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Data article

YPIBP: A repository for phosphoinositide-binding proteins in yeast

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ABSTRACT

Phosphoinositides (PIs) are a family of eight lipids consisting of phosphatidylinositol (PtdIns) and its seven phosphorylated forms. PIs have important regulatory functions in the cell including lipid signaling, protein transport, and membrane trafficking. Yeast has been recognized as a eukaryotic model system to study lipid-protein interactions. Hundreds of yeast PI-binding proteins have been identified, but this research knowledge remains scattered. Besides, the complete PI-binding spectrum and potential PI-binding domains have not been interlinked. No comprehensive databases are available to support the lipid-protein interaction research on phosphoinositides. Here we constructed the first knowledgebase of Yeast Phosphoinositide-Binding Proteins (YPIBP), a repository consisting of 679 PI-binding proteins collected from high-throughput proteome-array and lipid-array studies, QuickGO, and a rigorous literature mining. The YPIBP also contains protein domain information in categories of lipid-binding domains, lipid-related domains and other domains. The YPIBP provides search and browse modes along with two enrichment analyses (PI-binding enrichment analysis and domain enrichment analysis). An interactive visualization is given to summarize the PI-domain-protein interactome. Finally, three case studies were given to demonstrate the utility of YPIBP. The YPIBP knowledgebase consolidates the present knowledge and provides new insights of the PI-binding proteins by bringing comprehensive and in-depth interaction network of the PI-binding proteins. YPIBP is available at http://cosbi7.ee.ncku.edu.tw/YPIBP/.

1. Introduction

Phosphoinositides (PIs) are a family of eight lipids consisting of phosphatidylinositol (PtdIns) and its seven phosphorylated forms. Site-specific phosphorylation by a variety of lipid kinases on the 3, 4, and/or 5 hydroxyl positions of the inositol ring leads to the generation of seven different compounds (PI3P, PI4P, PI5P, PI(3,4)P2, PI(4,5)P2, PI(3,5)P2, and PI(3,4,5)P3) [1–3]. PIs play significant regulatory functions in the cell including lipid signaling, protein transport, and membrane trafficking [1,4,5]. The physiological and regulatory roles of PIs remain under extensive investigation [6,7].

The binding of PIs to their partner proteins defines their unique structural and regulatory functions. PI-protein interactions are critical for different membrane constituents, metabolism, and regulation [8,9]. PI-protein interactions are dynamic and diverse. They may occur in different cellular environments (organelles) which enable PIs to gain new structural and functional benefits [10,11].

Abbreviations: ANTH, AP180 N-terminal Homology; BAR, Bin-Amphiphysin-Rvs; CAFA, Critical Assessment of Functional Annotation; CRAL-TRIO, cellular retinaldehyde-binding protein (CRALBP) and TRIO guanine exchange factor; Cvt, Cytoplasm-to-vacuole targeting; ENTH, Epsin N-terminal Homology; FDR, False Discovery Rate; FYVE, Fab 1 (yeast orthologue of PIKfyve), YOTB, Vac 1 (vesicle transport protein), and EEA1; GO, Gene Ontology; ITC, Isothermal Titration Calorimetry; LBD, Lipid-Binding Domain; LMPD, LIPID MAPS Proteome Database; LMSD, LIPID MAPS Structure Database; LRD, Lipid-Related Domain; OMIM, Online Mendelian Inheritance in Man; OSBP, Oxysterol-Binding Protein; PH, Pleckstrin Homology; PIs, Phosphoinositides; PtdIns, Phosphatidylinositol; PI3P, phosphatidylinositol-3-phosphate; PI4P, phosphatidylinositol-4-phosphate; PI5P, phosphatidylinositol-5-phosphate; PI(3,4)P2, phosphatidylinositol-3,4-bisphosphate; PI(3,5)P2, phosphatidylinositol-3,5-bisphosphate; PI(4,5)P2, phosphatidylinositol-4,5-bisphosphate; PI(3,4,5)P3, phosphatidylinositol-3,4,5-trisphosphate; PMID, PubMed ID; PX, Phox Homology; QCM, Quartz Crystal Microbalance; SNX, Sorting Nexin; SPR, Surface Plasmon Resonance; YPIBP, Yeast Phosphoinositide-Binding Proteins.

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PI-protein interactions could reorganize the organelle-associated proteome. Therefore, it is impossible to understand the functions of PIs without the information of PI-binding proteins.

The techniques to study lipid-protein interactions were classified into two major categories: (i) low-throughput techniques which can identify one-to-one interaction and (ii) high-throughput technological platforms that can screen an array of candidates in a single experimental setup to identify many interactions. Most of the low-throughput traditional methods such as liposome-binding (pull-down) [12,13] and lipid-protein overlay assay [14] are widely used when the focus was on a single or few proteins to be explored for their possible interactions with lipids. These assays use immobilized individual lipids on a matrix (e.g., nitrocellulose membranes for the lipid-overlay assay and magnetic beads for the lipid pull-down method) and proteins that bind to the immobilized lipids can be immuno-detected [15] or identified by mass spectrometry [16,17]. However, these interaction assay techniques are qualitative as they just aim to detect the lipid-binding event. The quantification of binding which is defined by their affinity towards a specific lipid needs to be done using surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), and quartz crystal microbalance (QCM) [18]. Additionally, there are few microscopic techniques available which use a labeled tag to identify the co-localization of lipids and proteins in membranes [19]. Various simulation or computational docking as well as crystallographic studies of lipid-bound complexes could deduce the mode of interaction [20].

In recent years, various high-throughput array platforms such as lipid array [21], liposome microarray [22] and proteome microarray [6,23,24] have been developed to identify many lipid-protein interactions in a single probing experiment. Gallego *et al.* [21] developed an effective lipid array method to screen lipid-protein interactions in the yeast system. The lipid array platform contains an array of different lipids. Lipid array allows probing with only one protein of interest at a time. Multiple reports have indicated a critical requirement to define lipid-protein interactions at the proteome level to unravel the diversity of lipid-protein interactome in the cell [23–26]. The proteome array platform consists of individually purified proteins that are chemically immobilized on a chip. The lipid of interest is represented by the fluorescently labeled liposomes that interact with a specific protein at a unique location in the chip. Using a microarray chip scanner, the positive interaction signals identify the list of proteins which bind to the liposomal lipid of interest. It is noteworthy that all eight members of PIs have been probed on yeast proteome microarray, providing a complete spectrum of PI-binding proteins in the yeast system [6,23,24].

Yeast is a widely accepted eukaryotic model system that can support genome-scale investigations [27–30]. The availability of genome sequences and mutant libraries of *Saccharomyces cerevisiae* provides opportunities to design genome-scale experiments to understand the functional roles of lipids. *S. cerevisiae* shares multiple homologs with other higher eukaryotes including humans. Yeast genome possesses 23% homologous genes in the human genome [31,32]. Therefore, the budding yeast could be useful for studying lipid-protein interactions beyond yeast itself and paves the way towards understanding the role of lipids in cellular functions as well as human health and diseases. Across the lipid spectrum, the PIs have been studied extensively in the yeast system. The proteome microarray-based studies have covered all eight members of PIs (i.e., PtdIns, PI3P, PI4P, PI5P, PI(3,4)P2, PI(4,5)P2, PI(3,5)P2, and PI(3,4,5)P3). Zhu *et al.* [23], Lu *et al.* [24], and Herianto *et al.* [6] have comprehensively identified hundreds of yeast proteins which bind to PIs. Moreover, there are more than 200 publications in PUBMED mentioning hundreds of proteins interacting with a specific spectrum of PIs identified by diverse low throughput experiments. This broad interaction knowledge availability highlights the extensive research on yeast PI-binding proteins. However, the knowledge about these identified PI-binding proteins is widely scattered which cannot be easily accessed by researchers. Therefore, there is a need for a user-friendly database that comprehensively collects the PI-binding proteins from various web resources and published literature along with various enrichment tools for downstream analysis.

