DATASET BRIEF

Proteome reference map of *Drosophila melanogaster* head

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Drosophila melanogaster has been used as a genetic model organism to understand the fundamental molecular mechanisms in human biology including memory formation that has been reported involving protein synthesis and/or post-translational modification. In this study, we employed a proteomic platform based on fluorescent 2DE and MALDI-TOF MS to build a standard *D. melanogaster* head proteome map for proteome–proteome comparison. In order to facilitate the comparison, an interactive database has been constructed for systematically integrating and analyzing the proteomes from different conditions and further implicated to study human diseases related to *D. melanogaster* model. In summary, the fundamental head proteomic database and bioinformatic analysis will be useful for further elucidating the biological mechanisms such as memory formation and neurodegenerative diseases.

Keywords:
Animal proteomics / Brain / 2-D PAGE / *Drosophila melanogaster* / MALDI-TOF MS

For nearly a century, the fruit fly, *Drosophila melanogaster*, has been a powerful and most commonly utilized model organism for the study of complex biological problems. *Drosophila* serves as an invaluable model organism and offers many advantages for the studying of human diseases. Benefits include a faster time frame due to shorter life span of flies, numbers of progeny, availability of techniques, and tools for the manipulation of gene expression, and well-known anatomy and phenotypes [1]. Moreover, the sequence of human genome and the sequence of fly genome show unequivocally high degree of interrelatedness [2]. Therefore, in the last decade, numerous scientists have been trying to gain insights into neurodegenerative diseases by utilizing such model system. Many *Drosophila* transgenic strains have been developed and widely applied to study various human diseases such as Alzheimer's, Parkinson's, and Huntington's diseases, etc. and helped scientists immensely to advance the understanding of these complex disorders. However, no database for the protein expression profiles of normal *D. melanogaster* brain is currently available. In order to establish a high-throughput platform for identification of diseases, development, and memory-related gene expression changes, a comprehensive 2D gel-based fly head protein database was constructed so that the reference map can be used as a fundamental tool for comparative proteomics study to further increase our knowledge on the brain physiology of *D. melanogaster*. To illustrate the significance of this standard map, reference protein patterns and protein identification on 2D gel via MALDI-TOF MS analysis linked with searches in public protein databases (SwissProt, MSDB, and NCBI) have also been conveniently maintained as a 2D gel-based *D. melanogaster* brain database accessible as image maps via internet. Additionally, comparison of an experimental gel pattern with such a reference gel database can provide immediate information on protein identities and

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Colour Online: See the article online to view Figs. 1 and 2 in colour.

further apply to study human diseases related *Drosophila* model.

D. melanogaster normal control, *white*, used in this study was provided from the laboratory of Prof. Ann-Shing Chiang at the Brain Research Center, National Tsing Hua University, Taiwan. The red eye of *D. melanogaster* is rendered white by homozygous mutation of the *white* gene. P elements, naturally occurring transposable elements in *Drosophila*, can be modified to carry transgenes and used for mutagenesis by inserting into genomic regions [3]. The *white* gene is commonly used for the insertion of P elements serving as a positive marker for transgene incorporation [4]. The *white* flies (50% male and 50% female) were reared in the temperature-controlled incubator which maintained at the 25°C with 70% relative humidity on a cycle of 12 h of light and 12 h of dark conditions. Fly were raised and maintained on food containing culture medium (0.9% agar medium containing 10.5% dextrose, 5% cornmeal, 2.6% bakers yeast, and 0.23% tegocept, to which active yeast is added) in glass bottles. Flies were raised in culture medium for 4–7 days after eclosion. The matured flies were then transferred to a new culture medium and kept under same conditions for 2 days before sacrificed.

During sample preparation for proteomic analysis, *D. melanogaster*, *white*, was collected into centrifuge tubes (50 mL) followed by frozen with liquid nitrogen and shaken vigorously to separate heads from bodies and extremities. Fly heads were isolated using prechilled size exclusion sieves. The top sieve (Tyler equivalent 25 mesh#, 0.71 mm square) allowed the heads to pass to the bottom sieve (Tyler equivalent 40 mesh#, 0.42 mm square), which separated the extremities from the heads. The heads were collected and pulverized in liquid nitrogen with a prechilled mortar and pestle and then transferred to a microcentrifuge tube. All samples were then suspended in lysis buffer consisting of 7 M urea, 2 M thiourea, and 4% (w/v) CHAPS followed by purified proteins with methanol/chloroform precipitation. The purified head proteins were further resolved with fluorescent dye-labeled 2D-PAGE and identified with MALDI-TOF MS according to the protocol listed in [5].

