Advanced CryoTEM and Tomography for Two- and Three-Dimensional Nano-Characterisation of Soft Matter

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ABSTRACT

Recent advances in transmission electron microscopy (TEM) have led to unprecedented automated control over data acquisition for various applications. Improvement of methods for obtaining and reconstructing tilt series projections allow us to create three-dimensional tomographic representations of bulk materials at the nanoscale. We demonstrate the combination of the latest TEM technology and tomography, at cryogenic temperatures, for the observation of soft matter such as polymers, hybrid coatings, engineered proteins and nanoparticles for targeted gene and drug delivery, nanocomposites and biomaterials, in conjunction with state-of-the-art vitrification methods for specimen preparation. Using such advanced Cryo-TEM, we aim to understand the organization of molecules, macromolecules and bio-molecules, in order to control the properties and performance of materials and devices

Keywords: Reconstruction, tomography, cryo-TEM, soft matter, vitrobot

1 CRYO-SPECIMEN PREPARATION

The basis for cryo-TEM studies lies in the specimen preparation procedure and technique. The Vitrobot (FEI Company) is an advanced automated specimen preparation device that helps the user to obtain thin frozen films for transmission electron microscopy. It gives the user better control over the environmental temperature and humidity and is essential for the vitrification of frozen-hydrated samples in their 'natural' (native) state.

Environmental control, together with gentle and automatic blotting (adjustable blotting pressure), results in reproducible vitrification of a broad spectrum of specimens, e.g. aqueous and non aqueous samples with a range of viscosities.

These vitrified samples possess excellent imaging properties for low dose cryo-EM (see, for example, Figure 1).

Further developments of cryo-preparation and related technology, such as time-controlled vitrification, are underway as part of the mission of the consortium.

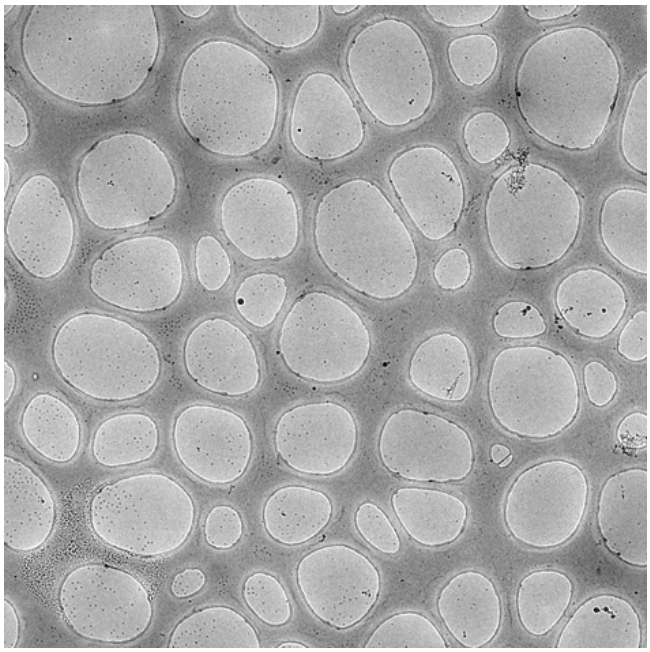

Figure 1: The quality of the overall specimen cleanness and ice thickness obtained after preparation of slam frozen specimen suspensions is shown by the overview. Typically fields of over a grid square, show vitrified ice of excellent quality and typically more than 80% of the grid surface is covered with such a thin vitrified film.

2 TARGETED GENE AND DRUG DELIVERY

Nanotechnology is of great importance in drug formulation. Nano-encapsulation opens the possibility to diminish the side effects of drug treatment while maintaining the desired major effect. The addition of further functionalities to nanocapsules, such as ligands for targeting or imaging (MRI, PET, etc) are attributed to the increased interest in nano-encapsulation.

A classical example of nano encapsulation is the liposomal package of the anti-cancer drug doxorubicine ('Doxil', 'Caelyx') in vesicles with some pegylated lipid ('Stealth' liposomes).

