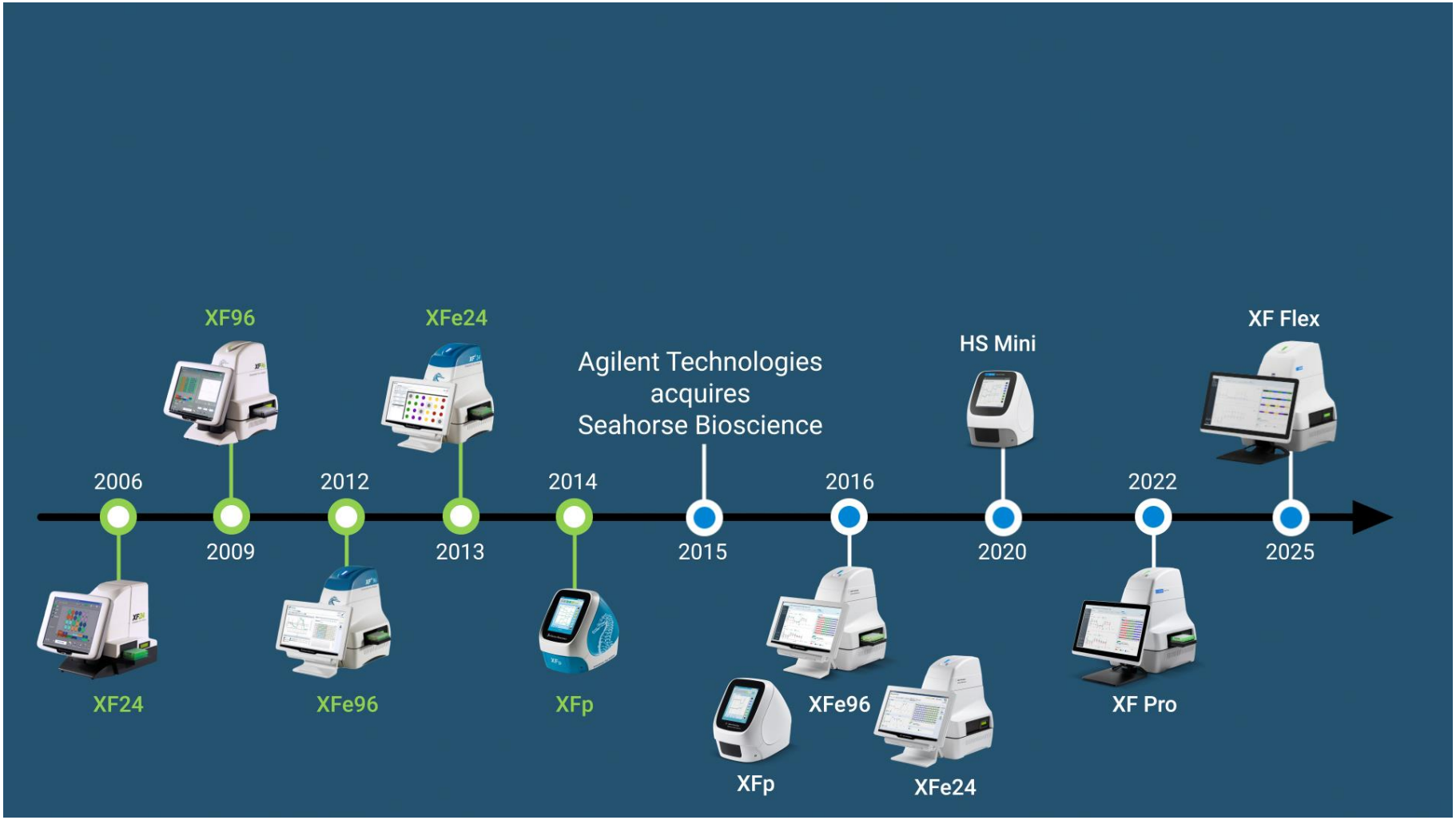
The World's Most Advanced Cell Metabolism Analyzer

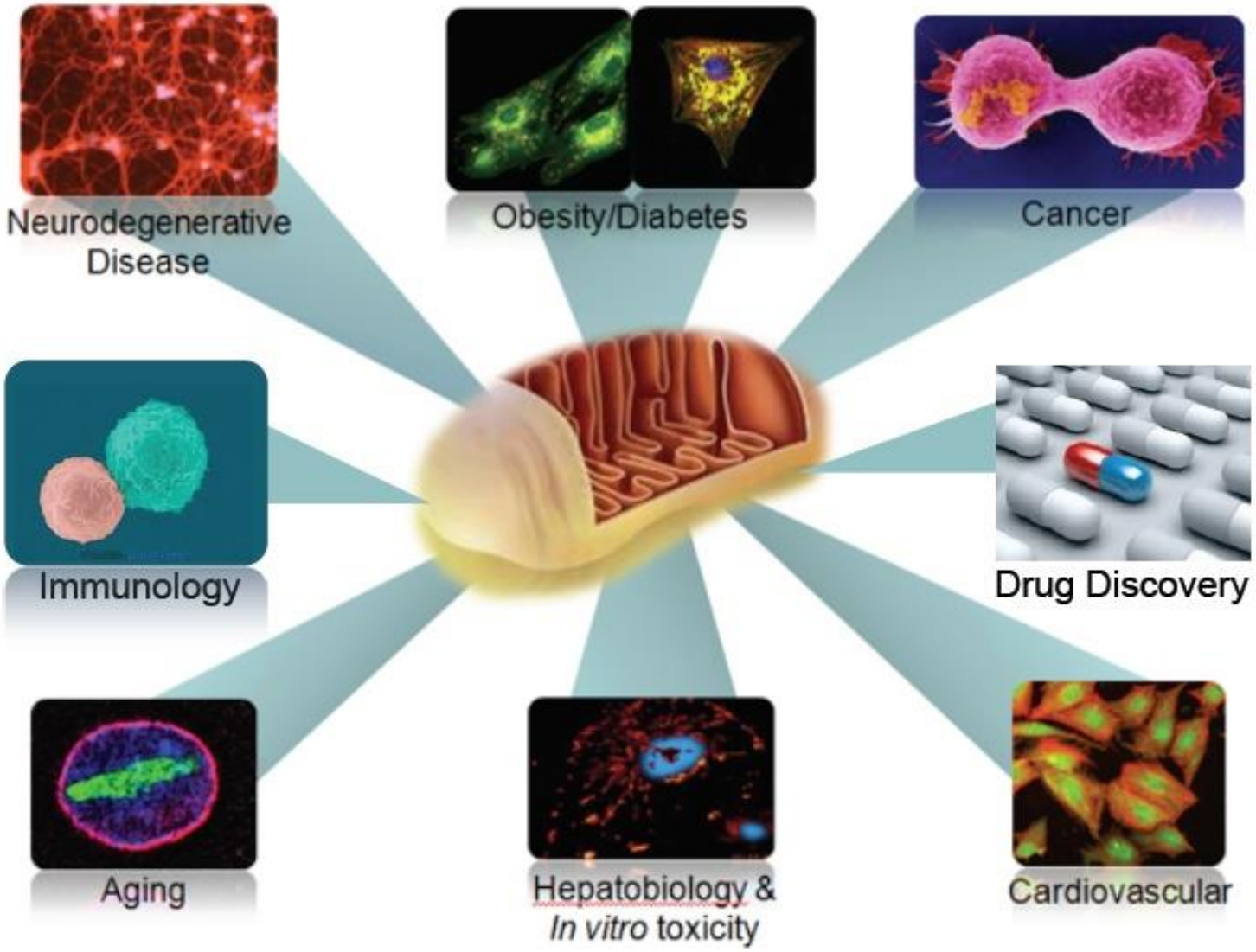
Seahorse Bioscience
A part of Agilent Technologies

Seahorse XF Analyzers:
20 Years Enabling Metabolic Measurements



Seahorse Bioscience
A part of Agilent Technologies

The Era of Cellular Bioenergetics



Seahorse Bioscience
A part of Agilent Technologies

The 16th World Congress on Targeting Mitochondria

Day 1 – Workshop

Mitochondrial Function & Mitochondrial Intelligence

Dynamics • AI
Resilience • Evaluation
Innovation

Day 2 – Understanding the System

Mitochondria: Energy & Communication Regulators

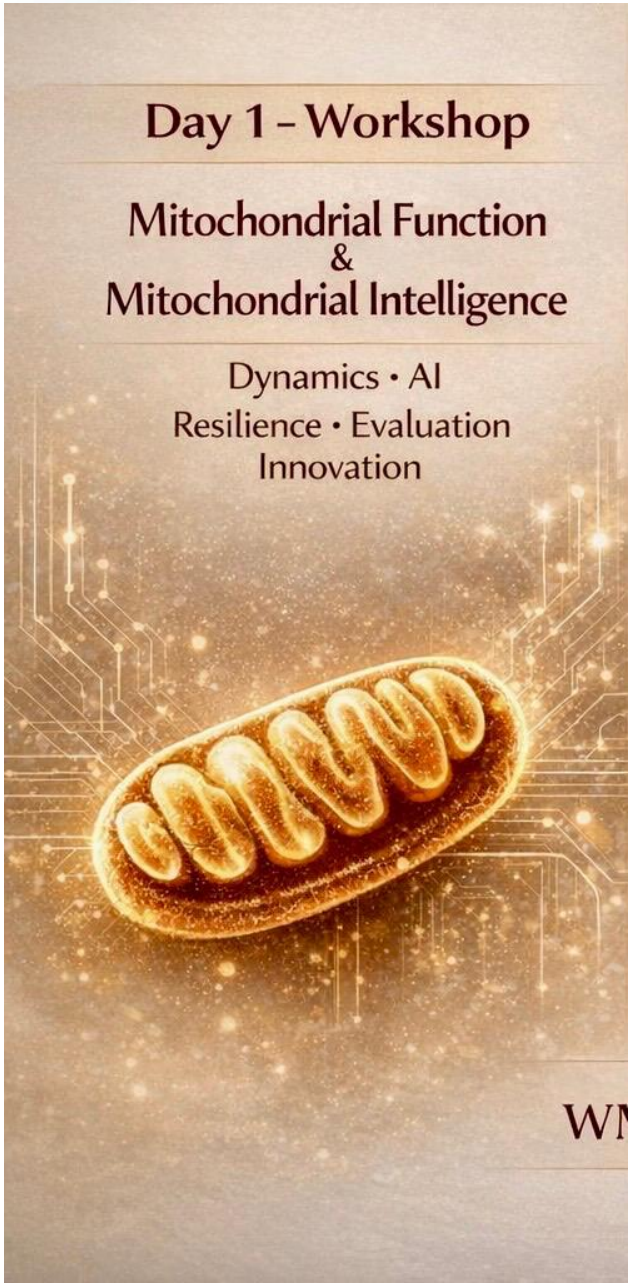
Energy • Systems Biology
Communication • AI Integration

Day 3 – From Knowledge to Action

Health • Prevention • Therapy • Longevity

Translation • Resilience • Intervention
Future Medicine

WMS 2026 Agenda at a Glance

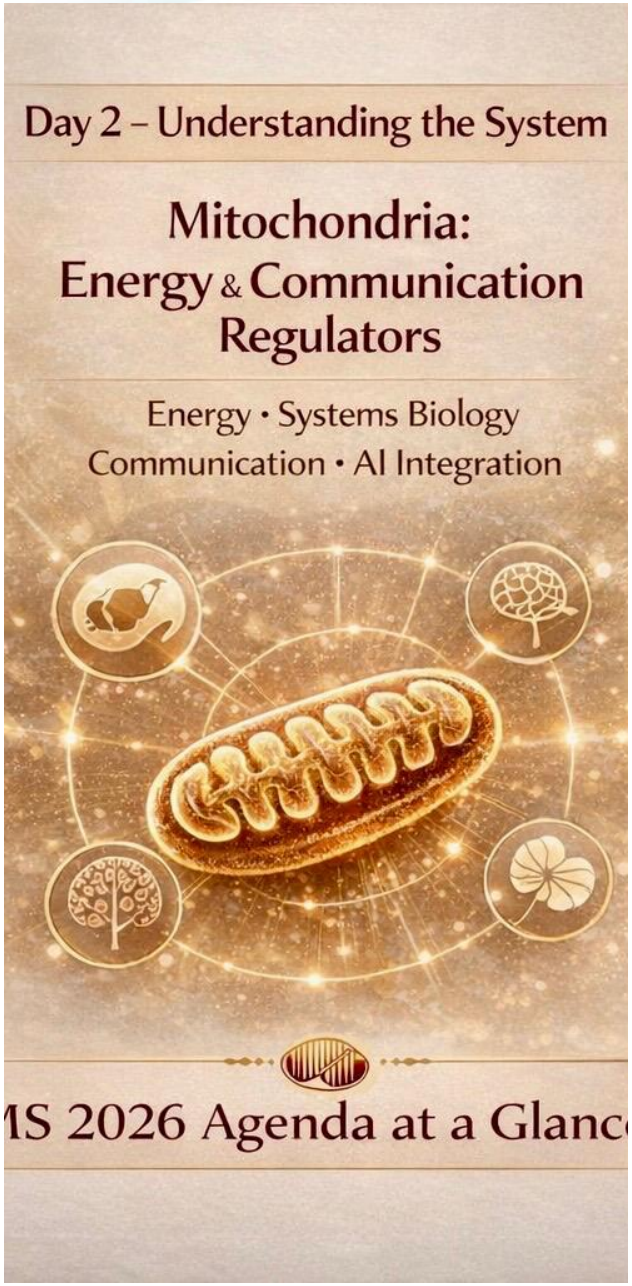


- **細胞分型與品管：**
通過 Seahorse 建立「代謝指紋圖譜」。不同的細胞具有不同的代謝特徵，可作為有效品管與細胞特性監控工具。

- **健康監控：** 作為一種非侵入性指標，透過分析 PBMC 的粒線體健康指數 (Mitochondrial Health Index, MHI)，來監測疾病進程或治療反應。

- **AI 藥物篩選：** 預測粒線體與疾病的關係，縮短粒線體藥物（Mito-targeted therapeutics）的開發週期。

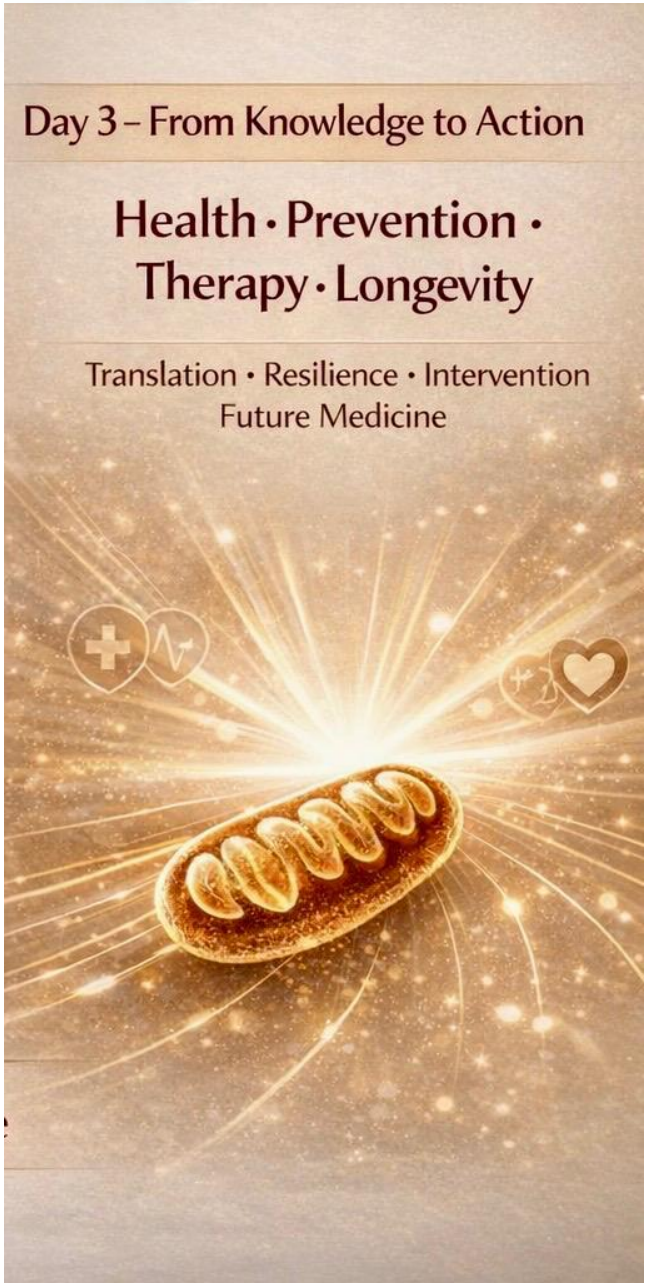
Seahorse Bioscience
A part of Agilent Technologies



- **Mito-EVs 載體技術：**
聚焦「粒線體外囊泡 (Extracellular Vesicles)」作為生物載體，實現跨細胞、跨器官的修復訊號傳遞，且可有效穿過腦血屏障。

- **精準修復：** 透過「粒線體移植與轉移 (Mitochondrial Transplantation)」技術，將健康的粒線體精準遞送至受損的心肌或神經細胞，重建生物能量網絡。

Seahorse Bioscience
A part of Agilent Technologies



- **代謝重塑 (Metabolic Reset)：**
從單純補充劑轉向「精準干預」。例如使用 NAD+ 促進劑 或 尿石素 A (Urolithin A) 來誘導粒線體自噬，提升健康壽命 (Healthspan)。

- **未來醫學實踐：** 臨床試驗進行中的 LHON 遺傳性視神經病變 的基因療法，以及其他針對特定器官（心、腦、肌肉）的靶向修復療法。

Seahorse Bioscience
A part of Agilent Technologies

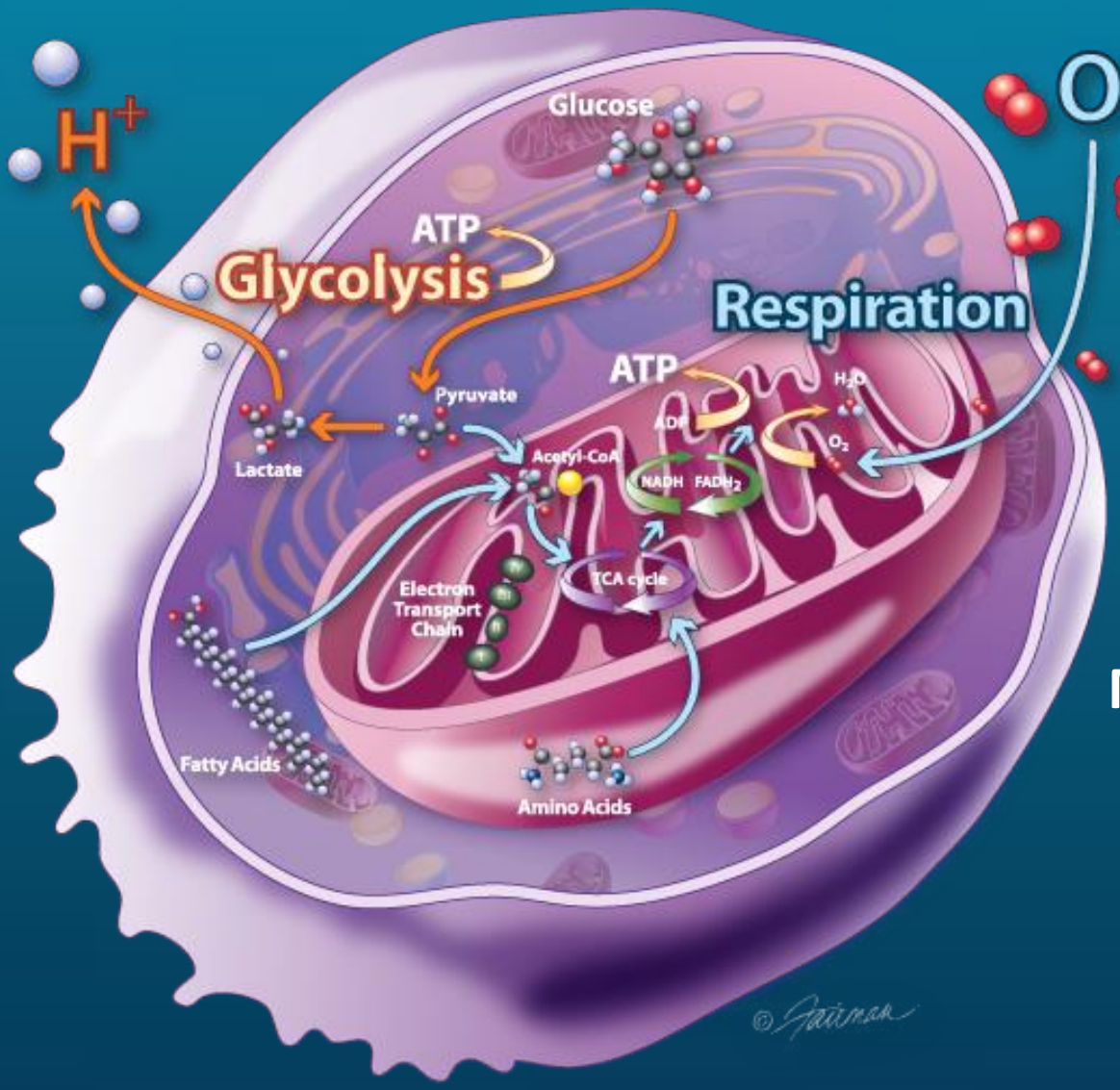

O2
O2
O2
O2
O2
O2
O2
O2
H+
H+
H+
H+
H+

Measure the Two Major Energy Pathways

Extracellular Acidification Rate (ECAR)

