CHEMICAL REVIEWS

REVIEW

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Bioinformatics and Systems Biology of the Lipidome

Shankar Subramaniam,[*,†,‡,§] Eoin Fahy,[†] Shakti Gupta,[†] Manish Sud,[§] Robert W. Byrnes,[§] Dawn Cotter,[§] Ashok Reddy Dinasarapu,[†] and Mano Ram Maurya[†]

[†]Department of Bioengineering and [‡]Departments of Chemistry and Biochemistry and of Cellular and Molecular Medicine, University of California at San Diego, 9500 Gilman Drive, La Jolla, California 92093, United States

[§]San Diego Supercomputer Center, 9500 Gilman Drive, La Jolla, California 92093, United States

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1. INTRODUCTION

Lipids play an important role in physiology and pathophysiology of living systems. Until a few decades ago, the number of lipid molecules that were chemically characterized was a few hundred at most, and they were cataloged in monographs and compendia.[1] Since the advent of the era of the genome and the proteome, there has been increasing recognition that other macromolecules such as lipids and polysaccharides in living systems display considerable structural diversity, and systematic efforts are under way to identify, characterize, and catalog these molecules. With mass spectrometric techniques coming of age, several thousand distinct molecular species have been identified from living species, and the roles of several of these are beginning to be characterized.[2] Unlike genes and proteins, whose defined alphabets provide the framework for ontologies and classification at the sequence level, lipids and polysaccharides have been characterized for the large part by popular names, with no foundations for systematic classification.

The past two decades have witnessed two major advances in lipid biology. First, mass spectrometry has enabled the identification of thousands of lipid molecular species from cells and tissues, and this has pointed to the important need for developing a

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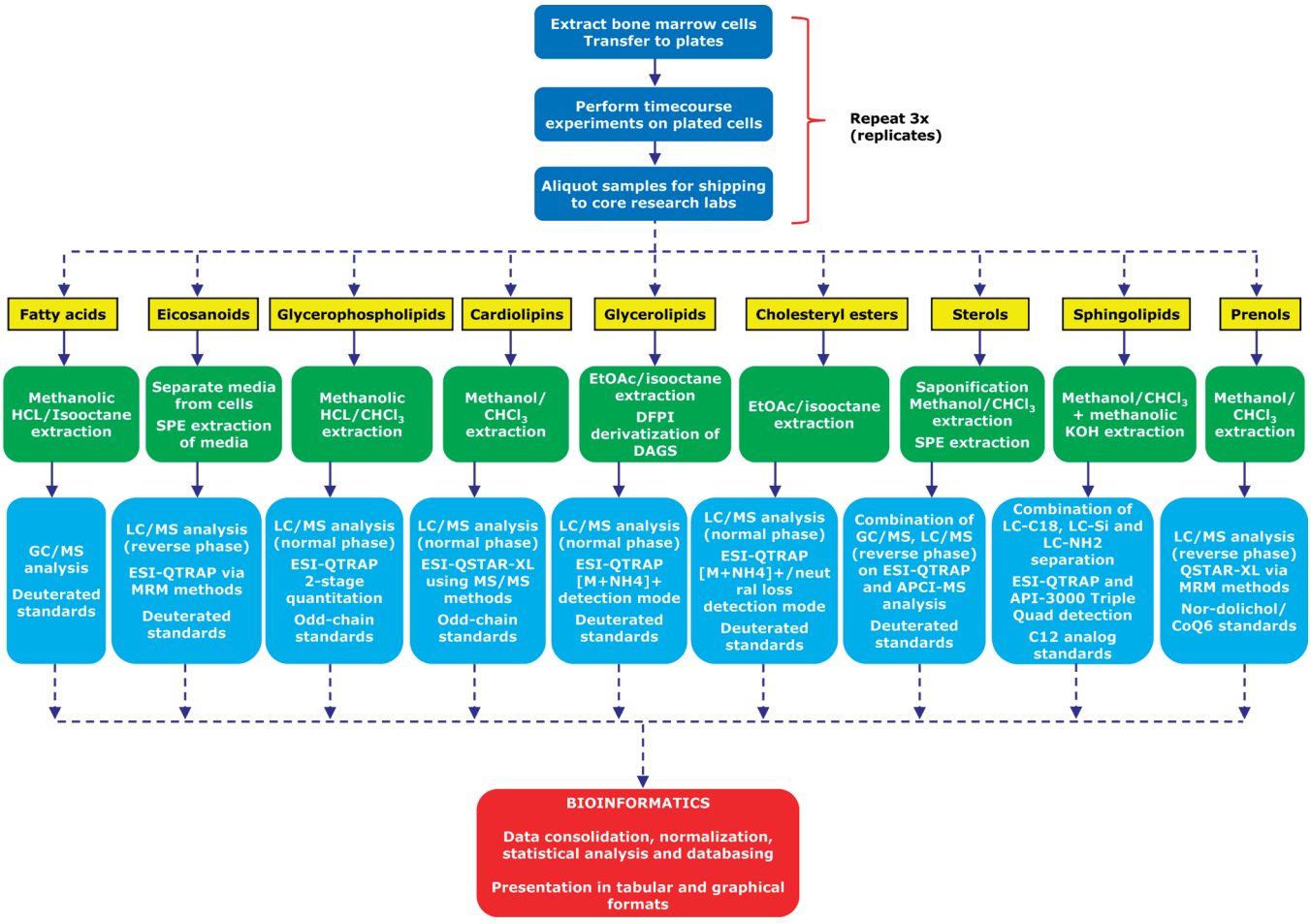

Figure 1. Overview of the process of performing a quantitative lipid analysis of a macrophage cell sample (in this example, a time-course experiment using bone marrow derived macrophages). Extraction methods, LC/GC purification methods, MS acquisition strategies, and quantitative standard approaches are optimized for each lipid class.[2,70,72]

systematic ontology that can rationally name and catalog the molecules. Second, the ability to investigate the functional roles of lipid molecules through systematic phenotypic studies has led to the identification of lipids as extremely important players in physiology and pathophysiology of living species.[3] In combination with proteins and nucleic acids, lipids are integrally involved in biochemical networks that lead to phenotypes such as homeostasis, differentiation, and death of cells and tissues. Any approach to systems characterization of living systems, of necessity, has to include lipids along with other macromolecules and all complex cellular pathways involving lipid molecular species. Systems biology is now extended in its scope to identify biosynthetic and metabolic lipid networks, cellular signaling networks that explicitly include lipid molecules and transcriptional and epigenetic networks where lipids play an integral role.[4]

Several large-scale projects to characterize lipids and their functional roles have been initiated as exemplified by the LIPID MAPS[5] effort. The LIPID MAPS is an exemplary systems biology project that measures cell-wide lipid changes in an attempt to reconstruct biochemical pathways associated with lipid processing and signaling. The cell-wide measurements of components of these pathways include mass spectrometric measurements of lipid changes in response to a stimulus in mammalian cells, changes in transcription profiles in response to a stimulus, and in select cases proteomic changes in response to a stimulus. Figure 1 shows a schematic of the LIPID MAPS experiments related to different lipid categories/pathways and the subsequent processing of the experimental data generated. Network reconstruction efforts rely on organization, analysis, and integration of these data, and this requires a strong bioinformatics and systems biology effort. The former has to include development of a systematic and universal classification and nomenclature system, design and development of lipid, lipid−gene, and lipid−protein databases with appropriate functional annotations, and development of efficient query and analysis systems that can be broadly useful to the biology research community. The latter has to include methods for analysis of large-scale lipid measurements in cells, reconstruction of lipid metabolic and biosynthetic pathways, and quantitative models of lipid fluxes in cells under varied perturbations. In this review, we provide a comprehensive summary of extant developments in lipid bioinformatics and systems biology and discuss the outlook for the future integration of lipidomics into cellular and organismic biology. The sections that follow are delineated into the informatics approaches specific to lipid biology followed by an overview and exemplary approach to analysis of large-scale lipidomic data toward a systems description of mammalian cells.

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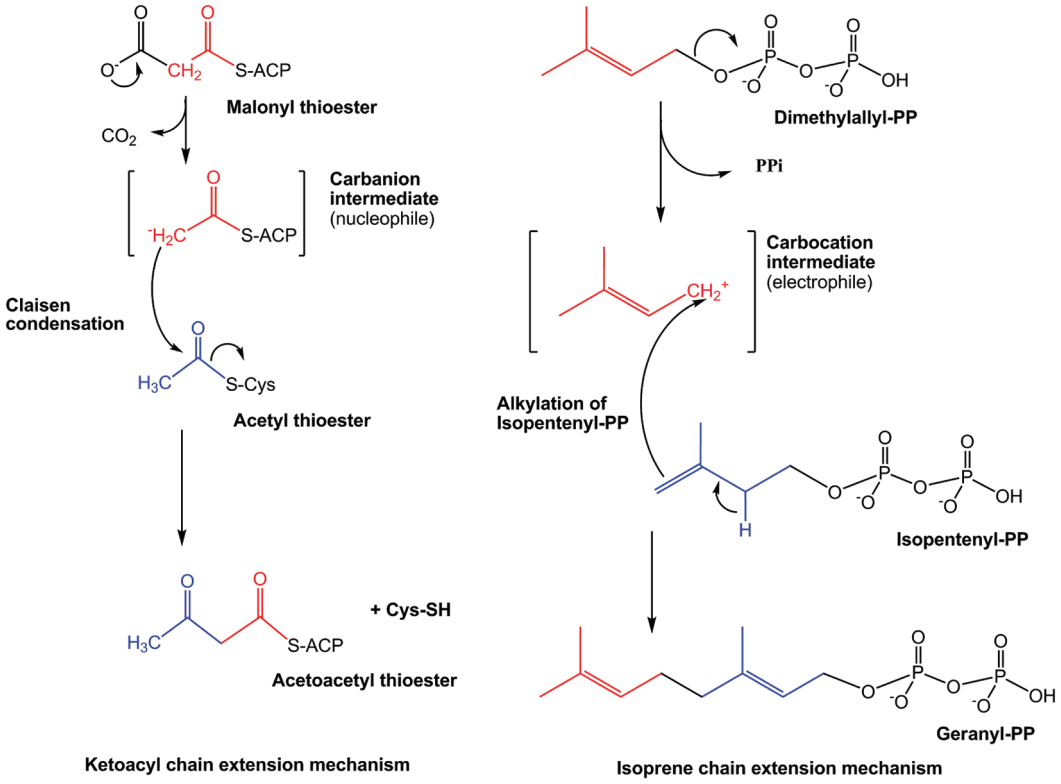

Figure 2. Mechanisms of lipid biosynthesis. Biosynthesis of ketoacyl- and isoprene-containing lipids proceeds by carbanion- and carbocation-mediated chain extension, respectively.[8] Reprinted with permission from ref 8. Copyright 2011 Elsevier Ltd.

2. CLASSIFICATION, ONTOLOGY, NOMENCLATURE, AND STRUCTURE REPRESENTATION OF LIPID MOLECULES

The first step toward classification of lipids is the establishment of an ontology that is extensible, flexible, and scalable. One must be able to classify, name, and represent these molecules in a logical manner which is amenable to data basing and computational manipulation. Lipids have been loosely defined as biological substances that are generally hydrophobic in nature and in many cases soluble in organic solvents.[6] These chemical features are present in a broad range of molecules such as fatty acids, phospholipids, sterols, sphingolipids, terpenes, and others. In view of the fact that lipids comprise an extremely heterogeneous collection of molecules from a structural and functional standpoint, it is not surprising that there are significant differences with regard to the scope and organization of current classification schemes.

2.1. Classification, Ontology, and Nomenclature

To address the lack of a consistent classification and nomenclature methodology for lipids, LIPID MAPS consortium members have developed a comprehensive classification system for lipids.[7] The consortium has taken a more chemistry-based approach and defines lipids as hydrophobic or amphipathic small molecules that may originate entirely or in part by carbanion-based condensations of thioesters (such as fatty acids and polyketides) and/or by carbocation-based condensations of isoprene units (such as prenols and sterols). Figure 2 shows the mechanisms of lipid biosynthesis.[8] On the basis of this classification system, lipids have been divided into eight categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides. Each category is further divided into classes and subclasses. Additionally, following the existing rules and recommendations proposed by the International Union of Biochemistry and Applied Chemists and the International Union of Biochemistry and Molecular Biology (IUPAC-IUBMB) Commission on Biochemical Nomenclature, a consistent nomenclature scheme has also been developed to provide systematic names for various classes and subclasses of lipids.[7]

All lipids in the LIPID MAPS Structure Database (LMSD) are classified and annotated using this comprehensive classification and nomenclature system developed by the LIPID MAPS consortium.

2.2. Structure Representation

Currently, different members of the lipids community draw lipid structures in distinct ways. The same lipid structure in one lipid database can appear quite different in another database.[9] Moreover, large and complex lipids are rather difficult to draw manually, which leads to proliferation of shorthand and other abbreviations to represent lipid structures. To address these issues, the LIPID MAPS consortium proposed a consistent framework for representing lipid structures.[7,10] In general, the acid/acyl group or its equivalent is drawn on the right side and the hydrophobic chain on the left. A number of structurally complex lipids—acylaminosugar glycans, polycyclic isoprenoids, and polyketides—cannot be drawn using these simple rules; these structures are drawn using commonly accepted representations. Structures of all lipids in LMSD adhere to the structure drawing rules proposed by the LIPID MAPS consortium. Figure 3 shows representative structures for each lipid category.

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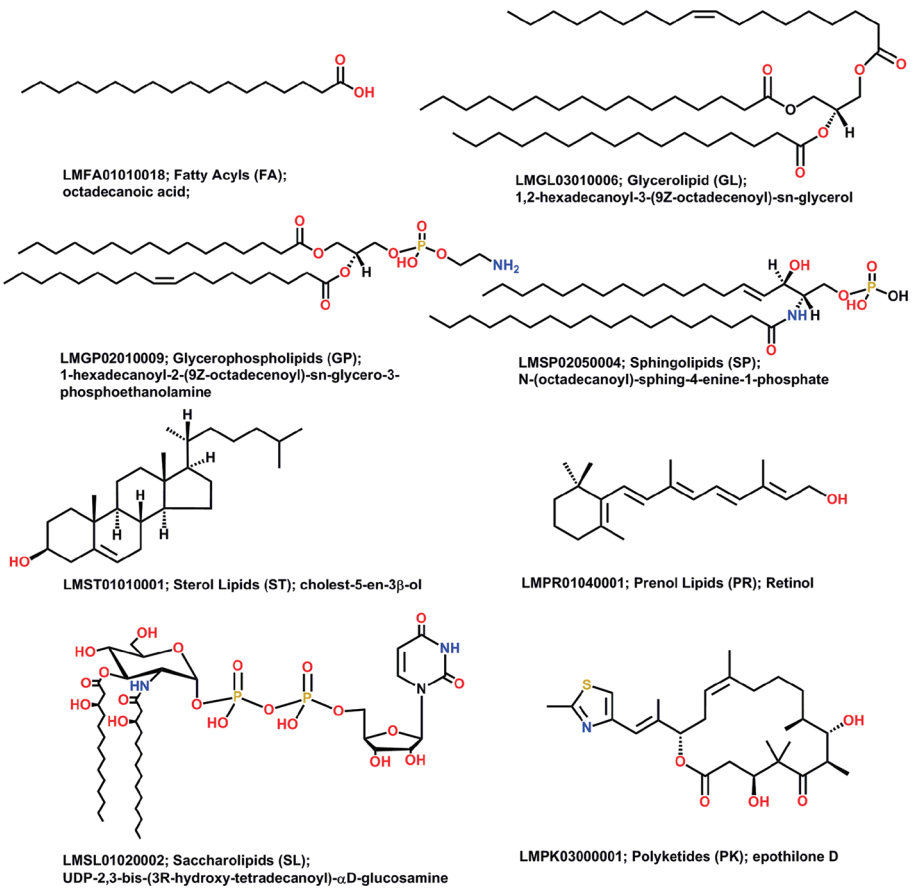

Figure 3. Representative structures from each lipid category shown with LM ID, category name, category abbreviation, and systematic name. Reprinted with permission from ref 10. Copyright 2007 Oxford University Press.

2.2.1. Structural Representation of Positional Isomers. LIPID MAPS core laboratories are engaged in identification, characterization, and quantification of known and new lipids using liquid chromatography (LC) and mass spectrometry (MS) experimental techniques. Information about various lipid standards developed for these experiments, along with the protocols used, is available on the Lipidomics Gateway Web site.[5] However, for some lipid categories such as glycerolipids and glycerophospholipids, it is not always straightforward to identify the positions of radyl (acyl, alkyl, or alkenyl) hydrocarbon chains at the *sn* carbons on the glycerol group. For example, MS/MS experiments might be able to identify the presence of three radyl hydrocarbon chains in a triacylglycerol, but their positions on the glycerol backbone would be unknown. Combinatorial enumeration of the three radyl chains at *sn* carbons leads to six possible isomeric structures. These positional isomers are stored in LMSD as one structure, and it is marked as a computationally generated structure. Structures for all other positional isomers are created on demand. To indicate the positional isomeric nature of the structure, the suffix "iso" followed by the number of isomers is also added to the abbreviation used as the common name. For example, entry LMGL03010043 in LMSD, with common name TG(16:0/16:1(9Z)/18:1(9Z))[iso6] and systematic name 1-hexadecanoyl-2-(9(Z)-hexadecenoyl)-3-(9(Z)-octadecenoyl)-*sn*-glycerol, represents a lipid structure with six possible positional isomers.

2.2.2. Structural Representation of Glycans in Glycosphingolipids. For structural representation of lipids in neutral and acidic glycosphingolipid main classes under the sphingolipid category, LMSD uses the symbol and text nomenclature as proposed by the Consortium for Functional Glycomics nomenclature committee on symbol and text representation of glycan structures.[11] In addition to using symbol and text representation for glycans, the last four digits of the LIPID MAPS identifier (LM ID) are further subdivided into two groups: The first two positions are used to differentiate glycan series within a subclass; the last two positions represent a unique ID. For the first two positions, only letters are used; the last two positions use combinations of numbers and letters.

2.3. Structure Drawing

The structures of large and complex lipids are difficult to represent in drawings, which leads to the use of many custom formats that often generate more confusion than clarity among members of the lipid research community. For example, usage of the Simplified Molecular Line Entry Specification (SMILES)[12] format to represent lipid structures, while being very compact and accurate in terms of bond connectivity, valence, and stereochemistry, does not contain information about atomic coordinates and causes problems when the structure is rendered. Different structure drawing tools end up generating different 2-dimensional structural layout corresponding to the same SMILES string for a lipid molecule. The structure drawing step is typically the most time-consuming process in creating molecular databases of lipids. However, many classes of lipids lend themselves to automated structure drawing paradigms, due to

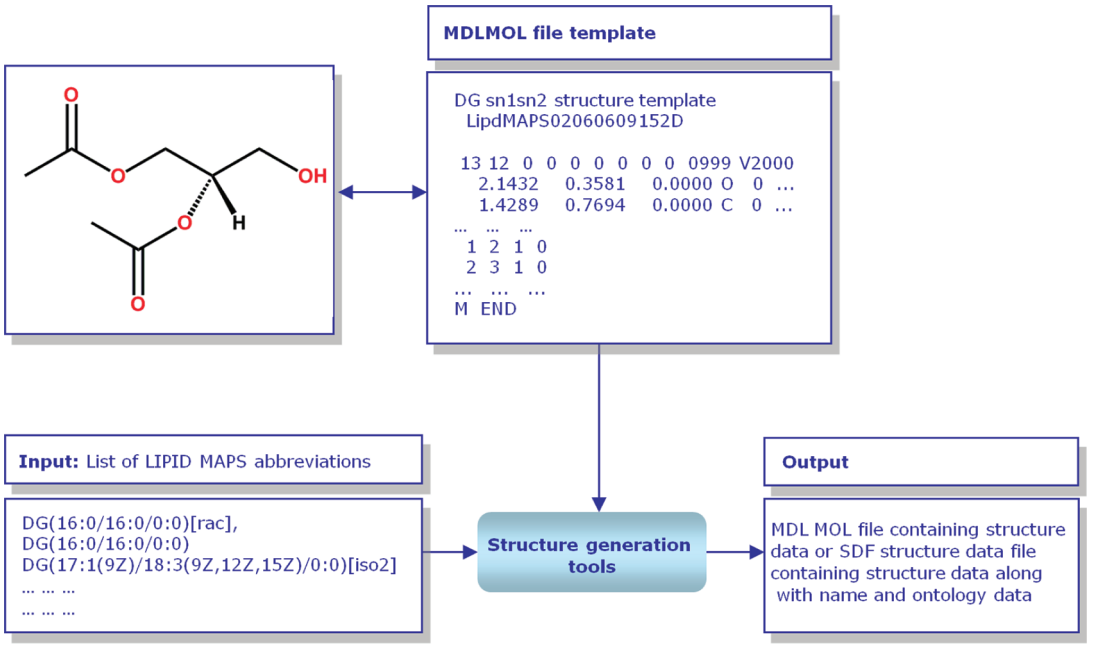

Figure 4. Overview of LIPID MAPS structure data generation methodology. Starting from specified abbreviations for lipids corresponding to the LIPID MAPS format, the structure generation tools select an appropriate lipid structure template internally stored in the MDL MOL file format, attach appropriate radyl chains, enumerate appropriate lipid structures, and generate an MDL MOL structure file or SDF file containing structural data along with name and other ontology data. Reprinted with permission from ref 13. Copyright 2007 Oxford University Press.

their consistent 2-dimensional layout. The LIPID MAPS consortium has developed and deployed a suite of structure drawing tools[13] that greatly increase the efficiency of data entry into lipid structure databases and permit "on-demand" structure generation. A consistent format is chosen for representing lipid structures[7,10] where, in the simplest case of the fatty acid derivatives, the acid group (or equivalent) is drawn on the right and the hydrophobic hydrocarbon chain is on the left. Similarly for glycerolipids, glycerophospholipids, and sphingolipids, the radyl hydrocarbon chains are drawn to the left and the head groups are depicted on the right. This approach enables a more consistent, error-free approach to drawing lipid structures and has been used extensively in populating the LMSD, which currently contains over 30 000 molecules.[10]

"Core" structures such as diacetylglycerol (glycerolipids) and formic acid (fatty acyls) are represented as text-based MDL MOL files,[14] and these MOL file templates are then manipulated to generate a variety of structures in MDL MOL files and Structure Data Format (SDF) files containing that core and other appropriate modifications (Figure 4). This manipulation is carried out by command-line or online programs written in the Perl[15] programming language.

The Lipidomics Gateway Web site[5] currently contains a suite of structure drawing tools for the following lipid categories: fatty acyls, glycerolipids, glycerophospholipids, cardiolipins, sphingolipids, sterols, and sphingolipid glycans. The online layout (Figure 5) consists of a core structure and pull-down menus arranged in locations appropriate for that structure. For example, in the case of the glycerophospholipid drawing tool, a central glycerol core is surrounded by pull-down menus allowing the end-user to choose from a list of head groups and *sn1* and *sn2* acyl side chains. The list of acyl chains represents the more common species found in mammalian cells and could easily be modified to include additional chains. The selected lipid structure is then generated via a server-side Perl script. The structure is rendered in the Web browser as a Java-based MarvinView applet[16] or Jmol[17] applet. Additionally, the structure may be viewed online with the Chemdraw ActiveX/Plugin[18] by users who have this component installed on their system. Current versions of the fatty acyl drawing tools are now capable of drawing chiral centers and ring structures. Molecules with correct stereochemistry are drawn by implementing the following method: (1) usage of a custom-developed module to define atoms, bonds, and neighbors; (2) a recursive algorithm which applies Cahn–Ingold–Prelog (CIP)[19] rules to a chiral center; (3) a scoring system to estimate substituent priority to assign chirality.

