

DESCRIPTION OF DRUGMATRIX DATABASE CONTENT

VERSION 5.0

DESCRIPTION OF DATABASE CONTENT FOR DRUGMATRIX 5.0

DrugMatrix[®] is a chemogenomics reference database designed and built specifically for applications in pharmaceutical research and development. It contains microarray gene expression data of organ tissues from drug treated rats and primary rat hepatocytes. Iconix has selected a subset of compounds among the known drugs, withdrawn and failed molecules, and biochemical and toxicological standards as benchmark molecules to build this reference database. In DM release 5.0, 660 compounds have been profiled by microarray gene expression. Each of the 660 compounds profiled by microarray gene expression is also profiled in a suite of 127 pharmacological assays and is associated with detailed compound curation. Each of the 5004 microarray experiments in the database is a biological triplicate and is associated with clinical chemistry, hematology, and organ histopathology data of the treated animal. The assembled chemogenomic profiles on each benchmark molecule are made available in an integrated informatics system that allows candidate compounds to be compared, analyzed and prioritized using sophisticated bio- and chemoinformatics tools, including 135 manually curated DrugMatrix pathways.

1. Summary of Major Data Domains

1a. Curated Information

- Chemical structures for 8359 compounds
- Molecular target identified for 3278 compounds
- Full curation, including chemical structure, solubility, protein binding, molecular target, indication, therapeutic class, product class, adverse effects, molecular/in vitro/clinical pharmacology, and animal toxicity data has been completed for 1064 compounds
- 135 DrugMatrix pathways representing molecular and cellular processes relevant to toxicity and pharmacology

1b. Experimental Information

- DM 5.0 contains in vitro Molecular Pharmacology profiles for 865 compounds. Each compound has been profiled in an *in vitro* molecular pharmacological bioassay panel of known target proteins, selected and screened in partnership with MDS Pharma Services. The list of assays that comprises the Profile is shown below and is available as the “DrugMatrixScreen” from MDS Pharma: <http://discovery.mdsps.com/Catalog/Assays/Packages/AssayPackage.aspx?id=89>.

Catalog #	Assay Name	Species
<u>200720</u>	Adenosine A ₃	Human
<u>200610</u>	Adenosine A _{2A}	Human
<u>200510</u>	Adenosine A ₁	Human
<u>203620</u>	Adrenergic α _{2A}	Human
<u>203710</u>	Adrenergic α _{2B}	Human

Catalog #	Assay Name	Species
<u>203800</u>	Adrenergic α_{2C}	Human
<u>203100</u>	Adrenergic α_{1A}	Rat
<u>203200</u>	Adrenergic α_{1B}	Rat
<u>203400</u>	Adrenergic α_{1D}	Human
<u>204110</u>	Adrenergic β_2	Human
<u>204200</u>	Adrenergic β_3	Human
<u>204010</u>	Adrenergic β_1	Human
<u>285010</u>	Androgen (Testosterone) AR	Rat
<u>210110</u>	Angiotensin AT ₂	Human
<u>211000</u>	Atrial Natriuretic Factor (ANF)	Guinea pig
<u>212610</u>	Bradykinin B ₂	Human
<u>213610</u>	Calcitonin	Human
<u>214510</u>	Calcium Channel L-Type, Benzothiazepine	Rat
<u>214600</u>	Calcium Channel L-Type, Dihydropyridine	Rat
<u>215000</u>	Calcium Channel L-Type, Phenylalkylamine	Rat
<u>217020</u>	Cannabinoid CB ₁	Human
<u>217550</u>	Chemokine CCR2B	Human
<u>217650</u>	Chemokine CCR4	Human
<u>217700</u>	Chemokine CCR5	Human
<u>244500</u>	Chemokine CXCR2 (IL-8R _B)	Human
<u>218010</u>	Cholecystokinin CCK ₁ (CCK _A)	Human
<u>219800</u>	Dopamine D ₃	Human
<u>219600</u>	Dopamine D _{2L}	Human
<u>219900</u>	Dopamine D _{4,2}	Human
<u>219500</u>	Dopamine D ₁	Human
<u>224010</u>	Endothelin ET _A	Human
<u>226010</u>	Estrogen ER α	Human
<u>226500</u>	GABA _A , Agonist Site	Rat
<u>226600</u>	GABA _A , Benzodiazepine, Central, Flunitrazepam	Rat
<u>226810</u>	GABA _A , Chloride Channel, TBOB	Rat
<u>232010</u>	Glucocorticoid	Human
<u>232600</u>	Glutamate, AMPA	Rat
<u>232700</u>	Glutamate, Kainate	Rat
<u>232810</u>	Glutamate, NMDA, Agonism	Rat
<u>233000</u>	Glutamate, NMDA, Phencyclidine	Rat
<u>239000</u>	Glycine, Strychnine-Sensitive	Rat
<u>239710</u>	Histamine H ₂	Human
<u>239610</u>	Histamine H ₁	Human
<u>241000</u>	Imidazoline I ₂ , Central	Rat
<u>243000</u>	Insulin	Rat
<u>243510</u>	Interleukin IL-1	Mouse
<u>250460</u>	Leukotriene, Cysteinyl CysLT ₁	Human
<u>251300</u>	Melanocortin MC ₃	Human
<u>251350</u>	Melanocortin MC ₄	Human
<u>251400</u>	Melanocortin MC ₅	Human
<u>252710</u>	Muscarinic M ₂	Human
<u>252810</u>	Muscarinic M ₃	Human
<u>252910</u>	Muscarinic M ₄	Human
<u>252610</u>	Muscarinic M ₁	Human

