REVIEW ARTICLE
Structures and target recognition modes of PDZ domains: recurring themes and emerging pictures

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PDZ domains are highly abundant protein–protein interaction modules and are often found in multidomain scaffold proteins. PDZ-domain-containing scaffold proteins regulate multiple biological processes, including trafficking and clustering receptors and ion channels at defined membrane regions, organizing and targeting signalling complexes at specific cellular compartments, interfacing cytoskeletal structures with membranes, and maintaining various cellular structures. PDZ domains, each with ∼90-amino-acid residues folding into a highly similar structure, are best known to bind to short C-terminal tail peptides of their target proteins. A series of recent studies have revealed that, in addition to the canonical target-binding mode, many PDZ–target interactions involve amino acid residues beyond the regular PDZ domain fold, which we refer to as extensions. Such extension sequences often form an integral structural and functional unit with the attached PDZ domain, which is defined as a PDZ supramodule. Correspondingly, PDZ-domain-binding sequences from target proteins are frequently found to require extension sequences beyond canonical short C-terminal tail peptides. Formation of PDZ supramodules not only affords necessary binding specificities and affinities demanded by physiological functions of PDZ domain targets, but also provides regulatory switches to be built in the PDZ–target interactions. At the 20th anniversary of the discovery of PDZ domain proteins, we try to summarize structural features and target-binding properties of such PDZ supramodules emerging from studies in recent years.

Key words: extended PDZ domain, PDZ domain, PDZ supramodule, protein assembly, scaffold protein, tandem repeat.

INTRODUCTION

PDZ domains, originally recognized as ∼90-amino-acid residue repeats in the three founding members of structural proteins called the PSD-95 (postsynaptic density-95), the *Drosophila* tumour suppressor protein Dlg-1 (discs large 1) and the tight junction protein ZO-1 (zonula occludens 1) [1–4], are now known as one of the most abundant protein–protein interaction modules found in all metazoans [5–9]. More than 150 PDZ domain-containing proteins with over 250 non-redundant PDZ domains have been recognized in the human proteome [8]. Several characteristic features can be readily extracted after the domain organization analysis of PDZ-domain-containing proteins (Figure 1). First, PDZ domains often coexist with a wide variety of modular domains including PDZ itself. Secondly, many PDZ proteins contain multiple repeats, and some of them can be very large [e.g. MUPP1 (multiple PDZ-domain-containing protein 1) contains 13 PDZ domains plus an L27 domain at the N-terminus] (Figure 1). Thirdly, most PDZ proteins lack intrinsic catalytic activities, and thus they largely function as pure scaffold proteins. PDZ domain proteins play critical roles in very broad biological processes including cell polarity establishment and maintenance, directed cell migrations, tissue growth and differentiation and embryonic development, through trafficking and clustering receptors and ion

channels at defined membrane regions, organizing and targeting signalling complexes or cell fate determinants at specific cellular compartments, and connecting cytoskeletal structures with membranes. Mutations of PDZ domain proteins and their interaction partners are frequently linked to various human diseases including many forms of cancers and neurological disorders [10–13].

A canonical PDZ domain contains six β-strands (βA to βF) and two α-helices (αA and αB), and the six β-strands form a partially opened barrel with each of its opening sides capped with one α-helix [14] (Figure 2). The best known function of PDZ domains is to bind to a short stretch of amino acid residues (approximately five to seven amino acids) at the C-termini of target proteins [5,6,15]. Peptide ligands bind to an extended groove formed by βB and αB of a PDZ domain via forming an antiparallel β-sheet with βB. The C-terminus of a peptide ligand binds to the so-called 'GLGF motif' at one end of the αB/βB groove (Figure 2). The specificities of PDZ-binding peptides are chiefly determined by the residues at the 0 and -2 positions of the peptide ligands, and thus PDZ-binding ligands are often categorized on the basis of the type of residues in these two positions [16]. Recent systematic PDZ–ligand interaction studies revealed that residues at the other positions (-1, -3, -4 and -5) of the peptide ligands also contribute to the binding specificities [17,18]. Some of the PDZ domain can also recognize
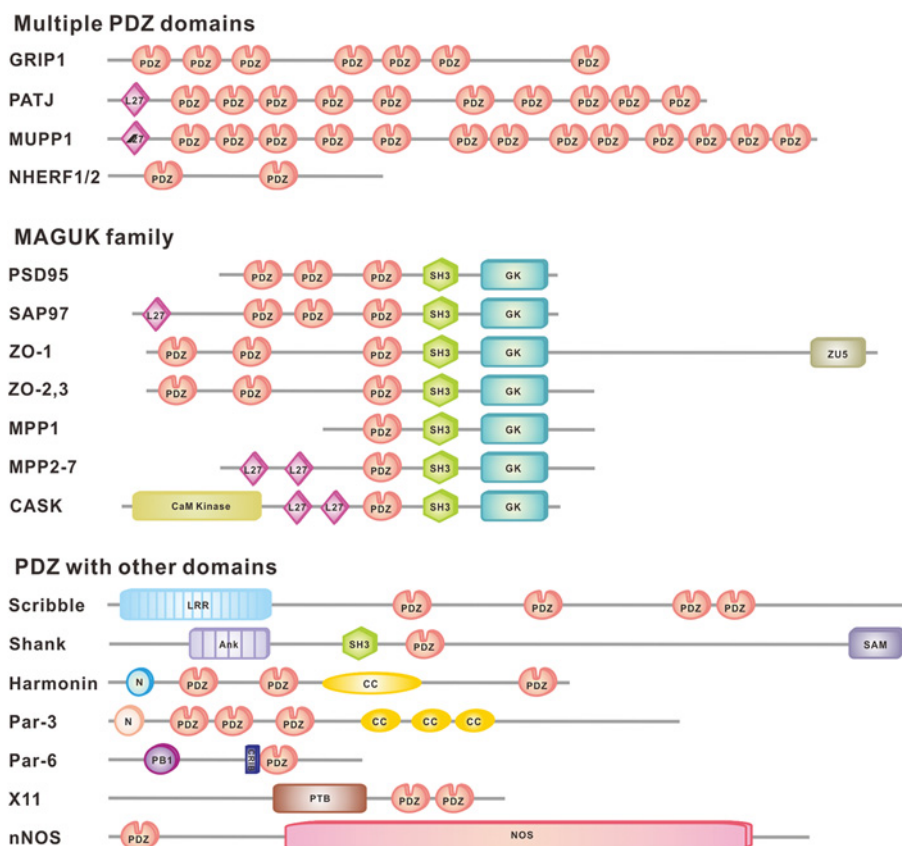
Figure 1   Domain organizations of a selected set of PDZ domain proteins

Proteins are grouped according to their domain organization patterns. PDZ domains are shown in orange. Ank, ankyrin repeats; CaM kinase, calmodulin-dependent kinase (CaMK)-like domain; CASK; calmodulin-associated serine/threonine kinase; CC, coiled-coil domain; CRIB, Cdc42/Rac-interactive binding domain; LRR, leucine-rich repeat; MPP, membrane protein, palmitoylated; N, N-terminal domain; Par, partitioning defective homologue; PATJ, PALS1-associated tight junction protein; PB1, Phox and Bem1p domain; SAP97, synapse-associated protein 97; ZU5, domain present in ZO-1 and UNC5-like netrin receptor.
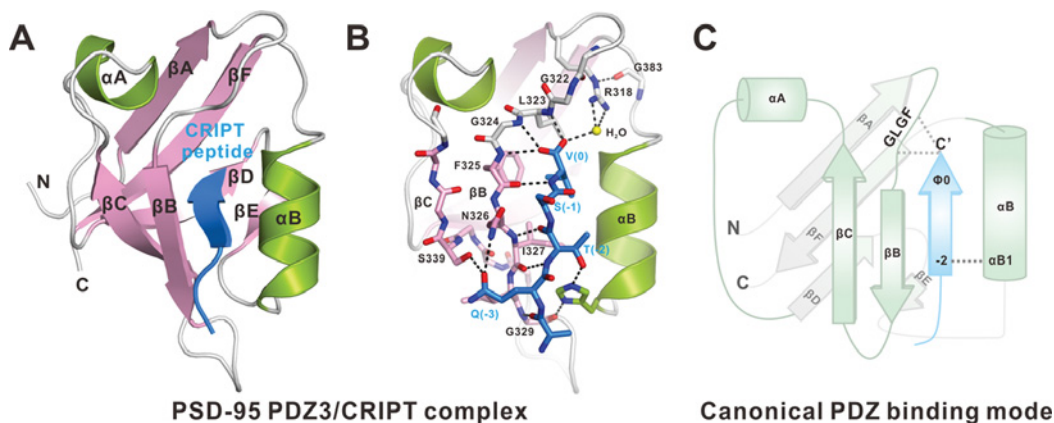

Figure 2   The structure and ligand-binding mode of a canonical PDZ domain

(**A**) Ribbon diagram showing the crystal structure of PSD-95 PDZ3 in complex with a target peptide (in blue) [14]. (**B**) Detailed view of the interaction between the peptide ligand (in blue stick model) and the αB/βB groove of PSD-95 PDZ3. The oxygen, nitrogen and carbon atoms of the peptide are shown in red, blue and grey respectively. A bridging water molecule is shown using a yellow sphere. The hydrogen bonds are indicated using black broken lines. (**C**) Schematic diagram showing the canonical target recognition mode of a PDZ domain.

the internal peptide sequences [19,20]. In addition to binding to the peptide ligands, a significant portion of PDZ domains can also specifically interact with phospholipids [21–25].

In the present review, we focus on a series of recent studies showing that, in addition to the well-documented canonical PDZ–target interaction mode shown in Figure 2, many PDZ domains employ amino acid sequences beyond the canonical PDZ domain fold, which we refer to as 'extended PDZ domains'. Such extension sequences often form an integral structural and functional unit with the regular PDZ domains, and we term the 'extended PDZ domains' as PDZ supramodules. Accordingly, PDZ-domain-binding regions from target proteins also often

require extension sequences (or even additional domains) beyond short C-terminal tail peptides. In the present review, we summarize the structural features of such PDZ supramodules and their complexes with cognate target peptides/proteins. Formation of PDZ supramodules often allow these PDZ domain proteins to bind to their targets with much higher specificities and affinities. An additional advantage of forming PDZ supramodules is that regulatory switches can be readily built in to modulate the interactions between PDZ domains and target proteins.

STRUCTURES FEATURES AND TARGET-BINDING PROPERTIES OF PDZ SUPRAMODULES

PDZ domains with short extensions at N- or C-termini

It is increasingly recognized that additional residues outside of the canonical PDZ domain sequences often play critical roles in modulating both the structure and function of a PDZ domain. A previous bioinformatics study showed that a very large proportion of PDZ domains ($\sim$40 %) contain extension sequences at the two termini of the canonical domain boundary, and these extensions are often highly conserved and predicted to form either α-helices or β-strands [8]. Interestingly, a number of disease-associated mutations has been found in the extension sequences of several PDZ domains [26–28], implying that these extension sequences may modulate the functions of the associated PDZ domains.

PSD-95 (also known as Dlg-4), a member of the MAGUK (membrane-associated guanylate kinase) family scaffold proteins, contains three PDZ domains, an SH3 (Src homolgy 3) domain and a GK (guanylate kinase-like) domain. PSD-95 is one of the most abundant proteins in postsynaptic densities and perhaps one of the best-characterized PDZ domain proteins. Notably, the first PDZ domain structure solved is the complex between PSD-95 PDZ3 and a C-terminal peptide from CRIPT (cysteine-rich interactor of PDZ3) [14]. Interestingly, the structure of PDZ3 contains an additional C-terminal α-helix extension distal to the ligand-binding groove of the domain and the α-helix extension directly packs with the core of PDZ (Figure 3A), although this structural feature was not discussed in the original publication [14]. Subsequent studies showed that removal of the C-terminal α-helix extension leads to a $\sim$25-fold decrease in the binding affinity to the CRIPT peptide (Figure 3C) [30]. NMR-based studies showed that the backbone motion of PDZ3 is largely unaffected, whereas many of the side chains of PDZ3 experience significantly increased flexibilities after removal of the C-terminal extension, indicating that the C-terminal extension plays a role in modulation of the target-binding affinity of the PDZ domain by lowering its conformational entropy [30] or possibly by directly contacting the target peptide [31,32]. Moreover, the C-terminal extension may also play a regulatory role in the PSD-95 PDZ3–target interaction. The aromatic ring of Tyr[397] located at the centre of the helix extension physically interacts with the PDZ core. It was shown that Tyr[397] can be phosphorylated *in vivo* [33], and phosphorylation of Tyr[397] can uncouple the helix extension from the PDZ3 core and thus lead to a weakened interaction with the target peptide [34].

