An Agent-Based System to Discover Protein–Protein Interactions, Identify Protein Complexes and Proteins with Multiple Peptide Mass Fingerprints

TZONG-YI LEE,[1] JORNG-TZONG HORNG,[2,3] HSUEH-FEN JUAN,[4] HSIEN-DA HUANG,[1] LI-CHENG WU,[2] MENG-FONG TSAI,[2] HSUAN-CHENG HUANG[5]

[1]*Department of Biological Science and Technology & Institute of Bioinformatics, National Chiao-Tung University, Taiwan, Republic of China*

[2]*Department of Computer Science and Information Engineering, National Central University, No. 320, Jung-do Road, Jungli 320, Taiwan, Republic of China*

[3]*Department of Life Science, National Central University, Taiwan, Republic of China*

[4]*Department of Life Science & Institute of Molecular and Cellular Biology, National Taiwan University, Taiwan, Republic of China*

[5]*Institute of Bioinformatics, National Yang-Ming University, Taiwan, Republic of China*

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Abstract: Proteins "work together" by actually binding to form multicomponent complexes that carry out specific functions. Proteomic analyses based on the mass spectrum are now key methods to determine the components in protein complexes. The protein–protein interaction or functional association may be known to exist among the extracted protein spots while analyzing the proteins on the 2D gel. In this study, we develop an agent-based system, namely AgentMultiProtIdent, which integrated two protein identification tools and a variety of databases storing relations among proteins and used to discover protein–protein interactions and protein functional associations, and identify protein complexes and proteins with multiple peptide mass fingerprints as input. The system takes Multiple Peptide Mass Fingerprints (PMFs) as a whole in the protein complex or protein identification. With the relations among proteins, it may greatly improve the accuracy of identification of protein complexes. Also, possible relationship of the multiple peptide mass fingerprints, such as ontology relation, can be discovered by our system, especially in the identification of protein complexes. The agent-based system is now available on the Web at http://dbms104.csie.ncu.edu.tw/~protein/NEW2/.

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Introduction

Proteomics is the study of all expressed proteins in an organism.[1] Proteins are the ultimate performers of important biological functions in every type of living organism.[2] Proteins "work together" by actually binding, to form multicomponent complexes that carry out specific functions.[3] Therefore, protein identification is fundamentally important to the study of proteomics. The principle of protein identification, using peptide mass fingerprints,[4] is based on comparing the list of experimental masses, with a database containing the theoretical peptide masses of known proteins.[2]

Protein Identification

Several proteomic experimental steps are involved in the identification of a protein. Unidentified proteins are separated by one- or two-dimensional (1D or 2D) gel electrophoresis, and some protein-specific attributes, such as molecular weight (MW) or isotopic points,[5] are measured. The separated proteins are digested with an enzyme and the proteolytic peptides are measured by mass spectrometry (MS), to obtain peptide mass fingerprints.[4]

A protein sequence database is then searched to identify the protein matching the PMF, MW and pI. Mass spectrometry, such as matrix-assisted laser desorption and ionization,[6] and electrospray ionization (ESI), as well as the newer spectrometers that are available, have made it possible to analyze proteins, in small concentrations, in a short time.[7]

Peptide Mass Fingerprinting is a protein identification technique, in which mass spectrometry is used to measure the masses of proteolytic peptide fragments. The protein is identified by

Correspondence to: J. Horng; e-mail: horng@db.csie.ncu.edu.tw

matching the measured peptide masses, with the corresponding peptide masses, from protein or nucleotide sequence databases. The simplest and most obvious scoring method for peptide mass fingerprinting is to count the number of measured peptide masses that have a corresponding entry in a list of calculated peptide masses, within the theoretical mass spectrum of each protein, in the database. Several protein identification tools, available on the Internet, use this method of ranking the proteins in a database, according to the number of matching peptides. For example, PeptIdent (http://us.expasy.org/tools/peptident.html),[1] PepSea (http://pepsea.protana.com/Pa_PepSeaForm.html),[8] and PepFrag (http://www.proteometrics.com/prow/PepFragch.html)[9] calculate a score for the proteins in the database, according to the number of matching peptides.[7]

Several of the available peptide mass fingerprinting programs have introduced more sophisticated scoring algorithms. These algorithms correct for scoring bias due to protein size, in which larger proteins give rise to a greater numbers of peptides, such as Mowse and MS-Fit (http://prospector.ucsf.edu/ucsfhtmle.2/msfit.htm).[6] They also correct for the tendency of smaller peptides in databases to have a greater number of matches with searched *m/z* values. Finally, some of these algorithms also apply probability-based statistics to better define the significance of protein identification, such as ProFound and Mascot.

In contrast to the mass spectra of peptide maps, which contain a protein's global information, peptide fragmentation mass spectra contain rich information on a small section of a protein.[2] The information on the sequence of each peptide enables identification of a protein from a single peptide. Tandem mass spectrometry (MS/MS) can further discover the actual peptide sequence and improve the success rate of protein identification. There are several approaches to using peptide fragment information for protein identification. For instance, SEQUEST (http://thompson.mbt.washington.edu/sequest/) uses data from uninterpreted peptide fragment mass spectra (i.e., information from the whole mass spectrum is used). A crosscorrelation function is calculated, between the measured fragment mass spectrum and the protein sequences in the database, and used to score the proteins in the database. PepFrag[9] uses peptide fragment mass information in combination with other mass spectrometric information, such as amino acid composition, to identify proteins. Mascot[10] uses the same probability-based scoring algorithm for fragment information as for peptide maps. It also supports the use of information from several fragment mass spectra in the database search.

Identifying the Components of Protein Complex

It is known that proteins "work together" by actually binding to form multicomponent complexes that carry out specific functions.[3] The association of proteins with each other in cellular systems has come primarily from two types of experiments. The first involves the immunoprecipitation of a protein interest, together with any associated proteins. The second major approach is the yeast two-hybrid system.[3]

Application of the MS-based proteomic analysis offers a new way to identify the components of multiprotein complexes.[11,12] There are two general approaches for MS analysis of protein–protein interactions and complexes. One is to resolve proteins on a 1D SDS-PAGE gel stain, and to select the protein bands, digest them, and analyze them via MALDI-TOF. Another approach is to digest them directly (without first separating them from each other) and then to analyze the peptide–digest mixture by MALDI-TOF MS or LC-MS-MS.[3] Other techniques in large-scale protein analysis identifying mixtures and multiple protein complex can also found in refs. 13–16. Our prototype system MultiProtIdent[17] tries to identified multiple protein simultaneously, but drawbacks include: single protein identification may not as well as other identification tool, performance issues, and lock of interaction due to few interaction databases. To further assist the identification of multiple proteins (ex: protein complex), we have developed a new tool namely AgentMultiProtIdent that can identify multiple proteins simultaneously with assistance of the protein–protein interaction information from DIP (http://dip.doe-mbi.ucla.edu),[18] STRING (http://www.bork.embl-heidelberg.de/STRING/),[19] BIND (http://www.blueprint.org/bind/bind.php),[20] and MINT (http://160.80.34.4/mint/)[21] databases.