To meet this need, we constructed YPIBP (Yeast Phosphoinositide-Binding Proteins) database which collects 679 PI-binding proteins in yeast from the literature. Users can search by protein names to check whether they are PI-binding proteins and if so, along with their PI-binding experimental evidence, possible lipid-binding domains, and cellular locations. Furthermore, users can browse YPIBP to retrieve eight protein lists, each of which contains the proteins which bind to a particular member of PIs, along with the enrichment analysis and PI-domain-protein network visualization. In summary, YPIBP could be a valuable resource for the scientific community to investigate functions of PIs through their binding proteins in yeast.

2. Materials and methods

2.1. The configuration of the YPIBP database

Fig. 1 illustrates the configuration of the YPIBP database. The web interface of YPIBP was developed in Python using the Django MTV framework. The 16 data sets (e.g., PI-binding proteins, protein domains, cellular components, human homologs, and protein–protein interaction) were deposited in MySQL. All the data sets are listed in Table S1. All tables were produced by JavaScript and feature-rich JavaScript libraries (jQuery and DataTables) to present data on the webpage. The graphics (i.e., network figures) were generated by vis.js (a browser-based graphic drawing library).

2.2. Collection of binding proteins of PIs from the literature

To collect PI-binding proteins, we used three kinds of data sources ranging from (i) high-throughput proteome microarray and lipid array studies, (ii) QuickGO [33] and (iii) literature mining from PubMed.

Firstly, we collected 8 sets of PI-binding yeast proteins that cover all eight members of PIs (i.e., PtdIns, PI3P, PI4P, PI5P, PI(3,4)P2, PI(4,5)P2, PI(3,5)P2, and PI(3,4,5)P3) from three proteome microarray studies. Zhu *et al.* [23] provided the binding protein lists for PI3P (51 proteins), PI4P (85 proteins), PI(3,4)P2 (71 proteins), PI(4,5)P2 (73 proteins), and PI(3,4,5)P3 (65 proteins), respectively. Lu *et al.* [24] provided 295 P(3,5)P2-binding proteins and 269 PtdIns-binding yeast proteins. Total 41 yeast PI5P-binding proteins were obtained from Herianto *et al.* [6]. Besides, Gallego *et al.* [21] used a lipid array to define the selected proteins' lipid-binding spectrum which covers PtdIns (32 proteins), PI3P (35 proteins), PI4P (48 proteins), PI(4,5)P2 (58 proteins), and PI(3,4,5)P3 (48 proteins), respectively.

Secondly, we collected PI-binding proteins using GO (Gene Ontology) terms in QuickGO [33]. QuickGO database is composed of SGD, GO_Central, InterPro, UniProt, Critical Assessment of Functional Annotation (CAFA) and Complex Portal entries. Here we used eight specific GO terms [phosphatidylinositol-binding (GO:0035091), phosphatidylinositol-3-phosphate binding (GO:0032266), phosphatidylinositol-4-phosphate binding (GO:0070273), phosphatidylinositol-5-phosphate binding (GO:0010314), phosphatidylinositol-3,4-bisphosphate binding (GO:0043325), phosphatidylinositol-3,5-bisphosphate binding (GO:0080025), phosphatidylinositol-4,5-bisphosphate binding

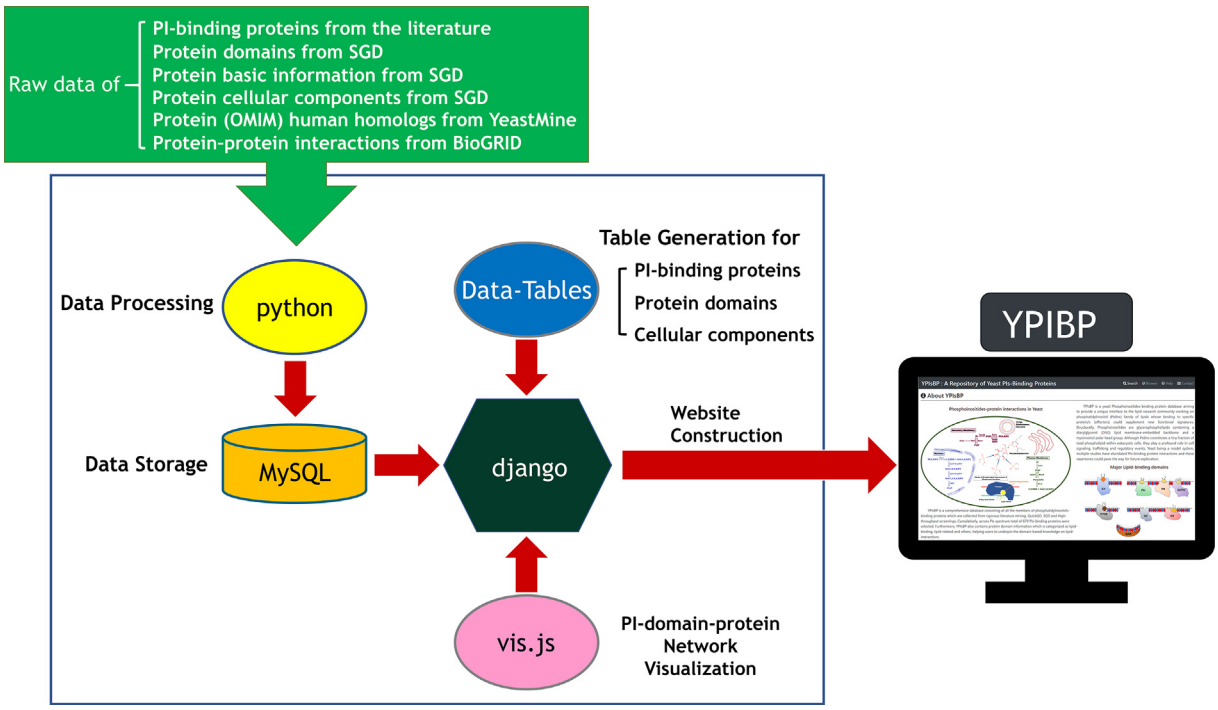

Fig. 1. The configuration of the YPIBP database.

(GO:0005546), phosphatidylinositol-3,4,5-trisphosphate binding (GO:0005547)] to obtain proteins defined with a particular phosphoinositide-binding annotation. A total of seven sets of PI-binding proteins were obtained except for GO:0005547. No proteins are annotated with the "phosphatidylinositol-3,4,5-trispho sphate binding" (GO:0005547) for the yeast taxon.

Thirdly, we collected research articles obtained by PubMed search engine using several keywords related to PI-binding (Table S2). The search results were obtained on April 8, 2020. A total of 869 research articles were collected (Table S2). After removing the redundancy, 203 papers were read and screened. Out of the 203 papers, 79 papers reported PI-binding proteins using traditional and high-throughput methodologies. For each study, the following information was collected: the PI-binding protein names, PI-binding spectrum, and experimental evidence (Table S3).

Table 1 provides the number of PI-binding proteins collected from different data sources. After removing the redundancy, we have collected a total of 679 PI-binding proteins, among which 457 have human homologs, including 181 OMIM human homologs (Table S4). The number of binding proteins of each member of PIs is as follows: PtdIns (240 proteins), PI3P (141 proteins), PI4P (169 proteins), PI5P (88 proteins), PI(3,4)P2 (107 proteins), PI(3,5)P2 (293 proteins), PI(4,5)P2 (162 proteins), and PI(3,4,5)P3 (123 proteins).

