BioMAP® Systems

Bridging the In Vitro to In Vivo Gap

BioSeek is a drug discovery alliance and technology development company that applies its unique BioMAP® platform of predictive human-based models to the discovery and development of safer and more efficacious therapeutics and chemicals. Our mission is to accelerate discovery and improve success rates for our partners by integrating pre-clinical human biology throughout the drug discovery process using our proprietary primary human cell-based assays and integrated bioinformatics analysis platform. These BioMAP® profiles provide actionable data to guide lead selection, lead optimization (SAR), mechanistic understanding of compound action, and nomination of candidates for preclinical development.

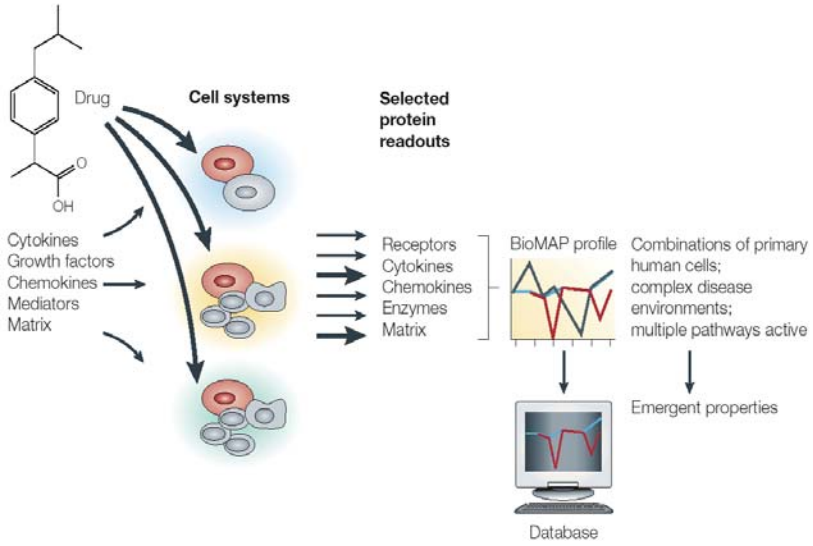

Figure 1. Compound evaluation using BioMAP® Systems (from E. Butcher, Nature Rev. Drug Discovery 4:461 (2005).

Our BioMAP® Systems generate a unique signature of human biological response for every active compound. Comparison of these signature profiles using proprietary algorithms to BioSeek's database containing profiles of thousands of experimental compounds and approved therapeutics, provides an enhanced understanding of the mechanism of action of compounds. This platform provides the ability to detect and distinguish a broad range of mechanistically diverse compound classes, and enables the rapid identification of lead compounds with activities that are most relevant for developing safe, effective therapeutics. BioMAP® Systems have been applied for lead selection and optimization, identification of mechanism of action, secondary and off-target activities, and clinical insights in the areas of inflammatory and autoimmune disease, cardiovascular disease and cancer.

FEATURES	BENEFITS
• **Human primary cell-based assays**	• **Intact regulatory and feedback mechanisms**
• **Human disease models**	• **More predictive of human biological responses**
• **Automated assay platform**	• **Reproducible within and between assays**
• **Proprietary database and computational platform**	• **Benchmarking vs. hundreds of known compounds, drugs and mechanisms of action**
• **Simultaneous interrogation of 100s of targets**	• **Broad coverage of human disease biology**
• **Identification of secondary or off-target activities**	• **Reveal unknown/potential assets and liabilities of test compounds**

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BioMAP® Systems: Human Primary Cell-Based Assay Systems

BioMAP® Systems are primary human cell-based disease models developed for specific therapeutic areas including inflammatory and autoimmune diseases, respiratory diseases, dermatological conditions, cardiovascular disease and cancer. In each model system a single cell type or defined mixtures of primary human cell types are stimulated in complex formats such that multiple, disease-relevant signaling pathways are simultaneously active. The choice of cell types and stimuli is guided by knowledge of both disease biology and the importance of pathway interactions and feedback control mechanisms to clinical outcomes. The ability to monitor disease-relevant signaling pathways in multiple cell types permits the association of biological activities detected in BioMAP® Systems with multiple disease indications. Primary cell types are superior to cell lines due to their ability to retain more of the normal regulatory processes of their in vivo counterparts. Drug effects are recorded by measuring a panel of protein readouts that are selected to provide optimal coverage of biological/disease space of interest (e.g., vascular inflammation, metabolism), maximize cellular information content, and provide the link to clinical responses.

