Organization and dynamics of PDZ-domain-related supramodules in the postsynaptic density

Wei Feng and Mingjie Zhang

Abstract | As the major components of the postsynaptic density of excitatory neuronal synapses, PDZ-domain-containing scaffold proteins regulate the clustering of surface glutamate receptors, organize synaptic signalling complexes, participate in the dynamic trafficking of receptors and ion channels, and coordinate cytoskeletal dynamics. These scaffold proteins often contain multiple PDZ domains, with or without other protein-binding modules, and they usually lack intrinsic enzymatic activities. Recent biochemical and structural studies have shown that tandemly arranged PDZ domains often serve as structural and functional supramodules that could regulate the organization and dynamics of synaptic protein complexes, thus contributing to the broad range of neuronal activity.

Scaffold protein
A protein characterized by its multiple protein–protein interaction domains that can assemble multiple proteins, through individual domain-mediated interactions, into functional complexes in diverse cellular processes.

Adaptor protein
A protein that links protein components of signalling pathways, thereby aiding intracellular signal transduction. The terms 'adaptor protein' and 'scaffold protein' are sometimes used interchangeably in the literature.

Department of Biochemistry, Molecular Neuroscience Center, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong. Correspondence to M.Z.
e-mail: mzhang@ust.hk
doi:10.1038/nrn2540

Excitatory glutamatergic synapses are characterized by an enrichment of presynaptic vesicles at the axon terminal and by postsynaptic electron-dense membrane thickenings (referred to as the postsynaptic density (PSD)) that are concentrated at the top of dendritic spines (for recent reviews, see REFS 1–5). On stimulation, glutamate is released from the presynaptic terminal and acts on the glutamate receptor channels (NMDA (*N*-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors) on the postsynaptic side to allow the influx of specific ions that depolarize postsynaptic membranes. Thus, neuronal signals are received, decoded and further propagated by the PSD, which can be viewed as a membrane-associated mega-organelle that is specialized for postsynaptic signal transduction and processing[1,6–8]. Thin-section electron microscopy (EM) studies of isolated PSDs from mammalian brain showed several decades ago that the PSD forms a disk-like shape ~200–800 nm wide and ~30–50 nm thick underneath the postsynaptic membrane[9–11]. Subsequent high-resolution SDS–PAGE separation of biochemically purified PSD, coupled with amino-acid sequencing, identified abundant components of the PSD, such as PSD95 (also known as DLG4), calcium/calmodulin-dependent protein kinase II (CaMKII), synaptic Ras GTPase activating protein 1 (SYNGAP1)and actin[12–14]. In parallel, a number of other PSD components were identified by affinity purification or by yeast two-hybrid screening, using the known PSD proteins as bait[15–17]. Recent developments in proteomics have provided a global catalogue of proteins in purified PSDs, many of which belong to previously unrecognized classes[18–24]. They have a wide range of functions and can be roughly classified into several categories: membrane receptors; cell-adhesion molecules; signalling proteins, including kinases and phosphatases, and their regulators; scaffold and adaptor proteins; cytoskeleton proteins and cytoskeletal regulators; membrane-trafficking proteins and molecular motors; and protein-synthesis machineries[20,23]. Mass spectrometry is being used not only for the identification of new PSD components, but also for the quantification of protein compositions, as it is possible to obtain absolute copy numbers of major PSD proteins once one or a few of the key components (for example, PSD95) have been quantified by other means[23,25] (this topic is comprehensively reviewed in REF. 1). Quantitative fluorescence microscopy using a green fluorescent protein signal as a calibrator has also been used to measure the copy numbers of PSD proteins in cultured neurons, and the results are remarkably consistent with those obtained from quantitative mass spectrometry[26]. These quantitative methods allow us to reconstruct PSDs in molecular terms, and help us to discern the signalling events that occur in the PSD on neuronal excitation (FIG. 1). As expected, both NMDA and AMPA receptors are found at the membrane surface of PSDs. In each synapse, the number of NMDA receptors is more or less fixed at ~20 and the number of AMPA receptors widely varies from ~5–200. A typical PSD contains ~300–400 PSD95-family membrane-associated guanylate kinase

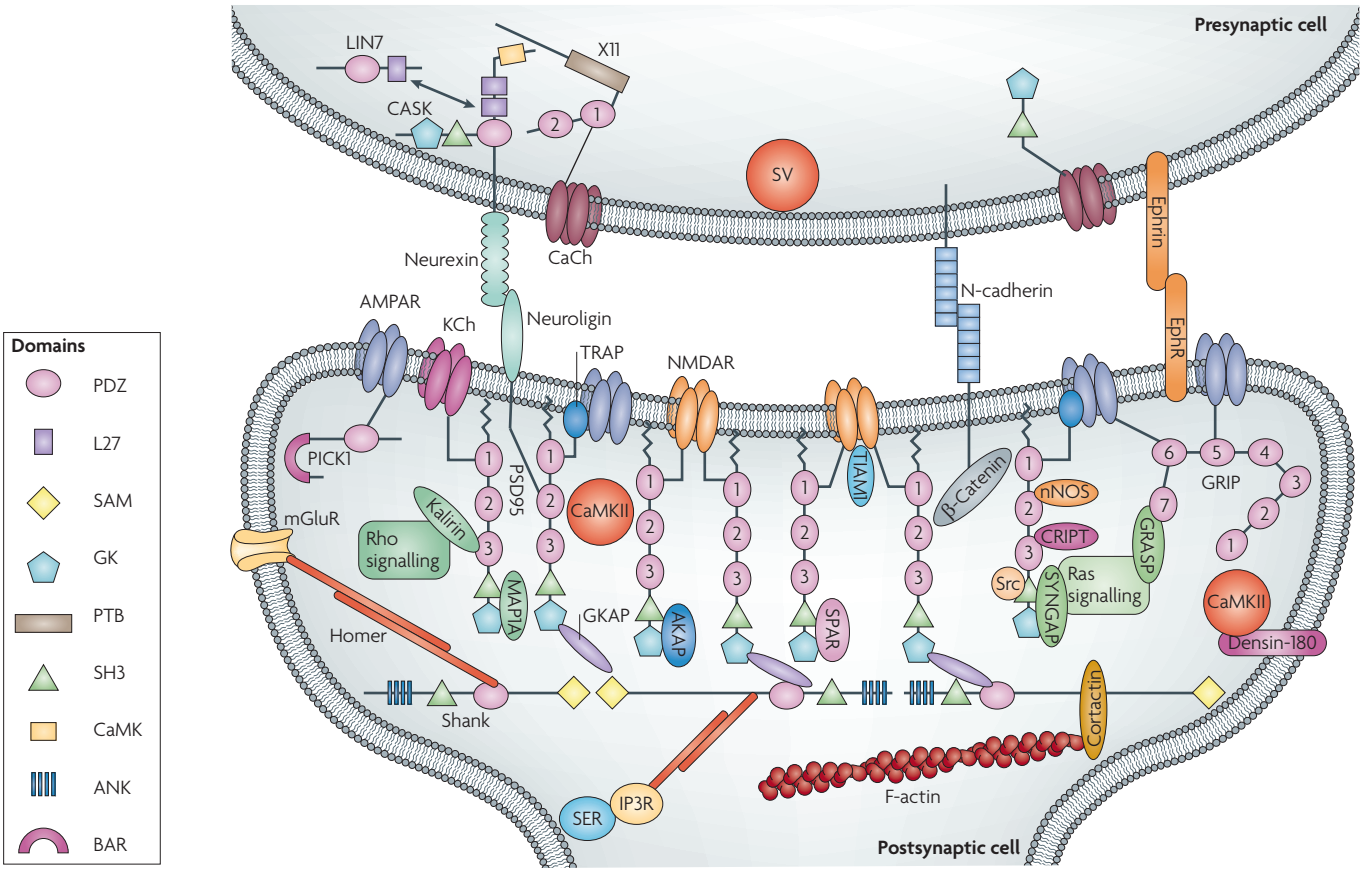

Figure 1 | **Protein complex organization in the postsynaptic density (PSD).** The postsynaptic density is comprised of membrane receptors and ion channels, scaffold and adaptor proteins, signalling proteins, cell-adhesion molecules and components of the cytoskeleton. Glutamate receptors, such as NMDARs (*N*-methyl-D-aspartate receptors) and AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors), are located at the postsynaptic membrane, with the NMDARs at the centre and the AMPARs more peripheral. The PDZ-domain-containing scaffold proteins PSD95 (also known as DLG4) and the Src-homology domain 3 (SH3) and multiple ankyrin repeat domains (Shank) family form a two-layer protein network below the postsynaptic membrane, which is bridged by guanylate kinase-associated protein (GKAP). PSD95 forms membrane-perpendicular and roughly equally spaced filamentous structures, with its amino terminus attached to the membrane. Other signalling molecules occupy the spaces in the PSD95–GKAP–Shank protein web. Shank-family scaffolds are further linked to actin filaments. The domains of PSD95 and Shank (PDZ, SH3, guanylate kinase (GK), sterile-alpha motif (SAM) and ankyrin repeats (ANK) (see key)) are shown; other proteins are represented by simple shapes and are labelled. The presynaptic and postsynaptic membranes are connected by cell-adhesion molecules. The domains of the presynaptic calcium/calmodulin-dependent serine protein kinase (CASK)–LIN7–X11 (also known as APBA1 and MINT) trafficking complex are also shown. AKAP, adenylate-kinase anchoring protein; CaCh, Ca^{2+} channel; CaMKII, calcium/calmodulin-dependent protein kinase II; CRIPT, cysteine-rich PDZ-binding protein; EphR, ephrin receptor; GKAP, guanylate kinase-associated protein; GRASP, GRIP-associated protein; GRIP, glutamate receptor interacting protein; IP3R, inositol-1,4,5-trisphosphate receptor; KCh, K^+ channel; MAP1A, microtubule-associated protein 1A; mGluR, metabotropic glutamate receptor; nNOS, neuronal nitric oxide synthase; PICK1, protein interacting with PRKCA1; SER, smooth endoplasmic reticulum; SPAR, spine-associated RAPGAP; SV, synaptic vesicle; SYNGAP, synaptic Ras GTPase-activating protein; TIAM1, T-cell lymphoma invasion and metastasis 1; TRAP, C-terminal receptor-binding region.

(MAGUK) proteins and ~150 SH3 and multiple ankyrin repeat domains (Shank) family members. It is important to note that the copy numbers of the PSD proteins vary depending on the type of neuron, and even vary in the same neuron in different developmental and physiological stages, consistent with the activity-dependent changes in protein composition that occur in synapses and that form the molecular basis of synaptic plasticity[27]. Finally, recent advances in high-resolution EM tomography coupled with specific antibody labelling have allowed us to visualize the anatomical structures of PSDs directly[28]: the first layer of a PSD mainly contains membrane receptors, ion channels and transmembrane cell-adhesion molecules, with NMDA receptors at the centre and AMPA receptors at the periphery; the second layer is enriched with scaffold proteins (MAGUK proteins, in particular PSD95), which are closely coupled to the membrane receptors and ion channels and are arranged perpendicular to the PSD membrane with their amino-terminal lipids attached to it; the third layer is comprised of Shank and guanylate kinase-associated protein (GKAP)-family proteins, which are linked to the carboxy-terminal Src

homology 3–guanylate kinase-like (SH3–GK) domains of the MAGUK proteins and are arranged in parallel to the PSD membrane. The proteins in this third layer are further connected to the actin cytoskeleton. All of these membrane receptors and scaffold proteins form a web-like protein network to which other cytoplasmic PSD proteins and enzymes can bind (FIG. 1).

