The Application of Cell-Based Impedance Technology in Drug Discovery

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Cell Biology and Assay Development
ACEA Biosciences

ACEA
Biosciences, Inc.

ACEA Biosciences

- **Founded in early 2002**

- **Located in San Diego, CA**

- **Mission: Integration of microelectronics with cell biology and molecular biology for providing innovative and cost-effective microelectronic biological analysis systems and applications for life science industry and clinical diagnostics**

- **ACEA's first product was marketed under the brand name of RT-CES system in 2004**

- **In November of 2007 Roche and ACEA Biosciences entered into an exclusive agreement for the development, supply and distribution for ACEA Bioscience's real-time cell assay technology. Under the terms of the agreement, RAS will exclusively market systems for real-time cell analysis, based on ACEA Bioscience's impedance-based technology**

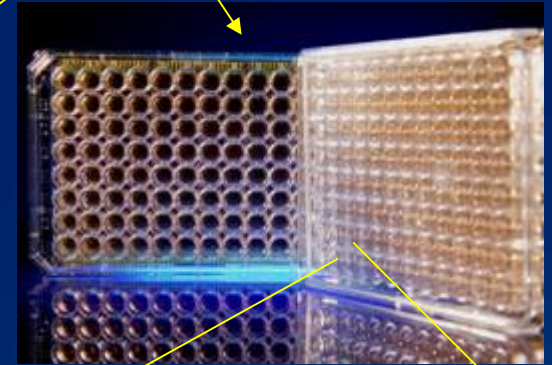- **The first joint Roche/ACEA product is marketed under the brand name of xCELLigence**

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xCELLigence RTCA SP System

Computer and Software

E-Plate

Analyzer

Plate Reader(RTCA SP) (in CO2 incubator)

Gold Microelectrode Covers 80% of Well Area

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Electronic Sensor Technology Applied to Cell Biology: Principle of Operation

Derivation of Cell Index

A dimensionless parameter termed Cell Index (CI) is derived as a relative change in measured electrical impedance to represent cell status.

Several features of the CI are summarized:

1. When cells are not present or are not well-adhered on the electrodes, then the CI is zero

2. Under the same physiological conditions when more cells are attached on the electrodes, then the CI values are larger. Thus, CI is a quantitative measure of cell number present in a well.

3. Additionally, change in a cell status, such as cell morphology, cell adhesion or cell viability will lead to a change in CI.

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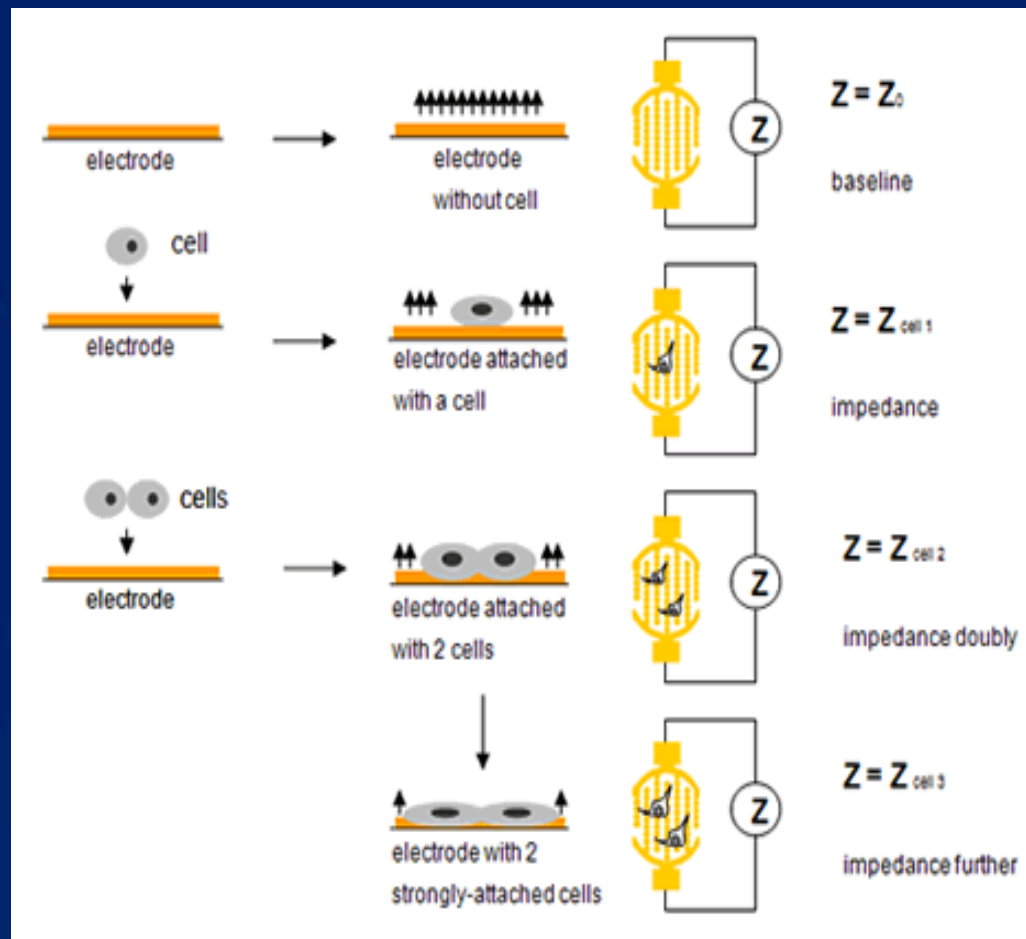
Advantages of xCELLigence System for Cell-Based Assays and Drug Discovery Applications

- **Label free, no reporters**
- **Non-invasive measurement**
- **Real-time Monitoring**
  - **-Short-term (milliseconds)**
  - **-Long-term (days and weeks)**
- **Continuous QC**

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Cell-based Assays:

Traditional Methods

Seed
cells

Treatment:
e.g.: drug compound

Traditional methods:
Labeling (e.g. optical)
& **End-point** Measurement

Initiate
Experiment

Black Box

1-24 Hours

Data
Analysis

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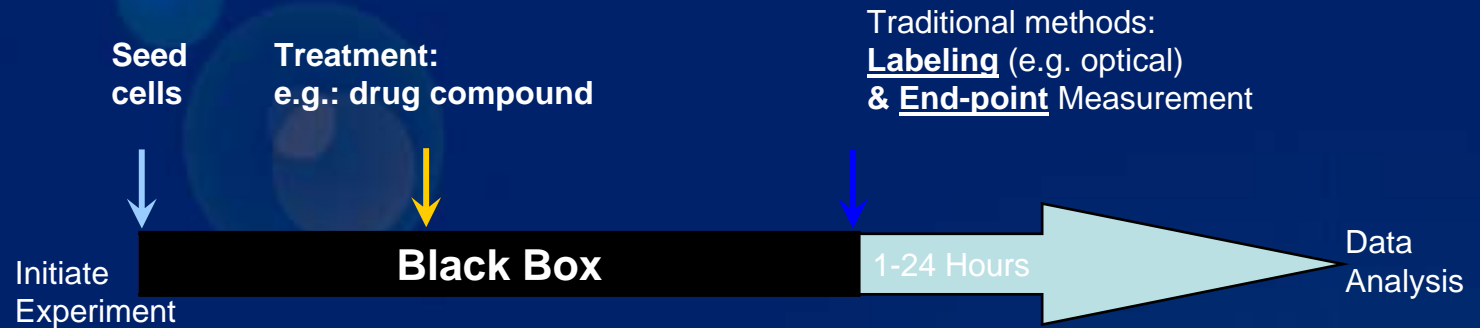
Cell-based Assays:

Traditional Methods vs xCELLigence System

Seed cells

Treatment: e.g.: drug compound

Traditional methods:
Labeling (e.g. optical)
& End-point Measurement

Initiate Experiment

| 18-24 Hours | 24-48 Hours | 1-24 Hours |

Data Analysis

Treatment

Normalized Cell Index

- Control
- Cmpd A
- Cmpd B
- Cmpd C
- Cmpd D

Time (Hours)
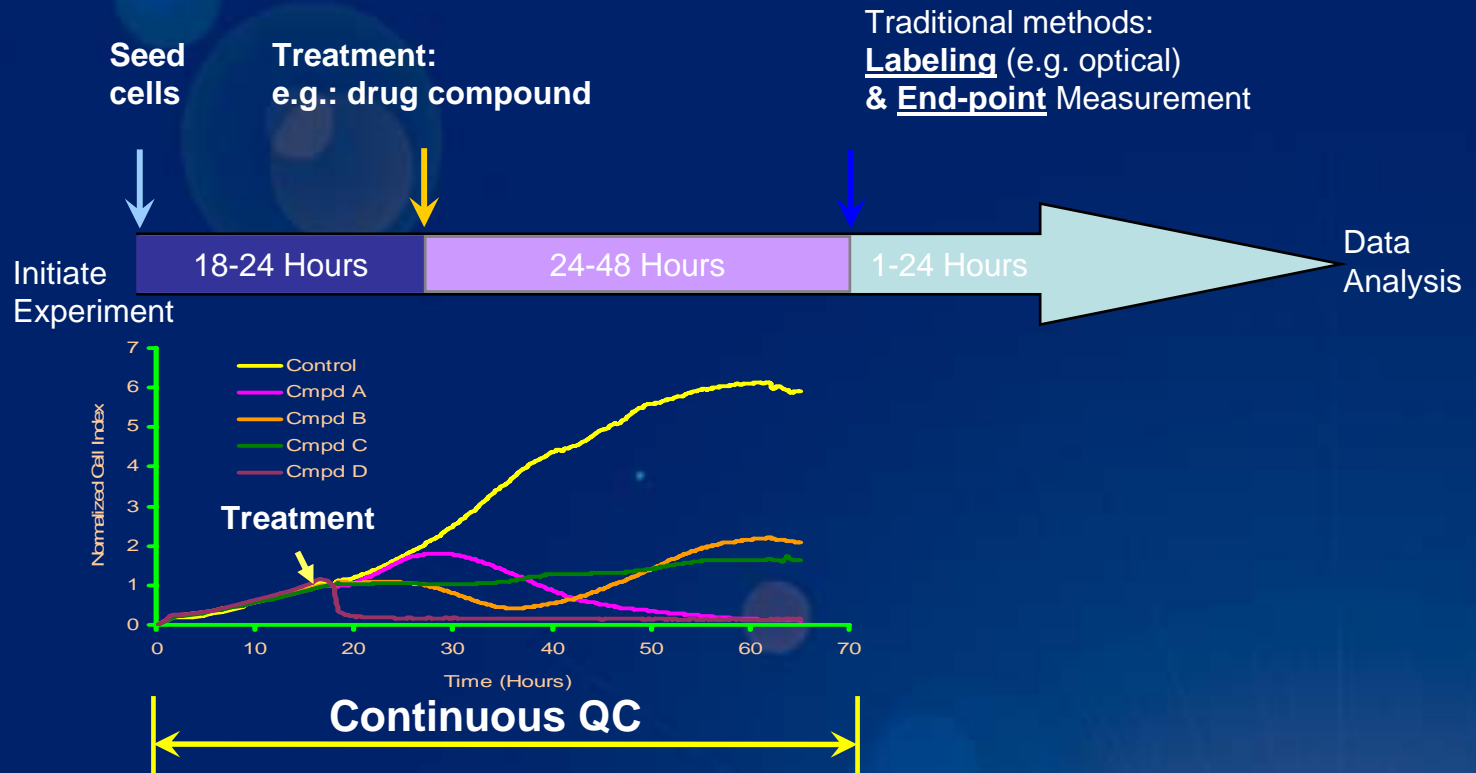
Continuous QC

xCELLigence System :

- **Label-free:** Electronics-based detection
- **Real-time:** Continuous measurement, data analysis and display
- **Therefore, both short term and long term compound effects can be captured**