Catalog #	Assay Name	Species
<u>253010</u>	Muscarinic M ₅	Human
<u>257110</u>	Neuropeptide Y Y ₂	Human
<u>257010</u>	Neuropeptide Y Y ₁	Human
<u>258590</u>	Nicotinic Acetylcholine	Human
<u>260110</u>	Opiate δ (OP1, DOP)	Human
<u>260210</u>	Opiate κ (OP2, KOP)	Human
<u>260410</u>	Opiate μ (OP3, MOP)	Human
<u>264500</u>	Phorbol Ester	Mouse
<u>265010</u>	Platelet Activating Factor (PAF)	Human
<u>265200</u>	Platelet-Derived Growth Factor (PDGF)	Mouse
<u>265600</u>	Potassium Channel [K _{ATP}]	Hamster
<u>268000</u>	Progesterone	Bovine
<u>268700</u>	Purinergic P _{2X}	Rabbit
<u>271910</u>	Serotonin (5-Hydroxytryptamine) 5-HT ₃	Human
<u>271700</u>	Serotonin (5-Hydroxytryptamine) 5-HT _{2B}	Human
<u>271800</u>	Serotonin (5-Hydroxytryptamine) 5-HT _{2C}	Human
<u>272000</u>	Serotonin (5-Hydroxytryptamine) 5-HT ₄	Guinea pig
<u>271110</u>	Serotonin (5-Hydroxytryptamine) 5-HT _{1A}	Human
<u>272200</u>	Serotonin (5-Hydroxytryptamine) 5-HT ₆	Human
<u>278200</u>	Sigma σ ₂	Rat
<u>278110</u>	Sigma σ ₁	Human
<u>279510</u>	Sodium Channel, Site 2	Rat
<u>255600</u>	Tachykinin NK ₂	Human
<u>255510</u>	Tachykinin NK ₁	Human
<u>202000</u>	Transporter, Adenosine	Guinea pig
<u>220320</u>	Transporter, Dopamine (DAT)	Human
<u>204410</u>	Transporter, Norepinephrine (NET)	Human
<u>274030</u>	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	Human
<u>286510</u>	Tumor Necrosis Factor (TNF), Non-Selective	Human
<u>286900</u>	Vascular Endothelial Growth Factor (VEGF)	Human
<u>287010</u>	Vasoactive Intestinal Peptide VIP ₁	Human
<u>287520</u>	Vasopressin V _{1A}	Human
<u>107000</u>	Aldose Reductase	Rat
<u>107710</u>	ATPase, Na ⁺ /K ⁺ , Heart, Pig	Pig
<u>126000</u>	Beta-Lactamase	Bacteria
<u>112000</u>	Carbonic Anhydrase	Human
<u>104010</u>	Cholinesterase, Acetyl, ACES	Human
<u>116020</u>	Cyclooxygenase COX-1	Human
<u>118010</u>	Cyclooxygenase COX-2	Human
<u>118050</u>	CYP450, 1A2	Human
<u>118070</u>	CYP450, 2C19	Human
<u>118060</u>	CYP450, 2C9	Human
<u>118080</u>	CYP450, 2D6	Human
<u>118090</u>	CYP450, 3A4	Human
<u>124000</u>	HMG-CoA Reductase	Rat
<u>132000</u>	Leukotriene LTC ₄ Synthase	Guinea pig
<u>138000</u>	Lipoxygenase 15-LO	Rabbit
<u>140010</u>	Monoamine Oxidase MAO-A	Human
<u>144000</u>	Nitric Oxide Synthase, Inducible (iNOS)	Mouse

