

Lipid sponge phases and nanoparticle dispersions able to entrap large biomolecules

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BIBAFOODS



Introduction

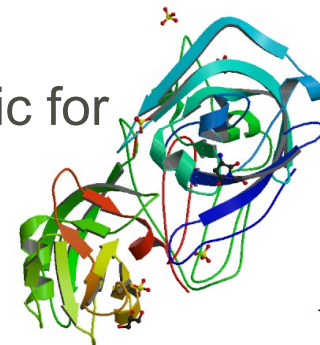
Preparation of lipid liquid crystal (LC) phases for the encapsulation of an enzyme in order to control enzyme activity and stability

1. Formation bicontinuous phases such as sponge or reverse cubic phases with large unit cell dimensions
2. Inclusion of aspartic protease and beta-galactosidase
3. Study of encapsulated enzyme activity, stability and release
4. Interactions between:
 - Lipid system and surfaces
 - Lipid system and the enzymes

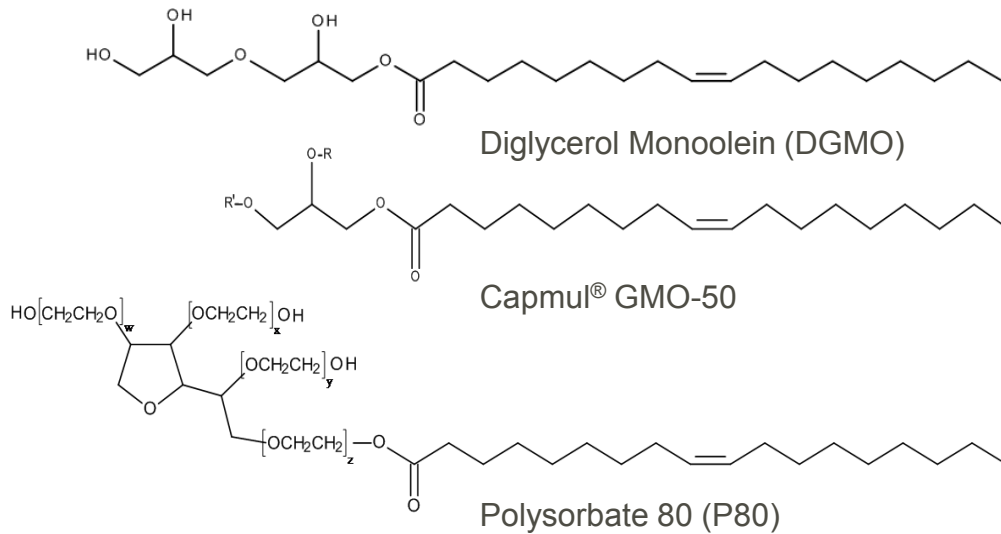


Enzymes

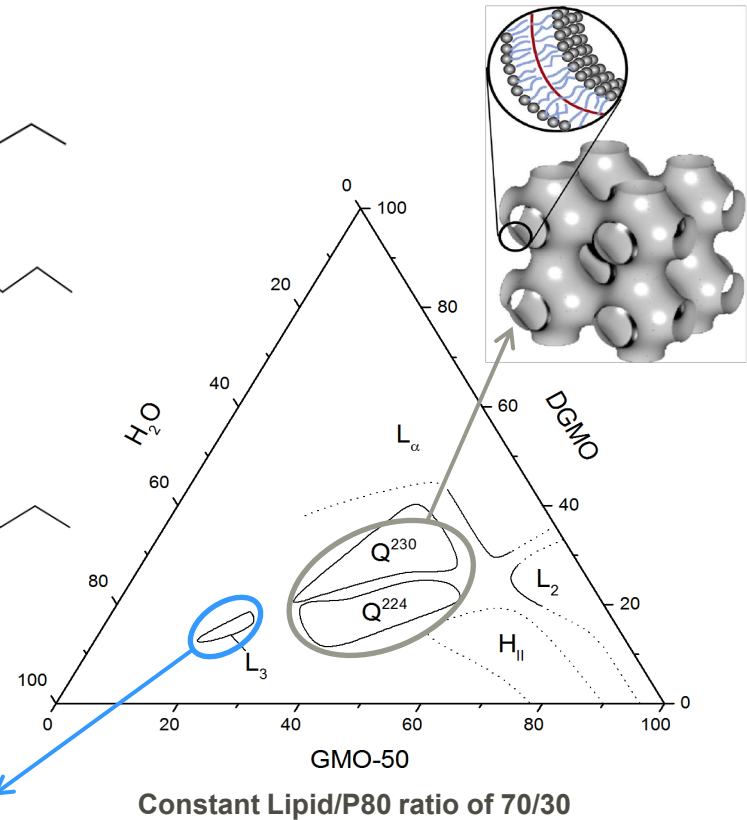
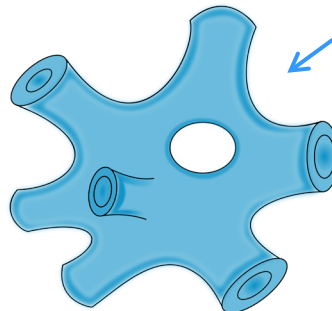
- Beta-galactosidase: $\approx 465\text{kDa}$ ($\approx 14\text{ nm}$)
 - Yeast *Kluyveromyces lactis*
 - Hydrolyses lactose to a mixture of glucose and galactose
 - Lactose free/reduced lactose products
→ Lactose intolerance
- Aspartic protease: $\approx 34\text{ kDa}$ ($\approx 5.7\text{ nm}$)
 - Fungus *Cryphonectria parasitica*
 - Milk-clotting enzymes which is highly specific for kappa-casein, resulting in curd formation.
 - Cheese ripening