The 3-D architecture of these particles can be reconstructed upon cryo-TEM tomography and an example is shown in figure 2. Apparent from a slab taken from the 3D reconstruction is the 27Å repeat of the Doxorubicine (liquid crystalline) lattice (Figure 3). A hint of twisting in this crystal can be inferred from the fact that the repeat is not visible throughout the crystal viewing it from one single angle.

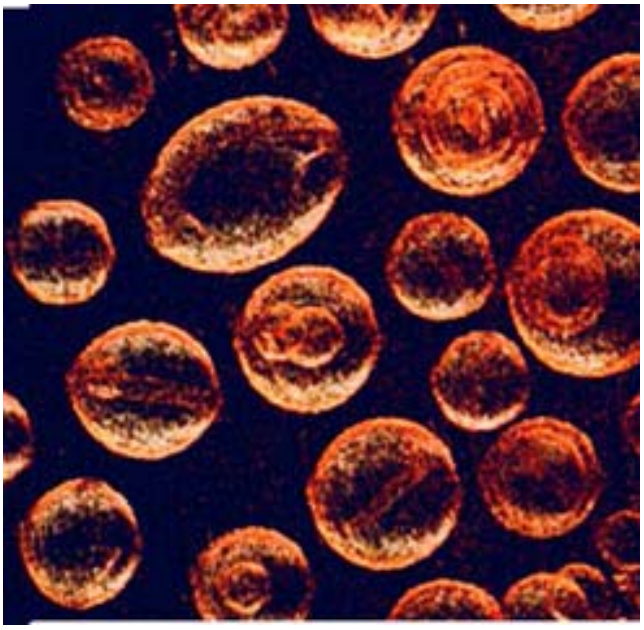

Figure 2 : Snapshot from Doxil/Caelyx drug-loaded vesicles. (Specimen courtesy of Alza)

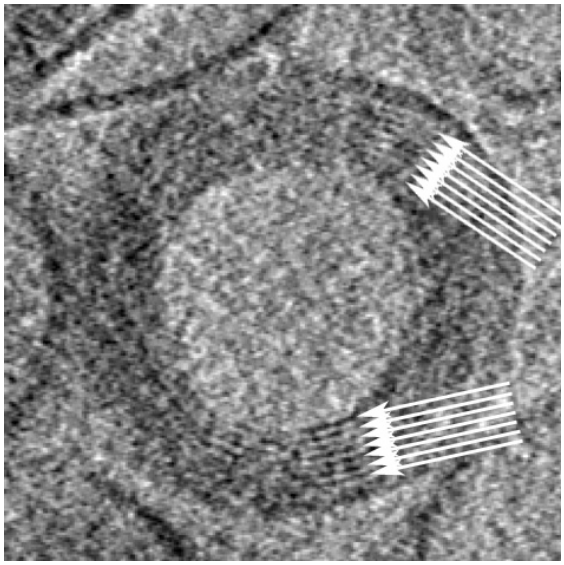

Figure 3 : The 27Å repeating distance of the Doxyl lattice is preserved even after cryo-tomography as is shown in the detail picked from the whole SIRT 3D reconstruction

An example of lipid-DNA complexes to be used for transfection is showed in figure 4. By mixing vesicles prepared with cationic lipids and DNA (plasmid coded for GFP) different particle morphologies can be created. The shape and size of the aggregates is largely determined by the charge ratio +/-, DNA being negatively charged and attracted to the surface of the positively charged bilayer. In the example shown, excess negatively charged DNA was added which can be observed as densely packed spikes at the bilayer surface, as well as freely suspended strands in the supporting medium.

