A High Efficiency Strategy for Binding Property Characterization of Peptide-binding Domains*

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A large proportion of protein-protein interactions is mediated by families of peptide-binding domains. Comprehensive characterization of each of these domains is critical for understanding the mechanisms and networks of protein interaction at the domain level. However, existing methods are all based on large scale screenings for each domain that are inefficient to deal with hundreds of members in major domain families. We developed a systematic strategy for efficient binding property characterization of peptide-binding domains based on high throughput validation screening of a specialized candidate ligand library using yeast two-hybrid mating array. Its outstanding feature is that the overall efficiency is dramatically improved compared with that of traditional screening, and it will be higher as the system cycles. PDZ domain family was first used to test the strategy. Five PDZ domains were rapidly characterized. Broader binding properties were identified compared with other methods, including novel recognition specificities that provided the basis for major revision of conventional PDZ classification. Several novel interactions were discovered, serving as significant clues for further functional investigation. This strategy can be easily extended to a variety of peptide-binding domains as a powerful tool for comprehensive analysis of domain binding property in proteomic scale. Molecular & Cellular Proteomics 5:1368–1381, 2006.

A substantial proportion of protein-protein interactions is mediated by families of protein interaction domains that recognize short peptide motifs in their binding partners, such as PDZ, SH3, WW, GYF, etc. (1). These peptide-binding domains are involved in a variety of functions such as subcellular localization, enzymatic activity, substrate specificity of regulatory proteins, and the assembly of multiprotein complexes (2, 3). Comprehensively investigating the binding properties of each peptide-binding domain family is critical for understanding the mechanisms and networks of protein interactions at the domain level (4, 5).

Several powerful methods, including oriented peptide library, SPOT synthesis, phage display, and yeast two-hybrid (Y2H) methods, have been successfully used for characterization of the peptide recognition specificities of individual domain families. Oriented peptide library approach was the initial foray into investigation of the consensus-binding sequences of domains (6, 7). The domain of interest is immobilized and incubated with soluble oriented peptides, and the adsorbed peptides are sequenced as a mixture to deconvolute the consensus motif in a statistical manner. This method is powerful for revealing preferences of the domain for certain amino acids at a given position; however, it can neither determine whether certain residues are forbidden at the particular position nor isolate actual sequences of binding peptides (6, 7). As an alternative strategy, oriented peptide array library integrates the oriented peptide library and array technologies (8). Hundreds of individual pools, each of them consisting of an oriented peptide library, are synthesized on solid supports, and the preferred amino acids at every position are read directly from arrays without protein sequencing. A disadvantage of this method is that the binding peptides are analyzed in a pool, making it impossible to obtain actual sequences of positive peptides and quantitatively compare affinities of defined peptides. SPOT synthesis is based on chemical synthesis of peptides on cellulose membranes and independent tests of the binding of these peptides to the domain of interest (9). In this approach, actual binding sequences can be obtained directly, but unexpected sequences will not be detected because synthesized sequences are dependent on a priori information, and the throughput is limited by the number of peptides that can be synthesized on a membrane of reasonable size in a realistic time (~10⁴ peptides) (10, 11). Phage display is a high throughput approach in which libraries of 10⁸–10¹⁰ random peptides can be displayed and screened (5, 12). High affinity ligands can be identified much more efficiently than can low affinity ones (13). All the above methods are in vitro and require purified protein(s) as well as artificial conditions for incubation and elution. As an in vivo approach,
Y2H assays are extensively used in a high throughput manner to find ligands for domains of interest (14–16). The advantages are that affinity competition can be avoided, transient interactions can be captured, and actual binding sequences can be isolated (14–16). However, cDNA libraries have traditionally been screened in Y2H assays with the result that low abundance ligands are easily overwhelmed by high abundance ligands (17). For comprehensive analysis of the binding properties for a certain domain, multiple libraries must be screened repetitively. Alternatively a random peptide library allows the identification of specific peptides to deduce the consensus-binding sequences in a single round of screening (18).

However, these methods are all based on large scale screenings for each bait domain that are relatively labor-intensive and time-consuming. Hundreds of domains have been predicted for each of the major domain families in the human proteome (smart.embl-heidelberg.de/), making it tremendously difficult to do individual large scale screening for each member of a domain family. Direct validation of preferred ligands from a special library that consists of highly diversified candidate ligands can dramatically improve the overall efficiency. It is very common in most domain families that one domain can bind many ligands (1) and that one ligand can be recognized by multiple domains (19, 20). In a given domain family, the ligands selected by some domain members are more likely to bind other members than unrelated random sequences. For more efficiently characterizing the binding properties of peptide-binding domains, here we present a systematic strategy based on high throughput validation screening of a specialized candidate ligand library using yeast two-hybrid mating array. The library was constructed mainly by collecting the ligand clones isolated from yeast two-hybrid screenings of random peptide libraries (RPy2Hs) with representative members of the selected domain family.

We first focused on one particular type of peptide-binding domain, PDZ (named after PSD-95/SAP90, DLG, and ZO-1) domain, and used it as an example for explanation and evaluation of the strategy. PDZ domain is one of the most abundant protein interaction modules and is involved in a variety of important cellular functions, such as intracellular routing of proteins, regulation of neurotransmitter transporter, and formation of multiprotein complexes (21, 22). Typically PDZ domain is specialized for binding the extreme carboxyl termini of its target proteins (7, 23). Based on the last four residues, PDZ binding motifs have been grouped conventionally into four classes: Class I, -(S/T)XpΨ; Class II, -φXpΨ; Class III, -(D/E/K/R)XpΨ; and Class IV, -ΨXp(D/E)Ψ where X is any amino acid, Ψ is a hydrophobic residue, p represents a charged residue, and * represents stop codons of peptide sequences (21). Some PDZ domains can create further functional diversities by other interaction modes, such as dimerization of PDZ-PDZ domains (24, 25) and recognition of internal sequences (26, 27).