Fly brains are difficult to obtain; therefore, our purpose of running the proteome is to show that there is a high overlap of proteins found in both the head and the brain, and we can simply substitute the head for the brain for our further studies. We analyzed the proteomes of heads, brains, and cuticles, and compared the difference in proteome expression patterns by the 2D-DIGE technique. Heads of fruit flies were carefully dissected and separated into two parts, brains and cuticles. The proteins from heads, brains, and cuticles were extracted according to the extraction method as mentioned above, and consequently labeled with fluorescence dyes (Cy2, Cy3, and Cy5). After separating by the 2D-PAGE, the CyDye™-labeled protein spots could be visualized by the fluorescence scanner. Proteins found in the heads and brains have ~90% of similarity (Supporting Information Fig. S1). Theoretically, the sum of proteins found in both the brain and the cuticle equals

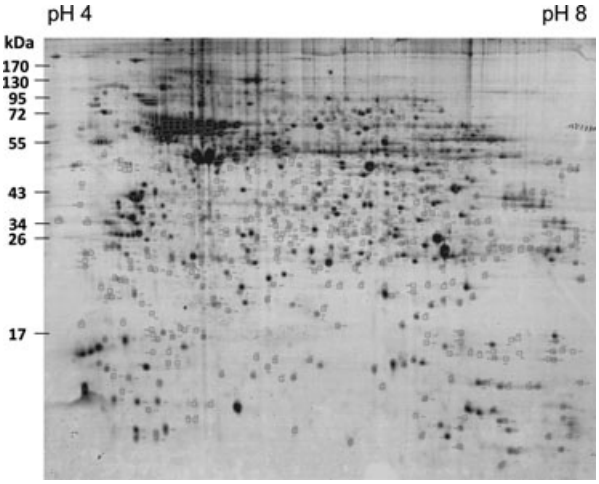


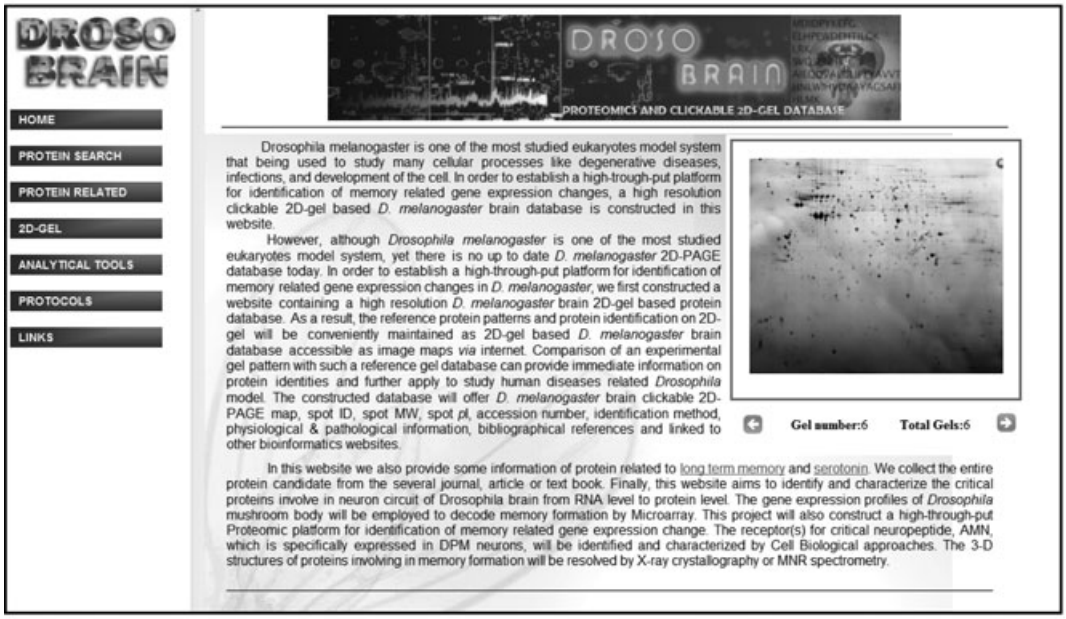
Figure 1. Two-dimensional *D. melanogaster* head protein profiling. Proteins extracted from 200 heads of wild-type *D. melanogaster* were separated by 2D-PAGE. After Coomassie blue staining, approximately 1500 protein spots could be detected on a 24-cm 12.5% SDS-PAGE gel. Red boxes indicate the selected and picked protein spots for protein identification.

to the total proteins found in the head. However, there is about 30% overlap of proteins found in the brain and cuticle (Supporting Information Fig. S2). In summary, these results showed that there are about 10% of protein difference between the head proteome and brain proteome. In order to facilitate the analysis of differential protein expression, brain proteomic analysis had been replaced by head proteomic analysis in this study to perform brain functions related proteome comparison experiments.