System and Methods

Data Warehousing

AgentMultiProtIdent integrated four databases, comprising protein relationships: DIP (http://dip.doe-mbi.ucla.edu),[18] STRING (http://www.bork.embl-heidelberg.de/STRING/),[19] BIND (http://www.blueprint.org/bind/bind.php),[20] and MINT (http://160.80.34.4/mint/).[21] The information contained in protein–protein interaction databases was used by AgentMultiProtIdent to analyze relationships among unknown proteins.

The DIP[18] database documents experimentally determined protein–protein interactions; up to June 2004, 44,349 interactions had been documented, among 17,048 proteins. STRING[19] is a database of known and predicted protein–protein interactions. STRING currently contains 444,238 genes in 110 species. BIND[20] contains archived information about interactions, molecular complexes, and pathways occurring among proteins, RNA, DNA, and genes; up to June 2004, 77,732 interactions had been documented, among 32,551 proteins. MINT[21] is a relational database, designed to store interactions between biological molecules. Presently, MINT contains 18,115 interactions among 42,481 proteins. Table 1 shows statistics relating to these four databases. Entries describing interactions among proteins from mammalian proteomes are fewer than those from yeast and fruit flies.

AgentMultiProtIdent also integrates gene and protein function databases that offer more relationships among these unknown proteins. The GO (http://www.geneontology.org/) database is used by AgentMultiProtIdent, and is briefly described as follows. The GO (Gene Ontology) database provides structured, controlled vocabularies and classifications that cover several domains of molecular and cellular biology, which are freely available for community use in the annotation of genes, gene products, and sequences. GOA (http://www.ebi.ac.uk/GOA) (Gene Ontology Annotation) is an integrated resource of GO annotations to the UniProt Knowledgebase. The GOA database uses the GO vocabulary to provide high-quality electronic and manual annotations for

Table 1. The Statistics of DIP, STRING, BIND, and MINT.

Database		DIP	STRING	BIND	MINT
Source		Known	Known and Predict	Known	Known
Proteins	Mammalian	about 1200	N/A	N/A	3039
	Total	17,048	444,238 (genes)	32,551	18,115
Interactions	Mammalian	about 1800	N/A	N/A	4367
	Total	44,349	4,611,520	77,732	42,481

gene products contained in UniProt (Swiss-Prot, TrEMBL, PIR-PSD).[5]

Agent-Based System

Because each protein identification tool had a different user interface, a program interface was created by integrating the summation of the query options of several protein identification tools, accessible on the Web, such as Mascot or PeptIdent. In this section, two major technique issues are discussed. One is how to create an agent to determine which parameters to search, in Mascot or PeptIdent; another is how to obtain the result and save it.

First, we must determine how an agent sets the parameters to be searched in Mascot or PeptIdent. An HTTP technique is used in solving the method. The search script in Mascot and PeptIdent Web server will only accept data in "HTTP MultipartPostMethod." Although most Internet packages such as the java.net package provides basic functionality for accessing resources via HTTP, it does not provide the full flexibility or functionality needed by our agent application of multipart POST applications. Thus, we adapted the freeware software package "Jakarta (http://jakarta.apache.org/commons/httpclient/) Commons *HttpClient* component" which provided an efficient, up-to-date, and feature-rich package to implement the client aspect of the most recent HTTP standards and recommendations. The *MultipartPostMethod* package in the *HttpClient* component accepted data in HTTP *MultipartPostMethod,* which solved this problem.

The second issue was how to obtain the result data from the protein identification tools. Mascot saves the search results in a ".dat" file; thus, a URL link could be created to link to the search result or retrieve the results at stage 2 of AgentMultiProtIdent. However, PeptIdent does not save search results. Thus, a direct URL link was not available. To overcome this problem, AgentMultiProtIdent parsed the search results in PeptIdent and created a URL link in AgentMultiProtIdent.

System Flow

We developed AgentMultiProtIdent, a proteomic tool, which identifies multiple proteins through the use of peptide mass fingerprints and possible relationships among proteins. Processing the AgentMultiProtIdent data consists of two stages: stage 1 is an agent-based system, which identifies PMFs into candidate proteins though Internet; stage 2 is a mining system, which is capable of mining relationships among candidate proteins, by using a data warehouse of protein–protein interaction databases, and gene function databases. We discuss each section in detail, as follows. The system flow, showing the two stages of AgentMultiProtIdent, can be seen in Figure 1a.

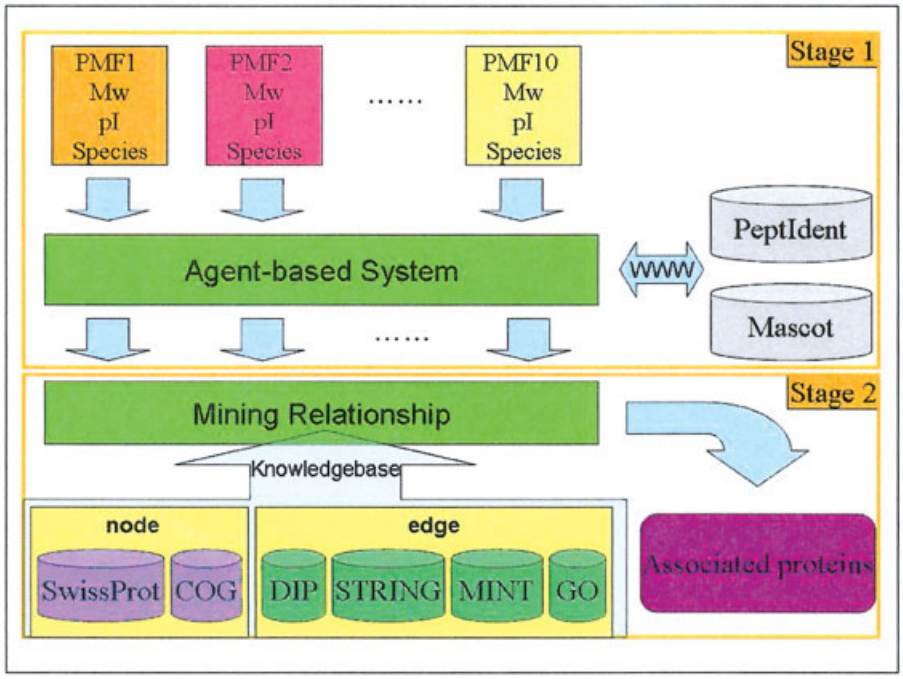
In Stage 1, we developed an agent-based system that takes the input of multiple PMFs and protein-specific attributes and initiates a protein identification search agent for each PMF, to identify the protein, through an Internet protein identification server, such as PeptIdent or Mascot. Only one of PeptIdent or Mascot can be used exclusively but not combine. The user can choose which identification to use.