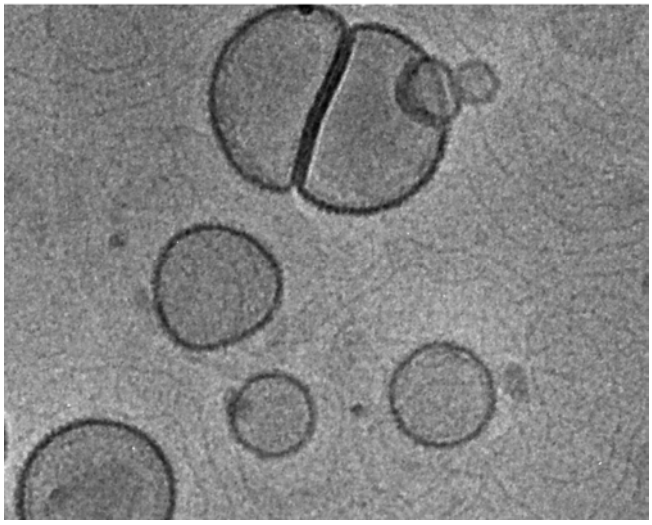

Figure 4 : Lipid-DNA complexes (specimen courtesey of R.K. Jellema Maastricht University). DNA strands are resolved both in suspension and as attached to the bilayer surface ('spikes')

3 DNA-BASED BIOMATERIALS COATINGS

We have studied the formation of layered complexes of DNA and a bis-ureido based cationic surfactant and their application as a biomaterial coating for implants. The interaction between the two components was studied using a Langmuir monolayer of the surfactant under which DNA was injected in the aqueous sub-phase. The monolayers were transferred to quantifoil grids positioned under the air-water interface by careful draining of the sub-phase. By using a dedicated glovebox, with control over temperature and humidity, we were able to transfer the grids at 100% humidity to the chamber of the Vitrobot. The grids were subsequently vitrified in liquid ethane (colder than -170 °C) before being transferred to an FEI Titan Krios TEM and studied at 300kV (see Figure 5).

3D information with regard to the organization of the DNA molecules was obtained using cryo-tomography. A tilt series (-70 º to + 70 º with a tilt increment of 1º) was recorded and reconstructed using SIRT. With this method we were able to visualize the individual DNA molecules that have a diameter of ~2.5 nm. The 3D volume showed that the DNA molecules did not smoothly adhere to the monolayer over their entire lengths, but instead contained many "dangling ends" that extended into the aqueous phase.

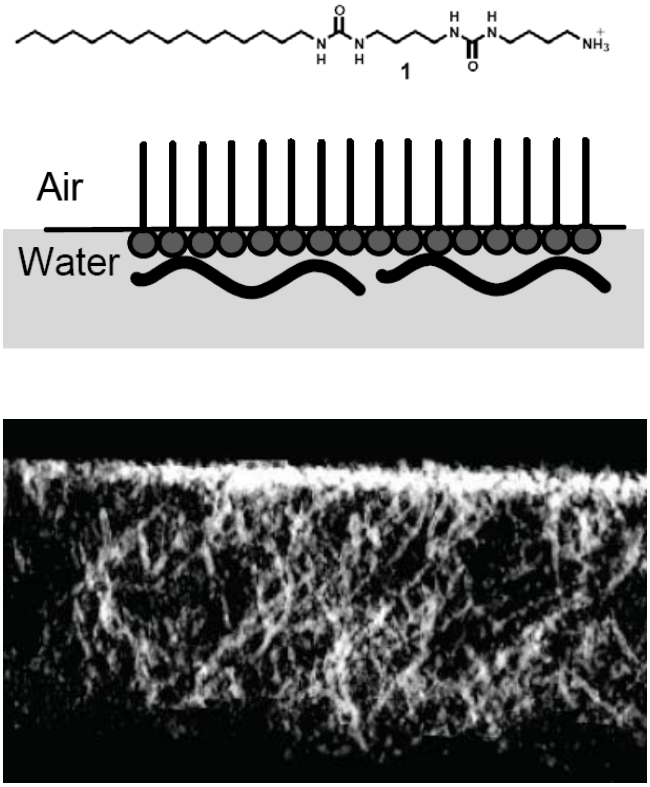

Figure 5 : (top) Molecular structure of a bis-urea surfactant (center) schematic representation of a DNA surfactant complex, in which DNA was injected underneath a preformed bis-urea surfactant monolayer and (bottom) part of a reconstruction of the cryo-tomogram showing DNA strands suspended from the monolayer at a resolution better than 2.5 nm