Over 30 BioMAP® Systems, covering a broad range of tissue and disease biology, have been fully optimized and automated. These include systems that utilize combinations of endothelial cells, blood leukocytes, macrophages, Th2-type T cells, bronchial epithelial cells, mast cells, smooth muscle cells, fibroblasts, and keratinocytes to cover a broad range of disease-specific biology relevant to asthma, allergy, COPD, skin inflammation/psoriasis, arthritis and fibrosis. Because these systems model complex cellular and molecular network responses, they are also sensitive to other disease-relevant areas, from metabolism to cancer (see Figure 2B). A detailed description of all BioMAP® Systems is available.

Primary Human Cell Types	Disease Relevance	BioMAP*
Endothelial cells (EC)	Th1and Th2 **inflammation**, allergy, asthma, dermatitis, **angiogenesis**, **wound healing**, restenosis, atherosclerosis	3C, 4H
EC+lymphocyte+monocytes	Th1 **inflammation**, psoriasis, COPD **fibrosis**, **monocyte and T cell responses**	LPS, SAg, HPNo
EC+Macrophages	**Macrophage responses,** arthritis, COPD, fibrosis	Mphg, BT
B cells + T cells	Asthma, allergy, dermatitis, fibrosis	
EC+Smooth Muscle Cells	**Vascular inflammation,** restenosis, atherosclerosis, **proliferation**	HSM3C, TH
EC+Th2 blasts	Allergy, asthma, **inflammation**	HDFT, HDF3CT
Fibroblasts	**Inflammation**, Allergy,	
Fibroblasts + PBMC	Arthritis, asthma, dermatitis, **fibrosis**, psoriasis, **wound healing**	HDF3C, HDF3CGF, HDFSAg
Myofibroblasts	**Fibrosis**, COPD, **wound healing**	K3CT, HDFNo, MyoF
Keratinocytes	Psoriasis, dermatitis, **wound healing**	
Keratinocytes+Fibroblasts	Psoriasis, dermatitis, **wound healing**	KF3CT, KFNo
Bronchial Epithelial Cells	Th1 and Th2 **inflammation** Allergy, asthma, **fibrosis**, COPD	BE3C, BE4T
Bronchial Epithelial Cells + Fibroblasts	Asthma, allergy, **fibrosis**, COPD	BF4T, CASM3C
Smooth Muscle Cells	**Vascular inflammation**, asthma, COPD, fibrosis	

*color indicates different disease-like environment

Figure 2: BioMAP® systems cover a broad range of human tissue and disease biology

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Compound Profiling with BioMAP® Systems

Each compound tested in BioMAP® Systems gives a unique, compound-specific functional profile. To create a functional profile, the levels of protein readouts in drug-treated BioMAP® Systems, Figure 2 shows an example of the BioMAP® profile of prednisolone, a glucocorticoid receptor agonist, which was generated by combining data from multiple BioMAP® Systems. The activities shown in this figure are consistent with biological activities observed in patients treated with corticosteroids. The breadth of signaling pathways activated in each BioMAP® System and the inherent connectivity of these pathways permits a broad range of active compounds to be detected. Combining data from multiple systems allows compound evaluation for multiple therapeutic indications in a common assay format, and also provides the statistical power to discriminate among compounds with very similar effects when evaluated by single-target assays.

