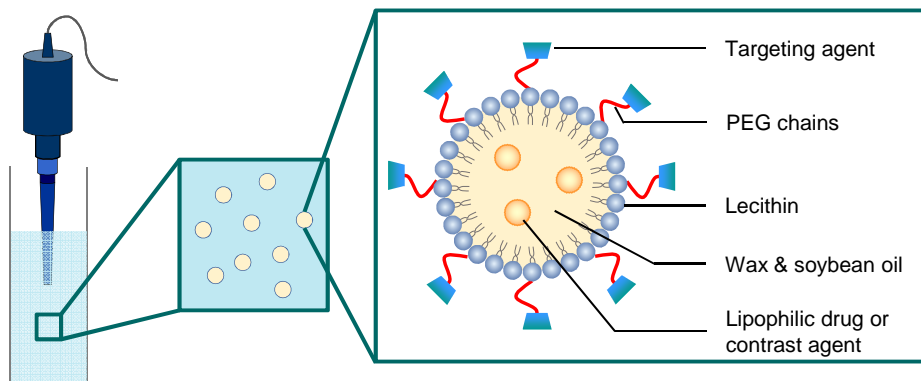


CONTRAST ENHANCEMENT FOR LNP CHARACTERIZATION USING TRANSMISSION ELECTRON MICROSCOPY

NanoSafe 2018 | Amandine Arnould | 05-09/11/2018



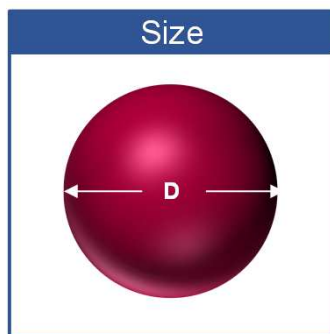
GLOBAL INFORMATION ABOUT LIPID NANOPARTICLES



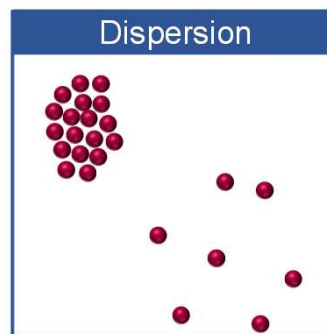
Ultrasonication process

- Completely biodegradable & biocompatible
- **Lipophilic** drug or contrast agent
- Amorphous particles
- Commonly characterized by DLS (size)
- F80 - NC75 (75% wax / 25% soybean oil): $D_h = 80 \text{ nm}$

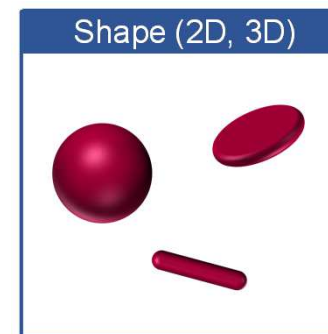
Robust characterization required:



- Blood capillaries
- EPR effect
- Cell internalization



Agglomerates or aggregates
of LNP less effective



Drug internalization

CHARACTERIZATION METHODS : DLS, AF4-LS AND TEM

M I C R O S C O P Y

Transmission Electron Microscopy (TEM)

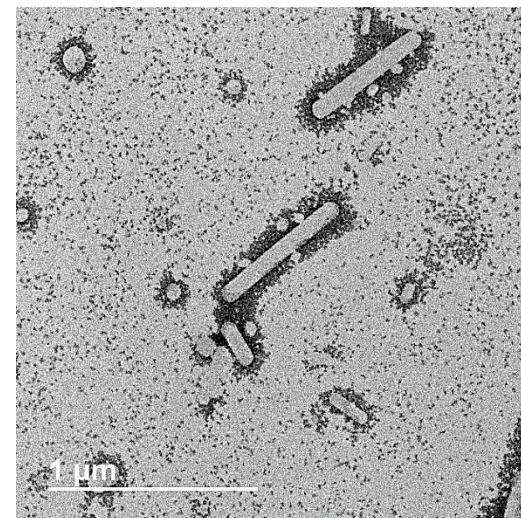
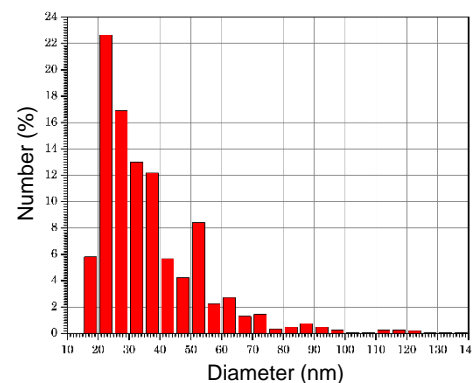


Dry process

Rapid freezing

In-situ liquid

- 2D and 3D imaging technique
- Suitable sample preparation required



S P E C T R O S C O P Y

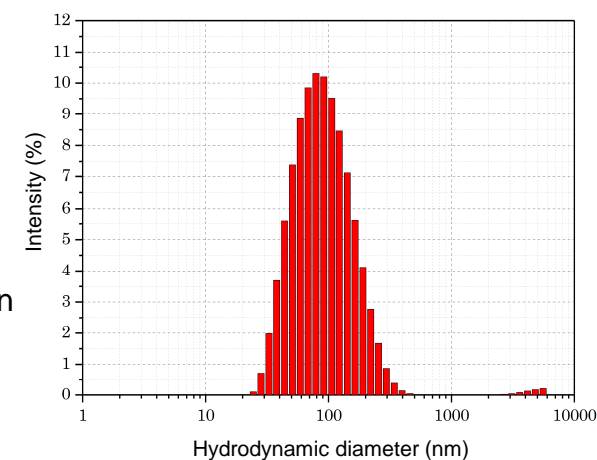
Light Scattering (LS)



DLS
(batch mode)

