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RaftProt V2: understanding membrane microdomain function through lipid raft proteomes

Ahmed Mohamed[1,2], Anup D. Shah[1], David Chen[3] and Michelle M. Hill[1,2,*]

[1]The University of Queensland Diamantina Institute, Faculty of Medicine, The University of Queensland, Translational Research Institute, Brisbane, QLD 4012, Australia, [2]QIMR Berghofer Medical Research Institute, Brisbane, QLD 4006, Australia and [3]School of Information and Communication Technology, Griffith University, Brisbane, QLD, Australia

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ABSTRACT

Cellular membranes feature dynamic submicrometer-scale lateral domains termed lipid rafts, membrane rafts or glycosphingolipid-enriched microdomains (GEM). Numerous proteomics studies have been conducted on the lipid raft proteome, however, interpretation of individual studies is limited by potential undefined contaminant proteins. To enable integrated analyses, we previously developed RaftProt (http://lipid-raft-database.di.uq.edu.au/), a searchable database of mammalian lipid raft-associated proteins. Despite being a highly used resource, further developments in annotation and utilities were required. Here, we present RaftProt V2 (http://raftprot.org), an improved update of RaftProt. Besides the addition of new datasets and re-mapping of all entries to both UniProt and UniRef IDs, we have implemented a stringent annotation based on experimental evidence level to assist in identification of possible contaminant proteins. RaftProt V2 allows for simultaneous search of multiple proteins/experiments at the cell/tissue type and UniRef/Gene level, where correlations, interactions or overlaps can be investigated. The web-interface has been completely re-designed to enable interactive data and subset selection, correlation analysis and network visualization. Overall, RaftProt aims to advance our understanding of lipid raft function through integrative analysis of datasets collected from diverse tissue and conditions. Database URL: http://raftprot.org.

INTRODUCTION

Biological membranes perform critical roles in compartmentalization and signal transduction between the external environment and the cell, as well as between intracellular organelles. The existence of lateral membrane subcompartments was initially suggested by the distinct glycolipid and glycosphingolipid-anchored proteins content on the apical and basal membranes of epithelial cells (1,2), leading to use of the term glycosphingolipid-enriched membranes (GEM). The 'lipid raft' hypothesis was developed in 1997 to explain the biophysical principles underlying the lateral membrane movements, and it was proposed that the interaction between specific lipids drive the formation of functionally important lateral membrane domains (3).

An important step towards understanding the molecular mechanisms and effectors of membrane raft function is the characterization of its proteomic compositions in various cell types and conditions. Given the dynamic nature and nanometer scale of lipid rafts, isolation of individual or pure lipid raft microdomains for proteomics analysis is beyond the capabilities of current techniques. On the other hand, numerous studies have reported the proteomic analysis of bulk lipid rafts prepared from diverse cell and tissue types, and these public datasets are a useful resource for knowledge-based data mining and integration. To facilitate this, we developed and reported RaftProt, a searchable database for mammalian lipid raft proteomics data (4). Since publication, RaftProt database has been used to compare with new lipid raft proteomics data from bovine lens (5), ovarian cancer cells (6) and mouse brain (7), as well as HeLa cell surface proteome (8) and in a computational study of single-pass transmembrane proteins (9). We also used RaftProt data to provide independent experimental support for raft localization of IQGAP1, its involvement in cancer metastasis (10).

*To whom correspondence should be addressed. Tel: +61 7 3845 3020; Fax: +61 7 3362 0102; Email: michelle.hill@qimrberghofer.edu.au
Present address: Anup D. Shah, Monash Biomedical Proteomics Facility, Monash Bioinformatics Platform, School of Biomedical Sciences, Monash University, Clayton, Victoria 3800, Australia.

