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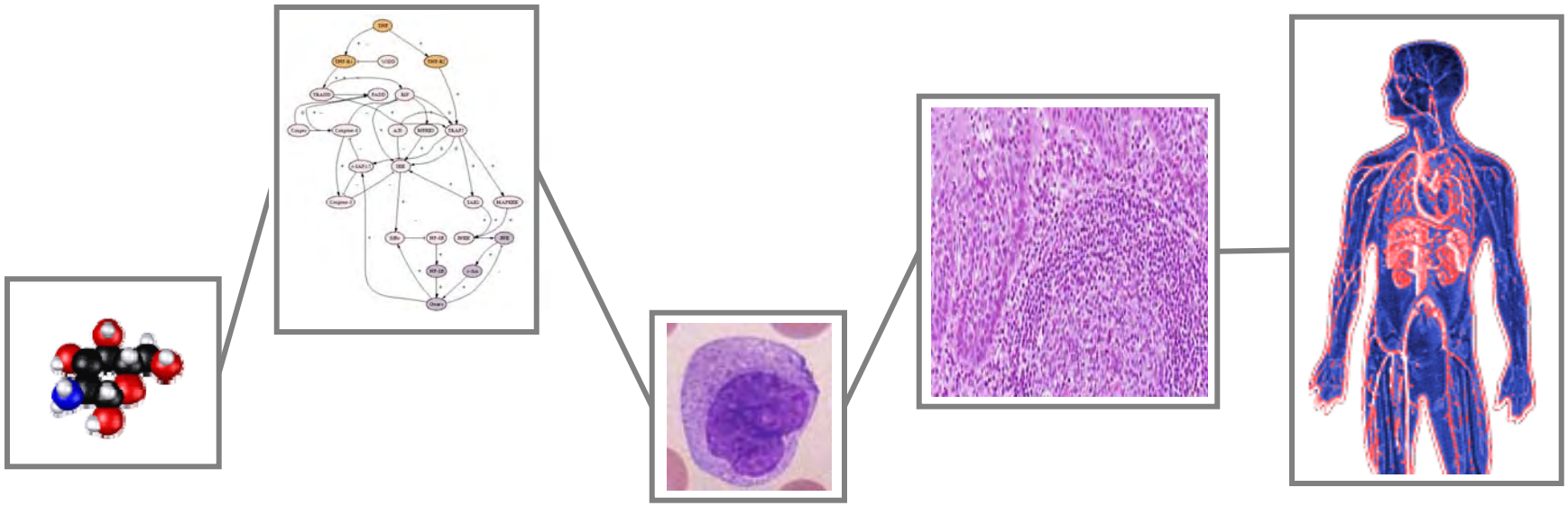
Complex Human Cell Systems for Understanding Toxicity Mechanisms

Ellen L. Berg, PhD

May 23, 2007

Biological Complexity

Modular design / Networked architecture



Molecular targets **Human exposure**

molecules | pathways | cells | tissues | humans

10^{-9} M, 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M, 10^{-1} M, 1 M

10^{-6} sec *10^{2} sec* *10^{4} sec* *10^{5} sec* *10^{8} sec*

Scale (meters) *(Time)*

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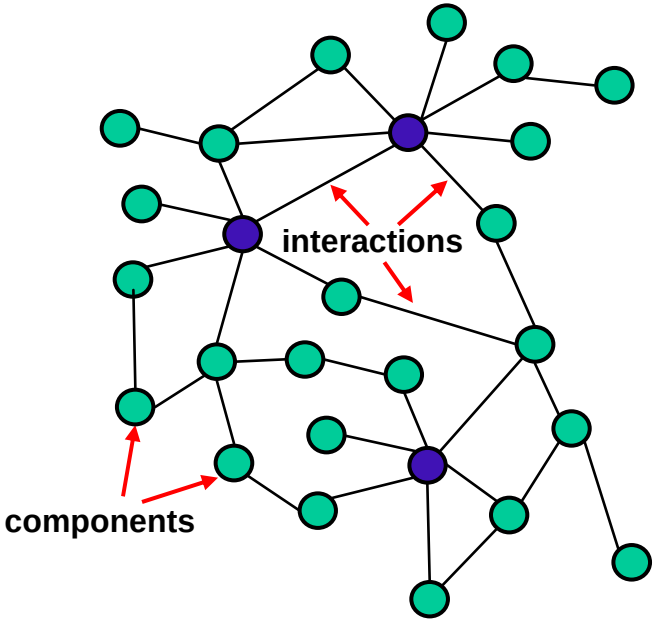
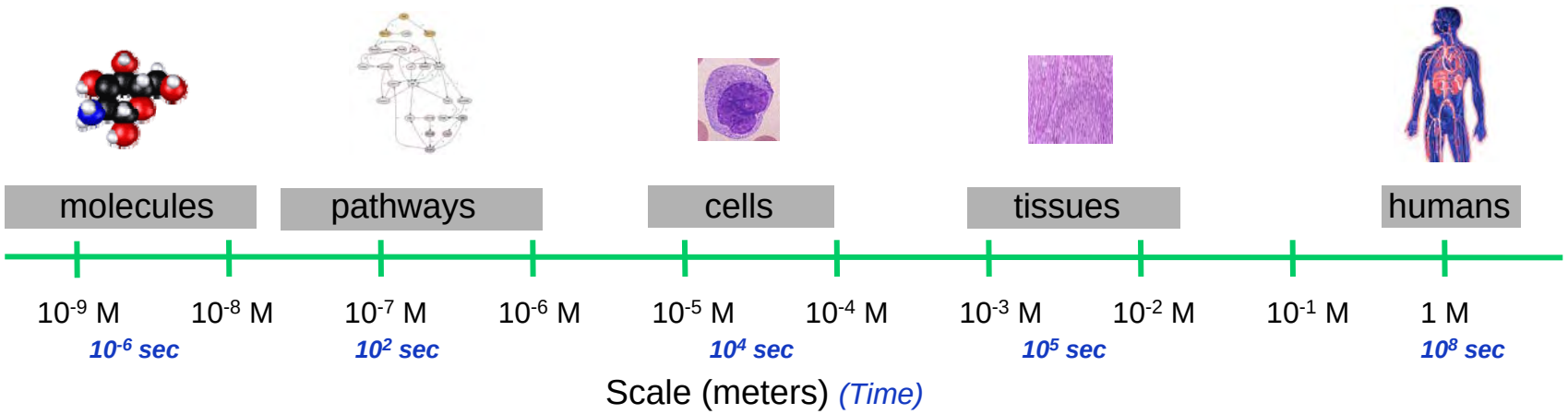
Biological Complexity

Modular Design



- **Great diversity from few components**
  - **A given component can contribute to "many" functions**
- **The ability to compartmentalize**
  - **Safety feature to limit damage**



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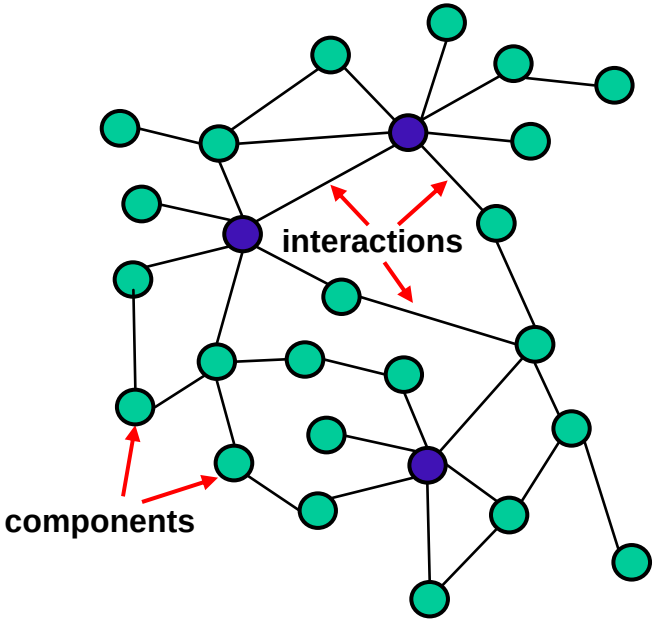
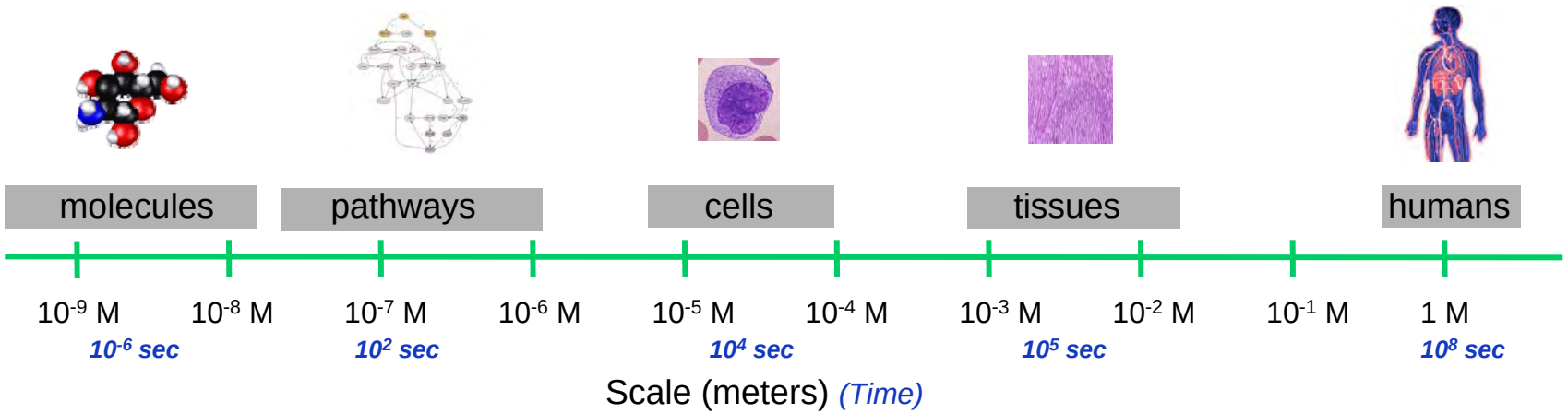
Biological Complexity

Networked Architecture



- **Rapid responses to environment**
  - **Efficient information flow**
- **Framework for control systems**
  - **Feedback mechanisms, etc.**
- **Many potential outcomes**
  - **System "wiring" determines outcome**



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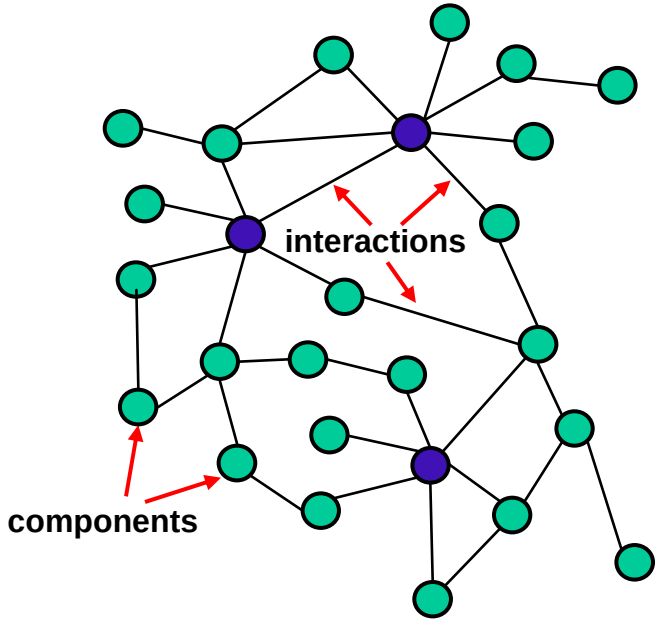
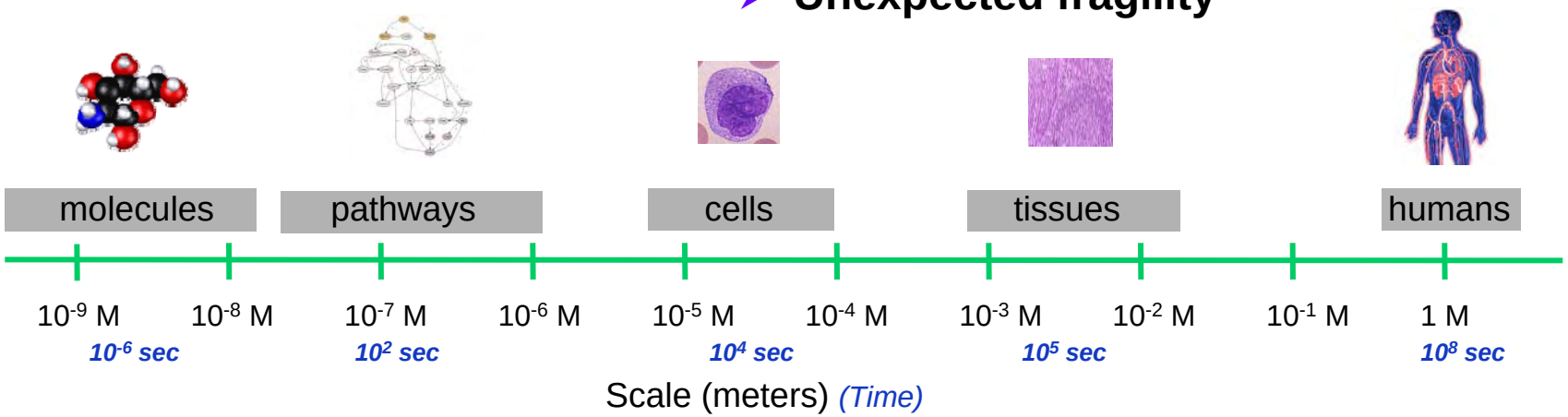
Biological Complexity

