Redox & Bioenergetics Core
Translational Metabolomics Shared Resource, MCW Cancer Center

OVERVIEW

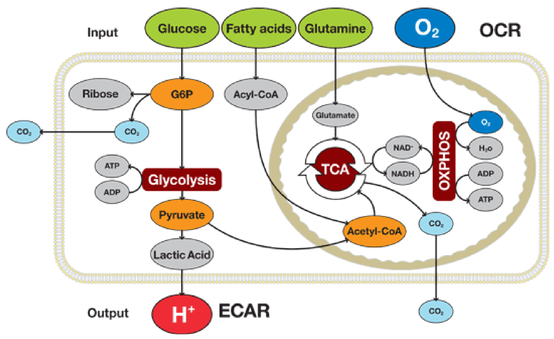
The Redox & Bioenergetics Core opened in 2012 to serve MCW researchers and clinicians in need of the expertise in cell bioenergetics and redox biology. The facility is located in the Center for Cancer Discovery, room N1130, and is a part of the Translational Metabolomics Shared Resource (TraMSR).

The purpose of the Redox & Bioenergetics Core is four-fold:

1. Investigate cancer cell metabolism and understand how cancer cells exploit redox and metabolic pathways for survival
2. Provide a better understanding of the redox and bioenergetic pathways in cancer metabolism during hypoxia and normoxia
3. Assess new metabolism-based strategies for cancer treatment
4. Promote increased collaboration in cancer research between basic scientists and clinical researchers

SERVICES PROVIDED

- Assessment of mitochondrial and glycolytic function
- Analysis of cellular metabolites
- Analysis of drugs targeting cell metabolism
- Longitudinal studies in tumorigenesis models
- Studies of mitochondrial toxicity of compounds
- Measurement of reactive oxygen species and redox biomarkers



Examples of Research Topics

- Modulation of redox and bioenergetic pathways by anticancer drugs in cancer and immune cells
- Selective accumulation of mitochondrial drugs in cancer cells and tumor tissues
- Synergistic antitumor inhibitory effect of experimental and FDA-approved drugs targeting cell metabolism
- Metabolic and bioenergetic effects of natural compounds in cancer cells
- Monitoring H_2O_2 production by cancer and immune cells
- Monitoring $ONOO^-$ production in tumor microenvironment

STRUCTURE AND CONTACT

Jacek Zielonka, PhD
Assistant Professor of Biophysics; Core Director
- Expert in cell bioenergetics, redox biology and chemistry, reactive oxygen species, mass spectrometry, HPLC

Monika Zielonka, MSc
Research Associate, Core Manager
- Expert in Seahorse XF-based bioenergetic analyses, analytical chemistry

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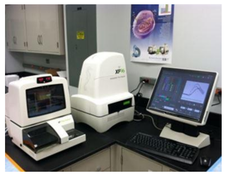
KEY EQUIPMENT

1. Seahorse Bioscience XF96, XFe96 and XFe24 Extracellular Flux Analyzer



Capabilities

- Measure mitochondrial function via oxygen consumption rates
- Measure glycolytic function via pH changes
- Measure fatty acid oxidation
- Add mitochondrial stressors such as oligomycin, FCCP, and antimycin A during the run
- Measure mitochondrial consequences of cancer cell treatments

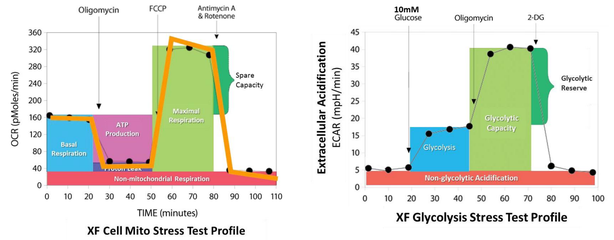


Figure 1: Bioenergetic profiles of Seahorse XF96 extracellular flux analyzer examining (A) mitochondrial and (B) glycolytic function.