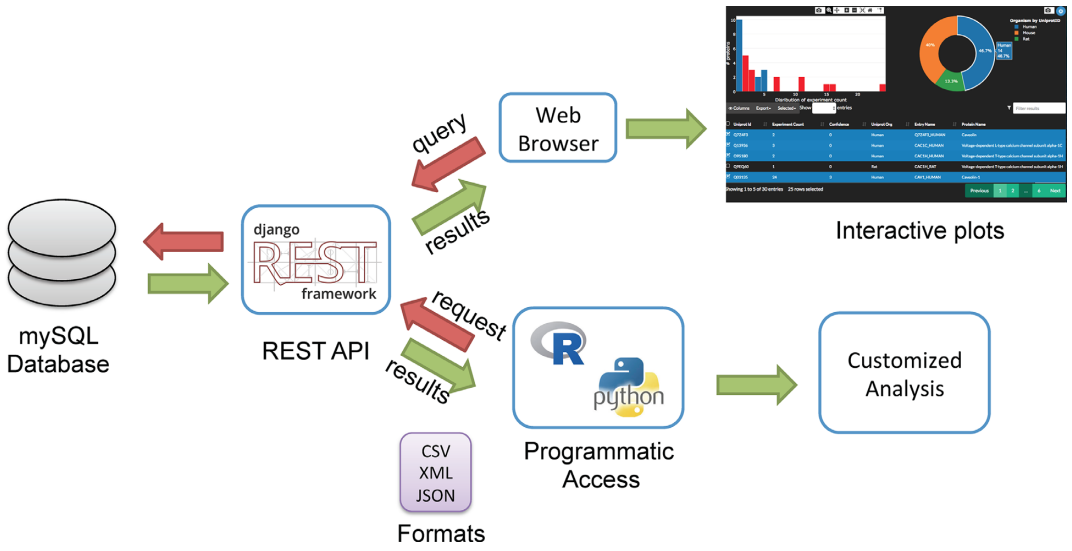

Figure 1. RaftProt V2 database architecture.

To further the utility of RaftProt, we implemented several new features. Firstly, we mapped each UniProt ID to their UniRef and GENE ID, to allow cross-species and gene-level interrogation. Furthermore, we re-designed the web-interface to provide interactive tools for dataset selection, analysis and visualization. A new experimental evidence level annotation has been developed and implemented, providing a simple summary score for each protein entry. In addition, the database has been relocated to a simpler domain name (http://raftprot.org).

DATABASE DESCRIPTION

RaftProt V2 has been completely re-developed, and the web address updated from http://lipid-raft-database.di.uq.edu.au/ to http://raftprot.org. We describe below improvements in the data content, visualization and usability.

Database architecture

RaftProt V2 is implemented with a set of RESTful Web APIs, as shown in Figure 1. When users use RaftProt directly via a web browser, all queries are issued to the database via this Web API. Furthermore, this Web API is open to public access, hence users can write their own programs (in the programming language of their choice) to retrieve the most up-to-date data from our database, and perform their own customized analysis. The Web API can return data in different formats such as CSV, XML or JSON. RaftProt V2 web site contains detailed API documentation, along with helpful code examples. In addition, users can browse the API and test different queries to help with their implementation.

Data collection and curation

New lipid raft proteomics datasets from July 2014 to June 2017 were identified through PubMed using the terms 'lipid raft' or 'microdomains' with 'proteomics' or 'proteome'. Data were downloaded from online supplemental information of articles, or requested from corresponding authors. RaftProt V2 currently includes 98 studies and 163 experiments from six species. Human lipid raft proteome is the most studied with more than 50% of experiments, followed by mouse and rat.

In addition to supplementing the database with new studies, we also updated the annotation for previously collected data. All protein IDs were remapped to the latest version of UniProt database. Obsolete UniProt IDs were updated (where possible) or removed. We flagged IDs as 'mismatching' whenever they belonged to a different species than that of the reporting experiment. Experiments with >50% mismatching IDs were also flagged. Flagged proteins and experiments were excluded from the subsequent evidence levels scoring. Finally, we added UniRef IDs for all mapped proteins, which clusters proteins according to their sequences, thereby allowing users to explore closely related proteins in the lipid rafts. Clickable external links are provided for each protein entry, directing users to UniProt (11), UniRef (12) or RefSeq (13) databases.

