# Innovation in Toxicology at NCATS

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*ADVERSE OUTCOME PATHWAYS: FROM RESEARCH TO REGULATION*

SEPTEMBER 3, 2014

NCATS

NIH National Center for Advancing Translational Sciences

# What is Translation?

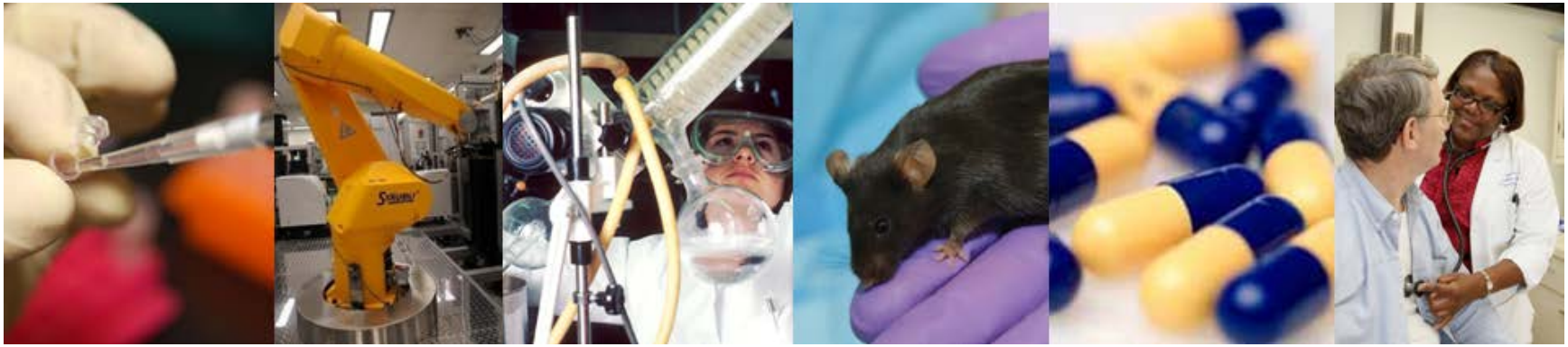
*Translation* is the process of turning observations in the laboratory and clinic into interventions that improve the health of individuals and the public - from diagnostics and therapeutics to medical procedures and behavioral changes.

# What is Translational Science?

*Translational Science* is the field of investigation focused on understanding the scientific and operational principles underlying each step of the translational process.

NCATS studies translation as a scientific and organizational problem.

# NCATS Mission



To catalyze the generation of innovative methods and technologies that will enhance the development, testing and implementation of diagnostics and therapeutics across a wide range of human diseases and conditions.

# Some of the *scientific* translational problems on NCATS' to-do list…

- Predictive toxicology
- Predictive efficacy
- Derisking undruggable targets/untreatable diseases
- Data interoperability
- Biomarker qualification process
- Clinical trial networks
- Patient recruitment
- Electronic Health Records for research
- Harmonized IRBs
- Clinical diagnostic criteria
- Clinical outcome criteria (e.g., PROs)
- Adaptive clinical trial designs
- Shortening time of intervention adoption
- Methods to better measure impact on health (or lack of)

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# NCATS Scientific Initiatives

- **Clinical Translational Science**
  - » Clinical and Translational Science Awards
  - » Rare Disease Clinical Research Network
  - » New Therapeutic Uses program
- **Preclinical Translational Science**
  - » NIH Chemical Genomics Center
  - » Therapeutics for Rare and Neglected Diseases program
  - » Bridging Interventional Development Gaps program
- **Re-engineering Translational Sciences**
  - » Toxicology in the 21st Century
  - » Tissue Chip program
  - » Office of Rare Diseases Research

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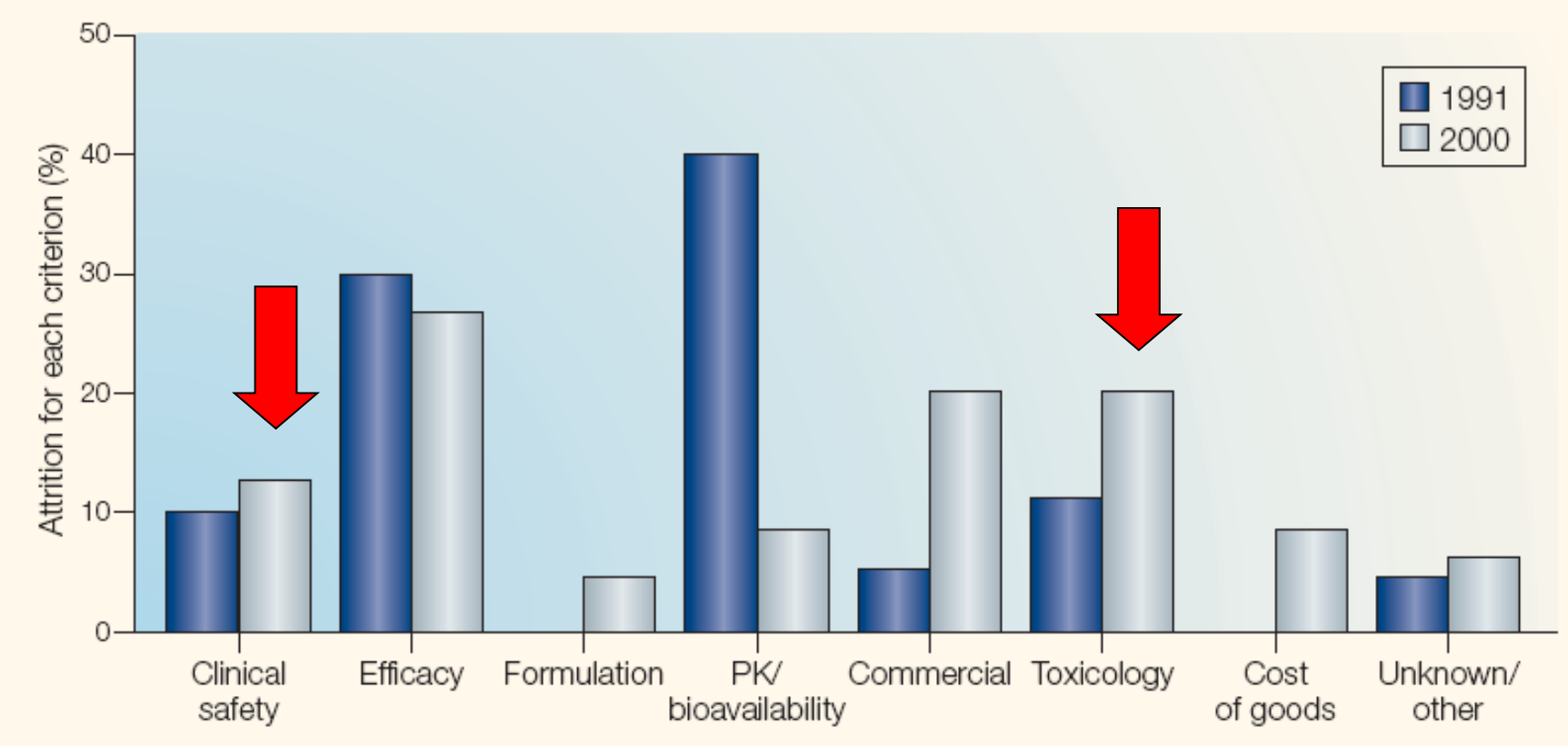
# Toxicity is a common reason for drug development failure

