PMI RESEARCH & DEVELOPMENT

Building Computable Biological Network Models and their Application to Product Risk Assessment.

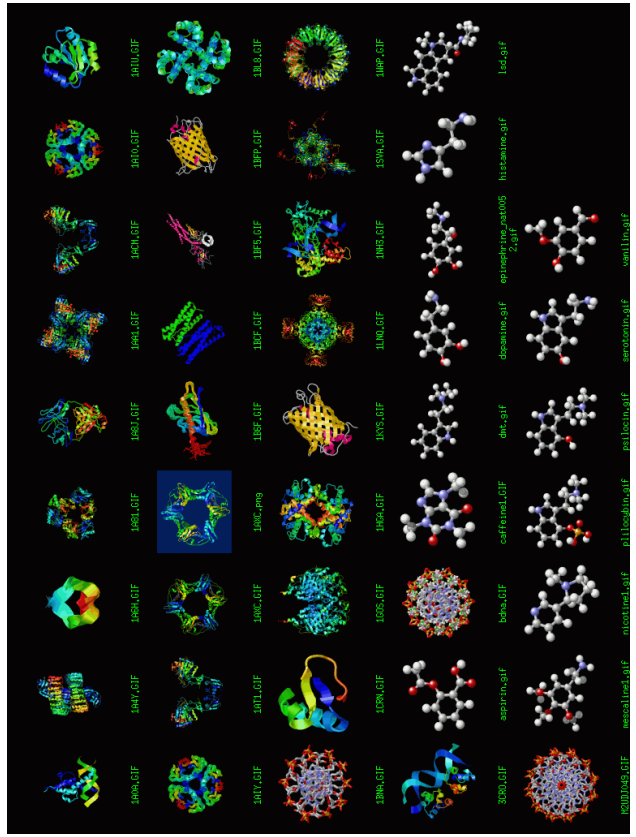
Systems Toxicology approach

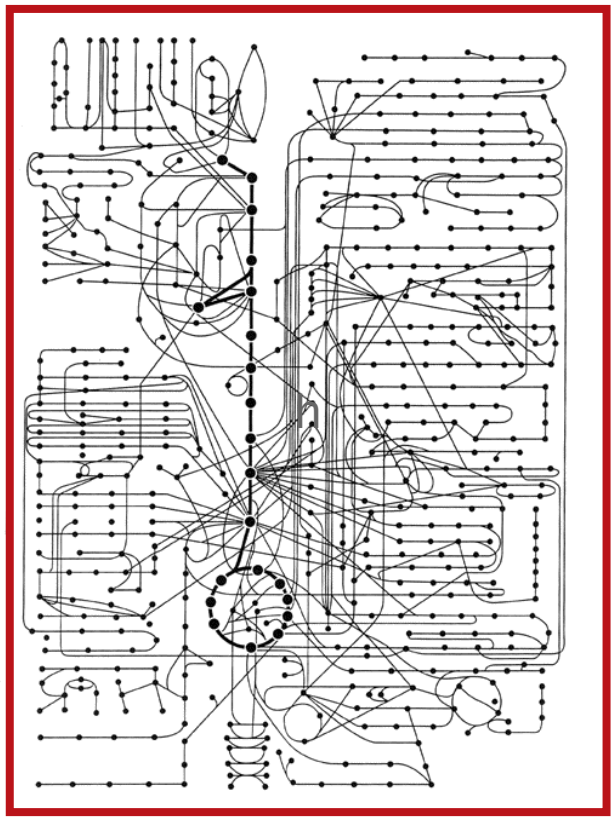
Nikolai V. Ivanov, Ph.D.

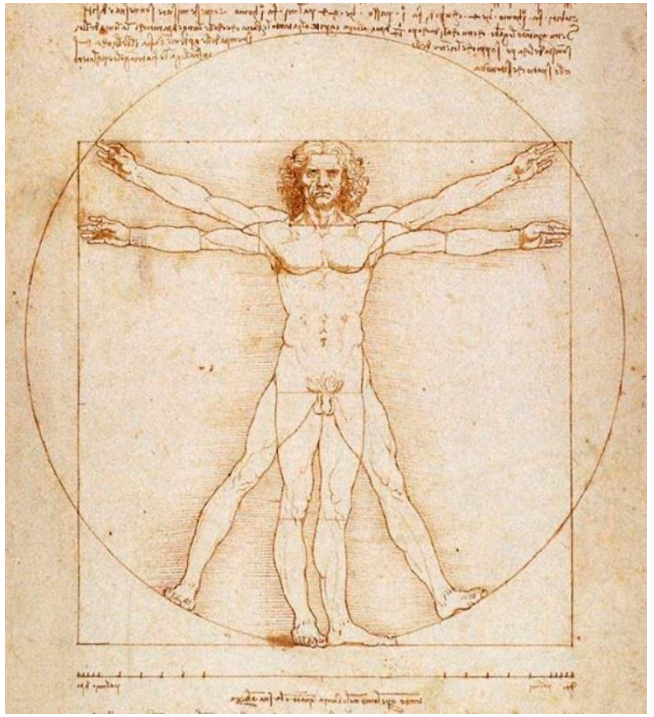
24 July 2015

From Systems Biology to Systems Toxicology

Genes/Proteins/Metabolites + Biological Networks = Organism







Descriptive Biochemistry
Functional Genomics

Systems Biology

Medicine

Adapted from SystemsX.ch

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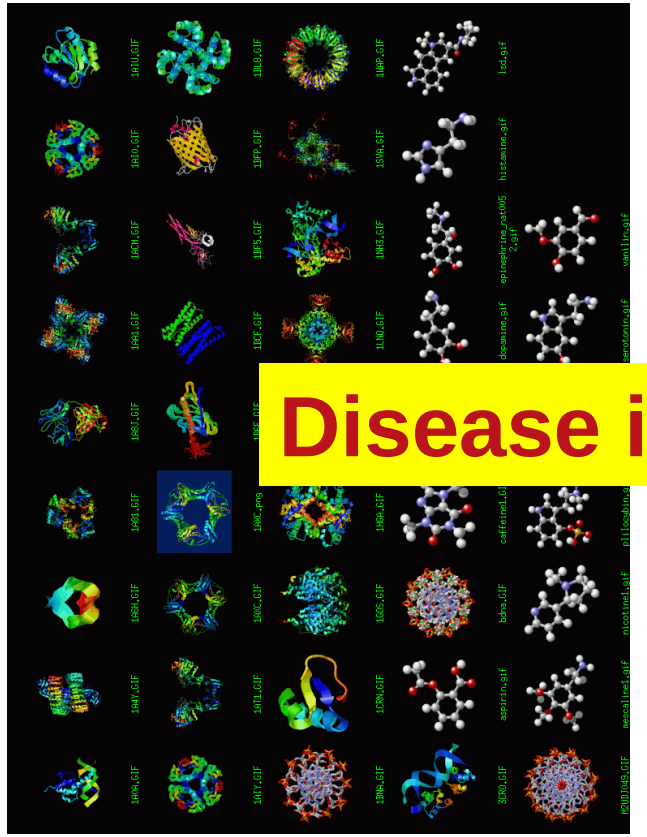
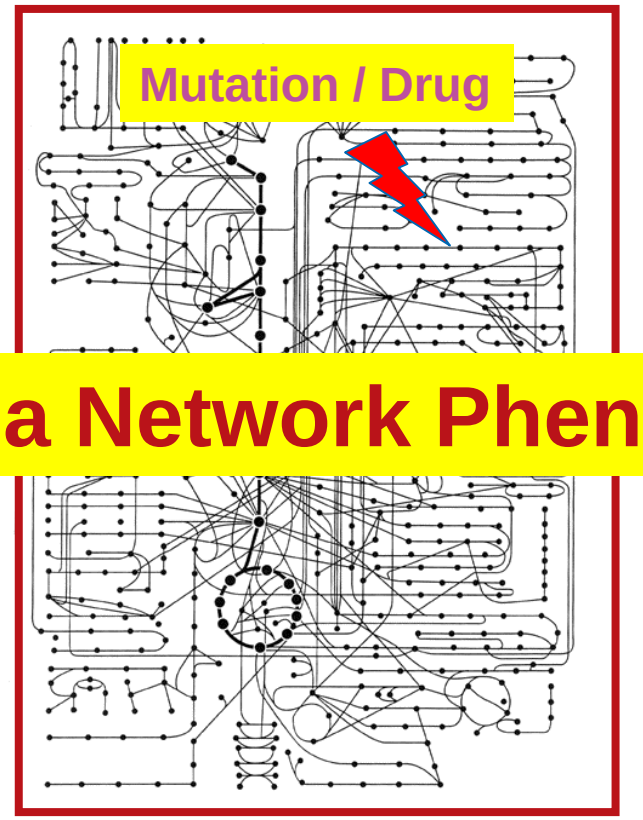
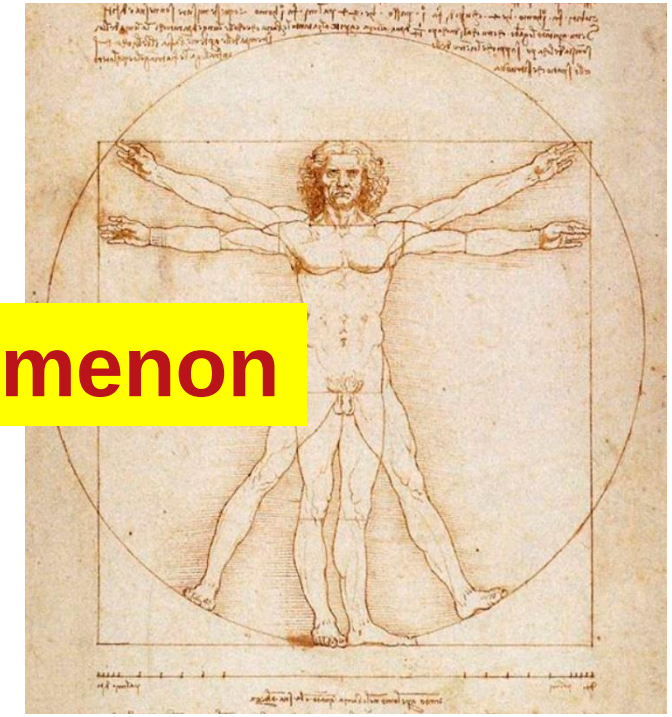
From Systems Biology to Systems Toxicology

Genes/Proteins/Metabolites + Biological Networks = Organism







Disease is a Network Phenomenon

Descriptive Biochemistry
Functional Genomics

Systems Biology

Medicine

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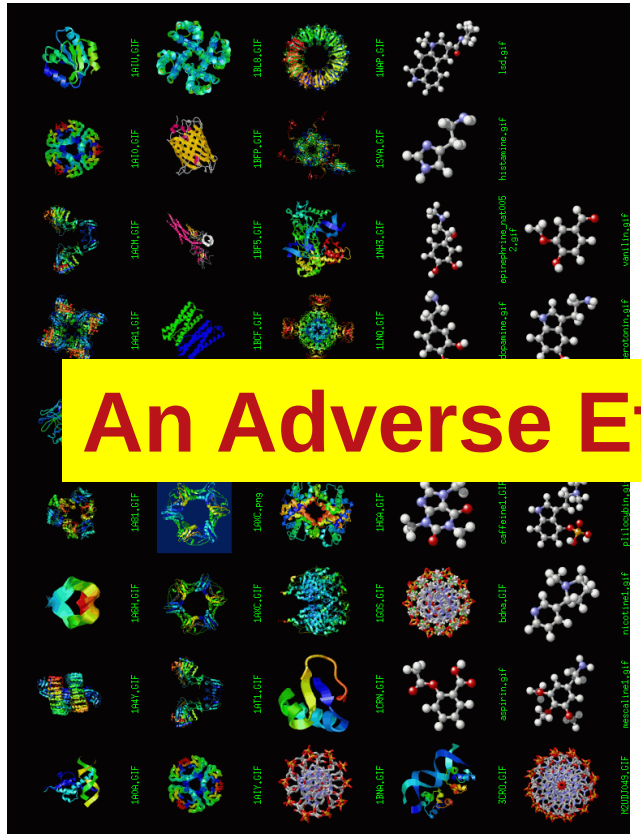
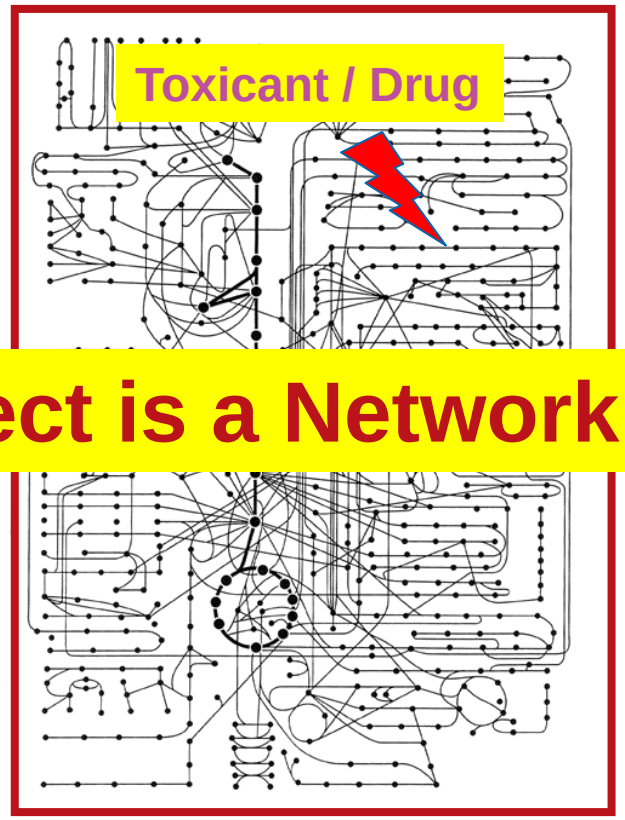
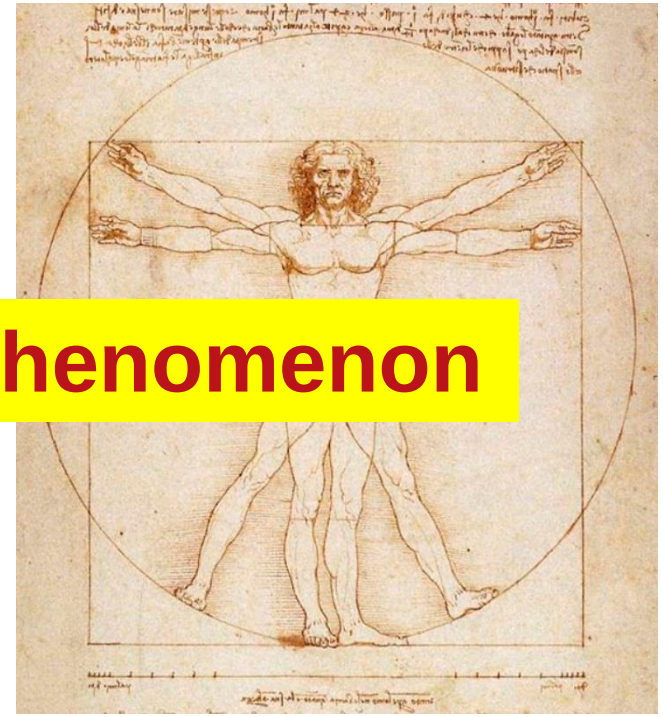
From Systems Biology to Systems Toxicology

Genes/Proteins/Metabolites + Biological Networks = Organism







An Adverse Effect is a Network Phenomenon

Descriptive Biochemistry
Functional Genomics

Systems Toxicology

Medicine

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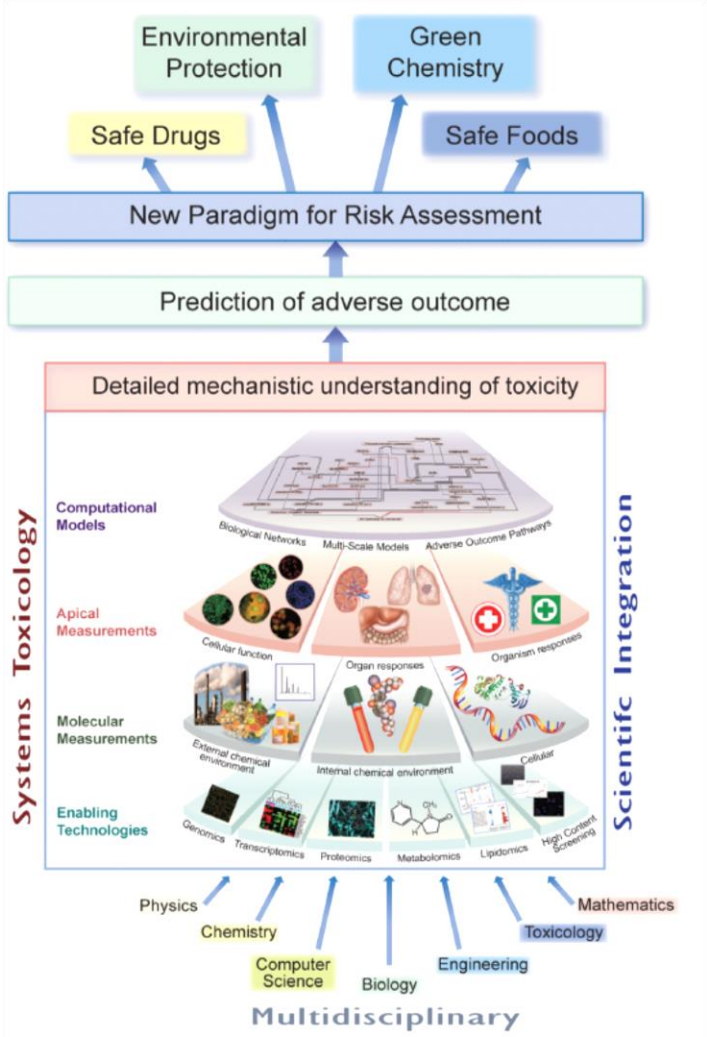
What is Systems Toxicology?



- Systems Toxicology is the integration of the classic toxicology paradigm with the quantitative analysis of many molecular and functional changes occurring across multiple levels of biological organization.
- SysTox Research is aimed at developing a detailed mechanistic as well as dynamic understanding of toxicological processes.
- SysTox Assessment leverages this detailed knowledge in a new paradigm of Product Assessment
- SysTox enables inter-species and system translation at the mechanistic level.

Sturla SJ, Boobis AR, FitzGerald RE, Hoeng J, Kavlock RJ, Schirmer K, Whelan M, Wilks MF and Peitsch MC (2014) Systems Toxicology: from basic research to risk assessment. Chem. Res. Toxicol., 27:314-329. (PMID: 24446777).

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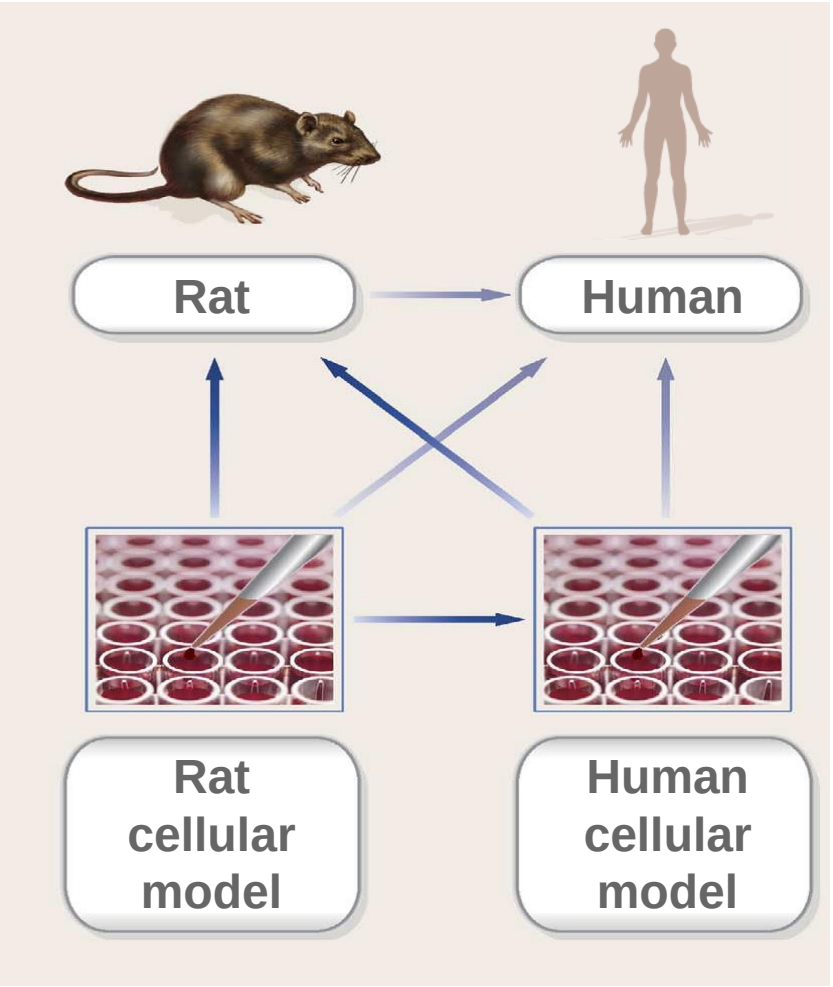
Framework of species and system translation



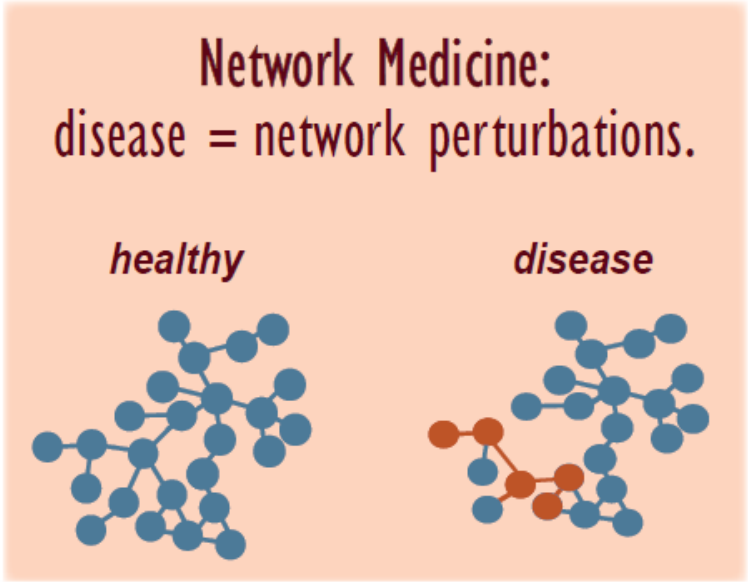
Translation:

- Are animal models useful in toxicology?
  - What biology can be applied across species?
- Which *in vitro* systems are necessary to recapitulate a meaningful part of whole body biology?

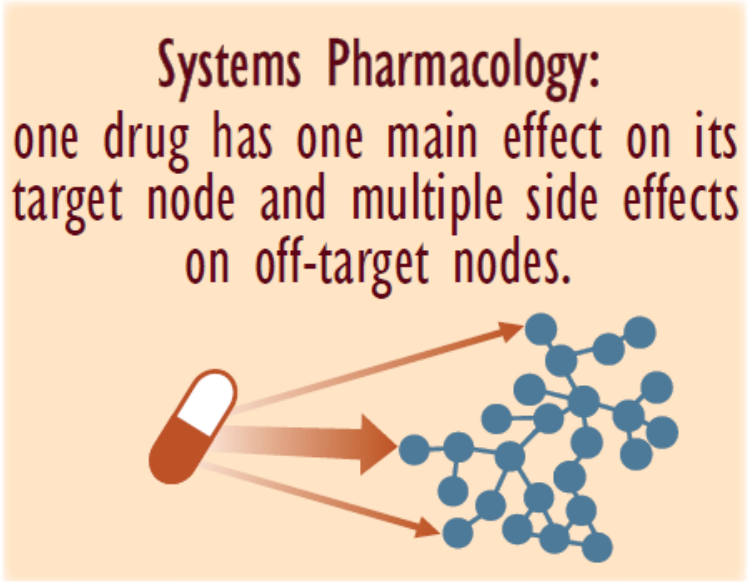
Poussin C, Mathis C, Alexopoulos LG, Messinis DE, Dulize RHJ, Belcastro V, Melas IN, Sakellaropoulos T, Rhrissorrakrai K, Bilal E, Meyer P, Talikka M, Boue S, Norel R, Rice JJ, Stolovitzky G, Ivanov NV, Peitsch MC and Hoeng J (2014) The Species Translation Challenge - A Systems Biology Perspective on Human and Rat Bronchial Epithelial Cells. **Scientific Data,** 1:140009

Biological Networks and Systems Toxicology

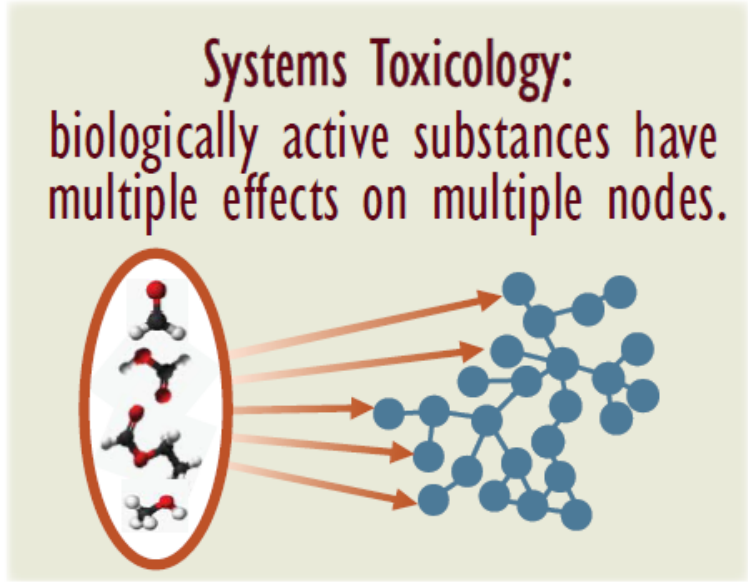
- Systems Toxicology can be viewed as an extension of Network Medicine and Systems Pharmacology.



