

# Integrated Biology

A Pathway-centric Approach  
to Multiomics Research  
Powered by GeneSpring  
Analytics

Nigel Skinner, PhD  
Global Segment Marketing Manager  
Life Science Research

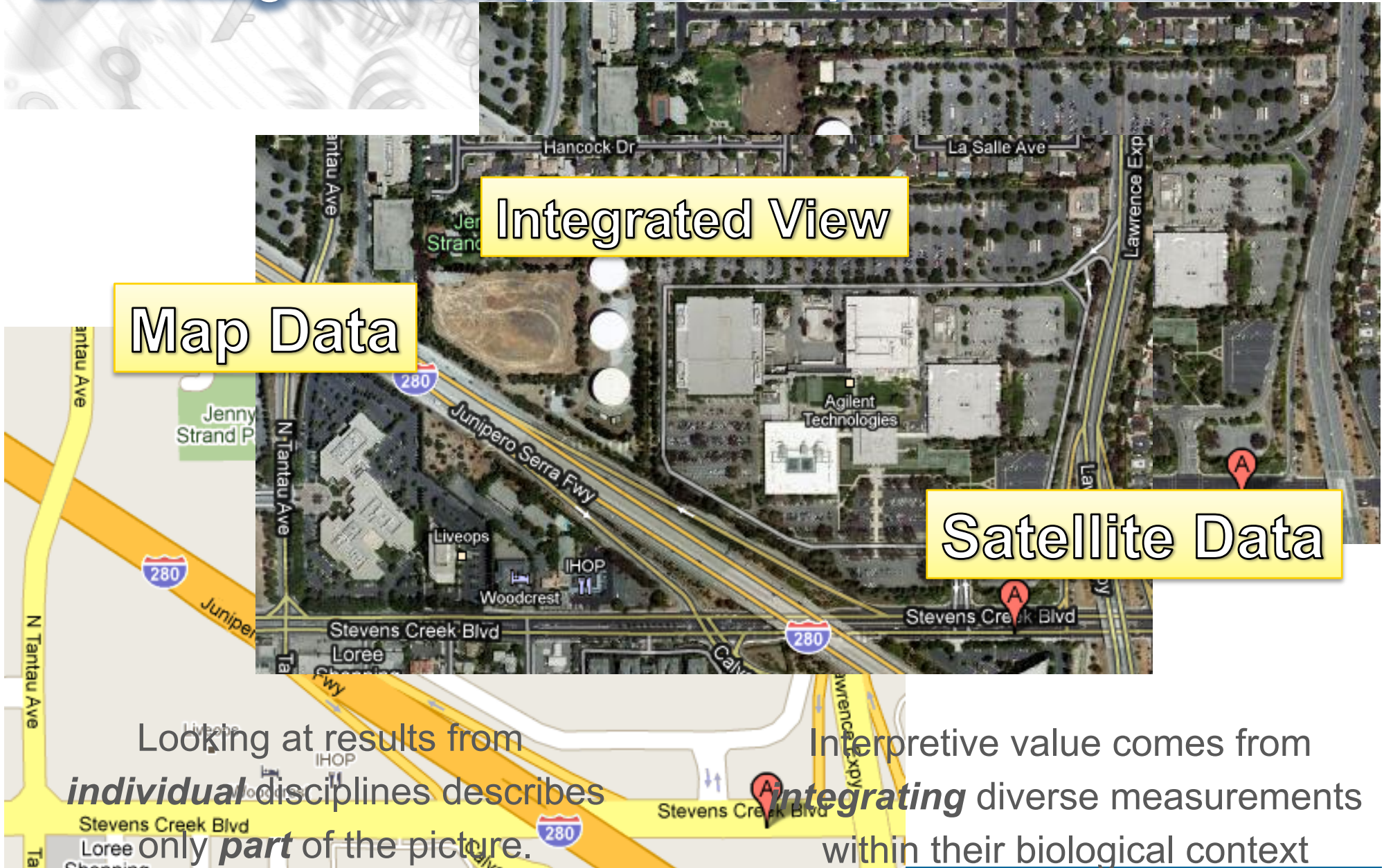


# Presentation Outline

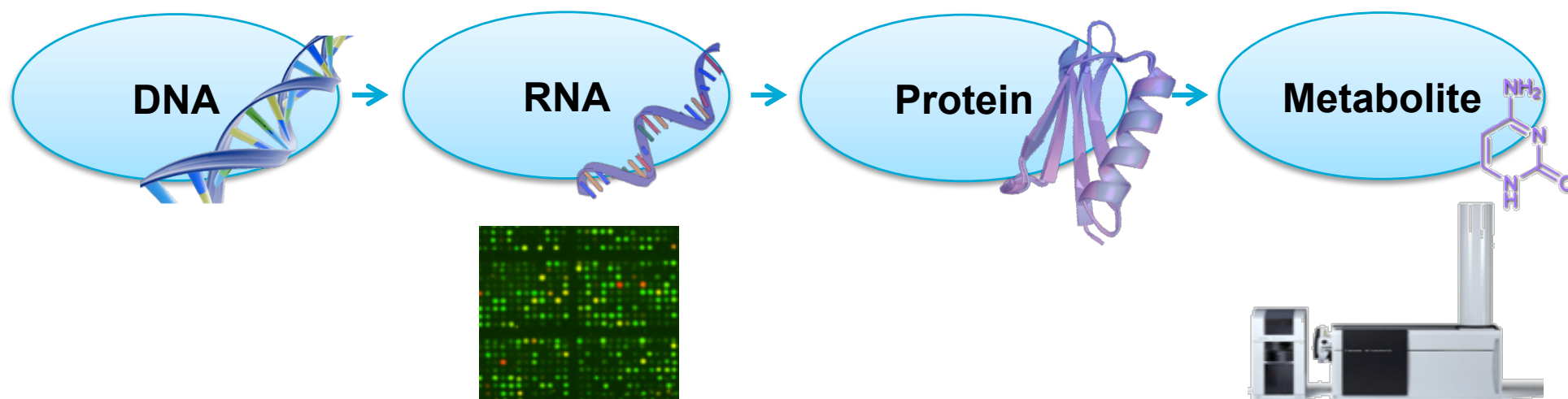
1. Multi-omics Integration: Why?
2. Multi-omics Research: How? (Case Studies)
  - Metabolomics + Transcriptomics → Targeted Proteomics : In search of new anti-TB drugs
  - Proteomics + Transcriptomics:
    - PD Biomarkers for Cancer Chemoprevention
3. Summary



# Data Integration: Maps and Geospatial Coordinates

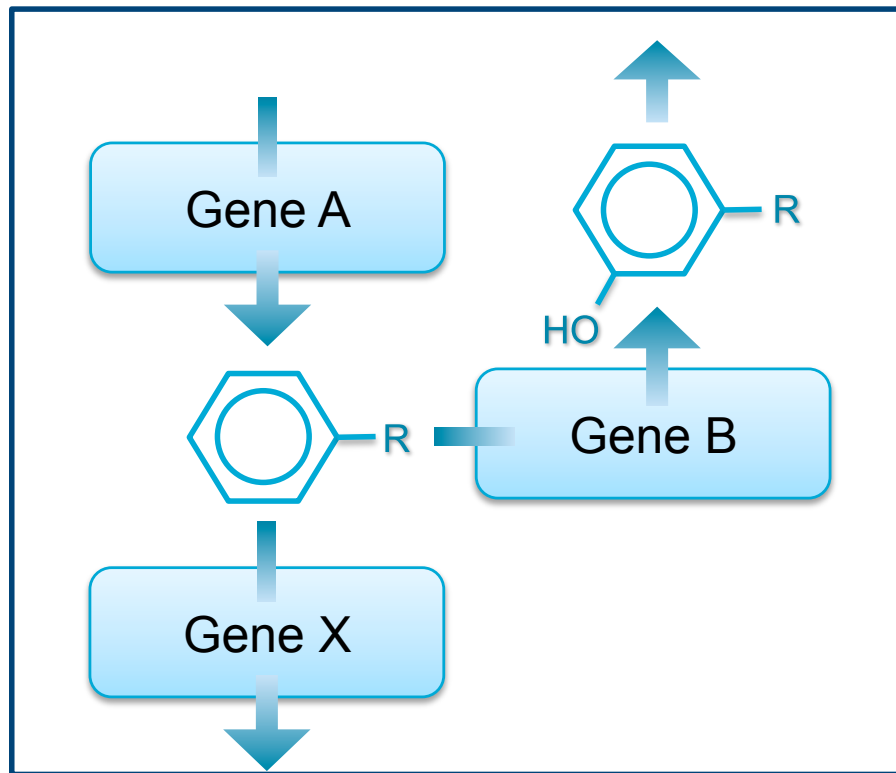


# “Omics”




What is the “common coordinate” that enables integrative analysis?


