

PDZ Domains:

Structural Modules for Protein Complex Assembly

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PDZ domains¹ are modular protein interaction domains that play a role in protein targeting and protein complex assembly. Once termed Discs-large homology regions (DHRs) or GLGF repeats (after a conserved Gly-Leu-Gly-Phe sequence found within the domain), these domains of ~ 90 amino acids are now primarily known by an acronym of the first three PDZ-containing proteins identified: the postsynaptic protein PSD-95/SAP90, the *Drosophila* septate junction protein Discs-large, and the tight junction protein ZO-1. Since their initial identification, PDZ and PDZ-like domains have been recognized in numerous proteins from organisms as diverse as bacteria, plants, yeast, metazoans, and *Drosophila* (1). In fact, they are among the commonest protein domains represented in sequenced genomes. Analysis of the human, *Drosophila*, and *Caenorhabditis elegans* genomes estimates the presence of 440 PDZ domains in 259 different proteins, 133 PDZ domains in 86 proteins, and 138 PDZ domains in 96 proteins, respectively (2).

The structural features of PDZ domains allow them to mediate specific protein-protein interactions which underlie the assembly of large protein complexes involved in signaling or subcellular transport. Not surprisingly, disrupting these interactions can play a role in human diseases. Mutations in a gene encoding harmonin, a PDZ-containing protein, cause Usher syndrome type 1C, an autosomal recessive disorder characterized by congenital sensorineural deafness, vestibular dysfunction, and blindness (3-5). This was the first mutation in a PDZ-encoding gene linked to a human disease. Subsequently, mutations in the periaxin gene, which also encodes a PDZ-containing protein, have been

identified as a cause of Dejerine-Sottas neuropathy, a severe demyelinating form of peripheral neuropathy (6,7).

The Structural Basis of PDZ Binding and Specificity

The notion that PDZ domains serve as protein interaction modules emerged from the finding that the first and second PDZ (PDZ1 and 2) domains of PSD-95 can bind the extreme carboxyl-terminal peptide sequence of Shaker-type K⁺ channels (8) and NMDA receptor NR2 subunits (9,10). PDZ3 of the protein tyrosine phosphatase FAP-1/PTP1E similarly was identified as a binding site for the C-terminus of the cell surface receptor Fas (11). These studies further demonstrated that PDZ domains maintained their activity and selectivity when expressed in heterologous proteins, establishing these motifs as modular domains that bind the C-termini of target proteins in a sequence-specific manner.

The structural basis for PDZ specificity became apparent with the solution of the X-ray crystallographic structure of PDZ domains complexed with their cognate peptide ligands. First solved for PDZ3 of PSD-95 (12,13), numerous additional PDZ crystal structures have been determined in recent years, including PDZ2 of PSD-95 (14), the single PDZ domain of CASK (15), syntrophin (16) and neuronal nitric oxide synthase (nNOS) (17), PDZ2 from human phosphatase hPTP1E (18), PDZ1 of the Na⁺/H⁺ exchanger regulatory factor (NHERF) (19), and PDZ1 of InaD (20). The common structure of PDZ domains comprises six β strands (β A- β F) and two α helices (α A and α B), which fold in an overall six-stranded β sandwich (Figure 1A). C-terminal peptides

bind as an antiparallel β -strand in a groove between the β B strand and the α B helix, in essence extending one of the β -sheets. The conserved Gly-Leu-Gly-Phe (GLGF) sequence of the PDZ domain is found within the β A- β B connecting loop, and is important for hydrogen bond coordination of the C-terminal carboxylate (COO^-) group. The N-terminus and C-terminus of the PDZ domain are located near each other on the opposite side of the PDZ domain from the peptide-binding site, a feature shared with other protein interaction modules such as SH2 domains.

PDZ-like domains identified in plants and bacteria have a similar overall secondary and tertiary structure, but show a different topology (Figure 1C). Specifically, the β A strand, derived from the N-terminal sequences in conventional PDZ domains, is formed by the C-terminus in the PDZ-like domain of the photosystem II D1 C-terminal protease (21). This circularly permuted fold, also found in the Tsp protease from *Escherichia coli*, retains the ability to bind C-terminal sequences (22).