NHERF (sodium–hydrogen exchange regulatory cofactor) is another case of a PDZ domain using extension sequences to modulate its target-binding properties. NHERF1 contains two PDZ domains connected in tandem and a C-terminal ezrin-binding motif. Both NHERF1 PDZ domains contain extension sequences at their two termini [35,36]. The C-terminal helical extension of NHERF1 PDZ2 forms two α-helices and directly couples with the core of the PDZ domain (Figure 3B). CFTR (cystic fibrosis transmembrane conductance regulator) interacts with NHERF1

PDZ2 in a C-terminal helical extension-dependent manner [35]. Similar to the C-terminal extension in PSD-95 PDZ3, structural coupling of NHERF1 PDZ2 C-terminal extension considerably reduces the conformational dynamics of the domain, allowing PDZ2 to interact with the CFTR peptide with higher affinity. Removal of the PDZ2 extension decreases the CFTR-binding affinity $\sim$10-fold (Figure 3C).

Given that numerous PDZ domains contain extension sequences with predicted secondary structures [8], we believe that many more additional examples of extended PDZ domains will be discovered in the near future (for a recent review on this topic see [9]). We further predict that the extension sequences of extended PDZ domains may play more direct roles in modulating target-binding specificities as well as affinities other than the relatively 'indirect' roles observed in PSD-95 PDZ3 and NHERF1 PDZ1 and PDZ2.

Homotypic PDZ supramodules

An obvious feature of many PDZ domain proteins is that they often contain multiple PDZ domains (Figure 1). Multiple PDZ domains in these PDZ proteins are arranged into closely linked tandem arrays with short and conserved interdomain sequences, implying that such interdomain connecting sequences may play an additional role other than domain connectors. Indeed, increasing examples discovered in recent years have revealed that multiple PDZ domains in tandem arrays often interact with each other to form integral structural units with target-binding properties distinct from individual PDZ domain or a simple sum of PDZ domains within a tandem. We term these PDZ tandem arrays with integrated structures as homotypic PDZ supramodules. In the present review, we discuss the structural features and target-binding properties of several known homotypic PDZ supramodules.

In several homotypic PDZ supramodules, one PDZ domain functions as a 'chaperone' to stabilize the folding of the neighbouring ligand-engaging PDZ domain through extensive interdomain couplings. The PDZ45 and PDZ12 tandems of GRIP1 (glutamate receptor-interacting protein 1) were examples of such homotypic supramodules [37,38]. GRIP1 and GRIP2, each containing six to seven PDZ domains, are first identified by their interactions with GluR2/3 (glutamate receptor 2/3) subunits of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors [39,40]. The C-terminal tail of the GluR2 subunit of the AMPA receptor interacts with the fifth PDZ domain of GRIP1. However, covalently connected PDZ4 is absolutely required for PDZ5 to bind to the GluR2 tail [41]. The structure of the GRIP1 PDZ45–GluR2 complex revealed that PDZ4 and PDZ5 tightly pack with each other, forming a structural supramodule. The conserved linker between the two PDZ domains plays a critical role in integrating the two PDZ domains together by forming a β-strand antiparallel to βA of PDZ5 and by directly interacting with the N-terminal extension of PDZ4 (Figure 4A, panel i) [37]. In the PDZ45 tandem, the αB/βB groove of PDZ4, although completely accessible, is distorted and thus predicted to be unfavourable for C-terminal peptide ligand binding. The isolated GRIP1 PDZ5 is completely unfolded in solution and not capable of binding to GluR2, and isolated PDZ4 is stably folded [37,41]. Thus it seems that the primary role of PDZ4 is to maintain the folding of PDZ5, thus supporting the PDZ45 supramodule to bind to GluR2 (Figure 4A, panel i). It was noted that the PDZ45 supramodule contains two highly conserved serine residues within the interdomain linker (Ser[567] and Ser[568] in human GRIP1). Replacing either one or both of the serine resides
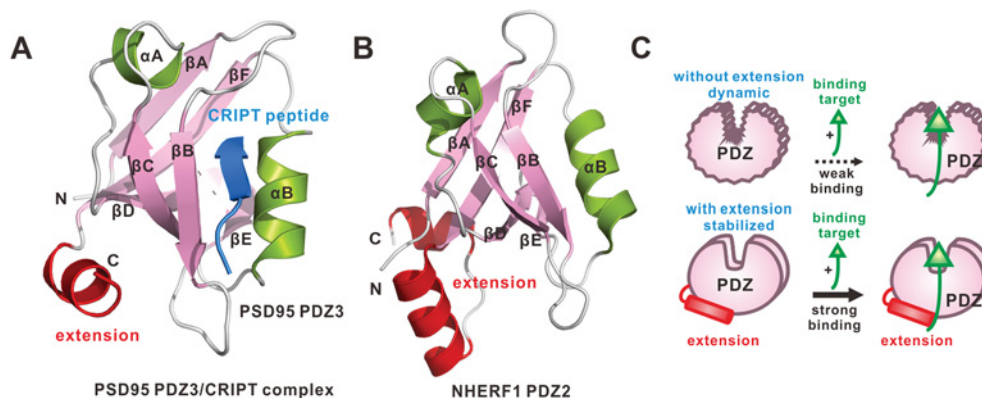
Figure 3   PDZ domains with short extensions at their two termini

(**A**) Ribbon diagram showing the complex structure of PSD-95 PDZ3 bound to the CRIPT peptide (blue) (PDB code 1BE9). (**B**) Ribbon diagram showing the target-free form of NHERF1 PDZ2 (PDB code 2KJD). The C-terminal α-helix extension of PSD-95 PDZ3 and that of NHERF1 PDZ2 are drawn in red. (**C**) Schematic diagram showing the truncated PDZ domain is dynamic, and inclusion of C-terminal α-helix extension reduces the conformational entropy of both PDZ domains and enhances their target-binding affinities.

with glutamic acid led to destabilization of PDZ5, suggesting that phosphorylation of the serine residues in the PDZ45 linker may play a regulatory role in the GRIP1–AMPA association (Figure 4A, panel i).

A similar interdomain chaperoning effect can be found in the GRIP1 PDZ12 tandem. The folding of PDZ1 strictly depends on the covalent attachment of PDZ2 (Figure 4B, panel ii). The interaction between Fras1 tail and GRIP1 PDZ1 requires the first two PDZ domains (PDZ1 and PDZ2) to be connected in tandem [38]. The crystal structure of GRIP1 PDZ12 in complex with the Fras1 C-terminal peptide reveals that the PDZ12 tandem forms a supramodule and interacts with Fras1 through the PDZ1 canonical peptide-binding groove [38] (Figure 4B, panel i). In the GRIP1 PDZ12 tandem, the target-binding groove of PDZ2 is completely occluded by the back side of PDZ1, suggesting that PDZ2 functions as PDZ1's folding partner. A highly conserved tyrosine residue (Tyr[134]) located at the interface between PDZ1 and PDZ2 plays a critical role in 'stitching' the two PDZ domains together. Mutation of the tyrosine residue to glutamic acid led to the unfolding of PDZ1 and subsequent dissociation of Fras1, suggesting that the interaction between GRIP1 PDZ12 and Fras1 might be regulated by phosphorylation of Tyr[134] [38] (Figure 4B, panel ii).

Recent structural, biochemical and functional studies of the PDZ45 tandem from *Drosophila* visual signalling scaffold protein INAD (inactivation no after potential D) reveals a number of previous unanticipated features of homotypic PDZ supramodules. Within the microvilli of fly photoreceptor cells, INAD organizes the core components of the phototransduction pathway into a supramolecular complex, involving the Ca^{2+}-permeant TRP (transient receptor potential) channel, PLCβ (phospholipase Cβ/norpA) and ePKC (eye-specific protein kinase C) via distinct PDZ domains [42]. A pair of cysteine residues in INAD PDZ5 (Cys[606] and Cys[645]) undergo light-dependent reversible oxidation and mediate the target interaction of PDZ45 (Figure 4C). When flies are kept in the dark, the two cysteine residues in PDZ5 are in the reduced form. On exposure to light, PDZ5 is converted into the oxidized state by forming intramolecular Cys[606]–Cys[645] disulfide bond [43], most likely leading to the dissociation of the PDZ5-binding target due to the disulfide-induced distortion of the ligand-binding groove. This light-dependent oxidation of INAD PDZ5 is required for the light-induced escape behaviour of flies [43]. Subsequent detailed biochemical and structural studies showed that the redox potential of INAD PDZ5 is allosterically regulated by a direct conformational couplings with

PDZ4 as well as a short C-terminal extension of PDZ5 [44] (Figure 4C). Unlike PDZ1 and PDZ5 in GRIP1, isolated INAD PDZ5 is highly stable in solution. Interestingly, the oxidized form (i.e. with the Cys[606]–Cys[645] disulfide bond) of isolated PDZ5 is highly stable. Formation of the PDZ45 supramodule dramatically raises the redox potential of the Cys[606]–Cys[645] disulfide pair and thus maintains PDZ5 in the reduced state capable of binding to its target with high affinity (Figure 4C, panel ii) [44]. The studies of the INAD PDZ45 reveal that, in addition to well-known passive scaffolding roles, scaffold proteins can actively modulate biological signalling processes (e.g. modulating photo-reception dynamics in fly eyes by forming a reversible INAD PDZ45 supramodule) [43,44]. The reversible conformational coupling of INAD PDZ45 is regulated by proton released by PIP_2 (phosphatidylinositol 4,5-bisphosphate) hydrolysis, a reaction catalysed by light-activated PLCβ in fly photoreceptors [44,45]. In the INAD PDZ45 supramodule, the C-terminal extension of PDZ5 wraps around the rear side of PDZ4, and physically staples the two PDZ domains together [44] (Figure 4C). Thr[669] located in the C-terminal tail forms a strong hydrogen bond with the imidazole ring of His[547] in PDZ4. Protonation of His[547], a process presumably mediated by PLCβ-controlled hydrolysis of PIP_2, breaks the hydrogen bond and thereby leads to uncoupling of the PDZ45 supramodule and subsequent oxidation of PDZ5 [44].