The lipid system

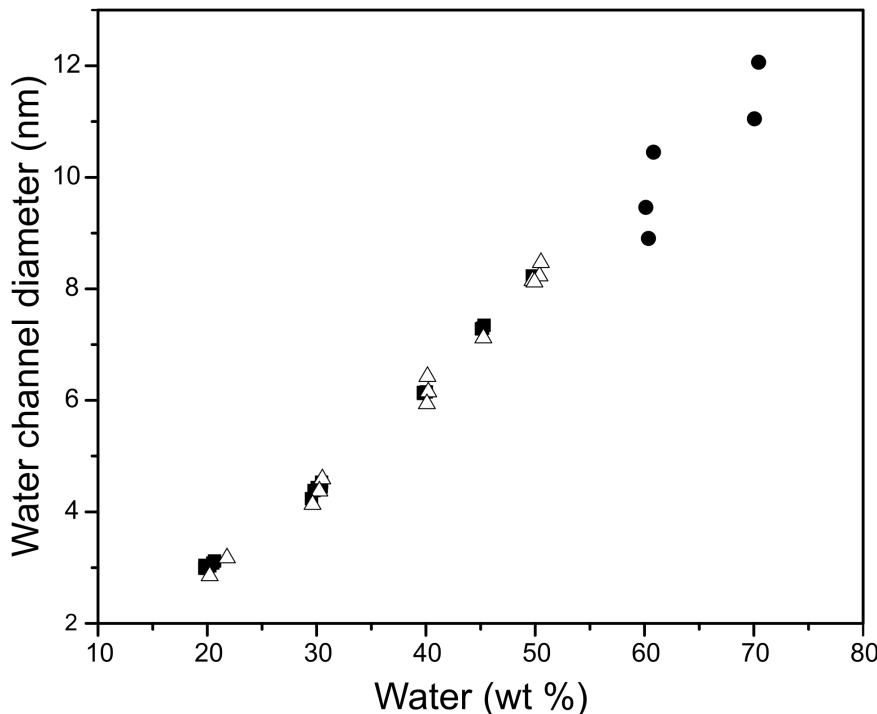


- Enhances swelling properties
- Stabilize nanoparticles



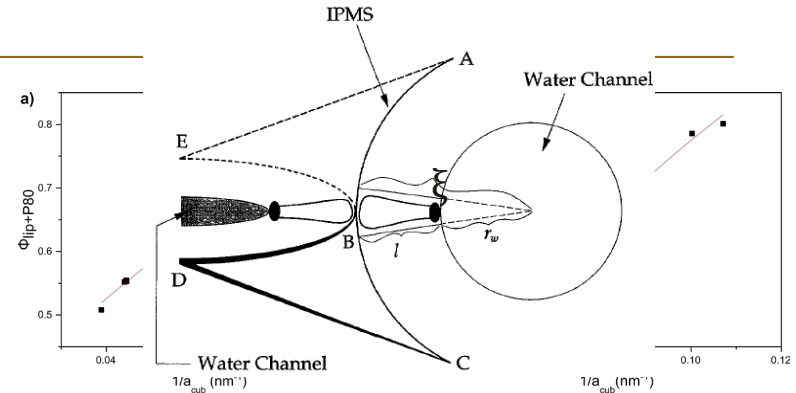
The lipid system

Monolayer thickness (l): a) 2.2 nm for Ia3d and b) 2.3 nm for Pn3m



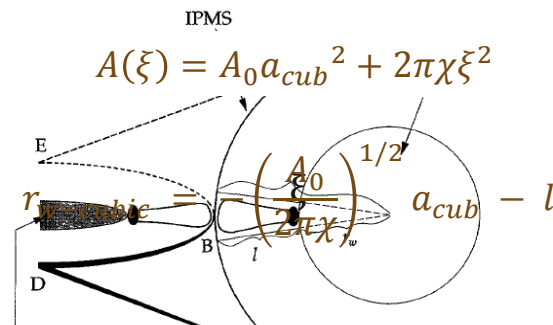
A_0 = ratio of the IPMS area to (unit cell volume)^{2/3}
 χ = Euler-Poincaré characteristics
 $A(\xi)$ = molecular cross sectional area

- [1] Briggs et al., J. Phys. II, vol. 6, no. 5, pp. 723–751, 1996.
 [2] Turner et al., J. Phys. II, vol. 2, no. 11, pp. 2039–2063, 1992.
 Valdeperas et al. *Langmuir* 2016 32 (34), 8650-8659



It was considered that inverse cubic phases are curved lipid bilayer draped on an Infinite Periodic Minimal Surface (IPMS):^{1,2}

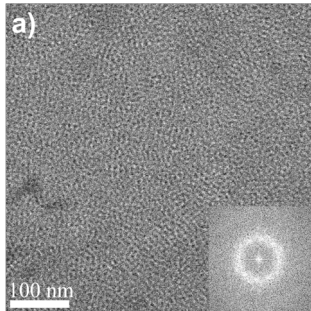
$$\Phi_{lip+P80} = 2A_0 \left(\frac{l}{a_{cub}} \right) + \frac{4\pi\chi}{3} \left(\frac{l}{a_{cub}} \right)^3$$



Water channels diameter up to 12 nm were achieved!

