Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Shanks — multidomain molecular scaffolds of the postsynaptic density

Petri Kursula[1,2]

The postsynaptic density (PSD) is a protein-rich assembly below the postsynaptic membrane, formed of large scaffolding proteins. These proteins carry a combination of protein interaction domains, which may interact with several alternative partners; the structure of the protein assembly can be regulated in an activity-dependent manner. A major scaffolding molecule in the PSD is Shank, a family of three main isoforms with highly similar domain structure. Proteins of the Shank family are targets of mutations in neurological disorders, such as autism and schizophrenia. All the predicted folded domains of Shank have now been crystallized. However, for an understanding of the structure and function of full-length Shank and its complexes in the supramolecular PSD assembly, novel complementary approaches and hybrid techniques must be employed.

Addresses
[1] Department of Biomedicine, University of Bergen, Jonas Lies vei 91, Bergen, Norway
[2] Faculty of Biochemistry and Molecular Medicine, University of Oulu, Aapistie 7, Oulu, Finland

Corresponding author: Kursula, Petri (petri.kursula@uib.no)

Current Opinion in Structural Biology 2019, **54**: 122–128

This review comes from a themed issue on **Proteins: 3D-structures**

Edited by **Gabrielle Rudenko** and **Hideto Takahashi**

For a complete overview see the Issue and the Editorial

Available online 6th March 2019

https://doi.org/10.1016/j.sbi.2019.01.007

The postsynaptic density (PSD) is an electron-dense supramolecular structure made of specific proteins linking the postsynaptic membrane to the neuronal cytoskeleton. The PSD contains 'scaffolding' proteins, which have multiple folded domains as well as regions predicted to be highly flexible. PSD scaffold proteins belong to several protein families, and they each carry distinct sets of protein interaction domains, which can be used not only to form multivalent contacts in the tight protein network of the PSD, but also for regulating the PSD molecular assembly in an activity-dependent manner. The latter is important for the participation of the PSD in long-term potentiation and depression. The structure of the PSD varies between excitatory and inhibitory synapses [1••].

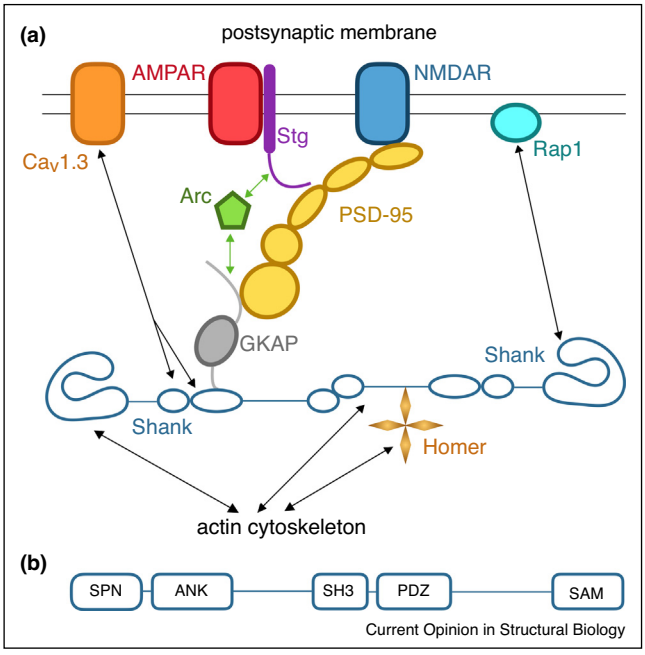
Different scaffold molecules are concentrated at different depths of the PSD from the postsynaptic membrane [2,3]. The distribution of individual proteins can be regulated by synaptic activity [4–7], which in turn may result in changes in post-translational modifications as well as interaction partners. While many interactions have been well-characterized at the domain level, information is lacking at the level of full-length scaffold proteins, concentrated into a dense phase at the PSD. Here, the structures of the folded domains of the Shank scaffolding proteins are reviewed, and aspects of full-length Shank structure are considered.

Shank as a PSD scaffold protein

PSD scaffolding proteins belong to different families, which differ in their modular domain composition. Through these scaffolds, the postsynaptic membrane and the receptors residing in it are linked to the cytoskeleton. The main PSD scaffolds include membrane-associated guanylate kinases (such as PSD-95), Shanks, calmodulin-dependent protein kinase II, Homer, synaptic Ras GTPase-activating protein 1, and guanylate kinase-associated protein (GKAP) (Figure 1a). For many of the individual domains in the scaffold proteins, structural data exist (see Table 1 for examples), but it is to a large extent still unclear, how interactions and higher-order assemblies form at the molecular level, and whether and how different domains within the same large scaffold protein might interact.