It should be pointed out that homotypic PDZ supramodules are not limited to the tightly coupled PDZ tandems. The first two PDZ domains of PSD-95, connected by a short and conserved linker, were shown to interact with each other rather weakly [46]. Such weak inter-PDZ interaction limits the conformational freedom of the two PDZ domains and positions the two C-terminal peptide-binding grooves of PSD-95 PDZ12 with an orientation favourable for binding to tails of multimeric transmembrane receptors or ion channels extending from the membrane bilayers (a phenomenon known as 'multivalency effect') [46,47] (Figure 4D). Increasing the spacing between PDZ1 and PDZ2 led to decreased binding affinity between PDZ12 and its dimeric targets. Moreover, isolated PDZ1 and PDZ2 have similar structures and target-binding properties. The bivalent PSD-95 PDZ12 supramodule not only can provide exquisite specificity and much enhanced affinity in binding to multimeric membrane targets, but also may indicate that the PSD-95 PDZ12 tandem can induce dimerization of receptors/ion channels [46]. The bivalent target interaction mode revealed in the PSD-95 PDZ12 supramodule also suggests that one might be able to develop bivalent low-molecular-mass
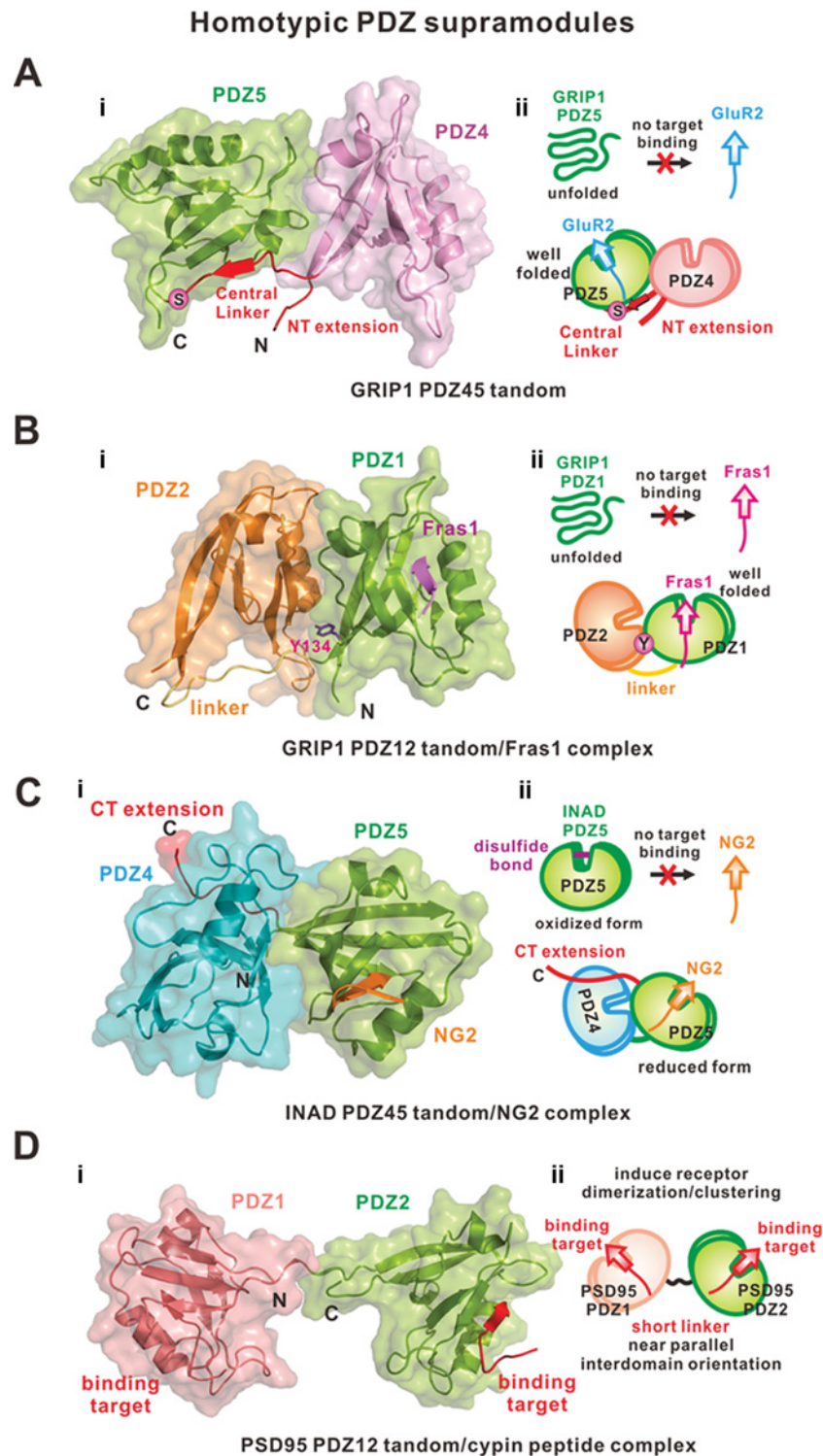
Figure 4   Structure and target-binding properties of homotypic PDZ supramodules

Ribbon diagrams combined with surface representations of GRIP1 PDZ45 (PDB code 1P1D) (**A**, panel i), GRIP1 PDZ12 (PDB code 2QT5) (**B**, panel i), INAD PDZ45 (PDB code 3R0H) (**C**, panel i) and PSD-95 PDZ12 (**D**, panel i). The Fras1 peptide in the GRIP1 PDZ12–Fras1 complex is shown as a purple ribbon and labelled. The NG2 peptide in the INAD PDZ45–NG2 complex is shown as an orange ribbon and labelled. The Cypin peptide in the PSD-95 PDZ12–Cypin complex is shown as a red ribbon and labelled. The potential phosphorylation sites in GRIP1 PDZ45, GRIP1 PDZ12 are highlighted and labelled. In GRIP1 PDZ45, the interdomain linker (red), which forms a β-strand antiparallel to βA of PDZ5 and directly interacts with the N-terminal extension of PDZ4 (red), is highlighted and labelled. In INAD PDZ45, the C-terminal extension of PDZ5 (red) which directly couples with PDZ4 is also highlighted. The Figure also shows schematic diagrams of distinct target-binding properties conferred by the supramodular organization of the PDZ tandems for the GRIP1 PDZ45 (**A**, panel ii), GRIP1 PDZ12 (**B**, panel ii), INAD PDZ45 (**C**, panel ii) and PSD95 PDZ12 (**D**, panel ii).

inhibitors targeting both PDZ1 and PDZ2 to modulate PSD-95–target interactions [48]. Indeed, several recent studies have described developments of such bivalent low-molecular-mass inhibitors that are very specific and potent towards the PSD-95 PDZ12 supramodule, and such inhibitors show promising potential in protecting stroke-induced ischaemic brain damage by disrupting the formation of the NMDA (N-methyl-D-aspartate) receptor–PSD-95–nNOS (neuronal nitric oxide synthase) ternary complex [49,50].

An increasing number of examples, including those described above, demonstrates that two or more PDZ domains connected in tandem often display distinct target-binding properties from each isolated domain or the simple sum of the isolated PDZ domains [37,38,44,46,51,52]. These distinct target-binding properties originate from the direct interactions of PDZ domains in the tandem, which is in contrast with the simple 'beads-on-a-string' model commonly viewed for multidomain scaffold proteins. As shown in the INAD PDZ45 tandem [44] and suggested for a number of other PDZ supramodules shown in Figure 4, an additional advantage of arranging multiple protein modules into a higher-order supramodule is to be able to build regulatory mechanisms into scaffold proteins, thereby enabling the scaffold proteins to actively control signalling events organized around them. It should be pointed out that, in addition to PDZ domain tandems, the supramodular organization is likely to be a common feature for many other multidomain proteins involved in diverse cellular signalling events.

Heterotypic PDZ supramodules

In addition to being arranged into closely spaced tandem arrays, PDZ domains are often found to coexist with diverse other protein-interacting domains or signalling modules (Figure 1). Emerging evidence suggests that some PDZ domains physically interact with such non-PDZ protein domains, forming structurally and functionally distinct higher-order units, which are termed heterotypic PDZ supramodules. Formation of heterotypic PDZ supramodules allows PDZ domains to function co-operatively with many other domains to perform their specific functions.

Harmonin [also known as USH1C (Usher syndrome 1C); a multi-PDZ domain protein] (Figure 1C) and Sans (USH1G) are two of the key scaffold proteins in the so-called USH1 (Usher syndrome 1) protein complex [11,53]. USH1 is the most severe form of hereditary hearing–vision loss disease in humans, and is caused by mutations of one or multiple of the five *USH1* genes including *USH1C* and *USH1G* [53]. Harmonin and Sans interact directly with each other regulating the development and maintenance of cilia in photoreceptors and hair cells [53,54]. Detailed biochemical studies showed that both the N-terminal domain proceeding PDZ1 and a stretch of extension sequence following PDZ1 are required to function together with the PDZ domain for harmonin to form a stable complex with Sans [55]. It turns out that the helical N-terminal domain and PDZ1 are tethered by the C-terminal PDZ1 extension, which forms a mini-domain composed of a β-hairpin followed by an α-helix, to form an integral structural supramodule for harmonin (referred to as NPDZ1) to interact with Sans [55] (Figure 5A). The isolated harmonin PDZ1 is unstable and not sufficient for binding to Sans. Interestingly, both the SAM (sterile α-motif) domain and PBM (PDZ-binding motif) of Sans (referred to as SAM-PBM) are required for Sans to form a high-affinity complex with harmonin [55] (Figure 5B). The structure of the harmonin NPDZ1–Sans SAM-PBM complex reveals that the canonical Sans PBM (the last four residues to be precise) binds to the $\alpha B/\beta B$ groove of harmonin PDZ1 and the upstream four residues (from the -4

to -7 positions) of PBM extensively interact with the mini-domain extension following PDZ1 (Figure 5B). Perhaps most unexpectedly, the Sans SAM domain binds directly to harmonin PDZ1 via a surface outside its $\alpha B/\beta B$ groove (i.e. forming a PDZ–SAM heterodimer; Figure 5B), revealing a previously unknown interaction mode both for the PDZ domain and for the SAM domain [55]. The canonical target-binding groove of PDZ1, the C-terminal mini-domain extension, and an additional binding surface in PDZ1 form an extensive interaction interface for Sans SAM-PBM (Figure 5B). Accordingly, the complex formed between harmonin NPDZ1 and Sans SAM-PBM is extremely tight (with a K_d value of $\sim$1 nM) [55], the most stable PDZ-domain-mediated protein complex known to date.

MAGUKs (Figure 1B) are a large family of PDZ domain scaffold proteins that play critical roles in diverse cellular processes including intercellular connections, cell polarity development and maintenance, neuronal plasticity, and cell survival in both metazoans and premetazoans [56,57]. The founding members of PDZ domain proteins (PSD-95, Dlg and ZO-1) are all members of the MAGUK family. Every member of the MAGUK family of proteins except for MAGI (membrane-associated guanylate kinase inverted) share a core structural module composed of a PDZ domain, an SH3 domain and a catalytically inactive GK domain arranged sequentially into a PDZ–SH3–GK complex. Such domain organization of MAGUKs is highly conserved throughout evolution, indicating that the signature PDZ–SH3–GK complex may form a structural and functional supramodule. This idea was confirmed recently in the PDZ–SH3–GK complex of ZO-1 [58,59]. The crystal structure of the ZO-1 PDZ3–SH3–GK tandem in complex with two different target peptides shows that the PDZ domain physically interacts with the SH3–GK tandem forming an integrated heterotypic PDZ supramodule with a rod-like shape (Figure 5C). As shown in the SH3–GK tandem of other MAGUKs [60–62], the SH3 and GK domains of ZO-1 interact with each other, forming an integral structure supramodule in which the last two β strands (βE and βF) of the SH3 domain come from the extreme N- and C-termini of the GK domain (Figure 5C). Furthermore, it was found that the interdomain linker between PDZ3 and SH3 forms an α-helix, which, together with a number of residues from αB and the $\beta B/\beta C$ loop of PDZ3, mediate the extensive domain–domain coupling between PDZ3 and SH3 domain (Figure 5C). Sequence analysis shows that the residues responsible for the inter-PDZ–SH3 domain interactions are highly conserved among different species, indicating that the supramodule is a feature important for ZO-1 function. The formation of PDZ3–SH3–GK supramodule significantly modifies the target-binding property of ZO-1 PDZ3 [58,59]. PDZ3–SH3 coupling reshapes the target-binding pocket of PDZ3 by generating an additional binding site for a target in the PDZ3–SH3 interface, and thus enhances the interaction between the PDZ3–SH3–GK complex and its targets. Mutations disrupting the interdomain coupling between PDZ3 and SH3 weaken the interaction between the PDZ–SH3–GK complex and its targets such as Cx45 (connexin45) [58].