MATERIALS AND METHODS

Preparation of Bait Plasmids—Yeast two-hybrid bait plasmids, human ZO-1 PDZ2-pMBa (Swiss-Prot accession number Q07157, amino acids 1–105) and PDZ3-pMBa (Swiss-Prot accession number Q07157, amino acids 401–500) plasmids, were kindly provided by Dr. Ben Giepmans (The Netherlands Cancer Institute), cDNA of Erbin PDZ domain (Swiss-Prot accession number Q96RT1, amino acids 1273–1371) was amplified by PCR with sense primer 5′-GAAGTTCGGCCAT- GAAGCTGAAACAAAG-3′ and antisense primer 5′-GAATTTCATT- GAGGAAAATCTCGTAC-3′ from human T cell cDNA library. The PCR product was digested with EcoRI and cloned into the EcoRI site of pGAL4 BD vector, pBridge, which carries Trp1 as a selection marker. cDNA of HtrA2 PDZ domain (Swiss-Prot accession number Q43464, amino acids 343–454) was obtained by PCR from human bone marrow cDNA library with the sense primer 5′-CCGGAGATCCCATGCT- GGGAGAAGAGAAAATT-3′ and antisense primer 5′-GGCGGATC- CAGGGTGCACATATAGGTCAG-3′. The PCR product was digested with BamHI and ligated into the BamHI site of pBridge. cDNA of LNX1 PDZ2 domain (Swiss-Prot accession number Q8TBB1, amino acids 371–473) was amplified by PCR with sense primer 5′-CCGGAGATC- GATGCTGCAACACCCCGAT-3′ and antisense primer 5′-CCGG- GATCCCCTACTGAAAGATGTCGGATCCGGCG-3′ using IMAGE 5164034 clone (Invitrogen) as template. The PCR product was digested with EcoRI and BamHI and cloned into GAL4 BD vector, pAS2-1, which carries Trp1 as a selection marker. All the constructed bait plasmids were confirmed by DNA sequencing.

Construction and Characterization of the Y2H Random Peptide Library—High diversity random peptide library was constructed by the improved methodology based on our previously described method (28). We choose pGADT7, which carries selection marker Leu2, as the GAL4 AD vector. We modified its reading frames as follows. 1) The reading frame at BamHI site was modified. Plasmid pGADT7 was digested by EcoRI, and the four protruding nucleotides at the 5′-end were made up by T4 DNA polymerase or cut by mung bean nuclease, and then the two blunt ends were ligated by T4 DNA ligase. Two new GAL4 AD vectors, pGADT7(+B) in which the reading frame at BamHI site was shifted one nucleotide backward and pGADT7(−B) in which the reading frame at BamHI site was shifted one nucleotide forward, were obtained. 2) The reading frame at EcoRI was modified. Oligonucleotides 5′-AAATGAATTCTGGG-3′ and 5′-AAATGACTTTG-3′ and antisense strand were synthesized. The fragment of double-stranded DNA was obtained by annealing the oligonucleotides and their respective antisense strands followed by subcloning into the EcoRI sites of pGADT7 individually. Another two new GAL4 AD vectors, pGADT7(+E) in which the reading frame in EcoRI site was shifted one nucleotide backward and pGADT7(−E) in which the reading frame in EcoRI site was shifted one nucleotide forward, were obtained.

Human genomic DNA was digested by Tsp509I overnight at 65 °C, and the fragments were cloned into the EcoRI site of pGADT7(+E) and pGADT7(−E), respectively. Human genomic DNA was digested by DpnII for 5 h at 37 °C, and the fragments were cloned into the BamHI site of pGADT7(+B) and pGADT7(−B), respectively. The same procedure was used with tobacco genomic DNA with the additional cloning into original pGADT7 vector. The constructed library plasmids were transformed into Escherichia coli DH10B, and the number of transformed clones was estimated before amplification. Finally we obtained 10 libraries (Supplemental Table 1). For characterization of the library, several single clones were selected randomly from each library, and the inserted fragments were analyzed by PCR and restriction enzyme digestion. A final random peptide library was obtained by pooling all 10 libraries.

Y2H Screening of Random Peptide Library—The GAL4 BD-PDZ fusion bait plasmid was transformed into the yeast strain CG1945
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using the lithium acetate protocol. The transformants were grown on SD/-Trp plates and spread on SD/-His-Trp plates for self-activation estimation. The transformants that had no background growth or had background growth but could be inhibited by 3-amino-1,2,4-triazole and were also negative for the LacZ assay were selected for the subsequent screening. The random peptide library was screened following the MATCHMAKER Two-Hybrid System protocol (Clontech). Approximate $10^7$ Trp’Leu’ transformants were selected on plates with SD/-His/-Leu’-Trp medium in the primary screening and then tested by the improved LacZ assay in the second screening. After rescue, the potential positive plasmids were isolated and re-transformed into the yeast strain CG1945 containing corresponding bait plasmid. Only the clones that were positive for all the reporter assays and confirmed by at least two independent tests were selected for specific interactions and sequenced.

Semiquantitative Binding Assay—The semiquantitative binding affinity of interaction between positive clone and corresponding PDZ bait was determined as β-galactosidase units using liquid culture β-galactosidase assay with o-nitrophenyl β-D-galactopyranoside (ONPG) as substrate. The assay protocol and the calculation of β-galactosidase units were based on the “MATCHMAKER Random Peptide Library User Manual” from Clontech. In brief, yeasts in appropriate selective medium were cultured overnight, and $A_{420}$ was recorded. Cells from 500 μl of culture were suspended in 100 μl of Z buffer and frozen in liquid nitrogen. Then 700 μl of Z buffer plus 0.27% β-mercaptoethanol and 160 μl of ONPG (4 mg/ml in Z buffer) were added. The reaction was terminated by adding 0.4 ml of 1 M Na$_2$CO$_3$ until yellow color developed. The incubation time and $A_{420}$ were recorded. Results were expressed as Miller units: β-galactosidase units = $1000 \times A_{420}/(A_{420} \times T)$ (incubation time in min) $\times V$ (volume of cell culture in ml). To reduce variability, three separate transformants were assayed, each in triplicate. Values represent the means ± S.D. of β-galactosidase units. The results were analyzed by the statistical software SPSS 11.0.

Construction of PDZ Ligand Library and High Throughput Yeast Mating Array—The PDZ ligand library was constructed by individually introducing all the non-redundant positive plasmids isolated from RPY2Hs, all the GAL4 AD-PDZ fusion plasmids, and other plasmids (as described under “Discussion”) into the yeast strain Y187 (RPY2Hs, all the GAL4 AD-PDZ fusion plasmids, and other plasmids introducing all the non-redundant positive plasmids isolated from the PDZ ligand library were constructed mainly by collecting all the PDZ binding clones isolated from all the RPY2Hs and all the PDZ domain clones (b) along with addition of known or potential PDZ binding clones such as the cDNA clones of PDZ2 ligand proteins and the synthesized clones of potential PDZ binding peptides (l). Step 3 is validation screening. A PDZ domain of interest was validated by validation screening of the PDZ ligand library using high throughput Y2H mating array (c). Both positive and negative binding sequences were read directly from arrays (d). Step 4 is supplemental RPY2H. Certain PDZ domains that did not interact with enough numbers of ligands from the ligand library were used as bait for de novo RPY2Hs in the traditional way. The selected clones were added to the PDZ ligand library to increase its diversity for subsequent studies of PDZ domains with similar preferences. Step 5 is characterization of binding properties. The precise binding properties of each PDZ were characterized by comparative analysis of both positive and negative sequences isolated from RPY2H and/or validation screening (f and g). Step 6 is prediction of candidate ligand proteins. The candidate ligand proteins were predicted by protein database searches with consensus-binding sequences followed by manually filtering with biological information such as subcellular localization and known functions (h). Step 7 is confirmation of potential interactions. The clones expressing carboxyl