A central PSD scaffold is made up of proteins belonging to the Shank family (Figure 1b). The three major isoforms of Shank, produced from different genes, are highly similar, and a molecular basis of their possible functional differences is currently lacking. Mutations related to neuropsychiatric or neurodevelopmental disorders, including autism spectrum disorders and schizophrenia, have been linked to the Shank proteins [8–13], but the molecular effects of the mutations remain in general unknown. Considering the nature of Shank as a large molecule with several interaction partners, it is likely the mutations will affect one or more of its molecular interactions in a way that will disturb normal formation and regulation of the PSD. Although the structural domain components of Shank are rather simple in isolation, it is the combination of them within the same polypeptide chain that allows for multivalent interactions with other

Figure 1



A Shank-centric view of the PSD assembly. **(a)** Simplified view of the organization of the PSD. **(b)** The domain structure of full-length Shank proteins.

proteins in the PSD network and enables protein phase separation to form the PSD assembly.

At the supramolecular level, Shank is known to both self-assemble into large scaffolds as well as to form networks with other PSD proteins [14,15]. Shank interaction partners, including GKAP, are able to bind several protein ligands, and regulatory proteins, such as Arc, may form transient interactions in an activity-dependent manner with different PSD proteins [16–18]. Different populations of Shank have been detected at different depths in the PSD [3].

The domains of Shank

While the Shank isoforms present themselves through different splice isoforms [19], conserved domains are readily identified. A conserved SPN (Shank/ProSAP N-terminal) domain at the Shank N terminus has been recognized, but only recent studies highlighted its functional and structural details [20••]. The SPN domain is followed by ankyrin repeats. The central part of Shank contains one copy each of the SH3 and PDZ domain — classical protein interaction domains recognizing short linear peptide motifs. The C terminus of Shank harbors a SAM domain, shown to form a supramolecular lattice at the PSD in a Zn-dependent manner [14].

While the SAM and PDZ domains have been structurally characterized some years ago, data on the N-terminal segment as well as the SH3 domain have now provided crucial new information on the Shank family at the molecular level (Figure 2). Recent research has highlighted a role for Shanks in integrin inactivation through small GTP-binding proteins [20••]. The N-terminal domain of Shank is directly involved in these processes through protein–protein interactions with Rap1 and R-Ras. The SPN domain is structurally homologous to the Rap1-binding domain in talin, which is a major regulator of integrin activity. The ASD-linked mutations in the SPN domain were shown to impair interactions with Rap1 [20••], indicating possible disease mechanisms related to protein interactions.

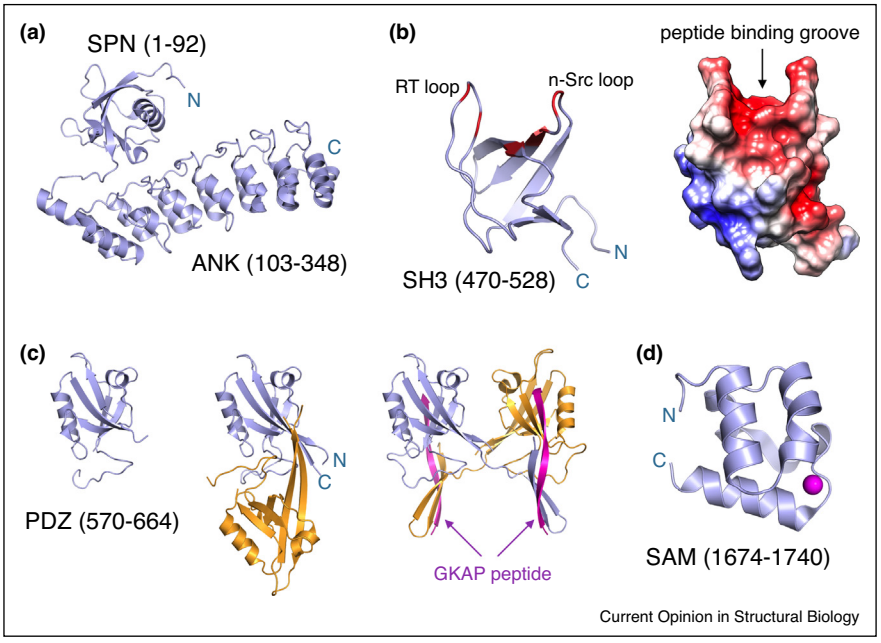
The Shank SH3 domain folds like a canonical SH3 domain (Figure 2b); however, the binding site for proline-rich peptides, comprised of highly conserved aromatic residues in SH3 domains, has been lost in Shank [21,22•]. Accordingly, a large-scale screen for binding motifs of human SH3 domains failed to find ligands for any of the Shank SH3 domains [23•]. Whether the SH3 domain could have specific ligands that do not conform to the PxxP motif, has remained unclear until very recently. Proline-rich partners for the Shank SH3 domain have