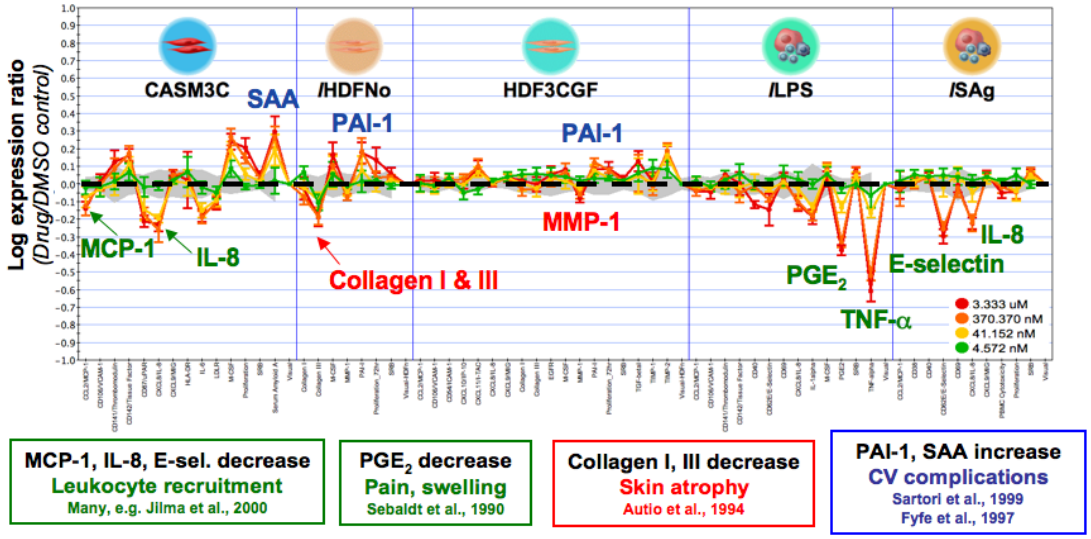

Figure 3. Example BioMAP® Profile. This dose-response BioMAP® Profile of prednisolone was generated by combining data from five BioMAP® systems. The data are expressed as log expression ratios $\log_{10}$ [readout value with drug/readout value of DMSO control]. Biomarker readouts measured are listed on the horizontal axis (x-axis). Values falling outside of the 99% significance envelope are significantly different from control. BioMAP® profiles are stored in BioSeek's proprietary BioMAP® Reference Database.

BioMAP® Reference Database

The reproducibility of BioMAP® profiles has enabled BioSeek to create a database representing over 3000 unique compounds and over 14000 compound profiles—a valuable asset for BioSeek partners. These reference compounds and profiles include a diverse set of approved therapeutics, clinical-stage compounds and marketed, off-patent drugs whose mechanisms of action are largely known. The BioMAP® Reference Database allows the function of test compounds to be readily compared to those of known drugs and therapeutics.

Inflammation / Autoimmune
- Calcineurin, TCR
- Glucocorticoid R
- Prostaglandin, leukotriene
- TNF-α
- IL-10, IL-4, IL-17
- NFκB
- IL-1, IFNβ, IFNγ
- p38 kinase
- Jak/Stat (Jak1, 2, 3, Tyk2)
- Lck kinase, PI-3Kδ, PCKθ
- mTOR
- JNK

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

Cardiovascular
- ACE
- β2 Adrenergic
- Ca++ Channel
- Cholesterol
- Antioxidant

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

Cancer
- HDAC
- Hsp90
- Proteasome
- EGFR
- NFκB
- PI-3Kα/β/AKT
- Mek
- CDK
- RAR/RXR
- Ras/MAPK
- TGFβ
- Microtubule
- Jak/Stat
- Tie2 R
- Mitochondrial function

Figure 4. Selected drug targets modulated by various compounds profiled in the BioMAP® Database (see comment one). The database contains BioMAP® profiles for diverse classes of compounds and disease indications from inflammation to cancer. All BioMAP® reference profiles have been generated from multi-dose assays in multiple BioMAP® Systems.

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Analysis of BioMAP® Activity Profiles

Integrated Data Management Platform. BioSeek's robust data management and analysis platform (Figure 5) provides for rapid analysis and interpretation of BioMAP® activity profiles for determining mechanisms of action, pathway information, identification of secondary activities, and clinical indication guidance.

