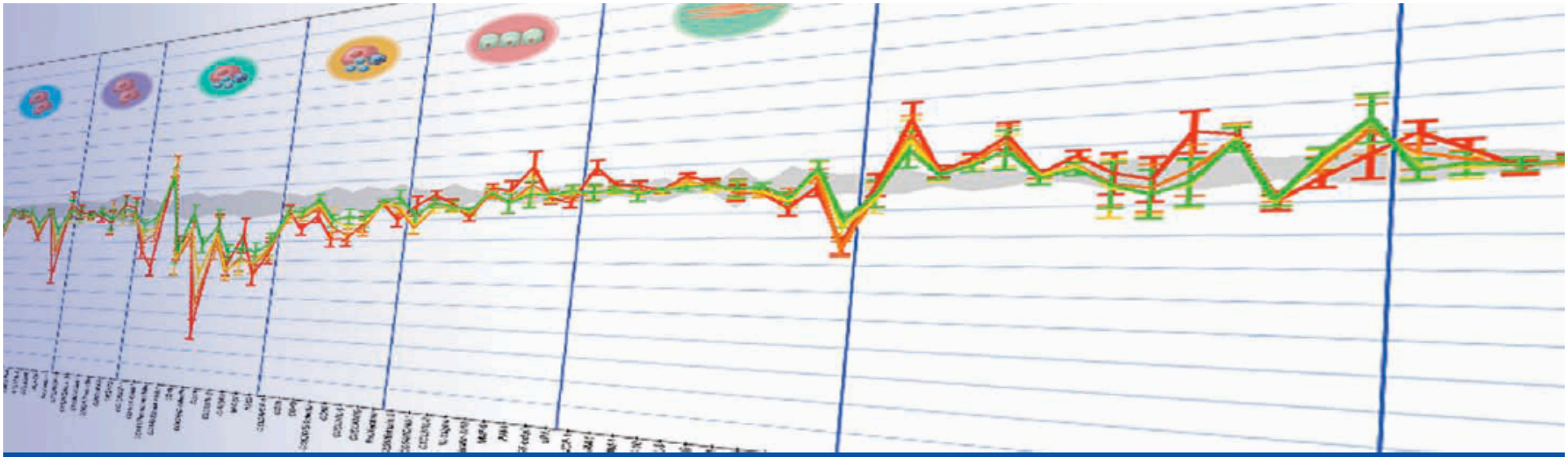


# BioSeek - ToxCast Phase I Project Update

**Ellen Berg, PhD, BioSeek, Inc.**

**EPA Chemical Prioritization Community of Practice**
**Monthly Meeting**

January 24, 2008

BioSeek

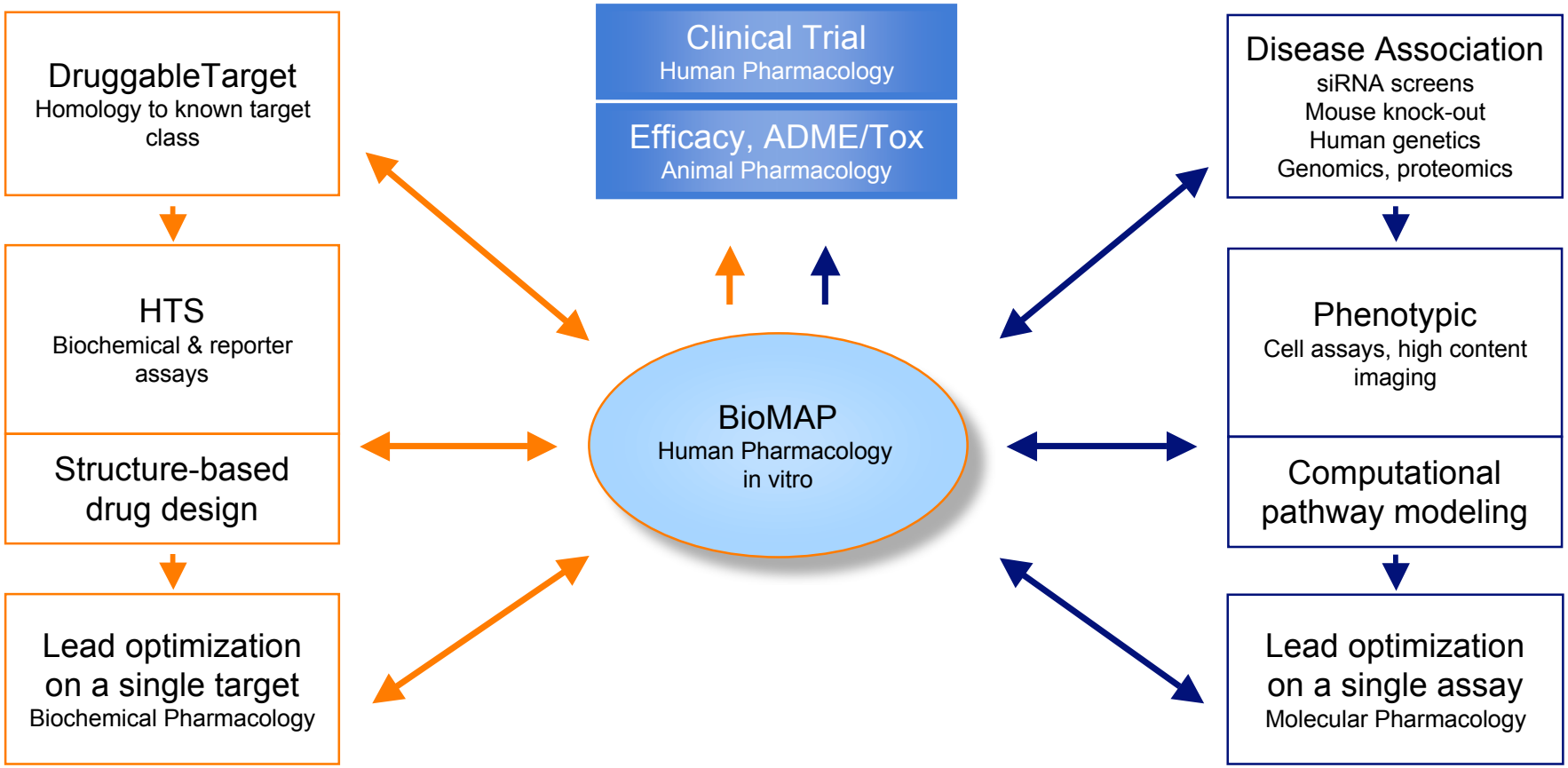
# Agenda

**Introduction to BioSeek's BioMAP Technology**

**Summary of results - Proof-of-Concept study**

**Next steps**

# Translating Chemistry into Human Pharmacology




***With BioMAP, pharmacological properties of compounds are addressed at each step, de-risking critical decision points***



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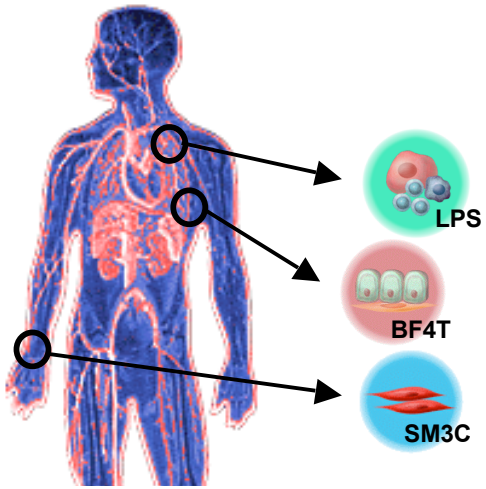
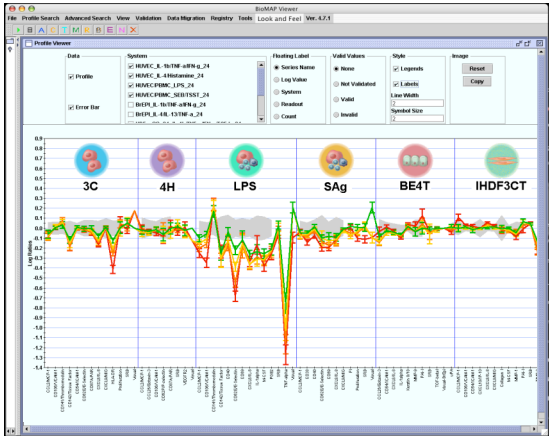
# BioMAP® Technology Platform