PSD dynamics

The architecture of the PSD is highly dynamic, not only during developmental processes but also in response to synaptic activity. During brain development, the subunit compositions of NMDA receptors change dramatically: NR1 and NR2B predominate in the early PSD and later become less abundant, whereas NR2A (which is important for synaptic activity) gradually increases in abundance[29–31]. The major scaffold proteins — for example, PSD95 and the GKAP and Shank families of proteins — are always present and also increase in abundance as the brain matures[32]. In response to synaptic activation, PSD proteins undergo short-term modifications (such as phosphorylation) to activate downstream signalling pathways or their own long-term turnover, thus changing the synaptic strength[27]. AMPA receptors, which are primarily localized at the periphery of the PSD, are particularly dynamic[28]. The clustering, trafficking and membrane insertion or removal of AMPA receptors are associated with various forms of synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), which are physiologically linked to learning and memory[2,33–36]. The surface mobility of membrane AMPA receptors can fine-tune synaptic transmission[37]. Although the numbers of NMDA receptors and PSD95 at the PSD are largely fixed after synapse maturation[38,39], recent studies have suggested that a rapid and reversible switch of synaptic NMDA receptors and PSD95 between the membrane and the cytosol occurs in young animals[40,41] (see REFS 33,34 for comprehensive reviews of the dynamics of glutamate receptors and MAGUK scaffold proteins). Although still somewhat controversial, these observations nevertheless indicate that postsynaptic proteins are dynamic and able to modulate the PSD both structurally and functionally. The morphology of the PSD is ultimately defined by the dynamics of the actin cytoskeleton. Indeed, the dynamics of PSD proteins are closely linked with regulators of the actin cytoskeleton[42].

PDZ-domain scaffold proteins in the PSD

Scaffold proteins are abundant components of the PSD and have been shown to have crucial roles in diverse PSD functions, including trafficking, anchoring and clustering of glutamate receptors; linking glutamate receptors with their downstream signalling proteins; organizing multiple components into large signalling complexes; and interfacing with and regulating the dynamics of cytoskeletal structures[1,7,16,34]. Interestingly, most of the PSD scaffold proteins contain multiple well-folded domains and often do not have intrinsic enzymatic activities, making them ideal for organizing protein networks (FIG. 2). Given that scaffold proteins, like the pillars of a building, are the central components of the PSD architecture, their dynamic organization and regulation are directly correlated with the morphology of the PSD and, hence, with the synaptic plasticity of neurons.

The PDZ (PSD95, DLG1 and ZO1) domain (BOX 1) is probably the most common protein–protein interaction module among the PSD scaffold proteins (FIG. 2). Many PSD scaffold proteins are constructed from multiple copies of PDZ domains with or without other protein modules. An advantage of having PDZ domains might be that they interact with small peptide fragments situated at the very carboxyl tail of their targets with relatively weak binding affinities[43]. We speculate, simply based on the physical chemistry of protein–protein interactions, that the high number of PDZ domains, which greatly outnumber glutamate receptors and other transmembrane targets, in the PSD ensures plenty of 'slots' for the various binding partners, thereby ensuring the dynamic range of synaptic responses. Additionally, densely packed PDZ scaffold proteins in the PSD might also regulate receptor and ion channel mobility in the synaptic membranes. For instance, NMDA receptors situated at the centre of the PSD membrane would have less mobility due to the higher density of interacting PDZ scaffolds at this part of the membrane than at the periphery[28,38] (FIG. 1). Densely packed PDZ 'grids' could also prevent disengaged receptors (that is, receptors that are not actively participating in signalling events) from freely diffusing away from the PSD. Such receptors would be able to quickly respond to synaptic inputs, without having to go through *de novo* trafficking and insertion into the PSD membrane, providing a physical-chemistry basis for the fast kinetics of neuronal responses to stimuli[44].

The abundance of PDZ scaffold proteins in synapses with overlapping interacting targets raises issues regarding the functional specificity and redundancy of the scaffolds. For example, multiple MAGUKs (PSD95, PSD93 (also known as DLG2), SAP102 (also known as DLG3) and SAP97 (also known as DLG1)) are expressed in excitatory synapses, and each of these MAGUKs can mediate the trafficking of glutamate receptors at different developmental stages[31,45]. Knockout studies in mice, combined with short hairpin RNA (shRNA)-mediated acute knock-down experiments, have revealed that MAGUKs have a remarkable ability to functionally compensate for each other[45,46]. Presumably, this highly interconnected MAGUK system is required for appropriate glutamate receptor expression at synapses[34].

Supramodules in multi-domain PSD proteins

A distinct feature of PDZ scaffold proteins is that they often contain multiple PDZ domains (FIG. 2a), and such domain-organization features are highly conserved throughout evolution, presumably owing to positive selections imposed by their functions. A number of recent studies have demonstrated that two or more PDZ domains connected in tandem often display target-binding properties that are distinct from those of each isolated domain or even the simple sum of the isolated PDZ domains. These distinct target-binding properties

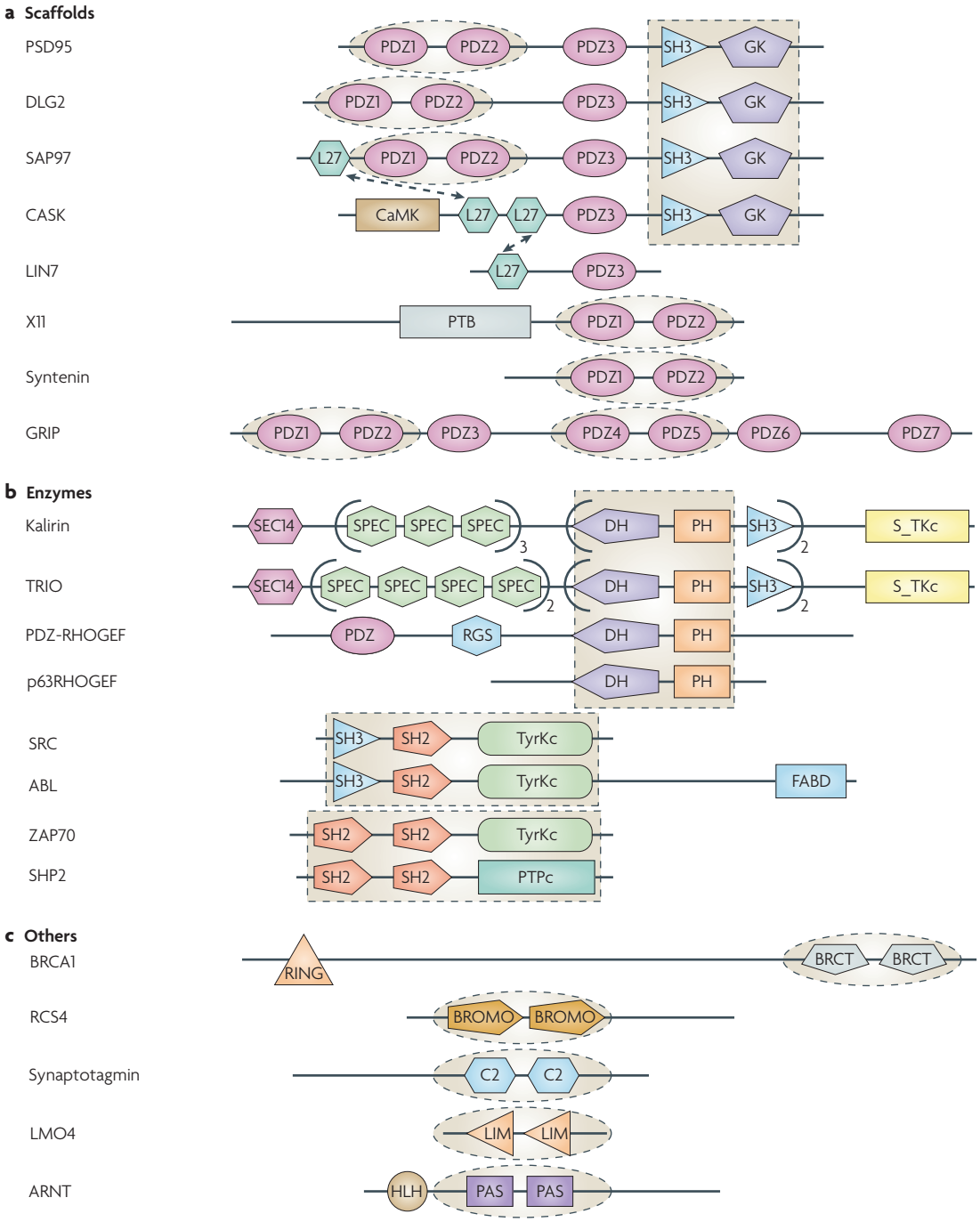

Figure 2 | **Selected proteins containing supramodules.** In addition to PDZ-domain-containing scaffold proteins (**a**), there are supramodular domain organizations in enzymes found in the postsynaptic density (PSD) (**b**) and in proteins in diverse cellular environments (**c**). Supramodules composed of multiple well-defined protein modules arranged in tandem are highlighted by dashed-line ovals or boxes. The arrows between the L27 domains indicate that these domains could form cognate complexes and thereby assemble the proteins into supramolecular complexes. ARNT, aryl hydrocarbon receptor nuclear translocator; BRCA1, breast cancer 1; BRCT, BRCA1 carboxy-terminal domain; CaMK, calmodulin-dependent kinase-like domain; CASK, calmodulin-associated serine/threonine kinase; C2, PKC conserved region 2; DH, Dbl homology; FABD, F-actin binding domain; GK, guanylate kinase-like domain; GRIP, glutamate receptor interacting protein; HLH, helix–loop–helix domain; LIM, LIN11–ISL1–MEC3 domain; LMO4, LIM domain only 4; PAS, PER–ARNT–SIM domain; PDZ, PSD95–DLG1–ZO1 domain; PH, pleckstrin homology domain; PTB, phosphotyrosine binding domain; PTPc, protein tyrosine phosphatase catalytic domain; RGS, regulator of G-protein signalling domain; RING, ring finger domain; RSC4, remodels the structure of chromatin complex subunit 4; SAP97, synapse-associated protein 97; SH2, Src homology 2 domain; SH3, Src homology 3 domain; SHP2, SH2-domain-containing protein tyrosine phosphatase 2; SPEC, spectrin repeats; S_TKc, serine/threonine protein kinases catalytic domain; TyrKc, tyrosine kinase catalytic domain; ZAP70, zeta chain-associated protein kinase 70 kDa.

Box 1 | **PDZ domains**

PDZ domains were originally identified as ~90-amino-acid repeats of unknown function in several structurally related proteins, including postsynaptic density 95 (also known as DLG4), the *Drosophila melanogaster* tumour-suppressor protein discs large 1 (DLG1) and the tight-junction protein zonula occludens 1 (ZO1; also known as TJP1)[12,120–122]. Analysis of the human genome estimates that there are 335 non-redundant PDZ domains, making PDZ domains one of the most abundant protein domains in multicellular eukaryotic genomes. PDZ domains coexist with a wide variety of modular signalling domains. Most PDZ-domain- containing proteins are 'pure' scaffold proteins, as they do not have intrinsic enzymatic activities (FIG. 2). A canonical PDZ domain contains six β-strands (βA to βF) and two α-helices (αA and αB). The six β-strands form a partially opened barrel, and the opening sides of the barrel are each capped with an α-helix[123]. PDZ domains most commonly function as protein-interaction modules that recognize a short stretch of amino-acid residues (~5–7 amino acids) at the carboxyl termini of target proteins[43,124,125]. Peptide ligands bind to an extended groove formed by the βB strand and the αB helix of PDZ domains. The carboxylate of peptide ligands binds to the 'carboxylate-binding loop' located at one end of the αB–βB groove. The specificities of the peptide ligands of the PDZ domain are chiefly determined by the residues at the 0 and −2 positions of the ligands, and thus PDZ-binding ligands are traditionally categorized based on the properties of the amino-acid side chains in these two positions[66]. Recent systematic PDZ–ligand interaction studies at the proteome scale revealed that residues at the −1, −3, −4 and −5 positions of the peptide ligands also contribute to the binding specificities[126,127], and this increases the number of potential PDZ-binding ligands. In addition to binding to the carboxyl tail of peptide ligands, PDZ domains can specifically interact with internal peptide sequences[128–131]. Recently, PDZ domains were shown to bind to phosphatidylinositol lipids, and these PDZ–lipid interactions have important roles in diverse cellular processes, such as cell polarity and receptor trafficking[47,86,87,132,133]. A systematic survey of PDZ domains showed that a significant portion (~20%) in the mammalian genome display phosphatidylinositide-dependent membrane-binding capacity, indicating that binding to lipid membranes is another general property of PDZ domains[87].

originate from the direct interactions between the PDZ domains in the tandem — that is, from the unique spatial arrangement of the PDZ domains (FIG. 2a). Such structural and functional units are referred to as PDZ supramodules. The target binding of these supramodules contrasts with that described by the simple 'beads-on-a-string' model that is commonly used for multi-domain scaffold proteins. Examples of PDZ-supramodule-containing proteins include the most abundant PSD scaffold protein, PSD95, the AMPA-receptor trafficking adaptor GRIP1 (glutamate-receptor interacting protein 1), the vesicle trafficking adaptor protein X11 (also known as APBA1 and MINT) and the synaptic adaptor syntenin[47–52].