Table 1

Structures of selected PSD scaffold protein domains. Note that for some domains, a number of structures are available, and not all individual structures are referred to below

Scaffold protein	Domains	PDB entry	Reference
GKAP	GH1 domain	4r0y	[51]
PSD-95	PDZ1-2	3zrt	[52]
	PDZ3	3i4w	[53]
	SH3+GK	1kjw, 5ypr	[54,55]
	GK bound to GKAP peptide	5ypo	[55]
	Full-length model from EM and SAXS	2xkx	[56]
Homer	EVH1 domain	1ddw	[57]
	Tetramerization domain	3cve	[15]
Shank	SPN-ANK	5g4x	[20••]
	PDZ	1q3p, 5izu, 3o5n, 3l4f, 3qjm, 5ova, 5ovc	[32,35,58–60]
	SH3	5o99, 6cpi, 6cpj, 6cpk	[21,22•]
	SAM	2f3n, 2f44	[14]

Figure 2



Crystal structures of all known folded domains of Shank. The sequence numbering for all domains corresponds to the rat Shank3 (UniProt Q9JLU4), as nearly all structural data are from this protein. N and C termini are indicated by blue letters. **(a)** The SPN-ankyrin unit [20••]. The SPN domain folds back onto the ankyrin repeats. **(b)** The Shank3 SH3 domain [22•]. Left: the high-resolution crystal structure [22•], with the residues mainly implicated in $Ca_V1.3$ peptide binding [21] in red. Right: Surface electrostatics indicate a highly polarized structure, with the $Ca_V1.3$ binding site having a strong negative charge. The canonical loops and the peptide binding groove of SH3 domains are indicated. **(c)** The PDZ domain. Left: The unliganded PDZ domain monomer [32]. Middle: Dimeric form of the PDZ domain [35]. Right: Another dimeric form induced by an elongated GKAP peptide (magenta, arrow) [34]. **(d)** The Zn-bound SAM domain [14].

been suggested [24,25], and an interaction between the Shank ankyrin domain and the SH3 domain has been reported, possibly linked to multimerization [26]. The structures of the Shank SH3 domains were solved by NMR, and a non-canonical direct interaction partner was identified as the cytoplasmic domain of the $Ca_V1.3$ channel [21]. This interaction is of electrostatic nature (Figure 2b), and could play a role in linking $Ca_V1.3$ channels to the PSD scaffold. $Ca_V1.3$ channels are known to be important for neuronal function, and they possibly play a role in PSD structure through interactions with PDZ domains of the scaffold proteins [27]. It is possible that the SH3 and PDZ domains both bind to the cytoplasmic part of $Ca_V1.3$ [21]. In general, it is likely that simultaneous interactions with two neighboring protein interaction domains will increase the affinity of binding, and the SH3-PDZ unit of Shank could be a functional interaction module, providing a large interaction surface-coupled to flexibility. Similarly, intramolecular interactions between PDZ and SH3 domains were reported for GKAP [28]. The finding of an unconventional SH3 domain that binds non-canonical ligands paves the way for the identification of additional non-PxxP ligands for SH3 domains, in which the aromatic binding site is not conserved, including the SH3 domain of Caskin [29]. Caskins are members of a presynaptic protein scaffold, and similarly to Shank, in addition to a non-canonical SH3 domain, they contain SAM domains [30], which can polymerize. The Caskin1 SH3 domain was shown to bind lipids, such as lysophosphatidic acid, instead of proteins [31]; such hypotheses are testable also in the case of Shank.

The PDZ domain in Shanks binds to typical class I ligands with high affinity [32]. In addition, recent screens have identified internal binding motifs, which broaden the ligand selection for the PDZ domain [33]. For known Shank PDZ domain class I ligands, side-chain interactions fine-tune the peptide affinity; the highest affinity thus far has been detected toward the GKAP C terminus [32]. In addition, an extended GKAP peptide ligand was shown to interact with another binding site in the linker between the SH3 and PDZ domains, resulting in an order of magnitude higher affinity [34] — hence, for full protein–protein complexes, affinities estimated through binding assays involving a single domain and a short linear peptide may provide misleading results in the biological context.

Shank PDZ domain dimerization has been observed in crystal structures both with and without bound ligand peptides [34,35]. The two observed dimerization modes

both involve residues from the SH3-PDZ linker region (Figure 2c). Whether physiological binding partners cause changes in full-length Shank oligomeric state and/or conformation, such as seen in the crystal structures of the extended PDZ domains, remains to be determined — if this would be the case, it would provide truly novel insights into the regulation of the PSD molecular assembly.