Similar supramodular properties of the PDZ–SH3–GK tandem may also exist in other MAGUKs. Dlg1, the mammalian homologue of the *Drosophila* Dlg tumour suppressor, is found to interact with the synaptic protein GKH (GK holder) to facilitate the organization of signal transduction pathways in the establishment and maintenance of epithelial polarity. Previous studies have demonstrated that functional interaction between the Dlg1 SH3–GK tandem and GKH depends on the presence of the PDZ3 domain of Dlg1 and this interaction is further regulated by the PDZ3-binding peptide [63,64]. It was also shown very recently that PDZ3 of PSD-95 is localized adjacent to the
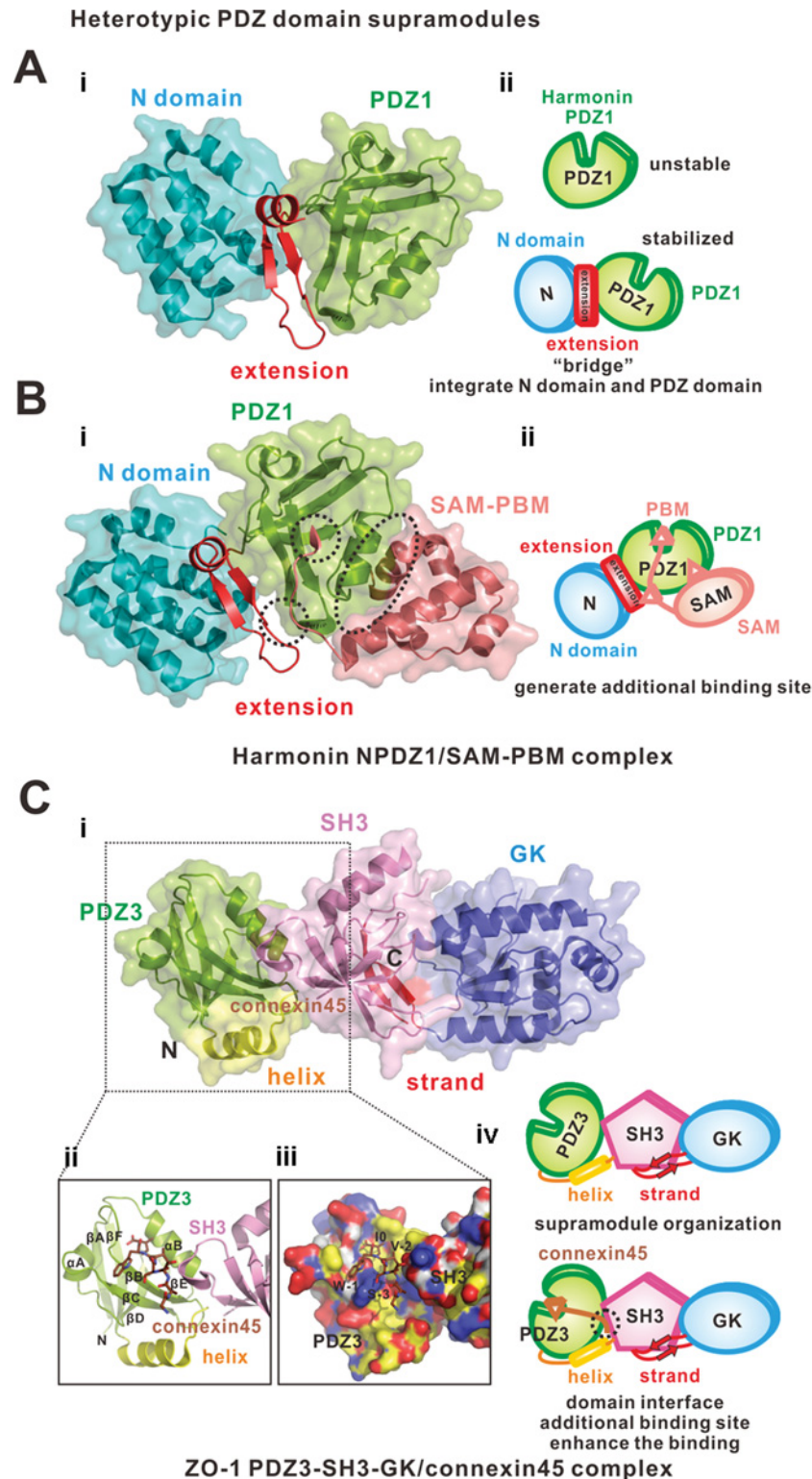
Heterotypic PDZ domain supramodules

Figure 5   Structure and target-binding properties of heterotypic PDZ supramodules

(**A** and **B**) Ribbon diagrams combined with surface representations of the harmonin NPDZ1 supramodule (**A**, panel i) and in complex with Sans SAM-PBM (PDB code 3K1R) (**B**, panel i). Schematic diagrams showing the NPDZ1 supramodule (**A**, panel ii) and its formation of complex with Sans SAM-PBM (**B**, panel ii). The black broken circles highlight three distinct target-binding sites available on the harmonin NPDZ1 supramodule. (**C**) Ribbon diagram combined with surface representation showing the structure of the ZO-1 PDZ3–SH3–GK/Cx45 peptide complex (**C**, panel i). Ribbon combined stick models showing the detailed interaction between ZO-1 PDZ3–SH3–GK and Cx45 (**C**, panel ii). Surface representation showing the detailed interaction between ZO-1 PDZ3–SH3–GK and Cx45 (**C**, panel iii). The positively charged amino acids of ZO-1 PDZ3–SH3–GK are highlighted in blue, the negatively charged residues are highlighted in red, the hydrophobic residues are highlighted in yellow and the others are highlighted in white. Schematic diagrams showing the ZO-1 PDZ3–SH3–GK supramodule organization and the formation of the ZO-1 PDZ3–SH3–GK/Cx45 complex (**C**, panel iv).

SH3–GK tandem, and directly interacts with the SH3 domain via PDZ peptide binding groove and the interdomain linker between PDZ3 and SH3, although the interaction between PDZ3 and the SH3–GK tandem seems to be very weak and transient [65,66]. Therefore this emerging evidence suggests that formation of the PDZ–SH3–GK supramodule may be a rather common feature for many of the MAGUK family members, although the detailed structural basis for the formation of PDZ3–SH3–GK supramodules and the consequent functional implications remain to be elucidated.

Non-canonical binding modes for canonical PDZ domains

The best known target-binding mode for a canonical PDZ domain is to recognize a short peptide fragment located at the extreme C-terminal tail of target proteins. However, accumulating evidence indicates that C-terminal tail peptides with only a few residues in length are too restrictive to fully account for the functional and structural characteristics of PDZ domains [17,18]. Large-scale systematic PDZ–target interaction screening studies [17,18] as well as many individual case studies (reviewed previously in [5,7]) have shown that PDZ domains both within the same class and sometimes even from different classes share overlapping bindings to short C-terminal peptides (i.e. the canonical PDZ–target interactions are rather promiscuous). However, such promiscuous PDZ–target interactions (i.e. with low specificities) are not compatible with highly specific cellular functions known for many PDZ domain proteins as well as their binding targets. Additionally, the majority of the reported PDZ–target peptide interactions display rather weak binding affinities (with K_d in the range of a few micromolar to tens of micromolar). It is again hard to rationalize specific functional roles for many PDZ scaffold–target protein complexes known to date. For example, many PDZ domain proteins with overlapping target-binding properties and a large number of receptors, ion channels or cell adhesion molecules containing PBMs are known to coexist in various specific cellular regions such as postsynaptic densities in neuronal synapses or intercellular junctions in polarized epithelia. It is conceptually challenging how PDZ–target interactions with rather poor specificities and weak affinities can support highly specific cellular functions of many PDZ-binding targets such as glutamate receptors, various ion channels including Ca^{2+} channels and many ligand-gated receptors. It is possible that our current understanding of PDZ–target interactions is partial, and many specific and strong PDZ–target interactions with new interaction modes have escaped our detection for various technical reasons. An increasing number of non-canonical target-binding modes revealed for canonical PDZ domains in recent years provide partial answers to the issue of target-binding specificities. Below, we summarize some of such non-canonical PDZ–target recognition modes.

It is now well-accepted that, in addition to binding to C-terminal peptides, some PDZ domains can specifically recognize internal peptide fragment from target proteins [19,20]. One such example is the Par (partitioning defective)-6 PDZ domain, which has been reported to bind to an internal peptide fragment from Pals1 as well as to conventional C-terminal peptides [19,67,68] (Figure 6A). The structure of the Par-6 PDZ–Pals1 peptide complex revealed that Pals1 internal ligand adopts an extended conformation to bind to the αB/βB groove of Par-6 PDZ. The side chain aspartic acid at the $+1$ position mimics the carboxy group of the canonical C-terminal peptide ligand in binding to the carboxy-group-binding loop of the PDZ domain [19]. Another example of recognizing internal peptide sequences is the interaction between the PDZ domains from β-syntrophin or PSD-95 and the internal β-hairpin

finger of the nNOS PDZ domain [20,69]. The PDZ domain of nNOS specifically heterodimerizes with the PDZ domains of β-syntrophin from muscle and with PSD-95 in neurons, and thereby regulates NO production and signalling in these tissues [70,71]. Structural analysis of nNOS PDZ in complex with β-syntrophin PDZ or with PSD-95 PDZ2 reveals that nNOS PDZ domain contains an unusual β-finger extension at its C-terminal end, and this β-finger extension is physically anchored to the back side (i.e. opposite to the αB/βB groove) of the nNOS PDZ itself via one of the β-strands. The other strand of the nNOS PDZ β-finger inserts into the αB/βB groove of β-syntrophin PDZ or PSD-95 PDZ2 [20,69] (Figure 6B). Analogous to the carboxy group from the canonical C-terminal peptides, the backbones of the residue in the turn connecting the two strands of the nNOS PDZ β-finger form hydrogen bonds with the carboxy-group-binding loop of the β-syntrophin–PSD-95 PDZ domains (Figure 6B, panel ii). A recent systematic study showed that a sizable portion of PDZ domains can form heterodimers with another PDZ domain [72], suggesting that dimerization is a rather general property of PDZ domains.

In addition to the residues at the extreme C-terminal ends, residues at the upstream positions (up to more than ten residues from the last C-terminal residue) are also important for many C-terminal PDZ targets to bind to their cognate PDZ domains ([73–77], and reviewed recently in [9]; therefore we only discuss this very briefly in the present review). For an example, Par-3 PDZ3 can bind to PTEN (phosphatase and tensin homologue deleted on chromosome 10) or VE-cadherin (vascular endothelial cadherin) with two discrete binding sites: a canonical PDZ–ligand interaction site and a distal charge–charge interaction site in the βB/βC loop (Figure 6C) [73,76]. This distinct target recognition mechanism confers the interaction specificity of Par-3 with its targets (e.g. PTEN), which is important for the cellular localization of PTEN at the junctional membranes of MDCK cells [73].

