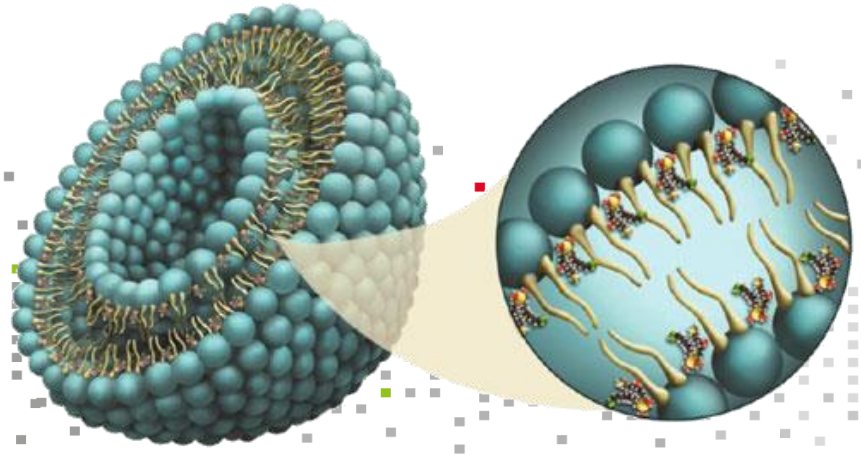
liten
cea tech



CHARACTERIZATION OF NANO CARRIERS FOR DRUG DELIVERY SYSTEMS: THE LIPIDOTS ®

NanoSafe 2016 | Amandine Arnould | 07-11/11/2016

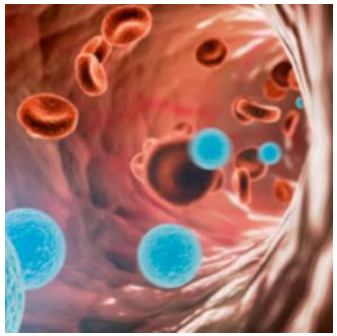
ibs
Institut de Biologie Structurale

AGENCE NATIONALE DE LA RECHERCHE
ANR
NanoID
NanoSécurité

UNIVERSITÉ
Grenoble
Alpes

CONTEXT : NANOPARTICLES IN SENSITIVE MEDIA

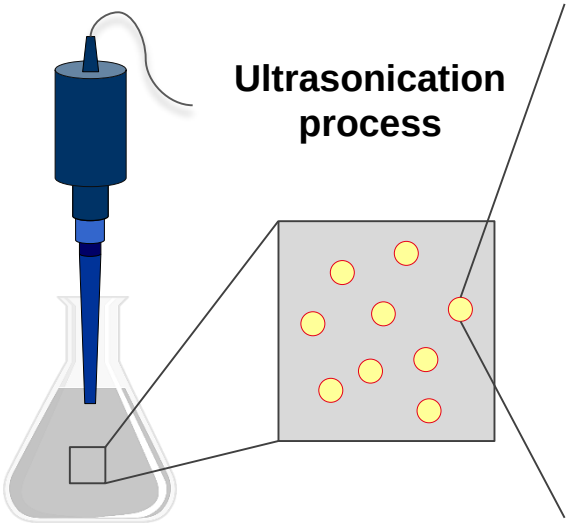
Nano-safety issue

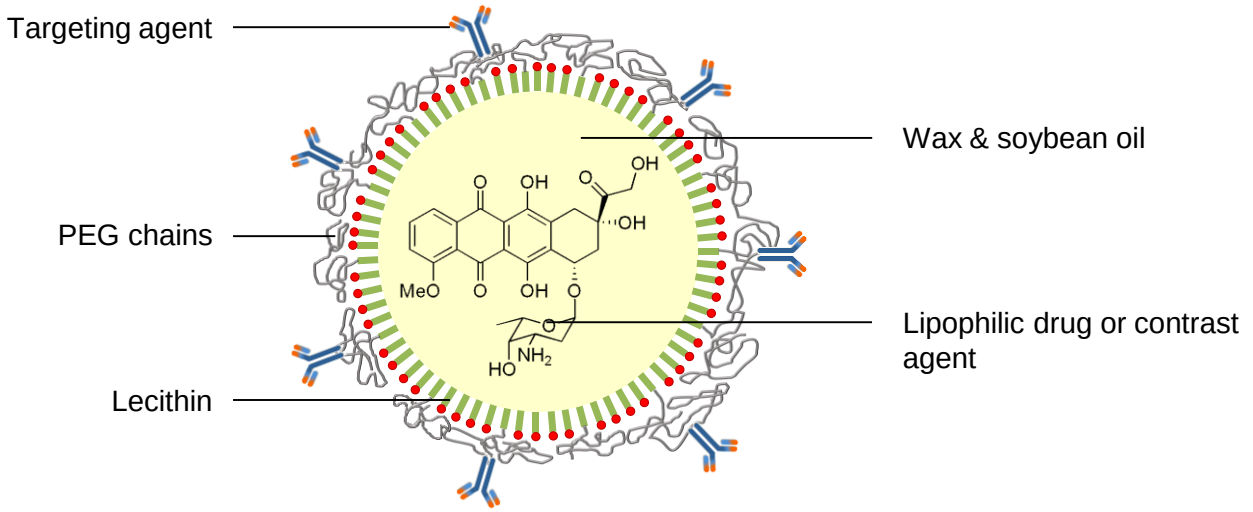
Environment	Medicine	Agribusiness	Cosmetic
			