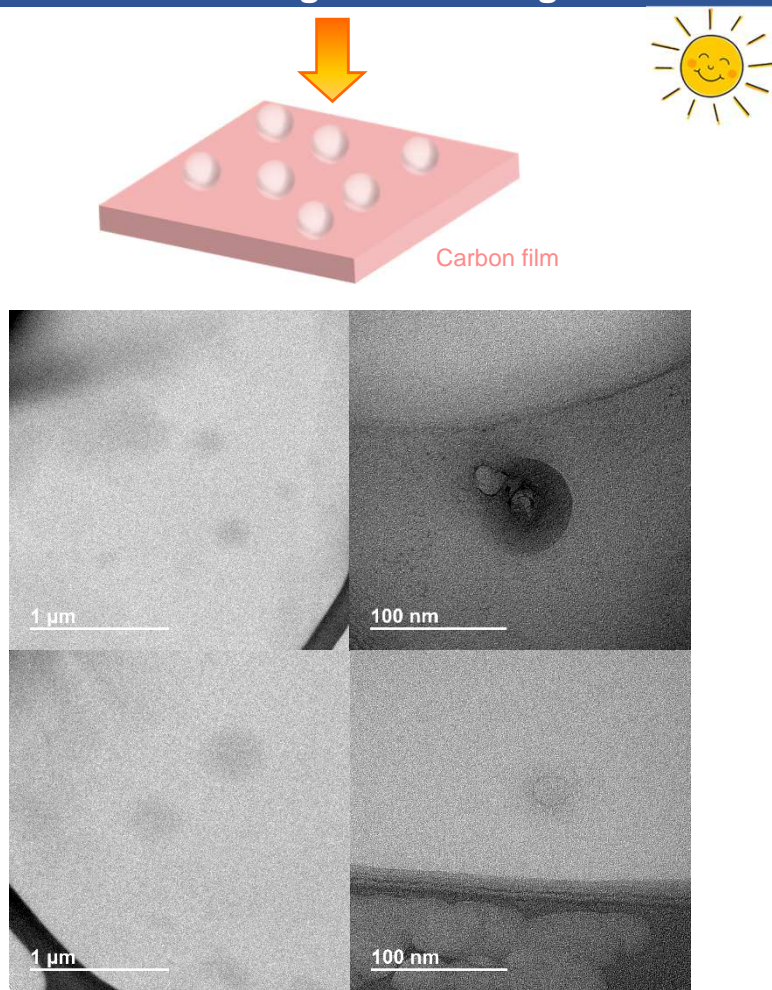
AF4-LS
(online mode)

- Commonly used technique
- Fast and cheap
- Only for spherical and monodispersed samples
- Fractionation before detection
- Detectors: DLS, MALS, ICP-MS, UV-Vis, ...
- MALS : shape based model



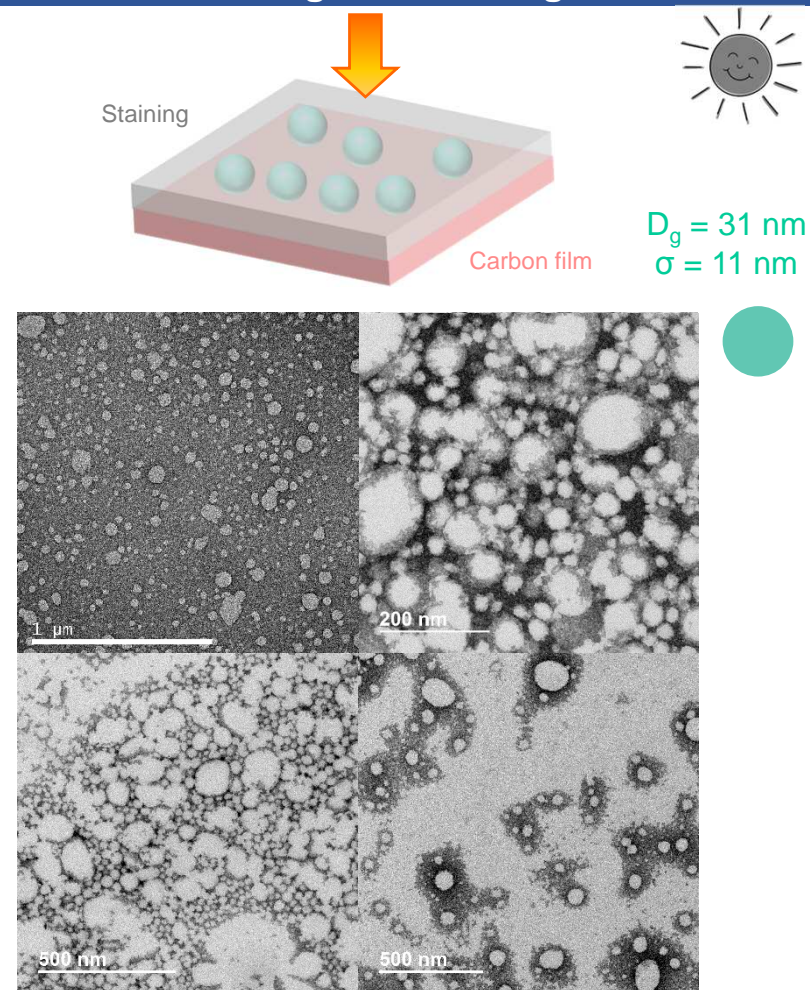
TEM SAMPLE PREPARATION AND RESULTS : DRYING

Without negative staining



Artefact on the carbon film or lack of contrast of LNPs ?

With negative staining



Good contrast but agglomeration and intern structure not visible

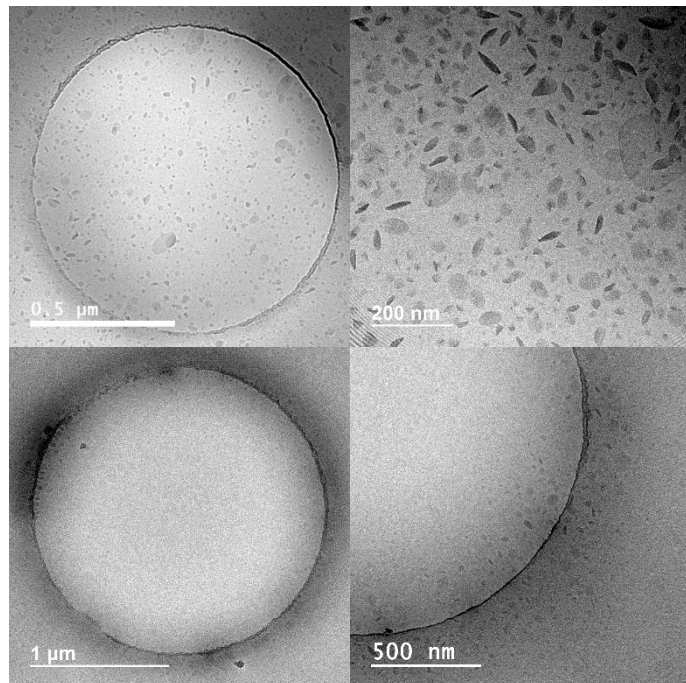
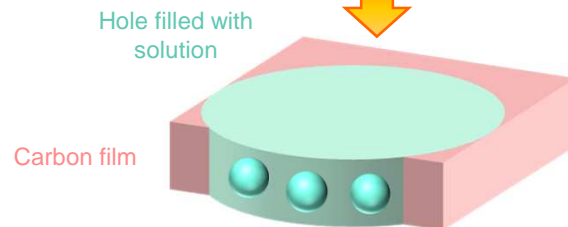
TEM SAMPLE PREPARATION AND RESULTS: CRYO