The surface properties of the SH3 and PDZ domains may give further clues to ligand interactions. Both domains are electrostatically highly polarized [22•,32], which may promote their interactions with each other, as well as with binding partners in the PSD. In addition, the SH3-PDZ unit may have specific properties resulting from the interplay of these domains connected by a linker, which is less flexible than predicted and participates in ligand protein binding [32,34]. It is likely that ligands binding to a Shank PDZ domain through their C terminus in addition have interactions with the SH3 domain and the SH3-PDZ linker.

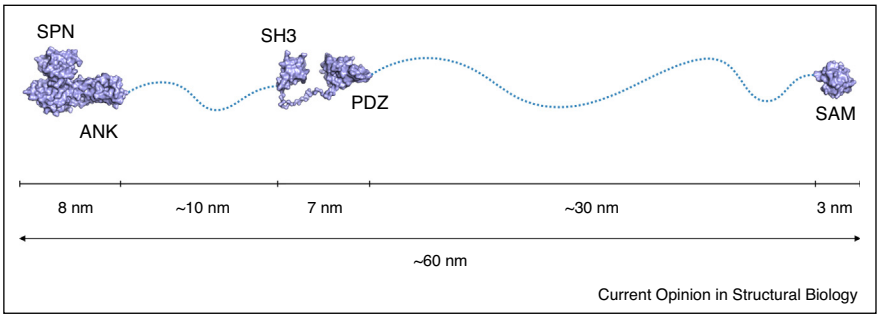
The C-terminal Shank SAM domain (Figure 2d) binds zinc and is able to form homo-oligomeric structures [14]. Only the SAM domains of Shank2 and Shank3 bind Zn, while Shank1 does not [36]. The SAM domain is expected to form filaments/polymers, and the form used for high-resolution structure determination was, indeed, mutated to prevent this phenomenon [14]. Current advances in cryo-EM data collection and processing would be tempting approaches to solve a high-resolution structure of this core assembly of the PSD.

From individual domains to full-length Shank

Considering full-length Shank, we must take into account long regions predicted to be disordered. Between the ankyrin repeats and the SH3 domain, a >100-residue linker is present, which in a random coil conformation would have an average length of ~10 nm. The 40-residue linker between the SH3 and PDZ domains was shown to be compact, and the SH3 and PDZ domains are close together in solution [32]. The longest disordered region is found between the PDZ and SAM domains, and its length of ~1000 residues would correspond to a random chain of ~30 nm in diameter. This proline-rich segment, although not likely to be folded, is an important protein interaction domain in Shank proteins. Taking into account known structures, and the linkers between them, one can estimate an average length of 60 nm for a single Shank molecule (Figure 3). Multimerization of Shanks via the SAM domain would, hence, generate huge assemblies of Shank, spanning large distances relative to the size of the PSD. Until we obtain more structural data on the linker regions and full-length Shank, we cannot be sure of the conformation(s) Shank will sample in solution and, more importantly, in the PSD. The median distance of Shank, labelled with antibodies recognizing N-terminal regions, from the postsynaptic membrane was ~50 nm, with large variations, changes upon K^+ or Ca^{2+} stimulation, and the presence of GKAP between Shank and the membrane [3]. Considering the possible dimensions of full-length Shank (Figure 3), it is possible that at least some activity-dependent changes [4,6] could reflect differences in Shank conformation and binding partners, rather than complete relocalization of Shank, especially if, for example, antibodies against one end of Shank are used for localization studies.

Phase separation in the PSD is a recent suggestion for the mechanism of formation of the PSD molecular network [37]. Phase separation, or the formation of so-called membraneless organelles, is an emerging theme in many biological processes, and can be induced by properties of individual macromolecules and/or complexes under specific conditions. The structural details of forming a separated phase centered on Shank and other PSD scaffolds still remain to be elucidated, although recent work using various truncated PSD proteins has shed light on the domains and interactions required for this phenomenon [38••].

Figure 3



Estimated dimensions of an extended full-length Shank molecule. Dimensions of the folded domains are based on crystal and SAXS structures [14,20••,32], while those of the linkers have been calculated based on random polymer theory [50].

Overall, more information will be required to obtain an accurate view on the structure and dynamics of full-length Shank. Whether the various domains interact, and if different segments of Shank – and its protein complexes – form 'supramodules', such as those detected in PSD-95 [39], are some of the questions that will require the use of multidisciplinary and hybrid techniques. Current technological advances in structural biology, microscopy, and biophysics may make solving the structure of the PSD reality, and several practical aspects of this process have been discussed [40].