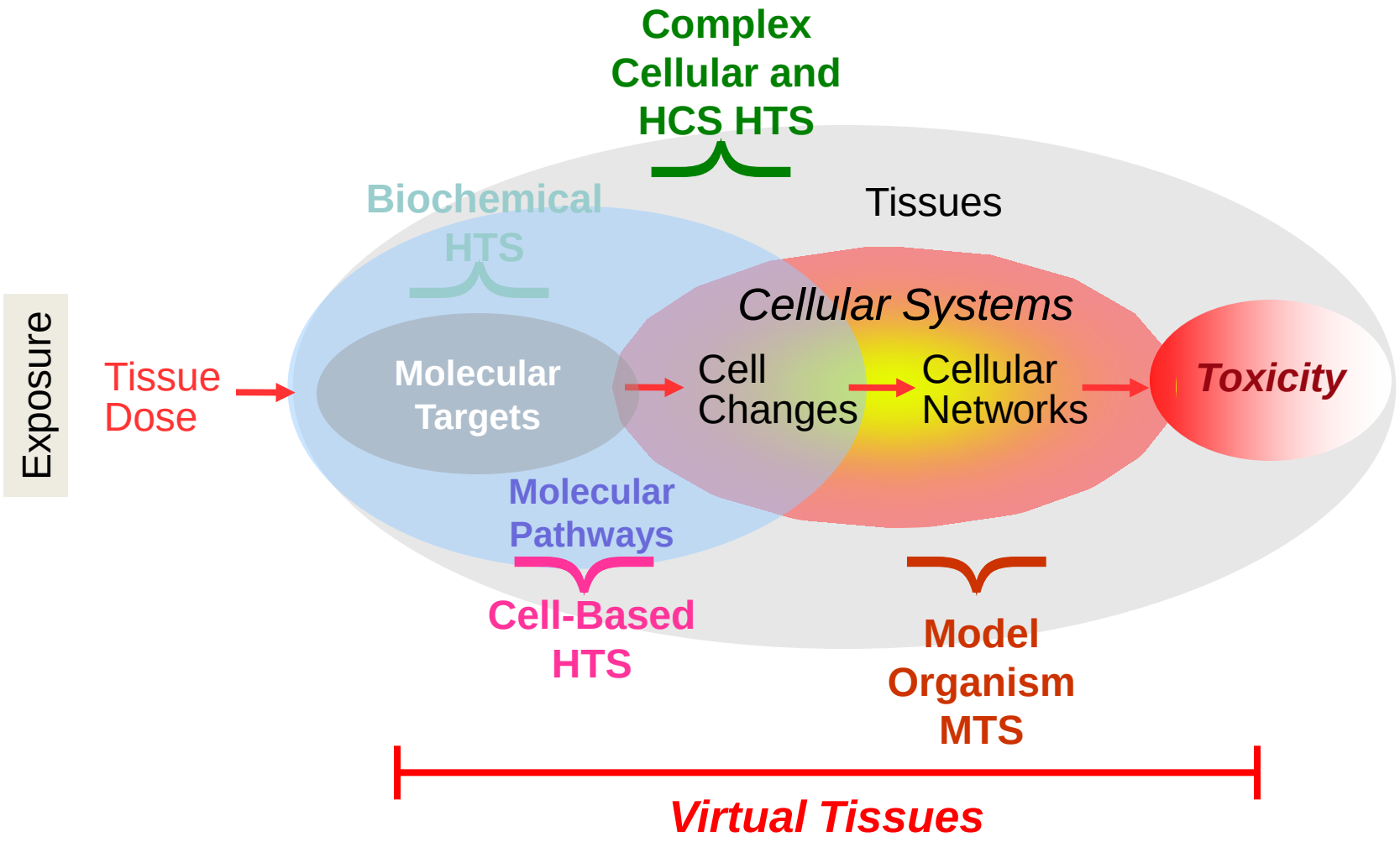



**Preclinical (21%) + Clinical (12%) Tox = 33% of all failures**



Kola and Landis, *Nature Reviews Drug Discovery* 3, 711-716, 2004.

# A Grand Challenge: Predicting Toxicity



# Toxicology Technology Development

## *The Tox21 Program*

FDA


Tox21

National Toxicology Program
Department of Health and Human Services

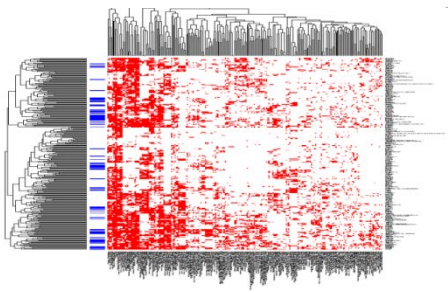
NIH National Institute of Environmental Health Sciences

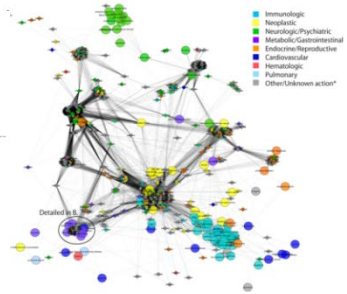
NIH National Center for Advancing Translational Sciences

NCGC NIH Chemical Genomics Center

# Tox21 Goals

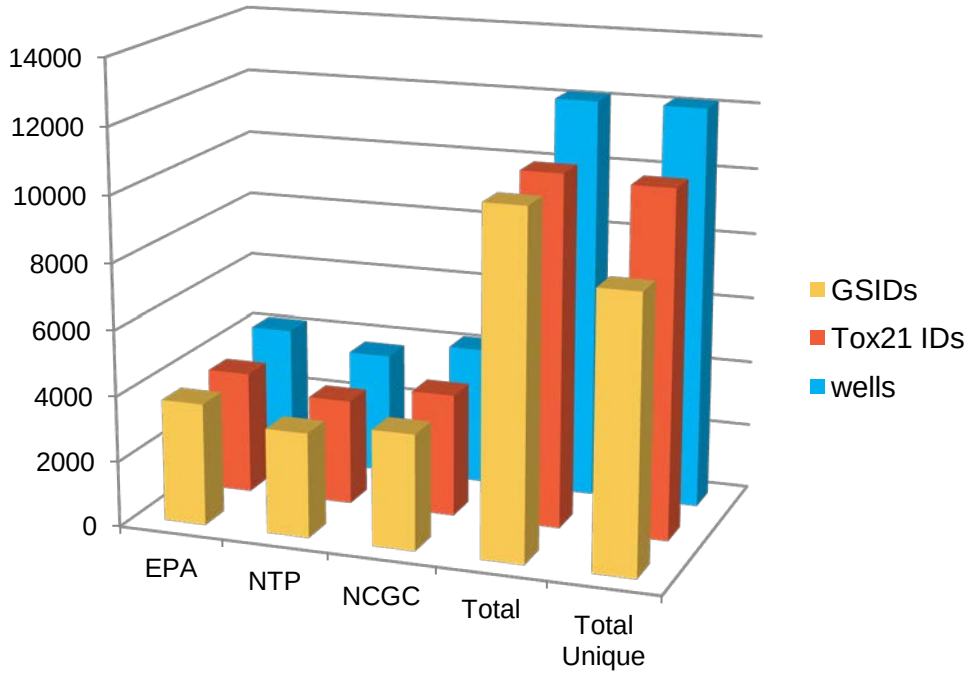
- Identify patterns of compound-induced biological response in order to:
  - » characterize toxicity/disease pathways
  - » facilitate cross-species extrapolation
  - » model low-dose extrapolation
- Prioritize compounds for more extensive toxicological evaluation
- Develop predictive models for biological response in humans