Cryo-TEM



Vitrobot parameters:

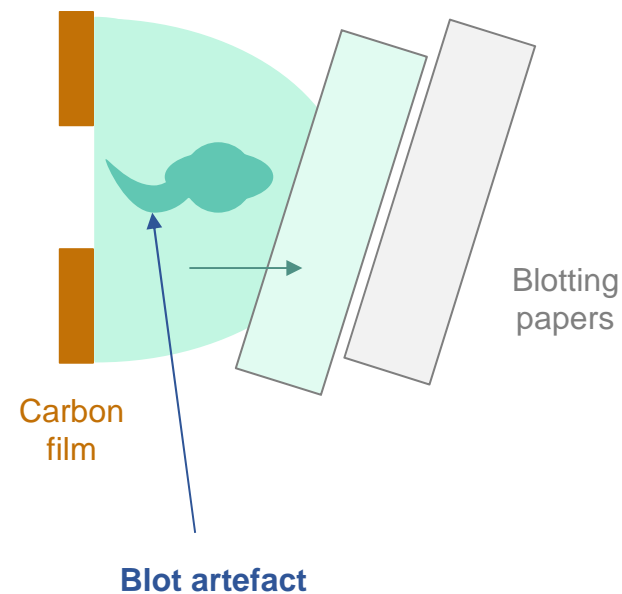
- Grid C-Flat-T
- Blot force: -5
- Blot time: 2s
- Drain time: 0s



