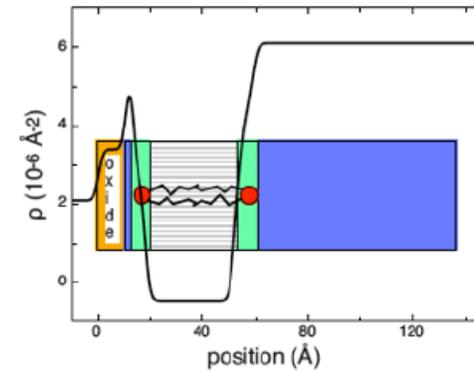
The analysis of the measured reflectivity curve leads to the determination of the scattering length density (SLD) profile (z) along the surface normal

SLD depends on the chemical composition along the z-direction

DMPC+25% Cholesterol bilayer

The data set from the different contrasts were fitted simultaneously under the constraint of obtaining identical structural parameters
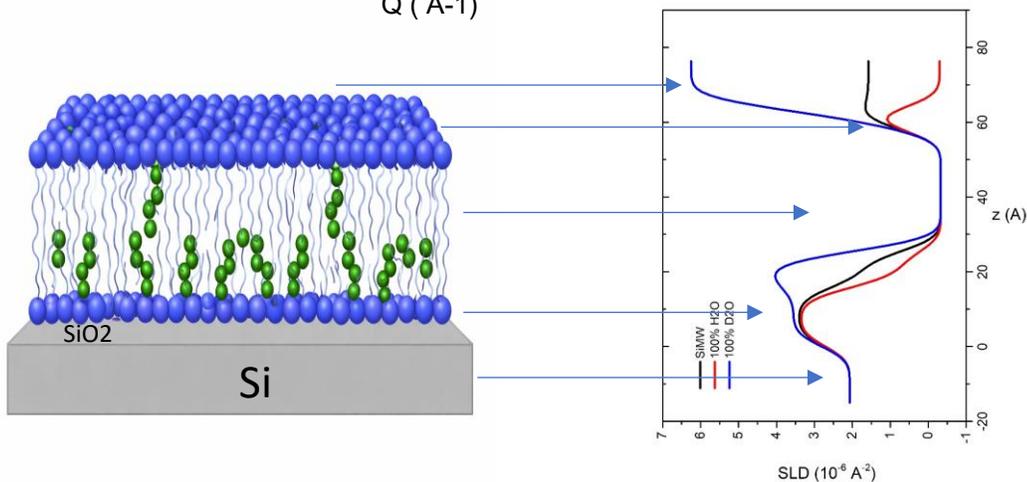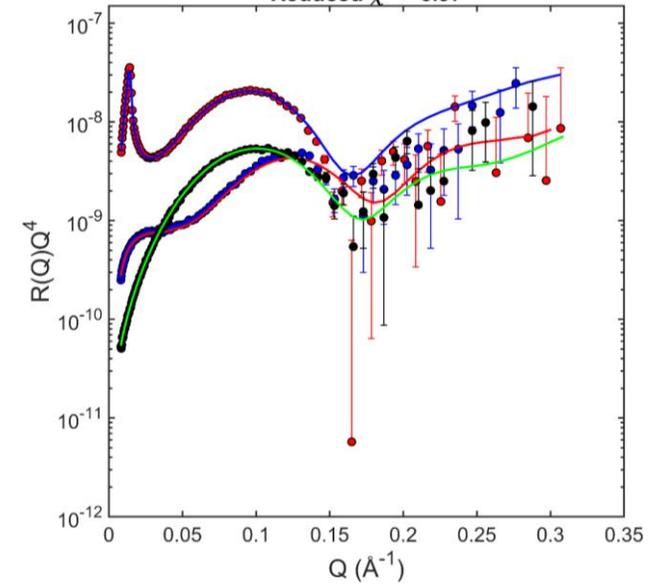
Measurements @ RT.
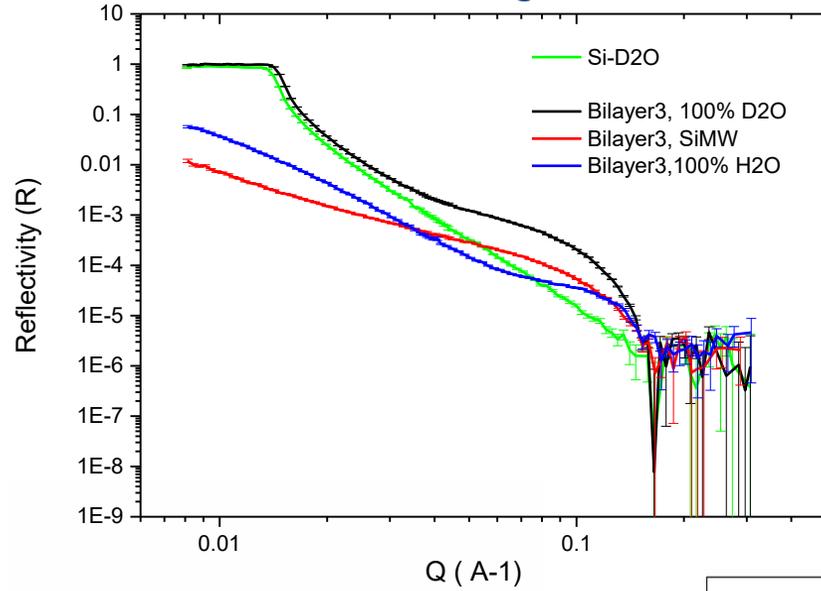
100 % D2O: *geometry of the system, thickness and interface*