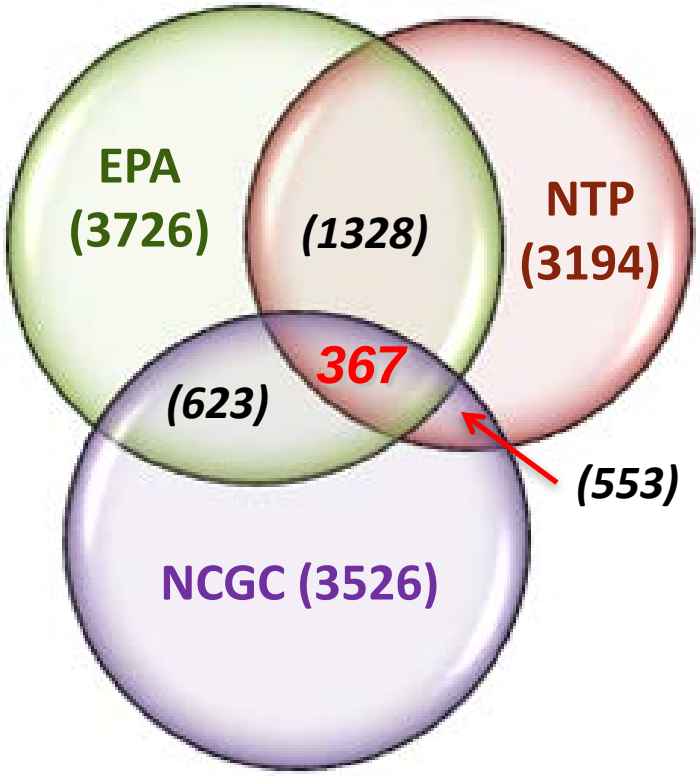





| Area of Expertise | NIEHS/NTP | NCATS | EPA | FDA |
|---|---|---|---|---|
| Lab Animal Toxicology | ✓ | | ✓ | ✓ |
| Human Toxicology/Exposure Assessment | ✓ | | ✓ | ✓ |
| Ultra High Throughput Screening | | ✓ | | |
| Low to Mid Throughput Assays | ✓ | ✓ | ✓ | ✓ |
| Stem Cell Assay Development | ✓ | ✓ | ✓ | ✓ |
| Epigenetic Assays | ✓ | ✓ | | |
| Engineered Tissue Models | ✓ | ✓ | ✓ | ✓ |
| ‘Omic Based Systems | ✓ | ✓ | ✓ | ✓ |
| Lower Organism Models | ✓ | | ✓ | ✓ |
| Genetic Variability in Response | ✓ | ✓ | | |
| Databases & Informatic Tools | ✓ | ✓ | ✓ | ✓ |
| Validation Experience | ✓ | ✓ | ✓ | ✓ |

# Tox21 10K Compound Library




| Unique | EPA | NTP | NCGC | Total | Total Unique | |
|---|---|---|---|---|---|---|
| GSIDs | 3726 | 3194 | 3524 | 10444 | 8307 | unique substances |
| Tox21 IDs | 3729 | 3210 | 3733 | 10672 | 10496 | unique solution IDs |
| wells | 4224 | 3726 | 4224 | 12174 | 12174 | total number of test cmpd wells |

88 single-sourced cmpds in duplicate on each plate

2255 replicate substances (GSIDs) across 3 inventories

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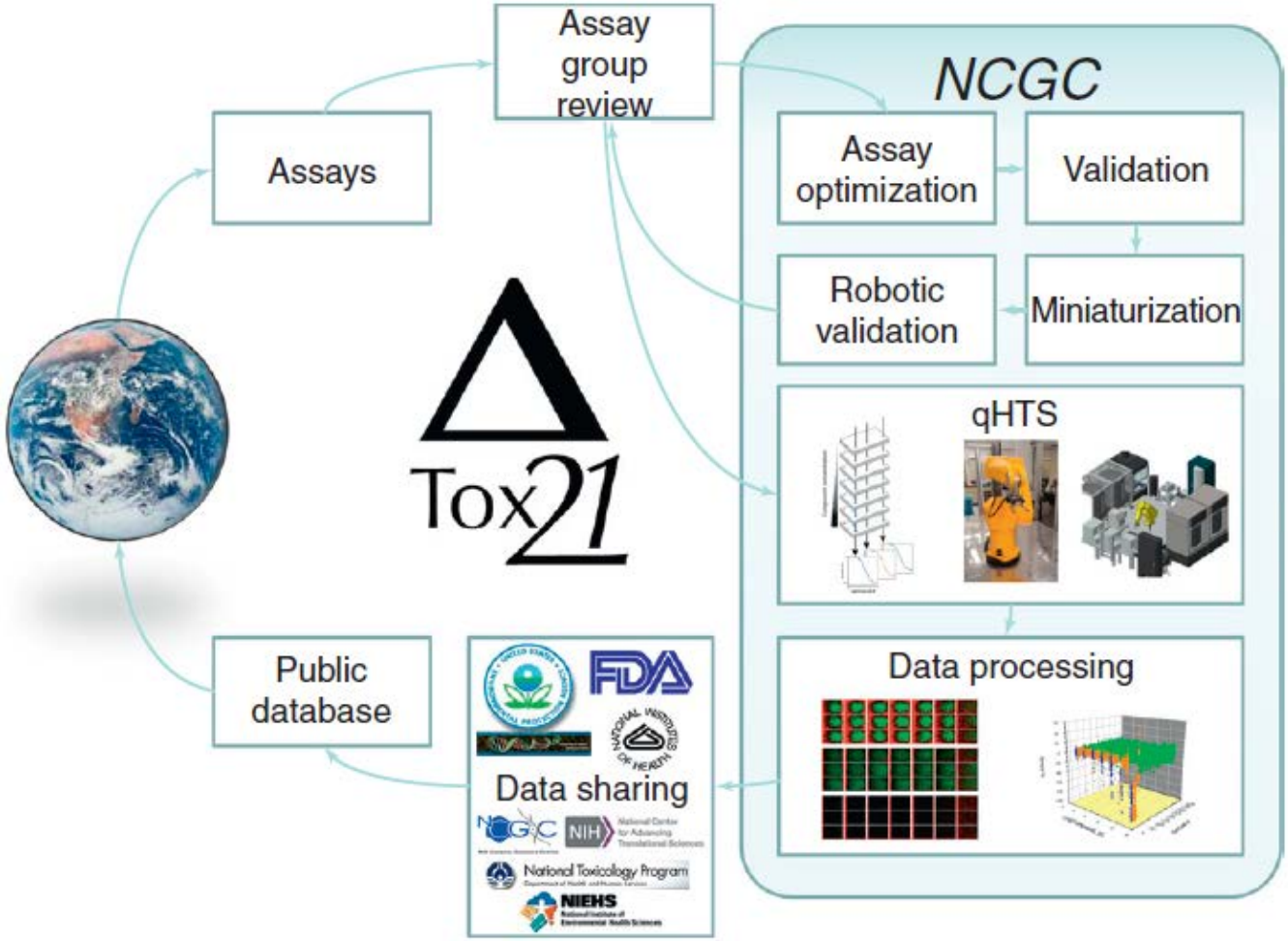
*Compound identity and structures available at http://www.epa.gov/ncct/dsstox/sdf_tox21s.html*

# Tox21 Screening Process




## Validation

- Positive controls
- Time course
- Signal to background

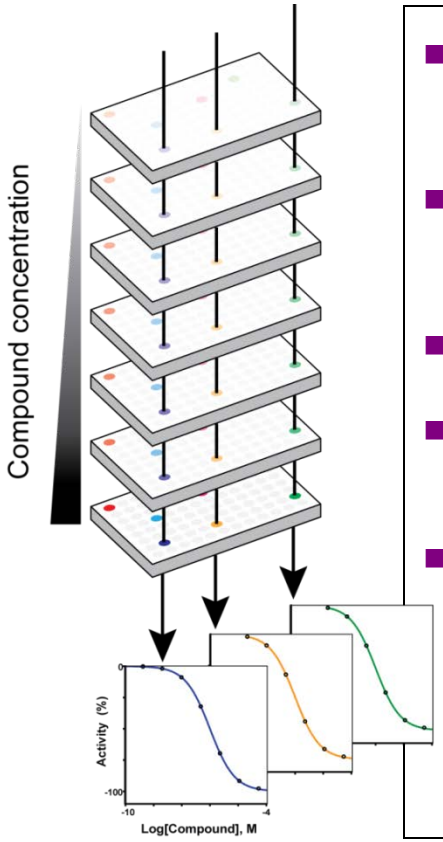
## Miniaturization

- Cell density per well
- Positive controls
- Signal to background ≥ 3
- CV <10%

Attene-Ramos et al., 2013, Drug Discovery Today 18:716-723

- CV (coefficient of variation) = standard deviation (SD) of compound area/median of compound area
- Z factor = 1-[3*(SD of compound area + SD of basal)/(median of compound area – median of basal)]