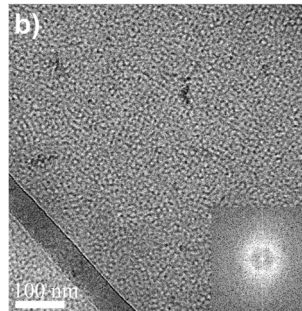


The lipid system



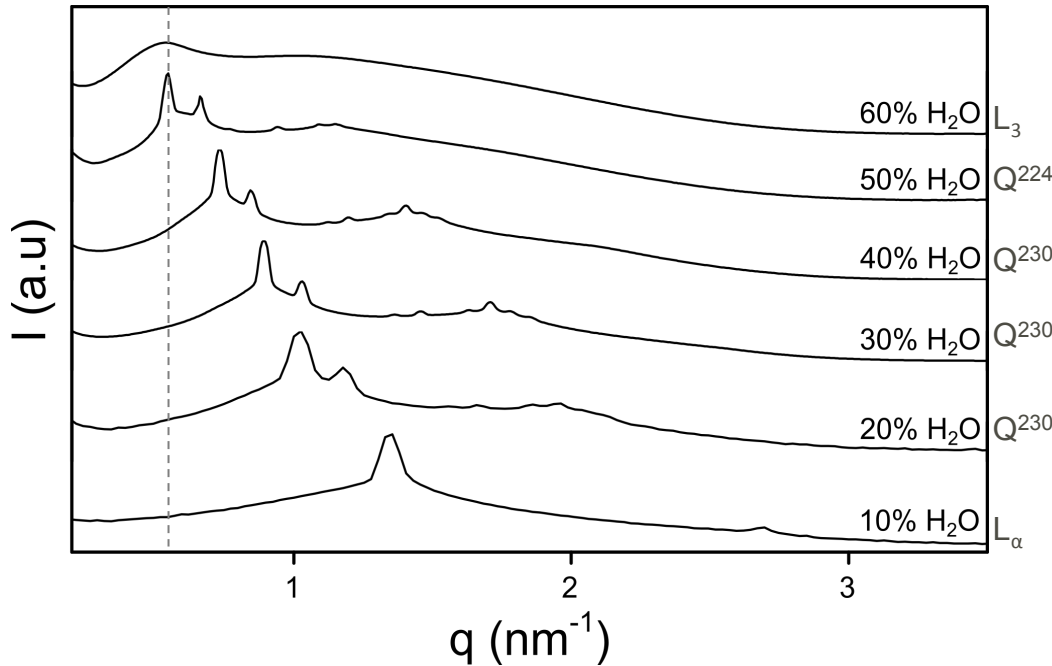
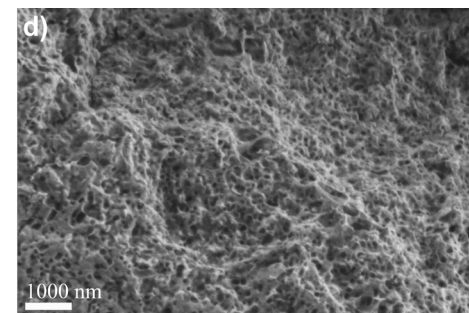
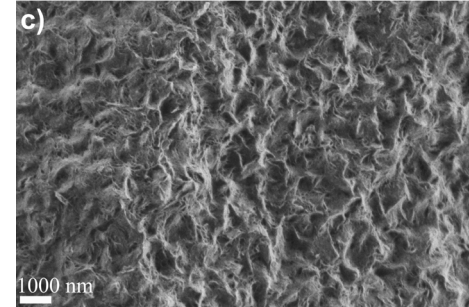
Cryo-TEM

- Highly dense and homogeneous structure
- FFT: Diffuse order

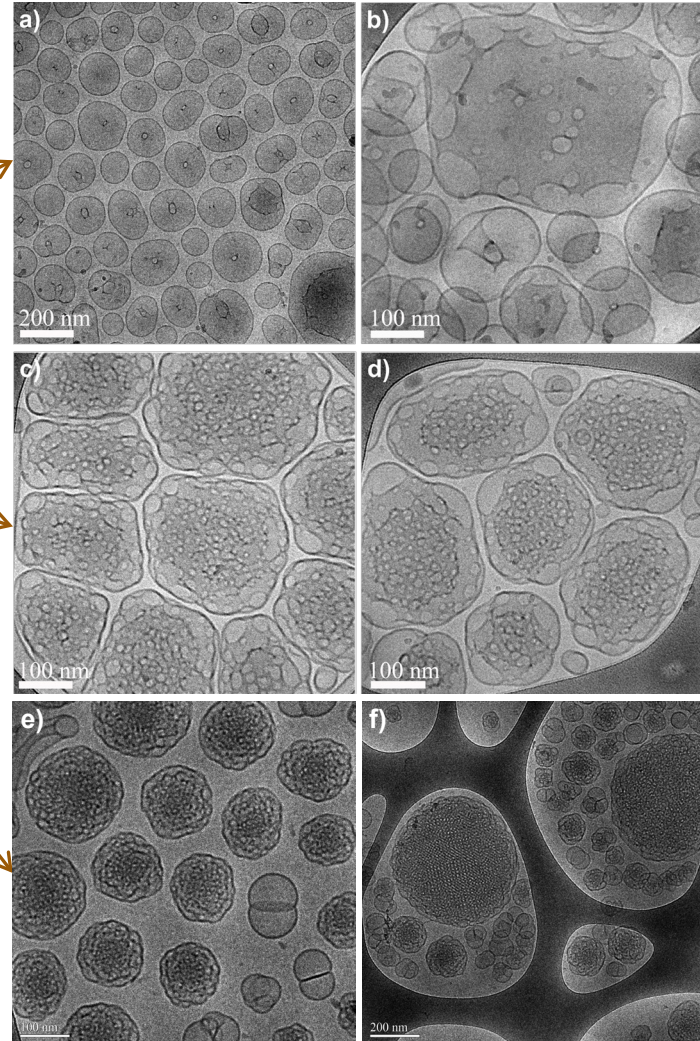
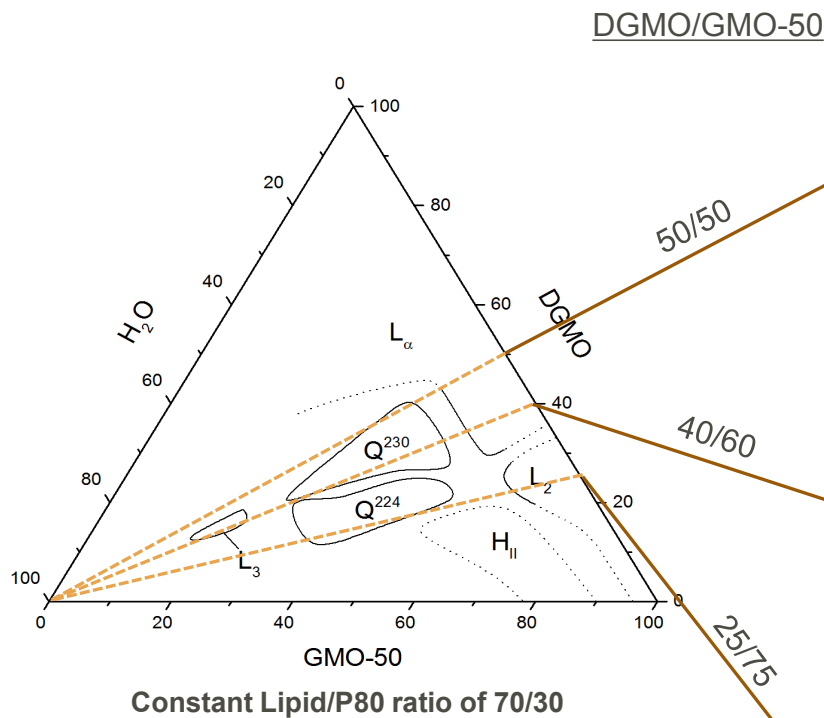