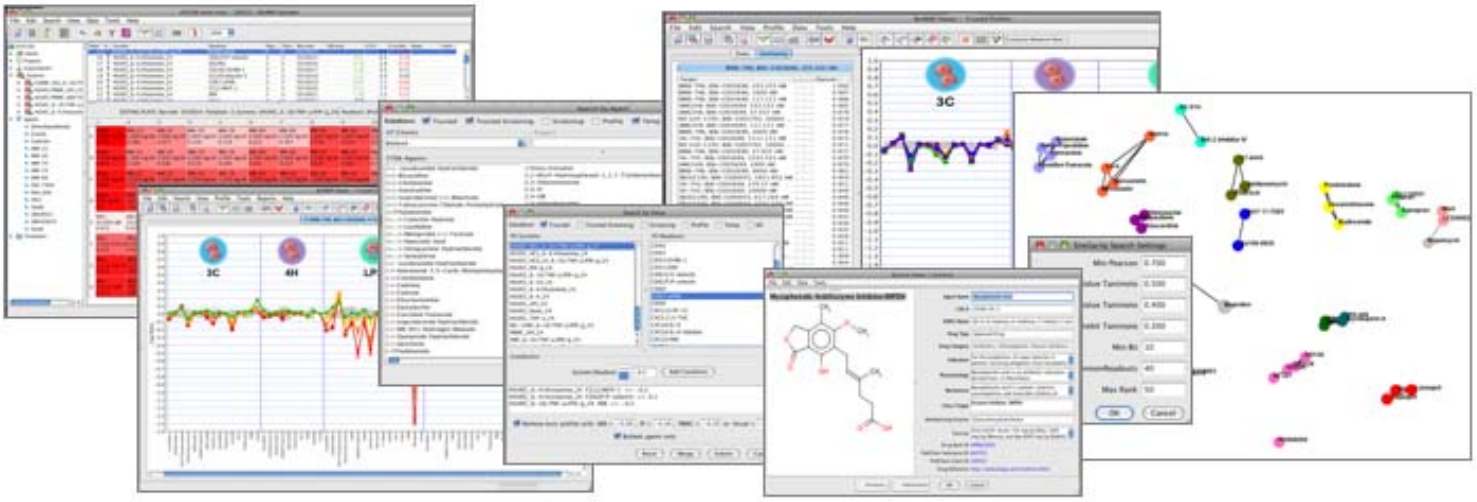

Figure 5. BioSeek's Data Management Components. For analysis of BioMAP® activity profiles, BioSeek has developed a proprietary data analysis and visualization tool (BioMAP® Viewer) that is integrated with software for data storage with specialized QA/QC (BioMAP® Uploader).

BioMAP® Profile Similarity Search. BioMAP® activity profiles can be compared to the BioMAP® Database of reference profiles and compounds or bioactive agents with statistically similar profiles can be identified. This is an efficient tool for mechanism of action studies, as the BioMAP® Reference Database contains compounds with 100s of different mechanisms of action. An example of the results of a BioMAP® Reference Database search is shown in Figure 6.