RESULTS

The High Efficiency Strategy for Binding Property Characterization of PDZ Domain Family

The newly developed strategy involves the following seven steps as illustrated in Fig. 1A. Step 1 is RPY2H. Individual RPY2Hs with representative PDZ domains as bait were performed to isolate a series of positive PDZ binding clones (a). Step 2 is construction of the ligand library. A specialized PDZ ligand library was constructed mainly by collecting all the PDZ binding clones isolated from all the RPY2Hs and all the PDZ domain clones (b) along with addition of known or potential PDZ binding clones such as the cDNA clones of PDZ2 ligand proteins and the synthesized clones of potential PDZ binding peptides (l). Step 3 is validation screening. A PDZ domain of interest was validated by validation screening of the PDZ ligand library using high throughput Y2H mating array (c). Both positive and negative binding sequences were read directly from arrays (d). Step 4 is supplemental RPY2H. Certain PDZ domains that did not interact with enough numbers of ligands from the ligand library were used as bait for de novo RPY2Hs in the traditional way. The selected clones were added to the PDZ ligand library to increase its diversity for subsequent studies of PDZ domains with similar preferences. Step 5 is characterization of binding properties. The precise binding properties of each PDZ were characterized by comparative analysis of both positive and negative sequences isolated from RPY2H and/or validation screening (f and g). Step 6 is prediction of candidate ligand proteins. The candidate ligand proteins were predicted by protein database searches with consensus-binding sequences followed by manually filtering with biological information such as subcellular localization and known functions (h). Step 7 is confirmation of potential interactions. The clones expressing carboxyl
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termini of the predicted candidate PDZ ligand proteins were constructed individually by synthesis of oligonucleotides and cotransformed with corresponding PDZ bait in one-to-one Y2H assays to confirm the interactions (i and k). The synthesized clones were then added to the PDZ ligand library (l).

This strategy is notably different from other approaches in several features. First, the overall efficiency is dramatically improved by validation screening instead of traditional screening. It can be further improved as the candidate ligand library expands. Second, we can achieve high throughput with the yeast two-hybrid mating array approach. Multiple bait domains can be tested in parallel, and thousands of candidate ligands can be screened on arrays simultaneously. Third, because the library consists of ligands of known sequences, actual positive and negative binding sequences, which are both very important for precisely defining binding properties, can be directly read from arrays without resequencing. Fourth, the clones of PDZ domains are also included in the PDZ ligand library, enabling study of PDZ-PDZ domain interactions at the same time. Fifth, the ligands having the highest affinity and the highly specific ligands capable of binding to one particular target PDZ domain but not to others will be ultimately identified. These ligands can provide the basis for the synthesis of mimetics to be used as research tools or therapeutic agents (18, 32).

Investigation of PDZ Domains Using the Newly Developed Strategy

Construction of a PDZ Ligand Library by RPY2Hs with Representative PDZ Domains—We first constructed 10 random peptide libraries individually (Supplemental Table 1) by subcloning the digested genomic DNA fragments into the yeast two-hybrid AD vectors with different reading frames (28). Because PDZ domain typically recognizes four residues at the extreme carboxyl termini of their targets, 1.6 × 10^5 (20^9) independent random clones are necessary for studying its recognition specificity. Each library contained approximately 1 × 10^7 transformed clones and was sufficient for expressing all the possibilities of the carboxyl-terminal random four residues. Taking into account that more than four residues are possibly involved in PDZ recognitions, we generated a larger random peptide library by pooling the 10 libraries together. The final library contained 1.5 × 10^8 transformed clones and was sufficient to cover all the possibilities of the carboxyl-terminal random six amino acids (6.4 × 10^7, 20^6).

We chose three human PDZ domains, PDZ1 and PDZ3 domains of ZO-1 (Swiss-Prot accession number Q07157) and PDZ domain of Erbin (Swiss-Prot accession number Q96RT1), to screen the final random peptide library individually using the Y2H approach. The specificities of ZO-1 PDZ1 and PDZ3 have not been characterized by large scale screening as shown by a limited number of known target proteins, ZO-1 PDZ1 binds ligands that conform to Class I (33, 34), Class II (35, 36), and Class III (35) motifs, and PDZ3 binds ligands conforming to Class II motif (35). Erbin PDZ domain, as determined by phage display experiments, binds peptides that belong to Class I motif (37). We reasoned that the binding sequences selected by these three PDZ domains could cover the major classes of PDZ motifs.

From RPY2Hs, we isolated 42, 37, and 35 non-redundant positive binding clones for ZO-1 PDZ1, ZO-1 PDZ3, and Erbin PDZ, respectively (Supplemental Tables 2–4). All the specific clones were positive for both reporter genes HIS3 and LacZ and confirmed by three independent cotransformation experiments of the candidate plasmids with their corresponding bait.

Next we generated the initial PDZ ligand library by collecting all the positive PDZ binding clones isolated from RPY2Hs and all the PDZ domain clones in our laboratory. The library was composed of 143 non-redundant clones (Supplemental Table 12). To examine whether the linear ligands in the library were representative of potential PDZ binding sequences, the class and length distributions were analyzed (Fig. 1, B and C). Besides all the four classes of traditional ligands, notably some unclassified ligands with Cys or hydrophilic amino acid residues at the extreme carboxyl termini were included, enabling discovery of atypical PDZ motifs. In detail, the numbers of Class I, Class II, Class III, Class IV, and unclassified ligands in the library were 72 (56.25%), 35 (27.34%), 6 (4.70%), 1 (0.80%), and 14 (10.90%), respectively. It has been reported that the majority of PDZ motifs are Class I and Class II, and Class III and Class IV are the minority (7, 21). The distribution of these traditional classes of ligands in the library was consistent with the general preference of PDZ domains. The lengths of linear ligands in the library varied from six to 91 amino acid residues with most of them in the range of 6–20, longer than the four residues required for typical PDZ recognition. Both analyses indicated that this PDZ ligand library was suitable to be used in validation screenings.

