# Elemental maps of low phosphorus content in Corynebacterium glutamicum ΔmcbR

Michael Epping[1], Rudolf Reichelt[2] and Helmut Kohl[1]

1. Physikalisches Institut und Interdisziplinäres Centrum für Elektronenmikroskopie und Mikroanalyse (ICEM)
Universität Münster, Wilhelm-Klemm-Str. 10, 48149 Münster, Germany

2. Institut für Medizinische Physik und Biophysik, Universitätsklinikum
Universität Münster, Robert-Koch-Str. 31, 48149 Münster, Germany

http://www.uni-muenster.de/Physik/PI/kohl m.epping@uni-muenster.de

MC 2009 Graz
Symposium L6, P240

## 1) Introduction

The Corynebacterium glutamicum is often used in genetic engineering. C. glutamicum ΔmcbR is an mutant where a regulator-gene is removed. It was assumed, that this regulator controls the biosynthesis of the amino acids L-methionine and L-cystein. Instead of a higher amount of these amino acids, high quantities of Adenosine triphosphate (ATP) have been found. X-ray microanalysis has shown accumulations with higher phosphorus- and sulfur-content [1]. To determine the distribution of phosphorus we calculated phosphorus maps from energy-loss-images of thin sections (about 30 to 50 nm), using a Zeiss EM902 (80 keV) and a Zeiss Libra 200FE (200 keV).

## 2) Positioning of the energy-loss-windows

For calculating the elemental maps we used the four-window-method, three pre-edge windows and one post-edge window. The width of each window was set to about 18 eV. This large energy window width allows to reduce the recording time thus reducing the influence of specimen-drift . The pre-edge windows were placed at 80 eV, 100 eV, and 120 eV. The post-edge window was recorded at a loss of 155 eV. The delayed edge of phosphorus starts at 132 eV and reaches its maximum at 156 eV.

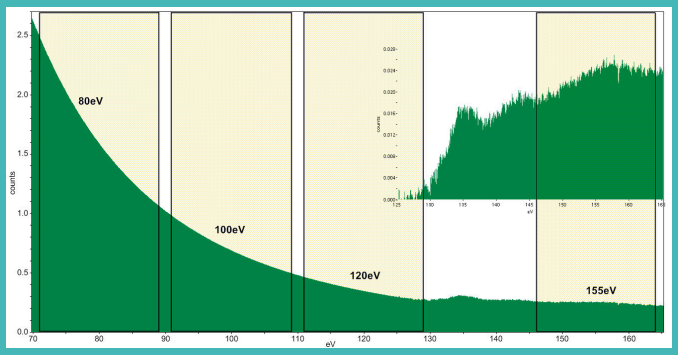


Fig. 1: Position of energy-loss-windows

## 3) Calculation of Phosphorus-maps

The images below were recorded with Binning 2 (2048x2048 pixel) and an acquisition time of 10 s for the energy-loss-images. For the calculation of the elemental map and the SNR we used a program by Gralla [2]. The drift between the images was corrected by using normalized cross-correlations. The background was calculated by using the power law model.

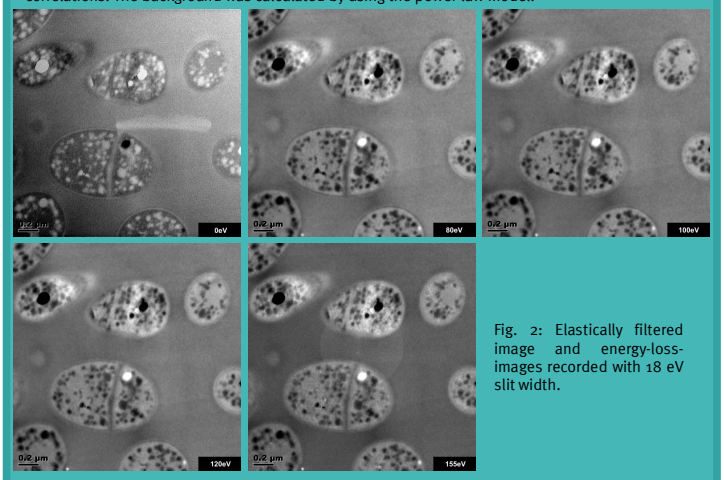


Fig. 2: Elastically filtered image and energy-loss-images recorded with 18 eV slit width.