2.3. Collection of the protein domain data (181 lipid-binding domains and 103 lipid-related domains), cellular components, and protein–protein interaction data

A complete list of 13,878 yeast protein domains was obtained from the SGD [31] database which has the proteome-wide collection of the identified domains in yeast proteins. Twenty-three known lipid-binding domain types (Table S5) were collected from Chiapparino *et al.* [34], DiNitto *et al.* [35], and Stahelin [36]. Among the 13,878 domains, 181 domains were defined as lipid-binding domains (LBDs) because their domain descriptions mention the known lipid-binding domain types. For example, PF01363 was defined as a LBD because its domain description (FYVE zinc finger) mentions the known lipid-binding domain type (FYVE). The detailed descriptions of these 181 LBDs are provided in Table S6. In addition, to define lipid-related domains (LRDs), we first col-

Table 1
The number of PI-binding proteins deposited in YPIBP. Quick GO, high-throughput methods (proteome microarray and lipid array) and literature mining are the three data sources used to collect yeast PI-binding proteins.

Phosphoinositide Name	Number of PI-binding proteins from different data sources				Number of collective PI-binding proteins
	Quick GO	High-throughput Methods		Literature Mining	
		Proteome Microarray	Lipid Array		
PtdIns	29	175	32	11	240
PI3P	35	51	35	67	141
PI4P	18	85	48	53	169
PI5P	10	45	0	37	88
PI(3,4)P2	3	71	0	35	107
PI(3,5)P2	14	241	0	46	293
PI(4,5)P2	17	73	58	50	162
PI(3,4,5)P3	0	65	48	15	123
Jointly YPIBP contains 679 PI-binding yeast proteins					

lected several lipid-related terms (Table S7). Then a domain was defined as a lipid-related domain (LRD) if its domain description contains any of the lipid-related terms. A total of 103 LRDs were extracted from the list of 13,878 domains. A detailed characterization of these 103 lipid-related domains of yeast proteins is provided in Table S8. The rest of the domains (13594 domains) were categorized as "Others".

The GO cellular component information of each protein was collected from SGD [31] which consists of cell organelle location descriptions along with evidence codes and references. The protein–protein interaction data was collected from BioGRID [37] to support lipid-domain-protein network visualization of the input protein list.

2.4. Testing the enrichment of the binding proteins of a specific phosphoinositide in the input proteins

For the input proteins, YPIBP tests whether they are enriched with the proteins which bind to the specific phosphoinositide under study. The *p-value* is calculated using the hypergeometric test [28,38] as follows

$$p\_value = \sum_{x \geq K}^{\min(A,I)} \frac{\binom{A}{x}\binom{G-A}{I-x}}{\binom{G}{I}}$$

where G = 6705 is the number of yeast genes which have protein products, A is the number of yeast proteins which bind to the specific phosphoinositide of interest (e.g. A = 240 for PtdIns, A = 107 for PI(3,4)P2, etc.), I is the number of input proteins, and K is the number of input proteins which bind to the specific phosphoinositide under study.

The *p-value* is then corrected by the Bonferroni correction or the FDR (false discovery rate) to represent the true alpha level in the multiple hypotheses testing. Finally, the input proteins are said to be enriched with the binding proteins of the specific phosphoinositide if the corrected *p-value* is less than the user-defined threshold (e.g., 0.01). Note that Bonferroni correction and FDR are two statistical methods for multiple hypotheses correction. Bonferroni correction is more conservative than FDR. That is, Bonferroni correction has a smaller type I error rate, resulting in a smaller power than FDR does.

2.5. Testing the enrichment of the protein domains in the input proteins

For the input proteins, YPIBP tests whether they are enriched with any protein domains. The *p-value* is calculated using the hypergeometric test [28,38] as follows

$$p\_value = \sum_{x \geq K}^{\min(A,I)} \frac{\binom{A}{x}\binom{G-A}{I-x}}{\binom{G}{I}}$$

where G = 6705 is the number of yeast genes which have protein products, A is the number of yeast proteins which have the protein domain of interest (e.g., A = 8 for SM00273, A = 40 for G3DSA:2.30.29.30, etc.), I is the number of the input proteins, and K is the number of input proteins which have the protein domain of interest. The *p-value* is then corrected by the Bonferroni correction or the FDR to represent the true alpha level in the multiple hypotheses testing. Finally, the input proteins are said to be enriched with the domain of interest if the corrected *p-value* is less than the user-defined threshold.

3. Results

3.1. Search mode

YPIBP provides two types of search modes. In the first search mode, a user can input a single protein name (systematic, standard, or alias name) [e.g., Vps17] (Fig. 2a). Once submitted, YPIBP yields an output page of four sections. The first section shows the basic information such as systematic and standard names, aliases, description of the input protein and a link to SGD [39]. This section also shows the input protein's human homolog(s) [e.g., SNX11] and OMIM (Online Mendelian Inheritance in Man) human homolog(s) [e.g., SNX10] (Fig. 2b). In the second section, a user can see the PI-binding evidence of the input protein if it is recognized as one of the 679 PI-binding proteins deposited in YPIBP (Fig. 2c). In the third section, YPIBP provides the categorized (color-coded) domain information as lipid-binding domains (highlighted in yellow), lipid-related domains (highlighted in green) and any other domain(s) found in the input protein [e.g., 6 LBD and 5 other domains identified in Vps17] (Fig. 2d). Each domain is provided with its name, description, source of nomenclature, protein domain coordinates, InterPro entry (hyperlinked to InterPro) and InterPro entry description. A lipid-binding domain name is hyperlinked to a description explaining its connection to a well-known lipid-binding domain type with reference(s). In the fourth section, YPIBP provides the cellular component information which allows a user to identify specific organelles or compartments where the input protein could be located [e.g., endosome for Vps17] (Fig. 2e).

In the second search mode, a user can input a list of proteins (Fig. 3a) that will be checked by the PI-binding evidence collected in the YPIBP. Based on the binding evidence, the PI-binding proteins will be identified from the input list. The number of phosphoinositide-wise binding evidence is given for each PI-binding protein (Fig. 3b). Moreover, two kinds of enrichment analyses are performed for the input list. The first enrichment analysis tests the enrichment of the binding proteins of each member of PIs in the input proteins. The members of PIs whose binding proteins are enriched in the input protein list will be identified along with the enrichment analysis details (*p-value*, expected ratio, and observed ratio) (Fig. 4a). The second enrichment analysis tests the enrichment of protein domains in the input proteins. The enriched domains in the input proteins will be identified along with the enrichment analysis details (*p-value*, expected ratio, and observed ratio) (Fig. 4b). Finally, an interactive visualization of the PI-domain-protein network is provided. When a user selects the enriched members of PIs and enriched domains of interest, YPIBP will extract the input proteins which either bind the selected members of PIs or contain the selected domains. Then the interactive visualization of the PI-domain-protein network is generated for a user to navigate. To add more biological information, YPIBP also provides the protein–protein interaction information among the proteins in the network (Fig. 4c).

