

Structural Studies on Protein-Protein Interactions in Vesicle Transport with Crystallography and SAXS

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Vesicle transport is responsible for protein trafficking between different organelles and plasma membrane, and is orchestrated by a network of dynamic protein-protein interactions. For vesicles to be formed in response to various signals, membranes have to be bent at the site of invagination/tabulation. There are several mechanisms to achieve this including clathrin coats, coatamer proteins, BAR domains, ESCRT complexes etc. Rab and Arf family proteins are involved in various stages of vesicle transport by interacting with effectors which are often multi domain proteins whose domains make transient but specific interactions. The small GTPases alternate between GTP- and GDP-bound states. They are activated by guanine nucleotide exchange factors (GEF) which replace their GDP with GTP. An example of structural studies of a plant Rab5 will be described where Rab5 will undergo a transformation from a domain swapped, inactive, dimer to a complex with its GEF for GDP-GTP exchange.

Once activated, the GTP-bound Arf or Rab can interact with a number of effectors for specific functions such as cellular localization, control of vesicle budding or fusion, and membrane remodeling. In most cases, the specificities of GTPase towards their cognate effectors are achieved by the surface areas including Switches I/II and interswitch antiparallel beta sheet. This will be illustrated using crystallographic and SAXS examples of Arf1 in complex with the N-terminal GAT domain of GGA, an adaptor protein essential for the protein trafficking and targeting of clathrin coated vesicles. GGA is a multi-domain protein comprised of VHS, GAT, and GAE domains each of which can interact with various partner proteins. How can GGA recognize its partners and how can GGA bind and release them during the protein trafficking processes? We will discuss the effect of binding of cognate peptides to the VHS domain on the overall domain arrangement of GGA in complex with Arf using a combination of X-ray crystallography and SAXS.