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Structural Organization of *Plasmodium chabaudi* Infected Erythrocytes: a Three-Dimensional Visualization Using Different Microscopy Approaches

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Plasmodium, the causal agent of malaria, is an obligatory intracellular parasite that infects erythrocytes causing a series of modifications in its internal organization to set up a novel trafficking system within and to the surface of host cell. These structures comprise micro and small vesicles that are spread throughout the cytoplasm and a complex group of adjacent membranes named Maurer's clefts. Parasite also modifies the surface of host erythrocyte by inducing the generation of knobs, presumably in regions where internal membranes are connected. Few data are available on structural organization of *Plasmodium chabaudi* infected erythrocytes, especially in a high resolution (EM) 3D level. *Plasmodium chabaudi* is an attractive model for structural studies because a synchronized life cycle, controlled by circadian period, can be obtained in mouse models, producing bleeds enriched in specific developmental forms of the parasite. Electron microscopy observation of ultrathin sections of *Plasmodium chabaudi* infected erythrocytes showed the presence of a membrane network in the host cell cytoplasm with different shapes originated from the parasitophorus vacuole membrane. Dynamics of the interaction and formation of pores within this membrane network were revealed by electron tomography. Three-dimensional reconstruction of tubulovesicular network showed that these structures are numerous and vary in shape and size. Using different approaches such as transmission electron microscopy, atomic force microscopy, field-emission scanning electron microscopy and focused ion beam associated with scanning electron microscopy we observed striking deformations, invaginations and knob formation on the surface of the erythrocyte. Alterations of the intracellular organization, such as formation of vesicles as a consequence of the presence of parasite, were observed. These results provide insights of the structural modifications that involve membrane trafficking upon *Plasmodium* infection and exemplify how front-end microscopy techniques can provide a better understanding of the mechanisms underlying the interaction of *Plasmodium chabaudi* with the host cell.

Keywords: Plasmodium, three dimensional reconstruction, Maure's clefts, tubulovesicular network