- Air decontamination - Water treatment	- Bactericide - Drug carriers	- Food colouring - Anticaking	- UV filter - Colouring
Fe, Al	**Ag, Liposomes**	**TiO_2, SiO_2**	**Core shell TiO_2**

Robust characterization on organic nanoparticles

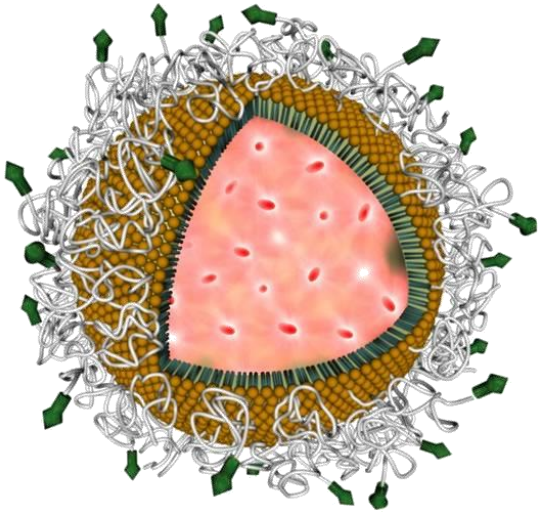
- Stability of the nanoparticles (aging)
- Interaction with biological media (toxicity)

GLOBAL INFORMATIONS ABOUT LIPIDOTS





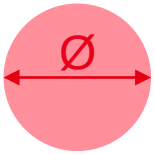
- Tunable diameter (lipid ratio) (30 to 120 nm)
- Completely biodegradable & biocompatible
- **Lipophilic** drug or contrast agent
- Amorphous particles



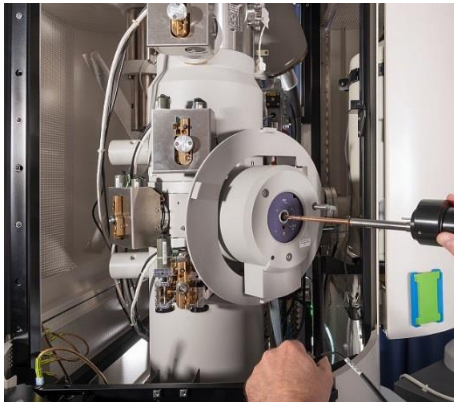
TEM OBSERVATIONS

Transmission Electron Microscopy

Size



Agglomeration, dispersion



- Drying process
- Negative staining + drying process
- Plunge freezing (cryo-TEM)
- In-situ liquid

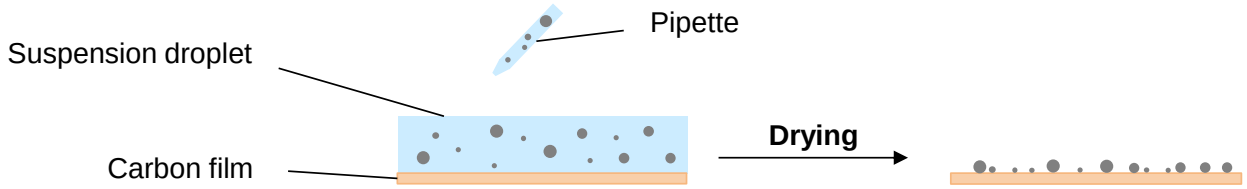
⇨ Well adapted for metallic particles

⇨ Optimizations for organic particles

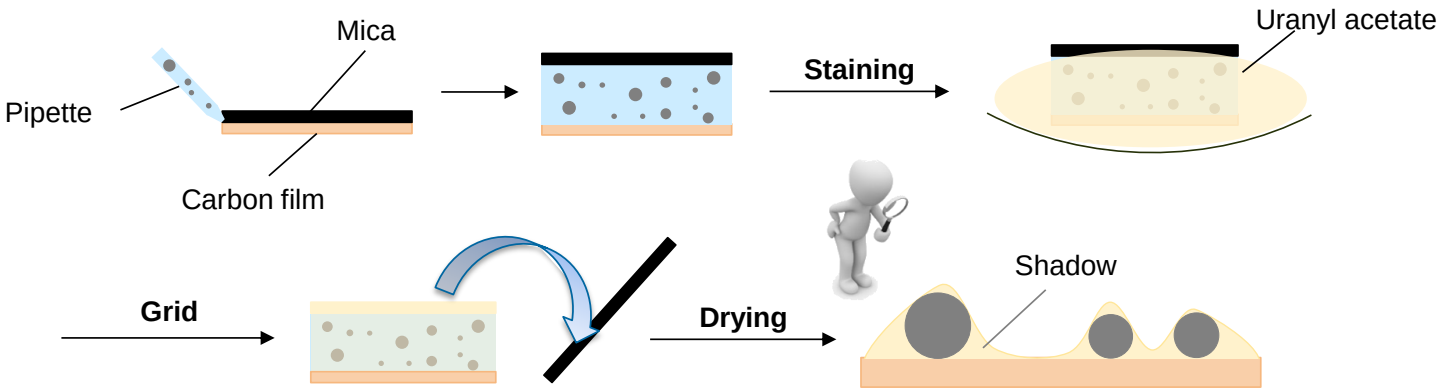
TEM OBSERVATIONS

Drying process: concentration adjustment

- Without staining:



