Shanks — multidomain molecular scaffolds of the postsynaptic density
Petri Kursula¹,²

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)

Shanks 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...
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 Px nipple motif, has remained unclear until very recently. Proline-rich partners for the Shank SH3 domain have...
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 Cα1.3 peptide binding [21] in red. Right: Surface electrostats indicate a highly polarized structure, with the Cα1.3 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 Cα1.3 channel [21]. This interaction is of electrostatic nature (Figure 2b), and could play a role in linking Cα1.3 channels to the PSD scaffold. Cα1.3 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 Cα1.3 [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 peptides, such as lysophosphatic 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/polymer, 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 Ca2+ 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 in 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, and 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


Using state-of-the-art microscopic techniques, molecular structures in different kinds of synapses were revealed.


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.


23. Kursula P: 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.


39. The PSD was reconstructed 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.


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


An excellent, informative review on the biological function of Shank's, as well as their disease linkages and future prospects of treatment.


