

# Understanding Structure Formation in Hierarchical Hybrid Materials using Cryogenic Electron Tomography and Liquid Phase Electron Microscopy

## Introduction

The formation of biological hierarchical materials, as well as of many synthetic ones occurs from aqueous media. In these environments, material formation proceeds through dynamic processes of nucleation, self-assembly, and crystal growth. The complex 3D character of many of these systems, together with the size and fast migration of the building units in solution, limits the range of tools that can provide nanoscale insight into the formation mechanisms. However, recent developments in liquid-phase electron microscopy (LPEM) and advances in cryogenic electron tomography (cryo-ET, 3D cryoTEM), are increasing our capabilities to monitor materials synthesis in solution at different length scales.(De Yoreo et al. 2016; Patterson et al. 2017). These two methods deliver complementary information: LPEM produces a movie of materials formation with limited control over solution conditions, whereas cryo-ET provides higher-resolution with 3D analysis at fixed solution

conditions. The combination of these two techniques may be used to characterize many materials systems, including biological materials, composite films, vesicles, macromolecules and nanoparticles. A time resolved cryo-ET experiment together with a LPEM experiment in silicon nitride flow chips or graphene liquid cells should be the most detailed microscopy analysis on hierarchical hybrid materials formation at this moment.

## Time resolved cryogenic electron tomography

In general, a cryo-TEM experiment involves the investigation of a thin vitrified film of solution/dispersion in which processes are arrested by plunging a thin layer of liquid on a copper mesh into an appropriate coolant. Accordingly, the objects under investigation become embedded in a solid amorphous film of the solvent. To ensure electron transparency of the vitrified films, they are best prepared with thicknesses of less than 100 nm. The vitrification process allows time-resolved sampling of

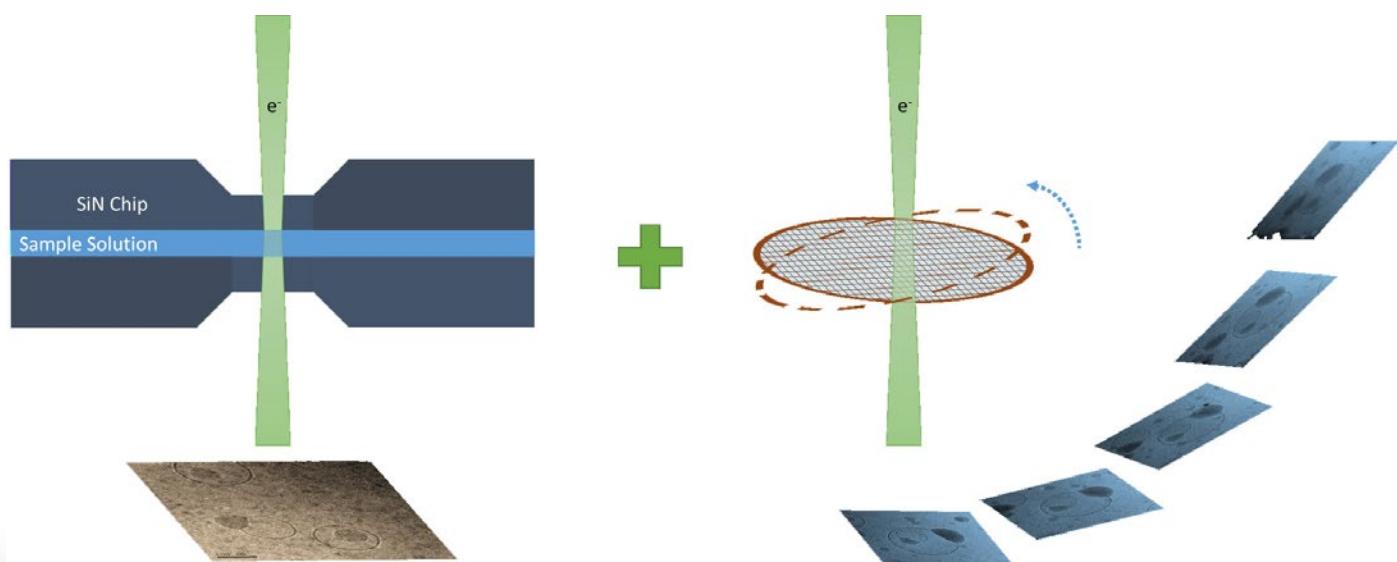
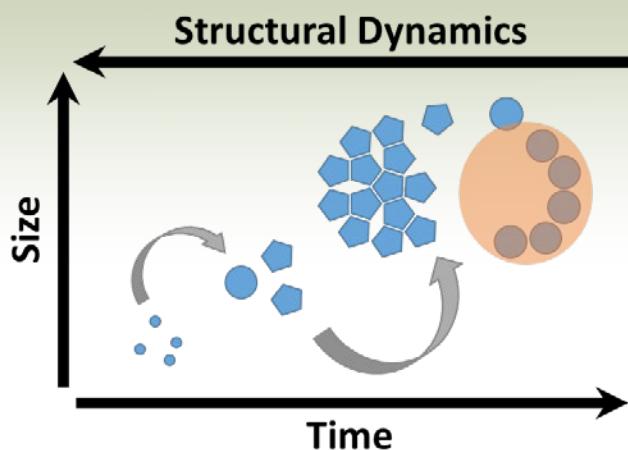