Advantages compared with alternatives

- Capable of running 96-well microplate in a high throughput format
- Decreased sample size compared to Clark electrode
- Capable of simultaneously measuring both mitochondrial function (Figure 1A) and glycolytic function (Figure 1B) via monitoring of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR)
- Capability of measuring mitochondrial function in response to the addition of drugs and mitochondrial inhibitors and protonophores during the experiment (Figure 1A)

2. Shimadzu Ultra-High Performance Liquid Chromatography / Mass Spectrometry 8030 System

Capabilities

- Measure metabolites pre-optimized on the instrument
- Measure intracellular uptake of cancer cell treatments
- Profile cellular oxidants using unique redox probes

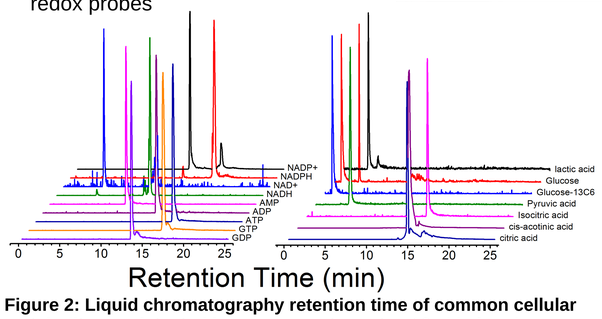


Figure 2: Liquid chromatography retention time of common cellular metabolites as detected by mass spectrometry. 10 µM of each compound was prepared into mobile phase and eluted on a Phenomenex C_{18} column. Specific daughter ion fragmentation patterns were used to identify each metabolite.

Advantages compared with alternatives

- Capable of measure bioenergetic metabolites in under 30 minutes
- Capable of screening numerous cellular metabolic pathways simultaneously (TCA, glycolysis)
- Profiling different products formed from chemical probes
- Tandem mass spec breaks parent ion into daughter ions for optimum identification of compounds and metabolites compared to other mass specs

EXAMPLES OF RESEARCH SUPPORTED

Example 1: 2D Map of bioenergetics in PDAC – susceptibility to glycolytic inhibitors

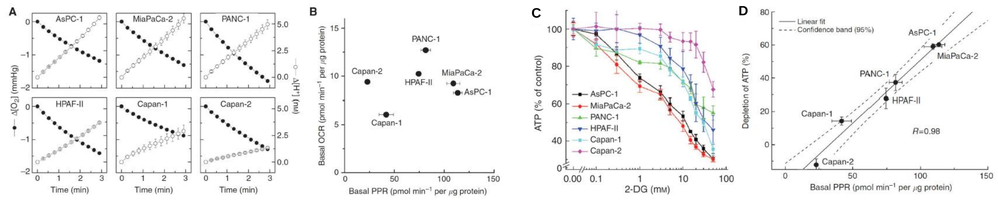


Figure 3: Relationship between OCR/ECAR and 2-DG-induced ATP depletion in various PDACs. (A) Oxygen consumption (ΔO_2) and proton production (ΔH^+) after normalization to 1 µg of protein. (B) 2D map of OCR and ECAR in PDAC cell lines. (C) Intracellular ATP levels in specified cell lines treated with 2-DG as indicated for 24 hours. (D) Relationship between basal ECAR value and 2-DG induced ATP loss (normalized to protein). Values are mean SD (n=4-6). [Cheng G et al. *Br J Cancer*. 2014. May:4-6].

Example 2: Regulation of cellular energetic function by FOXM1 in multiple myeloma

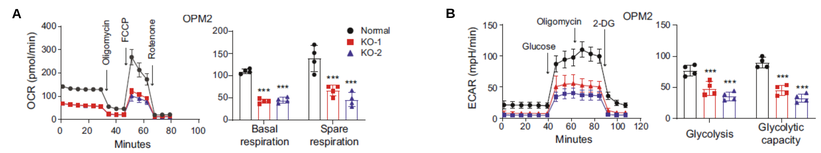


Figure 4: Measurements of mitochondrial (OCR) and glycolytic (ECAR) function in OPM2 cells in FOXM1^{Normal} and FOXM1^{KO} OPM2 multiple myeloma cells. (A) Line diagram depicting the oxygen consumption rate (OCR) of OPM2 cells containing normal levels of FOXM1 (control) or lacking FOXM1 (KO-1 and KO-2). Steady-state baseline conditions in the first 30 min were interrupted by addition of metabolic modulators (indicated in left panels by arrows pointing down that are labeled) to determine base and spare respiration capacity (right panels). (B) Line diagram of extracellular acidification rate (ECAR) under baseline conditions (left panel) challenged by small compounds that permit the determination of glycolysis and glycolytic capacity (right panel). [Cheng Y et al. Oncogene. 2022, 41, 3899-3911].