=

Glycolysis

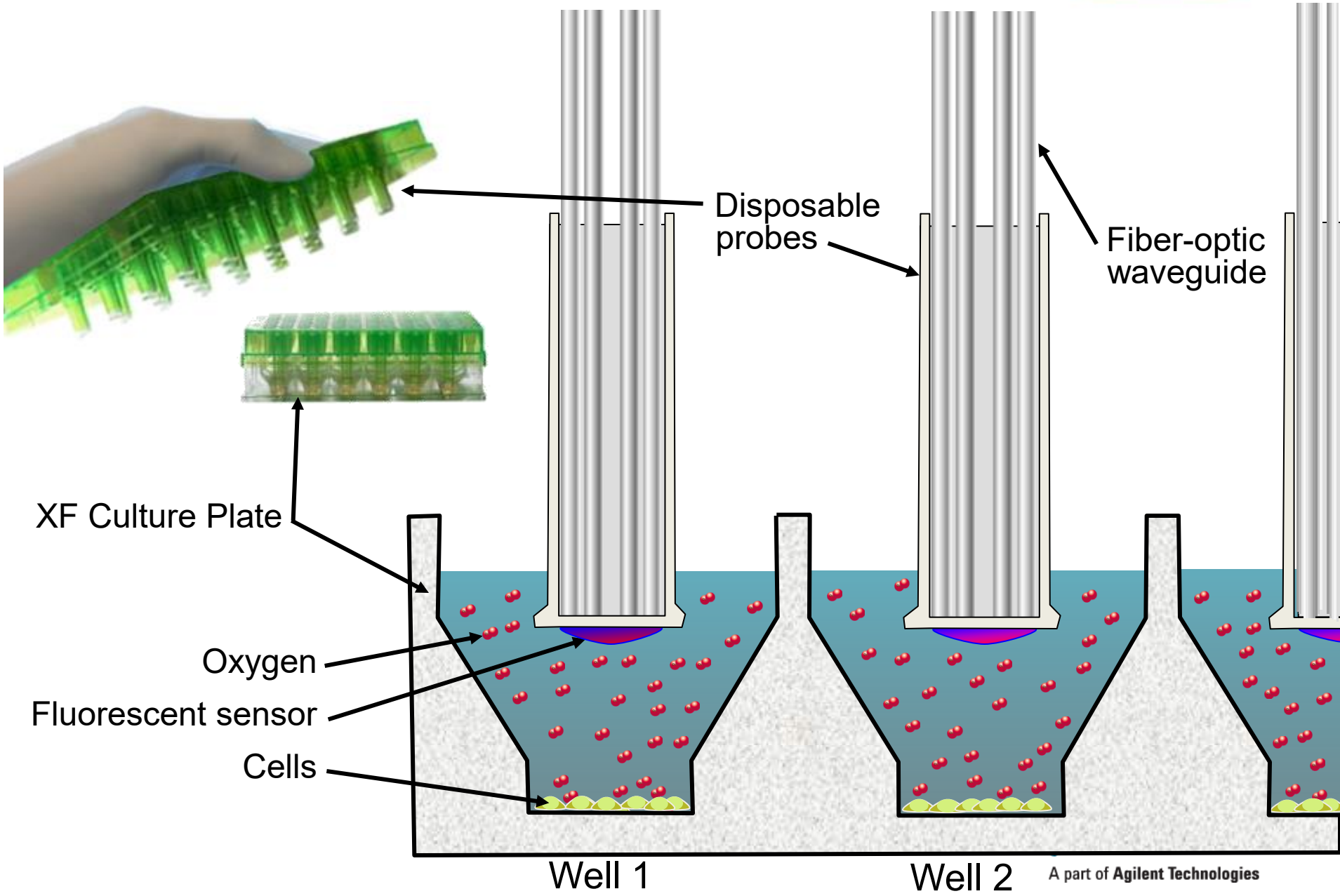


Oxygen Consumption Rate (OCR)

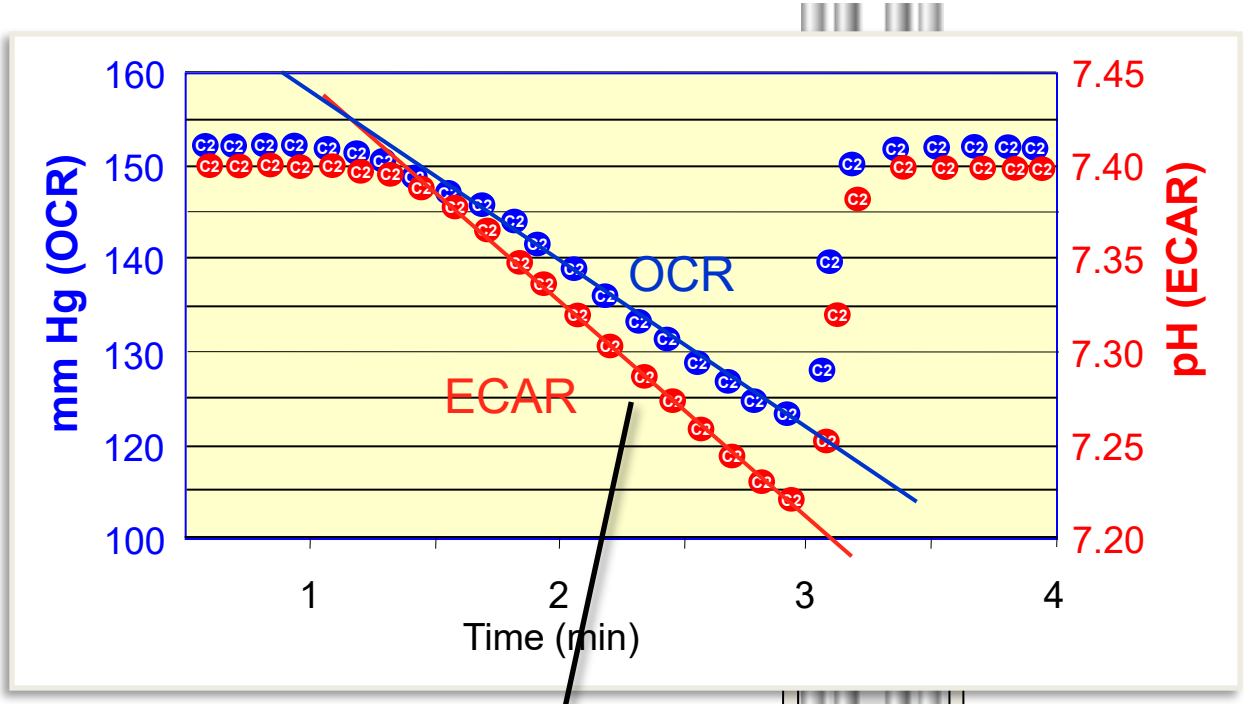
=

Mitochondrial Respiration

Measuring Mitochondrial Respiration and Glycolysis in a Microplate



Measuring Mitochondrial Respiration and Glycolysis in a Microplate



The rate is calculated from the slope

The bio-cartridge is

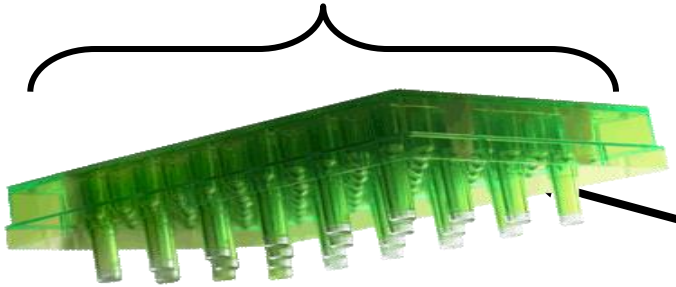
A temporary micro-chamber is formed

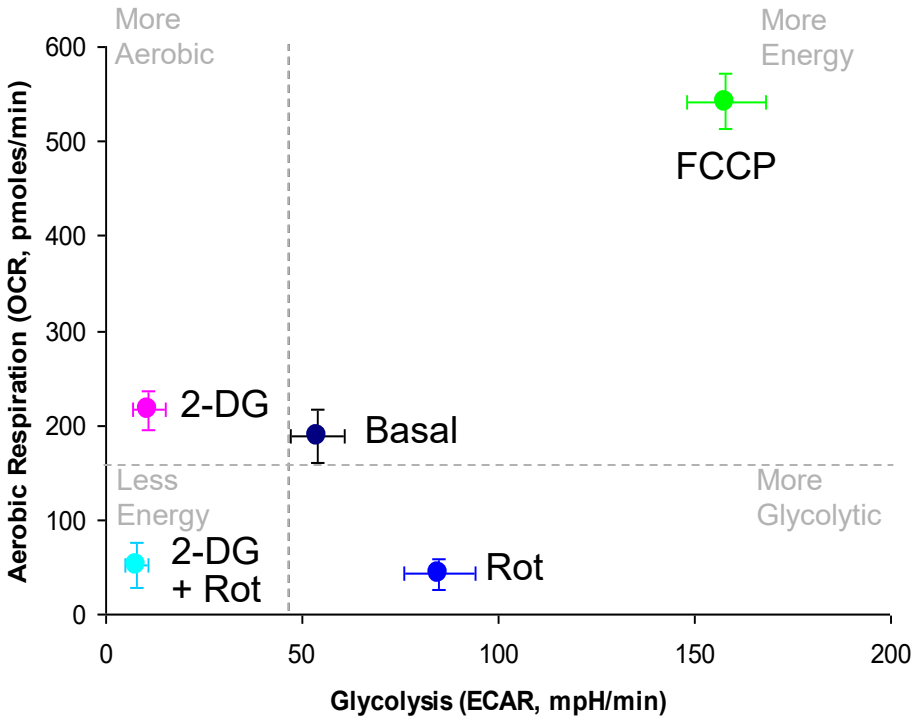
Well 1

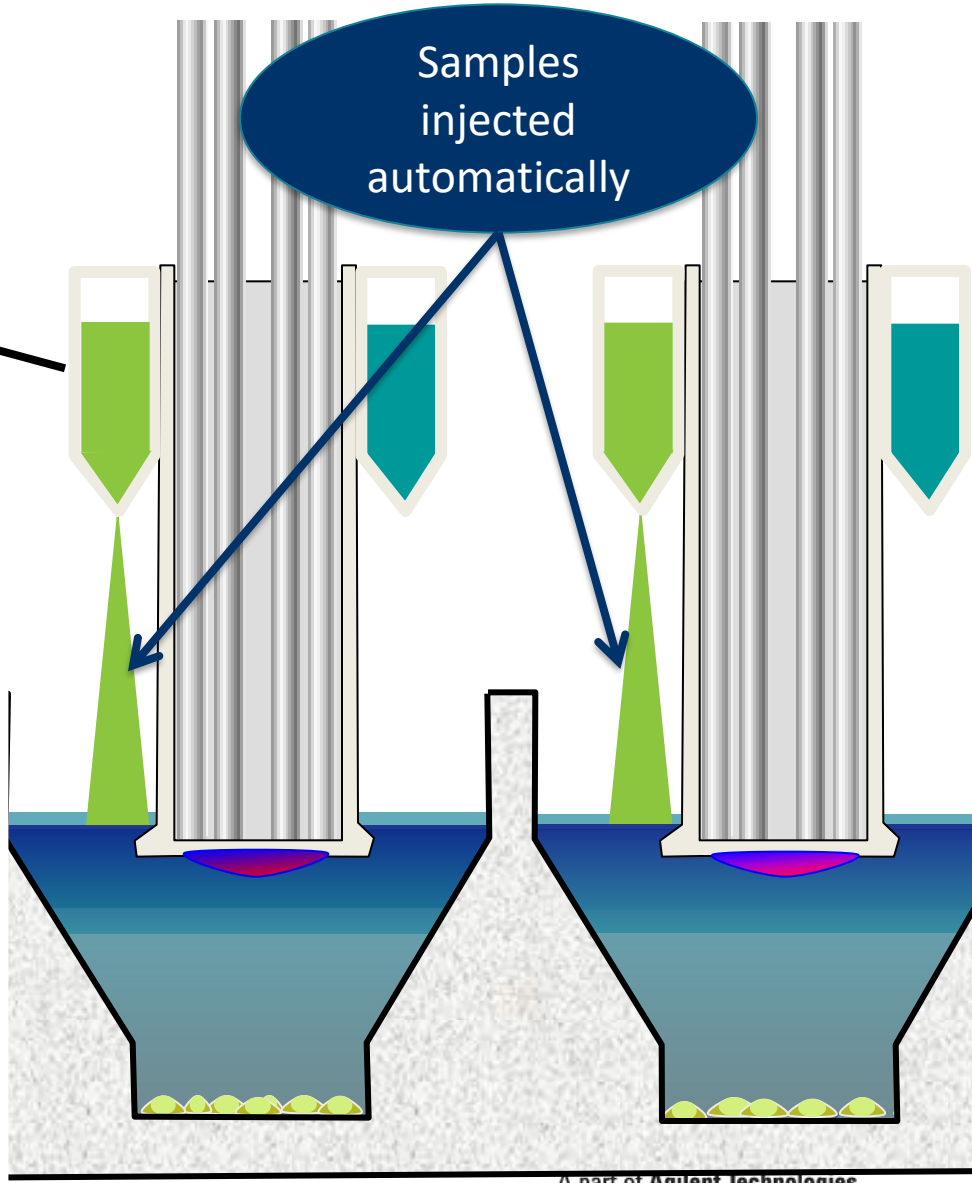
Well 2

Automated Drug Injection Ports Enable Kinetic, Functional Data

4 Injection Ports/Well

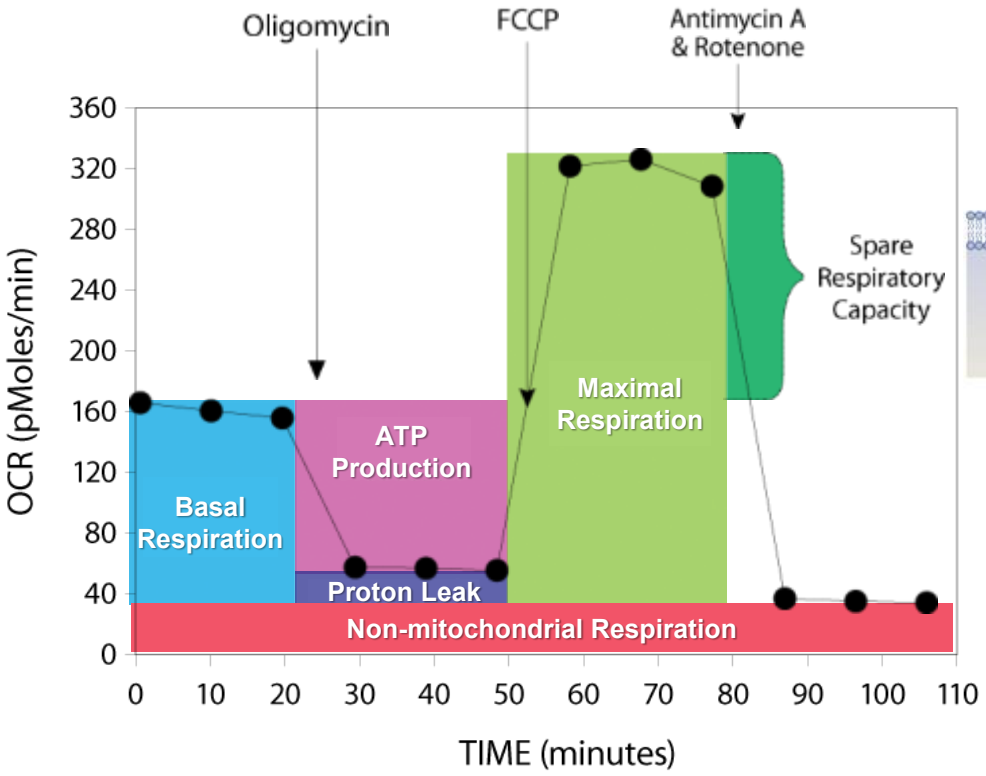




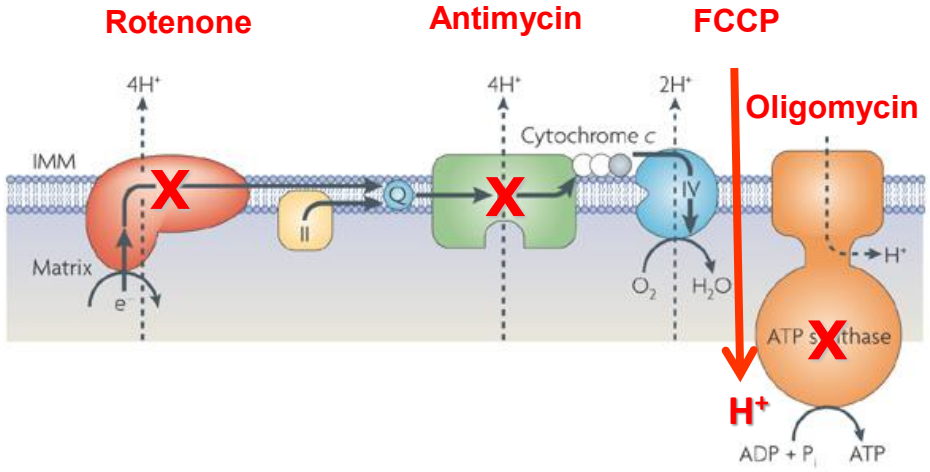


XF CELL MITO STRESS TEST

The Gold Standard That Makes You a Bioenergetics Expert



Mito Stress Test

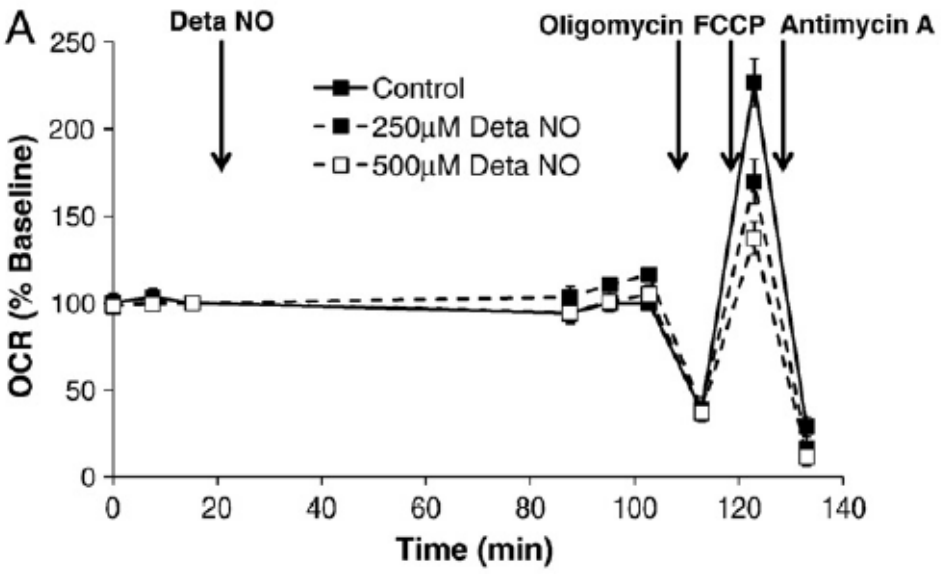


Seahorse Bioscience

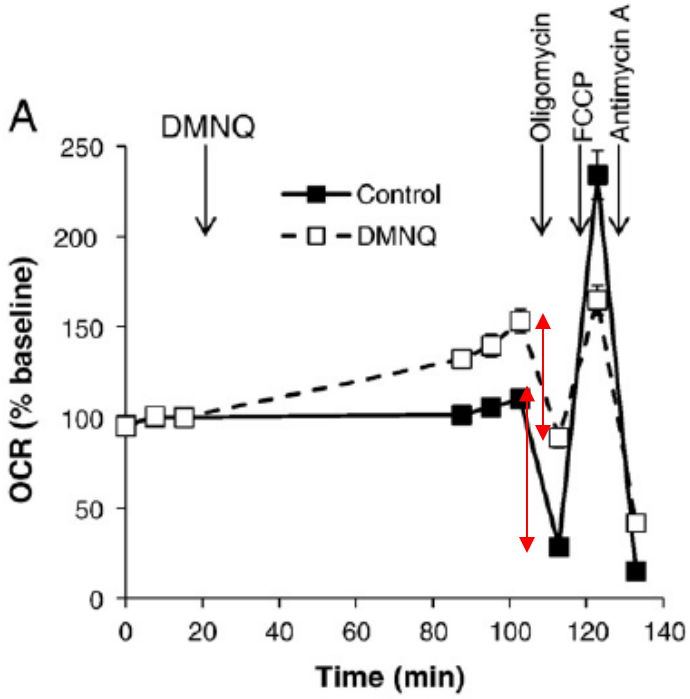
A part of Agilent Technologies

Mitochondrial reserve capacity in endothelial cells in responding to oxidative stress

BACE : bovine aortic endothelial cells



Deta NONOate：nitric oxide (NO) donor



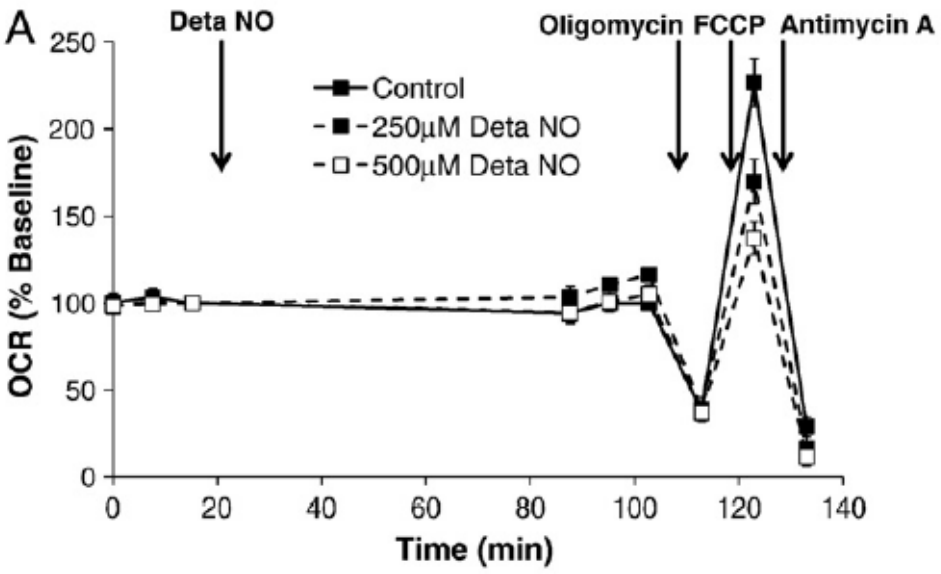
DMNQ： 15 uM
reactive oxygen species (ROS) donor

Mitochondrial reserve capacity in endothelial cells: The impact of nitric oxide and reactive oxygen species
Free Radic Biol Med; 2010 Apr 1. 48(7):905-914.

