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Three-dimensional Visualization of Ion Nanodomains in Subcellular Compartments

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Understanding mechanisms involved in osmoregulation control in protozoan parasites has been a challenge for many research groups. Over the past years, a number of key players in cell signaling in trypanosomatid parasites have been identified. Among these, inorganic polyphosphate (PolyP) polymers have proven to play important roles in cell physiology, both as an energy source, stored in its constituent phosphoanhydride bonds, and as a polyanion that might activate a number of physiological processes [1]. A number of methods for PolyP localization and quantification are available, including DAPI-staining followed by microscopic visualization and quantification, P-NMR analysis, enzymatic assay using recombinant exopolyphosphatases and analytical electron microscopy (AEM). From the AEM point of view, X-ray microanalysis combined with elemental mapping as well as energy filtered TEM have been the most employed techniques carried out to explore the two-dimensional composition and distribution of (poly)ions (including polyphosphate stores) within cells [2].

In this work, we used a combination of cutting edge electron microscopy techniques to map the 3D distribution of diffusible ions within the whole volume of ion-rich organelles present in the protozoan parasite *Trypanosoma cruzi*, at high resolution, using X-rays microanalysis. Cryofixed cells were analyzed by scanning transmission electron tomography (STEM-Tomography) combined with energy dispersive X-ray microanalysis (EDS), using the latest high performance setup of multiple X-ray detectors [3] to obtain 3D elemental maps (EDS tomography) of ion-rich organelles with nanoscale dimensions.

We showed a heterogeneous three-dimensional distribution of ions within the shell of polyphosphate polymers forming segregated nanochemical domains (figure 1). Pearson correlation analysis showed that phosphorus, present namely in the form of polyphosphate anions, appear homogeneously distributed along the sampling volume whereas cations such as magnesium, calcium, potassium and zinc display heterogeneous distribution with a self-excluding pattern (cations self-exclude themselves). This is the first direct evidence for the asymmetric distribution of cations bound to a polyphosphate polymer, raising questions about polyphosphate assembly mechanisms and its influence on the functional role of polyphosphate in cell physiology. In addition, these strategies were used here to explore the three-dimensional elemental distribution are novel for biological materials. We believe that the experimental pipeline shown here can be applied to a variety of models (synaptic vesicles, endoplasmic reticulum, etc) where ion mobilization plays a crucial role in the broad physiological processes.

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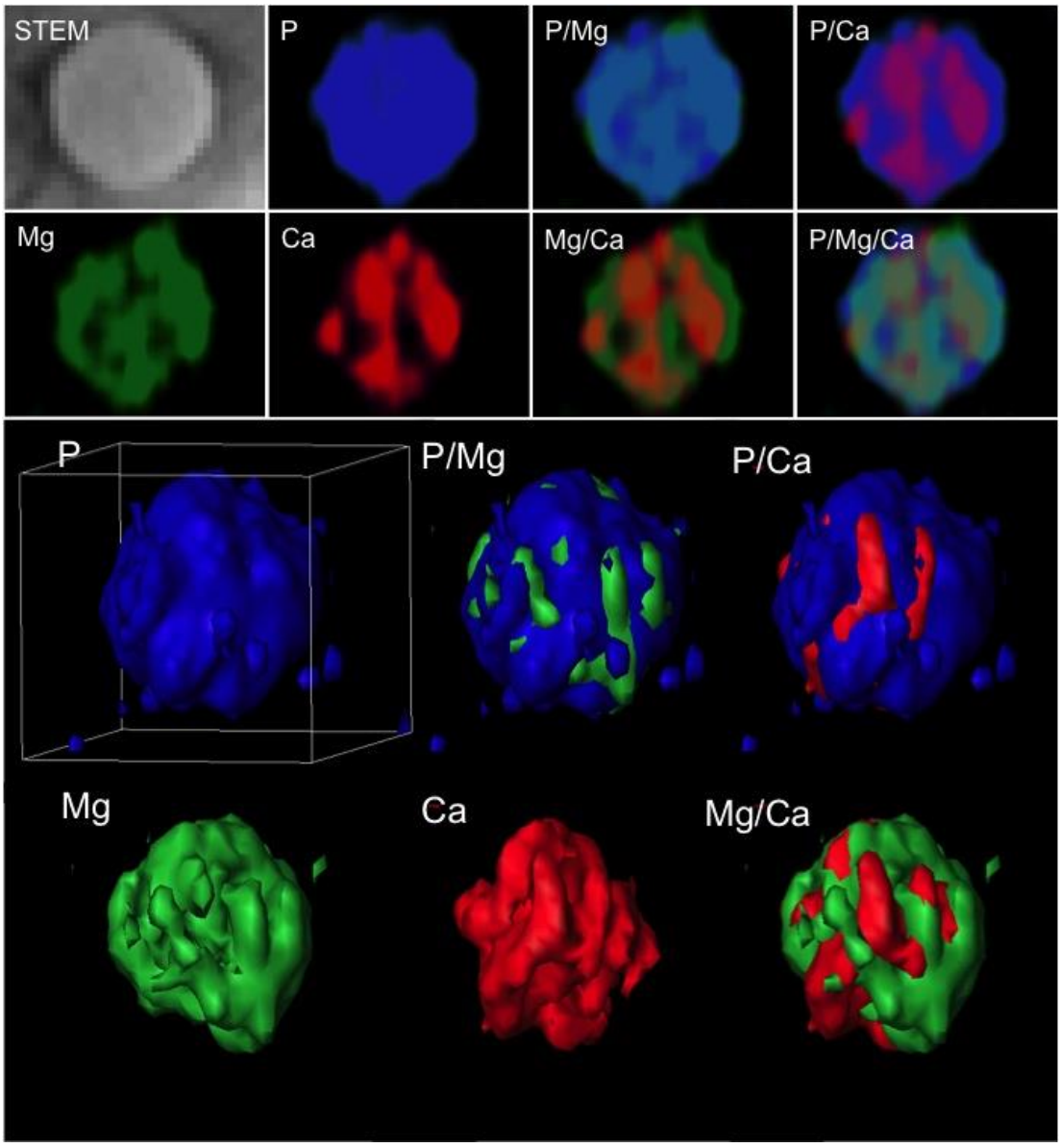

Figure 1. Analytical electron tomography of *T. cruzi* ion-rich organelle at nanoscale showing the 3D distribution of phosphorus, magnesium and calcium. The upper two lines show a central slice from the 3D mapping segmented by Voltex (Amira) of each element and the combination between these elements to visualize the degree of localization. 3D models from the same elements are also shown.

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