Catalog #	Assay Name	Species
<u>142000</u>	Nitric Oxide Synthase, Neuronal (nNOS)	Rat
<u>107300</u>	Peptidase, Angiotensin Converting Enzyme	Rabbit
<u>163000</u>	Peptidase, CASP1 (Caspase 1)	Human
<u>112510</u>	Peptidase, CTSG (Cathepsin G)	Human
<u>166000</u>	Peptidase, ELA2 (Neutrophil Elastase 2)	Human
<u>114110</u>	Peptidase, Matrix Metalloproteinase-1 (MMP-1)	Human
<u>114910</u>	Peptidase, Matrix Metalloproteinase-9 (MMP-9)	Human
<u>152000</u>	Phosphodiesterase PDE3	Human
<u>154000</u>	Phosphodiesterase PDE4	Human
<u>156000</u>	Phosphodiesterase PDE5	Human
<u>171120</u>	Protein Serine/Threonine Kinase, MAPK1 (ERK2)	Human
<u>176600</u>	Protein Serine/Threonine Kinase, MAPK14 (p38 α)	Human
<u>171000</u>	Protein Serine/Threonine Kinase, MAPK3 (ERK1)	Human
<u>180010</u>	Protein Serine/Threonine Kinase, PRKCA (PKC α)	Human
<u>188020</u>	Protein Serine/Threonine Phosphatase, PPP3CA (Calcineurin, PP2B)	Human
<u>170010</u>	Protein Tyrosine Kinase, EGF Receptor	Human
<u>174010</u>	Protein Tyrosine Kinase, ERBB2 (HER2)	Human
<u>172010</u>	Protein Tyrosine Kinase, Fyn	Bovine
<u>176010</u>	Protein Tyrosine Kinase, LCK	Human
<u>190010</u>	Protein Tyrosine Phosphatase, PTPRC (CD45)	Human
<u>194020</u>	Thromboxane Synthase	Human

Compounds with percent inhibition greater than 70% are profiled with a primary screen only, in duplicate at 10^{-5} M. For tests with >70% inhibition, an 8-point semi-quantitative concentration-response is performed to estimate the IC₅₀ using 1/2 log intervals between 10^{-5} and 10^{-6} , 10^{-7} , 3×10^{-9} M.

Each protein in the assay is linked to the gene identifier that codes for the protein.

- The database contains in vivo Pathology Profiles, including body and organ weights, clinical observations, clinical chemistry, hematology and histopathology data for 659 compounds representing 5245 compound-dose-time combinations (treatments) in biological triplicate. This includes 40 clinical pathology endpoints, 59 histopathology diagnoses in liver, 31 in kidney and 25 in heart.
- The database contains in vivo and in vitro Expression Profiles for 660 compounds, representing 1079 compound-tissue combinations, and 4103 compound-dose-time-tissue combinations (treatments) in biological triplicate:

Tissue	Compounds	Treatments
LIVER	346	1695
KIDNEY	249	906
HEART	209	629
BONE MARROW	73	325
SPLEEN	39	180
BRAIN	18	65
THIGH MUSCLE	14	29

Tissue	Compounds	Treatments
INTESTINE	7	20
PRIMARY HEPATOCYTE	124	254
Total	1079 (660 unique)	4103

2. Curated Compound Information

Compound-associated information is curated into database fields with predefined vocabulary and standardized units so that the curated properties are easily searchable in combination. For example, one can search for anti-emetics under therapeutic class, browse through indications, protein targets, and adverse effects associated with the retrieved compounds and select one that is most effective with the least side effects. DrugMatrix 5.0 includes:

- Chemical structures of 8359 compounds registered in the industry-standard MDL ISIS database.
- Molecular weight, molecular formula for all compounds with molecular structures.
- 56 compounds including TNF α , the interleukins, and some chemically undefined mixtures of compounds that were registered without chemical structures.
- Compound names, synonyms, CAS numbers
- Physical properties including CLogPs, calculated and experimental aqueous solubility data if available, as shown below.

