Direct measurements of cellular ATP levels using automated, quantitative live-cell analysis for mitochondrial toxicity screening

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Summary & Impact

- Mitochondrial toxicity has been implicated in several clinical trial failures and withdrawals
- Standard approaches to monitoring drug induced metabolic perturbations are limited to endpoint assays that provide population-based measurements and limited kinetic information
- We have developed a genetically encoded ATP sensor that enables direct, automated live cell analysis of cellular ATP levels using the Incucyte® Metabolism Optical Module
- Our live cell imaging approach to categorize compounds as nontoxic, cytotoxic, or mitotoxic using the glucose/galactose switch model was evaluated
- Substituting galactose for glucose in growth media blocks the ability of cells to generate ATP via glycolysis, conferring reliance on mitochondrial oxidative phosphorylation to generate ATP and enhancing sensitivity to mitochondrial-driven toxicity
- Cellular ATP levels were measured up to 24 hours following compound treatment. Nontoxic compounds resulted in little to no change in ATP, cytotoxic compounds conferred a decrease in ATP in both glucose and galactose conditions, and mitotoxic compounds displayed leftward shift in potency under galactose condition
- Reductions in ATP could be observed in minutes, and transient reductions followed by recovery highlight the sensitivity and value of kinetic data using our live cell imaging approach

Continuous Live-Cell Analysis: Methodology

Live-Cell Analysis System

A flexible assay platform that sits inside a standard tissue culture incubator. IncuCyte automatically and continuously acquires and analyzes HD phase and fluorescent images of living cells cultured in microplates, dishes, or flasks.

IncuCyte® Software

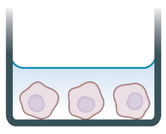
Fast, flexible, and powerful control hub for continuous live-cell analysis comprising image acquisition, processing, and date visualization.

IncuCyte® Reagents & Consumables

A suite of non-perturbing cell labeling and reporter reagents. Includes nuclear-targeted GFP and RFPs for cell counting, no-wash caspase 3/7 substrate for apoptosis, and cell kits for angiogenesis.

Quick guide

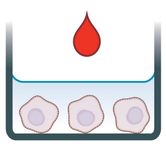
1 SEED CELLS



Cell Seeding

Seed cells in growth media and leave to adhere (4-24 hours). Cells should be 15-35% confluent at the time of transduction.

2 TRANSDUCE



Add IncuCyte® ATP sensor (binding or non-binding control sensor)

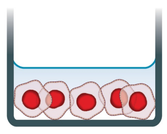
Add ATP lentivirus diluted in media ± Polybrene®. After 24 hours, remove transfection media and replace with fresh growth media. Monitor expression using the Incucyte® equipped with the Metabolism Optical Module.

3 APPLY SELECTION

Generate a Stable Population or Clone

Apply antibiotic selection to derive a stable, homogenous cell population or clone that expresses the cytoplasmic fluorescent sensor (Optional: Freeze cells and use for future assays).