3.2. Browse mode

YPIBP provides two browse modes (Fig. 5a). In the first browse mode, a user can go through all 679 yeast phosphoinositide-binding proteins and their phosphoinositide-binding evidence (Fig. 5b). In the second browse mode, a user can see the number of binding proteins of each member of PIs (Fig. 5c). After clicking "See more" of a specific member of PIs (e.g., PtdIns), a user can see (i) the names of binding proteins and their binding evidence of the selected member, (ii) all the enriched PI-binding and enriched domains in the proteins which bind to that specific mem-

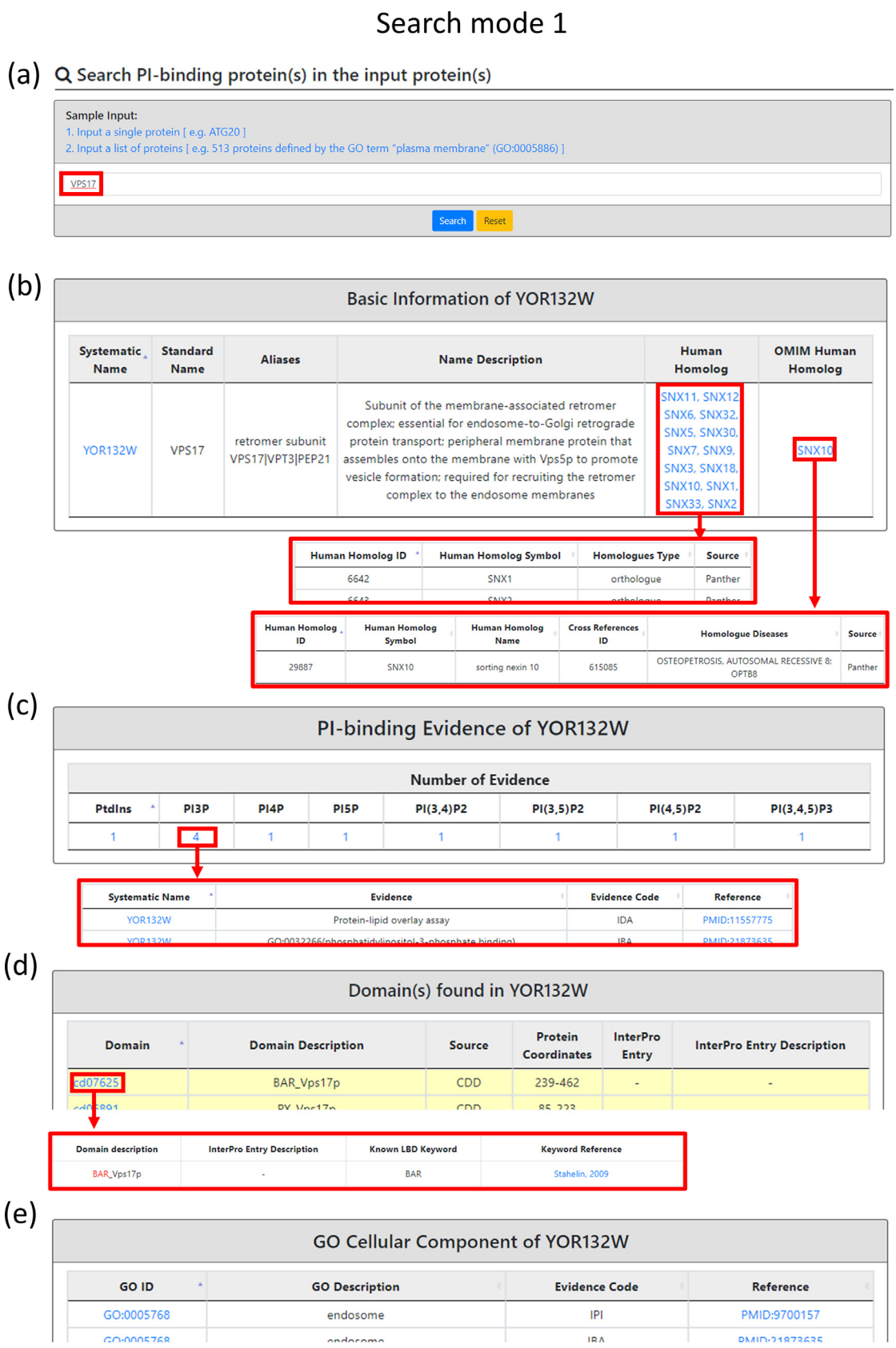

Fig. 2. The first search mode. (a) Input a single gene name VPS17. (b) The basic information of VPS17 shows that it has 14 human homologs and 1 OMIM human homolog. (c) VPS17's PI-binding evidence number and the evidence details. (d) Protein domains found in VPS17 along with lipid-binding domain descriptions (LBDs are highlighted with yellow background). (e) VPS17's GO cellular component details. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Search mode 2

(a) Search PI-binding protein(s) in the input protein(s)

Sample Input:
1. Input a single protein [e.g. ATG20]
2. Input a list of proteins [e.g. 513 proteins defined by the GO term "plasma membrane" (GO:0005886)]

YIL171W
YKR093W
YJR160C
YHR096C
YOL002C
YNL257C
YGR014W
YGL186C
YCL048W

Search Reset

(b) Search Result

84 out of 513 input proteins are PI-binding proteins

Show 10 entries Search: Download

Systematic Name	Standard Name	Number of Evidence							
		PtdIns	PI3P	PI4P	PI5P	PI(3,4)P2	PI(3,5)P2	PI(4,5)P2	PI(3,4,5)P3
YER125W	RSP5	1	1	1	2	1	1	1	1
YIL105C	SLM1	1	1	3	1	1	1	6	-
YNL047C	SLM2	1	1	1	1	1	1	3	-
YAL026C	DRS2	-	1	2	1	1	1	1	1
YPR165W	RHO1	-	1	1	1	1	1	1	1
YBL060W	YEL1	1	1	1	1	-	1	1	-
YDR153C	ENT5	1	1	1	-	-	2	1	1
YHR073W	OSH3	-	1	4	1	1	1	2	-
YBR129C	OPY1	-	1	3	1	2	1	3	-
YDL019C	OSH2	-	1	2	1	1	1	2	-

Showing 1 to 10 of 84 entries Previous 1 2 3 4 5 ... 9 Next

Systematic Name	Evidence	Evidence Code	Reference
YHR073W	Crystal structure analysis	ISS	PMID:23791945
YHR073W	Structural analysis	ISS	PMID:23823324
YHR073W	Lipid array	HDA	PMID:21119626
YHR073W	Protein lipid overlay assay	IDA	PMID:15023338

Fig. 3. The second search mode. (a) Input a list of 513 plasma membrane proteins defined by GO term (GO:0005886). (b) YPIBP identifies 84 (out of 513) input proteins are PI-binding proteins. The number "4" in the figure means that YHR073W have four experimental evidence showing that it is a PI4P-binding protein (Search output is continued in Fig. 4).

Fig. 4. The second search mode provides enrichment analysis and network visualization. (a) Phosphoinositide-binding enrichment analysis result. (b) Domain enrichment analysis result. (c) Visualization of the selected PI(4,5)P2-SM00273-protein interactome.

Fig. 5. Two browse modes. (a) Two browse modes are "Total 679 Phosphoinositide-binding Proteins" and "Browse by Phosphoinositide's Name". (b) The number of PI-binding evidence for each of 679 PI-binding proteins. (c) The number of binding proteins of each member of PIs. (d) The "See more" information of PtdIns including the names of all PtdIns-binding proteins, two enrichment analysis results, and the visualization of PI-domain-proteins interactome.

Fig. 6. The output results of querying ATG20. (a) ATG20 has a human homolog SNX4. (b) ATG20 could bind to all eight members of phosphoinositides. ATG20 shows the highest number of evidence for PI3P-binding. (c) ATG20 has 7 lipid-binding domains which belong to two important known lipid-binding domain types: Phox Homology (PX) and Bin-Amphiphysin-Rvs (BAR). (d) ATG20 is located in various membranes (pre-autophagosomal structure membranes, endosome membranes, and an extrinsic component of membranes).