In addition to PDZ domains, other protein domains connected in tandem have been shown to form structurally and functionally distinct supramodules. For example, the SH3–GK tandem found in all MAGUK-family scaffold proteins is likely to form a distinct supramodule through a direct intramolecular interaction between the SH3 and GK domains[53–56] (FIG. 2b). As well as scaffold proteins, supramodules are also found in a number of enzymes in the PSD (FIG. 2). Two well-known examples belong to the Rho GEF (guanine nucleotide exchange factor) family of proteins (for example, PDZ-RHOGEF (also known as ARHGEF11) and p63RHOGEF (also known as GEFT)), which contain a tandem Dbl homology–pleckstrin homology (DH–PH) supramodule[57–59], and the Src-family tyrosine kinases (for example, SRC and FYN), which contain a SH2–SH3 kinase supramodule locked in an auto-inhibited conformation (see REF. 60 for the details of the Src kinase supramodule). Finally, it should be noted that tandem-domain supramodules are frequently found in other multi-domain proteins involved in signal transduction and gene regulation. Some well-known examples include the tandem-BRCT, -BROMO, -C2, -LIM and -PAS-domain-containing proteins shown in FIG. 2c. Bioinformatic studies have suggested that a supramodular organization might be a common feature of a large portion of multi-domain proteins in all living organisms[61].

Beads-on-a-string model
A model in which individual domains in multi-domain scaffold proteins are viewed as linearly connected globular folds with no direct contacts. According to the model, each domain functions independently.

Tandem-PDZ supramodules

PSD95 PDZ12. The most abundant scaffold protein in the PSD, PSD95, contains three PDZ domains at its N terminus (FIG. 2). A large number of PSD proteins, including NMDA receptors, K^+ channels, nitric oxide synthase and the cytoskeletal protein cysteine-rich PDZ-binding protein (CRIPT), have been reported to bind to these domains[62–65]. The linking sequence between the first two PDZ domains (together referred to as PDZ12) is short (five residues), rigid and highly conserved, suggesting that it might restrain interdomain motion rather than merely function as a passive connector. Determining the structure of the PDZ12 tandem showed that the two PDZ domains indeed contact each other in a side-by-side manner, and that two of their target-binding grooves point in directions that are favourable for binding to the tails of multimeric transmembrane proteins extending from the membrane surface (FIG. 3Aa). Additionally, isolated PDZ1 and PDZ2 domains have similar structures and target-binding properties, and thus the PSD95 PDZ12 tandem can be viewed as a simple domain duplication[48]. The structure of PSD95 PDZ12 has direct implications for the function of the supramodule. The near-parallel orientation of the two target-binding grooves of the PDZ12 tandem provides a molecular basis for synergistic interactions with dimeric and/or multimeric membrane proteins, including NMDA receptors and K^+ channels (FIG. 3Ab). Such synergistic PDZ12 supramodule–multimeric target interaction not only enhances the binding affinity between the scaffold and its targets, as can be appreciated from the thermodynamic point of view, but (perhaps more importantly) also provides exquisite specificity in PDZ scaffold–target interactions. It is well known that single PDZ domain–target interactions are generally of modest affinity and specificity[43,66]. There are several hundred PDZ domains and PDZ-domain-binding targets in each mammalian genome. The fundamental and highly specific roles of PDZ proteins and their targets in diverse cellular processes are certainly not compatible with the promiscuous single PDZ–target interactions. By contrast, the PSD95 PDZ12 supramodule provides a molecular basis for highly specific PDZ–target interaction, as the PDZ12 tandem will be biased towards a multimeric target with two identical or highly similar PDZ-binding motifs (for example, two NR2 subunits in NMDA receptors; FIG. 3Ab).

Lengthening the interdomain linker impaired the supramodular nature of PSD95 PDZ12. This PSD95

mutant displayed weaker binding to dimeric targets and a decreased capacity in clustering K^+ channels[48]. Such PDZ-supramodule-mediated specific target interaction is certainly not limited to PSD95 PDZ12. Specific interaction between the kinase-deficient neurotrophin receptor isoform TRKCT1 and the PDZ scaffold protein tamalin (also known as GRASP) requires neurotrophin 3 binding to TRKCT1 (REF. 67). The PDZ domain of tamalin forms a homodimer[68]. The inactive monomeric TRKCT1 C-terminal tail has low binding affinity to tamalin PDZ domains. NT3-binding-induced dimerization of TRKCT1 dramatically increases the binding affinity between the dimeric TRKCT1 receptor and the tamalin PDZ dimer through a mechanism similar to that discussed above

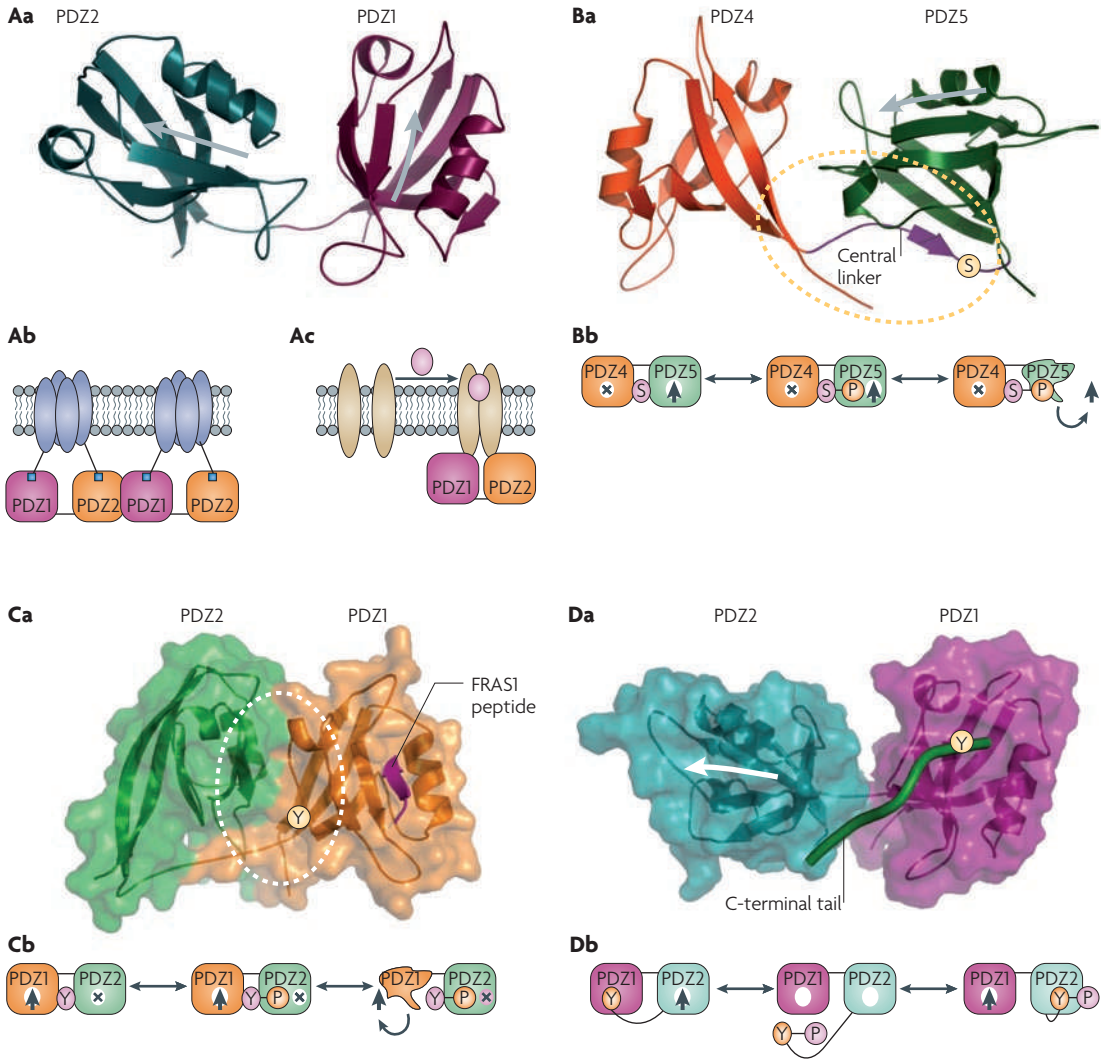

Figure 3 | **Structure and target-binding properties of PDZ-domain supramodules.** Ribbon diagrams or surface representations of postsynaptic density 95 (PSD95) PDZ12 (**Aa**), glutamate receptor interacting protein 1 (GRIP1) PDZ45 (Protein Data Bank (PDB) code: 1P1D) (**Ba**), GRIP1 PDZ12 (PDB code: 2QT5) (**Ca**), and X11 PDZ12C (**Da**) supramodules. The target-binding pockets of the PDZ domains in PSD95 PDZ12, GRIP1 PDZ45 and X11 PDZ12C are indicated by arrows. The FRAS1 peptide in the GRIP1 PDZ12–FRAS1 complex is shown in purple ribbon and labelled, and the carboxy-terminal tail of X11 PDZ12C is in worm model and labelled. The potential phosphorylation sites in GRIP1 PDZ45, GRIP1 PDZ12 and X11 PDZ12C are highlighted by yellow circles (and labelled S for Ser or Y for Tyr). Schematic diagrams showing distinct target-binding properties conferred by the supramodular organization of the PDZ tandems are shown for the GRIP1 PDZ45 (**Bb**), GRIP1 PDZ12 (**Cb**) and X11 PDZ12C (**Db**) structures. The PSD95 PDZ12 supramodule enables synergistic binding to C-terminal tails extending from multimeric transmembrane receptors and ion channels, a process that is crucial for both specific adaptor–receptor interaction and adaptor-facilitated clustering of the receptors (**Ab**). The bidentate nature of the PSD95 PDZ12 supramodule also allows for an activity-dependent interaction between the supramodule and receptors (**Ac**). Phosphorylation of residues in the interface of the PDZ domains leads to the unfolding of, and the subsequent dissociation of targets from, PDZ5 in GRIP1 PDZ45 (**Bb**) and PDZ1 in GRIP1 PDZ12 (**Cb**). The black cross indicates that the potential PDZ-binding pocket in GRIP1 PDZ2 and PDZ4 is not functional. On unfolding of PDZ1, the ligand-binding pocket in GRIP1 PDZ2 might be functional, indicated by a cross with a pink background. The penultimate Tyr in X11 might undergo phosphorylation and this might switch the inhibitory C-terminal tail of X11 from PDZ1 to PDZ2 (**Db**).

for the PSD95 PDZ12–dimeric target complex. The TRKCT1–tamalin PDZ interaction is dependent on NT3 binding and is therefore activity regulated (FIG. 3Ac). The ligand-mediated dimerization of receptors and their subsequent interaction with multi-PDZ-domain scaffold proteins could be a general mode of interaction in signal transduction involving PDZ proteins.

PDZ12 and PDZ45 in GRIP-family scaffolds. The GRIP-family proteins, GRIP1 and GRIP2, were originally identified by their interaction with GluR2 and GluR3 subunits of AMPA receptors[69–71]. GRIP proteins contain six to seven PDZ domains and no other identifiable protein modules (FIG. 2). Since their discovery, numerous proteins have been shown to interact with the GRIP-family proteins, and most interactions between GRIP proteins and their targets are mediated by the PDZ domains of GRIP. Accumulating data indicate that the GRIP-family proteins are ideally suited as adaptors, modulating the delivery and organization of transmembrane proteins at the surfaces of membranes[72–76]. Interruption of the GluR2–GRIP interaction leads to the inhibition of AMPA receptor accumulation at synapses[69,77]. The surface expression of the receptor tyrosine kinase EPHB2 was greatly reduced when endogenous GRIP1 was reduced by small interfering RNA[72]. Consistent with the protein-trafficking function of the GRIP-family proteins, the microtubule motor kinesin 1 was shown to bind directly to a specific region of GRIP1 (REF. 73).