First, the agent-based system in Stage 1 creates an agent for each PMF and protein-specific attribute and sends the information to the protein identification server through the Internet. Next, each agent receives the identification results from protein identification Web server. The system collects each agent's results and integrates them into lists of candidate proteins, associated with scores that match the input PMF in the protein sequence database.

An AgentMultiProtIdent Stage 1 example is shown in Figure 1b. Ten sets of peptide mass fingerprints (PMF1, PMF2, . . ., PMF10) and related protein-specific attributes were submitted to AgentMultiProtIdent Stage 1. AgentMultiProtIdent created an agent for each pair of PMFs and the protein-specific attributes and performed protein identification with an Internet protein identification server, such as PeptIdent or Mascot. The identification result was then returned to the agent, completing the identification process. The identification scores of PeptIdent and Mascot were different, because they had two different scoring schemes. In PeptIdent, the scores represented the number of measured peptide masses equal to the calculated peptide masses in the theoretical mass spectrum of each protein in the database. However, in Mascot, the candidate proteins were ranked with decreasing probability of being a random match to the experimental data.

Next, an agent-based system collected the search results from each agent. The search results were transformed into a uniform format; this comprised lists of candidate proteins associated with scores calculated by the PeptIdent or Mascot scoring function. In Figure 1b, the results represent 10 sets of candidate proteins and scores denoted S_{PMF1}, S_{PMF2}, . . ., S_{PMF10}, respectively. Each candidate protein contained a score calculated by the protein identification scoring algorithm. The candidate proteins in S_{PMF1} were denoted as *A_1, A_2, . . . , A_10* with scores S_{a_1}, S_{a_2}, . . . , S_{a_10}, respectively. Because the candidate protein with the first ranking in each PMF set may not be the correct protein corresponding to the PMF, the user can select some or

(a)



(b)

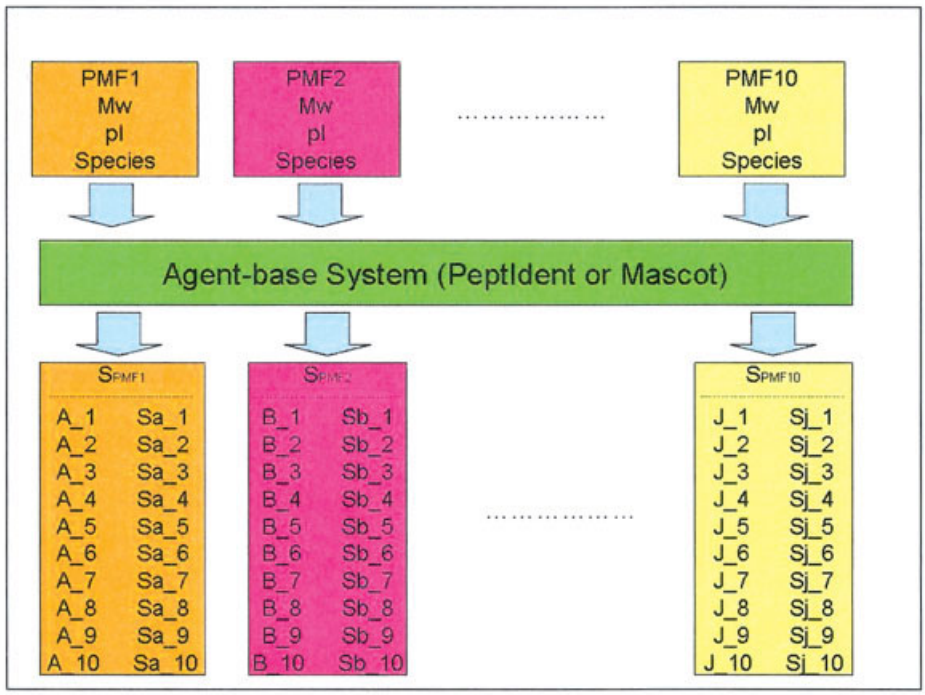


Figure 1. (a) The system flow of AgentMultiProtIdent. (b) An example of Stage 1 in AgentMultiProtIdent. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

all candidate proteins to be analyzed in Stage 2 of AgentMultiProtIdent.

Stage 2 of AgentMultiProtIdent analyzes the relationships among the sets of candidate proteins by using a protein relation knowledgebase. Relationships, such as protein–protein interactions or functional associations, may exist among these candidate proteins, for example, in a protein complex. With the integration and preprocessing of the information in the knowledgebase, protein–protein interactions or functional associations, between each pair of the candidate proteins, can be found if they exist. Figure 2a gives an example, showing the relationships among S_{PMF1}, S_{PMF2}, . . . , S_{PMF10}. S_{PMFn}, containing a set of candidate proteins, $n = 1, \ldots, 10$.

Figure 2b shows a detailed presentation of relationships among S_{PMF1}, S_{PMF4}, and S_{PMF7}. The DIP, STRING, MINT, and GO are represented by the black, red, blue, and green lines, respectively. The protein–protein interactions or functional associations are visualized as an undirected graph $G = (V, E)$, where $x, y \in V$ and $(x, y) \in E$. Let x and y represent proteins and $(x, y) \in E$ represent an interaction or association between proteins x and y.[22] In AgentMultiProtIdent, V refers to all proteins in Swiss-Prot, and E refers to all relationships in knowledgebase. To make the relationship search possible, we first found the subgraph of G, defined as follows,

Let $x', y' \in V'$ represent the proteins in candidate protein sets and $(x', y') \in E'$ represent a protein–protein interaction or functional association between proteins x' and y'. The graph $G' = (V', E')$, is a subgraph of G, where $V' \subset V$ and $E' \subset E$. In this example, the 10 sets of candidate proteins S_{PMF1}, S_{PMF2}, . . ., S_{PMF10} were subsets of V', that is, $S_{PMF1} \cup S_{PMF2}$ &cur; . . . $\cup S_{PMF10} = V'$. All the edges $(x', y') \in E'$ among candidate proteins V' were searched from knowledgebase E.

In addition to offering additional information among these unknown proteins, this information can help improve the accuracy of protein identification. Considering the relationships among S_{PMF1}, S_{PMF4}, and S_{PMF7}, vertexes *A_5*, *G_4*, *D_2*, and edges (*A_5, G_4*), (*G_4, D_2*) form a connected subgraph of G'. In general, *A_1*, *D_1*, and *G_1* were the first ranking, with the highest score in each candidate protein set. Because false positives do occur in traditional protein identification, due to the quality of MS spectra, parameters, and crosscontamination Keratins,[4] *A_1*, *D_1*, and *G_1* may not be the correct protein. However, *A_5*, *G_4*, and *D_2* are more likely to be the correct proteins responding to the PMFs, and protein–protein interactions may exist among them. We say that *A_5*, *G_4*, and *D_2* are associated proteins, because there are relationships between *A_5* and *G_4*, as well as between *G_4* and *D_2*.