Network medicine: a network-based approach to human disease, Barabási AL, Gulbahce N, Loscalzo J, Nat Rev Genet. 2011.



Network analyses in systems pharmacology, Berger SI, Iyengar R, Bioinformatics 2009.



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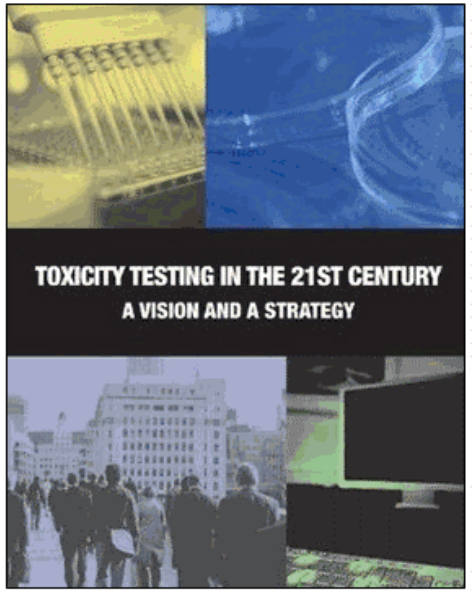
Relevance of Systems Toxicology beside "pure science": the regulatory context (*Toxicity Testing in the 21st Century* initiative and others)

Initiated by the US EPA (environmental protection agency) in 2007 as an alternative to "standard" animal-based toxicological testing.

1) Identify the toxicity pathways/networks (mode of action) = cellular response pathways/ networks that are significantly perturbed and that can cause adverse health effects in human population.

2) For each toxicity pathways/networks, quantify its perturbations at different doses (dose-response assessment) in *in vitro* cellular systems.



SEVENTH FRAMEWORK PROGRAMME

SEURAT-1
Towards the Replacement of in vivo Repeated Dose Systemic Toxicity Testing

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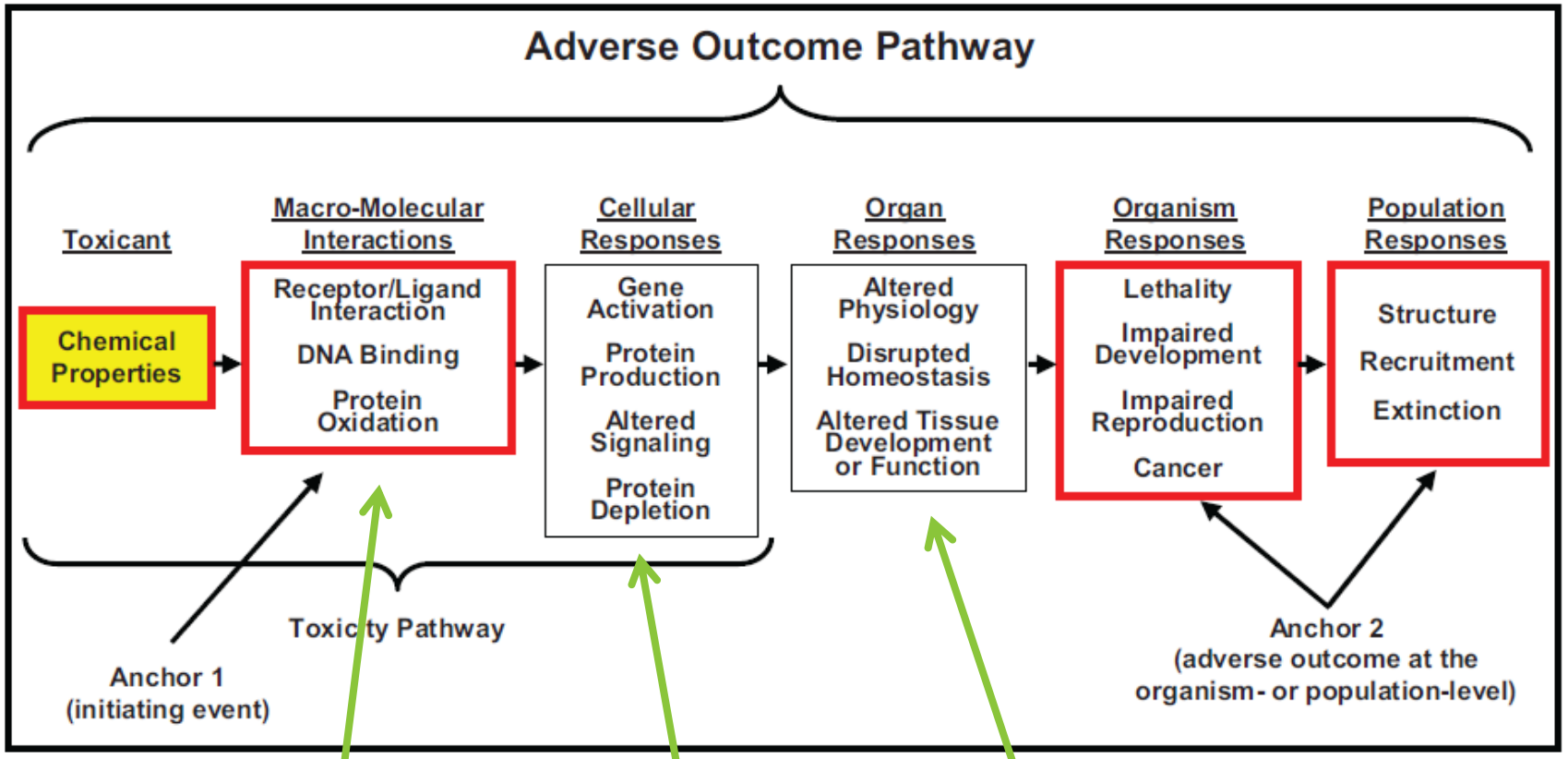
Biological Network Perturbations are Pivotal to Understand the Causal Link between Toxicants and Health Effects



Interaction between toxicant and biological molecules

Biological Networks Perturbations

Changes to cells tissue and organs

Ankley et al., (2010) AOPs: A conceptual Framework to Support Ecotoxicological Research and Risk Assessment. Environ. Toxicol. Chem. 29: 730-41.

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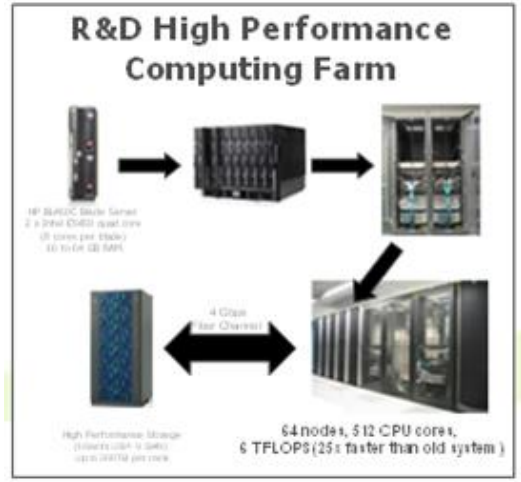
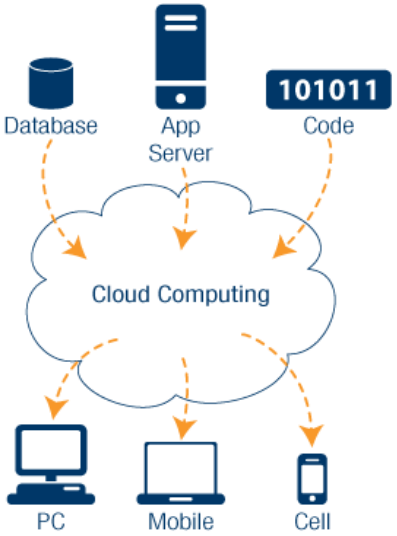
Systems Toxicology Requires a Robust Scientific Computing Environment

Computational approaches in systems biology require a robust Scientific Computing Environment (SCE) composed of:

- High performance computing infrastructure designed to provide multi-TFLOP **computing power**
- Scalable, high-available and high-performance shared file system with **massive storage** capacity
- Fast and reliable data **network infrastructure** to enable efficient data transfer and system access between R&D sites
- Access to cloud computing services to provide on-demand access to additional resources, enable efficient sharing of data and analyses, and support reproducibility

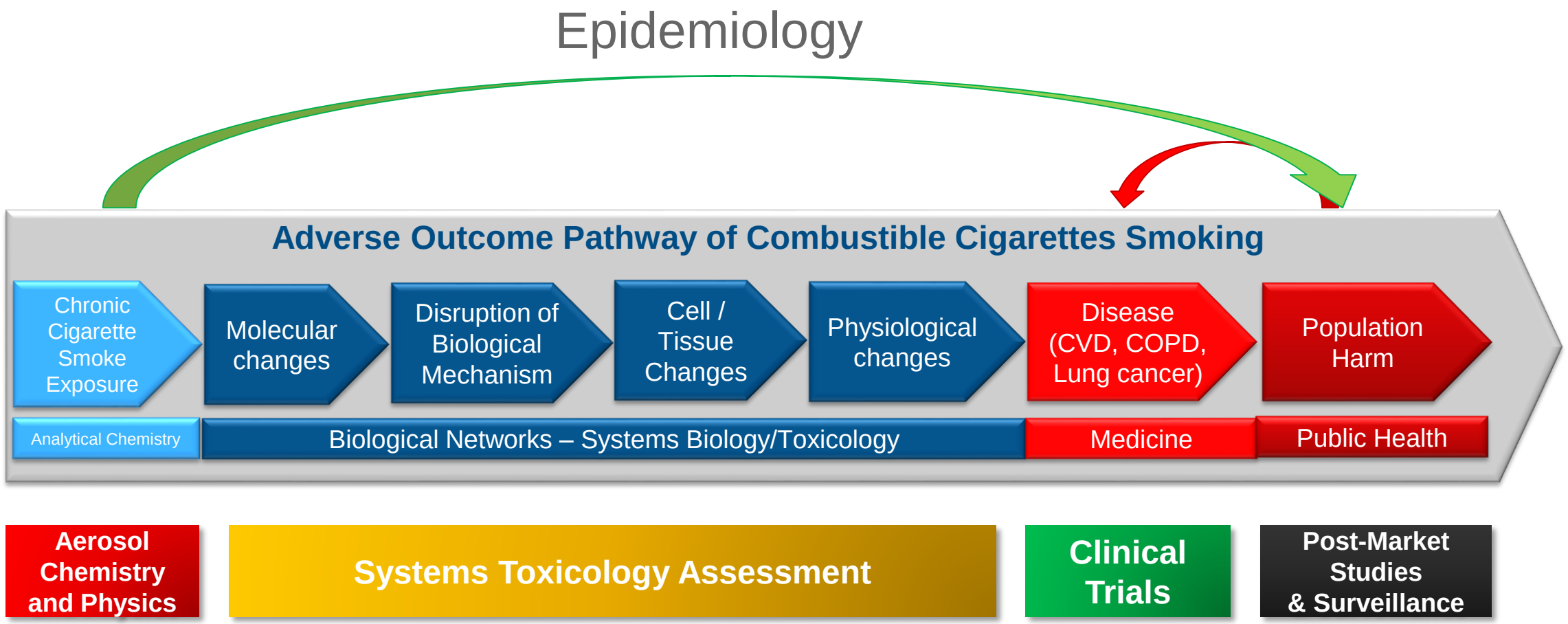




Background to case studies

- Smoking causes serious diseases such as cardiovascular diseases, lung cancer and chronic obstructive pulmonary disease.
- Philip Morris International is therefore developing novel products that may have the potential to reduce smoking-related disease risk compared to conventional combustible cigarettes.
- To determine whether such potentially reduced-risk products (RRP) have the potential to reduce disease risk, we compare their biological impact with that of a combustible reference cigarette (3R4F) on a mechanism-by-mechanism basis.

From Exposure to Population Harm: a Causal Chain of Events



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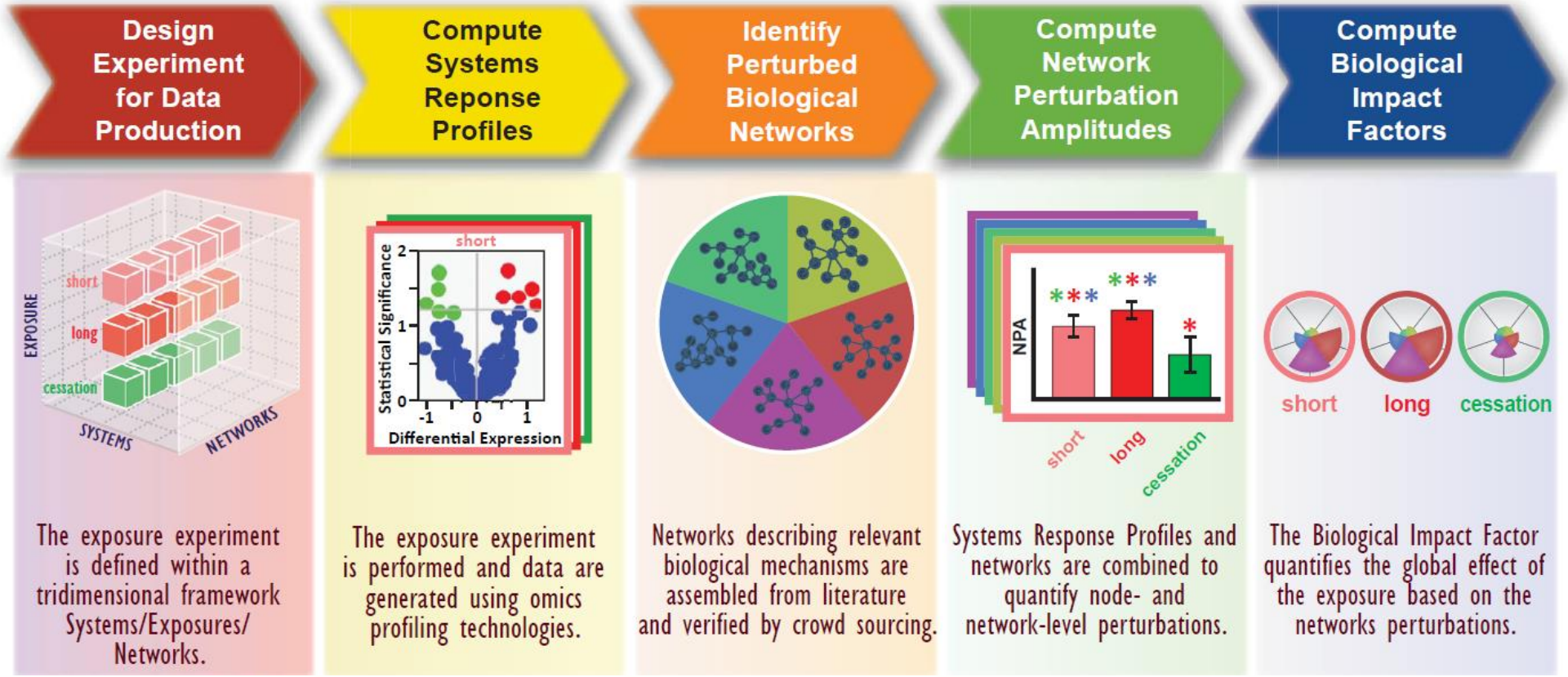
A Five Step Approach for Systems Toxicology-based Product Testing

Five-step Strategy implementing Network-based Systems Toxicology (@PMI)

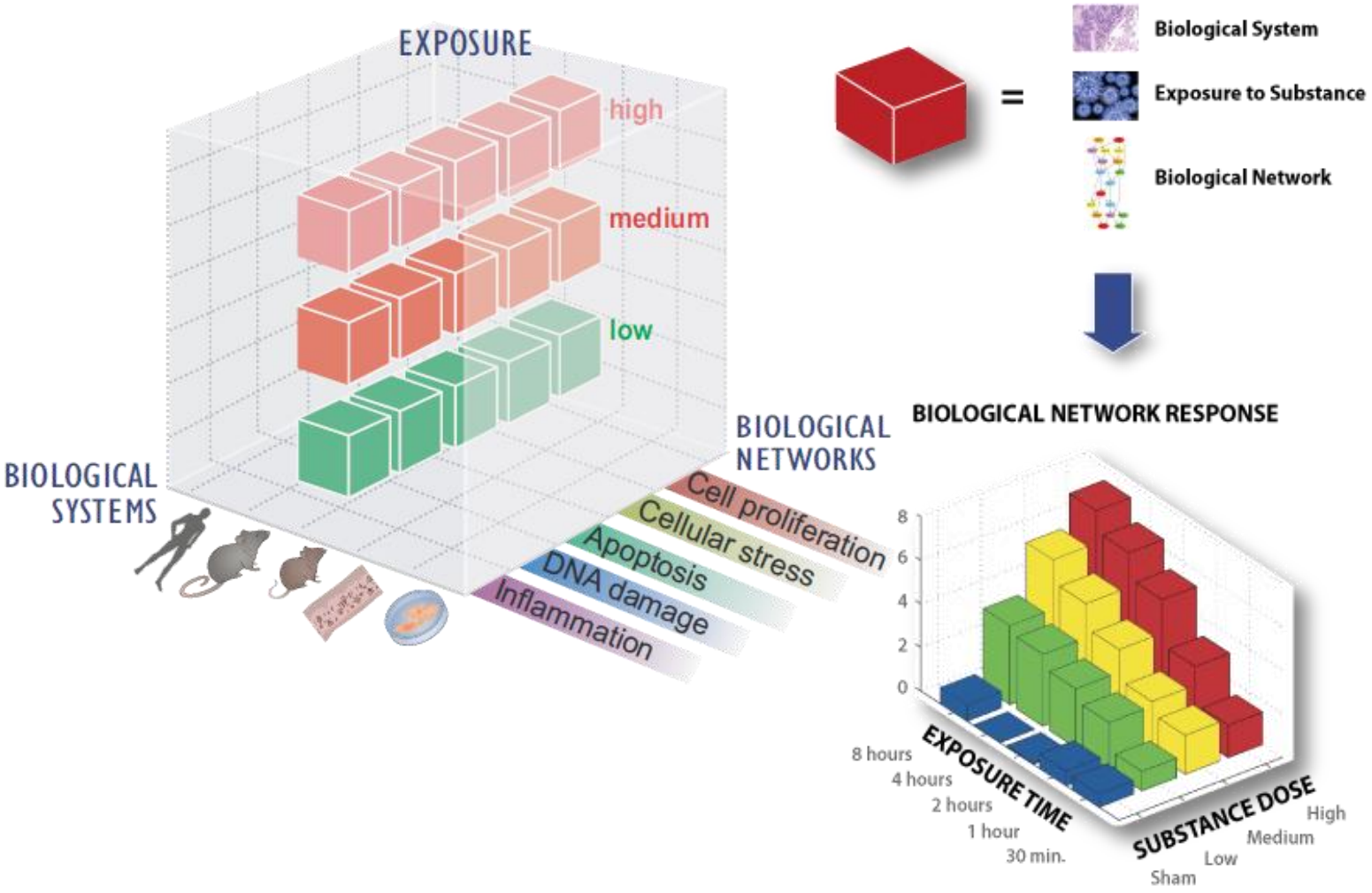
- Goal: enable product testing following the "*Toxicity Testing in the 21st Century*" guidelines.
- Result: this strategy has been implemented at PMI R&D over the last five years.



A network-based approach to quantifying the impact of biologically active substances, Hoeng J *et al*., Drug Discov Today, 2012.

Step 1: Design Experiments for Data Production

Experimental Data Production



Systematic experiments conducted in several test systems:

- adequate selection of systems:
  - Primary cells whenever possible.
  - Animal model of disease.
- Dose-responses
- Time-resolved
- Deep analysis of exposure
- Multi-omics to cover mechanisms of toxicity

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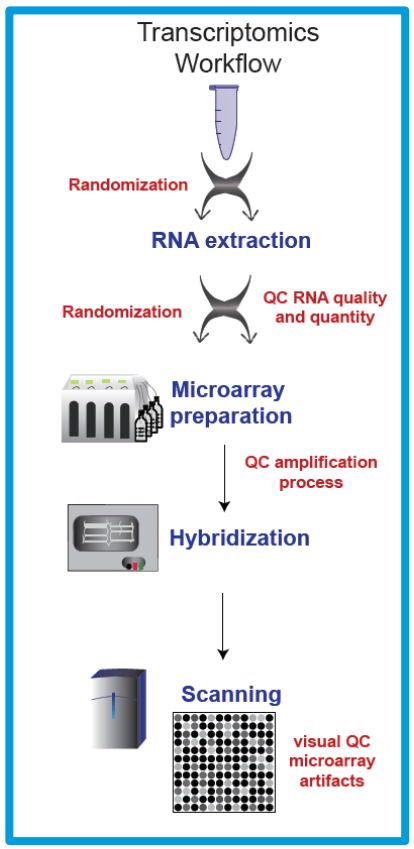
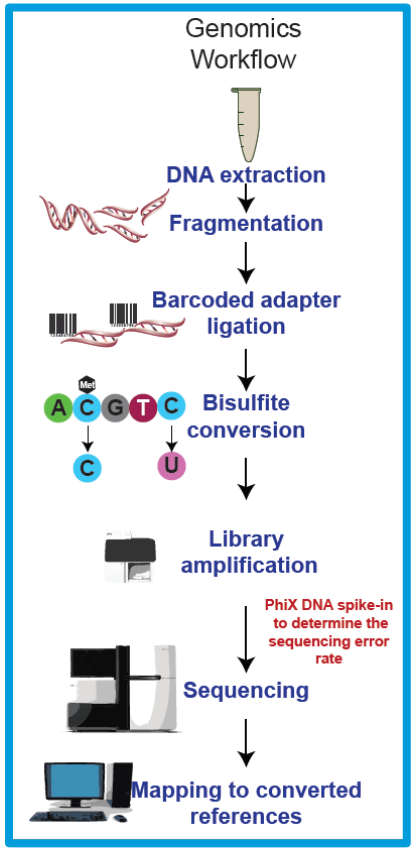
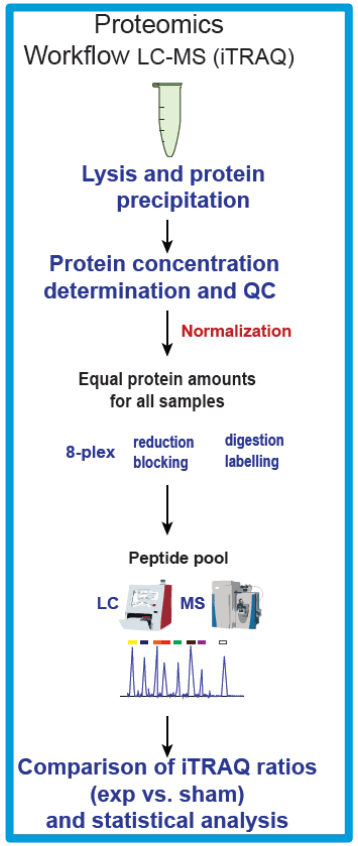
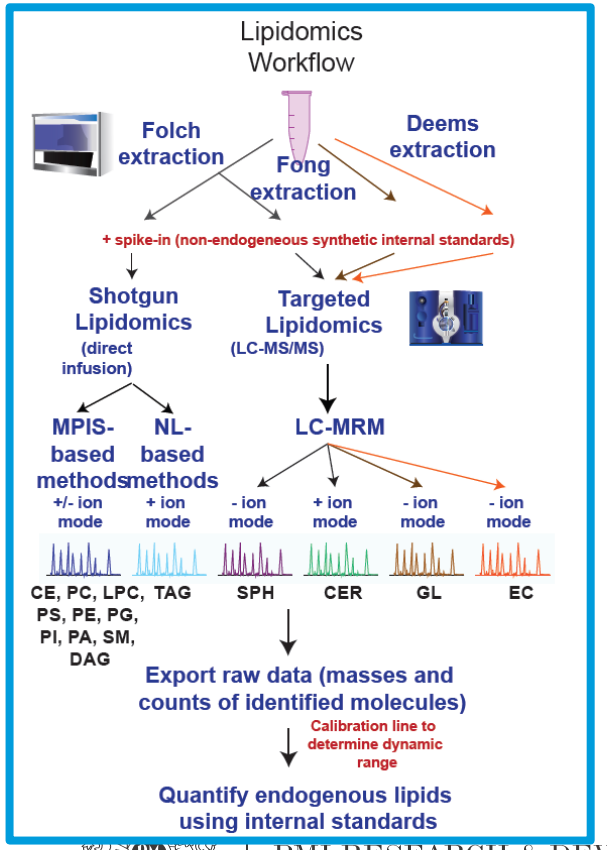
The "Systems" Part







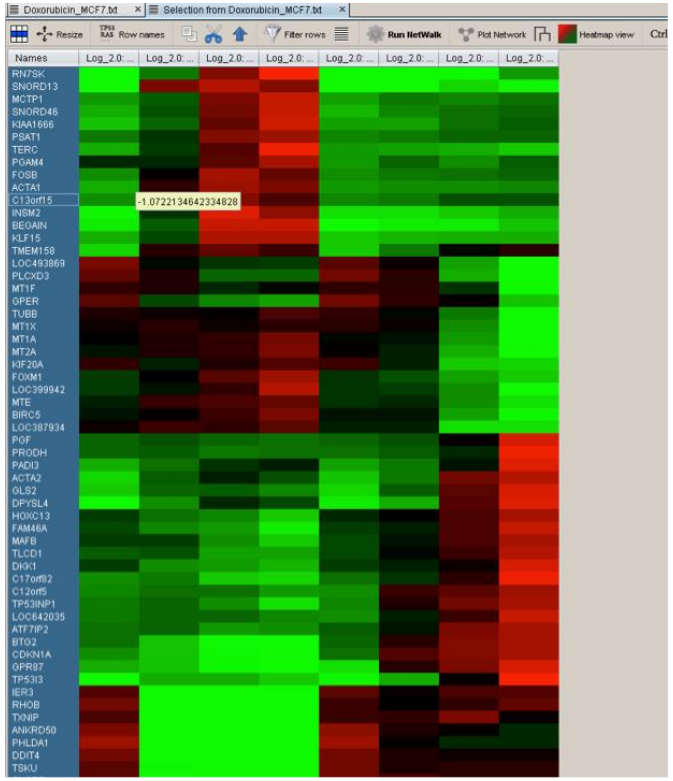


Titz B, Elamin A, Martin F et al. (2014) Proteomics for systems toxicology. Computational and Structural Biotechnology Journal 11:73-90