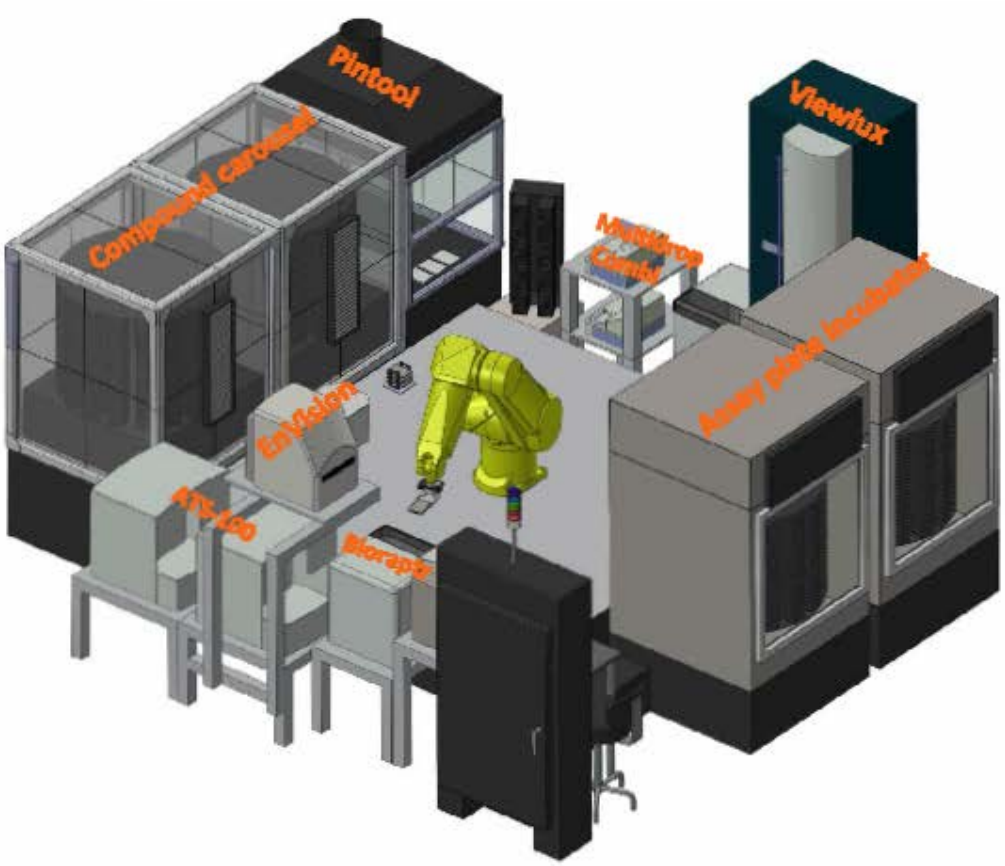
# Quantitative High-Throughput Screening (qHTS)




- Conventional screening done at one concentration
  - Not appropriate for toxicity testing – "dose makes the poison"
- qHTS tests compounds assayed at multiple concentrations
  - For Tox21, 14 concentrations over 4 logs (high:~ 100 uM)
- Miniaturized assay volumes 2-8 uL in 1536-well plate
- Informatics pipeline for data processing, curve fitting & classification, extraction of SAR
- Generates *toxicological actives* rather than statistical "hits"
  - Dramatically increases reliability
  - Dramatically reduces false positives and false negatives

*Inglese et al., Proc Natl Acad Sci 103:11473, 2006*

# Tox21 Robotic Screening System




### ViewLux Multilabel Reader

- Absorbance
- Fluorescence
- F.P.
- Luminescence
- TR-FRET
- Top reading

### EnVision Multilabel Reader

- Absorbance
- Fluorescence
- F.P.
- Luminescence
- TR-FRET
- AlphaScreen
- Top/Bottom reading

### BioRAPTR FRD Workstation

- Transfer size: 0.2 - 10 ul
- 0.5 ml dead volume
- 4 reagents

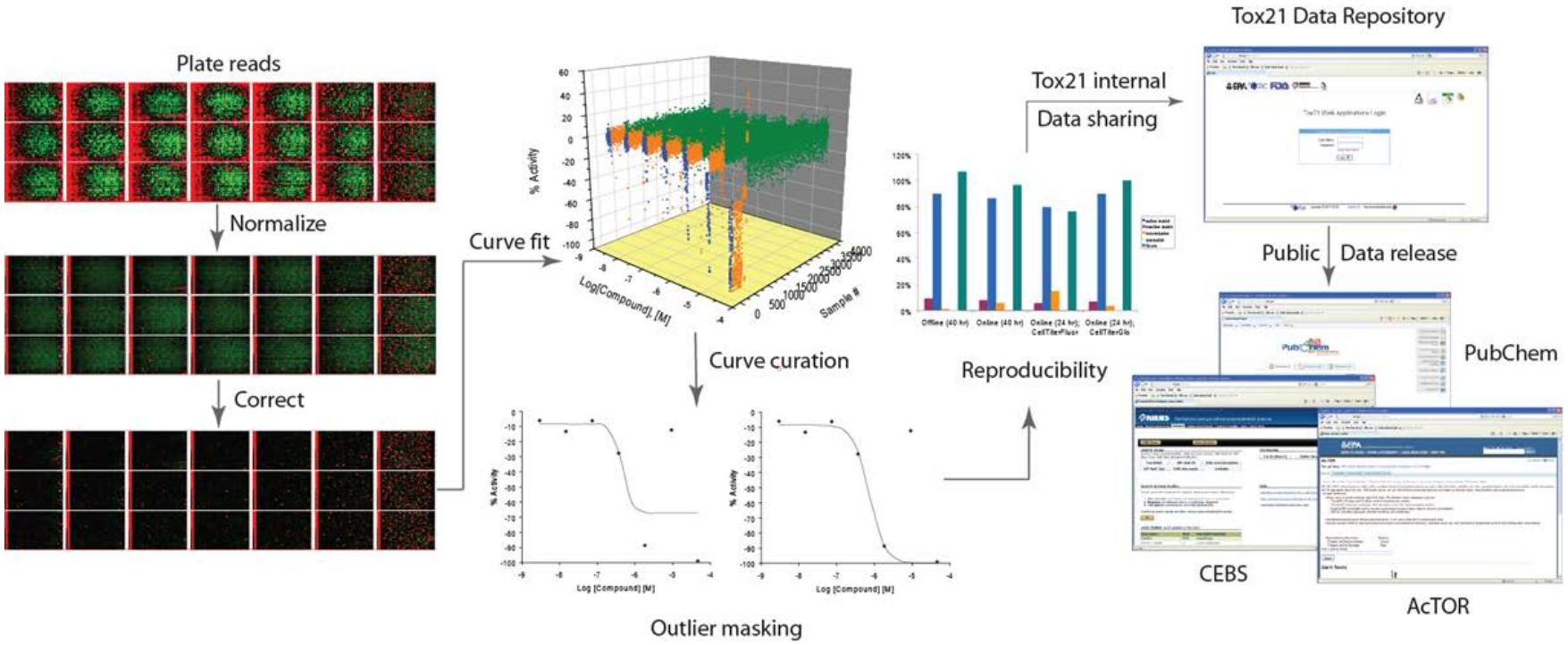
### Multidrop Combi

- Transfer size: 2 - 10 ul
- 10 ml dead volume
- 1 reagent

### Pintool Station

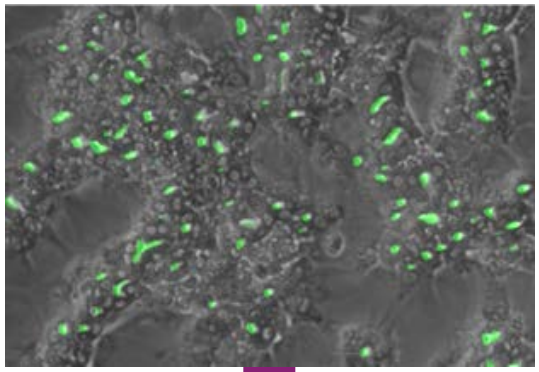
- Transfe
- Pins washed in 3 solvents

# Tox21 Informatics Analysis Process



# Tox21 Phase III

- Focused on increased pathway coverage, high content imaging assays, and high throughput gene expression platforms using
  - » cells capable of xenobiotic metabolism
  - » ES/iPSC derived differentiated cell populations (e.g., cardiomyocytes, neurocytes, hepatocytes)
- Integration of metabolite prediction models into hazard prediction models
- Secondary screens needed to bridge HTS to *in vivo* toxicology
- Expanded utilization of lower organisms (zebrafish, C. elegans)