Control Systems



- **Dominate in networks with demanding performance requirements**
- **Important for providing tolerance to perturbations (robustness)**
- **"Hidden nature"**
  - **Unexpected fragility**



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Modular, Networked Architecture

Consequences for Drug Discovery & Predictive Toxicology

- **Drug targets function in multiple biological processes**

  Different pathways, cell types, different environments

  Context, context, context

- **Hard to predict clinical trial efficacy / human exposure effects**

  Redundant mechanisms are difficult to detect

  Compensatory mechanisms often override drug effects

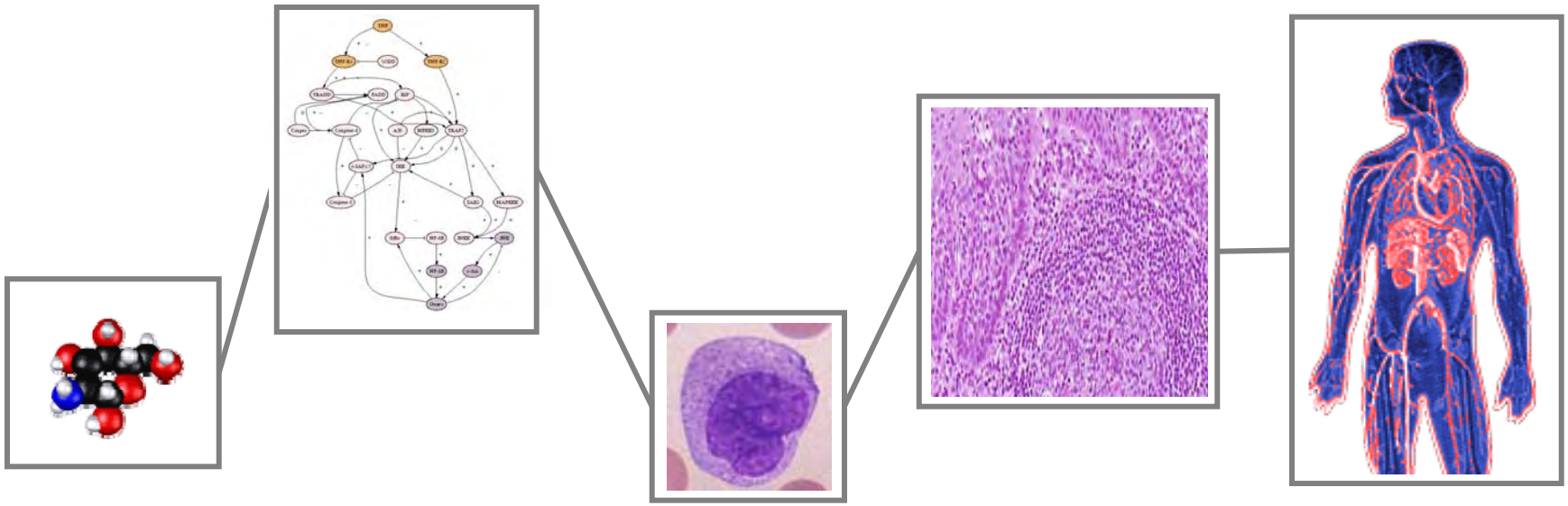
- **Drugs can have effects far downstream of the target**

  Unexpected side effects are often only discovered in clinical testing

- **Problems become amplified since most drugs have multiple targets**

BioMAP® Technology

Bridging the Gap



Molecular targets

BioMAP Technology

Human exposure

molecules | pathways | cells | tissues | humans

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Scale (meters) *(Time)*

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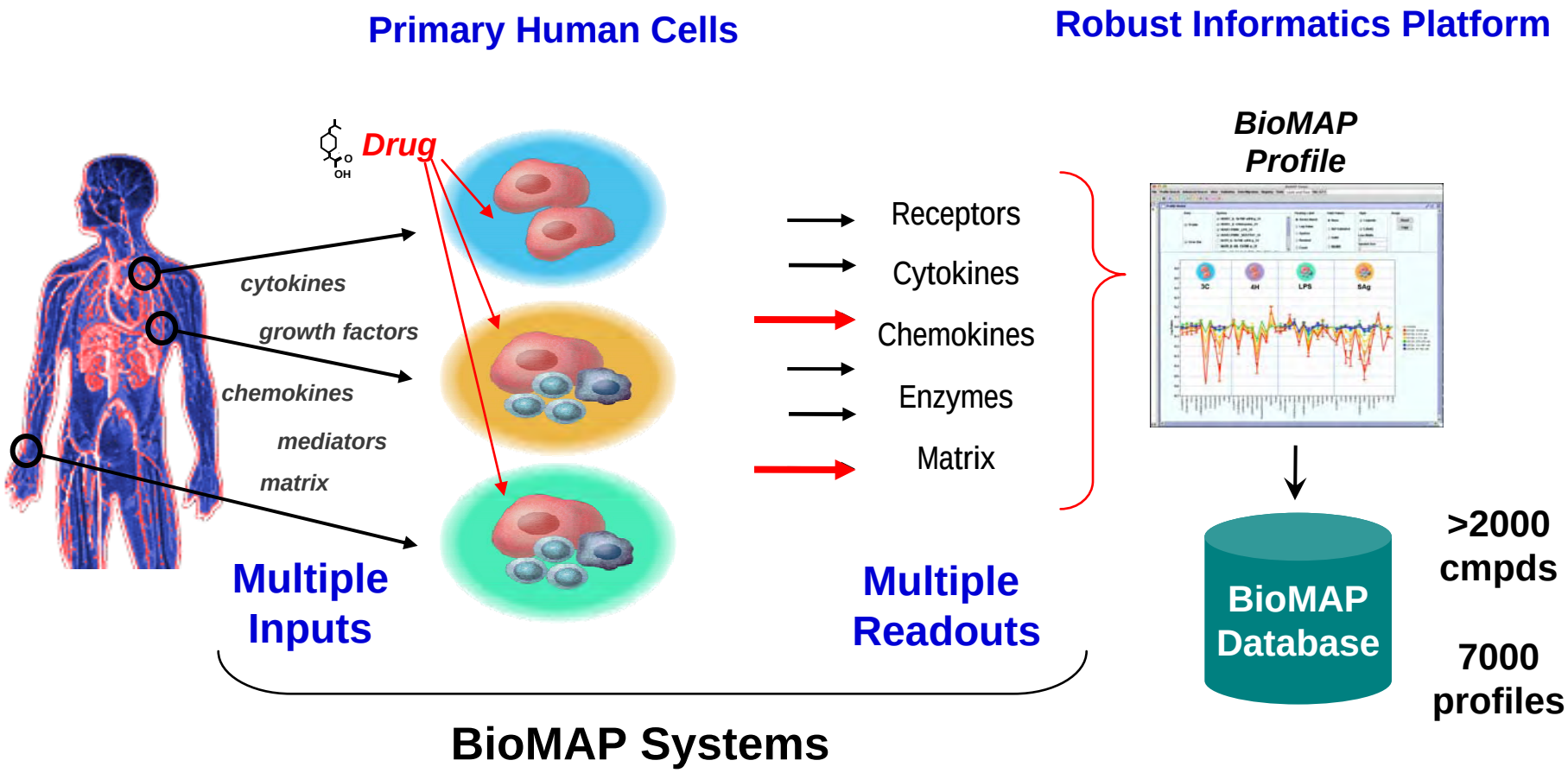
BioMAP® Systems

Complex Cell-Based In Vitro Models of Human Disease



- ***BioMAP Systems integrate biological complexity***

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Current BioMAP Systems

Inflammation / Autoimmune Disease / Respiratory / Cardiovascular

Primary Human Cell Types	Disease Relevance	BioMAP® System
Endothelial cells (EC)	Th1/ Th2 inflammation, allergy, asthma, dermatitis, angiogenesis, wound healing, restenosis, atherosclerosis (coronary art.)	3C, 4H, CA3C
EC + Peripheral Blood Mononuclear Cells	Th1 inflammation, psoriasis, COPD fibrosis, monocyte and T cell responses	LPS, SAg, HPNo
EC + Macrophages	Macrophage responses, arthritis, COPD, fibrosis	Mphg, MCIgE
EC + Mast cells	Asthma, allergy, dermatitis, fibrosis	HSM3C, HTh2
EC + Smooth Muscle Cells	Vascular biology, restenosis, atherosclerosis	HDFT, HDF3CT
EC + Th2 blasts	Allergy, asthma	HDF3C, HDFNo, HDF3CGF
Fibroblasts	Arthritis, asthma, dermatitis, fibrosis, psoriasis, wound healing	K3CT, MyoF
Myofibroblasts	Fibrosis, COPD, wound healing	KF3CT, KFNo
Keratinocytes	Psoriasis, dermatitis, wound healing	BE3C, BE4T
Keratinocytes + Fibroblasts	Psoriasis, dermatitis, wound healing	BF4T, SM3C, CASM3C
Bronchial Epithelial Cells	Th1 and Th2 inflammation Allergy, asthma, fibrosis, COPD	
Bronchial Epithelial Cells + Fibroblasts	Asthma, allergy, fibrosis, COPD	
Smooth Muscle Cells	Vascular inflammation, asthma, COPD, fibrosis (coronary artery SMC)	

Broad Coverage of Targets and Pathways

Detect and Distinguish Known and Novel Therapeutics

Inflammation / Autoimmune

- Calcineurin, TCR
- Glucocorticoid R
- Prostaglandin, leukotriene
- TNF-α
- IL-10
- NFκB
- IL-1, IFNγ
- p38 kinase
- Jak/Stat
- Lck kinase
- p38 kinase
- JNK

Asthma/Allergy

- H1-Receptor
- β2 Adrenergic
- cAMP/PDE
- PAF
- IL-4, IL-13

Cardiovascular

- ACE
- β2 Adrenergic
- Ca++ Channel
- Cholesterol
- Hyperlipidemia
- Restenosis
- Antioxidant

Metabolism

- PPARγ
- GR
- LXR
- FXR
- Estrogen receptor
- Androgen receptor
- HMG-CoA reductase
- AMPK
- GSK3β

Cancer

- HDAC
- Hsp90
- Proteasome
- NFκB
- PI-3K/AKT
- Mek
- CDK
- RAR/RXR
- Stat
- Ras/MAPK
- TGFβ
- Microtubule
- Jak/Stat
- Tie2 R
- TGFβ

….and many others (>2000 drugs/compounds in reference database)