The associated proteins among candidate proteins, such as *A_5*, *G_4*, and *D_2* in Figure 2b, can be seen as a connected subgraph problem, given n candidate protein sets as S_{PMF1}, S_{PMF2}, . . ., S_{PMFn} and $\cup S_{PMFi} = V'$ for $i = 1$ to n. Our goal was to find all subgraphs of G' denoted as $G'' = (V'', E'')$, which satisfy the following conditions:

1. G'' is a subgraph of G' $V'' \in V'$, $E'' \in E'$
2. Every vertex has an edge connected. Given $x_k \in V''$, $k = 1$ *to* n, there exists $y'' \in V'' - x_k$, such that $(x_k, y'') \in E''$
3. There are no two vertexes from the same candidate proteins set. For every l = 1 to n, given $x_l \in S_{PMFw}$, for some $w = n$ and $w > 0$ (see lemma 1). For every $y'' \in V'' - x_l$, $y'' \notin S_{PMFw}$,
4. The number of vertex is not more than the number of candidate proteins set. $N(V'') \leq n$.

The third condition uses a small obvious lemma as follows:

Lemma 1. Because

$$V' = \prod_{i=1}^{n} S_{PMFi},$$

For every $x'' \in V'$, there exist S_{PMFj} such that $x'' \in S_{PMFj}$, for some $j = n$ and $j > 0$.

In Figure 2b, vertices *A_5*, *G_4*, *D_2*, and edges (*A_5, G_4*), (*G_4, D_2*) form a connected subgraph of G', which matched the condition. Vertices *D_1*, *D_5*, *G_1*, and edges (*D_1, D_5*), (*D_5, G_1*) did not match the condition because *D_1* and *D_5* were from the same candidate proteins set S_{PMF4}. A weighted score of the edge (Relationship) between each pair of proteins was calculated by summing up the ranking scores of the two proteins. The score of each candidate protein is shown in Figure 1b, the weighted score of edge (*A_5, G_4*) was $2(S_{a\_5}+S_{g\_4})$, because there were two relationships from DIP and GO. However, (*D_5, G_1*) was $S_{d\_5} + S_{g\_1}$. The total score of the connected subgraph "vertices *A_5*, *G_4*, *D_2*, and edges (*A_5, G_4*), (*G_4, D_2*)" was $2(S_{a\_5}+ S_{g\_4}) + 2(S_{g\_4} + S_{d\_2})$. The score of the connected subgraph is defined as follows:

$$S = \sum We \quad (1)$$

where S is the score of the connected subgraph; and We is a weighted score of edge e. The score of the connected subgraph is the sum of all the weighted edge scores for all edges e in the connected subgraph.

Results

AgentMultiProtIdent is a Web-based system. A unified interface was designed so that the user could input more than one set of PMFs and protein specific attributes. Below, we present two groups of results to show how the AgentMultiProtIdent analyzed relationships among candidate proteins. One group shows the results after using the system with the MS spectra from mammalian and *Pseudomonas Aeruginosa* proteomes. The other group used simulated PMFs of the known components of cellular yeast complex from the MIPS database.

MS Spectra from Mammalian and Pseudomonas aeruginosa

One set of MS spectra was offered by Prof. Juan and another by Prof. Huang. The MS spectra TestSet_1 offered by Prof. Juan came from mammals, and the MS spectra TestSet_2 offered by Prof. Huang came from Pseudomonas aeruginosa. We had no prior knowledge of the test set, except for the source organism and the MS spectra. A description of TestSet_1 follows, in which the relationship among unidentified proteins was analyzed.

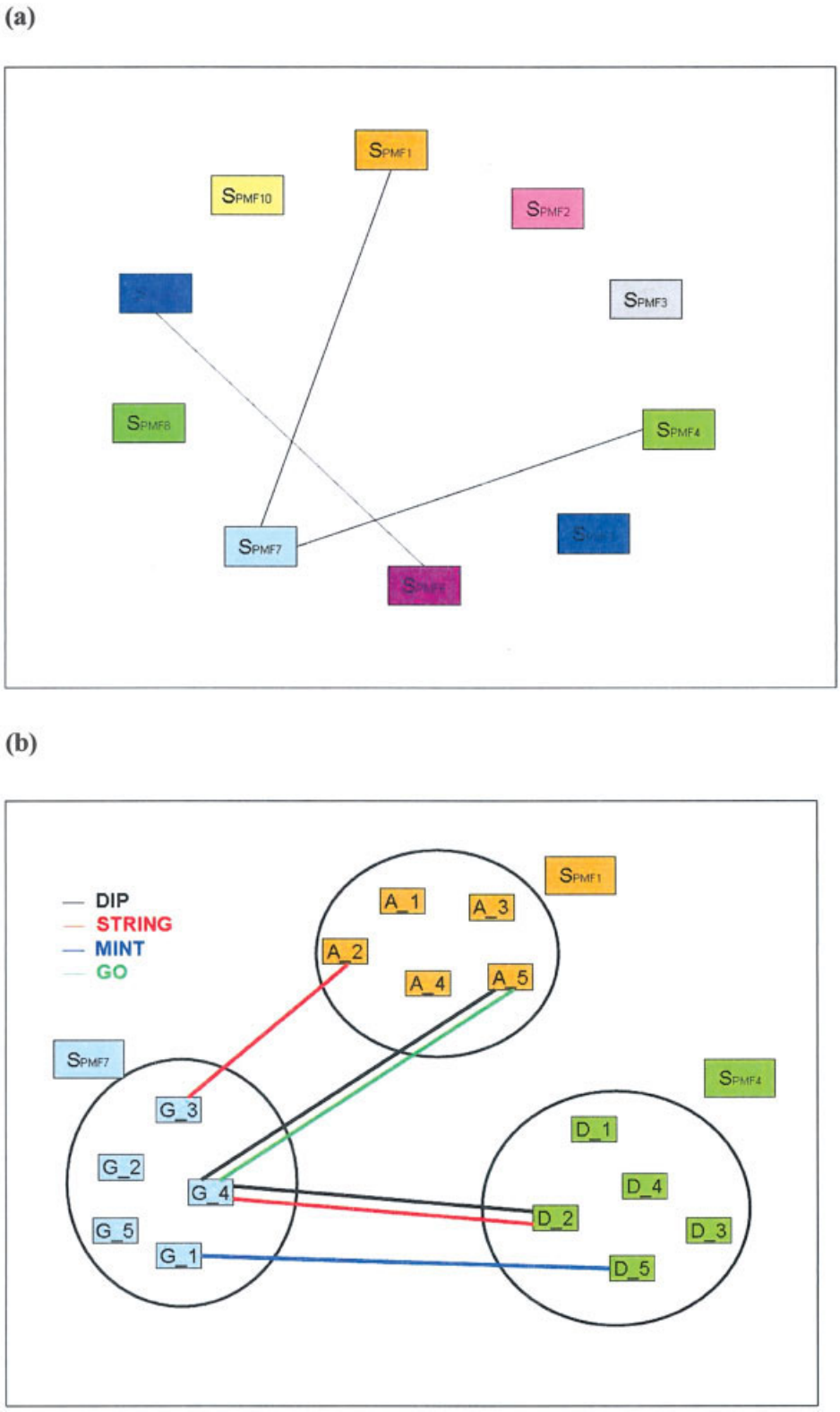


Figure 2. (a) An example to show the result from Stage 2 of AgentMultiProtIdent. (b) A detailed representation of relationships among sets of candidate proteins.