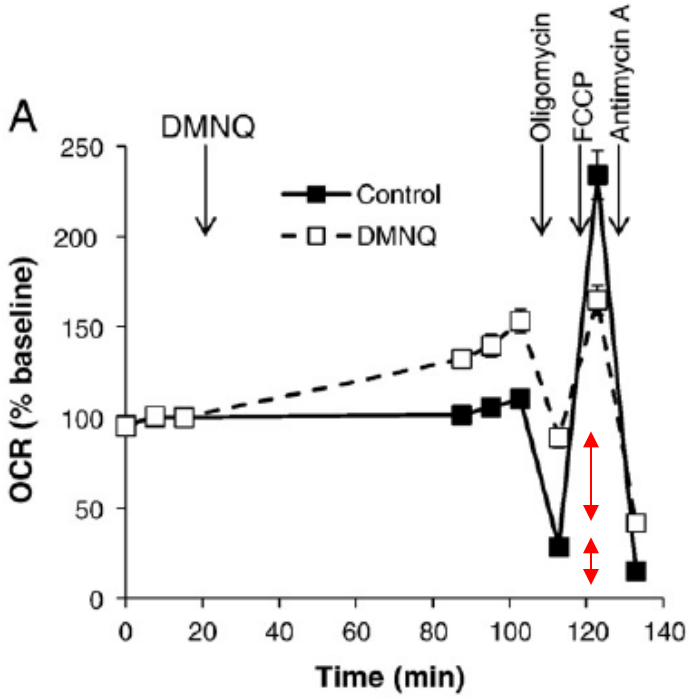
Seahorse Bioscience
A part of Agilent Technologies

Mitochondrial reserve capacity in endothelial cells in responding to oxidative stress

BACE : bovine aortic endothelial cells



Deta NONOate：nitric oxide (NO) donor



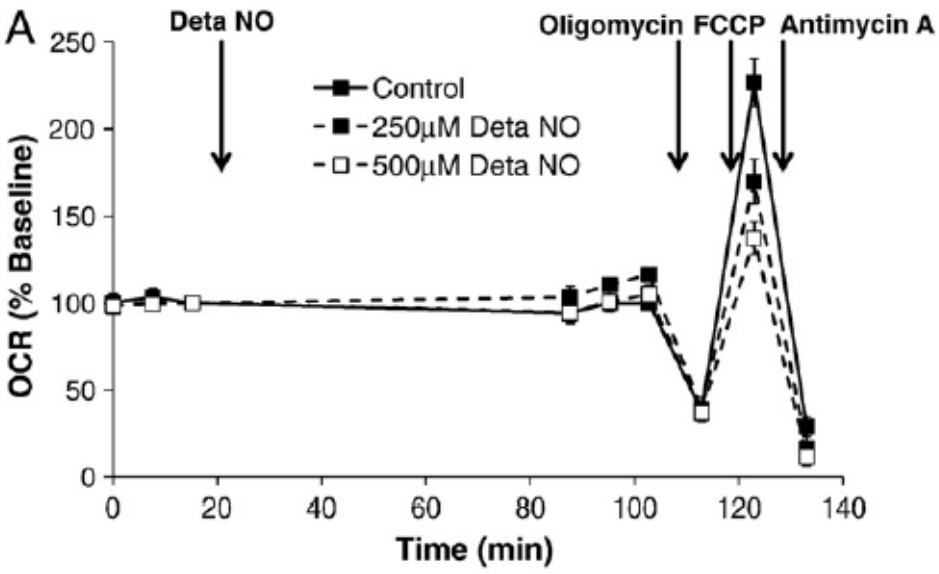
DMNQ： 15 uM
reactive oxygen species (ROS) donor

Mitochondrial reserve capacity in endothelial cells: The impact of nitric oxide and reactive oxygen species
Free Radic Biol Med; 2010 Apr 1. 48(7):905-914.

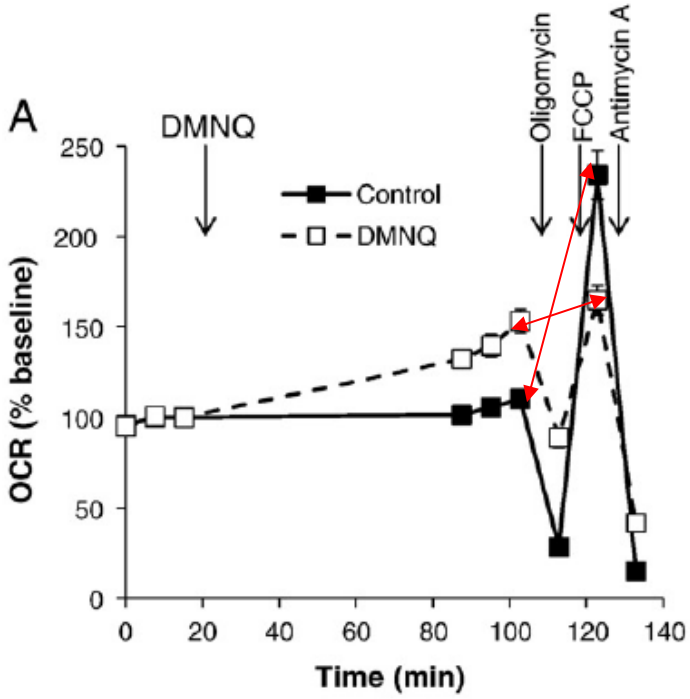
Seahorse Bioscience
A part of Agilent Technologies

Mitochondrial reserve capacity in endothelial cells in responding to oxidative stress

BACE : bovine aortic endothelial cells



Deta NONOate：nitric oxide (NO) donor

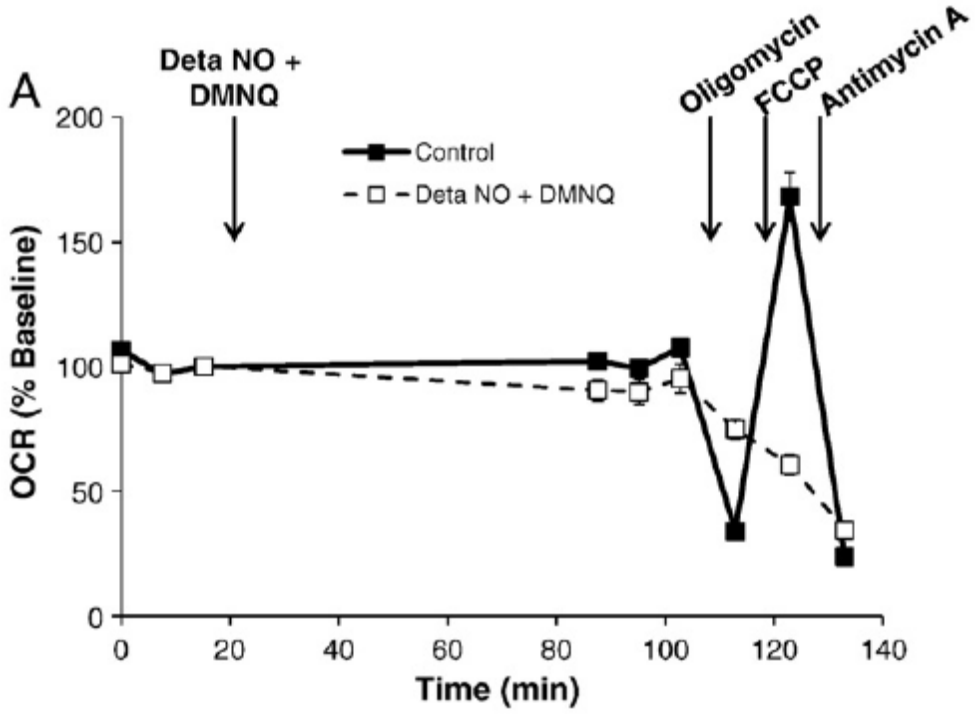


DMNQ： 15 uM
reactive oxygen species (ROS) donor

Mitochondrial reserve capacity in endothelial cells: The impact of nitric oxide and reactive oxygen species
Free Radic Biol Med; 2010 Apr 1. 48(7):905-914.

Seahorse Bioscience
A part of Agilent Technologies

Mitochondrial reserve capacity in endothelial cells in responding to oxidative stress



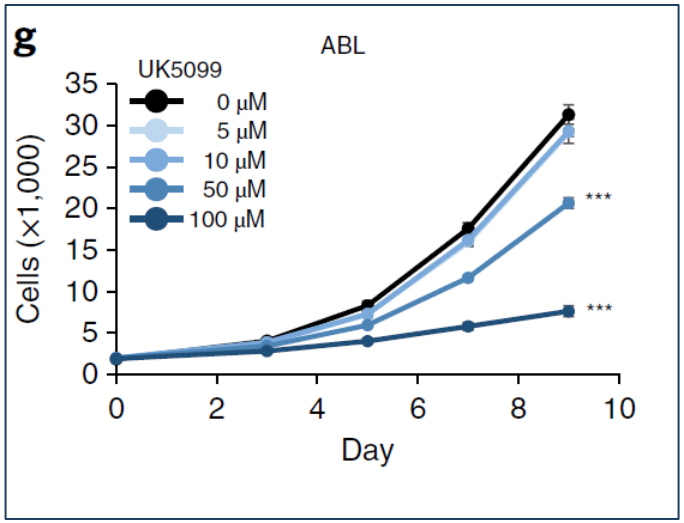
Deta NONOate：250 uM

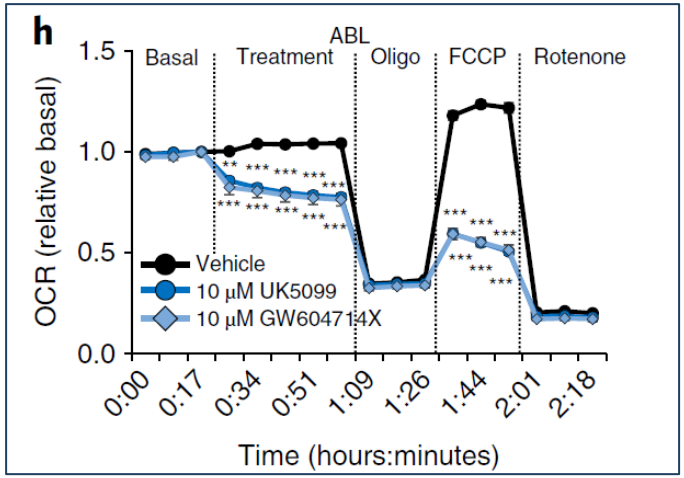
DMNQ： 15 uM

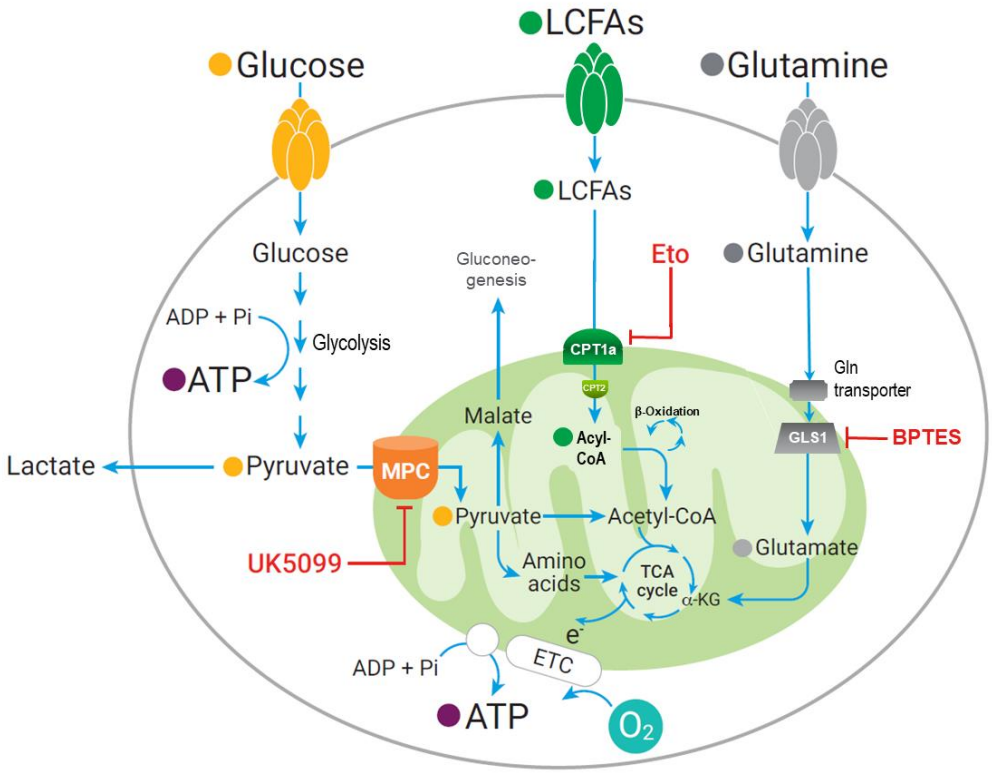
Mitochondrial reserve capacity in endothelial cells: The impact of nitric oxide and reactive oxygen species
Free Radic Biol Med; 2010 Apr 1. 48(7):905-914.

Seahorse Bioscience
A part of Agilent Technologies

Substrate Oxidation Stress Test







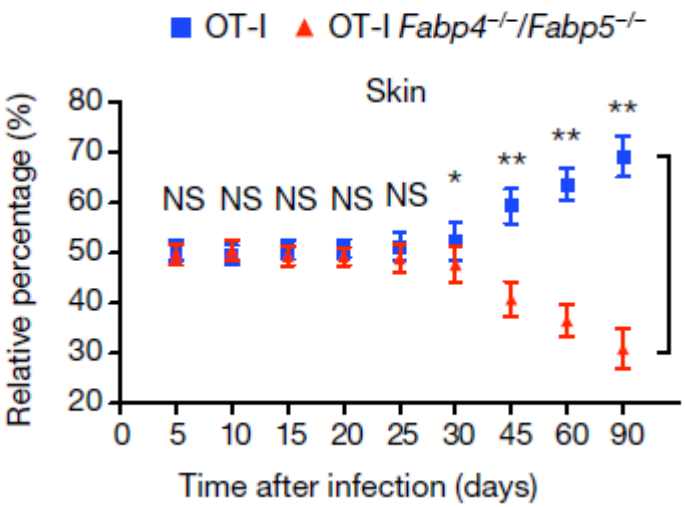
Androgen receptor (AR)-driven growth in prostate adenocarcinoma (PCa)

The MPC as a tractable therapeutic target in AR-driven prostate tumors.

Nat Metab. 2019 January ; 1(1): 70–85.
doi:10.1038/s42255-018-0002-y.

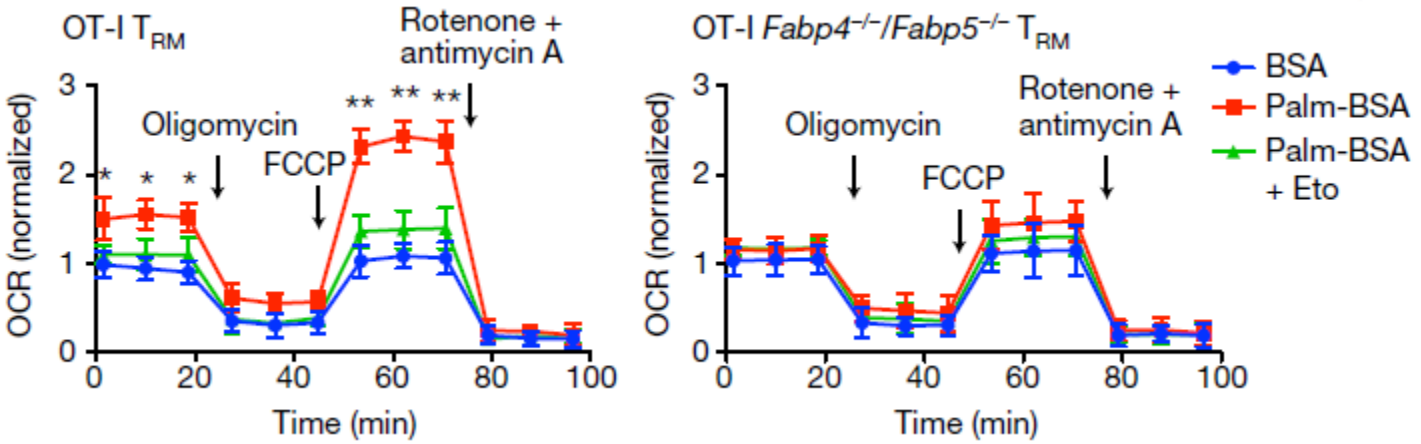
Seahorse Bioscience
A part of Agilent Technologies

Loss of *Fabp4 and Fabp5 decreases fatty-acid uptake* and metabolism by CD8+ TRM cells and impairs their long-term maintenance



***Fabp*:** Fatty-acid-binding proteins
RM: Resident Memory

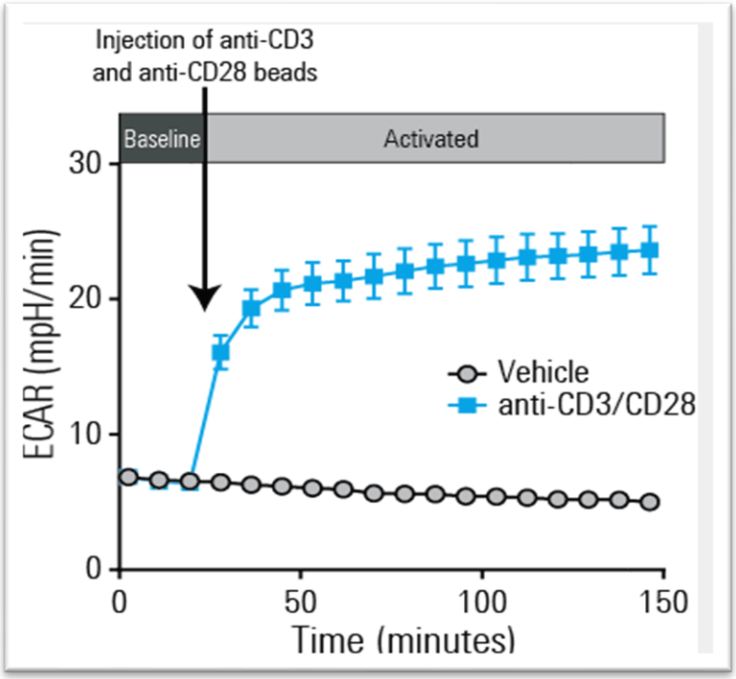
Freshly isolated and sorted T cells (2.5 × 10^5) were incubated for 30 min with fatty-acid oxidation assay medium. When required, cells were pre-treated with etomoxir for 15 min. Afterwards, BSA or palmitate-BSA was added to the medium, and the OCR was measured.

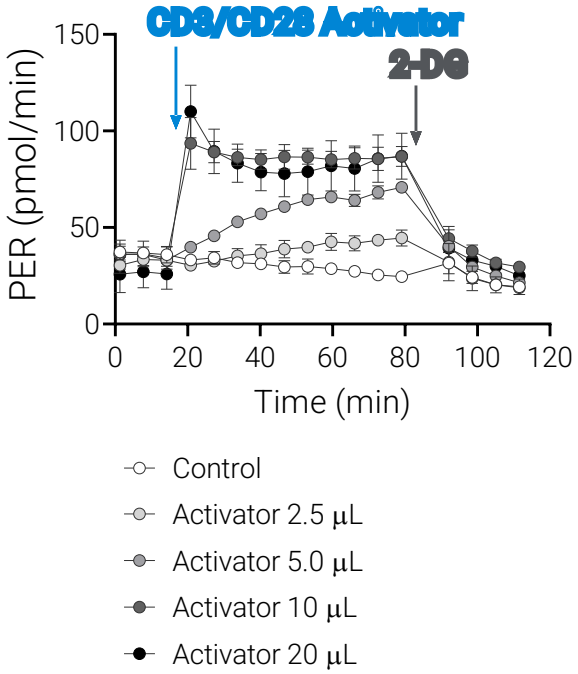


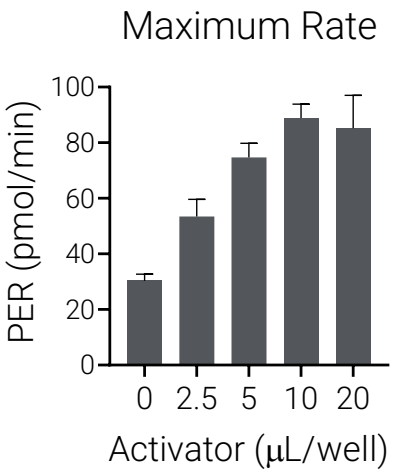
Survival of tissue-resident memory T cells requires exogenous lipid uptake and metabolism
Nature. 2017 Mar 09, 543 (7644):252-256.

