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Development of Fluorogenic Antioxidants to Monitor Reactive Oxygen Species in the Lipid Membrane of Live Cells.

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We have identified peroxyl radicals as key targets to monitor in our quest to reconcile the chemistry and biology of reactive oxygen species (ROS). In this context, we pioneered the development of lipophilic fluorogenic antioxidants for the spatiotemporal imaging of lipid peroxyl radicals in the membrane of live cells [1]. Our strategy involves preparing receptor-reporter probes that mimic the peroxyl radicalscavenging activity of α-tocopherol, the most active naturally occurring lipid soluble antioxidant present in mammalian tissues [2]. The receptor segment consists of the chromanol moiety of α-tocopherol, ensuring the probe reactivity towards peroxyl radicals is on par with that of the antioxidant. A tethered borondipyrromethane dye (BODIPY) reports structural changes at the receptor end following peroxyl radical scavenging. BODIPY dyes are an ideal choice because they are lipophilic, photostable and easily prepared. Initially non-emissive, the probes become fluorescent once oxidation of chromanol to chromanone deactivates an otherwise operational intramolecular photoinduced electron transfer (PET) process [1].

Building on this initial discovery, over the past few years we have tackled i) The elucidation of the mechanism of action of the first lipophilic fluorogenic antioxidant [3]; ii) the development of novel borodipyrromethane dyes (BODIPY dyes) with versatile functionalities and tunable redox properties [4]; iii) The preparation and characterization of second generation fluorogenic antioxidants with enhanced sensitivity and reactivity, to image Reactive Oxygen Species (ROS) in lipid membranes [5]; iv) The elaboration of high throughput assays to monitor how lipid unsaturation, peroxyl radical partitioning, and chromanol lipophilic tail affect the antioxidant activity of α-tocopherol [5]; v) The evaluation of the antioxidant activity of novel synthetic analogues of a-tocopherol intended as new drugs [5,6]; vi) The imaging of ROS in the lipid membrane of living cells [7]; and most recently, vii) the development of a new probe that specifically targets the mitochondria [8]; and viii) the visualization at the single molecule level of oxidative reactions in surface immobilized liposomes stained with our probes.

In this presentation I will discuss the general concepts behind the new probes and will focus on the reactivity and imaging opportunities in live cells arising from a novel α-tocopherol based fluorogenic probe that readily targets mitochondria (Mito-Bodipy-TOH) [8]. Here we will show that Mito-Bodipy-TOH targets the inner mitochondrial lipid membrane (IMM) and is sensitive to the presence of lipid peroxyl radicals, effective chain carriers in the lipid chain autoxidation. Mito-Bodipy-TOH enables monitoring of the antioxidant status, i.e., the antioxidant load and ability to prevent lipid chain autoxidation, within the inner mitochondrial membrane of live cells. The new probe consists of 3 segments: a receptor, a reporter, and a mitochondria-targeting element, constructed, respectively, from an α-tocopherol-like chromanol moiety, a BODIPY fluorophore, and a triphenylphosphonium cation (TPP). The chromanol moiety ensures reactivity akin to that of α-tocopherol, the most potent naturally–occurring lipid soluble antioxidant, while the BODIPY fluorophore and TPP ensure partitioning within the inner mitochondrial membrane [8].

We will present studies conducted on live fibroblast that show the antioxidant depletion in the presence of methyl viologen (paraquat), a known agent of oxidative stress and source of superoxide radical anion (and indirectly, a causative of lipid peroxidation) within the mitochondria matrix. A ca. 8-fold emission enhancement was recorded with Mito-Bodipy-TOH in cells stressed with methyl viologen whereas no enhancement was observed in control studies with untreated cells. Our findings underscore the potential of the new fluorogenic antioxidant Mito-Bodipy-TOH to study the chemical link between antioxidant load, lipid peroxidation and mitochondrial physiology [8].

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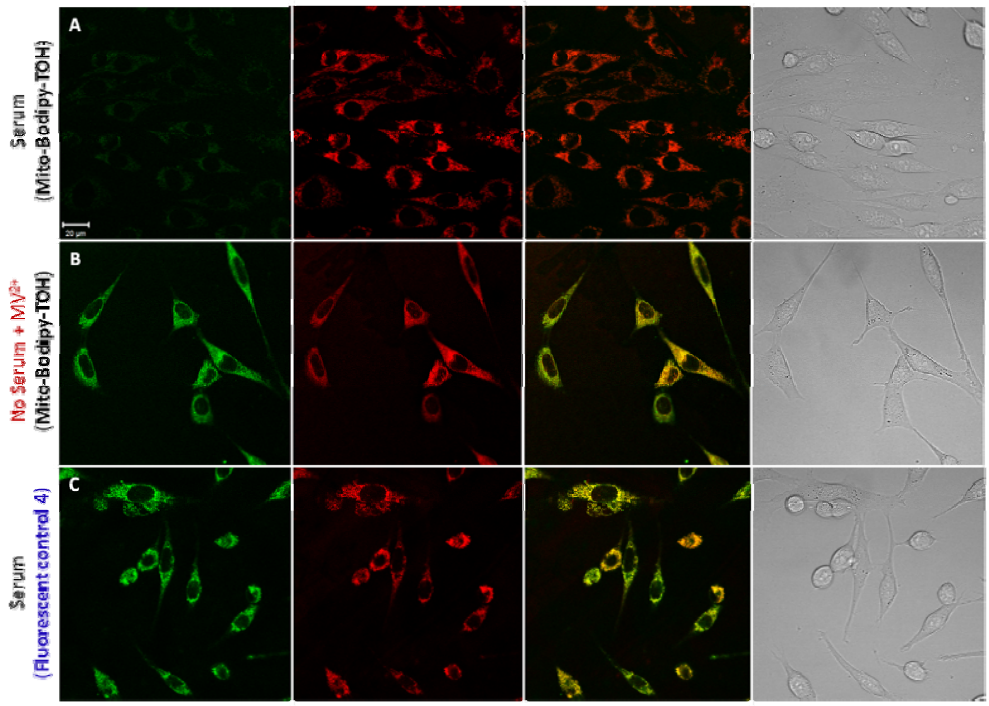
Figure 1. Detection of ROS with fluorogenic probe Mito-Bodipy-TOH in live NIH 3T3 mouse embryo fibroblast cells. Cells were incubated with 1 μM Mito-Bodipy-TOH and 200 nM MitoTracker Deep Red at 37 °C for 5 min. The panels show (left to right) confocal fluorescence images of 1 μM Mito-Bodipy-TOH, 200 nM MitoTracker Deep Red, overlay of the first two images, and bright field image of: A) Fibroblast cells in the presence of 10 % FBS; B) Fibroblast cells under oxidative stress conditions (deprived of growth factors and additionally stressed with 1mM MV^{2+} for 4 h prior to imaging); C) Fibroblast cells in the presence of 10 % FBS incubated with 1 μM control mitochondria-targeting fluorescent probe 4. Scale bar is 20 μm. From reference [8]



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