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Applications Developed on the xCELLigence System

- Cell Proliferation
- Cell Quality
- Compound-mediated Cytotoxicity
- Cell-mediated Cytotoxicity
- Cell Adhesion and Spreading
- Functional Monitoring of Receptor Tyrosine Kinase Signaling
- Functional Monitoring of GPCR Signaling
- IgE Receptor Function
- Cell Invasion and Migration
- Barrier Function
- Viral Cytopathogenecity

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Applications Developed on the xCELLigence System

- Cell Proliferation
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- Compound-mediated Cytotoxicity
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Time-Dependent Cell Response Profiling

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The Road to Cellular Cytotoxicity Takes Many Twists and Turns
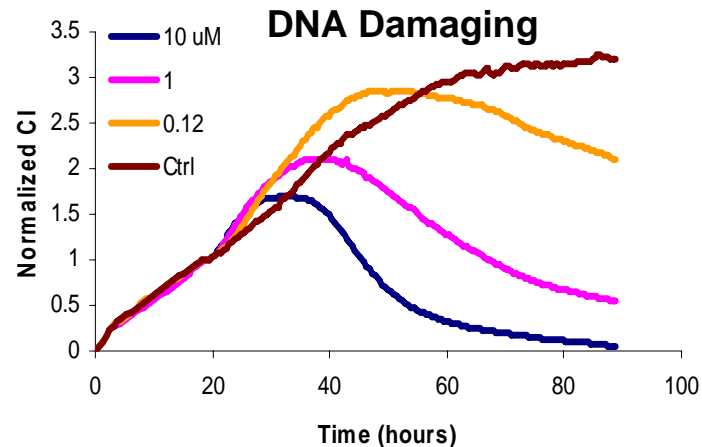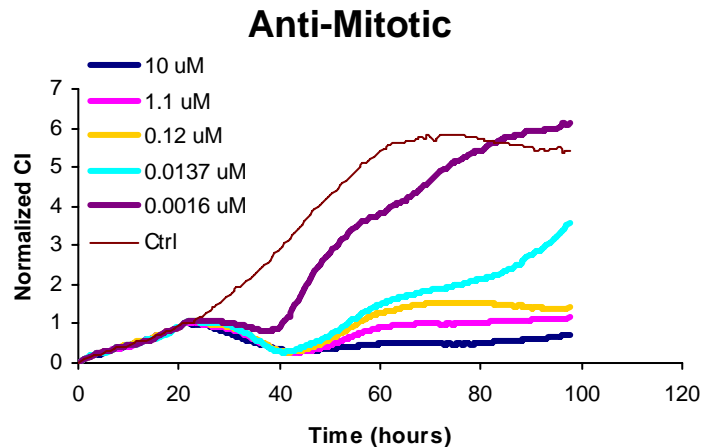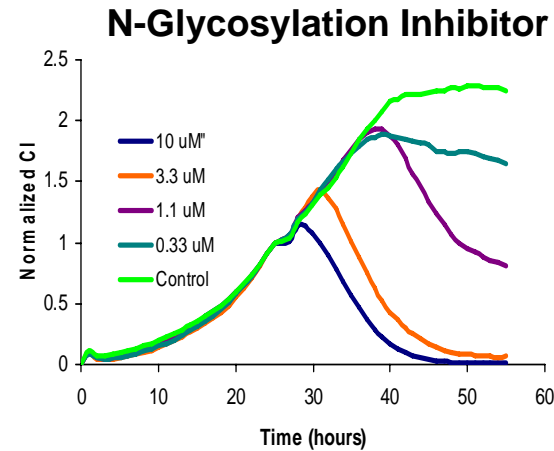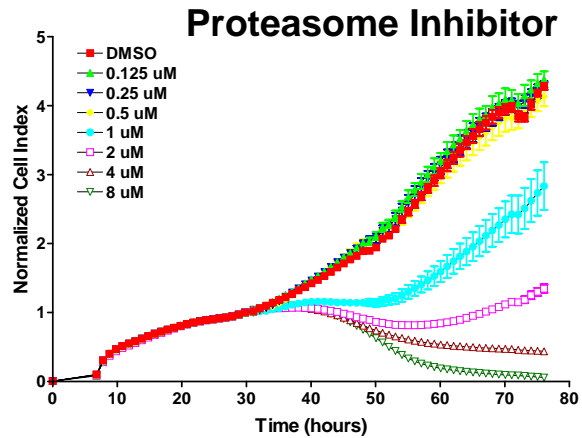
Are Impedance-Based Cell Response Profiles Predictive of Biological Mechanism ?

TCRP Approach

E-Plate

Add Cells → Monitor 20-24 hours → Add Compound → Monitor 48 hours

Interdigitated gold microelectrodes

Real-Time Continuous Monitoring

Impedance-based real-time cellular response profile
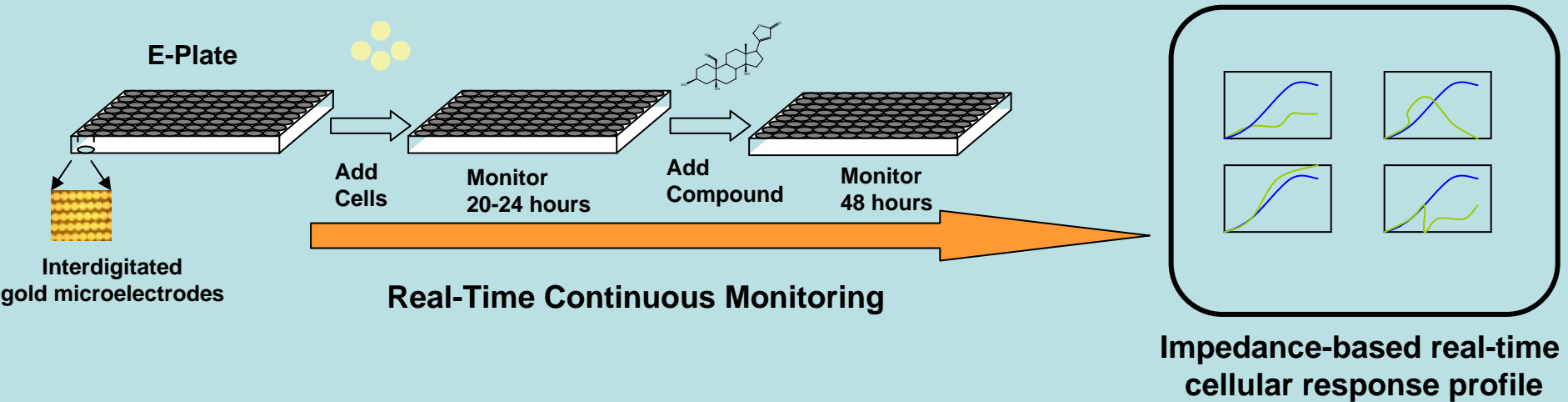
Seed 4000 A549 Cancer Cells in 96 well E-Plates

↓

Treat with Compounds at a final concentration Of 20 μM

Spectrum Compound Library from MS Discovery
(Collection of FDA approved drugs, nature compounds
Experimental compounds, insecticides and herbicides)

↓

Monitor the cellular response for 48 hours

↓

Compare cytological profiles

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Hit Selection and Clustering Analysis

TCRP from Screen

Short-term response
(w/in 1 hour)

Long Term Response
(1-48 hours)

Hit criteria
(25% of control)

Hit criteria
(25% and 40%)

TCRP with Known Mechanisms
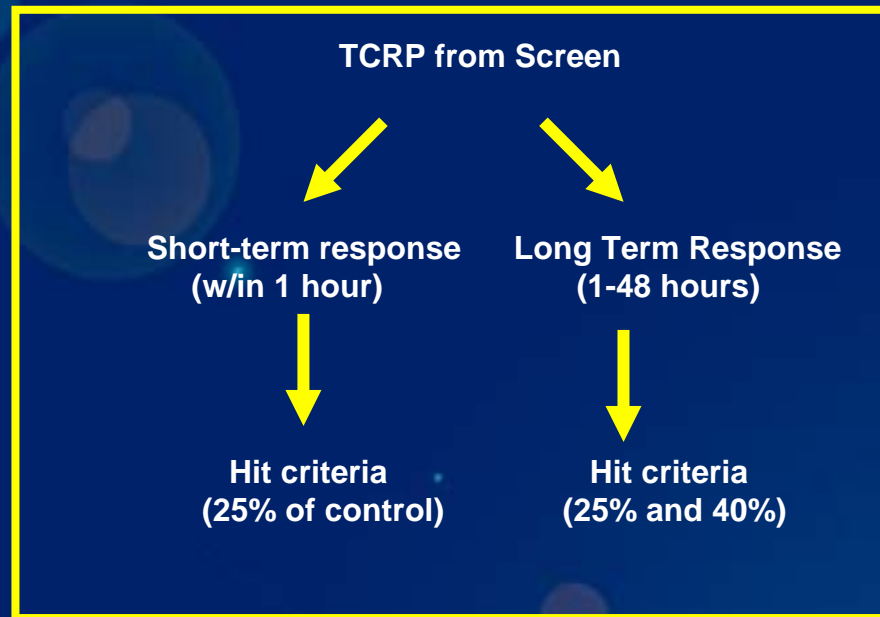
TCRP with Known Mechanisms