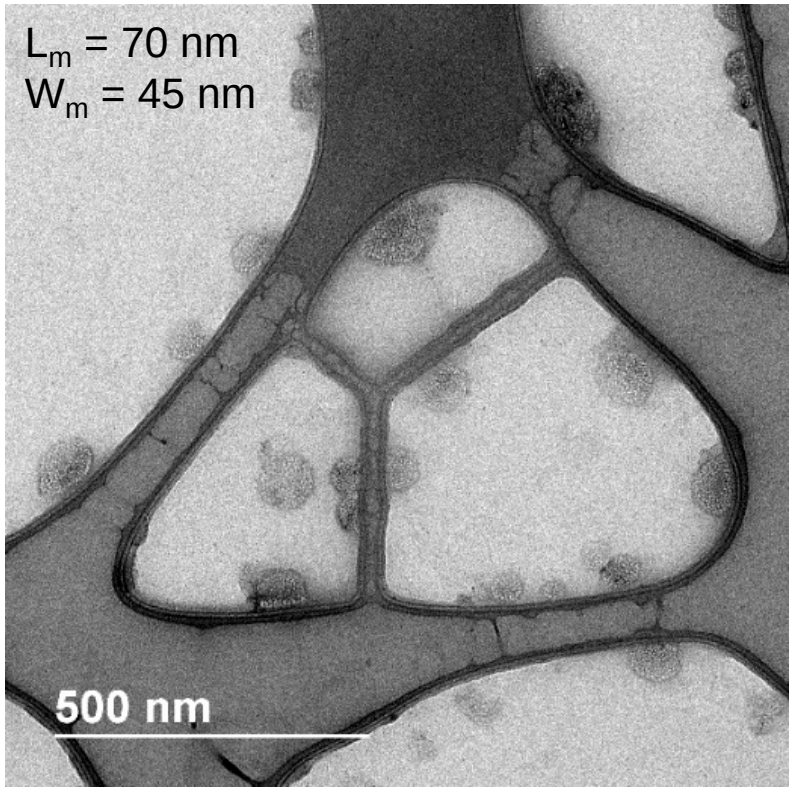
- With negative staining:



Quick methods

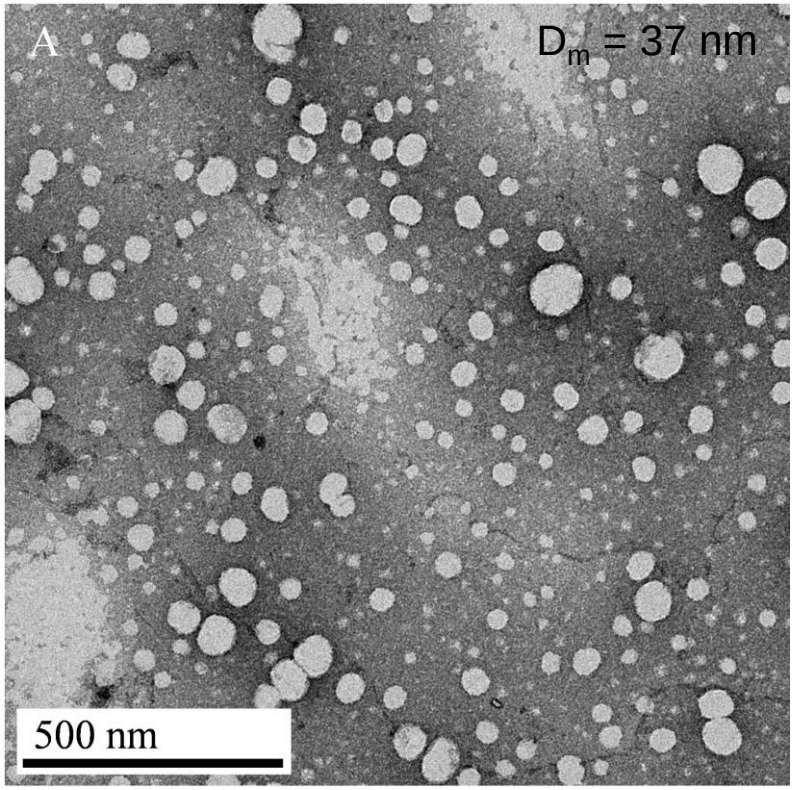
TEM OBSERVATIONS

Without staining



- Particle dehydration
- Poor contrast
- Concentration on the lacey carbon

With negative staining

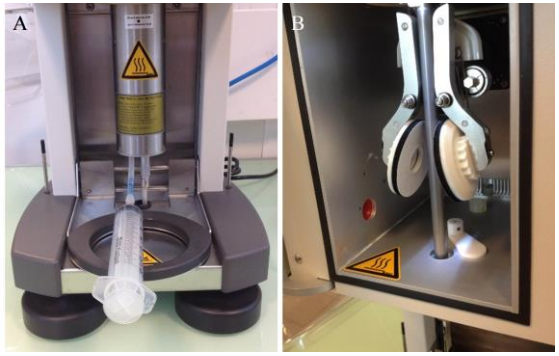


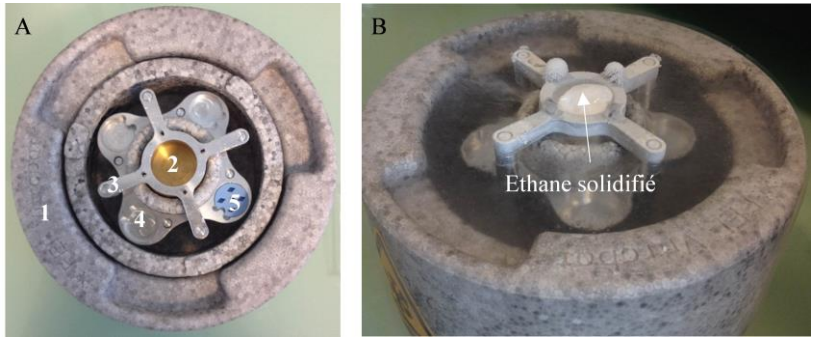
- Real shape or artefact ?
- Agglomeration
- Interior of the particle not visible