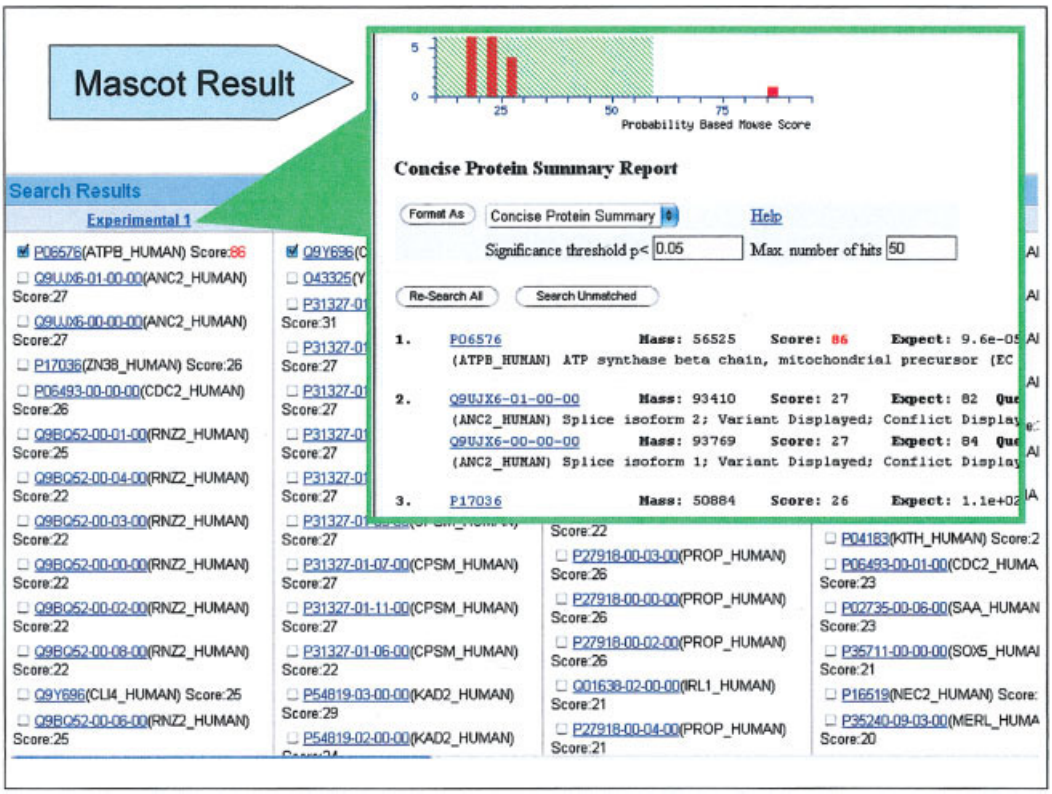


Figure 3. Result list of candidate proteins. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

There were 10 PMFs from Human liver in TestSet_1. The identification parameters of the 10 PMFs were given equally, as follows: the protein search identification tool used was Mascot; the searched database was Swiss-Prot; the selected species was *Homo sapiens* (human); the digested enzyme was Trypsin; the posttranslational modification was *Oxidation of Methionine* (M); the maximal tolerance for masses was within 1 dalton; and at most, one missed cleavage was allowed, with the maximum number to list the results being set to 30.[23] By default, AgentMultiProtIdent was set to pick the first ranking of candidate proteins in each result list, in order to analyze the relationship among them, as shown in Figure 3. User could choose more candidate proteins in each result, from the identification protein list. In this experiment, we only used the first ranking of candidate proteins.

In Figure 4a, several relationships can be seen among the 10 sets of candidate proteins; the proteins in PMF sets 2, 4, 7, and 10 seem to be isolated from the others, however. The user is able to view the detailed relationship information and the source of these relationships, as shown in Figure 4b.

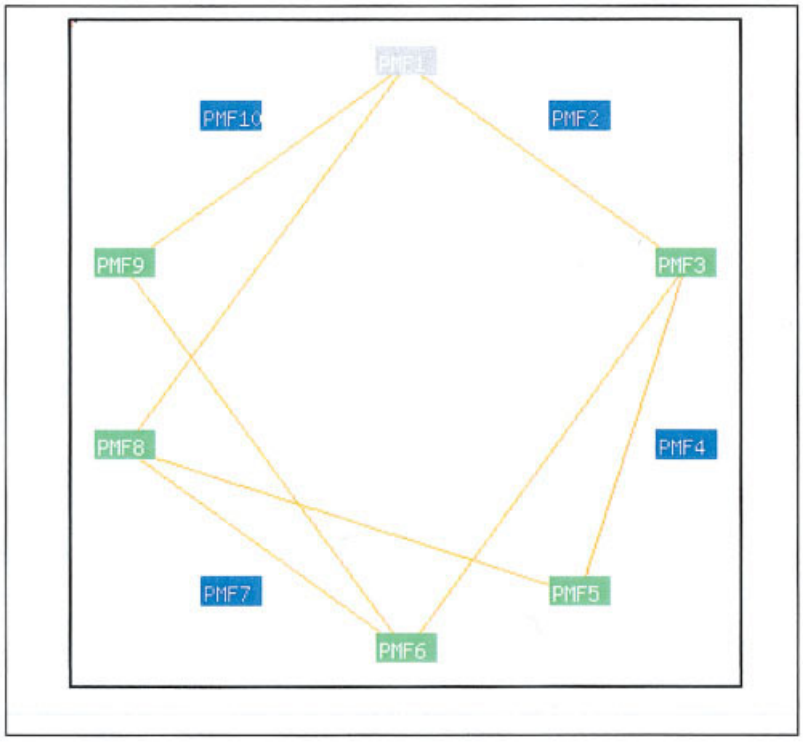
By pressing the PMF1 in Figure 4a, possible relations as Figure 4b are shown to demonstrate the relationship of proteins in PMF 3, 8, and 9 to the protein in PMF1 (ATPB_HUMAN). The left part of Figure 4b shows the basic protein information such as protein ID, protein accession number, and protein name. The right part shows the possible interaction between these proteins. Figure 4b indicates that all the relationships in this experiment came from the GO database. This means that although there were no known physical relationships (known protein–protein interactions) among these selected candidate proteins, functional relationships did exist among these proteins, based on the relationship information in the GO database. For example, the candidate protein ATPB_HUMAN of PMF1 had a similar protein function (GO:0005215) to the candidate protein FABL_HUMAN of PMF3. GO:0005215 had a transporter activity of molecular function; the annotated description of the relationship is "enables the directed movement of substances (such as macromolecules, small molecules, ions) into, out of, within or between cells."

