Nonappa, Nonappa; Engelhardt, Peter

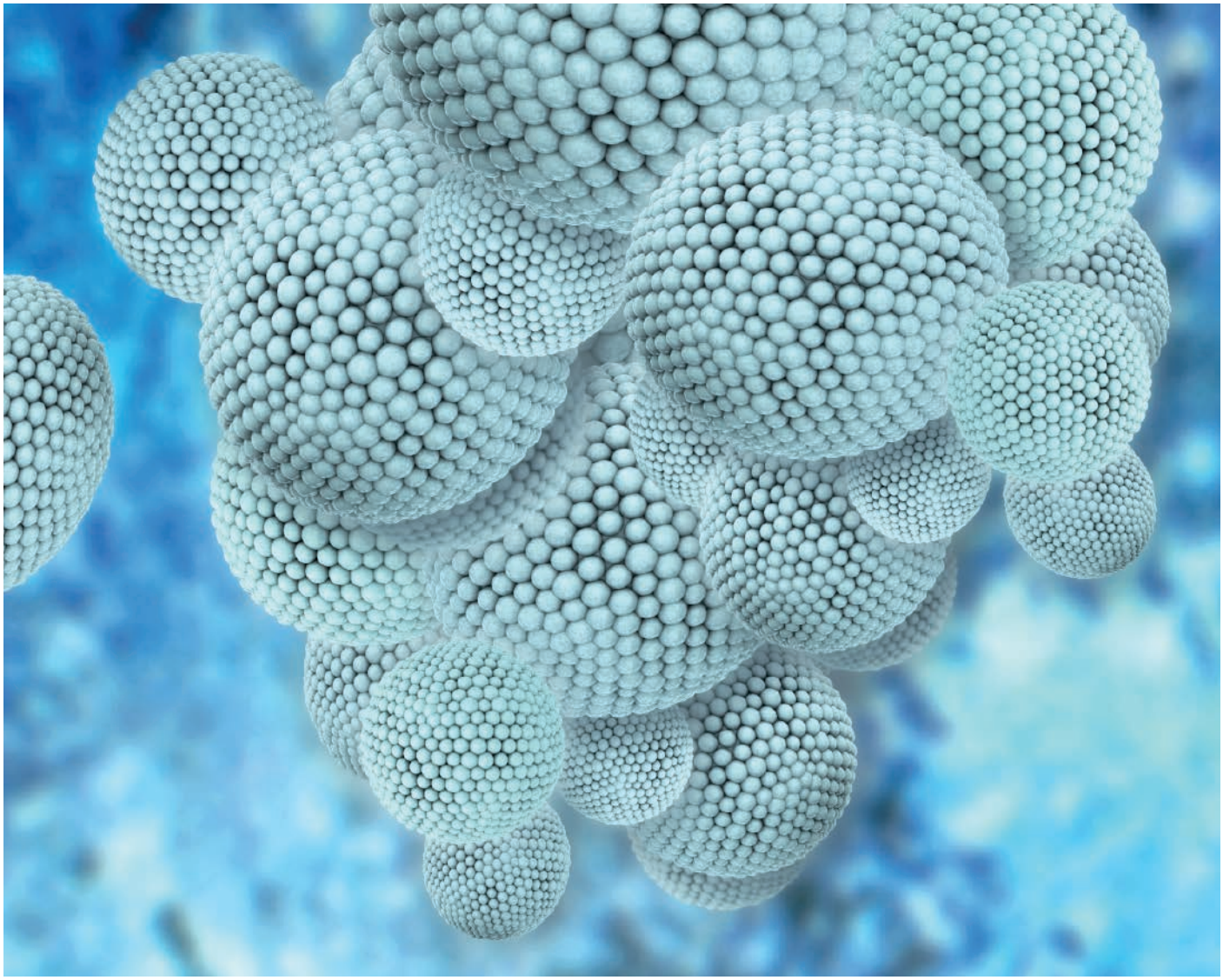
Electron Tomography of Whole Mounts

Published in:
Imaging & Microscopy

Published: 21/06/2019

Document Version
Publisher's PDF, also known as Version of record

Please cite the original version:
Nonappa, N., & Engelhardt, P. (2019). Electron Tomography of Whole Mounts. *Imaging & Microscopy*, *21*(2), 22-24. https://www.imaging-git.com/science/electron-and-ion-microscopy/electron-tomography-whole-mounts