High Throughput Validation Screening of PDZ Ligand Library Using Y2H Mating Array—We used the Y2H mating array approach for validation screening of the PDZ ligand library in which each bait PDZ was mated with every candidate ligand in a 96-well format. Positive interactions were identified first by the growth of colonies on selective medium followed by the appearance of blue stains in LacZ assays. From the mating array both positive and negative binding sequences could be directly read without resequencing. To assess the validation screening method, we first chose to study HtrA2 PDZ, which binds Class II motifs as determined by oriented peptide library (38). As a result, 38 positive binding sequences were rapidly identified (Supplemental Table 5). We also used ZO-1 PDZ1, ZO-1 PDZ3, and Erbin PDZ, which had been analyzed by RPY2H, to do validation screenings. Sixteen, zero, and three new positive sequences besides the ones found in RPY2H were discovered for each of them.
FIG. 1. A high efficiency strategy for binding property characterization of peptide-binding domains. A, the scheme of the strategy with focus on PDZ domain as an example. a, RPY2Hs with representative PDZ domains to isolate positive PDZ binding clones. b, construction of candidate ligand library mainly by collecting all the PDZ binding clones isolated from RPY2Hs and all the PDZ domain clones. c, study of PDZ domains by validation screening of PDZ ligand library using high throughput yeast two-hybrid mating array. d, identification of both positive and negative binding sequences by directly reading from arrays. e, supplemental RPY2H. For certain PDZ domains, if there are not enough positive sequences isolated for characterization of the binding properties, RPY2H is used to select positive clones. These clones will be added to PDZ ligand library to increase its diversity for subsequent studies of PDZ domains with similar preferences. f, identification of consensus-
respectively (Supplemental Tables 2–4). Negative sequences for all four PDZ domains were identified simultaneously (Supplemental Tables 2–4). Remarkably some negative sequences were quite similar to the positive ones. For example, -ETWV* was selected as positive by ZO-1 PDZ1 in RPY2H, whereas -DTWV* was identified as negative by mating array. Such a slight difference may present meaningful information to differentiate between binding and nonbinding sequences, suggesting that negative sequences are as important for precisely characterizing the PDZ binding properties as are the positive sequences.

New results were obtained for ZO-1 PDZ1, ZO-1 PDZ3, and Erbin PDZ from validation screenings, including negative and new positive sequences. They were useful complements to the RPY2H results. As the PDZ ligand library expands, each studied PDZ can be taken into a new cycle of validation screening for more comprehensive characterization of the binding properties.

Analysis of the Consensus-binding Sequences and Prediction of Candidate Ligand Proteins—We aligned the positive sequences for each PDZ. The results indicated strong consensus located at the carboxyl-terminal three to five amino acid residues (Supplemental Tables 2–5, shaded). Furthermore we comparatively analyzed both positive and negative sequences to deduce more precise consensus-binding sequences (Table I).

Next we searched Swiss-Prot or/and NR human database with the consensus-binding sequences to predict all the potential ligands in human proteome. For each PDZ studied, many native proteins were predicted to be its ligands, including the previously identified binding partners such as p0071, ß-catenin, and armadillo repeat protein deleted in velo-cardio-facial syndrome for Erbin PDZ (37, 39) as well as novel ones (Supplemental Tables 7–10). Then we used biological information such as subcellular localization and known functions to select the most promising candidate ligands (Table I).

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B

Class Distribution

Class I
Class II
Class III
Class IV
Others

Initial Library
Expanded Library

C

Length Distribution

Fig. 1—continued

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Confirmation of Candidate PDZ Interactions—We used one-to-one Y2H assay to confirm the potential interactions. The carboxyl termini of the most promising candidate ligand proteins were cloned and cotransformed into yeast cells with the corresponding PDZ bait. As shown in Table I, several novel ligand proteins were confirmed for each PDZ of interest. Then we added all the clones constructed here to the PDZ ligand library to expand its diversity.

A New Cycle: Investigation of LNX1 PDZ2 by Validation Screening of the Expanded PDZ Ligand Library—The initial PDZ ligand library was expanded by addition of synthesized clones of native proteins constructed for confirmations. By validation screening of the expanded library, we rapidly studied another PDZ domain, LNX1 PDZ2. Its binding properties have not been clarified, and it has been reported that it binds only one Class I ligand protein (40). Here we identified 83 positive clones (Supplemental Table 6), including 14 native protein clones (Table I). The direct isolation of native protein clones greatly improved the efficiency for discovering novel ligand proteins. We consequently deduced the consensus-binding sequences (Table I), predicted the potential native ligands (Supplemental Table 11), and confirmed the most promising interactions following the processes of the strategy. Finally we confirmed 10 more
novel ligand proteins selected from the protein database searches (Table I).

Characterization of the Binding Properties of PDZ Domain—
For each PDZ of interest, a series of positive and negative sequences were successfully identified from RPY2Hs and/or validation screenings of PDZ ligand library (Supplemental Tables 2–6). The occurrence of each amino acid type at positions of the positive sequences was calculated (Fig. 2, A, B, and C). A semiquantitative binding assay (ONPG) was used to assess the relative binding affinity of positive interactions (Supplemental Fig. 1, A and B).

The binding properties of ZO-1 PDZ1 and Erbin PDZ are similar. Both PDZ domains showed predominant preference for hydrophobic amino acids at the extreme carboxyl terminus (P0), especially Val (94.7%) for Erbin and Val (38.1%), Leu (38.1%), or Ile (14.3%) for ZO-1 PDZ1; whereas rarer Cys and Thr were also selected by PDZ1. At P-1, aromatic amino acids were overwhelmingly preferred, especially Trp (68.4%) for Erbin PDZ; however, fewer Asp and Glu were also tolerated by Erbin PDZ, and fewer Arg was tolerated by PDZ1. At P-2, Ser (23.8% for PDZ1; 23.7% for Erbin) and Thr (69.4% for PDZ1; 55.3% for Erbin) were the dominant selections, while acidic and hydrophobic residues such as Glu, Val, Ile, and Ala were also observed to a lesser extent. At P-3, for Erbin Glu (60.5%) and Asp (18.4%) were mostly selected, whereas for PDZ1 a more variable consensus was found, but Asp could not be tolerated (derived from negative sequences). At P-4, for Erbin PDZ, hydrophobic amino acids contributed to higher affinity than did other amino acids when the last four residues were the same (Supplemental Fig. 1B); for PDZ1, no specificity was observed beyond the four carboxyl-terminal residues.

ZO-1 PDZ3 showed unique properties quite different from ZO-1 PDZ1 and Erbin PDZ. At P0, hydrophobic amino acids, Val (29.7%), Leu (27%), Ile (13.5%), and Phe (13.5%), were preferred along with a rare occurrence of aromatic amino acids, Tyr and Trp, and hydrophilic amino acids, Lys, Asp, and Gin. At P-1, aromatic amino acids were predominant preferences, in particular Phe (48.6%) and Trp (43.2%). At P-2, no dominant preference was observed among the positive sequences, although there was a slight favor of hydrophobic residues over hydrophilic ones; however, the negative sequences revealed distinct intolerance for Ser and Thr. Notably PDZ3 showed specific preference for hydrophobic amino acid residues at P-3, especially Leu (56.8%), which is an unreported PDZ binding property.