$$D_g = 25 \text{ nm}$$

$$\sigma = 8 \text{ nm}$$

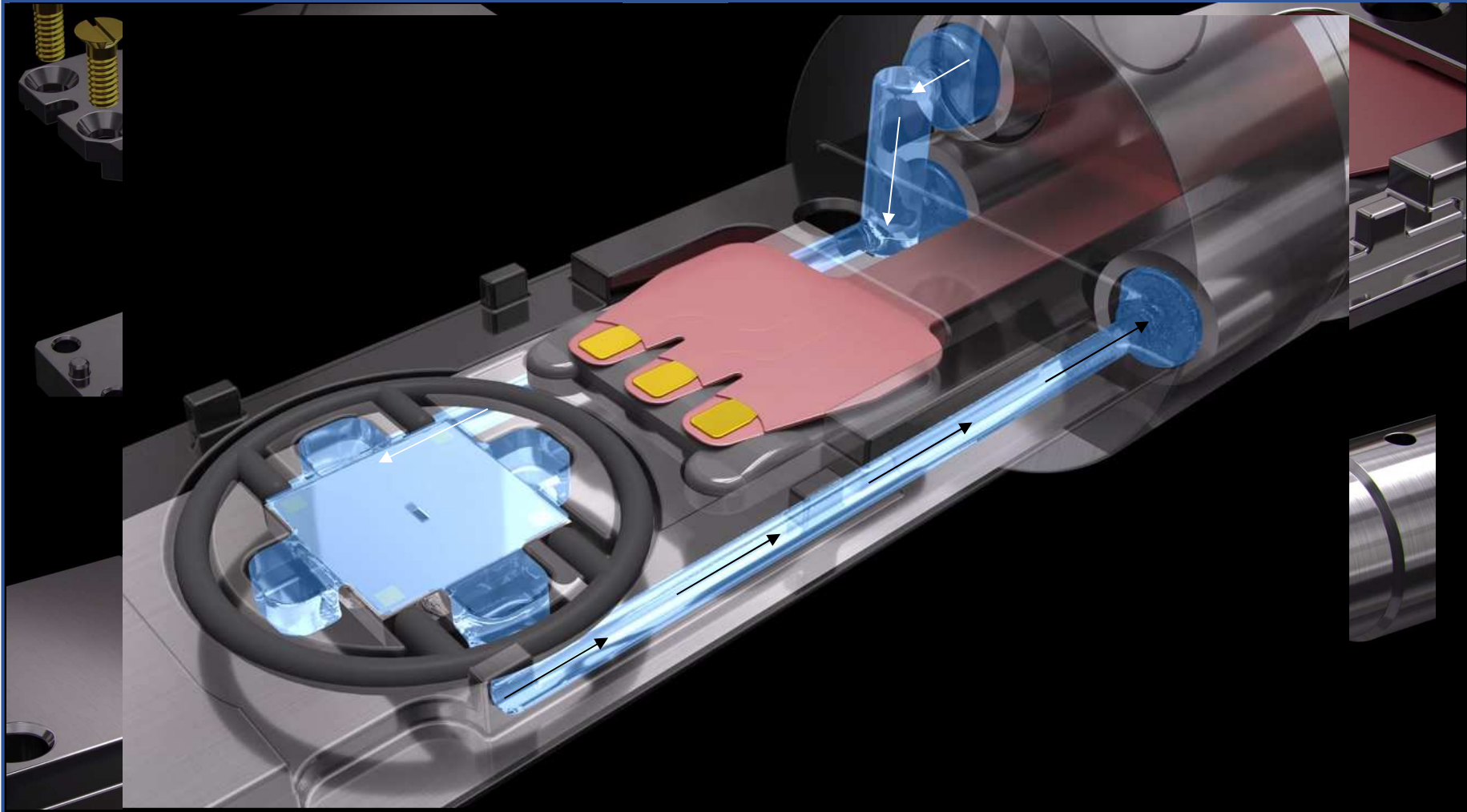
Blot process



Particle deformation (blot process), cryo-TEM not adapted

TEM SAMPLE PREPARATION AND RESULTS: IN-SITU

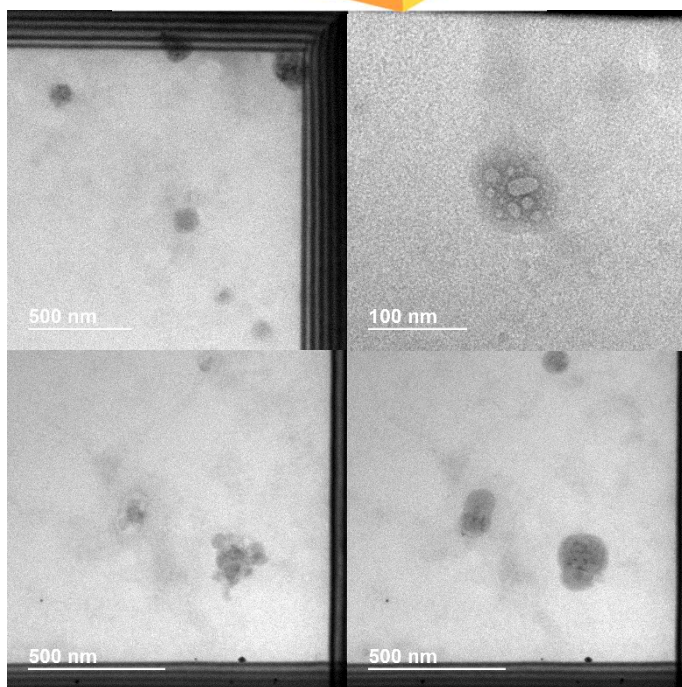
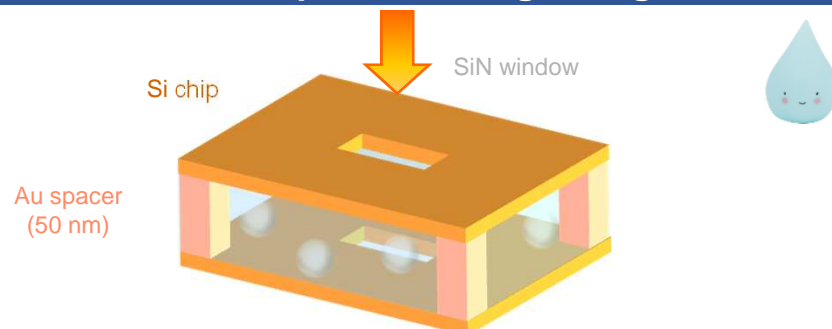
In-situ liquid TEM



Innovative technique

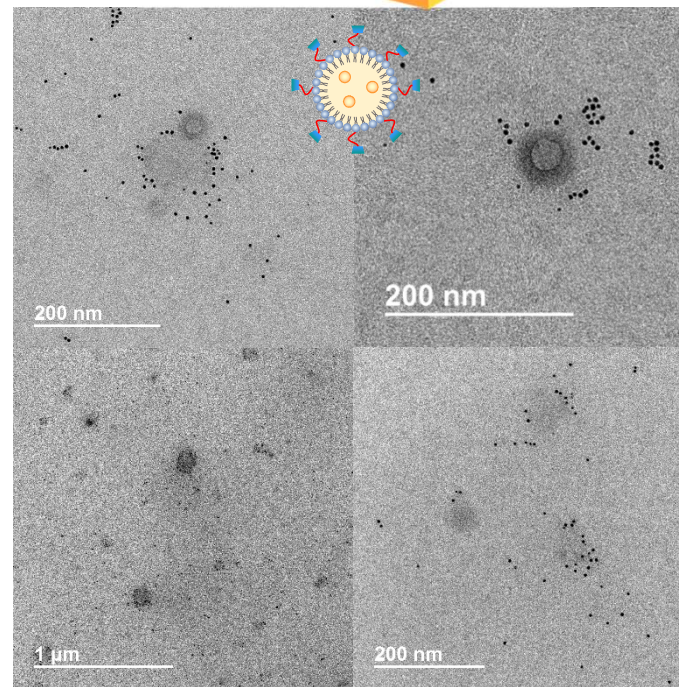
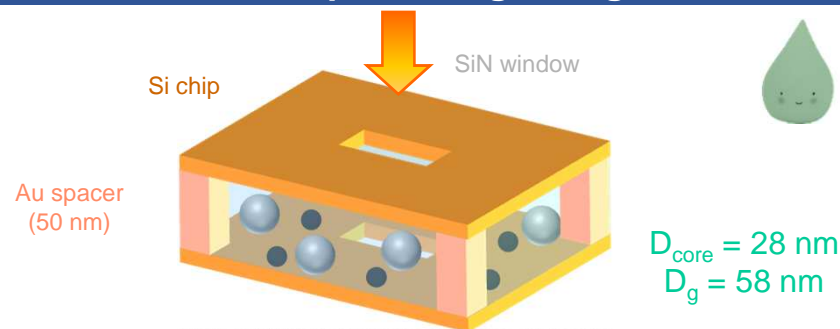
TEM SAMPLE PREPARATION AND RESULTS: IN-SITU