# Common Reference: Pathway Representation

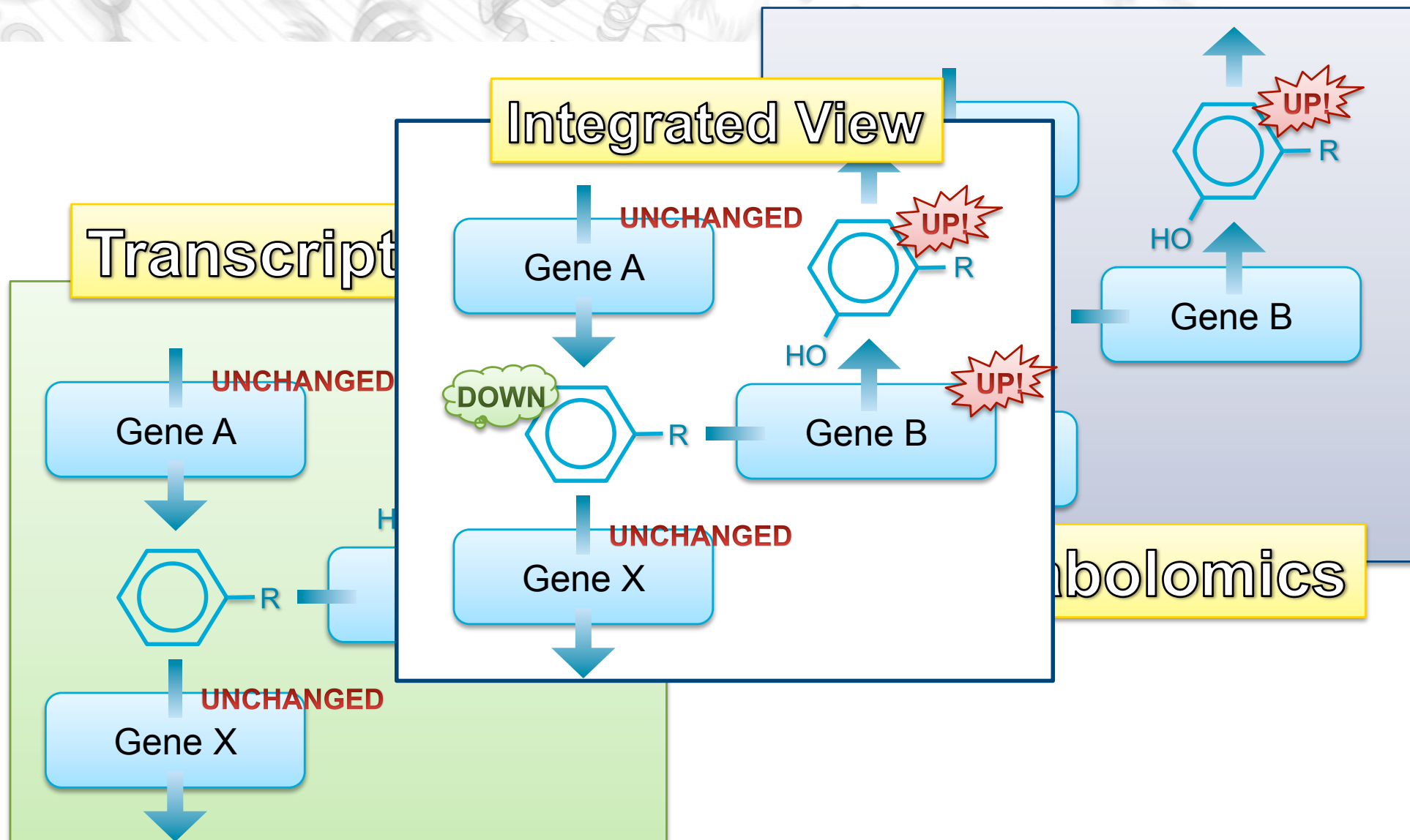


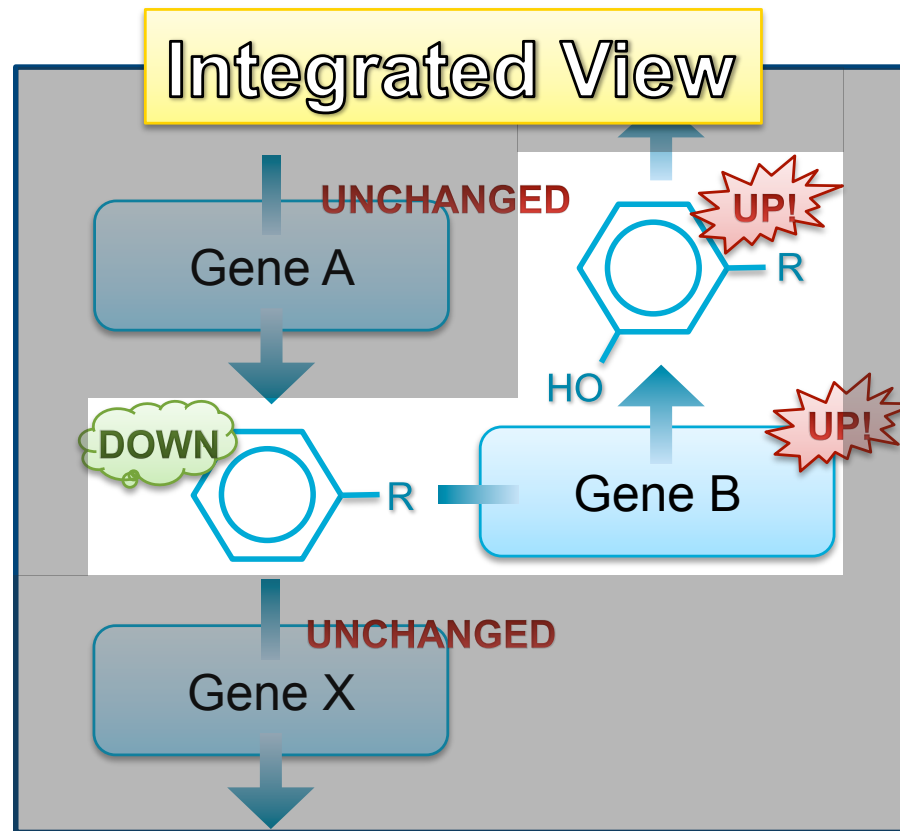
## Sources

- **WikiPathways** 
- BioCyc/MetaCyc
- Generalized BioPax
- KEGG

## Platforms

- **GeneSpring** 
- IPA
- MetaCore
- others....



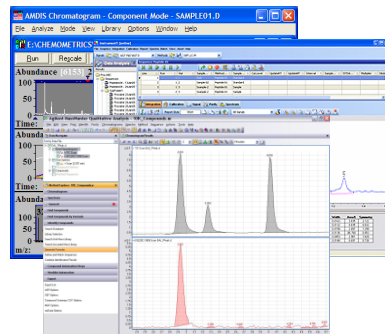


- Identifies **why** the pathway is active
- Suggests follow-on experiments

# Agilent Integrated Biology Workflows: Practically any –omics technology type imaginable



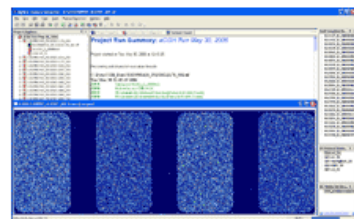
LC/MS  
GC/MS



MassHunter Qual/Quant  
ChemStation AMDIS



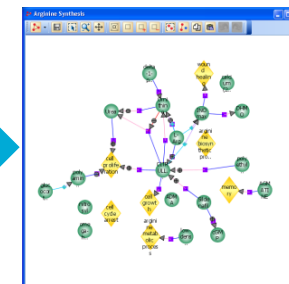
Microarrays



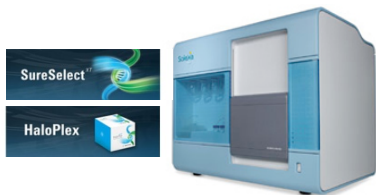
Feature Extraction



GeneSpring Platform



Biological Pathways



NGS

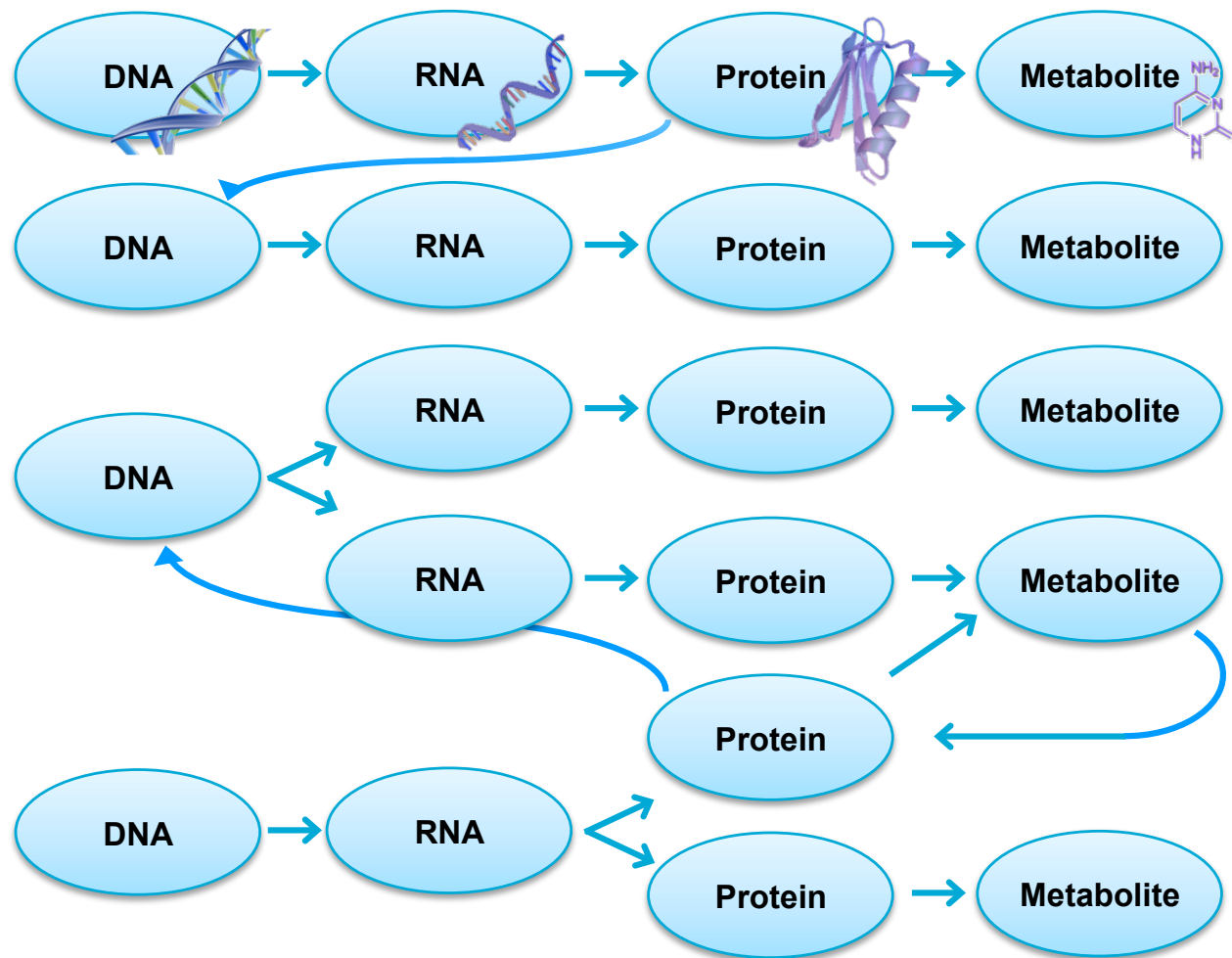


Alignment to Reference Genome

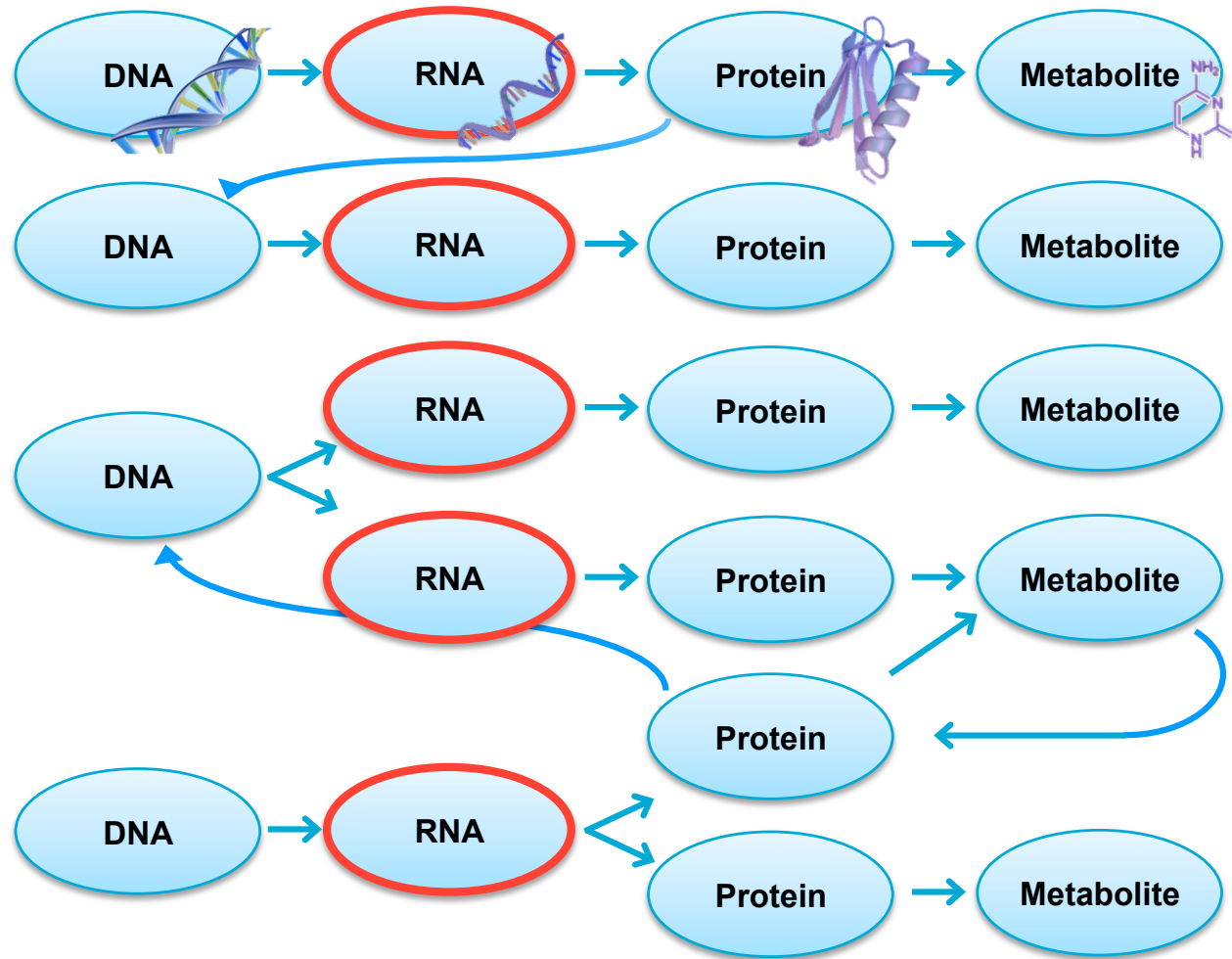
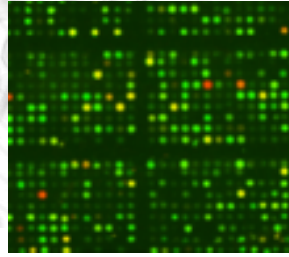