## Assays



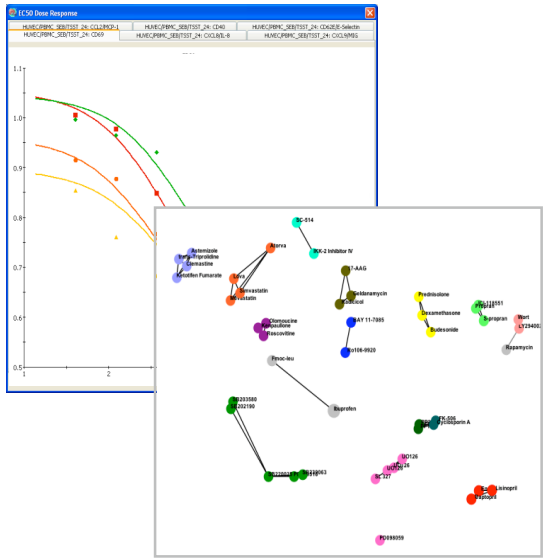

Human primary cells
Disease-like culture conditions

## Profile Database




Biological responses to drugs and stored in the database

## Informatics



Specialized informatics tools are used to mine and analyze biological data

***BioMAP is highly complementary to biochemical target and phenotypic screening***



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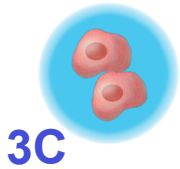
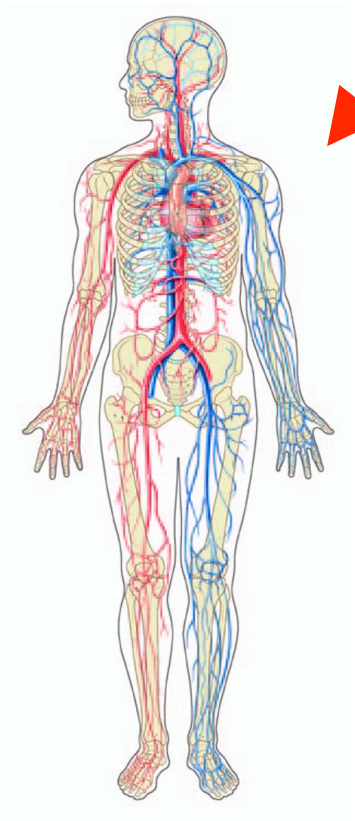
# Example Assay System – Vascular Inflammation

| System | Cell Type | Tissue Setting | Biomarkers |
|---|---|---|---|
|  3C | Endothelial Cells | Chronic Th1 Inflammation<br>IL-1$\beta$ + TNF$\alpha$ + IFN$\gamma$ | MCP-1, VCAM, CD141, CD142, ICAM, E-selectin, uPAR, IL-8, MIG, HLA-DR |





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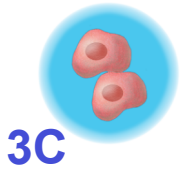
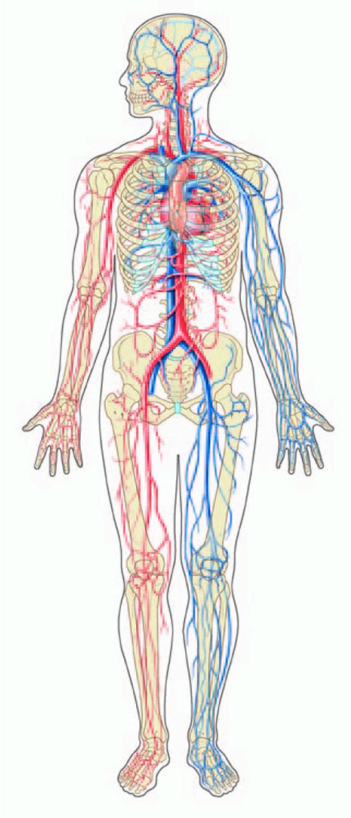
# Example Assay System – Vascular Inflammation

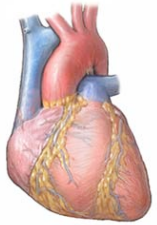
| System | Cell Type | Tissue Setting | Biomarkers |
|---|---|---|---|
|  3C | Endothelial Cells | Chronic Th1 Inflammation IL-1$\beta$ + TNF$\alpha$ + IFN$\gamma$ | MCP-1, VCAM, CD141, CD142, ICAM, E-selectin, uPAR, IL-8, MIG, HLA-DR |



Stroke

Multiple Sclerosis

Cardiovascular Disease



Autoimmune Disease

Arthritis

Respiratory Disease

Heart Disease

COPD



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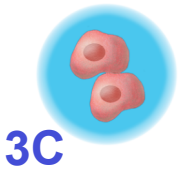
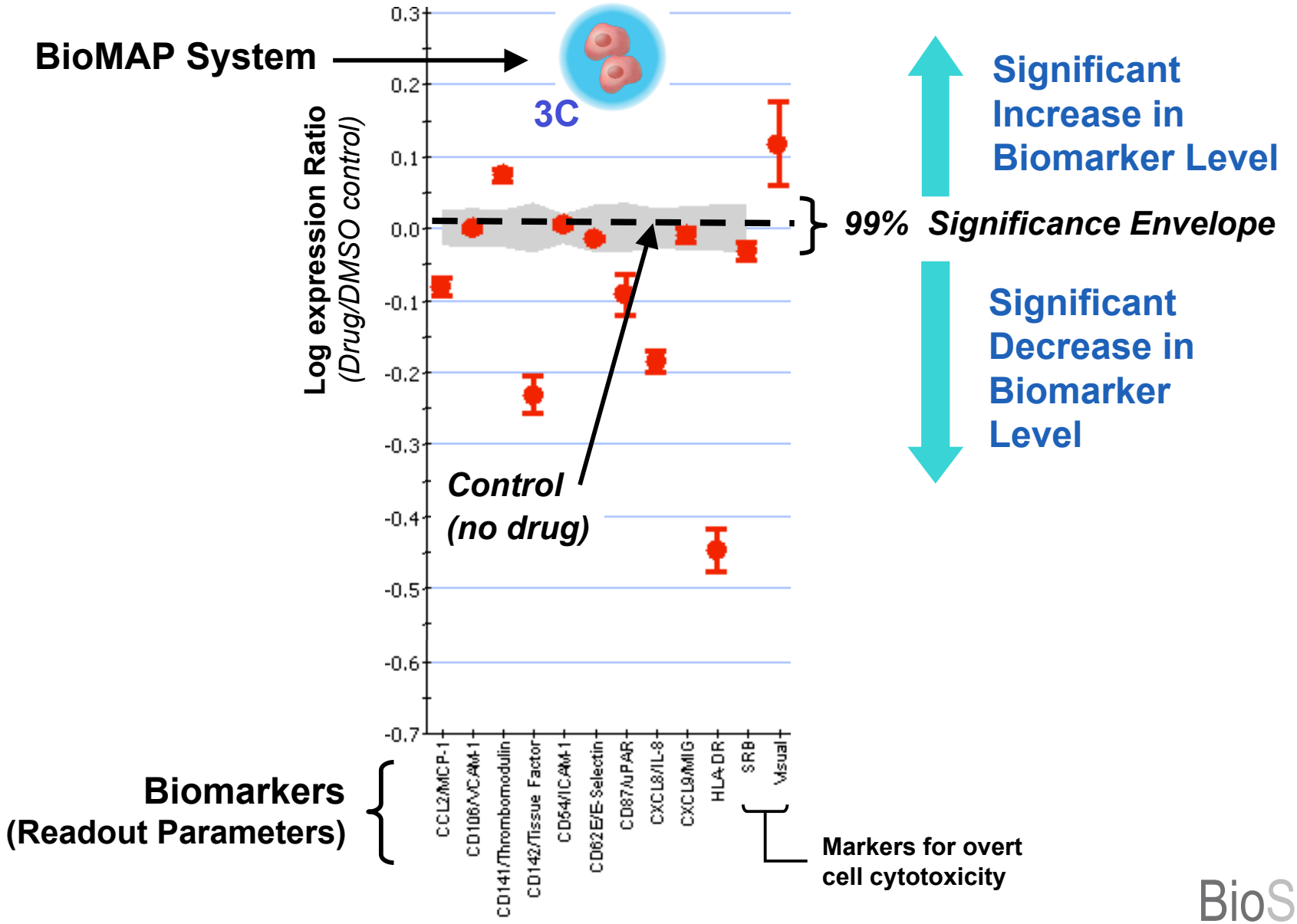
# Example Assay System – Vascular Inflammation

| System | Cell Type | Tissue Setting | Biomarkers |
|---|---|---|---|
|  3C | Endothelial Cells | Chronic Th1 Inflammation IL-1$\beta$ + TNF$\alpha$ + IFN$\gamma$ | MCP-1, VCAM, CD141, CD142, ICAM, E-selectin, uPAR, IL-8, MIG, HLA-DR |

| Biomarker | Biological Process |
|---|---|
| MCP-1 | Vascular inflammation, monocyte, T cell recruitment |
| VCAM | Chronic inflammation, redox stress; monocyte, T cell recruitment |
| CD141/Thrombomodulin | Fibrinolysis, thrombosis (-) |
| CD142/Tissue Factor | Angiogenesis, coagulation, thrombosis |
| ICAM | Acute and chronic inflammation; leukocyte adhesion and recruitment |
| E-Selectin | Leukocyte adhesion, neutrophil recruitment, skin T cell recruitment |
| CD87/uPAR | Cell migration, proliferation, angiogenesis |
| IL-8 | Acute inflammation, neutrophil recruitment, angiogenesis |
| MIG | Chronic inflammation, T cell recruitment, angiogenesis (-) |
| HLA-DR | Th1 immune responses, infection, antigen presentation |

