

(PLTR) is governed by metabolic memory of exposures that occurred during prenatal, and likely also during perinatal development.

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### Trapping and study of individual mitochondria using nanofluidic technology

Presenter: Katayoun Zand

Katayoun Zand<sup>a</sup>, Ted Pham<sup>a</sup>, Antonio Davila Jr.<sup>b</sup>, Douglas Wallace<sup>b</sup>, Peter Burke<sup>a</sup>

<sup>a</sup>Integrated Nanosystems Research Facility, University of California Irvine, Irvine, CA 92697, USA

<sup>b</sup>Center for Mitochondrial and Epigenomic Medicine, Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA

**Body of Abstract:** It has been shown that there is large morphological and functional heterogeneity within mitochondrial populations. It is important to study this heterogeneity in order to understand the mechanisms of mitochondrial functions. However, current technologies are mostly suitable for investigating the average behavior of a large number of mitochondria; the dynamics and behavior of individual mitochondria is lost. Microfluidic and nanofluidic technologies make it possible to control fluids in small volumes and enable assays requiring significantly less sample size, therefore they provide a powerful platform for investigation of individual mitochondria under different chemical conditions. Here we present a novel platform for interrogation of single isolated mitochondria. Nanofluidic devices are fabricated by soft lithography of polydimethylsiloxane (PDMS) over a patterned silicon mold through a simple, cost effective and reproducible method. The device consists of an array of 10 parallel nanochannels each with a cross section of 500 nm × 2 μm. Respiration buffer containing mitochondria isolated from human cervical cancer cell line HeLa is pumped in to the device using a syringe pump and individual mitochondria get immobilized along the channels physically due to the small size of the channels. Time lapsed fluorescence microscopy of JC-1 and TMRM stained mitochondria reveals that trapped mitochondria maintain their membrane potential. As a proof of concept effect of adding substrates and calcium on the membrane potential is studied and the results indicate that mitochondria remain vital and functional in this trapped state. Flickering of membrane potential in some substrate fed mitochondria is observed.

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### Therapy for mitochondrial diseases: An investigation into the potential to stimulate Parkin-mediated mitophagy

Presenter: Alicia M Pickrell

Alicia M Pickrell, Chiu-Hui Huang, Milena Pinto, Carlos T. Moraes, Richard J. Youle

National Institutes of Health/National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892, USA

Mitochondrial diseases characterized by genetic mutations in mitochondrial DNA (mtDNA) lead to declines in oxidative phosphorylation (OXPHOS) and ATP generation, affecting organs with a high bioenergetic demand such as brain, heart, and skeletal muscle. Clinical phenotypes arise when mutant loads in specific tissues reach particularly high thresholds.

PARK2 and PARK6, encoding Parkin and PINK1 proteins, respectively, first sparked interest in the research community when mutations found

in these genes were attributed to forms of familial Parkinson's disease (PD). Parkin and PINK1 have been shown to interact within the same pathway, affecting mitochondria and influencing mitochondrial morphology. Our group, and others, have found that this interaction extends further, in that Parkin and PINK1 promote the removal of dysfunctional mitochondria (mitophagy) by targeting those with low mitochondrial membrane potential ( $\Delta\psi_m$ ) and pathogenic mtDNA mutations. Although extensive research has been performed *in vitro*, it is still unclear whether Parkin-mediated mitophagy is active *in vivo*.

The POLG Mutator mouse has a catalytic proofreading-deficient domain in polymerase  $\gamma$ , a polymerase that replicates and transcribes mtDNA. This mouse model exhibits a premature aging phenotype with progressive mitochondrial dysfunction caused by accumulating mtDNA mutations. Parkin translocates to damaged mitochondria and stimulates mitophagy, removing the dysfunctional organelles. Mutator mice will be used to determine if Parkin mediates mitophagy *in vivo*. We have mated the Mutator mouse on a Parkin null background to understand how loss of Parkin affects mitochondrial homeostasis.

We allowed control, Parkin  $-/-$ , Mutator, and Mutator Parkin  $-/-$  animals to age to 48–52 weeks as it has been previously reported that this is the time point when Mutator mice undergo respiratory chain dysfunction in the CNS.

We isolated mitochondria from the brain and liver and measured  $\Delta\psi_m$  to confirm that Mutator or Mutator Parkin  $-/-$  mice have dysfunctional mitochondria. Mitochondria from both the cortex and liver of Mutator and Mutator Parkin  $-/-$  mice are depolarized.

We next wanted to observe changes in proteins involved in mitophagy. We blotted liver mitochondria and probed for PINK1, which stabilizes, translocates, and facilitates Parkin-mediated mitophagy. We found increased protein levels of PINK1 in Mutator mitochondria. Mitofusin1 and 2 proteins are ubiquitinated by Parkin and degraded during mitophagy. We also found a lower level of Mfn1 and Mfn2 in isolated mitochondria from Mutator mice. This data suggest that mitochondria from Mutator mice reach low levels of  $\Delta\psi_m$  possible to trigger a mitophagic response.

We then wanted to test if there were impairments in the nigrostriatal pathway of Mutator Parkin  $-/-$  mice. We examined axons and the cell bodies of dopaminergic (DA) neurons. We noted a loss of DA axons and neurons Mutator Parkin  $-/-$  mice that was absent in wt, Mutator, or Parkin  $-/-$  groups. We have found that the loss of Parkin in a mouse model of mitochondrial dysfunction causes neurodegeneration.

Our data suggests that endogenous Parkin-mediated mitophagy may occur *in vivo*. Future work will determine if Parkin-mediated mitophagy substantially and selectively clears dysfunctional mitochondria in hopes of treating mitochondrial diseases through the pharmacological stimulation of this pathway.

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### A human reprogrammed-cell model of MELAS

Presenter: Rajesh Ambasadhan (rajesh@sanfordburnham.org)

Rajesh Ambasadhan<sup>a</sup>, James Parker<sup>a</sup>, Carlos Ainsa<sup>b,c</sup>, Nima Dolatabadi<sup>a</sup>, Scott Ryan<sup>a</sup>, Thuy Le<sup>b,c</sup>, Stuart A Lipton<sup>a,b</sup>, Richard H Haas<sup>b,c</sup>

<sup>a</sup>Del E Webb Center for Neuroscience, Aging and Stem Cell Research, Sanford-Burnham Medical Research Institute, 10901 N. Torrey Pines Rd., La Jolla, CA 92037, USA

<sup>b</sup>Department of Neurosciences, University of California San Diego, La Jolla, CA 92039, USA

<sup>c</sup>The Mitochondrial and Metabolic Disease Center, University of California San Diego, La Jolla, CA 92039, USA