Table 2
PI-binding enrichment analysis results for the 513 input proteins used in case study 2.

Lipid Name	Expected Ratio	Observed Ratio	P-value
PI(4,5)P2	162 / 6705 (2.42%)	42 / 513 (8.19%)	5.5e-12
PI4P	169 / 6705 (2.52%)	32 / 513 (6.24%)	5.2e-06
PI3P	141 / 6705 (2.10%)	28 / 513 (5.46%)	6.2e-06
PI(3,4)P2	107 / 6705 (1.60%)	20 / 513 (3.90%)	2.6e-04
PI5P	88 / 6705 (1.31%)	17 / 513 (3.31%)	4.5e-04
PI(3,5)P2	293 / 6705 (4.37%)	39 / 513 (7.60%)	5.3e-04

ber (e.g., 240 proteins that bind to PtdIns), (iii) the interactive visualization of the PI-domain-protein network (Fig. 5d).

4. Discussion

To demonstrate the utility of YPIBP, we provide three case studies showing that the search modes of YPIBP can output biologically meaningful PI-binding information for the protein(s) of interest.

4.1. The first case study

The first case demonstrates a scenario of a single protein name submission. Autophagy-related protein ATG20 is a pivotal member of the sorting nexin (SNX) family known to be essential for the cytoplasm-to-vacuole targeting (Cvt) pathway as well as the sorting process of endosomes and selective autophagy [40–42]. Searching ATG20 in YPIBP returns the following information. First, ATG20 has a human homolog SNX4 (Fig. 6a). Second, ATG20 could bind to all eight members of PIs (Fig. 6b). ATG20 shows the highest number of evidence for PI3P-binding. The PI3P-binding evidence comes from QuickGO (GO:0032266, phosphatidylinositol-3-phosphate binding), a membrane-binding study and a protein-lipid overlay assay (Fig. 6b). Third, ATG20 has 7 lipid-binding domains which belong to two important known lipid-binding domain types: Phox Homology (PX) and Bin-Amphiphysin-Rvs (BAR) (Fig. 6c). Both PX and BAR domains in the ATG20 have been shown to be involved in autophagy and membrane remodeling [41]. Fourth, GO cellular component annotations show that ATG20 is located in various membranes (pre-autophagosomal structure membranes, endosome membranes, and an extrinsic component of membranes) (Fig. 6d). This is supported by the study of Nice *et al.* [43] which demonstrated the requirement of ATG20 in the Cvt pathway, and suggested that the PX domain of ATG20 binds PI3P which is essential for membrane localization to the pre-autophagosomal structures.

4.2. The second case study

The second case study illustrates an analysis of a protein list submission. It is known that lipid-binding characteristics are profoundly connected to the ability of proteins' membrane translocation either being membrane-bound proteins or integral membrane proteins. Therefore, we used a yeast protein list (containing 513 proteins) defined by the GO term "plasma membrane" (GO:0005886) as the input protein list submitted to YPIBP. The functions of plasma membrane proteins are usually related to ion/-electron transport and cell signaling. Yeast plasma membrane is constituted by PIs (17.7%), PtdSer (33.6%), PtdEtn (20.3%), PtdCho (16.8%), PA (3.9%), CL (0.2%), and other lipids (6.9%) [44]. The plasma membrane-located PIs play a vital role in protein translocation and cell signaling [45–47]. Therefore, it is interesting to know which members of PIs interact with these 513 plasma membrane proteins. YPIBP identified 84 PI-binding proteins from the input list of 513 proteins (Table S9). The enrichment analysis with FDR correction (*p-value* cutoff 0.01) identified that the input proteins are enriched with the binding proteins of six members of PIs (PI3P, PI4P, PI5P, PI(3,4)P2, PI(3,5)P2, and PI(4,5)P2) (Table 2). The highest enrichment (*p-value* = 5.5e-12) was found for PI(4,5)P2. The enrichment results corroborated with the previous studies indicating that the binding proteins of PI(4,5)P2, PI4P and PI3P are usually located at plasma membrane, and are involved in signaling, trafficking, membrane identity and ion channel activities [48,49]. Additionally, enrichment analysis with FDR correction (*p-value* cutoff 0.01) identified that the input 513 membrane proteins are enriched with proteins having any of the five lipid-binding domains (4 PH domains and one ENTH domain) (Table 3) and five lipid-related domains (PF16209, PF16212, TIGR01652, PF01735, and SM00022) (Table S10). Corroborating our results, various plasma membrane proteins are known to utilize PH and ENTH domains to interact with phosphoinositide(s) for their anchoring to the plasma membrane and shaping its curvature [50–53]. Finally, using the PI-domain-protein network visualization in YPIBP, we found that the ENTH domain (SM000273) is most likely associated with PI(4,5)P2 in comparison to the other members of PIs (Fig. 7). Six out of seven input proteins with ENTH domain can bind PI(4,5)P2. ENT3 is the only exception (Fig. 7a). ENT3 contains an N-terminal epsin-like domain (SM00273) and is known to be associated with clathrin recruitment and trafficking events between the Golgi and endosomes [54]. Using YPIBP, we found that ENT3 has PI3P and PI(3,5)P2 binding evidence (Table S11) which corroborated its association with the endosomal membrane structures rather than plasma membrane [54,55]. It is worthy to further investigate why among the ENTH domain-containing proteins, ENT1 and ENT2 can bind PI(4,5)P2 but ENT3 cannot bind PI(4,5)P2. The reason could be due to the poorly conserved ENTH domain residues in ENT3 (compared to ENT1 and ENT2) which enable ENT3 for non-canonical PI3P and PI(3,5)P2-binding instead of conventional PI(4,5)P2-binding [54].

4.3. The third case study

The third case study illustrates the domain enrichment analysis of phosphoinositide-binding protein lists. In a total of eight lists (Fig. 5c), each list represents the binding proteins of a particular member of PIs. Fig. 8 summarized the enriched LBDs found in each list. The analysis results showed that three LBDs [ANTH, FYVE, and ENTH (SM00273)] are enriched in the binding proteins of a particular member of PIs, and five LBDs [ENTH (SSF48464), CRAL-TRIO, OSBP, PH, and PX] are enriched in the binding proteins of multiple members of PIs.

Table 3
Five lipid-binding domains (one ENTH domain and 4 PH domains) enriched in the input 513 plasma membrane proteins.

Enriched Lipid-Binding Domain	Domain Description	Source	InterPro Entry	InterPro Entry Description	P-value
SM00273	ENTH; ENTH domain	SMART	IPR013809	ENTH domain	3.7E-05
PF00169	PH domain	Pfam	IPR001849	Pleckstrin homology domain	3.5E-06
SM00233	PH; Pleckstrin homology domain	SMART	IPR001849	Pleckstrin homology domain	4.2E-05
G3DSA:2.30.29.30	PH domain-like	Gene3D	IPR011993	PH domain-like	2.0E-04
SSF50729	PH domain-like	SUPERFAMILY	IPR011993	PH domain-like	4.0E-03