The Par-6 PDZ domain offers another example of non-canonical target-binding mode for a classical PDZ domain. The Par-6 PDZ domain contains a semi-CRIB [Cdc42 (cell division cycle 42)/Rac-interactive binding] sequence at its N-terminal end. Thus Par-6 PDZ is another example of PDZ domains with short extensions discussed above. The semi-CRIB sequence of Par-6 PDZ domain is known to binding to Cdc42 in a GTP-dependent manner, and the interaction require the semi-CRIB sequence to be covalently attached to the PDZ domain [78–80]. In the apo-form of Par-6 PDZ, the semi-CRIB extension is dynamic and unstructured [80]. Upon forming a complex with Cdc42, the semi-CRIB sequence forms a β-strand extension that is antiparallel to both β2 of Cdc42 and βA of Par-6 PDZ, thus integrating the two domains together by forming an eight-stranded β-sheet (Figure 6D). Cdc42 also makes direct contact with αA of Par-6 PDZ, leading to a conformational rearrangement of the helix and surrounding loops. Interestingly, binding of Cdc42 to the semi-CRIB sequence induces long-range allosteric conformational changes to the target-binding groove of the PDZ domain [80,81], and consequently enhances its target-binding affinities [67,82]. The Cdc42 binding-induced allosteric conformational change and the subsequent enhanced target binding have great implications for the regulation of PDZ domain-mediated protein complex organizations (see below for more details). Several recent studies have shed light on possible origins of the allosteric conformational couplings in PDZ domains. These studies provided evidence that a small set of amino acid residues in a PDZ domain are physically and energetically coupled with each other, most likely via their side chains, forming a long-range interaction network or networks spanning from the target-binding αB/βB groove to αA at the opposite side
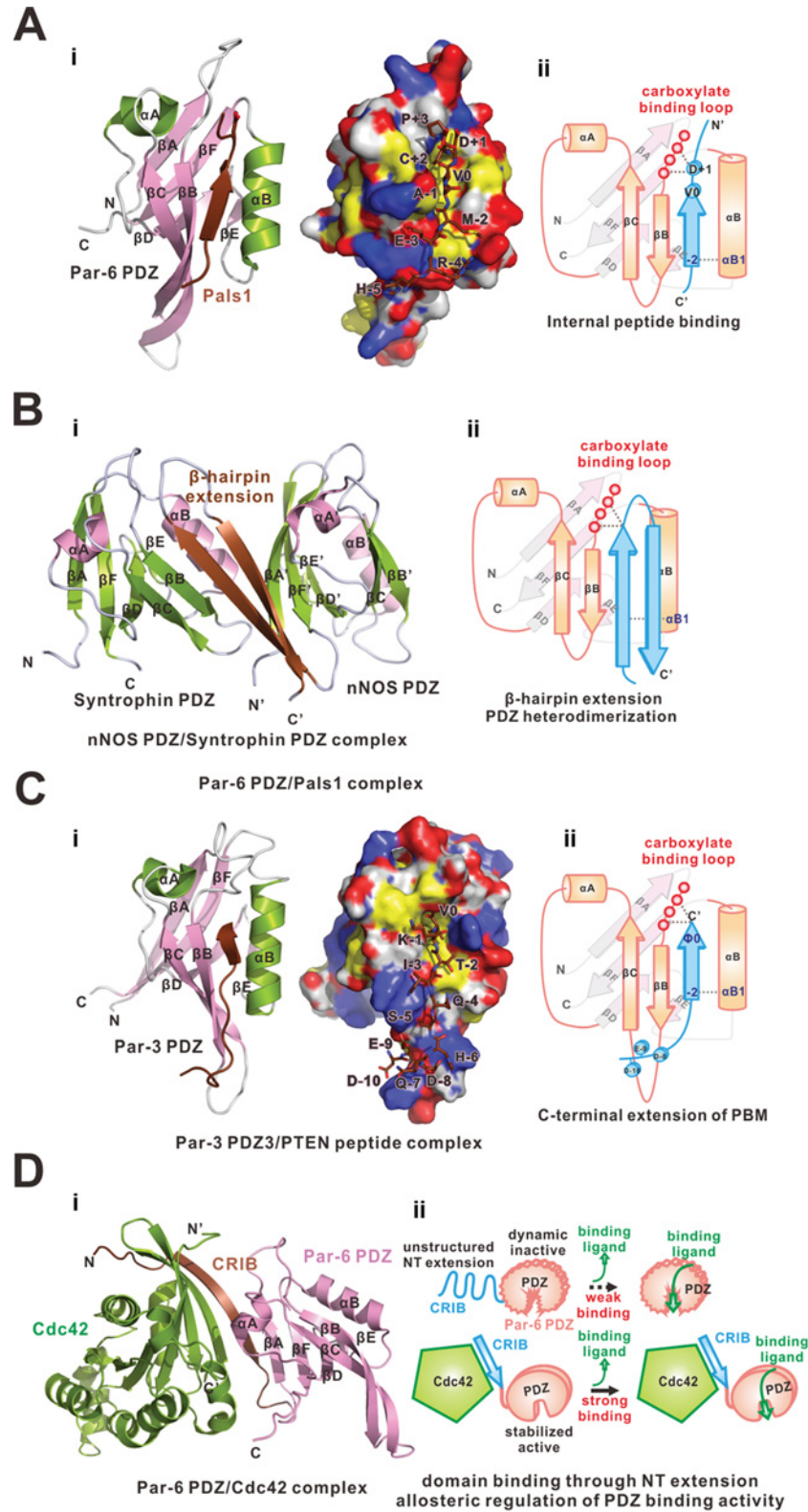
Figure 6   Non-canonical PDZ–target interactions

(**A**) Ribbon diagram combined with surface representation showing the Par-6 PDZ domain in complex with a Pals1 internal peptide (PDB code 1X8S). (**B**) The structure of the nNOS PDZ–syntrophin PDZ heterodimer (PDB code 1QAV). (**C**) The structure of Par-3 PDZ2 in complex with the PTEN peptide (PDB code 2K20). (**D**) The structure of the Cdc42–Par-6 PDZ complex (PDB code 1NF3). The colour coding of the surface diagrams is the same as that in Figure 5(C).
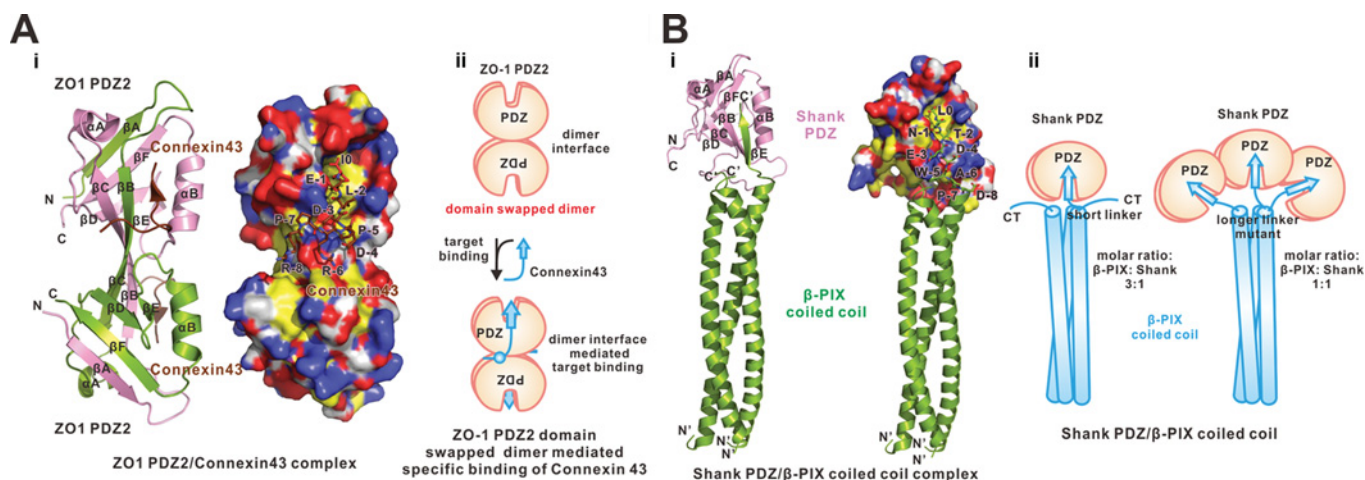
Figure 7   More examples of non-canonical PDZ–target interactions

(**A**) Domain-swapped dimerization of ZO-1 PDZ2 and its binding to the Cx43 peptide (PDB code 3CYY). The colour coding of the surface diagram is the same as that in Figure 5(C). (**B**) The structure of Shank PDZ in complex with β-PIX C-terminal tail coiled-coil domain (PDB code 3L4F). β-PIX forms a parallel trimer through the C-terminal leucine zipper and interact with one Shank PDZ domain forming a 3:1 stoichiometric complex.

of the domain [83–85]. Therefore it is possible that binding of a regulatory protein to a site opposite to the target-binding groove can influence target-binding property of a PDZ domain.

Another noteworthy case of non-canonical PDZ–target recognition is the interaction between ZO-1 PDZ2 and Cx43 (connexin43) involving the domain-swapped dimerization of ZO-1 PDZ2 [74]. ZO-1 plays critical functions in mediating the formation and maintenance of intercellular junctions, including tight junctions, adherens junctions and gap junctions, through direct binding to multiple junctional proteins including connexins, occludins, claudins, JAM1 (junctional adhesion molecule 1) and α-catenin [86,87]. The structure of ZO-1 PDZ2 and Cx43 complex reveals that ZO-1 PDZ2 domain undergoes a domain-swapped dimerization (Figure 7A), which is induced by the lack of the connecting residues between its βB and βC [74]. The formation of the domain-swapped dimer creates an additional highly charged target-binding site at the dimer interface distal to the canonical C-terminal peptide-binding groove (Figure 7A), thereby enhancing both the affinity and specificity of Cx43 in binding ZO-1 PDZ2 [74]. Disruption of the domain-swapped dimer formation by inserting a few flexible residues in the βB/βC loop of the PDZ domain abolishes the binding between ZO-1 PDZ2 and Cx43. The charge–charge interaction network formed by residues in the PDZ dimer interface and Cx43 not only confers the interaction specificity, but also may function as a regulatory switch for the interaction. Two completely conserved serine residues in the -9 and -10 positions of the Cx43 peptide have reported to be substrates of several kinases, including PKC (protein kinase C) and Akt [88]. Mutation of serine residues (-9 and -10 positions) to glutamate in the Cx43 to mimic their phosphorylations weakens the interaction between PDZ2 and Cx43, indicating that the phosphorylation may play a regulatory role for the dynamic assembly of the Cx43 gap junctions [74].

A somewhat intriguing PDZ–target-binding mode is an asymmetric interaction between the Shank PDZ monomer and the β-PIX (β-p21-activated kinase-interacting exchange factor) trimer [89]. The Shank family protein (Shank1, Shank2 and Shank3) are synaptic scaffolding proteins that play important roles in organizing multi-protein complexes in the PSD of excitatory glutamatergic synapses [90,91]. *SHANK* genes are known as causative genes for idiopathic autism spectrum disorders

[28,92,93]. β-PIX, a guanine-nucleotide-exchange factor for Rac1 and Cdc42 GTPases, is reported to interact with the PDZ domain of Shank to regulate synaptic development and plasticity [94]. Interestingly, both the C-terminal leucine zipper domain as well as the extreme C-terminal PBM of β-PIX is required for its Shank PDZ binding [94]. The complex structure of Shank PDZ and β-PIX shows that the C-terminal leucine zipper of β-PIX forms a parallel trimer with three PBMs pointing to the same direction. However, each β-PIX trimer binds to one Shank PDZ domain forming a 3:1 stoichiometric complex [89] (Figure 7B). In the Shank PDZ–β-PIX complex, only one of the three β-PIX PBMs binds to the canonical binding groove of Shank PDZ due to the steric hindrance between the bound PDZ domain and the C-terminal end of the β-PIX coiled-coil, and the remaining two are not involved in the binding and are unstructured (Figure 7B). Relief of the steric hindrance by insertion of a 15-residue flexible loop between the coiled coil and PBM allows β-PIX to bind to Shank PDZ by forming a 3:3 complex [89] (Figure 7B, panel ii). Nonetheless, it is rather perplexing as to why β-PIX needs to form a trimer to bind to a single Shank PDZ (i.e. the functional significance of this asymmetric Shank PDZ–β-PIX interaction is unclear).

Although numerous examples of PDZ–ligand interactions have now been identified, and structural bases governing many of such interactions have been elucidated to date, it is safe to predict that many more additional PDZ–target interaction modes remain to be discovered. We believe that various forms of PDZ extensions as well as non-canonical PBMs can generate diverse interaction modes for forming specific PDZ protein–target complexes demanded by physiological settings. Uncovering these new PDZ–target interaction modes and elucidation of the molecular mechanisms governing these new modes of interactions is an important research direction for the PDZ domain scaffold proteins in the coming years.

FUNCTIONAL REGULATIONS OF INTERACTIONS OF PDZ DOMAINS WITH THEIR TARGETS

Intuitively, the majority of PDZ domain–target interactions should be reversible. Thus it is expected that the formation of many of
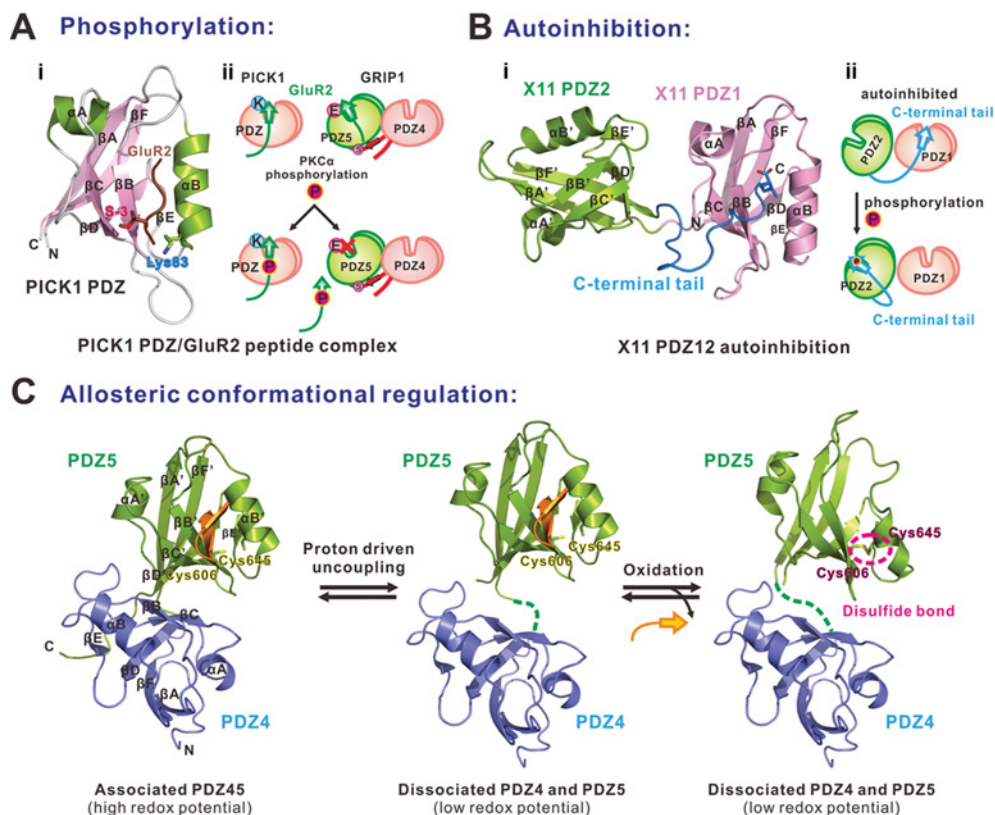
Figure 8   Regulations of PDZ–target interactions