100 % H2O: *lipids heads*
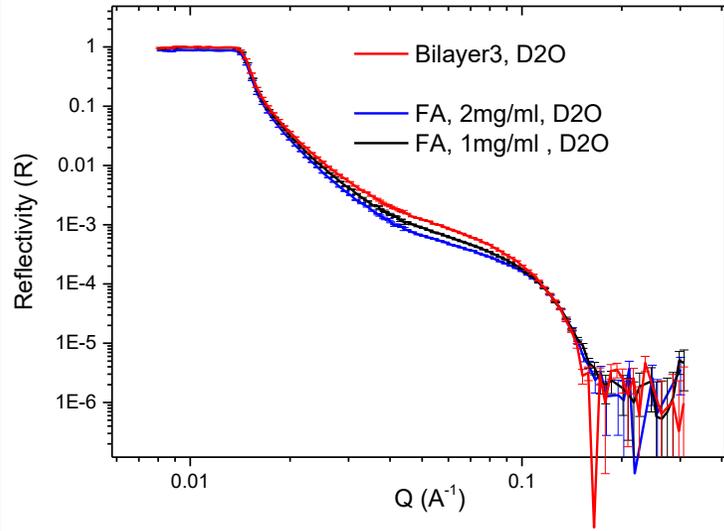
**35 % D2O /65% H2O** : *match the Si substrate.*

Reduced χ^2 = 6.97

Layer contents	Tickness (A)	SLD (10-6 A-2)	Water %	Roughness (A)
SiO2	15	3.47	5	3
Inner heads	9.7	1.88	52	3
Tails	32.5	-0.32	~ 0	3
Outer heads	5.4	1.88	~ 0	3

Interaction of the Fetească alba (FA) purified extracts with DMPC+25 % cholesterol bilayer

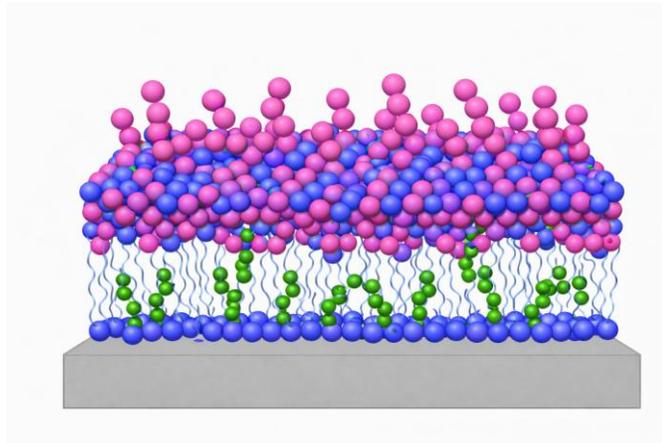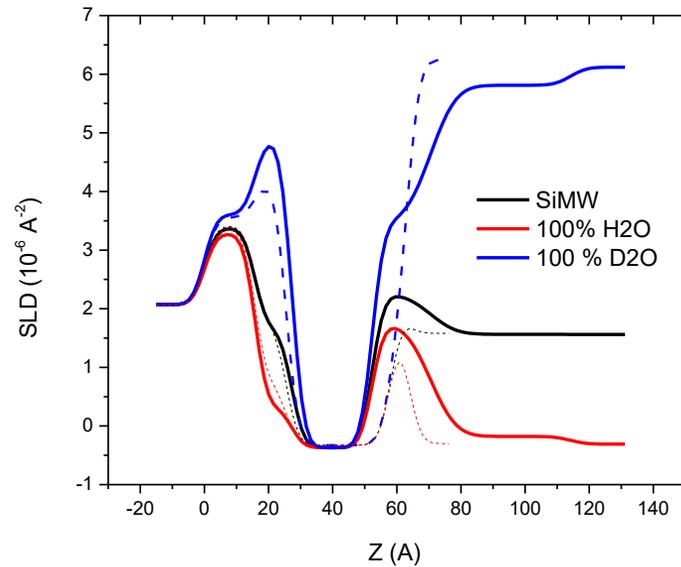Signal arising from Si, polar heads, core, hydration (geometry of the system)