The crystallographic data indicate that the C-terminal four residues of PDZ ligands interact directly with the peptide-binding groove. The main chain atoms of the β B strand form hydrogen bonds with the extended peptide ligand and stabilize the interaction, although they do not account for sequence specificity. The specific interaction of C-terminal sequences suggests that recognition of the carboxylate group is critical for PDZ binding. Indeed, a highly conserved positively-charged residue (e.g., arginine-318 of PDZ3 of PSD-95) and the main chain amides of the Gly-Leu-Gly-Phe motif form hydrogen bonds with the terminal carboxylate group (12). The side chain of the C-terminal residue (position 0) projects into a hydrophobic pocket, accounting for

PDZ domains binding preferentially to sequences ending with a hydrophobic residue (such as valine, isoleucine, or leucine; 23).

In the crystal structure of PSD-95 PDZ3 with its peptide ligand, the side chain of the residue at position -1 points away from the interaction surface (12). This correlates with the relative lack of PDZ specificity for recognition at the -1 position. Nevertheless, substitutions at this site can affect binding preference for individual PDZ domains, albeit to a lesser degree than the 0 and -2 positions (23-25). In contrast, the guanido group of arginine at position -1 of the cystic fibrosis transmembrane regulator (CFTR) C-terminus (C-terminal sequence $-DTRL$) forms two salt bridges and two hydrogen bonds with residues in PDZ1 of NHERF, indicating that in some cases, the -1 residue contributes directly to the specificity and affinity of the interaction (19). The crystal structure of the InaD PDZ1 with a C-terminal peptide from NorpA (C-terminal sequence $-EFCA$) further demonstrates a critical role for a cysteine residue at position -1 , as intermolecular disulfide bond formation of this residue with a cysteine residue in the PDZ domain is required for high-affinity interaction (20).

The binding specificity of PDZ domains is critically determined by the interaction of the first residue of helix αB (position $\alpha B1$) and the side chain of the -2 residue of the C-terminal ligand; this forms the basis for PDZ classification (Table 1) (23). In Class I PDZ interactions, such as those of PSD-95, a serine or threonine residue occupies the -2 position. The side chain hydroxyl group forms a hydrogen bond with the N-3 nitrogen of a histidine residue at position $\alpha B1$ (12), which is highly conserved among Class I PDZ domains. In contrast, class II PDZ interactions are characterized by hydrophobic residues at both the -2 position of the peptide ligand and the $\alpha B1$ position of the PDZ domain

(23). A third class of PDZ domains, such as nNOS, prefers negatively charged amino acids at the -2 position (24). This specificity is determined by the coordination of the hydroxyl group of a tyrosine residue at position α B1 with the side chain carboxylate of the -2 residue (24,26). Other classes of PDZ domain specificities are likely to be distinguished with further research.

PDZ domains vary in their range and stringency of specificity. For example, the PDZ domain of PICK1, which has a lysine residue at the α B1 position, can bind the C-termini of both protein kinase C (ending in -QSAV; 27) and the AMPA receptor subunit GluR2 (ending in -SVKI; 28). Thus, a single PDZ domain can show both Class I and Class II specificity; the three-dimensional structural basis of this promiscuity remains to be determined.

While the residue at the -2 position is a key determinant of PDZ-ligand interactions, more N-terminal residues in PDZ-binding peptides also contribute to specificity. Crystallographic data indicate that the -3 side chain also directly contacts the peptide binding groove (12,19,26), and the residue at position -3 is important in determining the binding of ligands selected from a peptide library (23). Moreover, several studies have demonstrated that residues beyond the last four amino acids are also important, up to position -8 (18,23,25).

Although many examples of PDZ-peptide ligand interactions have now been identified, uncertainty remains regarding the binding affinity of the PDZ domain for its ligand. Using solid phase methods such as surface plasmon resonance (Biacore) or modified ELISA assays, affinities of PDZ binding to their cognate peptides have been measured in the 10-100 nanomolar range. On the other hand, solution methods such as

fluorescence polarization suggest that the binding affinity may be weaker, in the low micromolar range (25,29). Likely, a wide range of affinities apply to the diversity of PDZ domain interactions *in vivo*. Moreover, especially because many PDZ ligands are membrane-associated and clustered, it is difficult to know whether solid phase or solution methods approaches better the *in vivo* situation.