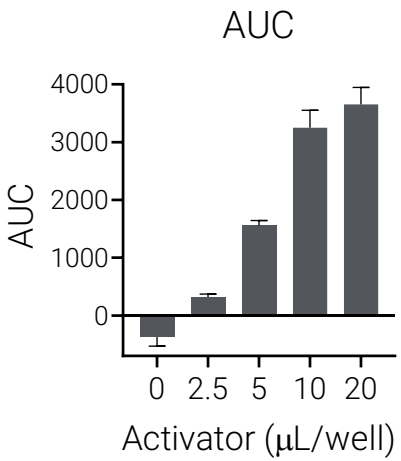
Seahorse Bioscience
A part of Agilent Technologies

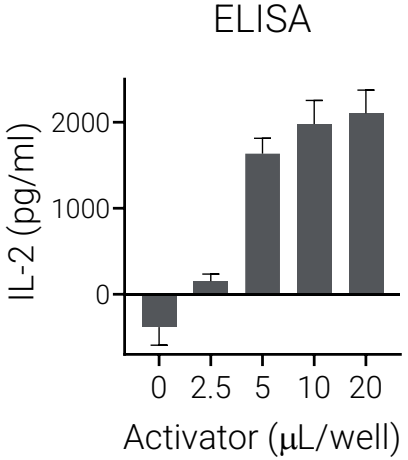
Hu T Cell Activation Assay





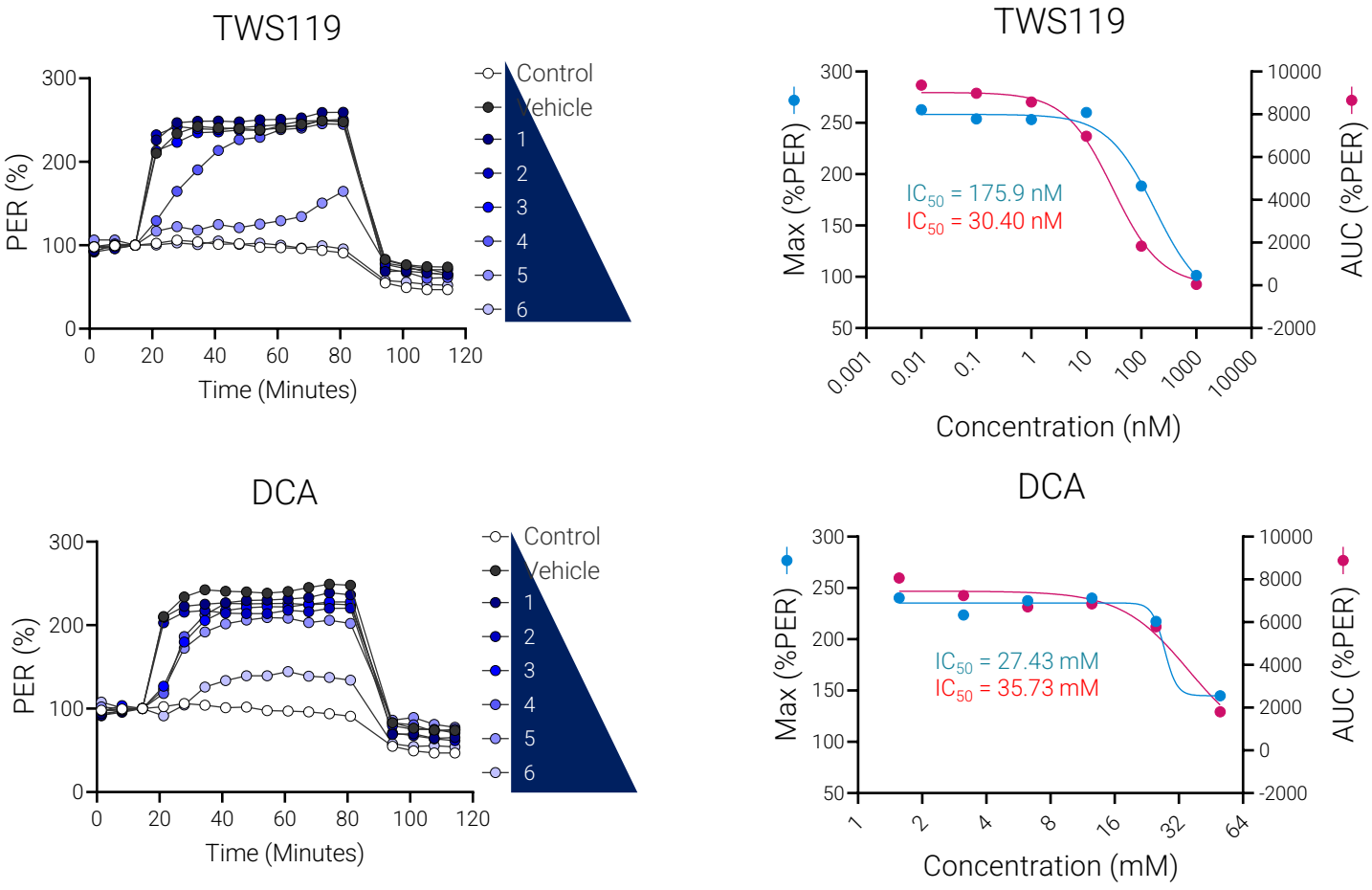






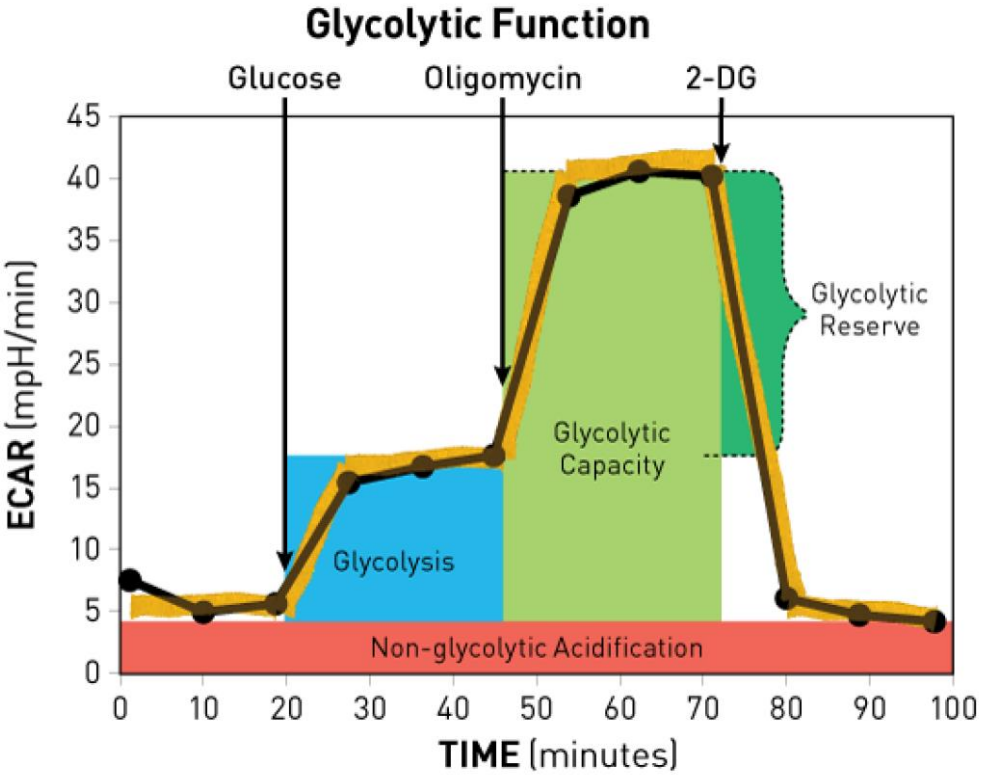
Bioscience
chnologies

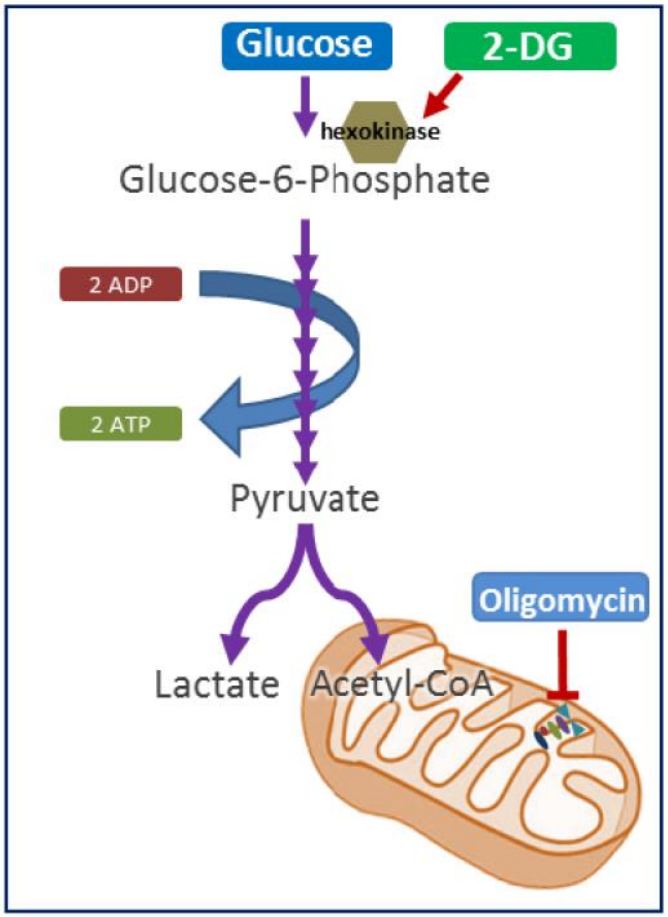
Effective immune modulator dosing can be measured by using metrics calculated from T cell activation kinetic data



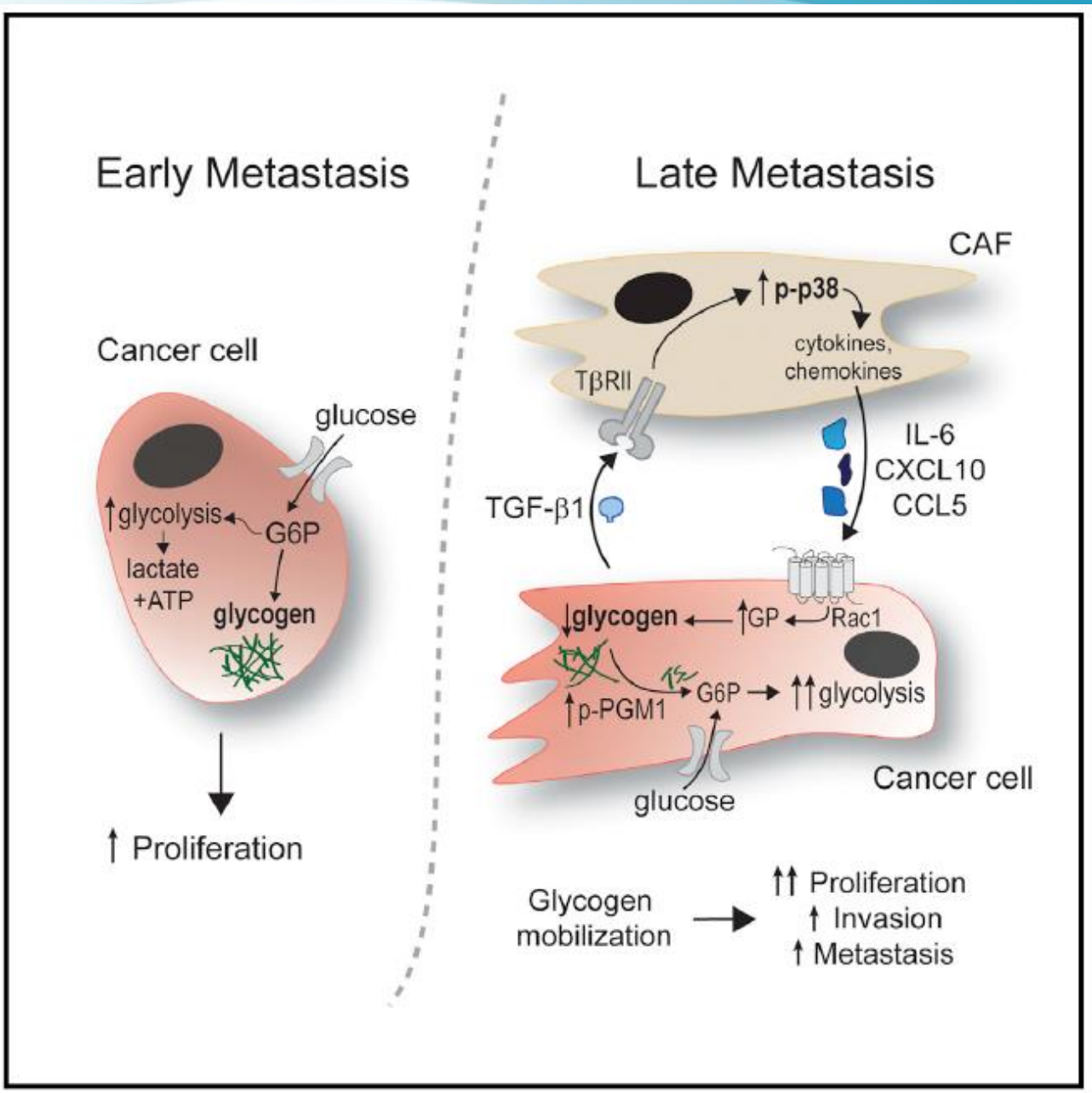
Modulator efficacy test using XF Hu T Cell Activation Assay Kit. Pre-activated CD4+ T cells were pretreated with different inhibitors at various concentrations for 30 min prior to the T Cell Activation Assay. The kinetic graphs detected dose-dependent suppression of PER increase induced by CD3/CD28 Activator showing kinetic response variation depending on the inhibitor type.

Glycolysis Stress Test Kit





Seahorse Bioscience
A part of Agilent Technologies



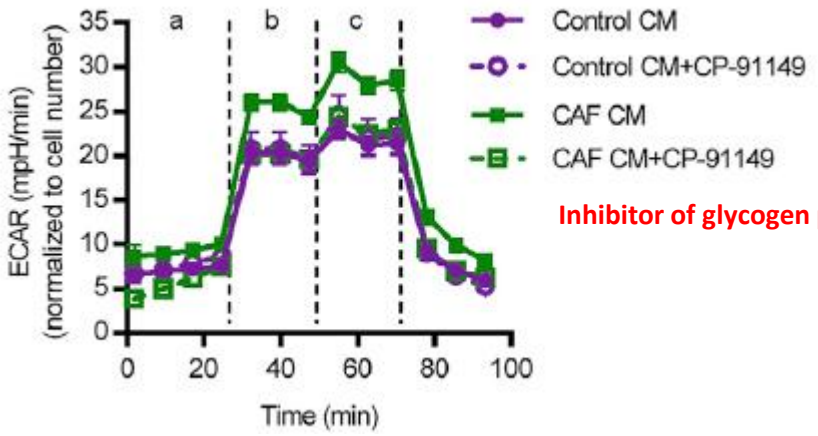
Cancer-Associated Fibroblasts

ovarian cancer cells

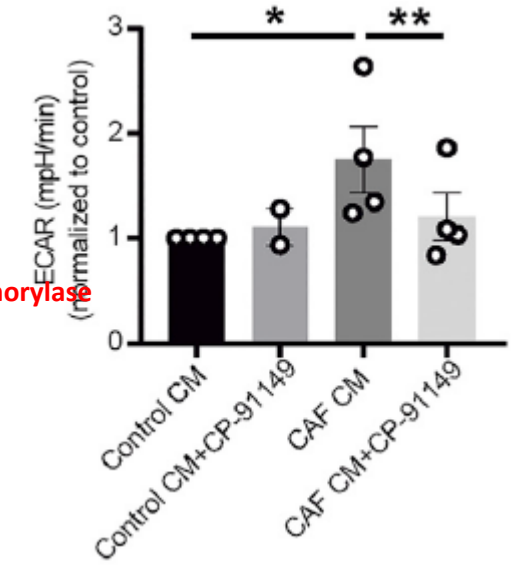
Fibroblasts Mobilize Tumor Cell Glycogen to Promote Proliferation and Metastasis
Cell Metab. 2018 Aug 28

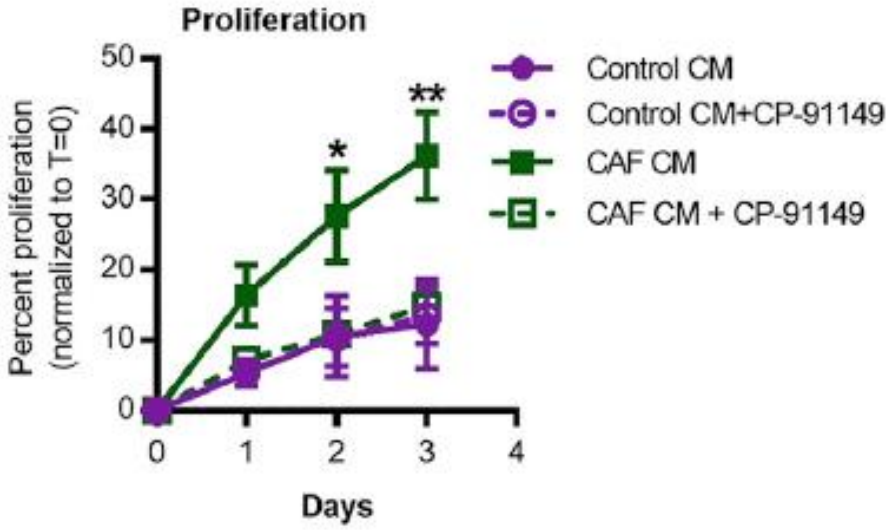
Glycogen Fuels Glycolysis to Promote Proliferation and Invasion

Control CM was generated by incubating DMEM supplemented with pen/strep and 0.1% BSA for 72hr in a dish without cells.

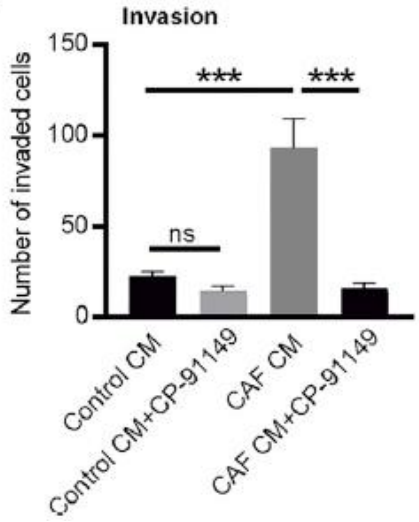


Inhibitor of glycogen phosphorylase





F

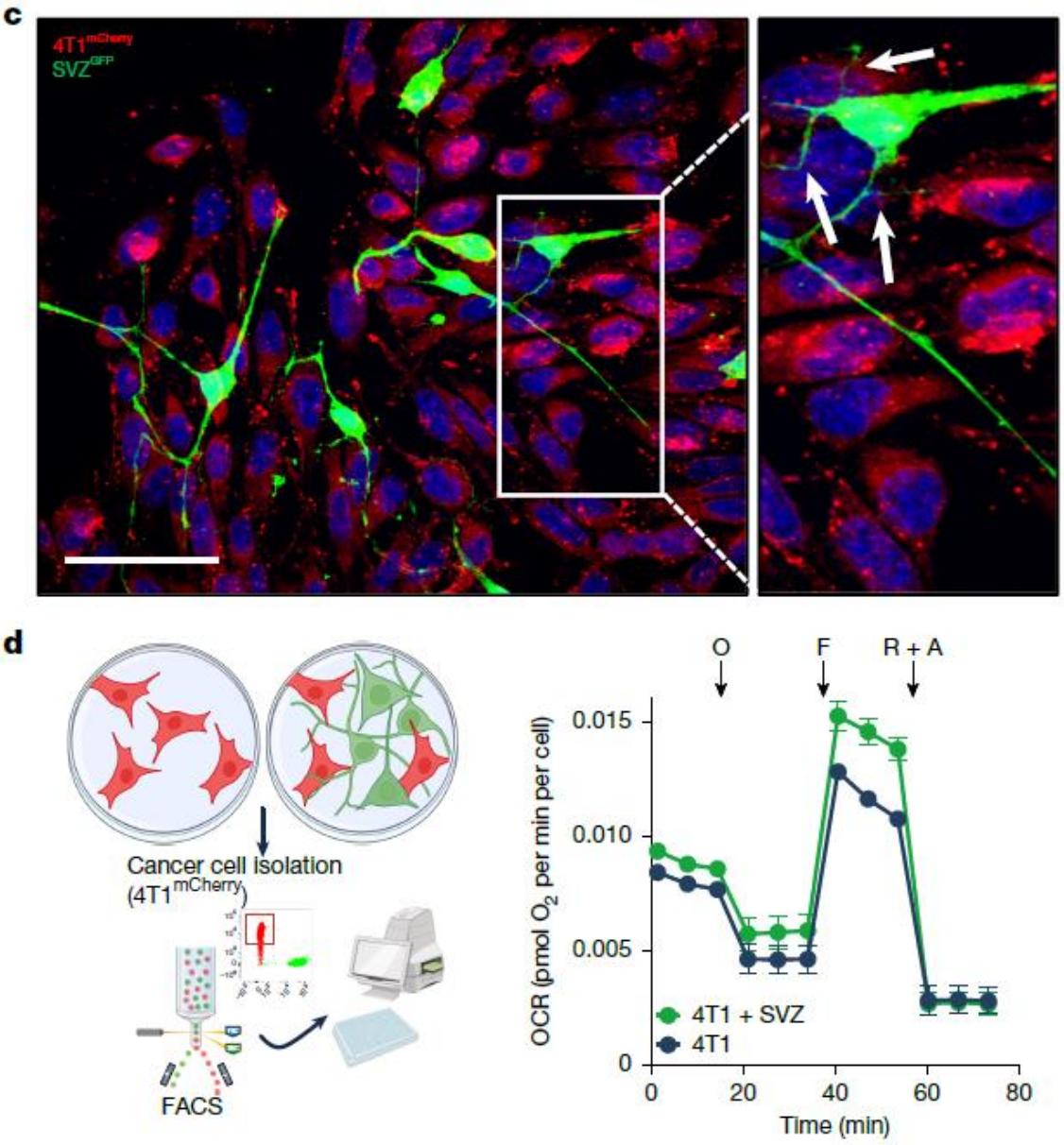


Fibroblasts Mobilize Tumor Cell Glycogen to Promote Proliferation and Metastasis
Cell Metab. 2018 Aug 28