The interaction between the GluR2 tail of AMPA receptors and GRIP1 differs from the conventional PDZ domain–ligand interactions, at least in terms of the binding stoichiometry, as the GRIP1 PDZ45 tandem is required to bind to just one GluR2 tail[69,78]. Structural studies of the GRIP1 PDZ45 tandem explained this odd observation[49]. The two PDZ domains pack closely together to form one structural unit — the short conserved inter-PDZ linker forms a β-strand and pairs with the first β-strand of PDZ5 (FIG. 3Ba). Although there are two potential target-binding pockets in the PDZ45 tandem, the pocket in PDZ4 is deformed and non-functional. It is only the PDZ5 pocket that can bind to the GluR2 tail. Interestingly, isolated PDZ5 is completely unfolded and therefore cannot bind to the GluR2 tail. Proper folding of PDZ5 and subsequent GluR2 binding require the covalent attachment of PDZ4 to form the PDZ45 supramodule, and thus PDZ4 serves as a chaperone for PDZ5. The requirement of PDZ4 for the structure and function of PDZ5 has the advantage of building a potential GRIP1–AMPA receptor interaction-modulating regulatory mechanism into the PDZ45 supramodule. Conceptually, the interaction between a trafficking adaptor (GRIP1 in this case) and its cargo (GluR2) should be reversible, so that the cargo can be loaded after its biogenesis and unloaded on reaching its destination. Sequence alignment of the PDZ45 supramodule showed that the linker contains two highly conserved Ser residues (Ser567 and Ser568 in human GRIP1 (FIG. 3Ba)). Substituting Glu for one or both of these Ser residues led to decreased stability of PDZ5 due to the weakened interaction between the linker and PDZ5 (M.Z., unpublished observations). One could envision that phosphorylation of the Ser residues in the PDZ45 linker might function as a switch in controlling AMPA receptor loading and unloading in GRIP1-mediated trafficking (FIG. 3Bb).

Similar interdomain chaperoning exists between GRIP1's first two PDZ domains. Binding of the carboxyl tail of the transmembrane cell matrix protein FRAS1 to GRIP1 requires the first two PDZ domains to be connected in tandem, as the folding of PDZ1 strictly depends on the covalent attachment of PDZ2. The structure of GRIP1 PDZ12 in complex with the FRAS1 carboxyl tail showed that the PDZ12 tandem forms a supramodule in which only the peptide-binding groove of PDZ1 binds to the FRAS1 peptide. The potential target-binding pocket of PDZ2 is completely occluded by the interdomain packing (FIG. 3Ca). Interestingly, the interface between PDZ1 and PDZ2 contains a completely conserved Tyr residue (Tyr134), and this Tyr is crucial for the stability of the PDZ12 supramodule. Substitution of Glu for this Tyr led to the unfolding of PDZ1 and to the subsequent dissociation of FRAS1 from GRIP1 (REF. 52). Structural and biochemical studies suggest that the GRIP1 PDZ12–FRAS1 interaction could be regulated by phosphorylation of this Tyr[52] (FIG. 3Cb). Further studies are required to validate whether such a switch is indeed used in a physiological setting.

X11 PDZ12C supramodule. X11 is a neuron-specific trafficking adaptor that directly interacts with calcium/calmodulin-dependent serine protein kinase (CASK) to form an evolutionarily conserved CASK–LIN7–X11 complex that mediates the cellular trafficking of NMDA receptors and other transmembrane proteins[79–82]. X11 contains two PDZ domains arranged in tandem followed by a conserved C-terminal PDZ-binding motif (together termed PDZ12C; FIG. 2). The highly conserved C terminus folds back and directly interacts with the first PDZ domain of the PDZ12 tandem[50]. Determining the structure of the PDZ12C supramodule showed that the target-binding pocket of PDZ1 is occupied by the C-terminal tail (PDZ12C thus adopts an auto-inhibited conformation), and that the target-binding pocket of PDZ2 is accessible to proteins, such as the kinesin motor KIF17 (FIG. 3Da). Interestingly, the X11 C-terminal tail also contains a highly conserved Tyr at the –1 position. This Tyr residue is essential for the tail to bind to PDZ1 (FIG. 3Da): when this Tyr was substituted with Glu, the tail was released from PDZ1, as one would expect from the structure of the complex. The surprising observation was that the tail carrying the mutation occupied the target-binding pocket of PDZ2 (REF. 50). Alteration of the auto-inhibited conformation of PDZ12C led to changes in the PDZ tandem's target-binding affinities, as well as to profound changes in the synaptic targeting of the adaptor itself[50]. We propose that the penultimate Tyr residue of X11 may function as a kinase-dependent switch that dynamically regulates the target-binding properties of the PDZ12C supramodule (FIG. 3Db). Further studies are required to prove whether such a kinase-dependent regulatory switch is used in living cells.

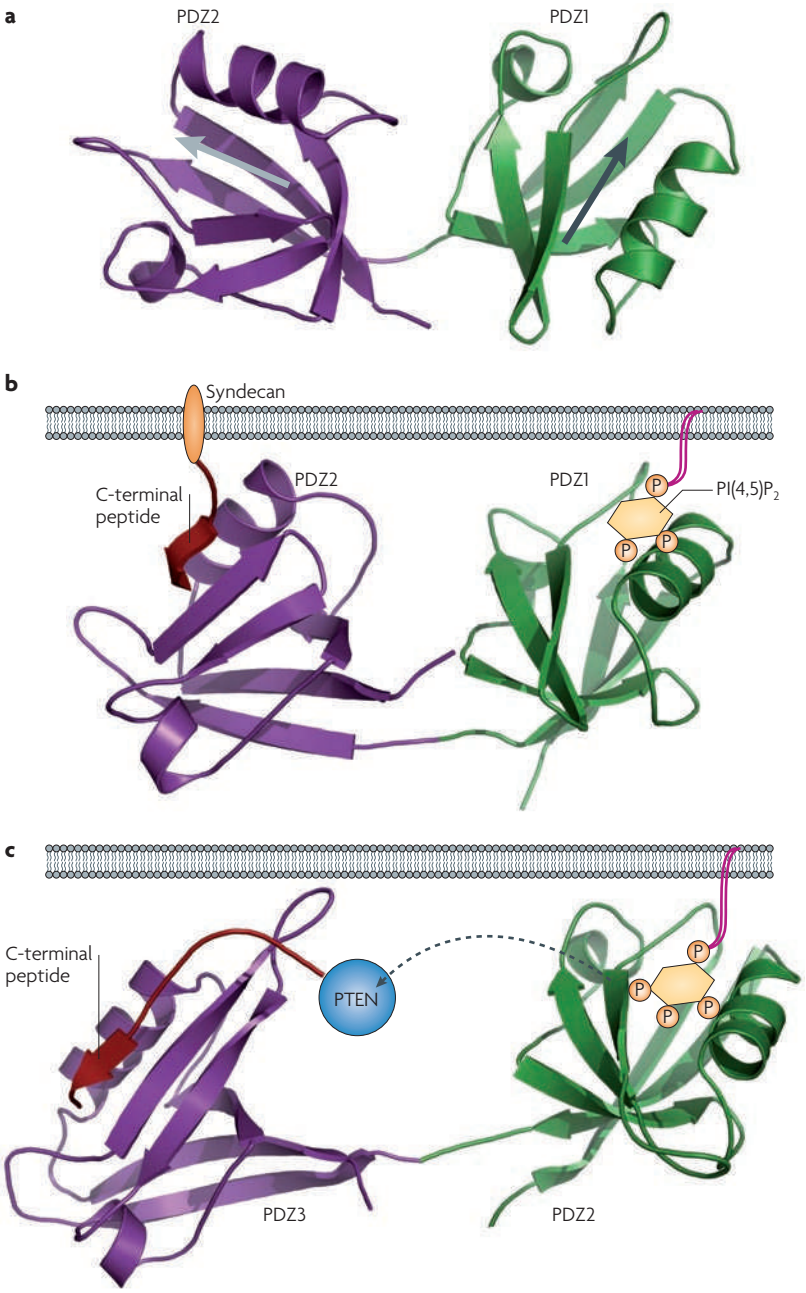

Figure 4 | **PDZ supramodules as integrators of phospholipid and protein signalling. a** | A ribbon diagram of the syntenin PDZ12 tandem (Protein Data Bank (PDB) code: 1N99). The target-binding pockets of PDZ1 and PDZ2 are indicated by arrows. The syntenin PDZ12 supramodule binds to protein targets in a similar manner to the postsynaptic density 95 PDZ12. **b** | In addition to binding to protein targets, the syntenin PDZ12 binds to phospholipids. This ribbon diagram shows the syntenin PDZ12 in complex with the peptide syndecan (PDB code: 1YBO) and bound to phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$). The binding of phospholipids to PDZ1 and of a protein ligand to PDZ2 couples signals from phosphatidylinositol lipids and membrane proteins. This binding mechanism may also allow the syntenin PDZ12 to function as a detector for the presence of both phosphatidylinositol lipids and transmembrane proteins in the same region of cell membrane. **c** | Ribbon diagram of a PAR3 PDZ23 structural model (constructed from PDB codes 2OGP and 2K20), showing the PDZ23 supramodule simultaneously binding to phospholipids and proteins. In this model, PDZ2 interacts with phosphatidylinositol lipids embedded in the membrane bilayers, whereas PDZ3 anchors the lipid phosphatase PTEN in the vicinity of the membrane by binding to its carboxy-terminal tail. Therefore, the PAR3 PDZ23 provides a feedback loop for maintaining a membrane gradient of phosphoinositide[87].

Syntenin PDZ12 and PAR3 PDZ23 supramodules. Syntenin is a small scaffold protein that contains two PDZ domains separated by four amino-acid residues[83]. The PDZ domains of syntenin bind to a diverse set of proteins, including several PSD proteins: AMPA receptors, ephrin and its receptors, neurofascin, the neurexins, SYNGAP1 and kalirin, although the functions of these interactions are not well characterized (see REF. 84 for a recent review). Similar to the PDZ12 tandem of PSD95, the two PDZ domains of syntenin have limited interdomain flexibility[51,83]. Analogous to what was found in the PSD95 PDZ12 supramodule, the peptide-binding grooves of the two syntenin PDZ domains are oriented such that they can interact cooperatively and specifically with two cytoplasmic tails extending from multimeric transmembrane proteins, such as syndecan[85] (FIG. 4a). Thus, the syntenin PDZ12 tandem is another example of a PDZ supramodule.