Regulation of PDZ Binding to C-terminal Sequences

The regulation of protein-protein interactions between modular elements such as SH3 domains and their binding partners is often critical for cell signaling (30). What regulates the binding of PDZ domains to their respective C-terminal ligands? One mode of control is the phosphorylation of residues within the C-terminal sequences that bind the PDZ domain. For example, serine phosphorylation at position -2 in the inward rectifier K⁺ channel Kir2.3 by protein kinase A disrupts binding to the PDZ domains of PSD-95 (31). The association of β_2 -adrenergic receptor with NHERF is similarly abolished by phosphorylation at position -2 by G-protein-coupled receptor kinase GRK5 (32). Intriguingly, binding of a single C-terminal peptide ligand to multiple PDZ domains can be differentially regulated. For instance, phosphorylation at serine-880 of the AMPA receptor subunit GluR2 (C-terminal sequence -SVKI) by protein kinase C inhibits binding to the PDZ domain of GRIP1 but not to PICK1 (33,34). PDZ interactions may also be regulated by extracellular signals, as the binding of the β_2 -adrenergic receptor C-terminus to NHERF is stimulated by β -adrenergic agonists (35).

Thus, it is likely that both intracellular and extracellular signals regulate the temporal and spatial organization of PDZ-based interactions.

Binding of PDZ Domains to Internal Sequences

Although binding to C-terminal peptides appears to be the typical mode of interaction, PDZ domains can also interact with internal peptide sequences. The best example of this is the interaction of neuronal nitric oxide synthase (nNOS) with the PDZ domain of PSD-95 or syntrophin (36,37). In the crystal structure of the nNOS-syntrophin PDZ complex, amino acid residues adjacent to the canonical PDZ domain of nNOS form a two-stranded β -hairpin “finger”, which docks in the peptide-binding groove of the syntrophin PDZ domain (Figure 1B) (14,26). The sharp β turn of the β -finger binds to the same site as the terminal carboxylate group of peptide ligands (36). Introduction of point mutations that destabilize the nNOS β -finger conformation results in decreased binding to the syntrophin PDZ domain, supporting the model that the β -finger is required for proper recognition (29). Screening of combinatorial phage libraries has also identified cyclic peptides as potential ligands for PDZ domains; PDZ binding of these peptides depends on intramolecular disulfide bond formation (38). These examples suggest that PDZ domains may interact with internal sequences that are conformationally constrained, structurally mimicking a free C-terminus.

In addition, PDZ domains can associate with other PDZ domains to form homo- and hetero-oligomers. Examples include GRIP (39,40), ZO-1 (41), InaD (42), and NHERF (43,44). PDZ domains are also reported to interact with other distinct protein-

binding motifs, including ankyrin repeats (45), spectrin repeats (46), and LIM domains (47). The structural bases for these interactions is not yet clear.

Structural Features of PDZ-containing Proteins

A fascinating feature of PDZ-containing proteins is that they often contain multiple PDZ domains, up to thirteen in the MUPP1 protein (48) (Figure 2). In many cases, the PDZ domains are closely grouped into tandem arrays, including pairs (e.g., PDZ1,2 of PSD-95) and triplets (e.g., PDZ1-3 and PDZ4-6 of GRIP). The significance of PDZ grouping is not known. However, there is some evidence to suggest that multiple domains can cooperate to enhance binding to target ligands. For instance, syntenin contains two PDZ domains in tandem. PDZ2 of syntenin binds to the C-terminus of syndecan, neuexin, and ephrin-B1 only when paired with PDZ1 (or another copy of PDZ2), but does not interact when presented in isolation (49). In addition, a recent report suggests that one PDZ domain may influence the folding of an adjacent PDZ domain (50). In this example, PDZ5 of GRIP alone was unstructured in solution by nuclear magnetic resonance and circular dichroism spectroscopy and failed to bind GluR2. However, when covalently connected to PDZ4, PDZ5 became highly structured and GluR2 binding was restored.

PDZ domains are also often found in proteins with other known interaction domains or signaling domains (Figure 2). The superfamily of proteins called membrane-associated guanylate kinases (MAGUKs), which includes PSD-95/SAP90, Dlg, and ZO-1, are characterized by one or more PDZ domains, an SH3 domain, and a catalytically

inactive guanylate kinase-like (GK) domain. PDZ domains also occur in proteins with WW, LIM, and CaMK-like domains, as well as ankyrin and leucine-rich repeats. Recently, a cytoplasmic protein, PDZ-RGS3, was identified that binds B-ephrins through its PDZ domain, and has a regulator of heterotrimeric G protein signaling (RGS) domain (51).