In-situ liquid without grafting



No contrast, hydrolysis

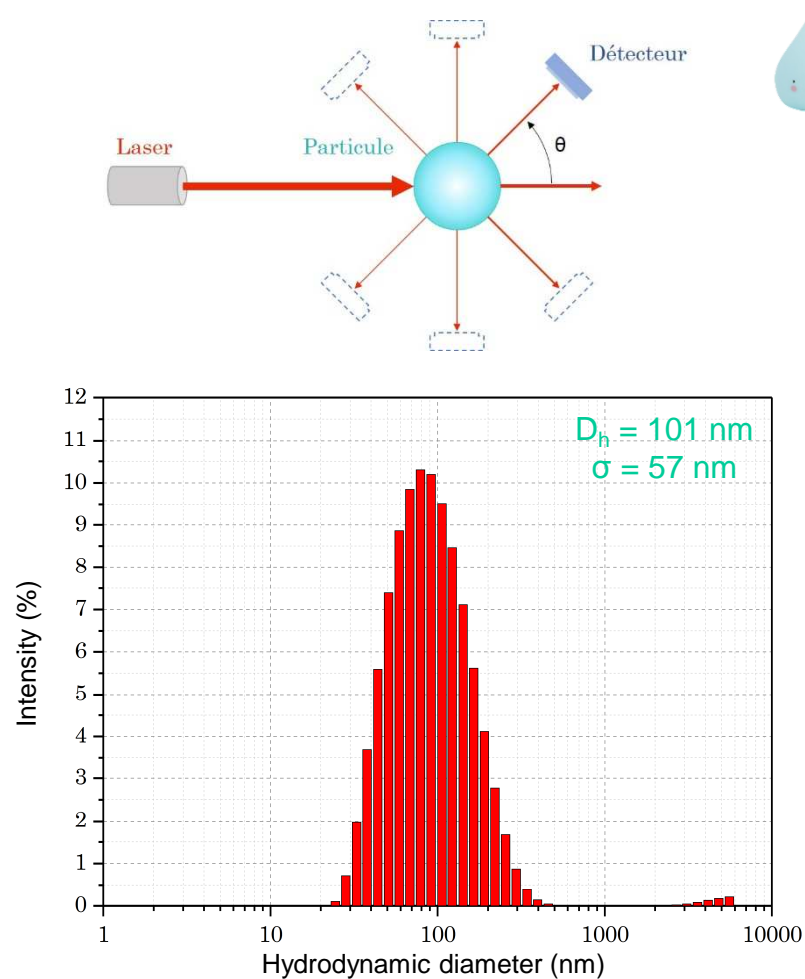
In-situ liquid with grafting



Contrast OK + Core/shell structure

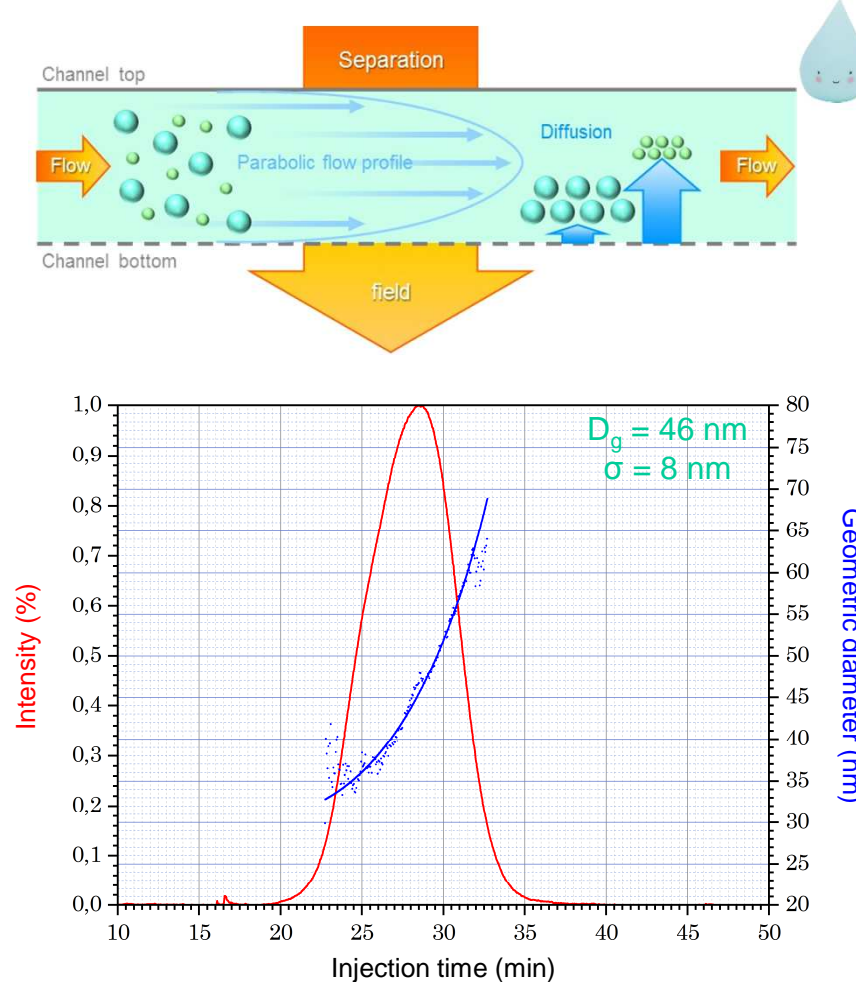
LIGHT SCATTERING RESULTS : DLS AND AF4-MALS

DLS (batch mode)



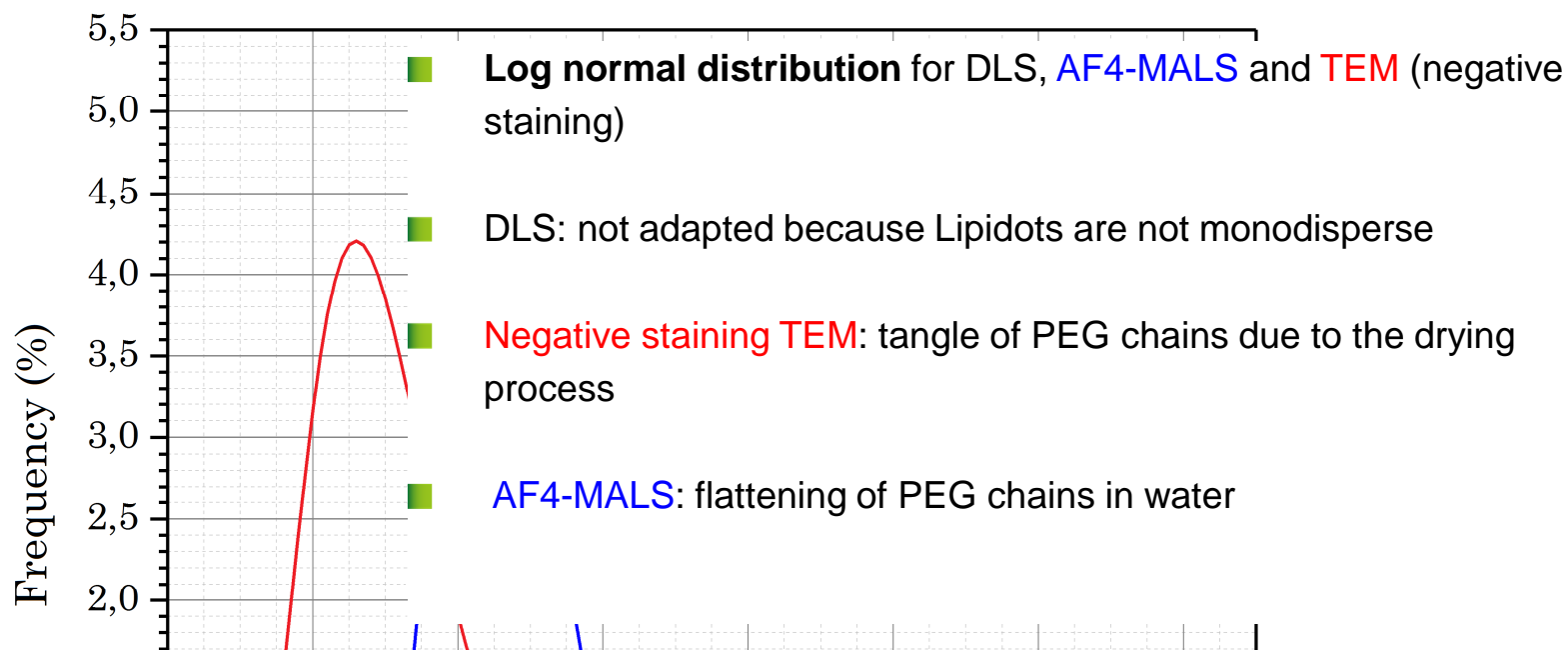
Smallest particles hidden by the largest ones