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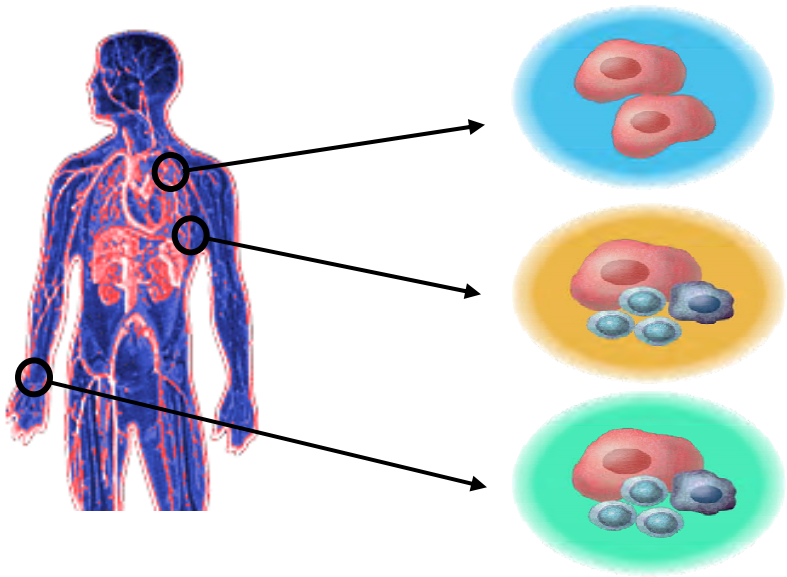
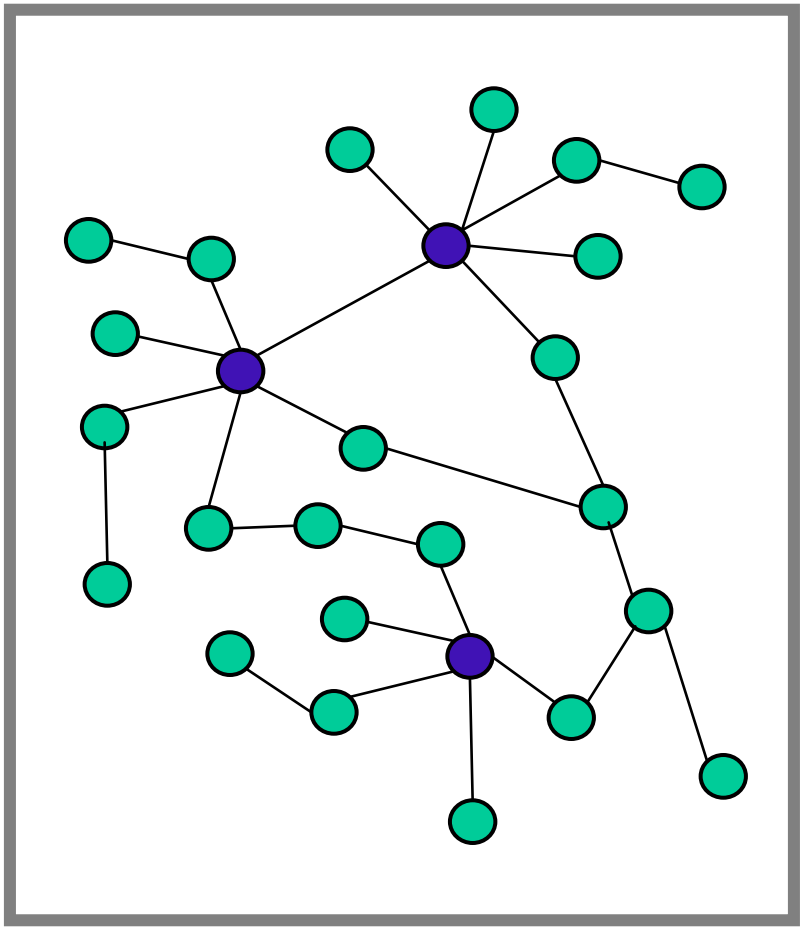
What Makes BioMAP Systems Different

Why Primary Cells?

Primary Human Cells



Primary Cells



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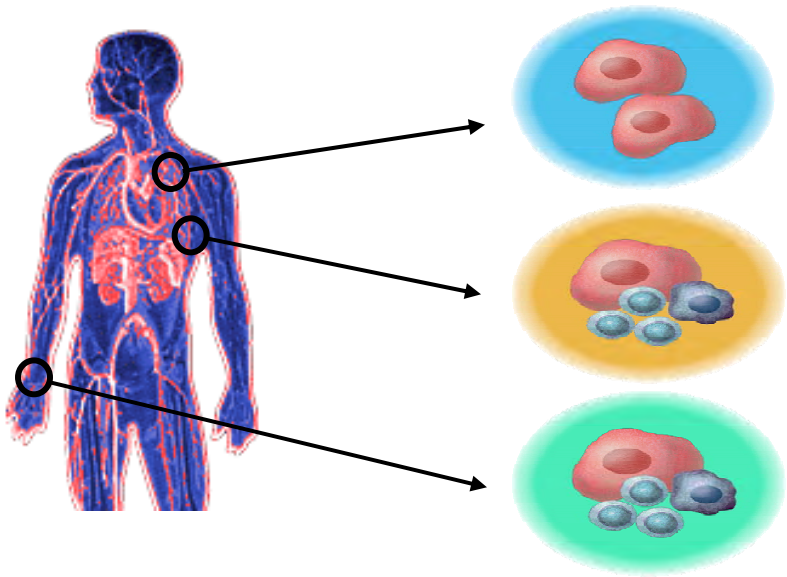
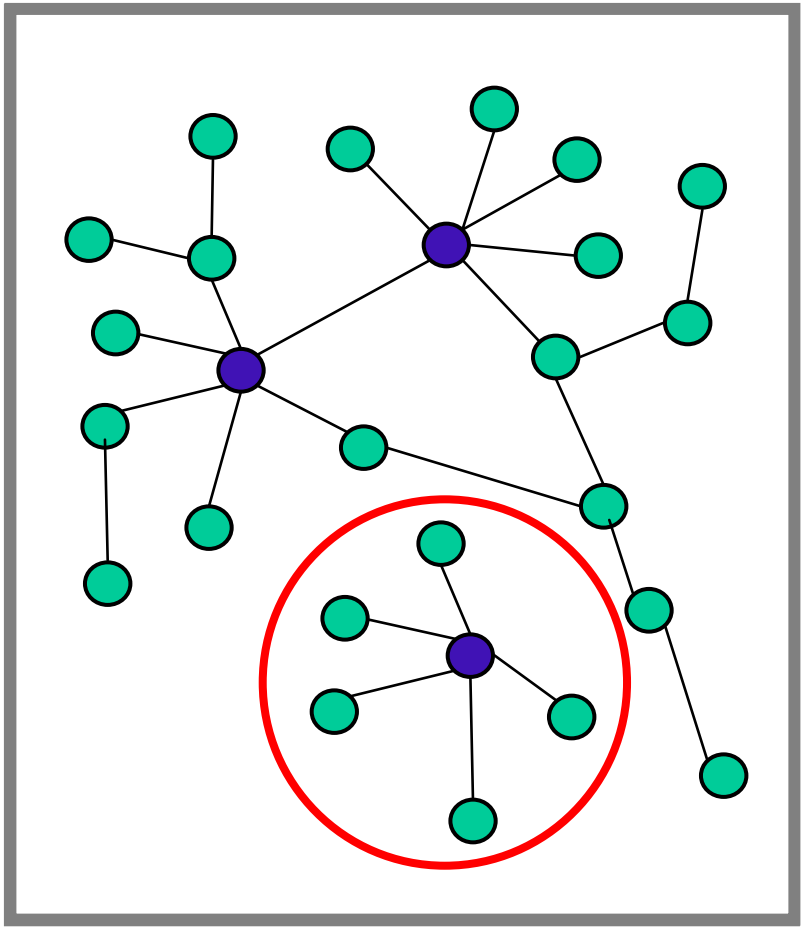
Why Primary Cells?

Primary Human Cells



Cell Line

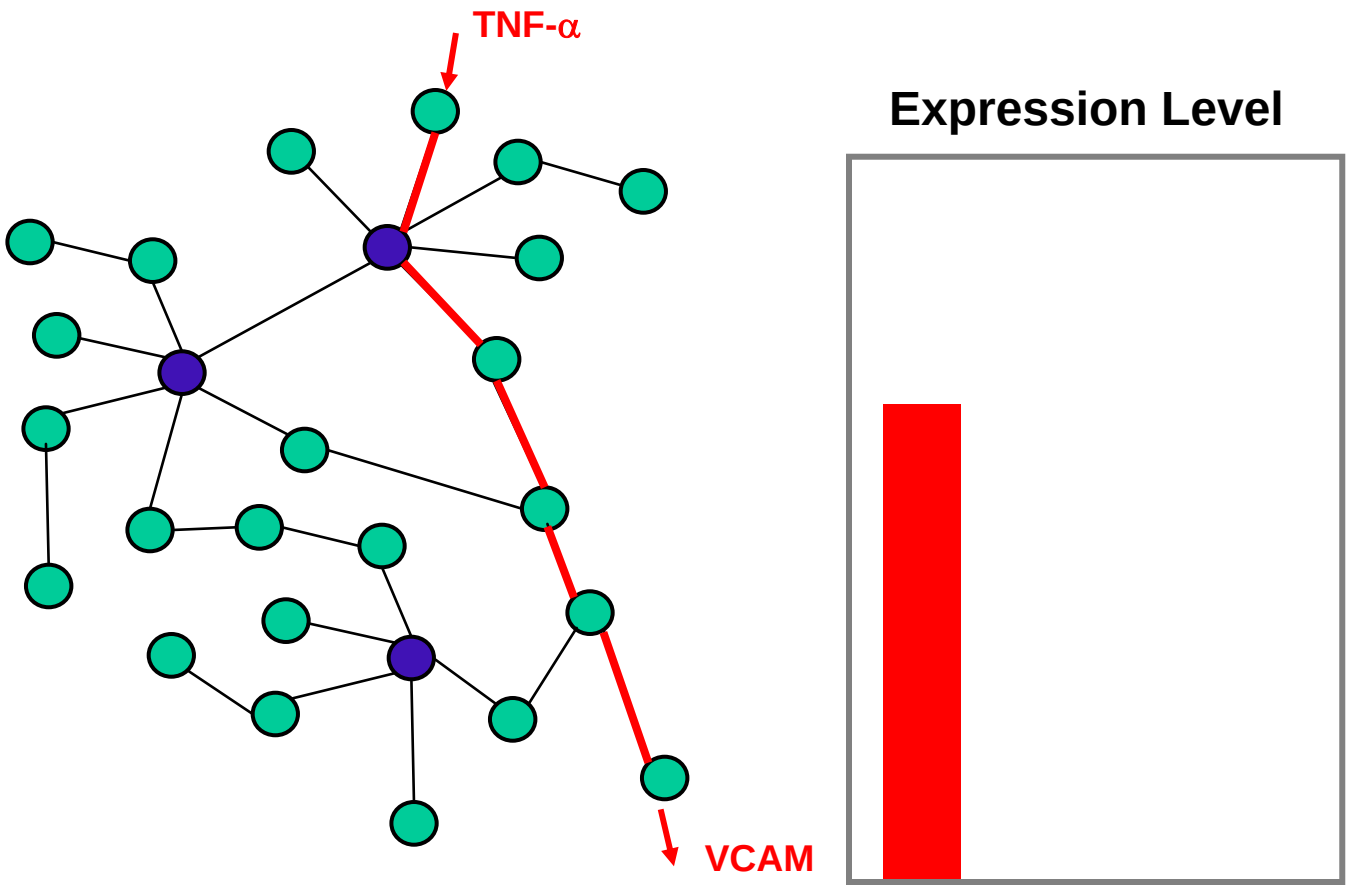


Primary cells retain regulatory networks and feedback mechanisms

What Makes BioMAP Systems Different

Why Activate Multiple Pathways?

One Pathway Active



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What Makes BioMAP Systems Different

Why Activate Multiple Pathways?