In addition to binding to peptide ligands, syntenin's PDZ1 was found to interact with phosphatidylinositol lipids, including phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$)[47], although the molecular basis of these interactions is not known. Interestingly, the covalent attachment of PDZ2 is essential for the $PI(4,5)P_2$-mediated membrane localization of syntenin[47,86]. PDZ2-mediated binding of syntenin to syndecan's C terminus (and perhaps to other transmembrane targets) can synergistically enhance syntenin's lipid-membrane binding *in vitro* and its plasma-membrane localization *in vivo*[47,86]. Therefore, the arrangement of the syntenin PDZ12 supramodule exemplifies a PDZ-domain arrangement in which PDZ1 is responsible for specific binding to phosphatidylinositol lipids embedded in the membrane bilayers whereas PDZ2 interacts with the C-terminal tail of protein ligands (FIG. 4b). The simultaneous binding of the syntenin PDZ12 supramodule to phospholipids and proteins not only provides a molecular explanation for the synergistic interactions of syntenin with lipids and proteins situated in the plasma membrane, it also, perhaps more importantly, contributes to integrating signals from phosphatidylinositol lipids and membrane proteins (for example, receptors and cell-adhesion proteins) (FIG. 4b). This idea is consistent with a recent study which showed that the PDZ23 tandem of PAR3 simultaneously binds to phosphatidylinositol lipids and to their metabolizing enzyme PTEN[87,88]. In the PAR3 PDZ23 supramodule, PDZ2 uses its part of the peptide-binding pocket to bind to phosphatidylinositol lipids with high affinity. PDZ2-mediated lipid-membrane binding is required for PAR3's membrane localization *in vivo*. The binding of PDZ3 to the C-terminal tail of PTEN physically positions the phosphatidylinositol-3,4,5-trisphosphate ($PI(3,4,5)P_3$)-hydrolyzing enzyme together with the phospholipids captured by PAR3 (FIG. 4c). Thus, the PAR3 PDZ23 supramodule is ideally suited for maintaining the $PI(3,4,5)P_3$ gradient in polarized cells, including neurons[87–89]. Taken together, syntenin's PDZ12 and PAR3's PDZ23 represent a class of supramodules that can interact with different types of targets (for example, lipids and proteins). Given that a large proportion of PDZ domains have been shown to bind to phospholipids[87], it will not be

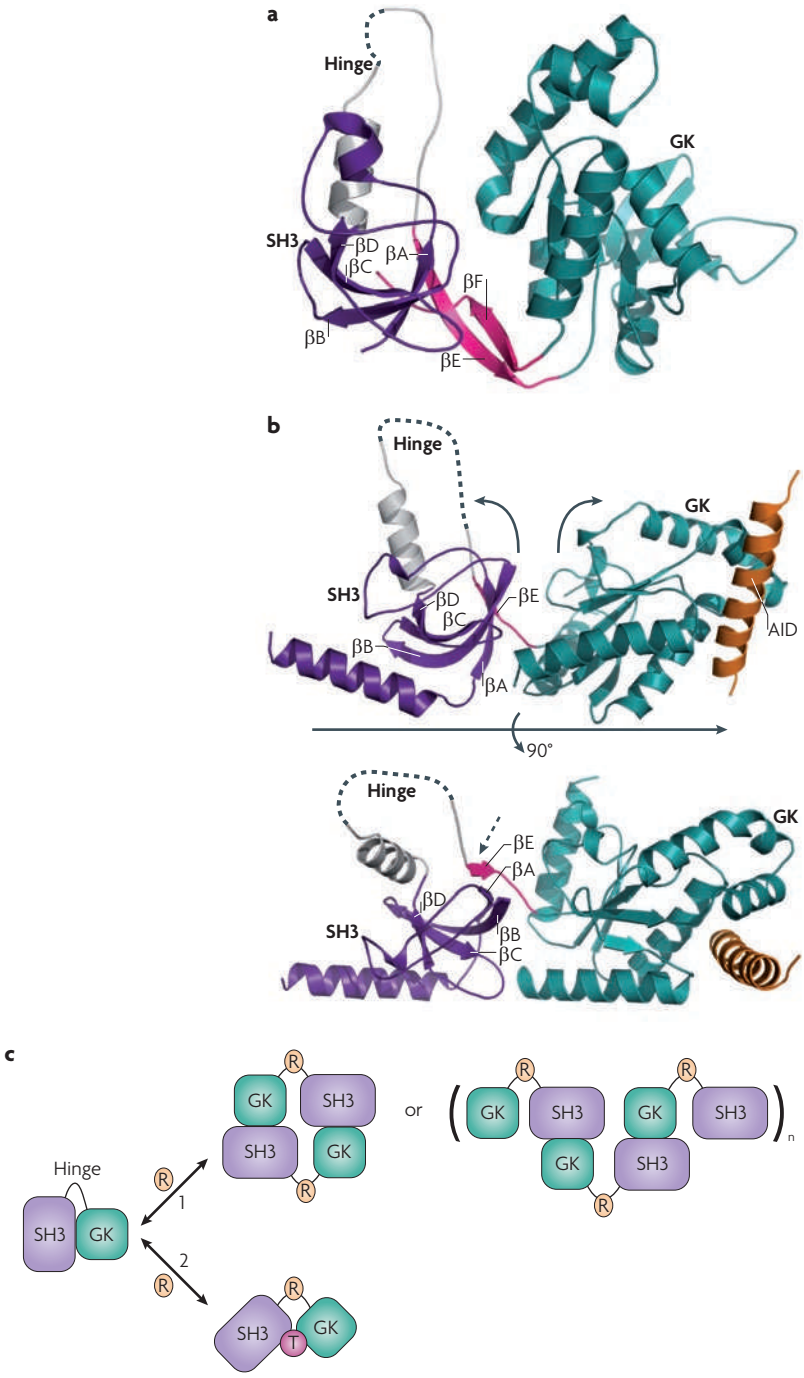

Figure 5 | **The SH3–GK supramodules. a,b** | Ribbon diagrams of the postsynaptic density 95 (PSD95) Src homology domain 3 (SH3)–guanylate kinase (GK) tandem (Protein Data Bank (PDB) code: 1KJW) (**a**) and of the auxiliary β-subunit of the voltage-gated Ca^{2+} channel ($Ca_v\beta$) SH3–GK tandem (PDB code: 1VYT) (**b**). The hinge region and interfacial strands between the SH3 domain and the GK domain are coloured in grey and pink, respectively. The secondary structures of the SH3 domain are labelled following the scheme of canonic SH3 domains (βA–βE). The interdomain orientation difference of the SH3–GK tandems between PSD95 and $Ca_v\beta$ are indicated by the black arrows. **c** | Schematic diagrams showing possible regulation modes of the SH3–GK supramodules. Following the binding of regulatory proteins (denoted R) to the hinge region, the SH3–GK tandem could open to form either a dimer or an oligomer (step 1), providing a mechanism for multimerization, which is observed in many membrane-associated guanylate kinase (MAGUK) scaffolds. Alternatively, binding of regulatory proteins to the hinge region may induce interdomain conformational changes, thereby exposing new binding surface for additional target proteins (denoted T) (step 2). AID, α-interacting domain.

surprising if more such lipid- and protein-binding PDZ supramodules are identified in the future.

SH3–GK supramodule in MAGUK scaffolds

PSD95-subfamily MAGUK proteins are key scaffold proteins that determine the steady-state as well as the activity-dependent changes of glutamate-receptor number in excitatory synapses[34]. The hallmark of the MAGUK-family proteins is that they all contain an SH3–GK tandem near their C-terminal tail (FIG. 2). The SH3–GK tandem of PSD95 binds to numerous proteins, including GKAP proteins, A-kinase-anchoring protein (AKAP) proteins, SYNGAP1, SPAR and microtubule-associated protein 1A (MAP1A), and these PSD95 SH3–GK-mediated interactions have been implicated in the regulation of both the size and the number of synapses[90–94]. The crystal structures of the PSD95 SH3–GK tandem revealed that the SH3 domain and the GK domain pack extensively with each other to form an integral structural unit in which the last two β-strands (βE and βF) of the SH3 domain are contributed by two peptide fragments from the extreme N and C termini of the GK domain[53,54] (FIG. 5a). Therefore, the SH3 domain of the SH3–GK tandem is a split protein domain, and the SH3–GK structure is an integral supramodule. The supramodular intramolecular SH3–GK packing is required for the proper functions of PSD95. Disruption of the SH3–GK interaction compromised PSD95-mediated clustering of $K_v1.4$ K^+ channels[95], and mutations of the only PSD95-family MAGUK in *Drosophila melanogaster* (DLG1) that disrupt SH3–GK packing led to a tumorigenic phenotype of larval imaginal discs[96]. The structure of the PSD95 SH3–GK supramodule shows that the canonical ligand-binding site of the SH3 domain is occluded by the long sequence inserted between βD and βE; this insertion is known as the hinge region in MAGUK-family proteins. It is interesting to note that the sequences of the hinges are highly diverse among the family members and can bind to various regulatory proteins, such as protein 4.1 (also known as EBP41) and calmodulin[97–99]. It is possible that binding of these regulatory proteins to the hinge can regulate the SH3–GK interaction, as the interdomain interaction is primarily mediated by the βE and βF strands of the SH3–GK tandem and thus is expected not to be tight. Indeed, substitution of the entire hinge region with a rigid five-Pro linker disrupted SH3–GK packing and resulted in the formation of mutant protein dimers in solution[53].

The structure of the auxiliary β-subunit of voltage-gated Ca^{2+} channels ($Ca_v\beta$s) was solved a few years ago. Unexpectedly, this subunit contains an SH3–GK supramodule. Although the orientation of the two domains to each other is different from that in the PSD95 SH3–GK tandem[55,56,100] (FIG. 5a,b), the topology of the $Ca_v\beta$ SH3–GK supramodule is remarkably similar to that of the PSD95 SH3–GK tandem. The first conserved domain in $Ca_v\beta$ (C1) constitutes part of the SH3 domain (βA to βD), and the last β-strand of the SH3 domain is contributed by the N-terminal end of the second conserved domain (C2), which also forms the GK domain (thus, the SH3 domain can be

characterized as a split protein module). The βD and βE strands of the SH3 domain are separated by an α-helix and a long variable loop called V2 (equivalent to the hinge sequence in the PSD95 SH3–GK tandem), and the canonical target-binding site of the SH3 domain is occluded by this hinge sequence (FIG. 5b). Although the α-interacting domain (AID) of the channel α-subunit binds to a hydrophobic groove opposite the SH3–GK interface (FIG. 5b), the interaction between SH3 and GK is absolutely required for both membrane-surface trafficking and the channel gating exerted by $Ca_v\beta$[101,102]. It was shown using a domain-complementation approach that both V2 and the last β-strand of SH3 (contributed by C2) are directly involved in the surface trafficking and channel-gating properties of $Ca_v\beta$[102].

The structures of the SH3–GK supramodules of PSD95 and $Ca_v\beta$, together with associated structural and functional studies, reveal a common theme for these MAGUK scaffolds: the orientation of SH3 and GK to one another is flexible owing to the small domain interface, and the hinge that splits the SH3 domain between βD and βE is likely to act as a structural and functional switch. As shown in FIG. 5c, it is possible that the binding of regulatory proteins to the hinge region of the split SH3 domain causes several changes to the SH3–GK supramodule. It might alter the rigidity of the hinge region, thereby converting the SH3–GK monomer into a dimer or oligomer. Alternatively, it might alter the orientation of SH3 and GK to one another, potentially exposing new binding sites in the SH3–GK supramodule, which could be in either the SH3 or GK domains or in the new interface between them.

DH–PH supramodule in GEFs

One of the most salient features of synaptic plasticity is the activity-dependent change in neuron morphology, which is reflected by changes in the number and shape of dendritic spines. Spine formation and subsequent changes in morphology are directly coupled to the dynamics of the actin cytoskeleton, which are intimately linked to the Rho-family small GTPases and their regulatory GEFs and GTPase-activating proteins (GAPs)[42,103]. Consistent with their crucial roles in spine dynamics, small GTPases and their regulators constitute a large proportion (~8%) of total PSD proteins[23]. GEFs catalyze the exchange of GDP for GTP of small GTPases, and the human Dbl family of Rho-GEFs (the most common GEFs) contains 69 distinct members[104]. A number of these Rho-GEFs, including βPIX (also known as ARHGEF7), kalirin, LFC (also known as ARHGEF2), ephexin 1 (also known as NGEF) and T-cell lymphoma invasion and metastasis 1 (TIAM1), have been shown to directly regulate dendritic spine morphogenesis, often in an activity-dependent manner[105–112]. Many of these GEFs are known to interact with PDZ scaffold proteins in the PSD[42].