AF4-MALS (online mode)



Analysis OK

COMPARISON OF THE CHARACTERIZATION METHODS

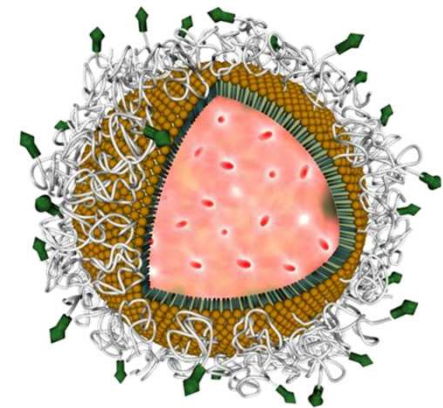


- Cryo-TEM : not adapted for LNPs (preparation artefact on the nanoparticle shape)
- *In-situ* liquid : weak statistic, core@shell structure visible
- AF4-MALS, *in-situ* TEM and negative staining well adapted for lipid nanoparticle characterization: REPEATABILITY, RELIABILITY

CONCLUSION AND PERSPECTIVES

- Set up of a characterization procedure for the study of LNPs
- Comparison of 5 techniques : DLS, AF4-MALS, negative staining TEM, cryo-TEM, *in-situ* liquid TEM
- **AF4-MALS & negative staining TEM** : best methods
- ***In-situ* liquid TEM**: core/shell structure visible BUT work on contrast enhancement (patent)

- Study of drug loaded nanoparticles, stability (aging, biological media) (article in review)
- ***In-situ*: interaction of nanoparticles with a protein-based media**



Thank you for your attention

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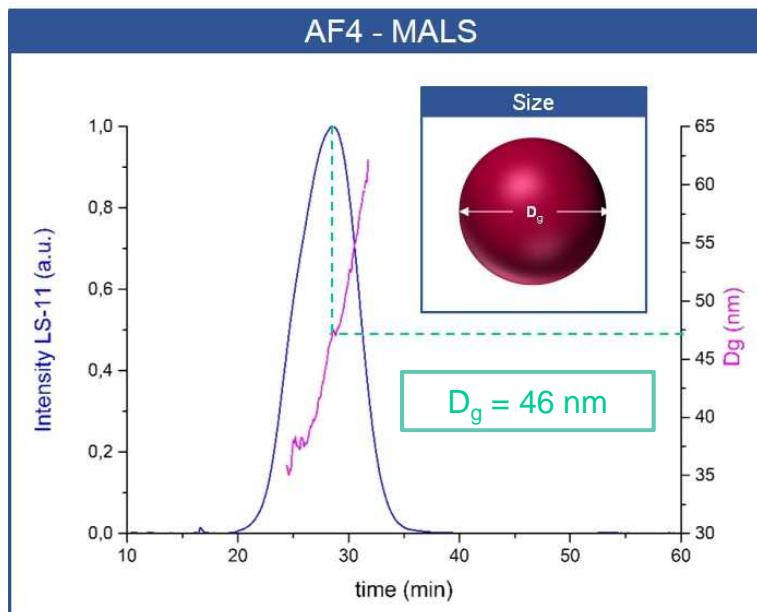
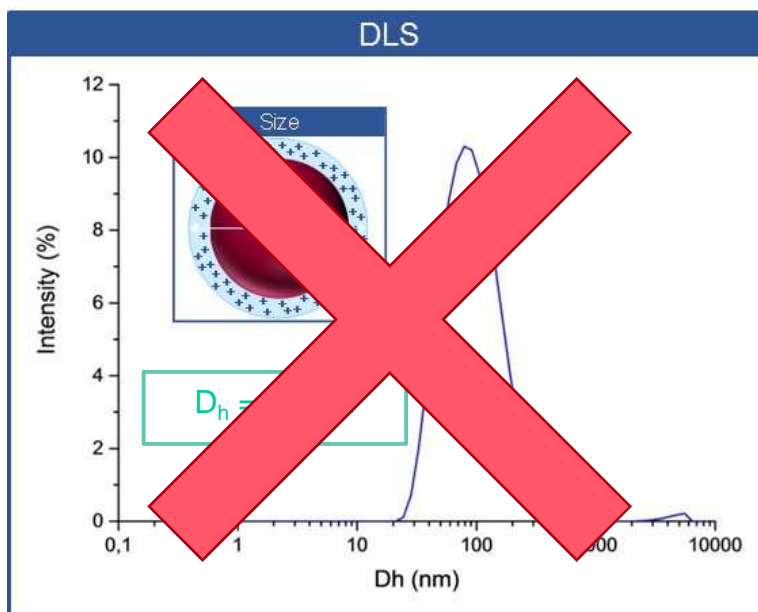
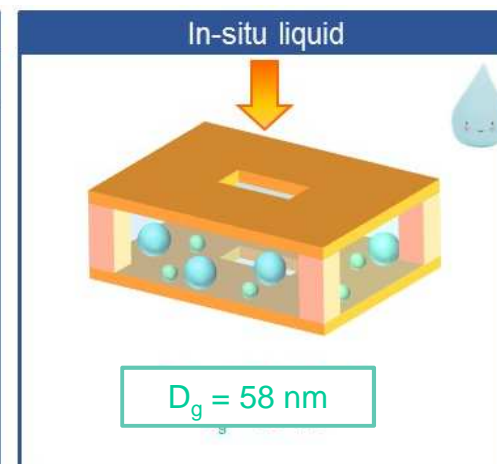
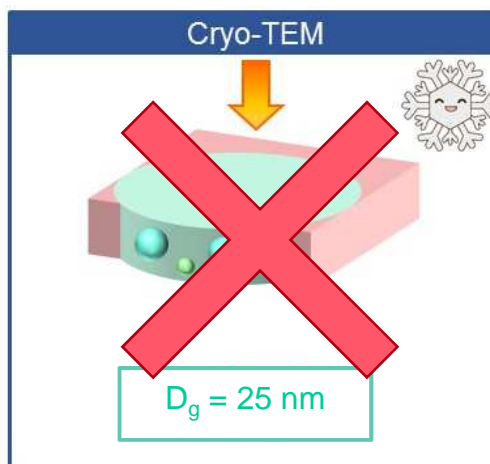
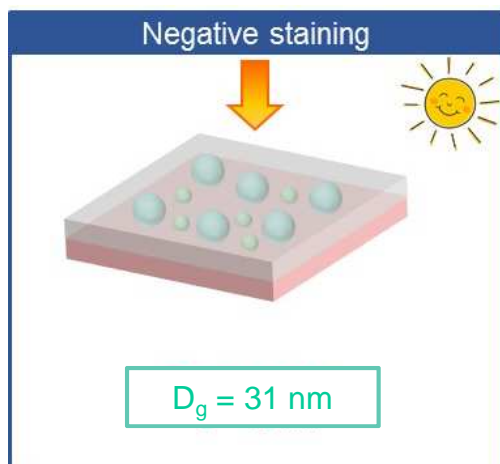




