# What is the true size of the mitochondrial intermembrane space? A study using high-pressure freezing and STEM tomography

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It is generally believed that an intermembrane space of about 40 to 100 nm separates inner and outer mitochondrial membrane and the membranes of the cristae; and according to textbooks this space bears a number of different proteins for oxidative phosphorylation and for control of apoptosis [1]. This concept is derived from electron microscopical samples chemically fixed with glutharaldehyde [2, and others]. The size of the intermembrane space is described to be rather constant, although form and shape of the mitochondria and of the cristae vary between cell types and physiological states [2]. Considerably smaller intermembrane spaces, however, have been observed when cryo-fixation methods have been used [3, and others].

We have found that after immobilization by high-pressure freezing (HPF) in a number of different cell types, inner and outer membranes are in very close apposition: (Figures 1 to 6): Figure 1 is a computed section of a scanning transmission electron microscopical (STEM) tomogram recorded at 300 kV, and Figure 2 is an ultrathin section of a mitochondrion of cultivated Panc 1 cells prepared by HPF and freeze substitution [4]. Figure 3 is a mitochondrion from a yeast cell (*Saccharomyces cerevisiae*) after HPF and freeze substitution. The thickness of the intermembrane space including both membranes was less than 20 nm, special contact sites with even closer apposition of the membranes were not observed. In order to exclude artefact formation during freeze-substitution, we compared the results with data from yeast after HPF and cryo-fracturing in the cryo-SEM [5] (Figures 4 to 6). Also in these samples we found inner and outer membranes in close apposition.

Our findings of a very small intermembrane gap in healthy mitochondria correspond well with older studies on cryofixed isolated mitochondria by spray freezing [6], or propane jet freezing [7]. When frozen in a metabolically active state, inner and outer membranes were in close apposition, whereas when respiration was blocked by antimycin A, the intermembrane space became large [6]. Based on these results it appears unlikely that dehydration of the intermembrane space by ice crystal formation in the cytoplasm due to insufficient freezing rates may have caused the collapse of the intermembrane space. The cell biological implication of these findings is, that in a respiratory active mitochondrion all intermembrane proteins must be in close touch with the membranes [8].

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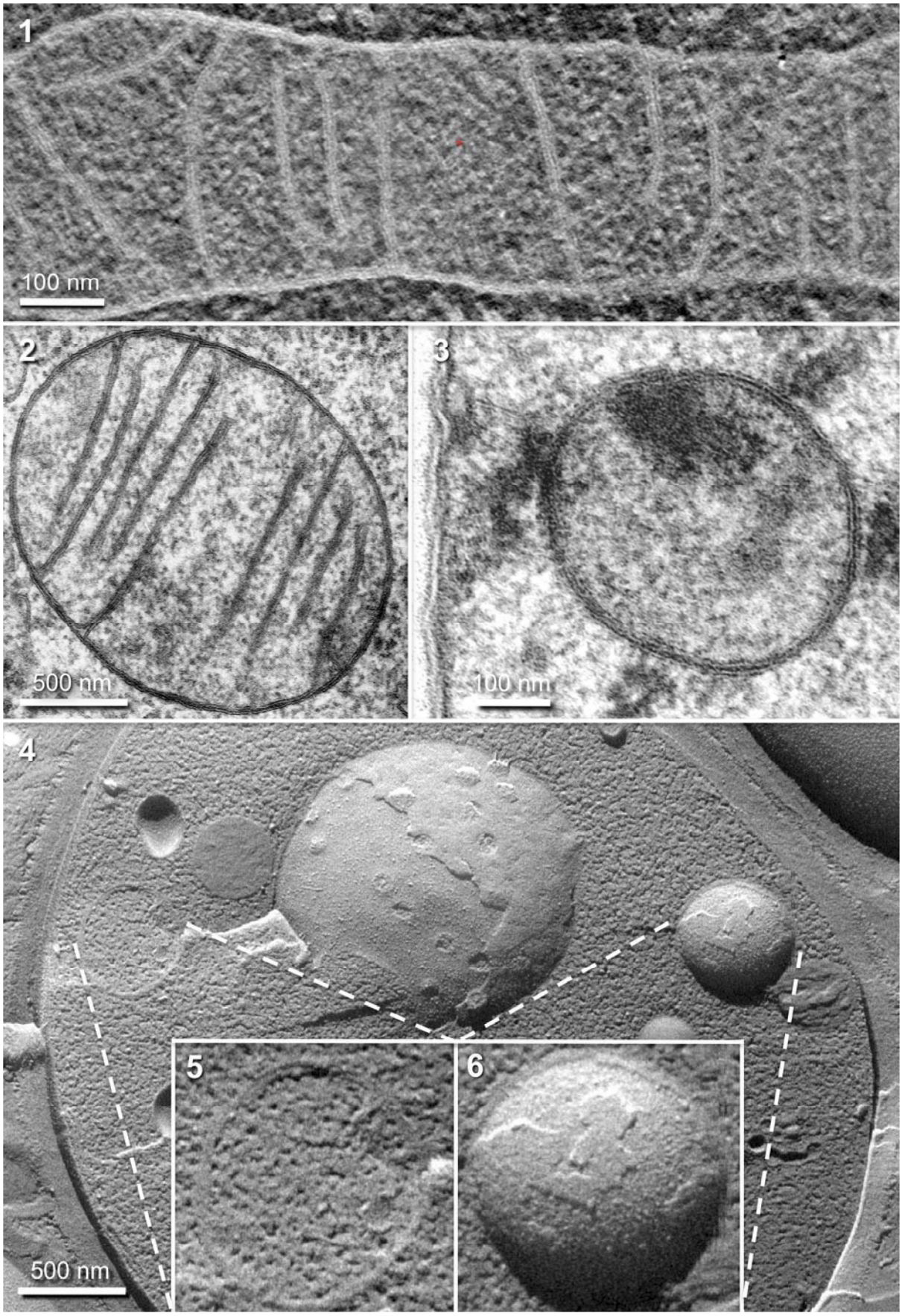


Mitochondria in high-pressure frozen Panc 1 cells (**Figures 1 and 2**) as well as in yeast cells (**Figures 3 to 6**) exhibit inner and outer membranes in close apposition.

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