Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in
Neurobiology

Phase separation in the multi-compartment organization of synapses

Shihan Zhu[1,2], Zeyu Shen[1,2], Xiandeng Wu[1] and
Mingjie Zhang[1]

A neuronal synapse is formed by juxtaposition of a transmitter releasing presynaptic bouton of one neuron with a transmitter receiving postsynaptic compartment such as a spine protrusion of another neuron. Each presynaptic bouton and postsynaptic spine, though very small in their volumes already, are further compartmentalized to micro-/nano-domains with distinct molecular organizations and synaptic functions. This review summarizes studies in recent years demonstrating that multivalent protein–protein interaction-induced phase separation underlies formation and coexistence of multiple distinct molecular condensates within tiny synapses. In post-synapses where synaptic compartmentalization via phase separation was first demonstrated, phase separation allows clustering of transmitter receptors into distinct nanodomains and renders postsynaptic densities to be regulated by synaptic stimulation signals for plasticity. In pre-synapses, such phase separation-mediated synaptic condensates formation allows SVs to be stored as distinct pools and directly transported for activity-induced transmitter release.

Addresses
[1] School of Life Sciences, Southern University of Science and Technology, Shenzhen 518055, China
[2] Division of Life Science, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, Kowloon, China

Corresponding author: Zhang, Mingjie (zhangmj@sustech.edu.cn)

Current Opinion in Neurobiology 2025, **90:**102975

This review comes from a themed issue on **Developmental Neuroscience 2025**

Edited by **Lisa Goodrich** and **Song-Hai Shi**

For complete overview of the section, please refer the article collection - Developmental Neuroscience 2025

Available online 1 February 2025

https://doi.org/10.1016/j.conb.2025.102975

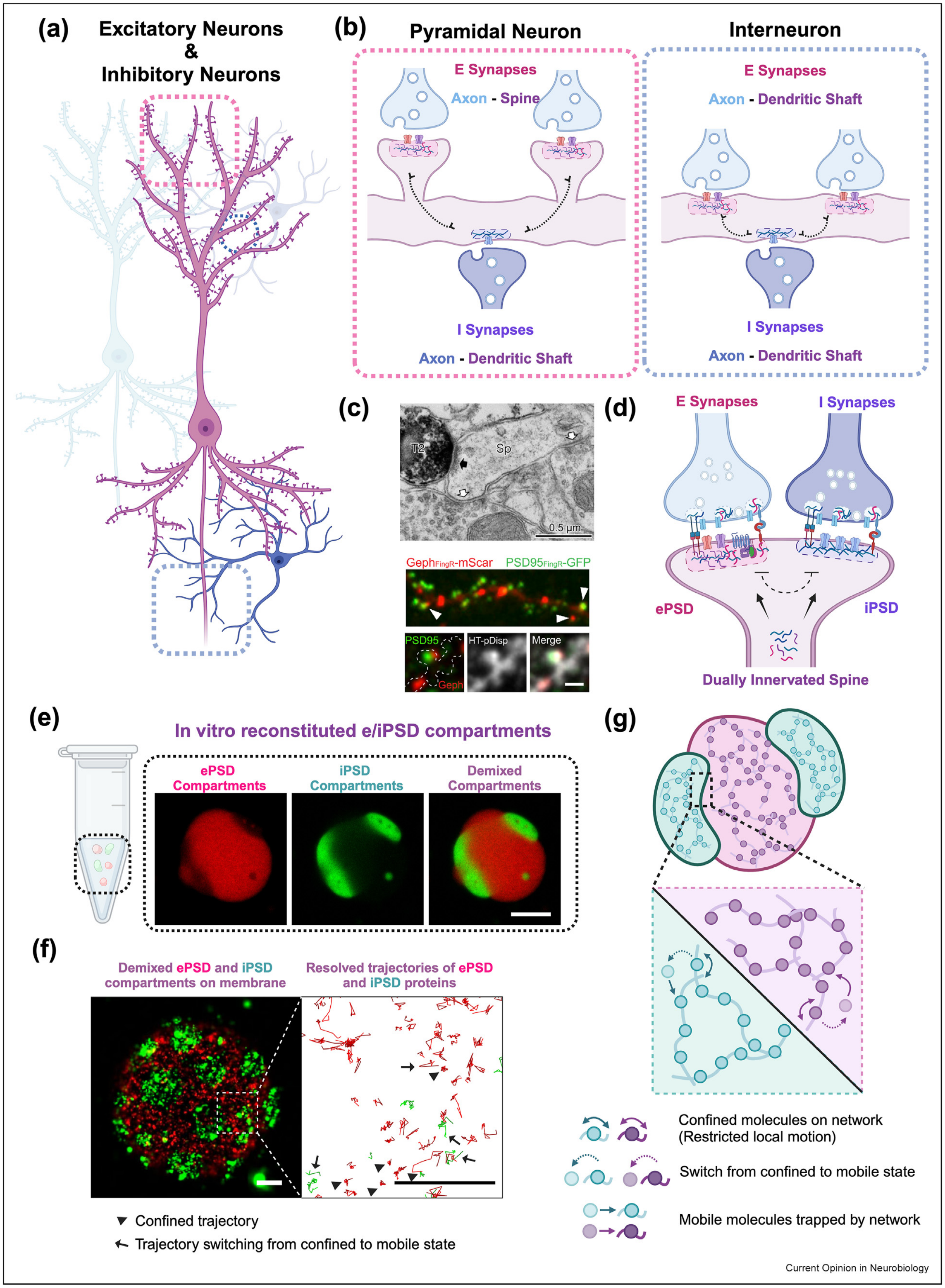
Introduction

Neurons are highly polarized and extremely compartmentalized. Such unique architectures of neurons are the foundation for forming diverse and precisely controlled neuronal networks responsible for essentially all physiological processes in animals including humans. One of the most basic steps for the establishment of a neuronal network is formation of a synapse, a micron-sized multi-compartment organization assembled via juxtaposed interactions between a signal-transmitting axonal terminal bouton of one neuron and a size-matched, signal-receiving dendritic compartment of another neuron (or a signal receiving compartment in target cells in peripheral nervous systems). Although the volume of a synapse is small (~1–2 μm^3), it could be further divided into at least three functional compartments: presynaptic bouton, postsynaptic dendrite and synaptic cleft.

Both pre- and post-synaptic termini are further sub-compartmentalized. Each presynaptic bouton contains many synaptic vesicles (SVs) clustered together and a protein-dense region known as the active zone (AZ) right beneath plasma membrane (PM). AZ is required for tethering, docking, priming, and fusion of SVs [1]. Under electron microscope (EM), AZ appears as an electron-dense zone and only a few SVs are directly attached to AZ. The majority of SVs are clustered as a distinct pool that is ~100 nm away from AZ. In the postsynaptic side, a highly electron-dense zone known as the postsynaptic density (PSD) is obvious under EM for both excitatory and inhibitory synapses from the central nervous system. The PSD of excitatory synapses (ePSD) has a thickness of 20–50 nm and the PSD of inhibitory synapses (iPSD) has a thickness of 10–15 nm [2]. A clear boundary between the PSD and spine cytoplasm exists as viewed from the EM micrographs of synapses. The ePSD appears to be further compartmentalized with a more electron-dense layer known as the PSD core right beneath the postsynaptic PM and a relatively less electron-dense layer further away from PM and facing spine cytoplasm (this layer is referred to as the PSD pallium) [3]. Such highly compartmentalized organizations of each synapse are not static. Instead, molecular constituents can exchange between synaptic sub-compartments in responses to synaptic stimulations [3].