# BioMAP Profiling: Example Profile

*Biomarkers affected by reference p38 MAPK Inhibitor*



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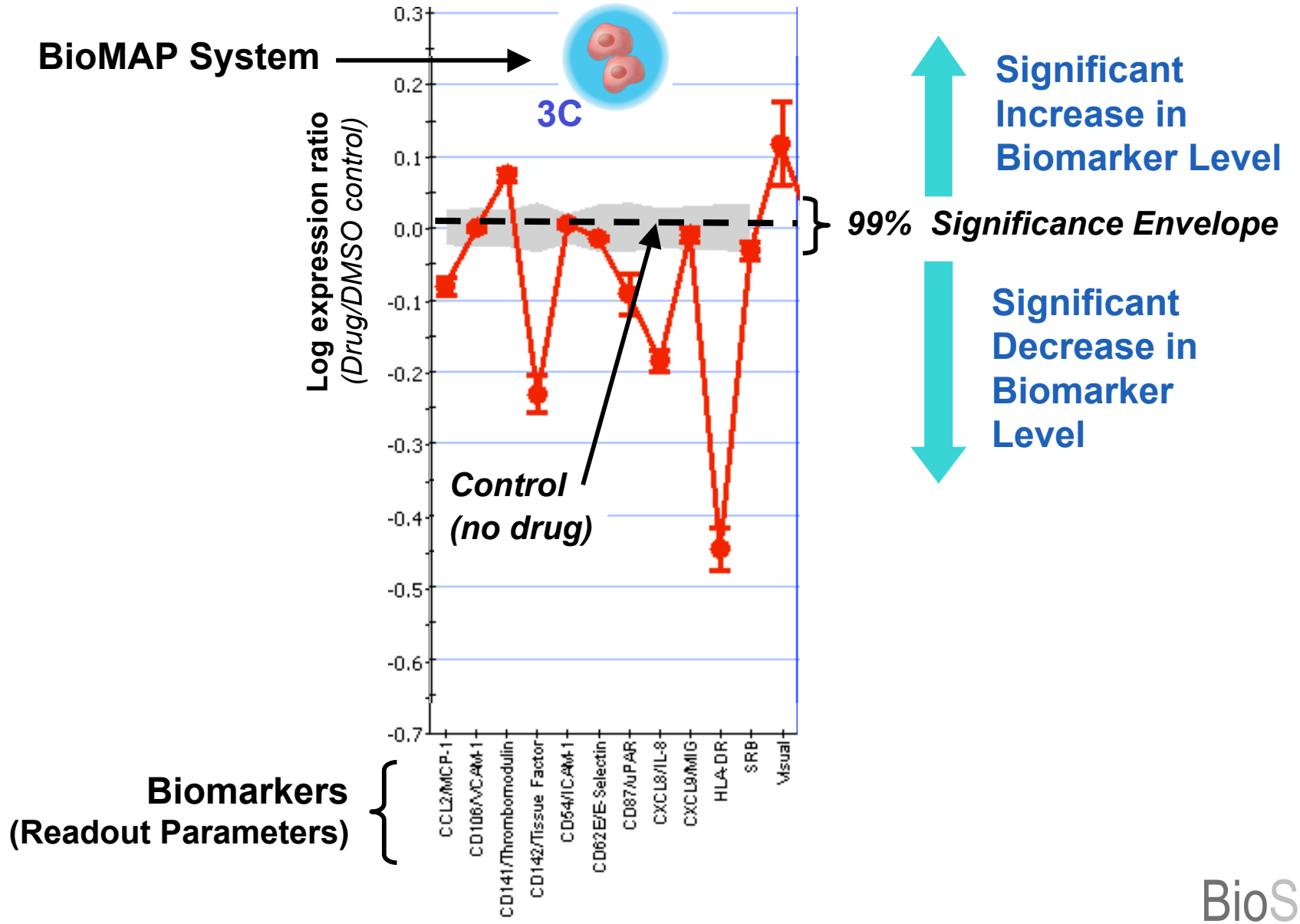
# BioMAP Profiling: Example Profile

*Pattern of biomarker activities creates a "profile"*



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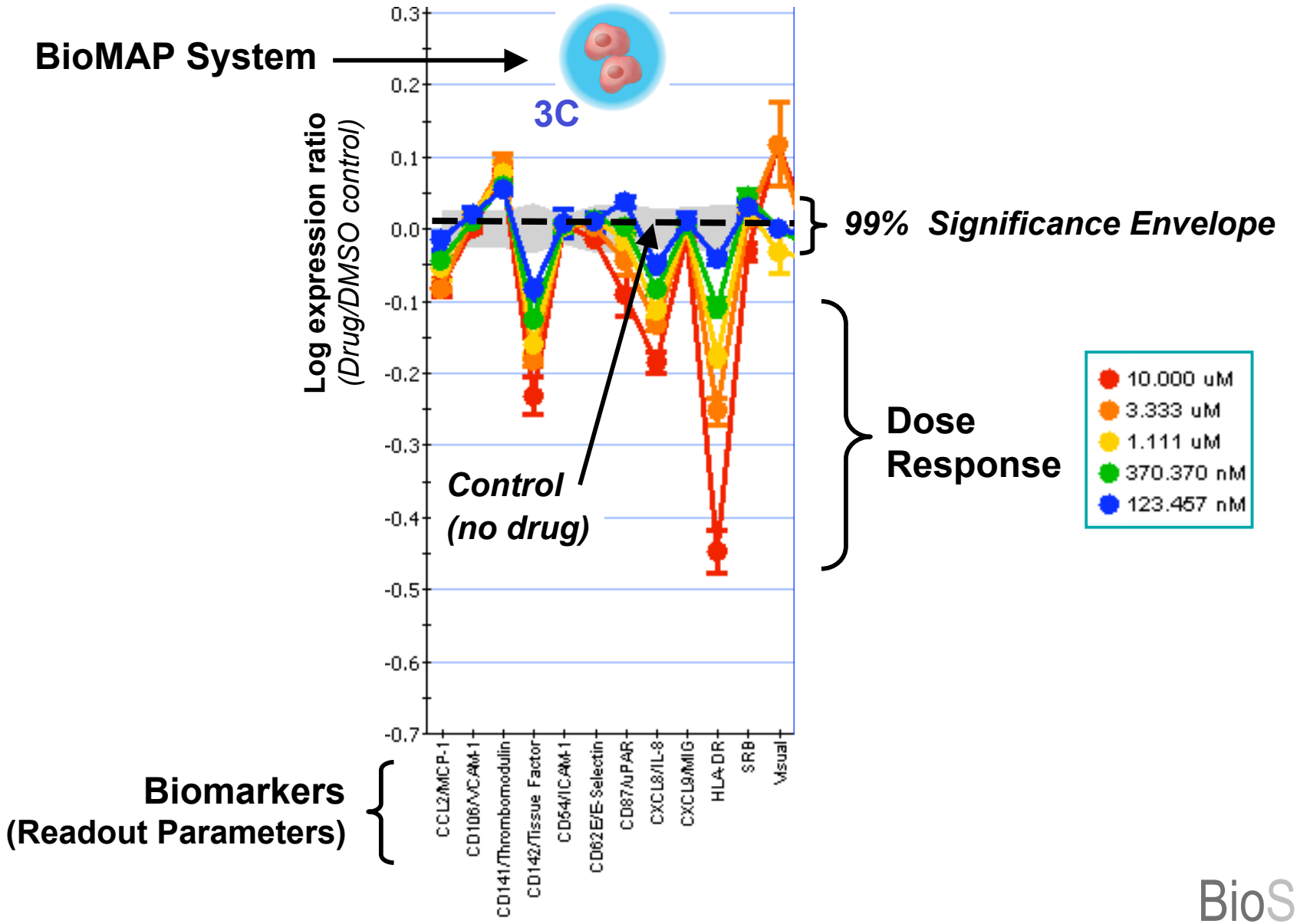
# BioMAP Profiling: Example Profile