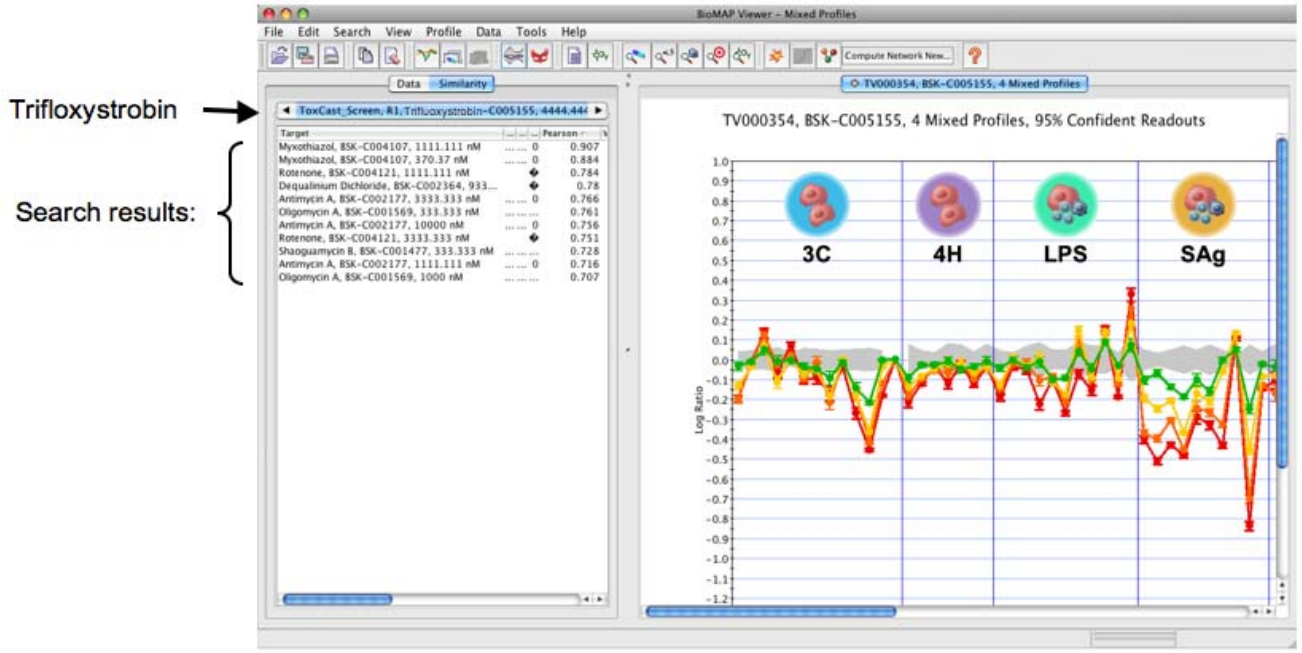

Figure 6. BioMAP® Profile Similarity Search. The BioMAP® computational platform generates a quantitative measure of similarity between the test compound and all other compounds in the database. A search for database compounds with similarity to trifloxystrobin, a strobilurin fungicide are shown ranked by similarity. This analysis shows that trifloxystrobin is most similar to myxathiazol, a Complex II inhibitor, consistent with its mode of action.

BioMAP® Function Similarity Maps. Comparison of a large set of BioMAP® profiles to each other allows creation of functional biological networks or "function similarity maps". These maps are graphic representations of functional similarities between different agents (compounds, biologics, natural products) tested in BioMAP® Systems. They enable extraction of biological information from large sets of profiling data, leading to determination of mechanisms, pathway effects, secondary activities and therapeutic hypotheses of test compounds.