© fotoliaxrender - Adobe-Stock.com

Electron Tomography of Whole Mounts

tert-Butanol Freeze-Dried Colloids

Nonappa[1,2] and Peter Engelhardt[1]

Rapid progress in the instrumentation, sample preparation methods, and computational power have triggered a revolution in electron tomography methods. Herein, we adapted a straightforward freeze-drying method using *tert*-butanol for electron tomography of whole mount colloids. This approach will overcome some of the common artifacts in electron microscopy specimen preparation.

Introduction

Electron microscopy (EM) is one of the powerful imaging techniques to study a diverse range of synthetic and biological materials [1]. EM images of three dimensional (3D) objects produce superimposed two dimensional (2D) orthogonal projections. Importantly, in transmission electron microscopy (TEM) images, the 2D projection often contains high resolution structural details. Because of the superimposed nature, limited amount of correct internal structural information is obtained from the 2D images. To overcome the above limitations, electron tomographic (ET) reconstruction (i.e. 3D reconstruction) or single particle reconstructions (SPR) methods have been developed and studied [2,3]. The success of 3D reconstruction relies on the quality of the data and which in turn depends entirely on the quality of the specimen. Thus, specimen preservation nearly close to its native state is the most significant part in EM imaging. Methods including, chemical fixation, negative staining and critical point drying (CPD) have been used to image whole mount samples [3]. Among several methods, cryo-TEM has emerged as a method to preserve specimen close to their native state [5-8]. While the cryo-sample preparation method is unrivaled by its ability to obtain 3D structures from non-crystalline samples, there exist several challenges to study soft colloidal particles beyond a particular size. Particularly, to study whole mount soft colloidal particles dispersed in aqueous media with specimen thickness above ~200 nm. The structures at this length scales are of great importance as self-assembled biomacromolecules, and cellular components fall within this regime. Under ambient drying (AD), soft organic or biological colloids produce drying artifacts (e.g.,

collapsed structures). It has been shown that in cryo-vitrification, the ice thickness varies from 70-120 nm depending on the type of TEM grid and the support film. Therefore, in cryo-TEM particles over ~200 nm thick often undergo flattening during vitrification. Therefore, to observe the unaltered structural features, the particles should be within the above-mentioned limits [9]. Thus, there is a need for other specimen preparation methods to study whole mount colloids. We have used simple *tert*-butanol freeze-drying (*t*BFD) method for TEM imaging and ET reconstruction of whole mount colloids (d > 200 nm). Further, we have compared the results obtained from *t*BFD method with AD, CPD, and cryo-vitrification. Our results suggest that morphological features are retained in *t*BFD and applicable to self-assembled synthetic and bio-based assemblies.

Materials and Methods

We show three categories of materials *viz.* i) capsid-like self-assembled cobalt nanoparticle superstructures. ii) super-micellar structures obtained from the self-assembly of star-like amphiphiles and iii) spherical particles resulted from the self-assembly of resilin-fusion proteins [10-13].

General Methods

For specimen preparation, 300 mesh gold grids with carbon support film (Ted Pella) were used. The grids were plasma cleaned and deposited with 5.0 nm fiducial gold markers before specimen preparation. TEM images were collected using JEM 3200FSC field emission microscope (JEOL) operated at 300 kV in bright field mode with Omega-type Zero-loss energy filter. The images were acquired with Gatan digital micrograph software while the specimen temperature was maintained at -187°C. Analytical grade methanol (≥99.9%, Sigma-Aldrich) and *tert*-butanol (≥99.0%, Fluka) were used. The distilled deionized water (MilliQ water,18.0 Ω) was filtered through 0.22 µm MF-Millipore membrane filter.