Short-Term Response

Anti-Histamine

Serotonin Receptor Antagonist

L-Type Voltage-gated Ca Channel Inhibitor

Long-Term Response

Anti-mitotics

Protein-Synthesis

DNA Damaging

Nuclear Hormone

HDAC Inhibitors
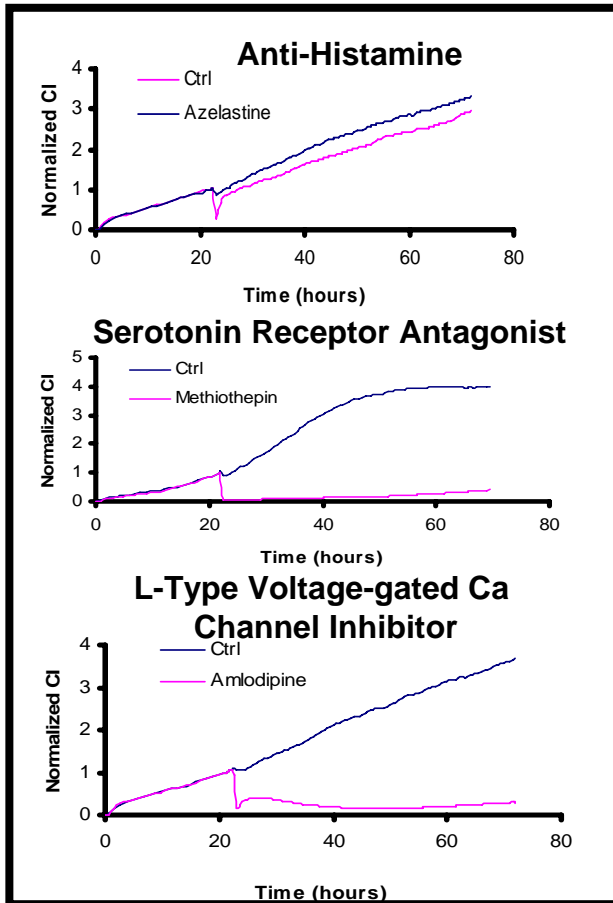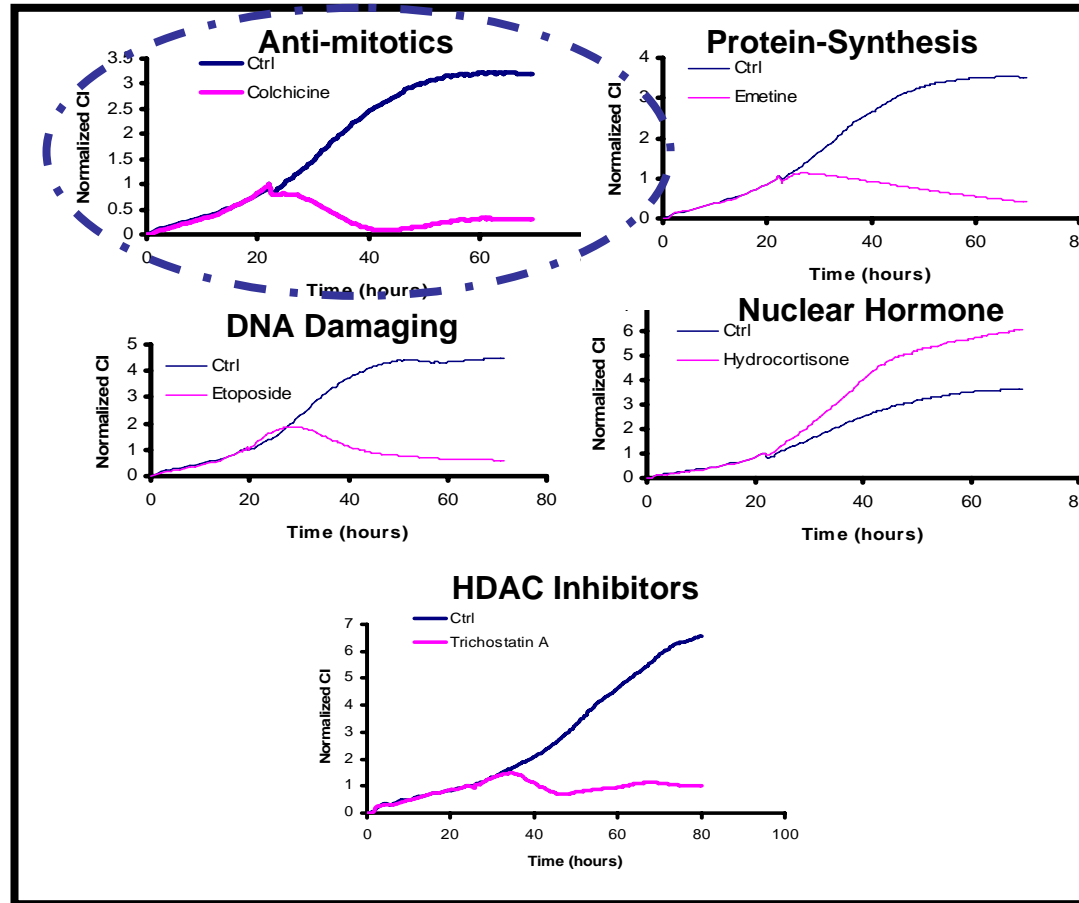
TCRP of Anti-mitotic Compounds
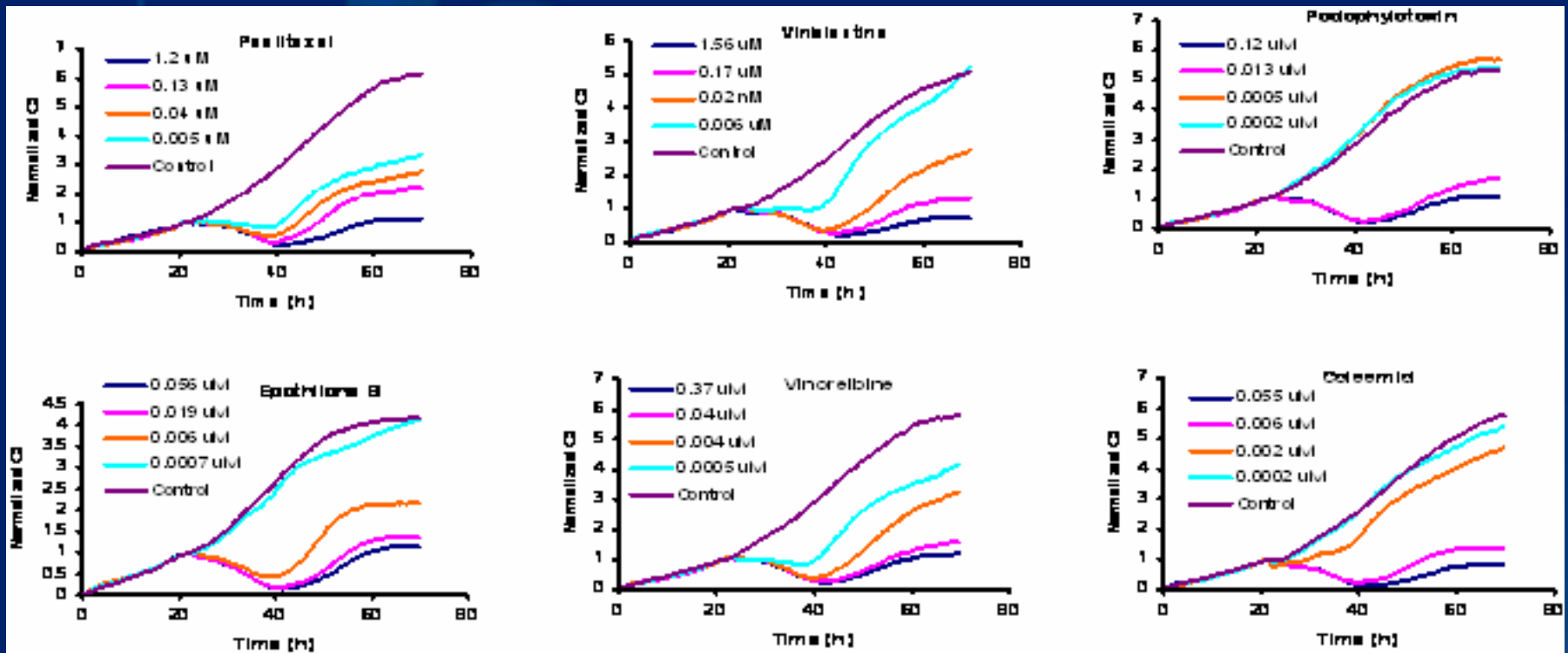
Characterization of Impedance-Based Anti-mitotic Profile

Ctrl

6 h (Phase I)

14 h (Phase II)

24 h (Phase III)

48 h (Phase IV)

Mitotic Index

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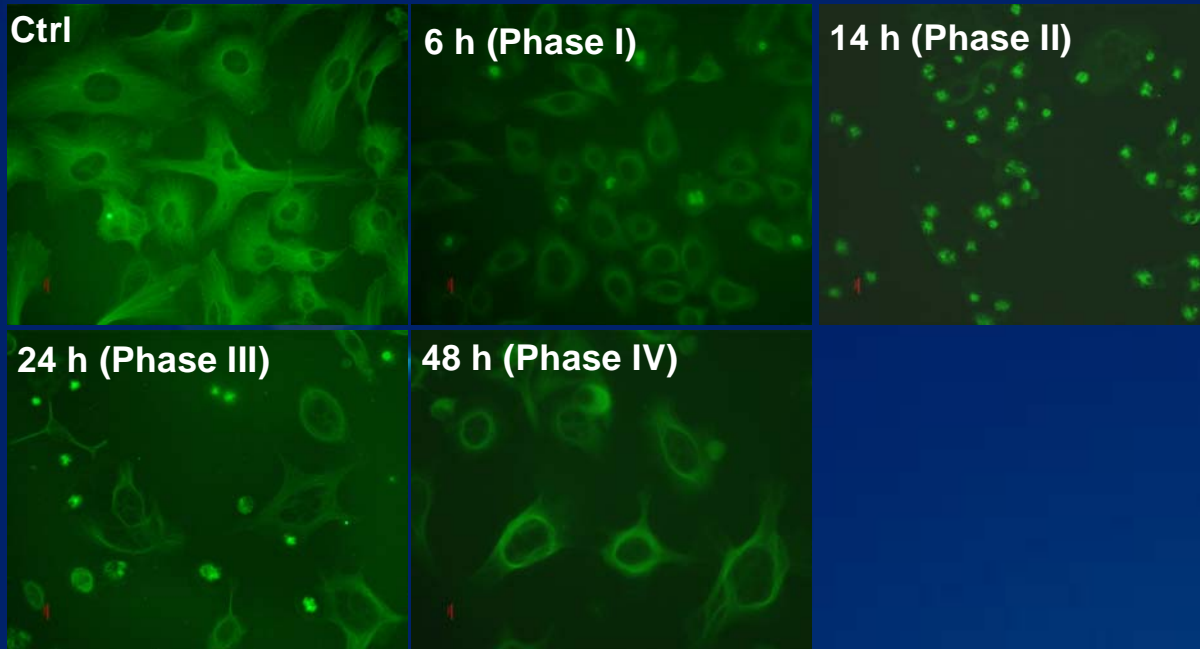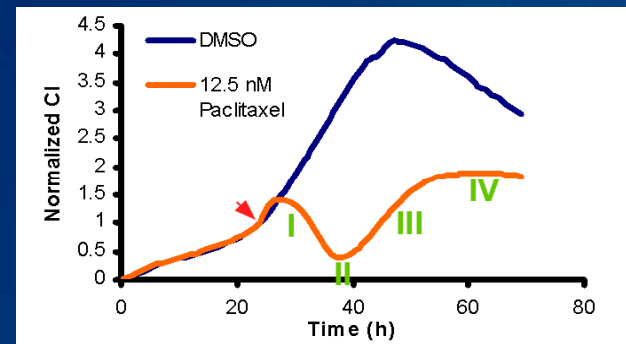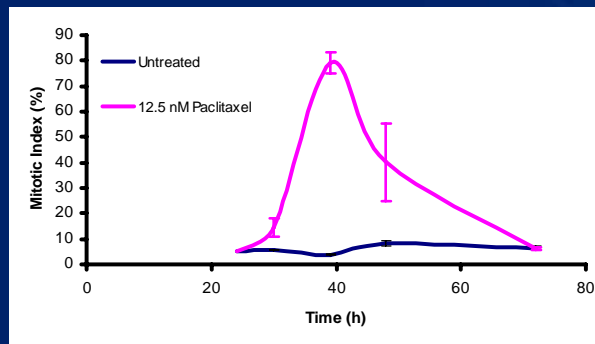
Validation of Mitotic Arrest Profile