Signal arising from the lipid heads
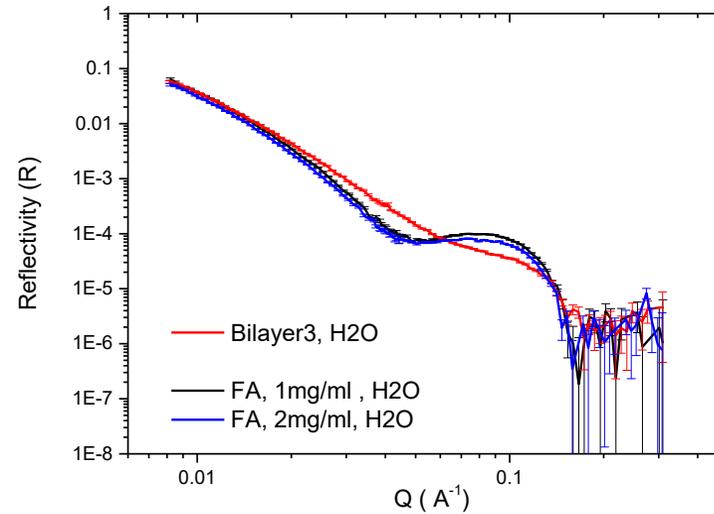
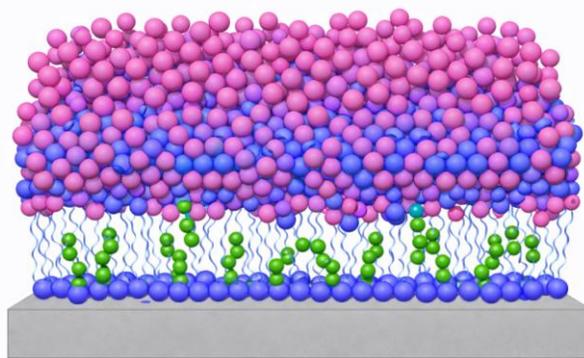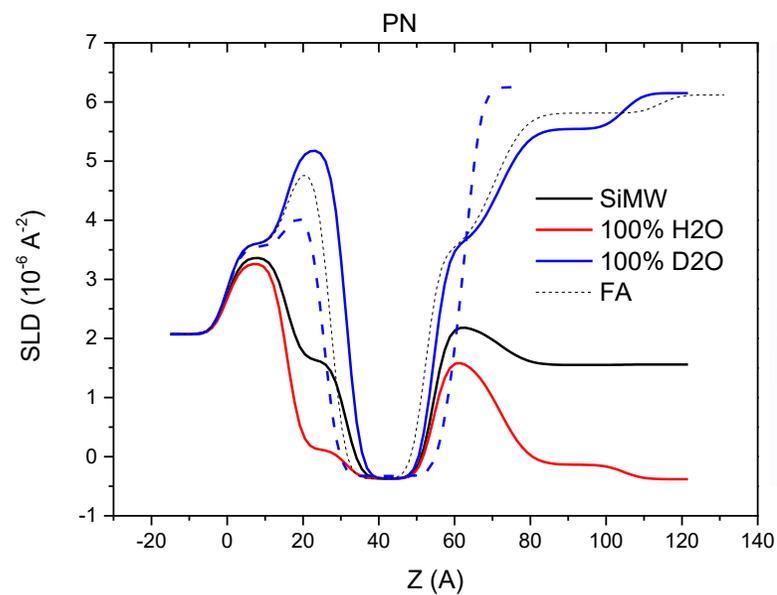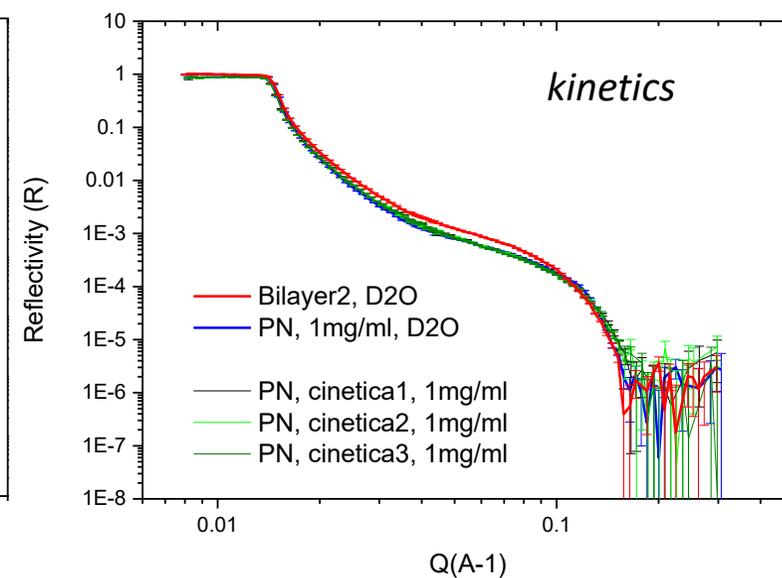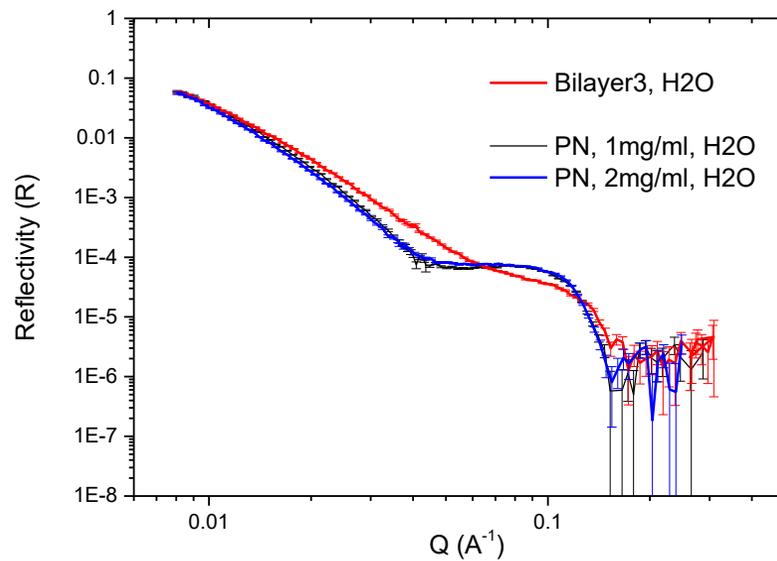Signal arising only from the structure of the lipid membrane (heads and tails)
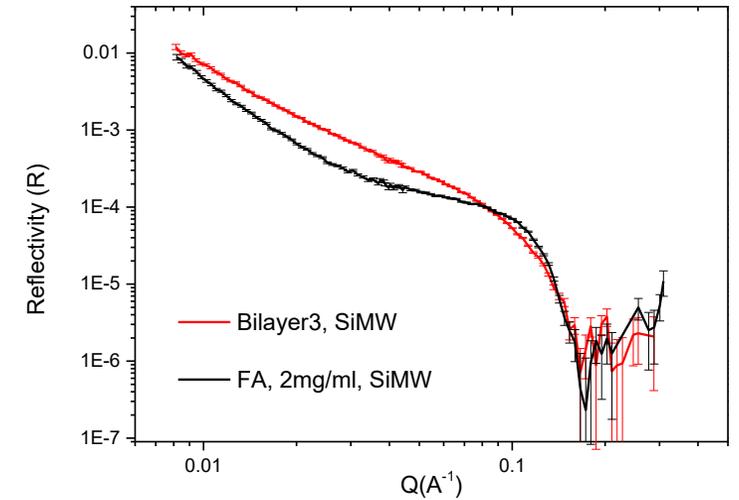
FA

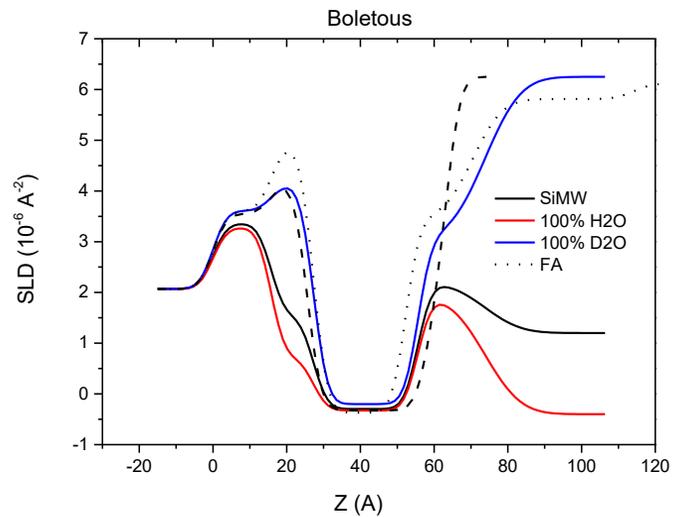| Layer contents	Tickness (A)	SLD (10-6 A-2)	Water %	Roughness (A)
SiO2	15	3.47	5	3
Inner heads	11.5	1.88	70	3
Tails	25	-0.32	~ 0	4
layer	18	2.5	26	6
Outer layer	43	1.6	93	4