HtrA2 PDZ recognized the Class I and Class III sequences besides the reported Class II sequences (38). Three consensus-binding sequences, -X(S/T)ΨΨΦ-*, -XΨΨΦΦ*, and -X(D/E)ΨΦΦ* (where X denotes any amino acids except the conditions described as follows) were identified. In contrast to traditional PDZ recognition specificities, HtrA2 PDZ exhibited restricted variability at P-3, namely the P-3 residue was selected according to the composition of the last three residues. Glu-3 was only present in the sequences of -E(T/V)(W/F)V*. (S/T)-3 were overwhelmingly preferred when hydrophobic or acidic residues were at P-2. Hydrophobic residues were selected with the consensus of -(S/T)HΦΦ*, and basic or large hydrophobic residues were necessary with -(S/T)YΦ*. Unique binding properties were observed at P0 of LNX1 PDZ2 for which two different kinds of residues, Val and Cys, were both predominantly preferred. When Cys was present at P0, the consensus sequence can be defined as -(S/T)XC*. While Val was at P0, aromatic amino acids, Trp and Phe, were the overwhelming preference at P-1, and no specificity was observed at P-2; the consensus sequence can be deduced as -(W/F)ΨΨΨ*. This suggested the traditional classification of PDZ
| PDZ       | Consensus sequence | Swiss-Prot accession no. | Carboxyl terminus | Candidate ligand                                              | Results |
|-----------|--------------------|--------------------------|-------------------|--------------------------------------------------------------|---------|
| ZO-1 PDZ1 | (S/T)Ψ(V/I/L/C)^*  | Q15760                   | SNPPNFTV          | Probable G protein-coupled receptor GPR19                   | ×       |
|           |                    | P56750                   | SKTSTSYV          | Claudin-17                                                  | ○       |
|           |                    | P32745                   | STMRISYL          | Somatostatin receptor type 3                                | ○       |
|           |                    | Q60229                   | GDFPSTYV          | Huntingtin-associated protein-interacting protein            | ○       |
|           |                    | Q9GZW8                   | KSSSRSWI          | Membrane-spanning 4-domains subfamily A member 7             | ×       |
|           |                    | Q9UL10                   | VHMFETFL          | Hypothetical protein KIAA1240                               | ×       |
|           |                    | Q86W26                   | ETPKNTYI          | NACH-, LRR- and PYD-containing protein 10                    | ○       |
|           | (D/E)ΨV^*          | Q95832                   | PSSGKDYV          | Claudin-1                                                    | □       |
|           |                    | O15551                   | GYDRKDYV          | Claudin-3                                                    | ×       |
|           |                    | O95471                   | SNSSKEYV          | Huntingtin-associated protein-interacting protein            | ○       |
|           |                    | Q92823-1                 | VEAQKSKV          | Vascular cell adhesion protein 1                            | ×       |
|           |                    | Q9UBN4                   | EDYVTTRL          | Short transient receptor potential channel 4                | ×       |
|           |                    | Q96J84                   | QGROMQTHV         | NEPH1 (kin of IRRE-like protein 1)                          | ×       |
| LNX1 PDZ2 | (S/T)(K/R/H)(V/I/L)^* | Q92599                   | ATWREGFL         | Vascular cell adhesion protein 1                            | ×       |
|           |                    | O43815                   | DALAKFV           | Striatin                                                     | ×       |
|           |                    | O14798                   | IVLLIVFV          | Tumor necrosis factor receptor superfamily member 10C       | ×       |
|           |                    | Q9YSU5                   | GRLGDLWV          | Tumor necrosis factor receptor superfamily member 18        | ○       |
|           |                    | Q86Y07                   | VFLALFFV          | Serine/threonine-protein kinase VRK2                         | ×       |
|           |                    | Q9H1J5                   | RWFQGYI           | Wnt-8a protein                                               | ×       |
|           |                    | Q8N6F1                   | SAAAREYV          | Claudin-19                                                   | X       |
|           |                    | Q96AC1                   | YKLTSGWV          | Pleckstrin homology domain-containing family C member 1; Mig-2 | ×       |
| Erbin PDZ | (D/E)(Q)(Ψ/S/T)(Ψ/W/F)^* | Q14232                   | DELIKLYL         | Translation initiation factor elf-2B α subunit              | X       |
|           |                    | Q99502                   | HALELEYL          | Eyes absent homolog 1                                         | ×       |
|           |                    | Q99504                   | LALEDFL          | Eyes absent homolog 3                                        | ×       |
|           |                    | Q12906-5                 | TAGYTGFV          | Interleukin enhancer binding factor 3 isofom c               | ×       |
|           |                    | Q8NHY3                   | PPEESWW           | GAS2-related protein isofom β                                | ○       |
|           |                    | P09619                   | AEAEDSFL         | PDGF-R-β                                                    | ×       |
|           |                    | O00141                   | APPTDSDL         | Serine/threonine-protein kinase Sgk1                         | X       |
|           |                    | O00192                   | PQPVDSWW         | Armadillo repeat protein deleted in velocardiofacial syndrome | ×       |
|           |                    | Q99966                   | TADFPSSC         | Cbp/p300-interacting transactivator 1                        | ○       |
|           |                    | Q9P2M7                   | SNLQTSSC         | Cingulin                                                     | X       |
|           |                    | Q99434                   | RLRVETD           | PDZ-binding kinase                                           | ×       |
|           |                    | Q99343                   | RLLVETD           | ARHGFEF16 protein                                            | ×       |
|           |                    | Q12906-5                 | TAGYTGFV          | Tumor necrosis factor receptor superfamily member 18        | ○       |
| HtrA2 PDZ | (D/E)(Ψ/V/I)LΨW*    | Q8TBB1                   | VSWPGFTL         | LNX1 protein                                                 | X       |
|           |                    | P46937                   | KESFLTWL         | Yes-associated protein                                       | ○       |
|           | (S/T)(Ψ/V/I)L^*     | P49815                   | VEDFTEVF         | Tuberin                                                     | ×       |
|           |                    | Q8N2R7                   | RARKSEW          | Connexin40.1                                                 | ×       |
|           | (S/T)(Ψ/V/I)L^*     | Q9Y4Z2                   | LAHLSDF           | Neurogenin 3                                                 | ×       |
|           | (Ψ/V/I)(Ψ/W/F)^*    | Q12906-5                 | TAGYTGFV          | Interleukin enhancer binding factor 3 isofom c               | ×       |
|           | (Ψ/V/I)L^*          | P43234                   | DSVSSIFV         | Cathepsin O                                                  | ○       |
|           | (Ψ/V/I)L^*          | Q99952                   | PPAEWTWRV        | Protein-tyrosine phosphatase, non-receptor type 18            | ○       |
|           | (Ψ/V/I)L^*          | P15498                   | EEDYSEYC         | Vav proto-oncogene                                           | X       |
|           | (Ψ/V/I)L^*          | P35961                   | CGGEFFYY         | Envelope glycoprotein (HIV)                                  | x       |
|           | (Ψ/V/I)L^*          | Q95835                   | KNRDLVYY         | WARTS protein kinase                                         | X       |
|           | (Ψ/V/I)L^*          | P49768                   | LAFHOFYI         | Presenilin-1 (PS1)                                           | X       |
|           | (Ψ/V/I)L^*          | Q96418                   | GLLPHSSC         | Tyrosine-protein kinase receptor TYRO3                       | □       |
|           | (Ψ/V/I)L^*          | Q99666                   | TADFPSSC         | Cbp/p300-interacting transactivator 1                        | ○       |
|           | (Ψ/V/I)L^*          | Q9P2M7                   | SNLQTSSC         | Cingulin                                                     | X       |
|           | (Ψ/V/I)L^*          | P43119                   | ASVACSLC         | Prostacyclin receptor                                         | □       |
domain was not suitable for description of the binding motifs of LNX1 PDZ2. In addition, when Thr was present at P domain was not suitable for description of the binding motifs