Physical Properties	Number of compounds
CLogP, ALogP, IALogP, Kowwin	6224
ALogpS, IALogpS	6224
Aqueous solubility (mg/L)	627
pKa	607

- Precomputed lists of structurally similar compounds using Tanimoto similarity score
- **Development Status**

The development status category tracks the status of a compound in a typical drug development pipeline. A vocabulary list of 61 terms in 16 categories has been developed for curation. A compound can be “FDA Approved”, “Marketed”, or “Withdrawn” at various development phases. Compounds which are not drugs may be “Toxicants” or “Biochemical” standards as summarized in the table below.

Development Status	Number of compounds
Biochemical standard	870

Development Status	Number of compounds
Botanical	6
Clinical, Phase I	1603
Clinical, Phase II/III	1031
Discontinued at Phase I	19
Discontinued at Phase II/III	83
Discontinued at preclinical toxicology	22
Environmental Pollutant	37
Laboratory Testing	759
Launched outside US, not approved by FDA	28
Launched outside US, not listed by FDA	612
Not approved by US FDA	29
Preclinical	2871
Preservative, US FDA approved	1
Toxicology standard	151
US FDA Approved	1314
Withdrawn by FDA	63
Withdrawn by Manufacturer	28

- **Structure Activity Classes assigned for 2415 compounds**

Each compound studied in DrugMatrix is assigned a structure activity class based on the molecular target associated with its approved clinical use, as reported in the literature. When the molecular target is not clearly defined, the compound is classified based on the accepted mechanism of action and/or clinical indications. When appropriate or necessary, a compound is classified with both its mechanism of action and molecular target in order to bin compounds into more general groups. When diverse chemical structure types are active against the same protein target, the compounds are sub-grouped into pharmacophore types, and a structure activity class is assigned to each sub-group based on the molecular target and chemical structure type.

A total of 409 structure-activity class terms have been assigned across 2415 compounds. The 660 compounds with gene expression profiles fall into 264 structure-activity classes.

- **Activity Class**

Each compound studied in DrugMatrix is grouped into an activity class, which represents a more generic compound annotation than structure activity class. Compounds are grouped together based on having structure activity class annotations that are related by a common therapeutic activity (i.e. anti-inflammatory) or toxicological activity (DNA damager). Structures are not considered when grouping compounds, such that unrelated structures that act through a common molecular target are grouped together. Likewise, compounds with distinct, but pharmacologically related targets, are also grouped together under a single activity class term. Compounds with a structure activity annotation

unrelated by therapeutic or toxicological activity are simply annotated with their structure activity until such time that related compounds are added to the database.

A total of 91 activity class terms have been assigned across the 264 structure activity classes for the 660 compounds with gene expression profiles.

- **Protein Target curated for 3278 of the compounds**

The direct molecular targets (the protein or proteins that a compound is known to bind directly) have been assigned for approximately half of the compounds. This information is curated from 1) Annual Reviews of Medicinal Chemistry for older and existing drugs, 2) Nature Review of Drug Discovery for drugs in discovery or under development, 3) pharmacology text books or primary literature for other compounds.

A total of 5000 compound-target pairs have been curated for 3278 compounds involving 442 protein targets mapped to 1527 genes.

- **Mode Class (compound's effect on its protein targets)**

A collection of 25 terms has been established to describe a compounds' effect on its protein target(s). Examples of mode classes are "Channel Blocker, selective", "Endogenous Receptor/Channel Ligand", "Enzyme Inhibitor, Non-selective", "Receptor Ligand Antagonist, Non-selective", and "Receptor Modulator".