The Dbl-family Rho-GEFs invariably contain a DH domain immediately followed by a C-terminal PH domain[104] (FIG. 2). Extensive studies have unequivocally shown that the DH–PH tandems in Rho-GEFs form distinct structural and functional supramodules[104]. It is well established that the DH domain is largely responsible for catalyzing nucleotide exchange and dictating GTPase binding specificity. The PH domain in the tandem has multiple functions, including regulating the exchange activities (both activation and inhibition) of the DH domain by directly contacting the GTPase; facilitating membrane attachment of the GEF by binding to phosphatidylinositides embedded in the membrane bilayers; and modulating the GEF's activities allosterically by interacting with phospholipids[59,104,113]. In the GTPase-unbound forms, the contact surface between the DH and PH domains is small and limited to the C-terminal end of the last α-helix (α6) of DH and the N-terminal end of PH, and therefore the interdomain orientation is flexible[114] (FIG. 6a). The phospholipid binding loops (the β1–β2 and β3–β4 loops) of the PH domain are flexible and freely accessible to lipid binding. In the GTPase-bound DH–PH complex, the PH domain is rotated ~10° clockwise with respect to the DH domain, such that the β3–β4 loop of the PH domain is in direct contact with the so-called switch 2 region of the GTPase, which helps to push Mg^{2+}-GDP out of the GTPase (accelerating the GEF activity of the DH domain; FIG. 6b,d)[58,104,115,116]. Thus, full GEF activity requires the coordinated engagement of both the DH and the PH domains of the DH–PH supramodule with the GTPase. Several factors can probably trigger the realignment of the DH and PH domains into a fully catalytically active orientation (for example, simple GTPase binding, the insertion of phospholipids into the lipid-binding pocket of the PH domain, or the binding of other proteins to the DH–PH tandem), as such realignment involves relatively small conformational changes and is not energy-demanding owing to the intrinsic interdomain flexibility of the DH–PH tandem[59,114,116].

In a subclass of Rho GEFs, called the Trio GEFs (examples include SOS1, PREX1, p63RHOGEF, TRIO and kalirin), the PH domain auto-inhibits the GEF activity of the DH domain[59,113,117]. Activation of these GEFs requires the binding of additional regulatory factors, including proteins and/or phospholipids, to the DH–PH tandem. One well-characterized example is p63RHOGEF, which is directly activated by the $G\alpha_q$ subfamily of heterotrimeric G proteins. Interestingly, p63RHOGEF lacks an RGS (regulator of G-protein signalling) domain for G-protein binding. Instead, $G\alpha_q$ binds directly to the DH–PH tandem of p63RHOGEF (FIG. 6c). In this complex, $G\alpha_q$ engages extensively with both the DH and the PH domains using residues that are conserved in the Trio family GEFs. The PH domain binds to $G\alpha_q$ using its last kinked α-helix and residues from the β2–β3 and β4–β5 loops. The DH domain uses a surface opposite its RHOA-binding site, and hence there is no direct interaction between RHOA and $G\alpha_q$. Compared with the structure of the N-terminal RAC1-bound DH–PH supramodule of the enzyme, which is not activated by $G\alpha_q$[116], binding of $G\alpha_q$ to a Trio GEF leads to an ~50° anti-clockwise rotation of PH from DH with respect to the bound RHOA (FIG. 6c,d). Presumably, this $G\alpha_q$-induced displacement of the PH domain prevents it inhibiting the DH domain (that is, it removes the

Split protein domain
A protein domain that is characterized by insertions of one or several autonomously folded protein modules into the middle of the domain's primary sequence. In most split domains with known structures, the splitting does not normally change the domain's overall folding but often modifies its functions.

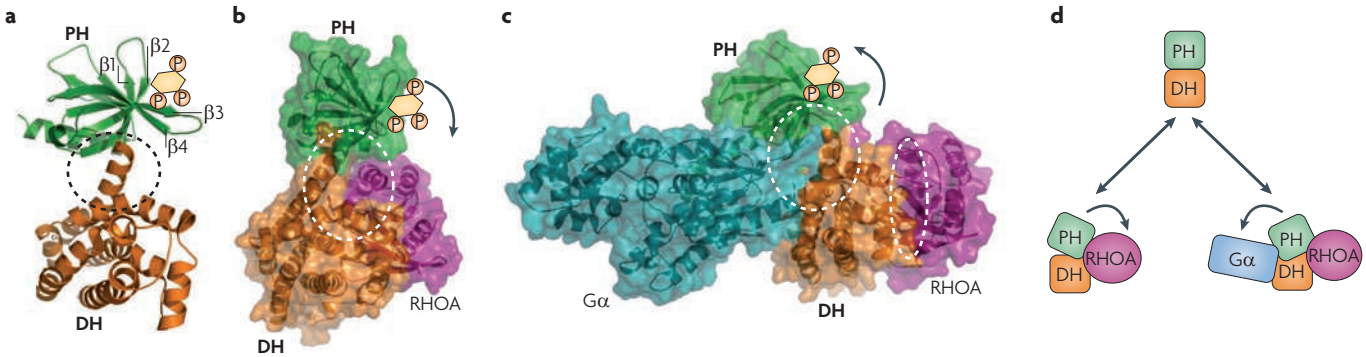

Figure 6 | **The DH–PH supramodules in RhoGEFs. a** | Ribbon diagram of the TRIO Dbl homology domain (DH)–pleckstrin homology domain (PH) (Protein Data Bank (PDB) code: 1NTY). **b** | Ribbon diagram combined with surface representation of the PDZ-RHOGEF (guanine nucleotide-exchange factor) DH–PH in complex with RHOA (PDB code: 1XCG). **c**| p63RHOGEF DH–PH in complex with RHOA and the G-protein subunit $G\alpha_q$ (PDB code: 2RGN). In these diagrams, the phosphatidylinositol-binding pockets formed by the β1–β2 and the β3–β4 loops of the PH domain are indicated by a phosphatidylinositol head group. The interfaces between different domains are highlighted by dashed-line circles. The orientation changes of the PH domain with respect to the DH domain on binding of the DH–PH to RHOA or $G\alpha_q$ are indicated by black arrows. **d** | A schematic diagram showing the domain-orientation changes of the DH–PH supramodule on binding to RHOA or $G\alpha_q$. The interdomain flexibility between the DH domain and the PH domain is crucial for the versatile functions of the DH–PH supramodule in tuning the nucleotide-exchange activities of the Rho small GTPases.

auto-inhibition). Again, the intrinsic flexibility of the domain interface plays a crucial part in the activity modulation of the p63RHOGEF DH–PH supramodule.

Concluding remarks

PDZ-domain proteins are the most abundant scaffold proteins found in the PSD. Through binding to their specific targets (proteins and phospholipids), PDZ-domain scaffolds have key roles at synapses, including clustering glutamate receptors, assembling signalling complexes, trafficking glutamate receptors and cytoskeletal remodelling of spines. In addition to having individual functions, the studies reviewed here suggest that two or more PDZ domains in multi-PDZ scaffold proteins often interact with each other to form supramodules with distinct spatial arrangements and target-binding properties. Building several PDZ domains into a supramodule not only provides distinct target-binding properties (such as enhanced binding affinity and unparalleled interaction specificity) for PDZ scaffolds, it also enables regulatory switches to be integrated into the PDZ scaffolds. This is an important concept, as increasing evidence suggests that catalytically inactive scaffold proteins are not mere passive molecular 'glue'[118,119] but, rather, active participants in the dynamic regulation of signalling events. Given the high abundance of multi-PDZ-domain scaffold proteins in the PSD, it would not be surprising if many other PDZ-supramodule-containing proteins help to regulate synaptic functions. In the future, PDZ supramodules in scaffolds should be studied as such, rather than as individual PDZ domains. It is important to point out that supramodules are likely to be a common feature of many multi-domain proteins, both in the PSD and in other cellular localizations. We have touched on only a few examples that are intimately related to the PDZ scaffolds in the PSD, and we expect many more supramodules to be identified and have their unique structural and functional properties uncovered. The prevalence of supramodules in multi-domain proteins also calls for scientists to interpret data obtained from individual domains with caution.

1. Sheng, M. & Hoogenraad, C. C. The postsynaptic architecture of excitatory synapses: a more quantitative view. *Annu. Rev. Biochem.* **76**, 823–847 (2007).
2. Shepherd, J. D. & Huganir, R. L. The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annu. Rev. Cell Dev. Biol.* **23**, 613–643 (2007).
3. Alvarez, V. A. & Sabatini, B. L. Anatomical and physiological plasticity of dendritic spines. *Annu. Rev. Neurosci.* **30**, 79–97 (2007).
4. Ziv, N. E. & Garner, C. C. Cellular and molecular mechanisms of presynaptic assembly. *Nature Rev. Neurosci.* **5**, 385–399 (2004).
5. Sudhof, T. C. The synaptic vesicle cycle. *Annu. Rev. Neurosci.* **27**, 509–547 (2004).
6. Okabe, S. Molecular anatomy of the postsynaptic density. *Mol. Cell. Neurosci.* **34**, 503–518 (2007).
7. Scannevin, R. H. & Huganir, R. L. Postsynaptic organization and regulation of excitatory synapses. *Nature Rev. Neurosci.* **1**, 133–141 (2000).
8. Kennedy, M. B. Signal-processing machines at the postsynaptic density. *Science* **290**, 750–754 (2000).
9. Cohen, R. S., Blomberg, F., Berzins, K. & Siekevitz, P. The structure of postsynaptic densities isolated from dog cerebral cortex. I. Overall morphology and protein composition. *J. Cell Biol.* **74**, 181–203 (1977).
10. Blomberg, F., Cohen, R. S. & Siekevitz, P. The structure of postsynaptic densities isolated from dog cerebral cortex. II. Characterization and arrangement of some of the major proteins within the structure. *J. Cell Biol.* **74**, 204–225 (1977).
11. Carlin, R. K., Grab, D. J., Cohen, R. S. & Siekevitz, P. Isolation and characterization of postsynaptic densities from various brain regions: enrichment of different types of postsynaptic densities. *J. Cell Biol.* **86**, 831–845 (1980).
12. Cho, K. O., Hunt, C. A. & Kennedy, M. B. The rat brain postsynaptic density fraction contains a homolog of the *Drosophila* discs-large tumor suppressor protein. *Neuron* **9**, 929–942 (1992).
13. Kim, J. H., Liao, D., Lau, L. F. & Huganir, R. L. SynGAP: a synaptic RasGAP that associates with the PSD-95/SAP90 protein family. *Neuron* **20**, 683–691 (1998).
14. Walsh, M. J. & Kuruc, N. The postsynaptic density: constituent and associated proteins characterized by electrophoresis, immunoblotting, and peptide sequencing. *J. Neurochem.* **59**, 667–678 (1992).
15. Kistner, U. *et al.* SAP90, a rat presynaptic protein related to the product of the *Drosophila* tumor suppressor gene *dlg*-A. *J. Biol. Chem.* **268**, 4580–4583 (1993).
16. Kim, E. & Sheng, M. PDZ domain proteins of synapses. *Nature Rev. Neurosci.* **5**, 771–781 (2004).
17. Funke, L., Dakoji, S. & Bredt, D. S. Membrane-associated guanylate kinases regulate adhesion and plasticity at cell junctions. *Annu. Rev. Biochem.* **74**, 219–245 (2005).
18. Walikonis, R. S. *et al.* Identification of proteins in the postsynaptic density fraction by mass spectrometry. *J. Neurosci.* **20**, 4069–4080 (2000).

