

**P011** X-ray crystal structural analysis of ALG-2/Alix peptide complex

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Alix/AIP1 was originally identified as an interacting protein of ALG-2, a member of the penta-EF-hand calcium-binding protein family. ALG-2 binds to a four-tandem PXY-repeat region in the C-terminal proline-rich domain of Alix. In the present study we solved the crystal structure of the complex between ALG-2 and a 16-residue Alix oligopeptide corresponding to Alix799-814. We determined critical residues in Alix and ALG-2 for binding by mutagenesis. The PPYP motif in Alix partly overlaps with the CEP55-binding GPP motif. The Alix peptide binds to ALG-2 at two hydrophobic pockets. Accessibility of PPYP to Pocket 1 is regulated by binding of  $\text{Ca}^{2+}$  to EF3 followed by the movement of the side chain of Arg125, which is present in the loop connecting EF3 and EF4. We propose a  $\text{Ca}^{2+}$ /EF3-driven arginine switch mechanism. The fact that ALG-2 forms a homodimer and each monomer has one peptide binding site indicates the possibility that ALG-2 bridges two interacting proteins, including Alix and TSG101, and functions as a  $\text{Ca}^{2+}$ -dependent adaptor protein, which is presented in the accompanying poster P010.