The discovery of liquid–liquid phase separation of mixtures of two abundant PSD proteins, PSD-95 and SynGAPα1, hinted that phase separation could serve as a mechanism for condensed PSD assembly formation [4]. Accumulating studies in recent years have further

Figure 1



Current Opinion in Neurobiology

demonstrated that scaffold proteins at PSDs and presynaptic boutons, via multivalent interactions among these proteins, could undergo phase separation to form highly condensed compartments capable of enriching functional constituents such as neurotransmitter receptors, ion channels, enzymes, SVs, etc (for details please refer to Refs. [4–11]). These findings begin to offer a new angle for understanding synaptic organization and function. In this short review, we focus on roles of phase separation in organizing multiple sub-compartments in PSDs and presynaptic boutons.

Segregation of distinct synaptic compartments via phase separation: ePSD versus iPSD

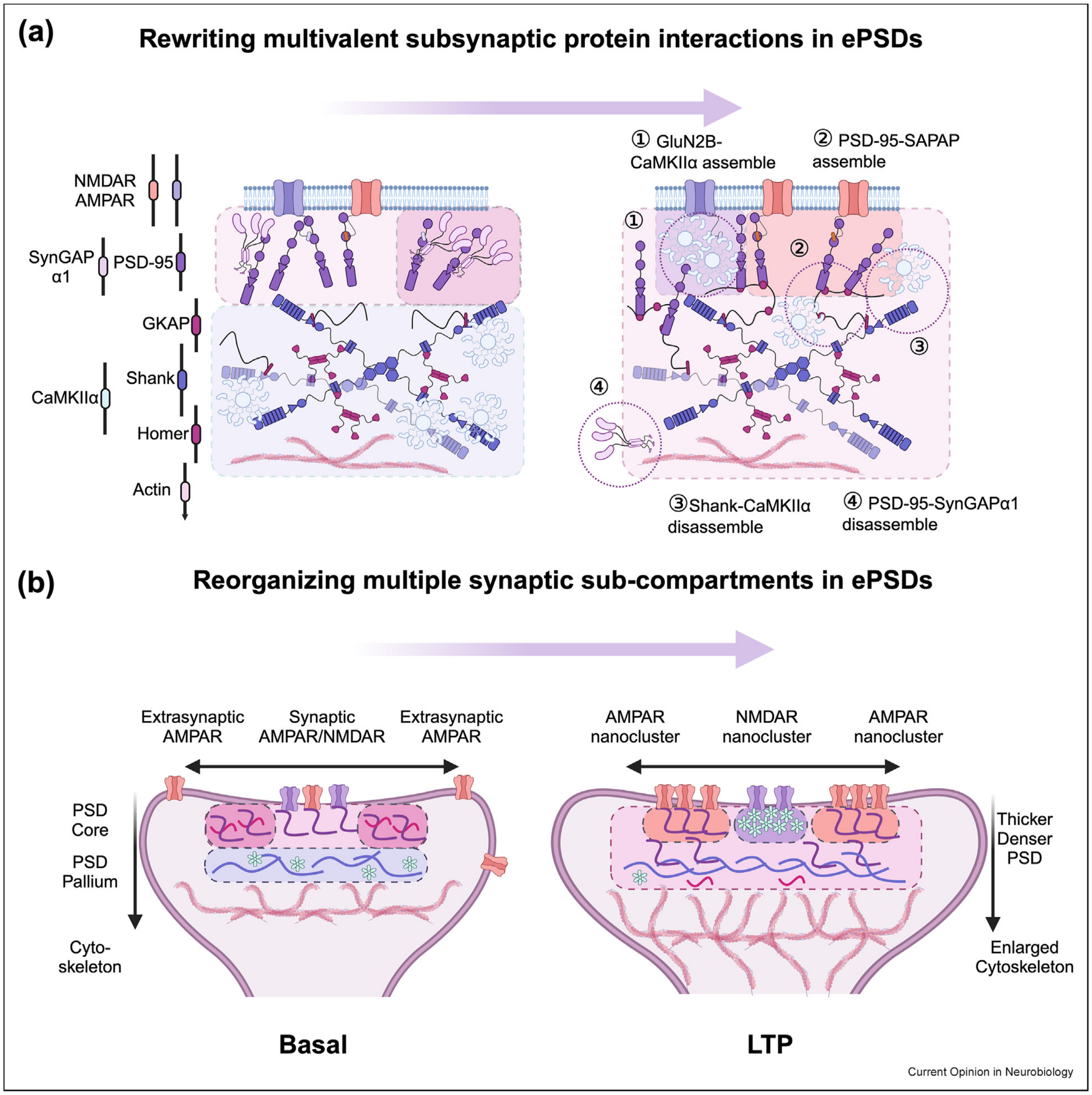
Balance of excitatory and inhibitory synaptic input/output is vitally important for neuronal circuit development and for proper functions of adult brains (Figure 1a). For individual neurons, the amount and types of synapses formed determine the signal input/output. For example, the majority of synapses (∼80 %) formed by cortical or hippocampal pyramidal neurons are excitatory in nature and are formed along dendrites as bulbous protrusions with head diameters of <1 μm [12]. The rest ∼20 % of synapses are inhibitory in nature and form on cell soma, dendrites, and axon initial segments. The segregation of excitatory and inhibitory signals into distinct sub-compartments in each neuron provides a foundation for E/I balance. A constrained ratio of ePSD/iPSD across different dendritic branches or along one branch has been observed in pyramidal neurons, suggesting a structural basis for E/I balance [13,14]. E/I imbalance is viewed as a major mechanism underlying various neurological and neurodegenerative disorders [15,16]. The location of inhibitory synapses has a profound effect on controlling neuronal excitability. The two extremes are those located on the axon initial segment and those located on dendritic spines. The former controls the overall firing of the neuron. The latter will selectively determine the effect of a single excitatory input by local shunting [17,18].

In pyramidal neurons, excitatory synapses are mainly localized in dendritic spine protrusions and thus are naturally segregated from inhibitory synapses that are chiefly localized on dendritic shafts or cell soma (Figure 1b). Nonetheless, a large proportion of inhibitory synapses are co-innervated onto the excitatory spine protrusions forming so called dually innervated spines (DiSs), which can account for ∼10 % of total inhibitory synapses in certain brain regions (Figure 1c and d) [13,19,20]. The anatomic structures of DiSs, their postsynaptic compartments, in particular, are interesting and intriguing (Figure 1c and d). Each of the submicron-sized postsynaptic protrusions is contacted by two presynaptic boutons, forming an ePSD and an iPSD. Electron microscopic and fluorescence microscopic studies have revealed that the ePSD and iPSD in each DiS form electron-dense/condensed and well-separated sub-compartments within each submicron-sized spine protrusion (Figure 1c) [19,21]. It is well established that ePSD and iPSD are formed by distinct sets of scaffold proteins capable of concentrating glutamate receptors and GABA/Glycine receptors ($GABA_AR$/GlyR), respectively, into dense clusters to conduct opposite electric currents [22–24]. Remarkably, the dense ePSD and iPSD molecular assemblies do not disperse and do not mix with each other despite the fact that they share a common dilute solution, which is the tiny spine cytoplasm of each DiS. In interneurons, ePSD and iPSD are densely distributed and well segregated with each other along dendrites (Figure 1b) [25]. The molecular basis governing spatial segregation of the ePSD and iPSD condensates in the nervous system is unclear until a very recent study [26].