Eg5 Small Molecule Inhibitor

	Ctrl	S-Trityl-L-Cysteine
anti-tubulin Ab		
anti- PH3		

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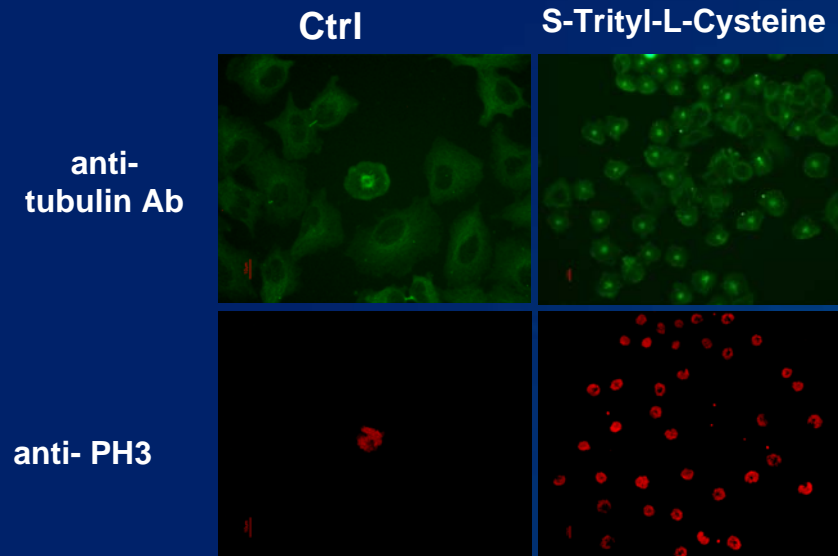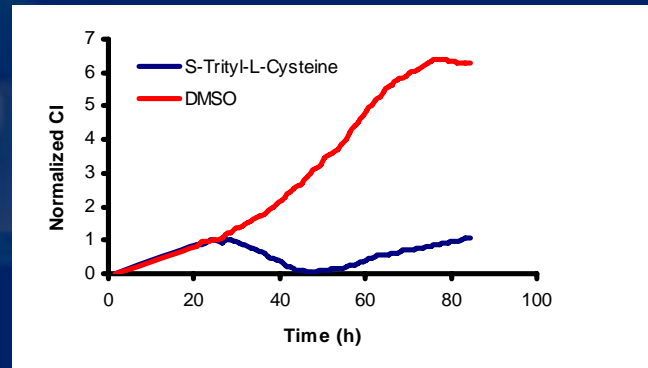
Compounds with Anti-mitotic Profile from the Spectrum Collection
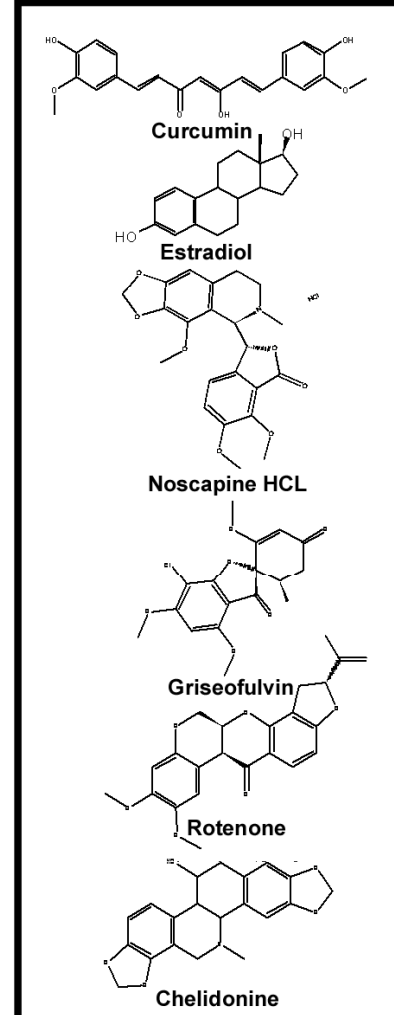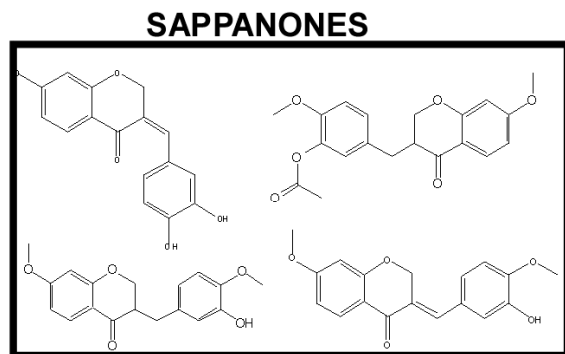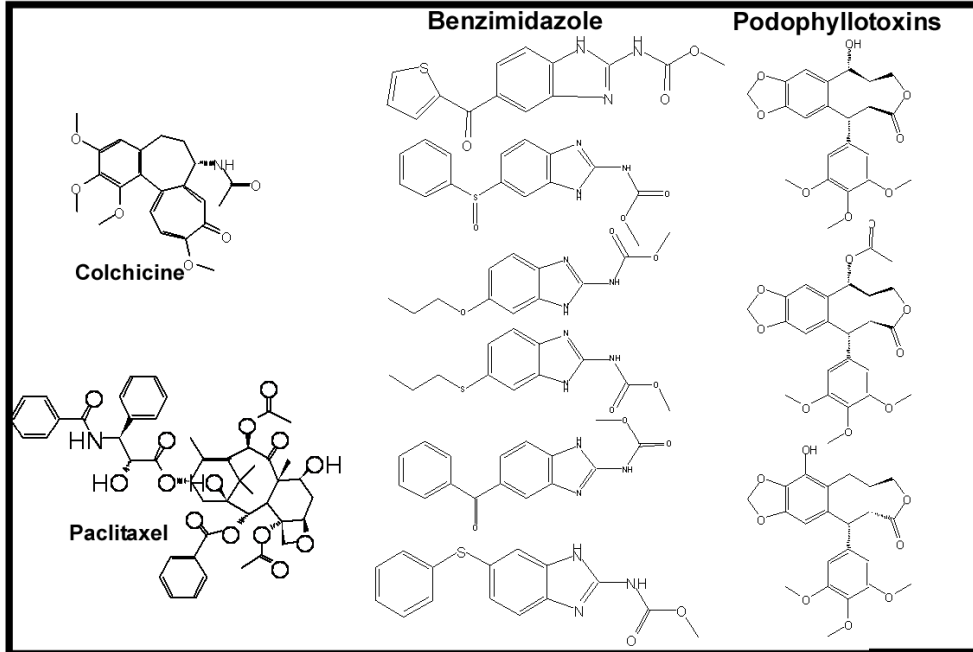
Systematic Analysis of Impedance-Based Cell Response Profiles

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Curve Classification Algorithm and Display