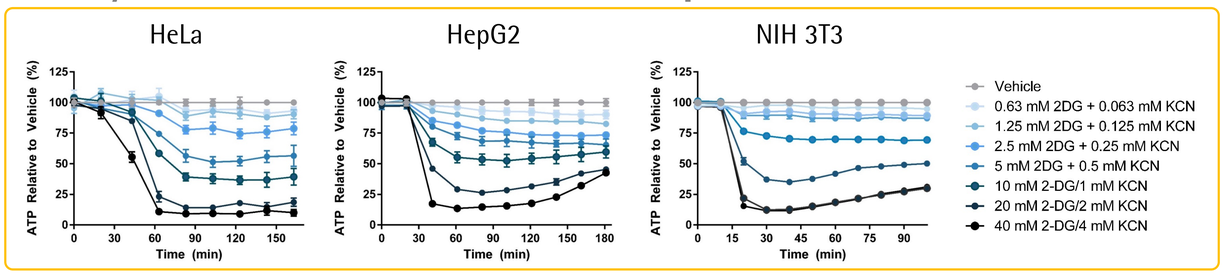
4 LIVE CELL FLUORESCENT IMAGING



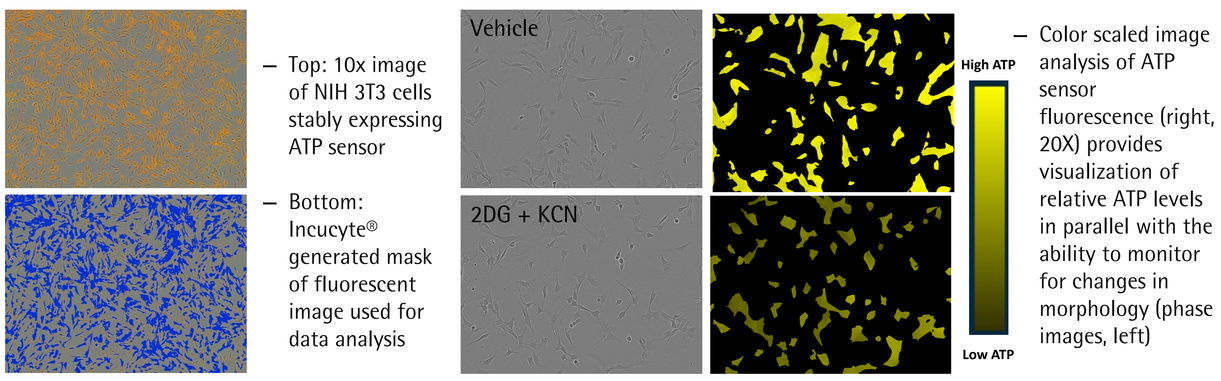
Automated Imaging and Quantitative Analysis

Capture images and analyse data in an Incucyte® equipped with the ATP Analysis Software Module.

Analysis and visualization of ATP depletion

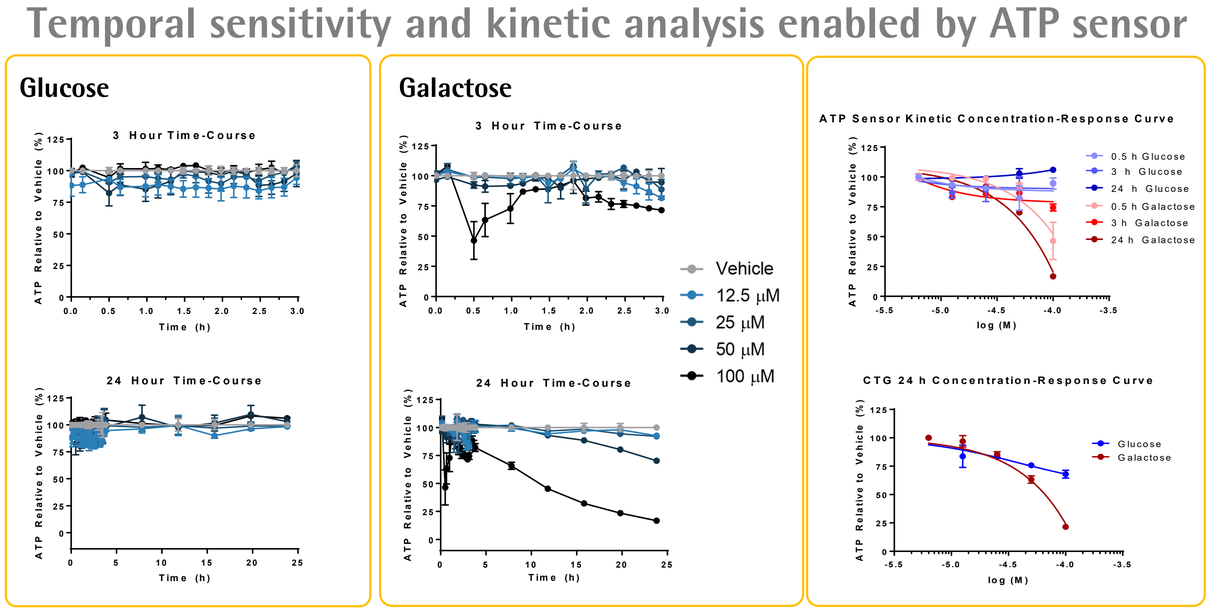


- HeLa, HepG2, and NIH 3T3 cell lines stably expressing ATP sensors were seeded overnight prior to experimentation
- Two sets of phase and fluorescent images (10X) were acquired prior to drug treatment to establish basal ATP levels, then every 15 min to monitor compound effects
- Quantification of ATP sensor fluorescence demonstrates a rapid, concentration-dependent decrease in ATP following concurrent blockade of glycolysis and OXPHOS