Concurrently, a generalized lipid abbreviation format[7] has been developed which enables structures, systematic names, and ontologies to be generated automatically from a single source format. Using this approach, a text file containing a list of lipid abbreviations may be submitted in batch mode to a drawing application which then generates structures (as MDL MOL files or SDF files), systematic names, and ontological information such as formula, molecular weight, number of rings, number of double/triple bonds, number of hydroxyl, amino, and keto groups, etc. In this way, thousands of lipid structures have been generated in a consistent fashion and deposited in the LMSD with considerable savings in time. Furthermore, the associated ontological information has been databased and used in various online search interfaces where end-users may search for structures by the presence (or number) of a functional group or other features.

2.3.1. Online Tools. A set of simple online interfaces have been developed to enable an end-user to rapidly generate a variety of lipid chemical structures, along with corresponding systematic names and ontological information. These are available in the "Tools" section of the Lipidomics Gateway Web site. The user interface is implemented using a combination of Perl and Hypertext Preprocessor (PHP)[20] scripts.

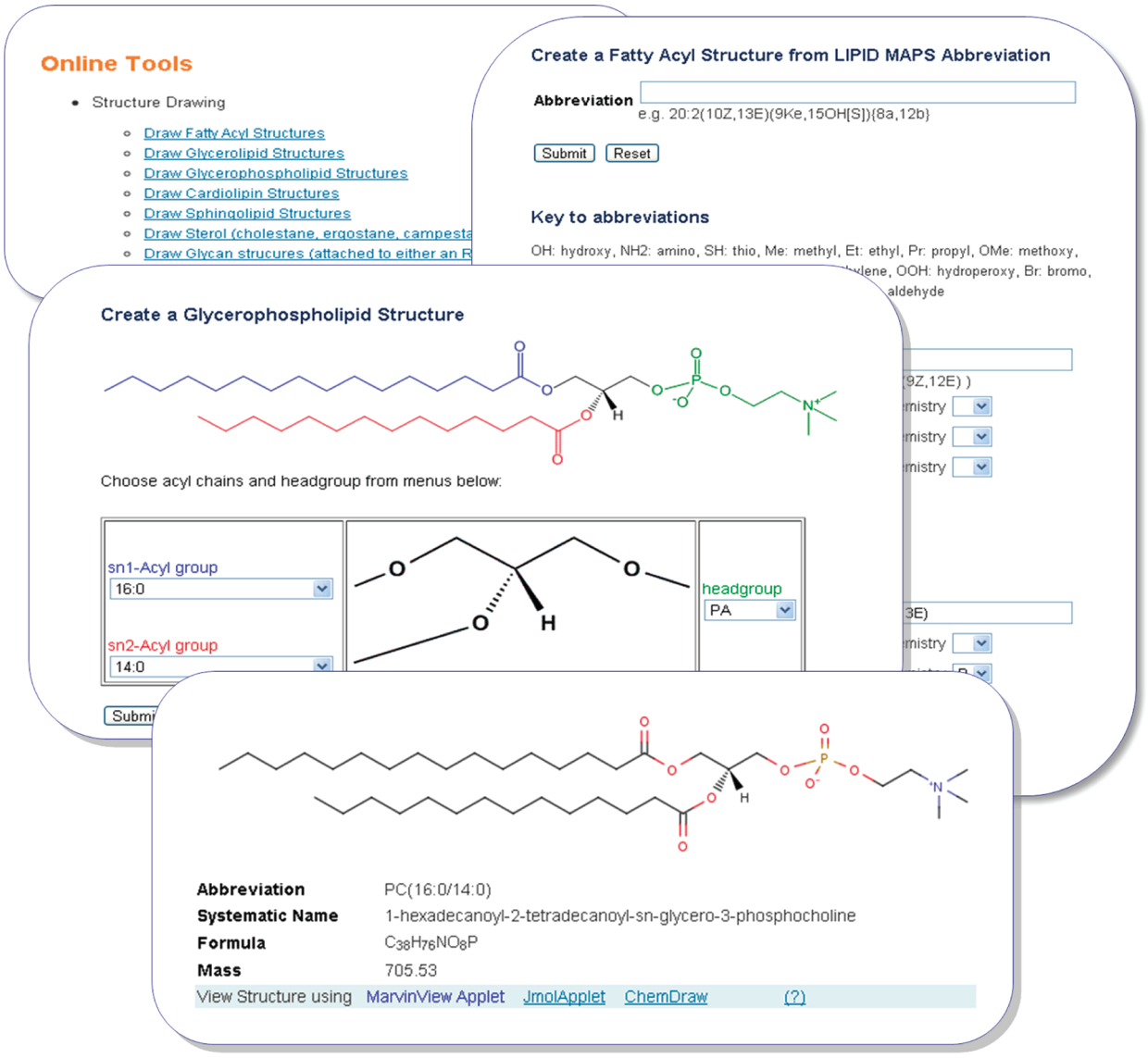

Figure 5. Montage of screen shots showing LIPID MAPS structure drawing tools. The top left box shows structure drawing tools available on the LIPID MAPS Web site. The top right box shows options available for generating a fatty acyl structure starting from either a complete abbreviation or individual specification of chain and substituent with its position and stereochemistry. The middle box shows an example of structure generation using acyl chains and head groups for glycerophospholipids. A comprehensive list of commonly occurring acyl chains and head groups is provided as a pull-down list. The button box shows an example of a structure generated for a glycerophospholipid along with other ontological information.

The lipid categories covered are fatty acyls, glycerolipids, glycerophospholipids (including cardiolipins as a special case), sphingolipids, and sterols. Using the glycerophospholipid structure drawing tool as an example, the user selects from a pull-down list of radyl chain abbreviations for the *sn1* and *sn2* positions and also from a list of head groups. The corresponding lipid structure is then generated in MDL MOL format and rendered in the Web browser using MarvinView applet,[16] which may alternatively be viewed using the JMol[17] applet or Chemdraw ActiveX/Plugin.[18] The fatty acyl structure drawing tool has a different user-input format where the user enters a valid fatty acyl LIPID MAPS abbreviation representing the acyl chain length, presence of double or triple bonds, and substituents on the acyl chain. Examples are "18:1(9Z)" (oleic acid) and "20:4(5Z,8Z,11E,14Z)(11OH[S])" (11(*S*)-hydroxy-5(*Z*),8(*Z*),11(*E*),14(*Z*)-eicosatetraenoic acid).

The sterol drawing tools currently support the generation of structures derived from cholestane, ergostane, campestane, and stigmastane sterol cores. In addition to double bond position specification, the user can choose to substitute atoms in the cholestane core by C, N, O, and H along with the stereochemistry specification of α or β for the substituted atom. Pull-down lists for position, stereochemistry, and atom specification are provided for up to four simultaneous substitutions.

All major lipid categories contain glycosylated forms whose glycan substituents can be challenging to draw in full chair conformation. The LIPID MAPS glycan structure drawing tools support the generation of a wide variety of glycan structures by specifying the constituent sugars using the Consortium for Functional Glycomics nomenclature.[11] The following sugar residues are supported: glucose (Glc), galactose (Gal), mannose (Man), *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine

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Table 1. Publicly Available LIPID MAPS Tools and Resources Discussed in This Review

name	URL
Pathway Editor	www.lipidmaps.org/pathways/pathwayeditor.html
Structure Database (LMSD)	www.lipidmaps.org/data/structure/
Proteome Database (LMPD)	www.lipidmaps.org/data/proteome/index.cgi
online structure drawing tools	www.lipidmaps.org/tools/
online mass MS tools	www.lipidmaps.org/tools/
command-line structure drawing tools package	www.lipidmaps.org/downloads/
command-line ontology generation package	www.lipidmaps.org/downloads/
stand-alone windows MS prediction tool	www.lipidmaps.org/downloads/
LMSD and LMPD data download	www.lipidmaps.org/downloads/
lipidomic and microarray data download	www.lipidmaps.org/data/index.html
lipidomic pathways download	www.lipidmaps.org/pathways/
experimental protocols	www.lipidmaps.org/protocols/

(GalNAc), xylose (Xyl), fucose (Fuc), acetylneuraminic acid (NeuAc), glycolylneuraminic acid (NeuGc), and deaminated neuraminic acid (KDN) as either the α or β anomer. Matched parentheses inside glycan chain specification indicate branched glycan chains, for example, GalNAcα1-3GalNAcβ1-3(Galβ1-3GalNAcβ1-4)Galα1-4Galβ1-4Glcb.

2.3.2. Standalone Command Line Tools. A suite of structure drawing tools in the form of Perl scripts have been developed which can generate a large number of structures relatively quickly using a command-line interface. These command-line tools are particularly useful in the area of bioinformatics because structures and related information such as formulas, masses, and abbreviations may be generated rapidly for large permutations of side-chain substituents. The tools are available from the Lipidomics Gateway Web site along with detailed documentation on the methods and functions used by these programs.

In addition to consistent structure representations from lipid abbreviations, the command-line tools developed by the LIPID MAPS consortium also generate ontological information such as the number of double bonds, chain lengths at different positions on the glycerol backbone, the number of various functional groups, and other structural characteristics. The ontological information is also loaded into LMSD. The IUPAC International Chemical Identifier[21] (InChI) string and InChIKeys for lipid structures are also generated using a command-line executable available from the InChI Web site and loaded into LMSD database tables. Table 1 provides a list of tools available from LIPID MAPS.

2.4. Ontology Generation

An issue of major importance in dealing with lipid structures is the huge diversity of chemical functional groups. This presents problems in explicitly classifying certain lipids containing multiple functional groups since assignment of a structure to a particular subclass may be somewhat subjective. For example, a fatty acid containing both epoxy and hydroxyl groups could be assigned to either the epoxy or hydroxy fatty acid subclass. To address this problem, the LIPID MAPS bioinformatics group has developed command-line tools which calculate the number of functional groups, the number of rings, and other structural information from an MDL MOL file representation of a molecular structure (Figure 6). These tools are available for download from the Lipidomics Gateway Web site. This approach may be performed in batch mode on the entire lipid structure database, thereby creating an "ontology" table which may then be incorporated into the database infrastructure. This in turn enables the use of an ontology-based search where a user may choose to search for lipids containing certain functional groups and a certain number of carbons, rings, etc., irrespective of their classification designation. A Web-based implementation of this type of ontology-based search has been implemented on the Lipidomics Gateway Web site.

3. LIPIDOME, LIPID GENOME, AND LIPID PROTEOME DATABASES

3.1. Lipid Databases and Other Small-Molecule Databases Containing Lipids

Lipids are generally hydrophobic in nature and soluble in organic solvents. However, lipid molecules show a remarkable structural and combinatorial diversity unlike other biological molecules such as nucleic acids and proteins. Chemical structures of lipids across different lipid categories are quite different and cover a wide range of chemical space. For example, sterol lipids are characterized by a four fused ring template consisting of three six-membered rings and one five-membered ring. Glycerolipids, on the other hand, typically do not contain any rings and contain radyl chains attached to *sn* carbons on the glycerol group. The radyl chains may be further unsaturated with varied double bond positions and geometry adding to the structural heterogeneity of lipids. Additionally, a large number of possible radyl chains at various *sn* carbons on the glycerol group along with different head groups lead to combinatorial isomeric positional diversity of lipid structures for various lipid categories such as glycerolipids, glycerophospholipids, and sphingolipids. Given the structural diversity of lipids and the importance of their role in the regulation and control of cellular function and disease, it is essential to have a database of lipids which not only facilitates the storage, retrieval, and dissemination of existing lipid structures and associated physiochemical properties data for the lipidomics community but also is extensible, flexible, and scalable to handle the vast amount of data being generated by new lipidomic studies. A well-designed lipid database must include a defined ontology which incorporates classification, nomenclature, structure representations, definitions, related biological/biophysical properties, cross-references, and physicochemical properties (formula, molecular weight, number of carbon atoms, number of various functional groups, etc.) of all objects stored in the database. This ontology can then be transformed into a well-defined schema that forms the foundation for a relational database of lipids. A large number of repositories (e.g., GenBank,[22] SwissProt,[23] ENSEMBL,[24] and GlycomeDB[25]) exist to support nucleic acid, protein, and carbohydrate databases; however, there are only a few specialized databases and resources

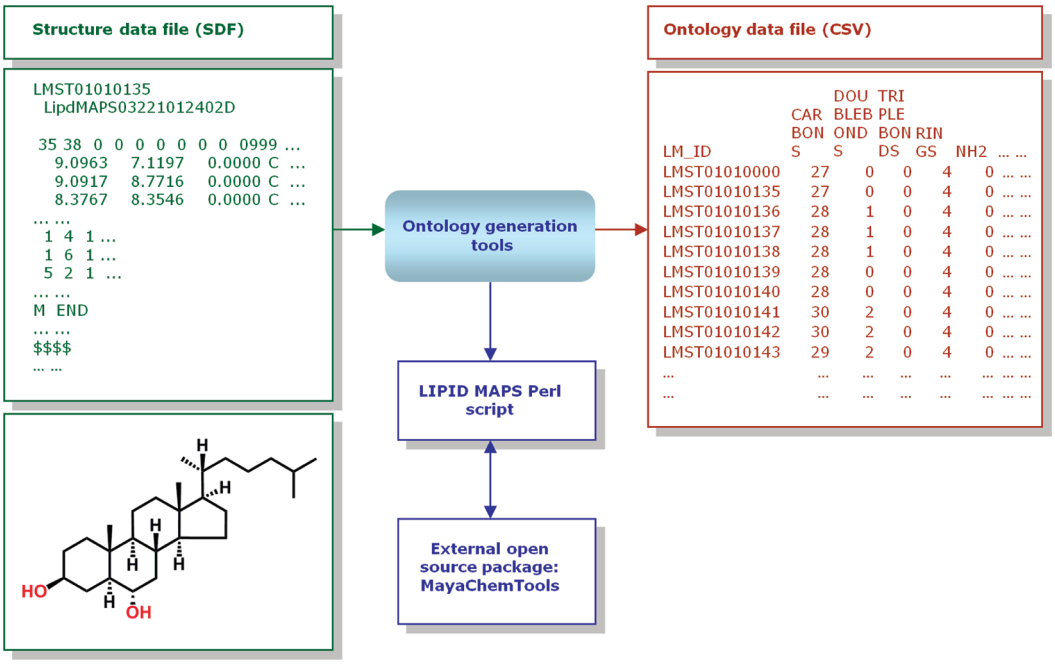


Figure 6. Overview of LIPID MAPS ontology data generation methodology from structure data.

Table 2. Resources and Databases Containing Information about Lipids

name	URL	comments/description
LMSD	www.lipidmaps.org	LIPID MAPS lipid structure database
LIPID BANK	www.lipidbank.jp	database of the Japanese Conference on the Biochemistry of Lipids
LIPIDAT	www.lipidat.tcd.ie	database of thermodynamic and associated information on lipids
Lipid Library	www.lipidlibrary.org	information about lipid chemistry, biology, technology, and analysis
Cyberlipids	www.cyberlipid.org	resource for lipid studies
HMDB	www.hmdb.ca	human metabololome database
DrugBank	www.drugbank.ca	drug data with target and action information
TTD	xin.cz3.nus.edu.sg/group/ttd/ttd.asp	therapeutic target database along with drug information
ChEBI	www.ebi.ac.uk/chebi	database and ontology for chemical entities of biological interest
ChemBank	chembank.broad.harvard.edu	small-molecule screening and cheminformatics resource database
PubChem	pubchem.nci.nih.gov	public repository for biological properties of small molecules including assay and screening data
ZINC	zinc.docking.org	commercially available compounds for virtual screening
ChemSpider	www.chemspider.com	chemical information resource
CAS	www.cas.org	small-molecule databases and associated information
eMolecules	www.emolecules.com	commercially available small molecules
Beilstein	www.reaxys.com/	small-molecule structures and other information
KEGG LIGAND	www.genome.jp/kegg/ligand.html	database of chemical compounds and reactions in biological pathways

(e.g., LMSD, LipidBank,[9c,d] LIPIDAT,[9a,b] Lipid Library,[9e] and Cyberlipids[9f]) that are dedicated to cataloging lipids. A variety of other small-molecule public and commercial databases (e.g., Human Metabolome Database (HMDB),[26] DrugBank,[27] Therapeutic Target Database (TTD),[28] Chemical Entities of Biological Interest (ChEBI),[29] ChemBank,[30] PubChem,[31] ZINC,[32] ChemSpider,[33] Chemical Abstract Service (CAS),[34] eMolecules,[35] Beilstein,[36] and Kyoto Encyclopedia of Genes and Genomes (KEGG) LIGAND[37]) also exist which provide information about lipid structures and their associated physicochemical properties.

While there has been no prior effort at systematic and comprehensive classification and nomenclature of lipid molecules, there are several small databases as mentioned in the previous paragraph which contain some or several lipid molecules. The LMSD database being developed by the LIPID MAPS consortium is one of the latest databases dedicated to lipids and provides comprehensive information about lipids. We provide an overview of the LMSD database, other lipid-specific databases, and small-molecule databases (Table 2) containing lipids in the rest of this section followed by a detailed description of the LMSD database.

The LMSD[10] is a relational database containing structures and annotations of biologically relevant lipids. It is being developed and maintained by the LIPID MAPS consortium and currently contains over 30 000 structures which are obtained from the

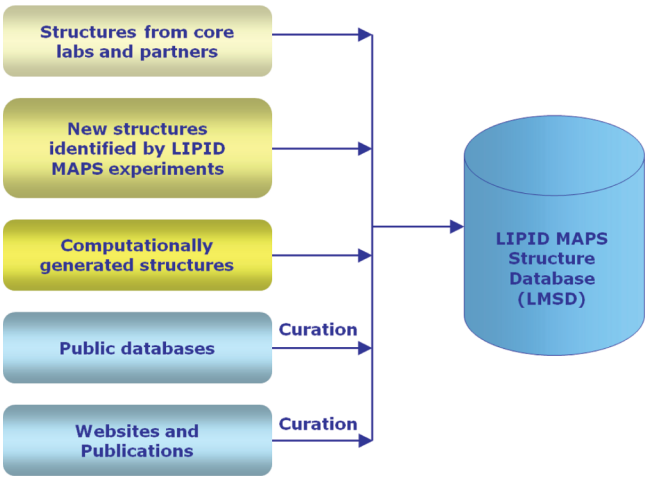


Figure 7. Overview of LMSD generation methodology.

following sources (Figure 7): LIPID MAPS consortium's core laboratories and partners; lipids identified by LIPID MAPS experiments; computationally generated structures for appropriate lipid classes; biologically relevant lipids manually curated from LIPID BANK, LIPIDAT, and other public databases; peer-reviewed journals and book chapters describing lipid structures.

The LIPID BANK is a lipid database of the Japanese Conference on the Biochemistry of Lipids (JCBL). It contains over 7000 lipids corresponding to the following main lipid classes: acylglycerol, bile acid, derived lipid, eicosanoid, ether-type lipid, fat-soluble vitamin, glycolipid, isoprenoid, lipid peroxide, lipoamino acid, lipopolysaccharide, lipoprotein, mycolic acid, phospholipid, steroid, and wax. In addition to classification-based browsing of lipids, the LIPID BANK supports text-based search and retrieval of lipid data using the name and other physicochemical properties; the structure-based search is not available. The search results along with structure and other basic information such as molecular weight, molecular formula, name, and common name provide the following additional information about a lipid: biological activity, physical and chemical properties, spectral data (ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), MS), chromatogram data, chemical synthesis, metabolism, genetic information, and references.

LIPIDAT is a relational database of thermodynamic and associated physicochemical property information on lipids. It contains over 20 000 lipids. Users can search the database using various physicochemical properties through more than two dozen available text-based query pages. The detailed search results page about a lipid includes the following information: structure, name, and formula along with other basic information; bibliographic information; experimental results and methods.

LIPID LIBRARY is not a database of lipids but an online resource about the chemistry, biology, technology, and analysis of lipids. The online pages provide information about lipids organized into the following sections: basic information, biochemistry and nutrition, lipid analysis, oils and fats, and latest news. The basic information section covers the structures, definitions, composition, biochemistry, and functions of the following lipid categories: fatty acids and eicosanoids, simple and complex glycerolipids and phospholipids, sphingolipids, and sterols. The biochemistry and nutrition section covers only plant lipid biochemistry. The lipid analysis section provides descriptions of both chromatographic and spectroscopic techniques used for analysis of lipids along with literature surveys of analytical methodologies. The oils and fats section covers the chemistry and technology of oils and fats along with the history of science and technology. The detailed information available for lipids covered in the basic information section provides the following details for each lipid: structure, name, source and occurrence, biochemistry, and function along with appropriate literature references.

Cyberlipids is an online resource for studies of lipids. It provides information about the definitions, source, compositions, and physicochemical properties of lipids along with a detailed review of various lipid analysis techniques. Users can retrieve detailed information about a lipid using its name for more than 900 lipids or get a list of all lipids with links to detailed information.

HMDB is a database containing information about small-molecule metabolites, including lipids, found in the human body. It contains over 7900 metabolite entries with links to over 7200 protein and deoxyribonucleic acid (DNA) sequences. The database provides links to three kinds of data: chemical data, clinical data, and molecular biology/biochemistry data. Users can search HMDB using text, chemical structures, and arbitrary relationships of available data fields. Database searching using spectral and chromatography data (MS, MS/MS, GC−MS, and NMR) is also available. Additionally, a variety of different data-browsing options are provided: class-based, pathway-based, and disease-based browsing and so on. The detailed information about each molecule is presented as a MetaboCard containing over 110 different data fields with two-thirds of the data fields containing information about chemical/clinical data and the rest about enzymatic and biological data. The links to other external data sources are also provided.

The DrugBank database provides detailed information about drugs, including lipids, along with the drug targets. The detailed drug information consists of chemical, pharmacological, and pharmaceutical information; the target information corresponds to the sequence, structure, and pathway. The database contains over 6800 drug entries covering the following types of drugs: over 1400 food and drug administration (FDA)-approved small-molecule drugs, over 130 FDA-approved biologics drugs, over 83 nutraceuticals, and over 5000 experimental drugs. Additionally, information for over 4000 nonredundant protein target sequences is linked to the drug entries. Users can search the DrugBank database using text, chemical structures, and arbitrary combinations of available data fields. A variety of different data-browsing options are also available: drug name, pathway, class name, and so on. The detailed information about each drug is presented as a DrugCard containing over 150 data fields with half the information covering drug/chemical data and the rest corresponding to the drug target.

TTD provides information about known targets along with information for associated diseases, pathways, and drugs for these targets. The TTD database contains information for over 1900 targets and over 5000 drugs with over 3000 small-molecule drugs. The drug information covers over 1500 approved drugs, over 1100 drugs in clinical trials, and over 2300 experimental drugs. The text-based database search provides searching using the target/disease name, drug name, function, and classification. The detailed search results page contains information about the target and disease, drug name and its function, and links to other external database containing information about targets and drugs.

The ChEBI database provides structural and ontological information about molecular entities focused on small-molecule compounds, including lipids. The molecular entities are either natural products or synthetic products used for biological intervention; nucleic acids are not included. The ChEBI database contains over 19 000 small molecules. The information about small molecules in ChEBI comes from the following four key sources: IntEnz[38]—the integrated relational enzyme database of the European Bioinformatics Institute (EBI), KEGG COMPOUND,[39] PDBeChem,[40] and ChEMBL.[41] Users can search the ChEBI database using text, chemical structures, and arbitrary combinations of available data fields. The structure-based search also supports similarity and substructure searching. The detailed search results along with structure and other basic information such as molecular weight, molecular formula, name, and common name provide the following additional information about a small molecule: ChEBI ontology, brand name, references to other databases, registry numbers corresponding to external sources (CAS, Beilstein, and Gmelin), and literature references.

