

RaftProt: mammalian lipid raft proteome database

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ABSTRACT

RaftProt (<http://lipid-raft-database.di.uq.edu.au/>) is a database of mammalian lipid raft-associated proteins as reported in high-throughput mass spectrometry studies. Lipid rafts are specialized membrane microdomains enriched in cholesterol and sphingolipids thought to act as dynamic signalling and sorting platforms. Given their fundamental roles in cellular regulation, there is a plethora of information on the size, composition and regulation of these membrane microdomains, including a large number of proteomics studies. To facilitate the mining and analysis of published lipid raft proteomics studies, we have developed a searchable database RaftProt. In addition to browsing the studies, performing basic queries by protein and gene names, searching experiments by cell, tissue and organisms; we have implemented several advanced features to facilitate data mining. To address the issue of potential bias due to biochemical preparation procedures used, we have captured the lipid raft preparation methods and implemented advanced search option for methodology and sample treatment conditions, such as cholesterol depletion. Furthermore, we have identified a list of high confidence proteins, and enabled searching only from this list of likely *bona fide* lipid raft proteins. Given the apparent biological importance of lipid raft and their associated proteins, this database would constitute a key resource for the scientific community.

INTRODUCTION

Lipid rafts are specialized cholesterol and glycosphingolipid-rich membrane microdomains thought to be abundant on most eukaryotic cell surfaces. The

concept came into light during the study of epithelial cell polarity, where the apical surface was found to be enriched with glycosphingolipids (1). The physicochemical properties of lipid rafts resemble densely packed liquid order phase, which is distinct from the loosely packed liquid disorder phase exhibited by the rest of the plasma membrane (2). Morphologically, these microdomains can be classified as planar rafts or flask-shaped invaginations called caveolae. The size and life span of lipid raft varies in plasma membrane. The results from *in vivo* studies on lipid rafts indicate the existence of lateral membrane heterogeneity which eventually led to a notion that these are fluctuating nanoscale assemblies. These structures can be stabilized to form specialized platforms that coordinate diverse biological processes (3).

Many different roles for these membrane platforms have emerged over the years (3). The most widely studied function of rafts is to provide a distinct environment for signalling molecules and receptors and therefore regulate downstream pathways (4). One potential mechanism for such modulation is the coalescing of lipid raft microdomains, bringing into proximity a new repertoire of protein–protein and protein–lipid interactions (5). Moreover, small changes in protein composition of lipid raft could lead to initialization and/or amplification of signalling cascades. For example, observations from cell signalling studies suggested that raft association is critical for receptors and many signalling molecules to perform their function (6,7). The presence of several protein transporters as well as drug efflux proteins in lipid raft highlighted roles for lipid rafts in the transport of substrates, and exogenous compounds both in and out of the cell (8–12). Likewise, different pathogens, including viruses and bacteria, use membrane lipid raft as a portal to enter the host cell (13–15). Growing evidence indicates that lipid rafts can act as sorting platforms involved in targeted membrane trafficking of various proteins. The presence of raft-like membranes on secreted extracellular vesicles, such as exosomes, indicate the involvement of rafts in sorting and release of exosomes from

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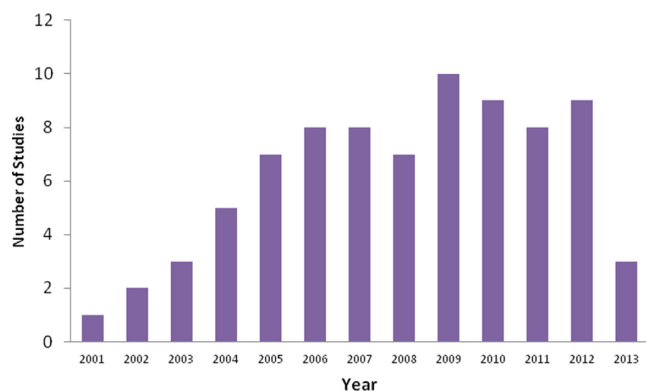


Figure 1. Trend of lipid raft proteomics studies published over the years.

cell surface (16). Moreover, clathrin-independent endocytic pathways are thought to be regulated by caveolae and non-caveolar lipid raft carriers (17).

Alterations in lipid rafts have been reported in many disorders (18). Due to their apparent importance as a signalling and sorting platform, membrane lipid rafts have been studied in several tumours, including prostate (19–21), breast (22,23), lung (24) and colon cancer (25,26). Likewise, these specialized membrane domains are also implicated in pathological conditions, such as Alzheimer's (27), Parkinson's (28), cardiovascular disorders (29) and HIV, etc. (30,31). As a result, rafts are proposed to be a therapeutic target to cure or prevent these disorders.

