TERRENCE G. FREY

Structure of Biological Macromolecules and Macromolecular Assemblies; Membrane Structure and Function; Electron Microscopy and Image Processing

Our research interests are the structural study of biological assemblies by application of biophysical and biochemical methods. More specifically, we apply techniques of high resolution electron microscopy and digital image processing to study the structures of biological macromolecules, macromolecular assemblies, and whole organelles. Currently we are studying the structure and function of mitochondria using state of the art microscopic techniques, principally Electron Tomography. Electron Tomography is a technique which calculates the three-dimensional structure from a series of electron micrographs of cells or cellular components tilted over a range of angles. Our study of mitochondria has led, along with the work of several other research groups, to a new paradigm of mitochondrial structure in which the inner membrane of mitochondria is divided into two components. The inner boundary membrane is the component that lies along the outer membrane separated from it by approximately 3-7 nanometers. At numerous sites we find 30 nm diameter tubular extensions of the inner membrane projecting into the matrix toward the center of the mitochondrion forming cristae, the second component of the inner membrane. Numerous tubular cristae often merge forming large disklike cristae. These results have raised a number of questions about mitochondrial structure and function that we are currently addressing in collaboration with groups at SDSU and other research institution.

(1) Is the mitochondrial inner membrane compartmentalized? Although the inner boundary membrane and the cristae membrane are one continuous surface, they are connected by tubular crista junctions that are only 30nm in diameter. This suggests that the functions of the inner membrane may be compartmentalized by controlling the distribution of inner membrane proteins We are testing this hypothesis by labeling various inner membrane proteins with specific monoclonal antibodies.

(2) Do cristae grow from the addition of membrane proteins and lipids at tubular crista junctions? To answer this question we are studying the growth of crista in *Neurospora* mitochondria containing few or no cristae owing to a deficiency in Tom20, a critical component in protein import. The function of Tom20 can be restored resulting in growth of crista that will be monitored by electron tomography.

(3) How are the sites of protein import distributed over the surface of mitochondria and what is their relationship to stable contact sites and crista junctions? We are studying the sites of protein import by microinjection of a protein construct into a giant mutant of *Neurospora*. The construct contains an N-terminal mitochondria target sequence, a stable folded protein domain to halt transport into mitochondria, and a colloidal gold particle to enable visualization in electron micrographs. The sites of protein import will be mapped and correlated with the positions of stable contact sites and of crista junctions

(4) What are the changes in mitochondria structure during apoptosis? Mitochondria play a key role in initiating the apoptosis program with the release of cytochrome *c* into the cytosol. We are using Electron tomography to study the structural changes in mitochondrial membranes in order to determine whether cytochrome *c* is released through specific pores or through rupture of the outer membrane following swelling of the matrix. In this context we are also studying the possible role of the mitochondrial permeability transition in cytochrome *c* release

5) What effects does hypoxia have on the structure of mitochondria? Prolonged severe hypoxia results in irreversible changes in cell structure that can lead to cell death. We will develop a cell culture model for hypoxia in cardiac myocytes in order to study the effects of long and short term hypoxia on mitchondrial structure and function which we will study by correlated light and electron microscopy.

Representative Publications

Perkins, G. A., Song, J. Y., Tarsa, L., Deerinck, T. J., Ellisman, M. H. and **Frey, T. G.** Electron Tomography of Mitochondria from Brown Adipocytes Reveals Crista Junctions. *J. Bioenerg. Biomembr.* **30**, 431-442 (1998).

Perkins, G., Renken, C., Martone, M.E., Young, S.J., Ellisman, M., and **Frey, T.** "Electron Tomography of Neuronal Mitochondria:Three-dimensional structure and organization of cristae and membrane contacts" *J. Struct. Biol.* **119**, 260-272 (1997).

Crum, J., Gruys, K.J., and **Frey, T.G.** "Electron microscopy of cytochrome *c* oxidase crystals: Labeling of subunit III with a monomaleimide undecagold cluster compound," *Biochemistry* **33**, 13719-13726 (1994).

Frey, T.G. and Murray, J.M. "Electron Microscopy of Cytochrome *c* Oxidase Crystals: Monomer-Dimer Relationship and Cytochrome *c* Binding Site," *J. Mol. Biol.* **237**, 275-297 (1994).

Frey, T.G. "Cytochrome *c* Oxidase: Structural Studies by Electron Microscopy of Two-Dimensional Crystals," *J. Elect. Microsc. Tech.* **27**, 319-332 (1994).

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