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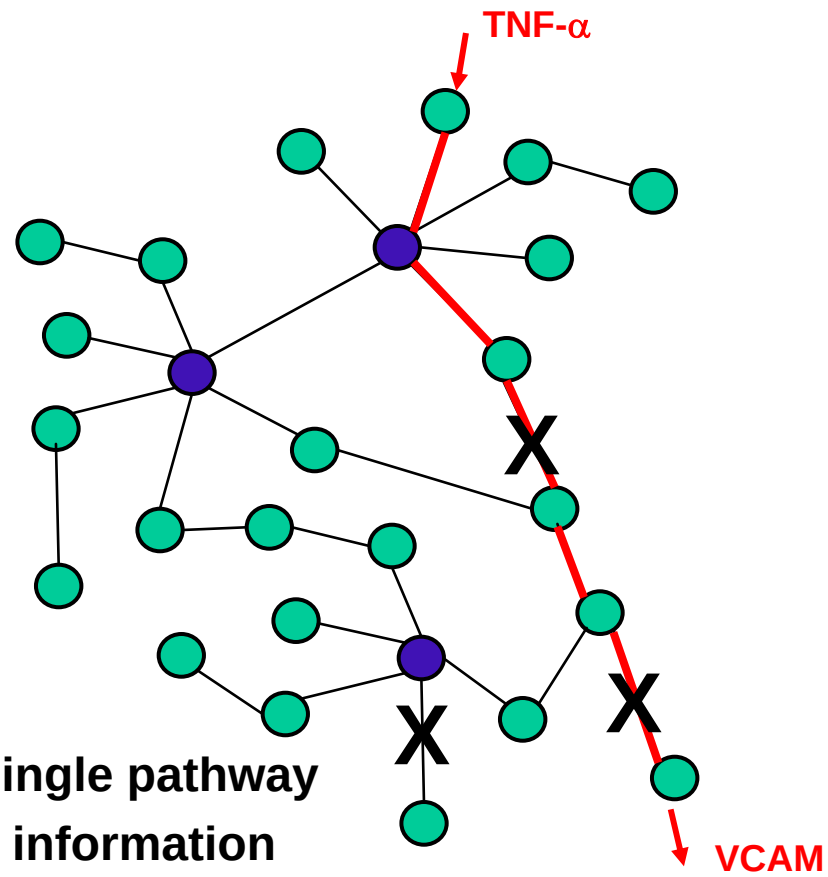
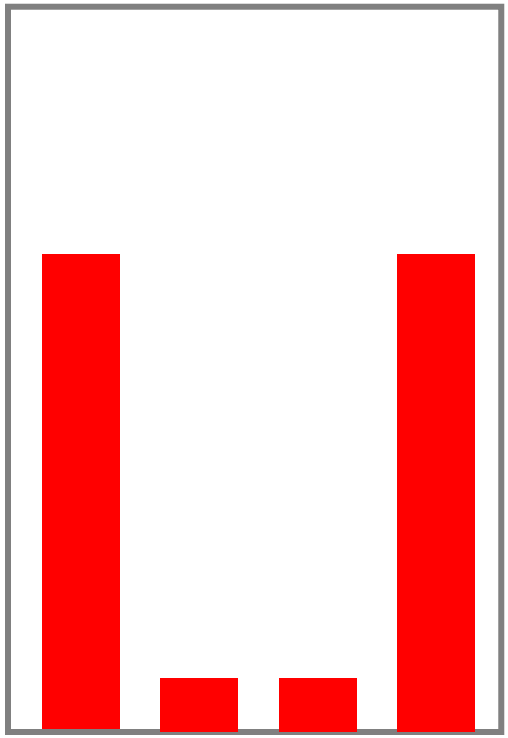


Expression Level



Activation of a single pathway provides limited information

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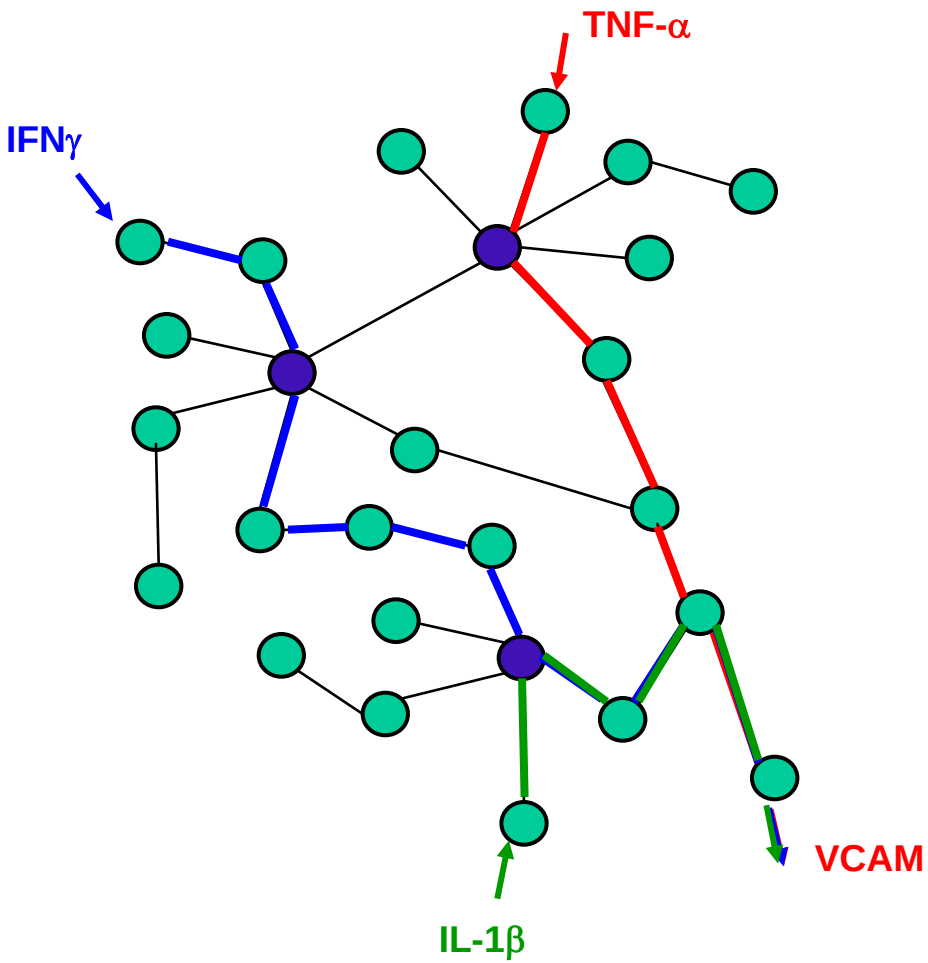
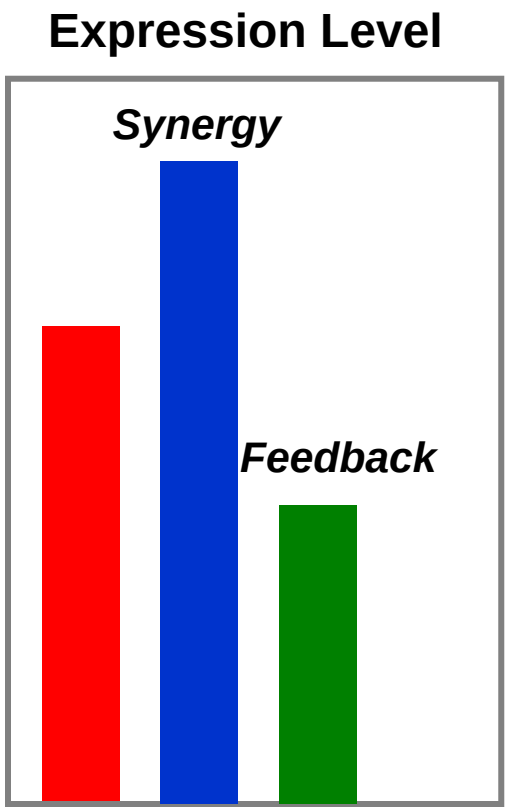
What Makes BioMAP Systems Different

Why Activate Multiple Pathways?

Multiple Pathways Active





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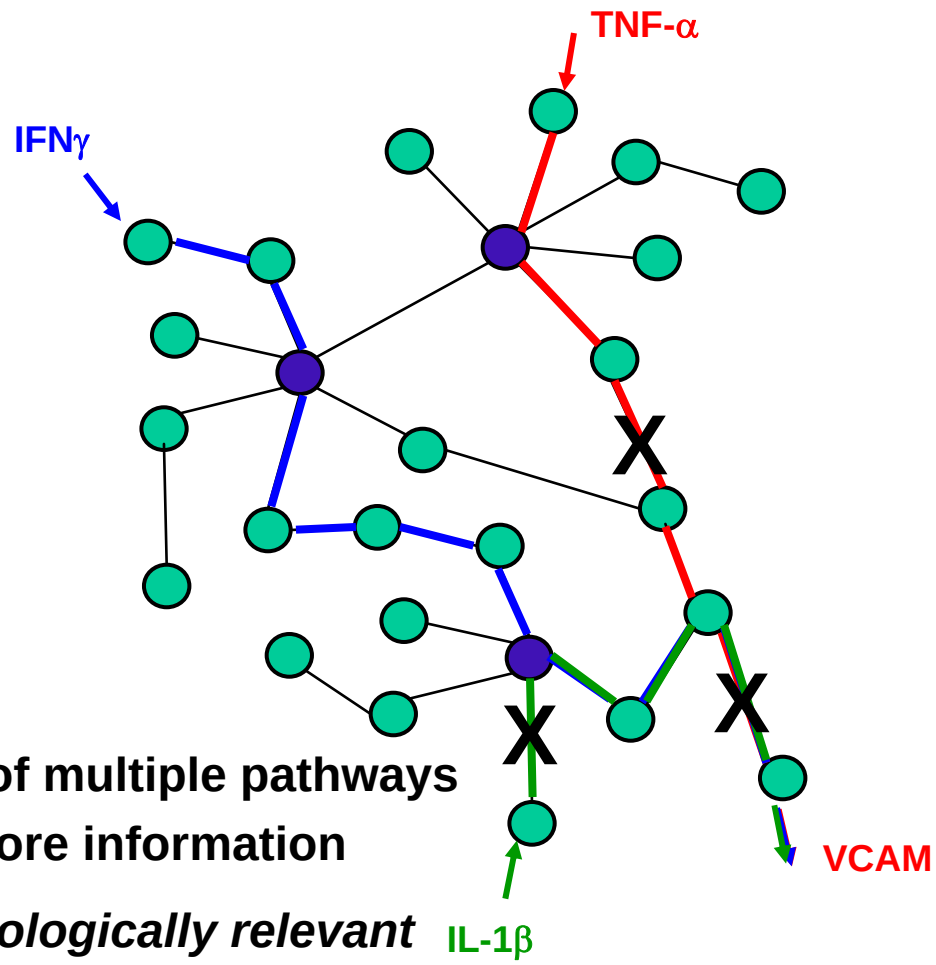
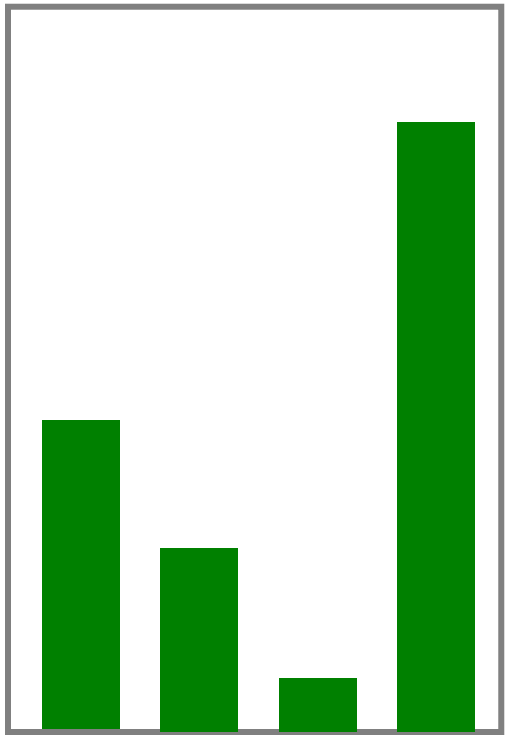
What Makes BioMAP Systems Different

Why Activate Multiple Pathways?