Experimental evidence level annotation

In the previous version of RaftProt, a searchable list of high confidence lipid raft proteins was generated, being the subset of proteins reported using more than one extraction method OR reported to be sensitive to methyl-β-cyclodextrin treatment (4). To improve the quality of confidence scores and increase flexibility of database search-

Table 1. Criteria for experimental evidence level scoring

Experimental evidence	Biochemical methods	Sensitive to raft perturbation techniques
☆☆☆	Less than two	Less than two
★☆☆	**Two or more**	Less than two
★★☆	Less than two	**Two or more**
★★★	**Two or more**	**Two or more**

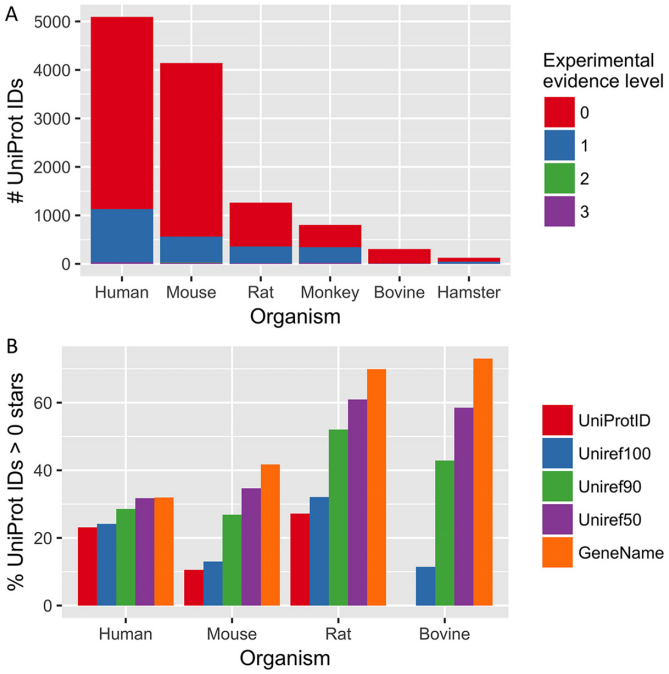

Figure 2. Distribution of lipid raft protein experimental evidence levels by organism. (**A**) Number of UniProt IDs with each experimental evidence level. (**B**) Percentage of UniProt IDs with more than zero stars for each calculated experimental level.

ing, we have now implemented a more stringent experimental evidence level rating system based on two key criteria: identification by two or more biochemical isolation methods and sensitivity to two or more raft perturbation techniques (Table 1). Each entry is annotated with upto three stars, where one star denotes identification by more than one biochemical extraction method, two stars denote sensitivity to more than one raft perturbation technique, and three stars denote compliance with both criteria. Zero star indicates that the protein has only been reported using a single biochemical extraction method (usually DRM) and has not been reported to be sensitive to at least two raft perturbation techniques (either due to lack of sufficient experiments, or actually being insensitive). Biochemical methods for raft isolation include detergent-resistant membrane (DRM) extraction (14), detergent-free membrane (15), and affinity isolation (16). Raft perturbation treatments manipulate specific raft lipids to selectively reduce the level of bona fide raft proteins in raft fractions, leaving artefactual proteins unaffected. While cholesterol depletion by methyl-β-cyclodextrin has been the most popular raft perturbation technique (17), other techniques have been reported and captured within RaftProt V2 database, including enzymatic lipid sequestration (18), blocking cholesterol biosynthesis (19) and genetic knockout of key raft genes such as caveolin-1 (20).

Figure 2A shows species-specific number of UniProt IDs for each experimental evidence level from 0 to 3. More than 30% of the reported human membrane raft proteins scored one star or more. Surprisingly, >80% of reported mouse, rat and bovine membrane raft proteins scored 0, possibly reflecting a large proportion of species-specific UniProt IDs being reported in only a small number of studies, and the exclusive use of DRM method.