Seahorse Bioscience
A part of Agilent Technologies

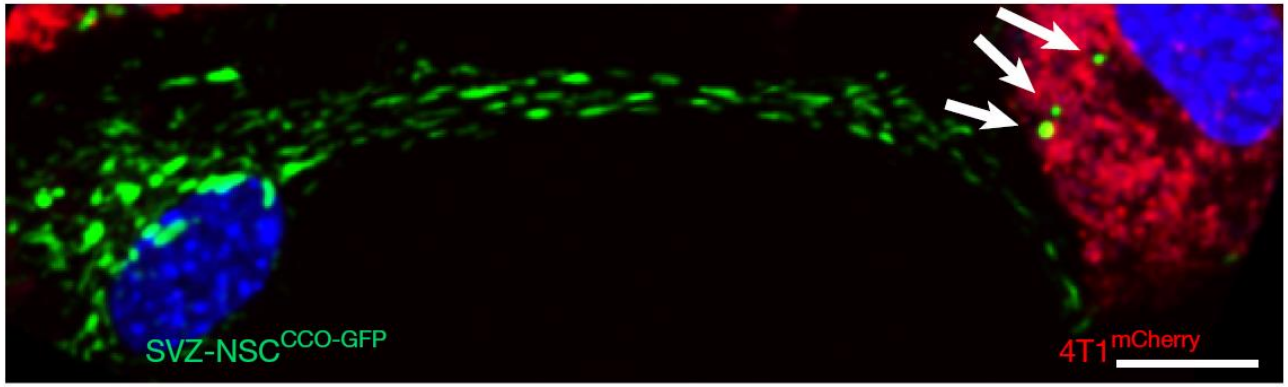
Nerve-to-cancer transfer of mitochondria during cancer metastasis

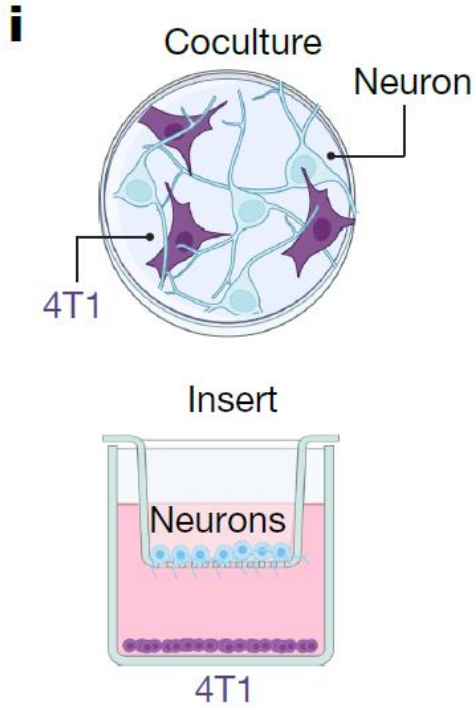
Nature **volume 644**, pages252–262 (2025)

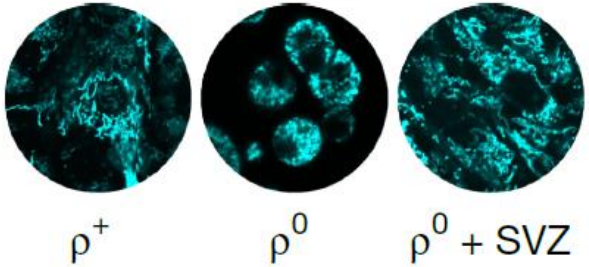


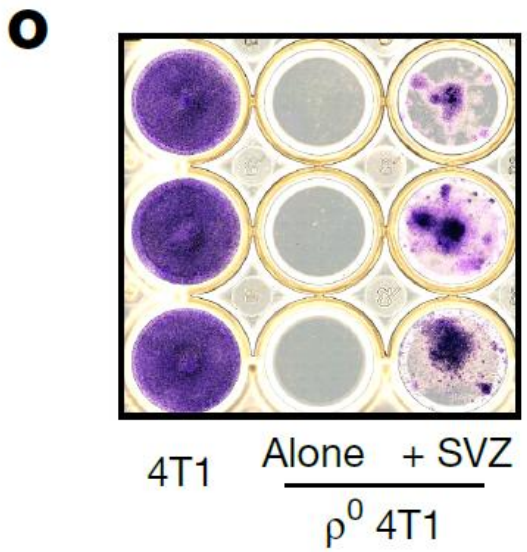
Nerve-to-cancer transfer of mitochondria during cancer metastasis

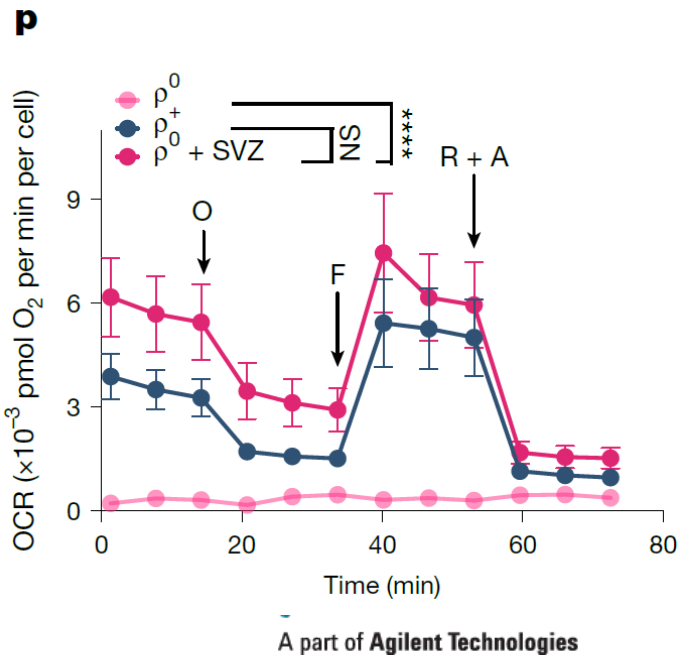
Nature **volume 644**, pages252–262 (2025)







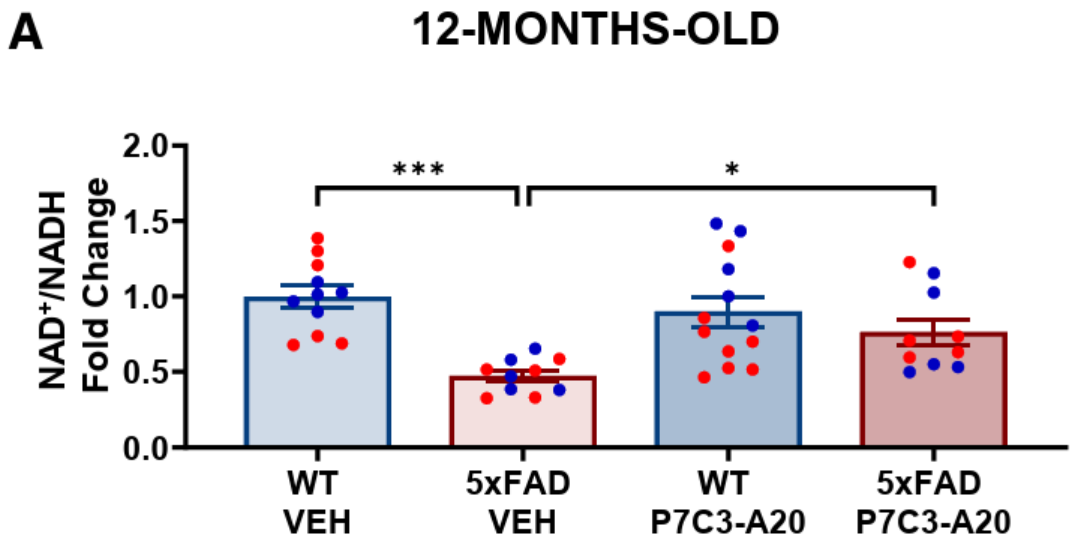


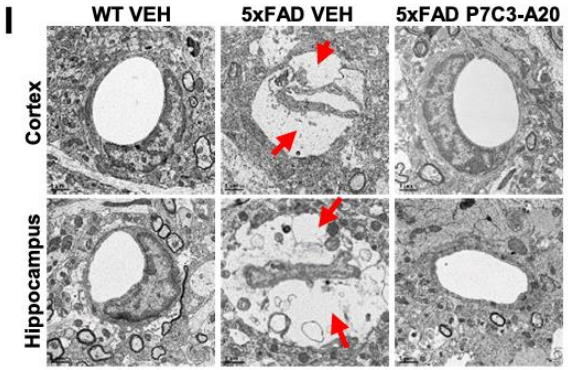


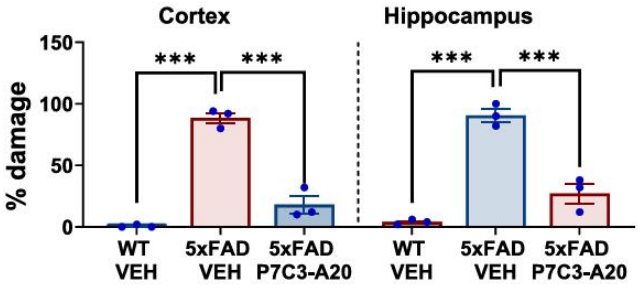
Cell Reports
Medicine

Article

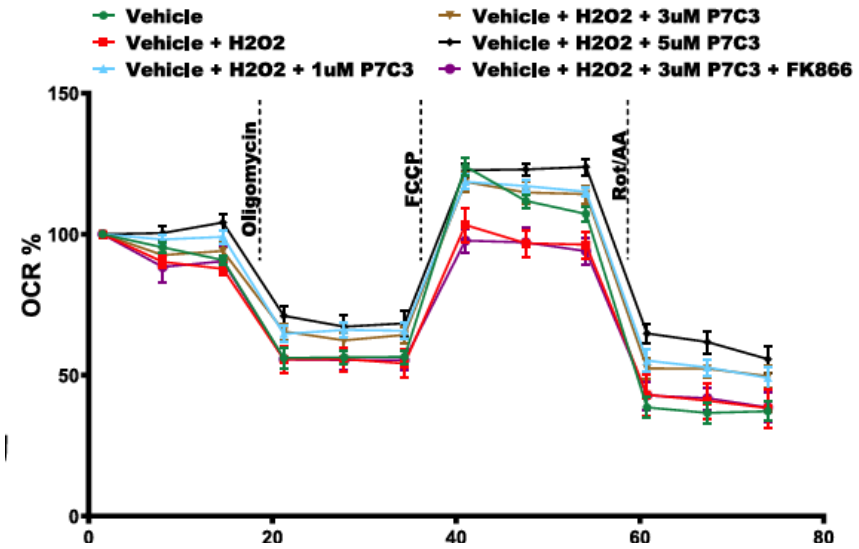
Pharmacologic reversal of advanced Alzheimer's disease in mice and identification of potential therapeutic nodes in human brain

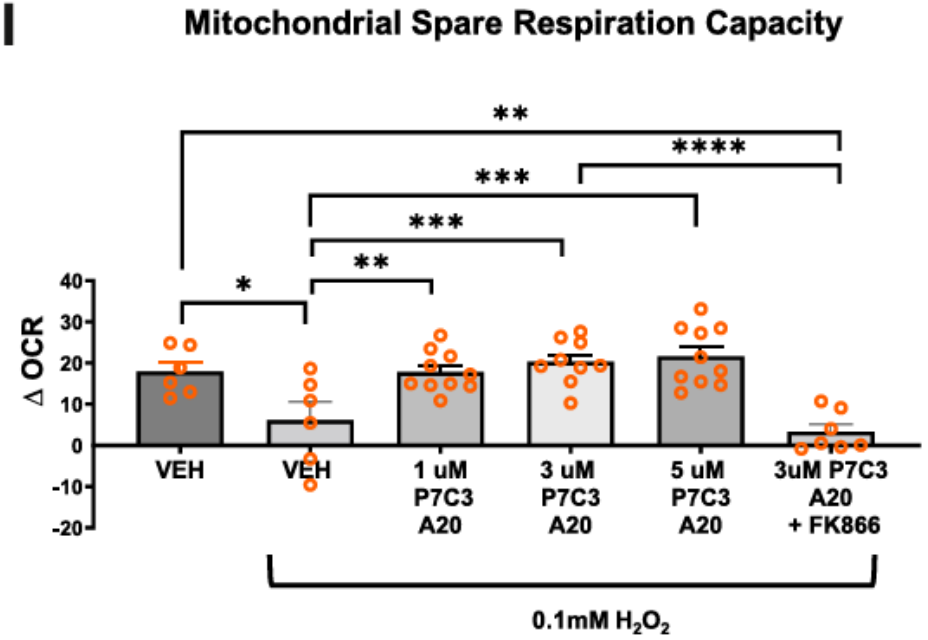






Human Brain Microvascular Endothelial Cells (HBMVEC)



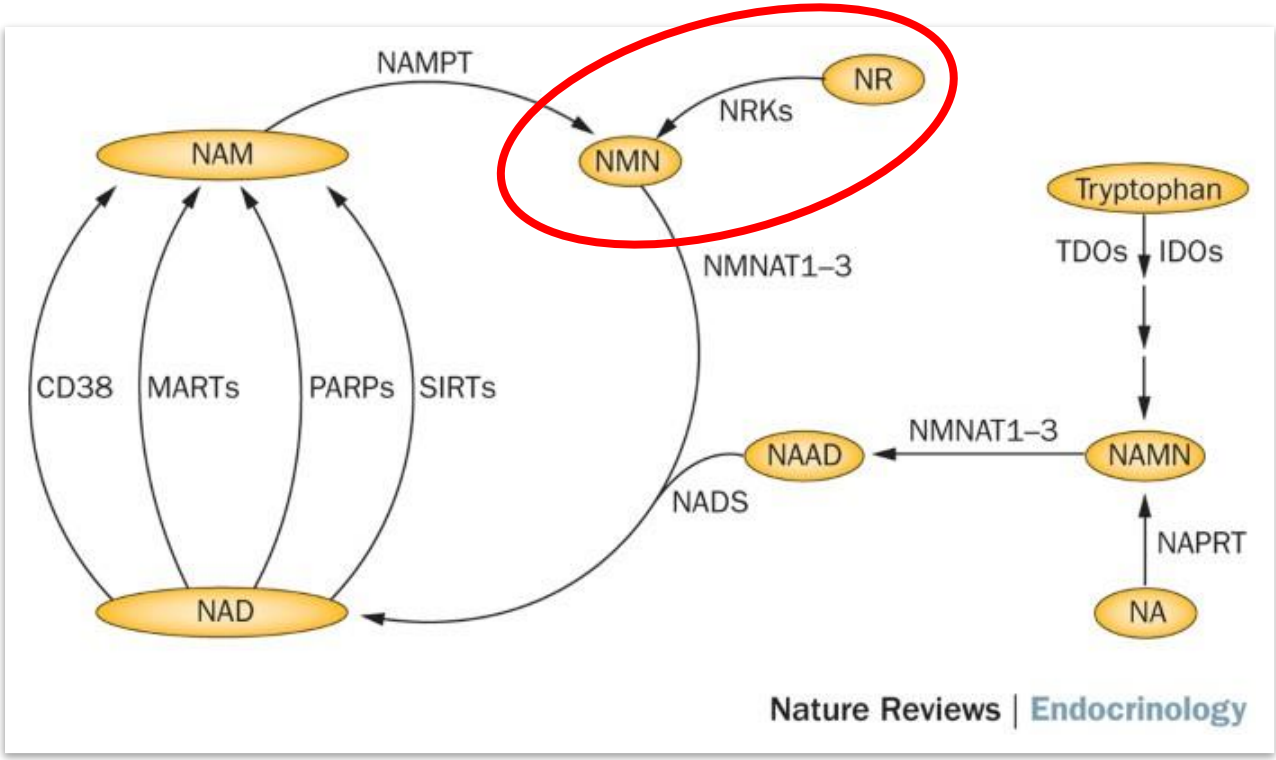


Review Article | Published: 28 July 2015

Physiological and pathophysiological roles of NAMPT and NAD metabolism

Antje Garten, Susanne Schuster, Melanie Penke, Theresa Gorski, Tommaso de Giorgis & Wieland Kiess

Nature Reviews Endocrinology **11**, 535–546 (2015) | Cite this article



NAD+ (Nicotinamide Adenine Dinucleotide)

NAM (Nicotinamide)

NMN (Nicotinamide Mononucleotide)

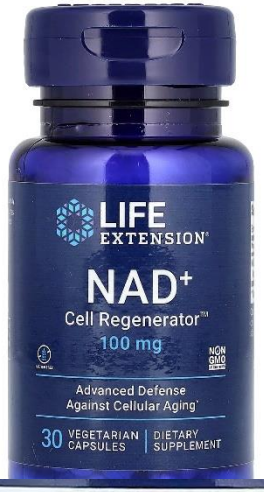
Seahorse Bioscience
A part of Agilent Technologies

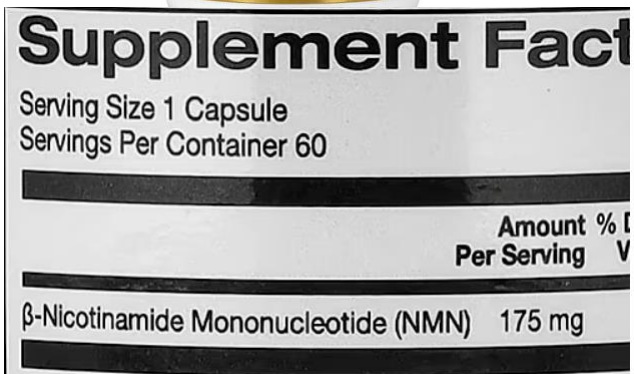
NAM (原料) $\xrightarrow{\text{NAMPT (關鍵機器)}}$ NMN (半成品) $\xrightarrow{\text{NMNAT (最後加工)}}$ NAD+ (能量貨幣)

1. **循環：** 細胞不斷消耗 **NAD+**，消耗後變回 **NAM**。
2. **補救：** **NAMPT** 把 **NAM** 抓回來，加工成 **NMN**。
3. **再生：** **NMN** 再度變回 **NAD+**。

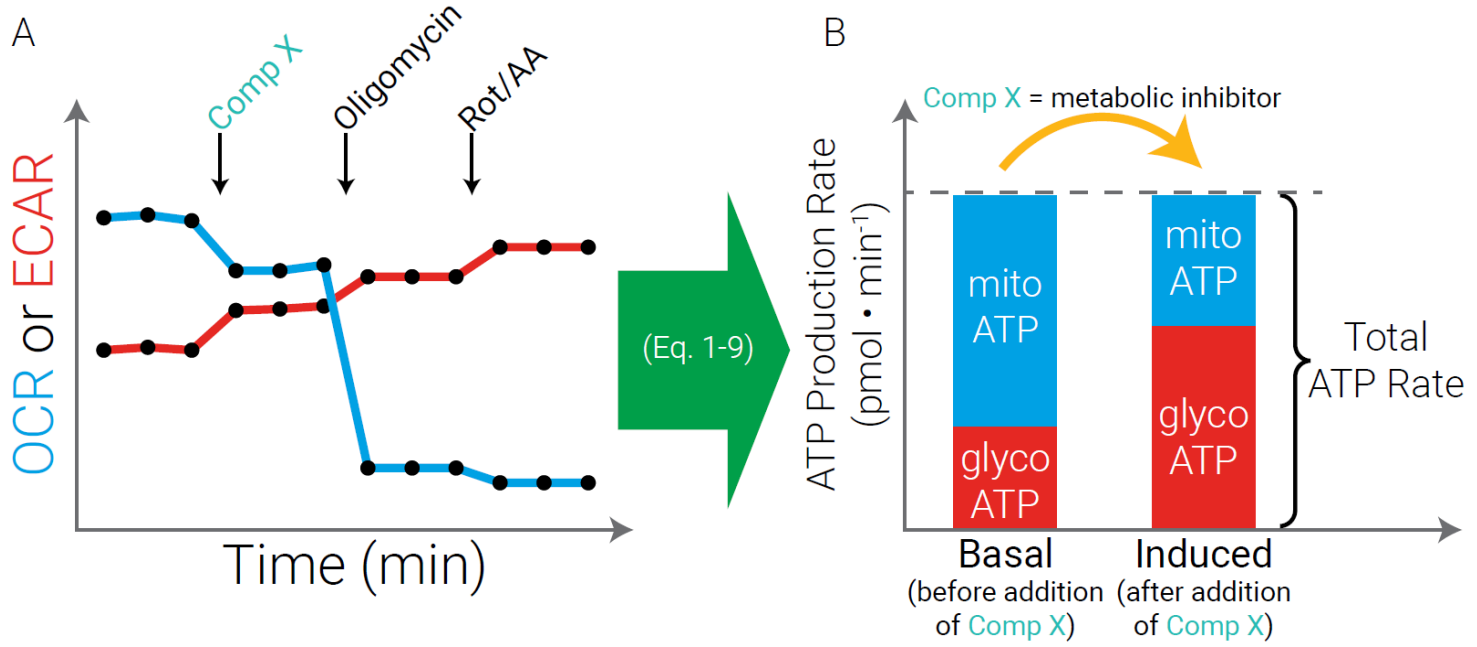
市場總值：2025 年全球 NMN 市場估值約在 **2.81 億至 6.18 億美元** 之間

NR 市場估值約在 **2.11 億**





Quantifying Cellular ATP Production Rate



Glucose + 2 ADP + 2 Pi ➔ 2 Lactate + 2 ATP + 2 H_2O + 2 H^+ (Eq. 1)

glycoATP Production Rate (pmol ATP/min) = glycoPER (pmol H^+/min) (Eq. 2)

glycoPER (pmol H^+/min) = PER (pmol H^+/min) – mitoPER (pmol H^+/min) (Eq. 3)

PER (pmol H^+/min) = ECAR (mpH/min) x BF (mmol H^+/L/pH) x Vol $_{XF\ microchamber}$ (µL) x Kvol (Eq. 4)

mitoPER (pmol H^+/min) = mitoOCR (pmol O_2/min) x CCF (pmol H^+/pmol O_2) (Eq. 5)