A total of 25 mode class terms have been assigned for 3591 compounds

- **Mechanism (compound's physiological effects)**

A collection of 74 terms have been established to describe a compounds' physiological or phenotypic effects. This is an approach that bins compounds with different protein targets into larger groups that may point to the same therapeutic use, as well as a providing a holding place for compounds with known physiological effects where the direct protein-target is not well defined. Examples of mechanisms are "Stimulate neural transmitter release", "Reduce bronchoconstriction", "Inhibit bacterial cell wall biosynthesis", "Trigger insulin release in pancreatic beta cells", and "Stimulate immune system cells".

The mechanisms of 3664 compounds have been curated.

- **Molecular and in vitro Pharmacology Data for 1582 compounds**

Curated molecular pharmacology and in vitro pharmacology data are reported in standardized units. Curated molecular pharmacology data include the IC₅₀, EC₅₀, K_i, K_b, or K_d against a compound's protein target. These are data obtained from cell-free enzyme assays or receptor/channel binding assays. Curated in vitro pharmacology data include MIC, MIC₅₀, MIC₉₀, IC₅₀, and EC₅₀ for a compounds' effect in a whole cell assay. The read-out of these assays may be the compound's effect on its protein target, a biochemical pathway, or a signal transduction cascade. Each curated molecular or in vitro pharmacology data point is accompanied by essential assay conditions such as source of the target

protein, radio-ligand used in a displacement assay, cell type, configuration of the whole-cell assay, and literature references.

- **Animal Toxicity Data for 1866 compounds**

Animal toxicity data is extracted from RTECS (<http://www.cdc.gov/niosh/rtecs/default.html>) if available, otherwise the information is curated from the primary literature or published NDAs.

- **Animal Pharmacology Data for 1077 compounds**

The effective dose against the compound's intended protein target and the AUC is curated from the primary literature.

- **Clinical (human) Pharmacology Data for 962 compounds**

The AUC, C_{max} , half-life, clearance and effective dose ranges is curated from the PDR, the compound NDA or the Clinical Pharmacology database (<http://www.clinicalpharmacology.com/Default.asp>).

- **Clinical Indication**

Approved (or proposed) clinical indications are curated from PDR, NDA, or the clinical pharmacology database. A comprehensive vocabulary list of 1822 has been created for curation.

A total of 6408 compound-indication associations have been curated including 1084 indication terms and 2925 compounds.

- **Therapeutic Class**

The purpose of the "therapeutic class" category is to classify a compound based on its therapeutic uses with respect to its indications. A comprehensive list of 120 therapeutic classes, such as "Anti-diabetic Agents", "General Anesthetics, Intravenous" or "Anti-bacterials, Systemic", has been established for this purpose. The same therapeutic class may be associated with several indications of a given compound. For example, the therapeutic class "Anti-bacterials, Systemic" may be associated with the indications "Mycobacterium tuberculosis", "Erythema Nodosum Leprosum (ENL)", "Leprosy", and "Atypical Mycobacterial Diseases" of the same compound.

A total of 105 therapeutic class terms have been associated with 3255 compounds.

- **Product Class**

Product class, such as "Hormones, Endocrine and Metabolic", "Central Nervous System (CNS)", or "Anti-infectives" is a general industry classification of the drug. A vocabulary list of 24 terms has been established for product class. Each therapeutic class is pre-associated with a product class in the curation database so that when a therapeutic class is selected by the curator, the product class is determined automatically. Toxicants and Biochemicals are examples of product classes with no associated therapeutic class.

A total 24 product class terms have been associated with 3705 compounds.

- **Adverse Effects**

A comprehensive vocabulary list of 1,600 adverse drug effects has been created for the purpose of curating adverse drug effects. Each of the adverse effects is associated with a tissue/organ ID and given an “Adverse Effect Group Name”. Each of the adverse effects is also assigned a severity index based on the seriousness of the adverse effect or clinical event. Genotoxicity, mutagenicity, carcinogenicity, and life threatening or potential life threatening events are given a score of “SSS”, serious but manageable events are given a score of “SS”, and measurable but not serious events are given a score of “S”.