ChemBank is a relational database containing data derived from small molecules, including lipids, and small-molecule screens along with tools for analyzing these data. The database contents include chemical structures and names, calculated molecular descriptors, human-curated information about small-molecule activities, raw experimental results from high-throughput biological assays, and metadata describing the screening experiments. The ChemBank database contains data for over 1.7 million compound samples with over 1.2 million unique small-molecule structures screened against more than 2500 assays covering more than 180 projects. Additionally, it contains information for over 1000 proteins, 500 cell lines, and 70 species associated with various assays. Users can search ChemBank using text, chemical structures, and arbitrary relationships of available data fields. Structure-based searching, in addition to substructure and exact match, also supports similarity searching. Database searching using information about high-throughput screens and small-molecule assays is also available. Additionally, a number of tools for analysis and visualization of small-molecule screening results are provided. The detailed search results along with structure and other basic information such as molecular weight, molecular formula, name, and common name provide the following additional information for a small molecule: a large number of calculated physicochemical properties; compound sample information; screening information, including project name, assay name, assay type, plate, well, and *z*-score.

The PubChem database is a database of chemical molecules and biological activities of molecules screened against various assays. It also contains information about lipids as the LIPID MAPS consortium uploads its LMSD database of lipids into PubChem on a regular basis. The PubChem database is divided into three main categories: The Compound database with over 32 million entries contains unique chemical substances derived from substance depositions, the Substance database with over 74 million entries consists of chemical compounds submitted by depositors corresponding to mixtures, extracts, and complexes, and the BioAssay database contains biological activity results from over 1600 high-throughput screening projects with several million measured values. The PubChem data deposition is open to the scientific community. The growing list of over 140 substance and 47 assay depositors represents all major sources, including commercial vendors, public nonprofit organizations, pharmaceutical companies, and individual contributors. Users can search PubChem compounds, substances, and bioassay databases using text, chemical structures, and arbitrary relationships of available data fields. Text-based searching supports the usage of a wide variety of parameters, including name, formula, physiochemical properties, stereochemistry specifications, elements, and so on. Structure-based searching provides support for substructure/superstructure search and identity/similarity search. The detailed search results page for compound along with structure and other basic information such as molecular weight, molecular formula, name, and common name provide the following additional information for a compound: synonyms, calculated physicochemical properties, substance information, biomedical annotation, pharmacological action and classification, chemical classification, safety and toxicology, links to exiting literature, and so on. The substance detailed results page, in addition to basic information such as chemical structure, name, and formula, contains the following additional information: link to data depositor, links to any bioactivity information and other structurally related substances, and links to other databases maintained by the National Center of Biotechnology Information (NCBI). A variety of analysis tools such as bioactivity structure−activity analysis and chemical structure clustering are also provided for the analysis of bioassay screening data.

The ZINC database contains commercially available small molecules for virtual screening. It contains over 13 million purchasable compounds, including lipids. Users can search the ZINC database using compound names, chemical structures/substructures, physicochemical properties, vendor catalog numbers/sources, and so on. The compound detailed search page includes chemical structure, name, formula, various calculated physicochemical properties, vendor and purchase information, and availability.

ChemSpider is a chemical database and an online resource linking together compound information across the Web. The compound information includes physical and chemical properties, chemical structure, systematic nomenclature spectral data, synthetic methods, known reactions, and safety information. ChemSpider contains over 25 million unique chemical compounds sourced and linked to over 400 separate data sources, including LIPID MAPS for lipids. The compound data are collected from over 50 different sources. Additionally, ChemSpider supports the uploading and curation of chemical structure and spectral data by the scientific community. Users can search the ChemSpider database using text, chemical structures, and arbitrary relationships of available data fields. Text-based searching supports the usage of a wide variety of parameters, including name, formula, physiochemical properties, literature search, and so on. The structure-based search supports chemical structure/substructure search along with arbitrary combinations of calculated physicochemical properties. The detailed search results page for a compound along with structure and other basic information such as molecular weight, molecular formula, name, and common name provide the following additional information: links to Wikipedia articles; associated data sources and commercial suppliers; patents; literature articles; calculated physicochemical properties; medical subject heading classification; pharmacological data; spectra; inks to other literature data. The ChemSpider online resource also hosts a variety of Web services such as chemical names to structure conversion, generation of InChI strings, and calculation of various physicochemical properties.

CAS is a comprehensive resource of chemical information combining databases with search and analysis tools available as chemical abstracts and chemical databases. CAS provides two main chemical databases: CAplus and CAS REGISTRY. The CAplus database consists of summaries and indices of scientific literature covering chemistry and chemistry-related topics such as proteomics, genomics, and so on. The CAplus database contains over 33 million references, and its coverage of scientific literature starts from the early 1800s and spans across 10 000 journals, technical reports, conference proceedings, and books in more than 60 languages; it also covers patent literature from over 60 countries. The CAS REGISTRY database contains over 52 million organic and inorganic chemical substances and over 62 million sequences. Its coverage of chemical substances also starts from the early 1800s and covers substances from patents, chemical catalogs, and various Web sources; the sequence data are retrieved from GenBank. In addition to basic compound information such as structure, name, formula, and molecular weight, the chemical substance record contains the following additional information: a unique CAS number, experimental and calculated physicochemical properties, ring analysis, and literature references. The CAS databases are searched using SciFinder, which supports both text-based and structure-based searching along with usage of other parameters during the search. In addition to CAplus and CAS REGISTRY, CAS provides the following three databases: CASREACT, CHEMLIST, and CHEMCATS. The CASREACT and CHEMLIST databases contain information about chemical synthesis and regulated chemicals, respectively. The CHEMCATS database contains over 44 million commercially available substances covering over 1200 catalogs from 1100 suppliers; it has over 12 million chemical substances with unique CAS numbers.

eMolecules is an online resource for commercially available chemical molecules, including lipids. It contains over 8 million unique molecules from a variety of commercial catalogs and other online data sources such as the National Institute of Standards and Technology (NIST), PubChem, DrugBank, and LIPID MAPS. Users can search the eMolecules database using molecule names, molecule structures/substructures, suppliers, and various physicochemical properties. In addition to basic molecule information such as structure, name, formula, and molecular weight, the molecule record contains information about suppliers and links to ordering chemicals.

The Beilstein database provides experimentally validated information about millions of chemical compounds uniquely identified by Beilstein Registry Numbers and chemical reactions compiled from the scientific literature starting from 1771. The original database was created using *Beilstein's Handbook of Organic Chemistry* and contains information about reactions, chemical substances, chemical structures, and physiochemical properties. The record for each substance has over 350 data fields corresponding to chemical and physical data along with appropriate literature references. Users can search the database using the Reaxys system using one of the following three search options: reaction searching, substance and property searching, and text searching. During reaction searching, a variety of other parameters such as starting materials, products, reaction conditions, and so on can also be specified. Substance and property searching provides structure/substructure search along with specification of various physical and chemical properties. The text-based search allows users to retrieve appropriate data using substance name, authors, and a variety of other parameters. The detailed search results page for substance along with structure and other basis information such as molecular weight, molecular formula, name, and common name provide the following additional information: calculated physicochemical properties, physical and spectral data, synthesis information, and links to the literature.

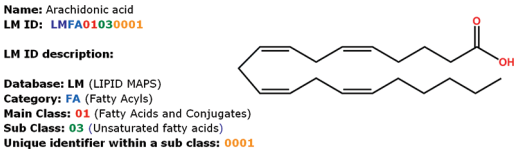


Figure 8. Description of the LIPID MAPS LM ID.

KEGG LIGAND is a database of chemical compounds and reactions involved in biological pathways. It is a composite database consisting of three other databases: KEGG COMPOUND, KEGG ENZYME, and KEGG REACTION. The KEGG COMPOUND database contains information for over 7000 metabolites and biologically relevant chemical compounds, including lipids, which are classified according to the LIPID MAPS classification system and made available through the KEGG BRITE database. The KEGG REACTION database contains information for over 5000 reactions corresponding to metabolic and other reactions. The KEGG ENZYME database has information for over 3800 enzymes involved in various transformations. Users can search KEGG LIGAND databases using text and chemical structures. The structure-based search supports structure/substructure search along with similarity searching. The detailed search results page for a compound along with structure and other basic information such as molecular weight, molecular formula, name, and common name provide the following additional information: links to ENZYME and REACTION databases, links to external data sources such as PubChem and CAS numbers.

3.1.1. Populating the Structure Database. An object-relational database of lipids containing structural, biophysical, and biochemical characteristics is available on the Lipidomics Gateway Web site with browsing and searching capabilities. LMSD currently contains over 30 000 structures which are obtained from a variety of sources: LIPID MAPS consortium's core laboratories and partners; lipids identified by LIPID MAPS experiments; computationally generated structures for appropriate lipid classes; biologically relevant lipids manually curated from LIPID BANK, LIPIDAT, and other public databases; peer-reviewed journals and book chapters describing lipid structures (Figure 7). All structures have been classified and redrawn according to LIPID MAPS guidelines. After lipids have been selected for inclusion into LMSD, they are classified following the LIPID MAPS classification scheme as explained earlier under the classification, ontology, and nomenclature of lipid molecules section (section 2). Structures of the lipids are either drawn manually or generated automatically by computational structure drawing tools developed by the LIPID MAPS consortium; the structure representation is consistent and adheres to the rules proposed by the LIPID MAPS consortium. On the basis of its classification, each lipid structure in LMSD is assigned a unique LM ID. The format of the LM ID (Figure 8) not only maintains the uniqueness of the ID but also provides the capability to add new categories, classes, and subclasses as the need arises.

In addition to import and manual curation of biologically relevant lipids from other database sources, LMSD also stores their original IDs to enable cross-referencing. LMSD lipid

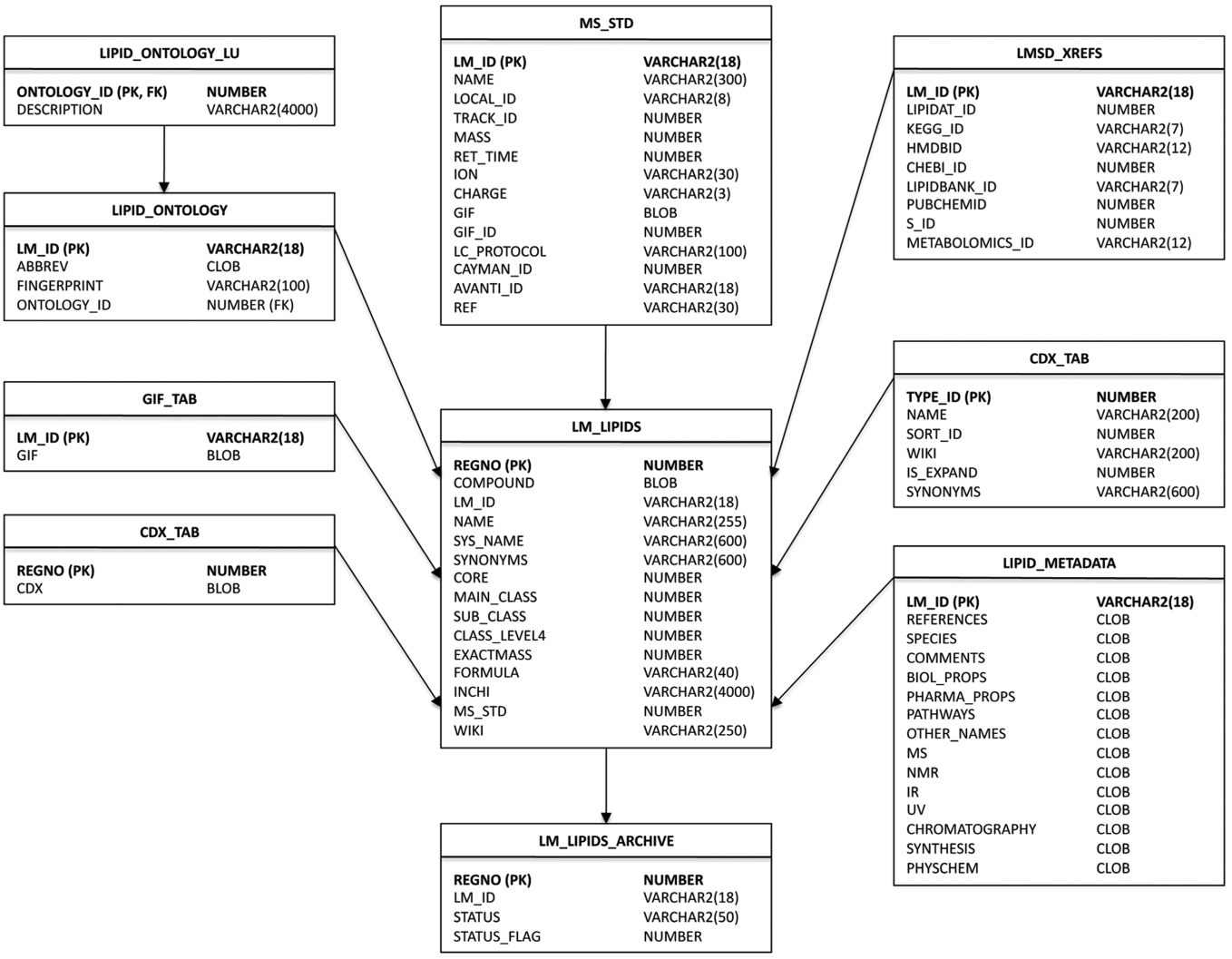


Figure 9. Entity relationship diagram for LMSD showing the Oracle database tables containing structural and classification information as well as annotations and ontological data. The unique LM ID identifier plays a central role as a primary key in this relational schema.

structures are deposited into the PubChem database periodically, and a link to the PubChem Substance ID (SID) is also maintained within LMSD. Access to the complete set of LMSD lipid structures in the PubChem database is also available.[42]

LMSD structures are either drawn manually using ChemDraw or generated automatically by structure drawing tools developed by the LIPID MAPS consortium for various subclasses in fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, and sterols. The structure drawing tools are Perl scripts which can generate a large number of structures relatively quickly via a command-line or Web-based interface. In addition to consistent structure representations from lipid abbreviations, these scripts also generate ontological information such as the number of double bonds, chain lengths at different positions on the glycerol backbone, the number of various functional groups, and other structural characteristics. The ontological information is also loaded into LMSD. The InChI string and InChIKeys for lipid structures are also generated using a command-line executable available from the InChI Web site and loaded into the Oracle database[43] tables. The database schema used for LMSD is outlined in an entity relationship diagram in Figure 9.

3.1.2. Searching the Structure Database. The Lipidomics Gateway Web site supports searching of the LMSD database in three different ways: classification-based, text/ontology-based, and structure-based search. Classification-based browsing provides the capability to retrieve lipids on the basis of the LIPID MAPS classification scheme. After the user selects one of the main categories of lipids, a listing of all lipids present in the selected category, along with a link to the set of lipids in each main class and subclass, is provided. The user may then select all lipids which belong to either a main class or a subclass and display the results as a results summary page.

In the case of lipids containing multiple functional groups, assignment of a structure to a particular subclass may be somewhat subjective. For example, a fatty acid containing both epoxy and hydroxy groups could be assigned to either the epoxy or hydroxy fatty acid subclass. To address this situation, an ontology-based search is also provided. The user may choose to search for lipids containing similar functionality, and all the lipids with the specific functionality, irrespective of their subclass designation, would be retrieved. The text/ontology-based query page allows the user to search LMSD by any combination of these data fields: LM ID, common or systematic name, mass along with a

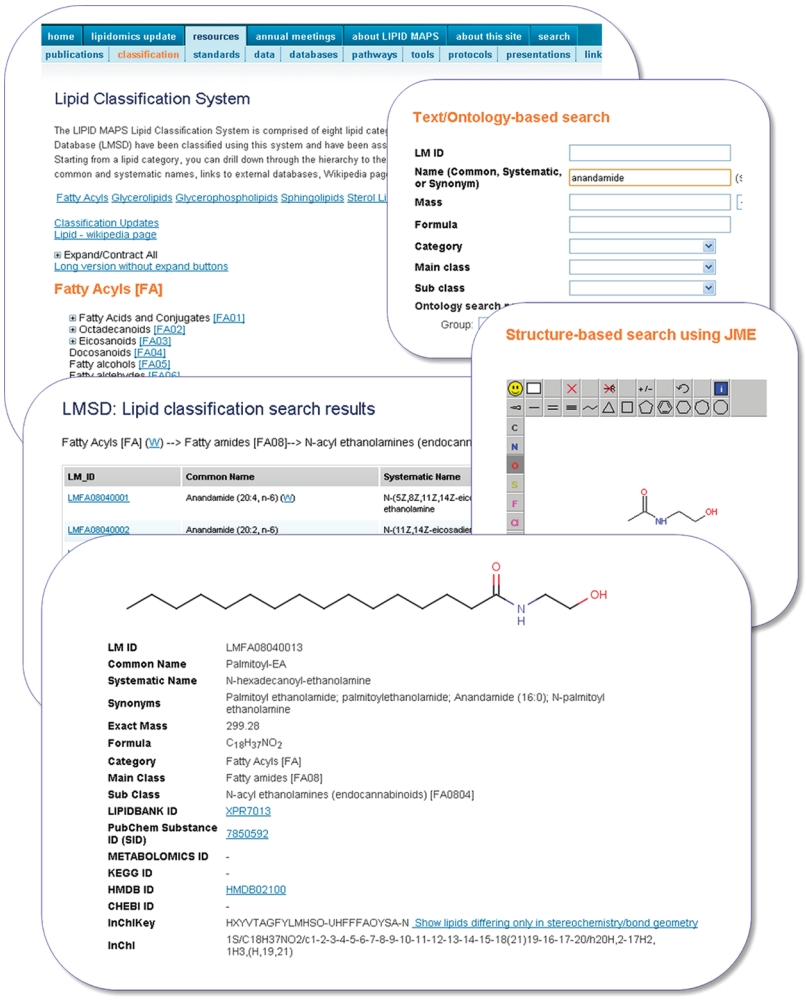


Figure 10. Selection of screen shots showing various options for searching LMSD and results summary for a specific LM ID.

tolerance value, formula, category, main class, subclass, and various combinations of ontology parameters. The structure-based search page provides the capability to search LMSD by performing a substructure or exact match using the structure drawn by the user. Three supported structure drawing tools are MarvinSketch,[16] JME,[44] and ChemDrawPro.[18] The first two of these structure drawing tools are Java applets and require only applet support in the browser. In addition to the structure, the user can also specify the LM ID and common or systematic name for the search.

The record details page, in addition to displaying the structure for the selected lipid, also contains all relevant information for that molecule such as common and systematic names, synonyms, molecular formula, exact mass, classification hierarchy, InChI-Key, and cross-references (if any) to other databases.

The default lipid detail page uses a Graphics Interchange Format (GIF) image for representing the structure of the lipid. The decision to use the GIF format for representing lipid structures in the Web browser was made due to its native support across all the browsers. The structure may also be viewed and manipulated using MarvinView,[16] JMol,[17] and the ChemDraw and ActiveX/Plugin[18] formats where structures may be manipulated, scaled, and saved in a number of high-resolution formats. Figure 10 shows screen shots of the LMSD user interface for lipid classification-based, text-based, and structure-based searching.

3.2. Lipid Proteome Databases

3.2.1. Populating the Proteome Database. To fully understand the roles of lipids, we must also understand the enzymes that catalyze lipid-related metabolic pathways, transcription factors and signaling agents involved in lipid regulation, and other proteins that affect lipid biochemistry by binding to or interacting with lipids. While Entrez Gene[45] and UniProt[46] provide annotations of proteins and their corresponding genes vis-à-vis their functional role, there was previously no database that comprehensively cataloged all lipid-associated proteins. The LIPID MAPS Proteome Database (LMPD)[47] developed by LIPID MAPS serves such a purpose.[5]

UniProt and Entrez Gene contain a significant part of the annotations of proteins and genes, respectively, and most of the known lipid-related proteins have been annotated in these databases. However, prior to the development of LMPD, there was no unique database of lipid-associated proteins that contained comprehensive and context-dependent annotations. LMPD was developed to fill this void by providing a catalog of genes and proteins involved in lipid metabolism and signaling. LMPD can be searched by database ID, keyword, KEGG pathway, or Gene Ontology (GO) term and is publicly available from the Lipidomics Gateway Web site.

LMPD is constructed as an object-relational database of lipid-associated protein sequences and annotations. The database

dx.doi.org/10.1021/cr200295k |*Chem. Rev.* 2011, 111, 6452–6490

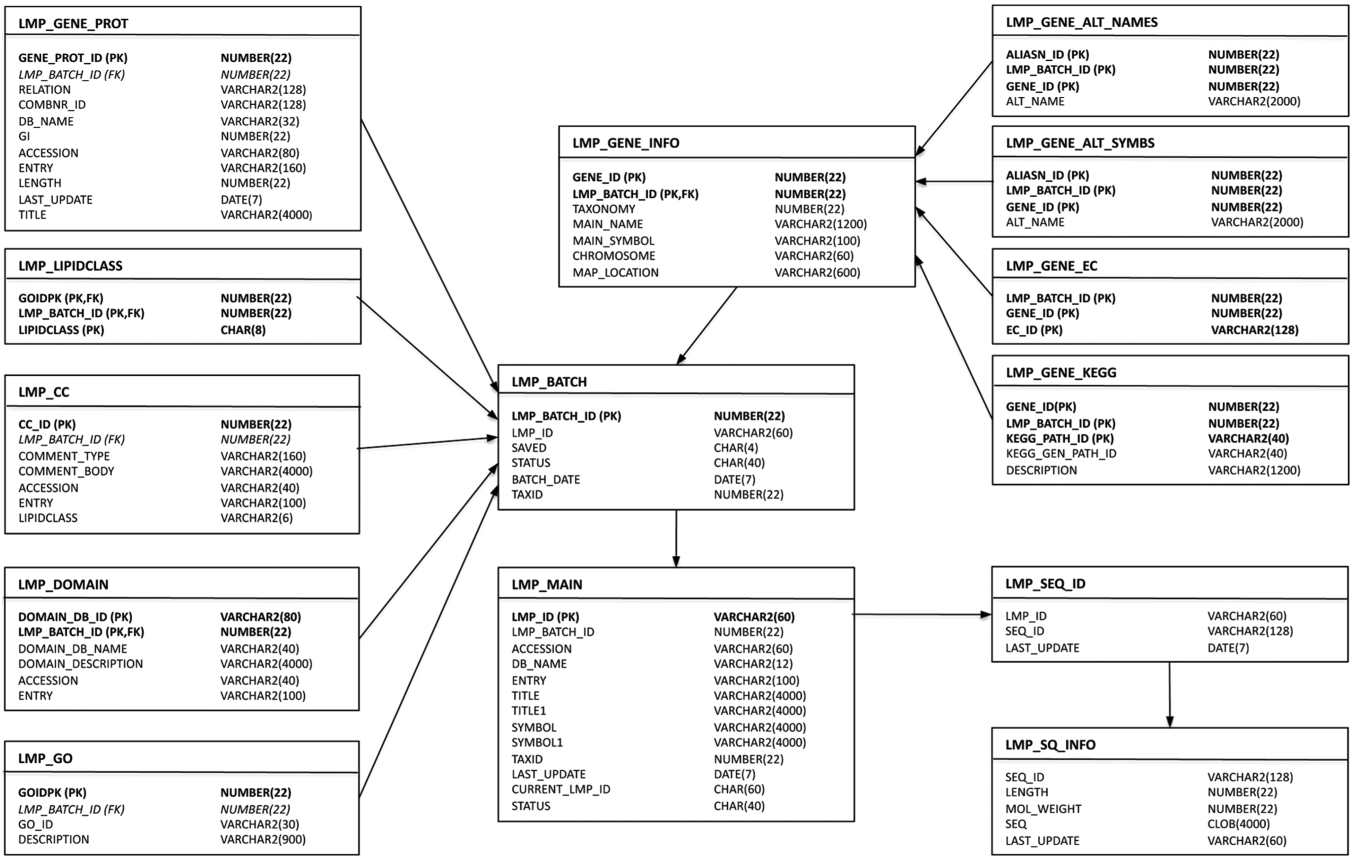


Figure 11. Entity relationship diagram for LMPD showing the Oracle database tables containing information pertaining to lipid-related genes and proteins for human and mouse species.

schema used for LMPD is outlined in an entity relationship diagram in Figure 11. The initial release of LMPD established a framework for creating a lipid-associated protein list, collecting relevant annotations, databasing this information, and providing an online user interface. A similar approach was used previously for development of the MitoProteome database.[48] The current release of LMPD contains approximately 1200 lipid-related proteins each for human and mouse species.