Previous biochemical and proteomic studies identified the major molecular components and their interaction networks within ePSDs and iPSDs, respectively [23,24]. Mixtures of main ePSD or iPSD scaffold proteins in vitro led to the spontaneous formation of ePSD or iPSD condensate via phase separation [5,9]. Mutations of PSD proteins found in patients with brain disorders can perturb PSD condensate formation. For example, mutations in the Shank Sterile alpha motif (SAM) domain found in autism patients would disrupt or decrease the polymerization of Shank and thus disturb its phase separation capacity [27–29]. The G375D mutants of Gephyrin found in a patient with Dravet-like syndrome is defective in phase separation and iPSD assembly [30]. Zhu et al. further found that mixing the

Phase separation-mediated segregation of ePSD from iPSD.
(a) Schematic diagram showing the wiring of excitatory a pyramidal neuron (purple cell) and an inhibitory interneuron (blue cell). **(b)** The subcellular localization of E/I synapses differs in pyramidal neurons (left panel) and interneurons (right panel). In pyramidal neurons, E synapses are localized to spines separated with I synapses on shafts. But in interneurons both E and I synapses are on dendritic shafts. **(c)** EM (top panel) and fluorescence images (bottom panel) showing the organization of e/iPSDs within DiSs. In the top panel, the black arrow indicates an ePSD, and the white arrows indicate iPSDs. Scale bar: 500 nm. In the bottom panel, the white arrowheads indicate DiSs. Scale bar: 1 μm. **(d)** Schematic diagram of a DiS. **(e)** Demixed e/iPSD compartments could be observed in test tubes upon mixing of major e/iPSD components. Scale bar: 5 μm. **(f)** Super-resolution image of demixed e/iPSD compartments on a membrane surface (left panel) and zoom-in view of resolved molecular trajectories of e/iPSD proteins (right panel). Scale bar: 1 μm. **(g)** Demixed e/iPSD compartments are supported by distinct percolated molecular networks trapping ePSD and iPSD proteins in each compartment.
c top panel was adapted from Ref. [21].
c bottom panel was adapted from Ref. [19].
e–f was modified from Ref. [26].
All schemes in this review are drawn using BioRender.com.

Figure 2



Multiple synaptic sub-compartments in ePSDs in the basal state and during LTP.
(a) ePSDs proteins are organized via multivalent protein–protein interactions and occupy certain synaptic sub-compartments forming layered structures (left panel). During LTP, the activation of CaMKIIα could induce dissociation of the Shank- CaMKIIα complex and translocation of CaMKIIα to the PSD core. Active CaMKIIα would stably interact with the cytoplasmic tail of GluN2B. Active CaMKIIα can phosphorylate SAPAP and promote its binding to PSD-95. Besides, phosphorylation of SynGAPα1 leads to its dispersion from the PSD core. **(b)** In the basal state, the compartments composed of PSD-95/SynGAPα1/AMPAR and SAPAP/Shank/Homer are separated into distinct PSD core/pallium. Formation of the PSD-95/SynGAPα1 compartment inhibits the clustering of synaptic AMPARs. During LTP, rewriting of synaptic protein interactome leads to a reorganization of synaptic sub-compartments in ePSDs. Phosphorylation of SAPAP causes merging of the pallium with the PSD core forming thicker and denser PSDs. Dispersion of PSD-95/SynGAPα1 compartment promotes formation of the PSD-95/AMPAR nanoclusters. Active CaMKIIα translocate to the PSD core and phase separates with GluN2B to form NMDAR-containing nano-signaling compartments.

ePSD and iPSD proteins together led to autonomous formation of two separate phases of condensates corresponding to ePSD and iPSD condensates, suggesting that segregation of ePSD from iPSD is an intrinsic property of the two types of condensates (Figure 1e) [26]. The G375D mutants of Gephyrin could also disrupt the demixing of ePSD with iPSD. Remarkably, the ePSD and iPSD condensates remained demixed even when Gephyrin and PSD-95 were forced to bind to each other by fusing a PSD-95 intrabody called PSD-95.FingR [31] to Gephyrin both in vitro and in living neurons, revealing that the demixed ePSD and iPSD condensates are extremely stable and can resist the binding force with nanomolar affinities [26]. This study also indicates that, in general, distinct biological condensates formed in cells have intrinsic tendencies to be segregated. Thus, cells can contain numerous membraneless organelles formed by phase separation in different sub-cellular regions.

What might be the molecular mechanism underlying the spontaneous segregation of the ePSD and iPSD condensates? Zhu et al. analyzed the diffusion behaviors of ePSD protein Stg and iPSD protein GlyR in the reconstituted condensates by single-molecule tracking (Figure 1f) [26]. They discovered that PSD proteins in the condensed phase do not follow the free diffusion law for molecules in homogeneous solutions. Instead, each of these proteins continuously switches between mobile states and confined states, an observation in line with previous studies showing that both GlyR and AMPAR could be trapped in dense sub-synaptic nanodomains with very low mobilities in living neurons (Figure 1f) [32,33]. The existence of confined states with extremely low diffusion rates for Stg or GlyR in the ePSD and iPSD condensates can be explained by the percolation theory upon condensate formation [29,34]. The strong and specific multivalent interactions among ePSD or among iPSD proteins led to the formation of system-spanning networks of ePSD and iPSD, respectively (Figure 1g). Such percolated ePSD or iPSD molecular network is with extremely large molecular mass, such that a client protein like Stg or GlyR in the network bound state is essentially immobile. Mixing of ePSD with iPSD would require breakup of at least one such percolated molecular network with numerous interaction nodes, a process that is extremely energy costly. Therefore, even a forced interaction between Gephyrin and PSD-95 with a nanomolar binding affinity cannot mix the ePSD and iPSD together.

ePSD proteins are organized in layers forming multiple synaptic sub-compartments

Apart from the segregation of ePSD from iPSD, molecules are assembled into discreate sub-compartments even within a single PSD. For example, immunogold labeling coupled with EM studies showed that PSD-95 is concentrated in the more condensed PSD core region close to the postsynaptic membrane with a median distance of ∼12 nm [24,35]. Shanks, another family of the ePSD scaffold proteins are distributed in the PSD pallium with a median distance of ∼53 nm from the postsynaptic plasma membrane [3,36]. The ePSD scaffold proteins SAPAPs, which constitutively interact with Shanks, are distributed in a narrow band with a median distance of ∼33 nm from the postsynaptic membrane [3,36]. Super-resolution fluorescence imaging studies also revealed that the ePSD proteins are distributed in the order of NMDAR/AMPAR, PSD-95, Shanks/CaMKIIs, and Homers from the postsynaptic plasma membranes to the spine cytosol (Figure 2a, left panel) [37]. Biochemical reconstitution in test tubes further showed that mixing of ePSD components leads to two immiscible condensates: one enriching AMPAR/PSD-95/SynGAPα1, and the other containing SAPAP1/Shank3/Homer1 [38].

The distinct sub-synaptic ePSD condensates may play unique roles in synaptic assembly and function (Table 1). For example, the PSD pallium condensate formed by SAPAP1/Shank3/Homer1 can recruit F-actin and promote actin bundling, a process required for long-term spine stabilization (Figure 2b left panel) [39]. The PSD core condensate formed by AMPAR and PSD-95 supports clustering of AMPAR receptors in PSD [40]. Recently, it was shown that an increasing level of SynGAPα1 can promote PSD-95/SynGAPα1 condensate formation and in return weaken AMPAR targeting in the PSD core via competing with TARP for binding to PSD-95 (Figure 2b left panel) [41]. Formation of PSD-95/SynGAP condensates leads to the repulsion of AMPARs from PSD-95 clusters, by which the anchoring of AMPARs in the PSD is weakened.