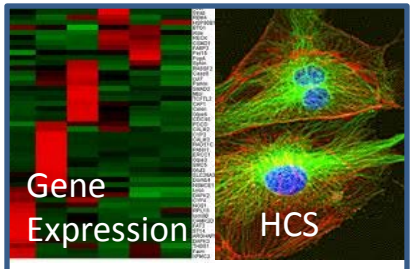
Targeted Assays

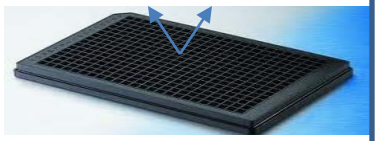
- High Content screening
  - Hoechst: Cell loss & nuclear size
  - DHE: Oxidative stress/ROS
  - p53: DNA damage
  - pH2A.X: Genotoxicity
  - JC-10: Mitochondrial damage (MMP)
  - Caspase 3: Apoptosis
  - Lipitox: Steatosis & Phospholipidosis
  - Reactive metabolites/ROS: GSH depletion
- Receptor Activation via Induction of gene expression
  - AhR, CAR, PXR, PPARα, FXR
- Necrosis
  - miR-122 leakage or LDH leakage

# Tox21 Focused on Secondary Screening Needed to Bridge HTS to in vivo Toxicology

qHTS

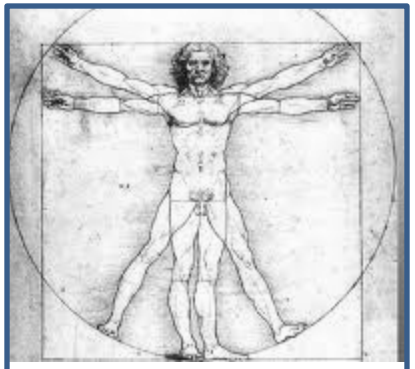
~10,000 cpds



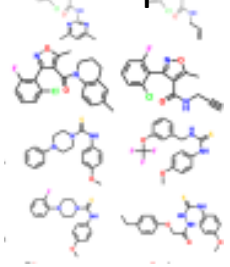
*In vitro* secondary assays

~100 cpds



Test in rats to predict human toxicity

5-10 cpds



# A working hypothesis

1. Any pathway important to normal function/physiology has the potential, when disrupted, to cause pathophysiology – i.e., toxicity
2. To reliably predict potential toxicity of a chemical, its activity on every pathway operant in mammalian (human, rodent) cells must be characterized
3. To allow this characterization, a complete/nonredundant list of all pathways operant in mammalian cells must be enumerated
4. A set of experimental assays, each of which covers ≥1 pathway in network space, could reliably characterize compound activities across pathway space with a desired degree of certainty

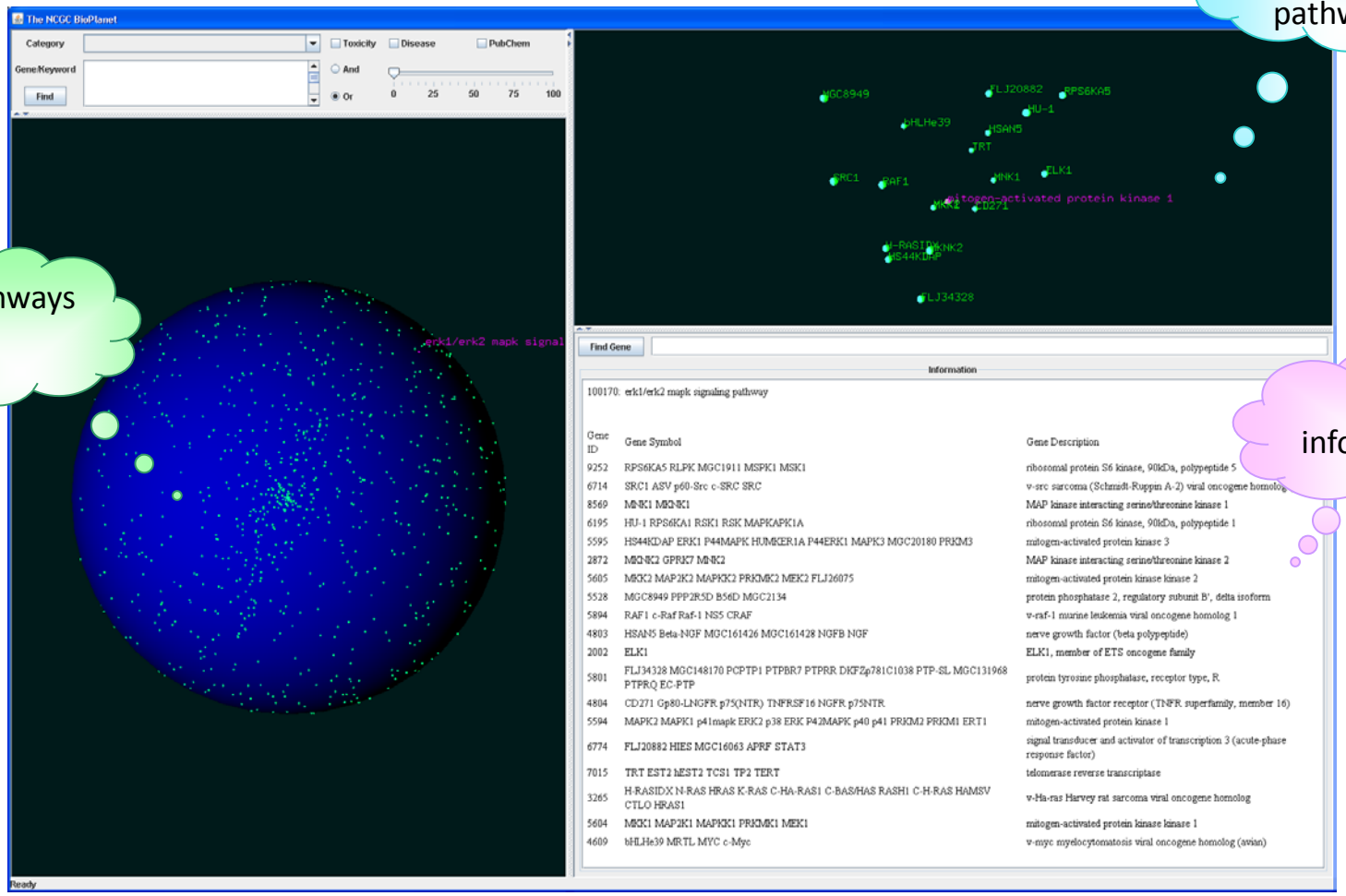
# The NCGC (NIH Chemical Genomics Center) BioPlanet™

- Hosts universe of pathways
  - Focus on human pathways (~2000 unique)
- All pathway annotations from manually curated, public sources
  - Integrates pathways from >10 different data sources
  - e.g. KEGG, WikiPathways, Reactome, Science Signaling
- Annotates pathways by source, species, biological function/process, disease/toxicity relevance, assay availability
- Easy visualization, browsing, analysis of pathways
- Facilitates pathway assay selection/prioritization for Tox21
- Web version in process for public release