# “Omics” ↔ Biology

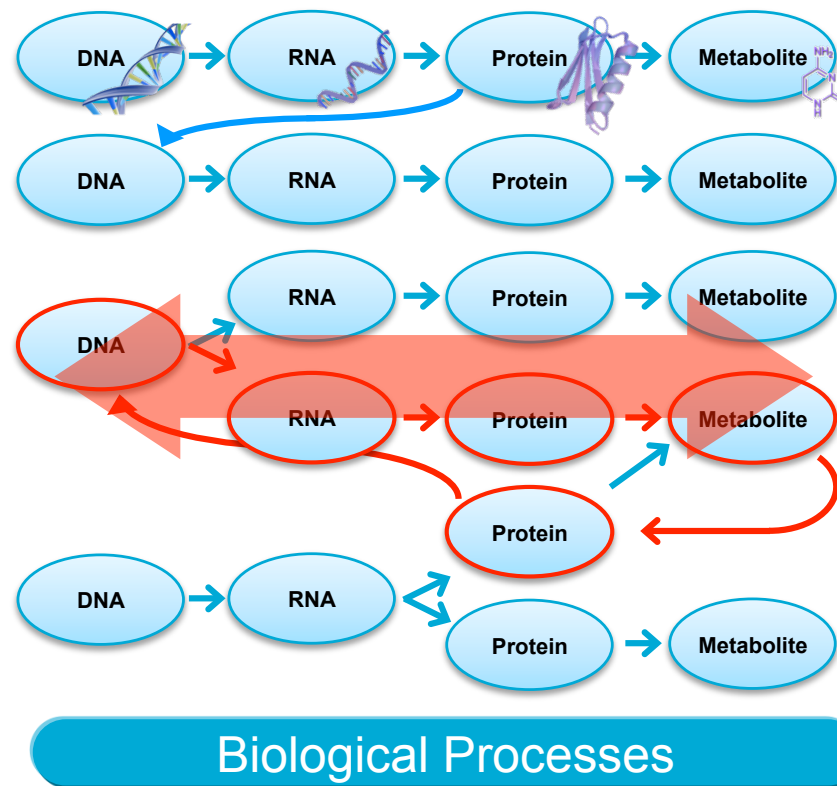
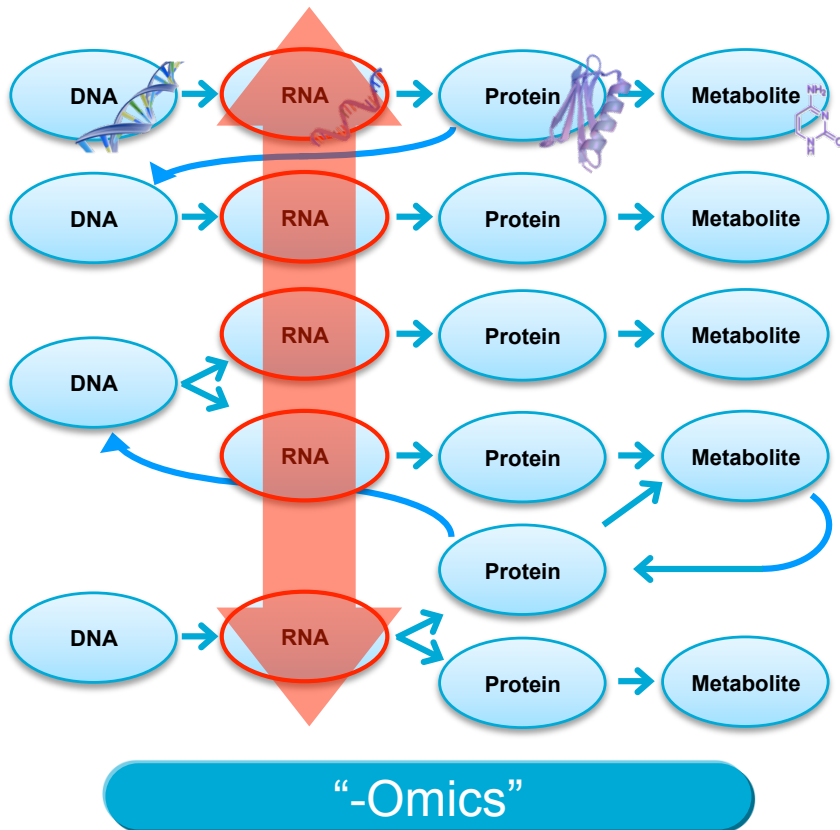


# “Omics”





# The Challenge

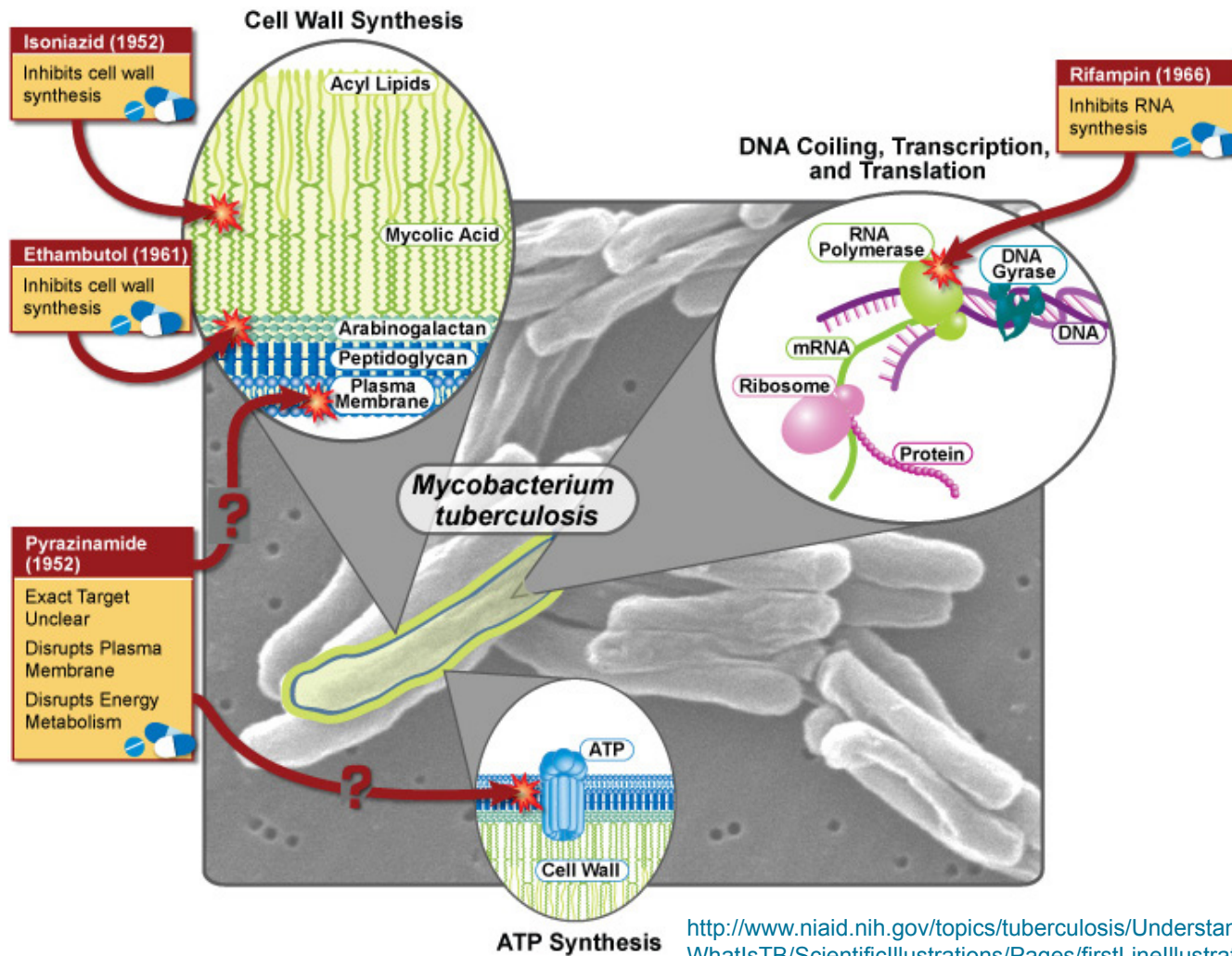


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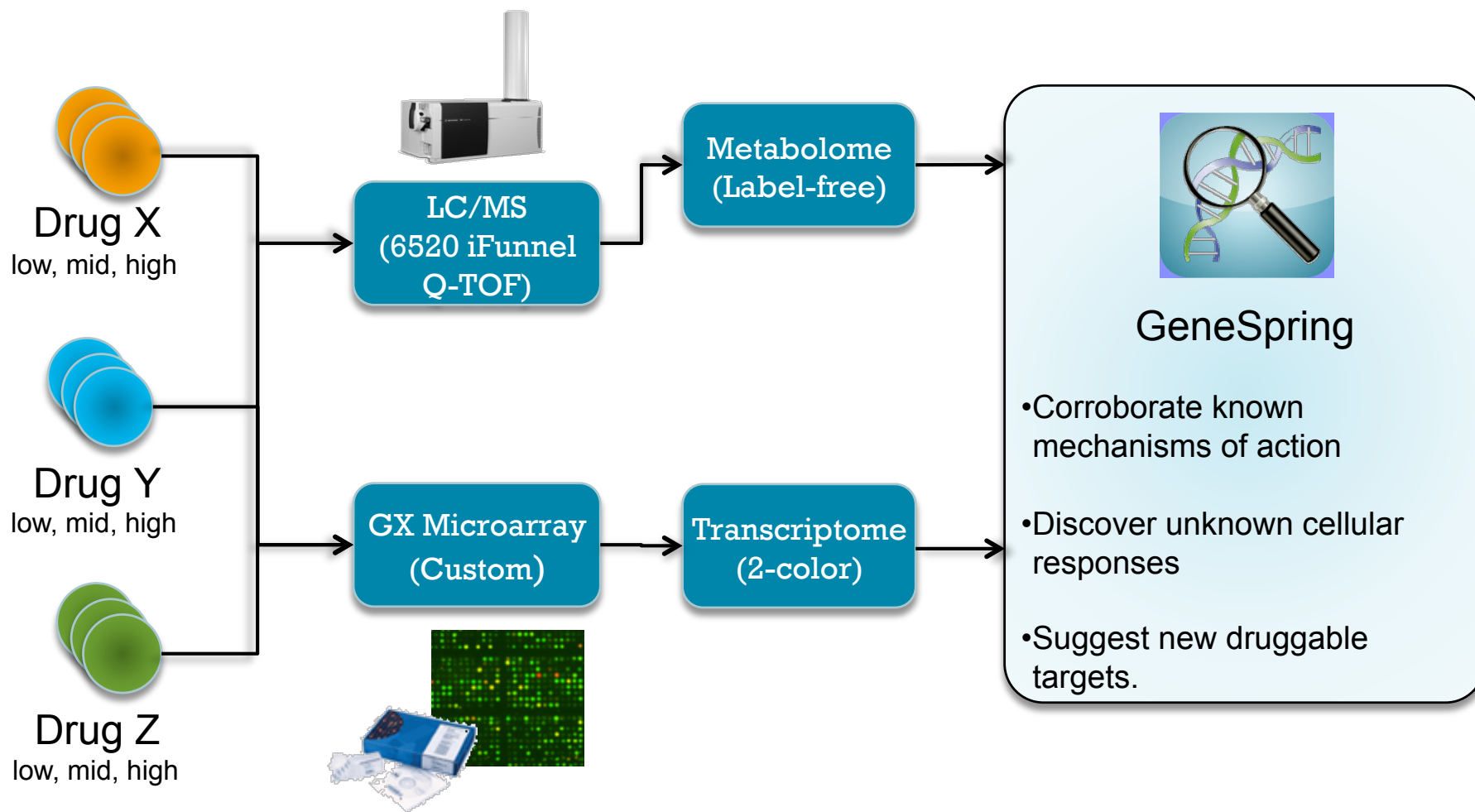
# Pathway-informed Drug Discovery for Tuberculosis (Dr. Kyu Rhee, Weill Cornell Medical College)