Reorganization of ePSD sub-compartments in response to synaptic signaling

The ePSDs, though quite condensed and stable, are dynamically regulated by synaptic signals. For example, ePSDs are known to enlarge during long-term potentiation (LTP) [3,42]. In cultured hippocampal neurons, the PSD pallium becomes more electron-dense after depolarization with K^+ or treatment with glutamate, likely due to the accumulation and redistribution of PSD proteins [3,42]. Ca^{2+}/Calmodulin-dependent kinase II α (CaMKIIα) has long been viewed as one of the most important proteins for synapse formation and plasticity [43,44]. Under basal condition, CaMKIIα is mainly localized in the PSD pallium revealed by immunogold-EM (Figure 2a left panel) [42,45]. The inactive CaMKIIα would phase separate with the PSD pallium protein Shank [10]. K^+- or glutamate-induced depolarization of cultured hippocampal neurons causes translocation of CaMKIIα from the PSD pallium to the PSD

Table 1

Key PSD scaffolds involved in synaptic sub-compartmentalization.

PSD scaffold	Valency	Interacting partners	Function in synapse	Synaptic regulators	Ref.
PSD-95	3 PDZ slots, Oligomerization of PSG	TARPs, NMDAR	Trap and cluster synaptic receptors; Maintain basal transmission and LTP	Arc (↓)[a] SynGAPα1 (↓)	[5,40,41,53]
		SynGAPα1	Decrease synaptic AMPARs; Inhibit synaptic maturation	SynGAPα1 phosphorylation by CaMKII (↓)	[4,41,51]
		SAPAPs, IRSp53	Link PSD core with PSD pallium	GBR phosphorylation by CaMKII (↑)	[5,38,80]
SAPAPs	2-5 GBRs	PSD-95, Shanks		GBR dephosphorylation by phosphatases (↓)	
Shanks	Oligomerization of SAM	SAPAPs, IRSp53			
		CaMKIIα	Anchor inactive CaMKIIα in PSDs	Activation of CaMKIIα (↓)	[10]
		Homers, Cortactin	Link PSD with cytoskeleton in spines	Homer1a (↓)	[5,39]
Homers	Tetramer of coiled-coil	Shanks, F-actin			
CaMKIIα	12/14-mer of the hub domain	Shanks	Anchor inactive CaMKIIα in PSDs	Activation of CaMKIIα (↓)	[10]
		NMDAR	Recruit active CaMKIIα for LTP induction	Activation of CaMKIIα (↑)	[10,11]
SynGAPα1	Trimer of coiled-coil	PSD-95	Decrease synaptic AMPAR; Inhibit synaptic maturation	SynGAPα1 phosphorylation by CaMKII (↓)	[4,41,51]

[a] (↑) indicates enhancing phase separation; (↓) indicates weakening phase separation.

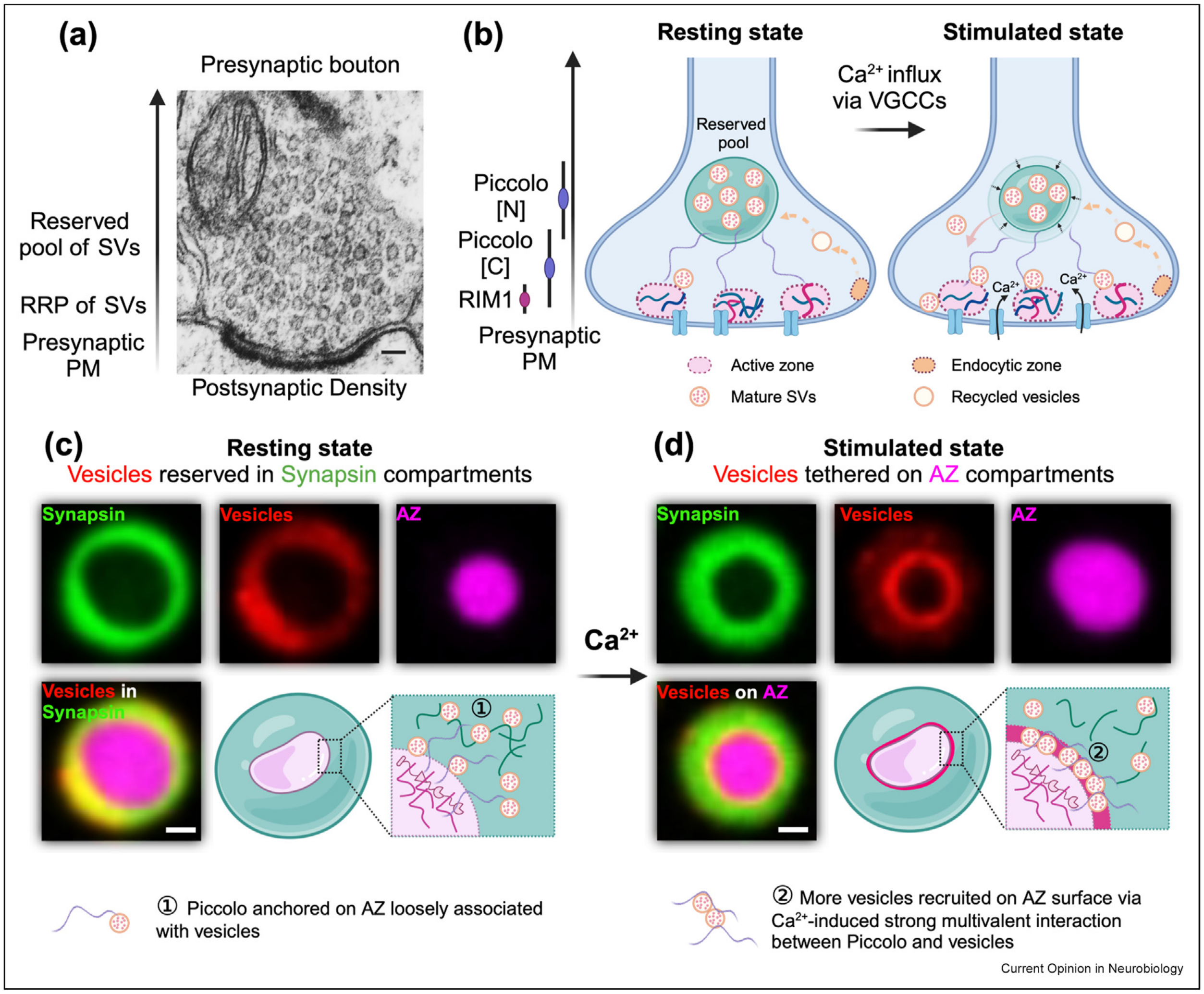
core (Figure 2a right panel) [42,45]. Ca^{2+}-induced activation of CaMKIIα leads to direct binding of the kinase to the cytoplasmic tail of GluN2B, forming a CaMKIIα/GluN2B nano-signaling compartment [10,11,46]. Interestingly, the CaMKIIα/GluN2B condensate is physically separated from the PSD-95/AMPAR condensate, implying that phase separation-mediated synaptic subcompartmentalization may underlie the segregation of AMPARs from NMDARs in the PSD and CaMKIIα plays a structural role in addition to acting as a protein kinase (Figure 2b right panel) [11,47]. Application of a CaMKII inhibitory peptide to block the interaction between CaMKII/GluN2B could disrupt the segregated distribution of NMDAR and AMPAR in synapses [11,47]. Two recent studies provided more direct evidence highlighting the structural role of CaMKIIα in synaptic transmission and plasticity via directly binding to GluN2B [48,49]. Such Ca^{2+}-regulated synaptic subcompartmentalization appears to be specific to CaMKIIα, as CaMKIIβ cannot undergo phase separation with Shank3 or GluN2B, nor can rescue the defects of CaMKIIα/β double knock out [10,50]. Consistently, CaMKIIα, but not CaMKIIβ, can rescue the synaptic transmission and plasticity defects of neurons in the CaMKIIα/β double knock-out mice brain [44,50].