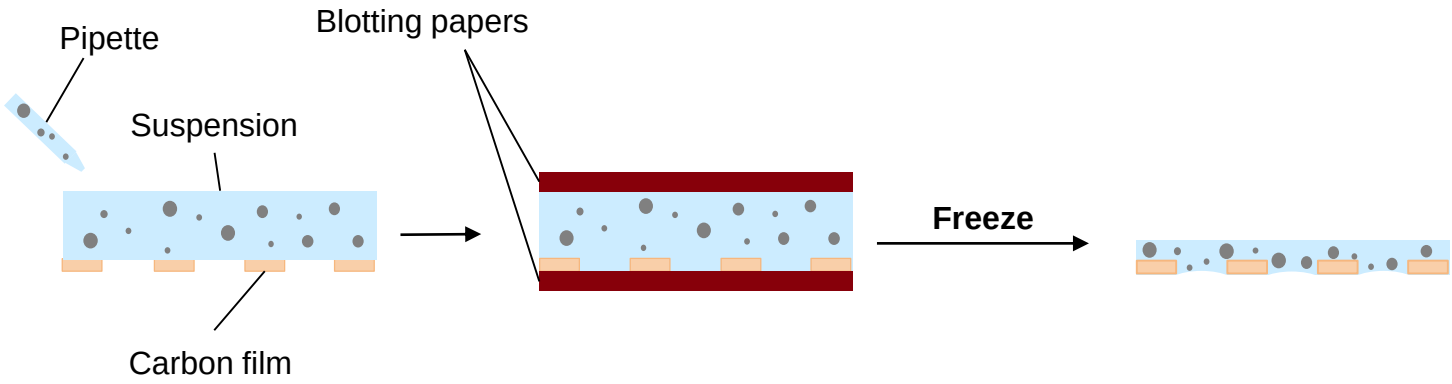
Not appropriate

TEM OBSERVATIONS

Cryogenics: preservation and 3D reconstruction





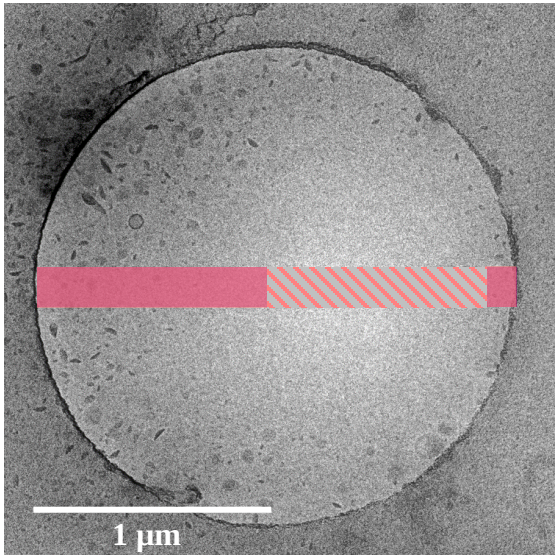


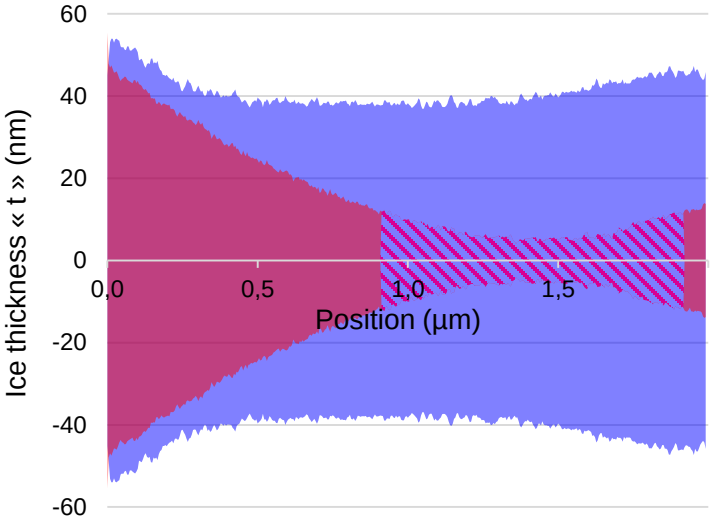
Amorphous & homogenous ice: optimizations