Clustering Analysis

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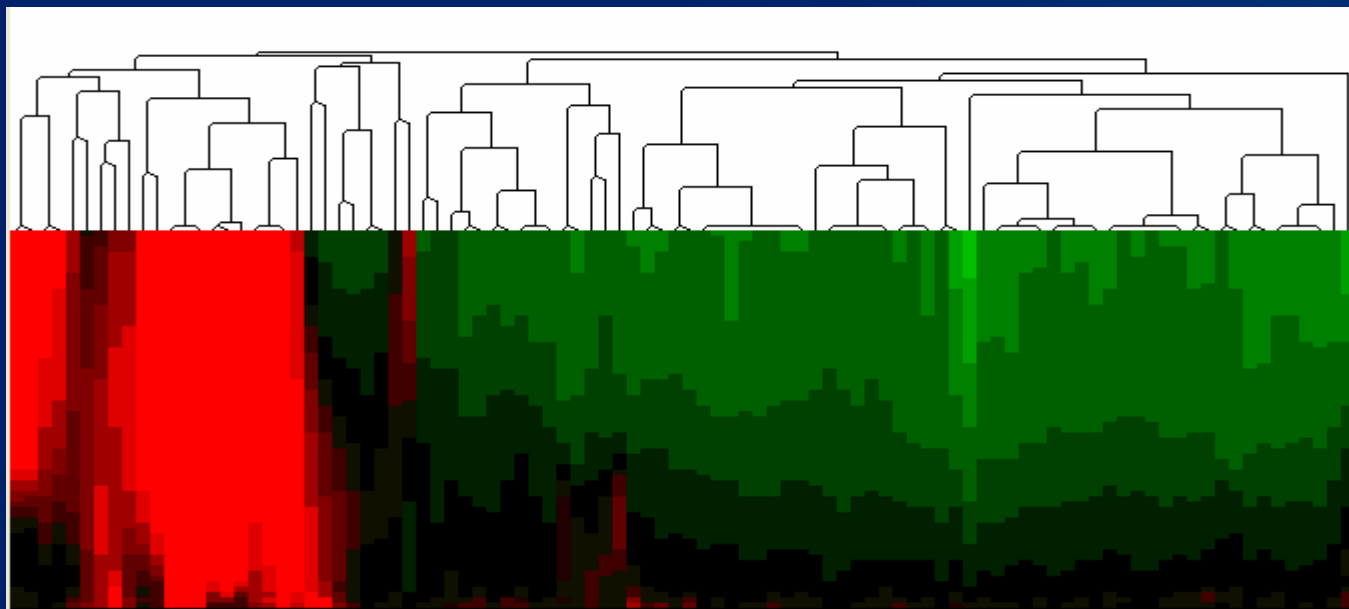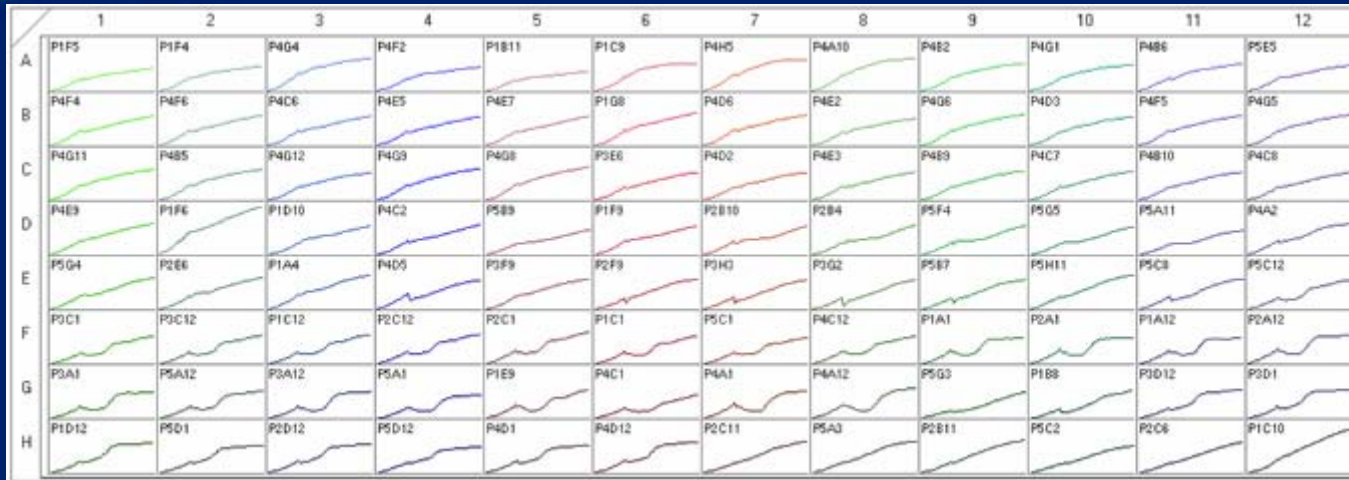
TCRP COX-2 Inhibitors
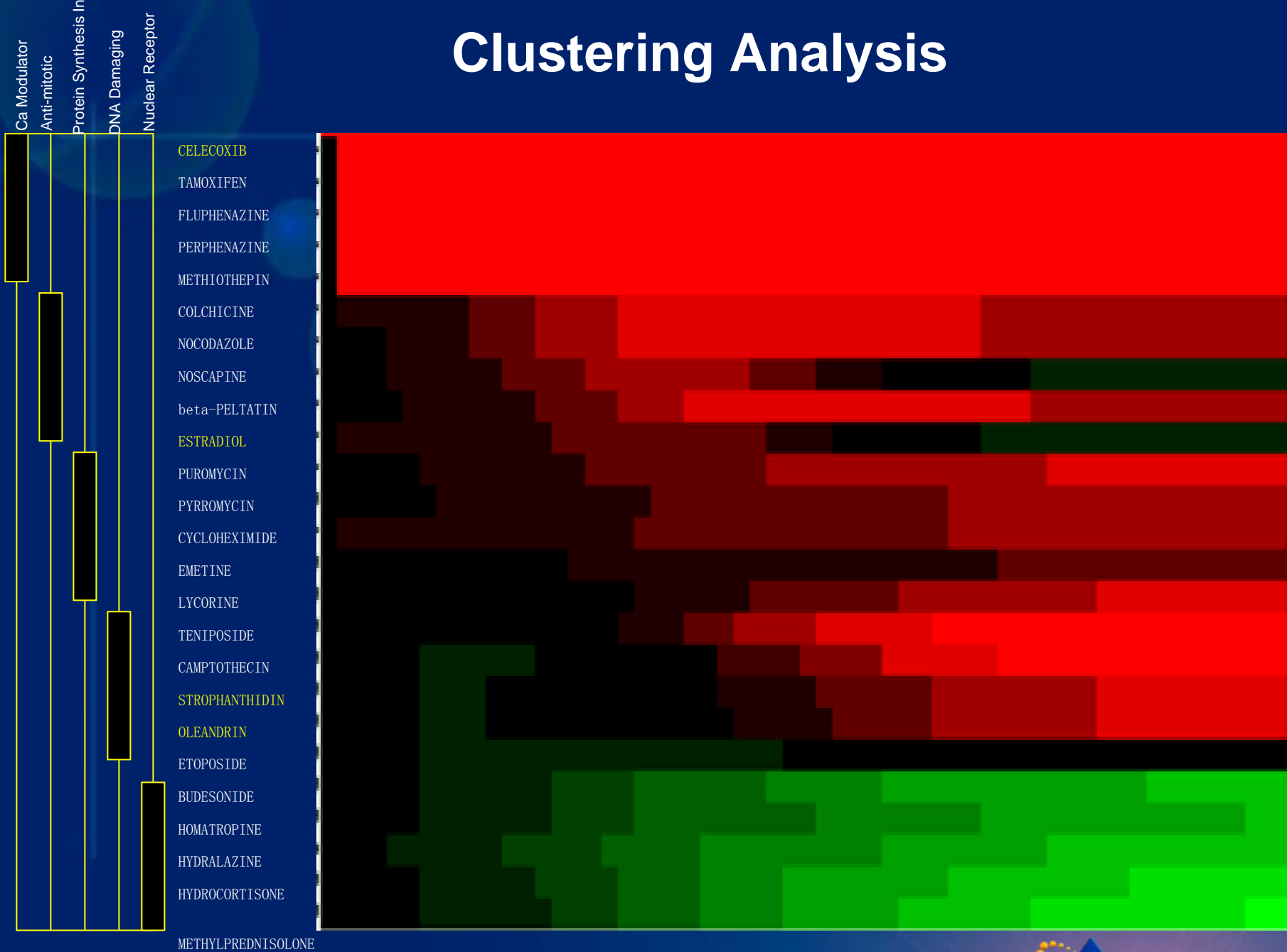
TCRP COX-2 Inhibitors
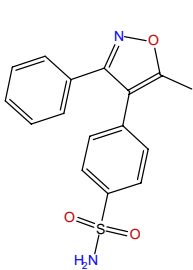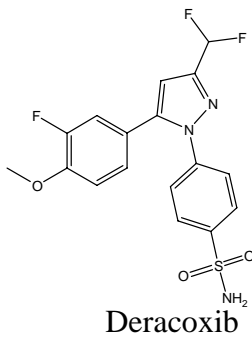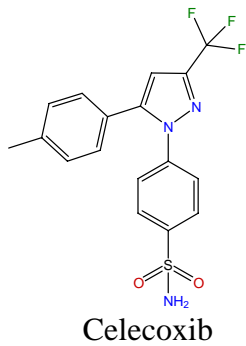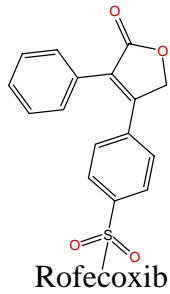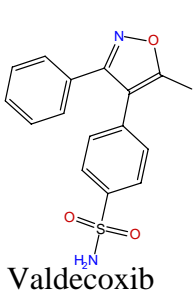
COX-2 Inhibitors: Valdecoxib, Rofecoxib, Celecoxib, Deracoxib with Normalized CI vs Time (hours) plots for Valdecoxib, Rofecoxib, Celecoxib, and Deracoxib.
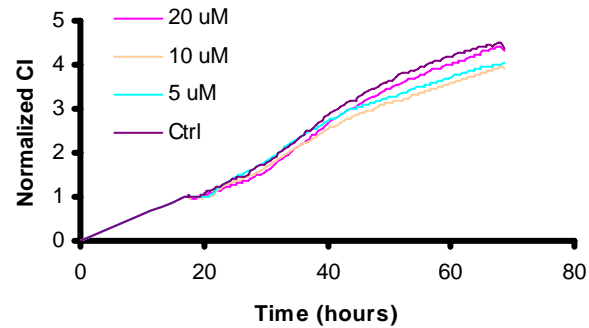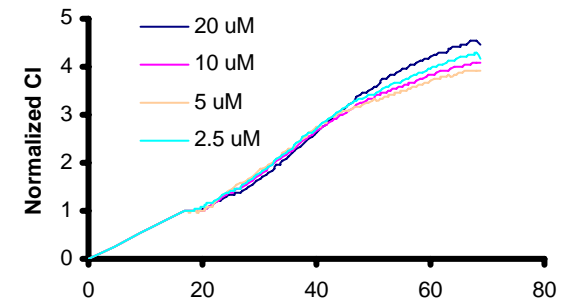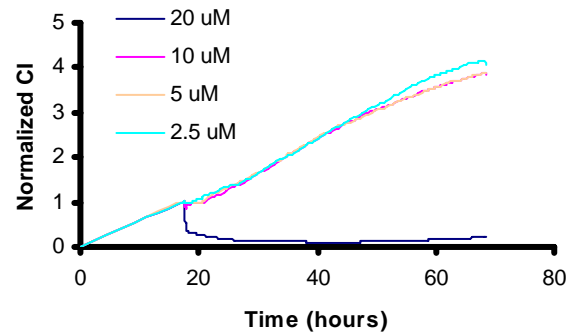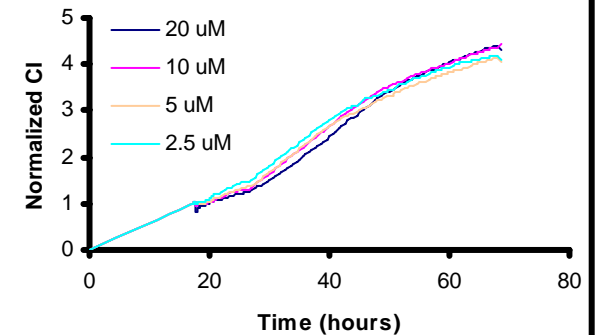
Validation of Celecoxib as Modulator of Intracellular Calcium Levels
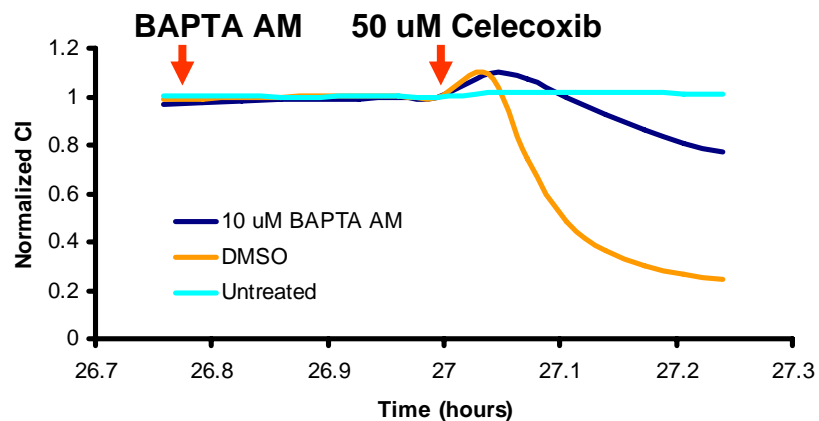
Validation of Celecoxib as Modulator of Intracellular Calcium Levels

Advantages of Short Term and Long Term Monitoring of Cellular Response Profiles
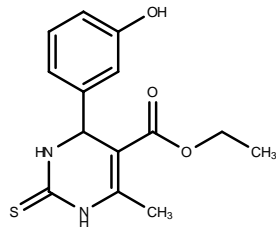
Monastrol

HeLa Cells
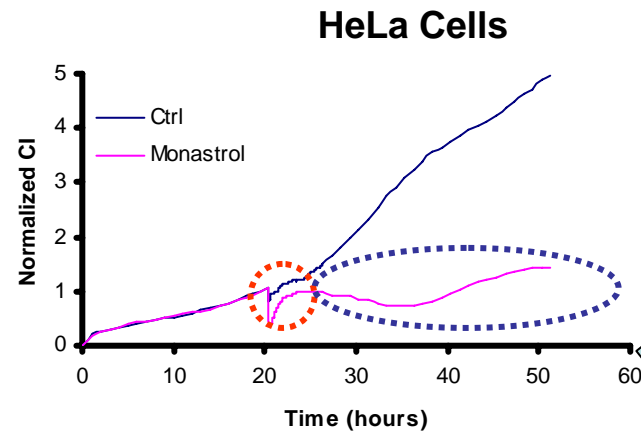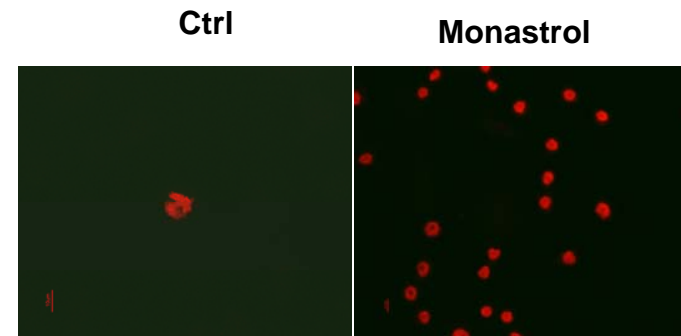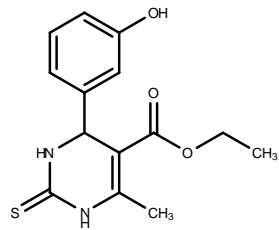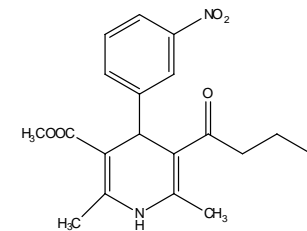
Advantages of Short Term and Long Term Monitoring of Cellular Response Profiles

Monastrol

HeLa Cells

Advantages of Short Term and Long Term Monitoring of Cellular Response Profiles

Monastrol

HeLa Cells

Advantages of Short Term and Long Term Monitoring of Cellular Response Profiles

Monastrol

HeLa Cells

Long Term

Ctrl **Monastrol**

Phospho-Histone H3

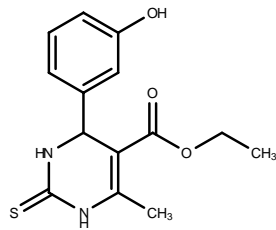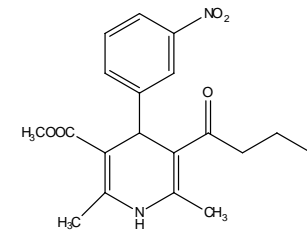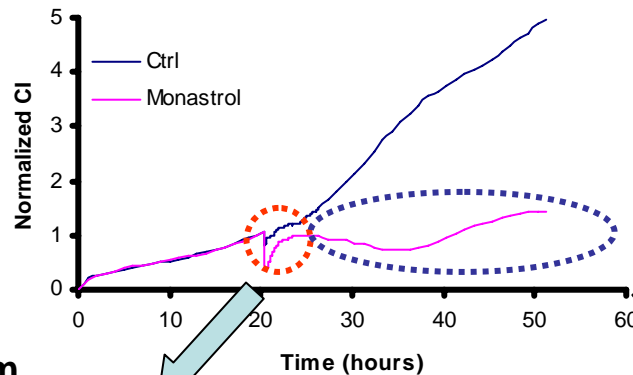# Advantages of Short Term and Long Term Monitoring of Cellular Response Profiles