Ambient Drying (AD)

Specimen preparation by drying under ambient conditions was carried out by adding 3.0 µL of the solution containing the colloidal particles on to a 300-mesh gold grid with carbon support film. The samples were allowed to dry under ambient condition for 48 hours prior to imaging.

Cryo-TEM

The Cryo-TEM samples were prepared by placing 3.0 µL sample on a 200-mesh copper grid with either holey carbon support film (CF-Quantifoil) and plunge freezed in 50/50 liquid propane/ethane mixture using Vitrobot (Thermo Fisher) with 2s blotting time under 100% humidity and cryo-transferred to the microscope.

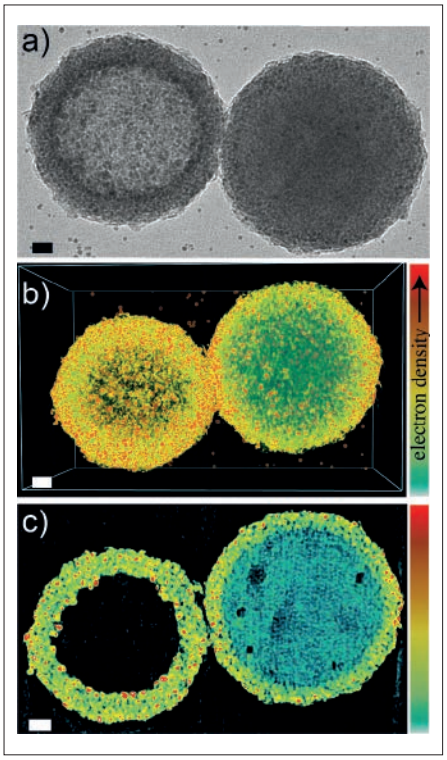


Fig. 1: TEM micrographs and ET reconstruction of cobalt nanoparticle superstructures. a) The TEM micrograph of self-assembled nanoparticle superstructures. b) 3D reconstruction shows that the capsids with a hollow interior (left) and filled with organic materials (right). c) A cross-sectional view of the particles reveals that both the particles contain ~20 nm thick layered shell. See also video S1. Scale bars (a-c) corresponds to 20 nm.

SerialEM and Electron Tomography

The tilt series (2D projections) were acquired using SerialEM-software package [14,15]. Samples were tilted between ± 69° angles with 2-3° increment steps under low dose mode. The acquired raw stack of images was first subjected for a series of pre-processing, coarse alignment, final alignment and further aligned using IMOD software package [16]. The images were binned two to four times to reduce noise and computation time. The final aligned file was then utilized for 3D reconstruction with custom made maximum entropy method (MEM) program with a regularization parameter value of $\lambda = 1.0e^{-3}$ on MacPro [14,17]. The 3D isosurface and solid colored images were produced using UCSF Chimera.

tert-Butanol Freeze Drying (tBFD) Method

The specimen preparation was done according to previously reported procedure in the literature [14]. In brief, the specimen was prepared by placing an aqueous dispersion (3.0 µL) of the colloids on the TEM grid. After two minutes of placing the sample, the grids were washed by dipping them into an Eppendorf containing water for 15 s. The grid was then subjected for dehydration by sequential washing with water:methanol (1:1), methanol (100%), methanol:*tert*-butanol (1:1) and *tert*-butanol (100%), three times for 10 s in each step using an anti-capillary tweezer. Finally, the specimen was placed in an Eppendorf tube (1.0 mL) containing 100 µL of *tert*-butanol. The excess *tert*-butanol was removed using micropipette before placing the specimen in a lyophilizer for 15-30 min. The dried specimen was used for imaging. It should be noted that the *tert*-butanol should be of highest purity as possible to avoid any contamination. Further, when the room temperature is below 25°C, the solidified *tert*-butanol should be heated to get a clear liquid and the temperature should be maintained above its melting point during specimen preparation.