Step 2: Compute Systems Response Profiles

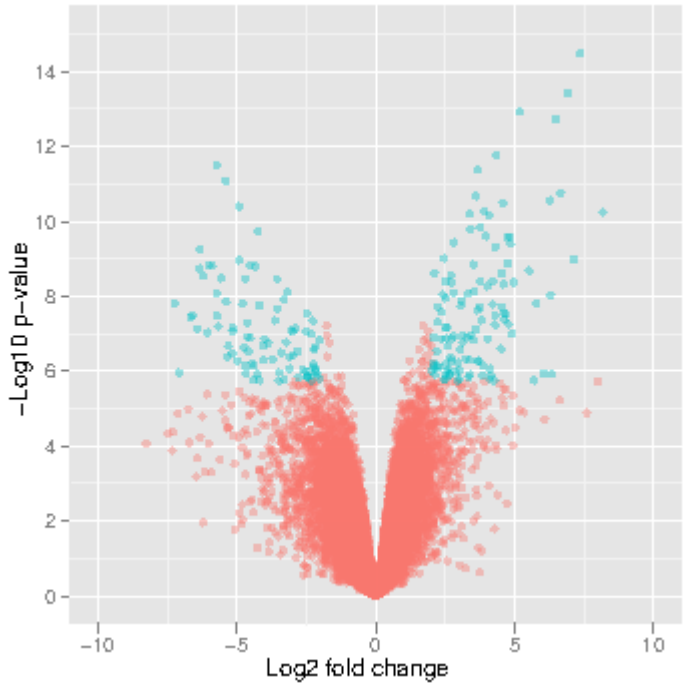
Differential gene expression (also proteomics, lipidomics, DNA methylation)

Heatmap Analysis



https://netwalkersuite.org/tutorials/doxorubicin/clustering-heatmap-analysis

Volcano plots of microarray data



http://bioinformatics.knowledgeblog.org/2011/06/21/volcano-plots-of-microarray-data/

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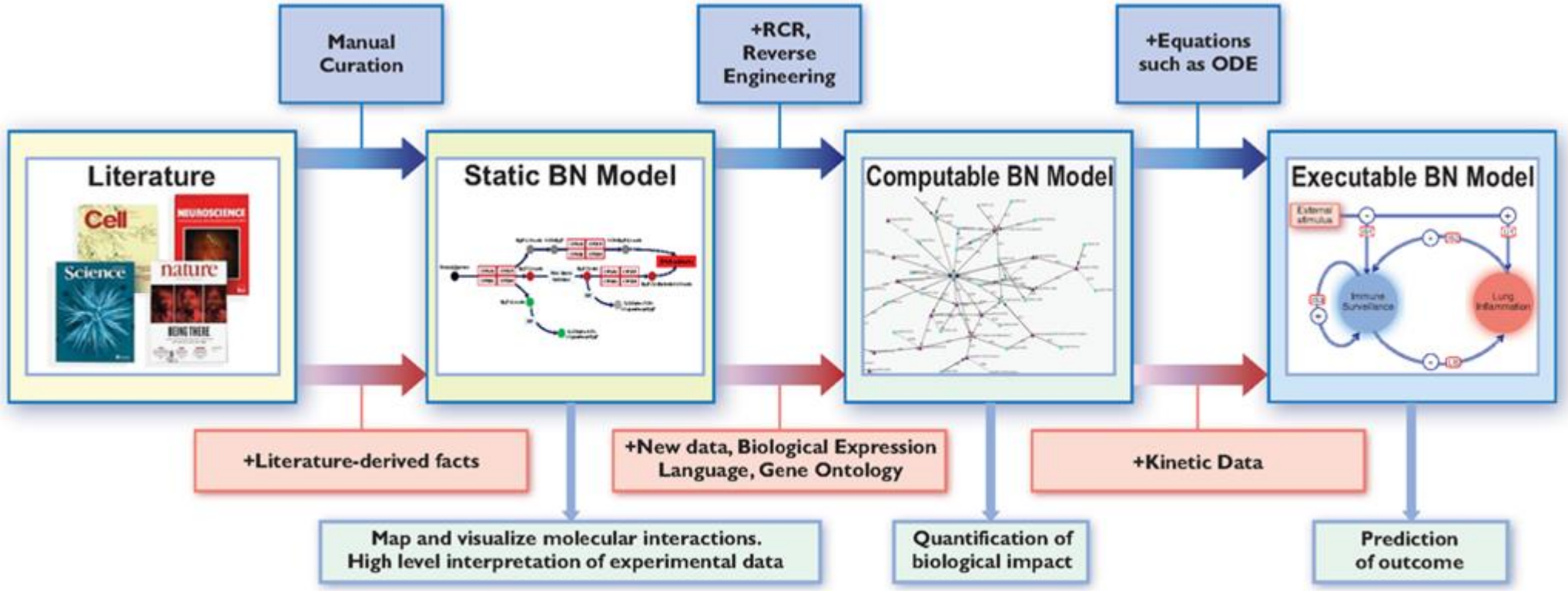
Step 3: Identify Perturbed Biological Networks

Biological network model-development process



Sturla SJ, Boobis AR, FitzGerald RE, Hoeng J, Kavlock RJ, Schirmer K, Whelan M, Wilks MF and Peitsch MC (2014) Systems Toxicology: from basic research to risk assessment. Chem. Res. Toxicol., 27:314-329. (PMID: 24446777).

Definition of the biological boundaries and context of the Network Models

- Goal: assemble networks describing the main biological processes perturbed in exposure experiments.

- Guidelines (for expert lung biologists):
  - define the boundaries of each network,
  - organize all the networks into a hierarchical structure: e.g. stress response → xenobiotic metabolism resonse → CYP1A1 activity,
  - try to be as objective as possible…

Example: network boundaries for the lung inflammation networks

In scope

- healthy processes
- inflammation triggered by cigarette smoke
- evidences can be collected from experiments performed in human, mouse, or rat.

Out of scope

- lung diseases: Chronic Obstructive Pulmonary Disease (COPD), lung cancer,…
- *in vitro* specific exclusion criteria: no tumor-derived/ immortalized/neuronal/ respiratory/vascular cell lines/types
- other species

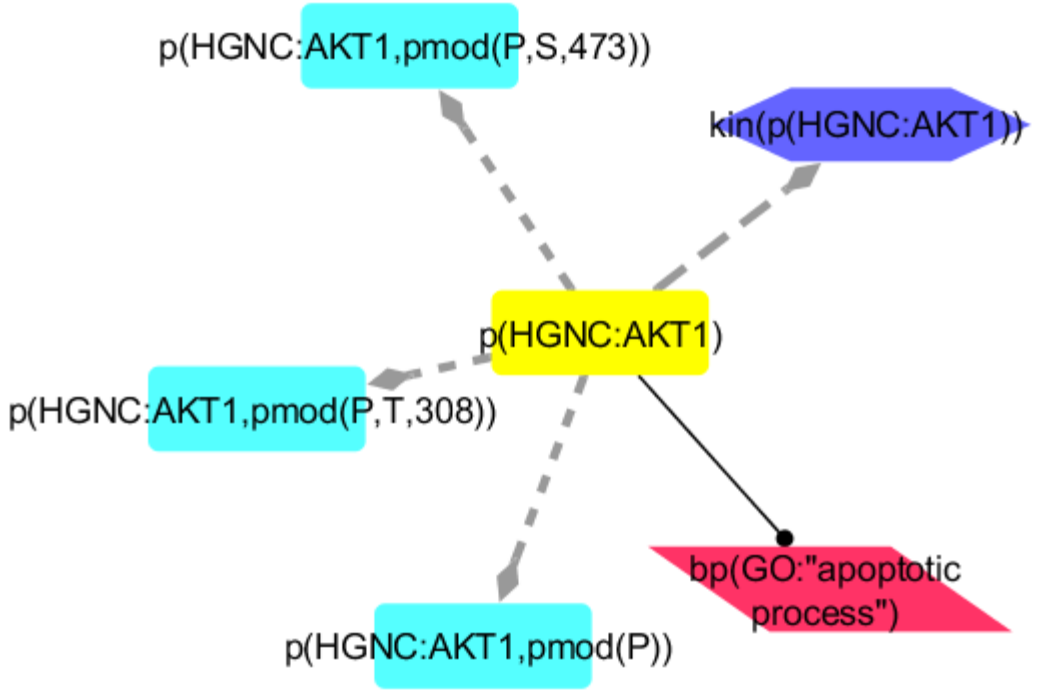
Encoding the content of the Biological Network Models in BEL

- BEL (=Biological Expression Language) represents scientific findings in life sciences in a computable format.

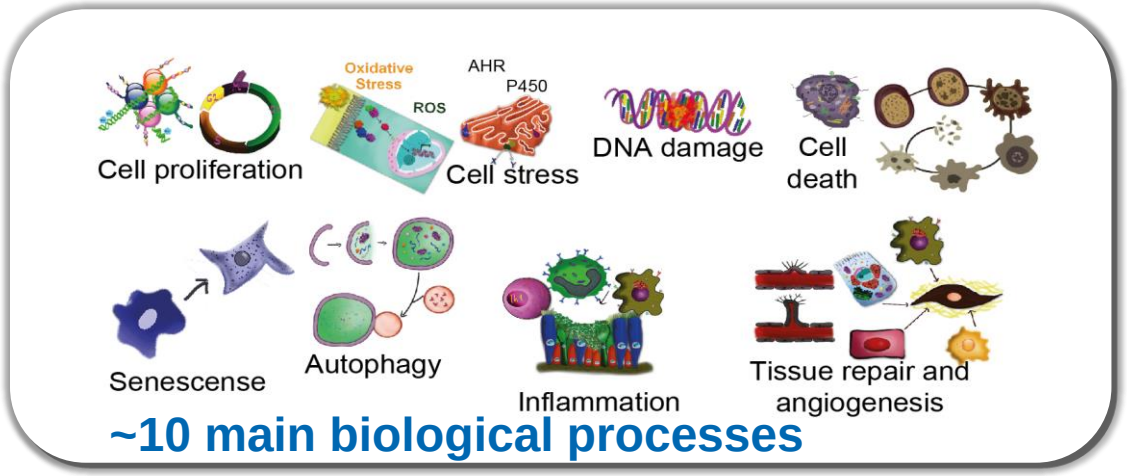
- We licensed the Selventa Knowledgebase (over 1.5 million nodes and over 7.5 million edges supported by literature evidences and encoded in BEL). A subset is freely available through the openBEL portal.

- Network nodes represent biological molecular mechanisms (“kinase activity of AKT1”), while edges encode signed causal relationships between the nodes (“increase/decrease”).

http://www.openbel.org

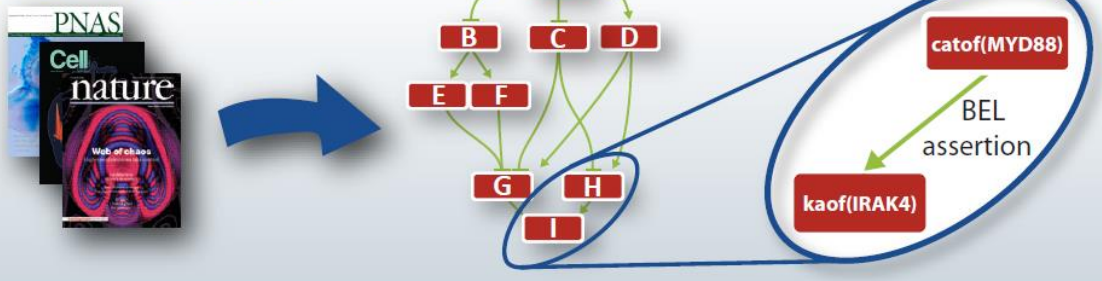


Building the Network Models

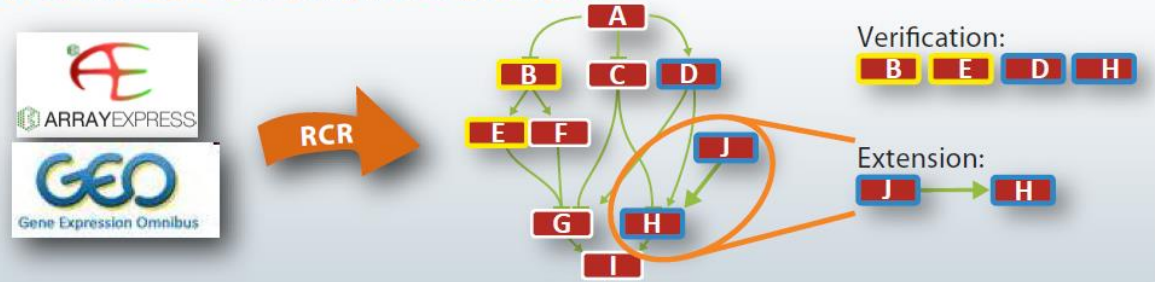


~10 main biological processes

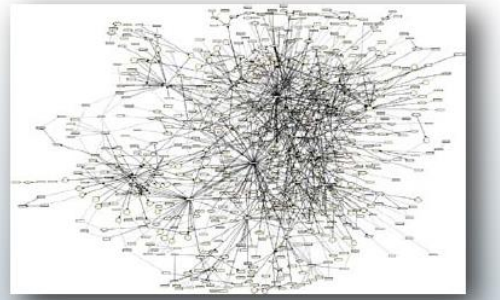
1. LITERATURE MODEL



2. DATA-BASED VERIFICATION AND EXTENSION



3. FINAL NETWORK MODEL



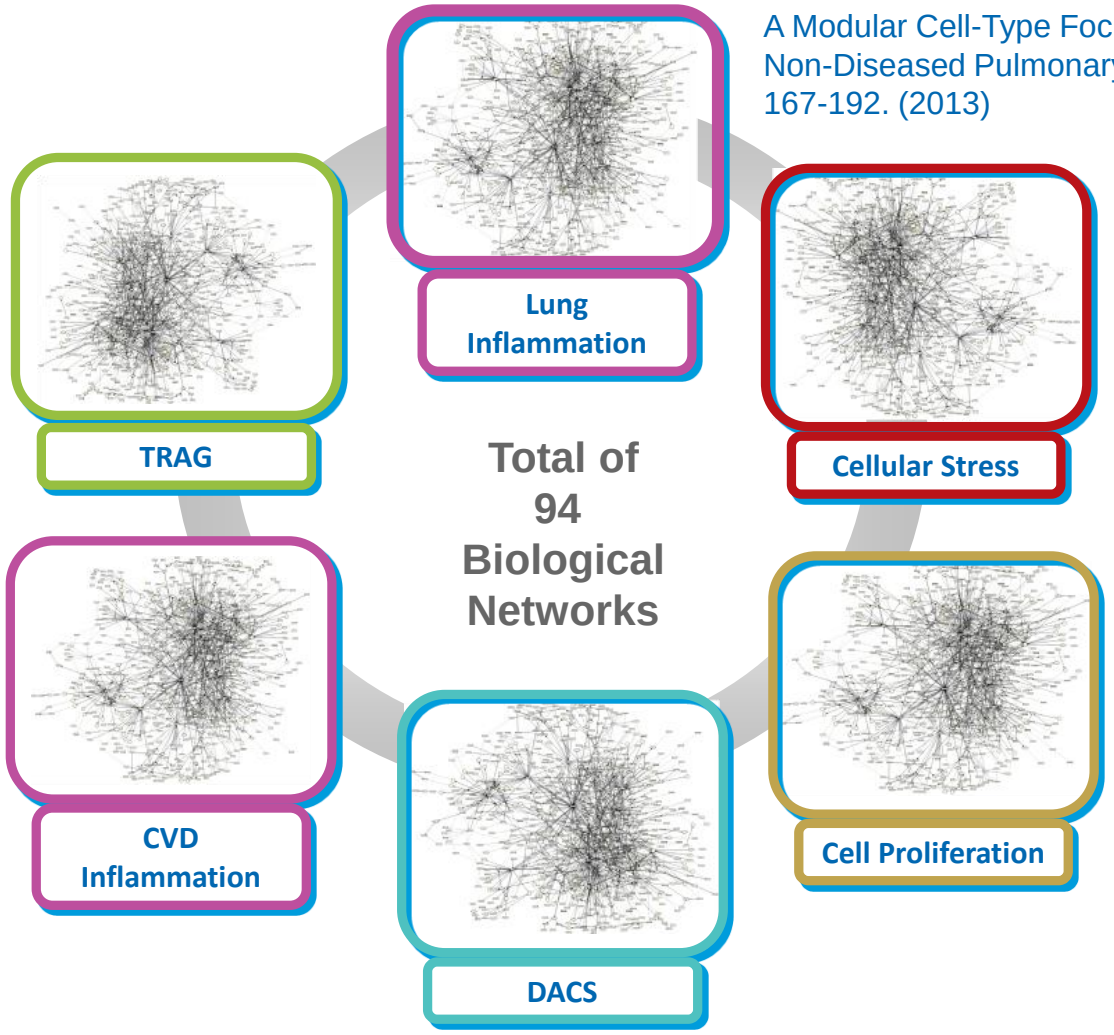
1000's of literature backed nodes and edges depicting the biology of interest

Network Models assembled so far

All available at http://causalbionet.com

Causal biological network database: a comprehensive platform of causal biological network models focused on the pulmonary and vascular systems, Boué S *et al.*, Database (Oxford) 2015

Construction of a Computable Network Model of Tissue Repair and Angiogenesis in the Lung. Journal of Clinical Toxicology, S12, 002. (2013)



A Modular Cell-Type Focused Inflammatory Process Network Model for Non-Diseased Pulmonary Tissue. Bioinformatics and Biology Insights, 7, 167-192. (2013)

A computable cellular stress network model for non-diseased pulmonary and cardiovascular tissue. BMC Systems Biology, 5, 168. (2011)

Construction of a Computable Cell Proliferation Network Focused on Non-Diseased Lung Cells. BMC Systems Biology, 5, 105. (2011)

Construction of a Computable Network Model for DNA Damage, Autophagy, Cell Death, and Senescence. Bioinformatics and Biology Insights, 7, 97-117. (2013)

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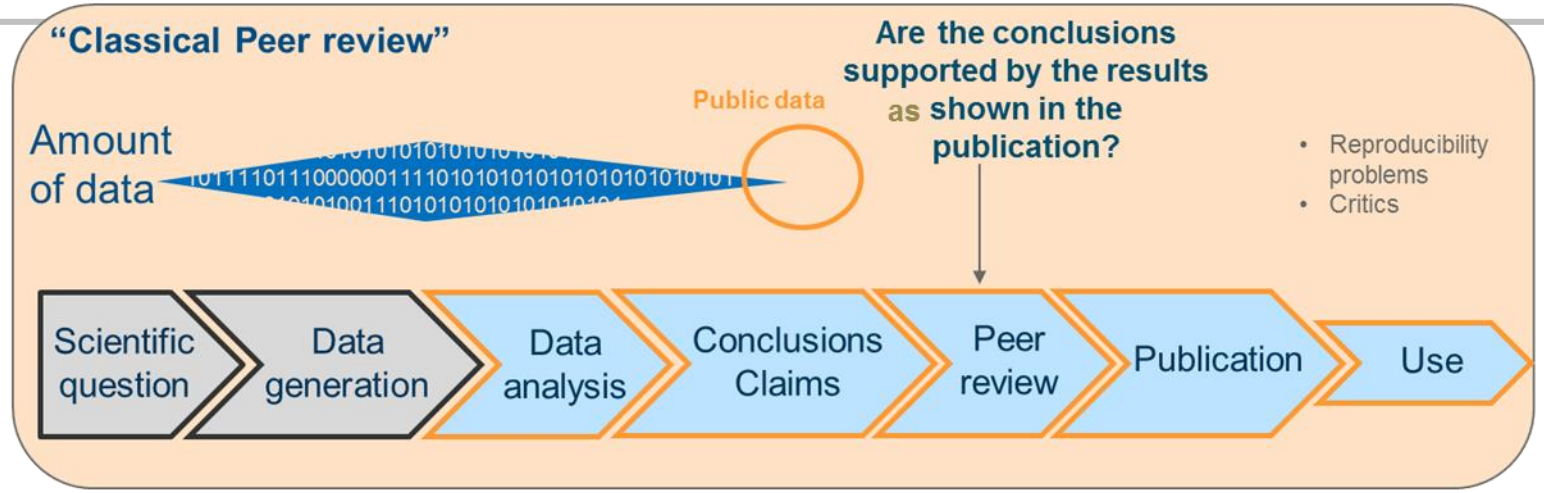
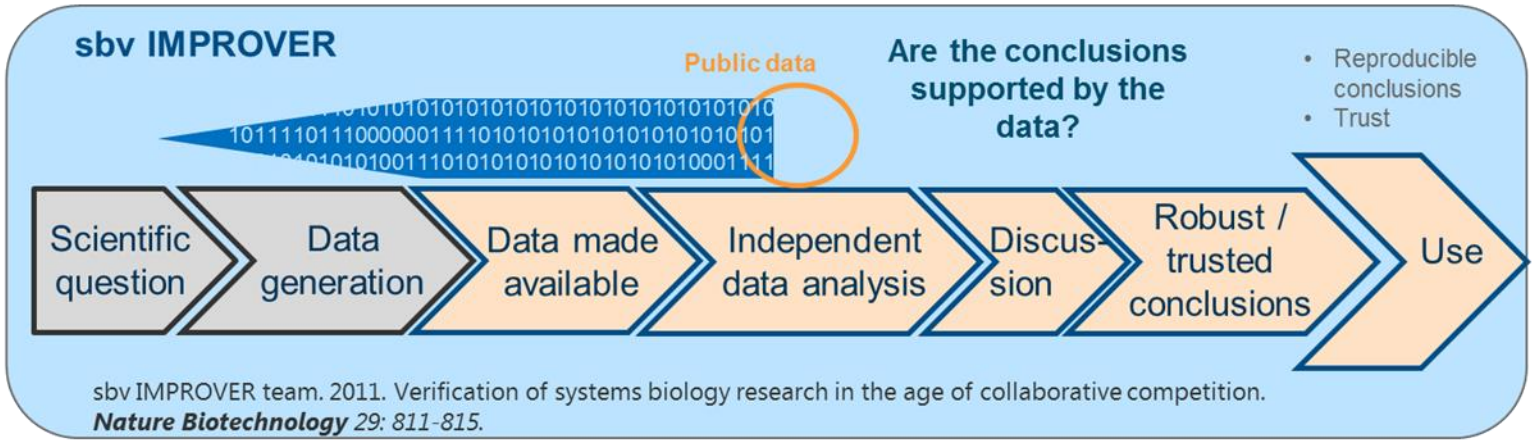
sbvIMPROVER: verification of Systems Biology data using crowd-sourcing

Anonymous

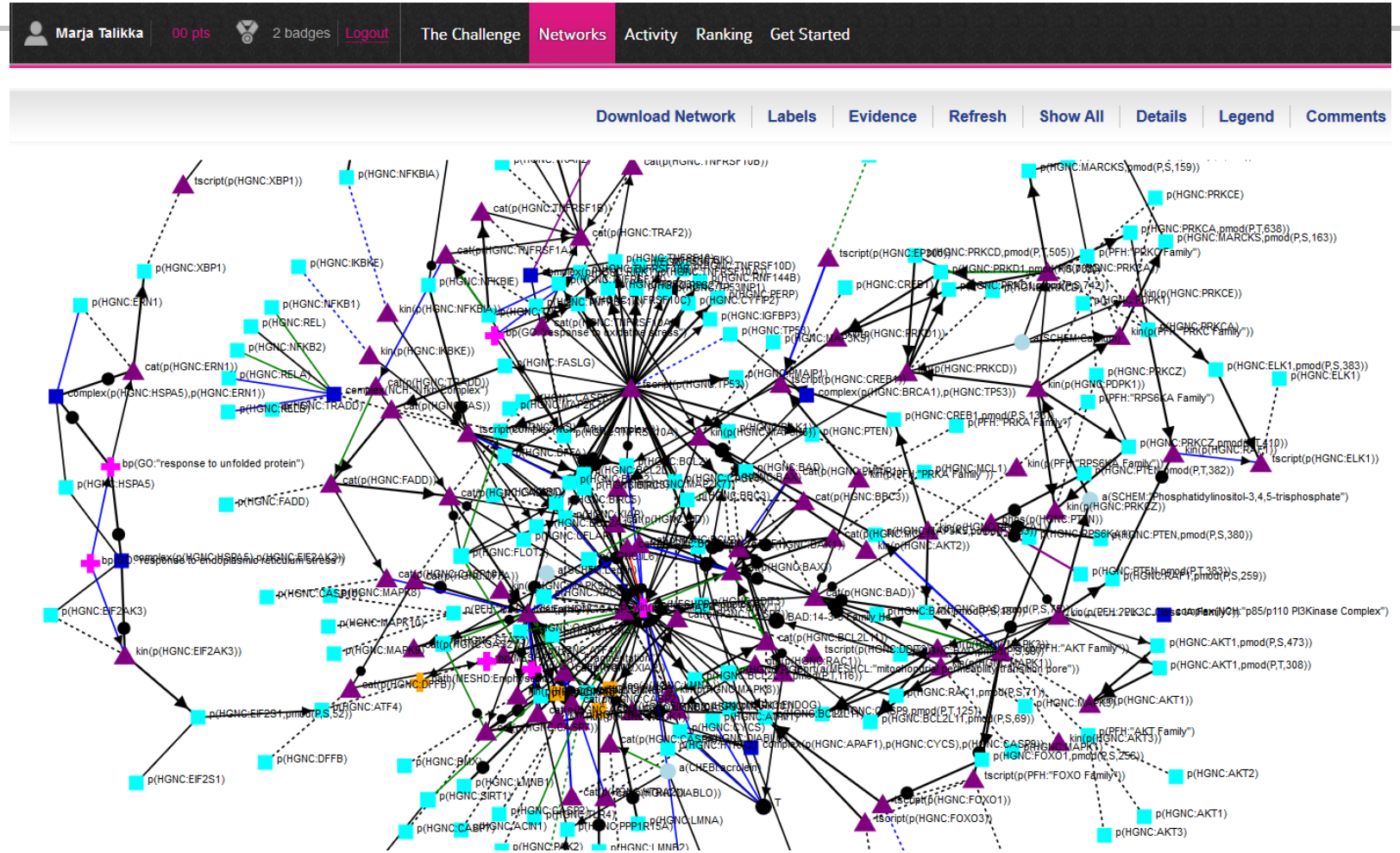


Leverages the collective expertise of the scientific community to provide the best answers

Known



sbvIMPROVER : crowd-verification of Biological Network Models



Enhancement of COPD biological networks using a web-based collaboration interface. F1000Research. 2015; 4: 32.
Binder J, et al. Reputation-based collaborative network biology. Pacific Symposium on Biocomputing Pacific Symposium on Biocomputing. 2014, p. 270-81.
SBV Improver Project Team. On Crowd-verification of Biological Networks. Bioinformatics and biology insights. 2013; 7: 307.