Example 3: Specific detection and fluorescence imaging of peroxynitrite production by macrophages

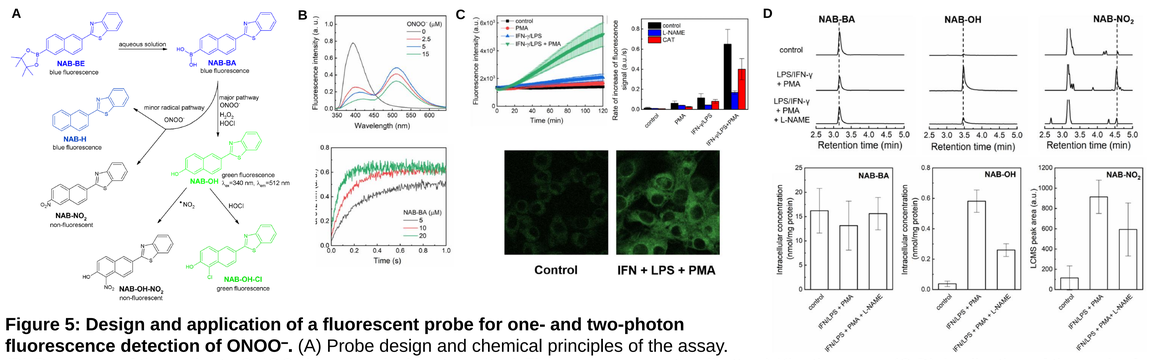


Figure 5: Design and application of a fluorescent probe for one- and two-photon fluorescence detection of $ONOO^-$. (A) Probe design and chemical principles of the assay. (B) Response of the probe to $ONOO^-$ in a chemical system. (*top*) Fluorescence spectra; (*bottom*) Kinetic curves (C,D) Application of the probe for the detection and monitoring (C, *top*) and imaging (C, *bottom*) of $ONOO^-$ formation in activated RAW 264.7 macrophages. (D) LC-MS-based profiling of the products generated from the probe in activated macrophages. [Grzelakowska A et al. *Free Radic. Biol. Med.* 2021, 169, 24-35].

SELECTED PUBLICATIONS USING THE RBC CORE

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3. Cheng G, Zielonka J, McAllister D, Tsai S, Dwinell MB, Kalyanaraman B. Profiling and targeting of cellular bioenergetics: inhibition of pancreatic cancer cell proliferation. Br J Cancer 2014(May):1–9.
4. Wu X, Garner J, Kittipongdaja W, Liu X, Komas SM, Hwang ST, Schieke SM. Rapamycin Suppresses Tumor Growth and Alters the Metabolic Phenotype in T-Cell Lymphoma. J Invest Dermatol. J Invest Dermatol 2015, 35(9):2301-2308.
5. Zhang Q, Pan J, Lubet R, Komas SM, Kalyanaraman B, Wang Y, You M. Enhanced antitumor inhibitory effect of 3-bromopyruvate in combination with rapamycin in vivo and in vitro. Cancer Prev Res (Phila) 2014, 8(4):318-26.
6. Roy I, McAllister DM, Gorse E, Dixon K, Zimmerman NP, Getschman AE, Tsai S, Engle DD, Evans DB, Volkman BF, Kalyanaraman B, Dwinell MB. Chemokine biased agonism regulates pancreatic cancer migration and metastasis through bioenergetic signaling. Cancer Res 2015, 75(17):3529.
7. Cheng G, Zielonka J, Ouari O, Lopez M, McAllister D, Boyle K, Barrios CS, Weber JJ, Johnson BD, Hardy M, Dwinell MB, Kalyanaraman B. Mitochondria-Targeted Analogues of Metformin Exhibit Enhanced Antiproliferative and Radiosensitizing Effects in Pancreatic Cancer Cells. Cancer Res. 2016 Jul 1;76(13):3904-15.
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11. AbuEid M, McAllister DM, McOlash L, Harwig MC, Cheng G, Drouillard D, Boyle KA, Hardy M, Zielonka J, Johnson BD, Hill RB, Kalyanaraman B, Dwinell MB. Synchronous effects of targeted mitochondrial complex I inhibitors on tumor and immune cells abrogate melanoma progression. iScience. 2021 May 25;24(6):102653.
12. Cheng G, Hardy M, Topchyan P, Zander R, Volberding P, Cui W, Kalyanaraman B. Mitochondria-targeted hydroxyurea inhibits OXPHOS and induces antiproliferative and immunomodulatory effects. iScience. 2021 May 31;24(6):102673.
13. Cheng Y, Sun F, Thornton K, Jing X, Dong J, Yun G, Pisano M, Zhan F, Kim SH, Katzenellenbogen JA, Katzenellenbogen BS, Hari P, Janz S. FOXM1 regulates glycolysis and energy production in multiple myeloma. Oncogene. 2022 Aug;41(32):3899-3911.
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