Cross-species analysis

For each protein entry, users can examine closely related proteins, i.e. sharing the same UniRef ID or gene name, using the Cross-species info tab. Users can further evaluate experiments reporting these proteins by directly clicking on the displayed search icons. To collate experimental evidence levels for proteins identified in several species, each UniProt entry was further annotated with experimental evidence levels based on UniRef IDs and gene name. The aggregation of experimental evidence across different species improves

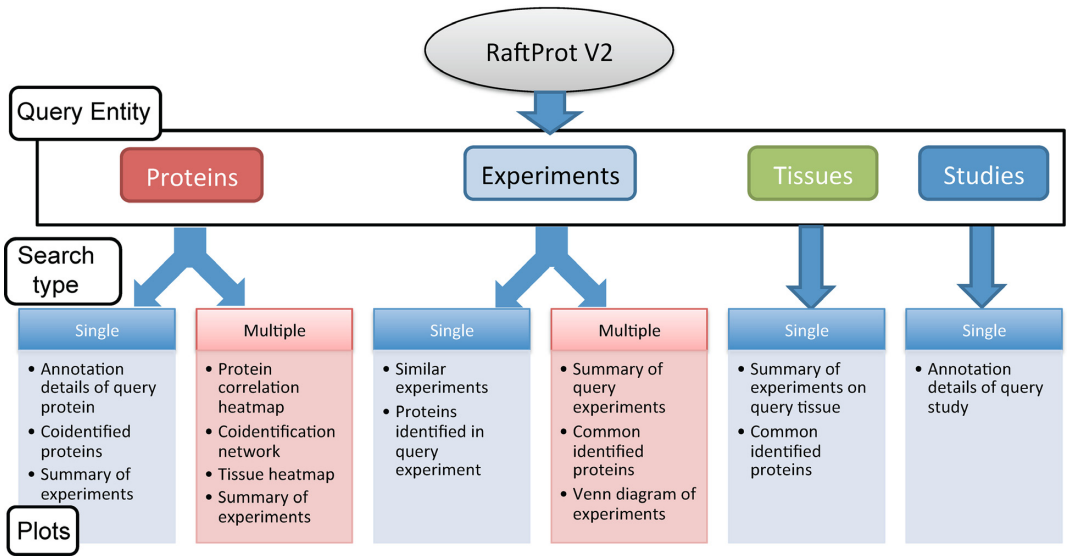

Figure 3. RaftProt V2 search options and data output.

the scores greatly, especially in species with limited number of experiments available in the database (Figure 2B).

DATABASE USAGE

The user interface has been completely re-designed to improve data analytics and visualization. All search results are now displayed as interactive plots using JavaScript libraries. The interactive plots and tables allow users to subset and select from search results using different parameters such as experimental evidence level, biochemical extraction or quantitative proteomics methods. Output tables and plots can be exported, and protein lists can be saved for use in later searches.

Searching

RaftProt V2 allows users to query the database using any of the four entities; proteins, experiments, studies or tissues, using two different search types, single and multiple (Figure 3). Single protein searches are performed using the 'Quick search' interface. Outputs from RaftProt single protein searches include statistics of experiments in which the selected protein was identified, where experiment subsetting can be performed by species or quantitation method, as well as the list of proteins co-identified with the query protein displayed in a table and interactive frequency graph.

The 'Advanced search' interface allows users to query a list of proteins, such as protein family or results of a new experiment, to investigate their relationship in membrane raft. In addition to providing a summary of experiments which report the list of proteins, RaftProt V2 offers three types of correlation tools for multi-protein searches; pairwise correlation, network visualization and protein-tissue heatmap. Use of these visualization tools is exemplified in Figure 4 with caveolin and cavin family proteins. Searching was performed using a list consisting of CAV1, CAV2, CAV3, CAVIN1, CAVIN2, CAVIN3 and MURC. The heat map in Figure 4A shows that CAV1, CAV2, CAVIN1, CAVIN2 and CAVIN3 are frequently co-identified in many membrane raft datasets, but CAV3 and MURC are less commonly co-identified with the other family members. These results are expected since CAV3 and MURC are both predominantly expressed in muscle, while the other family members have broad tissue expression (21). Extending beyond pairwise comparison, the network of the co-identification of these proteins are shown in Figure 4B as a static image. Using the interface, hovering over the nodes will displays the accession number and highlights the table row for the protein entry. The thickness of the edges correlates with the number of datasets that identified the connected nodes. Figure 4B shows two major hubs, one less densely connected than the other, representing the muscle and non-muscle caveolin-cavin complexes, respectively. Finally, the protein-tissue heatmap in Figure 4C further confirm the muscle-specific expression of CAV3 and MURC, and also reveal interesting expression patterns for the other family members.