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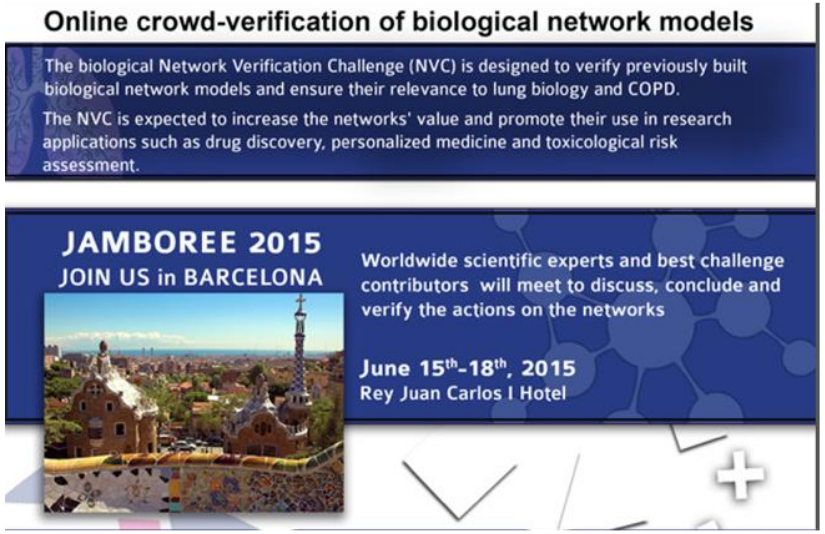
Second sbvIMPROVER Jamboree on crowd-verification of Network Models



sbv IMPROVER Challenges

2012 Diagnostic Signature Challenge

Designed to determine whether computational approaches and transcriptomics data could be used for phenotype prediction.

2013 Species Translation Challenge

Designed to address whether biological events observed in rodents were translatable to humans.

2014 2015 Network Verification Challenge

Designed to verify previously built biological network models and ensure their relevance to lung biology and COPD.

NOW Systems Toxicology Computational Challenge

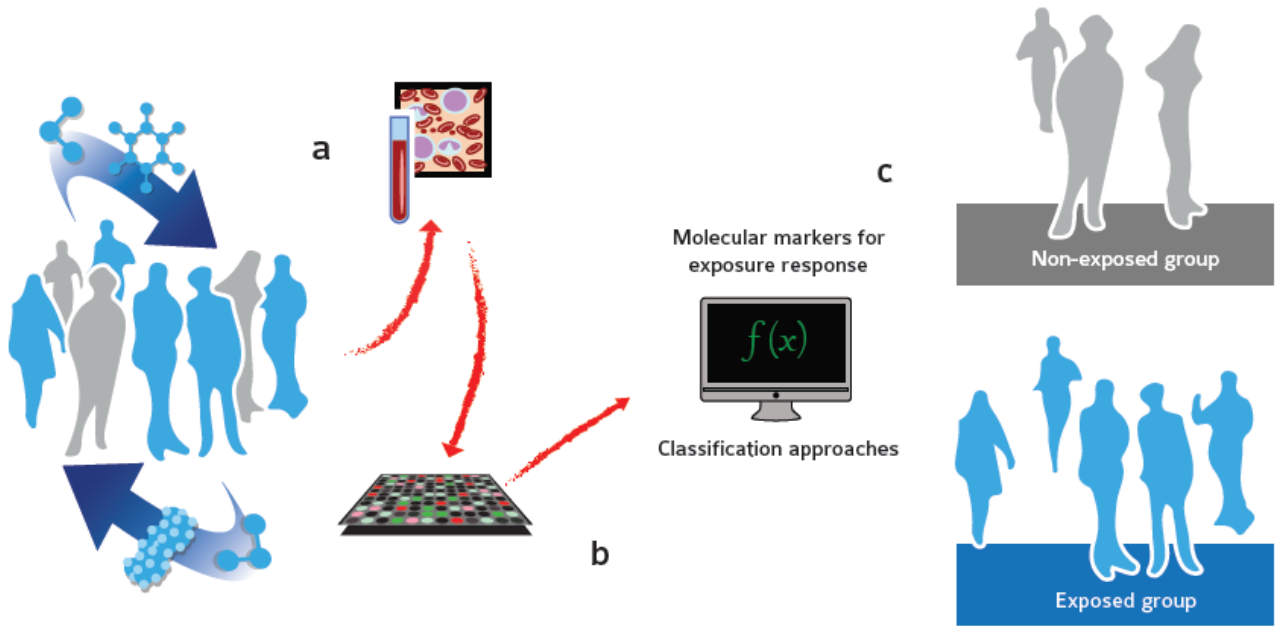
Designed to verify that a robust predictive signature can be extracted from gene expression data that differentiates smokers, former smokers, and never smoker subjects.

Who can be part of the Challenge?
Everyone – Knowledge in computational science is an asset

When?
Fall 2015 - Spring 2016

Contact
www.sbvimprover.com/discover
sbvimprover.rd@pmi.com

The Systems Toxicology Computational Challenge in a Nutshell



Markers of Exposure Response Identification

a. Blood samples are collected from human and mouse subjects belonging to exposed or non-exposed groups.
b. Gene expression profiles (GEX) are measured using microarray-based technology.
c. Participants are provided with GEX and asked to develop a classification approach that identifies a gene signature capable of associating subjects to the correct exposure group.

The Two Sub-Challenges

Sub-challenge1: Human blood signature as exposure response marker

Humans are constantly exposed to individual or mixtures of chemicals (e.g. cigarette smoke, polluants, pesticides, drugs) that may trigger molecular changes in their organism. The identification of specific response markers is important to assess the exposure status of an individual. The blood is an easily accessible matrix, however remains a complex biofluid to analyze.

Scientific Question

Are gene expression changes in blood sufficiently informative to extract a predictive gene signature for smoking exposure (Smoker vs Non-current smoker) or cessation (Former smoker vs Never smoker) in human?

Sub-challenge2: Species translatable blood signature as exposure response marker

Most of pre-clinical in vivo studies are conducted in rodents which raises the question of translatability and applicability of results to human.

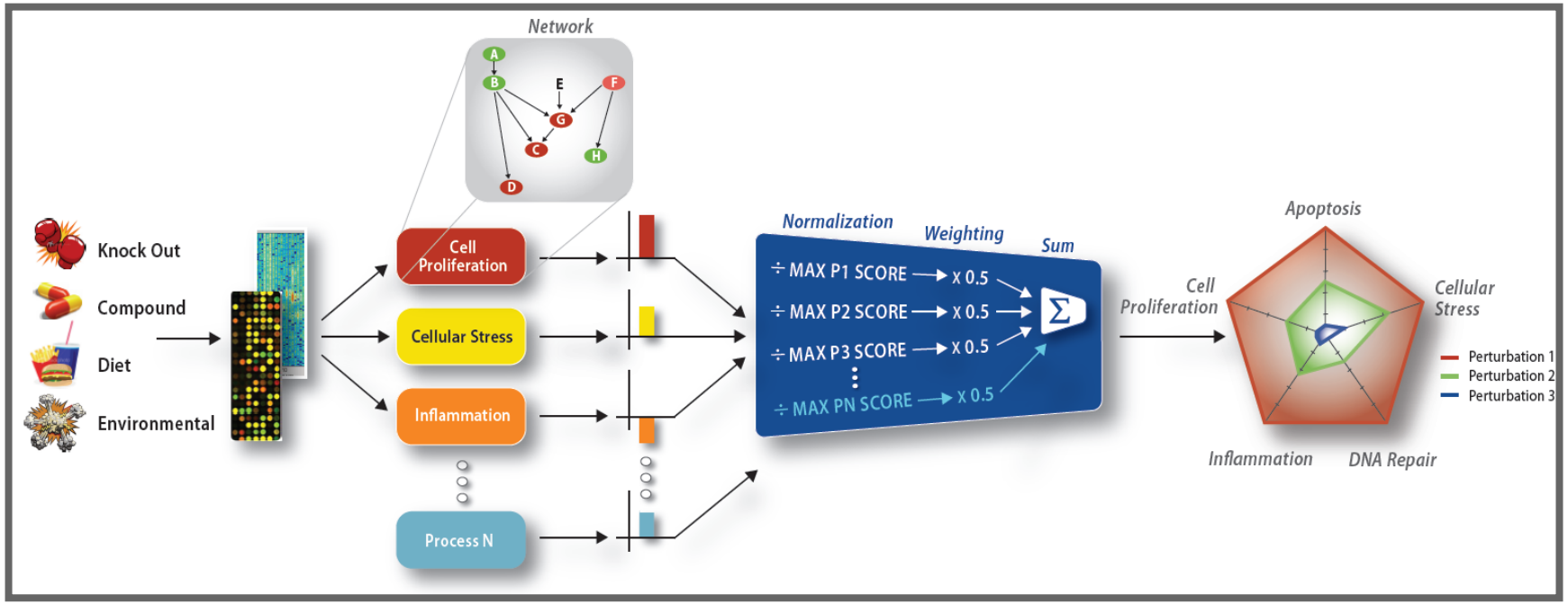
Scientific Question

Are gene expression changes in blood of humans and rodents sufficiently informative to define a unique rule or classifier to extract a specific gene signature predictive of smoking exposure (Smoker vs Non-current smoker) or cessation (Former smoker vs Never smoker) in both species?

OPMENT

Step 4: Compute Network Perturbation Amplitude

Quantifying Network Perturbations



Exposure → **Systems Biology Data** → **Network Perturbation Amplitude Scores** → **Biological Impact Factor (BIF)**

Biological Network Model — **Score Aggregation**

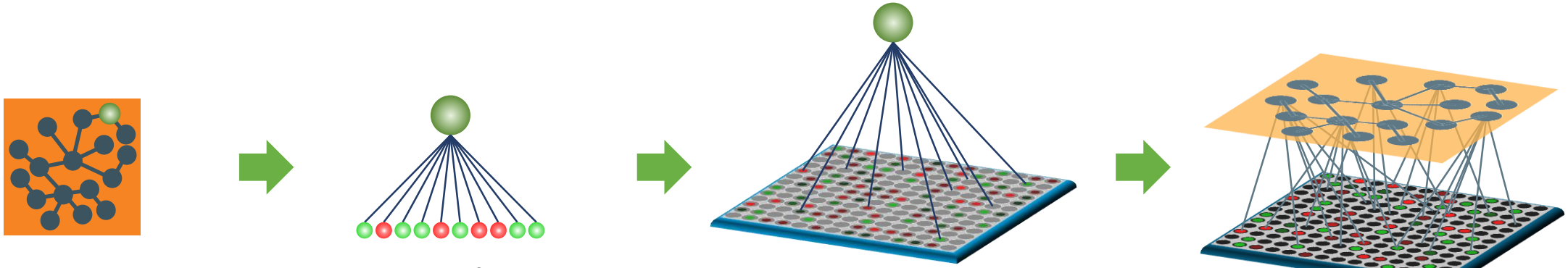
3 publications available upon request

PMI RESEARCH & DEVELOPMENT

Backward Reasoning connects Systems Response Profiles and Network Models

- Backward Reasoning views the measured Systems Response Profiles (=mRNA differential expressions) as the consequences of the perturbations of their upstream regulating mechanisms (=nodes of the biological network models)

- Backward Reasoning uses the concept of “transcriptional footprints” to causally relate one mechanism node and its downstream regulated mRNAs.



extract the “transcriptional footprints” from the Selventa Knowledgebase

assign the corresponding gene differential expression values

ready to calculate network perturbations!

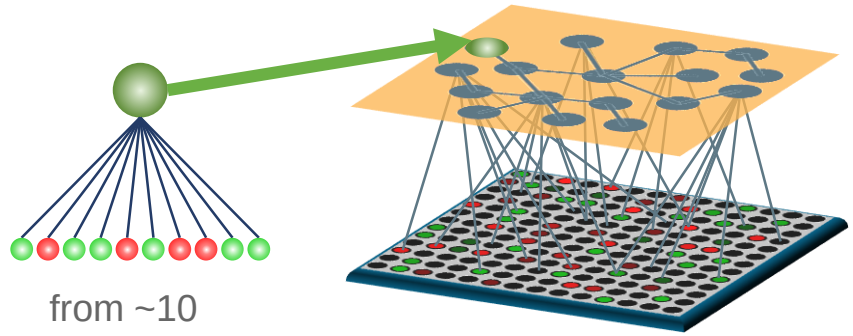
Reverse causal reasoning: applying qualitative causal knowledge to the interpretation of high-throughput data, Catlett NL *et al*., BMC Bioinformatics 2014

PMI RESEARCH & DEVELOPMENT

Aggregation Algorithm to calculate Node-level Perturbations

- Nodes-level perturbations are obtained by summing the values of the footprints differential expressions modulated by the sign∈{-1,+1} of the connecting edges.

- Associated statistics need to be determined to assess the significance of the obtained node-level perturbation.



from ~10
to ~1000 genes
per node

~100 nodes
(per network)

~1000s genes
(per network)

$$Strength = \frac{1}{N}\sum_{i=1}^{N} s_i \cdot \beta_i$$

$$\text{Confidence intervals } (\alpha=0.05): Strength \pm t_{Df}^{\alpha/2} \cdot Sd_{Strength},\ Df \approx \frac{\left(\sum_{i=1}^{n} k_i s_i^2\right)^2}{\sum_{i=1}^{n} \frac{(k_i s_i^2)^2}{\nu_i}}$$

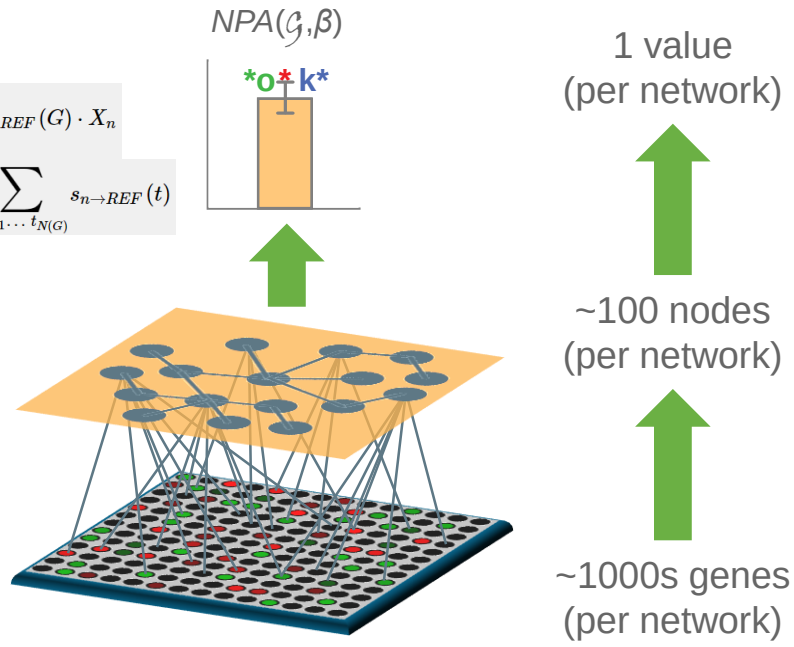
Assessment of network perturbation amplitudes by applying high-throughput data to causal biological networks, Martin F *et al*., BMC Syst Biol. 2012

Aggregation Algorithms to calculate Network-level Perturbations

- Most challenging step… (requires graph-theoretical concepts and properties)
- *SST* (sampling spanning trees) algorithm: sums the node-level perturbations modulated by suitable weights∈[-1,+1] (or ∈{+1,-1} in case of balanced/causally consistent/non-frustrated networks).
- *topoNPA* algorithm: the network-level perturbations are defined as a bilinear form on the node-level perturbations using the sign-inverted Laplacian matrix. Associated significance statistics are calculated by permuting the edges.

$$X_G = \sum_{n \in \text{Nodes}} S_{n \to REF}(G) \cdot X_n$$

$$S_{n \to REF}(G) = \frac{1}{N(G)} \sum_{t = t_1 \ldots t_{N(G)}} s_{n \to REF}(t)$$



$$TopoNPA(\mathscr{G}, \beta) = \frac{1}{C} \sum_{\substack{x \to y \\ \text{s.t} x,y \notin V_0}} (f(x) + sign(x \to y) f(y))^2 \cdot w(x,y)$$

An algorithm for score aggregation over causal biological networks based on random walk sampling, Vasilyev DM *et al*., BMC Res Notes 2014

Quantification of biological network perturbations for mechanistic insight and diagnostics using two-layer causal models, Martin F *et al*., BMC Bioinformatics 2014

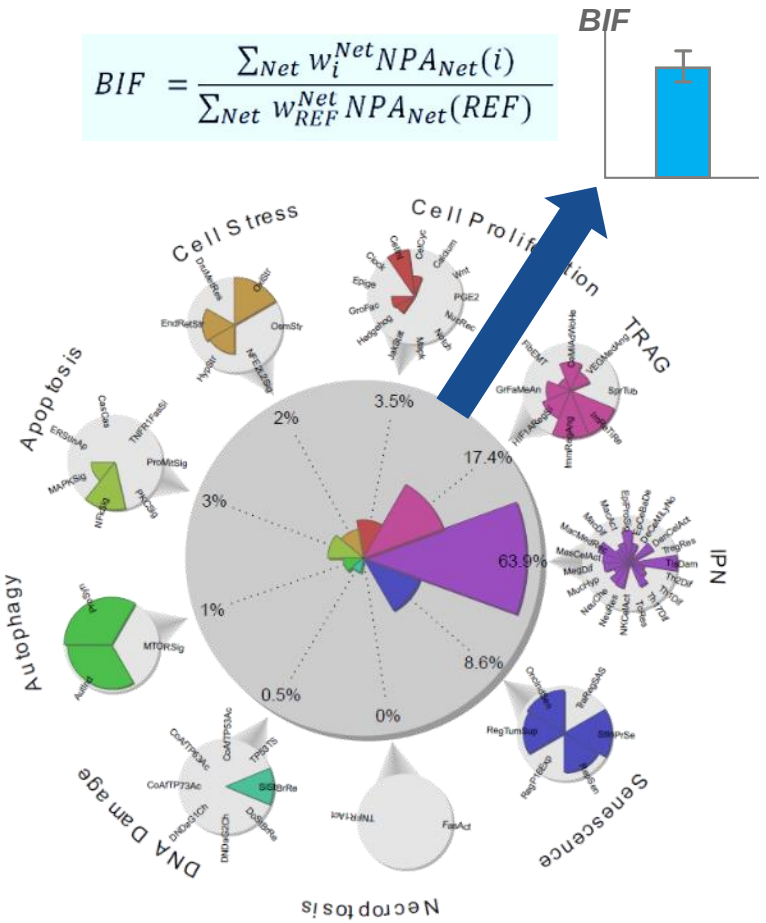
Step 5: Compute Biological Impact Factor

Aggregation Algorithm to calculate Biological Impact over sets of Networks

- The goal is to compute a systems-wide pan-mechanistic measure of the biological impact of the exposure to an active substance (named BIF=Biological Impact Factor).

- Network-level perturbation amplitudes are summed (and normalized) and displayed in “snowflake” plots reflecting the hierarchical structure of the network models.

$$BIF = \frac{\sum_{Net} w_i^{Net} NPA_{Net}(i)}{\sum_{Net} w_{REF}^{Net} NPA_{Net}(REF)}$$



1 value
(for the full collection of ~50 networks)

~10 values
(for each group of ~5 networks)

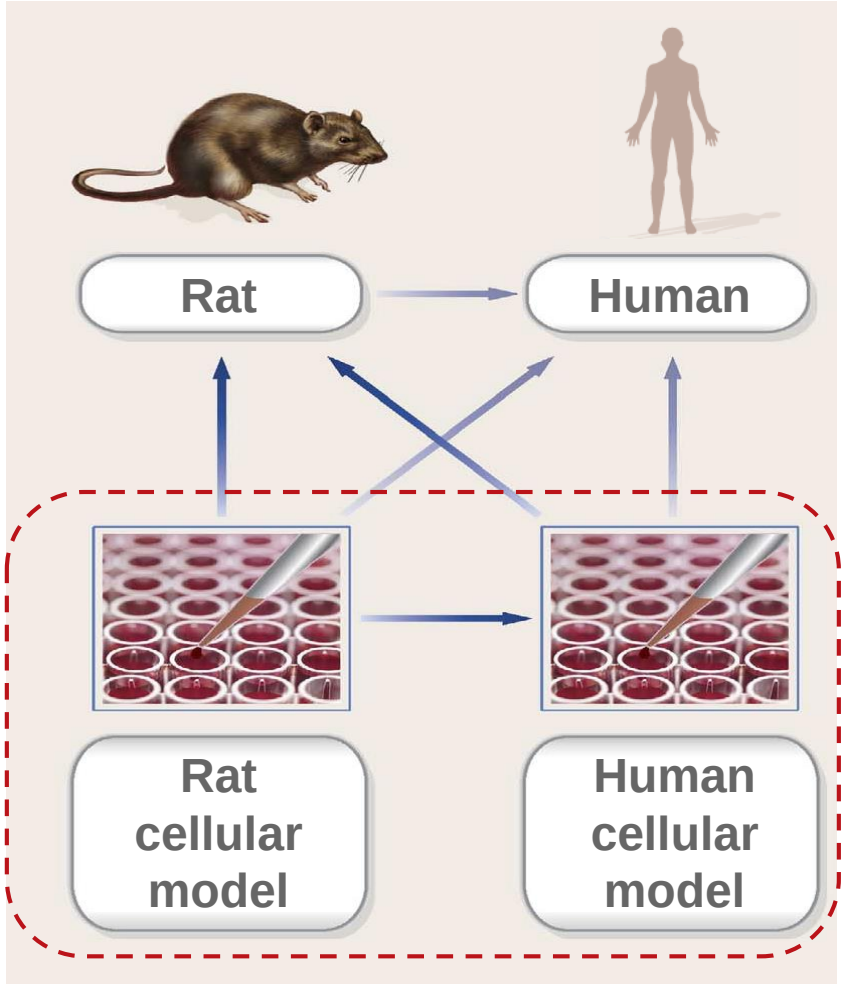
~50 values
(per network)

Quantitative assessment of biological impact using transcriptomic data and mechanistic network models, Thomson TM *et al*., Toxicol Appl Pharmacol. 2013

PMI RESEARCH & DEVELOPMENT

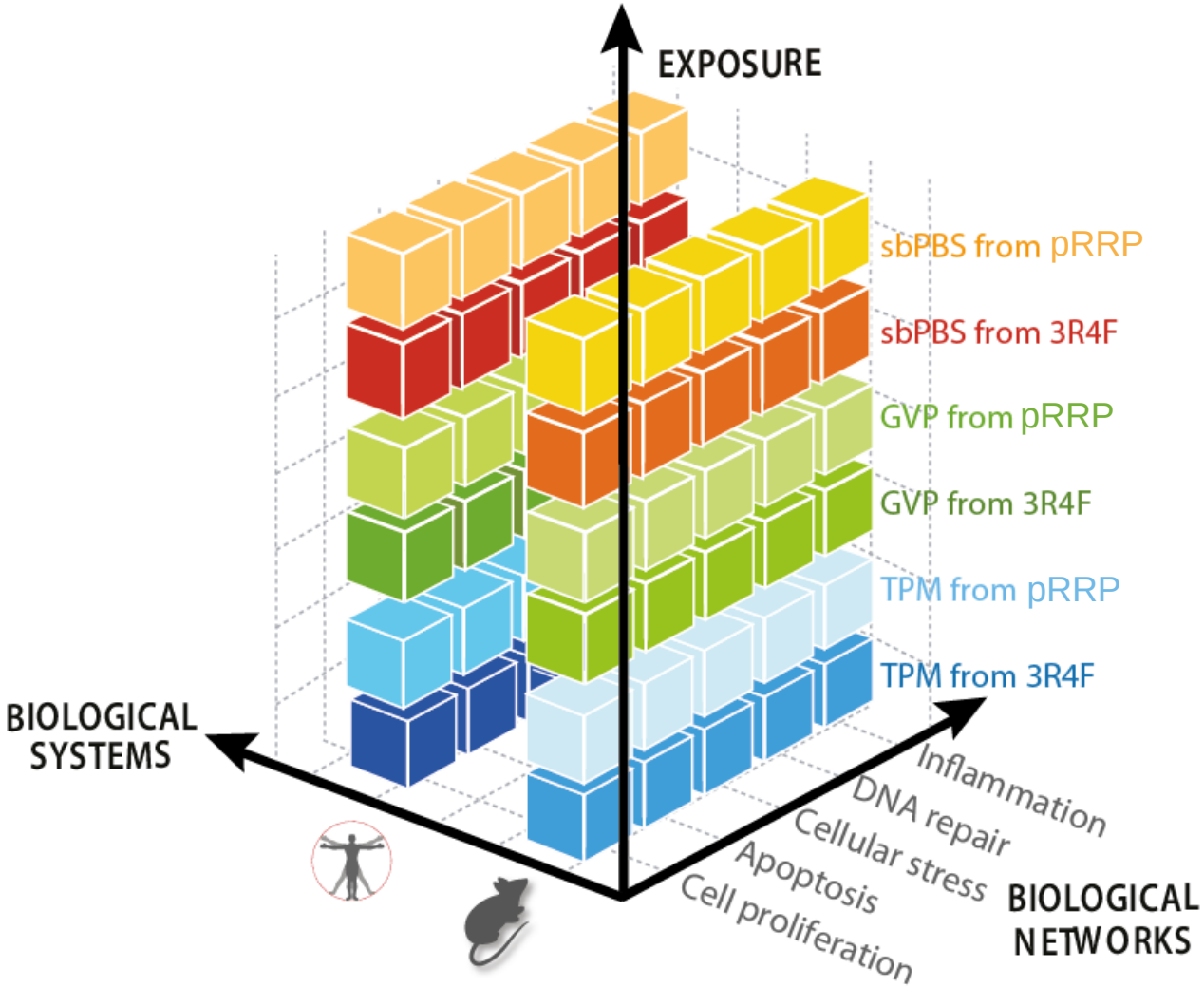
CASE STUDIES

Case study 1: *in vitro*



Apply Systems Toxicology *in vitro* to compare the biological impacts of a reference combustible cigarette (3R4F) and a prototypic RRP on primary human and rat lung epithelial cells.