TEM OBSERVATIONS

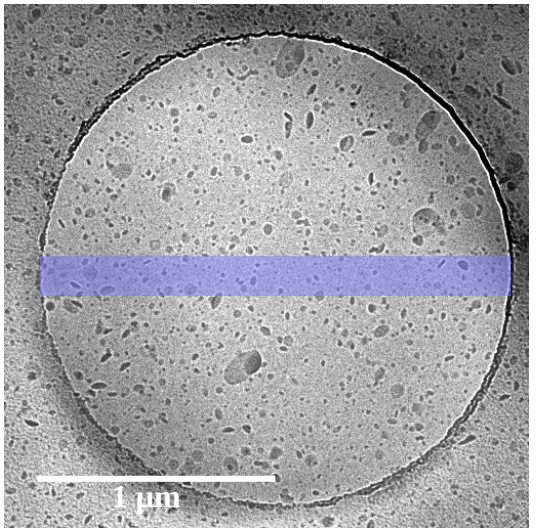
Cryogenics: ice thickness optimizations

Blot: 3s



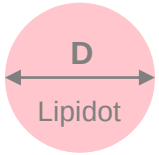


Blot: 1s



$t < D_{min}$

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

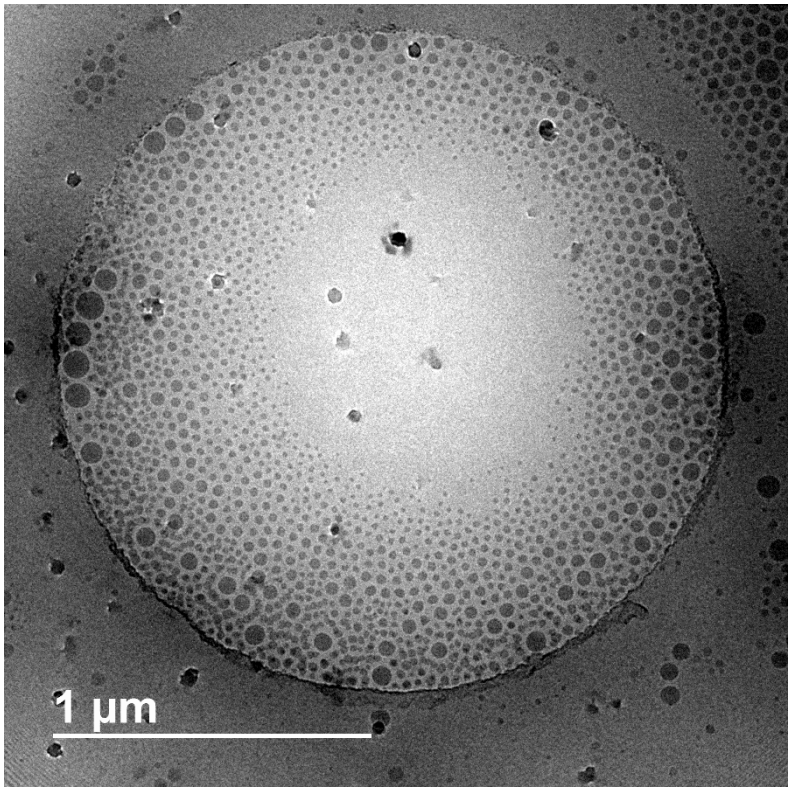


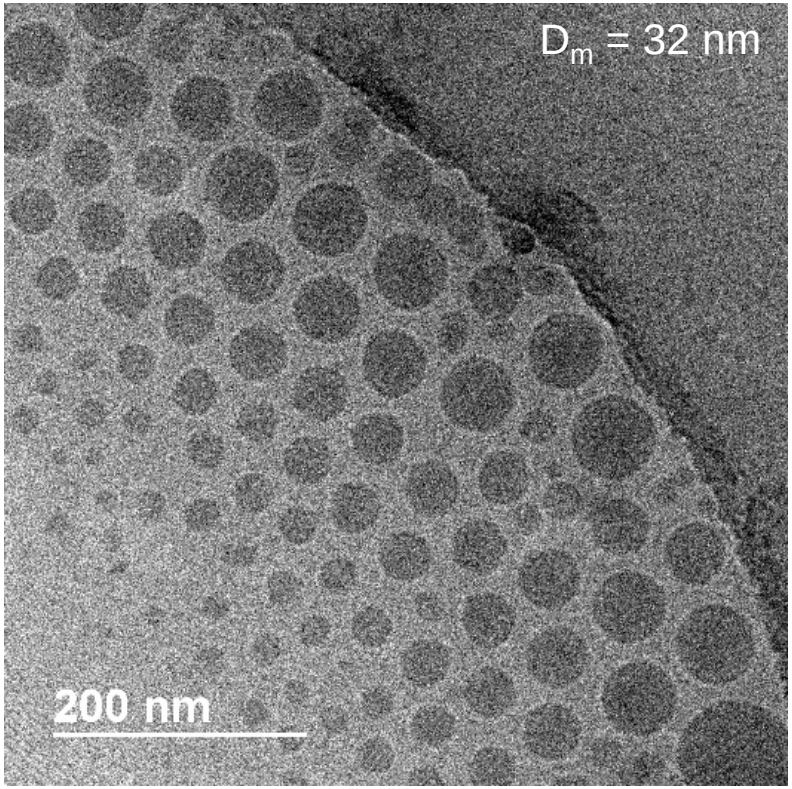
20 nm < D < 80 nm

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