*Multiple dose profile of reference p38 MAPK Inhibitor*



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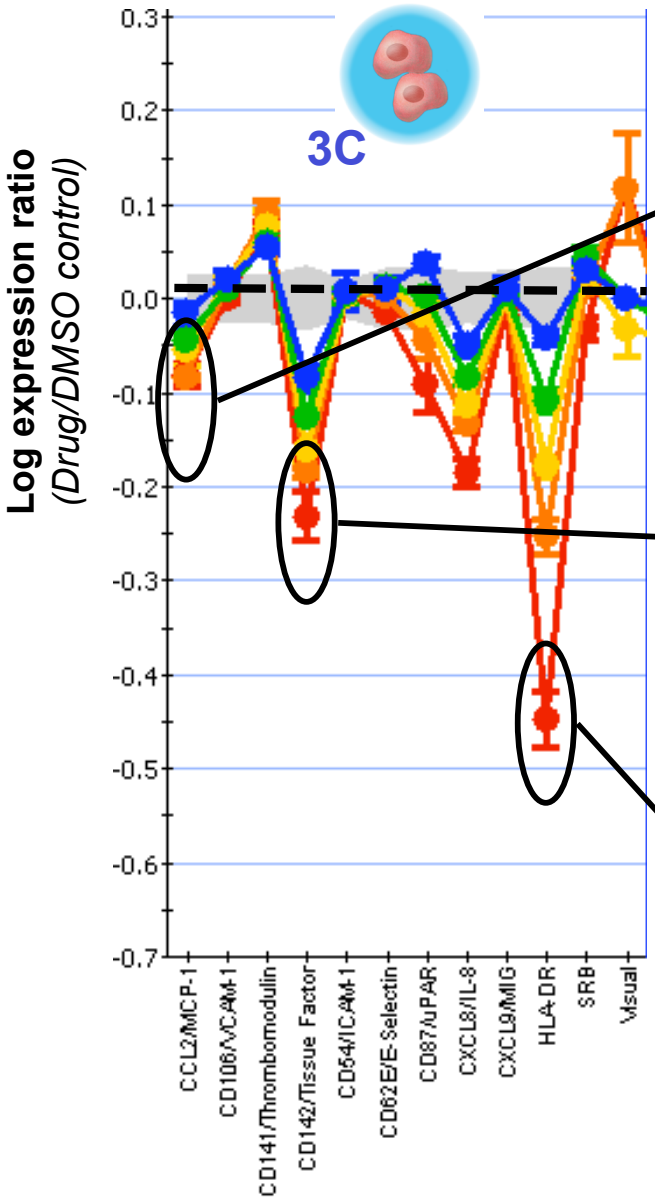
# BioMAP Profiling: Example Profile

## *Validation of activities identified for reference p38 Inhibitor*




| | |
|---|---|
| **MCP-1** | Sheryanna A, et al. Inhibition of p38 mitogen-activated protein kinase is effective in the treatment of experimental crescentic glomerulonephritis and suppresses monocyte chemoattractant protein-1 but not IL-1beta or IL-6. J Am Soc Nephrol. 2007, 18:1167-79. |
| **Tissue Factor** | Sakurai K, et al. Role of p38 mitogen-activated protein kinase in thrombus formation. J Recept Signal Transduct Res. 2004, 24:283-96. |
| **HLA-DR** | Wada T, et al. Reduction in chronic allograft nephropathy by inhibition of p38 mitogen-activated protein kinase. Am J Nephrol. 2006, 26:319-25. |



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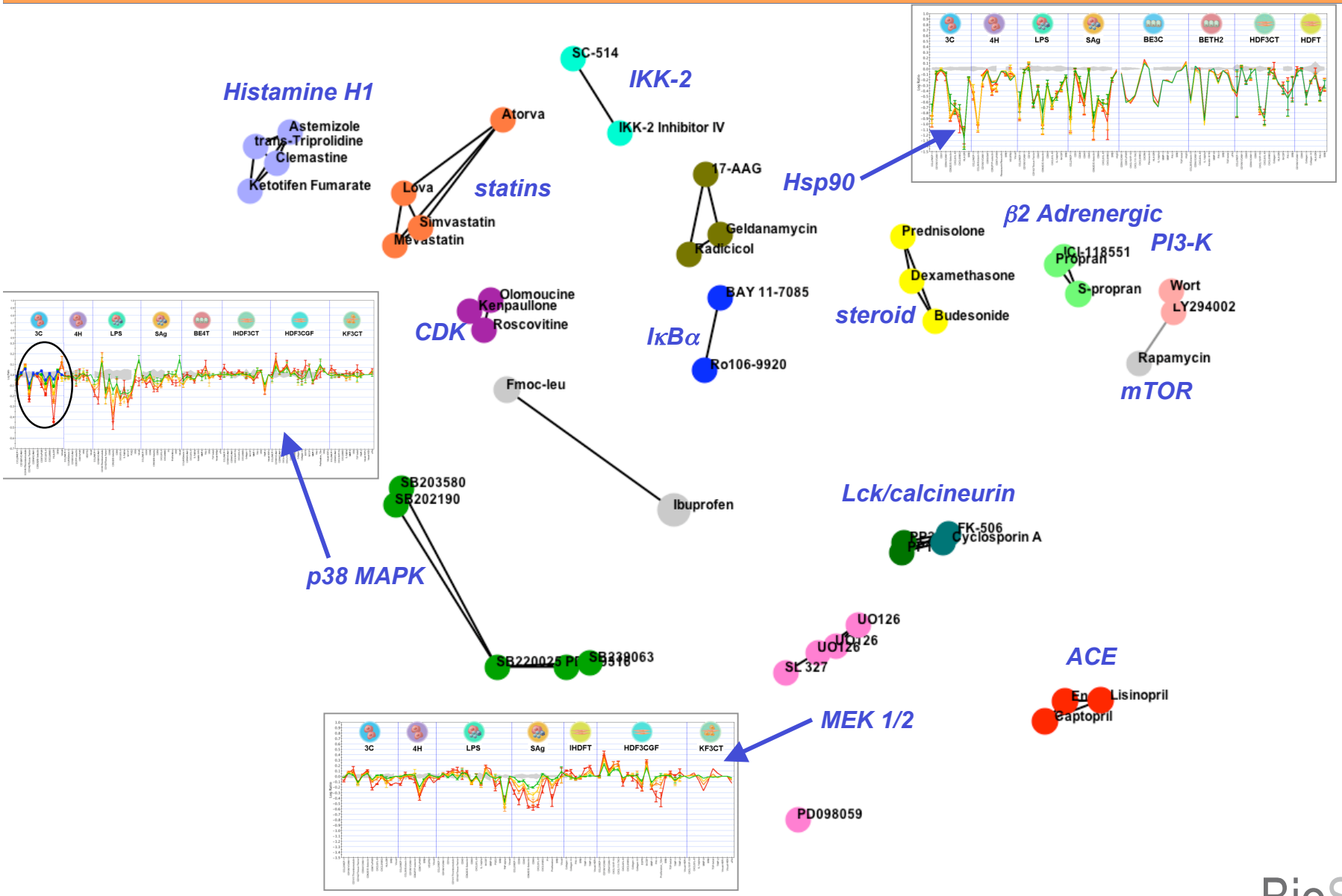
# BioMAP Classifies Drugs By Mechanism of Action

## *Multidimensional Scaling - Function Similarity Map*



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# Similarity Search for Mechanism of Action

## *BioMAP Database*

**Test compound:**
**PD169316**
**p38 MAPK inhibitor**

**Top matches: other p38 MAPK inhibitors**

BioMAP Viewer 7.1.2 - Trusted Profiles

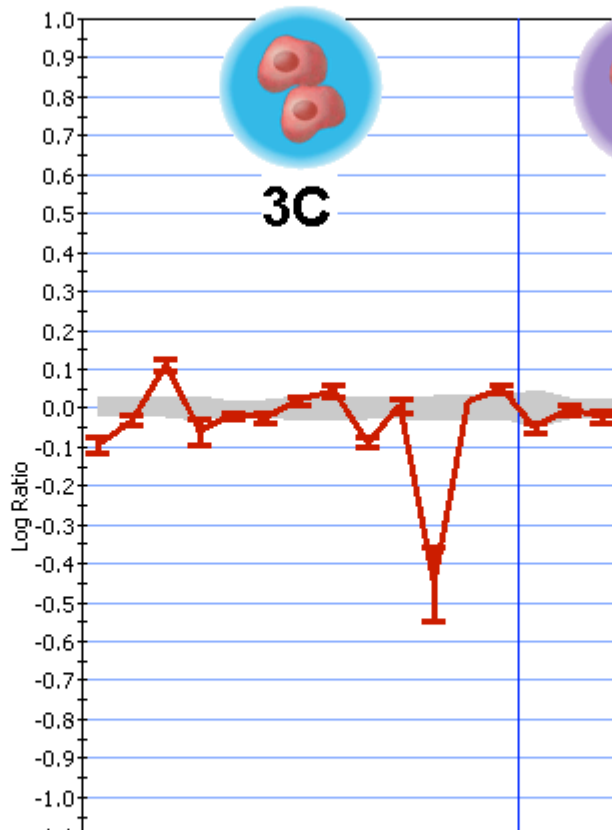
File Edit Search View Profile Data Registry Tools Help