Adverse effects information are curated from PDR, drug package insert, published NDA, or primary literature. A frequency score, 1- most frequent (>10% of the time), 2- less common (5-10% of the time), and 3- rare (<5% of the time) is associated with each compound-adverse effect pair according to the information in the documents. The “Adverse Effect” associated with each drug in DrugMatrix is thus the curated adverse effect terms for each drug prefixed with the associated tissue/organ ID and the frequency score (e.g., LIV_1_Increased Liver Enzymes; CVS_3_Bradycardia). If an “SSS” severity label is associated with an adverse effect, then the curated adverse effect appears in “Tissue Toxicity” of the compound, while the “Adverse Effect Group Name” appears in “Known Toxicity” of the compound.

A total of 19877 compound-adverse effect pairs have been curated involving 1366 adverse-effect terms and 1121 compounds.

- **Known Toxicity and Tissue Toxicity**

These two categories contain highlights and summary information of serious drug adverse effects from the Adverse Effect curation described above. If an “SSS” severity label is associated with an adverse effect, then the curated adverse effect appears in “Tissue Toxicity” of the compound, while the “Adverse Effect Group Name” appears in “Known Toxicity” of the compound.

794 compounds are associated with severe adverse effects including 507 FDA approved and 59 withdrawn drugs.

3. Curated Gene Information

Gene curation associated with probes on Affymetrix RG230-2, RAE230A, and RGU34A, and GE RU1 chips have been extracted from the NCBI gene database. This includes:

- Probe associated information includes RefSeqID (probe accession, mRNA sequence), GeneID (previously LocusID), UnigenID, and Affymetrix or GE probe ID/Name.
- Gene-associated information includes GeneID, UnigenID, Official Gene Name, Official Gene Symbol, and GeneOntology
- Links to pathways
- Links to compound protein target