To construct LMPD, a curated set of lipid-related keywords was created for each of the eight lipid categories. These keywords, containing terms such as "lipase", "cyclooxygenase", "ceramide", and "choline", were then used to search the name, description, and annotation information in publicly available UniProt,[46] Entrez Gene, GO,[49] and KEGG[50] data repositories for mouse and human species to identify proteins, genes, and related pathway and ontology information containing these terms. The GO terms identify proteins that are involved in particular anabolic, catabolic, and other metabolic processes, while proteins gathered from KEGG were identified as being involved in a lipid metabolic pathway. Experimental methods used in identifying these proteins included various enzyme assays, high-performance liquid chromatography (HPLC), polyacrylamide gel electrophoresis, and mass spectrometry. All protein lists generated by these automated methods were then manually curated, erroneous entries were deleted, known lipid-related proteins not identified by the methods above were added, and corresponding Entrez Gene IDs and annotations were generated for all Uniprot records. This process is illustrated in Figure 12.

The Signaling Gateway Molecule Pages (SGMP) database, another database containing states of proteins involving lipids, is a repository derived from a comprehensive signaling protein ontology that covers functional states of a protein, the transitions between those states, and the defined functions of a protein in a given cellular context.[51] The SGMP data are exported to the Biological Pathway Exchange (BioPAX)[52] and Systems Biology Markup Language (SBML).[53] The SGMP database contains information on several lipid binding and modifying proteins (Table 3).

3.2.2. Searching the Proteome Database. Multiple LMPD query interfaces are available, enabling users to search LMPD by database ID or keyword, by KEGG pathway, or by GO term. From the search results, one can access annotations relevant to each protein of interest, cross-linked to external databases. Annotations are organized by record overview, Gene/GO/KEGG information, protein domain information, SwissProt/UniProt annotations, and related proteins and LIPID MAPS experimental data (if any). The record overview contains LMPD ID, species, description, gene symbols, lipid categories, enzyme code (EC) number, molecular weight, sequence length, and protein sequence. Gene information includes Entrez Gene ID, chromosome, map location, primary name, primary symbol, and alternate names and symbols, GO IDs and descriptions, and KEGG pathway IDs and descriptions. UniProt annotations include primary accession number, entry name, and comments such as catalytic activity, and enzyme regulation, function, and similarity.

4. LIPID EXPERIMENTAL PROTOCOLS AND METADATA MANAGEMENT

The post genome sequencing era has heralded the beginning of a new phase of scientific discovery that is based on massive volumes of data generated by high-throughput technologies.[54] This exploratory, data-driven approach represents a paradigm shift from the traditional scientific discovery where an individual laboratory's effort is focused on a particular gene product and the pathway in which the gene product participates, i.e., a hypothesis-driven approach. Efforts to understand the detailed functioning of all the elements of the cellular machinery at the molecular level pose a major challenge that would require a large collective effort from a multidisciplinary organized team of scientists. If people working in academia were to engage in such an effort, the organization of the effort would perhaps require a consortium approach with laboratories having expertise in different areas such as cell biology, molecular biology, proteomics, functional genomics, and bioinformatics contributing to a joint and well-integrated effort.

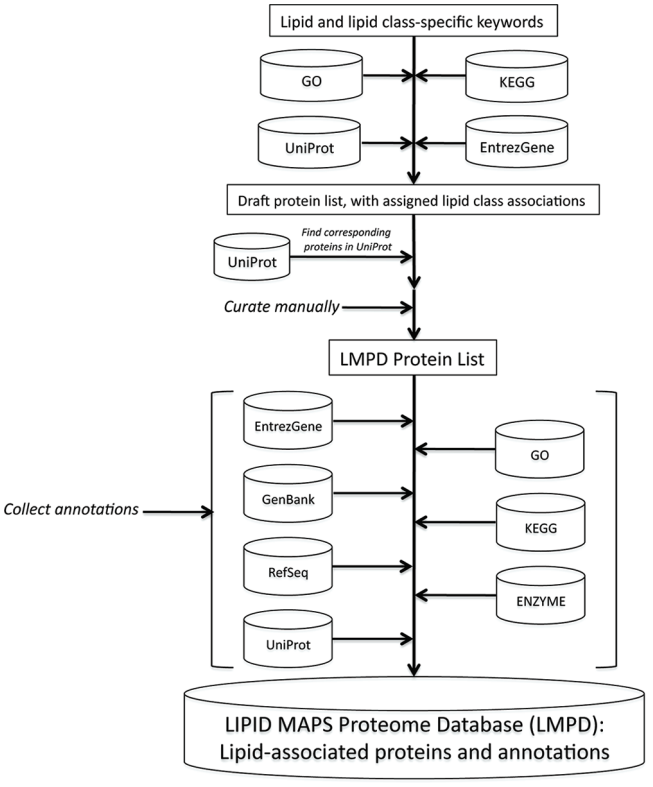


Figure 12. Overview of the bioinformatics process for creating LMPD.

Each high-throughput technique generates a large body of data to be recorded. It brings two data management issues to the fore: first, how the sheer amount of data from heterogeneous but related experiments from various laboratories will be handled and, second, how data will be shared and analyzed collectively among them and made available to the public at large. The laboratory notebook concept is insufficient to deal with the issues of data handling, structuring, and sharing.[55] For such a research endeavor, utilization of high-throughput techniques to explore complex biological systems is the norm rather than an exception. In a high-throughput setup the output from one experiment is the input of another. Situations like these create another set of issues to be dealt with, since samples will be passed from one laboratory to another in bulk quantities for subsequent handling and analysis. The samples are all necessarily coded such that the recipient laboratory could recover the information about the history of each received sample. Laboratory notebooks could be replaced by a relational database, which would facilitate data deposition from various laboratories to a common repository, and at the same time data could also be viewed by authorized personnel. The data structuring could be achieved by an appropriate database schema design, which could also enforce linking of the data from heterogeneous biological experiments, thus offering easy access to the data analysis en masse. The role of the pen will be replaced by graphical user interfaces (GUIs) and a keyboard; the GUI would enable the experiment to document the samples and their handling and directly deposit data to the database. There will be a separate GUI for each type of experiment, so the use can be guided as to what needs to be done. The GUI should be designed to check data validity prior to deposition into the database; this will minimize the manual data entry errors inherent in a notebook system. Data should be regularly backed up to guard against any kind of system failure. This scheme essentially represents a paper-free and scalable structured electronic notebook for data cataloging and automated incorporation of time stamps to record the data entry. After successful deposition of the experimental parameters to the database through a GUI, the user must be provided with a label to identify the sample container, which in biological experiments is often a tube or flask. The label should uniquely identify each experiment and contain meaningful information to facilitate deciphering its contents.

The data structuring, handling, and management requirements could be met by the use of a laboratory information management system (LIMS). Use of LIMSs is widespread in diverse industrial settings; they are used in pharmaceutical

Table 3. Representative List of Lipid-Related Signaling Proteins as Molecule Pages

SGMP ID	GenBank accession no.	molecule page name	molecule page category
A001757	AAH05636.1	phosphodiesterase 6D, cGMP-specific rod delta	lipid binding protein
A003319	NP_898977.2	DFCP1	lipid binding protein
A000010	NP_032892.1	acyl protein thioesterase 1	lipid modification, protein
A000095	NP_032733.1	protein N-myristoyltransferase 1	lipid modification, protein
A001778	NP_780565.1	phosphatidylinositol-4-kinase type III beta	kinase, lipid
A002220	NP_064395.2	sphingosine kinase 2	kinase, lipid
A001749	AAC37702.1	phosphodiesterase 1C, calmodulin dependent	phosphodiesterase
A001750	NP_001008548.1	phosphodiesterase 2A, cGMP stimulated	phosphodiesterase
A000046	BAC00906.1	phospholipase C epsilon	phospholipase
A001789	AAH45156.1	phospholipase A2, group IIA	phospholipase

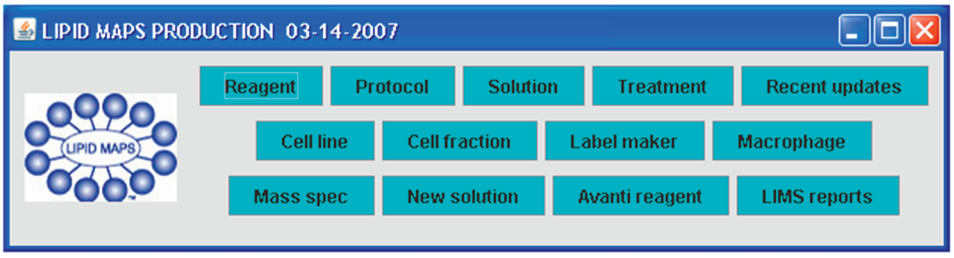


Figure 13. Main user interface of LIPID MAPS LIMS. Reprinted with permission from ref 57. Copyright 2007 Elsevier Ltd.

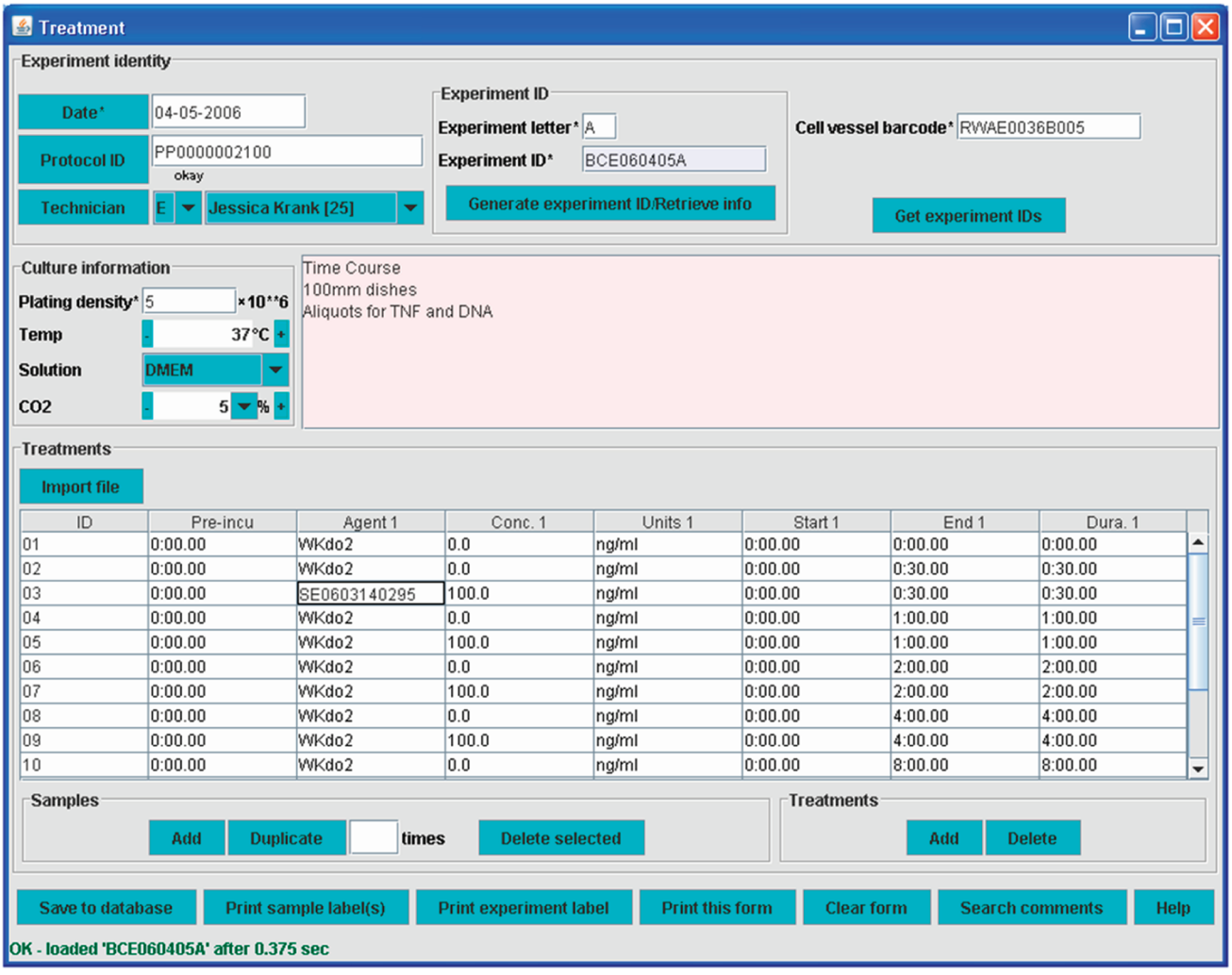
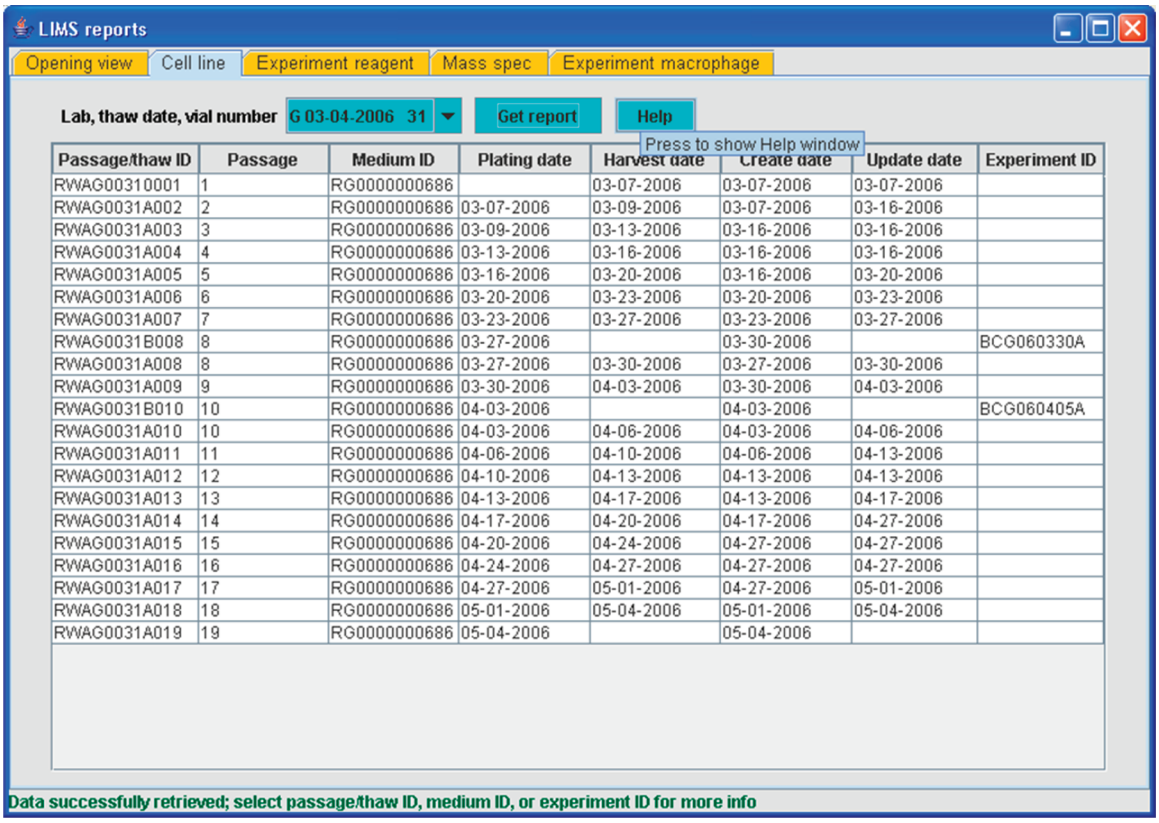


Figure 14. Treatment module of LIMS. Reprinted with permission from ref 57. Copyright 2007 Elsevier Ltd.

companies, forensic laboratories, environmental agencies, and the food and beverage industries, which have to follow strict quality assurance (QA)/quality control (QC) standards. Dozens of LIMSs are available in the market from commercial vendors; they are generally expensive and may not meet the specific needs of a particular project.

Apart from organizing data, a more important reason for laboratory information management systems in lipidomics is to minimize inherent variability in experimental data, as procedures, time, and personnel can all cause significant variation in the results. An LIMS should be organized in such a way as to minimize this variability and properly annotate the specific reagents and procedures utilized in a given experiment for future reference.

An LIMS must be usable by laboratory technicians and other personnel with limited bioinformatics experience. As much as possible, user interfaces must be engineered to provide important informational and contextual pointers for how they are intended to be used. Constraints on entries and readily understandable feedback messages should be provided in meaningful ways. In some cases, there may be no substitute for person-to-person interaction in providing assistance, and a person may be



Passage/thaw ID	Passage	Medium ID	Plating date	Harvest date	Create date	Update date	Experiment ID
RWAG00310001	1	RG0000000686		03-07-2006	03-07-2006	03-07-2006	
RWAG0031A002	2	RG0000000686	03-07-2006	03-09-2006	03-07-2006	03-16-2006	
RWAG0031A003	3	RG0000000686	03-09-2006	03-13-2006	03-16-2006	03-16-2006	
RWAG0031A004	4	RG0000000686	03-13-2006	03-16-2006	03-16-2006	03-16-2006	
RWAG0031A005	5	RG0000000686	03-16-2006	03-20-2006	03-16-2006	03-20-2006	
RWAG0031A006	6	RG0000000686	03-20-2006	03-23-2006	03-20-2006	03-23-2006	
RWAG0031A007	7	RG0000000686	03-23-2006	03-27-2006	03-23-2006	03-27-2006	
RWAG0031B008	8	RG0000000686	03-27-2006		03-30-2006		BCG060330A
RWAG0031A008	8	RG0000000686	03-27-2006	03-30-2006	03-27-2006	03-30-2006	
RWAG0031A009	9	RG0000000686	03-30-2006	04-03-2006	03-30-2006	04-03-2006	
RWAG0031B010	10	RG0000000686	04-03-2006		04-03-2006		BCG060405A
RWAG0031A010	10	RG0000000686	04-03-2006	04-06-2006	04-03-2006	04-06-2006	
RWAG0031A011	11	RG0000000686	04-06-2006	04-10-2006	04-06-2006	04-13-2006	
RWAG0031A012	12	RG0000000686	04-10-2006	04-13-2006	04-13-2006	04-13-2006	
RWAG0031A013	13	RG0000000686	04-13-2006	04-17-2006	04-13-2006	04-17-2006	
RWAG0031A014	14	RG0000000686	04-17-2006	04-20-2006	04-17-2006	04-27-2006	
RWAG0031A015	15	RG0000000686	04-20-2006	04-24-2006	04-27-2006	04-27-2006	
RWAG0031A016	16	RG0000000686	04-24-2006	04-27-2006	04-27-2006	04-27-2006	
RWAG0031A017	17	RG0000000686	04-27-2006	05-01-2006	04-27-2006	05-01-2006	
RWAG0031A018	18	RG0000000686	05-01-2006	05-04-2006	05-01-2006	05-04-2006	
RWAG0031A019	19	RG0000000686	05-04-2006		05-04-2006		

Figure 15. LIMS Reporter (reporting tool) module. Reprinted with permission from ref 57. Copyright 2007 Elsevier Ltd.

dedicated to providing help to other personnel. These features can foster the goal of achieving widespread user acceptance.

The LIPID MAPS project modified an earlier, highly developed LIMS system that had been constructed for the Alliance for Cell Signaling (AfCS).[56] The principles of lipidomics involve many of the same concepts as those associated with the broader category of metabolomics. That is, metabolomics studies often involve inducing perturbations to the ongoing state of living systems and subsequently monitoring changes at specific time points.[1b] The various lipid species are measured at different time points, and quantities are systematically determined. This may be performed within a single laboratory, or a number of laboratories may collaborate in the endeavor. In support of these aims, agreement must be reached among the persons performing the work on the experimental protocols at each step, and protocols and documents must be stored and made available to all. To accomplish transfer, centralized storage, and sharing of data among LIPID MAPS member laboratories, we have developed an LIMS to submit data to a central database and to obtain data from the same source.[57] To handle the large amounts of data, a relational database is an essential requirement. The information entered into the system is best entered by individual users or laboratories. A two- or three-tier platform may be deployed, and data entry forms may be presented in the form of a dedicated program or Web site.

The user interface of the LIPID MAPS LIMS consists of a number of discrete GUIs representing modules of functionality that are accessed from a single main window interface (Figure 13). The entire application is downloaded from a Web site as a Java Web Start application at the time of each use. These individual modules allow users to enter information and browse the LIMS database. After entering information, the user clicks a button to send information to a central Oracle database. The LIMS also allows tracking of laboratory materials and protocols via printed labels that may be scanned into modules using barcode readers, thus minimizing typing errors.

The LIPID MAPS LIMS is organized around cellular treatments and MS experiments. The LIMS enforces adherence to process controls in the form of exact control of experiments using strict solution and procedural protocols. A protocol ID is required by the majority of modules. The protocol ID refers to a document in the LIMS database that describes a laboratory procedure or solution composition. The user may use one of the protocol documents that are already within the LIMS for this purpose. In addition, any of the participating LIPID MAPS laboratories may upload a new protocol and generate a new protocol ID.

The Treatment module provides the essential lipidomics functionality of the LIMS (Figure 14). Into this form, details of the treatment conditions are entered. These include reagent or solution IDs, concentrations, and the start time, end time, and durations of both current treatment and pretreatment during an experiment with a particular cell preparation. These data are vital for studies of stimulus- and time-dependent alterations to lipid composition. Individual sample IDs are associated with cells receiving different treatments within an experiment.

A significant contribution to the functionality in the LIMS arises from close integration of modules. Each module has search functions that search database tables for information entered by that module. Another implementation of searching and user interaction occurs in the case of the Reporter, or the LIMS Reports, module. The Reporter module allows the user to construct high-level reports summarizing overall database content using certain key parameters as search terms. For example,

the user may obtain a summary table of cell vessel IDs that originate in a thaw of a particular vial of frozen cells used by a laboratory, along with the protocol ID that was used for thawing and passaging and the ID of any experiment in which a cell passage deriving from that vial was used (Figure 15). The history of a cell line from freezer to experiment is thus obtained.

The modules of the LIPID MAPS LIMS were intended to be used sequentially, with database identifiers from previous modules in list format made available to users for insertion into later modules. A flowchart published previously illustrates one potential usage sequence that begins with the Reagent module and ends with the Mass Spec module.[57]

While most of these modules are generic in nature, others have been engineered that are specific for the needs of LIPID MAPS. For example, the Avanti Reagent module allows the user to track reagents provided by supplier of molecular standards with the aim of ensuring that materials used for quantitation purposes remained within quality specifications. Among other actions, users can download a current, updated certificate of quality for any lot of material previously shipped to a consortium laboratory. This can be an important consideration when using standards that may possess abbreviated shelf lives. In LIPID MAPS, only Avanti Polar Lipids, Inc. (Alabaster, AL) can input such information, while all laboratories have access to downloading from this module.

On occasion, users may not have time to properly access all modules in succession. For example, the Solution module requires prior use of the Reagent module, along with the Protocol module to insert a protocol on solution composition. This step is of particular importance in mixing internal standards used in mass spectrometry. The New Solution module allows bypassing both these modules, with only a brief sketch of solution content required. During later data analysis, performed after the conclusion of an experiment, acceptance or rejection of a questionable datum may hinge on whether the information trail that includes the information entered by either of these modules provides sufficient detail that its reliability can be affirmed. Consequently, the New Solution module typically plays a role only in investigations limited in scope to a specific laboratory.

Analysis and mining of the metadata and associated data obtained with the assistance of this LIMS is conducted offline at the Bioinformatics core. LIMS metadata and the experimental data described by the metadata are available on the Internet for browsing and are directly linked to a public database of lipid structures that is curated by experts[10] and to a database of proteins known to be involved in lipid metabolism in mice and in humans.[47] Both are available from the Lipidomics Gateway Web site.[5] The availability of solution and procedure protocols as well as tools allowing searching and drawing of lipid structures are also featured at this site.