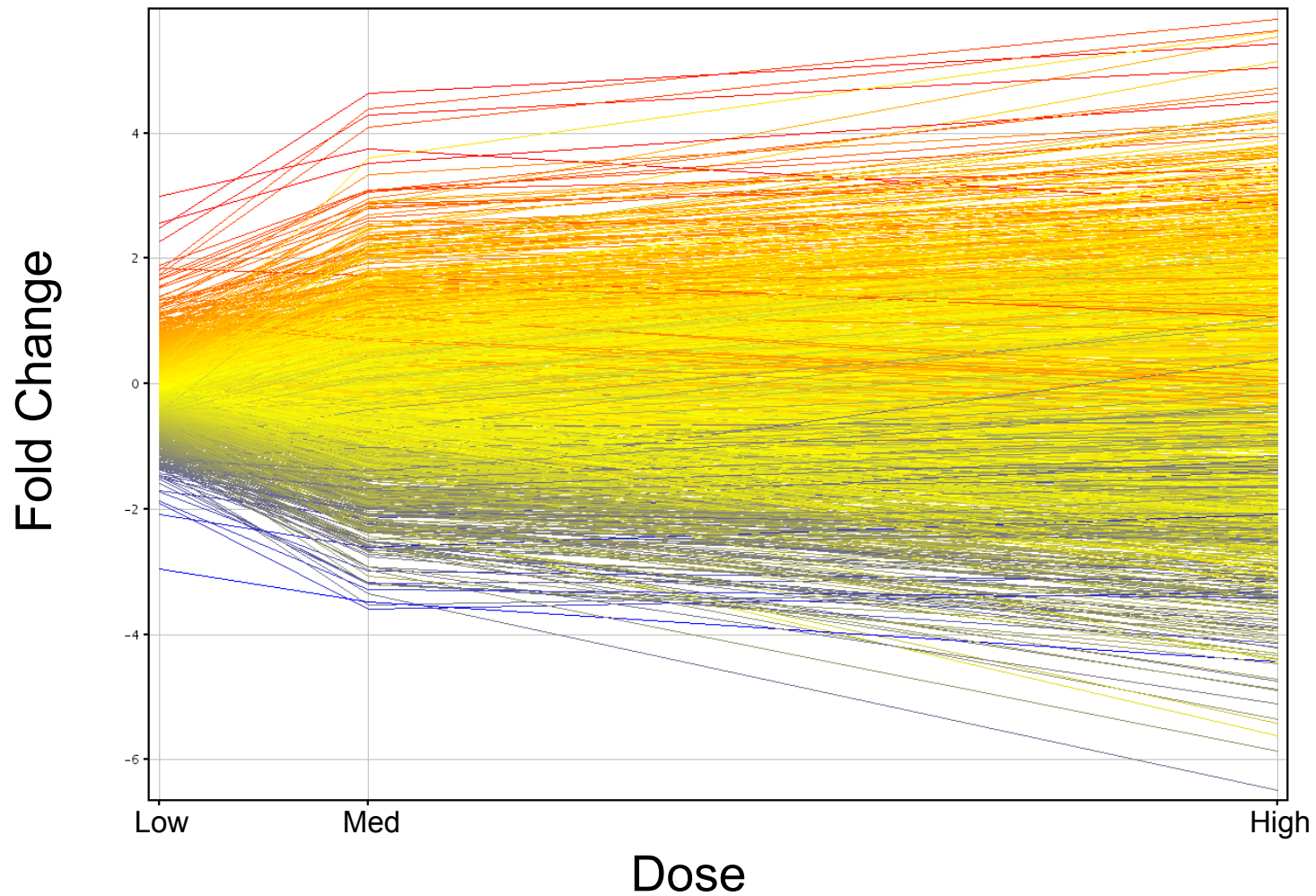
*“TB can usually be cured with a combination of first-line drugs taken for several months. Shown here are the four drugs in the standard regimen of first-line drugs and their modes of action. Also shown are the dates these four drugs were discovered—all more than 40 years ago.”*

NIAID

# Multi-omics Workflow: Transcriptomics + Metabolomics



# Example Analysis– Drug X

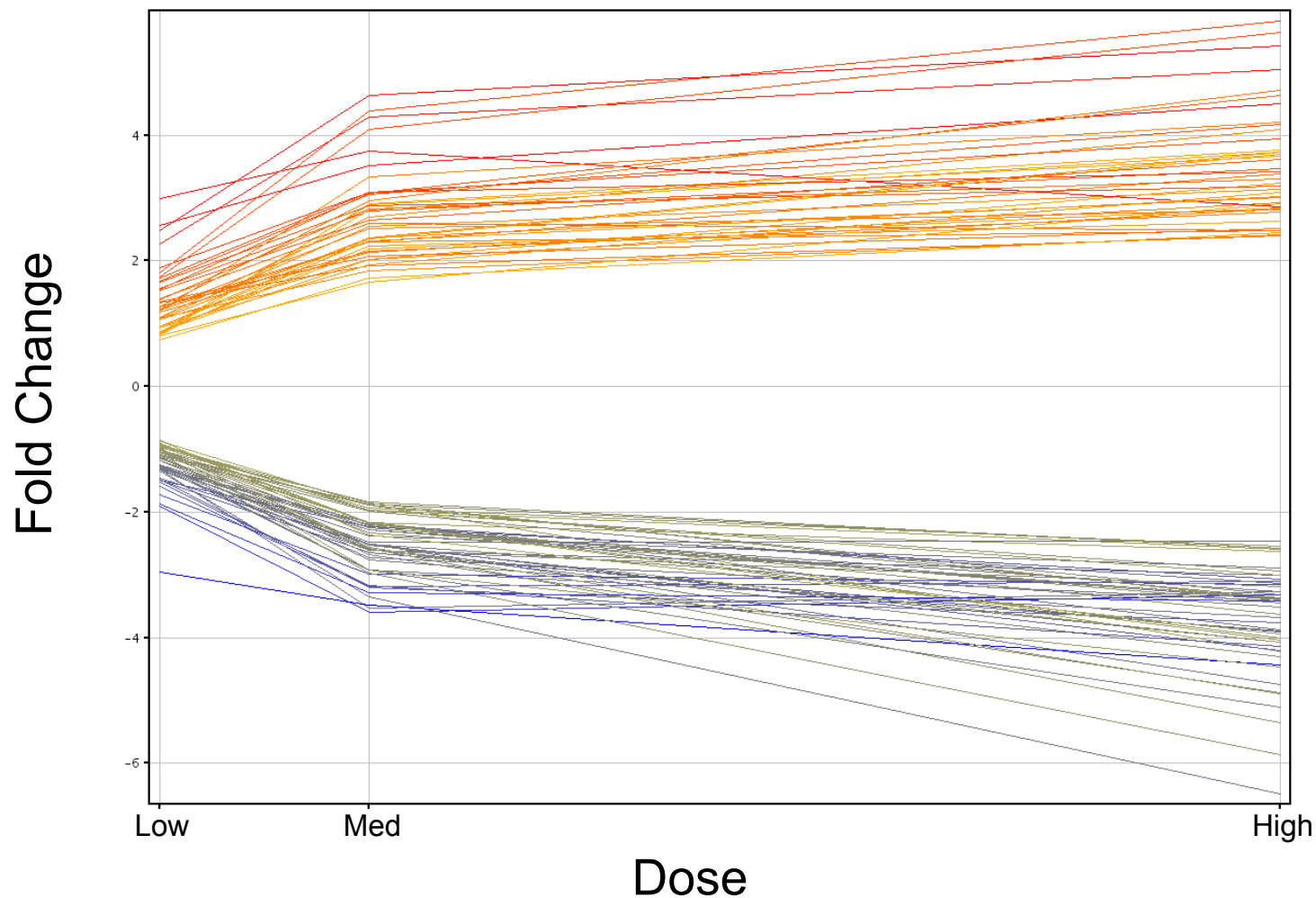


All Genes

(Note increased change with dose)



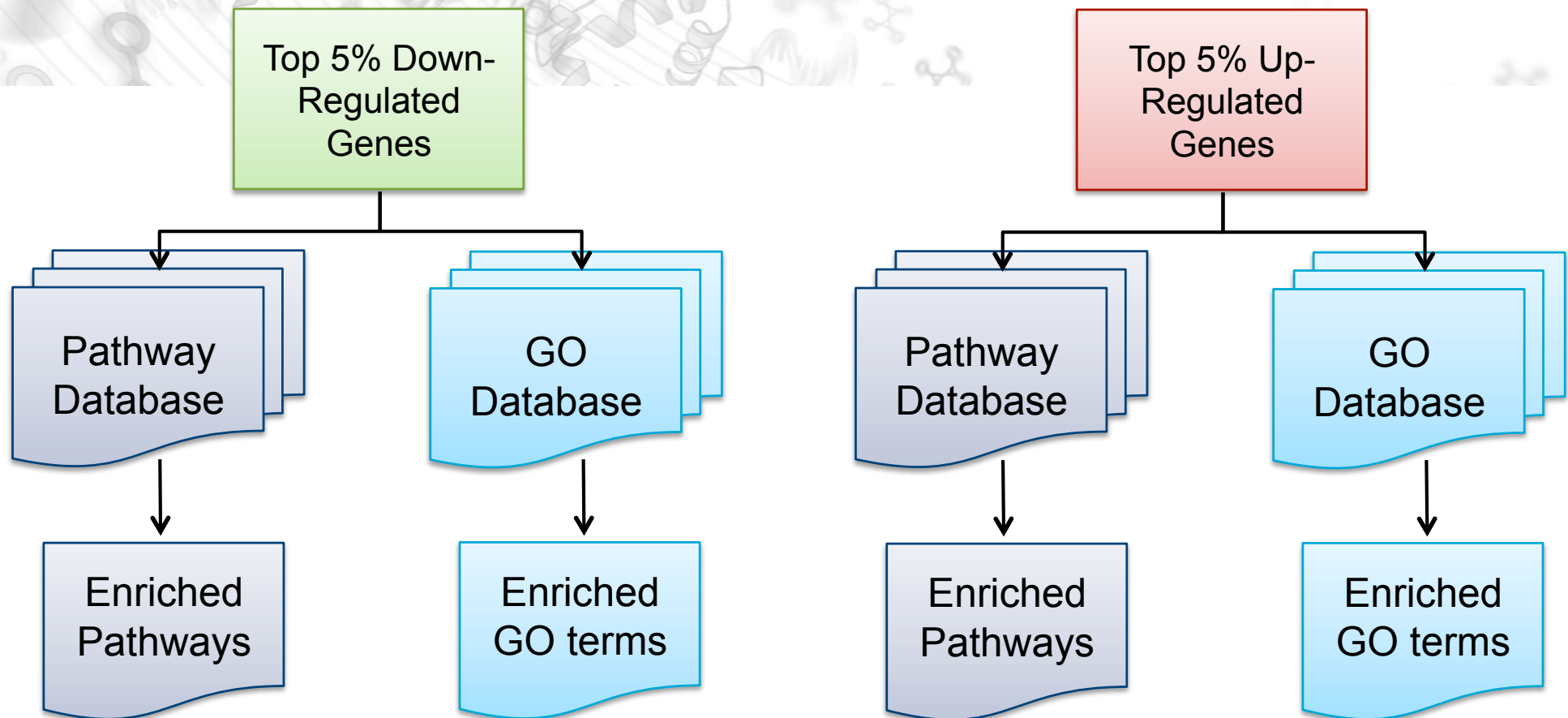
# Example Analysis– Drug X



Genes among top 5% up-regulated in all dosages

Genes among bottom 5% down-regulated in all dosages

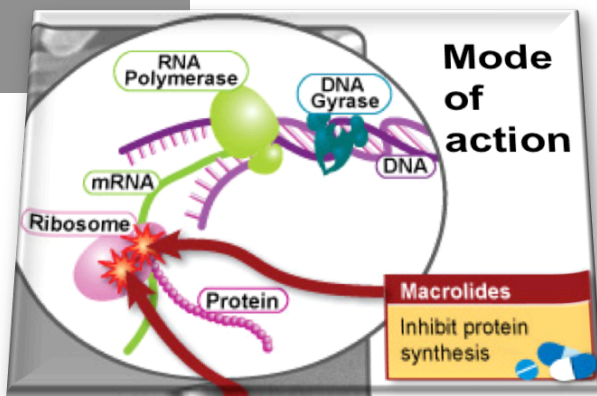
# Enrichment: Find “interesting” pathways