Results

First, we discuss the importance of the whole mount colloidal superstructures and their 3D reconstruction. As an example, capsid-like superstructures obtained by hydrogen bonding directed self-assembly of *p*-aminobenzoic acid capped cobalt nanoparticles (CoNPs) in apolar organic solvents is presented (fig. 1). The capsids are formed *in situ* under heating up synthesis using dicobalt octacarbonyl in the presence of *p*-aminobenzoic acid in 1,2-dichlorobenzene [12,13]. Figure 1a shows TEM micrograph (2D projection) of a capsid dimer, when the specimen was prepared under AD method (see supporting information). One of the capsids shows a clear difference in the core and the shell. However, the other capsid shows no difference in the contrast in the core and shell. Electron tomographic reconstruction and its cross-sectional view suggest that both the capsids contain layered shells. However, the interior of the capsid is either hollow or filled with organic material (fig. 1b, c, video S1) [12]. Further, ET also resolved the nanoparticle building blocks [14-17]. Therefore, whole mount stable colloidal superstructures are useful not only to study the internal structure but also to visualize the arrangement of individual subunits. However, AD method for soft organic or biological colloids dispersed in water leads to collapsed structures (i.e. drying artifacts, fig. 2a-c). To avoid such drying artifacts, we used *tert*-butanol freeze-drying method (*t*BFD) [14,18]. The *t*BFD method exploits the melting point (25.5°C) and sublimation of *tert*-butanol, allowing rapid drying (~15 min) under vacuum (see methods). As an example, we show

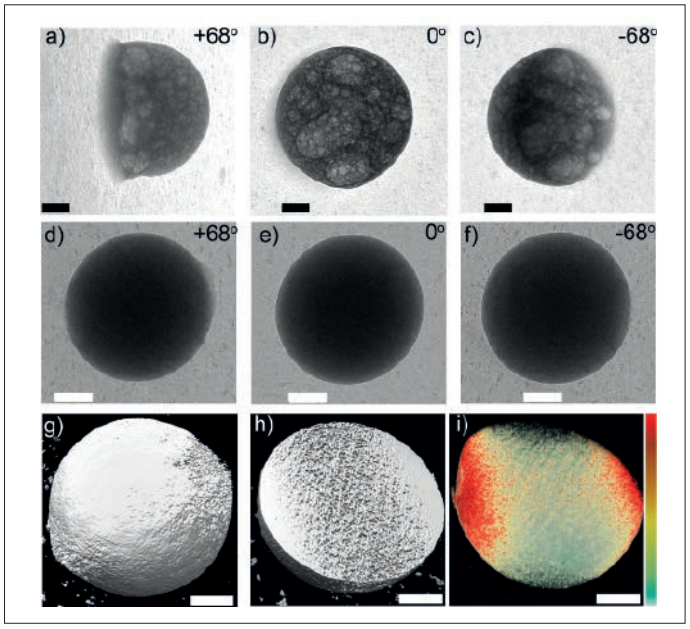


Fig. 2: Comparison of AD and tBFD methods. a-c) TEM images show that under AD method colloidal particles undergo deformation and beam damage. d-f) The tBFD method prevents deformation, and the sample appears to be stable under electron beam during collection of tilt series. g-i) shows 3D reconstruction and its cross-sections. g and h reproduced with permission from ref.12. Scale bars (a-i) corresponds to 100nm. g and h reproduced with permission from ref.12, Copyright 2017, Elsevier.