Shank in disease

Scaffolding proteins of the PSD are putative targets for mutations in neurological disorders [41]. In Shanks, mutations have been linked to neurodevelopmental and neuropsychiatric disorders, such as ASD [9,13,42,43]. Functional effects on protein interactions were described for the mutations in the SPN domain [20••]. A number of mouse mutant models have been used to decipher the *in vivo* roles of the Shank proteins in the PSD. Common features include changes in the molecular composition of the PSD and behavioral and learning defects [44•].

Can we use current structural data to understand the possible molecular mechanisms of disease? Is it possible to affect protein interactions in the PSD to treat such disorders? The Shank family and its interactions with other PSD proteins are considered to be promising targets for pharmacological intervention, with the aim of affecting neuropsychiatric disorders, such as ASD and schizophrenia [43,44•,45]. One important aspect hindering these approaches is the lack of adequate high-resolution structural information on both full-length Shank proteins as well as the protein–protein complexes they form at the PSD.

Conclusions

PSD is a highly complex molecular assembly, prone to regulation by synaptic activity. In addition to the large, multidomain scaffold proteins, the PSD contains regulatory elements, such as Arc, which interact with several PSD scaffold proteins and may be linked to activity-dependent changes of the synapse [16–18]. Methodological developments in many fields will allow visualizing PSD networks and events at the molecular level; such approaches include, but are not limited to, high-throughput proteomics, hybrid structural biology methods including cryo-EM and tomography [46,47], super-resolution microscopy coupled to specific nanobodies [48,49], as well as biophysical techniques for following protein interactions *in vivo*. The coming years will certainly bring about a much improved view on the structure and function of Shank and other PSD scaffold proteins.

Funding

This research did not receive any-specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

Nothing declared.

Acknowledgements

The author wishes to thank Srinivas Kumar Ponna and Erik Hallin for fruitful discussions.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Tao CL, Liu YT, Sun R, Zhang B, Qi L, Shivakoti S, Tian CL, Zhang P, Lau PM, Zhou ZH, Bi GQ: **Differentiation and characterization of excitatory and inhibitory synapses by cryo-electron tomography and correlative microscopy**. *J Neurosci* 2018, **38**:1493-1510.
Using state-of-the-art microscopic techniques, molecular structures in different kinds of synapses were revealed.

2. Valtschanoff JG, Weinberg RJ: **Laminar organization of the NMDA receptor complex within the postsynaptic density**. *J Neurosci* 2001, **21**:1211-1217.

3. Tao-Cheng JH, Yang Y, Reese TS, Dosemeci A: **Differential distribution of Shank and GKAP at the postsynaptic density**. *PLoS One* 2015, **10**:e0118750.

4. Tao-Cheng JH, Dosemeci A, Gallant PE, Smith C, Reese T: **Activity induced changes in the distribution of Shanks at hippocampal synapses**. *Neuroscience* 2010, **168**:11-17.

5. Tao-Cheng JH, Thein S, Yang Y, Reese TS, Gallant PE: **Homer is concentrated at the postsynaptic density and does not redistribute after acute synaptic stimulation**. *Neuroscience* 2014, **266**:80-90.

6. Tao-Cheng JH, Yang Y, Bayer KU, Reese TS, Dosemeci A: **NMDA-induced accumulation of Shank at the postsynaptic density is mediated by CaMKII**. *Biochem Biophys Res Commun* 2014, **450**:808-811.

7. Shin SM, Zhang N, Hansen J, Gerges NZ, Pak DT, Sheng M, Lee SH: **GKAP orchestrates activity-dependent postsynaptic protein remodeling and homeostatic scaling**. *Nat Neurosci* 2012, **15**:1655-1666.

8. Arons MH, Thynne CJ, Grabrucker AM, Li D, Schoen M, Cheyne JE, Boeckers TM, Montgomery JM, Garner CC: **Autism-associated mutations in ProSAP2/Shank3 impair synaptic transmission and neurexin-neuroligin-mediated transsynaptic signaling**. *J Neurosci* 2012, **32**:14966-14978.

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

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

11. Sato D, Lionel AC, Leblond CS, Prasad A, Pinto D, Walker S, O'Connor I, Russell C, Drmic IE, Hamdan FF *et al.*: **SHANK1 deletions in males with autism spectrum disorder**. *Am J Hum Genet* 2012, **90**:879-887.

12. Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, Nygren G, Rastam M, Gillberg IC, Anckarsäter H *et al.*: **Mutations in the gene encoding the synaptic scaffolding**

protein SHANK3 are associated with autism spectrum disorders. *Nat Genet* 2007, **39**:25-27.