- *Pathway source:* WikiPathways
- *Enrichment:* HG via GeneSpring
- *Identifier Mapping:* GeneSpring + BridgeDB

# Pathway & GO Enrichment: Drug X

- MOA
- Suggestion of secondary mechanisms
- Direction for further exploration



DOWNREGULATED			
Pathway	p-value (Genes)	GO Term	P-value
Mx_Oxidative_phosphorylation_WP1680_41452	1.38E-04	phospholipase C activity	3.34E-04
Mx_Peptidoglycan_biosynthesis_WP1685_41461	0.002111	lipase activity	2.01E-04
Mx_D-Glutamine_and_D-glutamate_metabolism_WP1643_41425	0.002212	phospholipase activity	0.001113
Mx_Ether_lipid_metabolism_WP1645_41436	0.00711		
Mx_Inositol_phosphate_metabolism_WP1664_41480	0.014402		
Mx_Riboflavin_metabolism_WP1696_41424	0.023819		

UPREGULATED			
Pathway	p-value (Genes)	GO Term	P-value
Mx_Glyoxylate_and_dicarboxylate_metabolism_WP1661_41479	0.030887	biological regulation	9.75E-06
	0.074453	regulation of biological process	7.11E-06
		response to chemical stimulus	3.50E-06
		response to stimulus	1.42E-05
		negative regulation of biological process	2.97E-05
		regulation of growth	8.20E-05
		negative regulation of growth	1.85E-04
		propionate catabolic process, 2-methylcitrate cycle	5.96E-04
		propionate metabolic process, methylcitrate cycle	5.96E-04
		regulation of metabolic process	4.66E-04

# Multi-omics Analysis: Learning more about significant genes, metabolites, paths, etc.

DOWNREGULATED			
Pathway	p-value (Genes)	GO Term	P-value
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		regulation of metabolic process	4.66E-04

Peptidoglycan biosynthesis

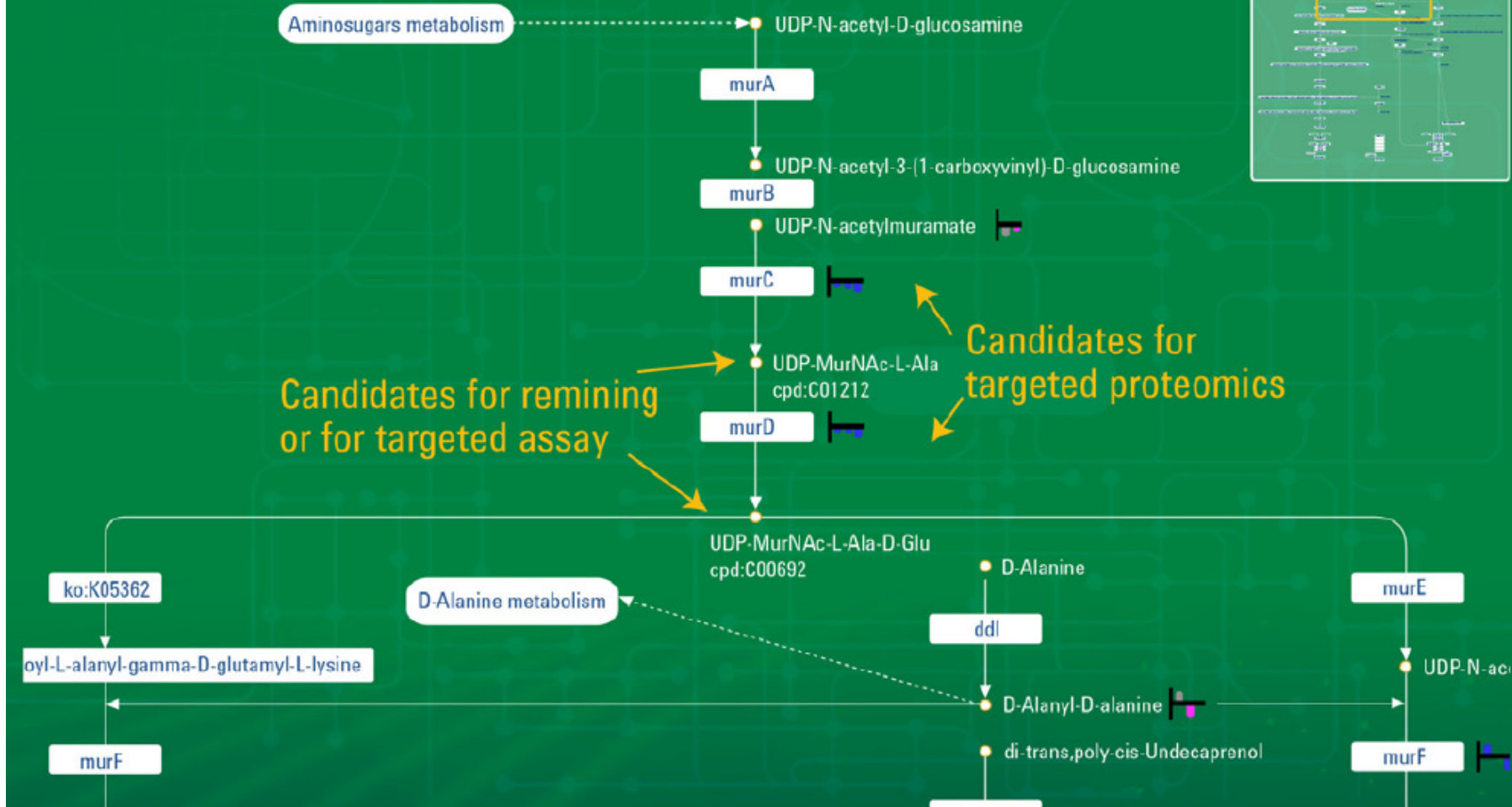
Question: How can I understand what is biologically relevant about this pathway?

Answer: Pathway visualization to find enriched paths



# Peptidoglycan Biosynthesis

## PATHWAY ANALYSIS



# Mapping Metabolites across Databases: UDP-N-acetylmuramate

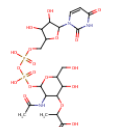
## METABOLITE DBs

**Human Metabolome Database** Version 3.5

Search:  Search type: Metabolites Search [Advanced]

HMDB has recently undergone some major changes, if you are experiencing problems please [click here](#) to provide us with feedback.

Showing metabocard for **UDP-N-acetylmuramate (HMDB11720)**

Metabolite Identification	
Common Name	UDP-N-acetylmuramate
Description	UDP-N-acetylmuramate is a nucleoside diphosphate sugar which is formed from UDP-N-acetylglucosamine and phosphoenolpyruvate. It serves as the building block upon which peptidoglycan is formed.
Structure	 Download: <a href="#">MOL</a>   <a href="#">SDF</a>   <a href="#">SMILES</a>   <a href="#">InChI</a> Display: <a href="#">2D Structure</a>   <a href="#">3D Structure</a>
Synonyms	<ol style="list-style-type: none"><li>1. N-Acetylmuramoyl-UDP</li><li>2. UDP-MurNAc</li><li>3. UDP-N-Acetyl-D-muramate</li><li>4. UDP-N-Acetylmuramate</li><li>5. UDP-N-Acetylmuramic acid</li><li>6. Uridine diphosphate N-acetylmuramic acid</li></ol>
Chemical Formula	C <sub>20</sub> H <sub>31</sub> N <sub>3</sub> O <sub>19</sub> P <sub>2</sub>
Average Molecular Weight	679.4164
Monoisotopic Molecular Weight	679.102698849

## PATHWAYS DBs

(1-carbol, ...)

murB

N-acetylmuran

murC

cpd:C01212

murD

**UDP-N-acetylmuramate**

Annotated with: C01050 (Kegg Compound)

Find pathways with UDP-N-acetylmuramate...

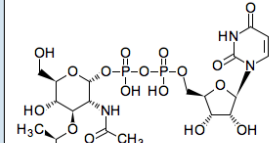
External references:

- ChEBI
- 17882
- HMDB
- Kegg Compound

WikiPathways

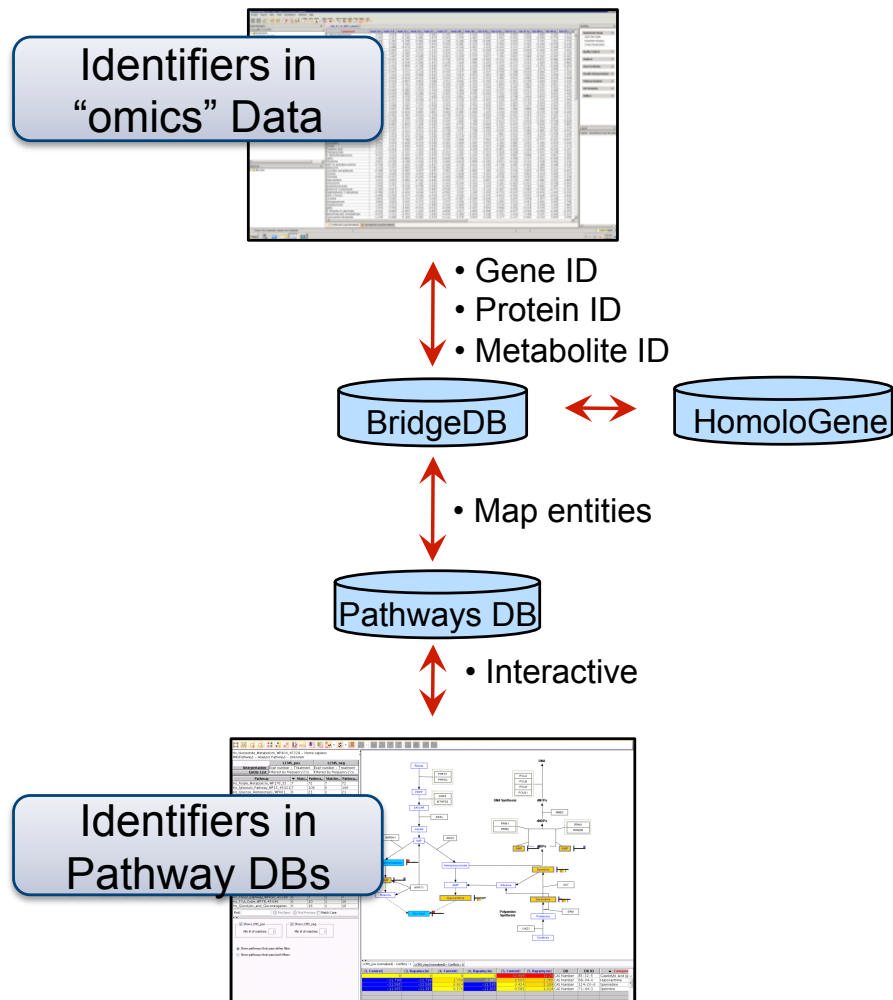


**KEGG** COMPOUND: C01050 Help

Entry	C01050	Compound
Name	UDP-N-acetylmuramate; UDP-N-acetyl-alpha-D-muramate; UDP-N-acetylmuramic acid; UDP-MurNAc	
Formula	C <sub>20</sub> H <sub>31</sub> N <sub>3</sub> O <sub>19</sub> P <sub>2</sub>	
Exact mass	679.1027	
Mol weight	679.4164	
Structure	 C01050 <a href="#">Mol file</a> <a href="#">KCF file</a> <a href="#">DB search</a> <a href="#">Jmol</a> <a href="#">KegDraw</a>	
Reaction	R03191 R03192 R03193	
Pathway	ko00471 D-Glutamine and D-glutamate metabolism ko00520 Amino sugar and nucleotide sugar metabolism ko00550 Peptidoglycan biosynthesis	