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COMPARISON OF THE CHARACTERIZATION METHODS

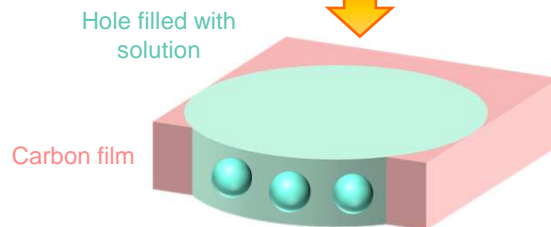


TEM SAMPLE PREPARATION AND RESULTS: CRYO

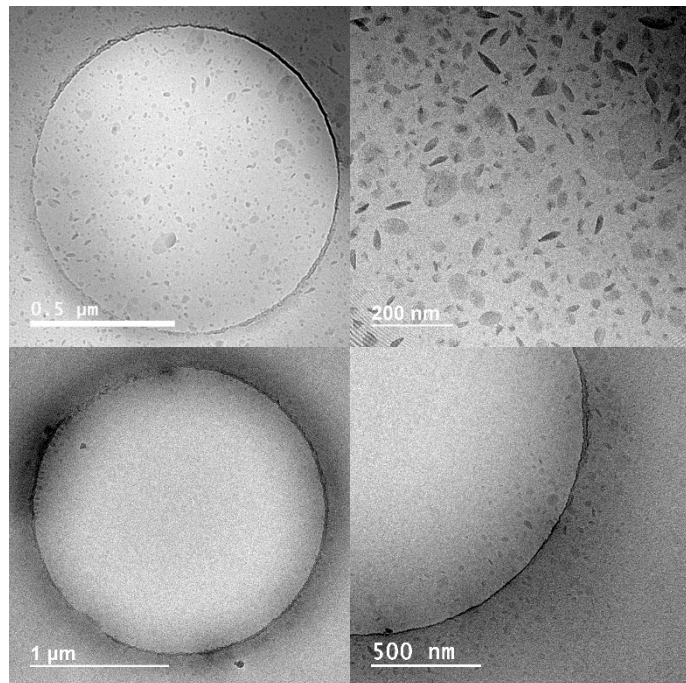
Cryo-TEM

Vitrobot parameters:

- Grid C-Flat-T
- Blot force: -5
- Blot time: 2s
- **Drain time: 0s**



Ice in the center of the holes
too thin ?



Thickness measurement techniques:



$$D_g = 25 \text{ nm}$$

$$\sigma = 8 \text{ nm}$$

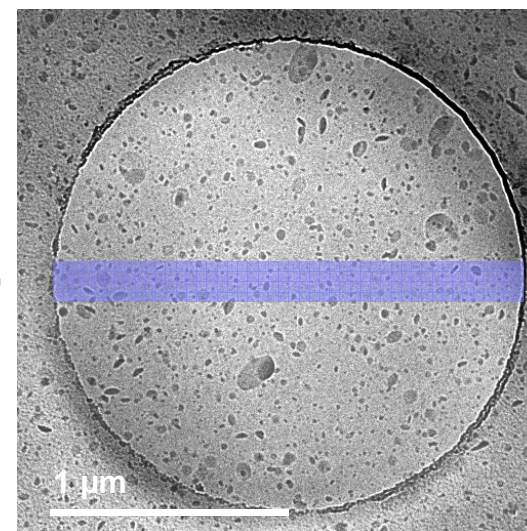
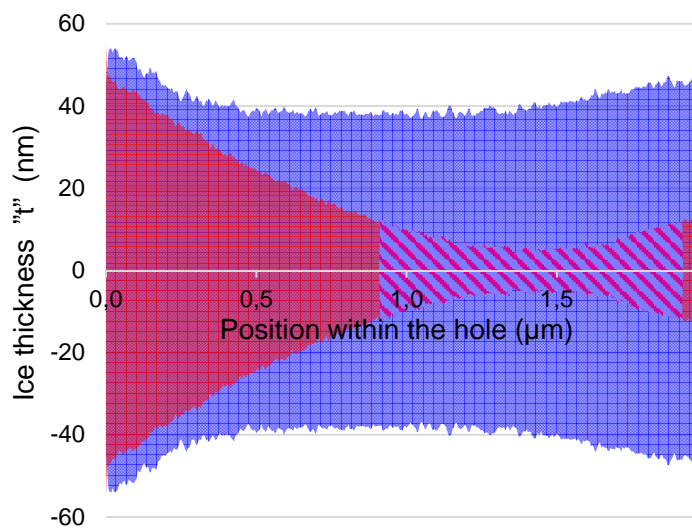
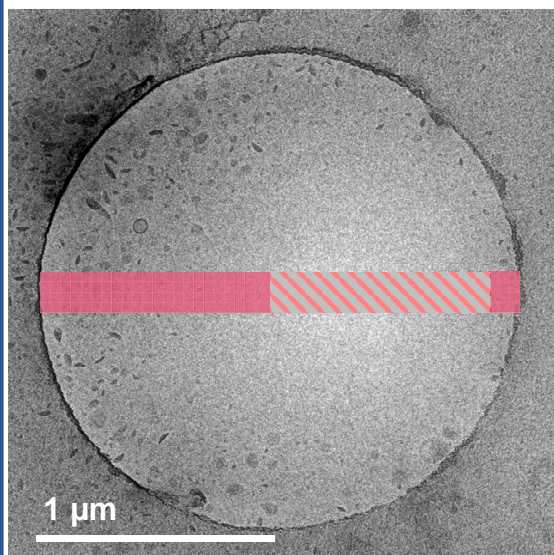
- CBED: crystalline material ~~✗~~
- Contamination spot: incompatible with ice ~~✗~~
- EDX: Zeta factor (complex) ~~✗~~
- EELS: t/λ quick to set up