Multiple Pathways Active



Expression Level



Activation of multiple pathways provides more information

More physiologically relevant

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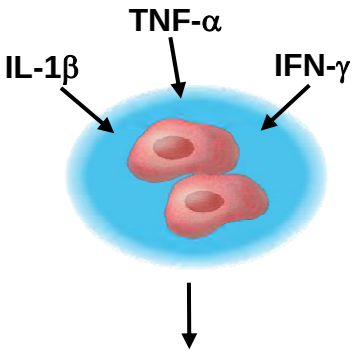
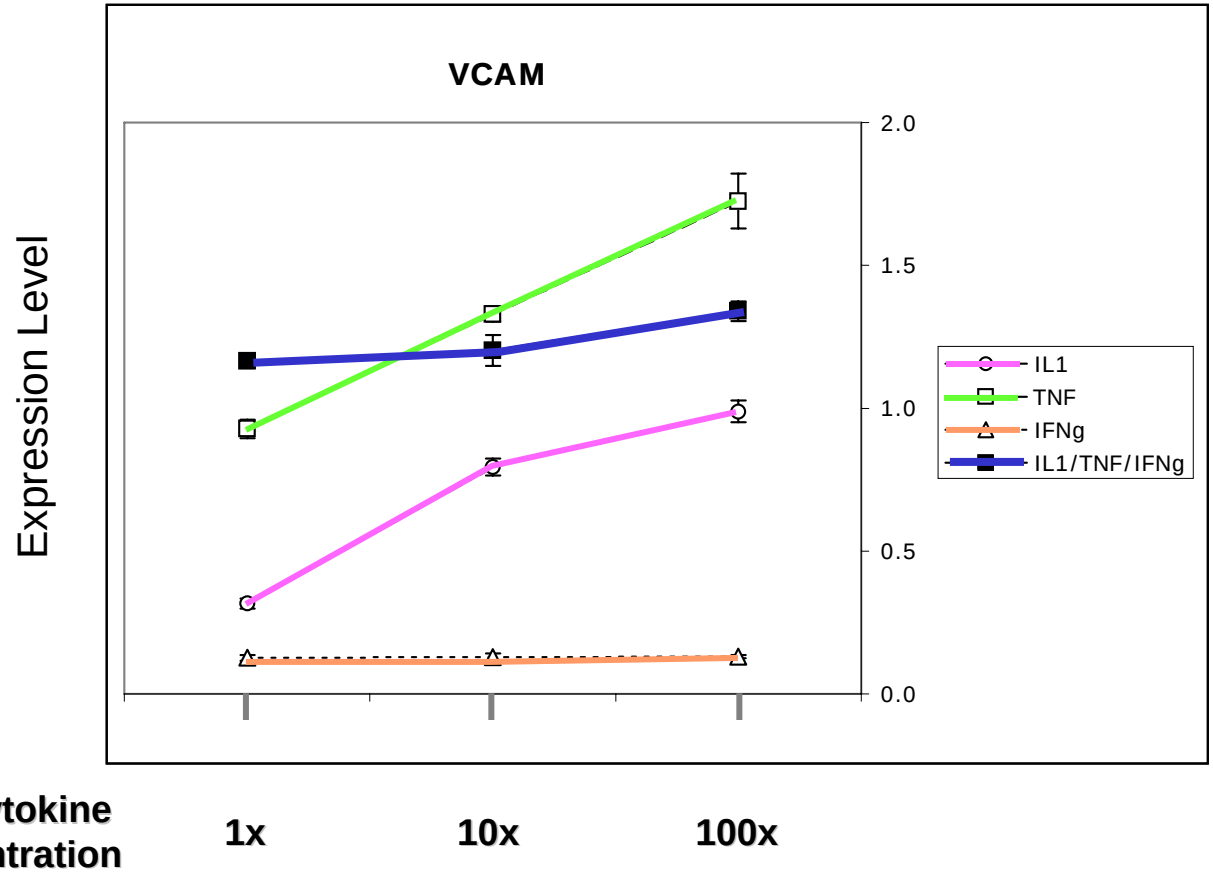
Complex Assays Capture "System" Properties

Primary Human Endothelial Cells





- **Constant level of expression over 2 orders of magnitude**
- **Complex assays demonstrate *robustness***

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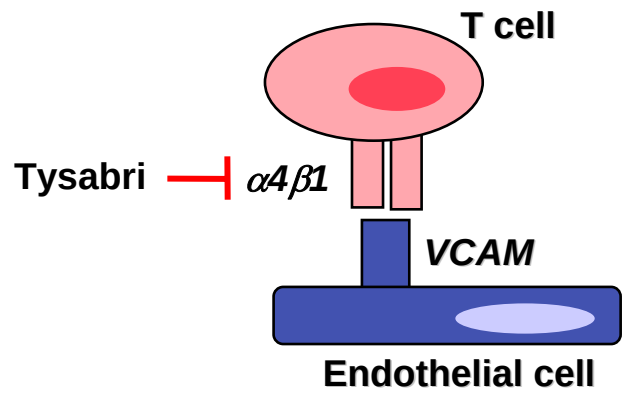
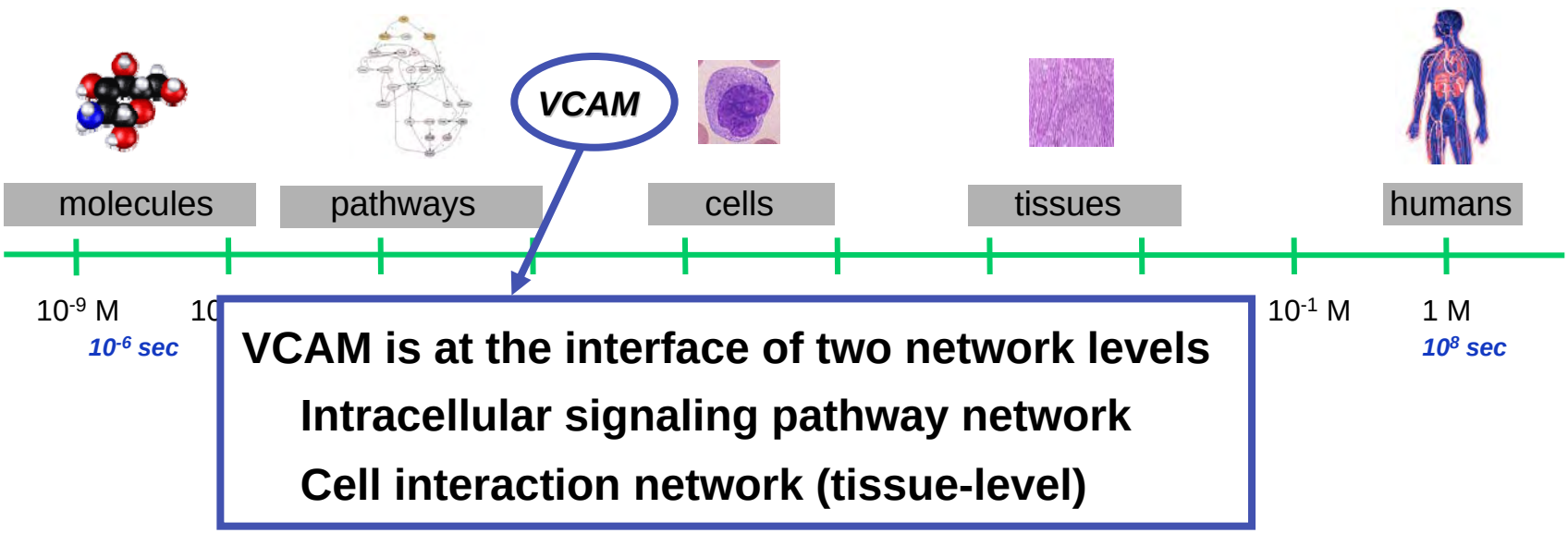
Is VCAM Special?

- **VCAM is an adhesion molecule and ligand for the T cell integrin α4β1**
  - **α4β1 is the target of the multiple sclerosis drug, Tysabri**





VCAM is at the interface of two network levels

- **Intracellular signaling pathway network**
- **Cell interaction network (tissue-level)**

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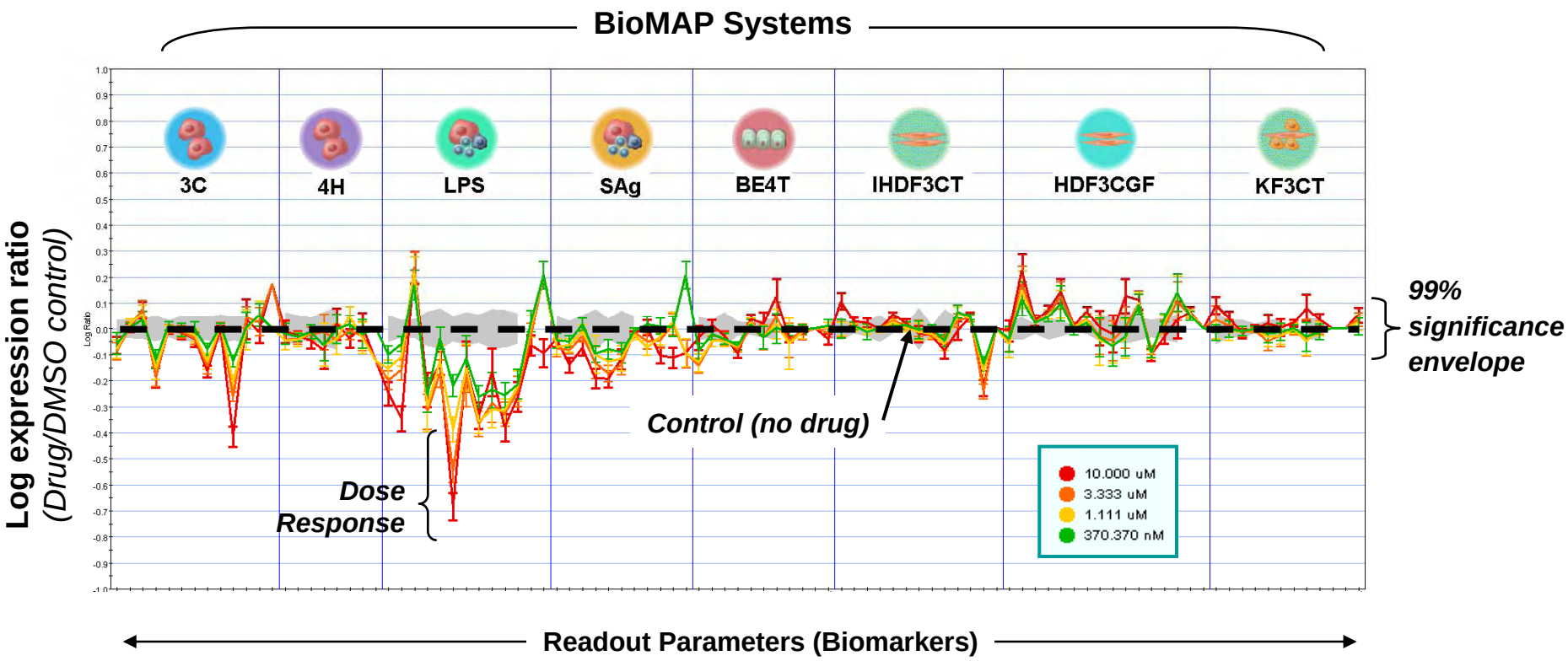
Example BioMAP Profile

Reference p38 Inhibitor



- **BioMAP activity profiles are robust and reproducible**
- **Profiles retain shape over multiple concentrations**