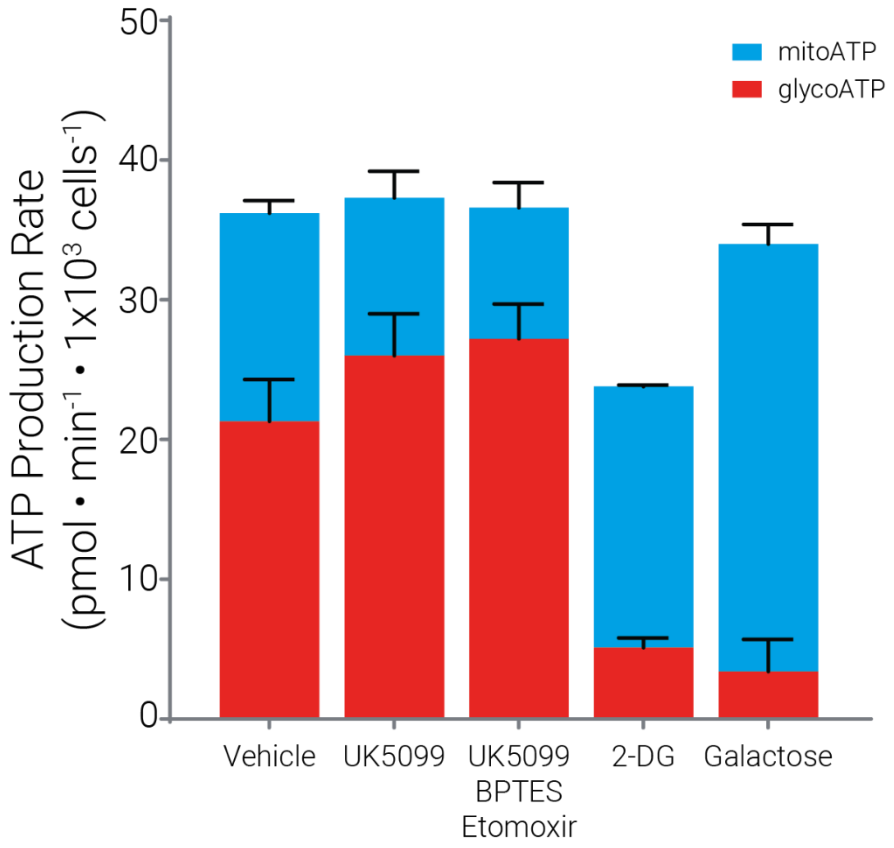
mitoOCR (pmol O_2/min) = OCR basal (pmol O_2/min) – OCR (Rot/AA) (pmol O_2/min) (Eq. 6)

OCR_{ATP} (pmol O_2/min) = OCR_{basal} (pmol O_2/min) – OCR_{Oligo} (pmol O_2/min) (Eq. 7)

mitoATP Production Rate (pmol ATP/min) = OCR_{ATP} (pmol O_2/min) * 2 (pmol O/pmol O_2) * P/O (pmol ATP/pmol O) (Eq. 8)

ATP Production Rate (pmol ATP/min) = glycoATP Production Rate (pmol ATP/min) + mitoATP Production Rate (pmol ATP/min) (Eq. 9)

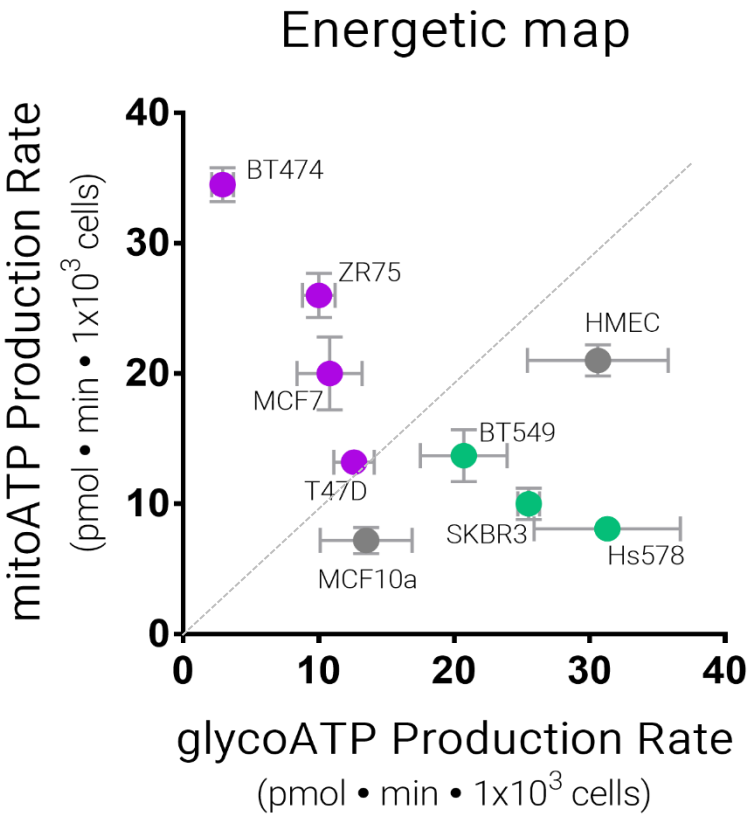
Fuel Dependencies for Energy Production Is Revealed Using XF Real-Time ATP Rate Assay

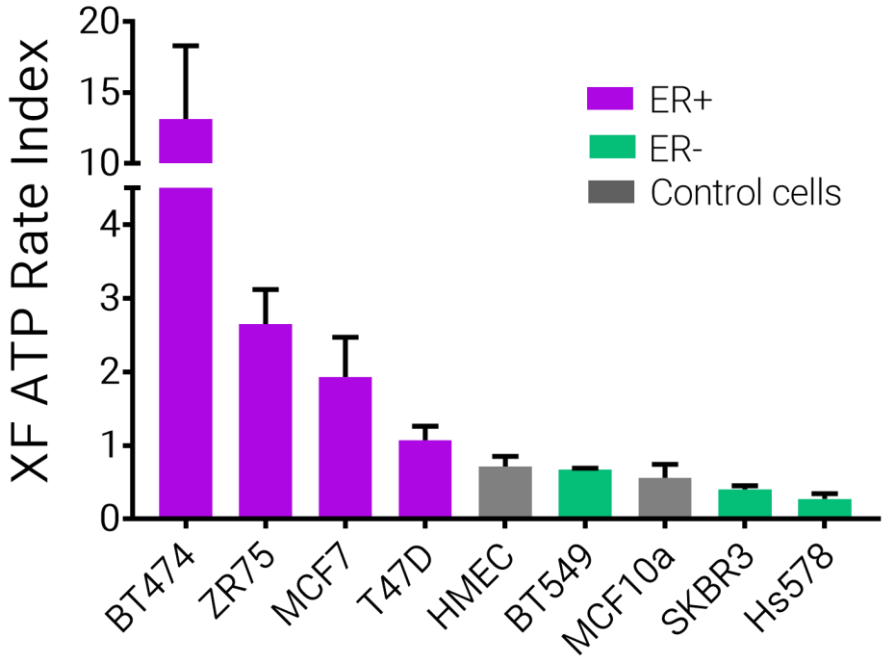


- Quantifies metabolic phenotype
- Allows identification of compounds that induce a metabolic switch
- Detects compounds that inhibit total ATP production (potentially affect cell viability)
- Enables the studies of dependency of cells on particular fuels for energy production

Seahorse Bioscience
A part of Agilent Technologies

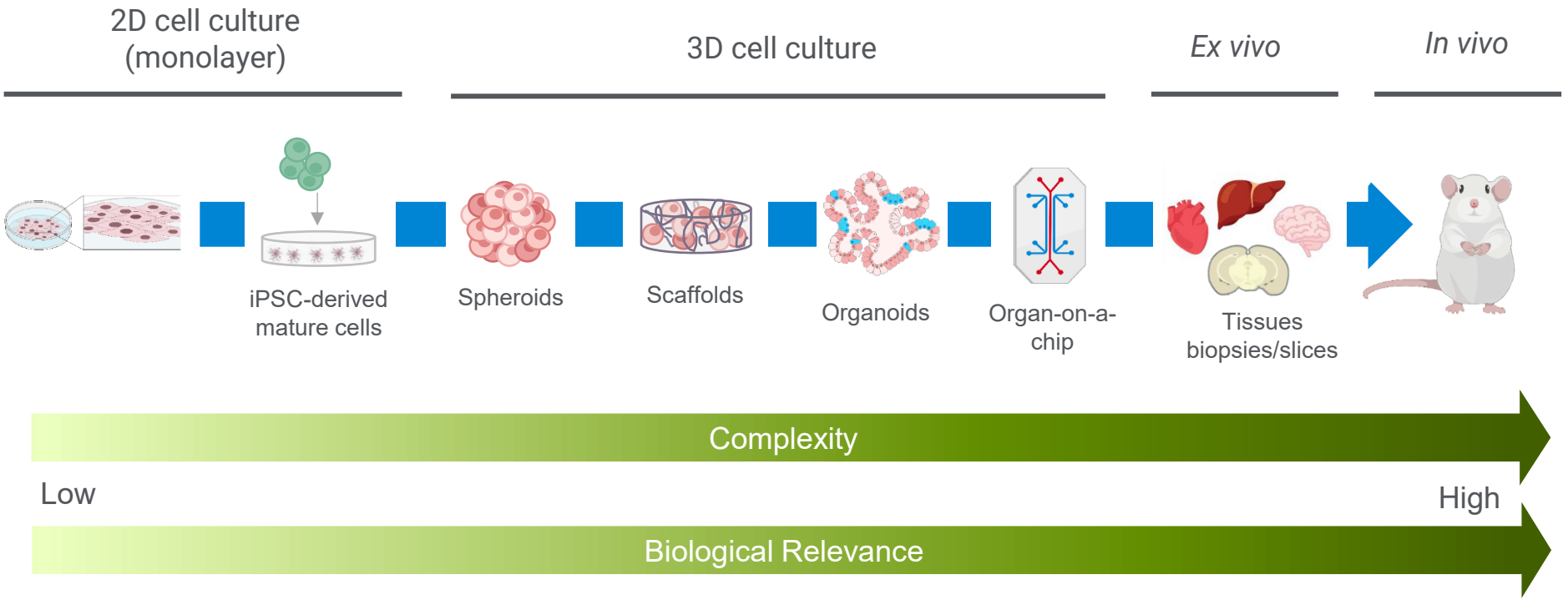
Estrogen Receptor Positive (ER+) Breast Cancer Cell Lines are More Oxidative than ER- or Control Cell Lines





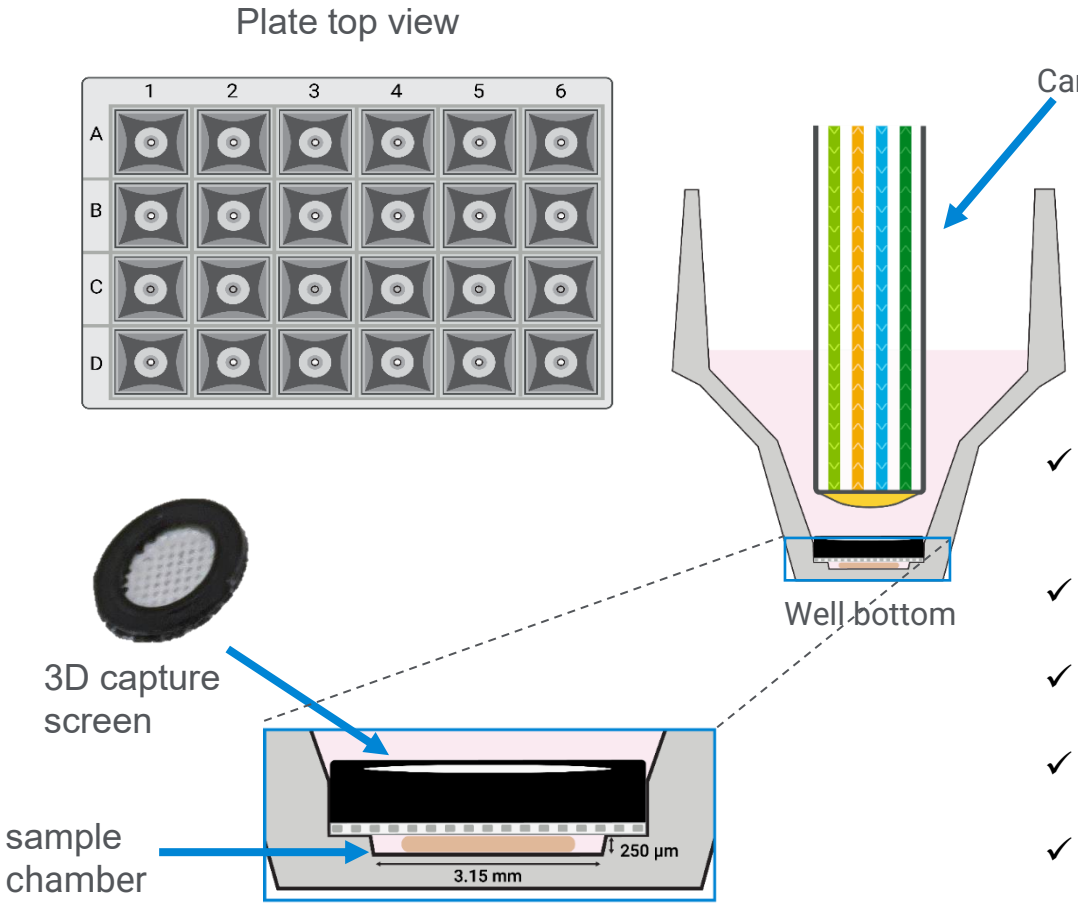
Seahorse Bioscience
A part of Agilent Technologies

Biological Models Used in Biomedical Research



Seahorse Bioscience
A part of Agilent Technologies

The XF Flex 3D Capture Microplate-L



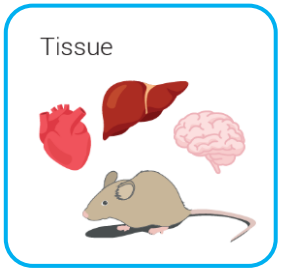
- ✓ Optimal microchamber dimension for better sensitivity
- ✓ Dual color ring/mesh for easy handling
- ✓ Large mesh size to reduce trapped bubbles
- ✓ Optimized instrument mix parameters
- ✓ Suitable for many material types (tissue, cell clusters, small organisms, etc.)

Seahorse Bioscience
A part of Agilent Technologies

Optimized 3D Tissue Workflow

Day of assay

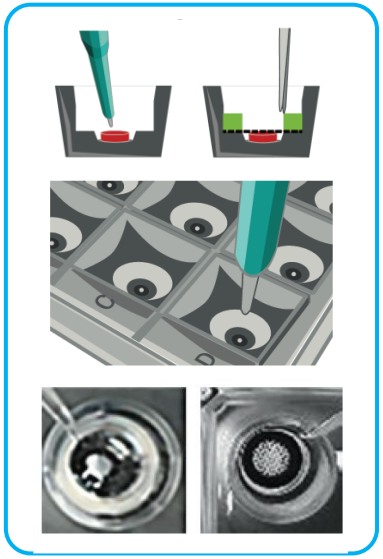
Dissect tissue of interest



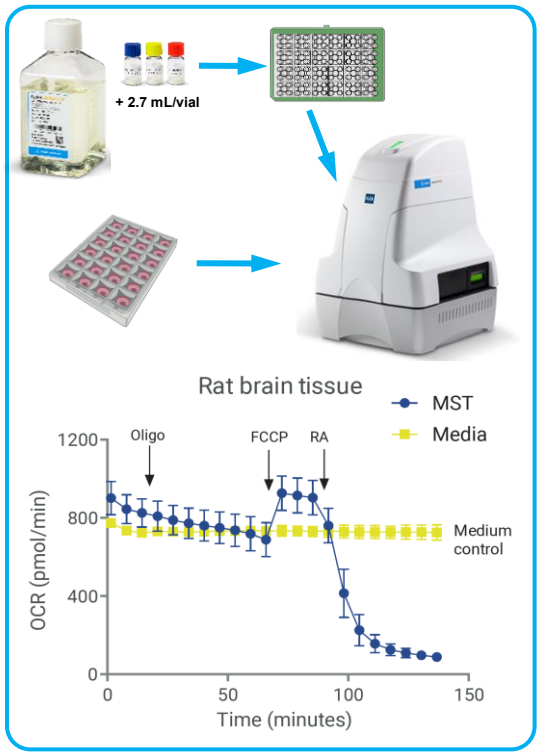
Prepare XF Assay Media

Prepare tissue slice and punch

Deliver tissue punch to well and place mesh/ring



Prepare compounds and perform XF assay



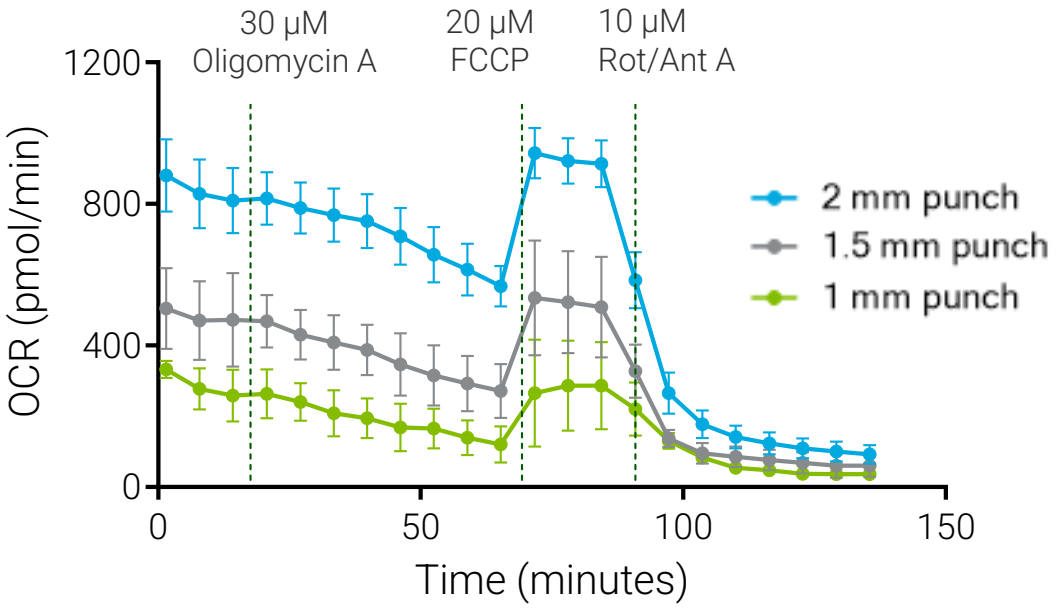
Seahorse Bioscience
A part of Agilent Technologies

Optimized 3D Tissue Workflow

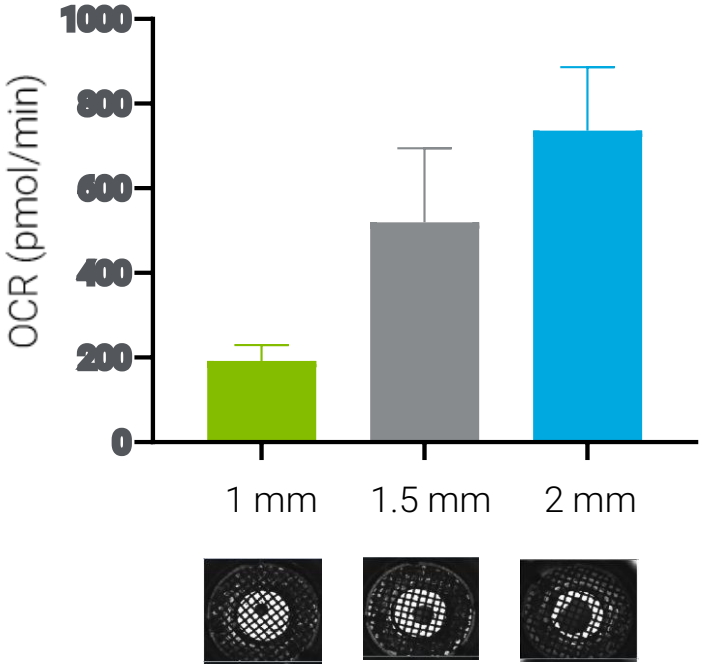
Seahorse Bioscience
A part of Agilent Technologies

Example Kinetic Data with Multiple Rat Brain Tissue Sizes

XF 3D Mito Stress Test

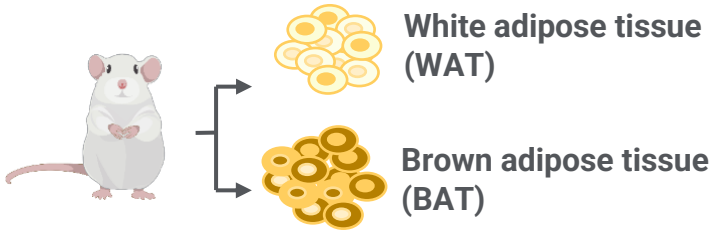


Mitochondrial Respiration

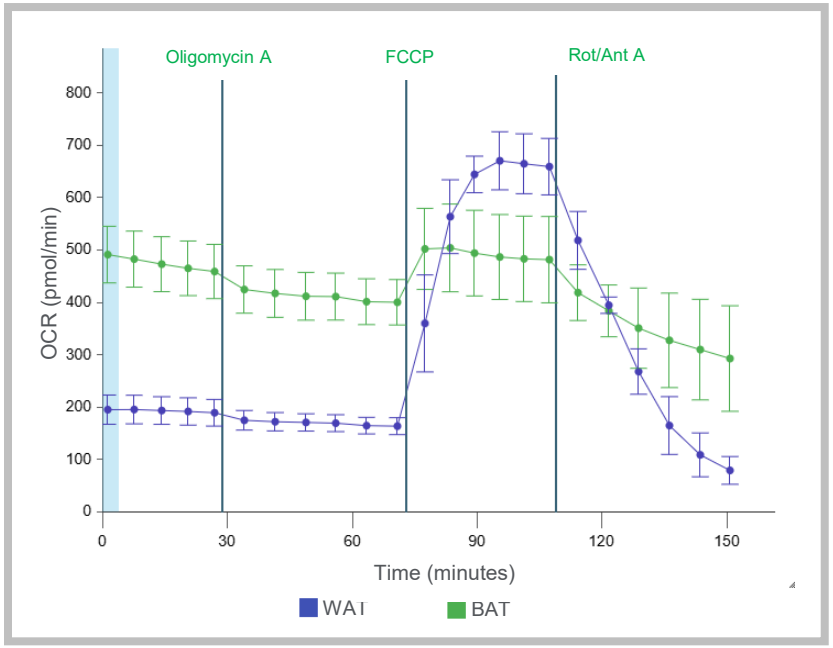


Seahorse Bioscience
A part of Agilent Technologies

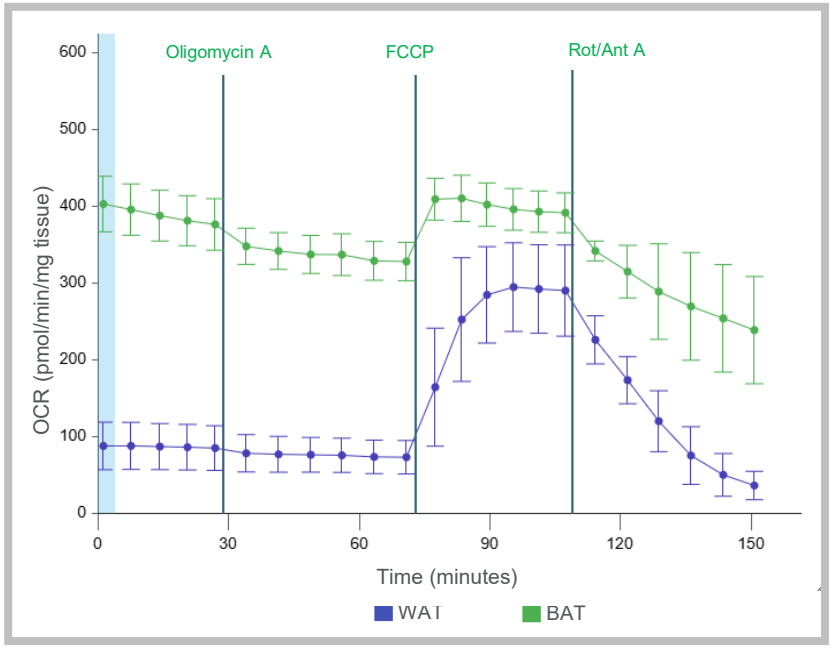
Mitochondrial function of white adipose and brown adipose tissue