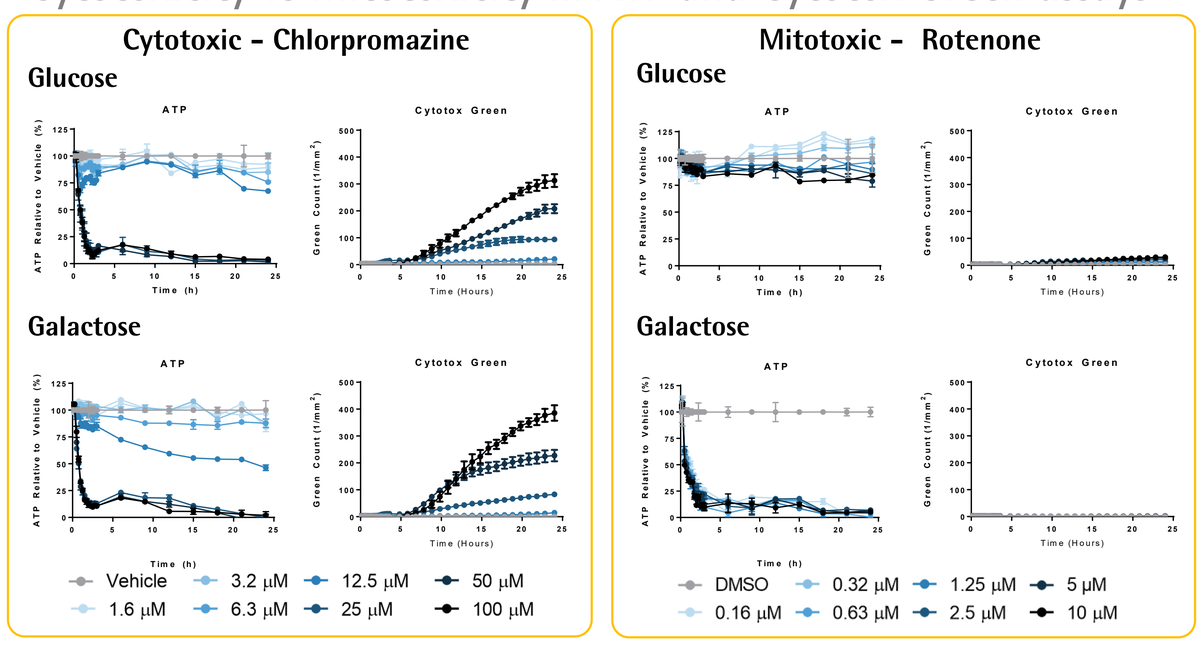
- Top: 10x image of NIH 3T3 cells stably expressing ATP sensor
- Bottom: Incucyte® generated mask of fluorescent image used for data analysis
- Color scaled image analysis of ATP sensor fluorescence (right, 20X) provides visualization of relative ATP levels in parallel with the ability to monitor for changes in morphology (phase images, left)