Monastrol

HeLa Cells
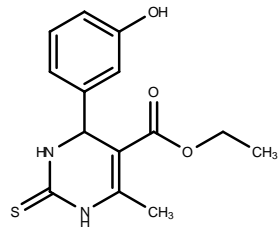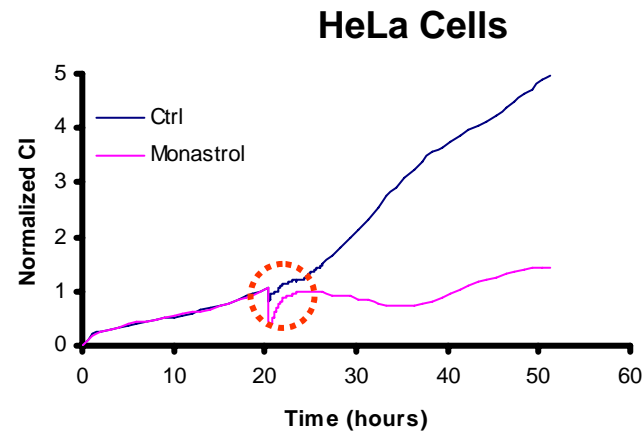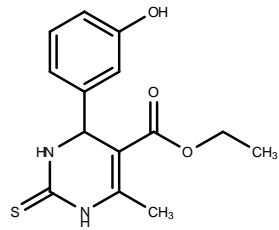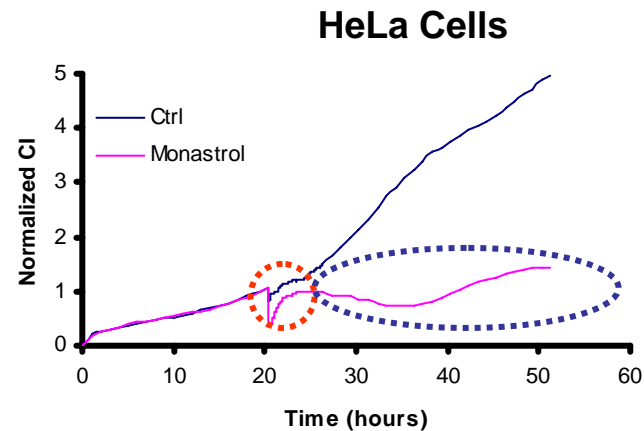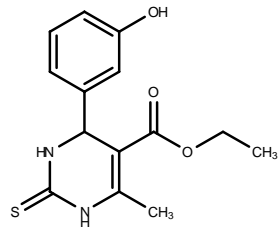
nifedipine

Long Term

Ctrl   Monastrol

Phospho-Histone H3

Advantages of Short Term and Long Term Monitoring of Cellular Response Profiles

Monastrol

HeLa Cells

nifedipine
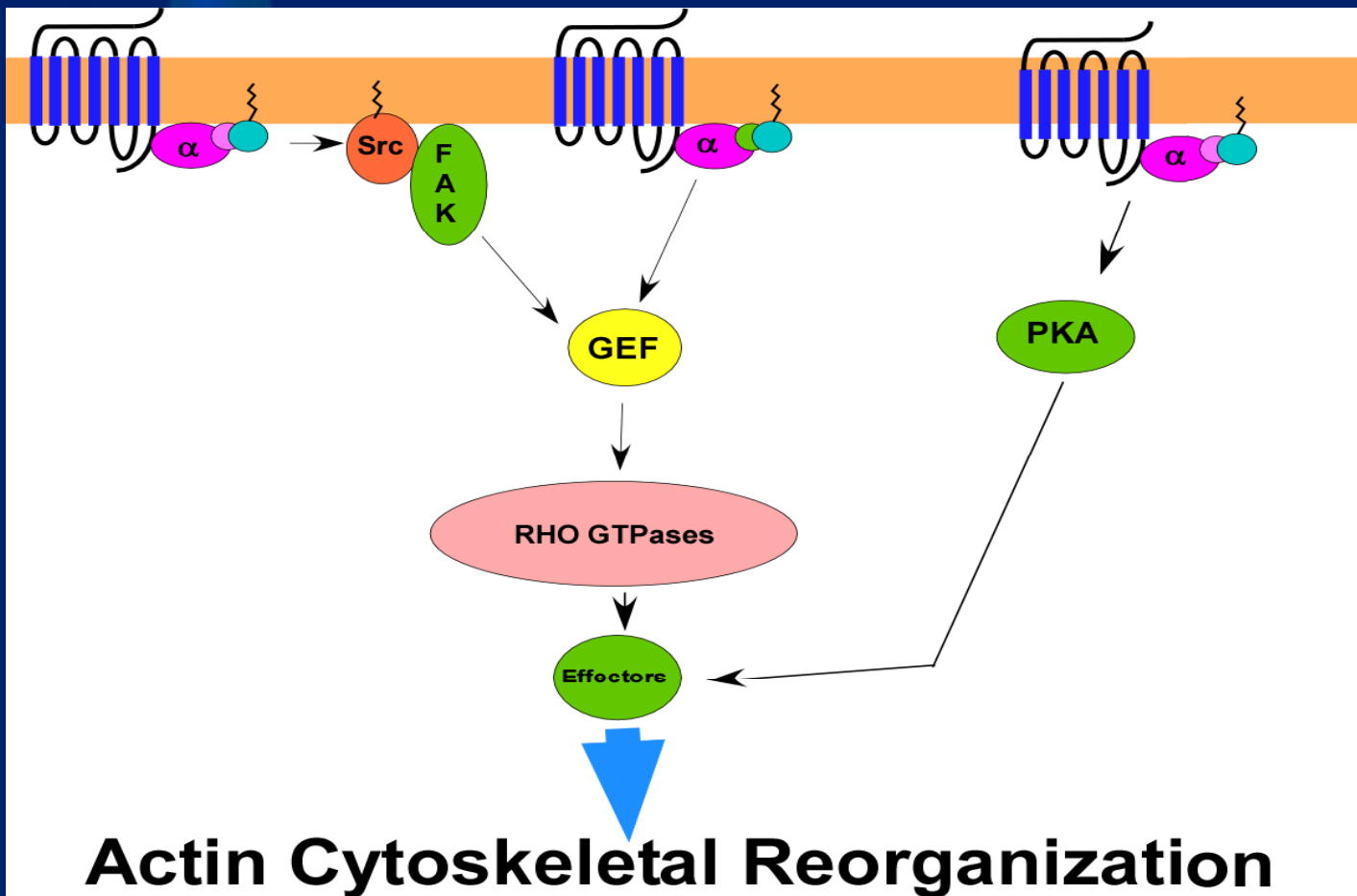
Short Term

Voltage-Gated Calcium Level Modulation

Long Term

Ctrl   Monastrol

Phospho-Histone H3

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Functional Monitoring of GPCR Signaling

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Biosciences, Inc.
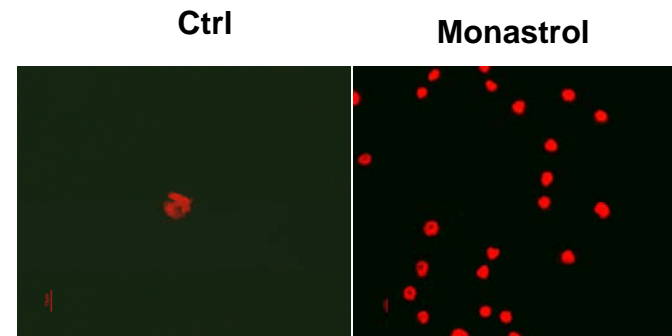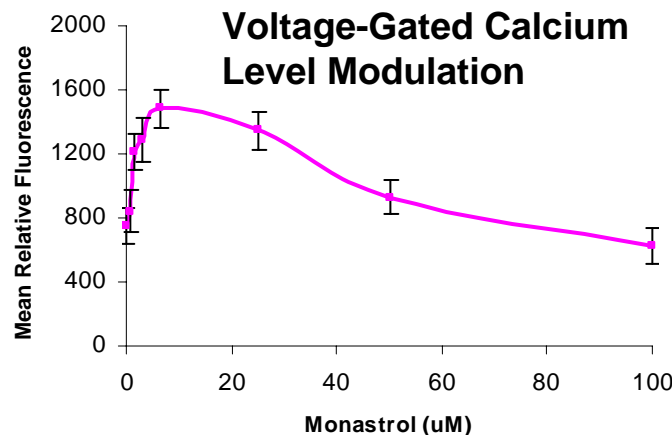
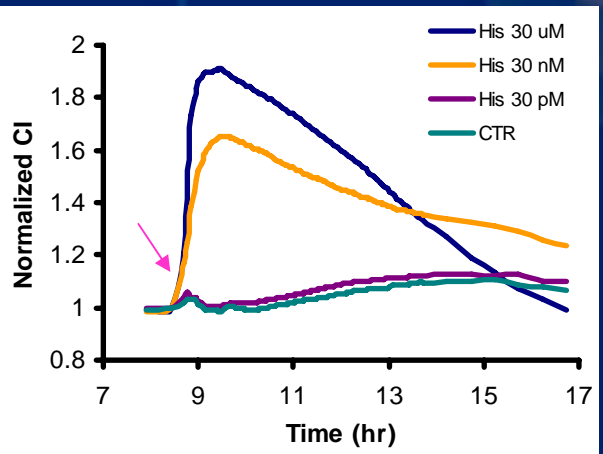# GPCR Activation Leads to Modulation of the Actin Cytoskeleton