Lipid rafts are perhaps the best-studied and thus most well-understood membrane microdomains, although our understanding of them is still far from complete. Protein components in those domains would have a marked impact on their structure, activities and interactions. Identification, characterization and quantitation of all or most of the proteins would therefore be critical in understanding functional organization of this complex biological system. Due to their biological importance, involvement in disease pathologies, and supposed ease of purification, lipid rafts have been a very popular target for proteomics studies (32). Numerous lipid raft proteomics studies have been published in the past decade with a continuing upwards trend as shown in Figure 1. They have captured both qualitative and quantitative aspects of membrane rafts. These studies were performed on a variety of cell/tissue types, and utilized different biochemical extraction methods to enrich membrane raft fraction (33). Therefore, the new challenge is a bioinformatic tool to collate and integrate the wealth of published lipid raft proteomics data. Here we present, for the first time, a dedicated online resource RaftProt comprising of comprehensive collection of searchable lipid raft proteomics studies for researchers.

DATABASE DESCRIPTION

Data collection and curation

RaftProt focuses on mammalian lipid raft proteomics results. The data set was obtained by searching the following terms in PubMed and Google Scholar: 'lipid raft', 'microdomains' and 'detergent-resistant membrane'. Only

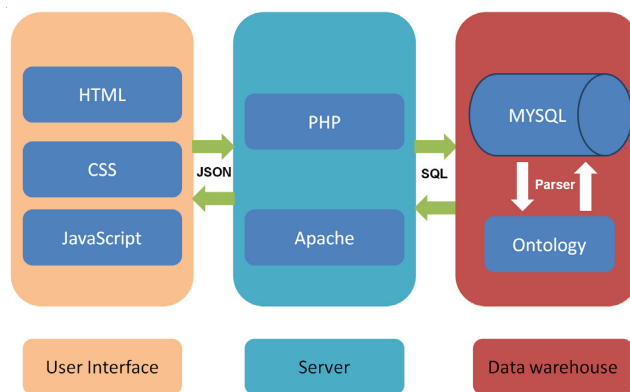


Figure 2. RaftProt database architecture.

studies focused on mammalian lipid raft proteomes were chosen for further analysis. We extracted protein information mentioned in various sections of the article. The proteins were then mapped to standard UniProtKB accessions, using Uniprot ID Mapping tool and customized scripts. We also kept the original annotation intact, so that user can always trace back the protein annotation from original article.

Some research papers reported multiple proteomics data sets; these were captured as 'experiments' in the database. Experiments were categorized as qualitative and quantitative studies. Various quantitative experiments attempted to explore time-dependent and post-translational changes in lipid raft proteome. Consequently, some studies explored dynamics compartmentalization of proteins into different subcellular organelles in response to external stimulus. From those studies only information about lipid raft fraction was extracted. Although we have performed an extensive search to extract as many lipid raft proteomic studies as possible, some relevant studies may still have been left out unintentionally. Therefore, we encourage researchers to submit their lipid raft proteomics data along with all related information.

Database architecture

The schematic representation of RaftProt architecture is shown in Figure 2. The database is deployed on Apache server 2.2.15 (<http://www.apache.org>). All the data were stored in a relational database schema in MySQL server 5.1.73 (<http://www.mysql.com>). The client side web interface was created using HTML and javascript. Graphical output of summary statistics of the database was implicated with the help of Google Visualization API. The server side logic was implemented using PHP (version 5.3.3).

RaftProt can be directly used by biologists. But in addition, we also envisage RaftProt to serve as a data source for other data analysis/mining systems. We have implemented RESTful like web-service approach for the transfer of data in JavaScript Object Notation format. This separation of data from presentation allows the potential for other data analysis/mining systems to pull data dynamically from RaftProt. Furthermore, this approach also allows RaftProt to be easily extended with addition data post-processing functionalities. Organization of data into different pheno-

typic categories, such as cell, tissue or organ, is one of the challenges for today's databases. One way of tackling this problem is allowing computers to logically manage that information by integrating biological ontologies (34). Ontologies specify how data, terminology, concepts and ideas all relate to each other in the system of interest. As the lipid raft proteomics studies have little overlap in specific cell type used, we wish to enable search for all experiments that used related cell types in order to derive a 'consensus' lipid raft proteome for specific cell types. To facilitate this search, we developed a placental mammalian cell ontology (PMCLO) which classifies cell lines from placental mammals based on cell-type, tissue and organs. This ontology is represented in standard Open Biological Ontology (OBO) format (familiar to users of the Gene Ontology) which was visualized and edited using OBO-Edit (35).