A widely publicized effort to standardize the content of metabolomics experiment informational resources to allow computerized searching has been proposed.[58] However, such standardization efforts seem not to have been widely pursued in metabolomics projects, at least partly because of difficulties in adequately comparing experiments performed using disparate technologies, such as NMR spectroscopy and mass spectrometry.[59]

5. ANALYSIS AND PRESENTATION OF LIPID MASS SPECTROMETRIC DATA

With the availability of sensitive analytical instrumentation such as mass spectrometry, it is now possible to obtain quantitative data on large numbers of lipid species under a variety of experimental conditions. MS methods for the characterization of lipid mixtures have also been published in recent years, most of them centered on the use of electrospray ionization (ESI) MS, atmospheric pressure chemical ionization (APCI) MS, and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS.[60] Currently, mass spectrometric analysis of lipids is mainly comprised of two complementary approaches which either employ direct infusion (shotgun lipidomics)[61] or use liquid chromatographic separations prior to mass spectrometric analysis (LC−MS). An advantage of shotgun lipidomics is that a mass spectrum displaying molecular ions of individual molecular species of a class of interest can be acquired at a constant concentration of the lipid solution during direct infusion. This unique feature of shotgun lipidomics allows researchers to perform precursor ion scans of the particular fragment ions and/or neutral loss scans of the interested neutrally lost fragments for identification and quantitation of the individual molecular species of a lipid class or a category of lipid. On the other hand, customized LC−MS techniques tailored to a particular lipid class of interest have the ability to resolve complex lipid mixtures during the LC step, allowing for more reliable identification during the MS step. From a bioinformatics standpoint, MS data analysis can be divided into a number of distinct phases: (a) processing of raw data files which may involve peak averaging, normalization, integration, isotope correction, and display of processed spectra; (b) peak identification using algorithms to match lipid ions against databases of known or computationally derived structures; (c) statistical analysis of MS data to quantify significant changes between different samples (lipidomic profiling) and between different lipid species in the same sample (correlation analysis) or within the same species over time (temporal analysis); (d) modeling of lipid data onto biological pathways as part of a systems-biology approach.

5.1. MS Analysis Software

In recent years there has been an urgent need for informatics solutions to efficiently process the large amounts of MS data generated by lipidomics experiments and deal with the unique complexities of lipid structures. The number of software packages has expanded considerably over the last 5 years, and they include a number of freely available applications that are capable of handling multiple tasks in the analysis pipeline (see Table 4). The Java-based MZmine[62] provides users with a modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data and is particularly useful for analyzing LC−MS experiments. Another recently released Java application is the Lipid Data Analyzer (LDA)[63] in which the authors have developed new algorithms for detection and quantification of minor lipid analytes from LC−MS data. Examples of lipidomics software implemented as Microsoft Excel add-ons are the Fatty Acid Analysis Tool (FAAT)[64] and Lipid Mass Spectrum Analysis (LIMSA).[65] FAAT has been optimized for analysis of high-resolution MS data generated by Fourier transform-ion cyclotron resonance (FT-ICR) mass spectra. The LIMSA tool is capable of performing isotopic correction and peak integration as well as mass matching to a user-supplied list of expected lipids. Commercial MS instrument vendors such as AB-SCIEX (www.absciex.com) are developing their own platform-specific lipid analysis approaches such as Lipid Profiler and LipidView,[66] but they suffer from the drawback that they must be used in conjunction with their proprietary Analyst software.

Table 4. Examples of Software for Performing Lipid MS Analysis

application	platform	comments, URL
Mzmine	Java	comprehensive package for lipidomics profiling, http://mzmine.sourceforge.net/
LDA	Java	novel algorithms for detection of minor lipid species, http://genome.tugraz.at/lda
LipidXplorer	Python	designed for shotgun lipidomics data, uses novel query language, http://sourceforge.net/projects/lipidxplorer/
LIMSA	Excel add-on	performs mass matching to a user-supplied list of expected lipids, http://www.helsinki.fi/science/lipids/software.html
FAAT	Excel add-on	designed for analysis of FT-ICR data, http://www.genomecenter.ucdavis.edu/leary
LipidView	Windows	proprietary AB-SCIEX package used in conjunction with Analyst software, http://www.absciex.com/products/software
LIPID MAPS	Web interface	set of online MS prediction tools tailored to different lipid classes, http://www.lipidmaps.org/tools/index.html

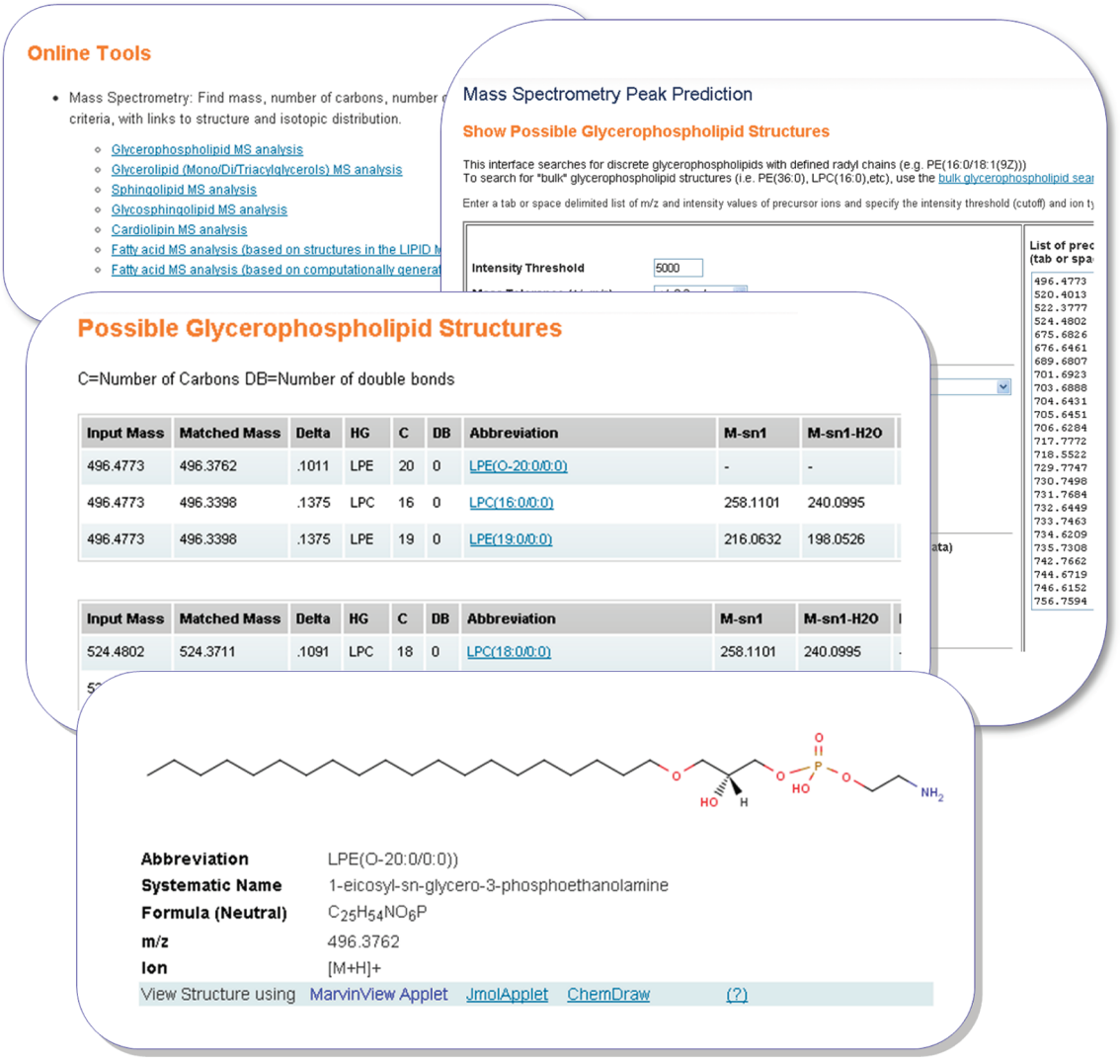


Figure 16. Montage of screen shots showing LIPID MAPS mass spectrometry tools.

A new open source Python programming language[67] application called LipidXplorer[68] is tailored toward the analysis of data from shotgun lipidomics experiments. LipidXplorer does not have a database of lipid masses for peak identification but instead enables the user to compose queries and constraints for lipid classes of interest using the novel concept of a Molecular Fragmentation Query Language (MFQL). The LIPID MAPS MS analysis tools (http://www.lipidmaps.org/tools/index.html) are a freely available set of online resources and focus on the simpler task of matching peak lists of precursor ions to predicted structures under a variety of experimental conditions. Certain classes of lipids such as acylglycerols and glycerophospholipids composed of an invariant core (glycerol and head groups) and one or more acyl/alkyl substituents are good candidates for MS computational analysis. These molecules tend to fragment in a predictable fashion in collision-induced experiments leading to loss of acyl side chains, neutral loss of fatty acids, and loss of water and other diagnostic ions[69] depending on the nature of the head group. It is possible to create a virtual database of permutations of the more common side chains for glycerolipids and

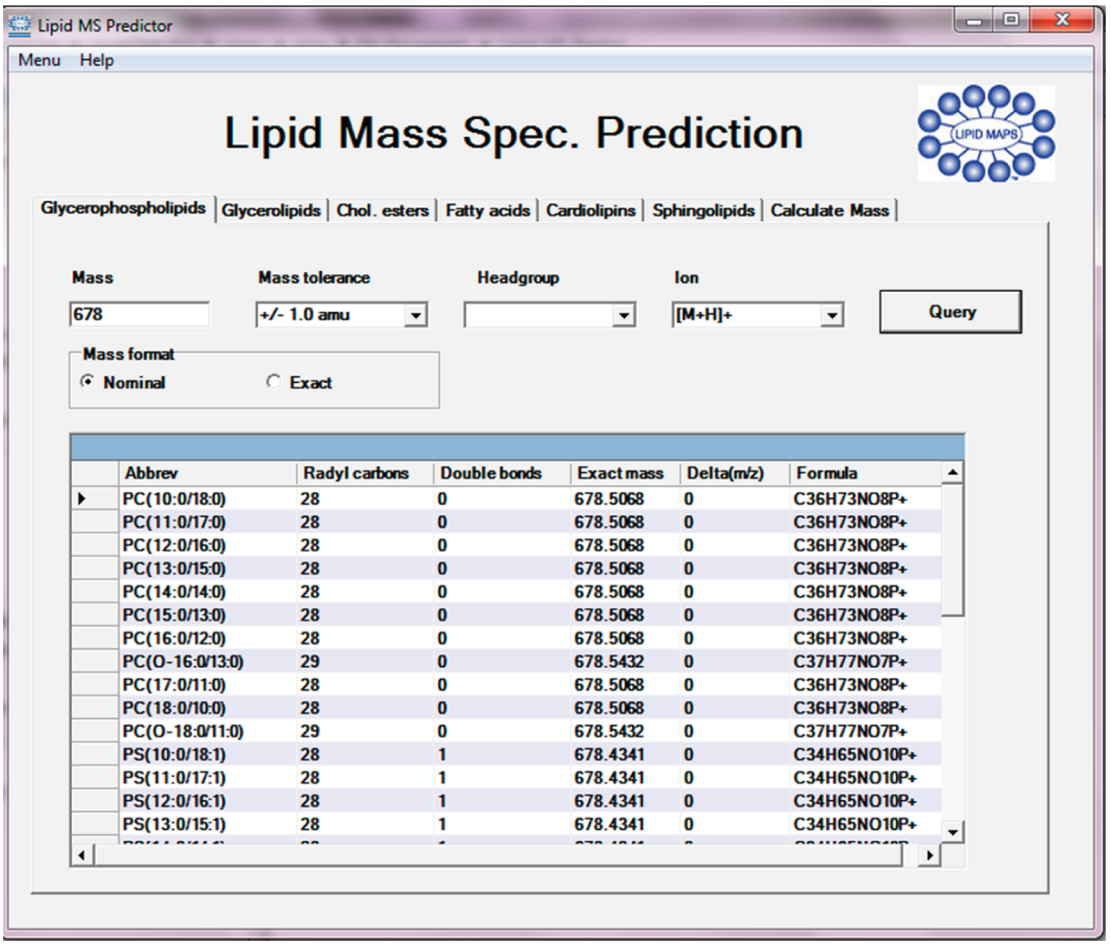


Figure 17. Stand-alone Windows application, LIPID MAPS MS prediction tools, for predicting possible molecular species for a given MS ion. The application enables a user to enter the m/z value of an unknown lipid ion and predict the most likely molecular species. It is available for download at www.lipidmaps.org/tools/index.html.

glycerophospholipids and calculate "high-probability" product ion candidates to compare the experimental data with predicted spectra. The LIPID MAPS group has developed a suite of search tools[13] that allow a user to enter an m/z value of interest and view a list of matching structure candidates, along with a list of calculated neutral loss ions and other high-probability product ions. The MS prediction tools are currently available for a number of different categories of lipids: glycerolipids, glycerophospholipids, cardiolipins, and sphingolipids. In each case, all possible structures corresponding to a list of likely head groups and acyl, alkyl ether, and vinyl ether chains have been expanded and enumerated by computational methods to generate a table containing the nominal and exact mass for each discrete structure as well as additional ontological information such as formula, abbreviation, and numbers of chain carbons and double bonds. This tabular data are then uploaded into category-specific database tables, making them amenable for online querying. The MS prediction tools for glycerolipids and glycerophospholipids have been extended by computing production ion masses for commonly observed fragments corresponding to acyl chain ions, neutral loss of acyl chains, loss of water, head group-specific fragmentations, and combinations of the above.

The MS prediction tools for glycerolipids, cardiolipins, and glycerophospholipids accept an m/z value from the user for the precursor ion and have a menu to allow selection of the ion mode ($[M + H]^+$, $[M + NH_4]^+$, $[M - H]^-$, etc.). In addition, a mass tolerance window and a head group (in the case of glycerophospholipids) may be specified to limit the number of matches. The list of matches may also be filtered by specifying a particular set of radyl chains (for example, only chains with even numbers of carbon atoms). On completion of a search, the output format (Figure 16) contains a list of structures (a) that satisfy the input criteria and (b) whose side chains belong to the list of radyl chains used to populate the database. The predicted masses of the fragment ions are computed at run-time by the online application. All entries in the result set are hyperlinked to the structure drawing application, enabling "on-demand" visualization of the molecular structures. Isotopic distribution profiles for each structure may also be viewed online. The online tools allow batch-mode searches of lists of precursor ions and intensity values which may be copied and pasted into the user interface. Users may perform searches where the matched ions are displayed in "bulk" format (e.g., PE(34:1), TG(54:2)) or as discrete molecular species (e.g., PE(16:0/18:1(9Z)), TG(18:0/18:1(9Z)/ 18:1(9Z))). Additionally, in the case of experimental samples where the relative amounts of the acyl groups of glycerolipids and glycerophospholipids are already known (e.g., from fatty acid methyl ester (FAME) analysis by GC), these data may be entered, and a scoring algorithm then ranks the matched species on the basis of the relative abundance of those acyl chains

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in each lipid. As mentioned above, the current versions of the LIPID MAPS MS prediction tools employ databases of mass permutations for the lipid classes of interest, but it is certainly possible to replace the database with user-specified lists of chains/head groups and perform all mass matching calculations in "real time". This type of option would be useful in cases where the sample of interest contains lipids with rare or unusual side chains such as those encountered in bacteria or invertebrates.

A stand-alone Windows application has also been developed (Figure 17) for predicting possible molecular species for a given MS ion. In contrast to the online tools which query a database table of masses corresponding to structural permutations for each lipid category, the stand-alone application (http://www.lipidmaps.org/tools/index.html) first computes these masses from first principles using a list of commonly occurring side chains and head groups typically found in mammalian versions of glycerolipids, glycerophospholipids (including cardiolipins), and sphingolipids. This application enables a user to enter the m/z value of an unknown lipid ion and predict the most likely molecular species. There are separate user interfaces for glycerolipids, glycerophospholipids, cardiolipins, sphingolipids, fatty acids, and cholesteryl esters. There is also a user interface to calculate the exact mass of glycerophospholipid and glycerolipid ions with defined side chains and head groups, along with a display of the isotopic distribution profile.

5.2. Presentation of MS Data

The LIPID MAPS consortium has placed an emphasis on online presentation of MS data to maximize the level of interactivity with other Web-based resources such as lipid/gene databases and experimental protocols. Recent studies by the LIPID MAPS consortium. have quantified over 550 different lipids from mouse macrophage cells[2] and almost 600 lipids from human plasma[70] using MS and statistical bioinformatics techniques. This ability to simultaneously assess the metabolic dynamics of hundreds of lipid species reveals a wealth of information regarding the cellular lipidome. On a more general scale, the LIPID MAPS consortium has embarked on a time-dependent study of a wide range of lipid classes in mouse macrophage cells, in response to stimulation by a number of agonists such as Kdo_2-lipid A (KLA), adenosine triphosphate (ATP), and 25-hydroxycholesterol. Large-scale integrated studies have been carried out on both cultured cells such as the RAW264.7 cell line and on primary cells such as thioglycolate-elicited peritoneal macrophages (TGEMs) and bone-marrow-derived macrophages (BMDMs). Quantitative data from these experiments are being used to validate existing lipid networks and elucidate novel interactions. MS quantitative measurements from time-course experiments on the various categories of lipids are obtained from the individual LIPID MAPS cores in Microsoft Excel or text format. These heterogeneous formats are then imported into a common data format prior to processing and conversion into Oracle database tables. Data on different cell samples (biological replicates) and/or different MS runs (technical replicates) for each lipid species are consolidated. A middleware layer composed of a Web server and PHP/Perl scripting has been deployed to create a Web-based user interface with the MS data stored in an Oracle database. All calculations used to display averages of technical and biological replicates, as well as all standard error of the mean (SEM) and standard deviation calculations are performed via Structured Query Language (SQL) code. All online data displays were integrated with the LIMS system (via sample barcodes) and the LIPID MAPS structure database (via LM ID identifiers where applicable), allowing seamless navigation across both data and metadata. A software drawing component called dynamic graphics (GD; http://www.boutell.com/gd/) was used to generate online graphs "on-the-fly", in response to user input. The database schema design was optimized for access speed and high data integrity. A set of online query and display tools were developed to allow the end-user to view MS time-course data in a number of different formats (Figure 18). These include tabular and graphical displays of data as averages of technical and biological replicates, as well as "drill-down" links to the corresponding LIMS metadata (cell samples) and structure/classification information (analytes). All lipidomic and gene array data generated by the LIPID MAPS consortium are available in the "Resources/Data" section of the Web site.[5] With a view to enabling lipidomics researchers to identify discrete lipid species, an online library of lipid standards, including tandem mass spectral data generated by the LIPID MAPS core facilities, has been made available on the LIPID MAPS Web site. This database currently consists of over 550 analytes spanning the 8 major lipid categories with annotated diagnostic product ion identifications and with links to molecular structures and MS acquisition protocols used to generate the raw spectra (http://www.lipidmaps.org/data/standards/index.html).

6. MODELS OF LIPID METABOLISM AND PATHWAYS

Pathways may be broadly described as models that characterize movement of material through a network of molecular species and processing steps. They serve as the basis upon which much of the new field of systems biology must be built. Many tools have become available over the past 10 years for enabling biological pathway construction.[71] Their construction has been stimulated by the growth in information resulting from adoption of new laboratory tools accompanying high-throughput data acquisition, such as mass spectrometry.[1b,72] The process of constructing pathways requires ready access to information in the form of experimental data of a quantitative nature. The use of reference model pathways as starting points for new work, as well as inclusion of well-characterized compounds in pathway schemes, is also of great importance.

Lipids play central roles in energy storage, cell membrane structure, cellular communication, and regulation of biological processes such as inflammatory response, neuronal signal transmission, and carbohydrate metabolism. Organizing these processes into useful, interactive pathways and networks represents a great bioinformatics challenge. The KEGG consortium maintains a collection of manually drawn pathway maps[73] representing current knowledge on the molecular interaction and reaction networks, several of which pertain to lipids, including fatty acid biosynthesis and degradation, sterol metabolism, and phospholipid pathways. Additionally, the KEGG Brite[74] collection of hierarchical classifications includes a section devoted to lipids where the user can select a lipid of interest and view reactions and pathways involving that molecule. A number of category-specific lipid pathways have been constructed, notably SphinGOMAP,[75] a pathway map of approximately 400 different sphingolipid and glycosphingolipid species.

In general, the field of metabolomics involves inducing perturbations to the ongoing state of living systems and subsequently monitoring changes to compounds at specific time points. The interactions among components of a pathway are

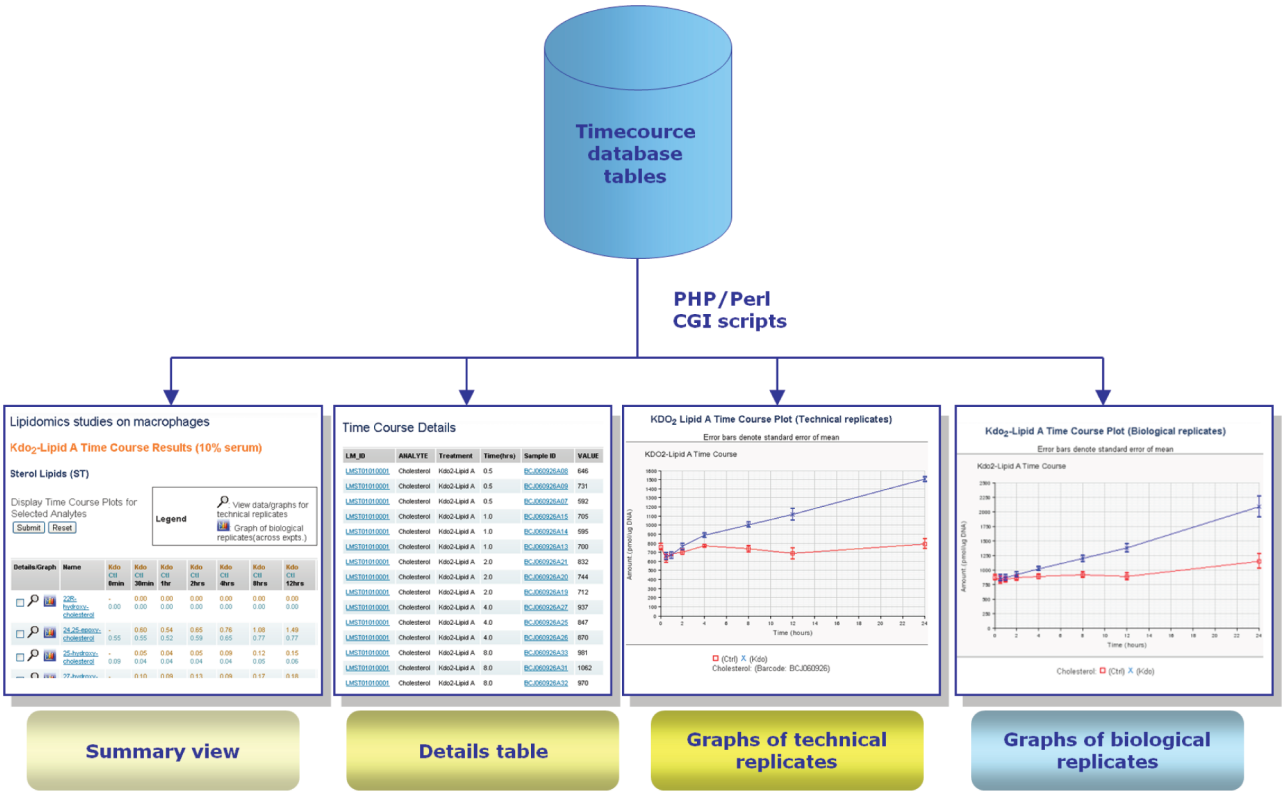

Figure 18. Scheme for online data display of time-course results.

then inferred by a variety of techniques, including metabolite fingerprinting and profiling, and by comparison between organisms that have been genetically perturbed or subjected to altered nutritional states.[71c,d]

A recent review of pathway editing tools[76] points out that a major function of pathway visualization tools is to enable new insights into biology. The choice of a program depends upon the task to be accomplished. For example, a tool may be selected on the basis of the nature of the data to be examined or whether mathematical modeling or statistical analysis is to be performed.