There are multiple substrates of CaMKIIα in ePSDs including scaffolds, enzymes, receptors, and adhesion molecules. Phosphorylation by CaMKIIα could rewrite the interactome in the ePSD, resulting in reorganization of synaptic sub-compartments (Figure 2a and b right panel). For example, the GK-binding repeats (GBRs) of SAPAPs could be phosphorylated by CaMKIIα, resulting in ∼1000-fold increase of the interaction between SAPAPs and PSD-95 (Figure 2a right panel) [38]. In test tubes, addition of active CaMKIIα into the immiscible condensates formed by PSD-95/SynGAPα1/AMPAR and SAPAP1/Shank3/Homer1 leads to fusion of the two compartments into one single PSD condensate because of the enhanced interaction between PSD-95 and SAPAP1. In synapses of striatal neurons in the Sapap3$^{-/-}$ mice brain, expressing SAPAP3 with forced binding with PSD-95 led to a narrower separation between PSD-95 and Homer1, an observation consistent with a merging of the PSD pallium with the PSD core (Figure 2b right panel) [38]. CaMKIIα has also been shown to phosphorylate SynGAP and AMPAR auxiliary subunits TARPs, thereby regulating PSD condensate dynamics and AMPAR synaptic clustering [51–53]. In addition to CaMKIIα, some immediate early gene products can also regulate PSD condensate formation or dispersion. For example, Arc can selectively disperse TARP/PSD-95 condensate by competing with TARP for binding to PSD-95 [53]. Homer1a, another product of immediate early gene and a monomeric isoform of the Homer1 family scaffold proteins, can disperse PSD condensate by lowering the valency of the PSD condensate network [5,39].

Sub-compartmentalization of presynaptic bouton contributes to SVs cycling

In addition to the ePSD, the presynaptic bouton is also highly compartmentalized. Multiple sub-compartments physically interact with each other, underling the organization and function of presynapses. The major physiological function of the presynaptic bouton is to store and release SVs in response to action potentials. Under EM, a large pool of clustered SVs (known as the reserve pool) away from PM could be easily identified (Figure 3a and b) [54]. In addition, several SVs are docked to AZ right beneath PM (known as the readily releasable pool, RRP) (Figure 3a and b) [1,54]. Extensive genetic studies identified a series of scaffold proteins required for maintaining the distinct pools of SVs. Knockout of Synapsin results in drastic impairment of the clustered SVs, suggesting a critical role of Synapsin in maintaining the reserve pool SVs [55]. Purified Synapsin1a undergoes phase separation and co-acervation with SVs, mimicking the clustering of the reserve pool SVs [6,56]. Recent single-molecule tracking analysis indicated the confined movement of SVs within Synapsin condensates

Figure 3



Multiple synaptic sub-compartments in presynaptic boutons organize and maintain distinct functional pools of SVs.
(**a**) EM images showing the organization of SVs in a presynaptic bouton. The majority of SVs are clustered in the reserved pool away from the presynaptic PM. Only a few SVs in RRP are close to PM. Scale bar: 200 nm. **(b)** In the resting state, several SVs are docked by the AZ compartments. Upon stimulation, Ca^{2+} influx via VGCCs could activate the giant protein Piccolo localized between the reserve pool and AZ for transporting SVs released from the reserve pool after partial dispersion of synapsin compartments to the AZ surface to replenish RRP. Besides, the endocytic zone localized on the presynaptic PM facilitates the recycling of vesicles. **(c)** The reconstituted presynaptic compartments recapitulate the distinct pools of SVs. In the resting state, purified Synapsin and AZ proteins form segregated compartments, and vesicles are clustered in the reserve pool composed of Synapsin. Scale bar: 1 μm. **(d)** By adding Ca^{2+} to mimic synaptic stimulation, Piccolo could specifically interact with the lipids on vesicles, and extract vesicles from Synapsin compartments. The Piccolo/Vesicles compartment could be recruited and merged with the AZ compartment. Vesicles associated with Piccolo are further coated on the AZ surface. Scale bar: 1 μm.
a is adapted from SynapseWeb (https://synapseweb.clm.utexas.edu/16-chemical-synapses-36).

[57]. Other proteins on SVs like Synaptophysin could further facilitate the compartmentalization of Synapsin1a and SVs [58,59]. In neurons from RIM$^{-/-}$/RIMBP$^{-/-}$ mice brain, the majority of RRP SVs are depleted but the reserve pool SVs remain intact, indicating that these two AZ proteins play a vital role in selectively tethering SVs to AZs [60]. In an in vitro reconstitution system, SVs coat on the surface of the RIM/RIMBP condensates. The interaction between the C2 domains of RIM and negatively charged lipids in vesicles is required for the coating of SVs on the surface of the RIM/RIMBP condensates [56]. Importantly, the Synapsin-clustered SVs and the RIM/RIMBP-organized SVs remain as two distinct phases when the two condensates were mixed in vitro (Figure 3c) [56,61], explaining how chemically similar SVs can be organized into distinct functional pools in presynaptic boutons.

Phase separation also contributes to the dynamic exchange of SVs between sub-bouton compartments. Since only a few SVs are attached to the surface of AZ, SVs need to be transported from the reserve pool to replenish RRP after releasing events to sustain continuous synaptic transmissions (Figure 3b). The reserve pool and the AZ-coated pool of SVs are separated only by ~100–200 nm. Such short-distance SV movements do not rely on conventional molecular motor-mediated transport, as the space between the reserve pool and AZ does not contain actin filaments or microtubules [62,63]. The presynaptic scaffold proteins Piccolo and Bassoon are indispensable for the replenishment of RRP SVs [64,65]. Piccolo and Basson are giant proteins with defined orientations in boutons: their N-termini facing the PM-distal region of the bouton reaching the reserve pool of SVs and their C-termini extend into AZ by binding to AZ proteins like ELKS and RIMBP (Figure 3b) [37,66]. Using a biochemical reconstitution approach, we recently demonstrated that SVs can be transported from the Synapsin-clustered reserve pool to AZ-tethered RRP in a Ca^{2+}-dependent manner via phase separation (Figure 3d) [61]. In the absence of Ca^{2+}, Piccolo is recruited to the Synapsin/SV compartment. Upon addition of Ca^{2+}, Piccolo undergoes phase separation with SVs relying on Ca^{2+}-dependent binding with lipids on SVs, and extracts SVs from the Synapsin/SV compartment. The Piccolo/SV condensate likely serves as an intermediate compartment to deliver SVs from the reserve pool to AZ. Via interacting with ELKS and RIMBP, Piccolo enters and enriches into the AZ compartment, while SVs retain on the surface of the AZ condensate (Figure 3d).

Following the action potential triggered influx of Ca^{2+} through the voltage-gated calcium channels (VGCCs), SVs in RRP undergo fusion with PM mediated by the SNARE machinery. The assembly of SNARE complexes requires Ca^{2+}-induced activation of Synaptotagmin on SVs. Therefore, the distance between VGCCs and the SNARE fusion machinery is crucial for the efficiency of SV fusion and synaptic transmission. Recent studies based on super-resolution imaging have revealed high-order assemblies of VGCCs, Synaptotagmin, and Munc-13, etc. [67–70], possibly also via phase separation. Endocytosis-mediated recycling of SVs, especially the ultrafast endocytosis, requires formation of dynamin 1A and Endophilin A1 condensates (Figure 3b) [71,72]. It is worthy to investigate whether these sub-compartments may interact with each other and how their interactions orchestrate SV fusion with PM. For example, both VGCCs and Munc-13 form nanoclusters by themselves and simultaneously interact with RIM [1], how do these sub-compartments co-assemble? Two recent studies showed that ablation of liprin-α, another key scaffold protein interacting with RIM in AZ, impairs the accumulation of Munc-13 at the release sites without affecting VGCCs, suggesting that RIM may form distinct sub-compartments with its different binding partners to organize Munc-13 and VGCCs, respectively [67,73].