Data | Similarity

PD169316, BSK-C000045, 1111.111 nM

| .. | Target | Tanimoto... | Pearson |
|---|---|---|---|
| ... | PD169316, BSK-C000045 , 1111.111 nM | 0.762 | 1.000 |
| ... | PD169316, BSK-C000045 , 3333.333 nM | 0.769 | 0.987 |
| ... | PD169316, BSK-C000045 , 370.37 nM | 0.625 | 0.986 |
| ... | SB239063, BSK-C000207 , 3333.333 nM | 0.515 | 0.980 |
| ... | SB239063, BSK-C000207 , 10000 nM | 0.574 | 0.961 |
| ... | SKF-86002, BSK-C000070 , 10000 nM | 0.621 | 0.959 |
| ... | BIRB-796, BSK-C003696 , 37.037 nM | 0.593 | 0.957 |
| ... | SB220025, BSK-C000142 , 1111.111 nM | 0.556 | 0.954 |
| ... | VX-745, BSK-C003698 , 1111.111 nM | 0.603 | 0.954 |
| ... | BIRB-796, BSK-C003696 , 111.111 nM | 0.638 | 0.948 |
| ... | RO 320-1195, BSK-C003702 , 10000 nM | 0.621 | 0.947 |
| ... | VX-745, BSK-C003698 , 370.37 nM | 0.603 | 0.947 |
| ... | VX-745, BSK-C003698 , 3333.333 nM | 0.610 | 0.945 |
| ... | RO 320-1195, BSK-C003702 , 3333.33... | 0.567 | 0.945 |
| ... | RO 320-1195, BSK-C003702 , 30000 nM | 0.621 | 0.943 |
| ... | BIRB-796, BSK-C003696 , 333.333 nM | 0.600 | 0.934 |
| ... | AMG548, BSK-C003699 , 333.333 nM | 0.644 | 0.933 |
| ... | AMG548, BSK-C003699 , 1000 nM | 0.655 | 0.932 |
| ... | AMG548, BSK-C003699 , 111.111 nM | 0.576 | 0.930 |
| ... | SB202190, BSK-C000055 , 5555.556 nM | 0.591 | 0.930 |
| ... | SB220025, BSK-C000142 , 3333.333 nM | 0.513 | 0.928 |
| ... | SB203580, BSK-C000054 , 16666.667 nM | 0.694 | 0.928 |
| ... | SB202190, BSK-C000055 , 1851.852 nM | 0.619 | 0.927 |
| ... | SB203580, BSK-C000054 , 617.284 nM | 0.613 | 0.926 |
| ... | SB203580, BSK-C000054 , 1851.852 nM | 0.629 | 0.925 |

PD169316, BSK-C000045, 5 Trust



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# BioSeek - EPA ToxCast Project

## BioSeek - EPA Proof-of Concept Project

- 320 compounds, 4 concentrations each
- 8 BioMAP Systems, 7-14 readouts (endpoints) per System
- Total:  87 readouts per compound
- Single well, screening format
- ~112,000 datapoints (348 datapoints per compound)

# BioMAP Systems for EPA ToxCast

| System | Cell Types | Environment | Readout Parameters |
|---|---|---|---|
| 3C | Endothelial cells | IL-1β+TNF-α+IFN-γ | MCP-1, VCAM-1, ICAM-1, Thrombomodulin, Tissue Factor, E-selectin, uPAR, IL-8, MIG, HLA-DR, Proliferation, Vis., SRB (13) |
| 4H | Endothelial cells | IL-4+histamine | VEGFRII, P-selectin, VCAM-1, uPAR, Eotaxin-3, MCP-1, SRB (7) |
| LPS | Peripheral blood mononuclear cells + Endothelial cells | TLR4 | CD40, VCAM-1,Tissue Factor, MCP-1, E-selectin, IL-1α, IL-8, M-CSF, TNF-α, PGE2, SRB (11) |
| SAg | Peripheral blood mononuclear cells + Endothelial cells | TCR | MCP-1, CD38, CD40, CD69, E-selectin, IL-8, MIG, PBMC Cytotox., SRB, Proliferation (10) |
| BE3C | Bronchial epithelial cells | I IL-1β+TNF-α+IFN-γ | uPAR, IP-10, MIG, HLA-DR, IL-1α, MMP-1, PAI-1, SRB, TGF-b1, tPA, uPA (11) |
| HDF3CGF | Fibroblasts | IL-1β+TNF-α+IFN-γ +bFGF+EGF+PDGF-BB | VCAM-1, IP-10, IL-8, MIG, Collagen III, M-CSF, MMP-1, PAI-1, Proliferation, TIMP-1, EGFR, SRB (12) |
| KF3CT | Keratinocytes + Fibroblasts | IL-1β+TNF-α+IFN-γ+TGF-β | MCP-1, ICAM-1, IP-10, IL-1α, MMP-9, TGF-β1, TIMP-2, uPA, SRB (9) |
| SM3C | Vascular smooth muscle cells | IL-1β+TNF-α+IFN-γ | MCP-1, VCAM-1, Thrombomodulin, Tissue Factor, IL-6, LDLR, SAA, uPAR, IL-8, MIG, HLA-DR, M-CSF, Prolif., SRB (14) |



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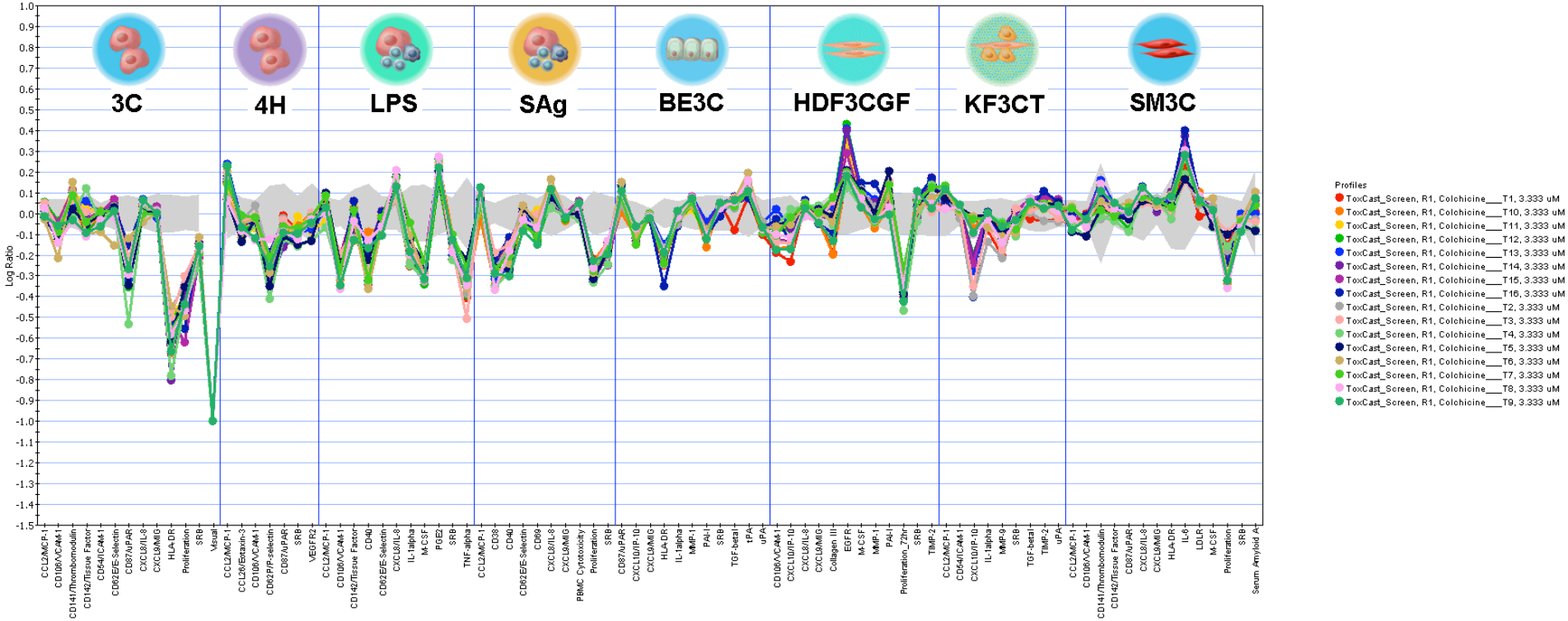
# Reproducibility of BioMAP Profiles

## *Positive Control (Colchicine) Replicates*




- **Overlay of BioMAP profiles of positive controls (colchicine)**
- **Each replicate represents a separate plate (template)**
- **99% Significance envelope is shown (grey shading)**