Interaction of the Pinot Noir (PN) purified extracts with DMPC+25 % cholesterol bilayer
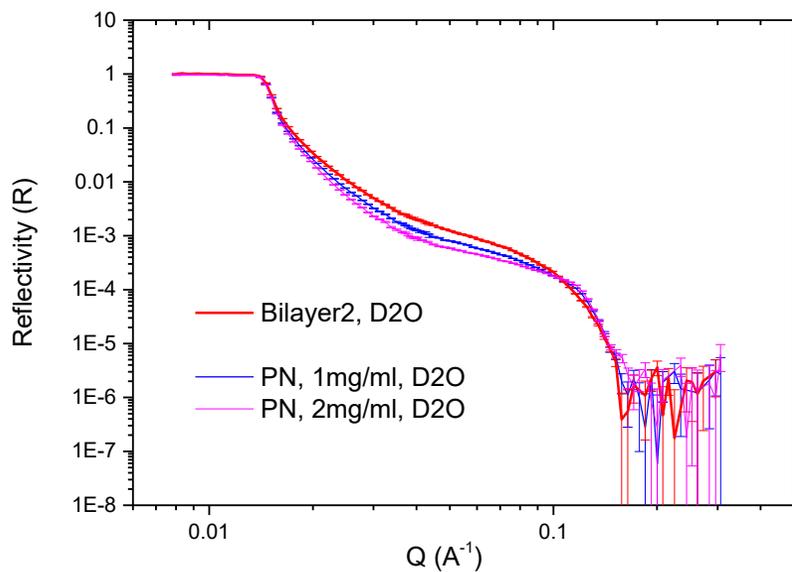
Layer contents	Tickness (A)	SLD (10-6 A-2)	Water %	Roughness (A)
SiO2	15	3.47	5	3
Inner heads	15	1.88	77	3
Tails	23	-0.32	~ 0	4
layer	17	2.5	28	6
Outer layer	33	1.5	86	3