(**A**) Phosphorylation-mediated regulation of PDZ–target interactions. (**A**, panel i) Ribbon diagram showing the complex structure of PICK1 PDZ with the GluR2 peptide (PDB code 2PKU). (**A**, panel ii) Schematic diagrams showing that the PKC phosphorylation of GluR2 differentially regulates its binding to GRIP1 PDZ45 and PICK1 PDZ. Lys[83] at the αB1 position of PICK1 PDZ is highlighted. (**B**) Auto-inhibition of X11 PDZ1 by its own C-terminal PBM. (**B**, panel i) Ribbon diagram showing the auto-inhibited conformation of X11α PDZ12C (PDB code 1U3B). (**B**, panel ii) Schematic diagram showing the phosphorylation-mediated switch of the inhibition of the two PDZ domains by the C-terminal PBM. The C-terminal tail of X11α folds back and occupies the target-binding groove of PDZ1, resulting a closed conformation of PDZ1. Phosphorylation of tyrosine residue (-1) releases PBM from PDZ1 and promotes its binding to PDZ2. (**C**) Ribbon diagrams showing that the redox potential of the Cys[606]–Cys[645] disulfide in INAD PDZ5 can be allosterically regulated through direct coupling of PDZ4 and the C-terminal tail of PDZ5 (PDB code 3R0H).

PDZ domain–target complexes is regulated. Compared with the wealth of knowledge on the molecular mechanisms governing PDZ–target interactions discussed above, our knowledge of the regulation of these interactions is scant. Nonetheless, several regulatory switches that can specifically modulate certain PDZ–target interactions have been discovered over the last few years. In the remaining part of the present review, we summarize this aspect of findings.

Phosphorylation-mediated regulation of PDZ–target interactions

PBMs are enriched with serine/threonine or tyrosine residues at positions -1, -2, -3, or even more upstream positions, and these phosphorylatable amino acid residues often directly participate in their binding to cognate PDZ domains. Thus phosphorylation of serine/threonine or tyrosine residues in PBMs are expected to weaken or even disrupt their PDZ-binding capacities [95–99]. One of the well-characterized phosphorylation-regulated PDZ target proteins is the GluR2 subunit of AMPA receptors. The GluR2 tail with a sequence of IESVKI is known to bind to the PDZ domain of PICK1 and PDZ45 of GRIP1 [100,101]. The serine residue in the -3 position of the GluR2 tail can be phosphorylated by PKC. Interestingly, the PKC phosphorylation of GluR2 differentially regulates its binding to GRIP1 and PICK1. The phosphorylated GluR2 has a much decreased binding affinity to GRIP1 PDZ45, but retains

a similar binding strength to PICK1 PDZ [98,99] (Figure 8A). Structural studies of the GluR2 tail peptide with PICK1 PDZ and with GRIP1 PDZ5 in the PDZ45 tandem revealed that a lysine residue (Lys[83]) at the αB1 position of PICK1 PDZ would be favourable for its binding to the phosphorylated GluR2 tail peptide. Instead, the corresponding αB1 residue of GRIP1 PDZ5 is a negatively charged glutamic acid, which would repel the phosphorylated GluR2 tail peptide and thus weaken its binding [102] (Figure 8).

The NR2 (NMDA receptor subunit 2) tail contains a conserved ESDV sequence that is known to bind to the N-terminal two PDZ domains of PSD-95. It was discovered in a previous study that protein kinase CK2 preferentially phosphorylates serine at position -2 of the NR2B tail. Since the side chain of serine (-2) of the NR2 tail is critical for binding to PSD-95 PDZ12, the phosphorylated NR2B has reduced binding to PSD-95 PDZ and thus is selectively removed from synapse via endocytosis [96]. Therefore the CK2 phosphorylation-mediated selective removal of NR2B subunit provides a means of determining the NR2 subunit composition of synaptic NMDA receptors.

Auto-inhibition

Auto-inhibition plays a significant role in the regulation of many proteins. A number of PDZ proteins contain a PBM at the very C-terminal end, which can bind to their own PDZ

domain and thus prevent its binding to other ligands. One such example is the C-terminal PBM-mediated auto-inhibition in X11α. The X11/Mint family of multidomain scaffold proteins, comprising X11α/Mint1, X11β/Mint2 and X11γ/Mint3, is involved in polarized trafficking of receptors and ion channels to plasma membranes. Each member of the X11 family contains a conserved PTB (phosphotyrosine-binding domain) domain followed by two C-terminal PDZ domains. X11s have been reported to bind to a number of proteins including presenilin, calcium channels, neurexin and AMPA receptors [103–107]. It was reported that the target-binding property of isolated PDZ domains is distinctly different from the target-binding property of two PDZ domains connected in tandem [104,106]. Structural analysis reveals that the two PDZ domains of X11α directly interact with each other forming a tandem PDZ supramodule, and the highly conserved C-terminal tail of X11α folds back and inserts into the target-binding groove of its own first PDZ domain, resulting in a closed conformation of PDZ1 [52] (Figure 8B). Interestingly, the auto-inhibited conformation of X11α can be regulated by phosphorylation. The X11α PBM contains a highly conserved tyrosine residue at the -1 position, which is essential for binding to PDZ1. Replacement of the tyrosine residue with glutamic acid abolishes the C-terminal tail's binding to PDZ1, and thus releases the auto-inhibition. Unexpectedly, the tyrosine-to-glutamic acid mutated PBM tail released from PDZ1 occupies the target-binding groove of PDZ2 in the PDZ12 tandem [52], indicating that the penultimate tyrosine residue of X11α may function as a phosphorylation-dependent switch dynamically regulating the target-binding properties of the PDZ12 supramodule (Figure 8B).

In addition to X11α, PBM-mediated auto-inhibition and the underlying structural basis of auto-inhibition have been described for the PDZ domain proteins of NHERF1 [35], NHERF3/PDZK1 [108] and Tamalin [109]. In these cases, the auto-inhibition is shown to invariably weaken the target binding of the corresponding inhibited PDZ domains.

Allosteric conformational regulation

Multiple studies provide evidence suggesting that long-range allosteric conformational changes can modulate target-binding properties of PDZ domains. For example, helical extensions of PDZ domains, such as those described previously for PSD-95 PDZ3 [30] and NHERF1 PDZ2 [35], modulate the internal dynamics as well as the side-chain conformation of PDZ domains and thereby enhance their target-binding affinities (Figure 3). Cdc42 directly contacts the αA helix of Par-6 PDZ, leading to a conformational rearrangement of the Par-6 PDZ domain, including the carboxy-group-binding loop and αB, allowing PDZ to bind to its ligand with higher affinities [80,82] (Figure 6D). In the case of INAD PDZ45, the redox potential of the Cys^{606}–Cys^{645} pair in the target-binding groove of PDZ5 is allosterically regulated by direct conformational coupling with PDZ4. The Cys^{606}–Cys^{645} pair in the uncoupled PDZ5 adopts the oxidized state due to a low redox potential. Formation of a PDZ45 locks PDZ5 in the reduced state by raising the redox potential of the Cys^{606}–Cys^{645} pair [43,44] (Figure 8B). The coupling of INAD PDZ45 is likely to be regulated by protons generated during photon-induced hydrolysis of PIP$_2$ by PLCβ. Taken together, the above examples demonstrate that the target-binding properties of the extended PDZ domains, as well as homo- or hetero-typic PDZ supramodules, can be regulated by allosteric conformational alterations rooted with different mechanisms. It is certainly possible that additional cases of allosteric-mediated PDZ–target interaction modes will be discovered in the future.

CONCLUSION

The year 2013 marks the 20th anniversary of the discovery of PDZ domains [1–3]. Enormous advances have been made over the last 20 years in our understanding of the functions and underlying mechanisms of PDZ domain proteins. To date, we know that PDZ domain proteins are one of the most abundant families of proteins in multicellular eukaryotes. PDZ domains play critical roles in very broad processes of physiological functions, and mutations of PDZ domain proteins or their binding targets are frequently associated with various human diseases including cancers, immune diseases and neuro-disorders. The present review tries to summarize the structural features of a number of PDZ domains and their target recognition properties beyond the well-established canonical PDZ domains that are extensively reviewed in the literature. We also discuss some of the known regulatory mechanisms of PDZ–target interactions. It is particularly interesting to note that the catalytically inactive scaffold proteins are not mere passive molecular 'glue', but can in fact actively regulate signalling events. We believe that the supramodular properties of PDZ domains, termed extended PDZ domains, homotypic PDZ supramodules and heterotypic PDZ supramodules in the present review, are the norm rather than special cases for a large portion of PDZ domain proteins. A more general lesson learnt from studies of PDZ domain proteins is that several domains in multidomain scaffold proteins can often form structural and functional supramodules, in contrast with the simple 'beads-on-a-string' model that is commonly perceived for each individual domain in these scaffold proteins. Finally, we believe that a large part of the general principles operated by the PDZ domain scaffold proteins reviewed in the present paper are likely to be applicable for other scaffold proteins as well.

REFERENCES

1   Cho, K. O., Hunt, C. A. and Kennedy, M. B. (1992) The rat brain postsynaptic density fraction contains a homolog of the *Drosophila* discs-large tumor suppressor protein. Neuron **9**, 929–942

2   Willott, E., Balda, M., Fanning, A., Jameson, B., Itallie, C. and Anderson, J. (1993) The tight junction protein ZO-1 is homologous to the *Drosophila* discs-large tumor suppressor protein of septate junctions. Proc. Natl. Acad. Sci. U.S.A. **90**, 7834–7838

3   Woods, D. F. and Bryant, P. J. (1993) ZO-1, DlgA and PSD-95/SAP90: homologous proteins in tight, septate and synaptic cell junctions. Mech. Dev. **44**, 85–89

4   Kennedy, M. B. (1995) Origin of PDZ (DHR, GLGF) domains. Trends Biochem. Sci. **20**, 350

5   Harris, B. Z. and Lim, W. A. (2001) Mechanism and role of PDZ domains in signaling complex assembly. J. Cell Sci. **114**, 3219–3231

6   Zhang, M. and Wang, W. (2003) Organization of signaling complexes by PDZ-domain scaffold proteins. Acc. Chem. Res. **36**, 530–538

7   Feng, W. and Zhang, M. (2009) Organization and dynamics of PDZ-domain-related supramodules in the postsynaptic density. Nat. Rev. Neurosci. **10**, 87–99

8   Wang, C. K., Pan, L., Chen, J. and Zhang, M. (2010) Extensions of PDZ domains as important structural and functional elements. Protein Cell **1**, 737–751

9   Luck, K., Charbonnier, S. and Travé, G. (2012) The emerging contribution of sequence context to the specificity of protein interactions mediated by PDZ domains. FEBS Lett. **586**, 2648–2661

10 Subbaiah, V. K., Kranjec, C., Thomas, M. and Banks, L. (2011) PDZ domains: the building blocks regulating tumorigenesis. Biochem. J. **439**, 195–205

11 Pan, L. and Zhang, M. (2012) Structures of Usher syndrome 1 proteins and their complexes. Physiology **27**, 25–42

12 Martin-Belmonte, F. and Perez-Moreno, M. (2012) Epithelial cell polarity, stem cells and cancer. Nat. Rev. Cancer **12**, 23–38

13 Facciuto, F., Cavatorta, A. L., Valdano, M. B., Marziali, F. and Gardiol, D. (2012) Differential expression of PDZ domain-containing proteins in human diseases: challenging topics and novel issues. FEBS J. **279**, 3538–3548