Samples with 40/60 DGMO/GMO-50
Lipid/P80 70/30
60% aqueous phase

Cryo-SEM: bicontinuous network

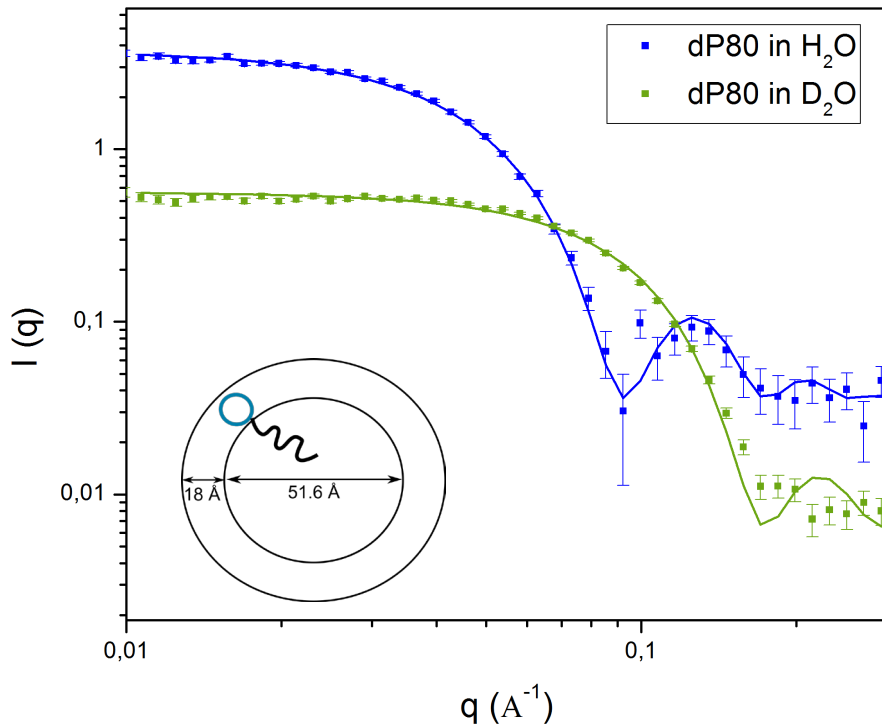


Sponge-like nanoparticles dispersion



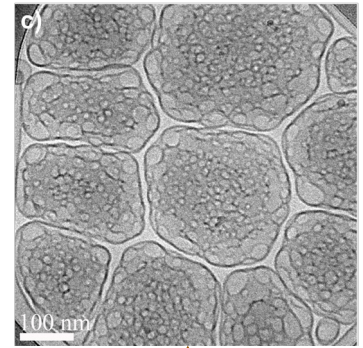
- Nanoparticles size varies between 130 and 280 nm
- Aqueous pores up to 13 nm were found