Conclusions and perspectives

Synapses are highly compartmentalized. Multivalent protein–protein interaction governed phase separation underlies formation of multiple sub-compartments with distinct functions in both presynaptic boutons and PSDs. Formation of multiple sub-compartments allows various synaptic molecules/components to be located and enriched in specific subsynaptic regions for synaptic transmission and synaptic plasticity. Phase separation-mediated molecular condensation and formation of multiple demixed condensates beautifully illustrate why distinct molecular assemblies can orderly exist in specific sub-compartments in both presynaptic boutons and postsynaptic densities instead of undergoing diffusion-governed homogenous mixing.

Although phase separation-mediated molecular compartmentalization has provided exciting new insights into synaptic organization and function, many questions remain to be answered. For example, how do different coexisting sub-compartments in pre- or post-synaptic regions communicate with each other? Whether and how synaptic adhesion molecules may actively participate in synaptic alignment and sub-compartmentalization [74]? A recent study showed that removing the extracellular domain of LRRTM2 leads to the disassembly of GluA1 clusters, suggesting that LRRTM2 is required for the condensed AMPAR compartment formation [75]. Synaptic adhesion molecules, via both trans- and cis-synaptic interactions across the synaptic cleft, are capable of forming high-order assemblies [76–78]). Thus, it is possible that synaptic adhesion molecules, either alone or together with interacting synaptic scaffold proteins, phase separate to form distinct condensates in aligning trans-synaptic structures such as transmitter releasing/receiving nanocolumns [79] and the global pre- and post-synaptic juxtaposition of each synapse.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgements

Research in our lab are supported by grants from the Key-Area Research and Development Program of Guangdong Province (2023B0303010001), the National Natural Science Foundation of China (82188101), the Ministry of Science and Technology of China (2019YFA0508402), Shenzhen Talent Program (KQTD20210811090115021), Shenzhen Science and Technology Basic Research Program (JCYJ20220818100215033), Guangdong Innovative and Entrepreneurial Research Team Program (2021ZT09Y104), Shenzhen Medical Research Fund (B2302039), and Shenzhen Science and Technology Program (ZDSYS20220402111000001) to MZ.

Data availability

Data will be made available on request.

References

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

- • of special interest
- •• of outstanding interest

1. Sudhof TC: **The presynaptic active zone**. *Neuron* 2012, **75**: 11–25.

2. Tao CL, Liu YT, Sun R, Zhang B, Qi L, Shivakoti S, Tian CL, Zhang P, Lau PM, Zhou ZH, *et al.*: **Differentiation and characterization of excitatory and inhibitory synapses by cryo-electron tomography and correlative microscopy**. *J Neurosci* 2018, **38**:1493–1510.

3. Dosemeci A, Weinberg RJ, Reese TS, Tao-Cheng JH: **The**
•• **postsynaptic density: there is more than meets the eye**. *Front Synaptic Neurosci* 2016, **8**:23.
Described the PSD core and pallium structures and reviewed the distribution of PSD proteins into the PSD core or pallium compartments.

4. 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 e1112.
This study is the first to demonstrate phase separation in the PSD formation.

5. 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 e1116.
Using biochemical reconsitution to show that ePSDs are formed via phase separation.

6. Milovanovic D, Wu Y, Bian X, De Camilli P: **A liquid phase of synapsin and lipid vesicles**. *Science* 2018, **361**: 604–607.

7. Wu X, Cai Q, Shen Z, Chen X, Zeng M, Du S, Zhang M: **RIM and RIM-BP form presynaptic active-zone-like condensates via phase separation**. *Mol Cell* 2019, **73**:971–984 e975.

8. McDonald NA, Fetter RD, Shen K: **Assembly of synaptic active zones requires phase separation of scaffold molecules**. *Nature* 2020, **588**:454–458.

9. Bai G, Wang Y, Zhang M: **Gephyrin-mediated formation of**
• **inhibitory postsynaptic density sheet via phase separation**. *Cell Res* 2021, **31**:312–325.
Biochemical reconstitution experiments to demonatrate iPSD formation by phase separation.

10. Cai Q, Zeng M, Wu X, Wu H, Zhan Y, Tian R, Zhang M:
•• **CaMKIIalpha-driven, phosphatase-checked postsynaptic plasticity via phase separation**. *Cell Res* 2021, **31**:37–51.
Demonstrated translocation of CaMKIIα from the PSD pallium to the PSD core upon activation by Ca^{2+}.

11. Hosokawa T, Liu PW, Cai Q, Ferreira JS, Levet F, Butler C,
•• Sibarita JB, Choquet D, Groc L, Hosy E, *et al.*: **CaMKII activation persistently segregates postsynaptic proteins via liquid phase separation**. *Nat Neurosci* 2021, **24**:777–785.
Demonstrated roles of CaMKIIα/GluN2B phase separation in maintaining segregated AMPAR and NMDAR nanoclusters.

12. Danielson E, Perez de Arce K, Cimini B, Wamhoff EC, Singh S, Cottrell JR, Carpenter AE, Bathe M: **Molecular diversity of glutamatergic and GABAergic synapses from multiplexed fluorescence imaging**. *eNeuro* 2021, **8**.

13. Iascone DM, Li Y, Sumbul U, Doron M, Chen H, Andreu V,
• Goudy F, Blockus H, Abbott LF, Segev I, *et al.*: **Whole-neuron synaptic mapping reveals spatially precise excitatory/inhibitory balance limiting dendritic and somatic spiking**. *Neuron* 2020, **106**:566–578 e568.
Whole-neuron mapping of distributions of excitatory and inhibitory synapses along the cell body and cell processes.

14. Liu G: **Local structural balance and functional interaction of excitatory and inhibitory synapses in hippocampal dendrites**. *Nat Neurosci* 2004, **7**:373–379.

15. Nelson SB, Valakh V: **Excitatory/inhibitory balance and circuit homeostasis in autism spectrum disorders**. *Neuron* 2015, **87**: 684–698.

16. Sohal VS, Rubenstein JLR: **Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders**. *Mol Psychiatr* 2019, **24**:1248–1257.

17. Chiu CQ, Lur G, Morse TM, Carnevale NT, Ellis-Davies GC, Higley MJ: **Compartmentalization of GABAergic inhibition by dendritic spines**. *Science* 2013, **340**:759–762.

18. Higley MJ: **Localized GABAergic inhibition of dendritic Ca(2+) signalling**. *Nat Rev Neurosci* 2014, **15**:567–572.

19. Kleinjan MS, Buchta WC, Ogelman R, Hwang IW, Kuwajima M, Hubbard DD, Kareemo DJ, Prikhodko O, Olah SL, Gomez Wulschner LE, *et al.*: **Dually innervated dendritic spines develop in the absence of excitatory activity and resist plasticity through tonic inhibitory crosstalk**. *Neuron* 2023, **111**:362–371 e366.

20. Chen JL, Villa KL, Cha JW, So PT, Kubota Y, Nedivi E: **Clustered dynamics of inhibitory synapses and dendritic spines in the adult neocortex**. *Neuron* 2012, **74**:361–373.

21. Kubota Y, Hatada S, Kondo S, Karube F, Kawaguchi Y: **Neocortical inhibitory terminals innervate dendritic spines targeted by thalamocortical afferents**. *J Neurosci* 2007, **27**: 1139–1150.

22. Crosby KC, Gookin SE, Garcia JD, Hahm KM, Dell'Acqua ML, Smith KR: **Nanoscale subsynaptic domains underlie the organization of the inhibitory synapse**. *Cell Rep* 2019, **26**: 3284–3297 e3283.

23. Uezu A, Kanak DJ, Bradshaw TW, Soderblom EJ, Catavero CM, Burette AC, Weinberg RJ, Soderling SH: **Identification of an elaborate complex mediating postsynaptic inhibition**. *Science* 2016, **353**:1123–1129.