4 3D ORGANISATION OF FUNCTIONAL POLYMERS

Polymeric semiconductor-based solar cells (PSCs) are potential low-cost devices for sustainable solar energy conversion. These devices incorporate photoactive layers based on at least two compounds which form bulk hetero-junctions. It is essential to understand and control morphology at the nanometer scale in order to optimise the photoactive layer's efficiency.

Techniques such as SPM, SEM and conventional TEM have proved useful for observation of the lateral organisation of the active layer. However, information on the 3-D organisation is needed, to better understand charge-carrier mobility and charge injection from the active layer into the electrodes, in relation to the morphology and the organisation of percolating networks within the bulk hetero-junction.

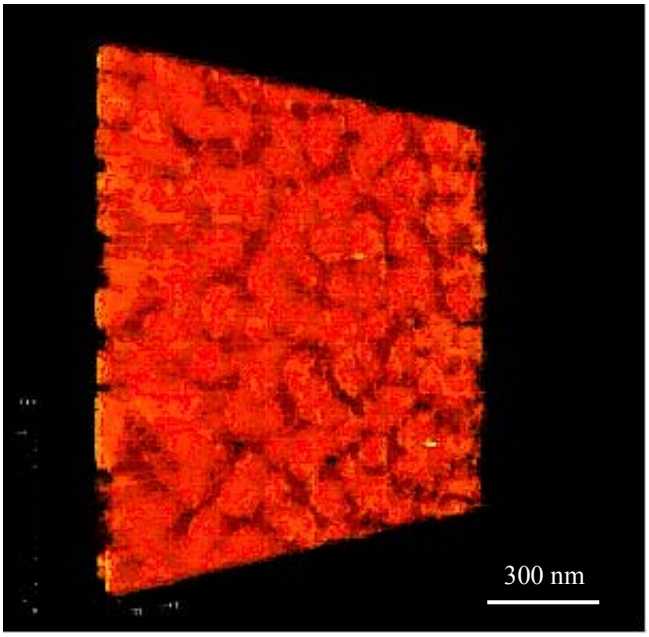

Fig 6. Snapshot from 3-dimensional TEM tomogram of the system MDMO-PPV/PCBM; the orange clusters represent the PCBM-rich phase.
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TEM tomography has been used to study the 3-D organisation of the active layer – thereby inaugurating the technique and demonstrating its benefit on unstained, low contrast and electron beam sensitive polymeric systems.

For example, by visualising the 3-D organisation of the system MDMO-PPV/PCBM having a PCBM content (80 wt%), optimum for high performance, detailed information about PCBM-rich domain sizes as well as connectivity of the PCBM domain network within the active layer could be obtained.

Figure 6 illustrates the morphology of the active layer, which was tuned for better contrast at the expense of performance; PCBM-active domains with sizes in the range 80-150nm are visible. Additional to revealing their size and shape, the tomogram also shows that the PCBM-rich domains are covered with MDMO-PPV, in agreement with SEM side views on active layer cross-sections [1].

These preliminary results allow deep insights in structure-processing-property relations in the research field of polymer electronics, in particular polymer solar cells, and further investigation on other systems are currently in progress.

5 CONCLUSIONS

Complete 3D insight in to highly complex architectures in suspension and bulk soft matter can be obtained by automated transmission electron tomography at room- and cryo-temperatures. Valuable information is retrieved which can be used to further optimize the assembly conditions in order to obtain the physical or chemical characteristics required.

REFERENCES

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[2] X. Yang and J. Loos, Toward High-Performance Polymer Solar Cells: The Importance of Morphology Control, *Macromolecules*; (Review) (2007) ASAP Article