13. Leblond CS, Nava C, Polge A, Gauthier J, Huguet G, Lumbroso S, Giuliano F, Stordeur C, Depienne C, Mouzat K *et al.*: **Meta-analysis of SHANK mutations in autism spectrum disorders: a gradient of severity in cognitive impairments**. *PLoS Genet* 2014, **10**:e1004580.

14. Baron MK, Boeckers TM, Vaida B, Faham S, Gingery M, Sawaya MR, Salyer D, Gundelfinger ED, Bowie JU: **An architectural framework that may lie at the core of the postsynaptic density**. *Science* 2006, **311**:531-535.

15. Hayashi MK, Tang C, Verpelli C, Narayanan R, Stearns MH, Xu RM, Li H, Sala C, Hayashi Y: **The postsynaptic density proteins Homer and Shank form a polymeric network structure**. *Cell* 2009, **137**:159-171.

16. Hallin EI, Eriksen MS, Baryshnikov S, Nikolaienko O, Grødem S, Hosokawa T, Hayashi Y, Bramham CR, Kursula P: **Structure of monomeric full-length ARC sheds light on molecular flexibility, protein interactions, and functional modalities**. *J Neurochem* 2018, **147**:323-343.

17. Nikolaienko O, Patil S, Eriksen MS, Bramham CR: **Arc protein: a flexible hub for synaptic plasticity and cognition**. *Semin Cell Dev Biol* 2018, **77**:33-42.

18. Zhang W, Wu J, Ward MD, Yang S, Chuang YA, Xiao M, Li R, Leahy DJ, Worley PF: **Structural basis of arc binding to synaptic proteins: implications for cognitive disease**. *Neuron* 2015, **86**:490-500.

19. Lim S, Naisbitt S, Yoon J, Hwang JI, Suh PG, Sheng M, Kim E: **Characterization of the Shank family of synaptic proteins. Multiple genes, alternative splicing, and differential expression in brain and development**. *J Biol Chem* 1999, **274**:29510-29518.

20. •• Lilja J, Zacharchenko T, Georgiadou M, Jacquemet G, De Franceschi N, Peuhu E, Hamidi H, Pouwels J, Martens V, Nia FH *et al.*: **SHANK proteins limit integrin activation by directly interacting with Rap1 and R-Ras**. *Nat Cell Biol* 2017, **19**:292-305.
The authors determined the structure of the N-terminal segment containing the SPN and ankyrin domains. This region was shown to bind small GTPases and regulate integrin activation. The study has opened up completely new directions for Shank research.

21. Ishida H, Skorobogatov A, Yamniuk AP, Vogel HJ: **Solution structures of the SH3 domains from Shank scaffold proteins and their interactions with Cav1.3 calcium channels**. *FEBS Lett* 2018, **592**:2786-2797.

22. • Ponna SK, Myllykoski M, Boeckers TM, Kursula P: **Structure of an unconventional SH3 domain from the postsynaptic density protein Shank3 at ultrahigh resolution**. *Biochem Biophys Res Commun* 2017, **490**:806-812.
The sub atomic resolution crystal structure showed for the first time the loss of the canonical proline-rich peptide binding site in the Shank SH3 domain.

23. • Teyra J, Huang H, Jain S, Guan X, Dong A, Liu Y, Tempel W, Min J, Tong Y, Kim PM *et al.*: **Comprehensive analysis of the human SH3 domain family reveals a wide variety of non-canonical specificities**. *Structure* 2017, **25**:1598-1610.e3.
The authors carried out a high-throughput screen for ligands of human SH3 domains. No ligands could be identified for the Shank SH3 domains, however.

24. Quitsch A, Berhörster K, Liew CW, Richter D, Kreienkamp HJ: **Postsynaptic shank antagonizes dendrite branching induced by the leucine-rich repeat protein Densin-180**. *J Neurosci* 2005, **25**:479-487.

25. Zhang H, Maximov A, Fu Y, Xu F, Tang TS, Tkatch T, Surmeier DJ, Bezprozvanny I: **Association of CaV1.3 L-type calcium channels with Shank**. *J Neurosci* 2005, **25**:1037-1049.

26. Romorini S, Piccoli G, Jiang M, Grossano P, Tonna N, Passafaro M, Zhang M, Sala C: **A functional role of postsynaptic density-95-guanylate kinase-associated protein complex in regulating Shank assembly and stability to synapses**. *J Neurosci* 2004, **24**:9391-9404.