# BridgeDb: Mapping Entities Onto Pathways

## Resolves Mapping Problem Between Databases



### Metabolite Identifiers

- KEGG
- HMDB
- ChEBI
- CAS

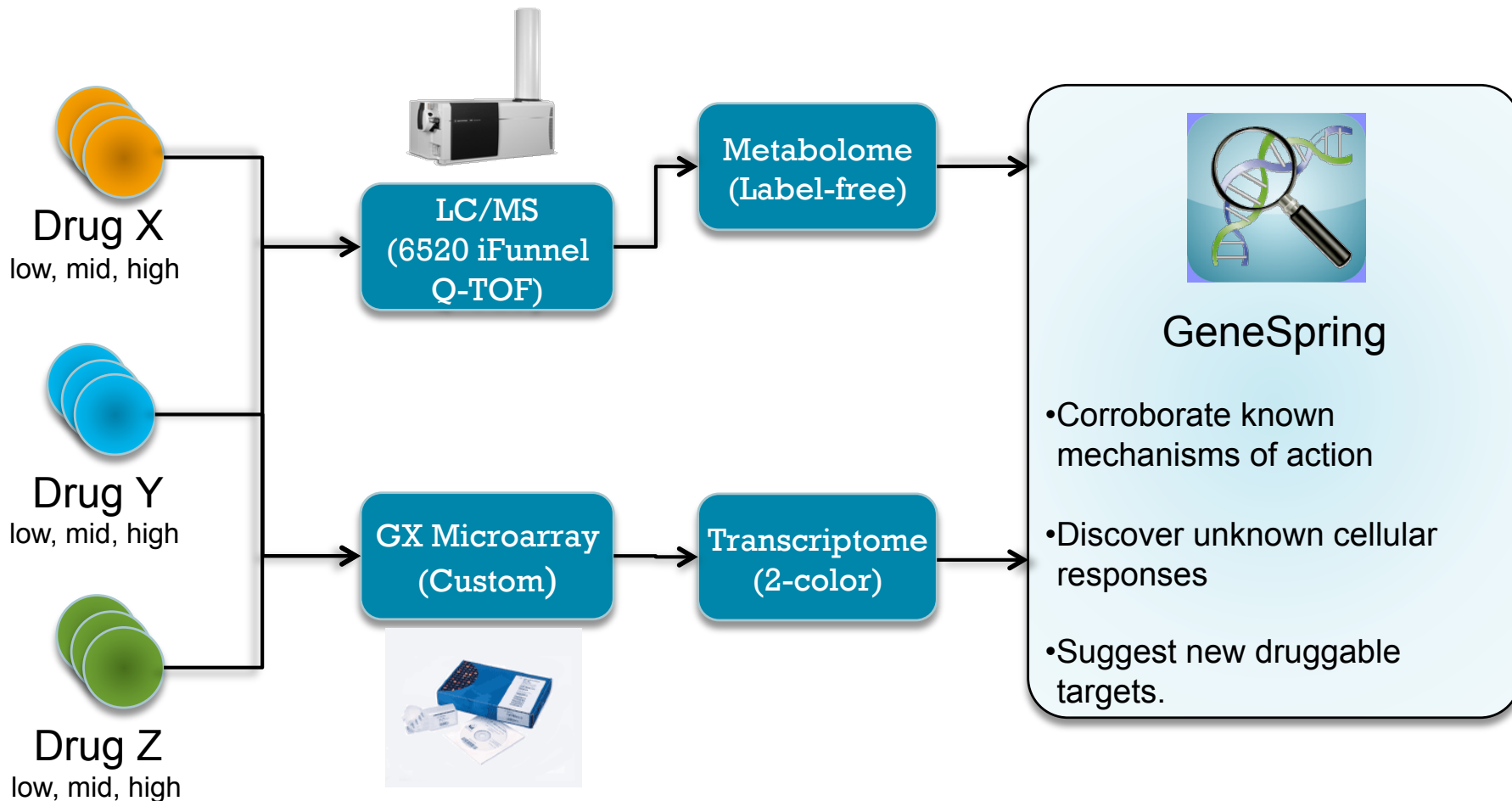
### Protein Identifiers:

- Swiss-Prot
- UniProt
- UniProt/TrEMBL

### Gene Identifiers :

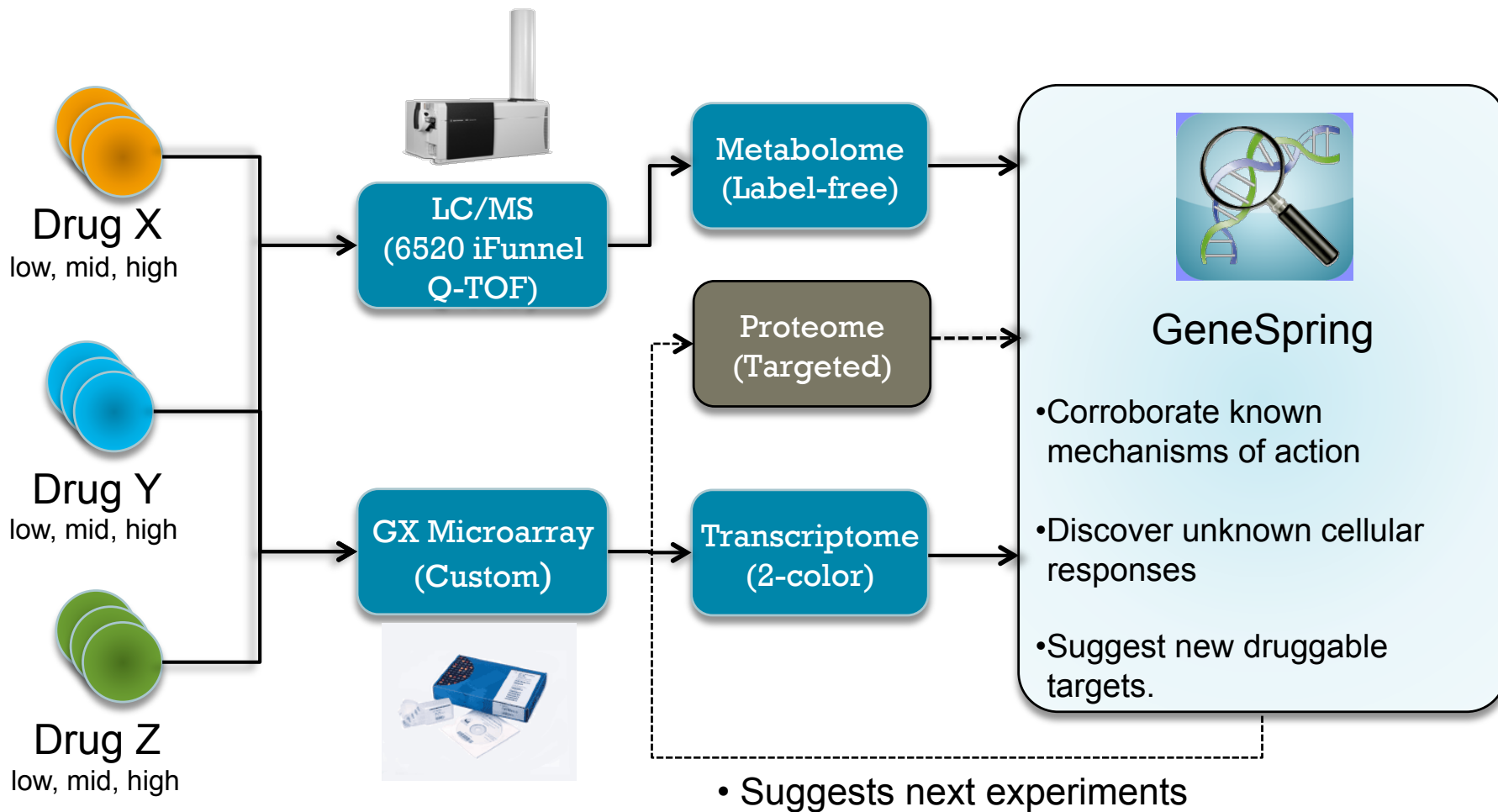
- Entrez Gene, GenBank, Ensembl
- EC Number, RefSeq, UniGene, HUGO
- HGNC, EMBL

# Multi-omics Workflow: Transcriptomics + Metabolomics

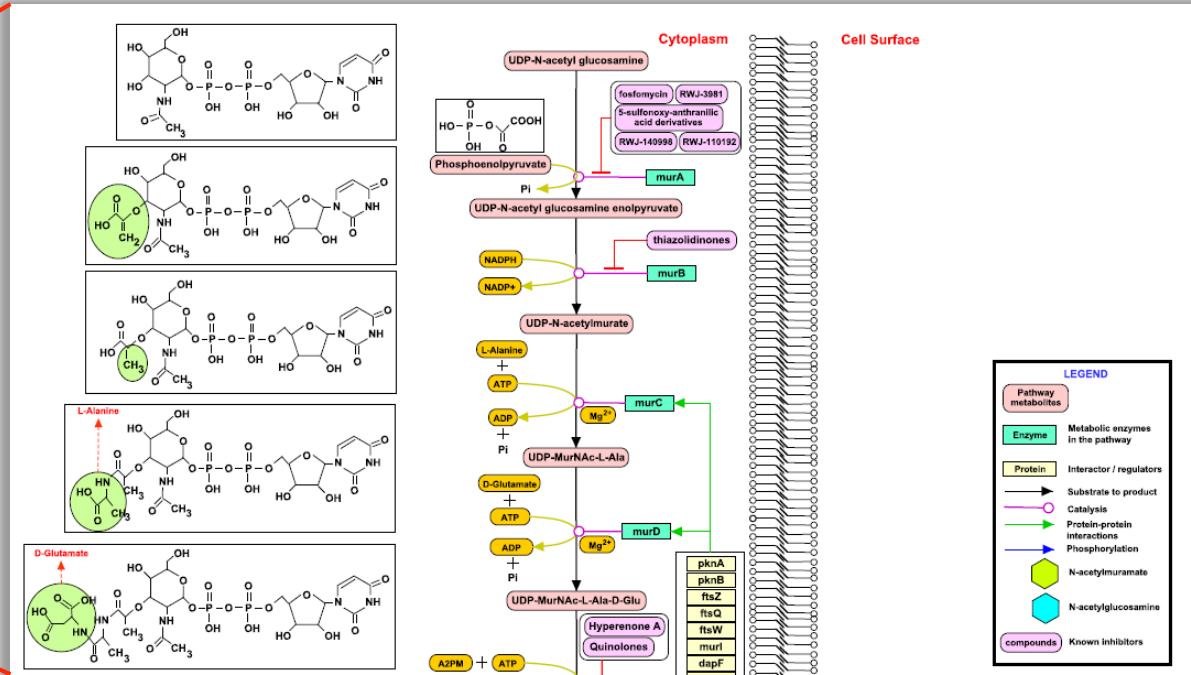
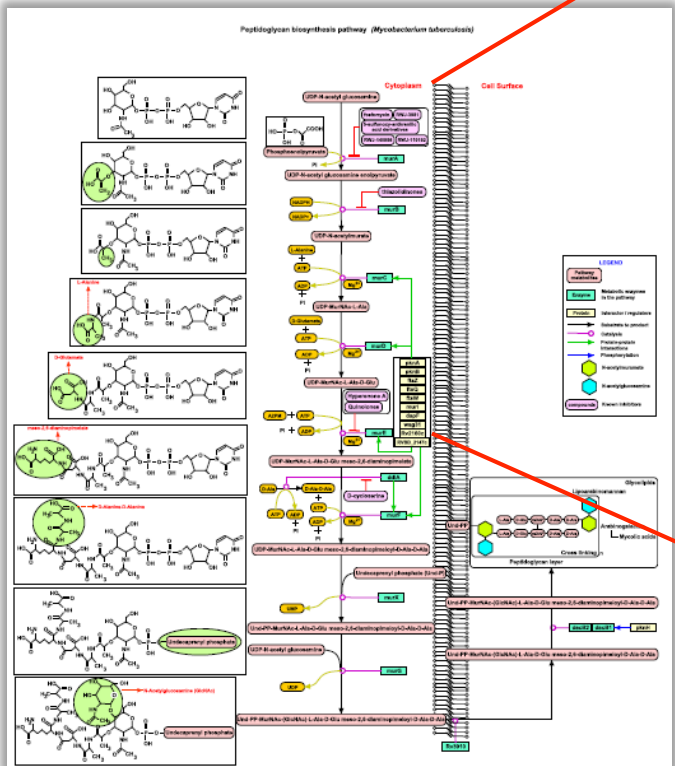