Non-normalized



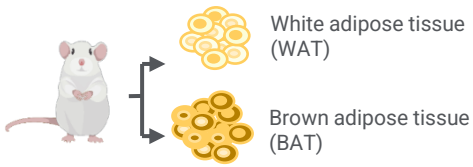
Normalized by weight

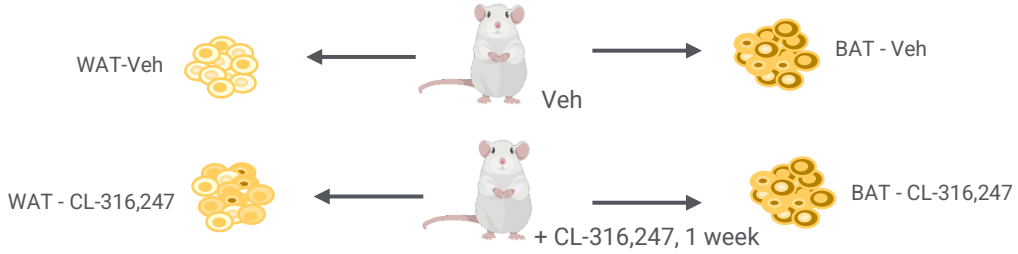


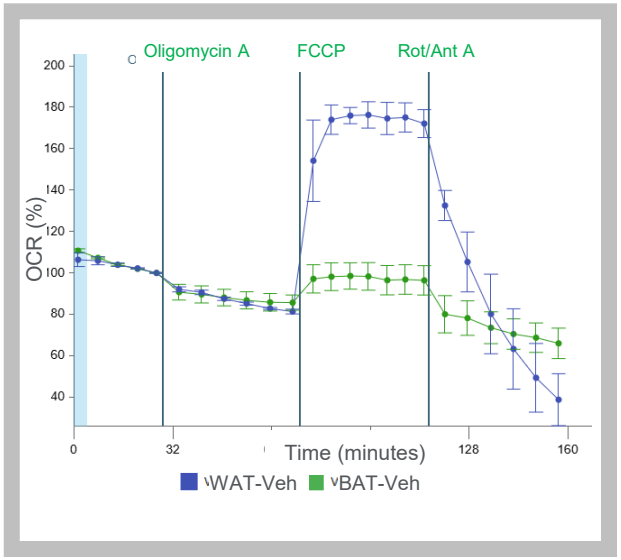
Dr. Yu (Aaron) An Lab – UT Health Science Center at Houston

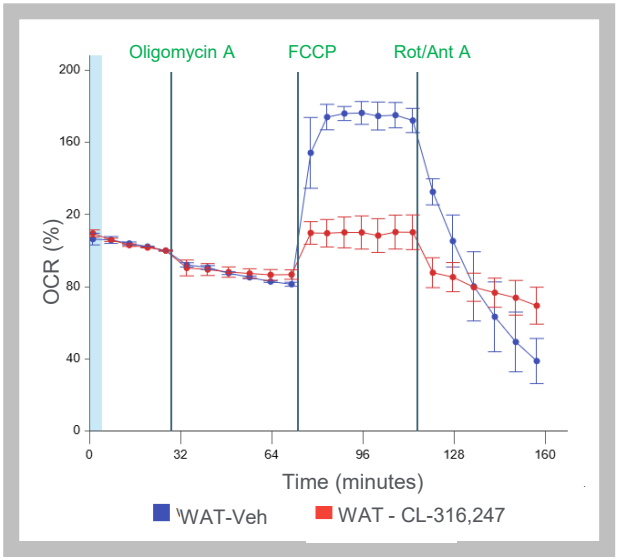
Seahorse Bioscience
A part of Agilent Technologies

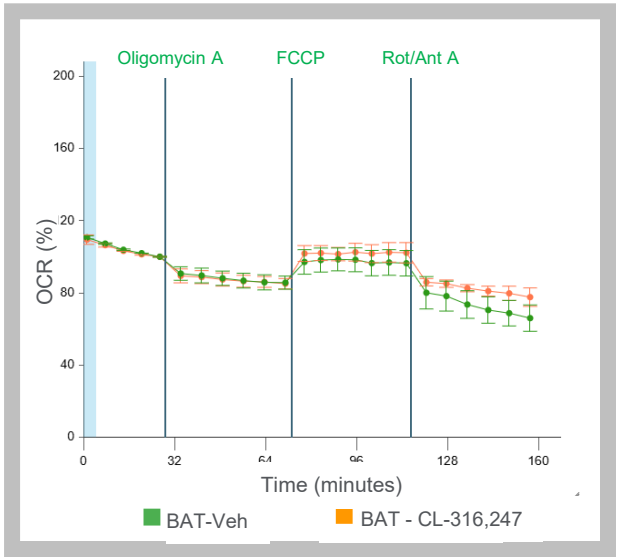
CL-316,247 induces "brown-like" mitochondrial phenotype in WAT











Dr. Yu (Aaron) An Lab – UT Health Science Center at Houston

Seahorse Bioscience
A part of Agilent Technologies

Seahorse XF Flex Organoid Microplate

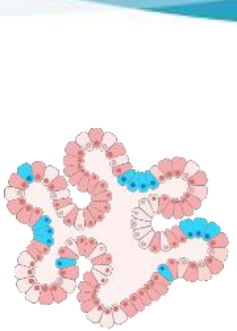
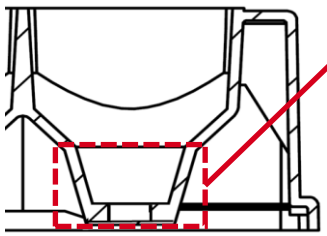
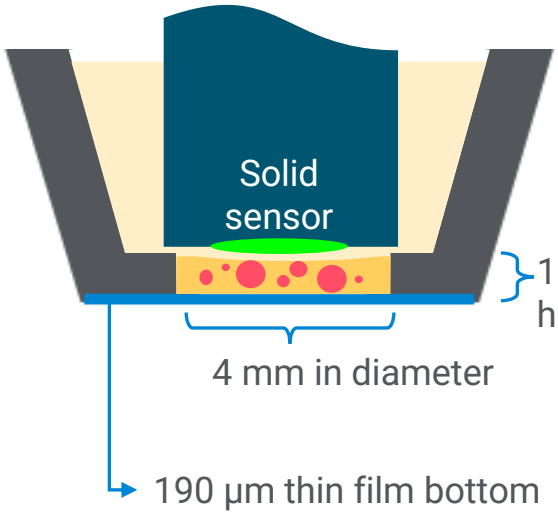


Plate top view

Angled view of a single well from top



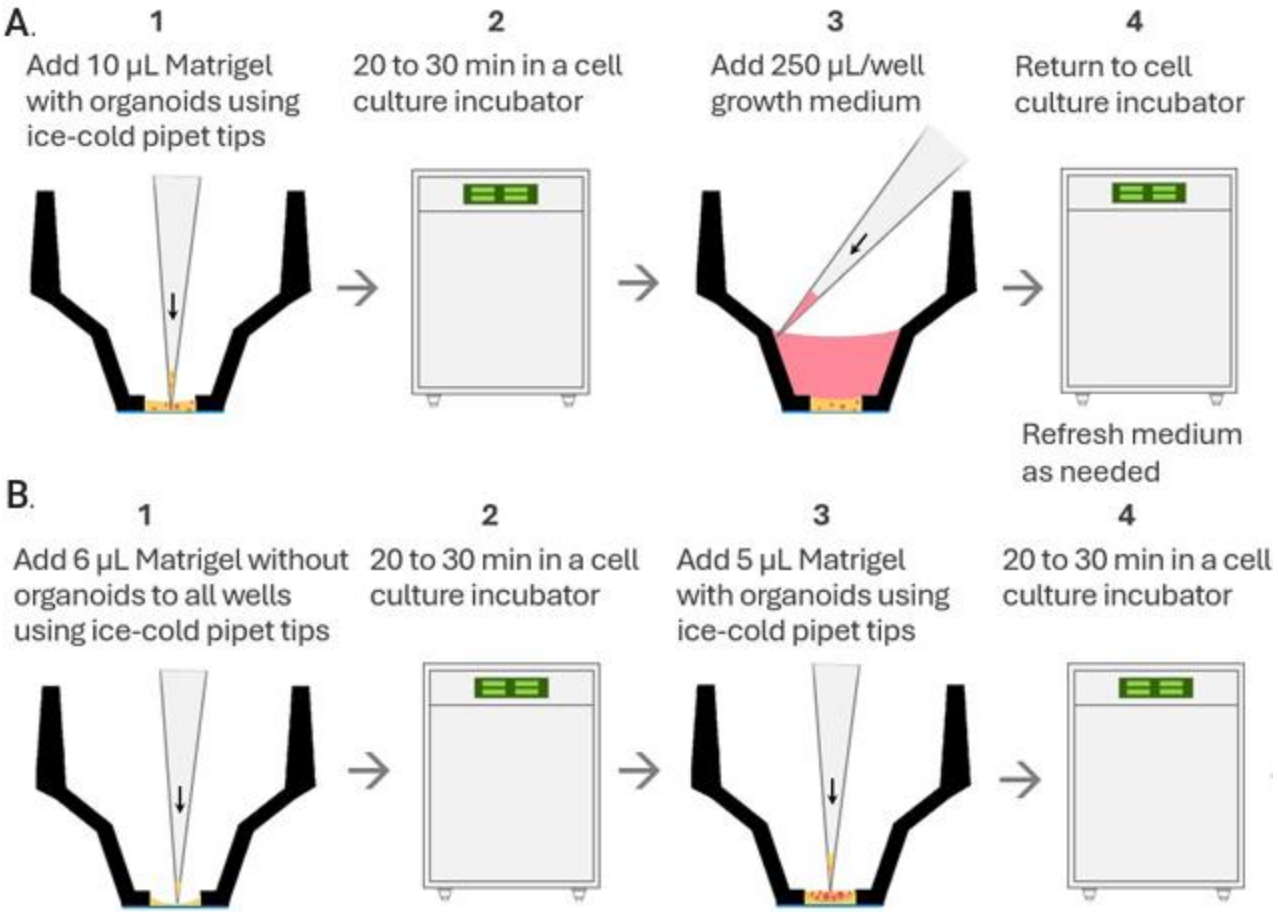
Side view of a single well



- ✓ Sample reservoir size: 1 mm (H) x 4 mm (Dia.), suitable for plating with 10 µL sample in matrix (min= 8 µL; max =12 µL)
- ✓ Thin film bottom enabling high-resolution imaging
- ✓ Flexible – supports several types of organoid cultures
- ✓ Robust workflow
- ✓ Only compatible with XF Flex (not with XFe24)

Seahorse Bioscience
A part of Agilent Technologies

Work with 2 plating strategies



Single Step

- ✓ Easy to handle
- ✓ Small and many organoid

Two Step

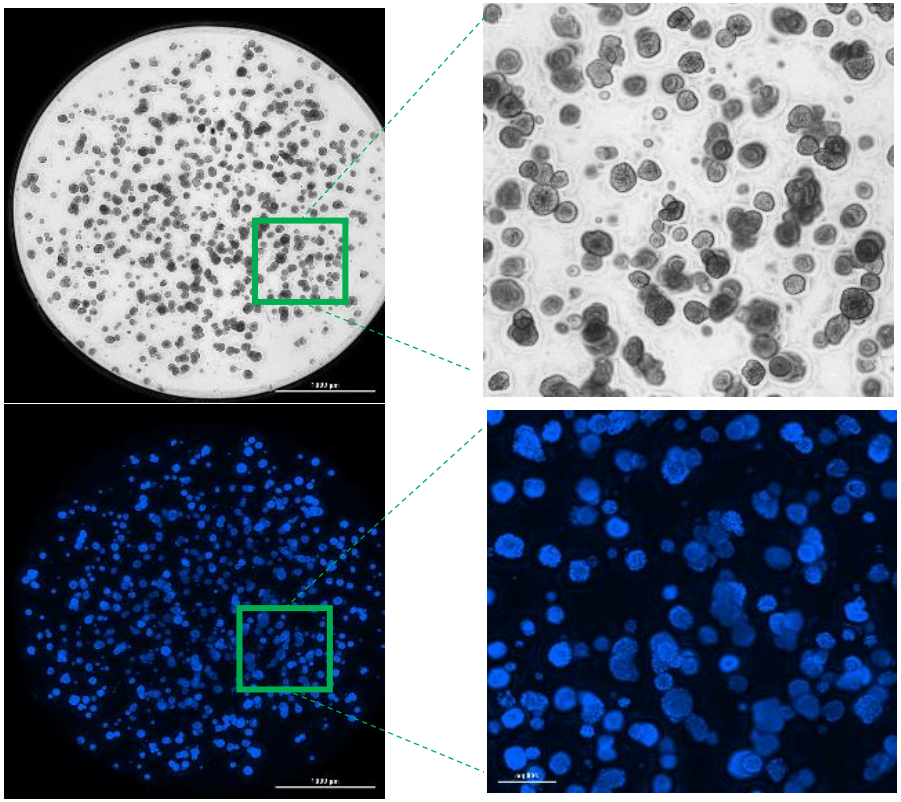
- ✓ Advanced handle
- ✓ Large and less organoid

Seahorse Bioscience
A part of Agilent Technologies

Example Data –
MCF10A Organoid Culture (8-day culture)

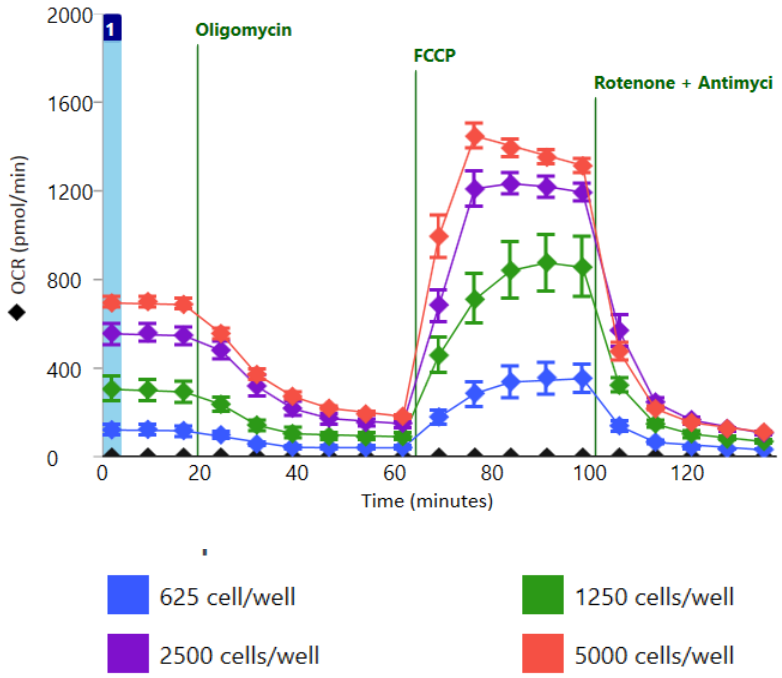
Cytation 5

4x, Brightfield



4x, Fluorescence, Hoechst

Typical XF Mito Stress Test Profile

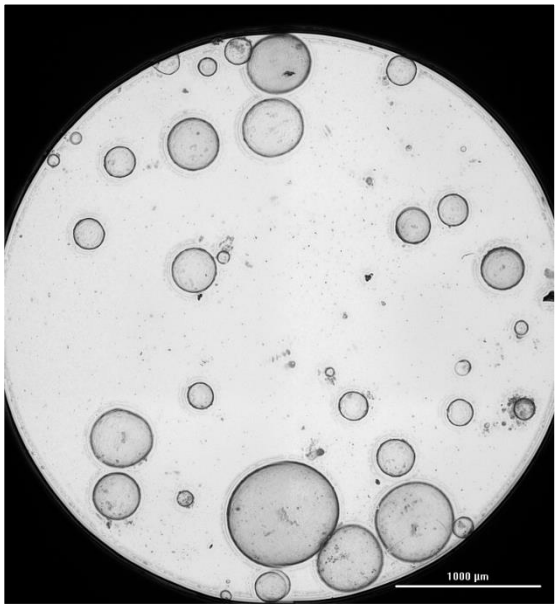


Seahorse Bioscience
A part of Agilent Technologies

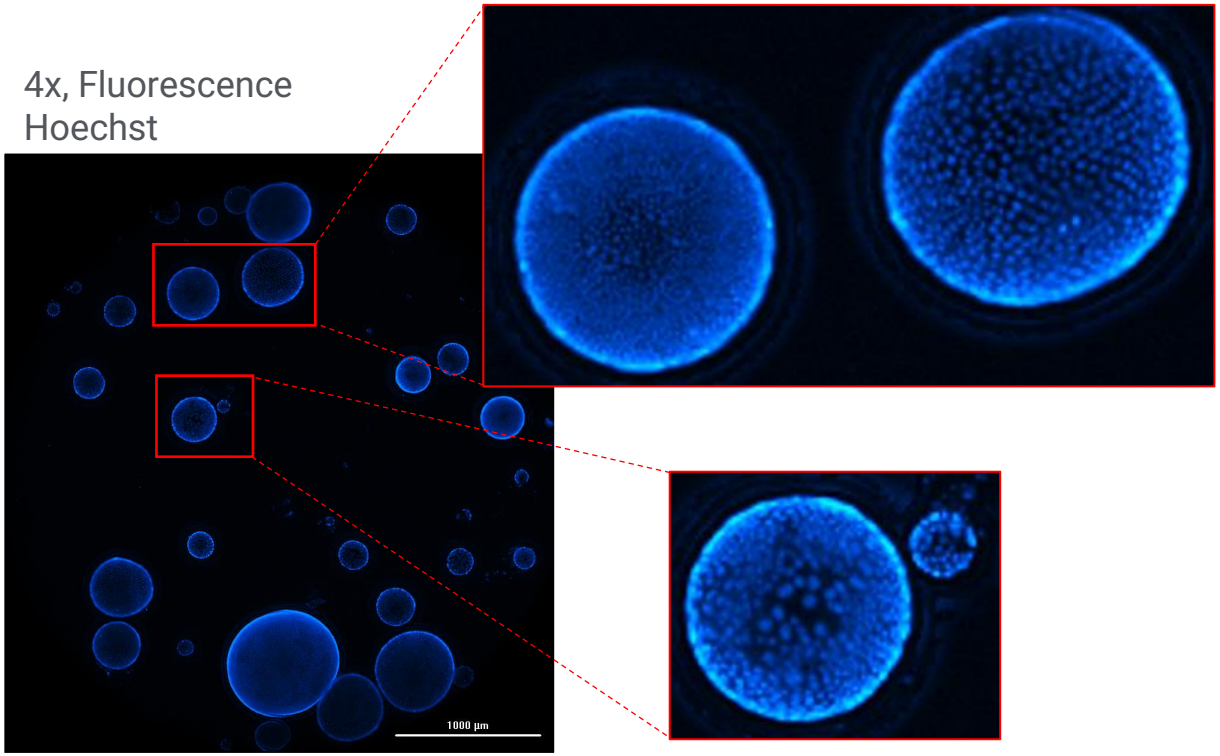
Example Data:
Stem Cell-Derived Mouse Liver Organoids (3-day culture)