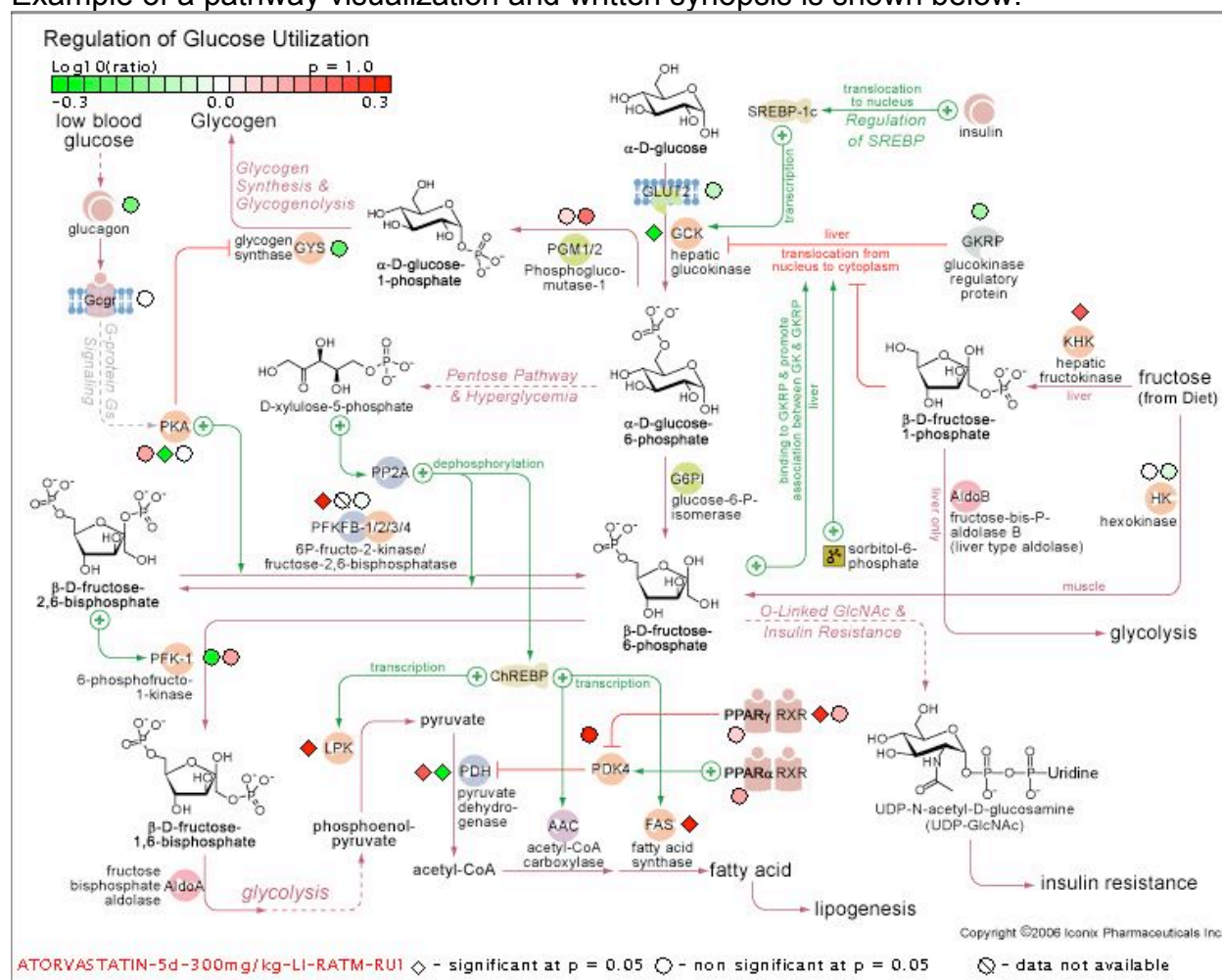
4. Curated Pathways

The suite of 135 DrugMatrix pathways have been designed to help users better understand the action and consequences of chemical exposure on mammalian systems at the molecular level. Thus, pathways associated with mammalian functions known to be affected by drug treatments are curated, including: 1) well defined signal transduction cascades, 2) biosynthesis of key signaling or building block molecules, and 3) regulation of specific biological processes or responses relevant to toxicity and pharmacology. Each DrugMatrix pathway is a functionally connected metabolic response or process derived from an in-depth literature review of the subject area. Details of concurrent or alternative processes of a complex physiological response are often curated into separate pathways. Wherever relevant and possible, physiological responses are included in the pathway drawings. Associated with each pathway drawing is the list of references used for curation and a 2000 - 4000 word synopsis written specifically for the pathway.

Existing pathways are revised as new or missed information is encountered during the curation process. Current articles in Science, Nature, Nature Review Drug Discovery, Nature Medicine, Cell, and Cell Metabolism are monitored for new pathway themes, and to keep the existing pathways up to date. DrugMatrix 5.0 includes:

- Pathway genes are mapped to probes on microarray chips.
- Mechanisms are in place to visualize a compound's effect on pathway as shown below.
- Inhibitors or activators of pathway proteins are curated into the pathway drawings.

Example of a pathway visualization and written synopsis is shown below.



After ingestion of carbohydrates, the hepatic portal vein glucose concentration increases to 10-15 mM. The glucose influx into hepatocytes is mediated by GLUT2 ($K_m = 17$ mM) and results in glucose concentration in the hepatocytes proportional to that in the portal vein. GLUT2 is constitutively expressed in liver, kidney, intestine, & pancreas (where glucose flux is high). In contrast to the other hexokinases, GK (glucokinase, hexokinase IV, liver & pancreatic beta cell specific) is not inhibited by its reaction product (glucose-6-phosphate, G6P), instead it is regulated by glucokinase regulatory protein (GKRP). The K_m for glucose in the GK/GKRP system is in the 15-20 mM range. The kinetic characteristic above implies that the rate of G6P formation is proportional to the plasma concentration of glucose. G6P can enter 3 major pathways: glycogen synthesis (see "Glycogen Synthesis & Glycogenolysis"), pentose pathway (see "Pentose Pathway & Hyperglycemia"), and glycolysis (see "Glycolysis" pathway). The major function of glycolysis in liver is to provide pyruvate for fatty acid synthesis. GKRP resides in the nucleus while GK translocates between the nucleus & the cytoplasm. GKRP appears to 1) sequester GK in the nucleus in the fasting state, 2) protecting GK from proteolytic degradation, and 3) maintaining a nuclear reserve of GK that can be quickly released. GKRP over expression in mice protects against the development of diet-induced diabetes.

This is a high level pathway containing different regulation points of glucose metabolism and refers to a number of other related pathways such as glycogen synthesis and glycogenolysis, and glycolysis within the pathway drawing. The expression data of an atorvastatin treatment is visualized on the image.