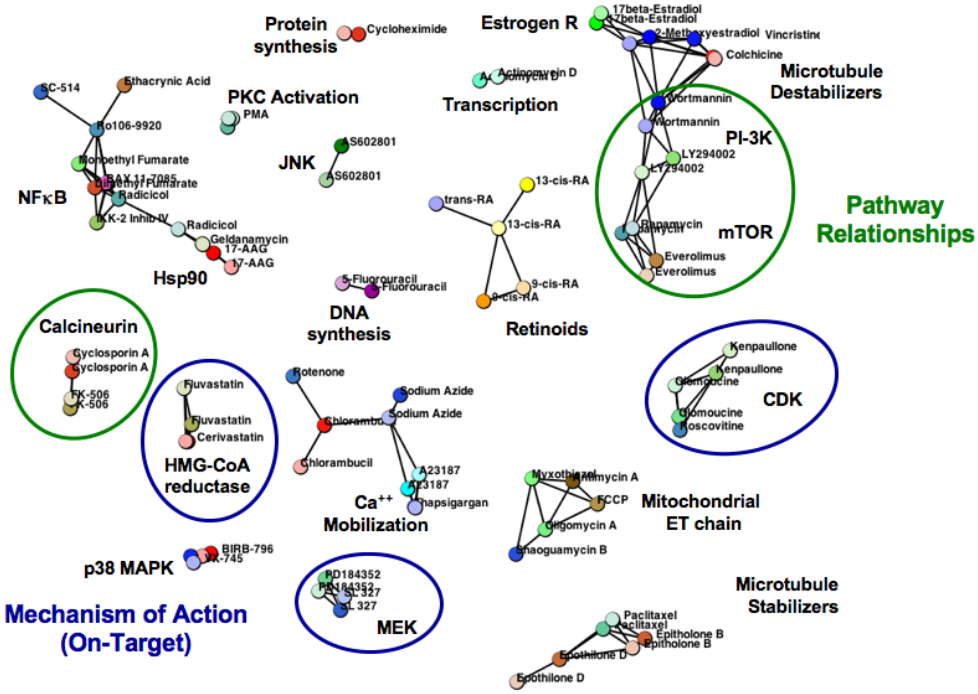

Figure 7. BioMAP® Function Similarity Map. Example of a function similarity map—here a clustering of 48 agents by similarity of BioMAP® profiles in four BioMAP® systems. Each circle represents a specific compound, colored according to its reported mechanism of action. The spatial arrangement of circles and connecting edges indicates similarity of function between different agents. For compounds that are not connected by a line, there is no similarity of function above a statistically significant threshold. Drugs specific for a common target cluster together (e.g., Anti-histamines, steroids); however, compounds with different off-target activities are readily discriminated (e.g., MEK inhibitors). In addition, compounds with targets that are closely linked may also be identified (e.g., PI3K inhibitors LY294002 and wortmannin + mTOR inhibitor rapamycin).

Applications of BioMAP® Systems in Drug Discovery

Lead Generation

- Innovative targets, novel biology

Lead Development: Selection, Optimization, Prioritization

- Discrimination of lead series
- Biology-driven lead optimization/structure-activity relationships

Toxicity Mechanisms

- Defining toxicity modes of action

BioMAP®-Driven Lead Generation. BioMAP® Systems offer a unique, proprietary and biology-driven approach to lead generation in which biological response—not single-target activity—is the primary driver of identification, optimization and selection of drug candidates. Here, BioMAP® Systems are used to screen smaller focused libraries or compound collections and subsequently applied to lead optimization/SAR and mechanism of action studies.

Compounds pre-selected for their drug-like chemical properties or biologics are profiled in sets of BioMAP® Systems chosen for their relevance to a target disease biology. Compounds with favorable dose responses are then benchmarked against known therapeutics in the BioMAP® Reference Database. A database match indicates a likely molecular target for the new compound, which can be confirmed in a separate biochemical assay.

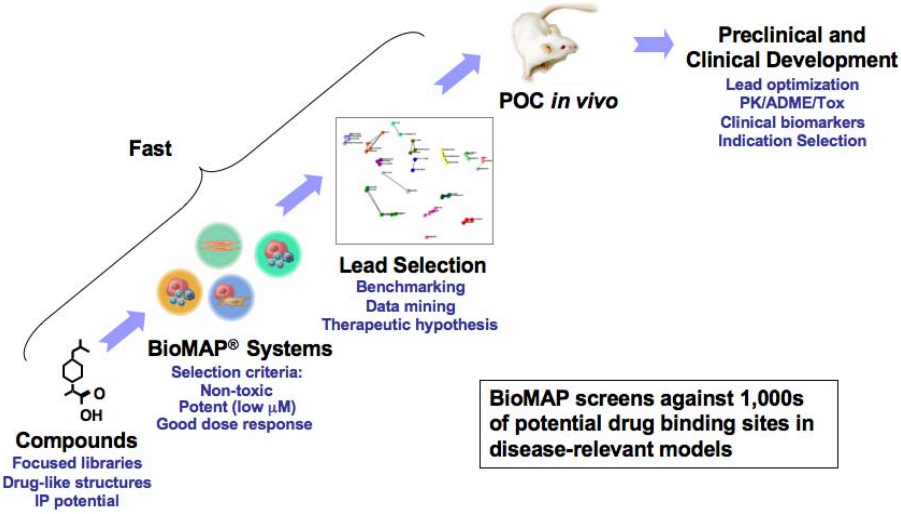

Figure 8. Biology-driven drug discovery using the BioMAP® Systems. BioMAP® profiling and benchmarking against reference compounds is used to select new leads. Benchmarking and biomarker analysis is used to establish therapeutic hypotheses that can then be confirmed in an appropriate animal model. Leads that are active in vivo are optimized for potency before moving into development. This approach effectively permits the simultaneous interrogation of hundreds to thousands of drug targets with a single compound.