Case study 1: Study Design



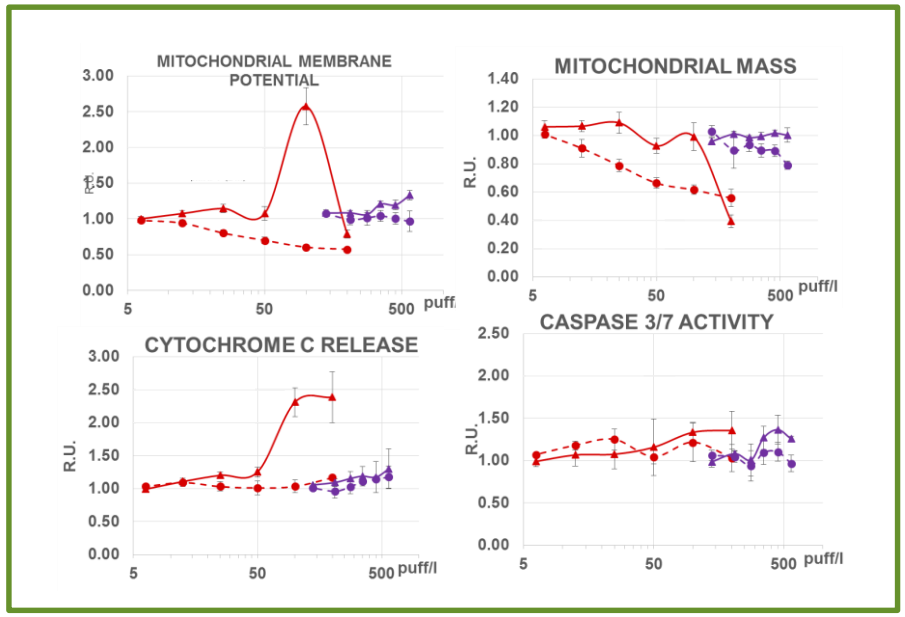
The cubic experimental design space recapitulates:

- the two biological systems (Normal Human Bronchial Epithelial cells (NHBE) and Normal Rat Bronchial Epithelial (NRBE) cells),
- the exposure regimen (GVP, TPM and sbPBS derived from 3R4F and pRRP smoke)
- the biological networks used to assess the biological impact of the exposure presented within this study.

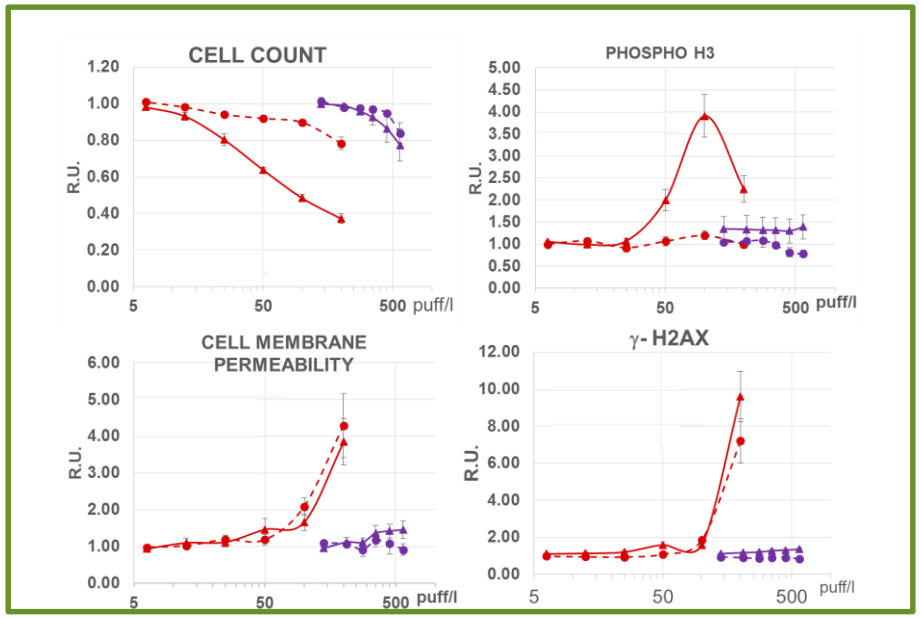
pRRP is prototypic RRP, TPM is total particulate matter, GVP is gas vapor phase, sbPBS is smoke-bubbled PBS

Case study 1: Results of High Content Screening with sbPBS

Apoptosis

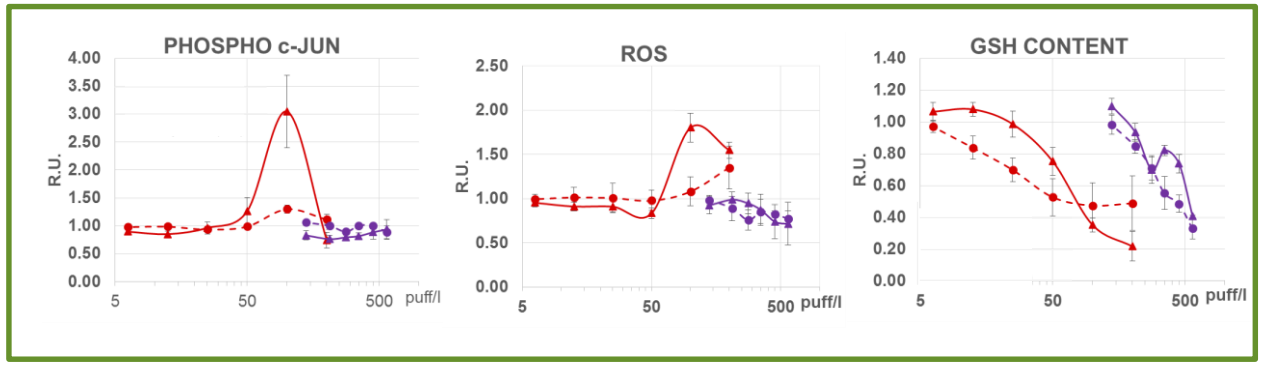


Cell count, Proliferation and Cell Death



3R4F (4h)
3R4F (24h)
pRRP (4h)
pRRP(24h)

Stress Response



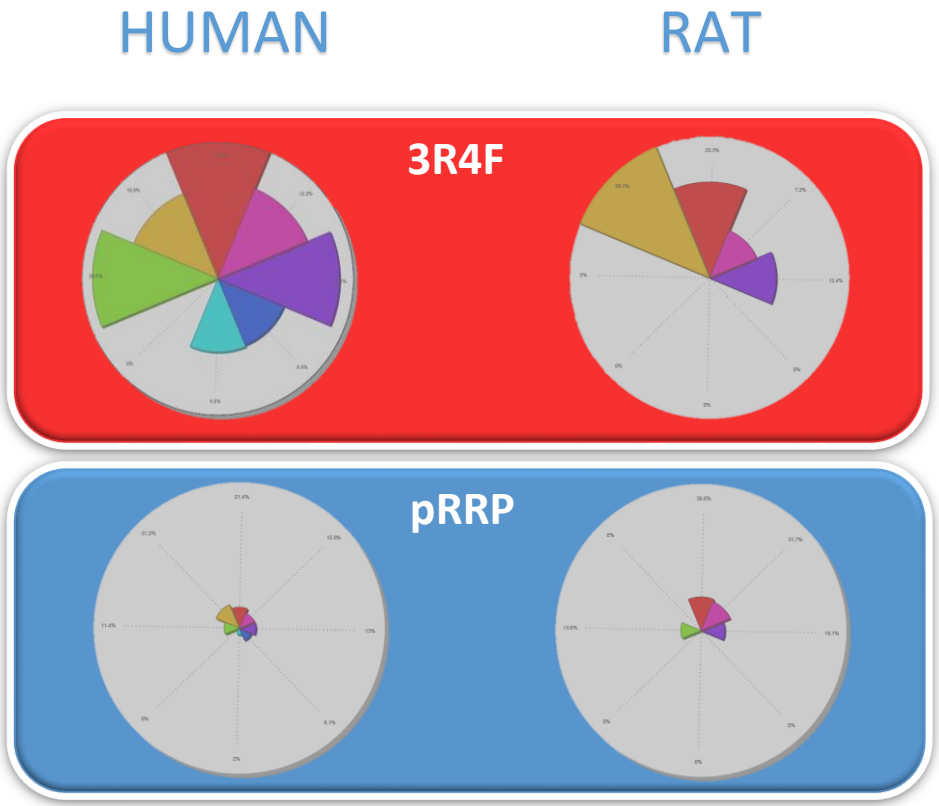
Note: These data alone do not represent a claim of reduced exposure or reduced risk. Combustible Cigarettes is a 3R4F reference cigarette.
pRRP is prototypic RRP, sbPBS is smoke-bubbled PBS

PMI RESEARCH & DEVELOPMENT

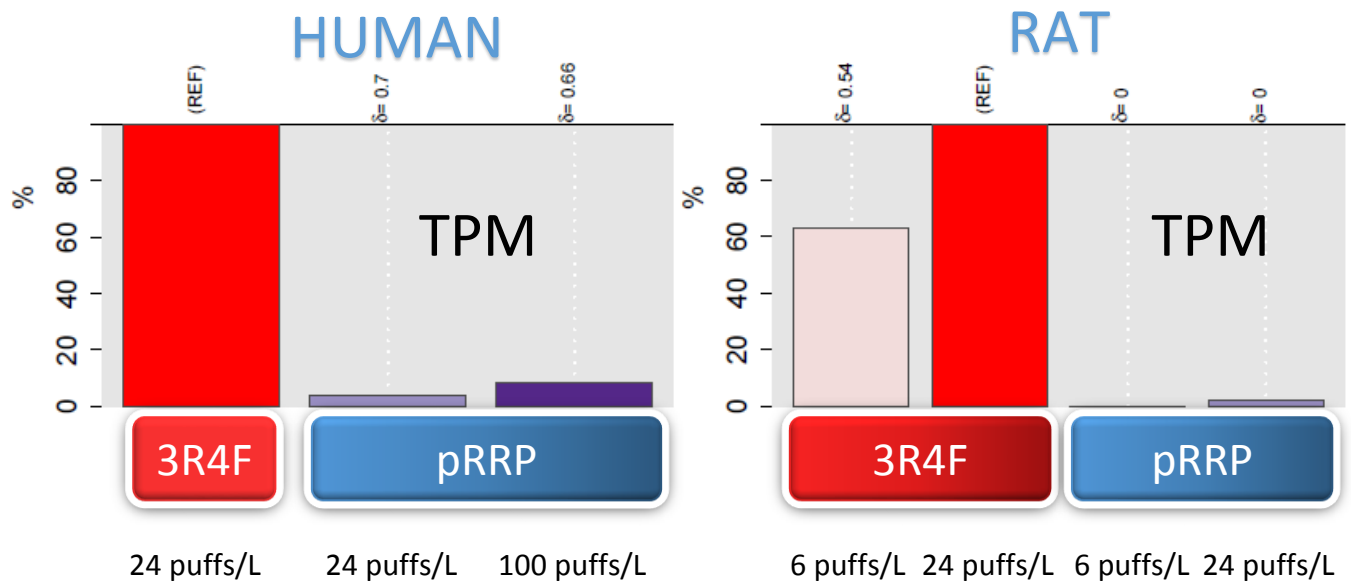
Case study 1: Results of Network Perturbation Amplitudes and Biological Impact Factor calculations

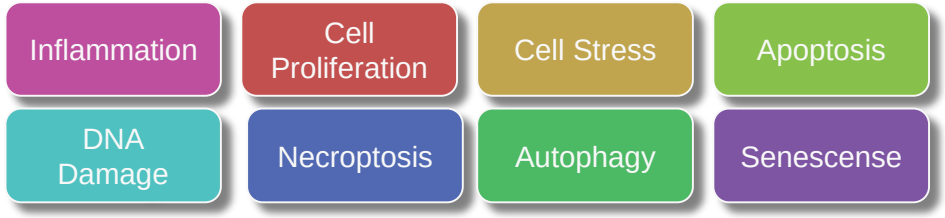
Network Perturbation Amplitudes

Common dose: 24 puffs/L TPM



Relative Biological Impact Factor



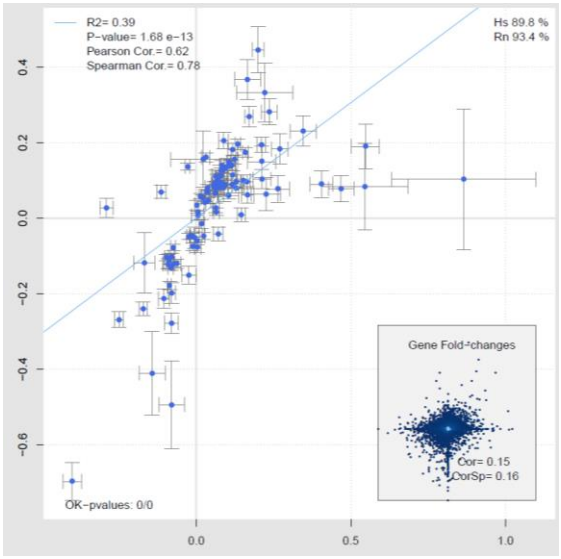


Note: These data alone do not represent a claim of reduced exposure or reduced risk. Combustible Cigarettes is a 3R4F reference cigarette pRRP is prototypic RRP, TPM is total particulate matter.

Case study 1: Results of Comparing Human and Rat Biological Network Perturbations

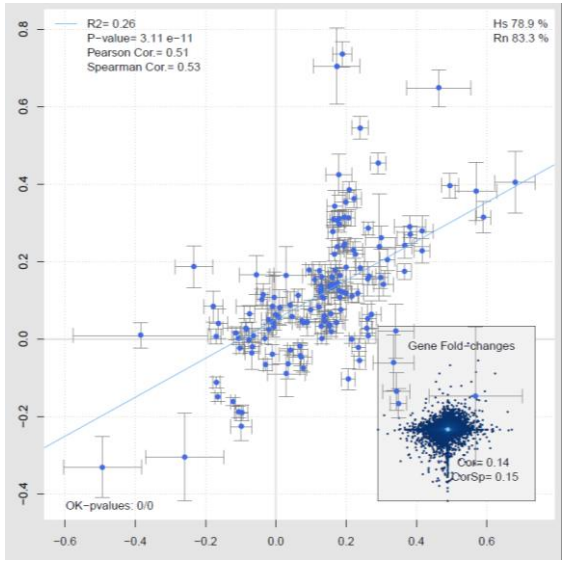
Cell Proliferation

A: Cell Proliferation / Cell Cycle



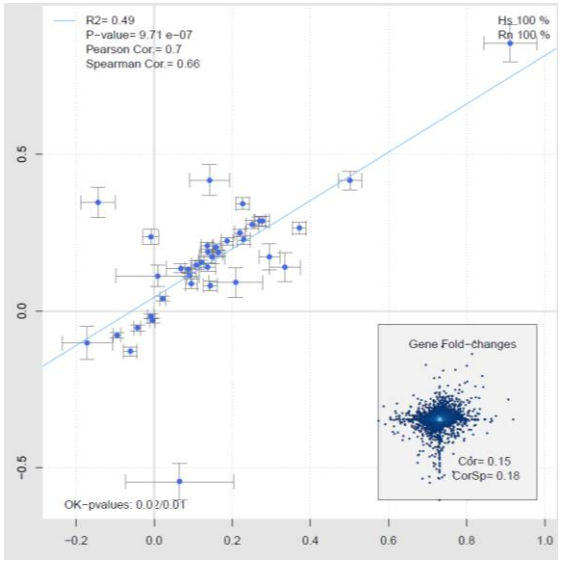
Cell Proliferation

B: Cell Proliferation / Growth Factor



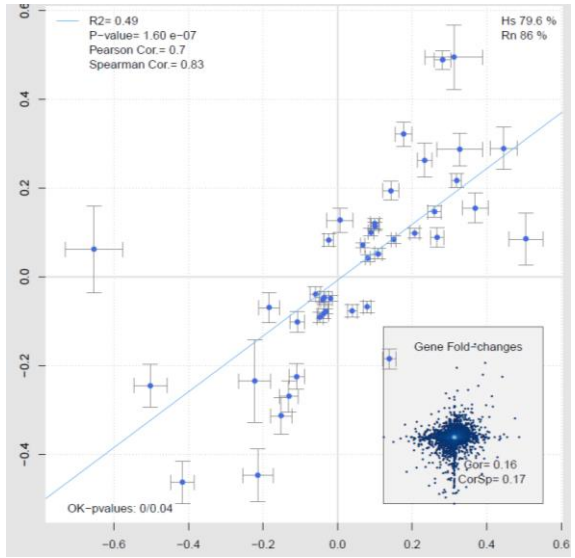
Cell Stress

C: Cell Stress / Xenobiotic Metabolism Response



Cell Stress

D: Cell Stress / NFE2L2 signaling

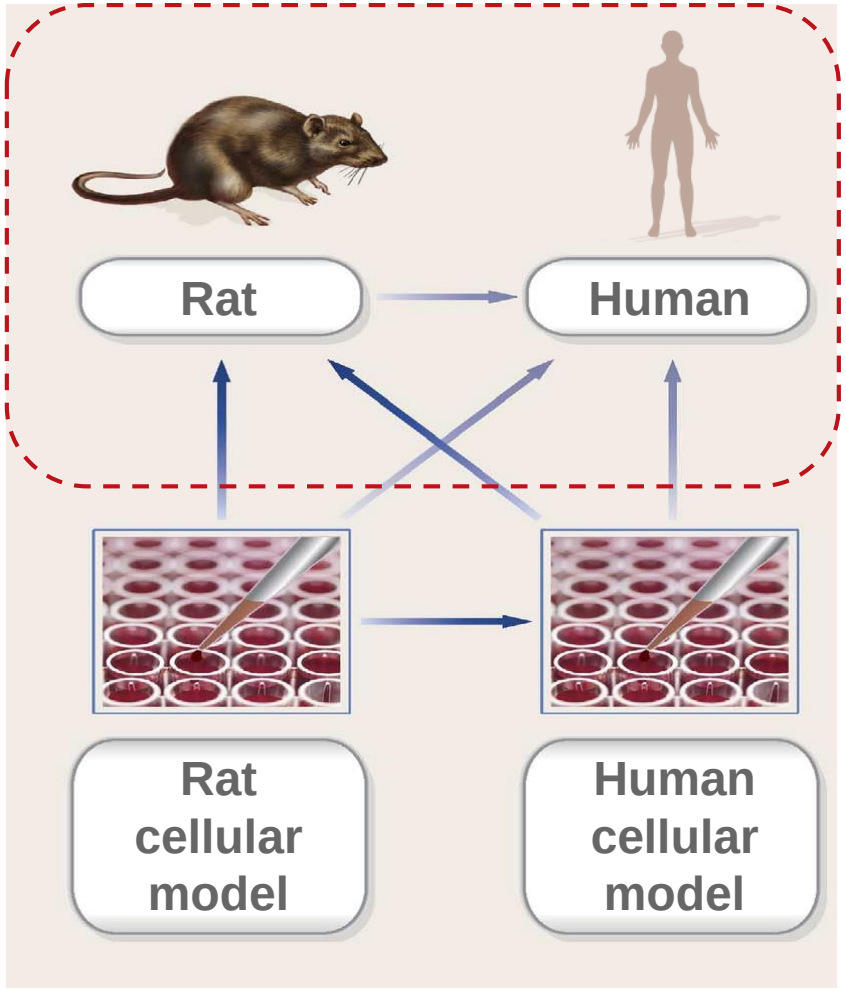


Rat backbone values

Human backbone values

PMI RESEARCH & DEVELOPMENT

Case study 2: *in vivo*

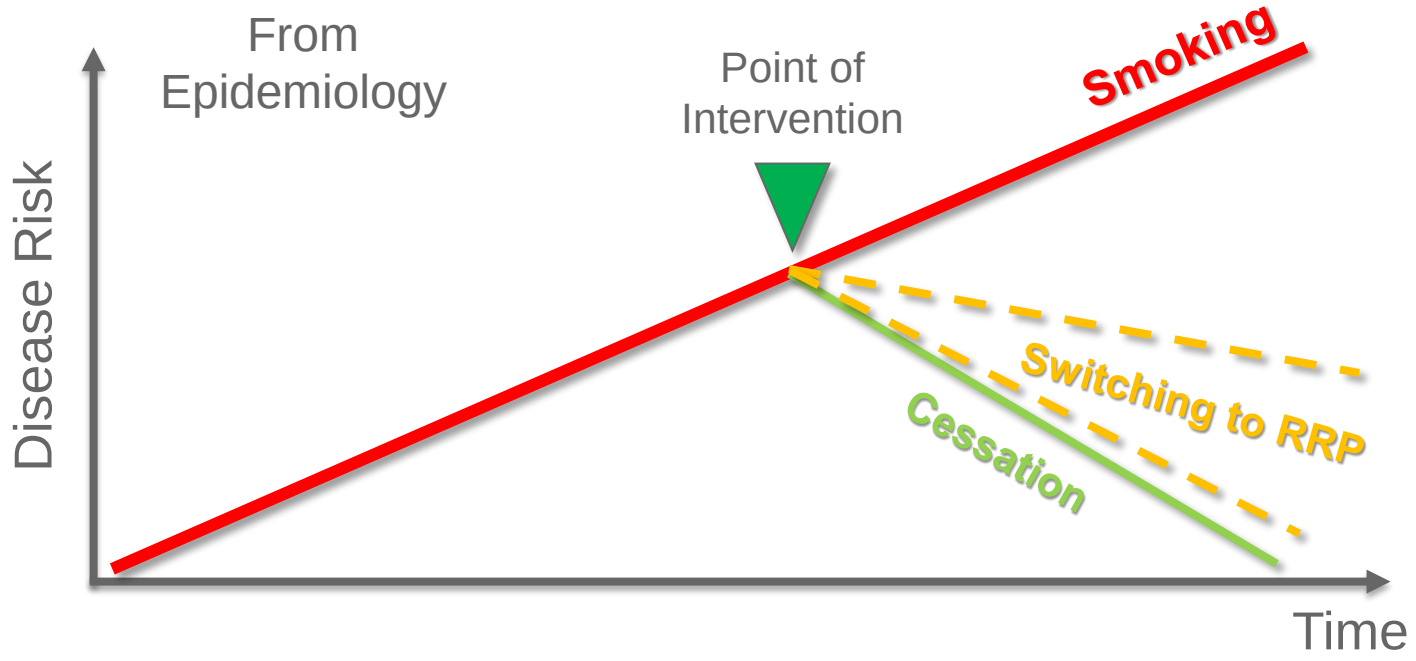


Apply Systems Toxicology *in vivo* to compare the biological impacts of a reference combustible cigarette (3R4F) and a prototypic RRP on the development of emphysema in C57Bl/6 mice.

Phillips B, et al. (2015) A 7-month cigarette smoke inhalation study in C57BL/6 mice demonstrates reduced lung inflammation and emphysema following smoking cessation or aerosol exposure from a prototypic modified risk tobacco product. **Food and Chemical Toxicology**, 80:328-345. (PMID: 25843363).

