A Proteoliposome Nanocarrier for Mitochondrial Gene Delivery

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A number of significant hurdles must be overcome to enable the manipulation of mitochondrial genetics. The mitochondrial genome is effectively sequestered within two lipid bilayers: the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM), making access to mitochondrial DNA (mtDNA) difficult. Cells contain hundreds to thousands of copies of mtDNA, and mutations in mitochondrial genes are typically heteroplasmic. Exceeding a threshold level of heteroplasmy (typically around 70% for many mtDNA mutations) can cause cells to rapidly degenerate from normal to a disease phenotype. An effective mitochondrial gene therapy requires technology to deliver complete, healthy mitochondrial genomes to the mitochondrial matrix, thereby shifting the level of heteroplasmy below the threshold level. Where possible, mimicking natural cellular mechanisms and components is desirable. We propose to engineer a nanocarrier with the capability to cross the plasma membrane and to fuse with both the OMM and the IMM, providing effective payload delivery to the mitochondrial matrix. This proteoliposome nanocarrier will consist of an outer liposomal shell enveloping inner concentric shells designed for sequential OMM and IMM fusion. Fusion of the OMMs of adjacent mitochondria is mediated by the transmembrane GTPases, mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2). The first part of the project, presented here, focuses specifically on developing a red fluorescently labelled proteoliposome incorporating recombinant Mfn2 for *in vitro* fusion with the OMM of green fluorescent protein (GFP)-labelled mitochondria isolated from stably transfected NIH-3T3 fibroblasts. Flow cytometry will be used to detect Mfn2-mediated liposomal-mitochondrial fusion through recognition of green-red fluorescence colocalization.