24. Sheng M, Hoogenraad CC: **The postsynaptic architecture of excitatory synapses: a more quantitative view**. *Annu Rev Biochem* 2007, **76**:823–847.

25. Shapson-Coe A, Januszewski M, Berger DR, Pope A, Wu Y,
• Blakely T, Schalek RL, Li PH, Wang S, Maitin-Shepard J, *et al.*: **A petavoxel fragment of human cerebral cortex reconstructed at nanoscale resolution**. *Science* 2024, **384**, eadk4858.

Used EM to visualize and annotate excitatory and inhibitory synapse distributions in neurons from a human brain tissue.

26. Zhu S, Shen Z, Wu X, Han W, Jia B, Lu W, Zhang M: **Demixing is a default process for biological condensates formed via phase separation**. *Science* 2024, **384**:920–928.
••
Demonstrated the role of phase separation in maintaining segregation of e/iPSD compartments.

27. De Rubeis S, Siper PM, Durkin A, Weissman J, Muratet F, Halpern D, Trelles MDP, Frank Y, Lozano R, Wang AT, *et al.*: **Delineation of the genetic and clinical spectrum of Phelan-McDermid syndrome caused by SHANK3 point mutations**. *Mol Autism* 2018, **9**:31.

28. Hassani Nia F, Woike D, Bento I, Niebling S, Tibbe D, Schulz K, Hirnet D, Skiba M, Honck HH, Veith K, *et al.*: **Structural deficits in key domains of Shank2 lead to alterations in postsynaptic nanoclusters and to a neurodevelopmental disorder in humans**. *Mol Psychiatr* 2024, **29**:1683–1697.

29. Shen Z, Jia B, Xu Y, Wessen J, Pal T, Chan HS, Du S, Zhang M: **Biological condensates form percolated networks with molecular motion properties distinctly different from dilute solutions**. *Elife* 2023, **12**, e81907.
••
Experimental demonstration of the percolated molecular network formation in biological condensates formed via phase separation.

30. Dejanovic B, Djemie T, Grunewald N, Suls A, Kress V, Hetsch F, Craiu D, Zemel M, Gormley P, Lal D, *et al.*: **Simultaneous impairment of neuronal and metabolic function of mutated gephyrin in a patient with epileptic encephalopathy**. *EMBO Mol Med* 2015, **7**:1580–1594.

31. Gross GG, Junge JA, Mora RJ, Kwon HB, Olson CA, Takahashi TT, Liman ER, Ellis-Davies GC, McGee AW, Sabatini BL, *et al.*: **Recombinant probes for visualizing endogenous synaptic proteins in living neurons**. *Neuron* 2013, **78**:971–985.

32. Dahan M, Levi S, Luccardini C, Rostaing P, Riveau B, Triller A: **Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking**. *Science* 2003, **302**:442–445.

33. Tardin C, Cognet L, Bats C, Lounis B, Choquet D: **Direct imaging of lateral movements of AMPA receptors inside synapses**. *EMBO J* 2003, **22**:4656–4665.

34. Mittag T, Pappu RV: **A conceptual framework for understanding phase separation and addressing open questions and challenges**. *Mol Cell* 2022, **82**:2201–2214.

35. Chen X, Winters C, Azzam R, Li X, Galbraith JA, Leapman RD, Reese TS: **Organization of the core structure of the postsynaptic density**. In *Proceedings of the national academy of sciences*, **105**; 2008:4453–4458.

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

37. Dani A, Huang B, Bergan J, Dulac C, Zhuang X: **Superresolution imaging of chemical synapses in the brain**. *Neuron* 2010, **68**: 843–856.

38. Wu H, Chen X, Shen Z, Li H, Liang S, Lu Y, Zhang M: **Phosphorylation-dependent membraneless organelle fusion and fission illustrated by postsynaptic density assemblies**. *Mol Cell* 2024, **84**:309–326 e307.
••
Demonstrated multi-compartments organization of PSD by phase separation and merging of the PSD core and pallium by active CaMKIIα.

39. Chen X, Jia B, Zhu S, Zhang M: **Phase separation-mediated actin bundling by the postsynaptic density condensates**. *Elife* 2023, **12**, e84446.

40. Zeng M, Diaz-Alonso J, Ye F, Chen X, Xu J, Ji Z, Nicoll RA, Zhang M: **Phase separation-mediated TARP/MAGUK complex condensation and AMPA receptor synaptic transmission**. *Neuron* 2019, **104**:529–543 e526.

41. Araki Y, Rajkovich KE, Gerber EE, Gamache TR, Johnson RC, Tran THN, Liu B, Zhu Q, Hong I, Kirkwood A: **SynGAP regulates synaptic plasticity and cognition independently of its catalytic activity**. *Science* 2024, **383**, eadk1291.

42. Dosemeci A, Tao-Cheng J-H, Vinade L, Winters CA, Pozzo-Miller L, Reese TS: **Glutamate-induced transient modification of the postsynaptic density**. In *Proceedings of the national academy of sciences*, **98**; 2001:10428–10432.

43. Yasuda R, Hayashi Y, Hell JW: **CaMKII: a central molecular organizer of synaptic plasticity, learning and memory**. *Nat Rev Neurosci* 2022, **23**:666–682.

44. Incontro S, Diaz-Alonso J, Iafrati J, Vieira M, Asensio CS, Sohal VS, Roche KW, Bender KJ, Nicoll RA: **The CaMKII/NMDA receptor complex controls hippocampal synaptic transmission by kinase-dependent and independent mechanisms**. *Nat Commun* 2018, **9**:2069.

45. Tao-Cheng JH: **Activity-dependent redistribution of CaMKII in the postsynaptic compartment of hippocampal neurons**. *Mol Brain* 2020, **13**:53.
•
Used immunogold-EM to show redistribution of CaMKII in the PSD of neurons at the basal and stimulated states.

46. Bayer K-U, De Koninck P, Leonard AS, Hell JW, Schulman H: **Interaction with the NMDA receptor locks CaMKII in an active conformation**. *Nature* 2001, **411**:801–805.

47. Goncalves J, Bartol TM, Camus C, Levet F, Menegolla AP, Sejnowski TJ, Sibarita JB, Vivaudou M, Choquet D, Hosy E: **Nanoscale co-organization and coactivation of AMPAR, NMDAR, and mGluR at excitatory synapses**. *Proc Natl Acad Sci U S A* 2020, **117**:14503–14511.

48. Tullis JE, Larsen ME, Rumian NL, Freund RK, Boxer EE, Brown CN, Coultrap SJ, Schulman H, Aoto J, Dell'Acqua ML, *et al.*: **LTP induction by structural rather than enzymatic functions of CaMKII**. *Nature* 2023, **621**:146–153.

49. Chen X, Cai Q, Zhou J, Pleasure SJ, Schulman H, Zhang M, Nicoll RA: **CaMKII autophosphorylation is the only enzymatic event required for synaptic memory**. *Proc Natl Acad Sci U S A* 2024, **121**, e2402783121.

50. Cai Q, Chen X, Zhu S, Nicoll RA, Zhang M: **Differential roles of CaMKII isoforms in phase separation with NMDA receptors and in synaptic plasticity**. *Cell Rep* 2023, **42**, 112146.

51. Araki Y, Zeng M, Zhang M, Huganir RL: **Rapid dispersion of SynGAP from synaptic spines triggers AMPA receptor insertion and spine enlargement during LTP**. *Neuron* 2015, **85**: 173–189.

52. Opazo P, Labrecque S, Tigaret CM, Frouin A, Wiseman PW, De Koninck P, Choquet D: **CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin**. *Neuron* 2010, **67**:239–252.