Interaction of the Boletous Eduli purified extracts with DMPC+25 % cholesterol bilayer
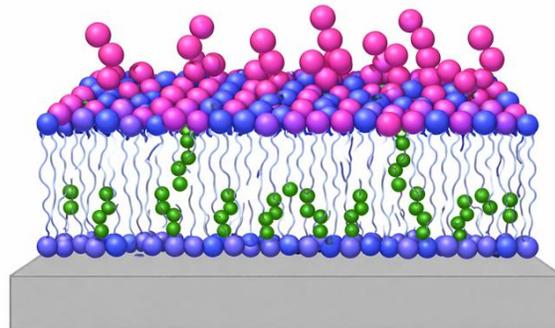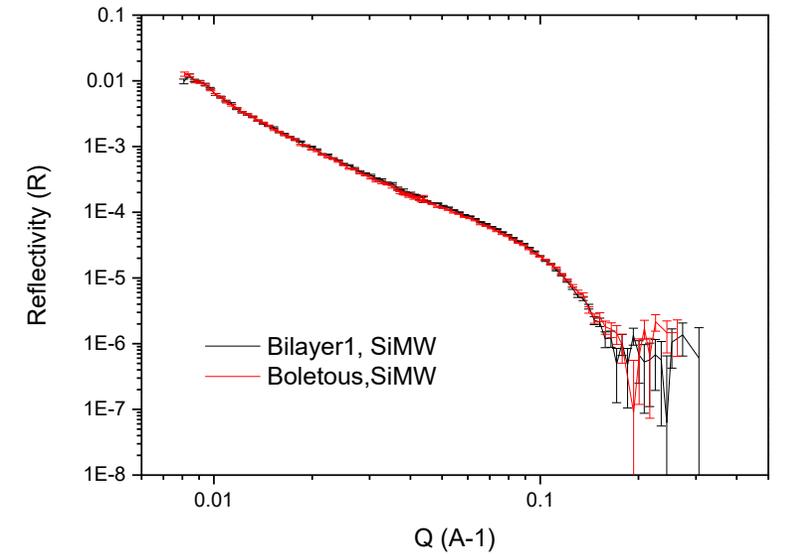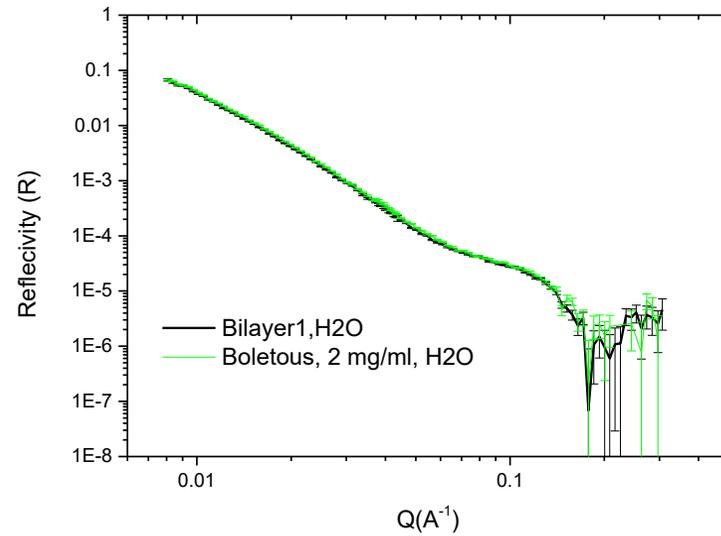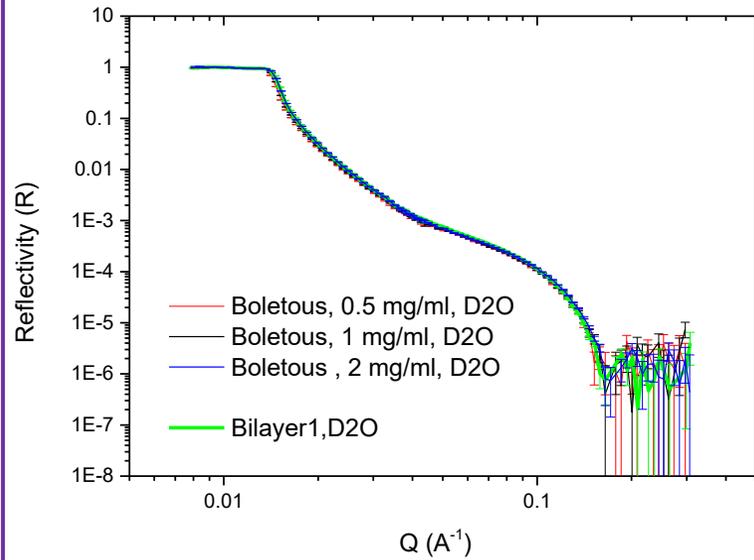
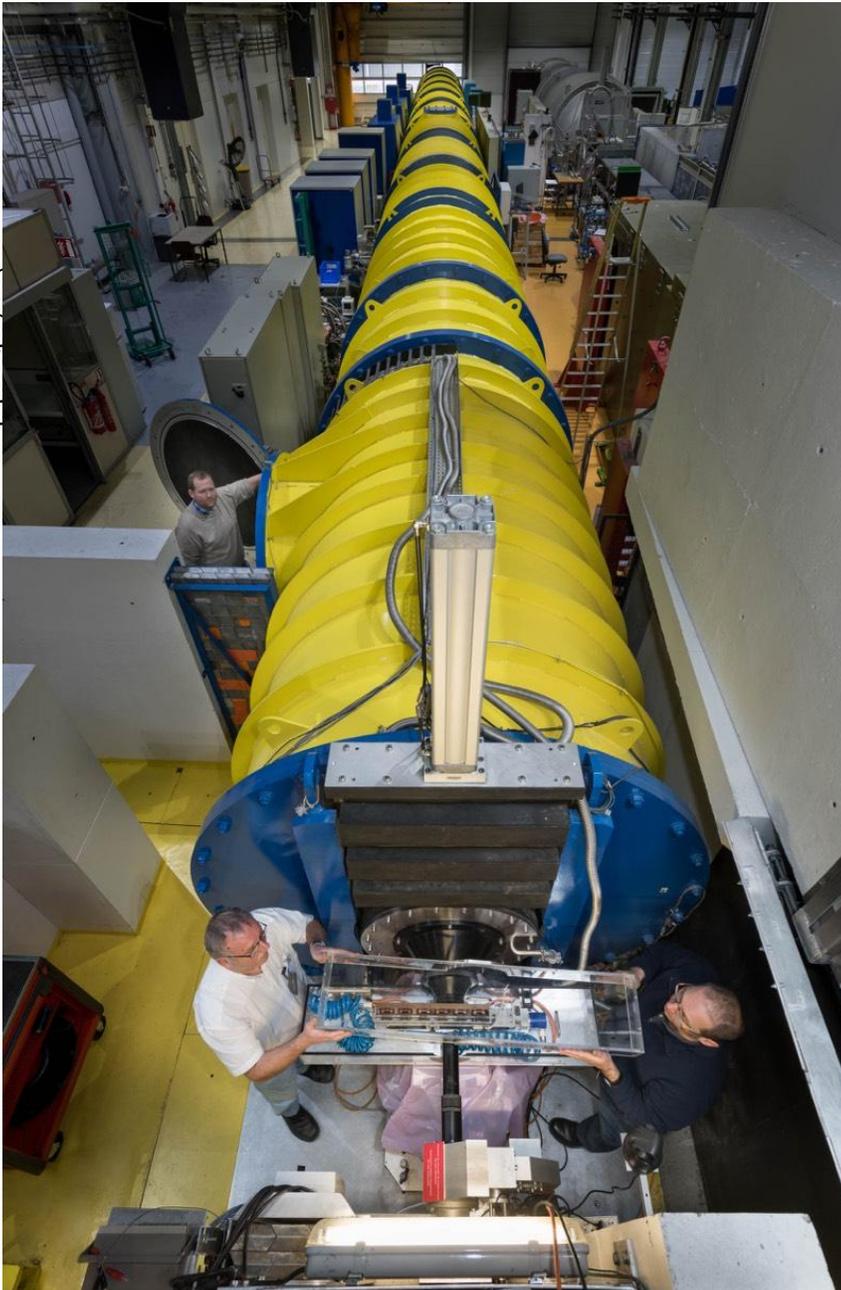| Layer contents	Tickness (A)	SLD (10-6 A-2)	Water %	Roughness (A)
SiO2	15	3.47	5	3
Inner heads	11	1.88	51	3
Tails	28	-0.32	~ 1	3
Outer heads	18	2.4	16	7