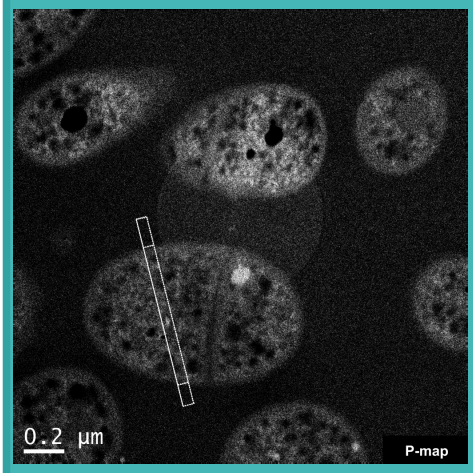


Fig. 3: Calculated P-map

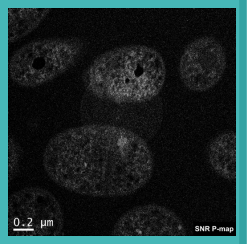


Fig. 4: SNR-image (5 to 11)

## 4) Silicon L-edge

Recording parallel spectra with the Libra 200FE, we could clearly identify phosphorus. Surprisingly, we found an additional silicon signal all over the specimen, whose origin is not yet clear.
The silicon L-edge is located at the energy-loss, where we placed one of our pre-edge windows. To verify our P-maps we calculated elemental maps by using only two pre edge windows (80 eV and 100 eV). The resulting maps showed the same distribution of phosphorus but they had a higher overall signal.

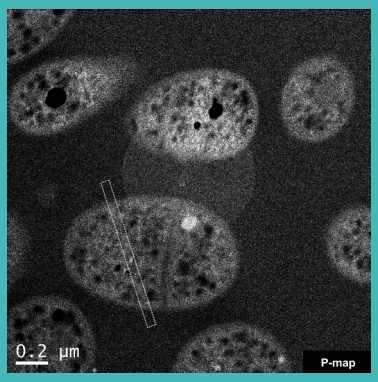


Fig. 6 (left): Calculated P-map (two pre-edge images)

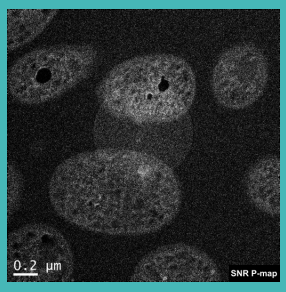


Fig. 7: SNR-image (5 to 11)

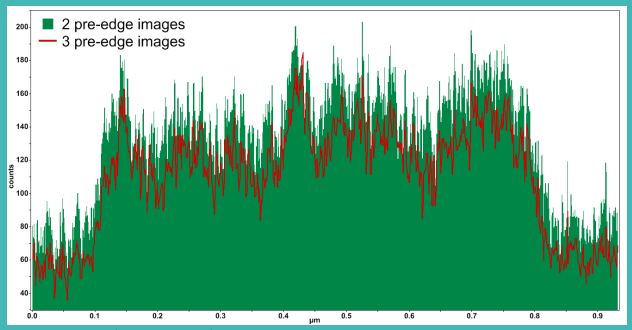


Fig. 8: Profile (50 pixel width) of the marked area in Fig. 6. For comparison the profile for the map with three pre-edge images is displayed too.

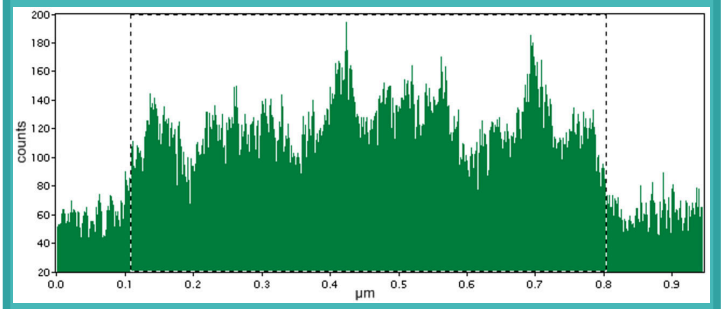


Fig. 5: Profile (50 pixel width) of the marked area in Fig. 3.

## 5) Conclusion

Without knowing about the interference of the silicon signal, we were able to calculate phosphorus-maps. With a SRV higher then 3 – at locations of high P-content even higher than 5 – we match the Rose-criterion for detection of phosphorus.
Calculating the elemental maps using only two pre-edge images we obtain a higher signal but also an increase of noise.

[1] J. O. Krömer et al., Microbiology (2008); 154: 3917-3930.
[2] B. Gralla, personal communication, Uni Münster

The provision of the specimen by C. Bolten (TU Braunschweig) is gratefully acknowledged.
For excellent specimen preparation we thank Mrs. U. Malkus (Uni Münster).

We express our gratitude to the DFG for funding the Libra 200FE within the “Großgeräteinitiative”.