SANS: role of P80

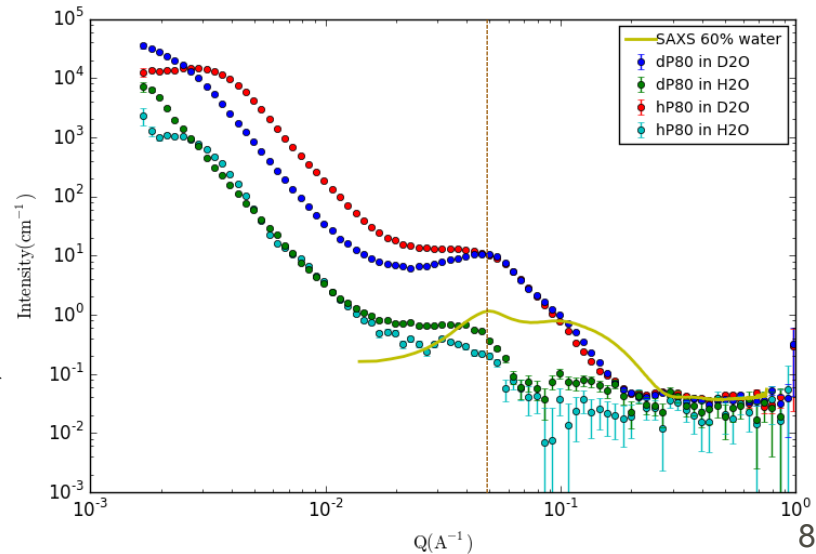
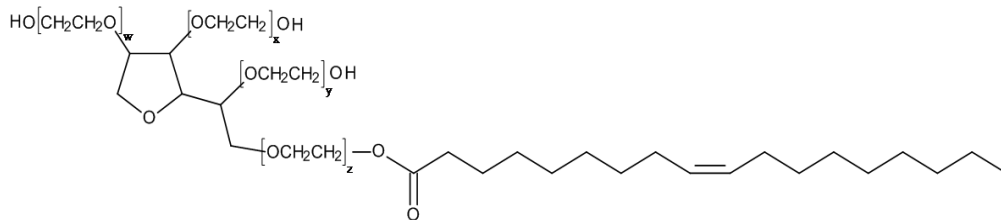


	dP80 in D ₂ O	dP80 in H ₂ O
SLD _{core} (Å ⁻² × 10 ⁻⁶)	0,770	-0,023
SLD _{shell} (Å ⁻² × 10 ⁻⁶)	6,517	1,009
SLD _{solvent} (Å ⁻² × 10 ⁻⁶)	6,350	-0,560

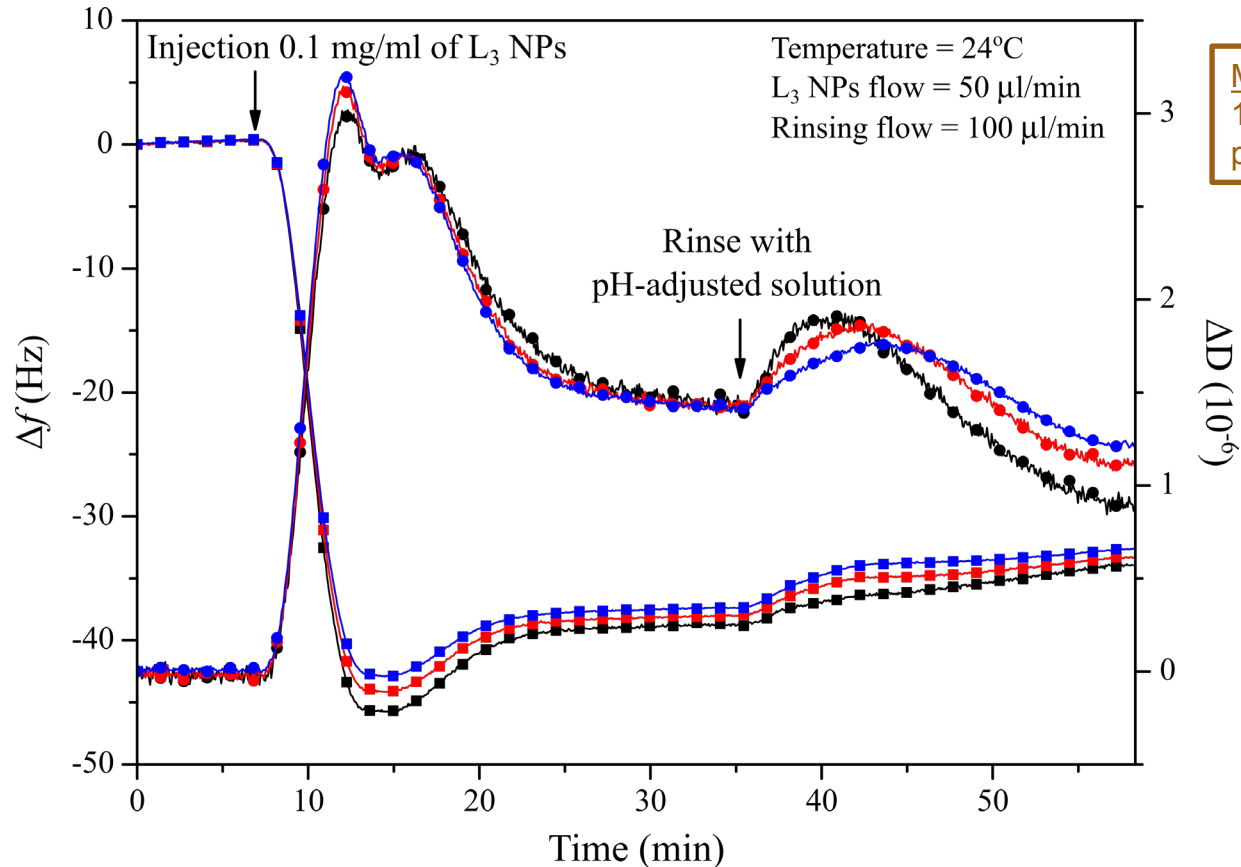
- SLD_{core} = 0.04498 × 10⁻⁶ Å⁻² (11.5% solvent penetration)
- SLD_{shell} = 7.17 × 10⁻⁶ Å⁻² (80% solvent penetration)