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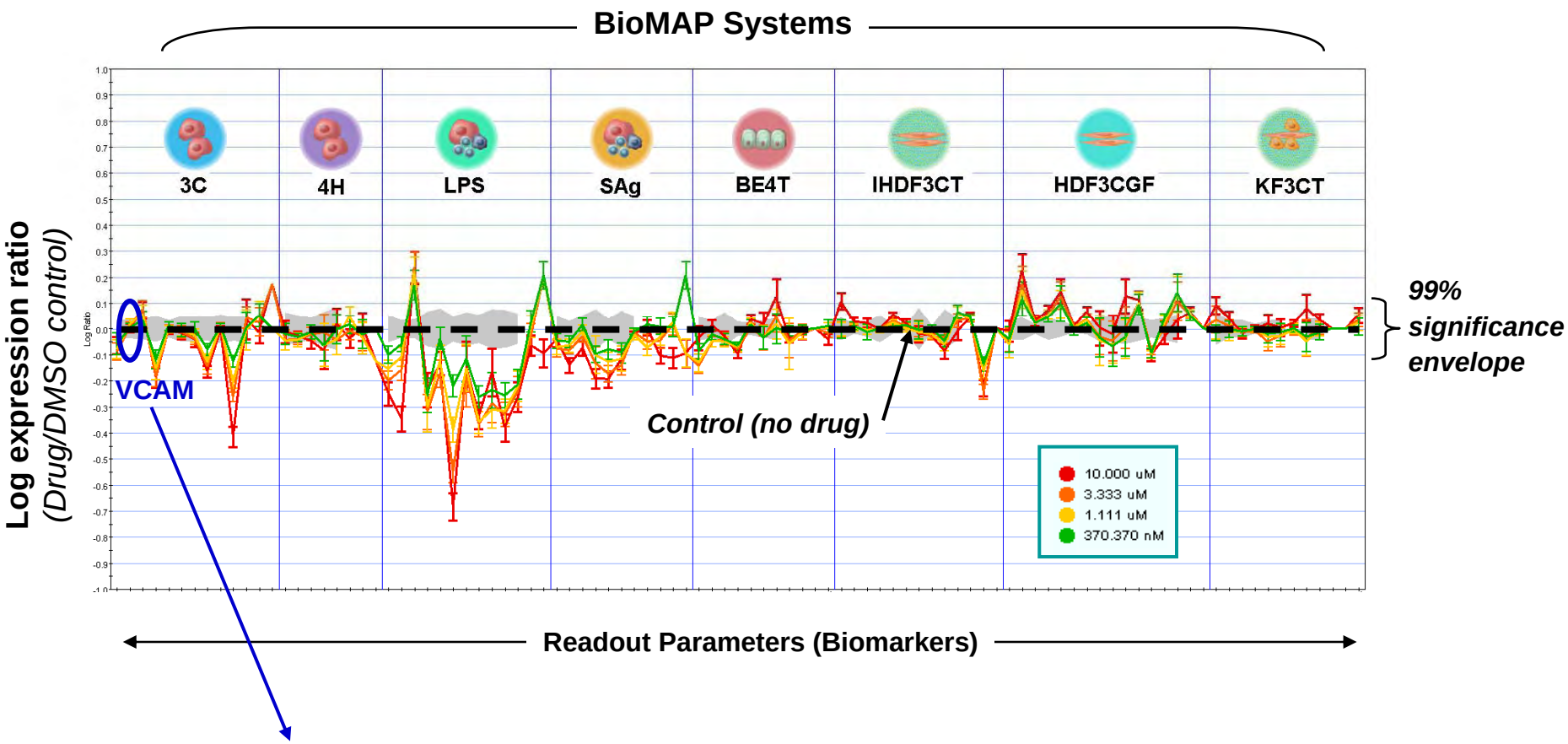
Example BioMAP Profile

Reference p38 Inhibitor



- **No inhibition of VCAM under "3C" conditions (IL-1β + TNF-α + IFNγ)**

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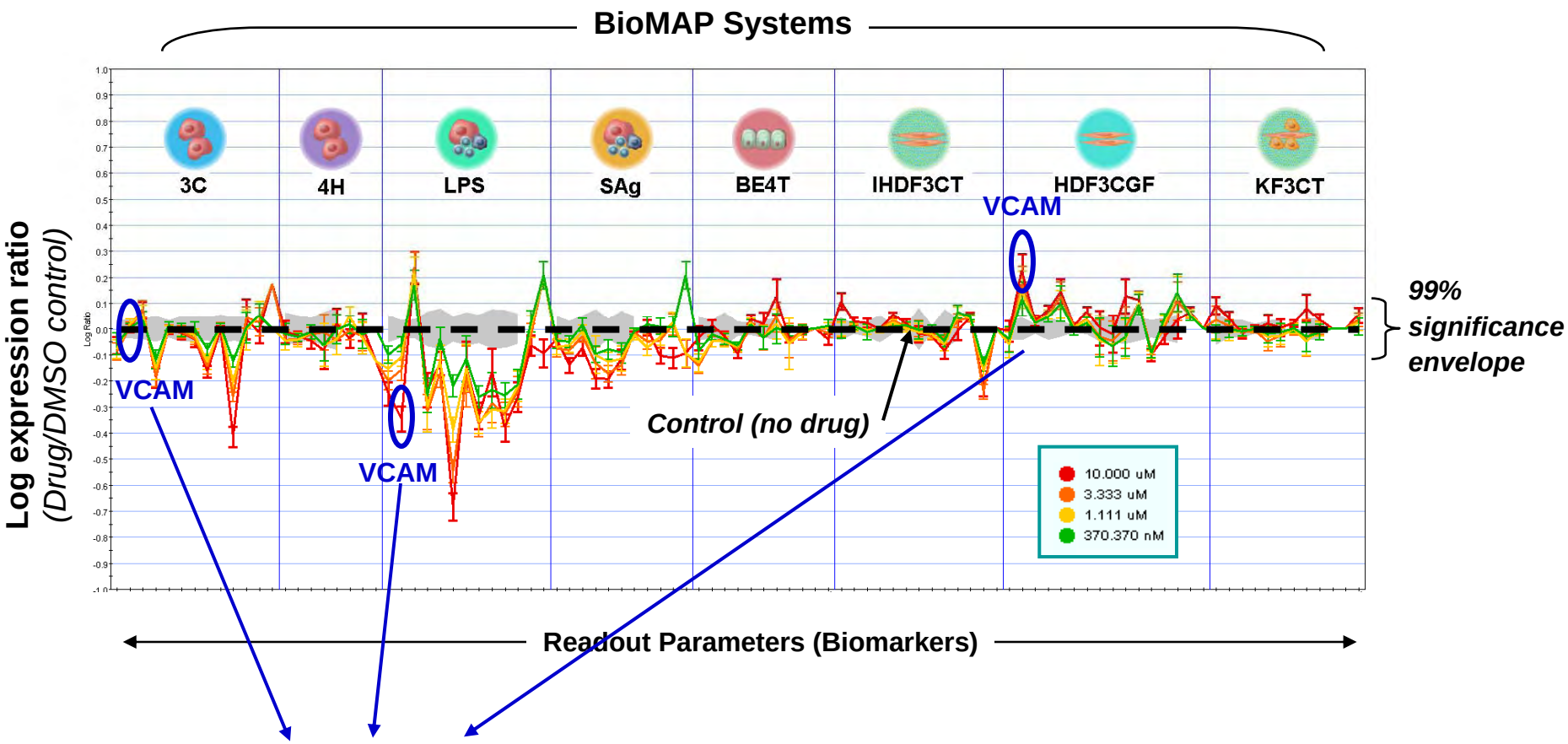
Example BioMAP Profile

Reference p38 Inhibitor



- **VCAM is differentially regulated**
- **Regulation of VCAM is "context dependent"**

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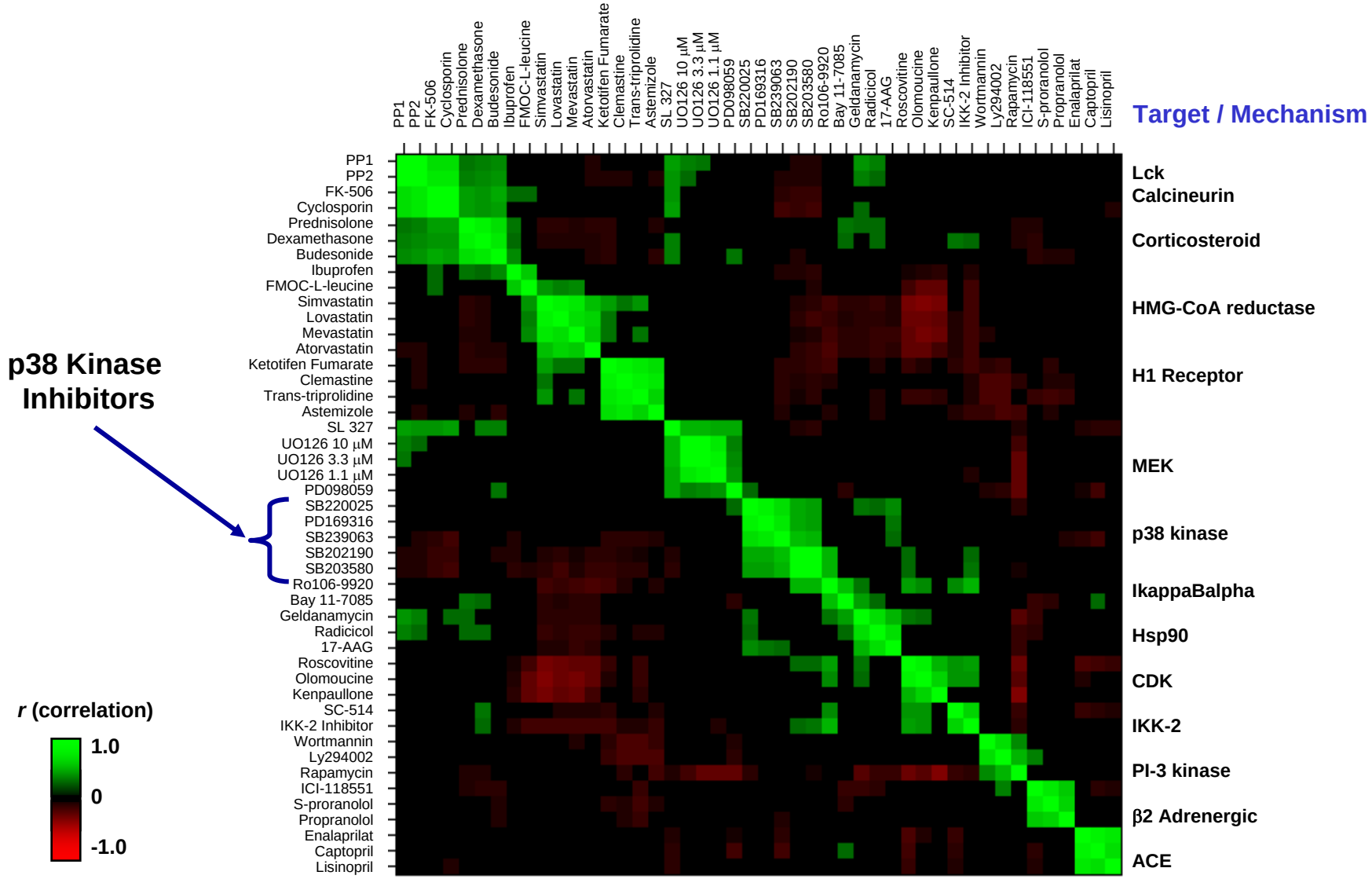
Classification of Compounds