Figure 1. Schematic of a liquid phase TEM experiment (left) in a SiN chip and of the collection of 2D projections from a frozen sample on a TEM grid at different tilt angles (right) to produce a 3D model of the existing objects through cryo-electron tomography



**Figure 2.** Schematic of a multi-scale self-assembly process from primary particles through material building block formation to macroscale crystal or confined arrangement coupling the structural dynamics to the evolution of size, and the relevant time resolution for investigation. Inspired by (Dey et al. 2010)

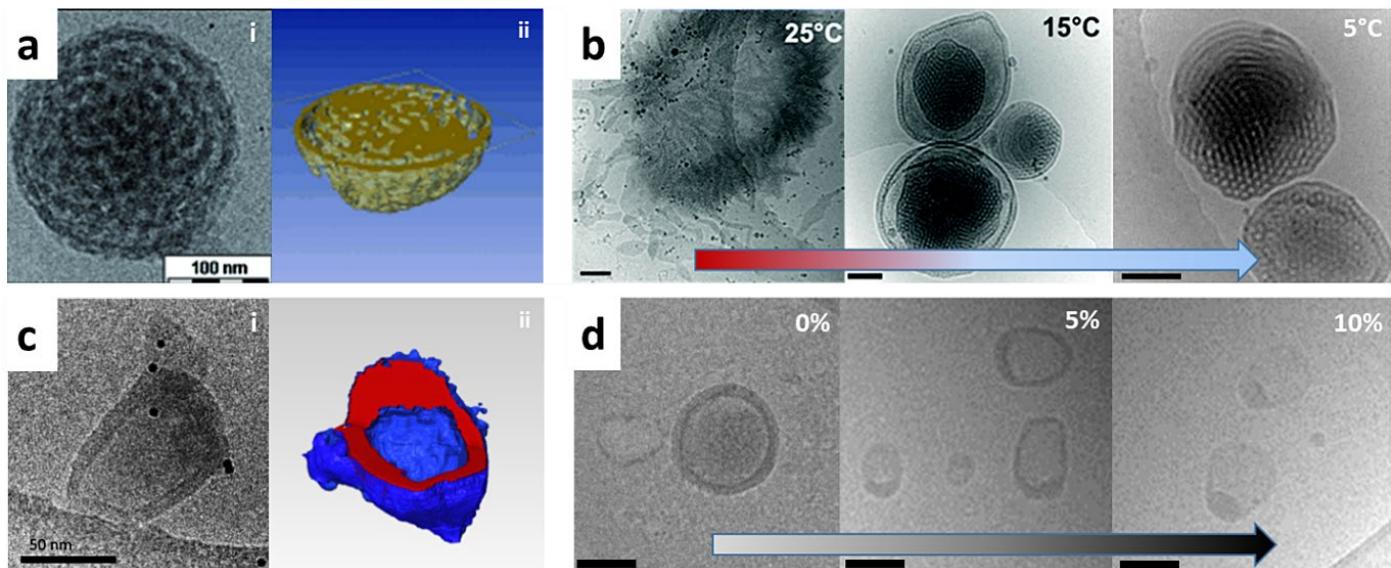
a reaction solution with a practical resolution of a few seconds. Arresting the sample dynamics enables the determination of 3D structure by cryo-ET, through the acquisition of a series of images from the same sample at different tilt angles and the reconstruction of the investigated volume. (Nudelman et al. 2011) Complex morphological transitions in macromolecular or multi-component systems are often difficult to follow in time resolved cryo-ET especially when the system is disperse and the structural evolution is not a single pathway for every particle (see Figure 2). In these cases, LPEM is an appropriate analysis tool to be used alongside cryo-ET.