40/60 DGMO/GMO-50



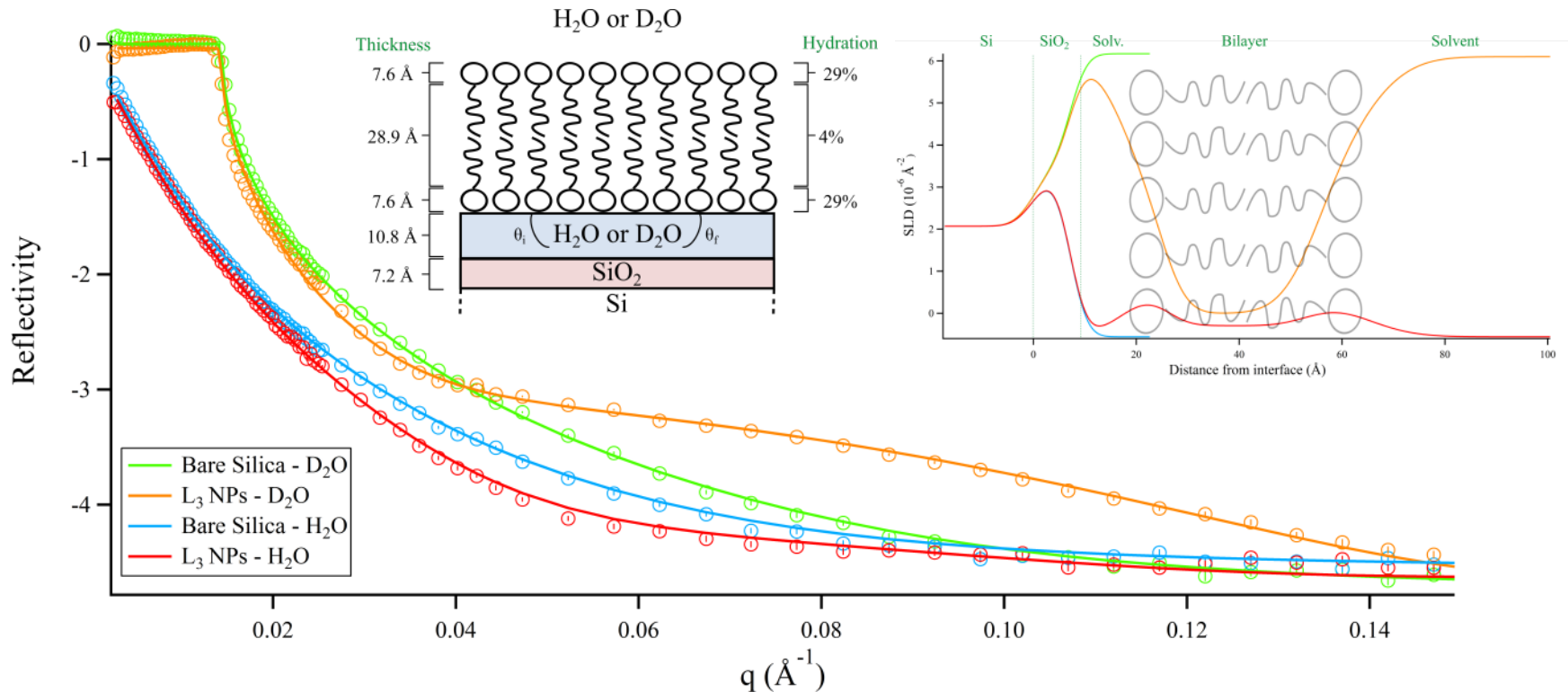
Adsorption studies: QCM-D



- A quite rigid and well-defined layer is adsorbed on hydrophilic silica
- There is reorganization of molecules on the interface



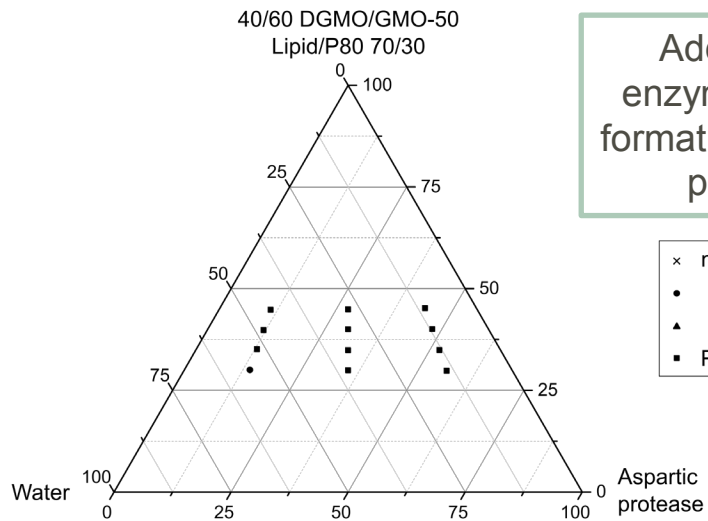
Adsorption studies: Neutron reflectometry



- L₃ NPs was found to form a bilayer of 44 Å on silica, with roughness up to 8 Å.
- Particles partially spread and rearrange themselves on this surface