19. Husi, H., Ward, M. A., Choudhary, J. S., Blackstock, W. P. & Grant, S. G. Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. *Nature Neurosci.* **3**, 661–669 (2000).
20. Peng, J. *et al.* Semiquantitative proteomic analysis of rat forebrain postsynaptic density fractions by mass spectrometry. *J. Biol. Chem.* **279**, 21003–21011 (2004).
21. Jordan, B. A. *et al.* Identification and verification of novel rodent postsynaptic density proteins. *Mol. Cell. Proteomics* **3**, 857–871 (2004).
22. Yoshimura, Y. *et al.* Molecular constituents of the postsynaptic density fraction revealed by proteomic analysis using multidimensional liquid chromatography-tandem mass spectrometry. *J. Neurochem.* **88**, 759–768 (2004).
23. Cheng, D. *et al.* Relative and absolute quantification of postsynaptic density proteome isolated from rat forebrain and cerebellum. *Mol. Cell. Proteomics* **5**, 1158–1170 (2006).
This paper presented an absolute quantification of proteins in PSDs purified from adult rat forebrain and cerebellum using proteomic approaches. The results were crucial for reconstructing PSDs at the molecular level.
24. Dosemeci, A., Tao-Cheng, J. H., Vinade, L. & Jaffe, H. Preparation of postsynaptic density fraction from hippocampal slices and proteomic analysis. *Biochem. Biophys. Res. Commun.* **339**, 687–694 (2006).
25. Chen, X. *et al.* Mass of the postsynaptic density and enumeration of three key molecules. *Proc. Natl Acad. Sci. USA* **102**, 11551–11556 (2005).
26. Sugiyama, Y., Kawabata, I., Sobue, K. & Okabe, S. Determination of absolute protein numbers in single synapses by a GFP-based calibration technique. *Nature Methods* **2**, 677–684 (2005).
27. Malenka, R. C. & Bear, M. F. LTP and LTD: an embarrassment of riches. *Neuron* **44**, 5–21 (2004).
28. Chen, X. *et al.* Organization of the core structure of the postsynaptic density. *Proc. Natl Acad. Sci. USA* **105**, 4453–4458 (2008).
This paper used EM tomography combined with immunogold labelling to delineate the organization of PSDs at glutamatergic synapses in rat hippocampal cultures. It provided images of the organization of PSDs from dendritic spines at the resolution of individual molecules.
29. Williams, K., Russell, S. L., Shen, Y. M. & Molinoff, P. B. Developmental switch in the expression of NMDA receptors occurs *in vivo* and *in vitro*. *Neuron* **10**, 267–278 (1993).
30. Quinlan, E. M., Olstein, D. H. & Bear, M. F. Bidirectional, experience-dependent regulation of *N*-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. *Proc. Natl Acad. Sci. USA* **96**, 12876–12880 (1999).
31. Sans, N. *et al.* A developmental change in NMDA receptor-associated proteins at hippocampal synapses. *J. Neurosci.* **20**, 1260–1271 (2000).
32. Petralia, R. S., Sans, N., Wang, Y. X. & Wenthold, R. J. Ontogeny of postsynaptic density proteins at glutamatergic synapses. *Mol. Cell. Neurosci.* **29**, 436–452 (2005).
33. Newpher, T. M. & Ehlers, M. D. Glutamate receptor dynamics in dendritic microdomains. *Neuron* **58**, 472–497 (2008).
34. Elias, G. M. & Nicoll, R. A. Synaptic trafficking of glutamate receptors by MAGUK scaffolding proteins. *Trends Cell Biol.* **17**, 343–352 (2007).
This review summarizes why the MAGUK family of PDZ scaffold proteins in PSDs are crucial in determining both the number and the mobility of glutamate receptors at the synapses.
35. Derkach, V. A., Oh, M. C., Guire, E. S. & Soderling, T. R. Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nature Rev. Neurosci.* **8**, 101–113 (2007).
36. Bredt, D. S. & Nicoll, R. A. AMPA receptor trafficking at excitatory synapses. *Neuron* **40**, 361–379 (2003).
37. Heine, M. *et al.* Surface mobility of postsynaptic AMPARs tunes synaptic transmission. *Science* **320**, 201–205 (2008).
38. Sharma, K., Fong, D. K. & Craig, A. M. Postsynaptic protein mobility in dendritic spines: long-term regulation by synaptic NMDA receptor activation. *Mol. Cell. Neurosci.* **31**, 702–712 (2006).
39. Blanpied, T. A., Kerr, J. M. & Ehlers, M. D. Structural plasticity with preserved topology in the postsynaptic protein network. *Proc. Natl Acad. Sci. USA* **105**, 12587–12592 (2008).
40. Bellone, C. & Nicoll, R. A. Rapid bidirectional switching of synaptic NMDA receptors. *Neuron* **55**, 779–785 (2007).
41. Gray, N. W., Weimer, R. M., Bureau, I. & Svoboda, K. Rapid redistribution of synaptic PSD-95 in the neocortex *in vivo*. *PLoS Biol.* **4**, e370 (2006).
42. Tada, T. & Sheng, M. Molecular mechanisms of dendritic spine morphogenesis. *Curr. Opin. Neurobiol.* **16**, 95–101 (2006).
43. Zhang, M. & Wang, W. Organization of signaling complexes by PDZ-domain scaffold proteins. *Acc. Chem. Res.* **36**, 530–538 (2003).
44. Bray, D. Signaling complexes: biophysical constraints on intracellular communication. *Annu. Rev. Biophys. Biomol. Struct.* **27**, 59–75 (1998).
45. Elias, G. M. *et al.* Synapse-specific and developmentally regulated targeting of AMPA receptors by a family of MAGUK scaffolding proteins. *Neuron* **52**, 307–320 (2006).
46. Migaud, M. *et al.* Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* **396**, 433–439 (1998).
47. Zimmermann, P. *et al.* PIP_2-PDZ domain binding controls the association of syntenin with the plasma membrane. *Mol. Cell* **9**, 1215–1225 (2002).
48. Long, J. F. *et al.* Supramodular structure and synergistic target binding of the N-terminal tandem PDZ domains of PSD-95. *J. Mol. Biol.* **327**, 203–214 (2003).
Determination of the structure of the N-terminal PDZ12 tandem of PSD95 for the first time directly showed that tandemly arranged PDZ domains function as supramodules with target-binding properties that are distinct from those of isolated PDZ domains or the simple sum of isolated PDZ domains.
49. Feng, W., Shi, Y., Li, M. & Zhang, M. Tandem PDZ repeats in glutamate receptor-interacting proteins have a novel mode of PDZ domain-mediated target binding. *Nature Struct. Biol.* **10**, 972–978 (2003).
By determining the structure of the PDZ45 tandem of GRIP1, this study showed that PDZ4 is needed for PDZ5 to bind to GluR2.
50. Long, J. F. *et al.* Autoinhibition of X11/Mint scaffold proteins revealed by the closed conformation of the PDZ tandem. *Nature Struct. Mol. Biol.* **12**, 722–728 (2005).
This study showed that the X11 PDZ12 tandem is auto-inhibited by the binding of the X11 C-terminal tail to PDZ1. The study also suggested that a regulatory switch modulates the conformational status of the X11 PDZ12 supramodule.
51. Grembecka, J. *et al.* The binding of the PDZ tandem of syntenin to target proteins. *Biochemistry* **45**, 3674–3683 (2006).
52. Long, J. *et al.* 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 (2008).
53. McGee, A. W. *et al.* 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 (2001).
54. Tavares, G. A., Panepucci, E. H. & Brunger, A. T. Structural characterization of the intramolecular interaction between the SH3 and guanylate kinase domains of PSD-95. *Mol. Cell* **8**, 1313–1325 (2001).
55. Van Petegem, F., Clark, K. A., Chatelain, F. C. & Minor, D. L. Structure of a complex between a voltage-gated calcium channel β-subunit and an α-subunit domain. *Nature* **429**, 671–675 (2004).
56. Chen, Y. H. *et al.* Structural basis of the α1–β subunit interaction of voltage-gated Ca^{2+} channels. *Nature* **429**, 675–680 (2004).
References 53–56 revealed that the SH3–GK tandems in MAGUKs and in $Ca_v\beta$ form structural and functional supramodules with potential regulatory switches in the interface between the SH3 and GK domains.
57. Skowronek, K. R., Guo, F., Zheng, Y. & Nassar, N. The C-terminal basic tail of RhoG assists the guanine nucleotide exchange factor trio in binding to phospholipids. *J. Biol. Chem.* **279**, 37895–37907 (2004).
58. Derewenda, U. *et al.* The crystal structure of RhoA in complex with the DH/PH fragment of PDZRhoGEF, an activator of the Ca^{2+} sensitization pathway in smooth muscle. *Structure* **12**, 1955–1965 (2004).
59. Lutz, S. *et al.* Structure of $G\alpha_q$-p63RhoGEF-RhoA complex reveals a pathway for the activation of RhoA by GPCRs. *Science* **318**, 1923–1927 (2007).
By determining the structure of the $G\alpha_q$–p63RHOGEF–RHOA complex, this study revealed the molecular basis of how the PH–DH supramodule of p63RHOGEF integrates G-protein coupled receptor signalling with small GTPase signalling pathways.
60. Harrison, S. C. Variation on an Src-like theme. *Cell* **112**, 737–740 (2003).
61. Vogel, C., Berzuini, C., Bashton, M., Gough, J. & Teichmann, S. A. Supra-domains: evolutionary units larger than single protein domains. *J. Mol. Biol.* **336**, 809–823 (2004).
62. Kornau, H. C., Schenker, L. T., Kennedy, M. B. & Seeburg, P. H. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* **269**, 1737–1740 (1995).
63. Kim, E., Niethammer, M., Rothschild, A., Jan, Y. N. & Sheng, M. Clustering of Shaker-type K^+ channels by interaction with a family of membrane-associated guanylate kinases. *Nature* **378**, 85–88 (1995).
64. Brenman, J. E. *et al.* Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and α1-syntrophin mediated by PDZ domains. *Cell* **84**, 757–767 (1996).
65. Niethammer, M. *et al.* CRIPT, a novel postsynaptic protein that binds to the third PDZ domain of PSD-95/SAP90. *Neuron* **20**, 693–707 (1998).
66. Songyang, Z. *et al.* Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. *Science* **275**, 73–77 (1997).
67. Esteban, P. F. *et al.* A kinase-deficient TrkC receptor isoform activates Arf6-Rac1 signaling through the scaffold protein tamalin. *J. Cell Biol.* **173**, 291–299 (2006).
68. Sugi, T. *et al.* Crystal structures of autoinhibitory PDZ domain of tamalin: implications for metabotropic glutamate receptor trafficking regulation. *EMBO J.* **26**, 2192–2205 (2007).
69. Dong, H. *et al.* GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. *Nature* **386**, 279–284 (1997).
70. Srivastava, S. *et al.* Novel anchorage of GluR2/3 to the postsynaptic density by the AMPA receptor-binding protein ABP. *Neuron* **21**, 581–591 (1998).
71. Wyszynski, M. *et al.* Association of AMPA receptors with a subset of glutamate receptor-interacting protein *in vivo*. *J. Neurosci.* **19**, 6528–6537 (1999).
72. Hoogenraad, C. C., Milstein, A. D., Ethell, I. M., Henkemeyer, M. & Sheng, M. GRIP1 controls dendrite morphogenesis by regulating EphB receptor trafficking. *Nature Neurosci.* **8**, 906–915 (2005).
73. Setou, M. *et al.* Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. *Nature* **417**, 83–87 (2002).
74. Fu, J., deSouza, S. & Ziff, E. B. Intracellular membrane targeting and suppression of Ser^{880} phosphorylation of glutamate receptor 2 by the linker I-set II domain of AMPA receptor-binding protein. *J. Neurosci.* **23**, 7592–7601 (2003).
75. Wyszynski, M. *et al.* Interaction between GRIP and liprin-α/SYD2 is required for AMPA receptor targeting. *Neuron* **34**, 39–52 (2002).
76. Takamiya, K. *et al.* A direct functional link between the multi-PDZ domain protein GRIP1 and the Fraser syndrome protein Fras1. *Nature Genet.* **36**, 172–177 (2004).
77. Osten, P. *et al.* Mutagenesis reveals a role for ABP/GRIP binding to GluR2 in synaptic surface accumulation of the AMPA receptor. *Neuron* **27**, 313–325 (2000).
78. Zhang, Q., Fan, J. S. & Zhang, M. Interdomain chaperoning between PSD-95, Dlg, and Zo-1 (PDZ) domains of glutamate receptor-interacting proteins. *J. Biol. Chem.* **276**, 43216–43220 (2001).
79. Rongo, C., Whitfield, C. W., Rodal, A., Kim, S. K. & Kaplan, J. M. LIN-10 is a shared component of the polarized protein localization pathways in neurons and epithelia. *Cell* **94**, 751–759 (1998).
80. Borg, J. P. *et al.* Identification of an evolutionarily conserved heterotrimeric protein complex involved in protein targeting. *J. Biol. Chem.* **273**, 31633–31636 (1998).
81. Butz, S., Okamoto, M. & Sudhof, T. C. A tripartite protein complex with the potential to couple synaptic vesicle exocytosis to cell adhesion in brain. *Cell* **94**, 773–782 (1998).
82. Setou, M., Nakagawa, T., Seog, D. H. & Hirokawa, N. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor- containing vesicle transport. *Science* **288**, 1796–1802 (2000).