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# Overall Summary

320 Compounds → 8 BioMAP Systems, 4 concentrations (87 parameters, screening format, n=1)

| | |
|---|---|
| Active | 219 (68%) |
| Inactive | 101 (32%) |

| Overt Cell Toxicity | Active, Non-toxic |
|---|---|
| 70 | 149 |



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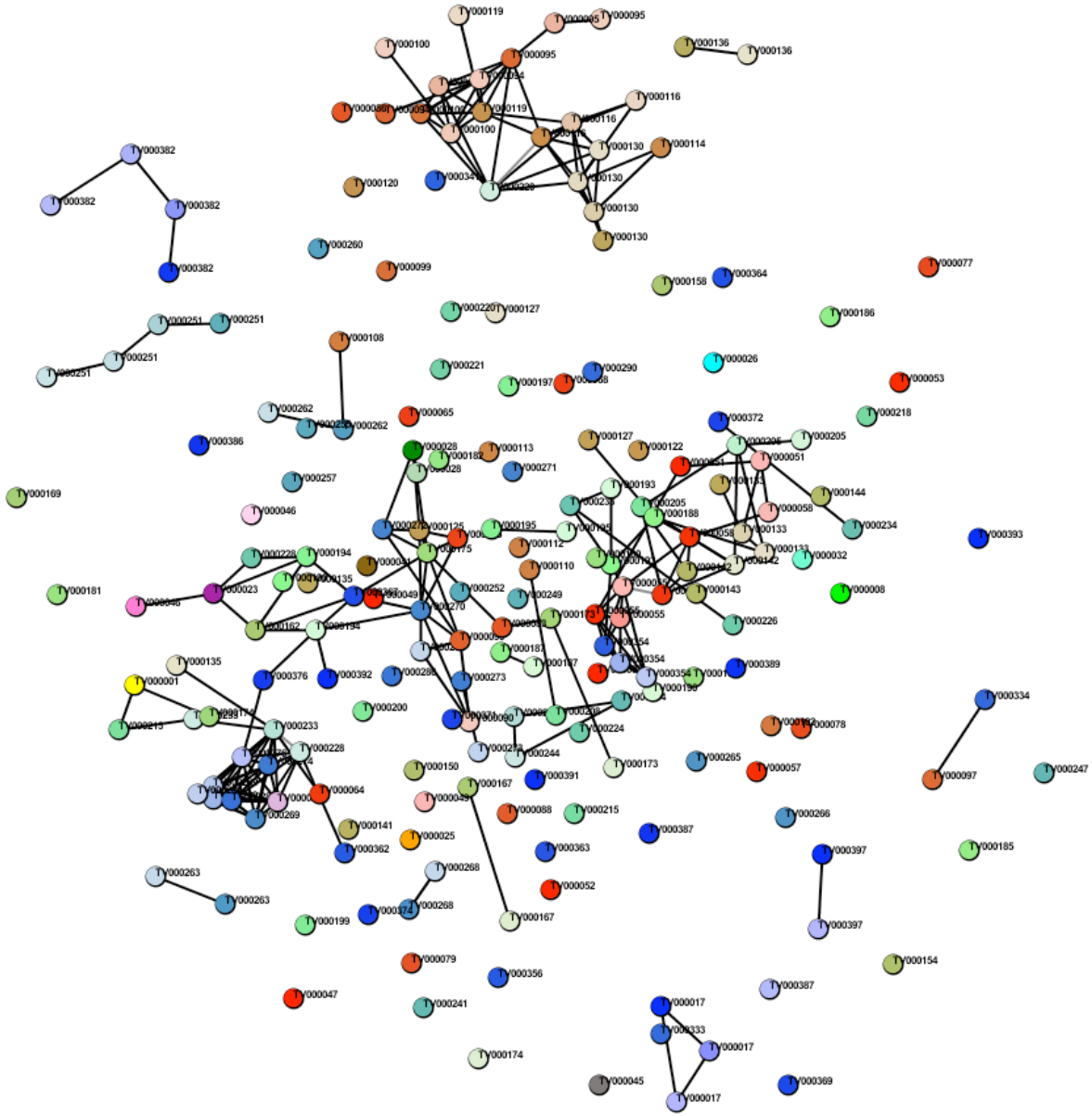
# MDS Analysis of Active Non-toxic Compounds

## *2D Visualization of Pairwise Correlation Analysis*





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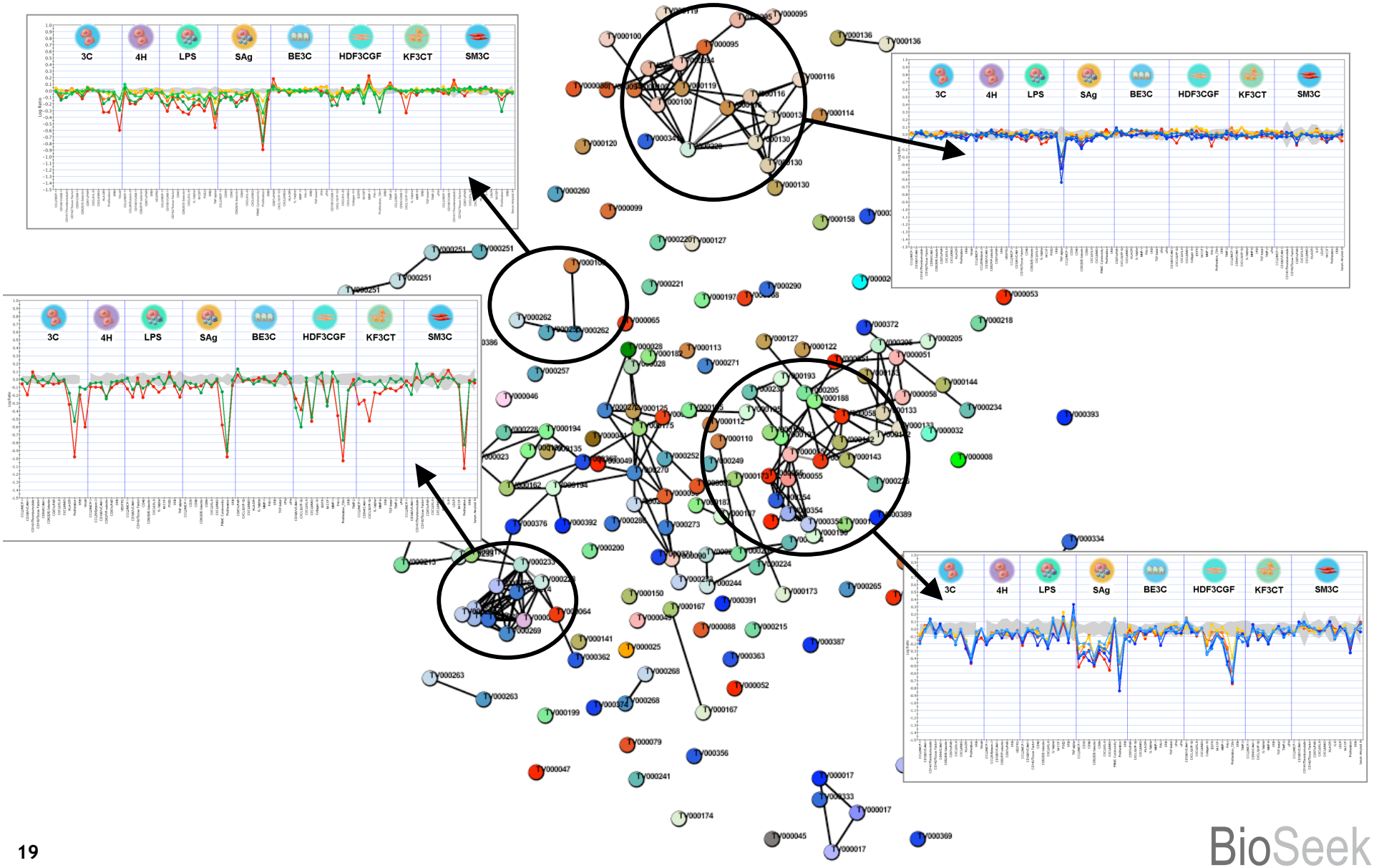
# Diversity of Mechanisms