An important function of pathway editor programs, in general, is to permit exchange of pathways. Different file format standards exist for this purpose. They include KEGG Markup Language (KGML),[77] SBML,[53b] BioPAX,[52] and CellML.[78]

To construct pathways, the LIPID MAPS Bioinformatics core is using two pathway editing tools: VANTED[79] and the LIPID MAPS Pathway Editor, which is based upon a toolkit referred to as the BioPathways Workbench.[53a,80] These tools read data from files and/or directly from databases and enable viewing of experimental data in the drawing panel. Most importantly, they enable setting node appearance on an individual basis, thus providing important visual clues as to the roles of the molecular species in the pathway. The Pathway Editor presents measurement data according to experiment and enables detailed viewing of data that may be selected on the basis of the treatment, reproducibility of the measurements, and other, more qualitative aspects, in the judgment of the user. Both Pathway Editor (Figure 19) and VANTED (Figure 20) have Java-based GUIs providing a comprehensive range of viewing and import/export formats.

Various methods are employed in constructing pathways. For example, a user may position a node in a pathway on the basis of whether the measured data that are presented meet expectations according to domain knowledge, including early or late responsiveness to a stimulus, and the magnitude of the response. Automated selection and layout, including filtering nodes based on quantitative or qualitative features, are also commonly used. The LIPID MAPS project has manually adapted mouse and human pathways relating to lipid metabolism from various sources and made them available for downloading through the Pathway Editor for viewing and modification.

7. STATISTICAL ANALYSIS, CORRELATIONS, AND INTEGRATION OF GENOMIC AND LIPIDOMIC DATA IN MACROPHAGES

From a systems perspective, the genome, metabolome, and proteome provide the complete parts list which can be used to reconstruct networks. However, in a given context, the entire parts list may not be of relevance. Hence, context-specific data, such as gene microarray or other types of genomic data, metabolomic data, and proteomic data obtained from specific experiments, can be used to obtain a refined (sub) parts list using various statistical analyses such as identification of significantly regulated genes and analysis of variance (ANOVA). Such a refined parts list serves as the starting point for network reconstruction by integration of experimental data and legacy knowledge.[81] The tools for network reconstruction include pathway enrichment analysis for studying pathway-level subglobal changes, motif discovery for coregulated genes, and correlation analysis for comparing different gene, proteins, or metabolites. Nextgen sequencing methods are now beginning to provide very accurate transcript measurements and will no doubt be used in gene expression studies. Once the transcriptomic changes are

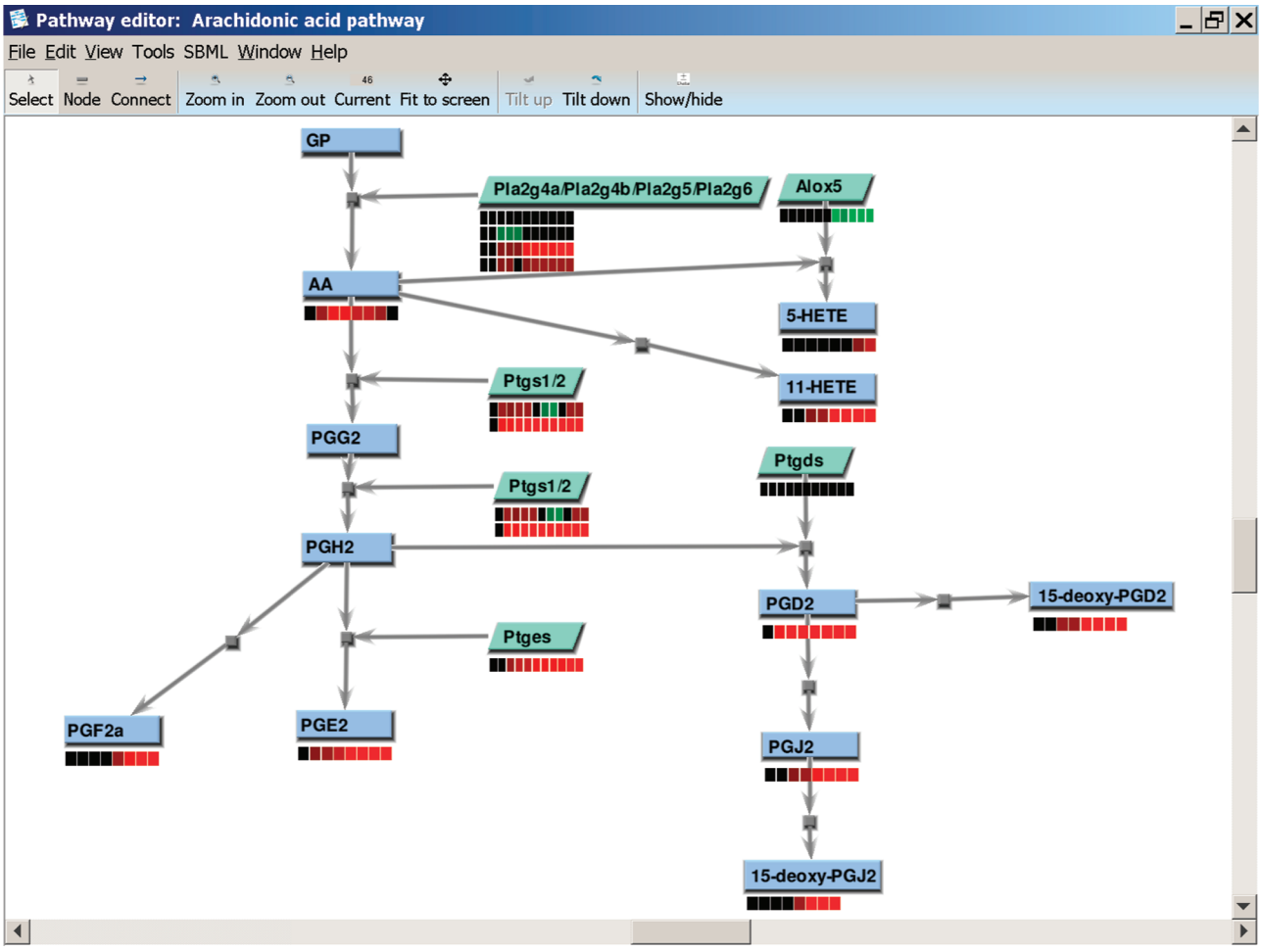

Figure 19. Pathway Editor showing the mouse arachidonate pathway and time-course data mapped in heat map format displayed under the lipid and enzyme (gene) nodes. Samples of RAW264.7 cells (a tissue cell line derived from mouse macrophages) were treated with KLA for times ranging from 0 to 24 h. Shown are ratios (pmol/μg of DNA) for metabolites and ratios of normalized intensity for RNA spots with respect to untreated control cells.[2,80]

deciphered from the mapping of sequence tags, strategies such as the ones described for analogue microarray experiments can be used.[82] In this section, various bioinformatics tools used for analyzing different types of data and their integration are discussed. Where appropriate, the data and studies from mouse macrophage RAW264.7 cells in LIPID MAPS have been used for illustrative purposes.

7.1. Identification of Significantly Regulated Genes

Gene microarray experiments provide a cost-effective way of studying the whole-genome-level response of the cell or tissue system. While there are about 30 000 genes in the mouse and human, in any experimental/treatment condition, only a small fraction of these genes show significant changes as compared to the normal (untreated) condition. The naive approach to identify which genes are significantly regulated would be to use a cutoff on the ratio of the intensities for treatment versus control conditions. However, due to the differences in the hybridization efficiency of different probes for the genes, a wide range of image intensity values are obtained across the whole genome. Coupled with the measurement noise and other effects, the large intensity range makes it difficult to use a single threshold for different genes on the ratio of the intensities between the treatment and control conditions. Hence, in the past 15 years, several approaches have been developed for the analysis of transcriptomic data to account for the wide intensity range across the gene chip. Variance modeling with prior exponentials (VAMPIRE),[83] CyberT,[84] and Linear Models for Microarray (LIMMA) data[85] techniques are commonly used to identify the significantly regulated genes. VAMPIRE involves modeling the global variance structure of array data in the context of a Bayesian framework. CyberT employs statistical analyses based on regularized *t* tests that use a Bayesian estimate of the local variance among gene measurements. Both VAMPIRE and CyberT are available as Web applications. LIMMA uses linear models for the analysis of differentially expressed genes and is available as a part of the Bioconductor project (http://www.bioconductor.org/) in R programming language (http://www.r-project.org/). These methods are able to detect gene expression changes with only two array replicates.

In the analysis of LIPID MAPS microarray data in RAW264.7 cells upon KLA and compactin (an HMG-CoA reductase inhibitor[86]) treatment, CyberT was applied.[2] Figure 21 shows the number of significantly regulated (up- or down-regulated) genes at various time points. In this analysis, a gene is identified as significantly regulated if its *p*-value is less than 0.01. Generally, multiple testing correction methods such as the false discovery rate (FDR) and Bonferroni correction are used for further refinement.[87]

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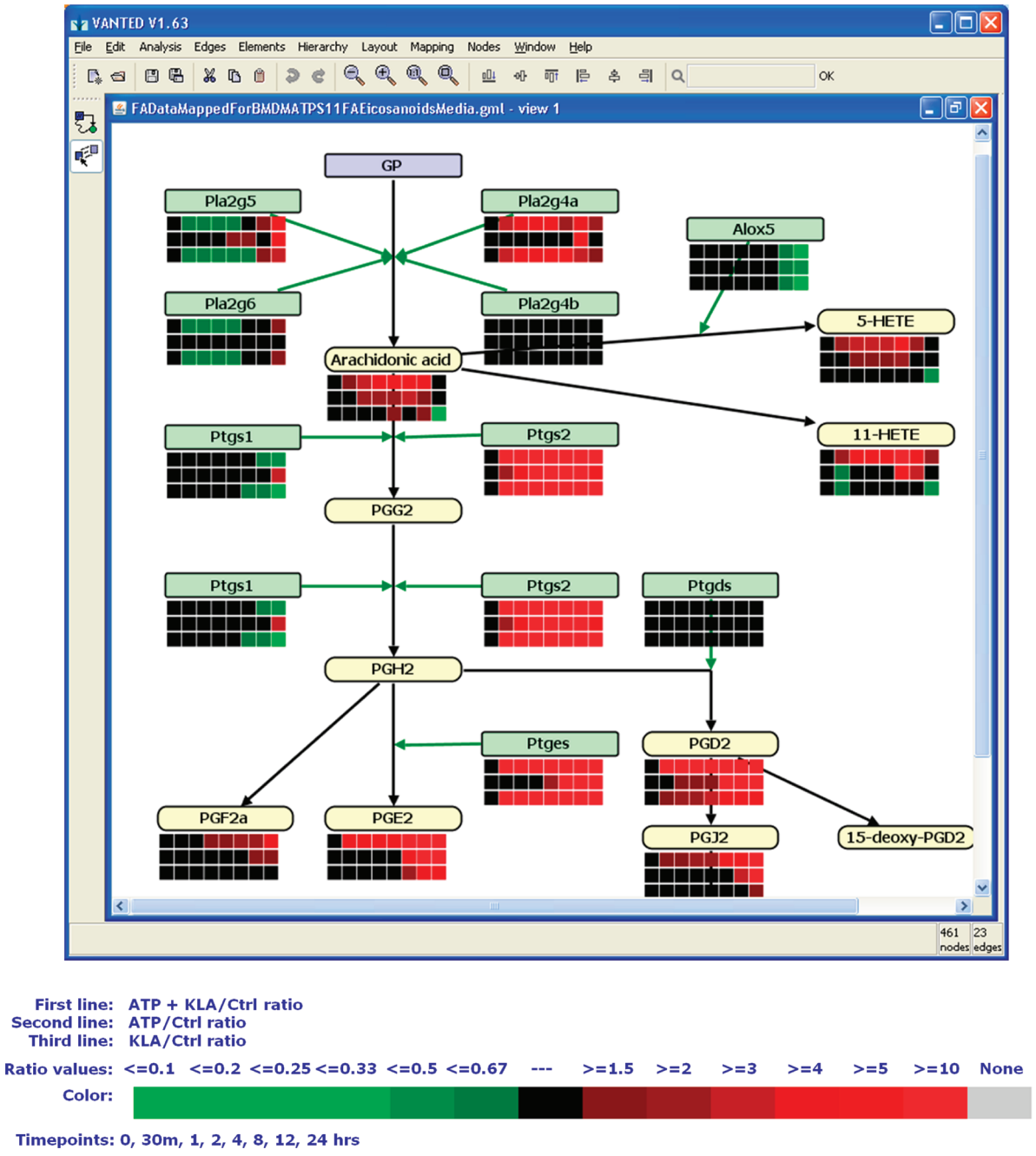

Figure 20. VANTED application showing the mouse arachidonate pathway and time-course data mapped in heat map format displayed under the lipid and enzyme (gene) nodes.

In this data set, compactin showed mild transcriptomic response. Bonferroni and FDR corrections were too stringent for this data set and resulted in no significantly regulated genes. Thus, to find the top significantly regulated genes, no further correction was applied. For further analysis, one may also use a cutoff of 2.0 on the fold change to generate a refined list of significantly regulated genes.

Ultimately, the utility of any combination of microarray platform and analytical method is determined by how well statistical predictions are matched by experimental validation. For expression analysis, quantitative polymerase chain reaction (QT-PCR) assays are performed. LIPID MAPS investigators have several hundred validated PCR primers for genes that are of particular interest to them. These primers are used to validate results of microarray experiments. While not comprehensive, sufficient probes are available to determine whether different analysis methods provide reliable results. Validation of microarray experiments in RAW264.7 cells for several genes using QT-PCR is discussed in a recent study.[2]

7.2. ANOVA

The *t* test is sufficient to compare between two conditions, namely, control or untreated samples and stimulated or treated samples. Hence, methods such as VAMPIRE, CyberT, and

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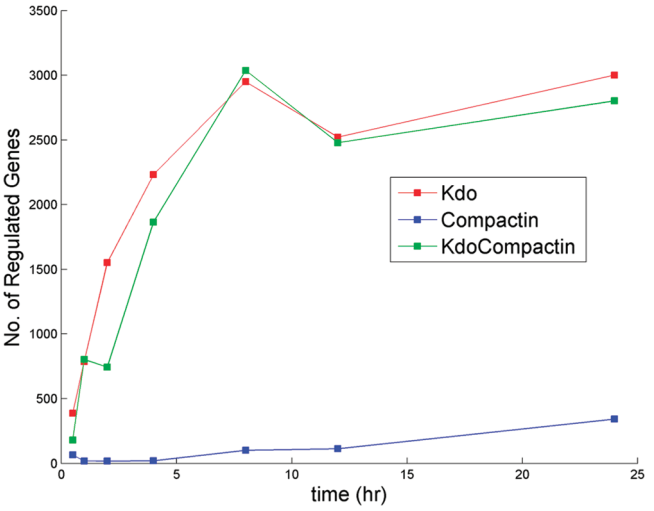

Figure 21. Time course of the number of regulated genes in RAW264.7 cells stimulated with KLA, compactin, and KLA + compactin.

LIMMA can identify the differentially regulated genes between two conditions corresponding to a single treatment. However, in the case of multiple treatment experiments or experiments at several time points, the above approaches cannot delineate the effect of different treatments on a particular gene or other measurements. It is necessary to separate the effect of different treatments or the time component to draw rational conclusions from the data. This task is accomplished by the ANOVA approach, which has been widely used to deconvolute the effect of different treatments. In ANOVA, the observed variance in the measured data is partitioned into the effect of individual factors or treatments.[88] If necessary, terms corresponding to the interactions among different factors can also be included in the variance partitioning model. Similar to statistical tests such as the *t* test (used by VAMPIRE, CyberT, etc.), in ANOVA, a *p*-value is assigned to the effect of different factors included in the model. ANOVA can be used to factor out the significance of different treatments or time effects on any experimental measurements such as genes,[89] proteins,[90] and metabolites.[2] ANOVA can also be used for the identification of significantly regulated genes as well[89] because for the case of one factor with only two possible values for the factor (e.g., control vs single treatment), ANOVA (called one-way ANOVA) and an unpaired *t* test are equivalent, although this cannot account for the effect of intensity range on the measure of variance, a hallmark of techniques such as CyberT and VAMPIRE. In LIPID MAPS studies, ANOVA was applied to transcriptomic and lipidomic data from RAW264.7 cells upon KLA and compactin treatment to separate the effect of KLA and compactin on the genes or lipids.[2] In a previous study relating to network reconstruction, ANOVA was applied on the measurement of phosphorylation states of signaling proteins and cytokines to find putative lumped connections from the stimuli to the signaling pathways or cytokine regulation.[90] Another study suggests that there is potential for further analysis of the ANOVA results by performing multivariate analyses such as principal component analysis (PCA) on the interaction terms for different factors[91] to find out if such interactions may be significant under certain conditions. Biplots from PCA may also aid in visualization and interpretation of results. More recently, the combined approach of ANOVA−PCA has gained considerable attention from statisticians, especially when three or more factors need to be analyzed.[92] Their utility for analyzing data with only two treatments may be limited.

7.3. Gene Ontology and Pathway Enrichment Analysis

The differentially regulated features obtained from any statistical test must be interpreted biologically. In this direction, GO and pathway enrichment analysis is prevailing significantly. These analyses identify which processes and pathways are affected significantly as compared to what would be expected by chance in the experiment. There are many tools available as software or Web applications. For example, AmiGO,[93] Goby (part of the VAMPIRE suite)[83a] and Database for Annotation, Visualization and Integrated Discovery (DAVID)[94] are available as Web applications. SubpathwayMiner is available as a part of the Bioconductor project in R programming language.[95] This database-driven application stores annotation data from several sources, namely, GO, KEGG, TRANSFAC[96] and Biocarta.[97] In addition, it can be easily updated with user-defined annotation lists.[83a] Most of these applications use hypergeometric distribution or the Fisher exact test to compute the enrichment likelihoods.

Goby was used extensively in the analysis of gene expression data in RAW264.7 macrophages.[2] Some of the results for the microarray data from RAW264.7 cells in the KLA/compactin study are listed in Table 5, which shows that the majority of the genes from the KEGG Toll-like Receptor (TLR) pathway are up-regulated. Other pathways relevant to inflammation, such as Jak-Stat, NF- κB, and cytokine−cytokine receptor interaction KEGG pathways, are also significantly enriched.

7.4. Sequence Motif Discovery

Identification of transcription factor binding sites (TFBSs) or motifs has been a challenge in the area of bioinformatics. The de novo discovery of the motifs requires the availability of TFBS databases and state of the art software tools. JASPAR[98] and TRANSFAC[96] have been good resources for obtaining the position weight matrices (PWMs) for several hundred transcription factors (TFs). There have been two approaches in the use of alignment for motif discovery. The first approach compares the TFBS alignment on the promoter sequence with the alignment on a random sequence based on the adenine (A), thymine (T), cytosine (C), and guanine (G) composition of the genome.[99] The second approach compares the enrichment of TFBS alignment in the target set with that in the background set.[100]

On the basis of the second approach, a novel computational method to identify regulatory motifs in coregulated genes was developed. The method builds on previous efforts to find DNA motifs that discriminate between the foreground (i.e., coregulated) and background promoter sequences, allowing both positive and negative binding information to be harnessed. The algorithm attempts to find a motif that has maximal enrichment in foreground sequences relative to background sequences. Enrichment is found by considering the overlap of genes in the foreground with genes that contain the motif, using the hypergeometric distribution to calculate the probability of this overlap by chance. The algorithm works by exhaustively checking short motifs of a given length for enrichment between foreground and background promoter sequences, keeping the highest scoring motifs. The highest scoring motifs are then used as seeds to a greedy optimization algorithm that creates degenerate probability matrices that maximize the enrichment of the

Table 5. Global KEGG Pathway (Mouse) Enrichment Analysis of RAW264.7 Cells Treated with KLA, Compactin, or KLA + Compactin[a]

Sr. no.	KEGG path ID	pathway name	total no. of genes	ligand	regulation type	30 min	1 h	2 h	4 h	8 h	12 h	24 h
1	04060	cytokine−cytokine receptor interaction	237	KLA	up	10	24	40	56	61	53	39
					down	1	2	7	11	8	9	13
				compactin	up					0	2	13
					down					7	4	3
				KLA + compactin	up	10	25	32	51	62	51	40
					down	0	1	4	8	10	11	13
2	04620	Toll-like receptor signaling pathway	96	KLA	up	9	17	25	30	33	27	24
					down	1	2	7	4	5	2	8
				compactin	up					0		7
					down					3		1
				KLA + compactin	up	9	19	19	31	32	26	25
					down	0	3	5	3	4	3	6
3	04010	MAPK signaling pathway	261	KLA	up	17	26	30	34	51	45	45
					down	1	6	8	19	21	13	15
				compactin	up							15
					down							1
				KLA + compactin	up	14	27	27	32	57	42	48
					down	0	6	7	13	12	10	12
4	04920	adipocytokine signaling pathway	64	KLA	up	5	11	13	11	13	13	17
					down	0	0	3	5	2	3	3
				compactin	up							
					down							
				KLA + compactin	up	3	11	10	11	15	14	15
					down	0	1	0	4	2	1	3
5	04621	NOD-like receptor signaling pathway	59	KLA	up	8	15	21	23	26	22	20
					down	0	1	1	1	2	1	2
				compactin	up							
					down							
				KLA + compactin	up	7	15	16	25	24	21	20
					down	0	1	1	0	0	1	2
6	04630	Jak-STAT signaling pathway	145	KLA	up	5	11	26	34	37	31	31
					down	1	2	6	7	1	2	5
				compactin	up						1	
					down						2	
				KLA + compactin	up	4	10	19	28	42	35	30
					down	0	4	2	6	2	1	4
7	04514	cell adhesion molecules (CAMs)	146	KLA	up			16	22	24	24	26
					down			1	6	8	6	8
				compactin	up			2			3	10
					down			0			0	1
				KLA + compactin	up			9	24	27	25	30
					down			1	6	7	8	8

[a] The number of genes regulated (up/down) is listed for each time point for the three experiments ($p \leq 0.05$).

motif in the positive set of sequences. This formulation requires surprisingly few assumptions, offering a natural description of motif quality that is applicable to a variety of problems such as finding binding sites that are associated with changes in gene expression or chromatin immunoprecipitation (ChIP) chip results. An application of this method to identify enriched motifs in the promoters of genes induced by KLA in RAW264.7 cells from the time-course experiment is shown in Figure 22.

Three of the most highly enriched motifs identified by this method correspond to binding sites for transcription factors that were previously established to mediate responses to TLR4 activation: NF-κB, interferon response factors (IRFs), and activator protein 1 (AP-1)/activating TF (ATF)/cAMP response element-binding (CREB) family members. Furthermore, many of the genes identified as having NF-κB, interferon-responsive sequence element (IRSE), or AP-1/CREB sites were shown to be direct targets of these TFs by conventional assays, providing one line of validation for this method. In contrast, conventional motif discovery methods failed to identify NF-κB or AP-1/ATF-1/CREB binding sites in transcriptionally

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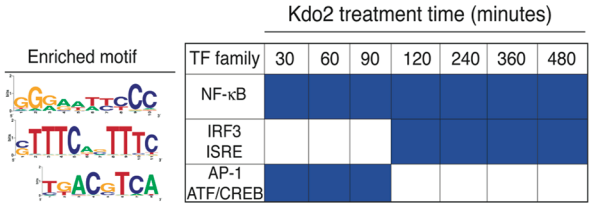

Figure 22. Identification of NF-κB, ISRE, and AP-1/ATF/CREB binding sites in KLA-stimulated genes in RAW macrophages. Blue color indicates significant enrichment of the motif in promoters of positively regulated genes for each time point.

activated genes. One of the interesting features of the enrichment plot illustrated in Figure 22 is the temporal windows in which IRF3/ISRE motifs and AP-1/ATF/CREB motifs appeared. These data have implications for understanding how the complex transcriptional response to TLR4 activation is regulated in a time-dependent manner. Several other sequence motifs are identified by this motif method in the set of lipopolysaccharide (LPS) responsive genes and provide the basis for a series of new studies to identify roles of other classes of transcription factors in regulating the genome-wide response to TLR4 signaling.