Lead Development: Selection, Optimization, Prioritization. BioSeek's partners are applying BioMAP® systems to compound evaluation at every step from lead generation to IND filing, including evaluation of clinical-stage, marketed and off-patent compounds for new indications. The reproducibility of BioMAP® assays, combined with their ability to detect and discriminate closely related compounds of the same class, provide pharmaceutical researchers with a very powerful tool for lead selection. Function similarity maps permit the intuitive extraction of relevant biological and comparative information from large data sets across multiple hits or lead candidates, pointing directly to testable hypotheses regarding relative potency, secondary effect and optimal clinical indications.

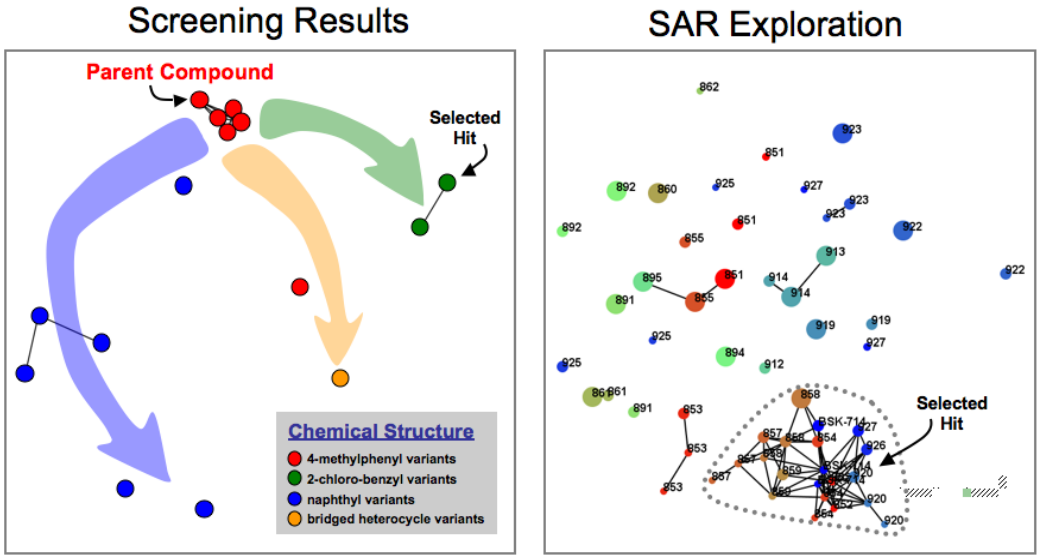

Figure 9. Example of a function similarity map—here a clustering of a series of chemically related compounds by similarity of BioMAP® profiles. Each circle represents a specific compound, colored according to chemical structure differences (e.g. having particular R groups, etc.). The spatial arrangement of circles and connecting edges indicates similarity of function between different agents. For compounds that are not connected by a line, there is no similarity of function above a statistically significant threshold.

In the same way, BioMAP® can be used to follow lead optimization/SAR, by mapping changes in biological and off-target effects to specific chemical modifications. Once a target-optimized lead has been identified, BioMAP® can again be used to guide the optimization of pharmacokinetic, metabolic and solubility properties of the molecule, assuring that on-target effects are retained in a further-optimized structure.

BioMAP® Profiling for Detecting and Defining Toxicity Mechanisms. Compound profiling in BioMAP® systems can help identify unexpected drug activities at an early stage. Separation of on- and off-target activities as well as detection of effects related to the chemical reactivity of drugs or metabolites can help with the identification of optimal clinical candidates and has the potential to reduce the number of preclinical and clinical stage failures. Compounds with various target, mechanisms associated with cellular and specific human toxicities can be distinguished by profiling in BioMAP Systems (Berg, 2006; Berg, 2009).

Safety-Related Activities

- Cell-type specific cytotoxicity (endothelial, epithelial, smooth muscle cell, etc.)
- Endothelial cell dysfunction (stress response)
- Cell cycle inhibition
- Immunosuppression (B cell, T cell, monocyte activation)
- Mitochondrial dysfunction
- Microtubule function

Safety-related targets / pathways

- PKC, PI-3K / AKT, CDKs
- Proteasome, NFκB, Hsp90, HDAC
- Tubulin, guanine synthesis
- Mitochondrial ATPase, electron transport chain complexes

Defining toxicity mechanisms by profiling in BioMAP® systems has been applied to environmental chemicals through the EPA ToxCast program (Houck, 2009). Figure 10 shows the clustering of environmental agents according to their BioMAP® profiles.