PDZ Domains as Organizers of Protein Complexes

The multidomain structure of PDZ-containing proteins enables them to interact with multiple binding partners simultaneously, thereby assembling larger protein complexes (recently reviewed in 52,53). PDZ-based complexes are often localized to specific subcellular compartments. PDZ-based scaffolds have been shown to organize signal transduction pathways such as phototransduction in *Drosophila*, where ion channels and signaling molecules are co-assembled by the multi-PDZ protein InaD (54-57). MAGUKs appear to play a similar role in the postsynaptic density, a specialized structure at excitatory synapses enriched in glutamate receptors and associated signaling proteins (58-60). In both the examples of InaD and MAGUKs, the ability of PDZ-containing proteins to interact with multiple binding partners creates a protein complex specialized for local signaling functions.

PDZ proteins have also been implicated in the establishment of cell polarity. In *Drosophila*, two PDZ-containing proteins, Bazooka and PAR-6, form a ternary complex with atypical protein kinase C that is required for proper establishment and maintenance of apical-basal polarity in epithelial tissues (reviewed in 61). The orthologues of these

binding partners in *C. elegans* are likewise necessary for polarization of the one-cell embryo. Importantly, depletion of any one of the proteins results in mislocalization of the other two, underscoring the functional importance of the complex. The LIN-2/LIN-7/LIN-10 tripartite complex also demonstrates a role for PDZ proteins in protein sorting (62,63). This complex specifies the basolateral targeting of the EGFR-like receptor LET-23 in *C. elegans*, and the mammalian homologs have been proposed to play a role in NMDA receptor trafficking through an interaction with the kinesin superfamily motor protein KIF17 (64).

These examples and others illustrate the role of PDZ-containing proteins in determining the subcellular location of their binding partners. Two recent studies also indicate that PDZ domains can modulate the function of their associated proteins as well as the localization, as interaction of the cystic fibrosis transmembrane conductance regulator (CFTR) with either the multi-PDZ adaptor protein CAP70 or NHERF increases chloride channel activity of the CFTR protein, perhaps by promoting multimerization of the channel protein (65,66). Thus, PDZ domains may have a functional role in modulating the activity of their target ion channels and membrane receptors (67).

Conclusions

It is now clear that PDZ domain proteins play an important role in the targeting of proteins to specific membrane compartments and their assembly into supramolecular complexes. There is also evidence that they can regulate the function of their ligands in addition to serving as scaffolds. Their ability to bind short extreme C-terminal sequences

offers a facile way for PDZ proteins to interact with target proteins without disrupting the overall structure and function of their ligands. Key questions remain in understanding how PDZ domains serve their function. For example, most PDZ domains seem to bind multiple ligands. What determines the interaction with specific binding partners, and how is this regulated spatially and temporally in the cell? How is ligand binding and protein stoichiometry affected by PDZ-based homo- and heteromultimerization, and what is the structural basis for multimer formation? What role does the tandem organization of PDZ domains play in determining scaffolding function? The answers to these questions and others await investigation in this exciting and evolving field.

¹Abbreviations used are: PDZ, PSD-95/Dlg/ZO-1 homology; PSD, postsynaptic density; SAP, synapse-associated protein; NMDA, N-methyl-D-aspartate; NHERF, Na⁺/H⁺ exchange regulatory factor; SH2, Src homology 2; SH3, Src homology 3; nNOS, neuronal nitric oxide synthase; PICK-1, protein interacting with C-kinase 1; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GRIP, glutamate receptor-interacting protein; InaD, inactivation-no afterpotential D; MUPP1, multi-PDZ containing protein 1; MAGUK, membrane-associated guanylate kinase; GK, guanylate kinase; CaMK, calcium-calmodulin dependent protein kinase.

Figure Legends

Figure 1. Structure of PDZ and PDZ-like domains. (A) Ribbon diagram of PDZ3 of PSD-95 complexed with a C-terminal peptide from CRIPT (12). The structure demonstrates the six β -strands (turquoise) and two α -helices (red), with the peptide (yellow) binding as a β -strand between the α B helix and β B strand. The amino and carboxyl termini are labeled. (B) The structure of α 1-syntrophin PDZ domain complexed to nNOS (green) (17). Note the overall similarity in PDZ structure with replacement of the C-terminal peptide ligand by a β -finger. (C) Ribbon diagram of the photosystem II D1 protease PDZ-like domain from *Scenedesmus obliquus* (21). Although the overall topology is similar to conventional PDZ domains, the β A strand is derived from the C-terminus of the domain.

Figure 2. Several examples of PDZ domain proteins illustrate their modular nature and variety of protein interaction domains. Numerous proteins contain multiple PDZ domains (shown in red), often organized in tandem repeats. The MAGUK superfamily of PDZ proteins (including PSD-95, Dlg, and ZO-1) have an SH3 domain (green) and guanylate kinase domain (blue), in addition to a variable number of PDZ domains. PDZ domains are also found in proteins which contain protein interaction modules such as LIM and WW domains, sterile α -motif (SAM), ankyrin (Ank) and leucine-rich (LRR) repeats, and a domain which regulates heterotrimeric G-protein signaling (RGS).