Case study 2: Cessation = the "Gold Standard"

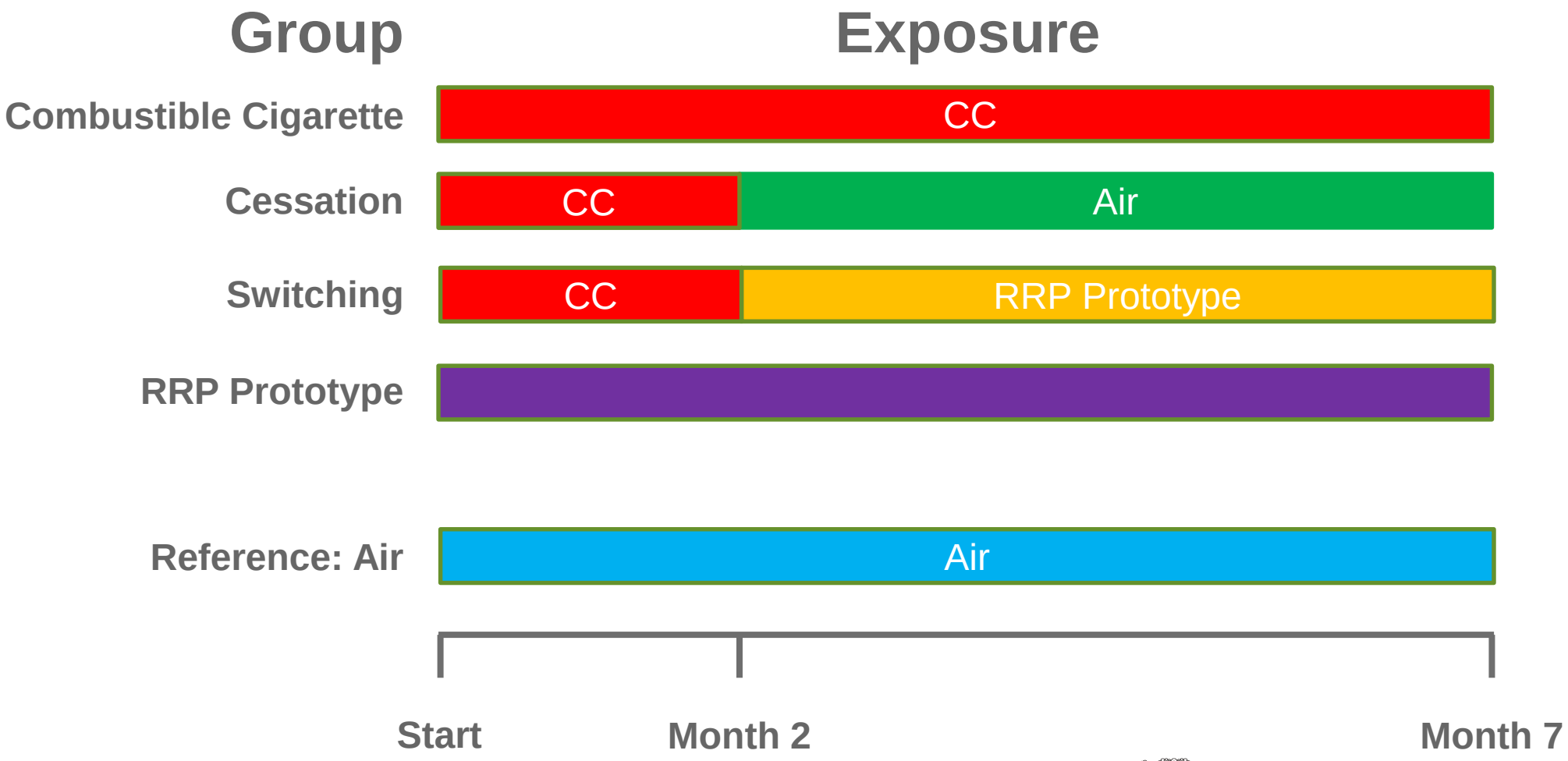
- We apply the US Institute of Medicine's "gold standard " for assessing risk reduction: comparability to cessation



Note: Reduced-Risk Products ("RRPs") is the term we use to refer to products that have the potential to reduce individual risk and population harm. The descriptions in the chart are for illustrative purposes only

Source: IOM (Institute of Medicine), 2012, Scientific Standards for Studies on Modified Risk Tobacco Products. Washington, DC: The National Academies Press

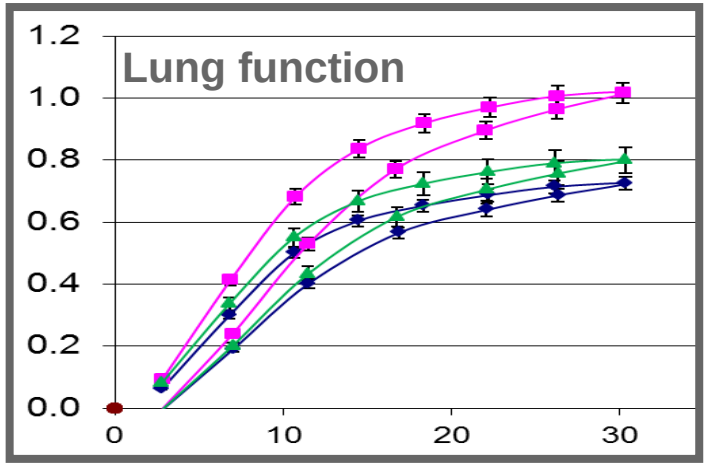
Case study 2: Design

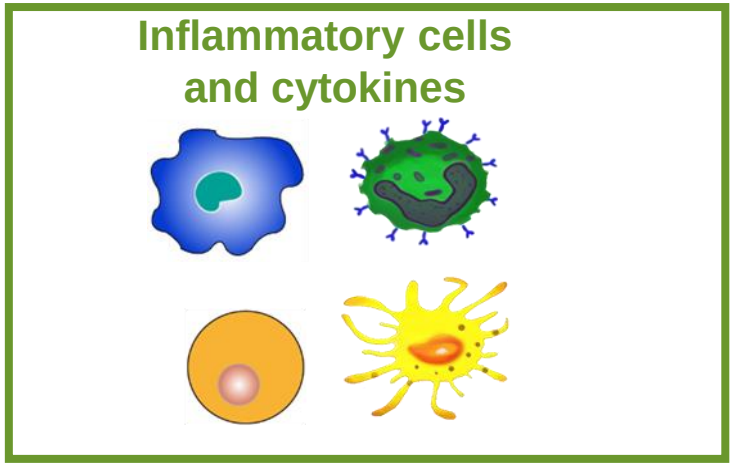


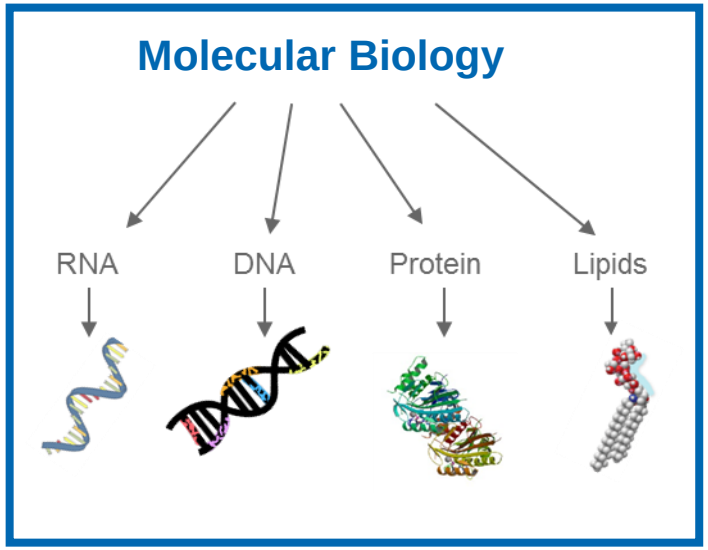
Note: CC is a 3R4F reference combustible cigarette

Source: PMI Research and Development

Case study 2: Multi-Parameter Assessment of Emphysema Progression (Endpoint analysis)

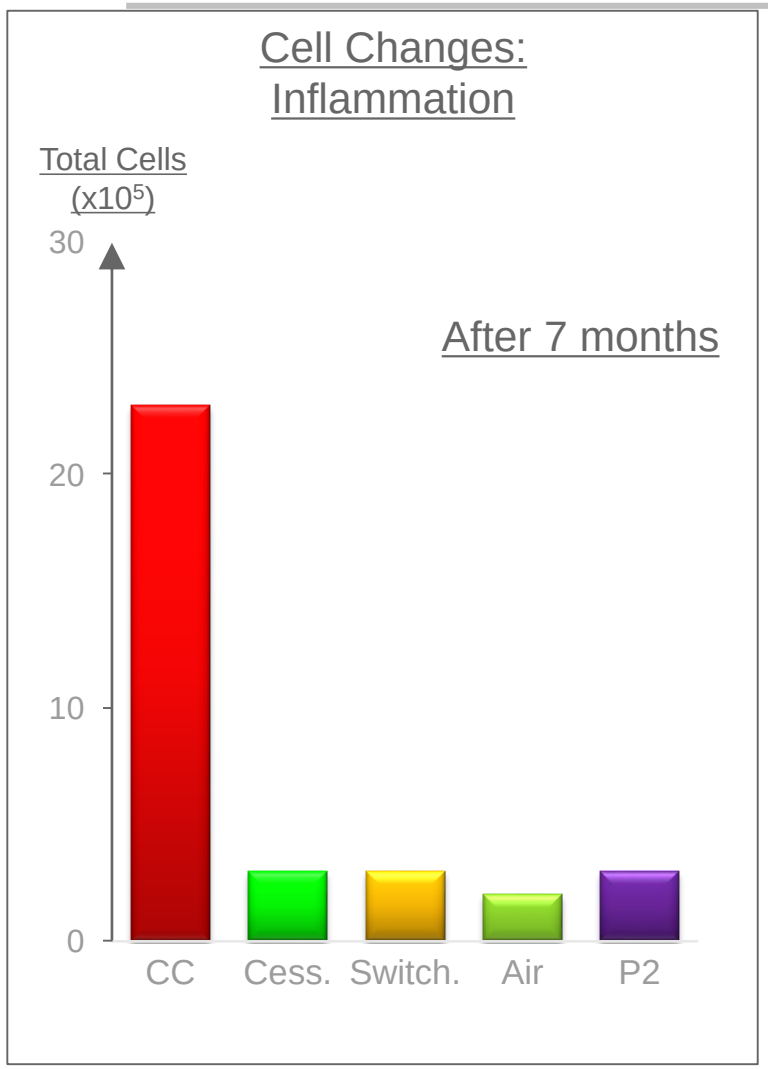


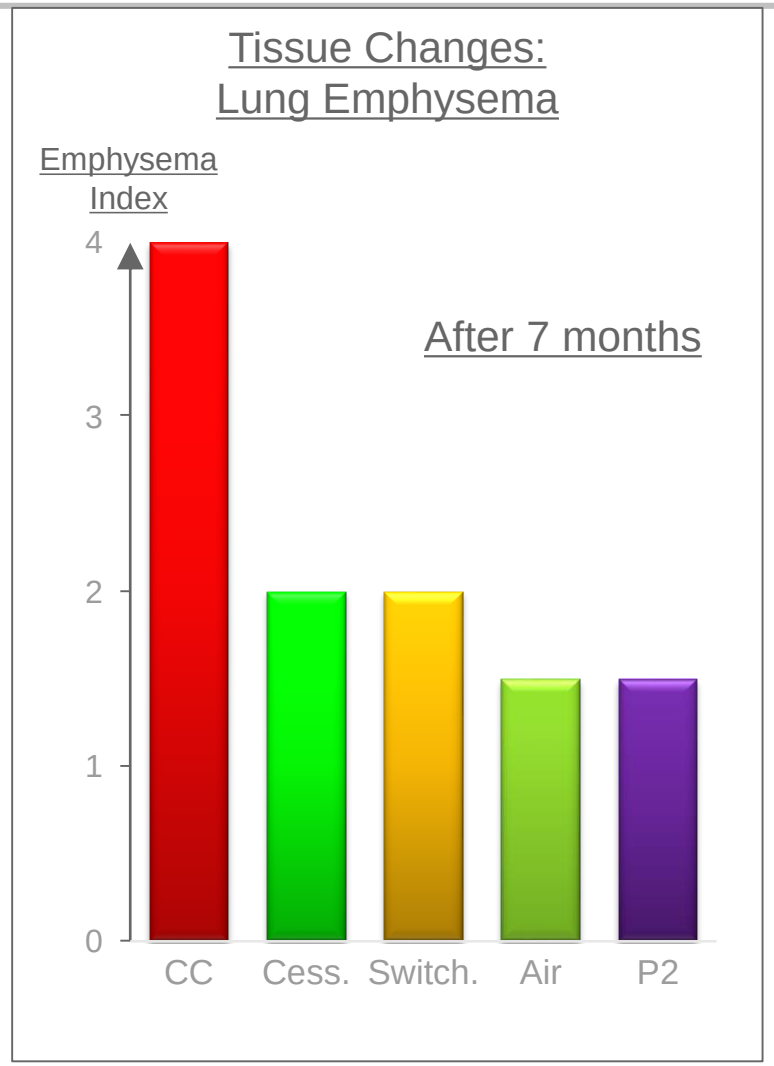


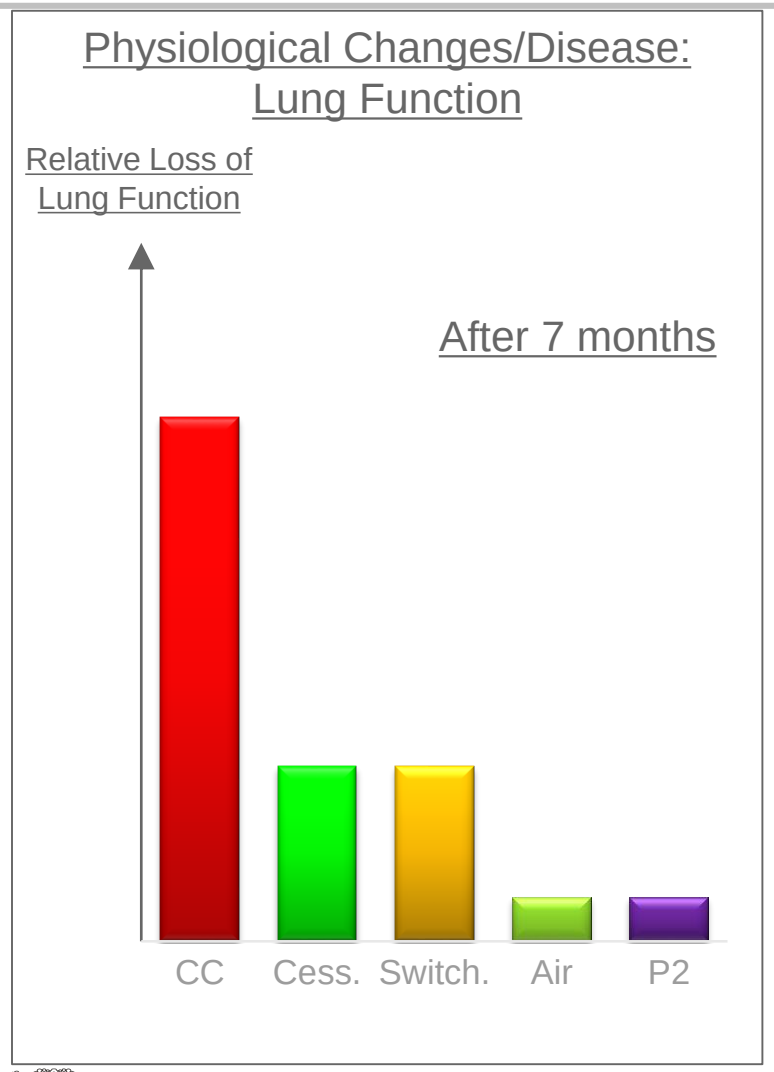


PMI RESEARCH & DEVELOPMENT

Case study 2: Results of cellular, histological and physiological endpoints



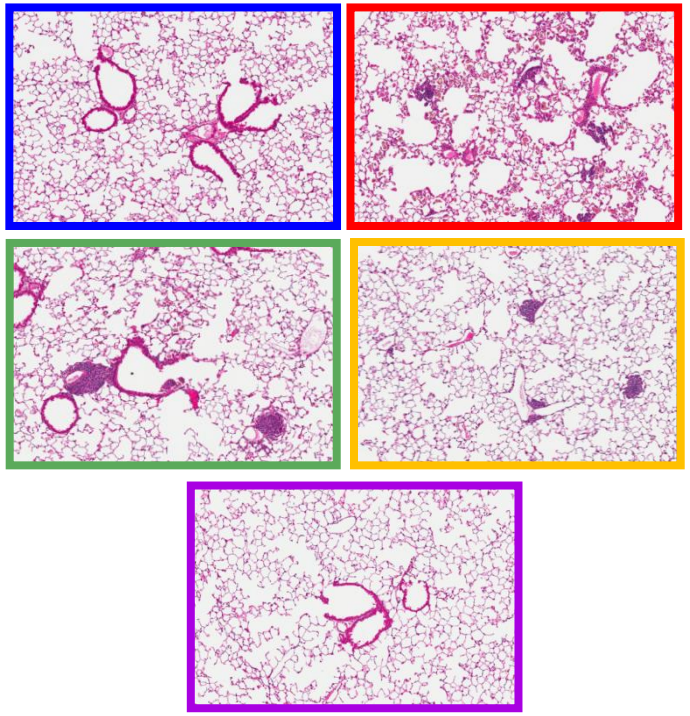




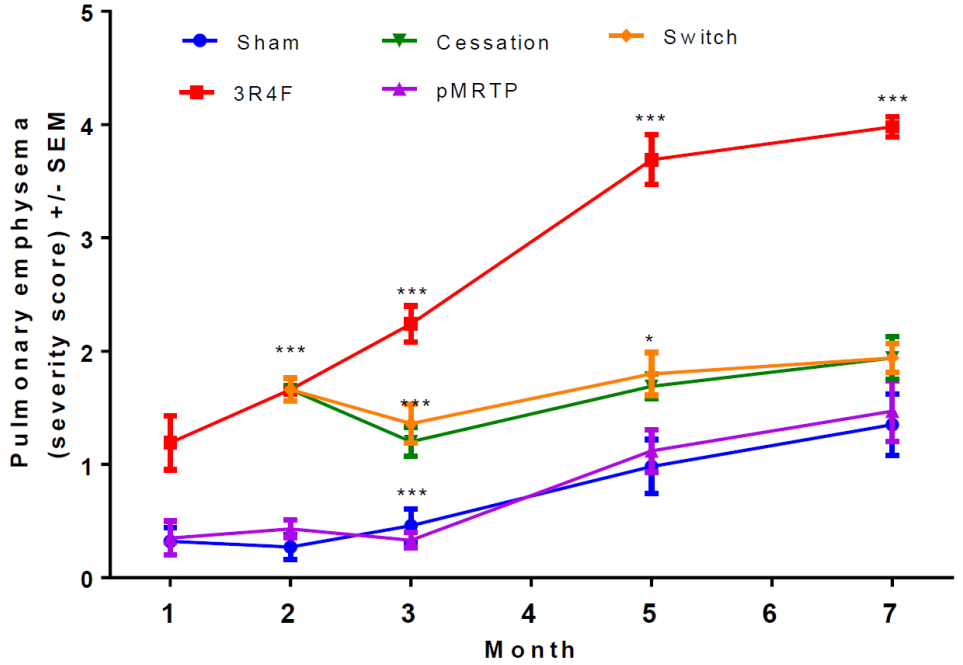
Note: These data alone do not represent a claim of reduced exposure or reduced risk. CC is a 3R4F reference cigarette, Cess. is Cessation, Switch. is Switching and P2 is RRP prototype

Case study 2: Emphysema Development Results

Lung histopathology at 7 Months



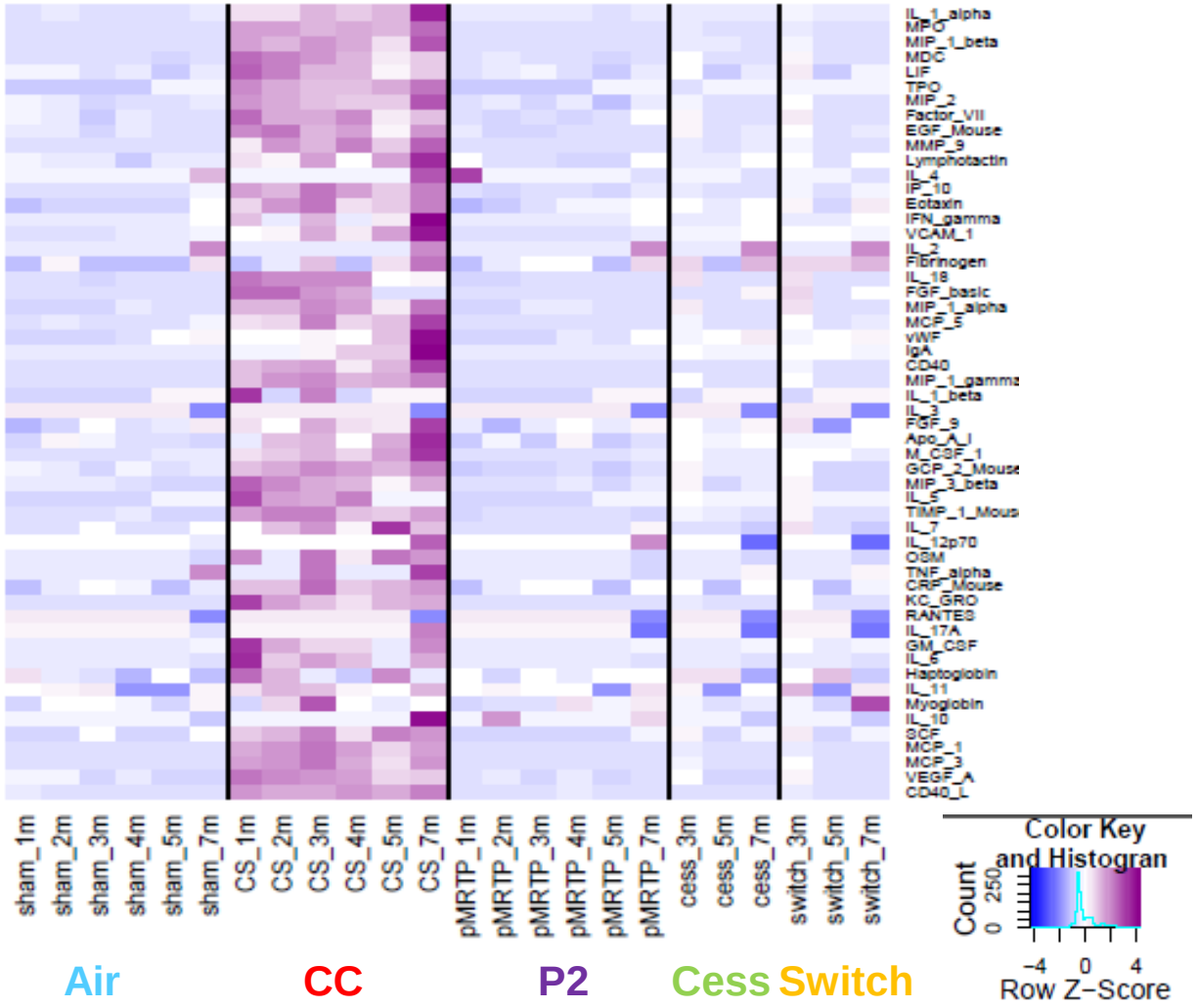
Severity of pulmonary emphysema at 7 months

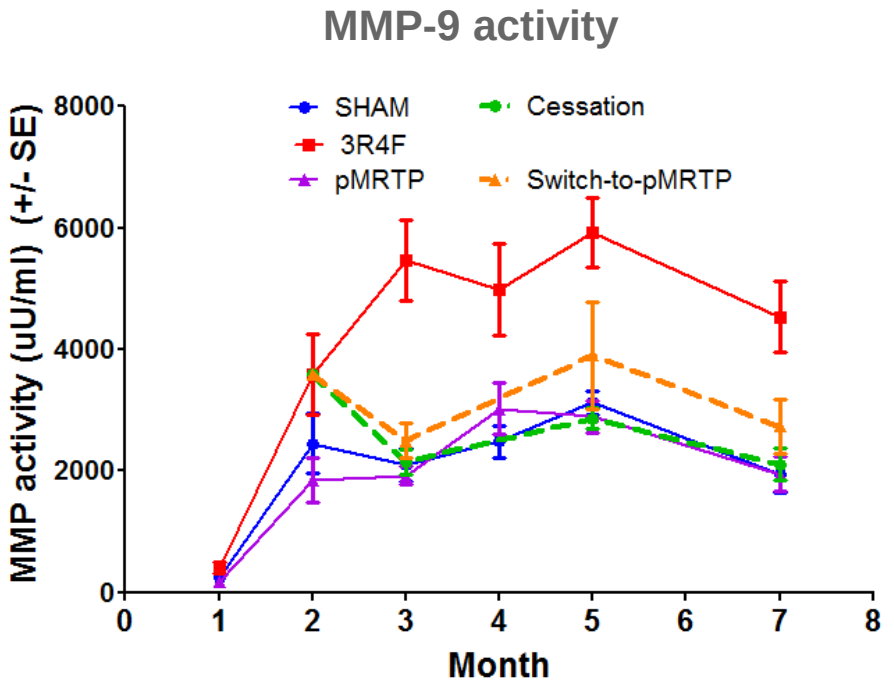


Emphysema is pathologically defined as an abnormal permanent enlargement of air spaces distal to the terminal bronchioles, accompanied by the destruction of alveolar walls and without obvious fibrosis.