14 Doyle, D. A., Lee, A., Lewis, J., Kim, E., Sheng, M. and MacKinnon, R. (1996) Crystal structures of a complexed and peptide-free membrane protein-binding domain: molecular basis of peptide recognition by PDZ. Cell **85**, 1067–1076

15 Sheng, M. and Sala, C. (2001) PDZ domains and the organization of supramolecular complexes. Annu. Rev. Neurosci. **24**, 1–29

16 Songyang, Z., Fanning, A. S., Fu, C., Xu, J., Marfatia, S. M., Chishti, A. H., Crompton, A., Chan, A. C., Anderson, J. M. and Cantley, L. C. (1997) Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. Science **275**, 73–77

17 Stiffler, M. A., Chen, J. R., Grantcharova, V. P., Lei, Y., Fuchs, D., Allen, J. E., Zaslavskaia, L. A. and MacBeath, G. (2007) PDZ domain binding selectivity is optimized across the mouse proteome. Science **317**, 364–369

18 Tonikian, R., Zhang, Y., Sazinsky, S. L., Currell, B., Yeh, J. H., Reva, B., Held, H. A., Appleton, B. A., Evangelista, M., Wu, Y. et al. (2008) A specificity map for the PDZ domain family. PLoS Biol. **6**, e239

19 Penkert, R. R., DiVittorio, H. M. and Prehoda, K. E. (2004) Internal recognition through PDZ domain plasticity in the Par-6–Pals1 complex. Nat. Struct. Mol. Biol. **11**, 1122–1127

20 Hillier, B. J., Christopherson, K. S., Prehoda, K. E., Bredt, D. S. and Lim, W. A. (1999) Unexpected modes of PDZ domain scaffolding revealed by structure of nNOS–syntrophin complex. Science **284**, 812–815

21 Zimmermann, P., Meerschaert, K., Reekmans, G., Leenaerts, I., Small, J. V., Vandekerckhove, J., David, G. and Gettemans, J. (2002) PIP(2)-PDZ domain binding controls the association of syntenin with the plasma membrane. Mol. Cell **9**, 1215–1225

22 Mortier, E., Wuytens, G., Leenaerts, I., Hannes, F., Heung, M. Y., Degeest, G., David, G. and Zimmermann, P. (2005) Nuclear speckles and nucleoli targeting by PIP2–PDZ domain interactions. EMBO J. **24**, 2556–2565

23 Yan, J., Wen, W., Xu, W., Long, J. F., Adams, M. E., Froehner, S. C. and Zhang, M. (2005) Structure of the split PH domain and distinct lipid-binding properties of the PH-PDZ supramodule of α-syntrophin. EMBO J. **24**, 3985–3995

24 Wu, H., Feng, W., Chen, J., Chan, L. N., Huang, S. and Zhang, M. (2007) PDZ domains of Par-3 as potential phosphoinositide signaling integrators. Mol. Cell **28**, 886–898

25 Chen, Y., Sheng, R., Kallberg, M., Silkov, A., Tun, M. P., Bhardwaj, N., Kurilova, S., Hall, R. A., Honig, B., Lu, H. and Cho, W. (2012) Genome-wide functional annotation of dual-specificity protein- and lipid-binding modules that regulate protein interactions. Mol. Cell **46**, 226–237

26 Roux, A. F., Faugere, V., Le Guedard, S., Pallares-Ruiz, N., Vielle, A., Chambert, S., Marlin, S., Hamel, C., Gilbert, B., Malcolm, S. and Claustres, M. (2006) Survey of the frequency of USH1 gene mutations in a cohort of Usher patients shows the importance of cadherin 23 and protocadherin 15 genes and establishes a detection rate of above 90%. J. Med. Genet. **43**, 763–768

27 Leblond, C. S., Heinrich, J., Delorme, R., Proepper, C., Betancur, C., Huguet, G., Konyukh, M., Chaste, P., Ey, E., Rastam, M. et al. (2012) Genetic and functional analyses of *SHANK2* mutations suggest a multiple hit model of autism spectrum disorders. PLoS Genet. **8**, e1002521

28 Jiang, Y. H. and Ehlers, M. D. (2013) Modeling autism by SHANK gene mutations in mice. Neuron **78**, 8–27

29 Reference deleted

30 Petit, C. M., Zhang, J., Sapienza, P. J., Fuentes, E. J. and Lee, A. L. (2009) Hidden dynamic allostery in a PDZ domain. Proc. Natl. Acad. Sci. U.S.A. **106**, 18249–18254

31 Chi, C. N., Haq, S. R., Rinaldo, S., Dogan, J., Cutruzzolà, F., Engström, Å., Gianni, S., Lundström, P. and Jemth, P. (2012) Interactions outside the boundaries of the canonical binding groove of a PDZ domain influence ligand binding. Biochemistry **51**, 8971–8979

32 Mostarda, S., Gfeller, D. and Rao, F. (2012) Beyond the binding site: the role of the $\beta_2-\beta_3$ loop and extra-domain structures in PDZ domains. PLoS Comput. Biol. **8**, e1002429

33 Ballif, B. A., Carey, G. R., Sunyaev, S. R. and Gygi, S. P. (2008) Large-scale identification and evolution indexing of tyrosine phosphorylation sites from murine brain. J. Proteome Res. **7**, 311–318

34 Zhang, J., Petit, C. M., King, D. S. and Lee, A. L. (2011) Phosphorylation of a PDZ domain extension modulates binding affinity and interdomain interactions in postsynaptic density-95 (PSD-95) protein, a membrane-associated guanylate kinase (MAGUK). J. Biol. Chem. **286**, 41776–41785

35 Bhattacharya, S., Dai, Z., Li, J., Baxter, S., Callaway, D. J., Cowburn, D. and Bu, Z. (2010) A conformational switch in the scaffolding protein NHERF1 controls autoinhibition and complex formation. J. Biol. Chem. **285**, 9981–9994

36 Bhattacharya, S., Ju, J. H., Orlova, N., Khajeh, J. A., Cowburn, D. and Bu, Z. (2013) Ligand-induced dynamic changes in extended PDZ domains from NHERF1. J. Mol. Biol. **425**, 2509–2528

37 Feng, W., Shi, Y., Li, M. and Zhang, M. (2003) Tandem PDZ repeats in glutamate receptor-interacting proteins have a novel mode of PDZ domain-mediated target binding. Nat. Struct. Biol. **10**, 972–978

38 Long, J., Wei, Z., Feng, W., Yu, C., Zhao, Y. X. and Zhang, M. (2008) Supramodular nature of GRIP1 revealed by the structure of its PDZ12 tandem in complex with the carboxyl tail of Fras1. J. Mol. Biol. **375**, 1457–1468

39 Dong, H., Zhang, P., Song, I., Petralia, R. S., Liao, D. and Huganir, R. L. (1999) Characterization of the glutamate receptor-interacting proteins GRIP1 and GRIP2. J. Neurosci. **19**, 6930–6941

40 Osten, P., Khatri, L., Perez, J. L., Kohr, G., Giese, G., Daly, C., Schulz, T. W., Wensky, A., Lee, L. M. and Ziff, E. B. (2000) Mutagenesis reveals a role for ABP/GRIP binding to GluR2 in synaptic surface accumulation of the AMPA receptor. Neuron **27**, 313–325

41 Zhang, Q., Fan, J. S. and Zhang, M. (2001) Interdomain chaperoning between PSD-95, Dlg, and Zo-1 (PDZ) domains of glutamate receptor-interacting proteins. J. Biol. Chem. **276**, 43216–43220

42 Yau, K. W. and Hardie, R. C. (2009) Phototransduction motifs and variations. Cell **139**, 246–264

43 Mishra, P., Socolich, M., Wall, M. A., Graves, J., Wang, Z. and Ranganathan, R. (2007) Dynamic scaffolding in a G protein-coupled signaling system. Cell **131**, 80–92

44 Liu, W., Wen, W., Wei, Z., Yu, J., Ye, F., Liu, C. H., Hardie, R. C. and Zhang, M. (2011) The INAD scaffold is a dynamic, redox-regulated modulator of signaling in the *Drosophila* eye. Cell **145**, 1088–1101

45 Huang, J., Liu, C. H., Hughes, S. A., Postma, M., Schwiening, C. J. and Hardie, R. C. (2010) Activation of TRP channels by protons and phosphoinositide depletion in *Drosophila* photoreceptors. Curr. Biol. **20**, 189–197

46 Long, J. F., Tochio, H., Wang, P., Fan, J. S., Sala, C., Niethammer, M., Sheng, M. and Zhang, M. (2003) Supramodular structure and synergistic target binding of the N-terminal tandem PDZ domains of PSD-95. J. Mol. Biol. **327**, 203–214

47 Wang, W., Weng, J., Zhang, X., Liu, M. and Zhang, M. (2008) Creating conformational entropy by increasing interdomain mobility in ligand binding regulation: a revisit to N-terminal tandem PDZ domains of PSD-95. J. Am. Chem. Soc. **131**, 787–796

48 Chi, C. N., Bach, A., Gottschalk, M., Kristensen, A. S., Stromgaard, K. and Jemth, P. (2010) Deciphering the kinetic binding mechanism of dimeric ligands using a potent plasma-stable dimeric inhibitor of postsynaptic density protein-95 as an example. J. Biol. Chem. **285**, 28252–28260

49 Bach, A., Clausen, B. H., Møller, M., Vestergaard, B., Chi, C. N., Round, A., Sørensen, P. L., Nissen, K. B., Kastrup, J. S., Gajhede, M. et al. (2012) A high-affinity, dimeric inhibitor of PSD-95 bivalently interacts with PDZ1–2 and protects against ischemic brain damage. Proc. Natl. Acad. Sci. U.S.A. **109**, 3317–3322

50 Sainlos, M., Tigaret, C., Poujol, C., Olivier, N. B., Bard, L., Breillat, C., Thiolon, K., Choquet, D. and Imperiali, B. (2011) Biomimetic divalent ligands for the acute disruption of synaptic AMPAR stabilization. Nat. Chem. Biol. **7**, 81–91

51 Zimmermann, J., Kuhne, R., Sylvester, M. and Freund, C. (2007) Redox-regulated conformational changes in an SH3 domain. Biochemistry **46**, 6971–6977

52 Long, J. F., Feng, W., Wang, R., Chan, L. N., Ip, F. C., Xia, J., Ip, N. Y. and Zhang, M. (2005) Autoinhibition of X11/Mint scaffold proteins revealed by the closed conformation of the PDZ tandem. Nat. Struct. Mol. Biol. **12**, 722–728

53 El-Amraoui, A. and Petit, C. (2005) Usher I syndrome: unravelling the mechanisms that underlie the cohesion of the growing hair bundle in inner ear sensory cells. J. Cell Sci. **118**, 4593–4603

54 Schwander, M., Kachar, B. and Muller, U. (2010) Review series: the cell biology of hearing. J. Cell Biol. **190**, 9–20

55 Yan, J., Pan, L., Chen, X., Wu, L. and Zhang, M. (2010) The structure of the harmonin/sans complex reveals an unexpected interaction mode of the two Usher syndrome proteins. Proc. Natl. Acad. Sci. U.S.A. **107**, 4040–4045

56 Funke, L., Dakoji, S. and Bredt, D. S. (2005) Membrane-associated guanylate kinases regulate adhesion and plasticity at cell junctions. Annu. Rev. Biochem. **74**, 219–245

57 Zhu, J., Shang, Y., Chen, J. and Zhang, M. (2012) Structure and function of the guanylate kinase-like domain of the MAGUK family scaffold proteins. Front. Biol. **7**, 379–396

58 Pan, L., Chen, J., Yu, J., Yu, H. and Zhang, M. (2011) The structure of the PDZ3–SH3–GuK tandem of ZO-1 protein suggests a supramodular organization of the membrane-associated guanylate kinase (MAGUK) family scaffold protein core. J. Biol. Chem. **286**, 40069–40074

59 Nomme, J., Fanning, A. S., Caffrey, M., Lye, M. F., Anderson, J. M. and Lavie, A. (2011) The Src homology 3 domain is required for junctional adhesion molecule binding to the third PDZ domain of the scaffolding protein ZO-1. J. Biol. Chem. **286**, 43352–43360