Homogenous repartition of the particles on the grid

TEM OBSERVATIONS



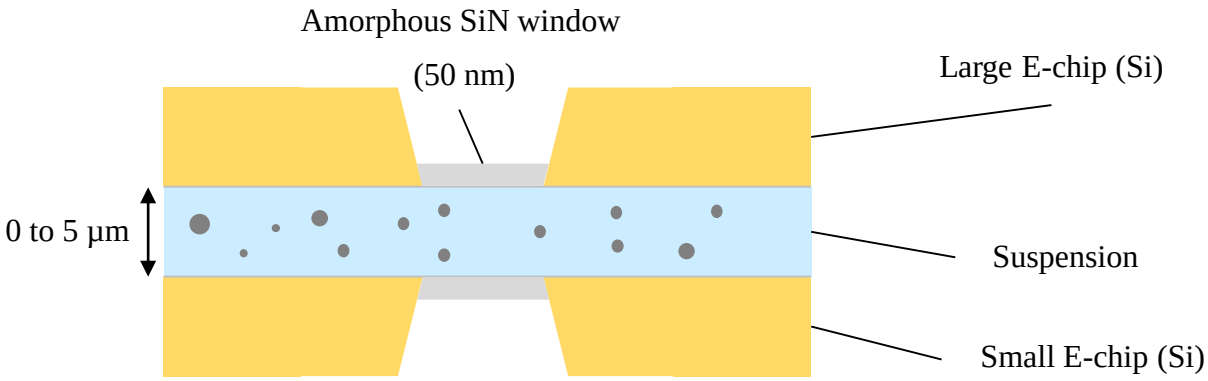


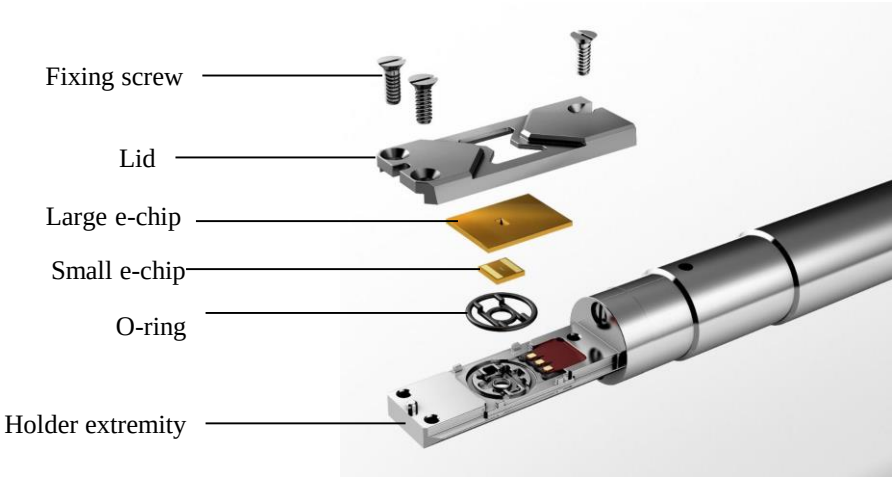
- Quite good contrast
- Ice contaminations
- Round shape

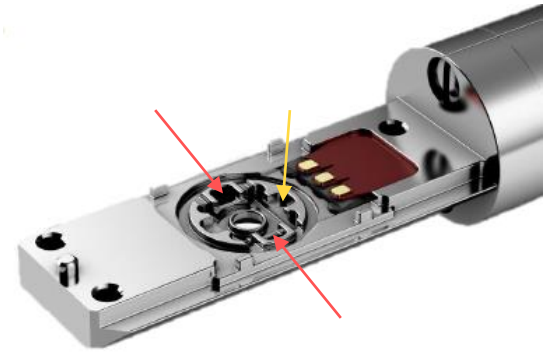
→ Native state cryofixed

TEM OBSERVATIONS

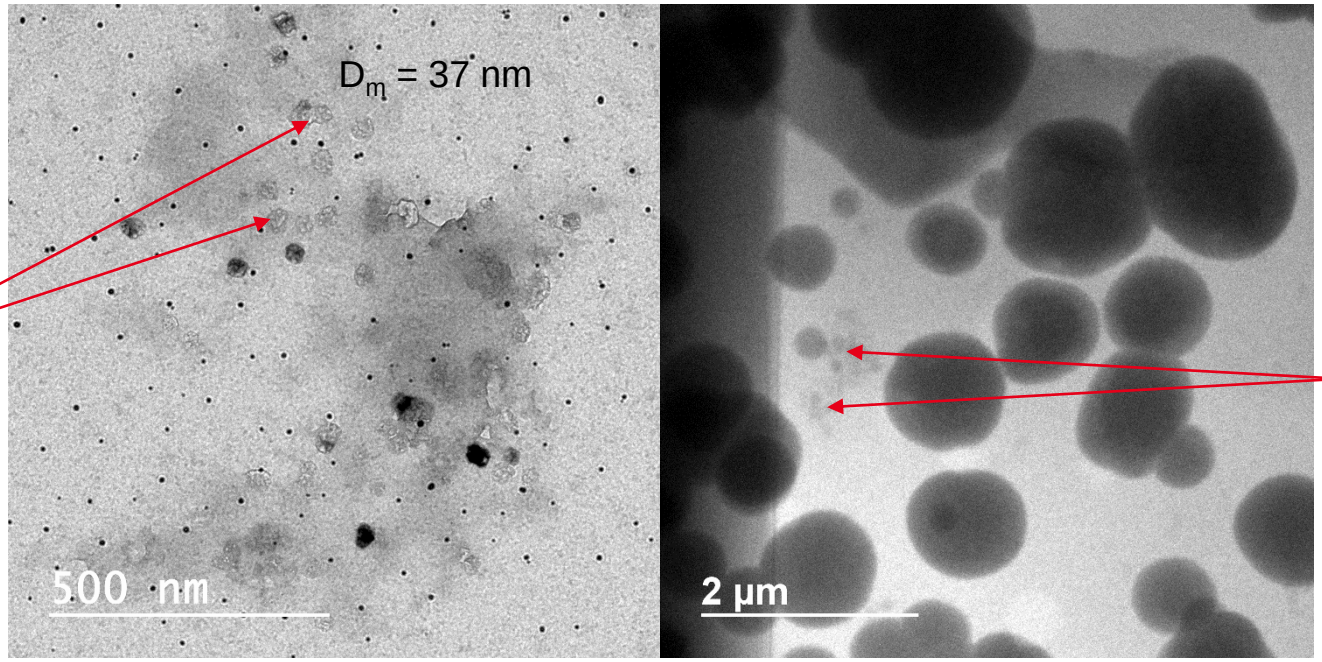
In-situ liquid: native state check-in for particles in suspension