# The Universe of Pathways

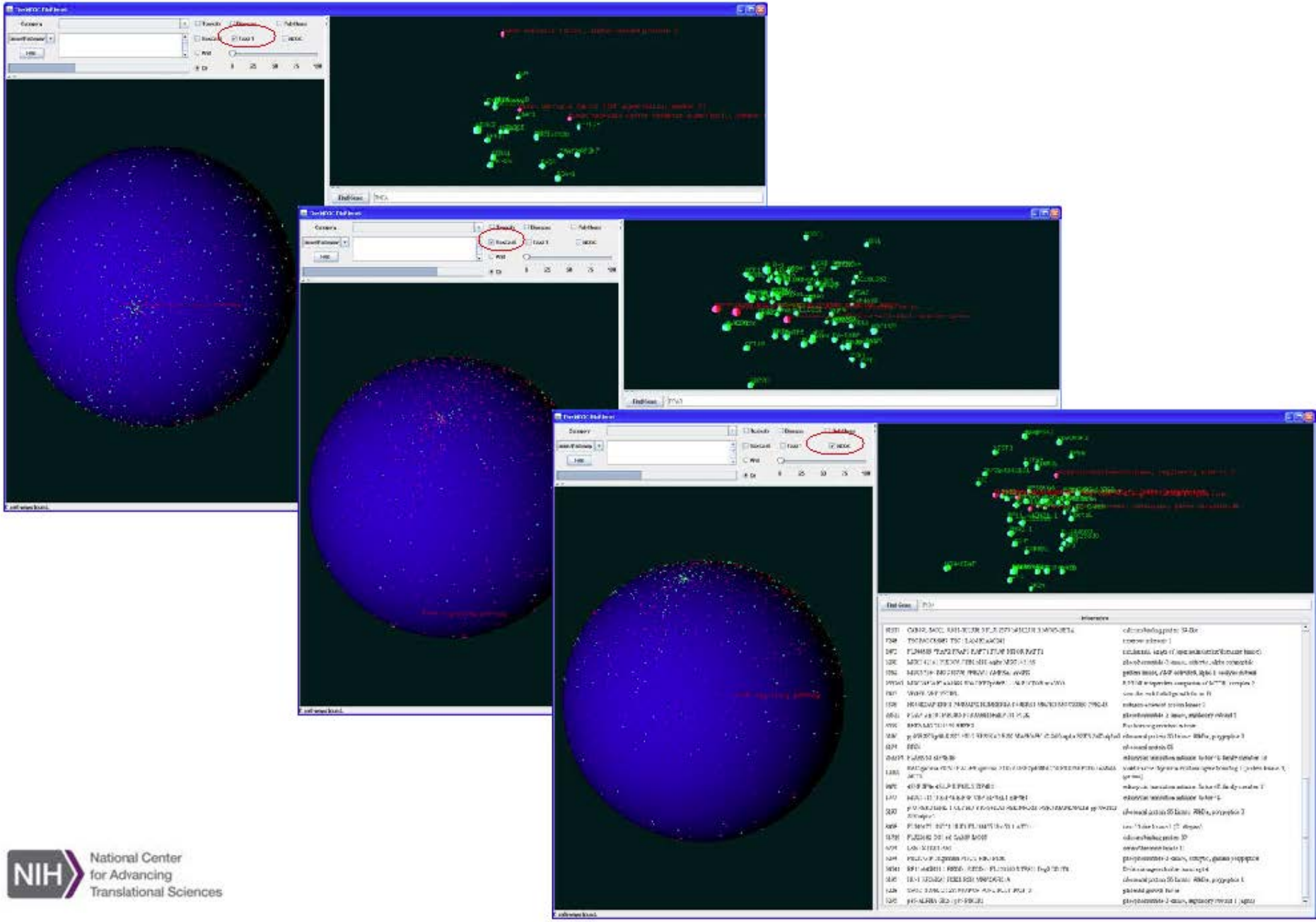
Detailed view of a pathway

Pathways




Gene information

# Pathways with available assays – Tox21, ToxCast, NCGC



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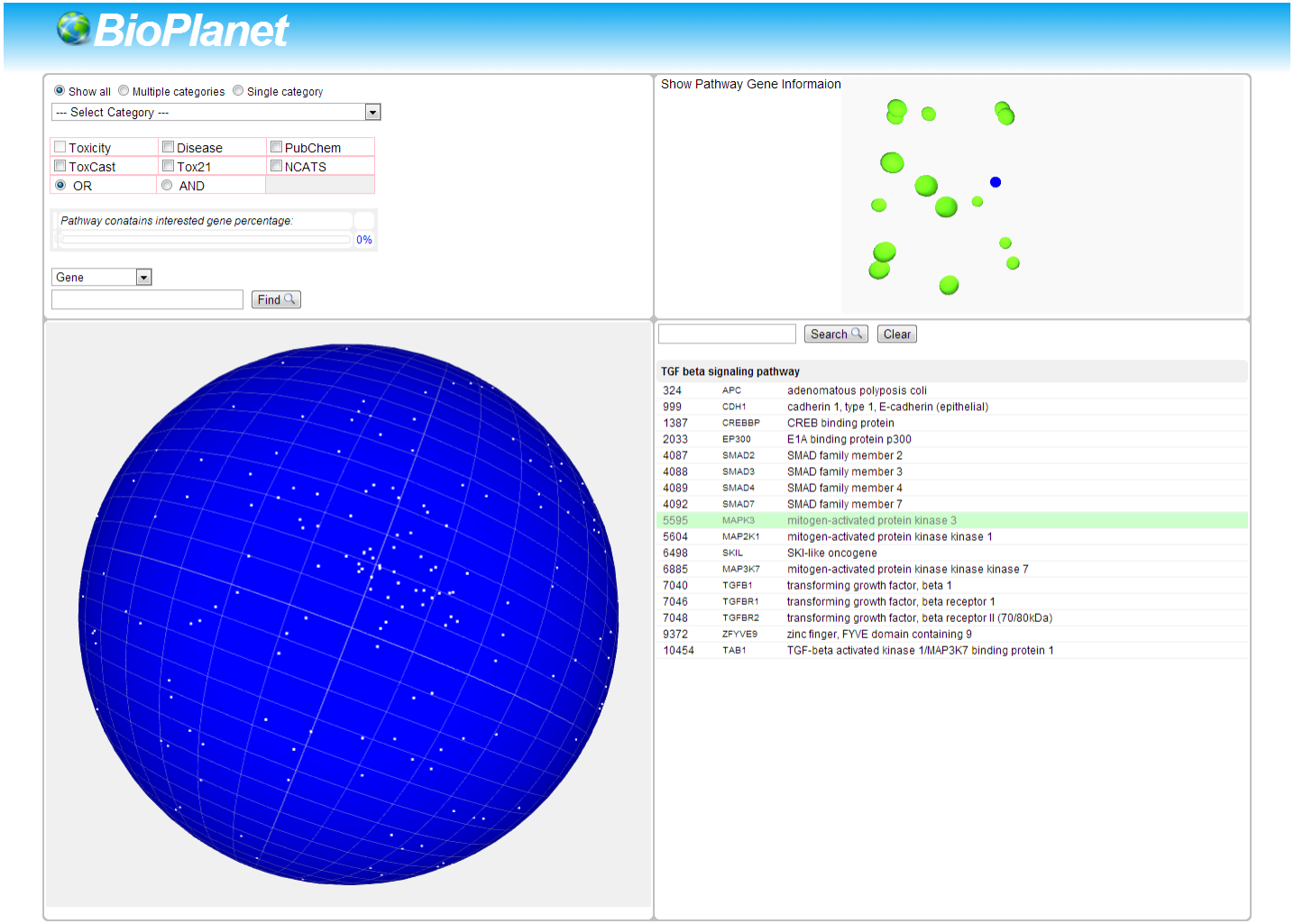
# Web-based version in development