Small angle neutron scattering: structure in solution

$$I(Q) \cong P(Q) * S(Q)$$
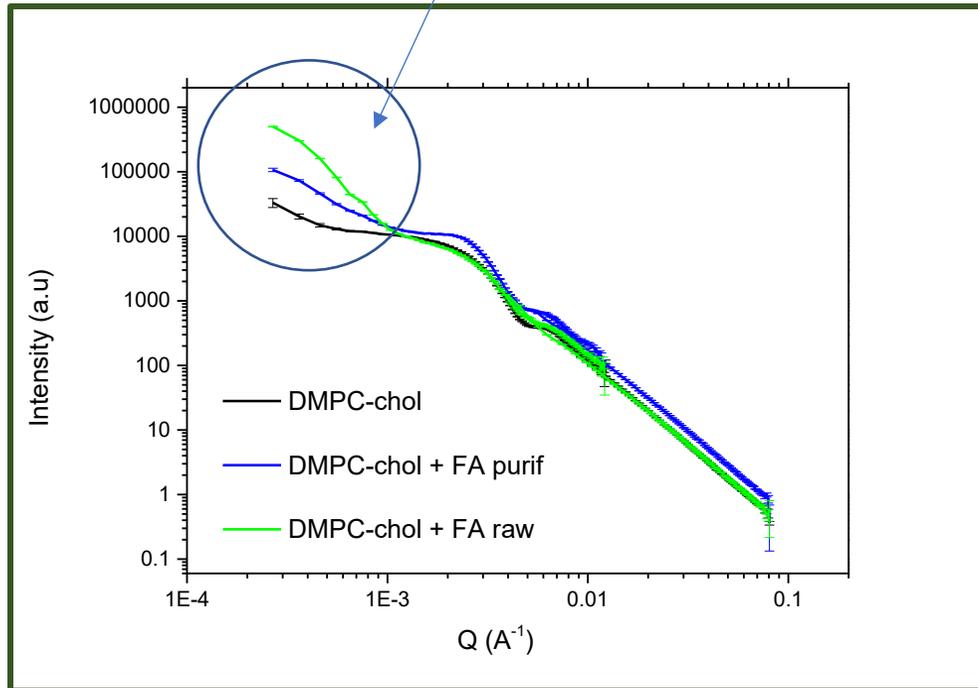
P(Q) : form factor (size and shape)

S(Q) : structure factor (interactions)

$Q=(4\pi\ sen\theta)/\lambda$

$\mathbf{k_f}$

2θ

$\mathbf{k_0}$

SANS Instrument layout

Institut Laue Langevin

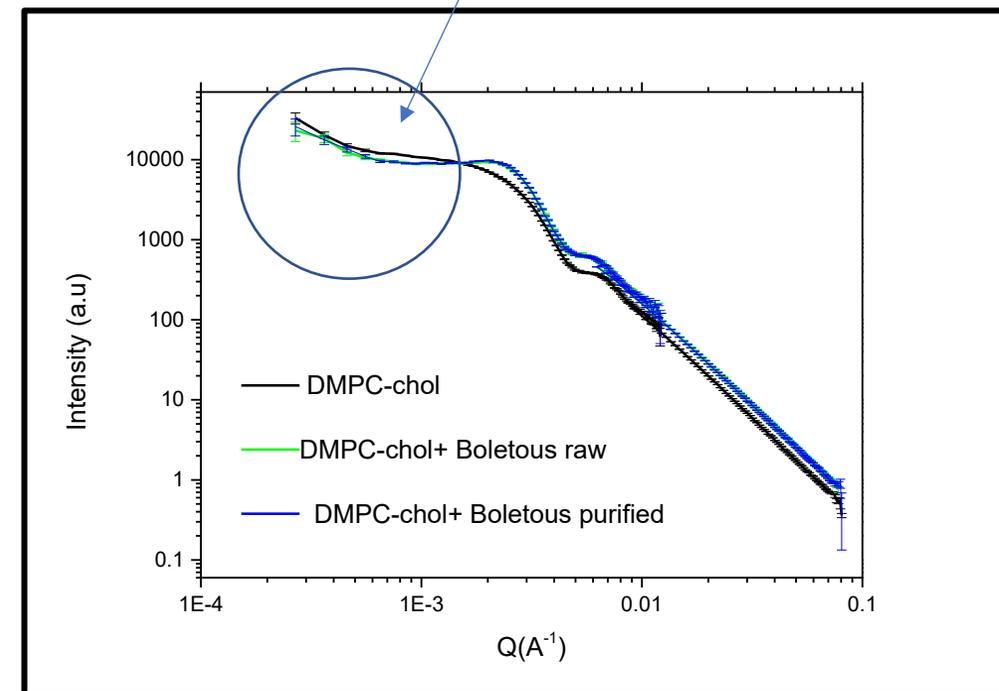**FA strongly affects the large-scale structure.**
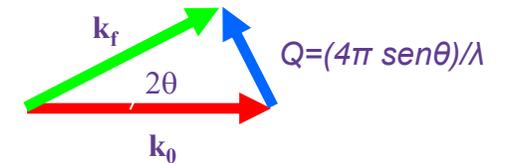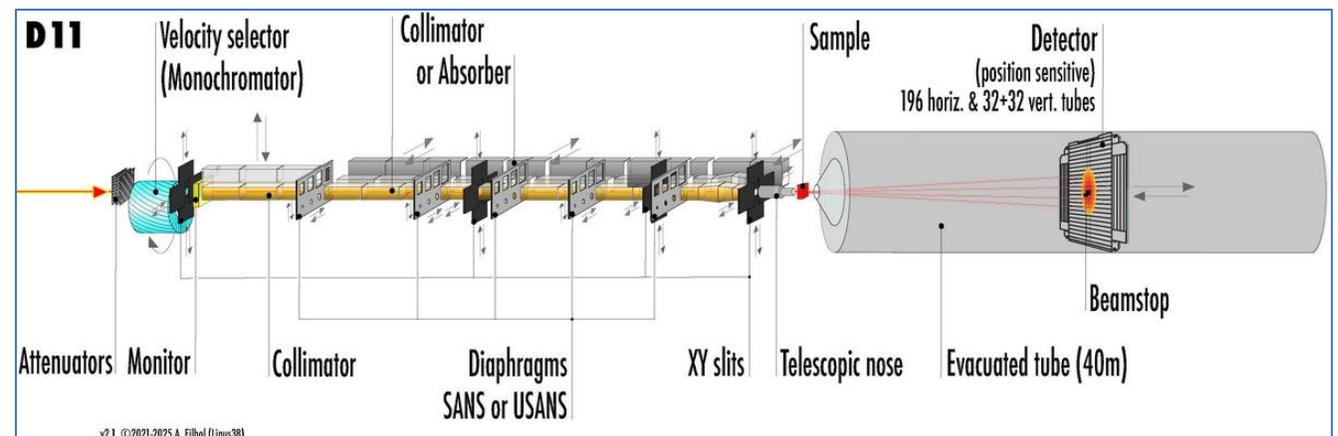
Boletous do not affects the large-scale structure.