Dynamic Monitoring of GPCR-mediated Morphological Dynamics Using the xCELLigence RTCA System
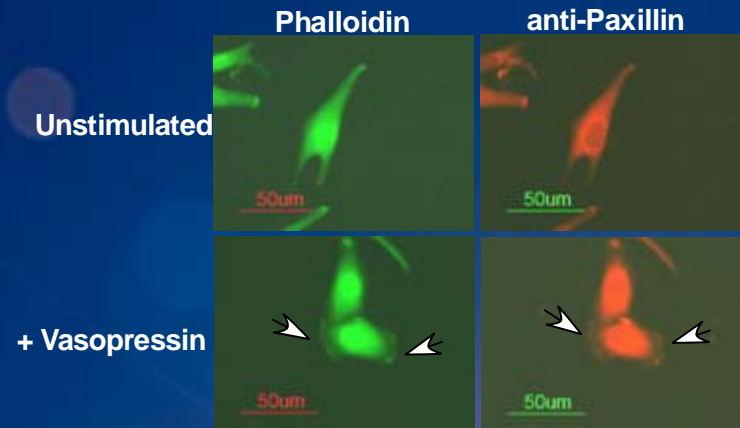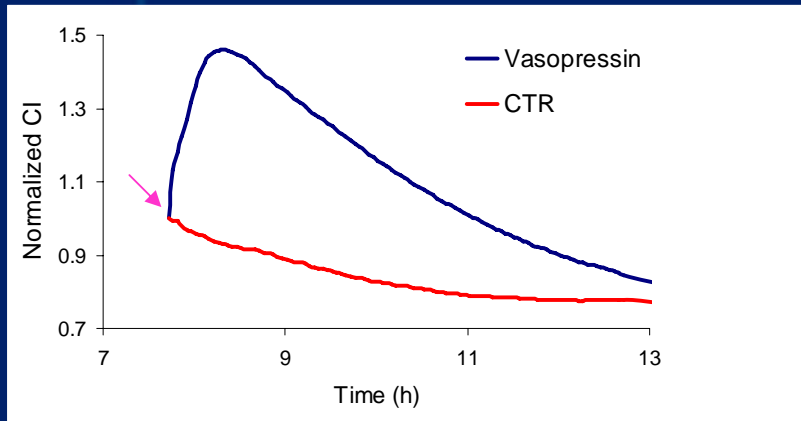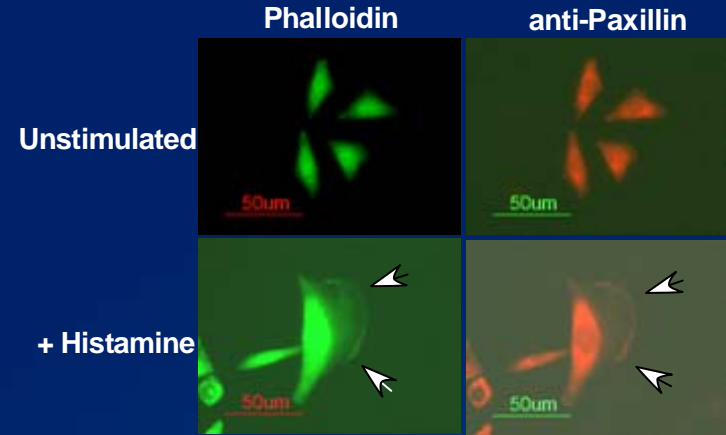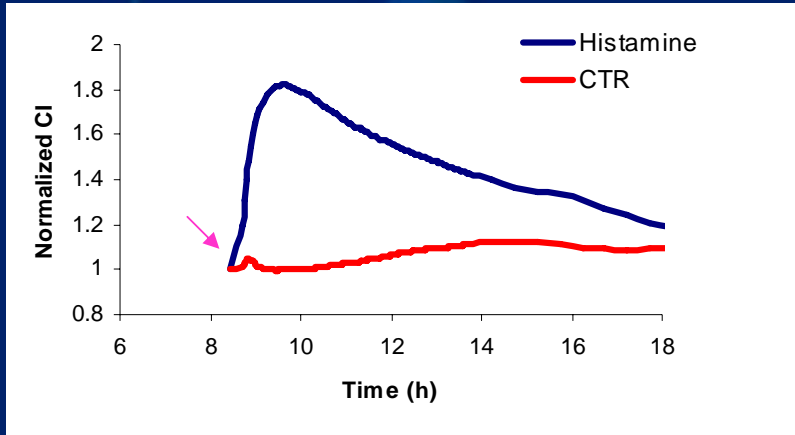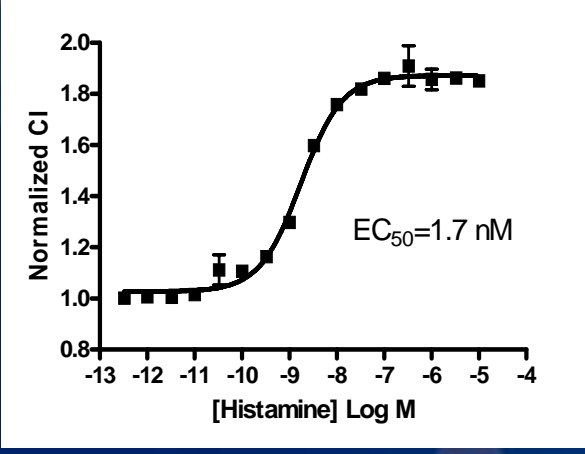
Functional Monitoring of Gq Coupled Receptors on the xCELLigence RT-CA System
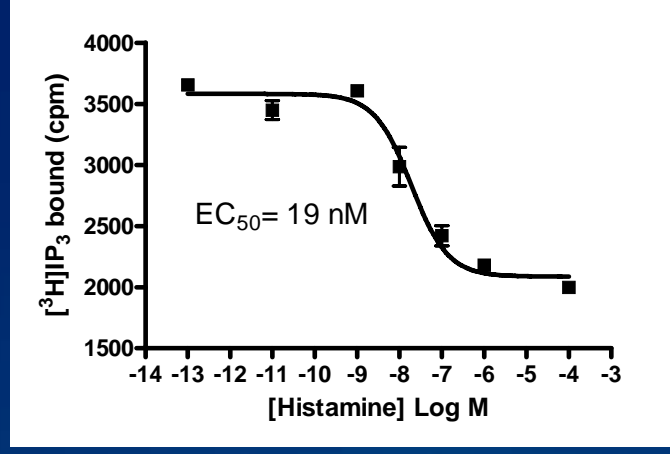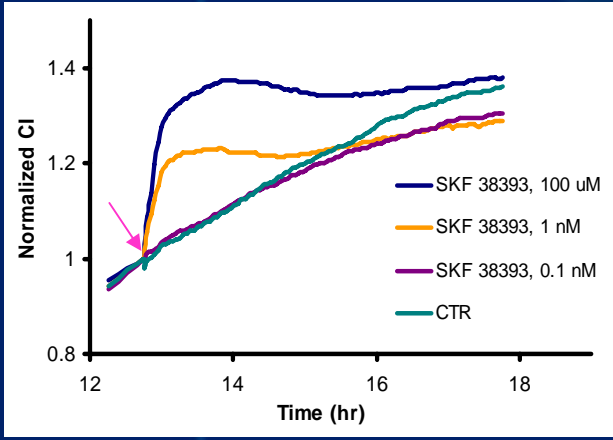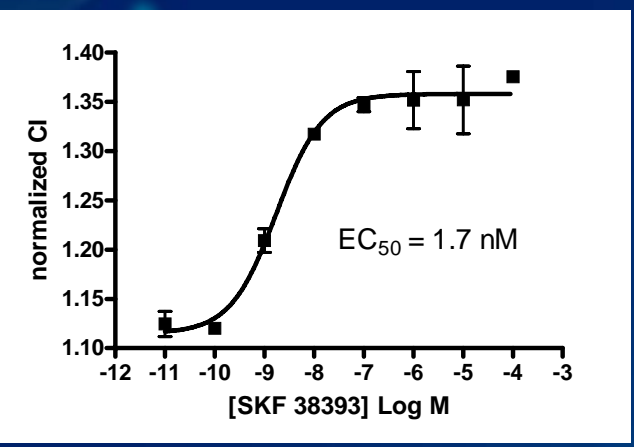
Functional Monitoring of G_s Coupled Receptors on the xCELLigence RT-CA System

Dopamine1 Receptor

RT-CA Assay

cAMP Assay

Normalized CI vs Time (hr), Dopamine1 Receptor plot with curves labeled: SKF 38393, 100 uM; SKF 38393, 1 nM; SKF 38393, 0.1 nM; CTR

RT-CA Assay: normalized CI vs [SKF 38393] Log M, $EC_{50} = 1.7$ nM

cAMP Assay: [cAMP] pmoles/well vs [SKF 38393] Log M, $EC_{50} = 1.9$ nM
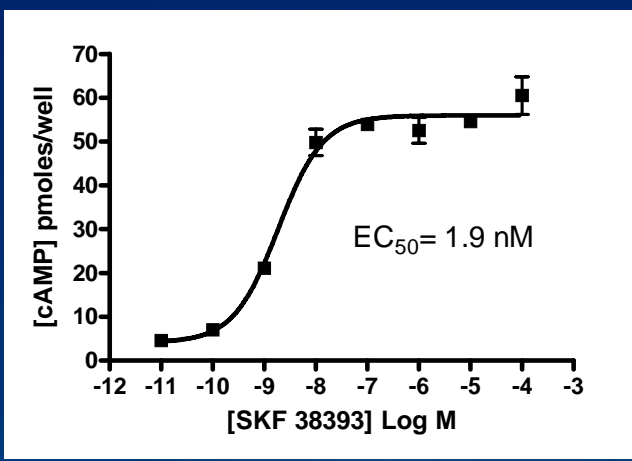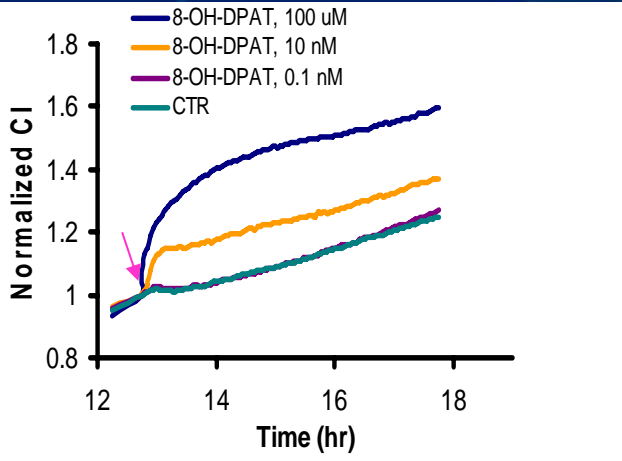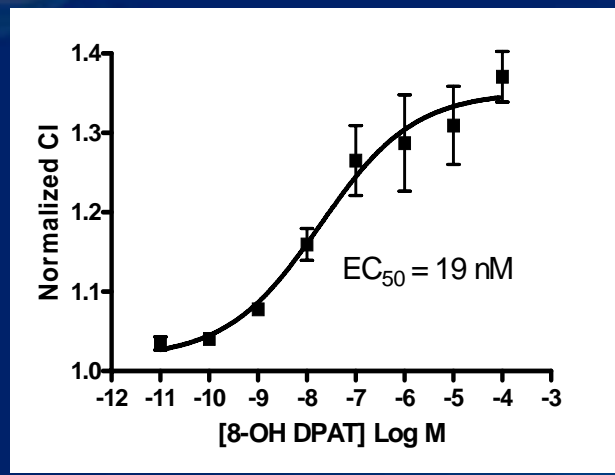
Functional Monitoring of G_i Coupled Receptors on the xCELLigence RT-CA System