### Liquid phase electron microscopy

LPEM provides a window into the dynamics of materials synthesis by allowing nucleation, growth and self-assembly to be controlled by reagent mixing, beam-induced deposition or thermally triggered reactions. Owing to this ability to probe processes occurring in the submicron-thick fluid films and provisions for both structural and compositional analysis, LPEM fills gaps left by cryo-ET experiments. (De Yoreo & N. A. J. M. 2016) Although the spatial resolution of LPEM is not comparable to cryo-TEM experiments, its temporal resolution is for example, higher than atomic force microscopy (AFM) which can only probe processes occurring on surfaces. LPEM is a fast growing technique, also from a chemical engineering point of view as it allows through the design of the cell/chip, a view into a nanoscale chemical reactor with microfluidic input and output.

### Control and synthesis of complex polymer structures

The use of cryo-ET can provide essential details regarding the structure and to a certain extent, formation of synthetic macromolecular systems. Although cryo-TEM is now a widespread technique for the characterization of organic self-assembled structures, cryo-ET is still a relatively young approach to investigate non-biological materials, with an early example being the study of bicontinuous polymer nanoparticles (Figure 3a). (McKenzie et al. 2010; Parry et al. 2008; McKenzie et al. 2015) Here, cryo-ET was used to see the pathway of bicontinuous structure formation for tripeptide-containing amphiphilic double-comb diblock copolymers as well as for the block copolymer poly(ethylene oxide)-b-poly(octadecyl methacrylate) (PEO-b-PODMA). In addition, cryoTEM was used to monitor the different stages of the polymer assembly process that



**Figure 3.** (a) A 2D cryo-TEM projection image (i) and a 3D rendering (ii) of a poly[norbornene oligo(ethylene oxide)]-b-[poly norbornene GLF peptide] bicontinuous nanoparticle, and (b) sequential cryo-TEM images of cooling PEO-b-PODMA dispersions. (c) a cryo-TEM projection image (i) and a 3D rendering (ii) of a protruded polymer vesicle synthesized by polymerization of ethylene glycol dimethacrylate (EGDMA) in/on a surfactant vesicle membrane using living radical polymerization. (d) cryo-TEM shows the effect of different weight % of EGDMA in the monomer feed on the resulting 2d nanocapsules (a) is adapted from (Parry et al. 2008) with permission from WILEY-VCH. (b) Reprinted with permission from (McKenzie et al. 2010) Copyright 2018 American Chemical Society. (c, d) Reprinted from (Moradi et al. 2018) with permission from Elsevier

involved changes in the solvent composition and temperature. This demonstrates that, contrary to expectations, the bicontinuous nature of the particles is not formed upon changing solvent composition, but results (after complete removal of the organic solvent) from a reversible temperature transition in pure water. (McKenzie et al. 2016)

Cryo-ET was also used to reveal the process of hybrid surfactant-polymer vesicle formation, where the vesicle structure evolves by varying the composition of monomers in a living radical polymerization at the surfactant vesicle surface (Figure 3b)(Moradi et al. 2018). Monitoring how protruded nanocapsule morphologies with different diffusion properties evolve from the living radical polymerization of a mixture of monomers with different hydrophobicity and reaction rates on a surfactant vesicle-surface further exemplifies how cryo-ET benefits materials synthesis. Understanding the details of these processes is the key to defining future research directions in the synthesis of these complex morphologies.

### Monitoring polymer controlled mineral formation

In many biominerization systems macromolecular assemblies control the nucleation and growth of different crystalline phases (Veis & Dorvee 2013). Charged biopolymers play a role in the stabilization of amorphous precursor phases from which many of these crystalline biominerals are formed. (Xu et al. 2018) However, their precise role in con-

trolling biominerization is still not well understood. This lack of understanding is partially due to the difficulty of studying biomimetic mineralization systems with sufficient temporal and spatial resolution.

As an interesting example, the nucleation and growth of  $\text{CaCO}_3$  in a matrix of polystyrene sulphonate (PSS) was visualized by LP-EM (figure 4), where the binding of calcium ions to form Ca-PSS globules is an essential step in the formation of metastable amorphous calcium carbonate (ACC) (Smeets et al. 2015). Here the method makes use of the fact that by analyzing particle development in time, the kinetics of the reaction could be extracted from which in turn the mechanism could be determined. The key finding was that ion binding can play a significant role in directing nucleation, and providing biology with a previously undiscovered tool to manipulate phase control, independent of any control over the free-energy barrier to nucleation.

