

# Imaging of cyanophycin in bacterial cells using energy filtering transmission electron microscopy

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For the local detection of chemical elements in a specimen electron energy loss spectroscopy (EELS) is a powerful technique, because it reveals an intensity distribution containing element specific ionization edges [1]. To get information about the local elemental distribution e.g. of nitrogen, the corresponding element specific intensities can be selected for the imaging process using an imaging energy filter. Nitrogen shows an ionization K-edge at an energy loss of about 401 eV, so selecting electrons within this energy loss region for the imaging process leads to an elemental map of nitrogen after subtraction of an uncharacteristic background.

In this project this method is used to examine bacterial cells and to detect a nitrogen-containing intracellular storage material called cyanophycin. Cyanophycin is a polymer that consists of equimolar amounts of arginine and aspartic acid; the sum formula of an arginine-aspartic acid monomer of the polymer is  $C_{10}H_{17}N_5O_4$ . It is synthesized naturally by most cyanobacteria (blue-green algae) and by a few noncyanobacteria (e.g. *Acinetobacter calcoaceticus*) under special culture conditions and appears as water-insoluble cell inclusions (granules). Their main function is the nitrogen storage [2].

Apart from natural cyanophycin producers there are noncyanobacteria that can be supplied with the genes for the synthesis of cyanophycin. Examples for those recombinant strains are *Ralstonia eutropha* H16-CGP, which stores not only cyanophycin but also poly(3-hydroxybutyrate) (PHB; it does not contain N), and *R. eutropha* PHB<sup>+</sup>4-CGP, which accumulates only cyanophycin [3]. It is highly interesting to detect and to quantify cyanophycin in these two recombinant strains as well as in *A. calcoaceticus* by recording elemental maps that show the local nitrogen distribution using nitrogen as a natural marker of cyanophycin.

Ultrathin sections of Spurr embedded specimens with a thickness of approximately 30 nm were stained with uranylacetate and examined by a ZEISS EM 902 transmission electron microscope equipped with a Castaing-Henry energy filter using an accelerating voltage of 80 kV. For the calculation of the elemental maps the micrographs were detected by a CCD-camera (Gatan MSC 794) using an energy loss window of 20 eV. The intensity in the elemental maps provides a direct measure of the concentration of the corresponding element.

Figure 1a shows the elastically filtered image of a *R. eutropha* H16-CGP specimen and Figure 2a the corresponding nitrogen map. Based on the Rose criterion [4] the granule with a diameter of about 0.3  $\mu\text{m}$  in the right cell can be characterized containing nitrogen; the nitrogen map gives a signal-to-noise ratio (SNR) of 3-5 in this region.

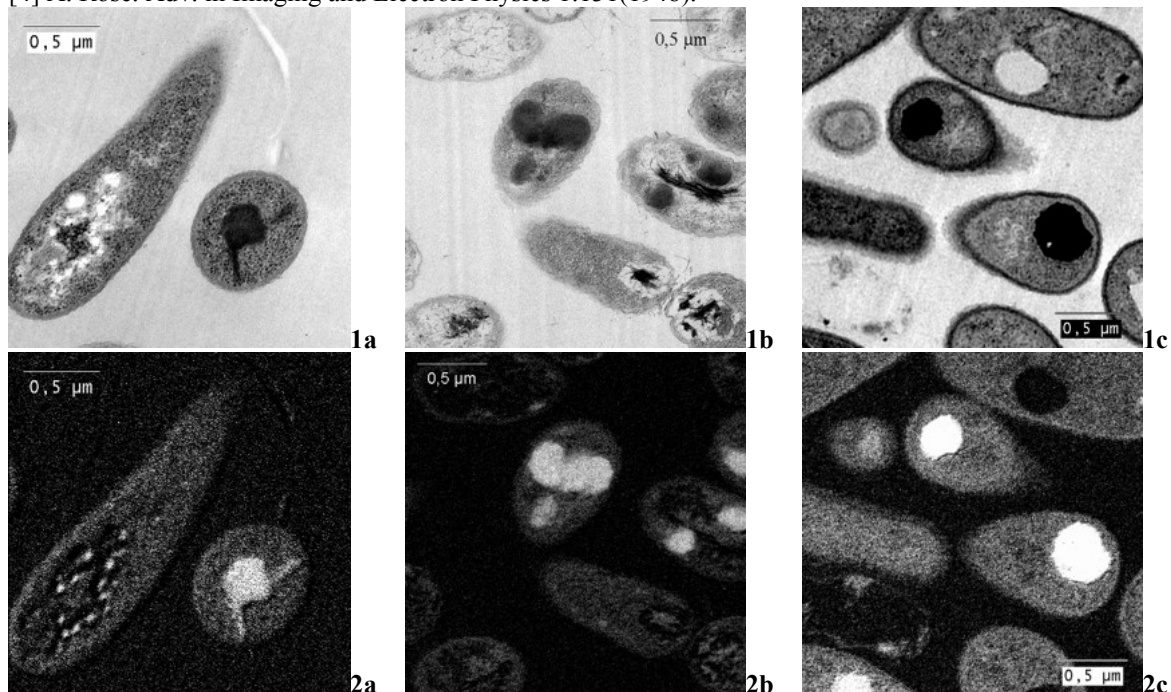
Regarding the Rose criterion this value is needed for a SNR to consider the corresponding element to be detected [4].

Figure 1b shows the elastically filtered image of an ultrathin section of *R. eutropha* PHB<sup>+</sup>4-CGP and Figure 2b the corresponding nitrogen map. In Figure 1b the dark granules with a diameter of 0.2-0.4  $\mu\text{m}$  appear bright in Figure 2b having a SNR of 5-8 which clearly indicates the presence of nitrogen.

The elastically filtered image of an *A. calcoaceticus* specimen is shown in Figure 1c; the dark granules have a diameter of 0.3-0.4  $\mu\text{m}$ . In the elemental map (Figure 2c) they appear as bright areas with a SNR of 6-8 ensuring that nitrogen is contained in these granules.

Comparing Figure 1c and Figure 2c another granule is visible in the uppermost cell, which according to the nitrogen distribution map does not contain nitrogen. This could be a polyphosphate granule, which represents another type of cell inclusion found in *A. calcoaceticus*. In future examinations this assumption could be verified by recording phosphorus maps. As mentioned above, also *R. eutropha* H16-CGP shows a second type of cell inclusion, which contains PHB. Further examinations could show clearly the differences between PHB and cyanophycin granules.

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**Figure 1.** Elastically filtered TEM-image of an ultrathin section of Spurr embedded a) *R. eutropha* H16-CGP, b) *R. eutropha* PHB<sup>+</sup>4-CGP, and c) *A. calcoaceticus*.  
**Figure 2a-c.** Nitrogen maps of the corresponding specimen areas shown in Figures 1a-c.