# BioPlanet™ Applications

- Assay selection/prioritization for Tox21
  - Toxicity pathways?
  - Disease pathways?
  - Assay availability?
  - Maximize pathway coverage?
- Future developments
  - Link compound activity data
  - Incorporate other data forms: sequence data, gene/protein expression data, etc.?
  - Other species: rat, mouse, etc.
  - Organize assays according to pathways/diseases/toxicity endpoints

# Tissue Chip for Drug Screening Program

- Goal
  - Develop organoids on chips to screen for compound toxicity, efficacy
    - Liver, heart, lung, other cell types
    - Integrate platform systems
    - Designed for multiple different readouts
- NIH, DARPA contributing ~$70M each over 5 years
  - NCATS and DARPA independently manage, fund separately but highly coordinated program
  - FDA provides regulatory science guidance
- Awards announced in 2012
  - Supporting the best ideas in engineering, biology, and toxicology

NIH National Institutes of Health
*Turning Discovery Into Health*

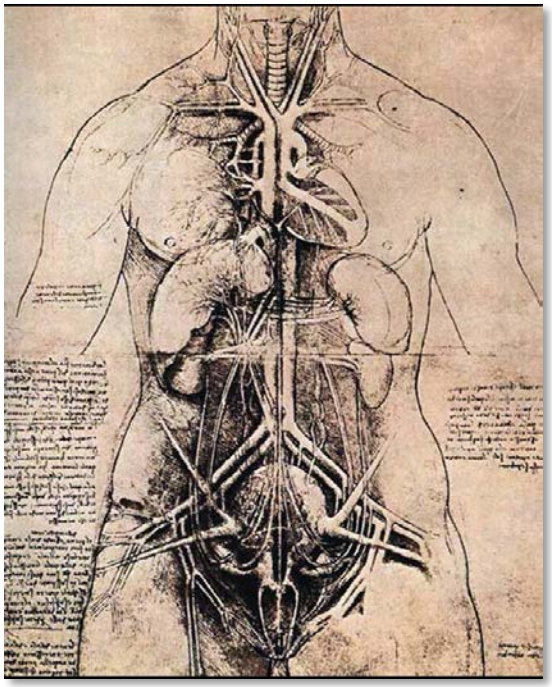
DARPA

FDA

NIH
National Center for Advancing Translational Sciences

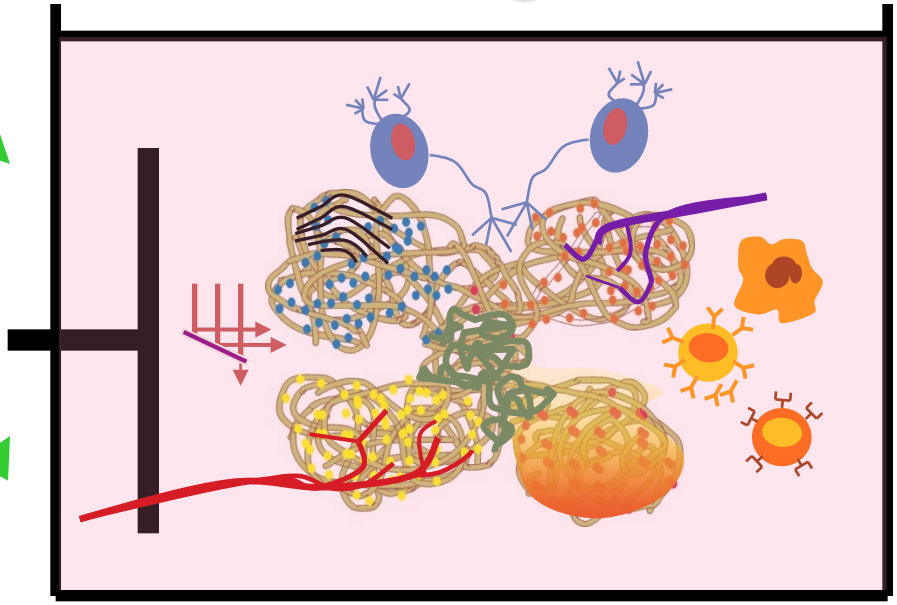
# Tissue Chip Program

**GOAL: Develop an *in vitro* platform that uses human tissues to evaluate the efficacy, safety and toxicity of promising therapies.**

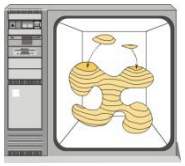


- All ten human physiological systems will be functionally represented by human tissue constructs:
  - Circulatory
  - Endocrine
  - Gastrointestinal
  - Immune
  - Integumentary
  - Musculoskeletal
  - Nervous
  - Reproductive
  - Respiratory
  - Urinary
- Physiologically relevant, genetically diverse, and pathologically meaningful.
- Modular, reconfigurable platform.
- Tissue viability for at least 4 weeks.
- Community-wide access.

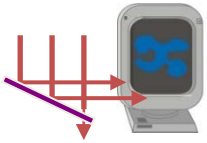
# Tissue Chips from Common Building Blocks



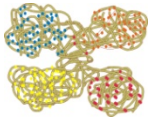
## Computational Design
- systems integration
- multi-scale modeling
- simulation
- feedback



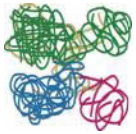
## Functional Readout
- real-time, label-free, non-destructive sensing
- imaging



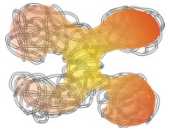
## Scaffold
- purified ECM
- synthetic polymers
- composites



## Cells
- stem/progenitor
- differentiated
- mixed cell types

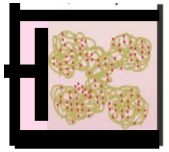


## Structure
- porosity
- topography
- stiffness

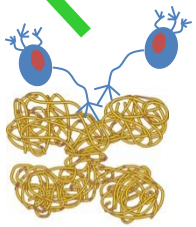


## Spatial/Temporal Patterning
- cytokine gradients
- controlled release

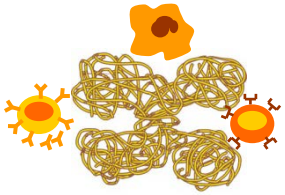
## Perfusion
- embedded channels
- vascularization



