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Using TMRM to monitor changes in mitochondrial membrane potential

White Paper

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Introduction

Mitochondrial function can be examined by measuring mitochondrial membrane potential ($\Delta\Psi_m$), a key marker of bioenergetics and cellular health. Any environmental or genetic factors affecting mitochondrial oxidative phosphorylation will alter the $\Delta\Psi_m$. Therefore, the ability to measure changes in $\Delta\Psi_m$ is extremely valuable in determining if a compound has mitochondrial toxicity or for unravelling mitochondrially associated pathologies[1]. Here we describe the use of the fluorescent dye, tetramethylrhodamine methyl ester (TMRM) to measure changes in $\Delta\Psi_m$ induced by chemical effectors[2].

Methods

HE293T cells were plated in 96 well plate format, incubated with TMRM for 30 mins and allowed to equilibrate. In some experiments Brilliant Black was added to quench extracellular TMRM fluorescence. Cells were treated with tool compounds known to influence $\Delta\Psi_m$ and fluorescence (ex: 548 nm / em: 574 nm) was measured. Fluorescence changes were measured kinetically using an atmosphere-controlled ClarioStar plate-reader.

Example Data

A kinetic trace of TMRM fluorescence changes is shown in Figure 2. CCCP, a protonophore that prevents mitochondrial permeability transition pore opening, increases $\Delta\Psi_m$. Oligomycin, an ATP synthase inhibitor that prevents backflow of H+, decreases $\Delta\Psi_m$.

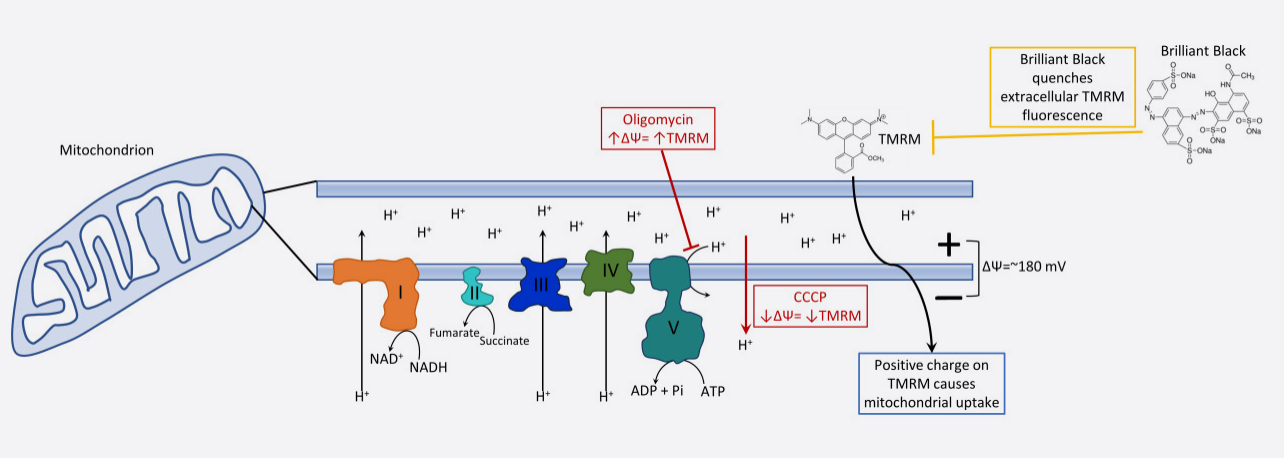

Figure 1: Schematic representation of TMRM $\Delta\Psi_m$ assay

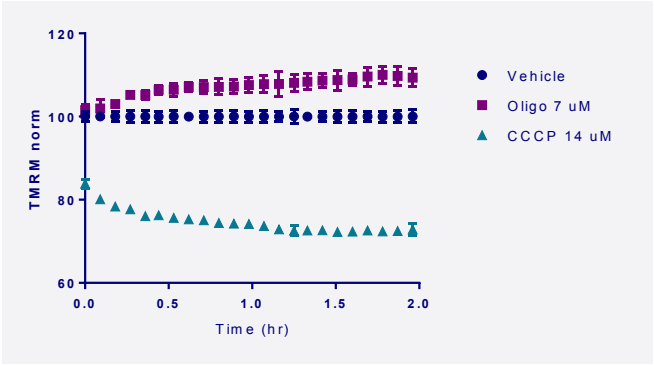

Figure 2: Time-course of TMRM fluorescence changes in HEK293T cells.

The effect of CCCP and oligomycin titrations on TMRM signal in HEK293T cells is shown in Figure 3.

The experiment was run in 2 modes: with Brilliant Black (Figure 3B), an impermeable dye which quenches extracellular fluorescence and increases assay window, or in the absence of Brilliant Black (Figure 3A), allowing multiplexing and kinetic data to be recorded. Compounds were incubated for up to 2 hours to ensure full equilibration.