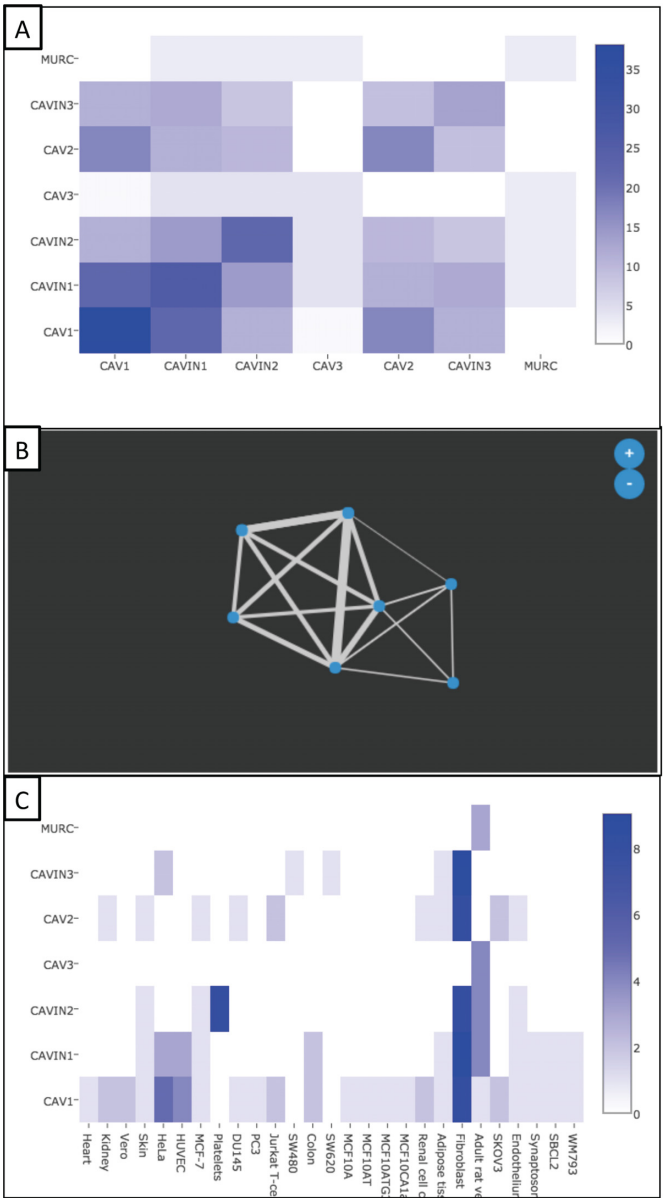

Figure 4. Example correlation analysis outputs using the list CAV1, CAV2, CAV3, CAVIN1, CAVIN2, CAVIN3, MURC. (**A**) Heatmap showing pairwise co-identification of proteins. (**B**) Network visualization for co-identification of proteins. (**C**) Protein-tissue heatmap showing co-identification in different tissues.

Browsing

Users can also browse experiments or studies according to species or tissue/cell type. The latter is visualized by an ontology tree which was developed for RaftProt (4), and updated with new tissue types. Experiments annotated with the selected tissue/cell type are displayed in a table with interactive donut graphs showing experimental organism and quantitation method. The user can easily select the segment of choice and subset those experiments using the Selected → Search selected option. Common proteins of the selected experiments are displayed in frequency a graph under the 'Commonly Identified Proteins' tab, while the 'Experiments

Venn' tab visualizes the overlap in the lipid raft proteome between the selected experiments. There is a limit of 10 experiments for the Venn diagram visualization.

DATABASE DOWNLOAD

All data included in RaftProt V2 can be downloaded from the website. Users can choose to either download the whole database, or a subset based on the organism and/or experimental evidence levels. Data can also be downloaded in several file formats including CSV and XML.

CONCLUSIONS AND FUTURE DIRECTIONS

RaftProt V2 is a searchable database for the integrated analysis of mammalian lipid raft proteomics datasets. Future expansion of RaftProt may include proteomics datasets uploaded into data repositories but not linked to a publication, non-mammalian lipid raft proteomic studies or lipidomics studies, as well as additional analytical and visualisation tools.

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