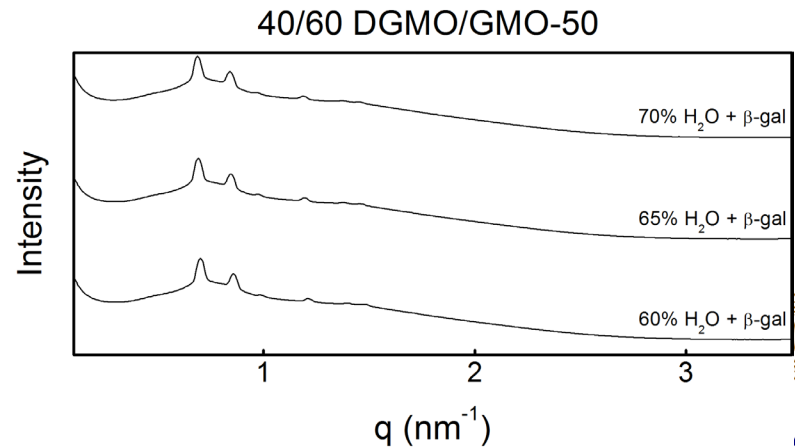
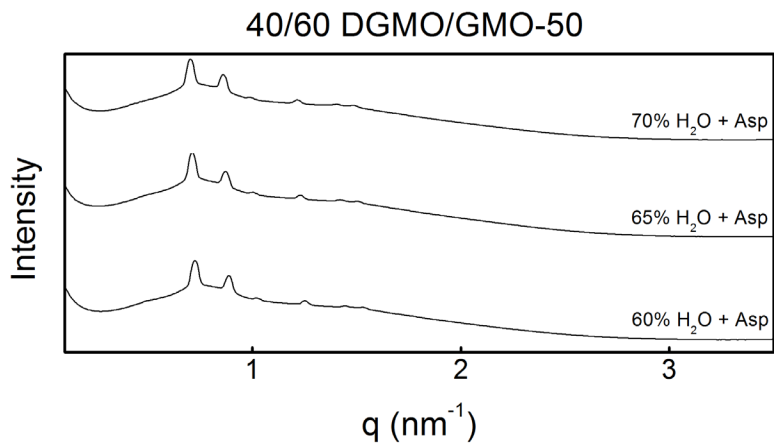
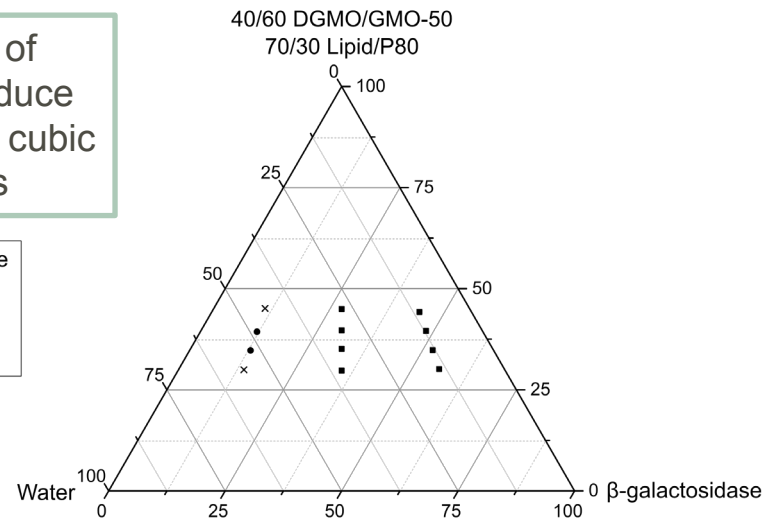


Structural changes on the lipid system

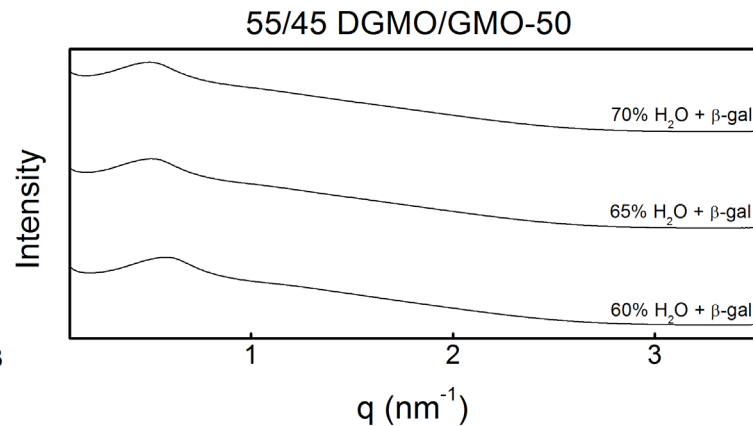
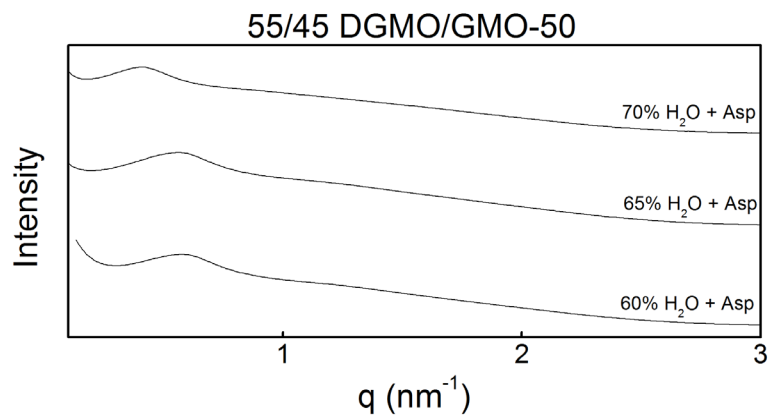
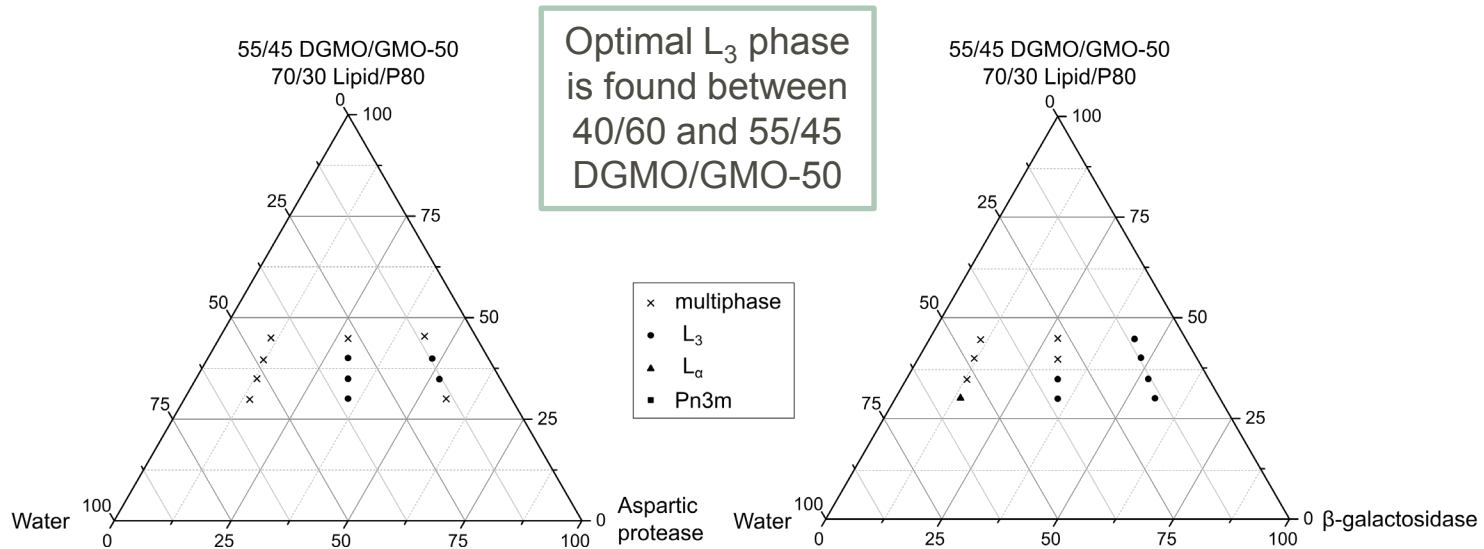