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Table 1. Examples of PDZ domains classified by specificity for C-terminal peptide ligands.

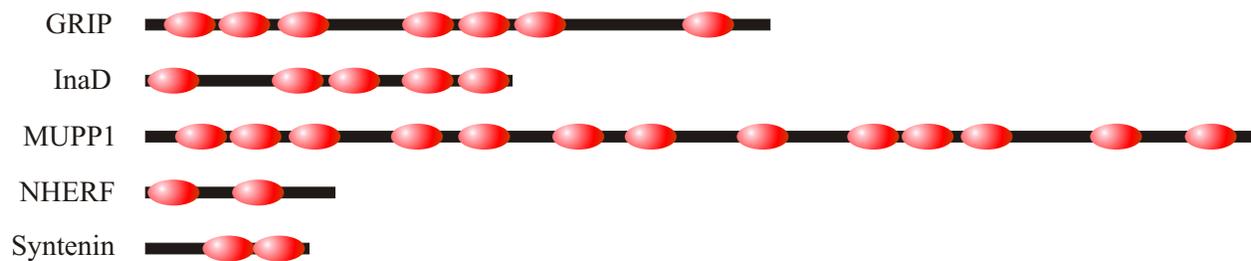
Class	C-terminal sequence*	Ligand protein	PDZ protein	Reference
Class I				
-X-S/T-X-Φ	-E-T-D-V	Shaker K ⁺ channel	PSD-95 (PDZ2)	8
	-E-S-D-V	NMDA receptor subunits NR2A/B	PSD-95 (PDZ2)	9
	-T-T-R-V	Neurologin	PSD-95 (PDZ3)	68
	-E-S-L-V	Voltage-gated Na ⁺ channel	Syntrophin	69
	-Q-S-A-V	Protein kinase C-α	PICK1	27
	-D-S-S-L	β ₂ -adrenergic receptor	NHERF (PDZ1)	35
	-D-T-R-L	CFTR	NHERF (PDZ1)	70
	-Q-T-R-L	GKAP	Shank/ProSAP	71
	-S-S-T-L	Metabotropic glutamate receptor subunit mGluR5	Shank/ProSAP	72
	Class II			
-X-Φ-X-Φ	-S-V-K-I	AMPA subunit GluR2	PICK1	28
			GRIP (PDZ5)	39
	-E-Y-F-I	Glycophorin C	Erythrocyte p55	73
	-E-Y-Y-V	Neurexin	CASK	74
	-E-F-Y-A	Syndecan-2	CASK	75
	-Y-Y-K-V	Ephrin B1	PICK1	76
			GRIP (PDZ6) Syntenin (PDZ2)	
Class III				
-X-D/E-X-Φ	-V-D-S-V	Melatonin receptor	nNOS	24
	-G-E-P-L	KIF17	mLIN10/Mint1/ X11	64

*Last four amino acids of the ligand proteins are shown, although specificity can involve more proximal residues as well (see text). Φ, hydrophobic amino acid; X, unspecified amino acid.

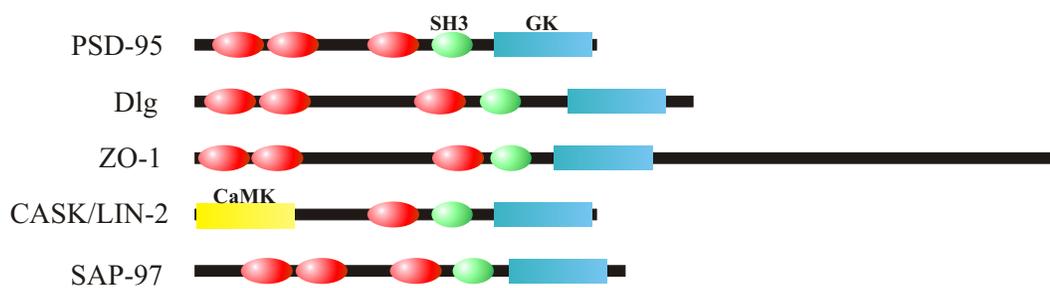
Hung and Sheng
Figure 1



Multi-PDZ proteins



MAGUKs



PDZ proteins with other interaction domains