53. Chen X, Jia B, Araki Y, Liu B, Ye F, Huganir R, Zhang M: **Arc weakens synapses by dispersing AMPA receptors from postsynaptic density via modulating PSD phase separation**. *Cell Res* 2022, **32**:914–930.

54. Rizzoli SO, Betz WJ: **Synaptic vesicle pools**. *Nat Rev Neurosci* 2005, **6**:57–69.

55. Rosahl TW, Spillane D, Missler M, Herz J, Selig DK, Wolff JR, Hammer RE, Malenka RC, Südhof TC: **Essential functions of synapsins I and II in synaptic vesicle regulation**. *Nature* 1995, **375**:488–493.

56. Wu X, Ganzella M, Zhou J, Zhu S, Jahn R, Zhang M: **Vesicle tethering on the surface of phase-separated active zone condensates**. *Mol Cell* 2021, **81**:13–24 e17.
••
Biochemical reconstitution to demonstrate organizations of different SV pools by phase separation.

57. Hoffmann C, Rentsch J, Tsunoyama TA, Chhabra A, Aguilar Perez G, Chowdhury R, Trnka F, Korobeinikov AA, Shaib AH, Ganzella M, *et al.*: **Synapsin condensation controls synaptic vesicle sequestering and dynamics**. *Nat Commun* 2023, **14**:6730.

58. Park D, Wu Y, Lee SE, Kim G, Jeong S, Milovanovic D, De Camilli P, Chang S: **Cooperative function of synaptophysin and synapsin in the generation of synaptic vesicle-like clusters in non-neuronal cells**. *Nat Commun* 2021, **12**:263.

59. Park D, Fujise K, Wu Y, Luján R, Del Olmo-Cabrera S, Wesseling JF, De Camilli P: **Overlapping role of synaptophysin**

and synaptogyrin family proteins in determining the small size of synaptic vesicles. In *Proceedings of the national academy of sciences*, **121**; 2024, e2409605121.

60. Acuna C, Liu X, Sudhof TC: **How to make an active zone: unexpected universal functional redundancy between RIMs and RIM-BPs**. *Neuron* 2016, **91**:792–807.

61. •• Qiu H, Wu X, Ma X, Li S, Cai Q, Ganzella M, Ge L, Zhang H, Zhang M: **Short-distance vesicle transport via phase separation**. *Cell* 2024, **187**:2175–2193. e2121.

Demonstrated phase separation-mediated, directional transport of SVs from the Synapsin-clustered compartment to the AZ compartment.

62. Fernandez-Busnadiego R, Zuber B, Maurer UE, Cyrklaff M, Baumeister W, Lucic V: **Quantitative analysis of the native presynaptic cytomatrix by cryoelectron tomography**. *J Cell Biol* 2010, **188**:145–156.

63. Tokuoka H, Goda Y: **Myosin light chain kinase is not a regulator of synaptic vesicle trafficking during repetitive exocytosis in cultured hippocampal neurons**. *J Neurosci* 2006, **26**:11606–11614.

64. Mukherjee K, Yang X, Gerber SH, Kwon H-B, Ho A, Castillo PE, Liu X, Südhof TC: **Piccolo and bassoon maintain synaptic vesicle clustering without directly participating in vesicle exocytosis**. In *Proceedings of the national academy of sciences*, **107**; 2010:6504–6509.

65. Ackermann F, Schink KO, Bruns C, Izsvak Z, Hamra FK, Rosenmund C, Garner CC: **Critical role for Piccolo in synaptic vesicle retrieval**. *Elife* 2019, **8**.

66. Limbach C, Laue MM, Wang X, Hu B, Thiede N, Hultqvist G, Kilimann MW: **Molecular in situ topology of Aczonin/Piccolo and associated proteins at the mammalian neurotransmitter release site**. *Proc Natl Acad Sci U S A* 2011, **108**:E392–E401.

67. •• Emperador-Melero J, Andersen JW, Metzbower SR, Levy AD, Dharmasri PA, de Nola G, Blanpied TA, Kaeser PS: **Distinct active zone protein machineries mediate Ca(2+) channel clustering and vesicle priming at hippocampal synapses**. *Nat Neurosci* 2024, **27**:1680–1694.

This study demonstrated phase separation governed multiple subcompartment organization of AZ in living neurons.

68. Mehta N, Mondal S, Watson ET, Cui Q, Chapman ER: **The juxtamembrane linker of synaptotagmin 1 regulates Ca(2+) binding via liquid-liquid phase separation**. *Nat Commun* 2024, **15**:262.

69. Zhu M, Xu H, Jin Y, Kong X, Xu B, Liu Y, Yu H: **Synaptotagmin-1 undergoes phase separation to regulate its calcium-sensitive oligomerization**. *JCB (J Cell Biol)* 2024, **223**, e202311191.

70. Sakamoto H, Ariyoshi T, Kimpara N, Sugao K, Taiko I, Takikawa K, Asanuma D, Namiki S, Hirose K: **Synaptic weight set by Munc13-1 supramolecular assemblies**. *Nat Neurosci* 2018, **21**:41–49.

71. Imoto Y, Raychaudhuri S, Ma Y, Fenske P, Sandoval E, Itoh K, Blumrich E-M, Matsubayashi HT, Mamer L, Zarebidaki F: **Dynamin is primed at endocytic sites for ultrafast endocytosis**. *Neuron* 2022, **110**:2815–2835. e2813.

72. Imoto Y, Xue J, Luo L, Raychaudhuri S, Itoh K, Ma Y, Craft GE, Kwan AH, Ogunmowo TH, Ho A, *et al.*: **Dynamin 1xA interacts with Endophilin A1 via its spliced long C-terminus for ultrafast endocytosis**. *EMBO J* 2024, **43**:3327–3357.

73. Marco de la Cruz B, Campos J, Molinaro A, Xie X, Jin G, Wei Z, Acuna C, Sterky FH: **Liprin-alpha proteins are master regulators of human presynapse assembly**. *Nat Neurosci* 2024, **27**: 629–642.

74. Biederer T, Kaeser PS, Blanpied TA: **Transcellular nanoalignment of synaptic function**. *Neuron* 2017, **96**:680–696.

75. Ramsey AM, Tang A-H, LeGates TA, Gou X-Z, Carbone BE, Thompson SM, Biederer T, Blanpied TA: **Subsynaptic positioning of AMPARs by LRRTM2 controls synaptic strength**. *Sci Adv* 2021, **7**, eabf3126.

76. Perez de Arce K, Schrod N, Metzbower SWR, Allgeyer E, Kong GK, Tang AH, Krupp AJ, Stein V, Liu X, Bewersdorf J, *et al.*: **Topographic mapping of the synaptic cleft into adhesive nanodomains**. *Neuron* 2015, **88**:1165–1172.

77. Trotter JH, Hao J, Maxeiner S, Tsetsenis T, Liu Z, Zhuang X, Sudhof TC: **Synaptic neurexin-1 assembles into dynamically regulated active zone nanoclusters**. *J Cell Biol* 2019, **218**: 2677–2698.

78. Fukata Y, Chen X, Chiken S, Hirano Y, Yamagata A, Inahashi H, Sanbo M, Sano H, Goto T, Hirabayashi M, *et al.*: **LGI1-ADAM22-MAGUK configures transsynaptic nanoalignment for synaptic transmission and epilepsy prevention**. *Proc Natl Acad Sci U S A* 2021, **118**.

79. Tang AH, Chen H, Li TP, Metzbower SR, MacGillavry HD, Blanpied TA: **A trans-synaptic nanocolumn aligns neurotransmitter release to receptors**. *Nature* 2016, **536**:210–214.

80. Feng Z, Lee S, Jia B, Jian T, Kim E, Zhang M: **IRSp53 promotes postsynaptic density formation and actin filament bundling**. *J Cell Biol* 2022, **221**.