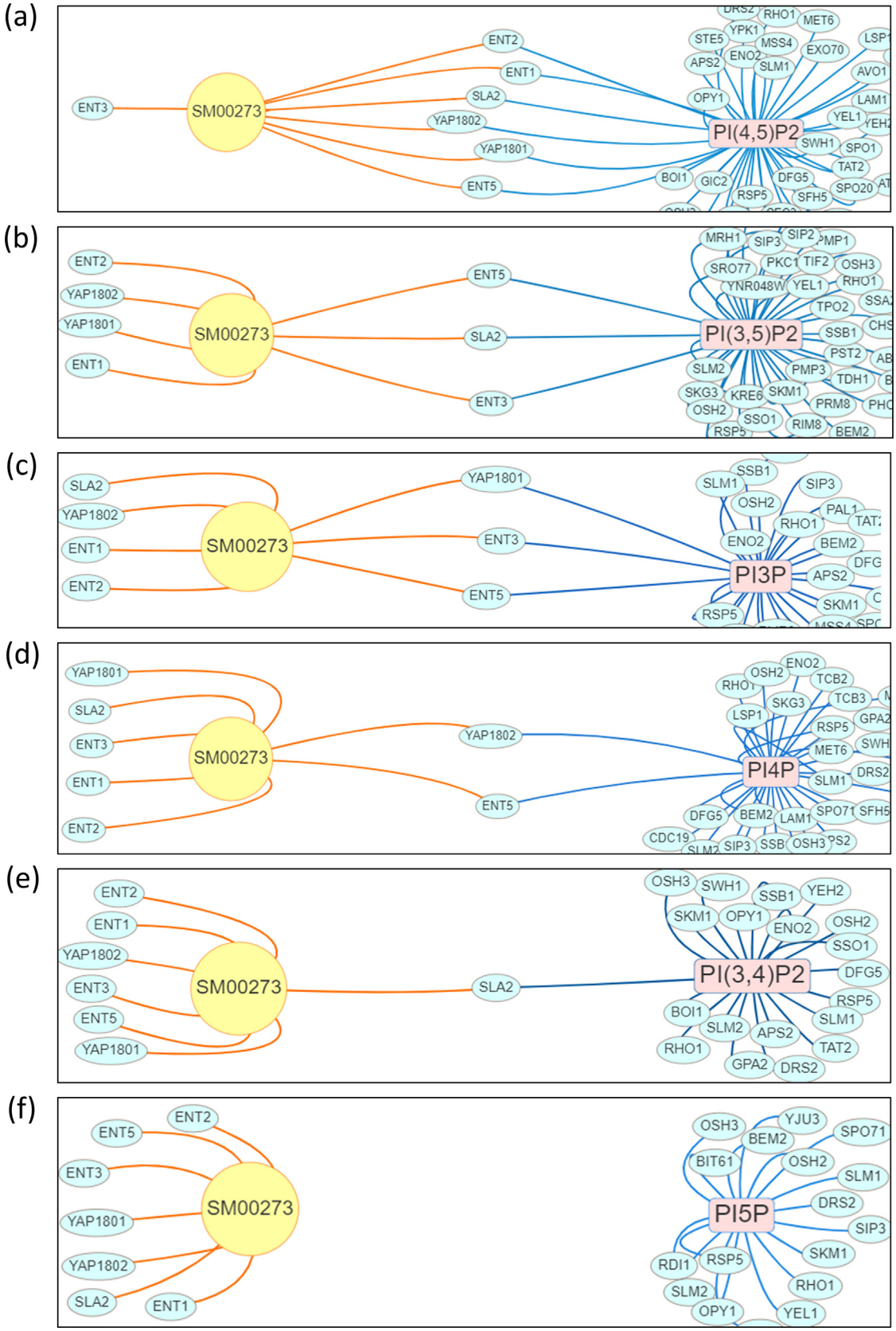

Fig. 7. PI-domain-protein network visualization. (a) PI(4,5)P2-SM000273-protein network. (b) PI(3,5)P2-SM000273-protein network. (c) PI3P-SM000273-protein network. (d) PI4P-SM000273-protein network. (e) PI(3,4)P2-SM000273-protein network. (f) PI5P-SM000273-protein network.

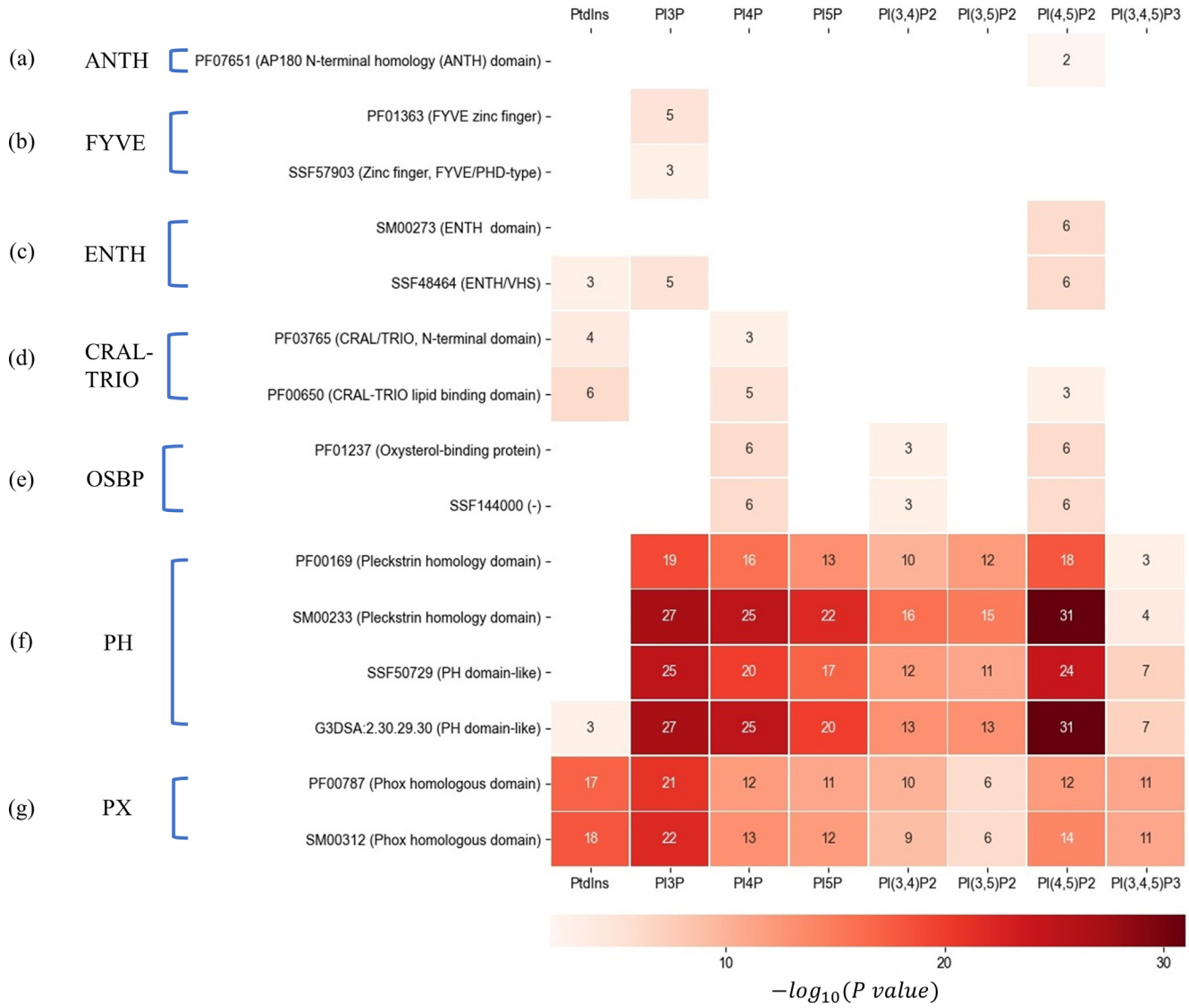

Fig. 8. The enriched LBDs found in the binding proteins of each of eight members of PIs The domain enrichment analysis shows that three LBDs [(a) ANTH, (b) FYVE, and (c) ENTH (SM00273)] are enriched in the binding proteins of a particular member of PIs. Five LBDs [(c) ENTH (SSF48464), (d) CRAL-TRIO, (e) OSBP, (f) PH, and (g) PX] are enriched in the binding proteins of multiple members of PIs.