5-HT1A Receptor

RT-CA Assay

cAMP Assay

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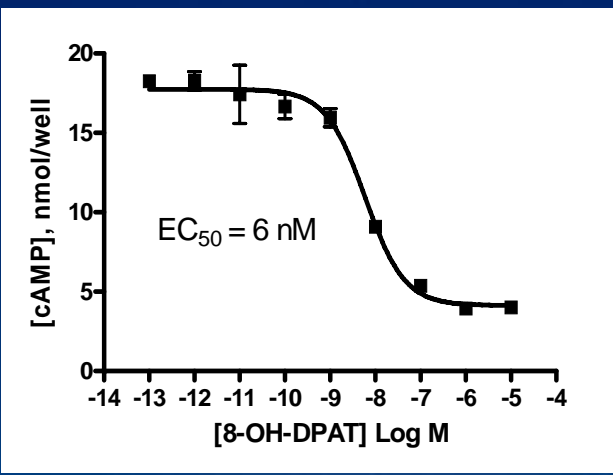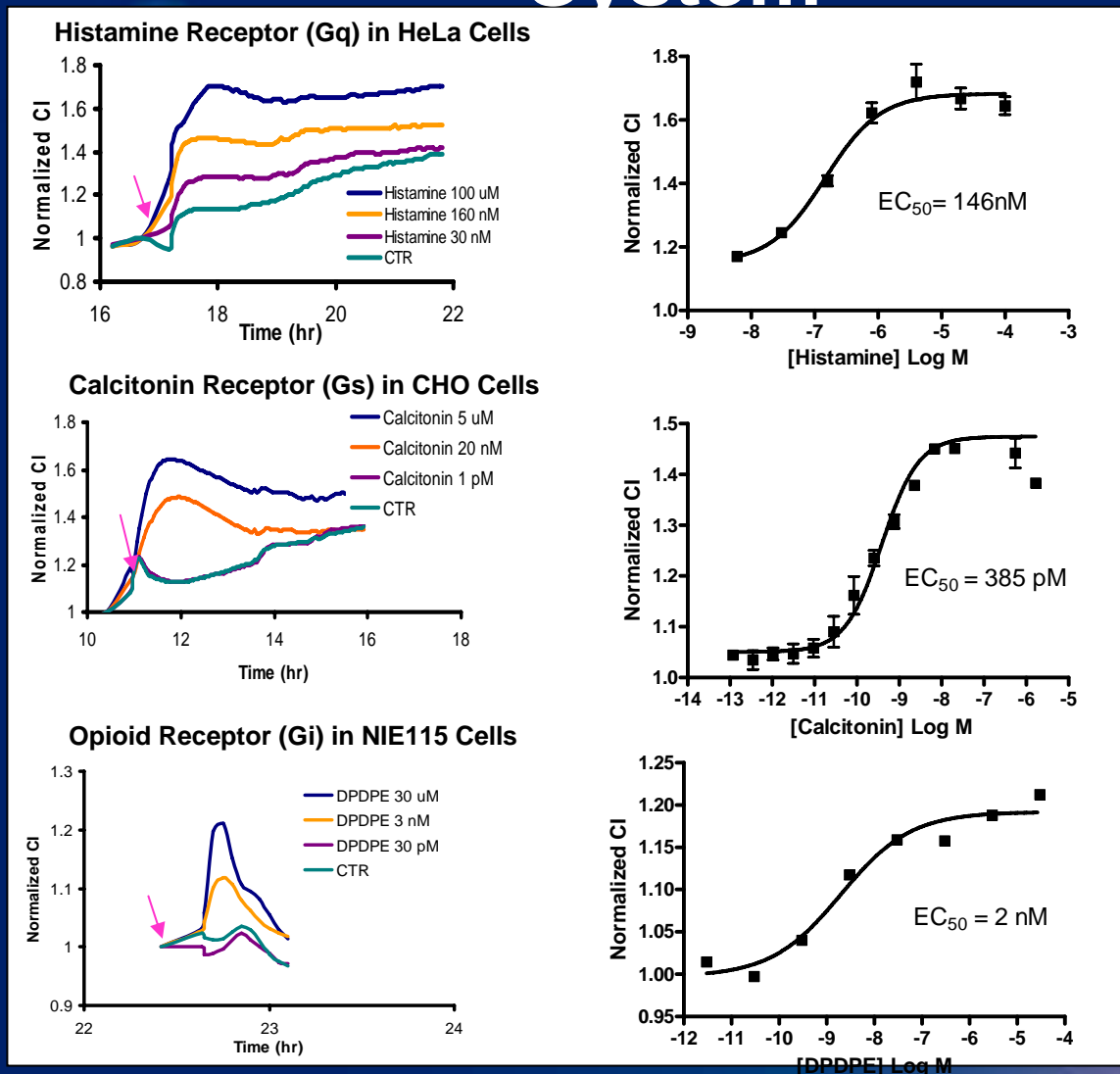
Dynamic Monitoring of Endogenous Receptors Using the xCELLigence System

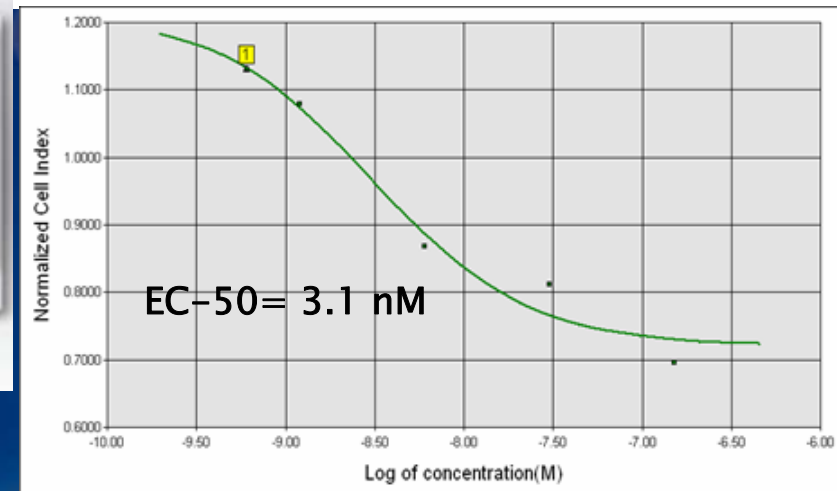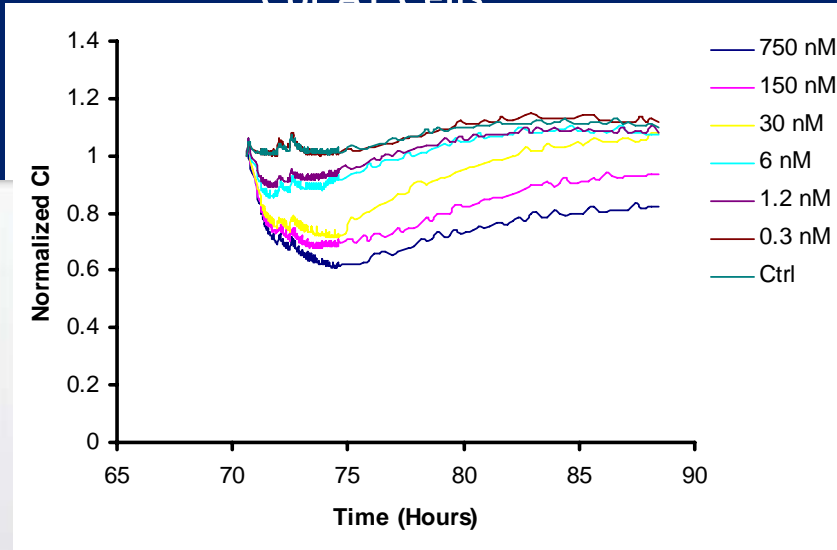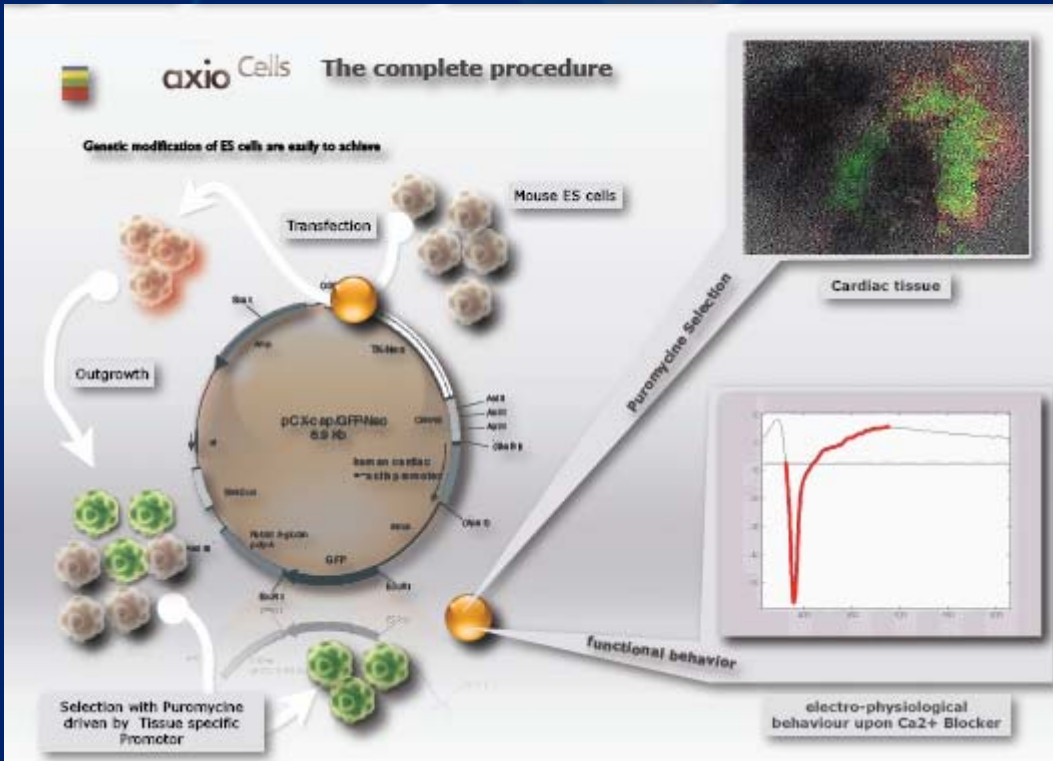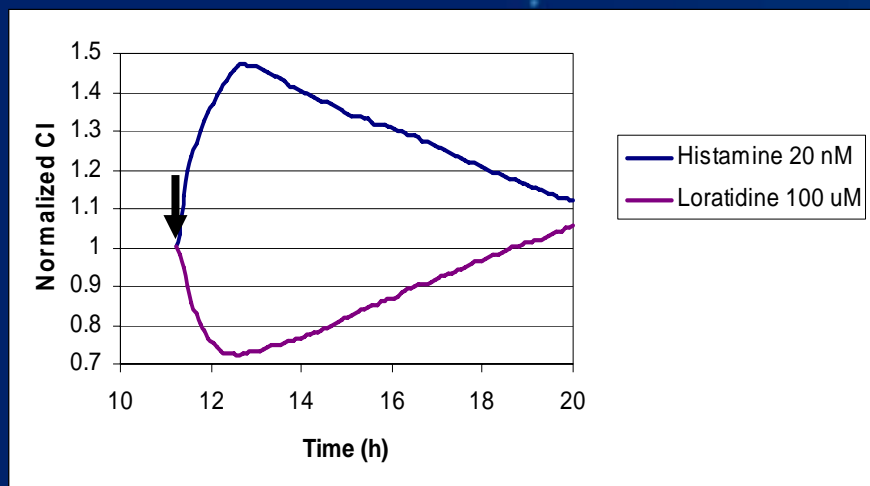# Dynamic Monitoring of Receptors in Disease-Relevant Cell Types

Cor.AT Cells from Axiogenesis

Mouse ES Cell-Derived Cardiomyocytes
100% Pure Population

β2 Adrenergic Receptor Activation in Cardiomyocyte–Differentiated Cor.AT Cells

EC-50= 3.1 nM

Identification of Histamine H1 Receptor Inverse Agonist using the xCELLigence RT-CA System
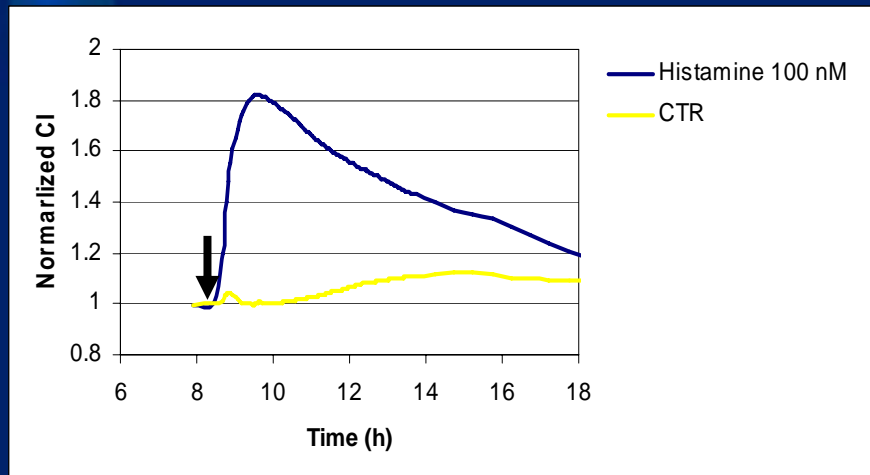
List of Receptors Functionally Monitored by the xCELLigence System

- GPCR
- RTK
- FcR (IgE and IgG)
- TCR
- Death Receptors (FasR, TNFR)
- Integrins
- Toll Receptors
- Nuclear Hormone Receptors

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Summary

- Impedance-based monitoring of cellular status using the xCELLigence platform allows for monitoring of both short term and long term responses

- The ability to monitor short and long term responses within the same experiment provides cytological profiles which can be predictive of mechanism of action

- The non-invasive nature of impedance readout provides the advantage of working with primary cells or disease relevant cells both for long term cytotoxicity studies and short term receptor responses

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