Pairwise Correlation Analysis of BioMAP Profiles



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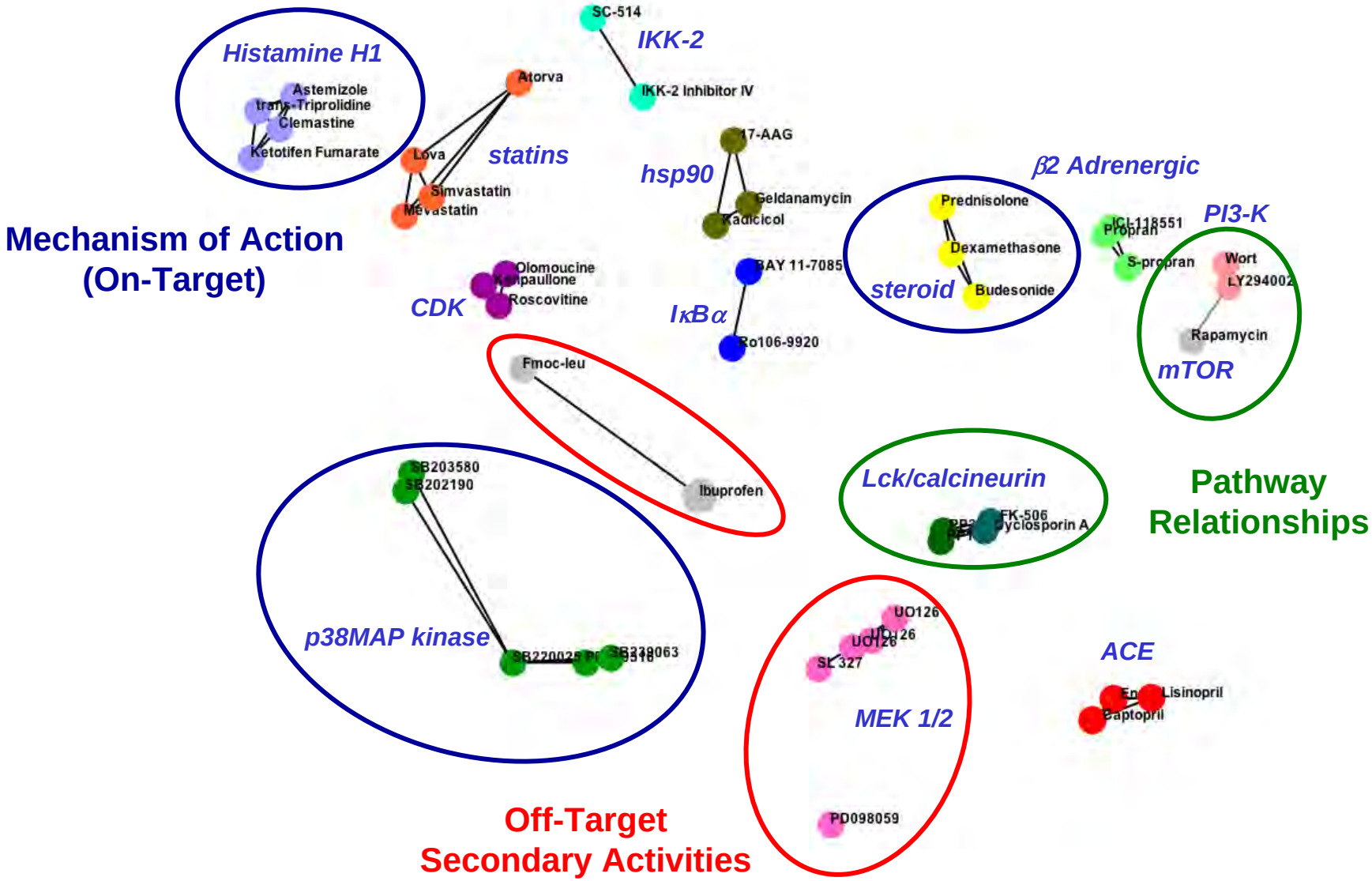
BioMAP Classifies Drugs By Mechanism of Action

Multidimensional Scaling - Function Similarity Map



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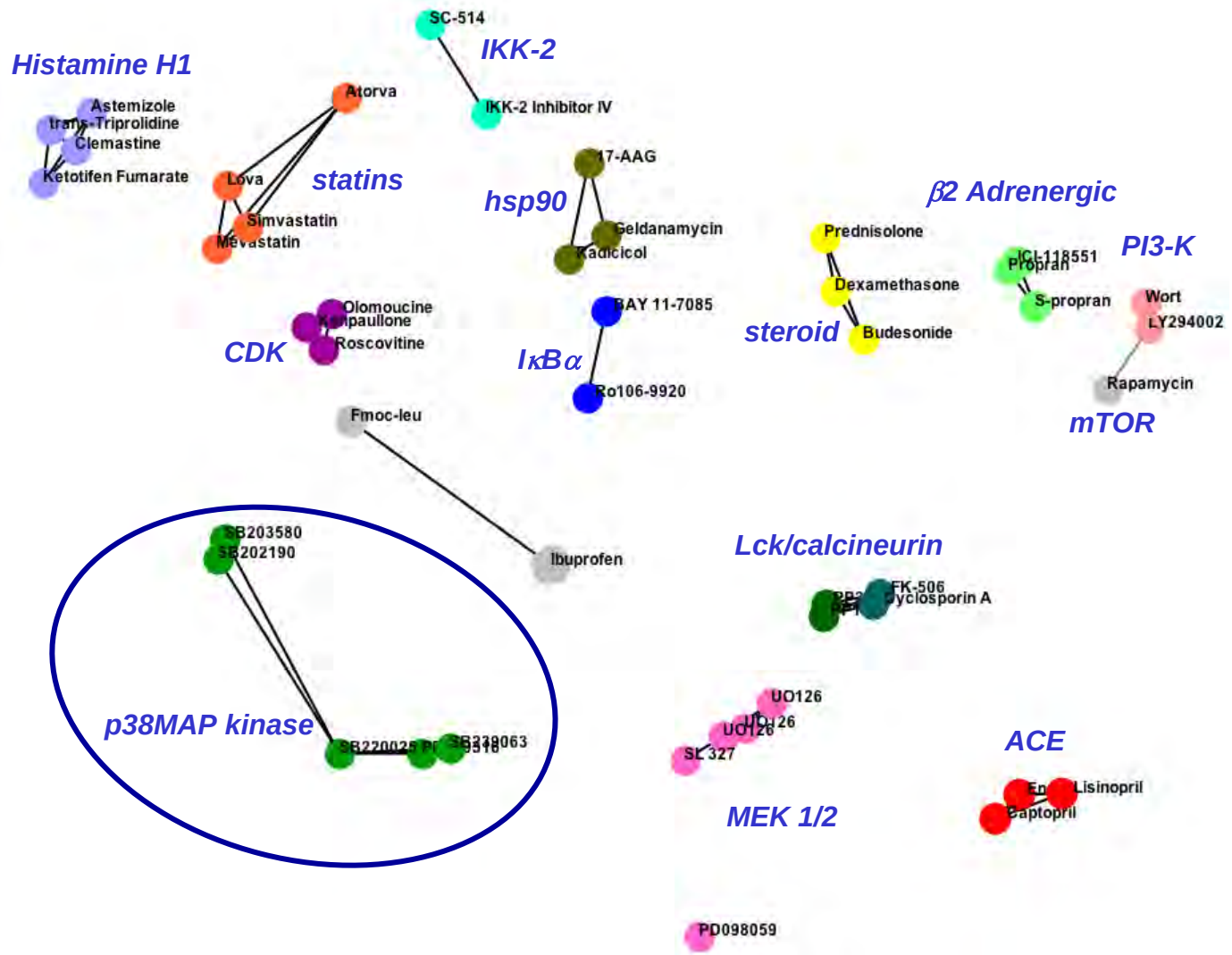

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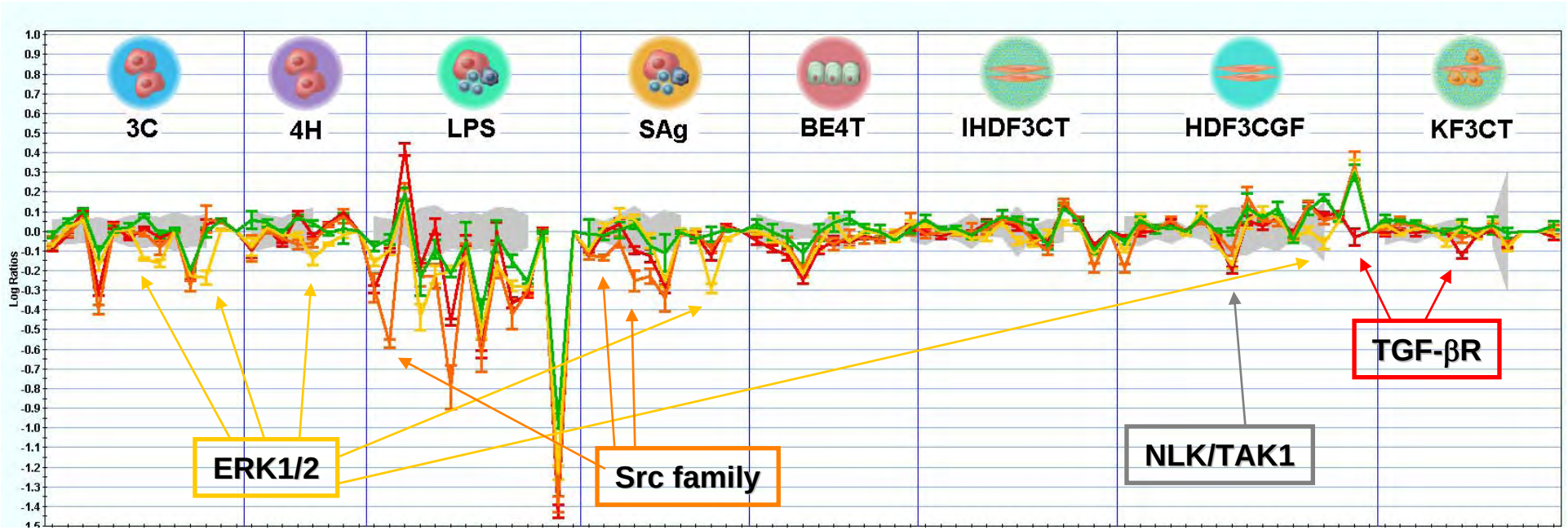
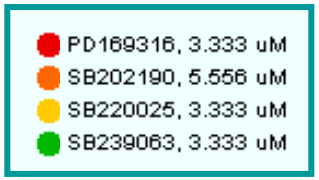
BioMAP for Structure Activity Relationships

p38 kinase Inhibitors – Correlation with Biochemical Data



Kinase Profiling 227 Kinases (Ambit)

Ambit Gene Symbol	SB202190	PD169316	SB220025	SB239063
ERK1	No hit	No hit	2.85	No hit
ERK2	No hit	No hit	2.1	No hit
JNK1	No hit	No hit	No hit	No hit
JNK2	0	0.15	1.65	0.1
JNK3	0.3	0.65	0.85	1.3
MAP4K4	No hit	11.2	7.05	5.1
MAP4K5	No hit	No hit	6.95	1.1
NLK	2.15	2.5	3.15	8.6
p38-alpha	0	0	0	0
p38-beta	0	0	0.4	0.3
p38-gamma	No hit	11.3	7.65	11.3
TGFBR1	15.35	5.8	10.1	No hit



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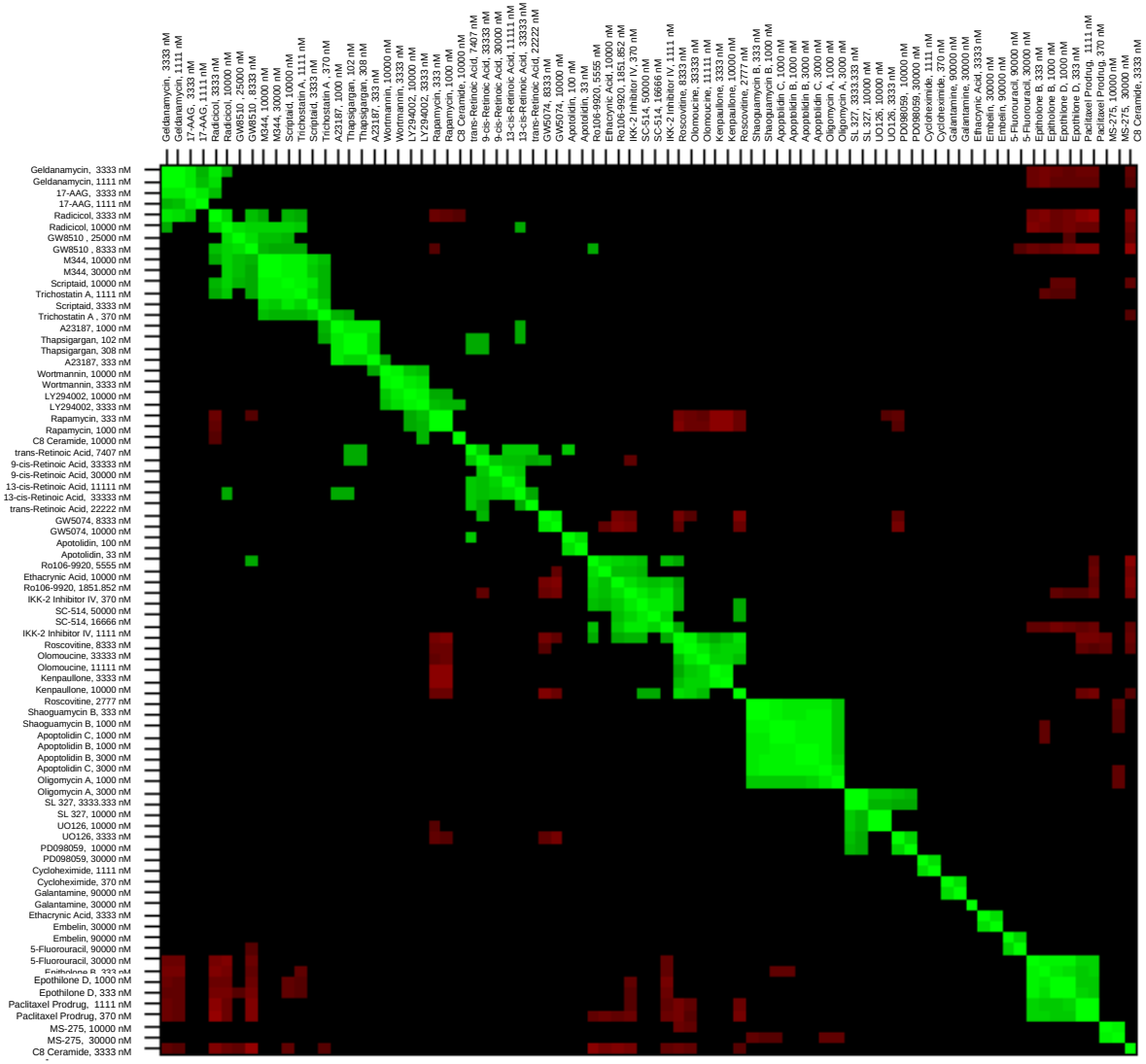
BioMAP Classifies Toxic Agents By Mechanism