Database utilities

RaftProt can be explored through the web interface using 'search' or 'browse' options. Data tables can also be downloaded via the 'downloads' option. Individual proteins can be searched by gene name, protein name or UniProt accession. Proteomics experiments, and their corresponding protein lists, can be retrieved by 'searching' or 'browsing' by several available parameters including organism, cell/tissue type, lipid raft preparation method and treatment conditions, such as cholesterol depletion. An 'advanced search' function is offered to search multiple fields at the same time. Outputs are visualized on the web interface, and can be downloaded in comma separated or pdf format. Comparison across experiments is available for the list of proteins in result output by accessing the 'analyse' option following 'search'.

Most of the databases offer searching against single cell line or experiments at a time. But with the integration of PMCLO to RaftProt, users can search for tissue- or organ-specific lipid raft proteome. The use of ontology permits the user to access all the cell lines associated with particular tissue or organ for which lipid raft proteomics data is available. Furthermore, we provide an option of category search of proteins and experiments identified from primary cells, perpetual cell lines and cancer-associated cell lines.

Statistics and analysis

Currently RaftProt comprises 27 773 entries from 117 proteomics experiments reported in 81 scientific publications. We are able to assign UniProt accession to 7959 proteins (~75% of total proteins). The data sets were generated from 69 different cell/tissue/organs belonging to six mammalian species. Among them T-cells, melanoma cells, fibroblasts, macrophages and brain tissues are the most common. Most of the studies were focused on human cell lines and/or tissues (Figure 3). Studies include qualitative and quantitative proteomics experiments, some with chemical or genetic perturbations. Detergent-resistant membrane was the most popular method for lipid raft preparation, used in ~80% experiments. Lipid raft isolation based on biochemical/biophysical properties may lead to co-isolation of contaminant, non-lipid raft pro-

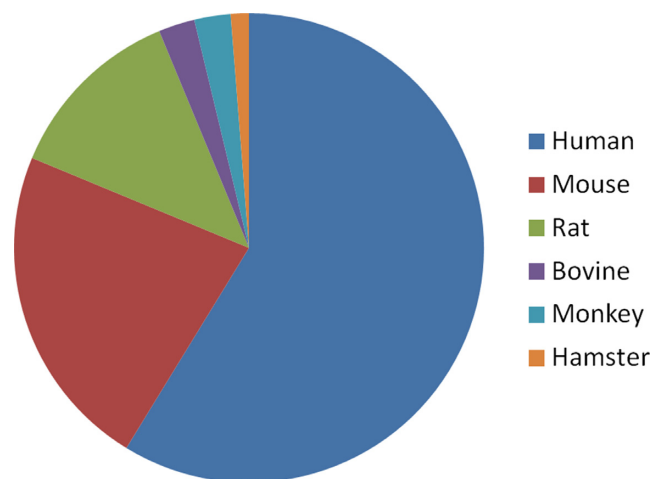


Figure 3. Distribution of experiments among the organisms.

teins. Sensitivity to cholesterol depletion using methyl- β -cyclodextrin is an established 'test' for lipid raft presence (32,33), however, only five experiments used this treatment. Therefore, we also consider that co-isolated contaminant proteins are likely to be specific to each biochemical isolation method, and used identification by more than one biochemical method as a second criteria for inclusion in the 'High Confidence' lipid raft protein list. Proteins that fulfill either criteria were added to the high confidence list of 2185 proteins (27.5% of total 7959 proteins of the database).

CONCLUSION

Cholesterol-rich membrane rafts regulate a myriad of cellular functions associated with health and disease. RaftProt is a unique resource providing the means to systematically analyse existing lipid raft proteomics data sets in an informed manner. Critical experimental information is captured in a searchable format. Combined with the use of a novel cell ontology, this user-friendly portal will enable biologists to distil useful lipid raft proteomics data from the potentially bewildering public data sets. Currently, there are limited number of studies for most cell types, specific treatments and preparation methods. With continued input from the research community to RaftProt, we will be able to increase our knowledge of the proteomic constituents and dynamic re-arrangement of mammalian lipid rafts.

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