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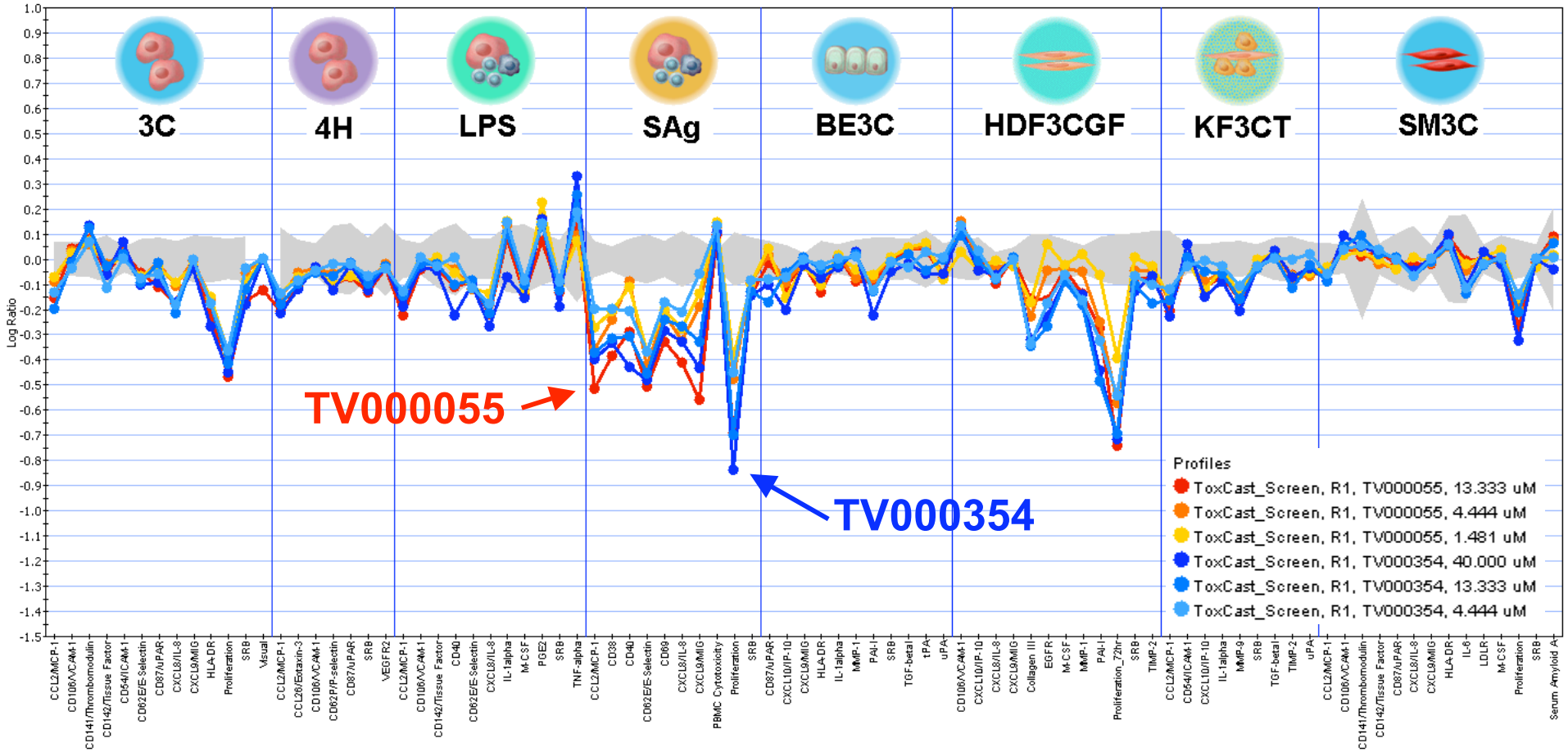
# TV00055 and TV000354




- **BioMAP Profiles of compounds that cluster in the MDS graph are highly similar to one another**



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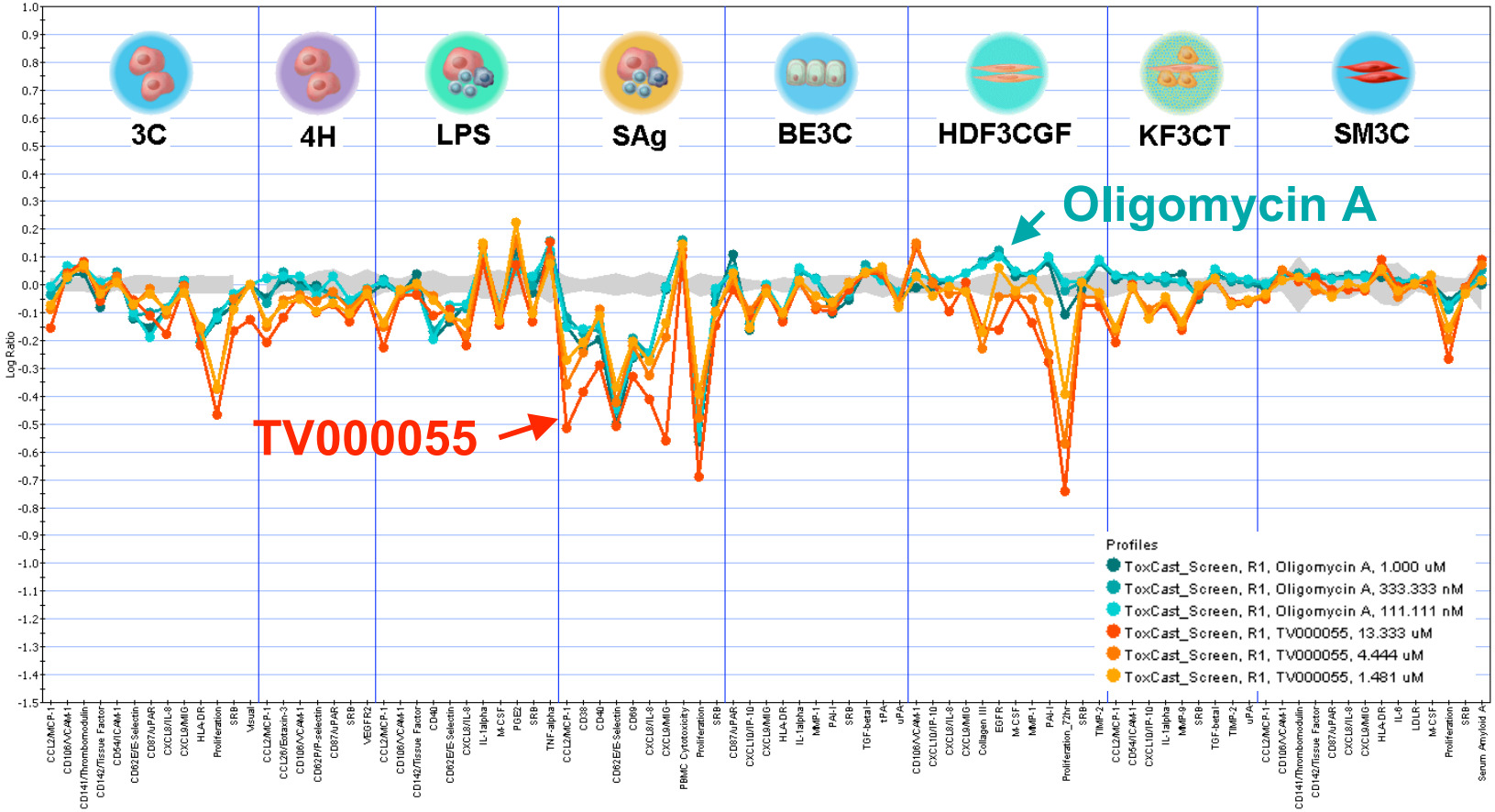
# BioMAP Profiles of Oligomycin A and TV000055




- **Oligomycin A is an inhibitor of mitochondrial ATPase**
- **Similarity suggests inhibition of mitochondrial function by TV000055**
  - (TV00005 is most similar to Complex I inhibitors)