### Conclusion

Understanding material formation processes in and from solutions/dispersions is a complicated but essential task for materials chemistry. As discussed, cryo-ET can be used to observe the structural evolution of a wide range of materials under a variety of environmental conditions such as heating/cooling cycles or the interaction with reacting monomers. The combination of cryo-ET with LPEM can be particularly powerful for the investigating the kinetics of these transformati-

ons and provide a deeper understanding of the evolution of these nanostructures.

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<https://www.4tu.nl/htm/en/new-horizons/understanding-structure-formation/>

### References

- Dey, A., de With, G. & Sommerdijk, N.A.J.M., 2010. *Chemical Society Reviews*, 39(2), pp. 397–409.
- McKenzie, B.E. et al., 2015. *Angewandte Chemie (International ed. in English)*, 54(8), pp.2457–61.
- McKenzie, B.E. et al., 2010. *Journal of the American Chemical Society*, 132(30), pp.10256–9.
- McKenzie, B.E. et al., 2016. *Soft Matter*, 12(18), pp.4113–4122.
- Moradi, M.-A. et al., 2018. *European Polymer Journal*, 108, pp.329–336.
- Nudelman, F., de With, G. & Sommerdijk, N.A.J.M., 2011. *Soft Matter*, 7(1), pp.17–24.
- Parry, A.L. et al., 2008. *Angewandte Chemie*, 120(46), pp.8991–8994.
- Patterson, J.P. et al., 2017. *Accounts of Chemical Research*, 50(7), pp.1495–1501.
- Smeets, P.J.M. et al., 2015. *Nature Materials*, 14, p.394.
- Veis, A. & Dorvee, J.R., 2013. *Calcified tissue international*, 93(4), pp.307–315.
- Xu, Y. et al., 2018. *Nature Communications*, 9(1), p.2582.
- De Yoreo, J.J. & Sommerdijk, N.A.J.M., 2016. *Nature Reviews Materials*, 1(8), p.16035.

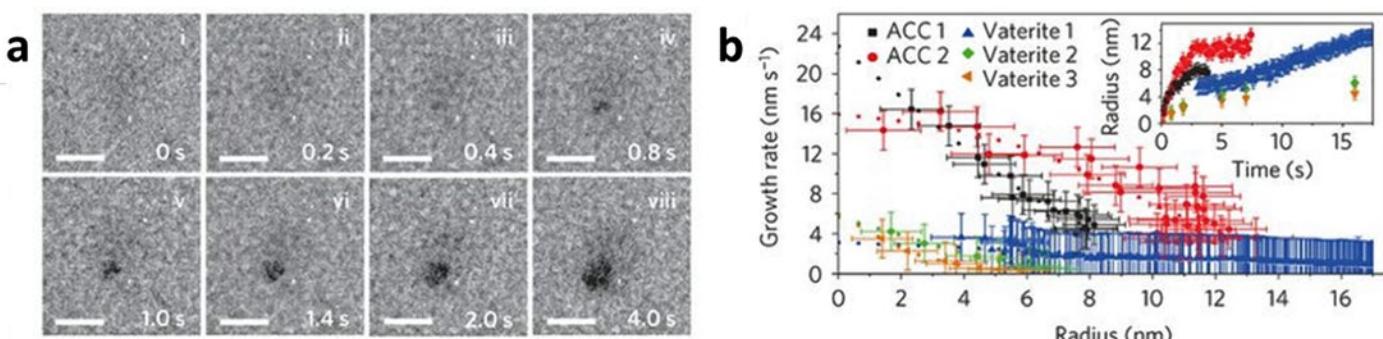


Figure 4. (a) Image sequence after 45 min of  $(\text{NH}_4)_2\text{CO}_3$  diffusion, showing initial nucleation and growth of a  $\text{CaCO}_3$  particle inside or on a primary Ca-PSS globule within 4 s (i–viii) (ACC 1; scale bars, 20 nm). (b) Extrapolated growth rates versus average radius for two ACC particles (ACC 1 and 2 with PSS) compared against those of three vaterite particles (without PSS). (a,b) Reprinted from (Smeets et al. 2015) with permission from Nature publishing group