7.5. Processing and Analysis of Proteomic Data

A novel, MS-based approach for the relative quantification of proteins, relying on the derivatization of primary amino groups in intact proteins using isobaric tags for relative and absolute quantitation (iTRAQ) was used to measure relative protein intensities in RAW264.7 cells in the presence or absence of KLA. The technique is based on chemically tagging the N-terminus of peptides generated from protein digests that were isolated from different samples, e.g., KLA-treated cells and control cells.[101] The two labeled samples are then combined, fractionated by nano-LC, and analyzed by tandem mass spectrometry. Database searching of the peptide fragmentation data allows identification of the labeled peptides and hence the corresponding proteins. Due to the isobaric mass design of the iTRAQ reagents, differentially labeled proteins do not differ in mass; accordingly, their corresponding proteolytic peptides appear as single peaks in MS scans. Fragmentation of the tag attached to the peptides generates a low molecular mass reporter ion that is unique to the tag used to label each of the digests. Measurement of the intensity of these reporter ions enables relative quantification of the peptides in each digest and hence the proteins from which they originate. The iTRAQ method was used to measure relative protein levels in three samples of RAW264.7 cells treated with KLA for 24 h and three corresponding control cell samples. Protein KLA/control (K/C)ratios were then compared to messenger ribonucleic acid (mRNA) ratios generated from gene array experiments on RAW264.7 cells. Statistical analyses using covariance plots of the 24 h protein ratio data with mRNA ratios at multiple time points established a maximal correlation at 18 h, as would be expected when one considers the time lag between transcription and translation (Figure 23).

A high correlation between mRNA and protein KLA/control ratios was observed for those proteins whose ratios were increased or decreased 2-fold or more. A disadvantage of the "shotgun" LC−MS approach used in these iTRAQ experiments is the lack of sensitivity for detection of low-abundance proteins. A tagged tryptic digest of the entire cell extract is applied to the

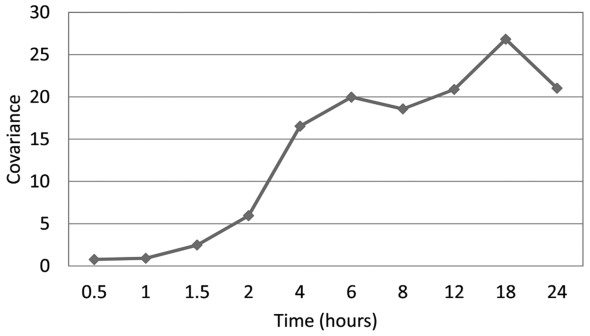

Figure 23. Covariance plot between 24 h protein data from iTRAQ measurements and gene array data at various time points (only proteins with a K/C ratio of >1.5 were chosen).

LC column, and due to sample complexity and a large range in protein concentrations, only about 25% of proteins (as compared to gene array experiments) are detected. A consequence is that many enzymes involved in lipid metabolism are not detected by the iTRAQ method. This disadvantage could be overcome by employing additional purification steps prior to LC−MS, such as subcellular fractionation and affinity chromatography. In addition, this methodology is capable of detecting proteins with post-translational modifications, providing another level of information with regard to function and activity.

7.6. Correlation Analysis

Pearson correlation is widely used to find which variables show similar changes across different experiments or time points.[102] Pearson correlation coefficients can also be used to perform hierarchical clustering[103] and generate correlation networks.[104] Such networks may capture some aspects of the causality among variables or factors. A more elaborate discussion on the issue of correlation versus causality is presented elsewhere.[105] Pearson correlation has also been used, at least conceptually, in various ways in data-driven network reconstruction[106] using an approach such as least-squares or principal component regression[90] and partial least-squares.[107] Correlation analysis has been applied to various biological systems to elucidate how different molecular components function in a network and to understand their phenotypic similarities and differences. Some examples are succinctly described below.

Fiehn and Weckwerth[108] have presented an interesting review on how the data on gene, protein, and metabolite measurements are correlated, resulting in complex networks. A related minireview is presented by Steuer et al.[109] They have also used metabolite−metabolite correlation analysis-based clustering and PCA to develop and visualize data-derived metabolic networks.[110] The visualization approach also includes a clique finding algorithm for improved interpretation. Recently, they have used PCA and partial least-squares analysis for feature extraction to differentiate between the responses of different metabolites in rice to a bacterial pathogen.[111] Schmitt et al.[112] have used correlation between time-lagged data on genes to develop gene interaction networks. They have used gene expression time-course data under different light conditions and were able to find several gene groups containing light-stimulated gene clusters, such as *Synechocystis* sp. photosystems I and II and carbon dioxide fixation pathways. Numata et al.[113] have used mutual information as a nonlinear correlation metric. They have shown that the mutual information-based analysis was able to

uncover some nonlinear relationships undetectable by the Pearson coefficient-based analysis in a data set from *Arabidopsis thaliana*. Fukushima et al.[104a] have also used correlation networks and a graph-clustering approach to find modules using data from three *Arabidopsis* genotypes, namely, Col-0 wild type, methionine overaccumulation 1, and transparent testa4, in samples of roots and aerial parts.

To analyze the LIPID MAPS data, Pearson correlation was used to find the similarity between two time courses.[102] In RAW264.7 cell experiments, the time course for gene data or lipid data consisted of eight time points (including the value at $t = 0$ h). The correlation value can be thought of as the cosine of the angle between the normalized time-course curves (z-scores). Some details previously used in such analyses are presented below.

7.6.1. Gene and Lipid Data. For the genes, the ratio of the value under the treatment condition to the value for the control condition was used at each time point. To compute the correlation between the time course for the lipids and the time courses for the genes in the same pathway (curated list of genes for each lipid pathway as listed on the LIPID MAPS Web site (http://www.lipidmaps.org/pathways/vanted.html) or the list of genes from KEGG pathways), the ratios to control values were used for the lipids as well. The time points for the lipids and the genes were 0, 0.5, 1, 2, 4, 8, 12, and 24 h.

7.6.2. Consideration of Time Delay. Since it is the enzyme or the protein level that may affect the time course of the lipid, in the absence of specific knowledge for individual genes, a time delay of 4 h corresponding to the time taken for mRNA translation, post-translational modification, and protein translocation was used for gene data.

7.6.3. Weighted Correlation. Since the measurements are taken at nonuniform time intervals (more frequently at the beginning and less frequently at later time points), a weighted correlation in which the time points were weighted proportional to the time interval is more appropriate than the raw correlation described above. Assuming a weight vector, $\mathbf{W} = [w_1, w_2, w_3, w_4, w_5, w_6, w_7, w_8]$, the weighted correlation was computed as follows.[2]

First, the weighted mean, weighted standard deviation, and weighted z-score ($n = 8$, the number of time points) were computed, and then the weighted dot product was computed:

$$\text{mean} : \bar{X}_w = \Big(\sum_{i=1}^{n} w_i x_i\Big)\Big/\sum_{i=1}^{n} w_i, \quad \bar{Y}_w = \Big(\sum_{i=1}^{n} w_i y_i\Big)\Big/\sum_{i=1}^{n} w_i$$

$$\text{standard deviation} : \bar{\sigma}_{x,w} = \sqrt{\Big(\sum_{i=1}^{n} w_i (x_i - \bar{X}_w)^2\Big)\Big/\sum_{i=1}^{n} w_i}; \quad \text{similarly, compute } \bar{\sigma}_{y,w}$$

$$z\text{-score} : X_{z,w} = (X - \bar{X}_w)/\bar{\sigma}_{x,w}, \quad Y_{z,w} = (Y - \bar{Y}_w)/\bar{\sigma}_{y,w}$$

$$\text{correlation} : r_w = \Big(\sum_{i=1}^{n} w_i (X_{z,w})_i (Y_{z,w})_i\Big)\Big/\sum_{i=1}^{n} w_i \qquad (1)$$

In the above, for convenience, the weight vector $\mathbf{W}$ was normalized to the unit sum as $w_i = w_i/\sum_{i=1}^{n} w_i$ at the beginning so that the division by $\sum_{i=1}^{n} w_i$ is not explicitly required in the above expressions.

The above equations are easily extended for two data matrices, $\mathbf{X}$ and $\mathbf{Y}$, with several rows in each where rows correspond to different genes or lipids and the columns correspond to different time points as in the above equations.

7.6.4. Data Values To Be Used with the Weighted Correlation. Linear interpolation of data in each time interval was used as an approximation to the scenario where data were measured at equal time intervals. Hence, the mean value of the data in the time interval (i.e., $(x_k + x_{k+1})/2$ for the kth time interval) was used.

7.6.5. Lipid and Gene Categories. Lipid−gene correlation was performed for six different lipid pathways, namely, eicosanoids in the media and sphingolipids, sterols, glycerolipids, glyrecophospholipids, and unsaturated fatty acids inside the cells. For the LIPID MAPS specific curated gene list, the pathways used included eicosanoid biosynthesis, sphingolipid biosynthesis, cholesterol biosynthesis, glycerolipid/glycerophospholipid biosynthesis, and fatty acid biosynthesis. In each selected pathway, only those genes which show significant regulation (differential expression) at one or more time points, computed using CyberT,[84] were used. More details can be found elsewhere.[2]

7.6.6. Display of the Data and Correlations. For the display of the data and the correlation, correlation-based hierarchical clustering[103] was used to lay out the variables (lipids and/or genes) so that the rows corresponding to the variables with high correlation were displayed near each other in the heat map for the data. The Statistics/Bioinformatics toolbox of Matlab[114] was used to perform the computations. Using the hierarchical clustering tools, clusters were identified (distance method = user-specified weighted correlation (eq 1), linkage method = average, cutoff criterion = distance, cutoff = 0.75). It can be noted that a correlation range of $[-1\ 1]$ corresponds to the equivalent distance range of $[2\ 0]$ ($d = 1 - r$). Therefore, the cutoff of 0.75 on the distance corresponds to a cutoff of 0.25 on the correlation. When applied to the lipid−gene data sets, each cluster may have one or more genes and lipids. Some clusters may include no genes or no lipids (but not empty).

The interesting clusters are those which have at least one gene and one lipid since they indicate that such genes and lipids are changing together and serve as a target for investigating causal relationships. In the case of lipid−gene correlations, the information flow was from the genes (proteins/enzymes) to the lipids (after accounting for the time delay). Using this strategy, it would be possible to generate correlation-based directed graphs. The links between two lipids or two genes would then be bidirectional.

For illustrative purposes, the heat map for the data for the eicosanoids (measured in the media) is shown in Figure 24. The prostaglandin lipids (e.g., prostaglandin (PG) E_2 (PGE_2), PGJ_2, and $PGF_{2\alpha}$) and the prostaglandin synthase genes (Ptgs2, Ptges) changed in a similar manner, resulting in strong correlation between them. The mechanistic relationship between these genes/enzymes and their corresponding products is shown in the pathway diagram of Figure 20; e.g., production of PGE_2 was catalyzed by the enzyme corresponding to the gene prostaglandin E synthase (Ptges). Similarly, the correlation analysis between various sterols and the genes for cholesterol biosynthesis suggested that its precursors and its several derivatives covary with the mRNA of HMG CoA reductase (Hmgcr) and cholesterol 25-hydroxylase (Ch25h). Correlation analysis between the sphingolipids and related genes has shown that several sphingolipids are coclustered with the important genes in the pathway, including serine palmitoyltransferase (Sptlc1, Sptlc2) and ceramide synthases (CerS) Lass4 and Lass6.[2] At a semisystemic level, these results had suggested that the joint-correlation analysis can potentially uncover such underlying physical mechanisms.

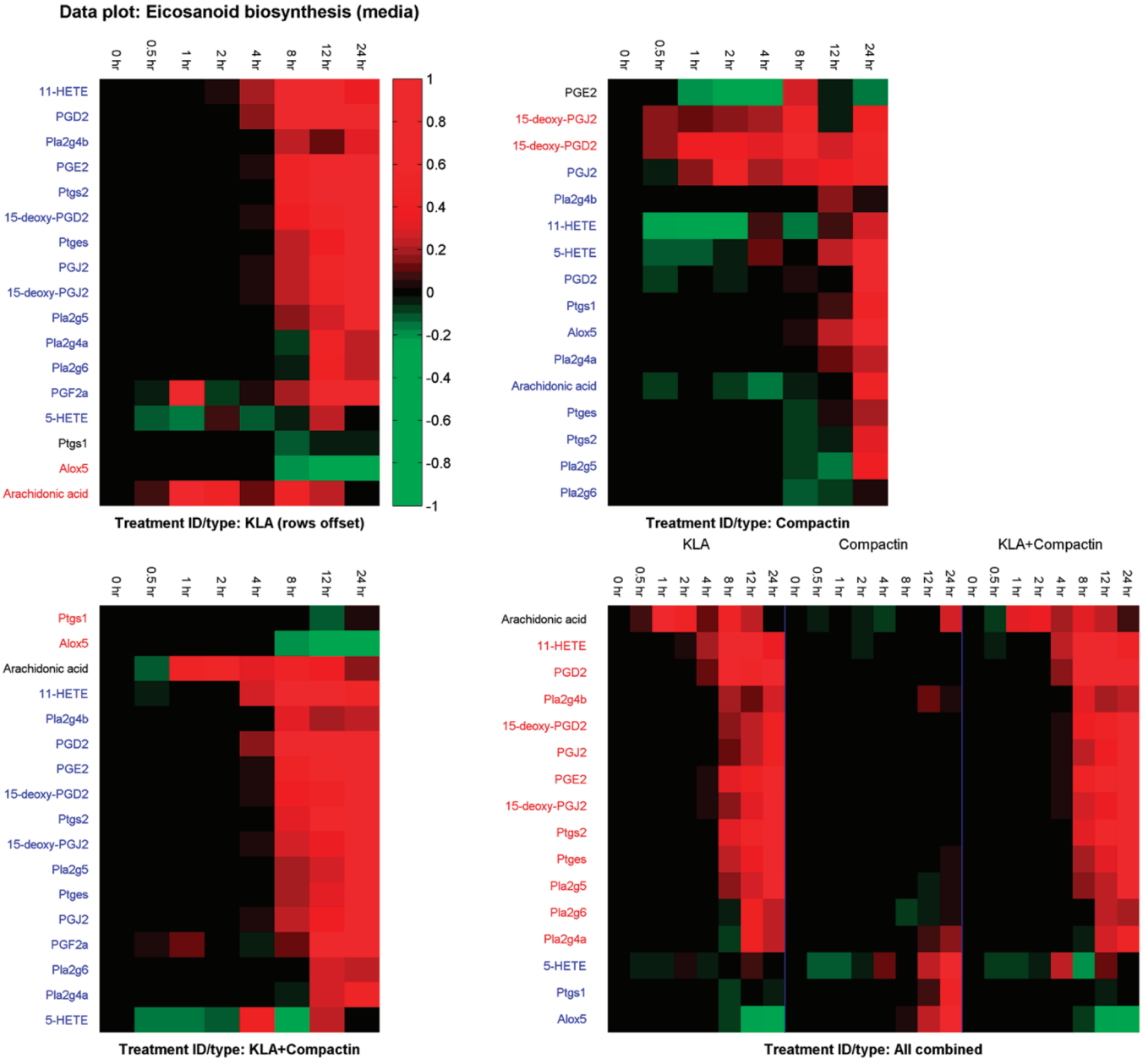

Figure 24. Heat map for the data for eicosanoids (measured in the media) and eicosanoid biosynthesis related genes. Reprinted with permission from ref 2. Copyright 2010 American Society for Biochemistry and Molecular Biology. The four panels correspond to (1) data based on the ratio of values for treatment with KLA to the values for control experiments, (2) the ratio of compactin treatment to the control, (3) the ratio of treatment with both KLA and compactin to the control, and (4) ratio data from (1)−(3) combined. The data in each row are scaled and offset by the $t = 0$ value. The names of the lipids/genes displayed on the *y*- and/or *x*-axis are listed in different colors to indicate the clusters.

8. QUANTITATIVE KINETIC MODELS OF LIPID METABOLISM

All biological processes are inherently dynamical systems. Thus, the use of systems biology approaches is becoming common in the study of metabolic and other networks to elucidate their functions and roles in human health and diseases. Toward this end, several software systems have been developed which allow various types of modeling and analysis, such as steady-state analysis, kinetic modeling, parameter estimation, sensitivity analysis, metabolic control analysis, stochastic simulation, and consideration of spatial variation (partial differential-equation-based modeling). An extensive list of such software systems is available at the SBML Web site.[53] Some of them are CellML (http://www.cellml.org/,[115] JSim (http://nsr.bioeng.washington.edu/jsim/docs/overview.html), VCell (http://www.nrcam.uchc.edu/;[116]), Systems Biology Workbench (http://sys-bio.org/;[117]), COPASI (http://www.copasi.org/;[118]), and MCEll (http://www.mcell.cnl.salk.edu/[119]). Their salient features are summarized in Table 6. All these software systems have some capability to plot and visualize the results of simulation. This comparison, although simple and concise, can help the modeler choose the appropriate software application. The majority of the software systems allow the modeling of signaling and metabolic pathways as a biochemical reaction

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Table 6. Representative List of Different Software Systems for Quantitative Simulation and Analysis of Biological Systems[a]

	tasks allowed (all these software systems have the capability of plotting/visualization of results)						
	deterministic simulation		stochastic simulation				
software	well-mixed system (ODEs)	spatial modeling (PDEs)	well-mixed system	spatial modeling (reaction diffusion)	sensitivity analysis	parameter scan/ estimation	submodel import
CellML/ OpenCell	√	√[b]					√
JSim	√	√			√	√	
Virtual cell	√	√	√		√	√	
COPASI	√		√		√	√	
MCell				√ (3D)			
SBW	√	√[b]	√		√	√	

[a] SBW = Systems Biology Workbench. [b] This capability is available by interfacing with other software systems.

Table 7. Representative List of Lipid Metabolism Related Enzymes for Which Kinetic Data Are Available in the BRENDA Database[136]

KEGG pathway	enzyme name (EC number)	specific activity [(μmol/min)/mg]
arachidonic acid metabolism	prostaglandin-D synthase (5.3.99.2)	14.9−434.0
	leukotriene-A4 hydrolase (3.3.2.6)	0.185−0.49
sphingolipid metabolism	3-dehydrosphinganine reductase (1.1.1.102)	0.000121
	serine *C*-palmitoyltransferase (2.3.1.50)	0.000044
	ceramide glucosyltransferase (2.4.1.80)	0.0000082
	sphinganine kinase (2.7.1.91)	2.0×10^{-6} to 2.4×10^{-4}
	ceramide kinase (2.7.1.138)	4.0×10^{-9} to 1.41×10^{-6}
steroid biosynthesis	sterol *O*-acyltransferase (2.3.1.26)	2.22×10^{-5} to 1.56×10^{-4}
glycerolipid metabolism	glycerol-3-phosphate *O*-acyltransferase (2.3.1.15)	1.3×10^{-4} to 0.192
	diacylglycerol *O*-acyltransferase (2.3.1.20)	3.0×10^{-4} to 0.169
glycerophospholipid metabolism	choline kinase (2.7.1.32)	0.000139
	acetylcholinesterase (3.1.1.7)	7700

system. Most of them have SBML import/export capability, although the information related to pathway/network visualization may be lost during SBML export, a common problem relating to the interoperability of most such software applications.

Among many metabolic pathways, there has been tremendous progress in modeling of glucose metabolic networks. Several researchers have developed genome-scale metabolic networks for different organisms such as *Saccharomyces cerevisiae*, *Escherichia coli*, and humans.[120] There have been efforts in the modeling of signaling pathways as well. Some of the examples include modeling of the mitogen-activated protein (MAP) kinase pathway,[121] regulation of the cell cycle,[122] and calcium signaling.[123] Some of the above approaches are also being used to study plant metabolism. Fiehn et al. have worked extensively on metabolite profiling and their analysis for *A. thaliana*.[124]

Due to the complexity of lipid metabolism, and the paucity of data for its many metabolites, there are only a few models of lipid metabolism available in the literature. For example, Callender et al. have developed a model of diacylglycerol dynamics in the RAW264.7 macrophage.[125] Yang et al. have developed a model of arachidonic acid (AA) metabolism in human polymorphonuclear leukocytes.[126] Only two models of sphingolipid metabolism are found in the literature, one by Alvarez-Vasquez et al.[127] for yeast and one by Henning et al.[128] (the cell system was not specified). All of these models suffer from the unavailability of sufficiently large data sets. Though there are several enzymes for which activity data are available (Table 7), their number is still significantly smaller than the numbers of enzymes in the pathways.

Toward a comprehensive study of lipid metabolism, the LIPID MAPS consortium[69] has quantified the global changes in lipid metabolites ("lipidomics"). Using LIPID MAPS data, context-specific pathway models were developed for several lipid categories by integrating the legacy knowledge and experimental data on lipid changes in macrophages upon KLA stimulation.[4a,129] A central question that can be addressed through quantitative measurements of lipids as a function of time is the flux of metabolites through the cellular network. This is possible as the rate of change of the metabolite concentrations, which can be computed directly from the time-course data, is related to its fluxes corresponding to the different reactions. This enables the development of kinetic models for several lipid pathways. Once the kinetic model is developed and the rate parameters are estimated, the reaction fluxes (and their relative distribution in different branches of the network) can be computed. It is useful to note that, in most kinetic modeling

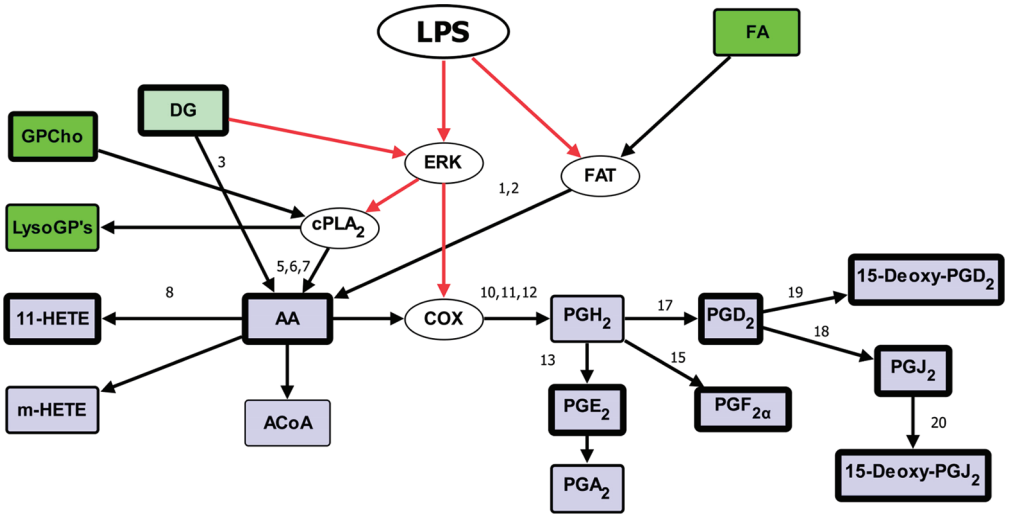

Figure 25. LPS/KLA-stimulated eicosanoid metabolism and signaling pathway. The numbers above the arrows are reaction numbers (Table 8), and default degradation reactions are not labeled. Black lines represent lipid metabolism, and red lines indicate signaling pathways. Metabolites and enzymes are represented as rectangular and oval boxes, respectively. The measured and unmeasured metabolites are differentiated by thick and thin borders, respectively. Purple color is used for eicosanoids and green for glycerolipids and glycerophospholipids. Reprinted with permission from ref 129. Copyright 2009 Elsevier Ltd.