- **Larger vesicles** (increased average radius) and
- **Inter-vesicle aggregation / clustering/ breaking**

- **Modification of the interactions:** Minor components (impurities, mixed chain lengths, charged species) promote **vesicle–vesicle attraction** *(FA-induced surface heterogeneity)*
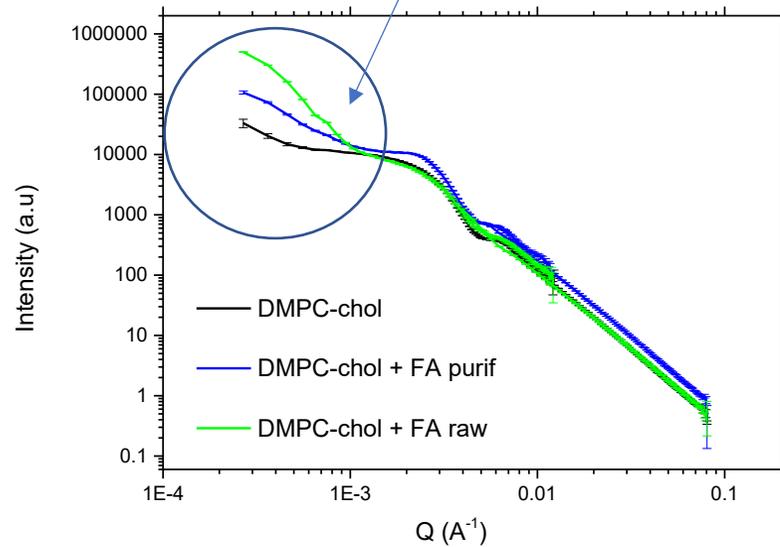
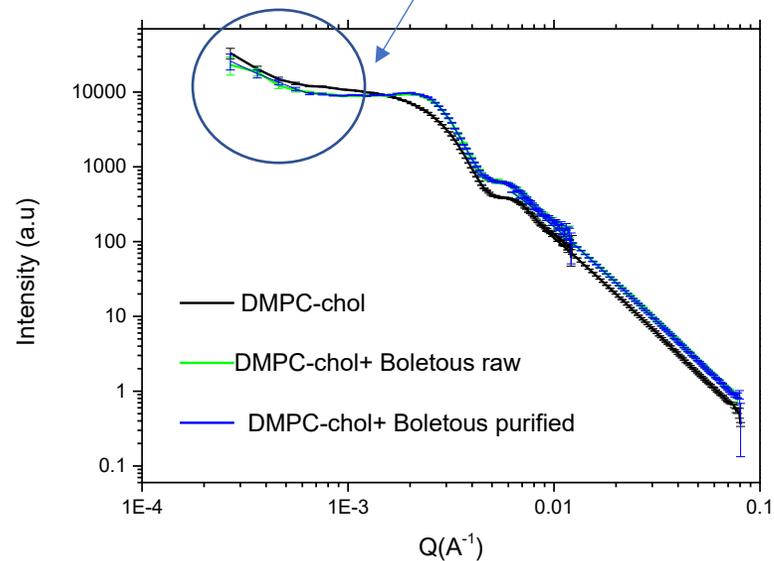Weak modification of the interactions : **Weak attraction**
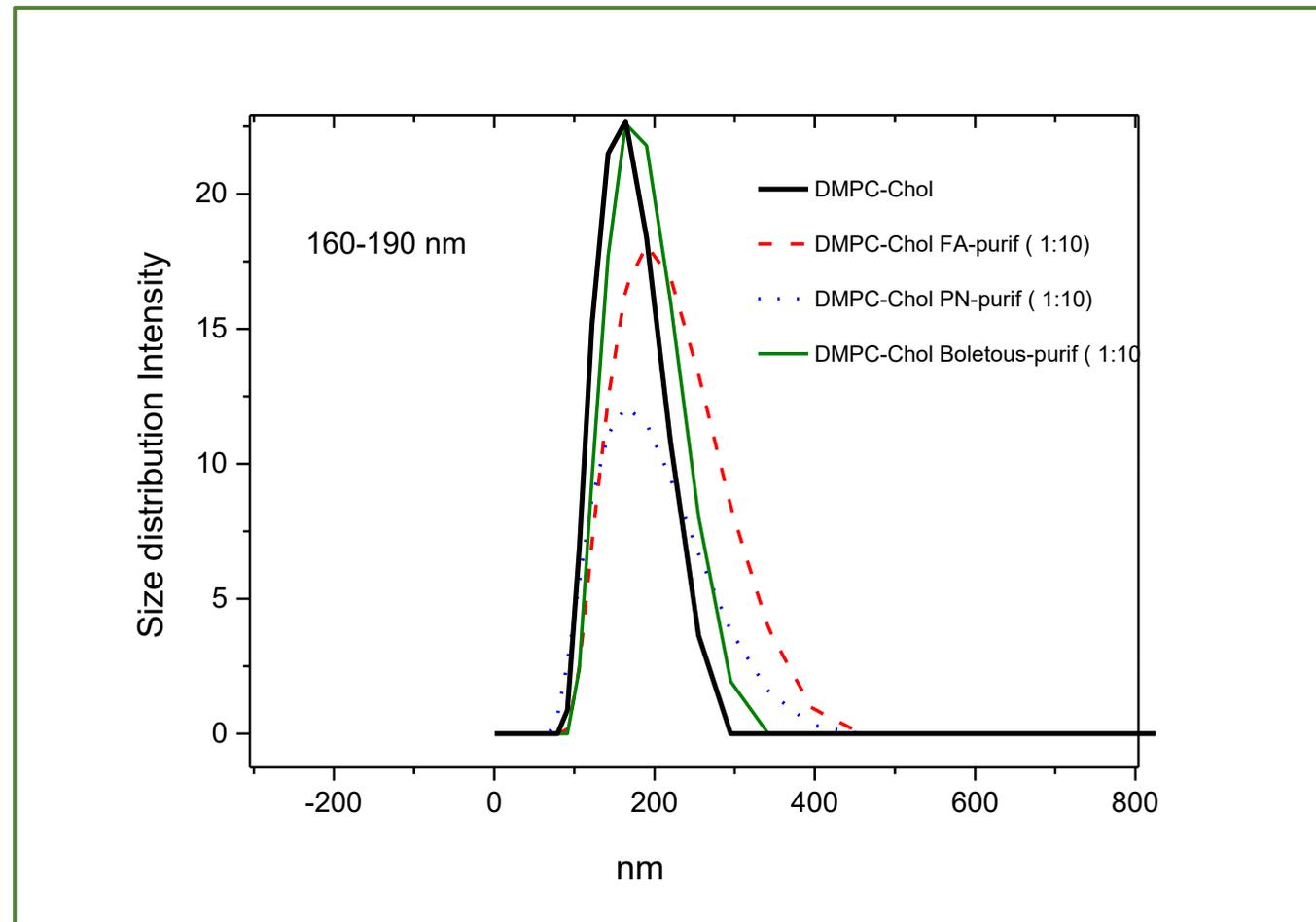
Vesicles remain **intact**