27. Stanika RI, Flucher BE, Obermair GJ: **Regulation of postsynaptic stability by the L-type calcium channel CaV1.3 and its interaction with PDZ proteins**. *Curr Mol Pharmacol* 2015, **8**:95-101.

28. Zhang J, Lewis SM, Kuhlman B, Lee AL: **Supertertiary structure of the MAGUK core from PSD-95**. *Structure* 2013, **21**:402-413.

29. Kwan JJ, Donaldson LW: **A lack of peptide binding and decreased thermostability suggests that the CASKIN2 scaffolding protein SH3 domain may be vestigial**. *BMC Struct Biol* 2016, **16**:14.

30. Stafford RL, Hinde E, Knight MJ, Pennella MA, Ear J, Digman MA, Gratton E, Bowie JU: **Tandem SAM domain structure of human Caskin1: a presynaptic, self-assembling scaffold for CASK**. *Structure* 2011, **19**:1826-1836.

31. Koprivanacz K, Tőke O, Besztercei B, Juhász T, Radnai L, Merő B, Mihály J, Péter M, Balogh G, Vígh L *et al.*: **The SH3 domain of Caskin1 binds to lysophosphatidic acid suggesting a direct role for the lipid in intracellular signaling**. *Cell Signal* 2017, **32**:66-75.

32. Ponna SK, Ruskamo S, Myllykoski M, Keller C, Boeckers TM, Kursula P: **Structural basis for PDZ domain interactions in the post-synaptic density scaffolding protein Shank3**. *J Neurochem* 2018, **145**:449-463.

33. Garrido-Urbani S, Garg P, Ghossoub R, Arnold R, Lembo F, Sundell GN, Kim PM, Lopez M, Zimmermann P, Sidhu SS, Ivarsson Y: **Proteomic peptide phage display uncovers novel interactions of the PDZ1-2 supramodule of syntenin**. *FEBS Lett* 2016, **590**:3-12.

34. Zeng M, Shang Y, Guo T, He Q, Yung WH, Liu K, Zhang M: **A binding site outside the canonical PDZ domain determines the specific interaction between Shank and SAPAP and their function**. *Proc Natl Acad Sci U S A* 2016, **113**:E3081-90.

35. Im YJ, Lee JH, Park SH, Park SJ, Rho SH, Kang GB, Kim E, Eom SH: **Crystal structure of the Shank PDZ-ligand complex reveals a class I PDZ interaction and a novel PDZ-PDZ dimerization**. *J Biol Chem* 2003, **278**:48099-48104.

36. Grabrucker AM, Knight MJ, Proepper C, Bockmann J, Joubert M, Rowan M, Nienhaus GU, Garner CC, Bowie JU, Kreutz MR *et al.*: **Concerted action of zinc and ProSAP/Shank in synaptogenesis and synapse maturation**. *EMBO J* 2011, **30**:569-581.

37. Zeng M, Shang Y, Araki Y, Guo T, Huganir RL, Zhang M: **Phase transition in postsynaptic densities underlies formation of synaptic complexes and synaptic plasticity**. *Cell* 2016, **166**:1163-1175.e12.

38. •• Zeng M, Chen X, Guan D, Xu J, Wu H, Tong P, Zhang M: **Reconstituted postsynaptic density as a molecular platform for understanding synapse formation and plasticity**. *Cell* 2018, **174**:1172-1187.e16.
The PSD was reconstituted from truncated variants of several scaffolding proteins, and key interactions were identified. Such approaches will be required for studies on full-length scaffolds in the future.

39. McCann JJ, Zheng L, Rohrbeck D, Felekyan S, Kühnemuth R, Sutton RB, Seidel CA, Bowen ME: **Supertertiary structure of the synaptic MAGuK scaffold proteins is conserved**. *Proc Natl Acad Sci U S A* 2012, **109**:15775-15780.

40. Gold MG: **A frontier in the understanding of synaptic plasticity: solving the structure of the postsynaptic density**. *Bioessays* 2012, **34**:599-608.

41. Soler J, Fañanás L, Parellada M, Krebs MO, Rouleau GA, Fatjó-Vilas M: **Genetic variability in scaffolding proteins and risk for schizophrenia and autism-spectrum disorders: a systematic review**. *J Psychiatry Neurosci* 2018, **43**:170066.

42. Homann OR, Misura K, Lamas E, Sandrock RW, Nelson P, McDonough SI, DeLisi LE: **Whole-genome sequencing in multiplex families with psychoses reveals mutations in the SHANK2 and SMARCA1 genes segregating with illness**. *Mol Psychiatry* 2016, **21**:1690-1695.

43. Sala C, Vicidomini C, Bigi I, Mossa A, Verpelli C: **Shank synaptic scaffold proteins: keys to understanding the pathogenesis of**

autism and other synaptic disorders. *J Neurochem* 2015, **135**:849-858.

