DANDRITE Topical Seminar

Tuesday 27 April 2021
13.00 – 14.00

Online via Zoom
If you have not received the link, please request it by emailing: karenb@mbg.au.dk

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Exploring the Role of the PDZ Domain in a Supramodule

The postsynaptic density (PSD) is a large, dense and membraneless compartment of proteins associated below the postsynaptic membrane bilayer, and which constantly undergoes morphological alteration in response to synaptic activity. Formation of PSD is associated with liquid-liquid phase separation of scaffold proteins in complex with other PSD proteins. PSD-95, one of the most abundant scaffold proteins, contains five domains: PDZ1, PDZ2, PDZ3, Src homology 3 (SH3), and guanylate kinase-like (GK) domain. The domains are functionally divided in two supramodules: PDZ1-PDZ2 and PDZ3-SH3-GK (PSG). Multi-domain proteins are characterized through their isolated domains in most studies and represented by "beads on a string" model, which means that the function of a single domain is independent of the context. The properties of PDZ3 and PSG are compared to elucidate how and when PSD-95 can been characterized by the simple "beads on a string" model.

Kinetic characterization of CRIPT binding to PDZ3 showed a two-state mechanism, but a more complex mechanism involving two conformational states upon binding to PSG. The results were consistent with recent structural findings of conformational changes in PSD-95, altogether showing that conformational transitions in supertertiary structures can shape the ligand-binding energy landscape and modulate protein- protein interactions. Next the allosteric networks in a PDZ:ligand complex were experimentally mapped, both in isolation and in the context of a supramodular structure. Data showed that allosteric networks in a PDZ3 domain has high dependency on the supertertiary structure. Furthermore, equilibrium and kinetic folding experiments were applied to demonstrate that the PDZ3 domain folds faster and independently from the SH3-GK tandem, which folds as one cooperative unit. However, concurrent folding of the PDZ3 domain slows down folding of SH3-GK by non-native interactions, resulting in an off-pathway folding intermediate. Finally, the interactome of PSG in PSD was mapped. PDZ3 and PSG show high specificity for peptides with type I PBM. Interestingly, two proteins called SynGap and AGRB1 only bind with high affinity to PSG and forms concentration dependent liquid droplets. The results show how context in terms of supertertiary structure alter affinity and function, and suggest a model for how PSD anchor to the postsynaptic membrane. Altogether, the findings in the thesis show that binding energy landscape, interactome, allosteric network, folding mechanism and phase separation are dependent on the context, which suggest that we need to be careful in interpretation of data obtained from isolated domains
in multi-domain proteins.

Host: Group Leader Poul Nissen, DANDRITE, Dept. Molecular Biology and Genetics, Aarhus University