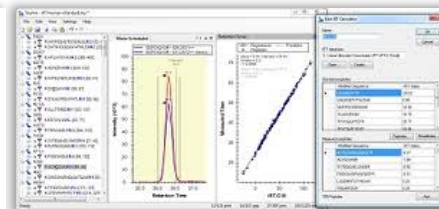
# Multi-omics Workflow: Transcriptomics + Metabolomics → Targeted Proteomics



# Enhancing Targeted Proteomics Experiments: Integrating Prior knowledge and Community-based Tools into the IB Ecosystem



Pathway courtesy of the Institute of Bioinformatics, Bangalore, India



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# Case Study: Looking for Evidence of Pathway Intervention to Advance the Search for New Breast Cancer Chemopreventives

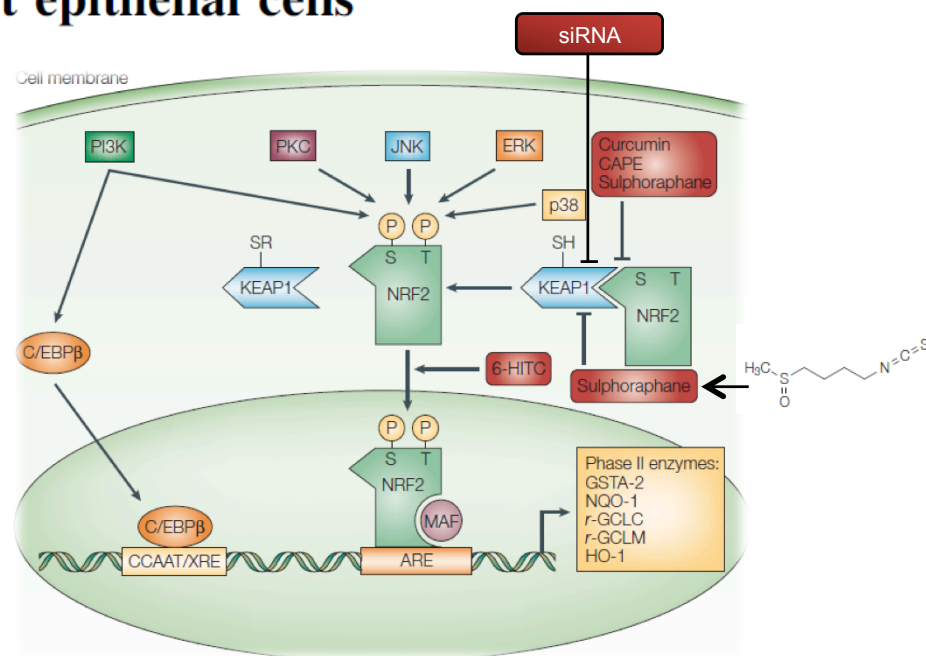
Breast Cancer Res Treat  
DOI 10.1007/s10549-011-1536-9

PRECLINICAL STUDY

## Transcriptomic and proteomic profiling of KEAP1 disrupted and sulforaphane-treated human breast epithelial cells reveals common expression profiles

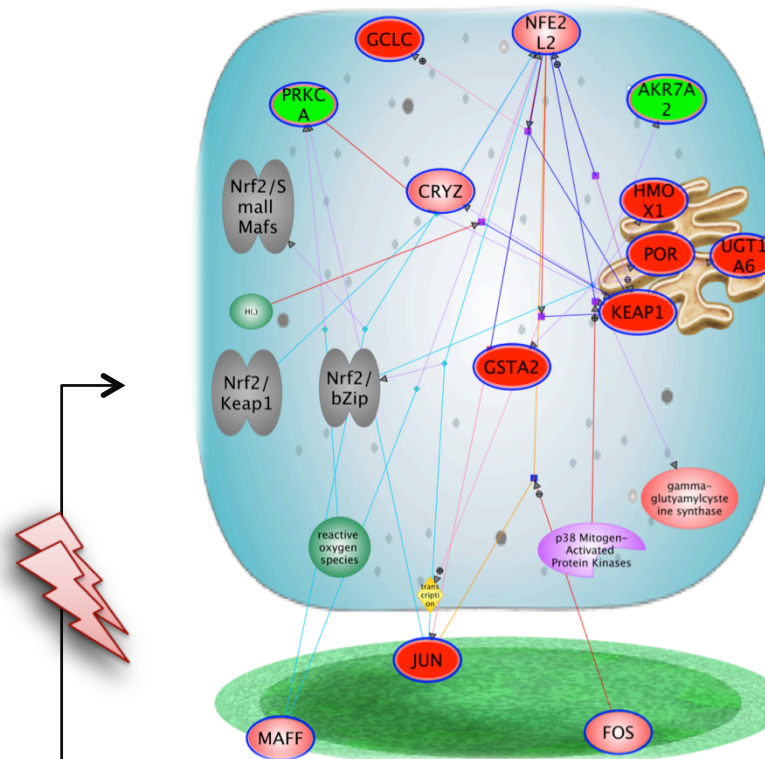
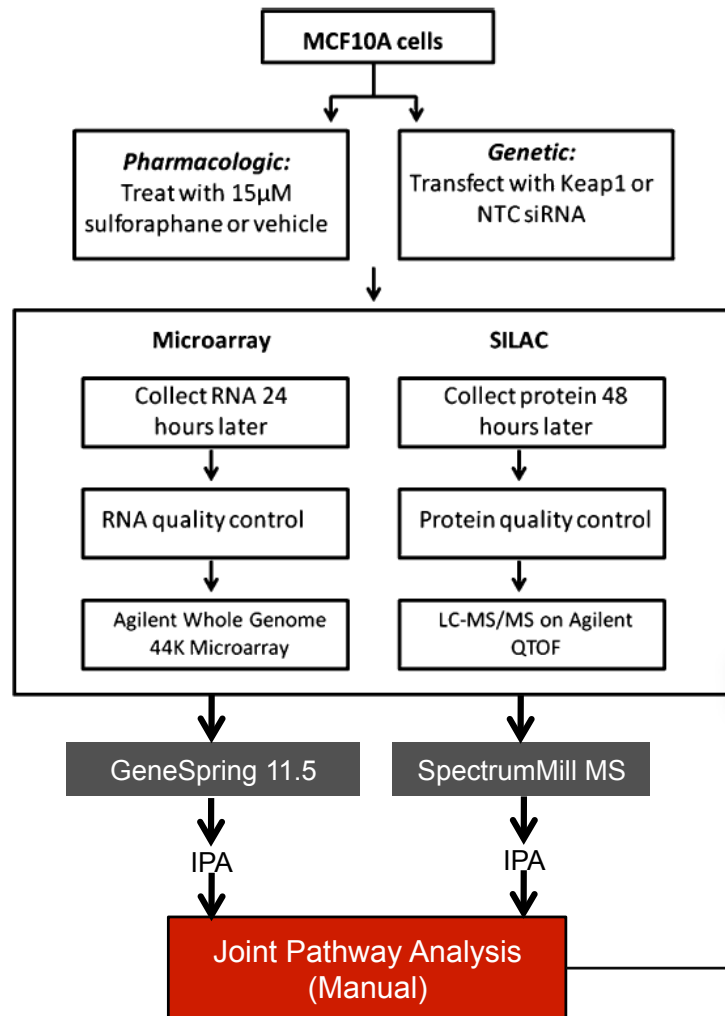
Abena S. Agyeman · Raghothama Chaerkady ·  
Patrick G. Shaw · Nancy E. Davidson · Kala Visvanathan ·  
Akhilesh Pandey · Thomas W. Kensler

Received: 15 April 2011 / Accepted: 17 April 2011  
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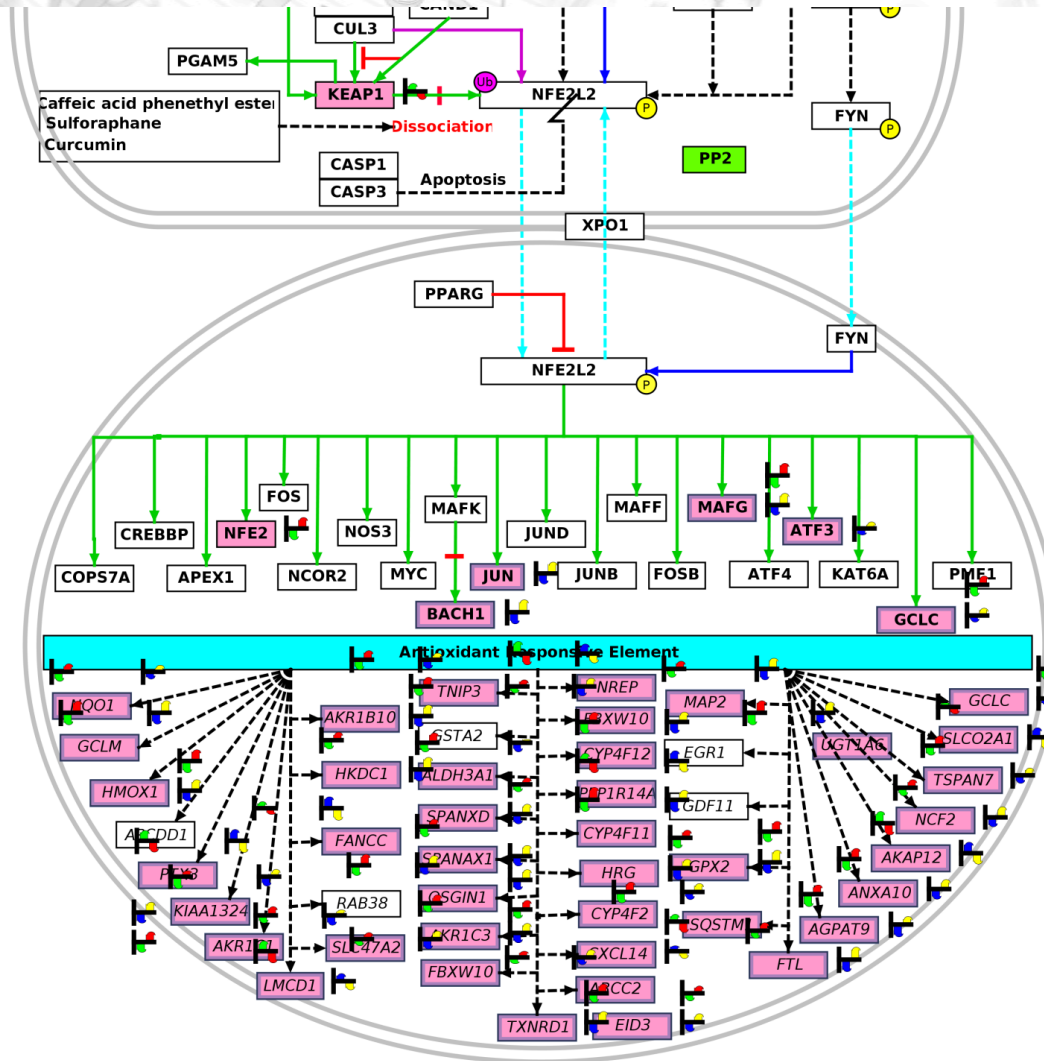


Suhr Y-J. *Nat. Rev. Cancer*, 2003, 768-80.