Temporal sensitivity and kinetic analysis enabled by ATP sensor



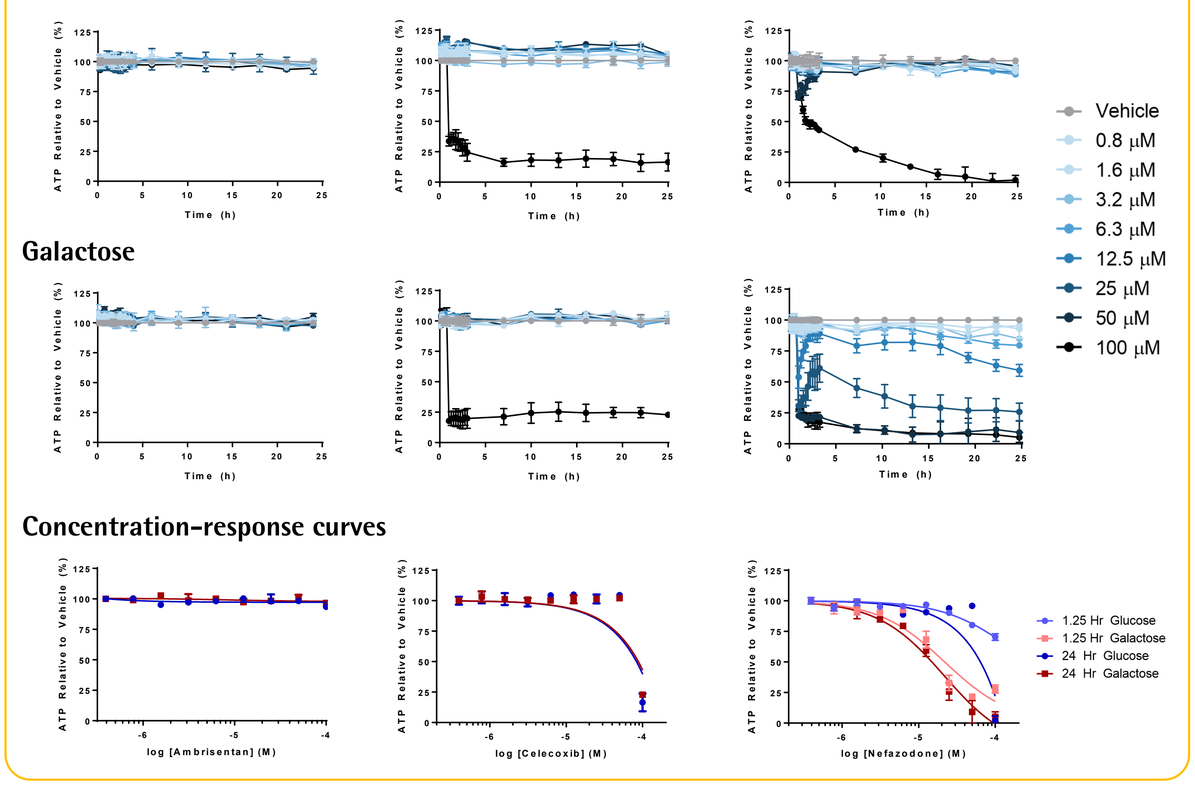
- NIH 3T3 cells stably expressing the ATP sensor were adapted to media containing either glucose or galactose prior to assay
- Cells (12K/well) were seeded overnight prior to treatment with Troglitazone
- Detection of drug effects can be observed within minutes of compound addition using the Incucyte® ATP assay
- Kinetic data allows the user to monitor drug effects, such as metabolic recovery, in real-time (top right)
- The ability to monitor changes in EC50 over time gives valuable insights into the kinetics of drug effects that may be missed with end-point biochemical assays, such as CellTiter Glo (CTG, bottom right)

Cytotoxicity vs Mitotoxicity in ATP and Cytotox Green assays



- NIH 3T3 cells (12K/well) adapted to glucose or galactose media were seeded overnight prior to experimentation
- The Incucyte® Cytotox Green and ATP sensor assays were run in parallel on duplicate plates
- ATP sensor data align with expected observations: cytotoxic compounds induce a similar reduction in ATP across media conditions, while cells grown in galactose are more sensitive to mitotoxic compounds
- Chlorpromazine treatment produces similar qualitative results in both assays, with the ATP sensor providing enhanced temporal resolution
- Rotenone induces little to no Cytotox Green response during the 24-h time course, highlighting the ATP sensor's ability to measure sensitive metabolic changes in response to mitochondrial toxicity

Mitochondrial toxicity screening application



- NIH 3T3 cells (12K/well) adapted to glucose or galactose were seeded overnight prior to experimentation
- Images (10X) were acquired every 15 minutes for 3 hours following compound addition to detect transient changes in ATP, then every 3 hours through 24 hours to monitor stability of more persistent responses
- Data represent typical screening results of compounds validated to be nontoxic, cytotoxic, and mitotoxic by additional methods
- Note that stability profiles of ATP depletion vary across compounds, and earlier time points can be useful for making comparisons between related compounds