The following gives the details of the three LBDs [ANTH, FYVE, and ENTH (SM00273)] that are enriched in the binding proteins of a particular member of PIs. First, ANTH domain (PF07651) is only enriched in PI(4,5)P2-binding proteins (Fig. 8a) supported by an existing paper showing that SLA2 specifically binds to PI(4,5)P2 through its ANTH domain [56]. Second, FYVE domain (PF01363 and SSF57903) is only enriched in PI3P-binding proteins (Fig. 8b) consistent with the knowledge that FYVE is a specific PI3P-binding domain [57–60]. Third, one type of ENTH domain (SM00273) is only enriched in PI(4,5)P2-binding proteins (Fig. 8c) supported by a recent study on ENTH domain showing that it is a sensitive reporter of PI(4,5)P2 [61].

The following gives the details of the five LBDs [ENTH domain (SSF48464), CRAL-TRIO, OSBP, PH, and PX) that are enriched in the binding proteins of multiple members of PIs. First, we found that another type of ENTH domain (SSF48464) is enriched in the binding proteins of PI(4,5)P2, PI3P, and PtdIns. The lack of PI(4,5)P2 binding specificity of SSF48464 may be explained by the variant of residues involved in lipid binding attribute [62]. Second, one type of CRAL-TRIO domain (PF03765) is enriched in the binding proteins of PtdIns and PI4P, while another type (PF00650) is additionally enriched in the binding proteins of PI(4,5)P2 (Fig. 8d). Gallego *et al.* [21] have experimentally confirmed that CRAL-TRIO domain can bind PtdIns and PI(3,4,5)P3. Moreover, a CRAL-TRIO domain-containing plant protein, patellin1 (PatL1), has been shown to bind to PI4P and PI(4,5)P2 [63]. Whether CRAL-TRIO domain can bind PI4P and PI(4,5)P2 in yeast is worth investigating further. Third, OSBP-domain (PF01237 and SSF144000) is enriched in the binding proteins of PI4P, PI(3,4)P2 and PI(4,5)P2 (Fig. 8e) consistent with its reported functions in a broad lipid-binding spectrum and its transport activity [64,65]. Fourth, three types of pH domain (PF00169, SM00233, and SSF50729) are enriched in the binding proteins of seven phosphorylated members of PIs. Finally, it is noteworthy that a special type of pH domain (G3DSA:2.30.29.30) and the PX domain (PF00787 and SM00312) are enriched in all eight members of PIs (Fig. 8f and Fig. 8g) consistent with their reported functions in a broad lipid-binding characteristics [66–69]. The possible reasons for non-specific binding of pH domain to all PIs could be its coincidence-sensing mechanism and lipid cooperativity between

various signaling lipids [70–72]. The lack of phosphoinositide-binding specificity of PX domain could be due to the multiple interaction mechanisms such as non-specific electrostatic and hydrophobic forces/interactions along with inositol headgroup recognition [73].

To investigate further, we retrieved all the 16 PX domain-containing proteins and 54 PH domain-containing proteins from YPIBP. The broad PI-binding spectrum could be seen in PX-containing proteins and PH-containing proteins (Table S12 and Table S13). All 16 PX domain-containing proteins have PI-binding evidence, and nine of them bind to all eight members of PIs (Fig. 9a). This could be explained by the studies on PX domain having canonical PI3P-binding and non-canonical diverse phospholipids-binding [68,69]. Moreover, PH domain-containing CLA4 was found to be a universal PI-binder (Table S13) supported by two studies showing that PH domains follow a broad PI-binding specificities to all seven phosphorylated members of PIs [66,67]. Further, it is noteworthy that out of 54 PH domain-containing yeast proteins, so far only 39 have been reported to be PI-binding proteins. The remaining 15 PH domain-containing yeast proteins could be candidates to decipher new phosphoinositide-protein interactions (Fig. 9b). Additionally, the cellular component analysis showed that 9 out of these 15 PH domain-containing proteins are associated with various membrane fractions of different cell organelles (Table S13). Therefore, these 9 PH domain-containing proteins are good candidates for experimentally testing

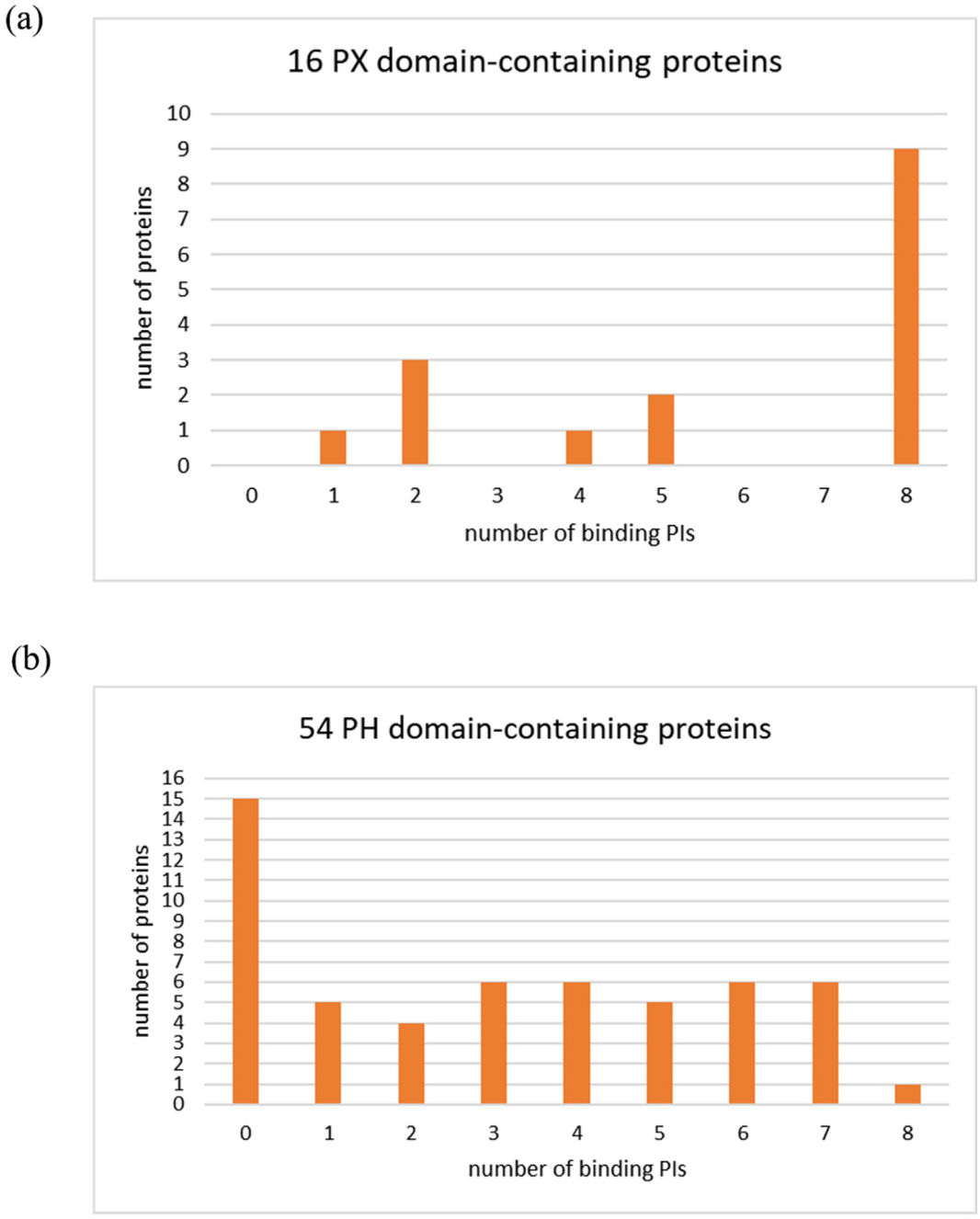

Fig. 9. The number of members of PIs that are bound by PX domain-containing proteins or PH domain-containing proteins. (a) All 16 PX domain-containing proteins are PI-binding proteins, and nine of them could bind all eight members of PIs. (b) Out of 54 PH domain-containing yeast proteins, so far only 39 are known PI-binding proteins. The remaining 15 PH domain-containing yeast proteins are good candidates for experimental testing their PI-binding capability.

their PI-binding capability. Ultimately, this signified the usability of YPIBP in systematic study of PI-protein interactions.