ample was the discovery of several novel interactions for the PDZ2 of LNX1, the first described PDZ domain-containing member of the E3 ubiquitin ligase family (47), which may provide important clues for further clarifying the roles of LNX1 in regulation of protein ubiquitin modification.

**DISCUSSION**

We developed a systematic strategy for characterization of the binding properties of PDZ domains based on high throughput validation screening of a specialized PDZ ligand library. The key of this integrated system is the PDZ ligand library. Besides collecting the binding clones isolated from RPY2Hs and predicted ligand clones constructed for confirmation, the library can also be further expanded by addition of other clones of other known or potential PDZ ligands. Up to the submission of this manuscript, our PDZ ligand library had been expanded to a total of 257 non-redundant PDZ ligands (Supplemental Table 12). Both class and length distributions of linear ligands in the library were analyzed (Fig. 1, B and C). The current library consists of four kinds of ligands, including PDZ binding peptides, carboxyl termini of native proteins, full-length native PDZ ligand proteins, and PDZ domains. The former three kinds are appropriate for studying the carboxyl-terminal binding properties of PDZ, and the last one enables study of the dimerization of PDZ domains. The native proteins in the library enable direct discovery of novel PDZ ligand proteins from a single round of validation screening. However, the internal PDZ binding sequences are not covered so far.

The high efficiency of the strategy is accounted for by the following features. 1) It is time-saving. 1 month at least is required for one cycle of traditional Y2H screening, whereas

**TABLE I—continued**

| PDZ Consensus sequence | Swiss-Prot accession no. | Carboxyl terminus | Candidate ligand | Protein description | Results |
|------------------------|--------------------------|-------------------|-----------------|---------------------|--------|
| DSWV*                  | Q9Y345                   | DLEEGSTQC         | Sodium- and chloride-dependent glycine transporter 2    |
|                        | P57058                   | ADGVTQC           | Hormonally up-regulated neu tumor-associated kinase     |
|                        | P48546                   | SRELEYC           | Gastric inhibitory polypeptide receptor                 |
|                        | O00192                   | POPVDSSWV         | Armadillo repeat protein deleted in velocardiofacial syndrome |
| (S/T/E)(W/Y/F)V*       | Q9U9Q3                   | PASPDSSWV         | Catenin-6-2                                             |
|                        | Q99569-2                 | PGSPPSWW          | p0071 (plakophilin-4)                                    |
|                        | PS6750                   | SKTSTSYY          | Claudin-16                                              |
|                        | O06229                   | GDPSTFYV          | Huntington-associated protein-interacting protein       |
|                        | Q8NH93                   | PEEESWV           | GAS2-related protein isoform β                           |
|                        | P49815                   | VEDFTFV           | Tuberin                                                  |
|                        | Q8NZR7                   | RARKSEWV          | Connexin40.1                                             |
| (G/I/L)(W/F)V*         | Q12906-5                 | TAYTGFV           | Interleukin enhancer binding factor 3 isoform c          |
|                        | P43234                   | DSVSSIFV          | Cathepsin O                                              |
|                        | Q9Y5U5                   | GRLGDLLW          | Tumor necrosis factor receptor superfamily member 18    |
| (V/F)(F/Y)(V/I/L)*     | Q86Y70                   | VFLALFFL          | Serine/threonine-protein kinase VRK2                     |
|                        | Q9H1JS                   | RWFGVYI           | Wnt-Ba protein                                            |
|                        | O43815                   | DALAKVVF          | Striatin                                                  |
| ET(D/E)V*              | P15385                   | AKADVTEV          | Potassium channel Kv1.4                                  |
|                        | Q99434                   | RLRVTEV           | ARHGGEF16 protein                                        |
|                        | P03409                   | KFHRTEV           | TAX_HTLY-1                                                |

* The ligand proteins identified directly from the validation screening of the expanded PDZ ligand library.
only 1 week is sufficient for one validation screening. 2) It is labor-saving. Current procedures of traditional screening are tedious, including large scale library transformation, isolation of candidate plasmids, cotransformation, and sequencing of positive clones, whereas only one step of mating assay is required for validation screening. Therefore, high throughput can be achieved. 3) There is a high success rate of prediction. By considering the negative binding sequences, more precise consensus-binding sequences can be deduced, thereby enhancing the success of prediction. Taking HtrA2 PDZ as an example, the comparative analysis of all the positive and negative sequences uncovered the unique preference of P<sup>−3</sup>. As a result, seven of 10 predicted candidate ligand proteins were confirmed.

The confidence of our results can be justified in several aspects. First, clear consensus-binding sequences are present at the carboxyl termini of positive sequences selected by each PDZ of interest. Second, 10 of 20 reported PDZ ligand proteins were recapitulated in our system (50%); this is much higher than currently expected overlap rates between data

**Fig. 3** Protein interaction map mediated by the PDZ domains of interest. A protein interaction map including the five PDZ domains of interest and all 50 interactions identified in this work including 40 novel interactions and 10 known interactions is shown. Colored dots represent the different biology processes according to HPRD annotation. Black lines represent novel interactions. Red lines represent the known interactions that were reconfirmed in this work. The small map at the bottom left represents an example of the subnetwork bordered by the shortest path length between protein pairs of novel interaction (between LNX1 and Cingulin); see Supplemental Fig. 2K. The shortest path length is defined as the minimum number of edges that is necessary to traverse from one node to the other; this gives a measure of how closely nodes are connected within the network.
sets (15, 16). Third, each protein pair of 32 interactions identified here can be mapped into a small network of protein interactions extracted from the HPRD reference data, suggesting that the two binding partners are biological relevant (41, 42). Links for the remaining 18 interactions were not found in the HPRD network probably because the current HPRD interaction data are far from completed.