83. Kang, B. S. *et al.* PDZ tandem of human syntenin: crystal structure and functional properties. *Structure* **11**, 459–468 (2003).
84. Beekman, J. M. & Coffer, P. J. The ins and outs of syntenin, a multifunctional intracellular adaptor protein. *J. Cell Sci.* **121**, 1349–1355 (2008).
85. Grootjans, J. J., Reekmans, G., Ceulemans, H. & David, G. Syntenin-syndecan binding requires syndecan-synteny and the co- operation of both PDZ domains of syntenin. *J. Biol. Chem.* **275**, 19933–19941 (2000).
86. Mortier, E. *et al.* Nuclear speckles and nucleoli targeting by PIP_2-PDZ domain interactions. *EMBO J.* **24**, 2556–2565 (2005).
This study showed that PI(4,5)P_2 and syndecan bind cooperatively to the PDZ12 tandem of syntenin 2, and that such coordinated lipid and protein ligand binding is required for the cellular functions of syntenin.
87. Wu, H. *et al.* PDZ domains of Par-3 as potential phosphoinositide signalling integrators. *Mol. Cell* **28**, 886–898 (2007).
This paper demonstrated that lipid binding is the second most prevalent interaction mode of PDZ domains known to date. It also demonstrated that the PDZ23 tandem of PAR3 has crucial roles in cell-polarity regulation, through the coordinated actions of PDZ2-mediated phosphatidylinositol-lipid binding and PDZ3-mediated PTEN interaction.
88. Feng, W., Wu, H., Chan, L. N. & Zhang, M. Par-3-mediated junctional localization of the lipid phosphatase PTEN is required for cell polarity establishment. *J. Biol. Chem.* **283**, 23440–23449 (2008).
89. Shi, S. H., Jan, L. Y. & Jan, Y. N. Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. *Cell* **112**, 63–75 (2003).
90. Kim, E. *et al.* GKAP, a novel synaptic protein that interacts with the guanylate kinase-like domain of the PSD-95/SAP90 family of channel clustering molecules. *J. Cell Biol.* **136**, 669–678 (1997).
91. Colledge, M. *et al.* Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. *Neuron* **27**, 107–119 (2000).
92. Kim, E. *et al.* Plasma membrane Ca^{2+} ATPase isoform 4b binds to membrane-associated guanylate kinase (MAGUK) proteins via their PDZ (PSD-95/Dlg/ZO-1) domains. *J. Biol. Chem.* **273**, 1591–1595 (1998).
93. Pak, D. T., Yang, S., Rudolph-Correia, S., Kim, E. & Sheng, M. Regulation of dendritic spine morphology by SPAR, a PSD-95-associated RapGAP. *Neuron* **31**, 289–303 (2001).
94. Brenman, J. E. *et al.* Localization of postsynaptic density-93 to dendritic microtubules and interaction with microtubule-associated protein 1A. *J. Neurosci.* **18**, 8805–8813 (1998).
95. Shin, H., Hsueh, Y. P., Yang, F. C., Kim, E. & Sheng, M. An intramolecular interaction between Src homology 3 domain and guanylate kinase-like domain required for channel clustering by postsynaptic density-95/SAP90. *J. Neurosci.* **20**, 3580–3587 (2000).
96. Woods, D. F., Hough, C., Peel, D., Callaini, G. & Bryant, P. J. Dlg protein is required for junction structure, cell polarity, and proliferation control in *Drosophila epithelia*. *J. Cell Biol.* **134**, 1469–1482 (1996).
97. Cohen, A. R. *et al.* Human CASK/LIN-2 binds syndecan-2 and protein 4.1 and localizes to the basolateral membrane of epithelial cells. *J. Cell Biol.* **142**, 129–138 (1998).
98. Lue, R. A., Brandin, E., Chan, E. P. & Branton, D. Two independent domains of hDlg are sufficient for subcellular targeting: the PDZ1–2 conformational unit and an alternatively spliced domain. *J. Cell Biol.* **135**, 1125–1137 (1996).
99. Masuko, N. *et al.* Interaction of NE-dlg/SAP102, a neuronal and endocrine tissue-specific membrane-associated guanylate kinase protein, with calmodulin and PSD- 95/SAP90. A possible regulatory role in molecular clustering at synaptic sites. *J. Biol. Chem.* **274**, 5782–5790 (1999).
100. Opatowsky, Y., Chen, C. C., Campbell, K. P. & Hirsch, J. A. Structural analysis of the voltage-dependent calcium channel β subunit functional core and its complex with the α1 interaction domain. *Neuron* **42**, 387–399 (2004).
101. Takahashi, S. X., Miriyala, J. & Colecraft, H. M. Membrane-associated guanylate kinase-like properties of β-subunits required for modulation of voltage-dependent Ca^{2+} channels. *Proc. Natl Acad. Sci. USA* **101**, 7193–7198 (2004).
102. Takahashi, S. X., Miriyala, J., Tay, L. H., Yue, D. T. & Colecraft, H. M. A $Ca_v\beta$ SH3/guanylate kinase domain interaction regulates multiple properties of voltage-gated Ca^{2+} channels. *J. Gen. Physiol.* **126**, 365–377 (2005).
103. Etienne-Manneville, S. & Hall, A. Rho GTPases in cell biology. *Nature* **420**, 629–635 (2002).
104. Rossman, K. L., Der, C. J. & Sondek, J. GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. *Nature Rev. Mol. Cell Biol.* **6**, 167–180 (2005).
This comprehensive review covers the structure and the catalytic and regulatory mechanisms of the PH–DH tandem-containing GEFs.
105. Saneyoshi, T. *et al.* Activity-dependent synaptogenesis: regulation by a CaM-kinase kinase/CaM-kinase I/βPIX signaling complex. *Neuron* **57**, 94–107 (2008).
106. Xie, Z. *et al.* Kalirin-7 controls activity-dependent structural and functional plasticity of dendritic spines. *Neuron* **56**, 640–656 (2007).
107. Zhang, H. & Macara, I. G. The polarity protein PAR-3 and TIAM1 cooperate in dendritic spine morphogenesis. *Nature Cell Biol.* **8**, 227–237 (2006).
108. Ryan, X. P. *et al.* The Rho-specific GEF Lfc interacts with neurabin and spinophilin to regulate dendritic spine morphology. *Neuron* **47**, 85–100 (2005).
109. Tolias, K. F. *et al.* The Rac1-GEF Tiam1 couples the NMDA receptor to the activity-dependent development of dendritic arbors and spines. *Neuron* **45**, 525–538 (2005).
110. Parnas, D., Haghighi, A. P., Fetter, R. D., Kim, S. W. & Goodman, C. S. Regulation of postsynaptic structure and protein localization by the Rho-type guanine nucleotide exchange factor dPix. *Neuron* **32**, 415–424 (2001).
111. Penzes, P. *et al.* The neuronal Rho-GEF kalirin-7 interacts with PDZ domain-containing proteins and regulates dendritic morphogenesis. *Neuron* **29**, 229–242 (2001).
112. Irie, F. & Yamaguchi, Y. EphB receptors regulate dendritic spine development via intersectin, Cdc42 and N-WASP. *Nature Neurosci.* **5**, 1117–1118 (2002).
113. Welch, H. C. *et al.* P-Rex1, a PtdIns(3,4,5)P3- and Gβγ-regulated guanine-nucleotide exchange factor for Rac. *Cell* **108**, 809–821 (2002).
114. Worthylake, D. K., Rossman, K. L. & Sondek, J. Crystal structure of the DH/PH fragment of Dbs without bound GTPase. *Structure* **12**, 1078–1086 (2004).
115. Worthylake, D. K., Rossman, K. L. & Sondek, J. Crystal structure of Rac1 in complex with the guanine nucleotide exchange region of Tiam1. *Nature* **408**, 682–688 (2000).
116. Chhatriwala, M. K., Betts, L., Worthylake, D. K. & Sondek, J. The DH and PH domains of Trio coordinately engage Rho GTPases for their efficient activation. *J. Mol. Biol.* **368**, 1307–1320 (2007).
117. Soisson, S. M., Nimnual, A. S., Uy, M., Bar-Sagi, D. & Kuriyan, J. Crystal structure of the Dbl and pleckstrin homology domains from the human Son of sevenless protein. *Cell* **95**, 259–268 (1998).
118. Mishra, P. *et al.* Dynamic scaffolding in a G protein-coupled signaling system. *Cell* **131**, 80–92 (2007).
This paper showed that, in addition to having a passive scaffolding role, the fifth PDZ domain in the photoreceptor scaffold protein INAD actively regulates visual signalling in fruit flies through light-dependent conformational cycling. The work demonstrated that, in addition to well-accepted assembling capacities, scaffold proteins can actively participate in the regulation of signalling events.
119. Zhang, M. Scaffold proteins as dynamic switches. *Nature Chem. Biol.* **3**, 756–757 (2007).
120. Willott, E. *et al.* The tight junction protein ZO-1 is homologous to the *Drosophila* discs- large tumor suppressor protein of septate junctions. *Proc. Natl Acad. Sci. USA* **90**, 7834–7838 (1993).
121. Woods, D. F. & Bryant, P. J. ZO-1, DlgA and PSD-95/SAP90: homologous proteins in tight, septate and synaptic cell junctions. *Mech. Dev.* **44**, 85–89 (1993).
122. Kennedy, M. B. Origin of PDZ (DHR, GLGF) domains. *Trends Biochem. Sci.* **20**, 350 (1995).
123. Doyle, D. A. *et al.* Crystal structures of a complexed and peptide-free membrane protein- binding domain: molecular basis of peptide recognition by PDZ. *Cell* **85**, 1067–1076 (1996).
124. Sheng, M. & Sala, C. PDZ domains and the organization of supramolecular complexes. *Annu. Rev. Neurosci.* **24**, 1–29 (2001).
125. Harris, B. Z. & Lim, W. A. Mechanism and role of PDZ domains in signaling complex assembly. *J. Cell Sci.* **114**, 3219–3231 (2001).
126. Stiffler, M. A. *et al.* PDZ domain binding selectivity is optimized across the mouse proteome. *Science* **317**, 364–369 (2007).
127. Tonikian, R. *et al.* A specificity map for the PDZ domain family. *PLoS Biol.* **6**, e239 (2008).
128. Hillier, B. J., Christopherson, K. S., Prehoda, K. E., Bredt, D. S. & Lim, W. A. Unexpected modes of PDZ domain scaffolding revealed by structure of nNOS-syntrophin complex. *Science* **284**, 812–815 (1999).
129. Tochio, H. *et al.* Formation of nNOS/PSD-95 PDZ dimer requires a preformed β-finger structure from the nNOS PDZ domain. *J. Mol. Biol.* **303**, 359–370 (2000).
130. Wong, H. C. *et al.* Direct binding of the PDZ domain of Dishevelled to a conserved internal sequence in the C-terminal region of Frizzled. *Mol. Cell* **12**, 1251–1260 (2003).
131. Penkert, R. R., DiVittorio, H. M. & Prehoda, K. E. Internal recognition through PDZ domain plasticity in the Par-6-Pals1 complex. *Nature Struct. Mol. Biol.* **11**, 1122–1127 (2004).
132. Yan, J. *et al.* Structure of the split PH domain and distinct lipid-binding properties of the PH-PDZ supramodule of α-syntrophin. *EMBO J.* **24**, 3985–3995 (2005).
133. Pan, L. F. *et al.* Clustering and synaptic targeting of PICK1 requires direct interaction between the PDZ domain and lipid membranes. *EMBO J.* **26**, 4576–4587 (2007).

Acknowledgements
We would like to apologize for not being able to cite many original papers from colleagues owing to space constraints. We thank N. Ip, J. Xia and members of M.Z.'s laboratory for comments and suggestions on the manuscript, and an anonymous reviewer for suggesting changes to figure 1. M.Z. is supported by grants from the Research Grants Council of Hong Kong.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
βPIX | CRIPT | DLG1 | ephexin 1 | ephrin | FRAS1 | GRIP1 | kalirin | LFC | MINT | neurofascin | p63RHOGEF | PAR3 | PDZ-RHOGEF | PREX1 | PSD93 | PSD95 | SAP97 | SAP102 | SOS1 | SYNGAP1 | syntenin | tamalin | TIAM1 | TRIO | X11 | ZO1

FURTHER INFORMATION
Mingjie Zhang's homepage: http://bcz102.ust.hk

ALL LINKS ARE ACTIVE IN THE ONLINE PDF