Pairwise Correlation Analysis



HSP90

HDAC

Ca++ mobilization

PI-3 kinase

mTOR

Retinoids

NFκB

CDK

Mitochondrial Uncoupling

MEK/Erk

Protein Synthesis

XIAP

DNA Synthesis

Microtubule stabilizer

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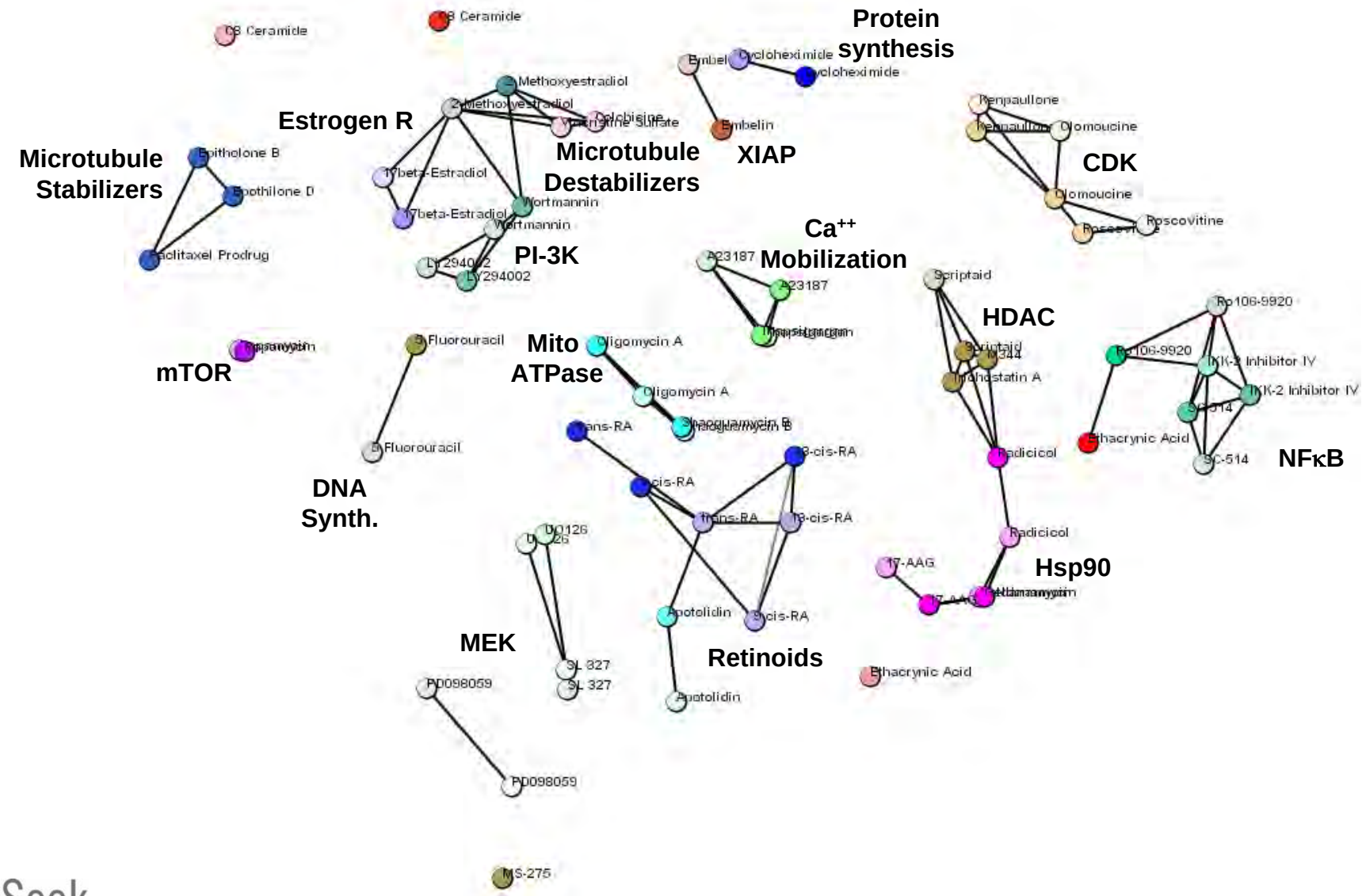
BioMAP Classifies Toxic Agents By Mechanism

Multidimensional Scaling - Function Similarity Map



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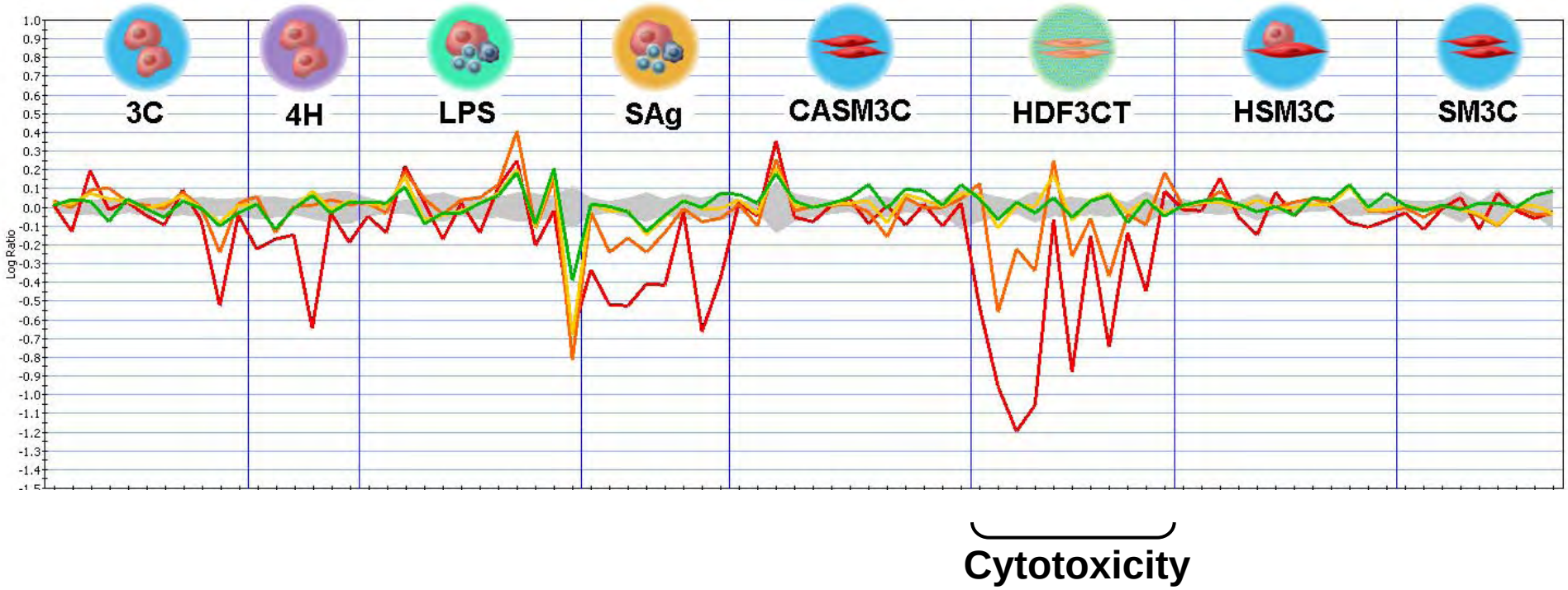
Retinoic Acid



- **Cell-type selective cytotoxicity**
- **Unexpected fragility**

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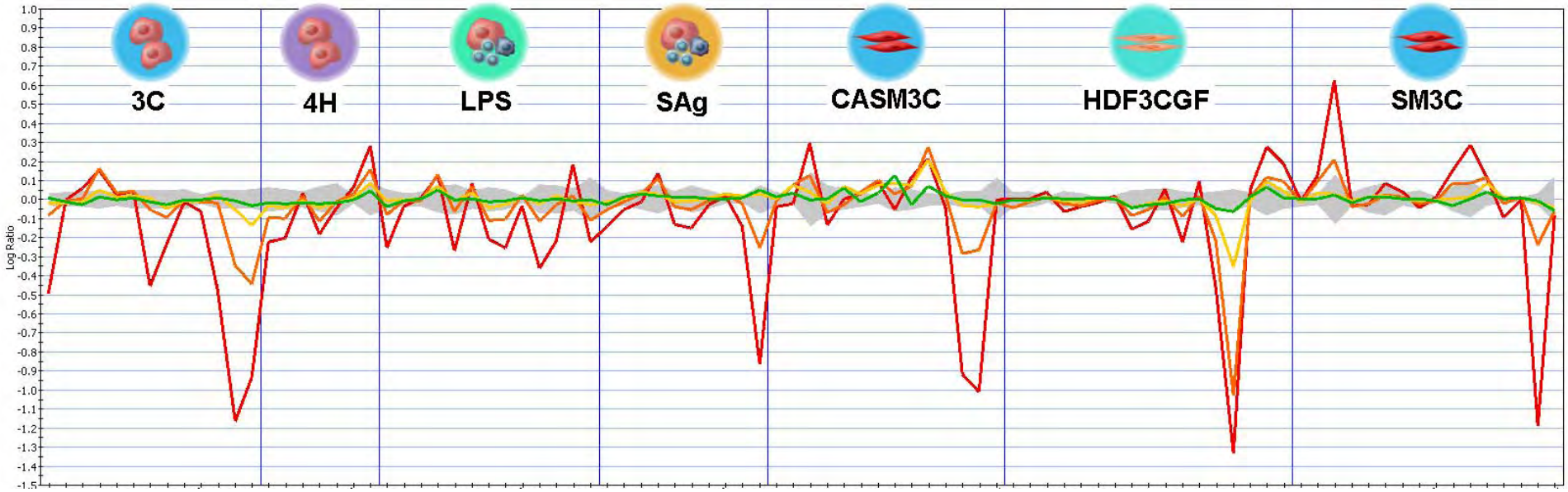
Terconazole



- **Profile shows similarity to PI-3 kinase inhibitors**
- **Cytochrome P450 products have been shown to regulate fibroblast proliferation via PI-3 kinase (Nieves, 2006, J. Lipid Res., 47:2681)**

Summary

- **Addressing biological complexity in assay design is important for understanding compound mechanisms**
  - **Much of the regulatory architecture is hidden**
  - **"Complexity for simplicity"**
    - **We know much less that we think we know**
- **Complex human cell systems:**
  - **Practical format for evaluating drug / compound mechanisms**
    - **Broad range of target mechanisms**
  - **Useful platform for integrating molecular and clinical information**

Acknowledgements

BioSeek

- Eric J. Kunkel
- Jonathan Bingham
- David Patterson
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- Jian Yang

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