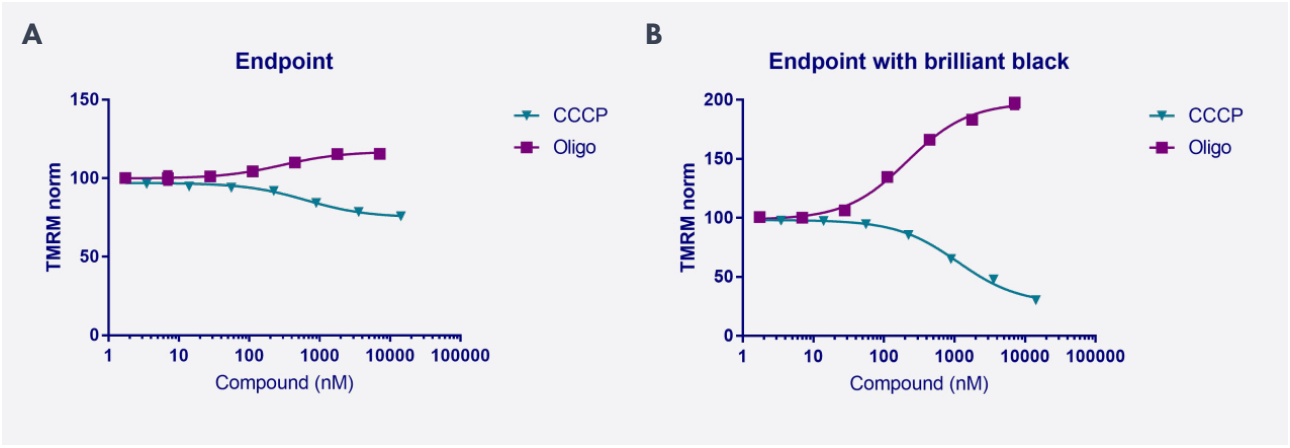

Figure 3: TMRM concentration-response curves of control $\Delta\Psi_m$ modifying reagents CCCP and oligomycin in the two assay formats (A: endpoint and B: endpoint with Brilliant Black)

References

1. Evaluating Mitochondrial Membrane Potential in Cells. Giancarlo Solaini, Gianluca Sgarbi, Giorgio Lenaz and Alessandra Baracca. Evaluating Mitochondrial Membrane Potential in Cells. *Biosci Rep.* **2007**; 27:11-22.

2. Measurement of Mitochondrial Membrane Potential Using Fluorescent Rhodamine Derivatives. Russell C. Scaduto, Lee W. Grotyohann. *Biophysical Journal.* **1999**; 76:469-477.

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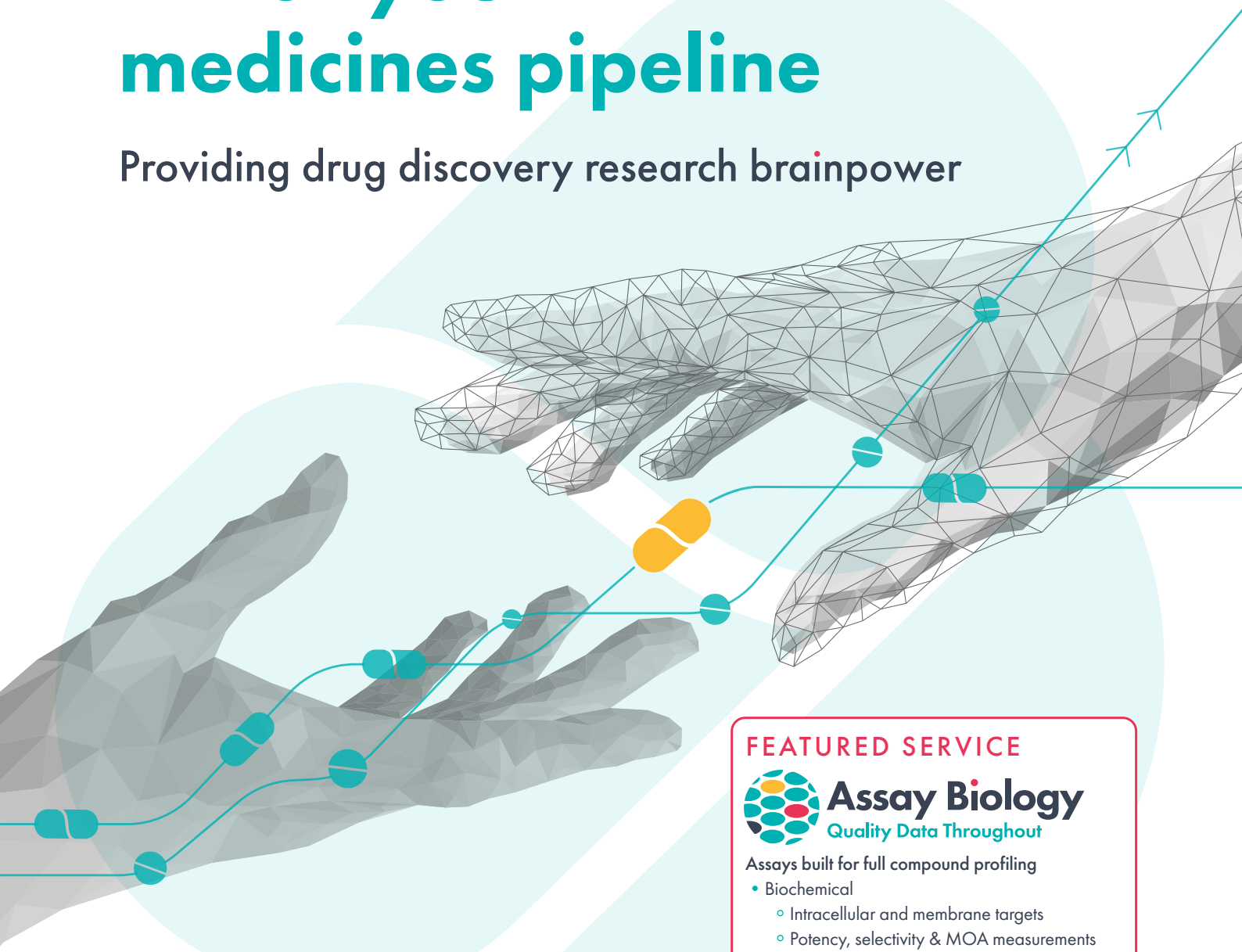

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