PMI RESEARCH & DEVELOPMENT

Case study 2: Results of Pulmonary Inflammation Mediators in Bronchoalveolar lavage fluid (BALF)



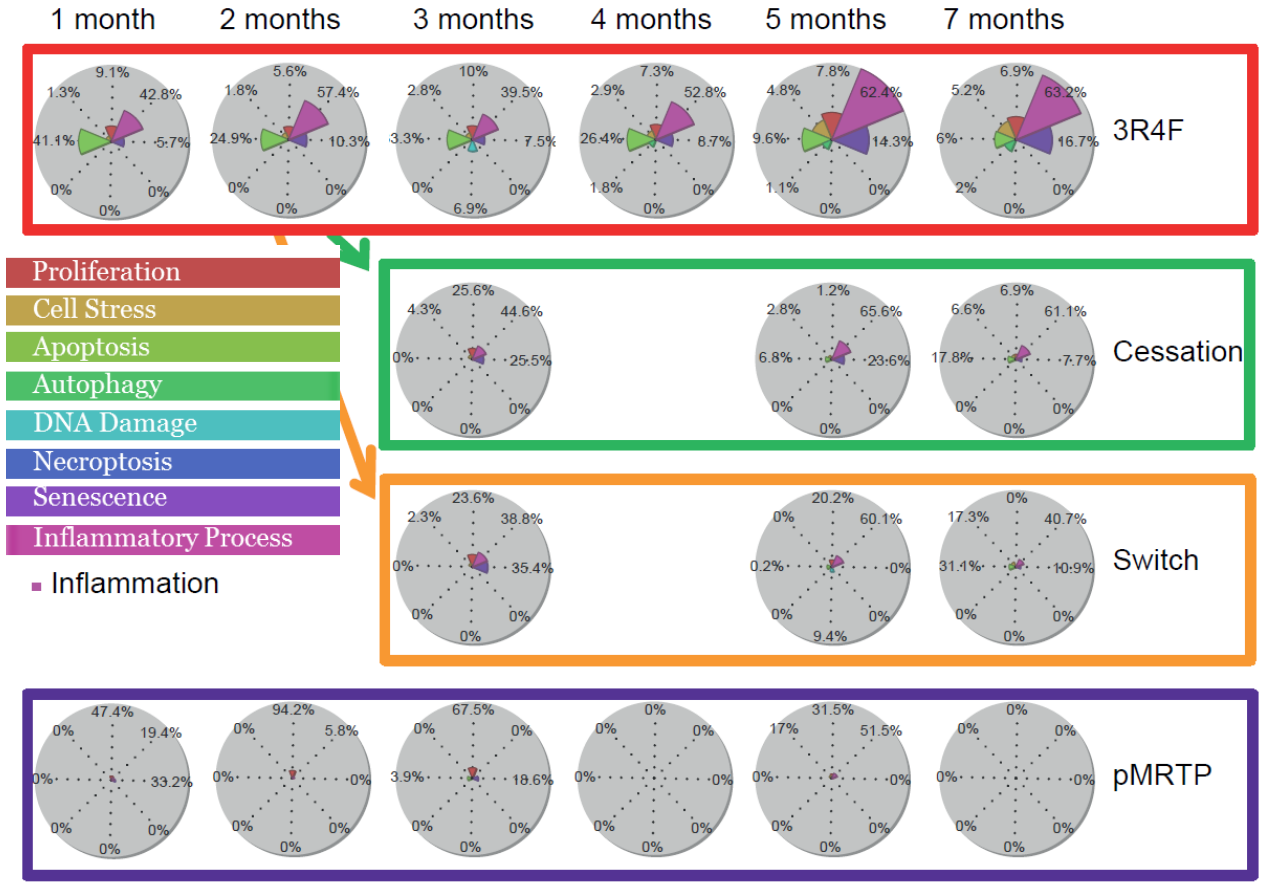


Gelatinolytic activity of proteinase

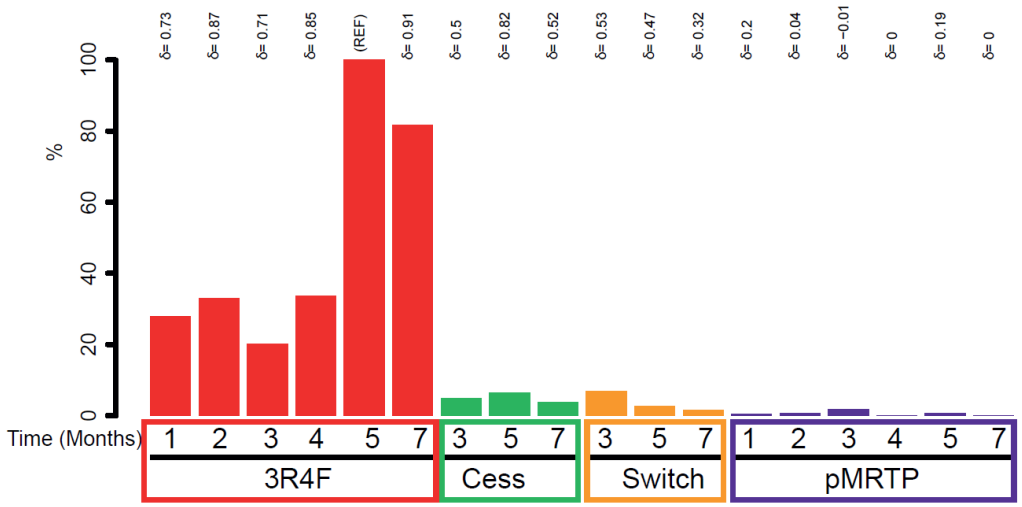
Note: These data alone do not represent a claim of reduced exposure or reduced risk. CC is a 3R4F reference cigarette, Cess. is Cessation, Switch. is Switching and P2 is RRP prototype

PMI RESEARCH & DEVELOPMENT

Case study 2: Results of Network Perturbation Amplitudes and Biological Impact Factors derived from Transcriptomics Data Analysis of Lung Tissue



Perturbation Amplitudes for the main Biological Networks affected by Cigarette Smoke

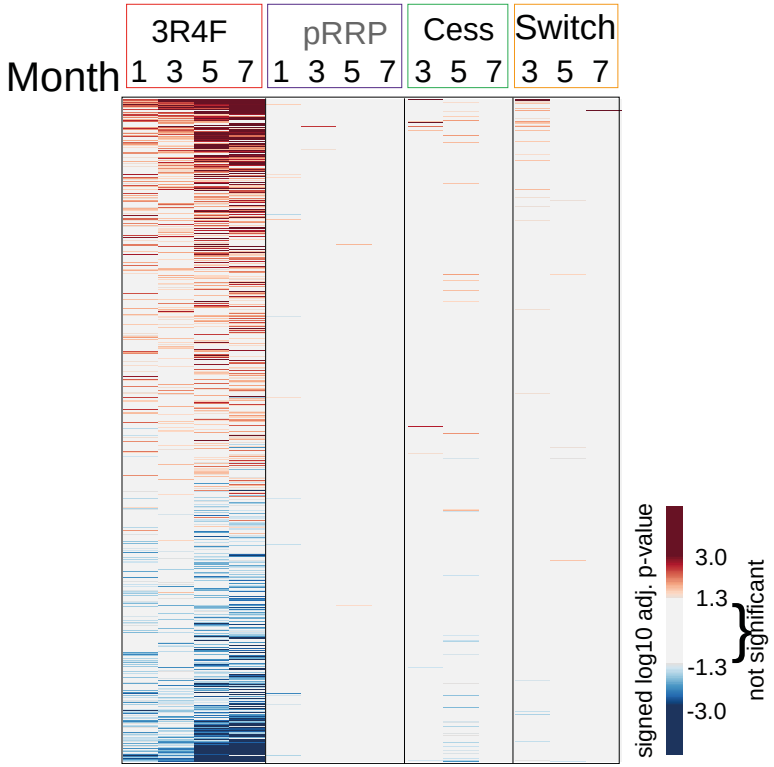


Network model-based computational analysis of the biological impact factor (BIF)

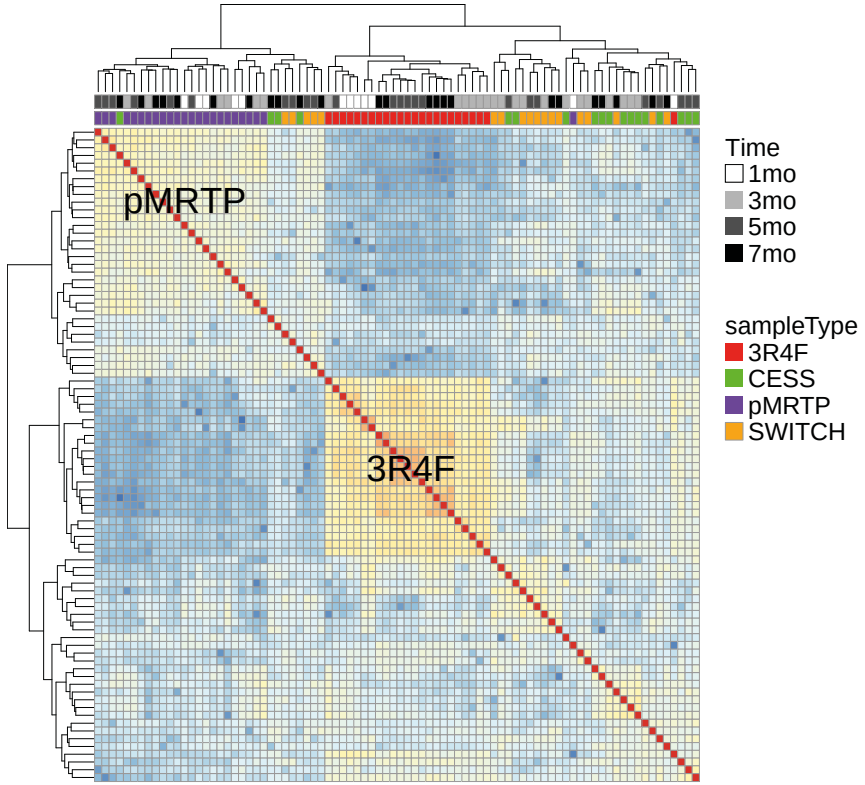
PMI RESEARCH & DEVELOPMENT

Case study 2: Results of Differential Proteomics of the Lung Tissue

Quantitative iTRAQ-based analysis treatment vs. sham exposed

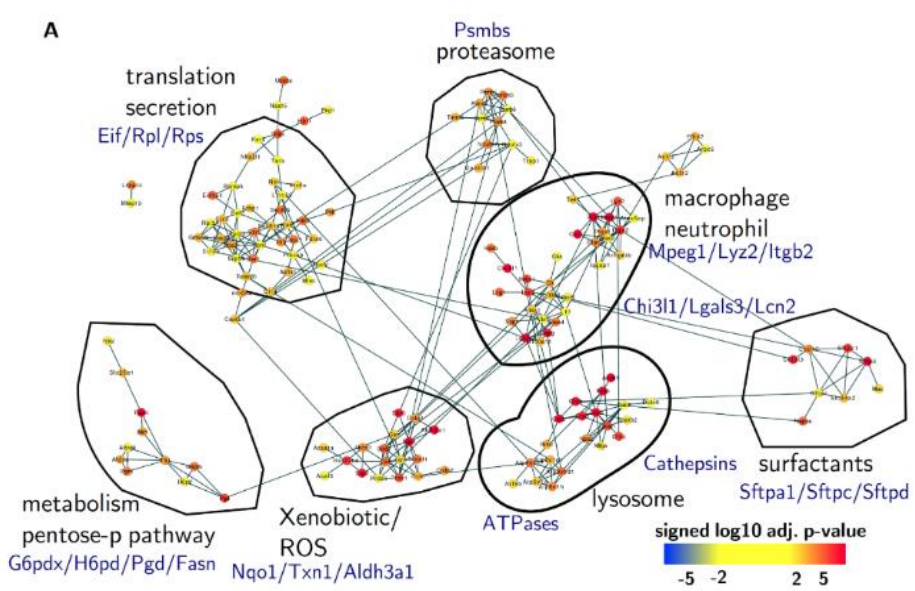


Unsupervised clustering of lung proteome alterations separates the types of exposure

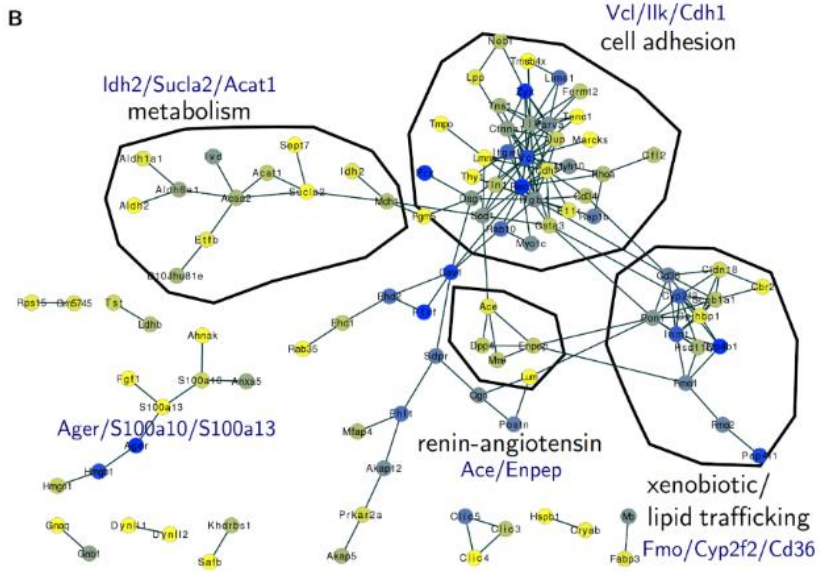


PMI RESEARCH & DEVELOPMENT

Case study 2: Results of Protein Interaction Networks in Mouse Lung tissue



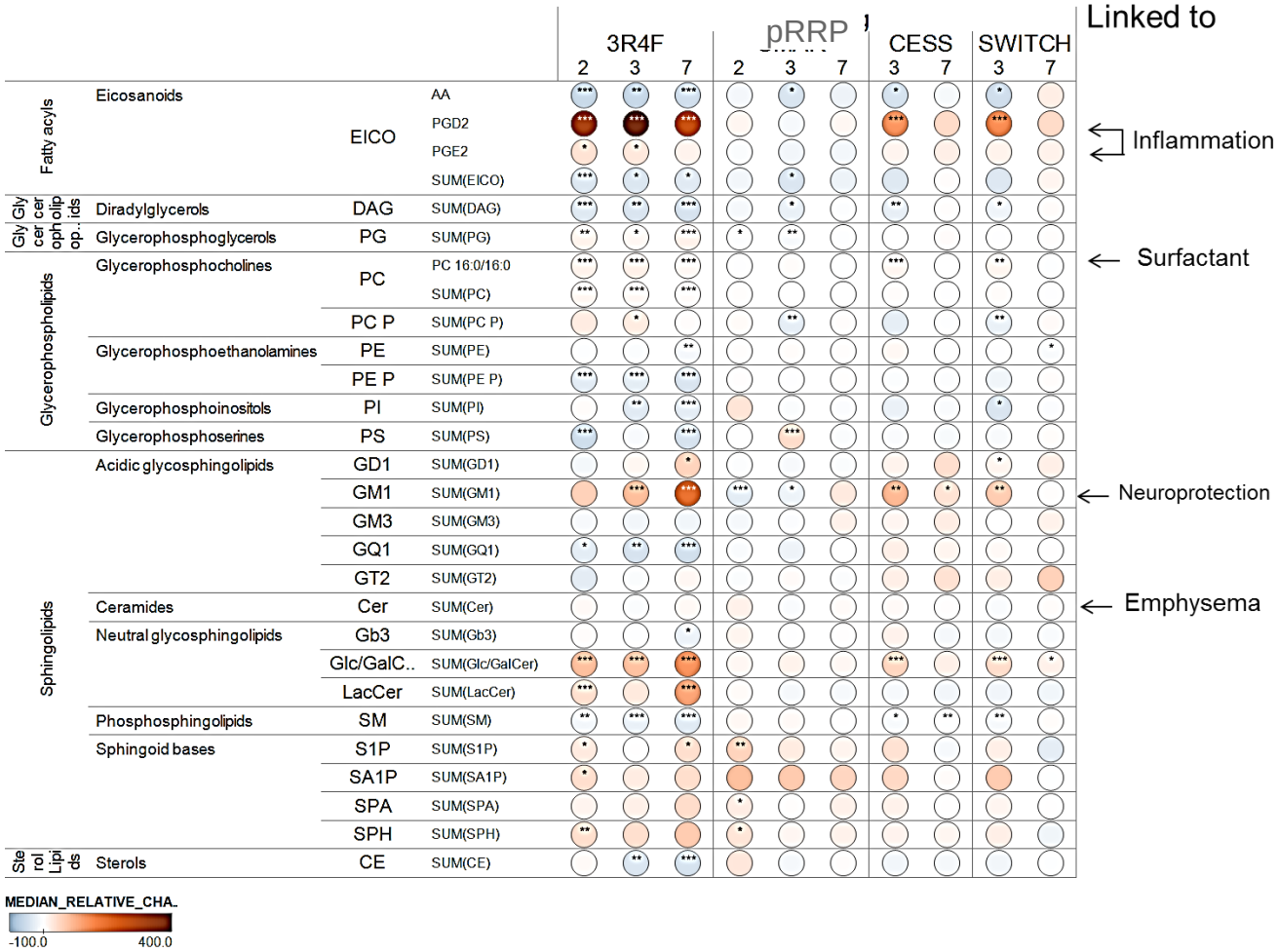
up-regulated proteins



down-regulated proteins

- the xenobiotic and macrophage/neutrophil clusters correspond to the observed network perturbations identified using transcriptomics data
- other functional clusters include surfactants and cellular energy metabolism therefore complementing our systems biology approach.

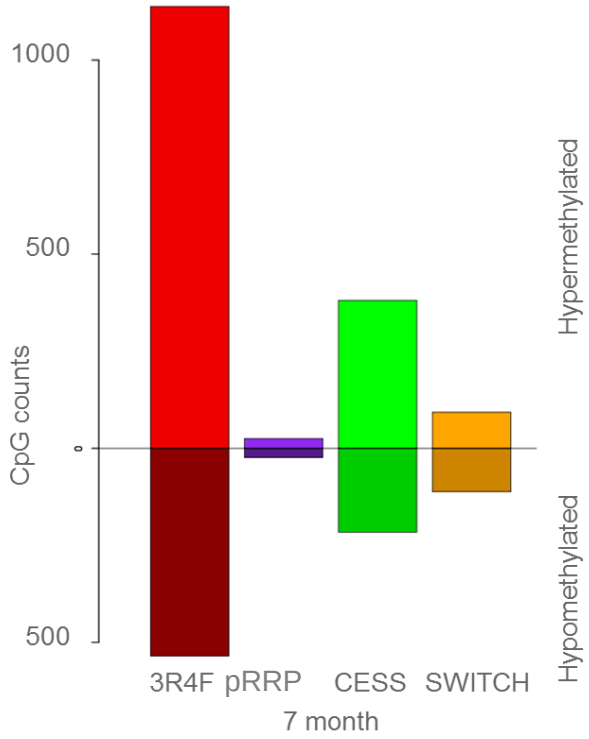
Case study 2: Results of Differential Lipidomics of the Lung Tissue

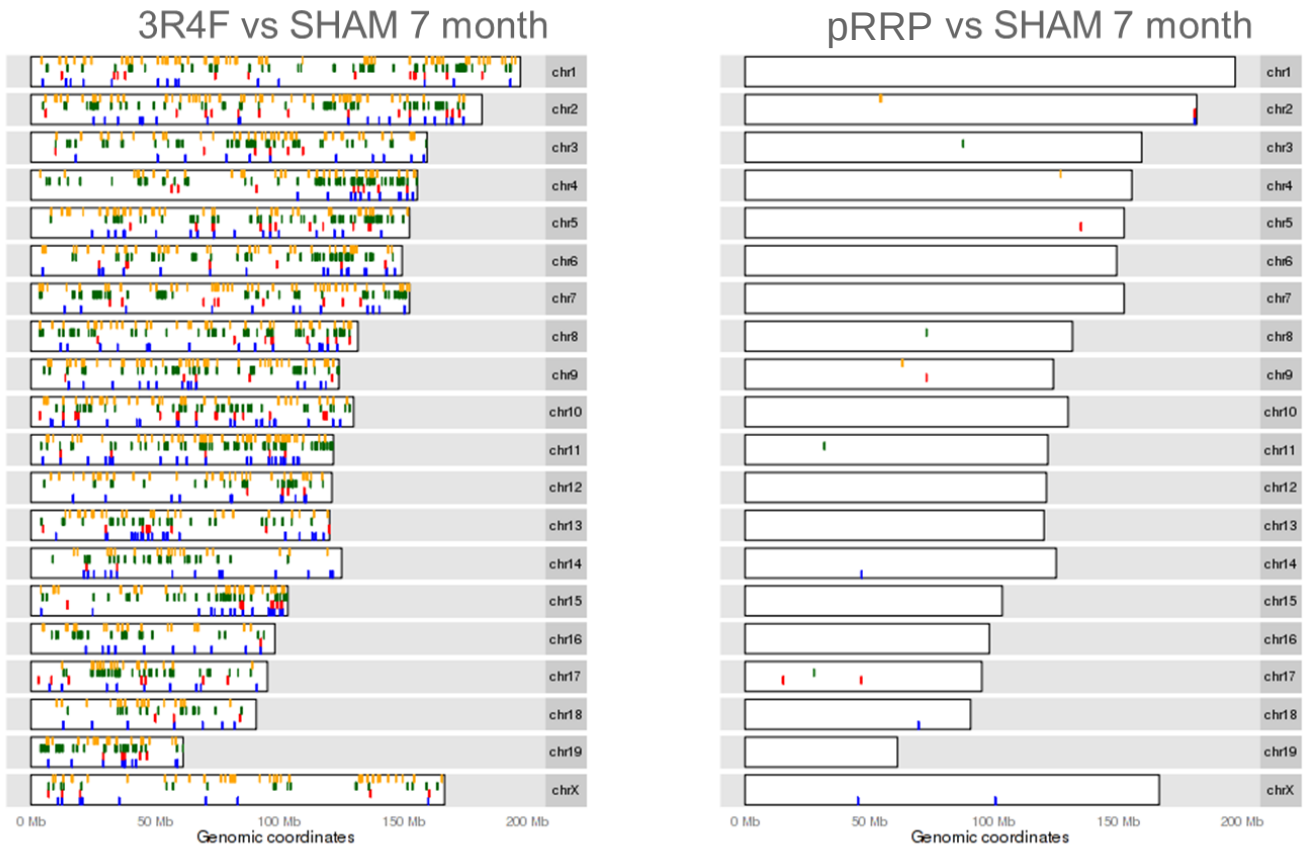


CS exposure impacts the levels of distinct long and very long chain ceramides, surfactant-characteristic lipids and inflammation-related eicosanoids in COPD mouse model

PMI RESEARCH & DEVELOPMENT

Case study 2 results: CpG Methylation in Lung Tissue





Hypermethylated CpG Upregulated gene

Hypomethylated CpG Downregulated gene

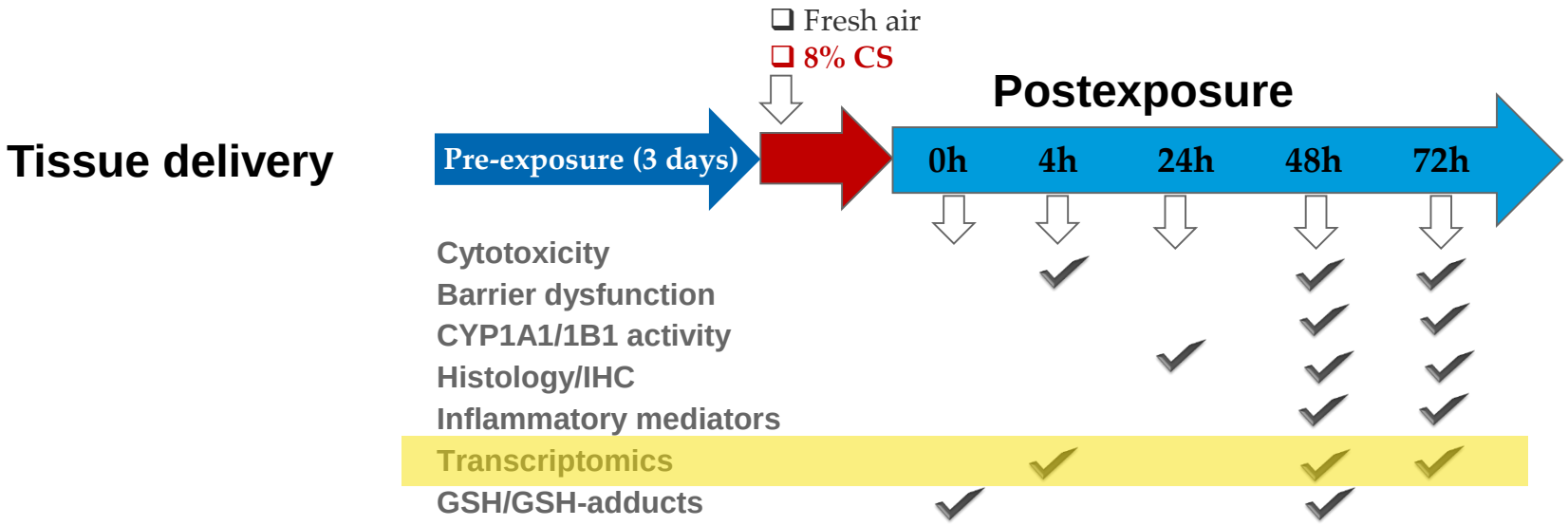
Overall DNA methylation

Correlation of DNA methylation and gene expression at different loci

PMI RESEARCH & DEVELOPMENT

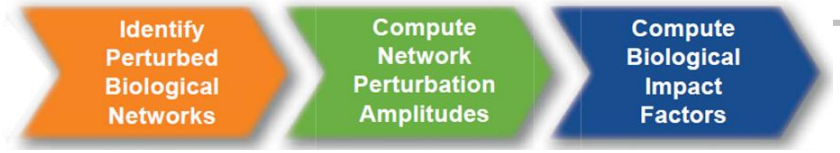
Case study 3: Exposure of Organotypic Nasal Tissue Cultures to Cigarette Smoke (8% CS)

Design Experiment for Data Production → Compute Systems Reponse Profiles

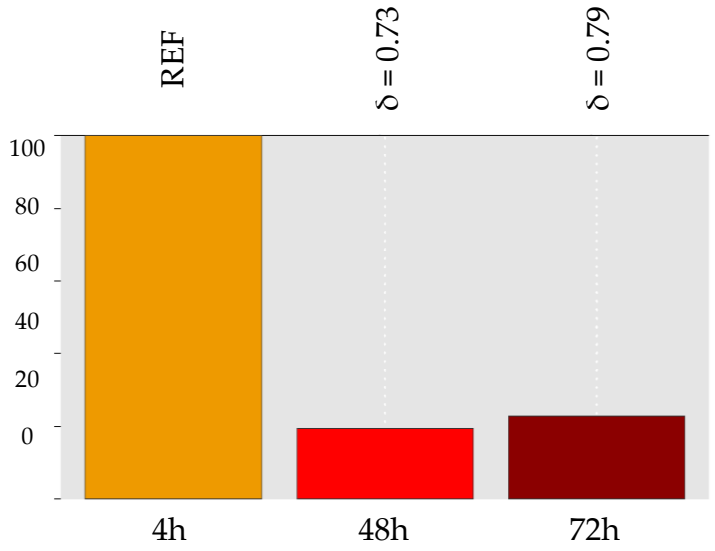


Impact Assessment of Repeated Exposure of Organotypic 3D Bronchial and Nasal Tissue Culture Models to Whole Cigarette Smoke
http://www.jove.com/video/52325/impact-assessment-repeated-exposure-organotypic-3d-bronchial-nasal