In summary, the three given case studies demonstrated the power of YPIBP for investigating phosphoinositide-domain-protein interactome. We anticipate that YPIBP will help lipid biologists specifically on PI-binding molecular dissection.

4.4. Comparison with existing lipid databases

Most of the existing lipidomics or lipid databases, such as LipidHome [74], LipidMatch [75] and LIPID MAPS Structure Database (LMSD) [76], provide information related to lipid classification, annotation, structural diversity, mass spectrometric identification as well as rudimentary published article mining. However, the knowledge from these conventional lipid databases is insufficient to explain the biological roles of lipids. Lipid-protein interactions are critical in functional characterization of lipids. Therefore, the knowledge of lipid-protein binding has been the core focus while constructing YPIBP. Only two existing databases provide lipid-associated proteins or lipid-binding proteins. They are LIPID MAPS Proteome Database (LMPD) [77] and SwissLipids [78]. However, both of them have their limitations to provide comprehensive yeast PI-binding proteins.

LMPD, a member of the LIPID MAPS consortium, contains lipid-associated proteins for multiple model organisms. The lipid-associated proteins were collected from UniProt IDs, KEGG and GO databases using broad lipid annotation terms defined by EntrezGene, ENZYME and other public resources [77]. LMPD possesses a total of 720 yeast lipid-associated proteins, among which only 16 are PI-related proteins. However, whether these 16 PI-related proteins can bind PIs is unknown. LMPD also provides protein domain information; however, whether these domains are related to lipid binding is unknown. On the contrary, YPIBP specifically provides a total of 679 yeast PI-binding proteins along with the PI-binding evidence, lipid-binding/lipid-related domains, and locations in the cell. Moreover, YPIBP provides enrichment analyses and PI-domain-protein interaction network visualization for a list of input proteins. All these useful enrichment analyses and network visualization cannot be found in LMPD.

The second database, SwissLipids [78], contains a vast diversity of lipid's information including structure, metabolism, and gene ontology. SwissLipids also provides lipid-protein binding information. However, this information is not comprehensive since it only has 8 PI-binding proteins in mouse and human (Table 4). Although fruitful PI-protein binding information in yeast is available, SwissLipids does not provide any yeast PI-binding proteins. On the contrary, YPIBP has 679 yeast PI-binding proteins along with lipid-binding/lipid-related domain information. For example, SwissLipids has no PtdIns-binding proteins, whereas YPIBP provides 240 yeast PtdIns-binding proteins. SwissLipids only provides less than 5 binding proteins for each member of PIs (Table 4) indicating that their collection is far from complete. It is noteworthy that YPIBP possess a comprehensive list of PI-binding proteins in yeast [PtdIns, 240 proteins; PI3P, 141 proteins; PI4P, 169 proteins; PI5P, 88 proteins; PI(3,4)P2, 107 proteins; PI(3,5)P2, 293 proteins; PI(4,5)P2, 162 proteins; PI(3,4,5)P3, 123 proteins] (Table 4).

Table 4
A comparison of the number of collected PI-binding proteins between SwissLipids and YPIBP.

Phosphoinositide	Number of collected PI-binding proteins in SwissLipids	Number of collected PI-binding proteins in YPIPB
PtdIns	None	240 yeast proteins
PI3P	0 yeast protein,3 human proteins (DDHD2, SEC23IP, SESTD1)	141 yeast proteins
PI4P	0 yeast protein,4 human proteins (DDHD2, OSBPL8, SEC23IP, SESTD1)	169 yeast proteins
PI5P	0 yeast protein,3 human proteins (DDHD2, SEC23IP, SESTD1)	88 yeast proteins
PI(3,4)P2	0 yeast protein,2 human proteins (SH3YL1, SESTD1)	107 yeast proteins
PI(3,5)P2	0 yeast protein,4 human proteins (CLVS1, NR5A2, SH3YL1, SESTD1),1 mouse protein (Nr5a1)	293 yeast proteins
PI(4,5)P2	0 yeast protein,2 human proteins (SH3YL1, SESTD1)	162 yeast proteins
PI(3,4,5)P3	0 yeast protein,2 human proteins (NR5A2, SH3YL1)	123 yeast proteins

5. Conclusion

Excellent genetic tractability and a plethora of ever-increasing experimental evidence-based knowledge make yeast (*S. cerevisiae*) a system of choice for understanding eukaryotic lipid biology. Based on the number of studies, yeast is the most popular model system to understand lipid-protein interactions. Here, we present the Yeast Phosphoinositide-Binding Proteins (YPIBP) database which is a repository of 679 PI-binding proteins obtained from QuickGO, high-throughput, and conventional methodologies as well as an extensive literature mining. To the best of our knowledge, YPIBP is the only database that contains comprehensive yeast PI-binding proteins along with various kinds of useful information (e.g., lipid-binding evidence, lipid-binding domains, and cellular components), enrichment analyses, and PI-domain-protein network visualization. Three case studies (a single protein ATG20, a list of 513 plasma membrane proteins, and a systematic protein domain enrichment study of PI-binding proteins) have been given to demonstrate that YPIBP can retrieve and visualize the PI-domain-protein interactome. Keeping YPIBP up to date, we will incorporate newly identified PI-binding proteins in yeast from all relevant sources. YPIBP will be maintained regularly; therefore, its long-term stability is guaranteed. We anticipate that YPIBP will expedite the research on phospholipid biology and disease pathophysiology.

CRediT authorship contribution statement

Jagat Rathod: Conceptualization, Investigation, Data curation, Project administration, Visualization, Writing - original draft, Writing - review & editing. **Han-Chen Yen:** Investigation, Software, Visualization. **Biqing Liang:** Writing - review & editing. **Yan-Yuan Tseng:** Conceptualization, Investigation, Supervision. **Chien-Sheng Chen:** Conceptualization, Investigation, Supervision, Writing - review & editing. **Wei-Sheng Wu:** Conceptualization, Investigation, Supervision, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

JR, YYT, WSW and CSC conceptualized the YPIBP. JR, HCY, WSW and CSC conceived the research topic. JR collected the PI-binding proteins as well as lipid-binding and lipid-related domain information from QuickGO, SGD and literature. HCY constructed the YPIBP website. HCY and JR prepared all the figures in the manuscript. JR and WSW wrote the first draft of the manuscript. JR, BL, CSC and WSW contributed to the final manuscript writing and editing. All authors read, edited, and approved the final manuscript.

Data availability

YPIBP provides a download page for a user to download all data at once. YPIBP is freely available at http://cosbi7.ee.ncku.edu.tw/YPIBP/. We also provide a backup site at http://cosbi4.ee.ncku.edu.tw/YPIBP/.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2021.06.035.

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