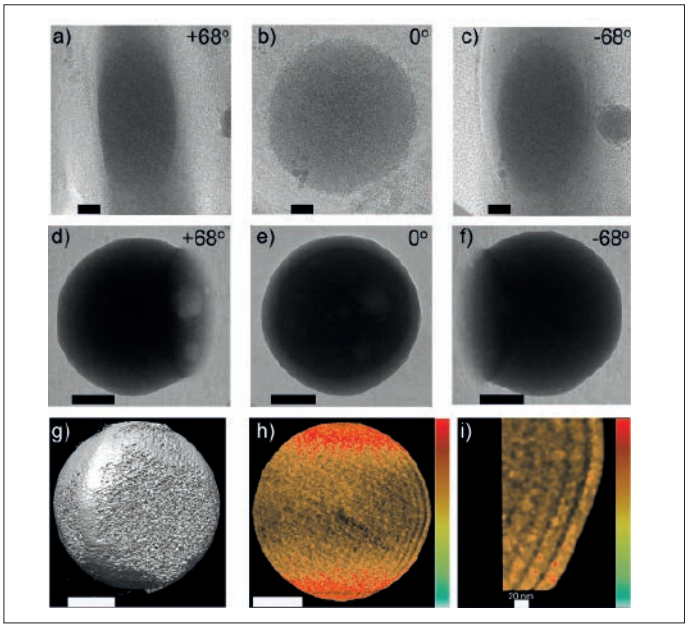


Fig. 3: Comparison of cryo-TEM and tBFD. a) Cryo-TEM images for a self-assembled resilin-fusion protein superstructure showing flattened particle under cryo-TEM conditions. d-f) tBFD method retains the spherical nature with improved contrast. g-i) 3D reconstruction and cross-sectional view reveals that the particles are composed of ordered structures. d-f reproduced with permission from ref.13. Scale bars (a-h) corresponds to 200 nm. d-f reproduced with permission from ref.13, Copyright 2018, Elsevier.

how specimen preparation methods affect the TEM imaging of self-assembled spherical structures (fig. 2). The spherical particles presented here are formed when star-like amphiphilic polymeric molecules appended with 2-ureido-4[1H]-pyrimidinone (UPy) units were solvent exchanged from dimethyl sulfoxide to water [12]. The specimen under AD collapsed, with severe beam damage when imaged (fig. 2d-f). The spherical structure of the particles was retained when *t*BFD method was applied as evidenced by the electron tomographic reconstruction (fig. 2g-i).

We then applied *t*BFD method for self-assembled proteins dispersed in aqueous media. Accordingly, colloidal particles obtained by the self-assembly of resilin-fusion proteins were tested [13]. Cryo-TEM images show that the samples are flattened (fig. 3a-c). Therefore, the cryo-TEM 3D reconstruction resulted in a disc-like structure with limited resolution. In *t*BFD method, the particle retained the morphological features (fig. 3d-f). The 3D reconstructions resolved the nanoscale structural details (fig. 3g-i).

Conclusions

The *t*BFD specimen preparation method for TEM imaging and electron tomographic reconstruction is simple and straightforward. Importantly, the structural features of the particles are retained to a large extent in addition to their electron beam stability and well resolved internal details. Here we have demonstrated this with synthetic and bio-based colloids. Furthermore, in this method, fixation or additional reagents are not necessary. However, the *t*BFD method might require modification or additional fixation depending on the nature of the materials.

Acknowledgements

This work was carried out under the Academy of Finland Centre of Excellence Programme (HYBER 2014-2019). We acknowledge the provision of facilities and technical support by Aalto University at OtaNano – Nanomicroscopy Center (Aalto-NMC).

Affiliations

[1]Department of Applied Phyiscs, Aalto University School of Science, Espoo, Finland

[2]Department of Bioproducts and Biosystems, Aalto University School of Chemical Engineering, Espoo, Finland

Contact

Dr. Nonappa

Department of Applied Phyiscs

Aalto University School of Science

Espoo, Finland

nonappa@aalto.fi