The binding properties of five PDZ domains were characterized in this study. All of the recognition specificities previously determined by large scale approaches were confirmed, but also some novel binding properties were discovered. The specificity of Erbin PDZ had previously been studied by phage-displayed random peptide library, and a carboxyl-terminal -(D/E)(S/T)W^V^ binding motif was identified (37). In this study, we identified more diverse consensus-binding sequences and observed a novel phenomenon that Asp and Glu could be selected at P^−^ in addition to Trp. The preferred binding sequences of HtrA2 PDZ have been determined previously by the oriented peptide library approach, and the Class II motif has been identified to be the most preferred (38). We found that HtrA2 PDZ also permitted Class I and Class III ligands. The broader binding properties characterized can probably be attributed to effectively avoiding both affinity competition and abundance suppression in our system. ZO-1 PDZ1 and PDZ3 had not been studied by large scale screening before. Several proteins, including Claudins (35), Connexin36 (36), NEPH1 (33), and TRPC4 (34), have been reported to bind ZO-1 PDZ1 through their carboxyl termini. Here all the reported native ligand proteins were retrieved except the Claudins with the sequences of -KNYV^ and -SQYV^*. For ZO-1 PDZ3, only one interactor, junction adhesion molecule, has been reported (48). However, the interaction was not confirmed in our system, even in one-to-one Y2H assay between PDZ3 and the carboxyl terminus of JAM. The reasons are not clear. Noticeably the reported ligand proteins we failed to identify carry carboxyl termini that were not consistent with the consensus-binding sequences. For LNX1 PDZ2, the current study is the first to comprehensively describe the binding properties of this domain. The results pave the way for functional characterization of LNX1 protein.

To date, PDZ domains have been conventionally grouped into four classes based on binding motifs (21). However, this simple classification may not be sufficient in differentiating all PDZ domains. For example, ZO-1 PDZ3 exhibited a unique characterization for specific selections at P^−^5 where hydrophobic amino acids, in particular Leu, were predominantly preferred; yet P^−^5 was not considered to contribute to PDZ recognition specificity before. The predominant preference of aromatic amino acids Trp, Phe, and Tyr at P^−^1 for all five PDZ domains studied was observed, whereas previously P^−^1 was not considered conserved and was regarded of only minor importance for PDZ binding specificity (49). At P^5^, besides typical hydrophobic amino acids, Cys was significantly selected by LNX1 PDZ2, and other polar or hydrophilic residues such as Lys, Asn, and Thr were also selected by different PDZ domains. In addition, all the PDZ domains of interest bind more than one conventional class of ligands as well as unclassified PDZ ligands. As increasing novel types of PDZ-ligand interactions are reported (50, 51), the traditional classification may need major revision.

We demonstrated the feasibility of the newly developed strategy by using it to investigate the PDZ domain interactions. It can be easily extended to a variety of domain families that recognize short peptides as long as an individual member in a certain family can engage different ligands and one ligand can be recognized by different domain members. A subset of peptide-binding domains are adoptable, including SH3 domain that recognizes PXXX motif (52, 53), WW domain that binds PXY motif (54), GYF domain that favors PPG(F/I/L/M/V) motif (3), EVH1 domain that prefers FPPPP(D/E) motif (55), and VPS-27, Hrs, and STAM domain that selects (D/E)XXL motif (56). For the domain families that recognize modified peptides, such as SH2 and phosphotyrosine-binding that bind phosphorylated tyrosine (67), the strategy is incompetent due to the lack of corresponding post-translational modifications in Y2H system. This limitation may be overcome by applying the yeast three-hybrid system in which an exogenous kinase can be used to phosphorylate the Tyr residues on the ligands.

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REFERENCES

1. Pawson, T., and Nash, P. (2003) Assembly of cell regulatory systems through protein interaction domains. Science 300, 445–452
2. Gmeiner, W. H., and Horita, D. A. (2001) Implications of SH3 domain structure and dynamics for protein regulation and drug design. Cell. Biochem. Biophys. 35, 127–140
3. Kofler, M., Motzny, K., and Freund, C. (2005) GYF domain proteomics reveals interaction sites in known and novel target proteins. Mol. Cell. Proteomics 4, 1797–1811
4. Deng, M., Mehta, S., Sun, F., and Chen, T. (2002) Inferring domain-domain interactions from protein-protein interactions. Genome Res. 12, 1540–1548
5. Landgraf, C., Panni, S., Montecchi-Palazzi, L., Castagnoli, L., Schneider-Mergener, J., Volkmer-Engert, R., and Cesareni, G. (2004) Protein inter-
Characterization of Domains by Novel Screening Method

9. Nourry, C., Grant, S. G., and Borg, J. P. (2003) PDZ domain proteins: plug and play! Sci. STKE 2003, RE7

25. Wong, H. C., Bourdelas, A., Krauss, A., Lee, H. J., Shao, Y., Wu, D., Mlodzik, M., Shi, D. L., and Zheng, J. (2003) Direct binding of the PDZ domain of Dishevelled to a conserved internal sequence in the C-terminal region of Frizzled. Mol. Cell 12, 1251–1260

24. Huang, H., and Gao, Y. (2003) A method for generation of arbitrary peptide libraries using genomic DNA. Mol. Biotechnol. 30, 135–142

23. Huang, H. M., Zhang, L., Cui, Q. H., Jiang, T. Z., Ma, S. C., and Gao, Y. H. (2004) Finding potential ligands for PDZ domains by tailiff, a JAVA program. Chin. Med. Sci. J. 19, 97–104

22. Yu, H., Zhu, X., Greenbaum, D., Karro, J., and Gerstein, M. (2004) TopNet: a tool for comparing biological sub-networks, correlating protein properties with topological statistics. Nucleic Acids Res. 32, 328–337

21. Boisguerin, P., Leben, R., Ay, B., Radziwill, G., Moelling, K., Dong, L., and Boisguerin, P., Leben, R., Ay, B., Radziwill, G., Moelling, K., Dong, L., and Vidal, M. (2005) Towards a proteome-scale map of the human protein-protein interaction network. J. Biol. Chem. 278, 3947–3947

20. Rousset, R., Fabre, S., Desbois, C., Bantignies, F., and Jalinot, P. (1998) Rousset, R., Fabre, S., Desbois, C., Bantignies, F., and Jalinot, P. (1998) Making protein interactions druggable: targeting PDZ domain networks. Nat. Rev. Drug Discov. 3, 1047–1056

19. Huber, T. B., Schmids, M., Gerke, P., Schermer, B., Zahn, A., Hartleben, B., Sellin, L., Walz, G., and Benzing, T. (2003) The carboxyl terminus of Neph family members binds to the PDZ domain protein zonula occludens-1. J. Biol. Chem. 278, 13417–13421

18. Song, X., Mao, Y., Narciscu, R., Hoss, K. J., Press, Y., Lee, S., and Brossnan, C. F. (2005) Canonical transient receptor potential channel 4 (TRPC4) co-localizes with the scaffolding protein ZO-1 in human fetal astrocytes in culture. Oiga 49, 418–429