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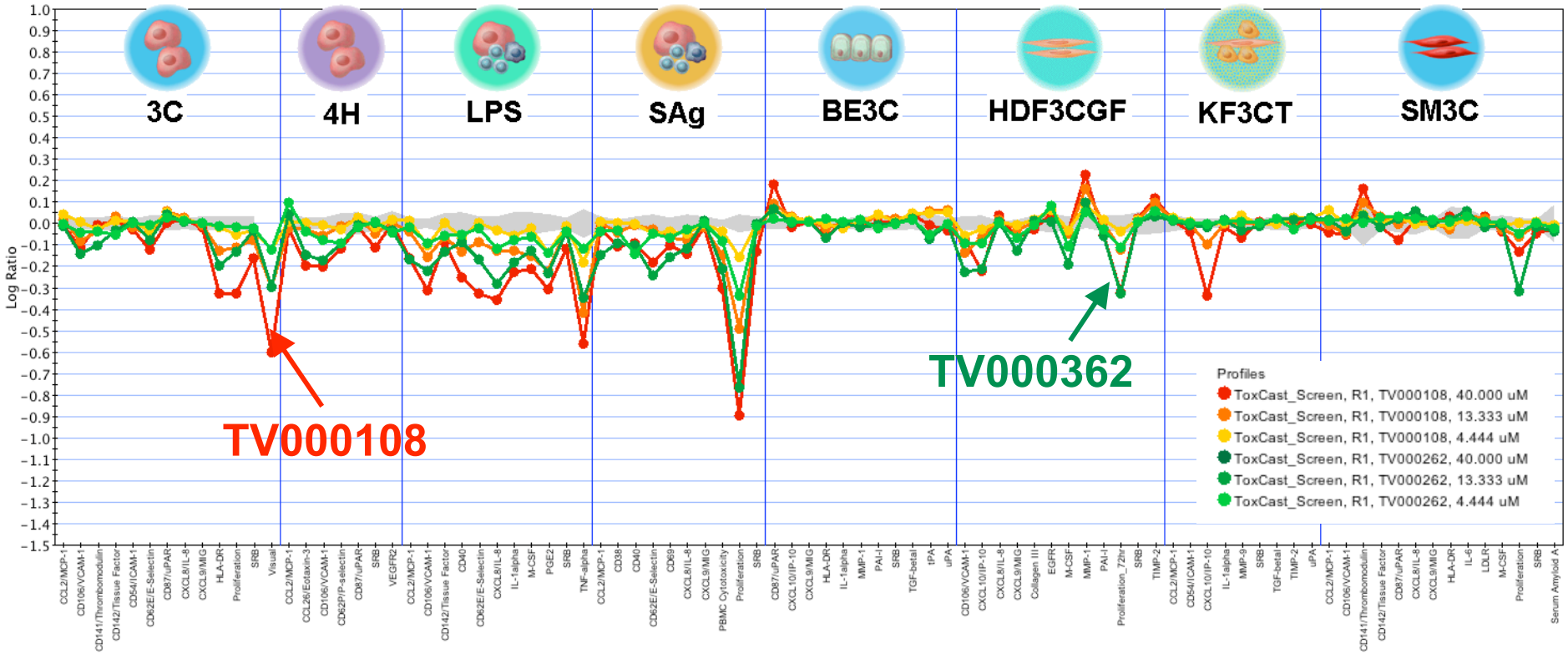
# TV000108 and TV000362




- **BioMAP Profiles of compounds that cluster in the MDS graph are highly similar to one another**



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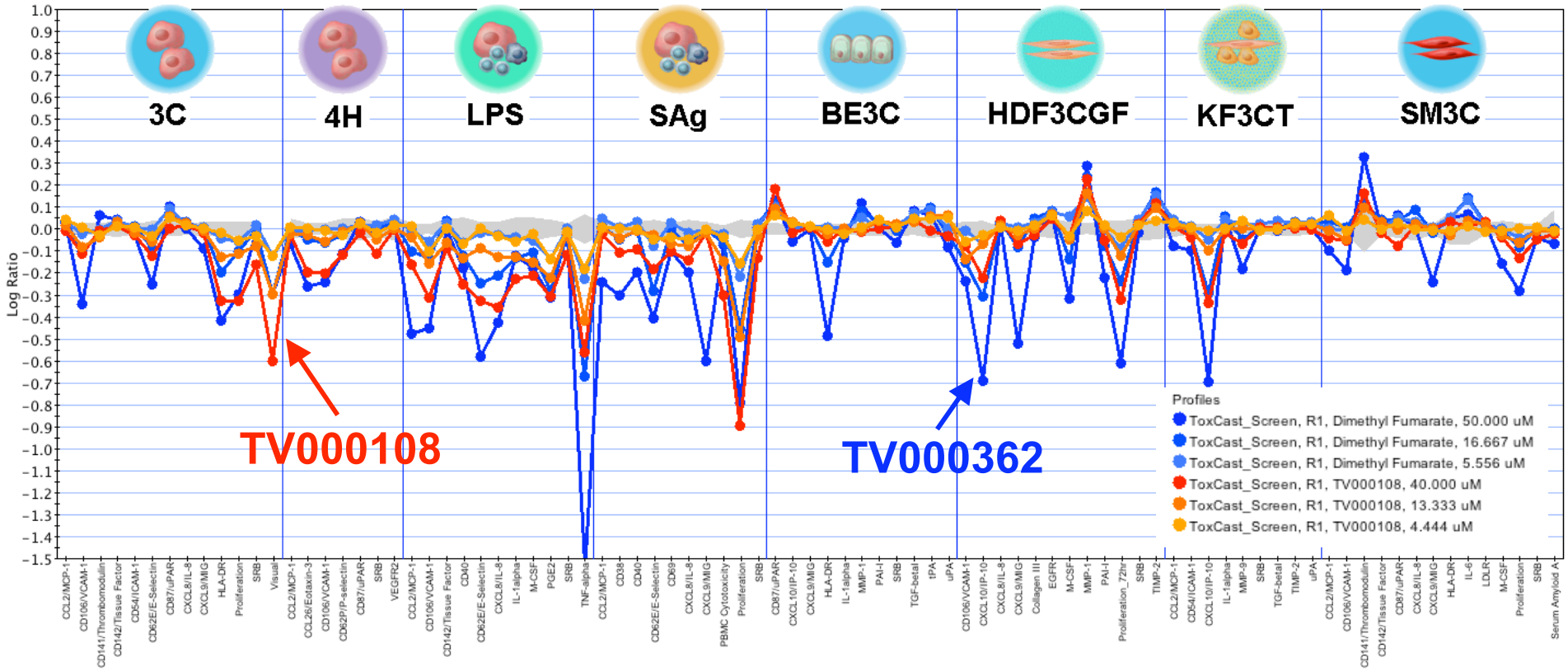
# BioMAP Profiles of Dimethyl Fumarate and TV000108




- **Dimethyl Fumarate is an inhibitor of NFkappaB translocation**



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# Classification of Compounds
## *Example Mechanism Classes*

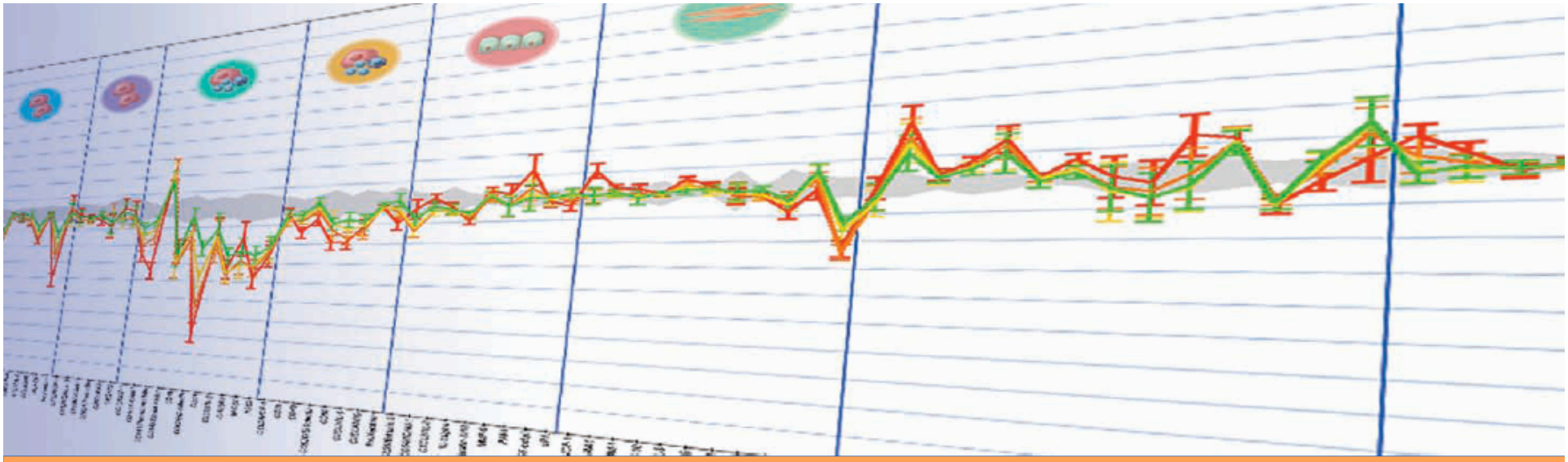
- **Mitochondria energy inhibition**
  - Electron transport chain inhibitors
- **ER stress (unfolded protein response)**
  - Proteasome inhibition
  - DNA damage
    - Chlorambucil (alkylating agent)
  - Inhibition of translation
    - Cycloheximide
- **Microtubule function inhibition**
  - Colchicine, vincristine, paclitaxel

# Classification of Compounds
*In Progress*

| Mechanisms | # Compounds |
|---|---|
| Mitochondrial Dysfunction | 41 |
| ER Stress | 23 |
| Microtubule Function Inhibition | 7 |
| PI3 Kinase Inhibition | 9 |
| cAMP Elevation | 12 |

# Next Steps

- Decode compound IDs
- Complete similarity analyses
- Correlate BioMAP data with other data types
- Build classifiers for specific mechanism classes



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