## Bioreactors
- optimized culture conditions
- biomechanical properties
- blood mimetics



## Innervation
- signal propagation
- coordinated response



## Host Response
- generalized inflammation
- specific immunity

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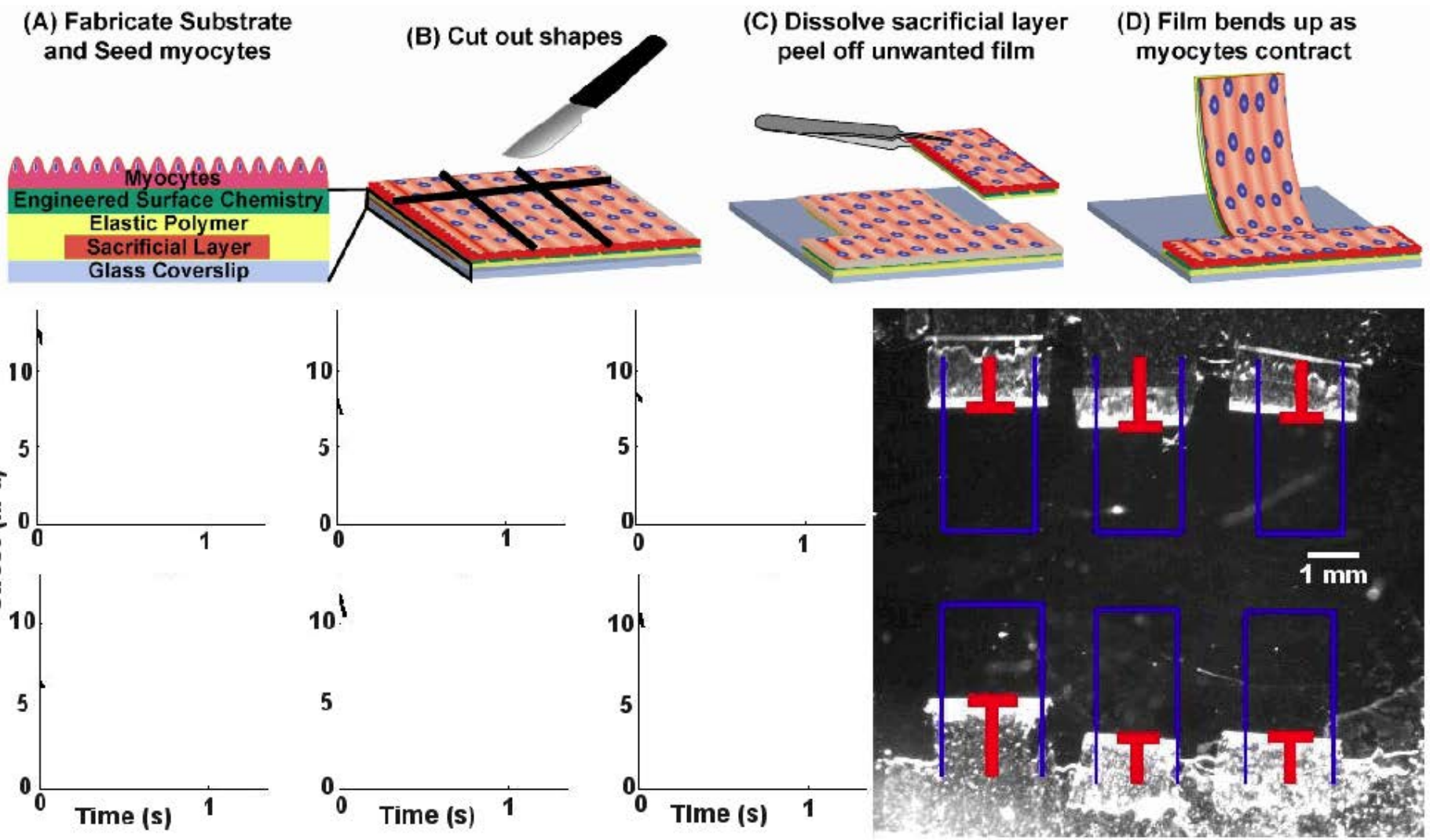
# Engineered Cardiac Muscular Thin Films




**Film length**
**Automatic projection tracking**

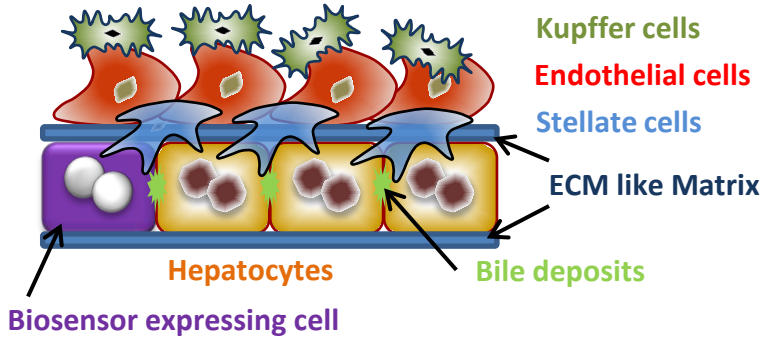
Data provided by Dr. Kit Parker, Wyss Institute

National Center for Advancing Translational Sciences

Science 2007;317:1366
Lab Chip 2011;11:4165

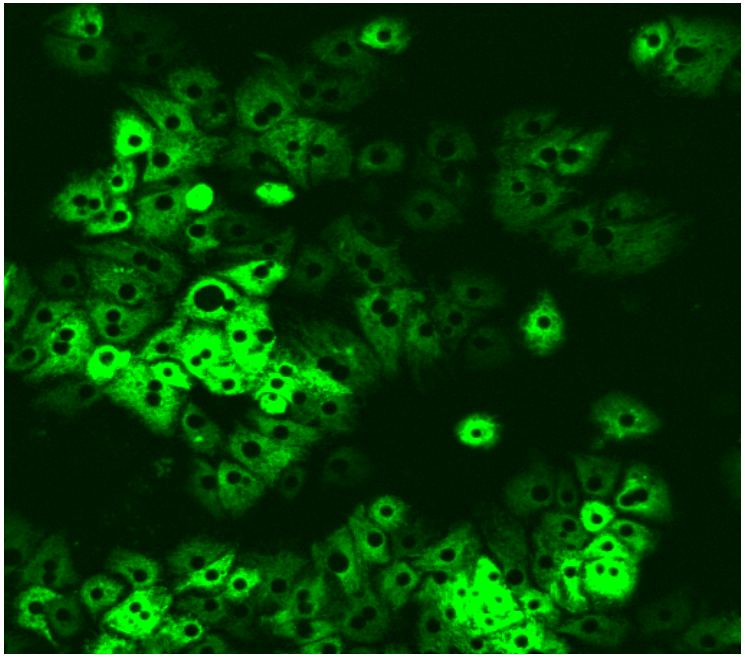
Biomaterials 2010;31:3613
J Pharm Tox Methods 2012;65:126

# 3-D biomimetic liver sinusoid construct



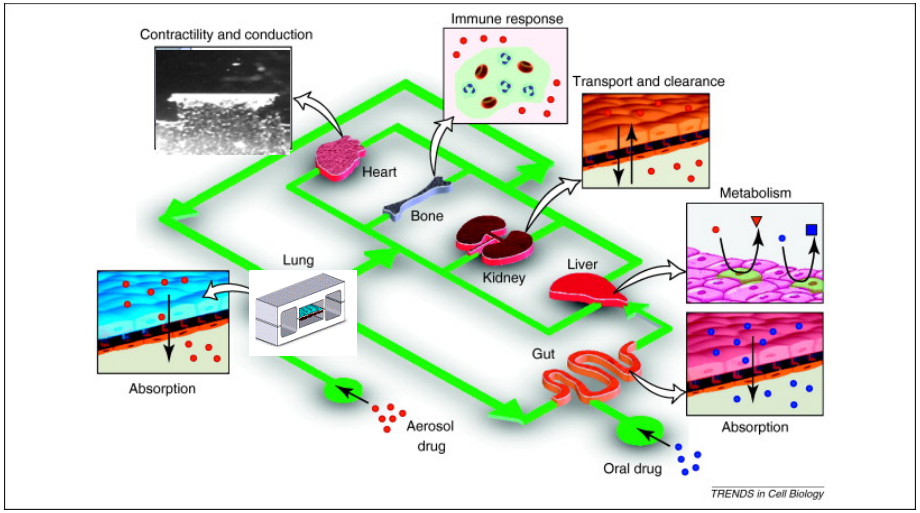

**Sentinel cells:** a subpopulation of hepatocytes, stellate and Kupffer cells that stably express biosensors to monitor key cell functions.

## Biosensors

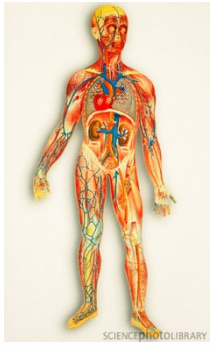


- **Cytochrome C released from mitochondria**
- **Exposed to 10µM Nefazadone**
- **Time-lapse of 16 hours**

# Body-on-a-Chip?




## In vivo Correlation



- Absorption
- Distribution
- Metabolism
- Excretion
- Conc(t)
- Effect(t)
- Toxicity(t)
- Rare toxicities

## Read outs

- Human biology
- Tissue/organ structure
- Cell histology
- Cell viability
- Mechanical properties
- Electrical properties
- Signaling pathways
- Cell metabolism
- Protein synthesis
- Gene expression
- Enzyme activities
- Ion channel properties

# Program Leads at NCATS

- Tox21: Anton Simeonov
  - anton.simeonov@nih.gov
- Tissue Chip: Dan Tagle
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