17. Barabasi, A. L., and Oltvai, Z. N. (2004) Network biology: understanding the cell as a network of networks. Nat. Rev. Drug Discov. 3, 101–113

16. Stelzl, U., Worm, U., Lalowski, M., Haenig, C., Brembeck, F. H., Goehler, H.,莫尔尼, F., Smuckler, E., Koeppen, S., Timm, J., Mintzlaff, S., Abraham, C., Bock, N., Kietzmann, S., Goede, A., Toksoz, E., Droegge, A., Korbtsch, K., Korn, B., Birmingham, W., Lehrach, H., and Wanker, E. E. (2005) A human protein-protein interaction network. Cell 122, 997–998

15. Izawa, I., Nishizawa, M., Tomono, Y., Ohtakara, K., Takahashi, T., and Inagaki, M. (2002) ERBB2 associates with p0071, an armadillo protein, at cell-cell junctions of epithelial cells. Genes Cells 7, 495–485

14. Barabasi, A. L., and Oltvai, Z. N. (2004) Network biology: understanding the cell as a network of networks. Nat. Rev. Drug Discov. 3, 101–113

13. Vaccaro, P., Brannetti, B., Montecchi-Palazzi, L., Philipp, S., Helmer Citric, M., Cesareni, G., and Dente, L. (2001) Distinct binding specificity of domains by novel screening method. J. Biol. Chem. 276, 42122–42130

12. TopNet: a tool for comparing biological sub-networks, correlating protein properties with topological statistics. Nucleic Acids Res. 32, 328–337

11. Weiser, A. A., Or-Guil, M., Tapia, V., Leichsenring, A., Schuchhardt, J., Weiser, A. A., Or-Guil, M., Tapia, V., Leichsenring, A., Schuchhardt, J., Boisguerin, P., Leben, R., Ay, B., Radziwill, G., Moelling, K., Dong, L., and Vidal, M. (2005) Towards a proteome-scale map of the human protein-protein interaction network. Nature 437, 1173–1178

10. Boisguerin, P., Leben, R., Ay, B., Radziwill, G., Moelling, K., Dong, L., and Boisguerin, P., Leben, R., Ay, B., Radziwill, G., Moelling, K., Dong, L., and Vidal, M. (2005) Towards a proteome-scale map of the human protein-protein interaction network. J. Biol. Chem. 278, 3947–3947

9. Nourry, C., Grant, S. G., and Borg, J. P. (2003) PDZ domain proteins: plug and play! Sci. STKE 2003, RE7

8. Schlieker, C., Mogk, A., and Bukau, B. (2004) A PDZ switch for a cellular function. J. Biol. Chem. 279, 48099–48104

7. TopNet: a tool for comparing biological sub-networks, correlating protein properties with topological statistics. Nucleic Acids Res. 32, 328–337

6. Rousset, R., Fabre, S., Desbois, C., Bantignies, F., and Jalilov, P. (1998) The C-terminus of the HTLV-I Tax oncoprotein mediates interaction with the PDZ domain of cellular proteins. Oncogene 16, 643–653

5. Rousset, R., Fabre, S., Desbois, C., Bantignies, F., and Jalilov, P. (1998) The C-terminus of the HTLV-I Tax oncoprotein mediates interaction with the PDZ domain of cellular proteins. Oncogene 16, 643–653

4. Rousset, R., Fabre, S., Desbois, C., Bantignies, F., and Jalilov, P. (1998) The C-terminus of the HTLV-I Tax oncoprotein mediates interaction with the PDZ domain of cellular proteins. Oncogene 16, 643–653

3. Rousset, R., Fabre, S., Desbois, C., Bantignies, F., and Jalilov, P. (1998) The C-terminus of the HTLV-I Tax oncoprotein mediates interaction with the PDZ domain of cellular proteins. Oncogene 16, 643–653

2. Rousset, R., Fabre, S., Desbois, C., Bantignies, F., and Jalilov, P. (1998) The C-terminus of the HTLV-I Tax oncoprotein mediates interaction with the PDZ domain of cellular proteins. Oncogene 16, 643–653

1. Rousset, R., Fabre, S., Desbois, C., Bantignies, F., and Jalilov, P. (1998) The C-terminus of the HTLV-I Tax oncoprotein mediates interaction with the PDZ domain of cellular proteins. Oncogene 16, 643–653
A comprehensive two-hybrid analysis to explore the yeast protein interactome. Proc. Natl. Acad. Sci. U. S. A. 98, 4569–4574

49. Schultz, J., Hoffmuller, U., Krause, G., Ashurst, J., Macias, M. J., Schmieder, P., Schneider-Mergener, J., and Oschkinat, H. (1998) Specific interactions between the syntrophin PDZ domain and voltage-gated sodium channels. Nat. Struct. Biol. 5, 19–24

50. Bezprozvanny, I., and Maximov, A. (2001) Classification of PDZ domains. FEBS Lett. 509, 457–462

51. Schneider, S., Buchert, M., Georgiev, O., Catimel, B., Halford, M., Stacker, S. A., Baechi, T., Moelling, K., and Hovens, C. M. (1999) Mutagenesis and selection of PDZ domains that bind new protein targets. Nat. Biotechnol. 17, 170–175

52. Feng, S., Kasahara, C., Rickles, R. J., and Schreiber, S. L. (1995) Specific interactions outside the proline-rich core of two classes of Src homology 3 ligands. Proc. Natl. Acad. Sci. U. S. A. 92, 12408–12415

53. Mayer, B. J. (2001) SH3 domains: complexity in moderation. J. Cell Sci. 114, 1253–1263

54. Hu, H., Columbus, J., Zhang, Y., Wu, D., Lian, L., Yang, S., Goodwin, J., Luczak, C., Carter, M., Chen, L., James, M., Davis, R., Sudol, M., Rodwell, J., and Herrero, J. J. (2004) A map of WW domain family interactions. Proteomics 4, 643–655

55. Niebuhr, K., Ebel, F., Frank, R., Reinhard, M., Domann, E., Carl, U. D., Walter, U., Gertler, F. B., Wehland, J., and Chakraborty, T. (1997) A novel proline-rich motif present in ActA of Listeria monocytogenes and cytoskeletal proteins is the ligand for the EVH1 domain, a protein module present in the Ena/VASP family. EMBO J. 16, 5433–5444

56. Shibata, T., Takatsu, H., Nogi, T., Matsugaki, N., Kawasaki, M., Igarashi, N., Suzuki, M., Kato, R., Earnest, T., Nakayama, K., and Wakatsuki, S. (2002) Structural basis for recognition of acidic-cluster dileucine sequence by GGA1. Nature 415, 937–941

57. Yaffe, M. B. (2002) Phosphotyrosine-binding domains in signal transduction. Nat. Rev. Mol. Cell. Biol. 3, 177–186