MS Spectra from Known Cellular Complex

To validate our approach, protein complexes were used for testing the analysis of multiple PMFs, which had protein–protein interactions or functional associations among them. In Table 2, we show five sets of test data, including cellular complexes from MIPS (http://mips.gsf.de/)[24] and some proteins, randomly chosen from *Saccharomyces cerevisiae*. Parameter given to AgentMultiProtIdent is identical to the Parameter of TestSet_1 and TestSet_2 except species. Here we adapted a simulation of the peptide mass fingerprint.

In Figure 5, we show that computer simulations have been performed to generate PMFs of component proteins in the cellular complex.[16] For example, the cellular complex cAMP-dependent

(a)



(b)

PMF	Protein Information				Interaction			
1	ID ATPB_HUMAN	AC P06576; Q14283;	Organism Homo sapiens (Human).		DIP	MINT	STRING	GO
	MW 56560 Da	pI 5.95	Score 86					
	Protein Name	ATP synthase beta chain, mitochondrial precursor (EC 3.6.3.14).						
	Gene Name	ATP5B OR ATPSB OR ATPMB.						
3	ID FABL_HUMAN	AC P07148;	Organism Homo sapiens (Human).					GO:0005215
	MW 14208 Da	pI 5.95	Score 45					
	Protein Name	Fatty acid-binding protein, liver (L-FABP).						
	Gene Name	FABP1 OR FABPL.						
6	ID FABL_HUMAN	AC P07148;	Organism Homo sapiens (Human).					GO:0005215
	MW 14208 Da	pI 5.95	Score 58					
	Protein Name	Fatty acid-binding protein, liver (L-FABP).						
	Gene Name	FABP1 OR FABPL.						
9	ID CYB5_HUMAN	AC P00167;	Organism Homo sapiens (Human).					GO:0005739 GO:0006091 GO:0016021
	MW 15199 Da	pI 5.95	Score 88					
	Protein Name	Cytochrome b5.						
	Gene Name	CYB5.						

Figure 4. (a)The relationships among the 10 sets of candidate proteins. (b) By pressing the PMF1 in (a), possible relations are shown to demonstrate relationship of proteins in PMF 3, 8, and 9 to the protein in PMF1 (ATPB_HUMAN). There are five GO relations between ATPB_HUMAN, FABL_HUMAN,FABL_HUMAN, and CYB5_HUMAN shown in the right of the page. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

protein kinase was composed of four entries: YIL033c, YJL164c, YKL166c, and YPL203w. Corresponding Swiss-Prot protein ID KAPA_YEAST, KAPB_YEAST, KAPC_YEAST, and KAPR_YEAST were retrieved in step 1 of the simulation. These protein sequences were submitted to theoretical tryptic digestion by PeptideMass (http://us.expasy.org/tools/peptide-

Table 2. Five Test Data Sets Include Cellular Complexes from the MIPS Database.

	Test data	Swiss-Prot ID of each Entries
1	2-oxoglutarate dehydrogenase (YDR148c, YFL018c, YIL125w)	ODO1_YEAST, ODO2_YEAST, DLDH_YEAST
2	cAMP-dependent protein kinase (YIL033c, YJL164c, YKL166c, YPL203w)	KAPA_YEAST, KAPB_YEAST, KAPC_YEAST, KAPR_YEAST
3	2-oxoglutarate dehydrogenase (YIL125w, YDR148c, YFL018c) and YMR105c	ODO1_YEAST, ODO2_YEAST, DLDH_YEAST, PGM2_YEAST
4	Anthranilate synthase (YER090w, YKL211c) and YDR256C	TRPE_YEAST, TRPG_YEAST, CATA_YEAST
5	Anthranilate synthase (YER090w, YKL211c) + Fatty acid synthetase cytoplasmic (YKL182w, YPL231w)	TRPE_YEAST, TRPG_YEAST, FAS1_YEAST, FAS2_YEAST

mass.html)[18] with parameters allowing two missed cleavages and with some posttranslational modifications in Step 2. To simulate a low coverage of 30%, three peptide masses with missed cleavages ≤ 1 and no posttranslational modification were randomly selected from the theoretical digested peptide masses in Step 3. Each of the randomly selected three peptide masses was added to a random value between -1 to $+1$ daltons in Step 4. Similar mass error tolerance can be found in prior studies.[23] On the other hand, seven peptide masses were randomly selected with missed cleavages $= 2$ or with posttranslational modifications (PTMs) from the theoretical digested peptide masses in Step 5. Finally, the total 10 peptide masses were treated as simulated PMFs for each protein. The simulated PMFs were then submitted to AgentMultiProtIdent for multiple PMF analysis. Each test data was executed five times, in our simulation.

The second test data was a cellular complex cAMP-dependent protein kinase composed of four entries: YIL033c, YJL164c, YKL166c, and YPL203w, whose corresponding Swiss-Prot protein IDs were KAPA_YEAST, KAPB_YEAST, KAPC_YEAST, and KAPR_YEAST, respectively. These four proteins were mixed with six other proteins, randomly selected from Swiss-Prot. A total of 10 simulated PMFs were generated by the previous simulation process. PMF1, PMF3, PMF6, and