No large-scale phase separation

FA **strongly affects the large-scale structure**.

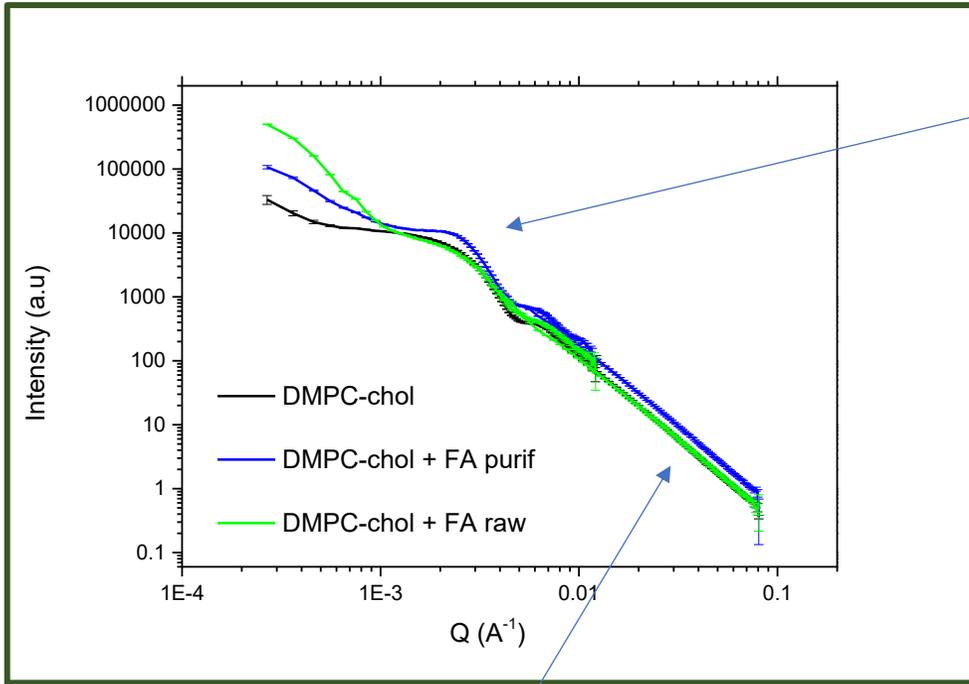Boletous do not **affects the large-scale structure**.

Supported by Dynamic Light scattering

160-190 nm

Shoulder region (≈ 10⁻³ – 5×10⁻³ Å⁻¹): bilayer thickness

- The shoulder shifts slightly with FA not with the Boletus
- FA purify shows a more pronounced shoulder FA raw

Interpretation
FA molecules are **inserting into the bilayer**, Boletous is interacting at the surface

Effects:

- **Increase of the bilayer thickening**
- Purified FA gives a more uniform insertion → sharper form-factor features

Raw FA → broader distribution → smeared shoulder.

Intermediate-q power law (≈ 0.005 – 0.05 Å⁻¹): membrane mechanics

FA and Boletous containing samples have **higher intensity**

Interpretation

Change in the **membrane undulations and stiffness**

FA addition **softens the membrane**, reducing the cholesterol's ordering effect and enhancing a more liquid-disordered–like behaviour. Boletous slightly soften the membrane and does not disorder the bilayer.

Highlights

Wine cane polyphenols extract show affinity with the lipid membrane hydrocarbon chains. They Insert into the bilayer, eventually pull out some lipids and induce membrane disorder and increasing water penetration.

At sufficiently high concentrations, they induce rapid disruption of unilamellar vesicles.

Boletous Eduli polyphenols extracts interact with lipid heads on the membrane surface. Do not promote bilayer aggregation

At high concentration they destabilize the unilamellar vescicules with a longer kinetc compare to the wine cane.

*.. Analysis in progress: **impact on bilayers with cholesterol and charged lipids***

For beam time allocation, local contact experimental support

4 peer-reviewed and approved proposals

NEUTRONS FOR SCIENCE®

NEXT proposal DEADLINE : February 15th

New projects in prospectives in collabotation with L. Dinu

FoodWaStop

Emanuel Vamanu and Laura Dinu

USAMV — UNIVERSITATEA DE ȘTIINȚE AGRONOMICE ȘI MEDICINĂ VETERINARĂ DIN BUCUREȘTI
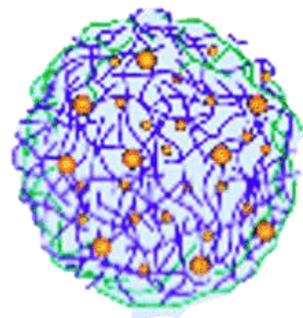
Polyphenols extracts

Jana Pekarkova.

Faculty of Agri-Sciences/Department of Chemistry and Biochemistry, University in Brno

PLGA NPs loaded polyphenolic extract

Investigations on the interaction between plant-waste derived extracellular vesicles (EVs) and biological membranes

E. Calzoni , C. Emiliani
Universily of Perugia , Italy

PDEVs as Natural Drug Delivery System — Plants and Fruits — PDEVs Natural Cargo — Exogenous Cargo — Anti-inflammatory, Antioxidant, Anticancer