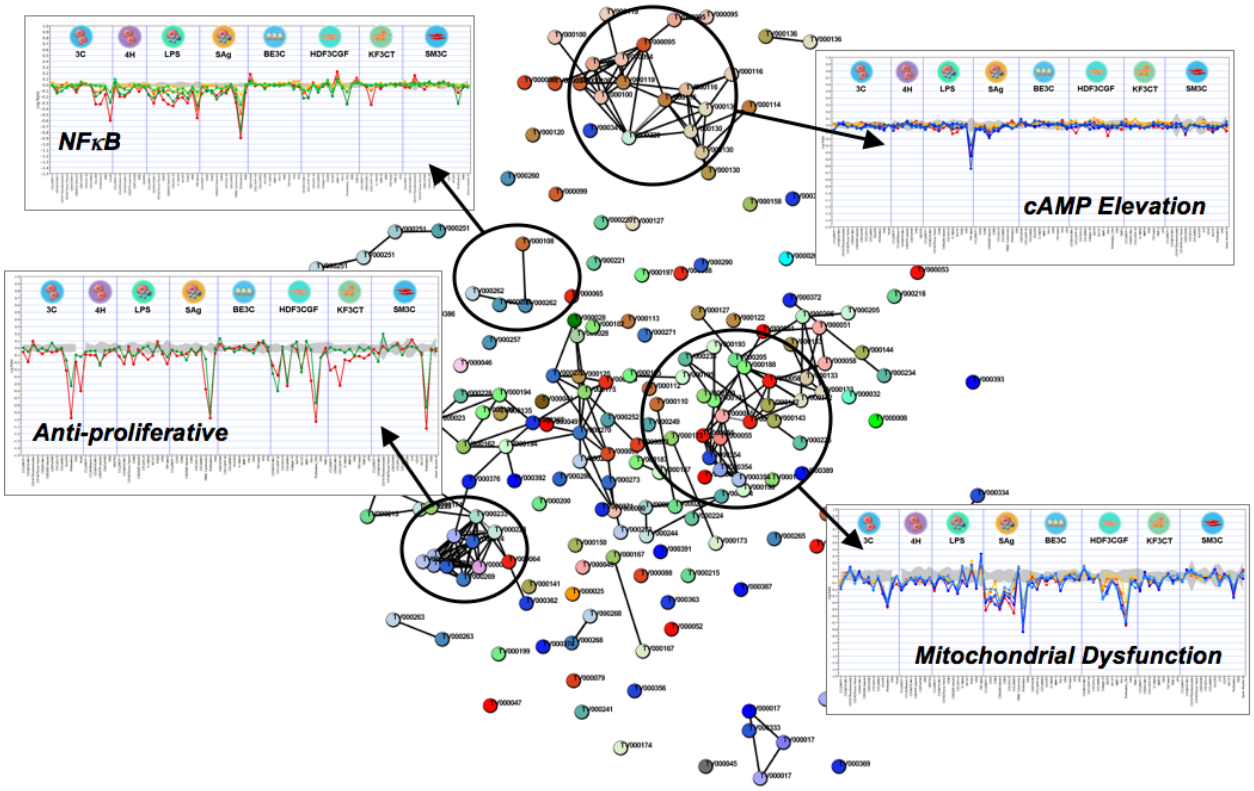

Figure 10. Mechanisms of toxicity defined by BioMAP® systems. Cluster analysis of BioMAP® profiles of EPA ToxCast™ compounds. BioMAP® activity profiles of 320 ToxCast™ compounds, generated in eight BioMAP® Systems, were subjected to pair wise correlation analysis, and visualization by multidimensional scaling. Lines connect compounds whose BioMAP® profiles are significantly similar. Compounds with similar mechanisms cluster together. Similarity analyses of compound profiles with reference compound resulted in identification of mechanism classes including the classes shown. Adapted from Houck, 2009.

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