Exploring the effect of phosphorylation on protein-protein interactions of PSD-95 using genetically encoded phosphorylated amino acids

Abstract
The postsynaptic density protein 95 (PSD-95) is a key scaffolding protein in the postsynaptic density (PSD) of excitatory glutamatergic neurons. Here it organizes signaling complexes primarily through interactions with its three postsynaptic density/discs large/zonula-occludens (PDZ) domains. PSD-95 is regulated by phosphorylation, but many of the implicated kinases are still unknown. Phosphorylation of PSD-95 has previously been studied on single PDZ domains using a semi-synthetic approach. However, this approach is limited to terminal sites and sequence restrictions, and hence the effect of phosphorylation of full-length PSD-95 and the contribution from the additional protein domains remains to be elucidated.

Here, site-specific phosphorylated Ser (pSer) was introduced at five different Ser residues in PDZ1, PDZ1-2 and PSD-95 using amber codon suppression. The feasibility of genetically introducing pSer was explored, revealing some sites to be more prone for amber codon suppression than others. A total of 11 phosphorylated protein variants were generated and site-specific pSer was verified by mass spectrometry. The effect of phosphorylation on ligand binding was tested by biochemical binding assays and phase separation. Binding affinity increased with protein size and the additional protein domains of PSD-95 were observed to contribute with compensatory mechanisms as the effect of phosphorylation was more potent for PDZ1 and PDZ1-2 than PSD-95. However, two new phosphorylation sites were identified and found to have opposing effects on ligand binding. Phosphorylation of Ser78 inhibited the interaction and phase separation with GluN2B and stargazin, whereas phosphorylation of Ser116 promoted phase separation with stargazin only.

An experimental protocol for site-specific Thr phosphorylation was also established. In contrast to the pSer approach, most attempted phosphorylations displayed low amber codon suppression efficiency, but phosphorylation of Thr19 in PSD-95 was achieved. Furthermore, novel kinases phosphorylating PSD-95 were identified in a large in vitro phosphorylation screen. A dozen of Ser/Thr/Tyr kinases were found to phosphorylate PSD-95, three of which were moreover verified.

In summary, the results presented in this thesis unravel the regulation of PSD-95 by phosphorylation from the bottom, by introducing site-specific phosphorylations and evaluating their effect, to the top, by identifying new and novel kinases phosphorylating the key scaffolding protein. Overall this provides further insight into PSD-95 regulation and the dynamics of the PSD.