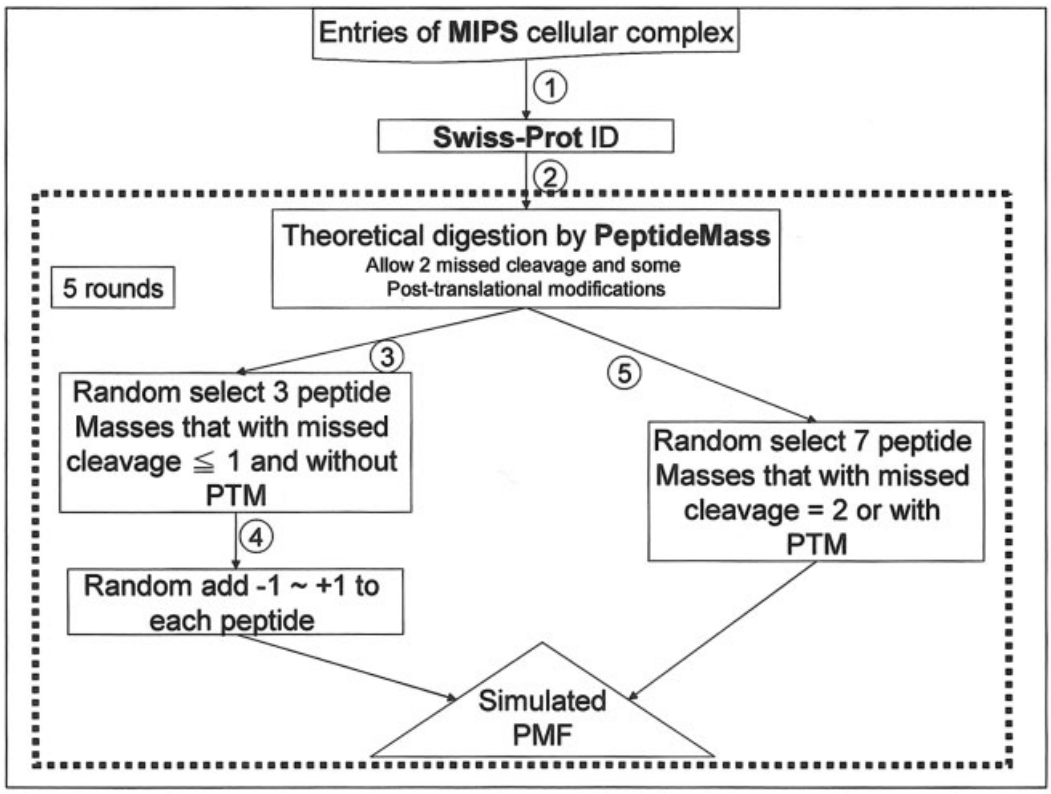
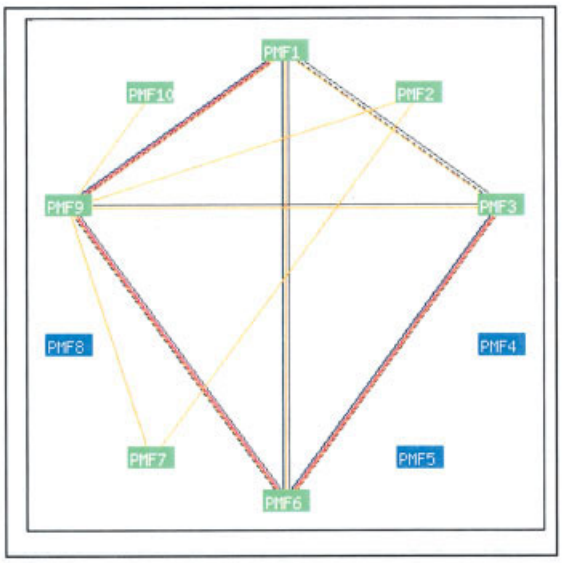


Figure 5. The flow of generating simulated PMFs. The processes pointed by the dotted line are repeated five times.

(a)



(b)

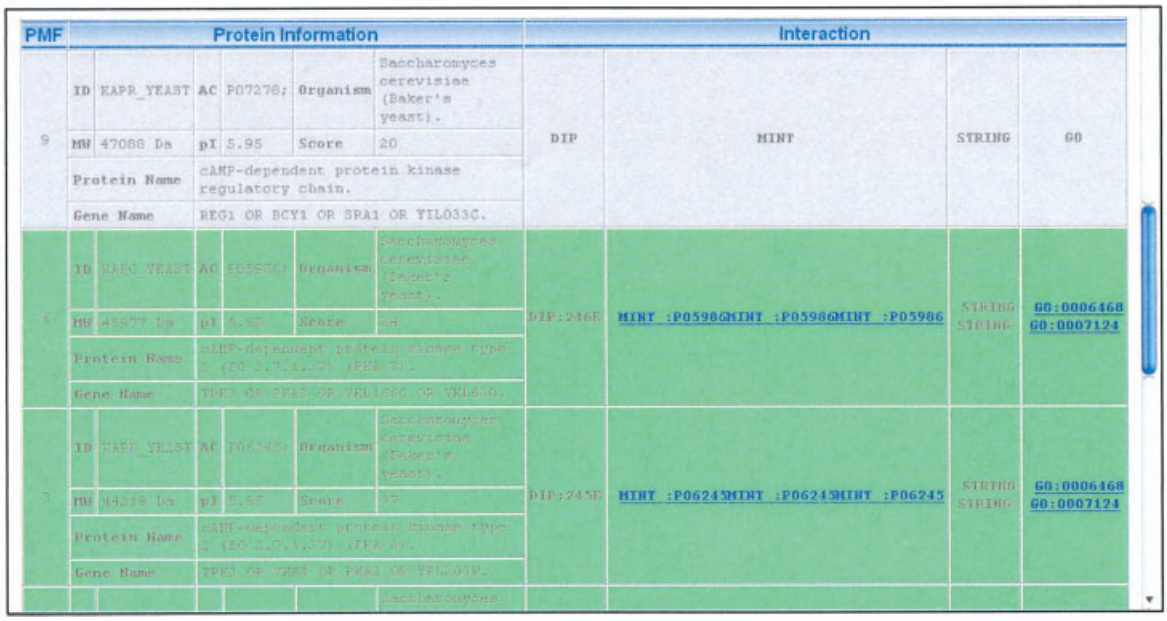
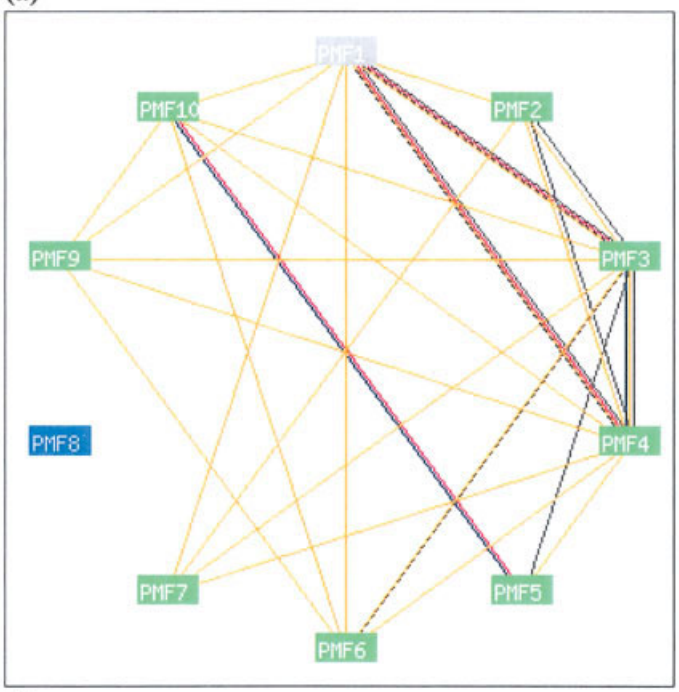


Figure 6. (a) A graph shows the relationship among these sets of candidate proteins. (b) Detailed information about relationships among these candidate proteins (KAPA_YEAST of PMF1 with DIP: 244E and MINT: P04244 is in the bottom of the Web page, which is now shown). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

PMF9 were represented by KAPA_YEAST, KAPB_YEAST, KAPC_YEAST, and KAPR_YEAST, respectively.