60 McGee, A. W., Dakoji, S. R., Olsen, O., Bredt, D. S., Lim, W. A. and Prehoda, K. E. (2001) Structure of the SH3-guanylate kinase module from PSD-95 suggests a mechanism for regulated assembly of MAGUK scaffolding proteins. Mol. Cell **8**, 1291–1301

61 Tavares, G. A., Panepucci, E. H. and Brunger, A. T. (2001) Structural characterization of the intramolecular interaction between the SH3 and guanylate kinase domains of PSD-95. Mol. Cell **8**, 1313–1325

62 Zhu, J., Shang, Y., Xia, C., Wang, W., Wen, W. and Zhang, M. (2011) Guanylate kinase domains of the MAGUK family scaffold proteins as specific phospho-protein-binding modules. EMBO J. **30**, 4986–4997

63 Qian, Y. and Prehoda, K. E. (2006) Interdomain interactions in the tumor suppressor discs large regulate binding to the synaptic protein GukHolder. J. Biol. Chem. **281**, 35757–35763

64 Newman, R. A. and Prehoda, K. E. (2009) Intramolecular interactions between the SRC homology 3 and guanylate kinase domains of discs large regulate its function in asymmetric cell division. J. Biol. Chem. **284**, 12924–12932

65 Zhang, J., Lewis, S. M., Kuhlman, B. and Lee, A. L. (2013) Supertertiary structure of the MAGUK core from PSD-95. Structure **21**, 402–413

66 McCann, J. J., Zheng, L., Rohrbeck, D., Felekyan, S., Kuhnemuth, R., Sutton, R. B., Seidel, C. A. and Bowen, M. E. (2012) Supertertiary structure of the synaptic MAGuK scaffold proteins is conserved. Proc. Natl. Acad. Sci. U.S.A. **109**, 15775–15780

67 Hurd, T. W., Gao, L., Roh, M. H., Macara, I. G. and Margolis, B. (2003) Direct interaction of two polarity complexes implicated in epithelial tight junction assembly. Nat. Cell Biol. **5**, 137–142

68 Lemmers, C., Michel, D., Lane-Guermonprez, L., Delgrossi, M. H., Medina, E., Arsanto, J. P. and Le Bivic, A. (2004) CRB3 binds directly to Par6 and regulates the morphogenesis of the tight junctions in mammalian epithelial cells. Mol. Biol. Cell **15**, 1324–1333

69 Tochio, H., Mok, Y. K., Zhang, Q., Kan, H. M., Bredt, D. S. and Zhang, M. (2000) Formation of nNOS/PSD-95 PDZ dimer requires a preformed β-finger structure from the nNOS PDZ domain. J. Mol. Biol. **303**, 359–370

70 Brenman, J. E., Chao, D. S., Xia, H., Aldape, K. and Bredt, D. S. (1995) Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy. Cell **82**, 743–752

71 Brenman, J. E., Chao, D. S., Gee, S. H., McGee, A. W., Craven, S. E., Santillano, D. R., Wu, Z., Huang, F., Xia, H., Peters, M. F., Froehner, S. C. and Bredt, D. S. (1996) Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and α1-syntrophin mediated by PDZ domains. Cell **84**, 757–767

72 Chang, B. H., Gujral, T. S., Karp, E. S., BuKhalid, R., Grantcharova, V. P. and MacBeath, G. (2011) A systematic family-wide investigation reveals that ∼30 % of mammalian PDZ domains engage in PDZ–PDZ interactions. Chem. Biol. **18**, 1143–1152

73 Feng, W., Wu, H., Chan, L. N. and Zhang, M. (2008) Par-3-mediated junctional localization of the lipid phosphatase PTEN is required for cell polarity establishment. J. Biol. Chem. **283**, 23440–23449

74 Chen, J., Pan, L., Wei, Z., Zhao, Y. and Zhang, M. (2008) Domain-swapped dimerization of ZO-1 PDZ2 generates specific and regulatory connexin43-binding sites. EMBO J. **27**, 2113–2123

75 Birrane, G., Chung, J. and Ladias, J. A. A. (2003) Novel mode of ligand recognition by the erbin PDZ domain. J. Biol. Chem. **278**, 1399–1402

76 Tyler, R. C., Peterson, F. C. and Volkman, B. F. (2010) Distal interactions within the par3–VE–cadherin complex. Biochemistry **49**, 951–957

77 Balana, B., Maslennikov, I., Kwiatkowski, W., Stern, K. M., Bahima, L., Choe, S. and Slesinger, P. A. (2011) Mechanism underlying selective regulation of G protein-gated inwardly rectifying potassium channels by the psychostimulant-sensitive sorting nexin 27. Proc. Natl. Acad. Sci. U.S.A. **108**, 5831–5836

78 Joberty, G., Petersen, C., Gao, L. and Macara, I. G. (2000) The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. Nat. Cell Biol. **2**, 531–539

79 Gao, L., Joberty, G. and Macara, I. G. (2002) Assembly of epithelial tight junctions is negatively regulated by Par6. Curr. Biol. **12**, 221–225

80 Garrard, S. M., Capaldo, C. T., Gao, L., Rosen, M. K., Macara, I. G. and Tomchick, D. R. (2003) Structure of Cdc42 in a complex with the GTPase-binding domain of the cell polarity protein, Par6. EMBO J. **22**, 1125–1133

81 Whitney, D. S., Peterson, F. C. and Volkman, B. F. (2011) A conformational switch in the CRIB–PDZ module of Par-6. Structure **19**, 1711–1722

82 Peterson, F. C., Penkert, R. R., Volkman, B. F. and Prehoda, K. E. (2004) Cdc42 regulates the Par-6 PDZ domain through an allosteric CRIB–PDZ transition. Mol. Cell **13**, 665–676

83 McLaughlin, Jr, R. N., Poelwijk, F. J., Raman, A., Gosal, W. S. and Ranganathan, R. (2012) The spatial architecture of protein function and adaptation. Nature **491**, 138–142

84 Chi, C. N., Elfstrom, L., Shi, Y., Snall, T., Engstrom, A. and Jemth, P. (2008) Reassessing a sparse energetic network within a single protein domain. Proc. Natl. Acad. Sci. U.S.A. **105**, 4679–4684

85 Hultqvist, G., Haq, S. R., Punekar, A. S., Chi, C. N., Engstrom, A., Bach, A., Stromgaard, K., Selmer, M., Gianni, S. and Jemth, P. (2013) Energetic pathway sampling in a protein interaction domain. Structure **21**, 1193–1202

86 Mitic, L. L. and Anderson, J. M. (1998) Molecular architecture of tight junctions. Annu. Rev. Physiol. **60**, 121–142

87 Adachi, M., Inoko, A., Hata, M., Furuse, K., Umeda, K., Itoh, M. and Tsukita, S. (2006) Normal establishment of epithelial tight junctions in mice and cultured cells lacking expression of ZO-3, a tight-junction MAGUK protein. Mol. Cell Biol. **26**, 9003–9015

88 Solan, J. L. and Lampe, P. D. (2005) Connexin phosphorylation as a regulatory event linked to gap junction channel assembly. Biochimi. Biophys. Acta **1711**, 154–163

89 Im, Y. J., Kang, G. B., Lee, J. H., Park, K. R., Song, H. E., Kim, E., Song, W. K., Park, D. and Eom, S. H. (2010) Structural basis for asymmetric association of the βPIX coiled coil and shank PDZ. J. Mol. Biol. **397**, 457–466

90 Naisbitt, S., Kim, E., Tu, J. C., Xiao, B., Sala, C., Valtschanoff, J., Weinberg, R. J., Worley, P. F. and Sheng, M. (1999) Shank, a novel family of postsynaptic density proteins that binds to the NMDA receptor/PSD-95/GKAP complex and cortactin. Neuron **23**, 569–582

91 Tu, J. C., Xiao, B., Naisbitt, S., Yuan, J. P., Petralia, R. S., Brakeman, P., Doan, A., Aakalu, V. K., Lanahan, A. A., Sheng, M. and Worley, P. F. (1999) Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. Neuron **23**, 583–592

92 Berkel, S., Marshall, C. R., Weiss, B., Howe, J., Roeth, R., Moog, U., Endris, V., Roberts, W., Szatmari, P., Pinto, D. et al. (2010) Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. Nat. Genet. **42**, 489–491

93 Durand, C. M., Betancur, C., Boeckers, T. M., Bockmann, J., Chaste, P., Fauchereau, F., Nygren, G., Rastam, M., Gillberg, I. C., Anckarsater, H. et al. (2007) Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat. Genet. **39**, 25–27

94 Park, E., Na, M., Choi, J., Kim, S., Lee, J. R., Yoon, J., Park, D., Sheng, M. and Kim, E. (2003) The Shank family of postsynaptic density proteins interacts with and promotes synaptic accumulation of the βPIX guanine nucleotide exchange factor for Rac1 and Cdc42. J. Biol. Chem. **278**, 19220–19229

95 Cohen, N. A., Brenman, J. E., Snyder, S. H. and Bredt, D. S. (1996) Binding of the inward rectifier K^+ channel Kir 2.3 to PSD-95 is regulated by protein kinase A phosphorylation. Neuron **17**, 759–767

96 Sanz-Clemente, A., Matta, J. A., Isaac, J. T. R. and Roche, K. W. (2010) Casein kinase 2 regulates the NR2 subunit composition of synaptic NMDA receptors. Neuron **67**, 984–996

97 Sulka, B., Lortat-Jacob, H., Terreux, R., Letourneur, F. and Rousselle, P. (2009) Tyrosine dephosphorylation of the syndecan-1 PDZ binding domain regulates syntenin-1 recruitment. J. Biol. Chem. **284**, 10659–10671

98 Chung, H. J., Xia, J., Scannevin, R. H., Zhang, X. and Huganir, R. L. (2000) Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. J. Neurosci. **20**, 7258–7267

99 Daw, M. I., Chittajallu, R., Bortolotto, Z. A., Dev, K. K., Duprat, F., Henley, J. M., Collingridge, G. L. and Isaac, J. T. (2000) PDZ proteins interacting with C-terminal GluR2/3 are involved in a PKC-dependent regulation of AMPA receptors at hippocampal synapses. Neuron **28**, 873–886

100 Xia, J., Zhang, X., Staudinger, J. and Huganir, R. L. (1999) Clustering of AMPA receptors by the synaptic PDZ domain-containing protein PICK1. Neuron **22**, 179–187

101 Dong, H., O'Brien, R. J., Fung, E. T., Lanahan, A. A., Worley, P. F. and Huganir, R. L. (1997) GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. Nature **386**, 279–284

102 Pan, L., Wu, H., Shen, C., Shi, Y., Jin, W., Xia, J. and Zhang, M. (2007) Clustering and synaptic targeting of PICK1 requires direct interaction between the PDZ domain and lipid membranes. EMBO J. **26**, 4576–4587

103 Stricker, N. L. and Huganir, R. L. (2003) The PDZ domains of mLin-10 regulate its trans-Golgi network targeting and the surface expression of AMPA receptors. Neuropharmacology **45**, 837–848

104 Biederer, T., Cao, X., Sudhof, T. C. and Liu, X. (2002) Regulation of APP-dependent transcription complexes by Mint/X11s: differential functions of Mint isoforms. J. Neurosci. **22**, 7340–7351

105 Biederer, T. and Sudhof, T. C. (2000) Mints as adaptors. Direct binding to neurexins and recruitment of munc18. J. Biol. Chem. **275**, 39803–39806

106 Lau, K. F., McLoughlin, D. M., Standen, C. and Miller, C. C. (2000) X11 α and x11 β interact with presenilin-1 via their PDZ domains. Mol. Cell. Neurosci. **16**, 557–565

107 Maximov, A., Sudhof, T. C. and Bezprozvanny, I. (1999) Association of neuronal calcium channels with modular adaptor proteins. J. Biol. Chem. **274**, 24453–24456

108 LaLonde, D. P. and Bretscher, A. (2009) The scaffold protein PDZK1 undergoes a head-to-tail intramolecular association that negatively regulates its interaction with EBP50. Biochemistry **48**, 2261–2271

109 Sugi, T., Oyama, T., Muto, T., Nakanishi, S., Morikawa, K. and Jingami, H. (2007) Crystal structures of autoinhibitory PDZ domain of Tamalin: implications for metabotropic glutamate receptor trafficking regulation. EMBO J. **26**, 2192–2205