Addition of enzyme induce formation of cubic phases

- × multiphase
- L_3
- ▲ L_α
- Pn3m

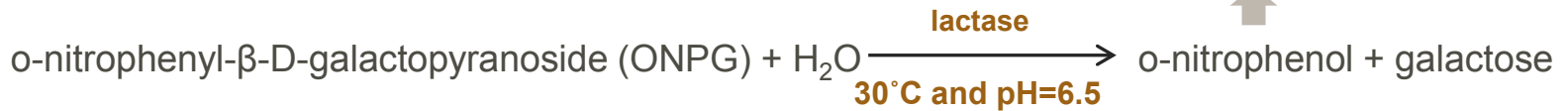


Structural changes on the lipid system



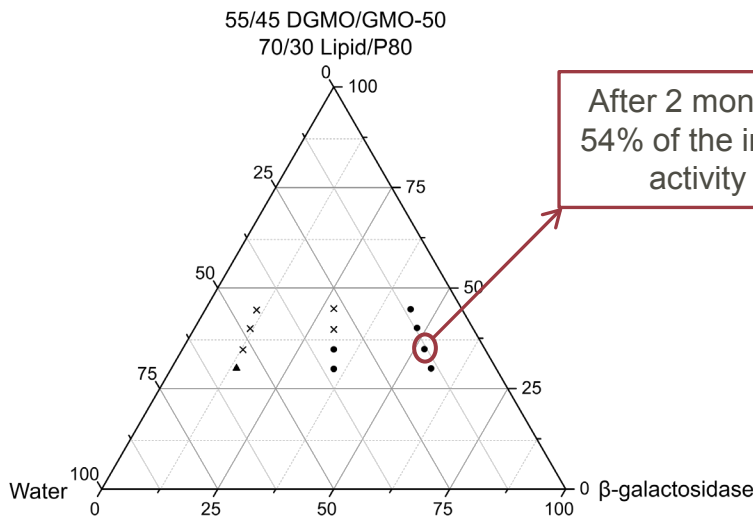
Activity test – Preliminary results

- β -galactosidase:



$$NLU/g = \frac{\Delta Abs \times 8 \times Total\ Dilution}{\epsilon \times 10 \times 1.30}$$

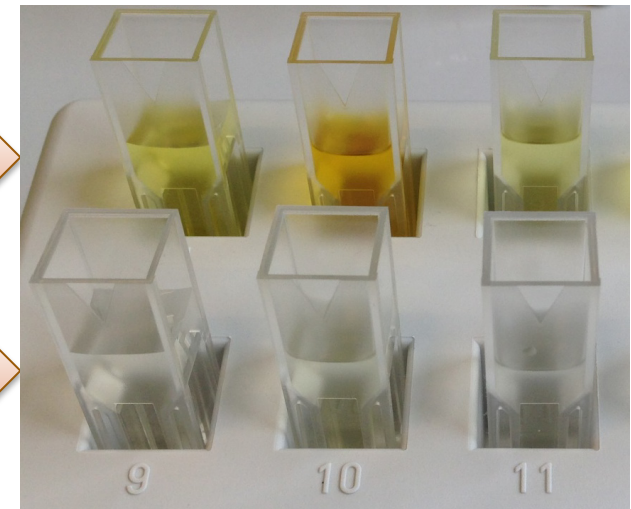
Detected at 420nm



After 2 months:
54% of the initial activity

Samples

Blind



Standard

2 week old sample
100x diluted

3 months old sample
100x diluted

Samples were kept at room temperature while enzyme reference solution was kept at 5°C to maintain its activity.

Conclusions and future work

- Addition of P80 to the DGMO/GMO-50/water system allows us to obtain highly swollen phases.
- Water channels up to 13 nm of diameter were achieved in the L_3 nanoparticles → suitable for entrapment of large biomolecules
- Addition of both enzymes induce a phase shift to the cubic phases.
- Adsorption studies suggest that the L_3 nanoparticles self-assemble on hydrophilic silica by forming a bilayer → possible use as drug delivery process
- Further work:
 - Check activity and stability of the encapsulated enzyme
 - Locate the enzyme and study the enzyme-lipid interactions by SANS and NR experiments.



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