Cytation 5

4x, Brightfield



4x, Fluorescence
Hoechst



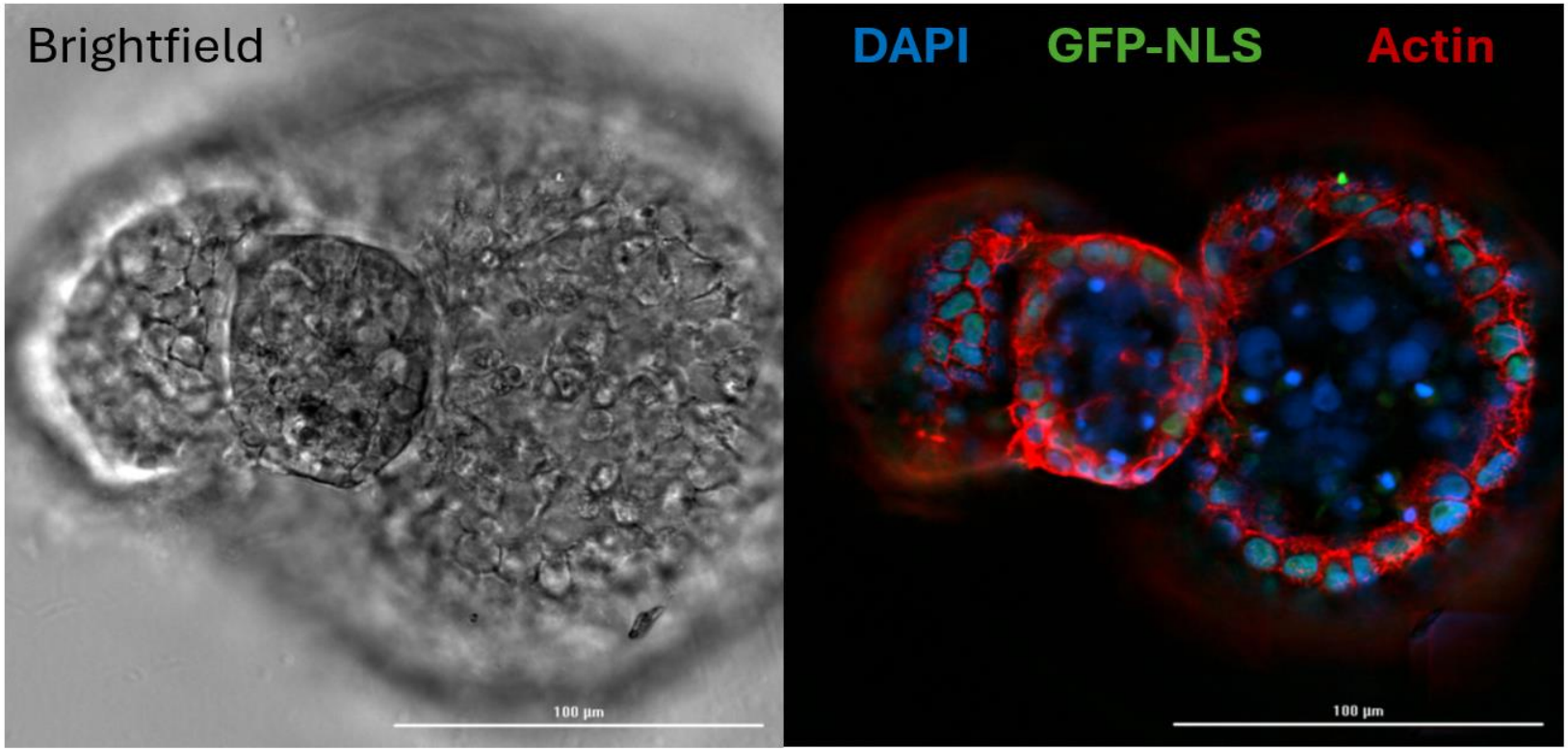
Seahorse Bioscience
A part of Agilent Technologies

Example Data:
MCF10A Breast Organoid Culture (6-day culture)

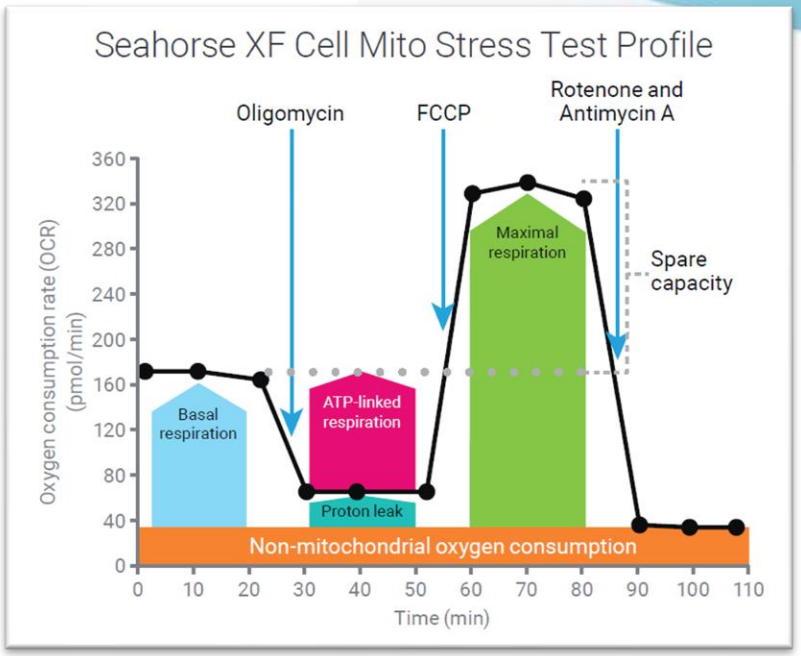
Cytation C10

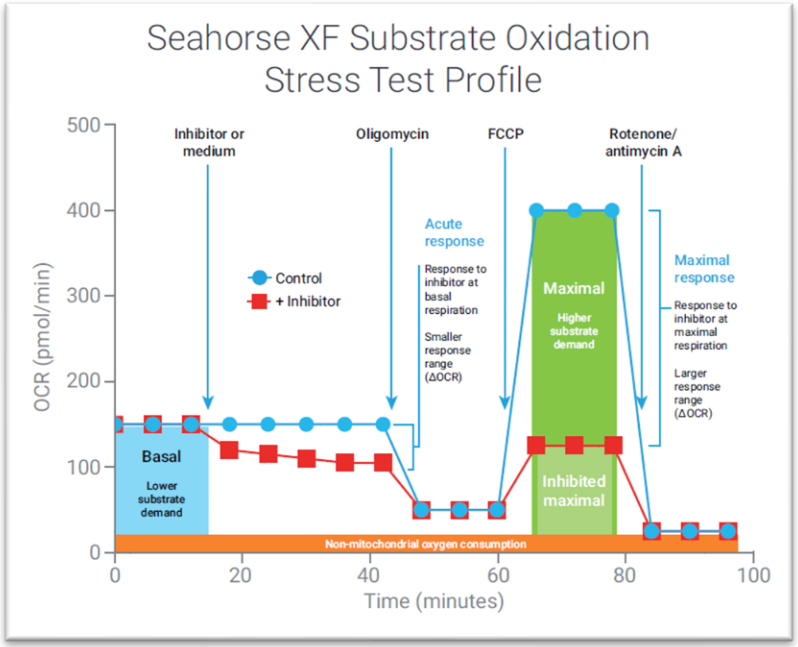
60x, Brightfield

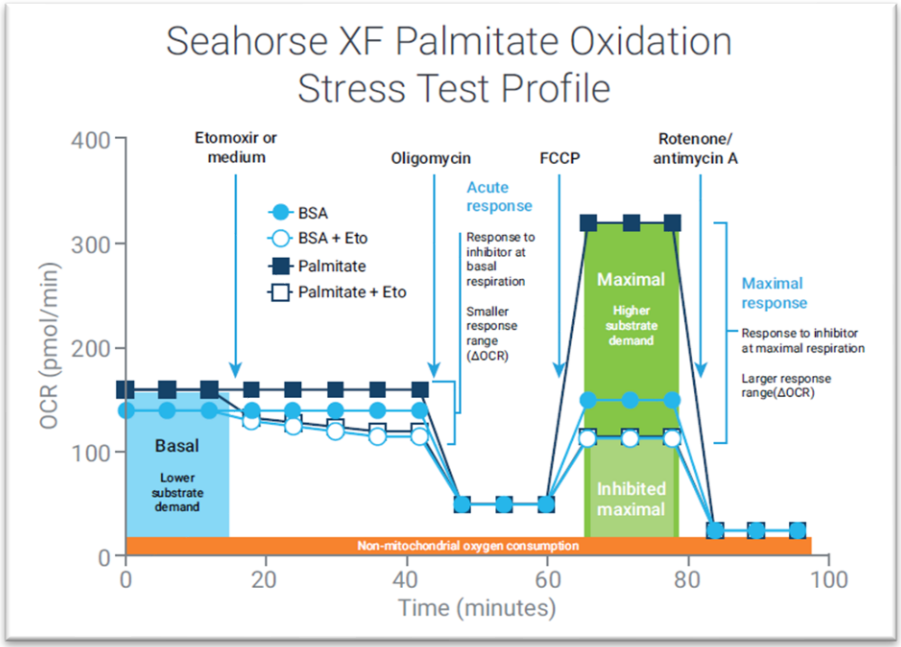
60x, Fluorescence, fixed sample

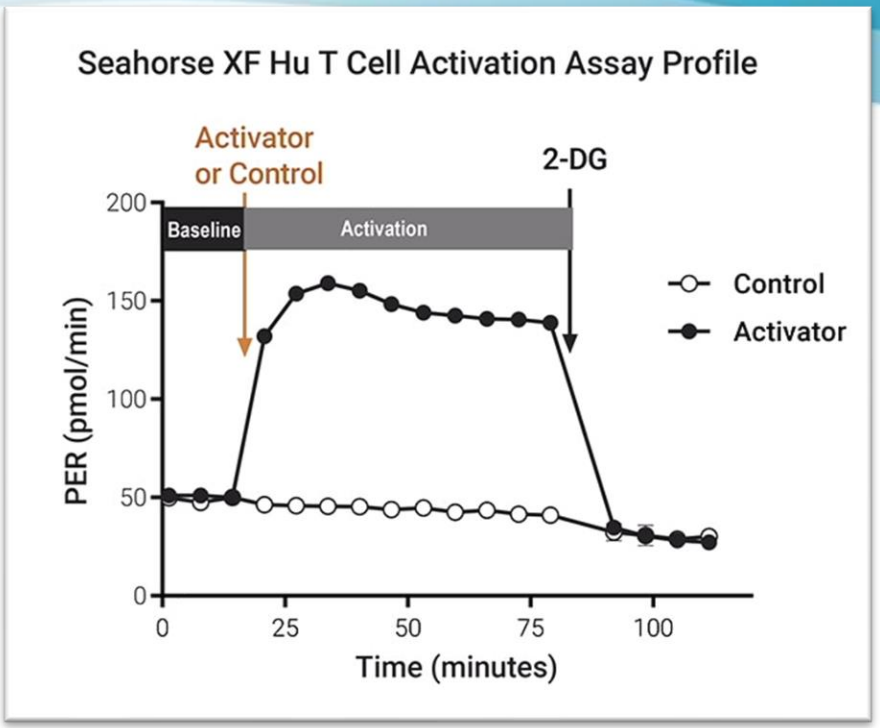


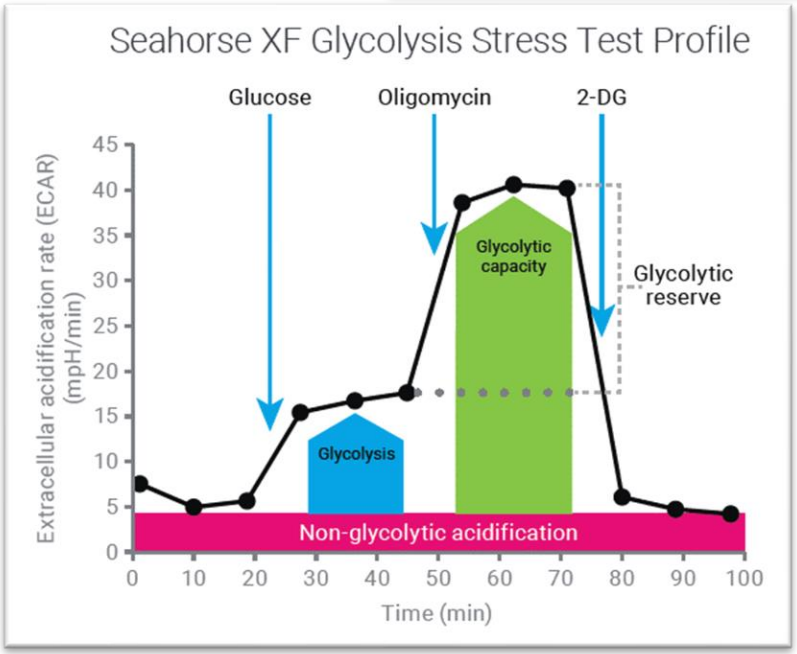
Seahorse Bioscience
A part of Agilent Technologies

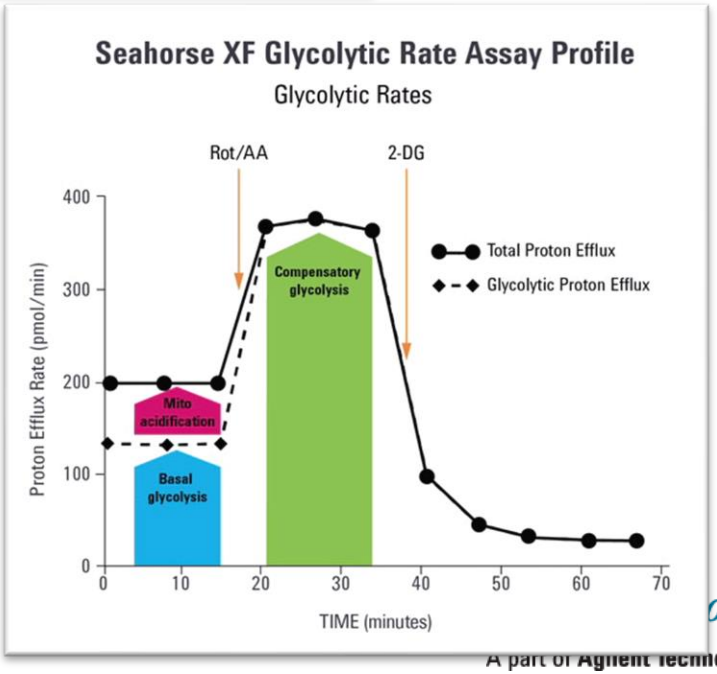

Seahorse XF Cell Mito Stress Test Profile
Oligomycin
FCCP
Rotenone and Antimycin A
Oxygen consumption rate (OCR) (pmol/min)
Basal respiration
ATP-linked respiration
Proton leak
Maximal respiration
Spare capacity
Non-mitochondrial oxygen consumption
Time (min)


Seahorse XF Substrate Oxidation Stress Test Profile
Inhibitor or medium
Oligomycin
FCCP
Rotenone/ antimycin A
OCR (pmol/min)
Control
+ Inhibitor
Acute response
Response to inhibitor at basal respiration
Smaller response range (ΔOCR)
Maximal
Higher substrate demand
Maximal response
Response to inhibitor at maximal respiration
Larger response range (ΔOCR)
Basal
Lower substrate demand
Inhibited maximal
Non-mitochondrial oxygen consumption
Time (minutes)


Seahorse XF Palmitate Oxidation Stress Test Profile
Etomoxir or medium
Oligomycin
FCCP
Rotenone/ antimycin A
OCR (pmol/min)
BSA
BSA + Eto
Palmitate
Palmitate + Eto
Acute response
Response to inhibitor at basal respiration
Smaller response range (ΔOCR)
Maximal
Higher substrate demand
Maximal response
Response to inhibitor at maximal respiration
Larger response range(ΔOCR)
Basal
Lower substrate demand
Inhibited maximal
Non-mitochondrial oxygen consumption
Time (minutes)

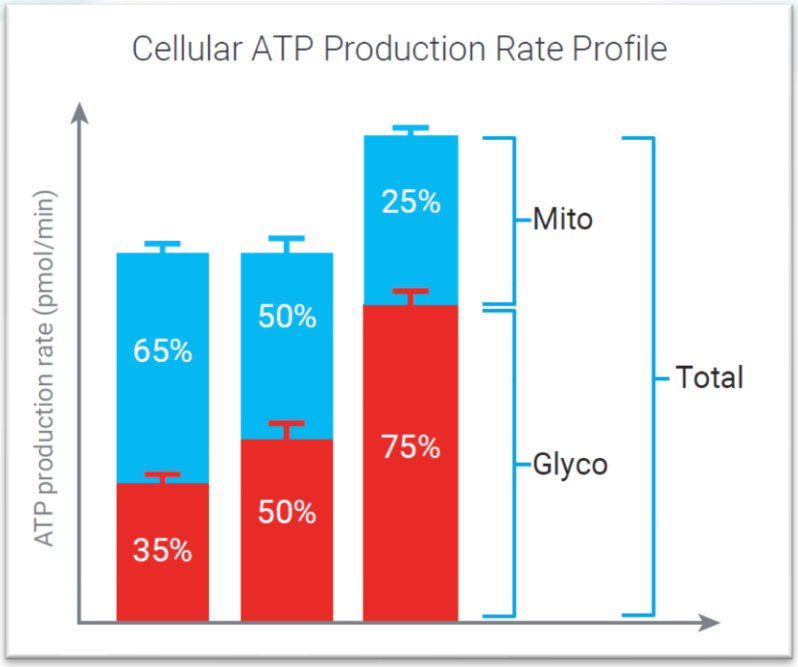


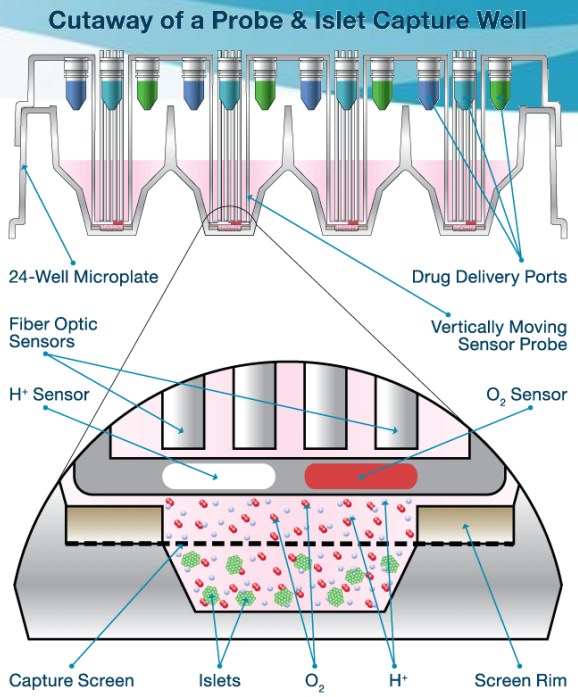


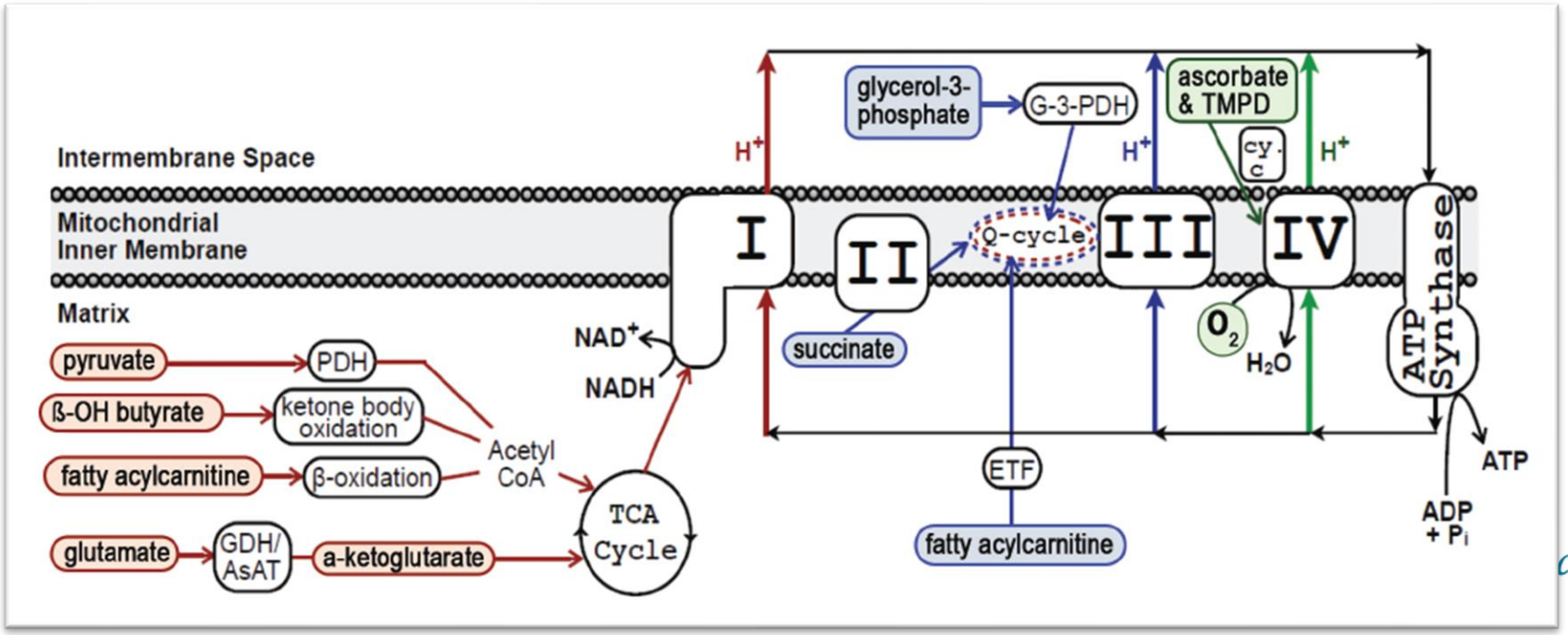


oscience

A part of Agilent Technologies


Cellular ATP Production Rate Profile
ATP production rate (pmol/min)
65%
35%
50%
50%
25%
75%
Mito
Glyco
Total


Cutaway of a Probe & Islet Capture Well
24-Well Microplate
Drug Delivery Ports
Fiber Optic Sensors
Vertically Moving Sensor Probe
H+ Sensor
O2 Sensor
Capture Screen
Islets
O2
H+
Screen Rim


Intermembrane Space
Mitochondrial Inner Membrane
Matrix
glycerol-3-phosphate
G-3-PDH
ascorbate & TMPD
cy c
H+
I
II
Q-cycle
III
IV
ATP Synthase
NAD+
NADH
succinate
O2
H2O
ATP
ADP + Pi
ETF
fatty acylcarnitine
pyruvate
PDH
ß-OH butyrate
ketone body oxidation
fatty acylcarnitine
ß-oxidation
Acetyl CoA
glutamate
GDH/ AsAT
a-ketoglutarate
TCA Cycle

Build Your Cell Analysis Lab with Agilent

Investigate dynamic cancer cell strategies with innovative cell analysis technologies

Seahorse Bioscience
A part of Agilent Technologies