In this study, the method for *D. melanogaster* head 2D-PAGE profiling has been successfully built up and optimized. More than 1500 fluorescent protein features were successfully resolved from 200 *D. melanogaster* heads and 650 protein features corresponding to 500 unique proteins were identified by MALDI-TOF MS (Fig. 1 and Supporting Information Table S1). Using functional information from the Swiss-Prot and KEGG pathway databases, numerous biological functions and subcellular locations were ascribed to the identified proteins. Supporting Information Fig. S3 shows these identified proteins were dominantly located in cytoplasm and nucleus while Supporting Information Fig. S4 demonstrates most of the identified proteins were involved in oxidation–reduction, ATP synthesis, transcription, and development.

To facilitate comparing the head proteomes with different conditions, we have constructed a website called Drosobrain (http://140.114.98.63/~drosobrain/flybrain/fb.html), which contains a database with high-resolution *D. melanogaster* head proteomes and several image analytical tools for analyzing the 2D gels (Fig. 2). This website mainly contains two parts, one is for proteomic groups to collect and analyze the proteomes from different conditions; the other is for biochemical groups

A



B

Figure 2. The interactive Drosobrain website for studying the head proteomes and the memory-related proteins in *Drosophila*. This website contains two databases, one is for proteomic groups to collect and analyze the proteomes from different conditions (2D-PAGE); the other is for biochemical groups to collect the protein sequence and the structural information about memory-related proteins (serotonin- and long-term memory-related proteins) (A). Two-dimensional head proteomes of fruit flies were resolved by 2DE and the ID of protein spots were identified by MS. The detail information of protein spots and hyperlink of other databases are shown in (B). The spots on the gel that have been identified by MS are indicated in red.

to collect the protein sequence and the structural information. As the result, the reference protein patterns and protein identification on 2D gel were conveniently maintained as 2D gel-based *D. melanogaster* head database accessible as image maps *via* internet. This map serves as an interactive reference database for researchers studying changes in protein levels in different physiological conditions. Comparison of an experimental gel pattern with such a reference gel database can provide immediate information on protein identities and further apply to study human diseases related *Drosophila* model. The constructed database offers *D. melanogaster* brain 2D-PAGE map, spot ID, spot MW, spot p*I*, accession number, identification method, physiological and pathological information, bibliographical references, and linked to other bioinformatic websites (Fig. 2). In addition to the collected and integrated results and information for *Drosophila* head proteomes, we also constructed several image analysis programs in this database (from Supporting Information Figs. S5 to S8). We have also collected and analyzed the information of the memory-related proteins including long-term memory-related proteins and serotonin metabolism and transportation proteins. The analytical results by bioinformatics are summarized in Supporting Information Fig. S8. This database would offer important information for studying protein function and protein structures in memory formation.

In our established interactive *D. melanogaster* head database, we not only constructed a reference head protein profile, but compared the head proteomes between male and female and head proteomes between two *D. melanogaster* normal strains, *white* and *2u*, with different memory score. The results showed that numerous protein spots were differentially expressed between male and female brain. For instance, vitellogenin is a significant protein observed in female *D. melanogaster* only. In addition, the proteome comparison shows that the protein difference between *2u* strain and *white* strain is about 20%. Most of the proteins (80%) are equally expressed in both two strains implying that these differential displayed proteins may influence the process of memory formation and even the behavior of *D. melanogaster* (data not shown).

Currently, there are several 2D-PAGE databases that have been established and these databases recruit the 2D-PAGE maps from 18 species including *Homo sapiens*, etc. (data from World-2DPAGE Portal last updated on June, 2011). To our knowledge, our *D. melanogaster* head 2DE proteome reference map is the first report in this field. The overall scope of the work may be of interest to the *D. melanogaster* research community especially in research on neurodegenerative diseases and memory formation.

In this study, we produced reference proteome maps of the *D. melanogaster* head. Detailed gel images, the locations of the identified protein spots and an interactive database are available from the Drosobrain website, in which the interactive website provides the proteomic information regarding expression profiles of 650 proteins in the *D. melanogaster* head. Additionally, the database has been constructed for systematically integrating and analyzing the proteomes from different conditions and further implicated to study human diseases related to *D. melanogaster* model.

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The authors have declared no conflict of interest.

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