# The Workflow: Manual

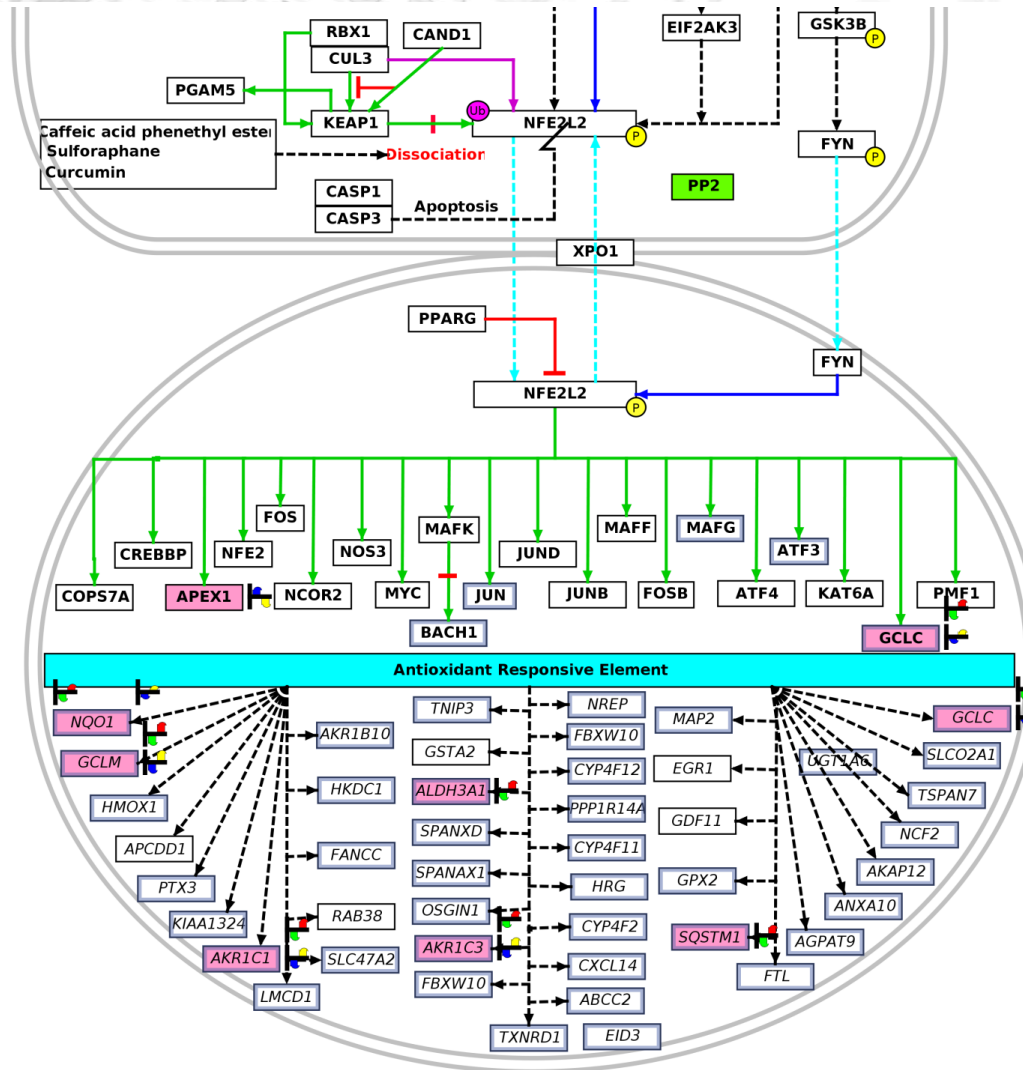


# Keap1- Nrf2 Pathway: Transcriptome in GeneSpring Integrated Biology

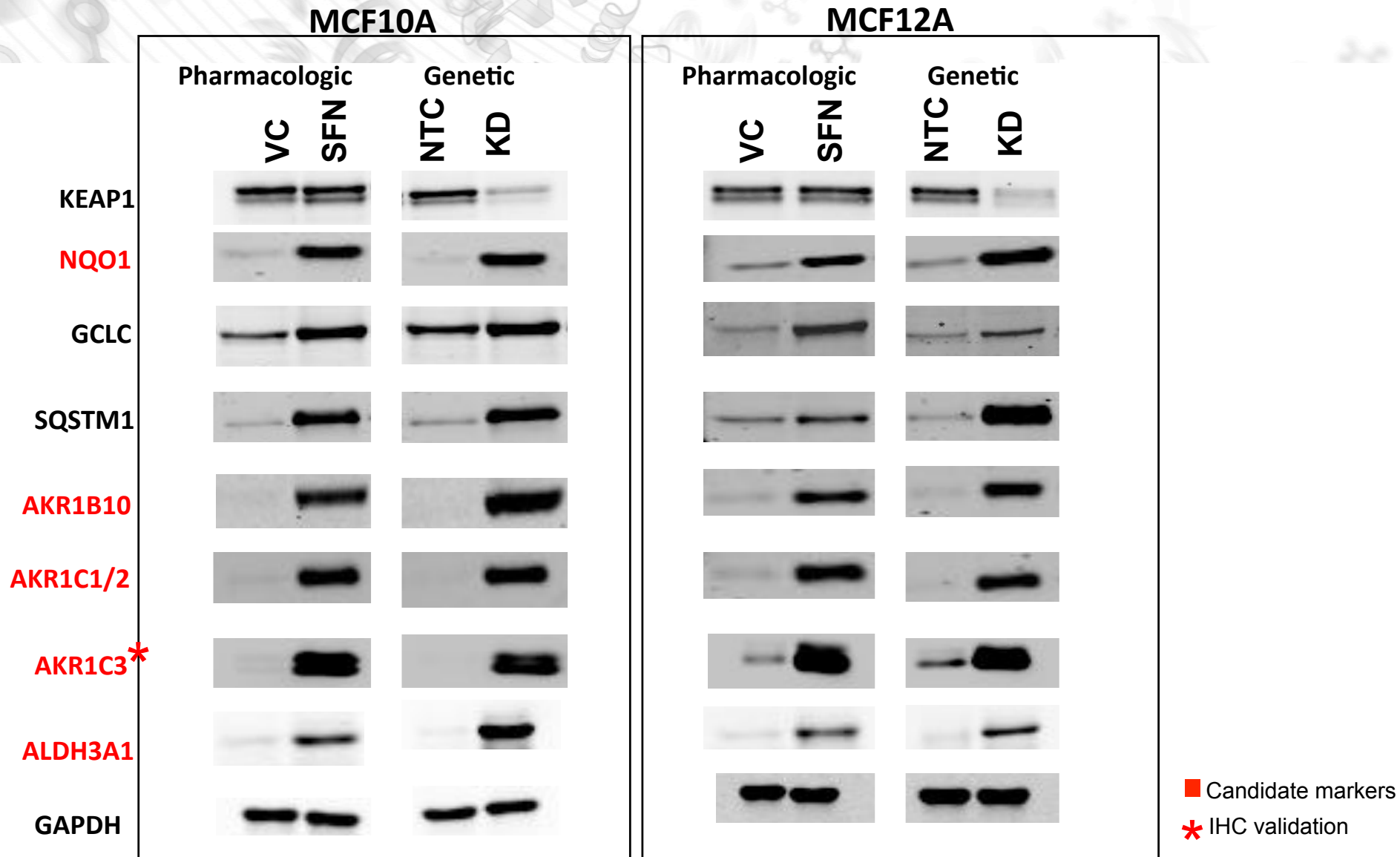


- Control
- Knockdown
- Control
- SFN treated

# Keap1- Nrf2 Pathway: Proteome in GeneSpring Integrated Biology



# Western blot validation of SILAC data





# Insights from Integrated Analysis

- “Big Picture” view
- Identify activated pathways  
...including known cytoprotective pathways
- Identify off-target behaviors, and theorize as to mechanism  
(or even toxicity)  
(e.g. SFN does more than inhibit KEAP1)

## Key Pathways from SFN Exploration

- Xenobiotic metabolism and antioxidants
- Glutathione metabolism
- Carbohydrate metabolism and NAD(P)H generation

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# Pathway-directed re-mining of data or designing the next experiment

Propose new experiments based on pathway analysis

1. Re-mine originally acquired (or legacy) untargeted metabolomics data based on pathway analysis—create db
2. Design new experiments (metabolite, protein or genes) based on pathway results interpretation

**PCDL**

**Build custom metabolite database**

**Multi-Omic Analysis in GeneSpring**

**Agilent Spectrum Mill - Peptide Selector - (none)**

**Spectrum Mill**

**Export protein IDs to Peptide Selector for targeted MS/MS**

**eArray**

**Upload select pathway genes for custom microarray or NGS design**

# Thank you!

## Acknowledgements

Prof. Kyu Rhee  
Cornell Weil Medical College

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Johns Hopkins University

## Agilent Technologies

- Steve Fischer, Metabolomics & Proteomics
- Chris Miller & Alex Apfel, Proteomics/IB
- Ravi Krovvidi, Agilent LSCI
- Antoni Wandycz & Team, GeneSpring
- Allan Kuchinsky, Agilent Labs

## Learn more!

New Agilent IB Website:

<http://biology.chem.agilent.com/>

The screenshot displays the Agilent Integrated Biology website. The top navigation bar includes 'PRODUCTS & SERVICES', 'SOLUTIONS', 'TECHNICAL SUPPORT', 'LIBRARY', and 'TRAINING & EVENTS'. The main content area is titled 'Integrated Biology' and features three video thumbnails with accompanying text:

- An Integrated Transcriptomic and Proteomic Analysis to Determine the Pharmacodynamic Action of Sulforaphane**  
Akhilesh Pandey, M.D., Ph.D.  
The Johns Hopkins University, USA
- Translational Proteomics and Metabolomics: A Case Study in Ankylosing Spondylitis**  
Benedikt M. Kessler, Ph.D.  
University of Oxford, UK
- Tuberculosis: A Global Health Pandemic**  
Kyu Rhee, M.D., Ph.D.  
Weill Cornell Medical College, USA

The right sidebar contains a 'NEWS' section with links for 'Product Announcements', 'Upcoming Events', and 'Special Offers'. Below this is a 'Connect with Agilent' section with social media icons for Facebook, Twitter, LinkedIn, YouTube, and RSS. At the bottom right, there is a promotional banner for 'ILLUMINATING PATHWAY-BASED DISCOVERY' with the text 'FREE ACCESS TO AGILENT SPONSORED PRESENTATIONS AT SOT 2012' and a 'WATCH NOW' button.