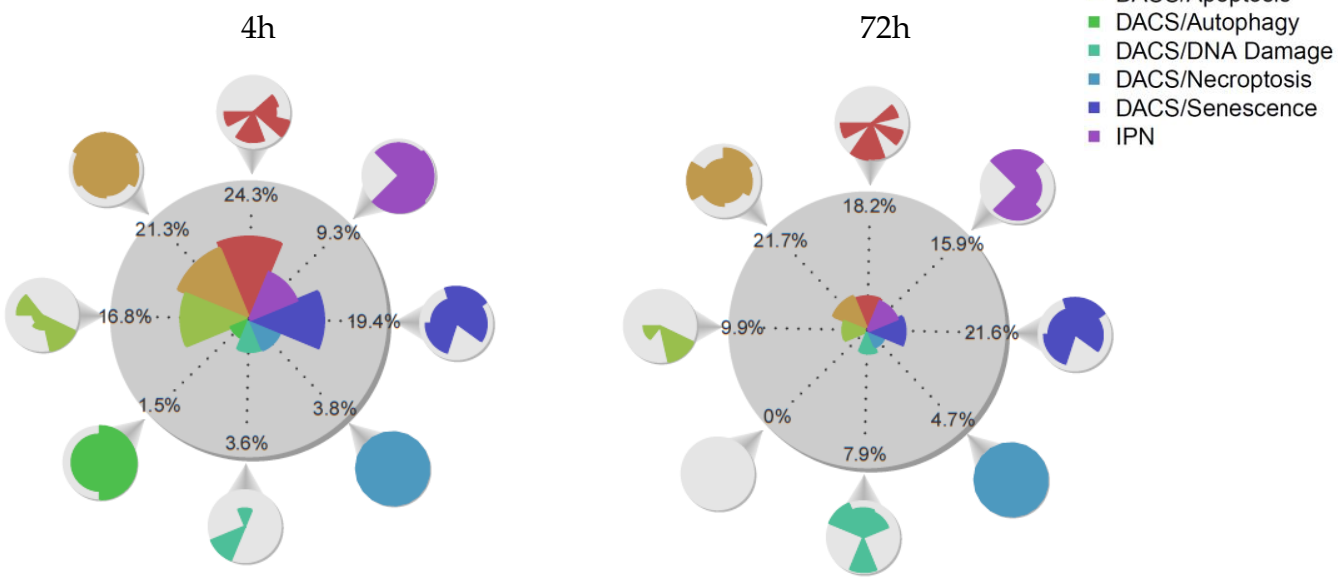
Case study 3: Biological Impact of 8% CS Exposure on Nasal Organotypic Tissue Cultures



Overall biological impact



Perturbations of biological networks



- The largest biological impact of whole CS exposure on nasal organotypic tissue cultures can be observed 4 hours after the exposure
- The most impacted biological processes are related to cell fate and stress

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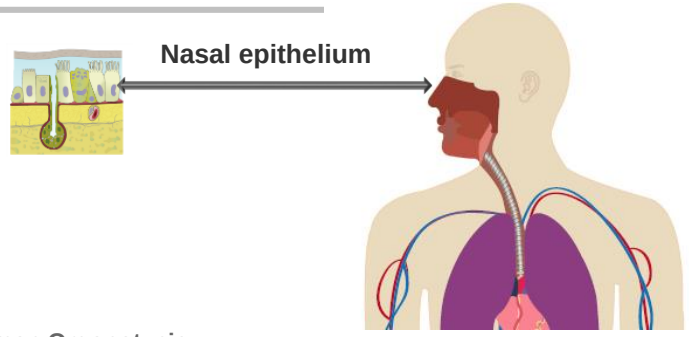
Case study 3: Comparison of Smokers' clinical Samples to CS-exposed nasal organotypic Cultures



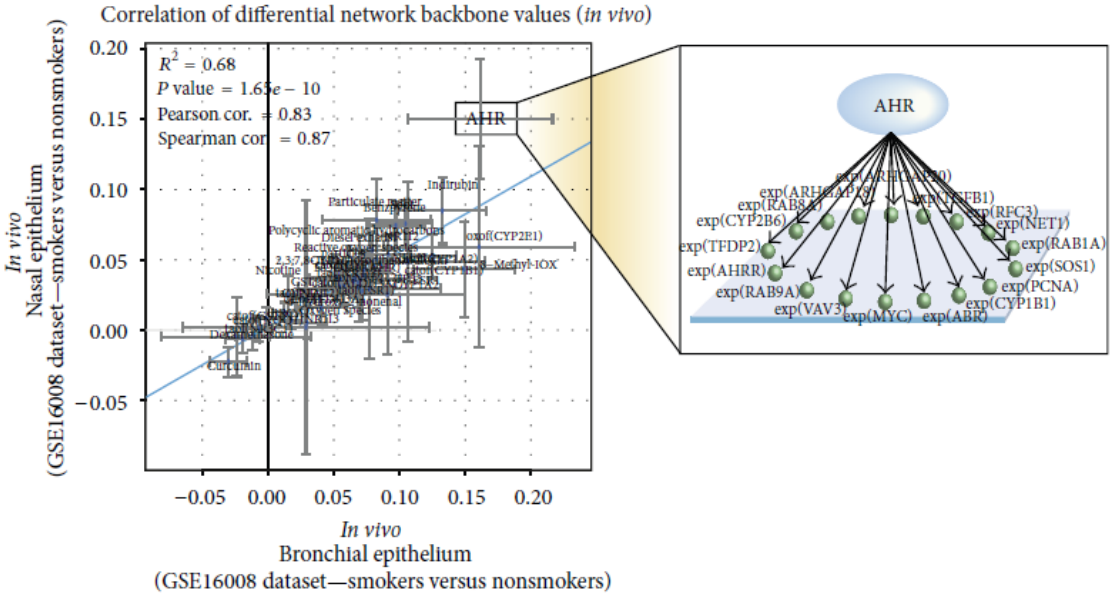
Human Organotypic *in vitro* models

Clinical samples

Healthy Non-smoker (n=14) Age	Healthy Smoker (n=13) Age	Smoker Pack-years
31.6 ± 10.8	35.4 ± 9.9	10.77 ± 9.3

(Zhang X. et al., Physiol. Genomics 2010)

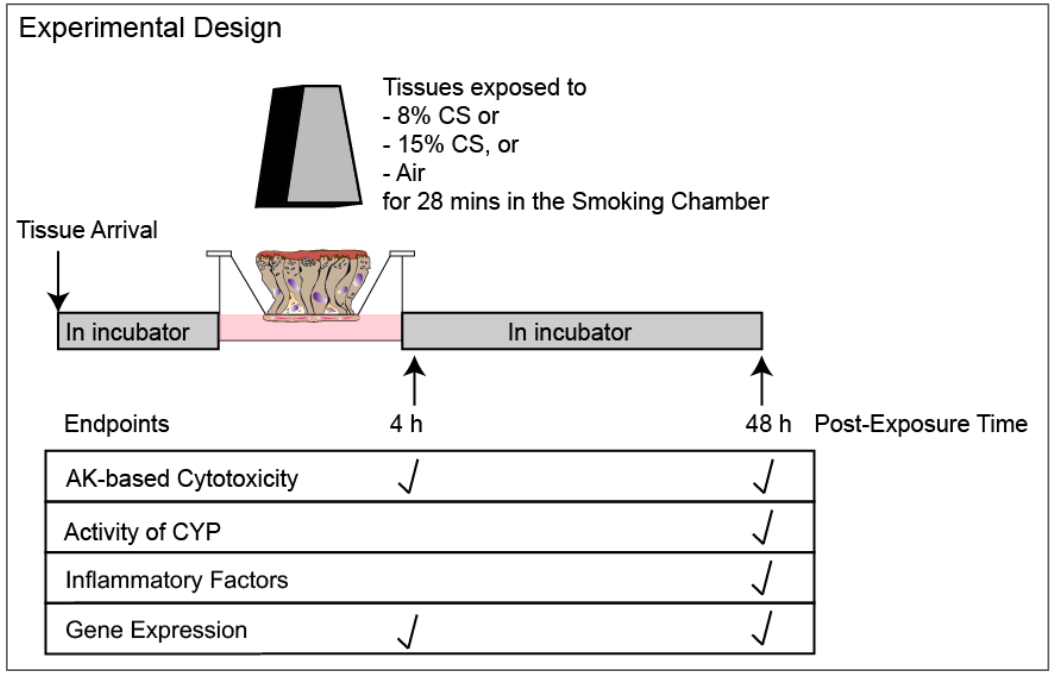
xenobiotic metabolism network model



Iskandar AR, Martin F, Talikka M et al. (2013) Systems approaches evaluating the perturbation of xenobiotic metabolism in response to cigarette smoke exposure in nasal and bronchial tissues. BioMed research international 2013

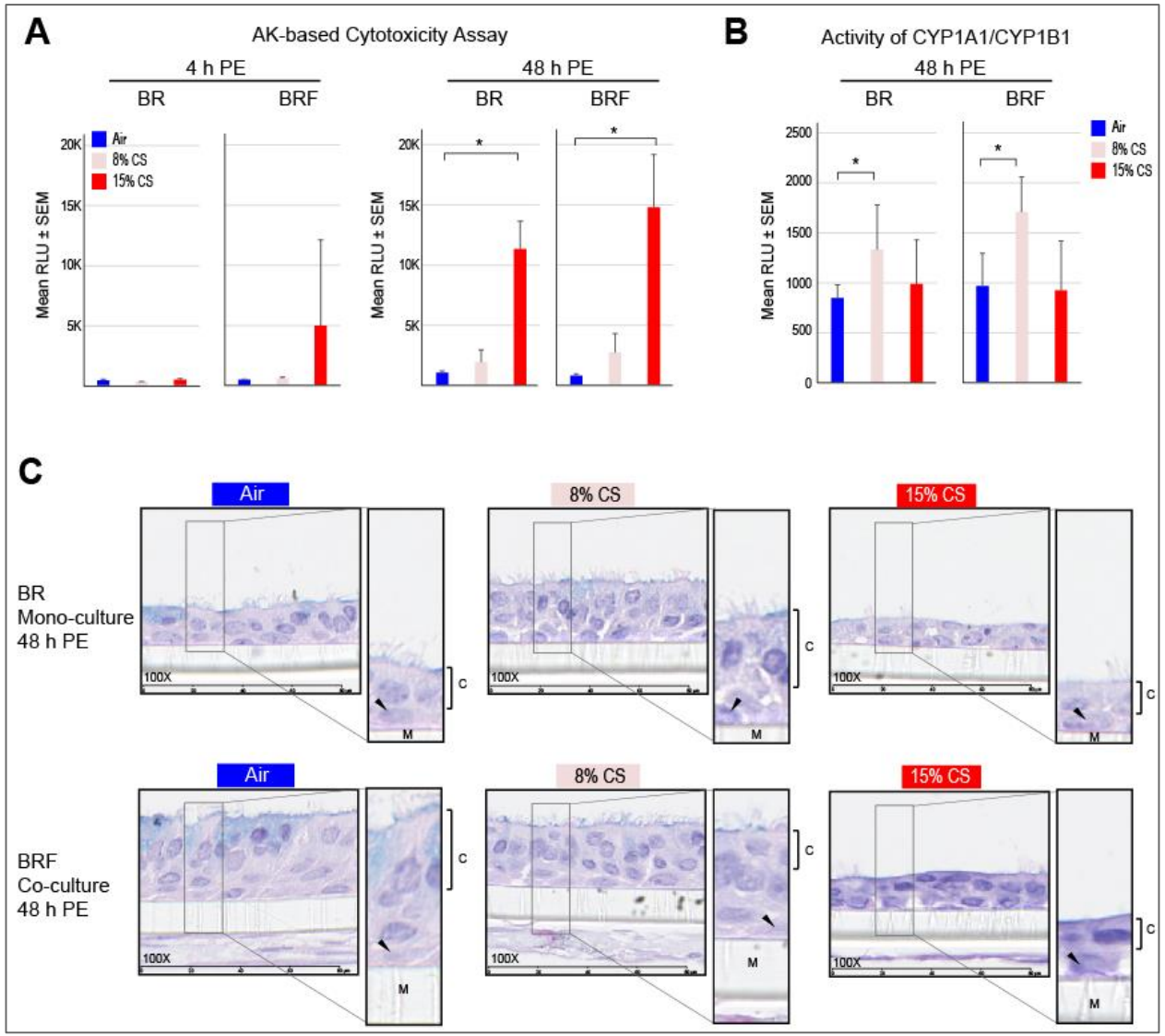
Case study 4: Impact assessment of cigarette smoke exposure on organotypic bronchial tissue culture

Experimental Design



Iskandar AR, Xiang Y, Frentzel S, Talikka M, Leroy P, Kuehn D, Guedj E, Martin F, Mathis C, Ivanov NV, Peitsch MC, Hoeng J. Impact assessment of cigarette smoke exposure on organotypic bronchial epithelial tissue cultures: a comparison of mono-culture and co-culture model containing fibroblasts. **Toxicol Sciences 2015** Jun 16. pii: kfv122. [Epub ahead of print] PubMed PMID: 26085348.

Impact of CS exposure on cytotoxicity, CYP1A1/1B1 activity, and bronchial tissue morphology.



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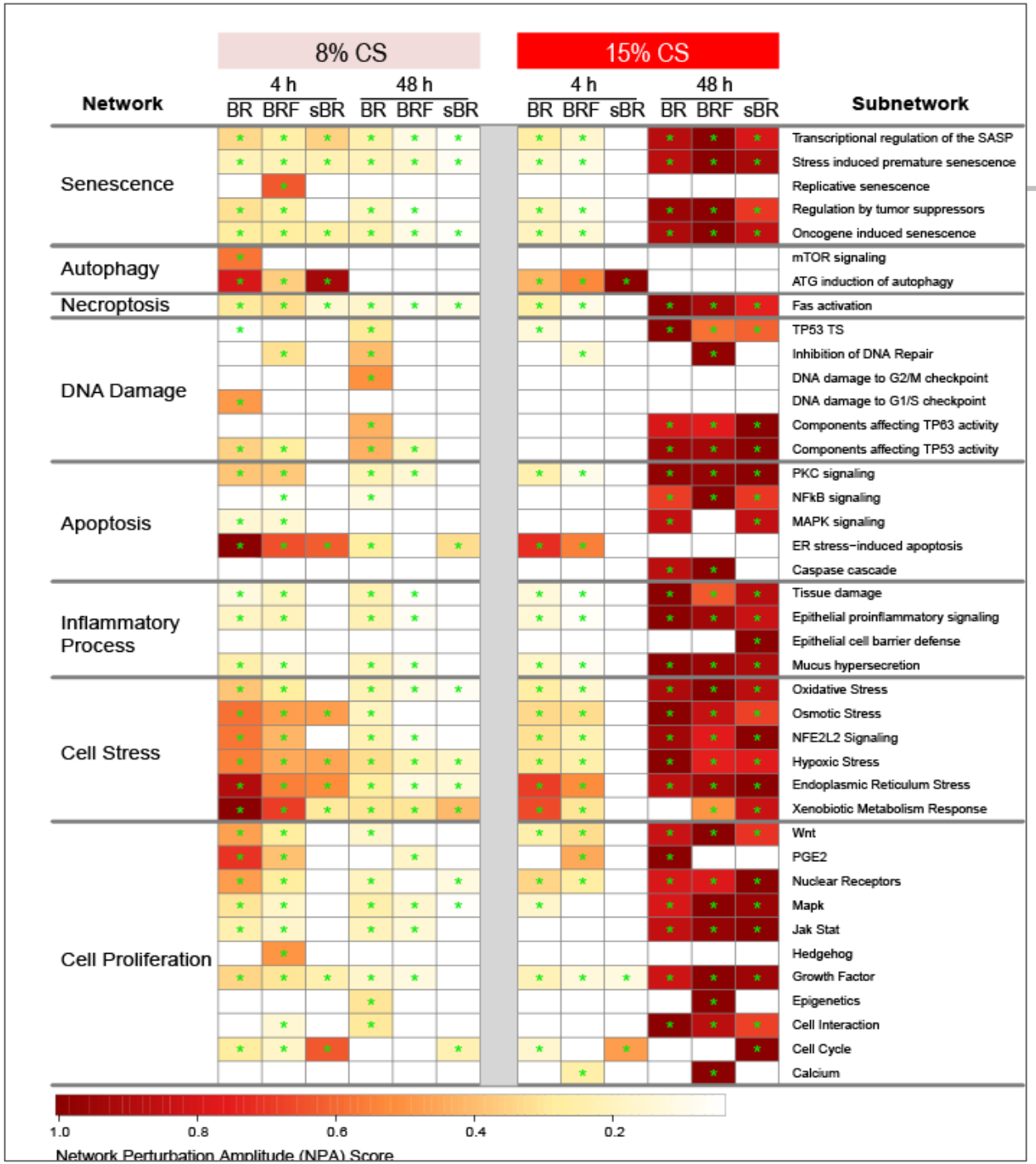

Impact of CS (NPA) on various biological processes analyzed using a network-based systems biology approach analyzed at 4 and 48 h post-exposure.

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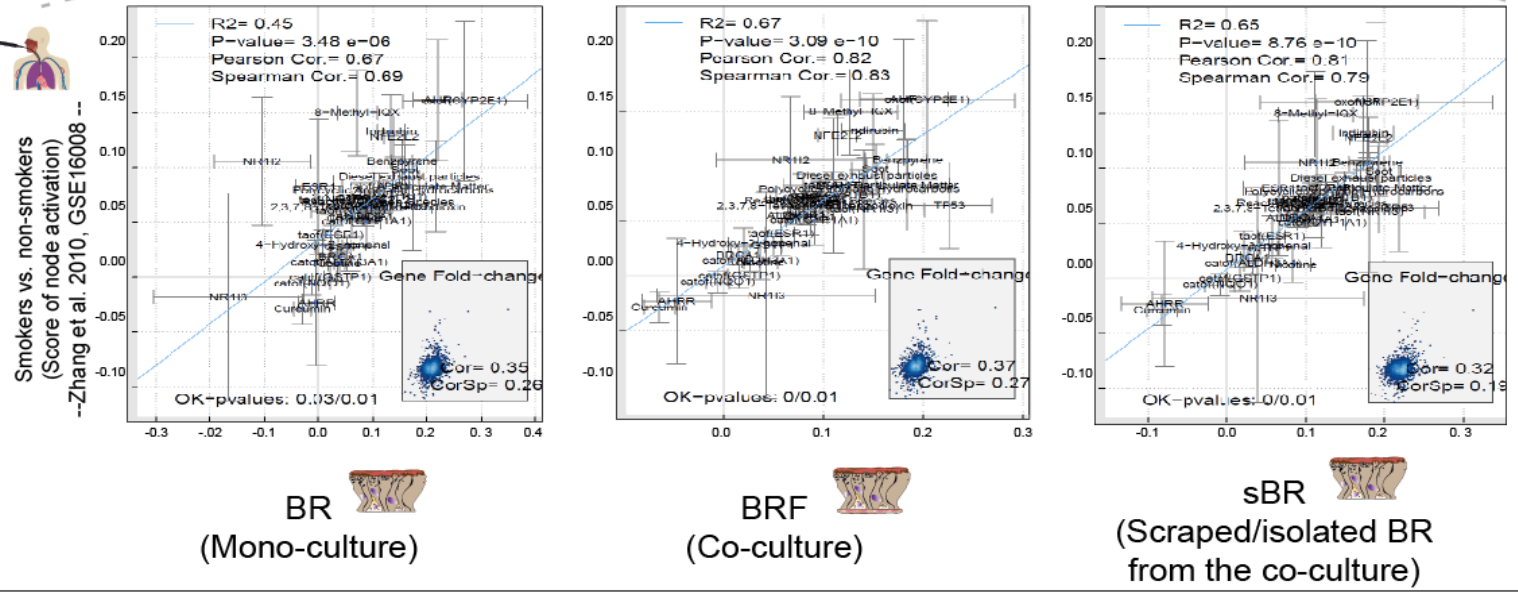
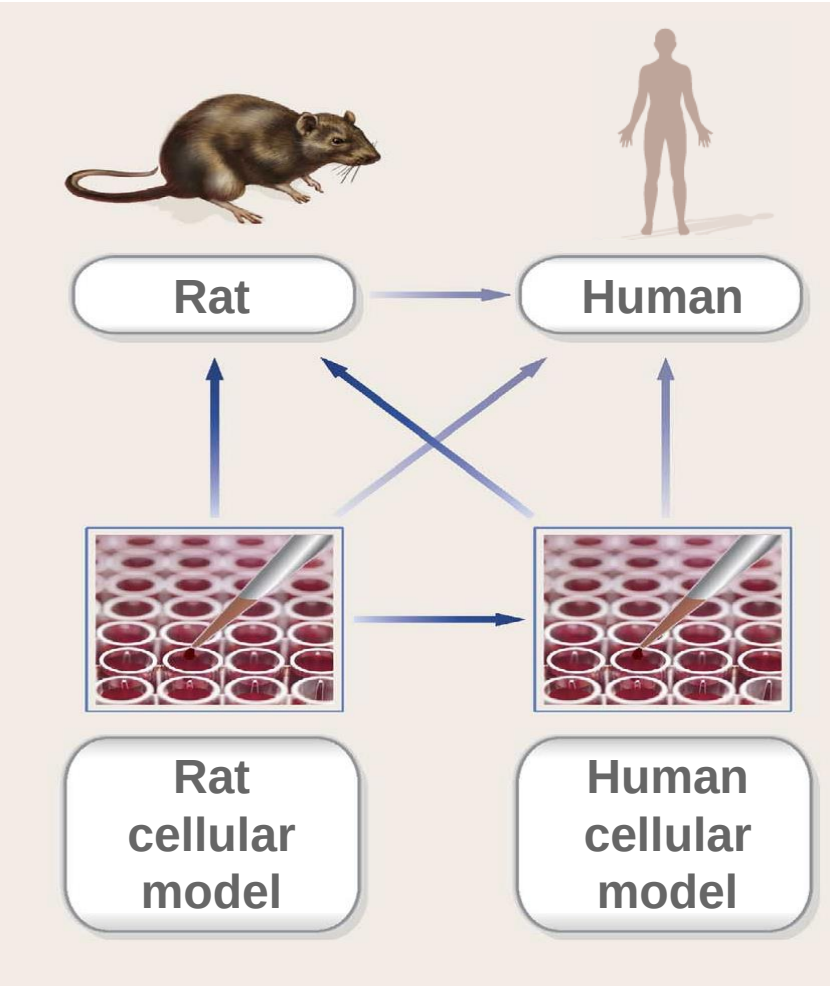
Correlations between the network backbone values of the nodes in the xenobiotic metabolism response network.

R^2		*In vitro* organotypic tissue models											
		BR	BRF	sBR	BR	BRF	sBR	BR	BRF	sBR	BR	BRF	sBR
		8% CS						15% CS					
In vivo brushing dataset		4 h post-exposure			48 h post-exposure			4 h post-exposure			48 h post-exposure		
Schembri, et al.	GSE14633	0.49	0.53	0.39	0.43	0.65	0.63	0.42	0.38	0.27	0.31	0.18	0.36
Zhang, et al.	GSE16008	0.37	0.45	0.3	0.45	0.67	0.65	0.3	0.29	0.15	0.34	0.25	0.45
Beane , et al.	GSE7895	0.42	0.39	0.37	0.42	0.65	0.59	0.38	0.24	0.31	0.2	0.11	0.26



The correlation plots showed that the *in vitro* xenobiotic responses following exposure of 8% CS (at 48 h post-exposure) to the BRF co-culture and sBR cells were comparable with the *in vivo* situation of apparently healthy smokers (R^2 = 0.59–0.67).

Species and system translation



Translation:

- Are animal models useful in toxicology?
  - What biology can be applied across species?
- Which *in vitro* systems are necessary to recapitulate a meaningful part of whole body biology?

Poussin C, Mathis C, Alexopoulos LG, Messinis DE, Dulize RHJ, Belcastro V, Melas IN, Sakellaropoulos T, Rhrissorrakrai K, Bilal E, Meyer P, Talikka M, Boue S, Norel R, Rice JJ, Stolovitzky G, Ivanov NV, Peitsch MC and Hoeng J (2014) The Species Translation Challenge - A Systems Biology Perspective on Human and Rat Bronchial Epithelial Cells. **Scientific Data,** 1:140009

Conclusions

- PMI R&D established a state-of-the-art approach for Systems Toxicology-based product testing
  - computational NPA/BIF methodology based on OMICS data
  - an *in vivo* model of cigarette smoke-induced diseases (e.g. COPD)
  - an *in vitro* model of cigarette smoke exposure (including organotypic cultures)
  - species and systems translation

- The systems toxicology approach adds a strongly supportive mechanistic layer to traditional toxicology end points

- The perturbations of major biological networks were markedly reduced following switching to potential RRP, very similar to cessation.

Acknowledgments

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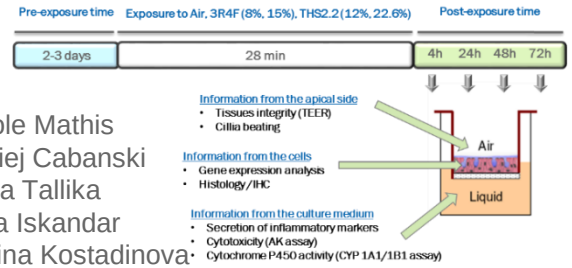

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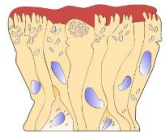

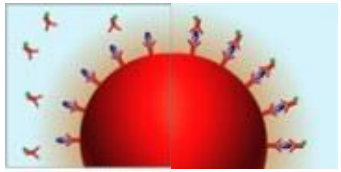

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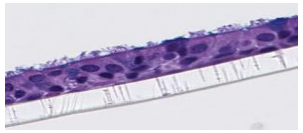

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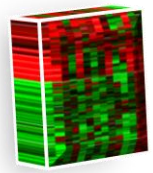

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