Table 8. Estimated Parameter Values for the Eicosanoid Model[a]

no.	reaction	parameter name	value
1	[LPS] FA → AA[b]	k_1	355.637
2	FA → AA	k_2	10^{-15}
3	DG → AA	k_3	10^{-15}
4	AA →[c,d]	k_4	10^{-15}
5	[DG] GPCho → AA[b]	k_5	10^{-15}
6	[LPS] GPCho → AA[b,e]	k_6	0.330
7	GPCho → AA	k_7	10^{-15}
8	AA → HETE	k_8	0.007
9	HETE →	k_9	0.187
10	[DG] AA → PGH2[f]	k_{10}	0.024
11	[LPS] AA → PGH2	k_{11}	0.111
12	AA → PGH2	k_{12}	0.098
13	PGH2 → PGE2	k_{13}	0.204
14	PGE2 →	k_{14}	10^{-15}
15	PGH2 → PGF2a	k_{15}	0.061
16	PGF2a →	k_{16}	10^{-15}
17	PGH2 → PGD2	k_{17}	3.116
18	PGD2 → PGJ2	k_{18}	0.054
19	PGD2 → dPGD2	k_{19}	0.029
20	dPGD2 →	k_{20}	0.014
21	PGJ2 → dPGJ2	k_{21}	0.034
22	dPGJ2 →	k_{22}	0.116

[a] Reprinted with permission from ref 129. Copyright 2009 Elsevier Ltd. [b] [DG] and [LPS] indicate the effect of signaling (molecules) in the reaction. [c] X → means default degradation of the metabolite X. [d] The unit of the first-order reaction is 1/h. [e] The unit of the second-order reaction is 1/h when it involves either FA or LPS as one of the metabolites as we have used a scaled profile for these variables. [f] The unit of the second-order reaction is μg of DNA/(ratio of intensity × h) when it involves DG as one of the metabolites.

studies on biochemical pathways, generic values for the rate parameters are used because system- and context-specific values are lacking. As we have illustrated in a previous review,[105] lack of such specific rate parameter values is a major challenge in computational systems biology. However, in the LIPID MAPS study, due to the availability of a large amount of data (about five data points per unknown rate constant), the rate constants were estimated with good accuracy.[129] A matrix-based approach and optimization was used to estimate the rate constants using experimental data and known network topology from the literature while ensuring that the rate constants are positive. Modeling of the eicosanoid pathway is presented as an example. More details can be found elsewhere.[129] The network model used, which includes only the measured metabolites, is presented in Figure 25.

8.1. Kinetic Model and Parameter Estimation

A kinetic model was developed for the simplified lipid network involving AA metabolism.[129] The reaction rates were described by linear or law of mass action kinetics. Thus, the flux expressions obtained from this scheme were linear in rate parameters and nonlinear in metabolite concentrations. The matrix-based approach to estimate the rate constants is described below in terms of the reaction numbers labeled in Figure 25 and listed in Table 8. The metabolite concentrations were known, and the rate parameters were unknown. Hence, the following ordinary differential equations (ODEs) describing the rate of change of concentrations of metabolites can be rearranged in a matrix format as shown in eq 2 for $[PGH_2]$ and $[PGD_2]$:

$$\frac{d[PGH_2]}{dt} = k_{10}[DG][AA] + k_{11}[LPS][AA] + k_{12}[AA] - k_{13}[PGH_2] - k_{15}[PGH_2] - k_{17}[PGH_2]$$

$$\frac{d[PGD_2]}{dt} = k_{17}[PGH_2] - k_{18}[PGD_2] - k_{19}[PGD_2]$$

where the rate constants k_i (i = 10, 11, 12, 13, 15, 17, 18, 19) are as defined in Table 8.

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$$\begin{bmatrix} \frac{d[PGH_2]}{dt} \\ \frac{d[PGD_2]}{dt} \end{bmatrix} = \begin{bmatrix} [DG][AA] & [LPS][AA] & [AA] & -[PGH_2] & -[PGH_2] & -[PGH_2] & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & [PGH_2] & -[PGD_2] & -[PGD_2] \end{bmatrix} \begin{bmatrix} k_{10} \\ k_{11} \\ k_{12} \\ k_{13} \\ k_{15} \\ k_{17} \\ k_{18} \\ k_{19} \end{bmatrix} \quad (2)$$

$$\mathbf{Y} = \mathbf{Xb}$$

X is completely known. The left-hand side of the equations (matrix **Y**) was computed using discretization and the experimental data. To avoid singularity during matrix inversion and to require positive values of the rate parameters, a constrained least-squares approach was used (Matlab[114] function *lsqlin*). The parameter values thus obtained were used as good initial values for further refinement by using generalized constrained nonlinear optimization (Matlab function *fmincon*). The objective function for use with *fmincon* was

$$\min_{K, X_0} \left(\begin{array}{l} w_1 \sum_{i=1}^{nsp} \left(\sum_{j=1}^{nt} \left(y_{i,j,\text{exptl}} - y_{i,j,\text{pred}}(K, X_0) \right)^2 \right) + \\ w_2 \sum_{i=1}^{nsp} \left(\sum_{j=1}^{nt} \left(\left(\frac{dy}{dt} \right)_{i,j,\text{exptl}} - \left(\frac{dy}{dt} \right)_{i,j,\text{pred}} (K, X_0) \right)^2 \right) \end{array} \right) \quad (3)$$

where nt is the number of time points, nsp is the number of species, K indicates the parameters (rate constants), and X_0 indicates the initial conditions (species concentrations). The first term represents the fit error between the experimental and predicted concentrations, and the second term represents the fit error between their experimental and predicted derivatives. Different weights (w_i) can be assigned to these two terms to improve the fit. The initial concentrations of the metabolites were also optimized in a narrow range around the experimental values. When data on more than one condition were available, then all the data were used to compute the fit error by simulating the model several times individually and minimizing the objective function collectively.

Table 8 lists the reactions and the corresponding estimated reaction rate parameters included in the model. Figure 26 shows the simulation results.[129] For most time points, the difference between the predicted and experimental data was within the SEM (Figure 26). Thus, a good fit to the data from both treatment and control conditions suggested that the topology of the simplified network was correct and captured the important metabolic and signaling effects. The model was validated by excluding the data on one of the intermediate metabolites from objective function minimization. The rate parameters were estimated, and the predictions were compared with the actual experimental data. There are two intermediate metabolites present in the network: PGD_2 and PGJ_2. The validation was performed on both of the metabolites, and satisfactory results were obtained. Parametric sensitivity analysis was also performed.[129] In short, for each parameter and each metabolite, a monotonic increase or decrease or no change was observed depending upon the respective location of the parameter and the metabolite chosen in the network. The change in the parameters belonging to the upper part of the network produced a larger change in almost all metabolites as compared to those for the parameters belonging to the lower part of the network.

8.2. Time-Scale Analysis

Time-scale characterization is important to understand the metabolite dynamics and its response time.[129] The analysis for the AA metabolism model was performed by computing eigenvalues and eigenvectors of the Jacobian matrix of ordinary differential equations at the steady-state conditions. Time-scale analysis has been used previously to find the slow and fast modes in nonlinear dynamical systems.[130] Characteristic time constants (time scales) are the inverse of the eigenvalues since the dynamic response of the system for small perturbation from the steady state consists of exponential terms such as $\exp(-\lambda t)$, λ being an eigenvalue.[131] As a consequence, if all the eigenvalues have a negative real part, then the dynamic system would be stable, and also, if some of the eigenvalues are complex, then the system will exhibit sustained or unsustained oscillatory response for small perturbations. In the time-scale analysis of the AA metabolism, the eigenvalues were split into three broad ranges. For each eigenvalue, the metabolites with substantial contribution to the corresponding eigenvector were identified. Depending upon the eigenvalues and metabolites significantly contributing to the corresponding eigenvectors, these metabolites were divided into three categories as listed in Table 9. Medium-time-scale metabolites go up and return to the basal levels in 24 h; however, the slow-time-scale metabolites show monotonic increases up to 24 h (Figure 26).

8.3. Comparison of Rate Parameters for the Enzymes

The values for the rate constant for the enzyme cyclooxygenase (COX) reported in the literature were based on in vitro measurements with partially purified proteins.[132] Thus, it was assumed that the literature values represented its basal activity, and these activities (flux through the enzyme) were compared with predicted activities of these enzymes in the "control" simulation. The computed value (10^{-13} (μM/min)/cell) and reported value (10^{-14} (μM/min)/cell) for COX are within 1 order of magnitude.[133]

8.4. Stable Isotope Labeling for Improved Characterization of Fluxes

Stable isotope labeling of one key metabolite in a given metabolic pathway introduces pointwise (specieswise) perturbation in the network. For system identification purposes, labeling is equivalent to exciting the system, which helps decipher the network topology. Stable isotope labeling can be used to differentiate, in the production of metabolites in the downstream parts of the above network (Figure 25), the contribution of the metabolite that is labeled from the contribution by other metabolites. The propagation network of the labeled metabolite is less complex than the original propagation network. Thus,

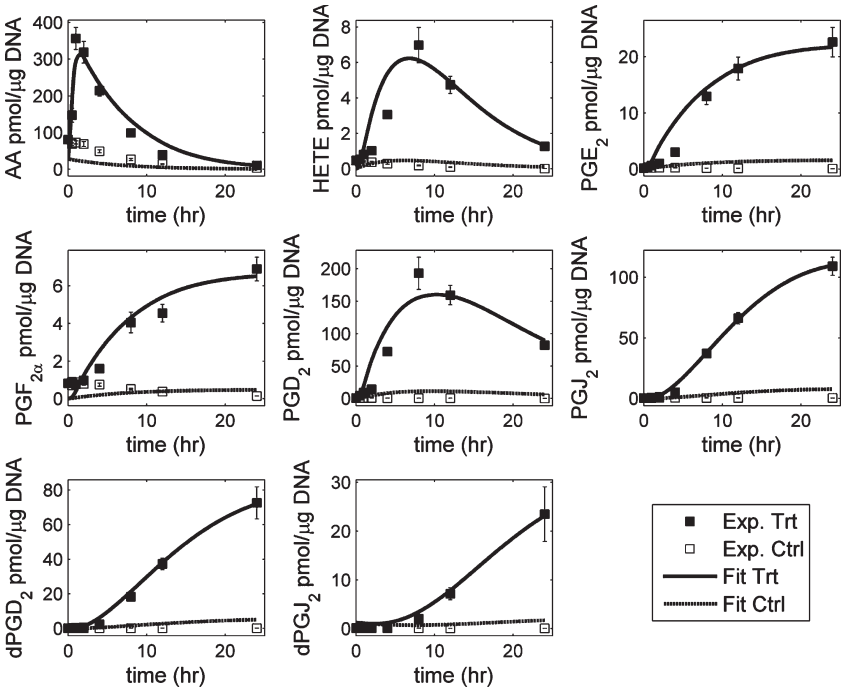

Figure 26. Simulation of kinetic modeling of the simplified lipid network: fit of the predicted response (control and treatment with KLA) to the corresponding experimental data. In the legend, "Ctrl" refers to control and "Trt" refers to KLA treatment of RAW264.7 cells. The error bars shown on the experimental data are the standard error of the mean. Reprinted with permission from ref 129. Copyright 2009 Elsevier Ltd.

Table 9. Results of Eigenvalue-Based Time-Scale Analysis of the Metabolites[a]

fast (∼1 h)	medium (∼10 h)	slow (∼50 h)
PGH_2	AA	PGE_2
	PGD_2	$PGF_{2\alpha}$
	HETE	PGJ_2
		$dPGD_2$
		$dPGJ_2$

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using labeled data, the reaction rate parameters can be estimated with better accuracy. Labeled data help identify alternate/new pathways.[134] Furthermore, it provides a more direct approach of computing fluxes and estimating the split ratios at branch points. Mass balance can be used to detect the leakage through unmodeled pathways, and potential connections between two different parts of the pathway can be detected. Deconvoluting the spectra in the context of lipid metabolites to identify peaks has been discussed previously.[134a] The main source of complexity in modeling labeled data is the presence of feedback loops.[135] When reactions result in elongation or breakdown of one or more chains of labeled carbon atoms or result in other structural changes, labeling of multiple carbon atoms changes even if all the carbon atoms in the original labeled metabolite were ^{13}C. These complexities need to be taken into account in using labeled data in kinetic modeling studies.

9. PERSPECTIVE AND FUTURE OF LIPIDOMICS

Although the field of lipidomics is relatively young, quantitative estimation of lipids over a wide dynamic range is already possible, and comparative analysis of lipid compositions and concentrations between normal and pathological tissues is beginning to yield rich insights into lipid-associated mechanisms of pathology. With next-generation mass spectrometers, methods for quantitative identification of lipid molecular species and context-specific association of lipid species with proteins involved in biosynthesis and metabolism and the concomitant genes encoding these proteins, several lipid-specific pathways will be reconstructed in the future. These pathways will help delineate the physiological function of cells and tissues, in conjunction with associated cellular signaling and transcriptional changes, in normal and pathological conditions. The early efforts serve as a harbinger for the integration of lipids as important molecular players in physiology and pathophysiology, leading to integrative systems biology approaches to describing function.

The challenges for lipidome bioinformatics and systems biology are manifold. With increasing ability to catalog lipids, the number and diversity of lipid species will increase dramatically. The classification of these lipids and their organization and most importantly characterizing their functional role will form a significant part of the lipidomics future. Most importantly, the quantification of lipids in a contextual manner, i.e., identifying small differences between lipids under two different conditions, normal and pathological or untreated and treated tissues, will form a significant challenge even with the availability of standards. Characterization of lipids in vivo is a daunting task, and despite advances in imaging mass spectrometry, image and data analysis to quantify specific lipids will require novel methods.

To study differences between normal and pathological samples, it is not adequate to merely measure and quantitate lipid species. It will be important to decipher and study the biochemical pathways associated with biosynthesis and metabolism of lipids and to study the fluxes associated with lipid changes with disease or treatment. The fluxes will also reveal hitherto uncharacterized pathways. Isotopomer experiments are one route to

deciphering the unknown pathways. Using labeled data, the reaction rate parameters can be estimated with better accuracy. Labeled data help identify alternate/new pathways.[134] Furthermore, they provide a more direct approach of computing fluxes and estimating the split ratios at branch points.

Proteins, genes, and lipids act in combination in pathways to create biological function. The key challenge for systems biology lies in the integration of proteomics, genomics, regulatory genomics, and metabolomics data to provide a context-specific systems-level perspective on phenotypic responses of living systems to stimuli. Identifying all the parts lists, such as the cellwide or tissuewide lipidome, is only a first step and needs to be significantly extended to identify interactions, mechanisms, and pathways. While traditional statistical methods can be applied to each type of data, e.g., gene expression, proteomics, or lipidomics, the integration across these data to provide mechanistically meaningful models continues to be a difficult challenge. Correlation methods and analyses suggest mechanistic connections, but have no foundation for causal relationships. Use of prior knowledge can provide useful constraints in developing network models, but also has the potential to bias the analyses of data to yield false connections and pathways. Dynamic measurements, when analyzed in context, can provide causal links, but for these to be accurate the density of measurements across time needs to be very high. Synergistic measurements of all components and "ome-integrated" reconstruction of pathways is essential for providing a mechanistic model. Even then, this model needs to have the dynamic element, which can only be obtained by time-varying measurements at necessary and sufficient granularity. Once such a dynamic model is created, the scope exists for quantitative modeling using physical principles to obtain predictive input−response relationships.

In developing computational models of biological processes, there is a growing realization that given the enormous complexity of biochemical interactions and paucity of data (as compared to how much data is required to uniquely identify the networks and parameters), unique networks would be seldom obtained in data-driven network identification. When manageable, this degeneracy in network reconstruction is not necessarily bad because it provides new and alternate hypotheses that can be further tested by knockout and pathway inhibition (intervention) studies, thus leading to the refinement of the network models. To date, most approaches to incorporate prior knowledge into network modeling are based on the Bayesian network or its variants. Can prior knowledge be systematically included in deterministic approaches (e.g., state−space formulation) as well? In all likelihood, the answer is yes. Such a framework must be able to operate on the network topology and the parameters simultaneously. It will require the ability to manipulate the topology, the complex expressions for the postulated cause−effect relationships, and the corresponding model parameters simultaneously. It is imperative that such an approach will require nonlinear optimization methods. Given the complexity of nonlinear optimization, stochastic-search-based approaches are expected to be more practical for such an application.[105]

It is anticipated that in the coming decades several models of lipid metabolic and signaling networks will be developed and systems biology approaches will provide predictive approaches to input−response relationships in cellular function. The tools of informatics and systems biology will be valuable in this research landscape.

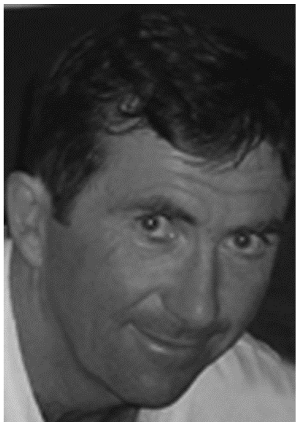
AUTHOR INFORMATION

Corresponding Author

*E-mail: shankar@ucsd.edu.

BIOGRAPHIES

Shankar Subramaniam is the Joan and Irwin Jacobs Endowed Chair in Bioengineering and Systems Biology and a Professor of Bioengineering, Bioinformatics and Systems Biology, Cellular and Molecular Medicine, Chemistry and Biochemistry, and Nanoengineering at the University of California, San Diego (UCSD). He was the founding director of the Bioinformatics and Systems Biology Program at UCSD. He received his B.S. and M.S. degrees from Osmania University in India and a Ph.D. in Chemistry from the Indian Institute of Technology, Kanpur, in 1982. He was a Professor of Biophysics, Biochemistry, Molecular and Integrative Physiology, Chemical Engineering, and Electrical and Computer Engineering at the University of Illinois at Urbana−Champaign prior to moving to UCSD. He has written over 200 articles and is on the editorial board of several journals. His awards include a Genome Technology All Star Award, Smithsonian Institution Award for Innovation, and Faculty Research Excellence Award. Research in his laboratory spans several areas of bioinformatics, systems biology, and systems medicine. In bioinformatics, he is involved in developing novel strategies for identifying protein interaction networks and identification of functional networks in cells. In systems biology, he is involved in deciphering mammalian cellular networks from high-throughput and phenotypic data and in developing strategies for modeling cellular signaling networks. In systems medicine, he is interested in mapping the circuitry of cells to mechanisms and phenotypes in physiology and pathology to develop quantitative models of cellular pathways.



Eoin Fahy has a B.Sc. in Biochemistry from University College Galway and a Ph.D. in Chemistry from the University of British Columbia, specializing in the structure elucidation of marine natural products. He completed a postdoctoral appointment at the Scripps Institution of Oceanography at the University of California, San Diego (UCSD). He has over 15 years of experience in the biotechnology industry in the areas of organic chemistry, drug target discovery, molecular biology, proteomics, genomics, and informatics. He joined the LIPID MAPS consortium in 2003 and serves as Project Coordinator for the Bioinformatics core at UCSD. His current interests include development of a lipid classification system as a member of the International Lipids Classification and Nomenclature Committee, design and development of database infrastructures for lipidomics, development of mass spectrometry software for lipid research, design of novel lipid structure drawing tools, and development of integrated pathway tools and resources.

Shakti Gupta received his bachelor degree in Chemical Engineering from the Indian institute of Technology, Kanpur, India, in 1999. He joined the Department of Chemical Engineering, University of Florida, in 2000 and received his Ph.D. in 2005. He worked at the Center for Disease Control, Atlanta, for one year before joining the University of California, San Diego (UCSD), in 2006 as a postdoctoral fellow. Presently, he is working as a Research Scientist in the San Diego Supercomputer Center and the Department of Bioengineering at UCSD. His research interests include bioinformatics analysis of high-throughput data, data-driven network reconstruction, and non-linear modeling of metabolic and signaling pathways.

Manish Sud is currently involved with the LIPID MAPS project at the San Diego Supercomputer Center/University of California, San Diego. His interests include research, development, and application of computational discovery tools. He has been working on development and usage of computational discovery tools at various small and large software development and drug discovery companies for over two decades.

Robert W. Byrnes is a Research Programmer in the San Diego Supercomputer Center and the Department of Bioengineering, University of California at San Diego. He maintains databases for the LIPID MAPS Pathway Editor program and the LIPID MAPS LIMS, writes software code, and provides user support for these programs. Previously, he worked on a grid portal interface for TeraGrid applications. He has a B.S. in Physics from the University of Rochester, New York, an M.S. in Natural Sciences and Mathematics from the State University of New York—Buffalo (SUNY—Buffalo), and a Ph.D. in Cellular and Molecular Biophysics from the Roswell Park Division, SUNY—Buffalo. Dr. Byrnes has also held a position of Staff Scientist in the Department of Chemistry, University of Wisconsin—Milwaukee, where he worked on metal biochemistry and oxidative DNA damage.

Dawn Cotter is a Senior Computational Scientist at the San Diego Supercomputer Center, University of California, San Diego (UCSD). She received a B.S. in Economics from the University of Illinois, Urbana−Champaign (UIUC) in 1989 and entered the Ph.D. program in Molecular and Integrative Physiology at UIUC in 1993. Dawn earned her M.S. in Physiology in 1996; her thesis title was "Dynamic Simulation Modeling of Changes in Human Body Composition". While pursuing her Ph.D., she also worked part-time for the Automated Learning and Education Groups at the National Center for Supercomputing Applications (NCSA), UIUC. In 1999, she accepted a full-time position with Dr. Shankar Subramaniam at NCSA and moved to

UCSD with Dr. Subramaniam that same year to continue work on the Biology Workbench. Dawn is interested in simulation modeling, data visualization, and metabolism, particularly as it pertains to regulation of human body composition.

Ashok Reddy Dinasarapu received his B.Sc. in Chemistry and Biology from Andhra Loyola College, Vijayawada, India, in 1997. Dr. Dinasarapu received an M.Sc. in Biochemistry from the University of Hyderabad, Hyderabad, India, in 2000 and an M. Tech. degree in Biotechnology from Anna University, Chennai, India, in 2002. Then Dr. Dinasarapu joined Shantha Biotechnics Pvt. Ltd., Hyderabad, India, and worked on gene cloning, expression, and purification of single-chain antibodies. Later in 2003, Dr. Dinasarapu moved back to the Department of Biochemistry at the University of Hyderabad for his doctoral research, in which he focused on the bioinformatics analysis of the promoter sequences of eukaryotes, and graduated in 2007. Since 2008, Dr. Dinasarapu has been a postdoctoral researcher in the Department of Bioengineering at the University of California, San Diego. His research interests include bioinformatics analysis of signal transduction and regulation and semantic integration and visualization of the life sciences data.

Mano Ram Maurya completed his B.Tech. in Chemical Engineering from IIT Bombay in 1998, M.E. in Chemical Engineering from the City College of New York in 1999, and Ph.D. in Chemical Engineering from Purdue University in 2003. Dr. Maurya was a postdoctoral researcher for three years in the Department of Bioengineering and the San Diego Supercomputer Center at the University of California, San Diego (UCSD). Then he worked in the Department of Bioengineering as an Assistant Scientist from October 2006 to November 2010. Since then, Dr. Maurya has been a Research Scientist in the San Diego Supercomputer Center and the Department of Bioengineering at UCSD. In 2005, Dr. Maurya received the Best Paper Award jointly with his Ph.D. advisor Dr. Venkat Venkatasubramanian and coadvisor Dr. Raghunathan Rengaswamy for their paper published in the journal *Engineering Applications of Artificial Intelligence* in 2004. In August 2011, he joined the editorial board of *ISRN Biophysics*. Dr. Maurya's current research interests include the study of complex biochemical processes and pathways using systems engineering/biology and bioinformatics approaches and their applications to biomedicine.

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