TEM OBSERVATIONS



Lipidots

Lipidots

- Contaminations
- Poor contrast
- Lack of statistic

Clean the holder & retry with smaller spacer (50 nm)

CONCLUSION

Comparison of the different sample preparation methods

Drying process

poor contrast

dehydration

L_m = 70 nm
W_m = 45 nm

flattening

concentration on the lacey carbon

Negative staining + drying

no special equipment required

quick

Preserved shape

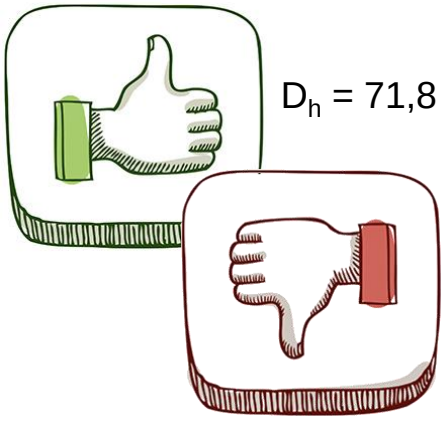
D_m = 37 nm

overstatement of the diameter

Interior of the particle non visible

agglomeration

DLS



D_h = 71,8 nm

Plunge freezing

lots of particles

D_m = 32 nm

good contrast

ice thickness

ice contamination

In-situ liquid

no preparation artefact

D_m = 37 nm

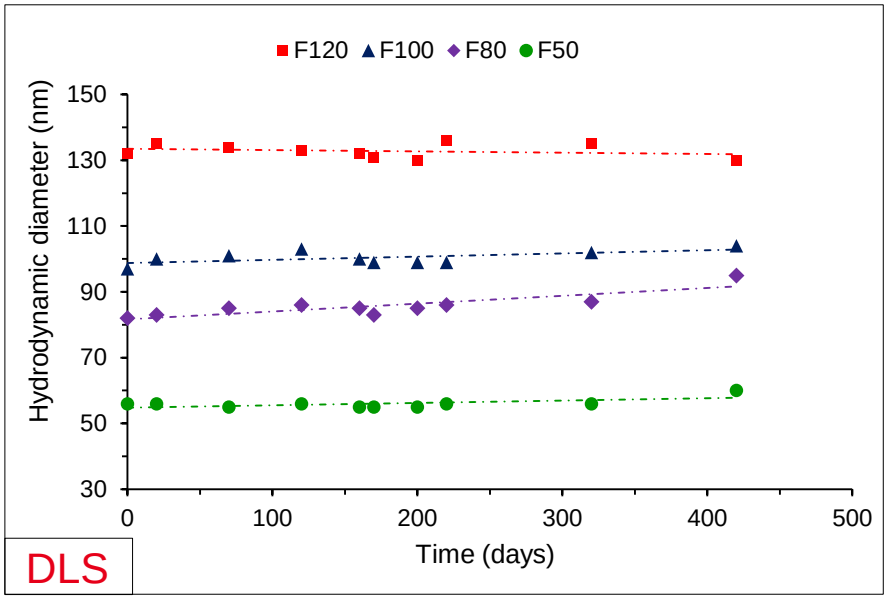
poor contrast

native state

time consuming

PERSPECTIVES

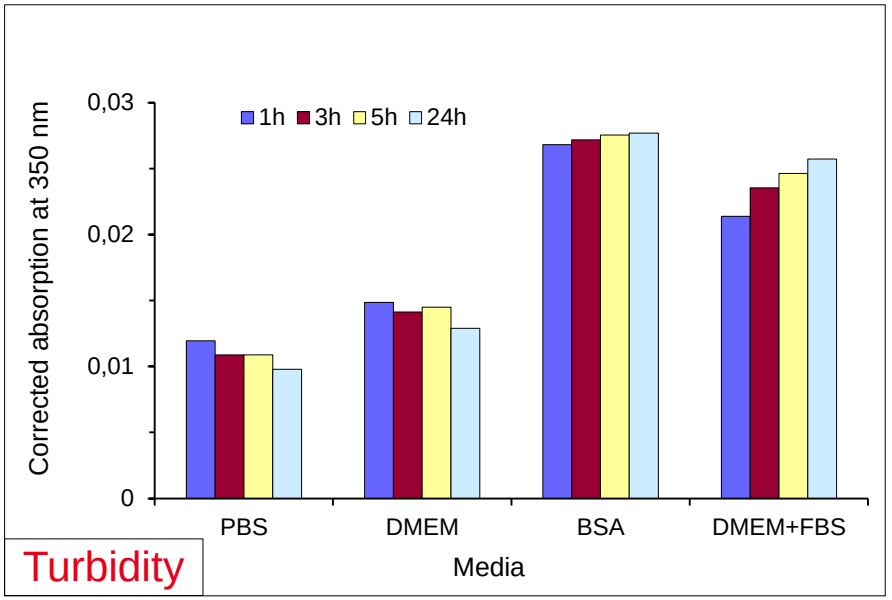
Stability of reference particles in PBS [1]