By default, the first ranking candidate protein in each result set was selected to analyze the relationships among them. Assuming it was not known which one was correct, the user could select the top five ranking candidate proteins for analysis. Figure 6a shows the relationships among these candidate protein sets. In Figure 6b, the cellular complex cAMP-dependent protein kinase, composed of KAPA_YEAST, KAPB_YEAST, KAPC_YEAST, and KAPR_YEAST, were successfully identified. KAPA_YEAST, KAPB_YEAST, KAPC_YEAST, and KAPR_YEAST were not the first ranking candidate proteins in each result set. Looking at the

(a)



(b)

PMF	Protein Information					Interaction			
1	ID ODO1_YEAST	AC P20967;		Organism	Saccharomyces cerevisiae (Baker's yeast).	DIP	MINT	STRING	GO
	MW 114416 Da	pI 5.95		Score	22				
	Protein Name	2-oxoglutarate dehydrogenase E1 component, mitochondrial precursor (EC 1.2.4.2) (Alpha-ketoglutarate dehydrogenase).							
	Gene Name	KGD1 OR YIL125W.							
4	ID [illegible]	AC [illegible]		Organism	Saccharomyces cerevisiae (Baker's yeast).	DIP:15361E	MINT :P09624	STRING STRING STRING STRING	GO:0005739 GO:0006096 GO:0016491 GO:0042645
	MW [illegible]	pI [illegible]		Score	[illegible]				
	Protein Name	Dihydrolipoamide dehydrogenase, mitochondrial precursor (EC 1.8.1.4).							
	Gene Name	[illegible]							
3	ID [illegible]	AC [illegible]		Organism	Saccharomyces cerevisiae (Baker's yeast).	DIP:1248	MINT :P19262MINT :P19262	STRING STRING	GO:0005739 GO:0008152 GO:0042645
	MW [illegible]	pI [illegible]		Score	[illegible]				
	Protein Name	Dihydrolipoamide succinyltransferase component of 2-oxoglutaratedehydrogenase complex, mitochondrial precursor (EC 2.3.1.61) (E2).							
	Gene Name	[illegible]							
	ID [illegible]	AC [illegible]		Organism	Saccharomyces cerevisiae (Baker's				

Figure 7. (a) A graph shows the relationships among these sets of candidate proteins. (b) Detailed information about relationships among these candidate proteins. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

relationship information from DIP, STRING, and MINT, the four candidate proteins KAPA_YEAST, KAPB_YEAST, KAPC_YEAST, and KAPR_YEAST, may be the correct proteins corresponding to PMF1, PMF3, PMF6, and PMF9, respectively.

The third test data was a cellular complex 2-oxoglutarate dehydrogenase composed of three entries: YIL125w, YDR148c, and YFL018c, corresponding to Swiss-Prot protein ID of ODO1_YEAST, ODO2_YEAST, and DLDH_YEAST,

Table 3. A Comparative Table of AgentMultiProtIdent and Other Popular Protein Identification Tools.[7]

Name	MS type	Other input	PTM	Note
PeptIdent	MS	None	Cys blocking and Met oxidation	making extensive use of database annotations
MultiIdent	MS	AA + sequence tag	Cys blocking and Met oxidation	None
MS-Fit	MS	AA	Predefine partial and complete	None
MOWSE	MS	AA + sequence tag	None	None
Mascot	MS and MS/MS	None	Predefine partial and complete	Probability based scoring function
CombSearch	MS	AA + sequence tag	Predefine partial and complete	Provide a unified interface to query several protein identification tools accessible on the Web
MultiProtIdent	MS and MS/MS	AA + sequence tag	Predefine partial and complete	1. Allow user to input more than one PMF and MS/MS spectra 2. Find relations among DIP and STRING
AgentMultiProtIdent	MS and MS/MS	AA + sequence tag	Predefine partial and complete	1. Agent base takes advantage of other identification tool 2. Allow user to input more than one PMF and MS/MS spectra 3. Find relations among candidate proteins on various databases

AA represents amino acid composition and MS/MS represents tandem mass spectrometry.

respectively. These three proteins were mixed with seven other proteins, randomly selected from Swiss-Prot. A total of 10 simulated PMFs were generated by the previous simulation process. PMF1, PMF3, and PMF4, were represented by ODO1_YEAST, ODO2_YEAST, and DLDH_YEAST, respectively.

Here, we also selected the top five ranking candidate proteins in each result set to analyze the relationships among these candidate proteins. Figure 7a shows the graphical representation of relationships among these candidate protein sets. There were many edges among PMF1, PMF3, and PMF4, which corresponded to ODO1_YEAST, ODO2_YEAST, and DLDH-_YEAST, respectively. By clicking on the block chart of PMF1 in Figure 7a, the user was able to observe the detailed information of the candidate protein relationships of PMF1, as shown in Figure 7b. There was information of several relationships from DIP, STRING, and MINT, so the three candidate proteins ODO1_YEAST, ODO2_YEAST, and DLDH_YEAST may be the correct proteins corresponding to PMF1, PMF3, and PMF4, respectively.

Another finding showed that the identification processes of the sets of five cellular complexes, from MIPS, were identified correctly. The results of multiple PMF analysis, using interaction data from DIP, MINT, and BIND databases, performed better than interaction results from GO and STRING databases. Because of the quantity and quality of the predicted functional associations of STRING and GO, there are still false positives in the identification of protein complexes. On the other hand, most of the protein–protein interactions of the DIP database focused, mainly, on *Drosophila melanogaster* and *Saccharomyces cerevisiae* (baker's yeast), as the protein–protein interactions of other organisms were lesser than these two.

Table 3 shows a comparison of the AgentMultiProtIdent and other popular protein identification tools. CombSearch (http://us.expasy.org/tools/CombSearch/) is capable of querying several protein identification tools, simultaneously, through the Web, including PeptIdent, MultiIdent, MS-Fit, Mowse, and ProFound. The major feature of the AgentMultiProtIdent is the ability to identify multiple proteins though Internet, with the assistance of protein–protein interaction. Instead of a small interaction network, slow performance, and proprietary identification engine of our prototype MultiProtIdent,[17] AgenttMultiProtIdent is a smart agent system that takes advantage of other high accuracy identification tools and variety of interaction databases.

Discussion and Conclusion

AgentMultiProtIdent is the first protein identification tool able to identify multiple proteins simultaneously, and combine the information of protein–protein interactions or functional associations in protein identification. Relationships such as protein–protein interactions or functional associations may exist among proteins excised from the same 1D/2D gel or when comparing two 2D gels. The results show that multiple PMF analysis has high precision, when applied to the identification of a protein complex. The results also show that an ontology relationship may be discovered via the AgentMultiProtIdent. Especially in the identification of protein complexes, the advantage of the existing protein–protein interaction databases can improve identification accuracy.

We plan to add more protein–protein interaction databases, such as MIPS and KEGG (http://www.genome.ad.jp/kegg/)[25] in the further works. The KEGG pathway database will be added as a first priority; this will offer a detailed biological process for identifying multiple proteins sharing undirected interactions. There are several aspects to be considered for future study. For multiplicity, the sequence tags of proteins can be submitted to the

system for identification of multiple proteins. For accuracy, the scoring function of our protein identification, and the weighting function of the interaction between proteins, will be refined; post-translational modification will also be considered. A visualization interface, showing the interaction, is also being considered, as well as a graph layout algorithm, to be used to draw the relationships of the multiple PMF analysis result.[26]

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