44. • Monteiro P, Feng G: **SHANK proteins: roles at the synapse and in autism spectrum disorder**. *Nat Rev Neurosci* 2017, **18**:147-157.
An excellent, informative review on the biological function of Shanks, as well as their disease linkages and future prospects of treatment.

45. de Bartolomeis A, Latte G, Tomasetti C, Iasevoli F: **Glutamatergic postsynaptic density protein dysfunctions in synaptic plasticity and dendritic spines morphology: relevance to schizophrenia and other behavioral disorders pathophysiology, and implications for novel therapeutic approaches**. *Mol Neurobiol* 2014, **49**:484-511.

46. Baker LA, Grange M, Grünewald K: **Electron cryo-tomography captures macromolecular complexes in native environments**. *Curr Opin Struct Biol* 2017, **46**:149-156.

47. Earl LA, Falconieri V, Milne JL, Subramaniam S: **Cryo-EM: beyond the microscope**. *Curr Opin Struct Biol* 2017, **46**:71-78.

48. Blom H, Widengren J: **Stimulated emission depletion microscopy**. *Chem Rev* 2017, **117**:7377-7427.

49. Pleiner T, Bates M, Trakhanov S, Lee CT, Schliep JE, Chug H, Böhning M, Stark H, Urlaub H, Görlich D: **Nanobodies: site-specific labeling for super-resolution imaging, rapid epitope-mapping and native protein complex isolation**. *Elife* 2015, **4**: e11349.

50. Fitzkee NC, Rose GD: **Reassessing random-coil statistics in unfolded proteins**. *Proc Natl Acad Sci U S A* 2004, **101**:12497-12502.

51. Tong J, Yang H, Eom SH, Chun C, Im YJ: **Structure of the GH1 domain of guanylate kinase-associated protein from Rattus norvegicus**. *Biochem Biophys Res Commun* 2014, **452**:130-135.

52. Bach A, Clausen BH, Møller M, Vestergaard B, Chi CN, Round A, Sørensen PL, Nissen KB, Kastrup JS, Gajhede M *et al.*: **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* 2012, **109**:3317-3322.

53. Cámara-Artigas A, Murciano-Calles J, Gavira JA, Cobos ES, Martínez JC: **Novel conformational aspects of the third PDZ domain of the neuronal post-synaptic density-95 protein revealed from two 1.4 A X-ray structures**. *J Struct Biol* 2010, **170**:565-569.

54. McGee AW, Dakoji SR, Olsen O, Bredt DS, Lim WA, Prehoda KE: **Structure of the SH3-guanylate kinase module from PSD-95 suggests a mechanism for regulated assembly of MAGUK scaffolding proteins**. *Mol Cell* 2001, **8**:1291-1301.

55. Zhu J, Zhou Q, Shang Y, Li H, Peng M, Ke X, Weng Z, Zhang R, Huang X, Li SSC *et al.*: **Synaptic targeting and function of SAPAPs mediated by phosphorylation-dependent binding to PSD-95 MAGUKs**. *Cell Rep* 2017, **21**:3781-3793.

56. Fomina S, Howard TD, Sleator OK, Golovanova M, O'Ryan L, Leyland ML, Grossmann JG, Collins RF, Prince SM: **Self-directed assembly and clustering of the cytoplasmic domains of inwardly rectifying Kir2.1 potassium channels on association with PSD-95**. *Biochim Biophys Acta* 2011, **1808**:2374-2389.

57. Beneken J, Tu JC, Xiao B, Nuriya M, Yuan JP, Worley PF, Leahy DJ: **Structure of the Homer EVH1 domain-peptide complex reveals a new twist in polyproline recognition**. *Neuron* 2000, **26**:143-154.

58. Im YJ, Kang GB, Lee JH, Park KR, Song HE, Kim E, Song WK, Park D, Eom SH: **Structural basis for asymmetric association of the betaPIX coiled coil and shank PDZ**. *J Mol Biol* 2010, **397**:457-466.

59. Lee JH, Park H, Park SJ, Kim HJ, Eom SH: **The structural flexibility of the shank1 PDZ domain is important for its binding to different ligands**. *Biochem Biophys Res Commun* 2011, **407**:207-212.

60. Saupe J, Roske Y, Schillinger C, Kamdem N, Radetzki S, Diehl A, Oschkinat H, Krause G, Heinemann U, Rademann J: **Discovery, structure-activity relationship studies, and crystal structure of nonpeptide inhibitors bound to the Shank3 PDZ domain**. *ChemMedChem* 2011, **6**:1411-1422.