NC75 (75% wax, 25% soybean oil), 4°C

- High stability (> 13 months)

Stability in biological media



F50 NC75, 0,2 % m/m, 37°C

- DMEM ⟷ PBS ; stable over 24h
- BSA: quite stable over 24h ; increased of the particle diameter compared to PBS ?
- DMEM + FBS: destabilization of the particles ? Adsorption of proteins at the surface of the particles ?

PBS (*Phosphate Buffered Saline*): Buffer solution

DMEM (*Dulbecco's Modified Eagle Medium*): ~ plasma without protein

BSA (*Bovine Serum Albumin*): ~ liver proteins

FBS (*Foetal Bovine Serum*): ~ plasma with plasma proteins & biomolecules

[1] Delmas, T. (2011). *Caractérisation physicochimique et compréhension des propriétés de vectorisation des nanoparticules lipidots pour les applications biomédicales* (PhD thesis).

ACKNOWLEDGMENTS

Thank you for your attention

Maria Bacia (IBS)

Fanny Caputo (CEA)

Anne-Claude Couffin (CEA)

Isabelle Texier-Nogues (CEA)

Constantin Mattei (CEA)

Benoit Gallet (IBS)

Jean-François Damlencourt (CEA)

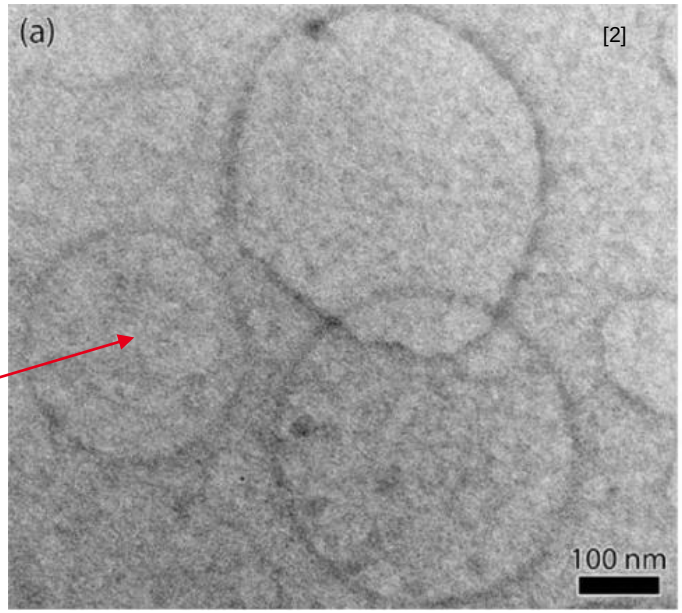
Romain Soulas (CEA)

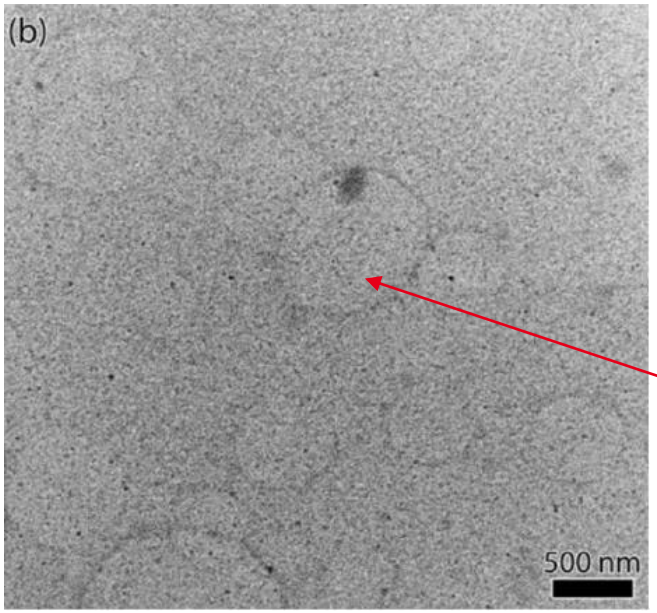
Delphine Boutry (CEA)

Stéphane Aguy (Eden Instrument)

TEM OBSERVATIONS

Liposome





Liposome

[2] Hoppe, S. M., Sasaki, D. Y., Kinghorn, A. N., & Hattar, K. (2013). In-situ transmission electron microscopy of liposomes in an aqueous environment.*Langmuir*, *29*(32), 9958-9961.

Commissariat à l'énergie atomique et aux énergies alternatives
17 rue des Martyrs | 38054 Grenoble Cedex
www-liten.cea.fr

Établissement public à caractère industriel et commercial | RCS Paris B 775 685 019