Problem with the particle distribution within the holes and with their morphology

TEM SAMPLE PREPARATION AND RESULTS: CRYO

Cryo-TEM

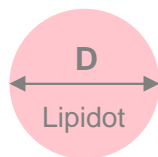
Ice thickness measurement



$$t < D_{\min}$$



$$D_{\min} < t < D_{\max}$$



$$20 \text{ nm} < D < 80 \text{ nm}$$



$$D_{\min} < t < D_{\max}$$

Particle distribution within the holes linked to ice thickness

Particles still deformed

CONTEXT : NANOCARRIERS FOR DRUG DELIVERY

■ Nanometer scale

■ Biocompatible

■ (Biodegradable)

■ Organic

■ Metallic

■ Semiconductor

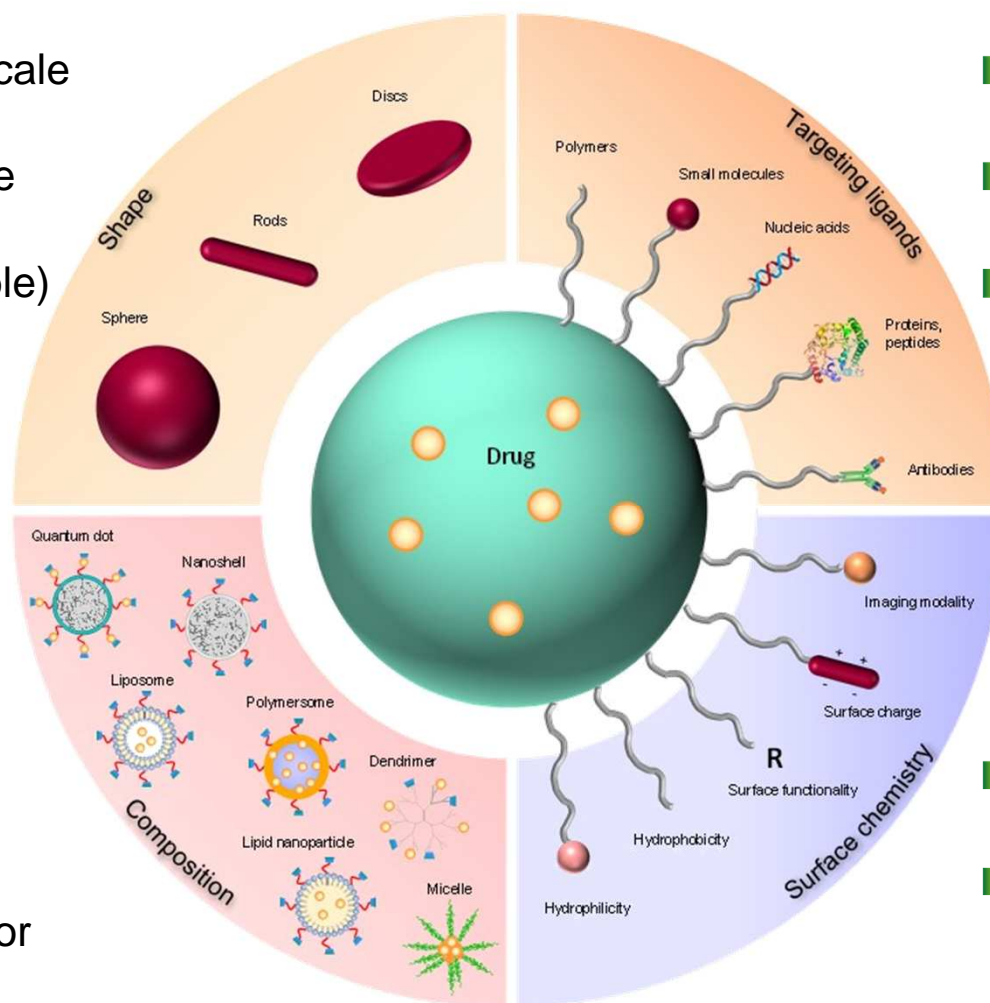
■ Active

■ Passive (EPR effect)

■ Fewer side effects

■ Diagnostic

■ Drug delivery



COMPARISON OF THE CHARACTERIZATION METHODS

Characterization method		Measure	Diameter (nm)	Pros	Cons
DLS		D_h	80	Quick method	Only for monodisperse solutions
AF4-MALS		D_g	46	Fractionation	Time consuming
TEM	Negative staining	D_g	31	Quick method	Mask of the particles
	Cryo		25	Cryofixation	Preparation artefact
	<i>In-situ</i> liquid		58	No preparation artifact	Contrast

- DLS : not adapted because Lipidots are not monodisperse
- Cryo-TEM : preparation artefact on the nanoparticle shape, distortion
- Negative staining : high statistic, tangle of PEG chains due to the drying process
- *In-situ* liquid : weak statistic, flattening of PEG chains, core@shell nanoparticles
- AF4-MALS, *in-situ* TEM and even negative staining well adapted for lipid nanoparticle characterization: REPEATABILITY, RELIABILITY